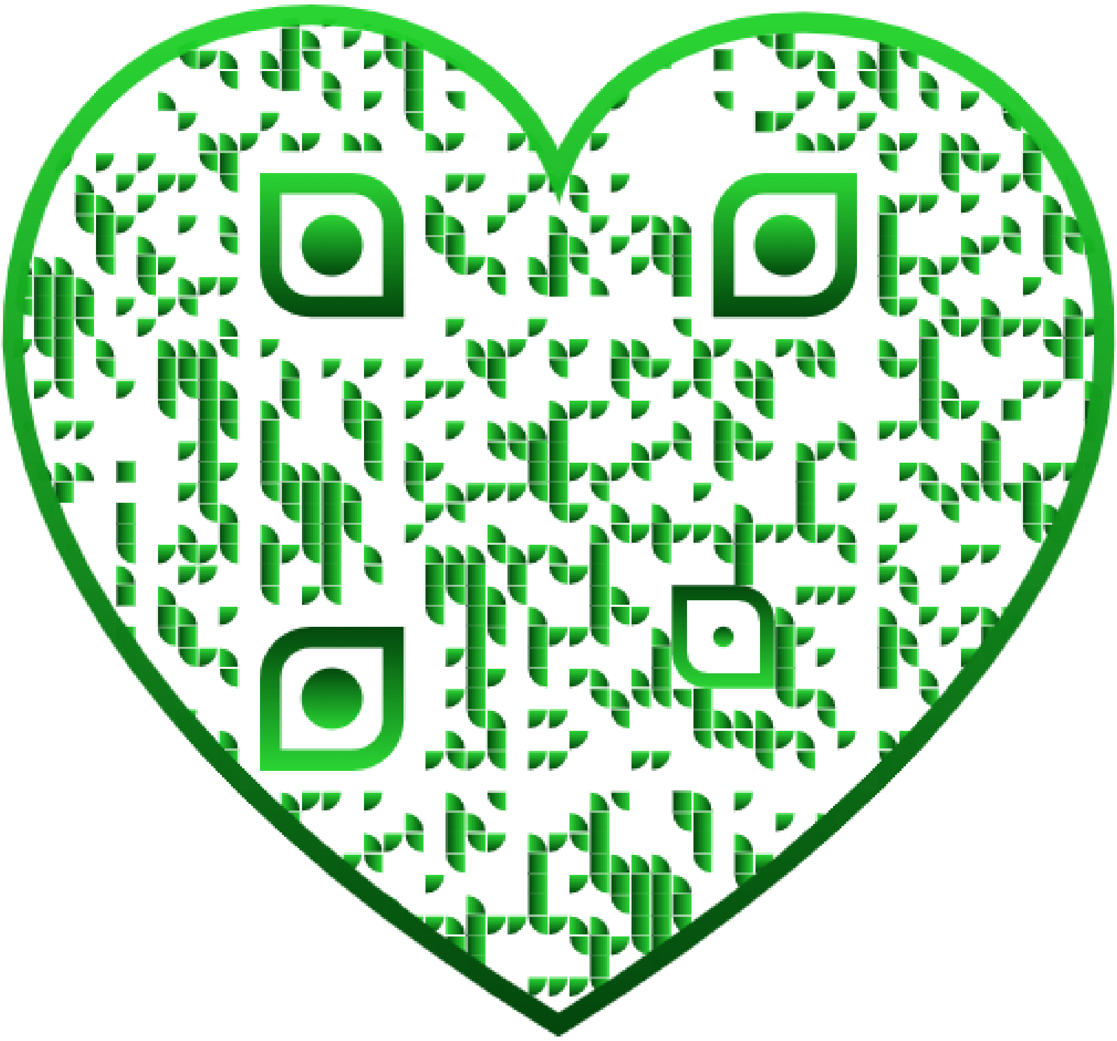
DIEU ET MON DROIT

British Pharmacopoeia



British Pharmacopoeia 2009

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See General Notices

Effective date: 1 January 2009

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British Pharmacopoeia Commission

The British Pharmacopoeia Commission is appointed, on behalf of the Secretary of State for Health, by the Appointments Commission, the body responsible for appointments to all of the Medicines Act 1968 Advisory Bodies.

The duties of the British Pharmacopoeia Commission are as follows:

(a) the preparation under section 99(1) of the Act of any new edition of the British Pharmacopoeia;

(b) the preparation under section 99(1) of the Act, as given effect by section 102(1) thereof, of any amendments of the edition of the British Pharmacopoeia published in 1968 or any new edition of it;

(c) the preparation under section 100 of the Act (which provides for the preparation and publication of lists of names to be used as headings to monographs in the British Pharmacopoeia) of any list of names and the preparation under that section as given effect by section 102(3) of the Act of any amendments of any published list;

(d) the preparation under section 99(6) of the Act, of any compendium, or any new edition thereof, containing information relating to substances and articles which are or may be used in the practice of veterinary medicine or veterinary surgery;

(e) to frame clear and unequivocal technical advice in order to discharge the Commission's responsibilities both for the British Pharmacopoeia, the British Pharmacopoeia (Veterinary) and British Approved Names and as the national pharmacopoeial authority with respect to the European Pharmacopoeia.

Members of the British Pharmacopoeia Commission are appointed for a (renewable) term of 4 years and, under the requirements laid down by the Office of the Commissioner for Public Appointments, can serve for a maximum of 10 years.

British Pharmacopoeia Laboratory Staff

Current members of staff of the British Pharmacopoeia Laboratory who have taken part in the production of this edition include:

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Preface

The British Pharmacopoeia 2009 is published for the Health Ministers on the recommendation of the Commission on Human Medicines in accordance with section 99(6) of the Medicines Act 1968.

The Commission on Human Medicines believes that the British Pharmacopoeia contributes significantly to the overall control of the quality of medicinal products by providing an authoritative statement of the quality that a product, material or article is expected to meet at any time during its period of use. The Pharmacopoeial standards, which are publicly available and legally enforceable, are designed to complement and assist the licensing and inspection processes and are part of the system for safeguarding purchasers and users of medicinal products.

The Commission on Human Medicines wishes to record its appreciation of the services of all those who have contributed to this important work.

Notices

Monographs of the European Pharmacopoeia are distinguished by a chaplet of stars against the title. The term European Pharmacopoeia, used without qualification, means the sixth edition of the European Pharmacopoeia comprising, unless otherwise stated, the main volume, published in 2007 as amended by any subsequent supplements and revisions.

Patents

In this Pharmacopoeia certain drugs and preparations have been included notwithstanding the existence of actual or potential patent rights. In so far as such substances are protected by Letters Patent their inclusion in this Pharmacopoeia neither conveys, nor implies, licence to manufacture.

Effective dates

New and revised monographs of national origin enter into force on 1 January 2009. Monographs of the European Pharmacopoeia have previously been published by the Council of Europe and have been brought into effect by means of Notices published in the Belfast, Edinburgh and London Gazettes.

Introduction

The British Pharmacopoeia 2009 supersedes the British Pharmacopoeia 2008. It has been prepared by the British Pharmacopoeia Commission, with the collaboration and support of its Expert Advisory Groups and Panels of Experts, and contains approximately 3200 monographs for substances, preparations and articles used in the practice of medicine. Some of these monographs are of national origin while others have been reproduced from the 6th edition of the European Pharmacopoeia. This edition, together with its companion edition, the British Pharmacopoeia (Veterinary) 2009, incorporates all the monographs of the 6th edition of the European Pharmacopoeia as amended by Supplements 6.1 and 6.2. The user of the British Pharmacopoeia thereby benefits by finding within this one, comprehensively indexed, compendium all current United Kingdom pharmacopoeial standards for medicines for human use. The new edition comprises five volumes as follows.

Volumes I and II	Medicinal Substances
Volume III	Formulated Preparations, Blood-related Products, Immunological Products, Radiopharmaceutical Preparations, Surgical Materials, Herbal Drugs and Herbal Drug Preparations and Materials for use in the Manufacture of Homoeopathic Preparations
Volume IV	Infrared Reference Spectra, Appendices, Supplementary Chapters and Index
Volume V	British Pharmacopoeia (Veterinary) 2009

Effective Date

The effective date for this edition is 1 January 2009.

Where a monograph which appeared previously in an earlier edition of the British Pharmacopoeia has not been included in this edition, it remains effective in accordance with Section 65(4) of the Medicines Act 1968.

General Notices

The British Pharmacopoeia General Notices (Part II) have been amended as follows.

Labelling This General Notice has been amended to reflect current legislative requirements described in title V of the EC Directive 2001/83/EC as amended.

Additions

A list of monographs included for the first time in the British Pharmacopoeia 2009 is given at the end of this introduction. It includes 42 new monographs of national origin and 55 new monographs reproduced from the 6th Edition of the European Pharmacopoeia as amended by Supplements 6.1 and 6.2.

Traditional Herbal Medicines

Work is continuing on the development of monographs for herbal materials and processed herbs used in Traditional Chinese Medicines. This new edition sees the publication of 6 new monographs. It is emphasized that, although requirements for the *quality* of the material are provided in the monograph to assist the registration scheme by the UK Licensing Authority, the British Pharmacopoeia Commission has not assessed the safety of the material in traditional use.

Homoeopathic Preparations

A further 4 new monographs for homoeopathic stocks and mother tinctures, that support the simplified registration scheme by the UK Licensing Authority, have been added to this edition.

Immunological Products

This new edition sees the publication of a new monograph for Haemophilus Type b and Meningococcal Group C Conjugate Vaccine, a vaccine widely used in the vaccination programme in the United Kingdom.

Unlicensed Medicines

With this new edition, 6 individual monographs for unlicensed formulations have been added. These individual monographs are characterised by a statement that they are unlicensed in the United Kingdom. The general and individual monographs are intended to apply to all types of Unlicensed Medicines, that is, those formulations manufactured under a specials licence and those prepared extemporaneously under the supervision of a pharmacist.

Revisions

National monographs which have been amended technically by means of this edition are listed at the end of this introduction. For the benefit of the reader this list indicates the section, or sections, of each monograph which has/have been revised.

The list is as comprehensive as practicable. However, to ensure that the reader uses the current standard, it is essential to refer to the full text of each individual monograph.

Titles

Monographs for medicinal substances and formulated preparations, the titles of which had been changed from the British Approved Names in the British Pharmacopoeia 2003, had previously included the statement '*The name [former title] was formerly used in the United Kingdom*'. These monographs have been amended to delete the statement in this edition. Only the International Nonproprietary Names have been retained. The exceptions are Adrenaline and Noradrenaline which continue to be dual-labelled in this new edition. Adrenaline and Noradrenaline are the terms used in the European Pharmacopoeia and therefore the official titles used in the 37 Member States party to the Convention on the Elaboration of a European Pharmacopoeia.

Reference Substances

A number of monographs for medicinal substances and formulated preparations of the BP have been amended to refer to new British Pharmacopoeia Chemical Reference Substances established by the British Pharmacopoeia Laboratory.

Tablets

The general monograph for Tablets of the British Pharmacopoeia has been amended to delete the requirement for tablet shape. As a consequence approximately 70 monographs for formulated preparations have been amended.

Unlicensed Medicines

The general monograph for Unlicensed Medicines has been amended to introduce a requirement under Production concerning the need to ensure that, in the manufacturing, packaging and storage of unlicensed oral liquids, the microbial quality meets Pharmacopoeial requirements.

Infrared Reference Spectra

As with the previous edition, the reference spectra are placed in alphabetical order within this edition. Seven new spectra have been added to the collection.

Appendices

A new Appendix XI B 2 (Water-soluble Extractive) has been introduced in this edition to support the monographs for traditional herbal medicines.

The title of Appendix XIV A (Biological Assay of Antibiotics) has been harmonised with the title of the European Pharmacopoeia Method 2.7.2 (Microbiological Assay of Antibiotics). As a consequence, approximately 40 monographs have been amended in the new edition to refer to the new title.

For this edition, 14 new Appendices have been created to incorporate the methods of the European Pharmacopoeia.

Supplementary Chapters

Changes in Monograph Titles

The Supplementary Chapter on Changes in Monograph Titles (Supplementary Chapter II A) has been amended to delete reference to the British Approved Names previously used in monograph titles in earlier editions of the British Pharmacopoeia.

Chromatograms for Information

As an additional service to users, the chromatograms and spectra, produced by the British Pharmacopoeia Laboratory, during evaluation of a number of British Pharmacopoeia monographs for formulated preparations are collated and published in this Supplementary Chapter (Supplementary Chapter IV I).

Editorial Changes

Chromatographic tests

The new format for chromatographic tests, introduced in the BP 2008, to delineate sample preparation, chromatographic conditions, system suitability and acceptance criteria has been refined. The format will continue to be harmonised in future editions for all BP monographs.

Monographs for Herbal and Complementary Medicines

The monographs for herbal and complementary medicines have been reorganised into two sections in Volume III of the new edition. These sections are entitled 'Herbal Drugs and Herbal Drug Preparations' and 'Materials for use in the Manufacture of Homoeopathic Preparations' respectively.

European Pharmacopoeia

In accordance with previous practice, all monographs and requirements of the European Pharmacopoeia are reproduced in this edition of the British Pharmacopoeia or, where appropriate, within its companion edition, the British Pharmacopoeia (Veterinary) 2009.

Where a monograph has been reproduced from the European Pharmacopoeia, this is signified by the presence of a European chaplet of stars alongside its title. Additionally, reference to the European Pharmacopoeia monograph number is included immediately below the title in italics in the form '*Ph Eur monograph xxx*'. Where the title in the British Pharmacopoeia is different from that in the European Pharmacopoeia, an approved synonym has been created (see Appendix XXI B) and the Ph Eur title is included before the monograph number. The entire European Pharmacopoeia text is then bounded by two horizontal lines bearing the symbol '*Ph Eur*'.

The European Pharmacopoeia texts have been reproduced in their entirety but, where deemed appropriate, additional statements of relevance to UK usage have been added (e.g. action and use statement, a list of BP preparations). It should be noted, however, that in the event of doubt of interpretation in any text of the European Pharmacopoeia, the text published in English under the direction of the Council of Europe should be consulted.

Correspondence between the general methods of the European Pharmacopoeia and the appendices of the British Pharmacopoeia is indicated in each appendix and by inclusion of a check list at the beginning of the appendices section.

Pharmacopoeial Requirements

It should be noted that any article intended for medicinal use which is described by a name at the head of a monograph in the current edition of the Pharmacopoeia must comply with that monograph '*whether or not it is referred to as BP*'.

It is also important to note that no requirement of the Pharmacopoeia can be taken in isolation. A valid interpretation of any particular requirement depends upon it being read in the context of (i) the monograph as a whole, (ii) the specified method of analysis, (iii) the relevant General Notices and (iv) where appropriate, the relevant general monograph(s). Familiarity with the General Notices of the Pharmacopoeia will facilitate the correct application of the requirements. Additional guidance and information on the basis of pharmacopoeial requirements is provided in Supplementary Chapter I. This non-mandatory text describes the general underlying philosophy and current approaches to particular aspects of pharmacopoeial control.

Forward Look

A new website, www.pharmacopoeia.gov.uk, is currently being developed. It is intended to increase the scope of information available to British Pharmacopoeia stakeholders and to improve the regulatory transparency of the British Pharmacopoeia.

Acknowledgements

The British Pharmacopoeia Commission is greatly indebted to the members of its Expert Advisory Groups and Panels of Experts without whose dedicated enthusiasm and assistance this edition could not have been prepared.

Close co-operation has continued with many organisations at home and overseas. These include the Medicines and Healthcare products Regulatory Agency, the National Institute for Biological Standards and Control, the Veterinary Medicines Directorate, the Royal Pharmaceutical Society of Great Britain, the Association of the British Pharmaceutical Industry, the British Association of Homoeopathic Manufacturers, the European Pharmacopoeia Commission and the European Directorate for the Quality of Medicines & HealthCare, the Therapeutic Goods Administration (Australia), the Health Protection Branch of the Canadian Department of Health and Welfare, the Committee of Revision of the United States Pharmacopeia, the Essential Drugs and Other Medicines Department of the World Health Organization (WHO) and the WHO Collaborating Centre for Chemical Reference Substances.

The British Pharmacopoeia Commission acknowledges the contribution of H Douglas, T Lau and S Matumo, of the Medicines and Healthcare products Regulatory Agency Laboratory, for their collaboration and advice in the practical evaluation of a number of monographs in this edition.

The British Pharmacopoeia Commission acknowledges the contribution of Professor Frederick A Senese, Department of Chemistry, Frostburg State University, USA, for his kind permission to reproduce the indicator colour chart.

The British Pharmacopoeia Commission also acknowledges the advice of the publishing team at The Stationery Office, in particular, Mr Robert Bullen, Mr Phil Halls, Dr Gill Hodgson and Ms Anna Wilson, in the production of this edition.

Additions

The following monographs of the British Pharmacopoeia 2009 were not included in the British Pharmacopoeia 2008.

Medicinal and Pharmaceutical Substances

Acemetacin*

Adrenaline*

Altizide*

Bisoprolol Fumarate*

Microcrystalline Cellulose and Carmellose Sodium*

Clopamide*

Dacarbazine*

Desflurane*

Dextranomer*

Dorzolamide Hydrochloride*

Felbinac*

Fexofenadine Hydrochloride*

Flavoxate Hydrochloride*

Flucloxacillin Magnesium Octahydrate*

Fluorescein*

Fluvoxamine Maleate*

Glycerol Monocaprylate*

Glycerol Monocaprylocaprate*

Indinavir Sulphate*

Lansoprazole*

Macrogol 40 Sorbitol Heptaoleate*

Anhydrous Magnesium Citrate*

Magnesium Gluconate*

Manganese Gluconate*

Methylergometrine Maleate*

Molsidomine*

Niflumic Acid*

Nilutamide*

Norgestimate*

Pantoprazole Sodium Sesquihydrate*

Racecadotril*

Ritonavir*

Ropivacaine Hydrochloride Monohydrate*

Sertraline Hydrochloride*

Sodium Clodronate Tetrahydrate*

Sodium Phenylbutyrate*

Sucrose Monopalmitate*

Sucrose Stearate*

Telmisartan*

Vinpocetine*

Formulated Preparations: Specific Monographs

Adrenaline and Cocaine Intranasal Solution

Prolonged-release Aminophylline Tablets

Prolonged-release Bezafibrate Tablets

Bupivacaine Heavy Injection

Captopril Oral Solution

Chlorhexidine Gluconate Gel

Clarithromycin Tablets

Prolonged-release Clarithromycin Tablets

Cocaine Paste

Desmopressin Tablets

Desogestrel Tablets

Dexamethasone Eye Drops, Suspension

Dexamethasone Sodium Phosphate Eye Drops, Solution

Prolonged-release Felodipine Tablets

Flucticasone Nasal Drops

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution

Hydrocortisone Sodium Phosphate Oral Solution

Ifosfamide Injection

Iron Sucrose Injection

Mupirocin Cream

Mupirocin Nasal Ointment

Ondansetron Injection

Ondansetron Tablets

Paracetamol, Codeine Phosphate and Caffeine Capsules

Paracetamol, Codeine Phosphate and Caffeine Tablets

Potassium Dihydrogen Phosphate Oral Solution

Prednisolone Sodium Phosphate Ear Drops

Pseuedoephedrine Oral Solution

Gastro-resistant Sulfasalazine Tablets

Vancomycin Oral Solution

Zopiclone Tablets

Blood-related Products

Human α-1-Proteinase Inhibitor*

Immunological Products

Cholera Vaccine (Inactivated, Oral)*

Haemophilus Type b and Meningococcal Group C Conjugate Vaccine

Radiopharmaceutical Preparations

Fluorodopa [^{18}F] Injection*

Herbal Drugs and Herbal Drug Preparations

Refined and Standardised Fresh Bilberry Fruit Dry Extract*

Bistort Rhizome*

Boldo Leaf Dry Extract*

Greater Burnet Root*

Devil's Claw Dry Extract*

Refined and Quantified Ginkgo Dry Extract*

Liquorice Dry Extract for Flavouring Purposes*

Notoginseng Root*

Safflower Flower*

Spanish Sage Oil*

Quantified St. John's Wort Dry Extract*

Salvia Miltiorrhiza Rhizome and Root for use in THM

Processed Salvia Miltiorrhiza Rhizome and Root for use in THMP

Terminalia Arjuna Stem Bark for use in THMP

Terminalia Belerica Fruit for use in THMP

Terminalia Chebula Fruit for use in THMP

Willow Bark Dry Extract*

Withania Somnifera Root for use in THMP

Materials for use in the Manufacture of Homoeopathic Preparations

Apomorphine Hydrochloride for Homoeopathic Preparations

Calcium Phosphate for Homoeopathic Preparations

Hydrated Iron (III) Phosphate for Homoeopathic Preparations

Hydrated Iron (II) and Iron (III) Phosphate for Homoeopathic Preparations

Omissions

The following monographs of the British Pharmacopoeia 2008 are not included in the British Pharmacopoeia 2009.

Medicinal and Pharmaceutical Substances

Acriflavinium Chloride[1]

Stanozolol[2]

Formulated Preparations: Specific Monographs

Stanozolol Tablets

Immunological Products

Pertussis Vaccine[2]

Technical Changes

The following monographs in the British Pharmacopoeia 2009 have been technically amended since the publication of the British Pharmacopoeia 2008. This list does not include revised monographs of the European Pharmacopoeia. An indication of the nature of the change or the section of the monograph that has been changed is given in *italic type* in the right hand column.

Medicinal and Pharmaceutical Substances

Diphenylpyraline Hydrochloride	*Assay*
Hydroxychloroquine Sulphate	*Assay*
Phenindamine Tartrate	*Assay*
Quillaia	*Identification; Extractive Soluble in Ethanol (45%)*
Squill	*Identification; Extractive Soluble in Ethanol (60%)*

Formulated Preparations: Specific Monographs

Amikacin Injection	*Kanamycin (deleted); Related substances; Assay*
Amoxicillin Injection	*Identification test C*
Concentrated Anise Water	*Definition; Extemporaneous preparation*
Beclometasone Pressurised Inhalation	*Related substances*
Gastro-resistant Bisacodyl Tablets	*Related substances; Assay*
Caffeine Citrate Oral Solution	*Acidity (deleted)*
Calamine Lotion	*Identification test A*
Cefotaxime Injection	*Related substances; Assay*
Co-proxamol Tablets	*Introductory statement*
Dantrolene Oral Suspension	*Dissolution*
Desmopressin Injection	*Acidity; Related substances; Assay*
Desmopressin Intranasal Solution	*Acidity; Related substances; Assay*
Erythromycin Lactobionate Intravenous Infusion	*Related substances*
Meloxicam Tablets	*Related substances*
Mercaptopurine Tablets	*Identification; Dissolution; Related substances*
Methotrexate Injection	*Related substances; Assay*
Methotrexate Tablets	*Related substances*
Camphorated Opium Tincture	*Definition; Extemporaneous preparation*
Concentrated Camphorated Opium Tincture	*Definition; Extemporaneous preparation*
Compound Orange Spirit	*Definition*
Pancuronium Injection	*Related substances*
Piperacillin Intravenous Infusion	*Identification test C*
Pyridostigmine Tablets	*Related substances*
Senna Tablets	*Loss on drying (deleted)*
Hydrogenated Vegetable Oil	*Heavy metals*

Herbal Drugs and Herbal Drug Preparations

Angelica Sinensis Root for use in THM	*Identification test C*
Processed Angelica Sinensis Root for use in THMP	*Identification test C*
Astragalus Root for use in THM	*Identification test C*
Processed Astragalus Root for use in THMP	*Identification test C*
White Peony Root for use in THM	*Identification tests A and D*
Processed White Peony Root for use in THMP	*Identification test D*

Changes in Title

The following list gives the alterations in the titles of monographs of the British Pharmacopoeia 2008 that have been retained in the British Pharmacopoeia 2009.

BRITISH PHARMACOPOEIA 2008	**BRITISH PHARMACOPOEIA 2009**
Medicinal and Pharmaceutical Substances	
Streptokinase Bulk Solution	Streptokinase Concentrated Solution

Reference Substances

A number of formulated preparation monographs of the British Pharmacopoeia 2009 have been revised to refer to British Pharmacopoeia Chemical Reference Substances (BPCRS). The following is a list of those reference standards that have been revised.

Amiodarone Hydrochloride

Amoxicillin Trihydrate

Ampicillin Trihydrate

Anhydrous Ampicillin

Betamethasone

Cefadroxil

Cefradine

Chlortetracycline Hydrochloride

Colecalciferol

Cloxacillin Sodium

Cortisone Acetate

Dexamethasone

Doxycycline Hyclate

Ergocalciferol

Erythromycin

Erythromycin A

Erythromycin B

Erythromycin C

Erythromycin Estolate

Erythromycin Ethyl Succinate

Flucloxacillin Sodium

Fluocinolone Acetonide

Fluoxetine Hydrochloride

Hydrocortisone

Hydrocortisone Acetate

Isopromethazine Hydrochloride

Isosorbide Dinitrate

Isosorbide Mononitrate

Isosorbide-2-nitrate

Lidocaine Hydrochloride

Medroxyprogesterone Acetate

Methylprednisolone

Nabumetone Impurity F

Prednisolone

Prednisolone Sodium Phosphate

Temazepam

Testosterone Propionate

Tetracycline Hydrochloride

Theophylline

Triamcinolone

Triamcinolone Acetonide

[*] denotes a monograph of the European Pharmacopoeia
[1] Monograph suppressed by European Pharmacopoeia Commission on 1 January 2008.
[2] Monograph suppressed by European Pharmacopoeia Commission on 1 April 2008.

British Pharmacopoeia Staff

Members of staff who have taken part in the production of this edition include:

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Expert Advisory Groups, Panels of Experts and Working Parties

Members of Expert Advisory Groups, Panels of Experts and Working Parties are appointed by the British Pharmacopoeia Commission.

The duties of the members are as follows:

(a) To collaborate in the preparation and revision of Monographs, Appendices and Supplementary Chapters for inclusion in the British Pharmacopoeia and British Pharmacopoeia (Veterinary).

(b) To collaborate in the preparation and revision of Monographs, Methods and General Chapters of the European Pharmacopoeia.

(c) To collaborate in the preparation and revision of the list of names to be used as titles for monographs of the British Pharmacopoeia and British Pharmacopoeia (Veterinary).

Members of Expert Advisory Groups, Panels of Experts and Working Parties are usually appointed for a (renewable) term of 4 years.

Foreword

Since 1864, the British Pharmacopoeia has been providing authoritative official standards for pharmaceutical substances and medicinal products. The 2009 edition (BP 2009) continues this tradition. It makes an important contribution, therefore, to the role of the Medicines and Healthcare products Regulatory Agency in protecting public health by setting publicly available standards for the quality of medicines.

In addition to expanding the numbers of monographs for licensed formulated products, this edition supports the new regulatory work in the fields of herbal and complementary medicines by providing new monographs for traditional herbal medicinal products and for homoeopathic stocks and mother tinctures. The 2009 edition continues to improve the control of the quality of unlicensed medicines by providing standards for extensively used unlicensed formulations together with legal and ethical guidance on the preparation and supply of these products.

The British Pharmacopoeia maintains close ties with the work of the European Pharmacopoeia and continues to play a significant role in the standard-setting process in Europe, participating in the activities of the European Directorate for the Quality of Medicines and HealthCare, and influencing the decisions of the European Pharmacopoeia Commission through the United Kingdom Delegation. The texts and monographs of the European Pharmacopoeia form an integral part of the BP 2009.

Annually updated, the British Pharmacopoeia is the only comprehensive collection of standards for UK medicinal substances. It is essential for all individuals and organisations involved in pharmaceutical research, development, manufacture, quality control and analysis.

Sir Alasdair Breckenridge

Chairman, MHRA

Part I

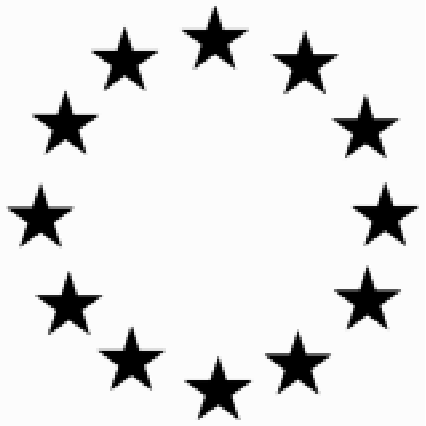
General Notices

Part I

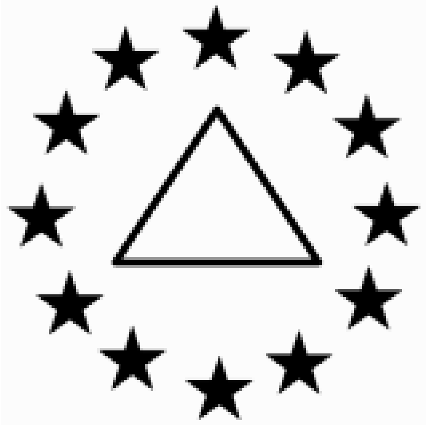
The British Pharmacopoeia comprises the entire text within this publication. The word official' is used in the Pharmacopoeia to signify 'of the Pharmacopoeia'. It applies to any title, substance, preparation, method or statement included in the general notices, monographs and appendices of the Pharmacopoeia. The abbreviation for British Pharmacopoeia is BP.

European Pharmacopoeia

Monographs of the European Pharmacopoeia are reproduced in this edition of the British Pharmacopoeia by incorporation of the text published under the direction of the Council of Europe (Partial Agreement) in accordance with the Convention on the Elaboration of a European Pharmacopoeia (Treaty Series No. 32 (1974) CMND 5763) as amended by the Protocol to the Convention (Treaty Series No. MISC16 (1990) CMND 1133). They are included for the convenience of users of the British Pharmacopoeia. In cases of doubt or dispute reference should be made to the Council of Europe text.



Monographs of the European Pharmacopoeia are distinguished by a chaplet of stars against the title and by reference to the European Pharmacopoeia monograph number included immediately below the title in italics. The beginning and end of text from the European Pharmacopoeia are denoted by means of horizontal lines with the symbol '*Ph Eur*' ranged left and right, respectively.



Inclusion of a triangle within the chaplet of stars denotes monographs that have been adopted by the European Pharmacopoeia Commission following their preparation according to a procedure of harmonisation agreed between the bodies responsible for the European Pharmacopoeia and those of Japan and the United States of America.

The general provisions of the European Pharmacopoeia relating to different types of dosage form are included in the appropriate general monograph in that section of the British Pharmacopoeia entitled Monographs: Formulated Preparations. These general provisions apply to all dosage forms of the type defined, whether an individual monograph is included in the British Pharmacopoeia or not.

Texts of the European Pharmacopoeia are governed by the General Notices of the European Pharmacopoeia. These are reproduced as Part III of these notices.

Part II

General Notices Part II

The following general notices apply to the statements made in the monographs of the British Pharmacopoeia other than those reproduced from the European Pharmacopoeia and to the statements made in the Appendices of the British Pharmacopoeia other than when a method, test or other matter described in an appendix is invoked in a monograph reproduced from the European Pharmacopoeia.

Official Standards

The requirements stated in the monographs of the Pharmacopoeia apply to articles that are intended for medicinal use but not necessarily to articles that may be sold under the same name for other purposes. An article intended for medicinal use that is described by means of an official title must comply with the requirements of the relevant monograph. A formulated preparation must comply throughout its assigned shelf-life (period of validity). The subject of any other monograph must comply throughout its period of use.

A monograph is to be construed in accordance with any general monograph or notice or any appendix, note or other explanatory material that is contained in this edition and that is applicable to that monograph. All statements contained in the monographs, except where a specific general notice indicates otherwise and with the exceptions given below, constitute standards for the official articles. An article is not of pharmacopoeial quality unless it complies with all of the requirements stated. This does not imply that a manufacturer is obliged to perform all the tests in a monograph in order to assess compliance with the Pharmacopoeia before release of a product. The manufacturer may assure himself that a product is of pharmacopoeial quality by other means, for example, from data derived from validation studies of the manufacturing process, from in-process controls or from a combination of the two. Parametric release in appropriate circumstances is thus not precluded by the need to comply with the Pharmacopoeia. The general notice on Assays and Tests indicates that analytical methods other than those described in the Pharmacopoeia may be employed for routine purposes.

Requirements in monographs have been framed to provide appropriate limitation of potential impurities rather than to provide against all possible impurities. Material found to contain an impurity not detectable by means of the prescribed tests is not of pharmacopoeial quality if the nature or amount of the impurity found is incompatible with good pharmaceutical practice.

The status of any statement given under the headings Definition, Production, Characteristics, Storage, Labelling or Action and use is defined within the general notice relating to the relevant heading. In addition to any exceptions indicated by one of the general notices referred to above, the following parts of a monograph do not constitute standards: (a) a graphic or molecular formula given at the beginning of a monograph; (b) a molecular weight; (c) a Chemical Abstracts Service Registry Number; (d) any information given at the end of a monograph concerning impurities known to be limited by that monograph; (e) information in any annex to a monograph. Any statement containing the word 'should' constitutes non-mandatory advice or recommendation.

The expression 'unless otherwise justified and authorised' means that the requirement in question has to be met, unless a competent authority authorises a modification or exemption where justified in a particular case. The term 'competent authority' means the national, supranational or international body or organisation vested with the authority for making decisions concerning the issue in question. It may, for example, be a licensing authority or an official control laboratory. For a formulated preparation that is the subject of monograph in the British Pharmacopoeia any justified and authorised modification to, or exemption from, the requirements of the relevant general monograph of the European Pharmacopoeia is stated in the individual monograph. For example, the general monograph for Tablets requires that Uncoated Tablets, except for chewable tablets, disintegrate within 15 minutes; for Calcium Lactate Tablets a time of 30 minutes is permitted.

Many of the general monographs for formulated preparations include statements and requirements additional to those of the European Pharmacopoeia that are applicable to the individual monographs of the British Pharmacopoeia. Such statements and requirements apply to all monographs for that dosage form included in the Pharmacopoeia unless otherwise indicated in the individual monograph.

Where a monograph on a biological substance or preparation refers to a strain, a test, a method, a substance, etc., using the qualifications 'suitable' or 'appropriate' without further definition in the text, the choice of such strain, test, method, substance, etc., is made in accordance with any international agreements or national regulations affecting the subject concerned.

Definition of Terms

Where the term "about" is included in a monograph or test it should be taken to mean approximately (fairly correct or accurate; near to the actual value).

Where the term "corresponds" is included in a monograph or test it should be taken to mean similar or equivalent in character or quantity.

Where the term "similar" is included in a monograph or test it should be taken to mean alike though not necessarily identical.

Further qualifiers (such as numerical acceptance criteria) for the above terms are not included in the BP. The acceptance criteria for any individual case is set based on the range of results obtained from known reference samples, the level of precision of the equipment or apparatus used and the level of accuracy required for the particular application. The user should determine the variability seen in his/her own laboratory and set in-house acceptance criteria that he/she judges to be appropriate based on the local operating conditions.

Expression of Standards

Where the standard for the content of a substance described in a monograph is expressed in terms of the chemical formula for that substance an upper limit exceeding 100% may be stated. Such an upper limit applies to the result of the assay calculated in terms of the equivalent content of the specified chemical formula. For example, the statement 'contains not less than 99.0% and not more than 101.0% of $C_{20}H_{24}N_2O_2$,HCl' implies that the result of the assay is not less than 99.0% and not more than 101.0%, calculated in terms of the equivalent content of $C_{20}H_{24}N_2O_2$,HCl.

Where the result of an assay or test is required to be calculated with reference to the dried, anhydrous or ignited substance, the substance free from a specified solvent or to the peptide content, the determination of loss on drying, water content, loss on ignition, content of the specified solvent or peptide content is carried out by the method prescribed in the relevant test in the monograph.

Temperature

The Celsius thermometric scale is used in expressing temperatures.

Weights and Measures

The metric system of weights and measures is employed; SI Units have generally been adopted. Metric measures are required to have been graduated at 20°C and all measurements involved in the analytical operations of the Pharmacopoeia are intended, unless otherwise stated, to be made at that temperature. Graduated glass apparatus used in analytical operations should comply with Class A requirements of the appropriate International Standard issued by the International Organization for Standardization.

Atomic Weights

The atomic weights adopted are the values given in the Table of Relative Atomic Weights 2001 published by the International Union of Pure and Applied Chemistry (Appendix XXV).

Constant Weight

The term 'constant weight', used in relation to the process of drying or the process of ignition, means that two consecutive weighings do not differ by more than 0.5 mg, the second weighing being made after an additional period of drying or ignition under the specified conditions appropriate to the nature and quantity of the residue (1 hour is usually suitable).

Expression of Concentrations

The term 'per cent' or more usually the symbol '%' is used with one of four different meanings in the expression of concentrations according to circumstances. In order that the meaning to be attached to the expression in each instance is clear, the following notation is used:

Per cent w/w (% w/w) (percentage weight in weight) expresses the number of grams of solute in 100 g of product.

Per cent w/v (% w/v) (percentage weight in volume) expresses the number of grams of solute in 100 ml of product.

Per cent v/v (% v/v) (percentage volume in volume) expresses the number of millilitres of solute in 100 ml of product.

Per cent v/w (% v/w) (percentage volume in weight) expresses the number of millilitres of solute in 100 g of product.

Usually the strength of solutions of solids in liquids is expressed as percentage weight in volume, of liquids in liquids as percentage volume in volume and of gases in liquids as percentage weight in weight.

When the concentration of a solution is expressed as parts per million (p.p.m.), it means weight in weight, unless otherwise specified.

When the concentration of a solution is expressed as parts of dissolved substance in parts of the solution, it means parts by weight (g) of a solid in parts by volume (ml) of the final solution; or parts by volume (ml) of a liquid in parts by volume (ml) of the final solution; or parts by weight (g) of a gas in parts by weight (g) of the final solution.

When the concentration of a solution is expressed in molarity designated by the symbol M preceded by a number, it denotes the number of moles of the stated solute contained in sufficient Purified Water (unless otherwise stated) to produce 1 litre of solution.

Water Bath

The term 'water bath' means a bath of boiling water, unless water at some other temperature is indicated in the text. An alternative form of heating may be employed providing that the required temperature is approximately maintained but not exceeded.

Reagents

The reagents required for the assays and tests of the Pharmacopoeia are defined in appendices. The descriptions set out in the appendices do not imply that the materials are suitable for use in medicine.

Indicators

Indicators, the colours of which change over approximately the same range of pH, may be substituted for one another but in the event of doubt or dispute as to the equivalence of indicators for a particular purpose, the indicator specified in the text is alone authoritative.

The quantity of an indicator solution appropriate for use in acid-base titrations described in assays or tests is 0.1 ml unless otherwise stated in the text.

Any solvent required in an assay or test in which an indicator is specified is previously neutralised to the indicator, unless a blank test is prescribed.

Caution Statements

A number of materials described in the monographs and some of the reagents specified for use in the assays and tests of the Pharmacopoeia may be injurious to health unless adequate precautions are taken. The principles of good laboratory practice and the provisions of any appropriate regulations such as those issued in the United Kingdom in accordance with the Health and Safety at Work etc. Act 1974 should be observed at all times in carrying out the assays and tests of the Pharmacopoeia.

Attention is drawn to particular hazards in certain monographs by means of an italicised statement; the absence of such a statement should not however be taken to mean that no hazard exists.

Titles

Subsidiary titles, where included, have the same significance as the main titles. An abbreviated title constructed in accordance with the directions given in Appendix XXI A has the same significance as the main title.

Titles that are derived by the suitable inversion of words of a main or subsidiary title, with the addition of a preposition if appropriate, are also official titles. Thus, the following are all official titles: Aspirin Tablets, Tablets of Aspirin; Ginger Tincture, Tincture of Ginger; Atropine Injection, Injection of Atropine.

A title of a formulated preparation that includes the full nonproprietary name of the active ingredient or ingredients, where this is not included in the title of the monograph, is also an official title. For example, the title Promethazine Hydrochloride Oral Solution has the same significance as Promethazine Oral Solution and the title Brompheniramine Maleate Tablets has the same significance as Brompheniramine Tablets.

Where the English title at the head of a monograph in the European Pharmacopoeia is different from that at the head of the text incorporated into the British Pharmacopoeia, an Approved Synonym has been declared in accordance with section 65(8) of the Medicines Act 1968. The titles and subsidiary titles, if any, are thus official titles. A cumulative list of such Approved Synonyms is provided in Appendix XXI B.

Where the names of pharmacopoeial substances, preparations and other materials occur in the text they are printed with capital initial letters and this indicates that materials of Pharmacopoeial quality must be used. Words in the text that name a reagent or other material, a physical characteristic or a process that is described or defined in an appendix are printed in italic type, for example, *methanol*, *absorbance*, *gas chromatography*, and these imply compliance with the requirements specified in the appropriate appendix.

Chemical Formulae

When the chemical composition of an official substance is known or generally accepted, the graphic and molecular formulae, the molecular weight and the Chemical Abstracts Service Registry Number are normally given at the beginning of the monograph for information. This information refers to the chemically pure substance and is not to be regarded as an indication of the purity of the official material. Elsewhere, in statements of standards of purity and strength and in descriptions of processes of assay, it is evident from the context that the formulae denote the chemically pure substances.

Where the absolute stereochemical configuration is specified, the International Union of Pure and Applied Chemistry (IUPAC) *R/S* and *E/Z* systems of designation have been used. If the substance is an enantiomer of unknown absolute stereochemistry the sign of the optical rotation, as determined in the solvent and under the conditions specified in the monograph, has been attached to the systematic name. An indication of sign of rotation has also been given where this is incorporated in a trivial name that appears on an IUPAC preferred list.

All amino acids, except glycine, have the L-configuration unless otherwise indicated. The three-letter and one-letter symbols used for amino acids in peptide and protein sequences are those recommended by the Joint Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry.

In the graphic formulae the following abbreviations are used:

Me	$-CH_3$	Bu^s-	$CH(CH_3)CH_2CH_3$
Et	$-CH_2CH_3$	Bu^n-	$CH_2CH_2CH_2CH_3$
Pr^i	$-CH(CH_3)_2$	Bu^t-	$C(CH_3)_3$
Pr^n	$-CH_2CH_2CH_3$	Ph–	C_6H_5
Bu^i	$-CH_2CH(CH_3)_2$	Ac–	$COCH_3$

Definition

Statements given under the heading Definition constitute an official definition of the substance, preparation or other article that is the subject of the monograph. They constitute instructions or requirements and are mandatory in nature.

Certain medicinal or pharmaceutical substances and other articles are defined by reference to a particular method of manufacture. A statement that a substance or article *is* prepared or obtained by a certain method constitutes part of the official definition and implies that other methods are not permitted. A statement that a substance *may be* prepared or obtained by a certain method, however, indicates that this is one possible method and does not imply that other methods are proscribed.

Additional statements concerning the definition of formulated preparations are given in the general notice on Manufacture of Formulated Preparations.

Production

Statements given under the heading Production draw attention to particular aspects of the manufacturing process but are not necessarily comprehensive. They constitute mandatory instructions to manufacturers. They may relate, for example, to source materials, to the manufacturing process itself and its validation and control, to in-process testing or to testing that is to be carried out by the manufacturer on the final product (bulk material or dosage form) either on selected batches or on each batch prior to release. These statements cannot necessarily be verified on a sample of the final product by an independent analyst. The competent authority may establish that the instructions have been followed, for example, by examination of data received from the manufacturer, by inspection or by testing appropriate samples.

The absence of a section on Production does not imply that attention to features such as those referred to above is not required. A substance, preparation or article described in a monograph of the Pharmacopoeia is to be manufactured in accordance with the principles of good manufacturing practice and in accordance with relevant international agreements and supranational and national regulations governing medicinal products.

Where in the section under the heading Production a monograph on a vaccine defines the characteristics of the vaccine strain to be used, any test methods given for confirming these characteristics are provided as examples of suitable methods. The use of these methods is not mandatory.

Additional statements concerning the production of formulated preparations are given in the general notice on Manufacture of Formulated Preparations.

Manufacture of Formulated Preparations

Attention is drawn to the need to observe adequate hygienic precautions in the preparation and dispensing of pharmaceutical formulations. The principles of good pharmaceutical manufacturing practice should be observed.

The Definition in certain monographs for pharmaceutical preparations is given in terms of the principal ingredients only. Any ingredient, other than those included in the Definition, must comply with the general notice on Excipients and the product must conform with the Pharmacopoeial requirements.

The Definition in other monographs for pharmaceutical preparations is presented as a full formula. No deviation from the stated formula is permitted except those allowed by the general notices on Colouring Agents and Antimicrobial Preservatives. Where additionally directions are given under the heading Extemporaneous Preparation these are intended for the extemporaneous preparation of relatively small quantities for short-term supply and use. When so prepared, no deviation from the stated directions is permitted. If, however, such a pharmaceutical preparation is manufactured on a larger scale with the intention that it may be stored, deviations from the stated directions are permitted provided that the final product meets the following criteria:

(1) compliance with all of the requirements stated in the monograph;

(2) retention of the essential characteristics of the preparation made strictly in accordance with the directions of the Pharmacopoeia.

Monographs for yet other pharmaceutical preparations include both a Definition in terms of the principal ingredients and, under the side-heading Extemporaneous Preparation, a full formula together with, in some cases, directions for their preparation. Such full formulae and directions are intended for the extemporaneous preparation of relatively small quantities for short-term supply and use. When so prepared, no deviation from the stated formula and directions is permitted. If, however, such a pharmaceutical preparation is manufactured on a larger scale with the intention that it may be stored, deviations from the formula and directions stated under the heading Extemporaneous Preparation are permitted provided that any ingredient, other than those included in the Definition, complies with the general notice on Excipients and that the final product meets the following criteria:

(1) accordance with the Definition stated in the monograph;

(2) compliance with all of the requirements stated in the monograph;

(3) retention of the essential characteristics of the preparation made strictly in accordance with the formula and directions of the Pharmacopoeia.

In the manufacture of any official preparation on a large scale with the intention that it should be stored, in addition to following any instruction under the heading Production, it is necessary to ascertain that the product is satisfactory with respect to its physical and chemical stability and its state of preservation over the claimed shelf-life. This applies irrespective of whether the formula of the Pharmacopoeia and any instructions given under the heading Extemporaneous Preparation are followed precisely or modified. Provided that the preparation has been shown to be stable in other respects, deterioration due to microbial contamination may be inhibited by the incorporation of a suitable antimicrobial preservative. In such circumstances the label states appropriate storage conditions, the date after which the product should not be used and the identity and concentration of the antimicrobial preservative.

Freshly and Recently Prepared

The direction, given under the heading Extemporaneous Preparation, that a preparation must be freshly prepared indicates that it must be made not more than 24 hours before it is issued for use. The direction that a preparation should be recently prepared indicates that deterioration is likely if the preparation is stored for longer than about 4 weeks at 15°C to 25°C.

Methods of Sterilisation

The methods of sterilisation used in preparing the sterile materials described in the Pharmacopoeia are given in Appendix XVIII. For aqueous preparations, steam sterilisation (heating in an autoclave) is the method of choice wherever it is known to be suitable. Any method of sterilisation must be validated with respect to both the assurance of sterility and the integrity of the product and to ensure that the final product complies with the requirements of the monograph.

Water

The term water used without qualification in formulae for formulated preparations means either potable water freshly drawn direct from the public supply and suitable for drinking or freshly boiled and cooled Purified Water. The latter should be used if the public supply is from a local storage tank or if the potable water is unsuitable for a particular preparation.

Excipients

Where an excipient for which there is a pharmacopoeial monograph is used in preparing an official preparation it shall comply with that monograph. Any substance added in preparing an official preparation shall be innocuous, shall have no adverse influence on the therapeutic efficacy of the active ingredients and shall not interfere with the assays and tests of the Pharmacopoeia. Particular care should be taken to ensure that such substances are free from harmful organisms.

Colouring Agents

If in a monograph for a formulated preparation defined by means of a full formula a specific colouring agent or agents is prescribed, suitable alternatives approved in the country concerned may be substituted.

Antimicrobial Preservatives

When the term 'suitable antimicrobial preservative' is used it is implied that the preparation concerned will be effectively preserved according to the appropriate criteria applied and interpreted as described in the test for *efficacy of antimicrobial preservation* (Appendix XVI C). In certain monographs for formulated preparations defined by means of a full formula, a specific antimicrobial agent or agents may be prescribed; suitable alternatives may be substituted provided that their identity and concentration are stated on the label.

Characteristics

Statements given under the heading Characteristics are not to be interpreted in a strict sense and are not to be regarded as official requirements. Statements on taste are provided only in cases where this property is a guide to the acceptability of the material (for example, a material used primarily for flavouring). The status of statements on solubility is given in the general notice on Solubility.

Solubility Statements on solubility given under the heading Characteristics are intended as information on the approximate solubility at a temperature between 15°C and 25°C, unless otherwise stated, and are not to be considered as official requirements.

Statements given under headings such as Solubility in ethanol express exact requirements and constitute part of the standards for the substances under which they occur.

The following table indicates the meanings of the terms used in statements of approximate solubilities.

Descriptive term	Approximate volume of solvent in millilitres per gram of solute
very soluble	less than 1
freely soluble	from 1 to 10
soluble	from 10 to 30
sparingly soluble	from 30 to 100
slightly soluble	from 100 to 1000
very slightly soluble	from 1000 to 10 000
practically insoluble	more than 10 000

The term 'partly soluble' is used to describe a mixture of which only some of the components dissolve.

Identification

The tests described or referred to under the heading Identification are not necessarily sufficient to establish absolute proof of identity. They provide a means of verifying that the identity of the material being examined is in accordance with the label on the container.

Unless otherwise prescribed, identification tests are carried out at a temperature between 15°C and 25°C.

Reference spectra Where a monograph refers to an infrared reference spectrum, this spectrum is provided in a separate section of the Pharmacopoeia. A sample spectrum is considered to be concordant with a reference spectrum if the transmission minima (absorption maxima) of the principal bands in the sample correspond in position, relative intensities and shape to those of the reference. Instrumentation software may be used to calculate concordance with a previously recorded reference spectrum.

When tests for infrared absorption are applied to material extracted from formulated preparations, strict concordance with the specified reference spectrum may not always be possible, but nevertheless a close resemblance between the spectrum of the extracted material and the specified reference spectrum should be achieved.

Assays and Tests

The assays and tests described are the official methods upon which the standards of the Pharmacopoeia depend. The analyst is not precluded from employing alternative methods, including methods of micro-analysis, in any assay or test if it is known that the method used will give a result of equivalent accuracy. Local reference materials may be used for routine analysis, provided that these are calibrated against the official reference materials. In the event of doubt or dispute, the methods of analysis, the reference materials and the reference spectra of the Pharmacopoeia are alone authoritative.

Where the solvent used for a solution is not named, the solvent is Purified Water.

Unless otherwise prescribed, the assays and tests are carried out at a temperature between 15°C and 25°C.

A temperature in a test for Loss on drying, where no temperature range is given, implies a range of ±2°C about the stated value.

Visual comparative tests, unless otherwise prescribed, are carried out using identical tubes of colourless, transparent, neutral glass with a flat base. The volumes of liquid prescribed are for use with tubes 16 mm in internal diameter; tubes with a larger internal diameter may be used but the volume of liquid examined must be increased so that the depth of liquid in the tubes is not less than that obtained when the prescribed volume of liquid and tubes 16 mm in internal diameter are used. Equal volumes of the liquids to be compared are examined down the vertical axis of the tubes against a white background or, if necessary, against a black background. The examination is carried out in diffuse light.

Where a direction is given that an analytical operation is to be carried out in subdued light', precautions should be taken to avoid exposure to direct sunlight or other strong light. Where a direction is given that an analytical operation is to be carried out 'protected from light', precautions should be taken to exclude actinic light by the use of low-actinic glassware, working in a dark room or similar procedures.

For preparations other than those of fixed strength, the quantity to be taken for an assay or test is usually expressed in terms of the active ingredient. This means that the quantity of the active ingredient expected to be present and the quantity of the preparation to be taken are calculated from the strength stated on the label.

In assays the approximate quantity to be taken for examination is indicated but the quantity actually used must not deviate by more than 10% from that stated. The quantity taken is accurately weighed or measured and the result of the assay is calculated from this exact quantity. Reagents are measured and the procedures are carried out with an accuracy commensurate with the degree of precision implied by the standard stated for the assay.

In tests the stated quantity to be taken for examination must be used unless any divergence can be taken into account in conducting the test and calculating the result. The quantity taken is accurately weighed or measured with the degree of precision implied by the standard or, where the standard is not stated numerically (for example, in tests for Clarity and colour of solution), with the degree of precision implied by the number of significant figures stated. Reagents are measured and the procedures are carried out with an accuracy commensurate with this degree of precision.

The limits stated in monographs are based on data obtained in normal analytical practice; they take account of normal analytical errors, of acceptable variations in manufacture and of deterioration to an extent considered acceptable. No further tolerances are to be applied to the limits prescribed to determine whether the article being examined complies with the requirements of the monograph.

In determining compliance with a numerical limit, the calculated result of a test or assay is first rounded to the number of significant figures stated, unless otherwise prescribed. The last figure is increased by 1 when the part rejected is equal to or exceeds one half-unit, whereas it is not modified when the part rejected is less than a half-unit.

In certain tests, the concentration of impurity is given in parentheses either as a percentage or in parts per million by weight (ppm). In chromatographic tests such concentrations are stated as a percentage irrespective of the limit. In other tests they are usually stated in ppm unless the limit exceeds 500 ppm. In those chromatographic tests in which a secondary spot or peak in a chromatogram obtained with a solution of the substance being examined is described as corresponding to a named impurity and is compared with a spot or peak in a chromatogram obtained with a reference solution of the same impurity, the percentage given in parentheses indicates the limit for that impurity. In those chromatographic tests in which a spot or peak in a chromatogram obtained with a solution of the substance being examined is described in terms other than as corresponding to a named impurity (commonly, for example, as any (other) *secondary spot* or *peak*) but is compared with a spot or peak in a chromatogram obtained with a reference solution of a named impurity, the percentage given in parentheses indicates an impurity limit expressed in terms of a nominal concentration of the named impurity. In chromatographic tests in which a comparison is made between spots or peaks in chromatograms obtained with solutions of different concentrations of the substance being examined, the percentage given in parentheses indicates an impurity limit expressed in terms of a nominal concentration of the medicinal substance itself. In some monographs, in particular those for certain formulated preparations, the impurity limit is expressed in terms of a nominal concentration of the active moiety rather than of the medicinal substance itself. Where necessary for clarification the terms in which the limit is expressed are stated within the monograph.

In all cases where an impurity limit is given in parentheses, the figures given are approximations for information only; conformity with the requirements is determined on the basis of compliance or otherwise with the stated test.

The use of a proprietary designation to identify a material used in an assay or test does not imply that another equally suitable material may not be used.

Biological Assays and Tests

Methods of assay described as Suggested methods are not obligatory, but when another method is used its precision must be not less than that required for the Suggested method.

For those antibiotics for which the monograph specifies a microbiological assay the potency requirement is expressed in the monograph in International Units (IU) per milligram. The material is not of pharmacopoeial quality if the upper fiducial limit of error is less than the stated potency. For such antibiotics the required precision of the assay is stated in the monograph in terms of the fiducial limits of error about the estimated potency.

For other substances and preparations for which the monograph specifies a biological assay, unless otherwise stated, the precision of the assay is such that the fiducial limits of error, expressed as a percentage of the estimated potency, are within a range not wider than that obtained by multiplying by a factor of 10 the square roots of the limits given in the monograph for the fiducial limits of error about the stated potency.

In all cases fiducial limits of error are based on a probability of 95% ($P = 0.95$).

Where the biological assay is being used to ascertain the purity of the material, the stated potency means the potency stated on the label in terms of International Units (IU) or other Units per gram, per milligram or per millilitre. When no such statement appears on the label, the stated potency means the fixed or minimum potency required in the monograph. This interpretation of stated potency applies in all cases except where the monograph specifically directs otherwise.

Where the biological assay is being used to determine the total activity in the container, the stated potency means the total number of International Units (IU) or other Units stated on the label or, if no such statement appears, the total activity calculated in accordance with the instructions in the monograph.

Wherever possible the primary standard used in an assay or test is the respective International Standard or Reference Preparation established by the World Health Organization for international use and the biological activity is expressed in International Units (IU).

In other cases, where Units are referred to in an assay or test, the Unit for a particular substance or preparation is, for the United Kingdom, the specific biological activity contained in such an amount of the respective primary standard as the appropriate international or national organisation indicates. The necessary information is provided with the primary standard.

Unless otherwise directed, animals used in an assay or a test are healthy animals, drawn from a uniform stock, that have not previously been treated with any material that will interfere with the assay or test. Unless otherwise stated, guinea-pigs weigh not less than 250 g or, when used in systemic toxicity tests, not less than 350 g. When used in skin tests they are white or light coloured. Unless otherwise stated, mice weigh not less than 17 g and not more than 22 g.

Certain of the biological assays and tests of the Pharmacopoeia are such that in the United Kingdom they may be carried out only in accordance with the Animals (Scientific Procedures) Act 1986. Instructions included in such assays and tests in the Pharmacopoeia, with respect to the handling of animals, are therefore confined to those concerned with the accuracy and reproducibility of the assay or test.

Reference Substances and Reference Preparations

Certain monographs require the use of a reference substance, a reference preparation or a reference spectrum. These are chosen with regard to their intended use as prescribed in the monographs of the Pharmacopoeia and are not necessarily suitable in other circumstances.

Any information necessary for proper use of the reference substance or reference preparation is given on the label or in the accompanying leaflet or brochure. Where no drying conditions are stated in the leaflet or on the label, the substance is to be used as received. No certificate of analysis or other data not relevant to the prescribed use of the product are provided. The products are guaranteed to be suitable for use for a period of three months from dispatch when stored under the appropriate conditions. The stability of the contents of opened containers cannot be guaranteed. The current lot is listed in the BP Laboratory website catalogue. Additional information is provided in Supplementary Chapter III E.

Chemical Reference Substances The abbreviation BPCRS indicates a Chemical Reference Substance established by the British Pharmacopoeia Commission. The abbreviation CRS or EPCRS indicates a Chemical Reference Substance established by the European Pharmacopoeia Commission. Some Chemical Reference Substances are used for the microbiological assay of antibiotics and their activity is stated, in International Units, on the label or on the accompanying leaflet and defined in the same manner as for Biological Reference Preparations.

Biological Reference Preparations The majority of the primary biological reference preparations referred to are the appropriate International Standards and Reference Preparations established by the World Health Organisation. Because these reference materials are usually available only in limited quantities, the European Pharmacopoeia has established Biological Reference Preparations (indicated by the abbreviation BRP or EPBRP) where appropriate. Where applicable, the potency of the Biological Reference Preparations is expressed in International Units. For some Biological Reference Preparations, where an international standard or reference preparation does not exist, the potency is expressed in European Pharmacopoeia Units.

Storage

Statements under the side-heading Storage constitute non-mandatory advice. The substances and preparations described in the Pharmacopoeia are to be stored under conditions that prevent contamination and, as far as possible, deterioration. Unless otherwise stated in the monograph, the substances and preparations described in the Pharmacopoeia are kept in well-closed containers and stored at a temperature not exceeding 25°. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat and light are indicated, where appropriate, in the monographs. Further precautions may be necessary when some materials are stored in tropical climates or under other severe conditions.

The expression 'protected from moisture' means that the product is to be stored in an airtight container. Care is to be taken when the container is opened in a damp atmosphere. A low moisture content may be maintained, if necessary, by the use of a desiccant in the container provided that direct contact with the product is avoided.

The expression 'protected from light' means that the product is to be stored either in a container made of a material that absorbs actinic light sufficiently to protect the contents from change induced by such light or in a container enclosed in an outer cover that provides such protection or stored in a place from which all such light is excluded.

The expression 'tamper-evident container' means a closed container fitted with a device that reveals irreversibly whether the container has been opened, whereas, the expression 'tamper-proof container' means a closed container in which access to the contents is prevented under normal conditions of use. The two terms are considered to be synonymous by the European Pharmacopoeia Commission.

Labelling

The labelling requirements of the Pharmacopoeia are not comprehensive, and the provisions of regulations issued in accordance with the requirements of the territory in which the medicinal product is to be used should be met.

Licensed medicines intended for use within the United Kingdom must comply with the requirements of the Medicines Act 1968 and EU Directive 2001/83/EC, Title V (as amended) in respect of their labelling and package leaflets, together with those regulations for the labelling of hazardous materials.

Best practice guidance on the labelling and packaging of medicines for use in the United Kingdom advises that certain items of information are deemed critical for the safe use of the medicine (see MHRA Guidance Note No. 25: Best Practice Guidance on Labelling and Packaging of Medicines). Further information and guidance on the labelling of medicinal products can be found in Supplementary Chapter I G.

Such matters as the exact form of wording to be used and whether a particular item of information should appear on the primary label and additionally, or alternatively, on the package or exceptionally in a leaflet are, in general, outside the scope of the Pharmacopoeia. When the term 'label' is used in Labelling statements of the Pharmacopoeia, decisions as to where the particular statement should appear should therefore be made in accordance with relevant legislation.

The label of every official formulated preparation other than those of fixed strength also states the content of the active ingredient or ingredients expressed in the terms required by the monograph. Where the content of active ingredient is required to be expressed in terms other than the weight of the official medicinal substance used in making the formulation, this is specifically stated under the heading Labelling. Unless otherwise stated in the monograph, the content of the active ingredient is expressed in terms of the official medicinal substance used in making the formulation.

These requirements do not necessarily apply to unlicensed preparations supplied in accordance with a prescription. For requirements for unlicensed medicines see the general monograph on Unlicensed Medicines.

Action and Use

The statements given under this heading in monographs are intended only as information on the principal pharmacological actions or the uses of the materials in medicine or pharmacy. It should not be assumed that the substance has no other action or use. The statements are not intended to be binding on prescribers or to limit their discretion.

Crude Drugs; Traditional Herbal and Complementary Medicines

Herbal and complementary medicines are classed as medicines under European Directive 2001/83/EC as amended. It is emphasised that, although requirements for the quality of the material are provided in the monograph to assist the registration scheme by the UK Licensing Authority, the British Pharmacopoeia Commission has not assessed the safety or efficacy of the material in traditional use.

Monograph Title For traditional herbal medicines, the monograph title is a combination of the established English name together with a description of use. Monographs for the material that has not been processed (the herbal drug) and the processed material (the herbal drug preparation) are published where possible. To distinguish between the two, the word 'Processed' is included in the relevant monograph title. The acronym 'THM' is used in the title to indicate 'Traditional Herbal Medicine'. This refers to the general use of the material that has not been processed. Where the acronym 'THMP' is used, this indicates the use of the processed herb in a 'Traditional Herbal Medicinal Product'.

Definition Under the heading Definition, the botanical name together with any synonym is given. Where appropriate, for material that has not been processed, information on the collection/harvesting and/or treatment/drying of the whole herbal drug may be given. For processed materials, the method of processing, where appropriate, will normally be given in a separate section.

Characteristics References to odour are included only where this is highly characteristic. References to taste are not included.

Control methods Where applicable, the control methods to be used in monographs are:

(a) macroscopical and microscopical descriptions and chemical/chromatographic tests for identification

(b) tests for absence of any related species

(c) microbial test to assure microbial quality

(d) tests for inorganic impurities and non-specific purity tests, including extractive tests, sulphated ash and heavy metals where appropriate

(e) test for Loss on drying or Water

(f) wherever possible, a method for assaying the active constituent(s) or suitable marker constituent(s).

The macroscopical characteristics include those features that can be seen by the unaided eye or by the use of a hand lens. When two species/subspecies of the same plant are included in the Definition, individual differences between the two are indicated where possible.

The description of the microscopical characteristics of the powdered drug includes information on the dominant or the most specific characters. Where it is considered to be an aid to identification, illustrations of the powdered drug may be provided.

The following aspects are controlled by the general monograph for Herbal Drugs, that is, they are required to be free from moulds, insects, decay, animal matter and animal excreta. Unless otherwise prescribed the amount of foreign matter is not more than 2% w/w. Microbial contamination should be minimal.

In determining the content of the active principle(s) or the suitable marker principle(s) measurements are made with reference to the dried or anhydrous herbal drug. In the tests for Acid-insoluble ash, Ash, Extractive soluble in ethanol, Loss on drying, Sulphated ash, Water, Water-soluble ash and Water-soluble extractive of herbal drugs, the calculations are made with reference to the herbal drug that has not been specifically dried unless otherwise prescribed in the monograph.

Homoeopathic Medicines

Homoeopathic medicines are classed as medicines under European Directive 2001/83/EC as amended. It is emphasised that, although requirements for the quality of the material are provided in the monograph to assist the simplified registration scheme by the UK Licensing Authority, the British Pharmacopoeia Commission has not assessed the safety or efficacy of the material in use.

The statements under Crude Drugs; Traditional Herbal and Complementary Medicines are also applicable to homoeopathic stocks and mother tinctures.

Specific BP monographs for homoeopathic medicines only apply to homoeopathic stocks and mother tinctures. These monographs also include general statements on the methods of preparation of homoeopathic stocks and mother tinctures. Specifications have not been set for final homeopathic products due to the high dilution used in their preparation and the subsequent difficulty in applying analytical methodology.

Identification tests rely predominantly on macroscopical description and thin-layer chromatography. Tests for foreign matter are included when there is the hazard of contamination.

Unlicensed Medicines

The General Monograph for Unlicensed Medicines applies to those formulations used in human medicine that are prepared under a manufacturing specials licence or prepared extemporaneously under the supervision of a pharmacist, whether or not there is a published monograph for the specific dosage form.

An article intended for medicinal use that is described by means of an official title must comply with the requirements of the relevant monograph. A formulated preparation must comply throughout its assigned shelf-life (period of validity). The subject of any other monograph must comply throughout its period of use.

Unlicensed medicines that are prepared under a manufacturing specials licence comply with the requirements of the general monograph and, where applicable, the requirements of the individual monograph for the specific dosage form.

Unlicensed medicines prepared extemporaneously under the supervision of a pharmacist comply with the requirements of the general monograph and, where applicable, the requirements of the individual monograph for the specific dosage form. While it is expected that extemporaneous preparations will demonstrate pharmacopoeial compliance when tested, it is recognised that it might not be practicable to carry out the pharmacopoeial tests routinely on such formulations. In the event of doubt or dispute, the methods of analysis, the reference materials and the reference spectra of the Pharmacopoeia are alone authoritative.

Part III

General Notices Part III

Monographs and other texts of the European Pharmacopoeia that are incorporated in this edition of the British Pharmacopoeia are governed by the general notices of the European Pharmacopoeia; these are reproduced below.

General Notices of the European Pharmacopoeia

1.1. General statements

The General Notices apply to all monographs and other texts of the European Pharmacopoeia.

The official texts of the European Pharmacopoeia are published in English and French. Translations in other languages may be prepared by the signatory States of the European Pharmacopoeia Convention. In case of doubt or dispute, the English and French versions are alone authoritative.

In the texts of the European Pharmacopoeia, the word 'Pharmacopoeia' without qualification means the European Pharmacopoeia. The official abbreviation Ph. Eur. may be used to indicate the European Pharmacopoeia.

The use of the title or the subtitle of a monograph implies that the article complies with the requirements of the relevant monograph. Such references to monographs in the texts of the Pharmacopoeia are shown using the monograph title and reference number in *italics*.

A preparation must comply throughout its period of validity; a distinct period of validity and/or specifications for opened or broached containers may be decided by the competent authority. The subject of any other monograph must comply throughout its period of use. The period of validity that is assigned to any given article and the time from which that period is to be calculated are decided by the competent authority in the light of experimental results of stability studies.

Unless otherwise indicated in the General Notices or in the monographs, statements in monographs constitute mandatory requirements. General chapters become mandatory when referred to in a monograph, unless such reference is made in a way that indicates that it is not the intention to make the text referred to mandatory but rather to cite it for information.

The active ingredients (medicinal substances), excipients (auxiliary substances), pharmaceutical preparations and other articles described in the monographs are intended for human and veterinary use (unless explicitly restricted to one of these uses). An article is not of Pharmacopoeia quality unless it complies with all the requirements stated in the monograph. This does not imply that performance of all the tests in a monograph is necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia before release of a product. The manufacturer may obtain assurance that a product is of Pharmacopoeia quality from data derived, for example, from validation studies of the manufacturing process and from in-process controls. Parametric release in circumstances deemed appropriate by the competent authority is thus not precluded by the need to comply with the Pharmacopoeia.

The tests and assays described are the official methods upon which the standards of the Pharmacopoeia are based. With the agreement of the competent authority, alternative methods of analysis may be used for control purposes, provided that the methods used enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used. In the event of doubt or dispute, the methods of analysis of the Pharmacopoeia are alone authoritative.

Certain materials that are the subject of a pharmacopoeial monograph may exist in different grades suitable for different purposes. Unless otherwise indicated in the monograph, the requirements apply to all grades of the material. In some monographs, particularly those on excipients, a list of functionality-related characteristics that are relevant to the use of the substance may be appended to the monograph for information. Test methods for determination of one or more of these characteristics may be given, also for information.

Quality Systems

The quality standards represented by monographs are valid only where the articles in question are produced within the framework of a suitable quality system.

General Monographs

Substances and preparations that are the subject of an individual monograph are also required to comply with relevant, applicable general monographs. Cross-references to applicable general monographs are not normally given in individual monographs.

General monographs apply to all substances and preparations within the scope of the Definition section of the general monograph, except where a preamble limits the application, for example to substances and preparations that are the subject of a monograph of the Pharmacopoeia.

General monographs on dosage forms apply to all preparations of the type defined. The requirements are not necessarily comprehensive for a given specific preparation and requirements additional to those prescribed in the general monograph may be imposed by the competent authority.

General monographs and individual monographs are complementary. If the provisions of a general monograph do not apply to a particular product, this is expressly stated in the individual monograph.

Validation of Pharmacopoeial Methods

The test methods given in monographs and general chapters have been validated in accordance with accepted scientific practice and current recommendations on analytical validation. Unless otherwise stated in the monograph or general chapter, validation of the test methods by the analyst is not required.

Conventional Terms

The term 'competent authority' means the national, supranational or international body or organisation vested with the authority for making decisions concerning the issue in question. It may, for example, be a national pharmacopoeia authority, a licensing authority or an official control laboratory.

The expression 'unless otherwise justified and authorised' means that the requirements have to be met, unless the competent authority authorises a modification or an exemption where justified in a particular case.

Statements containing the word 'should' are informative or advisory.

In certain monographs or other texts, the terms 'suitable' and 'appropriate' are used to describe a reagent, micro-organism, test method etc.; if criteria for suitability are not described in the monograph, suitability is demonstrated to the satisfaction of the competent authority.

Interchangeable Methods

Certain general chapters contain a statement that the text in question is harmonised with the corresponding text of the Japanese Pharmacopoeia and/or the United States Pharmacopeia and that these texts are interchangeable. This implies that if a substance or preparation is found to comply with a requirement using an interchangeable method from one of these pharmacopoeias it complies with the requirements of the European Pharmacopoeia. In the event of doubt or dispute, the text of the European Pharmacopoeia is alone authoritative.

References to Regulatory Documents

Monographs and general chapters may contain references to documents issued by regulatory authorities for medicines, for example directives and notes for guidance of the European Union. These references are provided for information for users for the Pharmacopoeia. Inclusion of such a reference does not modify the status of the documents referred to, which may be mandatory or for guidance.

1.2. Other provisions applying to general chapters and monographs

Quantities

In tests with numerical limits and assays, the quantity stated to be taken for examination is approximate. The amount actually used, which may deviate by not more than 10 per cent from that stated, is accurately weighed or measured and the result is calculated from this exact quantity. In tests where the limit is not numerical, but usually depends upon comparison with the behaviour of a reference substance in the same conditions, the stated quantity is taken for examination. Reagents are used in the prescribed amounts.

Quantities are weighed or measured with an accuracy commensurate with the indicated degree of precision. For weighings, the precision corresponds to plus or minus 5 units after the last figure stated (for example, 0.25 g is to be interpreted as 0.245 g to 0.255 g). For the measurement of volumes, if the figure after the decimal point is a zero or ends in a zero (for example, 10.0 ml or 0.50 ml), the volume is measured using a pipette, a volumetric flask or a burette, as appropriate; otherwise, a graduated measuring cylinder or a graduated pipette may be used. Volumes stated in microlitres are measured using a micropipette or microsyringe.

It is recognised, however, that in certain cases the precision with which quantities are stated does not correspond to the number of significant figures stated in a specified numerical limit. The weighings and measurements are then carried out with a sufficiently improved accuracy.

Apparatus and Procedures

Volumetric glassware complies with Class A requirements of the appropriate International Standard issued by the International Organisation for Standardisation.

Unless otherwise prescribed, analytical procedures are carried out at a temperature between 15 °C and 25 °C.

Unless otherwise prescribed, comparative tests are carried out using identical tubes of colourless, transparent, neutral glass with a flat base; the volumes of liquid prescribed are for use with tubes having an internal diameter of 16 mm, but tubes with a larger internal diameter may be used provided the volume of liquid used is adjusted *(2.1.5)*. Equal volumes of the liquids to be compared are examined down the vertical axis of the tubes against a white background, or if necessary against a black background. The examination is carried out in diffuse light.

Any solvent required in a test or assay in which an indicator is to be used is previously neutralised to the indicator, unless a blank test is prescribed.

Water-bath

The term 'water-bath' means a bath of boiling water unless water at another temperature is indicated. Other methods of heating may be substituted provided the temperature is near to but not higher than 100 °C or the indicated temperature.

Drying and Ignition to Constant Mass

The terms 'dried to constant mass' and 'ignited to constant mass' mean that 2 consecutive weighings do not differ by more than 0.5 mg, the 2nd weighing following an additional period of drying or of ignition respectively appropriate to the nature and quantity of the residue.

Where drying is prescribed using one of the expressions 'in a desiccator' or '*in vacuo*', it is carried out using the conditions described in chapter *2.2.32. Loss on drying*.

Reagents

The proper conduct of the analytical procedures described in the Pharmacopoeia and the reliability of the results depend, in part, upon the quality of the reagents used. The reagents are described in general chapter *4*. It is assumed that reagents of analytical grade are used; for some reagents, tests to determine suitability are included in the specifications.

Solvents

Where the name of the solvent is not stated, the term 'solution' implies a solution in water.

Where the use of water is specified or implied in the analytical procedures described in the Pharmacopoeia or for the preparation of reagents, water complying with the requirements of the monograph *Purified water (0008)* is used, except that for many purposes the requirements for bacterial endotoxins *(Purified water in bulk)* and microbial contamination *(Purified water in containers)* are not relevant. The term 'distilled water' indicates purified water prepared by distillation.

The term 'ethanol' without qualification means anhydrous ethanol. The term 'alcohol' without qualification means ethanol (96 per cent). Other dilutions of ethanol are indicated by the term 'ethanol' or 'alcohol' followed by a statement of the percentage by volume of ethanol (C_2H_6O) required.

Expression of Content

In defining content, the expression 'per cent' is used according to circumstances with one of 2 meanings:

— per cent *m/m* (percentage, mass in mass) expresses the number of grams of substance in 100 grams of final product;

— per cent *V/V* (percentage, volume in volume) expresses the number of millilitres of substance in 100 millilitres of final product.

The expression 'parts per million' (or ppm) refers to mass in mass, unless otherwise specified.

Temperature

Where an analytical procedure describes temperature without a figure, the general terms used have the following meaning:

— in a deep-freeze: below - 15 °C;

— in a refrigerator: 2 °C to 8 °C;

— cold or cool: 8 °C to 15 °C;

— room temperature: 15 °C to 25 °C.

1.3. General chapters

Containers

Materials used for containers are described in general chapter *3.1*. General names used for materials, particularly plastic materials, each cover a range of products varying not only in the properties of the principal constituent but also in the additives used. The test methods and limits for materials depend on the formulation and are therefore applicable only for materials whose formulation is covered by the preamble to the specification. The use of materials with different formulations, and the test methods and limits applied to them, are subject to agreement by the competent authority.

The specifications for containers in general chapter *3.2* have been developed for general application to containers of the stated category, but in view of the wide variety of containers available and possible new developments, the publication of a specification does not exclude the use, in justified circumstances, of containers that comply with other specifications, subject to agreement by the competent authority.

Reference may be made within the monographs of the Pharmacopoeia to the definitions and specifications for containers provided in chapter *3.2*. *Containers*. The general monographs for pharmaceutical dosage forms may, under the heading Definition/Production, require the use of certain types of container; certain other monographs may, under the heading Storage, indicate the type of container that is recommended for use.

1.4. Monographs

Titles

Monograph titles are in English and French in the respective versions and there is a Latin subtitle.

Relative Atomic and Molecular Masses

The relative atomic mass (A_r) or the relative molecular mass (M_r) is shown, as and where appropriate, at the beginning of each monograph. The relative atomic and molecular masses and the molecular and graphic formulae do not constitute analytical standards for the substances described.

Chemical Abstracts Service (CAS) Registry Number

CAS registry numbers are included for information in monographs, where applicable, to provide convenient access to useful information for users. CAS Registry Number® is a Registered Trademark of the American Chemical Society.

Definition

Statements under the heading Definition constitute an official definition of the substance, preparation or other article that is the subject of the monograph.

Limits of content Where limits of content are prescribed, they are those determined by the method described under Assay.

Herbal drugs In monographs on herbal drugs, the definition indicates whether the subject of the monograph is, for example, the whole drug or the drug in powdered form. Where a monograph applies to the drug in several states, for example both to the whole drug and the drug in powdered form, the definition states this.

Production

Statements under the heading Production draw attention to particular aspects of the manufacturing process but are not necessarily comprehensive. They constitute mandatory requirements for manufacturers, unless otherwise stated. They may relate, for example, to source materials; to the manufacturing process itself and its validation and control; to in-process testing; or to testing that is to be carried out by the manufacturer on the final article, either on selected batches or on each batch prior to release. These statements cannot necessarily be verified on a sample of the final article by an independent analyst. The competent authority may establish that the instructions have been followed, for example, by examination of data received from the manufacturer, by inspection of manufacture or by testing appropriate samples.

The absence of a Production section does not imply that attention to features such as those referred to above is not required.

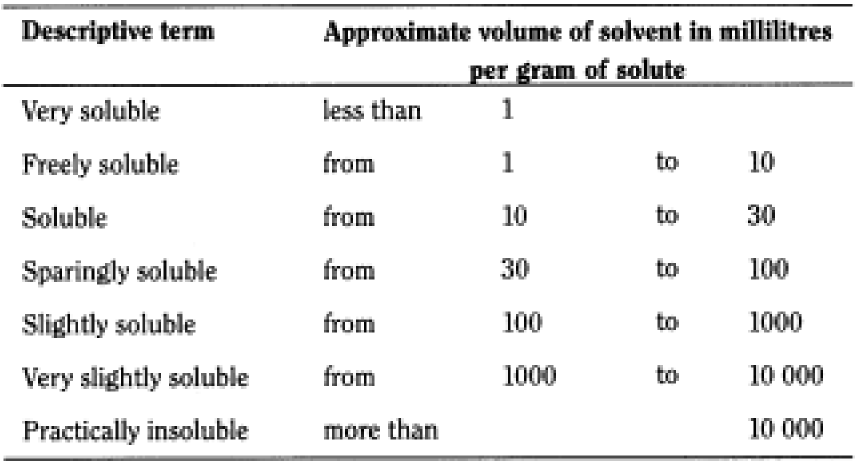
Choice of Vaccine Strain, Choice of Vaccine Composition

The Production section of a monograph may define the characteristics of a vaccine strain or vaccine composition. Unless otherwise stated, test methods given for verification of these characteristics are provided for information as examples of suitable methods. Subject to approval by the competent authority, other test methods may be used without validation against the method shown in the monograph.

Characters

The statements under the heading Characters are not to be interpreted in a strict sense and are not requirements.

Solubility In statements of solubility in the Characters section, the terms used have the following significance referred to a temperature between 15 °C and 25 °C.



Descriptive term	Approximate volume of solvent in millilitres per gram of solute			
Very soluble	less than	1		
Freely soluble	from	1	to	10
Soluble	from	10	to	30
Sparingly soluble	from	30	to	100
Slightly soluble	from	100	to	1000
Very slightly soluble	from	1000	to	10 000
Practically insoluble	more than			10 000

The term 'partly soluble' is used to describe a mixture where only some of the components dissolve. The term 'miscible' is used to describe a liquid that is miscible in all proportions with the stated solvent.

Identification

Scope The tests given in the Identification section are not designed to give a full confirmation of the chemical structure or composition of the product; they are intended to give confirmation, with an acceptable degree of assurance, that the article conforms to the description on the label.

First and second identifications Certain monographs have subdivisions entitled First identification' and 'Second identification'. The test or tests that constitute the 'First identification' may be used for identification in all circumstances. The test or tests that constitute the 'Second identification' may be used for identification provided it can be demonstrated that the substance or preparation is fully traceable to a batch certified to comply with all the other requirements of the monograph.

Powdered herbal drugs Monographs on herbal drugs may contain schematic drawings of the powdered drug. These drawings complement the description given in the relevant identification test.

Tests and Assays

Scope The requirements are not framed to take account of all possible impurities. It is not to be presumed, for example, that an impurity that is not detectable by means of the prescribed tests is tolerated if common sense and good pharmaceutical practice require that it be absent. See also below under Impurities.

Calculation Where the result of a test or assay is required to be calculated with reference to the dried or anhydrous substance or on some other specified basis, the determination of loss on drying, water content or other property is carried out by the method prescribed in the relevant test in the monograph. The words 'dried substance' or 'anhydrous substance' etc. appear in parentheses after the result.

Limits The limits prescribed are based on data obtained in normal analytical practice; they take account of normal analytical errors, of acceptable variations in manufacture and compounding and of deterioration to an extent considered acceptable. No further tolerances are to be applied to the limits prescribed to determine whether the article being examined complies with the requirements of the monograph.

In determining compliance with a numerical limit, the calculated result of a test or assay is first rounded to the number of significant figures stated, unless otherwise prescribed. The last figure is increased by one when the part rejected is equal to or exceeds one half-unit, whereas it is not modified when the part rejected is less than a half-unit.

Indication of permitted limit of impurities For comparative tests, the approximate content of impurity tolerated, or the sum of impurities, may be indicated for information only. Acceptance or rejection is determined on the basis of compliance or non-compliance with the stated test. If the use of a reference substance for the named impurity is not prescribed, this content may be expressed as a nominal concentration of the substance used to prepare the reference solution specified in the monograph, unless otherwise described.

Herbal drugs For herbal drugs, the sulphated ash, total ash, water-soluble matter, alcohol-soluble matter, water content, content of essential oil and content of active principle are calculated with reference to the drug that has not been specially dried, unless otherwise prescribed in the monograph.

Equivalents Where an equivalent is given, for the purposes of the Pharmacopoeia only the figures shown are to be used in applying the requirements of the monograph.

Culture media The culture media described in monographs and general chapters have been found to be satisfactory for the intended purpose. However, the components of media, particularly those of biological origin, are of variable quality, and it may be necessary for optimal performance to modulate the concentration of some ingredients, notably:

— peptones and meat or yeast extracts, with respect to their nutritive properties;

— buffering substances;

— bile salts, bile extract, deoxycholate, and colouring matter, depending on their selective properties;

— antibiotics, with respect to their activity.

Storage

The information and recommendations given under the heading Storage do not constitute a pharmacopoeial requirement but the competent authority may specify particular storage conditions that must be met.

The articles described in the Pharmacopoeia are stored in such a way as to prevent contamination and, as far as possible, deterioration. Where special conditions of storage are recommended, including the type of container (see section 1.3. General chapters) and limits of temperature, they are stated in the monograph.

The following expressions are used in monographs under Storage with the meaning shown.

In an airtight container means that the product is stored in an airtight container *(3.2)*. Care is to be taken when the container is opened in a damp atmosphere. A low moisture content may be maintained, if necessary, by the use of a desiccant in the container provided that direct contact with the product is avoided.

Protected from light means that the product is stored either in a container made of a material that absorbs actinic light sufficiently to protect the contents from change induced by such light, or in a container enclosed in an outer cover that provides such protection, or is stored in a place from which all such light is excluded.

Labelling

In general, labelling of medicines is subject to supranational and national regulation and to international agreements. The statements under the heading Labelling are not therefore comprehensive and, moreover, for the purposes of the Pharmacopoeia only those statements that are necessary to demonstrate compliance or non-compliance with the monograph are mandatory. Any other labelling statements are included as recommendations. When the term 'label' is used in the Pharmacopoeia, the labelling statements may appear on the container, the package, a leaflet accompanying the package, or a certificate of analysis accompanying the article, as decided by the competent authority.

Warnings

Materials described in monographs and reagents specified for use in the Pharmacopoeia may be injurious to health unless adequate precautions are taken. The principles of good quality control laboratory practice and the provisions of any appropriate regulations are to be observed at all times. Attention is drawn to particular hazards in certain monographs by means of a warning statement; absence of such a statement is not to be taken to mean that no hazard exists.

Impurities

A list of all known and potential impurities that have been shown to be detected by the tests in a monograph may be given. See also chapter *5.10. Control of impurities in substances for pharmaceutical use*. The impurities are designated by a letter or letters of the alphabet. Where a letter appears to be missing, the impurity designated by this letter has been deleted from the list during monograph development prior to publication or during monograph revision.

Functionality-related Characteristics of Excipients

Monographs on excipients may have a section on functionality-related characteristics. The characteristics, any test methods for determination and any tolerances are not mandatory requirements; they may nevertheless be relevant for use of the excipient and are given for information (see also section 1.1. General statements).

Reference Standards

Certain monographs require the use of reference standards (chemical reference substances, biological reference preparations, reference spectra). See also chapter *5.12. Reference standards.* The European Pharmacopoeia Commission establishes the official reference standards, which are alone authoritative in case of arbitration. These reference standards are available from the European Directorate for the Quality of Medicines & HealthCare (EDQM). Information on the available reference standards and a batch validity statement can be obtained via the EDQM website.

1.5. Abbreviations and Symbols

A	Absorbance
$[\alpha]_{D}^{20}$	Specific absorbance

A_r	Relative atomic mass
$[\alpha]_D^{20}$	Specific optical rotation
bp	Boiling point
BRP	Biological Reference Preparation
CRS	Chemical Reference Substance
d_{20}^{20}	Relative density
IU	International Unit
λ	Wavelength
M	Molarity
M_r	Relative molecular mass
mp	Melting point
n_D^{20}	Refractive index
Ph. Eur. U.	European Pharmacopoeia Unit
ppm	Parts per million
R	Substance or solution defined under *4. Reagents*
R_F	Retardation factor (see Chapter 2.2.46)
R_{st}	Used in chromatography to indicate the ratio of the distance travelled by a substance to the distance travelled by a reference substance
RV	Substance used as a primary standard in volumetric analysis (chapter *4.2.1*)

Abbreviations used in the monographs on immunoglobulins, immunosera and vaccines

LD_{50}	The statistically determined quantity of a substance that, when administered by the specified route, may be expected to cause the death of 50 per cent of the test animals within a given period
MLD	Minimum lethal dose
L+/10 dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, causes the death of the test animals within a given period

L+ dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 1 IU of antitoxin and administered by the specified route, causes the death of the test animals within a given period
lr/100 dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 0.01 IU of antitoxin and injected intracutaneously causes a characteristic reaction at the site of injection within a given period
Lp/10 dose	The smallest quantity of toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, causes paralysis in the test animals within a given period
Lo/10 dose	The largest quantity of a toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, does not cause symptoms of toxicity in the test animals within a given period
Lf dose	The quantity of toxin or toxoid that flocculates in the shortest time with 1 IU of antitoxin
CCID$_{50}$	The statistically determined quantity of virus that may be expected to infect 50 per cent of the cell cultures to which it is added
EID$_{50}$	The statistically determined quantity of virus that may be expected to infect 50 per cent of fertilised eggs into which it is inoculated
ID$_{50}$	The statistically determined quantity of a virus that may be expected to infect 50 per cent of the animals into which it is inoculated
PD$_{50}$	The statistically determined dose of a vaccine that, in the conditions of the test, may be expected to protect 50 per cent of the animals against a challenge dose of the micro-organisms or toxins against which it is active
ED$_{50}$	The statistically determined dose of a vaccine that, in the conditions of the test, may be expected to induce specific antibodies in 50 per cent of the animals for the relevant vaccine antigens
PFU	Pock-forming units or plaque-forming units
SPF	Specified-pathogen-free.

Collections of micro-organisms

ATCC American Type Culture Collection

10801 University Boulevard

Manassas, Virginia 20110-2209, USA

C.I.P. Collection de Bactéries de l'Institut Pasteur

B.P. 52, 25 rue du Docteur Roux

75724 Paris Cedex 15, France

IMI	International Mycological Institute Bakeham Lane Surrey TW20 9TY, Great Britain
I.P.	Collection Nationale de Culture de Microorganismes (C.N.C.M.) Institut Pasteur 25, rue du Docteur Roux 75724 Paris Cedex 15, France
NCIMB	National Collection of Industrial and Marine Bacteria Ltd 23 St Machar Drive Aberdeen AB2 1RY, Great Britain
NCPF	National Collection of Pathogenic Fungi London School of Hygiene and Tropical Medicine Keppel Street London WC1E 7HT, Great Britain
NCTC	National Collection of Type Cultures Central Public Health Laboratory Colindale Avenue London NW9 5HT, Great Britain
NCYC	National Collection of Yeast Cultures AFRC Food Research Institute Colney Lane Norwich NR4 7UA, Great Britain
S.S.I.	Statens Serum Institut 80 Amager Boulevard, Copenhagen, Denmark

1.6. Units of the international system (SI) used in the Pharmacopoeia and equivalence with other units

International System Of Units (SI)

The International System of Units comprises 3 classes of units, namely base units, derived units and supplementary units[1]. The base units and their definitions are set out in Table 1.6-1.

The derived units may be formed by combining the base units according to the algebraic relationships linking the corresponding quantities. Some of these derived units have special names and symbols. The SI units used in the European Pharmacopoeia are shown in Table 1.6-2.

Some important and widely used units outside the International System are shown in Table 1.6-3.

The prefixes shown in Table 1.6-4 are used to form the names and symbols of the decimal multiples and submultiples of SI units.

Notes

1. In the Pharmacopoeia, the Celsius temperature is used (symbol t). This is defined by the equation:

$$t = T - T_0$$

where T_0 = 273.15 K by definition. The Celsius or centigrade temperature is expressed in degree Celsius (symbol °C). The unit 'degree Celsius' is equal to the unit 'kelvin'.

2. The practical expressions of concentrations used in the Pharmacopoeia are defined in the General Notices.

3. The radian is the plane angle between two radii of a circle that cut off on the circumference an arc equal in length to the radius.

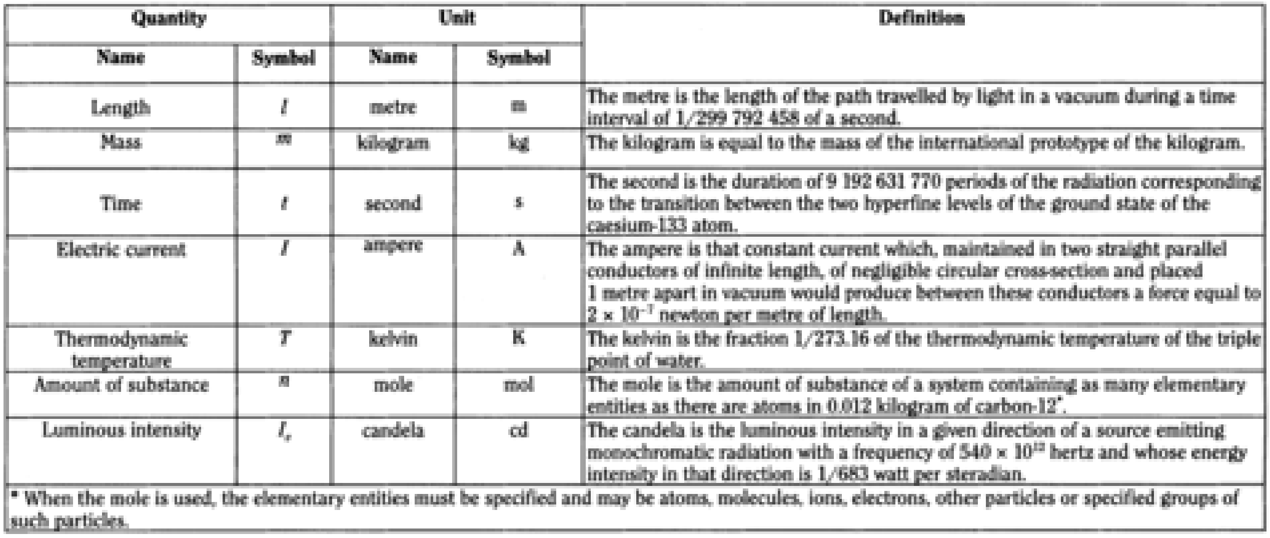
4. In the Pharmacopoeia, conditions of centrifugation are defined by reference to the acceleration due to gravity (g):

$$g = 9.80665\ m \bullet s^{-2}$$

5. Certain quantities without dimensions are used in the Pharmacopoeia: relative density *(2.2.5)*, absorbance *(2.2.25)*, specific absorbance *(2.2.25)* and refractive index *(2.2.6)*.

6. The microkatal is defined as the enzymic activity that, under defined conditions, produces the transformation (e.g. hydrolysis) of 1 micromole of the substrate per second.

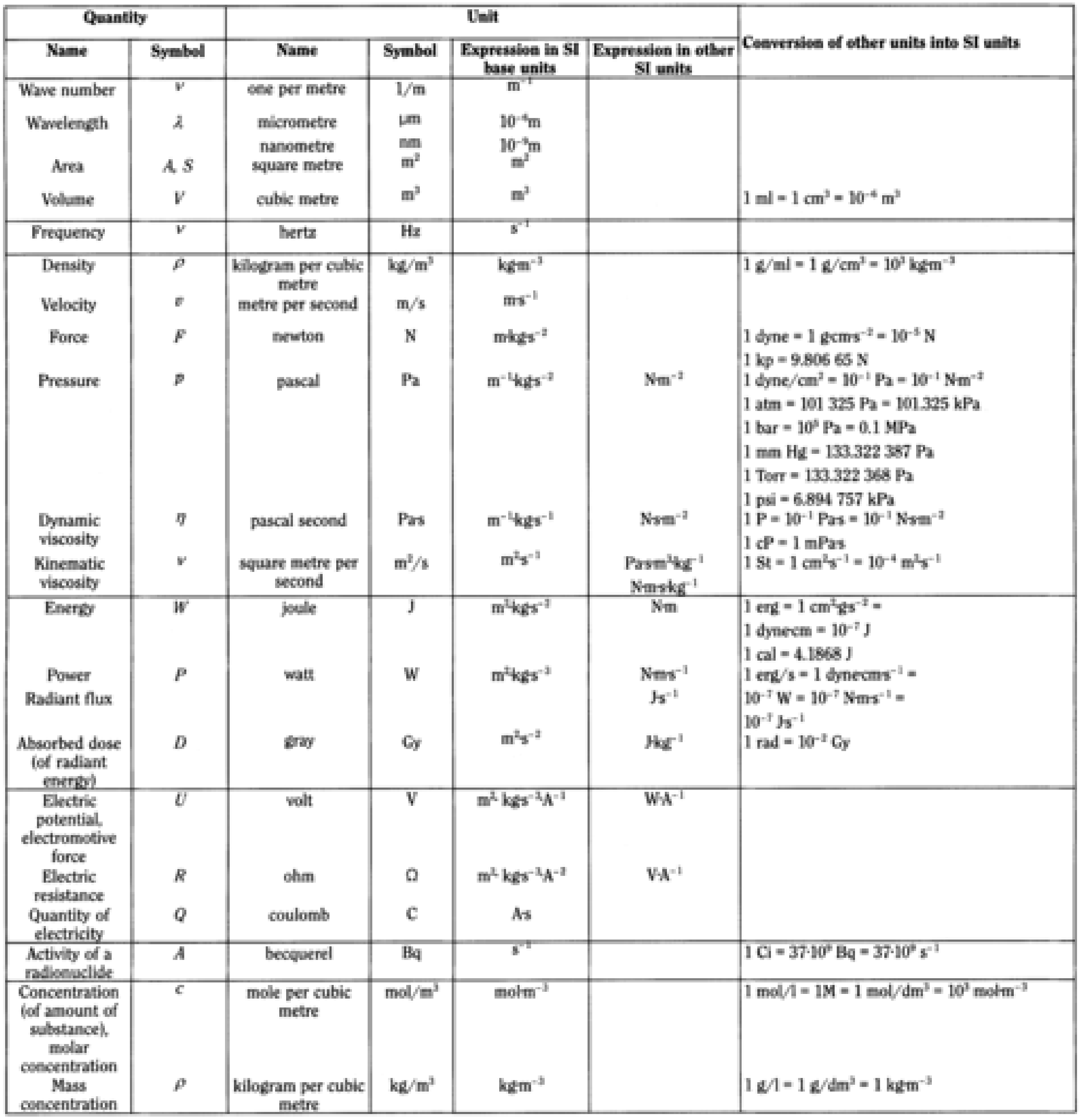
Table 1.6.-1. – *SI base units*



Quantity		Unit		Definition
Name	Symbol	Name	Symbol	
Length	l	metre	m	The metre is the length of the path travelled by light in a vacuum during a time interval of 1/299 792 458 of a second.
Mass	m	kilogram	kg	The kilogram is equal to the mass of the international prototype of the kilogram.
Time	t	second	s	The second is the duration of 9 192 631 770 periods of the radiation corresponding to the transition between the two hyperfine levels of the ground state of the caesium-133 atom.
Electric current	I	ampere	A	The ampere is that constant current which, maintained in two straight parallel conductors of infinite length, of negligible circular cross-section and placed 1 metre apart in vacuum would produce between these conductors a force equal to 2×10^{-7} newton per metre of length.
Thermodynamic temperature	T	kelvin	K	The kelvin is the fraction 1/273.16 of the thermodynamic temperature of the triple point of water.
Amount of substance	n	mole	mol	The mole is the amount of substance of a system containing as many elementary entities as there are atoms in 0.012 kilogram of carbon-12*.
Luminous intensity	I_v	candela	cd	The candela is the luminous intensity in a given direction of a source emitting monochromatic radiation with a frequency of 540×10^{12} hertz and whose energy intensity in that direction is 1/683 watt per steradian.

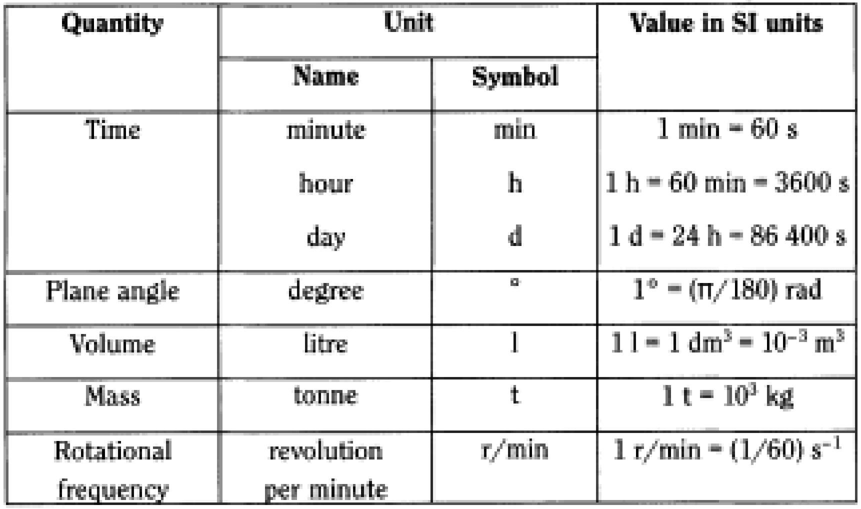
* When the mole is used, the elementary entities must be specified and may be atoms, molecules, ions, electrons, other particles or specified groups of such particles.

Table 1.6.-2. – *SI units used in the European Pharmacopoeia and equivalence with other units*



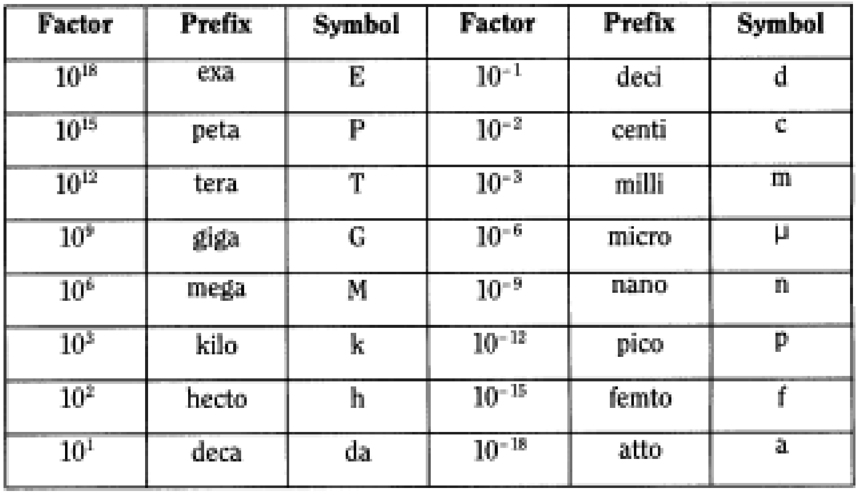
Quantity		Unit				Conversion of other units into SI units
Name	Symbol	Name	Symbol	Expression in SI base units	Expression in other SI units	
Wave number	ν	one per metre	1/m	$\mathrm{m^{-1}}$		
Wavelength	λ	micrometre nanometre	μm nm	$\mathrm{10^{-6}m}$ $\mathrm{10^{-9}m}$		
Area	A, S	square metre	$\mathrm{m^2}$	$\mathrm{m^2}$		
Volume	V	cubic metre	$\mathrm{m^3}$	$\mathrm{m^3}$		$\mathrm{1\ ml = 1\ cm^3 = 10^{-6}\ m^3}$
Frequency	ν	hertz	Hz	$\mathrm{s^{-1}}$		
Density	ρ	kilogram per cubic metre	$\mathrm{kg/m^3}$	$\mathrm{kg{\cdot}m^{-3}}$		$\mathrm{1\ g/ml = 1\ g/cm^3 = 10^3\ kg{\cdot}m^{-3}}$
Velocity	v	metre per second	m/s	$\mathrm{m{\cdot}s^{-1}}$		
Force	F	newton	N	$\mathrm{m{\cdot}kg{\cdot}s^{-2}}$		$\mathrm{1\ dyne = 1\ g{\cdot}cm{\cdot}s^{-2} = 10^{-5}\ N}$ 1 kp = 9.806 65 N
Pressure	p	pascal	Pa	$\mathrm{m^{-1}{\cdot}kg{\cdot}s^{-2}}$	$\mathrm{N{\cdot}m^{-2}}$	$\mathrm{1\ dyne/cm^2 = 10^{-1}\ Pa = 10^{-1}\ N{\cdot}m^{-2}}$ 1 atm = 101 325 Pa = 101.325 kPa $\mathrm{1\ bar = 10^5\ Pa = 0.1\ MPa}$ 1 mm Hg = 133.322 387 Pa 1 Torr = 133.322 368 Pa 1 psi = 6.894 757 kPa
Dynamic viscosity	η	pascal second	Pa·s	$\mathrm{m^{-1}{\cdot}kg{\cdot}s^{-1}}$	$\mathrm{N{\cdot}s{\cdot}m^{-2}}$	$\mathrm{1\ P = 10^{-1}\ Pa{\cdot}s = 10^{-1}\ N{\cdot}s{\cdot}m^{-2}}$ 1 cP = 1 mPa·s
Kinematic viscosity	ν	square metre per second	$\mathrm{m^2/s}$	$\mathrm{m^2{\cdot}s^{-1}}$	$\mathrm{Pa{\cdot}s{\cdot}m^3{\cdot}kg^{-1}}$ $\mathrm{N{\cdot}m{\cdot}s{\cdot}kg^{-1}}$	$\mathrm{1\ St = 1\ cm^2{\cdot}s^{-1} = 10^{-4}\ m^2{\cdot}s^{-1}}$
Energy	W	joule	J	$\mathrm{m^2{\cdot}kg{\cdot}s^{-2}}$	N·m	$\mathrm{1\ erg = 1\ cm^2{\cdot}g{\cdot}s^{-2} =}$ $\mathrm{1\ dyne{\cdot}cm = 10^{-7}\ J}$ 1 cal = 4.1868 J
Power Radiant flux	P	watt	W	$\mathrm{m^2{\cdot}kg{\cdot}s^{-3}}$	$\mathrm{N{\cdot}m{\cdot}s^{-1}}$ $\mathrm{J{\cdot}s^{-1}}$	$\mathrm{1\ erg/s = 1\ dyne{\cdot}cm{\cdot}s^{-1} =}$ $\mathrm{10^{-7}\ W = 10^{-7}\ N{\cdot}m{\cdot}s^{-1} =}$ $\mathrm{10^{-7}\ J{\cdot}s^{-1}}$
Absorbed dose (of radiant energy)	D	gray	Gy	$\mathrm{m^2{\cdot}s^{-2}}$	$\mathrm{J{\cdot}kg^{-1}}$	$\mathrm{1\ rad = 10^{-2}\ Gy}$
Electric potential, electromotive force	U	volt	V	$\mathrm{m^2{\cdot}kg{\cdot}s^{-3}{\cdot}A^{-1}}$	$\mathrm{W{\cdot}A^{-1}}$	
Electric resistance	R	ohm	Ω	$\mathrm{m^2{\cdot}kg{\cdot}s^{-3}{\cdot}A^{-2}}$	$\mathrm{V{\cdot}A^{-1}}$	
Quantity of electricity	Q	coulomb	C	A·s		
Activity of a radionuclide	A	becquerel	Bq	$\mathrm{s^{-1}}$		$\mathrm{1\ Ci = 37{\cdot}10^9\ Bq = 37{\cdot}10^9\ s^{-1}}$
Concentration (of amount of substance), molar concentration	c	mole per cubic metre	$\mathrm{mol/m^3}$	$\mathrm{mol{\cdot}m^{-3}}$		$\mathrm{1\ mol/l = 1M = 1\ mol/dm^3 = 10^3\ mol{\cdot}m^{-3}}$
Mass concentration	ρ	kilogram per cubic metre	$\mathrm{kg/m^3}$	$\mathrm{kg{\cdot}m^{-3}}$		$\mathrm{1\ g/l = 1\ g/dm^3 = 1\ kg{\cdot}m^{-3}}$

Table 1.6.-3. – *Units used with the International System*



Quantity	**Unit**		**Value in SI units**
	Name	**Symbol**	
Time	minute	min	1 min = 60 s
	hour	h	1 h = 60 min = 3600 s
	day	d	1 d = 24 h = 86 400 s
Plane angle	degree	°	1° = (π/180) rad
Volume	litre	l	1 l = 1 dm^3 = 10^{-3} m^3
Mass	tonne	t	1 t = 10^3 kg
Rotational frequency	revolution per minute	r/min	1 r/min = (1/60) s^{-1}

Table 1.6.-4. – *Decimal multiples and sub-multiples of units*



Factor	**Prefix**	**Symbol**	**Factor**	**Prefix**	**Symbol**
10^{18}	exa	E	10^{-1}	deci	d
10^{15}	peta	P	10^{-2}	centi	c
10^{12}	tera	T	10^{-3}	milli	m
10^{9}	giga	G	10^{-6}	micro	μ
10^{6}	mega	M	10^{-9}	nano	n
10^{3}	kilo	k	10^{-12}	pico	p
10^{2}	hecto	h	10^{-15}	femto	f
10^{1}	deca	da	10^{-18}	atto	a

[1] The definitions of the units used in the International System are given in the booklet "Le Système International d'Unités (SI)" published by the Bureau International des Poids et Mesures, Pavillion de Breteuil, F-92310 Sèvres.

Acacia

General Notices

(Ph Eur monograph 0307)

Action and use

Bulk-forming laxative; excipient.

When Powdered Acacia is prescribed or demanded, material complying with the requirements below with the exception of Identification test A shall be dispensed or supplied.

Ph Eur

DEFINITION

Air-hardened, gummy exudate flowing naturally from or obtained by incision of the trunk and branches of *Acacia senegal* L. Willdenow, other species of *Acacia* of African origin and *Acacia seyal Del*.

CHARACTERS

Acacia is almost completely but very slowly soluble, after about 2 h, in twice its mass of water leaving only a very small residue of vegetable particles; the liquid obtained is colourless or yellowish, dense, viscous, adhesive, translucent and weakly acid to blue litmus paper. Acacia is practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Acacia occurs as yellowish-white, yellow or pale amber, sometimes with a pinkish tint, friable, opaque, spheroidal, oval or reniform pieces (tears) of a diameter from about 1-3 cm, frequently with a cracked surface, easily broken into irregular, whitish or slightly yellowish angular fragments with conchoidal fracture and a glassy and transparent appearance. In the centre of an unbroken tear there is sometimes a small cavity.

B. Reduce to a powder (355) *(2.9.12)*. The powder is white or yellowish-white. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows the following diagnostic characters: angular, irregular, colourless, transparent fragments. Only traces of starch or vegetable tissues are visible. No stratified membrane is apparent.

C. Examine the chromatograms obtained in the test for glucose and fructose.

Results The chromatogram obtained with the test solution shows 3 zones due to galactose, arabinose and rhamnose. No other important zones are visible, particularly in the upper part of the chromatogram.

D. Dissolve 1 g of the powdered drug (355) *(2.9.12)* in 2 ml of *water R* by stirring frequently for 2 h. Add 2 ml of *ethanol (96 per cent) R*. After shaking, a white, gelatinous mucilage is formed which becomes fluid on adding 10 ml of *water R*.

TESTS

Solution S

Dissolve 3.0 g of the powdered drug (355) *(2.9.12)* in 25 ml of *water R* by stirring for 30 min. Allow to stand for 30 min and dilute to 30 ml with *water R*.

Insoluble matter

Maximum 0.5 per cent.

To 5.0 g of the powdered drug (355) *(2.9.12)* add 100 ml of *water R* and 14 ml of *dilute hydrochloric acid R*, boil gently for 15 min, shaking frequently and filter while hot through a tared sintered-glass filter *(2.1.2)*. Wash with hot *water R* and dry at 100-105 °C. The residue weighs a maximum of 25 mg.

Glucose and fructose

Thin-layer chromatography *(2.2.27)*.

Test solution To 0.100 g of the powdered drug (355) *(2.9.12)* in a thick-walled centrifuge tube add 2 ml of a 100 g/l solution of *trifluoroacetic acid R*, shake vigorously to dissolve the forming gel, stopper the tube and heat the mixture at 120 °C for 1 h. Centrifuge the hydrolysate, transfer the clear supernatant carefully into a 50 ml flask, add 10 ml of *water R* and evaporate the solution to dryness under reduced pressure. To the resulting clear film add 0.1 ml of *water R* and 0.9 ml of *methanol R*. Centrifuge to separate the amorphous precipitate. Dilute the supernatant, if necessary, to 1 ml with *methanol R*.

Reference solution Dissolve 10 mg of *arabinose R*, 10 mg of *galactose R*, 10 mg of *glucose R*, 10 mg of *rhamnose R* and 10 mg of *xylose R* in 1 ml of *water R* and dilute to 10 ml with *methanol R*.

Plate TLC silica gel plate R.

Mobile phase 16 g/l solution of *sodium dihydrogen phosphate R, butanol R, acetone R* (10:40:50 *V/V/V*).

Application 10 µl as bands.

Development A Over a path of 10 cm.

Drying A In a current of warm air for a few minutes.

Development B Over a path of 15 cm using the same mobile phase.

Drying B At 110 °C for 10 min.

Detection Spray with *anisaldehyde solution R* and heat at 110 °C for 10 min.

Results The chromatogram obtained with the reference solution shows 5 clearly separated coloured zones due to galactose (greyish-green or green), glucose (grey), arabinose (yellowish-green), xylose (greenish-grey or yellowish-grey) and rhamnose (yellowish-green), in order of increasing R_F value. The chromatogram obtained with the test solution shows no grey zone and no greyish-green zone between the zones corresponding to galactose and arabinose in the chromatogram obtained with the reference solution.

Starch, dextrin and agar

To 10 ml of solution S previously boiled and cooled add 0.1 ml of *0.05 M iodine*. No blue or reddish-brown colour develops.

Sterculia gum

A. Place 0.2 g of the powdered drug (355) *(2.9.12)* in a 10 ml ground-glass-stoppered cylinder graduated in 0.1 ml. Add 10 ml of *ethanol (60 per cent V/V) R* and shake. Any gel formed occupies a maximum of 1.5 ml.

B. To 1.0 g of the powdered drug (355) *(2.9.12)* add 100 ml of *water R* and shake. Add 0.1 ml of *methyl red solution R*. Not more than 5.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Tannins

To 10 ml of solution S add 0.1 ml of *ferric chloride solution R1.* A gelatinous precipitate is formed, but neither the precipitate nor the liquid are dark blue.

Tragacanth

Examine the chromatograms obtained in the test for glucose and fructose.

Results The chromatogram obtained with the test solution shows no greenish-grey or yellowish-grey zone corresponding to the zone of xylose in the chromatogram obtained with the reference solution.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 4.0 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli (2.6.13)*.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for acacia used as a viscosity-increasing agent and/or suspending agent in aqueous preparations.

Apparent viscosity

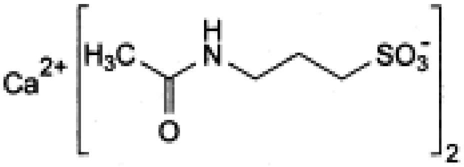
Determine the dynamic viscosity using a capillary viscometer *(2.2.9)* or a rotating viscometer *(2.2.10)* on a 100 g/l solution of acacia (dried substance).

Ph Eur

Acamprosate Calcium

General Notices

(Ph Eur monograph 1585)



$C_{10}H_{20}CaN_2O_8S_2$ 400.5 *77337-73-6*

Action and use

Treatment of alcoholism.

Ph Eur

DEFINITION

Calcium bis[3-(acetylamino)propane-1-sulphonate].

Content 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, practically insoluble in alcohol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of acamprosate calcium.

B. It gives reaction (a) of calcium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.5 to 7.0 for solution S.

Impurity A

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.40 g of the substance to be examined in *distilled water R* and dilute to 20.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *borate buffer solution pH 10.4 R*. Place 3.0 ml of the solution obtained in a 25 ml ground-glass-stoppered tube. Add 0.15 ml of a freshly prepared 5 g/l solution of *fluorescamine R* in *acetonitrile R*. Shake immediately and vigorously for 30 s. Place in a water-bath at 50 °C for 30 min. Cool under a stream of cold water. Centrifuge and filter the supernatant through a suitable membrane filter (0.45 µm), 25 mm in diameter.

Reference solution Dissolve 50 mg of *acamprosate impurity A CRS* in *distilled water R* and dilute to 200.0 ml with the same solvent. Dilute 0.4 ml of the solution to 100.0 ml with *borate buffer solution pH 10.4 R*. Treat 3.0 ml of this solution in the same way as the test solution

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm,

— *stationary phase: spherical octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 170 m2/g and a pore size of 12 nm.

Mobile phase *acetonitrile R, methanol R, 0.1 M phosphate buffer solution pH 6.5 R* (10:10:80 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 261 nm.

Injection 20 µl.

Run time 6 times the retention time of impurity A

Retention times Fluorescamine = about 4 min; impurity A = about 8 min; acamprosate is not detected by this system.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *distilled water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.4 per cent, determined on 1.000 g by drying in an oven at 105 °C.

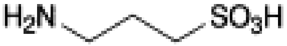
ASSAY

To 4 g of *cation exchange resin R* (75-150 µm) add 20 ml of *distilled water R* and stir magnetically for 10 min. Introduce this suspension into a glass column, 45 cm long and 2.2 cm in internal diameter, equipped with a polytetrafluoroethylene flow cap covered by a glass-wool plug. Allow a few millilitres of this solution to flow, then place a plug of glass wool over the resin. Pass 50 ml of *1 M hydrochloric acid* through the column. The pH of the eluate is close to 1. Wash with 3 quantities, each of 200 ml, of *distilled water R* to obtain an eluate at pH 6. Dissolve 0.100 g of the substance to be examined in 15 ml of *distilled water R*. Pass through the column and wash with 3 quantities, each of 25 ml, of *distilled water R*, collecting the eluate. Allow to elute until an eluate at pH 6 is obtained. Titrate the solution obtained with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* corresponds to 20.02 mg of $C_{10}H_{20}CaN_2O_8S_2$.

IMPURITIES

IMPURITIES



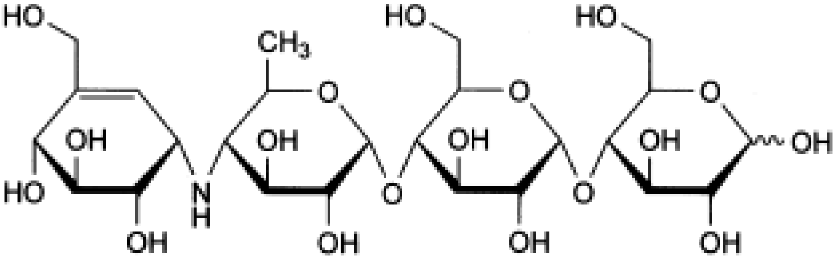
A. 3-aminopropane-1-sulphonic acid (homotaurine).

Ph Eur

Acarbose

General Notices

(Ph Eur monograph 2089)



$C_{25}H_{43}NO_{18}$ 646 *561-94-0*

Action and use

Alpha-glucosidase inhibitor; treatment of diabetes mellitus.

Ph Eur

DEFINITION

O-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose, which is produced by certain strains of *Actinoplanes utahensis*.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish, amorphous powder, hygroscopic.

Solubility

Very soluble in water, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison acarbose for identification CRS.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH *(2.2.3)*

5.5 to 7.5 for solution S.

Specific optical rotation *(2.2.7)*

+ 168 to + 183 (anhydrous substance).

Dilute 2.0 ml of solution S to 10.0 ml with *water R*.

Absorbance *(2.2.25)*

Maximum 0.15 at 425 nm for solution S.

Related substances.

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *acarbose CRS* in 5.0 ml of *water R*.

Reference solution (b) Dissolve 20 mg of *acarbose for peak identification CRS* (acarbose containing impurities A, B, C, D, E, F, G and H) in 1 ml of *water R*.

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with *water R*.

Column:
- *size: l* = 0.25 m, Ø = 4 mm,
- *stationary phase: aminopropylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 35 °C.

Mobile phase Mix 750 volumes of *acetonitrile R1* and 250 volumes of a solution containing 0.60 g/l of *potassium dihydrogen phosphate R* and 0.35 g/l of *disodium hydrogen phosphate dihydrate R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl of the test solution and reference solutions (b) and (c).

Run time 2.5 times the retention time of acarbose.

Identification of impurities Use the chromatogram supplied with *acarbose for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D, E, F, G and H.

Relative retention With reference to acarbose (retention time = about 16 min): impurity D = about 0.5; impurity H = about 0.6; impurity B = about 0.8; impurity A = about 0.9; impurity C = about 1.2; impurity E = about 1.7; impurity F = about 1.9; impurity G = about 2.2.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to acarbose,

— the chromatogram obtained is similar to the chromatogram supplied with *acarbose for peak identification CRS*.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.63; impurity D = 0.75; impurity E = 1.25; impurity F = 1.25; impurity G = 1.25;

— *impurity A*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent);

— *impurity B*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.5 per cent);

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *impurity E*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurities F, G*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *impurity H*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *any other impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.5 g in *water R* and dilute to 15 ml with the same solvent. If the solution is not clear, carry out prefiltration and use the filtrate. 10 ml complies with limit test E. Prepare the reference solution using 20 ml of *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

Maximum 4.0 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection   Test solution and reference solution (a).

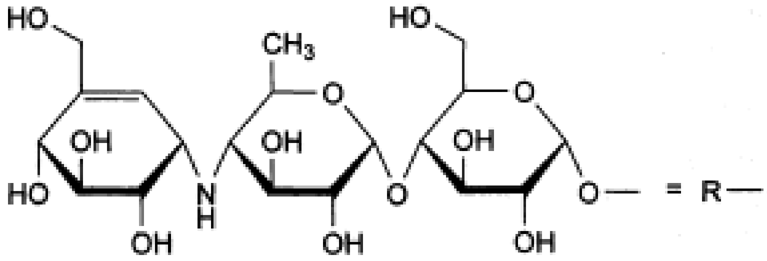
Calculate the percentage content of $C_{25}H_{43}NO_{18}$ from the areas of the peaks and the declared content of *acarbose CRS*.

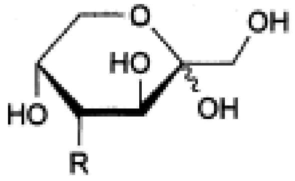
STORAGE

In an airtight container.

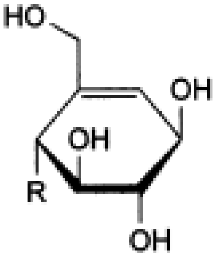
IMPURITIES

Specified impurities   *A, B, C, D, E, F, G, H.*

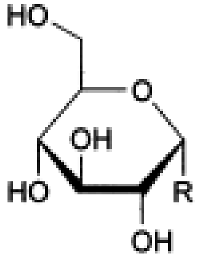




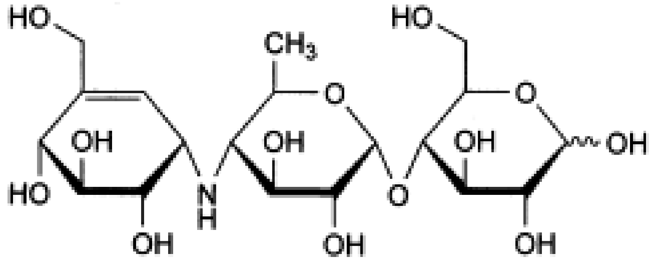
A. *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl] amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-*arabino*-hex-2-ulopyranose,



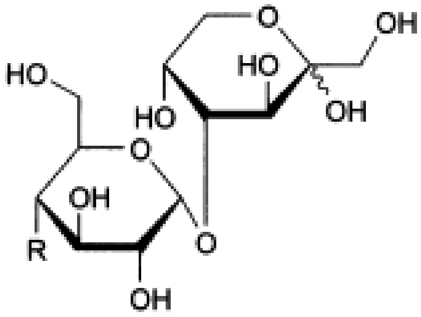
B. (1*R*,4*R*,5*S*,6*R*)-4,5,6-trihydroxy-2-(hydroxymethyl)cyclohex-2-enyl 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-α-D-glucopyranoside,



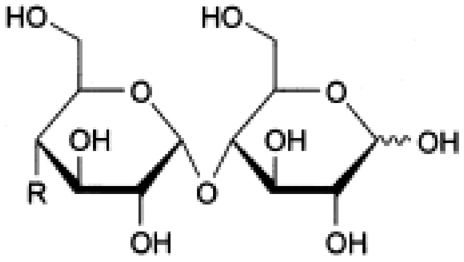
C. α-D-glucopyranosyl 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-α-D-glucopyranoside,



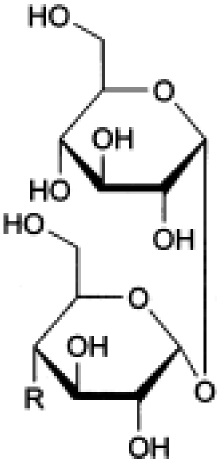
D. 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl] amino]-α-D-glucopyranosyl]-D-glucopyranose,



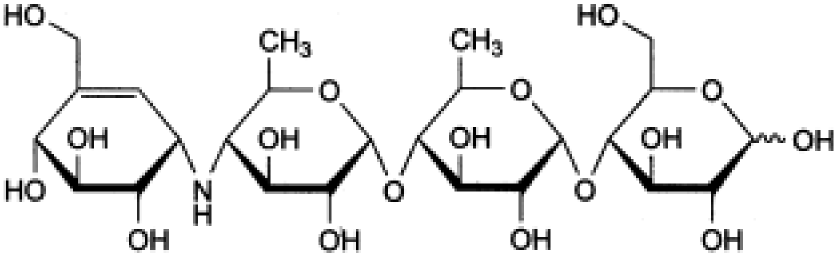
E. *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl] amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-*arabino*-hex-2-ulopyranose (4-*O*-α-acarbosyl-D-fructopyranose),



F. *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl] amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose (4-*O*-α-acarbosyl-D-glucopyranose),



G. α-D-glucopyranosyl *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl) cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranoside (α-D-glucopyranosyl α-acarboside),



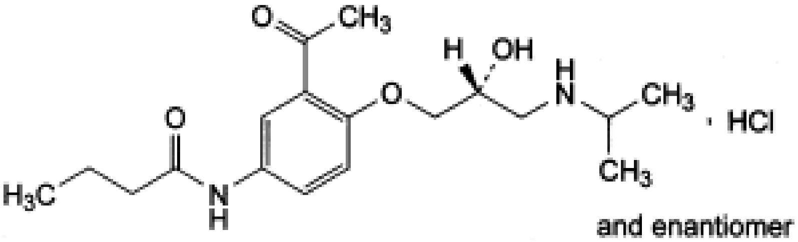
H. *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl] amino]-α-D-glucopyranosyl-(1→4)-*O*-6-deoxy-α-D-glucopyranosyl-(1→4)-D-glucopyranose.

Ph Eur

Acebutolol Hydrochloride

General Notices

(Ph Eur monograph 0871)



$C_{18}H_{28}N_2O_4$,HCl 372.9 *34381-68-5*

Action and use

Beta-adrenoceptor antagonist.

Preparations

Acebutolol Capsules

Acebutolol Tablets

Ph Eur

DEFINITION

N-[3-Acetyl-4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]butanamide hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in ethanol (96 per cent), very slightly soluble in acetone and in methylene chloride.

mp

About 143 °C.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 20.0 mg in a 0.1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid solution. Dilute 5.0 ml of this solution to 100.0 ml with a 0.1 per cent *V/V* solution of *hydrochloric acid R*.

Spectral range 220-350 nm.

Absorption maxima At 233 nm and 322 nm.

Specific absorbance at the absorption maximum 555 to 605 at 233 nm.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *acebutolol hydrochloride CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *acebutolol hydrochloride CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *pindolol CRS* in *methanol R* and dilute to 20 ml with the same solvent. To 1 ml of this solution add 1 ml of reference solution (a).

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *Perchloric acid R, methanol R, water R* (5:395:600 *V/V/V*).

Application 10 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Dissolve 0.5 g in *water R* and dilute to 10 ml with the same solvent.

pH *(2.2.3)*

5.0 to 7.0.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a) Dissolve 20.0 mg of the substance to be examined in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 0.5 ml of this solution to 50.0 ml with mobile phase A.

Reference solution (b) Dissolve the contents of a vial of *acebutolol impurity I CRS* in 1.0 ml of mobile phase A.

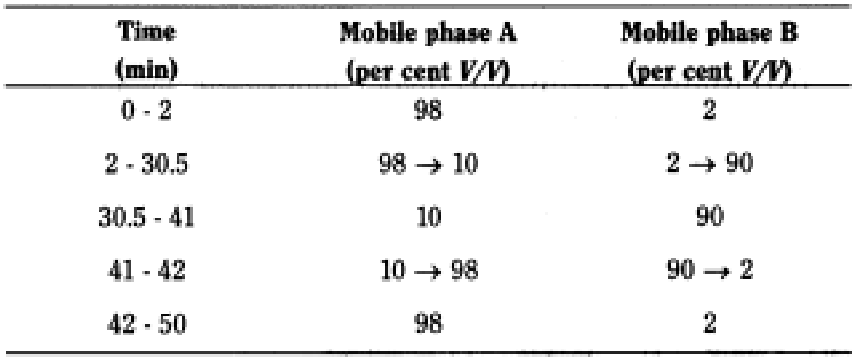
Reference solution (c) Mix 2.0 ml of reference solution (a) and 1.0 ml of reference solution (b) and dilute to 10.0 ml with mobile phase A.

Reference solution (d) Dissolve 5.0 mg of *acebutolol impurity C CRS* in 10 ml of *acetonitrile R* and dilute to 25.0 ml with mobile phase A. Dilute 0.5 ml of this solution to 50.0 ml with mobile phase A.

Reference solution (e) Dissolve 5.0 mg of *acebutolol impurity B CRS* in 10.0 ml of *acetonitrile R* and dilute to 25.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 50.0 ml with mobile phase A.

Column:
- *size: l* = 0.125 m, Ø = 4 mm,
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 2.0 ml of *phosphoric acid R*, and 3.0 ml of *triethylamine R* and dilute to 1000 ml with *water R*;
- *mobile phase B*: mix equal volumes of *acetonitrile R* and mobile phase A;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	98	2
2 - 30.5	98 → 10	2 → 90
30.5 - 41	10	90
41 - 42	10 → 98	90 → 2
42 - 50	98	2

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 25 µl.

System suitability Reference solution (c):

— *resolution*: minimum 7.0 between the peaks due to impurity I and acebutolol.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *impurity I*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 0.50 g in 20.0 ml of *water R*. The solution complies with test E. Prepare the reference solution by diluting 10.0 ml of *lead standard solution (1 ppm Pb) R* to 20.0 ml with *water R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *ethanol (96 per cent) R* and add 1 ml of *0.1 M hydrochloric acid* . Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

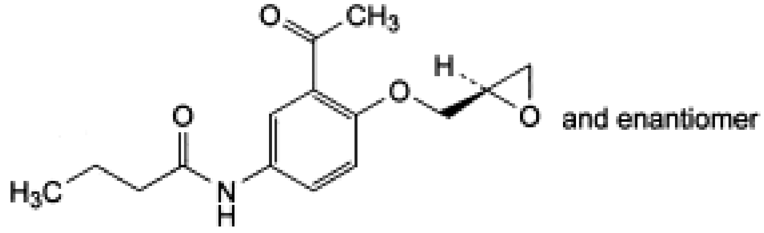
1 ml of *0.1 M sodium hydroxide* is equivalent to 37.29 mg of $C_{18}H_{29}ClN_2O_4$.

STORAGE

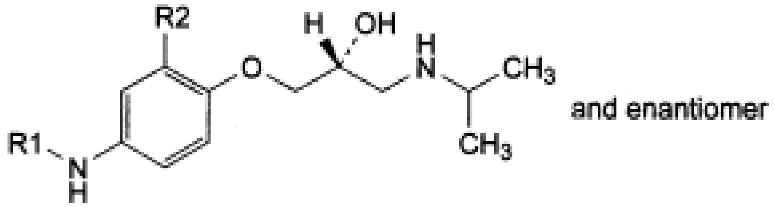
Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J, K.



A. *N*-[3-acetyl-4-[(2*RS*)-oxiran-2-ylmethoxy]phenyl]butanamide,



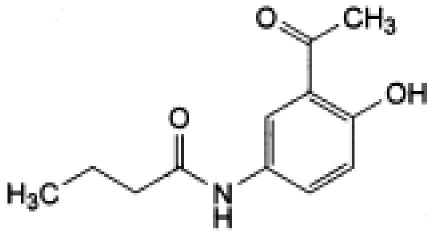
B. R1 = R2 = CO-CH_3: *N*-[3-acetyl-4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] phenyl]acetamide (diacetolol),

D. R1 = H, R2 = CO-CH_3: 1-[5-amino-2-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] phenyl]ethanone,

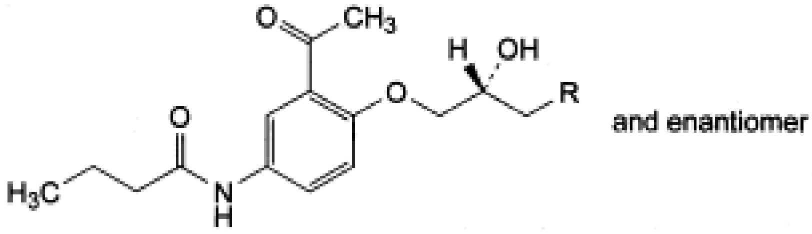
E. R1 = CO-CH_2-CH_2-CH_3, R2 = H: *N*-[4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] phenyl]butanamide,

J. R1 = CO-CH_2-CH_3, R2 = CO-CH_3: *N*-[3-acetyl-4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino] propoxy]phenyl]propanamide,

K. R1 = R2 = CO-CH_2-CH_2-CH_3: *N*-[3-butanoyl-4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino] propoxy]phenyl]butanamide,

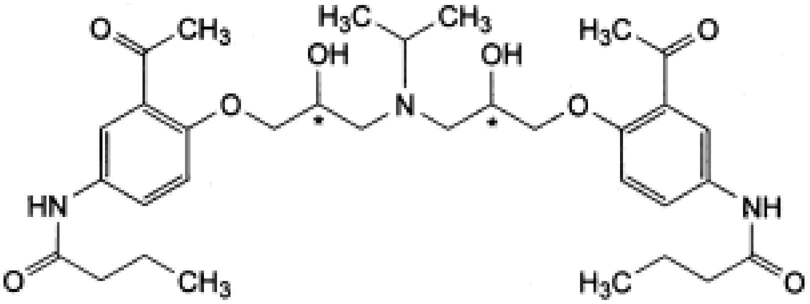


C. *N*-(3-acetyl-4-hydroxyphenyl)butanamide,

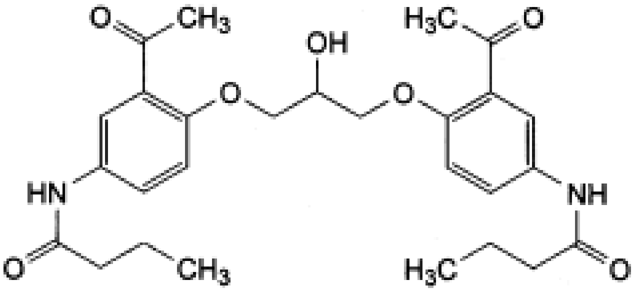


F. R = OH: *N*-[3-acetyl-4-[(2*RS*)-2,3-dihydroxypropoxy]phenyl]butanamide,

I. R = NH-CH_2-CH_3: *N*-[3-acetyl-4-[(2*RS*)-3-(ethylamino)-2-hydroxypropoxy]phenyl] butanamide,



G. *N,N′*-[[(1-methylethyl)imino]bis[(2-hydroxypropane-1,3-diyl)oxy(3-acetyl-1,4-phenylene)]] dibutanamide (biamine),



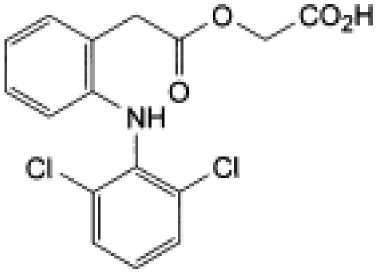
H. *N,N′*-[(2-hydroxypropane-1,3-diyl)bis[oxy(3-acetyl-1,4-phenylene)]]dibutanamide.

Ph Eur

Aceclofenac

General Notices

(Ph Eur monograph 1281)



$C_{16}H_{13}Cl_2NO_4$ 354.2 *89796-99-6*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

[[[2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl]oxy]acetic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B.

Second identification A, C.

A. Dissolve 50.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 50.0 ml with *methanol R*. Examined between 220 nm and 370 nm *(2.2.25)*, the solution shows an absorption maximum at 275 nm. The specific absorbance at the absorption maximum is 320 to 350.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of aceclofenac.*

C. Dissolve about 10 mg in 10 ml of *ethanol (96 per cent) R*. To 1 ml of the solution, add 0.2 ml of a mixture, prepared immediately before use, of equal volumes of a 6 g/l solution of *potassium ferricyanide R* and a 9 g/l solution of *ferric chloride R*. Allow to stand protected from light for 5 min. Add 3 ml of a 10.0 g/l solution of *hydrochloric acid R*. Allow to stand protected from light for 15 min. A blue colour develops and a precipitate is formed.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 50.0 mg of the substance to be examined in a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B and dilute to 25.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 21.6 mg of *diclofenac sodium CRS* (impurity A) in a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 2.0 ml of the test solution to 10.0 ml with a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Reference solution (c) Mix 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) and dilute to 100.0 ml with a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Reference solution (d) Dissolve 4.0 mg of *aceclofenac impurity F CRS* and 2.0 mg of *aceclofenac impurity H CRS* in a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B then dilute to 10.0 ml with the same mixture of solvents.

Reference solution (e) Mix 1.0 ml of reference solution (b) and 1.0 ml of reference solution (d) and dilute to 100.0 ml with a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Reference solution (f) Dissolve the contents of a vial of *diclofenac impurity A CRS* (aceclofenac impurity I) in 1.0 ml of a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B, add 1.5 ml of the same mixture of solvents and mix.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

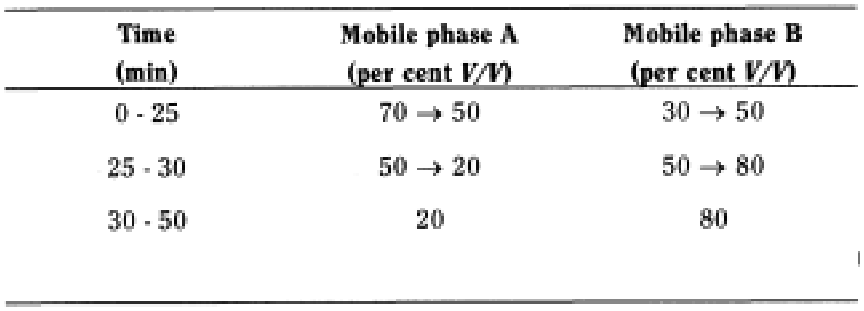
— *stationary phase: spherical end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent;

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: 1.12 g/l solution of *phosphoric acid R* adjusted to pH 7.0 using a 42 g/l solution of *sodium hydroxide R*;

— *mobile phase B*: *water R, acetonitrile R* (1:9 *V/V*);



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 25	70 → 50	30 → 50
25 - 30	50 → 20	50 → 80
30 - 50	20	80

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µl of the test solution and reference solutions (c), (e) and (f).

Relative retention Wth reference to aceclofenac (retention time = about 14 min): impurity A = about 0.8; impurity G = about 1.3; impurity H = about 1.5; impurity I = about 2.3; impurity D = about 2.6; impurity B = about 2.7; impurity E = about 2.8; impurity C = about 3.0; impurity F = about 3.2.

System suitability Reference solution (c):

— *resolution*: minimum 5.0 between the peaks due to impurity A and aceclofenac.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurities B, C, D, E, G*: for each impurity, not more than the area of the peak due to aceclofenac in the chromatogram obtained with reference solution (e) (0.2 per cent);

— *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (0.2 per cent);

— *impurity H*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (0.1 per cent);

— *impurity I*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.1 per cent);

— *unspecified impurities*: not more than 0.5 times the area of the peak due to aceclofenac in the chromatogram obtained with reference solution (e) (0.10 per cent);

— *total*: not more than 0.7 per cent;

— *disregard limit*: 0.1 times the area of the peak due to aceclofenac in the chromatogram obtained with reference solution (e) (0.02 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

To 2.0 g in a silica crucible, add 2 ml of *sulphuric acid R* to wet the substance. Heat progressively to ignition and continue heating until an almost white or at most a greyish residue is obtained. Carry out the ignition at a temperature not exceeding 800 °C. Allow to cool. Add 3 ml of *hydrochloric acid R* and 1 ml of *nitric acid R*. Heat and evaporate slowly to dryness. Cool and add 1 ml of a 100 g/l solution of *hydrochloric acid R* and 10.0 ml of *distilled water R*. Neutralise with a 1.0 g/l solution of *ammonia R* using *0.1 ml of phenolphthalein solution R* as indicator. Add 2.0 ml of a 60 g/l solution of *anhydrous acetic acid R* and dilute to

20 ml with *distilled water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 40 ml of *methanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

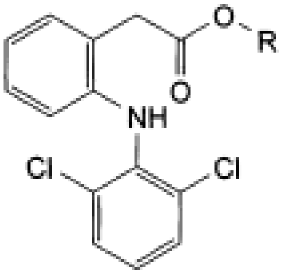
1 ml of *0.1 M sodium hydroxide* is equivalent to 35.42 mg of $C_{16}H_{13}Cl_2NO_4$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

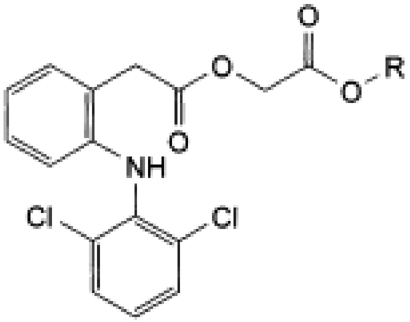
Specified impurities A, B, C, D, E, F, G, H, I.



A. R = H: [2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid (diclofenac),

B. R = CH_3: methyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetate (methyl ester of diclofenac),

C. R = C_2H_5: ethyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetate (ethyl ester of diclofenac),



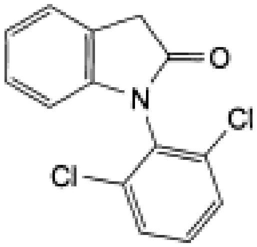
D. R = CH_3: methyl [[[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate (methyl ester of aceclofenac),

E. R = C_2H_5: ethyl [[[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate (ethyl ester of aceclofenac),

F. R = CH_2-C_6H_5: benzyl [[[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate (benzyl ester of aceclofenac),

G. R = CH_2-CO_2H: [[[[[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetyl]oxy]acetic acid (acetic aceclofenac),

H. R = CH_2-CO-O-CH_2-CO_2H: [[[[[[[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetyl]oxy]acetyl]oxy]acetic acid (diacetic aceclofenac),



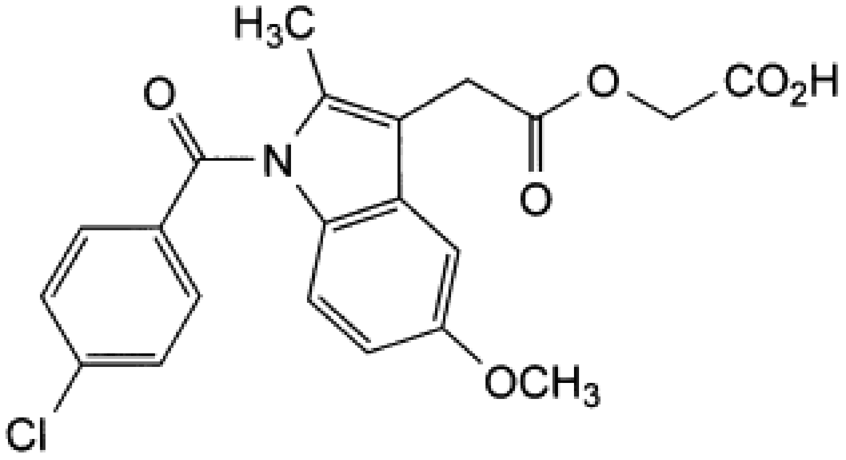
I. 1-(2,6-dichlorophenyl)-1,3-dihydro-2*H*-indol-2-one.

Ph Eur

Acemetacin

General Notices

(Ph Eur monograph 1686)



$C_{21}H_{18}ClNO_6$ 415.8 *53164-05-9*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

[[[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetyl]oxy]acetic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow or greenish-yellow, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone, slightly soluble in anhydrous ethanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison acemetacin CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *acetonitrile for chromatography R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of the test solution to 50.0 ml with *acetonitrile for chromatography R*. Dilute 1.0 ml of this solution to 100.0 ml with *acetonitrile for chromatography R.*

Reference solution (b) Dissolve 5.0 mg of *acemetacin impurity A CRS* and 10.0 mg of *indometacin CRS* (impurity B) in *acetonitrile for chromatography R*, and dilute to 50.0 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 20.0 ml with *acetonitrile for chromatography R*.

Reference solution (d) To 1 ml of reference solution (b), add 10 ml of the test solution and dilute to 20 ml with *acetonitrile for chromatography R.*

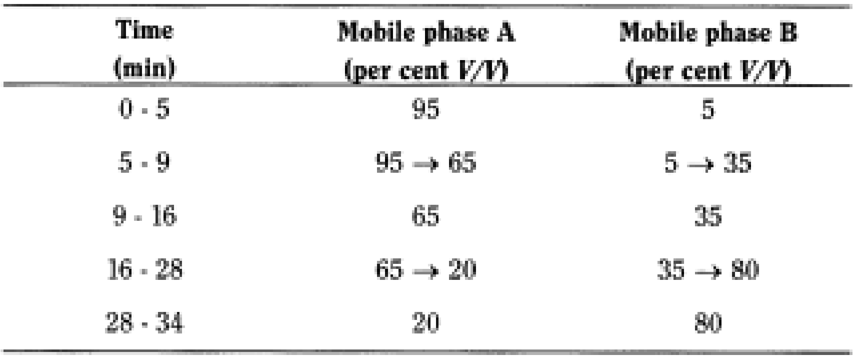
Reference solution (e) Dissolve the contents of a vial of *acemetacin impurity mixture CRS* (containing impurities C, D, E and F) in 2 ml of the test solution.

Column:

- — *size: l* = 0.25 m, Ø = 4 mm;
- — *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 40 °C.

Mobile phase:

- — *mobile phase A*: dissolve 1.0 g of *potassium dihydrogen phosphate R* in 900 ml of *water R*, adjust to pH 6.5 with *1 M sodium hydroxide* and dilute to 1000 ml with *water R*;
- — *mobile phase B*: *acetonitrile for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 9	95 → 65	5 → 35
9 - 16	65	35
16 - 28	65 → 20	35 → 80
28 - 34	20	80

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl.

Identification of impurities:

— use the chromatogram supplied with *acemetacin impurity mixture CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities C, D, E and F;

— use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention With reference to acemetacin (retention time = about 15 min): impurity A = about 0.7; impurity B = about 0.9; impurity F = about 1.2; impurity C = about 1.3; impurity D = about 1.5; impurity E = about 2.2.

System suitability Reference solution (d):

— *peak-to-valley ratio*: minimum 15, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to acemetacin.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.3; impurity D = 1.4; impurity F = 1.3;

— *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *impurities C, D, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals

Maximum 20 ppm.

Solvent mixture *methanol R*, *acetone R* (10:90 *V/V*).

Test solution Dissolve 0.250 g of the substance to be examined in 20 ml of the solvent mixture.

Reference solution Dilute 0.5 ml of *lead standard solution (10 ppm Pb) R* to 20 ml with the solvent mixture.

Blank solution 20 ml of the solvent mixture.

Monitor solution Dissolve 0.250 g of the substance to be examined in 0.5 ml of *lead standard solution (10 ppm Pb) R* and dilute to 20 ml with the solvent mixture.

To each solution, add 2 ml of *buffer solution pH 3.5 R.* Mix and add to 1.2 ml of *thioacetamide reagent R*. Mix immediately. Filter the solutions through a membrane filter (pore size 0.45 µm) *(2.4.8)*. Compare the spots on the filters obtained with the different solutions. The test is invalid if the reference solution does not show a slight brown colour compared to the blank solution. The substance to be examined complies with the test if the brown colour of the spot resulting from the test solution is not more intense than that of the spot resulting from the reference solution.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 20 ml of *acetone R* and add 10 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

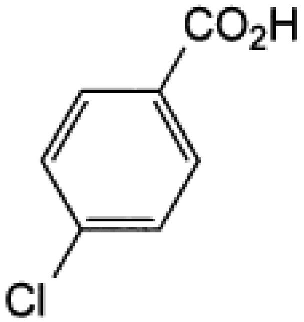
1 ml of *0.1 M sodium hydroxide* is equivalent to 41.58 mg of $C_{21}H_{18}ClNO_6$.

STORAGE

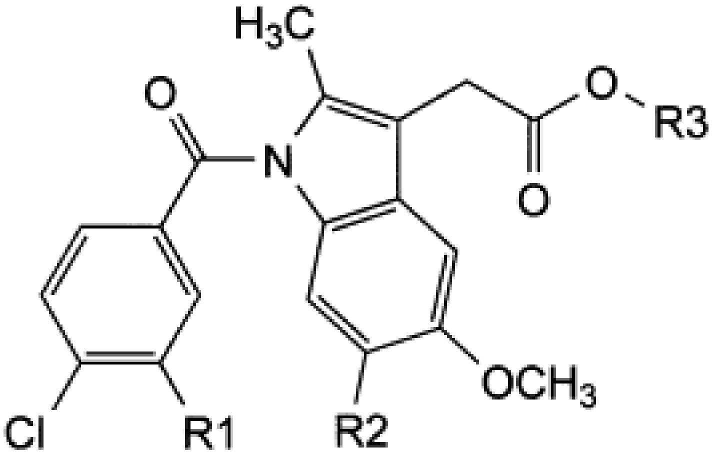
Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F*.



A. 4-chlorobenzoic acid,



B. R1 = R2 = R3 = H: indometacin,

C. R1 = Cl, R2 = H, R3 = CH_2-CO_2H: [[[1-(3,4-dichlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetyl]oxy]acetic acid,

D. R1 = H, R2 = $C(CH_3)_3$, R3 = CH_2-CO_2H: [[[1-(4-chlorobenzoyl)-6-(1,1-dimethylethyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetyl]oxy]acetic acid,

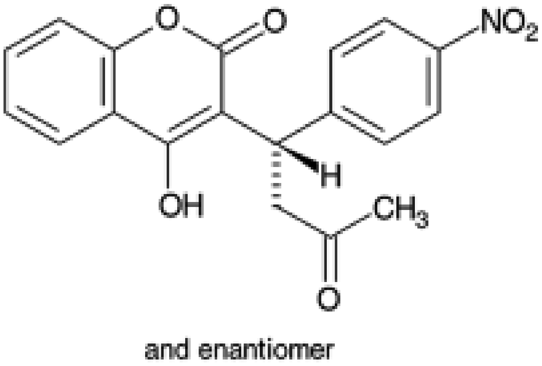
E. R1 = R2 = H, R3 = CH_2-CO-*O*-$C(CH_3)_3$: 1,1-dimethylethyl [[[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetyl]oxy]acetate,

F. R1 = R2 = H, R3 = CH_2-CO-*O*-CH_2-CO_2H: [[[[[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetyl]oxy]acetyl]oxy]acetic acid.

Ph Eur

Acenocoumarol

General Notices



$C_{19}H_{15}NO_6$ 353.3 *152-72-7*

Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant.

Preparation

Acenocoumarol Tablets

DEFINITION

Acenocoumarol is (*RS*)-4-hydroxy-3-(1-*p*-nitrophenyl-3-oxobutyl)coumarin. It contains not less than 98.5% and not more than 100.5% of $C_{19}H_{15}NO_6$, calculated with reference to the dried substance.

CHARACTERISTICS

An almost white to buff powder.

Practically insoluble in *water* and in *ether*; slightly soluble in *ethanol (96%).* It dissolves in aqueous solutions of the alkali hydroxides. It exhibits polymorphism.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of acenocoumarol *(RS 001)*. If the spectra are not concordant, dissolve 0.1 g of the substance being examined in 10 ml of *acetone* and add *water* drop wise until the solution becomes turbid. Heat on a water bath until the solution is clear and allow to stand. Filter, wash the crystals with a mixture of equal volumes of *acetone* and *water* and dry at 100° at a pressure of 2 kPa for 30 minutes. Prepare a new spectrum of the residue.

TESTS

Clarity and colour of solution

A. A 2.0% w/v solution in *acetone* is *clear*, Appendix IV A.

B. The *absorbance* of a 4-cm layer of a 2.0% w/v solution in *acetone* at 460 nm is not more than 0.12, Appendix II B.

C. A 2.0% w/v solution in 0.1M *sodium hydroxide* is *clear*, Appendix IV A, and yellow.

Light absorption

Absorbance of a 0.001% w/v solution in a mixture of 1 volume of 1M *hydrochloric acid* and 9 volumes of *methanol* at the maximum at 306 nm, 0.50 to 0.54, calculated with reference to the dried substance, Appendix II B.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 50 volumes of *dichloromethane*, 50 volumes of *cyclohexane* and 20 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 20 µl of each of two solutions of the substance being examined in *acetone* containing (1) 2.0% w/v and (2) 0.0020% w/v. After removal of the plate, allow it to dry in air and immediately examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

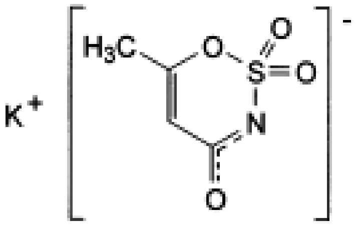
ASSAY

Dissolve 0.6 g in 50 ml of *acetone* and titrate with 0.1M *sodium hydroxide VS* using *bromothymol blue solution R3* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of sodium hydroxide required. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 35.33 mg of $C_{19}H_{15}NO_6$.

Acesulfame Potassium

General Notices

(Ph Eur monograph 1282)



$C_4H_4KNO_4S$ 201.2   *55589-62-3*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

Potassium 6-methyl-1,2,3-oxathiazin-4-olate 2,2-dioxide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Soluble in water, very slightly soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison acesulfame potassium CRS.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *acesulfame potassium CRS* in *water R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *acesulfame potassium CRS* and 5 mg of *saccharin sodium R* in *water R* and dilute to 5 ml with the same solvent.

Plate *Cellulose for chromatography R* as the coating substance.

Mobile phase *Concentrated ammonia R*, *acetone R*, *ethyl acetate R* (10:60:60 *V/V/V*).

Application 5 µl as bands.

Development Twice over a path of 15 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated bands.

Results The principal band in the chromatogram obtained with the test solution is similar in position and size to the principal band in the chromatogram obtained with reference solution (a).

C. 0.5 ml of solution S (see Tests) gives reaction (b) of potassium *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 20 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.80 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *acetylacetamide R* (impurity A) in *water R* and dilute to 25 ml with the same solvent. To 5 ml of the solution add 45 ml of *water R* and dilute to 100 ml with *methanol R*.

Reference solution (b) To 10 ml of reference solution (a) add 1 ml of the test solution and dilute to 20 ml with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *water R, ethanol (96 per cent) R, ethyl acetate R* (2:15:74 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air until the solvents are completely removed.

Detection Spray with *phosphoric vanillin solution R* and heat at 120 °C for about 10 min; examine in daylight.

System suitability The chromatogram obtained with reference solution (a) shows a clearly visible spot and the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.125 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 4.0 mg of *acesulfame potassium impurity B CRS* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 200.0 ml with *water R*.

Reference solution (b) Dissolve 0.100 g of the substance to be examined in reference solution (a) and dilute to 10.0 ml with the same solution.

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a 3.3 g/l solution of *tetrabutylammonium hydrogen sulphate R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 234 nm.

Injection 20 µl.

Run time 3 times the retention time of acesulfame.

Relative retention With reference to acesulfame

(retention time = about 5.3 min): impurity B = about 1.6.

System suitability:

— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to acesulfame in the chromatogram obtained with reference solution (b).

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (20 ppm),

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) except for the peak due to impurity B (0.05 per cent).

Fluorides

Maximum 3 ppm.

Potentiometry *(2.2.36, Method I)*.

Test solution Dissolve 3.000 g of the substance to be examined in *distilled water R*, add 15.0 ml of *total-ionic-strength-adjustment buffer R1* and dilute to 50.0 ml with *distilled water R*.

Reference solutions To 0.5 ml, 1.0 ml, 1.5 ml and 3.0 ml of *fluoride standard solution (10 ppm F) R* add 15.0 ml of *total-ionic-strength-adjustment buffer R1* and dilute to 50.0 ml with *distilled water R*.

Indicator electrode Fluoride-selective.

Reference electrode Silver-silver chloride.

Heavy metals *(2.4.8)*

Maximum 5 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

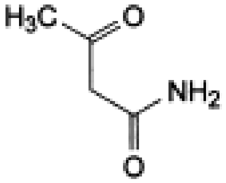
ASSAY

Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

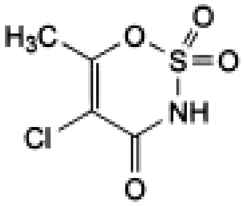
1 ml of *0.1 M perchloric acid* is equivalent to 20.12 mg of $C_4H_4KNO_4S$.

IMPURITIES

Specified impurities *A, B.*



A. 3-oxobutanamide (acetylacetamide),



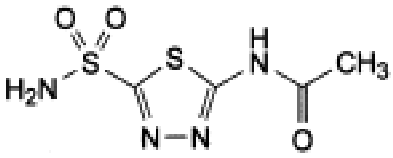
B. 5-chloro-6-methyl-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide.

Ph Eur

Acetazolamide

General Notices

(Ph Eur monograph 0454)



$C_4H_6N_4O_3S_2$ 222.2 *59-66-5*

Action and use

Carbonic anhydrase inhibitor; diuretic; treatment of glaucoma and ocular hypertension; treatment of mountain sickness.

Preparation

Acetazolamide Tablets

Ph Eur

DEFINITION

Acetazolamide contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of *N*-(5-sulphamoyl-1,3,4-thiadiazol-2-yl)acetamide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, slightly soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Dissolve 30.0 mg in *0.01 M sodium hydroxide* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M sodium hydroxide* (solution A). Examined between 230 nm and 260 nm *(2.2.25)*, solution A shows an absorption maximum at 240 nm. The specific absorbance at the maximum is 162 to 176. Dilute 25.0 ml of solution A to 100.0 ml with *0.01 M sodium hydroxide*. Examined between 260 nm and 350 nm, the solution shows an absorption maximum at 292 nm. The specific absorbance at the maximum is 570 to 620.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *acetazolamide CRS*. If the spectra obtained in the solid state with the substance to be examined and the reference substance show differences, dissolve separately the substance to be examined and the reference substance in *alcohol R*, evaporate to dryness, prepare new discs and record the spectra.

C. Introduce about 20 mg into a test-tube and add 4 ml of *dilute hydrochloric acid R* and 0.2 g of *zinc powder R*. Immediately place a piece of *lead acetate paper R* over the mouth of the tube. The paper shows a brownish-black colour.

D. Dissolve about 25 mg in a mixture of 0.1 ml of *dilute sodium hydroxide solution R* and 5 ml of *water R*. Add 0.1 ml of *copper sulphate solution R*. A greenish-blue precipitate is formed.

TESTS

Appearance of solution

Dissolve 1.0 g in 10 ml of *1 M sodium hydroxide*. The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution Y_5 or BY_5 *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 50 mg of the substance to be examined in a mixture of equal volumes of *alcohol R* and *ethyl acetate R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dilute 1 ml of the test solution to 100 ml with a mixture of equal volumes of *alcohol R* and *ethyl acetate R*.

Apply separately to the plate 20 µl of each solution. Use the tank without lining the walls and allow to saturate for 1 h before development. Develop over a path of 15 cm using a freshly prepared mixture of 20 volumes of *concentrated ammonia R*, 30 volumes of *ethyl acetate R* and 50 volumes of *2-propanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (1 per cent).

Sulphates *(2.4.13)*

To 0.4 g add 20 ml of *distilled water R* and dissolve by heating to boiling. Allow to cool with frequent shaking and filter. 15 ml of the filtrate complies with the limit test for sulphates (500 ppm).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 25 ml of *dimethylformamide R*. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 22.22 mg of $C_4H_6N_4O_3S_2$.

Ph Eur

Acetic Acid (33 per cent)

General Notices

Acetic Acid

Preparation

Acetic Acid (6 per cent)

DEFINITION

Acetic Acid (33 per cent) contains not less than 32.5% and not more than 33.5% w/w of acetic acid, $C_2H_4O_2$.

CHARACTERISTICS

A clear, colourless liquid.

Miscible with *water*, with *ethanol (96%)* and with *glycerol*.

IDENTIFICATION

A. Strongly acidic, even when diluted freely.

B. When neutralised, yields the reactions characteristic of *acetates*, Appendix VI.

TESTS

Weight per ml

1.040 to 1.042 g, Appendix V G.

Heavy metals

Evaporate 10.0 ml to dryness and add 20 ml of *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (2 ppm).

Chloride

Dilute 5.0 ml with sufficient *water* to produce 100 ml. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (70 ppm).

Sulphate

12.5 ml of the solution used in the test for Chloride, diluted to 15 ml with *water*, complies with the *limit test for sulphates*, Appendix VII (240 ppm).

Aldehydes

Distil 15 ml. To the first 5 ml of the distillate add 10 ml of a 5% w/v solution of *mercury(II) chloride*, make alkaline with 5M *sodium hydroxide*, allow to stand for 5 minutes and make acidic with 1M *sulphuric acid*. The solution shows not more than a faint turbidity.

Formic acid and oxidisable impurities

Mix 5 ml with 6 ml of *sulphuric acid* and cool to 20°. Add 2 ml of 0.0167M *potassium dichromate VS*, allow to stand for 1 minute, add 25 ml of *water* and 1 ml of freshly prepared *dilute potassium iodide solution* and titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Not less than 1.0 ml of 0.1M *sodium thiosulphate VS* is required.

Readily oxidisable impurities

To 5.0 ml add 20 ml of *water* and 0.2 ml of 0.02M *potassium permanganate VS* and allow to stand for 1 minute. The pink colour is not entirely discharged.

Non-volatile matter

When evaporated to dryness and dried at 105°, leaves not more than 0.01% w/w of residue.

ASSAY

Weigh 5 g into a stopper flask containing 50 ml of *water* and titrate with 1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 1M *sodium hydroxide VS* is equivalent to 60.05 mg of $C_2H_4O_2$.

Acetic Acid (6 per cent)

General Notices

Dilute Acetic Acid

DEFINITION

Acetic Acid (6 per cent) contains not less than 5.7% and not more than 6.3% w/w of acetic acid, $C_2H_4O_2$. It may be prepared by mixing 182 g of Acetic Acid (33 per cent) with 818 g of Purified Water.

IDENTIFICATION

A. Strongly acidic.

B. When neutralised, yields the reactions characteristic of *acetates*, Appendix VI.

TESTS

Weight per ml

About 1.005 g, Appendix V G.

Heavy metals

Evaporate 20.0 ml to dryness and add 20 ml of *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (1 ppm).

Chloride

Dilute 5.0 ml with sufficient *water* to produce 100 ml. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (70 ppm).

Sulphate

12.5 ml of the solution used in the test for Chloride, diluted to 15 ml with *water*, complies with the *limit test for sulphates*, Appendix VII (240 ppm).

Aldehydes

Distil 75 ml. To the first 5 ml of the distillate add 10 ml of a 5% w/v solution of *mercury(II) chloride*, make alkaline with 5M *sodium hydroxide*, allow to stand for 5 minutes and acidify with 1M *sulphuric acid*. The solution shows not more than a faint turbidity.

Formic acid and oxidisable impurities

Mix 5 ml with 6 ml of *sulphuric acid* and cool to 20°. Add 0.4 ml of 0.0167M *potassium dichromate VS*, allow to stand for 1 minute, add 25 ml of *water* and 1 ml of freshly prepared *dilute potassium iodide solution* and titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Not less than 0.2 ml of 0.1M *sodium thiosulphate VS* is required.

Readily oxidisable impurities

To 25 ml add 0.2 ml of 0.02M *potassium permanganate VS* and allow to stand for 1 minute. The pink colour is not entirely discharged.

Non-volatile matter

When evaporated to dryness and dried at 105°, leaves not more than 0.01% w/w of residue.

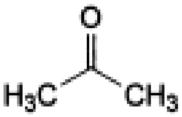
ASSAY

Add 30 ml of *water* to 20 g in a stopper flask and titrate with 1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 1M *sodium hydroxide VS* is equivalent to 60.05 mg of $C_2H_4O_2$.

Acetone

General Notices

(Ph Eur monograph 0872)



C_3H_6O 58.08 *67-64-1*

Ph Eur

DEFINITION

Propanone.

CHARACTERS

Appearance

Volatile, clear, colourless liquid.

Solubility

Miscible with water and with ethanol (96 per cent).

The vapour is flammable.

IDENTIFICATION

A. It complies with the test for relative density (see Tests).

B. To 1 ml, add 3 ml of *dilute sodium hydroxide solution R* and 0.3 ml of a 25 g/l solution of *sodium nitroprusside R*. An intense red colour is produced which becomes violet with the addition of 3.5 ml of *acetic acid R*.

C. To 10 ml of a 0.1 per cent *V/V* solution of the substance to be examined in *ethanol (50 per cent V/V) R*, add 1 ml of a 10 g/l solution of *nitrobenzaldehyde R* in *ethanol (50 per cent V/V) R* and 0.5 ml of *strong sodium hydroxide solution R*. Allow to stand for about 2 min and acidify with *acetic acid R*. A greenish-blue colour is produced.

TESTS

Appearance of solution

To 10 ml add 10 ml of *water R*. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 5 ml add 5 ml of *carbon dioxide-free water R*, 0.15 ml of *phenolphthalein solution R* and 0.5 ml of *0.01 M sodium hydroxide*. The solution is pink. Add 0.7 ml of *0.01 M hydrochloric acid* and 0.05 ml of *methyl red solution R*. The solution is red or orange.

Relative density *(2.2.5)*

0.790 to 0.793.

Reducing substances

To 30 ml add 0.1 ml of *0.02 M potassium permanganate* and allow to stand in the dark for 2 h. The mixture is not completely decolourised.

Related substances

Gas chromatography *(2.2.28)*.

Test solution The substance to be examined.

Reference solution (a) To 0.5 ml of *methanol R* add 0.5 ml of *2-propanol R* and dilute to 100.0 ml with the test solution. Dilute 1.0 ml of this solution to 10.0 ml with the test solution.

Reference solution (b) Dilute 100 µl of *benzene R* to 100.0 ml with the test solution. Dilute 0.20 ml of this solution to 100.0 ml with the test solution.

Column:

— *material*: fused silica,

— *size: l* = 50 m, Ø = 0.3 mm,

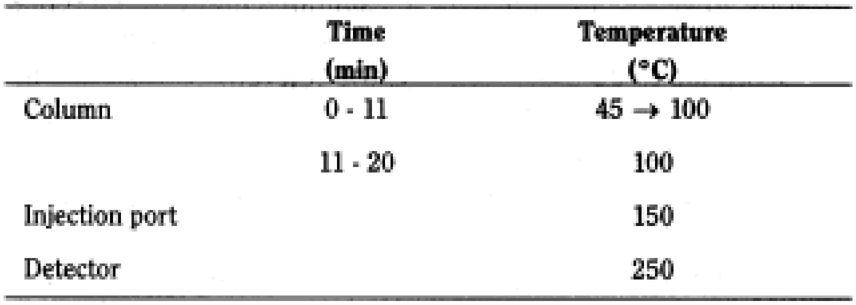
— *stationary phase: macrogol 20 000 R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 21 cm/s.

Split ratio 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 11	45 → 100
	11 - 20	100
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Retention time Impurity C = about 7.5 min.

System suitability:

— *resolution*: minimum 5.0 between the peak due to impurity A (2nd peak) and the peak due to impurity B (3rd peak) in the chromatogram obtained with reference solution (a),

— *signal-to-noise ratio*: minimum 5 for the peak due to impurity C in the chromatogram obtained with reference solution (b).

Limits:

— *impurities A, B*: for each impurity, not more than the difference between the areas of the corresponding peaks in the chromatogram obtained with reference solution (a) and the areas of the corresponding peaks in the chromatogram obtained with the test solution (0.05 per cent *V/V*),

— *impurity C*: not more than the difference between the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) and the area of the corresponding peak in the chromatogram obtained with the test solution (2 ppm *V/V*),

— *any other impurity*: for each impurity, not more than the difference between the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) and the area of the corresponding peak in the chromatogram obtained with the test solution (0.05 per cent *V/V*).

Matter insoluble in water

Dilute 1.0 ml to 20 ml with *water R*. The solution is clear *(2.2.1)*.

Residue on evaporation

Maximum 50 ppm.

Evaporate 20.0 g to dryness on a water-bath and dry at 100-105 °C. The residue weighs a maximum of 1 mg.

Water *(2.5.12)*

Maximum 3 g/l, determined on 10.0 ml. Use 20 ml of *anhydrous pyridine R* as solvent.

STORAGE

Protected from light.

IMPURITIES

Specified impurities   A, B, C.

A. CH_3-OH: methanol,

B. CH_3-CHOH-CH_3: propan-2-ol (isopropanol),

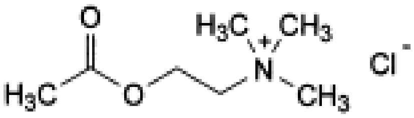
C. C_6H_6: benzene.

Ph Eur

Acetylcholine Chloride

General Notices

(Ph Eur monograph 1485)



$C_7H_{16}ClNO_2$ 181.7 *60-31-1*

Action and use

Cholinoceptor agonist.

Ph Eur

DEFINITION

2-(Acetyloxy)-*N,N,N*-trimethylethanaminium chloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder or colourless crystals, very hygroscopic.

Solubility

Very soluble in water, freely soluble in alcohol, slightly soluble in methylene chloride.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Melting point *(2.2.14)*: 149 °C to 152 °C.

Introduce the substance to be examined into a capillary tube. Dry in an oven at 100-105 °C for 3 h. Seal the tube and determine the melting point.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *acetylcholine chloride CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal band in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal band in the chromatogram obtained with reference solution (b).

D. To 15 mg add 10 ml of *dilute sodium hydroxide solution R*, 2 ml of *0.02 M potassium permanganate* and heat. The vapours formed change the colour of *red litmus paper R* to blue.

E. 0.5 ml of solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 or BY_6 *(2.2.2, Method II)*.

Acidity

Dilute 1 ml of solution S to 10 ml with *carbon dioxide-free water R*. Add 0.05 ml of *phenolphthalein solution R*. Not more than 0.4 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances

Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use*.

Test solution (a) Dissolve 0.30 g of the substance to be examined in *methanol R* and dilute to 3.0 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dilute 1 ml of test solution (a) to 100 ml with *methanol R*.

Reference solution (b) Dissolve 20.0 mg of *acetylcholine chloride CRS* in *methanol R* and dilute to 2.0 ml with the same solvent.

Reference solution (c) Dissolve 20 mg of *choline chloride R* in *methanol R*, add 0.4 ml of test solution (a) and dilute to 2.0 ml with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase Mix 20 volumes of a 40 g/l solution of *ammonium nitrate R*, 20 volumes of *methanol R* and 60 volumes of *acetonitrile R*.

Application 5 µl as bands of 10 mm by 2 mm.

Development Over 2/3 of the plate.

Detection Spray with *potassium iodobismuthate solution R3*.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated bands.

Limits:

— *any impurity*: any bands in the chromatogram obtained with test solution (a), apart from the principal band, are not more intense than the principal band in the chromatogram obtained with reference solution (a) (1 per cent).

Trimethylamine

Dissolve 0.1 g in 10 ml of *sodium carbonate solution R* and heat to boiling. No vapours appear which turn *red litmus paper R* blue.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

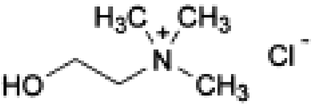
Dissolve 0.200 g in 20 ml of *carbon dioxide-free water R*. Neutralise with *0.01 M sodium hydroxide* using 0.15 ml of *phenolphthalein solution R* as indicator. Add 20.0 ml of *0.1 M sodium hydroxide* and allow to stand for 30 min. Titrate with *0.1 M hydrochloric acid* .

1 ml of *0.1 M sodium hydroxide* is equivalent to 18.17 mg of $C_7H_{16}ClNO_2$.

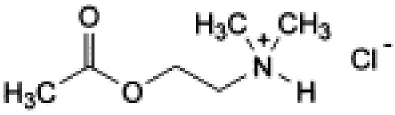
STORAGE

In ampoules, protected from light.

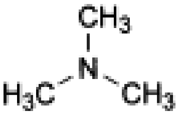
IMPURITIES



A. 2-hydroxy-*N,N,N*-trimethylethanaminium chloride (choline chloride),



B. 2-(acetyloxy)-*N,N*-dimethylethanaminium chloride,

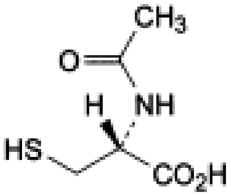


C .*N,N*-dimethylmethanamine.

Acetylcysteine

General Notices

(Ph Eur monograph 0967)



$C_5H_9NO_3S$ 163.2 *616-91-1*

Action and use

Sulphydryl donor; antidote to paracetamol poisoning; mucolytic.

Preparation

Acetylcysteine Injection

Ph Eur

DEFINITION

(2*R*)-2-(Acetylamino)-3-sulfanylpropanoic acid.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, C.

Second identification A, B, D, E.

A. Specific optical rotation (see Tests).

B. Melting point *(2.2.14)*: 104 °C to 110 °C.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison acetylcysteine CRS.

D. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

E. To 0.5 ml of solution S (see Tests) add 0.05 ml of a 50 g/l solution of *sodium nitroprusside R* and 0.05 ml of *concentrated ammonia R*. A dark violet colour develops.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

2.0 to 2.8.

To 2 ml of solution S add 8 ml of *carbon dioxide-free water R* and mix.

Specific optical rotation *(2.2.7)*

+ 21.0 to + 27.0 (dried substance).

In a 25 ml volumetric flask, mix 1.25 g with 1 ml of a 10 g/l solution of *sodium edetate R*. Add 7.5 ml of a 40 g/l solution of *sodium hydroxide R*, mix and dissolve. Dilute to 25.0 ml with *phosphate buffer solution pH 7.0 R2*.

Related substances

Liquid chromatography *(2.2.29)*. *Except where otherwise prescribed, prepare the solutions immediately before use.*

Test solution (a) Suspend 0.80 g of the substance to be examined in 1 ml of *1 M hydrochloric acid* and dilute to 100.0 ml with *water R*.

Test solution (b) Dilute 5.0 ml of test solution (a) to 100.0 ml with *water R*. Dilute 5.0 ml of this solution to 50.0 ml with *water R*.

Test solution (c) Use test solution (a) after storage for at least 1 h.

Reference solution (a) Suspend 4.0 mg of *acetylcysteine CRS*, 4.0 mg of *L-cystine R* (impurity A), 4.0 mg of *L-cysteine R* (impurity B), 4.0 mg of *acetylcysteine impurity C CRS* and 4.0 mg of *acetylcysteine impurity D CRS* in 1 ml of *1 M hydrochloric acid* and dilute to 100.0 ml with *water R*.

Reference solution (b) Suspend 4.0 mg of *acetylcysteine CRS* in 1 ml of *1 M hydrochloric acid* and dilute to 100.0 ml with *water R*.

Column:

— *size:* l = 0.25 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Stir 3 volumes of *acetonitrile R* and 97 volumes of *water R* in a beaker; adjust to pH 3.0 with *phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl, 3 times; inject *0.01 M hydrochloric acid* as a blank.

Run time 5 times the retention time of acetylcysteine (about 30 min).

Retention time Impurity A = about 2.2 min; impurity B = about 2.4 min; 2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution (c) = about 3.3 min; acetylcysteine = about 6.4 min; impurity C = about 12 min; impurity D = about 14 min.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurities A and B and minimum 2.0 between the peaks due to impurities C and D.

From the chromatogram obtained with test solution (a), calculate the percentage content of the known impurities (T_1) and the unknown impurities (T_2) using the following equations:

$$T_1 = \frac{A_1 \times m_2 \times 100}{A_2 \times m_1}$$

$$T_2 = \frac{A_3 \times m_3 \times 100}{A_4 \times m_1}$$

A_1 = peak area of individual impurity (impurity A, impurity B, impurity C and impurity D) in the chromatogram obtained with test solution (a);

A_2 = peak area of the corresponding individual impurity (impurity A, impurity B, impurity C and impurity D) in the chromatogram obtained with reference solution (a);

A_3 = peak area of unknown impurity in the chromatogram obtained with test solution (a);

A_4 = peak area of acetylcysteine in the chromatogram obtained with reference solution (b);

m_1 = mass of the substance to be examined in test solution (a);

m_2 = mass of the individual impurity in reference solution (a);

m_3 = mass of acetylcysteine in reference solution (b).

Limits:

- *impurities A, B, C, D*: for each impurity, maximum 0.5 per cent;
- *any other impurity*: for each impurity, maximum 0.5 per cent;
- *total*: maximum 0.5 per cent;
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak with a retention time of about 3.3 min due to 2-methyl-2-thiazoline-4-carboxylic acid.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard (10 ppm Pb) R*.

Zinc

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 1.00 g in *0.001 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solutions Prepare the reference solutions using *zinc standard solution (5 mg/ml Zn) R*, diluting with *0.001 M hydrochloric acid*.

Source Zinc hollow-cathode lamp.

Wavelength 213.8 nm.

Atomisation device Air-acetylene flame.

Use a correction procedure for non-specific absorption.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 70 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.140 g in 60 ml of *water R* and add 10 ml of *dilute hydrochloric acid R*. After cooling in iced water, add 10 ml of *potassium iodide solution R* and titrate with *0.05 M iodine*, using 1 ml of *starch solution R* as indicator.

1 ml of *0.05 M iodine* is equivalent to 16.32 mg of $C_5H_9NO_3S$.

STORAGE

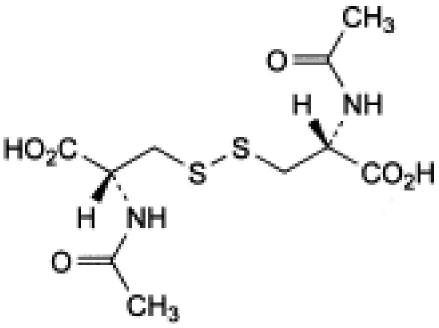
Protected from light.

IMPURITIES

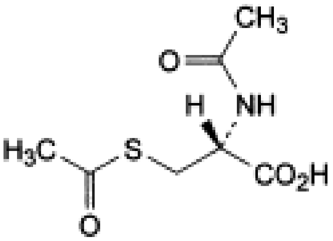
Specified impurities *A, B, C, D.*

A. L-cystine,

B. L-cysteine,



C. *N,N′*-diacetyl-l-cystine,



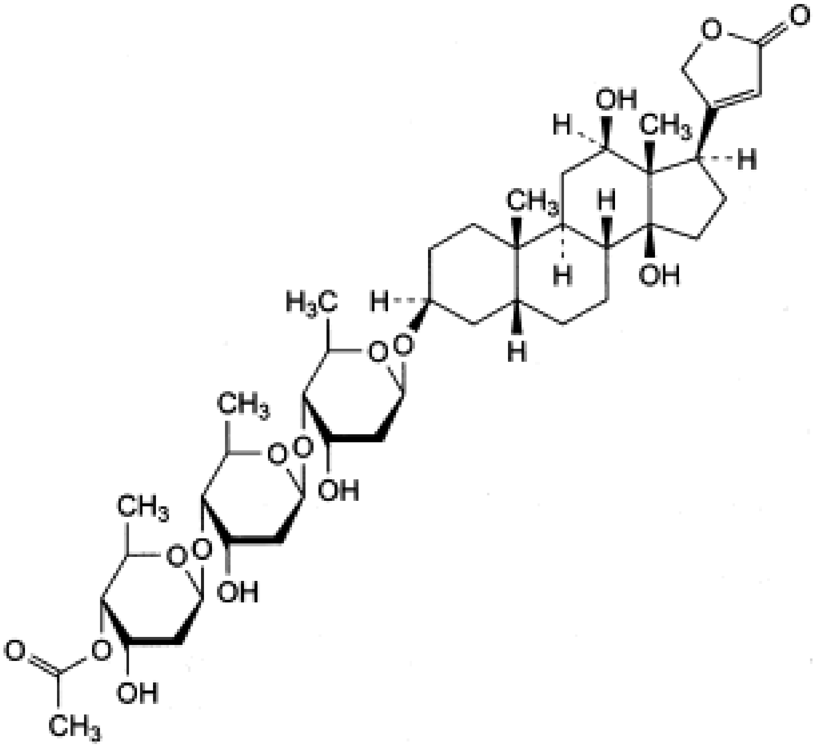
D. *N,S*-diacetyl-l-cysteine.

Ph Eur

Acetyldigoxin

General Notices

(β-Acetyldigoxin, Ph Eur monograph 2168)



$C_{43}H_{66}O_{15}$ 823 *5355-48-6*

Action and use

Cardiac Glycoside.

Ph Eur

DEFINITION

3β-[(4-*C*-Acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)- 2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20 (22)-enolide.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison β*-acetyldigoxin CRS.*

TESTS

Specific optical rotation *(2.2.7)*

+ 26.2 to + 28.2 (dried substance).

Dissolve 0.50 g in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 25.0 ml with the same mixture of solvents.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Solvent mixture Mix equal volumes of *methanol R2* and *acetonitrile for chromatography R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dissolve 10.0 mg of β*-acetyldigoxin CRS* in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *gitoxin CRS* (impurity D) in the solvent mixture and dilute to 100.0 ml with the solvent mixture. To 5.0 ml of this solution, add 0.5 ml of reference solution (a) and dilute to 100.0 ml with the solvent mixture.

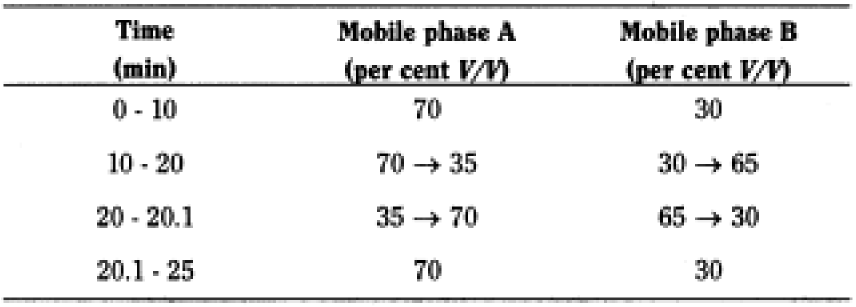
Reference solution (d) Dissolve 5.0 mg of β*-acetyldigoxin for peak identification CRS* (containing impurities A and B) in 10.0 ml of the solvent mixture.

Column:

- *size:* l = 0.125 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:

- *mobile phase A: water for chromatography R*;
- *mobile phase B: acetonitrile for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	70	30
10 - 20	70 → 35	30 → 65
20 - 20.1	35 → 70	65 → 30
20.1 - 25	70	30

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatograms obtained with reference solutions (c) and (d) to identify the peaks due to impurities A, B and D.

Relative retention With reference to β-acetyldigoxin (retention time = about 9 min): impurity about 0.3; impurity A = about 0.7; impurity D = about 1.2.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to β-acetyldigoxin and impurity D;

— *symmetry factor*: maximum 2.5 for the peak due to β-acetyldigoxin.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurity D*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *any other impurity*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *sum of impurities other than A, B and D*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Loss on drying *(2.2.32)*

Maximum 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{43}H_{66}O_{15}$ from the declared content of β*-acetyldigoxin CRS*.

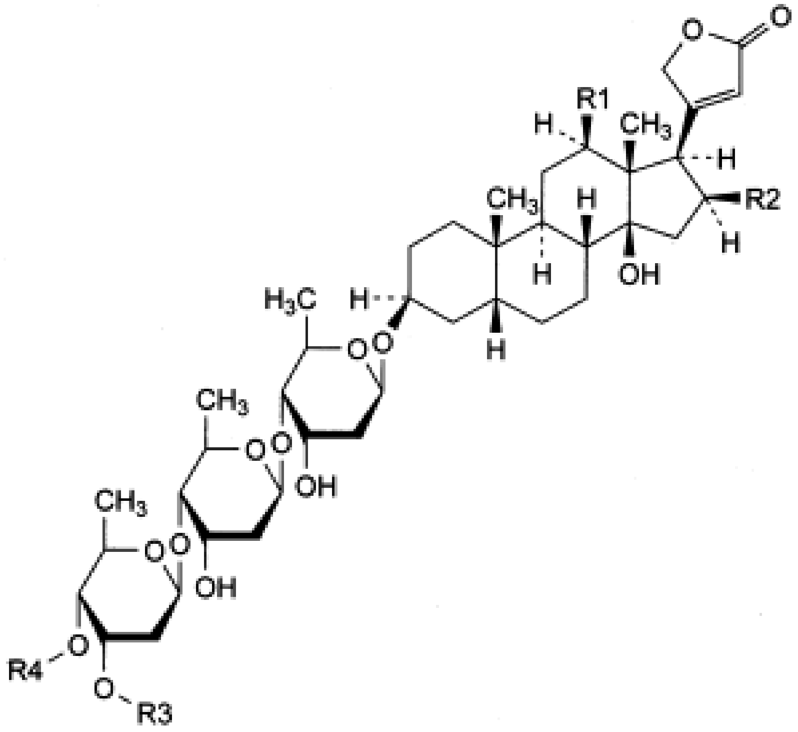
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, D.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C, E, F, G, H.*



A. R1 = OH, R2 = R4 = H, R3 = CO-CH$_3$: 3β-[(3-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (α-acetyldigoxin),

B. R1 = OH, R2 = R3 = R4 = H: digoxin,

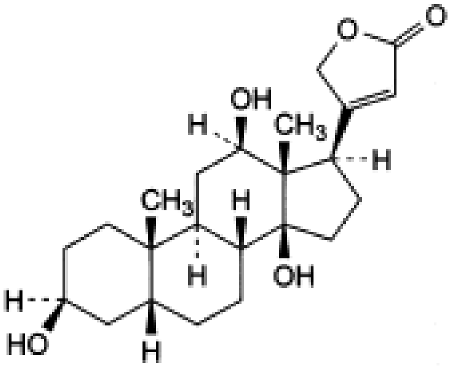
D. R1 = R3 = R4 = H, R2 = OH: 3β-[(2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14,16β-dihydroxy-5β-card-20(22)-enolide (gitoxin),

E. R1 = R2 = R3 = R4 = H: digitoxin,

H. R1 = R2 = R3 = H, R4 = CO-CH_3: β-acetyldigitoxin,

F. R1 = OH, R2 = H, R3 = R4 = CO-CH_3: 3β-[(3,4-*O*-diacetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (diacetyldigoxin),

G. R1 = R2 = R4 = H, R3 = CO-CH_3: 3β-[(3-*O*-acetyl- 2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxy-5β-card-20(22)-enolide (α-acetyldigitoxin),



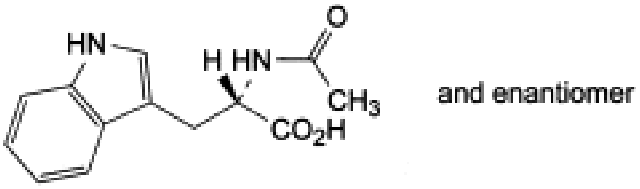
C. 3β,12β,14-trihydroxy-5β-card-20(22)-enolide (digoxigenin).

Ph Eur

Acetyltryptophan

General Notices

(*N*-Acetyltryptophan, Ph Eur monograph 1383)



$C_{13}H_{14}N_2O_3$ 246.3 *87-32-1*

Ph Eur

DEFINITION

(*RS*)-2-Acetylamino-3-(1*H*-indol-3-yl)propanoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

Tryptophan used for the production of *N*-acetyltryptophan complies with the test for impurity A and other related substances in the monograph on *Tryptophan (1272)*.

CHARACTERS

Appearance

White or almost white, crystalline powder, or colourless crystals.

Solubility

Slightly soluble in water, very soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

mp: about 205 °C.

IDENTIFICATION

First identification A, B.

Second identification A, C, D, E.

A. Optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *N-acetyltryptophan CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in 0.2 ml of *concentrated ammonia R* and dilute to 10 ml with *water R*.

Reference solution (a) Dissolve 50 mg of *N-acetyltryptophan CRS* in 0.2 ml of *concentrated ammonia R* and dilute to 10 ml with *water R*.

Reference solution (b) Dissolve 10 mg of *tryptophan R* in the test solution and dilute to 2 ml with the test solution.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (25:25:40 *V/V/V*).

Application 2 µl.

Development Over a path of 10 cm.

Drying In an oven at 100-105 °C for 15 min.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 2 mg in 2 ml of *water R*. Add 2 ml of *dimethylaminobenzaldehyde solution R6*. Heat on a water-bath. A blue or greenish-blue colour develops.

E. It gives the reaction of acetyl *(2.3.1)*. Proceed as described for substances hydrolysable only with difficulty.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 or GY_7 *(2.2.2, Method II)*.

Dissolve 1.0 g in a 40 g/l solution of *sodium hydroxide R* and dilute to 100 ml with the same alkaline solution.

Optical rotation *(2.2.7)*

- 0.1 ° to + 0.1 °.

Dissolve 2.50 g in a 40 g/l solution of *sodium hydroxide R* and dilute to 25.0 ml with the same alkaline solution.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the test and reference solutions immediately before use.*

Buffer solution pH 2.3 Dissolve 3.90 g of *sodium dihydrogen phosphate R* in 1000 ml of *water R*. Add about 700 ml of a 2.9 g/l solution of *phosphoric acid R* and adjust to pH 2.3 with the same acidic solution.

Solvent mixture *acetonitrile R, water R* (10:90 *V/V*).

Test solution Dissolve 0.10 g of the substance to be examined in a mixture of 50 volumes of *acetonitrile R* and 50 volumes of *water R* and dilute to 20.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dissolve 1.0 mg of *1,1′-ethylidenebis(tryptophan) CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (c) To 4.0 ml of reference solution (a), add 20.0 ml of reference solution (b) and dilute to 100.0 ml with the solvent mixture.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

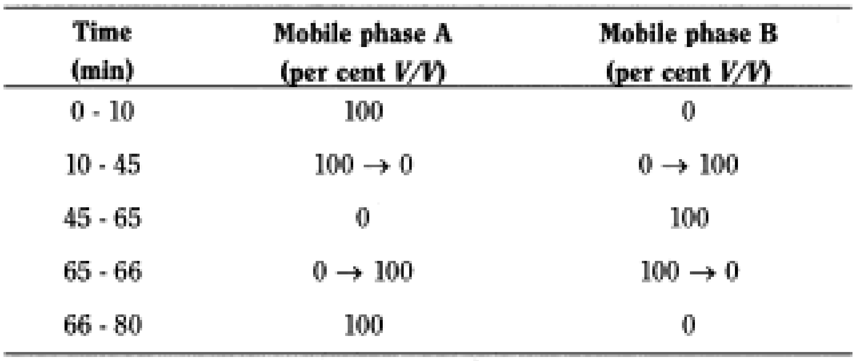
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A: acetonitrile R*, buffer solution pH 2.3 (115:885 *V/V*);

— *mobile phase B: acetonitrile R*, buffer solution pH 2.3 (350:650 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 45	100 → 0	0 → 100
45 - 65	0	100
65 - 66	0 → 100	100 → 0
66 - 80	100	0

Flow rate 0.7 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of the test solution and reference solutions (a) and (c).

Retention time *N*-acetyltryptophan = about 29 min; 1,1′-ethylidenebis(tryptophan) = about 34 min.

System suitability Reference solution (c):

— *resolution*: minimum 8.0 between the peaks due to *N*-acetyltryptophan and 1,1′-ethylidenebis(tryptophan); if necessary, adjust the time programme for the elution gradient (an increase in the duration of elution with mobile phase A produces longer retention times and a better resolution).

Limits:

— *impurities A, B, C, D, E, F, G, H, I, J, K, L*: for each impurity, not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.01 times the area of the principal peak the chromatogram obtained with reference solution (a) (0.01 per cent).

Ammonium ***(2.4.1, Method B)***

Maximum 200 ppm, determined on 0.10 g.

Prepare the standard using 0.2 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron ***(2.4.9)***

Maximum 10 ppm.

Dissolve 1.0 g in 50 ml of *hydrochloric acid R1*, with heating at 50 °C. Allow to cool. In a separating funnel, shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. Examine the aqueous layer.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 5 ml of *methanol R*. Add 50 ml of *anhydrous ethanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 24.63 mg of $C_{13}H_{14}N_2O_3$.

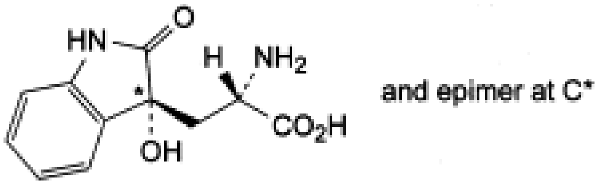
STORAGE

Protected from light.

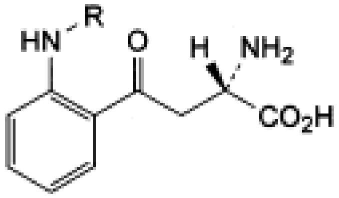
IMPURITIES

Specified impurities   A, B, C, D, E, F, G, H, I, J, K, L.

A. tryptophan,

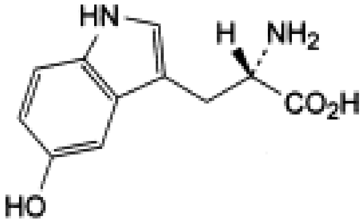


B. (S)-2-amino-3-[(3RS)-3-hydroxy-2-oxo-2,3-dihydro-1H-indol-3-yl]propanoic acid (dioxyindolylalanine),

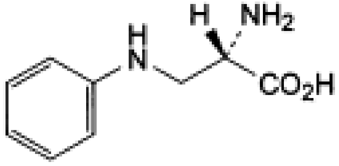


C. R = H: (S)-2-amino-4-(2-aminophenyl)-4-oxobutanoic acid (kynurenine),

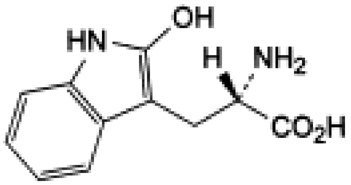
E. R = CHO: (S)-2-amino-4-[2-(formylamino)phenyl]-4-oxobutanoic acid (N-formylkynurenine),



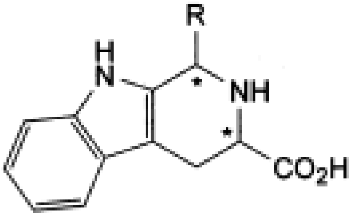
D. (S)-2-amino-3-(5-hydroxy-1H-indol-3-yl)propanoic acid (5-hydroxytryptophan),



F. (S)-2-amino-3-(phenylamino)propanoic acid (3-phenylaminoalanine),

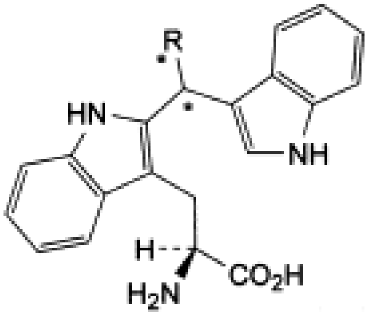


G. (S)-2-amino-3-(2-hydroxy-1H-indol-3-yl)propanoic acid (2-hydroxytryptophan),



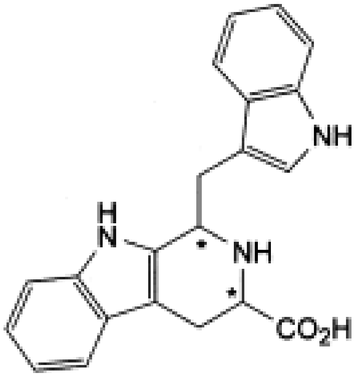
H. R = H: (3*RS*)-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylic acid,

I. R = CH_3: 1-methyl-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylic acid,



J. R = CHOH-CH_2-OH: (*S*)-2-amino-3-[2-[2,3-dihydroxy-1-(1*H*-indol-3-yl)propyl]-1*H*-indol-3-yl]propanoic acid,

K. R = H: (*S*)-2-amino-3-[2-(1*H*-indol-3-ylmethyl)-1*H*-indol-3-yl]propanoic acid,



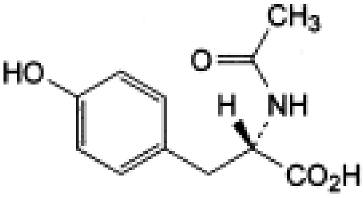
L. 1-(1*H*-indol-3-ylmethyl)-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylic acid.

Ph Eur

Acetyltyrosine

General Notices

(N-Acetyltyrosine, Ph Eur monograph 1384)



$C_{11}H_{13}NO_4$ 223.2 *537-55-3*

Ph Eur

DEFINITION

N-Acetyltyrosine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*S*)-2-(acetylamino)-3-(4-hydroxyphenyl)propanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, practically insoluble in cyclohexane.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *N-acetyltyrosine CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Solution S (see Tests) is strongly acid *(2.2.4)*.

TESTS

Solution S

Dissolve 2.50 g in *water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dilute 10.0 ml of solution S to 25.0 ml with *water R*. The specific optical rotation is + 46 to + 49, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel* F_{254} *plate R*.

Test solution (a) Dissolve 0.80 g of the substance to be examined in 6 ml of a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 10 ml with *ethanol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *ethanol R*.

Reference solution (a) Dissolve 80 mg of *N-acetyltyrosine CRS* in a mixture of 3 volumes of *water R*, 3 volumes of *glacial acetic acid R* and 94 volumes of *ethanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dilute 0.5 ml of test solution (b) to 10 ml with *ethanol R*.

Reference solution (c) Dissolve 40 mg of *tyrosine CRS* in 20 ml of a mixture of equal volumes of *water R* and *glacial acetic acid R* and dilute to 50 ml with *ethanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of *water R*, 15 volumes of *glacial acetic acid R* and 75 volumes of *ethyl acetate R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 10 min. Examine in daylight. Any spot corresponding to tyrosine is not more intense that the spot in the chromatogram obtained with reference solution (c) (1 per cent).

Chlorides *(2.4.4)*

Dilute 10 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dissolve 1.0 g in *distilled water R* and dilute to 20 ml with the same solvent. The solution complies with the limit test for sulphates (200 ppm).

Ammonium

Prepare a cell consisting of two watch-glasses 60 mm in diameter placed edge to edge. To the inner wall of the upper watch-glass stick a piece of *red litmus paper R 5* mm square and wetted with a few drops of *water R*. Finely powder the substance to be examined, place 50 mg in the lower watch-glass and dissolve in 0.5 ml of *water R*. To the solution add 0.30 g of *heavy magnesium oxide R*. Briefly triturate with a glass rod. Immediately close the cell by putting the two watch-glasses together. Heat at 40 °C for 15 min. The litmus paper is not more intensely blue coloured than a standard prepared at the same time and in the same manner using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*, 0.5 ml of *water R* and 0.30 g of *heavy magnesium oxide R* (200 ppm).

Iron ***(2.4.9)***

In a separating funnel, dissolve 0.5 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (20 ppm).

Heavy metals ***(2.4.8)***

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

Pyrogens ***(2.6.8)***

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1.0 ml of a freshly prepared solution in *water for injections R* containing per millilitre 10.0 mg of the substance to be examined and 9.0 mg of *pyrogen-free sodium chloride R*.

ASSAY

Dissolve 0.180 g in 50 ml of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

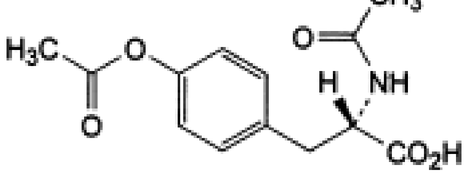
1 ml of *0.1 M sodium hydroxide* is equivalent to 22.32 mg of $C_{11}H_{13}NO_4$.

STORAGE

Store protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

IMPURITIES

A. tyrosine,



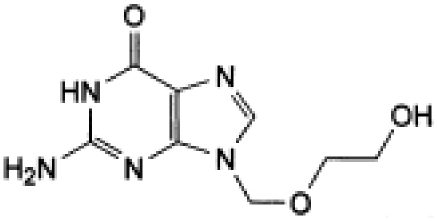
B. (2*S*)-2-(acetylamino)-3-[4-(acetoxy)phenyl]propanoic acid (diacetyltyrosine).

Ph Eur

Aciclovir

General Notices

(Ph Eur monograph 0968)



$C_8H_{11}N_5O_3$ 225.2 *59277-89-3*

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

Preparations

Aciclovir Cream

Aciclovir Eye Ointment

Aciclovir Intravenous Infusion

Aciclovir Oral Suspension

Aciclovir Tablets

Dispersible Aciclovir Tablets

Ph Eur

DEFINITION

Aciclovir contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water, freely soluble in dimethyl sulphoxide, very slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *aciclovir CRS*.

TESTS

Appearance of solution

Dissolve 0.25 g in *0.1 M sodium hydroxide* and dilute to 25 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Related substances

A. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Prepare the solutions immediately before use.

Test solution Dissolve 0.1 g of the substance to be examined in *dimethyl sulphoxide R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 5 mg of *aciclovir impurity A CRS* in *dimethyl sulphoxide R* and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *dimethyl sulphoxide R*.

Apply to the plate 10 µl of each solution. Keep the spots compact by drying in a current of warm air. Allow the plate to cool and develop over a path of 10 cm with a mixture of 2 volumes of *concentrated ammonia R*, 20 volumes of *methanol R* and 80 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spot with an R_F value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

B. Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in 10 ml of a mixture of 20 volumes of *glacial acetic acid R* and 80 volumes of *water R* and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *aciclovir CRS* and 5 mg of *aciclovir impurity A CRS* in a mixture of 20 volumes of *glacial acetic acid R* and 80 volumes of *water R* and dilute to 25.0 ml with the same mixture of solvents. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 7 mg of *guanine R* in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same solution. Dilute 1.0 ml to 20.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.10 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (3 µm),

— as mobile phase at a flow rate of 2 ml/min a mixture prepared as follows: dissolve 6.0 g of *sodium dihydrogen phosphate R* and 1.0 g of *sodium decanesulphonate R* in 900 ml of *water R* and adjust to pH 3 ± 0.1 with *phosphoric acid R*; add 40 ml of *acetonitrile R* and dilute to 1 litre with *water R*,

— as detector a spectrophotometer set at 254 nm,

— a loop injector.

Inject 20 µl of each solution. Record the chromatograms for 7 times the retention time of aciclovir. The test is not valid unless in the chromatogram obtained with reference solution (b), the number of theoretical plates calculated for the peak due to impurity A is at least 1500 and its mass distribution ratio is at least 7 (V_0 can be calculated using *dimethyl sulphoxide R*). In the chromatogram obtained with the test solution: the area of any peak corresponding to guanine is not greater than that of the peak in the chromatogram obtained with reference solution (c) (0.7 per cent); the area of any peak apart from the principal peak and any peak corresponding to guanine is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of such peaks is not greater than twice the area of the peak in the chromatogram obtained with reference solution (a) (1 per cent). Disregard any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (a).

Water *(2.5.12)*

Not more than 6.0 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

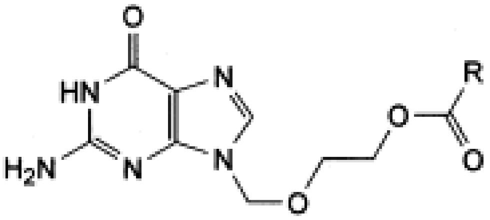
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

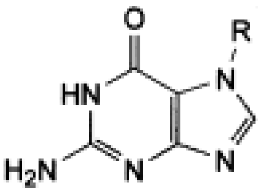
1 ml of *0.1 M perchloric acid* is equivalent to 22.52 mg of $C_8H_{11}N_5O_3$.

IMPURITIES



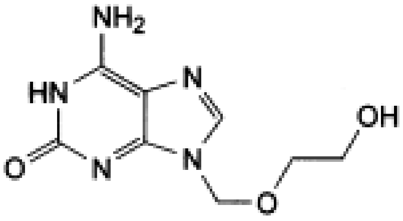
A. R = CH_3: 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate,

D. R = C_6H_5: 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl benzoate,

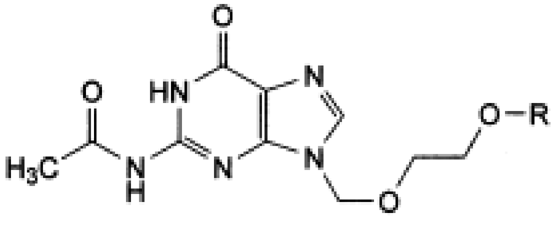


B. R = H: 2-amino-1,7-dihydro-6*H*-purin-6-one (guanine),

C. R = CH_2-O-CH_2-CH_2-OH: 2-amino-7-[(2-hydroxyethoxy)methyl]-1,7-dihydro-6*H*-purin-6-one,



E. 6-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-2*H*-purin-2-one,



F. R = H: *N*-[9-[(2-hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl]acetamide,

G. R = CO-CH_3: 2-[[2-(acetylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl acetate,

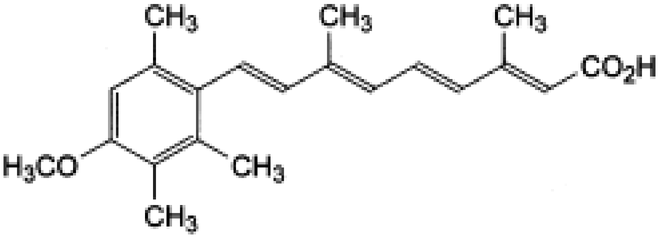
H. R = CO-C_6H_5: 2-[[2-(acetylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl benzoate.

Ph Eur

Acitretin

General Notices

(Ph Eur monograph 1385)



$C_{21}H_{26}O_3$ 326.4 *55079-83-9*

Action and use

Vitamin A analogue (retinoid); treatment of psoriasis; ichthyosis; Darier's disease.

Ph Eur

DEFINITION

(all-*E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow or greenish-yellow, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in tetrahydrofuran, slightly soluble in acetone and in ethanol (96 per cent), very slightly soluble in cyclohexane.

It is sensitive to air, heat and light, especially in solution.

Carry out all operations as rapidly as possible and avoid exposure to actinic light; use freshly prepared solutions.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 15.0 mg in 10 ml of *tetrahydrofuran R* and dilute immediately to 100.0 ml with the same solvent. Dilute 2.5 ml of this solution to 100.0 ml with *tetrahydrofuran R*.

Spectral range 300-400 nm.

Absorption maximum At 358 nm.

Specific absorbance at the absorption maximum 1350 to 1475.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *acitretin CRS.*

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)*. Maintain the sampler at 4 °C.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 5 ml of *tetrahydrofuran R* and dilute immediately to 100.0 ml with *anhydrous ethanol R*.

Test solution (b) Dilute 10.0 ml of test solution (a) to 25.0 ml with *anhydrous ethanol R*.

Reference solution (a) Dissolve 25.0 mg of *acitretin CRS* in 5 ml of *tetrahydrofuran R* and dilute immediately to 100.0 ml with *anhydrous ethanol R*. Dilute 10.0 ml of this solution to 25.0 ml with *anhydrous ethanol R*.

Reference solution (b) Dissolve 1.0 mg of *tretinoin CRS* in *anhydrous ethanol R* and dilute to 20.0 ml with the same solvent. Mix 5.0 ml of this solution with 2.5 ml of reference solution (a) and dilute to 100.0 ml with *anhydrous ethanol R*.

Reference solution (c) Dilute 2.5 ml of reference solution (a) to 50.0 ml with *anhydrous ethanol R*. Dilute 3.0 ml of this solution to 20.0 ml with *anhydrous ethanol R*.

Column:

— *size* l = 0.25 m, Ø = 4 mm;

— *stationary phase*: microparticulate *octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 200 m^2/g, a pore size of 15 nm and a carbon loading of 20 per cent;

— *temperature*: 25 °C.

Mobile phase A 0.3 per cent *V/V* solution of *glacial acetic acid R* in a mixture of 8 volumes of *water R* and 92 volumes of *anhydrous ethanol R*.

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 360 nm.

Injection 10 µl of test solution (a) and reference solutions (b) and (c).

Run time 2.5 times the retention time of acitretin.

Retention time Impurity A = about 4.8 min; tretinoin = about 5.2 min; acitretin = about 6.2 min; impurity B = about 10.2 min.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to acitretin and tretinoin; if necessary, adjust the concentration of *anhydrous ethanol R*.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the peak due to acitretin in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *total*: not more than the area of the peak due to acitretin in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c).

Palladium

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Introduce 2.0 g into a quartz beaker and add 3 ml of *magnesium nitrate solution R*. Heat in a muffle furnace to 350 °C at a rate of 40 °C/min to incinerate the content. Ignite at about 450 °C for 8 h and then at 550 ± 50 °C for a further hour. Dissolve the residue in a mixture of 0.75 ml of *hydrochloric acid R* and 0.25 ml of *nitric acid R*, warming gently. Cool, then transfer the solution into a volumetric flask containing *water R* and dilute to 50.0 ml with the same solvent.

Reference solution Dissolve 0.163 g of *heavy magnesium oxide R* in a mixture of 0.5 ml of *nitric acid R*, 1.5 ml of *hydrochloric acid R* and 50 ml of *water R*, add 2.0 ml of *palladium standard solution (20 ppm Pd R*) and dilute to 100.0 ml with *water R*.

Source Palladium hollow-cathode lamp.

Wavelength 247.6 nm.

Atomisation device Air-acetylene flame.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 100 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the assay protected from light, use amber volumetric flasks and prepare the solutions immediately before use.

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

System suitability:

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a); if necessary, adjust the integration parameters.

Calculate the percentage content of $C_{21}H_{26}O_3$ from the declared content of *acitretin CRS*.

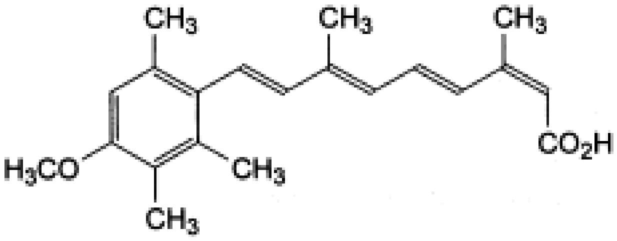
STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

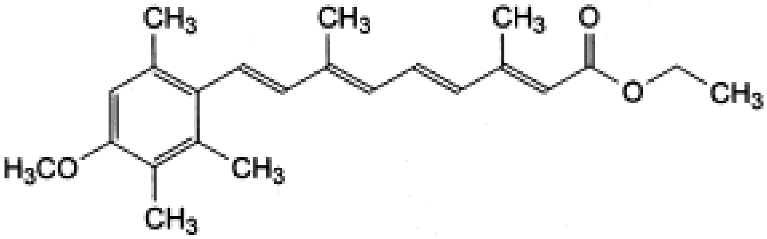
It is recommended that the contents of an opened container be used as soon as possible and any unused part be protected by an atmosphere of inert gas.

IMPURITIES

Specified impurities *A, B.*



A. (2*Z*,4*E*,6*E*,8*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid,



B. ethyl (all-*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoate.

Ph Eur

Activated Attapulgite

General Notices

Action and use

Antidiarrhoeal.

DEFINITION

Activated Attapulgite is a purified native hydrated magnesium aluminium silicate essentially consisting of the clay mineral palygorskite that has been carefully heated to increase its adsorptive capacity.

CHARACTERISTICS

A light, cream or buff, very fine powder, free or almost free from gritty particles.

IDENTIFICATION

Complies with tests A to D for Identification stated under Attapulgite.

TESTS

Acidity or alkalinity; Arsenic; Heavy metals; Acid-soluble matter; Water-soluble matter

Complies with the requirements stated under Attapulgite.

Adsorptive capacity

In a stoppered bottle shake 1.0 g, in *very fine powder*, with 50 ml of a 0.12% w/v solution of *methylene blue* for 5 minutes, allow to settle and centrifuge. The colour of the clear supernatant solution is not more intense than that of a 0.0012% w/v solution of *methylene blue*.

Loss on drying

When dried to constant weight at 105°, loses not more than 4.0% of its weight. Use 1 g.

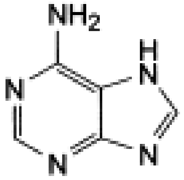
Loss on ignition

When ignited at 600°, loses not more than 9.0% of its weight. Use 1 g.

Adenine

General Notices

(Ph Eur monograph 0800)



$C_5H_5N_5$ 135.1 *73-24-5*

Action and use

Constituent of anticoagulant and preservative solutions for blood.

Ph Eur

DEFINITION

Adenine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7*H*-purin-6-amine, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, very slightly soluble in water and in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *adenine CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 1 g add 3.5 ml of *propionic anhydride R* and boil for 15 min with stirring. Cool. To the resulting crystalline mass add 15 ml of *light petroleum R* and heat to boiling with vigorous stirring. Cool and filter. Wash the precipitate with two quantities, each of 5 ml, of *light petroleum R*. Dissolve the precipitate in 10 ml of *water R* and boil for 1 min. Filter the mixture at 30 °C to 40 °C. Allow to cool. Filter, and dry the precipitate at 100 °C to 105 °C for 1 h. The melting point *(2.2.14)* of the precipitate is 237 °C to 241 °C.

TESTS

Solution S

Suspend 2.5 g in 50 ml of *distilled water R* and boil for 3 min. Cool and dilute to 50 ml with *distilled water R*. Filter. Use the filtrate as solution S.

Appearance of solution

Dissolve 0.5 g in *dilute hydrochloric acid R* and dilute to 50 ml with the same acid. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is blue. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is yellow.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dilute acetic acid R*, with heating if necessary, and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *dilute acetic acid R*.

Reference solution (a) Dissolve 10 mg of *adenine CRS* in *dilute acetic acid R*, with heating if necessary, and dilute to 10 ml with the same acid.

Reference solution (b) Dilute 1 ml of test solution (b) to 20 ml with *dilute acetic acid R*.

Reference solution (c) Dissolve 10 mg of *adenine CRS* and 10 mg of *adenosine R* in *dilute acetic acid R*, with heating if necessary, and dilute to 10 ml with the same acid.

Apply to the plate 5 µl of each solution. Develop over a path of 12 cm using a mixture of 20 volumes of *concentrated ammonia R*, 40 volumes of *ethyl acetate R* and 40 volumes of *propanol R*. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

To 10 ml of solution S add 1 ml of *concentrated ammonia R* and 3 ml of *silver nitrate solution R2*. Filter. Wash the precipitate with a little *water R* and dilute the filtrate to 15 ml with *water R*. The solution complies with the limit test for chlorides (100 ppm). When carrying out the test, add 2 ml of *dilute nitric acid R* instead of 1 ml of *dilute nitric acid R*.

Sulphates *(2.4.13)*

Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium

Prepare a cell consisting of two watch-glasses 60 mm in diameter placed edge to edge. To the inner wall of the upper watch-glass stick a piece of *red litmus paper R* 5 mm square and wetted with a few drops of *water R*. Finely powder the substance to be examined, place 0.5 g in the lower watch-glass and suspend in 0.5 ml of *water R*. To the suspension add 0.30 g of *heavy magnesium oxide R*. Briefly triturate with a glass rod. Immediately close the cell by putting the two watch-glasses together. Heat at 40 °C for 15 min. The litmus paper is not more intensely blue coloured than a standard prepared at the same time and in the same manner using 0.05 ml of *ammonium standard solution (100 ppm* NH_4*) R*, 0.5 ml of *water R* and 0.30 g of *heavy magnesium oxide R* (10 ppm).

Heavy metals ***(2.4.8)***

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in a mixture of 20 ml of *acetic anhydride R* and 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

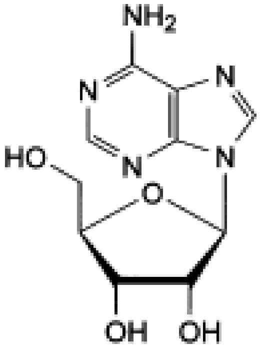
1 ml of *0.1 M perchloric acid* is equivalent to 13.51 mg of $C_5H_5N_5$.

Ph Eur

Adenosine

General Notices

(Ph Eur monograph 1486)



$C_{10}H_{13}N_5O_4$ 267.2 *58-61-7*

Action and use

Antiarrhythmic.

Ph Eur

DEFINITION

9-β-D-Ribofuranosyl-9*H*-purin-6-amine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, soluble in hot water, practically insoluble in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute mineral acids.

mp: about 234 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *adenosine CRS.*

TESTS

Solution S

Suspend 5.0 g in 100 ml of *distilled water R* and heat to boiling. Allow to cool, filter with the aid of vacuum and dilute to 100 ml with *distilled water R*.

Appearance of solution

Solution S is colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S, add *0.1 ml of bromocresol purple solution R* and 0.1 ml of *0.01 M hydrochloric acid*. The solution is yellow. Add 0.4 ml of *0.01 M sodium hydroxide*. The solution is violet-blue.

Specific optical rotation *(2.2.7)*

- 45 to - 49 (dried substance).

Dissolve 1.25 g in *1 M hydrochloric acid* and dilute to 50.0 ml with the same acid. Examine within 10 min of preparing the solution.

Related substances

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g of the substance to be examined in *dilute acetic acid R* with slight heating and dilute to 5 ml with the same acid.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *water R*.

Reference solution (b) Dissolve 10 mg of *adenosine CRS* and 10 mg of *adenine CRS* in *dilute acetic acid R*, with heating if necessary, and dilute to 10 ml with the same acid.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *water R, concentrated ammonia R, propanol R* (10:30:60 *V/V/V*).

Application 5 µl.

Development Over a path of 12 cm.

Drying In a current of warm air.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Detection A Examine in ultraviolet light at 254 nm.

Results A Any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent).

Detection B Spray with a 5 g/l solution of *potassium permanganate R* in *1 M sodium hydroxide*. Allow the plate to dry in a current of warm air and examine in daylight.

Results B Any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent).

B. Liquid chromatography *(2.2.29)*.

Solvent mixture Dissolve 6.8 g of *potassium hydrogen sulphate R* and 3.4 g of *tetrabutylammonium hydrogen sulphate R* in *water R*, adjust to pH 6.5 with a 60 g/l solution of *potassium hydroxide R* and dilute to 1000 ml with the same solvent. Use freshly prepared solvent mixture.

Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *adenine R* (impurity A) and 5 mg of *inosine R* (impurity G) in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 4 ml of this solution to 100 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R*, solvent mixture (40:60 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 1.5 times the retention time of adenosine.

Relative retention With reference to adenosine (retention time = about 13 min): impurity A = about 0.3; impurity G = about 0.4.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurities A and G.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity G = 1.4;

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides *(2.4.4)*

Maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 200 ppm, determined on solution S.

Ammonium *(2.4.1, Method B)*

Maximum 10 ppm, determined on 0.5 g.

Prepare the standard using 5 ml of *ammonium standard solution (1 ppm NH_4) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g, warming slightly if necessary, in a mixture of 20 ml of *acetic anhydride R* and 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 26.72 mg of $C_{10}H_{13}N_5O_4$.

IMPURITIES

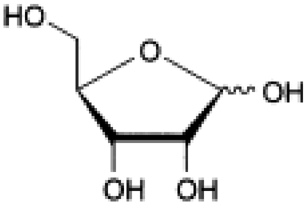
Specified impurities *A, B, C, D, E, G.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *F, H.*

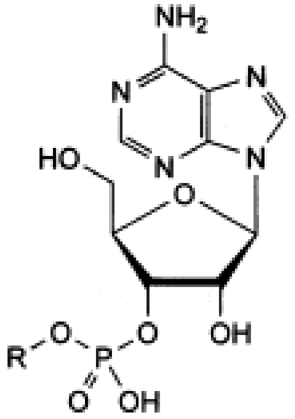
By thin-layer chromatography: B, C, D, E.

By liquid chromatography: A, F, G, H.

A. adenine,



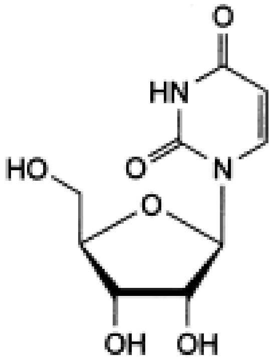
B. D-ribose,



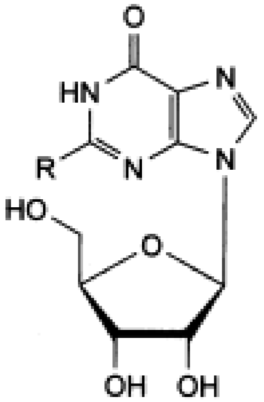
C. R = H: adenosine 3′-(dihydrogen phosphate),

D. R = PO_3H_2: adenosine 3′-(trihydrogen diphosphate),

E. R = PO_2H-O-PO_3H_2: adenosine 3′-(tetrahydrogen triphosphate),



F. 1-β-D-ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione (uridine),



G. R = H: 9-β-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (inosine),

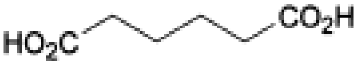
H. R = NH_2: 2-amino-9-β-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (guanosine).

Ph Eur

Adipic Acid

General Notices

(Ph Eur monograph 1586)



$C_6H_{10}O_4$ 146.1 *124-04-9*

Action and use

Excipient.

Ph Eur

DEFINITION

Hexanedioic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, soluble in boiling water, freely soluble in alcohol and in methanol, soluble in acetone.

IDENTIFICATION

A. Melting point *(2.2.14)*: 151 °C to 154 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison adipic acid CRS.

TESTS

Solution S

Dissolve 5.0 g with heating in *distilled water R* and dilute to 50 ml with the same solvent. Allow to cool and to crystallise. Filter through a sintered-glass filter (40) *(2.1.2)*. Wash the filter with *distilled water R*. Collect the filtrate and the washings until a volume of 50 ml is obtained.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.20 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 20 mg of *glutaric acid R* in 1.0 ml of the test solution and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase, dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Column:

— *size:* l = 0.125 m, Ø = 4.0 mm,

— *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m^2/g and a pore size of 10 nm,

— *temperature*: 30 °C.

Mobile phase Mix 3 volumes of *acetonitrile R* and 97 volumes of a 24.5 g/l solution of *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 209 nm.

Injection 20 µl.

Run time 3 times the retention time of adipic acid.

System suitability Reference solution (a):

— *resolution*: minimum 9.0 between the peaks due to glutaric acid and adipic acid.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides *(2.4.4)*

Maximum 200 ppm.

2.5 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides.

Nitrates

Maximum 30 ppm.

To 1 ml of solution S add 2 ml of *concentrated ammonia R*, 0.5 ml of a 10 g/l solution of *manganese sulphate R*, 1 ml of a 10 g/l solution of *sulfanilamide R* and dilute to 20 ml with *water R*. Add 0.10 g of *zinc powder R* and cool in iced water for 30 min; shake from time to time. Filter and cool 10 ml of the filtrate in iced water. Add 2.5 ml of *hydrochloric acid R1* and 1 ml of a 10 g/l solution of *naphthylethylenediamine dihydrochloride R*. Allow to stand at room temperature. After 15 min the mixture is not more intensely coloured than a standard prepared at the same time and in the same manner, using 1.5 ml of *nitrate standard solution (2 ppm* NO_3*) R* instead of 1 ml of solution S. The test is invalid if a blank solution prepared at the same time and in the same manner, using 1 ml of *water R* instead of 1 ml of solution S, is more intensely coloured than a 2 mg/l solution of *potassium permanganate R*.

Sulphates ***(2.4.13)***

Maximum 500 ppm.

3 ml of solution S diluted to 15 ml with *distilled water R* complies with the limit test for sulphates.

Iron ***(2.4.9)***

Maximum 10 ppm.

10 ml of solution S complies with the limit test for iron.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.2 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent.

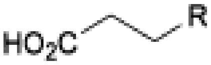
Melt 1.0 g completely over a gas burner, then ignite the melted substance with the burner. After ignition, lower or remove the flame in order to prevent the substance from boiling and keep it burning until completely carbonised. Carry out the test for sulphated ash using the residue.

ASSAY

Dissolve 60.0 mg in 50 ml of *water R*. Add 0.2 ml of *phenolphthalein solution R* and titrate with *0.1 M sodium hydroxide*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 7.31 mg of $C_6H_{10}O_4$.

IMPURITIES



A. R = CH_2-CO_2H: pentanedioic acid (glutaric acid),

B. R = CO_2H: butanedioic acid (succinic acid),

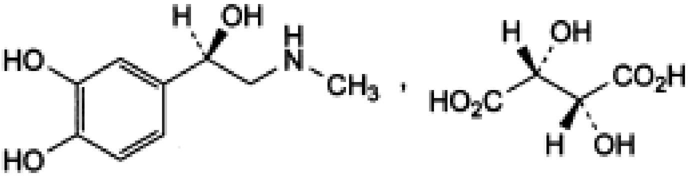
C. R = $[CH_2]_3$-CO_2H: heptanedioic acid (pimelic acid).

Ph Eur

Adrenaline Acid Tartrate / Epinephrine Acid Tartrate

General Notices

(Adrenaline Tartrate, Ph Eur monograph 0254)



$C_9H_{13}NO_3,C_4H_6O_6$ 333.3 *51-42-3*

Action and use

Adrenoceptor agonist.

Preparations

Adrenaline Injection/Epinephrine Injection

Dilute Adrenaline Injection (1 in 10,000)/Dilute Epinephrine Injection (1 in 10,000)

Adrenaline Solution/Epinephrine Solution

Adrenaline and Cocaine Intranasal Solution

Bupivacaine and Adrenaline Injection/Bupivacaine and Epinephrine Injection

Lidocaine and Adrenaline Injection/Lidocaine and Epinephrine Injection

Ph Eur

DEFINITION

(1*R*)-1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanol hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or greyish-white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 5 g in 50 ml of a 5 g/l solution of *sodium metabisulphite R* and make alkaline by addition of *ammonia R*. Keep the mixture at room temperature for at least 15 min and filter. Reserve the filtrate for identification test C. Wash the precipitate with 3 quantities, each of 10 ml, of *methanol R*. Dry at 80 °C. The specific optical rotation *(2.2.7)* of the residue (adrenaline base) is - 50 to - 53.5, determined using a 20.0 g/l solution in *0.5 M hydrochloric acid* .

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of adrenaline base prepared as described under identification test A.

Comparison Use adrenaline base prepared as described under identification test A from 50 mg of *adrenaline tartrate CRS* dissolved in 5 ml of a 5 g/l solution of *sodium metabisulphite R*. Keep the mixture at room temperature for at least 30 min. Filter through a sintered-glass filter *(2.1.2)*.

C. 0.2 ml of the filtrate obtained in identification test A gives reaction (b) of tartrates *(2.3.1)*.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Dissolve 0.5 g in *water R* and dilute to 10 ml with the same solvent. Examine the solution immediately.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions protected from light.*

Solvent mixture A Dissolve 5.0 g of *potassium dihydrogen phosphate R* and then 2.6 g of *sodium octanesulphonate R* in *water for chromatography R*, and dilute to 1000 ml with the same solvent (it is usually necessary to stir for at least 30 min to achieve complete dissolution) . Adjust to pH 2.8 with *phosphoric acid R*.

Solvent mixture B *acetonitrile R1*, solvent mixture A (130:870 *V/V*).

Test solution Dissolve 75 mg of the substance to be examined in 5 ml of *0.1 M hydrochloric acid* and dilute to 50 ml with solvent mixture B.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with solvent mixture B. Dilute 1.0 ml of this solution to 10.0 ml with solvent mixture B.

Reference solution (b) Dissolve 1.5 mg of *noradrenaline tartrate CRS* (impurity B) and 1.5 mg of *adrenalone hydrochloride R* (impurity C) in solvent mixture B, add 1.0 ml of the test solution and dilute to 100.0 ml with solvent mixture B.

Reference solution (c) Dissolve the contents of a vial of *adrenaline impurity mixture CRS* (impurities D and E) in 0.1 ml of *0.1 M hydrochloric acid* and 0.9 ml of solvent mixture B.

Reference solution (d) Dissolve 7.5 mg of *adrenaline tartrate with impurity A CRS* in 0.5 ml of *0.1 M hydrochloric acid* and dilute to 5.0 ml with solvent mixture B.

Blank solution *0.1 M hydrochloric acid* , solvent mixture B (1:9 *V/V*).

Column:

— *size: l* = 0.10 m, Ø = 4.6 mm;

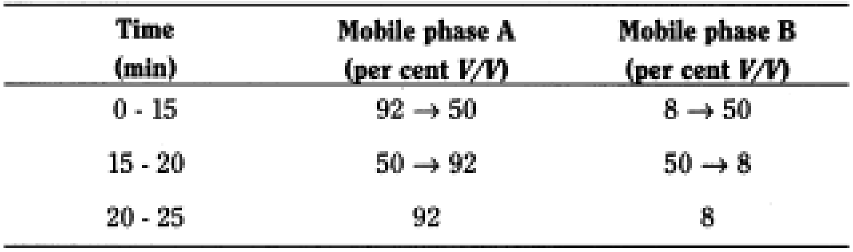
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 50 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R1*, solvent mixture A (5:95 *V/V*);

— *mobile phase B*: *acetonitrile R1*, solvent mixture A (45:55 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	92 → 50	8 → 50
15 - 20	50 → 92	50 → 8
20 - 25	92	8

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *adrenaline impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and E; use the chromatogram supplied with *adrenaline tartrate with impurity A CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity A.

Relative retention With reference to adrenaline

(retention time = about 4 min): impurity B = about 0.8; impurity C = about 1.3; impurity A = about 3.2; impurity D = about 3.3; impurity E = about 3.7.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to impurity B and adrenaline.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.7; impurity E = 0.6;

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities B, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* for 18 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*, heating gently if necessary. Titrate with *0.1 M perchloric acid* until a bluish-green colour is obtained, using 0.1 ml of *crystal violet solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 33.33 mg of $C_{13}H_{19}NO_9$.

STORAGE

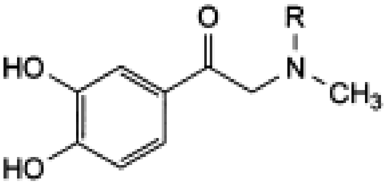
In an airtight container, or preferably in a sealed tube under vacuum or under an inert gas, protected from light.

IMPURITIES

Specified impurities A, B, C, D, E.

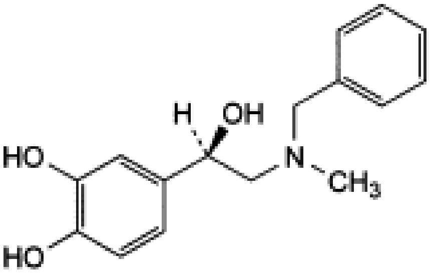
A. unknown structure,

B. noradrenaline,



C. R = H: 1-(3,4-dihydroxyphenyl)-2-(methylamino)ethanone (adrenalone),

E. R = CH_2-C_6H_5: 2-(benzylmethylamino)-1-(3,4-dihydroxyphenyl)ethanone,



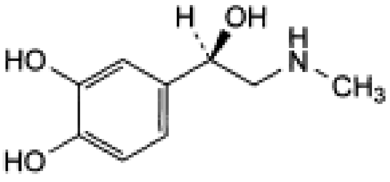
D. (1*R*)-2-(benzylmethylamino)-1-(3,4-dihydroxyphenyl)ethanol.

Ph Eur

Adrenaline / Epinephrine

General Notices

(Ph Eur monograph 2303)



$C_9H_{13}NO_3$ 183.2 *51-43-4*

Action and use

Adrenoceptor agonist.

Preparations

Adrenaline Eye Drops/Epinephrine Eye Drops

Dilute Adrenaline Injection (1 in 10,000)/Dilute Epinephrine Injection (1 in 10,000)

Ph Eur

DEFINITION

4-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]benzene-1,2-diol.

Synthetic product.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder, becoming coloured on exposure to air and light.

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride. It dissolves in hydrochloric acid.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *adrenaline CRS.*

B. Specific optical rotation (see Tests).

TESTS

Solution S

Dissolve 1.000 g in a 25.75 g/l solution of *hydrochloric acid R* and dilute to 50.0 ml with the same solvent. Examine the solution immediately.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

- 50.0 to - 54.0 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions protected from light.*

Solvent mixture A Dissolve 5.0 g of *potassium dihydrogen phosphate R* and 2.6 g of *sodium octanesulphonate R* in *water for chromatography R* and dilute to 1000 ml with the same solvent (it is usually necessary to stir for at least 30 min to achieve complete dissolution). Adjust to pH 2.8 with *phosphoric acid R*.

Solvent mixture B *acetonitrile R1*, solvent mixture A (13:87 *V/V*).

Test solution Dissolve 40 mg of the substance to be examined in 5 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with solvent mixture B.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with solvent mixture B. Dilute 1.0 ml of this solution to 10.0 ml with solvent mixture B.

Reference solution (b) Dissolve 1.5 mg of *noradrenaline tartrate CRS* (impurity B) and 1.5 mg of *adrenalone hydrochloride R* (impurity C) in solvent mixture B, add 1.0 ml of the test solution and dilute to 100 ml with solvent mixture B.

Reference solution (c) Dissolve the contents of a vial of *adrenaline impurity mixture CRS* (containing impurities D and E) in 1.0 ml of the blank solution.

Reference solution (d) Dissolve 4 mg of *adrenaline with impurity F CRS* in 0.5 ml of *0.1 M hydrochloric acid* and dilute to 5 ml with solvent mixture B.

Blank solution *0.1 M hydrochloric acid*, solvent mixture B (1:9 *V/V*).

Column:

— *size:* *l* = 0.10 m, Ø = 4.6 mm;

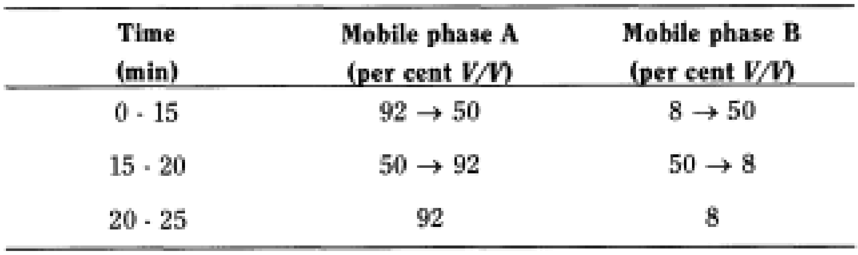
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 50 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R1,* solvent mixture A (5:95 *V/V*);

— *mobile phase B*: *acetonitrile R1,* solvent mixture A (45:55 *V/V*);



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 15	92 → 50	8 → 50
15 - 20	50 → 92	50 → 8
20 - 25	92	8

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *adrenaline impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and E; use the chromatogram supplied with *adrenaline with impurity F CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity F.

Relative retention With reference to adrenaline (retention time = about 4 min): impurity F = about 0.2; impurity B = about 0.8; impurity C = about 1.3; impurity D = about 3.3; impurity E = about 3.7.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to impurity B and adrenaline.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.7; impurity E = 0.6;

— *impurities B, C, F*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 18 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 18.32 mg of $C_9H_{13}NO_3$.

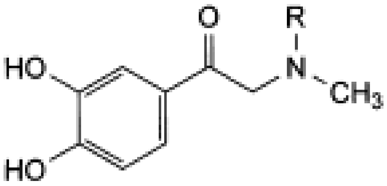
STORAGE

Under nitrogen, protected from light.

IMPURITIES

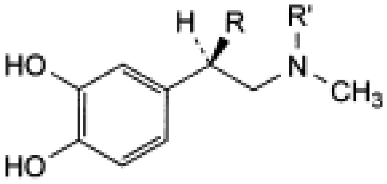
Specified impurities *B, C, D, E, F.*

B. noradrenaline,



C. R = H: 1-(3,4-dihydroxyphenyl)-2-(methylamino)ethanone (adrenalone),

E. R = CH_2-C_6H_5: 2-(benzylmethylamino)-1-(3,4-dihydroxyphenyl)ethanone,



D. R = OH, R′ = CH_2-C_6H_5: 4-[(1*R*)-2-(benzylmethylamino)-1-hydroxyethyl]benzene-1,2-diol,

F. R = SO_3H, R′ = H: (1*R*)-1-(3,4-dihydroxyphenyl)-2-(methylamino)ethanesulphonic acid.

Ph Eur

Agar

General Notices

(Ph Eur monograph 0310)

Action and use

Excipient.

Ph Eur

DEFINITION

Polysaccharides from various species of Rhodophyceae mainly belonging to the genus *Gelidium*. It is prepared by treating the algae with boiling water; the extract is filtered whilst hot, concentrated and dried.

CHARACTERS

Appearance

Powder or crumpled strips 2-5 mm wide or sometimes flakes, colourless or pale yellow, translucent, somewhat tough and difficult to break, becoming more brittle on drying.

Mucilaginous taste.

IDENTIFICATION

A. Examine under a microscope. When mounted in *0.005 M iodine*, the strips or flakes are partly stained brownish-violet. Magnified 100 times, they show the following diagnostic characters: numerous minute, colourless, ovoid or rounded grains on an amorphous background; occasional brown, round or ovoid spores with a reticulated surface, measuring up to 60 µm, may be present. Reduce to a powder, if necessary. The powder is yellowish-white. Examine under a microscope using *0.005 M iodine*. The powder presents angular fragments with numerous grains similar to those seen in the strips and flakes; some of the fragments are stained brownish-violet.

B. Dissolve 0.1 g with heating in 50 ml of *water R*. Cool. To 1 ml of the mucilage carefully add 3 ml of *water R* so as to form 2 separate layers. Add 0.1 ml of *0.05 M iodine*. A dark brownish-violet colour appears at the interface. Mix. The liquid becomes pale yellow.

C. Heat 5 ml of the mucilage prepared for identification test B on a water-bath with 0.5 ml of *hydrochloric acid R* for 30 min. Add 1 ml of *barium chloride solution R1*. A white turbidity develops within 30 min.

D. Heat 0.5 g with 50 ml of *water R* on a water-bath until dissolved. Only a few fragments remain insoluble. During cooling, the solution gels between 35 °C and 30 °C. Heat the gel thus obtained on a water-bath; it does not liquefy below 80 °C.

TESTS

Swelling index *(2.8.4)*

Minimum 10 and within 10 per cent of the value stated on the label, determined on the powdered drug (355) *(2.9.12)*.

Insoluble matter

Maximum 1.0 per cent.

To 5.00 g of the powdered drug (355) *(2.9.12)* add 100 ml of *water R* and 14 ml of dilute *hydrochloric acid R*. Boil gently for 15 min with frequent stirring. Filter the hot liquid through a tared, sintered-glass filter (160) *(2.1.2)*, rinse the filter with hot *water R* and dry at 100-105 °C. The residue weighs a maximum of 50 mg.

Gelatin

To 1.00 g add 100 ml of *water R* and heat on a water-bath until dissolved. Allow to cool to 50 °C. To 5 ml of this solution add 5 ml of *picric acid solution R*. No turbidity appears within 10 min.

Loss on drying *(2.2.32)*

Maximum 20.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate-count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

LABELLING

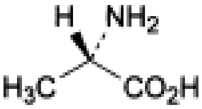
The label states the swelling index.

Ph Eur

Alanine

General Notices

(Ph Eur monograph 0752)



$C_3H_7NO_2$ 89.1 *56-41-7*

Action and use

Amino acid.

Ph Eur

DEFINITION

Alanine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2-aminopropanoic acid, calculated with reference to the dried substance.

CHARACTERS

White or almost white, crystalline powder or colourless crystals, freely soluble in water, very slightly soluble in alcohol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *alanine CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.5 g in a mixture of 1 ml of *water R*, 0.5 ml of a 100 g/l solution of *sodium nitrite R* and 0.25 ml of *hydrochloric acid R1*. Shake. Gas is given off. Add 2 ml of *dilute sodium hydroxide solution R*, followed by 0.25 ml of *iodinated potassium iodide solution R*. After about 30 min, a yellow precipitate with a characteristic odour is formed.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Dilute 10 ml of solution S to 20 ml with *water R*. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 2.50 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 13.5 to + 15.5, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *alanine CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *alanine CRS* and 10 mg of *glycine CRS* in *water R* and dilute to 25 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm with a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air. Spray with *ninhydrin solution R*. Heat the plate at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

Dilute 5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals *(2.4.8)*

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 80.0 mg in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Using 0.1 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid*, until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 8.91 mg of $C_3H_7NO_2$.

STORAGE

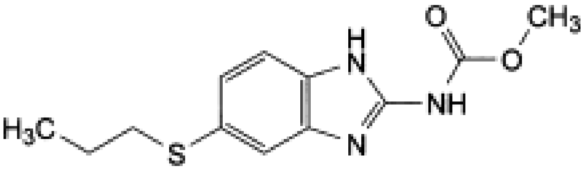
Store protected from light.

Ph Eur

Albendazole

General Notices

(Ph Eur monograph 1386)



$C_{12}H_{15}N_3O_2S$ 265.3 *54965-21-8*

Action and use

Benzimidazole antihelminthic.

Preparations

Albendazole Oral Suspension

Albendazole Oral Suspension with Minerals

Ph Eur

DEFINITION

Methyl [5-(propylsulphanyl)-1*H*-benzimidazol-2-yl]carbamate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous formic acid, very slightly soluble in methylene chloride, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *albendazole CRS.*

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Dissolve 0.10 g in a mixture of 1 volume of *anhydrous formic acid R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in 5 ml of *methanol R* containing 1 per cent *V/V* of *sulphuric acid R* and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of the substance to be examined in 10 ml of *methanol R* containing 1 per cent *V/V* of *sulphuric acid R* and dilute to 100.0 ml with the mobile phase. Dilute 0.5 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 50.0 mg of the substance to be examined and 50 mg of *oxibendazole CRS* in 5 ml of *methanol R* containing 1 per cent *V/V* of *sulphuric acid R* and dilute to 100.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *spherical end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Mix 300 volumes of a 1.67 g/l solution of *ammonium dihydrogen phosphate R* and 700 volumes of *methanol R*.

Flow rate 0.7 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 1.5 times the retention time of albendazole.

Relative retention With reference to albendazole: impurity D = about 0.40; impurities B and C = about 0.43; impurity E = about 0.47; impurity F = about 0.57; impurity A = about 0.80.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to albendazole and oxibendazole.

Limits:
— *impurities A, B, C, D, E, F*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating during the titration, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.250 g in 3 ml of *anhydrous formic acid R* and add 40 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

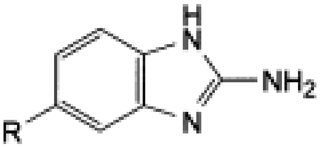
1 ml of *0.1 M perchloric acid* is equivalent to 26.53 mg of $C_{12}H_{15}N_3O_2S$.

STORAGE

Protected from light.

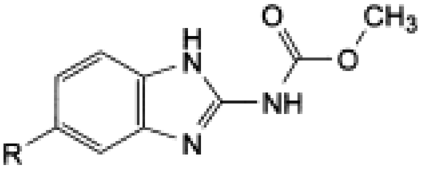
IMPURITIES

Specified impurities A, B, C, D, E, F.



A. R = $S\text{-}CH_2\text{-}CH_2\text{-}CH_3$: 5-(propylsulphanyl)-1*H*-benzimidazol-2-amine,

D. R = $SO_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$: 5-(propylsulphonyl)-1*H*-benzimidazol-2-amine,



B. R = $SO\text{-}CH_2\text{-}CH_2\text{-}CH_3$: methyl [5-(propylsulphinyl)-1*H*-benzimidazol-2-yl]carbamate,

C. R = $SO_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$: methyl [5-(propylsulphonyl)-1*H*-benzimidazol-2-yl]carbamate,

E. R = H: methyl (1*H*-benzimidazol-2-yl)carbamate,

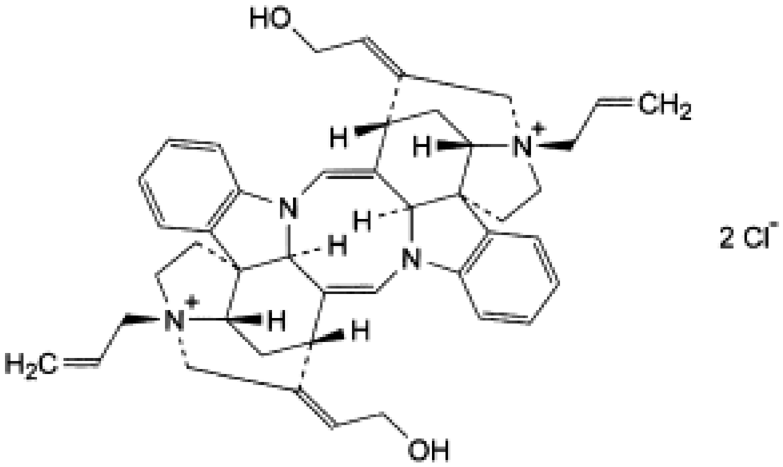
F. R = $S\text{-}CH_3$: methyl [5-(methylsulphanyl)-1*H*-benzimidazol-2-yl]carbamate.

Ph Eur

Alcuronium Chloride

General Notices

(Ph Eur monograph 1285)



$C_{44}H_{50}Cl_2N_4O_2$ 738.0 *151180-03-7*

Action and use

Non-depolarizing neuromuscular blocker.

Ph Eur

DEFINITION

(1*R*,3a*S*,10*S*,11a*S*,12*R*,14a*S*,19a*S*,20b*S*,21*S*,22a*S*,23*E*,26*E*)-23,26-bis(2-Hydroxyethylidene)-1,12-bis(prop-2-enyl)-2,3,11,11a,13,14,22,22a-octahydro-10*H*,21*H*-1,21:10,12-diethano-19a*H*,20b*H*-[1,5]diazocino[1,2,3-*lm*:5,6,7-*l′m′*]dipyrrolo[2′,3′-*d*:2′′,3′′:*d′*]dicarbazolediium dichloride (4,4′-didesmethyl-4,4′-bis(prop-2-enyl)toxiferin I dichloride).

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly greyish-white, crystalline powder.

Solubility

Freely soluble in water and in methanol, soluble in ethanol (96 per cent), practically insoluble in cyclohexane.

Carry out the identification, tests and assay as rapidly as possible avoiding exposure to actinic light.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison alcuronium chloride CRS.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *alcuronium chloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase Mix 15 volumes of a 58.4 g/l solution of *sodium chloride R*, 35 volumes of *dilute ammonia R2* and 50 volumes of *methanol R*.

Application 10 µl.

Development Over a path of 15 cm.

Drying In air for 10 min.

Detection Spray with *0.1 M ammonium and cerium nitrate*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.250 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6, BY_6 or B_6 *(2.2.2, Method I)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M hydrochloric acid* . The solution is red. Add 0.4 ml of *0.01 M sodium hydroxide*. The solution is yellow.

Specific optical rotation *(2.2.7)*

- 430 to - 451 (anhydrous substance), determined on solution S.

Propan-2-ol ***(2.4.24, System A)***

Maximum 1.0 per cent.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture Mix 100 ml of *methanol R*, 200 ml of *acetonitrile R* and 200 ml of a 6.82 g/l solution of *potassium dihydrogen phosphate R*. Dissolve 1.09 g of *sodium laurylsulphonate for chromatography R* in the mixture and adjust the apparent pH to 8.0 with a 100 g/l solution of *sodium hydroxide R*.

Test solution Dissolve 0.20 g of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dilute 0.5 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 4.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (d) To 5.0 ml of the test solution add 5.0 mg of *allylstrychnine bromide CRS*, dissolve in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Column:
- *size: l* = 0.25 m, Ø = 4 mm;
- *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 200 ml of *methanol R*, 400 ml of *acetonitrile R* and 400 ml of a 6.82 g/l solution of *potassium dihydrogen phosphate R*. Dissolve 2.18 g of *sodium laurylsulphonate for chromatography R* in the mixture and adjust the apparent pH to 5.4 with a 100 g/l solution of *phosphoric acid R*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time Twice the retention time of alcuronium.

System suitability Reference solution (d):
- *resolution*: minimum 4.0 between the peaks due to *N*-allylstrychnine and alcuronium.

Limits:
- *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than one of the peaks has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water ***(2.5.12)***

Maximum 5.0 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g by stirring in 70 ml of *acetic anhydride R* for 1 min. Titrate with *0.1 M perchloric acid* until the colour changes from violet-blue to greenish-blue, using 0.1 ml of *crystal violet solution R* as indicator.

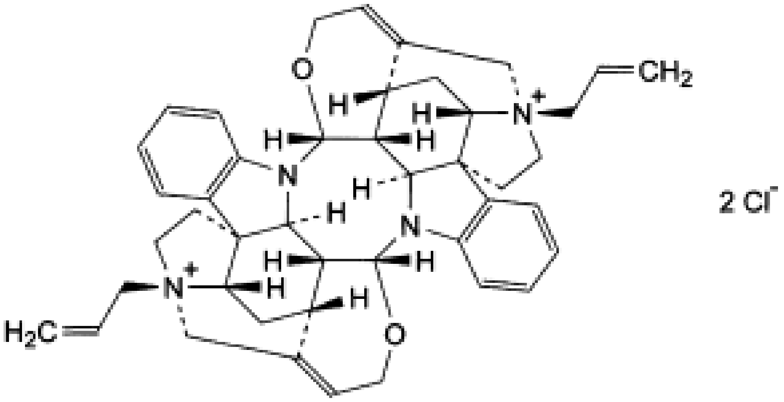
1 ml of *0.1 M perchloric acid* is equivalent to 36.9 mg of $C_{44}H_{50}Cl_2N_4O_2$.

STORAGE

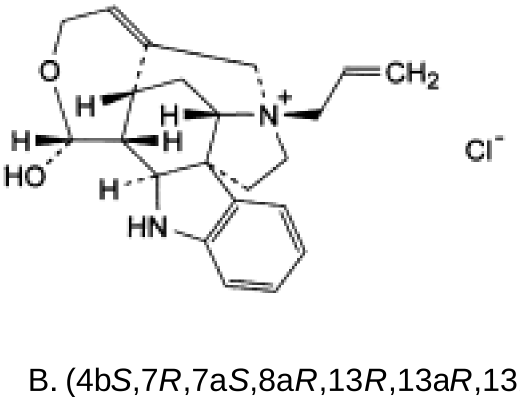
In an airtight container under nitrogen, protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES

Specified impurities *A, B.*



A. (1*R*,3a*S*,9*R*,9a*R*,10*R*,11a*S*,12*R*,14a*S*,19a*S*,20*R*, 20a*R*,20b*S*,21*R*,22a*S*)-1,12-bis(prop-2-enyl)-2,3,9a,11,11a,13,14,19a,20a,21,22,22a-dodecahydro-10*H*,20b*H*-1,23:12,27-dimethano-9,10:20,21-bis(epoxyprop[2]eno)-9*H*,20*H*-[1,5]diazocino[1,2,3-*lm*:5,6,7-*l′m′*]dipyrrolo[2′,3′-*d*:2′′,3′′:*d′*]dicarbazolediium dichloride (4,4′-diallylcaracurin V dichloride),



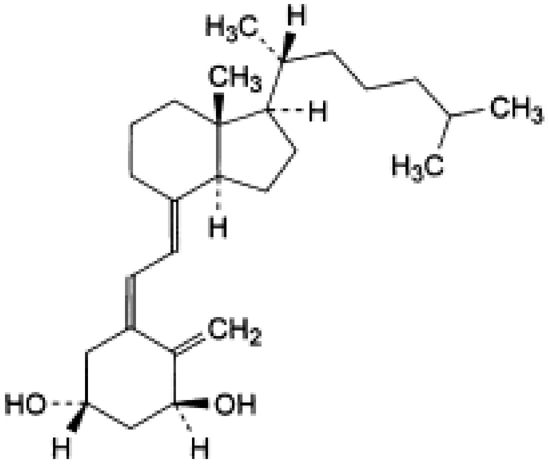
B. (4b*S*,7*R*,7a*S*,8a*R*,13*R*,13a*R*,13b*S*)-13-hydroxy-7-(prop-2-enyl)-5,6,7a,8,8a,11,13,13a,13b,14-decahydro-7,9-methano-7*H*-oxepino[3,4-*a*]pyrrolo[2,3-*d*]carbazolium chloride ((4*R*,17*R*)-4-allyl-17,18-epoxy-17-hydroxy-19,20-didehydrocuranium chloride).

Ph Eur

Alfacalcidol

General Notices

(Ph Eur monograph 1286)



$C_{27}H_{44}O_2$ 400.6 *41294-56-8*

Action and use

Vitamin D analogue.

Ph Eur

DEFINITION

(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-triene-1α,3β-diol.

Content

97.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white crystals.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in fatty oils.

It is sensitive to air, heat and light.

A reversible isomerisation to pre-alfacalcidol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of alfacalcidol.*

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)*: use the normalisation procedure. *Carry out the test as rapidly as possible, avoiding exposure to actinic light and air.*

Test solution Dissolve 1.0 mg of the substance to be examined without heating in 10.0 ml of the mobile phase.

Reference solution (a) Dissolve 1.0 mg of *alfacalcidol CRS* without heating in 10.0 ml of the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Heat 2 ml of reference solution (a) in a water-bath at 80 °C under a reflux condenser for 2 h and cool.

Column:
— *size:* *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R2* (5 µm).

Mobile phase *ammonia R, water R, acetonitrile R* (1:200:800 *V/V/V*).

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 100 µl of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of alfacalcidol.

Relative retention With reference to alfacalcidol: pre-alfacalcidol = about 1.3.

System suitability Reference solution (c):

— *resolution*: minimum 4.0 between the peaks due to pre-alfacalcidol and alfacalcidol; if necessary, adjust the proportions of the constituents of the mobile phase.

Limits:
— *impurities A, B, C*: for each impurity, maximum 0.5 per cent;

— *total*: maximum 1.0 per cent;

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); disregard the peak due to pre-alfacalcidol.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection The test solution and reference solutions (a) and (c).

System suitability Reference solution (c):

— *repeatability*: maximum relative standard deviation of 1 per cent for the peak due to alfacalcidol after 6 injections.

Calculate the percentage content of $C_{27}H_{44}O_2$ from the declared content of *alfacalcidol CRS*.

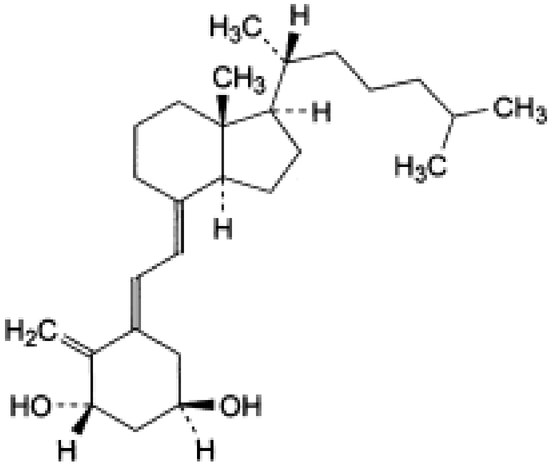
STORAGE

Under nitrogen, in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

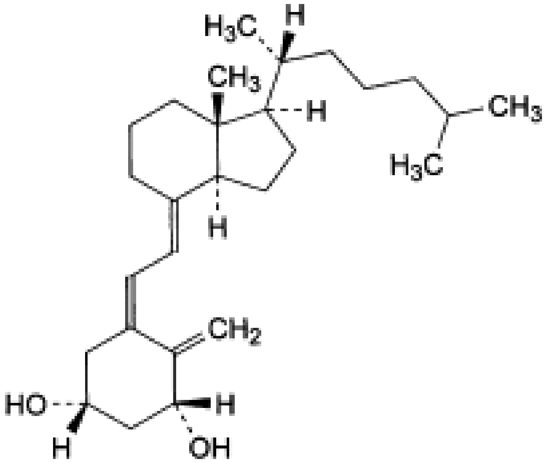
The contents of an opened container are to be used immediately.

IMPURITIES

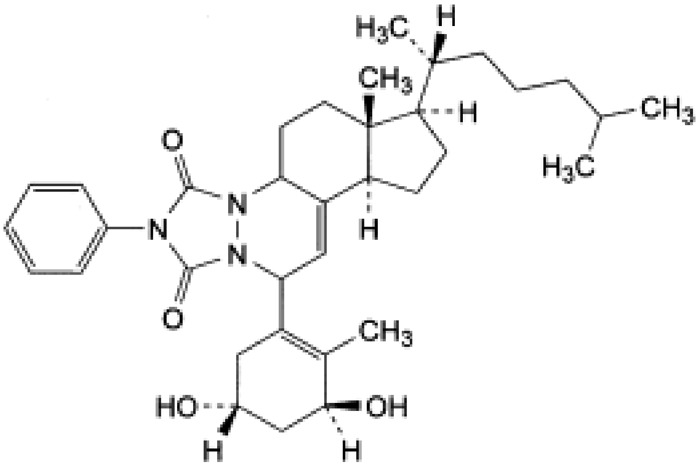
Specified impurities A, B, C.



A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1α,3β-diol (*trans*-alfacalcidol),



B. (5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1β,3β-diol (1β-calcidol),



C. triazoline adduct of pre-alfacalcidol.

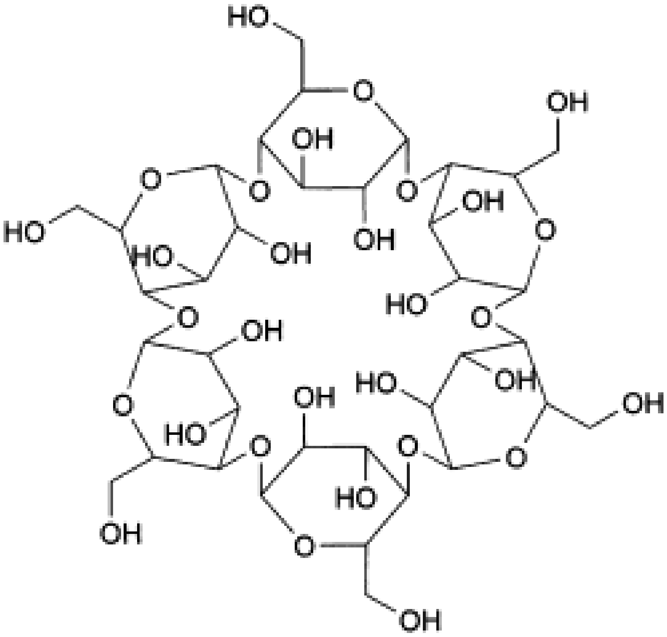
Ph Eur

Alfadex

General Notices

Alphacyclodextrin

(Ph Eur monograph 1487)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$[C_6H_{10}O_5]_6$ 973 *10016-20-3*

Action and use

Cyclodextran; carrier molecule for drug delivery systems.

Ph Eur

DEFINITION

Cyclohexakis-(1→4)-(α-D -glucopyranosyl) (cyclomaltohexaose or α-cyclodextrin).

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, amorphous or crystalline powder.

Solubility

Freely soluble in water and in propylene glycol, practically insoluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Examine the chromatograms obtained in the assay.

Results   The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

C. Dissolve 0.2 g in 2 ml of *iodine solution R4* by warming on a water-bath, and allow to stand at room temperature; a yellowish-brown precipitate is formed.

TESTS

Solution S

Dissolve 1.000 g in *carbon dioxide-free water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

pH *(2.2.3)*

5.0 to 8.0.

Mix 1 ml of a 223.6 g/l solution of *potassium chloride R* and 30 ml of solution S.

Specific optical rotation *(2.2.7)*

+ 147 to + 152 (dried substance), determined on solution S.

Reducing sugars

Maximum 0.2 per cent.

Test solution   To 1 ml of solution S add 1 ml of *cupri-tartaric solution R4*. Heat on a water-bath for 10 min, cool to room temperature. Add 10 ml of *ammonium molybdate reagent R1* and allow to stand for 15 min.

Reference solution   Prepare a reference solution at the same time and in the same manner as the test solution, using 1 ml of a 0.02 g/l solution of *glucose R*.

Measure the absorbance *(2.2.25)* of the test solution and the reference solution at the absorption maximum at 740 nm using *water R* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution.

Light-absorbing impurities

Examine solution S between 230 nm and 750 nm. Between 230 nm and 350 nm, the absorbance *(2.2.25)* is not greater than 0.10. Between 350 nm and 750 nm, the absorbance *(2.2.25)* is not greater than 0.05.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 0.25 g of the substance to be examined in *water R* with heating, cool and dilute to 25.0 ml with the same solvent.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with *water R*.

Reference solution (a) Dissolve 25.0 mg of *betadex CRS* (impurity A), 25.0 mg of *gammacyclodextrin CRS* (impurity B) and 50.0 mg of *alfadex CRS* in *water R*, then dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water R*.

Reference solution (c) Dissolve 25.0 mg of *alfadex CRS* in *water R* and dilute to 25.0 ml with the same solvent.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase *methanol R, water R* (10:90 *V/V*).

Flow rate 1.5 ml/min.

Detection Differential refractometer.

Equilibration With the mobile phase for about 3 h.

Injection 50 µl of test solution (a) and reference solutions (a) and (b).

Run time 3.5 times the retention time of alfadex.

Relative retention With reference to alfadex (retention time = about 10 min): impurity B = about 0.7; impurity A = about 2.2.

System suitability Reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to impurity B and alfadex; if necessary, adjust the concentration of methanol in the mobile phase.

Limits:
- *impurities A, B*: for each impurity, not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *sum of impurities other than A and B*: not more than 0.5 times the area of the peak due to alfadex in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 11 per cent, determined on 1.000 g by drying in an oven at 120 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solutions (a) and (c).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 2.0 per cent for the peak due to alfadex after 5 injections.

Calculate the percentage content of $[C_6H_{10}O_5]_6$ from the declared content of *alfadex CRS.*

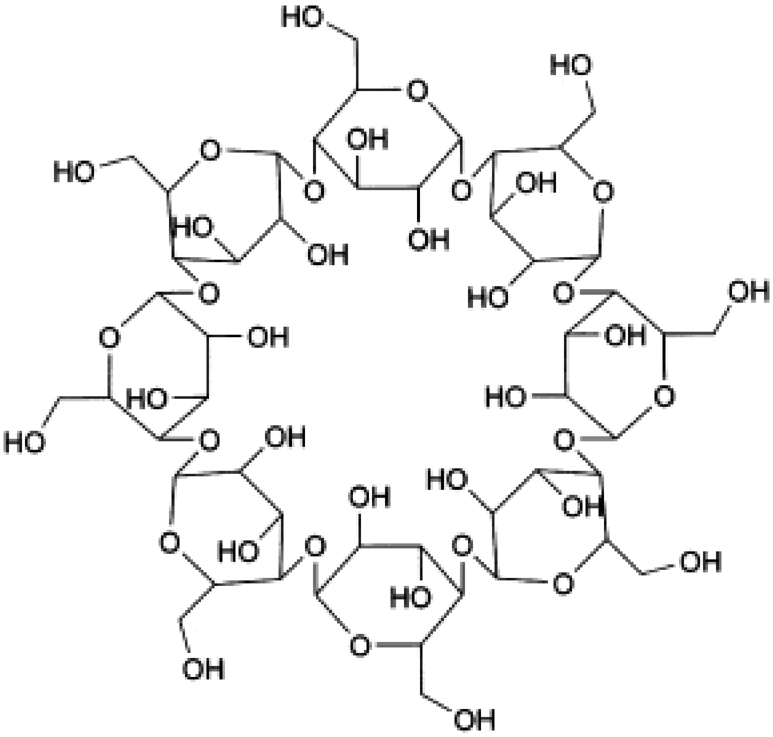
STORAGE

In an airtight container.

IMPURITIES

Specified impurities A, B.

A. betadex,



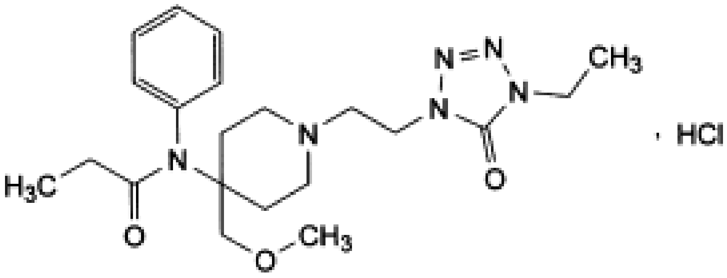
B. cyclooctakis-(1→4)-(α-D-glucopyranosyl) (cyclomaltooctaose or γ-cyclodextrin).

Ph Eur

Alfentanil Hydrochloride

General Notices

(Ph Eur monograph 1062)



$C_{21}H_{32}N_6O,HCl$ 453.0 *69049-06-5*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

N-[1-[2-(4-Ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)piperidin-4-yl]-*N*-phenylpropanamide hydrochloride.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, in ethanol (96 per cent) and in methanol.

mp

About 140 °C, with decomposition.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of alfentanil hydrochloride.*

B. Dissolve 50 mg in a mixture of 0.4 ml of *ammonia R* and 2 ml of *water R*. Mix, allow to stand for 5 min and filter. Acidify the filtrate with *dilute nitric acid R*. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.2 g in *water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

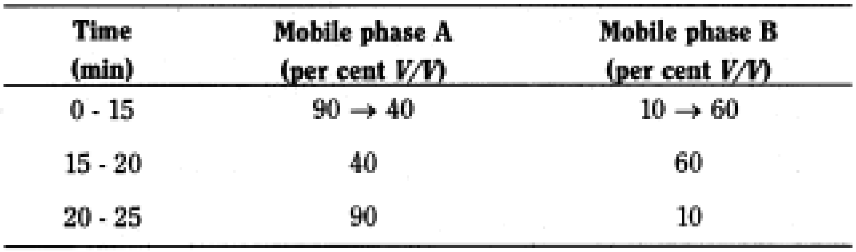
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) In order to produce impurity E *in situ*, dissolve 10 mg of the substance to be examined in 10.0 ml of *dilute hydrochloric acid R*. Heat on a water-bath under a reflux condenser for 4 h. Neutralise with 10.0 ml of *dilute sodium hydroxide solution R*. Evaporate to dryness on a water-bath. Cool and take up the residue in 10 ml of *methanol R*. Filter.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol R*.

Column:
- *size:* *l* = 0.1 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: 5 g/l solution of *ammonium carbonate R* in a mixture of 10 volumes of *tetrahydrofuran R* and 90 volumes of *water R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 40	10 → 60
15 - 20	40	60
20 - 25	90	10

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Impurity E = about 6 min; alfentanil = about 7 min.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity E; disregard any other peak.

System suitability Reference solution (a):

- — *resolution*: minimum 4.0 between the peaks due to alfentanil and impurity E; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear-gradient elution.

Limits:

- — *impurities A, B, C, D, E, F, G, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- — *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- — *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Water *(2.5.12)*

3.0 per cent to 4.0 per cent, determined on 0.500 g.

ASSAY

Dissolve 0.350 g in 50 ml of a mixture of 1 volume of *ethanol (96 per cent) R* and 4 volumes of *water R* and add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Read the volume added between the 2 points of inflexion.

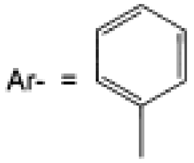
1 ml of *0.1 M sodium hydroxide* is equivalent to 45.30 mg of $C_{21}H_{33}ClN_6O_3$.

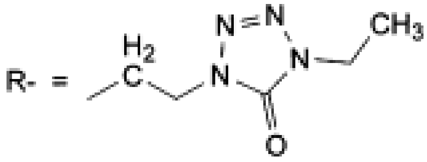
STORAGE

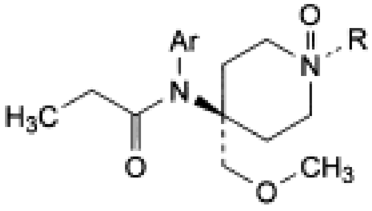
Protected from light.

IMPURITIES

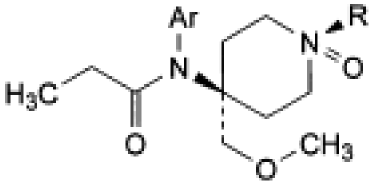
Specified impurities *A, B, C, D, E, F, G, H.*



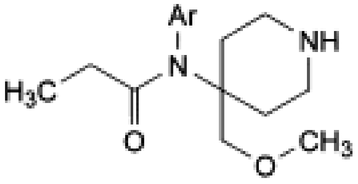




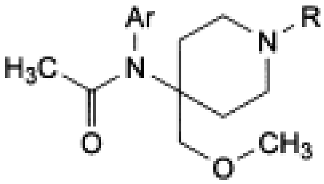
A. *cis*-*N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)piperidin-4-yl]-*N*-phenylpropanamide *N*-oxide,



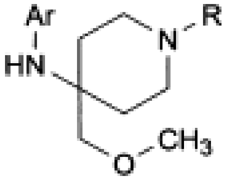
B. *trans*-*N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl) piperidin-4-yl]-*N*-phenylpropanamide *N*-oxide,



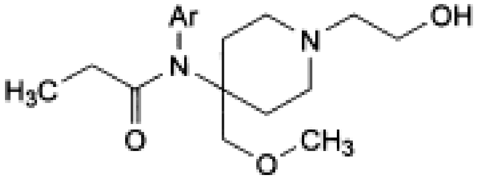
C. *N*-[4-(methoxymethyl)piperidin-4-yl]-*N*-phenylpropanamide,



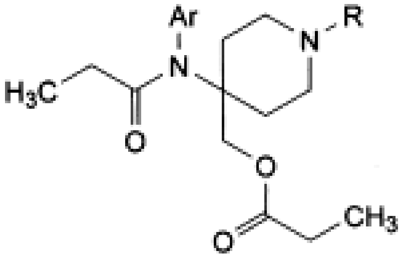
D. *N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)piperidin-4-yl] -*N*-phenylacetamide,



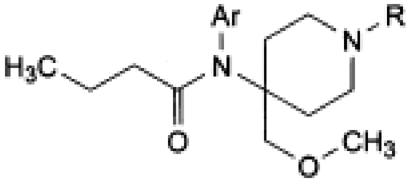
E. 1-ethyl-1,4-dihydro-4-[2-[[4-(methoxymethyl)-4-phenylamino]piperidin-1-yl]ethyl]-5*H*-tetrazol-5-one,



F. *N*-[1-(2-hydroxyethyl)-4-(methoxymethyl)piperidin-4-yl]-*N*-phenylpropanamide,



G. *N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(propanoyloxymethyl) piperidin-4-yl]-*N*-phenylpropanamide,



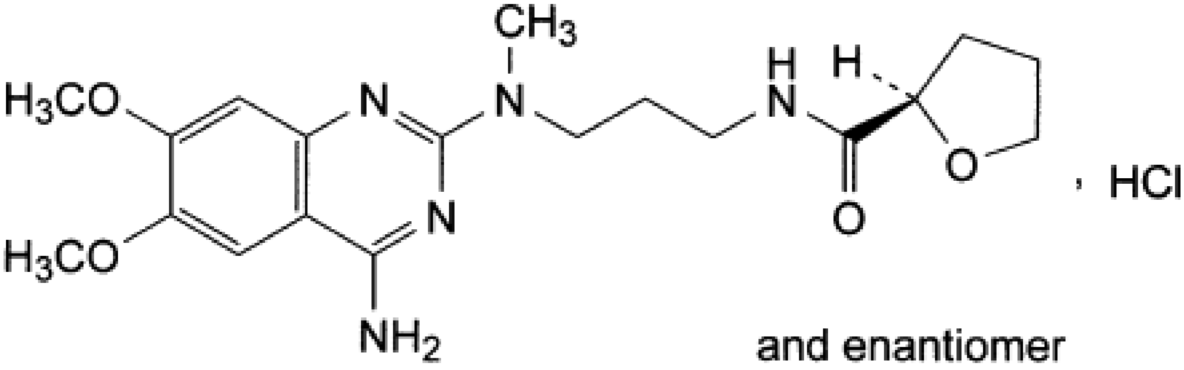
H. *N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)piperidin-4-yl]-*N*-phenylbutanamide.

Ph Eur

Alfuzosin Hydrochloride

General Notices

(Ph Eur monograph 1287)



$C_{19}H_{27}N_5O_4$,HCl 425.9 *81403-68-1*

Action and use

Alpha$_1$-adrenoceptor antagonist.

Ph Eur

DEFINITION

(2*RS*)-*N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)methylamino]propyl]tetrahydrofuran-2-carboxamide hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, slightly hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *alfuzosin hydrochloride CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)

4.0 to 5.5.

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent. Use a freshly prepared solution.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 4 mg of *alfuzosin for system suitability CRS* (containing impurities A and D) in the mobile phase and dilute to 10 ml with the mobile phase.

Column:
- — *size*: *l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 1 volume of *tetrahydrofuran R*, 20 volumes of *acetonitrile R* and 80 volumes of a solution prepared as follows: dilute 5.0 ml of *perchloric acid R* in 900 ml of *water R*, adjust to pH 3.5 with *dilute sodium hydroxide solution R* and dilute to 1000 ml with *water R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time Twice the retention time of alfuzosin.

Identification of impurities Use the chromatogram supplied with alfuzosin for system suitability CRS and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and D.

Relative retention With reference to alfuzosin (retention time = about 8 min): impurity D = about 0.4; impurity A = about 1.2.

System suitability Reference solution (b):

- — *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to alfuzosin.

Limits:
- — *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.000 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 40 ml of *anhydrous acetic acid R* and 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 42.59 mg of $C_{19}H_{28}ClN_5O_4$.

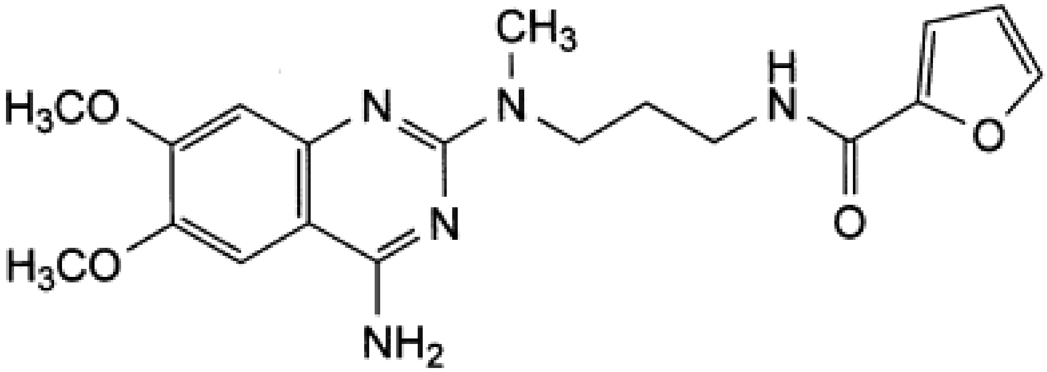
STORAGE

In an airtight container, protected from light.

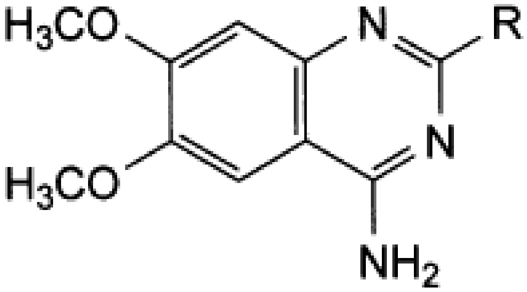
IMPURITIES

Specified impurities *D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, E.*



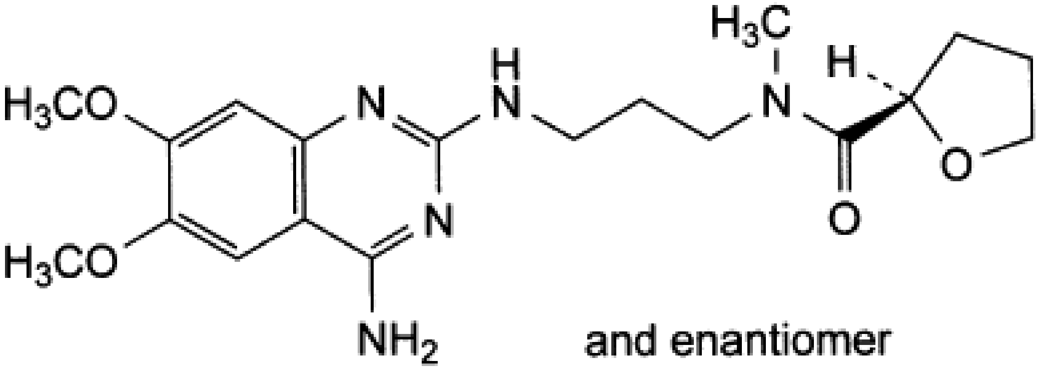
A. *N*-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)methylamino]propyl]furan-2-carboxamide



B. R = Cl: 2-chloro-6,7-dimethoxyquinazolin-4-amine,

D. R = $N(CH_3)$-$[CH_2]_3$-NH_2: *N*-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*N*-methylpropane-1,3-diamine,

E. R = $N(CH_3)$-$[CH_2]_3$-NH-CO-H: *N*-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)methylamino] propyl]formamide,



C. (2*RS*)-*N*-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)amino]propyl]-*N*-methyltetrahydrofuran-2-carboxamide.

Ph Eur

Alginic Acid

General Notices

(Ph Eur monograph 0591)

Action and use

Treatment of gastro-oesophageal reflux disease; excipient; thickening agent.

Ph Eur

DEFINITION

Mixture of polyuronic acids [$(C_6H_8O_6)_n$] composed of residues of D-mannuronic and L-guluronic acids, obtained mainly from algae belonging to the Phaeophyceae. A small proportion of the carboxyl groups may be neutralised.

Content

19.0 per cent to 25.0 per cent of carboxyl groups ($-CO_2H$) (dried substance).

CHARACTERS

Appearance

White or pale yellowish-brown, crystalline or amorphous powder.

Solubility

Very slightly soluble or practically insoluble in ethanol (96 per cent), practically insoluble in organic solvents. It swells in water but does not dissolve; it dissolves in solutions of alkali hydroxides.

IDENTIFICATION

A. To 0.2 g add 20 ml of *water R* and 0.5 ml of *sodium carbonate solution R*. Shake and filter. To 5 ml of the filtrate add 1 ml of *calcium chloride solution R*. A voluminous gelatinous mass is formed.

B. To 5 ml of the filtrate obtained in identification test A add 0.5 ml of a 123 g/l solution of *magnesium sulphate R*. No voluminous gelatinous mass is formed.

C. To 5 mg add 5 ml of *water R*, 1 ml of a freshly prepared 10 g/l solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and 5 ml of *hydrochloric acid R*. Boil gently for 3 min, cool, add 5 ml of *water R*, and shake with 15 ml of *di-isopropyl ether R*. Carry out a blank test. The upper layer obtained with the substance to be examined exhibits a deeper bluish-red colour than that obtained with the blank.

TESTS

Chlorides

Maximum 1,0 per cent.

To 2.50 g add 50 ml of *dilute nitric acid R*, shake for 1 h and dilute to 100.0 ml with *dilute nitric acid R*. Filter. To 50.0 ml of the filtrate add 10.0 ml of *0.1 M silver nitrate* and 5 ml of *toluene R*. Titrate with *0.1 M ammonium thiocyanate*, using 2 ml of *ferric ammonium sulphate solution R2* as indicator and shaking vigorously towards the end-point.

1 ml of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 0.1000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 8.0 per cent (dried substance), determined on 0.100 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^2 micro-organisms per gram, determined by plate-count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

To 0.2500 g add 25 ml of *water R*, 25.0 ml of *0.1 M sodium hydroxide* and 0.2 ml of *phenolphthalein solution R*. Titrate with *0.1 M hydrochloric acid*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 4.502 mg of carboxyl groups ($-CO_2H$).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.The following characteristics may be relevant for alginic acid used as disintegrant and/or binder.

Particle-size distribution *(2.9.31* or *2.9.38)*.

Settling volume

Place 75 ml of *water R* in a 100 ml graduated cylinder and add 1.5 g of the substance to be examined in 0.5 g portions, shaking vigorously after each addition. Dilute to 100.0 ml with *water R* and shake again until the substance is homogeneously distributed. Allow to stand for 4 h and determine the volume of the settled mass.

The following characteristic may be relevant for alginic acid used as gelling agent or viscosity-increasing agent.

Apparent viscosity

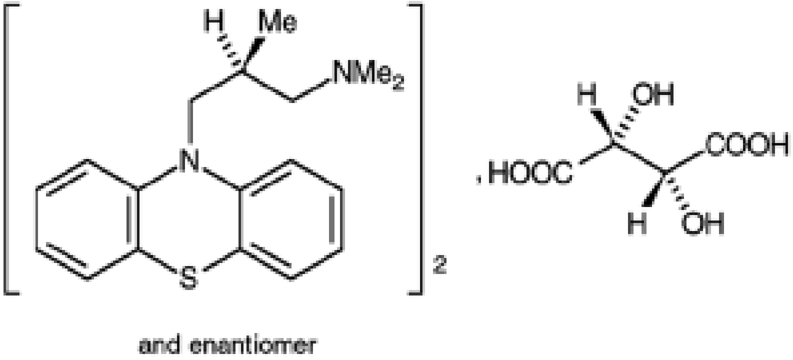
Determine the dynamic viscosity using a rotating viscometer *(2.2.10)*.

Prepare a 20 g/l suspension of alginic acid (dried substance) and add *0.1 M sodium hydroxide* until a solution is obtained.

Ph Eur

Alimemazine Tartrate

General Notices



$(C_{18}H_{22}N_2S)_2,C_4H_6O_6$ 747.0   *4330-99-8*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparations

Paediatric Alimemazine Oral Solution

Strong Paediatric Alimemazine Oral Solution

Alimemazine Tablets

DEFINITION

Alimemazine Tartrate is (*RS*)-dimethyl (2-methyl-3-phenothiazin-10-ylpropyl)amine (2*R*,3*R*)-tartrate. It contains not less than 99.0% and not more than 101.0% of $(C_{18}H_{22}N_2S)_2,C_4H_6O_6$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or slightly cream powder. It darkens on exposure to light.

Freely soluble in *water*; sparingly soluble in *ethanol (96%)*; very slightly soluble in *ether* .

IDENTIFICATION

A. Dissolve 0.1 g in 10 ml of *water* and add 2 ml of 1M *sodium hydroxide*. Extract with 25 ml of *ether* , wash the extract with 5 ml of *water*, dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 1 ml of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the reference spectrum of alimemazine *(RS 005).*

B. *Melting point*, 159° to 163°, Appendix V A.

TESTS

Acidity

pH of a 2% w/v solution, 5.0 to 6.5, Appendix V L.

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A*.

Loss on drying

When dried to constant weight at 100° at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 1 g and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 37.35 mg of $(C_{18}H_{22}N_2S)_2,C_4H_6O_6$.

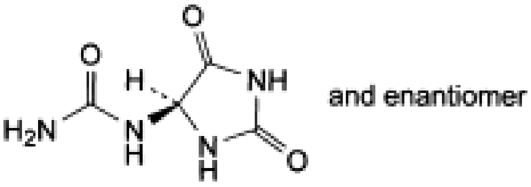
STORAGE

Alimemazine Tartrate should be protected from light.

Allantoin

General Notices

(Ph Eur monograph 1288)



$C_4H_6N_4O_3$ 158.1 *97-59-6*

Action and use

Astringent; keratolytic.

Ph Eur

DEFINITION

Allantoin contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*RS*)-(2,5-dioxoimidazolidin-4-yl)urea.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water, very slightly soluble in alcohol.

It melts at about 225 °C, with decomposition.

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *allantoin CRS*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Boil 20 mg with a mixture of 1 ml of *dilute sodium hydroxide solution R* and 1 ml of *water R*. Allow to cool. Add 1 ml of *dilute hydrochloric acid R*. To 0.1 ml of the solution add 0.1 ml of a 100 g/l solution of *potassium bromide R*, 0.1 ml of a 20 g/l solution of *resorcinol R* and 3 ml of *sulphuric acid R*. Heat for 5 min to 10 min on a water-bath. A dark blue colour develops, which becomes red after cooling and pouring into about 10 ml of *water R*.

D. Heat about 0.5 g. Ammonia vapour is evolved, which turns *red litmus paper R* blue.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R*, with heating if necessary, and dilute to 100 ml with the same solvent.

Acidity or alkalinity

To 5 ml of solution S add 5 ml of *carbon dioxide-free water R*, 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Optical rotation *(2.2.7)*

The angle of optical rotation, determined on solution S, is - 0.10° to + 0.10°.

Reducing substances

Shake 1.0 g with 10 ml of *water R* for 2 min. Filter. Add 1.5 ml of *0.02 M potassium permanganate*. The solution must remain violet for at least 10 min.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable *cellulose for chromatography R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 5.0 ml of *water R* with heating. Allow to cool. Dilute to 10 ml with *methanol R*. *Use the solution immediately after preparation.*

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *methanol R* and 1 volume of *water R*.

Reference solution (a) Dissolve 10 mg of *allantoin CRS* in a mixture of 1 volume of *methanol R* and 1 volume of *water R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *urea R* in 10 ml of *water R*. Dilute 1 ml of this solution to 10 ml with *methanol R*.

Reference solution (c) Mix 1 ml of reference solution (a) and 1 ml of reference solution (b).

Apply to the plate 10 µl of test solution (a) and 5 µl each of test solution (b), reference solution (a), reference solution (b) and reference solution (c). Develop over a path of 10 cm using a mixture of 15 volumes of *glacial acetic acid R*, 25 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air. Spray the plate with a 5 g/l solution of *dimethylaminobenzaldehyde R* in a mixture of 1 volume of *hydrochloric acid R* and 3 volumes of *methanol R*. Dry the plate in a current of hot air. Examine in daylight after 30 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Loss on drying *(2.2.32)*

Not more than 0.1 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

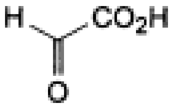
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 120.0 mg in 40 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 15.81 mg of $C_4H_6N_4O_3$.

IMPURITIES



A. glyoxylic acid,

B. urea.

Ph Eur

Allergen Products

General Notices

(Ph Eur monograph 1063)

Ph Eur

DEFINITION

Allergen products are pharmaceutical preparations derived from extracts of naturally occurring source materials containing allergens, which are substances that cause or provoke allergic (hypersensitivity) disease. The allergenic components are most often of a proteinaceous nature. Allergen products are intended for *in vivo* diagnosis or treatment of allergic (hypersensitivity) diseases attributed to these allergens.

Allergen products are available as finished products, as bulk preparations in dried form, solutions or suspensions intended to be further concentrated or diluted prior to use or as final preparations in solutions, suspensions or freeze-dried. Allergen products intended for parenteral, bronchial and conjunctival administration are sterile.

For *diagnostic use*, allergen products are usually prepared as unmodified extracts in a 50 per cent *V/V* solution of glycerol for skin-prick testing. For intradermal diagnosis or for provocation tests by nasal, ocular or bronchial routes, suitable dilutions of allergen products may be prepared by dilution of aqueous or glycerinated extracts, or by reconstitution immediately before use of unmodified freeze-dried extracts.

For *immunotherapy*, allergen products may be either unmodified extracts or extracts modified chemically and/or by adsorption onto different carriers (for example, aluminium hydroxide, calcium phosphate or tyrosine).

This monograph does not apply to: chemicals that are used solely for diagnosis of contact dermatitis; chemically synthesised products; allergens derived by rDNA technology; finished products used on a named-patient basis. It does not necessarily apply to allergen products for veterinary use.

PRODUCTION

Allergen products are derived from a wide range of allergenic source materials. They are often prepared as bulk products intended to be further diluted or concentrated prior to use. They may be treated to modify or reduce the allergenic activity or remain unmodified.

Where allergen products are manufactured using materials of human or animal origin, the requirements of chapter *5.1.7. Viral safety* apply.

SOURCE MATERIALS

Source materials for the preparation of allergen products are mostly pollens, moulds, mites, animal epithelia, hymenoptera venoms and certain foods.

They are described by their origin, nature, method of collection or production and pretreatment, and are stored under defined conditions that minimise deterioration.

The collection or production, as well as the handling of the source materials are such that uniform qualitative and quantitative composition is ensured as far as possible from batch to batch.

Pollens

Potential chemical contaminants, such as pesticides and heavy metals, must be minimised. Pollens contain not more than 1 per cent of foreign pollens as determined by microscopic examination. Pollens contain not more than 1 per cent of mould spores as determined by microscopic examination.

Mites and moulds

Biologically active contaminants such as mycotoxins in moulds must be minimised and any presence justified. Care must be taken to minimise any allergenic constituents of the media used for the cultivation of mites and moulds as source materials. Culture media that contain substances of human or animal origin must be justified and, when required must be suitably treated to ensure the inactivation or elimination of possible transmissible agents of disease.

Animal epithelia

Animal epithelia must be obtained from healthy animals selected to avoid possible transmissible agents of disease.

MANUFACTURING PROCESS

Allergen products are generally obtained by extraction, and may be purified, from the source materials using appropriate methods shown to preserve the biological properties of the allergenic components. Allergen products are manufactured under conditions designed to minimise microbial growth and enzymatic degradation.

A purification procedure, if any, is designed to minimise the content of any potential irritant low-molecular-mass components or other non-allergenic components.

Allergen products may contain a suitable antimicrobial preservative. The nature and the concentration of the antimicrobial preservative have to be justified.

The manufacturing process comprises various stages.

Native allergen extracts result after separation from the extracted source materials.

Intermediate allergen products are obtained by further processing or modification of the native allergen extracts. The modification may be achieved by chemical processes (chemical conjugation) or physical processes (physical adsorption onto different carriers, for example, aluminium hydroxide, calcium phosphate or tyrosine). They may also be modified by inclusion into such vehicles as liposomes or microspheres, or by the addition of other biologically active agents to enhance efficacy or safety. Intermediate allergen products may be freeze-dried.

Bulk allergen preparations consist of products in solution or suspension which will not be further processed or modified, and are ready for dilution or filling into final containers.

IN-HOUSE REFERENCE PREPARATION

An appropriate representative preparation is selected as the In-House Reference Preparation (IHRP), characterised and used to verify batch-to-batch consistency. The IHRP is stored in suitably sized aliquots under conditions ensuring its stability, usually freeze-dried.

Characterisation of the In-House Reference Preparation

The extent of characterisation of the IHRP depends on the nature of the allergenic source material, knowledge of the allergenic components and availability of suitable reagents, as well as the intended use. The characterised IHRP is used as reference in the batch control of native allergen extracts or intermediate allergen products and, if possible, in the batch control of final allergen preparations.

The In-House Reference Preparation (IHRP) is characterised by the protein content determination and a protein profile using relevant methods (such as isoelectric focusing, polyacrylamide gel electrophoresis, immunoelectrophoresis or molecular-mass profiling). Allergenic components may be detected by appropriate methods (for example, immunoblotting or crossed radio-immunoelectrophoresis). Characterisation of the allergenic components may include identification of relevant allergens based on serological or other techniques using a pool or individual sera from allergic patients, or allergen-specific polyclonal or monoclonal antibodies. When allergen reference substances are available, determination of the content of individual allergens may be performed. Individual allergens are identified according to internationally established nomenclature whenever possible.

Where possible, the biological potency of the IHRP is established by *in vivo* techniques such as skin testing, and expressed in units of biological activity. If not, for certain extracts, potency may be established by suitable immunoassays (for example, those based on the inhibition of the binding capacity of specific immunoglobulin E antibodies) or by quantitative techniques for a single major component.

IDENTIFICATION

Identity is confirmed at the intermediate or other applicable stage by comparison with the IHRP using protein profiling by appropriate methods (for example, isoelectric focusing, sodium dodecyl sulphate-polyacrylamide gel electrophoresis or immunoelectrophoresis).

TESTS

Various biochemical and immunological tests have been developed in order to characterise allergens qualitatively and quantitatively. However, some of the methods, particularly for the determination of allergenic activity and allergen profile, are not applicable to all products at present. This is because knowledge of the allergenic components or the required reagents is not available. Accordingly, allergen products have been classified in different categories with increasing test requirements, according to quality and intended use.

Where possible the following tests are applied to the final preparations. If not, they must be performed on the extracts as late as possible in the manufacturing process, for example, at the stage immediately prior to that stage (modification, dilution etc.) which renders the test not feasible on the final preparation.

Water *(2.5.12)*

Not more than 5 per cent for freeze-dried products.

Sterility *(2.6.1)*

Allergen products intended for parenteral, bronchial and conjunctival administration comply with the test for sterility.

Protein content

80 per cent to 120 per cent of the stated content of a given batch. If the biological potency can be determined then the test for protein content may be omitted.

Protein profile

The protein composition determined by suitable methods corresponds to that of the IHRP.

Abnormal toxicity *(2.6.9)*

Allergen products obtained from moulds and intended for parenteral administration (except skin-prick tests) comply with the test for abnormal toxicity for immunosera and vaccines for human use.

Various additional tests, some with increasing selectivity, depending on the allergen product concerned can be applied, but in any case for allergen products intended for therapeutic use, a validated test measuring the potency (total allergenic activity, determination of individual allergens or any other justified tests) must be applied.

Aluminium *(2.5.13)*

Not less than 80 per cent and not more than 120 per cent of the stated amount but in any case not more than 1.25 mg per human dose unless otherwise justified and authorised, when aluminium hydroxide or aluminium phosphate is used as adsorbent.

Calcium *(2.5.14)*

Not less than 80 per cent and not more than 120 per cent of the stated amount when calcium phosphate is used as adsorbent.

Antigen profile

The antigens are identified by means of suitable techniques using antigen-specific animal antibodies.

Allergen profile

Relevant allergenic components are identified by means of suitable techniques using allergen-specific human antibodies.

Total allergenic activity

The activity is 50 per cent to 200 per cent of the stated amount as assayed by inhibition of the binding capacity of specific immunoglobulin E antibodies or a suitable equivalent *in vitro* method.

Individual allergens

50 per cent to 200 per cent of the stated amount, determined by a suitable method.

STORAGE

Adsorbed allergen products should not be frozen.

LABELLING

The label states:

— the biological potency and/or the protein content and/or the extraction concentration;

— the route of administration and the intended use;

— the storage conditions;

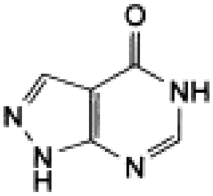
- where applicable, the name and amount of added antimicrobial preservative;
- for freeze-dried preparations:
  - the name, composition and volume of the reconstituting liquid to be added
  - the period of time within which the preparation is to be used after reconstitution;
- where applicable, that the preparation is sterile;
- where applicable, the name and amount of adsorbent.

Ph Eur

Allopurinol

General Notices

(Ph Eur monograph 0576)



$C_5H_4N_4O$ 136.1 *315-30-0*

Action and use

Xanthine oxidase inhibitor; treatment of gout and hyperuricaemia.

Preparation

Allopurinol Tablets

Ph Eur

DEFINITION

1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 10 mg in 1 ml of a 4 g/l solution of *sodium hydroxide R* and dilute to 100.0 ml with a 10.3 g/l solution of *hydrochloric acid R*. Dilute 10.0 ml of this solution to 100.0 ml with a 10.3 g/l solution of *hydrochloric acid R*.

Spectral range 220-350 nm.

Absorption maximum At 250 nm.

Absorption minimum At 231 nm.

Absorbance ratio A_{231}/A_{250} = 0.52 to 0.62.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *allopurinol CRS.*

C. Dissolve 0.3 g in 2.5 ml of *dilute sodium hydroxide solution R* and add 50 ml of *water R*. Add slowly and with shaking 5 ml of *silver nitrate solution R1*. A white precipitate is formed which does not dissolve on the addition of 5 ml of *ammonia R*.

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *concentrated ammonia R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *allopurinol CRS* in concentrated *ammonia R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous ethanol R, methylene chloride R* (40:60 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Use freshly prepared solutions. Store and inject them at 8 °C, using a cooled autosampler.*

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 2.5 ml of a 4 g/l solution of *sodium hydroxide R* and dilute immediately to 50.0 ml with the mobile phase.

Test solution (b) Dissolve 20.0 mg of the substance to be examined in 5.0 ml of a 4 g/l solution of *sodium hydroxide R* and dilute immediately to 250.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *allopurinol impurity A CRS*, 5 mg of *allopurinol impurity B CRS* and 5.0 mg of *allopurinol impurity C CRS* in 5.0 ml of a 4 g/l solution of *sodium hydroxide R* and dilute immediately to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 20.0 mg of *allopurinol CRS* in 5.0 ml of a 4 g/l solution of *sodium hydroxide R* and dilute immediately to 250.0 ml with the mobile phase.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 1.25 g/l solution of *potassium dihydrogen phosphate R*.

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (b).

Run time Twice the retention time of allopurinol.

Elution order Impurity A, impurity B, impurity C, allopurinol.

Retention time allopurinol = about 10 min.

System suitability Reference solution (b):
- *resolution*: minimum 1.1 between the peaks due to impurities B and C.

Limits:
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *sum of impurities other than A, B and C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurities D and E

Liquid chromatography *(2.2.29)*. *Use freshly prepared solutions. Store and inject them at 8 °C, using a cooled autosampler.*

Solution A 1.25 g/l solution of *potassium dihydrogen phosphate R*.

Test solution Dissolve 50.0 mg of the substance to be examined in 5.0 ml of a 4 g/l solution of *sodium hydroxide R* and dilute immediately to 100.0 ml with solution A.

Reference solution Dissolve 5.0 mg of *allopurinol impurity D CRS* and 5.0 mg of *allopurinol impurity E CRS* in 5.0 ml of a 4 g/l solution of *sodium hydroxide R* and dilute immediately to 100.0 ml with solution A. Dilute 1.0 ml of this solution to 100.0 ml with solution A.

Column:
- *size: l* = 0.05 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase *methanol R*, 1.25 g/l solution of *potassium dihydrogen phosphate R* (10:90 *V/V*)
.

Flow rate 2 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 1.5 times the retention time of impurity E.

Retention times Impurity D = about 3.6 min; impurity E = about 4.5 min.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the peaks due to impurities D and E.

Limits:

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent);

— *impurity E*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Impurity F

Liquid chromatography *(2.2.29)*.

Under the following conditions, any hydrazine in the sample reacts with benzaldehyde to give benzaldehyde azine.

Solvent mixture Mix equal volumes of *dilute sodium hydroxide solution R* and *methanol R*.

Solution A Dissolve 2.0 g of *benzaldehyde R* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Prepare immediately before use.

Test solution Dissolve 250.0 mg of the substance to be examined in 5 ml of the solvent mixture. Add 4 ml of solution A, mix and allow to stand for 2.5 h at room temperature. Add 5.0 ml of *hexane R* and shake for 1 min. Allow the layers to separate and use the upper layer.

Reference solution Dissolve 10.0 mg of *hydrazine sulphate R* in the solvent mixture by sonicating for about 2 min and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml to 20.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture. To 5.0 ml of the solution obtained, add 4 ml of solution A, mix and allow to stand for 2.5 h at room temperature. Add 5.0 ml of *hexane R* and shake for 1 min. Allow the layers to separate and use the upper layer.

Blank solution To 5 ml of the solvent mixture add 4 ml of solution A, mix and allow to stand for 2.5 h at room temperature. Add 5.0 ml of *hexane R* and shake for 1 min. Allow the layers to separate and use the upper layer.

Column:

— *size: l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *cyanosilyl silica gel for chromatography R* (5 µm) with a pore size of 100 nm;

— *temperature*: 30 °C.

Mobile phase *2-propanol R, hexane R* (5:95 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 310 nm.

Injection 20 µl.

Relative retention With reference to benzaldehyde (retention time = about 2.8 min): benzaldehyde azine = about 0.8.

System suitability Reference solution:

— *resolution*: minimum 2 between the peaks due to benzaldehyde azine and benzaldehyde;

— *signal-to-noise ratio*: minimum 20 for the peak due to benzaldehyde azine.

Limit:

— *impurity F*: the area of the peak due to benzaldehyde azine in the chromatogram obtained with the test solution is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm of hydrazine sulphate equivalent to 2.5 ppm of hydrazine).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

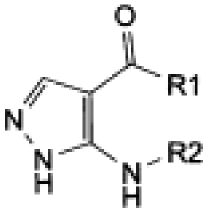
Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_5H_4N_4O$ from the declared content of *allopurinol CRS*.

IMPURITIES

Specified impurities *A, B, C, D, E, F.*

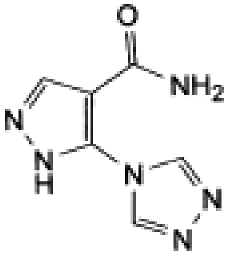


A. R1 = NH_2, R2 = H: 5-amino-1*H*-pyrazole-4-carboxamide,

B. R1 = NH_2, R2 = CHO: 5-(formylamino)-1*H*-pyrazole-4-carboxamide,

D. R1 = O-C_2H_5, R2 = H: ethyl 5-amino-1*H*-pyrazole-4-carboxylate,

E. R1 = O-C_2H_5, R2 = CHO: ethyl 5-(formylamino)-1*H*-pyrazole-4-carboxylate,



C. 5-(4*H*-1,2,4-triazol-4-yl)-1*H*-pyrazole-4-carboxamide,

F. H_2N-NH_2: diazane (hydrazine).

Almagate

General Notices

(Ph Eur monograph 2010)

$Al_2Mg_6C_2O_{20}H_{14},4H_2O$ 630 *66827-12-1*

Action and use

Antacid.

Ph Eur

DEFINITION

Hydrated aluminium magnesium hydroxycarbonate.

Content:

- — aluminium: 15.0 per cent to 17.0 per cent (calculated as Al_2O_3),
- — magnesium: 36.0 per cent to 40.0 per cent (calculated as MgO),
- — carbonic acid: 12.5 per cent to 14.5 per cent (calculated as CO_2).

CHARACTERS

Appearance

White or almost white, fine crystalline powder.

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride. It dissolves with effervescence and heating in dilute mineral acids.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of almagate.

B. Dissolve 150 mg in *dilute hydrochloric acid R* and dilute to 20 ml with the same acid. 2 ml of the solution gives the reaction of aluminium *(2.3.1)*.

C. 2 ml of the solution prepared under identification test B gives the reaction of magnesium *(2.3.1)*.

TESTS

pH *(2.2.3)*

9.1 to 9.7.

Disperse 4.0 g in 100 ml of *carbon dioxide-free water R*, stir for 2 min and filter.

Neutralising capacity

Carry out the test at 37 °C. Disperse 0.5 g in 100 ml of *water R*, heat, add 100.0 ml of *0.1 M hydrochloric acid*, previously heated and stir continuously; the pH *(2.2.3)* of the solution between 5 min and 20 min is not less than 3.0 and not greater than 4.5. Add 10.0 ml of *0.5 M hydrochloric acid*, previously heated, stir continuously for 1 h and titrate with *0.1 M sodium hydroxide* to pH 3.5; not more than 20.0 ml of *0.1 M sodium hydroxide* is required.

Chlorides *(2.4.4)*

Maximum 0.1 per cent.

Dissolve 0.33 g in 5 ml of *dilute nitric acid R* and dilute to 100 ml with *water R*. 15 ml of the solution complies with the limit test for chlorides. Prepare simultaneously the standard by diluting 0.7 ml of *dilute nitric acid R* to 5 ml with *water R* and adding 10 ml of *chloride standard solution (5 ppm Cl) R*.

Sulphates *(2.4.13)*

Maximum 0.4 per cent.

Dissolve 0.25 g in 5 ml of *dilute hydrochloric acid R* and dilute to 100 ml with *distilled water R*. 15 ml of the solution complies with the limit test for sulphates. Prepare simultaneously the standard by adding 0.8 ml of *dilute hydrochloric acid R* to 15 ml of *sulphate standard solution (10 ppm* SO_4*) R*.

Sodium

Maximum 1.50 × 102 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 0.25 g in 50 ml of a 103 g/l solution of *hydrochloric acid R*.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with a 103 g/l solution of *hydrochloric acid R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 20.0 ml with the same acid. 12 ml of the solution complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

43.0 per cent to 49.0 per cent, determined on 1.000 g by ignition at 900 ± 50 °C.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 103 micro-organisms per gram determined by plate count. It complies with the tests for *Escherichia coli* and *Pseudomonas aeruginosa (2.6.13)*.

ASSAY

Aluminium

Dissolve 1.000 g in 5 ml of *hydrochloric acid R*, heating if necessary. Allow to cool to room temperature and dilute to 100.0 ml with *water R* (solution A). Introduce 10.0 ml of solution A into a 250 ml conical flask, add 25.0 ml of *0.05 M sodium edetate*, 20 ml of *buffer solution pH* 3.5 R, 40 ml of *ethanol R* and 2 ml of a freshly prepared 0.25 g/l solution of *dithizone R* in *ethanol R*. Titrate the excess of sodium edetate with *0.05 M zinc sulphate* until the colour changes from greenish-violet to pink.

1 ml of *0.05 M sodium edetate* is equivalent to 2.549 mg of Al_2O_3.

Magnesium

Introduce 10.0 ml of solution A prepared in the assay of aluminium into a 500 ml conical flask, add 200 ml of *water R*, 20 ml of *triethanolamine R* with shaking, 10 ml of *ammonium chloride buffer solution pH 10.0 R* and 50 mg of *mordant black 11 triturate R*. Titrate with *0.05 M sodium edetate* until the colour changes from violet to pure blue.

1 ml of 0.05 M sodium edetate is equivalent to 2.015 mg of MgO.

Carbonic acid

12.5 per cent to 14.5 per cent.

Test sample Place 7.00 mg of the substance to be examined in a tin capsule. Seal the capsule.

Reference sample Place 7.00 mg of *almagate CRS* in a tin capsule. Seal the capsule.

Introduce separately the test sample and the reference sample into a combustion chamber of a CHN analyser purged with *helium for chromatography R* and maintained at a temperature of 1020 °C. Simultaneously, introduce *oxygen R* at a pressure of 40 kPa and a flow rate of 20 ml/min and allow complete combustion of the sample. Sweep the combustion gases through a reduction reactor and separate the gases formed by gas chromatography *(2.2.28)*.

Column:
— *size: l* = 2 m, Ø = 4 mm,

— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R1*.

Carrier gas *helium for chromatography R.*

Flow rate 100 ml/min.

Temperature:
— *column*: 65 °C,

— *detector*: 190 °C.

Detection Thermal conductivity.

Run time 16 min.

System suitability:
— average percentage of carbon in 5 reference samples must be within ± 0.2 per cent of the value assigned to the CRS. The difference between the upper and the lower values of the percentage of carbon in these samples must be below 0.2 per cent.

Calculate the percentage content of carbonic acid in the test sample according to the following formula:

$$C \times K \times \frac{A}{m}$$

C = percentage content of carbonic acid in the reference sample,

K = mean value for the 5 reference samples of the ratio of the mass in milligrams to the area of the peak due to carbonic acid,

A = area of the peak due to carbonic acid in the chromatogram obtained with the test sample,

m = sample mass, in milligrams.

STORAGE

In an airtight container.

Ph Eur

Aloxiprin

General Notices

9014-67-9

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

Preparation

Aloxiprin Tablets

DEFINITION

Aloxiprin is a polymeric condensation product of aluminium oxide and *O*-acetylsalicylic acid. It contains not less than 7.5% and not more than 8.5% of aluminium, Al, and not less than 79.0% and not more than 87.4% of total salicylates, calculated as *O*-acetylsalicylic acid, $C_9H_8O_4$, both calculated with reference to the dried substance.

CHARACTERISTICS

A fine, white or slightly pink powder.

Practically insoluble in *water*; practically insoluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. Boil 1 g with 20 ml of 2M *hydrochloric acid*, cool, filter and reserve the filtrate. Dissolve the residue in 10 ml of 0.1M *sodium hydroxide* and neutralise with 1M *acetic acid*. 1 ml of the resulting solution yields reaction A characteristic of *salicylates*, Appendix VI.

B. The filtrate reserved in test A yields the reaction characteristic of *aluminium salts*, Appendix VI.

TESTS

Heavy metals

Carefully ignite 2.0 g at a low temperature until completely charred, cool, add 2 ml of *nitric acid* and 0.25 ml of *sulphuric acid*, heat cautiously until white fumes are evolved and ignite at 500° to 600°. Cool, add 2 ml of *hydrochloric acid*, evaporate to dryness on a water bath and carry out the procedure for *limit test C for heavy metals*, Appendix VII, beginning at the words 'Dissolve the residue...'. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Free acetylsalicylic acid

To a quantity containing the equivalent of 1.0 g of total salicylates add 50 ml of dry *ether* and shake for 30 minutes. Filter quickly through fluted filter paper, wash the paper with several portions of dry *ether* and dilute the combined filtrate and washings to 100 ml with dry *ether*. The *absorbance* of the solution at the maximum at 278 nm is not more than 0.36, Appendix II

B (0.5%, calculated with reference to the content of total salicylates).

Salicylic acid

The *absorbance* of the solution used in the test for Free acetylsalicylic acid at the maximum at 308 nm is not more than 0.50, Appendix II B (0.15%, calculated with reference to the content of total salicylates).

Combined salicylate

Not more than 9.5%, calculated as salicylic acid, $C_7H_6O_3$, with reference to the content of total salicylates calculated as *O*-acetylsalicylic acid when determined in the following manner. To 0.1 g add 40 ml of a 0.5% w/v solution of *sodium fluoride* in 0.1M *hydrochloric acid* and shake for 5 minutes. Allow the solution to stand for 10 minutes, shaking at frequent intervals. Extract with six 20 ml quantities of *dichloromethane*, filter the combined extracts through a layer of *anhydrous sodium sulphate*, wash with 30 ml of *dichloromethane* and dilute the combined filtrate and washings to 200 ml with *dichloromethane*. Dilute 20 ml of the solution to 50 ml with *dichloromethane* and measure the *absorbance* of the resulting solution at the maximum at 308 nm, Appendix II B. Calculate the content of $C_7H_6O_3$ taking 293 as the value of A (1%, 1 cm) at the maximum at 308 nm.

Loss on drying

When dried to constant weight over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, loses not more than 2.0% of its weight. Use 1 g.

ASSAY

For aluminium

Ignite 2 g in a tared silica crucible, heat gently until the organic matter is destroyed and then ignite to constant weight at 1000°. Each g of residue is equivalent to 0.5292 g of Al.

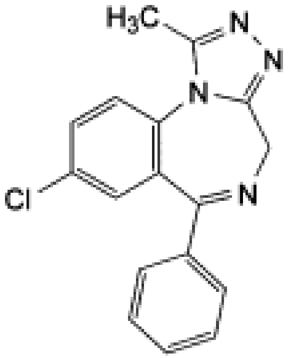
For total salicylates

To 0.25 g add 50 ml of 1M *sodium hydroxide* and boil gently until dissolved. Cool, add 50 ml of *water*, adjust the pH to between 2.40 and 2.50 with 1M *hydrochloric acid* and dilute to 500 ml with *water*. To 5 ml add 4 ml of *iron(III) chloride solution*, allow to stand for 30 minutes, dilute to 50 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 530 nm, Appendix II B, using in the reference cell a solution prepared by diluting 4 ml of *iron (III) chloride solution* to 50 ml with *water*. Calculate the content of total salicylates as $C_9H_8O_4$ from the *absorbance* obtained by repeating the procedure using 4 ml of a 0.05% w/v solution of *salicylic acid* in place of the solution being examined and beginning at the words add 4 ml of *iron(III) chloride solution*...'. Each g of salicylic acid is equivalent to 1.305 g of $C_9H_8O_4$.

Alprazolam

General Notices

(Ph Eur monograph 1065)



$C_{17}H_{13}ClN_4$ 308.8 *28981-97-7*

Action and use

Benzodiazepine.

Ph Eur

DEFINITION

8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]-benzodiazepine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone and in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification B.

Second identification *A, C.*

A. Dissolve the substance to be examined in the smallest necessary quantity of *ethyl acetate R* and evaporate to dryness on a water-bath. Thoroughly mix 5.0 mg of the substance to be examined with 5.0 mg of *alprazolam CRS*. The melting point *(2.2.14)* of the mixture does not differ by more than 2 °C from the melting point of the substance to be examined.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *alprazolam CRS .*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethyl acetate R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *alprazolam CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *alprazolam CRS* and 10 mg of *midazolam CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *glacial acetic acid R, water R, methanol R, ethyl acetate R* (2:15:20:80 *V/V/V/V*).

Application 5 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separately spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Buffer solution Dissolve 7.7 g of *ammonium acetate R* in 1000 ml of *water R* and adjust to pH 4.2 with *glacial acetic acid R*.

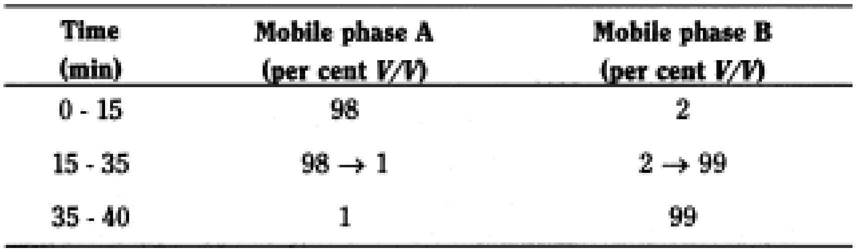
Test solution Dissolve 0.100 g of the substance to be examined in *dimethylformamide R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *alprazolam CRS* and 2 mg of *triazolam CRS* in *dimethylformamide R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *dimethylformamide R*. Dilute 0.5 ml of this solution to 10.0 ml with *dimethylformamide R*.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: phenylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase:
- *mobile phase A*: buffer solution, *methanol R* (44:56 *V/V*);
- *mobile phase B*: buffer solution, *methanol R* (5:95 *V/V*);
- *temperature*: 40 °C;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	98	2
15 - 35	98 → 1	2 → 99
35 - 40	1	99

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl; inject *dimethylformamide R* as a blank.

Retention time triazolam = about 9 min; alprazolam = about 10 min.

System suitability Reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to triazolam and alprazolam.

Limits:
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

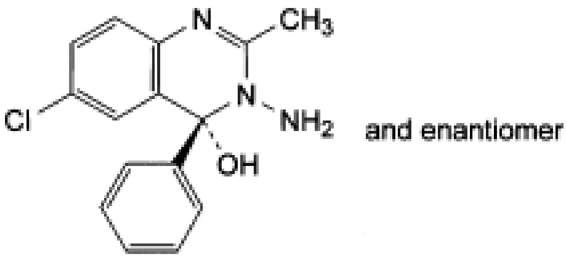
Dissolve 0.140 g in 50 ml of a mixture of 2 volumes of *acetic anhydride R* and 3 volumes of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Titrate to the 2nd point of inflexion.

1 ml of *0.1 M perchloric acid* is equivalent to 15.44 mg of $C_{17}H_{13}ClN_4$.

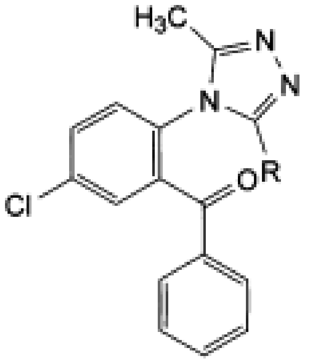
STORAGE

Protected from light.

IMPURITIES


N
CH3
Cl
N
NH2
OH
and enantiomer

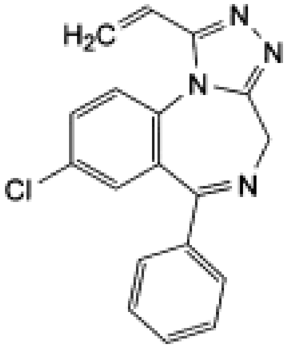
A. (4*RS*)-3-amino-6-chloro-2-methyl-4-phenyl-3,4-dihydroquinazolin-4-ol,


H3C
N
N
N
Cl
O
R

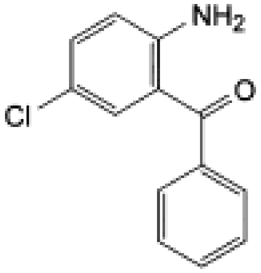
B. R = CH_2OH: [5-chloro-2-[3-(hydroxymethyl)-5-methyl-4*H*-1,2,4-triazol-4-yl]phenyl] phenylmethanone,

C. R = H: [5-chloro-2-[3-methyl-4*H*-1,2,4-triazol-4-yl]phenyl]phenylmethanone,

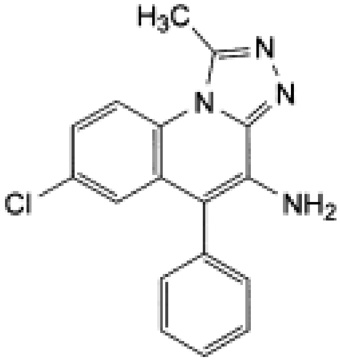
F. R = CH_2Cl: [5-chloro-2-[3-(chloromethyl)-5-methyl-4*H*-1,2,4-triazol-4-yl]phenyl] phenylmethanone,



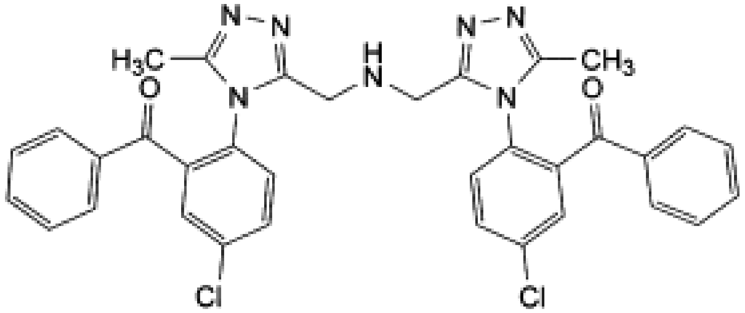
D. 8-chloro-1-ethenyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine,



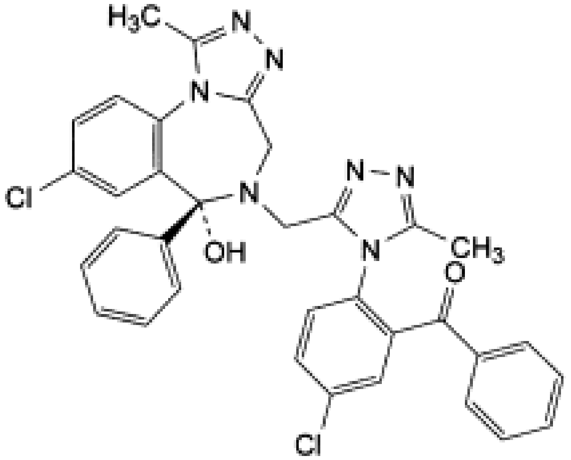
E. (2-amino-5-chlorophenyl)phenylmethanone,



G. 7-chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-*a*]quinolin-4-amine,

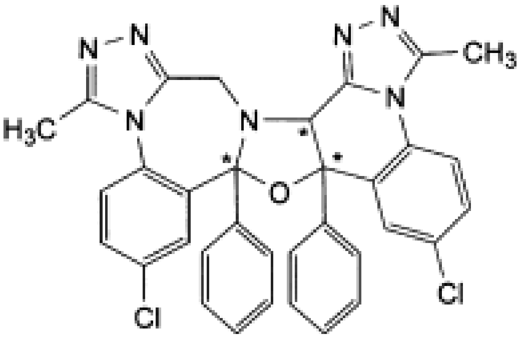


H. bis[[4-(2-benzoyl-4-chlorophenyl)-5-methyl-4*H*-1,2,4-triazol-3-yl]methyl]amine,



and enantiomer

I. [5-chloro-2-[3-[[(6*RS*)-8-chloro-6-hydroxy-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-5(6*H*)-yl]methyl]-5-methyl-4*H*-1,2,4-triazol-4-yl]phenyl]phenylmethanone,



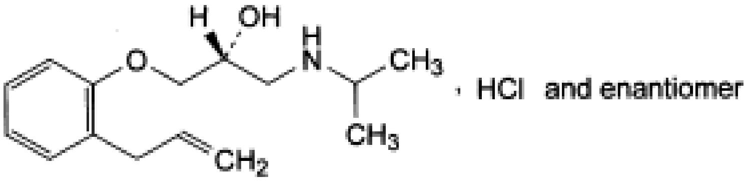
J. 2,17-dichloro-6,13-dimethyl-18b,19a-diphenyl-8b,19adihydro-1C*H*,18b*H*-[1,2,4]triazolo[4‴,3‴:1″,2″]quinolo[3″,4″:4′,5′]oxazolo[3′,2′-*d*]-1,2,4-triazolo[4,3-*a*][1,4]benzodiazepine.

Ph Eur

Alprenolol Hydrochloride

General Notices

(Ph Eur monograph 0876)



$C_{15}H_{23}NO_2$,HCl 285.8 *13707-88-5*

Action and use

Beta-adrenoceptor antagonist.

Ph Eur

DEFINITION

(2*RS*)-1-[(1-Methylethyl)amino]-3-[2-(prop-2-enyl)phenoxy]propan-2-ol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 108 °C to 112 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison alprenolol hydrochloride CRS.

C. Examine the chromatograms obtained in test A for related substances.

Detection Examine in daylight, after exposure to iodine vapour for 30 min.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_9 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M hydrochloric acid* ; the solution is red. Add 0.4 ml of *0.01 M sodium hydroxide*; the solution is yellow.

Impurity C

Maximum 0.1 per cent.

Dissolve 0.25 g in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent. The absorbance *(2.2.25)* measured at 297 nm is not greater than 0.20.

Related substances

A. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *methanol R*.

Reference solution (a) Dissolve 10 mg of *alprenolol hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *alprenolol hydrochloride CRS* and 10 mg of *oxprenolol hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dilute 5 ml of test solution (b) to 50 ml with *methanol R*.

Plate *TLC silica gel G plate R.*

Mobile phase Place 2 beakers each containing 30 ml of *ammonia R* at the bottom of the tank containing a mixture of 5 volumes of *methanol R* and 95 volumes of *ethyl acetate R*.

Application 5 µl.

Development Over a path of 15 cm in a tank saturated for at least 1 h.

Drying At 100 °C for 15 min.

Detection Expose to iodine vapour for up to 6 h.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Limits Test solution (a):

— *impurity D*: any spot with an R_F value greater than that of the principal spot is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

B. Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 4.0 mg of *alprenolol hydrochloride CRS* and 0.8 mg of *4-isopropylphenol R* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 4.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
— *size: l* = 0.15 m, Ø = 4 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 0.656 g of *sodium octanesulphonate R* with 150 ml of *acetonitrile R* and dilute to 500 ml with phosphate buffer pH 2.8 prepared as follows: mix 1.78 g of *phosphoric acid R* and 15.6 g of *sodium dihydrogen phosphate R* and dilute to 2000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Equilibration With the mobile phase for about 1 h.

Injection 20 µl.

Run time Twice the retention time of alprenolol.

Retention time Alprenolol = about 11 min; 4-isopropylphenol = about 18 min.

System suitability Reference solution (a):

— *resolution*: minimum 5 between the peaks due to alprenolol and 4-isopropylphenol; if necessary, adjust the concentration of sodium octanesulphonate and/or acetonitrile in the mobile phase (increase the concentration of sodium octanesulphonate to increase the retention time of alprenolol and increase the concentration of acetonitrile to decrease the retention times of both compounds).

Limits:
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.04 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at a pressure not exceeding 2.7 kPa.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 25 ml of a mixture of equal volumes of *anhydrous ethanol R* and *water R*. Add 10 ml of *0.01 M hydrochloric acid* . Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 28.58 mg of $C_{15}H_{24}ClNO_2$.

STORAGE

Protected from light.

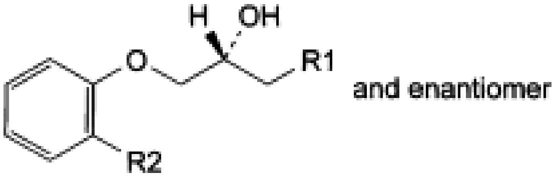
IMPURITIES

Specified impurities *C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B.*

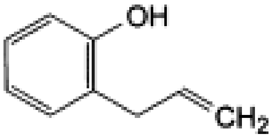
By thin-layer chromatography *D.*

By liquid chromatography *A, B.*

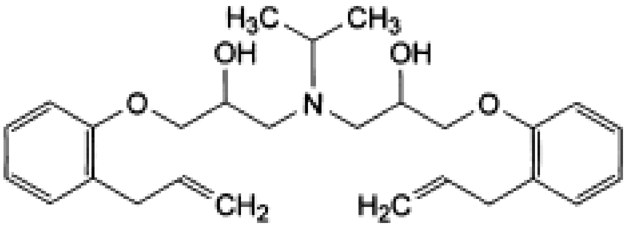


A. R1 = OH, R2 = CH_2-CH=CH_2: (2*RS*)-3-[2-(prop-2-enyl)phenoxy]propan-1,2-diol,

C. R1 = NH-CH$(CH_3)_2$, R2 = CH=CH-CH_3: (2*RS*)-1-[(1-methylethyl)amino]-3-[2-(prop-1-enyl) phenoxy]propan-2-ol,



B. 2-(prop-2-enyl)phenol,



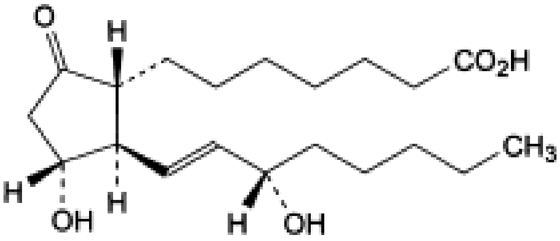
D. 1,1′-[(1-methylethyl)imino]bis[3-[2-(prop-2-enyl)phenoxy]propan-2-ol].

Ph Eur

Alprostadil

General Notices

(Ph Eur monograph 1488)



$C_{20}H_{34}O_5$ 354.5 *745-65-3*

Action and use

Prostaglandin E_1 (PGE_1).

Ph Eur

DEFINITION

7-[(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]heptanoic acid.

Content

95.0 per cent to 102.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in alcohol, soluble in acetone, slightly soluble in ethyl acetate.

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: - 60 to - 70 (anhydrous substance).

Immediately before use, dissolve 50 mg in *alcohol R* and dilute to 10.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *alprostadil CRS.*

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions protected from light.*

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R1* and *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 100 µl of the test solution to 20.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (b) Dissolve 1.0 mg of *dinoprostone impurity C CRS* (alprostadil impurity H) and 1.0 mg of *alprostadil CRS* in a mixture of equal volumes of *acetonitrile R1* and *water R* and dilute to 20.0 ml with the same mixture of solvents.

Reference solution (c) In order to prepare in situ the degradation compounds (impurity A and impurity B), dissolve 1 mg of the substance to be examined in 100 µl of *1 M sodium hydroxide* (the solution becomes brownish-red), wait for 3 min and add 100 µl of *1 M phosphoric acid* (yellowish-white opalescent solution); dilute to 5.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

System A

Column:

— *size:* *l* = 0.25 m, Ø = 4.0 mm,

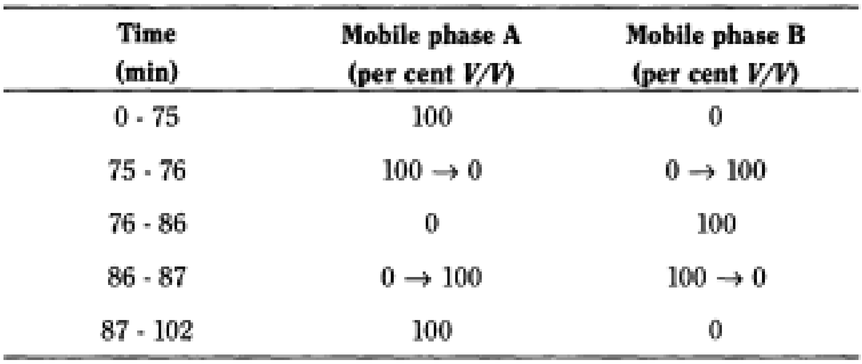
— *stationary phase*: *octylsilyl base-deactivated silica gel for chromatography R* (4 µm) with a pore size of 6 nm,

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*. Dissolve 3.9 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000.0 ml with the same solvent; adjust to pH 2.5 with a 2.9 g/l solution of *phosphoric acid R* (approximately 600 ml is required); to 740 ml of the buffer solution add 260 ml of *acetonitrile R1*;

— *mobile phase B*. Dissolve 3.9 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000.0 ml with the same solvent; adjust to pH 2.5 with a 2.9 g/l solution of *phosphoric acid R* (approximately 600 ml is required); to 200 ml of the buffer solution add 800 ml of *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 75	100	0
75 - 76	100 → 0	0 → 100
76 - 86	0	100
86 - 87	0 → 100	100 → 0
87 - 102	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 200 nm.

Injection 20 µl loop injector.

System suitability:

— *retention time*: alprostadil = about 63 min,

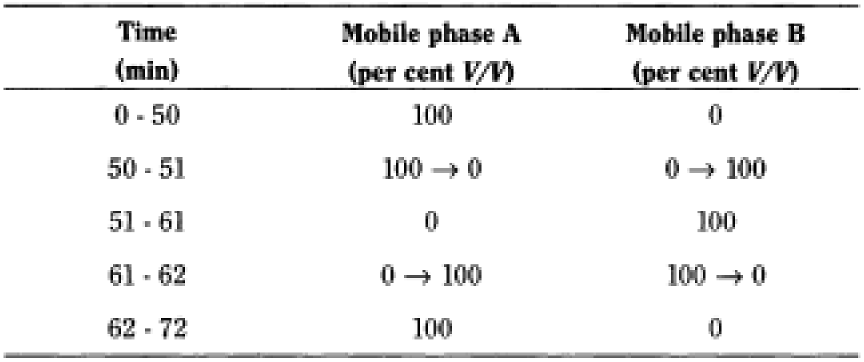
— *resolution*: minimum of 1.5 between the peaks due to impurity H and alprostadil in the chromatogram obtained with reference solution (b).

System B

Use the same conditions as for system A with the following mobile phase and elution programme:

— *mobile phase A*. Dissolve 3.9 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000.0 ml with the same solvent; adjust to pH 2.5 with a 2.9 g/l solution of *phosphoric acid R* (approximately 600 ml is required); to 600 ml of the buffer solution add 400 ml of *acetonitrile R1*;

— *mobile phase B*. Use mobile phase B as described under system A;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	100	0
50 - 51	100 → 0	0 → 100
51 - 61	0	100
61 - 62	0 → 100	100 → 0
62 - 72	100	0

System suitability:

— *relative retentions* with reference to alprostadil (retention time = about 7 min): impurity A = about 2.4; impurity B = about 2.6,

— *resolution*: minimum of 1.5 between the peaks due to impurity A and impurity B in the chromatogram obtained with reference solution (c).

Carry out the test according to system A and B.

Limits:

— *correction factors*: multiply the areas of the corresponding peaks using the correction factors in Table 1488.-1 to obtain the corrected areas,

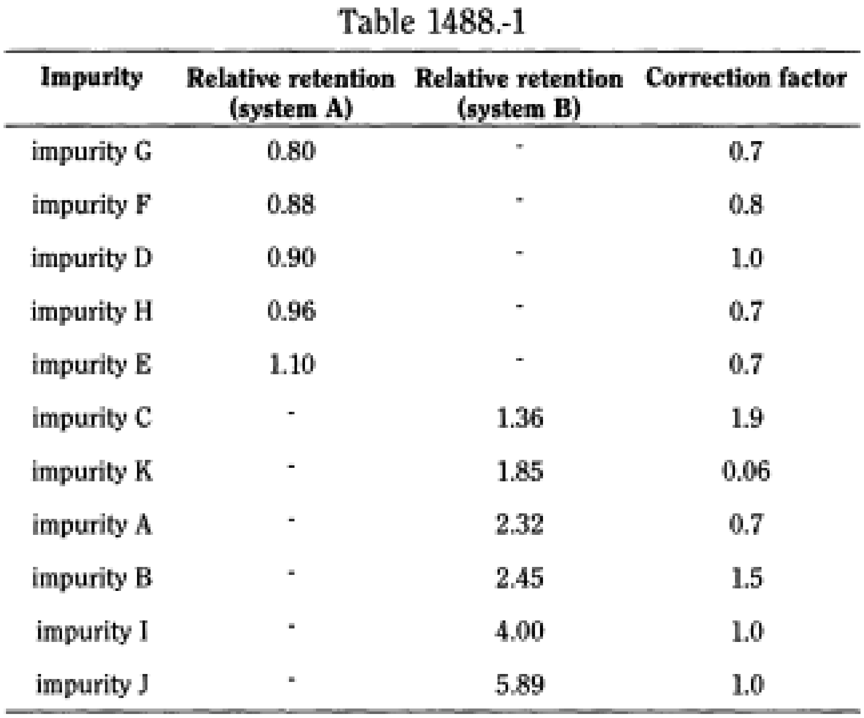


Table 1488.-1

Impurity	Relative retention (system A)	Relative retention (system B)	Correction factor
impurity G	0.80	-	0.7
impurity F	0.88	-	0.8
impurity D	0.90	-	1.0
impurity H	0.96	-	0.7
impurity E	1.10	-	0.7
impurity C	-	1.36	1.9
impurity K	-	1.85	0.06
impurity A	-	2.32	0.7
impurity B	-	2.45	1.5
impurity I	-	4.00	1.0
impurity J	-	5.89	1.0

— *impurity A (corrected area)*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent),

— *impurity B (corrected area)*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *any other impurity (corrected area)*: not more than 1.8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.9 per cent), and not more than 1 such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Evaluate impurities appearing at relative retentions less than 1.2 by system A and impurities appearing at relative retentions greater than 1.2 by system B,

— *total (corrected area)*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.32)*

Maximum 0.5 per cent, determined on 50 mg.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances, system A. *Prepare the solutions protected from light.*

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R1* and *water R* and dilute to 25.0 ml with the same mixture of solvents. Dilute 3.0 ml of the solution to 20.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution   Dissolve 10.0 mg of *alprostadil CRS* in a mixture of equal volumes of *acetonitrile R1* and *water R* and dilute to 25.0 ml with the same mixture of solvents. Dilute 3.0 ml of the solution to 20.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

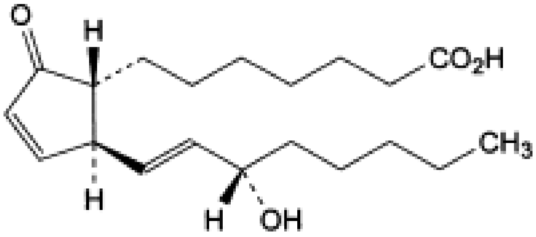
Injection   20 µl.

Calculate the percentage content of $C_{20}H_{34}O_5$.

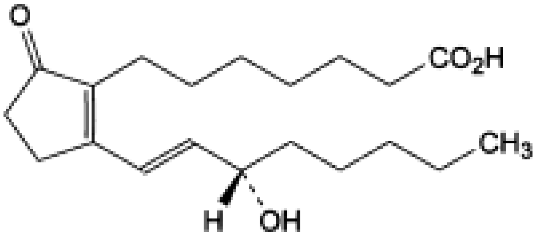
STORAGE

At a temperature of 2 °C to 8 °C.

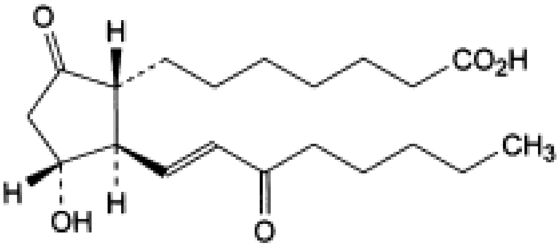
IMPURITIES



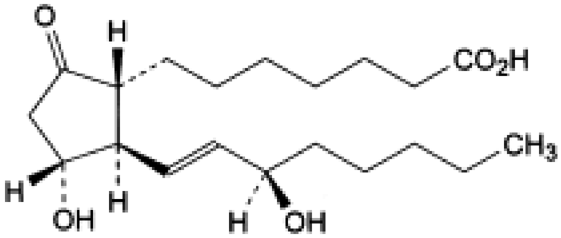
A. 7-[(1*R*,2*S*)-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-3-enyl]heptanoic acid (prostaglandin A_1),



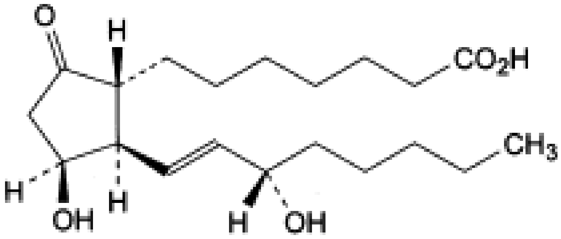
B. 7-[2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-1-enyl]heptanoic acid (prostaglandin B_1),



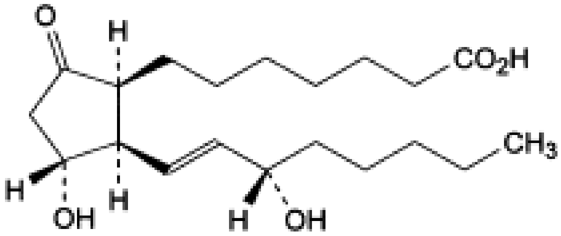
C. 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(1*E*)-3-oxooct-1-enyl]-5-oxocyclopentyl]heptanoic acid (15-oxoprostaglandin E_1),



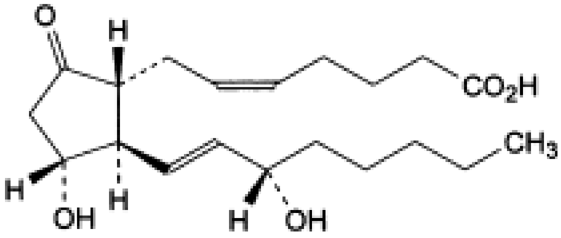
D. 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(1*E*,3*R*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]heptanoic acid (15-epiprostaglandin E_1),



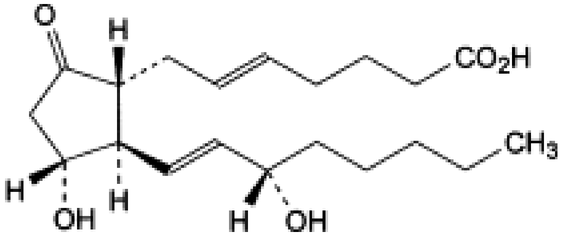
E. 7-[(1*R*,2*R*,3*S*)-3-hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]heptanoic acid (11-epiprostaglandin E_1),



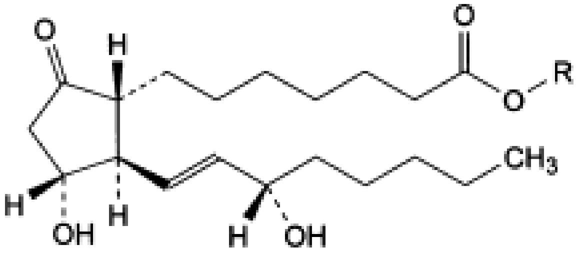
F. 7-[(1*S*,2*R*,3*R*)-3-hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]heptanoic acid (8-epiprostaglandin E_1),



G. (5*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (dinoprostone),

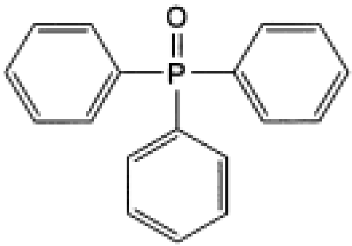


H. (5*E*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid ((5*E*)-prostaglandin E_2),



I. R = CH_2-CH_3: ethyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]heptanoate (prostaglandin E_1, ethyl ester),

J. R = $CH(CH_3)_2$: 1-methylethyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]heptanoate (prostaglandin E_1, isopropyl ester),



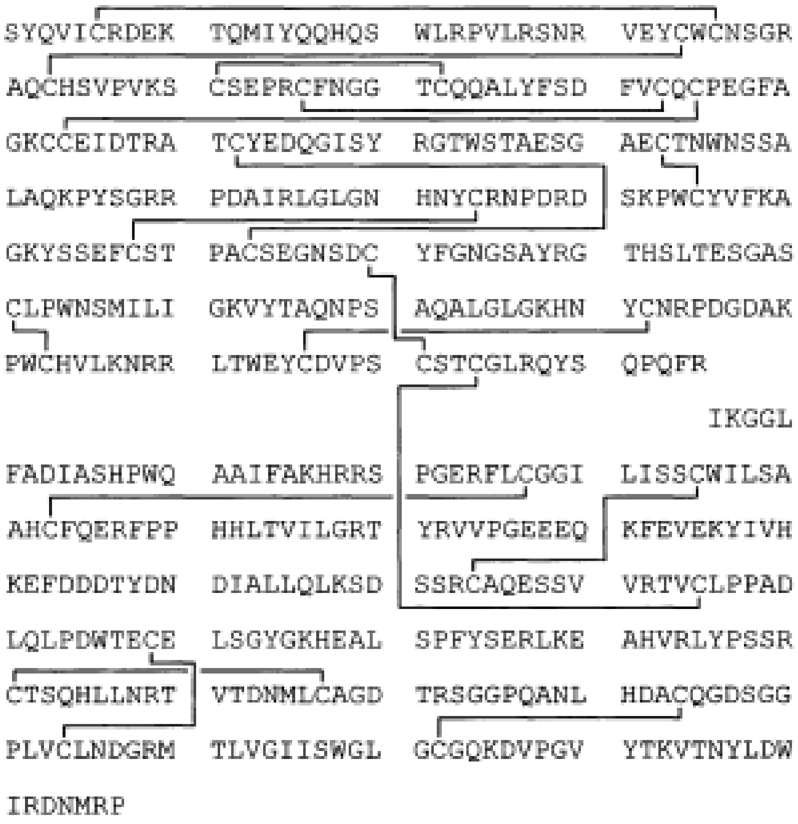
K. triphenylphosphine oxide.

Ph Eur

Alteplase for Injection

General Notices

(Ph Eur monograph 1170)


SYQVICRDEK TQMIYQQHQS WLRPVLRSNR VEYCWCNSGR
AQCHSVPVKS CSEPRCFNGG TCQQALYFSD FVCQCPEGFA
GKCCEIDTRA TCYEDQGISY RGTWSTAESG AECTNWNSSA
LAQKPYSGRR PDAIRLGLGN HNYCRNPDRD SKPWCYVFKA
GKYSSEFCST PACSEGNSDC YFGNGSAYRG THSLTESGAS
CLPWNSMILI GKVYTAQNPS AQALGLGKHN YCNRPDGDAK
PWCHVLKNRR LTWEYCDVPS CSTCGLRQYS QPQFR
IKGGL
FADIASHPWQ AAIFAKHRRS PGERFLCGGI LISSCWILSA
AHCFQERFPP HHLTVILGRT YRVVPGEEEQ KFEVEKYIVH
KEFDDDTYDN DIALLQLKSD SSRCAQESSV VRTVCLPPAD
LQLPDWTECE LSGYGKHEAL SPFYSERLKE AHVRLYPSSR
CTSQHLLNRT VTDNMLCAGD TRSGGPQANL HDACQGDSGG
PLVCLNDGRM TLVGIISWGL GCGQKDVPGV YTKVTNYLDW
IRDNMRP

$C_{2736}H_{4174}N_{914}O_{824}S_{45}$ (non-glycosylated protein) *105857-23-6*

Action and use

Tissue-type plasminogen activator; fibrinolytic.

Ph Eur

DEFINITION

Alteplase for injection is a sterile, freeze-dried preparation of alteplase, a tissue plasminogen activator produced by recombinant DNA technology. It has a potency of not less than 500 000 IU per milligram of protein.

Tissue plasminogen activator binds to fibrin clots and activates plasminogen, leading to the generation of plasmin and to the degradation of fibrin clots or blood coagulates.

Alteplase consists of 527 amino acids with a calculated relative molecular mass of 59 050 without consideration of the carbohydrate moieties attached at positions Asn 117, Asn 184 and Asn 448. The total relative molecular mass is approximately 65 000. Alteplase is cleaved by plasmin between amino-acids 275 and 276 into a two-chain form (A chain and B chain) that are connected by a disulphide bridge between Cys 264 and Cys 395. The single-chain form and the two-chain form show comparable fibrinolytic activity *in vitro*.

PRODUCTION

Alteplase is produced by recombinant DNA synthesis in cell culture; the fermentation takes place in serum-free medium.

The purification process is designed to remove efficiently potential impurities, such as antibiotics, DNA and protein contaminants derived both from the host cell and from the production medium, and potential viral contaminants.

If alteplase is stored in bulk form, stability (maintenance of potency) in the intended storage conditions must be demonstrated.

The production, purification and product consistency are checked by a number of analytical methods described below, carried out routinely as in-process controls.

Protein content

The protein concentration of alteplase solutions is determined by measuring the absorbance *(2.2.25)* of the protein solution at 280 nm and at 320 nm, using formulation buffer as the compensation liquid. If dilution of alteplase samples is necessary, the samples are diluted in formulation buffer. For the calculation of the alteplase concentration the absorbance value (A_{280} - A_{320}) is divided by the specific absorption coefficient for alteplase of 1.9.

Potency

The potency of alteplase is determined in an *in-vitro* clot-lysis assay as described under Assay. The specific activity of bulk alteplase is approximately 580 000 IU per milligram of alteplase.

N-terminal sequence

N-terminal sequencing is applied to determine the correct *N*-terminal sequence and to determine semiquantitatively additional cleavage sites in the alteplase molecule, for example at position AA 275-276 or at position AA 27-28. The *N*-terminal sequence must conform with the sequence of human tissue plasminogen activator.

Isoelectric focusing

The consistency in the microheterogeneity of glycosylation of the alteplase molecule can be demonstrated by isoelectric focusing (IEF). A complex banding pattern with ten major and several minor bands in the pH range 6.5-8.5 is observed. Denaturing conditions are applied to achieve a good separation of differently charged variants of alteplase. The broad charge distribution observed is due to a population of molecules, which differ in the fine structure of biantenary and triantenary complex-type carbohydrate residues, with different degrees of substitution with sialic acids. The banding pattern of alteplase test samples must be consistent with the pattern of alteplase reference standard.

Single-chain alteplase content

The alteplase produced by CHO (Chinese hamster ovary) cells in serum-free medium is predominantly single-chain alteplase. The single-chain form can be separated from the two-chain form by gel-permeation liquid chromatography under reducing conditions as described under Single-chain content (see Tests). The single-chain alteplase content in bulk samples must be higher than 60 per cent.

Tryptic-peptide mapping

The primary structure of the alteplase molecule is verified by tryptic-peptide mapping as described under Identification B. The reduced and carboxymethylated molecule is cleaved by trypsin into about fifty peptides, which are separated by reverse-phase liquid chromatography. A characteristic chromatogram (fingerprint) is obtained. The identity of the tryptic-peptide map of a given alteplase sample with the profile of a well-characterised reference standard is an indirect confirmation of the amino-acid sequence, because even single amino-acid exchanges in individual peptides can be detected by this sensitive technique. In addition, complex peaks of the glycopeptides can be isolated from the tryptic-peptide map and separated in a second dimension, either by reverse-phase liquid chromatography under modified conditions or by capillary electrophoresis. By this two-dimensional separation of glycopeptide variants, lot-to-lot consistency of the microheterogeneity of glycosylation can be demonstrated.

The tryptic-peptide map of alteplase samples must be consistent with the tryptic-peptide map of alteplase reference standard.

Monomer content

The monomer content of alteplase is measured by gel-permeation liquid chromatography under non-reduced conditions as described under Monomer content (see Tests). The monomer content of alteplase bulk samples must be higher than 95 per cent.

Type I/Type II alteplase content

CHO cells produce two glycosylation variants of alteplase. Type I alteplase contains one polymannose-type glycosylation at position Asn 117 and two complex-type glycosylation sites at positions Asn 184 and Asn 448. Type II alteplase is only glycosylated at positions Asn 117 and Asn 448.

The ratio of Type I/Type II alteplase is constant in the range of 45 to 65 per cent of Type I and 35 to 55 per cent of Type II. The content of alteplase Type I and Type II can be determined by a densitometric scan of SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) gel. Plasmin-treated samples of alteplase, which are reduced and carboxymethylated before loading on the gel, are separated into three bands: Type I alteplase A-chain (AA 1-275), Type II alteplase A-chain (AA 1-275) and alteplase B-chain (AA 276-527). The ratio of Type I/Type II alteplase is determined from a calibration curve, which is obtained by a densitometric scan of defined mixtures of purified Type I alteplase and Type II alteplase standards.

SDS-PAGE

SDS-PAGE (silver staining) is used to demonstrate purity of the alteplase bulk material and the integrity of the alteplase molecule. For alteplase bulk samples, no additional protein bands compared to reference standard or degradation products must occur in SDS-PAGE gels at a loading amount of 2.5 µg alteplase protein per lane and a limit of detection of 5 ng per protein (BSA) band.

Bacterial endotoxins *(2.6.14)*

Less than 1 IU per milligram of alteplase.

Sialic acids

Dialyse samples and alteplase reference standard against enzyme buffer (8.9 g/l *sodium chloride R*, 4.1 g/l *sodium acetate R*, pH 5.5) using a membrane with a cut-off point corresponding to a relative molecular mass of 10 000 for globular proteins. After dialysis, determine the protein concentration. Add 5 µl of calcium chloride solution (19.98 per cent *m/m calcium chloride R*) to 1 ml of protein solution. Add 10 milliunits of neuraminidase per milligram of protein. Incubate this solution at 37 °C for about 17 h.

Prepare standard dilutions between 1.56 mg/ml and 25.0 mg/ml from an *N-acetylneuraminic acid R* reference stock solution with a concentration of 50 mg/ml. Pipette 0.2 ml of the sample and of the protein reference standard in duplicate into reagent tubes. Pipette also 0.2 ml of the standard dilutions into reagent tubes. Add 0.25 ml of periodate reagent (5.4 g/l solution of *sodium periodate R* in a 1.25 per cent *V/V* solution of *sulphuric acid R*), mix and incubate for 30 min at 37 °C. Add 0.2 ml of arsenite reagent (20 g/l solution of *sodium arsenite R* in a 1.55 per cent *V/V* solution of *hydrochloric acid R*) and mix. A yellowish-brown colour develops and disappears. Add 2.0 ml of a 28.9 g/l solution of *thiobarbituric acid R* and mix. Heat the closed tubes in boiling water for 7.5 min and then cool them in an ice-bath for 5 min. Add 2.0 ml of a mixture of *butanol R* and *hydrochloric acid R* (95:5) and mix. Centrifuge the tubes at 3000 r/min for 3 min. Measure the absorbance of the butanol-HCl layer at 552 nm within 30 min using the butanol-HCl mixture as the compensation solution. Perform a linear-regression analysis for the *N*-acetylneuraminic acid standard. The molar content of *N*-acetylneuraminic acid for the samples and for alteplase reference standard is calculated from the calibration curve. The sialic acids content for the test samples must be within the range 70 to 130 per cent of alteplase reference standard, which contains about 3 moles of sialic acids per mole of alteplase.

Neutral sugars

Dilute alteplase samples and the reference standard in the assay buffer, containing 34.8 g/l of *arginine R*, 0.1 g/l of *polysorbate 80 R* and adjusted to pH 7.4 with *phosphoric acid R*, to a protein concentration of 50 µg/ml. Prepare the following concentrations of mannose in the same assay buffer for a calibration curve: 20, 30, 40, 50 and 60 µg/ml. Pipette 2 ml of alteplase samples and reference standard, as well as 2 ml of each mannose concentration in duplicate in reagent tubes. Add 50 µl of *phenol R*, followed by 5 ml of *sulphuric acid R* in each reagent tube. Incubate the mixture for 30 min at room temperature. Measure the absorbance at 492 nm for each tube. Read the content of neutral sugars from the mannose calibration curve. The neutral sugar content is expressed in moles of neutral sugar per mole of alteplase, taking into account the dilution factor for alteplase samples and reference standard and using a relative molecular mass of 180.2 for mannose and a relative molecular mass of 59 050 for the alteplase protein moiety. The neutral sugar content of the alteplase samples must be in the range of 70 to 130 per cent compared to alteplase reference standard, which contains about 12 moles of neutral sugar per mole of alteplase.

CHARACTERS

A white or slightly yellow powder or solid friable mass.

Reconstitute the preparation as stated on the label immediately before carrying out the Identification, Tests (except those for solubility and water) and Assay.

IDENTIFICATION

A. The assay serves also to identify the preparation.

B. Tryptic-peptide mapping. Examine by liquid chromatography *(2.2.29)*.

Test solution Dilute the preparation to be examined with *water R* to obtain a solution containing about 1 mg of alteplase per millilitre. Dialyse about 2.5 ml of the solution for at least

12 h into a solution containing 480 g/l of *urea R*, 44 g/l of *tris(hydroxymethyl)aminomethane R* and 1.5 g/l of *disodium edetate R* and adjusted to pH 8.6, using a membrane with a cut-off point corresponding to a relative molecular mass of 10 000 for globular proteins. Measure the volume of the solution, transfer it to a clean test-tube and add per millilitre 10 µl of a 156 g/l solution of *dithiothreitol R*. Allow to stand for 4 h, cool in iced water and add per millilitre of solution 25 µl of a freshly prepared 190 g/l solution of *iodoacetic acid R*. Allow to stand in the dark for 30 min. Add per millilitre 50 µl of dithiothreitol solution to stop the reaction. Dialyse for 24 h against an 8 g/l solution of *ammonium hydrogen carbonate R*. Add 1 part of *trypsin for peptide mapping R* to 100 parts of the protein and allow to stand for 6 h to 8 h. Repeat the addition of trypsin and allow to stand for a total of 24 h.

Reference solution Prepare as for the test solution using a suitable reference standard instead of the preparation to be examined.

The chromatographic procedure may be carried out using:

— a column 0.1 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm to 10 µm);

Mobile phase A A 8 g/l solution of *sodium dihydrogen phosphate R*, adjusted to pH 2.85 with *phosphoric acid R*, filtered and degassed;

Mobile phase B *Acetonitrile R* 75 per cent *V/V* in mobile phase A;

— as detector a spectrophotometer set at 210 nm.

Equilibrate the system with mobile phase A at a flow rate of 1 ml/min. After injection of the solution, increase the proportion of mobile phase B at a rate of 0.44 per cent per minute until the ratio of mobile phase A to mobile phase B is 60:40, then increase the proportion of mobile phase B at a rate of 1.33 per cent per minute until the ratio of mobile phase A to mobile phase B is 20:80 and then continue elution with this mixture for a further 10 min. Record the chromatogram for the reference solution: the test is not valid unless the resolution of peaks 6 (peptides 268-275) and 7 (peptides 1-7) is at least 1.5; $b_{0.5a}$ and $b_{0.5b}$ are not more than 0.4 min. Inject about 100 µl of the test solution and record the chromatogram. Verify the identity of the peaks by comparison with the chromatograms of the reference solution. There should not be any additional significant peaks or shoulders, a significant peak or shoulder being defined as one with an area response equal to or greater than 5 per cent of peak 19 (peptides 278-296); no significant peak is missing. A type chromatogram for identification of the peaks cited is given at the end of the monograph (see Figure 1170.-1).

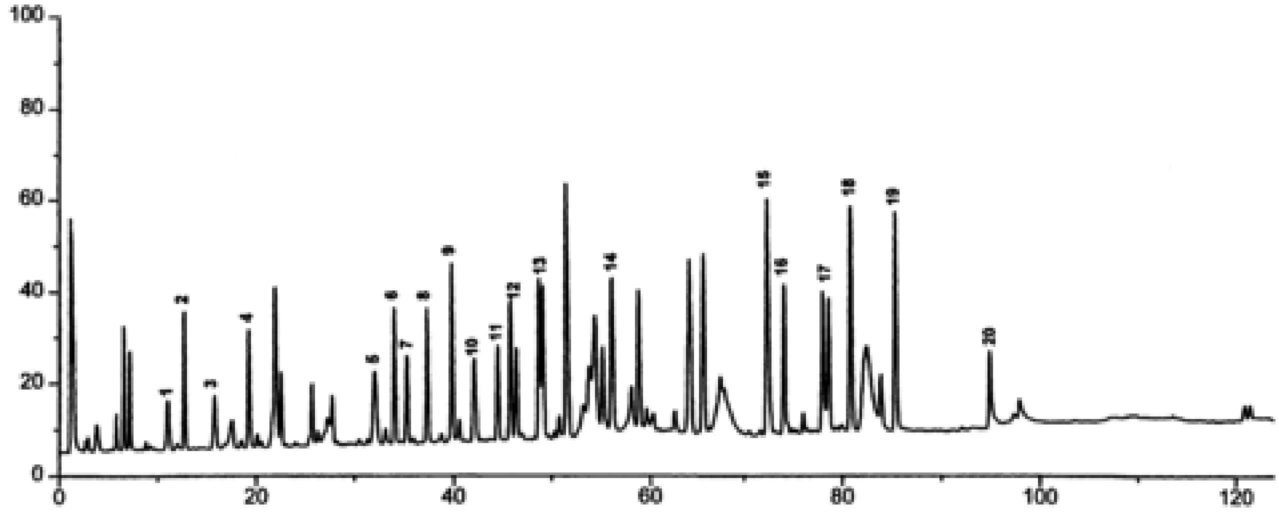


Figure 1170.-1. – *Chromatogram for tryptic-peptide mapping of alteplase*

TESTS

Appearance of solution

The reconstituted preparation is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

7.1 to 7.5.

Solubility

Add the volume of the liquid stated on the label. The preparation dissolves completely within 2 min at 20 °C to 25 °C.

Protein content

Prepare a solution of the substance to be examined with an accurately known concentration of about 1 g/l. Using a 34.8 g/l solution of *arginine R* adjusted to pH 7.3 with *phosphoric acid R*, dilute an accurately measured volume of the solution of the substance to be examined so that the absorbance measured at the maximum at about 280 nm is 0.5 to 1.0 (*test solution)*. Measure the absorbance *(2.2.25)* of the solution at the maximum at about 280 nm and at 320 nm using the arginine solution as the compensation liquid. Calculate the protein content in the portion of alteplase taken from the following expression:

$$\frac{V\,(A_{280} - A_{320})}{1.9}$$

in which *V* is the volume of arginine solution required to prepare the test solution, A_{280} is the absorbance at the maximum at about 280 nm and A_{320} is the absorbance at 320 nm.

Single-chain content

Examine by liquid chromatography*(2.2.29)*.

Test solution Dissolve the preparation to be examined in *water R* to obtain a solution containing about 1 mg of alteplase per millilitre. Place about 1 ml of the solution in a tube, add 3 ml of a 3 g/l solution of *dithiothreitol R* in the mobile phase, place a cap on the tube and heat at about 80 °C for 3 min to 5 min.

The chromatographic procedure may be carried out using:

— a column 0.6 m long and 7.5 mm in internal diameter packed with silica-based rigid, hydrophilic gel with spherical particles 10 µm to 13 µm in diameter, suitable for size-exclusion chromatography;

— as mobile phase at a flow rate of 0.5 ml/min a solution containing 30 g/l of *sodium dihydrogen phosphate R* and 1 g/l of *sodium dodecyl sulphate R*, adjusted to pH 6.8 with *dilute sodium hydroxide solution R*;

— as detector a spectrophotometer set at 214 nm.

Inject about 50 µl of the test solution and record the chromatogram. The chromatogram shows two major peaks corresponding to single-chain and two-chain alteplase. Calculate the relative amount of single-chain alteplase from the peak area values.

The test is not valid unless: the number of theoretical plates calculated on the basis of the single-chain alteplase peak is at least 1000. The content of single-chain alteplase is not less than 60 per cent of the total amount of alteplase-related substances found.

Monomer content

Examine by liquid chromatography *(2.2.29)*.

Test solution Reconstitute the preparation to be examined to obtain a solution containing about 1 mg per millilitre.

The chromatographic procedure may be carried out using:

— a column 0.6 m long and 7.5 mm in internal diameter packed with silica-based rigid, hydrophilic gel with spherical particles 10 µm to 13 µm in diameter, suitable for size-exclusion chromatography;

— as mobile phase at a flow rate of 0.5 ml/min a solution containing 30 g/l of *sodium dihydrogen phosphate R* and 1 g/l of *sodium dodecyl sulphate R*, adjusted to pH 6.8 with *dilute sodium hydroxide solution R*;

— as detector a spectrophotometer set at 214 nm.

Inject the test solution and record the chromatogram. The test is not valid unless the number of theoretical plates calculated for the alteplase monomer peak is at least 1000. Measure the response for all peaks, i.e. peaks corresponding to alteplase species of different molecular masses. Calculate the relative content of monomer from the area values of these peaks. The monomer content for alteplase must be at least 95 per cent.

Water *(2.5.12)*

Not more than 4.0 per cent, determined by the semi-micro determination of water.

Bacterial endotoxins *(2.6.14)*

Less than 1 IU per milligram of protein.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

The potency of alteplase is determined by comparing its ability to activate plasminogen to form plasmin with the same capacity of a reference preparation calibrated in International Units. The formation of plasmin is measured by the determination of the lysis time of a fibrin clot in given conditions.

The International Unit is the activity of a stated quantity of the International Standard of alteplase. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Solvent buffer A solution containing 1.38 g/l of *sodium dihydrogen phosphate monohydrate R*, 7.10 g/l of *anhydrous disodium hydrogen phosphate R*, 0.20 g/l of *sodium azide R* and 0.10 g/l of *polysorbate 80 R*.

Human thrombin solution A solution of *human thrombin R* containing 33 IU/ml in solvent buffer.

Human fibrinogen solution A 2 g/l solution of *fibrinogen R* in solvent buffer.

Human plasminogen solution A 1 g/l solution of *human plasminogen R* in solvent buffer.

Test solutions Using a solution of the substance to be examined containing 1 g/l, prepare serial dilutions using solvent buffer, for example 1:5000, 1:10 000, 1:20 000.

Reference solutions Using a solution of a suitable reference standard having an accurately known concentration of about 1 g/l (580 000 IU of alteplase per millilitre), prepare five serial dilutions using *water R* to obtain reference solutions having known concentrations in the range 9.0 IU/ml to 145 IU/ml.

To each of a set of labelled glass test-tubes, add 0.5 ml of human thrombin solution. Allocate each test and reference solution to a separate tube and add to each tube 0.5 ml of the solution allocated to it. To each of a second set of labelled glass tubes, add 20 µl of human plasminogen solution, and 1 ml of human fibrinogen solution, mix and store on ice. Beginning with the reference/thrombin mixture containing the lowest number of International Units per millilitre, record the time and separately add 200 µl of each of the thrombin mixtures to the test tubes containing the plasminogen-fibrinogen mixture. Using a vortex mixture, intermittently mix the contents of each tube for a total of 15 s and carefully place in a rack in a circulating water-bath at 37 °C. A visibly turbid clot forms within 30 s and bubbles subsequently form within the clot. Record the clot-lysis time as the time between the first addition of alteplase solution and the moment when the last bubble rises to the surface. Using a least-squares fit, determine the equation of the line using the logarithms of the concentrations of the reference preparation in International Units per millilitre versus the logarithms of the values of their clot-lysis times in seconds, according to the following equation:

$$\log t = a + b\,(\log U_s)$$

in which t is the clot-lysis time, U_S the activity in International Units per millilitre of the reference preparation, b is the slope and a the y-intercept of the line. The test is not valid unless the correlation coefficient is - 0.9900 to - 1.0000. From the line equation and the clot-lysis time for the test solution, calculate the logarithm of the activity U_A from the following equation:

$$\log U_A = \frac{[(\log t) - a]}{b}$$

Calculate the alteplase activity in International Units per millilitre from the following expression:

$$D \times U_A$$

in which D is the dilution factor for the test solution. Calculate the specific activity in the portion of the substance to be examined from the following expression:

$$\frac{U_A}{P}$$

in which P is the concentration of protein obtained in the test for protein content.

The estimated potency is not less than 90 per cent and not more than 110 per cent of the stated potency.

STORAGE

Store in a colourless, glass container, under vacuum or under an inert gas, protected from light, at a temperature of 2 °C to 30 °C.

LABELLING

The label states:

— the number of International Units per container;

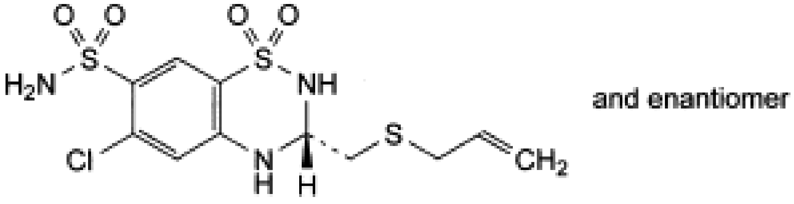
— the amount of protein per container.

Ph Eur

Altizide

General Notices

(Ph Eur monograph 2185)



$C_{11}H_{14}ClN_3O_4S_3$ 383.9 *5588-16-9*

Ph Eur

DEFINITION

(3*RS*)-6-Chloro-3-[(prop-2-enylsulphanyl)methyl]-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in methanol, practically insoluble in dichloromethane.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison altizide CRS.

If the spectra obtained show differences, dissolve 50 mg of the substance to be examined and 50 mg of the reference substance separately in 2 ml of *acetone R* and evaporate the solvent. Precipitate by adding 1 ml of *methylene chloride R*. Evaporate to dryness and record new spectra using the residues.

TESTS

Impurity B

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.200 g of the substance to be examined in *acetone R* and dilute to 2.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *altizide impurity B CRS* in *acetone R* and dilute to 25.0 ml with the same solvent.

Reference solution (b) To 1.0 ml of reference solution (a) add 1.0 ml of the test solution.

Reference solution (c) Dilute 5.0 ml of reference solution (a) to 10.0 ml with *acetone R*.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *acetone R, methylene chloride R*

(25:75 *V/V*).

Application 10 µl of the test solution and reference solutions (b) and (c).

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a mixture of equal volumes of a 10 g/l solution of *potassium permanganate R* and a 50 g/l solution of *sodium carbonate R*, prepared immediately before use. Allow to stand for 30 min and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Limit Any spot due to impurity B is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use, except reference solution (b)*.

Test solution Dissolve 50 mg of the substance to be examined in 5 ml of *acetonitrile R* and dilute to 25 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) In order to produce impurity A in situ, dissolve 50 mg of the substance to be examined in 5 ml of *acetonitrile R* and dilute to 25 ml with *water R*. Allow to stand for 30 min.

Reference solution (c) Dissolve 4 mg of *furosemide CRS* in 2 ml of *acetonitrile R*, add 2 ml of the test solution and dilute to 100 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R, water R* previously adjusted to pH 2.0 with *perchloric acid R* (25:75 *V/V*).

Flow rate 0.7 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 5 µl.

Run time Twice the retention time of altizide.

Relative retention With reference to altizide (retention time = about 25 min): impurity A = about 0.15; furosemide = about 1.05.

System suitability Reference solution (c):

— *resolution*: minimum 1.0 between the peaks due to altizide and furosemide.

Limits:

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.32)*

Maximum 0.5 per cent, determined on 50.0 mg.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances, with the following modifications.

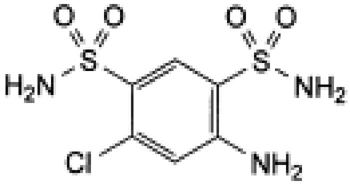
Test solution Dissolve 25.0 mg of the substance to be examined in 2 ml of *acetonitrile R* and dilute to 25.0 ml with the mobile phase.

Reference solution Dissolve 25.0 mg of *altizide CRS* in 2 ml of *acetonitrile R* and dilute to 25.0 ml with the mobile phase.

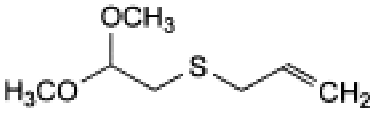
Calculate the percentage content of $C_{11}H_{14}ClN_3O_4S_3$ from the declared content of *altizide CRS*.

IMPURITIES

Specified impurities *A, B.*



A. 4-amino-6-chlorobenzene-1,3-disulphonamide,



B. 3-[(2,2-dimethoxyethyl)sulphanyl]prop-1-ene.

Ph Eur

Alum

General Notices

Potash Alum; Aluminium Potassium Sulphate

(Ph Eur monograph 0006)

$AlK(SO_4)_2,12H_2O$ 474.4 *7784-24-9*

Action and use

Astringent.

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent of $AlK(SO_4)_2,12H_2O$.

CHARACTERS

Appearance

Granular powder or colourless, transparent, crystalline masses.

Solubility

Freely soluble in water, very soluble in boiling water, soluble in glycerol, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives the reactions of sulphates *(2.3.1)*.

B. Solution S gives the reaction of aluminium *(2.3.1)*.

C. Shake 10 ml of solution S with 0.5 g of *sodium bicarbonate R* and filter. The filtrate gives reaction (a) of potassium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

3.0 to 3.5.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Ammonium *(2.4.1)*

Maximum 0.2 per cent.

To 1 ml of solution S add 4 ml of *water R*. Dilute 0.5 ml of this solution to 14 ml with *water R*.

Iron *(2.4.9)*

Maximum 100 ppm.

Dilute 2 ml of solution S to 10 ml with *water R*. Use in this test 0.3 ml of *thioglycollic acid R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 0.900 g in 20 ml of *water R* and carry out the complexometric titration of aluminium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 47.44 mg of $AlK(SO_4)_2,12H_2O$.

Ph Eur

Aluminium Chloride Hexahydrate

General Notices

(Ph Eur monograph 0971)

$AlCl_3,6H_2O$ 241.4 *7784-13-6*

Action and use

Astringent.

Preparation

Aluminium Chloride Solution

Ph Eur

DEFINITION

Content

95.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or slightly yellow, crystalline powder or colourless crystals, deliquescent.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), soluble in glycerol.

IDENTIFICATION

A. Dilute 0.1 ml of solution S2 (see Tests) to 2 ml with *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

B. Dilute 0.3 ml of solution S2 to 2 ml with *water R*. The solution gives the reaction of aluminium *(2.3.1)*.

TESTS

Solution S1

Dissolve 10.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Solution S2

Dilute 50 ml of solution S1 to 100 ml with *water R*.

Appearance of solution

Solution S2 is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

Sulphates *(2.4.13)*

Maximum 100 ppm, determined on solution S1.

Iron *(2.4.9)*

Maximum 10 ppm, determined on solution S1.

Alkali and alkaline-earth metals

Maximum 0.5 per cent.

To 20 ml of solution S2 add 100 ml of *water R* and heat to boiling. To the hot solution add 0.2 ml of *methyl red solution R*. Add *dilute ammonia R1* until the colour of the indicator changes to yellow and dilute to 150 ml with *water R*. Heat to boiling and filter. Evaporate 75 ml of the filtrate to dryness on a water-bath and ignite to constant mass. The residue weighs a maximum of 2.5 mg.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S1 complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Water *(2.5.12)*

42.0 per cent to 48.0 per cent, determined on 50.0 mg.

ASSAY

Dissolve 0.500 g in 25.0 ml of *water R*. Carry out the complexometric titration of aluminium *(2.5.11)*. Titrate with *0.1 M zinc sulphate* until the colour of the indicator changes from greyish-green to pink. Carry out a blank titration.

1 ml of *0.1 M sodium edetate* is equivalent to 24.14 mg of $AlCl_3,6H_2O$.

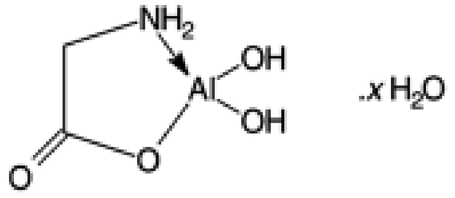
STORAGE

In an airtight container.

Ph Eur

Aluminium Glycinate

General Notices



$C_2H_6AlNO_4,xH_2O$ 135.1   *41354-48-7*

Action and use

Antacid.

DEFINITION

Aluminium Glycinate is a basic aluminium monoglycinate, partly hydrated. It contains not less than 34.5% and not more than 38.5% of Al_2O_3 and not less than 9.9% and not more than 10.8% of N, both calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Practically insoluble in *water* and in organic solvents. It dissolves in dilute mineral acids and in aqueous solutions of the alkali hydroxides.

IDENTIFICATION

A. Add 0.1 g to 10 ml of a solution prepared by dissolving 0.84 g of *citric acid* in 8 ml of *1M sodium hydroxide* and diluting to 20 ml with *water*. Add 0.5 ml of a 0.1% w/v solution of *ninhydrin* in *methanol* and warm. A purple colour is produced.

B. Suspend 1 g in 25 ml of *0.5M hydrochloric acid* and heat gently until a clear solution is produced. Reserve half of the solution. To 2 ml of the solution add 0.15 ml of *liquefied phenol*, shake and add carefully without shaking 5 ml of *dilute sodium hypochlorite solution*. A blue colour is produced.

C. The solution reserved in test B yields the reaction characteristic of *aluminium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a suspension of 1 g in 25 ml of *carbon dioxide-free water*, 6.5 to 7.5, Appendix V L.

Neutralising capacity

Shake 0.2 g vigorously with 25 ml of *0.1M hydrochloric acid* for 5 minutes and allow to stand for 5 minutes. The pH of the mixture is greater than 3.0, Appendix V L.

Arsenic

Dissolve 2.0 g in 18 ml of *brominated hydrochloric acid* and 32 ml of *water*. 25 ml of the resulting solution complies with the *limit test for arsenic*, Appendix VII (1 ppm).

Heavy metals

Dissolve 1.5 g in 20 ml of *2M hydrochloric acid* and 10 ml of *water*, add 0.5 ml of *nitric acid* and boil for about 30 seconds. Cool, add 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with two 10 ml quantities of a mixture of equal parts of *isoamyl alcohol* and *ether*. To the aqueous layer add 2 g of *citric acid*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (20 ppm).

Mercuric salts

Dissolve 2.0 g in 10 ml of *1M sulphuric acid*, transfer to a separating funnel with the aid of *water*, dilute to about 50 ml with *water* and add 50 ml of *0.5M sulphuric acid*. Add 100 ml of *water*, 2 g of *hydroxylamine hydrochloride*, 1 ml of *0.05M disodium edetate* and 1 ml of *glacial acetic acid*. Add 5 ml of *chloroform*, shake, allow to separate and discard the chloroform layer. Titrate the aqueous layer with a solution of *dithizone* in *chloroform* containing 8 µg per ml until the chloroform layer remains green. After each addition, shake vigorously, allow the layers to separate and discard the chloroform layer. Repeat the operation using a solution prepared by diluting 1 ml of *mercury standard solution (5 ppm Hg)* to 100 ml with *0.5M sulphuric acid* and beginning at the words 'Add 100 ml of *water* …'. The volume of the dithizone solution required by the substance being examined does not exceed that required by the mercury standard solution.

Chloride

Dissolve 1.0 g in 10 ml of *2M nitric acid* and dilute to 100 ml with *water*. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Loss on drying

When dried to constant weight at 130°, loses not more than 12.0% of its weight. Use 1 g.

ASSAY

For Al_2O_3

Dissolve 0.25 g in a mixture of 3 ml of *1M hydrochloric acid* and 50 ml of *water*, add 50 ml of *0.05M disodium edetate VS* and neutralise with *1M sodium hydroxide* using *methyl red solution* as indicator. Heat the solution to boiling, allow to stand for 10 minutes on a water bath, cool rapidly, add about 50 mg of *xylenol orange triturate* and 5 g of *hexamine* and titrate the excess of disodium edetate with *0.05M lead nitrate VS* until the solution becomes red. Each ml of *0.05M disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

Aluminium Magnesium Silicate

General Notices

(Ph Eur monograph 1388)

12511-31-8

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of particles with colloidal particle size of montmorillonite and saponite, free from grit and non-swellable ore.

Content:

— *aluminium* (Al; A_r 26.98): 95.0 per cent to 105.0 per cent of the value stated on the label;

— *magnesium* (Mg; A_r 24.30): 95.0 per cent to 105.0 per cent of the value stated on the label.

CHARACTERS

Appearance

Almost white powder, granules or plates.

Solubility

Practically insoluble in water and in organic solvents.

It swells in water to produce a colloidal dispersion.

IDENTIFICATION

A. Fuse 1 g with 2 g of *anhydrous sodium carbonate R*. Warm the residue with *water R* and filter. Acidify the filtrate with *hydrochloric acid R* and evaporate to dryness on a water-bath. 0.25 g of the residue gives the reaction of silicates *(2.3.1)*.

B. Dissolve the remainder of the residue obtained in identification test A in a mixture of 5 ml of *dilute hydrochloric acid R* and 10 ml of *water R*. Filter and add *ammonium chloride buffer solution pH 10.0 R*. A white, gelatinous precipitate is formed. Centrifuge and keep the supernatant for identification C. Dissolve the remaining precipitate in *dilute hydrochloric acid R*. The solution gives the reaction of aluminium *(2.3.1)*.

C. The supernatant liquid obtained after centrifugation in identification test B gives the reaction of magnesium *(2.3.1)*.

TESTS

pH *(2.2.3)*

9.0 to 10.0.

Disperse 5.0 g in 100 ml of *carbon dioxide-free water R*.

Arsenic *(2.4.2, Method A)*

Maximum 3 ppm.

Transfer 16.6 g to a 250 ml beaker containing 100 ml of *dilute hydrochloric acid R*. Mix, cover with a watch glass and boil gently, with occasional stirring, for 15 min. Allow the insoluble matter to settle and decant the supernatant liquid through a rapid-flow filter paper into a 250 ml volumetric flask, retaining as much sediment as possible in the beaker. To the residue in the beaker add 25 ml of hot *dilute hydrochloric acid R*, stir, heat to boiling, allow the insoluble matter to settle and decant the supernatant liquid through the filter into the volumetric flask. Repeat the extraction with 4 additional quantities, each of 25 ml, of hot *dilute hydrochloric acid R*, decanting each supernatant liquid through the filter into the volumetric flask. At the last extraction, transfer as much of the insoluble matter as possible onto the filter. Allow the combined filtrates to cool to room temperature and dilute to 250.0 ml with *dilute hydrochloric acid R*. Dilute 5.0 ml of this solution to 25.0 ml with *dilute hydrochloric acid R*.

Lead

Maximum 15.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Transfer 10.0 g to a 250 ml beaker containing 100 ml of *dilute hydrochloric acid R*. Mix, cover with a watch glass and boil for 15 min. Allow to cool to room temperature and allow the insoluble matter to settle. Decant the supernatant liquid through a rapid-flow filter paper into a 400 ml beaker. To the insoluble matter in the 250 ml beaker add 25 ml of hot *water R*. Stir, allow the insoluble matter to settle and decant the supernatant liquid through the filter into the 400 ml beaker. Repeat the extraction with 2 additional quantities, each of 25 ml, of *water R*, decanting each time the supernatant liquid through the filter into the 400 ml beaker. Wash the filter with 25 ml of hot *water R*, collecting this filtrate in the 400 ml beaker. Concentrate the combined filtrates to about 20 ml by gently boiling. If a precipitate appears, add about 0.1 ml of *nitric acid R*, heat to boiling and allow to cool to room temperature. Filter the concentrated extracts through a rapid-flow filter paper into a 50 ml volumetric flask. Transfer the remaining contents of the 400 ml beaker through the filter paper and into the flask with *water R*. Dilute this solution to 50.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *lead standard solution (10 ppm Pb) R*, diluted as necessary with *water R*.

Source Lead hollow-cathode lamp.

Wavelength 217 nm.

Atomisation device Oxidising air-acetylene flame.

Loss on drying *(2.2.32)*

Maximum 8.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli (2.6.13)*.

ASSAY

Aluminium

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution In a platinum crucible mix 0.200 g with 1.0 g of *lithium metaborate R*. Heat slowly at first and ignite at 1000-1200 °C for 15 min. Allow to cool, then place the crucible in a 100 ml beaker containing 25 ml of *dilute nitric acid R* and add an additional 50 ml of *dilute nitric acid R*, filling and submerging the crucible. Place a polytetrafluoroethylene-coated magnetic stirring bar in the crucible and stir gently with a magnetic stirrer until dissolution is complete. Pour the contents into a 250 ml beaker and remove the crucible. Warm the solution and transfer through a rapid-flow filter paper into a 250 ml volumetric flask, wash the filter and beaker with *water R* and dilute to 250.0 ml with *water R* (solution A). To 20.0 ml of solution A add 20 ml of a 10 g/l solution of *sodium chloride R* and dilute to 100.0 ml with *water R*.

Reference solutions Dissolve, with gentle heating, 1.000 g of *aluminium R* in a mixture of 10 ml of *hydrochloric acid R* and 10 ml of *water R*. Allow to cool, then dilute to 1000.0 ml with *water R* (1 mg of aluminium per millilitre). Into 3 identical volumetric flasks, each containing 0.20 g of *sodium chloride R*, introduce 2.0 ml, 5.0 ml and 10.0 ml of this solution respectively, and dilute to 100.0 ml with *water R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309 nm.

Atomisation device Oxidising acetylene-nitrous oxide flame.

Magnesium

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dilute 25.0 ml of solution A, prepared in the assay for aluminium, to 50.0 ml with *water R*. To 5.0 ml of this solution add 20.0 ml of *lanthanum nitrate solution R* and dilute to 100.0 ml with *water R*.

Reference solutions Place 1.000 g of *magnesium R* in a 250 ml beaker containing 20 ml of *water R* and carefully add 20 ml of *hydrochloric acid R*, warming if necessary to dissolve. Transfer the solution to a volumetric flask and dilute to 1000.0 ml with *water R* (1 mg of magnesium per millilitre). Dilute 5.0 ml of this solution to 250.0 ml with *water R*. Into 4 identical volumetric flasks, introduce 5.0 ml, 10.0 ml, 15.0 ml and 20.0 ml of the solution respectively. To each flask add 20.0 ml of *lanthanum nitrate solution R* and dilute to 100.0 ml with *water R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285 nm.

Atomisation device Reducing air-acetylene flame.

LABELLING

The label states the content of aluminium and magnesium.

Ph Eur

Aluminium Phosphate Gel

General Notices

(Ph Eur monograph 2166)

Al(OH)x(PO_4)y 122.0 (anhydrous) *7784-30-7 (anhydrous)*

Action and use

Antacid; vaccine adjuvant.

Ph Eur

DEFINITION

Hydrated $AlPO_4$ in gel form.

Content

19.0 per cent to 21.0 per cent of $AlPO_4$.

CHARACTERS

Appearance

Gel.

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute solutions of mineral acids.

IDENTIFICATION

A. Solution S (see Tests) gives reaction (b) of phosphates *(2.3.1)*.

B. Solution S gives the reaction of aluminium *(2.3.1)*.

C. It complies with the assay.

TESTS

Solution S

Dissolve 2.00 g in *dilute hydrochloric acid R* and dilute to 100 ml with the same acid.

pH *(2.2.3)*

6.0 to 8.0.

Peroxides

Maximum 150 ppm, expressed as hydrogen peroxide.

Test solution Dissolve with heating 1.0 g of the substance to be examined in 5 ml of *dilute hydrochloric acid R*, then add 5 ml of *water R* and 2 ml of *divanadium pentoxide solution in sulphuric acid R*.

Reference solution Dilute 1.0 ml of *dilute hydrogen peroxide solution R* to 200.0 ml with *water R*. To 1 ml of this solution add 9 ml of *water R* and 2 ml of *divanadium pentoxide solution in sulphuric acid R*.

The test solution is not more intensely coloured than the reference solution.

Chlorides ***(2.4.4)***

Maximum 500 ppm.

Dissolve 1.3 g in 5 ml of *dilute nitric acid R* and dilute to 200 ml with *water R*.

Soluble phosphates

Maximum 0.5 per cent, expressed as PO_4.

Test solution Centrifuge 10.0 g until a clear supernatant is obtained. To 2.00 ml of the supernatant add 20.0 ml of a 10.3 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with *water R*. To 10.0 ml of this solution add 10.0 ml of *nitro-molybdovanadic reagent R* and dilute to 50.0 ml with *water R*. Allow to stand protected from light for 15 min.

Reference solution Add 10.0 ml of *nitro-molybdovanadic reagent R* to 10.0 ml of a 143 mg/l solution of *potassium dihydrogen phosphate R* and dilute to 50.0 ml with *water R*. Allow to stand protected from light for 15 min.

Measure the absorbances *(2.2.25)* of the 2 solutions at 400 nm. The absorbance of the test solution is not greater than that of the reference solution.

Sulphates ***(2.4.13)***

Maximum 0.2 per cent.

Dilute 25 ml of solution S to 100 ml with *distilled water R*.

Soluble aluminium

Maximum 50 ppm.

To 16.0 g add 50 ml of *water R*. Heat to boiling for 5 min. Cool and centrifuge. Separate the supernatant. Wash the residue with 20 ml of *water R* and centrifuge. Separate the supernatant and add to the first supernatant. To the combined supernatants add 5 ml of *hydrochloric acid R* and 20 ml of *water R*. Introduce all of this solution into a 500 ml conical flask and carry out the complexometric titration of aluminium *(2.5.11)* using *0.01 M sodium edetate*.

Arsenic ***(2.4.2, Method A)***

Maximum 1 ppm, determined on 1.0 g.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 4.0 g in *dilute hydrochloric acid R* and dilute to 20 ml with the same acid. 12 ml of the solution complies with test A. Prepare the reference solution using l*ead standard solution (2 ppm Pb) R*.

Acid neutralising capacity

Add 2.0 g to 30 ml of *0.1 M hydrochloric acid R* heated to 37 °C and maintain at 37 °C while shaking. Determine the pH after 15 min. The pH *(2.2.3)* of the mixture is 2.0 to 2.5.

Residue on ignition

19.0 per cent to 23.0 per cent.

Heat 0.500 g at 50 °C for 5 hours, then ignite at 500 ± 50 °C until constant mass.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count. It complies with the test for enterobacteria and certain other gram-negative bacteria and with the test for *Escherichia coli (2.6.13)*.

ASSAY

Dissolve with heating 0.300 g in 5 ml of *dilute hydrochloric acid R*. Add 45 ml of *water R*, 10.0 ml of *0.1 M sodium edetate* and 30 ml of a mixture of equal volumes of *ammonium acetate solution R* and *dilute acetic acid R*. Heat to boiling and maintain boiling for 3 min. Cool, then add 25 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M zinc sulphate*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M zinc sulphate* is equivalent to 12.2 mg of $AlPO_4$.

STORAGE

In an airtight container.

Ph Eur

Aluminium Powder

General Notices

Al 26.98 *7429-90-5*

Action and use

Topical protective.

Preparation

Compound Aluminium Paste

DEFINITION

Aluminium Powder consists mainly of metallic aluminium in the form of very small flakes, usually with an appreciable proportion of aluminium oxide; it is lubricated with stearic acid to prevent oxidation. It contains not less than 86.0% of Al, calculated with reference to the substance freed from lubricant and volatile matter.

CHARACTERISTICS

A silvery grey powder.

Practically insoluble in *water* and in *ethanol (96%)*. It dissolves in dilute acids and in aqueous solutions of alkali hydroxides, with the evolution of hydrogen.

IDENTIFICATION

A solution in *2M hydrochloric acid* yields the reaction characteristic of *aluminium salts*, Appendix VI.

TESTS

Surface-covering power

Not less than 4000 cm^2 per g when determined by the following method. Fill with *water* a shallow trough measuring approximately 60 cm × 12 cm × 1.5 cm, fitted with a movable partition so constructed that it is a sliding fit and can be used to divide the trough into two rectangular areas. Place the movable partition near one end and sprinkle 50 mg of the substance being examined on the surface of the liquid confined in the smaller area. Using a glass rod, spread the powder evenly over the liquid surface until an unbroken film covers the entire surface. Move the partition so as to increase the area confined and again spread the powder to cover the increased surface. Continue this process and determine the maximum unbroken surface area obtained. The surface-covering power is the area covered per g of the powder at the breaking point of the film.

Iron

Dissolve 10 mg in 20 ml of *2M hydrochloric acid* and dilute to 100 ml with *water*. 10 ml of the resulting solution complies with the *limit test for iron*, Appendix VII (1.0%).

Lead

Use two solutions prepared in the following manner. For solution (1) boil 0.40 g with 20 ml of 2M *hydrochloric acid* and 10 ml of *water* until effervescence ceases, add 0.5 ml of *nitric acid*, boil for 30 seconds and cool; add 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate*, extract with three 10 ml quantities of a mixture of equal volumes of *amyl alcohol* and *ether*, discard the extracts and add 2 g of *citric acid*. For solution (2) dissolve 2 g of *citric acid* in 10 ml of 2M *hydrochloric acid* and add 4 ml of *lead standard solution (10 ppm Pb)*. Make solutions (1) and (2) alkaline with 5M *ammonia* and to each add 1 ml of *potassium cyanide solution PbT*. The solutions should be not more than faintly opalescent. If the colours of the solutions differ, equalise by the addition of about 0.2 ml of a highly diluted solution of burnt sugar or other non-reactive substance. Dilute each solution to 50 ml with *water*, add 0.1 ml of a 10% w/v solution of *sodium sulphide* to each and mix thoroughly. The colour produced in solution (1) is not more intense than that produced in solution (2), when viewed against a white background (100 ppm).

Other metals

Dissolve 2 g in 40 ml of 2M *hydrochloric acid*. Dilute 20 ml of the solution to 100 ml with *water*, make alkaline to *litmus paper* by the addition of 5M *ammonia*, boil and filter. Evaporate the filtrate to dryness, add 0.05 ml of *sulphuric acid* and ignite. The residue weighs not more than 2 mg.

Lubricant

To 2 g add 100 ml of hot *water*, cover and add, drop wise, sufficient of a mixture of equal volumes of *hydrochloric acid* and *water* to dissolve the metal almost completely. Heat to complete dissolution, cool, filter through a hardened filter paper and wash the vessel and filter paper thoroughly with *water*; dry both the vessel and paper at room temperature. Extract the paper with three 100-ml quantities of boiling, freshly distilled *acetone*, using the original vessel to contain the solvent and then wash the paper with five 10-ml quantities of freshly distilled *acetone*. Evaporate the combined filtrate and washings to dryness using a rotary evaporator. The residue, after drying at 105° for 30 minutes and allowing to cool, weighs 10 to 60 mg.

When the basin containing the residue is floated in a beaker of water suitably stirred and heated, the residue melts between 40° and 60°. The residue is almost completely soluble, with effervescence, in hot *dilute sodium carbonate solution*.

Volatile matter

When heated to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

Transfer 0.2 g, previously freed from lubricant by successive washing with *acetone* and drying, to a three-necked 500 ml flask fitted with a 150 ml dropping funnel, an inlet tube connected to a cylinder of *carbon dioxide* and an outlet tube dipping into a water trap. Add 60 ml of *water* and disperse the substance being examined; replace the air by *carbon dioxide* and add 100 ml of a solution containing 56 g of *ammonium iron(III) sulphate* and 7.5 ml of *sulphuric acid* in *water*. While maintaining an atmosphere of *carbon dioxide* in the flask, heat to boiling, boil for 5 minutes after the sample has dissolved, cool rapidly to 20° and dilute to 250 ml with *water*. To 50 ml add 15 ml of *orthophosphoric acid* and titrate with 0.02M *potassium permanganate VS*. Each ml of 0.02M *potassium permanganate VS* is equivalent to 0.8994 mg of Al.

Aluminium Sulphate

General Notices

(Ph Eur monograph 0165)

Preparation

Aluminium Acetate Ear Drops

Ph Eur

DEFINITION

Content

51.0 per cent to 59.0 per cent of $Al_2(SO_4)_3$.

It contains a variable quantity of water of crystallisation.

CHARACTERS

Appearance

Colourless, lustrous crystals or crystalline masses.

Solubility

Soluble in cold water, freely soluble in hot water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of sulphates *(2.3.1)*.

B. Solution S gives the reaction of aluminium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* and is colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

2.5 to 4.0.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Alkali and alkaline-earth metals

Maximum 0.4 per cent.

To 20 ml of solution S add 100 ml of *water R*, heat and add 0.1 ml of *methyl red solution R*. Add *dilute ammonia R1* until the colour of the indicator changes to yellow. Dilute to 150 ml with *water R*, heat to boiling and filter. Evaporate 75 ml of the filtrate to dryness on a water-bath and ignite. The residue weighs a maximum of 2 mg.

Ammonium *(2.4.1)*

Maximum 500 ppm.

Dilute 0.4 ml of solution S to 14 ml with *water R*.

Iron *(2.4.9)*

Maximum 100 ppm.

Dilute 2 ml of solution S to 10 ml with *water R*. Use 0.3 ml of *thioglycollic acid R* in this test.

Heavy metals *(2.4.8)*

Maximum 50 ppm.

Dilute 8 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 0.500 g in 20 ml of *water R*. Carry out the complexometric titration of aluminium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 17.11 mg of $Al_2(SO_4)_3$.

STORAGE

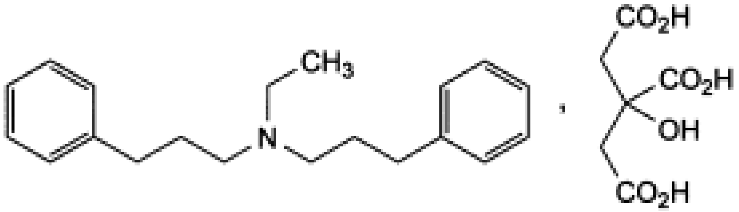
In an airtight container.

Ph Eur

Alverine Citrate

General Notices

(Ph Eur monograph 2156)



$C_{20}H_{27}N,C_6H_8O_7$ 473.6 *5560-59-8*

Action and use

Smooth muscle relaxant; antispasmodic.

Preparation

Alverine Capsules

Ph Eur

DEFINITION

N-Ethyl-3-phenyl-*N*-(3-phenylpropyl)propan-1-amine dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water and in methylene chloride, sparingly soluble in ethanol (96 per cent).

mp

About 104 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *alverine citrate CRS.*

TESTS

pH *(2.2.3)*

3.5 to 4.5.

Dissolve 0.250 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure. *Use freshly prepared solutions.*

Test solution Dissolve 0.250 g of the substance to be examined in *water R* and dilute to 20 ml with the same solvent. Add 2 ml of *concentrated ammonia R* and shake with 3 quantities, each of 15 ml, of *methylene chloride R*. To the combined lower layers add *anhydrous sodium sulphate R*, shake, filter, and evaporate the filtrate at a temperature not exceeding 30 °C, using a rotary evaporator. Take up the residue with *methylene chloride R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *alverine impurity D CRS* (impurity D citrate) in 5 ml of *water R*, add 1 ml of *concentrated ammonia R* and shake with 3 quantities, each of 5 ml, of *methylene chloride R*. To the combined lower layers add *anhydrous sodium sulphate R*, shake, filter, and evaporate the filtrate at a temperature not exceeding 30 °C, using a rotary evaporator. Take up the residue with *methylene chloride R*, add 0.2 ml of the test solution and dilute to 2 ml with *methylene chloride R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methylene chloride R*. Dilute 1.0 ml of this solution to 20.0 ml with *methylene chloride R*.

Reference solution (c) Dissolve the contents of a vial of *alverine for peak identification* CRS (containing impurities C and E) in 1 ml of *methylene chloride R*.

Column:

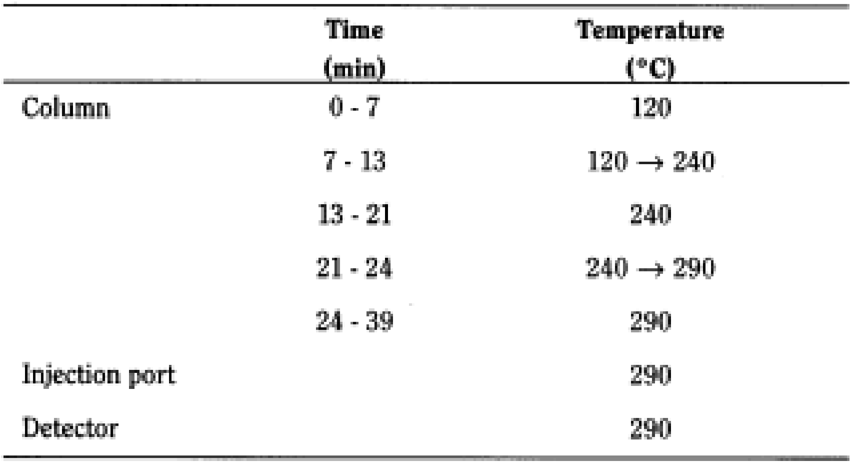
- *material*: fused silica;
- *size:* l = 25 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.45 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.2 ml/min.

Split ratio 1:11.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 7	120
	7 - 13	120 → 240
	13 - 21	240
	21 - 24	240 → 290
	24 - 39	290
Injection port		290
Detector		290

Detection Flame ionisation.

Injection 1 µl.

Identification of impurities Use the chromatogram supplied with *alverine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C and E.

Relative retention With reference to alverine (retention time = about 16 min): impurity A = about 0.28; impurity B = about 0.29; impurity C = about 0.46; impurity D = about 0.97; impurity E = about 1.7.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to impurity D and alverine.

Limits:

— *impurities A, B*: for each impurity, maximum 0.1 per cent;

— *impurity C*: maximum 0.2 per cent;

— *impurities D, E*: for each impurity, maximum 0.3 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 1.0 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

0.5 g complies with test G. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 80 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.375 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

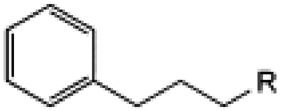
1 ml of *0.1 M perchloric acid* is equivalent to 47.36 mg of $C_{26}H_{35}NO_7$.

STORAGE

Protected from light.

IMPURITIES

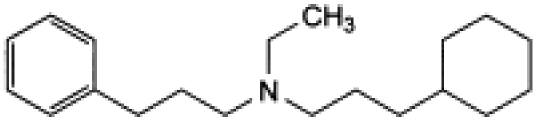
Specified impurities *A, B, C, D, E.*



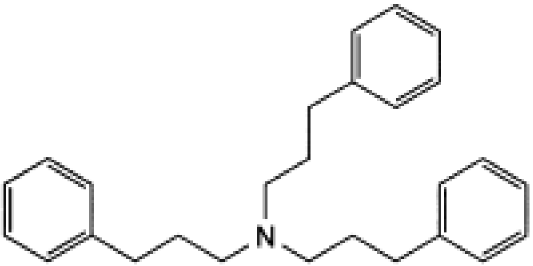
A. R = Cl: 1-chloro-3-phenylpropane,

B. R = OH: 3-phenylpropan-1-ol,

C. R = NH-C_2H_5: *N*-ethyl-3-phenylpropan-1-amine,



D. *N*-(3-cyclohexylpropyl)-*N*-ethyl-3-phenylpropan-1-amine,



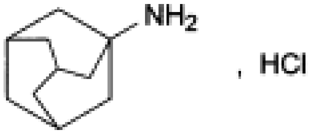
E. 3-phenyl-*N*,*N*-bis(3-phenylpropyl)propan-1-amine.

Ph Eur

Amantadine Hydrochloride

General Notices

(Ph Eur monograph 0463)



$C_{10}H_{17}N,HCl$ 187.7 *665-67-7*

Action and use

Viral replication inhibitor (influenza A); dopamine receptor agonist; treatment of influenza and Parkinson's disease.

Preparations

Amantadine Capsules

Amantadine Oral Solution

Ph Eur

DEFINITION

Tricyclo[3.3.1.1^{3,7}]decan-1-amine hydrochloride.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in ethanol (96 per cent).

It sublimes on heating.

IDENTIFICATION

First identification A, D.

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *amantadine hydrochloride CRS* .

B. To 0.1 g add 1 ml of *pyridine R*, mix and add 0.1 ml of *acetic anhydride R*. Heat to boiling for about 10 s. Pour the hot solution into 10 ml of *dilute hydrochloric acid R*, cool to 5 °C and filter. The precipitate, washed with *water R* and dried *in vacuo* at 60 °C for 1 h, melts *(2.2.14)* at 147 °C to 151 °C.

C. Dissolve 0.2 g in 1 ml of *0.1 M hydrochloric acid*. Add 1 ml of a 500 g/l solution of *sodium nitrite R*. A white precipitate is formed.

D. 1 ml of solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Acidity or alkalinity

Dilute 2 ml of solution S to 10 ml with *carbon dioxide-free water R*. Add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 0.10 g of the substance to be examined in 2 ml of *water R*. Add 2 ml of a 200 g/l solution of *sodium hydroxide R* and 2 ml of *chloroform R*. Shake for 10 min. Separate the chloroform layer, dry over *anhydrous sodium sulphate R* and filter.

Column:

— *material*: glass;

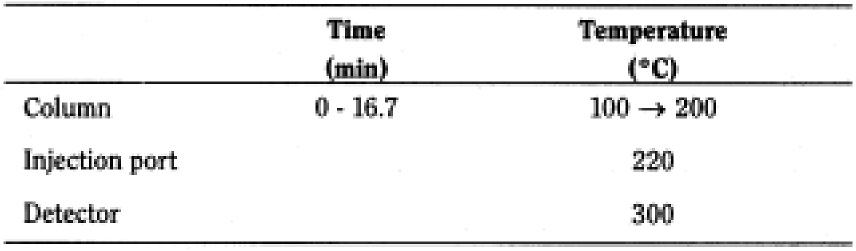
— *size:* *l* = 1.8 m, Ø = 2 mm;

— *stationary phase*: mix 19.5 g of *silanised diatomaceous earth for gas chromatography R* with 60 ml of a 3.3 g/l solution of *potassium hydroxide R* in *methanol R* and evaporate the solvent under reduced pressure while rotating the mixture slowly (support); dissolve 0.4 g of *low-vapour-pressure hydrocarbons (type L) R* in 60 ml of *toluene R* (dissolution requires up to 5 h), add this solution to the support and evaporate the solvent under reduced pressure while rotating the mixture slowly.

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 16.7	100 → 200
Injection port		220
Detector		300

Detection Flame ionisation.

Injection 1 µl or the chosen volume.

Run time At least 2.5 times the retention time of amantadine.

Limits:

— *any impurity*: for each impurity, maximum 0.3 per cent;

— *total*: maximum 1 per cent;

— *disregard limit*: disregard the peak due to the solvent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 2.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

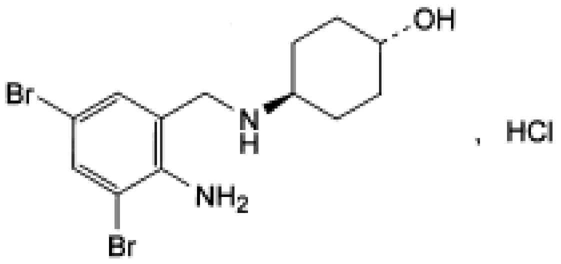
1 ml of *0.1 M sodium hydroxide* is equivalent to 18.77 mg of $C_{10}H_{18}ClN$.

Ph Eur

Ambroxol Hydrochloride

General Notices

(Ph Eur monograph 1489)



$C_{13}H_{18}Br_2N_2O,HCl$ 414.6 *23828-92-4*

Action and use

Mucolytic expectorant.

Ph Eur

DEFINITION

trans-4-[(2-Amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish crystalline powder.

Solubility

Sparingly soluble in water, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Dissolve 20.0 mg in *0.05 M sulphuric acid* and dilute to 100.0 ml with the same acid. Dilute 2.0 ml of the solution to 10.0 ml with *0.05 M sulphuric acid*. Examined between 200 nm and 350 nm *(2.2.25)*, the solution shows two absorption maxima at 245 nm and 310 nm.

The ratio of the absorbance measured at 245 nm to that measured at 310 nm is 3.2 to 3.4.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ambroxol hydrochloride CRS* .

C. Examine by thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 50 mg of *ambroxol hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *concentrated ammonia R*, *1-propanol R*, *ethyl acetate R*, *hexane R* (1:10:20:70 *V/V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 25 mg in 2.5 ml of *water R*, mix with 1.0 ml of *dilute ammonia R1* and allow to stand for 5 min. Filter and acidify the filtrate with *dilute nitric acid R*. The filtrate gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.75 g in *methanol R* and dilute to 15 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 6.0.

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of the test solution to 250.0 ml with *water R*. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of the substance to be examined in 0.2 ml of *methanol R* and add 0.04 ml of a mixture of 1 volume of *formaldehyde solution R* and 99 volumes of *water R*. Heat at 60 °C for 5 min. Evaporate to dryness under a current of nitrogen. Dissolve the residue in 5 ml of *water R* and dilute to 20 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase A mixture of equal volumes of *acetonitrile R* and a solution prepared as follows: dissolve 1.32 g of *ammonium phosphate R* in 900 ml of *water R*, adjust to pH 7.0 with *phosphoric acid R* and dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 248 nm.

Injection 20 µl.

Sensitivity Reference solution (a).

Run time 3 times the retention time of the principal peak in the chromatogram obtained with the test solution.

System suitability:

- *resolution*: minimum of 4.0 between the peaks due to impurity B and ambroxol in the chromatogram obtained with reference solution (b).

Limits:

- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

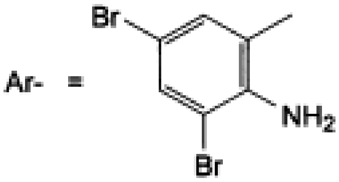
Dissolve 0.300 g in 70 ml of *alcohol R* and add 5 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 41.46 mg of $C_{13}H_{19}Br_2ClN_2O$.

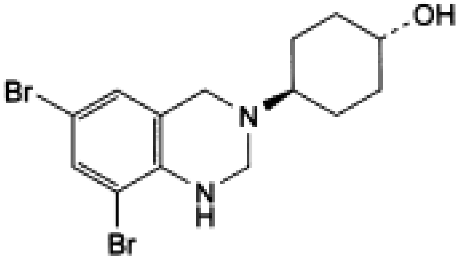
STORAGE

Store protected from light.

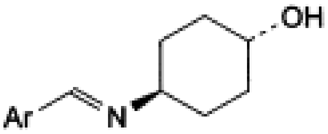
IMPURITIES



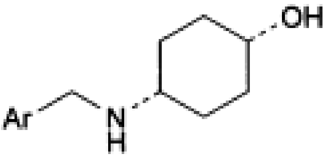
A. Ar-CH_2OH: (2-amino-3,5-dibromophenyl)methanol,



B. *trans*-4-(6,8-dibromo-1,4-dihydroquinazolin-3(2*H*)-yl)cyclohexanol,



C. *trans*-4-[[(*E)*-2-amino-3,5-dibromobenzyliden]amino]cyclohexanol,



D. *cis*-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol,

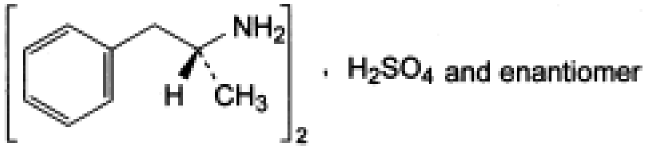
E. Ar-CH=O: 2-amino-3,5-dibromobenzaldehyde.

Ph Eur

Amfetamine Sulphate

General Notices

(Ph Eur monograph 0368)



$C_{18}H_{26}N_2,H_2SO_4$ 368.5 *60-13-9*

Action and use

Releases dopamine; central nervous system stimulant.

Ph Eur

DEFINITION

Bis[(2*RS*)-1-phenylpropan-2-amine] sulphate.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A Optical rotation *(2.2.7)*: - 0.04° to + 0.04° (measured in a 2 dm tube), determined on solution S (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Mulls in *liquid paraffin R*.

Comparison *Ph. Eur. reference spectrum of amfetamine sulphate.*

C. To 50 ml of solution S add 5 ml of *strong sodium hydroxide solution R* and 0.5 ml of *benzoyl chloride R* and shake. Continue to add *benzoyl chloride R* in portions of 0.5 ml until no further precipitate is formed. Filter, wash the precipitate with *water R*, recrystallise twice from a mixture of equal volumes of *ethanol (96 per cent) R* and *water R*, then dry at 100-105 °C. The crystals melt *(2.2.14)* at 131 °C to 135 °C.

D. To about 2 mg add 1 ml of *sulphuric acid-formaldehyde reagent R*. An orange colour develops and quickly becomes dark-brown.

E. Solution S gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 25 ml of solution S add 0.1 ml of *methyl red solution R*. Not more than 0.1 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 36.85 mg of $C_{18}H_{28}N_2O_4S$.

STORAGE

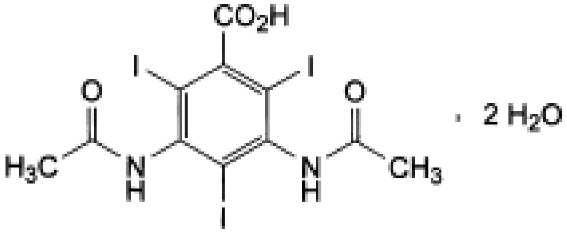
Protected from light.

Ph Eur

Amidotrizoic Acid Dihydrate

General Notices

(Ph Eur monograph 0873)



$C_{11}H_9I_3N_2O_4,2H_2O$ 650 *50978-11-5*

Action and use

Iodinated contrast medium.

Preparation

Meglumine Amidotrizoate Injection

Ph Eur

DEFINITION

Amidotrizoic acid dihydrate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 3,5-bis(acetylamino)-2,4,6-triiodobenzoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water and in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *amidotrizoic acid dihydrate CRS* .

B. Examine the chromatograms obtained in the test for related substances (see Tests). The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

C. Heat 50 mg gently in a small porcelain dish over a naked flame. Violet vapour is evolved.

TESTS

Appearance of solution

Dissolve 1.0 g in *dilute sodium hydroxide solution R* and dilute to 20 ml with the same solvent. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.50 g of the substance to be examined in a 3 per cent *V/V* solution of *ammonia R* in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a 3 per cent *V/V* solution of *ammonia R* in *methanol R*.

Reference solution (a) Dilute 1 ml of test solution (b) to 50 ml with a 3 per cent *V/V* solution of *ammonia R* in *methanol R*.

Reference solution (b) Dissolve 50 mg of *amidotrizoic acid dihydrate CRS* in a 3 per cent *V/V* solution of *ammonia R* in *methanol R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 2 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *anhydrous formic acid R*, 25 volumes of *methyl ethyl ketone R* and 60 volumes of *toluene R*. Allow the plate to dry until the solvents have evaporated and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent).

Halides

Dissolve 0.55 g in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 15 ml of *water R*. Add 6 ml of *dilute nitric acid R* and filter. 15 ml of the filtrate complies with the limit test for chlorides *(2.4.4)* (150 ppm expressed as chloride).

Free aromatic amines

Maintain the solutions and reagents in iced water protected from bright light. To 0.50 g in a 50 ml volumetric flask add 15 ml of *water R*. Shake and add 1 ml of *dilute sodium hydroxide solution R*. Cool in iced water, add 5 ml of a freshly prepared 5 g/l solution of *sodium nitrite R* and 12 ml of *dilute hydrochloric acid R*. Shake gently and allow to stand for exactly 2 min after adding the hydrochloric acid. Add 10 ml of a 20 g/l solution of *ammonium sulphamate R*. Allow to stand for 5 min, shaking frequently, and add 0.15 ml of a 100 g/l solution of *α-naphthol R* in *alcohol R*. Shake and allow to stand for 5 min. Add 3.5 ml of *buffer solution pH 10.9 R*, mix and dilute to 50.0 ml with *water R*. The absorbance *(2.2.25)*, measured within 20 min at 485 nm using as the compensation liquid a solution prepared at the same time and in the same manner but omitting the substance to be examined, is not greater than 0.30.

Heavy metals *(2.4.8)*

Dissolve 2.0 g in 4 ml of *dilute sodium hydroxide solution R* and dilute to 20 ml with *water R*. 12 ml of this solution complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Loss on drying *(2.2.32)*

4.5 per cent to 7.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

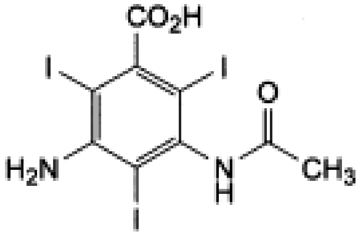
To 0.150 g in a 250 ml round-bottomed flask add 5 ml of *strong sodium hydroxide solution R*, 20 ml of *water R*, 1 g of *zinc powder R* and a few glass beads. Boil under a reflux condenser for 30 min. Allow to cool and rinse the condenser with 20 ml of *water R,* adding the rinsings to the flask. Filter through a sintered-glass filter *(2.1.2)* and wash the filter with several quantities of *water R*. Collect the filtrate and washings. Add 40 ml of *dilute sulphuric acid R* and titrate immediately with *0.1 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20)*, using a suitable electrode system such as silver-mercurous sulphate.

1 ml of *0.1 M silver nitrate* is equivalent to 20.47 mg of $C_{11}H_9I_3N_2O_4$.

STORAGE

Store protected from light.

IMPURITIES



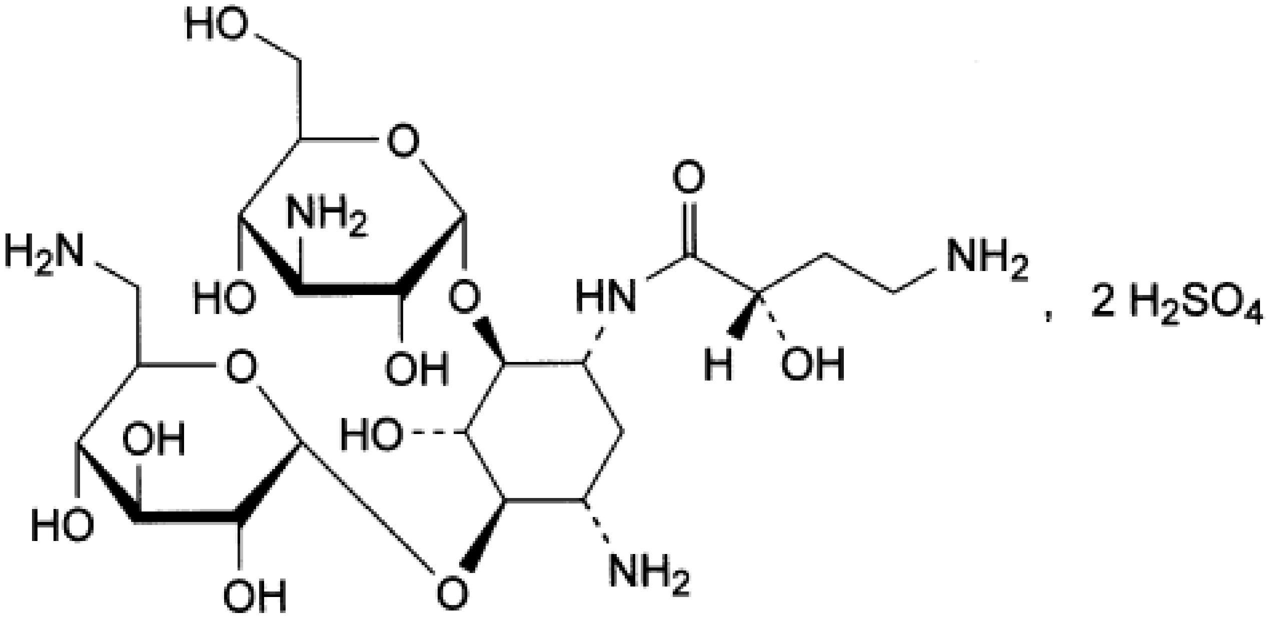
A. 3-(acetylamino)-5-amino-2,4,6-triiodobenzoic acid.

Ph Eur

Amikacin Sulphate

General Notices

(Ph Eur monograph 1290)



$C_{22}H_{43}N_5O_{13},2H_2SO_4$ 782 *149022-22-0*

Action and use

Aminoglycoside antibacterial.

Preparation

Amikacin Injection

Ph Eur

DEFINITION

6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine sulphate.

Antimicrobial substance obtained from kanamycin A.

Semi-synthetic product derived from a fermentation product.

Content

96.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *amikacin sulphate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *amikacin sulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *kanamycin monosulphate CRS* in 1 ml of the test solution and dilute to 10 ml with *water R.*

Plate *TLC silica gel plate R*.

Mobile phase The lower layer of a mixture of equal volumes of *concentrated ammonia R*, *methanol R* and *methylene chloride R.*

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

pH (*2.2.3*)

2.0 to 4.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 76 to + 84 (dried substance).

Dissolve 0.50 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Maintain the solutions at 10 °C.*

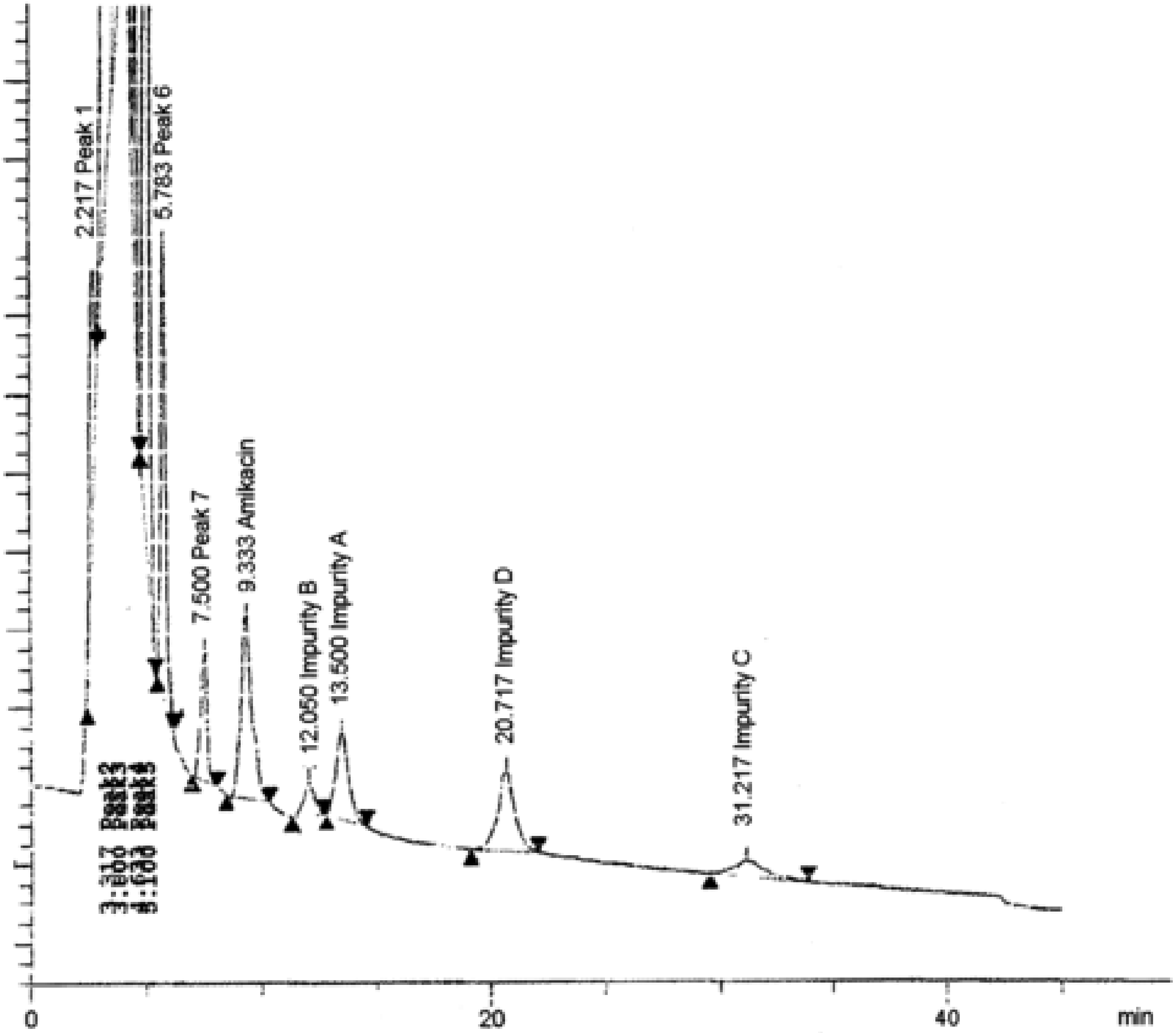


Figure 1290.-1. – *Chromatogram for the test for related substances of amikacin sulphate*

Test solution (a) Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent. In a ground-glass-stoppered vial, add 0.2 ml of this solution to 2.0 ml of a 10 g/l solution of *2,4,6-trinitrobenzene sulphonic acid R*. Then add 3.0 ml of *pyridine R* and close the vial tightly. Shake vigorously for 30 s and heat on a water-bath at 75 °C for 2 h. Cool in cold water for 2 min and add 2 ml of *glacial acetic acid R*. Shake vigorously for 30 s.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

Reference solution (a) Dissolve 5.0 mg of *amikacin impurity A CRS* in *water R* and dilute to 50.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

Reference solution (b) Dissolve 50.0 mg of *amikacin sulphate CRS* in *water R* and dilute to 50.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

Reference solution (c) Dissolve 2 mg of *amikacin sulphate CRS* and 2 mg of *amikacin impurity A CRS* in *water R* and dilute to 20 ml with the same solvent. Then prepare as prescribed for test solution (a).

Blank solution Prepare as described for test solution (a) using 0.2 ml of water R.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 30 volumes of a 2.7 g/l solution of *potassium dihydrogen phosphate R*, adjusted to pH 6.5 with a 22 g/l solution of *potassium hydroxide R*, and 70 volumes of *methanol R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 340 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (c).

Run time 4 times the retention time of amikacin.

System suitability Reference solution (c):

— *resolution*: minimum 3.5 between the peaks due to amikacin and impurity A (see Figure 1290.-1).

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *sum of impurities other than A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard any peak due to the blank and any peak eluting before the principal peak.

Sulphate

23.3 per cent to 25.8 per cent (dried substance).

Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 ml of *ethanol (96 per cent) R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of sulphate (SO_4).

Loss on drying (*2.2.32*)

Maximum 13.0 per cent, determined on 0.500 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa for 3 h.

Pyrogens (*2.6.8*)

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 5 ml of a solution containing 25 mg of the substance to be examined in *water for injections R*.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (b).

System suitability:

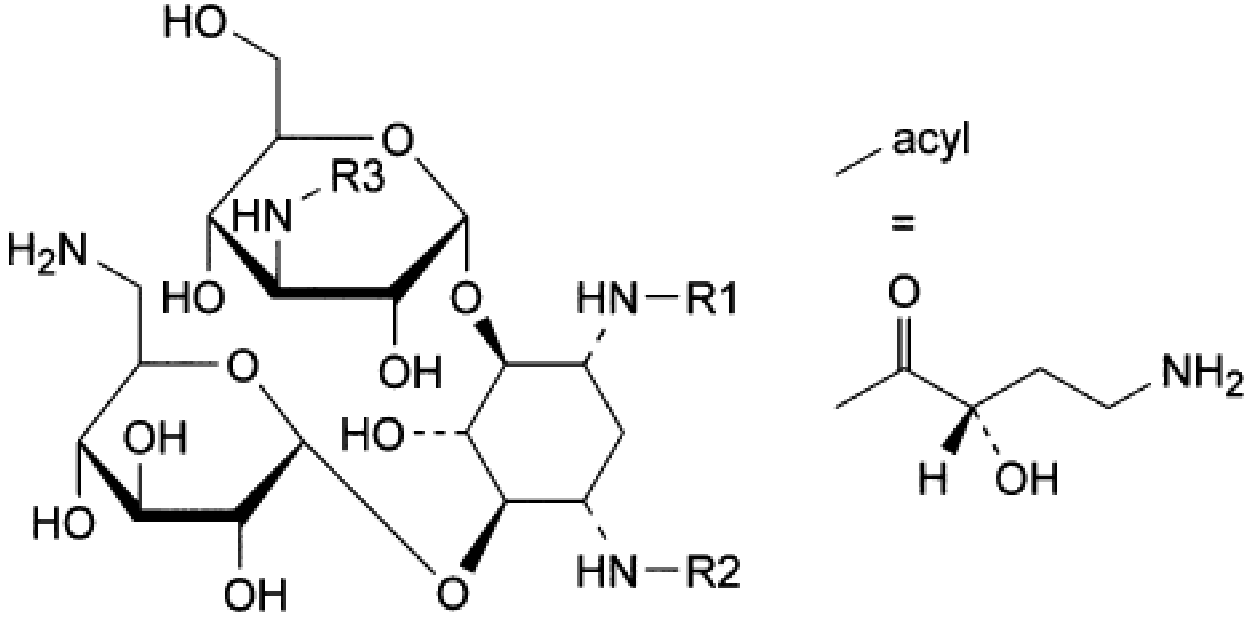
— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (b).

Calculate the percentage content of $C_{22}H_{47}N_5O_{21}S_2$ from the declared content of *amikacin sulphate CRS.*

STORAGE

If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES



A. R1 = R3 = H, R2 = acyl: 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine,

B. R1 = R2 = acyl, R3 = H: 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1,3-*N*-bis[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine,

C. R1 = R2 = H, R3 = acyl: 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-6-*O*-[3-[[(2*S*)-4-amino-2-hydroxybutanoyl]amino]-3-deoxy-α-D-glucopyranosyl]-2-deoxy-D-streptamine,

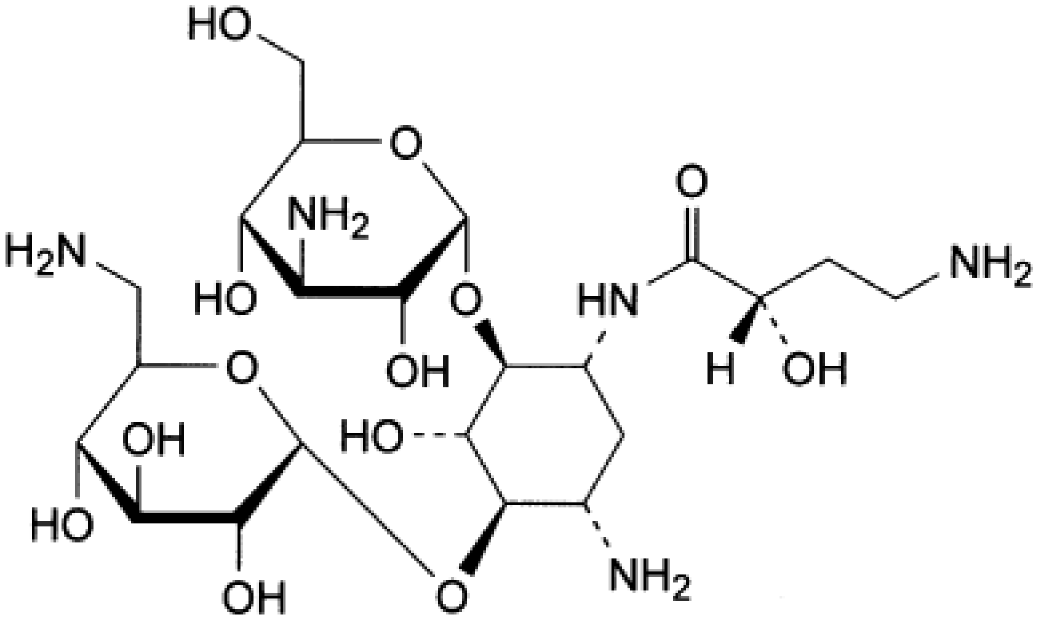
D. R1 = R2 = R3 = H: kanamycin.

Ph Eur

Amikacin

General Notices

(Ph Eur monograph 1289)



$C_{22}H_{43}N_5O_{13}$ 585.6 *37517-28-5*

Action and use

Aminoglycoside antibacterial.

Ph Eur

DEFINITION

6-*C*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-4-*C*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine.

Antimicrobial substance obtained from kanamycin A.

Semi-synthetic product derived from a fermentation product.

Content

96.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Sparingly soluble in water, slightly soluble in methanol, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *amikacin CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *amikacin CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *kanamycin monosulphate CRS* in 1 ml of the test solution and dilute to 10 ml with *water R*.

Plate *TLC silica gel plate R.*

Mobile phase The lower layer of a mixture of equal volumes of *concentrated ammonia R*, *methanol R* and *methylene chloride R.*

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

pH (*2.2.3*)

9.5 to 11.5.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 97 to + 105 (anhydrous substance).

Dissolve 0.50 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Maintain the solutions at 10 °C.*

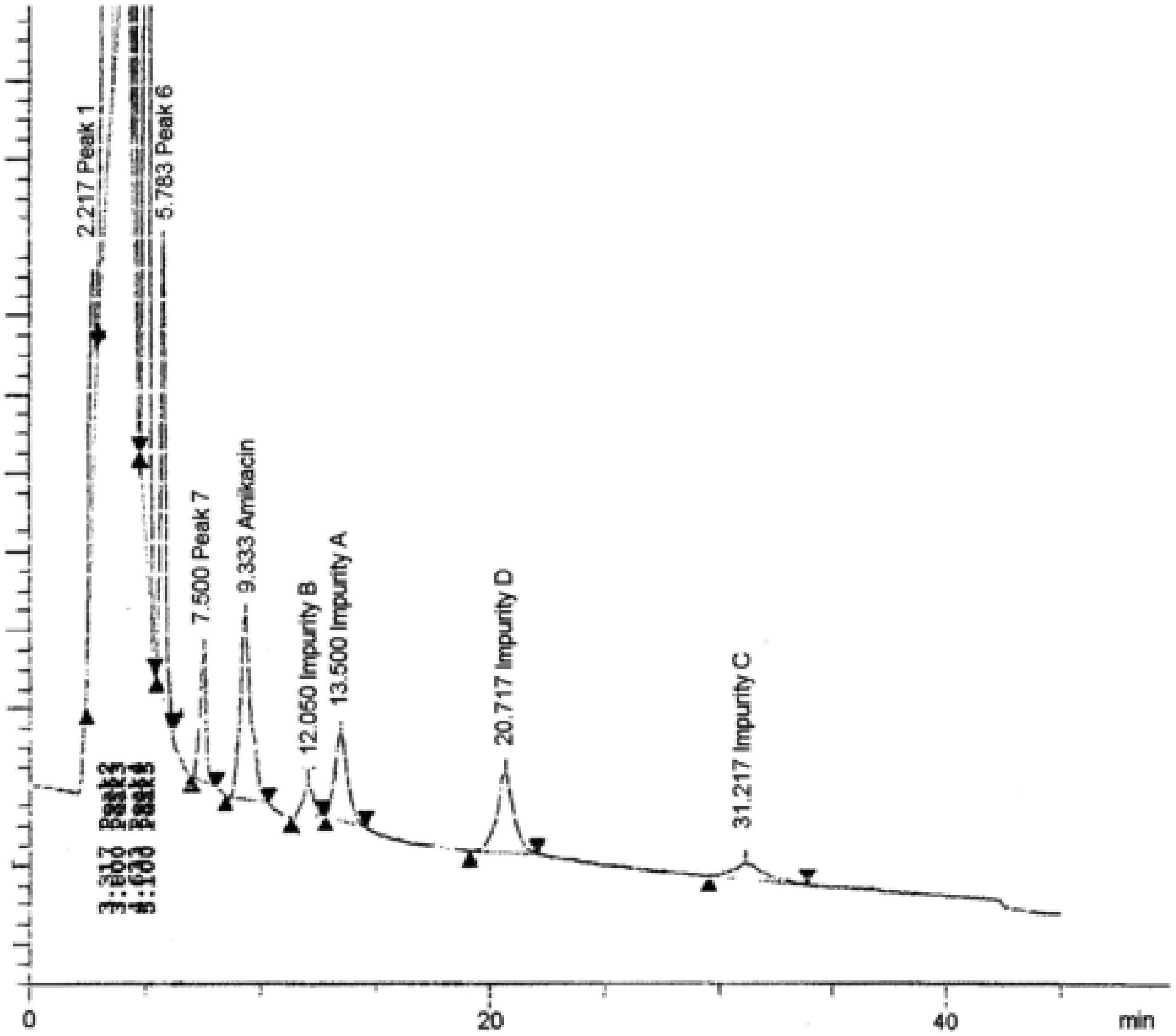


Figure 1289.-1. – *Chromatogram for the test for related substances of amikacin*

Test solution (a) Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent. In a ground-glass-stoppered vial, add 0.2 ml of this solution to 2.0 ml of a 10 g/l solution of *2,4,6-trinitrobenzene sulphonic acid R*. Then add 3.0 ml of *pyridine R* and close the vial tightly. Shake vigorously for 30 s and heat in a water-bath at 75 °C for 45 min. Cool in cold water for 2 min and add 2 ml of *glacial acetic acid R*. Shake vigorously for 30 s.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

Reference solution (a) Dissolve 5.0 mg of *amikacin impurity A CRS* in *water R* and dilute to 50.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

Reference solution (b) Dissolve 50.0 mg of *amikacin CRS* in *water R* and dilute to 50.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

Reference solution (c) Dissolve 2 mg of *amikacin CRS* and 2 mg of *amikacin impurity A CRS* in *water R* and dilute to 20 ml with the same solvent. Then prepare as prescribed for test solution (a).

Blank solution Prepare as described for test solution (a) using 0.2 ml of *water R.*

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 30 volumes of a 2.7 g/l solution of *potassium dihydrogen phosphate R*, adjusted to pH 6.5 with a 22 g/l solution of *potassium hydroxide R*, and 70 volumes of *methanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 340 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (c).

Run time 4 times the retention time of amikacin.

System suitability Reference solution (c):

— *resolution*: minimum 3.5 between the peaks due to amikacin and impurity A (see Figure 1289.-1).

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *sum of impurities other than A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard any peak due to the blank.

Water (*2.5.12*)

Maximum 8.5 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

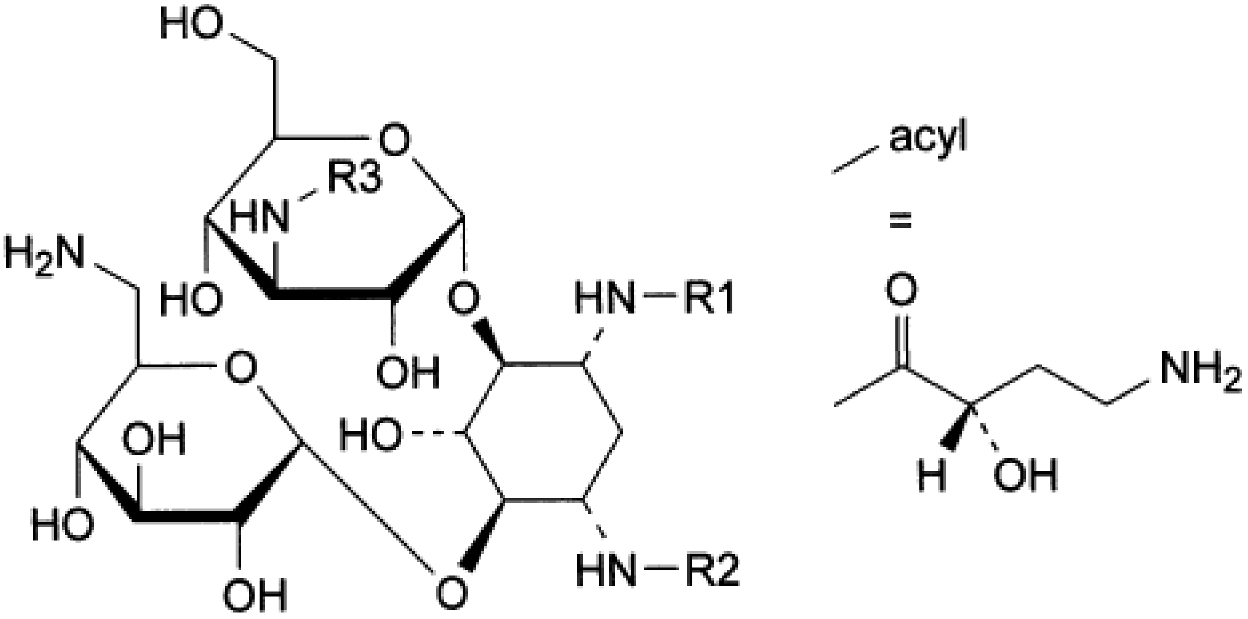
Injection Test solution (b) and reference solution (b).

System suitability:

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (b).

Calculate the percentage content of $C_{22}H_{43}N_5O_{13}$ from the declared content of *amikacin CRS*.

IMPURITIES



A. R1 = R3 = H, R2 = acyl: 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine,

B. R1 = R2 = acyl, R3 = H: 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1,3-*N*-bis[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine,

C. R1 = R2 = H, R3 = acyl: 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-6-*O*-[3-[[(2S)-4-amino-2-hydroxybutanoyl]amino]-3-deoxy-α-D-glucopyranosyl]-2-deoxy-D-streptamine,

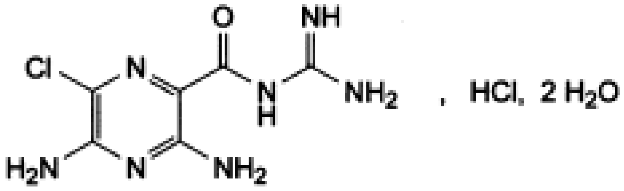
D. R1 = R2 = R3 = H: kanamycin.

Ph Eur

Amiloride Hydrochloride

General Notices

(Ph Eur monograph 0651)



$C_6H_8ClN_7O,HCl,2H_2O$ 302.1 *17440-83-4*

Action and use

Sodium channel blocker; potassium-sparing diuretic.

Preparations

Amiloride Tablets

Co-amilofruse Tablets

Co-amilozide Oral Solution

Co-amilozide Tablets

Ph Eur

DEFINITION

3,5-Diamino-*N*-carbamimidoyl-6-chloropyrazine-2-carboxamide hydrochloride dihydrate.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Pale yellow or greenish-yellow powder.

Solubility

Slightly soluble in water and in anhydrous ethanol.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *amiloride hydrochloride CRS* .

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 40 mg of *amiloride hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *dilute ammonia R1*, *water R*, *dioxan R* (6:6:88 *V/V/V*); freshly prepared mixture.

Application 5 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 10 mg in 10 ml of *water R*. Add 10 ml of a 200 g/l solution of *cetrimide R*, 0.25 ml of *dilute sodium hydroxide solution R* and 1 ml of *bromine water R*. A greenish-yellow colour is produced. Add 2 ml of *dilute hydrochloric acid R*. The solution becomes deep yellow and shows blue fluorescence in ultraviolet light at 365 nm.

D. It gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Free acid

Dissolve 1.0 g in a mixture of 50 ml of *methanol R* and 50 ml of *water R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Not more than 0.3 ml of *0.1 M sodium hydroxide* is required to reach the end-point.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R*.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R*.

Reference solution (c) Dissolve 5.0 mg of *amiloride impurity A CRS* in a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R* and dilute to 5.0 ml with the same mixture of solvents. Dilute 1.0 ml of this solution to 100.0 ml with a mixture of 1 volume of *acetonitrile R* and 3 volumes of *water R*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 5 volumes of *tetramethylammonium hydroxide solution R*, 250 volumes of *acetonitrile R* and 745 volumes of *water R*; adjust to pH 7.0 with a mixture of 1 volume of *phosphoric acid R* and 9 volumes of *water R*. Adjust the concentration of acetonitrile in the mobile phase so that the retention time of impurity A is 5-6 min (an increase in the concentration of acetonitrile results in a shorter retention time). Adjust the concentration of tetramethylammonium hydroxide and of phosphoric acid keeping the pH at 7.0 so that the retention time of amiloride is 9-12 min (an increase in the concentration results in a shorter retention time for amiloride).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 5 times the retention time of amiloride.

System suitability Reference solution (b):

— *signal-to-noise ratio*: minimum 5.0 for the peak due to amiloride.

Limits:

— *total*: not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water *(2.5.12)*

11.0 per cent to 13.0 per cent, determined on 0.200 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

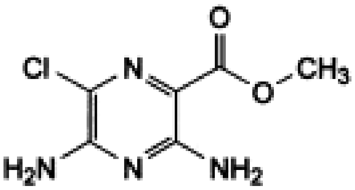
Dissolve 0.200 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 26.61 mg of $C_6H_9Cl_2N_7O$.

STORAGE

Protected from light.

IMPURITIES



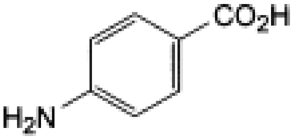
A. methyl 3,5-diamino-6-chloropyrazine-2-carboxylate.

Ph Eur

Aminobenzoic Acid

General Notices

(4-Aminobenzoic Acid, Ph Eur monograph 1687)



$C_7H_7NO_2$ 137.1 *150-13-0*

Action and use

Skin protective.

Ph Eur

DEFINITION

4-Aminobenzoic acid.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Melting point *(2.2.14)*: 186 °C to 189 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *4-aminobenzoic acid CRS .*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *4-aminobenzoic acid CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *4-nitrobenzoic acid R* in 10 ml of reference solution (a).

Plate Suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm as the coating substance.

Mobile phase *glacial acetic acid R*, *hexane R*, *methylene chloride R* (5:20:75 *V/V/V*).

Application 1 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_5 *(2.2.2, Method II)*.

Dissolve 1.0 g in *alcohol R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution Dissolve 25.0 mg of *4-nitrobenzoic acid R* and 25.0 mg of *benzocaine R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.12 m, Ø = 4.0 mm,

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of a mixture of 70 volumes of *acetonitrile R* and 80 volumes of *methanol R*, and 80 volumes of a solution containing 1.5 g/l of *potassium dihydrogen phosphate R* and 2.5 g/l of *sodium octanesulphonate R* adjusted to pH 2.2 with *phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µl.

Run time 11 times the retention time of 4-aminobenzoic acid.

Relative retention With reference to 4-aminobenzoic acid (retention time = about 3 min): impurity A = about 4; impurity B = about 9.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.2 per cent),

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.2 per cent),

— *any other impurity*: not more than 0.5 times the area of the peak due to impurity A in the chromatogram obtained with the reference solution (0.1 per cent),

— *total*: not more than 2.5 times the area of the peak due to impurity A in the chromatogram obtained with the reference solution (0.5 per cent),

— *disregard limit*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with the reference solution (0.02 per cent).

Impurity C and impurity D

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 20.0 mg of *lauric acid R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Test solution Dissolve 1.000 g of the substance to be examined in 10.0 ml of an 84 g/l solution of *sodium hydroxide R* and extract with 2 quantities, each of 10 ml, of *methylene chloride R*. Combine and wash with 5 ml of *water R*; filter through *anhydrous sodium sulphate R*. Wash the filter with *methylene chloride R*. Evaporate in a water-bath at 50-60 °C to obtain a volume of about 1-5 ml. Add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *methylene chloride R*.

Reference solution (a) Dissolve 20.0 mg of *aniline R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dissolve 20.0 mg of *p-toluidine R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Reference solution (c) Dilute 0.50 ml of reference solution (a), 0.50 ml of reference solution (b) and 10.0 ml of the internal standard solution to 100.0 ml with *methylene chloride R*.

Column:

— *material*: fused silica,

— *size*: *l* = 30 m, Ø = 0.32 mm,

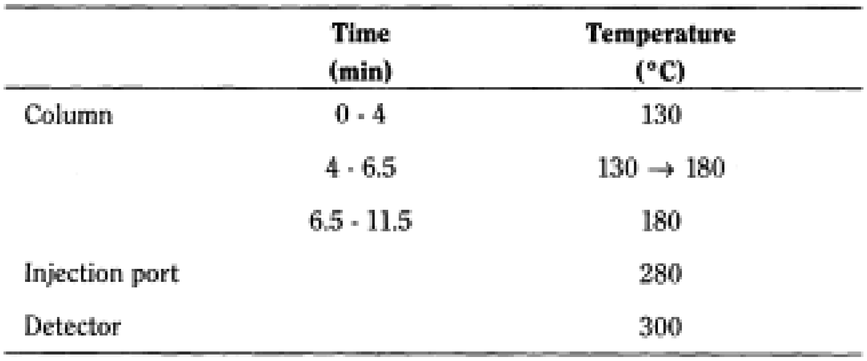
— *stationary phase*: *poly[methyl(95)phenyl(5)] siloxane R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.0 ml/min.

Split ratio 1:10.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 4	130
	4 - 6.5	130 → 180
	6.5 - 11.5	180
Injection port		280
Detector		300

Detection Flame ionisation.

Injection 2 µl; inject the test solution and reference solution (c).

Retention time Internal standard = about 9.5 min.

Limits:

— *impurity C*: calculate the ratio (*R*) of the area of the peak due to impurity C to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (c); calculate the ratio of the area of the peak due to impurity C to the area of the peak due to the internal standard from the chromatogram obtained with the test solution: this ratio is not greater than *R* (10 ppm),

— *impurity D*: calculate the ratio (*R*) of the area of the peak due to impurity D to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (c); calculate the ratio of the area of the peak due to impurity D to the area of the peak due to the internal standard from the chromatogram obtained with the test solution: this ratio is not greater than *R* (10 ppm).

Iron *(2.4.9)*

Maximum 40 ppm.

Dissolve 0.250 g in 3 ml of *alcohol R* and dilute to 10.0 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 0.2 per cent, determined on 1.00 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

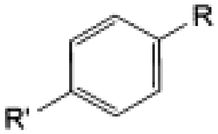
Dissolve 0.100 g with heating in 50 ml of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 13.71 mg of $C_7H_7NO_2$.

STORAGE

Protected from light.

IMPURITIES



A. R = CO_2H, R′ = NO_2: 4-nitrobenzoic acid,

B. R = CO-O-C_2H_5, R′ = NH_2: benzocaine,

C. R = H, R′ = NH_2: aniline,

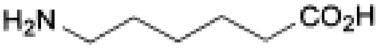
D. R = CH_3, R′ = NH_2: 4-methylaniline (*p*-toluidine).

Ph Eur

Aminocaproic Acid

General Notices

(Ph Eur monograph 0874)



$C_6H_{13}NO_2$ 131.2 *60-32-2*

Action and use

Antifibrinolytic.

Ph Eur

DEFINITION

Aminocaproic acid contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 6-aminohexanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in alcohol.

It melts at about 205 °C with decomposition.

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *aminocaproic acid CRS* . Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with the test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.5 g in 4 ml of a mixture of equal volumes of *dilute hydrochloric acid R* and *water R*. Evaporate to dryness by heating on a water-bath. Dry the residue in a desiccator. Dissolve the residue in about 2 ml of boiling *ethanol R*. Allow to cool and maintain at 4 °C to 8 °C for 3 h. Filter under reduced pressure. The residue washed with about 10 ml of *acetone R* and dried at 60 °C for 30 min, melts *(2.2.14)* at 131 °C to 133 °C.

D. Dissolve about 5 mg in 0.5 ml of *distilled water R*. Add 3 ml of *dimethylformamide R* and 2 ml of *ascorbic acid solution R*. Heat on a water-bath. An orange colour develops.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is colourless *(2.2.2, Method II)* and remains clear *(2.2.1)* on standing for 24 h.

pH *(2.2.3)*

The pH of solution S is 7.5 to 8.0.

Absorbance *(2.2.25)*.

A. The absorbance of solution S at 287 nm is not more than 0.10 and at 450 nm is not more than 0.03.

B. Place 2.0 g in an even layer in a shallow dish 9 cm in diameter, cover and allow to stand at 98 °C to 102 °C for 72 h. Dissolve in *water R* and dilute to 10.0 ml with the same solvent. The absorbance of the solution at 287 nm is not more than 0.15 and at 450 nm is not more than 0.03.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *aminocaproic acid CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *aminocaproic acid CRS* and 10 mg of *leucine CRS* in *water R* and dilute to 25 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Dry the plate in a current of warm air. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with the test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Heavy metals *(2.4.8)*

12 ml of solution S complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 20 ml of *anhydrous acetic acid R*. Using 0.1 ml of *crystal violet solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from bluish-violet to bluish-green.

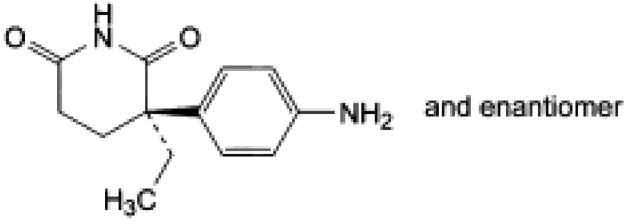
1 ml of *0.1 M perchloric acid* is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

Ph Eur

Aminoglutethimide

General Notices

(Ph Eur monograph 1291)



$C_{13}H_{16}N_2O_2$ 232.3 *125-84-8*

Action and use

Inhibitor of adrenal corticosteroid synthesis; used in chemical adrenalectomy.

Preparation

Aminoglutethimide Tablets

Ph Eur

DEFINITION

(3*RS*)-3-(4-Aminophenyl)-3-ethylpiperidine-2,6-dione.

Content

98.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in methanol.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Melting point *(2.2.14)*: 150 °C to 154 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *aminoglutethimide CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in *acetone R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *aminoglutethimide CRS* in *acetone R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 25 mg of *aminoglutethimide CRS* and 25 mg of *glutethimide CRS* in *acetone R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *glacial acetic acid R*, *methanol R*, *ethyl acetate R* (0.5:15:85 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separed spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 1.0 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.10 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *acetate buffer solution pH 5.0 R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 5.0 mg of *aminoglutethimide impurity A CRS* in a mixture of equal volumes of *methanol R* and *acetate buffer solution pH 5.0 R* and dilute to 25.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with a mixture of equal volumes of *methanol R* and *acetate buffer solution pH 5.0 R*.

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *methanol R* and *acetate buffer solution pH 5.0 R*.

Reference solution (d) Dilute 1.0 ml of the test solution to 10.0 ml with reference solution (a).

Column:

— *size:* l = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (4 µm);

— *temperature*: 40 °C.

Mobile phase Mix 27 volumes of *methanol R* and 73 volumes of *acetate buffer solution pH 5.0 R*.

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl of the test solution and reference solutions (b), (c) and (d).

Run time 4 times the retention time of aminoglutethimide.

Retention time aminoglutethimide = about 9 min; impurity A = about 12 min.

System suitability Reference solution (d):

— *resolution*: minimum 2.0 between the peaks due to aminoglutethimide and impurity A.

Limits:

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent);

— *total*: maximum 2.0 per cent for the sum of the contents of all impurities;

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Impurity D

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light. Use shaking, not sonication or heat, to dissolve the reference substance and the substance to be examined.*

Test solution Dissolve 0.100 g of the substance to be examined in *dimethyl sulphoxide R* and dilute to 100.0 ml with the same solvent.

Reference solution Dissolve 3.0 mg of *aminoglutethimide impurity D CRS* in *dimethyl sulphoxide R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *dimethyl sulphoxide R*.

Column:

— *size:* l = 0.12 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.285 g of *sodium edetate R* in *water R*, add 7.5 ml of *dilute acetic acid R* and 50 ml of *0.1 M potassium hydroxide* and dilute to 1000 ml with *water R*; adjust to pH 5.0 with *glacial acetic acid R*; mix 350 ml of this solution with 650 ml of *methanol R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 328 nm.

Injection 10 µl.

System suitability Test solution:

— *number of theoretical plates*: minimum 3300, calculated for the principal peak;

— *mass distribution ratio*: 2.0 to 5.0 for the principal peak;

— *symmetry factor*: maximum 1.2 for the principal peak.

Limit:
— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (300 ppm).

Sulphates *(2.4.13)*

Maximum 500 ppm.

Dilute 6 ml of solution S to 15 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in 15 ml of *acetone R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 ml of *acetone R* and 5 ml of *water R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.180 g in 50 ml of *anhydrous acetic acid R* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 23.23 mg of $C_{13}H_{16}N_2O_2$.

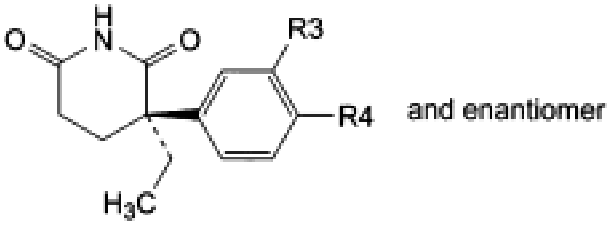
IMPURITIES

Specified impurities *A, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph

Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*)

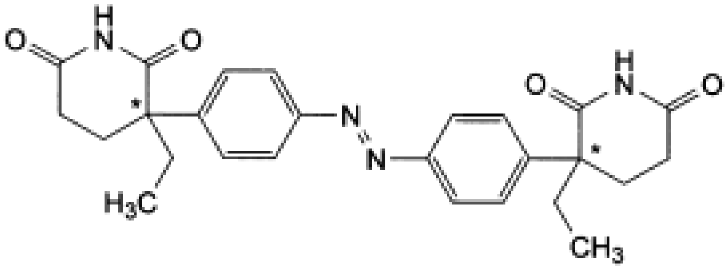
B, C.



A. R3 = NH_2, R4 = H: (3*RS*)-3-(3-aminophenyl)-3-ethylpiperidine-2,6-dione (3-aminoglutethimide),

B. R3 = NO_2, R4 = H: (3*RS*)-3-ethyl-3-(3-nitrophenyl)piperidine-2,6-dione,

C. R3 = H, R4 = NO_2: (3*RS*)-3-ethyl-3-(4-nitrophenyl)piperidine-2,6-dione,



D. 3,3′-[diazenediylbis(4,1-phenylene)]bis(3-ethylpiperidine-2,6-dione) (azoglutethimide).

Ph Eur

Aminophylline Hydrate

General Notices

(Theophylline-ethylenediamine Hydrate, Ph Eur monograph 0301)

5877-66-5

Action and use

Non-selective phosphodiesterase inhibitor; treatment of reversible airways obstruction.

Preparation

Aminophylline Injection

Ph Eur

DEFINITION

Theophylline-ethylenediamine hydrate contains not less than 84.0 per cent and not more than the equivalent of 87.4 per cent of theophylline ($C_7H_8N_4O_2$; M_r 180.2) and not less than 13.5 per cent and not more than the equivalent of 15.0 per cent of ethylenediamine ($C_2H_8N_2$; M_r 60.1), both calculated with reference to the anhydrous substance.

CHARACTERS

A white or slightly yellowish powder, sometimes granular, freely soluble in water (the solution becomes cloudy through absorption of carbon dioxide), practically insoluble in ethanol.

IDENTIFICATION

First identification *B, C, E.*

Second identification *A, C, D, E, F.*

Dissolve 1.0 g in 10 ml of *water R* and add 2 ml of *dilute hydrochloric acid R* dropwise with shaking. Filter. Use the precipitate for identification tests A, B, D and F and the filtrate for identification test C.

A. The precipitate, washed with *water R* and dried at 100 °C to 105 °C, melts *(2.2.14)* at 270 °C to 274 °C.

B. Examine the precipitate, washed with *water R* and dried at 100 °C to 105 °C, by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *theophylline CRS* .

C. To the filtrate add 0.2 ml of *benzoyl chloride R,* make alkaline with *dilute sodium hydroxide solution R* and shake vigorously. Filter the precipitate, wash with 10 ml of *water R*, dissolve in 5 ml of hot *alcohol R* and add 5 ml of *water R*. A precipitate is formed, which when washed and dried at 100 °C to 105 °C, melts *(2.2.14)* at 248 °C to 252 °C.

D. Heat about 10 mg of the precipitate with 1.0 ml of a 360 g/l solution of *potassium* *hydroxide R* in a water-bath at 90 °C for 3 min, then add 1.0 ml of *diazotised sulphanilic acid solution R*. A red colour slowly develops. Carry out a blank test.

E. It contains 3.0 per cent to 8.0 per cent of water (see Tests).

F. The precipitate gives the reaction of xanthines *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 0.5 g with gentle warming in 10 ml of *carbon dioxide-free water R*. The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution GY_6 *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution Dissolve 0.2 g of the substance to be examined in 2 ml of *water R* with heating and dilute to 10 ml with *methanol R*.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R*, 30 volumes of *acetone R*, 30 volumes of *chloroform R* and 40 volumes of *butanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

3.0 per cent to 8.0 per cent, determined on 0.50 g dissolved in 20 ml of *pyridine R*, by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Ethylenediamine

Dissolve 0.250 g in 30 ml of *water R*. Add 0.1 ml of *bromocresol green solution R*. Titrate with *0.1 M hydrochloric acid* until a green colour is obtained.

1 ml of *0.1 M hydrochloric acid* is equivalent to 3.005 mg of $C_2H_8N_2$.

Theophylline

Heat 0.200 g to constant mass in an oven at 135 °C. Dissolve the residue with heating in 100 ml of *water R*, allow to cool, add 20 ml of *0.1 M silver nitrate* and shake. Add 1 ml of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

STORAGE

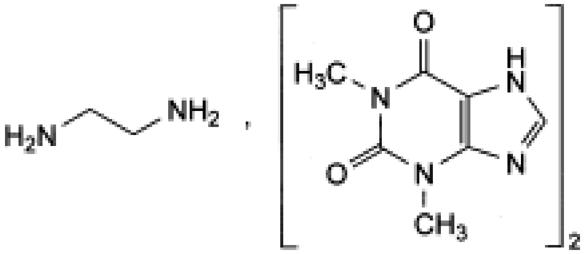
Store in a well-filled airtight container , protected from light.

Ph Eur

Aminophylline

General Notices

(Theophylline-ethylenediamine, Ph Eur monograph 0300)



$C_{16}H_{24}N_{10}O_4$ 420.4 *317-34-0*

Action and use

Non-selective phosphodiesterase inhibitor; treatment of reversible airways obstruction.

Preparations

Aminophylline Injection

Aminophylline Tablets

Prolonged-release Aminophylline Tablets

Ph Eur

DEFINITION

Theophylline-ethylenediamine contains not less than 84.0 per cent and not more than the equivalent of 87.4 per cent of theophylline ($C_7H_8N_4O_2$; M_r 180.2) and not less than 13.5 per cent and not more than the equivalent of 15.0 per cent of ethylenediamine ($C_2H_8N_2$; M_r 60.1), both calculated with reference to the anhydrous substance.

CHARACTERS

A white or slightly yellowish powder, sometimes granular, freely soluble in water (the solution becomes cloudy through absorption of carbon dioxide), practically insoluble in ethanol.

IDENTIFICATION

First identification *B, C, E.*

Second identification *A, C, D, E, F.*

Dissolve 1.0 g in 10 ml of *water R* and add 2 ml of *dilute hydrochloric acid R* dropwise with shaking. Filter. Use the precipitate for identification tests A, B, D and F and the filtrate for identification test C.

A. The precipitate, washed with *water R* and dried at 100 °C to 105 °C, melts *(2.2.14)* at 270 °C to 274 °C.

B. Examine the precipitate, washed with *water R* and dried at 100 °C to 105 °C, by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *theophylline CRS*.

C. To the filtrate add 0.2 ml of *benzoyl chloride R*, make alkaline with *dilute sodium hydroxide solution R* and shake vigorously. Filter the precipitate, wash with 10 ml of *water R*, dissolve in 5 ml of hot *alcohol* R and add 5 ml of *water R*. A precipitate is formed, which when washed and dried at 100 °C to 105 °C, melts *(2.2.14)* at 248 °C to 252 °C.

D. Heat about 10 mg of the precipitate with 1.0 ml of a 360 g/l solution of *potassium hydroxide R* in a water-bath at 90 °C for 3 min, then add 1.0 ml of *diazotised sulphanilic acid solution R*. A red colour slowly develops. Carry out a blank test.

E. It complies with the test for water (see Tests).

F. The precipitate gives the reaction of xanthines *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 0.5 g with gentle warming in 10 ml of *carbon dioxide-free water R*. The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution GY_6 *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution Dissolve 0.2 g of the substance to be examined in 2 ml of *water R* with heating and dilute to 10 ml with *methanol R*.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *methanol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R*, 30 volumes of *acetone R*, 30 volumes of *chloroform R* and 40 volumes of *butanol R.* Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Not more than 1.5 per cent, determined on 2.00 g dissolved in 20 ml of *anhydrous pyridine R*, by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Ethylenediamine

Dissolve 0.250 g in 30 ml of *water R*. Add 0.1 ml of *bromocresol green solution R*. Titrate with *0.1 M hydrochloric* acid until a green colour is obtained.

1 ml of *0.1 M hydrochloric acid* is equivalent to 3.005 mg of $C_2H_8N_2$.

Theophylline

Heat 0.200 g to constant mass in an oven at 135 °C. Dissolve the residue with heating in 100 ml of *water R*, allow to cool, add 20 ml of *0.1 M silver nitrate* and shake. Add 1 ml of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

STORAGE

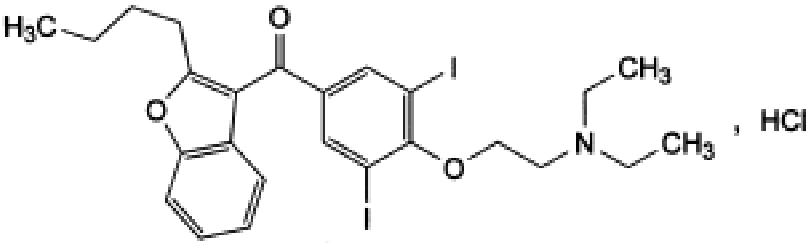
Store in airtight container , protected from light.

Ph Eur

Amiodarone Hydrochloride

General Notices

(Ph Eur monograph 0803)



$C_{25}H_{29}I_2NO_3$,HCl 682 *199774-82-4*

Action and use

Potassium channel blocker; class III antiarrhythmic.

Preparations

Amiodarone Intravenous Infusion

Amiodarone Tablets

Ph Eur

DEFINITION

(2-Butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, fine, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in methylene chloride, soluble in methanol, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *amiodarone hydrochloride CRS* .

B. It gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution GY_5 or BY_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *methanol R* and dilute to 20 ml with the same solvent.

pH *(2.2.3)*

3.2 to 3.8.

Dissolve 1.0 g in *carbon dioxide-free water R*, heating at 80 °C, cool and dilute to 20 ml with the same solvent.

Impurity H

Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use and keep protected from bright light.*

Test solution Dissolve 0.500 g of the substance to be examined in *methylene chloride R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *(2-chloroethyl)diethylamine hydrochloride R* (impurity H) in *methylene chloride R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with *methylene chloride R*.

Reference solution (b) Mix 2.0 ml of the test solution and 2.0 ml of reference solution (a).

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *anhydrous formic acid R*, *methanol R*, *methylene chloride R* (5:10:85 *V/V/V*).

Application 50 µl of the test solution and reference solution (a); 100 µl of reference solution (b).

Development Over 2/3 of the plate.

Drying In a current of cold air.

Detection Spray with *potassium iodobismuthate solution R1* and then with *dilute hydrogen peroxide solution R*; examine immediately in daylight.

System suitability Reference solution (b):

- the spot due to impurity H is clearly visible.

Limit:

- *impurity H*: any spot with the same R_F as the spot due to impurity H in the chromatogram obtained with reference solution (b), is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.02 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Buffer solution pH 4.9 To 800 ml of *water R* add 3.0 ml of *glacial acetic acid R*, adjust to pH 4.9 with *dilute ammonia R1* and dilute to 1000 ml with *water R*.

Test solution Dissolve 0.125 g of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 25.0 ml with the same mixture of solvents.

Reference solution Dissolve 5 mg of *amiodarone impurity D CRS* , 5 mg of *amiodarone impurity E CRS* and 5.0 mg of *amiodarone hydrochloride CRS* in *methanol R* and dilute to 25.0 ml with the same solvent. Dilute 1.0 ml of this solution to 20.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *buffer solution pH 4.9*, *methanol R*, *acetonitrile R* (30:30:40 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl.

Run time Twice the retention time of amiodarone.

Relative retention With reference to amiodarone (retention time = about 24 min): impurity A = about 0.26; impurity D = about 0.29; impurity E = about 0.37; impurity B = about 0.49; impurity C = about 0.55; impurity G = about 0.62; impurity F = about 0.69.

System suitability Reference solution:

— *resolution*: minimum 3.5 between the peaks due to impurities D and E.

Limits:
— *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the peak due to amiodarone in the chromatogram obtained with the reference solution (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the peak due to amiodarone in the chromatogram obtained with the reference solution (0.10 per cent);

— *total*: not more than 2.5 times the area of the peak due to amiodarone in the chromatogram obtained with the reference solution (0.5 per cent);

— *disregard limit*: 0.25 times the area of the peak due to amiodarone in the chromatogram obtained with the reference solution (0.05 per cent).

Iodides

Maximum 150 ppm.

Prepare the test and reference solutions simultaneously.

Solution A Add 1.50 g of the substance to be examined to 40 ml of *water R* at 80 °C and shake until completely dissolved. Cool and dilute to 50.0 ml with *water R*.

Test solution To 15.0 ml of solution A add 1.0 ml of *0.1 M hydrochloric acid* and 1.0 ml of *0.05 M potassium* i*odate*. Dilute to 20.0 ml with *water R*. Allow to stand protected from light for 4 h.

Reference solution To 15.0 ml of solution A add 1.0 ml of *0.1 M hydrochloric acid*, 1.0 ml of an 88.2 mg/l solution of *potassium iodide R* and 1.0 ml of *0.05 M potassium iodate*. Dilute to 20.0 ml with *water R*. Allow to stand protected from light for 4 h.

Measure the absorbances *(2.2.25)* of the solutions at 420 nm, using a mixture of 15.0 ml of solution A and 1.0 ml of *0.1 M hydrochloric acid* diluted to 20.0 ml with *water R* as the compensation liquid. The absorbance of the test solution is not greater than half the absorbance of the reference solution.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying at 50 °C at a pressure not exceeding 0.3 kPa for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.600 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 75 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

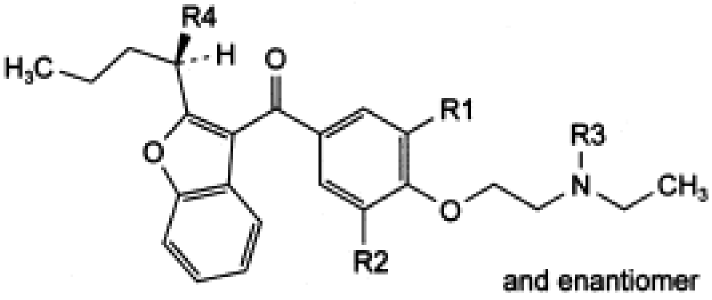
1 ml of *0.1 M sodium hydroxide* is equivalent to 68.18 mg of $C_{25}H_{30}ClI_2NO_3$.

STORAGE

Protected from light, at a temperature not exceeding 30 °C.

IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H.*

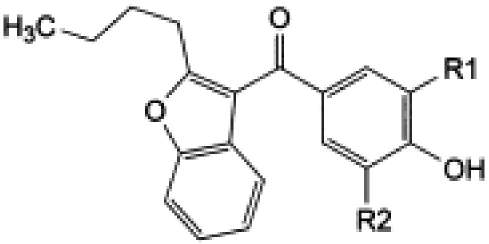


A. R1 = R2 = R4 = H, R3 = C_2H_5: (2-butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]phenyl] methanone,

B. R1 = R2 = I, R3 = R4 = H: (2-butylbenzofuran-3-yl)[4-[2-(ethylamino)ethoxy]-3,5-diiodophenyl]methanone,

C. R1 = I, R2 = R4 = H, R3 = C_2H_5: (2-butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]-3-iodophenyl]methanone,

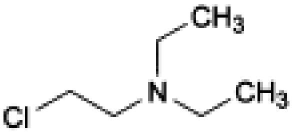
G. R1 = R2 = I, R3 = C_2H_5, R4 = OCH_3: [2-[(1*RS*)-1-methoxybutyl]benzofuran-3-yl][4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone,



D. R1 = R2 = I: (2-butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone,

E. R1 = R2 = H: (2-butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone,

F. R1 = I, R2 = H: (2-butylbenzofuran-3-yl)(4-hydroxy-3-iodophenyl)methanone,



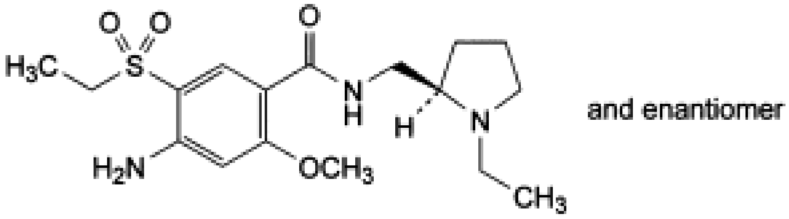
H. 2-chloro-*N*,*N*-diethylethanamine (2-chlorotriethylamine,(2-chloroethyl)diethylamine).

Ph Eur

Amisulpride

General Notices

(Ph Eur monograph 1490)



$C_{17}H_{27}N_3O_4S$ 369.5 *71675-85-9*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

4-Amino-*N*-[[(2*RS*)-1-ethylpyrrolidin-2-yl]methyl]-5-(ethylsulphonyl)-2-methoxybenzamide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in anhydrous ethanol.

mp

About 126 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison amisulpride CRS .

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 1.0 g in 3 ml of a mixture of 1 volume of *acetic acid R* and 4 volumes of *water R* and dilute to 20 ml with *water R*.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 5.0 g in *dimethylformamide R* and dilute to 50.0 ml with the same solvent.

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *sulpiride impurity A CRS* (amisulpride impurity A) in *methanol R* and dilute to 25 ml with the same solvent. Dilute 2 ml of the solution to 20 ml with *methanol R*.

Reference solution (b) Dilute 1 ml of the test solution to 10 ml with reference solution (a).

Plate *TLC silica gel G plate R*.

Mobile phase The upper layer obtained after shaking a mixture of a 50 per cent *V/V* solution of *concentrated ammonia R*, *anhydrous ethanol R* and *di-isopropyl ether R* (10:25:65 *V/V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Limit:

— *impurity A*: any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent).

Related substances

Examine by liquid chromatography (*2.2.29*).

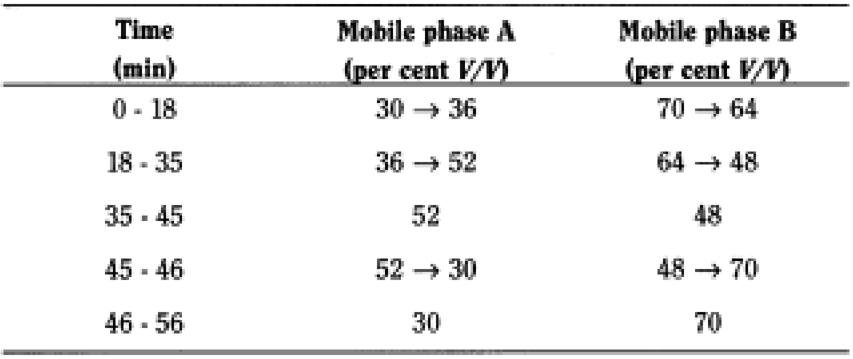
Test solution Dissolve 0.10 g in 30 ml of *methanol R* and dilute to 100.0 ml with mobile phase B.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B. Dilute 1.0 ml of the solution to 25.0 ml with a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Reference solution (b) Dissolve 5 mg of *amisulpride impurity B CRS* in 5 ml of the test solution and dilute to 50 ml with a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B. Dilute 1 ml of the solution to 10 ml with a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm) with a carbon loading of 16 per cent, a specific surface area of 330 m2/g and a pore size of 7.5 nm.

Mobile phase:
- *mobile phase A*: *methanol R*,
- *mobile phase B*: 0.7 g/l solution of *sodium octanesulphonate R* in a 0.25 per cent *V/V* solution of *dilute sulphuric acid R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 18	30 → 36	70 → 64
18 - 35	36 → 52	64 → 48
35 - 45	52	48
45 - 46	52 → 30	48 → 70
46 - 56	30	70

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to amisulpride and impurity B.

Limits:
- *any impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Chlorides *(2.4.4)*

Maximum 200 ppm.

Shake 0.5 g with 30 ml of *water R* for 10 min. Filter. 15 ml of the filtrate complies with the test.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 4.0 g by gently heating in 5 ml of *dilute acetic acid R*. Allow to cool and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash ***(2.4.14)***

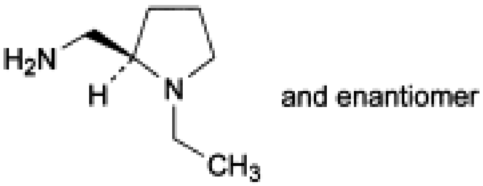
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

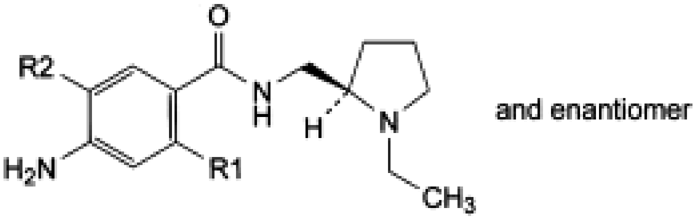
Dissolve 0.300 g with shaking in a mixture of 5 ml of *acetic anhydride R* and 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 36.95 mg of $C_{17}H_{27}N_3O_4S$.

IMPURITIES



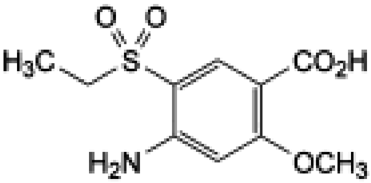
A. [(2*RS*)-1-ethylpyrrolidin-2-yl]methanamine,



B. R1 = OH, R2 = SO_2-CH_2-CH_3: 4-amino-*N*-[[(2*RS*)-1-ethylpyrrolidin-2-yl]methyl]-5-(ethylsulphonyl)-2-hydroxybenzamide,

C. R1 = OCH_3, R2 = I: 4-amino-*N*-[[(2*RS*)-1-ethylpyrrolidin-2-yl]methyl]-5-iodo-2-methoxybenzamide,

D. R1 = OCH_3, R2 = SO_2-CH_3: 4-amino-*N*-[[(2*RS*)-1-ethylpyrrolidin-2-yl]methyl]-2-methoxy-5-(methylsulphonyl)benzamide,

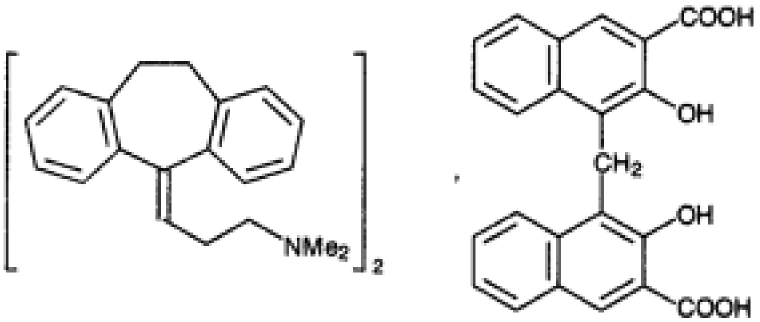


E. 4-amino-5-(ethylsulphonyl)-2-methoxybenzoic acid.

Ph Eur

Amitriptyline Embonate

General Notices



$(C_{20}H_{23}N)_2,C_{23}H_{16}O_6$ 943.2   *17086-03-2*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Amitriptyline Embonate is 3-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohept-5-ylidene) propyldimethylamine 4,4′-methylenebis(3-hydroxy-2-naphthoate). It contains not less than 98.5% and not more than 101.0% of $(C_{20}H_{23}N)_2,C_{23}H_{16}O_6$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A pale yellow to brownish yellow powder.

Practically insoluble in *water*; slightly soluble in *ethanol (96%).*

IDENTIFICATION

A. Dissolve 40 mg in 100 ml of *methanol*. To 1 ml of the solution add 1 ml of a 2.5% w/v solution of *sodium hydrogen carbonate*, 1 ml of a 2.0% w/v solution of *sodium periodate* and 1 ml of a 0.3% w/v solution of *potassium permanganate*, shake and allow to stand for 15 minutes. Acidify with 1M *sulphuric acid*, extract with 10 ml of *2,2,4-trimethylpentane* and filter. The *light absorption* of the filtrate, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 265 nm.

B. Dissolve 0.2 g in 10 ml of *dichloromethane*, add 5 ml of 1.25M *sodium hydroxide* and shake. The aqueous layer exhibits a green fluorescence when examined under *ultraviolet light (365 nm)*.

TESTS

Chloride

Not more than 0.2% when determined by the following method. Dissolve 1 g in a mixture of 50 ml of *acetone* and 50 ml of *water*, add 2 ml of *nitric acid* and 75 ml of *acetate buffer pH 5.0* and titrate with 0.01M *silver nitrate VS* determining the end point potentiometrically. Each ml of 0.01M *silver nitrate VS* is equivalent to 0.3545 mg of Cl.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using *silica gel G* as the coating substance and a mixture of 3 volumes of *diethylamine*, 15 volumes of *ethyl acetate* and 85 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application in an unlined tank. Apply separately to the plate 10 µl of each of three solutions in *chloroform* containing (1) 3.3% w/v of the substance being examined, (2) 0.0010% w/v of *dibenzosuberone EPCRS* and (3) 0.0040% w/v of *cyclobenzaprine hydrochloride EPCRS.* After removal of the plate, allow it to dry in air, spray with a freshly prepared mixture of 4 volumes of *formaldehyde* and 96 volumes of *sulphuric acid* , heat at 100° to 105° for 10 minutes and examine under *ultraviolet light (365 nm)*. Any spot corresponding to dibenzosuberone in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.05%, with reference to amitriptyline). Examine the plate under *ultraviolet light (254 nm)*. Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%, with reference to amitriptyline).

Sulphated ash

Not more than 0.2%, Appendix IX A.

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

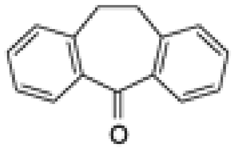
ASSAY

Dissolve 0.6 g in 50 ml of *acetic anhydride* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each ml of *0.1M perchloric acid VS* is equivalent to 47.16 mg of $(C_{20}H_{23}N)_2,C_{23}H_{16}O_6$.

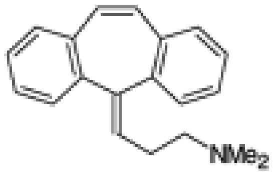
STORAGE

Amitriptyline Embonate should be protected from light.

IMPURITIES



A. dibenzosuberone

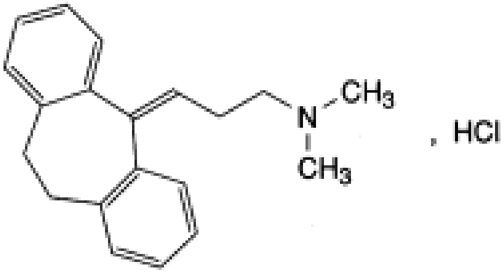


B. cyclobenzaprine.

Amitriptyline Hydrochloride

General Notices

(Ph Eur monograph 0464)



$C_{20}H_{23}N,HCl$ 313.9 *549-18-8*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Amitriptyline Tablets

Ph Eur

DEFINITION

3-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*,*N*-dimethylpropan-1-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or colourless crystals.

Solubility

Freely soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *amitriptyline hydrochloride CRS* .

B. 50 mg gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

Dissolve 1.25 g in *water R* and dilute to 25 ml with the same solvent.

Acidity or alkalinity

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. Add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid* . The solution is red.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *dibenzosuberone CRS* (impurity A) and 5.0 mg of *cyclobenzaprine hydrochloride CRS* (impurity B) in 5.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Column:

- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 5.23 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 7.0 with *phosphoric acid R*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Run time 3 times the retention time of amitriptyline.

Relative retention With reference to amitriptyline (retention time = about 14 min): impurity B = about 0.9; impurity A = about 2.2.

System suitability Reference solution (a):

- *resolution*: minimum 2.0 between the peaks due to impurity B and amitriptyline.

Limits:

- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *impurity A*: not more than 0.5 times the area of the corresponding peak in the

chromatogram obtained with reference solution (b) (0.05 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to amitriptyline in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the peak due to amitriptyline in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the peak due to amitriptyline in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 30 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.39 mg of $C_{20}H_{24}ClN$.

STORAGE

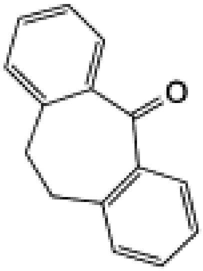
Protected from light.

IMPURITIES

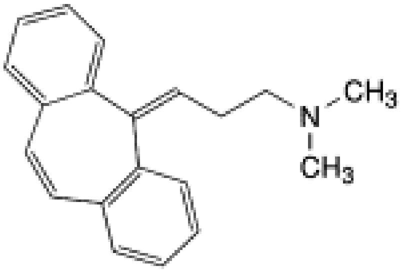
Specified impurities   *A, B.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*

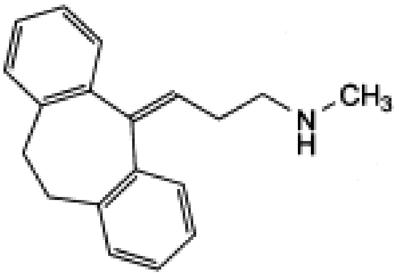
C, D, E, F, G.



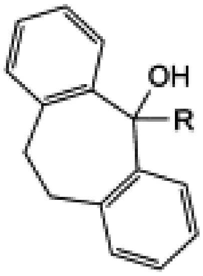
A. 10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-one (dibenzosuberone),



B. 3-(5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*,*N*-dimethylpropan-1-amine (cyclobenzaprine),

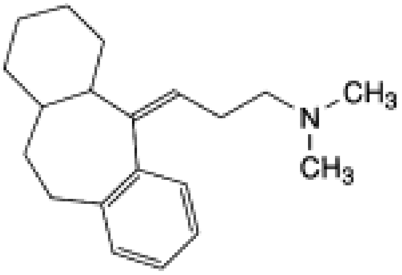


C. 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*-methylpropan-1-amine (nortriptyline),

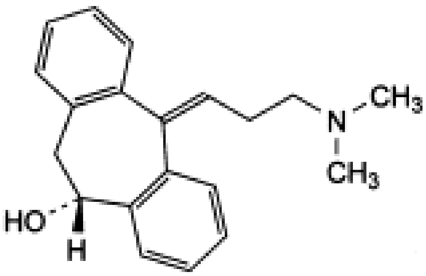


D. R = CH_2-CH_2-CH_2-$N(CH_3)_2$: 5-[3-(dimethylamino)propyl]-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ol,

G. R = H: 10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ol (dibenzosuberol),



E. *N*,*N*-dimethyl-3-(1,2,3,4,4a,10,11,11a-octahydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)propan-1-amine,


its (E)-isomer and
their enantiomers

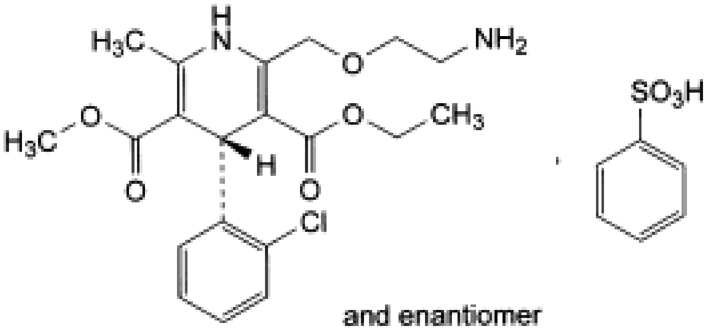
F. (5*EZ*,10*RS*)-5-[3-(dimethylamino)propylidene]-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-10-ol.

Ph Eur

Amlodipine Besilate

General Notices

(Ph Eur monograph 1491)



$C_{20}H_{25}ClN_2O_5,C_6H_6O_3S$ 567.1 *111470-99-6*

Action and use

Calcium channel blocker.

Ph Eur

DEFINITION

3-Ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulphonate.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in methanol, sparingly soluble in ethanol, slightly soluble in 2-propanol.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *amlodipine besilate CRS* .

Preparation Mulls.

B. Examine the chromatograms obtained in test A for related substances in ultraviolet light at 366 nm.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (b).

C. Dissolve 5.0 mg in a 1 per cent *V/V* solution of *0.1 M hydrochloric acid* in *methanol R* and dilute to 100.0 ml with the same acid solution. Examined between 300 nm and 400 nm *(2.2.25)*, the solution shows an absorption maximum at 360 nm. The specific absorbance at the maximum is 113 to 121.

TESTS

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 0.250 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

A. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.140 g of the substance to be examined in *methanol R* and dilute to 2.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol R*.

Reference solution (a) Dissolve 70.0 mg of *amlodipine besilate CRS* in 1.0 ml of *methanol R*.

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with *methanol R*.

Reference solution (c) Dilute 3.0 ml of test solution (b) to 100.0 ml with *methanol R*.

Reference solution (d) Dilute 1.0 ml of test solution (b) to 100.0 ml with *methanol R*.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase The upper layer of a mixture of *glacial acetic acid R*, *water R* and *methyl isobutyl ketone R* (25:25:50 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying For 15 min at 80 °C.

Detection Examine in ultraviolet light at 254 nm and 366 nm.

System suitability The chromatogram obtained with reference solution (a) shows 2 clearly separated minor spots with R_F values of about 0.18 and 0.22.

Limits In the chromatogram obtained with test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.3 per cent) and at most 2 spots are more intense than the spot in the chromatogram obtained with reference solution (d) (0.1 per cent).

B. Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b) Dilute 5.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *amlodipine besilate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 3.0 ml of test solution (a) to 100.0 ml with the mobile phase and dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 5 mg of the substance to be examined in 5 ml of *strong hydrogen peroxide solution R*. Heat at 70 °C for 45 min.

Column:
— *size:* l = 0.15 m, Ø = 3.9 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 15 volumes of *acetonitrile R*, 35 volumes of *methanol R* and 50 volumes of a solution prepared as follows: dissolve 7.0 ml of *triethylamine R* in 1 litre of *water R* and adjust to pH 3.0 ± 0.1 with *phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 237 nm.

Injection 10 µl; inject test solution (a) and reference solutions (b) and (c).

Run time 3 times the retention time of amlodipine.

Relative retention With reference to amlodipine (retention time = about 7 min): impurity D = about 0.5.

System suitability Reference solution (c):

— *resolution*: minimum 4.5 between the peaks corresponding to amlodipine and impurity D.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 2,

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *total of other impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent); disregard any peak due to benzene sulphonate (relative retention = about 0.2),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 3.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

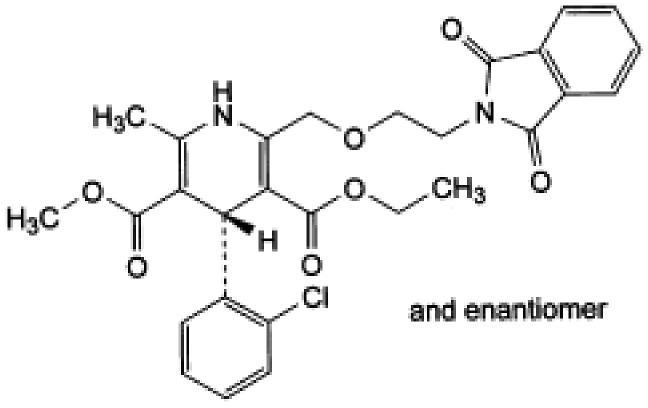
Injection Test solution (b), reference solution (a).

Calculate the percentage content of amlodipine besilate from the areas of the peaks and the declared content of $C_{26}H_{31}ClN_{2}0_{8}S$ in *amlodipine besilate CRS* .

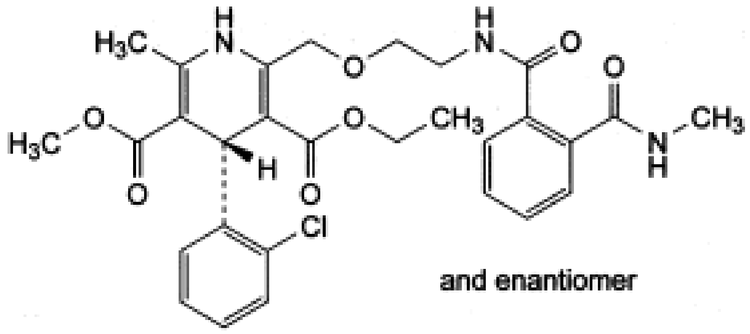
STORAGE

In an airtight container , protected from light.

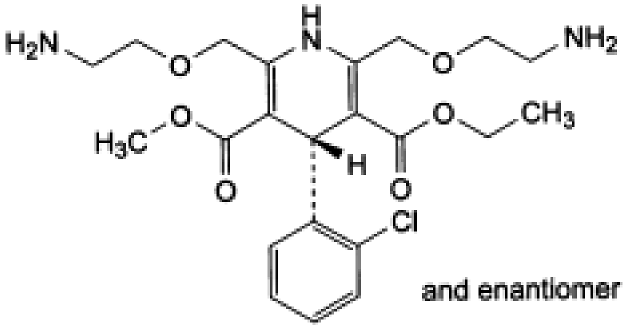
IMPURITIES



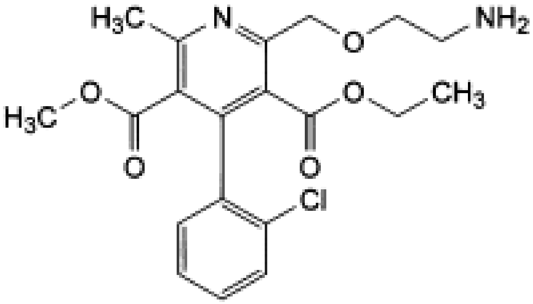
A. 3-ethyl 5-methyl (4*RS*)-4-(2-chlorophenyl)-2-[[2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) ethoxy]methyl]-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate,



B. 3-ethyl 5-methyl (4*RS*)-4-(2-chlorophenyl)-6-methyl-2-[[2-[[2-(methylcarbamoyl)benzoyl] amino]ethoxy]methyl]-1,4-dihydropyridine-3,5-dicarboxylate,



C. ethyl methyl (4*RS*)-2,6-bis[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate,



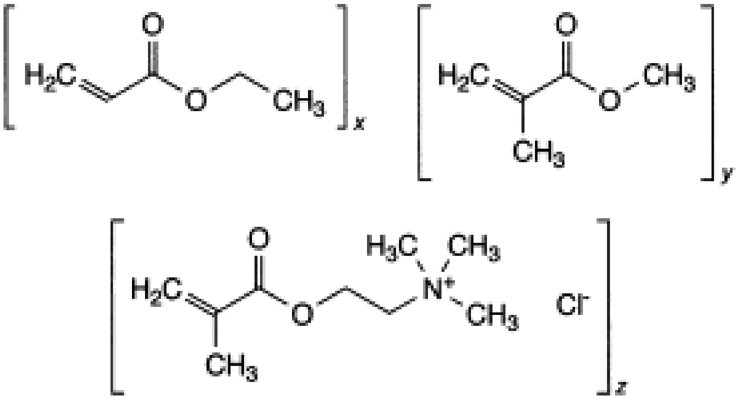
D. 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate.

Ph Eur

Ammonio Methacrylate Copolymer (Type A)

General Notices

(Ph Eur monograph 2081)



Action and use

Excipient.

Ph Eur

DEFINITION

Poly(ethyl propenoate-co-methyl 2-methylpropenoate-co-2-(trimethylammonio)ethyl 2-methylpropenoate) chloride having a mean relative molecular mass of about 150 000.

The ratio of ethyl propenoate groups to methyl 2-methylpropenoate groups to 2-(trimethylammonio)ethyl 2-methylpropenoate groups is about 1:2:0.2.

Content of ammonio methacrylate groups 8.9 per cent to 12.3 per cent (dried substance).

CHARACTERS

Appearance

Colourless to white or almost white granules or powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol and in methylene chloride giving clear to cloudy solutions. Due to the polymeric nature of the substance, a stirring time of up to 5 h may be necessary.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of ammonio methacrylate copolymer (type A).

B. Viscosity (see Tests).

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve a quantity of the substance to be examined corresponding to 12.5 g of the dried substance in a mixture of 35.0 g of *acetone R* and 52.5 g of *2-propanol R*.

Viscosity *(2.2.10)*

Maximum 15 mPa·s, determined on solution S.

Apparatus Rotating viscometer.

Dimensions:
- *spindle*: diameter = 25.15 mm; height = 90.74 mm; shaft diameter = 4.0 mm;
- *cylinder*: diameter = 27.62 mm; height = 0.135 m.

Stirring speed 30 r/min.

Volume of solution 16 ml of solution S.

Temperature 20 °C.

Appearance of a film

Spread 2 ml of solution S evenly on a glass plate. Upon drying a clear film is formed.

Monomers

Liquid chromatography *(2.2.29)*.

Solution A Dissolve 3.5 g of *sodium perchlorate R* in *water for chromatography R* and dilute to 100 ml with the same solvent.

Test solution Dissolve 5.00 g of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent. To 10.0 ml of this solution add 5.0 ml of solution A, dropwise, while continuously stirring. Remove the precipitated polymer by centrifugation. Use the clear supernatant solution.

Reference solution Dissolve 50.0 mg of *ethyl acrylate R* and 10.0 mg of *methyl methacrylate R* in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Add 10 ml of this solution to 5 ml of solution A.

Column:
- *size: l* = 0.12 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Dilute phosphoric acid R with *water for chromatography R* to obtain a solution at pH 2.0; mix 800 ml of this solution and 200 ml of *methanol R*, filter and degas.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 202 nm.

Injection 50 µl.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to impurity A and impurity B.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (100 ppm);

— *impurity B:* not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (50 ppm).

Methanol *(2.4.24, System A)*

Maximum 1.5 per cent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2.0 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 3.0 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 5 h.

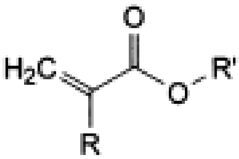
ASSAY

Dissolve 1.000 g in a mixture of 3 ml of *anhydrous formic acid R* and 30 ml of *anhydrous acetic acid R* and heat to dissolve. Add 20 ml *of acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 20.77 mg of $C_9H_{18}O_2NCl$ (ammonio methacrylate groups).

IMPURITIES

Specified impurities *A, B*.



A. R = H, R′ = C_2H_5: ethyl propenoate (ethyl acrylate),

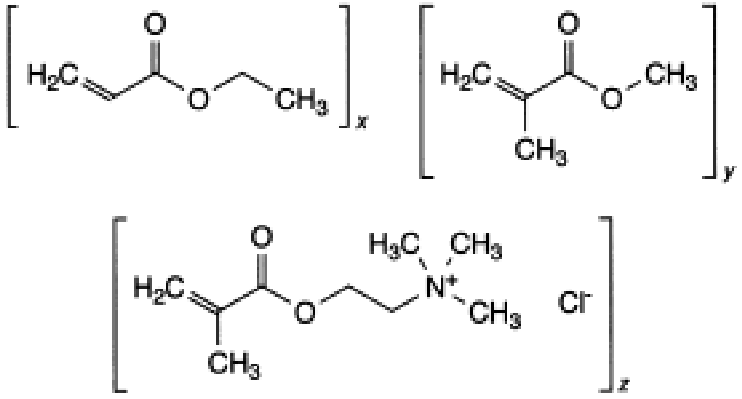
B. R = R′ = CH_3: methyl 2-methylpropenoate (methyl methacrylate).

Ph Eur

Ammonio Methacrylate Copolymer (Type B)

General Notices

(Ph Eur monograph 2082)



Action and use

Excipient.

Ph Eur

DEFINITION

Poly(ethyl propenoate-co-methyl 2-methylpropenoate-co-2-(trimethylammonio)ethyl 2-methylpropenoate) chloride having a mean relative molecular mass of about 150 000.

The ratio of ethyl propenoate groups to methyl 2-methylpropenoate groups to 2-(trimethylammonio)ethyl 2-methylpropenoate groups is about 1:2:0.1.

Content of ammonio methacrylate groups 4.5 per cent to 7.0 per cent (dried substance).

CHARACTERS

Appearance

Colourless to white or almost white granules or powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol and in methylene chloride giving clear to cloudy solutions. Due to the polymeric nature of the substance, a stirring time of up to 5 h may be necessary.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of ammonio methacrylate copolymer (type B) .*

B. Viscosity (see Tests).

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve a quantity of the substance to be examined corresponding to 12.5 g of the dried substance in a mixture of 35.0 g of *acetone R* and 52.5 g of *2-propanol R*.

Viscosity *(2.2.10)*

Maximum 15 mPa·s, determined on solution S.

Apparatus Rotating viscometer.

Dimensions:

— *spindle*: diameter = 25.15 mm; height = 90.74 mm; shaft diameter = 4.0 mm;

— *cylinder*: diameter = 27.62 mm; height = 0.135 m.

Stirring speed 30 r/min.

Volume of solution 16 ml of solution S.

Temperature 20 °C.

Appearance of a film

Spread 2 ml of solution S evenly on a glass plate. Upon drying a clear film is formed.

Monomers

Liquid chromatography *(2.2.29)*.

Solution A Dissolve 3.5 g of *sodium perchlorate R* in *water for chromatography R* and dilute to 100 ml with the same solvent.

Test solution Dissolve 5.00 g of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent. To 10.0 ml of this solution add 5.0 ml of solution A, dropwise, while continuously stirring. Remove the precipitated polymer by centrifugation. Use the clear supernatant solution.

Reference solution Dissolve 50.0 mg of *ethyl acrylate R* and 10.0 mg of *methyl methacrylate R* in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Add 10 ml of this solution to 5 ml of solution A.

Column:

— *size:* l = 0.12 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Dilute *phosphoric acid R* with *water for chromatography R* to obtain a solution at pH 2.0; mix 800 ml of this solution and 200 ml of *methanol R*, filter and degas.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 202 nm.

Injection 50 µl.

System suitability Reference solution:

- — *resolution*: minimum 1.5 between the peaks due to impurity A and impurity B.

Limits:

- — *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (100 ppm);
- — *impurity B*: not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (50 ppm).

Methanol *(2.4.24, System A)*

Maximum 1.5 per cent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2.0 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 3.0 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 5 h.

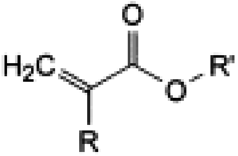
ASSAY

Dissolve 2.000 g in a mixture of 3 ml of *anhydrous formic acid R* and 30 ml of *anhydrous acetic acid R* and heat to dissolve. Add 20 ml of *acetic anhydride R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 20.77 mg of $C_9H_{18}O_2NCl$ (ammonio methacrylate groups).

IMPURITIES

Specified impurities *A, B*.



A. R = H, R′ = C_2H_5: ethyl propenoate (ethyl acrylate),

B. R = R′ = CH_3: methyl 2-methylpropenoate (methyl methacrylate).

Ph Eur

Ammonium Bicarbonate

General Notices

(Ammonium Hydrogen Carbonate, Ph Eur monograph 1390)

NH_4HCO_3 79.1 *1066-33-7*

Action and use

Expectorant.

Preparations

Aromatic Ammonia Solution

Strong Ammonium Acetate Solution

Aromatic Ammonia Spirit

Ph Eur

DEFINITION

Content

98.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

Fine, white or almost white, crystalline powder or white or almost white crystals, slightly hygroscopic.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

It volatilises rapidly at 60 °C. The volatilisation takes place slowly at ambient temperatures if the substance is slightly moist. It is in a state of equilibrium with ammonium carbamate.

IDENTIFICATION

A. It gives the reaction of carbonates and bicarbonates *(2.3.1)*.

B. Dissolve 50 mg in 2 ml of *water R*. The solution gives the reaction of ammonium salts *(2.3.1)*.

TESTS

Solution S

Dissolve 14.0 g in 100 ml of *distilled water R.* Boil to remove the ammonia, allow to cool and dilute to 100.0 ml with *distilled water R*.

Chlorides *(2.4.4)*

Maximum 70 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 70 ppm, determined on solution S.

Iron *(2.4.9)*

Maximum 40 ppm.

Dilute 1.8 ml of solution S to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve cautiously 2.5 g in 25 ml of *1 M hydrochloric acid* . 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve cautiously 1.0 g in 20.0 ml of *0.5 M sulphuric acid* and dilute to 50 ml with *water R*. Boil, cool and titrate the excess of acid with *1 M sodium hydroxide*, using 0.1 ml of *methyl red solution R* as indicator.

1 ml of *0.5 M sulphuric acid* is equivalent to 79.1 mg of NH_4HCO_3.

STORAGE

In an airtight container .

Ph Eur

Ammonium Bromide

General Notices

(Ph Eur monograph 1389)

NH_4Br 97.9 *12124-97-9*

Ph Eur

DEFINITION

Content

98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in alcohol.

It becomes yellow when exposed to light or air.

IDENTIFICATION

A. It gives reaction (a) of bromides *(2.3.1)*.

B. 10 ml of solution S (see Tests) gives the reaction of ammonium salts *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromates

To 10 ml of solution S add 1 ml of *starch solution R*, 0.1 ml of a 100 g/l solution of *potassium iodide R* and 0.25 ml of *0.5 M sulphuric acid* and allow to stand protected from light for 5 min. No blue or violet colour develops.

Chlorides

Maximum 0.6 per cent.

In a conical flask, dissolve 1.000 g in 20 ml of *dilute nitric acid R*. Add 5 ml of *strong hydrogen peroxide solution R* and heat on a water-bath until the solution is completely decolorised. Wash down the sides of the flask with a little *water R* and heat on a water-bath for 15 min. Allow to cool, dilute to 50 ml with *water R* and add 5.0 ml of *0.1 M silver nitrate* and 1 ml of *dibutyl phthalate R*. Shake and titrate with *0.1 M ammonium thiocyanate* using 5 ml of *ferric ammonium sulphate solution R2* as indicator. Not more than 1.7 ml of *0.1 M silver nitrate* is used. Note the volume of *0.1 M silver nitrate* used (see Assay). Carry out a blank test.

Iodides

To 5 ml of solution S add 0.15 ml of *ferric chloride solution R1* and 2 ml of *methylene chloride R*. Shake and allow to separate. The lower layer is colourless *(2.2.2, Method I)*.

Sulphates *(2.4.13)*

Maximum 100 ppm.

15 ml of solution S complies with the limit test for sulphates.

Iron *(2.4.9)*

Maximum 20 ppm.

5 ml of solution S diluted to 10 ml with *water R* complies with the limit test for iron.

Magnesium and alkaline-earth metals *(2.4.7)*

Maximum 200 ppm, calculated as Ca.

10.0 g complies with the limit test for magnesium and alkaline-earth metals. The volume of *0.01 M sodium edetate* used does not exceed 5.0 ml.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 1.500 g in *water R* and dilute to 100.0 ml with the same solvent. To 10.0 ml of the solution add 50 ml of *water R*, 5 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R*. Shake. Titrate with *0.1 M ammonium thiocyanate* using 2 ml of *ferric* *ammonium sulphate solution R2* as indicator and shaking vigorously towards the end-point.

1 ml of *0.1 M silver nitrate* is equivalent to 9.794 mg of NH_4Br.

Calculate the percentage content of NH_4Br from the expression:

$$a - 2.763\,b$$

a = percentage content of NH_4Br and NH_4Cl obtained in the assay and calculated as NH_4Br,

b = percentage content of Cl obtained in the test for chlorides.

STORAGE

In an airtight container, protected from light.

Ph Eur

Ammonium Chloride

General Notices

(Ph Eur monograph 0007)

NH_4Cl 53.49 *12125-02-9*

Action and use

Used for the acidification of urine and to correct metabolic alkalosis.

Preparation

Ammonium Chloride Mixture

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water.

IDENTIFICATION

A. It gives the reactions of chlorides *(2.3.1)*.

B. 10 ml of solution S (see Tests) gives the reaction of ammonium salts *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.5 ml *of 0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides and iodides

To 10 ml of solution S add 0.1 ml of *dilute hydrochloric acid R* and 0.05 ml of *chloramine solution R*. After 1 min, add 2 ml of *chloroform R* and shake vigorously. The chloroform layer remains colourless *(2.2.2, Method I)*.

Sulphates *(2.4.13)*

Maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Calcium *(2.4.3)*

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Iron *(2.4.9)*

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 1.000 g in 20 ml of *water R* and add a mixture of 5 ml of *formaldehyde solution R*, previously neutralised to *phenolphthalein solution R*, and 20 ml of *water R*. After 1-2 min, titrate slowly with *1 M sodium hydroxide*, using a further 0.2 ml of the same indicator.

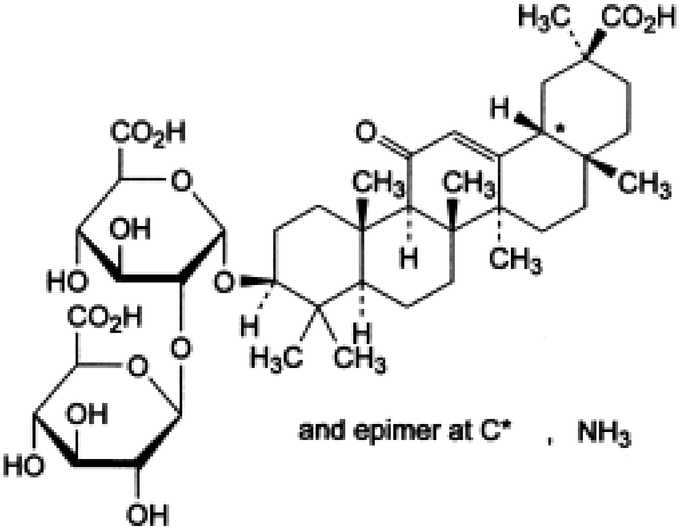
1 ml of *1 M sodium hydroxide* is equivalent to 53.49 mg of NH_4Cl.

Ph Eur

Ammonium Glycyrrhizinate

General Notices

(Ammonium Glycyrrhizate, Ph Eur monograph 1772)



$C_{42}H_{65}NO_{16}$ *840* *53956-04-0*

Ph Eur

DEFINITION

Mixture of ammonium 18α- and 18β-glycyrrhizate (ammonium salt of (20β)-3β-[[2-*O*-(β-D-glucopyranosyluronic acid)-α-D-glucopyranosyluronic acid]oxy]-11-oxoolean-12-en-29-oic acid), the 18β-isomer being the main component.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish-white, hygroscopic powder.

Solubility

Slightly soluble in water, very slightly soluble in anhydrous ethanol, practically insoluble in acetone. It dissolves in dilute solutions of acids and of alkali hydroxides.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ammonium glycyrrhizate CRS*.

B. Dissolve 0.1 g in 20 ml of *water R*, add 2 ml of *dilute sodium hydroxide solution R* and heat cautiously. On heating, the solution gives off vapours that may be identified by the alkaline reaction of wet litmus paper *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method I)*.

Dissolve 1.0 g in *ethanol (20 per cent V/V) R* and dilute to 100.0 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 49.0 to + 54.0 (anhydrous substance).

Dissolve 0.5 g in *ethanol (50 per cent V/V) R* and dilute to 50.0 ml with the same solvent.

Related substances Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 50 mg of *ammonium glycyrrhizate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Column:
- *size: l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase *glacial acetic acid R*, *acetonitrile R*, *water R* (6:380:614 *V/V/V*).

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 3 times the retention time of 18β-glycyrrhizic acid.

Relative retention With reference to 18β-glycyrrhizic acid (retention time = about 8 min): impurity A = about 0.8; 18α-glycyrrhizic acid = about 1.2.

System suitability Reference solution (b):

- *resolution*: minimum 2.0 between the peaks due to 18β-glycyrrhizic acid and 18α-glycyrrhizic acid.

Limits:
- *18α-glycyrrhizic acid*: not more than twice the sum of the areas of the peaks in the chromatogram obtained with reference solution (a) (10.0 per cent),

— *impurity A*: not more than the sum of the areas of the peaks in the chromatogram obtained with reference solution (a) (5.0 per cent),

— *any other impurity*: for each impurity, not more than 0.4 times the sum of the areas of the peaks in the chromatogram obtained with reference solution (a) (2.0 per cent),

— *sum of other impurities*: not more than 1.4 times the sum of the areas of the peaks in the chromatogram obtained with reference solution (a) (7.0 per cent),

— *disregard limit*: 0.04 times the sum of the areas of the peaks in the chromatogram obtained with reference solution (a) (0.2 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 6.0 per cent, determined on 0.250 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

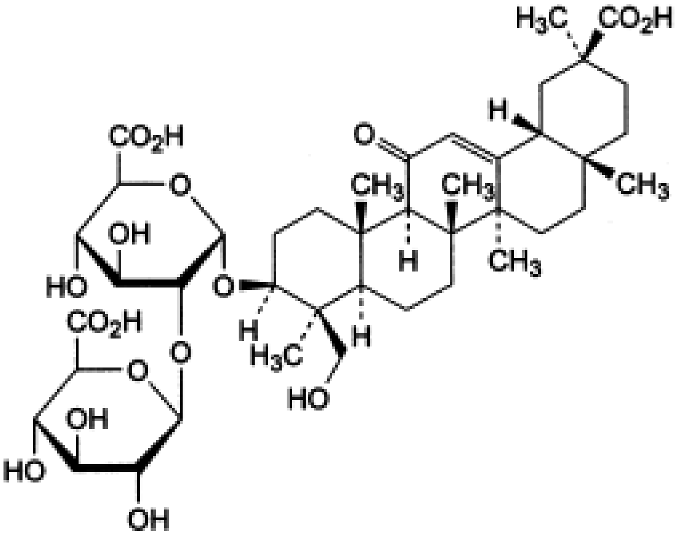
Dissolve 0.600 g in 60 ml of *anhydrous acetic acid R* heating at 80 °C if necessary. Cool. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 84.0 mg of $C_{42}H_{65}NO_{16}$.

STORAGE

In an airtight container.

IMPURITIES



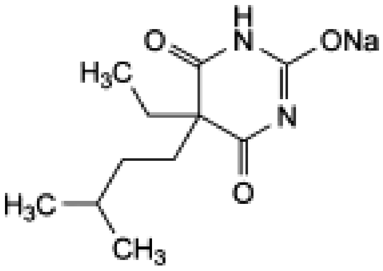
A. (4β,20β)-3β-[[2-O-(β-D-glucopyranosyluronic acid)-α-D-glucopyranosyluronic acid]oxy]-23-hydroxy-11-oxoolean-12-en-29-oic acid (24-hydroxyglycyrrhizinic acid).

Ph Eur

Amobarbital Sodium

General Notices

(Ph Eur monograph 0166)



$C_{11}H_{17}N_2NaO_3$ 248.3 *64-43-7*

Action and use

Barbiturate.

Ph Eur

DEFINITION

Amobarbital sodium contains not less than 98.5 per cent and not more than the equivalent of 102.0 per cent of sodium derivative of 5-ethyl-5-(3-methylbutyl)pyrimidin-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, granular powder, hygroscopic, very soluble in carbon dioxide-free water (a small fraction may be insoluble), freely soluble in alcohol.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. Acidify 10 ml of solution S (see Tests) with *dilute hydrochloric acid R* and shake with 20 ml of *ether R*. Separate the ether layer, wash with 10 ml of *water R*, dry over *anhydrous sodium sulphate R* and filter. Evaporate the filtrate to dryness and dry the residue at 100 °C to 105 °C (test residue). Repeat the operations using 0.1 g of *amobarbital sodium CRS* (reference residue). Determine the melting point *(2.2.14)* of the test residue. Mix equal parts of the test residue and the reference residue and determine the melting point of the mixture. The difference between the melting points (which are about 157 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing the spectrum obtained with the reference residue prepared from *amobarbital sodium CRS* with that obtained with the test residue (see identification test A).

C. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 0.1 g of *amobarbital sodium CRS* in *alcohol R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates *(2.3.1)*.

E. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *alcohol (50 per cent V/V) R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent. Disregard any slight residue. The pH of the solution is not more than 11.0.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *alcohol R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine the plate immediately in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent). Disregard any spot at the starting-point.

Loss on drying *(2.2.32)*

Not more than 3.0 per cent, determined on 0.50 g by drying in an oven at 130 °C.

ASSAY

Dissolve 0.200 g in 5 ml of *ethanol R*. Add 0.5 ml of *thymolphthalein solution R* and 10 ml of *silver nitrate solution in pyridine R*. Titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained. Carry out a blank titration.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 24.83 mg of $C_{11}H_{17}N_2NaO_3$.

STORAGE

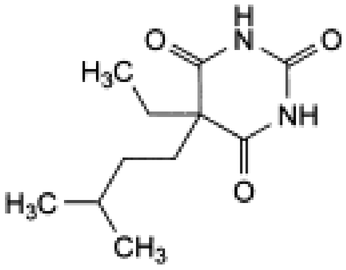
Store in an airtight container .

Ph Eur

Amobarbital

General Notices

(Ph Eur monograph 0594)



$C_{11}H_{18}N_2O_3$ 226.3 *57-43-2*

Action and use

Barbiturate.

Ph Eur

DEFINITION

Amobarbital contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 5-ethyl-5-(3-methylbutyl)pyrimidin-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, freely soluble in alcohol, soluble in methylene chloride. It forms water-soluble compounds with alkali hydroxides and carbonates and with ammonia.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Determine the melting point *(2.2.14)* of the substance to be examined. Mix equal parts of the substance to be examined and *amobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 157 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *amobarbital CRS* .

C. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 0.1 g of *amobarbital CRS* in *alcohol R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer from a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 6 ml of *water R*. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 1.0 g add 50 ml of *water R* and boil for 2 min. Allow to cool and filter. To 10 ml of the filtrate add 0.15 ml of *methyl red solution R* and 0.1 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.2 ml of *0.01 M hydrochloric acid*. The solution is red.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *alcohol R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer from a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine the plate immediately in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution. Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 5 ml of *pyridine R*. Add 0.5 ml of *thymolphthalein solution R* and 10 ml of *silver nitrate solution* in *pyridine R*. Titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained. Carry out a blank titration.

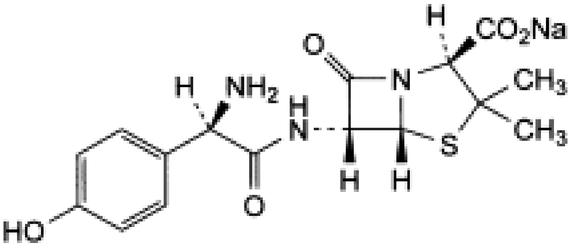
1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 11.31 mg of $C_{11}H_{18}N_2O_3$.

Ph Eur

Amoxicillin Sodium

General Notices

(Ph Eur monograph 0577)



$C_{16}H_{18}N_3NaO_5S$ *387.4* 34642-77-8

Action and use

Penicillin antibacterial.

Preparations

Amoxicillin Injection

Co-amoxiclav Injection

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

89.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, very hygroscopic, powder.

Solubility

Very soluble in water, sparingly soluble in anhydrous ethanol, very slightly soluble in acetone.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 0.250 g in 5 ml of *water R*, add 0.5 ml of *dilute acetic acid R*, swirl and allow to stand for 10 min in iced water. Filter the crystals and wash with 2-3 ml of a mixture of 1 volume of *water R* and 9 volumes of *acetone R*, then dry in an oven at 60 °C for 30 min.

Comparison *amoxicillin trihydrate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 10 ml of *sodium hydrogen carbonate solution R*.

Reference solution (a) Dissolve 25 mg of *amoxicillin trihydrate CRS* in 10 ml of *sodium hydrogen carbonate solution R.*

Reference solution (b) Dissolve 25 mg of *amoxicillin trihydrate CRS* and 25 mg of *ampicillin trihydrate CRS* in 10 ml of *sodium hydrogen carbonate solution R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 10 volumes of *acetone R* and 90 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube in a water-bath for 1 min; a dark yellow colour develops.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)*, it may show an initial, but transient, pink colour, and after 5 min, its absorbance *(2.2.25)* at 430 nm is not greater than 0.20.

Dissolve 1.0 g in *water R* and dilute to 10.0 ml with the same solvent. Examine immediately after dissolution.

pH *(2.2.3)*

8.0 to 10.0.

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 240 to + 290 (anhydrous substance).

Dissolve 62.5 mg in a 4 g/l solution of *potassium hydrogen phthalate R* and dilute to 25.0 ml with the same solution.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 30.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Test solution (b) Dissolve 30.0 mg of the substance to be examined in mobile phase A and dilute to 20.0 ml with mobile phase A. *Prepare immediately before use.*

Reference solution (a) Dissolve 30.0 mg of *amoxicillin trihydrate CRS* in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (b) Dissolve 4.0 mg of *cefadroxil CRS* in mobile phase A and dilute to 50 ml with mobile phase A. To 5.0 ml of this solution add 5.0 ml of reference solution (a) and dilute to 100 ml with mobile phase A.

Reference solution (c) Dilute 2.0 ml of reference solution (a) to 20.0 ml with mobile phase A. Dilute 5.0 ml of this solution to 20.0 ml with mobile phase A.

Reference solution (d) To 0.20 g of *amoxicillin trihydrate R* add 1.0 ml of *water R*. Shake and add dropwise *dilute sodium hydroxide solution R* to obtain a solution. The pH of the solution is about 8.5. Store the solution at room temperature for 4 h. Dilute 0.5 ml of this solution to 50.0 ml with mobile phase A.

Column:

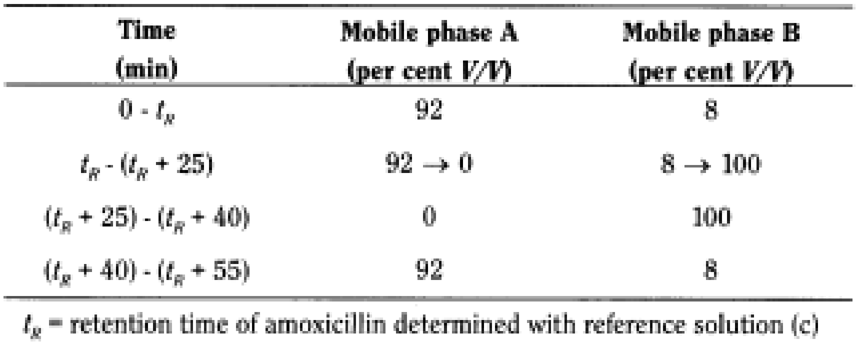
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 1 volume of *acetonitrile R* and 99 volumes of a 25 per cent *V/V* solution of *0.2 M potassium dihydrogen phosphate R* adjusted to pH 5.0 with *dilute sodium hydroxide solution R*;

— *mobile phase B*: mix 20 volumes of *acetonitrile R* and 80 volumes of a 25 per cent *V/V* solution of *0.2 M potassium dihydrogen phosphate R* adjusted to pH 5.0 with *dilute sodium hydroxide solution R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	92	8
t_R - (t_R + 25)	92 → 0	8 → 100
(t_R + 25) - (t_R + 40)	0	100
(t_R + 40) - (t_R + 55)	92	8

t_R = retention time of amoxicillin determined with reference solution (c)

If the mobile phase has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 50 µl of reference solutions (b) and (c) with isocratic elution at the initial mobile phase composition and 50 µl of test solution (b) and reference solution (d) according to the elution gradient described under Mobile phase; inject mobile phase A as a blank according to the elution gradient described under Mobile phase.

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the 3 principal peaks eluted after the main peak corresponding to impurity C, amoxicillin dimer (impurity J; n = 1) and amoxicillin trimer (impurity J; n = 2).

Relative retention With reference to amoxicillin: impurity C = about 3.4; impurity J (n = 1) = about 4.1; impurity J (n = 2) = about 4.5.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to amoxicillin and cefadroxil; if necessary, adjust the ratio A:B of the mobile phase.

Limits:

— *impurity J (n = 1)*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3 per cent);

— *any other impurity: for each impurity*, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2 per cent);

— *total*: not more than 9 times the area of the principal peak in the chromatogram obtained with reference solution (c) (9 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

N*,*N*-Dimethylaniline *(2.4.26, Method A or B)

Maximum 20 ppm.

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.8 per cent m/m.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 0.400 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

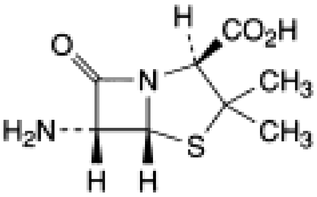
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of amoxicillin sodium by multiplying the percentage content of amoxicillin by 1.060.

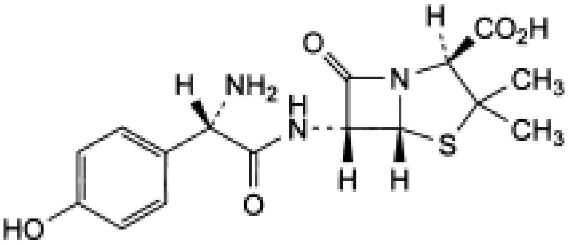
STORAGE

In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

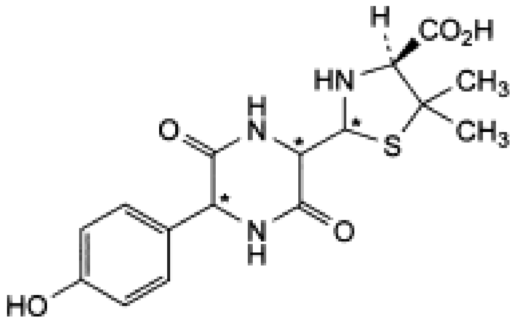
IMPURITIES



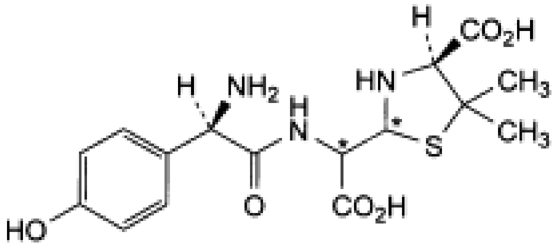
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



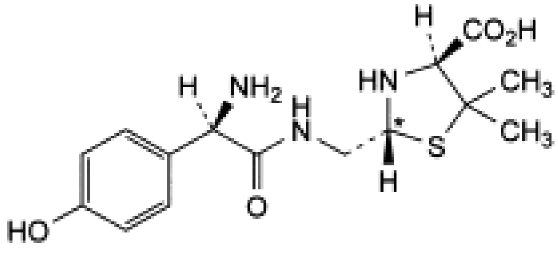
B. (2*S*,5*R*,6*R*)-6-[[(2*S*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (L-amoxicillin),


H
CO2H
HN
CH3
CH3
S
O
N
H
*
*
*
N
H
O
HO

C. (4*S*)-2-[5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid (amoxicillin diketopiperazines),

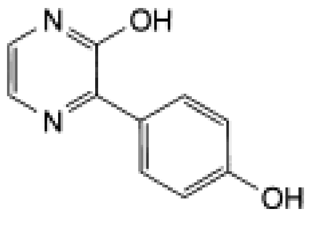

H
CO2H
H
NH2
HN
CH3
H
N
*
*
S
CH3
O
CO2H
HO

D. (4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin),

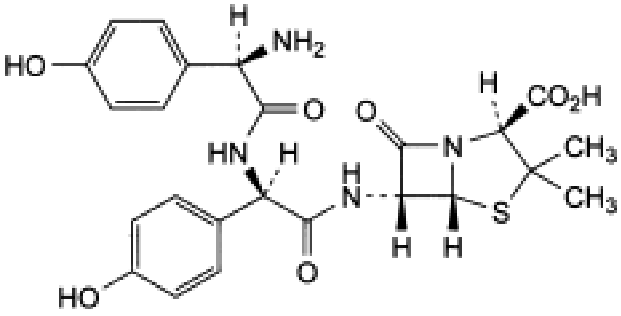

H
CO2H
H
NH2
HN
CH3
H
N
*
S
CH3
H
O
HO

and epimer at C*

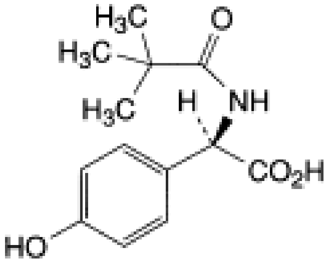
E. (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of amoxicillin),



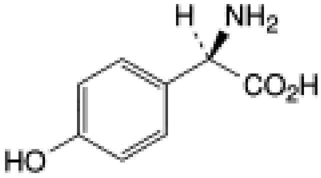
F. 3-(4-hydroxyphenyl)pyrazin-2-ol,



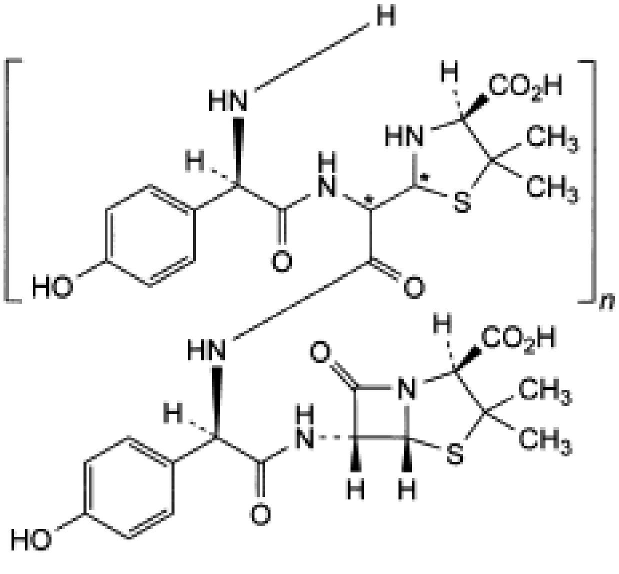
G. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane2-carboxylic acid (D-(4-hydroxyphenyl)glycylamoxicillin),



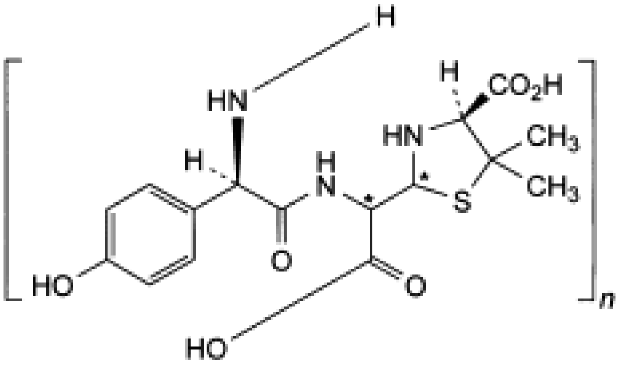
H. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-(4-hydroxyphenyl)acetic acid,



I. (2*R*)-2-amino-2-(4-hydroxyphenyl)acetic acid,



J. co-oligomers of amoxicillin and penicilloic acids of amoxicillin,



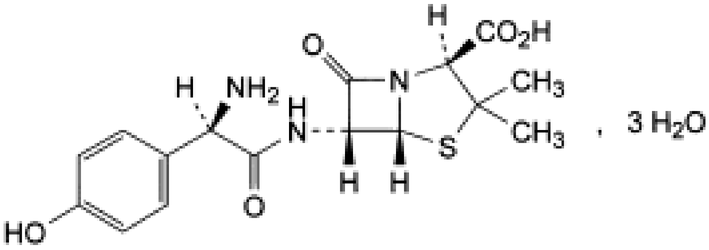
K. oligomers of penicilloic acids of amoxicillin.

Ph Eur

Amoxicillin Trihydrate

General Notices

(Ph Eur monograph 0260)



$C_{16}H_{19}N_3O_5S,3H_2O$ 419.4 *61336-70-7*

Action and use

Penicillin antibacterial.

Preparations

Amoxicillin Capsules

Amoxicillin Oral Suspension

Co-amoxiclav Tablets

Ph Eur

DEFINITION

(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in fatty oils. It dissolves in dilute acids and dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *amoxicillin trihydrate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 10 ml of *sodium hydrogen carbonate solution R.*

Reference solution (a) Dissolve 25 mg of *amoxicillin trihydrate CRS* in 10 ml of *sodium hydrogen carbonate solution R.*

Reference solution (b) Dissolve 25 mg of *amoxicillin trihydrate CRS* and 25 mg of *ampicillin trihydrate CRS* in 10 ml of *sodium hydrogen carbonate solution R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 10 volumes of *acetone R* and 90 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube in a water-bath for 1 min; a dark yellow colour develops.

TESTS

Solution S

With the aid of ultrasound or gentle heating, dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

The solutions are not more opalescent than reference suspension II *(2.2.1)*.

Dissolve 1.0 g in 10 ml of *0.5 M hydrochloric acid*. Dissolve separately 1.0 g in 10 ml of *dilute ammonia R2*. Examine immediately after dissolution.

pH ***(2.2.3)***

3.5 to 5.5 for solution S.

Specific optical rotation ***(2.2.7)***

+ 290 to + 315 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Buffer solution pH 5.0 To 250 ml of *0.2 M potassium dihydrogen phosphate R* add *dilute sodium hydroxide solution R* to pH 5.0 and dilute to 1000.0 ml with *water R*.

Test solution (a) Dissolve 30.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Test solution (b) Dissolve 30.0 mg of the substance to be examined in mobile phase A and dilute to 20.0 ml with mobile phase A. *Prepare immediately before use.*

Reference solution (a) Dissolve 30.0 mg of *amoxicillin trihydrate CRS* in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (b) Dissolve 4.0 mg of *cefadroxil CRS* in mobile phase A and dilute to 50 ml with mobile phase A. To 5.0 ml of this solution add 5.0 ml of reference solution (a) and dilute to 100 ml with mobile phase A.

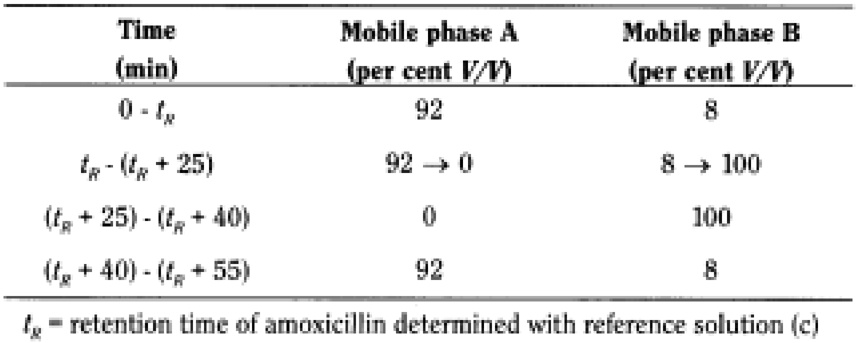
Reference solution (c) Dilute 2.0 ml of reference solution (a) to 20.0 ml with mobile phase A. Dilute 5.0 ml of this solution to 20.0 ml with mobile phase A.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *acetonitrile R*, buffer solution pH 5.0 (1:99 *V/V*);

— *mobile phase B*: *acetonitrile R*, buffer solution pH 5.0 (20:80 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	92	8
t_R - (t_R + 25)	92 → 0	8 → 100
(t_R + 25) - (t_R + 40)	0	100
(t_R + 40) - (t_R + 55)	92	8

t_R = retention time of amoxicillin determined with reference solution (c)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 50 µl of reference solutions (b) and (c) with isocratic elution at the initial mobile phase composition and 50 µl of test solution (b) according to the elution gradient described under Mobile phase; inject mobile phase A as a blank according to the elution gradient described under Mobile phase.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to amoxicillin and cefadroxil; if necessary, adjust the ratio A:B of the mobile phase.

Limit:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent).

N*,*N*-Dimethylaniline** ***(2.4.26, Method A or B)

Maximum 20 ppm.

Water ***(2.5.12)***

11.5 per cent to 14.5 per cent, determined on 0.100 g.

Sulphated ash ***(2.4.14)***

Maximum 1.0 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

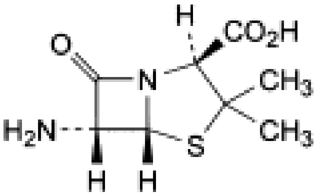
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{16}H_{19}N_3O_5S$ from the declared content of *amoxicillin trihydrate CRS*.

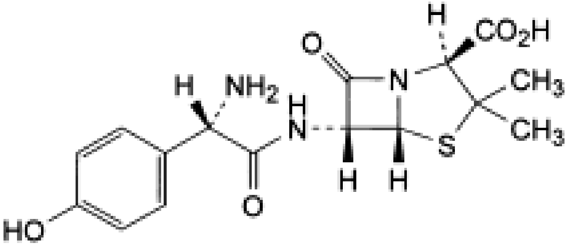
STORAGE

In an airtight container.

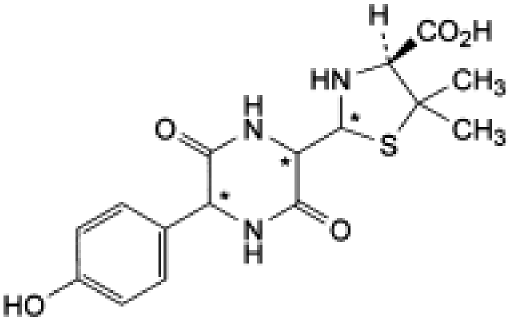
IMPURITIES



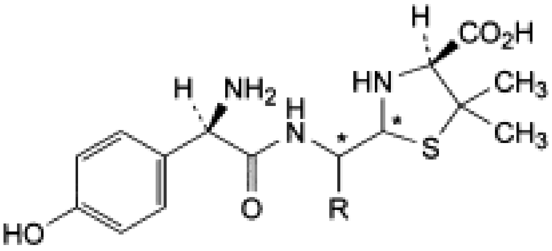
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



B. (2*S*,5*R*,6*R*)-6-[[(2*S*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (L-amoxicillin),

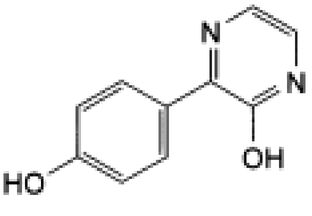


C. (4*S*)-2-[5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid (amoxicillin diketopiperazines),

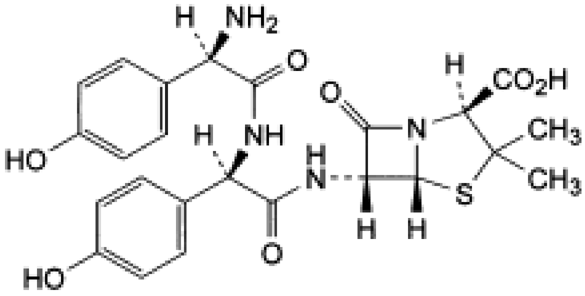


D. R = CO_2H: (4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin),

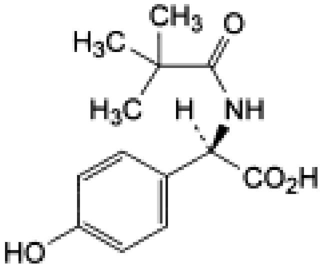
E. R = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of amoxicillin),



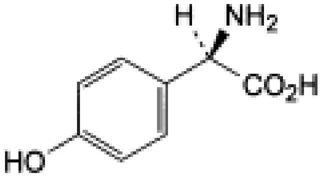
F. 3-(4-hydroxyphenyl)pyrazin-2-ol,



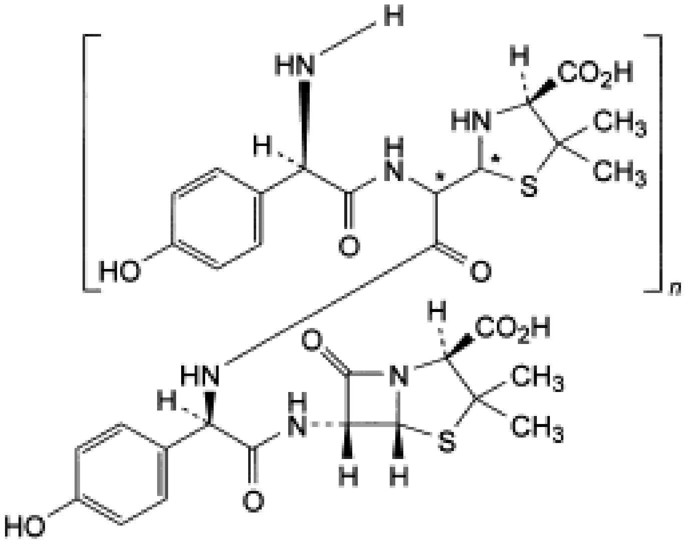
G. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-2-(4-hydroxyphenyl)acetyl]-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid (D-(4-hydroxyphenyl)glycylamoxicillin),



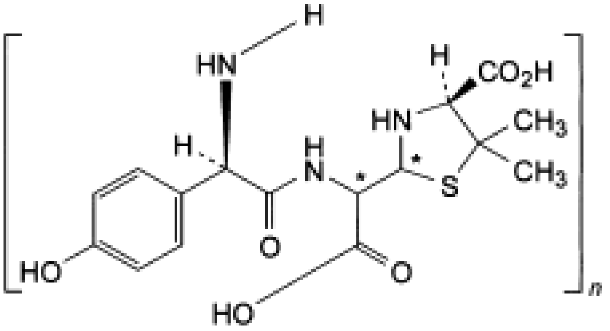
H. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-(4-hydroxyphenyl)acetic acid,



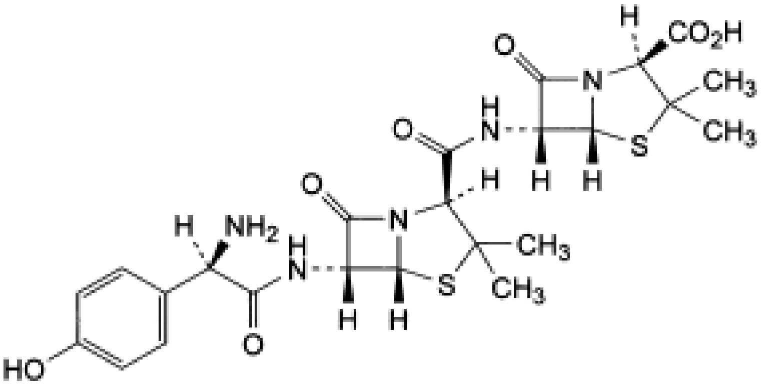
I. (2*R*)-2-amino-2-(4-hydroxyphenyl)acetic acid,



J. co-oligomers of amoxicillin and of penicilloic acids of amoxicillin,



K. oligomers of penicilloic acids of amoxicillin,



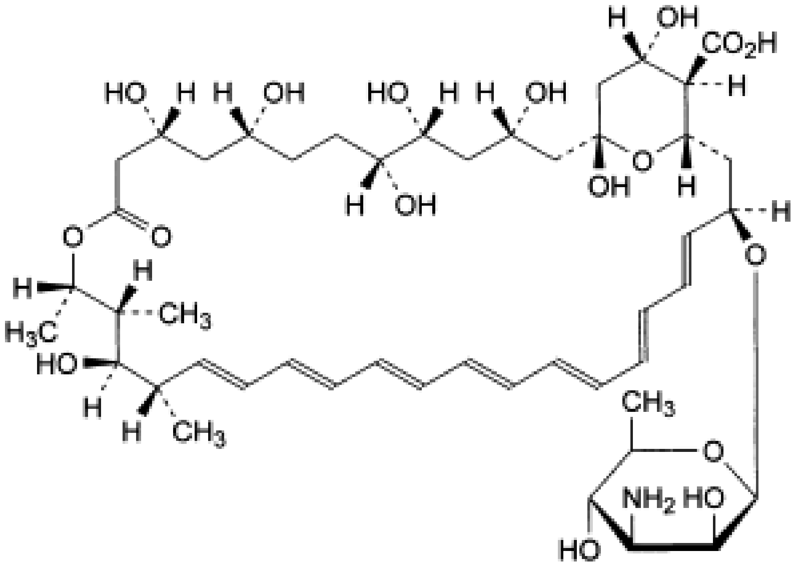
L. (2*S*,5*R*,6*R*)-6-[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA amoxicillin amide).

Ph Eur

Amphotericin

General Notices

(Amphotericin B, Ph Eur monograph 1292)



$C_{47}H_{73}NO_{17}$ 924 *1397-89-3*

Action and use

Antifungal.

Preparation

Amphotericin Lozenges

Amphotericin Oral Suspension

Ph Eur

DEFINITION

Amphotericin B is a mixture of antifungal polyenes produced by the growth of certain strains of *Streptomyces nodosus* or by any other means. It consists mainly of amphotericin B which is (1*R*,3*S*,5*R*,6*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,23*E*, 25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*, 37*S*)-33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15, 16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid. The potency is not less than 750 IU/mg, calculated with reference to the dried substance.

CHARACTERS

A yellow or orange powder, practically insoluble in water, soluble in dimethyl sulphoxide and in propylene glycol, slightly soluble in dimethylformamide, very slightly soluble in methanol, practically insoluble in alcohol.

It is sensitive to light in dilute solutions and is inactivated at low pH values.

IDENTIFICATION

A. Dissolve 25 mg in 5 ml of *dimethyl sulphoxide R* and dilute to 50 ml with *methanol R*. Dilute 2 ml of the solution to 200 ml with *methanol R*. Examined between 300 nm and 450 nm *(2.2.25)*, the solution shows 3 absorption maxima at 362 nm, 381 nm and 405 nm. The ratio of the absorbance measured at 362 nm to that measured at 381 nm is 0.57 to 0.61. The ratio of the absorbance measured at 381 nm to that measured at 405 nm is 0.87 to 0.93.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *amphotericin B CRS* . If the spectra obtained show differences dry the substance to be examined at 60 °C at a pressure not exceeding 0.7 kPa for 1 h and prepare a new spectrum.

C. To 1 ml of a 0.5 g/l solution in *dimethyl sulphoxide R*, add 5 ml of *phosphoric acid R* to form a lower layer, avoiding mixing the 2 liquids. A blue ring is immediately produced at the junction of the liquids. Mix, an intense blue colour is produced. Add 15 ml of *water R* and mix; the solution becomes pale yellow.

TESTS

Content of tetraenes

Not more than 10.0 per cent and not more than 5.0 per cent if intended for use in the manufacture of parenteral dosage forms, determined by the following method.

Test solution Dissolve 50.0 mg in 5 ml of *dimethyl sulphoxide R* and dilute to 50.0 ml with *methanol R*. Dilute 4.0 ml of the solution to 50.0 ml with *methanol R*.

Reference solution (a) Dissolve 50.0 mg of *amphotericin B CRS* in 5 ml of *dimethyl sulphoxide R* and dilute to 50.0 ml with *methanol R*. Dilute 4.0 ml of the solution to 50.0 ml with *methanol R*.

Reference solution (b) Dissolve 25.0 mg of *nystatin CRS* in 25 ml of *dimethyl sulphoxide R* and dilute to 250.0 ml with *methanol R*. Dilute 4.0 ml of the solution to 50.0 ml with *methanol R*.

Measure the absorbances *(2.2.25)* of the test solution and of reference solutions (a) and (b) at the maxima at 282 nm and 304 nm respectively, using a 0.8 per cent *V/V* solution of *dimethyl sulphoxide R* in *methanol R* as the compensation liquid. Calculate the specific absorbance of the substance being examined, of *nystatin CRS* and of *amphotericin B CRS* at both wavelengths, each with reference to the dried substance, and calculate the percentage content of tetraenes using the following expression:

$$F + \frac{100\,(B_1S_2 - B_2S_1)}{(N_2B_1 - N_1B_2)}$$

S_1 and S_2 = specific absorbances of the substance to be examined at 282 nm and 304 nm respectively,

N_1 and N_2 = specific absorbance of *nystatin CRS* at 282 nm and 304 nm respectively,

B_1 and B_2 = specific absorbance of *amphotericin B CRS* at 282 nm and 304 nm respectively,

F = declared content of tetraenes in *amphotericin B CRS*.

Loss on drying ***(2.2.32)***

Not more than 5.0 per cent, determined on 1.000 g by drying in an oven at 60 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash ***(2.4.14)***

Not more than 3.0 per cent and not more than 0.5 per cent if intended for use in the manufacture of parenteral dosage forms, determined on 1.0 g.

Bacterial endotoxins ***(2.6.14)***

Less than 1.0 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Protect all solutions from light throughout the assay. Dissolve 25.0 mg in *dimethyl sulphoxide R* and dilute, with shaking, to 25.0 ml with the same solvent. Under constant stirring of this stock solution, dilute with *dimethyl sulphoxide R* to obtain solutions of appropriate concentrations (the following concentrations have been found suitable: 44.4, 66.7 and 100 IU/ml). Final solutions are prepared by diluting 1 to 20 with 0.2 M phosphate buffer solution pH 10.5 so that they all contain 5 per cent *V/V* of dimethyl sulphoxide. Prepare the reference and the test solutions simultaneously. Carry out the microbiological assay of antibiotics *(2.7.2)*.

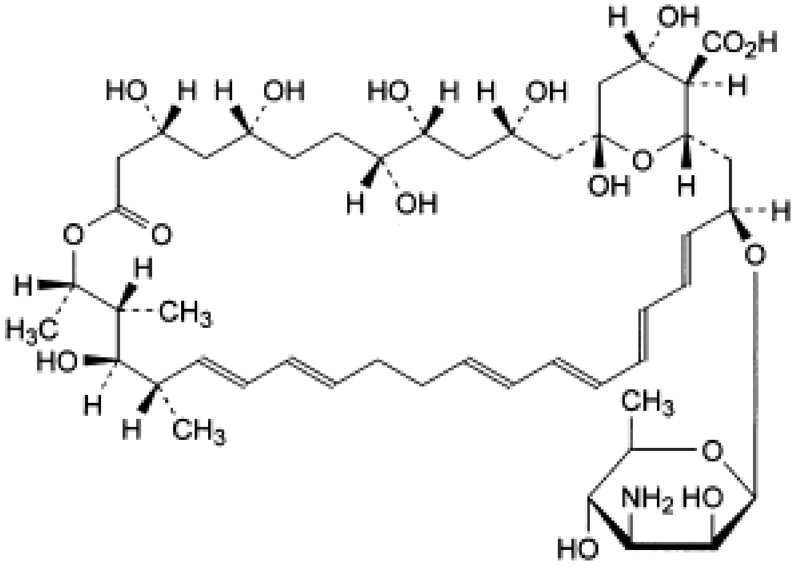
STORAGE

Store protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES



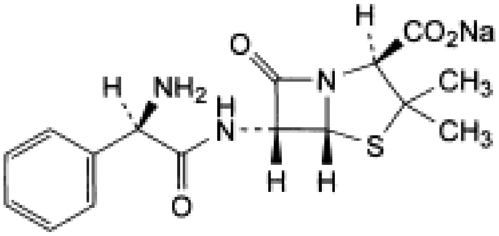
A. amphotericin A (tetraene).

Ph Eur

Ampicillin Sodium

General Notices

(Ph Eur monograph 0578)



$C_{16}H_{18}N_3NaO_4S$ 371.4   *69-52-3*

Action and use

Penicillin antibacterial.

Preparation

Ampicillin Injection

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

91.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in acetone, practically insoluble in fatty oils and in liquid paraffin.

IDENTIFICATION

First identification   *A, D.*

Second identification   *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation   Dissolve 0.250 g in 5 ml of *water R*, add 0.5 ml of *dilute acetic acid R*, swirl and allow to stand for 10 min in iced water. Filter the crystals through a small sintered-glass filter (40) *(2.1.2)*, applying suction, wash with 2-3 ml of a mixture of 1 volume of *water R* and 9 volumes of *acetone R*, then dry in an oven at 60 °C for 30 min.

Comparison   *ampicillin trihydrate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution   Dissolve 25 mg of the substance to be examined in 10 ml of *sodium hydrogen carbonate solution R.*

Reference solution (a)   Dissolve 25 mg of *ampicillin trihydrate CRS* in 10 ml of *sodium hydrogen carbonate solution R*.

Reference solution (b)   Dissolve 25 mg of *amoxicillin trihydrate CRS* and 25 mg of *ampicillin trihydrate CRS* in 10 ml of *sodium hydrogen carbonate solution R.*

Plate   *TLC silanised silica gel plate R*.

Mobile phase   Mix 10 volumes of *acetone R* and 90 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Application   1 µl.

Development   Over a path of 15 cm.

Drying   In air.

Detection   Expose to iodine vapour until the spots appear and examine in daylight.

System suitability   Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results   The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube in a water-bath for 1 min; a dark yellow colour develops.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Appearance of solution

Solutions A and B are not more opalescent than reference suspension II *(2.2.1)* and the absorbance *(2.2.25)* of solution B at 430 nm is not greater than 0.15.

Place 1.0 g in a conical flask and add slowly and with continuous swirling 10 ml of *1 M hydrochloric acid* (solution A). Separately dissolve 1.0 g in *water R* and dilute to 10.0 ml with the same solvent (solution B). Examine immediately after dissolution.

pH *(2.2.3)*

8.0 to 10.0.

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. Measure 10 min after dissolution.

Specific optical rotation *(2.2.7)*

+ 258 to + 287 (anhydrous substance).

Dissolve 62.5 mg in a 4 g/l solution of *potassium hydrogen phthalate R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Test solution (b) Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A. *Prepare immediately before use*.

Reference solution (a) Dissolve 27.0 mg of *anhydrous ampicillin CRS* in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (b) Dissolve 2.0 mg of *cefradine CRS* in mobile phase A and dilute to 50 ml with mobile phase A. To 5.0 ml of this solution add 5.0 ml of reference solution (a).

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

Reference solution (d) To 0.20 g of the substance to be examined add 1.0 ml of *water R*. Heat the solution at 60 °C for 1 h. Dilute 0.5 ml of this solution to 50.0 ml with mobile phase A.

Column:

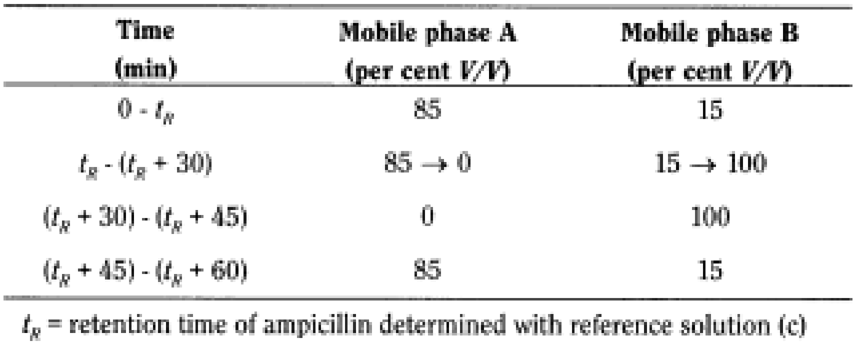
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase* A: mix 0.5 ml of *dilute acetic acid R*, 50 ml of *0.2 M potassium dihydrogen phosphate* R and 50 ml of *acetonitrile R*, then dilute to 1000 ml with *water R*;

— *mobile phase B*: mix 0.5 ml of *dilute acetic acid R,* 50 ml of *0.2 M potassium dihydrogen phosphate R* and 400 ml of *acetonitrile R*, then dilute to 1000 ml with *water R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	85	15
t_R - (t_R + 30)	85 → 0	15 → 100
(t_R + 30) - (t_R + 45)	0	100
(t_R + 45) - (t_R + 60)	85	15

t_R = retention time of ampicillin determined with reference solution (c)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 50 µl of reference solutions (b) and (c) with isocratic elution at the initial mobile phase composition and 50 µl of test solution (b) and reference solution (d) according to the elution gradient described under Mobile phase; inject mobile phase A as a blank according to the elution gradient described under Mobile phase.

Identification of peaks Use the chromatogram obtained with reference solution (d) to identify the peaks due to ampicillin and ampicillin dimer.

Relative retention With reference to ampicillin: ampicillin dimer = about 2.8.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to ampicillin and cefradin; if necessary adjust the ratio A:B of the mobile phase.

Limits:

— *ampicillin dimmer*: not more than 4.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4.5 per cent);

— *any other impurity*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2 per cent).

N*,*N*-Dimethylaniline *(2.4.26, Method B)

Maximum 20 ppm.

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.8 per cent m/m.

Methylene chloride

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 1.0 ml of *ethylene chloride R* in *water R* and dilute to 500.0 ml with the same solvent.

Test solution (a) Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 1.0 g of the substance to be examined in *water R*, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *water R*.

Reference solution Dissolve 1.0 ml of *methylene chloride R* in *water R* and dilute to 500.0 ml with the same solvent. To 1.0 ml of this solution add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *water R*.

Column:

— *material*: glass;

— *size: l* = 1.5 m, Ø = 4 mm;

— *stationary phase*: *diatomaceous earth for gas chromatography R* impregnated with 10 per cent *m/m* of *macrogol 1000 R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 40 ml/min.

Temperature:
- — *column*: 60 °C;
- — *injection port*: 100 °C;
- — *detector*: 150 °C.

Detection Flame ionisation.

Calculate the content of methylene chloride taking its density at 20 °C to be 1.325 g/ml.

Limit:
- — *methylene chloride*: maximum 0.2 per cent m/m.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.300 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.15 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

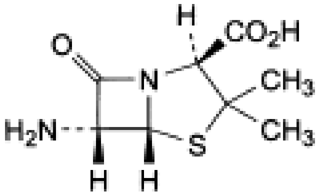
- — *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of ampicillin sodium by multiplying the percentage content of ampicillin by 1.063.

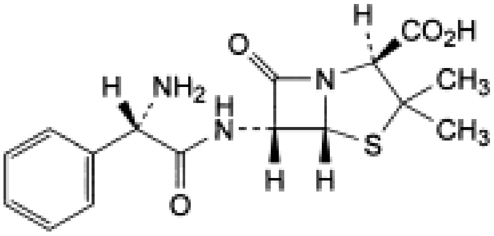
STORAGE

In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

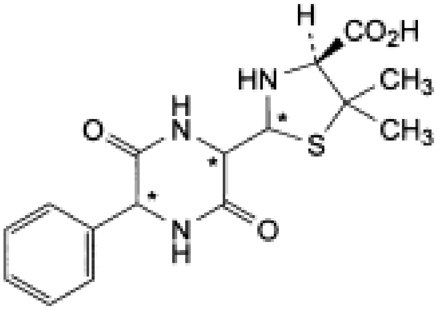
IMPURITIES



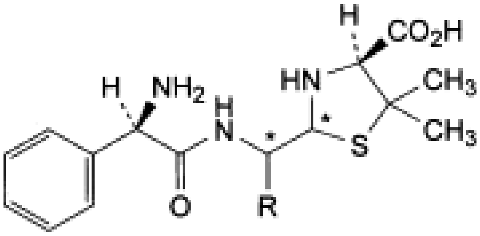
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),


H
CO2H
O
N
CH3
H
NH2
H
N
CH3
S
H
H
O

B. (2*S*,5*R*,6*R*)-6-[[(2*S*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (L-ampicillin),

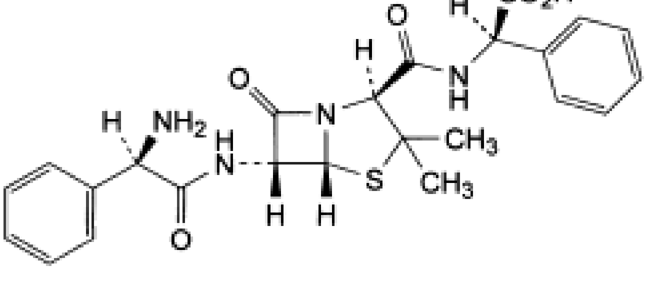

H
CO2H
HN
CH3
H
N
CH3
O
*
*
S
*
N
H
O

C. (4*S*)-2-(3,6-dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid (diketopiperazines of ampicillin),

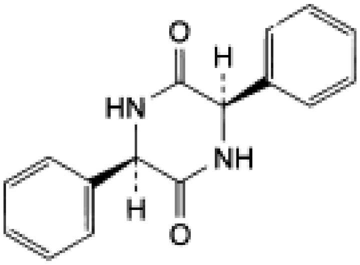

H
CO2H
H
NH2
HN
CH3
H
N
*
*
S
CH3
O
R

D. R = CO_2H: (4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of ampicillin),

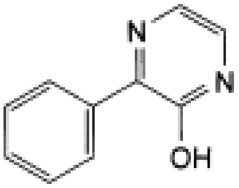
F. R = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of ampicillin),


O
H
CO2H
H
N
H
O
N
H
NH2
CH3
H
N
S
CH3
H
H
O

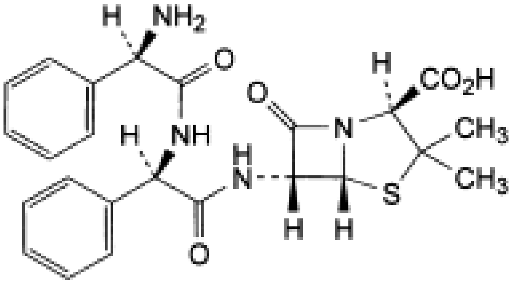
E. (2*R*)-2-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-2-phenylacetic acid (ampicillinyl-D-phenylglycine)
,



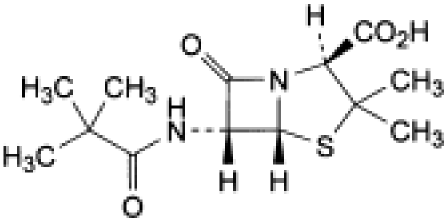
G. (3*R*,6*R*)-3,6-diphenylpiperazine-2,5-dione,



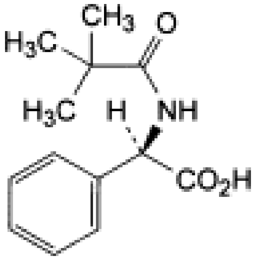
H. 3-phenylpyrazin-2-ol,


H
NH₂
O
H
NH
H
N
O
H
CO₂H
N
CH₃
CH₃
S
H
H
O

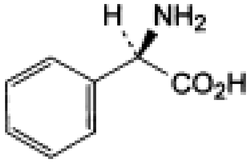
I. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia1-azabicyclo[3.2.0]heptane-2-carboxylic acid (D-phenylglycylampicillin),


H₃C
CH₃
H₃C
H
N
O
O
N
H
CO₂H
CH₃
CH₃
S
H
H

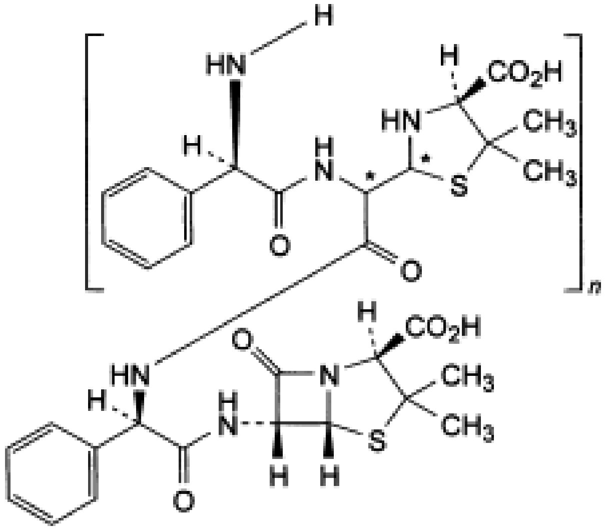
J. (2*S*,5*R*,6*R*)-6-[(2,2-dimethylpropanoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo
[3.2.0]heptane-2-carboxylic acid,



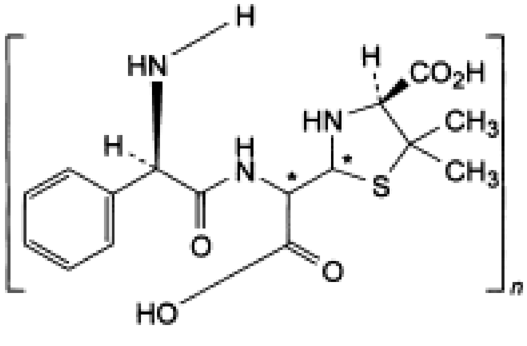
K. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-phenylacetic acid,



L. (2*R*)-2-amino-2-phenylacetic acid (D-phenylglycine),



M. co-oligomers of ampicillin and of penicilloic acids of ampicillin,



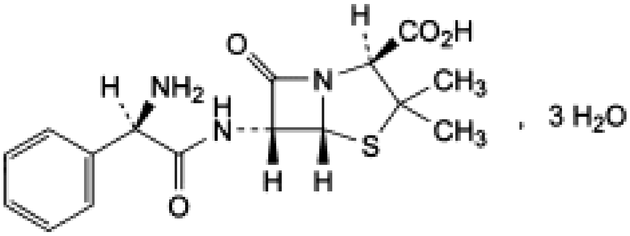
N. oligomers of penicilloic acids of ampicillin.

Ph Eur

Ampicillin Trihydrate

General Notices

(Ph Eur monograph 0168)



$C_{16}H_{19}N_3O_4S,3H_2O$ 403.5 *7177-48-2*

Action and use

Penicillin antibacterial.

Preparations

Ampicillin Capsules

Ampicillin Oral Suspension

Co-fluampicil Capsules

Co-fluampicil Oral Suspension

Ph Eur

DEFINITION

(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, practically insoluble in ethanol (96 per cent) and in fatty oils. It dissolves in dilute solutions of acids and of alkali hydroxides.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ampicillin trihydrate CRS* .

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 10 ml of *sodium hydrogen carbonate solution R.*

Reference solution (a) Dissolve 25 mg of *ampicillin trihydrate CRS* in 10 ml of *sodium hydrogen carbonate solution R.*

Reference solution (b) Dissolve 25 mg of *amoxicillin trihydrate CRS* and 25 mg of *ampicillin trihydrate CRS* in 10 ml of *sodium hydrogen carbonate solution R*.

Plate *TLC silanised silica gel plate R*.

Mobile phase Mix 10 volumes of *acetone R* and 90 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube in a water-bath for 1 min; a dark yellow colour develops.

D. Water (see Tests).

TESTS

Appearance of solution

The solutions are not more opalescent than reference suspension II *(2.2.1)*.

Dissolve 1.0 g in 10 ml of *1 M hydrochloric acid* . Separately dissolve 1.0 g in 10 ml of *dilute ammonia R2*. Examine immediately after dissolution.

pH *(2.2.3)*

3.5 to 5.5.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 40 ml with the same solvent.

Specific optical rotation ***(2.2.7)***

+ 280 to + 305 (anhydrous substance).

Dissolve 62.5 mg in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Test solution (b) Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A*. Prepare immediately before use*.

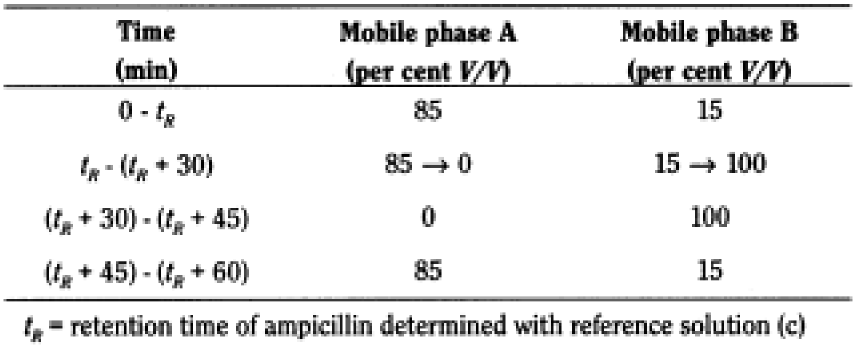
Reference solution (a) Dissolve 27.0 mg of *anhydrous ampicillin CRS* in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (b) Dissolve 2 mg of *cefradine CRS* in mobile phase A and dilute to 50 ml with mobile phase A. To 5 ml of this solution, add 5 ml of reference solution (a).

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase* A: mix 0.5 ml of *dilute acetic acid R*, 50 ml of *0.2 M potassium dihydrogen phosphate R* and 50 ml of *acetonitrile R*, then dilute to 1000 ml with *water R*;
- *mobile phase B*: mix 0.5 ml of *dilute acetic acid R,* 50 ml of *0.2 M potassium dihydrogen phosphate R* and 400 ml of *acetonitrile R*, then dilute to 1000 ml with *water R*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - t_R	85	15
t_R - (t_R + 30)	85 → 0	15 → 100
(t_R + 30) - (t_R + 45)	0	100
(t_R + 45) - (t_R + 60)	85	15

t_R = retention time of ampicillin determined with reference solution (c)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 50 µl of reference solutions (b) and (c) with isocratic elution at the initial mobile phase composition and 50 µl of test solution (b) according to the elution gradient described

under Mobile phase; inject mobile phase A as a blank according to the elution gradient described under Mobile phase.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to ampicillin and cefradin; if necessary, adjust the ratio A:B of the mobile phase.

Limit:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

N*,*N*-Dimethylaniline** ***(2.4.26, Method B)

Maximum 20 ppm.

Water ***(2.5.12)***

12.0 per cent to 15.0 per cent, determined on 0.100 g.

Sulphated ash ***(2.4.14)***

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

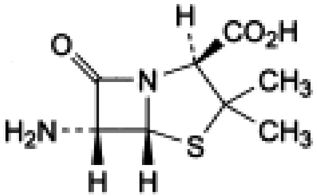
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of ampicillin from the declared content of *anhydrous ampicillin CRS* .

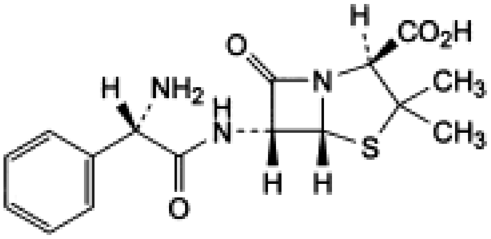
STORAGE

In an airtight container .

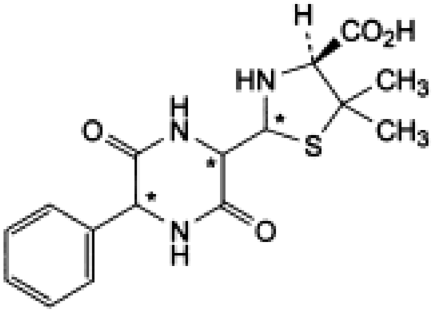
IMPURITIES



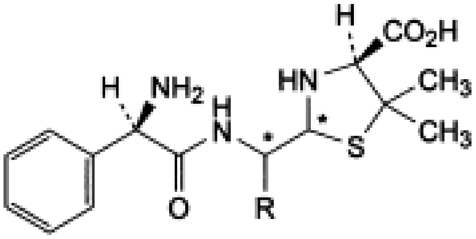
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



B. (2*S*,5*R*,6*R*)-6-[[(2*S*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (L-ampicillin),

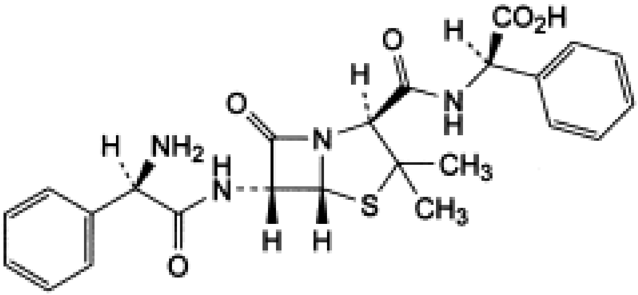


C. (4*S*)-2-(3,6-dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid (diketopiperazines of ampicillin),

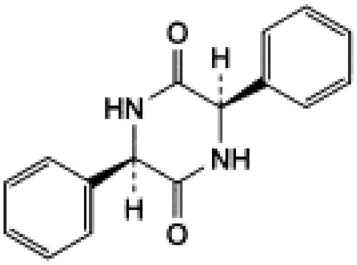


D. R = CO_2H: (4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of ampicillin),

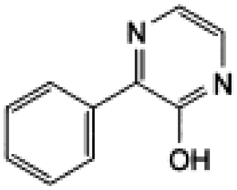
F. R = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of ampicillin),



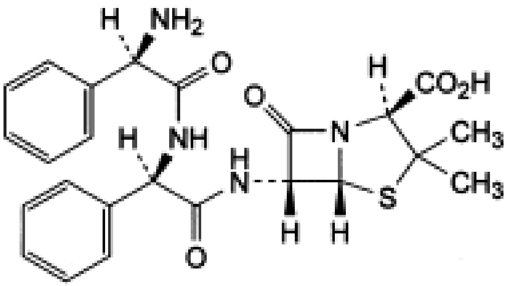
E. (2*R*)-2-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-2-phenylacetic acid (ampicillinyl-D-phenylglycine)
,



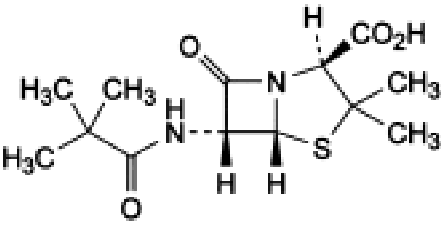
G. (3*R*,6*R*)-3,6-diphenylpiperazine-2,5-dione,



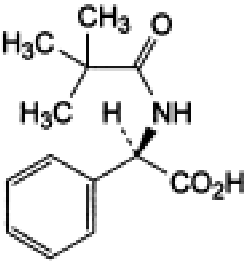
H. 3-phenylpyrazin-2-ol,



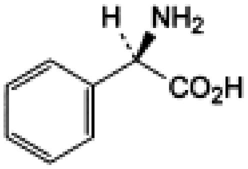
I. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (D-phenylglycylampicillin)
,



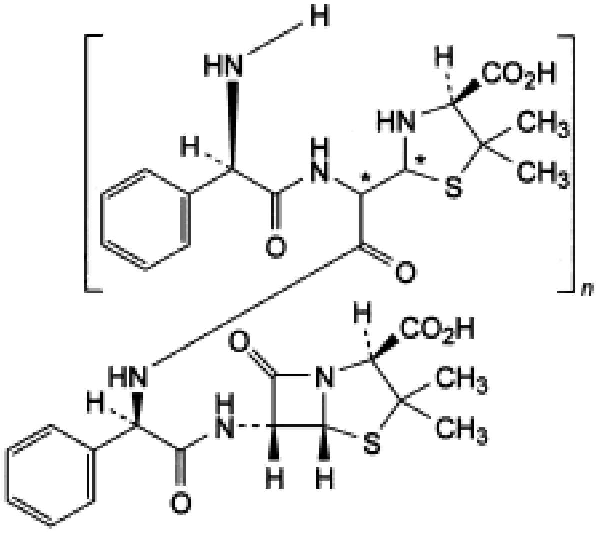
J. (2*S*,5*R*,6*R*)-6-[(2,2-dimethylpropanoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo
[3.2.0]heptane-2-carboxylic acid,



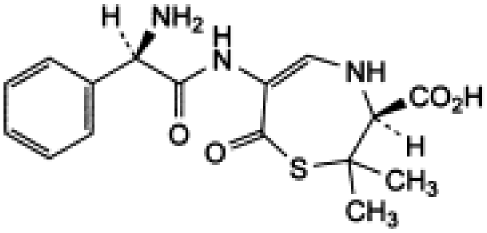
K. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-phenylacetic acid,



L. (2*R*)-2-amino-2-phenylacetic acid (D-phenylglycine),



M. co-oligomers of ampicillin and of penicilloic acids of ampicillin,



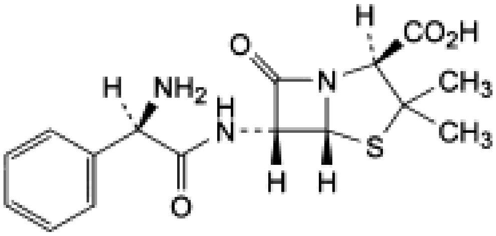
N. (3*S*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2,2-dimethyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine-3-carboxylic acid.

Ph Eur

Ampicillin

General Notices

(Anhydrous Ampicillin, Ph Eur monograph 0167)



$C_{16}H_{19}N_3O_4S$ 349.4 *69-53-4*

Action and use

Penicillin antibacterial.

Preparations

Ampicillin Capsules

Ampicillin Oral Suspension

Ph Eur

DEFINITION

(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, practically insoluble in acetone, in ethanol (96 per cent) and in fatty oils. It dissolves in dilute solutions of acids and of alkali hydroxides.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *anhydrous ampicillin CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 10 ml of *sodium hydrogen carbonate solution R.*

Reference solution (a) Dissolve 25 mg of *anhydrous ampicillin CRS* in 10 ml of *sodium hydrogen carbonate solution R.*

Reference solution (b) Dissolve 25 mg of *amoxicillin trihydrate CRS* and 25 mg of *anhydrous ampicillin CRS* in 10 ml of *sodium hydrogen carbonate solution R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 10 volumes of *acetone R* and 90 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube in a water-bath for 1 min; a dark yellow colour develops.

D. Water (see Tests).

TESTS

Appearance of solution

The solutions are not more opalescent than reference suspension II *(2.2.1)*.

Dissolve 1.0 g in 10 ml of *1 M hydrochloric acid*. Separately dissolve 1.0 g in 10 ml of *dilute ammonia R2*. Examine immediately after dissolution.

pH *(2.2.3)*

3.5 to 5.5.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 40 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 280 to + 305 (anhydrous substance).

Dissolve 62.5 mg in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 27.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Test solution (b) Dissolve 27.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A. *Prepare immediately before use*.

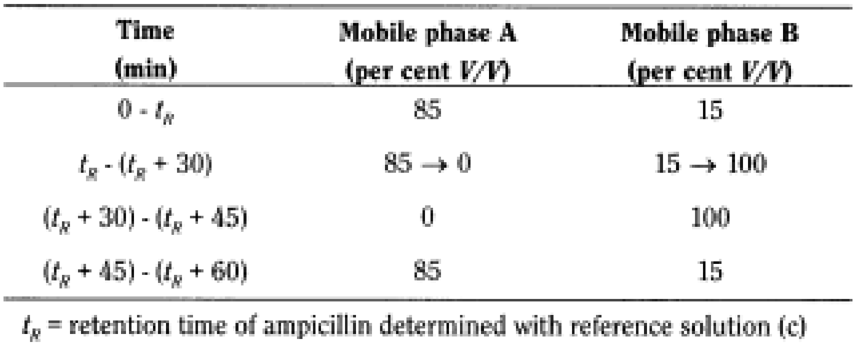
Reference solution (a) Dissolve 27.0 mg of *anhydrous ampicillin CRS* in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (b) Dissolve 2.0 mg of *cefradine CRS* in mobile phase A and dilute to 50 ml with mobile phase A. To 5.0 ml of this solution add 5.0 ml of reference solution (a).

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: mix 0.5 ml of *dilute acetic acid R*, 50 ml of *0.2 M potassium dihydrogen phosphate R* and 50 ml of *acetonitrile R*, then dilute to 1000 ml with *water R*;
- *mobile phase B*: mix 0.5 ml of *dilute acetic acid R*, 50 ml of *0.2 M potassium dihydrogen phosphate R* and 400 ml of *acetonitrile R*, then dilute to 1000 ml with *water R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	85	15
t_R - (t_R + 30)	85 → 0	15 → 100
(t_R + 30) - (t_R + 45)	0	100
(t_R + 45) - (t_R + 60)	85	15

t_R = retention time of ampicillin determined with reference solution (c)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 50 µl of reference solutions (b) and (c) with isocratic elution at the initial mobile phase composition and 50 µl of test solution (b) according to the elution gradient described

under Mobile phase; inject mobile phase A as a blank according to the elution gradient described under Mobile phase.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to ampicillin and cefradin; if necessary, adjust the ratio A:B of the mobile phase.

Limit:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

N*,*N*-Dimethylaniline *(2.4.26, Method B)

Maximum 20 ppm.

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

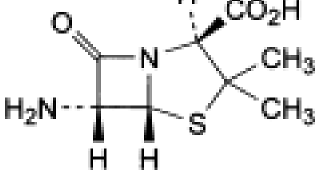
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{16}H_{19}N_3O_4S$ from the declared content of *anhydrous ampicillin CRS*.

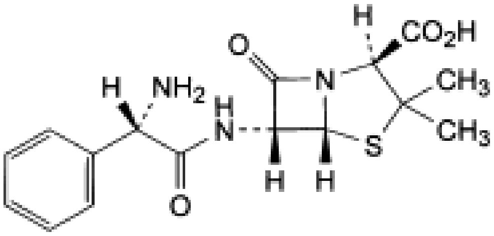
STORAGE

In an airtight container, at a temperature not exceeding 30 °C.

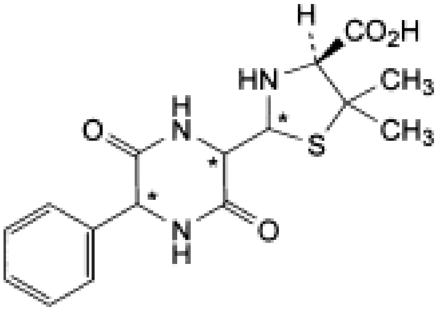
IMPURITIES



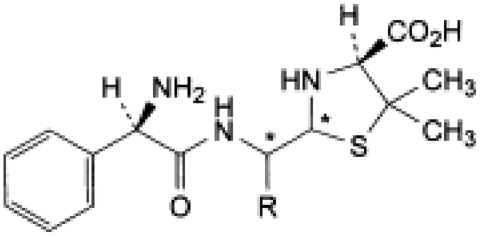
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



B. (2*S*,5*R*,6*R*)-6-[[(2S)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (L-ampicillin),

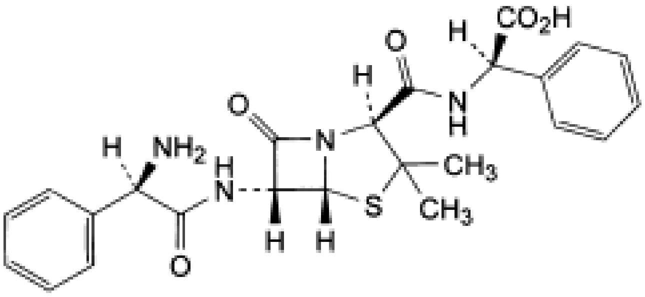


C. (4*S*)-2-(3,6-dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid (diketopiperazines of ampicillin),

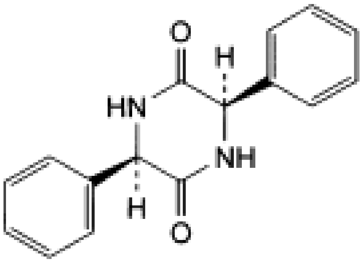


D. R = CO_2H: (4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of ampicillin),

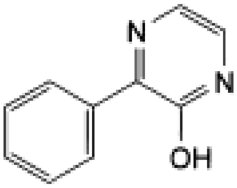
F. R = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of ampicillin),



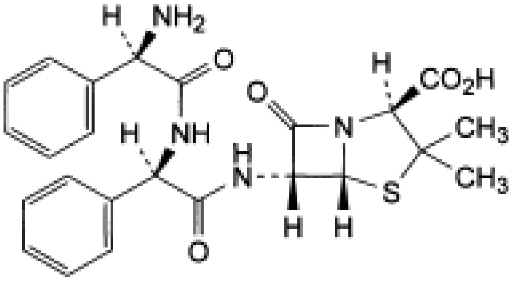
E. (2*R*)-2-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-2-phenylacetic acid (ampicillinyl-d-phenylglycine),



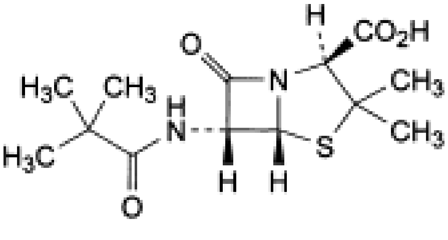
G. (3*R*,6*R*)-3,6-diphenylpiperazine-2,5-dione,



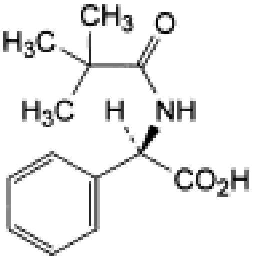
H. 3-phenylpyrazin-2-ol,



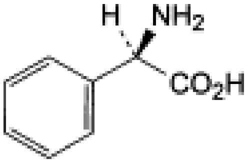
I. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (D-phenylglycylampicillin)
,



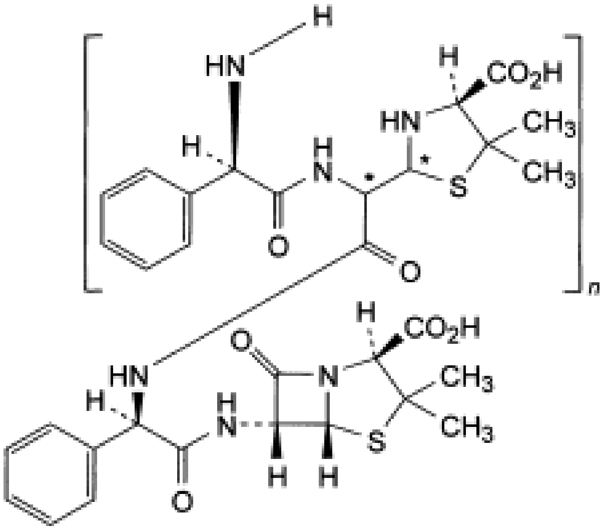
J. (2*S*,5*R*,6*R*)-6-[(2,2-dimethylpropanoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid,



K. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-phenylacetic acid,



L. (2*R*)-2-amino-2-phenylacetic acid (D-phenylglycine),

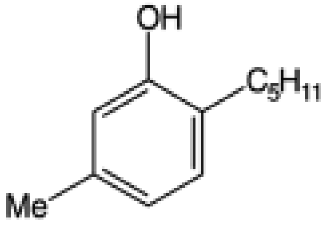


M. co-oligomers of ampicillin and of penicilloic acids of ampicillin.

Ph Eur

Amylmetacresol

General Notices



$C_{12}H_{18}O$ 178.3 *1300-94-3*

Action and use

Antiseptic.

DEFINITION

Amylmetacresol is 5-methyl-2-pentylphenol. It contains not less than 96.0% and not more than 104.0% of $C_{12}H_{18}O$.

CHARACTERISTICS

A clear or almost clear liquid or a solid crystalline mass, colourless or slightly yellow when freshly prepared, which darkens on storage.

Practically insoluble in *water*; soluble in *ethanol (96%)*, in *ether* and in fixed and volatile oils.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of amylmetacresol *(RS 014)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.004% w/v solution in *absolute ethanol* containing 2% v/v of *0.1M hydrochloric acid* exhibits a maximum at 278 nm and a less well-defined maximum at 286 nm. The *absorbance* at the maximum at 278 nm is about 0.53.

C. To 5 ml of a 0.5% v/v solution in *methanol* add 5 ml of *bromine water*. A yellow precipitate is produced.

D. *Freezing point*, about 22°, Appendix V B.

TESTS

Weight per ml

0.947 to 0.953 g, Appendix V G.

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Solution (1) contains 1% w/v of the substance being examined in *chloroform*. Solution (2) is the substance being examined.

The chromatographic procedure may be carried out using a glass column (1.8 m × 3 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 10% w/w of silicone grease (Apiezon L is suitable) and maintained at 180°.

In the chromatogram obtained with solution (2) the area of any *secondary peak* is not greater than 2% and the sum of the areas of any *secondary peaks* is not greater than 3% by *normalisation*.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.2 g in *absolute ethanol* containing 2% v/v of *0.1M hydrochloric acid* and dilute to 100 ml with the same solvent. Dilute 2 ml of the solution to 100 ml with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{12}H_{18}O$ taking 132 as the value of A(1%, 1 cm) at the maximum at 278 nm.

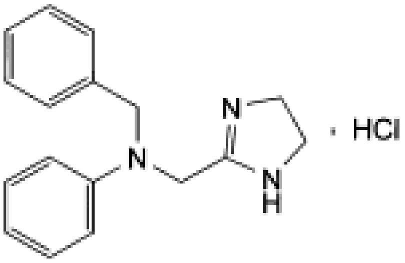
STORAGE

Amylmetacresol should be protected from light.

Antazoline Hydrochloride

General Notices

(Ph Eur monograph 0972)



$C_{17}H_{19}N_3$,HCl 301.8 *2508-72-7*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

Antazoline hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of *N*-benzyl-*N*-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]aniline hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, sparingly soluble in water, soluble in alcohol, slightly soluble in methylene chloride.

It melts at about 240 °C, with decomposition.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *antazoline hydrochloride CRS* . Examine the substances as discs prepared using *potassium chloride R*.

B. Examine the chromatograms obtained in the test for related substances in daylight after spraying. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (b).

C. To 5 ml of solution S (see Tests) add, drop by drop, *dilute sodium hydroxide solution R* until an alkaline reaction is produced. Filter. The precipitate, washed with two quantities, each of 10 ml, of *water R* and dried in a desicator under reduced pressure, melts *(2.2.14)* at 119 °C to 123 °C.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* prepared from *distilled water R*, heating at 60 °C if necessary. Allow to cool and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.2 ml of *methyl red solution R*. Not more than 0.1 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance. Heat the plate at 110 °C for 15 min before using.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 5 ml with *methanol R*.

Reference solution (a) Dilute 0.5 ml of test solution (a) to 100 ml with *methanol R*.

Reference solution (b) Dissolve 20 mg of *antazoline hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (c) Dissolve 20 mg of *xylometazoline hydrochloride CRS* in 1 ml of test solution (a) and dilute to 5 ml with *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *diethylamine R*, 10 volumes of *methanol R* and 85 volumes of *ethyl acetate R*. Dry the plate in a current of warm air for 15 min. Examine in ultraviolet light at 254 nm. The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots. Spray with a mixture of equal volumes of a 200 g/l solution of *ferric chloride R* and a 5 g/l solution of *potassium ferricyanide R*. Examine immediately in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

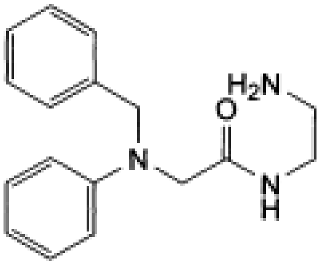
Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 0.250 g in 100 ml of *alcohol R*. Add 0.1 ml of *phenolphthalein solution R1*. Titrate with *0.1 M alcoholic potassium hydroxide*.

1 ml of *0.1 M alcoholic potassium hydroxide* is equivalent to 30.18 mg of $C_{17}H_{20}ClN_3$.

IMPURITIES



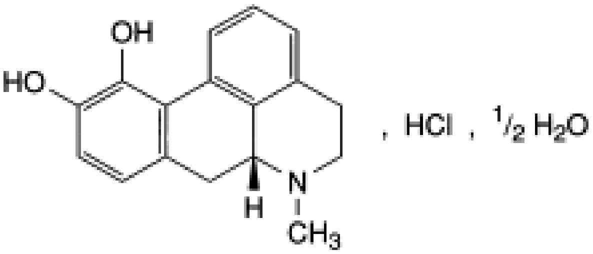
A. *N*-(2-aminoethyl)-2-(benzylphenylamino)acetamide.

Ph Eur

Apomorphine Hydrochloride

General Notices

(Ph Eur monograph 0136)



$C_{17}H_{17}NO_2,HCl,½H_2O$ 312.8 *41372-20-7*

Action and use

Dopamine receptor agonist; treatment of Parkinson's disease.

Preparation

Apomorphine Hydrochloride for Homoeopathic Preparations

Ph Eur

DEFINITION

(6a*R*)-6-Methyl-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de*,*g*]quinoline-10,11-diol hydrochloride hemihydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish-brown or green-tinged greyish, crystalline powder or crystals; on exposure to air and light, the green tinge becomes more pronounced.

Solubility

Sparingly soluble in water and in alcohol, practically insoluble in toluene.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Dissolve 10.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 273 nm and a shoulder at 300 nm to 310 nm. The specific absorbance at the maximum is 530 to 570.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of apomorphine hydrochloride* .

C. To 5 ml of solution S (see Tests) add a few millilitres of *sodium hydrogen carbonate solution R* until a permanent, white precipitate is formed. The precipitate slowly becomes greenish. Add 0.25 ml of *0.05 M iodine* and shake. The precipitate becomes greyish-green. Collect the precipitate. The precipitate dissolves in *ether R* giving a purple solution, in *methylene chloride R* giving a violet-blue solution and in *alcohol R* giving a blue solution.

D. To 2 ml of solution S add 0.1 ml of *nitric acid R*. Mix and filter. The filtrate gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.25 g without heating in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 or GY_5*(2.2.2, Method II)*.

pH *(2.2.3)*

4.0 to 5.0 for solution S.

Specific optical rotation *(2.2.7)*

- 48 to - 52 (dried substance).

Dissolve 0.25 g in *0.02 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.25 g of the substance to be examined in a 1 per cent *V/V* solution of *glacial acetic acid R* and dilute to 100.0 ml with the same solution.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with a 1 per cent *V/V* solution of *glacial acetic acid R*. Dilute 1.0 ml to 100.0 ml with a 1 per cent *V/V* solution of *glacial acetic acid R*.

Reference solution (b) Dissolve 25 mg of *boldine R* in a 1 per cent *V/V* solution of *glacial acetic acid R* and dilute to 10.0 ml with the same solvent. To 1 ml of this solution, add 1 ml of the test solution and dilute to 10.0 ml with a 1 per cent *V/V* solution of *glacial acetic acid R*.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm,

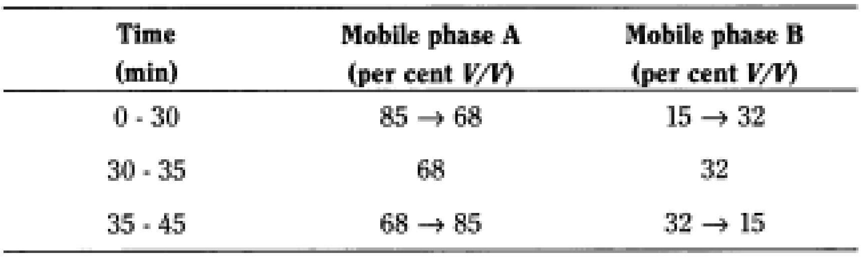
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: 1.1 g/l solution of *sodium octanesulphonate R*, adjusted to pH 2.2 using a 50 per cent *m/m* solution of *phosphoric acid R*,

— *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	85 → 68	15 → 32
30 - 35	68	32
35 - 45	68 → 85	32 → 15

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µl.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to boldine and apomorphine.

Limits:

— *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *total*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent),

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

2.5 per cent to 4.2 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

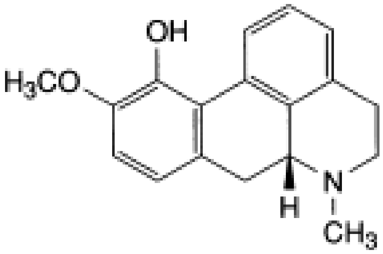
Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the first 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 30.38 mg of $C_{17}H_{18}ClNO_2$.

STORAGE

In an airtight container , protected from light.

IMPURITIES



A. (6a*R*)-10-methoxy-6-methyl-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de*,*g*]quinolin-11-ol (apocodeine),

B. morphine.

Ph Eur

Aprotinin Concentrated Solution

General Notices

(Ph Eur monograph 0579)

Action and use

Antifibrinolytic.

Preparation

Aprotinin Injection

Ph Eur

DEFINITION

Aprotinin concentrated solution is a solution of aprotinin, a polypeptide consisting of a chain of 58 amino acids, which inhibits stoichiometrically the activity of several proteolytic enzymes such as chymotrypsin, kallikrein, plasmin and trypsin. It contains not less than 15.0 Ph. Eur. U. of aprotinin activity per millilitre.

PRODUCTION

The animals from which aprotinin is derived must fulfil the requirements for the health of animals suitable for human consumption to the satisfaction of the competent authority.

The manufacturing process is validated to demonstrate suitable inactivation or removal of any contamination by viruses or other infectious agents.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following tests.

Abnormal toxicity *(2.6.9)*

Inject into each mouse a quantity of the preparation to be examined containing 2 Ph. Eur. U. diluted with a sufficient quantity of *water for injections R* to give a volume of 0.5 ml.

Histamine *(2.6.10)*

Maximum 0.2 µg of histamine base per 3 Ph. Eur. U.

CHARACTERS

Appearance

Clear, colourless liquid.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Solution S (see Tests).

Reference solution *Aprotinin solution BRP.*

Plate *TLC silica gel G plate R.*

Mobile phase *water R, glacial acetic acid R* (80:100 *V/V*) containing 100 g/l of *sodium acetate R*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with a solution of 0.1 g of *ninhydrin R* in a mixture of 6 ml of a 10 g/l solution of *cupric chloride R*, 21 ml of *glacial acetic acid R* and 70 ml of *anhydrous ethanol R*. Dry the plate at 60 °C.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Determine the ability of the preparation to be examined to inhibit trypsin activity using the method described below.

Test solution Dilute 1 ml of solution S to 50 ml with *buffer solution pH 7.2 R*.

Trypsin solution Dissolve 10 mg of *trypsin BRP* in *0.002 M hydrochloric acid* and dilute to 100 ml with the same acid.

Casein solution Dissolve 0.2 g of *casein R* in *buffer solution pH 7.2 R* and dilute to 100 ml with the same buffer solution.

Precipitating solution Mix 1 volume of *glacial acetic acid R*, 49 volumes of *water R* and 50 volumes of *anhydrous ethanol R*.

Mix 1 ml of the test solution with 1 ml of the trypsin solution. Allow to stand for 10 min and add 1 ml of the casein solution. Incubate at 35 °C for 30 min. Cool in iced water and add 0.5 ml of the precipitating solution. Shake and allow to stand at room temperature for 15 min. The solution is cloudy. Carry out a blank test under the same conditions using *buffer solution pH 7.2 R* instead of the test solution. The solution is not cloudy.

TESTS

Solution S

Prepare a solution containing 15 Ph. Eur. U./ml, if necessary by dilution on the basis of the activity stated on the label.

Appearance of solution

Solution S is clear *(2.2.1)*.

Absorbance *(2.2.25)*

Maximum 0.80 by measuring at the absorption maximum at 277 nm.

Prepare from the concentrated solution a dilution containing 3.0 Ph. Eur. U./ml.

Protein impurities of higher molecular mass

Size-exclusion chromatography *(2.2.30)*.

Freeze-dry the preparation to be examined using a pressure of 2.7 Pa and a temperature of -30 °C; the operation, including freeze-drying and a period of drying at 15-25 °C, takes 6-12 h.

Use *cross-linked dextran for chromatography R2*. Use a 180 g/l solution of *anhydrous acetic acid R* to swell the gel and as the eluent. Prepare a column of gel 0.8 m to 1.0 m long and 25 mm in diameter, taking care to avoid the introduction of air bubbles. Place at the top of the column a quantity of the preparation to be examined containing 300 Ph. Eur. U. dissolved in 1 ml of a 180 g/l solution of *anhydrous acetic acid R* and allow to elute. Collect the eluate in fractions of 2 ml. Measure the absorbance *(2.2.25)* of each fraction at the absorption maximum at 277 nm and plot the values on a graph. The chromatogram obtained does not present an absorption maximum before the elution of the aprotinin.

Specific activity of the dry residue

Minimum 3.0 Ph. Eur. U. of aprotinin activity per milligram of dry residue.

Evaporate 25.0 ml to dryness in a water-bath, dry the residue at 110 °C for 15 h and weigh. From the mass of the residue and the activity determined as described below, calculate the number of European Pharmacopoeia Units per milligram of dry residue.

Bacterial endotoxins *(2.6.14)*

Less than 0.14 IU per European Pharmacopoeia Unit of aprotinin, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

The activity of aprotinin is determined by measuring its inhibitory action on a solution of trypsin of known activity. The inhibiting activity of the aprotinin is calculated from the difference between the initial activity and the residual activity of the trypsin.

The inhibiting activity of aprotinin is expressed in European Pharmacopoeia Units. 1 Ph. Eur. U. inhibits 50 per cent of the enzymatic activity of 2 microkatals of trypsin.

Use a reaction vessel with a capacity of about 30 ml and provided with:

— a device that will maintain a temperature of 25 ± 0.1 °C;

— a stirring device, such as a magnetic stirrer;

— a lid with 5 holes for accommodating the electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of the reagents.

An automatic or manual titration apparatus may be used. In the latter case the burette is graduated in 0.05 ml and the pH-meter is provided with a wide reading scale and glass and calomel electrodes.

Test solution With *0.0015 M borate buffer solution pH 8.0 R* prepare an appropriate dilution (*D*) of the concentrated solution expected on the basis of the stated potency to contain 1.67 Ph. Eur. U./ml.

Trypsin solution Prepare a solution of *trypsin BRF* containing about 0.8 microkatals per millilitre (about 1 mg/ml), using *0.001 M hydrochloric acid* as the solvent. Use a freshly prepared solution and keep in iced water.

Trypsin and aprotinin solution To 4.0 ml of the trypsin solution add 1.0 ml of the test solution. Dilute immediately to 40.0 ml with *0.0015 M borate buffer solution pH 8.0 R*. Allow to stand at room temperature for 10 min and then keep in iced water. Use within 6 h of preparation.

Dilute trypsin solution Dilute 0.5 ml of the trypsin solution to 10.0 ml with *0.0015 M borate buffer solution pH 8.0 R*. Allow to stand at room temperature for 10 min and then keep in iced water.

Maintain an atmosphere of nitrogen in the reaction flask and stir continuously; introduce 9.0 ml of *0.0015 M borate buffer solution pH 8.0 R* and 1.0 ml of a freshly prepared 6.9 g/l solution of *benzoylarginine ethyl ester hydrochloride R*. Adjust to pH 8.0 with *0.1 M sodium hydroxide*. When the temperature has reached equilibrium at 25 ± 0.1 °C, add 1.0 ml of the trypsin and aprotinin solution and start a timer. Maintain at pH 8.0 by the addition of *0.1 M sodium hydroxide* and note the volume added every 30 s. Continue the reaction for 6 min. Determine the number of millilitres of *0.1 M sodium hydroxide* used per second (n_1 ml). Carry out, under the same conditions, a titration using 1.0 ml of the dilute trypsin solution. Determine the number of millilitres of *0.1 M sodium hydroxide* used per second (n_2 ml).

Calculate the aprotinin activity in European Pharmacopoeia Units per millilitre using the following expression:

$$4000(2n_2 - n_1) \times D$$

D = dilution factor of the aprotinin concentrated solution to be examined in order to obtain a solution containing 1.67 Ph. Eur. U./ml.

The estimated activity is not less than 90 per cent and not more than 110 per cent of the activity stated on the label.

STORAGE

In an airtight, tamper-proof container, protected from light.

LABELLING

The label states the number of European Pharmacopoeia Units of aprotinin activity per millilitre.

Ph Eur

Aprotinin

General Notices

(Ph Eur monograph 0580)

Action and use

Antifibrinolytic.

Preparation

Aprotinin Injection

Ph Eur

DEFINITION

Aprotinin is a polypeptide consisting of a chain of 58 amino acids. It inhibits stoichiometrically the activity of several proteolytic enzymes such as chymotrypsin, kallikrein, plasmin and trypsin. It contains not less than 3.0 Ph. Eur. U. of aprotinin activity per milligram, calculated with reference to the dried substance.

PRODUCTION

The animals from which aprotinin is derived must fulfil the requirements for the health of animals suitable for human consumption to the satisfaction of the competent authority.

The manufacturing process is validated to demonstrate suitable inactivation or removal of any contamination by viruses or other infectious agents.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following tests.

Abnormal toxicity *(2.6.9)*

Inject into each mouse a quantity of the substance to be examined containing 2 Ph. Eur. U. dissolved in a sufficient quantity of *water for injections R* to give a volume of 0.5 ml.

Histamine *(2.6.10)*

Maximum 0.2 µg of histamine base per 3 Ph. Eur. U.

CHARACTERS

Appearance

Almost white powder, hygroscopic.

Solubility

Soluble in water and in isotonic solutions, practically insoluble in organic solvents.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Solution S (see Tests).

Reference solution *Aprotinin solution BRP*.

Plate *TLC silica gel G plate R*.

Mobile phase *water R*, *glacial acetic acid R* (80:100 *V/V*) containing 100 g/l of *sodium acetate R*.

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with a solution of 0.1 g of *ninhydrin R* in a mixture of 6 ml of a 10 g/l solution of *cupric chloride R*, 21 ml of *glacial acetic acid R* and 70 ml of *ethanol R*. Dry the plate at 60 °C.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Determine the ability of the substance to be examined to inhibit trypsin activity using the method described below.

Test solution Dilute 1 ml of solution S to 50 ml with *buffer solution pH 7.2 R*.

Trypsin solution Dissolve 10 mg of *trypsin BRP* in *0.002 M hydrochloric acid* and dilute to 100 ml with the same acid.

Casein solution Dissolve 0.2 g of *casein R* in *buffer solution pH 7.2 R* and dilute to 100 ml with the same buffer solution.

Precipitating solution Mix 1 volume of *glacial acetic acid R*, 49 volumes of *water R* and 50 volumes of *ethanol R*.

Mix 1 ml of the test solution with 1 ml of the trypsin solution. Allow to stand for 10 min and add 1 ml of the casein solution. Incubate at 35 °C for 30 min. Cool in iced water and add 0.5 ml of the precipitating solution. Shake and allow to stand at room temperature for 15 min. The solution is cloudy. Carry out a blank test under the same conditions using *buffer solution pH 7.2 R* instead of the test solution. The solution is not cloudy.

TESTS

Solution S

Prepare a solution of the substance to be examined containing 15 Ph. Eur. U./ml, calculated from the activity stated on the label.

Appearance of solution

Solution S is clear *(2.2.1)*.

Absorbance *(2.2.25)*

Maximum 0.80 by measuring at the absorption maximum at 277 nm.

Prepare a solution of the substance to be examined containing 3.0 Ph. Eur. U./ml.

Protein impurities of higher molecular mass

Size-exclusion chromatography *(2.2.30)*.

Use *cross-linked dextran for chromatography R2*. Use a 180 g/l solution of *anhydrous acetic acid R* to swell the gel and as the eluent. Prepare a column of gel 0.8 m to 1.0 m long and 25 mm in diameter, taking care to avoid the introduction of air bubbles. Place at the top of the column a quantity of the substance to be examined containing 300 Ph. Eur. U. dissolved in 1 ml of a 180 g/l solution of *anhydrous acetic acid R* and allow to elute. Collect the eluate in fractions of 2 ml. Measure the absorbance *(2.2.25)* of each fraction at the absorption maximum at 277 nm and plot the values on a graph. The chromatogram obtained does not present an absorption maximum before the elution of the aprotinin.

Loss on drying *(2.2.32)*

Maximum 6.0 per cent, determined on 0.100 g by drying *in vacuo*.

Bacterial endotoxins *(2.6.14)*

Less than 0.14 IU per European Pharmacopoeia Unit of aprotinin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

The activity of aprotinin is determined by measuring its inhibitory action on a solution of trypsin of known activity. The inhibiting activity of the aprotinin is calculated from the difference between the initial activity and the residual activity of the trypsin.

The inhibiting activity of aprotinin is expressed in European Pharmacopoeia Units. 1 Ph. Eur. U. inhibits 50 per cent of the enzymatic activity of 2 microkatals of trypsin.

Use a reaction vessel with a capacity of about 30 ml and provided with:

— a device that will maintain a temperature of 25 ± 0.1 °C;

— a stirring device, such as a magnetic stirrer;

— a lid with 5 holes for accommodating the electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of the reagents.

An automatic or manual titration apparatus may be used. In the latter case the burette is graduated in 0.05 ml and the pH-meter is provided with a wide reading scale and glass and calomel electrodes.

Test solution Prepare a solution of the substance to be examined in *0.0015 M borate buffer solution pH 8.0 R* expected to contain 1.67 Ph. Eur. U./ml (about 0.6 mg (m mg) per millilitre).

Trypsin solution Prepare a solution of *trypsin BRP* containing about 0.8 microkatals per millilitre (about 1 mg/ml), using *0.001 M hydrochloric acid* as the solvent. Use a freshly prepared solution and keep in iced water.

Trypsin and aprotinin solution To 4.0 ml of the *trypsin solution* add 1.0 ml of the test solution. Dilute immediately to 40.0 ml with *0.0015 M borate buffer solution pH 8.0 R*. Allow to stand at room temperature for 10 min and then keep in iced water. Use within 6 h of preparation.

Dilute trypsin solution Dilute 0.5 ml of the trypsin solution to 10.0 ml with *0.0015 M borate buffer solution pH 8.0 R*. Allow to stand at room temperature for 10 min and then keep in iced water.

Maintain an atmosphere of nitrogen in the reaction flask and stir continuously; introduce 9.0 ml of *0.0015 M borate buffer solution pH 8.0 R* and 1.0 ml of a freshly prepared 6.9 g/l solution of *benzoylarginine ethyl ester hydrochloride R*. Adjust to pH 8.0 with *0.1 M sodium hydroxide*. When the temperature has reached equilibrium at 25 ± 0.1 °C, add 1.0 ml of the trypsin and aprotinin solution and start a timer. Maintain at pH 8.0 by the addition of *0.1 M sodium hydroxide* and note the volume added every 30 s. Continue the reaction for 6 min. Determine the number of millilitres of *0.1 M sodium hydroxide* used per second (n_1 ml). Carry out, under the same conditions, a titration using 1.0 ml of the dilute trypsin solution. Determine the number of millilitres of *0.1 M sodium hydroxide* used per second (n_2 ml).

Calculate the aprotinin activity in European Pharmacopoeia Units per milligram from the expression:

$$\frac{4000\,(2n_2 - n_1)}{m}$$

The estimated activity is not less than 90 per cent and not more than 110 per cent of the activity stated on the label.

STORAGE

In an airtight, tamper-proof container, protected from light.

LABELLING

The label states the number of European Pharmacopoeia Units of aprotinin activity per milligram.

Arachis Oil

General Notices

Peanut Oil

(Refined Arachis Oil, Ph Eur monograph 0263)

Preparation

Arachis Oil Enema

Ph Eur

DEFINITION

The refined fatty oil obtained from the shelled seeds of *Arachis hypogaea* L. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, yellowish, viscous liquid.

Solubility

Very slightly soluble in ethanol (96 per cent), miscible with light petroleum.

Relative density

About 0.915.

It solidifies at about 2 °C.

IDENTIFICATION

It complies with the identification test for fatty oils by thin-layer chromatography *(2.3.2)*. The chromatogram obtained is similar to the typical chromatogram for arachis oil.

TESTS

Acid value *(2.5.1)*

Maximum 0.5, determined on 10.0 g.

Peroxide value *(2.5.5)*

Maximum 5.0.

Unsaponifiable matter *(2.5.7)*

Maximum 1.0 per cent, determined on 5.0 g.

Alkaline impurities *(2.4.19)*

It complies with the test for alkaline impurities in fatty oils.

Composition of fatty acids

Gas chromatography *(2.4.22, Method A)*. Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty acid fraction of the oil:

— *saturated fatty acids of chain length less than* C_{16}: maximum 0.4 per cent,

— *palmitic acid*: 7.0 per cent to 16.0 per cent,

— *stearic acid*: 1.3 per cent to 6.5 per cent,

— *oleic acid* (equivalent chain length on polyethyleneglycol adipate 18.3): 35.0 per cent to 72.0 per cent,

— *linoleic acid* (equivalent chain length on polyethyleneglycol adipate 18.9): 13.0 per cent to 43.0 per cent,

— *linolenic acid* (equivalent chain length on polyethyleneglycol adipate 19.7): maximum 0.6 per cent,

— *arachidic acid*: 0.5 per cent to 3.0 per cent,

— *eicosenoic acid* (equivalent chain length on polyethyleneglycol adipate 20.3): 0.5 per cent to 2.1 per cent,

— *behenic acid*: 1.0 per cent to 5.0 per cent,

— *erucic acid* (equivalent chain length on polyethyleneglycol adipate 22.3): maximum 0.5 per cent,

— *lignoceric acid*: 0.5 per cent to 3.0 per cent.

Water *(2.5.12)*

Maximum 0.3 per cent, determined on 3.00 g, if intended for use in the manufacture of parenteral dosage forms.

STORAGE

In a well-filled container, protected from light.

LABELLING

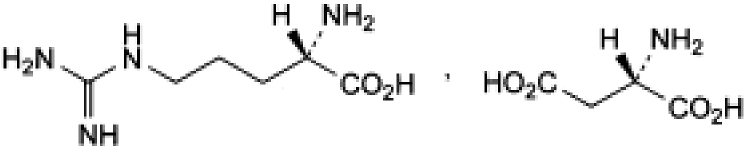
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Arginine Aspartate

General Notices

(Ph Eur monograph 2096)



$C_6H_{14}N_4O_2,C_4H_7NO_4$ 307.3   *7675-83-4*

Action and use

Amino acid; nutrient.

Ph Eur

DEFINITION

(2*S*)-2-Amino-5-guanidinopentanoic acid (2*S*)-2-aminobutanedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white granules or powder.

Solubility

Very soluble in water, practically insoluble in alcohol and in methylene chloride.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   arginine aspartate CRS .

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results   The 2 principal spots in the chromatogram obtained with test solution (b) are similar in position, colour and size to the 2 principal spots in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

6.0 to 7.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 25 to + 27 (dried substance).

Dissolve 2.50 g in *dilute hydrochloric acid R* and dilute to 25.0 ml with the same acid.

Ninhydrin-positive substances

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *water R*.

Reference solution (a) Dissolve 25 mg of *arginine R* and 25 mg of *aspartic acid R* in *water R* and dilute to 25 ml with the same solvent.

Reference solution (b) Dilute 2 ml of reference solution (a) to 50 ml with *water R*.

Plate *TLC silica gel G plate R*.

Mobile phase *ammonia R*, *propanol R* (36:64 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying At 100-105 °C for 10 min.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Limit Test solution (a):

— *any impurity*: any spots, apart from the 2 principal spots, are not more intense than each of the 2 principal spots in the chromatogram obtained with reference solution (b) (0.2 per cent).

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 300 ppm.

To 0.5 g add 2.5 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. Examine after 30 min.

Ammonium ***(2.4.1)***

Maximum 100 ppm, determined on 100 mg.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 60 °C for 24 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 80.0 mg in 2 ml of *anhydrous formic acid R*. Add 50 ml of anhydrous acetic acid R. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

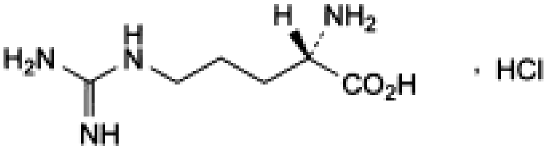
1 ml of *0.1 M perchloric acid* is equivalent to 10.24 mg of $C_{10}H_{21}N_5O_6$.

Ph Eur

Arginine Hydrochloride

General Notices

(Ph Eur monograph 0805)



$C_6H_{14}N_4O_2$,HCl 210.7 *1119-34-2*

Action and use

Amino acid; nutrient.

Preparation

Arginine Hydrochloride Intravenous Infusion

Ph Eur

DEFINITION

Arginine hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of the hydrochloride of (*S*)-2-amino-5-guanidinopentanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, very slightly soluble in alcohol.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *arginine hydrochloride CRS* . Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 25 mg in 2 ml of *water R*. Add 1 ml of α-*naphthol solution R* and 2 ml of a mixture of equal volumes of *strong sodium hypochlorite solution R* and *water R*. A red colour develops.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 2.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 21.0 to + 23.5, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R.*

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R.*

Reference solution (a) Dissolve 10 mg of *arginine hydrochloride CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *arginine hydrochloride CRS* and 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 25 ml with the same solvent.

Apply to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 30 volumes of *concentrated ammonia R* and 70 volumes of *2-propanol R*. Dry the plate at 100 °C to 105 °C until the ammonia disappears completely. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Sulphates *(2.4.13)*

Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals *(2.4.8)*

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.180 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Using 0.1 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 21.07 mg of $C_6H_{15}ClN_4O_2$.

STORAGE

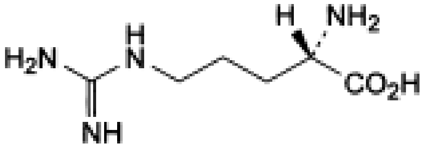
Store protected from light.

Ph Eur

Arginine

General Notices

(Ph Eur monograph 0806)



$C_6H_{14}N_4O_2$ 174.2 *74-79-3*

Action and use

Amino acid; nutrient.

Ph Eur

DEFINITION

Arginine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2-amino-5-guanidinopentanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, very slightly soluble in alcohol.

IDENTIFICATION

First identification A, C.

Second identification A, B, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Solution S (see Tests) is strongly alkaline *(2.2.4)*.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *arginine CRS* . Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

E. Dissolve about 25 mg in 2 ml of *water R*. Add 1 ml of α-*naphthol solution R* and 2 ml of a mixture of equal volumes of *strong sodium hypochlorite solution R* and water. A red colour develops.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 2.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 25.5 to + 28.5, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dilute hydrochloric acid R* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *arginine CRS* in *0.1 M hydrochloric acid* and dilute to 50 ml with the same acid.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *arginine CRS* and 10 mg of *lysine hydrochloride CRS* in *0.1 M hydrochloric acid* and dilute to 25 ml with the same acid.

Apply to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 30 volumes of *concentrated ammonia R* and 70 volumes of *2-propanol R*. Dry the plate at 100 °C to 105 °C until the ammonia disappears completely. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

To 5 ml of solution S add 0.5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

To 10 ml of solution S, add 1.7 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron ***(2.4.9)***

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1,* shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals ***(2.4.8)***

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 50 ml of *water R*. Using 0.2 ml of *methyl red mixed solution R* as indicator, titrate with *0.1 M hydrochloric acid* until the colour changes from green to violet-red.

1 ml of *0.1 M hydrochloric acid* is equivalent to 17.42 mg of $C_6H_{14}N_4O_2$.

STORAGE

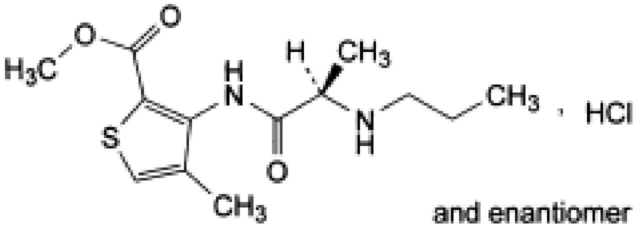
Store protected from light.

Ph Eur

Articaine Hydrochloride

General Notices

(Ph Eur monograph 1688)



$C_{13}H_{20}N_2O_3S,HCl$ 320.8 *23964-57-0*

Action and use

Local anaesthetic.

Ph Eur

DEFINITION

Methyl 4-methyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylate hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in alcohol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 50.0 mg in a 1 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of the solution to 100.0 ml with a 1 g/l solution of *hydrochloric acid R*. Examined between 200 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 272 nm. The specific absorbance at the maximum is 290 to 320.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Place dropwise 20 µl of the test solution on 300 mg discs.

Test solution Dissolve 0.1 g in 5 ml of *water R*, add 3 ml of a saturated solution of *sodium hydrogen carbonate R* and shake twice with 2 ml of *methylene chloride R*. Combine the methylene chloride layers, dilute to 5.0 ml with *methylene chloride R* and dry over *anhydrous sodium sulphate R.*

Comparison articaine hydrochloride CRS .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in 5 ml of *alcohol R*.

Reference solution Dissolve 20 mg of *articaine hydrochloride CRS* in 5 ml of *alcohol R*.

Plate TLC silica gel F_{254} *plate R.*

Mobile phase triethylamine R, *ethyl acetate R*, *heptane R* (10:35:65 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection .Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.50 g in *water R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method I)*.

pH *(2.2.3)*

4.2 to 5.2.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *articaine impurity A CRS* and 5.0 mg of *articaine impurity E CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (c) Add 1.0 ml of reference solution (b) to 50.0 mg of *articaine hydrochloride CRS* and dilute to 50 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of reference solution (b) to 50.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 335 m2/g and a carbon loading of 19 per cent,

— *temperature*: 45 °C.

Mobile phase Mix 25 volumes of *acetonitrile R* and 75 volumes of a solution prepared as follows: dissolve 2.02 g of *sodium heptanesulphonate R* and 4.08 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000 ml with the same solvent. Adjust to pH 2.0 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 276 nm.

Injection 10 µl; inject the test solution and reference solutions (a), (c) and (d).

Run time 5 times the retention time of articaine.

Relative retentions With reference to articaine (retention time = about 9.3 min): impurity B = about 0.6; impurity D = about 0.7; impurity A = about 0.8; impurity E = about 0.86; impurity F = about 0.9; impurity G = about 1.7; impurity H = about 2.1; impurity I = about 2.6; impurity C = about 3.6; impurity J = about 4.0.

System suitability Reference solution (c):

— *resolution*: minimum 1.2 between the peaks due to impurity A and impurity E.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.2 per cent),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total of other impurities*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 5 ppm.

Dissolve 4.0 g in 20.0 ml of *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)* using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 32.08 mg of $C_{13}H_{21}ClN_2O_3S$.

STORAGE

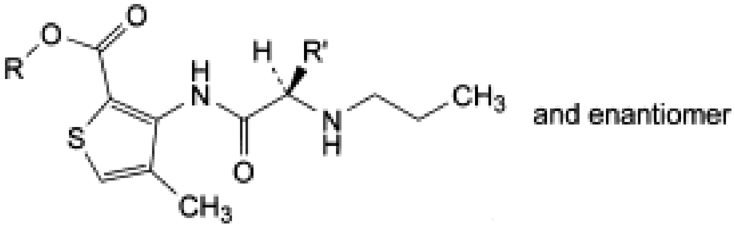
Protected from light.

IMPURITIES

Specified impurities *A, B, C.*

Other detectable impurities

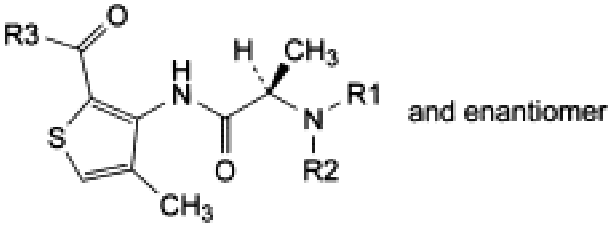
D, E, F, G, H, I, J.



A. R = CH_3, R′ = H: methyl 3-[[2-(propylamino)acetyl]amino]-4-methylthiophene-2-carboxylate (acetamidoarticaine),

B. R = H, R′ = CH_3: 4-methyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylic acid (articaine acid),

C. R = $CH(CH_3)_2$, R′ = CH_3: 1-methylethyl 4-methyl-3-[[(2*RS*)-2-(propylamino)propanoyl] amino]thiophene-2-carboxylate (articaine isopropyl ester),



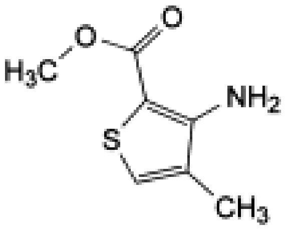
D. R1 = CH_2-CH_3, R2 = H, R3 = OCH_3: methyl 3-[[(2*RS*)-2-(ethylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate (ethylarticaine),

E. R1 = $CH(CH_3)_2$, R2 = H, R3 = OCH_3: methyl 4-methyl-3-[[(2*RS*)-2-[(1-methylethyl)amino] propanoyl]amino]thiophene-2-carboxylate (isopropylarticaine),

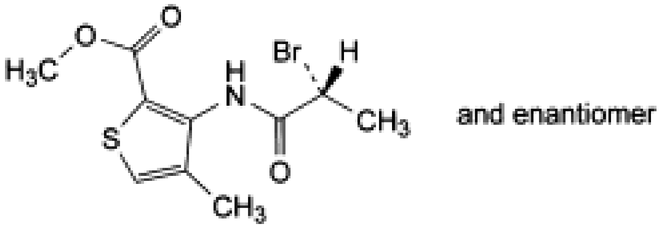
F. R1 = CH_2-CH_2-CH_3, R2 = H, R3 = NH-CH_2-CH_2-CH_3: 4-methyl-N-propyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxamide (articaine acid propionamide),

G. R1 = $(CH_2)_3$-CH_3, R2 = H, R3 = OCH_3: methyl 3-[[(2*RS*)-2-(butylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate (butylarticaine),

H. R1 = R2 = CH_2-CH_2-CH_3, R3 = OCH_3: methyl 3-[[(2*RS*)-2-(dipropylamino)propanoyl] amino]-4-methylthiophene-2-carboxylate (dipropylarticaine),



I. methyl 3-amino-4-methylthiophene-2-carboxylate (3-aminoarticaine),



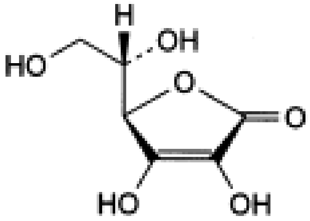
J. methyl 3-[[(2*RS*)-2-bromopropanoyl]amino]-4-methylthiophene-2-carboxylate (bromo compound).

Ph Eur

Ascorbic Acid

General Notices

(Ph Eur monograph 0253)



$C_6H_8O_6$ 176.1 *50-81-7*

Action and use

Vitamin C.

Preparations

Ascorbic Acid Injection

Ascorbic Acid Tablets

Paediatric Vitamins A, C and D Oral Drops

Vitamins B and C Injection

When Vitamin C is prescribed or demanded, Ascorbic Acid shall be dispensed or supplied.

Ph Eur

DEFINITION

Ascorbic acid contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of (5*R*)-5-[(1*S*)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, becoming discoloured on exposure to air and moisture, freely soluble in water, soluble in ethanol (96 per cent).

It melts at about 190 °C, with decomposition.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D.*

A. Dissolve 0.10 g in *water R* and dilute immediately to 100.0 ml with the same solvent. To 10 ml of *0.1 M hydrochloric acid* add 1.0 ml of the solution and dilute to 100.0 ml with *water R*. Measure the absorbance *(2.2.25)* at the maximum at 243 nm immediately after dissolution. The specific absorbance at the maximum is 545 to 585.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *ascorbic acid CRS*. Examine the substance prepared as discs containing 1 mg.

C. The pH *(2.2.3)* of solution S (see Tests) is 2.1 to 2.6.

D. To 1 ml of solution S add 0.2 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2*. A grey precipitate is formed.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 20.5 to + 21.5.

Oxalic acid

Dissolve 0.25 g in 5 ml of *water R.* Neutralise to *red litmus paper R* using *dilute sodium hydroxide solution R* and add 1 ml of *dilute acetic acid R* and 0.5 ml of *calcium chloride solution R* (test solution). Prepare a reference solution as follows: dissolve 70 mg of *oxalic acid R* in *water R* and dilute to 500 ml with the same solvent; to 5 ml of this solution add 1 ml of *dilute acetic acid R* and 0.5 ml of *calcium chloride solution R* (reference solution). Allow the solutions to stand for 1 h. Any opalescence in the test solution is not more intense than that in the reference solution (0.2 per cent).

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Copper

Not more than 5.0 ppm of Cu, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 2.0 g of the substance to be examined in *0.1 M nitric acid* and dilute to 25.0 ml with the same acid.

Reference solutions Prepare reference solutions containing 0.2 ppm, 0.4 ppm and 0.6 ppm of Cu by diluting *copper standard solution (10 ppm Cu) R* with *0.1 M nitric acid*.

Measure the absorbance at 324.8 nm using a copper hollow-cathode lamp as a source of radiation and an air-acetylene flame. Adjust the zero of the apparatus using *0.1 M nitric acid*.

Iron

Not more than 2.0 ppm of Fe, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 5.0 g of the substance to be examined in *0.1 M nitric acid* and dilute to 25.0 ml with the same acid.

Reference solutions Prepare reference solutions containing 0.2 ppm, 0.4 ppm and 0.6 ppm of Fe by diluting *iron standard solution (20 ppm Fe) R* with *0.1 M nitric acid*.

Measure the absorbance at 248.3 nm using an iron hollow-cathode lamp as a source of radiation and an air-acetylene flame. Adjust the zero of the apparatus using *0.1 M nitric acid*.

Heavy metals *(2.4.8)*

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A (10 ppm). Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in a mixture of 10 ml of *dilute sulphuric acid R* and 80 ml of *carbon dioxide-free water R*. Add 1 ml of *starch solution R*. Titrate with *0.05 M iodine* until a persistent violet-blue colour is obtained.

1 ml of *0.05 M iodine* is equivalent to 8.81 mg of $C_6H_8O_6$.

STORAGE

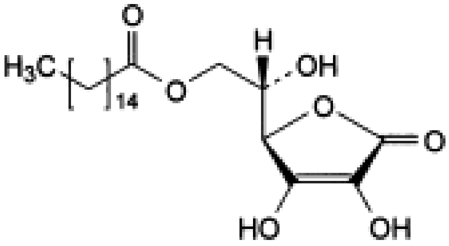
Store in a non-metallic container, protected from light.

Ph Eur

Ascorbyl Palmitate

General Notices

(Ph Eur monograph 0807)



$C_{22}H_{38}O_7$ 414.5 *137-66-6*

Action and use

Excipient.

Ph Eur

DEFINITION

(2*S*)-2-[(5*R*)-3,4-Dihydroxy-5-oxo-2,5-dihydrofuran-2-yl]-2-hydroxyethyl hexadecanoate.

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent) and in methanol, practically insoluble in methylene chloride and in fatty oils.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison ascorbyl palmitate CRS .

C. Dissolve about 10 mg in 5 ml of *methanol R*. The solution decolourises *dichlorophenolindophenol standard solution R*.

TESTS

Solution S

Dissolve 2.50 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_4 *(2.2.2, Method I)*.

Specific optical rotation *(2.2.7)*

+ 21 to + 24 (dried substance), determined on solution S.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 5 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g in 50 ml of *methanol R*. Add 30 ml of *water R* and 1 ml of *starch solution R*. Titrate with *0.05 M iodine* until a persistent violet-blue colour is obtained.

1 ml of *0.05 M iodine* is equivalent to 20.73 mg of $C_{22}H_{38}O_7$.

STORAGE

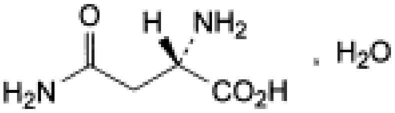
In an airtight container , protected from light.

Ph Eur

Asparagine Monohydrate

General Notices

(Ph Eur monograph 2086)



$C_4H_8N_2O_3,H_2O$ 150.1 *5794-13-8*

Action and use

Amino acid.

Ph Eur

DEFINITION

(2*S*)-2,4-Diamino-4-oxobutanoic acid monohydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder or colourless crystals.

Solubility

Slightly soluble in water, practically insoluble in alcohol and in methylene chloride.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *asparagine monohydrate CRS .*

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in

position, colour and size to the principal spot in the chromatogram obtained with reference solution (c).

TESTS

Solution S

Dissolve with heating 2.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.0 to 6.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 33.7 to + 36.0 (dried substance).

Dissolve 2.50 g in a 309.0 g/l solution of *hydrochloric acid R* and dilute to 25.0 ml with the same acid.

Ninhydrin-positive substances

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.25 g of the substance to be examined in *water R*, heating to not more than 40 °C, and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *water R*.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 200 ml with *water R*.

Reference solution (b) Dissolve 25 mg of *glutamic acid R* in *water R*, add 1 ml of test solution (a) and dilute to 10 ml with *water R*.

Reference solution (c) Dissolve 25 mg of *asparagine monohydrate CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *glacial acetic acid R*, *water R*, *butanol R* (25:25:50 *V/V/V*).

Application 5 µl.

Development Over half of the plate.

Drying At 110 °C for 15 min.

Detection Spray with *ninhydrin solution R* and heat at 110 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Limit Test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Chlorides ***(2.4.4)***

Maximum 200 ppm.

Dilute 12.5 ml of solution S to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 200 ppm.

To 0.75 g add 2.5 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. Examine after 30 min.

Ammonium ***(2.4.1, Method B)***

Maximum 0.1 per cent, determined on 10 mg.

Iron ***(2.4.9)***

Maximum 10 ppm.

Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 10 ml with the same acid. Shake 3 times with 10 ml of *methyl isobutyl ketone R1* for 3 min. Wash the combined organic phases with 10 ml of *water R* for 3 min. The aqueous phase complies with the limit test for iron.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 3 ml of *dilute hydrochloric acid R* and 15 ml of *water R* with gentle warming if necessary. Dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

10.5 per cent to 12.5 per cent, determined on 1.000 g by drying in an oven at 60 °C for 24 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.110 g in 5 ml of *anhydrous formic acid R*. Add 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 13.21 mg of $C_4H_8N_2O_3$.

IMPURITIES

Specified impurities *A, B.*

A. aspartic acid,

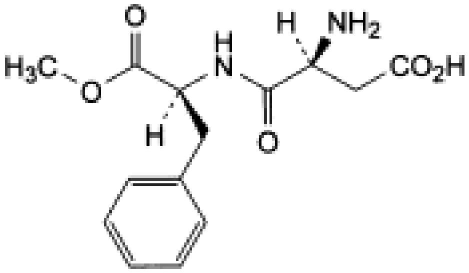
B. glutamic acid.

Ph Eur

Aspartame

General Notices

(Ph Eur monograph 0973)



$C_{14}H_{18}N_2O_5$ 294.3 *53906-69-7*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

(3*S*)-3-Amino-4-[[(1*S*)-1-benzyl-2-methoxy-2-oxoethyl]amino]-4-oxobutanoic acid.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic, crystalline powder.

Solubility

Sparingly soluble or slightly soluble in water and in ethanol (96 per cent), practically insoluble in hexane and in methylene chloride.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 0.1 g in *ethanol (96 per cent) R* and dilute to 100 ml with the same solvent.

Spectral range 230-300 nm.

Absorption maxima At 247 nm, 252 nm, 258 nm and 264 nm.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *aspartame CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 15 mg of the substance to be examined in 2.5 ml of *water R* and dilute to 10 ml with *acetic acid R*.

Reference solution Dissolve 15 mg of *aspartame CRS* in 2.5 ml of *water R* and dilute to 10 ml with *acetic acid R*.

Plate *TLC silica gel G plate R.*

Mobile phase *water R*, *anhydrous formic acid R*, *methanol R*, *methylene chloride R* (2:4:30:64 *V/V/V/V*).

Application 20 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

Results The spot in the chromatogram obtained with the test solution is similar in position, colour and size to the spot in the chromatogram obtained with the reference solution.

D. Dissolve about 20 mg in 5 ml of *methanol R* and add 1 ml of *alkaline hydroxylamine solution R1*. Heat on a water-bath for 15 min. Allow to cool and adjust to about pH 2 with *dilute hydrochloric acid R*. Add 0.1 ml of *ferric chloride solution R1*. A brownish-red colour is produced.

TESTS

Solution S

Dissolve 0.8 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution GY_6 *(2.2.2, Method II)*.

Conductivity *(2.2.38)*

Maximum 30 $\mu S \cdot cm^{-1}$.

Dissolve 0.80 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent. Measure the conductivity of the solution (C_1) and that of the water used for preparing the solution (C_2). The readings must be stable within 1 per cent over a period of 30 s.

Calculate the conductivity of the solution of the substance to be examined using the following expression:

$$C_1 - 0.992\ C_2$$

Specific optical rotation ***(2.2.7)***

+ 14.5 to + 16.5 (dried substance).

Dissolve 2.00 g in a 690 g/l solution of *anhydrous formic acid R* and dilute to 50.0 ml with the same solution. Measure within 30 min of preparation.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.60 g of the substance to be examined in a mixture of 1.5 volumes of *glacial acetic acid R* and 98.5 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 4.5 mg of *aspartame impurity A CRS* in a mixture of 1.5 volumes of *glacial acetic acid R* and 98.5 volumes of *water R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (b) Dissolve 30.0 mg of *phenylalanine R* (impurity C) in a mixture of 15 volumes of *glacial acetic acid R* and 85 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Reference solution (c) Dilute 5.0 ml of the test solution to 10.0 ml with *water R*. Dilute 3.0 ml of this solution to 100.0 ml with *water R*.

Reference solution (d) Dissolve 30.0 mg of *L-aspartyl-L-phenylalanine R* (impurity B) in a mixture of 15 volumes of *glacial acetic acid R* and 85 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 1.0 ml of the solution to 10.0 ml with *water R*. Mix 1.0 ml of this solution with 1.0 ml of reference solution (b).

Column:
- *size:* *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Mix 10 volumes of *acetonitrile R* and 90 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.7 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time Twice the retention time of aspartame.

System suitability Reference solution (d):

- *resolution*: minimum 3.5 between the peaks due to impurities B and C.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained

with reference solution (a) (1.5 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *sum of impurities other than A and C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.5 per cent);

— *disregard limit*: disregard any peak due to the solvent.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

1.0 g complies with test C. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 4.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 1.5 ml of *anhydrous formic acid R* and 60 ml of *anhydrous acetic acid R*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities *A, C.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*)

B.

A. 2-(5-benzyl-3,6-dioxopiperazin-2-yl)acetic acid (diketopiperazine),

B. L-aspartyl-L-phenylalanine,

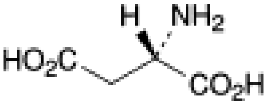
C. phenylalanine.

Ph Eur

Aspartic Acid

General Notices

(Ph Eur monograph 0797)



$C_4H_7NO_4$ 133.1 *6899-03-2*

Action and use

Amino acid.

Ph Eur

DEFINITION

Aspartic acid contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of (2*S*)-2-aminobutanedioic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. It complies with the test for specific optical rotation (see Tests).

B. A suspension of 1 g in 10 ml of *water R* is strongly acid *(2.2.4)*.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *aspartic acid CRS* . Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 10 ml with the same acid. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation ***(2.2.7)***

Dissolve 2.000 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 24.0 to + 26.0, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 2 ml of *ammonia R* and dilute to 10 ml with *water R*.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *aspartic acid CRS* in 2 ml of *dilute ammonia R1* and dilute to 50 ml with *water R*.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *aspartic acid CRS* and 10 mg of *glutamic acid CRS* in 2 ml of *dilute ammonia R1* and dilute to 25 ml with *water R*.

Apply separately to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R*. Heat at 100-105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated principal spots.

Chlorides ***(2.4.4)***

Dissolve 0.25 g in 3 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution, to which 1 ml of *water R* is added instead of *dilute nitric acid R*, complies with the limit test for chlorides (200 ppm).

Sulphates ***(2.4.13)***

Dissolve 0.5 g in 4 ml of *hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm). Carry out the evaluation of the test after 30 min.

Ammonium.*(2.4.1)*

50 mg complies with limit test B (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R.*

Iron ***(2.4.9)***

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals ***(2.4.8)***

2.0 g complies with limit test D (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 50 ml of *carbon dioxide-free water R*, with slight heating if necessary. Cool and add *0.1 ml of bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide* until the colour changes from yellow to blue.

1 ml of *0.1 M sodium hydroxide* is equivalent to 13.31 mg of $C_4H_7NO_4$.

STORAGE

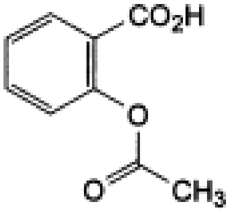
Protected from light.

Ph Eur

Aspirin

General Notices

(Acetylsalicylic Acid, Ph Eur monograph 0309)



$C_9H_8O_4$ 180.2 *50-78-2*

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor;antipyretic; analgesic; anti-inflammatory.

Preparations

Aspirin Tablets

Dispersible Aspirin Tablets

Effervescent Soluble Aspirin Tablets

Gastro-resistant Aspirin Tablets

Aspirin and Caffeine Tablets

Co-codaprin Tablets

Dispersible Co-codaprin Tablets

Ph Eur

DEFINITION

2-(Acetyloxy)benzoic acid.

Content

99.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Slightly soluble in water, freely soluble in ethanol (96 per cent).

mp: About 143 °C (instantaneous method).

IDENTIFICATION

First identification *A, B.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *acetylsalicylic acid CRS.*

B. To 0.2 g add 4 ml of *dilute sodium hydroxide solution R* and boil for 3 min. Cool and add 5 ml of *dilute sulphuric acid R*. A crystalline precipitate is formed. Filter, wash the precipitate and dry at 100-105 °C. The melting point *(2.2.14)* is 156 °C to 161 °C.

C. In a test tube mix 0.1 g with 0.5 g of *calcium hydroxide R*. Heat the mixture and expose to the fumes produced a piece of filter paper impregnated with 0.05 ml of *nitrobenzaldehyde solution R*. A greenish-blue or greenish-yellow colour develops on the paper. Moisten the paper with *dilute hydrochloric acid R*. The colour becomes blue.

D. Dissolve with heating about 20 mg of the precipitate obtained in identification test B in 10 ml of *water R* and cool. The solution gives reaction (a) of salicylates *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in 9 ml of *ethanol (96 per cent) R*.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 0.10 g of the substance to be examined in *acetonitrile for chromatography R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *salicylic acid R* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *salicylic acid R* in the mobile phase and dilute to 10.0 ml with the mobile phase. To 1.0 ml of this solution add 0.2 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *phosphoric acid R, acetonitrile for chromatography R, water R* (2:400:600 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 237 nm.

Injection 10 µl.

Run time 7 times the retention time of acetylsalicylic acid.

System suitability Reference solution (b):

— *resolution*: minimum 6.0 between the 2 principal peaks.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in 12 ml of *acetone R* and dilute to 20 ml with *water R*. 12 ml of this solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 6 volumes of *water R* and 9 volumes of *acetone R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo*.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

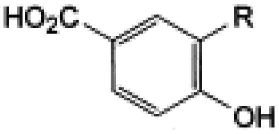
In a flask with a ground-glass stopper, dissolve 1.000 g in 10 ml of *ethanol (96 per cent) R*. Add 50.0 ml of *0.5 M sodium hydroxide*. Close the flask and allow to stand for 1 h. Using 0.2 ml of *phenolphthalein solution R* as indicator, titrate with *0.5 M hydrochloric acid*. Carry out a blank titration.

1 ml of *0.5 M sodium hydroxide* is equivalent to 45.04 mg of $C_9H_8O_4$.

STORAGE

In an airtight container.

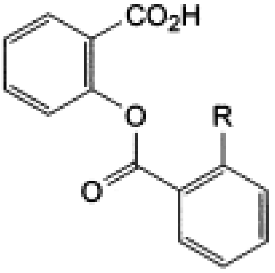
IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

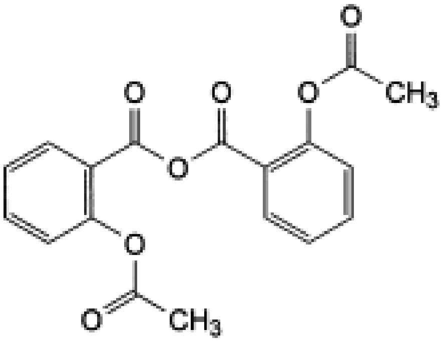
B. R = CO_2H: 4-hydroxybenzene-1,3-dicarboxylic acid (4-hydroxyisophthalic acid),

C. salicylic acid,



D. R = O-CO-CH_3: 2-[[2-(acetyloxy)benzoyl]oxy]benzoic acid (acetylsalicylsalicylic acid),

E. R = OH: 2-[(2-hydroxybenzoyl)oxy]benzoic acid (salicylsalicylic acid),



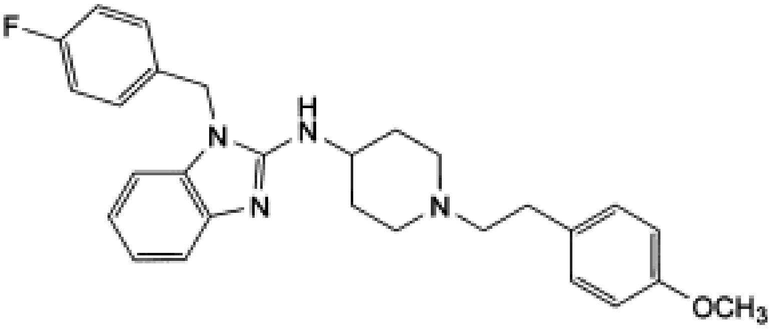
F. 2-(acetyloxy)benzoic anhydride (acetylsalicylic anhydride).

Ph Eur

Astemizole

General Notices

(Ph Eur monograph 1067)



$C_{28}H_{31}FN_4O$ 458.6 *68844-77-9*

Action and use

Histamine H_1 receptor antagonist.

Ph Eur

DEFINITION

1-(4-Fluorobenzyl)-*N*-[1-[2-(4-methoxyphenyl)ethyl]piperidin-4-yl]-1*H*-benzimidazol-2-amine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride and in methanol, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 175 °C to 178 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *astemizole CRS.*

C. Thin layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 30 mg of *astemizole CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 30 mg of *astemizole CRS* and 30 mg of *ketoconazole CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *ammonium acetate solution R*, *dioxan R*, *methanol R* (20:40:40 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose the plate to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank solution prepared in the same manner. The test solution is yellow and the blank solution is red.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and is not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Dissolve 0.2 g in *methanol R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *astemizole CRS* and 25 mg of *ketoconazole CRS* in *methanol R* and dilute to 100.0 ml with the same solvent.

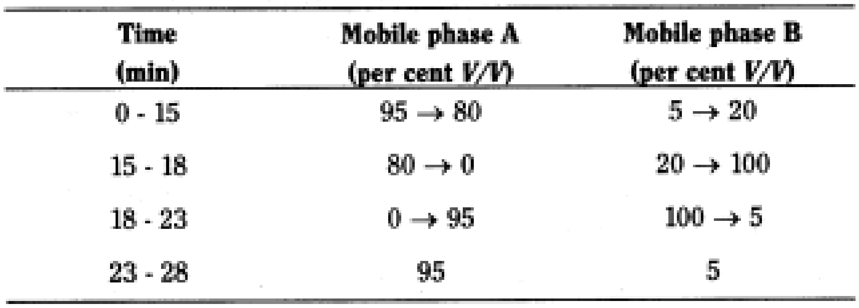
Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol R*.

Column:
— *size:* l = 0.1 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: a 17 g/l solution of *tetrabutylammonium hydrogen sulphate R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	95 → 80	5 → 20
15 - 18	80 → 0	20 → 100
18 - 23	0 → 95	100 → 5
23 - 28	95	5

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 278 nm.

Equilibration With *acetonitrile R* for at least 30 min then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time ketoconazole = about 8 min; astemizole = about 9 min.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to ketoconazole and astemizole; if necessary, adjust the final concentration of acetonitrile or the percentage of tetrabutylammonium hydrogen sulphate in the mobile phase or adjust the time programme for the linear gradient elution.

Limits:
— *impurities A, B, C, D, E, F, G, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak obtained with the blank.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution R* as indicator.

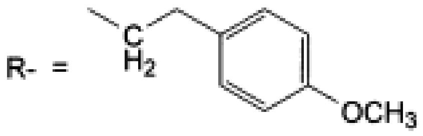
1 ml of *0.1 M perchloric acid* is equivalent to 22.93 mg of $C_{28}H_{31}FN_4O$.

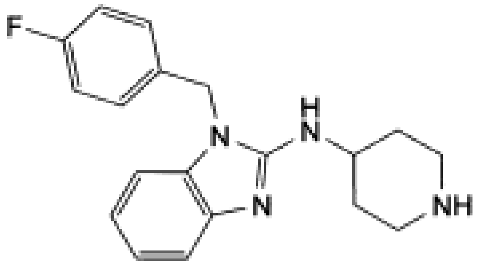
STORAGE

Protected from light.

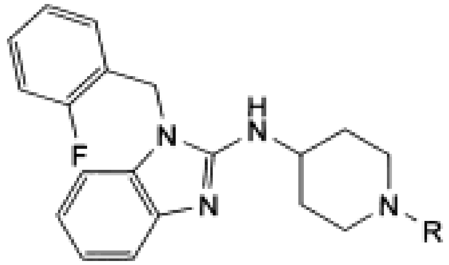
IMPURITIES

Specified impurities A, B, C, D, E, F, G, H.

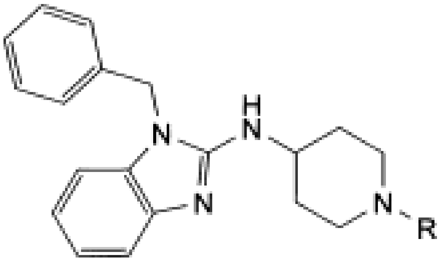




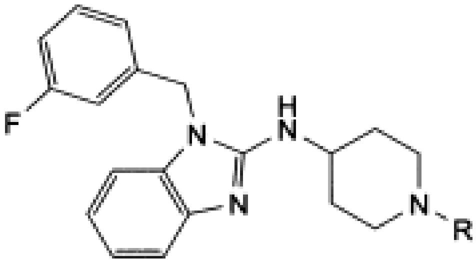
A. 1-(4-fluorobenzyl)-*N*-(piperidin-4-yl)-1*H*-benzimidazol-2-amine,



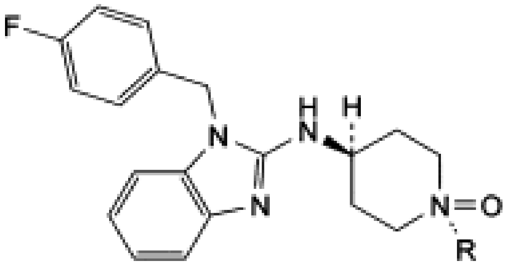
B. 1-(2-fluorobenzyl)-*N*-[1-[2-(4-methoxyphenyl)ethyl]piperidin-4-yl]-1*H*-benzimidazol-2-amine,



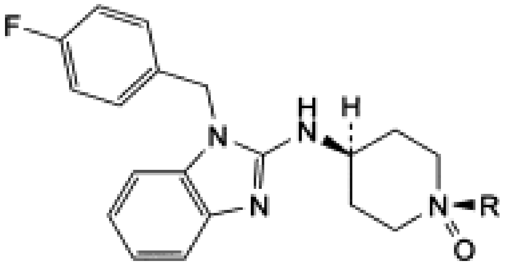
C. 1-benzyl-*N*-[1-[2-(4-methoxyphenyl)ethyl]piperidin-4-yl]-1*H*-benzimidazol-2-amine,



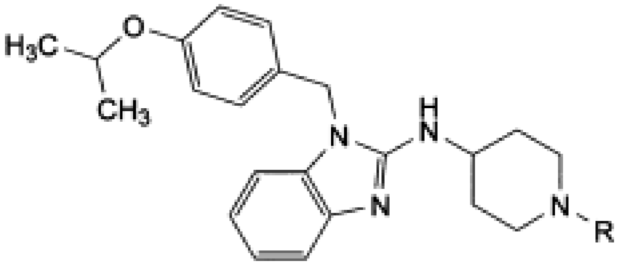
D. 1-(3-fluorobenzyl)-*N*-[1-[2-(4-methoxyphenyl)ethyl]piperidin-4-yl]-1*H*-benzimidazol-2-amine,



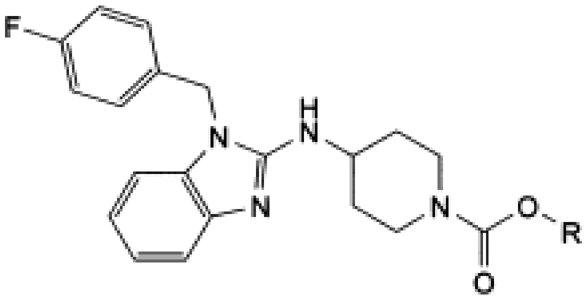
E. 1-(4-fluorobenzyl)-*N*-[*cis*-1-[2-(4-methoxyphenyl)ethyl]piperidin-4-yl 1-oxide]-1*H*-benzimidazol-2-amine,



F. 1-(4-fluorobenzyl)-*N*-[*trans*-1-[2-(4-methoxyphenyl)ethyl]piperidin-4-yl 1-oxide]-1*H*-benzimidazol-2-amine,



G. *N*-[1-[2-(4-methoxyphenyl)ethyl]piperidin-4-yl]-1-[4-(1-methylethoxy)benzyl]-1*H*-benzimidazol-2-amine,



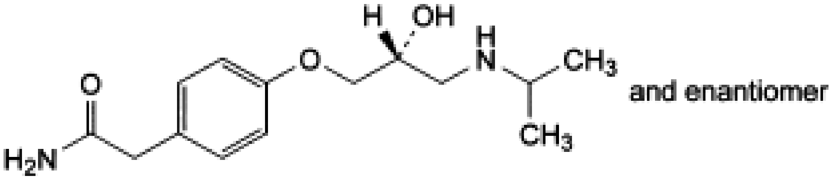
H. 2-(4-methoxyphenyl)ethyl 4-[[1-(4-fluorobenzyl)-1*H*-benzimidazol-2-yl]amino]piperidin-1-carboxylate.

Ph Eur

Atenolol

General Notices

(Ph Eur monograph 0703)



$C_{14}H_{22}N_2O_3$ 266.3 *29122-68-7*

Action and use

Beta-adrenoceptor antagonist.

Preparations

Atenolol Injection

Atenolol Oral Solution

Atenolol Tablets

Co-tenidone Tablets

Ph Eur

DEFINITION

Atenolol contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 2-[4-[(2 *RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]acetamide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, sparingly soluble in water, soluble in ethanol, slightly soluble in methylene chloride.

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Melting point *(2.2.14)*: 152 °C to 155 °C.

B. Dissolve 0.100 g in *methanol R* and dilute to 100 ml with the same solvent. Dilute 10.0 ml of this solution to 100 ml with *methanol R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows two absorption maxima, at 275 nm and 282 nm. The ratio of the absorbance measured at the maximum at 275 nm to that measured at the maximum at 282 nm is 1.15 to 1.20.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *atenolol CRS* .

D. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 10 mg of the substance to be examined in 1 ml of *methanol R*.

Reference solution Dissolve 10 mg of *atenolol CRS* in 1 ml of *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R1* and 99 volumes of *methanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 0.10 g in *water R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than degree 6 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

Determined on solution S, the angle of optical rotation is + 0.10° to - 0.10°.

Related substances

Examine by liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in 20 ml of the mobile phase and dilute to 25.0 ml with the mobile phase.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in 0.1 ml of *dimethyl sulphoxide R*, if necessary applying gentle heat by placing the containing vessel in a water-bath for a few seconds, and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 0.5 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 50.0 mg of *atenolol for column validation CRS* in 0.1 ml of *dimethyl sulphoxide R*, if necessary applying gentle heat by placing the containing vessel in a water-bath for a few seconds, and dilute to 25.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.15 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture prepared as follows: dissolve 1.0 g of *sodium octanesulphonate R* and 0.4 g of *tetrabutylammonium hydrogen sulphate R* in 1 litre of a mixture of 20 volumes of *tetrahydrofuran R*, 180 volumes of *methanol R* and 800 volumes of a 3.4 g/l solution of *potassium dihydrogen phosphate R*; adjust to pH 3.0 with *phosphoric acid R*,

— as detector a spectrophotometer set at 226 nm.

Equilibrate the column with the mobile phase at a flow rate of 1.0 ml/min for about 30 min.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 10 µl of reference solution (a) is at least 50 per cent of the full scale of the recorder.

Inject 10 µl of reference solution (b). The resulting chromatogram is similar to that of the specimen chromatogram provided with *atenolol for column validation CRS* in that the peak due to bis-ether precedes and is separated from that due to tertiary amine which normally appears as a doublet. If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase. Increasing the concentration of sodium octanesulphonate increases the retention time of the tertiary amine.

Inject separately 10 µl of test solution (a) and reference solution (a). Continue the chromatography for four times the retention time of the principal peak. In the chromatogram obtained with test solution (a): the area of any peak apart from the principal peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Disregard any peak with an area less than 0.1 times of that of the principal peak in the chromatogram obtained with reference solution (a).

If the substance to be examined is found to contain more than 0.15 per cent of bis-ether, its compliance is confirmed by repeating the chromatography using 10 µl of test solution (b).

Chlorides *(2.4.4)*

Dissolve 50 mg in a mixture of 1 ml of *dilute nitric acid R* and 15 ml of *water R*. The solution, without further addition of *dilute nitric acid R*, complies with the limit test for chlorides (0.1 per cent).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

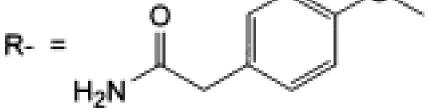
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

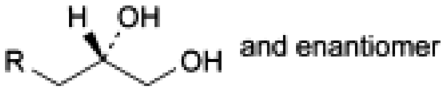
Dissolve 0.200 g in 80 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 26.63 mg of $C_{14}H_{22}N_2O_3$.

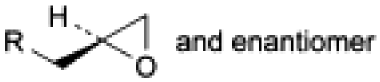
IMPURITIES



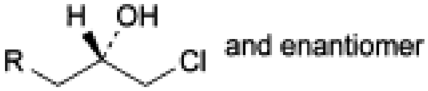
A. R-H: 2-(4-hydroxyphenyl)acetamide,


H
OH
R
OH
and enantiomer

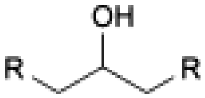
B. 2-[4-[(2*RS*)-2,3-dihydroxypropoxy]phenyl]acetamide,


H
R
O
and enantiomer

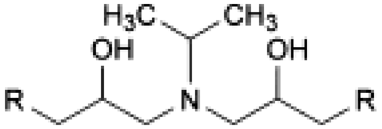
C. 2-[4-[[(2*RS*)-oxiran-2-yl]methoxy]phenyl]acetamide,


H
OH
R
Cl
and enantiomer

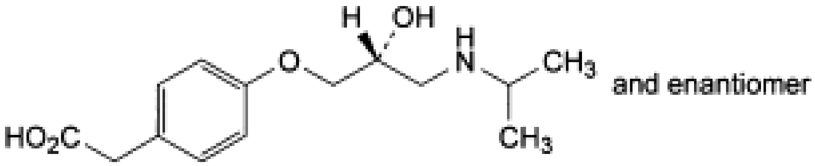
D. 2-[4-[(2*RS*)-3-chloro-2-hydroxypropoxy]phenyl]acetamide,



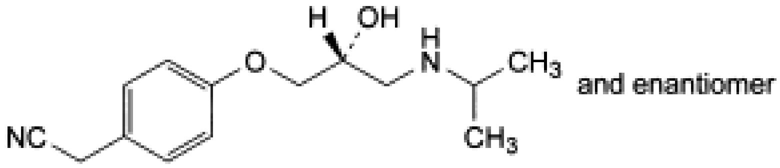
E. 2,2′-[2-hydroxypropan-1,3-diylbis(oxy-4,1-phenylene)]diacetamide,


H3C
CH3
OH
OH
R
N
R

F. 2,2′-[(1-methylethyl)iminobis(2-hydroxypropan-3,1-diyloxy-4,1-phenylene)]diacetamide,


H
OH
O
H
N
CH3
and enantiomer
HO2C
CH3

G. 2-[4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]acetic acid,

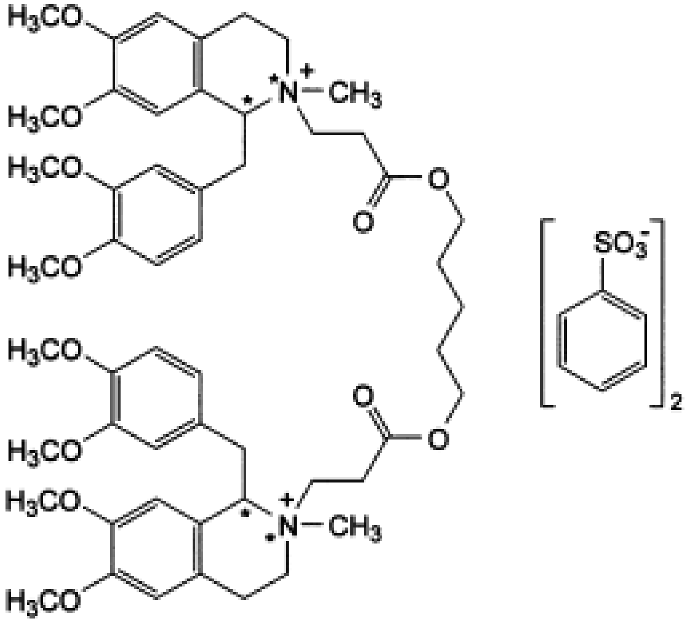

H
OH
O
H
N
CH3
and enantiomer
NC
CH3

H. 2-[4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]acetonitrile.

Atracurium Besilate

General Notices

(Ph Eur monograph 1970)



$C_{65}H_{82}N_2O_{18}S_2$ 1243 *64228-81-5*

Action and use

Non-depolarizing neuromuscular blocker.

Ph Eur

DEFINITION

Mixture of the *cis-cis*, *cis-trans* and *trans-trans* isomers of 2,2′-[pentane-1,5-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium] dibenzenesulphonate.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White to yellowish-white powder, slightly hygroscopic.

Solubility

Soluble in water, very soluble in acetonitrile, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison atracurium besilate CRS.

B. Examine the chromatograms obtained in the assay.

Results The 3 principal isomeric peaks in the chromatogram obtained with test solution (a) are similar in retention time to those in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 1.00 g in *water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Test solution (b) Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a) Dissolve 50.0 mg of *atracurium besilate CRS* in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 100.0 ml with mobile phase A.

Reference solution (c) Dissolve 20.0 mg of *methyl benzenesulphonate R* in *acetonitrile R* and dilute to 100.0 ml with the same solvent. Dilute 50 µl of the solution to 100.0 ml with mobile phase A.

Reference solution (d) Dissolve 2.0 mg of *atracurium for peak identification CRS* (containing impurities A1, A2, B, C1, C2, D1, D2, E, G and K) in 2.0 ml of mobile phase A.

Reference solution (e) Dissolve 2.0 mg of *atracurium for impurity F identification CRS* in 2.0 ml of mobile phase A.

Column:

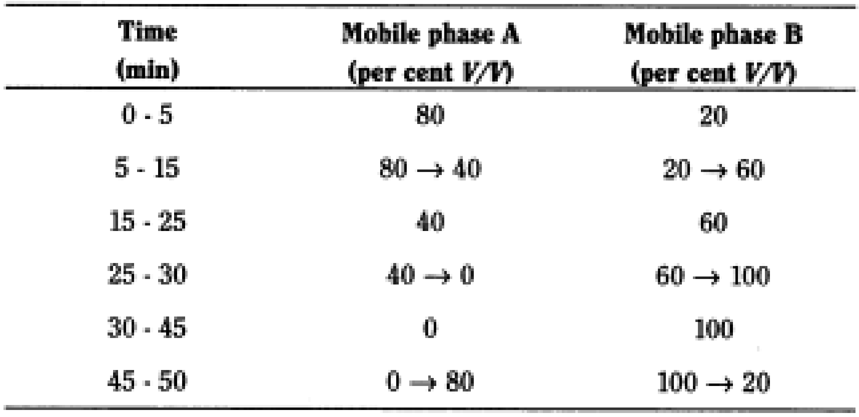
— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 5 volumes of *methanol R*, 20 volumes of *acetonitrile R* and 75 volumes of a 10.2 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.1 with *phosphoric acid R*,

— *mobile phase B*: mix 20 volumes of *acetonitrile R*, 30 volumes of *methanol R* and 50 volumes of a 10.2 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.1 with *phosphoric acid R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 15	80 → 40	20 → 60
15 - 25	40	60
25 - 30	40 → 0	60 → 100
30 - 45	0	100
45 - 50	0 → 80	100 → 20

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl of test solution (a) and reference solutions (a), (b), (d) and (e).

Relative retention With reference to the atracurium *cis-cis* isomer (retention time = about 30 min): impurity E = about 0.2; impurity F = about 0.25; impurity G = about 0.3; impurity D1 = about 0.45; impurity D2 = about 0.5; atracurium *trans-trans* isomer = about 0.8; atracurium *cis-trans* isomer = about 0.9; impurity A1 = about 1.04; impurity I1 = about 1.07; impurity H1 = about 1.07 (shoulder on the front of peak A2); impurity A2 (major isomer) = about 1.08; impurity K1 = about 1.09 (shoulder on the tail of peak A2); impurity I2 (major isomer) = about 1.12; impurity H2 (major isomer) = about 1.12; impurity K2 (major isomer) = about 1.12; impurity B = about 1.15; impurity C1 = about 1.2; impurity C2 (major isomer) = about 1.3.

Identification of impurities:

— use the chromatogram obtained with reference solution (d) and the chromatogram supplied *with atracurium for peak identification CRS* to identify the peaks due to impurities A1, A2, B, C1, C2, D1, D2, E, G and K;

— use the chromatogram obtained with reference solution (e) and the chromatogram supplied with *atracurium for impurity F identification CRS* to identify the peak due to impurity F.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to the atracurium *trans-trans* isomer and the atracurium *cis-trans* isomer, and minimum 1.5 between the peaks due to the atracurium *cis-trans* isomer and the atracurium *cis-cis* isomer.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity G by 0.5,

— *impurity E*: not more than 1.5 times the sum of the areas of the peaks due to the atracurium *cis-cis*, *trans-trans* and *cis-trans* isomers in the chromatogram obtained with reference solution (b) (1.5 per cent),

— *impurities A, D*: for each impurity, for the sum of the areas of the 2 isomer peaks, not more than 1.5 times the sum of the areas of the peaks due to the atracurium *cis-cis*, *trans-trans* and *cis-trans* isomers in the chromatogram obtained with reference solution (b) (1.5 per cent),

— *impurity C*: for the sum of the areas of the 2 isomer peaks, not more than the sum of the areas of the peaks due to the atracurium *cis-cis*, *trans-trans* and *cis-trans* isomers in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *impurities F, G*: for each impurity, not more than the sum of the areas of the peaks due to the atracurium *cis-cis*, *trans-trans* and *cis-trans* isomers in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *impurities H, I, K*: for the sum of the areas of the isomer peaks of these impurities, not more than the sum of the areas of the peaks due to the atracurium *cis-cis*, *trans-trans* and *cis-trans* isomers in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *any other impurity*: for each impurity, not more than 0.1 times the sum of the areas of the peaks due to the atracurium *cis-cis*, *trans-trans* and *cis-trans* isomers in the chromatogram obtained with reference solution (b) (0.1 per cent),

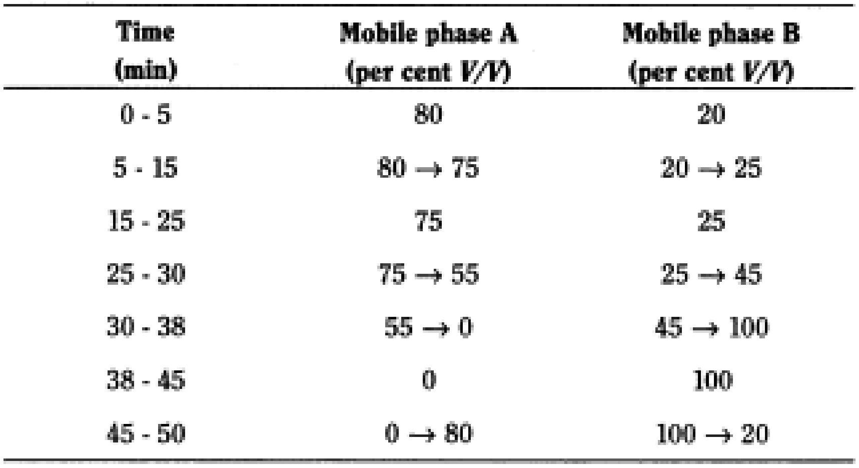
— *total*: not more than 3.5 times the sum of the areas of the peaks due to the atracurium *cis-cis*, *trans-trans* and *cis-trans* isomers in the chromatogram obtained with reference solution (b) (3.5 per cent),

— *disregard limit*: 0.05 times the sum of the areas of the peaks due to the atracurium *cis-cis*, *trans-trans* and *cis-trans* isomers in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity J

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase:



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 15	80 → 75	20 → 25
15 - 25	75	25
25 - 30	75 → 55	25 → 45
30 - 38	55 → 0	45 → 100
38 - 45	0	100
45 - 50	0 → 80	100 → 20

Detection Spectrophotometer at 217 nm.

Injection 100 µl of test solution (b) and reference solution (c).

Retention time Impurity J = about 25 min; atracurium *trans-trans* isomer = about 38 min.

Limit:

— *impurity J*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (10 ppm).

Isomer composition

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications. Use the normalisation procedure.

Injection Test solution (a).

Limits:

— *atracurium cis-cis isomer*: 55.0 per cent to 60.0 per cent,

— *atracurium cis-trans isomer*: 34.5 per cent to 38.5 per cent,

— *atracurium trans-trans isomer*: 5.0 per cent to 6.5 per cent.

Water ***(2.5.12)***

Maximum 5.0 per cent, determined on 1.000 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (a) and reference solution (a).

Calculate the percentage content of $C_{65}H_{82}N_2O_{18}S_2$ from the sum of the areas of the peaks due to the 3 isomers in test solution (a) and reference solution (a).

STORAGE

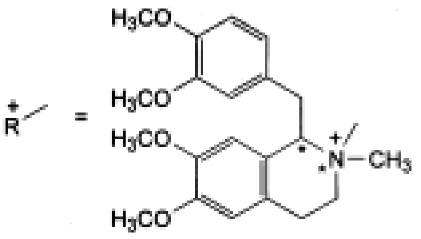
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

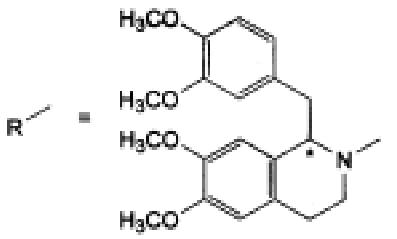
IMPURITIES

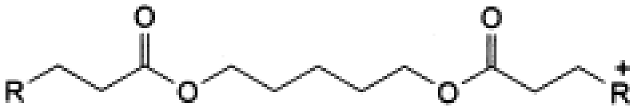
Specified impurities A, C, D, E, F, G, H, I, J, K.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*)

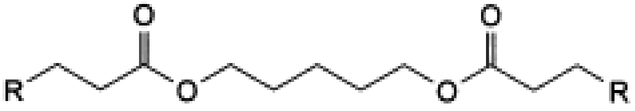
B.



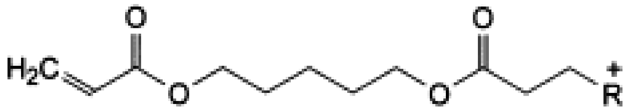




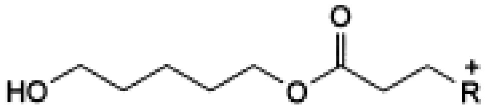
A. 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium (A1 = *cis-trans* isomer, A2 = *cis-cis* isomer),



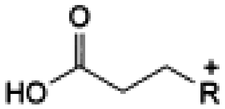
B. pentane-1,5-diyl bis[3-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2 (1*H*)-yl]propanoate],



C. 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium (C1 = *trans* isomer, C2 = *cis* isomer),



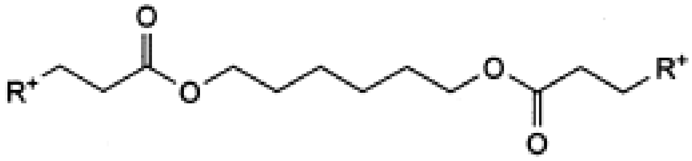
D. 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl)oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium (D1 = *trans* isomer, D2 = *cis* isomer),



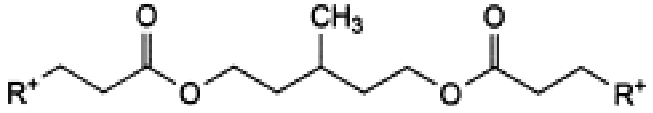
E. 3-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinio] propanoate,

F. R+-CH_3: 1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium,

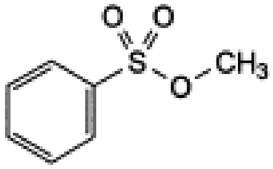
G. R-CH_3: 1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline,



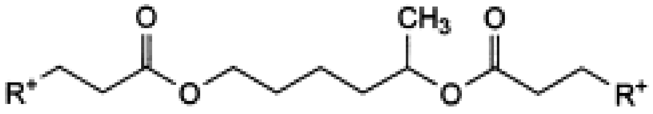
H. 2,2′-[hexane-1,6-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium] (H1 = *cis-trans* isomer, H2 = *cis-cis* isomer),



I. 2,2′-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium] (I1 = *cis-trans* isomer, I2 = *cis-cis* isomer),



J. methyl benzenesulphonate,



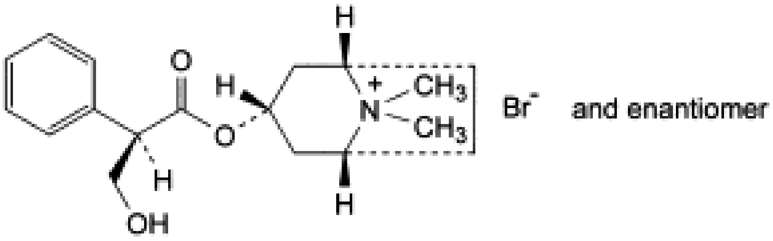
K. 2,2′-[(hexane-1,5)-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium]).

Ph Eur

Atropine Methobromide

General Notices

(Methylatropine Bromide, Ph Eur monograph 0511)



$C_{18}H_{26}BrNO_3$ 384.3 *2870-71-5*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

Methylatropine bromide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (1*R*,3*r*,5*S*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, sparingly soluble in alcohol.

It melts at about 219 °C, with decomposition.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. It complies with the test for optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *methylatropine bromide CRS*.

C. To 5 ml of solution S (see Tests) add 2 ml of *dilute sodium hydroxide solution R*. No precipitate is formed.

D. To about 1 mg add 0.2 ml of *fuming nitric acid R* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.1 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

E. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_9 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*; the solution is colourless. Add 0.5 ml of *0.01 M sodium hydroxide*; the solution is red.

Optical rotation *(2.2.7)*

Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent. The angle of optical rotation, measured in a 2 dm tube, is - 0.25° to + 0.05°.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel G plate R*.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with a mixture of 1 volume of *water R* and 9 volumes of *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R*, 15 volumes of *anhydrous formic acid R*, 15 volumes of *water R* and 60 volumes of *ethyl acetate R*. Dry the plate at 100 °C to 105 °C until the solvent has evaporated, allow to cool and spray with *dilute potassium iodobismuthate solution R* until the spots appear. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Apomethylatropine

Dissolve 0.10 g in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Measure the absorbances *(2.2.25)* at the maxima at 252 nm and 257 nm. The ratio of the absorbance at 257 nm to that at 252 nm is at least 1.19.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*, warming slightly if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 38.43 mg of $C_{18}H_{26}BrNO_3$.

STORAGE

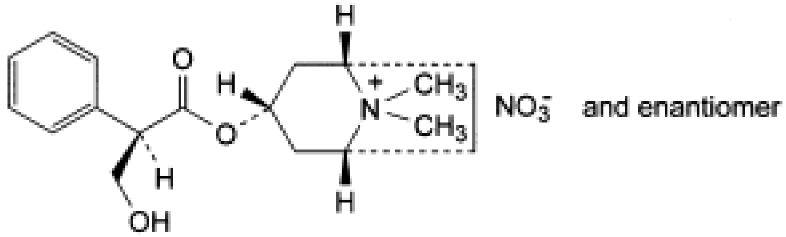
Store protected from light.

Ph Eur

Atropine Methonitrate

General Notices

(Methylatropine Nitrate, Ph Eur monograph 0512)



$C_{18}H_{26}N_2O_6$ 366.4 *52-88-0*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

Methylatropine nitrate contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (1*R*,3*r*,5*S*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane nitrate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, soluble in alcohol.

It melts at about 167 °C.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. It complies with the test for optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *methylatropine nitrate CRS*.

C. To a mixture of 2.5 ml of solution S (see Tests) and 2.5 ml of *water R* add 2 ml of *dilute sodium hydroxide solution R*. No precipitate is formed.

D. To about 1 mg add 0.2 ml of *fuming nitric acid R* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.25 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

E. To 0.05 ml of *diphenylamine solution R* add 0.05 ml of a 1 in 10 dilution of solution S. An intense blue colour is produced.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_9 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*; the solution is colourless. Add 0.5 ml of *0.01 M sodium hydroxide*; the solution is red.

Optical rotation *(2.2.7)*

Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent. The angle of optical rotation, measured in a 2 dm tube, is - 0.25° to + 0.05°.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with a mixture of 1 volume of *water R* and 9 volumes of *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R*, 15 volumes of *anhydrous formic acid R*, 15 volumes of *water R* and 60 volumes of *ethyl acetate R*. Dry the plate at 100 °C to 105 °C until the solvent has evaporated, allow to cool and spray with *dilute potassium iodobismuthate solution R* until the spots appear. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Apomethylatropine

Dissolve 0.10 g in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Measure the absorbances *(2.2.25)* at the maxima at 252 nm and 257 nm. The ratio of the absorbance at 257 nm to that at 252 nm is not less than 1.17.

Halides

15 ml of solution S complies with the limit test for chlorides *(2.4.4)*. Prepare the standard using 1.5 ml of *chloride standard solution (5 ppm Cl) R* (10 ppm).

Silver

Dissolve 1.0 g in 10 ml of *water R* and add 0.1 ml of *sodium sulphide solution R*. Allow to stand for 2 min. The solution is not more intensely coloured than reference solution B_8 *(2.2.2, Method II)* (10 ppm).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 36.64 mg of $C_{18}H_{26}N_2O_6$.

STORAGE

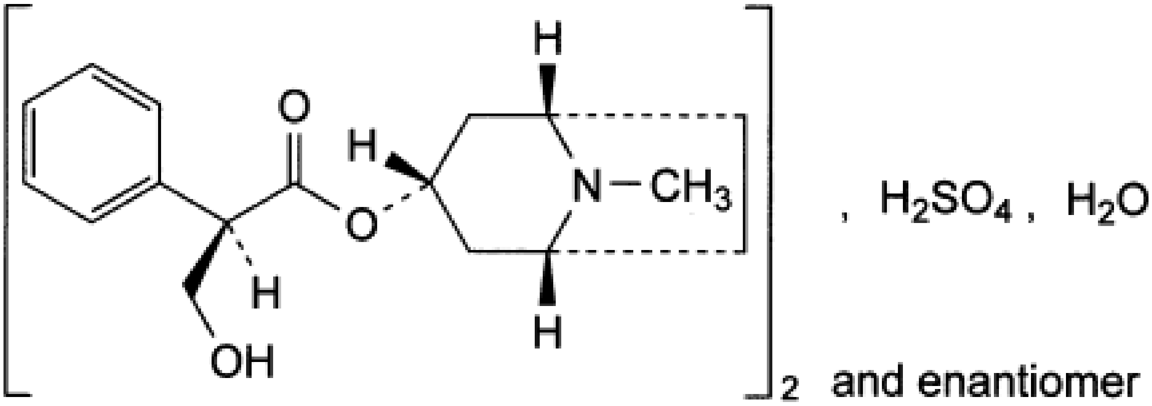
Store protected from light.

Ph Eur

Atropine Sulphate

General Notices

(Ph Eur monograph 0068)



$C_{34}H_{46}N_2O_6,H_2SO_4,H_2O$ 695   *5908-99-6*

Action and use

Anticholinergic.

Preparations

Atropine Eye Drops

Atropine Eye Ointment

Atropine Injection

Atropine Tablets

Morphine and Atropine Injection

Ph Eur

DEFINITION

Bis[(1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate] sulphate monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B, E.*

Second identification *C, D, E, F.*

A. Optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *atropine sulphate CRS.*

C. Dissolve about 50 mg in 5 ml of *water R* and add 5 ml of *picric acid solution R*. The precipitate, washed with *water R* and dried at 100-105 °C for 2 h, melts (*2.2.14*) at 174 °C to 179 °C.

D. To about 1 mg add 0.2 ml of *fuming nitric acid R* and evaporate to dryness in a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.1 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

E. It gives the reactions of sulphates (*2.3.1*).

F. It gives the reaction of alkaloids (*2.3.1*).

TESTS

pH (*2.2.3*)

4.5 to 6.2.

Dissolve 0.6 g in *carbon dioxide-free water R* and dilute to 30 ml with the same solvent.

Optical rotation (*2.2.7*)

- 0.50° to + 0.05° (measured in a 2 dm tube).

Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 24 mg of the substance to be examined in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 5 mg of *atropine impurity B CRS* in the test solution and dilute to 20 ml with the test solution. Dilute 5 ml of this solution to 25 ml with mobile phase A.

Reference solution (c) Dissolve 5 mg of *atropine for peak identification CRS* (containing impurities A, B, D, E, F, G and H) in mobile phase A and dilute to 20 ml with mobile phase A.

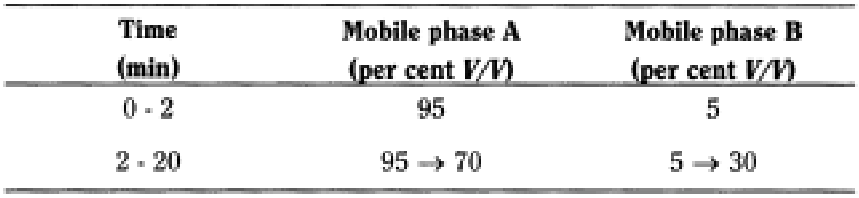
Reference solution (d) Dissolve 5 mg of *tropic acid R* (impurity C) in mobile phase A and dilute to 10 ml with mobile phase A. Dilute 1 ml of the solution to 100 ml with mobile phase A. Dilute 1 ml of this solution to 10 ml with mobile phase A.

Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

- *mobile phase A*: dissolve 3.5 g of *sodium dodecyl sulphate R* in 606 ml of a 7.0 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.3 with *0.05 M phosphoric acid*, and mix with 320 ml of *acetonitrile R1*;
- *mobile phase B*: *acetonitrile R1;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	95	5
2 - 20	95 → 70	5 → 30

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *atropine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, D, E, F, G and H. Use the chromatogram obtained with reference solution (d) to identify the peak due to impurity C.

Relative retention With reference to atropine (retention time = about 11 min): impurity C = about 0.2; impurity E = about 0.67; impurity D = about 0.73; impurity F = about 0.8; impurity B = about 0.89; impurity H = about 0.93; impurity G = about 1.1; impurity A = about 1.7.

System suitability Reference solution (b):

- *resolution*: minimum 2.5 between the peaks due to impurity B and atropine.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity C = 0.6;
- *impurities E, H*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurities A, B, C, D, F, G*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

2.0 per cent to 4.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 30 ml of *anhydrous acetic acid R*, warming if necessary. Cool the solution. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

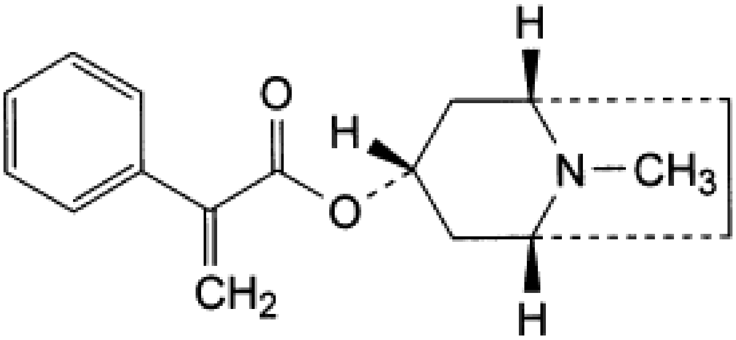
1 ml of *0.1 M perchloric acid* is equivalent to 67.68 mg of $C_{34}H_{48}N_2O_{10}S$.

STORAGE

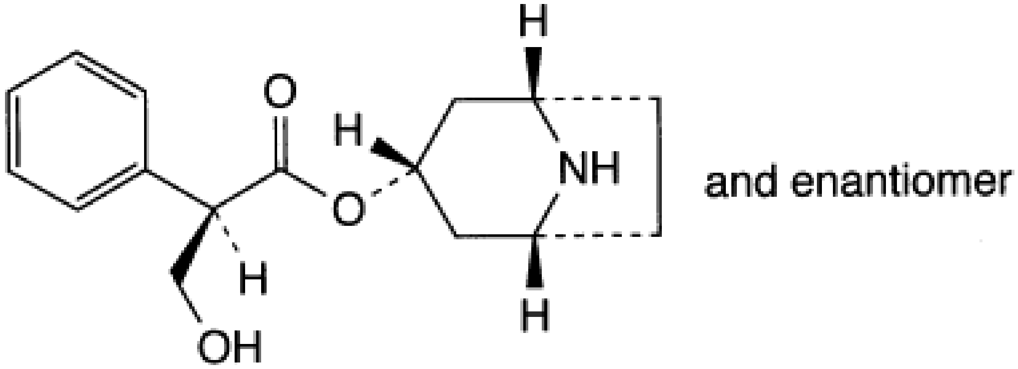
Protected from light.

IMPURITIES

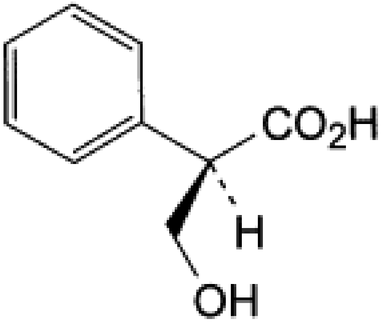
Specified impurities A, B, C, D, E, F, G, H.



A. (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-phenylpropenoate (apoatropine),

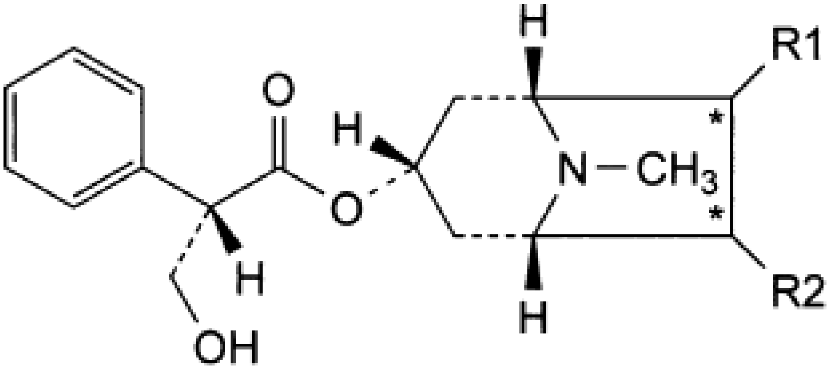


B. (1*R*,3*r*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate (noratropine),



and enantiomer

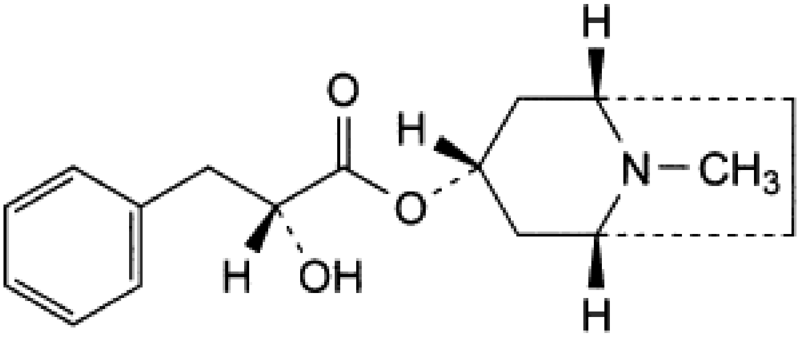
C. (2*RS*)-3-hydroxy-2-phenylpropanoic acid (tropic acid),



D. R1 = OH, R2 = H: (1*R*,3*S*,5*R*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2S)-3-hydroxy-2-phenylpropanoate (6-hydroxyhyoscyamine),

E. R1 = H, R2 = OH: (1*S*,3*R*,5*S*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (7-hydroxyhyoscyamine),

F. hyoscine,



and enantiomer

G. (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-3-phenylpropanoate (littorine).

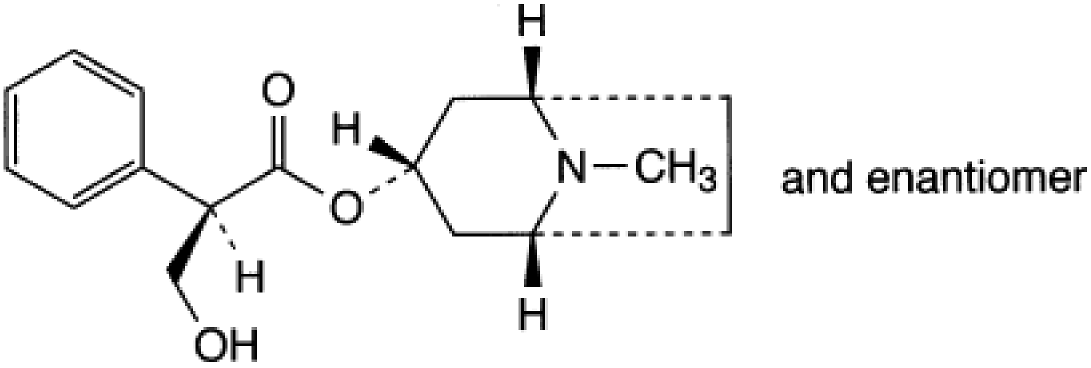
H. unknown structure.

Ph Eur

Atropine

General Notices

(Ph Eur monograph 2056)



$C_{17}H_{23}NO_3$ 289.4 *51-55-8*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification A, B, E.

Second identification *A, C, D, E.*

A. Melting point (*2.2.14*): 115 °C to 119 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *atropine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *atropine CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R*, *water R*, *acetone R* (3:7:90 *V/V/V*).

Application 10 µl.

Development Over half of the plate.

Drying At 100-105 °C for 15 min.

Detection After cooling, spray with *dilute potassium iodobismuthate solution R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Place about 3 mg in a porcelain crucible and add 0.2 ml of *fuming nitric acid R*. Evaporate to dryness on a water-bath. Dissolve the residue in 0.5 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*; a violet colour develops.

E. Optical rotation (see Tests).

TESTS

Optical rotation (*2.2.7*)

- 0.70° to + 0.05° (measured in a 2 dm tube).

Dissolve 1.25 g in *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 24 mg of the substance to be examined in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 5 mg of *atropine impurity B CRS* in the test solution and dilute to 20 ml with the test solution. Dilute 5 ml of this solution to 25 ml with mobile phase A.

Reference solution (c) Dissolve 5 mg of *atropine for peak identification CRS* (containing impurities A, B, D, E, F, G and H) in mobile phase A and dilute to 20 ml with mobile phase A.

Reference solution (d) Dissolve 5 mg of *tropic acid R* (impurity C) in mobile phase A and dilute to 10 ml with mobile phase A. Dilute 1 ml of the solution to 100 ml with mobile phase A. Dilute 1 ml of this solution to 10 ml with mobile phase A.

Column:

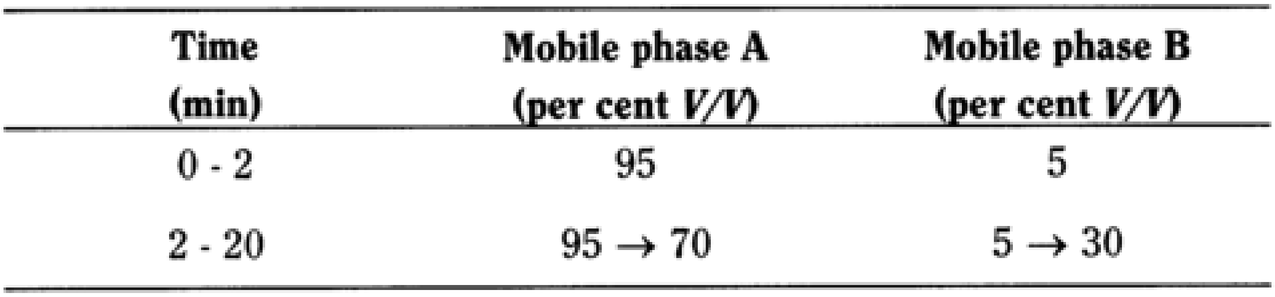
— *size*: *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: dissolve 3.5 g of *sodium dodecyl sulphate R* in 606 ml of a 7.0 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.3 with *0.05 M phosphoric acid*, and mix with 320 ml of *acetonitrile R1*;

— *mobile phase B*: *acetonitrile R1;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	95	5
2 - 20	95 → 70	5 → 30

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *atropine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, D, E, F, G and H. Use the chromatogram obtained with reference solution (d) to identify the peak due to impurity C.

Relative retention With reference to atropine (retention time = about 11 min): impurity C = about 0.2; impurity E = about 0.67; impurity D = about 0.73; impurity F = about 0.8; impurity B = about 0.89; impurity H = about 0.93; impurity G = about 1.1; impurity A = about 1.7.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to impurity B and atropine.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity C = 0.6;

— *impurities E, H*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities A, B, C, D, F, G*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.2 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

ASSAY

Dissolve 0.250 g in 40 ml of *anhydrous acetic acid R*, warming if necessary. Allow the solution to cool. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

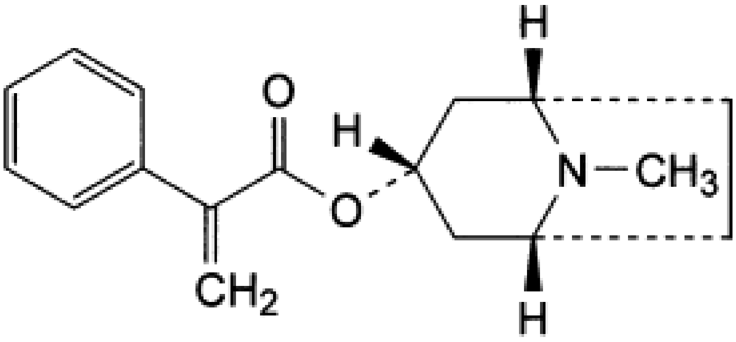
1 ml of *0.1 M perchloric acid* is equivalent to 28.94 mg of $C_{17}H_{23}NO_3$.

STORAGE

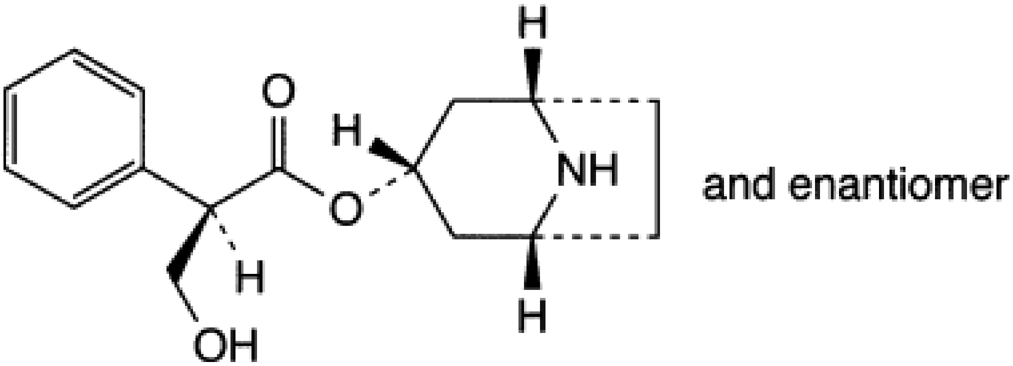
Protected from light.

IMPURITIES

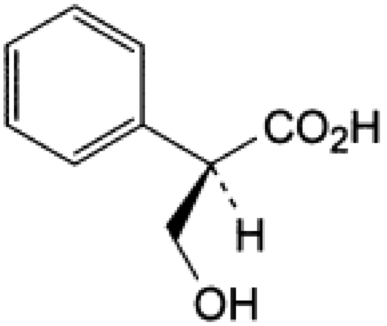
Specified impurities A, B, C, D, E, F, G, H.



A. (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-phenylpropenoate (apoatropine),

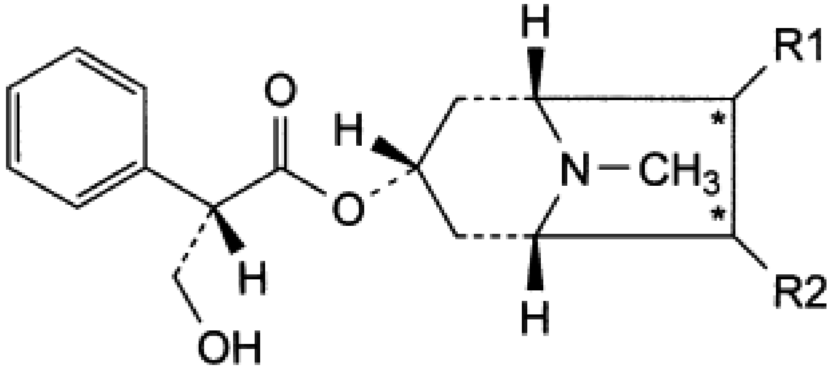


B. (1*R*,3*r*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate (noratropine),



CO2H
H
OH
and enantiomer

C. (2*RS*)-3-hydroxy-2-phenylpropanoic acid (tropic acid),

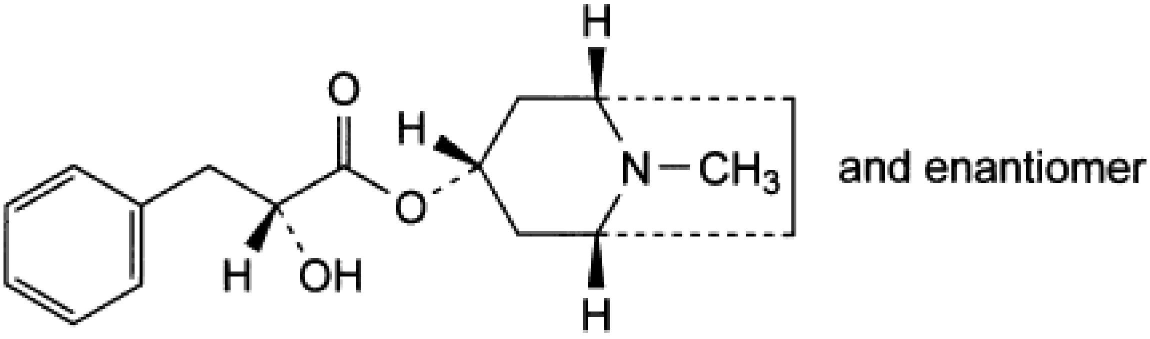


O
H
O
H
OH
H
N−CH3
H
R1
R2

D. R1 = OH, R2 = H: (1*R*,3*S*,5*R*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (6-hydroxyhyoscyamine),

E. R1 = H, R2 = OH: (1*S*,3*R*,5*S*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (7-hydroxyhyoscyamine),

F. hyoscine,



O
H
O
H
OH
H
N−CH3
H
and enantiomer

G. (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-3-phenylpropanoate (littorine),

H. unknown structure.

Ph Eur

Attapulgite

General Notices

Action and use

Excipient.

DEFINITION

Attapulgite is a purified native hydrated magnesium aluminium silicate essentially consisting of the clay mineral palygorskite.

CHARACTERISTICS

A light, cream or buff, very fine powder, free or almost free from gritty particles.

IDENTIFICATION

A. Ignite 0.5 g with 2 g of *anhydrous sodium carbonate* for 20 minutes, cool and extract with 25 ml of boiling *water*. Cool, filter, wash the residue with *water* and add the washings to the filtrate. Reserve the residue for test B. Cautiously acidify the combined filtrate and washings with *hydrochloric acid*, evaporate to dryness, moisten the residue with 0.2 ml of *hydrochloric acid*, add 10 ml of *water* and stir. A white, gelatinous precipitate is produced.

B. Wash the residue reserved in test A with *water* and dissolve in 10 ml of *2M hydrochloric acid*. To 2 ml of the solution add a 10% w/v solution of *ammonium thiocyanate*. An intense red colour is produced.

C. To 2 ml of the solution obtained in test B add 1 ml of *strong sodium hydroxide solution* and filter. To the filtrate add 3 ml of *ammonium chloride solution*. A gelatinous white precipitate is produced.

D. To 2 ml of the solution obtained in test B add *ammonium chloride* and an excess of *13.5M ammonia* and filter. To the filtrate add 0.15 ml of *magneson reagent* and an excess of *5M sodium hydroxide*. A blue precipitate is produced.

TESTS

Acidity or alkalinity

pH of a 5% w/v suspension in *carbon dioxide-free water*, after shaking for 5 minutes, 7.0 to 9.5, Appendix V L.

Adsorptive capacity

Moisture adsorption, 5 to 14% when determined by the following method. Dry in air and powder a sufficient quantity of the substance being examined and pass through a sieve with a nominal mesh aperture of 150 µm. Spread 0.5 g as a thin layer on a previously weighed piece of aluminium foil (60 mm × 50 mm) of nominal gauge 17.5 µm and transfer to a desiccator containing a dish of sodium chloride crystals partially immersed in saturated brine at 25°. After 4 hours, remove from the desiccator and weigh immediately. Dry in an oven at 110° for 4 hours, allow to cool in a desiccator and weigh. The *moisture adsorption* is the gain in weight of the substance being examined expressed as a percentage of its oven-dried weight.

Arsenic

To 0.13 g add 5 ml of *water*, 2 ml of *sulphuric acid* and 10 ml of *sulphur dioxide solution* and evaporate on a water bath until the sulphur dioxide solution is removed and the volume reduced to about 2 ml. Transfer the solution to the generator flask with the aid of 5 ml of *water*. The resulting solution complies with the *limit test for arsenic*, Appendix VII (8 ppm).

Heavy metals

A. Not more than 20 ppm when determined by the following method. Shake 6.0 g with 40 ml of *0.5M hydrochloric acid* at 37° for 30 minutes, cool and filter. Wash the residue with *water* and dilute the combined filtrate and washings to 50 ml with *water*. To 20 ml add 2 g each of *ammonium chloride* and *ammonium thiocyanate* and dissolve. Shake the solution with 80 ml of a mixture of equal volumes of *ether* and *isoamyl alcohol* and separate, retaining the aqueous layer. Extract with a further 80 ml of the mixture. To the aqueous layer add 2 g of *citric acid*, neutralise with *13.5M ammonia* and dilute to 25 ml with *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard.

B. Not more than 10 ppm when determined by the following method. Shake 6.0 g with 40 ml of *0.5M sodium hydroxide* at 37° for 30 minutes, cool and filter. Wash the residue with *water* and dilute the combined filtrate and washings to 50 ml with *water*. Neutralise 20 ml of the solution with *hydrochloric acid* and dilute to 25 ml with *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Acid-soluble matter

Boil 2 g with 100 ml of *0.2M hydrochloric acid* under a reflux condenser for 5 minutes, cool and filter. Evaporate 50 ml of the filtrate to dryness. The residue, after ignition at about 600° for 30 minutes, weighs not more than 0.25 g.

Water-soluble matter

Boil 10 g with 100 ml of *water* under a reflux condenser for 5 minutes, cool and filter. Evaporate 50 ml of the filtrate to dryness. The residue, after ignition at 600° for 30 minutes, weighs not more than 50 mg.

Loss on drying

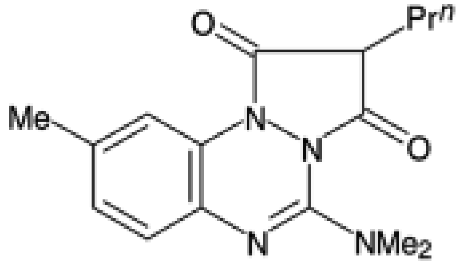
When dried to constant weight at 105°, loses not more than 17.0% of its weight. Use 1 g.

Loss on ignition

When ignited at 600°, loses 15.0 to 27.0% of its weight. Use 1 g.

Azapropazone

General Notices



$C_{16}H_{20}N_4O_2,2H_2O$ 336.4 *13539-59-8 (anhydrous)*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Azapropazone Capsules

Azapropazone Tablets

DEFINITION

Azapropazone is 5-dimethylamino-9-methyl-2-propylpyrazolo[1,2-*a*][1,2,4]benzotriazine-1,3 (2*H*)-dione dihydrate. It contains not less than 99.0% and not more than 101.0% of $C_{16}H_{20}N_4O_2$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white to pale yellow, crystalline powder.

Very slightly soluble in *water*; soluble in *ethanol (96%)*; it dissolves in solutions of alkali hydroxides.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of azapropazone *(RS 016)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.0008% w/v solution in 0.1M *sodium hydroxide* exhibits two maxima, at 255 nm and 325 nm. The *absorbances* at 255 nm and 325 nm are about 0.86 and 0.18 respectively.

TESTS

Acetic acid

Not more than 0.2%, determined by the following method. Dissolve 10 g in 25 ml of *methanol*, add 75 ml of *water* and carry out a *potentiometric titration*, Appendix VIII B, using *0.1M sodium hydroxide VS* as titrant to a pH of 5.9. Each ml of *0.1M sodium hydroxide VS* is equivalent to 6.005 mg of acetic acid, $C_2H_4O_2$.

Related substances

Carry out the following operations in subdued light using low-actinic glassware without delay. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 1 volume of *phosphate buffer pH 4.0* and 3 volumes of *methanol*. Solution (1) contains 0.10% w/v of the substance being examined. Solution (2) contains 0.00010% w/v of *azapropazone impurity A BPCRS.* Solution (3) contains 0.00025% w/v of *azapropazone impurity B BPCRS*. Solution (4) contains 0.00025% w/v of *azapropazone impurity C BPCRS.* Solution (5) contains 0.0001% w/v of the substance being examined. Solution (6) contains 0.00005% w/v of the substance being examined. Solution (7) contains 0.1% w/v of *azapropazone impurity standard BPCRS.*

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 1 volume of *glacial acetic acid*, 36 volumes of *methanol* and 63 volumes of a 0.068% w/v solution of *sodium butanesulphonate* in *water* as the mobile phase with a flow rate of 2.5 ml per minute and (c) a detection wavelength of 254 nm.

Inject solution (7) and continue the chromatography for 5 times the retention time of the principal peak. The test is not valid unless the chromatogram obtained with solution (7) closely resembles the reference chromatogram supplied with the azapropazone impurity standard. If necessary adjust the proportion of methanol in the mobile phase to give the required retention times.

In the chromatogram obtained with solution (1) the areas of any peaks corresponding to azapropazone impurities A, B or C are not greater than the areas of the corresponding peaks in the chromatograms obtained with solutions (2), (3) and (4) respectively (0.1%, 0.25% and 0.25%). The area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (5) (0.1%). Calculate the content of impurities A, B and C using the respective reference solutions and the content of any unnamed impurities using solution (5). The total nominal content of impurities is not greater than 0.5%. Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (6) (0.05%).

Heavy metals

Dissolve 3.125 g in 20 ml of *water*, boil for 10 minutes, dilute to 50 ml and filter (solution A). 12 ml of solution A complies with *limit test A for heavy metals*, Appendix VII. Use 10 ml of *lead standard solution (1 ppm Pb)* to prepare the standard (16 ppm).

Chloride

A mixture of 10 ml of solution A and 5 ml of *water* complies with the limit *test for chlorides*, Appendix VII (80 ppm).

Sulphate

A mixture of 10 ml of solution A and 5 ml of *water* complies with the *limit test for sulphates*, Appendix VII (240 ppm).

Water

10.0 to 11.5% w/w, Appendix IX C. Use 0.25 g.

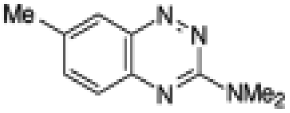
Sulphated ash

Not more than 0.1%, Appendix IX A.

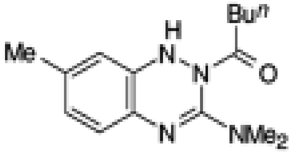
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 g and determining the end point potentiometrically. Each ml of *0.1M perchloric acid VS* is equivalent to 30.04 mg of $C_{16}H_{20}N_4O_2$.

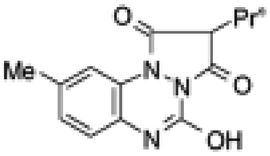
IMPURITIES



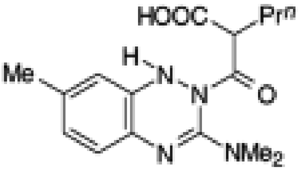
1. 3-dimethylamino-7-methyl-1,2,4-benzotriazine *(impurity A)*,



2. 3-dimethylamino-1,2-dihydro-7-methyl-2-valeryl-1,2,4-benzotriazine,



3. 5-hydroxy-9-methyl-2-propylpyrazolo[1,2-*a*][1,2,4]benzotriazine-1,3(2*H*)-dione *(impurity B)*,

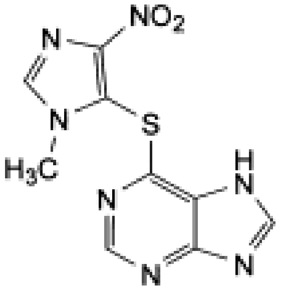


4. α-(3-dimethylamino-7-methyl-1,2-dihydro-1,2,4-benzotriazin-2-ylcarbonyl)valeric acid *(impurity C)*.

Azathioprine

General Notices

(Ph Eur monograph 0369)



$C_9H_7N_7O_2S$ 277.3 *446-86-6*

Action and use

Immunosuppressant.

Preparation

Azathioprine Tablets

Ph Eur

DEFINITION

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)sulfanyl]-7*H*-purine.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Pale yellow powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It is soluble in dilute solutions of alkali hydroxides and sparingly soluble in dilute mineral acids.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 0.150 g in 30 ml of *dimethyl sulphoxide R* and dilute to 500.0 ml with *0.1 M hydrochloric acid*. Dilute 25.0 ml of the solution to 1000.0 ml with *0.1 M hydrochloric acid*.

Spectral range 230-350 nm.

Absorption maximum At 280 nm.

Specific absorbance at the absorption maximum 600 to 660.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *azathioprine CRS*.

C. To about 20 mg add 100 ml of *water R*, heat and filter. To 5 ml of the filtrate add 1 ml of *hydrochloric acid R* and about 10 mg of *zinc powder R*. Allow to stand for 5 min. The solution becomes yellow. Filter, cool in iced water, add 0.1 ml of *sodium nitrite solution R* and 0.1 g of *sulphamic acid R* and shake until the bubbles disappear. Add 1 ml of *β-naphthol solution R*. A pale pink precipitate is formed.

TESTS

Acidity or alkalinity

To 0.5 g add 25 ml of *carbon dioxide-free water R*, shake for 15 min and filter. To 20 ml of the filtrate add 0.1 ml of *methyl red solution R.* Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chloromethylnitroimidazole and mercaptopurine

Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 0.2 g of the substance to be examined in *dilute ammonia R1* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *chloromethylnitroimidazole CRS* in *dilute ammonia R1* and dilute to 50 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *mercaptopurine R* in *dilute ammonia R1* and dilute to 50 ml with the same solvent.

Plate *cellulose for chromatography F_{254} R* as the coating substance.

Mobile phase *butanol R* saturated with *dilute ammonia R1*.

Application 5 µl.

Development Over a path of 15 cm.

Drying At 50 °C.

Detection Examine in ultraviolet light at 254 nm.

Limits:

— *chloromethylnitroimidazole*: any spot due to chloromethylnitroimidazole is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *mercaptopurine*: any spot due to mercaptopurine is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.50 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 25 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 27.73 mg of $C_9H_7N_7O_2S$.

STORAGE

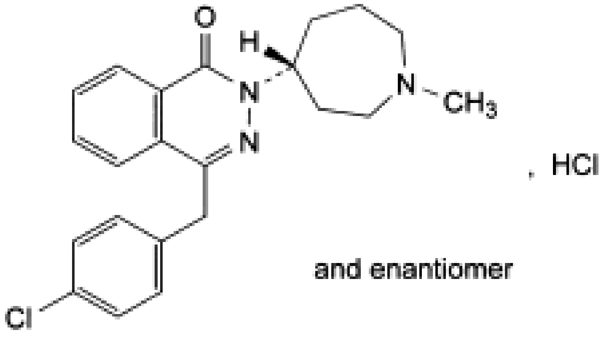
Protected from light.

Ph Eur

Azelastine Hydrochloride

General Notices

(Ph Eur monograph 1633)



$C_{22}H_{24}ClN_3O,HCl$ 418.4 *79307-93-0*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

4-(4-Chlorobenzyl)-2-[(4*RS*)-1-methylhexahydro-1*H*-azepin-4-yl]phthalazin-1(2*H*)-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, soluble in ethanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison azelastine hydrochloride CRS .

B. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.2 ml of *bromothymol blue solution R1*. Not more than 0.1 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the solution.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile for chromatography R*, *water R* (45:55 *V/V*).

Test solution Dissolve 0.125 g of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 1 mg of *azelastine impurity B CRS* , 1 mg of *azelastine impurity D CRS* and 1 mg of *azelastine impurity E CRS* in the test solution and dilute to 20 ml with the test solution.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *nitrile silica gel for chromatography R* (10 µm),

— *temperature*: 30 °C.

Mobile phase Dissolve 2.16 g of *sodium octanesulphonate R* and 0.68 g of *potassium dihydrogen phosphate R* in 740 ml of *water for chromatography R*, adjust to pH 3.0-3.1 with *dilute phosphoric acid R*, add 260 ml of *acetonitrile for chromatography R* and mix.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Run time Twice the retention time of azelastine.

Relative retention With reference to azelastine (retention time = about 8-9 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.4; impurity D = about 0.6; impurity E = about 1.4.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to impurity B and impurity D,

— the peaks due to impurity D and impurity E are baseline separated from the principal

---

peak.

Limits:

- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 3.6; impurity D = 0.7; impurity E = 2.1;
- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

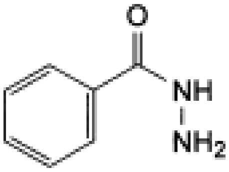
In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.300 g in 5 ml of *anhydrous formic acid R*. Add 30 ml of *acetic anhydride R*. Titrate quickly with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

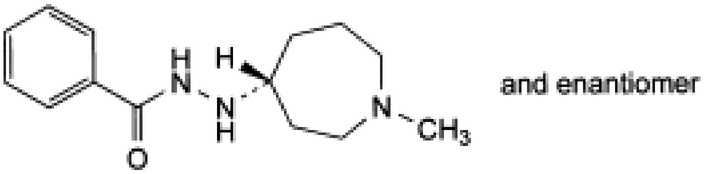
1.0 ml of *0.1 M perchloric acid* is equivalent to 41.84 mg of $C_{22}H_{25}Cl_2N_3O$.

IMPURITIES

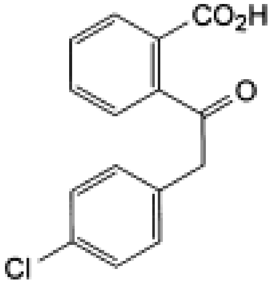
Specified impurities *A, B, C, D, E.*



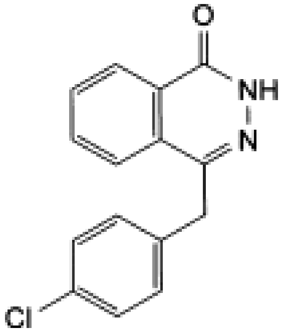
A. benzoyldiazane (benzohydrazide),



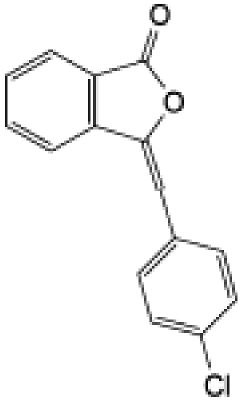
B. 1-benzoyl-2-[(4*RS*)-1-methylhexahydro-1*H*-azepin-4-yl]diazane,



C. 2-[(4-chlorophenyl)acetyl]benzoic acid,



D. 4-(4-chlorobenzyl)phthalazin-1(2*H*)-one,



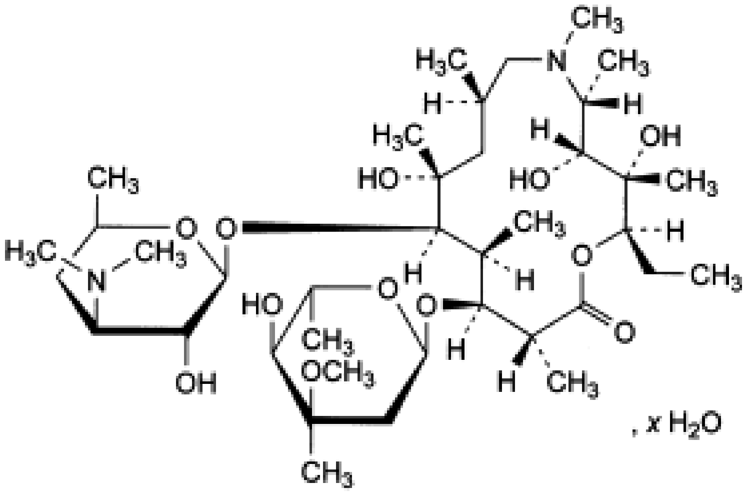
E. 3-(4-chlorobenzylidene)isobenzofuran-1(3*H*)-one.

Ph Eur

Azithromycin

General Notices

(Ph Eur monograph 1649)



$C_{38}H_{72}N_2O_{12},xH_20$ 749 *83905-01-5*

With *x* = 1 or 2 (anhydrous)

Action and use

Macrolide antibacterial.

Ph Eur

DEFINITION

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. The degree of hydration is 1 or 2.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *azithromycin CRS* .

If the spectra obtained in the solid state show differences, prepare further spectra using 90 g/l solutions in *methylene chloride R*.

TESTS

Solution S

Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

9.0 to 11.0.

Dissolve 0.100 g in 25.0 ml of *methanol R* and dilute to 50.0 ml with *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7)*

- 45 to - 49 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture Prepare a 1.73 g/l solution of *ammonium dihydrogen phosphate R* adjusted to pH 10.0 with *ammonia R*. Transfer 350 ml of this solution to a suitable container. Add 300 ml of *acetonitrile R1* and 350 ml of *methanol R1*. Mix well.

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *azithromycin for system suitability CRS* (containing impurities F, H and J) in 1.0 ml of the solvent mixture and sonicate for 5 min.

Reference solution (c) Dissolve 8.0 mg of *azithromycin for peak identification CRS* (containing impurities A, B, C, E, F, G, I, J, L, M, N, O, P) in 1.0 ml of the solvent mixture.

Reference solution (d) Dissolve the contents of a vial of *azithromycin impurity B CRS* in 1.0 ml of the solvent mixture.

Column:
 — *size:* *l* = 0.25 m, Ø = 4.6 mm;

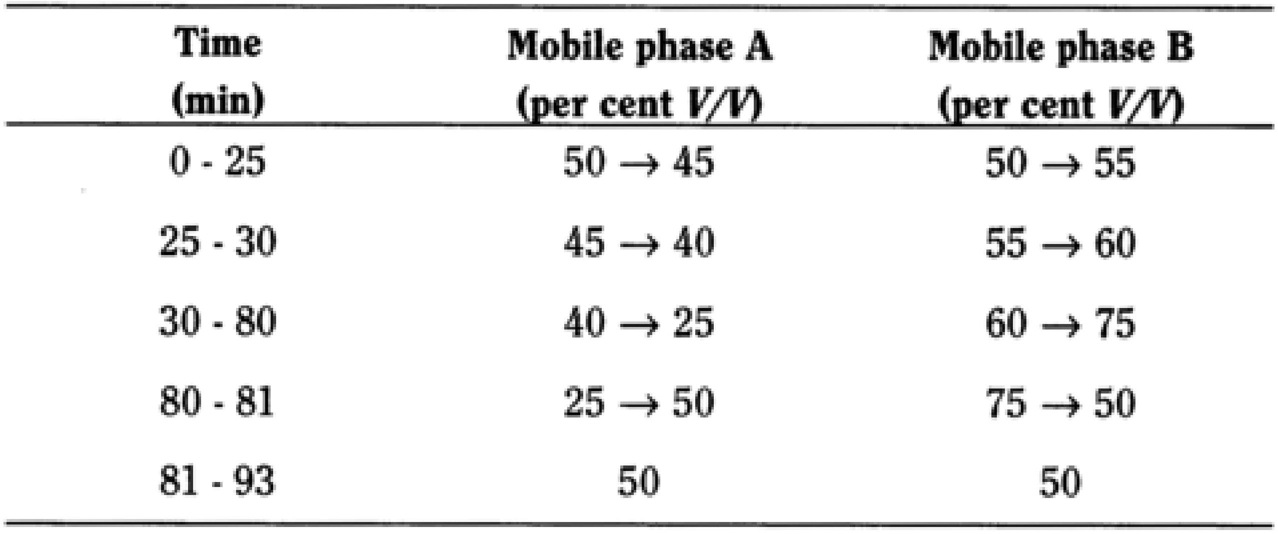
— *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for mass spectrometry R* (5 µm);

— *temperature*: 60 °C.

Mobile phase:

— *mobile phase A*: 1.80 g/l solution of *anhydrous disodium hydrogen phosphate R* adjusted to pH 8.9 with *dilute phosphoric acid R* or with *dilute sodium hydroxide solution R*;

— *mobile phase B*: *methanol R1*, *acetonitrile R1* (250:750 *V/V*);



Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 25	50 → 45	50 → 55
25 - 30	45 → 40	55 → 60
30 - 80	40 → 25	60 → 75
80 - 81	25 → 50	75 → 50
81 - 93	50	50

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µl.

Relative retention With reference to azithromycin (retention time = 45-50 min): impurity L = about 0.29; impurity M = about 0.37; impurity E = about 0.43; impurity F = about 0.51; impurity D = about 0.54; impurity J = about 0.54; impurity I = about 0.61; impurity C = about 0.73; impurity N = about 0.76; impurity H = about 0.79; impurity A = about 0.83; impurity P = about 0.92; impurity O = about 1.23; impurity G = about 1.26; impurity B = about 1.31.

Identification of impurities Use the chromatogram supplied with *azithromycin for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, C, E, F, G, I, J, L, M, N, O and P, the chromatogram obtained with reference solution (d) to identify the peak due to impurity B and the chromatogram obtained with reference solution (b) to identify the peak due to impurity H.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 1.4, where H_p = height above the baseline of the peak due to impurity J and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity F = 0.3; impurity H = 0.1; impurity L = 2.3; impurity M = 0.6; impurity N = 0.7;

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *impurities A, C, E, F, G, H, I, L, M, N, O, P*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *sum of impurities D and J*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard the peaks eluting before impurity L and after impurity B.

Heavy metals *(2.4.8)*

Maximum 25 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *anhydrous ethanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2.5 ppm Pb) obtained by *diluting lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *anhydrous ethanol R*.

Water *(2.5.12)*

1.8 per cent to 6.5 per cent, determined on 0.200 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)*.

Solution A Mix 60 volumes of *acetonitrile R1* and 40 volumes of a 6.7 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *phosphoric acid R*.

Test solution Dissolve 53.0 mg of the substance to be examined in 2 ml of *acetonitrile R1* and dilute to 100.0 ml with solution A.

Reference solution (a) Dissolve 53.0 mg of *azithromycin CRS* in 2 ml of *acetonitrile R1* and dilute to 100.0 ml with solution A.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *azithromycin impurity A CRS* in 0.5 ml of *acetonitrile R1* and dilute to 10 ml with solution A.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl vinyl polymer for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 60 volumes of *acetonitrile R1* and 40 volumes of a 6.7 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 11.0 with a 560 g/l solution of *potassium hydroxide R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Run time 1.5 times the retention time of azithromycin.

Retention time Azithromycin = about 10 min.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to impurity A and azithromycin.

Calculate the percentage content of $C_{38}H_{72}N_2O_{12}$ from the declared content of *azithromycin CRS* .

STORAGE

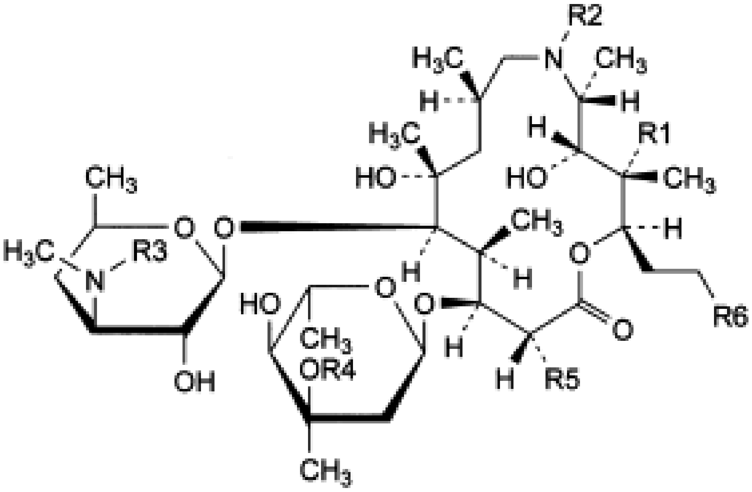
In an airtight container .

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J, L, M, N, O, P.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*)

K.



A. R1 = OH, R2 = R6 = H, R3 = R4 = R5 = CH_3: 6-demethylazithromycin,

B. R1 = R6 = H, R2 = R3 = R4 = R5 = CH_3: 3-deoxyazithromycin (azithromycin B),

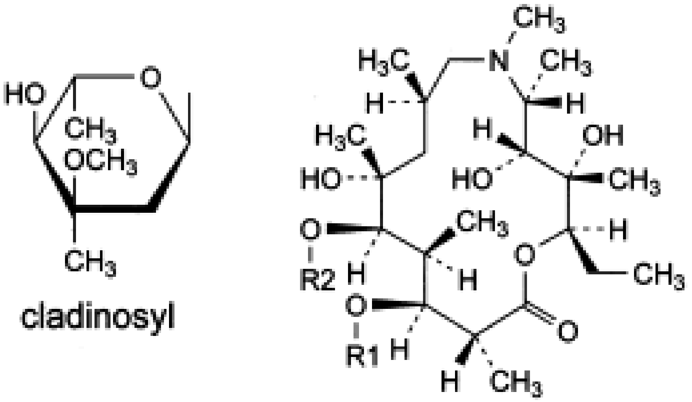
C. R1 = OH, R2 = R3 = R5 = CH_3, R4 = R6 = H: 3″-*O*-demethylazithromycin (azithromycin C),

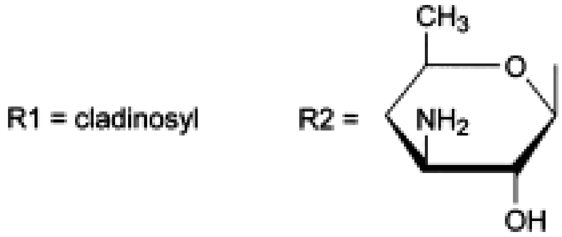
D. R1 = OH, R2 = R3 = R4 = CH_3, R5 = CH_2OH, R6 = H: 14-demethyl-14-(hydroxymethyl) azithromycin (azithromycin F),

F. R1 = OH, R2 = R4 = R5 = CH_3, R3 = CHO, R6 = H: 3′-*N*-demethyl-3′-*N*-formylazithromycin,

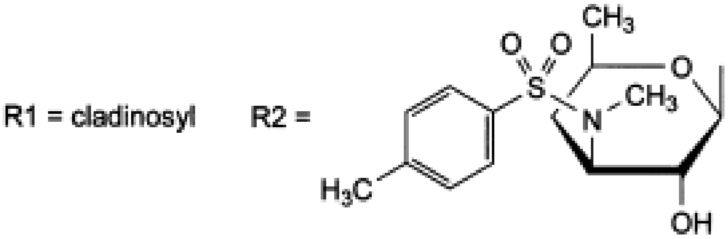
I. R1 = OH, R2 = R4 = R5 = CH_3, R3 = R6 = H: 3′-*N*-demethylazithromycin,

O. R1 = OH, R2 = R3 = R4 = R5 = R6 = CH_3: 2-desethyl-2-propylazithromycin,

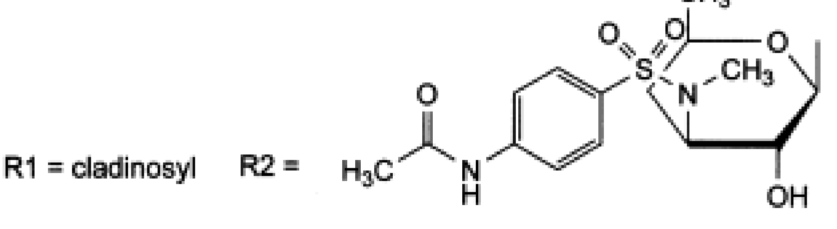

cladinosyl


R1 = cladinosyl
R2 =

E. 3′-(*N*,*N*-didemethyl)azithromycin (aminoazithromycin),


R1 = cladinosyl
R2 =

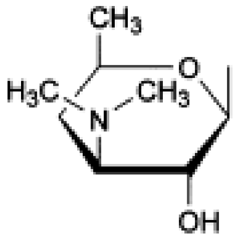
G. 3′-*N*-demethyl-3′-*N*-[(4-methylphenyl)sulphonyl]azithromycin,


R1 = cladinosyl
R2 =

H. 3′-*N*-[[4-(acetylamino)phenyl]sulphonyl]-3′-*N*-demethylazithromycin,

R1 = H

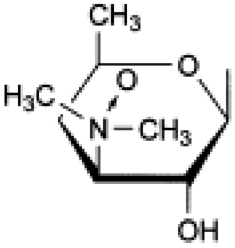
R2 =


CH$_3$
O
H$_3$C
CH$_3$
N
OH

J. 13-*C*-decladinosylazithromycin,

R1 = cladinosyl

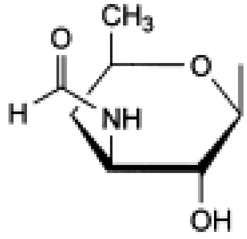
R2 =


CH$_3$
O
H$_3$C
O
N
CH$_3$
OH

L. azithromycin 3′- *N*-oxide,

R1 = cladinosyl

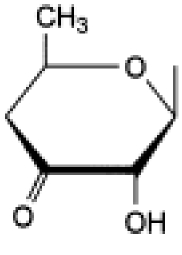
R2 =


O
CH$_3$
O
H
NH
OH

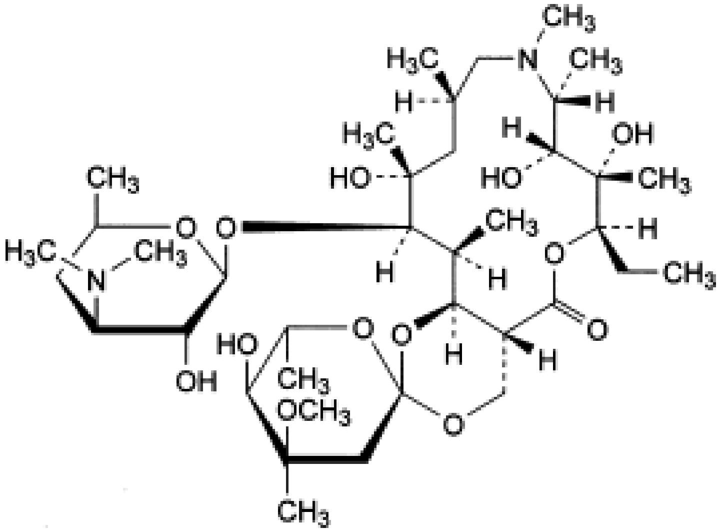
M. 3′-(*N*,*N*-didemethyl)-3′-*N*-formylazithromycin,

R1 = cladinosyl

R2 =



N. 3′-de(dimethylamino)-3′-oxoazithromycin,



K. C^{14},1″-epoxyazithromycin (azithromycin E),

P. unknown structure.

Ph Eur

Dried Aluminium Hydroxide

General Notices

(Hydrated Aluminium Oxide, Ph Eur monograph 0311)

Action and use

Antacid.

Preparations

Aluminium Hydroxide Tablets

Co-magaldrox Oral Suspension

Co-magaldrox Tablets

Compound Magnesium Trisilicate Tablets

Ph Eur

DEFINITION

Content

47.0 per cent to 60.0 per cent of Al_2O_3 (M_r 102.0).

CHARACTERS

Appearance

White or almost white, amorphous powder.

Solubility

Practically insoluble in water. It dissolves in dilute mineral acids and in solutions of alkali hydroxides.

IDENTIFICATION

Solution S (see Tests) gives the reaction of aluminium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in 15 ml of *hydrochloric acid R*, heating on a water-bath. Dilute to 100 ml with *distilled water R*.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution GY_6 *(2.2.2, Method II)*.

Alkaline impurities

Shake 1.0 g with 20 ml of *carbon dioxide-free water R* for 1 min and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. Any pink colour disappears on the addition of 0.3 ml of *0.1 M hydrochloric acid* .

Neutralising capacity

Carry out the test at 37 °C. Disperse 0.5 g in 100 ml of *water R*, heat, add 100.0 ml of *0.1 M hydrochloric acid* , previously heated, and stir continuously; the pH *(2.2.3)* of the solution after 10 min, 15 min and 20 min is not less than 1.8, 2.3 and 3.0 respectively and is at no time greater than 4.5. Add 10.0 ml of *0.5 M hydrochloric acid* , previously heated, stir continuously for 1 h and titrate with *0.1 M sodium hydroxide* to pH 3.5; not more than 35.0 ml of *0.1 M sodium hydroxide* is required.

Chlorides *(2.4.4)*

Maximum 1 per cent.

Dissolve 0.1 g with heating in 10 ml of *dilute nitric acid R* and dilute to 100 ml with *water R*. Dilute 5 ml of the solution to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 1 per cent.

Dilute 4 ml of solution S to 100 ml with *distilled water R*.

Arsenic *(2.4.2, Method A)*

Maximum 4 ppm, determined on 10 ml of solution S.

Heavy metals *(2.4.8)*

Maximum 60 ppm.

Neutralise 20 ml of solution S with *concentrated ammonia R*, using *metanil yellow solution R* as an external indicator. Filter, if necessary, and dilute to 30 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R*.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count. It complies with the tests for enterobacteria and certain other gram-negative bacteria and with the test for *Escherichia coli (2.6.13)*.

ASSAY

Dissolve 0.800 g in 10 ml of *hydrochloric acid R1*, heating on a water-bath. Cool and dilute to 50.0 ml with *water R*. To 10.0 ml of the solution, add *dilute ammonia R1* until a precipitate begins to appear. Add the smallest quantity of *dilute hydrochloric acid R* needed to dissolve the precipitate and dilute to 20 ml with *water R*. Carry out the complexometric titration of aluminium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 5.098 mg of Al_2O_3.

STORAGE

In an airtight container, at a temperature not exceeding 30 °C.

Ph Eur

Dried Aluminium Phosphate

General Notices

(Hydrated Aluminium Phosphate, Ph Eur monograph 1598)

$AlPO_4,xH_2O$ 122.0 *7784-30-7*
(anhydrous) *(anhydrous)*

Action and use

Antacid.

Ph Eur

DEFINITION

Content

94.0 per cent to 102.0 per cent of $AlPO_4$ (M_r 122.0) (ignited substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

A. Solution S (see Tests) gives reaction (b) of phosphates *(2.3.1)*.

B. Solution S gives the reaction of aluminium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.00 g in *dilute hydrochloric acid R* and dilute to 100 ml with the same acid.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.5 to 7.2

Shake 4.0 g with *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Chlorides *(2.4.4)*

Maximum 1.3 per cent.

Dissolve 50.0 mg in 10 ml of *dilute nitric acid R* and dilute to 200 ml with *water R*. 15 ml of the solution complies with the limit test for chlorides.

Soluble phosphates

Maximum 1.0 per cent, calculated as PO_4^{3-}.

Test solution Stir 5.0 g with 150 ml of *water R* for 2 h. Filter and wash the filter with 50 ml of *water R*. Combine the filtrate and the washings and dilute to 250.0 ml with *water R*. Dilute 10.0 ml of this solution to 100.0 ml with *water R*.

Reference solution (a) Dissolve 2.86 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dilute 1 ml of reference solution (a) to 5 ml with *water R*.

Reference solution (c) Dilute 3 ml of reference solution (a) to 5 ml with *water R*.

Treat each solution as follows. To 5.0 ml add 4 ml of *dilute sulphuric acid R*, 1 ml of *ammonium molybdate solution R*, 5 ml of *water R* and 2 ml of a solution containing 0.10 g of *4-methylaminophenol sulphate R*, 0.5 g of *anhydrous sodium sulphite R* and 20.0 g of *sodium metabisulphite R* in 100 ml of *water R*. Shake and allow to stand for 15 min. Dilute to 25.0 ml with *water R* and allow to stand for a further 15 min. Measure the absorbance *(2.2.25)* at 730 nm. Calculate the content of soluble phosphates from a calibration curve prepared using reference solutions (a), (b) and (c) after treatment.

Sulphates *(2.4.13)*

Maximum 0.6 per cent.

Dilute 8 ml of solution S to 100 ml with *distilled water R*. 15 ml of the solution complies with the limit test for sulphates.

Arsenic *(2.4.2)*

Maximum 1 ppm.

1.0 g complies with limit test A.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 20 ml with the same acid. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

10.0 per cent to 20.0 per cent, determined on 1.000 g at 800 ± 50 °C.

Neutralising capacity

Add 0.50 g to 30 ml of *0.1 M hydrochloric acid* previously heated to 37 °C and maintain at this temperature for 15 min while stirring. The pH *(2.2.3)* of the mixture after 15 min at 37 °C is 2.0

to 2.5.

ASSAY

Dissolve 0.400 g in 10 ml of *dilute hydrochloric acid R* and dilute to 100.0 ml with *water R*. To 10.0 ml of the solution, add 10.0 ml of *0.1 M sodium edetate* and 30 ml of a mixture of equal volumes of *ammonium acetate solution R* and *dilute acetic acid R*. Boil for 3 min, then cool. Add 25 ml of *alcohol R* and 1 ml of a freshly prepared 0.25 g/l solution of *dithizone R* in *alcohol R*. Titrate the excess of sodium edetate with *0.1 M zinc sulphate* until the colour changes to pink.

1 ml of *0.1 M sodium edetate* is equivalent to 12.20 mg of $AlPO_4$.

STORAGE

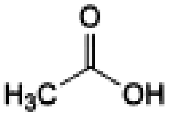
In an airtight container.

Ph Eur

Glacial Acetic Acid

General Notices

(Ph Eur monograph 0590)



$C_2H_4O_2$ 60.1 *64-19-7*

Ph Eur

DEFINITION

Content

99.0 per cent *m/m* to 100.5 per cent *m/m*.

CHARACTERS

Appearance

Crystalline mass or clear, colourless, volatile liquid.

Solubility

Miscible with water, with ethanol (96 per cent) and with methylene chloride.

IDENTIFICATION

A. A 100 g/l solution is strongly acid *(2.2.4)*.

B. To 0.03 ml add 3 ml of *water R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives reaction (b) of acetates *(2.3.1)*.

TESTS

Solution S

Dilute 20 ml to 100 ml with *distilled water R*.

Appearance

The substance to be examined is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Freezing point *(2.2.18)*

Minimum 14.8 °C.

Reducing substances

To 5.0 ml add 10.0 ml of *water R* and mix. To 5.0 ml of this solution add 6 ml of *sulphuric acid R*, cool and add 2.0 ml of *0.0167 M potassium dichromate*. Allow to stand for 1 min and add 25 ml of *water R* and 1 ml of a freshly prepared 100 g/l solution of *potassium iodide R*. Titrate with *0.1 M sodium thiosulphate*, using 1.0 ml of *starch solution R* as indicator. Not less than 1.0 ml of *0.1 M sodium thiosulphate solution* is required.

Chlorides *(2.4.4)*

Maximum 25 mg/l.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 50 mg/l, determined on solution S.

Iron *(2.4.9)*

Maximum 5 ppm.

Dilute 5.0 ml of solution A obtained in the test for heavy metals to 10.0 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 5 ppm.

Dissolve the residue obtained in the test for residue on evaporation by heating with 2 quantities, each of 15 ml, of *water R* and dilute to 50.0 ml (solution A). 12 ml of solution A complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Residue on evaporation

Maximum 0.01 per cent.

Evaporate 20 g to dryness on a water-bath and dry at 100-105 °C. The residue weighs a maximum of 2.0 mg.

ASSAY

Weigh accurately a conical flask with a ground-glass stopper containing 25 ml of *water R*. Add 1.0 ml of the substance to be examined and weigh again accurately. Add 0.5 ml of *phenolphthalein solution R* and titrate with *1 M sodium hydroxide*.

1 ml of *1 M sodium hydroxide* is equivalent to 60.1 mg of $C_2H_4O_2$.

STORAGE

In an airtight container.

Ph Eur

Hydrated Aluminium Hydroxide for Adsorption

General Notices

(Ph Eur monograph 1664)

$[AlO(OH)],nH_2O$

Ph Eur

DEFINITION

Content

90.0 per cent to 110.0 per cent of the content of aluminium stated on the label.

NOTE: shake the gel vigorously for at least 30 s immediately before examining.

CHARACTERS

Appearance

White or almost white, translucent, viscous, colloidal gel. A supernatant may be formed upon standing.

Solubility

A clear or almost clear solution is obtained with alkali hydroxide solutions and mineral acids.

IDENTIFICATION

Solution S (see Tests) gives the reaction of aluminium.

To 10 ml of solution S add about 0.5 ml of *dilute hydrochloric acid R* and about 0.5 ml of *thioacetamide reagent R*. No precipitate is formed. Add dropwise 5 ml of *dilute sodium hydroxide solution R*. Allow to stand for 1 h. A gelatinous white precipitate is formed which dissolves upon addition of 5 ml of *dilute sodium hydroxide solution R*. Gradually add 5 ml of *ammonium chloride solution R* and allow to stand for 30 min. The gelatinous white precipitate is re-formed.

TESTS

Solution S

Add 1 g to 4 ml of *hydrochloric acid R*. Heat at 60 °C for 1 h, cool, dilute to 50 ml with *distilled water R* and filter if necessary.

pH (*2.2.3*)

5.5 to 8.5.

Adsorption power

Dilute the substance to be examined with *distilled water R* to obtain an aluminium concentration of 5 mg/ml. Prepare *bovine albumin R* solutions with the following concentrations of bovine albumin: 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 5 mg/ml and 10 mg/ml. If necessary, adjust the gel and the *bovine albumin R* solutions to pH 6.0 with *dilute hydrochloric acid R* or *dilute sodium hydroxide solution R*.

For adsorption, mix 1 part of the diluted gel with 4 parts of each of the solutions of *bovine albumin R* and allow to stand at room temperature for 1 h. During this time shake the mixture vigorously at least 5 times. Centrifuge or filter through a non-protein-retaining filter. Immediately determine the protein content (*2.5.33, Method 2*) of either the supernatant or the filtrate.

It complies with the test if no bovine albumin is detectable in the supernatant or filtrate of the 2 mg/ml *bovine albumin R* solution (maximum level of adsorption) and in the supernatant or filtrate of *bovine albumin R* solutions of lower concentrations. Solutions containing 3 mg/ml, 5 mg/ml and 10 mg/ml *bovine albumin R* may show bovine albumin in the supernatant or filtrate, proportional to the amount of bovine albumin in the solutions.

Sedimentation

If necessary, adjust the substance to be examined to pH 6.0 using *dilute hydrochloric acid R* or *dilute sodium hydroxide solution R.* Dilute with *distilled water R* to obtain an aluminium concentration of approximately 5 mg/ml. If the aluminium content of the substance to be examined is lower than 5 mg/ml, adjust to pH 6.0 and dilute with a 9 g/l solution of *sodium chloride R* to obtain an aluminium concentration of about 1 mg/ml. After shaking for at least 30 s, place 25 ml of the preparation in a 25 ml graduated cylinder and allow to stand for 24 h.

It complies with the test if the volume of the clear supernatant is less than 5 ml for the gel with an aluminium content of about 5 mg/ml.

It complies with the test if the volume of the clear supernatant is less than 20 ml for the gel with an aluminium content of about 1 mg/ml.

Chlorides (*2.4.4*)

Maximum 0.33 per cent.

Dissolve 0.5 g in 10 ml of *dilute nitric acid R* and dilute to 500 ml with *water R*.

Nitrates

Maximum 100 ppm.

Place 5 g in a test-tube immersed in ice-water, add 0.4 ml of a 100 g/l solution of *potassium chloride R*, 0.1 ml of *diphenylamine solution R* and, dropwise with shaking, 5 ml of *sulphuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 5 ml of *nitrate standard solution (100 ppm* NO_3*) R.*

Sulphates (*2.4.13*)

Maximum 0.5 per cent.

Dilute 2 ml of solution S to 20 ml with *water R*.

Ammonium (*2.4.1, Method B*)

Maximum 50 ppm, determined on 1.0 g.

Prepare the standard using 0.5 ml of *ammonium standard solution (100 ppm NH_4) R*.

Arsenic (*2.4.2, Method A*)

Maximum 1 ppm, determined on 1 g.

Iron (*2.4.9*)

Maximum 15 ppm, determined on 0.67 g.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dissolve 2.0 g in 10 ml of *dilute nitric acid R* and dilute to 20 ml with *water R.* The solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Bacterial endotoxins (*2.6.14*)

Less than 5 IU of endotoxin per milligram of aluminium, if intended for use in the manufacture of an adsorbed product without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 2.50 g in 10 ml of *hydrochloric acid R*, heating for 30 min at 100 °C on a water-bath. Cool and dilute to 20 ml with *water R*. To 10 ml of the solution, add *concentrated ammonia R* until a precipitate is obtained. Add the smallest quantity of *hydrochloric acid R* needed to dissolve the precipitate and dilute to 20 ml with *water R*. Carry out the complexometric titration of aluminium (*2.5.11*). Carry out a blank titration.

STORAGE

At a temperature not exceeding 30 °C. Do not allow to freeze. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states the declared content of aluminium.

Ph Eur

Hydrogenated Arachis Oil

General Notices

Hydrogenated Peanut Oil
(Ph Eur monograph 1171)

Ph Eur

DEFINITION

Oil obtained by refining, bleaching, hydrogenating and deodorising oil obtained from the shelled seeds of *Arachis hypogaea* L. Each type of hydrogenated arachis oil is characterised by its nominal drop point.

CHARACTERS

Appearance

White or faintly yellowish, soft mass which melts to a clear, pale yellow liquid when heated.

Solubility

Practically insoluble in water, freely soluble in methylene chloride and in light petroleum (bp: 65-70 °C), very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, B.

Second identification A, C.

A. Drop point (see Tests).

B. Identification of fatty oils by thin-layer chromatography *(2.3.2)*.

Results The chromatogram obtained is similar to the typical chromatogram for arachis oil.

C. Composition of fatty acids (see Tests).

TESTS

Drop point *(2.2.17)*

32 °C to 43 °C, and within 3 °C of the nominal value.

Acid value *(2.5.1)*

Maximum 0.5.

Dissolve 10.0 g in 50 ml of the prescribed solvent by heating on a water-bath.

Peroxide value ***(2.5.5, Method A)***

Maximum 5.0.

Dissolve 5.0 g in 30 ml of the prescribed solvent by heating on a water-bath.

Unsaponifiable matter ***(2.5.7)***

Maximum 1.0 per cent.

Alkaline impurities in fatty oils ***(2.4.19)***

It complies with the test.

Composition of fatty acids ***(2.4.22, Method A)***

Use the mixture of calibrating substances in Table 2.4.22.-3.

Column:
- *material:* fused silica;
- *size*: *l* = 25 m, Ø = 0.25 mm;
- *stationary phase*: *poly(cyanopropyl)siloxane R* (film thickness 0.2 µm).

Carrier gas *helium for chromatography R.*

Flow rate 0.7 ml/min.

Split ratio 1:100.

Temperature:
- *column*: 180 °C for 20 min;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Composition of the fatty-acid fraction of the oil:
- *saturated fatty acids of chain length less than C_{14}*: maximum 0.5 per cent;
- *myristic acid*: maximum 0.5 per cent;
- *palmitic acid*: 7.0 per cent to 16.0 per cent;
- *stearic acid*: 3.0 per cent to 19.0 per cent;
- *oleic acid and isomers*: 54.0 per cent to 78.0 per cent;
- *linoleic acid and isomers*: maximum 10.0 per cent;
- *arachidic acid*: 1.0 per cent to 3.0 per cent;
- *eicosenoic acids*: maximum 2.1 per cent;
- *behenic acid*: 1.0 per cent to 5.0 per cent;
- *erucic acid and isomers*: maximum 0.5 per cent;
- *lignoceric acid*: 0.5 per cent to 3.0 per cent.

Nickel

Maximum 1.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Into a platinum or silica crucible previously tared after ignition introduce 5.0 g. Cautiously heat and introduce into the substance a wick formed from twisted ashless filter paper. Ignite the wick. When the substance has ignited stop heating. After combustion, ignite in a muffle furnace at about 600 ± 50 °C. Continue ignition until white ash is obtained. After cooling, take up the residue with 2 quantities, each of 2 ml, of *dilute hydrochloric acid R* and transfer into a 25 ml graduated flask. Add 0.3 ml of *nitric acid R* and dilute to 25.0 ml with *water R*.

Reference solutions Prepare 3 reference solutions by adding 1.0 ml, 2.0 ml and 4.0 ml of *nickel standard solution (0.2 ppm Ni) R* to 2.0 ml of the test solution and diluting to 10.0 ml with *water R*.

Source Nickel hollow-cathode lamp.

Wavelength 232 nm.

Atomisation device Graphite furnace.

Carrier gas *argon R.*

Water ***(2.5.12)***

Maximum 0.3 per cent, determined on 1.000 g.

STORAGE

Protected from light.

LABELLING

The label states the nominal drop point.

Ph Eur

Medical Air

General Notices

(Medicinal Air, Ph Eur monograph 1238)

When Medical Air is intended for use in a room in which magnetic resonance imaging (MRI) is being performed, the cylinder and fittings should be made from suitable non-ferromagnetic materials and labelled accordingly.

Ph Eur

DEFINITION

Compressed ambient air.

Content

20.4 per cent *V/V* to 21.4 per cent *V/V* of oxygen (O_2).

CHARACTERS

A colourless, odourless gas.

Solubility

At a temperature of 20 °C and a pressure of 101 kPa, 1 volume dissolves in about 50 volumes of water.

PRODUCTION

Carbon dioxide

Maximum 500 ppm *V/V*, determined using an infrared analyser *(2.5.24)*.

Gas to be examined Use the substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) Use a mixture of 79 per cent *V/V* of *nitrogen R1* and 21 per cent *V/V* of *oxygen R* containing less than 1 ppm *V/V* of *carbon dioxide R1*.

Reference gas (b) Use a mixture of 79 per cent *V/V* of *nitrogen R1* and 21 per cent *V/V* of *oxygen R* containing 500 ppm *V/V* of *carbon dioxide R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon dioxide in the gas to be examined.

Carbon monoxide

Maximum 5 ppm *V/V*, determined using an infrared analyser *(2.5.25)*.

Gas to be examined Use the substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) Use a mixture of 79 per cent *V/V* of *nitrogen R1* and 21 per cent *V/V* of *oxygen R* containing less than 1 ppm *V/V* of *carbon monoxide R*.

Reference gas (b) Use a mixture of 79 per cent *V/V* of *nitrogen R1* and 21 per cent *V/V* of *oxygen R* containing 5 ppm *V/V* of *carbon monoxide R*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon monoxide in the gas to be examined.

Sulphur dioxide

Maximum 1 ppm *V/V*, determined using an ultraviolet fluorescence analyser (Figure 1238.-1).

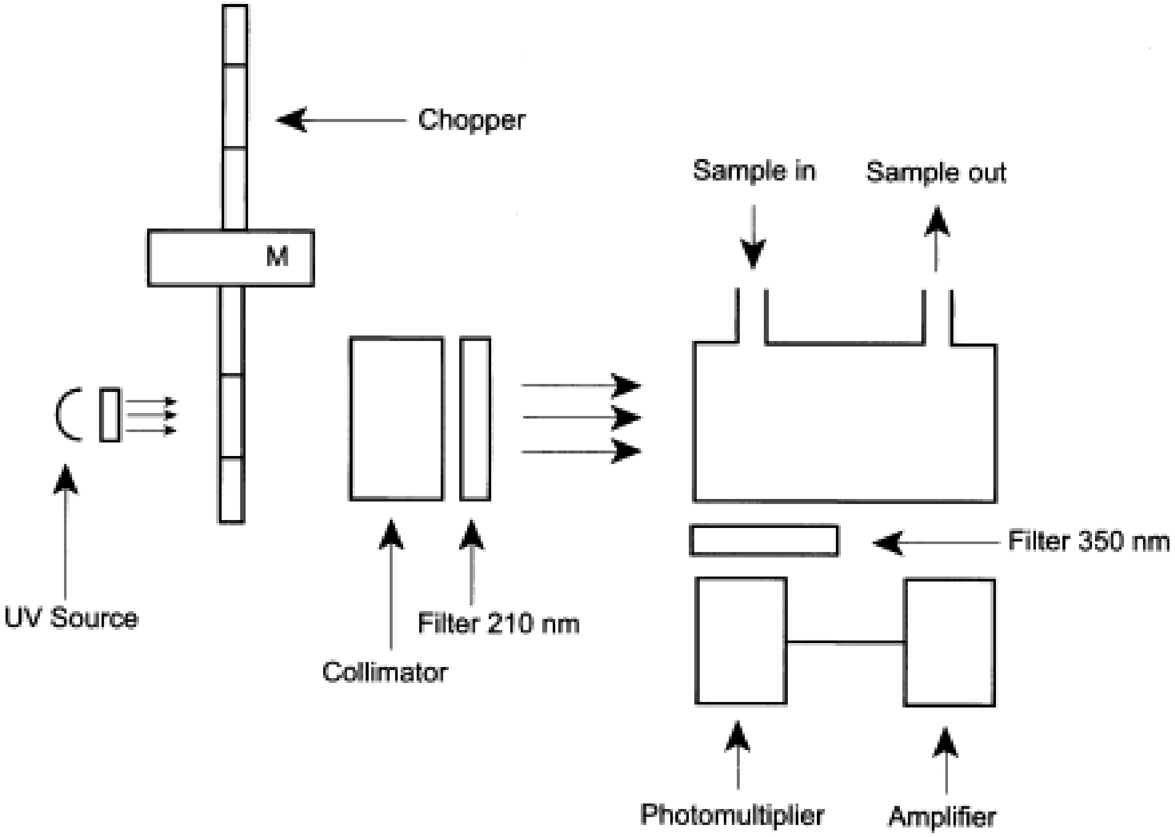


Figure 1238.-1. – *UV fluorescence analyser*

The apparatus consists of the following:

- — a system generating ultraviolet radiation with a wavelength of 210 nm, made up of an ultraviolet lamp, a collimator, and a selective filter; the beam is blocked periodically by a chopper rotating at high speeds;
- — a reaction chamber, through which flows the gas to be examined;
- — a system that detects radiation emitted at a wavelength of 350 nm, made up of a selective filter, a photomultiplier tube and an amplifier.

Gas to be examined Use the substance to be examined. It must be filtered.

Reference gas (a) Use a mixture of 79 per cent *V/V* of *nitrogen R1* and 21 per cent *V/V* of *oxygen R*.

Reference gas (b) Use a mixture of 79 per cent *V/V* of *nitrogen R1* and 21 per cent *V/V* of *oxygen R* containing 0.5 ppm *V/V* to 2 ppm *V/V* of *sulphur dioxide R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of sulphur dioxide in the gas to be examined.

Oil

Maximum 0.1 mg/m^3 calculated for atmospheric pressure and at 0 °C, determined using a measuring system such as described in Figure 1238.-2.

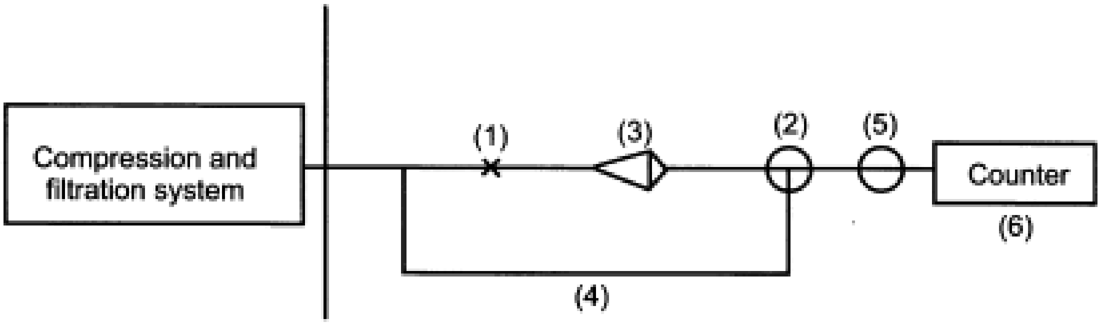


Figure 1238.-2. – *Measuring system for oil*

The apparatus consists of the following:

- an on-off valve (1),
- a three-way valve (2),
- an oil cone (3),
- a bypass line (4),
- a pressure-regulator (5),
- a flow-metering device (6).

All of the apparatus is cleaned beforehand, using trichlorotrifluoroethane R free from oil and grease.

Place a micro fibre-glass filter in the oil cone (3); this filter has the following characteristics: 100 per cent borosilicate glass without binder; resistant to heat treatment at 500 °C (to eliminate organic traces); 99.999 per cent retention efficiency for NaCl particles with a diameter of 0.6 µm. Close the on-off valve (1); the substance to be examined enters the bypass line (4) and purges the three-way valve (2), the pressure-regulator (5) and the flow-metering device (6). Close the inlet valve of the compression and filtration system: open the on-off valve (1) and set the three-way valve (2) to the position allowing passage between the oil cone and the pressure-regulator. Open the inlet valve and set the pressure-regulator (5) so that the flow indicated by the flow-metering device (6) is 20 litres/min. Pass 100.0 litres of the substance to be examined through the system.

Test solution Remove the micro fibre-glass filter and place in an airtight container. Carefully cut up the micro fibre-glass filter and place the pieces in 25.0 ml of *trichlorotrifluoroethane R*.

Reference solutions Prepare the reference solutions with quantities of oil (used for the lubrication of the compression system) ranging from 0.05 µg/ml to 0.5 µg/ml in *trichlorotrifluoroethane R*.

Measure the absorbance of the test solution and the reference solutions, using an appropriate infrared spectrophotometer, at 2960.3 cm^{-1}, 2927.7 cm^{-1} and 2855.0 cm^{-1}. The sum of the 3 absorbances gives the absorbance of the oil. Use potassium bromide cells with a pathlength of several centimetres.

Plot the calibration curve from the absorbances obtained with the reference solutions and determine the quantity of oil from this curve.

Nitrogen monoxide and nitrogen dioxide

Maximum 2 ppm *V/V* in total, determined using a chemiluminescence analyser *(2.5.26)*.

Gas to be examined Use the substance to be examined.

Reference gas (a) Use a mixture of 79 per cent *V/V* of *nitrogen R1* and 21 per cent *V/V* of *oxygen R* containing less than 0.05 ppm *V/V* of nitrogen monoxide and nitrogen dioxide.

Reference gas (b) Use a mixture of 2 ppm *V/V* of *nitrogen monoxide R* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of nitrogen monoxide and nitrogen dioxide in the gas to be examined.

Water

Maximum 67 ppm *V/V*, determined using an electrolytic hygrometer *(2.5.28)*, except where the competent authority decides that the following limit applies to medicinal air generated on-site and distributed in pipe-line systems operating at a pressure not greater than 10 bars and a temperature not less than 5 °C: maximum 870 ppm *V/V*, determined using an electrolytic hygrometer *(2.5.28)*.

Assay

Determine the concentration of oxygen in air using a paramagnetic analyser *(2.5.27)*.

IDENTIFICATION

First identification *C.*

Second identification *A, B.*

A. In a conical flask containing the substance to be examined, place a glowing wood splinter. The splinter remains glowing.

B. Use a gas burette (Figure 1238.-3) of 25 ml capacity in the form of a chamber in the middle of which is a tube graduated in 0.2 per cent between 19.0 per cent and 23.0 per cent, and isolated at each end by a tap with a conical barrel. The lower tap is joined to a tube with an olive-shaped nozzle and is used to introduce the gas into the apparatus. A cylindrical funnel above the upper tap is used to introduce the absorbent solution. Wash the burette with *water R* and dry. Open the 2 taps. Connect the nozzle to the source of the substance to be examined and set the flow rate to 1 litre/min. Flush the burette by passing the substance to be examined through it for 1 min. Close the lower tap of the burette and immediately afterwards the upper tap. Rapidly disconnect the burette from the source of the substance to be examined. Rapidly give a half turn to the upper tap to eliminate any excess pressure in the burette. Keeping the burette vertical, fill the funnel with a freshly prepared mixture of 21 ml of a 560 g/l solution of *potassium hydroxide R* and 130 ml of a 200 g/l solution of *sodium dithionite R*. Open the upper tap slowly. The solution absorbs the oxygen and enters the burette. Allow to stand for 10 min without shaking. Read the level of the liquid meniscus on the graduated part of the burette. This figure represents the percentage *V/V* of oxygen. The value read is 20.4 to 21.4.

C. It complies with the limits of the assay.

TESTS

Carbon dioxide

Maximum 500 ppm *V/V*, determined using a carbon dioxide detector tube *(2.1.6)*.

Sulphur dioxide

Maximum 1 ppm *V/V*, determined using a sulphur dioxide detector tube *(2.1.6)*.

Oil

Maximum 0.1 mg/m^3, determined using an oil detector tube *(2.1.6)*.

Nitrogen monoxide and nitrogen dioxide

Maximum 2 ppm *V/V*, determined using a nitrogen monoxide and nitrogen dioxide detector tube *(2.1.6)*.

Carbon monoxide

Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube *(2.1.6)*.

Water vapour

Maximum 67 ppm *V/V*, determined using a water vapour detector tube *(2.1.6)*, except where the competent authority decides that the following limit applies to medicinal air generated on-site and distributed in pipe-line systems operating at a pressure not greater than 10 bars and a temperature not less than 5 °C: maximum 870 ppm *V/V*, determined using a water vapour detector tube *(2.1.6)*.

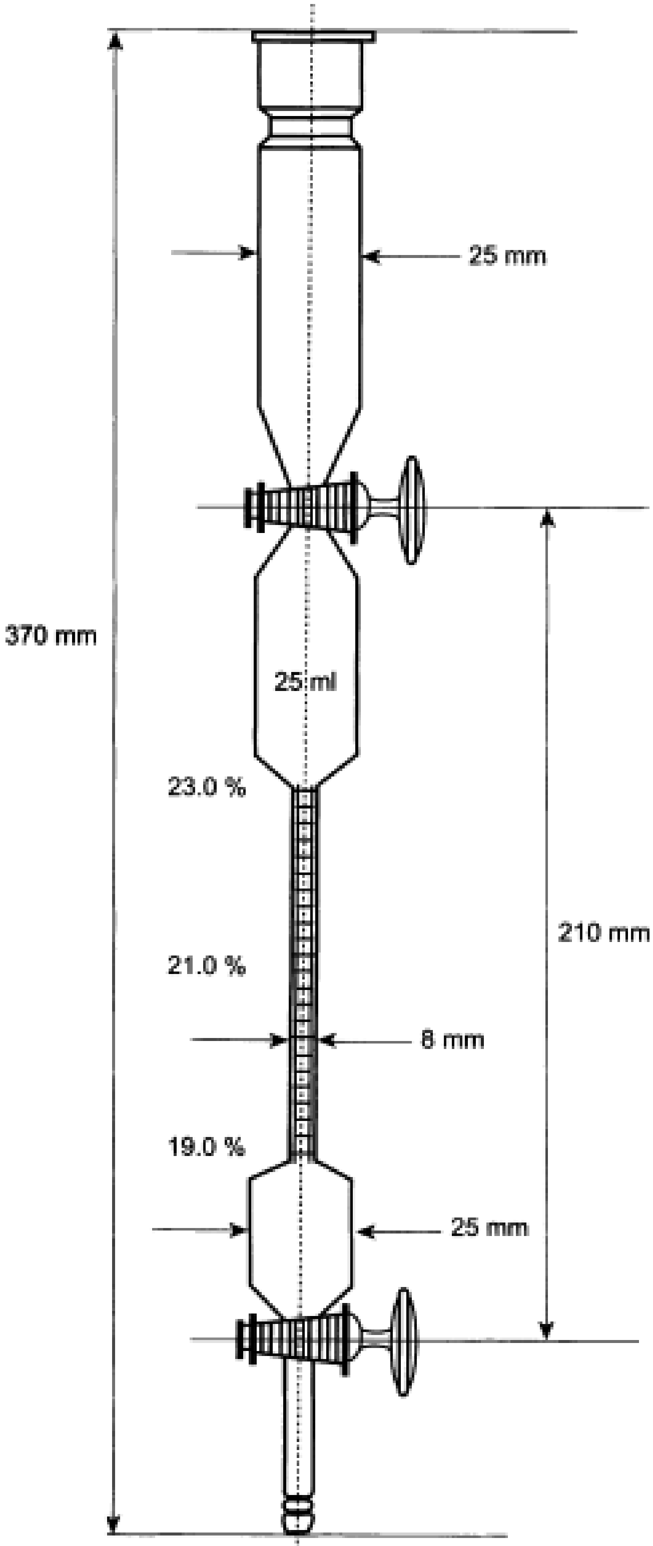

25 mm
370 mm
25 ml
23.0 %
210 mm
21.0 %
8 mm
19.0 %
25 mm

Figure 1238.-3. – *Gas burette*

STORAGE

As a gas, in suitable containers complying with the legal regulations or as a gas supplied by a pipe network.

IMPURITIES

A. carbon dioxide,

B. sulphur dioxide,

C. nitrogen monoxide,

D. nitrogen dioxide,

E. oil,

F. carbon monoxide,

G. water.

Ph Eur

Refined Almond Oil

General Notices

(Ph Eur monograph 1064)

Ph Eur

DEFINITION

Fatty oil obtained from the ripe seeds of *Prunus dulcis* (Miller) D.A. Webb var. *dulcis* or *Prunus dulcis* (Miller) D.A. Webb var. *amara* (D.C.) Buchheim or a mixture of both varieties by cold expression. It is then refined. A suitable antioxidant may be added.

CHARACTERS

Appearance

Pale yellow, clear liquid.

Solubility

Slightly soluble in ethanol (96 per cent), miscible with light petroleum.

Relative density

About 0.916.

It solidifies at about - 18 °C.

IDENTIFICATION

A. Carry out the identification of fatty oils by thin-layer chromatography *(2.3.2)*. The chromatogram obtained is similar to the type chromatogram for almond oil.

B. Composition of fatty acids (see Tests).

TESTS

Specific absorbance *(2.2.25)*

0.2 to 6.0, determined at the absorption maximum between 264 nm and 276 nm.

Dissolve 0.100 g in *cyclohexane R* and dilute to 10.0 ml with the same solvent.

Acid value *(2.5.1)*

Maximum 0.5, determined on 5.0 g.

Peroxide value *(2.5.5)*

Maximum 5.0.

Unsaponifiable matter ***(2.5.7)***

Maximum 0.9 per cent, determined on 5.0 g.

Composition of fatty acids

Gas chromatography *(2.4.22, Method A)*. Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

- *saturated fatty acids of chain length less than C_{16}*: maximum 0.1 per cent,
- *palmitic acid* : 4.0 per cent to 9.0 per cent,
- *palmitoleic acid* (equivalent chain length on polyethyleneglycol adipate 16.3): maximum 0.8 per cent,
- *margaric acid*: maximum 0.2 per cent,
- *stearic acid* : maximum 3.0 per cent,
- *oleic acid* (equivalent chain length on polyethyleneglycol adipate 18.3): 62.0 per cent to 86.0 per cent,
- *linoleic acid* (equivalent chain length on polyethyleneglycol adipate 18.9): 20.0 per cent to 30.0 per cent,
- *linolenic acid* (equivalent chain length on polyethyleneglycol adipate 19.7): maximum 0.4 per cent,
- *arachidic acid*: maximum 0.2 per cent,
- *eicosenoic acid* (equivalent chain length on polyethyleneglycol adipate 20.3): maximum 0.3 per cent,
- *behenic acid*: maximum 0.2 per cent,
- *erucic acid* (equivalent chain length on polyethyleneglycol adipate 22.3): maximum 0.1 per cent.

Sterols ***(2.4.23)*****.**

Composition of the sterol fraction of the oil:

- *cholesterol* : maximum 0.7 per cent,
- *campesterol*: maximum 5.0 per cent,
- *stigmasterol* : maximum 4.0 per cent,
- β-*sitosterol* : 73.0 per cent to 87.0 per cent,
- Δ*5-avenasterol*: minimum 5.0 per cent,
- Δ*7-stigmastenol*: maximum 3.0 per cent,
- Δ*7-avenasterol*: maximum 3.0 per cent,
- *brassicasterol*: maximum 0.3 per cent.

Water ***(2.5.32)***

Maximum 0.1 per cent, determined on 5.000 g, if intended for use in the manufacture of parenteral dosage forms.

STORAGE

In a well-filled container, protected from light.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Spray-dried Acacia

General Notices

(Ph Eur monograph 0308)

Ph Eur

DEFINITION

Spray-dried acacia is obtained from a solution of acacia.

CHARACTERS

It dissolves completely and rapidly, after about 20 min, in twice its mass of water. The liquid obtained is colourless or yellowish, dense, viscous, adhesive, translucent and weakly acid to blue litmus paper. Spray-dried acacia is practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Examined under a microscope, in *ethanol (96 per cent) R*, the powder is seen to consist predominantly of spheroidal particles about 4-40 µm in diameter, with a central cavity containing 1 or several air-bubbles; a few minute flat fragments are present. Only traces of starch granules are visible. No vegetable tissue is seen.

B. Examine the chromatograms obtained in the test for glucose and fructose.

Results The chromatogram obtained with the test solution shows 3 zones due to galactose, arabinose and rhamnose. No other important zones are visible, particularly in the upper part of the chromatogram.

C. Dissolve 1 g of the drug to be examined in 2 ml of *water R* by stirring frequently for 20 min. Add 2 ml of *ethanol (96 per cent) R*. After shaking a white gelatinous mucilage is formed which becomes fluid on adding 10 ml of *water R*.

TESTS

Solution S

Dissolve 3.0 g of the drug to be examined in 25 ml of *water R* by stirring for 10 min. Allow to stand for 20 min and dilute to 30 ml with *water R*.

Glucose and fructose

Thin-layer chromatography *(2.2.27)*.

Test solution To 0.100 g in a thick-walled centrifuge tube add 2 ml of a 100 g/l solution of *trifluoroacetic acid R*, shake vigorously to dissolve the forming gel, stopper the tube and heat the mixture at 120 °C for 1 h. Centrifuge the hydrolysate, transfer the clear supernatant carefully into a 50 ml flask, add 10 ml of *water R* and evaporate to dryness under reduced pressure. To the resulting clear film add 0.1 ml of *water R* and 0.9 ml of *methanol R*. Centrifuge to separate the amorphous precipitate. Dilute the supernatant, if necessary, to 1 ml with *methanol R*.

Reference solution Dissolve 10 mg of *arabinose R*, 10 mg of *galactose R*, 10 mg of *glucose R*, 10 mg of *rhamnose R* and 10 mg of *xylose R* in 1 ml of *water R* and dilute to 10 ml with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase 16 g/l solution of *sodium dihydrogen phosphate R, butanol R, acetone R* (10:40:50 *V/V/V*).

Application 10 µl as bands.

Development A Over a path of 10 cm.

Drying A In a current of warm air for a few minutes.

Development B Over a path of 15 cm using the same mobile phase.

Detection Spray with *anisaldehyde solution R* and heat at 110 °C for 10 min.

Results The chromatogram obtained with the reference solution shows 5 clearly separated coloured zones due to galactose (greyish-green or green), glucose (grey), arabinose (yellowish-green), xylose (greenish-grey or yellowish-grey) and rhamnose (yellowish-green), in order of increasing R_F value. The chromatogram obtained with the test solution shows no grey zone and no greyish-green zone between the zones corresponding to galactose and arabinose in the chromatogram obtained with the reference solution.

Starch, dextrin and agar

To 10 ml of solution S previously boiled and cooled add 0.1 ml of *0.05 M iodine*. No blue or reddish-brown colour develops.

Sterculia gum

A. Place 0.2 g in a 10 ml ground-glass-stoppered cylinder graduated in 0.1 ml. Add 10 ml of *ethanol (60 per cent V/V) R* and shake. Any gel formed occupies not more than 1.5 ml.

B. To 1.0 g add 100 ml of *water R* and shake. Add 0.1 ml of *methyl red solution R*. Not more than 5.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Tannins

To 10 ml of solution S add 0.1 ml of *ferric chloride solution R1*. A gelatinous precipitate is formed, but neither the precipitate nor the liquid shows a dark blue colour.

Tragacanth

Examine the chromatograms obtained in the test for Glucose and fructose.

Results The chromatogram obtained with the test solution shows no greenish-grey or yellowish-grey zone corresponding to the zone of xylose in the chromatogram obtained with the reference solution.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 4.0 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli (2.6.13)*.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for spray-dried acacia used as a viscosity-increasing agent and/or suspending agent in aqueous preparations.

Apparent viscosity

Determine the dynamic viscosity using a capillary viscometer *(2.2.9)* or a rotating viscometer *(2.2.10)* on a 100 g/l solution of spray-dried acacia (dried substance).

Ph Eur

Strong Ammonia Solution

General Notices

(Concentrated Ammonia Solution, Ph Eur monograph 0877)

Preparation

Dilute Ammonia Solution

Ph Eur

DEFINITION

Content

25.0 per cent m/m to 30.0 per cent *m/m*.

CHARACTERS

Appearance

Clear, colourless liquid, very caustic.

Solubility

Miscible with water and with ethanol (96 per cent).

IDENTIFICATION

A. Relative density *(2.2.5)*: 0.892 to 0.910.

B. It is strongly alkaline *(2.2.4)*.

C. To 0.5 ml add 5 ml of *water R*. Bubble air through the solution and lead the gaseous mixture obtained over the surface of a solution containing 1 ml of *0.1 M hydrochloric acid* and 0.05 ml of *methyl red solution R*. The colour changes from red to yellow. Add 1 ml of *sodium cobaltinitrite solution R*. A yellow precipitate is formed.

TESTS

Solution S

Evaporate 220 ml almost to dryness on a water-bath. Cool, add 1 ml of *dilute acetic acid R* and dilute to 20 ml with *distilled water R*.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

To 2 ml add 8 ml of *water R*.

Oxidisable substances

Cautiously add, whilst cooling, 8.8 ml to 100 ml of *dilute sulphuric acid R*. Add 0.75 ml of *0.002 M potassium permanganate*. Allow to stand for 5 min. The solution remains faintly pink.

Pyridine and related substances

Maximum 2 ppm, calculated as pyridine.

Measure the absorbance *(2.2.25)* at 252 nm using *water R* as the compensation liquid. The absorbance is not greater than 0.06.

Carbonates

Maximum 60 ppm.

To 10 ml in a test-tube with a ground-glass neck add 10 ml of *calcium hydroxide solution R*. Stopper immediately and mix. Any opalescence in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of a 0.1 g/l solution of *anhydrous sodium carbonate R*.

Chlorides *(2.4.4)*

Maximum 1 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 5 ppm.

Dilute 3 ml of solution S to 15 ml with *distilled water R*.

Iron *(2.4.9)*

Maximum 0.25 ppm.

Dilute 4 ml of solution S to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 1 ppm.

Dilute 4 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Residue on evaporation

Maximum 20 mg/l.

Evaporate 50 ml to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs a maximum of 1 mg.

ASSAY

Weigh accurately a flask with a ground-glass neck containing 50.0 ml of *1 M hydrochloric acid*. Add 2 ml of the substance to be examined and re-weigh. Add 0.1 ml of *methyl red solution R* as indicator. Titrate with *1 M sodium hydroxide* until the colour changes from red to yellow.

1 ml of *1 M hydrochloric acid* is equivalent to 17.03 mg of NH_3.

STORAGE

Protected from air, at a temperature not exceeding 20 °C.

Ph Eur

Substances for Pharmaceutical Use

General Notices

(Ph Eur monograph 2034)

Ph Eur

The statements in this monograph are intended to be read in conjunction with individual monographs on substances in the Pharmacopoeia. Application of the monograph to other substances may be decided by the competent authority.

DEFINITION

Substances for pharmaceutical use are any organic or inorganic substances that are used as active substances or excipients for the production of medicinal products for human or veterinary use. They may be obtained from natural sources or produced by extraction from raw materials, fermentation or synthesis.

This monograph does not apply to herbal drugs, herbal drug preparations or extracts, which are the subject of separate general monographs [*Herbal drugs (1433), Herbal drug preparations (1434), Extracts (0765)*].

Where medicinal products are manufactured using substances for pharmaceutical use of human or animal origin, the requirements of chapter *5.1.7. Viral safety* apply.

Substances for pharmaceutical use may be used as such or as starting materials for subsequent formulation to prepare medicinal products. Depending on the formulation, certain substances may be used either as active substances or as excipients. Solid substances may be compacted, coated, granulated, powdered to a certain fineness or processed in other ways. Processing with addition of excipients is permitted only where this is specifically stated in the Definition of the individual monograph.

Substance for pharmaceutical use of special grade Unless otherwise indicated or restricted in the individual monographs, a substance for pharmaceutical use is intended for human and veterinary use, and is of appropriate quality for the manufacture of all dosage forms in which it can be used.

Polymorphism Individual monographs do not usually specify crystalline or amorphous forms, unless bioavailability is affected. All forms of a substance for pharmaceutical use comply with the requirements of the monograph, unless otherwise indicated.

PRODUCTION

Substances for pharmaceutical use are manufactured by procedures that are designed to ensure a consistent quality and comply with the requirements of the individual monograph or approved specification.

The provisions of general chapter *5.10* apply to the control of impurities in substances for pharmaceutical use.

Whether or not it is specifically stated in the individual monograph that the substance for pharmaceutical use:

— is a recombinant protein or another substance obtained as a direct gene product based on genetic modification, where applicable, the substance also complies with the requirements of the general monograph on *Products of recombinant DNA technology (0784)*;

— is obtained from animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge, where applicable, the substance also complies with the requirements of the general monograph on *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)*;

— is a substance derived from a fermentation process, whether or not the micro-organisms involved are modified by traditional procedures or recombinant DNA (rDNA) technology, where applicable, the substance complies with the requirements of the general monograph on *Products of fermentation (1468)*.

If solvents are used during production, they are of suitable quality. In addition, their toxicity and their residual level are taken into consideration *(5.4)*. If water is used during production, it is of suitable quality.

If substances are produced or processed to yield a certain form or grade, that specific form or grade of the substance complies with the requirements of the monograph. Certain functionality-related tests may be described to control properties that may influence the suitability of the substance and subsequently the properties of dosage forms prepared from it.

Powdered substances may be processed to obtain a certain degree of fineness *(2.9.12)*.

Compacted substances are processed to increase the particle size or to obtain particles of a specific form and/or to obtain a substance with a higher bulk density.

Coated active substances consist of particles of the active substance coated with one or more suitable excipients.

Granulated active substances are particles of a specified size and/or form produced from the active substance by granulation directly or with one or more suitable excipients.

If substances are processed with excipients, these excipients comply with the requirements of the relevant monograph or, where no such monograph exists, the approved specification.

CHARACTERS

The statements under the heading Characters (e.g. statements about the solubility or a decomposition point) are not to be interpreted in a strict sense and are not requirements. They are given for information.

Where a substance may show polymorphism, this may be stated under Characters in order to draw this to the attention of the user who may have to take this characteristic into consideration during formulation of a preparation.

IDENTIFICATION

Where under Identification an individual monograph contains subdivisions entitled *First identification* and *Second identification*, the test or tests that constitute the *First identification* may be used in all circumstances. The test or tests that constitute the *Second identification* may be used for identification, provided it can be demonstrated that the substance is fully traceable to a batch certified to comply with all the other requirements of the monograph.

TESTS

Polymorphism *(5.9)*

If the nature of a crystalline or amorphous form imposes restrictions on its use in preparations, the nature of the specific crystalline or amorphous form is identified, its morphology is adequately controlled and its identity is stated on the label.

Related substances

Unless otherwise prescribed or justified and authorised, organic impurities in active substances are to be reported, identified wherever possible, and qualified as indicated in Table 2034.-1.

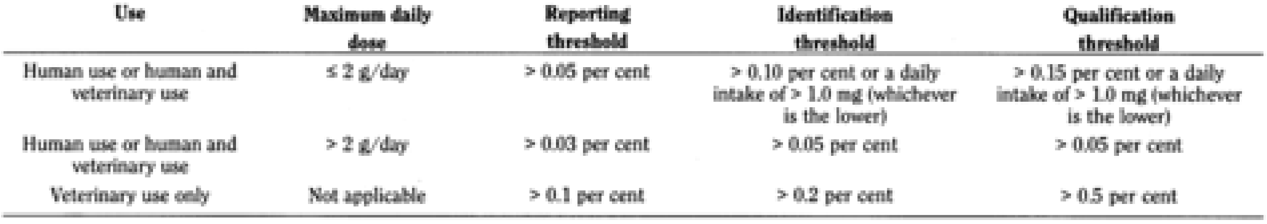


Table 2034.-1. – *Reporting, identification and qualification of organic impurities in active substances*

Use	Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Human use or human and veterinary use	≤ 2 g/day	> 0.05 per cent	> 0.10 per cent or a daily intake of > 1.0 mg (whichever is the lower)	> 0.15 per cent or a daily intake of > 1.0 mg (whichever is the lower)
Human use or human and veterinary use	> 2 g/day	> 0.03 per cent	> 0.05 per cent	> 0.05 per cent
Veterinary use only	Not applicable	> 0.1 per cent	> 0.2 per cent	> 0.5 per cent

Specific thresholds may be applied for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects.

If the individual monograph does not provide suitable control for a new impurity, a suitable test for control must be developed and included in the specification for the substance.

The requirements above do not apply to biological and biotechnological products, peptides, oligonucleotides, radiopharmaceuticals, products of fermentation and semi-synthetic products derived therefrom, to crude products of animal or plant origin or herbal products.

Residual solvents

Are limited according to the principles defined in chapter *5.4*, using general method *2.4.24* or another suitable method. Where a quantitative determination of a residual solvent is carried out and a test for loss on drying is not carried out, the content of residual solvent is taken into account for calculation of the assay content of the substance, the specific optical rotation and the specific absorbance.

Microbiological quality

Individual monographs give acceptance criteria for microbiological quality wherever such control is necessary. Table 5.1.4.-2. – *Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use in chapter* 5.1.4*. Microbiological quality of pharmaceutical preparations* gives recommendations on microbiological quality that are of general relevance for substances subject to microbial contamination. Depending on the nature of the substance and its intended use, different acceptance criteria may be justified.

Sterility *(2.6.1)*

If intended for use in the manufacture of sterile dosage forms without a further appropriate sterilisation procedure, or if offered as sterile grade, the substance for pharmaceutical use complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

If offered as bacterial endotoxin-free grade, the substance for pharmaceutical use complies with the test for bacterial endotoxins. The limit and test method (if not gelation method A) are stated in the individual monograph. The limit is calculated in accordance with *Test for bacterial endotoxins: guidelines (2.6.14)*, unless a lower limit is justified from results from production batches or is required by the competent authority. Where a test for bacterial endotoxins is prescribed, a test for pyrogens is not required.

Pyrogens *(2.6.8)*

If the test for pyrogens is justified rather than the test for bacterial endotoxins and if a pyrogen-free grade is offered, the substance for pharmaceutical use complies with the test for pyrogens. The limit and test method are stated in the individual monograph or approved by the competent authority. Based on appropriate test validation for bacterial endotoxins and pyrogens, the test for bacterial endotoxins may replace the test for pyrogens.

Additional properties

Control of additional properties (e.g. physical characteristics, functionality-related characteristics) may be necessary for individual manufacturing processes or formulations. Grades (such as sterile, endotoxin-free, pyrogen-free) may be produced with a view to manufacture of preparations for parenteral administration or other dosage forms and appropriate requirements may be specified in an individual monograph.

ASSAY

Unless justified and authorised, contents of substances for pharmaceutical use are determined. Suitable methods are used.

LABELLING

In general, labelling is subject to supranational and national regulation and to international agreements. The statements under the heading Labelling therefore are not comprehensive and, moreover, for the purposes of the Pharmacopoeia only those statements that are necessary to demonstrate compliance or non-compliance with the monograph are mandatory. Any other labelling statements are included as recommendations. When the term 'label' is used in the Pharmacopoeia, the labelling statements may appear on the container, the package, a leaflet accompanying the package or a certificate of analysis accompanying the article, as decided by the competent authority.

Where appropriate, the label states that the substance is:

— intended for a specific use;

— of a distinct crystalline form;

— of a specific degree of fineness;

— compacted;

— coated;

— granulated;

— sterile;

— free from bacterial endotoxins;

— free from pyrogens;

— containing gliding agents.

Where applicable, the label states:

— the degree of hydration,

— the name and concentration of any added substance (for example, an antimicrobial preservative or an antioxidant).

Where an active substance is processed with addition of an excipient or excipients, the label states the excipient(s) used and the content of active substance and excipient(s).

Ph Eur

Synthetic Air

General Notices

(Synthetic Medicinal Air, Ph Eur monograph 1684)

When Synthetic Air is intended for use in a room in which magnetic resonance imaging (MRI) is being performed, the cylinder and fittings should be made from suitable non-ferromagnetic materials and labelled accordingly.

Ph Eur

DEFINITION

Mixture of *Nitrogen (1247)* and *Oxygen (0417)*.

Content

95.0 per cent to 105.0 per cent of the nominal value which is between 21.0 per cent *V/V* to 22.5 per cent *V/V* of oxygen (O_2).

CHARACTERS

Colourless and odourless gas.

Solubility

At a temperature of 20 °C and a pressure of 101 kPa, 1 volume dissolves in about 50 volumes of water.

PRODUCTION

Water *(2.5.28)*

Maximum 67 ppm *V/V*.

Assay *(2.5.27)*

Carry out the determination of oxygen in gases.

IDENTIFICATION

First identification *C.*

Second identification *A, B.*

A. In a conical flask containing the substance to be examined, place a glowing splinter of wood. The splinter remains glowing.

B. Use a gas burette (Figure 1684.-1) of 25 ml capacity in the form of a chamber, in the middle of which is a tube graduated in 0.2 per cent between 19.0 per cent and 23.0 per cent, and isolated at each end by a tap with a conical barrel. The lower tap is joined to a tube with an olive-shaped nozzle and is used to introduce the gas into the apparatus. A cylindrical funnel above the upper tap is used to introduce the absorbent solution. Wash the burette with *water R* and dry. Open both taps. Connect the nozzle to the source of the substance to be examined and set the flow rate to 1 litre/min. Flush the burette by passing the substance to be examined through it for 1 min. Close the lower tap of the burette and immediately afterwards the upper tap. Rapidly disconnect the burette from the source of the substance to be examined. Rapidly give a half turn of the upper tap to eliminate any excess pressure in the burette. Keeping the burette vertical, fill the funnel with a freshly prepared mixture of 21 ml of a 560 g/l solution of *potassium hydroxide R* and 130 ml of a 200 g/l solution of *sodium dithionite R*. Open the upper tap slowly. The solution absorbs the oxygen and enters the burette. Allow to stand for 10 min without shaking. Read the level of the liquid meniscus on the graduated part of the burette. This figure represents the percentage *V/V* of oxygen. The value read is 95.0 per cent to 105.0 per cent of the nominal value.

C. It complies with the limits of the assay.

TESTS

Water vapour

Maximum 67 ppm *V/V*, determined using a water vapour detector tube *(2.1.6)*.

STORAGE

As a compressed gas in suitable containers complying with the legal regulations or as a compressed gas supplied by a pipe network, after mixing of the components.

LABELLING

The label states the nominal content of O_2 in per cent *V/V*.

IMPURITIES

A. water.

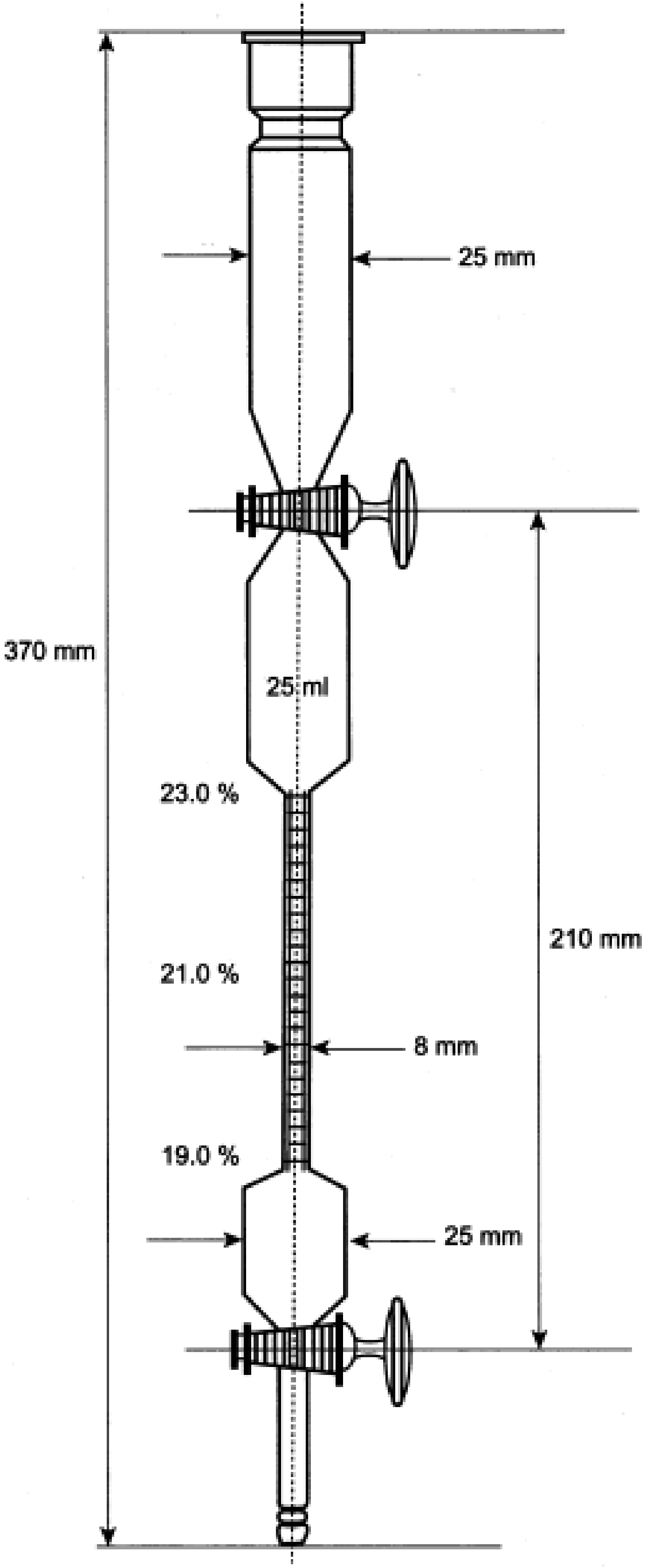

25 mm
370 mm
25 ml
23.0 %
210 mm
21.0 %
8 mm
19.0 %
25 mm

Figure 1684.-1.– *Gas burette*

Ph Eur

Virgin Almond Oil

General Notices

Almond Oil
(Ph Eur monograph 0261)

Preparation

Almond Oil Ear Drops

Ph Eur

DEFINITION

Fatty oil obtained by cold expression from the ripe seeds of *Prunus dulcis* (Miller) D.A. Webb var. *dulcis* or *Prunus dulcis* (Miller) D.A. Webb var. *amara* (D.C.) Buchheim or a mixture of both varieties.

CHARACTERS

Appearance

Yellow, clear liquid.

Solubility

Slightly soluble in ethanol (96 per cent), miscible with light petroleum.

Relative density

About 0.916.

It solidifies at about - 18 °C.

IDENTIFICATION

First identification A, C.

Second identification A, B.

A. It complies with the test for absorbance (see Tests).

B. Carry out the identification of fatty oils by thin-layer chromatography *(2.3.2)*. The chromatogram obtained is comparable with the typical chromatogram for almond oil.

C. It complies with the test for composition of fatty acids (see Tests).

TESTS

Absorbance *(2.2.25)*

Maximum 0.2 by measuring at the absorption maximum between 264 nm and 276 nm. The ratio of the absorbance measured at 232 nm to that measured at 270 nm is greater than 7.

Dissolve 0.100 g in *cyclohexane R* and dilute to 10.0 ml with the same solvent.

Acid value *(2.5.1)*

Maximum 2.0, determined on 5.0 g.

Peroxide value *(2.5.5)*

Maximum 15.0.

Unsaponifiable matter *(2.5.7)*

Maximum 0.9 per cent, determined on 5.0 g.

Composition of fatty acids

Gas chromatography *(2.4.22, Method A)*. Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

— *saturated fatty acids of chain length less than* C_{16}: maximum 0.1 per cent,

— *palmitic acid* : 4.0 per cent to 9.0 per cent,

— *palmitoleic acid* (equivalent chain length on polyethyleneglycol adipate 16.3): maximum 0.8 per cent,

— *margaric acid*: maximum 0.2 per cent,

— *stearic acid* : maximum 3.0 per cent,

— *oleic acid* (equivalent chain length on polyethyleneglycol adipate 18.3): 62.0 per cent to 86.0 per cent,

— *linoleic acid* (equivalent chain length on polyethyleneglycol adipate 18.9): 20.0 per cent to 30.0 per cent,

— *linolenic acid* (equivalent chain length on polyethyleneglycol adipate 19.7): maximum 0.4 per cent,

— *arachidic acid*: maximum 0.2 per cent,

— *eicosenoic acid* (equivalent chain length on polyethyleneglycol adipate 20.3): maximum 0.3 per cent,

— *behenic acid*: maximum 0.2 per cent,

— *erucic acid* (equivalent chain length on polyethyleneglycol adipate 22.3): maximum 0.1 per cent.

Sterols *(2.4.23)*.

Composition of sterol fraction of the oil:

— *cholesterol* : maximum 0.7 per cent,

— *campesterol*: maximum 4.0 per cent,

— *stigmasterol* : maximum 3.0 per cent,

— β-*sitosterol* : 73.0 per cent to 87.0 per cent,

— Δ*5-avenasterol*: minimum 10.0 per cent,

— Δ*7-stigmastenol*: maximum 3.0 per cent,

— Δ*7-avenasterol*: maximum 3.0 per cent,

— *brassicasterol*: maximum 0.3 per cent.

STORAGE

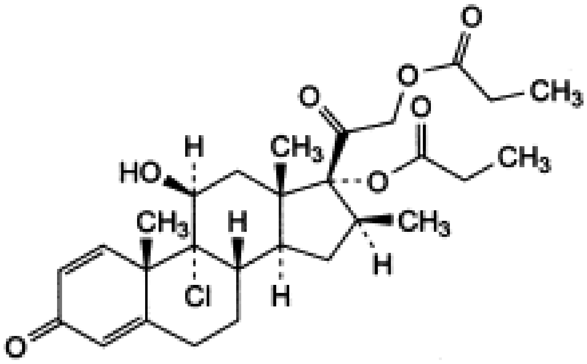
In a well-filled container, protected from light.

Ph Eur

Anhydrous Beclometasone Dipropionate

General Notices

(Ph Eur monograph 0654)



$C_{28}H_{37}ClO_7$ 521.1 *5534-09-8*

Action and use

Glucocorticoid.

Preparations

Beclometasone Cream

Beclometasone Nasal Spray

Beclometasone Ointment

Beclometasone Powder for Inhalation

Beclometasone Pressurised Inhalation

Ph Eur

DEFINITION

9-Chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate.

Content

96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *anhydrous beclometasone dipropionate CRS*.

B. Treat 25 mg by the oxygen-flask method *(2.5.10)*. Use a mixture of 1 ml of *1 M sodium hydroxide* and 20 ml of *water R* to absorb the combustion products. The solution gives reaction (a) of chlorides *(2.3.1)*.

C. Loss on drying (see Tests).

TESTS

Specific optical rotation *(2.2.7)*:

+ 108 to + 115 (dried substance).

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29).*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 50.0 ml with *acetonitrile R.*

Reference solution (a) Dilute 5.0 ml of test solution (b) to 100.0 ml with *acetonitrile R*.

Reference solution (b) Dissolve 5 mg of *beclometasone dipropionate for system suitability CRS* (containing impurity D) in *acetonitrile R* and dilute to 5 ml with the same solvent.

Reference solution (c) Dissolve 5 mg of *beclometasone dipropionate for peak identification CRS* (containing impurities A, B, C, L and M) in *acetonitrile R* and dilute to 5 ml with the same solvent.

Reference solution (d) Dissolve 50.0 mg of *anhydrous beclometasone dipropionate CRS* in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml to 50.0 ml with *acetonitrile R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R, water R* (45:55 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 238 nm.

Injection 20 µl of test solution (a) and reference solutions (a), (b) and (c).

Run time 2.5 times the retention time of beclometasone dipropionate.

Identification of impurities Use the chromatogram supplied with *beclometasone dipropionate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, L and M; use the chromatogram supplied with *beclometasone dipropionate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity D; if necessary, use the responses of impurities D and M detected at 284 nm to distinguish the 2 impurities: the response of impurity D decreases and that of impurity M increases.

Relative retention With reference to beclometasone dipropionate (retention time = about 29 min): impurity A = about 0.3; impurity B = about 0.6; impurity D = about 1.1; impurity M = about 1.1; impurity L = about 1.3; impurity C = about 1.6.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to beclometasone dipropionate.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 1.3;

— *impurities B, L*: for each impurity, not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent);

— *impurity M*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurities A, D*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity C:* not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— t*otal*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase *water R, acetonitrile R* (40:60 *V/V*).

Flow rate 1.0 ml/min.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{28}H_{37}ClO_7$.

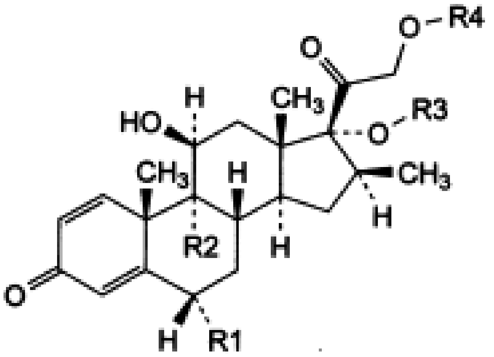
STORAGE

Protected from light.

IMPURITIES

Specified impurities: A, B, C, D, L, M.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F, G, H, I, J, K, N, O, P, Q, R.*



A. R1 = R3 = H, R2 = Cl, R4 = CO-C_2H_5: 9-chloro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl propanoate (beclometasone 21-propionate),

B. R1 = H, R2 = Cl, R3 = CO-C_2H_5, R4 = CO-CH_3: 21-(acetyloxy)-9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate (beclometasone 21-acetate 17-propionate),

C. R1 = H, R2 = Cl, R3 = CO-C_2H_5, R4 = CO-CH_2-CH_2-CH_3: 9-chloro-11β-hydroxy-16β-methyl-3,20-dioxo-17-(propanoyloxy)-pregna-1,4-dien-21-yl butanoate (beclometasone 21-butyrate 17-propionate),

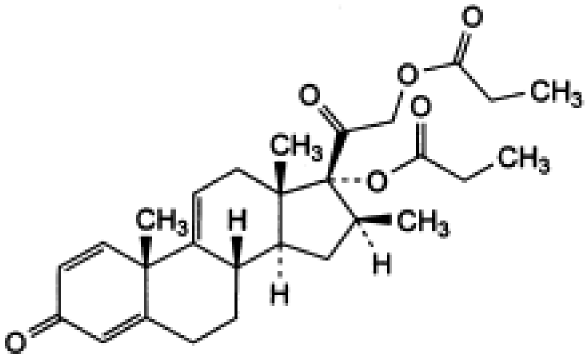
D. R1 = H, R2 = Br, R3 = R4 = CO-C_2H_5: 9-bromo-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

E. R1 = R2 = Cl, R3 = R4 = CO-C_2H_5: 6α,9-dichloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

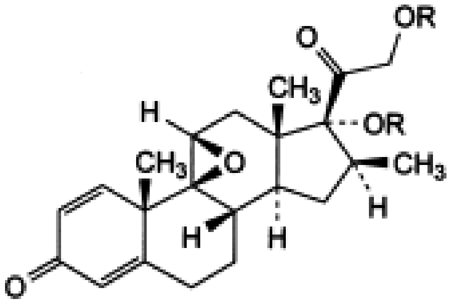
F. R1 = Br, R2 = Cl, R3 = R4 = CO-C_2H_5: 6α-bromo-9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

G. R1 = R3 = R4 = H, R2 = Cl: 9-chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3, 20-dione (beclometasone),

H. R1 = R4 = H, R2 = Cl, R3 = CO-C_2H_5: 9-chloro-11β,21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate (beclometasone 17-propionate),

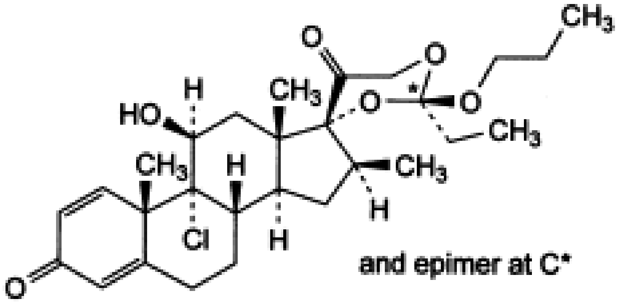

O
O
CH3
O
O
CH3
CH3
O
CH3
CH3
H
CH3
H
H
O

I. 16β-methyl-3,20-dioxopregna-1,4,9(11)-triene-17,21-diyl dipropanoate,


OR
O
CH3
H
OR
CH3
O
CH3
H
H
H
O

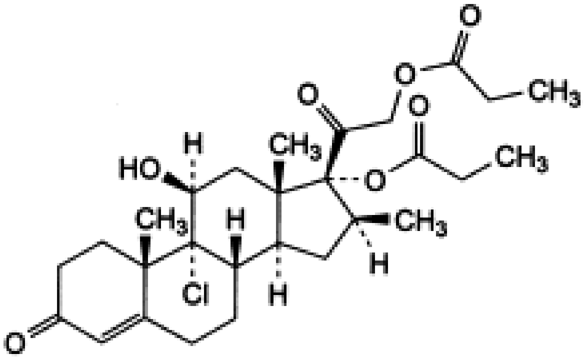
J. R = CO-C_2H_5: 9,11β-epoxy-16β-methyl-3,20-dioxo-9β-pregna-1,4-diene-17,21-diyl dipropanoate,

R. R = H: 9,11β-epoxy-17,21-dihydroxy-16β-methyl-9β-pregna-1,4-diene-3,20-dione,

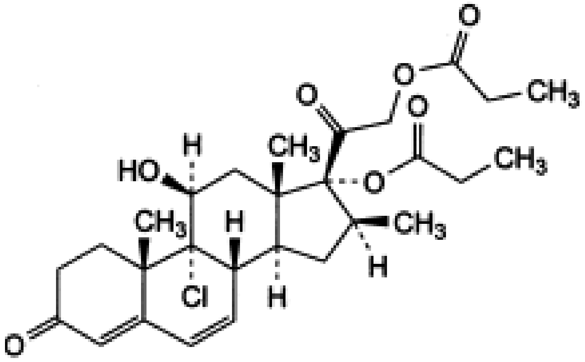

CH3
O
O
*
O
HO
H
CH3
O
CH3
CH3
H
CH3
Cl
H
H
O

and epimer at C*

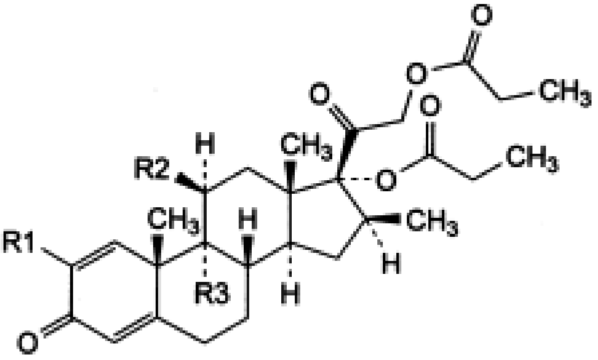
K. (2′*RS*,4′*R*)-9-chloro-2′-ethyl-11β-hydroxy-16β-methyl-2′-propoxyspiro[androsta-1,4-diene-17,4′-[1,3]dioxan]-3,5′-dione (beclometasone propyl 17,21-orthopropionate),



L. 9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregn-4-ene-17,21-diyl dipropanoate,



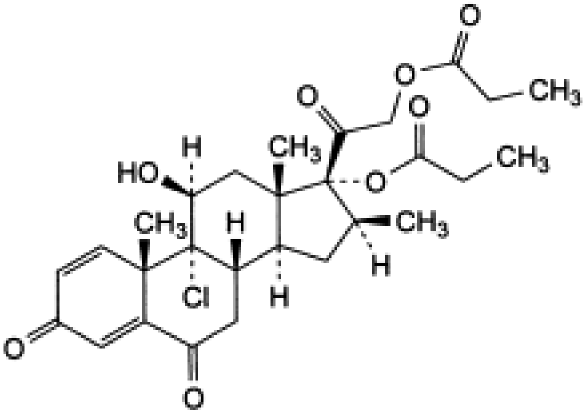
M. 9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-4,6-diene-17,21-diyl dipropanoate,



N. R1 = Br, R2 = OH, R3 = Cl: 2-bromo-9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

O. R1 = H, R2 = R3 = Cl: 9,11β-dichloro-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

Q. R1 = R2 = R3 = H: 16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,



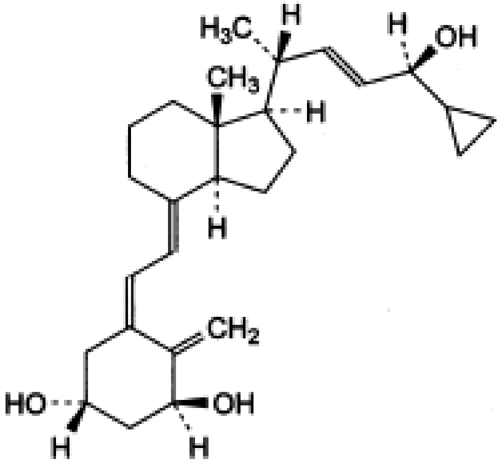
P. 9-chloro-11β-hydroxy-16β-methyl-3,6,20-trioxopregna-1,4-diene-17,21-diyl dipropanoate.

Ph Eur

Anhydrous Calcipotriol

General Notices

(Ph Eur monograph 2011)



$C_{27}H_{40}O_3$ 412.6 *112828-00-9*

Action and use

Vitamin D analogue.

Ph Eur

DEFINITION

(*5Z,7E,22E,24S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.

Content

95.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It is sensitive to heat and light.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of anhydrous calcipotriol.*

B. Loss on drying (see Tests).

Carry out the tests for related substances and the assay as rapidly as possible and protected from actinic light and air.

TESTS

Related substances

A. Thin-layer chromatography *(2.2.27)* .

Solution A To 1 ml of *triethylamine R* add 9 ml of *chloroform R* .

Test solution Dissolve 1 mg of the substance to be examined in 100 µl of solution A.

Reference solution (a) To 10 µl of the test solution add 990 µl of solution A.

Reference solution (b) To 250 µl of reference solution (a) add 750 µl of solution A.

Reference solution (c) To 100 µl of reference solution (a) add 900 µl of solution A.

Reference solution (d) Place 2 mg of the substance to be examined in a vial and dissolve in 200 µl of solution A. Close the vial and keep it in a water bath at 60 °C for 2 h.

Plate *TLC silica gel F_{254} plate R .*

Mobile phase *2-methylpropanol R, methylene chloride R* (20:80 V/V).

Application 10 µl of the test solution and reference solutions (b), (c) and (d).

Development Over 2/3 of the plate.

Drying In air, then at 140 °C for 10 min.

Detection Spray the hot plate with an *alcoholic solution of sulphuric acid R* , dry at 140 °C for not more than 1 min and examine in ultraviolet light at 366 nm.

Relative retention With reference to calcipotriol (R_F = about 0.4): impurity G = about 0.4; impurity H = about 0.4; pre-calcipotriol = about 0.9; impurity A = about 1.2.

System suitability Reference solution (d):

— the chromatogram shows a secondary spot due to pre-calcipotriol.

Limits:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent),

— *impurities G, H*: any spot due to impurity G or H is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent for the sum),

— *any other impurity*: any other spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

B. Liquid chromatography *(2.2.29)* .

Solution A. Dissolve 1.32 g of *ammonium phosphate R* in *water R* and dilute to 10.0 ml with the same solvent.

Solvent mixture solution A, *water R, methanol R* (3:297:700 *V/V/V*).

Test solution (a) Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Test solution (b) Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the same solvent mixture.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 1.0 mg of *calcipotriol monohydrate CRS* (containing impurities B, C and D) in the solvent mixture and dilute to 2.5 ml with the solvent mixture.

Reference solution (d) Dissolve 2.00 mg of *calcipotriol monohydrate CRS* in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.10 m, Ø = 4.0 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase *water R, methanol R* (30:70 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 264 nm.

Injection 20 µl of test solution (a) and reference solutions (a), (b) and (c).

Run time Twice the retention time of calcipotriol.

Relative retention With reference to calcipotriol (retention time = about 13.5 min): impurity B = about 0.86; impurity C = about 0.92; impurity D = about 1.3.

System suitability Reference solution (c):

— *peak-to-valley ratio* : minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to calcipotriol,

— the chromatogram obtained is similar to the chromatogram supplied with *calcipotriol monohydrate CRS*.

Limits:

— *impurity B*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *impurities C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying

Maximum 1.0 per cent, determined on 5 mg by thermogravimetry *(2.2.34)* . Heat to 105 °C at a rate of 10 °C/min and maintain at 105 °C for 60 min.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{27}H_{40}O_3$ from the areas of the peaks and the declared content of *calcipotriol monohydrate CRS*.

STORAGE

In an airtight container , protected from light at - 20 °C or below.

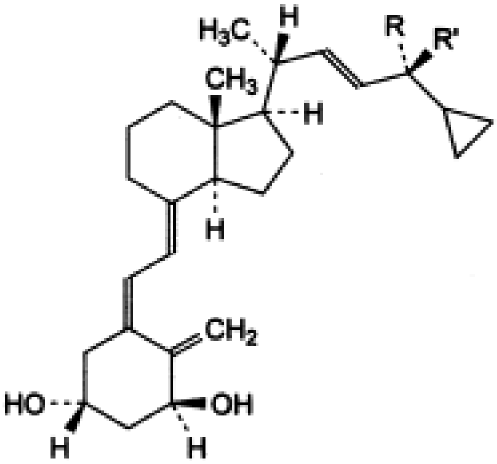
IMPURITIES

Specified impurities A, B, C, D, G, H.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F, I.*

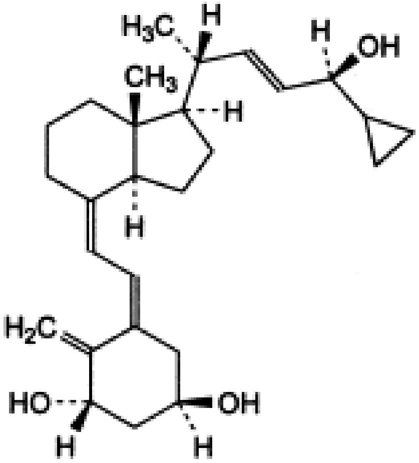
By thin-layer chromatography A, G, H, I.

By liquid chromatography B, C, D, E, F.

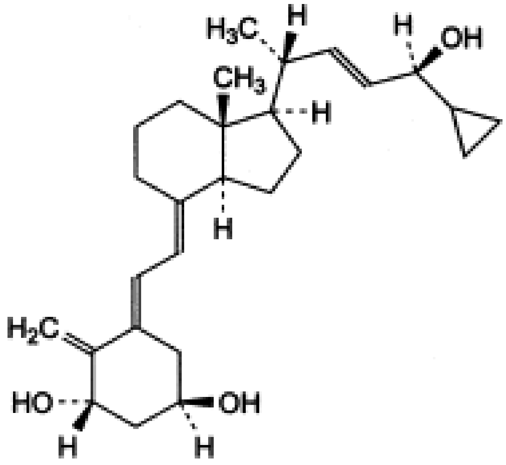

H₃C
H
R
R'
CH₃
H
H
CH₂
HO
OH
H
H

A. R + R′ = O: (*5Z,7E,22E*)-24-cyclopropyl-1α,3β-dihydroxy-9,10-secochola-5,7,10(19),22-tetraen-24-one,

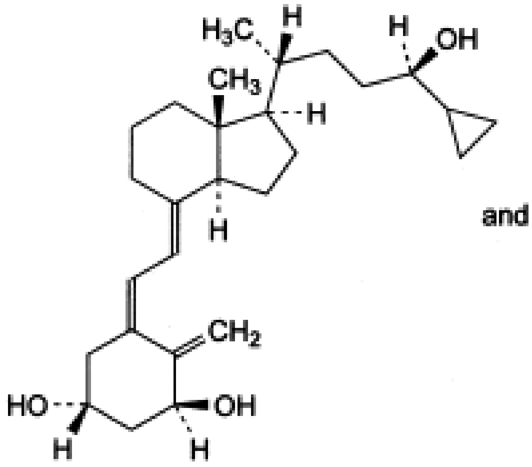
D. R = OH, R′ = H: (5*Z*,7*E*,22*E*,24*R*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol (24-*epi*-calcipotriol),


H₃C
H
H
OH
CH₃
H
H
H₂C
HO
OH
H
H

B. (5*Z*,7*Z*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol ((7*Z*)-calcipotriol),

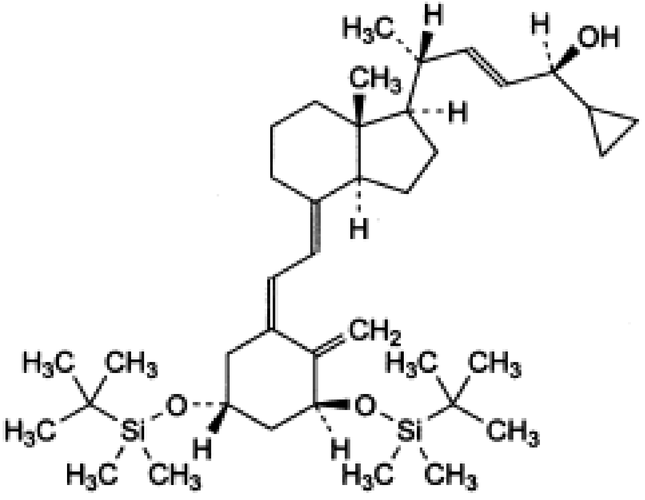


C. (5*E*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol ((5*E*)-calcipotriol),

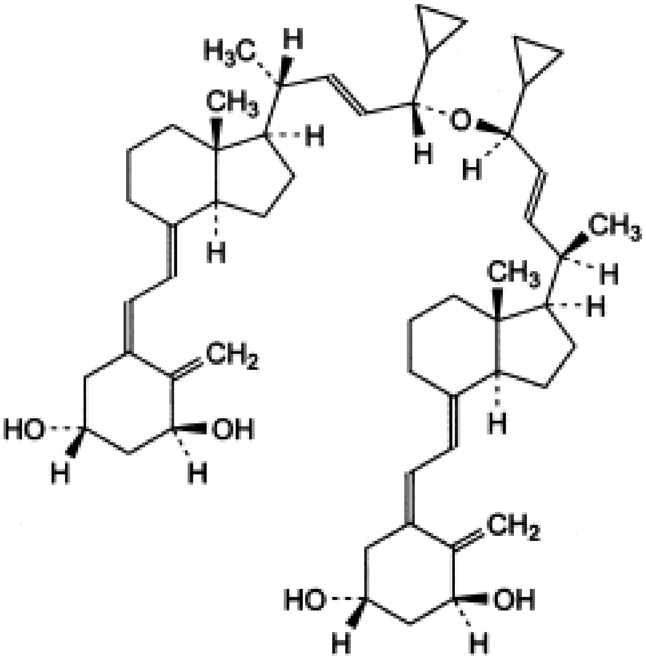


and enantiomer

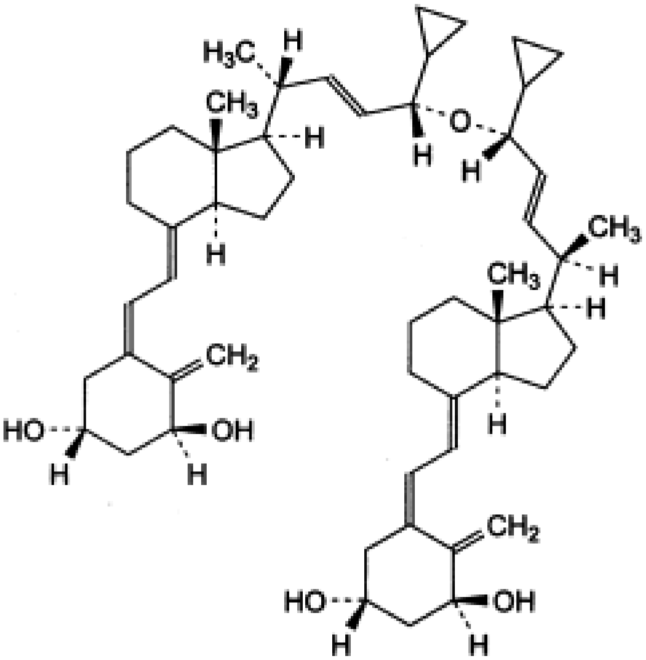
E. *rac*-(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19)-triene-1α,3β,24-triol,



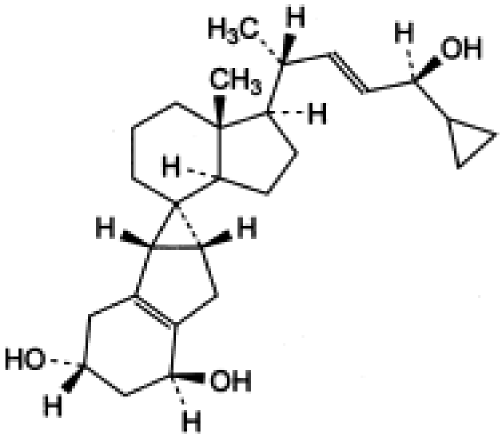
F. (5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-9,10-secochola-5,7,10(19),22-tetraen-24-ol,


H₃C
H
CH₃
H
H
O
H
H
CH₂
HO
OH
H
H
CH₃
H
CH₃
H
H
CH₂
HO
OH
H
H

G. 24,24′-oxybis[(5*Z*,7*E*,22*E*,24*S)*-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α, 3β-diol],


H₃C
H
CH₃
H
H
O
H
H
CH₂
HO
OH
H
H
CH₃
H
CH₃
H
H
CH₂
HO
OH
H
H

H. (5*Z*,7*E*,22*E*,24*R*)-24-cyclopropyl-24-[[(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-dihydroxy-9, 10-secochola-5,7,10(19),22-tetraen-24-yl]oxy]-9,10-secochola-5,7,10(19),22-tetraene-1α, 3β-diol,



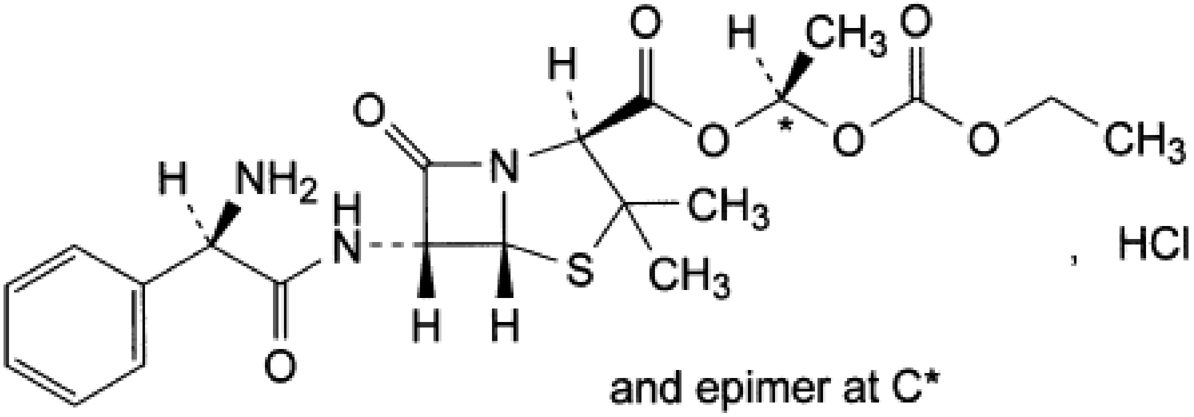
I. (6*S*,7*R*,8*R*,22*E*,24*S*)-24-cyclopropyl-6,8:7,19-dicyclo-9,10-secochola-5(10),22-diene-1α, 3β,24-triol (suprasterol of calcipotriol).

Ph Eur

Bacampicillin Hydrochloride

General Notices

(Ph Eur monograph 0808)



and epimer at C*

$C_{21}H_{27}N_3O_7S$, HCl 502.0 *37661-08-8*

Action and use

Penicillin antibacterial.

Ph Eur

DEFINITION

(1*RS*)-1-[(Ethoxycarbonyl)oxy]ethyl (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate hydrochloride.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder or granules, hygroscopic.

Solubility

Soluble in water, freely soluble in ethanol (96 per cent), soluble in methylene chloride.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *bacampicillin hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 2 ml of *methanol R.*

Reference solution (a) Dissolve 10 mg of *bacampicillin hydrochloride CRS* in 2 ml of *methanol R.*

Reference solution (b) Dissolve 10 mg of *bacampicillin hydrochloride CRS,* 10 mg of *talampicillin hydrochloride CRS* and 10 mg of *pivampicillin CRS* in 2 ml of *methanol R.*

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 10 volumes of a 272 g/l solution of *sodium acetate R* adjusted to pH 5.0 with *glacial acetic acid R*, 40 volumes of *water R* and 50 volumes of *ethanol (96 per cent) R.*

Application 1 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with *ninhydrin solution R1* and heat at 60 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube on a water-bath for 1 min; a dark yellow colour develops.

D. Dissolve about 25 mg in 2 ml of *water R*. Add 2 ml of *dilute sodium hydroxide solution R* and shake. Wait a few minutes and add 3 ml of *dilute nitric acid R* and 0.5 ml of *silver nitrate solution R1*. A white precipitate is formed. Add 0.5 ml of *concentrated ammonia R*. The precipitate dissolves.

TESTS

Appearance of solution

Dissolve 0.200 g in 20 ml of *water R*; the solution is not more opalescent than reference suspension II (*2.2.1*). Dissolve 0.500 g in 10 ml of *water R*; the absorbance (*2.2.25*) of the solution at 430 nm is not greater than 0.10.

pH (*2.2.3*)

3.0 to 4.5.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 175 to + 195 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the test solution and reference solutions (a), (b) and (d) immediately before use.*

Phosphate buffer A Dissolve 1.4 g of *sodium dihydrogen phosphate monohydrate R* in *water R* and dilute to about 800 ml with the same solvent. Adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000.0 ml with *water R*.

Phosphate buffer B Dissolve 2.75 g of *sodium dihydrogen phosphate monohydrate R* and 2.3 g of *disodium hydrogen phosphate dihydrate R* in *water R* and dilute to about 1800 ml with the same solvent. Adjust to pH 6.8, if necessary, using *dilute phosphoric acid R* or *dilute sodium hydroxide solution R* and dilute to 2000.0 ml with *water R*.

Test solution Dissolve 30.0 mg of the substance to be examined in phosphate buffer A and dilute to 100.0 ml with phosphate buffer A.

Reference solution (a) Dissolve 30.0 mg of *bacampicillin hydrochloride CRS* in phosphate buffer A and dilute to 100.0 ml with phosphate buffer A.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with phosphate buffer A.

Reference solution (c) Dissolve 30 mg of the substance to be examined in phosphate buffer B and dilute to 100 ml with phosphate buffer B. Heat at 80 °C for about 30 min.

Reference solution (d) Dissolve 20 mg of *ampicillin trihydrate CRS* (impurity I) in phosphate buffer A and dilute to 250 ml with phosphate buffer A. Dilute 5 ml of this solution to 100 ml with phosphate buffer A.

Column:

- *size*: *l* = 0.05 m, Ø = 3.9 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *acetonitrile R1* and 70 volumes of a 0.06 per cent *m/m* solution of *tetrahexylammonium hydrogen sulphate R* in phosphate buffer B.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of the test solution and reference solutions (b), (c) and (d).

Run time 3.5 times the retention time of bacampicillin.

System suitability:

- the peak due to impurity I is separated from the peaks due to the solvent in the chromatogram obtained with reference solution (d);
- *relative retention* with reference to bacampicillin: degradation product eluting just after bacampicillin = 1.12 to 1.38 in the chromatogram obtained with reference solution (c); if

necessary, adjust the concentration of tetrahexylammonium hydrogen sulphate in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Butyl acetate and ethyl acetate (*2.4.24, System A*)

Maximum 2.0 per cent of butyl acetate, maximum 4.0 per cent of ethyl acetate and maximum 5.0 per cent for the sum of the contents.

Sample solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Use the method of standard additions.

Static head-space conditions that may be used:

— *equilibration temperature*: 60 °C;

— *equilibration time*: 20 min.

N,N-Dimethylaniline (*2.4.26, Method A*)

Maximum 20 ppm.

Water (*2.5.12*)

Maximum 0.8 per cent, determined on 0.300 g.

Sulphated ash (*2.4.14*)

Maximum 1.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

System suitability Reference solution (a):

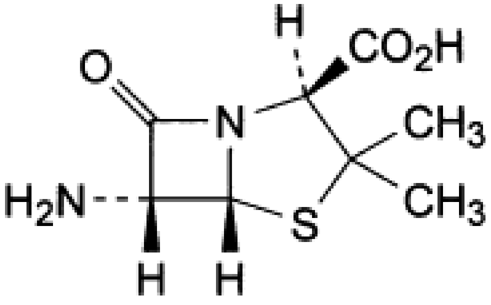
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{21}H_{28}ClN_3O_7S$ from the declared content of *bacampicillin hydrochloride CRS.*

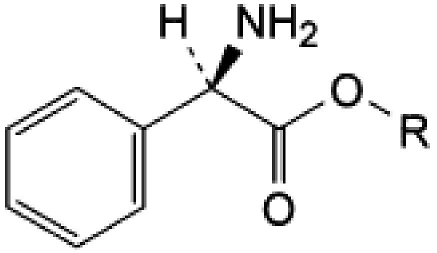
STORAGE

In an airtight container .

IMPURITIES

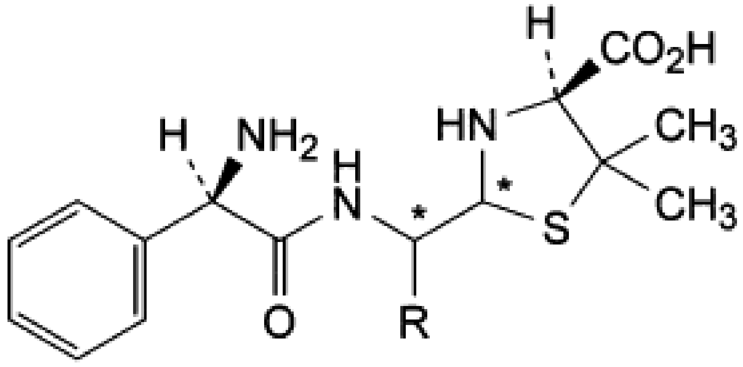


A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



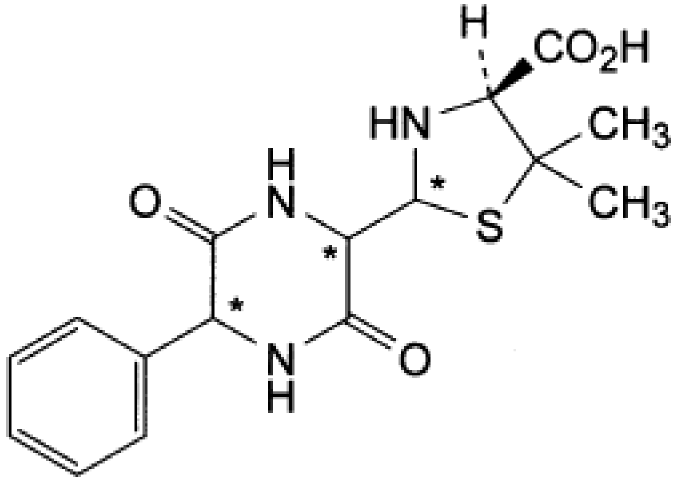
B. R = H: (2*R*)-2-amino-2-phenylacetic acid (D-phenylglycine),

G. R = CH3: methyl (2*R*)-2-amino-2-phenylacetate (methyl D-phenylglycinate),

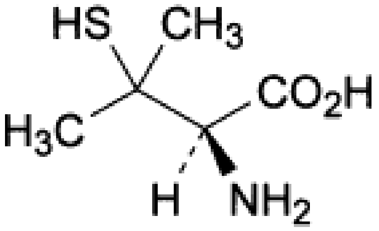


C. R = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of ampicillin),

D. R = CO_2H: (4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of ampicillin),

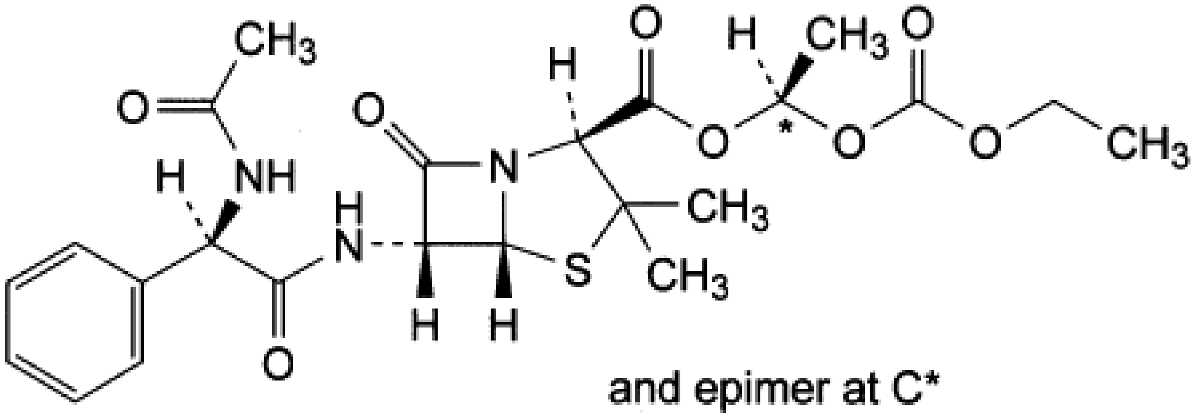


E. (4*S*)-2-(3,6-dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid (diketopiperazines of ampicillin),



and enantiomer

F. (2*RS*)-2-amino-3-methyl-3-sulphanylbutanoic acid (DL-penicillamine),



and epimer at C*

H. (1*RS*)-1-[(ethoxycarbonyl)oxy]ethyl (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-(acetylamino)-2-phenylacetyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (*N*-acetylbacampicillin),

I. ampicillin.

Ph Eur

Bacitracin Zinc

General Notices

(Ph Eur monograph 0466)

1405-49-6

Action and use

Polypeptide antibacterial.

Preparation

Polymyxin and Bacitracin Eye Ointment

Ph Eur

DEFINITION

Zinc complex of bacitracin, which consists of a mixture of antimicrobial polypeptides produced by certain strains of *Bacillus licheniformis* or *Bacillus subtilis*, the main components being bacitracins A, B1, B2 and B3.

Content

Minimum 60 IU/mg (dried substance).

CHARACTERS

Appearance

White or light yellowish-grey powder, hygroscopic.

Solubility

Slightly soluble in water and in alcohol.

IDENTIFICATION

First identification *B*, *C*.

Second identification *A*, *C*.

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in 0.5 ml of *dilute hydrochloride acid R* and dilute to 1.0 ml with *water R.*

Reference solution Dissolve 10 mg of *bacitracin zinc CRS* in 0.5 ml of *dilute hydrochloric acid R* and dilute to 1.0 ml with *water R.*

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R*

(1:2:4 *V/V/V*).

Application 10 µl.

Development Over half of the plate.

Drying At 100-105 °C.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The spots in the chromatogram obtained with the test solution are similar in position, size and colour to the spots in the chromatogram obtained with the reference solution.

B. It complies with the test for composition (see Tests).

C. Ignite about 0.15 g, allow to cool and dissolve the residue in 1 ml of *dilute hydrochloric acid R.* Add 4 ml of *water R*. The solution gives the reaction of zinc *(2.3.1)*.

TESTS

pH *(2.2.3)*

6.0 to 7.5.

Shake 1.0 g for about 1 min with 10 ml of *carbon dioxide-free water R* and filter.

Composition

Liquid chromatography *(2.2.29)*: use the normalisation procedure*. Prepare the solutions immediately before use.*

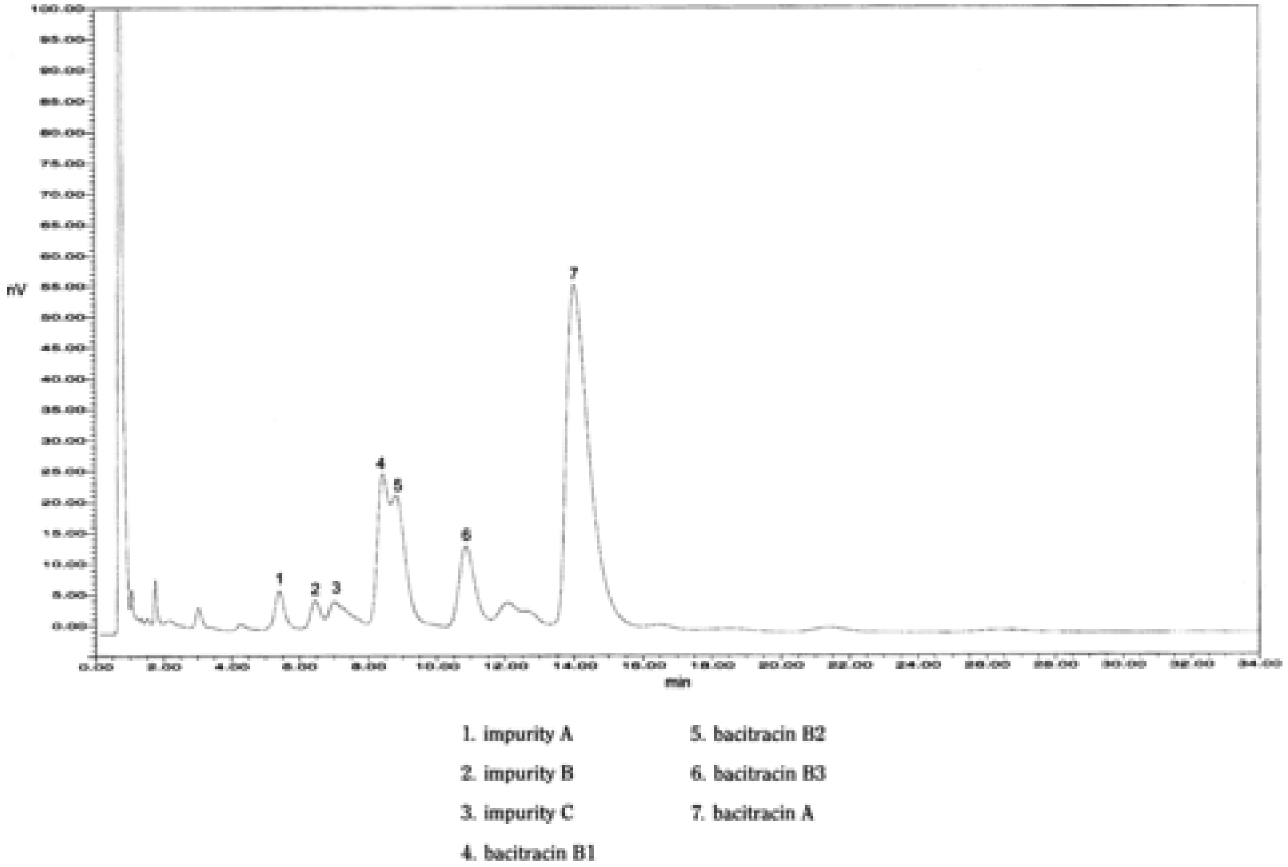


Figure 0466.-1. – *Chromatogram of the test for composition in bacitracin zinc obtained with the test solution at 254 nm*

Test solution Dissolve 0.100 g of the substance to be examined in 50.0 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*.

Reference solution (a) Dissolve 20.0 mg of *bacitracin zinc CRS* in 10.0 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *water R.*

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with *water R*.

Reference solution (d) Dissolve 50.0 mg of the substance to be examined in 25.0 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*. Heat in a boiling water-bath for 30 min. Cool to room temperature.

Blank solution A 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with dilute *sodium hydroxide R.*

Column:

— size: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Add 520 volumes of *methanol R1*, 40 volumes of *acetonitrile R* and 300 volumes of *water R* to 100 volumes of a 34.8 g/l solution of *dipotassium hydrogen phosphate R*, adjusted to pH 6.0 with a 27.2 g/l solution of *potassium dihydrogen phosphate R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 100 µl; inject the blank, the test solution and reference solutions (a) and (c).

Run time 3 times the retention time of bacitracin A.

Relative retention With reference to bacitracin A (retention time = 15 min to 25 min): bacitracin B1 = about 0.6; bacitracin B3 = about 0.8; impurity E = about 2.5.

If necessary, adjust the composition of the mobile phase by changing the amount of organic modifier whilst keeping the ratio constant between methanol and acetonitrile.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum of 1.2, where H_p = height above the baseline of the peak due to bacitracin B1 and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B2.

Limits:

— *bacitracin A:* minimum 40.0 per cent,

— *sum of bacitracins A, B1, B2 and B3*: minimum 70.0 per cent.

— *disregard limit*: the area of the peak due to bacitracin A in the chromatogram obtained with reference solution (c) (0.5 per cent); disregard any peak observed in the blank run.

Related peptides

Liquid chromatography *(2.2.29)* as described in the test for composition.

See Figure 0466.-1.

Limit:

— *sum of the areas of all peaks eluting before the peak due to bacitracin B1*: maximum 20.0 per cent.

Impurity E

Liquid chromatography *(2.2.29)* as described in the test for composition.

See Figure 0466.-2.

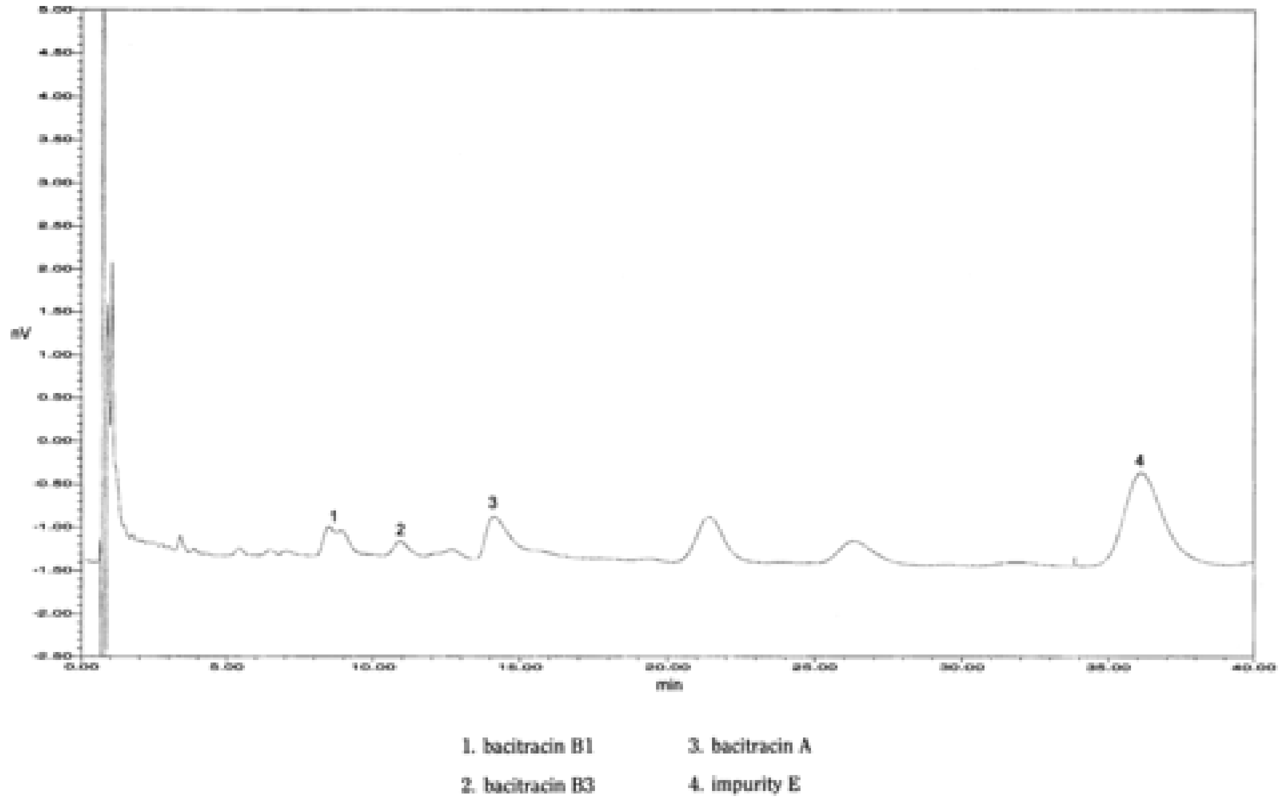


Figure 0466.-2. – *Chromatogram of the test for impurity E in bacitracin zinc obtained with reference solution (d) at 300 nm*

Detection Spectrophotometer at 254 nm; spectrophotometer at 300 nm for reference solution (d).

Injection Test solution and reference solutions (b) and (d).

Limit:

— *impurity E*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent).

Zinc

4.0 per cent to 6.0 per cent (dried substance).

Dissolve 0.200 g in a mixture of 2.5 ml of *dilute acetic acid R* and 2.5 ml of water. Add 50 ml of *water R,* 50 mg of *xylenol orange triturate R* and sufficient *hexamethylenetetramine R* to produce a red colour. Add 2 g of *hexamethylenetetramine R* in excess. Titrate with *0.01 M sodium edetate* until a yellow colour is obtained.

1 ml of *0.01 M sodium edetate* is equivalent to 0.654 mg of Zn.

Loss on drying ***(2.2.32)***

Maximum 5.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa for 3 h.

Sterility ***(2.6.1)***

If intended for administration by spraying into internal body cavities without a further appropriate sterilisation procedure, it complies with the test for sterility.

Pyrogens *(2.6.8)*

If intended for administration by spraying into internal body cavities without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 ml of the supernatant liquid obtained by centrifuging a suspension containing 11 mg per millilitre in a 9 g/l solution of *sodium chloride R*.

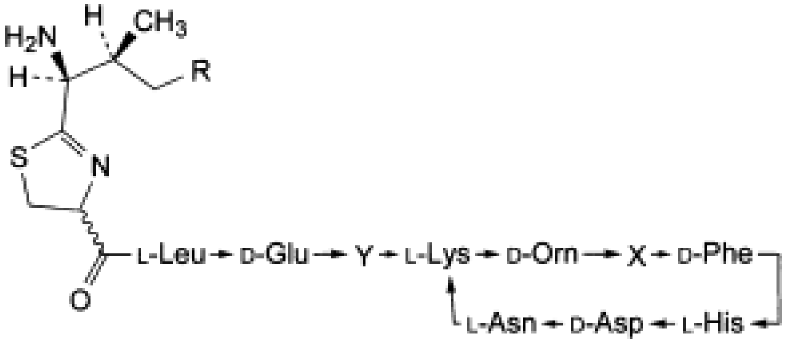
ASSAY

Suspend 50.0 mg in 5 ml of *water R*, add 0.5 ml of *dilute hydrochloric acid R* and dilute to 100.0 ml with *water R*. Allow the solution to stand for 30 min. Carry out the microbiological assay of antibiotics *(2.7.2)*.

STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

IMPURITIES

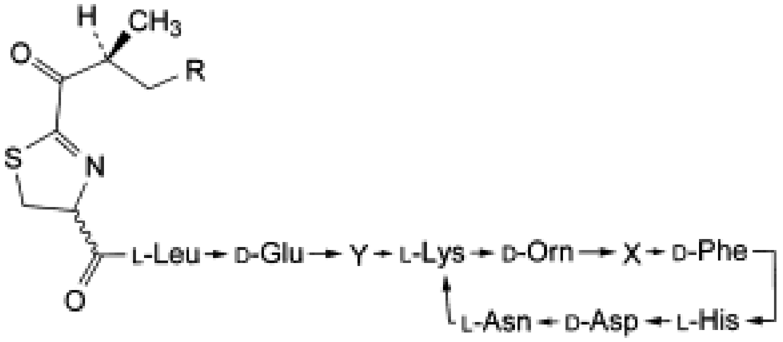

H₂N
H
CH₃
H
R
S
N
O
L-Leu
D-Glu
Y
L-Lys
D-Orn
X
D-Phe
L-Asn
D-Asp
L-His

A. X = L-Val, Y = L-Ile, R = H: bacitracin C1,

B. = L-Ile, Y = L-Val, R = H: bacitracin C2,

C. = Y = L-Val, R = CH_3 bacitracin C3,

D. X = Y = L-Val, R = H: bacitracin E,


H
CH₃
O
R
S
N
O
L-Leu
D-Glu
Y
L-Lys
D-Orn
X
D-Phe
L-Asn
D-Asp
L-His

E. = Y = L-Ile, R = CH_3: bacitracin F,

F. X = Y = L-Ile, R = H: bacitracin H1,

G. X = L-Val, Y = L-Ile, R = CH_3: bacitracin H2,

H. X = L-Ile, Y = L-Val, R = CH_3: bacitracin H3,

I. = L-Val, Y = L-Ile, R = H: bacitracin I1,

J. X = L-Ile, Y = L-Val, R = H: bacitracin I2,

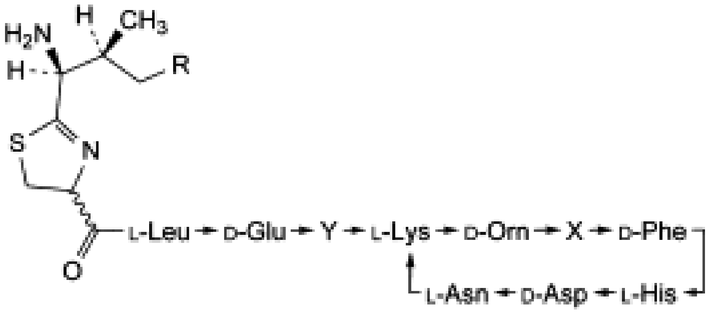
K. X = Y = L-Val, R = CH_3: bacitracin I3.

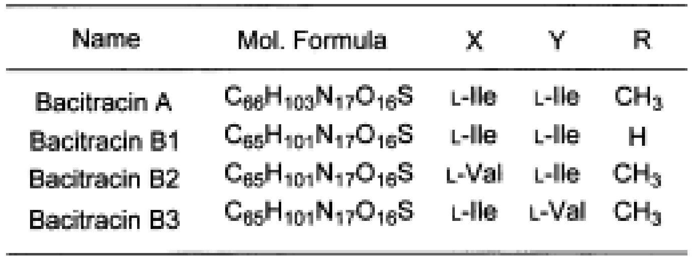
Ph Eur

Bacitracin

General Notices

(Ph Eur monograph 0465)





Name	Mol. Formula	X	Y	R
Bacitracin A	$C_{66}H_{103}N_{17}O_{16}S$	L-Ile	L-Ile	CH_3
Bacitracin B1	$C_{65}H_{101}N_{17}O_{16}S$	L-Ile	L-Ile	H
Bacitracin B2	$C_{65}H_{101}N_{17}O_{16}S$	L-Val	L-Ile	CH_3
Bacitracin B3	$C_{65}H_{101}N_{17}O_{16}S$	L-Ile	L-Val	CH_3

1405-87-4

Action and use

Polypeptide antibacterial.

Ph Eur

DEFINITION

Mixture of antimicrobial polypeptides produced by certain strains of *Bacillus licheniformis* or *Bacillus subtilis*, the main components being bacitracins A, B1, B2 and B3.

Content

Minimum 60 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Freely soluble in water and in alcohol.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C.*

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in a 3.4 g/l solution of *hydrochloric acid R* and dilute to 1.0 ml with the same solution.

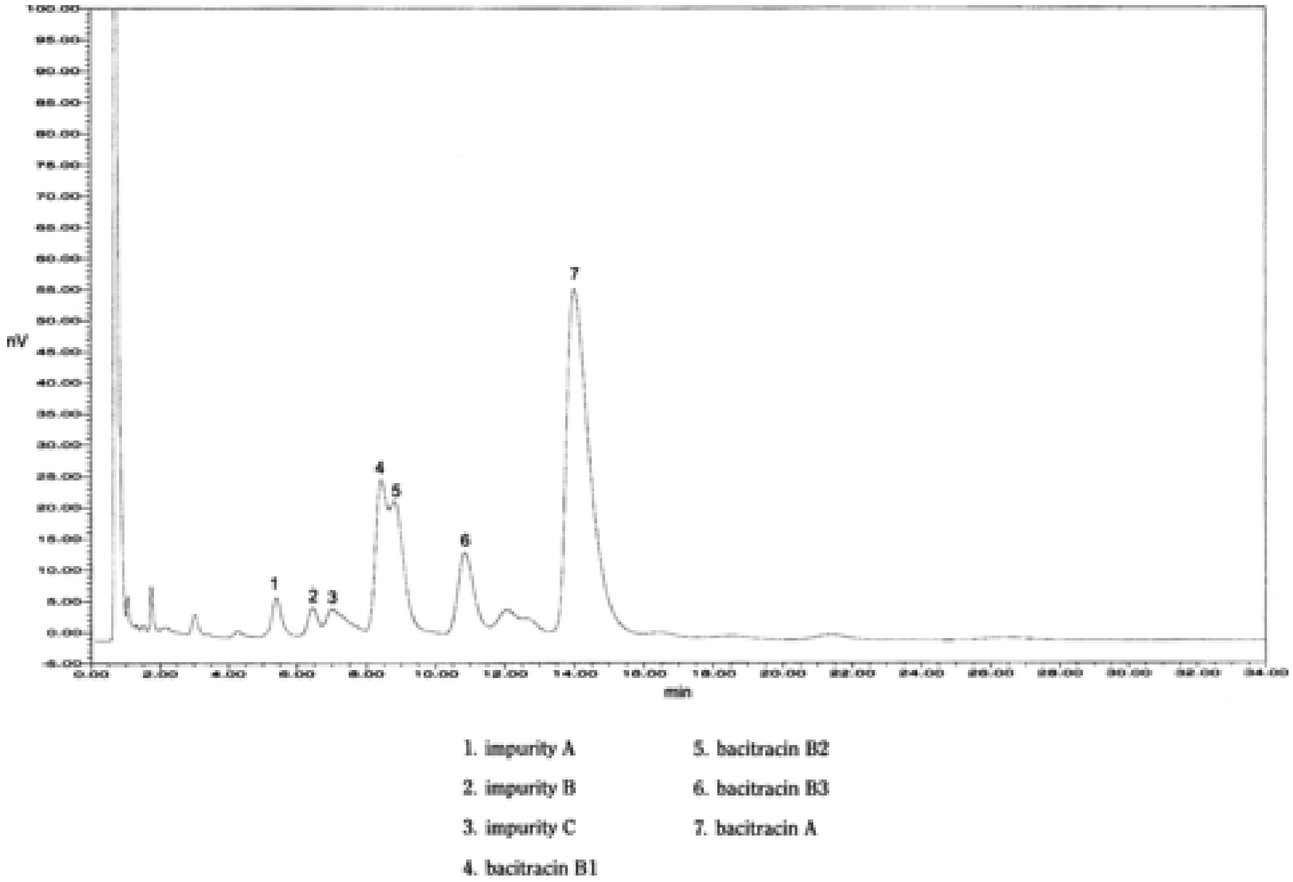


Figure 0465.-1. – *Chromatogram of the test for composition in bacitracin obtained with the test solution at 254 nm*

Reference solution Dissolve 10 mg of *bacitracin zinc CRS* in a 3.4 g/l solution of *hydrochloric acid R* and dilute to 1.0 ml with the same solution.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R*

(1:2:4 *V/V/V*).

Application 10 µl.

Development Over half of the plate.

Drying At 100-105 °C.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The spots in the chromatogram obtained with the test solution are similar in position, size and colour to the spots in the chromatogram obtained with the reference solution.

B. It complies with the test for composition (see Tests).

C. Ignite 0.2 g. An insignificant residue remains which is not yellow at high temperature. Allow to cool. Dissolve the residue in 0.1 ml *of dilute hydrochloric acid R*. Add 5 ml of *water R* and 0.2 ml of *strong sodium hydroxide solution R*. No white precipitate is formed.

TESTS

Solution S

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

pH *(2.2.3)*

6.0 to 7.0 for solution S.

Composition

Liquid chromatography *(2.2.29)*: use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution Dissolve 0.100 g of the substance to be examined in 50.0 ml of the mobile phase.

Reference solution (a) Suspend 20.0 mg of *bacitracin zinc CRS* in *water R*, add 0.2 ml of *dilute hydrochloric acid R* and dilute to 10.0 ml with *water R*.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

Reference solution (d) Dissolve 50.0 mg of the substance to be examined in 25.0 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*. Heat in a boiling water-bath for 30 min. Cool to room temperature.

Blank solution A 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*.

Column:

— size: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Add 520 volumes of *methanol R1*, 40 volumes of *acetonitrile R* and 300 volumes of *water R* to 100 volumes of a 34.8 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 6.0 with a 27.2 g/l solution of *potassium dihydrogen phosphate R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 100 µl; inject the blank, the test solution and reference solutions (a) and (c).

Run time   3 times the retention time of bacitracin A.

Relative retention with reference to bacitracin A (retention time = 15 min to 25 min): bacitracin B1 = about 0.6; bacitracin B3 = about 0.8; impurity E = about 2.5.

If necessary, adjust the composition of the mobile phase by changing the amount of organic modifier whilst keeping the ratio constant between methanol and acetonitrile.

System suitability   Reference solution (a):

— *peak-to-valley ratio*: minimum of 1.2, where H_p = height above the baseline of the peak due to bacitracin B1 and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B2.

Limits:

— *bacitracin A*: minimum 40.0 per cent,

— *sum of bacitracins A, B1, B2 and B3*: minimum 70.0 per cent,

— *disregard limit*: the area of the peak due to bacitracin A in the chromatogram obtained with reference solution (c) (0.5 per cent); disregard any peak observed in the blank run.

Related peptides

Liquid chromatography *(2.2.29)* as described in the test for composition.

See Figure 0465.-1.

Limit:

— *sum of the areas of all peaks eluting before the peak due to bacitracin B1*: maximum 20.0 per cent.

Impurity E

Liquid chromatography *(2.2.29)* as described in the test for composition.

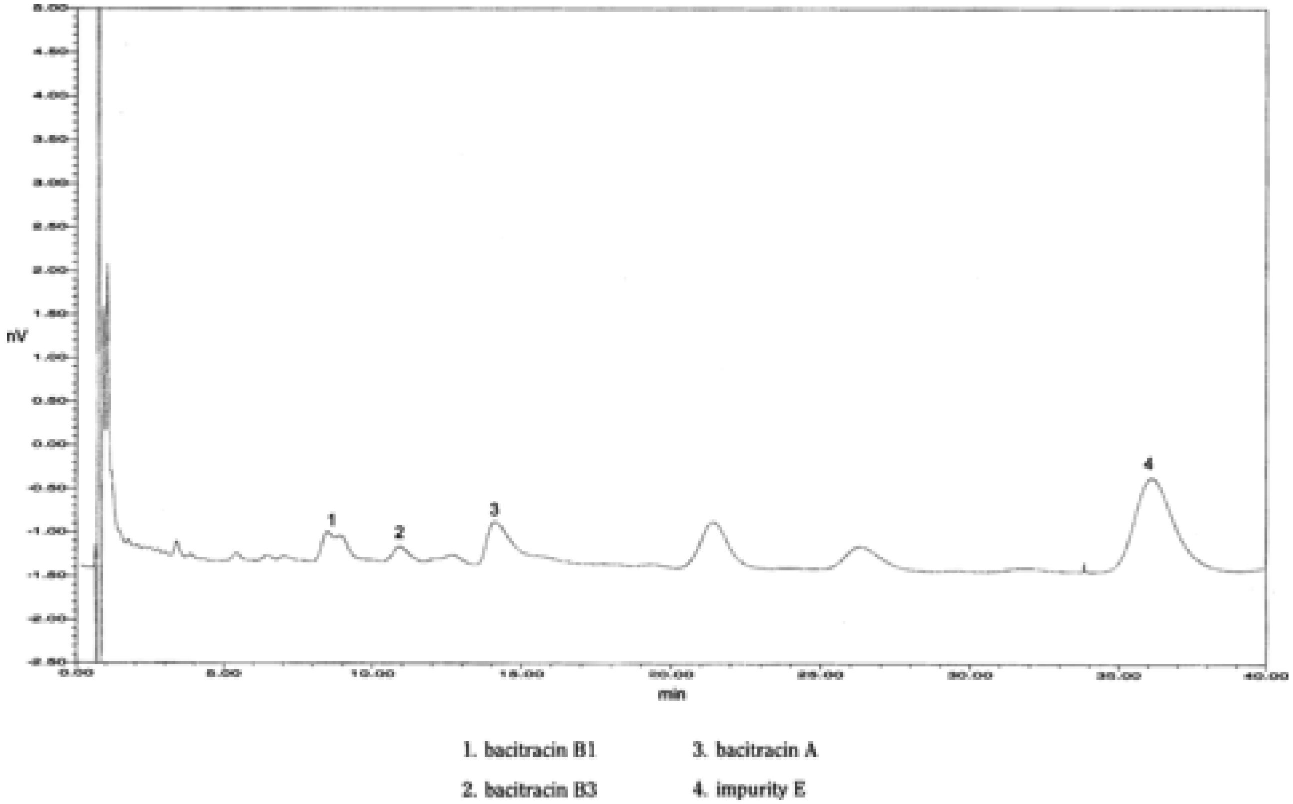


Figure 0465.-2. – *Chromatogram of the test for impurity E in bacitracin obtained with reference solution (d) at 300 nm*

Detection Spectrophotometer at 254 nm; spectrophotometer at 300 nm for reference solution (d).

Injection Test solution and reference solutions (b) and (d).

Limit:

— *impurity E*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent).

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 1.0 per cent, determined on 1.0 g.

Sterility *(2.6.1)*

If intended for the preparation of ophthalmic dosage forms without a further appropriate sterilisation procedure, it complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 0.8 IU/mg, if intended for use in the manufacture of ophthalmic dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

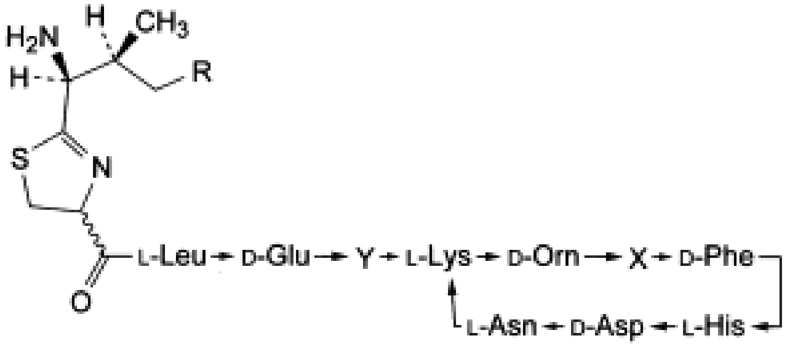
ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)*. Use *bacitracin zinc CRS* as the reference substance.

STORAGE

In an airtight container at 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

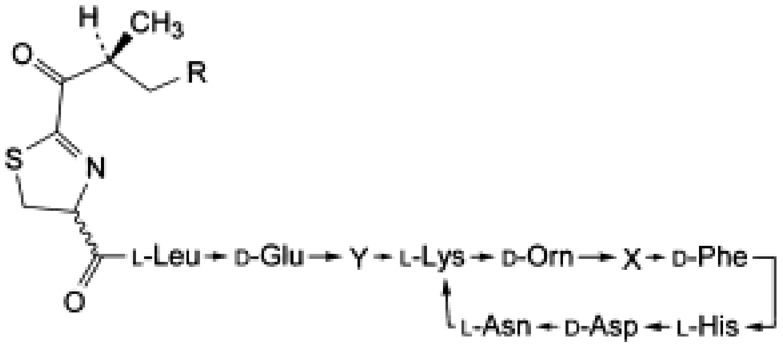


A. X = L-Val, Y = L-Ile, R = H: bacitracin C1,

B. X = L-Ile, Y = L-Val, R = H: bacitracin C2,

C. X = Y = L-Val, R = CH_3: bacitracin C3,

D. X = Y = L-Val, R = H: bacitracin E,



E. X = Y = L-Ile, R = CH_3: bacitracin F,

F. X = Y = L-Ile, R = H: bacitracin H1,

G. X = L-Val, Y = L-Ile, R = CH_3: bacitracin H2,

H. X = L-Ile, Y = L-Val, R = CH_3: bacitracin H3,

I. X = L-Val, Y = L-Ile, R = H: bacitracin I1,

J. X = L-Ile, Y = L-Val, R = H: bacitracin I2,

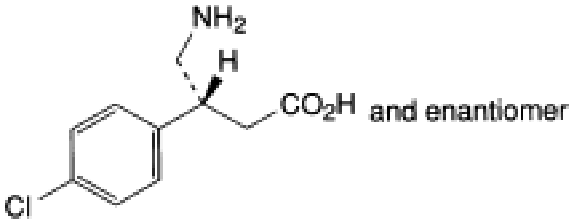
K. X = Y = L-Val, R = CH_3: bacitracin I3.

Ph Eur

Baclofen

General Notices

(Ph Eur monograph 0653)



$C_{10}H_{12}ClNO_2$ 213.7 *1134-47-0*

Action and use

Skeletal muscle relaxant.

Preparations

Baclofen Oral Solution

Baclofen Tablets

Ph Eur

DEFINITION

(3RS)-4-Amino-3-(4-chlorophenyl)butanoic acid.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in acetone. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 70 mg in *water R* and dilute to 100.0 ml with the same solvent.

Spectral range 220-320 nm.

Absorption maxima At 259 nm, 266 nm and 275 nm.

Resolution (2.2.25) Minimum 1.5 for the absorbance ratio.

Specific absorbance at the absorption maxima:

— at 259 nm: 9.8 to 10.8;

— at 266 nm: 11.5 to 12.7;

— at 275 nm: 8.4 to 9.3.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs prepared using 3 mg of substance and 300 mg of *potassium bromide R*.

Comparison *baclofen CRS.*

If the spectra obtained in the solid state show differences, dissolve 0.1 g of each of the substances separately in 1 ml of *dilute sodium hydroxide solution R* and add 10 ml of *ethanol (96 per cent) R* and 1 ml of *dilute acetic acid R*. Allow to stand for 1 h. Filter, wash the precipitate with *ethanol (96 per cent) R* and dry *in vacuo*. Prepare new discs and record the spectra.

C. Thin-layer chromatography *(2.2.27).*

Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution Dissolve 10 mg of *baclofen CRS* in the mobile phase and dilute to 10 ml with the mobile phase.

Plate *TLC silica gel G plate R.*

Mobile phase *anhydrous formic acid R, water R, methanol R, chloroform R, ethyl acetate R* (5:5:20:30:40 *V/V/V/V/V*).

Application 5 µl.

Development Over a path of 12 cm.

Drying Allow the solvents to evaporate.

Detection Spray with *ninhydrin solution R3* until the plate is slightly wet. Place in an oven maintained at 100 °C for 10 min. Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Dissolve 0.50 g in *1 M sodium hydroxide* and dilute to 25 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *baclofen impurity A CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (d) Dilute 2.0 ml of the test solution and 2.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.0 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Dissolve 1.822 g of *sodium hexanesulphonate R* in 1 litre of a mixture of 560 volumes of *water R*, 440 volumes of *methanol R* and 5 volumes of *glacial acetic acid R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 266 nm.

Injection 20 µl of the test solution and reference solutions (b), (c) and (d).

Run time 5 times the retention time of baclofen.

System suitability Reference solution (d):

— *resolution:* minimum 2.0 between the peaks due to baclofen and impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

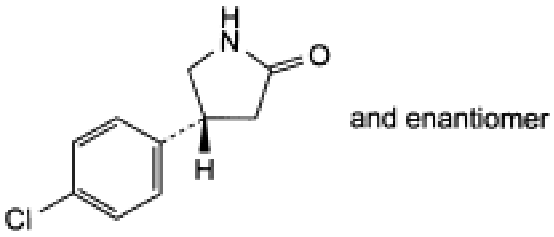
Dissolve 0.1500 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 21.37 mg of $C_{10}H_{12}ClNO_2$.

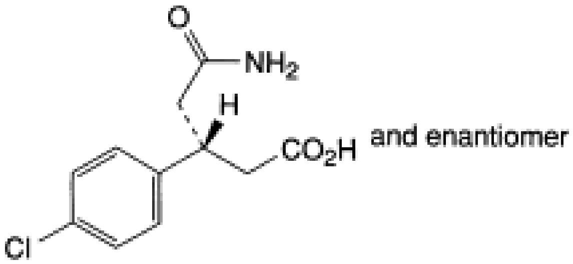
IMPURITIES

Specified impurities A.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use):B.*



A. (4*RS*)-4-(4-chlorophenyl)pyrrolidin-2-one,



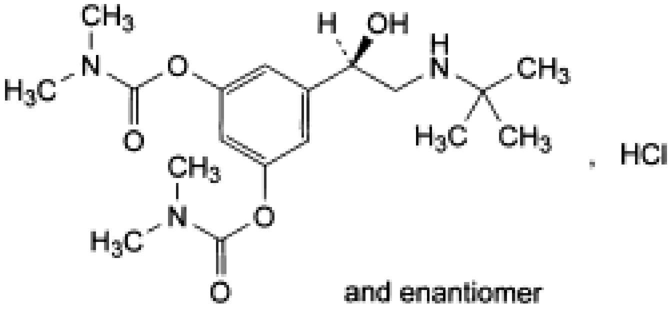
B. (3*RS*)-5-amino-3-(4-chlorophenyl)-5-oxopentanoic acid.

Ph Eur

Bambuterol Hydrochloride

General Notices

(Ph Eur monograph 1293)



$C_{18}H_{29}N_3O_5,HCl$ 403.9 *81732-46-9*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Ph Eur

DEFINITION

5-[(1*RS*)-2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-1,3-phenylene bis(dimethylcarbamate) hydrochloride.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *bambuterol hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in a mixture of 1 volume of *water R* and 6 volumes of *acetone R*, cool in ice to precipitate and dry both precipitates *in vacuo* at 50 °C to constant weight. Record new spectra using the residues.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 4.0 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Acidity or alkalinity

To 10 ml of solution S add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M hydrochloric acid*. The solution is red. Add 0.4 ml of *0.01 M sodium hydroxide*. The solution is yellow.

Optical rotation *(2.2.7)*

– 0.10 ° to + 0.10 °.

Dilute 1 ml of solution S to 10 ml with *carbon dioxide-free water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 5.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 1.0 mg of *formoterol fumarate dihydrate CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Mix 0.8 ml of this solution with 0.4 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.3 g of *sodium octanesulphonate R* in 430 ml of a mixture of 25 volumes of *acetonitrile R1* and 75 volumes of *methanol R*; then mix this solution with 570 ml of 0.050 M phosphate buffer pH 3.0 prepared as follows: dissolve 6.90 g of *sodium dihydrogen phosphate monohydrate R* in *water R* and dilute to 1000 ml with *water R*, adjust to pH 3.0 with a 50 g/l solution of *dilute phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl; inject the mobile phase as a blank.

Run time 1.5 times the retention time of bambuterol.

Retention time formoterol = about 7 min; bambuterol = about 9 min. If necessary, adjust the composition of the mobile phase; increase the content of phosphate buffer to increase the retention time.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to bambuterol and formoterol.

Limits:

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the mobile phase.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

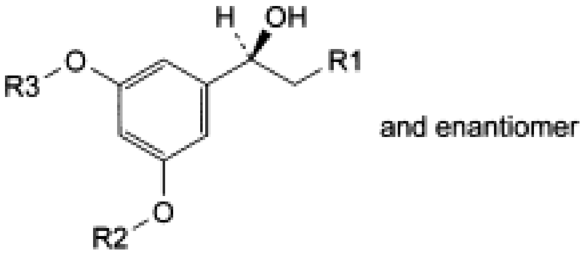
ASSAY

Dissolve 0.320 g in 50 ml of *ethanol (96 per cent) R* and add 5 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (2.2.20), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 40.39 mg of $C_{18}H_{30}ClN_3O_5$.

IMPURITIES

Specified impurities: A, B, C, D, E, F.

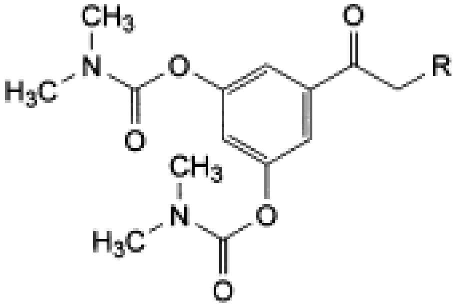


A. R1 = NH-C$(CH_3)_3$, R2 = R3 = H: (1*RS*)-1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl) amino]ethanol (terbutaline),

B. R1 = OH, R2 = R3 = CO-N$(CH_3)_2$: 5-[(1*RS*)-1,2-dihydroxyethyl]-1,3-phenylene bis (dimethylcarbamate),

C. R1 = NH-C$(CH_3)_3$, R2 = H, R3 = CO-N$(CH_3)_2$: 3-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-5-hydroxyphenyl dimethylcarbamate,

D. R1 = H, R2 = R3 = CO-N$(CH_3)_2$: 5-[(1*RS*)-1-hydroxyethyl]-1,3-phenylene bis (dimethylcarbamate),



E. R = H: 5-acetyl-1,3-phenylene bis(dimethylcarbamate),

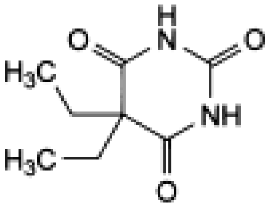
F. R = $NH-C(CH_3)_3$: 5-[[(1,1-dimethylethyl)amino]acetyl]-1,3-phenylene bis (dimethylcarbamate).

Ph Eur

Barbital

General Notices

(Ph Eur monograph 0170)



$C_8H_{12}N_2O_3$ 184.2 *57-44-3*

Action and use

Barbiturate.

Ph Eur

DEFINITION

Barbital contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 5,5-diethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, slightly soluble in water, soluble in boiling water and in alcohol. It forms water-soluble compounds with alkali hydroxides and carbonates and with ammonia.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Determine the melting point *(2.2.14)* of the substance to be examined. Mix equal parts of the substance to be examined and *barbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 190 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *barbital CRS*.

C. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 75 mg of the substance to be examined in *alcohol R* and dilute to 25 ml with the same solvent.

Reference solution Dissolve 75 mg of *barbital CRS* in alcohol R and dilute to 25 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 6 ml of *water R*. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity

Boil 1.0 g with 50 ml of *water R* for 2 min, allow to cool and filter. To 10 ml of the filtrate add 0.15 ml of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *alcohol R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 85.0 mg in 5 ml of *pyridine R*. Add 0.5 ml of *thymolphthalein solution R* and 10 ml of *silver nitrate solution in pyridine R*. Titrate with 0.1 M *ethanolic sodium hydroxide* until a pure blue colour is obtained. Carry out a blank titration.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 9.21 mg of $C_8H_{12}N_2O_3$.

Ph Eur

Barium Sulphate for Suspension

General Notices

Action and use

Radio-opaque preparation used in the investigation of the gastro-intestinal tract.

Preparation

Barium Sulphate Oral Suspension

DEFINITION

Barium Sulphate for Suspension is a dry mixture of Barium Sulphate with a suitable dispersing agent and may contain suitable flavours and suitable antimicrobial preservatives.

Content of barium sulphate, $BaSO_4$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A fine, white or creamy white powder.

IDENTIFICATION

A. Ignite 1 g to constant weight. To 0.2 g of the residue add 5 ml of a 50% w/v solution of *sodium carbonate* and boil for 5 minutes. Add 10 ml of *water* and filter. Reserve the residue for test B. Acidify a portion of the filtrate with *2M hydrochloric acid*. The solution yields the reactions characteristic of *sulphates*, Appendix VI.

B. Wash the residue reserved in test A with *water*, add 5 ml of *2M hydrochloric acid*, mix well and filter. Add 0.3 ml of *1M sulphuric acid* to the filtrate. A white precipitate is produced which is insoluble in *2M hydrochloric acid*.

TESTS

Acidity or alkalinity

pH of an aqueous suspension containing the equivalent of 60% w/w of Barium Sulphate or, for lower strengths, the aqueous suspension at the strength of intended use, 3.5 to 8.5, Appendix V L.

Loss on drying

When dried at 105° for 4 hours, loses not more than 1.0% of its weight. Use 1 g.

ASSAY

To a quantity containing 0.6 g of Barium Sulphate in a platinum dish add 5 g of *sodium carbonate* and 5 g of *potassium carbonate sesquihydrate* and mix. Heat to 1000° and maintain at this temperature for 15 minutes. Allow to cool and suspend the residue in 150 ml of *water*. Wash the dish with 2 ml of *6M acetic acid* and add the washings to the suspension. Cool in ice and decant the supernatant liquid, transferring as little of the solid matter as possible to the filter. Wash the residue with successive quantities of a 2% w/v solution of *sodium carbonate* until the washings are free from sulphate and discard the washings. Add 5 ml of *2M hydrochloric acid* to the filter, wash through into the vessel containing the bulk of the solid matter with *water*, add 5 ml of *hydrochloric acid* and dilute to 100 ml with *water*. Add 10 ml of a 40% w/v solution of *ammonium acetate*, 25 ml of a 10% w/v solution of *potassium dichromate* and 10 g of *urea*. Cover and digest in a hot-air oven at 80° to 85° for 16 hours. Filter whilst still hot through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable), washing the precipitate initially with a 0.5% w/v solution of *potassium dichromate* and finally with 2 ml of *water*. Dry to constant weight at 105°. Each g of the residue is equivalent to 0.9213 g of barium sulphate, $BaSO_4$.

Barium Sulphate

General Notices

(Ph Eur monograph 0010)

$BaSO_4$ 233.4 *7727-43-7*

Action and use

Radio-opaque substance used in the investigation of the gastro-intestinal tract.

Preparation

Barium Sulphate for Suspension

Ph Eur

CHARACTERS

Appearance

Fine, white or almost white powder, free from gritty particles.

Solubility

Practically insoluble in water and in organic solvents. It is very slightly soluble in acids and in solutions of alkali hydroxides.

IDENTIFICATION

A. Boil a suspension of 0.2 g with 5 ml of a 500 g/l solution of *sodium carbonate R* for 5 min, add 10 ml of *water R*, filter and acidify a part of the filtrate with *dilute hydrochloric acid R*. The solution gives the reactions of sulphates *(2.3.1)*.

B. Wash the residue collected in the preceding test with 3 successive small quantities of *water R*. To the residue add 5 ml of *dilute hydrochloric acid R*, filter and add to the filtrate 0.3 ml of *dilute sulphuric acid R*. A white precipitate is formed that is insoluble in *dilute sodium hydroxide solution R*.

TESTS

Solution S

To 20.0 g add 40 ml of *distilled water R* and 60 ml of *dilute acetic acid R*. Boil for 5 min, filter and dilute the cooled filtrate to 100 ml with *distilled water R*.

Acidity or alkalinity

Heat 5.0 g with 20 ml of *carbon dioxide-free water R* on a water-bath for 5 min and filter. To 10 ml of the filtrate add 0.05 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Acid-soluble substances

Maximum 0.3 per cent.

Evaporate 25 ml of solution S to dryness on a water-bath and dry to constant mass at 100-105 °C. The residue weighs a maximum of 15 mg.

Oxidisable sulphur compounds

Shake 1.0 g with 5 ml of *water R* for 30 s and filter. To the filtrate add 0.1 ml of *starch solution R*, dissolve 0.1 g of *potassium iodide R* in the mixture, add 1.0 ml of a freshly prepared 3.6 mg/l solution of *potassium iodate R* and 1 ml of *1 M hydrochloric acid* and shake well. The colour of the solution is more intense than that of a standard prepared at the same time and in the same manner, but omitting the potassium iodate.

Soluble barium salts

Maximum 10 ppm.

To 2.5 ml of a 0.2 mg/l solution of *barium nitrate R* in a mixture of 30 volumes of *ethanol (96 per cent) R* and 70 ml of *water R*, add 10 ml of *dilute sulphuric acid R.* Shake and allow to stand for 5 min. To 1 ml of this solution, add 10 ml of solution S. Prepare a standard in the same manner using 10 ml of *barium standard solution (2 ppm Ba) R* instead of solution S.

After 10 min, any opalescence in the test solution is not more intense than that in the standard.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

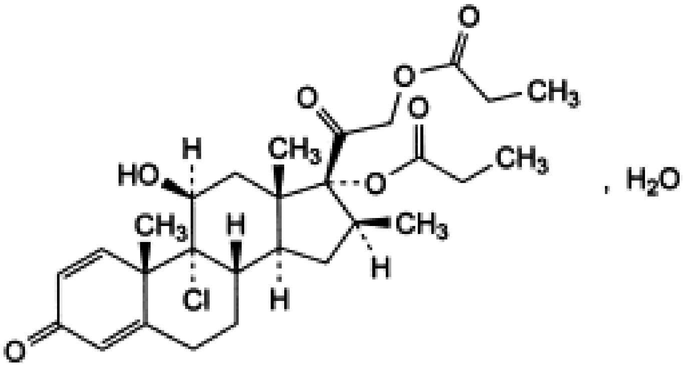
Maximum 2.0 per cent, determined on 1.0 g at 600 ± 50 °C.

Ph Eur

Beclometasone Dipropionate Monohydrate

General Notices

(Ph Eur monograph 1709)



$C_{28}H_{37}ClO_7,H_2O$ 539.1 *5534-09-8*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

9-Chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate monohydrate.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *beclometasone dipropionate monohydrate CRS*.

B. Treat 25 mg by the oxygen-flask method *(2.5.10)*. Use a mixture of 1 ml of *1 M sodium hydroxide* and 20 ml of *water R* to absorb the combustion products. The solution gives reaction (a) of chlorides *(2.3.1)*.

C. Loss on drying (see Tests).

TESTS

Specific optical rotation *(2.2.7)*

+ 108 to + 115 (dried substance).

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 50.0 ml with *acetonitrile R*.

Reference solution (a) Dilute 5.0 ml of test solution (b) to 100.0 ml with *acetonitrile R*.

Reference solution (b) Dissolve 5 mg of *beclometasone dipropionate for system suitability CRS* (containing impurity D) in *acetonitrile R* and dilute to 5 ml with the same solvent.

Reference solution (c) Dissolve 5 mg of *beclometasone dipropionate for peak identification CRS* (containing impurities A, B, C, L and M) in *acetonitrile R* and dilute to 5 ml with the same solvent.

Reference solution (d) Dissolve 50.0 mg of *beclometasone dipropionate anhydrous CRS* in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml to 50.0 ml with *acetonitrile R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R, water R* (45:55 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 238 nm.

Injection 20 µl of test solution (a) and reference solutions (a), (b) and (c).

Run time 2.5 times the retention time of beclometasone dipropionate.

Identification of impurities Use the chromatogram supplied with *beclometasone dipropionate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, L and M; use the chromatogram supplied with *beclometasone dipropionate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity D; if necessary, use the responses of impurities D and M detected at 284 nm to distinguish the 2 impurities: the response of impurity D decreases and that of impurity M increases.

Relative retention With reference to beclometasone dipropionate (retention time = about 29 min): impurity A = about 0.3; impurity B = about 0.6; impurity D = about 1.1; impurity M = about 1.1; impurity L = about 1.3; impurity C = about 1.6.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to beclometasone dipropionate.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 1.3;

— *impurities B, L*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurities A, C, D, M*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

2.8 per cent to 3.8 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase *water R, acetonitrile R* (40:60 *V/V*).

Flow rate 1.0 ml/min.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{28}H_{37}ClO_7$.

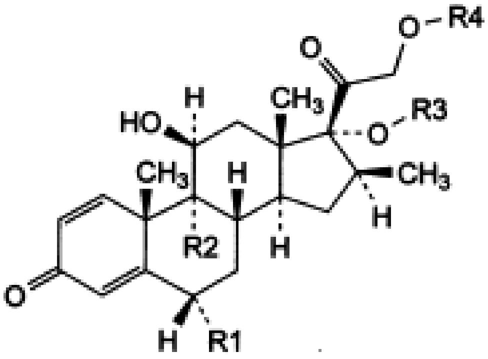
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, L, M.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034*). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances* *for pharmaceutical use): E, F, G, H, I, J, K, N, O, P, Q, R.*



A. R1 = R3 = H, R2 = Cl, R4 = CO-C_2H_5: 9-chloro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl propanoate (beclometasone 21-propionate),

B. R1 = H, R2 = Cl, R3 = CO-C_2H_5, R4 = CO-CH_3: 21-(acetyloxy)-9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate (beclometasone 21-acetate 17-propionate),

C. R1 = H, R2 = Cl, R3 = CO-C_2H_5, R4 = CO-CH_2-CH_2-CH_3: 9-chloro-11β-hydroxy-16β-methyl-3,20-dioxo-17-(propanoyloxy)-pregna-1,4-dien-21-yl butanoate (beclometasone 21-butyrate 17-propionate),

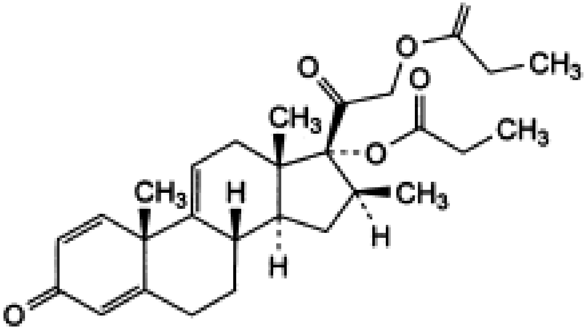
D. R1 = H, R2 = Br, R3 = R4 = CO-C_2H_5: 9-bromo-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

E. R1 = R2 = Cl, R3 = R4 = CO-C_2H_5: 6α,9-dichloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

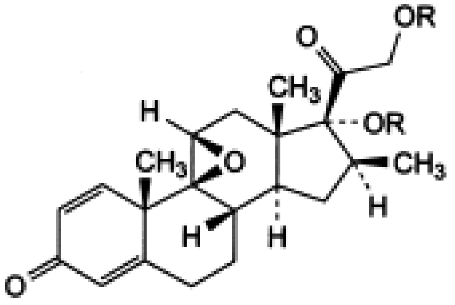
F. R1 = Br, R2 = Cl, R3 = R4 = CO-C_2H_5: 6α-bromo-9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

G. R1 = R3 = R4 = H, R2 = Cl: 9-chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3, 20-dione (beclometasone),

H. R1 = R4 = H, R2 = Cl, R3 = CO-C_2H_5: 9-chloro-11β,21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate (beclometasone 17-propionate),

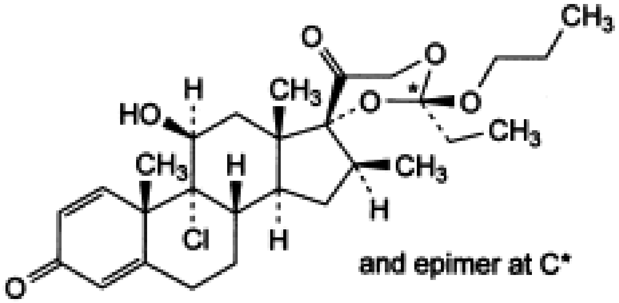


I. 16β-methyl-3,20-dioxopregna-1,4,9(11)-triene-17,21-diyl dipropanoate,

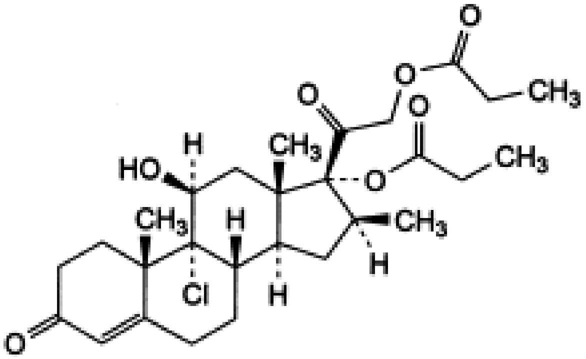


J. R = CO-C_2H_5: 9,11β-epoxy-16β-methyl-3,20-dioxo-9β-pregna-1,4-diene-17,21-diyl dipropanoate,

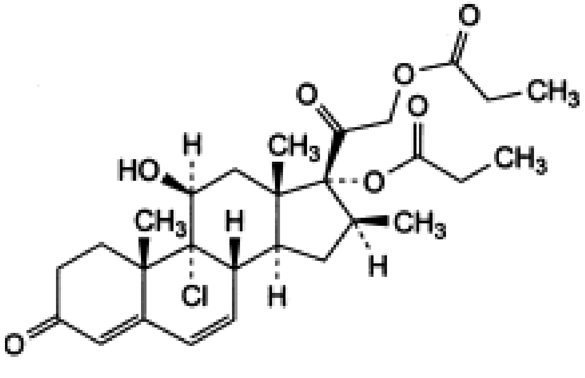
R. R = H: 9,11β-epoxy-17,21-dihydroxy-16β-methyl-9β-pregna-1,4-diene-3,20-dione,



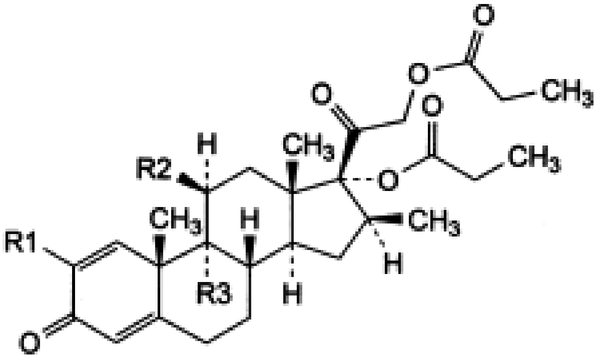
K. (2′*RS*,4′*R*)-9-chloro-2′-ethyl-11β-hydroxy-16β-methyl-2′-propoxyspiro[androsta-1,4-diene-17,4′-[1,3]dioxan]-3,5′-dione (beclometasone propyl 17,21-orthopropionate),



L. 9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregn-4-ene-17,21-diyl dipropanoate,



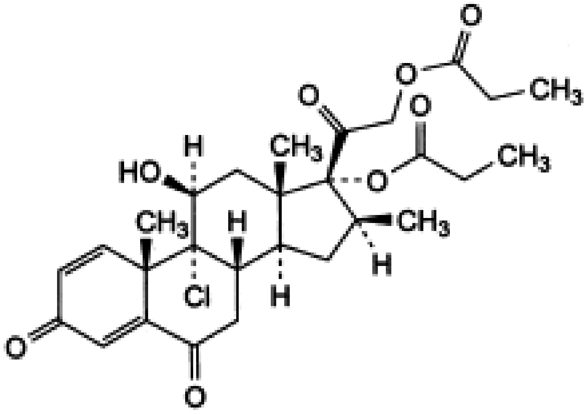
M. 9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-4,6-diene-17,21-diyl dipropanoate,



N. R1 = Br, R2 = OH, R3 = Cl: 2-bromo-9-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

O. R1 = H, R2 = R3 = Cl: 9,11β-dichloro-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,

Q. R1 = R2 = R3 = H: 16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate,



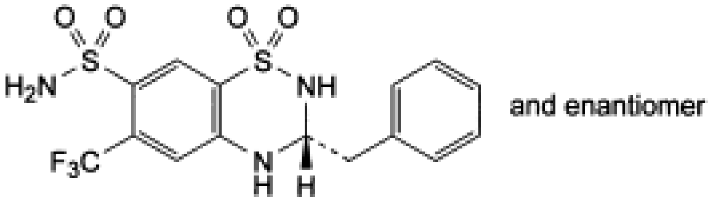
P. 9-chloro-11β-hydroxy-16β-methyl-3,6,20-trioxopregna-1,4-diene-17,21-diyl dipropanoate.

Ph Eur

Bendroflumethiazide

General Notices

(Ph Eur monograph 0370)



$C_{15}H_{14}F_3N_3O_4S_2$ 421.4 *73-48-3*

Action and use

Thiazide diuretic.

Preparation

Bendroflumethiazide Tablets

Ph Eur

DEFINITION

(3RS)-3-Benzyl-6-(trifluoromethyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1, 1-dioxide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison bendroflumethiazide CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Prepare the solutions immediately before use.

Solvent mixture Mix 40 volumes of *methanol R* and 60 volumes of a 2.0 g/l solution of *citric acid R*.

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *bendroflumethiazide impurity A CRS* and 2.5 mg of *altizide CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture. Mix 1 ml of this solution with 1 ml of the test solution and dilute to 100 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 3.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 15 volumes of *tetrahydrofuran R*, 25 volumes of *methanol R* and 60 volumes of a 2.0 g/l solution of *citric acid R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µl.

Run time Twice the retention time of bendroflumethiazide.

Relative retention With reference to bendroflumethiazide (retention time = about 8 min): impurity A = about 0.2; altizide = about 0.5.

System suitability Reference solution (a):

- *resolution*: minimum 10 between the peaks due to altizide and bendroflumethiazide.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

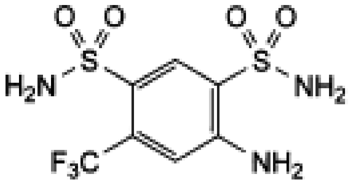
ASSAY

Dissolve 0.150 g in 50 ml of *dimethyl sulphoxide R*. Titrate to the 2nd point of inflexion with *0.1 M tetrabutylammonium hydroxide in 2-propanol*, determining the end-point potentiometrically (2.2.20). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 21.07 mg of $C_{15}H_{14}F_3N_3O_4S_2$.

IMPURITIES

Specified impurities A.



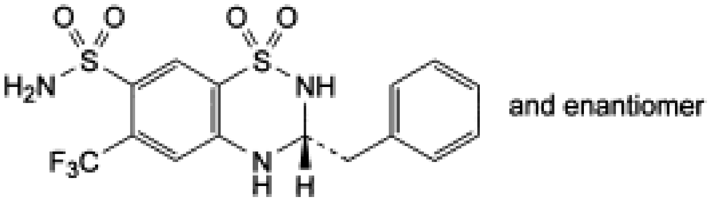
A. 4-amino-6-(trifluoromethyl)benzene-1,3-disulphonamide.

Ph Eur

Bendroflumethiazide

General Notices

(Ph Eur monograph 0370)



$C_{15}H_{14}F_3N_3O_4S_2$ 421.4 *73-48-3*

Action and use

Thiazide diuretic.

Preparation

Bendroflumethiazide Tablets

Ph Eur

DEFINITION

(3RS)-3-Benzyl-6-(trifluoromethyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1, 1-dioxide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *bendroflumethiazide CRS*.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Prepare the solutions immediately before use.

Solvent mixture Mix 40 volumes of *methanol R* and 60 volumes of a 2.0 g/l solution of *citric acid R*.

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *bendroflumethiazide impurity A CRS* and 2.5 mg of *altizide CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture. Mix 1 ml of this solution with 1 ml of the test solution and dilute to 100 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

— *size*: l = 0.15 m, Ø = 3.0 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 15 volumes of *tetrahydrofuran R*, 25 volumes of *methanol R* and 60 volumes of a 2.0 g/l solution of *citric acid R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µl.

Run time Twice the retention time of bendroflumethiazide.

Relative retention With reference to bendroflumethiazide (retention time = about 8 min): impurity A = about 0.2; altizide = about 0.5.

System suitability Reference solution (a):

— *resolution*: minimum 10 between the peaks due to altizide and bendroflumethiazide.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

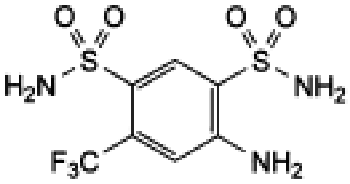
ASSAY

Dissolve 0.150 g in 50 ml of *dimethyl sulphoxide R*. Titrate to the 2nd point of inflexion with *0.1 M tetrabutylammonium hydroxide in 2-propanol*, determining the end-point potentiometrically (2.2.20). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 21.07 mg of $C_{15}H_{14}F_3N_3O_4S_2$.

IMPURITIES

Specified impurities A.

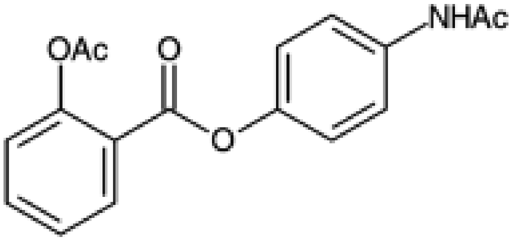


A. 4-amino-6-(trifluoromethyl)benzene-1,3-disulphonamide.

Ph Eur

Benorilate

General Notices



$C_{17}H_{15}NO_5$ 313.3 *5003-48-5*

Action and use

Salicylate-paracetamol derivative; antipyretic; analgesic; anti-inflammatory.

Preparations

Benorilate Oral Suspension

Benorilate Tablets

DEFINITION

Benorilate is 4-acetamidophenyl *O*-acetylsalicylate. It contains not less than 99.0% and not more than 100.5% of $C_{17}H_{15}NO_5$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Practically insoluble in *water*; soluble in *acetone*; sparingly soluble in *ethanol (96%)* and in *methanol*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of benorilate *(RS 023)*.

B. To 10 mg add 10 ml of 6M *hydrochloric acid* and boil until completely dissolved. To 5 ml of the resulting solution add 0.1 ml of *strong 1-naphthol solution*, mix and add sufficient 1M *sodium hydroxide* to make the solution just alkaline. A blue colour is produced which is extracted into *butan-1-ol*.

C. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in *absolute ethanol* exhibits a maximum only at 240 nm. The *absorbance* at 240 nm is about 0.74.

TESTS

Melting point

178° to 181°, Appendix V A.

Heavy metals

1.0 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (20 ppm).

4-Aminophenol

Shake 2.5 g with 100 ml of *water* for 15 minutes and filter. To 20 ml of the filtrate add 0.2 ml of *sodium nitroprusside-carbonate solution*, mix and allow to stand for 30 minutes. The solution is not more intensely coloured than a solution prepared at the same time and in the same manner but using 2 ml of a solution of *4-aminophenol* containing 5 µg per ml and 18 ml of *water* in place of the filtrate (20 ppm).

Salicylic acid

Shake 0.50 g with 20 ml of *water* for 15 minutes and filter. Transfer 10 ml of the filtrate to a *Nessler cylinder*, dilute to 50 ml with *water*, add 0.2 ml of a 0.5% w/v solution of *iron(III) chloride* and allow to stand for 1 minute. The colour obtained is not more intense than that of a solution prepared at the same time by adding 0.2 ml of a 0.5% w/v solution of *iron(III) chloride* to a mixture of 1 ml of a 0.025% w/v solution of *salicylic acid* in *ethanol (96%)* and sufficient *water* to produce 50 ml (0.1%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel HF_{254} precoated plate (Analtech plates are suitable) and a mixture of 80 volumes of *dichloromethane*, 15 volumes of *ether* and 5 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 10 µl of each of four solutions in a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol* containing (1) 4.0% w/v of the substance being examined, (2) 0.040% w/v of the substance being examined, (3) 0.0080% w/v of the substance being examined and (4) 0.0080% w/v of *paracetamol*. After removal of the plate, allow it to dry in air and develop in a second mobile phase consisting of a mixture of 45 volumes of *ether*, 45 volumes of *2,2,4-trimethylpentane* and 10 volumes of *formic acid*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

Any spot corresponding to paracetamol in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.2%). Any *secondary spot* in the chromatogram obtained with solution (1) with an Rf value slightly higher than that of the principal spot is not more intense than the principal spot in the chromatogram obtained with solution (2) (1%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

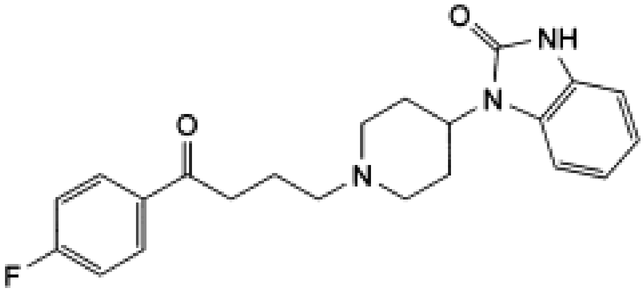
ASSAY

Carry out Method I for the *determination of nitrogen*, Appendix VIII H, using 0.6 g and 10 ml of *nitrogen-free sulphuric acid*. Each ml of 0.05M *sulphuric acid VS* is equivalent to 31.33 mg of $C_{17}H_{15}NO_5$.

Benperidol

General Notices

(Ph Eur monograph 1172)



$C_{22}H_{24}FN_3O_2$ 381.4 *2062-84-2*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

1-[1-[4-(4-Fluorophenyl)-4-oxobutyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide, soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *benperidol CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methyl isobutyl ketone R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a) Dissolve 30 mg of *benperidol CRS* in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b) Dissolve 30 mg of *benperidol CRS* and 30 mg of *droperidol CRS* in the mobile phase and dilute to 10 ml with the mobile phase.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *acetone R, methanol R* (1:9 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b)

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 5 ml of *anhydrous ethanol R*. Add 0.5 ml of *dinitrobenzene solution R* and 0.5 ml of *2 M alcoholic potassium hydroxide R*. A violet colour is produced which becomes brownish-red after 20 min.

D. Mix about 5 mg with 45 mg of heavy *magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 0.10 g of the substance to be examined in *dimethylformamide R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *benperidol CRS* and 2.5 mg of *droperidol CRS* in *dimethylformamide R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide R*. Dilute 5.0 ml of this solution to 20.0 ml with *dimethylformamide R*.

Column:

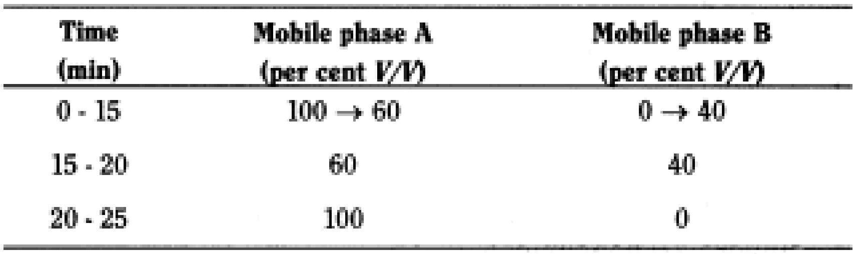
— *size*: *l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 10 g/l solution of *tetrabutylammonium hydrogen sulphate R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100 → 60	0 → 40
15 - 20	60	40
20 - 25	100	0

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 275 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *dimethylformamide R* as a blank.

Retention time Benperidol = about 6.5 min; droperidol = about 7 min.

System suitability Reference solution (a)

— *resolution*: minimum 2.0 between the peaks due to benperidol and droperidol; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient.

Limits:

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R* and titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution R* as indicator.

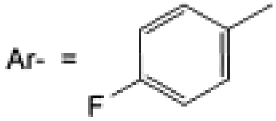
1 ml of *0.1 M perchloric acid* is equivalent to 38.14 mg of $C_{22}H_{24}FN_3O_2$.

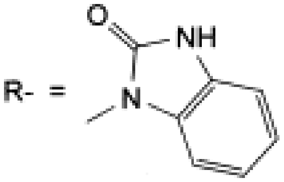
STORAGE

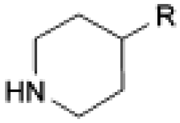
Protected from light.

IMPURITIES

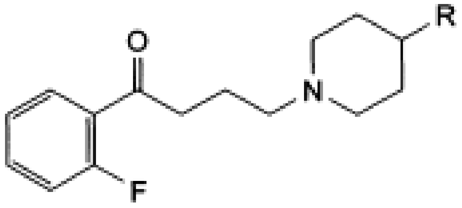
Specified impurities A, B, C, D, E.



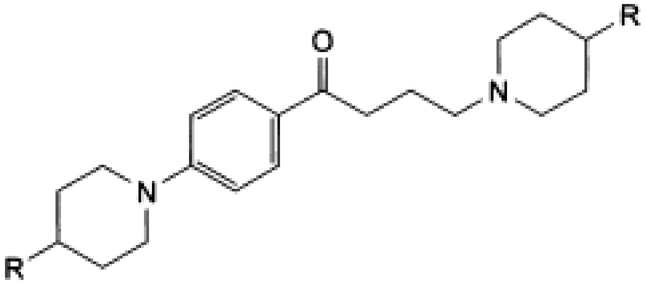




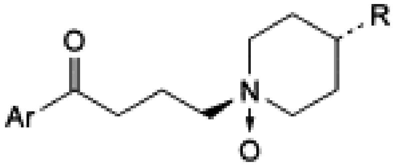
A. 1-(piperidin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one,



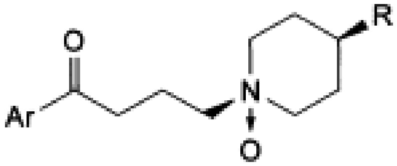
B. 1-[1-[4-(2-fluorophenyl)-4-oxobutyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



C. 1-[1-[4-oxo-4-[4-[4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl]phenyl]butyl] piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



D. *cis*-1-[1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl 1-oxide]-1,3-dihydro-2H-benzimidazol-2-one,



E. *trans*-1-[1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl 1-oxide]-1,3-dihydro-2H-benzimidazol-2-one.

Ph Eur

Bentonite

General Notices

(Ph Eur monograph 0467)

1302-78-9

Ph Eur

DEFINITION

Natural clay containing a high proportion of montmorillonite, a native hydrated aluminium silicate in which some aluminium and silicon atoms may be replaced by other atoms such as magnesium and iron.

CHARACTERS

Appearance

Very fine, homogeneous, greyish-white powder with a more or less yellowish or pinkish tint.

Solubility

Practically insoluble in water and in aqueous solutions.

It swells with a little water forming a malleable mass.

IDENTIFICATION

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate R* and 3 g of *sodium carbonate R* and heat until the mixture melts. Allow to cool. To this residue add 20 ml of boiling *water R*, mix and filter. Wash the insoluble residue with 50 ml of *water R*. To this residue add 1 ml of *hydrochloric acid R* and 5 ml of *water R*. Filter. To the filtrate add 1 ml of *strong sodium hydroxide solution R* and filter. To this filtrate add 3 ml of *ammonium chloride solution R*. A gelatinous white precipitate is formed.

B. Swelling power with water (see Tests).

C. 0.25 g gives the reaction of silicates *(2.3.1)*.

TESTS

Alkalinity

To 2 g add 100 ml of *carbon dioxide-free water R* and shake for 5 min. To 5 ml of this suspension add 0.1 ml of *thymolphthalein solution R*. The liquid becomes bluish. Add 0.1 ml of *0.1 M hydrochloric acid*. The liquid is decolourised within 5 min.

Coarse particles

Maximum 0.5 per cent.

To 20 g add 1000 ml of *water R* and mix for 15 min using a high-speed mixer capable of operating at not less than 5000 r/min. Transfer the suspension to a wet sieve (75), tared after drying at 100-105 °C. Wash with 3 quantities, each of 500 ml, of *water R*, ensuring that any agglomerates have been dispersed. Dry the sieve at 100-105 °C and weigh. The particles on the sieve weigh a maximum of 0.1 g.

Sedimentation volume

To 6.0 g add 200 ml of *water R* and mix for 20 min using a high-speed mixer capable of operating at 10 000 r/min. Transfer 100 ml of this suspension to a graduated cylinder. Allow to stand for 24 h. The volume of the clear supernatant liquid is not greater than 2 ml.

Swelling power with water

Add 2.0 g in 20 portions to 100 ml of a 10 g/l solution of *sodium laurilsulfate R* in a 100 ml graduated cylinder about 30 mm in diameter. Allow 2 min between additions for each portion to settle. Allow to stand for 2 h. The apparent volume of the sediment is not less than 22 ml.

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

To 5.0 g add 7.5 ml of *dilute hydrochloric acid R* and 27.5 ml of *water R*. Boil for 5 min. Centrifuge and filter the supernatant liquid. Wash the centrifugation residue with *water R* and filter. Dilute the combined filtrates to 50.0 ml with *water R*. To 5 ml of this solution add 5 ml of *water R*, 10 ml of *hydrochloric acid R* and 25 ml of *methyl isobutyl ketone R* and shake for 2 min. Separate the layers. Evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *acetic acid R*, dilute to 25 ml with *water R* and filter. 12 ml of the filtrate complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 15 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 103 micro-organisms per gram, determined by plate-count.

Ph Eur

Bentonite

General Notices

(Ph Eur monograph 0467)

1302-78-9

Ph Eur

DEFINITION

Natural clay containing a high proportion of montmorillonite, a native hydrated aluminium silicate in which some aluminium and silicon atoms may be replaced by other atoms such as magnesium and iron.

CHARACTERS

Appearance

Very fine, homogeneous, greyish-white powder with a more or less yellowish or pinkish tint.

Solubility

Practically insoluble in water and in aqueous solutions.

It swells with a little water forming a malleable mass.

IDENTIFICATION

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate R* and 3 g of *sodium carbonate R* and heat until the mixture melts. Allow to cool. To this residue add 20 ml of boiling *water R*, mix and filter. Wash the insoluble residue with 50 ml of *water R*. To this residue add 1 ml of *hydrochloric acid R* and 5 ml of *water R*. Filter. To the filtrate add 1 ml of *strong sodium hydroxide solution R* and filter. To this filtrate add 3 ml of *ammonium chloride solution R*. A gelatinous white precipitate is formed.

B. Swelling power with water (see Tests).

C. 0.25 g gives the reaction of silicates *(2.3.1)*.

TESTS

Alkalinity

To 2 g add 100 ml of *carbon dioxide-free water R* and shake for 5 min. To 5 ml of this suspension add 0.1 ml of *thymolphthalein solution R*. The liquid becomes bluish. Add 0.1 ml of *0.1 M hydrochloric acid*. The liquid is decolourised within 5 min.

Coarse particles

Maximum 0.5 per cent.

To 20 g add 1000 ml of *water R* and mix for 15 min using a high-speed mixer capable of operating at not less than 5000 r/min. Transfer the suspension to a wet sieve (75), tared after drying at 100-105 °C. Wash with 3 quantities, each of 500 ml, of *water R*, ensuring that any agglomerates have been dispersed. Dry the sieve at 100-105 °C and weigh. The particles on the sieve weigh a maximum of 0.1 g.

Sedimentation volume

To 6.0 g add 200 ml of *water R* and mix for 20 min using a high-speed mixer capable of operating at 10 000 r/min. Transfer 100 ml of this suspension to a graduated cylinder. Allow to stand for 24 h. The volume of the clear supernatant liquid is not greater than 2 ml.

Swelling power with water

Add 2.0 g in 20 portions to 100 ml of a 10 g/l solution of *sodium laurilsulfate R* in a 100 ml graduated cylinder about 30 mm in diameter. Allow 2 min between additions for each portion to settle. Allow to stand for 2 h. The apparent volume of the sediment is not less than 22 ml.

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

To 5.0 g add 7.5 ml of *dilute hydrochloric acid R* and 27.5 ml of *water R*. Boil for 5 min. Centrifuge and filter the supernatant liquid. Wash the centrifugation residue with *water R* and filter. Dilute the combined filtrates to 50.0 ml with *water R*. To 5 ml of this solution add 5 ml of *water R*, 10 ml of *hydrochloric acid R* and 25 ml of *methyl isobutyl ketone R* and shake for 2 min. Separate the layers. Evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *acetic acid R*, dilute to 25 ml with *water R* and filter. 12 ml of the filtrate complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 15 per cent, determined on 1.000 g by drying in an oven at 105 °C.

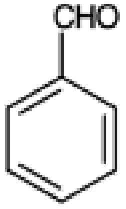
Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 103 micro-organisms per gram, determined by plate-count.

Ph Eur

Benzaldehyde

General Notices



C_7H_6O 106.1 *100-52-7*

Action and use

Flavour.

DEFINITION

Benzaldehyde contains not less than 98.0% w/w and not more than 100.5% w/w of C_7H_6O.

CHARACTERISTICS

A clear, colourless liquid.

Slightly soluble in *water*; miscible with *ethanol (96%)* and with *ether*.

TESTS

Refractive index

1.544 to 1.546, Appendix V E.

Weight per ml

1.043 to 1.049 g, Appendix V G.

Free acid

Not more than 1.0% w/v, calculated as benzoic acid, $C_7H_6O_2$, when determined by the following method. To 10 ml add 20 ml of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 12.21 mg of $C_7H_6O_2$.

Chlorinated compounds

Not more than 0.05% w/v, calculated as Cl, when determined by the following method. To 5 ml add 50 ml of *isoamyl alcohol* and 3 g of *sodium* and boil under a reflux condenser for 1 hour. Cool, add 50 ml of *water* and 15 ml of *nitric acid*, cool, add 5 ml of 0.1M *silver nitrate VS*, shake and titrate the excess silver nitrate with 0.1M *ammonium thiocyanate VS* using *ammonium iron(III) sulphate solution R2* as indicator. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of silver nitrate required. Each ml of 0.1M *silver nitrate VS* is equivalent to 3.545 mg of Cl.

ASSAY

Carry out the method for *determination of aldehydes*, Appendix X K, using 0.5 g. Each ml of *0.5M potassium hydroxide in ethanol (60%) VS* is equivalent to 53.06 mg of C_7H_6O.

STORAGE

Benzaldehyde should be kept in a well-filled container, protected from light and stored at a temperature not exceeding 15°.

Benzalkonium Chloride Solution

General Notices

(Ph Eur monograph 0371)

Action and use

Antiseptic.

Ph Eur

DEFINITION

Benzalkonium chloride solution is an aqueous solution of a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups having chain lengths of C_8 to C_{18}. Benzalkonium chloride solution contains not less than 475 g/l and not more than 525 g/l of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$ (M_r 354.0). The solution may contain alcohol.

CHARACTERS

A clear, colourless or slightly yellowish liquid, miscible with water and with alcohol. It froths copiously when shaken.

IDENTIFICATION

A. Dilute 0.3 ml to 100 ml with *water R*. Examined between 220 nm and 350 nm *(2.2.25)*, the solution shows three absorption maxima, at 257 nm, 263 nm and 269 nm, and a shoulder at about 250 nm.

B. To 0.05 ml add 2 ml of *water R*, 0.1 ml of *glacial acetic acid R* and, dropwise, *1 ml of sodium tetraphenylborate solution R*. A white precipitate is formed. Filter. Dissolve the precipitate in a mixture of 1 ml of *acetone R* and 5 ml of *alcohol R*, heating to not more than 70 °C. Add *water R* dropwise to the warm solution until a slight opalescence forms. Heat gently until the solution is clear and allow to cool. White crystals separate. Filter, wash with three quantities, each of 10 ml, of *water R* and dry *in vacuo* over *diphosphorus pentoxide R* or *anhydrous silica gel R* at a temperature not exceeding 50 °C. The crystals melt *(2.2.14)* at 127 °C to 133 °C.

C. To 5 ml of *dilute sodium hydroxide solution R* add 0.1 ml of *bromophenol blue solution R1* and 5 ml of *chloroform R* and shake. The chloroform layer is colourless. Add 0.05 ml of the solution to be examined and shake. The chloroform layer becomes blue.

D. To 0.05 ml add 1 ml of *dilute nitric acid R*. A white precipitate is formed which dissolves on the addition of 5 ml of *alcohol R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dilute 2.0 g to 100 ml with *carbon dioxide-free water R*.

Appearance of solution.

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 50 ml of solution S add 0.1 ml of *bromocresol purple solution R*. Not more than 0.1 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Amines and amine salts

Mix 10.0 g, while heating, with 20 ml of a mixture of 3 volumes of *1 M hydrochloric acid* and 97 volumes of *methanol R* and add 100 ml of *2-propanol R*. Pass a stream of *nitrogen R* slowly through the solution. Gradually add 12.0 ml of *0.1 M tetrabutylammonium hydroxide* and record the potentiometric titration curve *(2.2.20)*. If the curve shows two points of inflexion, the volume of titrant added between the two points is not greater than 5.0 ml. If the curve shows no point of inflexion, the solution to be examined does not comply with the test. If the curve shows one point of inflexion, repeat the test but add 3.0 ml of a 25.0 g/l solution of *dimethyldecylamine R* in *2-propanol R* before the titration. If the titration curve after the addition of 12.0 ml of the titrant shows only one point of inflexion, the solution to be examined does not comply with the test.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Determine the density *(2.2.5)* of the solution to be examined. Dilute 4.00 g to 100.0 ml with *water R*. Transfer 25.0 ml of the solution to a separating funnel, add 25 ml of *chloroform R*, 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 50 g/l *solution of potassium iodide R*. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous layer with three quantities, each of 10 ml, of *chloroform R* and discard the chloroform layers. To the aqueous layer add 40 ml of *hydrochloric acid R*, allow to cool and titrate with *0.05 M potassium iodate* until the deep-brown colour is almost discharged. Add 2 ml of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 10.0 ml of the freshly prepared 50 g/l solution of *potassium iodide R*, 20 ml of *water R* and 40 ml of *hydrochloric acid R*.

1 ml of *0.05 M potassium iodate* is equivalent to 35.4 mg of $C_{22}H_{40}ClN$.

LABELLING

The label states the content of alcohol, if any.

Ph Eur

Benzalkonium Chloride

General Notices

(Ph Eur monograph 0372)

8001-54-5

Action and use

Antiseptic.

Ph Eur

DEFINITION

Benzalkonium chloride is a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups having chain lengths of C_8 to C_{18}. It contains not less than 95.0 per cent and not more than the equivalent of 104.0 per cent of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$ (M_r 354.0) with reference to the anhydrous substance.

CHARACTERS

A white or yellowish-white powder or gelatinous, yellowish-white fragments, hygroscopic, soapy to the touch, very soluble in water and in alcohol. On heating it forms a clear molten mass. An aqueous solution froths copiously when shaken.

IDENTIFICATION

A. Dissolve 80 mg in *water R* and dilute to 100 ml with the same solvent. Examined between 220 nm and 350 nm *(2.2.25)*, the solution shows three absorption maxima, at 257 nm, 263 nm and 269 nm, and a shoulder at about 250 nm.

B. To 2 ml of solution S (see Tests) add 0.1 ml of *glacial acetic acid R* and, dropwise, 1 ml of *sodium tetraphenylborate solution R*. A white precipitate is formed. Filter. Dissolve the precipitate in a mixture of 1 ml of *acetone R* and 5 ml of *alcohol R*, heating to not more than 70 °C. Add *water R* dropwise to the warm solution until a slight opalescence forms. Heat gently until the solution is clear and allow to cool. White crystals separate. Filter, wash with three quantities, each of 10 ml, of *water R* and dry in vacuo over *diphosphorus pentoxide R* or *anhydrous silica gel R* at a temperature not exceeding 50 °C. The crystals melt *(2.2.14)* at 127 °C to 133 °C.

C. To 5 ml of *dilute sodium hydroxide solution R* add 0.1 ml of *bromophenol blue solution R1* and 5 ml of *chloroform R* and shake. The chloroform layer is colourless. Add 0.1 ml of solution S and shake. The chloroform layer becomes blue.

D. To 2 ml of solution S add 1 ml of *dilute nitric acid R*. A white precipitate is formed which dissolves on the addition of 5 ml of *alcohol R*. The solution gives reaction (a) of chlorides *(2.3.1).*

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 50 ml of solution S add 0.1 ml of *bromocresol purple solution R*. Not more than 0.1 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Amines and amine salts

Dissolve 5.0 g with heating in 20 ml of a mixture of 3 volumes of *1 M hydrochloric acid* and 97 volumes of *methanol R* and add 100 ml of *2-propanol R*. Pass a stream of *nitrogen R* slowly through the solution. Gradually add 12.0 ml of *0.1 M tetrabutylammonium hydroxide* and record the potentiometric titration curve *(2.2.20)*. If the curve shows two points of inflexion, the volume of titrant added between the two points is not greater than 5.0 ml. If the curve shows no point of inflexion, the substance to be examined does not comply with the test. If the curve shows one point of inflexion, repeat the test but add 3.0 ml of a 25.0 g/l solution of *dimethyldecylamine R* in *2-propanol R* before the titration. If the titration curve after addition of 12.0 ml of the titrant shows only one point of inflexion, the substance to be examined does not comply with the test.

Water *(2.5.12)*

Not more than 10 per cent, determined on 0.300 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 2.00 g in *water R* and dilute to 100.0 ml with the same solvent. Transfer 25.0 ml of the solution to a separating funnel, add 25 ml of *chloroform R*, 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 50 g/l solution of *potassium iodide R*. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous layer with three quantities, each of 10 ml, of *chloroform R* and discard the chloroform layers. To the aqueous layer add 40 ml of *hydrochloric acid R*, allow to cool and titrate with 0.05 M potassium iodate until the deep-brown colour is almost discharged. Add 2 ml of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 10.0 ml of the freshly prepared 50 g/l solution of *potassium iodide R*, 20 ml of *water R* and 40 ml of *hydrochloric acid R*.

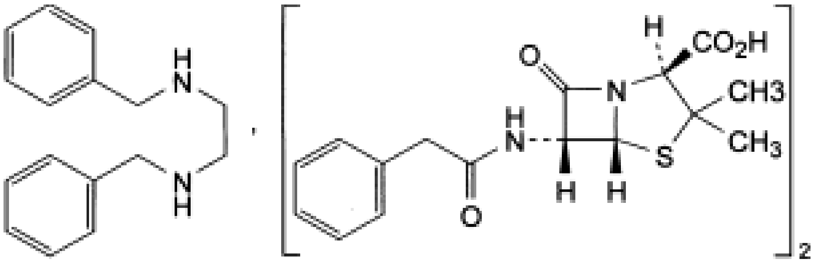
1 ml of *0.05 M potassium iodate* is equivalent to 35.4 mg of $C_{22}H_{40}ClN$.

Ph Eur

Benzathine Benzylpenicillin

General Notices

(Ph Eur monograph 0373)



$(C_{16}H_{18}N_2O_4S)_2C_{16}H_{20}N_2$ *909* 1538-09-6

Action and use

Penicillin antibacterial.

Ph Eur

DEFINITION

N,N′-Dibenzylethane-1,2-diamine compound (1:2) with (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Substance produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Content

- *benzathine benzylpenicillin*: 96.0 per cent to 102.0 per cent (anhydrous substance);
- *N,N′-dibenzylethylenediamine* (benzathine $C_{16}H_{20}N_2$; M_r 240.3): 24.0 per cent to 27.0 per cent (anhydrous substance).

It contains a variable quantity of water. Dispersing or suspending agents may be added.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, freely soluble in dimethylformamide and in formamide, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *benzathine benzylpenicillin CRS*.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *methanol R*.

Reference solution Dissolve 25 mg of *benzathine benzylpenicillin CRS* in 5 ml of *methanol R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R* adjusted to pH 7.0 with *ammonia R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution

— The chromatogram shows 2 clearly separated spots.

Results The 2 principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the 2 principal spots in the chromatogram obtained with the reference solution.

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube on a water-bath for 1 min; a reddish-brown colour develops.

D. To 0.1 g add 2 ml of *1 M sodium hydroxide* and shake for 2 min. Shake the mixture with 2 quantities, each of 3 ml, of *ether R*. Evaporate the combined ether layers to dryness and dissolve the residue in 1 ml of *ethanol (50 per cent V/V) R*. Add 5 ml of *picric acid solution R*, heat at 90 °C for 5 min and allow to cool slowly. Separate the crystals and recrystallise from *ethanol (25 per cent V/V) R* containing 10 g/l of *picric acid R*. The crystals melt *(2.2.14*) at about 214 °C.

TESTS

Acidity or alkalinity

To 0.50 g add 100 ml of *carbon dioxide-free water R* and shake for 5 min. Filter through a sintered-glass filter (2.1.2). To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1*. The solution is green or yellow. Not more than 0.2 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use, using sonication (for about 2 min) to dissolve the samples. Avoid any overheating during the sample preparation.*

Test solution Dissolve 70.0 mg of the substance to be examined in 25 ml of *methanol R* and dilute to 50.0 ml with a solution containing 6.8 g/l of *potassium dihydrogen phosphate R* and 1.02 g/l of *disodium hydrogen phosphate R*.

Reference solution (a) Dissolve 70.0 mg of *benzathine benzylpenicillin CRS* in 25 ml of *methanol R* and dilute to 50.0 ml with a solution containing 6.8 g/l of *potassium dihydrogen phosphate R* and 1.02 g/l of *disodium hydrogen phosphate R*.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with mobile phase A.

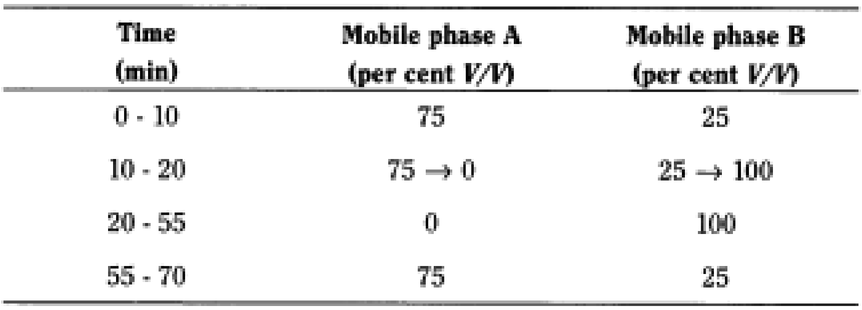
Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R (5 µm);*

— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: mix 10 volumes of a 34 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with *phosphoric acid R*, 30 volumes of *methanol R* and 60 volumes of *water R*;

— *mobile phase B*: mix 10 volumes of a 34 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with *phosphoric acid R*, 30 volumes of *water R* and 60 volumes of *methanol R;*



Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 10	75	25
10 - 20	75 → 0	25 → 100
20 - 55	0	100
55 - 70	75	25

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

System suitability Reference solution (a):

— *relative retention* with reference to benzylpenicillin: benzathine = 0.3 to 0.4 impurity C = about 2.4; if necessary, adjust the concentration of methanol in the mobile phase.

Limits:
— *impurity C*: not more than twice the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (2 per cent);

— *any other impurity*: for each impurity, not more than the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.05 times the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water ***(2.5.12)***

5.0 per cent to 8.0 per cent, determined on 0.300 g.

Bacterial endotoxins ***(2.6.14, Method E)***

Less than 0.13 IU/ml, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

Suspend 20 mg in 20 ml of a solution of *0.1 M sodium hydroxide* diluted 1 to 100, shake thoroughly and centrifuge. Examine the supernatant.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase *phosphate buffer solution pH 3.5 R, methanol R, water R* (10:35:55 *V/V/V*).

Injection Test solution and reference solution (a).

Calculate the percentage contents of benzathine and benzathine benzylpenicillin. Calculate the percentage content of benzathine benzylpenicillin by multiplying the percentage content of benzylpenicillin by 1.36.

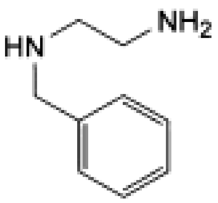
STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

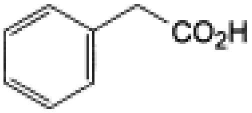
IMPURITIES

Specified impurities *C.*

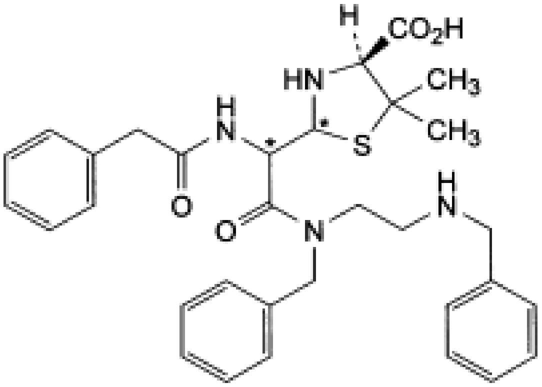
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034*). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, D, E, F.*



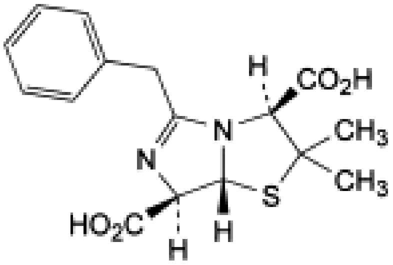
A. monobenzylethylenediamine,



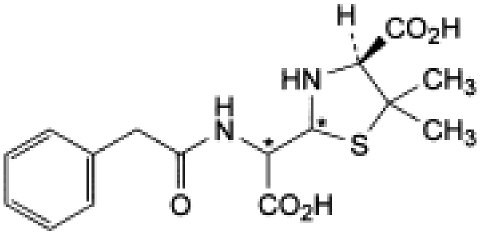
B. phenylacetic acid,



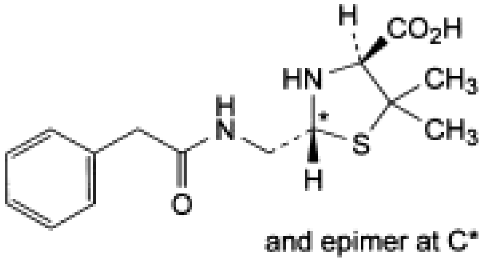
C. benzylpenicilloic acids benzathide,



D. (3*S*,7*R*,7a*R*)-5-benzyl-2,2-dimethyl-2,3,7,7a-tetrahydroimidazo[5,1-b]thiazole-3,7-dicarboxylic acid (penillic acid of benzylpenicillin),



E. (4*S*)-2-[carboxy[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of benzylpenicillin),

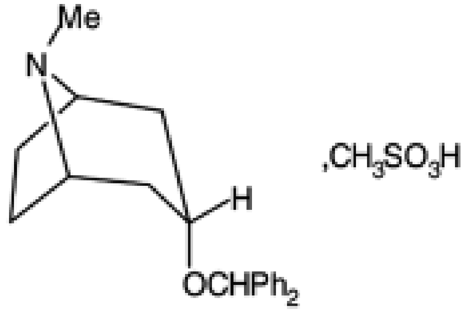

and epimer at C*

F. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin).

Ph Eur

Benzatropine Mesilate

General Notices



$C_{21}H_{25}NO,CH_4O_3S$ 403.5 *132-17-2*

Action and use

Anticholinergic.

Preparations

Benzatropine Injection

Benzatropine Tablets

DEFINITION

Benzatropine Mesilate is (1*R*,3*R*,5*S*)-3-benzhydryloxytropane methanesulphonate. It contains not less than 98.0% and not more than 100.5% of $C_{21}H_{25}NO,CH_4O_3S$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder. It melts at about 144°.

Very soluble in *water*; freely soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. Dry the substance at 105° for 3 hours. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of benzatropine mesilate (*RS 026*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.1% w/v solution in *2M hydrochloric acid* exhibits two maxima, at 253 and 258 nm. The *absorbance* at 253 nm is about 0.96 and at 258 nm is about 1.1.

C. Dissolve 10 mg in 2 ml of *water*, pour into 5 ml of hot *picric acid solution R1* and allow to cool. The *melting point* of the precipitate, after drying at 105°, is about 185°, Appendix V A.

TESTS

Tropine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 75 volumes of *ethanol (96%)* and 15 volumes of *13.5M ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in *acetone* containing (1) 4.0% w/v of the substance being examined and (2) 0.020% w/v of *tropine*. After removal of the plate, allow it to dry in air and spray with *sodium iodobismuthate solution* and then with a 0.4% w/v solution of *sulphuric acid*. Any spot corresponding to tropine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix with the aid of ultrasound 50 mg of the substance being examined with 15 ml of mobile phase A, dilute to 50 ml with the same solvent and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with mobile phase A and further dilute 1 volume of the resulting solution to 5 volumes with the same solvent. For solution (3) mix with the aid of ultrasound 50 mg of *desmethyl benzatropine hydrochloride BPCRS* with 15 ml of mobile phase A, dilute to 100 ml and dilute 1 volume of the resulting solution to 100 volumes with the same solvent. Solution (4) contains 0.01% w/v each of *benzatropine mesilate BPCRS* and *desmethyl benzatropine hydrochloride BPCRS* in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-Phenyl 5µ is suitable). Carry out a linear gradient elution with a flow rate of 1 ml per minute using the following conditions. Use a detection wavelength of 220 nm.

Mobile phase A A mixture of 5 volumes of a 1M potassium phosphate buffer prepared as described for mobile phase B, 20 volumes of *acetonitrile* and 75 volumes of *water*.

Mobile phase B A mixture of 35 volumes of *water*, 60 volumes of *acetonitrile* and 5 volumes of a 1M potassium phosphate buffer prepared in the following manner: dissolve 136.1 g of *potassium dihydrogen orthophosphate* in 900 ml of *water*, add 5 ml of *orthophosphoric acid (85%)* and dilute to 1000 ml.

Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v
0	70	30
20	30	70
30	0	100
50	0	100
55	70	30
65	70	30

Inject 20 µl of solution (4). The test is not valid unless the *resolution factor* between the two principal peaks is at least 1. If necessary adjust the concentration of acetonitrile or adjust the time program of the linear gradient elution.

Inject separately 20 µl of mobile phase A as a blank and 20 µl each of solutions (1), (2) and

(3). In the chromatogram obtained with solution (1) the area of any peak corresponding to desmethyl benzatropine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%), the area of any other *secondary peak* is not greater that the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). In solution (1) disregard any peaks corresponding to the peaks in the chromatogram obtained with the blank solution.

Loss on drying

When dried to constant weight at 105°, loses not more than 5.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

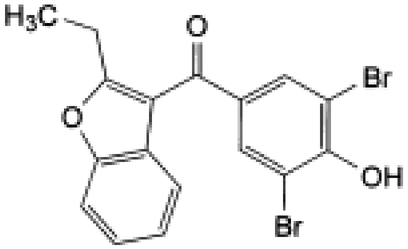
ASSAY

Dissolve 0.6 g in 25 ml of *water*, add 5 ml of *dilute sodium carbonate solution* and extract with four 10 ml quantities of *chloroform*. Wash the combined extracts with 10 ml of *water*, extract the washings with 5 ml of *chloroform* and add the chloroform to the combined extracts. Filter and wash the filter with 5 ml of *chloroform*. To the combined filtrate and washings add 25 ml of *1,4-dioxan* and titrate with *0.1M perchloric acid VS* using 0.15 ml of a 0.1% w/v solution of *methyl red* in *methanol* as indicator. Each ml of *0.1M perchloric acid VS* is equivalent to 40.35 mg of $C_{21}H_{25}NO,CH_4O_3S$.

Benzbromarone

General Notices

(Ph Eur monograph 1393)



$C_{17}H_{12}Br_2O_3$ 424.1 *3562-84-3*

Action and use

Uricosuric; treatment of hyperuricaemia.

Ph Eur

DEFINITION

(3,5-Dibromo-4-hydroxyphenyl)(2-ethylbenzofuran-3-yl)methanone.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in methylene chloride, sparingly soluble in ethanol (96 per cent).

mp: about 152 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *benzbromarone CRS*.

B. By means of a copper wire, previously ignited, introduce a small amount of the substance to be examined into the non-luminous part of a flame. The colour of the flame becomes green.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II).*

Dissolve 1.25 g in *dimethylformamide R* and dilute to 25 ml with the same solvent.

Acidity or alkalinity

Shake 0.5 g with 10 ml of *carbon dioxide-free water R* for 1 min and filter. To 2.0 ml of the filtrate add 0.1 ml of *methyl red solution R* and 0.1 ml of *0.01 M hydrochloric acid*. The solution is red. Add 0.3 ml of *0.01 M sodium hydroxide*. The solution is yellow.

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 0.125 g of the substance to be examined in 30 ml of *methanol R* and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *benzarone CRS* (impurity C) in the mobile phase and dilute to 20 ml with the mobile phase. To 5 ml of this solution add 1 ml of the test solution and dilute to 100 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography* R (5 µm).

Mobile phase *glacial acetic acid R, acetonitrile R, water R, methanol R* (5:25:300:990 *V/V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 231 nm.

Injection 20 µl.

Run time 2.5 times the retention time of benzbromarone.

Relative retention With reference to benzbromarone: impurity A = about 0.6; impurity B = about 2.

System suitability Reference solution (b).

— *resolution*: minimum 10.0 between the peaks due to impurity C (1st peak) and benzbromarone (2nd peak).

Limits:

— *impurity A*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *impurity B*: not more than 10 times the area of the principal peak in the chromatogram

obtained with reference solution (a) (1.0 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities* other than A and B: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Halides expressed as chlorides *(2.4.4)*

Maximum 400 ppm.

Shake 1.25 g with a mixture of 5 ml of *dilute nitric acid R* and 15 ml of *water R*. Filter. Rinse the filter with *water R* and dilute the filtrate to 25 ml with the same solvent. Dilute 2.5 ml of this solution to 15 ml with *water R*.

Iron *(2.4.9)*

Maximum 125 ppm.

Moisten the residue obtained in the test for sulphated ash with 2 ml of *hydrochloric acid R* and evaporate to dryness on a water-bath. Add 0.05 ml of *hydrochloric acid R* and 10 ml of *water R,* heat to boiling and maintain boiling for 1 min. Allow to cool. Rinse the crucible with *water R*, collect the rinsings and dilute to 25 ml with *water R*. Dilute 2 ml of this solution to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

0.5 g complies with test C. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 50 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 60 ml of *methanol R*. Stir until completely dissolved and add 10 ml of *water R.* Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 42.41 mg of $C_{17}H_{12}Br_2O_3$.

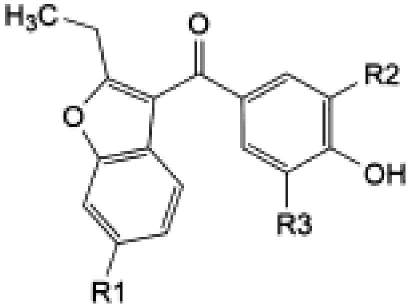
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034*). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C.*



A. R1 = R2 = H, R3 = Br: (3-bromo-4-hydroxyphenyl)(2-ethylbenzofuran-3-yl)methanone,

B. R1 = R2 = R3 = Br: (6-bromo-2-ethylbenzofuran-3-yl)(3,5-dibromo-4-hydroxyphenyl) methanone,

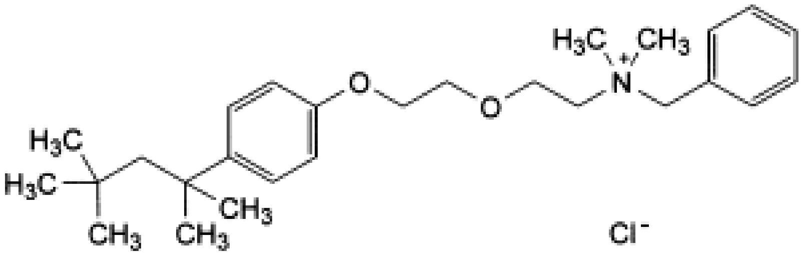
C. R1 = R2 = R3 = H: (2-ethylbenzofuran-3-yl)(4-hydroxyphenyl)methanone (benzarone).

Ph Eur

Benzethonium Chloride

General Notices

(Ph Eur monograph 0974)



$C_{27}H_{42}ClNO_2$ 448.1 *121-54-0*

Action and use

Antiseptic.

Ph Eur

DEFINITION

N-Benzyl-*N,N*-dimethyl-2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethanaminium chloride.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Very soluble in water and in ethanol (96 per cent), freely soluble in methylene chloride.

An aqueous solution froths copiously when shaken.

IDENTIFICATION

A. Melting point *(2.2.14)*: 158 °C to 164 °C, after drying at 105 °C for 4 h.

B. Thin-layer chromatography *(2.2.27).*

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 25 mg of *benzethonium chloride CRS* in *water R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R, water R, methanol R* (5:5:100 *V/V/V*).

Application 20 µl.

Development Over a path of 12 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 5 ml of *dilute sodium hydroxide solution R* add 0.1 ml of *bromophenol blue solution R1* and 5 ml of *methylene chloride R* and shake. The lower layer is colourless. Add 0.1 ml of solution S (see Tests) and shake. A blue colour develops in the lower layer.

D. To 2 ml of solution S add 1 ml of *dilute nitric acid R*. A white precipitate is formed which dissolves upon addition of 5 ml of *ethanol (96 per cent) R*. The solution gives reaction (a) of chlorides *(2.3.1).*

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II).*

Acidity or alkalinity

To 25 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Add 0.3 ml of *0.01 M sodium hydroxide*. The solution is pink. Add 0.1 ml of *methyl red solution R* and 0.5 ml of *0.01 M hydrochloric acid*. The solution is orange-red.

Volatile bases and salts of volatile bases *(2.4.1,*

Method B)

Maximum 50 ppm, determined on 0.20 g.

Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R.* Replace heavy magnesium oxide by 2.0 ml of *strong sodium hydroxide solution* R.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 2.000 g in *water R* and dilute to 100.0 ml with the same solvent. Transfer 25.0 ml of the solution to a separating funnel, add 10 ml of a 4 g/l solution of *sodium hydroxide R*, 10.0 ml of a freshly prepared 50 g/l solution of *potassium iodide R* and 25 ml of *methylene chloride R*. Shake vigorously, allow to separate and discard the lower layer. Shake the upper layer with 3 quantities, each of 10 ml, of *methylene chloride R* and discard the lower layers. To the upper layer add 40 ml of *hydrochloric acid R*, allow to cool and titrate with *0.05 M potassium iodate* until the deep brown colour is almost discharged. Add 4 ml of *methylene chloride R* and continue the titration, shaking vigorously, until the lower layer is no longer brown. Carry out a blank titration using a mixture of 10.0 ml of a freshly prepared 50 g/l solution of *potassium iodide R,* 20 ml of *water R* and 40 ml of *hydrochloric acid R*.

1 ml of *0.05 M potassium iodate* is equivalent to 44.81 mg of $C_{27}H_{42}ClNO_2$.

STORAGE

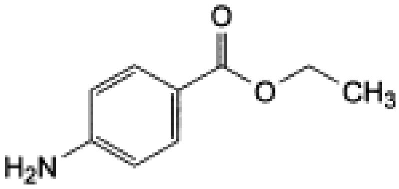
Protected from light.

Ph Eur

Benzocaine

General Notices

(Ph Eur monograph 0011)



$C_9H_{11}NO_2$ *165.2* 94-09-7

Action and use

Local anaesthetic.

Ph Eur

DEFINITION

Ethyl 4-aminobenzoate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very slightly soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 89 °C to 92 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison benzocaine CRS.

C. To about 50 mg in a test tube add 0.2 ml of a 500 g/l solution of *chromium trioxide R*. Cover the mouth of the tube with a piece of filter paper moistened with a freshly prepared mixture of equal volumes of a 50 g/l solution of *sodium nitroprusside R* and a 200 g/l solution of *piperazine hydrate R*. Boil gently for at least 30 s. A blue colour develops on the filter paper.

D. Dissolve about 50 mg in *ethanol (96 per cent) R* and dilute to 100 ml with the same solvent. 2 ml of the solution gives the reaction of primary aromatic amines *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 20 ml with the same solvent.

Acidity or alkalinity

Dissolve 0.5 g in 10 ml of *ethanol (96 per cent) R* previously neutralised to 0.05 ml of *phenolphthalein solution R*. Add 10 ml of *carbon dioxide-free water R*. The solution remains colourless and not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.00 g by drying *in vacuo*.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the determination of primary aromatic amino-nitrogen *(2.5.8)*, using 0.400 g dissolved in a mixture of 25 ml of *hydrochloric acid R* and 50 ml of *water R*.

1 ml of *0.1 M sodium nitrite* is equivalent to 16.52 mg of $C_9H_{11}NO_2$.

STORAGE

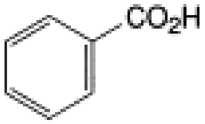
Protected from light.

Ph Eur

Benzoic Acid

General Notices

(Ph Eur monograph 0066)



$C_7H_6O_2$ *122.1* 65-85-0

Action and use

Antimicrobial preservative.

Preparations

Compound Benzoic Acid Ointment

Benzoic Acid Solution

Ph Eur

DEFINITION

Benzenecarboxylic acid.

Content

99.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Slightly soluble in water, soluble in boiling water, freely soluble in ethanol (96 per cent) and in fatty oils.

IDENTIFICATION

A. Melting point *(2.2.14)*: 121 °C to 124 °C.

B. Solution S (see Tests) gives reaction (a) of benzoates *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *ethanol (96 per cent) R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Carbonisable substances

Dissolve 0.5 g with shaking in 5 ml of *sulphuric acid R*. After 5 min, the solution is not more intensely coloured than reference solution Y_5 *(2.2.2, Method I)*.

Oxidisable substances

Dissolve 0.2 g in 10 ml of boiling *water R*. Cool, shake and filter. To the filtrate add 1 ml of *dilute sulphuric acid R* and 0.2 ml of *0.02 M potassium permanganate*. After 5 min, the solution is still coloured pink.

Halogenated compounds and halides

Maximum 300 ppm.

All glassware used must be chloride-free and may be prepared by soaking overnight in a 500 g/l solution of nitric acid R, rinsed with water R and stored full of water R. It is recommended that glassware be reserved for this test.

Solution (a) Dissolve 6.7 g in a mixture of 40 ml of *1 M sodium hydroxide* and 50 ml of *ethanol (96 per cent) R* and dilute to 100.0 ml with *water R*. To 10.0 ml of this solution add 7.5 ml of *dilute sodium hydroxide solution R* and 0.125 g of *nickel-aluminium alloy R* and heat on a water-bath for 10 min. Allow to cool to room temperature, filter into a 25 ml volumetric flask and wash with 3 quantities, each of 2 ml, of *ethanol (96 per cent) R*. Dilute the filtrate and washings to 25.0 ml with *water R*. This solution is used to prepare solution A.

Solution (b) In the same manner, prepare a similar solution without the substance to be examined. This solution is used to prepare solution B.

In four 25 ml volumetric flasks, place separately 10 ml of solution (a), 10 ml of solution (b), 10 ml of *chloride standard solution (8 ppm Cl) R* (used to prepare solution C) and 10 ml of *water R*. To each flask add 5 ml of *ferric ammonium sulphate solution R5*, mix and add dropwise and with swirling 2 ml of *nitric acid R* and 5 ml *of mercuric thiocyanate solution R*. Shake. Dilute the contents of each flask to 25.0 ml with *water R* and allow the solutions to stand in a water-bath at 20 °C for 15 min. Measure at 460 nm the absorbance *(2.2.25)* of solution A using solution B as the compensation liquid, and the absorbance of solution C using the solution obtained with 10 ml of *water R* as the compensation liquid. The absorbance of solution A is not greater than that of solution C.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test B. Prepare the reference solution using a mixture of 5 ml of *lead standard solution (1 ppm Pb) R* and 5 ml of *ethanol (96 per cent) R*.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

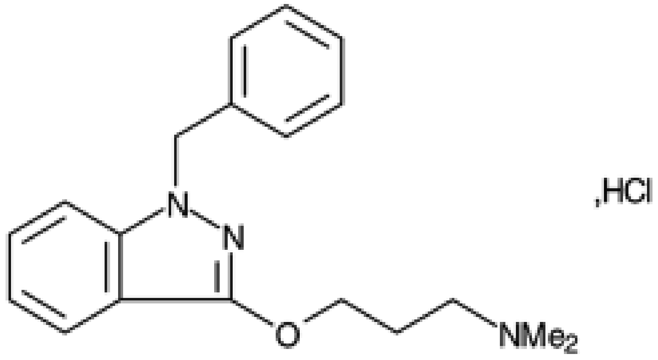
Dissolve 0.200 g in 20 ml of *ethanol (96 per cent) R* and titrate with *0.1 M sodium hydroxide*, using 0.1 ml of *phenol red solution R* as indicator, until the colour changes from yellow to violet-red.

1 ml of *0.1 M sodium hydroxide* is equivalent to 12.21 mg of $C_7H_6O_2$.

Ph Eur

Benzydamine Hydrochloride

General Notices



$C_{19}H_{23}N_3O,HCl$ 345.9 *132-69-4*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Benzydamine Cream

Benzydamine Mouthwash

Benzydamine Oromucosal Spray

DEFINITION

Benzydamine Hydrochloride is 3-(1-benzylindazol-3-yloxy)propyldimethylamine hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{19}H_{23}N_3O,HCl$, calculated with reference to the dried substance.

PRODUCTION

The method of manufacture is such that the level of 3-chloropropyl(dimethyl)amine hydrochloride is not more than 5 ppm when determined by a suitable method.

CHARACTERISTICS

A white crystalline powder.

Very soluble in *water*; freely soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference* *spectrum* of benzydamine hydrochloride *(RS 027)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Clarity and colour of solution

A 10.0% w/v solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* Y_6, Appendix IV B, Method II.

Acidity

pH of a 10% w/v solution, 4.0 to 5.5, Appendix V L.

Heavy metals

12 ml of a 10% w/v solution complies with *limit test A for heavy metals*, Appendix VII (10 ppm) . Use 1 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (50%)*. Solution (1) contains 0.25% w/v of the substance being examined. Solution (2) contains 0.0005% w/v of *3-dimethylaminopropyl 2-benzylaminobenzoate hydrochloride BPCRS* (impurity A) and 0.00125% w/v of 3-(*1,5-dibenzyl-1*H*-indazole-3-yl) oxypropyldimethylamine hydrochloride BPCRS* (impurity B). Solution (3) contains 0.00025% w/v of *1-benzyl-1*H*-indazol-3-ol BPCRS* (impurity C). Solution (4) contains 0.00025% w/v of the substance being examined. Solution (5) contains equal volumes of solutions (1), (2) and (3).

The chromatographic procedure may be carried out using a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Suplex pKB-100 is suitable), fitted with a stainless steel guard column (2 cm × 4.6 mm) packed with the same material. Use as the initial mobile phase with a flow rate of 1.5 ml per minute, a mixture of 50 volumes of solution A and 50 volumes of *methanol*. Solution A contains *0.01M potassium dihydrogen orthophosphate* and *0.005M sodium octyl sulphate* in *water*, adjusted to pH 3.0 ± 0.1 with *orthophosphoric acid*. Carry out a linear gradient elution increasing the percentage of *methanol* to 70% over 20 minutes from the moment of injection and then decreasing the percentage of *methanol* to 50% over 2 minutes and maintain the final mobile phase until the completion of that run. Use a detection wavelength of 320 nm.

Inject 20 µl of solution (5) and modify the rate of change of the mobile phase, if necessary, to obtain a retention time of about 10 minutes for the substance being examined.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between any two adjacent peaks is at least 2.5.

Inject 20 µl of solution (1) and allow the chromatography to proceed for 30 minutes. In the chromatogram obtained the areas of any peak corresponding to impurity A or impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.2% of impurity A and 0.5% of impurity B), the area of any peak corresponding to impurity C is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.1%) and the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.1%). The sum of the areas of any such peaks is not greater than 1%.

Primary amines

Dissolve 50 mg of the substance being examined in 10 ml of *ethanol (96%)*, add 0.1 ml of *hydrochloric acid* and 2 ml of a 5% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (96%)*. The yellow colour obtained is not more intense than that obtained by treating 10 ml of a 0.00005% w/v solution of *2-aminobenzoic acid* in *ethanol (96%)* in the same manner.

Loss on drying

When dried for 3 hours at 100° to 105° at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

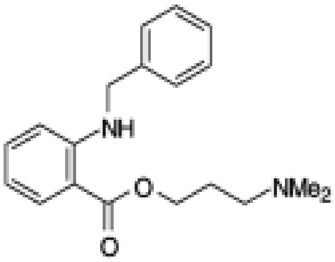
Sulphated ash

Not more than 0.1%, Appendix IX A. Use 1 g.

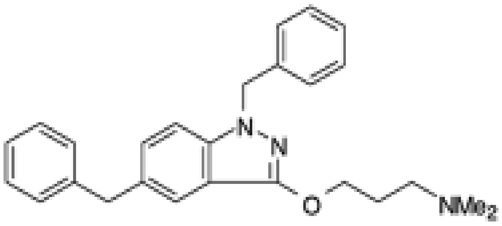
ASSAY

Dissolve 0.3 g in 100 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of *0.1M perchloric acid VS* is equivalent to 34.59 mg of $C_{19}H_{23}N_3O,HCl$.

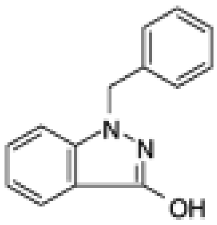
IMPURITIES



A. 3-dimethylaminopropyl 2-benzylaminobenzoate



B. 3-(1,5-dibenzyl-1*H*-indazol-3-yl)oxypropyldimethylamine

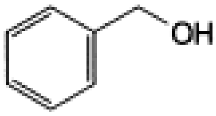


C. 1-benzyl-1*H*-indazol-3-ol

Benzyl Alcohol

General Notices

(Ph Eur monograph 0256)



C_7H_8O 08.1 *100-51-6*

Action and use

Local anaesthetic; disinfectant.

Ph Eur

DEFINITION

Phenylmethanol.

Content

98.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

Clear, colourless, oily liquid.

Solubility

Soluble in water, miscible with ethanol (96 per cent) and with fatty and essential oils.

Relative density

1.043 to 1.049.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24)*.

Comparison benzyl alcohol CRS.

TESTS

Appearance of solution

Shake 2.0 ml with 60 ml of *water R*. It dissolves completely. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II*).

Acidity

To 10 ml add 10 ml of *ethanol (96 per cent) R* and 1 ml of *phenolphthalein solution R*. Not more than 1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Refractive index *(2.2.6)*

1.538 to 1.541.

Peroxide value *(2.5.5)*:

Maximum 5.

Related substances

Gas chromatography *(2.2.28)*.

Test solution The substance to be examined.

Standard solution (a) Dissolve 0.100 g of *ethylbenzene R* in the test solution and dilute to 10.0 ml with the same solution. Dilute 2.0 ml of this solution to 20.0 ml with the test solution.

Standard solution (b) Dissolve 2.000 g of *dicyclohexyl R* in the test solution and dilute to 10.0 ml with the same solution. Dilute 2.0 ml of this solution to 20.0 ml with the test solution.

Reference solution (a) Dissolve 0.750 g of *benzaldehyde R* and 0.500 g of *cyclohexylmethanol R* in the test solution and dilute to 25.0 ml with the test solution. Add 1.0 ml of this solution to a mixture of 2.0 ml of standard solution (a) and 3.0 ml of standard solution (b) and dilute to 20.0 ml with the test solution.

Reference solution (b) Dissolve 0.250 g of *benzaldehyde R* and 0.500 g of *cyclohexylmethanol R* in the test solution and dilute to 25.0 ml with the test solution. Add 1.0 ml of this solution to a mixture of 2.0 ml of standard solution (a) and 2.0 ml of standard solution (b) and dilute to 20.0 ml with the test solution.

Column:

— *material*: fused silica;

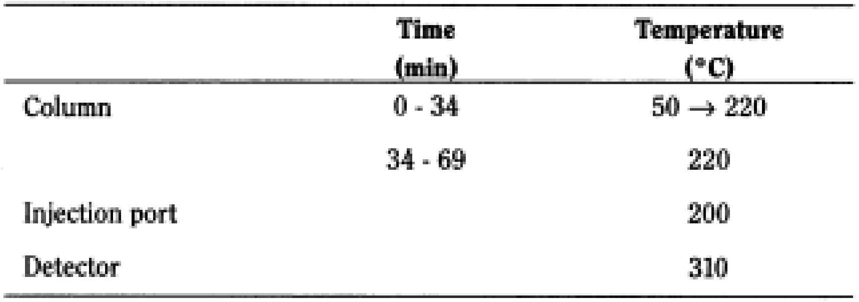
— *size*: *l* = 30 m, Ø = 0.32 mm;

— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 25 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 34	50 → 220
	34 - 69	220
Injection port		200
Detector		310

Detection Flame ionisation.

Benzyl alcohol not intended for parenteral use

Injection Without air-plug, 0.1 µl of the test solution and reference solution (a).

Relative retention With reference to benzyl alcohol (retention time = about 26 min): ethylbenzene = about 0.28; dicyclohexyl = about 0.59; impurity A = about 0.68; impurity B = about 0.71.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to impurity A and impurity B.

In the chromatogram obtained with the test solution, verify that there are no peaks with the same retention time as ethylbenzene and dicyclohexyl.

Limits:

— *impurity A*: not more than the difference between the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) and the area of the peak due to impurity A in the chromatogram obtained with the test solution (0.15 per cent);

— *impurity B*: not more than the difference between the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) and the area of the peak due to impurity B in the chromatogram obtained with the test solution (0.10 per cent);

— *sum of other peaks with a relative retention less than that of benzyl alcohol*: not more than 4 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (a) (0.04 per cent);

— *sum of peaks with a relative retention greater than that of benzyl alcohol*: not more than the area of the peak due to dicyclohexyl in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.01 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (a) (0.0001 per cent).

Benzyl alcohol intended for parenteral use

Injection Without air-plug, 0.1 µl of the test solution and reference solution (b).

Relative retention With reference to benzyl alcohol (retention time = about 26 min): ethylbenzene = about 0.28; dicyclohexyl = about 0.59; impurity A = about 0.68; impurity B = about 0.71.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to impurity A and impurity B.

In the chromatogram obtained with the test solution, verify that there are no peaks with the same retention time as the standards.

Limits:

— *impurity A*: not more than the difference between the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) and the area of the peak due to impurity A in the chromatogram obtained with the test solution (0.05 per cent);

— *impurity B*: not more than the difference between the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) and the area of the peak due to impurity B in the chromatogram obtained with the test solution (0.10 per cent);

— *sum of other peaks with a relative retention less than that of benzyl alcohol*: not more than twice the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (b) (0.02 per cent);

— *sum of peaks with a relative retention greater than that of benzyl alcohol*: not more than the area of the peak due to dicyclohexyl in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard limit*: 0.01 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (b) (0.0001 per cent).

Residue on evaporation

Maximum 0.05 per cent.

After ensuring that the substance to be examined complies with the test for peroxide value, evaporate 10.0 g to dryness in a tared quartz or porcelain crucible or platinum dish on a hot plate at a temperature not exceeding 200 °C. Ensure that the substance to be examined does not boil during evaporation. Dry the residue on the hot plate for 1 h and allow to cool in a desiccator. The residue weighs a maximum of 5 mg.

ASSAY

To 0.900 g (*m* g) add 15.0 ml of a freshly prepared mixture of 1 volume of *acetic anhydride R* and 7 volumes of *pyridine R* and boil under a reflux condenser on a water-bath for 30 min. Cool and add 25 ml of *water R*. Using 0.25 ml of *phenolphthalein solution R* as indicator, titrate with *1 M sodium hydroxide* (n_1 ml). Carry out a blank titration (n_2 ml).

Calculate the percentage content of C_7H_8O using the following expression:

$$\frac{10.81\left(n_2 - n_1\right)}{m}$$

STORAGE

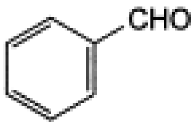
In an airtight container, under nitrogen, protected from light and at a temperature between 2 °C and 8 °C.

LABELLING

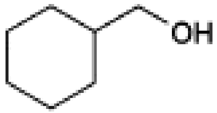
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES

Specified impurities A, B.



A. benzaldehyde,



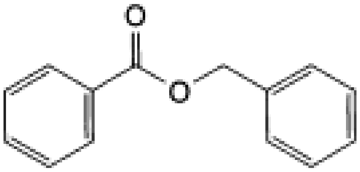
B. cyclohexylmethanol.

Ph Eur

Benzyl Benzoate

General Notices

(Ph Eur monograph 0705)



$C_{14}H_{12}O_2$ *212.2* 120-51-4

Action and use

Used topically in the treatment of scabies.

Preparation

Benzyl Benzoate Application

Ph Eur

DEFINITION

Phenylmethyl benzoate.

Content

99.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

Colourless or almost colourless crystals or colourless or almost colourless, oily liquid.

Solubility

Practically insoluble in water, miscible with ethanol (96 per cent), with methylene chloride and with fatty and essential oils.

Eb: about 320 °C.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   Ph. Eur. reference spectrum of benzyl benzoate.

B. To 2 g add 25 ml of *alcoholic potassium hydroxide solution R* and boil under a reflux condenser for 2 h. Remove the ethanol on a water-bath, add 50 ml of *water R* and distill. Collect about 25 ml of distillate and use it for identification test C. Acidify the liquid remaining in the distillation flask with *dilute hydrochloric acid R*. A white precipitate is formed that, when washed with *water R* and dried in vacuo melts *(2.2.14)* at 121 °C to 124 °C.

C. To the distillate obtained in identification test B add 2.5 g of *potassium permanganate R* and 5 ml of *dilute sodium hydroxide solution R*. Boil under a reflux condenser for 15 min, cool and filter. Acidify the filtrate with *dilute hydrochloric acid R*. A white precipitate is formed that, when washed with *water R* and dried *in vacuo*, melts *(2.2.14)* at 121 °C to 124 °C.

TESTS

Acidity

Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent. Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution R* as indicator. Not more than 0.2 ml is required to change the colour of the indicator to pink.

Relative density *(2.2.5)*

1.118 to 1.122.

Refractive index *(2.2.6)*

1.568 to 1.570.

Freezing point *(2.2.18)*

Minimum 17.0 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 2.000 g add 50.0 ml of *0.5 M alcoholic potassium hydroxide* and boil gently under a reflux condenser for 1 h. Titrate the hot solution with *0.5 M hydrochloric acid* using 1 ml of *phenolphthalein solution R* as indicator. Carry out a blank determination.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 106.1 mg of $C_{14}H_{12}O_2$.

STORAGE

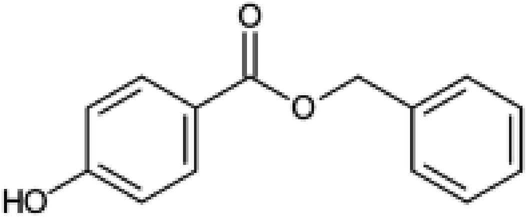
In an airtight, well-filled container, protected from light.

Ph Eur

Benzyl Hydroxybenzoate

General Notices

Benzylparaben



$C_{14}H_{12}O_3$ 228.3 *94-18-8*

Action and use

Antimicrobial preservative.

DEFINITION

Benzyl Hydroxybenzoate is benzyl 4-hydroxybenzoate. It contains not less than 99.0% and not more than 101.0% of $C_{14}H_{12}O_3$.

CHARACTERISTICS

A white to creamy white, crystalline powder.

Practically insoluble in *water*; freely soluble in *ethanol (96%)* and in *ether*. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of benzyl hydroxybenzoate *(RS 028)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in *ethanol (96%)* exhibits a maximum only at 260 nm. The *absorbance* at the maximum at 260 nm is about 0.76.

C. Dissolve 0.1 g in 2 ml of *ethanol (96%)*, boil and add 0.5 ml of *nitric acid solution of mercury*. A precipitate is produced slowly and the supernatant liquid becomes red.

D. *Melting point*, about 112°, Appendix V A.

TESTS

Acidity

Dissolve 0.2 g in 10 ml of *ethanol (50%)* previously neutralised to *methyl red solution* and titrate with 0.1M *sodium hydroxide VS* using *methyl red solution* as indicator. Not more than 0.1 ml of *0.1M sodium hydroxide VS* is required to change the colour of the solution.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a plate precoated with silica gel F_{254}, the surface of which has been modified with chemically-bonded octadecylsilyl groups (Whatman KC18F plates are suitable) and a mixture of 70 volumes of *methanol*, 30 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase. Apply separately to the plate 2 µl of each of two solutions of the substance being examined in *acetone* containing (1) 1.0% w/v and (2) 0.010% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Sulphated ash

Not more than 0.1%, Appendix IX A.

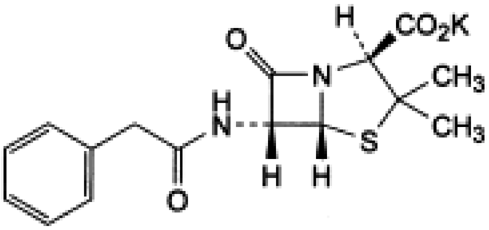
ASSAY

Gently boil 0.12 g under a reflux condenser with 20 ml of *2M sodium hydroxide* for 30 minutes. Cool and extract with three 20-ml quantities of *1,2-dichloroethane*. Wash the combined extracts with 20 ml of *0.1M sodium hydroxide* and add the washings to the main aqueous phase, discarding the organic layer. To the aqueous solution add 25 ml of *0.0333M potassium bromate VS*, 5 ml of a 12.5% w/v solution of *potassium bromide* and 10 ml of *hydrochloric acid* and immediately stopper the flask. Shake for 15 minutes and allow to stand for 15 minutes. Add 25 ml of *dilute potassium iodide solution* and shake vigorously. Titrate the liberated iodine with *0.1M sodium thiosulphate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of potassium bromate required. The volume of *0.0333M potassium bromate VS* used is equivalent to half of the volume of *0.1M sodium thiosulphate VS* required for the titration. Each ml of *0.0333M potassium bromate VS* is equivalent to 7.608 mg of $C_{14}H_{12}O_3$.

Benzylpenicillin Potassium

General Notices

(Ph Eur monograph 0113)



$C_{16}H_{17}KN_2O_4S$ 372.5 *113-98-4*

Action and use

Penicillin antibacterial.

Preparation

Benzylpenicillin Injection

Ph Eur

DEFINITION

Potassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Substance produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Content

96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, practically insoluble in fatty oils and in liquid paraffin.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *benzylpenicillin potassium CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *water R*.

Reference solution (a) Dissolve 25 mg of *benzylpenicillin potassium CRS* in 5 ml of *water R*.

Reference solution (b) Dissolve 25 mg of *benzylpenicillin potassium CRS* and 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 ml of *water R*.

Plate *TLC silanised silica gel plate R*.

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube on a water-bath for 1 min; a reddish-brown colour develops.

D. It gives reaction (a) of potassium *(2.3.1)*.

TESTS

pH *(2.2.3)*

5.5 to 7.5.

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 270 to + 300 (dried substance).

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Absorbance *(2.2.25)*

Dissolve 94.0 mg in *water R* and dilute to 50.0 ml with the same solvent. Measure the absorbance of the solution at 325 nm, 280 nm and at the absorption maximum at 264 nm, diluting the solution, if necessary, for the measurement at 264 nm. The absorbances at 325 nm and 280 nm do not exceed 0.10 and that at the absorption maximum at 264 nm is 0.80 to 0.88, calculated on the basis of the undiluted (1.88 g/l) solution. Verify the resolution of the apparatus *(2.2.25)*; the ratio of the absorbances is at least 1.7.

Related substances

Liquid chromatography *(2.2.29)*.

Prepare the solutions immediately before use.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dissolve 80.0 mg of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

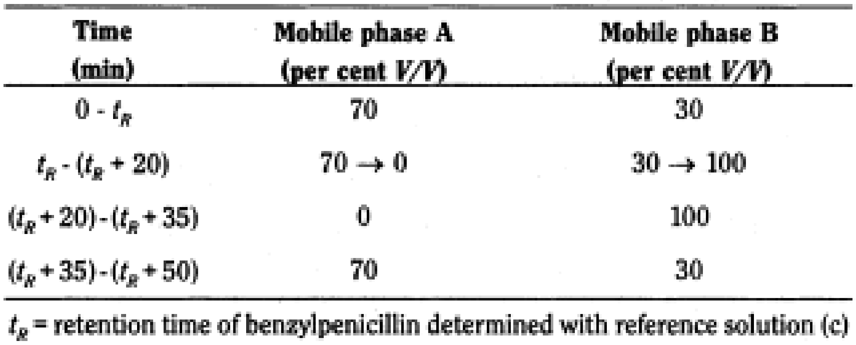
Reference solution (a) Dissolve 50.0 mg of *benzylpenicillin sodium CRS* in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *benzylpenicillin sodium CRS* and 10 mg of *phenylacetic acid R* (impurity B) in *water R*, then dilute to 50 ml with the same solvent.

Reference solution (c) Dilute 4.0 ml of reference solution (a) to 100.0 ml with *water R*.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: mix 10 volumes of a 68 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with a 500 g/l solution of *dilute phosphoric acid R*, 30 volumes of *methanol R* and 60 volumes of *water R*;
- *mobile phase B*: mix 10 volumes of a 68 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with a 500 g/l solution of *dilute phosphoric acid R*, 40 volumes of *water R* and 50 volumes of *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	70	30
t_R - (t_R + 20)	70 → 0	30 → 100
(t_R + 20) - (t_R + 35)	0	100
(t_R + 35) - (t_R + 50)	70	30

t_R = retention time of benzylpenicillin determined with reference solution (c)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl of reference solutions (b) and (c) with isocratic elution at the initial mobile phase composition and 20 µl of test solution (b) according to the elution gradient described

under Mobile phase; inject *water R* as a blank according to the elution gradient described under Mobile phase.

System suitability Reference solution (b):

— *resolution*: minimum 6.0 between the peaks due to impurity B and benzylpenicillin; if necessary, adjust the ratio A:B of the mobile phase.

Limit:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent).

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Bacterial endotoxins ***(2.6.14, Method E)***

Less than 0.16 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

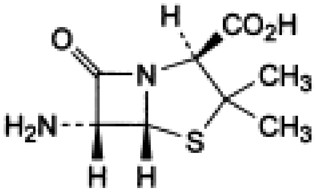
Injection Test solution (a) and reference solution (a).

Calculate the percentage content of $C_{16}H_{17}KN_2O_4S$ by multiplying the percentage content of benzylpenicillin sodium by 1.045.

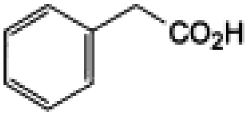
STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

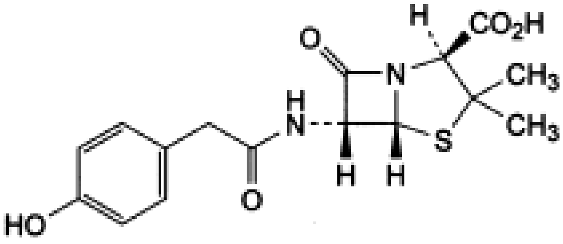
IMPURITIES



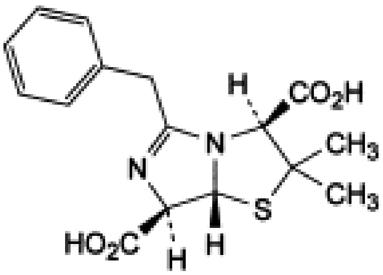
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



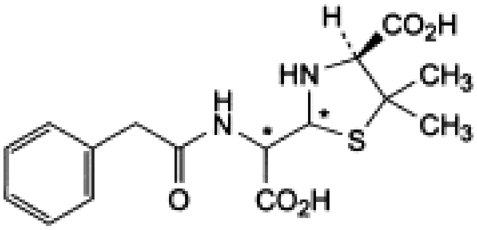
B. phenylacetic acid,



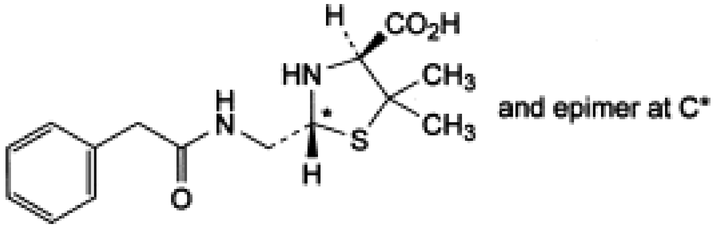
C. (2*S*,5*R*,6*R*)-6-[[(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid,



D. (3*S*,7*R*,7a*R*)-5-benzyl-2,2-dimethyl-2,3,7,7a-tetrahydroimidazo[5,1-*b*]thiazole-3,7-dicarboxylic acid (penillic acid of benzylpenicillin),



E. (4*S*)-2-[carboxy[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of benzylpenicillin),



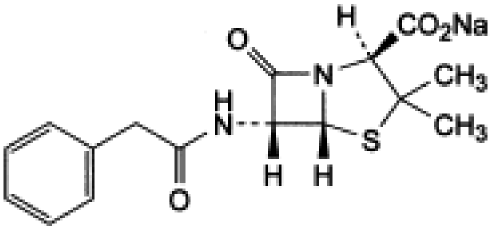
F. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin).

Ph Eur

Benzylpenicillin Sodium

General Notices

(Ph Eur monograph 0114)



$C_{16}H_{17}N_2NaO_4S$ 356.4   *69-57-8*

Action and use

Penicillin antibacterial.

Preparation

Benzylpenicillin Injection

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Substance produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Content

96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, practically insoluble in fatty oils and in liquid paraffin.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *benzylpenicillin sodium CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *water R*.

Reference solution (a) Dissolve 25 mg of *benzylpenicillin sodium CRS* in 5 ml of *water R*.

Reference solution (b) Dissolve 25 mg of *benzylpenicillin sodium CRS* and 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 ml of *water R*.

Plate *TLC silanised silica gel plate R*.

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results

The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube on a water-bath for 1 min; a reddish-brown colour develops.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

pH *(2.2.3)*

5.5 to 7.5.

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 285 to + 310 (dried substance).

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Absorbance *(2.2.25)*

Dissolve 90.0 mg in *water R* and dilute to 50.0 ml with the same solvent. Measure the absorbance of the solution at 325 nm, at 280 nm and at the absorption maximum at 264 nm, diluting the solution, if necessary, for the measurement at 264 nm. The absorbances at 325 nm and 280 nm are not greater than 0.10 and the absorbance at the absorption maximum at 264 nm is 0.80 to 0.88, calculated on the basis of the undiluted (1.80 g/l) solution. Verify the resolution of the apparatus *(2.2.25)*; the ratio of the absorbances is at least 1.7.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dissolve 80.0 mg of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

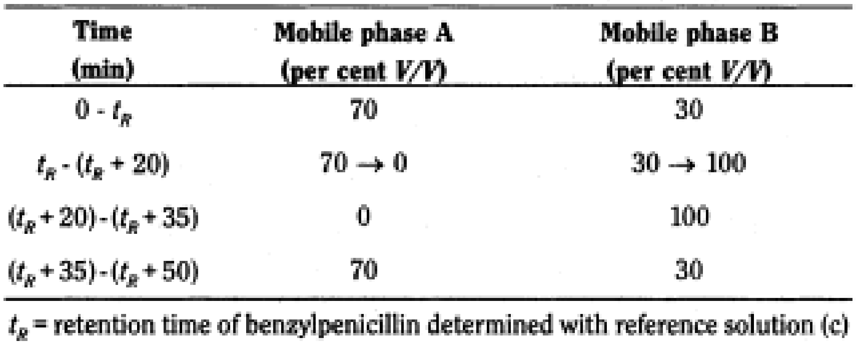
Reference solution (a) Dissolve 50.0 mg of *benzylpenicillin sodium CRS* in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *benzylpenicillin sodium CRS* and 10 mg of *phenylacetic acid R* (impurity B) in *water R*, then dilute to 50 ml with the same solvent.

Reference solution (c) Dilute 4.0 ml of reference solution (a) to 100.0 ml with *water R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: mix 10 volumes of a 68 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with a 500 g/l solution of *dilute phosphoric acid* R, 30 volumes of *methanol R* and 60 volumes of *water R*;
- *mobile phase B*: mix 10 volumes of a 68 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with a 500 g/l solution of *dilute phosphoric acid R*, 40 volumes of *water R* and 50 volumes of *methanol R*;



Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - t_R	70	30
t_R - (t_R + 20)	70 → 0	30 → 100
(t_R + 20) - (t_R + 35)	0	100
(t_R + 35) - (t_R + 50)	70	30

t_R = retention time of benzylpenicillin determined with reference solution (c)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl of reference solutions (b) and (c) with isocratic elution at the initial mobile phase composition and 20 µl of test solution (b) according to the elution gradient described under Mobile phase; inject *water R* as a blank according to the elution gradient described under Mobile phase.

System suitability Reference solution (b):

— *resolution*: minimum 6.0 between the peaks due to impurity B and benzylpenicillin; if necessary, adjust the ratio A:B of the mobile phase.

Limit:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent).

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.5 per cent *m/m*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Bacterial endotoxins *(2.6.14, Method E)*

Less than 0.16 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

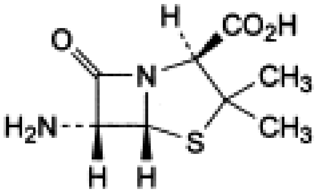
Injection Test solution (a) and reference solution (a).

Calculate the percentage content of $C_{16}H_{17}N_2NaO_4S$ from the declared content of *benzylpenicillin sodium CRS*.

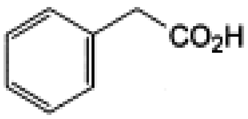
STORAGE

In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

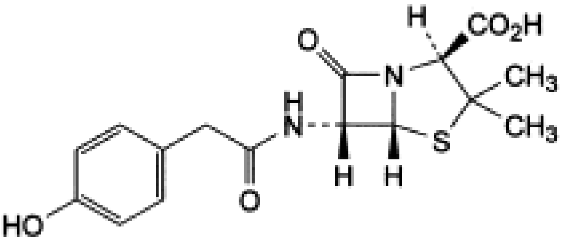
IMPURITIES



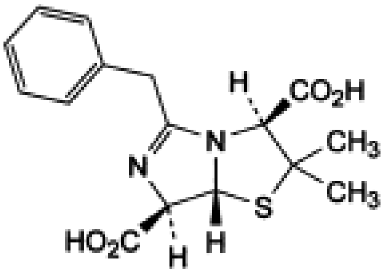
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



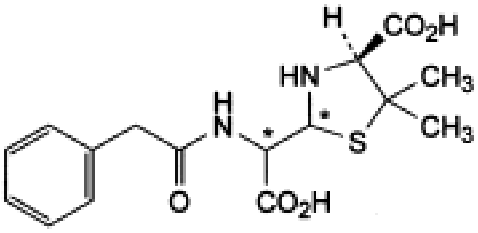
B. phenylacetic acid,



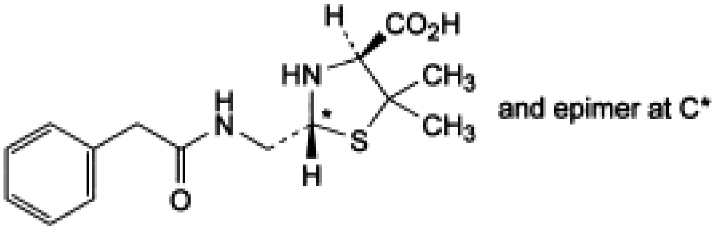
C. (2*S*,5*R*,6*R*)-6-[[(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia- 1-azabicyclo [3.2.0]heptane-2-carboxylic acid,



D. (3*S*,7*R*,7a*R*)-5-benzyl-2,2-dimethyl-2,3,7,7a-tetrahydroimidazo[5,1-*b*]thiazole-3,7-dicarboxylic acid (penillic acid of benzylpenicillin),



E. (4*S*)-2-[carboxy[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of benzylpenicillin),



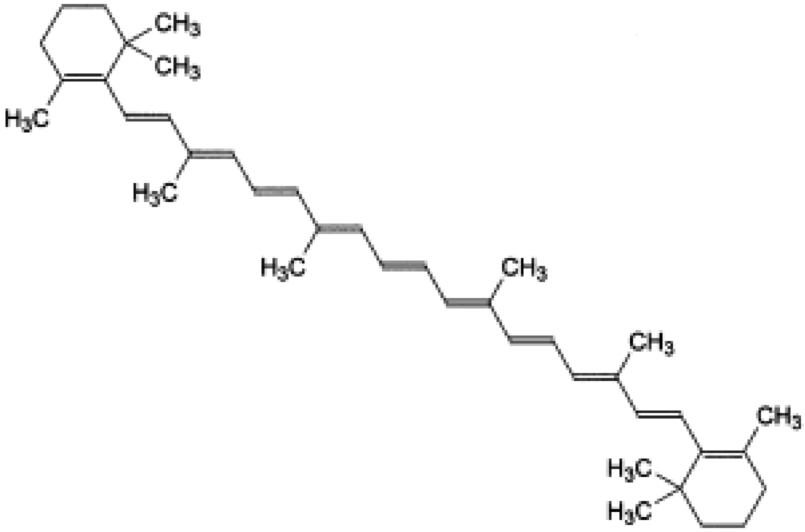
F. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin).

Ph Eur

Betacarotene

General Notices

(Ph Eur monograph 1069)



$C_{40}H_{56}$ *536.9* 7235-40-7

Action and use

Precursor of vitamin A.

Ph Eur

DEFINITION

(all-*E*)-3,7,12,16-Tetramethyl-1,18-bis(2,6,6-trimethylcyclohex-1-enyl)octadeca-1,3,5,7,9,11,13,15,17-nonaene.

Content

96.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Brown-red or brownish-red, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in cyclohexane, practically insoluble in anhydrous ethanol.

It is sensitive to air, heat and light, especially in solution.

Carry out all operations as rapidly as possible avoiding exposure to actinic light; use freshly prepared solutions.

IDENTIFICATION

Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution (a) Dissolve 50.0 mg in 10 ml of *chloroform R* and dilute immediately to 100.0 ml with *cyclohexane R*. Dilute 5.0 ml of this solution to 100.0 ml with *cyclohexane R*.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with *cyclohexane R*.

Absorption maximum At 455 nm for test solution (b).

Absorbance ratio A_{455} / A_{483} = 1.14 to 1.18 for test solution (b).

TESTS

Related substances

Determine the absorbance *(2.2.25)* of test solutions (b) and (a) used in Identification, at 455 nm and at 340 nm respectively.

Absorbance ratio A_{455} / A_{340}: minimum 1.5.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.2 per cent, determined on 1.000 g by drying *in vacuo* over *diphosphorus pentoxide R* at 40 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g, moistened with a mixture of 2 ml of *dilute sulphuric acid R* and 5 ml of *ethanol (96 per cent) R*.

ASSAY

Measure the absorbance *(2.2.25)* of test solution (b) used in Identification at the absorption maximum at 455 nm, using *cyclohexane R* as the compensation liquid.

Calculate the content of $C_{40}H_{56}$ taking the specific absorbance to be 2500.

STORAGE

In an airtight container , protected from light, at a temperature not exceeding 25 °C.

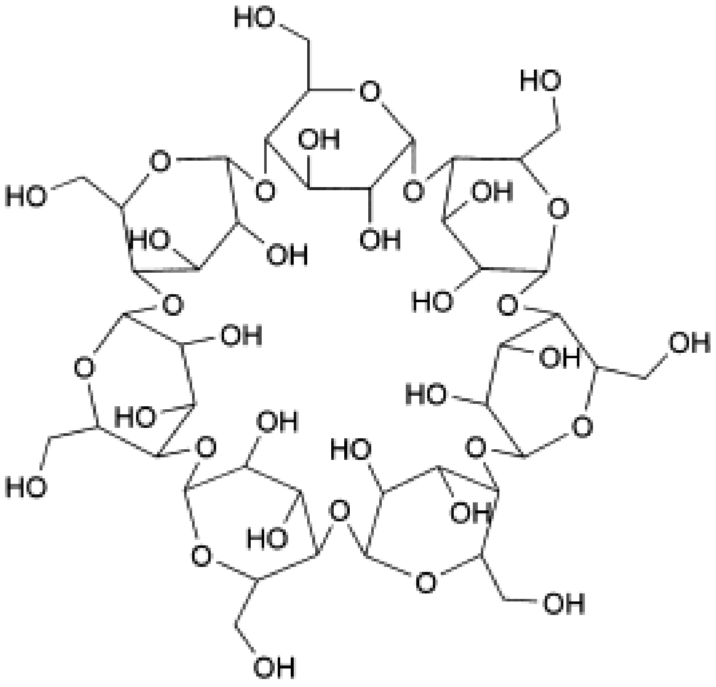
Ph Eur

Betadex

General Notices

Betacyclodextrin

(Ph Eur monograph 1070)



$[C_6H_{10}O_5]_7$ *1135* 68168-23-0

(monohydrate)

Action and use

Carrier molecule for drug delivery systems.

Ph Eur

DEFINITION

Cyclo-α-(1→4)-D-heptaglucopyranoside (betacyclodextrin).

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, amorphous or crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in propylene glycol, practically insoluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

C. Dissolve 0.2 g in 2 ml of *iodine solution R4* by warming on a water-bath, and allow to stand at room temperature. A yellowish-brown precipitate is formed.

TESTS

Solution S

Dissolve 1.000 g in *carbon dioxide-free water R* with heating, allow to cool and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

pH *(2.2.3)*

5.0 to 8.0.

To 10 ml of solution S add 0.1 ml of a saturated solution of *potassium chloride R*.

Specific optical rotation *(2.2.7)*

+ 160 to + 164 (dried substance), determined on solution S.

Reducing sugars

Maximum 0.2 per cent.

Test solution To 1 ml of solution S add 1 ml of *cupri-tartaric solution R4*. Heat on a water-bath for 10 min, cool to room temperature. Add 10 ml of *ammonium molybdate reagent R1* and allow to stand for 15 min.

Reference solution Prepare a reference solution at the same time and in the same manner as the test solution, using 1 ml of a 0.02 g/l solution of *glucose R*.

Measure the absorbance *(2.2.25)* of the test solution and the reference solution at the absorption maximum at 740 nm using *water R* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution.

Light-absorbing impurities

Examine solution S between 230 nm and 750 nm. Between 230 nm and 350 nm, the absorbance *(2.2.25)* is not greater than 0.10. Between 350 nm and 750 nm, the absorbance *(2.2.25)* is not greater than 0.05.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 0.25 g of the substance to be examined in *water R* with heating, cool and dilute to 25.0 ml with the same solvent.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with *water R*.

Reference solution (a) Dissolve 25.0 mg of *alfadex CRS* (impurity A), 25.0 mg of *gammacyclodextrin CRS* (impurity B) and 50.0 mg of *betadex CRS* in *water R*, then dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water R*.

Reference solution (c) Dissolve 25.0 mg of *betadex CRS* in *water R* and dilute to 25.0 ml with the same solvent.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase: octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase *methanol R, water R* (10:90 *V/V*).

Flow rate 1.5 ml/min.

Detection Differential refractometer.

Equilibration With the mobile phase for about 3 h.

Injection 50 µl of test solution (a) and reference solutions (a) and (b).

Run time 1.5 times the retention time of betadex.

Relative retention With reference to betadex (retention time = about 10 min): impurity B = about 0.3; impurity A = about 0.45.

System suitability Reference solution (a):
- — *resolution*: minimum 1.5 between the peaks due to impurities B and A; if necessary, adjust the concentration of methanol in the mobile phase.

Limits:
- — *impurities A, B*: for each impurity, not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- — *sum of impurities other than A and B*: not more than 0.5 times the area of the peak due to betadex in the chromatogram obtained with reference solution (b) (0.5 per cent).

Residual solvents

Head-space gas chromatography *(2.2.28)*: use the standard additions method.

Internal standard *ethylene chloride R*.

Test solutions In each of 4 identical 20 ml flasks, dissolve 0.5 g of the substance to be examined in *water R* and add 0.10 g of *calcium chloride R* and 30 µl of α-*amylase solution R*.

Add 1 ml of reference solutions (a), (b), (c) and (d), adding a different solution to each flask. Dilute to 10 ml with *water R*.

Reference solutions Prepare a 10 µl/l solution of *ethylene chloride R* (reference solution (a)). Prepare reference solutions (b), (c) and (d) from reference solution (a) to contain respectively, per litre, 5 µl, 10 µl and 15 µl of both *trichloroethylene R* and *toluene R*.

Column:
— *material*: fused silica;

— *size*: *l* = 25 m, Ø = 0.32 mm;

— *stationary phase: macrogol 20 000 R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Static head-space conditions which may be used:
— *equilibration temperature: 45 °C;*

— *equilibration time: 2 h.*

Temperature:
— *column*: 50 °C;

— *injection port*: 140 °C;

— *detector*: 280 °C.

Detection Flame ionisation.

Injection 200 µl of the head space, at least 3 times.

Retention time toluene = about 10 min.

System suitability:
— *resolution*: minimum 1.1 between the peaks due to trichloroethylene and toluene; minimum 1.1 between the peaks due to toluene and ethylene chloride;

— *repeatability*: maximum relative standard deviations of the ratios of the areas of the peaks due to trichloroethylene and toluene to that of the peak due to ethylene chloride of 5 per cent.

Calculate the content of trichloroethylene and of toluene taking their relative densities to be 1.46 and 0.87, respectively.

Limits:
— *trichloroethylene*: maximum 10 ppm;

— *toluene*: maximum 10 ppm.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

1.0 g complies with test C. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 16.0 per cent, determined on 1.000 g by drying in an oven at 120 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solutions (a) and (c).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of the area of the peak due to betadex of 2.0 per cent.

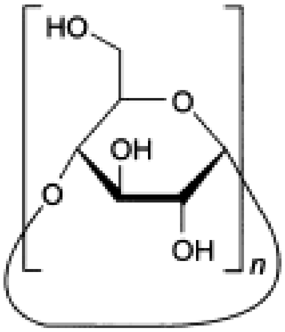
Calculate the percentage content of $[C_6H_{10}O_5]_7$ from the declared content of *betadex CRS*.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities *A, B*.



A. *n* = 6: alfadex,

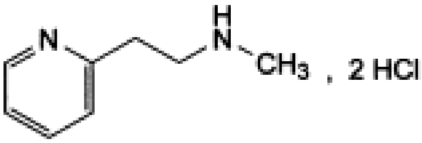
B. *n* = 8: gammacyclodextrin.

Ph Eur

Betahistine Dihydrochloride

General Notices

(Ph Eur monograph 1665)



$C_8H_{12}N_2$,2HCl 209.1 *5579-84-0*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Betahistine Dihydrochloride Tablets

Ph Eur

DEFINITION

N-Methyl-2-(pyridin-2-yl)ethanamine dihydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White to slightly yellow powder, very hygroscopic.

Solubility

Very soluble in water, soluble in ethanol (96 per cent), practically insoluble in 2-propanol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 150 °C to 154 °C.

B. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *betahistine dihydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27).*

Test solution Dissolve 10 mg of the substance to be examined in 2 ml of *ethanol (96 per cent) R*.

Reference solution Dissolve 10 mg of *betahistine dihydrochloride CRS* in 2 ml of *ethanol (96 per cent) R*.

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *concentrated ammonia R, ethyl acetate R, methanol R* (0.75:15:30 *V/V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Drying At 110 °C for 10 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R*, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_8 *(2.2.2, Method II).*

pH *(2.2.3)*

2.0 to 3.0 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 10 mg of *betahistine dihydrochloride CRS* and 10 mg of *2-vinylpyridine R* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 3.0 mm,

— *stationary phase*: *end-capped octadecylsilyl base deactivated silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 2.0 g of *sodium dodecyl sulphate R* in a mixture of 15 ml of a 10 per cent *V/V* solution of *sulphuric acid R*, 35 ml of a 17 g/l solution of *tetrabutylammonium hydrogen sulphate R* and 650 ml of *water R*; adjust to pH 3.3 using *dilute sodium hydroxide solution R* and mix with 300 ml of *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µl.

Run time 4 times the retention time of betahistine.

Relative retention With reference to betahistine (retention time = about 7 min): impurity B = about 0.2; impurity A = about 0.3; impurity C = about 3.

System suitability: reference solution (a):

— *resolution*: minimum 3.5 between the peaks due to 2-vinylpyridine and betahistine.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 0.4;

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times of the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 80.0 mg in 50 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Read the volume added to reach the second point of inflexion.

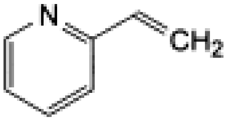
1 ml of *0.1 M sodium hydroxide* is equivalent to 10.46 mg of $C_8H_{14}Cl_2N_2$.

STORAGE

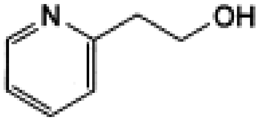
In an airtight container .

IMPURITIES

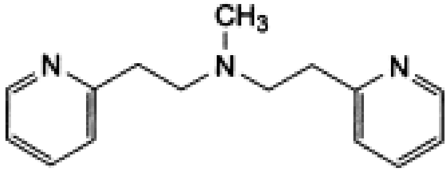
Specified impurities   A, B, C.



A. 2-ethenylpyridine (2-vinylpyridine),



B. 2-(pyridin-2-yl)ethanol,



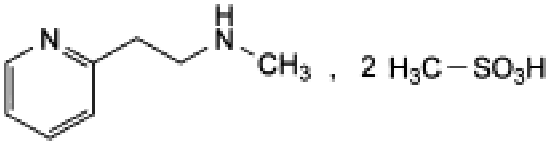
C. *N*-methyl-2-(pyridin-2-yl)-*N*-[2-(pyridin-2-yl)ethyl]ethanamine.

Ph Eur

Betahistine Mesilate

General Notices

(Ph Eur monograph 1071)



$C_8H_{12}N_2,2CH_3SO_3H$ *328.4* 54856-23-4

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

N-Methyl-2-(pyridin-2-yl)ethanamine bis(methanesulphonate).

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

White or almost white, crystalline powder, very hygroscopic.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), very slightly soluble in 2-propanol.

IDENTIFICATION

First identification B.

Second identification *A, C, D*.

A. Melting point *(2.2.14)*: 108 °C to 112 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *betahistine mesilate CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 2 ml with the same solvent.

Reference solution Dissolve 10 mg of *betahistine mesilate CRS*, in *ethanol (96 per cent) R* and dilute to 2 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *concentrated ammonia R, ethyl acetate R, methanol R* (0.75:15:30 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying At 110 °C for 10 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 0.1 g add 5 ml of *dilute hydrochloric acid R* and shake for about 5 min. Add 1 ml of *barium chloride solution R1*. The solution remains clear. To a further 0.1 g add 0.5 g of *anhydrous sodium carbonate R*, mix and ignite until a white residue is obtained. Allow to cool and dissolve the residue in 7 ml of *water R*. The solution gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R*, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

2.0 to 3.0 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 10 mg of *betahistine mesilate CRS* and 10 mg of *2-vinylpyridine R* (impurity A) in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 2.0 g of *sodium dodecyl sulphate R* in a mixture of 15 volumes of a 10 per cent V/V solution of *sulphuric acid R*, 35 volumes of a 17 g/l solution of *tetrabutylammoniumhydrogen sulphate R* and 650 volumes of *water R*; adjust to pH 3.3 using *dilute sodium hydroxide solution R* and mix with 300 volumes of *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µl.

Run time 3 times the retention time of betahistine mesilate.

Retention time betahistine mesilate = about 8 min.

System suitability Reference solution (a):

- *resolution*: minimum 3.5 between the peaks due to impurity A and betahistine mesilate.

Limits:

- *impurity A:* not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.025 per cent).

2-Propanol ***(2.4.24)***

Maximum 0.5 per cent.

Chlorides ***(2.4.4)***

Maximum 35 ppm.

To 14 ml of solution S add 1 ml of *water R*.

Sulphates ***(2.4.13)***

Maximum 250 ppm.

Dilute 6 ml of solution S to 15 ml with *distilled water R*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 2.0 per cent, determined on 0.50 g.

ASSAY

Dissolve 0.140 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

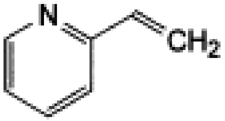
1 ml of *0.1 M perchloric acid* is equivalent to 16.42 mg of $C_{10}H_{20}N_2O_6S_2$.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities A.



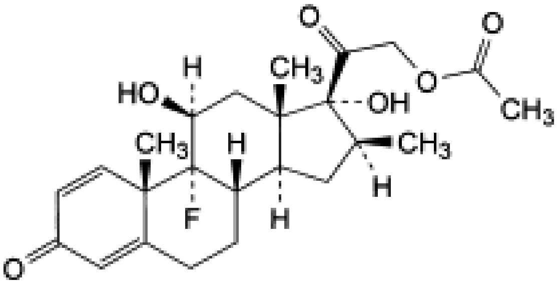
A. 2-ethenylpyridine.

Ph Eur

Betamethasone Acetate

General Notices

(Ph Eur monograph 0975)



$C_{24}H_{31}FO_6$ *434.5* 987-24-6

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

9-Fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-21-yl acetate.

Content

97.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D, E, F*.

A. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* measured at 419 nm is not greater than 0.10.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *betamethasone acetate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *betamethasone acetate CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *prednisolone acetate CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a deep reddish-brown colour develops. Add this solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

E. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S* *solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

F. About 10 mg gives the reaction of acetyl *(2.3.1)*.

TESTS

Specific optical rotation *(2.2.7)*

+ 120 to + 128 (anhydrous substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in 4 ml of *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *betamethasone acetate CRS* and 2 mg of *dexamethasone acetate CRS* (impurity B) in the mobile phase, then dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 380 ml of acetonitrile R with 550 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time 2.5 times the retention time of betamethasone acetate.

Retention time betamethasone acetate = about 19 min; impurity B = about 22 min.

System suitability Reference solution (a):

- *resolution*: minimum 3.3 between the peaks due to betamethasone acetate and impurity B; if necessary, adjust slightly the concentration of acetonitrile in the mobile phase.

Limits:

- *impurities A, B, C, D*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.25 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water ***(2.5.12)***

Maximum 4.0 per cent, determined on 0.100 g.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (2.2.25) at the absorption maximum at 240 nm.

Calculate the content of $C_{24}H_{31}FO_6$ taking the specific absorbance to be 350.

STORAGE

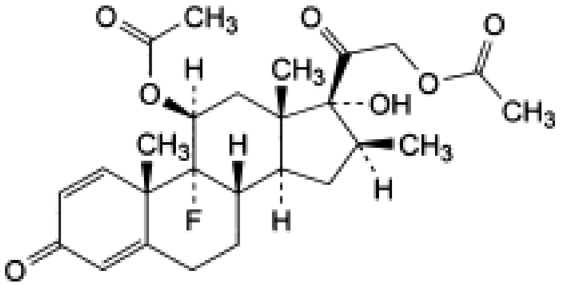
Protected from light.

IMPURITIES

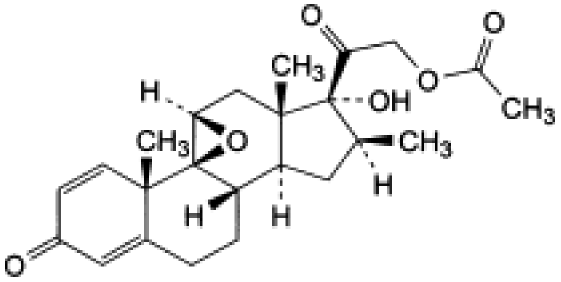
Specified impurities   *A, B, C, D.*

A. betamethasone,

B. dexamethasone acetate,



C. betamethasone 11,21-diacetate,



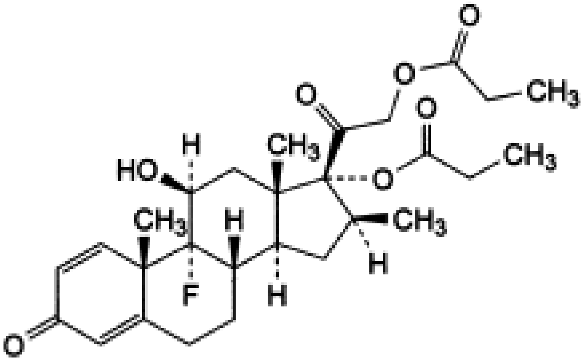
D. 9,11β-epoxy-17-hydroxy-16β-methyl-3,20-dioxo-9β-pregna-1,4-diene-21-yl acetate.

Ph Eur

Betamethasone Dipropionate

General Notices

(Ph Eur monograph 0809)



$C_{28}H_{37}FO_7$ *504.6* 5593-20-4

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

9-Fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in methylene chloride, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B, C.

Second identification *A, D, E, F.*

A. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* measured at 419 nm is not more than 0.10.

B. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *betamethasone dipropionate CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *betamethasone dipropionate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *desoxycortone acetate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture. Dilute 5 ml of this solution to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

D. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* with gentle heating and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Test solution (b) Transfer 2 ml of solution A to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 2 h. Allow to cool.

Reference solution (a) Dissolve 25 mg of *betamethasone dipropionate CRS* in *methanol R* with gentle heating and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Reference solution (b) Transfer 2 ml of solution B to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 2 h. Allow to cool.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes *of methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) has an R_F value distinctly lower than that of the principal spots in each of the chromatograms obtained with test solution (a) and reference solution (a).

E. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a deep reddish-brown colour develops. Add this solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

F. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml *of phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Specific optical rotation *(2.2.7)*

+ 63 to + 70 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 62.5 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of *betamethasone dipropionate CRS* and 2.5 mg of *anhydrous beclometasone dipropionate CRS* in the mobile phase and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix carefully 350 ml of *water R* with 600 ml of *acetonitrile R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 45 min.

Injection 20 µl.

Run time 2.5 times the retention time of betamethasone dipropionate.

Retention time betamethasone diproponiate = about 9 min; beclometasone dipropionate = about 10.7 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to betamethasone dipropionate and beclometasone dipropionate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent), and not more than 1 such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 50.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 240 nm.

Calculate the content of $C_{28}H_{37}FO_7$ taking the specific absorbance to be 305.

STORAGE

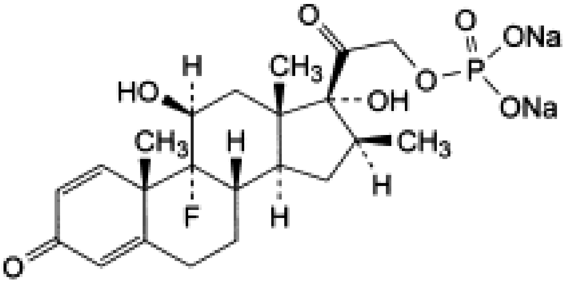
Protected from light.

Ph Eur

Betamethasone Sodium Phosphate

General Notices

(Ph Eur monograph 0810)



$C_{22}H_{28}FNa_2O_8P$ *516.4* 151-73-5

Action and use

Glucocorticoid.

Preparations

Betamethasone Eye Drops

Betamethasone Injection

Betamethasone Sodium Phosphate Tablets

Ph Eur

DEFINITION

9-Fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-21-yl disodium phosphate.

Content

96.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, very hygroscopic.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in

methylene chloride.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D, E, F.*

A. Dissolve 10.0 mg in 5 ml of *water R* and dilute to 100.0 ml with *anhydrous ethanol R*. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* measured at the absorption maximum at 450 nm is not more than 0.10.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *betamethasone sodium phosphate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27).*

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *betamethasone sodium phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *prednisolone sodium phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent. Dilute 5 ml of this solution to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, an intense reddish-brown colour develops. Add the solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

E. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml *of alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

F. To about 40 mg add 2 ml of *sulphuric acid R* and heat gently until white fumes are evolved. Add *nitric acid R* dropwise, continue the heating until the solution is almost colourless and cool. Add 2 ml of *water R*, heat until white fumes are again evolved, cool, add 10 ml of *water R* and neutralise to *red litmus paper R* with *dilute ammonia R1*. The solution gives reaction (a) of sodium *(2.3.1)* and reaction (b) of phosphates *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

7.5 to 9.0.

Dilute 1 ml of solution S to 5 ml with *carbon dioxide-free water* R.

Specific optical rotation *(2.2.7)*

+ 98 to + 104 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 62.5 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 25 mg of *betamethasone sodium phosphate CRS* and 25 mg of *dexamethasone sodium phosphate CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 25.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 250 ml conical flask, weigh 1.360 g of *potassium dihydrogen phosphate R* and 0.600 g of *hexylamine R*, mix and allow to stand for 10 min and then dissolve in 185 ml of *water R*; add 65 ml of *acetonitrile R*, mix and filter (0.45 µm).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 45 min.

Injection 20 µl.

Run time Twice the retention time of betamethasone sodium phosphate.

Retention time Betamethasone sodium phosphate = about 14 min; dexamethasone sodium phosphate = about 15.5 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to betamethasone sodium phosphate and dexamethasone sodium phosphate; if necessary, increase the concentration of acetonitrile or increase the concentration of water in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent), and not more than 1 such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *total:* not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Inorganic phosphate Maximum 1 per cent.

Dissolve 50 mg in *water R* and dilute to 100 ml with the same solvent. To 10 ml of this solution add 5 ml of *molybdovanadic reagent R*, mix and allow to stand for 5 min. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of *phosphate standard solution (5 ppm PO4) R*.

Water *(2.5.12)*

Maximum 8.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.100 g in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 250.0 ml with *water R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 241 nm.

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ taking the specific absorbance to be 297.

STORAGE

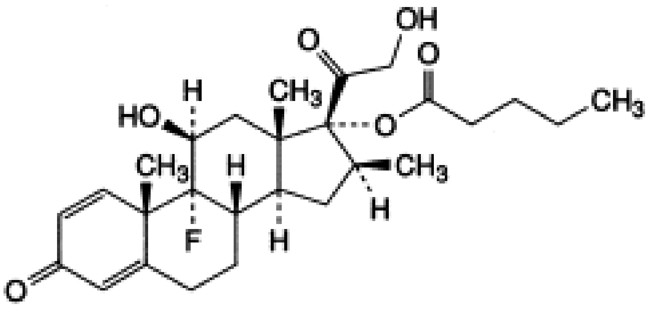
In an airtight container, protected from light.

Ph Eur

Betamethasone Valerate

General Notices

(Ph Eur monograph 0811)



$C_{27}H_{37}FO_6$ *476.6* 2152-44-5

Action and use

Glucocorticoid.

Preparations

Betamethasone Valerate Scalp Application

Betamethasone Valerate Cream

Betamethasone and Clioquinol Cream

Betamethasone Valerate Lotion

Betamethasone Valerate Ointment

Betamethasone and Clioquinol Ointment

Ph Eur

DEFINITION

Betamethasone valerate contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of 9-fluoro-11β,21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl pentanoate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone and in methylene chloride, soluble in ethanol (96 per cent).

It melts at about 192 °C, with decomposition.

IDENTIFICATION

First identification C, D.

Second identification A, B, E, F, G.

A. It complies with the test for specific optical rotation (see Tests).

B. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* of the solution measured at 419 nm is not more than 0.10.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *betamethasone 17-valerate CRS*. If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *chloroform R*, evaporate to dryness on a water-bath and record new spectra using the residues.

D. Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dissolve 10 mg of *betamethasone 17-valerate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 5 mg of *betamethasone 21-valerate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents, then dilute to 10 ml with reference solution (a).

Apply to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*. Develop over a path of 15 cm. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Spray the plate with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

E. Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* with gentle heating and dilute to 5 ml with the same solvent. This solution is also used to prepare test solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Test solution (b) Transfer 2 ml of the solution obtained during preparation of test solution (a) to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 3 h. Allow to cool.

Reference solution (a) Dissolve 25 mg of *betamethasone 17-valerate CRS* in *methanol R* with gentle heating and dilute to 5 ml with the same solvent. This solution is also used to prepare reference solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Reference solution (b) Transfer 2 ml of the solution obtained during preparation of reference solution (a) to a 15 ml glass tube with a ground glass-stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 3 h. Allow to cool.

Apply to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*. Develop over a path of 15 cm. Allow the plate to dry in air and examine under ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatograms obtained with the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) has an R_F value distinctly lower than that of the principal spots in each of the chromatograms obtained with test solution (a) and reference solution (a).

F. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a deep reddish-brown colour develops. Add the solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

G. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank solution is red.

TESTS

Specific optical rotation *(2.2.7)*

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 75 to + 82, calculated with reference to the dried substance.

Related substances

Examine by liquid chromatography *(2.2.29)*.

Solution A To 1000 ml of the mobile phase add 1 ml of *glacial acetic acid R* and mix carefully.

Test solution Dissolve 62.5 mg of the substance to be examined in solution A and dilute to 25.0 ml with solution A.

Reference solution (a) Dissolve 2 mg of *betamethasone 17-valerate CRS* and 2 mg of *betamethasone 21-valerate CRS* in solution A and dilute to 50.0 ml with solution A.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with solution A.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm);

— as mobile phase at a flow rate of 1 ml/min a mixture prepared as follows: mix 350 ml of *water R* with 600 ml of *acetonitrile R* and allow to equilibrate; adjust the volume to 1000 ml with *water R* and mix again;

— as detector a spectrophotometer set at 254 nm.

Equilibrate the column with the mobile phase for about 45 min.

Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with reference solution (b) is 70 per cent to 90 per cent of the full scale of the recorder.

Inject 20 µl of reference solution (a). When the chromatograms are recorded in the prescribed conditions, the retention times are: betamethasone 17-valerate, about 7 min; betamethasone 21-valerate, about 9 min. The test is not valid unless the resolution between the peaks due to betamethasone 17-valerate and betamethasone 21-valerate is at least 5.0; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject 20 µl of the test solution and 20 µl of reference solution (b). Continue the chromatography for 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak apart from the principal peak is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Disregard any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 50.0 ml with *ethanol (96 per cent) R* . Measure the absorbance *(2.2.25)* at the maximum at 240 nm.

Calculate the content of $C_{27}H_{37}FO_6$ taking the specific absorbance to be 325.

STORAGE

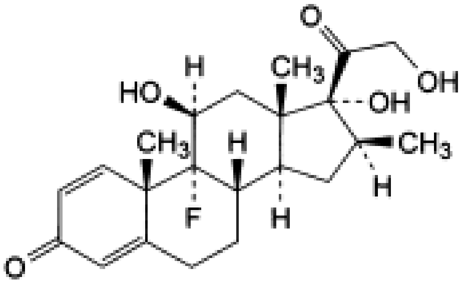
Protected from light.

Ph Eur

Betamethasone

General Notices

(Ph Eur monograph 0312)



$C_{22}H_{29}FO_5$ *392.5* 378-44-9

Action and use

Glucocorticoid.

Preparation

Betamethasone Tablets

Ph Eur

DEFINITION

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene 3,20-dione.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in anhydrous ethanol, very slightly soluble in methylene chloride.

IDENTIFICATION

First identification B, C.

Second identification A, C, D, E.

A. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Place 2.0 ml of this solution in a stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* measured at 419 nm is not greater than 0.10.

B. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *betamethasone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methylene chloride R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *betamethasone CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *dexamethasone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *butanol R* saturated with *water R, toluene R, ether R* (5:10:85 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml *of water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml *of zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

E. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a deep reddish-brown colour develops. Add this solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

TESTS

Specific optical rotation *(2.2.7)*

+ 118 to + 126 (dried substance).

Dissolve 0.125 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *methanol R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 2 mg of *betamethasone CRS* and 2 mg of *methylprednisolone CRS* in mobile phase A, then dilute to 100.0 ml with mobile phase A.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: in a 1000 ml volumetric flask mix 250 ml of *acetonitrile R* with 700 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 40	100 → 0	0 → 100
40 - 41	0 → 100	100 → 0
41 - 46	100	0

Flow rate 2.5 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With mobile phase B for at least 30 min and then with mobile phase A for 5 min. For subsequent chromatograms, use the conditions described from 40 min to 46 min.

Injection 20 µl; inject the mixture of equal volumes of *acetonitrile R* and *methanol R* as a blank.

Retention time methylprednisolone = about 11.5 min; betamethasone = about 12.5 min.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to methylprednisolone and betamethasone; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Limits:

— *impurities A, B, C, D, E, F, G, H, I, J*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), and not more than 1 such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 238.5 nm.

Calculate the content of $C_{22}H_{29}FO_5$ taking the specific absorbance to be 395.

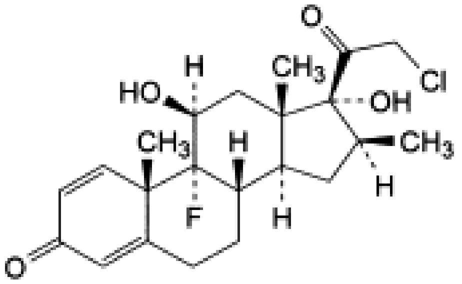
STORAGE

Protected from light.

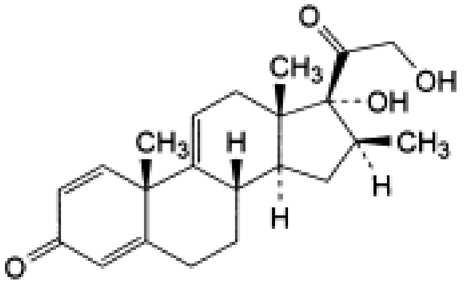
IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J.

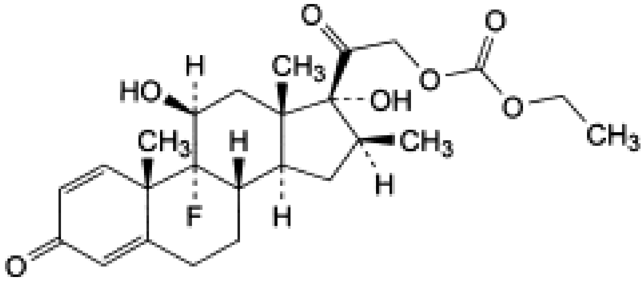
A. dexamethasone,



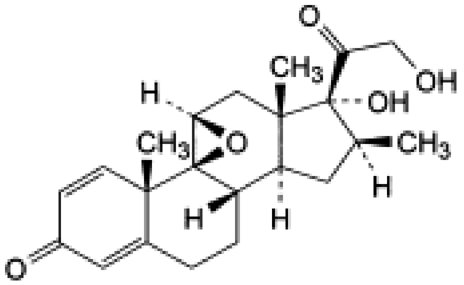
B. 21-chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione,



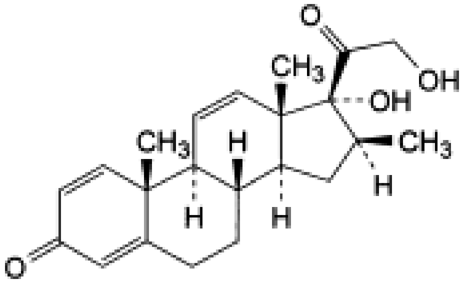
C. 17,21-dihydroxy-16β-methylpregna-1,4,9(11)-triene-3,20-dione,



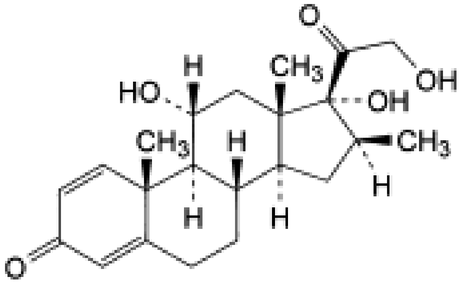
D. 9-fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl ethoxycarboxylate,



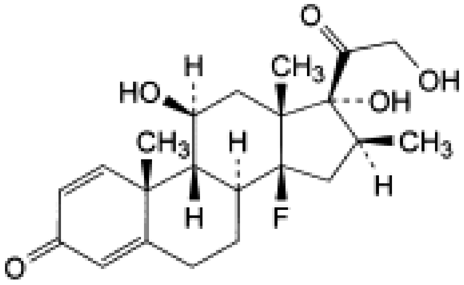
E. 9,11β-epoxy-17,21-dihydroxy-16β-methyl-9β-pregna-1,4-diene-3,20-dione,



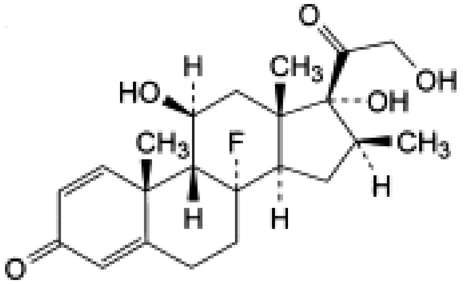
F. 17,21-dihydroxy-16β-methylpregna-1,4,11-triene-3,20-dione,



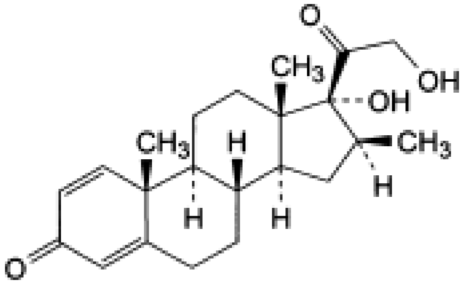
G. 11α,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione,



H. 14-fluoro-11β,17,21-trihydroxy-16β-methyl-8α,9β,14β-pregna-1,4-diene-3,20-dione,



I. 8-fluoro-11β,17,21-trihydroxy-16β-methyl-8α,9β-pregna-1,4-diene-3,20-dione,



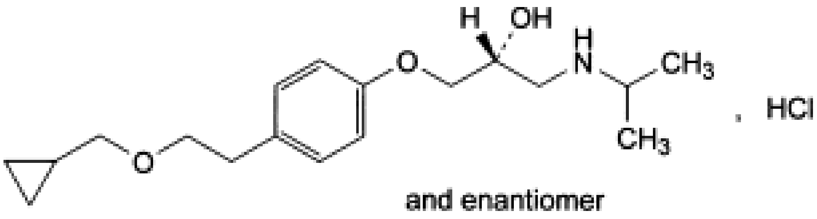
J. 17,21-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione.

Ph Eur

Betaxolol Hydrochloride

General Notices

(Ph Eur monograph 1072)



$C_{18}H_{29}NO_3$ HCl 343.9 *63659-19-8*

Action and use

Beta-adrenoceptor antagonist.

Preparations

Betaxolol Eye Drops, Solution

Betaxolol Eye Drops, Suspension

Ph Eur

DEFINITION

(2*RS*)-1-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]propan-2-ol hydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), soluble in methylene chloride.

IDENTIFICATION

First identification B, D.

Second identification *A, C, D.*

A. Melting point *(2.2.14)*: 113 °C to 117 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *betaxolol hydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in 1 ml of *methanol R*.

Reference solution (a) Dissolve 20 mg of *betaxolol hydrochloride CRS* in 2 ml of *methanol R*.

Reference solution (b) Dissolve 10 mg of *oxprenolol hydrochloride CRS* in 1 ml of reference solution (a).

Plate *TLC octadecylsilyl silica gel F_{254} plate R*.

Mobile phase *perchloric acid R, methanol R, water R* (0.5:50:50 *V/V/V*).

Application 2 µl.

Development Over a path of 10 cm.

Drying In air.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with a 50 g/l solution of *vanillin R* in a mixture of 5 volumes of *sulphuric acid R*, 10 volumes of *glacial acetic acid R* and 85 volumes of *methanol R*. Heat at 100-105 °C until the colour of the spots reaches maximum intensity (10-15 min). Examine in daylight.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.5 g in *water R* and dilute to 25 ml with the same solvent.

Acidity or alkalinity

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. Add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M hydrochloric acid*. The solution is red. Add 0.4 ml of *0.01 M sodium hydroxide*. The solution is yellow.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (a) Dissolve 8 mg of the substance to be examined and 4 mg of *betaxolol impurity A CRS* in 20.0 ml of the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4 mm;
- *stationary phase: octylsilyl silica gel for chromatography R (5 µm).*

Mobile phase Mix 175 ml of *acetonitrile R* with 175 ml of *methanol R* and dilute the mixture to 1 litre with a 3.4 g/l solution of *potassium dihydrogen phosphate R*, previously adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µl.

Run time 4 times the retention time of betaxolol.

System suitability Reference solution (a):

- *resolution*: minimum 2.0 between the peaks due to impurity A and betaxolol.

Limits:

- *impurities A, B, C, D, E*: for each impurity, not more than 0.3 times the area of the peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *total*: not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.025 times the area of the peak in the chromatogram obtained with reference solution (b) (0.025 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 10.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (2.2.20), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

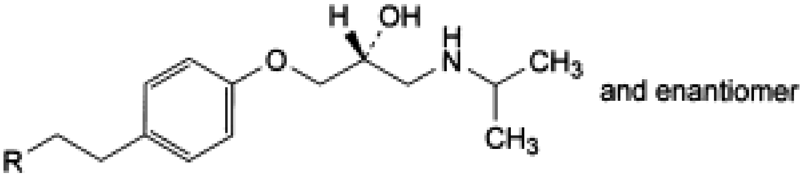
1 ml of *0.1 M sodium hydroxide* is equivalent to 34.39 mg of $C_{18}H_{30}ClNO_3$.

STORAGE

Protected from light.

IMPURITIES

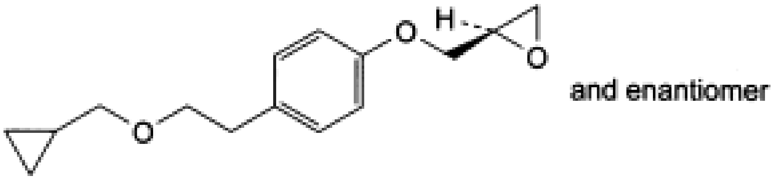
Specified impurities   A, B, C, D, E.


H
OH
H
N
O
CH3
CH3
R
and enantiomer

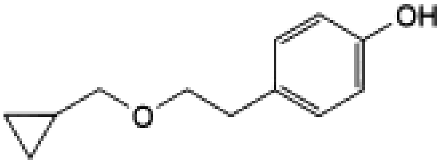
A. R = H: (2*RS*)-1-(4-ethylphenoxy)-3-[(1-methylethyl)amino]propan-2-ol,

B. R = OH: (2*RS*)-1-[4-(2-hydroxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

E. R = O-CH_2-CH_2-C_{H2}-CH_3: (2*RS*)-1-[4-(2-butoxyethyl)phenoxy]-3-[(1-methylethyl)amino] propan-2-ol,


H
O
O
O
and enantiomer

C. 2-[[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]methyl]oxirane,



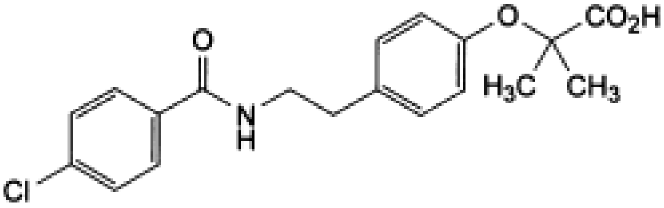
D. 4-[2-(cyclopropylmethoxy)ethyl]phenol.

Ph Eur

Bezafibrate

General Notices

(Ph Eur monograph 1394)



$C_{19}H_{20}ClNO_4$ 361.8 *41859-67-0*

Action and use

Fibrate; lipid-regulating drug.

Preparations

Bezafibrate Tablets

Prolonged-release Bezafibrate Tablets

Ph Eur

DEFINITION

2-[4-[2-[(4-Chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoic acid.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide, sparingly soluble in acetone and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification A, B.

Second identification A, C.

A. Melting point *(2.2.14)*: 181 °C to 185 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *bezafibrate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R* and evaporate to dryness. Dry the residues in vacuo at 80 °C for 1 h and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 10 mg of *bezafibrate CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *glacial acetic acid R, methyl ethyl ketone R, xylene R* (2.7:30:60 *V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying At 120 °C for at least 15 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 1.0 g in *dimethylformamide R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 10.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c) To 1 ml of the test solution, add 1 ml of *0.1 M hydrochloric acid* and evaporate to dryness on a hot plate. Dissolve the residue in 20 ml of the mobile phase.

Column:
- *size: l* = 0.125 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of a 2.72 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 2.3 with *phosphoric acid R*, and 60 volumes of *methanol R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 228 nm.

Injection 20 µl.

Run time The time necessary to detect the ester, which, depending on the route of synthesis, may be impurity C, D or E.

Retention time Impurity A = about 3 min; impurity B = about 3.5 min; bezafibrate = about 6.0 min; impurity C = about 9 min; impurity D = about 14 min; impurity E = about 37 min.

System suitability:
- *resolution*: minimum 5.0 between the 2 principal peaks in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:
- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides *(2.4.4)*

Maximum 300 ppm.

Dilute 10 ml of solution S to 50 ml with *water R*. Filter the resultant suspension through a wet filter previously washed with *water R* until free from chlorides. Prepare the standard using 9 ml of *chloride standard solution (5 ppm Cl) R* and 6 ml of *water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

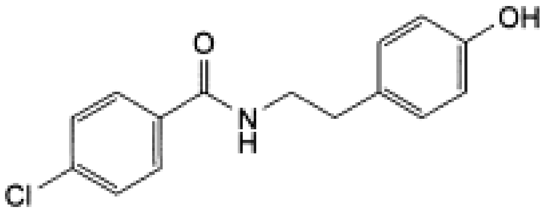
ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 25 volumes of *water R* and 75 volumes of *ethanol (96 per cent) R*. Using 0.1 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a pink colour is obtained. Carry out a blank titration.

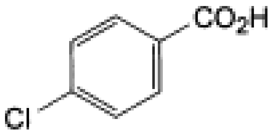
1 ml *of 0.1 M sodium hydroxide* is equivalent to 36.18 mg of $C_{19}H_{20}ClNO_4$.

IMPURITIES

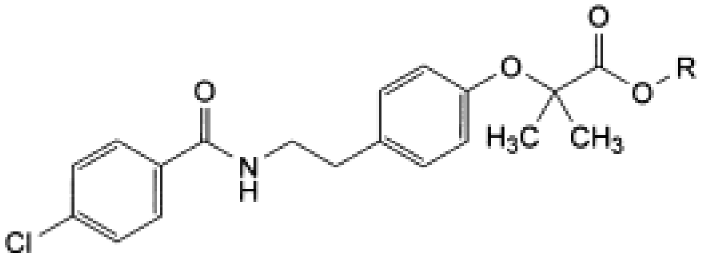
Specified impurities   A, B, C, D, E.



A. 4-chloro-*N*-[2-(4-hydroxyphenyl)ethyl]benzamide (chlorobenzoyltyramine),



B. 4-chlorobenzoic acid,



C. R = CH_3: methyl 2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2 methylpropanoate,

D. R = CH_2-CH_3: ethyl 2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoate,

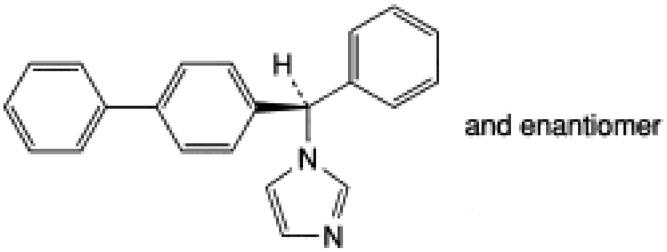
E. R = CH_2-CH_2-CH_2-CH_3: butyl 2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoate.

Ph Eur

Bifonazole

General Notices

(Ph Eur monograph 1395)



$C_{22}H_{18}N_2$ *310.4* *60628-96-8*

Action and use

Antifungal.

Ph Eur

DEFINITION

1-[(*RS*)-(Biphenyl-4-yl)phenylmethyl]-1*H*-imidazole.

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in anhydrous ethanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison bifonazole CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *2-propanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 0.20 g in 20.0 ml of *methanol R*.

Related substances

Liquid chromatography *(2.2.29)*.

Buffer solution pH 3.2 Mix 2.0 ml of *phosphoric acid R* with *water R* and dilute to 1000.0 ml with the same solvent. Adjust to pH 3.2 *(2.2.3)* with *triethylamine R.*

Test solution Dissolve 50.0 mg of the substance to be examined in 25 ml of *acetonitrile R* and dilute to 50.0 ml with buffer solution pH 3.2.

Reference solution (a) Dilute 0.25 ml of the test solution to 50.0 ml with buffer solution pH 3.2.

Reference solution (b) Dissolve 25.0 mg of *imidazole R* (impurity C) in *acetonitrile R* and dilute to 25.0 ml with the same solvent. Dilute 0.25 ml of this solution to 100.0 ml with buffer solution pH 3.2.

Reference solution (c) Dissolve 5.0 mg of *bifonazole impurity B CRS* in *acetonitrile R* and dilute to 5.0 ml with the same solvent.

Reference solution (d) Mix 0.25 ml of the test solution and 0.25 ml of reference solution (c) and dilute to 50.0 ml with buffer solution pH 3.2.

Column:

— *size: l* = 0.125 m, Ø = 4.6 mm;

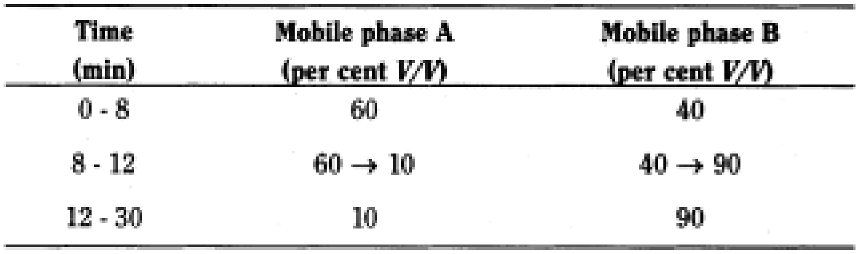
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R1*, buffer solution pH 3.2 (20:80 *V/V*);

— *mobile phase B*: buffer solution pH 3.2, *acetonitrile R1* (20:80 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	60	40
8 - 12	60 → 10	40 → 90
12 - 30	10	90

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µl of the test solution and reference solutions (a), (b) and (d).

Retention time Impurity B = about 4 min; bifonazole = about 4.5 min.

System suitability Reference solution (d):

— *resolution*: minimum 2.5 between the peaks due to impurity B and bifonazole.

Limits:

— *impurity B*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *impurities A, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

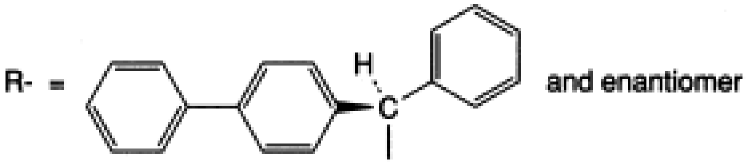
ASSAY

Dissolve 0.250 g in 80 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

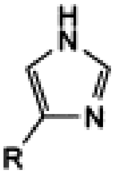
1 ml of *0.1 M perchloric acid* is equivalent to 31.04 mg of $C_{22}H_{18}N_2$.

IMPURITIES

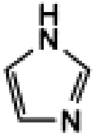
Specified impurities A, B, C, D.



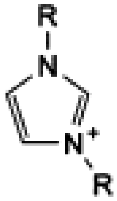
A. R-OH: (*RS*)-(biphenyl-4-yl)phenylmethanol,



B. 4-[(*RS*)-(biphenyl-4-yl)phenylmethyl]-1*H*-imidazole,



C. 1*H*-imidazole,



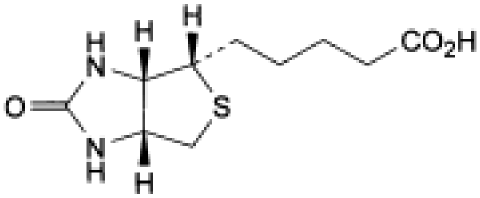
D. 1,3-bis[(biphenyl-4-yl)phenylmethyl]-1*H*-imidazolium ion.

Ph Eur

Biotin

General Notices

(Ph Eur monograph 1073)



$C_{10}H_{16}N_2O_3S$ 244.3 *58-85-5*

Action and use

Vitamin.

Ph Eur

DEFINITION

Biotin contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white crystalline powder or colourless crystals, very slightly soluble in water and in alcohol, practically insoluble in acetone. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *biotin CRS*.

B. Examine the chromatograms obtained in the test for related substances (see Tests). The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 20 ml of *water R* with heating. Allow to cool. Add 0.1 ml of *bromine water R*. The bromine water is decolourised.

TESTS

Solution S

Dissolve 0.250 g in a 4 g/l solution of *sodium hydroxide R* and dilute to 25.0 ml with the same alkaline solution.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

The specific optical rotation is + 89 to + 93, determined on solution S and calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel (5 µm). *Prepare the solutions immediately before use and keep protected from bright light.*

Test solution (a) Dissolve 50 mg of the substance to be examined in *glacial acetic acid R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *glacial acetic acid R*.

Reference solution (a) Dissolve 5 mg of *biotin CRS* in *glacial acetic acid R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 20 ml with *glacial acetic acid R*.

Reference solution (c) Dilute 1 ml of test solution (b) to 40 ml with *glacial acetic acid R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *methanol R*, 25 volumes of *glacial acetic acid R* and 75 volumes of *toluene R*. Dry the plate in a current of warm air. Allow to cool and spray with *4-dimethylaminocinnamaldehyde solution R*. Examine immediately in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

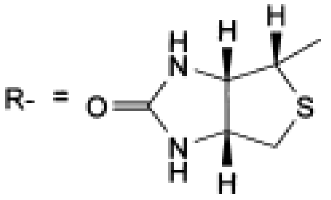
Suspend 0.200 g in 5 ml of *dimethylformamide R*. Heat until the substance has dissolved completely. Add 50 ml of *ethanol R* and titrate with 0.1 *M tetrabutylammonium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

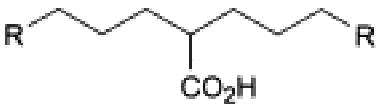
1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 24.43 mg of $C_{10}H_{16}N_2O_3S$.

STORAGE

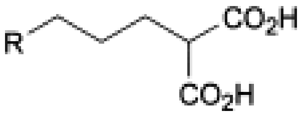
Store protected from light.

IMPURITIES

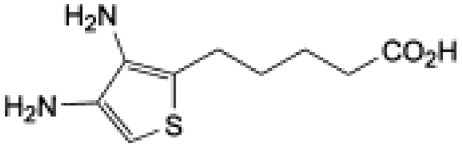




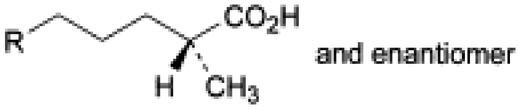
A. di[3-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]propyl]acetic acid,



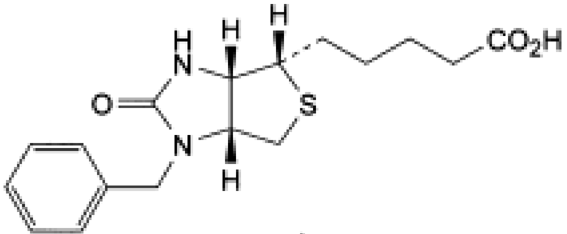
B. 4-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]butane-1,1-dicarboxylic acid,



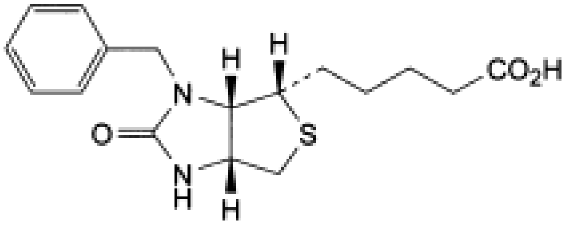
C. 5-(3,4-diamino-2-thienyl)pentanoic acid,



D. 2-methyl-5-[(3a*S*,4S,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid,



and



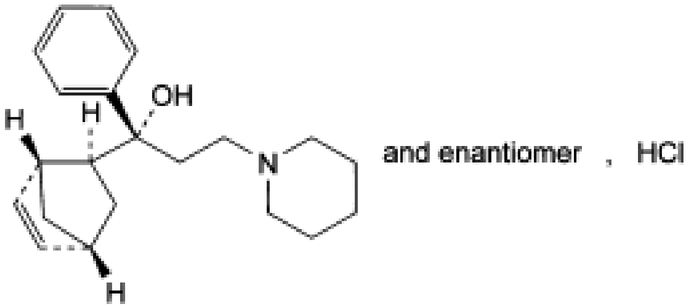
E. 5-[(3a*S*,4*S*,6a*R*)-3-benzyl-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid and 5-[(3a*S*,4*S*,6a*R*)-1-benzyl-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid.

Ph Eur

Biperiden Hydrochloride

General Notices

(Ph Eur monograph 1074)



$C_{21}H_{29}NO, HCl$ 347.9 *1235-82-1*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

(1*RS*)-1-[(1*RS*,2*SR*,4*RS*)-Bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water and in alcohol, very slightly soluble in methylene chloride.

mp

About 280 °C, with decomposition.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *biperiden hydrochloride CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *biperiden hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *biperiden impurity A CRS* in reference solution (a) and dilute to 2 ml with the same solution.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *diethylamine R, methanol R, toluene R* (1:1:20 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *dilute potassium iodobismuthate solution R* and then with *sodium nitrite solution R* and examine in daylight.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

C. To about 20 mg add 5 ml of *phosphoric acid R*. A green colour develops.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.10 g in *carbon dioxide-free water R*, heating gently if necessary, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and is colourless *(2.2.2, Method II)*.

pH ***(2.2.3)***

5.0 to 6.5 for solution S.

Related substances

Gas chromatography *(2.2.28).*

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 0.5 ml of the test solution to 100 ml with *methanol R*. Dilute 10 ml of this solution to 50 ml with *methanol R*.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *biperiden impurity A CRS* in *methanol R* and dilute to 5 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *methanol R.*

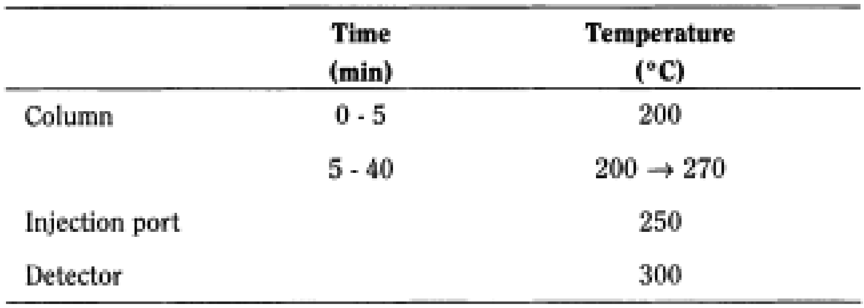
Column:
- *material*: fused silica,
- *size*: *l* = 50 m, Ø = 0.25 mm,
- *stationary phase: poly(dimethyl)(diphenyl)(divinyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 0.4 ml/min.

Split ratio 1:250.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	200
	5 - 40	200 → 270
Injection port		250
Detector		300

Detection Flame ionisation.

Injection 2 µl.

Run time Twice the retention time of biperiden.

Relative retention With reference to biperiden: impurities A, B and C = between 0.95 and 1.05.

System suitability:
- *resolution*: minimum 2.5 between the peak due to biperiden (1st peak) and the peak due to impurity A (2nd peak) in the chromatogram obtained with reference solution (b),
- *signal-to-noise ratio*: minimum 6 for the principal peak in the chromatogram obtained with reference solution (a).

Limits:

— *impurities A, B, C*: for each impurity, maximum 0.50 per cent of the area of the principal peak,

— *any other impurity*: for each impurity, maximum 0.10 per cent of the area of the principal peak,

— *total of impurities A, B and C*: maximum 1.0 per cent of the area of the principal peak,

— *total of impurities other than A, B and C*: maximum 0.50 per cent of the area of the principal peak,

— *disregard limit*: 0.05 per cent of the area of the principal peak.

Impurity F *(2.4.24)*

Maximum 2 ppm.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 60 ml of *alcohol R*. In a closed vessel, titrate with *0.1 M alcoholic potassium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M alcoholic potassium hydroxide* is equivalent to 34.79 mg of $C_{21}H_{30}ClNO$.

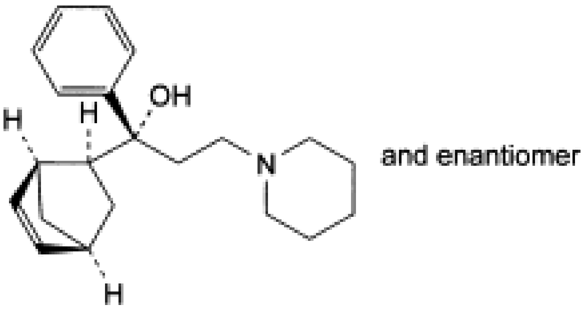
STORAGE

In an airtight container, protected from light.

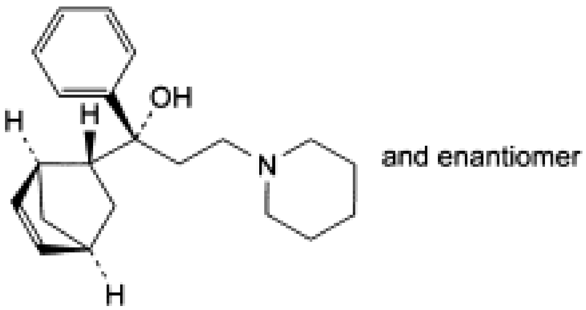
IMPURITIES

Specified impurities *A, B, C, F.*

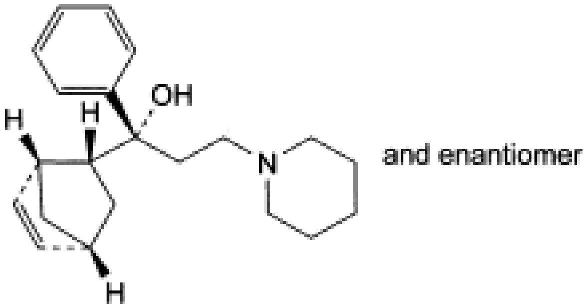
Other detectable impurities: D, E.



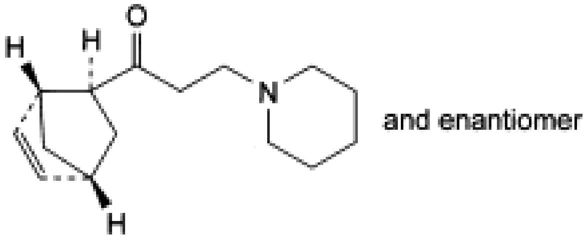
A. (1*RS*)-1-[(1*SR*,2*SR*,4*SR*)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol (*endo* form),


H
H
OH
N
and enantiomer
H

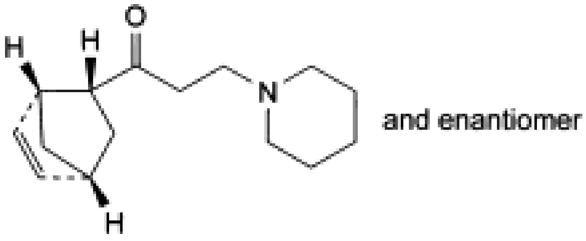
B. (1*RS*)-1-[(1*SR*,2*RS*,4*SR*)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol,


H
H
OH
N
and enantiomer
H

C. (1*RS*)-1-[(1*RS*,2*RS*,4*RS*)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol,


O
H
H
N
and enantiomer
H

D. 1-[(1*R*S,2*SR*,4*RS*)-bicyclo[2.2.1]hept-5-en-2-yl]-3-(piperidin-1-yl)propan-1-one,


O
H
H
N
and enantiomer
H

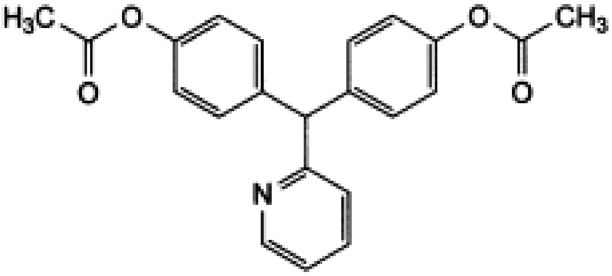
E. 1-[(1*RS*,2*RS*,4*RS*)-bicyclo[2.2.1]hept-5-en-2-yl]-3-(piperidin-1-yl)propan-1-one,

F. benzene.

Bisacodyl

General Notices

(Ph Eur monograph 0595)



$C_{22}H_{19}NO_4$ 361.4 *603-50-9*

Action and use

Stimulant laxative.

Preparations

Bisacodyl Suppositories

Bisacodyl Tablets

Ph Eur

DEFINITION

4,4′-(Pyridin-2-ylmethylene)diphenyl diacetate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION

First identification *C.*

Second identification *A, B, D.*

A. Melting point *(2.2.14)*: 131 °C to 135 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 10.0 mg in a 6 g/l solution of *potassium hydroxide R* in *methanol R* and dilute to 100.0 ml with the same solution. Dilute 10.0 ml of this solution to 100.0 ml with a 6 g/l solution of *potassium hydroxide R* in *methanol R*.

Spectral range 220-350 nm.

Absorption maximum At 248 nm.

Shoulder At 290 nm.

Specific absorbance at the absorption maximum *632 to 672.*

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *bisacodyl CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *chloroform R*, evaporate to dryness and record new spectra using the residues.

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *bisacodyl CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *methyl ethyl ketone R, xylene R* (50:50 *V/V*).

Application 10 µl.

Development Over a path of 10 cm.

Drying In air, if necessary heating at 100-105 °C.

Detection Spray with a mixture of equal volumes of *0.05 M iodine* and *dilute sulphuric acid* R.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Acidity or alkalinity

To 1.0 g add 20 ml of *carbon dioxide-free water R*, shake, heat to boiling, cool and filter. Add 0.2 ml of 0.01 M *sodium hydroxide* and 0.1 ml of *methyl red solution R*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances

Liquid chromatography *(2.2.29)*.

Prepare the solutions immediately before use.

Solvent mixture *glacial acetic acid R, acetonitrile R, water R* (4:30:66 *V/V/V*).

Test solution Dissolve 50 mg of the substance to be examined in 25 ml of *acetonitrile R* and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 2.0 mg of *bisacodyl for system suitability CRS* (containing impurities A, B, C, D and E) in 1.0 ml of *acetonitrile R* and dilute to 2.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *bisacodyl for peak identification CRS* (containing impurity F) in 2.5 ml of *acetonitrile R* and dilute to 5.0 ml with the solvent mixture.

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 45 volumes of *acetonitrile R* and 55 volumes of a 1.58 g/l solution of *ammonium formate R* previously adjusted to pH 5.0 with *anhydrous formic acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 20 µl.

Run time 3.5 times the retention time of bisacodyl.

Identification of impurities Use the chromatogram supplied with *bisacodyl for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to bisacodyl (retention time = about 13 min): impurity A = about 0.2; impurity B = about 0.4; impurity C = about 0.45; impurity D = about 0.8; impurity E = about 0.9; impurity F = about 2.6.

System suitability Reference solution (b):

- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to bisacodyl.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.7;
- *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *impurities C, E*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurity D*: not more than twice the area of the principal peak in the chromatogram

obtained with reference solution (a) (0.2 per cent);

— *impurity F*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

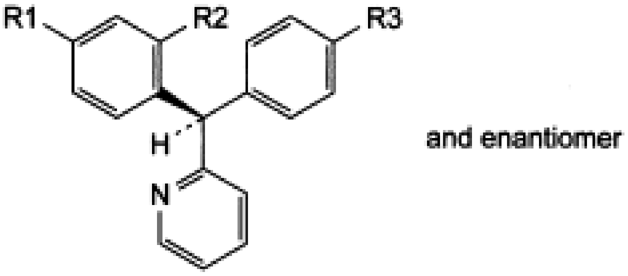
1 ml of *0.1 M perchloric acid* is equivalent to 36.14 mg of $C_{22}H_{19}NO_4$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F.



A. R1 = R3 = OH, R2 = H: 4,4′-(pyridin-2-ylmethylene)diphenol,

B. R1 = H, R2 = R3 = OH: 2-[(*RS*)-(4-hydroxyphenyl)(pyridin-2-yl)methyl]phenol,

C. R1 = OH, R2 = H, R3 = O-CO-CH_3: 4-[(*RS*)-(4-hydroxyphenyl)(pyridin-2-yl)methyl]phenyl acetate,

E. R1 = H, R2 = R3 = O-CO-CH_3: 2-[(*RS*)-[4-(acetyloxy)phenyl](pyridin-2-yl)methyl]phenyl acetate,

D. unknown structure,

F. unknown structure.

Bismuth Subcarbonate

General Notices

Bismuth Carbonate

(Ph Eur monograph 0012)

Ph Eur

DEFINITION

Content

80.0 per cent to 82.5 per cent of Bi (A_r 209.0) (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves with effervescence in mineral acids.

IDENTIFICATION

A. It gives the reaction of carbonates *(2.3.1)*.

B. It gives the reactions of bismuth *(2.3.1)*.

TESTS

Solution S

Shake 5.0 g with 10 ml of *water R* and add 20 ml of *nitric acid R*. Heat to dissolve, cool and dilute to 100 ml with *water R*.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and is colourless *(2.2.2, Method II)*.

Chlorides *(2.4.4)*

Maximum 500 ppm.To 6.6 ml of solution S add 4 ml of *nitric acid R* and dilute to 50 ml with *water R*.

Nitrates

Maximum 0.4 per cent.

To 0.25 g in a 125 ml conical flask, add 20 ml of *water R*, 0.05 ml of *indigo carmine solution R1* and then, as a single addition but with caution, 30 ml of *sulphuric acid R*. Titrate immediately with *indigo carmine solution R1* until a stable blue colour is obtained. Not more than *n* ml of the titrant is required, *n* being the volume corresponding to 1 mg of NO_3.

Alkali and alkaline-earth metals

Maximum 1.0 per cent.

To 1.0 g add 10 ml of *water R* and 10 ml of *acetic acid R*. Boil for 2 min, cool and filter. Wash the residue with 20 ml of *water R*. To the combined filtrate and washings add 2 ml of *dilute hydrochloric acid R* and 20 ml of *water R*. Boil and pass *hydrogen sulphide R* through the boiling solution until no further precipitate is formed. Filter, wash the residue with *water R*, evaporate the combined filtrate and washings to dryness on a water-bath and add 0.5 ml of *sulphuric acid R*. Ignite gently and allow to cool. The residue weighs a maximum of 10 mg.

Arsenic ***(2.4.2, Method A)***

Maximum 5 ppm.

To 0.5 g in a distillation flask add 5 ml of *water R* and 7 ml of *sulphuric acid R*, allow to cool and add 5 g of *reducing mixture R* and 10 ml of *hydrochloric acid R*. Heat the contents of the flask to boiling gradually over 15-30 min and continue heating at such a rate that the distillation proceeds steadily until the volume in the flask is reduced by half or until 5 min after the air-condenser has become full of steam. It is important that distillation be discontinued before fumes of sulphur trioxide appear. Collect the distillate in a tube containing 15 ml of *water R* cooled in ice-water. Wash down the condenser with *water R* and dilute the distillate to 25 ml with the same solvent. Prepare the standard using a mixture of 2.5 ml of *arsenic standard solution (1 ppm As) R* and 22.5 ml of *water* R.

Copper

Maximum 50 ppm.

To 5 ml of solution S, add 2 ml of *ammonia R* and dilute to 50 ml with *water R*. Filter. To 10 ml of the filtrate add 1 ml of a 1 g/l solution of *sodium diethyldithiocarbamate R*. The solution is not more intensely coloured than a standard prepared at the same time in the same manner using a mixture of 0.25 ml of *copper standard solution (10 ppm Cu) R* and 9.75 ml of *water R* instead of 10 ml of the filtrate.

Lead

Maximum 20.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 12.5 g in 75 ml of a mixture of equal volumes of *lead-free nitric acid R* and *water R*. Boil for 1 min, cool and dilute to 100.0 ml with *water R*.

Reference solutions Prepare the reference solutions using appropriate quantities of lead standard solution and a 37 per cent *V/V* solution of *lead-free nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm (depending on the apparatus, the line at 217.0 nm may be used).

Atomisation device Air-acetylene flame.

Silver

Maximum 25 ppm.

To 2.0 g add 1 ml of *water R* and 4 ml of *nitric acid R*. Heat gently until dissolved and dilute to 11 ml with *water R*. Cool and add 2 ml of *1 M hydrochloric acid*. Allow to stand protected from light for 5 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 10 ml of *silver standard solution (5 ppm Ag) R*, 1 ml of *nitric acid R* and 2 ml of *1 M hydrochloric acid*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.500 g in 3 ml of *nitric acid R* and dilute to 250 ml with *water R*. Carry out the complexometric titration of bismuth *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 20.90 mg of Bi.

STORAGE

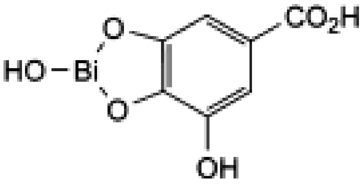
Protected from light.

Ph Eur

Bismuth Subgallate

General Notices

(Ph Eur monograph 1493)



$C_7H_5BiO_6$ 394.1 *149-91-7*

Ph Eur

DEFINITION

Complex of bismuth and gallic acid.

Content

48.0 per cent to 51.0 per cent of Bi (A_r 209.0) (dried substance).

CHARACTERS

Appearance

Yellow powder.

Solubility

Practically insoluble in water and in alcohol. It dissolves in mineral acids with decomposition and in solutions of alkali hydroxides, producing a reddish-brown liquid.

IDENTIFICATION

A. Mix 0.1 g with 5 ml of *water R* and 0.1 ml of *phosphoric acid R*. Heat to boiling and maintain boiling for 2 min. Cool and filter. To the filtrate, add 1.5 ml of *ferric chloride solution R1*, a blackish-blue colour develops.

B. It gives reaction (b) of bismuth *(2.3.1)*.

TESTS

Solution S

In a porcelain or quartz dish, ignite 1.0 g, increasing the temperature very gradually. Heat in a muffle furnace at 600 ± 50 °C for 2 h. Cool and dissolve the residue with warming in 4 ml of a mixture of equal volumes of *lead-free nitric acid R* and *water R* and dilute to 20 ml with *water R*.

Acidity

Shake 1.0 g with 20 ml of *water R* for 1 min and filter. To the filtrate add 0.1 ml of *methyl red solution R*. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Chlorides ***(2.4.4)***

Maximum 200 ppm.

To 0.5 g add 10 ml of *dilute nitric acid R*. Heat on a water-bath for 5 min and filter. Dilute 5 ml of the filtrate to 15 ml with *water R*.

Nitrates

Maximum 0.2 per cent.

To 1.0 g add 25 ml of *water R* then 25 ml of a mixture of 2 volumes of *sulphuric acid R* and 9 volumes of *water R*. Heat at about 50 °C for 1 min with stirring and filter. To 10 ml of the filtrate, carefully add 30 ml of *sulphuric acid R*. The solution is not more intensely brownish-yellow coloured than a reference solution prepared at the same time as follows: to 0.4 g of *gallic acid R*, add 20 ml of *nitrate standard solution (100 ppm* NO_3*) R* and 30 ml of a mixture of 2 volumes of *sulphuric acid R* and 9 volumes of *water R*, then filter; to 10 ml of the filtrate, carefully add 30 ml of *sulphuric acid R*.

Copper

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I).*

Test solution Solution S.

Reference solutions Prepare the reference solutions using *copper standard solution (10 ppm Cu) R* and diluting with a 6.5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Copper hollow-cathode lamp.

Wavelength 324.7 nm.

Atomisation device Air-acetylene flame.

Lead

Maximum 20.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *lead standard solution (10 ppm Pb) R* and diluting with a 6.5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm (depending on the apparatus, the line at 217.0 nm may be used).

Atomisation device Air-acetylene flame.

Silver

Maximum 25.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *silver standard solution (5 ppm Ag) R* and diluting with a 6.5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Silver hollow-cathode lamp.

Wavelength 328.1 nm.

Atomisation device Air-acetylene flame.

Substances not precipitated by ammonia

Maximum 1.0 per cent.

In a porcelain or quartz dish, ignite 2.0 g, increasing the temperature very gradually to 600 ± 50 °C; allow to cool. Moisten the residue with 2 ml of *nitric acid R*, evaporate to dryness on a water-bath and carefully heat and ignite once more at 600 ± 50 °C. After cooling, dissolve the residue in 5 ml of *nitric acid R* and dilute to 20 ml with *water R*. To 10 ml of this solution, add *concentrated ammonia R* until alkaline and filter. Wash the residue with *water R*, evaporate the combined filtrate and washings to dryness on a water-bath and add 0.3 ml of *dilute sulphuric acid R*. Ignite, the residue weighs a maximum of 10 mg.

Loss on drying *(2.2.32)*

Maximum 7.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

To 0.300 g add 10 ml of a mixture of equal volumes of *nitric acid R* and *water R*, heat to boiling and maintain boiling for 2 min. Add 0.1 g of *potassium chlorate R*, heat to boiling and maintain boiling for 1 min. Add 10 ml of *water R* and heat until the solution becomes colourless. To the hot solution, add 200 ml of *water R* and 50 mg of *xylenol orange triturate R*. Titrate with *0.1 M sodium edetate* until a yellow colour is obtained.

1 ml of *0.1 M sodium edetate* is equivalent to 20.90 mg of Bi.

STORAGE

Protected from light.

Ph Eur

Bismuth Subsalicylate

General Notices

(Ph Eur monograph 1495)

$C_7H_5BiO_4$ 362.1 *14882-18-9*

Ph Eur

DEFINITION

Complex of bismuth and salicylic acid.

Content

56.0 per cent to 59.4 per cent of Bi (A_r 209.0) (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in alcohol. It dissolves in mineral acids with decomposition.

IDENTIFICATION

A. To 0.5 g add 10 ml *of hydrochloric acid R1*. Heat on a boiling water-bath for 5 min. Cool and filter. Retain the filtrate for identification test B. Wash the residue with *dilute hydrochloric acid R* and then with *water R*. Dissolve the residue in 0.5-1 ml of *dilute sodium hydroxide solution R*. Add 15 ml of *water R.* Neutralise with *dilute hydrochloric acid R*. The solution gives reaction (a) of salicylates *(2.3.1)*.

B. The filtrate obtained in identification test A gives reaction (b) of bismuth *(2.3.1)*.

TESTS

Solution S

In a porcelain or quartz dish, ignite 1.0 g, increasing the temperature very gradually. Heat in a muffle furnace at 600 ± 25 °C for 2 h. Cool and dissolve the residue with warming in 4 ml of a mixture of equal volumes of *lead-free nitric acid R* and *water R* and dilute to 20 ml with *water R.*

Acidity

Shake 2.0 g with 30 ml of *ether R* for 1 min and filter. To the filtrate add 30 ml of *alcohol R* and 0.1 ml of *thymol blue solution R*. Not more than 0.35 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dissolve 0.250 g in a mixture of 2 ml of *nitric acid R*, 5 ml of *water R* and 8 ml of *methanol R*.

Nitrates

Maximum 0.4 per cent.

To 0.1 g add 10 ml of *water R* and, with caution, 20 ml of *sulphuric acid R* and stir. The solution is not more intensely yellow coloured than a reference solution prepared at the same time using 0.1 g of *salicylic acid R*, 6 ml of *water R*, 4 ml of *nitrate standard solution* (100 ppm NO_3) R and 20 ml of *sulphuric acid R.*

Copper

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *copper standard solution (10 ppm Cu) R* and diluting with a 6.5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Copper hollow-cathode lamp.

Wavelength 324.7 nm.

Atomisation device Air-acetylene flame.

Lead

Maximum 20.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *lead standard solution (10 ppm Pb) R* and diluting with a 6.5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm (depending on the apparatus, the line at 217.0 nm may be used).

Atomisation device Air-acetylene flame.

Silver

Maximum 25.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *silver standard solution (5 ppm Ag) R* and diluting with a 6.5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Silver hollow-cathode lamp.

Wavelength 328.1 nm.

Atomisation device Air-acetylene flame.

Soluble bismuth

Maximum 40.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Suspend 5.0 g in 100 ml of *water R*. Stir constantly for 2 h at 20-23 °C. Filter through filter paper (slow filtration) then through a cellulose micropore membrane filter (0.1 µm). To 10.0 ml of clear filtrate, add 0.1 ml of *nitric acid R*.

Reference solutions Prepare the reference solutions using *bismuth standard solution (100 ppm Bi) R* and diluting with a mixture of equal volumes of *dilute nitric acid R* and *water R*.

Source Bismuth hollow-cathode lamp.

Wavelength 223.06 nm.

Atomisation device Air-acetylene flame.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve with heating 0.300 g in 10 ml of a mixture of 2 volumes of *perchloric acid R* and 5 volumes of *water R*. To the hot solution, add 200 ml of water R and 50 mg of *xylenol orange triturate R*. Titrate with *0.1 M sodium edetate* until a yellow colour is obtained.

1 ml of 0*.1 M sodium edetate* is equivalent to 20.90 mg of Bi.

STORAGE

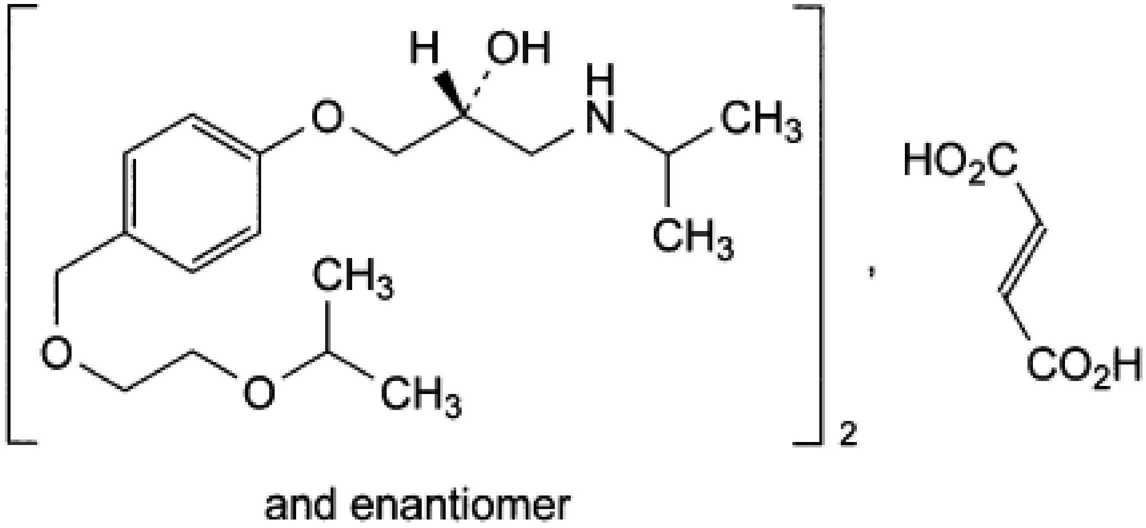
Protected from light.

Ph Eur

Bisoprolol Fumarate

General Notices

(Ph Eur monograph 1710)



$C_{40}H_{66}N_2O_{12}$ 767 *66722-44-9*

Action and use

Beta-adrenoceptor antagonist.

Ph Eur

DEFINITION

(*RS*)-1-[4-[[2-(1-Methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]propan-2-ol fumarate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic powder.

Solubility

Very soluble in water, freely soluble in methanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *bisoprolol fumarate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate and dry the residue at 60 °C at a pressure not exceeding 700 Pa and record new spectra using the residues.

TESTS

Related substances

A. Impurities A and E. Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25 mg of the substance to be examined in mobile phase A and dilute to 25.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve the contents of a vial of *bisoprolol for system suitability method A CRS* (containing impurities A, B and E) in 1.0 ml of mobile phase A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

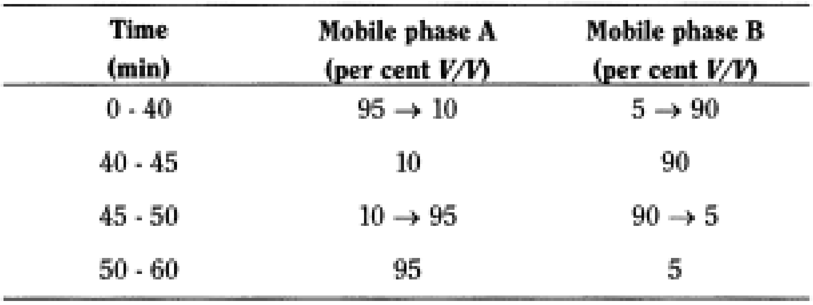
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: mix 10 volumes of *acetonitrile R1* and 90 volumes of a solution containing 0.4 ml/l of *triethylamine R1* and 3.12 g/l of *sodium dihydrogen phosphate R*, previously adjusted to pH 4.2 with *dilute phosphoric acid R;*

— *mobile phase B*: mix 25 volumes of a solution containing 0.4 ml/l of *triethylamine R1* and 3.12 g/l of *sodium dihydrogen phosphate R*, previously adjusted to pH 4.2 with *dilute phosphoric acid R* and 75 volumes of *acetonitrile R1;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	95 → 10	5 → 90
40 - 45	10	90
45 - 50	10 → 95	90 → 5
50 - 60	95	5

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *bisoprolol for system suitability method A CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to fumaric acid and impurities A, B and E.

Relative retention With reference to bisoprolol (retention time = about 14.5 min): impurity A = about 0.25; impurity G = about 1.05; impurity B = about 1.1; impurity E = about 1.3.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to bisoprolol and impurity B.

Limits:

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity E*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to fumaric acid and any peak due to impurity G.

B. Impurities A and G. Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1*, *water for chromatography R* (20:80 *V/V*).

Test solution Dissolve 25 mg of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *bisoprolol for system suitability method B CRS* (containing impurities A and G) in 1.0 ml of the solvent mixture.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

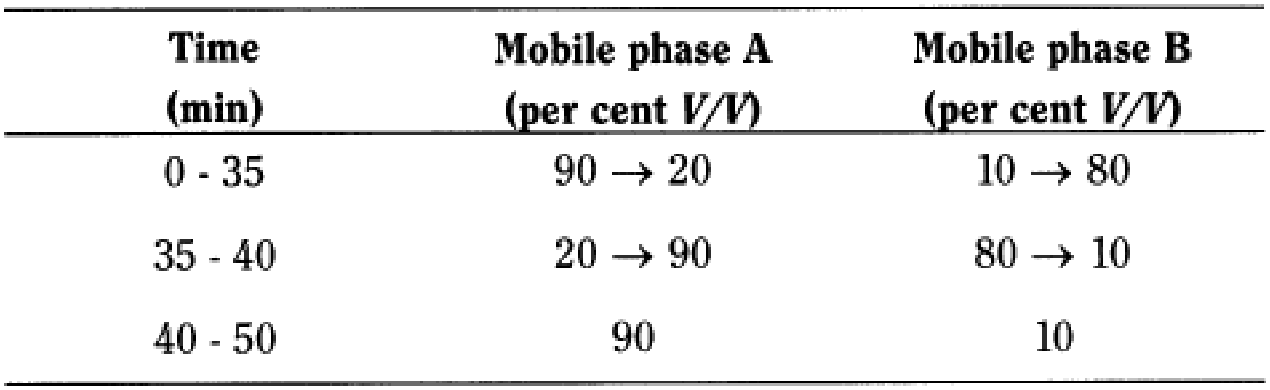
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: 10 g/l solution of *phosphoric acid R*;

— *mobile phase B*: 10 g/l solution of *phosphoric acid R* in *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	90 → 20	10 → 80
35 - 40	20 → 90	80 → 10
40 - 50	90	10

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *bisoprolol for system suitability method B CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to fumaric acid and impurities A and G.

Relative retention with reference to bisoprolol (retention time = about 13.4 min): impurity A = about 0.4; impurity G = about 1.02; impurity E = about 1.2.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity G, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to bisoprolol.

Limits:

— *impurity G*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to fumaric acid and any peak due to impurity E.

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 1.000 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 38.35 mg of $C_{40}H_{66}N_2O_{12}$.

STORAGE

In an airtight container, protected from light.

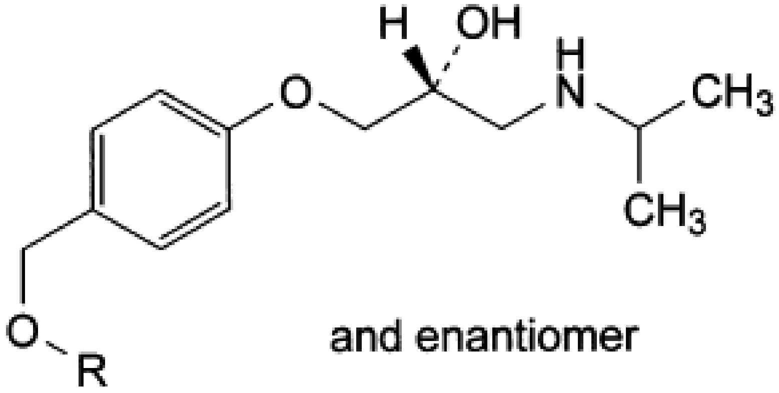
IMPURITIES

Specified impurities A, E, G.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*):

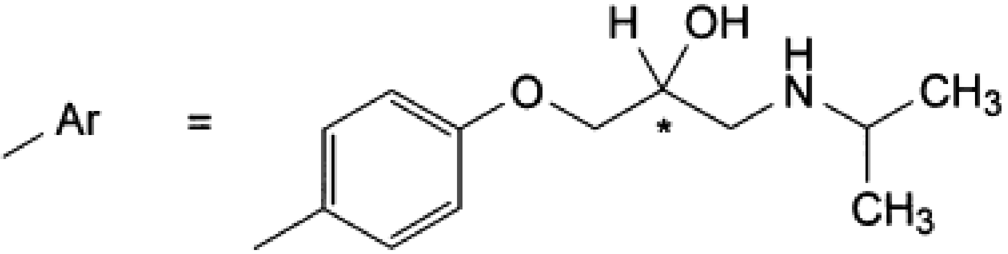
— by method A: B, C, D, F;

— by method B: B, K, L, N, Q, R, S, T, U.



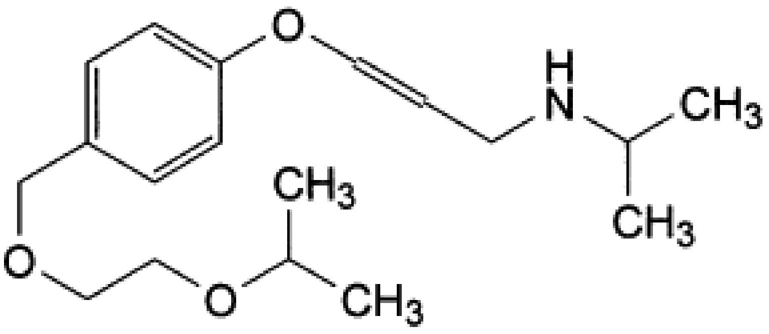
A. R = H: (*RS*)-1-(4-hydroxymethyl-phenoxy)-3-isopropylaminopropan-2-ol,

B. R = CH_2-CH_2-*O*-$[CH_2]_2$-CH_3: (*RS*)-1-isopropylamino-3-[4-(2-propoxy-ethoxymethyl) phenoxy]propan-2-ol,

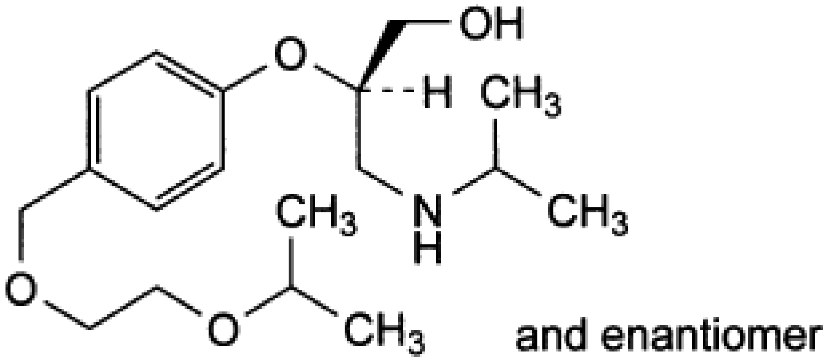


C. Ar-CH_2-Ar: (*RS*)-1-[4-[4-(2-hydroxy-3-isopropylamino-propoxy)benzyl]phenoxy]-3-isopropylaminopropan-2-ol,

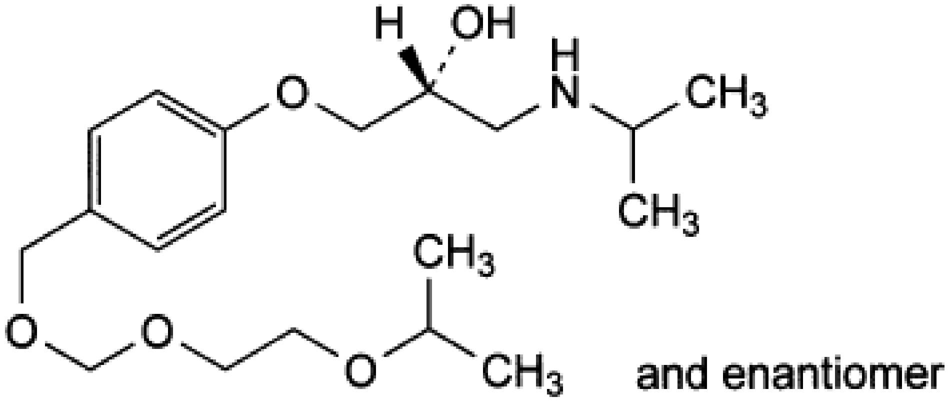
D. Ar-CH_2-*O*-CH_2-Ar: (*RS*)-1-[4-[4-(2-hydroxy-3-isopropylaminopropoxy)benzyloxylmethyl] phenoxy]-3-isopropylaminopropan-2-ol,



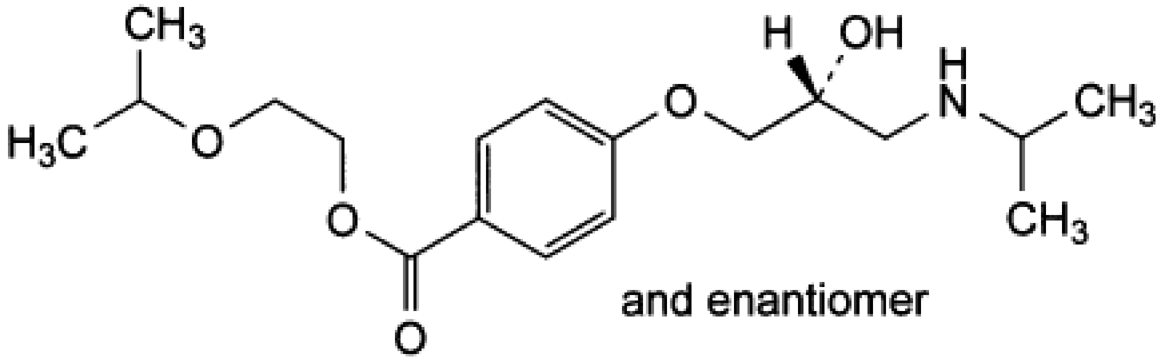
E. (*EZ*)-[3-[4-(2-isopropoxy-ethoxymethyl)phenoxy]allyl] isopropylamine,



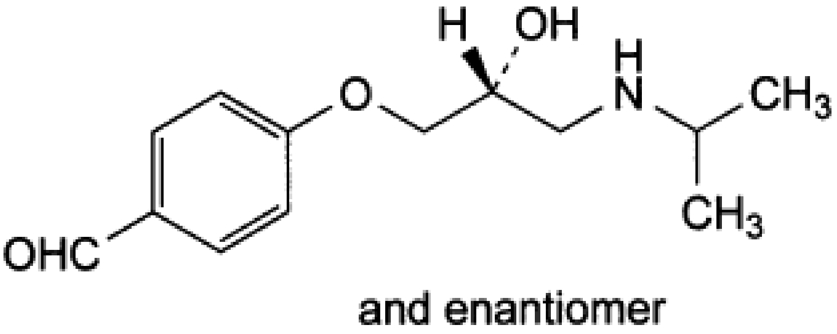
F. (*RS*)-2-[4-(2-isopropoxy-ethoxymethyl)phenoxy]-3-isopropylaminopropan-2-ol,



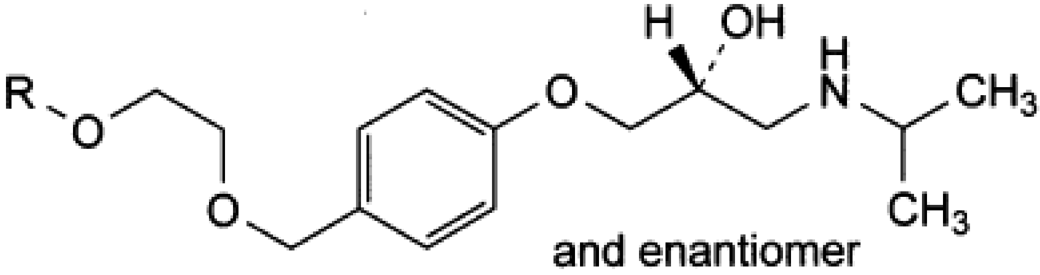
G. (2*RS*)-1-[4-[[(2-isopropoxyethoxy)methoxy]methyl] phenoxy]-3-isopropylaminopropan-2-ol,


and enantiomer

K. 2-isopropoxyethyl 4-[[(2*RS*)-2-hydroxy-3-(isopropylamino)propyl]oxy]benzoate,

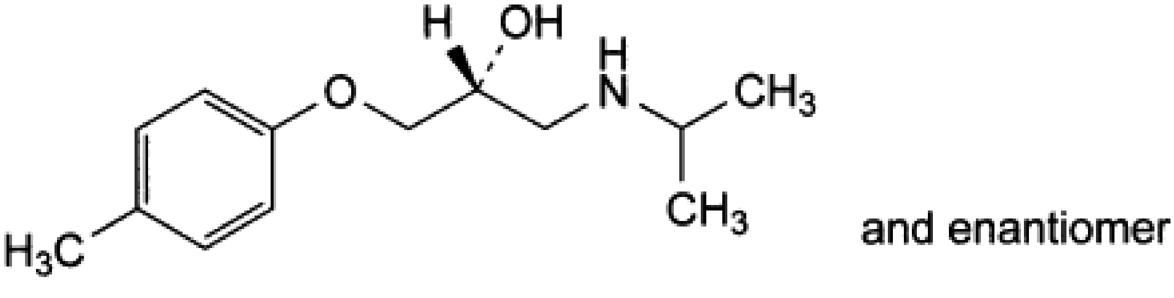

and enantiomer

L. 4-[[(2*RS*)-2-hydroxy-3-(isopropylamino)propyl]oxy]benzaldehyde,

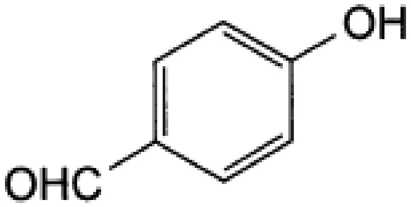

and enantiomer

N. R = C_2H_5: [(2*RS*)-1-[4-[(2-ethoxyethoxy)methyl]phenoxy]-3-isopropylaminopropan-2-ol,

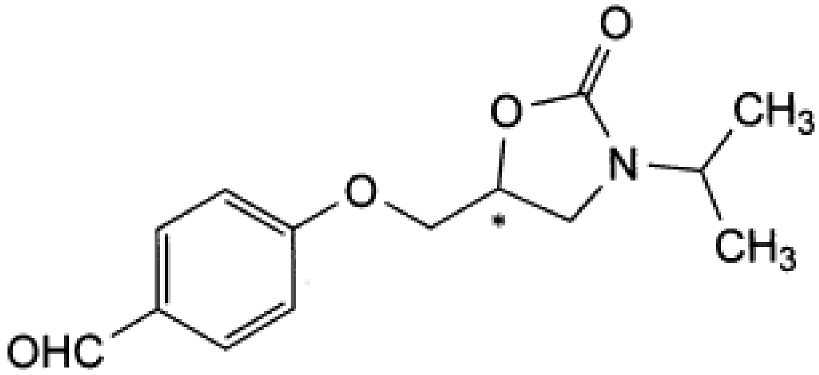
Q. R = CH_3: (2*RS*)-1-(isopropylamino)-3-[4-(2-methoxyethoxy)methyl]phenoxypropan-2-ol,


and enantiomer

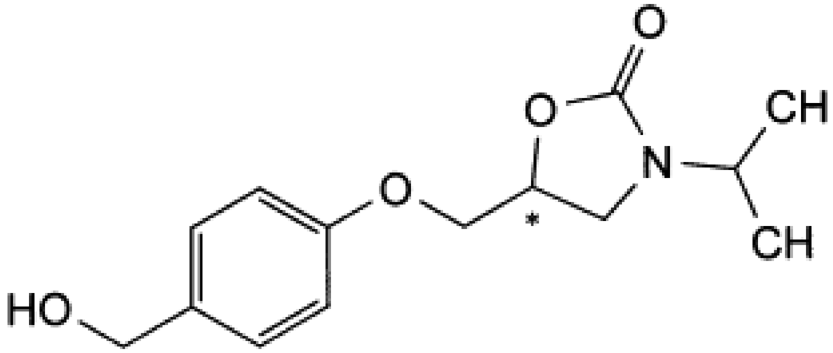
R. (2*RS*)-1-(isopropylamino)-3-(4-methylphenoxy)propan-2-ol,



S. 4-hydroxybenzaldehyde,



T. 4-[(3-isopropyl-2-oxo-1,3-oxazolidin-5-yl)methoxy]benzaldehyde,



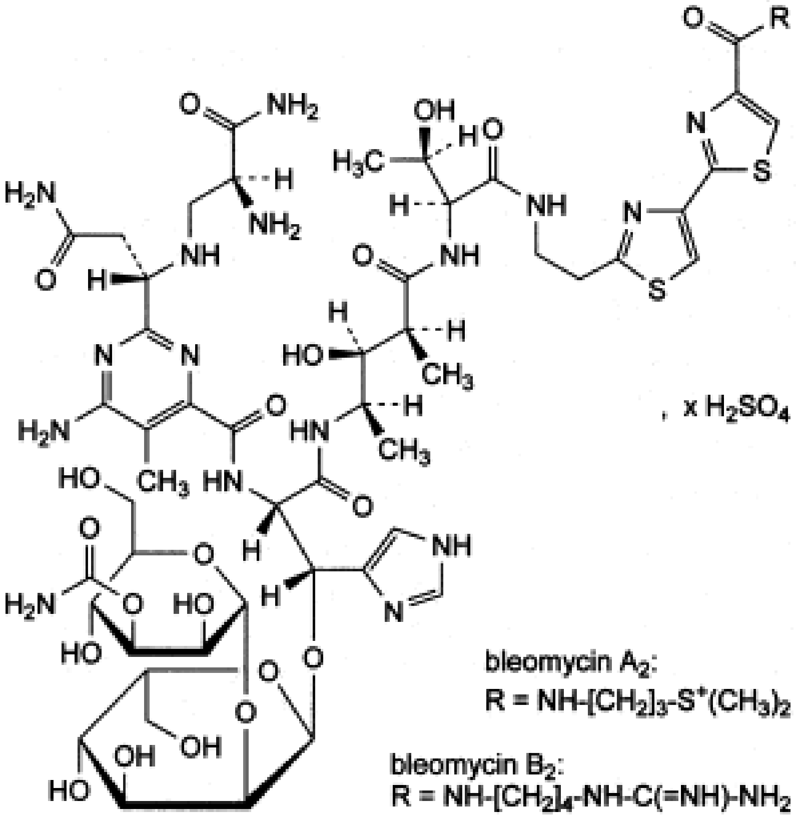
U. 5-[[4-(hydroxymethyl)phenoxy]methyl]-3-isopropyl-1,3-oxazolidin-2-one.

Ph Eur

Bleomycin Sulphate

General Notices

(Ph Eur monograph 0976)


, x H_2SO_4
bleomycin A_2:
$R = NH\text{-}[CH_2]_3\text{-}S^+(CH_3)_2$
bleomycin B_2:
$R = NH\text{-}[CH_2]_4\text{-}NH\text{-}C(=NH)\text{-}NH_2$

9041-93-4

Action and use

Cytotoxic antibacterial.

Preparation

Bleomycin Injection

Ph Eur

DEFINITION

Sulphate of a mixture of glycopeptides produced by *Streptomyces verticillus* or by any other means; the 2 principal components of the mixture are *N*-[3-(dimethylsulphonio)propyl]bleomycinamide (bleomycin A_2) and *N*-[4-(carbamimidoylamino)butyl]bleomycinamide (bleomycin B_2).

Potency Minimum 1500 IU/mg (dried substance).

CHARACTERS

Appearance

White or yellowish-white, very hygroscopic powder.

Solubility

Very soluble in water, slightly soluble in anhydrous ethanol, practically insoluble in acetone.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for composition.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the 2 principal peaks in the chromatogram obtained with reference solution (a).

B. It gives the reactions of sulphates *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and its absorbance *(2.2.25)* at 430 nm is not greater than 0.10.

Dissolve 0.200 g in *water R* and dilute to 10.0 ml with the same solvent.

pH *(2.2.3)*

4.5 to 6.0.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Composition

Liquid chromatography *(2.2.29)*: use the normalisation procedure.

Test solution Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 25.0 mg of *bleomycin sulphate CRS* in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 1.5 ml of reference solution (a) to 100.0 ml with *water R*.

Column:

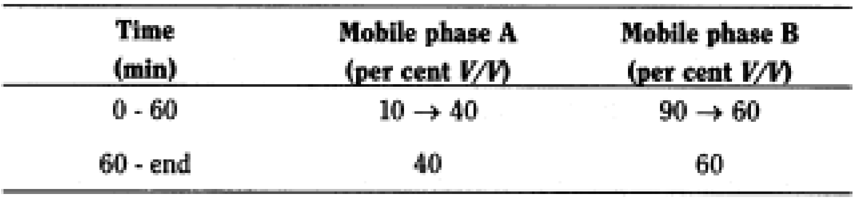
— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase:

— *mobile phase A*: *methanol R*;

— *mobile phase B:* dissolve 0.960 g of *sodium pentanesulphonate R* in 900 ml of acetic acid (4.8 g/l $C_2H_4O_2$), add 1.86 g of *sodium edetate R*, dilute to 1000 ml with the same solvent and adjust to pH 4.3 with *ammonia R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	10 → 40	90 → 60
60 - end	40	60

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time Until impurity D is eluted (about 80 min).

Relative retention With reference to bleomycin A_2: impurity D = 1.5 to 2.5.

System suitability:

— *resolution*: minimum 5 between the peaks due to bleomycin A_2 (1st principal peak) and bleomycin B_2 (2nd principal peak) in the chromatogram obtained with reference solution (a);

— *signal-to-noise ratio*: minimum 20 for the principal peak in the chromatogram obtained with reference solution (b);

— *repeatability*: maximum relative standard deviation of 2 per cent for the principal peak after 6 injections of reference solution (a).

Limits:

— *bleomycin* A_2: 55 per cent to 70 per cent;

— *bleomycin* B_2: 25 per cent to 32 per cent;

— *sum of bleomycin* A_2 *and* B_2: minimum 85 per cent;

— *impurity D:* maximum 5.5 per cent;

— *sum of impurities other than D*: maximum 9.5 per cent;

— *disregard limit*: 0.1 per cent of the total.

Copper

Maximum 2.00×10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 50 mg in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution Dilute 1.0 ml of *copper standard solution (10 ppm Cu) R* to 10.0 ml with *water R*.

Source Copper hollow-cathode lamp.

Wavelength 324.7 nm.

Atomisation device Air-acetylene flame.

Loss on drying ***(2.2.32)***

Maximum 3.0 per cent, determined on 50 mg by drying at 60 °C at a pressure not exceeding 0.67 kPa for 3 h.

Bacterial endotoxins ***(2.6.14)***

Less than 5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)*, using the diffusion method. Use *bleomycin sulphate CRS* as the chemical reference substance.

STORAGE

In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities D.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C*.

A. R = OH: bleomycinic acid,

B. R = $NH-[CH_2]_3-NH-[CH_2]_4-NH_2$: bleomycin A_5,

C. R = $NH-[CH_2]_4-NH-C(=NH)-NH-[CH_2]_4-NH-C(=NH)-NH_2$: bleomycin B_4,

D. R = $NH-[CH_2]_3-S-CH_3$: demethylbleomycin A_2.

Ph Eur

Borax

General Notices

Sodium Borate; Sodium Tetraborate

(Ph Eur monograph 0013)

$Na_2B_4O_7,10H_2O$ 381.4 *1303-96-4*

Ph Eur

DEFINITION

Disodium tetraborate decahydrate.

Content

99.0 per cent to 103.0 per cent of $Na_2B_4O_7,10H_2O$.

CHARACTERS

Appearance

White or almost white, crystalline powder, colourless crystals or crystalline masses, efflorescent.

Solubility

Soluble in water, very soluble in boiling water, freely soluble in glycerol.

IDENTIFICATION

A. To 1 ml of solution S (see Tests) add 0.1 ml of *sulphuric acid R* and 5 ml of *methanol R* and ignite. The flame has a green border.

B. To 5 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution is red. On the addition of 5 ml of *glycerol (85 per cent) R* the colour disappears.

C. Solution S gives the reactions of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 4.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

9.0 to 9.6 for solution S.

Sulphates ***(2.4.13)***

Maximum 50 ppm, determined on solution S.

Use in this test 1.0 ml of *acetic acid R*. Prepare the standard using a mixture of 3 ml of *sulphate standard solution (10 ppm* SO_4*) R* and 12 ml of *distilled water R.*

Ammonium ***(2.4.1)***

Maximum 10 ppm.

Dilute 6 ml of solution S to 14 ml with *water R.* Prepare the standard using a mixture of 2.5 ml of *ammonium standard solution (1 ppm* NH_4*) R* and 7.5 ml of *water R*.

Arsenic ***(2.4.2, Method A)***

Maximum 5 ppm, determined on 5 ml of solution S.

Calcium ***(2.4.3)***

Maximum 100 ppm, determined on solution S.

Prepare the standard using a mixture of 6 ml of *calcium standard solution (10 ppm Ca) R* and 9 ml of *distilled water R.*

Heavy metals ***(2.4.8)***

Maximum 25 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

ASSAY

Dissolve 20 g of *mannitol R* in 100 ml of *water R*, heating if necessary, cool and add 0.5 ml of *phenolphthalein solution R* and neutralise with 0.1 *M sodium hydroxide* until a pink colour is obtained. Add 3.00 g of the substance to be examined, heat until dissolution is complete, cool, and titrate with *1 M sodium hydroxide* until the pink colour reappears.

1 ml of *1 M sodium hydroxide* is equivalent to 0.1907 g of $Na_2B_4O_7,10H_2O$.

Ph Eur

Boric Acid

General Notices

(Ph Eur monograph 0001)

H_3BO_3 61.8 *10043-35-3*

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder, colourless, shiny plates greasy to the touch, or white or almost white crystals.

Solubility

Soluble in water and in ethanol (96 per cent), freely soluble in boiling water and in glycerol (85 per cent).

IDENTIFICATION

A. Dissolve 0.1 g by gently heating in 5 ml of *methanol R*, add 0.1 ml of *sulphuric acid R* and ignite the solution. The flame has a green border.

B. Solution S (see Tests) is acid *(2.2.4)*.

TESTS

Solution S

Dissolve 3.3 g in 80 ml of boiling *distilled water R*, cool and dilute to 100 ml with *carbon dioxide-free water R* prepared from *distilled water R*.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

3.8 to 4.8 for solution S.

Solubility in ethanol (96 per cent)

The solution is not more opalescent than reference suspension II *(2.2.1)* and is colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in 10 ml of boiling *ethanol (96 per cent) R*.

Organic matter

It does not darken on progressive heating to dull redness.

Sulphates *(2.4.13)*

Maximum 450 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 15 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using a mixture of 2.5 ml of *lead standard solution (2 ppm Pb) R* and 7.5 ml of *water R*.

ASSAY

Dissolve 1.000 g with heating in 100 ml of *water R* containing 15 g of *mannitol R*. Titrate with *1 M sodium hydroxide*, using 0.5 ml of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

1 ml of *1 M sodium hydroxide* is equivalent to 61.8 mg of H_3BO_3.

Ph Eur

Botulinum Toxin Type A For Injection

General Notices

(Ph Eur monograph 2113)

Ph Eur

DEFINITION

Botulinum toxin type A for injection is a dried preparation containing purified botulinum neurotoxin type A which may be present in the form of a complex with haemagglutinins and non-toxic proteins. Botulinum neurotoxin type A or its haemagglutinin complex is prepared by a suitable purification process of the liquid supernatant from a broth-culture of a suitable strain of *Clostridium botulinum* type A.

The purified complexes consist of several proteins and can be of various sizes. The largest complex (relative molecular mass of about 900 000) consists of a 150 000 relative molecular mass neurotoxin, a 130 000 relative molecular mass non-toxic protein and various haemagglutinins ranging between relative molecular mass 14 000 and 43 000. The purified toxin moiety is composed of only the same 150 000 relative molecular mass neurotoxin as is found in the 900 000 relative molecular mass neurotoxin complex, which is initially produced as a single chain and further cleaved (nicked) by endogenous proteases into a fully active, disulphide-linked, 54 000 relative molecular mass light chain and a 97 000 relative molecular mass heavy chain.

The preparation is reconstituted before use, as stated on the label.

PRODUCTION

GENERAL PROVISIONS

Production of the toxin is based on seed cultures, managed in a defined seed-lot system in which the ability to produce toxin is conserved. The production method must be shown to yield consistently product of activity and profile comparable to that of lots shown in clinical studies to be of adequate safety and efficacy.

The production method is validated to demonstrate that the product, if tested, would comply with the general test of abnormal toxicity *(2.6.9)* using not less than the maximum human clinical dose, in the presence of a suitable amount of specific botulinum type A antitoxin used for neutralisation.

The production method and stability of the finished product and relevant intermediates are evaluated using the tests below. Such tests include the specific toxin activity per milligram of protein of purified toxin in an appropriate functional model of toxin activity and may be supported by tests confirming the presence of botulinum toxin type A, and, if appropriate, associated non-toxic proteins.

BACTERIAL SEED LOTS

A highly toxigenic strain of *C. botulinum* of known toxin type A and confirmed absence of genes encoding other botulinum toxins (particularly botulinum toxin type B), with known origin and history, is grown using suitable media. The bacterial strain, used for the master seed lot, shall be identified by historical records that include information on its origin and the tests used to characterise the strain. These will include morphological, cultural, biochemical, genetic and serological properties of the strain. The master seed lot and the working seed lot, where applicable, must be demonstrated to have identical profiles. Only a seed lot that complies with the following requirements may be used.

Identification

Each seed lot is identified as containing pure cultures of *C. botulinum* type A bacteria with no extraneous bacterial or fungal contamination.

Microbial purity

Each seed lot complies with the requirements for absence of contaminating micro-organisms. The purity of bacterial cultures is verified by methods of suitable sensitivity. These may include inoculation into suitable media and examination of colony morphology.

Phenotypic parameters

Each seed lot must have a known fatty acid profile, sugar fermentation profile (glucose, lactose, mannose, etc.) and proteolytic activity and must demonstrate relevant lipase, lecithinase and gelatinase activity.

Genetic purity

Each seed lot must have information on the toxin gene sequence and comply with requirements for the absence of other genes encoding other toxin serotypes.

Production of active toxin

A bacterial strain producing a high yield of active toxin, as determined by an acute toxicity assay, is suitable. Seed lots should demonstrate a capability of producing at least a minimum toxicity level appropriate for the manufacturing process and scale.

MANUFACTURER'S REFERENCE PREPARATIONS

During development, reference preparations are established for subsequent verification of batch consistency during production and for control of the bulk purified toxin and finished product. They are derived from representative batches of botulinum toxin type A that are characterised as described under Bulk Purified Toxin.

The reference preparations are suitably characterised for their intended purpose and are stored in suitably sized aliquots under conditions ensuring their suitability.

BULK PURIFIED TOXIN

C. botulinum type A strain is grown anaerobically, in suitable media, from which cultures are selected for step-up incubations under a suitably controlled anaerobic atmosphere through the seed culture and bulk fermentation stages to allow maximum production of toxin. The toxin is purified by suitable methods to remove nucleic acids and components likely to cause adverse reactions.

Only a purified toxin that complies with the following requirements may be used in the preparation of the final bulk. For each test and for each product, limits of acceptance are established and each new purified toxin must comply with these limits.

Residual reagents

Removal of residual reagents used in purification steps is confirmed by suitable limit tests or by validation of the process.

Nucleic acids

Removal of nucleic acids is confirmed by suitable limit tests or by validation of the process.

Immunological identity

The presence of specific type A toxin is confirmed by a suitable immunochemical method *(2.7.1)*.

Specific activity

The specific activity is confirmed in a mouse model of toxicity or by *in vivo/ex vivo* methods validated with respect to the LD50 assay and expressed in mouse LD50 units per milligram of protein. Specific activity must not be less than 1×10^8 mouse LD50 units per milligram of protein for the 150 000 relative molecular mass neurotoxin and must not be less than 1×10^7 mouse LD50 units per milligram of protein for the 900 000 relative molecular mass neurotoxin complex.

Protein

The total protein concentration is determined by a suitable method. An acceptable value is established for the product and each batch must be shown to comply with the limits.

Protein profile

Identity and protein composition are determined by polyacrylamide gel electrophoresis *(2.2.31)* under reducing or non-reducing conditions or by other suitable physicochemical methods such as size-exclusion chromatography *(2.2.30)*, comparing with suitable reference standards.

Total viable count

It complies with the limits approved for the particular product.

FINAL BULK

The final bulk is prepared by adding approved excipients to the bulk purified toxin. The solution is filtered through a bacteria-retentive filter. If human albumin is added, it complies with the monograph on *Human albumin solution (0255)*.

FINAL LOT

The final bulk is distributed aseptically into sterile, tamper-proof containers. Uniformity of fill is verified during filling and the test for uniformity of content *(2.9.6)* is not required. The containers are closed so as to prevent contamination.

Only a final lot that is within the limits approved for the particular product and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

pH *(2.2.3)*

The pH of the reconstituted product is within ± 0.5 pH units of the limit approved for the particular product.

Water

Not more than the limit approved for the particular product.

IDENTIFICATION

The presence of botulinum toxin type A is confirmed by a suitable immunochemical method *(2.7.1)*.

TESTS

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU per vial.

ASSAY

The potency of the reconstituted product is determined by an LD50 assay in mice or by a method validated with respect to the LD50 assay. The potency is expressed in terms of the LD50 for mice or relative to the reference preparation.

For determination of the LD50, graded doses of the product are injected intraperitoneally into groups of mice and the LD50 is calculated by the usual statistical methods (*5.3*) from the mouse lethality in each group. A suitable reference preparation is assayed in parallel; the potency of the toxin is expressed relative to the reference or the value found for the reference is within suitable limits defined in terms of the assigned potency.

After validation with respect to the LD50 assay (reference method), the product may also be assayed by other methods that are preferable in terms of animal welfare, including 1 of the following:

1. endopeptidase assay *in vitro*;
2. *ex vivo* assay using the mouse phrenic nerve diaphragm;
3. mouse bioassay using paralysis as the end-point.

For these other methods, the potency is calculated with respect to a suitable reference preparation calibrated in mouse LD50 units.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

The test may be repeated but when more than 1 test is performed, the results of all valid tests must be combined in the estimate of potency.

LABELLING

The label states:

— the number of units of toxin per vial with a statement that units are product specific and not applicable to other preparations containing botulinum toxin type A;

— the name and the volume of the diluent to be added for reconstitution of a dried product.

Bovine Serum

General Notices

(Ph Eur monograph 2262)

Ph Eur

DEFINITION

Liquid fraction of blood obtained from the ox (*Bos taurus* L.) and from which cells, fibrin and clotting factors have been removed.

Different types of bovine serum are used:

— *adult bovine serum* obtained at slaughter from cattle that are declared fit for human consumption;

— *calf serum* obtained at slaughter from animals, fit for human consumption, before the age of 12 months;

— *new-born calf serum* obtained at slaughter from animals before the age of 20 days;

— *foetal bovine serum* obtained from normal foetuses from dams fit for human consumption;

— *donor bovine serum* obtained by repeated bleeding of donor animals from controlled donor herds.

This monograph provides a general quality specification for bovine serum. Various measures are applied during the production of bovine serum aimed at obtaining a product that is acceptable as regards viral safety. No single measure, nor the combination of measures outlined below can guarantee complete viral safety but they rather reduce the risk involved in the use of serum in the manufacture of medicinal products. It is therefore necessary for the manufacturer of a medicinal product to take account of this when choosing the serum for a particular use by making a risk assessment.

PRODUCTION

All stages of serum production are submitted to a suitable quality assurance system.

Traceability of serum is maintained from the final container to the abattoir of origin (for blood collected from slaughtered animals) or to the herd of origin (for blood collected from donor animals).

Further guarantee of the safety and quality of serum may be ensured by the use of a controlled donor herd. Where serum is obtained from such a herd, the animals are subjected to regular veterinary examination to ascertain their health status. Animals introduced into the herd are traceable as regards source, breeding and rearing history. The introduction of animals into the herd follows specified procedures, including defined quarantine measures. During the quarantine period the animals are observed and tested to establish that they are free from all agents and antibodies from which the donor herd is claimed to be free. It may be necessary to test the animals in quarantine for freedom from additional agents, depending on factors such as information available on their breeding and rearing history. It is recommended that animals in the herd should not be vaccinated against bovine viral diarrhoea virus. Tests are carried out for any agent and/or antibody from which the herd is claimed to be free.

Serum is obtained by separation of the serum from blood cells and clot under conditions designed to minimise microbial contamination. Serum from a number of animals is pooled and a batch number is allocated to the pool. Appropriate steps are taken to ensure homogeneity of the harvested material, intermediate pools and the final batch. Suitable measures (for example filtration) are taken to ensure sterility or a low bioburden. Before further processing, the serum is tested for sterility or bioburden. General and specific tests for viral contaminants are carried out as described below.

A step or steps for virus inactivation/removal are applied to serum intended for production of immunological veterinary medicinal products. Unless otherwise justified and authorised for a particular medicinal product, a step or steps for virus inactivation/removal are applied to serum intended for production of human and non-immunological veterinary medicinal products.

INACTIVATION

The inactivation procedure applied is validated with respect to a suitable representative range of viruses covering different types (enveloped, non-enveloped, DNA, RNA viruses). The optimal choice of relevant and model viruses depends strongly on the specific inactivation/ removal procedure; representative viruses with different degrees of resistance to the type of treatment must be included. Bovine viral diarrhoea virus must be included in the viruses used for validation. Serum free from antibodies against bovine viral diarrhoea virus is used in part or all of the validation studies.

For bovine serum intended for use in immunological veterinary medicinal products, for inactivation by gamma irradiation a minimum dose of 30 kGy is applied, unless otherwise justified and authorised.

Critical parameters for the method of virus inactivation/removal are established and the parameters used in the validation study are strictly adhered to during subsequent application of the procedures to each batch of serum.

For inactivation by gamma irradiation, critical parameters include:

— the temperature;

— packaging configuration;

— distribution of dosimeters to assess the effective dose received by the product whatever its position;

— the minimum and maximum dose received.

QUALITY CONTROL TESTS APPLIED TO EACH BATCH

A suitable sample size for each batch is established. Specific tests for viral contaminants are validated with respect to sensitivity and specificity. The cell cultures used for general tests for viral contaminants are shown to be sensitive to a suitable range of potential contaminants. Control cells used in the tests are cultivated, where relevant, with a bovine serum controlled and inactivated as described in this monograph. Serum free from antibodies to bovine viral diarrhoea virus is required for validation of the effect of antibodies on the detection limits for bovine viral diarrhoea virus.

Tests carried out on the batch prior to treatment

The following tests are carried out on the serum (before any virus inactivation/removal steps, where applicable).

Tests for viral contaminants General tests supplemented by specific tests are carried out.

General tests Validated tests are carried out by inoculation of the serum on at least 2 distinct cell lines, one of which is of bovine origin. The cell lines used are suitable for detecting haemadsorbing viruses such as bovine parainfluenza virus 3 and cytopathic agents such as bovine herpesvirus 1.

Specific tests For viral contaminants (if not detected by general tests), where relevant in view of the country of origin of the serum: bluetongue virus, bovine adenovirus, bovine parvovirus, bovine respiratory syncytial virus, bovine viral diarrhoea virus, rabies virus and reovirus. Depending on the country of origin, specific tests for other viruses may be needed. The animal health status of countries is defined by the 'Office International des Epizooties' (OIE).

For serum to be subjected to a virus inactivation/removal procedure, if evidence of viral contamination is found in any of the tests described above, the serum is acceptable only if the virus is identified and shown to be present in an amount that has been shown in a validation study to be effectively inactivated.

For serum that is not to be subjected to a virus inactivation/removal procedure, if evidence of viral contamination is found in any of the tests described above, the serum is not acceptable.

A test for bovine viral diarrhoea virus antibodies is carried out; an acceptance criterion for the titre is established taking account of the risk assessment.

Composition The content of a suitable selection of the following components is determined and shown to be within the expected range for the type of serum: cholesterol, α-, β- and γ-globulin, albumin, creatinine, bilirubin, glucose, serum aspartate transaminase (SAST, formerly SGOT - serum glutamic-oxaloacetic transaminase), serum alanine transaminase (SALT, formerly SGPT - glutamic-pyruvic transaminase), phosphorus, potassium, calcium, sodium and pH.

Tests carried out on the batch post-treatment

If bovine viral diarrhoea virus was detected before virus inactivation/removal, the following test for bovine viral diarrhoea virus is carried out after virus inactivation/removal.

Test for bovine viral diarrhoea virus A validated test for bovine viral diarrhoea virus is carried out, for example by inoculation into susceptible cell cultures, followed by not fewer than 3 subcultures and detection by immunostaining. No evidence of the presence of bovine viral diarrhoea virus is found.

IDENTIFICATION

A. The electrophoretic pattern corresponds to that for serum and is consistent with the type (foetal or other) of bovine serum.

B. Bovine origin is confirmed by a suitable immunochemical method *(2.7.1)*.

TESTS

Osmolality *(2.2.35)*

280 mosmol/kg to 365 mosmol/kg for foetal bovine serum and 240 mosmol/kg to 340 mosmol/kg for other types.

Total protein *(2.5.33)*

30 mg/ml to 45 mg/ml for foetal bovine serum and minimum 35 mg/ml for other types.

Haemoglobin

Maximum 4 mg/ml, determined by a validated method, such as spectrophotometry.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/ml for donor bovine serum, less than 25 IU/ml for foetal bovine serum, less than 100 IU/ml for other types.

Sterility *(2.6.1)*

It complies with the test. Use 10 ml for each medium.

Mycoplasmas *(2.6.7)*

It complies with the test.

STORAGE

Frozen at - 10 °C or below.

LABELLING

The label states:

— the type of serum;

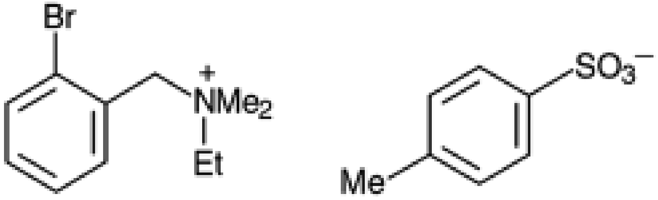
— where applicable, that the serum has been inactivated and the inactivation method;

— where the serum has been inactivated by gamma irradiation, the target minimum dose of the irradiation procedure.

Ph Eur

Bretylium Tosilate

General Notices



$C_{18}H_{24}BrNO_3S$ 414.4 *61-75-6*

Action and use

Antiarrhythmic.

Preparation

Bretylium Injection

DEFINITION

Bretylium Tosilate is 2-bromobenzyl-*N*-ethyldimethylammonium-*p*-toluenesulphonate. It contains not less than 99.0% and not more than 101.0% of $C_{18}H_{24}BrNO_3S$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder. It melts at about 98°. It exhibits polymorphism.

Freely soluble in *water*, in *ethanol (96%)* and in *methanol*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of bretylium tosilate *(RS 030)*. If the spectra are not concordant, dissolve a quantity of the substance being examined in the minimum volume of *acetone* by heating on a water bath at 50°, evaporate to dryness at room temperature under a current of nitrogen and prepare a new spectrum of the residue.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck plates are suitable) and a mixture of 15 volumes of *glacial acetic acid*, 30 volumes of *water* and 75 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in *water* containing (1) 0.5% w/v of the substance being examined and (2) 0.5% w/v of *bretylium tosilate BPCRS*. After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) the two principal spots correspond to those in the

chromatogram obtained with solution (2).

TESTS

Acidity

pH of a 5.0% w/v solution, 5.0 to 6.5, Appendix V L.

Clarity and colour of solution

A 5.0% w/v solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in the mobile phase containing (1) 0.20% w/v of the substance being examined, (2) 0.002% w/v of the substance being examined and (3) 0.05% w/v of *bretylium tosilate BPCRS* and 0.05% w/v of *2-bromobenzyldimethylamine hydrochloride BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemically bonded phenyl groups (5 µm) (Spherisorb Phenyl is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 0.5 volume of *triethylamine*, 2 volumes of *glacial acetic acid*, 19 volumes of *acetonitrile* and 81 volumes of *0.01M sodium octanesulphonate* and (c) a detection wavelength of 265 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 6.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than half the area of the peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard the peak due to tosilate (retention time, about 2 minutes) and any peak with an area less than 0.05 times the area of the peak in the chromatogram obtained with solution (2) (0.05%).

Loss on drying

When dried to constant weight over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa, loses not more than 3.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A. Use 1 g.

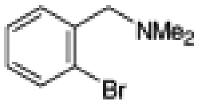
ASSAY

Dissolve 0.2 g in 50 ml of *1,4-dioxan* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *0.025M perchloric acid VS* as titrant and determining the end-point potentiometrically. Each ml of *0.025M perchloric acid VS* is equivalent to 10.36 mg of $C_{18}H_{24}BrNO_3S$.

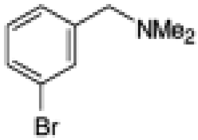
STORAGE

Bretylium Tosilate should be kept in an airtight container and protected from light.

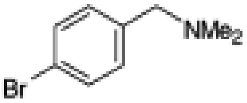
IMPURITIES



A. 2-bromobenzyldimethylamine



B. 3-bromobenzyldimethylamine



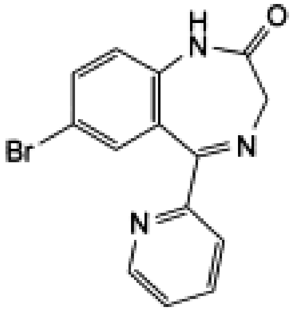
C. 4-bromobenzyldimethylamine

Browse: British Pharmacopoeia 2009
British Pharmacopoeia Volume I & II
Monographs: Medicinal and Pharmaceutical Substances
Bromazepam

Bromazepam

General Notices

(Ph Eur monograph 0879)



$C_{14}H_{10}BrN_3O$ 316.2 *1812-30-2*

Action and use

Benzodiazepine.

Ph Eur

DEFINITION

7-Bromo-5-(pyridin-2-yl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble or sparingly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison bromazepam CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 10.0 mg of the substance to be examined in 9 ml of a mixture of 1 volume of *acetonitrile R* and 8 volumes of *methanol R*. Dilute to 20.0 ml with an 11.33 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with a 100 g/l solution of *potassium hydroxide R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *bromazepam for system suitability* CRS (containing impurities A, B, C, D and E) in 5 ml of a mixture of 1 volume of *acetonitrile R* and 8 volumes of *methanol R*. Dilute to 10.0 ml with an 11.33 g/l solution *of potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with a 100 g/l solution of *potassium hydroxide R*.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R (3.5 µm);*

— temperature: 50 °C.

Mobile phase Mix 5 volumes of *acetonitrile R*, 45 volumes of *methanol R* and 50 volumes of an 11.33 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with a 100 g/l solution of *potassium hydroxide R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl.

Run time 4 times the retention time of bromazepam.

Identification of impurities Use the chromatogram supplied with *bromazepam for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to bromazepam (retention time = about 5 min): impurity D = about 1.4; impurity A = about 1.5; impurity C = about 1.6; impurity E = about 2.1; impurity B = about 2.2.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to bromazepam and impurity D and minimum 1.2 between the peaks due to impurities A and C.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.3; impurity B = 1.8; impurity E = 2.1;

— *impurities A, B, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.2 per cent, determined on 1.000 g by drying at 80 °C at a pressure not exceeding 2.7 kPa for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 20 ml of *anhydrous acetic acid R*. Add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 31.62 mg of $C_{14}H_{10}BrN_3O$.

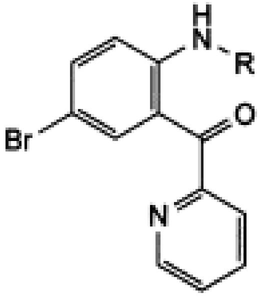
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, E.*

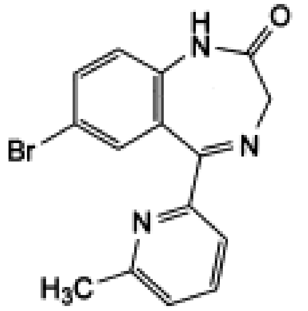
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C, D.*



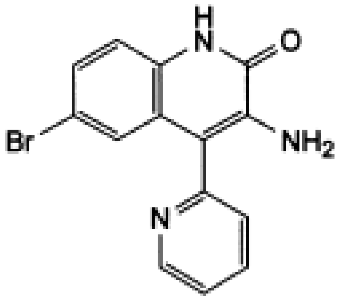
A. R = H: (2-amino-5-bromophenyl)(pyridin-2-yl)methanone,

B. R = CO-CH_2-Cl: *N*-[4-bromo-2-(pyridin-2-ylcarbonyl)phenyl]-2-chloroacetamide,

E. R = CO-CH_2-Br: 2-bromo-*N*-[4-bromo-2-(pyridin-2-ylcarbonyl)phenyl]acetamide,



C. 7-bromo-5-(6-methylpyridin-2-yl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



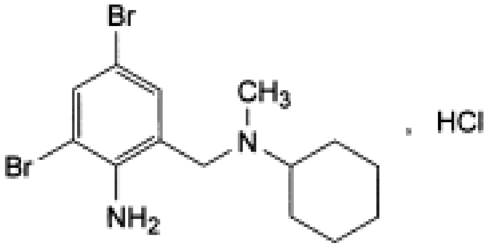
D. 3-amino-6-bromo-4-(pyridin-2-yl)quinolin-2(1*H*)-one.

Ph Eur

Bromhexine Hydrochloride

General Notices

(Ph Eur monograph 0706)



$C_{14}H_{20}Br_2N_2$,HCl 412.6 *611-75-6*

Action and use

Mucolytic.

Ph Eur

DEFINITION

N-(2-Amino-3,5-dibromobenzyl)-*N*-methylcyclohexanamine hydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, slightly soluble in alcohol and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification A, E.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *bromhexine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *bromhexine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (17:17:66 *V/V/V*).

Application 20 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 25 mg in a mixture of 1 ml of *dilute sulphuric acid R* and 50 ml of *water R*. Add 2 ml of *methylene chloride R* and 5 ml of *chloramine solution R* and shake. A brownish-yellow colour develops in the lower layer.

D. Dissolve about 1 mg in 3 ml of *0.1 M hydrochloric acid*. The solution gives the reaction of primary aromatic amines *(2.3.1)*.

E. Dissolve about 20 mg in 1 ml of *methanol R* and add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 0.6 g in *methanol R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *bromhexine impurity C CRS* in *methanol R*, add 1.0 ml of the test solution and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R.* Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Column:

— *size: l* = 0.12 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R (3 µm).*

Mobile phase Mix 0.50 ml of *phosphoric acid R* in 950 ml of *water R*, adjust to pH 7.0 with *triethylamine R* (about 1.5 ml) and dilute to 1000 ml with *water R*; mix 20 volumes of this solution with 80 volumes of *acetonitrile R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 248 nm.

Injection 10 µl.

Run time 2.5 times the retention time of bromhexine.

Relative retention With reference to bromhexine (retention time = about 11 min): impurity A = about 0.1; impurity B = about 0.2; impurity C = about 0.4; impurity D = about 0.5.

System suitability Reference solution (a):

— *resolution*: minimum 12.0 between the peaks due to impurity C and bromhexine.

Limits:

— *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), and not more than 1 such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 70 ml of *alcohol R* and add 1 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 41.26 mg of $C_{14}H_{21}Br_2ClN_2$.

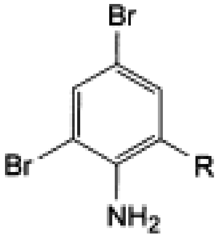
STORAGE

Protected from light.

IMPURITIES

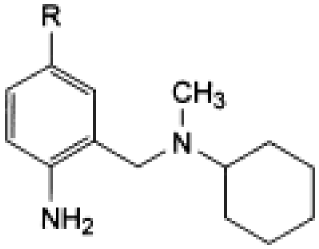
Specified impurities A, B, C, D.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E.*



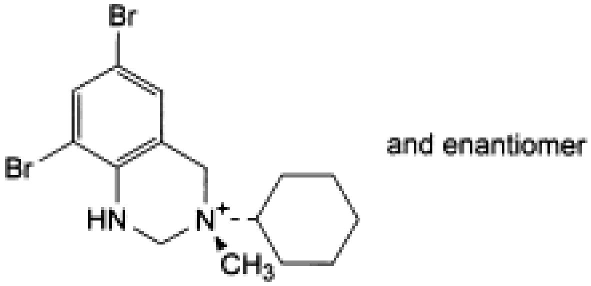
A. R = CH_2OH: (2-amino-3,5-dibromophenyl)methanol,

B. R = CHO: 2-amino-3,5-dibromobenzaldehyde,



C. R = H: *N*-(2-aminobenzyl)-*N*-methylcyclohexanamine,

D. R = Br: *N*-(2-amino-5-bromobenzyl)-*N*-methylcyclohexanamine,



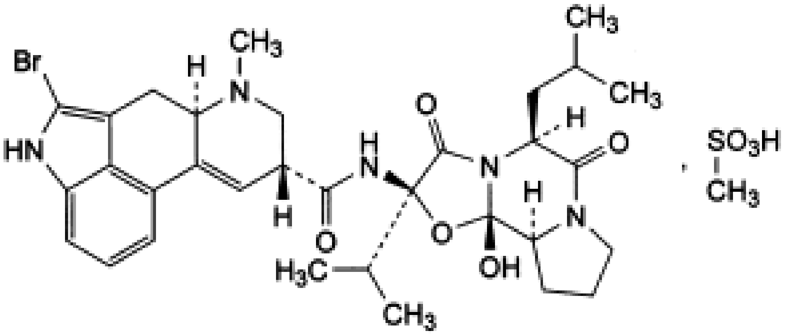
E. (3*RS*)-6,8-dibromo-3-cyclohexyl-3-methyl-1,2,3,4-tetrahydroquinazolin-3-ium.

Ph Eur

Bromocriptine Mesilate

General Notices

(Ph Eur monograph 0596)



$C_{32}H_{40}BrN_5O_5S,CH_4SO_3$ 751   *22260-51-1*

Action and use

Dopamine receptor agonist.

Preparations

Bromocriptine Capsules

Bromocriptine Tablets

Ph Eur

DEFINITION

(6a*R,*9*R*)-5-Bromo-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2-(1-methylethyl)-5-(2-methylpropyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide monomethanesulphonate.

Content

98.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

White or slightly coloured, fine crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methanol, soluble in ethanol (96 per cent), sparingly soluble in methylene chloride.

It is very sensitive to light.

The identification, tests and assay are to be carried out as rapidly as possible, protected from light.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D, E.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 10.0 mg in 10 ml of *methanol R* and dilute to 200.0 ml with 0*.01 M hydrochloric acid*.

Spectral range 250-380 nm.

Absorption maximum At 305 nm.

Absorption minimum At 270 nm.

Specific absorbance at the absorption maximum 120 to 135 (dried substance).

B. Infrared absorption spectrophotometry *(2.2.24*).

Comparison *bromocriptine mesilate CRS.*

C. Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use.*

Solvent mixture *ethanol (96 per cent) R, methanol R, methylene chloride* R (30:30:40 *V/V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution Dissolve 10 mg of *bromocriptine mesilate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, water R, 2-propanol R, methylene chloride R, ether R* (0.1:1.5:3:88:100 *V/V/V/V/V*).

Application 10 µl.

Development Immediately in an unsaturated tank, over a path of 15 cm.

Drying In a current of cold air for 2 min.

Detection Spray with *ammonium molybdate solution R3* and dry at 100 °C until the spots appear (about 10 min).

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 0.1 g add 5 ml of *dilute hydrochloric acid R* and shake for about 5 min. Filter and add 1 ml of *barium chloride solution R1*. The filtrate remains clear. To a further 0.1 g add 0.5 g of *anhydrous sodium carbonate R*, mix and ignite until a white residue is obtained. Allow to cool and dissolve the residue in 7 ml of *water R* (solution A). Solution A gives reaction (a) of sulphates *(2.3.1)*.

E. Solution A obtained in identification test D gives reaction (a) of bromides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_5, BY_5 or Y_5 *(2.2.2, Method II)*.

Dissolve 0.25 g in *methanol R* and dilute to 25 ml with the same solvent.

pH *(2.2.3)*

3.1 to 3.8.

Dissolve 0.2 g in a mixture of 2 volumes of *methanol R* and 8 volumes of *carbon dioxide-free water R* and dilute to 20 ml with the same mixture of solvents.

Specific optical rotation *(2.2.7)*

+ 95 to + 105 (dried substance).

Dissolve 0.100 g in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10.0 ml with the same mixture of solvents.

Related substances.

Liquid chromatography *(2.2.29)*.

Solvent mixture *buffer solution pH 2.0 R, methanol R* (50:50 *V/V*).

Test solution Dissolve 0.500 g of the substance to be examined in 5.0 ml of *methanol R* and dilute to 10.0 ml with *buffer solution pH 2.0 R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve the contents of a vial of *bromocriptine mesilate for system suitability CRS* (containing impurities A and B) in 1.0 ml of the solvent mixture.

Column:

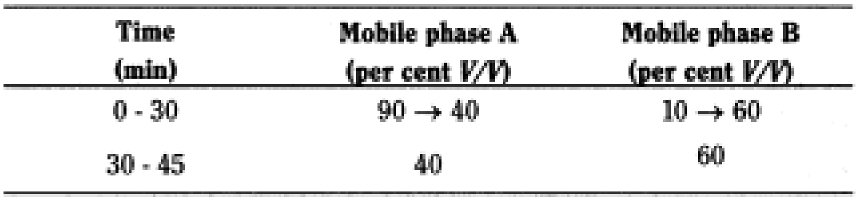
— *size:* l = 0.12 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: 0.791 g/l solution of *ammonium carbonate R*;

— *mobile phase B*: *acetonitrile R;*



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 30	90 → 40	10 → 60
30 - 45	40	60

Flow rate 2 ml/min.

Detection Spectrophotometer at 300 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *bromocriptine mesilate* for *system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to bromocriptine: impurity C = about 1.2.

System suitability Reference solution (c):

— *resolution*: minimum 1.1 between the peaks due to impurities A and B.

Limits:

— *impurity A:* not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent);

— *impurity C*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *impurities B, D, E, F, G*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and not more than 1 such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent), apart from the peak due to impurity A.

Loss on drying *(2.2.32)*

Maximum 3.0 per cent, determined on 0.500 g by drying *in vacuo* at 80 °C for 5 h.

ASSAY

Dissolve 0.500 g in 80 ml of a mixture of 10 volumes of *anhydrous acetic acid R* and 70 volumes of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

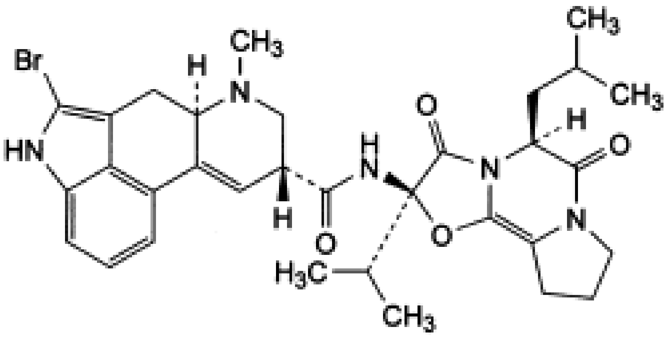
1 ml of *0.1 M perchloric acid* is equivalent to 75.1 mg of $C_{33}H_{44}BrN_5O_8S$.

STORAGE

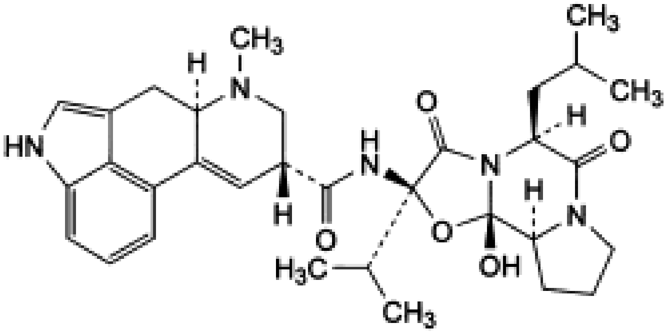
In an airtight container , protected from light, at a temperature not exceeding - 15 °C.

IMPURITIES

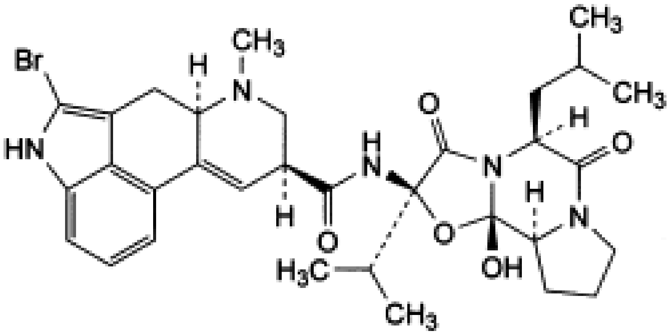
Specified impurities *A, B, C, D, E, F, G.*



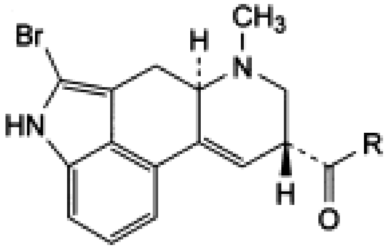
A. (6a*R*,9*R*)-5-bromo-*N*-[(2*R*,5*S*)-2-(1-methylethyl)-5-(2-methylpropyl)-3,6-dioxo-2,3,5,6,9,10-hexahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (2-bromodehydro-α-ergocriptine),



B. (6a*R*,9*R)*-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2-(1-methylethyl)-5-(2-methylpropyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (α-ergocriptine),

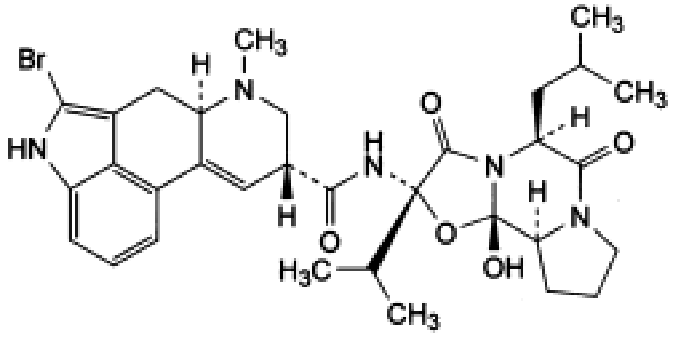


C. (6a*R*,9*S*)-5-bromo-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2-(1-methylethyl)-5-(2-methylpropyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide ((9S)-2-bromo-α-ergocriptine),

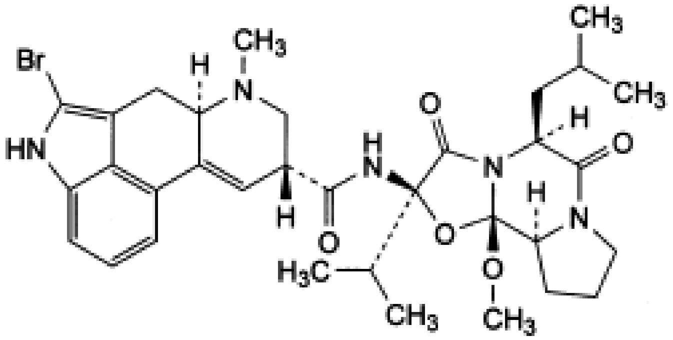

Br
HN
H
CH3
N
R
H
O

D. R = OH: (6a*R*,9*R*)-5-bromo-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,

E. R = NH_2: (6a*R,*9*R*)-5-bromo-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide,


Br
HN
H
CH3
N
H
O
H
N
O
H3C
CH3
O
N
CH3
CH3
H
O
H
N
O
OH

F. (6a*R*,9*R*)-5-bromo-*N*-[(2*S*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2-(1-methylethyl)-5-(2-methylpropyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide ((2′S)-2-bromo-α-ergocriptine),


Br
HN
H
CH3
N
H
O
H
N
O
H3C
CH3
O
N
CH3
CH3
H
O
H
N
O
CH3

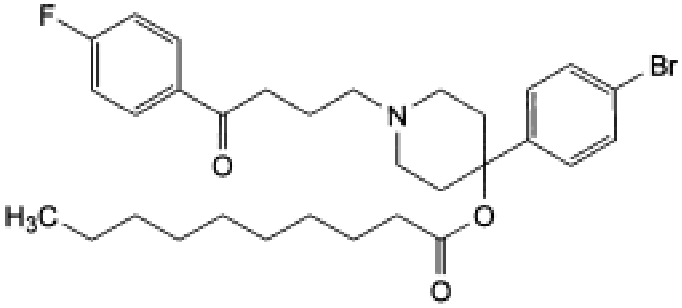
G. (6a*R*,9*R*)-5-bromo-*N*-[(2*R*,5*S*,10a*S,*10b*S*)-10b-methoxy-2-(1-methylethyl)-5-(2-methylpropyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (2-bromo-10′b-O-methyl-α-ergocriptine).

Ph Eur

Bromperidol Decanoate

General Notices

(Ph Eur monograph 1397)



$C_{31}H_{41}BrFNO_3$ 574.6 *75067-66-2*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

4-(4-Bromophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, soluble in ethanol (96 per cent).

mp: about 60 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Mulls in *liquid paraffin R*.

Comparison *bromperidol decanoate CRS.*

B. To 0.1 g in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of bromides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_5 *(2.2.2, Method II)*.

Dissolve 2.0 g in *methylene chloride R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg *of bromperidol decanoate CRS* and 2.5 mg of *haloperidol decanoate CRS* in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Column:

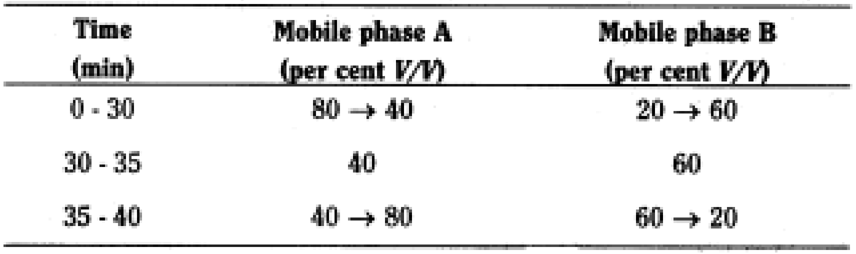
— *size:* *l* = 0.1 m, Ø = 4.0 mm;

— *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 27 g/l solution of *tetrabutylammonium hydrogen sulphate R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	80 → 40	20 → 60
30 - 35	40	60
35 - 40	40 → 80	60 → 20

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Haloperidol decanoate = about 24 min; bromperidol decanoate = about 24.5

min.

System suitability   Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to haloperidol decanoate and bromperidol decanoate; if necessary, adjust the gradient or the time programme for the linear gradient elution.

Limits:

— *impurities A, B, C, D, E, F, G, H, I, J, K*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 30 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.450 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid* using 0.2 ml of *naphtholbenzein solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 57.46 mg of $C_{31}H_{41}BrFNO_3$.

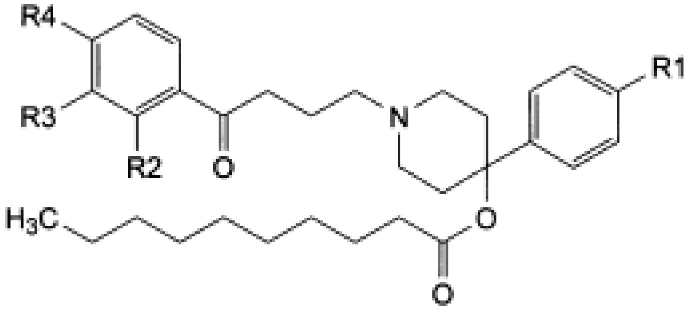
STORAGE

At a temperature below 25 °C, protected from light.

IMPURITIES

Specified impurities   *A, B, C, D, E, F, G, H, I, J, K.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): L.*

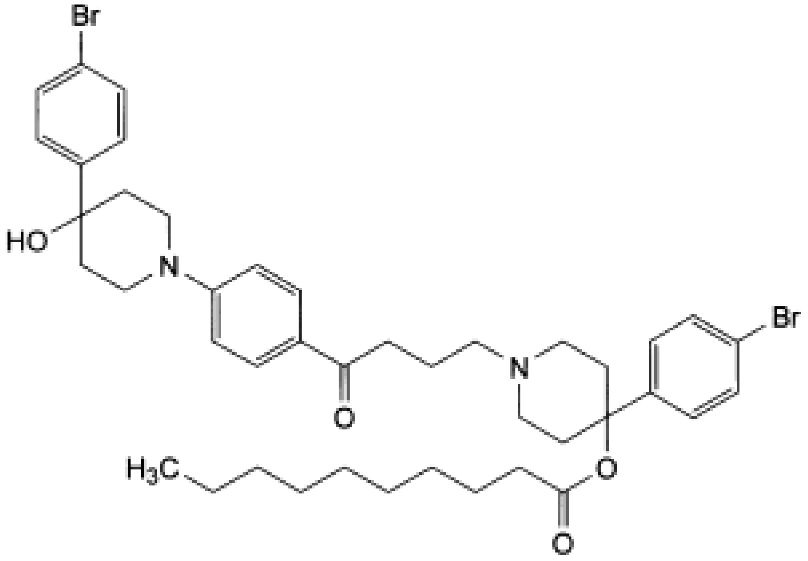


A. R1 = R2 = R3 = H, R4 = F: 1-[4-(4-fluorophenyl)-4-oxobutyl]-4-phenylpiperidin-4-yl decanoate,

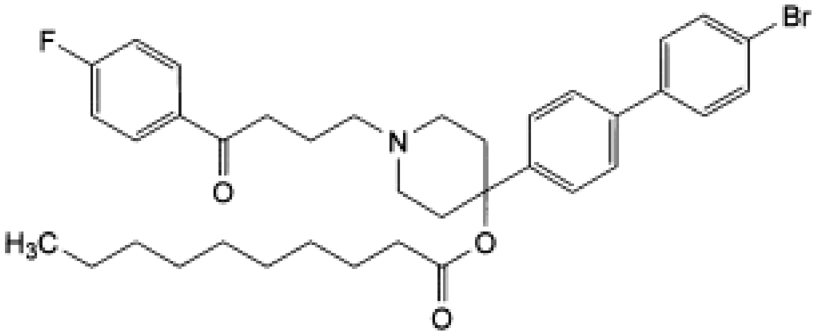
B. R1 = Br, R2 = F, R3 = R4 = H: 4-(4-bromophenyl)-1-[4-(2-fluorophenyl)-4-oxobutyl]-piperidin-4-yl decanoate,

C. R1 = Br, R2 = H, R3 = C_2H_5, R4 = F: 4-(4-bromophenyl)-1-[4-(3-ethyl-4-fluorophenyl)-4-oxobutyl]-piperidin-4-yl decanoate,

F. R1 = C_6H_5, R2 = R3 = H, R4 = F: 4-(biphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl] piperidin-4-yl decanoate,

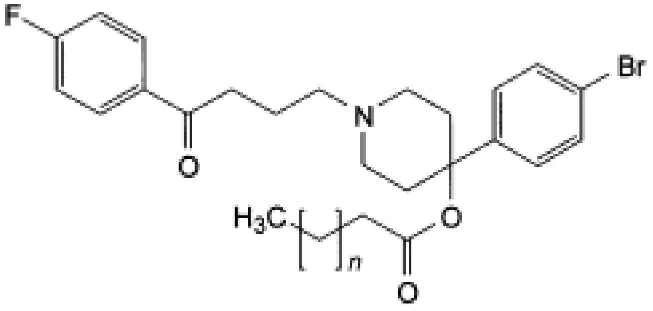


D. 4-(4-bromophenyl)-1-[4-[4-[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]phenyl]-4-oxobutyl] piperidin-4-yl decanoate,



E. 4-(4′-bromobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate,

G. bromperidol,

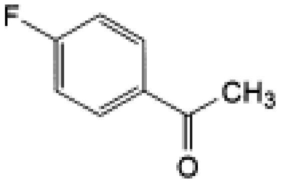


H. *n* = 5: 4-(4-bromophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl octanoate,

I. *n* = 6: 4-(4-bromophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl nonanoate,

J. *n* = 8: 4-(4-bromophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl undecanoate,

K. *n* = 9: 4-(4-bromophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl dodecanoate,



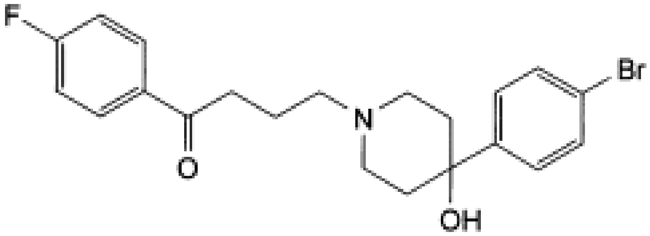
L. 1-(4-fluorophenyl)ethanone.

Ph Eur

Bromperidol

General Notices

(Ph Eur monograph 1178)



$C_{21}H_{23}BrFNO_2$ 420.3 *10457-90-6*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

4-[4-(4-Bromophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, sparingly soluble in methanol and in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. Melting point *(2.2.14)*: 156 °C to 159 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *bromperidol CRS.*

C. Thin layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *bromperidol CRS in methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *bromperidol CRS* and 10 mg *of haloperidol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *tetrahydrofuran R, methanol R*, 58 g/l solution of *sodium chloride R* (10:45:45 *V/V/V*).

Application 1 µl.

Development In an unsaturated tank over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 5 ml of *anhydrous ethanol R*. Add 0.5 ml of *dinitrobenzene solution R* and 0.5 ml of *2 M alcoholic potassium hydroxide solution R*. A violet colour is produced that becomes brownish-red after 20 min.

E. To 0.1 g in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of bromides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Dissolve 0.2 g in 20 ml of a 1 per cent *V/V* solution of *lactic acid R.*

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *bromperidol CRS* and 5.0 mg of *haloperidol CRS* in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R.*

Column:

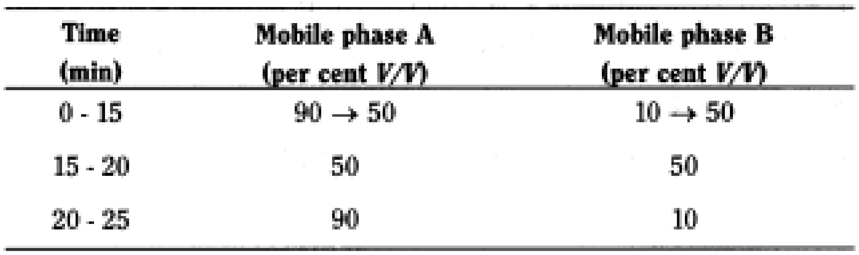
— *size: l* = 0.1 m, Ø = 4.0 mm;

— *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A:* 17 g/l solution of *tetrabutylammonium hydrogen sulphate R;*

— *mobile phase B: acetonitrile R;*



Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 15	90 → 50	10 → 50
15 - 20	50	50
20 - 25	90	10

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time haloperidol = about 5.5 min; bromperidol = about 6 min.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to haloperidol and bromperidol; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient elution.

Limits:

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes *of methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, using 0.2 ml *of naphtholbenzein solution R* as indicator.

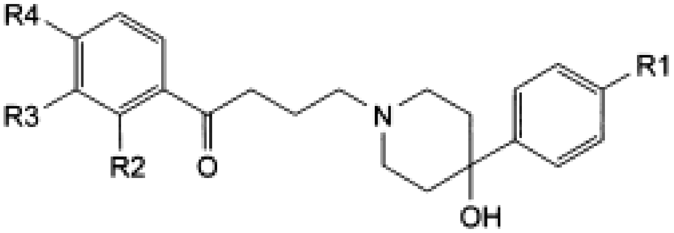
1 ml of *0.1 M perchloric acid* is equivalent to 42.03 mg of $C_{21}H_{23}BrFNO_2$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F.

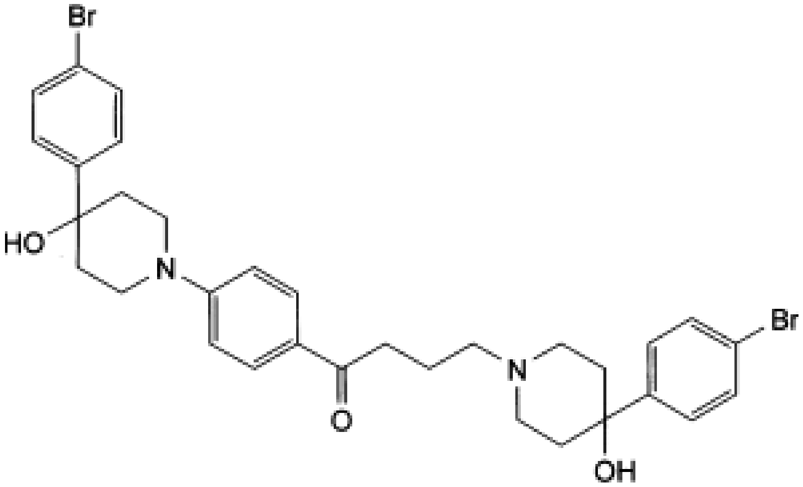


A. R1 = R2 = R3 = H, R4 = F: 1-(4-fluorophenyl)-4-(4-hydroxy-4-phenylpiperidin-1-yl)butan-1-one,

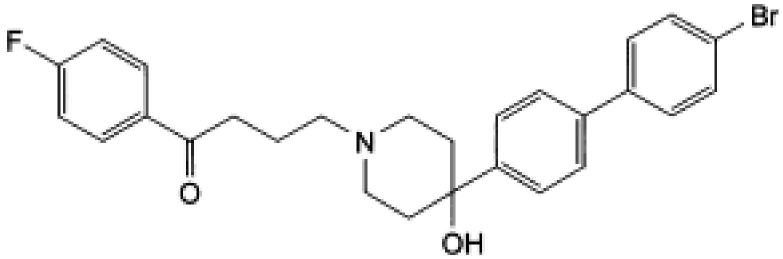
B. R1 = Br, R2 = F, R3 = R4 = H: 4-[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]-1-(2-fluorophenyl)butan-1-one,

C. R1 = C_6H_5. R2 = R3 = H, R4 = F: 4-[4-(biphenyl-4-yl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one,

D. R1 = Br, R2 = H, R3 = C_2H_5, R4 = F: 4-[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]-1-(3-ethyl-4-fluorophenyl)butan-1-one,



E. 4-[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]-1-[4-[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]phenyl]butan-1-one,



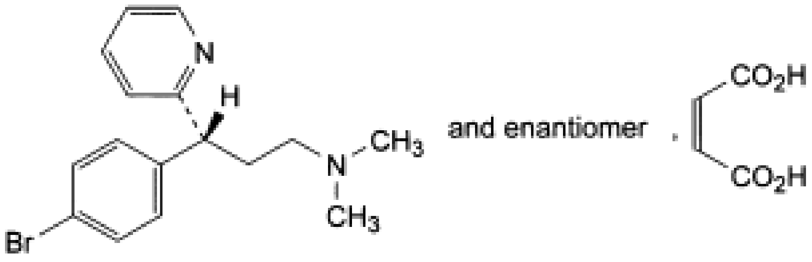
F. 4-[4-(4′-bromobiphenyl-4-yl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one.

Ph Eur

Brompheniramine Maleate

General Notices

(Ph Eur monograph 0977)



$C_{20}H_{23}BrN_2O_4$ 435.3 *980-71-2*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Brompheniramine Tablets

Ph Eur

DEFINITION

(3*RS*)-3-(4-Bromophenyl)-*N,N*-dimethyl-3-(pyridin-2-yl)propan-1-amine (*Z*)-butenedioate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in ethanol (96 per cent), in methanol and in methylene chloride.

IDENTIFICATION

First identification A, B, C, D, E.

Second identification *A, B, E, F.*

A. Melting point *(2.2.14)*: 130 °C to 135 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25).*

Test solution Dissolve 65 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*.

Spectral range 220-320 nm.

Absorption maximum At 265 nm.

Specific absorbance at the absorption maximum 190 to 210.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *brompheniramine maleate CRS.*

D. Examine the chromatograms obtained in the test for related substances.

System suitability Reference solution (c):

— the chromatogram shows 2 principal peaks with retention times corresponding to the retention times of the peaks obtained with reference solutions (a) and (b).

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

E. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution Dissolve 56 mg of *maleic acid R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *water R, anhydrous formic acid R, methanol R, di-isopropyl ether* R (3:7:20:70 *V/V/V/V*).

Application 5 µl.

Development Over a path of 12 cm.

Drying In a current of air for a few minutes.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution shows 2 clearly separated spots. The upper spot is similar in position and size to the spot in the chromatogram obtained with the reference solution.

F. To 0.15 g in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue in 10 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of bromides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *2.2.2, Method II*).

Dissolve 2.0 g in *methanol R* and dilute to 20 ml with the same solvent.

pH *(2.2.3)*

4.0 to 5.0.

Dissolve 0.20 g in 20 ml of *carbon dioxide-free water R*.

Optical rotation *(2.2.7)*

- 0.2° to + 0.2° (measured in a 2 dm tube).

Dissolve 2.5 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Gas chromatography *(2.2.28)*.

Test solution Dissolve 0.10 g of the substance to be examined in 10 ml of *methylene chloride R.*

Reference solution (a) Dissolve 10 mg of *brompheniramine maleate CRS* in *methylene chloride R* and dilute to 1 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *chlorphenamine maleate CRS* (impurity A) in *methylene chloride R* and dilute to 1 ml with the same solvent.

Reference solution (c) To 0.5 ml of the test solution add 0.5 ml of reference solution (b).

Column:

— *material*: glass;

— *size*: *l* = 2.3 m, Ø = 2 mm;

— *stationary phase*: acid- and base-washed *silanised diatomaceous earth for gas chromatography R* (135-175 µm) impregnated with 3 per cent *m/m* of *polymethylphenylsiloxane R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 20 ml/min.

Temperature:

— *column*: 205 °C;

— *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 2.5 times the retention time of brompheniramine.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to brompheniramine and impurity A.

Limits:

- *impurities A, B, C*: for each impurity, maximum 0.4 per cent of the area of the principal peak;
- *total*: maximum 1 per cent of the area of the principal peak;
- *disregard limit*: 0.1 per cent of the area of the principal peak.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.260 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.2.20).

1 ml of *0.1 M perchloric acid* is equivalent to 21.77 mg of $C_{20}H_{23}BrN_2O_4$.

STORAGE

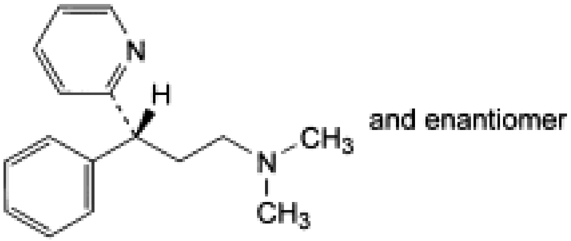
Protected from light.

IMPURITIES

Specified impurities *A, B, C.*

A. chlorphenamine,

B. dexchlorpheniramine,

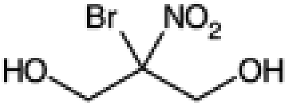


C. (3*RS*)-*N,N*-dimethyl-3-phenyl-3-(pyridin-2-yl)propan-1-amine (pheniramine).

Ph Eur

Bronopol

General Notices



$C_3H_6BrNO_4$ 200.0 *52-51-7*

Action and use

Antibacterial preservative.

DEFINITION

Bronopol is 2-bromo-2-nitropropane-1,3-diol. It contains not less than 99.0% and not more than 101.0% of $C_3H_6BrNO_4$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

White or almost white crystals or crystalline powder.

Freely soluble in *water* and in *ethanol (96%)*; slightly soluble in *glycerol* and in *liquid paraffin*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of bronopol *(RS 031)*.

B. Dissolve 0.1 g in 10 ml of *water*, add 10 ml of 7.5M *sodium hydroxide* and, carefully with constant stirring and cooling, 0.5 g of *nickel-aluminium alloy*. Allow the reaction to subside, filter and carefully neutralise with *nitric acid*. The resulting solution yields reaction A characteristic of *bromides*, Appendix VI.

C. *Melting point*, after drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, about 130°, Appendix V A.

TESTS

Acidity or alkalinity

pH of a 1% w/v solution, 5.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. Solution (1) contains 0.2% w/v of the substance being examined in the mobile phase. For solution (2) dilute a volume of solution (1) with sufficient mobile phase to produce a solution containing 0.0002% w/v of the substance being examined. Solution (3) contains 0.001% w/v each of *2-methyl-2-nitropropan-1,3-diol* and *tris(hydroxymethyl)nitromethane* in the mobile phase. Solution (4) contains 0.0002% w/v each of *2-methyl-2-nitropropane-1,3-diol, 2-nitroethanol, sodium bromide* and *tris(hydroxymethyl)nitromethane* and 0.2% w/v of the substance being examined in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C18 (2) is suitable) and maintained at 35°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 189 volumes of *water,* 10 volumes of *acetonitrile* and 1 volume of a 10% v/v solution of *orthophosphoric acid ,* adjusting the pH to 3.0 using 2M *sodium hydroxide* and (c) a detection wavelength of 214 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to sodium bromide and tris(hydroxymethyl)nitromethane is at least 1.0 and the *resolution factor* between the peaks due to tris(hydroxymethyl)nitromethane and 2-nitroethanol is at least 1.5.

For solution (1) continue the chromatography for 3 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the areas of any peaks corresponding to 2-methyl-2-nitropropane-1,3-diol and tris(hydroxymethyl)nitromethane are not greater than the area of the corresponding peaks in the chromatogram obtained with solution (3) (0.5% of each) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2)

Sulphated ash

Not more than 0.1%, Appendix IX A.

Water

Not more than 0.5% w/w, Appendix IX C, Method I B. Use 5 g.

ASSAY

In a flask fitted with a reflux condenser dissolve 0.4 g in 15 ml of *water* and add 15 ml of *7.5M sodium hydroxide*. Slowly, with caution, add 2 g of *nickel-aluminium alloy* through the reflux condenser, agitating the flask whilst cooling under running water. Allow the mixture to stand for 10 minutes and boil for 1 hour. Cool and filter under reduced pressure, washing the condenser, flask and residue with 150 ml of *water*. Combine the filtrate and washings, add 25 ml of *nitric acid* and 40 ml of *0.1M silver nitrate VS*, shake vigorously and titrate with *0.1M ammonium thiocyanate VS* using *ammonium iron(III) sulphate solution R2* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of silver nitrate required. Each ml of *0.1M silver nitrate VS* is equivalent to 20.00 mg of $C_3H_6BrNO_4$.

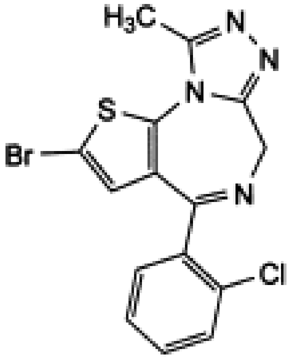
STORAGE

Bronopol should be protected from light.

Brotizolam

General Notices

(Ph Eur monograph 2197)



$C_{15}H_{10}BrClN_4S$ 393.7 *57801-81-7*

Action and use

Benzodiazepine.

Ph Eur

DEFINITION

2-Bromo-4-(2-chlorophenyl)-9-methyl-6*H*-thieno-[3,2-*i*][1,2,4]-triazolo[4,3-*a*][1,4]diazepine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish powder.

Solubility

Practically insoluble in water, sparingly soluble or slightly soluble in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison brotizolam CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light and prepare the solutions immediately before use.*

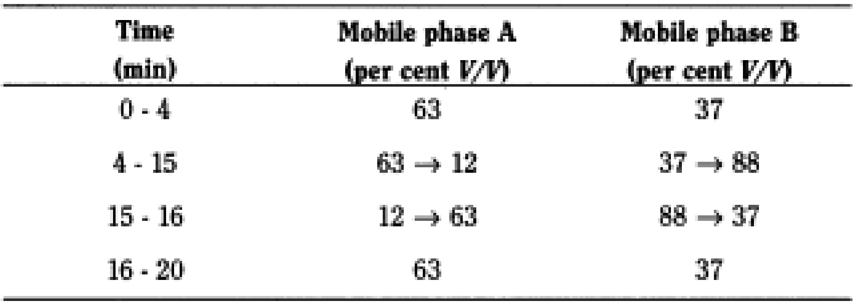
Test solution Dissolve 50.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml of *acetonitrile R*. Dilute 1.0 ml of this solution to 10.0 ml with *acetonitrile R.*

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *brotizolam impurity B CRS* in 50 ml of *acetonitrile R*. Dilute 2 ml of this solution to 20 ml with *acetonitrile R*.

Column:
- — *size: l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 40 °C.

Mobile phase:
- — *mobile phase A*: 2 g/l solution of *sodium heptanesulphonate monohydrate R;*
- — *mobile phase B*: mix 25 volumes of a 2 g/l solution of *sodium heptanesulphonate R* and 75 volumes of *acetonitrile R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	63	37
4 - 15	63 → 12	37 → 88
15 - 16	12 → 63	88 → 37
16 - 20	63	37

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 242 nm.

Injection 5 µl.

Relative retention With reference to brotizolam (retention time = about 7.4 min): impurity A = about 0.5; impurity B = about 0.9.

System suitability Reference solution (b):

- — — *resolution*: minimum 5.0 between the peaks due to impurity B and brotizolam.

Limits:
- — *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in

the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides ***(2.4.4)***

Maximum 100 ppm.

Dissolve 0.67 g in 20.0 ml of *methanol R*, mix and filter.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

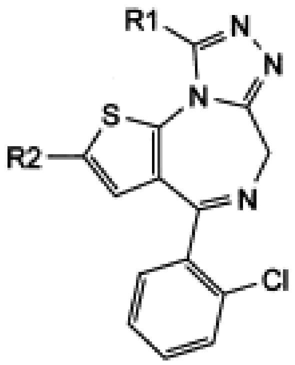
Dissolve 0.150 g in a mixture of 25 ml of *glacial acetic acid R* and 50 ml of *acetic anhydride R*. Titrate to the second point of inflexion with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 19.68 mg of $C_{15}H_{10}BrClN_4S$.

IMPURITIES

Specified impurities B.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A.*

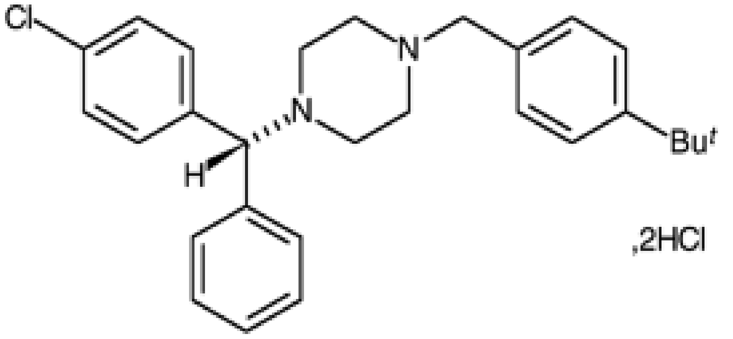


A. R1 = CH_3, R2 = H: 4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4] diazepine (desbromobrotizolam),

B. R1 = H, R2 = Br: 2-bromo-4-(2-chlorophenyl)-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4] diazepine (desmethylbrotizolam).

Buclizine Hydrochloride

General Notices



$C_{28}H_{33}ClN_2,2HCl$ 506.0 *129-74-8*

Action and use

Histamine H_1 receptor antagonist; antiemetic.

DEFINITION

Buclizine Hydrochloride is (*RS*)-1-(4-*tert*-butylbenzyl)-4-(4-chlorobenzhydryl)piperazine dihydrochloride. It contains not less than 99.0% and not more than 100.5% of $C_{28}H_{33}ClN_2$, 2HCl, calculated with reference to the dried substance.

CHARACTERISTICS

A white or slightly yellowish, crystalline powder.

Practically insoluble in *water*; sparingly soluble in *propane-1,2-diol*; very slightly soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of buclizine hydrochloride *(RS 032)*.

B. A 0.25% w/v solution in *ethanol (50%)* yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using four solutions in the initial mobile phase containing (1) 0.0010% w/v of the substance being examined, (2) 0.50% w/v of the substance being examined, (3) 0.0010% w/v of *1,4-bis(4-chlorobenzhydryl)* *piperazine BPCRS* and (4) 0.50% w/v of *buclizine hydrochloride impurity standard BPCRS*.

The chromatographic procedure may be carried out using a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable). Use as the initial mobile phase *0.01M sodium heptanesulphonate* in a mixture of 55 volumes of *water* and 45 volumes of *acetonitrile* and as the final mobile phase *0.01M sodium heptanesulphonate* in a mixture of 20 volumes of *water* and 80 volumes of *acetonitrile*. Before use, adjust the pH of both the initial and final mobile phases to 4.0 with *1M orthophosphoric acid*. Carry out a linear gradient elution with a flow rate of 2 ml per minute for 30 minutes and maintain the final mobile phase for 10 minutes with the same flow rate. Use a detection wavelength of 230 nm.

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *buclizine hydrochloride impurity standard BPCRS*.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 1,4-bis (4-chlorobenzhydryl)-piperazine is not greater than the area of the peak obtained in the chromatogram with solution (3) and the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (1).

Loss on drying

When dried to constant weight at 100° to 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.4 g and determining the end point potentiometrically. Each ml of *0.1M perchloric acid VS* is equivalent to 25.30 mg of $C_{28}H_{33}ClN_2$,2HCl.

IMPURITIES

A. 1,4-bis(4-chlorobenzhydryl)piperazine,

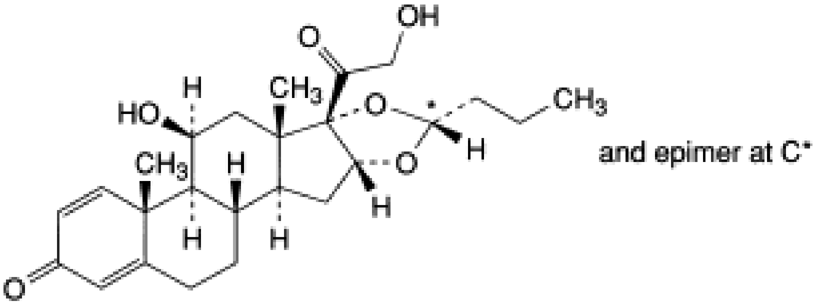
B. 4-chlorobenzhydrol, 1-(4-chlorobenzhydryl)piperazine,

4-chlorobenzophenone.

Budesonide

General Notices

(Ph Eur monograph 1075)



$C_{25}H_{34}O_6$ 430.5 *51333-22-3*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

Budesonide contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of a mixture of the C-22*S* (epimer A) and the C-22*R* (epimer B) epimers of 16α,17-[(1*RS*)-butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in alcohol.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *budesonide CRS*. Examine the substances prepared as discs.

B. Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution. Dissolve 25 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dissolve 25 mg *budesonide CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 12.5 mg *triamcinolone acetonide CRS* in reference solution (a) and dilute to 5 ml with the same solution.

Apply separately to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*. Develop over a path of 15 cm. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve about 2 mg in 2 ml of *sulphuric acid R*. Within 5 min, a yellow colour develops. Within 30 min, the colour changes to brown or reddish-brown. Add cautiously the solution to 10 ml of *water R* and mix. The colour fades and a clear solution remains.

D. Dissolve about 1 mg in 2 ml of a solution containing 2 g of *phosphomolybdic acid R* dissolved in a mixture of 10 ml of dilute *sodium hydroxide solution R*, 15 ml of *water R* and 25 ml of *glacial acetic acid R*. Heat for 5 min on a water-bath. Cool in iced water for 10 min and add 3 ml of dilute *sodium hydroxide solution R.* The solution is blue.

TESTS

Related substances

Examine by liquid chromatography *(2.2.29)* as described under Assay.

Inject 20 µl of reference solution (a). Adjust the sensitivity of the system so that the height of the peak corresponding to epimer B (the first of the two principal peaks) in the chromatogram obtained is not less than 50 per cent of the full scale of the recorder. Inject 20 µl of the test solution, 20 µl of reference solution (a) and 20 µl of reference solution (b). Continue the chromatography for 1.5 times the retention time of epimer B. In the chromatogram obtained with the test solution: the area of any peak, apart from the peaks corresponding to epimer A and epimer B, is not greater than the sum of the areas of the epimer peaks in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of any such peaks is not greater than the sum of the areas of the epimer peaks in the chromatogram obtained with reference solution (a) (1.5 per cent). Disregard any peak with an area less than 0.1 times the sum of the areas of the epimer peaks in the chromatogram obtained with reference solution (b).

Epimer A

Examine by liquid chromatography *(2.2.29)* as described under Assay.

Inject 20 µl of the test solution. The content of epimer A (second peak) is 40.0 per cent to 51.0 per cent of the sum of the areas of the two epimer peaks of budesonide.

Methanol

Not more than 0.1 per cent, determined by head-space gas chromatography using the standard addition method *(2.2.28)*.

Test solution Dissolve 2.000 g of the substance to be examined in *dimethylacetamide R* and dilute to 20.0 ml with the same solvent.

Reference solution Dilute 0.500 g of *methanol R in dimethylacetamide R* and dilute to 100.0 ml with the same solvent.

The following head-space conditions may be used:
— equilibration temperature: 80 °C,

— equilibration time: 30 min,

— transfer-line temperature: 85 °C,

— pressurisation time: 10 s,

— injection time: 10 s.

The chromatographic procedure may be carried out using:

— a fused-silica capillary column 30 m long and 0.32 mm in internal diameter coated with *macrogol 20 000 R* (film thickness 1 µm),

— *nitrogen for chromatography R* as the carrier gas at a pressure of 55 kPa,

— a flame-ionisation detector,

Maintaining the temperature of the column at 50 °C for 5 min, then raising the temperature at a rate of 30 °C per minute to 220 °C and maintaining at 220 °C for 2 min, and maintaining the temperature of the injection port at 250 °C and that of the detector at 300 °C.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Examine by liquid chromatography *(2.2.29)*. *Protect the solutions from light throughout the assay.*

Test solution Dissolve 25.0 mg of the substance to be examined in 15 ml of *acetonitrile R* and dilute to 50.0 ml with *phosphate buffer solution pH 3.2 R*. Allow to stand for at least 15 min before use.

Reference solution (a) Dilute 15.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *budesonide CRS* in 15 ml of *acetonitrile R* and dilute to 50.0 ml with *phosphate buffer solution pH 3.2 R.*

The chromatographic procedure may be carried out using:
— a stainless steel column 0.12 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.5 ml/min a mixture of 32 volumes of *acetonitrile R* and

68 volumes of *phosphate buffer solution pH 3.2 R*,

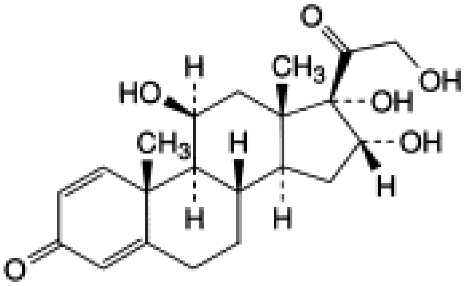
— as detector a spectrophotometer set at 240 nm.

Inject 20 µl of reference solution (c). Adjust the sensitivity of the system so that the height of the peak corresponding to epimer B (the first of the two principal peaks) in the chromatogram obtained is not less than 50 per cent of the full scale of the recorder. When the chromatograms are recorded in the prescribed conditions, the retention time for epimer B is about 16 min. If necessary, adjust the concentration of acetonitrile in the mobile phase (increasing the concentration to decrease the retention time). The test is not valid unless: in the chromatogram obtained with reference solution (c), the resolution between the peaks corresponding to epimer A and epimer B is not less than 1.5; the number of theoretical plates determined from the epimer B peak is at least 4000; the symmetry factor for the same peak is less than 1.5.

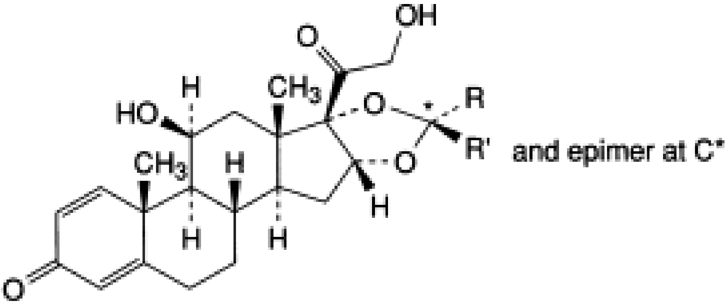
Inject 20 µl of reference solution (c) six times. The assay is not valid unless the relative standard deviation of the sum of the peak areas of the two epimers is at most 1.0 per cent. Inject alternatively the test solution and reference solution (c).

Calculate the percentage content of $C_{25}H_{34}O_6$ from the sum of the areas of the two epimer peaks.

IMPURITIES

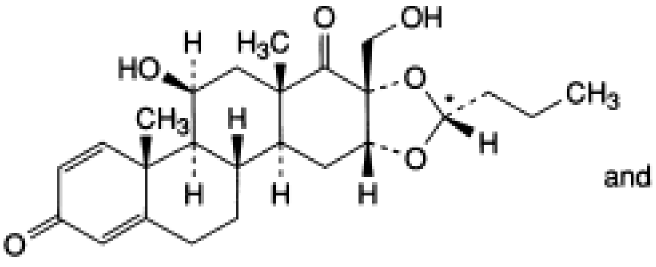


A. 11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione,



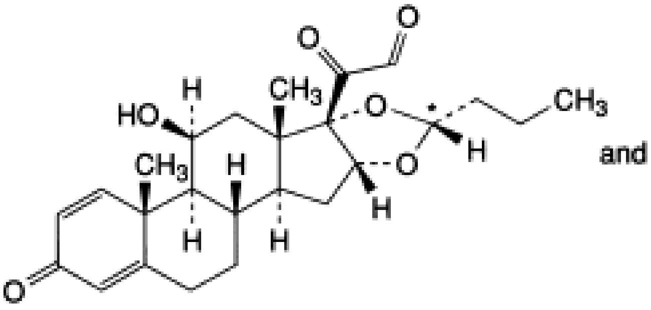
B. R = CH_3, R′ = H: 16α,17-[(1*RS*)-ethylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione,

F. R = R′ = CH_3: 16α,17-[1-methylethylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione,



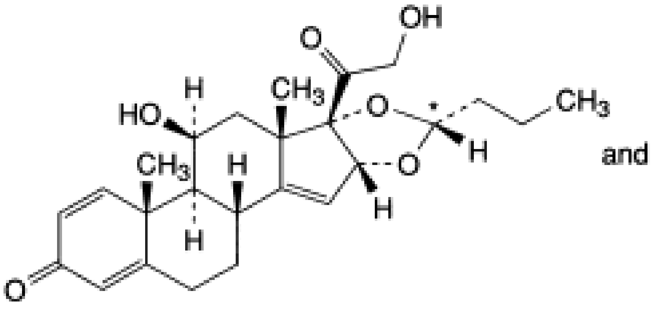
and epimer at C*

C. 16α,17-[(1*RS*)-butylidenebis(oxy)]-11β-hydroxy-17-(hydroxymethyl)-*D*-homoandrosta-1,4-diene-3,17a-dione,



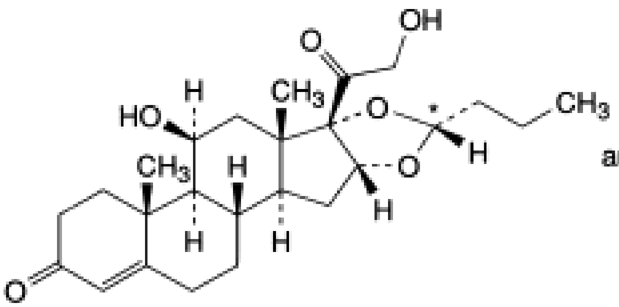
and epimer at C*

D. 16α,17-[(1*RS*)-butylidenebis(oxy)]-11β-hydroxy-3,20-dioxopregna-1,4-dien-21-al,



and epimer at C*

E. 16α,17-[(1*RS*)-butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4,14-triene-3,20-dione,



and epimer at C*

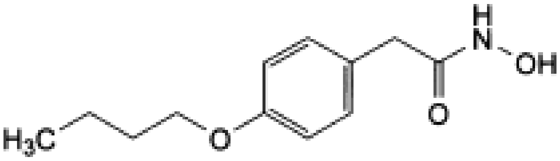
G. 16α,17-[(1*RS*)-butylidenebis(oxy)]-11β,21-dihydroxypregn-4-ene-3,20-dione.

Ph Eur

Bufexamac

General Notices

(Ph Eur monograph 1179)



$C_{12}H_{17}NO_3$ 223.3 *2438-72-4*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

2-(4-Butoxyphenyl)-*N*-hydroxyacetamide.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in dimethylformamide, slightly soluble in ethyl acetate and in methanol.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 20 mg in *methanol R* and dilute to 20 ml with the same solvent. Dilute 1 ml of this solution to 50 ml with *methanol R*.

Spectral range 210-360 nm.

Absorption maxima At 228 nm, 277 nm and 284 nm.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *bufexamac CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *bufexamac CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *salicylic acid R* in reference solution (a) and dilute to 5 ml with the same solution.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *glacial acetic acid R, dioxan R, toluene R* (4:20:90 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of the test solution to 25.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *bufexamac CRS* and 5 mg of *salicylic acid R* in the mobile phase and dilute to 10 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with the mobile phase.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m^2/g and a pore size of 10 nm.

Mobile phase Mix 30 volumes of a 1.4 g/l solution of *dipotassium hydrogen phosphate R* and 70 volumes of *methanol R*, then adjust to pH 3.6 with *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µl.

Run time 4 times the retention time of bufexamac.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to salicylic acid and bufexamac.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in vacuo at 80 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *dimethylformamide R*. Titrate with *0.1 M lithium methoxide*, determining the end-point potentiometrically *(2.2.20)*.

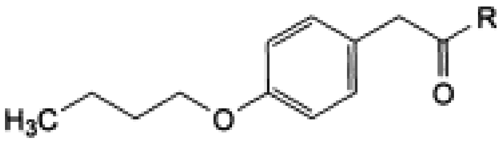
1 ml of *0.1 M lithium methoxide* is equivalent to 22.33 mg of $C_{12}H_{17}NO_3$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D.*



A. R = OH: 2-(4-butoxyphenyl)acetic acid,

B. R = OCH_3: methyl 2-(4-butoxyphenyl)acetate,

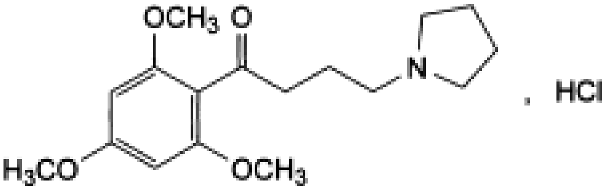
C. R = OC_4H_9: butyl 2-(4-butoxyphenyl)acetate,

D. R = NH_2: 2-(4-butoxyphenyl)acetamide.

Buflomedil Hydrochloride

General Notices

(Ph Eur monograph 1398)



$C_{17}H_{25}NO_4,HCl$ 343.9 *35543-24-9*

Action and use

Vasodilator.

Ph Eur

DEFINITION

4-(Pyrrolidin-1-yl)-1-(2,4,6-trimethoxyphenyl)butan-1-one hydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, microcrystalline powder.

Solubility

Freely soluble in water, soluble in alcohol, very slightly soluble in acetone.

mp

About 195 °C, with decomposition.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Dissolve 25.0 mg in *alcohol R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml to

20.0 ml with *alcohol R*. Examined between 220 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 275 nm. The specific absorbance at the maximum is 143 to 149.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *buflomedil hydrochloride CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 2 ml with the same solvent.

Reference solution Dissolve 40 mg of *buflomedil hydrochloride CRS* in *methanol R* and dilute to 2 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *triethylamine R, 2-propanol R, toluene R* (5:50:50 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.0 to 6.5 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 0.5 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 2 mg of *buflomedil impurity B CRS* in the mobile phase. Add 0.5 ml of the test solution and dilute to 100 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 40 °C.

Mobile phase Mix 45 volumes of *acetonitrile R* and 55 volumes of a 9.25 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 2.5 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Run time Twice the retention time of buflomedil.

Retention time buflomedil = about 5 min.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to buflomedil and impurity B.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent),

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

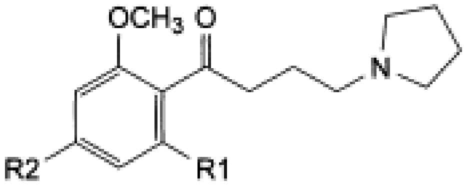
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 15 ml of *anhydrous acetic acid* R and add 35 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 34.39 mg of $C_{17}H_{26}ClNO_4$.

IMPURITIES



A. R1 = OH, R2 = OCH_3: 4-(pyrrolidin-1-yl)-1-(2-hydroxy-4,6-dimethoxyphenyl)butan-1-one,

B. R1 = OCH_3, R2 = OH: 4-(pyrrolidin-1-yl)-1-(4-hydroxy-2,6-dimethoxyphenyl)butan-1-one,

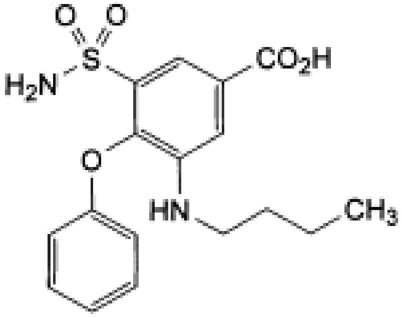
C. R1 = R2 = OH: 4-(pyrrolidin-1-yl)-1-(2,4-dihydroxy-6-methoxyphenyl)butan-1-one.

Ph Eur

Bumetanide

General Notices

(Ph Eur monograph 1076)



$C_{17}H_{20}N_2O_5S$ *364.4* 28395-03-1

Action and use

Loop diuretic.

Preparations

Bumetanide Injection

Bumetanide Oral Solution

Bumetanide Tablets

Bumetanide and Slow Potassium Tablets

Ph Eur

DEFINITION

3-(Butylamino)-4-phenoxy-5-sulphamoylbenzoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone and in alcohol, slightly soluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism *(5.9)*.

mp

About 233 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison bumetanide CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.1 g in a 6 g/l solution of *potassium hydroxide R* and dilute to 20 ml with the same solution.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 2 mg of *bumetanide impurity A CRS* and 2 mg of *bumetanide impurity B CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase Mix 70 volumes of *methanol R*, 25 volumes of water for *chromatography R* and 5 volumes of a 27.2 g/l solution of *potassium dihydrogen phosphate* R previously adjusted to pH 7.0 with a 280 g/l solution of *potassium hydroxide R*; add *tetrahexylammonium bromide R* to this mixture to obtain a concentration of 2.17 g/l.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 5 times the retention time of bumetanide.

Relative retention With reference to bumetanide (retention time = about 6 min): impurity B =

about 0.4; impurity A = about 0.6; impurity D = about 2.5; impurity C = about 4.4.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and impurity B.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *other impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *alcohol R*. Add 0.1 ml of *phenol red solution R*. Titrate with *0.1 M sodium hydroxide* until a violet-red colour is obtained. Carry out a blank titration.

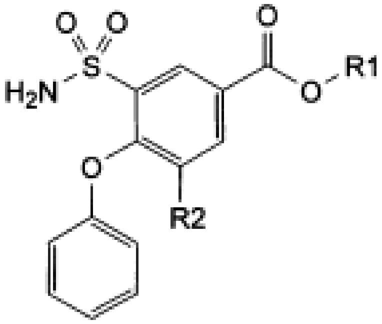
1 ml of *0.1 M sodium hydroxide* is equivalent to 36.44 mg of $C_{17}H_{20}N_2O_5S$.

STORAGE

Protected from light.

IMPURITIES

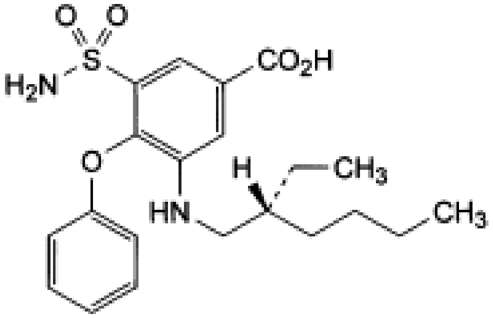
Specified impurities *A, B, C, D.*



A. R1 = H, R2 = NO_2: 3-nitro-4-phenoxy-5-sulphamoylbenzoic acid,

B. R1 = H, R2 = NH_2: 3-amino-4-phenoxy-5-sulphamoylbenzoic acid,

C. R1 = C_4H_9, R2 = NH-C_4H_9: butyl 3-(butylamino)-4-phenoxy-5-sulphamoylbenzoate,



and enantiomer

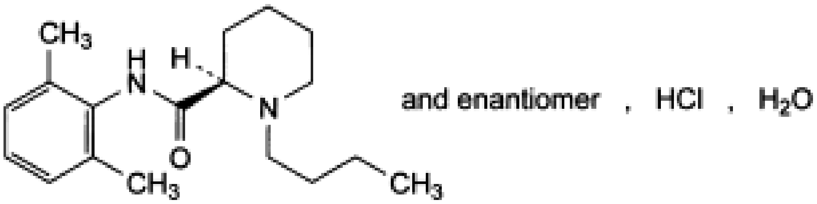
D. 3-[[(2*RS*)-2-ethylhexyl]amino]-4-phenoxy-5-sulphamoylbenzoic acid.

Ph Eur

Bupivacaine Hydrochloride

General Notices

(Ph Eur monograph 0541)



$C_{18}H_{28}N_2O,HCl,H_2O$ 342.9 *14252-80-3*

Action and use

Local anaesthetic.

Preparations

Bupivacaine Injection

Bupivacaine Heavy Injection

Bupivacaine and Adrenaline Injection/Bupivacaine and Epinephrine Injection

Ph Eur

DEFINITION

(2*RS*)-1-Butyl-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide hydrochloride monohydrate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Soluble in water, freely soluble in alcohol.

mp

About 254 °C, with decomposition.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *bupivacaine hydrochloride CRS.*

B. Thin-layer chromatography *(2.2.27).*

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 25 mg of *bupivacaine hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R, methanol R* (0.1:100 *V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *dilute potassium iodobismuthate solution R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 0.1 g in 10 ml of *water R,* add 2 ml of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 15 ml, of *ether R*. Dry the combined ether layers over *anhydrous sodium sulphate R* and filter. Evaporate the ether, recrystallise the residue from *alcohol (90 per cent V/V) R* and dry under reduced pressure. The crystals melt (*2.2.14)* at 105 °C to 108 °C.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.2 ml of *0.01 M sodium hydroxide*; the pH *(2.2.3)* is not less than 4.7. Add 0.4 ml of *0.01 M hydrochloric acid*; the pH is not greater than 4.7.

Related substances

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 25 mg of *methyl behenate R* in *methylene chloride R* and dilute to 500 ml with the same solvent.

Test solution Dissolve 50.0 mg of the substance to be examined in 2.5 ml of *water R*, add 2.5 ml of *dilute sodium hydroxide solution R* and extract with 2 quantities, each of 5 ml, of the internal standard solution. Filter the lower layer.

Reference solution (a) Dissolve 10 mg of the substance to be examined, 10 mg of *bupivacaine impurity B CRS* and 10 mg of *bupivacaine impurity E CRS* in 2.5 ml of *water R*, add 2.5 ml of *dilute sodium hydroxide solution R* and extract with 2 quantities, each of 5 ml, of the internal standard solution. Filter the lower layer and dilute to 20 ml with the internal standard solution.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the internal standard solution.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 10.0 ml with the internal standard solution.

Reference solution (d) Dilute 1.0 ml of reference solution (b) to 10.0 ml with the internal standard solution.

Column:

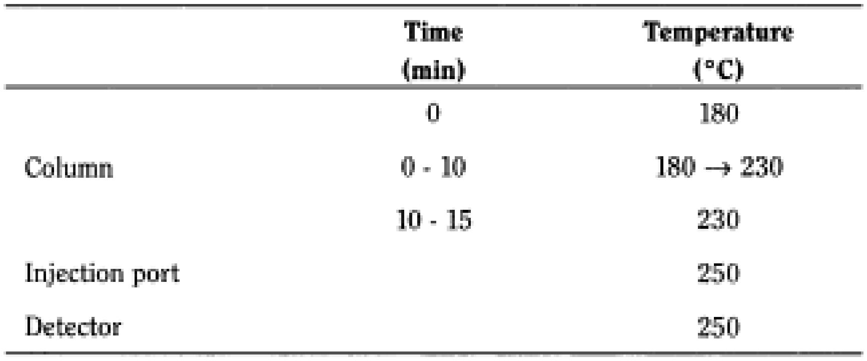
- *material*: fused silica,
- *size*: *l* = 30 m, Ø = 0.32 mm,
- *stationary phase: poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.5 ml/min.

Split ratio 1:12.

Temperature:



	Time (min)	**Temperature (°C)**
Column	0	180
	0 - 10	180 → 230
	10 - 15	230
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to bupivacaine (retention time = about 10 min): impurity C = about 0.5; impurity A = about 0.6; impurity B = about 0.7; impurity D = about 0.8; impurity E = about 1.1; internal standard = about 1.4.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to bupivacaine and impurity E.

Limits:

— *impurity B*: calculate the ratio (R) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (c); from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to impurity B to the area of the peak due to the internal standard: this ratio is not greater than R (0.5 per cent),

— *any other impurity*: calculate the ratio (R) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (d); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak, apart from the principal peak, the peak due to impurity B and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than R (0.1 per cent),

— *total*: calculate the ratio (R) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than R (1.0 per cent),

— *disregard limit*: ratio less than 0.01 times R (0.01 per cent).

2,6-Dimethylaniline

Maximum 100 ppm.

Dissolve 0.50 g in *methanol R* and dilute to 10 ml with the same solvent. To 2 ml of the solution add 1 ml of a freshly prepared 10 g/l solution of *dimethylaminobenzaldehyde R* in *methanol R* and 2 ml *of glacial acetic acid R* and allow to stand for 10 min. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 2 ml of a 5 mg/l solution of *2,6-dimethylaniline R* in *methanol R.*

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *methanol R.*

Loss on drying *(2.2.32)*

4.5 per cent to 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

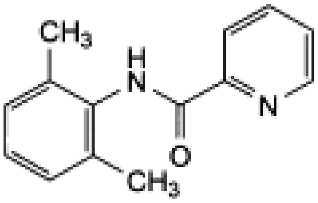
Dissolve 0.250 g in a mixture of 20 ml of *water R* and 25 ml of *alcohol R*. Add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20),* using *0.1 M ethanolic sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 32.49 mg of $C_{18}H_{29}ClN_2O$.

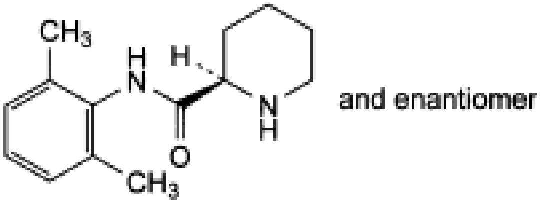
STORAGE

Protected from light.

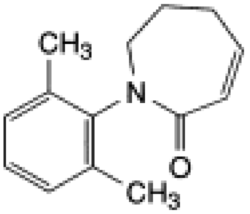
IMPURITIES



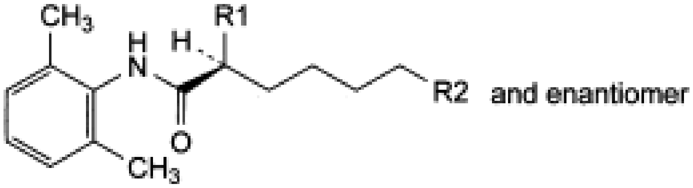
A. *N*-(2,6-dimethylphenyl)pyridine-2-carboxamide,



B. (2*RS*)-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide,

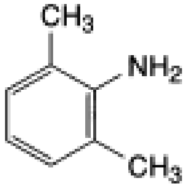


C. 1-(2,6-dimethylphenyl)-1,5,6,7-tetrahydro-2*H*-azepin-2-one,



D. R1 = R2 = Cl: (2*RS*)-2,6-dichloro-*N*-(2,6-dimethylphenyl)hexanamide,

E. R1 = H, R2 = NH-$(CH_2)_3$-CH_3: 6-(butylamino)-*N*-(2,6-dimethylphenyl)hexanamide,



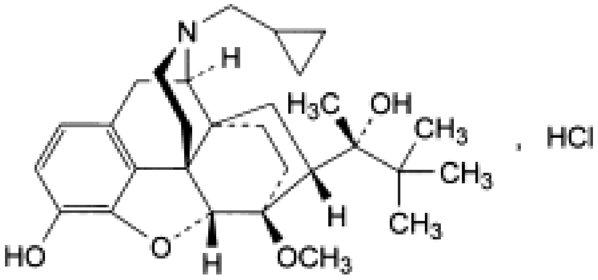
F. 2,6-dimethylaniline.

Ph Eur

Buprenorphine Hydrochloride

General Notices

(Ph Eur monograph 1181)



$C_{29}H_{41}NO_4,HCl$ 504.1 *53152-21-9*

Action and use

Opioid receptor partial agonist; analgesic.

Ph Eur

DEFINITION

Buprenorphine hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*S*)-2-[17-(cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, sparingly soluble in water, freely soluble in methanol, soluble in alcohol, practically insoluble in cyclohexane.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the *Ph. Eur. reference spectrum of buprenorphine hydrochloride.*

B. 3 ml of (see Tests) gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 0.250 g in 5.0 ml of *methanol R* and while stirring dilute to 25.0 ml with *carbon dioxide-free water R* .

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II*).

Acidity or alkalinity

To 10.0 ml of add 0.05 ml of *methyl red solution R* . Not more than 0.2 ml of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation *(2.2.7)*

Dissolve 0.100 g in *methanol R* and dilute to 10.0 ml with the same solvent. The specific optical rotation is - 92 to - 98, calculated with reference to the dried substance.

Related substances

Examine by liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of the substance to be examined in 2.0 ml of *methanol R* . Add 0.25 ml of *2 M hydrochloric acid R.*

Reference solution (b) Dilute 0.5 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (c) Dilute 0.65 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (d) Dilute 4.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

— as the mobile phase at a flow rate of about 1 ml/min, a mixture of 10 volumes of a 10 g/l solution of *ammonium acetate R* and 60 volumes of *methanol R* ,

— as detector a spectrophotometer set at 288 nm,

maintaining the temperature of the column at 40 °C.

Inject 20 µl of reference solution (a). Adjust the flow rate so that the retention time of the peak corresponding to buprenorphine is about 15 min. The test is not valid unless the chromatogram obtained with reference solution (a) presents two peaks, the first having a retention time of 0.93 relative to the second peak (buprenorphine). Inject separately 20 µl of each solution. Record the chromatogram of the test solution for 2.5 times the retention time of the main peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent) and the sum of the areas of such peaks is not greater than the area of the peak in the chromatogram obtained with reference solution (c) (0.65 per cent). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with reference solution (d).

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by heating in an oven at 115-120 °C.

ASSAY

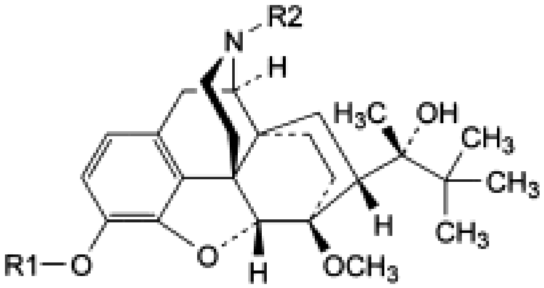
Dissolve 0.400 g in 40 ml of *anhydrous acetic acid R* and add 10 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 50.41 mg of $C_{29}H_{42}ClNO_4$.

STORAGE

Store protected from light.

IMPURITIES



A. R1 = H, R2 = CH_2-CH_2-CH=CH_2: (2*S*)-2-[17-(but-3-enyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol,

B. R1 = H, R2 = H: (2*S*)-2-(4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl)-3,3-dimethylbutan-2-ol,

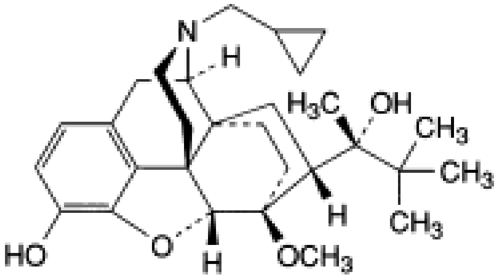
C. R1 = CH_3, R2 = CN: 4,5α-epoxy-7α-[(1*S*)-1-hydroxy-1,2,2-trimethylpropyl]-3,6-dimethoxy-6α,14-ethano-14α-morphinan-17-carbonitrile.

Ph Eur

Buprenorphine

General Notices

(Ph Eur monograph 1180)



$C_{29}H_{41}NO_4$ 467.6 *52485-79-7*

Action and use

Opioid receptor partial agonist; analgesic.

Ph Eur

DEFINITION

Buprenorphine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*S*)-2-[17-(cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, freely soluble in acetone, soluble in methanol, slightly soluble in cyclohexane. It dissolves in dilute solutions of acids.

It melts at about 217 °C.

IDENTIFICATION

Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the *Ph. Eur. reference spectrum of buprenorphine.*

TESTS

Solution S

Dissolve 0.250 g in *ethanol R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

- 103 to - 107, determined on and calculated with reference to the dried substance.

Related substances

Examine by liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of the substance to be examined in 2.0 ml of *methanol R* . Add 0.25 ml of *2 M hydrochloric acid R* .

Reference solution (b) Dilute 0.5 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (c) Dilute 0.65 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (d) Dilute 4.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of about 1 ml/min, a mixture of 10 volumes of a 10 g/l solution of *ammonium acetate R* and 60 volumes of *methanol R* ,

— as detector a spectrophotometer set at 288 nm,

maintaining the temperature of the column at 40 °C.

Inject 20 µl of reference solution (a). Adjust the flow rate so that the retention time of the peak corresponding to buprenorphine is about 15 min. The test is not valid unless the chromatogram obtained with reference solution (a) presents 2 peaks, the first having a retention time of 0.93 relative to the second peak (buprenorphine). Inject 20 µl of each solution. Record the chromatogram of the test solution for 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of such peaks is not greater than the area of the peak in the chromatogram obtained with reference solution (c) (0.65 per cent). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with reference solution (d).

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

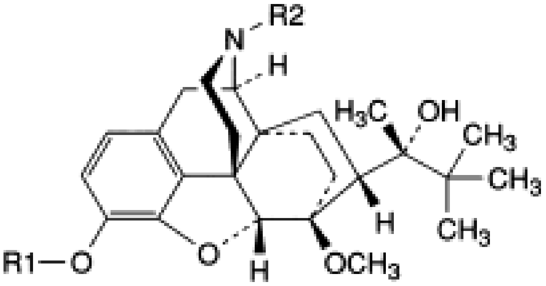
Dissolve 0.400 g in 40 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* until the colour changes from violet-blue to green, using 0.1 ml of *crystal violet solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 46.76 mg of $C_{29}H_{41}NO_4$.

STORAGE

Store protected from light.

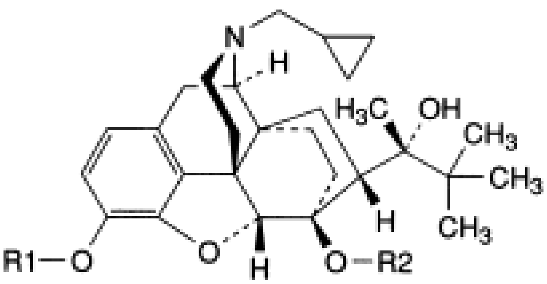
IMPURITIES



A. R1 = H, R2 = CH_2-CH_2-CH=CH_2: (2*S*)-2-[17-(but-3-enyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol,

B. R1 = R2 = H: (2*S*)-2-(4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl)-3,3-dimethylbutan-2-ol,

C. R1 = CH_3, R2 = CN: 4,5α-epoxy-7α-[(1*S*)-1-hydroxy-1,2,2-trimethylpropyl]-3,6-dimethoxy-6α,14-ethano-14α-morphinan-17-carbonitrile,



D. R1 = R2 = CH_3: (2*S*)-2-[17-(cyclopropylmethyl)-4,5α-epoxy-3,6-dimethoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol (3-*O*-methylbuprenorphine),

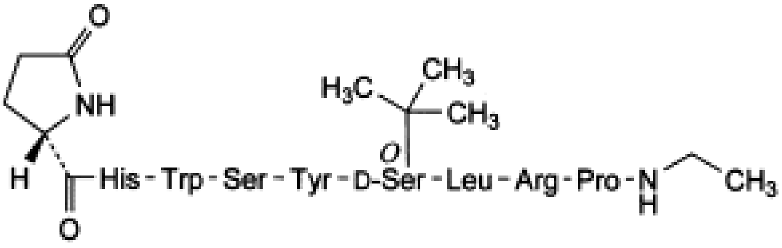
E. R1 = R2 = H: (2*S*)-2-[17-(cyclopropylmethyl)-4,5α-epoxy-3,6-dihydroxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol (6-*O*-desmethylbuprenorphine).

Ph Eur

Buserelin

General Notices

(Ph Eur monograph 1077)



$C_{60}H_{86}N_{16}O_{13}$ 1239 *68630-75-1*

Action and use

Gonadotrophin releasing hormone (gonadorelin) analogue; treatment of prostate cancer.

Ph Eur

DEFINITION

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-*O*-(1,1-dimethylethyl)-D-seryl-L-leucyl-L-arginyl-L-prolylethylamide.

Synthetic nonapeptide analogue of human gonadotrophin-releasing hormone GnRH with agonistic activity to gonadorelin. It is obtained by chemical synthesis and is available as an acetate.

Content

95.0 per cent to 102.0 per cent (anhydrous, acetic acid-free substance).

CHARACTERS

Appearance

White or slightly yellowish powder, hygroscopic.

Solubility

Sparingly soluble in water and in dilute acids.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

B. Nuclear magnetic resonance spectrometry *(2.2.33).*

Preparation 4 mg/ml solution in a mixture of 20 volumes of *deuterated acetic acid R* and 80 volumes of *deuterium oxide R* .

Comparison 4 mg/ml solution of *buserelin CRS* in a mixture of 20 volumes of *deuterated acetic acid R* and 80 volumes of *deuterium oxide R* (dissolve the contents of a vial of *buserelin CRS* in this solvent mixture to obtain the desired concentration).

Operating conditions Field strength: minimum 300 MHz.

Results The ^{1}H NMR spectrum obtained is qualitatively similar to the ^{1}H NMR spectrum obtained with *buserelin CRS* .

C. Amino acid analysis *(2.2.56)* . For protein hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of glutamic acid, histidine, tyrosine, leucine, arginine and proline as equal to one. The values fall within the following limits: serine 1.4 to 2.0; proline 0.8 to 1.2; glutamic acid 0.9 to 1.1; leucine 0.9 to 1.1; tyrosine 0.9 to 1.1; histidine 0.9 to 1.1; arginine 0.9 to 1.1. Not more than traces of other amino acids are present, with the exception of tryptophan.

TESTS

Appearance of solution

A 10 g/l solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

- 49 to - 58 (anhydrous, acetic acid-free substance), determined on a 10 g/l solution.

Specific absorbance *(2.2.25)*

49 to 56, measured at the absorption maximum at 278 nm (anhydrous, acetic acid-free substance).

Dissolve 10.0 mg in 100.0 ml of *0.01 M hydrochloric acid* .

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 5.0 mg of the substance to be examined in 5.0 ml of the mobile phase.

Reference solution (a) Dissolve the contents of a vial of *D -His-buserelin CRS* in the mobile phase. Dilute an appropriate volume of this solution in the mobile phase to obtain a final concentration of 1 mg/ml. Add 1.0 ml of the test solution to 1.0 ml of this solution.

Reference solution (b) Dissolve the contents of a vial of *buserelin CRS* in the mobile phase. Dilute an appropriate volume of this solution in the mobile phase to obtain a final concentration of 1.0 mg/ml.

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 200 ml of *acetonitrile R* and 700 ml of an 11.2 g/l solution *of phosphoric acid R* and adjust to pH 2.5 with *triethylamine R.*

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl of the test solution, reference solution (a) and reference solution (c).

Relative retention With reference to buserelin (retention time = about 36 min): impurity B = about 0.76; impurity C = about 0.83; impurity A = about 0.90; impurity D = about 0.94; impurity E = about 0.94.

System suitability Reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to impurity A and buserelin.

Limits:

- *sum of impurities D and E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3 per cent);
- *any other impurity*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Acetic acid ***(2.5.34)***

3.0 per cent to 7.0 per cent.

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of solvents.

Water ***(2.5.12)***

Maximum 4.0 per cent, determined on 80.0 mg.

Bacterial endotoxins ***(2.6.14)***

Less than 55.5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection 10 µl of the test solution and reference solution (b).

Calculate the content of buserelin ($C_{60}H_{86}N_{16}O_{13}$) using the areas of the peaks in the chromatograms obtained and the declared content of $C_{60}H_{86}N_{16}O_{13}$ in *buserelin CRS*.

STORAGE

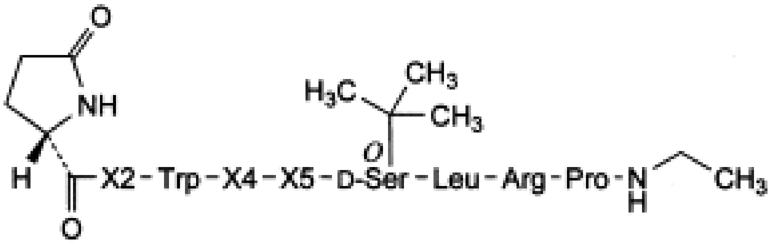
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in an airtight, sterile, tamper-proof container.

LABELLING

The label states the mass of peptide in the container.

IMPURITIES

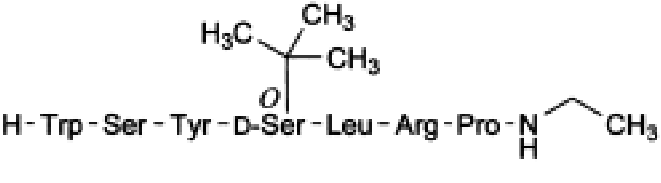
Specified impurities *A, B, C, D, E.*


O
NH
H
H3C
CH3
CH3
O
X2 - Trp - X4 - X5 - D-Ser - Leu - Arg - Pro - N
H
CH3
O

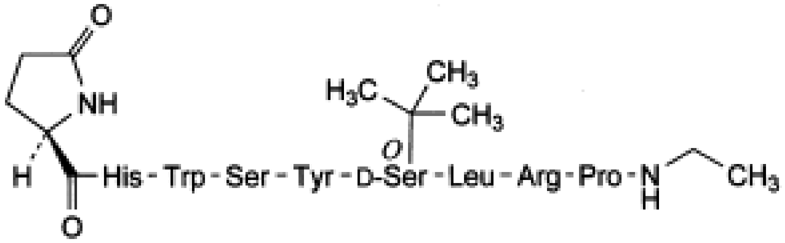
A. X2 = D-His, X4 = L-Ser, X5 = L-Tyr: [2-D-histidine]buserelin,

B. X2 = L-His, X4 = D-Ser, X5 = L-Tyr: [4-D-serine]buserelin,

D. X2 = L-His, X4 = L-Ser, X5 = D-Tyr: [5-D-tyrosine]buserelin,


H3C
CH3
CH3
O
H - Trp - Ser - Tyr - D-Ser - Leu - Arg - Pro - N
H
CH3

C. buserelin-(3-9)-peptide,



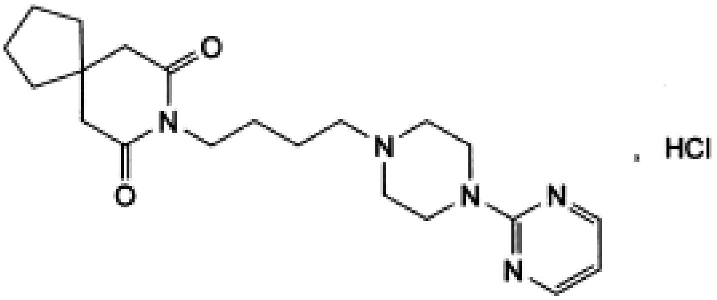
E. [1-(5-oxo-D-proline)]buserelin.

Ph Eur

Buspirone Hydrochloride

General Notices

(Ph Eur monograph 1711)



$C_{21}H_{31}N_5O_2,HCl$ 422.0 *33386-08-2*

Non-benzodiazepine hypnotic; treatment of anxiety.

Ph Eur

DEFINITION

8-[4-[4-(Pyrimidin-2-yl)piperazin-1-yl]butyl]-8-azaspiro[4.5]decane-7,9-dione hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, practically insoluble in acetone.

It shows polymorphism *(5.9)* .

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *buspirone hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R* , evaporate to dryness on a water-bath and record new spectra using the residues.

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Related substances

Liquid chromatography (*2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in mobile phase A and dilute to 25.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve the contents of a vial of *buspirone for system suitability CRS* (containing impurities E, G, J, L and N) in 2.0 ml of mobile phase A and sonicate for 10 min.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R (5 µm),*

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: mix 950 volumes of a solution containing 6.8 g/l of *potassium dihydrogen phosphate R* and 0.93 g/l of *sodium hexanesulphonate monohydrate R* , previously adjusted to pH 3.4 with *phosphoric acid R* and 50 volumes of *acetonitrile R1;*

— *mobile phase B*: mix 250 volumes of a solution containing 3.4 g/l of *potassium dihydrogen phosphate R* and 3.52 g/l of *sodium hexanesulphonate monohydrate R* , previously adjusted to pH 2.2 with *phosphoric acid R* and 750 volumes of *acetonitrile R1* ,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	90	10
6 - 34	90 → 42	10 → 58
34 - 45	42	58
45 - 55	42 → 0	58 → 100
55 - 56	0 → 100	100 → 0
56 - 60	100	0
60 - 61	100 → 90	0 → 10

Flow rate 1 ml/min.

Detection Variable wavelength spectrophotometer capable of operating at 240 nm and at 210 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *buspirone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities E, G, J, L and N.

Relative retention at 240 nm with reference to buspirone (retention time = about 25 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.6; impurity D = about 0.7; impurity E = about 0.8; impurity F = about 0.9; impurity G = about 1.05; impurity H = about 1.1; impurity I = about 1.2; impurity J = about 1.5.

Relative retention at 210 nm with reference to buspirone (retention time = about 25 min): impurity K = about 0.6; impurity L = about 1.7; impurity M = about 1.8; impurity N = about 1.9.

System suitability Reference solution (b):

— *peak-to-valley ratio at 240 nm*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to buspirone;

— *resolution at 210 nm:* minimum 4.0 between the peaks due to impurity L and impurity N;

— the chromatograms obtained are similar to the chromatograms supplied with *buspirone for system suitability CRS.*

Limits Spectrophotometer at 240 nm:

— *correction factor*: for the calculation of content, multiply the peak area of impurity J by 2,

— *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),

— *impurity J*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Limits Spectrophotometer at 210 nm:

— *impurity K*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *any other impurity eluting with a relative retention greater than 1.6*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 10 ml of *glacial acetic acid R* and add 50 ml of *acetic anhydride R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 21.10 mg of $C_{21}H_{32}ClN_5O_2$.

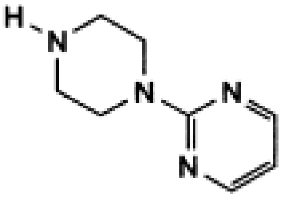
STORAGE

Protected from light.

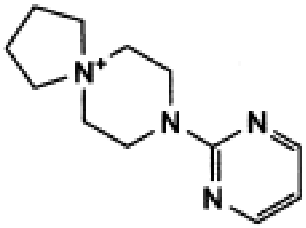
IMPURITIES

Specified impurities *E, J, K.*

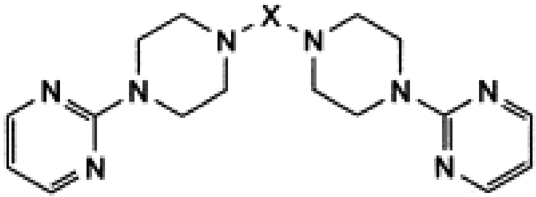
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034*). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, D, F, G, H, I, L, M, N.*



A. 2-(piperazin-1-yl)pyrimidine,

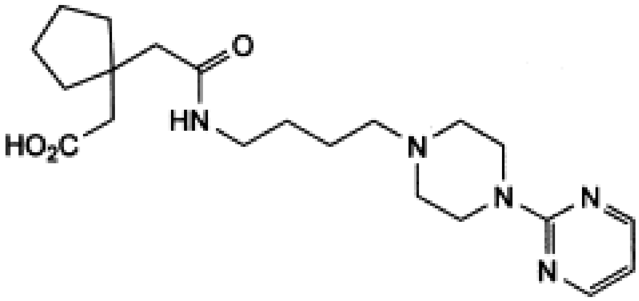


B. 8-(pyrimidin-2-yl)-8-aza-5-azoniaspiro[4.5]decane,

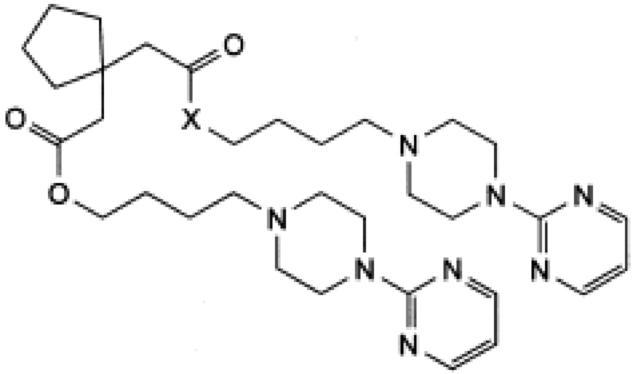


C. X = $[CH_2]_4$: 2,2′-[butane-1,4-diylbis(piperazine-1,4-diyl)]dipyrimidine,

D. X = $[CH_2]_4$-O-$[CH_2]_4$: 2,2′-[oxybis[butane-1,4-diyl(piperazine-1,4-diyl)]]dipyrimidine,

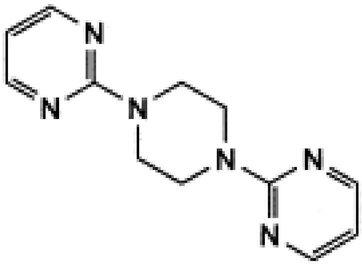


E. [1-[2-oxo-2-[[4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl]amino]ethyl]cyclopentyl]acetic acid,

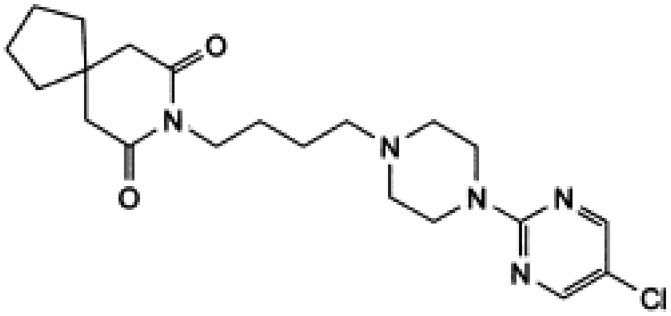


F. X = NH: 4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl [1-[2-oxo-2-[[4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl]amino]ethyl]cyclopentyl]acetate,

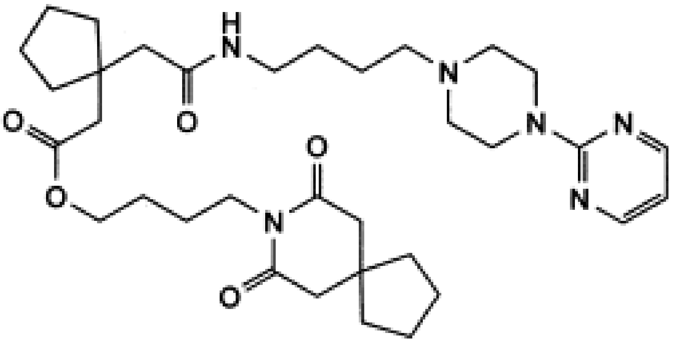
H. X = O: bis[4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl] (cyclopentane-1,1-diyl)diacetate,



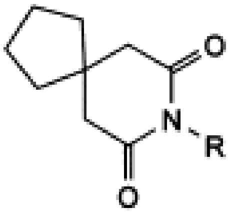
G. 2,2′-(piperazine-1,4-diyl)dipyrimidine,



I. 8-[4-[4-(5-chloropyrimidin-2-yl)piperazin-1-yl]butyl]-8-azaspiro[4.5]decane-7,9-dione,



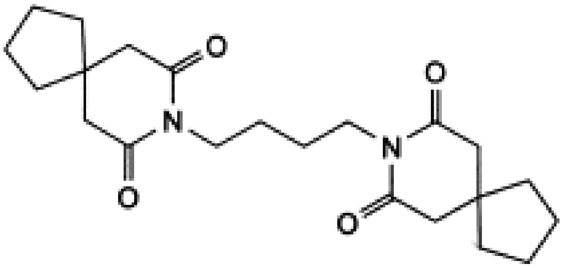
J. 4-(7,9-dioxo-8-azaspiro[4.5]dec-8-yl)butyl [1-[2-oxo-2-[[4-[4-(pyrimidin-2-yl)piperazin-1-yl] butyl]amino]ethyl]cyclopentyl]acetate,



K. R = H: 8-azaspiro[4.5]decane-7,9-dione,

L R = $[CH_2]_4$-Cl: 8-(4-chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione,

M. R = $[CH_2]_4$-Br: 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione,



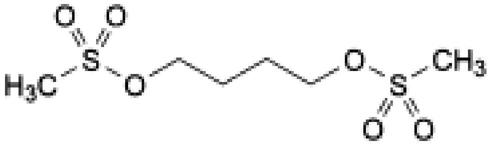
N. 8,8′-(butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).

Ph Eur

Busulfan

General Notices

(Ph Eur monograph 0542)



$C_6H_{14}O_6S_2$ 246.3 *55-98-1*

Action and use

Cytotoxic alkylating agent.

Preparation

Busulfan Tablets

Ph Eur

DEFINITION

Butane-1,4-diyl di(methanesulphonate).

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in acetone and in acetonitrile, very slightly soluble in ethanol (96 per cent).

mp: about 116 °C.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *busulfan CRS.*

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 20 mg of the substance to be examined in 2 ml of *acetone R.*

Reference solution Dissolve 20 mg of *busulfan CRS* in 2 ml of *acetone R.*

Plate *TLC silica gel G plate R.*

Mobile phase *acetone R, toluene R* (50:50 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with *anisaldehyde solution R* and heat at 120 °C.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 0.1 g add 5 ml of *1 M sodium hydroxide* . Heat until a clear solution is obtained. Allow to cool. To 2 ml of the solution add *0.1 ml of potassium permanganate solution R* . The colour changes from purple through violet to blue and finally to green. Filter and add *1 ml of ammoniacal silver nitrate solution R* . A precipitate is formed.

D. To 0.1 g add 0.1 g of *potassium nitrate R* and 0.25 g of *sodium hydroxide R* , mix and heat to fusion. Allow to cool and dissolve the residue in 5 ml of *water R* . Adjust to pH 1-2 using *dilute hydrochloric acid R* . The solution gives reaction (a) of sulphates *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

Dissolve 0.25 g in 20 ml of *acetonitrile R* , dilute to 25 ml with *water R* and examine immediately.

Acidity

Dissolve 0.20 g with heating in 50 ml of *anhydrous ethanol R* . Add 0.1 ml of *methyl red solution R .* Not more than 0.05 ml of 0.*1 M sodium hydroxide* is required to change the colour of the indicator.

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 0.250 g add 50 ml of *water R*. Shake. Boil under a reflux condenser for 30 min and, if necessary, make up to the initial volume with *water R*. Allow to cool. Using 0.3 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of 0.*1 M sodium hydroxide* is equivalent to 12.32 mg of $C_6H_{14}O_6S_2$.

STORAGE

In an airtight container, protected from light.

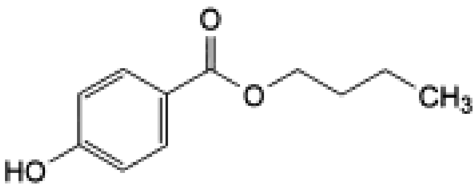
Ph Eur

Butyl Hydroxybenzoate

General Notices

Butylparaben

(Butyl Parahydroxybenzoate, Ph Eur monograph 0881)



$C_{11}H_{14}O_3$ 194.2 *94-26-8*

Action and use

Excipient.

Ph Eur

DEFINITION

Butyl 4-hydroxybenzoate.

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very slightly soluble in water, freely soluble in alcohol and in methanol.

IDENTIFICATION

First identification A, B.

Second identification *A, C, D.*

A. Melting point *(2.2.14)*

68 °C to 71 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *butyl parahydroxybenzoate CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

D. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution R* , boil for 30 s and cool (solution A). To a further 10 mg in a similar test-tube add 1 ml of *sodium carbonate solution R* ; the substance partly dissolves (solution B). Add at the same time to solution A and solution B 5 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* and mix. Solution B is yellow to orange-brown. Solution A is orange to red, the colour being clearly more intense than any similar colour which may be obtained with solution B.

TESTS

Solution S

Dissolve 1.0 g in *alcohol R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity

To 2 ml of solution S add 3 ml of *alcohol R* , 5 ml of *carbon dioxide-free water R* and 0.1 ml of *bromocresol green solution R* . Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Thin-layer chromatography *(2.2.27)*

Test solution (a) Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *acetone R* .

Reference solution (a) Dilute 0.5 ml of test solution (a) to 100 ml with *acetone R.*

Reference solution (b) Dissolve 10 mg of *butyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of *propyl parahydroxybenzoate R* in 1 ml of test solution (a) and dilute to 10 ml with *acetone R.*

Plate Suitable octadecylsilyl silica gel with a fluorescent indicator having an optimal intensity at 254 nm as the coating substance.

Mobile phase *glacial acetic acid R, water R, methanol R* (1:30:70 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated principal spots.

Limits:

— *any impurity*: any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Sulphated ash ***(2.4.14)***

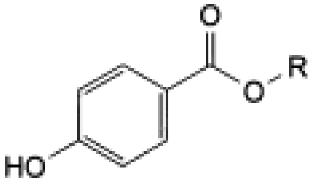
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 1.000 g add 20.0 ml of *1 M sodium hydroxide.* Heat at about 70 °C for 1 h. Cool rapidly in an ice bath. Prepare a blank in the same manner. Carry out the titration on the solutions at room temperature. Titrate the excess sodium hydroxide with *0.5 M sulphuric acid ,* continuing the titration until the second point of inflexion and determining the end-point potentiometrically *(2.2.20).*

1 ml of *1 M sodium hydroxide* is equivalent to 194.2 mg of $C_{11}H_{14}O_3$.

IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

B. R = CH_3: methyl 4-hydroxybenzoate,

C. R = CH_2-CH_3: ethyl 4-hydroxybenzoate,

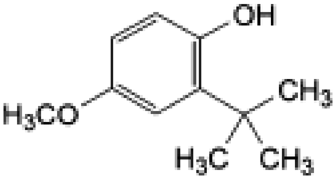
D. R = CH_2-CH_2-CH_3: propyl 4-hydroxybenzoate.

Ph Eur

Butylated Hydroxyanisole

General Notices

(Butylhydroxyanisole, Ph Eur monograph 0880)



$C_{11}H_{16}O_2$ 180.3 *25013-16-5*

Action and use

Antioxidant.

Ph Eur

DEFINITION

Butylhydroxyanisole is 2-(1,1-dimethylethyl)-4-methoxyphenol containing not more than 10 per cent of 3-(1,1-dimethylethyl)-4-methoxyphenol.

CHARACTERS

A white, yellowish or slightly pinkish, crystalline powder, practically insoluble in water, very soluble in methylene chloride, freely soluble in alcohol and in fatty oils. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To 0.5 ml of solution S (see Tests) add 10 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* . Mix and add 10 ml of *methylene chloride R* . Shake vigorously. After separation, the organic layer is red.

C. Dissolve about 10 mg in 2 ml of *alcohol R.* Add 1 ml of a 1 g/l solution of *testosterone propionate R* in *alcohol R* and 2 ml of *dilute sodium hydroxide solution R* . Heat in a water-bath at 80 °C for 10 min and allow to cool. A red colour develops.

TESTS

Solution S

Dissolve 2.5 g in *alcohol R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.25 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methylene chloride R*.

Reference solution (a) Dissolve 25 mg of *butylhydroxyanisole CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of reference solution (a) to 20 ml with *methylene chloride* R.

Reference solution (c) Dissolve 50 mg of *hydroquinone R* in 5 ml of *alcohol R* and dilute to 100 ml with *methylene chloride R*. Dilute 1 ml of this solution to 10 ml with *methylene chloride R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using *methylene chloride R*. Allow the plate to dry in air and spray with a freshly prepared mixture of 10 volumes of *potassium ferricyanide solution R*, 20 volumes of *ferric chloride solution R1* and 70 volumes of *water R*. In the chromatogram obtained with test solution (a): any violet-blue spot with an R_F value of about 0.35 (corresponding to 3-(1,1-dimethylethyl)-4-methoxyphenol) is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (10 per cent); any spot corresponding to hydroquinone is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent); any spot, apart from the principal spot and any spots corresponding to 3-(1,1-dimethylethyl)-4-methoxyphenol and hydroquinone, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R*.

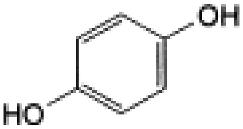
Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

STORAGE

Store protected from light.

IMPURITIES



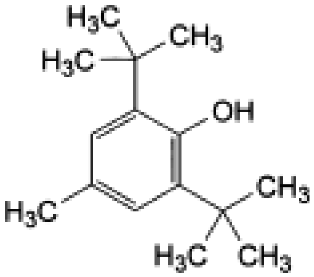
benzene-1,4-diol (hydroquinone).

Ph Eur

Butylated Hydroxytoluene

General Notices

(Butylhydroxytoluene, Ph Eur monograph 0581)



$C_{15}H_{24}O$ 220.4 *128-37-0*

Action and use

Antioxidant.

Ph Eur

DEFINITION

Butylhydroxytoluene is 2,6-bis(1,1-dimethylethyl)-4-methylphenol.

CHARACTERS

A white or yellowish-white, crystalline powder, practically insoluble in water, very soluble in acetone, freely soluble in alcohol and in vegetable oils.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D.*

A. It complies with the test for the freezing-point (see Tests).

B. Dissolve 0.500 g in *ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *ethanol R* . Examined between 230 nm and 300 nm *(2.2.25)* , the solution shows an absorption maximum at 278 nm. The specific absorbance at the maximum is 80 to 90.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *butylhydroxytoluene CRS.*

D. Dissolve about 10 mg in 2 ml of *alcohol R*. Add 1 ml of a 1 g/l solution of *testosterone propionate R* in *alcohol R* and 2 ml of *dilute sodium hydroxide solution R*. Heat in a water-bath at 80 °C for 10 min and allow to cool. A blue colour develops.

TESTS

Appearance of solution

Dissolve 1.0 g in *methanol R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 or BY_5 *(2.2.2, Method II)*.

Freezing-point *(2.2.18)*

69 °C to 70 °C.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution Dilute 1 ml of the test solution to 200 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using *methylene chloride R*. Dry the plate in air and spray with a freshly prepared mixture of 10 volumes of *potassium ferricyanide solution R*, 20 volumes of *ferric chloride solution R1* and 70 volumes of *water R*. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

Ph Eur

Calcium Carbonate

General Notices

(Ph Eur monograph 0014)

$CaCO_3$ 100.1 *471-34-1*

Action and use

Antacid.

Preparations

Calcium Carbonate Chewable Tablets

Calcium and Colecalciferol Tablets

Ph Eur

DEFINITION

Content

98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water.

IDENTIFICATION

A. It gives the reaction of carbonates *(2.3.1)* .

B. 0.2 ml of solution S (see Tests) gives the reactions of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 5.0 g in 80 ml of *dilute acetic acid R* . When the effervescence ceases, boil for 2

min. Allow to cool, dilute to 100 ml with *dilute acetic acid R* and filter, if necessary, through a sintered-glass filter *(2.1.2)*.

Substances insoluble in acetic acid

Maximum 0.2 per cent.

Wash any residue obtained during the preparation of solution S with 4 quantities, each of 5 ml, of hot *water R* and dry at 100-105 °C for 1 h. The residue weighs a maximum of 10 mg.

Chlorides ***(2.4.4)***

Maximum 330 ppm.

Dilute 3 ml of solution S to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 0.25 per cent.

Dilute 1.2 ml of solution S to 15 ml with *distilled water R*.

Arsenic ***(2.4.2, Method A)***

Maximum 4 ppm, determined on 5 ml of solution S.

Barium

To 10 ml of solution S add 10 ml of *calcium sulphate solution R*. After at least 15 min, any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution S and 10 ml of *distilled water R*.

Iron ***(2.4.9)***

Maximum 200 ppm.

Dissolve 50 mg in 5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*.

Magnesium and alkali metals

Maximum 1.5 per cent.

Dissolve 1.0 g in 12 ml of *dilute hydrochloric acid R*. Boil the solution for about 2 min and add 20 ml of *water R*, 1 g of *ammonium chloride R* and 0.1 ml of *methyl red solution R*. Add *dilute ammonia R1* until the colour of the indicator changes and then add 2 ml in excess. Heat to boiling and add 50 ml of hot *ammonium oxalate solution R*. Allow to stand for 4 h, dilute to 100 ml with *water R* and filter through a suitable filter. To 50 ml of the filtrate add 0.25 ml of *sulphuric acid R*. Evaporate to dryness on a water-bath and ignite to constant mass at 600 ± 50 °C. The residue weighs a maximum of 7.5 mg.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 200 ± 10 °C.

ASSAY

Dissolve 0.150 g in a mixture of 3 ml of *dilute hydrochloric acid R* and 20 ml of *water R* . Boil for 2 min, allow to cool and dilute to 50 ml with *water R* . Carry out the complexometric titration of calcium *(2.5.11)* .

1 ml of *0.1 M sodium edetate* is equivalent to 10.01 mg of $CaCO_3$.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.The following characteristics may be relevant for calcium carbonate used as filler in tablets and capsules.

Particle-size distribution ***(2.9.31 or 2.9.38)*****.**

Powder flow ***(2.9.36)*** **.**

Ph Eur

Calcium Carbonate

General Notices

(Ph Eur monograph 0014)

$CaCO_3$ 100.1 *471-34-1*

Action and use

Antacid.

Preparations

Calcium Carbonate Chewable Tablets

Calcium and Colecalciferol Tablets

Ph Eur

DEFINITION

Content

98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water.

IDENTIFICATION

A. It gives the reaction of carbonates *(2.3.1)* .

B. 0.2 ml of solution S (see Tests) gives the reactions of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 5.0 g in 80 ml of *dilute acetic acid R* . When the effervescence ceases, boil for 2

min. Allow to cool, dilute to 100 ml with *dilute acetic acid R* and filter, if necessary, through a sintered-glass filter *(2.1.2)* .

Substances insoluble in acetic acid

Maximum 0.2 per cent.

Wash any residue obtained during the preparation of solution S with 4 quantities, each of 5 ml, of hot *water R* and dry at 100-105 °C for 1 h. The residue weighs a maximum of 10 mg.

Chlorides ***(2.4.4)***

Maximum 330 ppm.

Dilute 3 ml of solution S to 15 ml with *water R* .

Sulphates ***(2.4.13)***

Maximum 0.25 per cent.

Dilute 1.2 ml of solution S to 15 ml with *distilled water R* .

Arsenic ***(2.4.2, Method A)***

Maximum 4 ppm, determined on 5 ml of solution S.

Barium

To 10 ml of solution S add 10 ml of *calcium sulphate solution R* . After at least 15 min, any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution S and 10 ml of *distilled water R* .

Iron ***(2.4.9)***

Maximum 200 ppm.

Dissolve 50 mg in 5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R* .

Magnesium and alkali metals

Maximum 1.5 per cent.

Dissolve 1.0 g in 12 ml of *dilute hydrochloric acid R* . Boil the solution for about 2 min and add 20 ml of *water R* , 1 g of *ammonium chloride R* and 0.1 ml of *methyl red solution R* . Add *dilute ammonia R1* until the colour of the indicator changes and then add 2 ml in excess. Heat to boiling and add 50 ml of hot *ammonium oxalate solution R* . Allow to stand for 4 h, dilute to 100 ml with *water R* and filter through a suitable filter. To 50 ml of the filtrate add 0.25 ml of *sulphuric acid R* . Evaporate to dryness on a water-bath and ignite to constant mass at 600 ± 50 °C. The residue weighs a maximum of 7.5 mg.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R* .

Loss on drying ***(2.2.32)***

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 200 ± 10 °C.

ASSAY

Dissolve 0.150 g in a mixture of 3 ml of *dilute hydrochloric acid R* and 20 ml of *water R* . Boil for 2 min, allow to cool and dilute to 50 ml with *water R* . Carry out the complexometric titration of calcium *(2.5.11)* .

1 ml of *0.1 M sodium edetate* is equivalent to 10.01 mg of $CaCO_3$.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.The following characteristics may be relevant for calcium carbonate used as filler in tablets and capsules.

Particle-size distribution ***(2.9.31 or 2.9.38)*****.**

Powder flow ***(2.9.36)*** **.**

Ph Eur

Heavy Bismuth Subnitrate

General Notices

(Ph Eur monograph 1494)

$4[BiNO_3(OH)_2],BiO(OH)$ 1462 *1304-85-4*

Ph Eur

DEFINITION

Content

71.0 per cent to 74.0 per cent of Bi (A_r 209.0) (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in mineral acids with decomposition.

IDENTIFICATION

A. Dilute 1 ml of solution S1 (see Tests) to 5 ml with *water R* and add 0.3 ml of *potassium iodide solution R*. A black precipitate is formed which dissolves into an orange solution with the addition of 2 ml *of potassium iodide solution R*.

B. It gives reaction (b) of bismuth *(2.3.1)*.

C. It gives the reaction of nitrates *(2.3.1)*.

D. pH *(2.2.3)*: maximum 2.0 for solution S2 (see Tests).

TESTS

Solution S1

Shake 5.0 g by gently heating in 10 ml of *water R* and add 20 ml of *nitric acid R*. Heat until dissolution, cool and dilute to 100 ml with *water R*.

Solution S2

Place 1.00 g in a 20 ml volumetric flask and add 2.0 ml of *lead-free nitric acid R*. Allow acid attack to take place without heating and if necessary warm slightly at the end to completely dissolve the test sample. Add 10 ml of *water R*, shake and add, in small fractions, 4.5 ml of *lead-free ammonia R*; shake and allow to cool. Dilute to 20.0 ml with *water R*, shake again and allow the solids to settle. The clear supernatant solution is solution S2.

Acidity

Suspend 1.0 g in 15 ml of *water R* and shake several times. Allow to stand for 5 min and filter. To 10 ml of the filtrate, add 0.5 ml of *phenolphthalein solution R1*. Not more than 0.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Chlorides *(2.4.4)*

Maximum 200 ppm.

To 5.0 ml of solution S1, add 3 ml of *nitric acid R* and dilute to 15 ml with *water R*.

Copper

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S2.

Reference solutions Prepare the reference solutions using *copper standard solution (10 ppm Cu) R* and diluting with a 37 per cent *V/V* solution *of lead-free nitric acid R*.

Source Copper hollow-cathode lamp.

Wavelength 324.7 nm.

Atomisation device Air-acetylene flame.

Lead

Maximum 20.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Solution S2.

Reference solutions Prepare the reference solutions using *lead standard solution (10 ppm Pb) R* and diluting with a 37 per cent *V/V* solution of *lead-free nitric acid R.*

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm (depending on the apparatus, the line at 217.0 nm may be used).

Atomisation device Air-acetylene flame.

Silver

Maximum 25.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S2.

Reference solutions Prepare the reference solutions using *silver standard solution (5 ppm Ag) R* and diluting with a 37 per cent *V/V* solution *of lead-free nitric acid R*.

Source Silver hollow-cathode lamp.

Wavelength 328.1 nm.

Atomisation device Air-acetylene flame.

Substances not precipitated by ammonia

Maximum 1.0 per cent.

To 20 ml of solution S1, add *concentrated ammonia R* until an alkaline reaction is produced and filter. Wash the residue with *water R*, and evaporate the combined filtrate and washings to dryness on a water-bath. To the residue, add 0.3 ml of *dilute sulphuric acid R* and ignite. The residue weighs a maximum of 10 mg.

Loss on drying *(2.2.32)*

Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve with heating 0.250 g in 10 ml of a mixture of 2 volumes of *perchloric acid R* and 5 volumes of *water R*. To the hot solution, add 200 ml of *water R* and 50 mg of *xylenol orange triturate R*. Titrate with *0.1 M sodium edetate* until a yellow colour is obtained.

1 ml of *0.1 M sodium edetate* is equivalent to 20.90 mg of Bi.

Ph Eur

White Beeswax

General Notices

(Ph Eur monograph 0069)

Action and use

Excipient.

Ph Eur

DEFINITION

Wax obtained by bleaching yellow beeswax.

CHARACTERS

Appearance

White or yellowish-white pieces or plates, translucent when thin, with a fine-grained, matt and non-crystalline fracture; when warmed in the hand they become soft and malleable.

It has an odour similar to that of yellow beeswax, though fainter and never rancid. It is tasteless and does not stick to the teeth.

Solubility

Practically insoluble in water, partially soluble in hot ethanol (90 per cent *V/V*) and completely soluble in fatty and essential oils.

Relative density

About 0.960.

TESTS

Drop point *(2.2.17)*

61 °C to 66 °C.

Melt the beeswax by heating on a water-bath, pour onto a glass plate and allow to cool to a semi-solid mass. Fill the metal cup by inserting the wider end into the beeswax and repeating the procedure until beeswax extrudes from the narrow opening. Remove the excess with a spatula and insert the thermometer immediately. Remove the beeswax displaced. Allow to stand at room temperature for at least 12 h before determining the drop point.

Acid value

17.0 to 24.0.

To 2.00 g (m g), in a 250 ml conical flask fitted with a reflux condenser, add 40 ml of *xylene R* and a few glass beads. Heat until the substance is dissolved. Add 20 ml of *ethanol (96 per cent) R* and 0.5 ml of *phenolphthalein solution R1* and titrate the hot solution with *0.5 M alcoholic potassium hydroxide* until a red colour persists for at least 10 s (n_1 ml). Carry out a blank test (n_2 ml).

$$\text{Acid value} = \frac{28.05\,(n_1 - n_2)}{m}$$

Ester value ***(2.5.2)***

70 to 80.

Saponification value

87 to 104.

To 2.00 g (m g), in a 250 ml conical flask fitted with a reflux condenser, add 30 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *xylene R* and a few glass beads. Heat until the substance is dissolved. Add 25.0 ml of 0.5 M *alcoholic potassium hydroxide* and heat under a reflux condenser for 3 h. Titrate the hot solution immediately with *0.5 M hydrochloric acid*, using 1 ml of *phenolphthalein solution R1* as indicator (n_1 ml). Reheat the solution to boiling several times during the course of the titration. Carry out a blank test (n_2 ml).

$$\text{Saponification value} = \frac{28.05\,(n_2 - n_1)}{m}$$

Ceresin, paraffins and certain other waxes

To 3.0 g, in a 100 ml round-bottomed flask, add 30 ml of a 40 g/l solution of *potassium hydroxide R* in *aldehyde-free alcohol R* and boil gently under a reflux condenser for 2 h. Remove the condenser and immediately insert a thermometer. Place the flask in a water-bath at 80 °C and allow to cool, swirling the solution continuously. No precipitate is formed until 65 °C, although the solution may be slightly opalescent. Beginning at 65 °C, the solution may become cloudy and precipitates may be formed. At 59 °C, the solution is cloudy.

Glycerol and other polyols

Maximum 0.5 per cent *m/m*, calculated as glycerol.

To 0.20 g add 10 ml of *alcoholic potassium hydroxide solution R* and heat on a water-bath under a reflux condenser for 30 min. Add 50 ml of *dilute sulphuric acid* R, cool and filter. Rinse the flask and the filter with *dilute sulphuric acid R*. Combine the filtrate and washings and dilute to 100.0 ml with *dilute sulphuric acid R*. Place 1.0 ml of the solution in a test-tube, add 0.5 ml of a 10.7 g/l solution of *sodium periodate R*, mix and allow to stand for 5 min. Add 1.0 ml of *decolorised fuchsin solution R* and mix. Any precipitate disappears. Place the tube in a beaker containing water at 40 °C. During cooling observe for 10-15 min. Any violet-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 1.0 ml of a 10 mg/l solution of *glycerol R* in *dilute sulphuric acid R.*

Ph Eur

Yellow Beeswax

General Notices

(Ph Eur monograph 0070)

Action and use

Excipient.

Ph Eur

DEFINITION

Wax obtained by melting the walls of the honeycomb made by the honey-bee, *Apis mellifera* L., with hot water and removing foreign matter.

CHARACTERS

Appearance

Yellow or light brown pieces or plates with a fine-grained, matt and non-crystalline fracture; when warmed in the hand they become soft and malleable.

It has a faint odour, characteristic of honey. It is tasteless and does not stick to the teeth.

Solubility

Practically insoluble in water, partially soluble in hot ethanol (90 per cent *V/V*) and completely soluble in fatty and essential oils.

Relative density: about 0.960.

TESTS

Drop point *(2.2.17)*

61 °C to 66 °C.

Melt the beeswax by heating on a water-bath, pour onto a glass plate and allow to cool to a semi-solid mass. Fill the metal cup by inserting the wider end into the beeswax and repeating the procedure until beeswax extrudes from the narrow opening. Remove the excess with a spatula and insert the thermometer immediately. Remove the beeswax displaced. Allow to stand at room temperature for at least 12 h before determining the drop point.

Acid value

17.0 to 22.0.

To 2.00 g (*m* g), in a 250 ml conical flask fitted with a reflux condenser, add 40 ml of *xylene R* and a few glass beads. Heat until the substance is dissolved. Add 20 ml of *ethanol (96 per cent) R* and 0.5 ml of *phenolphthalein solution R1* and titrate the hot solution with *0.5 M* *alcoholic potassium hydroxide* until a red colour persists for at least 10 s (n_1 ml). Carry out a blank test (n_2 ml).

$$\text{Acid value} = \frac{28.05\,(n_1 - n_2)}{m}$$

Ester value *(2.5.2)*

70 to 80.

Saponification value

87 to 102.

To 2.00 g (m g), in a 250 ml conical flask fitted with a reflux condenser, add 30 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *xylene R* and a few glass beads. Heat until the substance is dissolved. Add 25.0 ml of *0.5 M alcoholic potassium hydroxide* and heat under a reflux condenser for 3 h. Titrate the hot solution immediately with *0.5 M hydrochloric acid ,* using 1 ml of *phenolphthalein solution R1* as indicator (n_1 ml). Reheat the solution to boiling several times during the course of the titration. Carry out a blank test (n_2 ml).

$$\text{Saponification value} = \frac{28.05\,(n_2 - n_1)}{m}$$

Ceresin, paraffins and certain other waxes

To 3.0 g, in a 100 ml round-bottomed flask, add 30 ml of a 40 g/l solution of *potassium hydroxide R* in *aldehyde-free alcohol R* and boil gently under a reflux condenser for 2 h. Remove the condenser and immediately insert a thermometer. Place the flask in a water-bath at 80 °C and allow to cool, swirling the solution continuously. No precipitate is formed until 65 °C, although the solution may be slightly opalescent. Beginning at 65 °C, the solution may become cloudy and precipitates may be formed. At 59 °C, the solution is cloudy.

Glycerol and other polyols

Maximum 0.5 per cent *m/m*, calculated as glycerol.

To 0.20 g add 10 ml of *alcoholic potassium hydroxide solution R* and heat on a water-bath under a reflux condenser for 30 min. Add 50 ml of *dilute sulphuric acid R*, cool and filter. Rinse the flask and the filter with *dilute sulphuric acid R*. Combine the filtrate and washings and dilute to 100.0 ml with *dilute sulphuric acid R*. Place 1.0 ml of the solution in a test-tube, add 0.5 ml of a 10.7 g/l solution of *sodium periodate R*, mix and allow to stand for 5 min. Add 1.0 ml of *decolorised fuchsin solution R* and mix. Any precipitate disappears. Place the tube in a beaker containing water at 40 °C. During cooling observe for 10-15 min. Any violet-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 1.0 ml of a 10 mg/l solution of *glycerol R* in *dilute sulphuric acid R.*

Ph Eur

Activated Charcoal

General Notices

Decolourising Charcoal
(Ph Eur monograph 0313)

Action and use

Adsorbent.

Ph Eur

DEFINITION

Obtained from vegetable matter by suitable carbonisation processes intended to confer a high adsorption power.

CHARACTERS

Appearance

Black, light powder free from grittiness.

Solubility

Practically insoluble in all usual solvents.

IDENTIFICATION

A. When heated to redness it burns slowly without a flame.

B. Adsorption power (see Tests).

TESTS

Solution S

To 2.0 g in a conical flask with a ground-glass neck add 50 ml of *dilute hydrochloric acid R*. Boil gently under a reflux condenser for 1 h, filter and wash the filter with *dilute hydrochloric acid R*. Evaporate the combined filtrate and washings to dryness on a water-bath, dissolve the residue in *0.1 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Acidity or alkalinity

To 2.0 g add 40 ml of *water R* and boil for 5 min. Cool, restore to the original mass with *carbon dioxide-free water R* and filter. Reject the first 20 ml of the filtrate. To 10 ml of the filtrate add 0.25 ml of *bromothymol blue solution R1* and 0.25 ml of *0.02 M sodium hydroxide*. The solution is blue. Not more than 0.75 ml of *0.02 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid-soluble substances

Maximum 3 per cent.

To 1.0 g add 25 ml of *dilute nitric acid R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (10) *(2.1.2)* and wash with 10 ml of hot *water R*. Evaporate the combined filtrate and washings to dryness on a water-bath, add to the residue 1 ml of *hydrochloric acid R,* evaporate to dryness again and dry the residue to constant mass at 100-105 °C. The residue weighs a maximum of 30 mg.

Alkali-soluble coloured substances

To 0.25 g add 10 ml of *dilute sodium hydroxide solution R* and boil for 1 min. Cool, filter and dilute the filtrate to 10 ml with *water R*. The solution is not more intensely coloured than reference solution GY_4 *(2.2.2, Method II*).

Ethanol (96 per cent) soluble substances

Maximum 0.5 per cent.

To 2.0 g add 50 ml of *ethanol (96 per cent) R* and boil under a reflux condenser for 10 min. Filter immediately, cool, and dilute to 50 ml with *ethanol (96 per cent) R*. The filtrate is not more intensely coloured than reference solution Y_6 or BY_6 *(2.2.2, Method II)*. Evaporate 40 ml of the filtrate to dryness and dry to constant mass at 100-105 °C. The residue weighs a maximum of 8 mg.

Fluorescent substances

In an intermittent-extraction apparatus, treat 10.0 g with 100 ml of *cyclohexane R1* for 2 h. Collect the liquid and dilute to 100 ml with *cyclohexane R1*. Examine in ultraviolet light at 365 nm. The fluorescence of the solution is not more intense than that of a solution of 83 µg of *quinine R in* 1000 ml of *0.005 M sulphuric acid* examined under the same conditions.

Sulphides

To 1.0 g in a conical flask add 5 ml of *hydrochloric acid R1* and 20 ml of *water R*. Heat to boiling. The fumes released do not turn *lead acetate paper R* brown.

Copper

Maximum 25.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use solution S.

Reference solutions Prepare the reference solutions using *copper standard solution (0.1 per cent Cu) R* and diluting with *0.1 M hydrochloric acid*.

Source Copper hollow-cathode lamp.

Wavelength 325.0 nm.

Atomisation device Air-acetylene flame.

Lead

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use solution S.

Reference solutions Prepare the reference solutions using *lead standard solution (100 ppm Pb) R* and diluting with *0.1 M hydrochloric acid*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm; 217.0 nm may be used depending on the apparatus.

Atomisation device Air-acetylene flame.

Zinc

Maximum 25.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use solution S.

Reference solutions Prepare the reference solutions using *zinc standard solution (100 ppm Zn) R* and diluting with *0.1 M hydrochloric acid*.

Source Zinc hollow-cathode lamp.

Wavelength 214.0 nm.

Atomisation device Air-acetylene flame.

Loss on drying *(2.2.32)*

Maximum 15 per cent, determined on 1.00 g by drying in an oven at 120 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 5.0 per cent, determined on 1.0 g.

Adsorption power

To 0.300 g in a 100 ml ground-glass-stoppered conical flask add 25.0 ml of a freshly prepared solution of 0.5 g of *phenazone R* in 50 ml of *water R*. Shake thoroughly for 15 min. Filter and reject the first 5 ml of filtrate. To 10.0 ml of the filtrate add *1.0 g of potassium bromide R* and 20 ml of *dilute hydrochloric acid R*. Using 0.1 ml of *methyl red solution R* as indicator, titrate with 0.*0167 M potassium bromate* until the red colour is discharged. Titrate slowly (1 drop every 15 s) towards the end of the titration. Carry out a blank titration using 10.0 ml of the phenazone solution.

Calculate the quantity of phenazone adsorbed per 100 g of activated charcoal from the following expression:

$$\frac{2.353\,(a-b)}{m}$$

a = number of millilitres of *0.0167 M potassium bromate* used for the blank

b = number of millilitres of *0.0167 M potassium bromate* used for the test

m = mass in grams of the substance to be examined.

Minimum 40 g of phenazone is adsorbed per 100 g of activated charcoal, calculated with reference to the dried substance.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count.

STORAGE

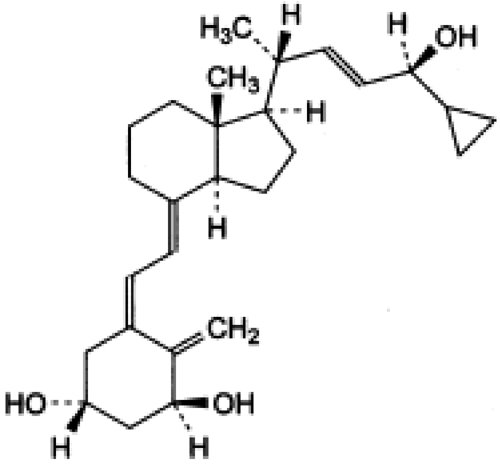
In an airtight container .

Ph Eur

Anhydrous Calcipotriol

General Notices

(Ph Eur monograph 2011)



$C_{27}H_{40}O_3$ 412.6 *112828-00-9*

Action and use

Vitamin D analogue.

Ph Eur

DEFINITION

(*5Z,7E,22E,24S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.

Content

95.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It is sensitive to heat and light.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of anhydrous calcipotriol.*

B. Loss on drying (see Tests).

Carry out the tests for related substances and the assay as rapidly as possible and protected from actinic light and air.

TESTS

Related substances

A. Thin-layer chromatography *(2.2.27)* .

Solution A To 1 ml of *triethylamine R* add 9 ml of *chloroform R* .

Test solution Dissolve 1 mg of the substance to be examined in 100 µl of solution A.

Reference solution (a) To 10 µl of the test solution add 990 µl of solution A.

Reference solution (b) To 250 µl of reference solution (a) add 750 µl of solution A.

Reference solution (c) To 100 µl of reference solution (a) add 900 µl of solution A.

Reference solution (d) Place 2 mg of the substance to be examined in a vial and dissolve in 200 µl of solution A. Close the vial and keep it in a water bath at 60 °C for 2 h.

Plate *TLC silica gel F_{254} plate R .*

Mobile phase *2-methylpropanol R, methylene chloride R* (20:80 V/V).

Application 10 µl of the test solution and reference solutions (b), (c) and (d).

Development Over 2/3 of the plate.

Drying In air, then at 140 °C for 10 min.

Detection Spray the hot plate with an *alcoholic solution of sulphuric acid R* , dry at 140 °C for not more than 1 min and examine in ultraviolet light at 366 nm.

Relative retention With reference to calcipotriol (R_F = about 0.4): impurity G = about 0.4; impurity H = about 0.4; pre-calcipotriol = about 0.9; impurity A = about 1.2.

System suitability Reference solution (d):

— the chromatogram shows a secondary spot due to pre-calcipotriol.

Limits:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent),

— *impurities G, H*: any spot due to impurity G or H is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent for the sum),

— *any other impurity*: any other spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

B. Liquid chromatography *(2.2.29)* .

Solution A. Dissolve 1.32 g of *ammonium phosphate R* in *water R* and dilute to 10.0 ml with the same solvent.

Solvent mixture solution A, *water R, methanol R* (3:297:700 *V/V/V*).

Test solution (a) Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Test solution (b) Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the same solvent mixture.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 1.0 mg of *calcipotriol monohydrate CRS* (containing impurities B, C and D) in the solvent mixture and dilute to 2.5 ml with the solvent mixture.

Reference solution (d) Dissolve 2.00 mg of *calcipotriol monohydrate CRS* in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.10 m, Ø = 4.0 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase *water R, methanol R* (30:70 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 264 nm.

Injection 20 µl of test solution (a) and reference solutions (a), (b) and (c).

Run time Twice the retention time of calcipotriol.

Relative retention With reference to calcipotriol (retention time = about 13.5 min): impurity B = about 0.86; impurity C = about 0.92; impurity D = about 1.3.

System suitability Reference solution (c):

— *peak-to-valley ratio* : minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to calcipotriol,

— the chromatogram obtained is similar to the chromatogram supplied with *calcipotriol monohydrate CRS*.

Limits:

— *impurity B*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *impurities C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying

Maximum 1.0 per cent, determined on 5 mg by thermogravimetry *(2.2.34)* . Heat to 105 °C at a rate of 10 °C/min and maintain at 105 °C for 60 min.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{27}H_{40}O_3$ from the areas of the peaks and the declared content of *calcipotriol monohydrate CRS*.

STORAGE

In an airtight container , protected from light at - 20 °C or below.

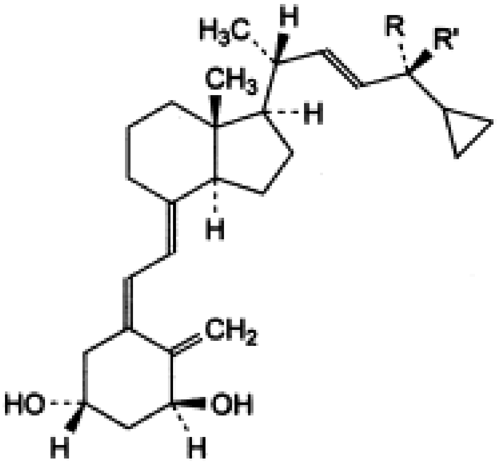
IMPURITIES

Specified impurities *A, B, C, D, G, H.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F, I.*

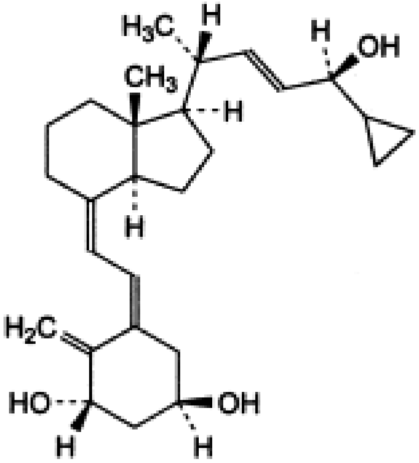
By thin-layer chromatography *A, G, H, I.*

By liquid chromatography *B, C, D, E, F.*

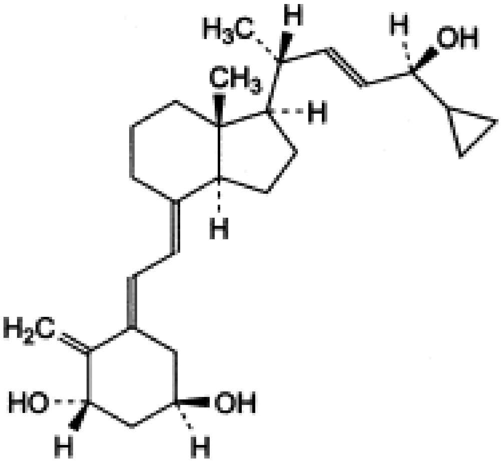

H$_3$C
H
R
R'
CH$_3$
H
H
CH$_2$
HO
OH
H
H

A. R + R′ = O: (*5Z,7E,22E*)-24-cyclopropyl-1α,3β-dihydroxy-9,10-secochola-5,7,10(19),22-tetraen-24-one,

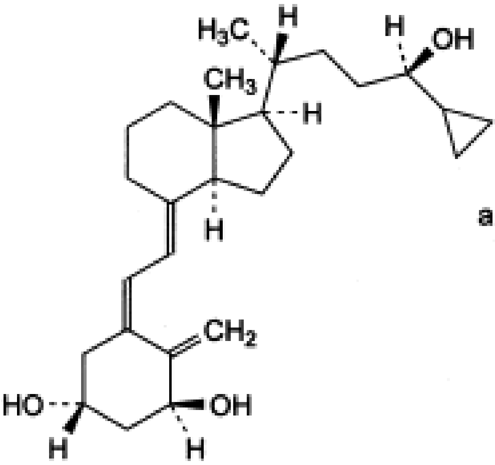
D. R = OH, R′ = H: (5*Z*,7*E*,22*E*,24*R*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol (24-*epi*-calcipotriol),


H$_3$C
H
H
OH
CH$_3$
H
H
H$_2$C
HO
OH
H
H

B. (5*Z*,7*Z*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol ((7*Z*)-calcipotriol),

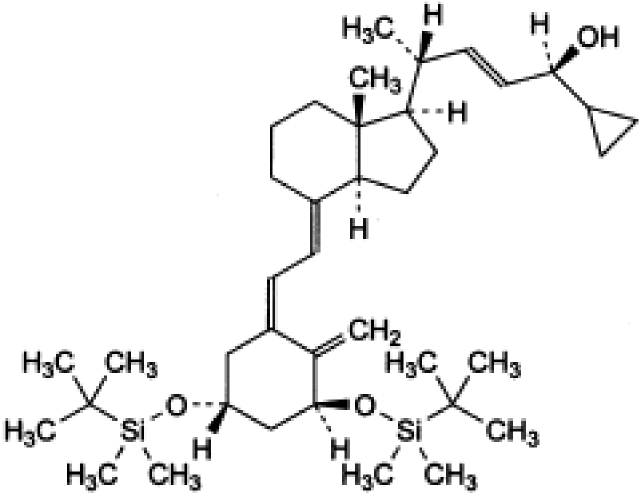


C. (5*E*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol ((5*E*)-calcipotriol),

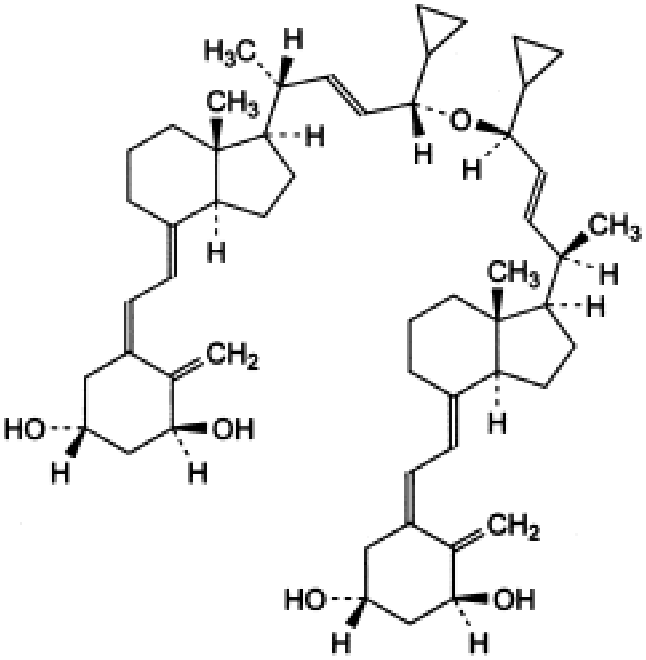


and enantiomer

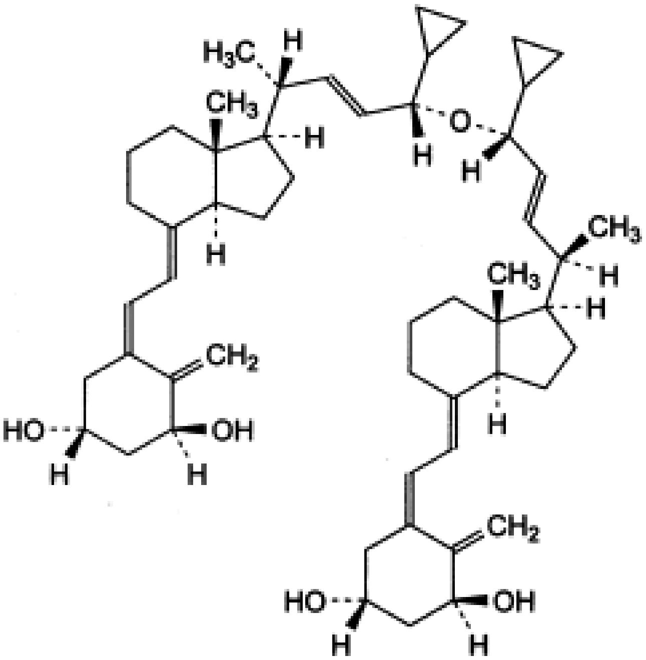
E. *rac*-(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19)-triene-1α,3β,24-triol,



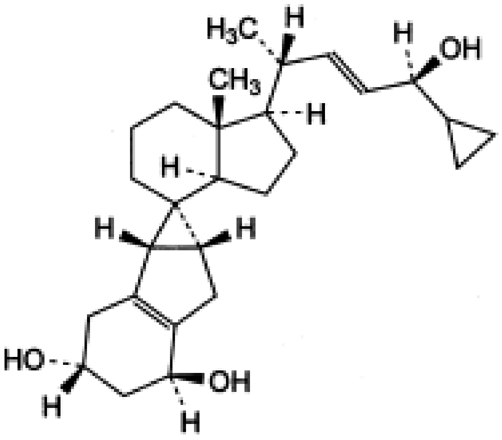
F. (5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-9,10-secochola-5,7,10(19),22-tetraen-24-ol,



G. 24,24′-oxybis[(5*Z*,7*E*,22*E*,24*S)*-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α, 3β-diol],



H. (5*Z*,7*E*,22*E*,24*R*)-24-cyclopropyl-24-[[(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-dihydroxy-9,10-secochola-5,7,10(19),22-tetraen-24-yl]oxy]-9,10-secochola-5,7,10(19),22-tetraene-1α, 3β-diol,



I. (6*S*,7*R*,8*R*,22*E*,24*S*)-24-cyclopropyl-6,8:7,19-dicyclo-9,10-secochola-5(10),22-diene-1α, 3β,24-triol (suprasterol of calcipotriol).

Ph Eur

Anhydrous Calcium Hydrogen Phosphate

General Notices

(Ph Eur monograph 0981)

$CaHPO_4$ 136.1 *7757-93-9*

Ph Eur

DEFINITION

Content

98.0 per cent to 103.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder, or colourless crystals.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute hydrochloric acid and in dilute nitric acid.

IDENTIFICATION

A. Dissolve with heating 0.1 g in 10 ml of *dilute hydrochloric acid R* . Add 2.5 ml of *dilute ammonia R1* shake, and add 5 ml of a 35 g/l solution of *ammonium oxalate R* . A white precipitate is produced.

B. Dissolve 0.1 g in 5 ml of *dilute nitric acid R* , add 2 ml of *ammonium molybdate solution R* and heat at 70 °C for 2 min. A yellow precipitate is produced.

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 2.5 g in 20 ml of *dilute hydrochloric acid R* , filter if necessary and add *dilute ammonia R1* until a precipitate is formed. Add just sufficient *dilute hydrochloric acid R* to dissolve the precipitate and dilute to 50 ml with *distilled water R* .

Acid-insoluble substances

Maximum 0.2 per cent.

Dissolve 5.0 g in 40 ml of *water R,* add 10 ml of *hydrochloric acid R* and heat to boiling for 5 min. Cool, then collect the insoluble substances using ashless filter paper. Wash with *water R* until turbidity is no longer produced when *silver nitrate solution R2* is added. Ignite at 600 ± 50 °C. The residue weighs not more than 10 mg.

Carbonates

Shake 0.5 g with 5 ml of *carbon dioxide-free water R* and add 1 ml of *hydrochloric acid R* . No effervescence is produced.

Chlorides

Maximum 0.25 per cent.

Test solution Dissolve 0.20 g in a mixture of 20 ml of *water R* and 13 ml of *dilute nitric acid R* , dilute to 100 ml with *water R* and filter if necessary. Use 50 ml of this solution.

Reference solution To 0.70 ml of *0.01 M hydrochloric acid* , add 6 ml of *dilute nitric acid R* and dilute to 50 ml with *water R* .

Add 1 ml of *silver nitrate solution R2* to the test solution and to the reference solution and mix. After standing for 5 min protected from light, any opalescence in the test solution is not more intense than that in the reference solution.

Fluorides

Maximum 100 ppm.

Potentiometry *(2.2.36, Method II)*.

Chelating solution Dissolve 45 g of *cyclohexylenedinitrilotetra-acetic acid R* in 75 ml of *sodium hydroxide solution R* and dilute to 250 ml with *water R* .

Test solution Dissolve 1.000 g in 4 ml *of hydrochloric acid R1* , add 20 ml of chelating solution, 2.7 ml of *glacial acetic acid R* and 2.8 g of *sodium chloride R* , adjust to pH 5-6 with *sodium hydroxide solution* R and dilute to 50.0 ml with *water R* .

Reference solution Dissolve 4.42 g of *sodium fluoride R* , previously dried at 300 °C for 12 h, in *water R* and dilute to 1000.0 ml with the same solvent. Dilute 50.0 ml of this solution to 500.0 ml with *total-ionic-strength-adjustment buffer R* (200 ppm F).

Indicator electrode Fluoride-selective.

Reference electrode Silver-silver chloride.

Carry out the measurement on 20.0 ml of the test solution. Add at least 3 times 0.10 ml of the reference solution and carry out the measurement after each addition. Calculate the concentration of fluorides using the calibration curve.

Sulphates

Maximum 0.5 per cent.

Test solution Dissolve 0.5 g in a mixture of 5 ml of *water R* and 5 ml of *dilute hydrochloric acid R* and dilute to 100 ml with *water R.* Filter if necessary. To 20 ml of this solution, add 1 ml of *dilute hydrochloric acid R* and dilute to 50 ml with *water R* .

Reference solution To 1.0 ml of *0.005 M sulphuric acid* , add 1 ml of *dilute hydrochloric acid R* and dilute to 50 ml with *water* R. Filter if necessary.

To the test solution and to the reference solution, add 2 ml of a 120 g/l solution of *barium chloride R* and allow to stand for 10 min. Any opalescence in the test solution is not more intense than that in the reference solution.

Arsenic *(2.4.2, Method A)*

Maximum 10 ppm, determined on 2 ml of solution S.

Barium

To 0.5 g, add 10 ml of *water R* and heat to boiling. While stirring, add 1 ml of *hydrochloric acid R* dropwise. Allow to cool and filter if necessary. Add 2 ml of a 10 g/l solution of *dipotassium sulphate R* and allow to stand for 10 min. No turbidity is produced.

Iron *(2.4.9)*

Maximum 400 ppm.

Dilute 0.5 ml of solution S to 10 ml with *water R* .

Heavy metals *(2.4.8)*

Maximum 40 ppm.

Dilute 10 ml of solution S to 20 ml with *water R* . 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R* .

Loss on ignition

6.6 per cent to 8.5 per cent, determined on 1.000 g to constant mass at 800-825 °C.

ASSAY

Dissolve 0.4 g in 12 ml of *dilute hydrochloric acid R* and dilute to 200 ml with *water R* . To 20.0 ml of this solution add 25.0 ml of *0.02 M sodium edetate* , 50 ml of *water R* , 5 ml of *ammonium chloride buffer solution pH 10.7 R* and about 25 mg of *mordant black 11 triturate R*. Titrate the excess of sodium edetate with *0.02 M zinc sulphate* . Carry out a blank titration.

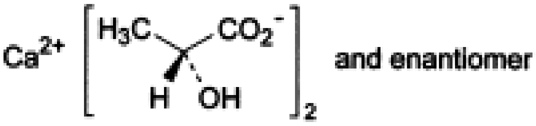
1 ml of *0.02 M sodium edetate* is equivalent to 2.72 mg of $CaHPO_4$.

Ph Eur

Anhydrous Calcium Lactate

General Notices

(Ph Eur monograph 2118)



$C_6H_{10}CaO_6$ 218.2 *814-80-2*

Action and use

Used in treatment of calcium deficiency.

Ph Eur

DEFINITION

Calcium bis(2-hydroxypropanoate) or mixture of calcium (2*R*)-, (2*S*)- and (2*RS*)-2-hydroxypropanoates.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance:

White or almost white, crystalline or granular powder.

Solubility

Soluble in water, freely soluble in boiling water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. It complies with the test for loss on drying (see Tests).

B. It gives the reaction of lactates *(2.3.1)* .

C. It gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 5.0 g with heating in *carbon dioxide-free water R* prepared from *distilled water R* , allow to cool and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R* and 0.5 ml of *0.01 M hydrochloric acid* . The solution is colourless. Not more than 2.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *water R* .

Sulphates *(2.4.13)*

Maximum 400 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R* .

Barium

To 10 ml of solution S add 1 ml of *calcium sulphate solution R* . Allow to stand for 15 min. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Iron *(2.4.9)*

Maximum 50 ppm.

Dilute 4 ml of solution S to 10 ml with *water R* .

Magnesium and alkali salts

Maximum 1 per cent.

To 20 ml of solution S add 20 ml of *water R* , 2 g of *ammonium chloride R* and 2 ml of *dilute ammonia R1* . Heat to boiling and rapidly add 40 ml of hot *ammonium oxalate solution R* . Allow to stand for 4 h, dilute to 100.0 ml with *water R* and filter. To 50.0 ml of the filtrate add 0.5 ml of *sulphuric acid R* . Evaporate to dryness and ignite the residue to constant mass at 600 ± 50 °C. The residue weighs a maximum of 5 mg.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 3.0 per cent, determined on 0.500 g by drying in an oven at 125 °C.

ASSAY

Dissolve 0.200 g in *water R* and dilute to 300 ml with the same solvent. Carry out the complexometric titration of calcium *(2.5.11)*.

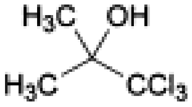
1 ml of *0.1 M sodium edetate* is equivalent to 21.82 mg of $C_6H_{10}CaO_6$.

Ph Eur

Anhydrous Chlorobutanol

General Notices

(Ph Eur monograph 0382)



$C_4H_7Cl_3O$ 177.5 *57-15-8*

Action and use

Disinfectant preservative.

Ph Eur

DEFINITION

1,1,1-Trichloro-2-methylpropan-2-ol.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white crystalline powder or colourless crystals, sublimes readily.

Solubility

Slightly soluble in water, very soluble in ethanol (96 per cent), soluble in glycerol (85 per cent).

mp: about 95 °C (without previous drying).

IDENTIFICATION

A. Add about 20 mg to a mixture of 1 ml of *pyridine R* and 2 ml of *strong sodium hydroxide solution R*. Heat in a water-bath and shake. Allow to stand. The pyridine layer becomes red.

B. Add about 20 mg to 5 ml of *ammoniacal silver nitrate solution R* and warm slightly. A black precipitate is formed.

C. To about 20 mg add 3 ml of *1 M sodium hydroxide* and shake to dissolve. Add 5 ml of *water R* and then, slowly, 2 ml of *iodinated potassium iodide solution R*. A yellowish precipitate is formed.

D. Water (see Tests).

TESTS

Solution S

Dissolve 5 g in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Acidity

To 4 ml of solution S add 15 ml of *ethanol (96 per cent) R* and 0.1 ml of *bromothymol blue solution R1*. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Chlorides *(2.4.4)*

Maximum 300 ppm.

Dissolve 0.17 g in 5 ml of *ethanol (96 per cent) R* and dilute to 15 ml with *water R*. When preparing the standard, replace the 5 ml of *water R* by 5 ml of *ethanol (96 per cent) R*.

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 2.00 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 20 ml of *ethanol (96 per cent) R*. Add 10 ml of *dilute sodium hydroxide solution R*, heat in a water-bath for 5 min and cool. Add 20 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R* and shake vigorously. Add 2 ml of *ferric ammonium sulphate solution R2* and titrate with *0.1 M ammonium thiocyanate* until an orange colour is obtained.

1 ml of *0.1 M silver nitrate* is equivalent to 5.92 mg of $C_4H_7Cl_3O$.

STORAGE

In an airtight container .

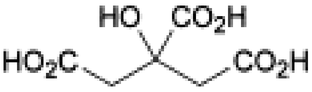
Ph Eur

Anhydrous Citric Acid

General Notices

Citric Acid

(Ph Eur monograph 0455)



$C_6H_8O_7$ 192.1 *77-92-9*

Ph Eur

DEFINITION

2-Hydroxypropane-1,2,3-tricarboxylic acid.

Content

99.5 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, colourless crystals or granules.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

mp: about 153 °C, with decomposition.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Dissolve 1 g in 10 ml of *water R*. The solution is strongly acidic *(2.2.4)*.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dry the substance to be examined and the reference substance at 100-105 °C for 2 h.

Comparison *anhydrous citric acid CRS*.

C. Add about 5 mg to a mixture of 1 ml of *acetic anhydride R* and 3 ml of *pyridine R*. A red colour develops.

D. Dissolve 0.5 g in 5 ml of *water R*, neutralise using *1 M sodium hydroxide* (about 7 ml), add 10 ml of *calcium chloride solution R* and heat to boiling. A white precipitate is formed.

E. Water (see Tests).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7, BY_7 or GY_7 *(2.2.2, Method II)*.

Dissolve 2.0 g in *water R* and dilute to 10 ml with the same solvent.

Readily carbonisable substances

To 1.0 g in a cleaned test tube add 10 ml of *sulphuric acid R* and immediately heat the mixture in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly immediately afterwards. The solution is not more intensely coloured than a mixture of 1 ml of red primary solution and 9 ml of yellow primary solution *(2.2.2, Method I)*.

Oxalic acid

Maximum 360 ppm, calculated as anhydrous oxalic acid.

Dissolve 0.80 g in 4 ml of *water R*. Add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules. Boil for 1 min. Allow to stand for 2 min. Transfer the supernatant liquid to a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of a 50 g/l solution of *potassium ferricyanide R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 4 ml of a 0.1 g/l solution of *oxalic acid R*.

Sulphates *(2.4.13)*

Maximum 150 ppm.

Dissolve 2.0 g in *distilled water R* and dilute to 30 ml with the same solvent.

Aluminium *(2.4.17)*

Maximum 0.2 ppm, if intended for use in the manufacture of dialysis solutions.

Prescribed solution Dissolve 20 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R.*

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 5.0 g in several portions in 39 ml of *dilute sodium hydroxide solution R* and dilute to 50 ml with *distilled water R.* 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 2.000 g.

Sulphated ash ***(2.4.14*****)**

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.550 g in 50 ml of *water R*. Titrate with *1 M sodium hydroxide*, using 0.5 ml of *phenolphthalein solution R* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 64.03 mg of $C_6H_8O_7$.

LABELLING

The label states, where applicable, that the substance is intended for use in the manufacture of dialysis solutions.

Ph Eur

Anhydrous Copper Sulphate

General Notices

(Ph Eur monograph 0893)

$CuSO_4$ 159.6 *7758-98-7*

Action and use

Used in treatment of copper deficiency.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Greenish-grey powder, very hygroscopic.

Solubility

Freely soluble in water, slightly soluble in methanol, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Add several drops of *dilute ammonia R2* to 1 ml of solution S (see Tests). A blue precipitate is formed. On further addition of *dilute ammonia R2* the precipitate dissolves and a dark blue colour is produced.

B. Loss on drying (see Tests).

C. Dilute 1 ml of solution S to 5 ml with *water R*. The solution gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 1.6 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

Chlorides ***(2.4.4)***

Maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *water R.*

Iron

Maximum 1.50 × 10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 0.32 g in 10 ml of *water R*, add 2.5 ml of *lead-free nitric acid R* and dilute to 25.0 ml with *water R.*

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, adding 2.5 ml of *lead-free nitric acid R* and diluting to 25.0 ml with *water R*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Copper may form explosive acetylides with acetylene. Therefore, clean the burner thoroughly before any residues become dry.

Lead

Maximum 80.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 1.6 g in 10 ml of *water R*, add 2.5 ml of *lead-free nitric acid R* and dilute to 25.0 ml with *water R.*

Reference solutions Prepare the reference solutions using *lead standard solution (100 ppm Pb) R*, adding 2.5 ml of *lead-free nitric acid R* and diluting to 25.0 ml with *water R.*

Source Lead hollow-cathode lamp.

Wavelength 217.0 nm.

Atomisation device Air-acetylene flame.

Copper may form explosive acetylides with acetylene. Therefore, clean the burner thoroughly before any residues become dry.

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 250 ± 10 °C.

ASSAY

Dissolve 0.125 g in 50 ml of *water R*. Add 2 ml of *sulphuric acid R* and 3 g of *potassium iodide R*. Titrate with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution R*, added towards the end of the titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 15.96 mg of $CuSO_4$.

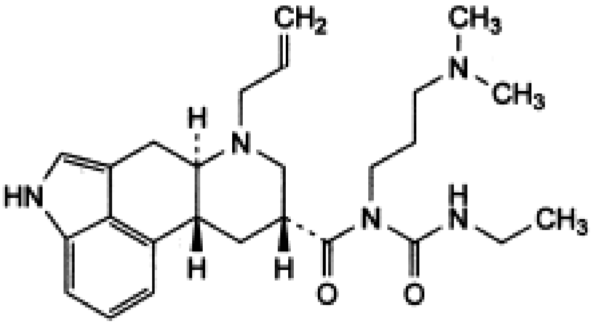
STORAGE

In an airtight container .

Cabergoline

General Notices

(Ph Eur monograph 1773)



$C_{26}H_{37}N_5O_2$ 451.6 *81409-90-7*

Action and use

Dopamine D2 receptor agonist.

Ph Eur

DEFINITION

1-Ethyl-3-[3-(dimethylamino)propyl]-3-[[(6a*R*,9*R*,10a*R*)-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]carbonyl]urea.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), very slightly soluble in hexane. It is slightly soluble in *0.1 M hydrochloric acid* .

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *cabergoline CRS.*

If the spectra obtained in the solid state show differences, dissolve 50 mg of the substance to be examined and 50 mg of the reference substance separately in 1 ml of *ethanol (96 per cent) R* , evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation *(2.2.7)*

- 77 to - 83 (anhydrous substance).

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use and protected from light.*

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 30.0 mg of *cabergoline CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) Suspend 50 mg of the substance to be examined in 10 ml of *0.1 M sodium hydroxide* . Stir for about 15 min. To 1 ml of the suspension add 1 ml of *0.1 M hydrochloric acid* and dilute to 10 ml with the mobile phase. Sonicate until dissolution is complete. The main degradation product obtained is impurity A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 16 volumes of *acetonitrile R* and 84 volumes of a freshly prepared 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R* . Add 0.2 volumes of *triethylamine R* .

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time 4 times the retention time of cabergoline.

Relative retention With reference to cabergoline (retention time = about 12 min): impurity D = about 0.3; impurity B = about 0.6; impurity A = about 0.8; impurity C = about 2.9.

System suitability   Reference solution (c):

— *resolution*: minimum 3.0 between the peaks due to cabergoline and impurity A.

Limits:

— *impurities A, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities B, D*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.000 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection   Test solution and reference solution (a).

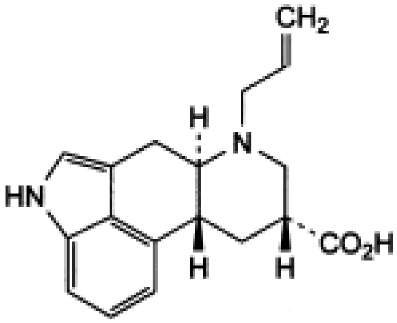
Calculate the percentage content of $C_{26}H_{37}N_5O_2$ from the areas of the peaks and the declared content of *cabergoline CRS.*

STORAGE

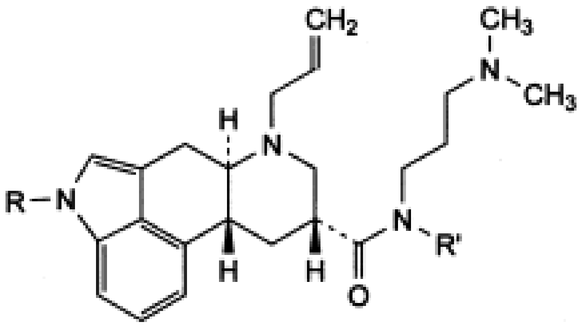
Protected from light.

IMPURITIES

Specified impurities   *A, B, C, D.*



A. (6a*R*,9*R*,10a*R*)-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,



B. R = CO-NH-C_2H_5, R′ = H: (6a*R*,9*R,*10a*R*)-*N* ^{9}-[3-(dimethylamino)propyl]-*N* ^{4}-ethyl-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6*H*)-dicarboxamide,

C. R = R' = CO-NH-C_2H_5: (6a*R*,9*R*,10a*R*)-*N* ^{9}-[3-(dimethylamino)propyl]-*N* ^{4}-ethyl-*N* ^{9}-(ethylcarbamoyl)-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6*H*)-dicarboxamide,

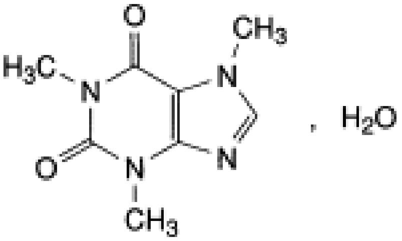
D. R = R′ = H: (6a*R*,9*R*,10a*R*)-*N*-[3-(dimethylamino)propyl]-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide.

Ph Eur

Caffeine Hydrate

General Notices

(Caffeine Monohydrate, Ph Eur monograph 0268)



$C_8H_{10}N_4O_2,H_2O$ 212.2   5743-12-4

Action and use

Central nervous system stimulant.

Ph Eur

DEFINITION

Caffeine monohydrate contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of 1,3,7-trimethyl-3,7-dihydro-1*H*-purine-2,6-dione, calculated with reference to the dried substance.

CHARACTERS

White or almost white, crystalline powder or silky crystals, sublimes readily, sparingly soluble in water, freely soluble in boiling water, slightly soluble in ethanol. It dissolves in concentrated solutions of alkali benzoates or salicylates.

IDENTIFICATION

First identification *A, B, E.*

Second identification *A, C, D, E, F.*

A. Melting point *(2.2.14)* 234 °C to 239 °C, determined after drying at 100-105 °C.

B. Dry the substance to be examined at 100-105 °C. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *caffeine CRS* .

C. To 2 ml of a saturated solution add 0.05 ml of *iodinated potassium iodide solution R*. The solution remains clear. Add 0.1 ml of *dilute hydrochloric acid R*. A brown precipitate is formed. Neutralise with *dilute sodium hydroxide solution R*. The precipitate dissolves.

D. In a glass-stoppered tube, dissolve about 10 mg in 0.25 ml of a mixture of 0.5 ml of *acetylacetone R* and 5 ml of *dilute sodium hydroxide solution R*. Heat in a water-bath at 80 °C for 7 min. Cool and add 0.5 ml of *dimethylaminobenzaldehyde solution R2*. Heat again in a water-bath at 80 °C for 7 min. Allow to cool and add 10 ml of *water R*. An intense blue colour develops.

E. It complies with the test for loss on drying (see Tests).

F. It gives the reaction of xanthines *(2.3.1)*.

TESTS

Solution S.

Dissolve 0.5 g with heating in 50 ml of *carbon dioxide-free water R* prepared from *distilled water R,* cool and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity

To 10 ml of solution S add 0.05 ml of *bromothymol blue solution R1*. The solution is green or yellow. Not more than 0.2 ml of 0*.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Examine by thin-layer chromatography *(2.2.27)* using *silica gel GF$_{254}$ R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 4 volumes of *methanol R* and 6 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with a mixture of 4 volumes of *methanol R* and 6 volumes of *methylene chloride R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R*, 30 volumes of *acetone R*, 30 volumes of *methylene chloride R* and 40 volumes of *butanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Sulphates ***(2.4.13)***

15 ml of solution S complies with the limit test for sulphates (500 ppm). Prepare the standard using a mixture of 7.5 ml of *sulphate standard solution (10 ppm SO_4) R* and 7.5 ml of *distilled water R.*

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R* .

Loss on drying *(2.2.32)*

5.0 per cent to 9.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.170 g, previously dried at 100-105 °C, with heating in 5 ml of *anhydrous acetic acid R* . Allow to cool, add 10 ml of *acetic anhydride R* and 20 ml of *toluene R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* .

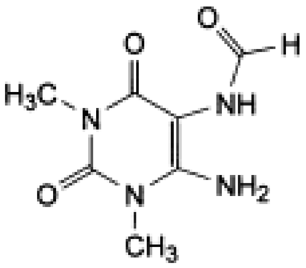
1 ml of *0.1 M perchloric acid* is equivalent to 19.42 mg of $C_8H_{10}N_4O_2$.

IMPURITIES

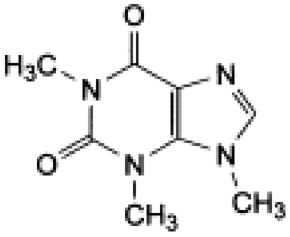
Specified impurities *A.*

Other detectable impurities *B, C.*

A. theophylline,



B. *N*-[6-amino-1,3-dimethyl-2,4(1*H*, 3*H*)-dioxopyrimidin-5-yl]formamide,



C. 1,3,9-trimethyl-3,9-dihydro-1*H*-purine-2,6-dione (isocaffeine).

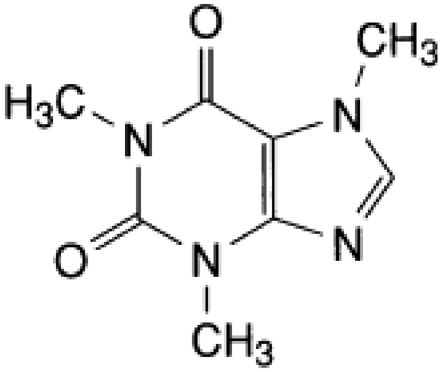
Ph Eur

Caffeine

General Notices

Anhydrous Caffeine

(Ph Eur monograph 0267)



$C_8H_{10}N_4O_2$ 194.2 *58-08-2*

Action and use

Central nervous system stimulant.

Preparations

Aspirin and Caffeine Tablets

Caffeine Citrate Injection

Caffeine Citrate Oral Solution

Paracetamol, Codeine Phosphate and Caffeine Capsules

Paracetamol, Codeine Phosphate and Caffeine Tablets

Ph Eur

DEFINITION

1,3,7-Trimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or silky, white or almost white, crystals.

Solubility

Sparingly soluble in water, freely soluble in boiling water, slightly soluble in ethanol (96 per cent). It dissolves in concentrated solutions of alkali benzoates or salicylates.

It sublimes readily.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E, F.

A. Melting point (*2.2.14*): 234 °C to 239 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison caffeine CRS.

C. To 2 ml of a saturated solution add 0.05 ml of *iodinated potassium iodide solution R*. The solution remains clear. Add 0.1 ml of *dilute hydrochloric acid R*; a brown precipitate is formed. Neutralise with *dilute sodium hydroxide solution R*; the precipitate dissolves.

D. In a ground-glass-stoppered tube, dissolve about 10 mg in 0.25 ml of a mixture of 0.5 ml of *acetylacetone R* and 5 ml of *dilute sodium hydroxide solution R*. Heat in a water-bath at 80 °C for 7 min. Cool and add 0.5 ml of *dimethylaminobenzaldehyde solution R2*. Heat again in a water-bath at 80 °C for 7 min. Allow to cool and add 10 ml of *water R*; an intense blue colour develops.

E. Loss on drying (see Tests).

F. It gives the reaction of xanthines (*2.3.1*).

TESTS

Solution S

Dissolve 0.5 g with heating in 50 ml of *carbon dioxide-free water R* prepared from *distilled water R*, cool and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity

To 10 ml of solution S add 0.05 ml of *bromothymol blue solution R1*; the solution is green or yellow. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *caffeine for system suitability CRS* (containing impurities A, C, D and F) in the mobile phase and dilute to 5 ml with the mobile phase. Dilute 2 ml of this solution to 10 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.64 g of *anhydrous sodium acetate R* in *water R* and dilute to 2000 ml with the same solvent. Adjust 1910 ml of this solution to pH 4.5 with *glacial acetic acid R* and add 50 ml of *acetonitrile R* and 40 ml of *tetrahydrofuran R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µl.

Run time 1.5 times the retention time of caffeine.

Identification of impurities Use the chromatogram supplied with *caffeine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, C, D and F.

Retention time Caffeine = about 8 min.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to impurities C and D and minimum 2.5 between the peaks due to impurities F and A.

Limits:

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphates (*2.4.13*)

Maximum 500 ppm, determined on 15 ml of solution S.

Prepare the standard using a mixture of 7.5 ml of *sulphate standard solution (10 ppm SO_4) R* and 7.5 ml of *distilled water R.*

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

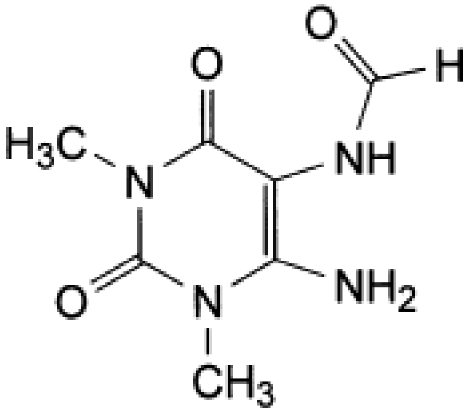
Dissolve 0.170 g with heating in 5 ml of *anhydrous acetic acid R*. Allow to cool, add 10 ml of *acetic anhydride R* and 20 ml of *toluene R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 19.42 mg of $C_8H_{10}N_4O_2$.

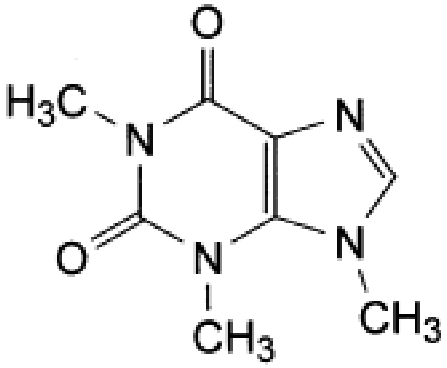
IMPURITIES

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F.*

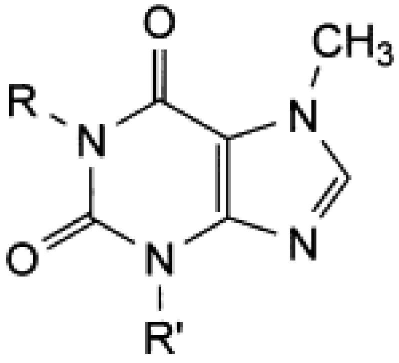
A. theophylline,



B. *N*-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide,

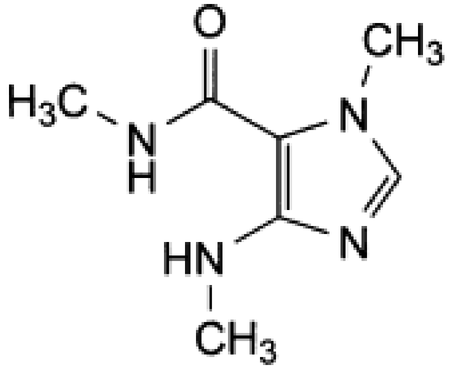


C. 1,3,9-trimethyl-3,9-dihydro-1*H*-purine-2,6-dione (isocaffeine),



D. R = H, R′ = CH_3: theobromine,

F. R = CH_3, R′ = H: 1,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,



E. *N*,1-dimethyl-4-(methylamino)-1*H*-imidazole-5-carboxamide (caffeidine).

Ph Eur

Calamine

General Notices

Prepared Calamine

Action and use

Antipruritic.

Preparations

Aqueous Calamine Cream

Calamine Lotion

Calamine Ointment

Calamine and Coal Tar Ointment

DEFINITION

Calamine is a basic zinc carbonate suitably coloured with iron(III) oxide.

CHARACTERISTICS

An amorphous, impalpable, pink or reddish brown powder, the colour depending on the variety and amount of iron(III) oxide present and the process by which it is incorporated.

Practically insoluble in *water*. It dissolves with effervescence in *hydrochloric acid* .

IDENTIFICATION

A. Yields the reactions characteristic of *carbonates* , Appendix VI.

B. To 2 g add 5 ml of *hydrochloric acid* and heat to boiling; if necessary, add *hydrochloric acid* drop wise until a bright yellow solution is obtained. Cool and add *13.5 M ammonia* until the first sign of precipitate (solution A). The solution yields reaction B characteristic of *iron salts* , Appendix VI. Dilute 1 ml of solution A to 5 ml with *water*; the solution yields the reaction characteristic of *zinc salts* , Appendix VI.

TESTS

Calcium

Dissolve 0.50 g in a mixture of 10 ml of *water* and 2.5 ml of *glacial acetic acid* and filter. To 0.5 ml of the filtrate add 15 ml of *5 M ammonia* and 2 ml of a 2.5% w/v solution of *ammonium oxalate* and allow to stand for 2 minutes. The solution remains clear.

Soluble barium salts

To the remainder of the filtrate obtained in the test for Calcium add 2 ml of *1 M sulphuric acid* and allow to stand for 5 minutes. The solution remains clear.

Lead

Not more than 150 ppm when determined by *atomic absorption spectrophotometry*, Appendix II D, Method II, measuring at 283.3 nm or 217 nm and using an air-acetylene flame. Carefully add 5 g of the substance being examined to 25 ml of *hydrochloric acid* and allow to stand for 18 hours. Add 5 ml of *nitric acid* and sufficient *water* to produce 200 ml. Use *lead standard solution (100 ppm Pb)* suitably diluted with a 3.5% v/v solution of *nitric acid* to prepare the standard solution.

Chloride

Dissolve 0.15 g in *water* with the addition of 1 ml of *nitric acid*, filter and dilute to 30 ml with *water*. The resulting solution complies with the *limit test for chlorides*, Appendix VII (0.07%).

Sulphate

Dissolve 0.1 g in *water* with the addition of 3 ml of *2 M hydrochloric acid*, filter and dilute to 60 ml with *water*. The resulting solution complies with the *limit test for sulphates*, Appendix VII (0.6%).

Ethanol-soluble dyes

Shake 1.0 g with 10 ml of *ethanol (90%)* and filter. The filtrate is *colourless*, Appendix IV B, Method II.

Matter insoluble in hydrochloric acid

Dissolve 1 g in 20 ml of warm *2 M hydrochloric acid* and filter. The residue, when washed with *water* and dried to constant weight at 105°, weighs not more than 10 mg.

Water-soluble dyes

Shake 1.0 g with 10 ml of *water* and filter. The filtrate is *colourless*, Appendix IV B, Method II.

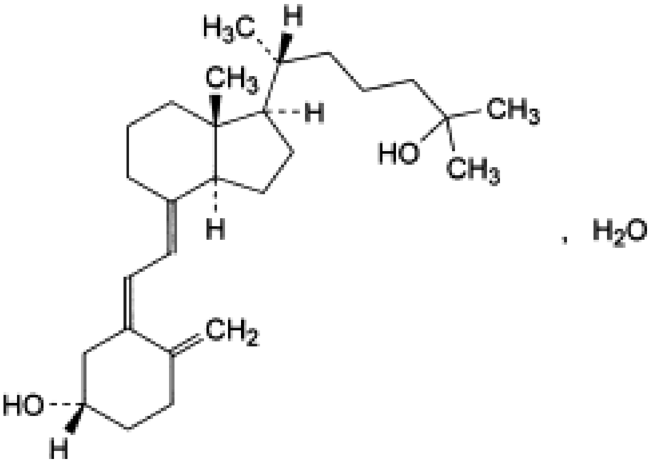
Residue on ignition

68.0 to 74.0%, when ignited at a temperature not lower than 900° until, after further ignition, two successive weighings do not differ by more than 0.2% of the weight of the residue. Use 1 g.

Calcifediol

General Notices

(Ph Eur monograph 1295)



$C_{27}H_{44}O_2,H_2O$ 418.7 *63283-36-3*

Action and use

Vitamin D analogue.

Ph Eur

DEFINITION

(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-triene-3β,25-diol monohydrate.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white crystals.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in fatty oils.

It is sensitive to air, heat and light.

A reversible isomerisation to pre-calcifediol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Mix 2 mg of the substance to be examined and 225 mg of *potassium bromide R* .

Comparison *Ph. Eur. reference spectrum of calcifediol.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)* : use the normalisation procedure. *Carry out the test as rapidly as possible, avoiding exposure to actinic light and air.*

Test solution Dissolve 1.0 mg of the substance to be examined without heating in 10.0 ml of the mobile phase.

Reference solution (a) Dissolve 1.0 mg of *calcifediol CRS* without heating in 10.0 ml of the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Heat 2 ml of reference solution (a) in a water-bath at 80 °C under a reflux condenser for 2 h and cool.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: octylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase *water R, methanol R* (200:800 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 50 µl of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of calcifediol.

Relative retention With reference to calcifediol (retention time = about 11 min): pre-calcifediol = about 1.3.

System suitability Reference solution (c):

— *resolution*: minimum 5.0 between the peaks due to pre-calcifediol and calcifediol; if necessary, adjust the proportions of the constituents in the mobile phase.

Limits:

— *impurities A, B, C, D*: for each impurity, maximum 0.5 per cent;

— *total*: maximum 1.0 per cent;

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); disregard the peak due to pre-alfacalcidiol.

Water ***(2.5.32)***

3.8 per cent to 5.0 per cent, determined on 10.0 mg.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection The test solution and reference solutions (a) and (c).

System suitability Reference solution (c):

— *repeatability*: maximum relative standard deviation of 1 per cent for the peak due to calcifediol after 6 injections.

Calculate the percentage content of $C_{27}H_{44}O_2$ from the declared content of *calcifediol CRS* .

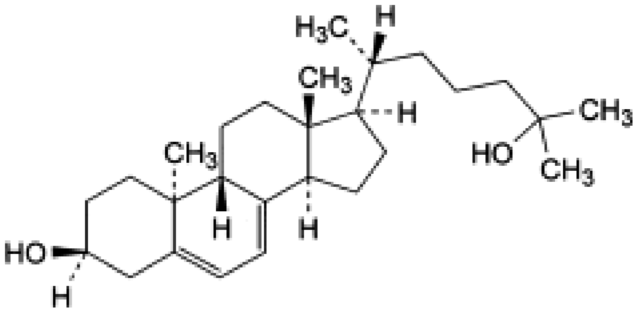
STORAGE

Under nitrogen, in an airtight container , protected from light, at a temperature of 2 °C to 8 °C.

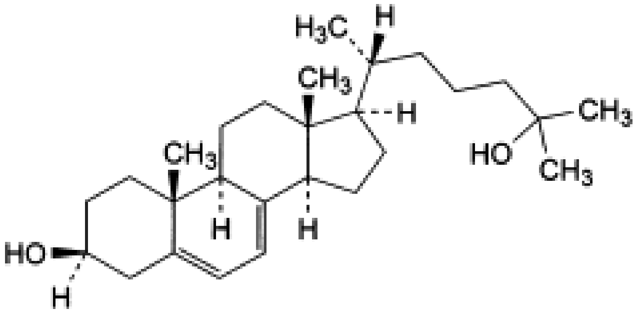
The contents of an opened container are to be used immediately.

IMPURITIES

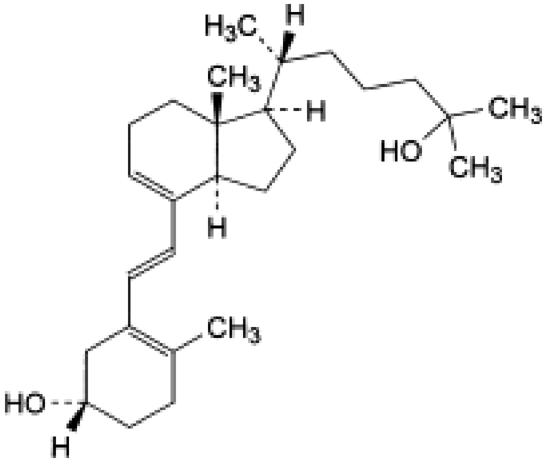
Specified impurities *A, B, C, D.*



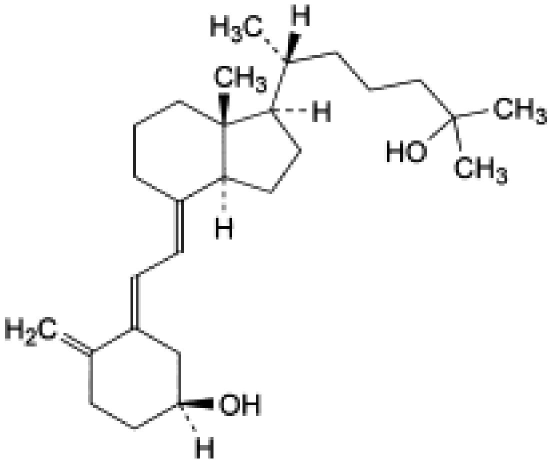
A. 9β,10α-cholesta-5,7-diene-3β,25-diol,



B. cholesta-5,7-diene-3β,25-diol,



C. (6*E*)-9,10-secocholesta-5(10),6,8-triene-3β,25-diol,



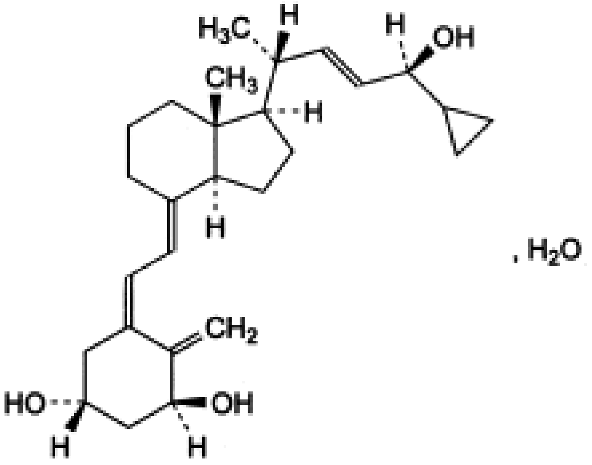
D. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-3β,25-diol.

Ph Eur

Calcipotriol Monohydrate

General Notices

(Ph Eur monograph 2284)



$C_{27}H_{40}O_3,H_2O$ 430.6 *112965-21-6*

Action and use

Vitamin D analogue.

Ph Eur

DEFINITION

(5*Z*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol monohydrate.

Content:

95.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It is sensitive to light.

A reversible isomerisation to pre-calcipotriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of calcipotriol monohydrate.*

B. Water (see Tests).

Carry out the tests for related substances and the assay as rapidly as possible and protected from actinic light and air.

TESTS

Related substances

A. Thin-layer chromatography *(2.2.27).*

Solution A To 1 ml of *triethylamine R* add 9 ml of *chloroform R* .

Test solution Dissolve 1 mg of the substance to be examined in 100 µl of solution A.

Reference solution (a) To 10 µl of the test solution add 990 µl of solution A.

Reference solution (b) To 250 µl of reference solution (a) add 750 µl of solution A.

Reference solution (c) To 100 µl of reference solution (a) add 900 µl of solution A.

Reference solution (d) Place 2 mg of the substance to be examined in a vial and dissolve in 200 µl of solution A. Close the vial and keep it in a water bath at 60 °C for 2 h.

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *2-methylpropanol R, methylene chloride R* (20:80 *V/V*).

Application 10 µl of the test solution and reference solutions (b), (c) and (d).

Development Over 2/3 of the plate.

Drying In air, then at 140 °C for 10 min.

Detection Spray the hot plate with an *alcoholic solution of sulphuric acid R* , dry at 140 °C for not more than 1 min and examine in ultraviolet light at 366 nm.

Relative retention With reference to calcipotriol (R_F = about 0.4): impurity G = about 0.4; impurity H = about 0.4; pre-calcipotriol = about 0.9; impurity A = about 1.2.

System suitability Reference solution (d):

— the chromatogram shows a secondary spot due to pre-calcipotriol.

Limits:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent),

— *impurities G, H*: any spot due to impurity G or H is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent for the sum),

— *any other impurity*: any other spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

B. Liquid chromatography *(2.2.29)* .

Solution A Dissolve 1.32 g of *ammonium phosphate R* in *water R* and dilute to 10.0 ml with the same solvent.

Solvent mixture solution A, *water R, methanol R* (3:297:700 *V/V/V*).

Test solution (a) Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Test solution (b) Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the same solvent mixture.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 1.0 mg of *calcipotriol monohydrate CRS* (containing impurities B, C and D) in the solvent mixture and dilute to 2.5 ml with the solvent mixture.

Reference solution (d) Dissolve 2.00 mg of *calcipotriol monohydrate CRS* in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.10 m, Ø = 4.0 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase *water R, methanol R* (30:70 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 264 nm.

Injection 20 µl of test solution (a) and reference solutions (a), (b) and (c).

Run time Twice the retention time of calcipotriol.

Relative retention With reference to calcipotriol (retention time = about 13.5 min): impurity B = about 0.86; impurity C = about 0.92; impurity D = about 1.3.

System suitability Reference solution (c):

— *peak-to-valley ratio* : minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to calcipotriol,

— the chromatogram obtained is similar to the chromatogram supplied with *calcipotriol monohydrate CRS.*

Limits:

— *impurity B:* not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *impurities C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water ***(2.5.12)***

3.3 per cent to 5.0 per cent, determined on 0.100 g .

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{27}H_{40}O_3$ from the areas of the peaks and the declared content of *calcipotriol monohydrate CRS*.

STORAGE

In an airtight container , protected from light.

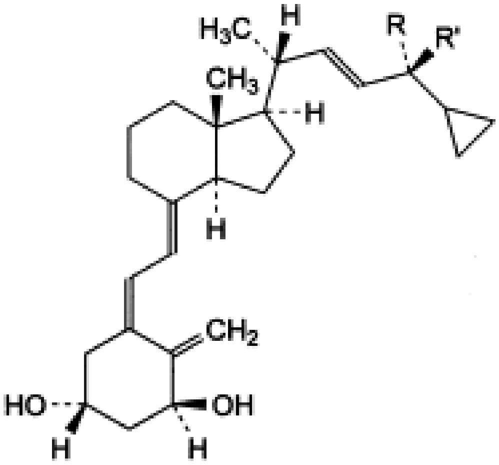
IMPURITIES

Specified impurities *A, B, C, D, G, H.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F, I.*

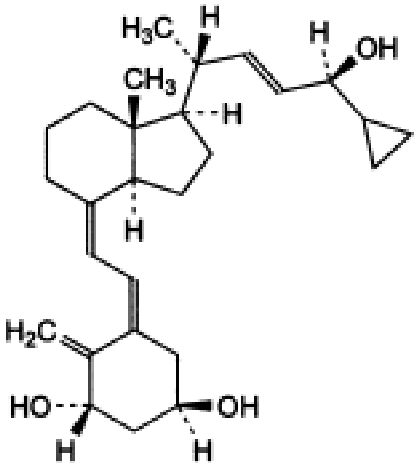
By thin-layer chromatography *A, G, H, I.*

By liquid chromatography *B, C, D, E, F.*

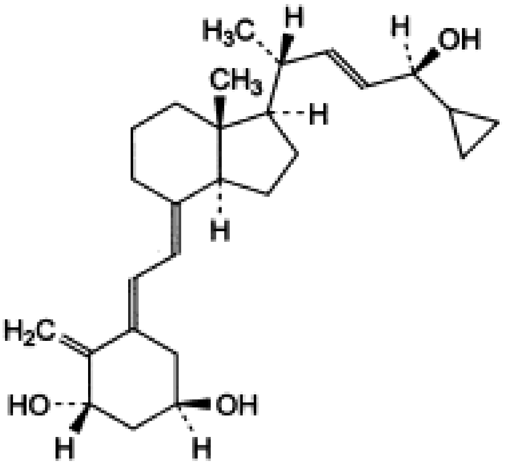


A. R + R′ = O: (5*Z*,7*E*,22*E*)-24-cyclopropyl-1α,3β-dihydroxy-9,10-secochola-5,7,10(19),22-tetraen-24-one,

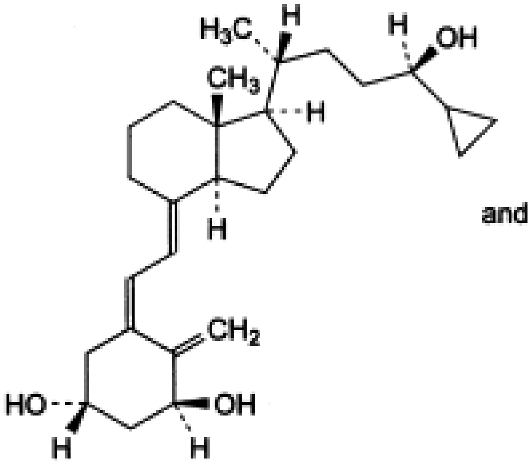
D. R = OH, R′ = H: (5*Z*,7*E*,22*E*,24*R*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol (24-*epi*-calcipotriol),



B. (5*Z*,7*Z*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol ((7*Z*)-calcipotriol),

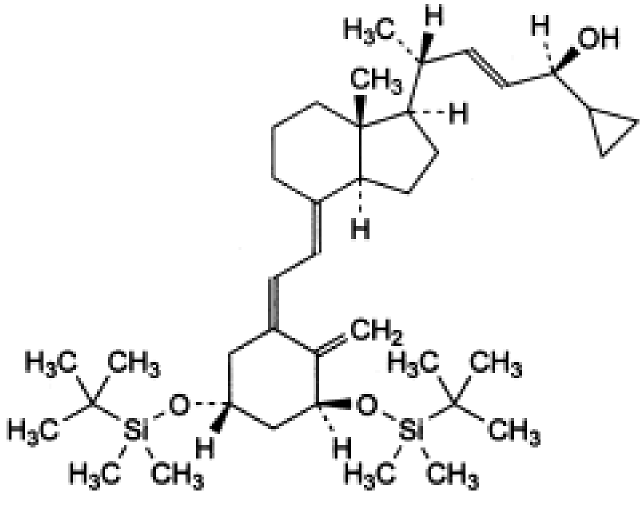


C. (5*E*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol ((5*E*)-calcipotriol),

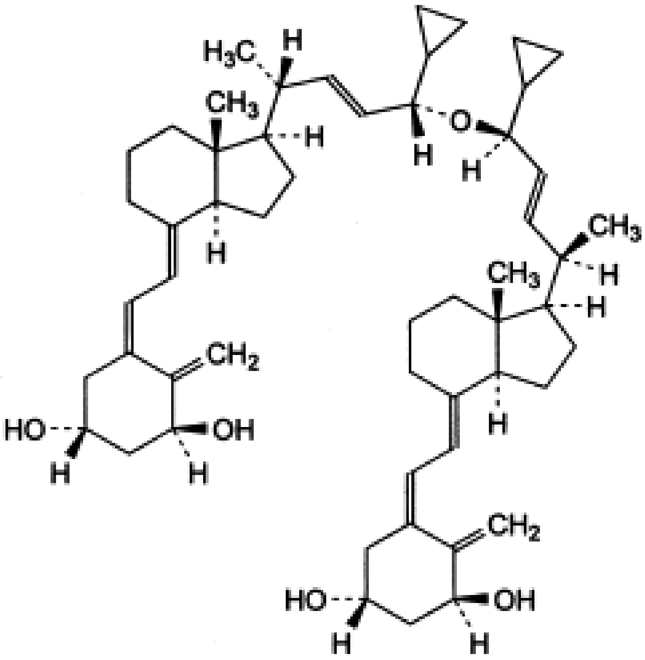


and enantiomer

E. *rac*-(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19)-triene-1α,3β,24-triol,



F. (5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-9,10-secochola-5,7,10(19),22-tetraen-24-ol,


H₃C
H
CH₃
H
H
O
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H
CH₃
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CH₃
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CH₂
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OH
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CH₂
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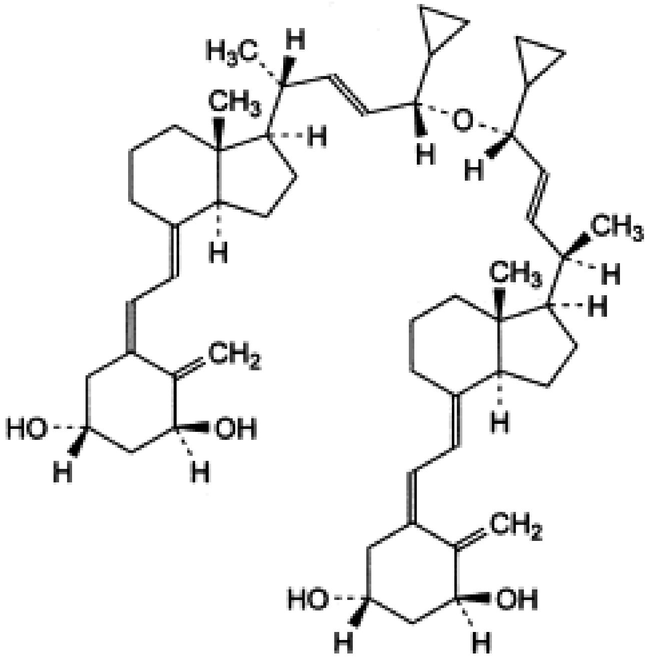
G. 24,24′-oxybis[(5*Z*,7*E*,22*E*,24S)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α, 3β-diol],


H₃C
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CH₂
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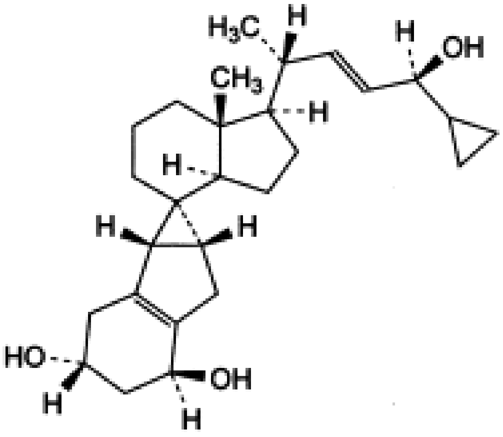
H. (5*Z*,7*E*,22*E*,24*R*)-24-cyclopropyl-24-[[(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-dihydroxy-9, 10-secochola-5,7,10(19),22-tetraen-24-yl]oxy]-9,10-secochola-5,7,10(19),22-tetraene-1α, 3β-diol,



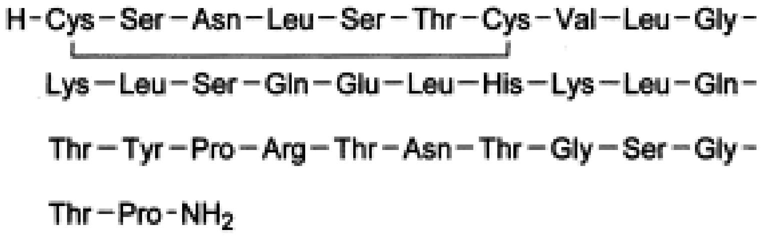
I. (6*S*,7*R*,8*R*,22*E*,24*S*)-24-cyclopropyl-6,8:7,19-dicyclo-9,10-secochola-5(10),22-diene-1α,3β,24-triol (suprasterol of calcipotriol).

Ph Eur

Calcitonin (Salmon)

General Notices

(Ph Eur monograph 0471)



$C_{145}H_{240}N_{44}O_{48}S_2$ 3432 *47931-85-1*

Action and use

Hormone.

Preparation

Calcitonin (Salmon) Injection

Ph Eur

DEFINITION

Polypeptide having the structure determined for salmon calcitonin I. It lowers the calcium concentration in plasma of mammals by diminishing the rate of bone resorption. It is obtained by chemical synthesis or by a method based on recombinant DNA (rDNA) technology. It is available as an acetate.

Content

90.0 per cent to 105.0 per cent of the peptide $C_{145}H_{240}N_{44}O_{48}S_2$ (anhydrous and acetic acid-free substance).

By convention, for the purpose of labelling calcitonin (salmon) preparations, 1 mg of calcitonin (salmon) ($C_{145}H_{240}N_{44}O_{48}S_2$) is equivalent to 6000 IU of biological activity.

PRODUCTION

The following requirements apply only to calcitonin (salmon) produced by a method based on *rDNA technology.*

Prior to release the following tests are carried out on each batch of final bulk product unless exemption has been granted by the competent authority.

Host-cell-derived proteins

The limit is approved by the competent authority.

Host-cell or vector-derived DNA

The limit is approved by the competent authority.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water.

IDENTIFICATION

A. examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

The following requirement applies only to calcitonin (salmon) obtained by chemical synthesis.

B. Amino acid analysis *(2.2.56).*

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking as equivalent to 1 the sum, divided by 20, of the number of moles of aspartic acid, glutamic acid, proline, glycine, valine, leucine, histidine, arginine and lysine. The values fall within the following limits: aspartic acid: 1.8 to 2.2; glutamic acid: 2.7 to 3.3; proline: 1.7 to 2.3; glycine: 2.7 to 3.3; valine: 0.9 to 1.1; leucine: 4.5 to 5.3; histidine: 0.9 to 1.1; arginine: 0.9 to 1.1; lysine: 1.8 to 2.2; serine: 3.2 to 4.2; threonine: 4.2 to 5.2; tyrosine: 0.7 to 1.1; half-cystine: 1.4 to 2.1.

The following requirement applies only to calcitonin (salmon) produced by a method based on rDNA technology.

C. Peptide mapping *(2.2.55)* .

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a 1 mg/ml solution of the substance to be examined. Transfer 1.0 ml to a clean tube. Add 100 µl of *1 M tris-hydrochloride buffer solution pH 8.0 R* and 20 µl of a freshly prepared 1.0 mg/ml solution of *trypsin for peptide mapping R* . Allow to stand at 2-8 °C for 16-20 h. Stop the reaction by adding 10 µl of a 50 per cent *V/V* solution of *trifluoroacetic acid R* . Cap the vial and mix. Centrifuge the vials to remove air bubbles.

Reference solution Prepare at the same time and in the same manner as for the test solution but using *calcitonin (salmon) CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION

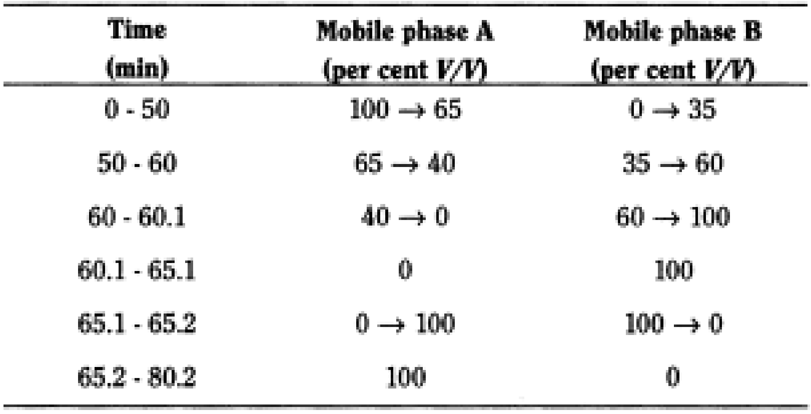
Liquid chromatography *(2.2.29)* .

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R (5 µm) with a pore size of 30 nm.*

Mobile phase:

- *mobile phase A*: mix 1 ml of *trifluoroacetic acid R* and 1000 ml of *water R* ; filter and degas;
- *mobile phase B*: mix 0.850 ml of *trifluoroacetic acid R* , 200 ml of *water R* and 800 ml of *acetonitrile for chromatography R* ; filter and degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	100 → 65	0 → 35
50 - 60	65 → 40	35 → 60
60 - 60.1	40 → 0	60 → 100
60.1 - 65.1	0	100
65.1 - 65.2	0 → 100	100 → 0
65.2 - 80.2	100	0

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.

Injection 20 µl.

System suitability The chromatograms obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of calcitonin (salmon) digest supplied with *calcitonin (salmon) CRS.*

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution: the retention times of the fragment peaks in the chromatogram obtained with the test solution are within 5 per cent of the retention times of the fragments obtained with the reference solution; the peak area ratios of the fragment peaks in the chromatogram obtained with the test solution, normalised to the area of peak T_2, are within 5 per cent of the corresponding peak ratios in the chromatogram obtained with the reference solution.

TESTS

Acetic acid *(2.5.34)*

4.0 per cent to 15.0 per cent.

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Related substances

Liquid chromatography *(2.2.29)* : use the normalisation procedure.

The following requirement applies to calcitonin (salmon), whether obtained by chemical synthesis or by a method based on rDNA technology.

A. *Test solution*

Prepare a 1.0 mg/ml solution of the substance to be examined in mobile phase A.

Reference solution Dissolve the contents of a vial of *calcitonin (salmon) CRS* in mobile phase A to obtain a concentration of 1.0 mg/ml.

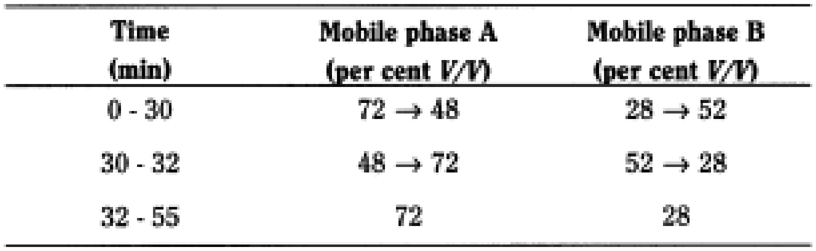
Resolution solution Dissolve the contents of a vial of *N-acetyl-Cys ^{1} -calcitonin CRS* in 400 µl of mobile phase A and add 100 µl of the test solution.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 65 °C.

Mobile phase

— *mobile phase A*: dissolve 3.26 g of *tetramethylammonium hydroxide R* in 900 ml of *water R* , adjust to pH 2.5 with *phosphoric acid R* and mix with 100 ml of *acetonitrile for chromatography R* ; filter and degas;

— *mobile phase B*: dissolve 1.45 g of *tetramethylammonium hydroxide R* in 400 ml of *water R* , adjust to pH 2.5 with *phosphoric acid* R and mix with 600 ml of acetonitrile for *chromatography R;* filter and degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	72 → 48	28 → 52
30 - 32	48 → 72	52 → 28
32 - 55	72	28

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Relative retention With reference to calcitonin (salmon) (retention time = about 20 min):

impurity B = about 0.8; impurity C = about 0.9; impurity D = about 1.05; impurity A = about 1.15.

System suitability Resolution solution:

— *resolution*: minimum 5.0 between the peaks due to calcitonin (salmon) and impurity A,

— *symmetry factor* : maximum 2.5 for the peak due to impurity A .

Limits:

— *impurities A, B, C, D*: for each impurity, maximum 3.0 per cent; other unidentified, specified impurities may occur that co-elute with impurities A, B, C and D; the acceptance criterion applies irrespective of whether these impurities co-elute;

— *total*: maximum 5.0 per cent;

— *disregard limit*: 0.1 per cent.

The following requirement applies only to calcitonin (salmon) produced by a method based on rDNA technology.

B. *Test solution*. Prepare a 0.5 mg/ml solution of the substance to be examined. To 1.0 ml of this solution add 100 µl of *0.25 M citrate buffer solution pH 3.0 R*.

Resolution solution Prepare a 1 mg/ml solution of the substance to be examined. Mix 1 volume of the solution and 1 volume of *calcitonin-Gly CRS*. To 1.0 ml of this mixture add 100 µl of *0.25 M citrate buffer solution pH 3.0 R.*

Column:

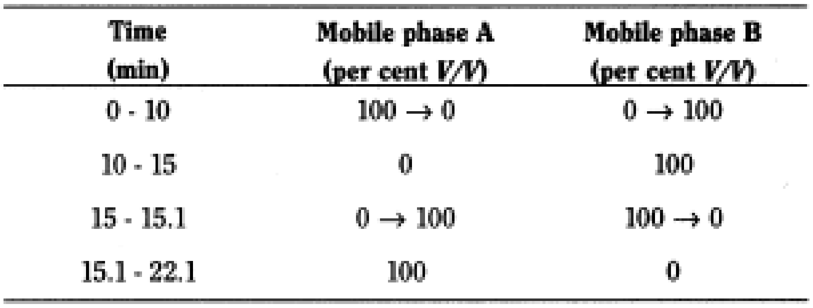
— *size*: *l* = 0.20 m, Ø = 4.6 mm;

— *stationary phase:* a suitable polysulphoethylaspartamide ion-exchange gel (5 µm).

Mobile phase:

— *mobile phase A*: mix 15 volumes of *acetonitrile for chromatography R* and 85 volumes of a 2.72 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 5.0 with a 600 g/l solution of *potassium hydroxide R* ;

— *mobile phase B*: mix 15 volumes of *acetonitrile for chromatography R* and 85 volumes of a solution containing 2.72 g/l of *potassium dihydrogen phosphate R* and 29.22 g/l of *sodium chloride R* adjusted to pH 4.6 with a 600 g/l solution of *potassium hydroxide R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100 → 0	0 → 100
10 - 15	0	100
15 - 15.1	0 → 100	100 → 0
15.1 - 22.1	100	0

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µl; rinse the injector with a 40 per cent *V/V* solution of *acetonitrile for chromatography R* .

Relative retention With reference to calcitonin (salmon) (retention time = about 9 min):

impurity G = about 0.4; impurity F = about 0.6; impurity E = about 0.9.

System suitability Resolution solution:

— *resolution*: minimum 3.0 between the peaks due to impurity E and calcitonin (salmon).

Limits:

— *impurity E*: maximum 0.6 per cent;

— *impurities F, G*: for each impurity, maximum 0.2 per cent.

Water ***(2.5.32)***

Maximum 10.0 per cent.

Acetic acid and water

Maximum 20 per cent, calculated by adding together the percentage contents of acetic acid and water determined by the methods described above.

Bacterial endotoxins ***(2.6.14)***

Less than 25 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances. Use method A for calcitonin (salmon) obtained by chemical synthesis and method B for calcitonin (salmon) obtained by a method based on rDNA technology.

Calculate the content of calcitonin (salmon) ($C_{145}H_{240}N_{44}O_{48}S_2$) from the area of the principal peak in each of the chromatograms obtained with the test solution and the reference solution and the declared content of $C_{145}H_{240}N_{44}O_{48}S_2$ in *calcitonin (salmon) CRS* . Proceed with tangential integration of the peak areas.

STORAGE

Protected from light at a temperature between 2 °C and 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

LABELLING

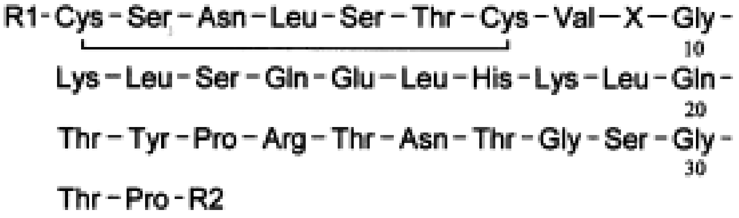
The label states:

— the calcitonin peptide content ($C_{145}H_{240}N_{44}O_{48}S_2$);

— the origin: synthetic or rDNA technology.

IMPURITIES

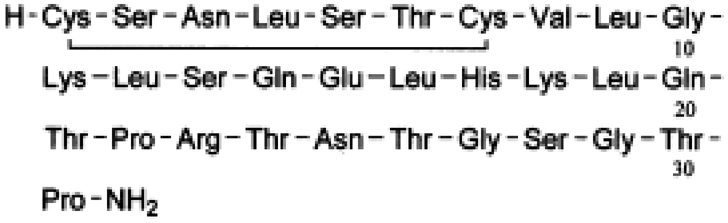
Specified impurities *A, B, C, D, E, F, G.*



A. R1 = CO-CH_3, R2 = NH_2, X = L-Leu: acetylcalcitonin (salmon),

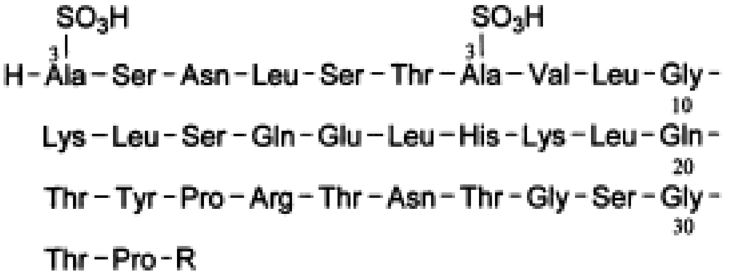
B. R1 = H, R2 = NH_2, X = D-Leu: [9-D-leucine]calcitonin (salmon),

E. R1 = H, R2 = NH-CH_2-CO_2H, X = L-Leu: salmon calcitoninylglycine,



C. des-22-tyrosine-calcitonin (salmon),

D. *C*-acetylated calcitonin (salmon),



F. R = NH_2: [1,7-bis(3-sulpho-L-alanine)]calcitonin (salmon),

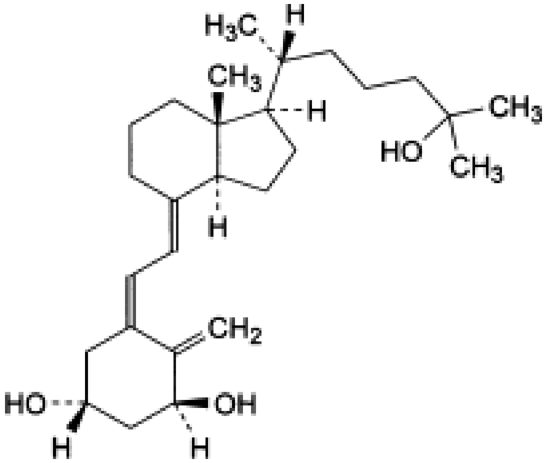
G. R = NH-CH_2-CO_2H: [1,7-bis(3-sulpho-L-alanine)]calcitoninylglycine (salmon).

Ph Eur

Calcitriol

General Notices

(Ph Eur monograph 0883)



$C_{27}H_{44}O_3$ 416.6 *32222-06-3*

Action and use

Vitamin D analogue.

Preparation

Calcitriol Capsules

Ph Eur

DEFINITION

(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-triene-1α,3β,25-triol.

Content

97.0 per cent to 103.0 per cent.

CHARACTERS

Appearance

White or almost white crystals.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in fatty oils.

It is sensitive to air, heat and light.

A reversible isomerisation to pre-calcitriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of calcitriol.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)* : use the normalisation procedure. *Carry out the test as rapidly as possible, avoiding exposure to actinic light and air.*

Test solution Dissolve 1.000 mg of the substance to be examined without heating in 10.0 ml of the mobile phase.

Reference solution (a) Dissolve 1.000 mg of *calcitriol CRS* without heating in 10.0 ml of the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Heat 2 ml of reference solution (a) at 80 °C for 30 min.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octylsilyl silica gel for chromatography R1* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 450 volumes of a 1.0 g/l solution of *tris(hydroxymethyl)aminomethane R* adjusted to pH 7.0-7.5 with *phosphoric acid R* , and 550 volumes of *acetonitrile R* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 50 µl.

Run time Twice the retention time of calcitriol.

Relative retention With reference to calcitriol (retention time = about 14 min): pre-calcitriol = about 0.9.

System suitability:

— *resolution*: minimum 3.5 between the peaks due to calcitriol and pre-calcitriol in the chromatogram obtained with reference solution (c);

— *number of theoretical plates* : minimum 10 0000, calculated for the peak due to calcitriol in the chromatogram obtained with reference solution (a).

Limits:

— *impurities A, B, C*: for each impurity, maximum 0.5 per cent;

— t*otal*: maximum 1.0 per cent;

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); disregard the peak due to pre-calcitriol.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection The test solution and reference solution (a).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1 per cent for the peak due to calcitriol after 6 injections.

Calculate the percentage content of $C_{27}H_{44}O_3$ from the declared content of *calcitriol CRS* .

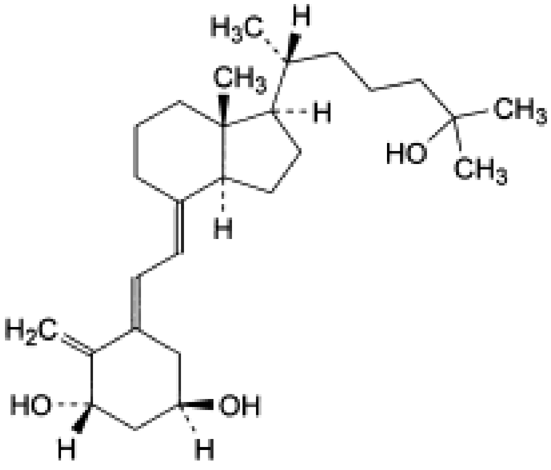
STORAGE

Under nitrogen, in an airtight container , protected from light, at a temperature of 2 °C to 8 °C.

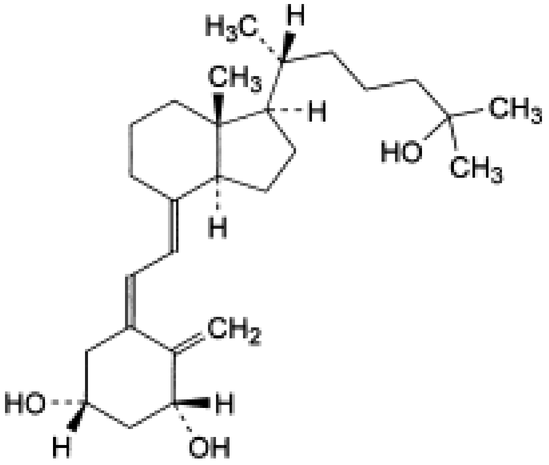
The contents of an opened container are to be used immediately.

IMPURITIES

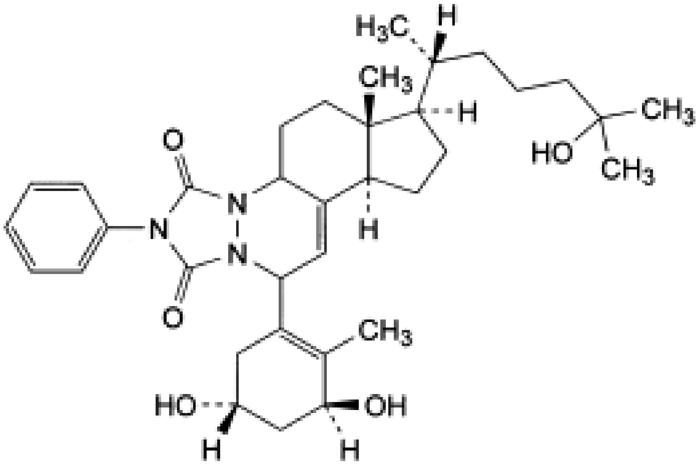
Specified impurities *A, B, C.*



A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1α,3β,25-triol (*trans*-calcitriol),



B. (5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1β,3β,25-triol (1β-calcitriol),



C. (6a*R*,7*R*,9a*R*)-11-[(3*S*,5*R*)-3,5-dihydroxy-2-methylcyclohex-1-enyl]-7-[(1*R*)-5-hydroxy-1,5-dimethylhexyl]-6a-methyl-2-phenyl-5,6,6a,7,8,9,9a,11-octahydro-1*H*,4a*H*-cyclopenta[*i*]1,2,4] triazolo[1,2-a]cinnoline-1,3(2*H*)-dione (triazoline adduct of pre-calcitriol).

Ph Eur

Calcium Acetate

General Notices

(Ph Eur monograph 2128)

$Ca^{2+}\ \left[H_3C-CO_2^-\right]_2$

$C_4H_6CaO_4$ 158.2 *62-54-4*

Action and use

Used in solutions for haemodialysis and peritoneal dialysis.

Ph Eur

DEFINITION

Calcium diacetate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives reaction (b) of calcium *(2.3.1)* .
B. It gives reaction (b) of acetates *(2.3.1)* .

TESTS

pH *(2.2.3)*

7.2 to 8.2.

Dissolve 0.50 g in 10.0 ml of *carbon dioxide-free water R*.

Readily oxidisable substances

Dissolve 2.0 g in boiling *water R* and dilute to 100 ml with boiling *water R*, add a few glass beads, 6 ml of *5 M sulphuric acid* and 0.3 ml of *0.02 M potassium permanganate*, mix, boil gently for 5 min and allow the precipitate to settle. The pink colour in the supernatant is not completely discharged.

Chlorides ***(2.4.4)***

Maximum 330 ppm.

Dissolve 0.15 g in *water R* and dilute to 15 ml with the same solvent.

Nitrates

Dissolve 1.0 g in *water R* and dilute to 10 ml with the same solvent. Add 5 mg of *sodium chloride R*, 0.05 ml of *indigo carmine solution R* and add with stirring, 10 ml of *nitrogen-free sulphuric acid R*. The blue colour remains for at least 10 min.

Sulphates ***(2.4.13)***

Maximum 600 ppm.

Dissolve 0.25 g in *distilled water R* and dilute to 15 ml with the same solvent.

Aluminium ***(2.4.17)***

Maximum 1 ppm.

Test solution Dissolve 4.0 g of the substance to be examined in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml *of aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R.*

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Arsenic ***(2.4.2)***

Maximum 2 ppm.

0.5 g complies with limit test A.

Barium

Maximum 50.0 ppm.

Atomic emission spectrometry *(2.2.22, Method II)*.

Test solution Dissolve 5.00 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *barium standard solution (0.1 per* *cent Ba) R*, diluted as necessary with *water R.*

Wavelength 455.4 nm.

Magnesium

Maximum 5.00×10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *magnesium standard solution (0.1 per cent Mg) R,* diluted as necessary with *water R* .

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Air-acetylene flame.

Potassium

Maximum 0.10 per cent.

Atomic emission spectrometry *(2.2.22, Method II*).

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *potassium standard solution (0.2 per cent K) R* , diluted as necessary with *water R* .

Wavelength 766.7 nm.

Sodium

Maximum 0.50 per cent.

Atomic emission spectrometry *(2.2.22, Method II)*.

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R* , diluted as necessary with *water R* .

Wavelength 589.0 nm.

Strontium

Maximum 5.00×10^2 ppm.

Atomic emission spectrometry *(2.2.22, Method II)*.

Test solution Dissolve 2.00 g of the substance to be examined in *water R* and dilute to

100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *strontium standard solution (1.0 per cent Sr) R*, diluted as necessary with *water R*.

Wavelength 460.7 nm.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Water *(2.5.12)*

Maximum 7.0 per cent, determined on 0.100 g. Add 2 ml of *anhydrous acetic acid R* to the titration vessel in addition to the methanol. Clean the titration vessel after each determination.

ASSAY

Dissolve 0.150 g in 15 ml of *water R*, add 5 ml of *diethylamine R*. Titrate with *0.1 M sodium edetate*, determining the end-point using *methylthymol blue mixture R*.

1 ml of *0.1 M sodium edetate* is equivalent to 15.82 mg of $C_4H_6CaO_4$.

STORAGE

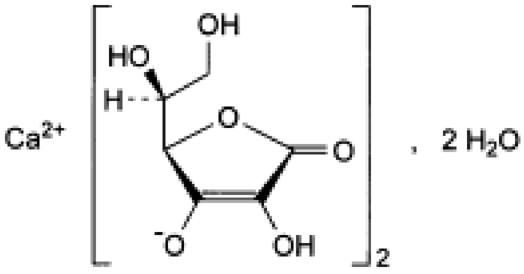
In an airtight container.

Ph Eur

Calcium Ascorbate

General Notices

(Ph Eur monograph 1182)



$C_{12}H_{14}CaO_{12},2H_2O$ 426.3 *5743-28-2*

Action and use

Excipient.

Ph Eur

DEFINITION

Calcium di[(*R*)-2-[(*S*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2*H*-furan-3-olate] dihydrate.

Content

99.0 per cent to 100.5 per cent of $C_{12}H_{14}CaO_{12},2H_2O$.

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, B, E.

Second identification *A, C, D, E.*

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24*).

Comparison *Ph. Eur. reference spectrum of calcium ascorbate.*

C. Dilute 1 ml of solution S (see Tests) to 10 ml with *water R* . To 2 ml of the solution add 0.2 ml of a 100 g/l solution of *ferrous sulphate R* . A deep violet colour develops.

D. To 1 ml of solution S add 0.2 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2* . A grey precipitate is formed.

E. The substance gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 5.00 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II*). Examine the colour of the solution immediately after preparation of the solution.

pH *(2.2.3)*

6.8 to 7.4 for solution S.

Specific optical rotation *(2.2.7)*

+ 95 to + 97 (dried substance), determined using freshly prepared solution S.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Fluorides:

Maximum 10.0 ppm.

Potentiometry *(2.2.36, Method I)*.

Test solution In a 50 ml volumetric flask, dissolve 1.000 g in a 10.3 g/l solution of *hydrochloric acid R* , add 5.0 ml of *fluoride standard solution (1 ppm F) R* and dilute to 50.0 ml with a 10.3 g/l solution of *hydrochloric acid R* . To 20.0 ml of the solution add 20.0 ml of *total-ionic-strength-adjustment buffer R* and 3 ml of an 82 g/l solution of *anhydrous sodium acetate R.* Adjust to pH 5.2 with *ammonia R* and dilute to 50.0 ml with *distilled water R.*

Reference solutions To 0.25 ml, 0.5 ml, 1.0 ml, 2.0 ml and 5.0 ml of *fluoride standard solution (10 ppm F) R* add 20.0 ml of *total-ionic-strength-adjustment buffer R* and dilute to 50.0 ml with *distilled water R.*

Indicator electrode Fluoride selective.

Reference electrode Silver-silver chloride.

Take into account the addition of fluoride to the test solution for the calculation.

Copper

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 2.0 g in a 9.7 g/l solution of *nitric acid R* and dilute to 25.0 ml with the same acid solution.

Reference solutions Prepare the reference solutions using *copper standard solution (10 ppm Cu) R* , diluting with a 9.7 g/l solution of *nitric acid R* .

Source Copper hollow-cathode lamp.

Wavelength 324.8 nm.

Atomisation device Air-acetylene flame.

Iron

Maximum 2.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 5.0 g in a 9.7 g/l solution of *nitric acid R* and dilute to 25.0 ml with the same acid solution.

Reference solutions Prepare the reference solutions using *iron standard solution (10 ppm Fe) R*, diluting with a 9.7 g/l solution of *nitric acid R* .

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2.0 ml of *lead standard solution (10 ppm Pb) R .*

Loss on drying *(2.2.32)*

Maximum 0.1 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

ASSAY

Dissolve 80.0 mg in a mixture of 10 ml of *dilute sulphuric acid R* and 80 ml of *carbon dioxide-free water R* . Add 1 ml of *starch solution R* . Titrate with 0.*05 M iodine* until a persistent violet-blue colour is obtained.

1 ml of 0.*05 M iodine* is equivalent to 10.66 mg of $C_{12}H_{14}CaO_{12},2H_2O$.

STORAGE

In a non-metallic container, protected from light.

Ph Eur

Calcium Carbonate

General Notices

(Ph Eur monograph 0014)

$CaCO_3$ 100.1 *471-34-1*

Action and use

Antacid.

Preparations

Calcium Carbonate Chewable Tablets

Calcium and Colecalciferol Tablets

Ph Eur

DEFINITION

Content

98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water.

IDENTIFICATION

A. It gives the reaction of carbonates *(2.3.1)* .

B. 0.2 ml of solution S (see Tests) gives the reactions of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 5.0 g in 80 ml of *dilute acetic acid R* . When the effervescence ceases, boil for 2

min. Allow to cool, dilute to 100 ml with *dilute acetic acid R* and filter, if necessary, through a sintered-glass filter *(2.1.2)*.

Substances insoluble in acetic acid

Maximum 0.2 per cent.

Wash any residue obtained during the preparation of solution S with 4 quantities, each of 5 ml, of hot *water R* and dry at 100-105 °C for 1 h. The residue weighs a maximum of 10 mg.

Chlorides ***(2.4.4)***

Maximum 330 ppm.

Dilute 3 ml of solution S to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 0.25 per cent.

Dilute 1.2 ml of solution S to 15 ml with *distilled water R*.

Arsenic ***(2.4.2, Method A)***

Maximum 4 ppm, determined on 5 ml of solution S.

Barium

To 10 ml of solution S add 10 ml of *calcium sulphate solution R*. After at least 15 min, any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution S and 10 ml of *distilled water R*.

Iron ***(2.4.9)***

Maximum 200 ppm.

Dissolve 50 mg in 5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*.

Magnesium and alkali metals

Maximum 1.5 per cent.

Dissolve 1.0 g in 12 ml of *dilute hydrochloric acid R*. Boil the solution for about 2 min and add 20 ml of *water R*, 1 g of *ammonium chloride R* and 0.1 ml of *methyl red solution R*. Add *dilute ammonia R1* until the colour of the indicator changes and then add 2 ml in excess. Heat to boiling and add 50 ml of hot *ammonium oxalate solution R*. Allow to stand for 4 h, dilute to 100 ml with *water R* and filter through a suitable filter. To 50 ml of the filtrate add 0.25 ml of *sulphuric acid R*. Evaporate to dryness on a water-bath and ignite to constant mass at 600 ± 50 °C. The residue weighs a maximum of 7.5 mg.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 200 ± 10 °C.

ASSAY

Dissolve 0.150 g in a mixture of 3 ml of *dilute hydrochloric acid R* and 20 ml of *water R*. Boil for 2 min, allow to cool and dilute to 50 ml with *water R*. Carry out the complexometric titration of calcium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 10.01 mg of $CaCO_3$.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.The following characteristics may be relevant for calcium carbonate used as filler in tablets and capsules.

Particle-size distribution ***(2.9.31 or 2.9.38)*****.**

Powder flow ***(2.9.36)*** **.**

Ph Eur

Calcium Chloride Dihydrate

General Notices

(Ph Eur monograph 0015)

$CaCl_2,2H_2O$ 147.0 *10035-04-8*

Preparations

Calcium Chloride Injection

Compound Sodium Lactate Intravenous Infusion

Ph Eur

DEFINITION

Content

97.0 per cent to 103.0 per cent of $CaCl_2,2H_2O$.

CHARACTERS

Appearance

White or almost white, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)* .

B. It gives the reactions of calcium *(2.3.1)* .

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of freshly prepared solution S add 0.1 ml of *phenolphthalein solution R*. If the solution is red, not more than 0.2 ml of *0.01 M hydrochloric acid* is required to discharge the colour and if the solution is colourless, not more than 0.2 ml of *0.01 M sodium hydroxide* is required to turn it red.

Sulphates ***(2.4.13)***

Maximum 300 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Aluminium

To 10 ml of solution S add 2 ml of *ammonium chloride solution R* and 1 ml of *dilute ammonia R1* and boil the solution. No turbidity or precipitate is formed.

If intended for use in the manufacture of dialysis solutions, the above test is replaced by the following test for aluminium *(2.4.17)*: maximum 1 ppm.

Prescribed solution Dissolve 4 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R.*

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R.*

Barium

To 10 ml of solution S add 1 ml of *calcium sulphate solution R*. After at least 15 min, any opalescence in the solution is not more intense than that in a mixture of 1 ml of distilled *water R* and 10 ml of solution S.

Iron ***(2.4.9)***

Maximum 10 ppm, determined on solution S.

Magnesium and alkali metals

Maximum 0.5 per cent.

To a mixture of 20 ml of solution S and 80 ml of *water R* add 2 g of *ammonium chloride R* and 2 ml of *dilute ammonia R1*, heat to boiling and pour into the boiling solution a hot solution of 5 g of *ammonium oxalate R* in 75 ml of *water R*. Allow to stand for 4 h, dilute to 200 ml with *water R* and filter through a suitable filter. To 100 ml of the filtrate add 0.5 ml of *sulphuric acid R*. Evaporate to dryness on a water-bath and ignite to constant mass at 600 ± 50 °C. The residue weighs a maximum of 5 mg.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R* .

ASSAY

Dissolve 0.280 g in 100 ml of *water R* and carry out the complexometric titration of calcium (2.5.11).

1 ml of *0.1 M sodium edetate* is equivalent to 14.70 mg of $CaCl_2,2H_2O$.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

STORAGE

In an airtight container .

Ph Eur

Calcium Chloride Hexahydrate

General Notices

(Ph Eur monograph 0707)

$CaCl_2,6H_2O$ 219.1 *7774-34-7*

Ph Eur

DEFINITION

Content

97.0 per cent to 103.0 per cent of $CaCl_2,6H_2O$.

CHARACTERS

Appearance

White or almost white, crystalline mass or colourless crystals.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

It solidifies at about 29 °C.

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)* .

B. It gives the reactions of calcium *(2.3.1)* .

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 15.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of freshly prepared solution S add 0.1 ml of *phenolphthalein solution R*. If the solution is red, not more than 0.2 ml of *0.01 M hydrochloric acid* is required to discharge the colour and if the solution is colourless, not more than 0.2 ml of *0.01 M sodium hydroxide* is required to turn it red.

Sulphates ***(2.4.13)***

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Aluminium

To 10 ml of solution S add 2 ml of *ammonium chloride solution R* and 1 ml of *dilute ammonia R1*. Heat to boiling. No turbidity or precipitate is formed.

If intended for use in the manufacture of dialysis solutions, the above test is replaced by the following test for aluminium (*2.4.17*): maximum 1 ppm.

Prescribed solution Dissolve 6 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R.*

Barium

To 10 ml of solution S add 1 ml of *calcium sulphate solution R*. After at least 15 min, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Iron ***(2.4.9)***

Maximum 7 ppm, determined on solution S.

Magnesium and alkali metals

Maximum 0.3 per cent.

To a mixture of 20 ml of solution S and 80 ml of *water R* add 2 g of *ammonium chloride R* and 2 ml of *dilute ammonia R1*, heat to boiling and pour into the boiling solution a hot solution of 5 g of *ammonium oxalate R* in 75 ml of *water R*. Allow to stand for 4 h, dilute to 200 ml with *water R* and filter through a suitable filter. To 100 ml of the filtrate add 0.5 ml of *sulphuric acid R*. Evaporate to dryness on a water-bath and ignite to constant mass at 600 ± 50 °C. The residue weighs a maximum of 5 mg.

Heavy metals ***(2.4.8)***

Maximum 15 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard*

solution (2 ppm Pb) R.

ASSAY

Dissolve 0.200 g in 100 ml of *water R*. Carry out the complexometric titration of calcium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 21.91 mg of $CaCl_2,6H_2O$.

LABELLING

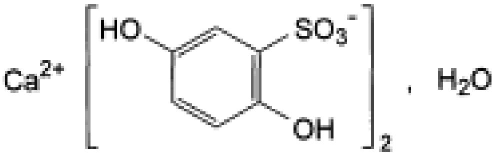
The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

Ph Eur

Calcium Dobesilate Monohydrate

General Notices

(Ph Eur monograph 1183)



$C_{12}H_{10}CaO_{10}S_2,H_2O$ 436.4 *20123-80-2*
(anhydrous)

Ph Eur

DEFINITION

Calcium di(2,5-dihydroxybenzenesulphonate) monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Very soluble in water, freely soluble in anhydrous ethanol, very slightly soluble in 2-propanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 0.100 g in *water R* and dilute to 200.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R* .

Spectral range 210-350 nm.

Absorption maxima At 221 nm and 301 nm.

Specific absorbance at the absorption maximum at 301 nm 174 to 181.

B. Mix 1 ml of *ferric chloride solution R2* , 1 ml of a freshly prepared 10 g/l solution of *potassium ferricyanide R* and 0.1 ml of *nitric acid R* . To this mixture add 5 ml of freshly prepared solution S (see Tests): a blue colour and a precipitate are immediately produced.

C. 2 ml of freshly prepared solution S gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S, when freshly prepared, is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH ***(2.2.3)***

4.5 to 6.0 for solution S.

Related substances

Liquid chromatography *(2.2.29)* . *Keep all solutions at 2-8 °C*.

Buffer solution Dissolve 1.2 g of *anhydrous sodium dihydrogen phosphate R* in 900 ml of *water for chromatography R* , adjust to pH 6.5 with *disodium hydrogen phosphate solution R* and dilute to 1000 ml with *water for chromatography R* .

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *water R* . Dilute 1.0 ml of this solution to 10.0 ml with *water R* .

Reference solution (b) Dissolve 10 mg of the substance to be examined and 10 mg of *hydroquinone R* (impurity A) in *water R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 100 ml with *water R* .

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R1* , buffer solution (10:90 *V/V*).

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Run time 2.5 times the retention time of dobesilate.

Relative retention With reference to dobesilate (retention time = about 6 min): impurity A =

about 1.7.

System suitability Reference solution (b):

— *resolution*: minimum 8.0 between the peaks due to dobesilate and impurity A.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.6;

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 15 ppm.

1.0 g complies with test C. Prepare the reference solution using 1.5 ml of *lead standard solution (10 ppm Pb) R* .

Iron *(2.4.9)*

Maximum 10 ppm, determined on 10 ml of solution S.

Water *(2.5.12)*

4.0 per cent to 6.0 per cent, determined on 0.500 g.

ASSAY

Dissolve 0.200 g in a mixture of 10 ml of *water R* and 40 ml of *dilute sulphuric acid R* . Titrate with *0.1 M cerium sulphate* , determining the end-point potentiometrically *(2.2.20)* .

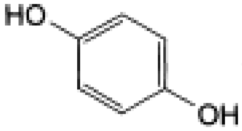
1 ml of *0.1 M cerium sulphate* is equivalent to 10.45 mg of $C_{12}H_{10}CaO_{10}S_2$.

STORAGE

In an airtight container , protected from light.

IMPURITIES

Specified impurities A.



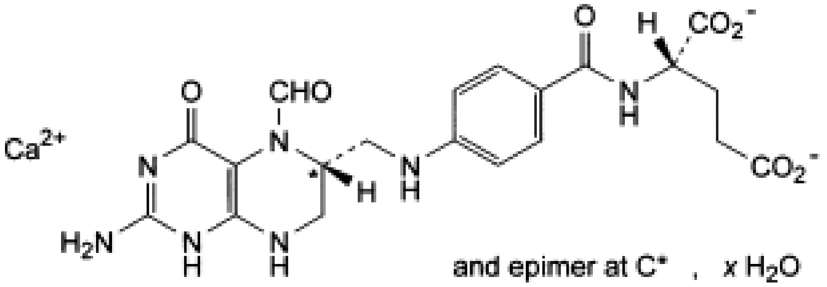
A. benzene-1,4-diol (hydroquinone).

Ph Eur

Calcium Folinate

General Notices

(Ph Eur monograph 0978)



$C_{20}H_{21}CaN_7O_7,xH_2O$ 511.5 *1492-18-8*
(anhydrous*) (anhydrous)*

Action and use

Antidote to folic acid antagonists.

Preparations

Calcium Folinate Injection

Calcium Folinate Tablets

Ph Eur

DEFINITION

Calcium (2*S*)-2-[[4-[[[(6*RS*)-2-amino-5-formyl-4-oxo1,4,5,6,7,8-hexahydropteridin-6-yl]methyl] amino]benzoyl]amino]pentanedioate.

Content:

— *calcium folinate* ($C_{20}H_21CaN_7O_7$): 97.0 per cent to 102.0 per cent (anhydrous substance);

— *calcium* (Ca; *A* $_r$ 40.08): 7.54 per cent to 8.14 per cent (anhydrous substance).

It contains a variable amount of water.

CHARACTERS

Appearance

White or light yellow, amorphous or crystalline powder.

Solubility

Sparingly soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

The amorphous form may produce supersaturated solutions in water.

IDENTIFICATION

First identification *A, B, D.*

Second identification *A, C, D.*

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *calcium folinate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R* and add dropwise sufficient *acetone R* to produce a precipitate. Allow to stand for 15 min, collect the precipitate by centrifugation, wash the precipitate with 2 small quantities of *acetone R* and dry. Record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 15 mg of the substance to be examined in a 3 per cent *V/V* solution of *ammonia R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 15 mg of *calcium folinate CRS* in a 3 per cent *V/V* solution of *ammonia R* and dilute to 5 ml with the same solvent.

Plate *cellulose for chromatography* F_{254} *R* as the coating substance.

Mobile phase The lower layer of a mixture of 1 volume of *isoamyl alcohol R* and 10 volumes of a 50 g/l solution of *citric acid R* previously adjusted to pH 8 with *ammonia R* .

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (b) of calcium *(2.3.1)* .

Carry out the tests and the assay as rapidly as possible, protected from actinic light.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R*, heating at 40 °C if necessary, and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 420 nm is not greater than 0.60. Use *water R* as the compensation liquid.

pH *(2.2.3)*

6.8 to 8.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 14.4 to + 18.0 (anhydrous substance), determined on solution S.

Acetone, ethanol and methanol

Head-space gas chromatography *(2.2.28)* : use the standard additions method.

Test solution Dissolve 0.25 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution Dilute 0.125 g of *acetone R*, 0.750 g of *anhydrous ethanol R* and 0.125 g of *methanol R* in *water R* and dilute to 1000.0 ml with *water R*.

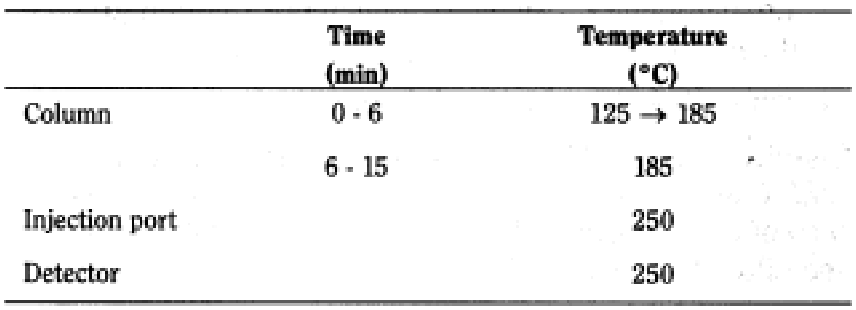
Column:
- — *material*: fused silica;
- — *size*: *l* = 10 m, Ø = 0.32 mm;
- — *stationary phase: styrene-divinylbenzene copolymer R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 4 ml/min.

Static head-space conditions that may be used:
- — *equilibration temperature*: 80 °C;
- — *equilibration time*: 20 min;
- — *pressurisation time*: 30 s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	125 → 185
	6 - 15	185
Injection port		250
Detector		250

Detection Flame ionisation.

Injection At least 3 times.

Limits:
- — *acetone* : maximum 0.5 per cent;
- — *ethanol* : maximum 3.0 per cent;
- — *methanol* : maximum 0.5 per cent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 10.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *calcium folinate CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water R.*

Reference solution (c) Dissolve 10.0 mg of *formylfolic acid CRS* (impurity D) in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with *water* R.

Reference solution (d) Dilute 1.0 ml of reference solution (b) to 10.0 ml with *water R* .

Reference solution (e) Dilute 5.0 ml of reference solution (c) to 10.0 ml with reference solution (b).

Column:
- — *size*: *l* = 0.25 m, Ø = 4 mm;
- — *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 40 °C.

Mobile phase Mix 220 ml of *methanol R* and 780 ml of a solution containing 2.0 ml of *tetrabutylammonium hydroxide solution (400 g/l) R* and *2.2 g of disodium hydrogen phosphate R* , previously adjusted to pH 7.8 with *phosphoric acid R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µl of the test solution and reference solutions (b), (c), (d) and (e).

Run time 2.5 times the retention time of folinate.

System suitability Reference solution (e):

— *resolution*: minimum 2.2 between the peaks due to folinate and impurity D.

Limits:

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent);

— *impurities A, B, C, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *sum of impurities other than D*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Chlorides ***(2.4.4)***

Maximum 0.5 per cent.

Dissolve 67 mg in 10 ml of *water R* and add 3 ml of *acetic acid R* . Filter and wash the precipitate with 5 quantities, each of 5 ml, of *water R* . Collect the filtrate and washings and dilute to 100 ml with *water R* .

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

1.0 g complies with test F. Prepare the reference solution using 5 ml of *lead standard solution (10 ppm Pb) R.*

Platinum

Maximum 20.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *platinum standard solution (30 ppm Pt) R*, diluted as necessary with a mixture of 1 volume of *nitric acid R* and 99 volumes of *water R* .

Source Platinum hollow-cathode lamp.

Wavelength 265.9 nm.

Water ***(2.5.12)***

Maximum 17.0 per cent, determined on 0.200 g (ground to a very fine powder). Stir the substance to be examined in the titration solvent for about 6 min before titrating and use a suitable titrant that does not contain pyridine.

Bacterial endotoxins ***(2.6.14)***

Less than 0.5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Calcium

Dissolve 0.400 g in 150 ml of *water R* and dilute to 300 ml with the same solvent. Carry out the complexometric titration of calcium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

Calcium folinate

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

System suitability:

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (a).

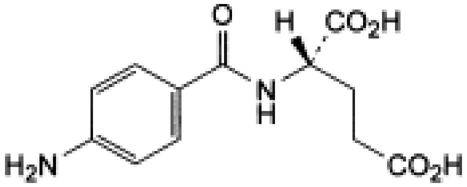
Calculate the percentage content of $C_{20}H_{21}CaN_7O_7$ from the declared content of *calcium folinate CRS* .

STORAGE

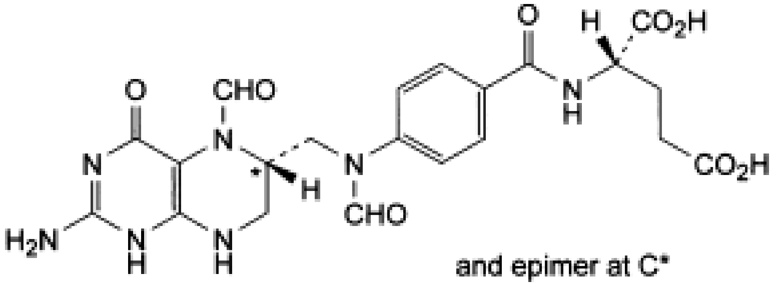
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities A, B, C, D, E, F, G.

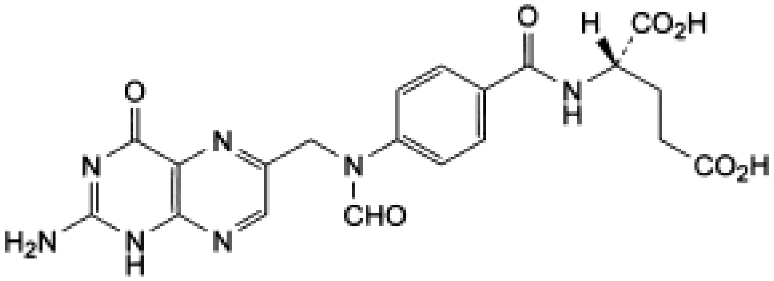


A. (2*S*)-2[(4-aminobenzoyl)amino]pentanedioic acid,

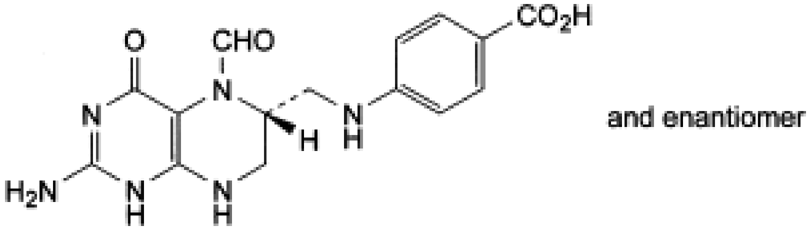


B. (2*S*)-2-[[4-[[[(6*RS*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl] formylamino]benzoyl]amino]pentanedioic acid (5,10-diformyltetrahydrofolic acid),

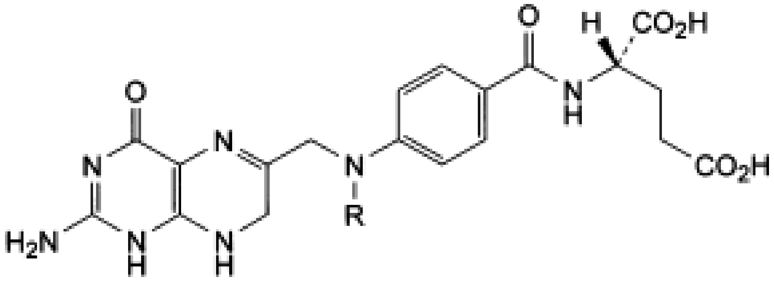
C. folic acid,



D. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]formylamino]benzoyl]amino] pentanedioic acid (10-formylfolic acid),



E. 4-[[[(6*RS*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]amino] benzoic acid (5-formyltetrahydropteroic acid),



F. R = CHO: (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]formylamino] benzoyl]amino]pentanedioic acid (10-formyldihydrofolic acid),

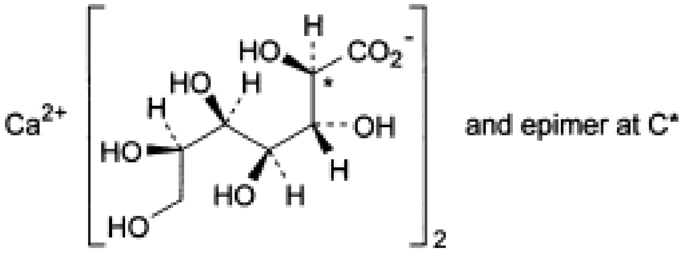
G. R = H: (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]amino]benzoyl] amino]pentanedioic acid (dihydrofolic acid).

Ph Eur

Calcium Glucoheptonate

General Notices

(Ph Eur monograph 1399)



$C_{14}H_{26}CaO_{16}$ 490.4 *29039-00-7*

Action and use

Used in treatment of calcium deficiency.

Ph Eur

DEFINITION

Mixture in variable proportions, of calcium di(D-*glycero*-D-*gulo*-heptonate) and calcium di(D-*glycero*-D-*ido*-heptonate).

Content

98.0 per cent to 102.0 per cent of calcium 2,3,4,5,6,7-hexahydroxyheptanoate (dried substance).

CHARACTERS

Appearance

White or very slightly yellow, amorphous powder, hygroscopic.

Solubility

Very soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 20 mg of the substance to be examined in 1 ml of *water R* .

Reference solution (a) Dissolve 20 mg of *calcium glucoheptonate CRS* in 1 ml of *water R.*

Reference solution (b) Dissolve 10 mg of *calcium gluconate CRS* in 0.5 ml of the test solution and dilute to 1 ml with *water R* .

Plate *cellulose for chromatography R1* as the coating substance.

Mobile phase A freshly prepared mixture of 20 volumes of *anhydrous formic acid R* , 20 volumes of *water R* , 30 volumes of *acetone R* and 30 volumes of *butanol R* .

Application 10 µl as bands of 20 mm by 2 mm.

Development In a tank previously allowed to saturate for 10 min, over a path of 12 cm.

Drying In air.

Detection Spray with *0.02 M potassium permanganate* .

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. 0.2 ml of (see Tests) gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution.

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

6.0 to 8.0 for solution S.

Reducing sugars

Maximum 1 per cent, expressed as glucose.

Dissolve 1.0 g in 5 ml of *water R* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine* . With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* until the precipitate dissolves, titrate the excess of iodine with *0.05 M sodium thiosulphate* using *1 ml of starch solution R* added towards the end of the titration, as indicator. Not less than 12.6 ml of *0.05 M sodium thiosulphate* is required.

Cyanide

Dissolve 5.0 g in 50 ml of *water R* and add 2.0 g of *tartaric acid R*. Place this solution in a distillation apparatus (2.2.11). The plain bend adapter attached to the end of the condenser has a vertical part that is long enough to extend to 1 cm from the bottom of a 50 ml test-tube used as a receiver. Place 10 ml of *water R* and 2 ml of *0.1 M sodium hydroxide* into the receiver. Distil, collect 25 ml of distillate and dilute to 50 ml with *water R*. To 25 ml of this solution add 25 mg of *ferrous sulphate R* and boil for a short time. After cooling to about 70 °C add 10 ml of *hydrochloric acid R1*. After 30 min, filter the solution and wash the filter. A yellow spot appears on the filter; there is no blue or green spot.

Chlorides ***(2.4.4)***

Maximum 100 ppm.

To 5 ml of solution S, add 10 ml of *water R*.

Sulphates ***(2.4.13)***

Maximum 100 ppm, determined on solution S.

Iron ***(2.4.9)***

Maximum 40 ppm.

Dilute 2.5 ml of to 10 ml with *water R.*

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 2.0 g in 10 ml of *buffer solution pH 3.5 R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Bacterial endotoxins ***(2.6.14)***

Less than 167 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.800 g in a mixture of 150 ml of *water R* and 2 ml of *3 M hydrochloric acid R*. While stirring, add 12.5 ml of *0.1 M sodium edetate*, 15 ml of *1 M sodium hydroxide* and 0.3 g of *hydroxynaphthol blue*, *sodium salt R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

1 ml of *0.1 M sodium edetate* is equivalent to 49.04 mg of $C_{14}H_{26C}aO_{16}$.

STORAGE

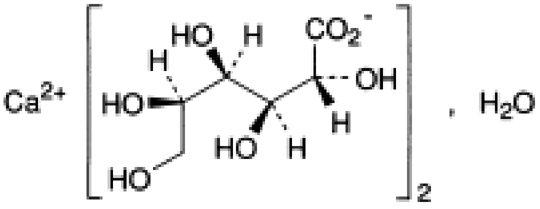
In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

Ph Eur

Calcium Gluconate for Injection

General Notices

(Ph Eur monograph 0979)



$C_{12}H_{22}CaO_{14},H_2O$ 448.4 *18016-24-5*

Action and use

Used in treatment of calcium deficiency.

Preparation

Calcium Gluconate Injection

Ph Eur

DEFINITION

Calcium D-gluconate monohydrate.

Content

99.0 per cent to 101.0 per cent of $C_{12}H_{22}CaO_{14},H_{2O}$.

CHARACTERS

Appearance

White or almost white, crystalline or granular powder.

Solubility

Sparingly soluble in water, freely soluble in boiling water.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 20 mg of the substance to be examined in 1 ml of *water R* , heating if necessary in a water-bath at 60 °C.

Reference solution Dissolve 20 mg of *calcium gluconate CRS* in 1 ml of *water R,* heating if necessary in a water-bath at 60 °C.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, ethyl acetate R, water R, ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying At 100 °C for 20 min and allow to cool.

Detection Spray with a 50 g/l solution of *potassium dichromate R* in a 40 per cent *m/m* solution of *sulphuric acid R.*

Results After 5 min, the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. About 20 mg gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

To 10.0 g add 90 ml of boiling *distilled water R* and boil with stirring, for not more than 10 s, until completely dissolved, then dilute to 100.0 ml with the same solvent.

Appearance of solution

At 60 °C, solution S is not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*. After cooling to 20 °C, it is not more opalescent than reference suspension II *(2.2.1)* .

pH ***(2.2.3)***

6.4 to 8.3.

Dissolve 1.0 g in 20 ml of *carbon dioxide-free water R* , heating on a water-bath.

Organic impurities and boric acid

Introduce 0.5 g into a porcelain dish previously rinsed with *sulphuric acid R* and placed in a bath of iced water. Add 2 ml of cooled *sulphuric acid R* and mix. No yellow or brown colour develops. Add 1 ml of *chromotrope II B solution R* . A violet colour develops and does not become dark blue. The solution is not more intensely coloured than that of a mixture of 1 ml of *chromotrope II B solution R* and 2 ml of cooled *sulphuric acid R* .

Oxalates

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 1.00 g of the substance to be examined in *water for chromatography R* and dilute to 100.0 ml with the same solvent.

Reference solution Dissolve 1.00 g of the substance to be examined in *water for chromatography R,* add 0.5 ml of a 0.152 g/l solution of *sodium oxalate R* in water for *chromatography R* and dilute to 100.0 ml with the same solvent.

Guard column:
- *size*: *l* = 30 mm, Ø = 4 mm;
- *stationary phase*: suitable strong anion exchange resin (30-50 µm).

Columns 1 and 2:
- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: suitable strong anion exchange resin (30-50 µm).

Anion-suppresser column Connected in series with the guard and analytical columns and equipped with a micromembrane that separates the mobile phase from the suppressor regeneration solution, flowing countercurrent to the mobile phase.

Mobile phase Dissolve 0.212 g of *anhydrous sodium carbonate R* and 63 mg of *sodium hydrogen carbonate R* in *water for chromatography R* and dilute to 1000.0 ml with the same solvent.

Flow rate of the mobile phase 2 ml/min.

Suppressor regeneration solution 1.23 g/l solution of *sulphuric acid R* in *water for chromatography R* .

Flow rate of the suppressor regeneration solution 4 ml/min.

Detection Conductance.

Injection 50 µl.

System suitability Reference solution:

- *repeatability*: maximum relative standard deviation of the area of the peak due to oxalate of 2.0 per cent after 5 injections.

Inject 50 µl of each solution 3 times. Calculate the content of oxalates in parts per million using the following expression:

$$\frac{S_T \times 50}{S_R - S_T}$$

S_T = area of the peak due to oxalate in the chromatogram obtained with the test solution,

S_R = area of the peak due to oxalate in the chromatogram obtained with the reference solution.

Limit:
- *oxalates*: maximum 100 ppm.

Sucrose and reducing sugars

Dissolve 0.5 g in a mixture of 2 ml of *hydrochloric acid R1* and 10 ml of *water R*. Boil for 5 min, allow to cool, add 10 ml of *sodium carbonate solution R* and allow to stand for 10 min. Dilute to 25 ml with *water R* and filter. To 5 ml of the filtrate add 2 ml of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 2 min. No red precipitate is formed.

Chlorides ***(2.4.4)***

Maximum 50 ppm.

To 10 ml of previously filtered solution S add 5 ml of *water R.*

Phosphates ***(2.4.11)***

Maximum 100 ppm.

Dilute 1 ml of solution S to 100 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 50 ppm, determined on previously filtered solution S.

Prepare the standard using a mixture of 7.5 ml of *sulphate standard solution (10 ppm SO4) R* and 7.5 ml of *distilled water R*.

Iron

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Introduce 2.0 g into a 100 ml polytetrafluoroethylene beaker and add 5 ml of *nitric acid R*. Boil, evaporating almost to dryness. Add 1 ml of *strong hydrogen peroxide solution R* and evaporate again almost to dryness. Repeat the hydrogen peroxide treatment until a clear solution is obtained. Using 2 ml of *nitric acid R*, transfer the solution into a 25 ml volumetric flask. Dilute to 25.0 ml with *dilute hydrochloric acid R*. In the same manner, prepare a compensation solution using 0.65 g of *calcium chloride R1* instead of the substance to be examined.

Reference solutions Prepare the reference solutions from *iron solution (20 ppm Fe) R* diluted with *dilute hydrochloric acid R.*

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Carry out a basic correction using a deuterium lamp.

Magnesium and alkali metals

Maximum 0.4 per cent.

To 0.50 g add a mixture of 1.0 ml of *dilute acetic acid R* and 10.0 ml of *water R* and rapidly boil, whilst shaking, until completely dissolved. To the boiling solution add 5.0 ml of *ammonium oxalate solution R* and allow to stand for at least 6 h. Filter through a sintered-glass filter (1.6) *(2.1.2)* into a porcelain crucible. Carefully evaporate the filtrate to dryness and ignite. The residue weighs not more than 2 mg.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Bacterial endotoxins *(2.6.14)*

Less than 167 IU/g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 102 micro-organisms per gram, determined by plate count. It complies with the tests for *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus (2.6.13)*.

ASSAY

Dissolve 0.350 g in 20 ml of hot *water R*, allow to cool and dilute to 300 ml with *water R*. Carry out the complexometric titration of calcium *(2.5.11)*. Use 50 mg of *calconecarboxylic acid triturate R.*

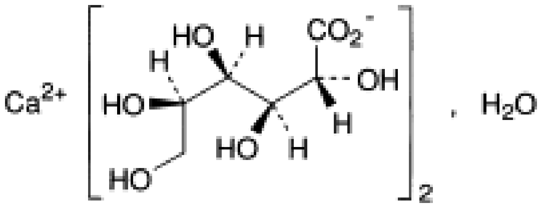
1 ml of *0.1 M sodium edetate* is equivalent to 44.84 mg of $C_{12}H_{22}CaO_{14},H_{2O}$.

Ph Eur

Calcium Gluconate

General Notices

(Ph Eur monograph 0172)



$C_{12}H_{22}CaO_{14},H_2O$ 448.4 *18016-24-5*

Action and use

Used in treatment of calcium deficiency.

Preparations

Calcium Gluconate Tablets
Effervescent Calcium Gluconate Tablets

Ph Eur

DEFINITION

Calcium D-gluconate monohydrate.

Content

98.5 per cent to 102.0 per cent of $C_{12}H_{22}CaO_{14},H_2O$.

CHARACTERS

Appearance

White or almost white, crystalline or granular powder.

Solubility

Sparingly soluble in water, freely soluble in boiling water.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 20 mg of the substance to be examined in 1 ml of *water R* , heating if necessary in a water-bath at 60 °C.

Reference solution Dissolve 20 mg of *calcium gluconate CRS* in 1 ml of *water R* , heating if necessary in a water-bath at 60 °C.

Plate *TLC silica gel G plate R* .

Mobile phase *ethyl acetate R, concentrated ammonia R, water R, ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying At 100 °C for 20 min. Allow to cool.

Detection Spray with a 50 g/l solution of *potassium dichromate R* in a 40 per cent *m/m* solution of *sulphuric acid R.*

Results After 5 min, the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Solution S (see Tests) gives the reactions of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 1.0 g in *water R* heated to 60 °C and dilute to 50 ml with the same solvent.

Appearance of solution

At 60 °C, solution S is not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*. After cooling, it is not more opalescent than reference suspension II *(2.2.1)* .

Organic impurities and boric acid

Introduce 0.5 g into a porcelain dish previously rinsed with *sulphuric acid R* and placed in a bath of iced water. Add 2 ml of cooled *sulphuric acid R* and mix. No yellow or brown colour develops. Add *1 ml of chromotrope II B solution R* . A violet colour develops and does not become dark blue. The solution is not more intensely coloured than that of a mixture of *1 ml of chromotrope II B solution R* and 2 ml of cooled *sulphuric acid R.*

Sucrose and reducing sugars

Dissolve 0.5 g in a mixture of 2 ml of *hydrochloric acid R1* and 10 ml of *water R* . Boil for 5 min, allow to cool, add 10 ml of *sodium carbonate solution R* and allow to stand. Dilute to 25 ml with *water R* and filter. To 5 ml of the filtrate add 2 ml of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 2 min. No red precipitate is formed.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 12.5 ml of solution S to 15 ml with *water R.*

Sulphates ***(2.4.13)***

Maximum 100 ppm.

Dissolve 10.0 g with heating in a mixture of 10 ml of *acetic acid R* and 90 ml of *distilled water R .*

Magnesium and alkali metals

Maximum 0.4 per cent.

Dissolve 1.00 g in 100 ml of *boiling water R* , add 10 ml *of ammonium chloride solution R* , 1 ml of *ammonia R* and, dropwise, 50 ml of hot *ammonium oxalate solution R* . Allow to stand for 4 h, dilute to 200 ml with *water R* and filter. Evaporate 100 ml of the filtrate to dryness and ignite. The residue weighs a maximum of 2 mg.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test D. Heat the substance to be examined gradually and with care until it is almost completely transformed into a white mass and then ignite. Prepare the reference solution using 2 ml *of lead standard solution (10 ppm Pb) R.*

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 10^3 micro-organisms per gram, determined by plate count.

ASSAY

Dissolve 0.8000 g in 20 ml of hot *water R* , allow to cool and dilute to 300 ml with *water R* . Carry out the complexometric titration of calcium *(2.5.11)* .

1 ml of *0.1 M sodium edetate* is equivalent to 44.84 mg of $C_{12}H_{22}CaO_{14},H_{2O}$.

Ph Eur

Calcium Glycerophosphate

General Notices

(Ph Eur monograph 0980)

$C_3H_7CaO_6P$ 210.1 *27214-00-2*

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture in variable proportions of the calcium salt of (*RS*)-2,3-dihydroxypropyl phosphate and of 2-hydroxy-1-(hydroxymethyl)ethyl phosphate which may be hydrated.

Content

18.6 per cent to 19.4 per cent of Ca (dried substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Sparingly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Mix 1 g with *1 g of potassium hydrogen sulphate R* in a test tube fitted with a glass tube. Heat strongly and direct the white vapour towards a piece of filter paper impregnated with a freshly prepared 10 g/l solution of *sodium nitroprusside R* . The filter paper develops a blue colour in contact with *piperidine R.*

B. Ignite 0.1 g in a crucible. Take up the residue with 5 ml of *nitric acid R* and heat on a water-bath for 1 min. Filter. The filtrate gives reaction (b) of phosphates *(2.3.1)*.

C. It gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 1.5 g at room temperature in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 150 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* .

Acidity or alkalinity

To 100 ml of solution S add *0.1 ml of phenolphthalein solution R* . Not more than 1.5 ml of *0.1 M hydrochloric acid* or 0.5 ml *of 0.1 M sodium hydroxide* is required to change the colour of the indicator.

Citric acid

Shake 5.0 g with 20 ml of *carbon dioxide-free water R* and filter. To the filtrate add 0.15 ml of *sulphuric acid R* and filter again. To the filtrate add 5 ml of *mercuric sulphate solution R* and heat to boiling. Add 0.5 ml of a 3.2 g/l solution of *potassium permanganate R* and again heat to boiling. No precipitate is formed.

Glycerol and ethanol (96 per cent)-soluble substances

Maximum 0.5 per cent.

Shake 1.000 g with 25 ml of *ethanol (96 per cent) R* for 1 min. Filter. Evaporate the filtrate on a water-bath and dry the residue at 70 °C for 1 h. The residue weighs a maximum of 5 mg.

Chlorides ***(2.4.4)***

Maximum 500 ppm.

Dissolve 0.1 g in a mixture of 2 ml of *acetic acid R* and 8 ml of *water R* and dilute to 15 ml with *water R.*

Phosphates ***(2.4.11)***

Maximum 400 ppm.

Dilute 2.5 ml of solution S to 100 ml with *water R.*

Sulphates ***(2.4.13)***

Maximum 0.1 per cent, determined on solution S.

Arsenic ***(2.4.2, Method A)***

Maximum 3 ppm.

Dissolve 0.33 g in *water R* and dilute to 25 ml with the same solvent.

Iron ***(2.4.9)***

Maximum 50 ppm, detemined on 0.20 g.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

Dissolve 2.0 g in 10 ml of *buffer solution pH 3.5 R* and dilute to 20 ml with *water R* . 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R* .

Loss on drying ***(2.2.32)***

Maximum 12.0 per cent, determined on 1.000 g by drying in an oven at 150 °C for 4 h.

ASSAY

Dissolve 0.200 g in *water R.* Carry out the complexometric titration of calcium *(2.5.11)* .

1 ml of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

Ph Eur

Calcium Hydrogen Phosphate

General Notices

Dibasic Calcium Phosphate

(Calcium Hydrogen Phosphate Dihydrate, Ph Eur monograph 0116)

$CaHPO_4,2H_2O$ 172.1 *7789-77-7*

Ph Eur

DEFINITION

Content

98.0 per cent to 105.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in cold water and in ethanol (96 per cent). It dissolves in dilute hydrochloric acid and in dilute nitric acid.

IDENTIFICATION

A. Dissolve with heating 0.1 g in 10 ml of *dilute hydrochloric acid R*. Add 2.5 ml of *dilute ammonia R1*, shake, and add 5 ml of a 35 g/l solution of *ammonium oxalate R*. A white precipitate is produced.

B. Dissolve 0.1 g in 5 ml of *dilute nitric acid R*, add 2 ml of *ammonium molybdate solution R* and heat at 70 °C for 2 min. A yellow precipitate is produced.

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 2.5 g in 20 ml of *dilute hydrochloric acid R*, filter if necessary and add *dilute ammonia R1* until a precipitate is formed. Add just sufficient *dilute hydrochloric acid R* to dissolve the precipitate and dilute to 50 ml with *distilled water R.*

Acid-insoluble substances

Maximum 0.2 per cent.

Dissolve 5.0 g in 40 ml of *water R,* add 10 ml of *hydrochloric acid R* and heat to boiling for 5 min. Cool, then collect the insoluble substances using ashless filter paper. Wash with *water R* until turbidity is no longer produced when *silver nitrate solution R2* is added to the filtrate. Ignite at 600 ± 50 °C. The residue weighs not more than 10 mg.

Carbonates

Shake 0.5 g with 5 ml of *carbon dioxide-free water R* and add 1 ml of *hydrochloric acid R* . No effervescence is produced.

Chlorides

Maximum 0.25 per cent.

Test solution Dissolve 0.20 g in a mixture of 20 ml of *water R* and 13 ml of *dilute nitric acid R* , dilute to 100 ml with *water R* and filter if necessary. Use 50 ml of this solution.

Reference solution To 0.70 ml of *0.01 M hydrochloric acid*, add 6 ml of *dilute nitric acid R* and dilute to 50 ml with *water R* .

Add 1 ml of *silver nitrate solution R2* to the test solution and to the reference solution and mix. After standing for 5 min protected from light, any opalescence in the test solution is not more intense than that in the reference solution.

Fluorides

Maximum 100 ppm.

Potentiometry *(2.2.36, Method II)*.

Chelating solution Dissolve 45 g of *cyclohexylenedinitrilotetra-acetic acid R* in 75 ml of *sodium hydroxide solution R* and dilute to 250 ml with *water R* .

Test solution Dissolve 1.000 g in 4 ml of *hydrochloric acid R1,* add 20 ml of chelating solution, 2.7 ml of *glacial acetic acid R* and 2.8 g of *sodium chloride R* , adjust to pH 5-6 with *sodium hydroxide solution R* and dilute to 50.0 ml with *water R* .

Reference solution Dissolve 4.42 g of *sodium fluoride R* , previously dried at 300 °C for 12 h, in *water R* and dilute to 1000.0 ml with the same solvent. Dilute 50.0 ml of this solution to 500.0 ml with *total-ionic-strength-adjustment buffer R* (200 ppm F).

Indicator electrode Fluoride-selective.

Reference electrode Silver-silver chloride.

Carry out the measurement on 20.0 ml of the test solution. Add at least 3 times 0.10 ml of the reference solution and carry out the measurement after each addition. Calculate the concentration of fluorides using the calibration curve.

Sulphates

Maximum 0.5 per cent.

Test solution Dissolve 0.5 g in a mixture of 5 ml of *water R* and 5 ml of *dilute hydrochloric acid R* and dilute to 100 ml with *water R*. Filter if necessary. To 20 ml of this solution, add 1 ml of *dilute hydrochloric acid R* and dilute to 50 ml with *water R*.

Reference solution To 1.0 ml of *0.005 M sulphuric acid*, add 1 ml of *dilute hydrochloric acid R* and dilute to 50 ml with *water R*. Filter if necessary.

To the test solution and to the reference solution, add 2 ml of a 120 g/l solution of *barium chloride R* and allow to stand for 10 min. Any opalescence in the test solution is not more intense than that in the reference solution.

Arsenic ***(2.4.2, Method A)***

Maximum 10 ppm, determined on 2 ml of solution S.

Barium

To 0.5 g, add 10 ml of *water R* and heat to boiling. While stirring, add 1 ml of *hydrochloric acid R* dropwise. Allow to cool and filter if necessary. Add 2 ml of a 10 g/l solution of *dipotassium sulphate R* and allow to stand for 10 min. No turbidity is produced.

Iron ***(2.4.9)***

Maximum 400 ppm.

Dilute 0.5 ml of solution S to 10 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 40 ppm.

Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

24.5 per cent to 26.5 per cent, determined on 1.000 g by ignition to constant mass at 800-825 °C.

ASSAY

Dissolve 0.4 g in 12 ml of *dilute hydrochloric acid R* and dilute to 200 ml with *water R*. To 20.0 ml of this solution add 25.0 ml of *0.02 M sodium edetate*, 50 ml of *water R*, 5 ml of *ammonium chloride buffer solution pH 10.7 R* and about 25 mg of *mordant black 11 triturate R*. Titrate the excess of sodium edetate with *0.02 M zinc sulphate*. Carry out a blank titration.

1 ml of *0.02 M sodium edetate* is equivalent to 3.44 mg of $CaHPO_4,2H_2O$.

Ph Eur

Calcium Hydroxide

General Notices

(Ph Eur monograph 1078)

$Ca(OH)_2$ 74.1 *1305-62-0*

Preparation

Calcium Hydroxide Solution

Ph Eur

DEFINITION

Content

95.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, fine powder.

Solubility

Practically insoluble in water.

IDENTIFICATION

A. To 0.80 g in a mortar, add 10 ml of *water R* and 0.5 ml of *phenolphthalein solution R* and mix. The suspension turns red. On addition of 17.5 ml of *1 M hydrochloric acid*, the suspension becomes colourless without effervescing. The red colour occurs again when the mixture is triturated for 1 min. On addition of a further 6 ml of *1 M hydrochloric acid* and triturating, the solution becomes colourless.

B. Dissolve about 0.1 g in *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. 5 ml of the solution give reaction (b) of calcium *(2.3.1)*.

TESTS

Matter insoluble in hydrochloric acid

Maximum 0.5 per cent.

Dissolve 2.0 g in 30 ml of *hydrochloric acid R*. Boil the solution and filter. Wash the residue with hot *water R*. The residue weighs a maximum of 10 mg.

Carbonates

Maximum 5.0 per cent of $CaCO_3$.

Add 5.0 ml of *1 M hydrochloric acid* to the titrated solution obtained under Assay and titrate with *1 M sodium hydroxide* using 0.5 ml of *methyl orange solution R* as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 50.05 mg of $CaCO_3$.

Chlorides ***(2.4.4)***

Maximum 330 ppm.

Dissolve 0.30 g in a mixture of 2 ml of *nitric acid R* and 10 ml of *water R* and dilute to 30 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 0.4 per cent.

Dissolve 0.15 g in a mixture of 5 ml of *dilute hydrochloric acid R* and 10 ml of *distilled water R* and dilute to 60 ml with *distilled water R.*

Arsenic ***(2.4.2, Method A)***

Maximum 4 ppm.

Dissolve 0.50 g in 5 ml of *brominated hydrochloric acid R* and dilute to 50 ml with *water R*. Use 25 ml of this solution.

Magnesium and alkali metals

Maximum 4.0 per cent calculated as sulphates.

Dissolve 1.0 g in a mixture of 10 ml of *hydrochloric acid R* and 40 ml of *water R*. Boil and add 50 ml of a 63 g/l solution of *oxalic acid R*. Neutralise with *ammonia R* and dilute to 200 ml with *water R*. Allow to stand for 1 h and filter through a suitable filter. To 100 ml of the filtrate, add 0.5 ml of *sulphuric acid R*. Cautiously evaporate to dryness and ignite. The residue weighs a maximum of 20 mg.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

Dissolve 1.0 g in 10 ml of *hydrochloric acid R1* and evaporate to dryness on a water-bath. Dissolve the residue in 20 ml of *water R* and filter. 12 ml of the filtrate complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

ASSAY

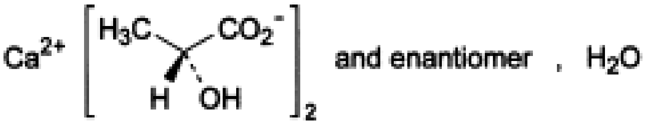
To 1.500 g in a mortar, add 20-30 ml of *water R* and 0.5 ml of *phenolphthalein solution R*. Titrate with *1 M hydrochloric acid* by triturating the substance until the red colour disappears. The final solution is used in the tests for carbonates.

1 ml of *1 M hydrochloric acid* is equivalent to 37.05 mg of $Ca(OH)_2$.

Calcium Lactate Monohydrate

General Notices

(Ph Eur monograph 2117)



$C_6H_{10}CaO_6,H_2O$ 236.0 *41372-22-9*

Action and use

Used in treatment of calcium deficiency.

Ph Eur

DEFINITION

Calcium bis(2-hydroxypropanoate) or mixture of calcium (2*R*)-, (2*S*)- and (2*RS*)-2-hydroxypropanoates monohydrates.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline or granular powder.

Solubility

Soluble in water, freely soluble in boiling water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. It complies with the test for loss on drying (see Tests).

B. It gives the reaction of lactates *(2.3.1)* .

C. It gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 5.4 g (equivalent to 5.0 g of the dried substance) with heating in *carbon dioxide-free water R* prepared from *distilled water R*, allow to cool and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II).*

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R* and 0.5 ml of *0.01 M hydrochloric acid*. The solution is colourless. Not more than 2.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 400 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*.

Barium

To 10 ml of solution S add 1 ml of *calcium sulphate solution R*. Allow to stand for 15 min. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Iron *(2.4.9)*

Maximum 50 ppm.

Dilute 4 ml of solution S to 10 ml with *water R*.

Magnesium and alkali salts

Maximum 1 per cent.

To 20 ml of solution S add 20 ml of *water R*, 2 g of *ammonium chloride R* and 2 ml of *dilute ammonia R1*. Heat to boiling and rapidly add 40 ml of hot *ammonium oxalate solution R.* Allow to stand for 4 h, dilute to 100.0 ml with *water R* and filter. To 50.0 ml of the filtrate add 0.5 ml of *sulphuric acid R*. Evaporate to dryness and ignite the residue to constant mass at 600 ± 50 °C. The residue weighs a maximum of 5 mg.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve a quantity equivalent to 2.0 g of the dried substance in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R* .

Loss on drying ***(2.2.32)***

5.0 per cent to 8.0 per cent, determined on 0.500 g by drying in an oven at 125 °C.

ASSAY

Dissolve a quantity equivalent to 0.200 g of the dried substance in *water R* and dilute to 300 ml with the same solvent. Carry out the complexometric titration of calcium *(2.5.11)* .

1 ml of 0.*1 M sodium edetate* is equivalent to 21.82 mg of $C_6H_{10}CaO_6$.

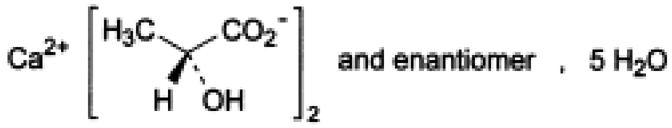
Ph Eur

Calcium Lactate Pentahydrate

General Notices

Calcium Lactate

(Ph Eur monograph 0468)



$C_6H_{10}CaO_6,5H_2O$	218.2	*814-80-2*
(*approx.*)	(anhydrous)	*(anhydrous)*

Action and use

Used in treatment of calcium deficiency.

Preparations

Calcium and Ergocalciferol Tablets

Calcium Lactate Tablets

Ph Eur

DEFINITION

Calcium bis(2-hydroxypropanoate) or mixture of calcium (2*R*)-, (2*S*)- and (2*RS*)-2-hydroxypropanoates pentahydrates.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline or granular powder, slightly efflorescent.

Solubility

Soluble in water, freely soluble in boiling water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. It complies with the test for loss on drying (see Tests).

B. It gives the reaction of lactates *(2.3.1)* .

C. It gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 7.1 g (equivalent to 5.0 g of the dried substance) with heating in *carbon dioxide-free water R* prepared from *distilled water R* , allow to cool and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R* and 0.5 ml of *0.01 M hydrochloric acid* . The solution is colourless. Not more than 2.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *water R.*

Sulphates *(2.4.13)*

Maximum 400 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R* .

Barium

To 10 ml of solution S add 1 ml of *calcium sulphate solution R* . Allow to stand for 15 min. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Iron *(2.4.9)*

Mmaximum 50 ppm.

Dilute 4 ml of solution S to 10 ml with *water R.*

Magnesium and alkali salts

Maximum 1 per cent.

To 20 ml of solution S add 20 ml of *water R*, 2 g of *ammonium chloride R* and 2 ml of *dilute ammonia R1*. Heat to boiling and rapidly add 40 ml of hot *ammonium oxalate solution R*. Allow to stand for 4 h, dilute to 100.0 ml with *water R* and filter. To 50.0 ml of the filtrate add 0.5 ml of *sulphuric acid R*. Evaporate to dryness and ignite the residue to constant mass at 600 ± 50 °C. The residue weighs a maximum of 5 mg.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve a quantity equivalent to 2.0 g of the dried substance in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

22.0 per cent to 27.0 per cent, determined on 0.500 g by drying in an oven at 125 °C.

ASSAY

Dissolve a quantity equivalent to 0.200 g of the dried substance in *water R* and dilute to 300 ml with the same solvent. Carry out the complexometric titration of calcium *(2.5.11)*.

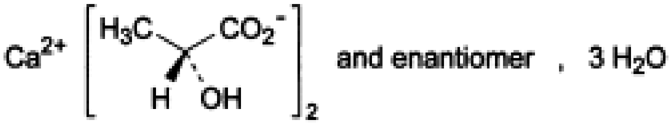
1 ml of *0.1 M sodium edetate* is equivalent to 21.82 mg of $C_6H_{10}CaO_6$.

Ph Eur

Calcium Lactate Trihydrate

General Notices

(Ph Eur monograph 0469)



$C_6H_{10}CaO_6,3H_2O$	218.2	*814-80-2*
(*approx.*)	(anhydrous)	*(anhydrous)*

Action and use

Used in treatment of calcium deficiency.

Preparation

Calcium Lactate Tablets

Ph Eur

DEFINITION

Calcium bis(2-hydroxypropanoate) or mixture of calcium (2*R*)-, (2*S*)- and (2*RS*)-2-hydroxypropanoates trihydrates.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline or granular powder.

Solubility

Soluble in water, freely soluble in boiling water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. It complies with the test for loss on drying (see Tests).

B. It gives the reaction of lactates *(2.3.1)* .

C. It gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 6.2 g (equivalent to 5.0 g of the dried substance) with heating in *carbon dioxide-free water R* prepared from *distilled water R* , allow to cool and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R* and 0.5 ml of *0.01 M hydrochloric acid* . The solution is colourless. Not more than 2.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *water R* .

Sulphates *(2.4.13)*

Maximum 400 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R* .

Barium

To 10 ml of solution S add 1 ml of *calcium sulphate solution R* . Allow to stand for 15 min. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Iron *(2.4.9)*

Maximum 50 ppm.

Dilute 4 ml of solution S to 10 ml with *water R* .

Magnesium and alkali salts

Maximum 1 per cent.

To 20 ml of solution S add 20 ml of *water R* , 2 g of *ammonium chloride R* and 2 ml of *dilute* *ammonia R1* . Heat to boiling and rapidly add 40 ml of hot *ammonium oxalate solution R* . Allow to stand for 4 h, dilute to 100.0 ml with *water R* and filter. To 50.0 ml of the filtrate add *0.5 ml of sulphuric acid R* . Evaporate to dryness and ignite the residue to constant mass at 600 ± 50 °C. The residue weighs a maximum of 5 mg.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve a quantity equivalent to 2.0 g of the dried substance in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R .*

Loss on drying *(2.2.32)*

15.0 per cent to 20.0 per cent, determined on 0.500 g by drying in an oven at 125 °C.

ASSAY

Dissolve a quantity equivalent to 0.200 g of the dried substance in *water R* and dilute to 300 ml with the same solvent. Carry out the complexometric titration of calcium *(2.5.11)* .

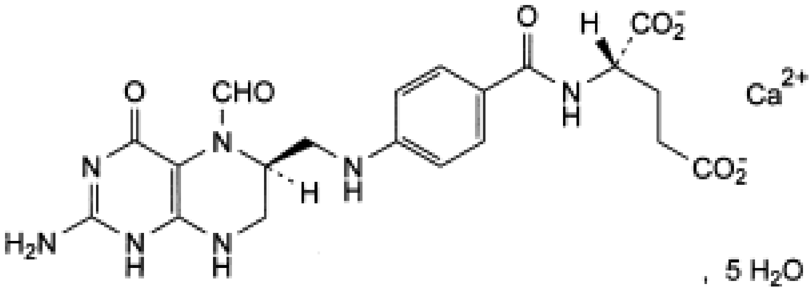
1 ml of *0.1 M sodium edetate* is equivalent to 21.82 mg of $C_6H_{10}CaO_6$.

Ph Eur

Calcium Levofolinate Pentahydrate

General Notices

(Ph Eur monograph 1606)



$C_{20}H_{21}CaN_7O_7,5H_2O$ 511.5 *80433-71-2*
(*anhydrous*)

Action and use

Antidote to folic acid antagonists.

Ph Eur

DEFINITION

Calcium (2*S*)-2-[[4-[[[(6*S*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl] amino]benzoyl]amino]pentanedioate pentahydrate.

Content:

- *calcium levofolinate* ($C_{20}H_{21}CaN_7O_7$; M_r 511.5): 97.0 per cent to 102.0 per cent (anhydrous substance);
- *calcium* (Ca; A_r 40.08): 7.54 per cent to 8.14 per cent (anhydrous substance).

CHARACTERS

Appearance

White or light yellow, amorphous or crystalline powder, hygroscopic.

Solubility

Slightly soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B, D.*

Second identification *A, C, D.*

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *calcium folinate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum quantity of *water R* and add dropwise sufficient *acetone R* to produce a precipitate. Allow to stand for 15 min, collect the precipitate by centrifugation, wash the precipitate twice with a minimum quantity of *acetone R* and dry. Record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 15 mg of the substance to be examined in a 3 per cent *V/V* solution of *ammonia R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 15 mg of *calcium folinate CRS* in a 3 per cent *V/V* solution of *ammonia R* and dilute to 5 ml with the same solvent.

Plate *cellulose for chromatography* F_{254} *R* as the coating substance.

Mobile phase the lower layer of a mixture of 1 volume of *isoamyl alcohol R* and 10 volumes of a 50 g/l solution of *citric acid R* previously adjusted to pH 8 with *ammonia R* .

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (b) of calcium *(2.3.1)* .

Carry out the tests and the assay as rapidly as possible, protected from bright light.

TESTS

Solution S

Dissolve 0.40 g in *carbon dioxide-free water R* , heating at 40 °C if necessary, and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 420 nm has a maximum of 0.25.

pH *(2.2.3)*

7.5 to 8.5 for solution S.

Specific optical rotation *(2.2.7)*

- 10 to - 15 (anhydrous substance), measured at 25 °C.

Dissolve 0.200 g in *tris(hydroxymethyl)aminomethane solution R* previously adjusted to pH 8.1 with *sodium hydroxide solution R* or *hydrochloric acid R1* and dilute to 20.0 ml with the same solvent.

Acetone and ethanol

Head-space gas chromatography *(2.2.28)* : use the standard additions method.

Test solution Dissolve 0.25 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 0.125 g of *acetone R* and 0.750 g of *anhydrous ethanol R* in *water R* and dilute to 1000.0 ml with *water R.*

Column:

— *material:* fused silica;

— *size:* *l* = 10 m, Ø = 0.32 mm;

— *stationary phase: styrene-divinylbenzene copolymer R* .

Carrier gas *nitrogen for chromatography R.*

Flow rate 4 ml/min.

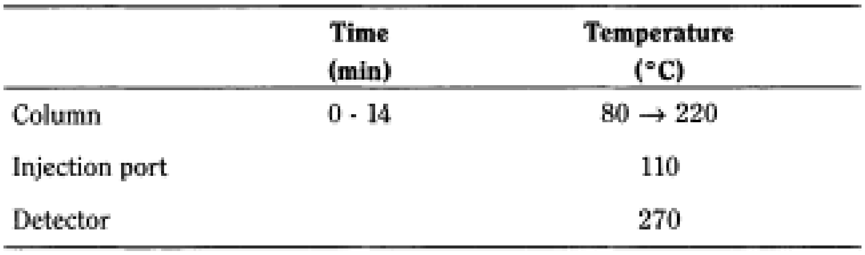
Static head-space conditions which may be used:

— *equilibration temperature*: 80 °C;

— *equilibration time*: 20 min;

— *pressurisation time*: 30 s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
Injection port		110
Detector		270

Detection Flame ionisation.

Injection At least 3 times.

Limits:

— *acetone* : maximum 0.5 per cent,

— *ethanol* : maximum 3.0 per cent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 10.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *calcium folinate CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water R* .

Reference solution (c) Dissolve 10.0 mg of *formylfolic acid CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with *water R* .

Reference solution (d) Dilute 1.0 ml of reference solution (b) to 20.0 ml with *water R* .

Reference solution (e) Dilute 5.0 ml of reference solution (c) to 10.0 ml with reference solution (b).

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 220 ml of *methanol R* and 780 ml of a solution containing 2.0 ml of *tetrabutylammonium hydroxide solution (400 g/l) R* and 2.2 g of *disodium hydrogen phosphate R* previously adjusted to pH 7.8 with *phosphoric acid R* . If necessary adjust the concentration of *methanol R* to achieve the prescribed resolution.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µl.

Run time 2.5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

System suitability Reference solution (e):

— *resolution*: minimum of 2.2 between the peaks due to folinate and to impurity D.

Limits:

— *impurity D*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.8 per cent);

— *any other impurity*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);

— *sum of other impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Impurity H

Liquid chromatography *(2.2.29)* : use the normalisation procedure.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *calcium folinate CRS* in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water R.*

Column:
— *size*: *l* = 0.15 m, Ø = 4 mm;

— *stationary phase: human albumin coated silica gel for chromatography R* (5 µm);

— *temperature:* 40 °C.

Mobile phase Dissolve 9.72 g of *sodium dihydrogen phosphate R* in 890 ml of *water R* and adjust to pH 5.0 with *sodium hydroxide solution R* ; add 100 ml of *2-propanol R* and 10 ml of *acetonitrile R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 286 nm.

Injection 10 µl.

Retention times Levofolinate = about 9 min; impurity H = about 19 min.

System suitability:
— *resolution*: minimum of 5.0 between the peaks due to levofolinate and to impurity H in the chromatogram obtained with reference solution (a). The sum of the areas of the 2 peaks is 100 per cent. The peak area of impurity H is 48 per cent to 52 per cent. In the chromatogram obtained with reference solution (b) 2 clearly visible peaks are obtained.

Limit:
— *impurity H*: maximum 0.5 per cent.

Chlorides

Maximum 0.5 per cent.

Dissolve 0.300 g in 50 ml of *water R* heating at 40 °C if necessary. Add 10 ml of *2 M nitric acid* and titrate with 0.*005 M silver nitrate* determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.005 M silver nitrate* is equivalent to 0.177 mg of Cl.

Platinum

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II).*

Test solution Dissolve 1.0 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *platinum standard solution (30 ppm Pt) R*, diluted as necessary with a mixture of 1 volume of *nitric acid R* and 99 volumes of *water R.*

Source Platinum hollow-cathode lamp.

Wavelength 265.9 nm.

Heavy metals *(2.4.8)*

Maximum 50 ppm.

1.0 g complies with test F. Prepare the reference solution using 5 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

10.0 per cent to 17.0 per cent, determined on 0.200 g (ground to a very fine powder). Stir the substance to be examined in the titration solvent for about 15 min before titrating and use *iodosulphurous reagent R* as titrant.

Bacterial endotoxins *(2.6.14)*

Less than 0.5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Calcium

Dissolve 0.400 g in 150 ml of *water R* and dilute to 300 ml with the same solvent. Carry out the complexometric titration of calcium *(2.5.11)* .

1 ml of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

Calcium folinate

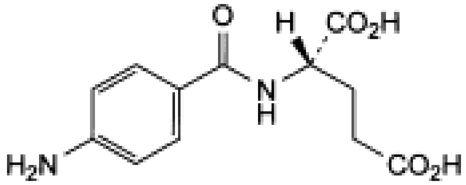
Liquid chromatography *(2.2.29)* as described in the test for related substances.

Calculate the percentage content of $C_{20}H2_{1}CaN_{7}O_{7}$ from the areas of the peaks in the chromatograms obtained with the test solution and reference solution (a) and the declared content of *calcium folinate CRS* .

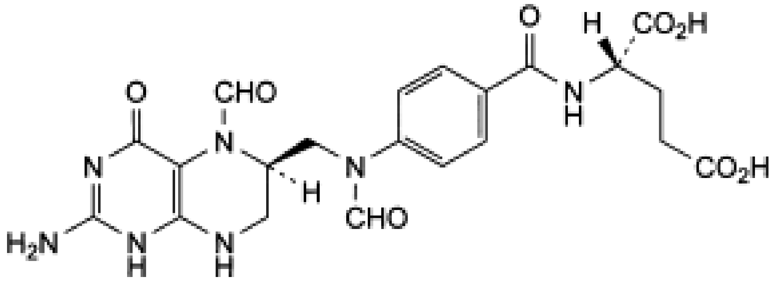
STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

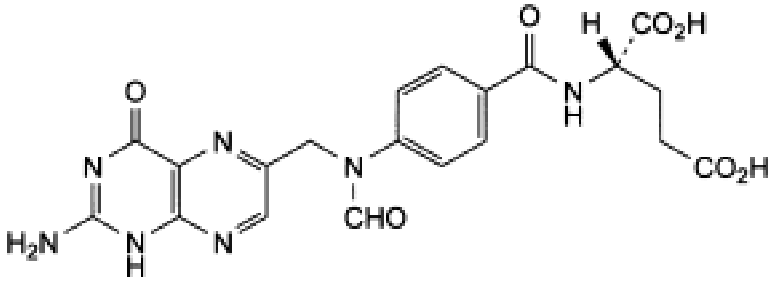


A. (2*S*)-2-[(4-aminobenzoyl)amino]pentanedioic acid,

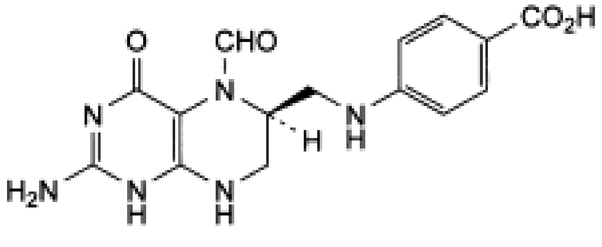


B. (2*S*)-2-[[4-[[[(6*R*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]
formylamino]benzoyl]amino]pentanedioic acid (5,10-diformyltetrahydrofolic acid),

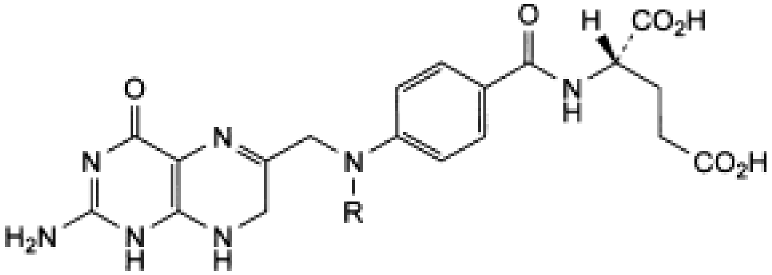
C. folic acid,



D. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]formylamino]benzoyl]amino]
pentanedioic acid (10-formylfolic acid),

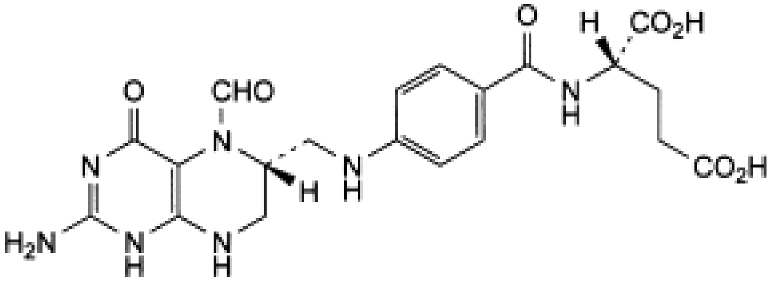


E. 4-[[[(6*S*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]amino]benzoic
acid (5-formyltetrahydropteroic acid),



F. R = CHO: (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]formylamino]benzoyl]amino]pentanedioic acid (10-formyldihydrofolic acid),

G. R = H: (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid (dihydrofolic acid),



H. (2*S*)-2-[[4-[[[(6*R*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]amino]benzoyl]amino]pentanedioic acid

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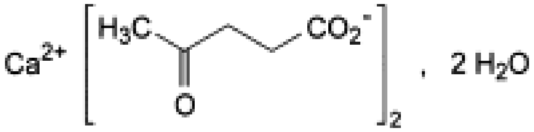
Calcium Levulinate Dihydrate

General Notices

(Ph Eur monograph 1296)



$C_{10}H_{14}CaO_6,2H_2O$ 306.3 *5743-49-7*

Action and use

Source of calcium.

Ph Eur

DEFINITION

Calcium di(4-oxopentanoate) dihydrate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification *A, D, E.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *calcium levulinate dihydrate CRS.*

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 60 mg of the substance to be examined in *water R* and dilute to 1 ml with the same solvent.

Reference solution Dissolve 60 mg of *calcium levulinate dihydrate CRS* in *water R* and dilute to 1 ml with the same solvent.

Plate *TLC silica gel plate R* .

Mobile phase *concentrated ammonia R, ethyl acetate R, water R, ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 10 µl.

Development Over a path of 10 cm.

Drying At 100-105 °C for 20 min and allow to cool.

Detection Spray with a 30 g/l solution of *potassium permanganate R* . Dry in a current of warm air for about 5 min or until the spots become yellow. Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 1 ml of solution S (see Tests), add 20 ml of a 2.5 g/l solution of *dinitrophenylhydrazine R* in *dilute hydrochloric acid R* . Allow to stand for 15 min. Filter, wash the precipitate with *water R.* Dry the precipitate in an oven at 100-105 °C. The melting point *(2.2.14)* is 203 °C to 210 °C.

D. It gives reaction (b) of calcium *(2.3.1)* .

E. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

6.8 to 7.8 for solution S.

Oxidisable substances

To 1 ml of solution S, add 10 ml of *water R* , 1 ml of *dilute sulphuric acid R* and 0.25 ml of a 3.0 g/l solution of *potassium permanganate R* . Mix. After 5 min, the violet colour of the mixture is still visible.

Sucrose and reducing sugars

To 5 ml of solution S add 2 ml of *hydrochloric acid R1* and dilute to 10 ml with *water R*. Heat to boiling for 5 min and allow to cool. Add 10 ml of *sodium carbonate solution R*. Allow to stand for 5 min, dilute to 25 ml with *water R* and filter. To 5 ml of the filtrate add 2 ml of *cupri-tartaric solution R* and heat to boiling for 1 min. No red precipitate is formed.

Chlorides *(2.4.4)*

Maximum 50 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*.

Magnesium and alkali metals

Maximum 1.0 per cent.

To 10 ml of solution S, add 80 ml of *water R*, 10 ml of *ammonium chloride solution R* and 1 ml of *ammonia R*. Heat to boiling. To the boiling solution, add dropwise 50 ml of warm *ammonium oxalate solution R*. Allow to stand for 4 h, then dilute to 200 ml with *water R* and filter. To 100 ml of the filtrate, add 0.5 ml of *sulphuric acid R*. Evaporate to dryness on a water-bath and ignite to constant mass at 600 ± 50 °C. The residue weighs a maximum of 5.0 mg.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

11.0 per cent to 12.5 per cent, determined on 0.200 g by drying at 105 °C.

Pyrogens *(2.6.8)*

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 4 ml of a solution containing per millilitre 50 mg of the substance to be examined.

ASSAY

Dissolve 0.240 g in 50 ml of *water R*. Carry out the complexometric titration of calcium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 27.03 mg of $C_{10}H_{14}CaO_6$.

STORAGE

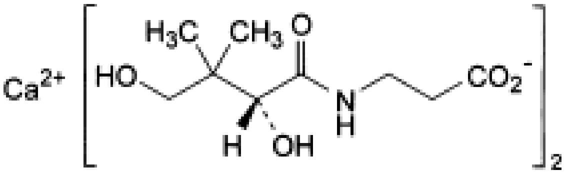
Protected from light.

Ph Eur

Calcium Pantothenate

General Notices

(Ph Eur monograph 0470)



$C_{18}H_{32}CaN_2O_{10}$ 476.5 *137-08-6*

Action and use

Component of vitamin B.

Ph Eur

DEFINITION

Calcium pantothenate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of calcium bis[3-[[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl]amino] propanoate], calculated with reference to the dried substance.

CHARACTERS

A. white or almost white powder, slightly hygroscopic, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Examine the chromatograms obtained in the test for 3-aminopropionic acid. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 1 ml of solution S (see Tests) add 1 ml of *dilute sodium hydroxide solution R* and 0.1 ml of *copper sulphate solution R* . A blue colour develops.

D. It gives reaction (a) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH ***(2.2.3)***

The pH of solution S is 6.8 to 8.0.

Specific optical rotation ***(2.2.7)***

+ 25.5 to + 27.5, determined on solution S and calculated with reference to the dried substance.

3-Aminopropionic acid

Examine by thin-layer chromatography *(2.2.27)* , using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *water R* .

Reference solution (a) Dissolve 20 mg of *calcium pantothenate CRS* in *water R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *3-aminopropionic acid R* in *water R* and dilute to 50 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 12 cm using a mixture of 35 volumes of *water R* and 65 volumes of *ethanol R* . Dry the plate in a current of air and spray with *ninhydrin solution R1* . Heat at 110 °C for 10 min. Any spot corresponding to 3-aminopropionic acid in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides ***(2.4.4)***

5 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides (200 ppm).

Heavy metals ***(2.4.8)***

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R* .

Loss on drying ***(2.2.32)***

Not more than 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.180 g in 50 ml of *anhydrous acetic acid R* . Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 23.83 mg of $C_{18}H_{32}CaN_2O_{10}$.

STORAGE

Store in an airtight container .

Ph Eur

Calcium Phosphate

General Notices

Tribasic Calcium Phosphate

(Ph Eur monograph 1052)

Action and use

Excipient.

Preparation

Calcium and Ergocalciferol Tablets

Calcium Phosphate for Homoeopathic Preparations

Ph Eur

DEFINITION

Mixture of calcium phosphates.

Content

35.0 per cent to 40.0 per cent of Ca (A_r 40.08).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water. It dissolves in dilute hydrochloric acid and in dilute nitric acid.

IDENTIFICATION

A. Dissolve 0.1 g in 5 ml of a 25 per cent *V/V* solution of *nitric acid R* . The solution gives reaction (b) of phosphates *(2.3.1)* .

B. It gives reaction (b) of calcium *(2.3.1)* . Filter before adding *potassium ferrocyanide solution R.*

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 2.50 g in 20 ml of *dilute hydrochloric acid R*. If the solution is not clear, filter it. Add *dilute ammonia R1* dropwise until a precipitate is formed. Dissolve the precipitate by adding *dilute hydrochloric acid R* and dilute to 50 ml with *distilled water R.*

Chlorides *(2.4.4)*

Maximum 0.15 per cent.

Dissolve 0.22 g in a mixture of 1 ml of *nitric acid R* and 10 ml of *water R* and dilute to 100 ml with *water R*.

Fluorides

Maximum 75 ppm.

Potentiometry *(2.2.36, Method II)*.

Test solution Dissolve 0.250 g in *0.1 M hydrochloric acid*, add 5.0 ml of *fluoride standard solution (1 ppm F) R* and dilute to 50.0 ml with *0.1 M hydrochloric acid*. To 20.0 ml of this solution add 20.0 ml of *total-ionic-strength-adjustment buffer R* and 3 ml of an 82 g/l solution of *anhydrous sodium acetate R*. Adjust to pH 5.2 with *ammonia R* and dilute to 50.0 ml with *distilled water R.*

Reference solution *Fluoride standard solution (10 ppm F) R.*

Indicator electrode Fluoride-selective.

Reference electrode Silver-silver chloride.

Carry out the measurements on the test solution, then add at least 3 quantities, each of 0.5 ml, of the reference solution, carrying out a measurement after each addition. Calculate the concentration of fluorides using the calibration curve, taking into account the addition of fluoride to the test solution.

Sulphates *(2.4.13)*

Maximum 0.5 per cent.

Dilute 1 ml of solution S to 25 ml with *distilled water R.*

Arsenic *(2.4.2, Method A)*

Maximum 4 ppm, determined on 5 ml of solution S.

Iron *(2.4.9)*

Maximum 400 ppm.

Dilute 0.5 ml of solution S to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 30 ppm.

Dilute 13 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Acid-insoluble matter

Maximum 0.2 per cent.

Dissolve 5.0 g in a mixture of 10 ml of *hydrochloric acid R* and 30 ml of *water R*. Filter, wash the residue with *water R* and dry to constant mass at 100-105 °C. The residue weighs a maximum of 10 mg.

Loss on ignition

Maximum 8.0 per cent, determined on 1.000 g by ignition at 800 ± 50 °C for 30 min.

ASSAY

Dissolve 0.200 g in a mixture of 1 ml of *hydrochloric acid R1* and 5 ml of *water R*. Add 25.0 ml of *0.1 M sodium edetate* and dilute to 200 ml with *water R*. Adjust to about pH 10 with *concentrated ammonia R*. Add 10 ml of *ammonium chloride buffer solution pH 10.0 R* and a few milligrams of *mordant black 11 triturate R*. Titrate the excess sodium edetate with *0.1 M zinc sulphate* until the colour changes from blue to violet.

1 ml of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

Ph Eur

Calcium Polystyrene Sulphonate

General Notices

Action and use

Used in the treatment of hyperkalaemia.

DEFINITION

Calcium Polystyrene Sulphonate is a cation-exchange resin prepared in the calcium form containing not less than 6.5% w/w and not more than 9.5% w/w of calcium, calculated with reference to the dried substance. Each g exchanges not less than 1.3 mEq and not more than 2.0 mEq of potassium, calculated with reference to the dried substance.

CHARACTERISTICS

A cream to light brown, fine powder.

Practically insoluble in *water* and in *ethanol (96%).*

IDENTIFICATION

A. The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of calcium polystyrene sulphonate *(RS 037)* .

B. Yields reaction C characteristic of *calcium salts* , Appendix VI.

TESTS

Particle size

Not more than 1% w/w is retained on a 150-µm sieve, Appendix XVII B. Use 20 g and sieve for 5 minutes.

Potassium

Not more than 0.1% of K when determined by *atomic emission spectrophotometry* , Appendix II D, measuring at 766.5 nm and using a solution prepared in the following manner. To 1.1 g of the substance being examined add 5 ml of *hydrochloric acid* , heat to boiling, cool and add 10 ml of *water*. Filter, wash the filter and residue with *water* and dilute the filtrate and washings to 25 ml with *water*. Use *potassium standard solution (100 ppm K)* , suitably diluted with *water*, to prepare the standard solutions.

Sodium

Not more than 0.1% of Na when determined by *atomic emission spectrophotometry* , Appendix II D, measuring at 589.0 nm and using a solution prepared in the following manner.

To 1.1 g of the substance being examined add 5 ml of *hydrochloric acid*, heat to boiling, cool and add 10 ml of *water*. Filter, wash the filter and residue with *water* and dilute the filtrate and washings to 25 ml with *water*. Use *sodium solution (200 ppm Na)*, suitably diluted with *water*, to prepare the standard solutions.

Arsenic

1 g complies with the *limit test for arsenic*, Appendix VII (1 ppm).

Heavy metals

Heat 4 g until charred, cool, add 4 ml of *lead-free nitric acid* and 0.5 ml of *sulphuric acid* drop wise and heat cautiously until white fumes are no longer evolved. Ignite in a muffle furnace at 500° to 600° until a white residue is obtained. Cool, add 4 ml of *hydrochloric acid* and dilute to 20 ml. The resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Styrene

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 10 g of the substance being examined with 10 ml of *acetone* for 30 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.0001% w/v of *styrene* in *acetone*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (µBondapak C18 is suitable), (b) a mixture of equal volumes of *acetonitrile* and *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Inject separately 20 µl of each solution. The area of the peak corresponding to styrene in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1 ppm).

Potassium exchange capacity

To 3 g of the substance being examined in a dry 250 ml glass-stoppered flask add 100 ml of a solution containing 0.7455% w/v of *potassium chloride* and 0.4401% w/v of *potassium hydrogen carbonate* in *water* (solution A), stopper and shake for 15 minutes. Filter and dilute 2 ml of the filtrate to 1000 ml with *water*. Determine the concentration of unbound potassium in this solution by *atomic emission spectrophotometry*, Appendix II D, measuring at 766.5 nm and using solution A suitably diluted with *water*, to prepare the standard solutions. Calculate the potassium exchange capacity of the substance being examined in milliequivalents taking the concentration of potassium in solution A as 144 milliequivalents of K per litre.

Loss on drying

When dried at 70° at a pressure not exceeding 0.7 kPa for 16 hours, loses not more than 8.0% of its weight. Use 2 g.

Microbial contamination

Carry out a quantitative evaluation for Enterobacteria and certain other Gram-negative bacteria, Appendix XVI B1. 0.01 g of the substance being examined gives a negative result, Table I (most probable number of bacteria per gram fewer than 10^2).

ASSAY

For calcium

Carefully heat 1 g in a platinum crucible until a white ash is obtained and dissolve in 10 ml of *2 M hydrochloric acid* with the aid of heat. Transfer the resulting solution to a conical flask using 20 ml of *water*. Add 50 ml of *0.05 M disodium edetate VS*, 20 ml of *ammonia buffer pH 10.9* and titrate the excess of disodium edetate with *0.02 M zinc sulphate VS*, using a 0.5% w/v solution of *mordant black 11* in *ethanol (96%)* as indicator to a red purple end point. Each ml of *0.05 M disodium edetate VS* is equivalent to 2.004 mg of Ca.

STORAGE

Calcium Polystyrene Sulphonate should be kept in an airtight container.

Calcium Stearate

General Notices

(Ph Eur monograph 0882)

1592-23-0

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of calcium salts of different fatty acids consisting mainly of stearic (octadecanoic) acid [$(C_{17}H_{35}COO)_2Ca$; *M* r 607] and palmitic (hexadecanoic) acid [$(C_{15}H_{31}COO)_2Ca$; *M* r 550.9] with minor proportions of other fatty acids.

Content:

- — *calcium*: 6.4 per cent to 7.4 per cent (A_r 40.08) (dried substance),
- — *stearic acid in the fatty acid fraction*: minimum 40.0 per cent,
- — *sum of stearic acid and palmitic acid in the fatty acid fraction*: minimum 90.0 per cent.

CHARACTERS

Appearance

Fine, white or almost white, crystalline powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification C, D.

Second identification A, B, D.

A. Freezing point *(2.2.18)*

Minimum 53 °C, for the residue obtained in the preparation of solution S (see Tests).

B. Acid value *(2.5.1)*

195 to 210.

Dissolve 0.200 g of the residue obtained in the preparation of solution S in 25 ml of the prescribed mixture of solvents.

C. Examine the chromatograms obtained in the test for fatty acid composition.

Results The retention times of the principal peaks in the chromatogram obtained with the test solution are approximately the same as those of the principal peaks in the chromatogram obtained with the reference solution.

D. Neutralise 5 ml of solution S to *red litmus paper R* using *strong sodium hydroxide solution R .* The solution gives reaction (b) of calcium *(2.3.1)* .

TESTS

Solution S

To 5.0 g add 50 ml of *peroxide-free ether R* , 20 ml of *dilute nitric acid R* and 20 ml of *distilled water R* . Boil under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 5 ml, of *distilled water R* . Combine the aqueous layers, wash with 15 ml of *peroxide-free ether R* and dilute the aqueous layer to 50 ml with *distilled water R* (solution S). Evaporate the ether layer to dryness and dry the residue at 100-105 °C. Keep the residue for identification tests A and B.

Acidity or alkalinity

To 1.0 g add 20 ml of *carbon dioxide-free water R* and boil for 1 min with continuous shaking. Cool and filter. To 10 ml of the filtrate add 0.05 ml of *bromothymol blue solution R1* . Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides ***(2.4.4)***

Maximum 0.1 per cent.

Dilute 0.5 ml of solution S to 15 ml with *water R.*

Sulphates ***(2.4.13)***

Maximum 0.3 per cent.

Dilute 0.5 ml of solution S to 15 ml with *distilled water R* .

Cadmium

Maximum 3.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Place 50.0 mg in a polytetrafluoroethylene digestion bomb and add 0.5 ml of a mixture of 1 volume of *hydrochloric acid R* and 5 volumes of *cadmium- and lead-free nitric acid R* . Allow to digest at 170 °C for 5 h. Allow to cool. Dissolve the residue in *water R* and dilute to 5.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *cadmium standard solution (10 ppm Cd) R* , diluted if necessary with a 1 per cent *V/V* solution of *hydrochloric acid R* .

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Graphite furnace.

Lead

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Use the solution described in the test for cadmium.

Reference solutions Prepare the reference solutions using *lead standard solution (10 ppm Pb) R* , diluted if necessary with *water R.*

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm; 217.0 nm may be used depending on the apparatus.

Atomisation device Graphite furnace.

Nickel

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Use the solution described in the test for cadmium.

Reference solutions Prepare the reference solutions using *nickel standard solution (10 ppm Ni) R* , diluted if necessary with *water R* .

Source Nickel hollow-cathode lamp.

Wavelength 232.0 nm.

Atomisation device Graphite furnace.

Loss on drying *(2.2.32)*

Maximum 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli (2.6.13)* .

ASSAY

Calcium

To 0.500 g in a 250 ml conical flask add 50 ml of a mixture of equal volumes of *butanol R* and *anhydrous ethanol R*, 5 ml of *concentrated ammonia R*, 3 ml of *ammonium chloride buffer solution pH 10.0 R*, 30.0 ml of *0.1 M sodium edetate* and 15 mg of *mordant black 11 triturate R*. Heat to 45-50 °C until the solution is clear. Cool and titrate with *0.1 M zinc sulphate* until the colour changes from blue to violet. Carry out a blank titration.

1 ml of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

Composition of fatty acids

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution In a conical flask fitted with a reflux condenser, dissolve 0.10 g of the substance to be examined in 5 ml of *boron trifluoride-methanol solution R*. Boil under a reflux condenser for 10 min. Add 4 ml of *heptane R* through the condenser. Boil under a reflux condenser for 10 min. Allow to cool. Add 20 ml of a *saturated sodium chloride solution R*. Shake and allow the layers to separate. Remove about 2 ml of the organic layer and dry over 0.2 g of *anhydrous sodium sulphate R*. Dilute 1.0 ml of the solution to 10.0 ml with *heptane R.*

Reference solution Prepare the reference solution in the same manner as the test solution using 50.0 mg of *palmitic acid CRS* and 50.0 mg of *stearic acid CRS* instead of calcium stearate.

Column:

— *material: fused silica*,

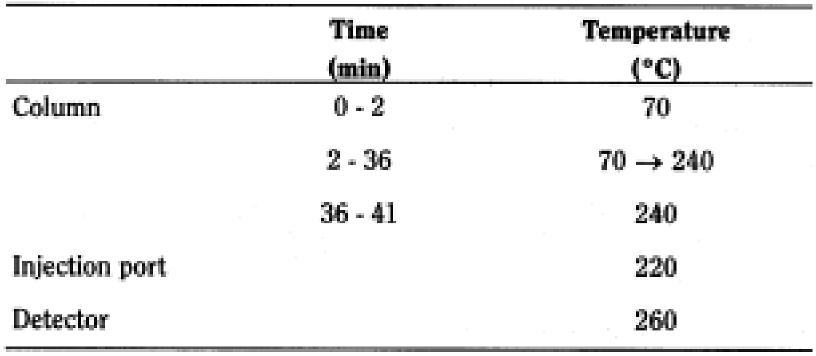
— *size*: *l* = 30 m, Ø = 0.32 mm,

— *stationary phase: macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.4 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	70
	2 - 36	70 → 240
	36 - 41	240
Injection port		220
Detector		260

Detection Flame ionisation.

Injection: 1 µl.

Relative retention With reference to methyl stearate: methyl palmitate = about 0.88.

System suitability Reference solution:

— *resolution*: minimum 5.0 between the peaks due to methyl stearate and methyl palmitate.

Calculate the content of palmitic acid and stearic acid. Disregard the peak due to the solvent.

Ph Eur

Calcium Sulphate Dihydrate

General Notices

(Ph Eur monograph 0982)

$CaSO_4,2H_2O$ 172.2 *10101-41-4*

Action and use

Excipient.

Ph Eur

DEFINITION

Content

98.0 per cent to 102.0 per cent of $CaSO_4,2H_2O$.

CHARACTERS

Appearance

White or almost white fine powder.

Solubility

Very slightly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Loss on ignition (see Tests).

B. Solution S (see Tests) gives reaction (a) of sulphates *(2.3.1)* .

C. Solution S gives reaction (a) of calcium *(2.3.1)* .

TESTS

Solution S

Dissolve 1.0 g in 50 ml of a 10 per cent *V/V* solution of *hydrochloric acid R* by heating at 50 °C for 5 min. Allow to cool.

Acidity or alkalinity

Shake 1.5 g with 15 ml of *carbon dioxide-free water R* for 5 min. Allow to stand for 5 min and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R* and 0.25 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.30 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.2 ml of *methyl red solution R*. The solution is reddish-orange.

Chlorides ***(2.4.4)***

Maximum 300 ppm.

Shake 0.5 g with 15 ml of *water R* for 5 min. Allow to stand for 15 min and filter. Dilute 5 ml of the filtrate to 15 ml with *water R.*

Arsenic ***(2.4.2, Method A)***

Maximum 10 ppm, determined on 5 ml of solution S.

Iron ***(2.4.9)***

Maximum 100 ppm.

To 0.25 g add a mixture of 5 ml of *hydrochloric acid R* and 20 ml of *water R*. Heat to boiling, cool and filter.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

To 2.5 g add a mixture of 2 ml of *hydrochloric acid R* and 15 ml of *water R*. Heat to boiling. Cool and then add 0.5 ml of *phenolphthalein solution R*. Cautiously add *concentrated ammonia R* until the colour changes to pink. Add 0.5 ml of *glacial acetic acid R* and dilute to 25 ml with *water R*. Filter. 12 ml of the filtrate complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Loss on ignition

18.0 per cent to 22.0 per cent, determined on 1.000 g by ignition to constant mass at 800 ± 50 °C.

ASSAY

Dissolve 0.150 g in 120 ml of *water R*. Carry out the complexometric titration of calcium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 17.22 mg of $CaSO_4,2H_2O$.

Ph Eur

Caprylocaproyl Macrogolglycerides

General Notices

(Ph Eur monograph 1184)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols with a mean relative molecular mass between 200 and 400.

They are obtained by partial alcoholysis of medium-chain triglycerides using macrogol or by esterification of glycerol and macrogol with caprylic (octanoic) acid and capric (decanoic) acid or a mixture of glycerol esters and condensates of ethylene oxide with caprylic acid and capric acid. They may contain free macrogols.

CHARACTERS

Appearance

Pale-yellow, oily liquid.

Solubility

Dispersible in hot water, freely soluble in methylene chloride.

Density

About 1.0 at 20 °C.

Refractive index

About 1.4 at 20 °C.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R* .

Mobile phase *hexane R*, *ether R* (30:70 *V/V*).

Application 50 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9 (R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.7), to 1,2-diglycerides (R_{st} 0.6), to monoglycerides (R_{st} 0.1) and to esters of macrogol (R_{st} 0).

B. Hydroxyl value (see Tests).

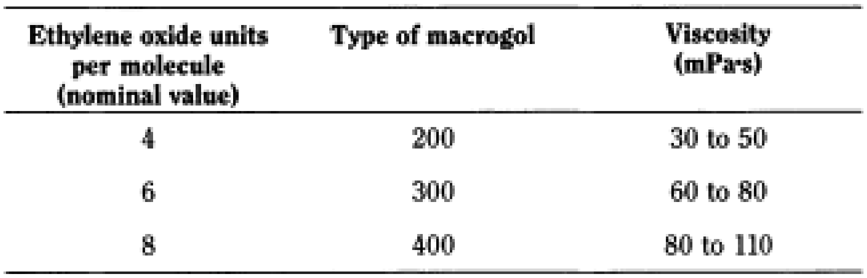
C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

TESTS

Viscosity ***(2.2.9)***

Carry out the determination at 20 ± 0.5 °C.



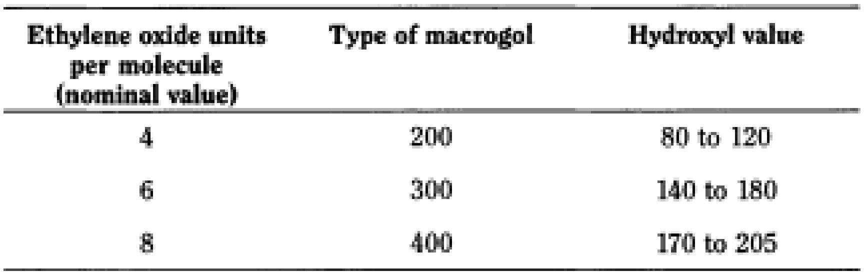
Ethylene oxide units per molecule (nominal value)	Type of macrogol	Viscosity (mPa·s)
4	200	30 to 50
6	300	60 to 80
8	400	80 to 110

Acid value ***(2.5.1)***

Maximum 2.0, determined on 2.0 g.

Hydroxyl value ***(2.5.3, Method A)***

Use 1.0 g.



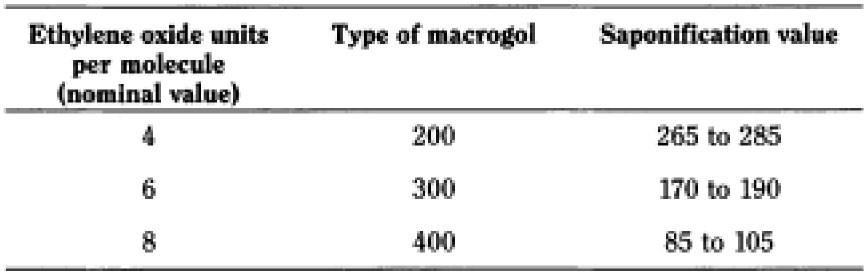
Ethylene oxide units per molecule (nominal value)	Type of macrogol	Hydroxyl value
4	200	80 to 120
6	300	140 to 180
8	400	170 to 205

Peroxide value ***(2.5.5, Method A)***

Maximum 6.0, determined on 2.0 g.

Saponification value ***(2.5.6)***

Use 2.0 g.



Ethylene oxide units per molecule (nominal value)	Type of macrogol	Saponification value
4	200	265 to 285
6	300	170 to 190
8	400	85 to 105

Alkaline impurities

Introduce 5.0 g into a test-tube and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 ml of *water R* and 10 ml of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 ml of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol

Maximum 5.0 per cent.

Dissolve 1.20 g in 25.0 ml of *methylene chloride R*. Heat if necessary. After cooling, add 100 ml of *water R*. Shake and add 25.0 ml of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 ml of a 75 g/l solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 ml of *starch solution R.* Titrate the iodine with *0.1 M sodium thiosulphate*. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids ***(2.4.22, Method A).***

Composition of the fatty-acid fraction of the substance:

— *caproic acid* : maximum 2.0 per cent;

— *caprylic acid* : 50.0 per cent to 80.0 per cent;

— *capric acid*: 20.0 per cent to 50.0 per cent;

— *lauric acid* : maximum 3.0 per cent;

— *myristic acid* : maximum 1.0 per cent.

Ethylene oxide and dioxan ***(2.4.25)***

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 1.0 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash *(2.4.16)*

Maximum 0.1 per cent.

LABELLING

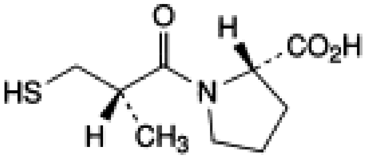
The label states the type of macrogol used (mean relative molecular mass) or the number of ethylene oxide units per molecule (nominal value).

Ph Eur

Captopril

General Notices

(Ph Eur monograph 1079)



$C_9H_{15}NO_3S$ 217.3 *62571-86-2*

Action and use

Angiotensin converting enzyme inhibitor.

Preparations

Captopril Oral Solution

Captopril Tablets

Ph Eur

DEFINITION

(2*S*)-1-[(2*S*)-2-Methyl-3-sulphanylpropanoyl]pyrrolidine-2-carboxylic acid.

Content

98.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, in methylene chloride and in methanol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *captopril CRS.*

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

2.0 to 2.6 for solution S.

Specific optical rotation *(2.2.7)*

- 127 to - 132 (dried substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of the substance to be examined in the mobile phase, add 0.25 ml of *0.05 M iodine* and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.125 m, Ø = 4 mm,

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *phosphoric acid R, methanol R, water R* (0.05:50:50 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 3 times the retention time of captopril.

System suitability Reference solution (b):

— the chromatogram shows 3 peaks,

— *resolution*: minimum 2.0 between the last 2 eluting principal peaks.

Limits:

- — *any impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),
- — *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent),
- — *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Disregard any peak with a retention time less than 1.4 min.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying under high vacuum at 60 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

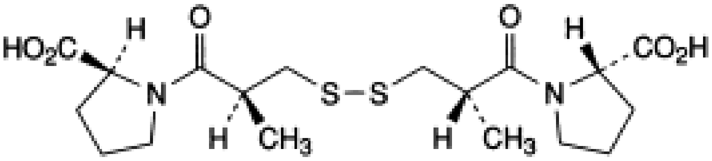
Dissolve 0.150 g in 30 ml of *water R* . Titrate with *0.05 M iodine* , determining the end-point potentiometrically *(2.2.20)* . Use a combined platinum electrode.

1 ml of *0.05 M iodine* is equivalent to 21.73 mg of $C_9H_{15}NO_3S$.

STORAGE

In an airtight container .

IMPURITIES



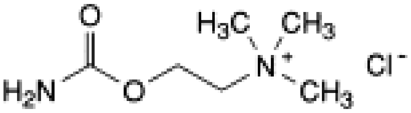
A. (2*S*,2′*S*)-1,1′-[disulphanediylbis[(2*S*)-2-methyl-1-oxopropane-3,1-diyl]-bis[pyrrolidine-2-carboxylic] acid (captopril-disulphide).

Ph Eur

Carbachol

General Notices

(Ph Eur monograph 1971)



$C_6H_{15}ClN_2O_2$ 182.7 *51-83-2*

Action and use

Cholinoceptor agonist.

Ph Eur

DEFINITION

2-(Carbamoyloxy)-*N,N,N*-trimethylethanaminium chloride.

Content

99.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline, hygroscopic powder.

Solubility

Very soluble in water, sparingly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison carbachol CRS.

B. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. 0.5 ml of solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Acidity or alkalinity

To 2.0 ml of solution S, add 0.05 ml of *methyl red mixed solution R* . Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Thin-layer chromatography *(2.2.27)* .

Prepare the solutions immediately before use.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Test solution (b) Dilute 2.0 ml of test solution (a) to 20.0 ml with *methanol R* .

Reference solution (a) Dissolve 20 mg of *carbachol CRS* in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dissolve 8 mg of *choline chloride R* and 8 mg of *acetylcholine chloride CRS* in *methanol R* and dilute to 10.0 ml with the same solvent. Dilute 5.0 ml to 10.0 ml with *methanol R* .

Plate *cellulose for chromatography R* as the coating substance.

Mobile phase *water R, methanol R* (10:90 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Detection Spray with *potassium iodobismuthate solution R3* .

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Limits In the chromatogram obtained with test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than one or other of the 2 principal spots in the chromatogram obtained with reference solution (b) (1 per cent). Compare the spots with the spot of the most appropriate colour in the chromatogram obtained with reference solution (b).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R* .

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g of the residue obtained in the test for loss on drying.

ASSAY

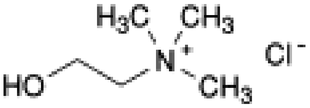
Dissolve 0.150 g in a mixture of 10 ml of *anhydrous acetic acid R* and 40 ml of *acetic anhydride R* . Titrate with *0.1 M perchloric acid* . Determine the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 18.27 mg of $C_6H_{15}ClN_2O_2$.

STORAGE

In an airtight container , protected from light.

IMPURITIES



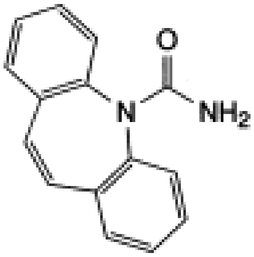
A. 2-hydroxy-*N,N,N*-trimethylethanaminium chloride (choline chloride).

Ph Eur

Carbamazepine

General Notices

(Ph Eur monograph 0543)



$C_{15}H_{12}N_2O$ 236.3 *298-46-4*

Action and use

Antiepileptic.

Preparation

Carbamazepine Tablets

Ph Eur

DEFINITION

5*H*-Dibenzo[*b*,*f*]azepine-5-carboxamide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in methylene chloride, sparingly soluble in acetone and in ethanol (96 per cent).

It shows polymorphism (*5.9*). The acceptable crystalline form corresponds to *carbamazepine CRS.*

IDENTIFICATION

A. Melting point *(2.2.14)* : 189 °C to 193 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *carbamazepine CRS.*

Preparation Examine the substances as discs without prior treatment.

TESTS

Acidity or alkalinity

To 1.0 g add 20 ml of *carbon dioxide-free water R* , shake for 15 min and filter. To 10 ml of the filtrate add 0.05 ml *of phenolphthalein solution R1* and 0.5 ml of *0.01 M sodium hydroxide* ; the solution is red. Add 1.0 ml of *0.01 M hydrochloric acid* ; the solution is colourless. Add 0.15 ml of *methyl red solution R* ; the solution is red.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution (a) Dissolve 60.0 mg of the substance to be examined in *methanol R2* and dilute to 20.0 ml with the same solvent. Sonicate. Dilute 10.0 ml of this solution to 20.0 ml with *water R.*

Test solution (b) Dilute 10.0 ml of test solution (a) to 50.0 ml with a mixture of equal volumes of *methanol R2* and *water R.*

Reference solution (a) Dissolve 7.5 mg of *carbamazepine CRS* , 7.5 mg of *carbamazepine impurity A CRS* and 7.5 mg of *iminodibenzyl R* (impurity E) in *methanol R2* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with a mixture of equal volumes of *methanol R2* and *water R* .

Reference solution (b) Dissolve 60.0 mg of *carbamazepine CRS* in *methanol R2* and dilute to 20.0 ml with the same solvent. Sonicate. Dilute 5.0 ml of this solution to 50.0 ml with a mixture of equal volumes of *methanol R2* and *water R.*

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: nitrile silica gel for *chromatography R1* (10 µm).

Mobile phase *tetrahydrofuran R, methanol R2, water R* (3:12:85 *V/V/V*); to 1000 ml of this solution add 0.2 ml of *anhydrous formic acid R* and 0.5 ml of *triethylamine R* .

Flow rate 2.0 ml/min.

Detection A spectrophotometer at 230 nm.

Injection 20 µl of test solution (a) and reference solution (a).

Run time 6 times the retention time of carbamazepine.

Relative retention With reference to carbamazepine (retention time = about 10 min): impurity A = about 0.9; impurity E = about 5.1.

System suitability:

— *resolution*: minimum 1.7 between the peaks due to carbamazepine and impurity A in the chromatogram obtained with reference solution (a).

Limits:

— *impurities A, E*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: not more than the area of the peak due to carbamazepine in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the peak due to carbamazepine in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the peak due to carbamazepine in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides ***(2.4.4)***

Maximum 140 ppm.

Suspend 0.715 g in 20 ml of *water R* and boil for 10 min. Cool and dilute to 20 ml with *water R* . Filter through a membrane filter (nominal pore size: 0.8 µm). Dilute 10 ml of the filtrate to 15 ml with *water R* . This solution complies with the limit test for chlorides.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

Injection Test solution (b) and reference solution (b).

System suitability:

— *repeatability*: reference solution (b).

Calculate the percentage content *m/m* of dried substance.

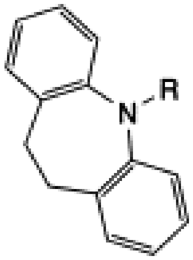
STORAGE

In an airtight container .

IMPURITIES

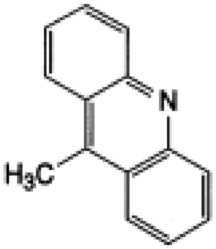
Specified impurities *A, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*) : *B, C, D, F.*

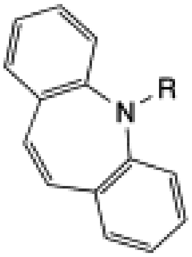


A. R = CO-NH_2: 10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (10,11-dihydrocarbamazepine),

E. R = H: 10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (iminodibenzyl),



B. 9-methylacridine,



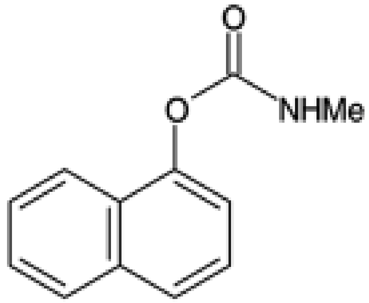
C. R = CO-NH-CO-NH_2: (5*H*-dibenzo[*b*,*f*]azepin-5-ylcarbonyl)urea (*N*-carbamoylcarbamazepine),

D. R = H: 5*H*-dibenzo[*b*,*f*]azepine (iminostilbene),

F. R = CO-Cl: 5*H*-dibenzo[*b*,*f*]azepine-5-carbonyl chloride (5-chlorocarbonyliminostilbene).

Carbaryl

General Notices



$C_{12}H_{11}NO_2$ 201.2 *63-25-2*

Action and use

Insecticide.

Preparation

Carbaryl Lotion

DEFINITION

Carbaryl is 1-naphthyl methylcarbamate. It contains not less than 98.0% and not more than 102.0% of $C_{12}H_{11}NO_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to off-white or light grey powder, which darkens on exposure to light. It melts at about 142°.

Very slightly soluble in *water*; soluble in *acetone* and in *ethanol (96%)* .

IDENTIFICATION

The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of carbaryl *(RS 039)* .

TESTS

1-Naphthol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.1% w/v of the substance being examined in *acetonitrile*. Solution (2) contains 0.001% w/v of *1-naphthol* in a 75% v/v solution of *acetonitrile* in *water*. Solution (3) contains 0.005% w/v of the substance being examined and 0.005% w/v of *1-naphthol* in the mobile phase.

The chromatographic conditions described under Assay may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 1-naphthol is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried to constant weight over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of the substance being examined in *methanol*. Solution (2) contains 0.005% w/v of *carbaryl BPCRS* in *methanol*. Solution (3) contains 0.005% w/v of the substance being examined and 0.005% w/v of *1-naphthol* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of 1 volume of *glacial acetic acid*, 25 volumes of *acetonitrile* and 75 volumes of *water* as the mobile phase with a flow rate of 2.5 ml per minute and (c) a detection wavelength of 280 nm.

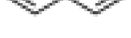
Inject 20 µl of each solution. The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the content of $C_{12}H_{11}NO_2$ using the declared content of $C_{12}H_{11}NO_2$ in *carbaryl BPCRS*.

STORAGE

Carbaryl should be protected from light.

IMPURITIES

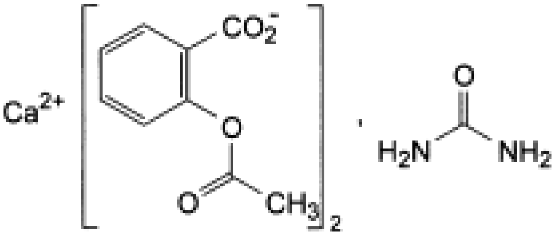


A. 1-naphthol

Carbasalate Calcium

General Notices

(Ph Eur monograph 1185)



$C_{19}H_{18}CaN_2O_9$ 458.4 *5749-67-7*

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

Equimolecular compound of calcium di[2-(acetyloxy)benzoate] and urea.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in dimethylformamide, practically insoluble in acetone and in anhydrous methanol.

Protect the substance from moisture during handling. Examination in aqueous solutions has to be performed immediately after preparation.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.250 g in *water R* and dilute to 100.0 ml with the same solvent. To 1.0 ml of the solution add 75 ml of *water R* and 5 ml of *dilute hydrochloric acid R*, mix and dilute to 100.0 ml with *water R*. Examine immediately.

Spectral range 220-350 nm.

Absorption maxima At 228 nm and 276 nm.

Specific absorbances at the absorption maxima:
— at 228 nm: 363 to 379,

— at 276 nm: 49 to 53.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of carbasalate calcium.*

C. Dissolve 0.1 g in 10 ml of *water R*, boil for 2 min and cool. The solution gives reaction (a) of salicylates *(2.3.1)*.

D. Heat 0.2 g with 0.2 g of *sodium hydroxide R*; a yellow or yellowish-brown colour is produced and the vapour turns *red litmus paper* R blue.

E. It gives reaction (a) of calcium *(2.3.1)*.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1)* and is colourless *(2.2.2, Method II)*.

Dissolve 2.5 g in 50 ml of *water R.*

Related substances

Maximum 0.1 per cent, expressed as acetylsalicylsalicylic acid.

In a 100 ml volumetric flask, dissolve 0.150 g in 10 ml of *0.1 M tetrabutylammonium hydroxide in 2-propanol*. Allow to stand for 10 min shaking occasionally. Add 8.0 ml of *0.1 M hydrochloric acid* and 20.0 ml of a 19 g/l solution of *disodium tetraborate R* and mix. While swirling continuously, add 2.0 ml of a 10 g/l solution of *aminopyrazolone R* and 2.0 ml of a 10 g/l solution of *potassium ferricyanide R*. Allow to stand for 2 min, dilute to 100.0 ml with *water R ,* mix and allow to stand for 20 min. Measure the absorbance *(2.2.25)* of the solution at the absorption maximum at 505 nm using *water R* as the compensation liquid. The absorbance is not greater than 0.125.

Impurity C

Maximum 0.5 per cent.

In a 100 ml volumetric flask, dissolve 0.200 g in 80 ml of *water R* and add 10 ml of a 10 g/l solution of *ferric nitrate R* in a 80 g/l solution of *dilute nitric acid R* . Dilute to 100.0 ml with *water R* . Immediately after preparation, measure the absorbance *(2.2.25)* of the solution at the absorption maximum of 525 nm using *water R* as the compensation liquid. The absorbance is not greater than 0.115.

Sodium

Maximum 0.10 per cent.

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution Dissolve 1.0 g in 500.0 ml of *water R* .

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in 8 ml of *water R* with heating, cool and add 12 ml of *acetone R* . 12 ml of the solution complies with test B. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R* .

Water *(2.5.12)*

Maximum 0.1 per cent, determined on 1.000 g. Use a mixture of 15 ml of *anhydrous methanol R* and 15 ml of *dimethylformamide R* as the solvent.

ASSAY

In a flask with a ground-glass stopper, dissolve 0.400 g in 25 ml of *water R.* Add 25.0 ml of *0.1 M sodium hydroxide* . Close the flask and allow to stand for 2 h. Titrate the excess of alkali with *0.1 M hydrochloric acid* , using 0.2 ml of *phenolphthalein solution R* . Carry out a blank titration.

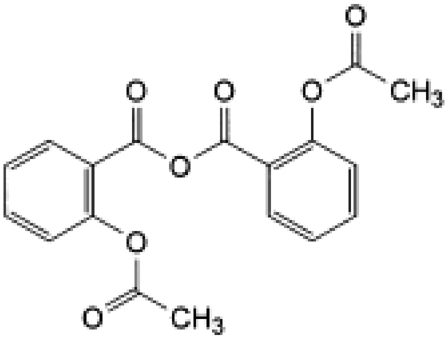
1 ml of *0.1 M sodium hydroxide* is equivalent to 22.92 mg of $C_{19}H_{18}CaN_2O_9$.

STORAGE

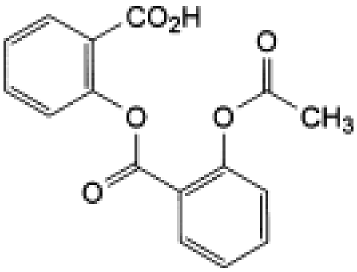
In an airtight container .

IMPURITIES

Specified impurities *A, B, C.*



A. 2-(acetyloxy)benzoic anhydride,



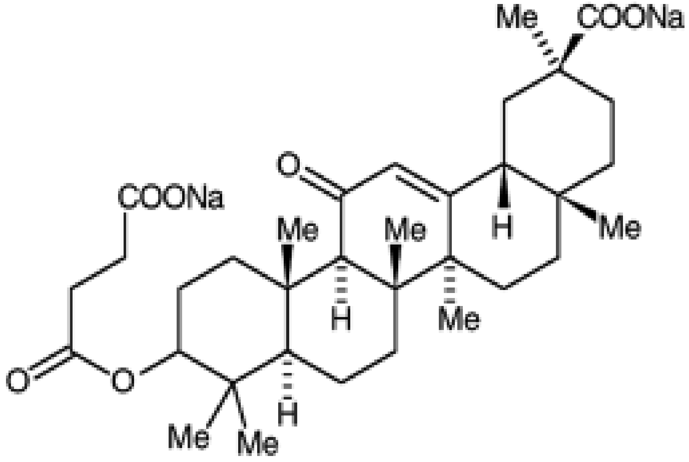
B. 2-[[2-(acetyloxy)benzoyl]oxy]benzoic acid (acetylsalicylsalicylic acid),

C. 2-hydroxybenzoic acid (salicylic acid).

Ph Eur

Carbenoxolone Sodium

General Notices



$C_{34}H_{48}Na_2O_7$ 614.7 *7421-40-1*

Action and use

Treatment of peptic ulcer.

DEFINITION

Carbenoxolone Sodium is disodium 3β-(3-carboxylatopropionyloxy)-11-oxo-olean-12-en-30-oate. It contains not less than 97.0% and not more than 103.0% of $C_{34}H_{48}Na_2O_7$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white or pale cream powder; hygroscopic.

Freely soluble in *water*; sparingly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. Dissolve 0.1 g in 5 ml of *water* and make just acid with *2 M hydrochloric acid*, stir well and filter. Wash the residue with *water* until the washings are no longer acidic and dry to constant weight at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of carbenoxolone *(RS 041)*.

B. Yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

pH of a 10% w/v solution, 8.0 to 9.2, Appendix V L.

Specific optical rotation

In a 1% w/v solution in a mixture of equal volumes of *methanol* and *0.02 M sodium carbonate*, +132 to +140, calculated with reference to the anhydrous substance, Appendix V F.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 60 volumes of *ethyl acetate*, 20 volumes of *methanol*, 11 volumes of *water* and 1 volume of *13.5 M ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions of the substance being examined in *methanol* containing (1) 1.50% w/v and (2) 0.030% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Spray with a 1.5% w/v solution of *vanillin* in *sulphuric acid (60%)* and heat at 105° for 10 to 15 minutes. By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%).

Water

Not more than 4.0% w/w, Appendix IX C. Use 0.6 g.

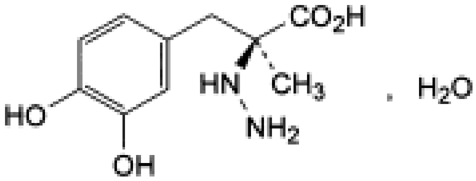
ASSAY

Dissolve 1 g in 30 ml of *water*, add 30 ml of *chloroform* and 15 ml of a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *water*, shake and allow to separate. Add the chloroform layer to 40 ml of a 20% w/v solution of *sodium chloride*, shake and allow to separate. Repeat the extraction with four 15 ml quantities of *chloroform*, combine the chloroform extracts and add sufficient *chloroform* to produce 100 ml. Evaporate 25 ml, dry the residue at 100° at a pressure of 2 kPa, dissolve in 10 ml of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and *thymol blue solution* as indicator. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 30.73 mg of $C_{34}H_{48}Na_2O_7$.

Carbidopa

General Notices

(Ph Eur monograph 0755)



$C_{10}H_{14}N_2O_4,H_2O$ 244.2 *38821-49-7*

Action and use

Dopa decarboxylase inhibitor.

Preparation

Co-careldopa Tablets

Ph Eur

DEFINITION

(2*S*)-3-(3,4-Dihydroxyphenyl)-2-hydrazino-2-methylpropanoic acid monohydrate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Slightly soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in dilute solutions of mineral acids.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D, E.*

A. Specific optical rotation (see Tests).

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 50.0 mg in a 8.5 g/l solution of *hydrochloric acid R* in *methanol R* and dilute to 100.0 ml with the same solution. Dilute 10.0 ml of this solution to 100.0 ml with a 8.5 g/l solution of *hydrochloric acid R* in *methanol R* .

Spectral range 230-350 nm.

Absorption maximum At 283 nm.

Specific absorbance at the absorption maximum 135 to 150 (dried substance).

C. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *carbidopa CRS.*

D. Shake vigorously about 5 mg with 10 ml of *water R* for 1 min and add 0.3 ml of *ferric chloride solution R2* . An intense green colour is produced, which quickly turns to reddish-brown.

E. Suspend about 20 mg in 5 ml of *water R* and add 5 ml of *cupri-tartaric solution R* . On heating, the colour of the solution changes to dark brown and a red precipitate is formed.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 or B_6 *(2.2.2, Method II)*.

Dissolve 0.25 g in 25 ml of *1 M hydrochloric ac*id.

Specific optical rotation *(2.2.7)*

- 22.5 to - 26.5 (dried substance).

With the aid of an ultrasonic bath, dissolve completely 0.250 g in *aluminium chloride solution R* and dilute to 25.0 ml with the same solution.

Hydrazine

Thin-layer chromatography *(2.2.27)* .

Test solution (a) Dissolve 0.50 g in *dilute hydrochloric acid R* and dilute to 2.0 ml with the same acid.

Test solution (b) Place 25 g of *strongly basic anion exchange resin R* into each of 2 conical flasks with ground-glass stoppers. To each, add 150 ml of *carbon dioxide-free water R* and shake from time to time during 30 min. Decant the liquid from both flasks and repeat the process with further quantities, each of 150 ml, of *carbon dioxide-free water R.*

Take two 100 ml measuring cylinders 3.5-4.5 cm in internal diameter and label these A and B. Into cylinder A, transfer as completely as possible the resin from 1 conical flask using 60 ml of *carbon dioxide-free water R* ; into cylinder B, transfer the 2nd quantity of resin, this time using 20 ml of *carbon dioxide-free water R.*

Into each cylinder, insert a gas-inlet tube, the end of which has an internal diameter of 2-3 mm and which reaches almost to the bottom of the cylinder. Pass a rapid stream of *nitrogen for chromatography R* through each mixture so that homogeneous suspensions are formed. After 30 min, without interrupting the gas flow, add 1.0 ml of test solution (a) to cylinder A; after 1 min stop the gas flow into cylinder A and transfer the contents, through a moistened filter paper, into cylinder B. After 1 min, stop the gas flow to cylinder B and pour the solution immediately through a moistened filter paper into a freshly prepared mixture of 1 ml of a 200 g/l solution of *salicylaldehyde R* in *methanol R* and 20 ml of *phosphate buffer solution pH 5.5 R* in a conical flask; shake thoroughly for 1 min and heat in a water-bath at 60 °C for 15 min. The liquid becomes clear. Allow to cool, add 2.0 ml of *toluene R* and shake vigorously for 2 min. Transfer the mixture into a centrifuge tube and centrifuge.

Separate the toluene layer in a 100 ml separating funnel and shake vigorously with 2 quantities, each of 20 ml, of a 200 g/l solution of *sodium metabisulphite R* and finally with 2 quantities, each of 50 ml, of *water R* . Separate the toluene layer.

Reference solution (a) Dissolve 10 mg of *hydrazine sulphate R* in *dilute hydrochloric acid* R and dilute to 50 ml with the same acid. Dilute 1.0 ml of this solution to 10.0 ml with *dilute hydrochloric acid R* .

Reference solution (b) Prepare the solution at the same time and in the same manner as described for test solution (b) using 1.0 ml of reference solution (a) instead of 1.0 ml of test solution (a).

Plate *TLC silanised silica gel plate R* .

Mobile phase *water R, methanol R* (10:20 *V/V*).

Application 10 µl of test solution (b) and reference solution (b).

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Limit:

— *hydrazine*: any spot showing a yellow fluorescence is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (20 ppm).

Methyldopa and methylcarbidopa

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.100 g of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 10.0 ml with the same acid.

Reference solution (a) Dissolve the contents of a vial of *methylcarbidopa CRS* in *0.1 M hydrochloric acid* , add 1 mg of *methyldopa CRS* and dilute to 20.0 ml with the same acid.

Reference solution (b) Dissolve 5 mg of *carbidopa CRS* and 5 mg of *methyldopa CRS* in

0.1 M hydrochloric acid and dilute to 10.0 ml with the same acid.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, 14 g/l solution of *potassium dihydrogen phosphate R* (2:98 *V/V*) .

Flow rate 1 ml/min.

Detection Spectrophotometer at 282 nm.

Injection 20 µl.

System suitability Reference solution (b):

- *resolution*: minimum 4.0 between the peaks due to methyldopa and carbidopa.

Limits:

- *methyldopa and methylcarbidopa*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

6.9 per cent to 7.9 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g with gentle heating in 75 ml of *anhydrous acetic acid R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 22.62 mg of $C_{10}H_{14}N_2O_4$.

STORAGE

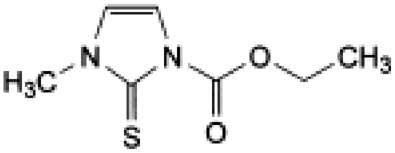
Protected from light.

Ph Eur

Carbimazole

General Notices

(Ph Eur monograph 0884)



$C_7H_{10}N_2O_2S$ 186.2 *22232-54-8*

Action and use

Thionamide antithyroid drug.

Preparation

Carbimazole Tablets

Ph Eur

DEFINITION

Ethyl 3-methyl-2-thioxo-2,3-dihydro-1*H*-imidazole-1-carboxylate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Slightly soluble in water, soluble in acetone and in alcohol.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 122 °C to 125 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *carbimazole CRS.*

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *carbimazole CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel GF_{254} plate R*.

Mobile phase *acetone R, methylene chloride R* (20:80 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air for 30 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in a mixture of 50 ml of *water R* and 0.05 ml of *dilute hydrochloric acid R* . Add 1 ml of *potassium iodobismuthate solution R* . A red precipitate is formed.

TESTS

Impurity A and other related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 5.0 mg of the substance to be examined in 10.0 ml of a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* . Use this solution within 5 min of preparation.

Reference solution (a) Dissolve 5 mg of *thiamazole R* and 0.10 g of *carbimazole CRS* in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* .

Reference solution (b) Dissolve 5.0 mg of *thiamazole R* in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 10.0 ml with the same mixture of solvents. Dilute 1.0 ml of this solution to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R.*

Column:

— *size*: *l* = 0.15 m, Ø = 3.9 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water R* (10:90 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 1.5 times the retention time of carbimazole.

Retention time Carbimazole = about 6 min.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to impurity A and carbimazole.

Limits:

— *impurity A*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *any other impurity*: not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 24 h.

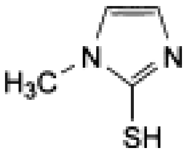
Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 50.0 mg in *water R* and dilute to 500.0 ml with the same solvent. To 10.0 ml add 10 ml of *dilute hydrochloric acid R* and dilute to 100.0 ml with *water R.* Measure the absorbance *(2.2.25)* at the maximum at 291 nm. Calculate the content of $C_7H_{10}N_2O_2S$ taking the specific absorbance to be 557.

IMPURITIES



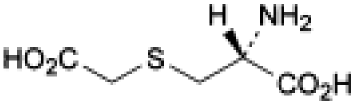
A. 1-methyl-1*H*-imidazole-2-thiol (thiamazole).

Ph Eur

Carbocisteine

General Notices

(Ph Eur monograph 0885)



$C_5H_9NO_4S$ 179.2 *2387-59-9*

Action and use

Mucolytic.

Ph Eur

DEFINITION

Carbocisteine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*R*)-2-amino-3-[(carboxymethyl)sulphanyl]propanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white crystalline powder, practically insoluble in water and in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *carbocisteine CRS* . Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g in 4.5 ml of *dilute sodium hydroxide solution R*. Heat on a water-bath for 10 min. Cool and add 1 ml of a 25 g/l solution of *sodium nitroprusside R*. A dark red colour is produced, which changes to brown and then to yellow within a few minutes.

TESTS

Solution S

Disperse 5.00 g in 20 ml of *water R* and add dropwise with shaking 2.5 ml of *strong sodium hydroxide solution R*. Adjust to pH 6.3 with *1 M sodium hydroxide* and dilute to 50.0 ml with *water R*.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

Shake 0.2 g with 20 ml of *carbon dioxide-free water R*. The pH of the suspension is 2.8 to 3.0.

Specific optical rotation *(2.2.7)*

- 32.5 to - 35.5, determined on solution S and calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dilute ammonia R2* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *carbocisteine CRS* in *dilute ammonia R2* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *carbocisteine CRS* and 10 mg of *arginine hydrochloride CRS* in 5 ml of *dilute ammonia R2* and dilute to 25 ml with *water R*.

Apply separately to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Dry the plate in a current of warm air. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Chlorides *(2.4.4)*

Dissolve 33 mg in 5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution, without further addition of nitric acid, complies with the limit test for chlorides (0.15 per cent).

Sulphates ***(2.4.13)***

Dissolve 0.5 g in 5 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Heavy metals ***(2.4.8)***

2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Not more than 0.3 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 10 ml of *anhydrous formic acid R* with slight heating and shake until dissolution is complete. Add 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 17.92 mg of $C_5H_9NO_4S$.

STORAGE

Store protected from light.

Ph Eur

Carbomers

General Notices

(Ph Eur monograph 1299)

Action and use

Stabilizer in pharmaceutical products.

Preparation

Carbomer Eye Drops

Ph Eur

DEFINITION

High-molecular-mass polymers of acrylic acid cross-linked with polyalkenyl ethers of sugars or polyalcohols.

Content

56.0 per cent to 68.0 per cent of carboxylic acid ($-CO_2H$) groups (dried substance).

CHARACTERS

Appearance

White or almost white, fluffy, hygroscopic, powder.

Solubility

Swells in water and in other polar solvents after dispersion and neutralisation with sodium hydroxide solution.

IDENTIFICATION

First identification *A, E.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Main bands At 1710 ± 5 cm^{-1}, 1454 ± 5 cm^{-1}, 1414 ± 5 cm^{-1}, 1245 ± 5 cm^{-1}, 1172 ± 5 cm^{-1}, 1115 ± 5 cm^{-1} and 801 ± 5 cm^{-1}, with the strongest band at 1710 ± 5 cm^{-1}.

B. Adjust a 10 g/l dispersion to about pH 7.5 with *1 M sodium hydroxide* . A highly viscous gel is formed.

C. Add 2 ml of a 100 g/l solution of *calcium chloride R* with continuous stirring to 10 ml of the gel from identification test B. A white precipitate is immediately produced.

D. Add 0.5 ml of *thymol blue solution R* to 10 ml of a 10 g/l dispersion; an orange colour is produced. Add 0.5 ml of *cresol red solution R* to 10 ml of a 10 g/l dispersion. A yellow colour is produced.

E. It complies with the nominal apparent viscosity indicated on the label.

TESTS

Apparent viscosity

The nominal apparent viscosity is between 300 mPa·s and 115 000 mPa·s. For a product with a nominal apparent viscosity of 20 000 mPa·s or greater, the apparent viscosity is 70.0 per cent to 130.0 per cent of the value stated on the label; for a product with a nominal apparent viscosity of less than 20 000 mPa·s, the apparent viscosity is 50.0 per cent to 150.0 per cent of the value stated on the label.

Dry the substance to be examined *in vacuo* at 80 °C for 1 h. Carefully add 2.50 g of the previously dried substance to be examined to 500 ml of *water R* in a 1000 ml beaker while stirring continuously at 1000 ± 50 r/min, with the stirrer shaft set at an angle of 60° to one side of the beaker. Add the previously dried substance over a period of 45-90 s, at a uniform rate, ensuring that loose aggregates of powder are broken up, and continue stirring at 1000 ± 50 r/min for 15 min. Remove the stirrer and place the beaker containing the dispersion in a water-bath at 25 ± 0.2 °C for 30 min. Insert the stirrer to a depth necessary to ensure that air is not drawn into the dispersion and, while stirring at 300 ± 25 r/min, titrate with a glass-calomel electrode system to pH 7.3-7.8 by adding a 180 g/l solution of *sodium hydroxide R* below the surface, determining the end-point potentiometrically (*2.2.20*). The total volume of the 180 g/l solution of *sodium hydroxide R* used is about 6.2 ml. Allow 2-3 min before the final pH determination. If the final pH exceeds 7.8, discard the preparation and prepare another using a smaller amount of sodium hydroxide for titration. Return the neutralised preparation to the water-bath at 25 °C for 1 h, then perform the viscosity determination without delay to avoid slight viscosity changes that occur 75 min after neutralisation. Determine the viscosity (*2.2.10*) using a rotating viscometer with a spindle rotating at 20 r/min, using a spindle suitable for the expected apparent viscosity.

Free acrylic acid

Liquid chromatography (*2.2.29*).

Test solution Mix 0.125 g of the substance to be examined with a 25 g/l solution of *aluminium potassium sulphate R* and dilute to 25.0 ml with the same solution. Heat the suspension at 50 °C for 20 min with shaking. Then shake the suspension at room temperature for 60 min. Centrifuge and use the clear supernatant solution as the test solution.

Reference solution Dissolve 62.5 mg of *acrylic acid R* in a 25 g/l solution of *aluminium potassium sulphate R* and dilute to 100.0 ml with the same solution. Dilute 1.0 ml of this solution to 50.0 ml with a 25 g/l solution of *aluminium potassium sulphate R.*

Column:

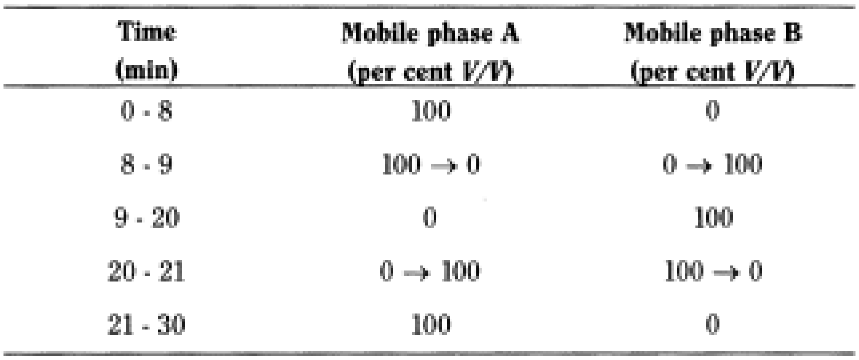
— *size*: *l* = 0.12 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: 1.361 g/l solution of *potassium dihydrogen phosphate R*, adjusted to pH 2.5 using *dilute phosphoric acid R*;

— *mobile phase B*: 1.361 g/l solution of *potassium dihydrogen phosphate R*, *acetonitrile for chromatography R* (50:50 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	100	0
8 - 9	100 → 0	0 → 100
9 - 20	0	100
20 - 21	0 → 100	100 → 0
21 - 30	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl.

Retention time Acrylic acid = about 6.0 min.

Limit:

— *acrylic acid*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.25 per cent).

Benzene

Gas chromatography (*2.4.24, System A*).

Solution A Dissolve 0.100 g of *benzene R* in *dimethyl sulphoxide R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R.*

Test solution Weigh 50.0 mg of the substance to be examined into an injection vial and add 5.0 ml of *water R* and 1.0 ml of *dimethyl sulphoxide R.*

Reference solution Weigh 50.0 mg of the substance to be examined into an injection vial and add 4.0 ml of *water R*, 1.0 ml of *dimethyl sulphoxide R* and 1.0 ml of solution A.

Close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous dispersion.

Static head-space conditions that may be used:

— *equilibration temperature*: 80 °C;

— *equilibration time*: 60 min;

— *transfer line temperature*: 90 °C.

Injection 1 ml of the gaseous phase of the test solution and 1 ml of the gaseous phase of the reference solution; repeat these injections twice more.

System suitability:

— *repeatability*: maximum relative standard deviation of the differences in area between the analyte peaks obtained from the 3 replicate pair injections of the reference solution and the test solution is 15 per cent.

Limit:

— *benzene* : the mean area of the peak due to benzene in the chromatograms obtained with the test solution is not greater than 0.5 times the mean area of the peak due to benzene in the chromatograms obtained with the reference solution (2 ppm).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 3.0 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 60 min.

Sulphated ash (*2.4.14*)

Maximum 4.0 per cent, determined on 1.0 g.

ASSAY

Slowly add 50 ml of *water R* to 0.120 g whilst stirring and heating at 60 °C for 15 min. Stop heating, add 150 ml of *water R* and continue stirring for 30 min. Add 2 g of *potassium chloride R* and titrate with *0.2 M sodium hydroxide* , determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.2 M sodium hydroxide* is equivalent to 9.0 mg of carboxylic acid (-CO_2H) groups.

STORAGE

In an airtight container.

LABELLING

The label states the nominal apparent viscosity.

Ph Eur

Carbon Dioxide

General Notices

(Ph Eur monograph 0375)

CO_2 44.01 *124-38-9*

Carbon Dioxide should be kept in approved metal cylinders which are painted grey and carry a label stating 'Carbon Dioxide'. In addition, 'Carbon Dioxide' or the symbol 'CO_2' should be stencilled in paint on the shoulder of the cylinder.

Ph Eur

DEFINITION

Content

Minimum 99.5 per cent *V/V* of CO_2 in the gaseous phase.

This monograph applies to carbon dioxide for medicinal use.

CHARACTERS

Appearance

Colourless gas.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 1 volume of water.

PRODUCTION

Examine the gaseous phase.

If the test is performed on a cylinder of gas, keep the cylinder of the substance to be examined at room temperature for not less than 6 h before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost.

Carbon monoxide

Gas chromatography *(2.2.28)* .

Gas to be examined The substance to be examined.

Reference gas A mixture containing 5 ppm *V/V* of *carbon monoxide R* in *nitrogen R1.*

Column:

— *material*: stainless steel,

— *size*: l = 2 m, Ø = 4 mm,

— *stationary phase*: an appropriate molecular sieve for chromatography (0.5 nm).

Carrier gas *helium for chromatography R* .

Flow rate 60 ml/min.

Temperature:
— *column*: 50 °C,

— *injection port and detector*: 130 °C.

Detection Flame ionisation with methaniser.

Injection Loop injector.

Adjust the injected volumes and the operating conditions so that the height of the peak due to carbon monoxide in the chromatogram obtained with the reference gas is at least 35 per cent of the full scale of the recorder.

Limit:
— *carbon monoxide* : not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (5 ppm *V/V*).

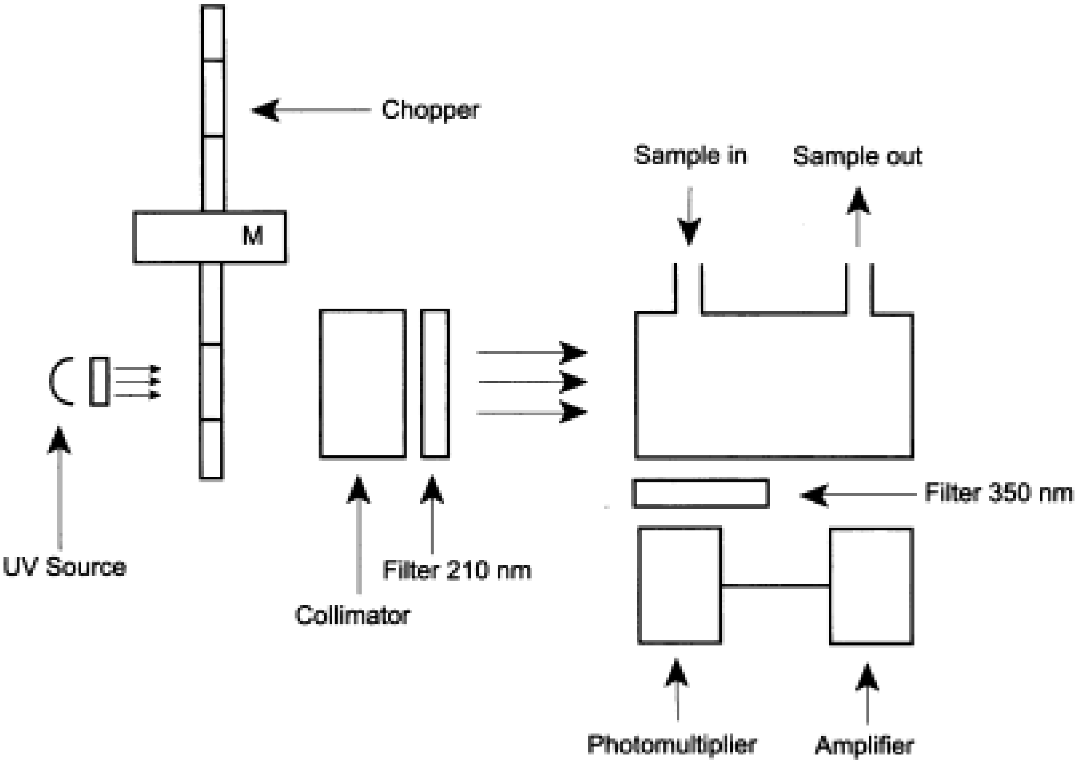

Chopper
M
Sample in
Sample out
UV Source
Collimator
Filter 210 nm
Filter 350 nm
Photomultiplier
Amplifier

Figure 0375.-1.– *UV Fluorescence Analyser*

Nitrogen monoxide and nitrogen dioxide

Maximum 2 ppm *V/V* in total, determined using a chemiluminescence analyser *(2.5.26)* .

Gas to be examined The substance to be examined.

Reference gas (a) *Carbon dioxide R1.*

Reference gas (b) A mixture containing 2 ppm *V/V* of *nitrogen monoxide R* in *carbon dioxide R1* or in *nitrogen R1* .

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of nitrogen monoxide and nitrogen dioxide in the gas to be examined.

If nitrogen is used instead of carbon dioxide in reference gas (b), multiply the result obtained by the quenching correction factor in order to correct the quenching effect on the analyser response caused by the carbon dioxide matrix effect.

The quenching correction factor is determined by applying a known reference mixture of nitrogen monoxide in carbon dioxide and comparing the actual content with the content indicated by the analyser which has been calibrated with a NO/N_2 reference mixture.

$$\text{Quenching correction factor} = \frac{\text{actual nitrogen monoxide content}}{\text{indicated nitrogen monoxide content}}$$

Total sulphur

Maximum 1 ppm *V/V*, determined using an ultraviolet fluorescence analyser after oxidation of the sulphur compounds by heating at 1000 °C (Figure 0375.-1).

The apparatus consists of the following:

— a system generating ultraviolet radiation with a wavelength of 210 nm, made up of an ultraviolet lamp, a collimator, and a selective filter; the beam is blocked periodically by a chopper rotating at high speed,

— a reaction chamber through which flows the previously filtered gas to be examined,

— a system that detects radiation emitted at a wavelength of 350 nm, made up of a selective filter, a photomultiplier tube and an amplifier.

Gas to be examined The substance to be examined.

Reference gas (a) *Carbon dioxide R1.*

Reference gas (b) A mixture containing between 0.5 ppm *V/V* and 2 ppm *V/V* of *hydrogen sulphide R1* in *carbon dioxide R1* .

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Pass the gas to be examined through a quartz oven heated to 1000 °C. *Oxygen R* is circulated in the oven at a tenth of the flow rate of the gas to be examined. Measure the sulphur dioxide content in the gaseous mixture leaving the oven.

Water

Maximum 67 ppm *V/V*, determined using an electrolytic hygrometer *(2.5.28)* .

ASSAY

Infrared analyser *(2.5.24)* .

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Carbon dioxide R1.*

Reference gas (b) A mixture containing 95.0 per cent *V/V* of *carbon dioxide R1* and 5.0 per cent *V/V* of *nitrogen R1* .

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon dioxide in the gas to be examined.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of carbon dioxide.*

B. Place a glowing splinter of wood in an atmosphere of the substance to be examined. It is extinguished.

C. Pass a stream of the substance to be examined through *barium hydroxide solution R* . A white precipitate is formed which dissolves with effervescence in *dilute acetic acid R* .

TESTS

Examine the gaseous phase.

If the test is performed on a cylinder of gas, keep the cylinder of the substance to be examined at room temperature for not less than 6 h before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost.

Carbon monoxide

Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube *(2.1.6)*.

Hydrogen sulphide

Maximum 1 ppm *V/V*, determined using a hydrogen sulphide detector tube *(2.1.6)*.

Nitrogen monoxide and nitrogen dioxide

Maximum 2 ppm *V/V* in total, determined using a nitrogen monoxide and nitrogen dioxide detector tube *(2.1.6)*.

Sulphur dioxide

Maximum 2 ppm *V/V*, determined using a sulphur dioxide detector tube *(2.1.6)*.

Water vapour

Maximum 67 ppm *V/V*, determined using a water vapour detector tube *(2.1.6)*.

STORAGE

Store liquefied under pressure in suitable containers complying with the legal regulations.

IMPURITIES

A. nitrogen monoxide,

B. nitrogen dioxide,

C. carbon monoxide,

D. total sulphur,

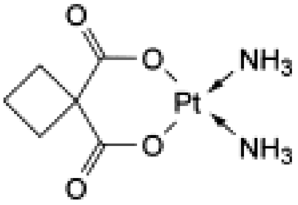
E. water.

Ph Eur

Carboplatin

General Notices

(Ph Eur monograph 1081)



$C_6H_{12}N_2O_4Pt$ 371.3   *41575-94-4*

Action and use

Platinum-containing cytotoxic.

Preparation

Carboplatin Injection

Ph Eur

DEFINITION

(*SP*-4-2)-Diammine[cyclobutan-1,1-dicarboxylato(2-)-*C*,*C′*]platin.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Colourless, crystalline powder.

Solubility

Sparingly soluble in water, very slightly soluble in acetone and in ethanol (96 per cent).

mp: about 200 °C, with decomposition.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of carboplatin.*

TESTS

Solution S1

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Solution S2

Dissolve 1.0 g in *water R* , heating slightly if necessary, and dilute to 40 ml with the same solvent; filter if necessary.

Appearance of solution

Solution S1 is clear *(2.2.1)* and colourless (*2.2.2, Method II)*.

Impurity B and acidity

Maximum 0.5 per cent, calculated as impurity B.

To 10 ml of solution S1 add 0.1 ml of *phenolphthalein solution R1* . The solution is colourless. Not more than 0.7 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R and water R* , then dilute to 20.0 ml with the same mixture of solvents.

Reference solution Dilute 0.5 ml of the test solution to 200.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: aminopropylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R, acetonitrile R* (13:87 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

Run time 2.5 times the retention time of carboplatin.

System suitability Test solution:

- *number of theoretical plates* : minimum 5000; if necessary, adjust the concentration of acetonitrile in the mobile phase;

— *mass distribution ratio* : minimum 4.0; if necessary, adjust the concentration of acetonitrile in the mobile phase;

— *symmetry factor* : maximum 2.0; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Chlorides ***(2.4.4)***

Maximum 100 ppm.

Dilute 10 ml of solution S2 to 15 ml with *water R* . Prepare the standard using 5 ml of *chloride standard solution (5 ppm Cl) R.*

Ammonium *(2.4.1, Method B)*

Maximum 100 ppm, determined on 0.20 g.

Prepare the standard using 0.2 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Silver

Maximum 10.0 ppm.

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution Dissolve 0.50 g in a 1 per cent *V/V* solution of *nitric acid R* and dilute to 50.0 ml with the same solution.

Reference solutions Prepare the reference solutions using *silver standard solution (5 ppm Ag) R* , diluting with a 1 per cent *V/V* solution of *nitric acid R.*

Wavelength 328.1 nm.

Soluble barium

Maximum 10.0 ppm.

Atomic emission spectrometry *(2.2.22, Method I*).

Test solution Use the solution described in the test for silver.

Reference solutions Prepare the reference solutions using *barium standard solution (50 ppm Ba) R* , diluting with a 1 per cent *V/V* solution of *nitric acid R.*

Wavelength 455.4 nm.

Heavy metals *(2.4.8*)

Maximum 20 ppm.

12 ml of solution S2 complies with test A. Prepare the reference solution using 5 ml of *lead standard solution (1 ppm Pb R)*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Use the residue obtained in the test for loss on drying.

Ignite 0.200 g of the residue to constant mass at 800 ± 50 °C.

1 mg of the residue is equivalent to 1.903 mg of $C_6H_{12}N_2O_4Pt$.

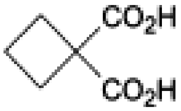
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B.

A. cisplatin,



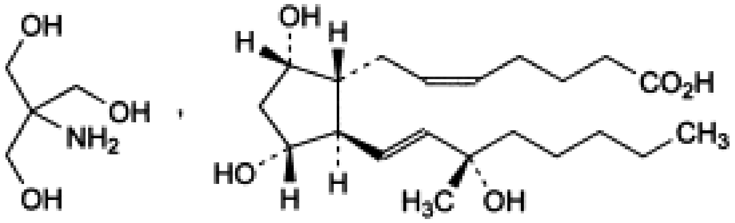
B. cyclobutane-1,1-dicarboxylic acid.

Ph Eur

Carboprost Trometamol

General Notices

(Ph Eur monograph 1712)



$C_{25}H_{47}NO_8$ 489.7 *58551-69-2*

Action and use

Prostaglandin ($PGF_{2\alpha}$) analogue.

Ph Eur

DEFINITION

2-Amino-2-(hydroxymethyl)propane-1,3-diol (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(1*E*,3*S*)-3-hydroxy-3-methyloct-1-enyl]cyclopentyl]hept-5-enoate ((15*S*)-15-methyl-PGF_2).

Content

94.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of carboprost trometamol.*

TESTS

Specific optical rotation *(2.2.7)*

+ 18 to + 24 (anhydrous substance).

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29*).

Test solution Dissolve 15.0 mg of the substance to be examined in a mixture of 23 volumes of *acetonitrile R* and 77 volumes of *water for chromatography R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 15.0 mg of *carboprost trometamol CRS* (containing impurity A) in a mixture of 23 volumes of *acetonitrile R* and 77 volumes of *water for chromatography R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of reference solution (a) and 0.15 ml of *(15R)-15-methylprostaglandin F* $_{2\alpha}$ *R* (impurity B) to 100.0 ml with a mixture of 23 volumes of *acetonitrile R* and 77 volumes of *water for chromatography R .*

Reference solution (c) Dilute 2.0 ml of the test solution to 20.0 ml with a mixture of 23 volumes of *acetonitrile R* and 77 volumes of *water for chromatography R* . Dilute 2.0 ml of this solution to 20.0 ml with a mixture of 23 volumes of *acetonitrile R* and 77 volumes of *water for chromatography R.*

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 8-10 nm and a carbon loading of 12-19 per cent.

Mobile phase Mix 23 volumes of *acetonitrile R1* and 77 volumes of a 2.44 g/l solution of *sodium dihydrogen phosphate R* in *water for chromatography R* previously adjusted to pH 2.5 with *phosphoric acid R .*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 200 nm.

Injection 20 µl.

Run time 1.3 times the retention time of carboprost.

Relative retention With reference to carboprost (retention time = about 80 min): impurity B = about 0.85; impurity A = about 0.9.

Identification of impurities Use the chromatogram obtained with reference solution (a) and the chromatogram supplied with *carboprost trometamol CRS* to identify the peak due to impurity A.

System suitability:

— *resolution:* minimum 3.4 between the peaks due to impurity B and carboprost in the chromatogram obtained with reference solution (b);

— *peak-to-valley ratio* : minimum 3.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B in the chromatogram obtained with reference solution (a).

Limits:

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3.0 per cent),

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

— *any other impurity*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4.0 per cent),

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water *(2.5.32)*

Maximum 0.5 per cent, determined on 50 mg.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Mix 27 volumes of *acetonitrile R1* and 73 volumes of a 2.44 g/l solution of *sodium dihydrogen phosphate R* in *water for chromatography R* previously adjusted to pH 2.5 with *phosphoric acid R* .

Injection Test solution and reference solution (a).

Run time 1.2 times the retention time of carboprost.

Retention time Carboprost = about 29 min.

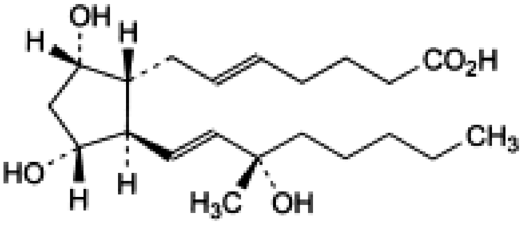
Calculate the percentage content of $C_{25}H_{47}NO_8$ using the declared content of *carboprost trometamol CRS* .

STORAGE

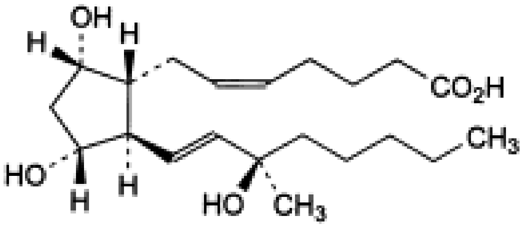
At a temperature below - 15 °C.

IMPURITIES

Specified impurities A, B.



A. (5*E*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(1*E*,3*S*)-3-hydroxy-3-methyloct-1-enyl] cyclopentyl]hept-5-enoic acid,



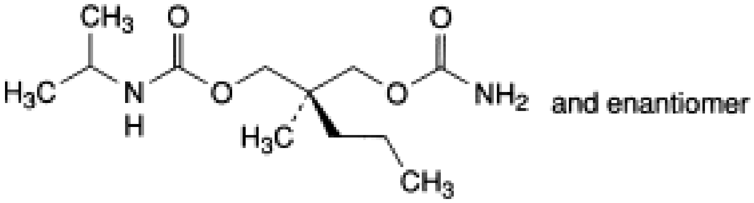
B. (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(1*E*,3*R*)-3-hydroxy-3-methyloct-1-enyl] cyclopentyl]hept-5-enoic acid.

Ph Eur

Carisoprodol

General Notices

(Ph Eur monograph 1689)



$C_{12}H_{24}N_2O_4$ 260.3 *78-44-4*

Action and use

Skeletal muscle relaxant.

Ph Eur

DEFINITION

(2*RS*)-2-[(Carbamoyloxy)methyl]-2-methylpentyl (1-methylethyl)carbamate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, fine powder.

Solubility

Very slightly soluble in water, freely soluble in acetone, in alcohol and in methylene chloride.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point *(2.2.14)* : 92 °C to 95 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *carisoprodol CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results: The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (d).

D. Dissolve 0.2 g in 15 ml of a 28 g/l solution of *potassium hydroxide R* in *alcohol R* and boil under a reflux condenser for 15 min. Add 0.5 ml *of glacial acetic acid R* and 1 ml of a 50 g/l solution of *cobalt nitrate R in ethanol R* . An intense blue colour develops.

TESTS

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 2.5 g in *alcohol R* and dilute to 25.0 ml with the same solvent.

Related substances

Thin-layer chromatography *(2.2.27).*

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methylene chloride R* .

Reference solution (a) Dissolve 5.0 mg *of meprobamate CRS* in *methylene chloride R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 50 ml with *methylene chloride R.*

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with *methylene chloride R.*

Reference solution (d) Dissolve 20 mg of *carisoprodol CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (e) Dissolve 10 mg of *carisoprodol impurity A CRS* in 5 ml of reference solution (d) and dilute to 50 ml with *methylene chloride R* .

Plate *TLC silica gel plate R* .

Mobile phase *acetone R, methylene chloride R* (20:80 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air for 15 min.

Detection Spray with a solution prepared as follows: dissolve 5 g of *phosphomolybdic acid R* in a mixture of 50 ml of *glacial acetic acid R* and 10 ml of *sulphuric acid R* , and dilute to 100 ml with *glacial acetic acid R* . Heat the plate at 100-105 °C for 30 min.

System suitability:

— the chromatogram obtained with reference solution (c) shows 1 clearly visible spot,

— the chromatogram obtained with reference solution (e) shows 2 clearly separated spots.

Limits In the chromatogram obtained with test solution (a):

— *impurity D*: any spot due to impurity D is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *any other impurity*: any spot, apart from the principal spot and any spot due to impurity D, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R* .

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g *in vacuo* at 60 °C for 3 h.

Sulphated ash ***(2.4.14)***

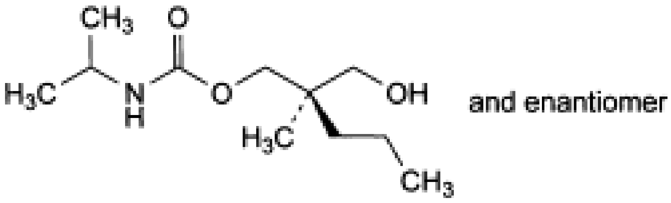
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

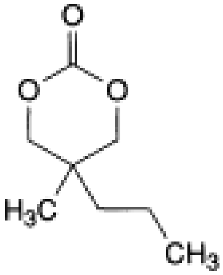
Dissolve 0.100 g in 15 ml of a 25 per cent *V/V* solution of *sulphuric acid R* and boil under a reflux condenser for 3 h. Cool, dissolve by cautiously adding 30 ml of *water R* , cool again and place in a steam-distillation apparatus. Add 40 ml of *strong sodium hydroxide solution R* and distil immediately by passing steam through the mixture. Collect the distillate into 40 ml of a 40 g/l solution of *boric acid R* until the total volume in the receiver reaches about 200 ml. Add 0.25 ml of *methyl red mixed solution R* . Titrate with *0.1 M hydrochloric acid* , until the colour changes from green to violet. Carry out a blank titration.

1 ml of *0.1 M hydrochloric acid* is equivalent to 13.02 mg of $C_{12}H_{24}N_2O_4$.

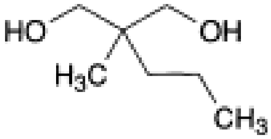
IMPURITIES



A. (2*RS*)-2-(hydroxymethyl)-2-methylpentyl (1-methylethyl)carbamate,



B. 5-methyl-5-propyl-1,3-dioxan-2-one,



C. 2-methyl-2-propylpropane-1,3-diol,

D. meprobamate.

Ph Eur

Carmellose Calcium

General Notices

(Ph Eur monograph 0886)

9050-04-8

Action and use

Excipient in pharmaceutical products; bulk laxative.

Ph Eur

DEFINITION

Calcium salt of a partly *C*-carboxymethylated cellulose.

CHARACTERS

Appearance

White or yellowish-white powder, hygroscopic after drying.

Solubility

Practically insoluble in acetone, in alcohol and in toluene. It swells with water to form a suspension.

IDENTIFICATION

A. Shake 0.1 g thoroughly with 10 ml of *water R* . Add 2 ml of *dilute sodium hydroxide solution R* and allow to stand for 10 min (solution A). Dilute 1 ml of solution A to 5 ml with *water R* . To 0.05 ml add 0.5 ml of a 0.5 g/l solution of chromotropic acid, *sodium salt R* in a 75 per cent *m/m* solution of *sulphuric acid R* and heat on a water-bath for 10 min. A reddish-violet colour develops.

B. Shake 5 ml of solution A obtained in identification test A with 10 ml of *acetone R* . A white, flocculent precipitate is produced.

C. Shake 5 ml of solution A obtained in identification test A with 1 ml of *ferric chloride solution R1* . A brown, flocculent precipitate is formed.

D. Ignite 1 g and dissolve the residue in a mixture of 5 ml of *acetic acid R* and 10 ml of *water R* . Filter if necessary and boil the filtrate for a few minutes. Cool and neutralise with *dilute ammonia R1* . The solution gives reaction (a) of calcium *(2.3.1)* .

TESTS

Solution S

Shake 1.0 g with 50 ml of *distilled water R*, add 5 ml of *dilute sodium hydroxide solution R* and dilute to 100 ml with *distilled water R.*

Alkalinity

Shake 1.0 g thoroughly with 50 ml of *carbon dioxide-free water R* and add 0.05 ml of *phenolphthalein solution R*. No red colour develops.

Chlorides *(2.4.4)*

Maximum 0.36 per cent.

Heat 28 ml of solution S with 10 ml of *dilute nitric acid R* on a water-bath until a flocculent precipitate is produced. Cool, centrifuge and separate the supernatant liquid. Wash the precipitate with 3 quantities, each of 10 ml, of *water R,* centrifuging each time. Combine the supernatant liquid and the washings and dilute to 100 ml with *water R*. To 25 ml add 6 ml of *dilute nitric acid R* and dilute to 50 ml with *water R*. Dilute 10 ml of the solution to 15 ml with *water R.*

Sulphates *(2.4.13)*

Maximum 1 per cent.

Heat 20 ml of solution S with 1 ml of *hydrochloric acid R* on a water-bath until a flocculent precipitate is produced. Cool, centrifuge and separate the supernatant liquid. Wash the precipitate with 3 quantities, each of 10 ml, of *distilled water R*, centrifuging each time. Combine the supernatant liquid and the washings and dilute to 100 ml with *distilled water R*. To 25 ml add 1 ml of *dilute hydrochloric acid R* and dilute to 50 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

10.0 per cent to 20.0 per cent, determined on 1.0 g in a platinum crucible.

STORAGE

In an airtight container.

Ph Eur

Carmellose Sodium

General Notices

(Ph Eur monograph 0472)

9004-32-4

Action and use

Excipient; bulk laxative.

Preparation

Carmellose Sodium Eye Drops

Ph Eur

DEFINITION

Carmellose sodium (carboxymethylcellulose sodium) is the sodium salt of a partly *O*-carboxymethylated cellulose. It contains not less than 6.5 per cent and not more than 10.8 per cent of sodium (Na), calculated with reference to the dried substance.

CHARACTERS

A white or almost white, granular powder, hygroscopic after drying, practically insoluble in acetone, in ethanol and in toluene. It is easily dispersed in water giving colloidal solutions.

IDENTIFICATION

A. To 10 ml of solution S (see Tests) add 1 ml of *copper sulphate solution R* . A blue, cotton-like precipitate is formed.

B. Boil 5 ml of solution S for a few minutes. No precipitate is formed.

C. The solution prepared from the sulphated ash in the test for heavy metals gives the reactions of sodium *(2.3.1)* .

TESTS

Solution S

Sprinkle a quantity of the substance to be examined equivalent to 1.0 g of the dried substance onto 90 ml of *carbon dioxide-free water R* at 40 °C to 50 °C stirring vigorously. Continue stirring until a colloidal solution is obtained, cool and dilute to 100 ml with *carbon dioxide-free water R.*

Appearance of solution

Solution S is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH ***(2.2.3)***

The pH of solution S is 6.0 to 8.0.

Apparent viscosity

While stirring, introduce a quantity of the substance to be examined equivalent to 2.00 g of the dried substance into 50 ml of *water R* heated to 90 °C. For a product of low viscosity, use if necessary, the quantity required to give the concentration indicated on the label. Allow to cool, dilute to 100.0 ml with *water R* and stir until dissolution is complete. Determine the viscosity *(2.2.10)* using a rotating viscometer at 20 °C and a shear rate of 10 s^{-1}. If it is impossible to obtain a shear rate of exactly 10 s^{-1}, use a shear rate slightly higher and a rate slightly lower and interpolate. The apparent viscosity is not less than 75 per cent and not more than 140 per cent of the value stated on the label.

Sodium glycollate

Place a quantity of the substance to be examined equivalent to 0.500 g of dried substance in a beaker. Add 5 ml of *acetic acid R* and 5 ml of *water R*. Stir until dissolution is complete (about 30 min). Add 80 ml of *acetone R* and 2 g of *sodium chloride R*. Filter through a fast filter paper impregnated with *acetone R* into a volumetric flask, rinse the beaker and filter with *acetone R* and dilute the filtrate to 100.0 ml with the same solvent. Allow to stand for 24 h without shaking. Use the clear supernatant liquid to prepare the test solution.

In a volumetric flask, dissolve 0.310 g of *glycollic acid R*, previously dried *in vacuo* over *diphosphorus pentoxide R*, in *water R* and dilute to 1000.0 ml with the same solvent. Place 5.0 ml of this solution in a volumetric flask, add 5 ml of *acetic acid R* and allow to stand for about 30 min. Add 80 ml of *acetone R* and 2 g of *sodium chloride R* and dilute to 100.0 ml with *acetone R*. Use this solution to prepare the reference solution.

Place 2.0 ml of each solution in a separate 25 ml volumetric flask. Heat on a water-bath to eliminate acetone. Cool to room temperature and add 5.0 ml of *2,7-dihydroxynaphthalene solution R* to each flask. Shake and add 15.0 ml of *2,7-dihydroxynaphthalene solution R*. Close the flasks with aluminium foil and heat on a water-bath for 20 min. Cool under running water and dilute to 25.0 ml with *sulphuric acid R.* Within 10 min, transfer 10.0 ml of each solution to a flat-bottomed tube. Examine the solutions viewing vertically. The test solution is not more intensely coloured than the reference solution (0.4 per cent).

Chlorides ***(2.4.4)***

Dilute 2 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (0.25 per cent).

Heavy metals ***(2.4.8)***

To the residue obtained in the determination of the sulphated ash, add 1 ml of *hydrochloric acid R* and evaporate on a water-bath. Take up the residue in 20 ml of *water R*. 12 ml of the solution complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Not more than 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

20.0 per cent to 33.3 per cent, determined on 1.0 g using a mixture of equal volumes of *sulphuric acid R* and *water R* and calculated with reference to the dried substance. These limits correspond to a content of 6.5 per cent to 10.8 per cent of sodium (Na).

LABELLING

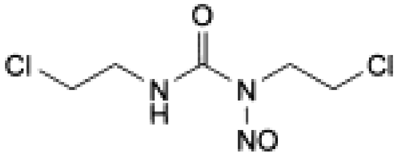
The label states the apparent viscosity in millipascal seconds for a 20 g/l solution; for a product of low viscosity, the label states the concentration of the solution to be used and the apparent viscosity in millipascal seconds.

Ph Eur

Carmustine

General Notices

(Ph Eur monograph 1187)



$C_5H_9Cl_2N_3O_2$ 214.1 *154-93-8*

Action and use

Cytotoxic alkylating agent.

Ph Eur

DEFINITION

Carmustine contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 1,3-bis(2-chloroethyl)-1-nitrosourea, calculated with reference to the anhydrous substance.

CHARACTERS

A yellowish, granular powder, very slightly soluble in water, very soluble in methylene chloride, freely soluble in ethanol.

It melts at about 31 °C with decomposition.

IDENTIFICATION

Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the *Ph. Eur. reference spectrum of carmustine*. Examine the melted substances prepared as films.

TESTS

1,3-bis(2-chloroethyl)urea (impurity A)

Examine by thin-layer chromatography *(2.2.27)* , using a suitable silica gel as the coating substance.

Test solution Dissolve 0.10 g of the substance to be examined in *methylene chloride R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *carmustine impurity A CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of the test solution to 10 ml with *methylene chloride R*. To 5 ml of this solution, add 5 ml of reference solution (a).

Apply separately to the plate 2 µl of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of *methanol R* and 90 volumes of *methylene chloride R*. Allow the plate to dry in air. Spray with *diethylamine R* and heat at 125 °C for 10 min. Allow to cool and spray with *silver nitrate solution R2*. Expose to ultraviolet light at 365 nm until brown to black spots appear. Any spot corresponding to carmustine impurity A in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Water *(2.5.12)*

Not more than 1.0 per cent, determined on 0.50 g by the semi-micro determination of water.

ASSAY

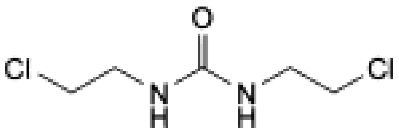
Dissolve 0.100 g in 30 ml of *ethanol R* and dilute to 100.0 ml with *water R*. Dilute 3.0 ml of the solution to 100.0 ml with *water R*. Measure the absorbance (*2.2.25*) at the maximum at 230 nm.

Calculate the content of $C_5H_9C_{l2}N_3O_2$ taking the specific absorbance to be 270.

STORAGE

Store in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES



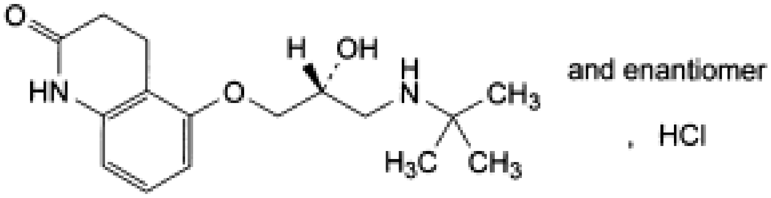
A. 1,3-bis(2-chloroethyl)urea.

Ph Eur

Carteolol Hydrochloride

General Notices

(Ph Eur monograph 1972)



$C_{15}H_{25}N_2O_3$,HCl 328.8 *51781-21-6*

Action and use

Beta-adrenoceptor antagonist.

Preparation

Carteolol Eye Drops

Ph Eur

DEFINITION

5-[(2*RS*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydroquinolin-2(1*H*)-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystals or crystalline powder.

Solubility

Soluble in water, sparingly soluble in methanol, slightly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of carteolol hydrochloride.*

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.300 g in *water R* and dilute to 10 ml with the same solvent.

pH *(2.2.3)*

5.0 to 6.0.

Dissolve 0.250 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 10 mg of *carteolol for system suitability CRS* in the mobile phase and dilute to 5 ml with the mobile phase.

Reference solution (d) Dilute 5.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 1 volume of *methanol R2* , 20 volumes of *acetonitrile R* and 79 volumes of a 2.82 g/l solution of *sodium hexanesulphonate R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 252 nm.

Injection 20 µl.

System suitability:

— the chromatogram obtained with reference solution (c) is similar to the chromatogram provided with *carteolol for system suitability CRS* ; the peaks due to impurity H and carteolol show base-line separation,

— *signal-to-noise ratio* : minimum 10 for the principal peak in the chromatogram obtained with reference solution (d),

— *number of theoretical plates* : minimum 6000, calculated for the principal peak in the chromatogram obtained with reference solution (a).

Limits Locate impurity H by comparison with the chromatogram provided with *carteolol for system suitability CRS,*

— *impurity H*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

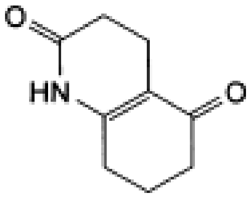
Dissolve 0.250 g in 60 ml of *alcohol R* . Add 5.0 ml of *0.01 M hydrochloric acid* . Carry out a potentiometric titration *(2.2.20)* , using *0.1 M sodium hydroxide* . Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 32.88 mg of $C_{16}H_{25}N_2O_3Cl$.

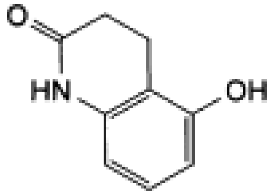
STORAGE

In an airtight container.

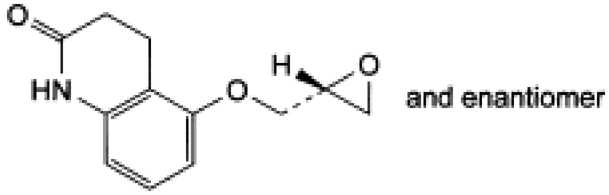
IMPURITIES



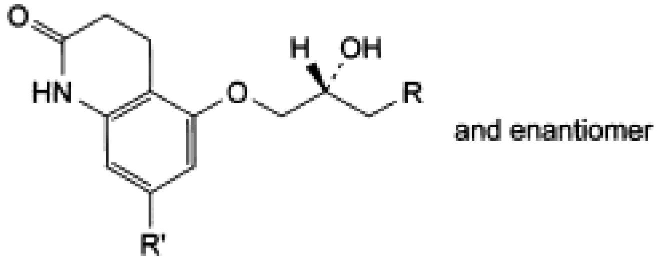
A. 4,6,7,8-tetrahydroquinoline-2,5(1*H*,3*H*)-dione,



B. 5-hydroxy-3,4-dihydroquinolin-2(1*H*)-one,



C. 5-[[(2*RS*)-oxiran-2-yl]methoxy]-3,4-dihydroquinolin-2(1*H*)-one,

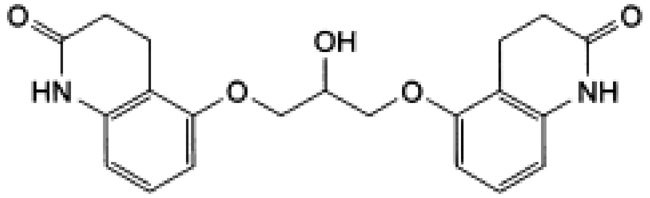


D. R = Cl, R′ = H: 5-[(2*RS*)-3-chloro-2-hydroxypropoxy]-3,4-dihydroquinolin-2(1*H*)-one,

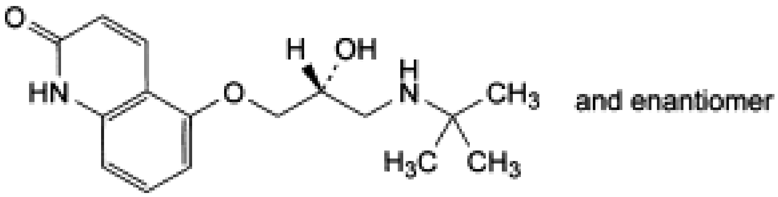
F. R = OCH_3, R′ = H: 5-[(2*RS*)-2-hydroxy-3-methoxypropoxy]-3,4-dihydroquinolin-2(1*H*)-one,

G. R = OH, R′ = H: 5-[(2*RS*)-2,3-dihydroxypropoxy]-3,4-dihydroquinolin-2(1*H*)-one,

I. R = NH-$C(CH_3)_3$, R′ = Br: 7-bromo-5-[(2*RS*)-3-[(1,1-(dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydroquinolin-2(1*H*)-one,



E. 5,5′-[(2-hydroxypropan-1,3-diyl)bis(oxy)]bis(3,4-dihydroquinolin-2(1*H*)-one),


and enantiomer

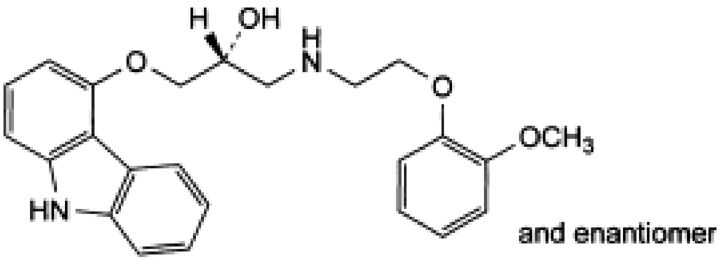
H. 5-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]quinolin-2(1*H*)-one.

Ph Eur

Carvedilol

General Notices

(Ph Eur monograph 1745)



$C_{24}H_{26}N_2O_4$ 406.5 *72956-09-3*

Action and use

Beta-adrenoceptor antagonist; arteriolar vasodilator.

Ph Eur

DEFINITION

(2*RS*)-1-(9*H*-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in alcohol, practically insoluble in dilute acids.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of carvedilol*.

If the spectrum obtained shows differences, dissolve the substance to be examined in *2-propanol R* , evaporate to dryness and record a new spectrum using the residue.

TESTS

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *carvedilol impurity C CRS* in 5.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.125 m, Ø = 4.6 mm,

— *stationary phase: octylsilyl silica gel for chromatography R (5 µm),*

— *temperature*: 55 °C.

Mobile phase Dissolve 1.77 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 650 ml with the same solvent; adjust to pH 2.0 with *phosphoric acid R* and add 350 ml of *acetonitrile R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time 8 times the retention time of carvedilol.

Relative retention With reference to carvedilol (retention time = about 4 min): impurity A = about 0.6; impurity C = about 3.5; impurity B = about 6.7.

System suitability Reference solution (b):

— *resolution*: minimum 17 between the peaks due to carvedilol and to impurity C.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 2,

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *impurity C*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.01 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2.0 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

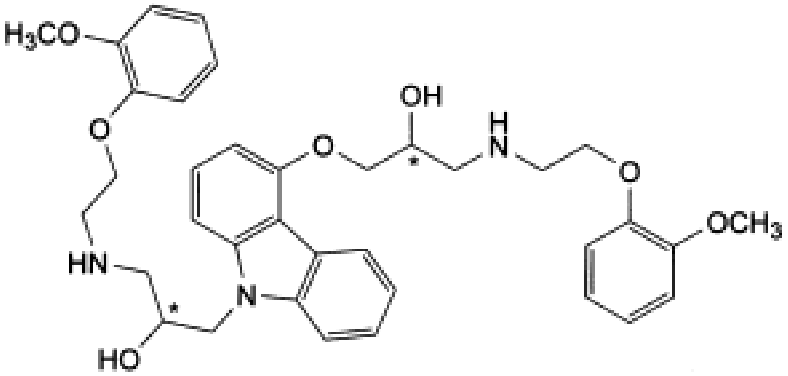
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

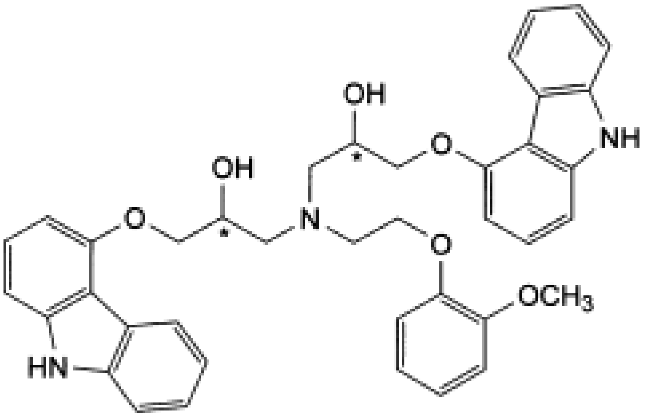
Dissolve 0.350 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 40.65 mg of $C_{24}H_{26}N_2O_4$.

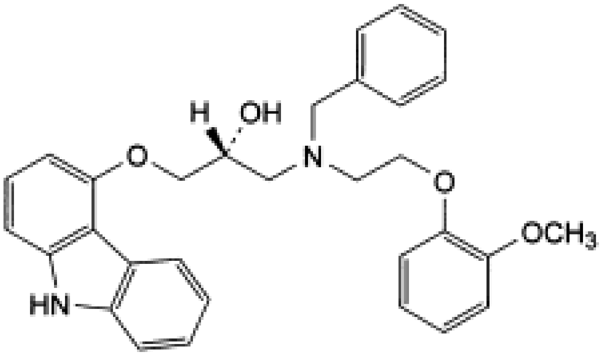
IMPURITIES



A. 1-[[9-[2-hydroxy-3-[[2-(2-methoxyphenoxy)ethyl]amino]propyl]-9*H*-carbazol-4-yl]oxy]-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol,


OH
OH
NH
N
O
O
O
OCH3
HN

B. 1,1´-[[2-(2-methoxyphenoxy)ethyl]nitrilo]bis[3-(9*H*-carbazol-4-yloxy)propan-2-ol],


H
OH
O
N
O
OCH3
HN

and enantiomer

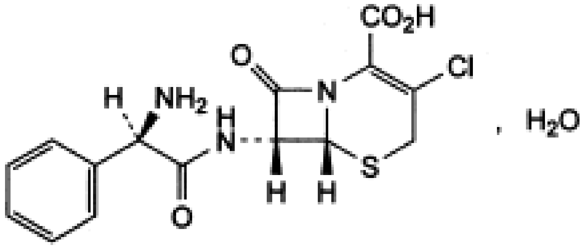
C. (2*RS*)-1-[benzyl[2-(2-methoxyphenoxy)ethyl]amino]-3-(9*H*-carbazol-4-yloxy)propan-2-ol.

Ph Eur

Cefaclor

General Notices

(Ph Eur monograph 0986)



$C_{15}H_{14}ClN_3O_4S,H_2O$ 385.8 *53994-73-3*

Action and use

Cephalosporin antibacterial.

Preparations

Cefaclor Capsules

Cefaclor Oral Suspension

Prolonged-release Cefaclor Tablets

Ph Eur

DEFINITION

(6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent of $C_{15}H_{14}ClN_3O_4S$ (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow powder.

Solubility

Slightly soluble in water, practically insoluble in methanol and in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefaclor CRS.*

TESTS

pH *(2.2.3)*

3.0 to 4.5.

Suspend 0.250 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 101 to + 111 (anhydrous substance).

Dissolve 0.250 g in a 10 g/l solution of *hydrochloric acid R* and dilute to 25.0 ml with the same solution.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in 10.0 ml of a 2.7 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 2.5 with *phosphoric acid R.*

Reference solution (a) Dissolve 2.5 mg of *cefaclor CRS* and 5.0 mg of *delta-3-cefaclor CRS* (impurity D) in 100.0 ml of a 2.7 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 2.5 with *phosphoric acid R.*

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a 2.7 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 2.5 with *phosphoric acid R.*

Column:

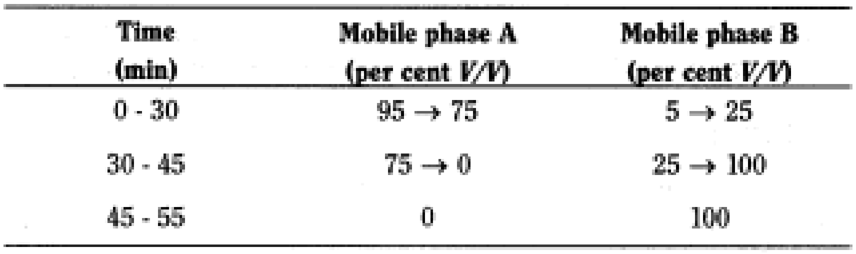
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: 7.8 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 4.0 with *phosphoric acid R;*

— *mobile phase B*: mix 450 ml of *acetonitrile R* with 550 ml of mobile phase A;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	95 → 75	5 → 25
30 - 45	75 → 0	25 → 100
45 - 55	0	100

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

System suitability Reference solution (a):

— *resolution*: minimum 2 between the peaks due to cefaclor and impurity D; if necessary, adjust the acetonitrile content in the mobile phase;

— *symmetry factor* : maximum 1.2 for the peak due to cefaclor; if necessary, adjust the acetonitrile content in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals ***(2.4.8)***

Maximum 30 ppm.

1.0 g complies with test C. Prepare the reference solution using 3 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

3.0 per cent to 6.5 per cent, determined on 0.200 g.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 15.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 15.0 mg of *cefaclor CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 3.0 mg of *cefaclor CRS* and 3.0 mg of *delta-3-cefaclor CRS* (impurity D) in the mobile phase and dilute to 10.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R (5* µm).

Mobile phase Add 220 ml of *methanol R* to a mixture of 780 ml of *water R,* 10 ml of *triethylamine R* and 1 g of *sodium pentanesulphonate R* , then adjust to pH 2.5 with *phosphoric acid R* .

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 20 µl.

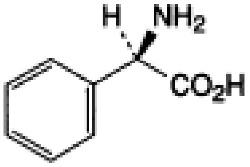
System suitability:

— *resolution*: minimum 2.5 between the peaks due to cefaclor and impurity D in the chromatogram obtained with reference solution (b); if necessary, adjust the concentration of methanol in the mobile phase;

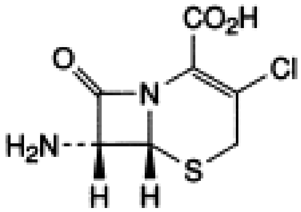
— *symmetry factor* : maximum 1.5 for the peak due to cefaclor in the chromatogram obtained with reference solution (b);

— *repeatability:* maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

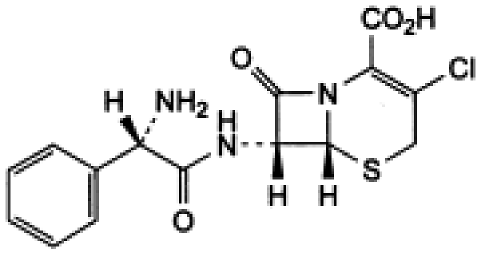
IMPURITIES



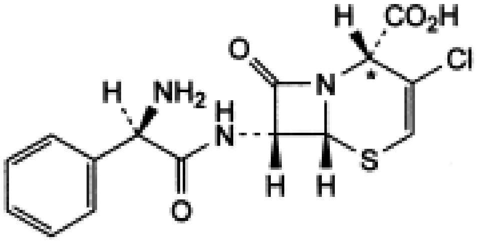
A. (2*R*)-2-amino-2-phenylacetic acid (phenylglycine),



B. (6*R*,7*R*)-7-amino-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

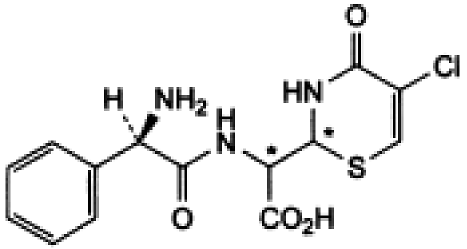


C. (6*R*,7*R*)-7-[[(2*S*)-2-amino-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

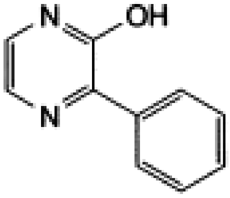

H
CO₂H
Cl
O
N
H
NH₂
H
N
S
O
H
H

and epimer at C*

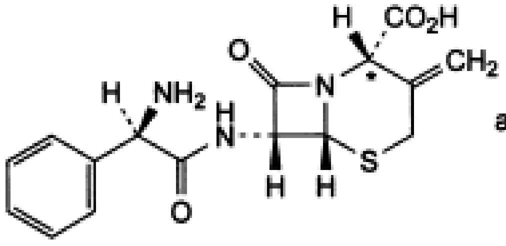
D. (*2R*,6*R*,7*R*)- and (2*S*,6*R*,7*R*)-7-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia- 1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid (delta-3-cefaclor),


O
Cl
H
NH₂
HN
H
N
S
O
CO₂H

E. 2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-(5-chloro-4-oxo-3,4-dihydro-2H-1,3-thiazin-2-yl)acetic acid,

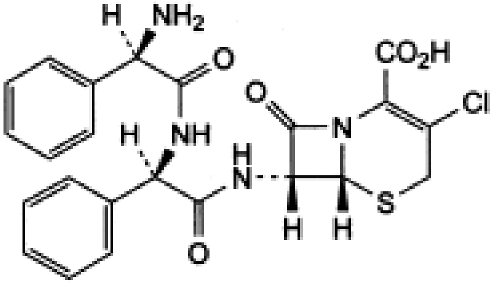


F. 3-phenylpyrazin-2-ol.


H
CO₂H
O
CH₂
H
NH₂
N
H
N
S
O
H
H

and epimer at C*

G. (2*R*,6*R*,7*R*)- and (2*S*,6*R*,7*R*)-7-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-methylene-8-oxo-5-thia-1-azabicyclo[4.2.0]octane-2-carboxylic acid (isocefalexine),



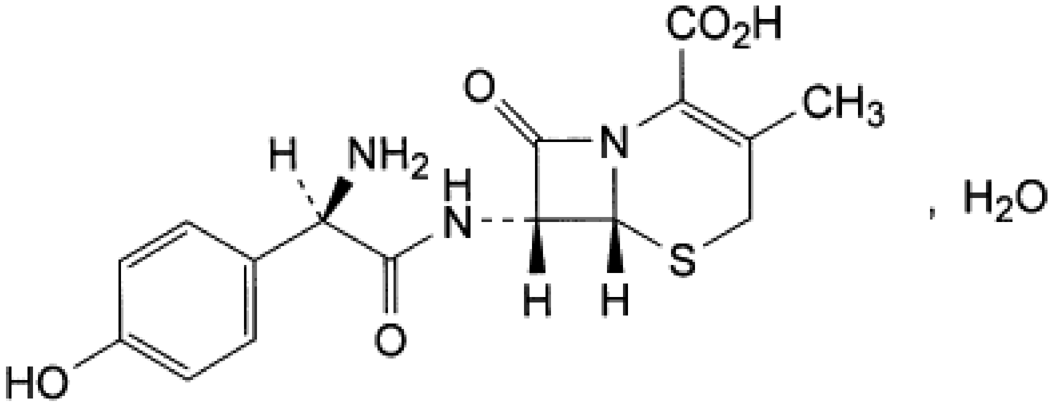
H. (6*R*,7*R*)-7-[[(2*R*)-2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (*N*-phenylglycyl cefaclor).

Ph Eur

Cefadroxil Monohydrate

General Notices

(Ph Eur monograph 0813)



$C_{16}H_{17}N_3O_5S,H_2O$ 381.4   *50370-12-2*

Action and use

Cephalosporin antibacterial.

Preparations

Cefadroxil Capsules

Cefadroxil Oral Suspension

Ph Eur

DEFINITION

(6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *cefadroxil CRS.*

TESTS

pH (*2.2.3*)

4.0 to 6.0.

Suspend 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 165 to + 178 (anhydrous substance).

Dissolve 0.500 g in *water R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a) Dissolve 10.0 mg of *D-α-(4-hydroxyphenyl)glycine CRS* (impurity A) in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 10.0 mg of *7-aminodesacetoxycephalosporanic acid CRS* (impurity B) in phosphate buffer solution pH 7.0 R5 and dilute to 10.0 ml with the same buffer solution.

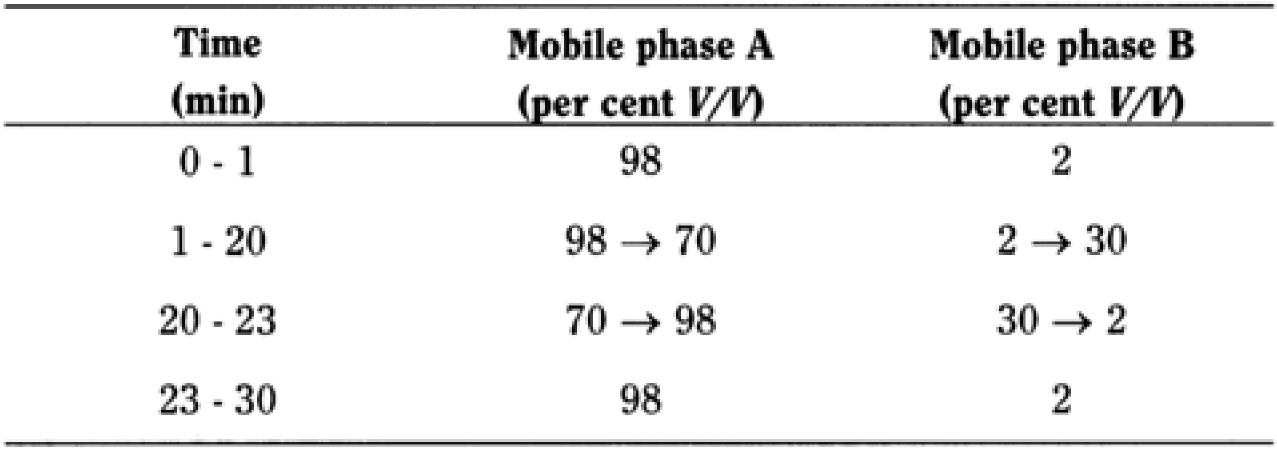
Reference solution (c) Dilute 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 100.0 ml with mobile phase A.

Reference solution (d) Dissolve 10 mg of *dimethylformamide R* and 10 mg of *dimethylacetamide R* in mobile phase A and dilute to 10.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 100.0 ml with mobile phase A.

Reference solution (e) Dilute 1.0 ml of reference solution (c) to 25.0 ml with mobile phase A.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm,
— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *phosphate buffer solution pH 5.0 R,*
— *mobile phase B*: *methanol R2,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	98	2
1 - 20	98 → 70	2 → 30
20 - 23	70 → 98	30 → 2
23 - 30	98	2

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of the test solution and reference solutions (c), (d) and (e).

Relative retention With reference to cefadroxil (retention time = about 6 min): dimethylformamide = about 0.4; dimethylacetamide = about 0.75.

System suitability:
— *resolution*: minimum 5.0 between the peaks due to impurities A and B in the chromatogram obtained with reference solution (c),

— *signal-to-noise ratio* : minimum 10 for the 2nd peak in the chromatogram obtained with reference solution (e).

Limits:
— *impurity A*: not more than the area of the 1st peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

— *any other impurity*: for each impurity, not more than the area of the 2nd peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

— *total*: not more than 3 times the area of the 2nd peak in the chromatogram obtained with reference solution (c) (3.0 per cent),

— *disregard limit*: 0.05 times the area of the 2nd peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peaks due to dimethylformamide and dimethylacetamide.

_N,N_-Dimethylaniline (_2.4.26, Method B_)

Maximum 20 ppm.

Water (_2.5.12_)

4.0 per cent to 6.0 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *cefadroxil CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *cefadroxil CRS* and 50 mg of *amoxicillin trihydrate CRS* in the mobile phase and dilute to 100 ml with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, a 2.72 g/l solution of *potassium dihydrogen phosphate R* (4:96 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

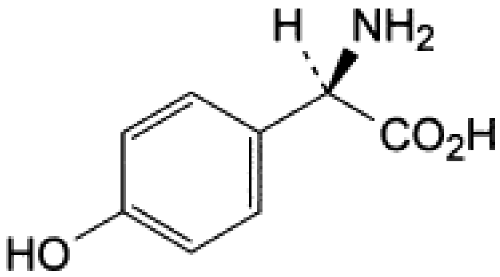
System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to cefadroxil and to amoxicillin.

Calculate the percentage content of cefadroxil.

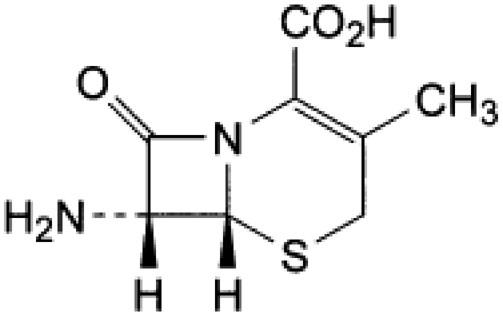
STORAGE

Protected from light.

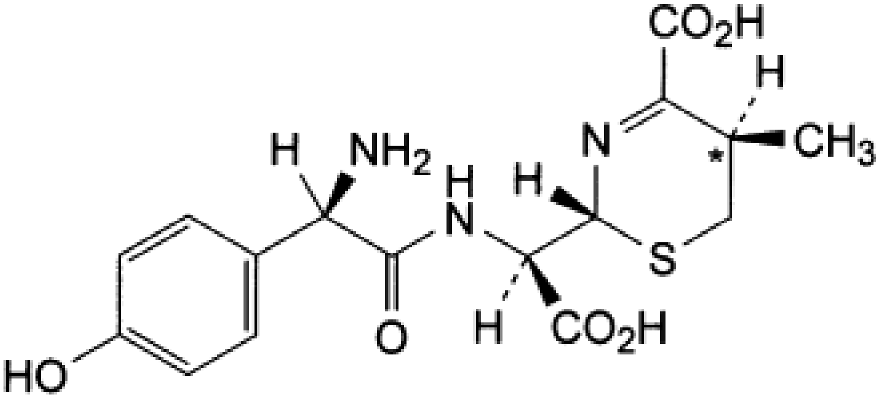
IMPURITIES



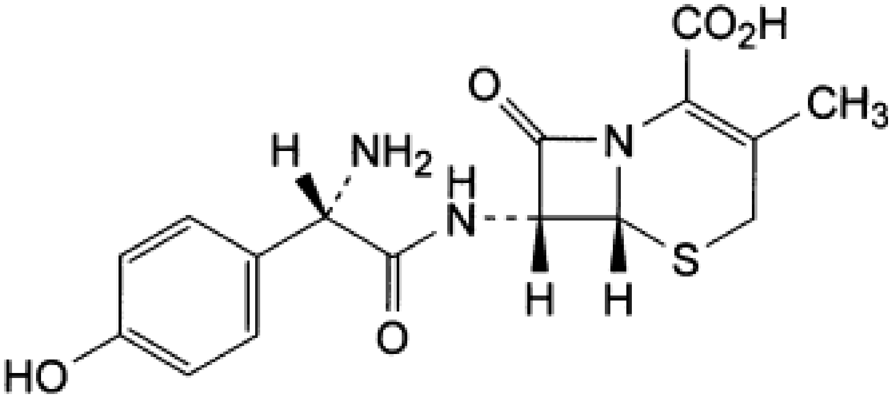
A. (2*R*)-2-amino-2-(4-hydroxyphenyl)acetic acid,



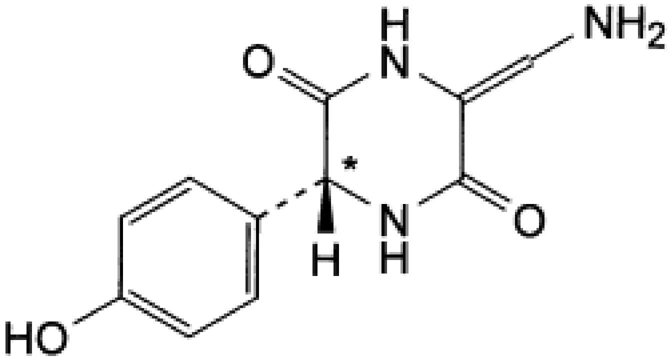
B. (6*R*,7*R*)-7-amino-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ADCA),



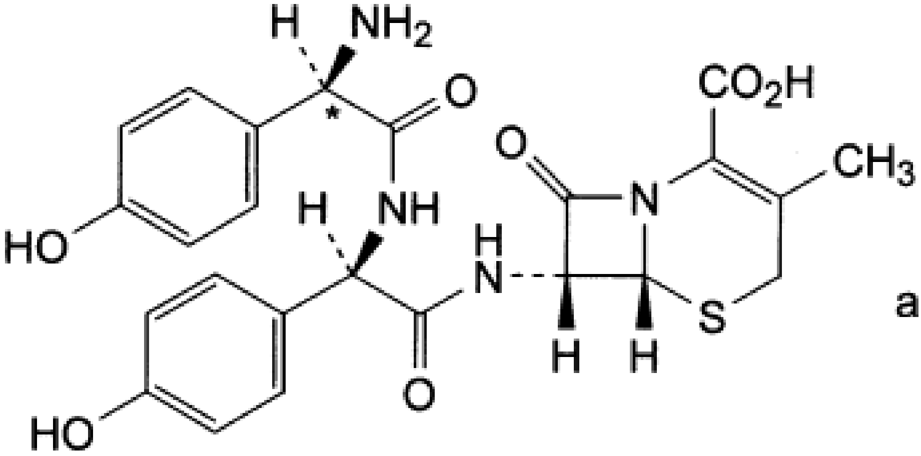
C. (2*R*,5*RS*)-2-[(*R*)-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]carboxymethyl]-5-methyl-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid,



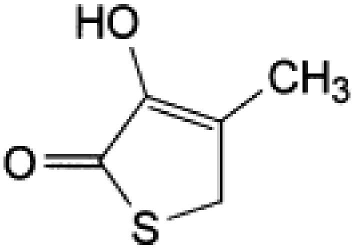
D. (6*R*,7*R*)-7-[[(2*S*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (l-cefadroxil),



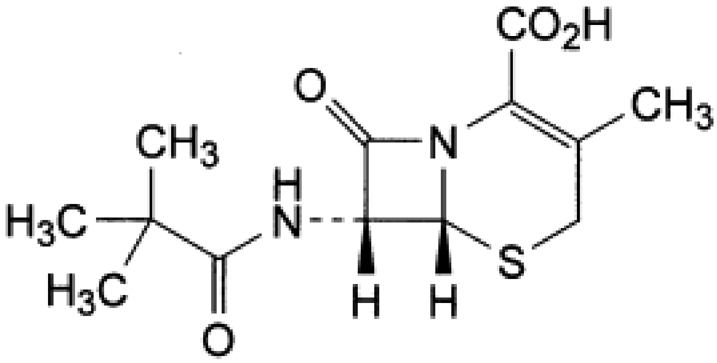
E. (6*RS*)-3-(aminomethylene)-6-(4-hydroxyphenyl)piperazine-2,5-dione,



F. (6*R*,7*R*)-7-[[(2*R*)-2-[[(2*RS*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,



G. 3-hydroxy-4-methylthiophen-2(5*H*)-one,



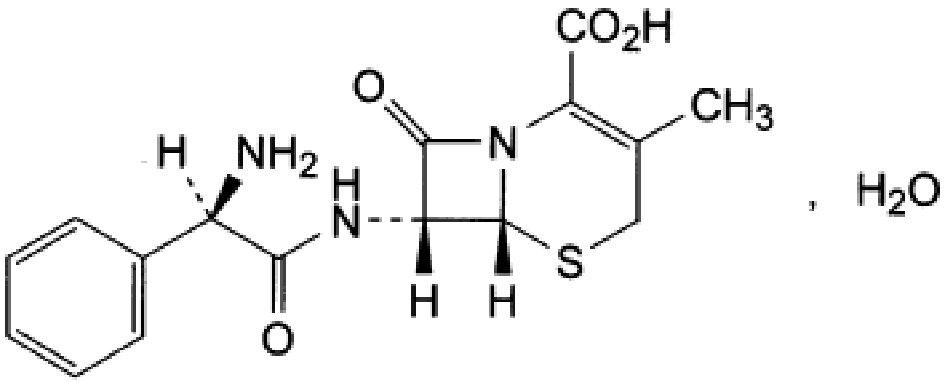
H. (6*R*,7*R*)-7-[(2,2-dimethylpropanoyl)amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ADCA pivalamide).

Ph Eur

Cefalexin Monohydrate

General Notices

(Ph Eur monograph 0708)



$C_{16}H_{17}N_3O_4S,H_2O$ 365.4 *23325-78-2*

Action and use

Cephalosporin antibacterial.

Preparations

Cefalexin Capsules

Cefalexin Oral Suspension

Cefalexin Tablets

Ph Eur

DEFINITION

(6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *cefalexin monohydrate CRS.*

TESTS

pH (*2.2.3*)

4.0 to 5.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 149 to + 158 (anhydrous substance).

Dissolve 0.125 g in *phthalate buffer solution pH 4.4 R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a) Dissolve 10.0 mg of *D-phenylglycine R* in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 10.0 mg of *7-aminodesacetoxycephalosporanic acid CRS* in *phosphate buffer solution pH 7.0 R5* and dilute to 10.0 ml with mobile phase A.

Reference solution (c) Dilute 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 100.0 ml with mobile phase A.

Reference solution (d) Dissolve 10 mg of *dimethylformamide R* and 10 mg of *dimethylacetamide R* in mobile phase A and dilute to 10.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 100.0 ml with mobile phase A.

Reference solution (e) Dilute 1.0 ml of reference solution (c) to 20.0 ml with mobile phase A.

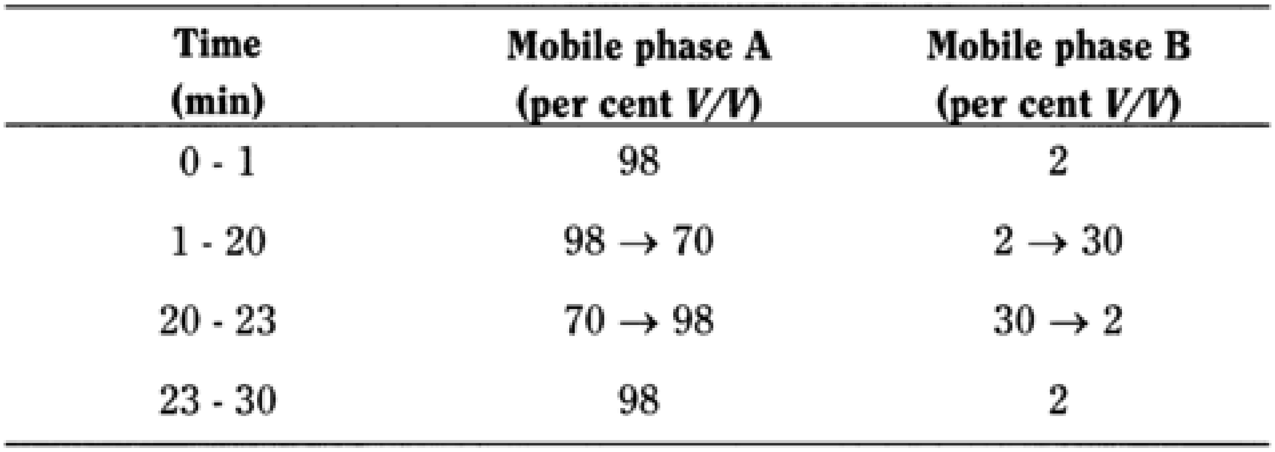
Reference solution (f) Dissolve 10 mg of *cefotaxime sodium CRS* in mobile phase A and dilute to 10.0 ml with mobile phase A. To 1.0 ml of this solution add 1.0 ml of the test solution and dilute to 100 ml with mobile phase A.

Column:

- *size: l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: *phosphate buffer solution pH 5.0 R;*
- *mobile phase B*: *methanol R2;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	98	2
1 - 20	98 → 70	2 → 30
20 - 23	70 → 98	30 → 2
23 - 30	98	2

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of the test solution and reference solutions (c), (d), (e) and (f).

System suitability:

- *resolution*: minimum 2.0 between the peaks due to impurities A and B in the chromatogram obtained with reference solution (c) and minimum 1.5 between the peaks due to cefalexin and cefotaxime in the chromatogram obtained with reference solution (f).

Limits:

- *impurity B*: not more than the area of the 2nd peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *any other impurity*: not more than the area of the 1st peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *total*: not more than 3 times the area of the 1st peak in the chromatogram obtained with reference solution (c) (3.0 per cent);
- *disregard limit*: the area of the 2nd peak in the chromatogram obtained with reference solution (e) (0.05 per cent); disregard any peaks due to dimethylformamide or dimethylacetamide.

***N,N*-Dimethylaniline (*2.4.26, Method B*)**

Maximum 20 ppm.

Water (*2.5.12*)

4.0 per cent to 8.0 per cent, determined on 0.300 g.

Sulphated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *cefalexin monohydrate CRS* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *cefradine CRS* in 20 ml of reference solution (a) and dilute to 100 ml with *water R.*

Column:
- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *acetonitrile R*, 13.6 g/l solution of *potassium dihydrogen phosphate R*, *water R* (2:5:10:83 *V/V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

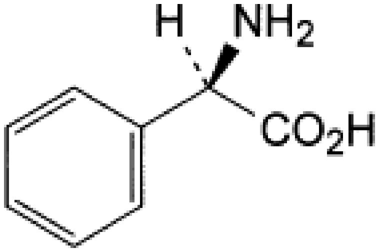
System suitability Reference solution (b):
- *resolution*: minimum 4.0 between the peaks due to cefalexin and cefradine.

Calculate the percentage content of cefalexin monohydrate.

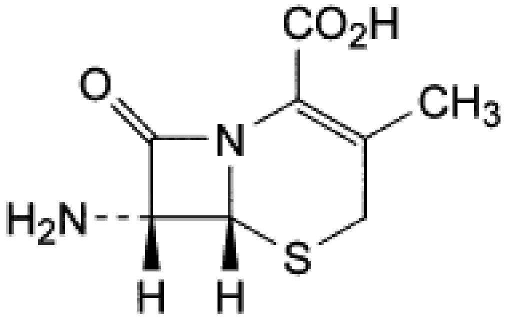
STORAGE

Protected from light.

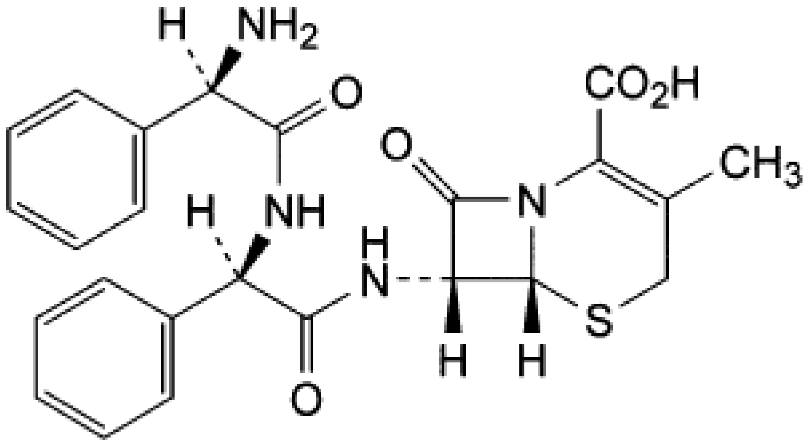
IMPURITIES



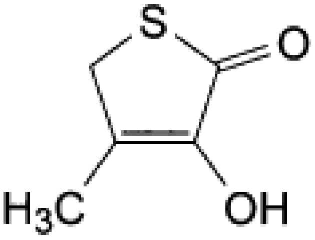
A. (2*R*)-2-amino-2-phenylacetic acid (D-phenylglycine),



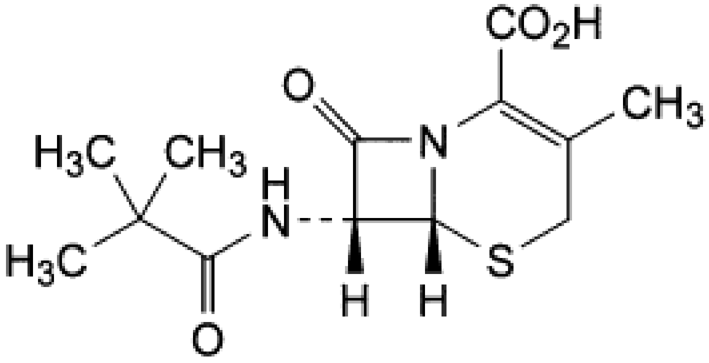
B. (6*R*,7*R*)-7-amino-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-aminodesacetoxycephalosporanic acid, 7-ADCA),



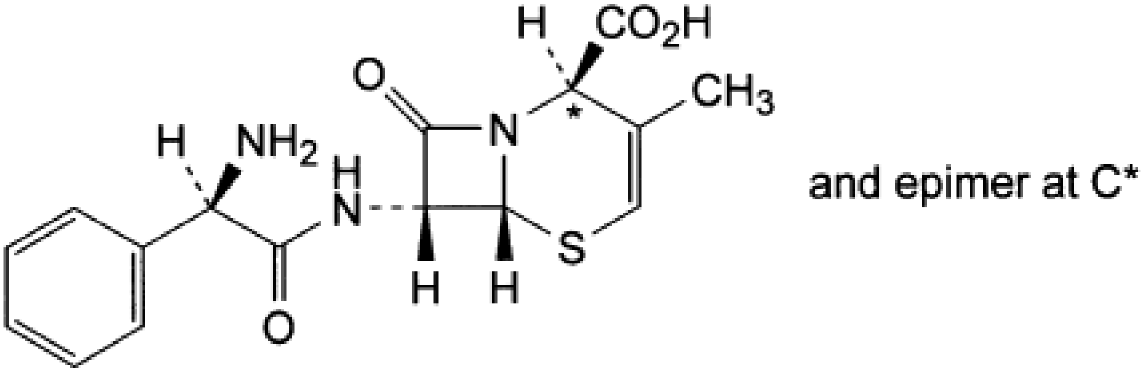
C. (6*R*,7*R*)-7-[[(2*R*)-2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,



D. 3-hydroxy-4-methylthiophen-2(5*H*)-one,



E. (6*R*,7*R*)-7-[(2,2-dimethylpropanoyl)amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ADCA pivalamide),



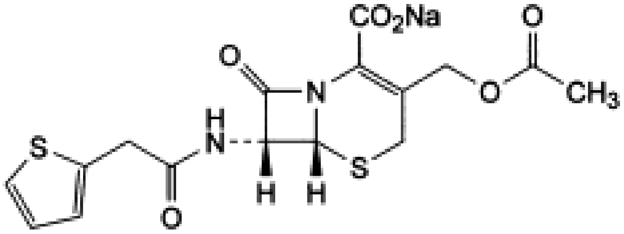
F. (2*RS*,6*R*,7*R*)-7-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-3-ene-2-carboxylic acid (delta-2-cefalexin).

Ph Eur

Cefalotin Sodium

General Notices

(Ph Eur monograph 0987)



$C_{16}H_{15}N_2NaO_6S_2$ 418.4 *58-71-9*

Action and use

Cephalosporin antibacterial.

Ph Eur

DEFINITION

Sodium (6*R*,7*R*)-3-[(acetyloxy)methyl]-8-oxo-7-[(thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefalotin sodium CRS.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 450 nm is not greater than 0.20.

pH *(2.2.3)*

4.5 to 7.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 124 to + 134 (anhydrous substance).

Dissolve 1.25 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*

Prepare the solutions immediately before use.

Test solution (a) Dissolve 75.0 mg of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with *water R* .

Reference solution (a) Dissolve 75.0 mg of *cefalotin sodium CRS* in *water R* and dilute to 25.0 ml with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with *water R.*

Reference solution (b) Dilute 1.0 ml of test solution (a) to 100.0 ml with *water R* .

Reference solution (c) Mix 1 ml of test solution (a), 1 ml of *hydrochloric acid R1* and 8 ml of *water R* . Heat at 60 °C for 12 min and cool to room temperature in iced water. Inject immediately.

Reference solution (d) Dissolve 5 mg of *cefalotin for impurity B identification CRS* in *water R* and dilute to 5 ml with the same solvent.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

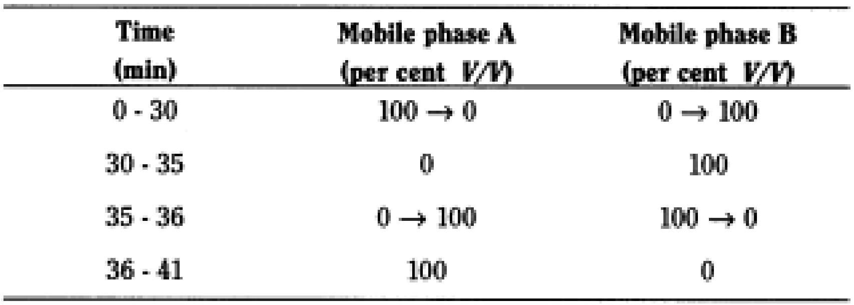
— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: mix 3 volumes of *acetonitrile R1* and 97 volumes of a 1.742 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 2.5 with *phosphoric acid*

R ;

— *mobile phase B:* mix 40 volumes of *acetonitrile R1* and 60 volumes of a 1.742 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 2.5 with *phosphoric acid R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	100 → 0	0 → 100
30 - 35	0	100
35 - 36	0 → 100	100 → 0
36 - 41	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (a) and reference solutions (b), (c) and (d).

Relative retention With reference to cefalotin (retention time = about 26 min): impurity C = about 0.2; impurity B = about 0.7; impurity A = about 0.8; impurity D = about 0.9.

System suitability Reference solution (c):

— *resolution*: minimum 7.0 between the peaks due to impurity D and cefalotin.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurity D:* not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

N*,*N*-Dimethylaniline *(2.4.26, Method B)

Maximum 20 ppm.

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.5 per cent.

Water *(2.5.12)*

Maximum 1.5 per cent, determined on 0.500 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.13 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Mix 14 volumes of *acetonitrile R* and 86 volumes of a 6.967 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 6.0 with *phosphoric acid R.*

Detection Spectrophotometer at 260 nm.

Injection 5 µl of test solution (b) and reference solution (a).

Run time 1.5 times the retention time of cefalotin (retention time = about 10 min).

Calculate the percentage content of $C_{16}H_{15}N_2NaO_6S_2$ using the chromatogram obtained with reference solution (a) and the declared content of *cefalotin sodium CRS.*

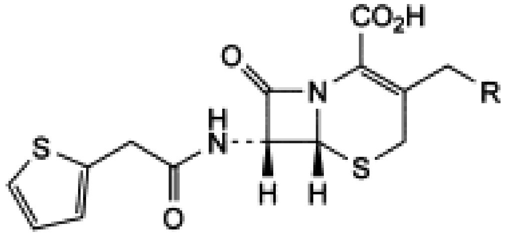
STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

IMPURITIES

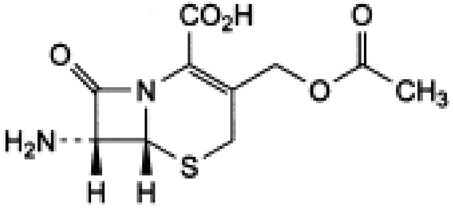
Specified impurities *B, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*) : *A, C.*

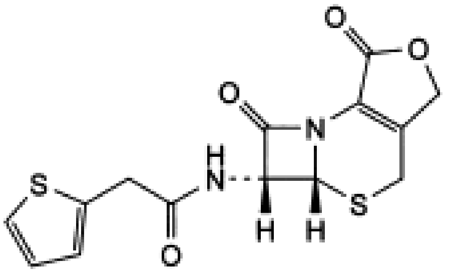


A. R = H: (6*R*,7*R*)-3-methyl-8-oxo-7-[(thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (deacetoxycefalotin),

B. R = OH: (6*R*,7*R*)-3-(hydroxymethyl)-8-oxo-7-[(thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (deacetylcefalotin),



C. (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA),



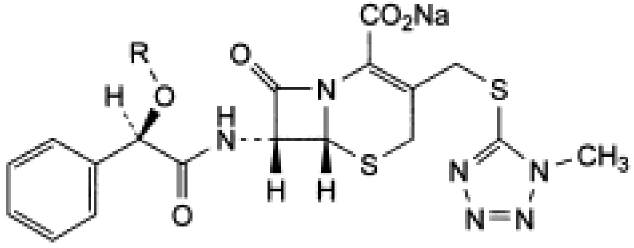
D. (5a*R*,6*R*)-6-[(thiophen-2-ylacetyl)amino]-5a,6-dihydro-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione (cefalotin lactone).

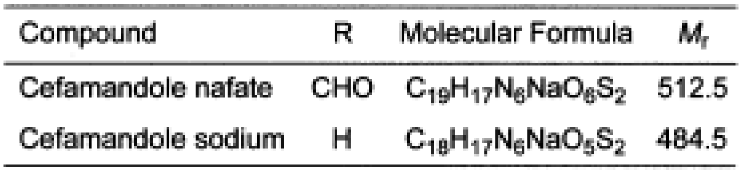
Ph Eur

Cefamandole Nafate

General Notices

(Ph Eur monograph 1402)





Compound	R	Molecular Formula	M_r
Cefamandole nafate	CHO	$C_{19}H_{17}N_6NaO_6S_2$	512.5
Cefamandole sodium	H	$C_{18}H_{17}N_6NaO_5S_2$	484.5

$C_{19}H_{17}N_6NaO_6S_2$ 512.5 *42540-40-9*

Action and use

Cephalosporin antibacterial.

Ph Eur

DEFINITION

Mixture of sodium (6*R*,7*R*)-7-[[(2*R*)-2-(formyloxy)-2-phenylacetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate and sodium (6*R*,7*R*)-7-[[(2*R*)-2-hydroxy-2-phenylacetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl) sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (cefamandole sodium), with sodium carbonate.

Semi-synthetic product derived from a fermentation product.

Content:

— *cefamandole nafate* ($C_{19}H_{17}N_6NaO_6S_2$): 93.0 per cent to 102.0 per cent (anhydrous and sodium carbonate-free substance), for the sum of the content of cefamandole nafate and cefamandole sodium expressed as cefamandole nafate;

— *cefamandole sodium* ($C_{18}H_{17}N_6NaO_5S_2$): maximum 10.0 per cent (anhydrous and sodium carbonate-free substance);

— *sodium carbonate* (Na_2CO_3): 4.8 per cent to 6.4 per cent.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, sparingly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefamandole nafate CRS.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 475 nm is not greater than 0.03.

pH

6.0 to 8.0 for solution S, measured after 30 min.

Specific optical rotation *(2.2.7)*

- 35.0 to - 45.0 (anhydrous and sodium carbonate-free substance).

Dissolve 1.00 g in *acetate buffer solution pH 4.7 R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Prepare the solutions immediately before use.

Solvent mixture Mix 18 volumes of *acetonitrile R* and 75 volumes of a 10 per cent *V/V* solution of *triethylamine R* previously adjusted to pH 2.5 with *phosphoric acid R.*

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dilute 1 ml of the test solution to 10 ml with the solvent mixture, then heat at 60 °C for 30 min.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

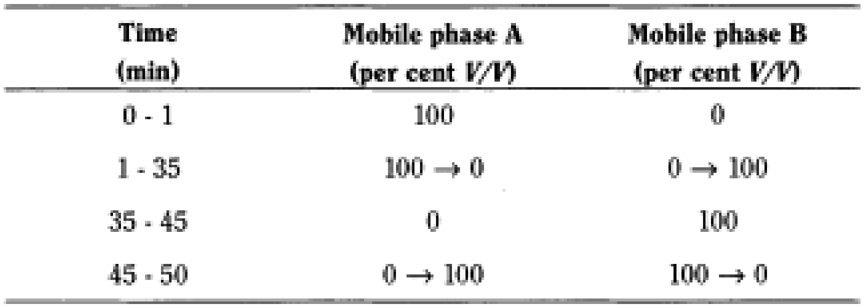
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *triethylamine phosphate solution*: dissolve 2.0 g of *sodium pentanesulphonate R* in 350 ml of *water R,* add 40 ml of *triethylamine R*, adjust to pH 2.5 with *phosphoric acid R* and dilute to 700 ml with *water R*;

— *mobile phase A:* mix 1 volume of the triethylamine phosphate solution and 2 volumes of *water R;*

— *mobile phase B*: mix equal volumes of the triethylamine phosphate solution, *methanol R and acetonitrile R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	100	0
1 - 35	100 → 0	0 → 100
35 - 45	0	100
45 - 50	0 → 100	100 → 0

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl loop injector.

Relative retention With reference to cefamandole nafate (retention time = about 24 min): cefamandole = about 0.8.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to cefamandole and cefamandole nafate.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent);

— *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

2-Ethylhexanoic acid ***(2.4.28)***

Maximum 0.3 per cent *m/m*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.500 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.15 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Cefamandole nafate

Liquid chromatography *(2.2.29)* .

Prepare the solutions immediately before use.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *cefamandole nafate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1 ml of the test solution to 10 ml with the mobile phase, then heat at 60 °C for 30 min.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of *acetonitrile R* and 75 volumes of a 10 per cent *V/V* solution of *triethylamine R* previously adjusted to pH 2.5 with *phosphoric acid R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl loop injector.

System suitability:

- *resolution*: minimum 7.0 between the 2 principal peaks in the chromatogram obtained with reference solution (b);
- *repeatability*: maximum relative standard deviation of 0.8 per cent after a series of single injections of not less than 3 freshly prepared reference solutions (a).

Calculate the percentage content of cefamandole nafate ($C_{19}H_{17}N_6NaO_6S_2$) from the sum of the contents of cefamandole nafate and cefamandole sodium expressed as cefamandole nafate, using the declared content of *cefamandole nafate CRS* .

1 mg of cefamandole sodium is equivalent to 1.0578 mg of cefamandole nafate

Sodium carbonate

Dissolve 0.500 g in 50 ml of *water R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M hydrochloric acid* is equivalent to 5.3 mg of Na_2CO_3.

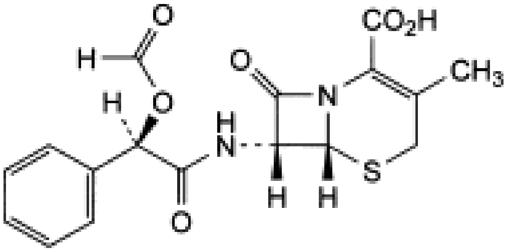
STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

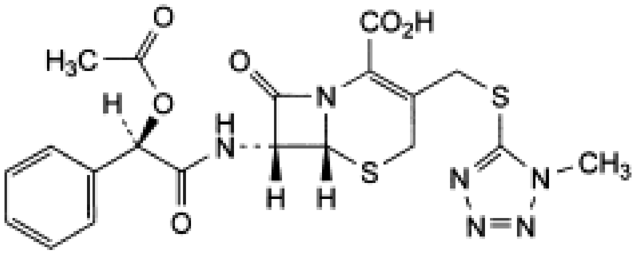
LABELLING

The label states that the substance contains sodium carbonate.

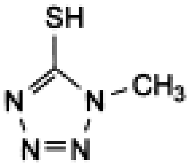
IMPURITIES



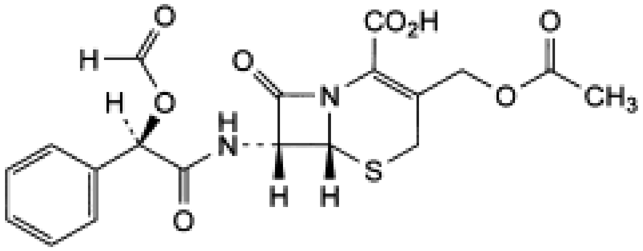
A. (6*R*,7*R*)-7-[[(2*R*)-2-(formyloxy)-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (formylmandeloyl-7-amino-desacetoxy-cephalosporanic acid),



C. (6*R*,7*R*)-7-[[(2*R*)-2-(acetyloxy)-2-phenylacetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (*O*-acetylcefamandole),



D. 1-methyl-1*H*-tetrazole-5-thiol,


O
H
O
H
CO$_2$H
O
O
N
O
CH$_3$
H
N
S
H
H
O

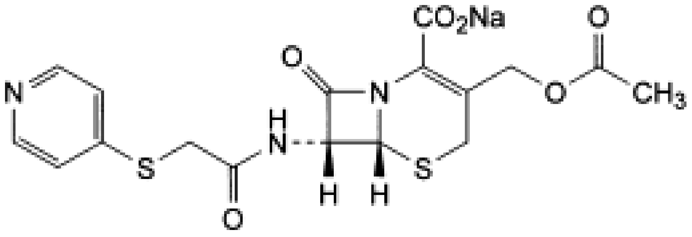
E. (6*R*,7*R*)-7-[[(2*R*)-2-(formyloxy)-2-phenylacetyl]amino]-3-[(acetyloxy)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (formylmandeloyl-7-ACA).

Ph Eur

Cefapirin Sodium

General Notices

(Ph Eur monograph 1650)



$C_{17}H_{16}N_3NaO_6S_2$ 445.5 *24356-60-3*

Action and use

Cephalosporin antibacterial.

Ph Eur

DEFINITION

Sodium (6*R*,7*R*)-3-[(acetyloxy)methyl]-8-oxo-7-[[[(pyridin-4-yl)sulphanyl]acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or pale yellow powder.

Solubility

Soluble in water, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefapirin sodium CRS.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Appearance of solution

Dissolve 2.0 g in *water R* and dilute to 10.0 ml with the same solvent. The solution is clear *(2.2.1)* . Dilute 5.0 ml to 10.0 ml with *water R* . The absorbance *(2.2.25)* of this solution at 450 nm is not greater than 0.25.

pH *(2.2.3)*

6.5 to 8.5.

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 150 to + 165 (anhydrous substance).

Dissolve 0.500 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Prepare the solutions immediately before use.

Test solution Dissolve 42 mg of the substance to be examined in the mobile phase and dilute to 200.0 ml with the mobile phase.

Reference solution (a) Dissolve 42 mg of *cefapirin sodium CRS* in the mobile phase and dilute to 200.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 20.0 ml with the mobile phase.

Reference solution (d) Mix 1 ml of the test solution, 8 ml of the mobile phase and 1 ml of *hydrochloric acid R1* . Heat at 60 °C for 10 min.

Column:

— *size*: *l* = 0.30 m, Ø = 4 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 80 ml of *dimethylformamide R,* 4.0 ml of *glacial acetic acid R* and 20 ml of a 4.5 per cent (m/m) solution of *potassium hydroxide R* . Dilute to 2 litres with *water R* .

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (b), (c) and (d).

Run time Twice the retention time of cefapirin.

Relative retention With reference to cefapirin (retention time = about 13 min): impurity B = about 0.3; impurity C = about 0.5; impurity A = about 0.75.

System suitability Reference solution (d):

— *resolution:* minimum 2.0 between the peaks due to cefapirin and impurity A.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), and not more than 1 such peak has an area greater than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

N,N-Dimethylaniline ***(2.4.26, Method B)***

Maximum 20 ppm.

2-Ethylhexanoic acid ***(2.4.28)***

Maximum 0.5 per cent.

Water ***(2.5.12)***

Maximum 2.0 per cent, determined on 0.300 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.17 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

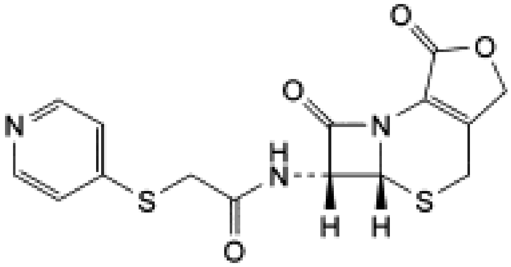
Calculate the percentage content of $C_{17}H_{16}N_3NaO_6S_2$.

STORAGE

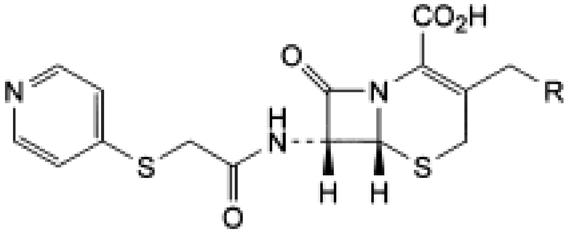
Protected from light. If the substance is sterile, store in a sterile, tamper-proof container.

IMPURITIES

Specified impurities *A, B, C.*



A. (5a*R*,6*R*)-6-[[[(pyridin-4-yl)sulphanyl]acetyl]amino]-5a,6-dihydro-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione (deacetylcefapirin lactone),



B. R = OH: (6*R*,7*R*)-3-(hydroxymethyl)-8-oxo-7-[[[(pyridin-4-yl)sulphanyl]acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (deacetylcefapirin),

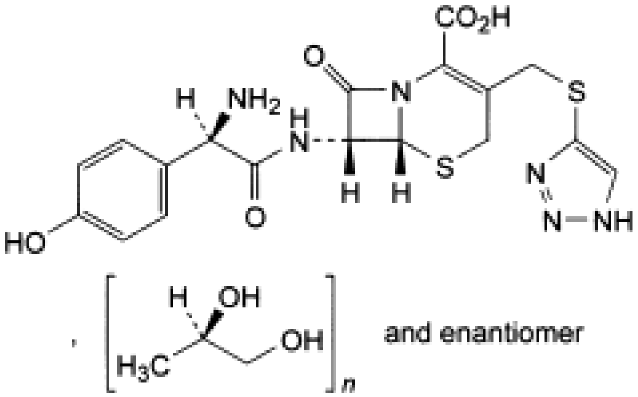
C. R = H: (6*R*,7*R*)-3-methyl-8-oxo-7-[[[(pyridin-4-yl)sulphanyl]acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (deacetoxycefapirin).

Ph Eur

Cefatrizine Propylene Glycol

General Notices

(Ph Eur monograph 1403)



$C_{18}H_{18}N_6O_5S_2,(C_3H_8O_2)_n$ 462.5 *64217-62-5*
(cefatrizine)

Action and use

Cephalosporin antibacterial.

Ph Eur

DEFINITION

Mixture of (6*R*,7*R*)-7-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-8-oxo-3-[[(1*H*-1,2,3-triazol-4-yl)sulphanyl]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid and propane-1,2-diol in molecular proportions of about 1:1.

Content: 95.0 per cent to 102.0 per cent of $C_{18}H_{18}N_6O_5S_2$ (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefatrizine propylene glycol CRS.*

B. Examine the chromatograms obtained in the test for propylene glycol.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

TESTS

Specific optical rotation *(2.2.7)*

+ 63 to + 69 (anhydrous substance).

Dissolve 0.400 g in *1 M hydrochloric acid* and dilute to 20.0 ml with the same acid.

Propylene glycol

Gas chromatography *(2.2.28)* .

Solvent mixture

acetone R, water R (20:80 *V/V*).

Internal standard solution Dissolve 1.0 g of *dimethylacetamide R* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Test solution Introduce 0.40 g of the substance to be examined into a ground-glass-stoppered test-tube. Add 3.0 ml of the internal standard solution, 1.0 ml of the solvent mixture and 2.0 ml of *hydrochloric acid R.* Seal the test-tube and shake.

Reference solution (a) Dissolve 2.0 g of *propylene glycol R* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (b) Introduce into a ground-glass-stoppered test-tube 1.0 ml of reference solution (a) and 1.0 ml of the internal standard solution.

Column:

— *material*: stainless steel;

— *size*: *l* = 2 m, Ø = 2 mm;

— *stationary phase: ethylvinylbenzene-divinylbenzene copolymer R* (150-180 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:

— *column*: 200 °C;

— *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µl of the test solution and reference solution (b).

Limit:

— *propylene glycol* : 13.0 per cent to 18.0 per cent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 60.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 60.0 mg of *cefatrizine propylene glycol CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 30.0 mg of *cefatrizine impurity A CRS in buffer solution pH 7.0 R* and dilute to 100.0 ml with the same buffer solution.

Reference solution (c) Dilute 0.6 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of reference solution (b) to 100.0 ml with *buffer solution pH 7.0 R* .

Reference solution (e) To 1.0 ml of reference solution (a) add 1.0 ml of reference solution (b) and dilute to 10.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 5 volumes of *acetonitrile R* and 95 volumes of a 2.72 g/l solution of *potassium dihydrogen phosphate R in water R.*

Flow rate 2 ml/min.

Detection Spectrophotometer at 272 nm.

Injection 20 µl of the test solution and reference solutions (c), (d) and (e).

Run time At least twice the retention time of cefatrizine.

System suitability Reference solution (e):

— *resolution*: minimum 5.0 between the peaks due to cefatrizine and impurity A.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent);

— *sum of impurities* other than A: not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (2.1 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent).

Water ***(2.5.12)***

Maximum 1.5 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

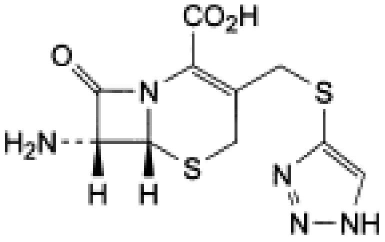
System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{18}H_{18}N_6O_5S_2$ from the declared content of $C_{18}H_{18}N_6O_5S_2$ in *cefatrizine propylene glycol CRS* .

IMPURITIES

Specified impurities *A*



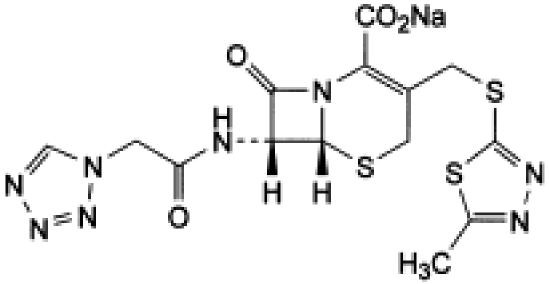
A. (6*R*,7*R*)-7-amino-8-oxo-3-[[(1*H*-1,2,3-triazol-4-yl)sulphanyl]methyl]-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid (7-ACA triazole).

Ph Eur

Cefazolin Sodium

General Notices

(Ph Eur monograph 0988)



$C_{14}H_{13}N_8NaO_4S_3$ 476.5 *27164-46-1*

Action and use

Cephalosporin antibacterial.

Preparation

Cefazolin Injection

Ph Eur

DEFINITION

Sodium (6*R*,7*R*)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)sulphanyl]methyl]-8-oxo-7-[(1*H*-tetrazol-1-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, very hygroscopic.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)* .

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Dissolve 0.150 g in 5 ml of *water R* , add 0.5 ml of *dilute acetic acid R* , swirl and allow to stand for 10 min in iced water. Filter the precipitate and rinse with 1-2 ml of *water R* . Dissolve in a mixture of 1 volume of *water R* and 9 volumes of *acetone R.* Evaporate the solvent almost to dryness, then dry in an oven at 60 °C for 30 min.

Comparison *cefazolin CRS.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 430 nm is not greater than 0.15.

pH *(2.2.3)*

4.0 to 6.0 for solution S.

Specific optical rotation *(2.2.7)*

- 15 to - 24 (anhydrous substance).

Dissolve 1.25 g in *water R* and dilute to 25.0 ml with the same solvent.

Absorbance *(2.2.25)*

Dissolve 0.100 g in *water R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *sodium hydrogen carbonate solution R* . Examined between 220 nm and 350 nm, the solution shows an absorption maximum at 272 nm. The specific absorbance at the maximum is 260 to 300 (anhydrous substance).

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 20.0 ml with the same mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b) Dissolve 20 mg of the substance to be examined in 10 ml of a 2 g/l solution of *sodium hydroxide R* . Allow to stand for 15-30 min. Dilute 1.0 ml of the solution to 20 ml with mobile phase A.

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

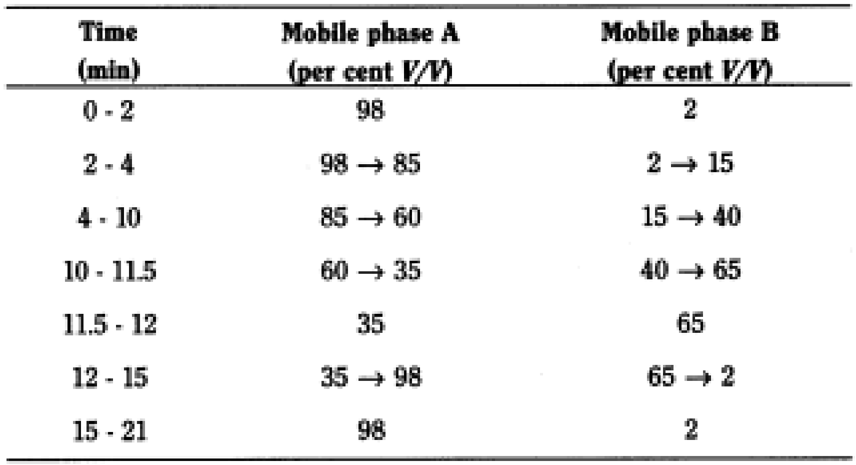
— *stationary phase: octadecylsilyl silica gel for chromatography* R (3 µm);

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A:* solution containing 14.54 g/l of *disodium hydrogen phosphate R* and 3.53 g/l of *potassium dihydrogen phosphate R;*

— *mobile phase B*: *acetonitrile for chromatography R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	98	2
2 - 4	98 → 85	2 → 15
4 - 10	85 → 60	15 → 40
10 - 11.5	60 → 35	40 → 65
11.5 - 12	35	65
12 - 15	35 → 98	65 → 2
15 - 21	98	2

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 5 µl.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to cefazolin and impurity L (see Figure 0988.-1).

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *total:* not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

N,N-Dimethylaniline ***(2.4.26, Method B)***

Maximum 20 ppm.

Water ***(2.5.12)***

Maximum 6.0 per cent, determined on 0.300 g.

Bacterial endotoxins *(2.6.14)*

less than 0.15 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *cefazolin CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *cefuroxime sodium CRS* in 10.0 ml of reference solution (a) and dilute to 100.0 ml with the mobile phase.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 10 volumes of *acetonitrile R* and 90 volumes of a solution containing 2.77 g/l of *disodium hydrogen phosphate R* and 1.86 g/l of *citric acid R* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to cefazolin and cefuroxime.

Calculate the percentage content of cefazolin sodium by multiplying the percentage content of cefazolin by 1.048.

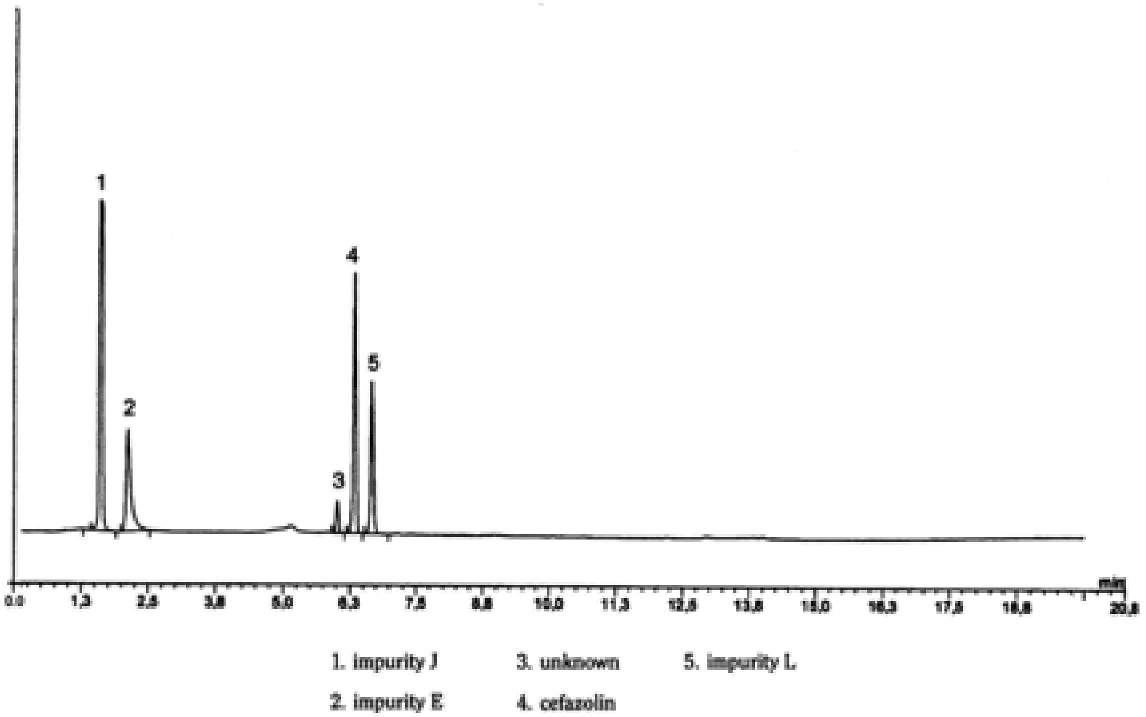
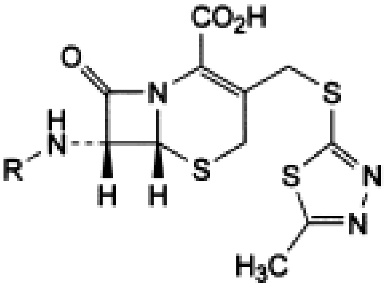


Figure 0988.-1. – *Chromatogram for the test for related substances of cefazolin sodium: reference solution (b) (in situ degradation)*

STORAGE

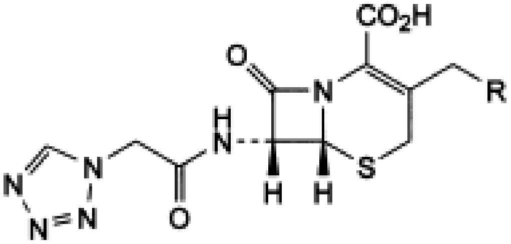
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES



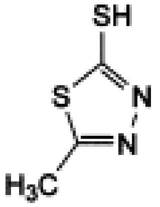
A. R = H: (6*R*,7*R*)-7-amino-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

B. R = CO-C$(CH_3)_3$: (6*R*,7*R*)-7-[(2,2-dimethylpropanoyl)amino]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

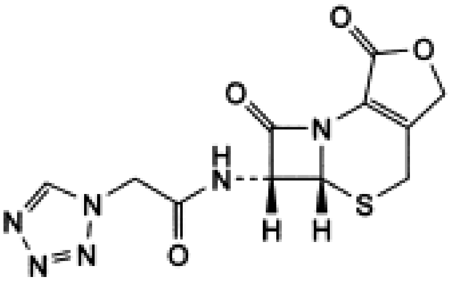


C. R = H: (6*R*,7*R*)-3-methyl-8-oxo-7-[(1*H*-tetrazol-1-ylacetyl)amino]-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid,

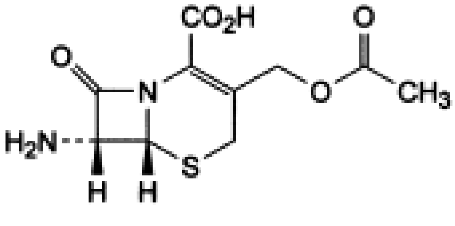
D. R = O-CO-CH_3: (6*R*,7*R*)-3-[(acetyloxy)methyl]-8-oxo-7-[(1*H*-tetrazol-1-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,



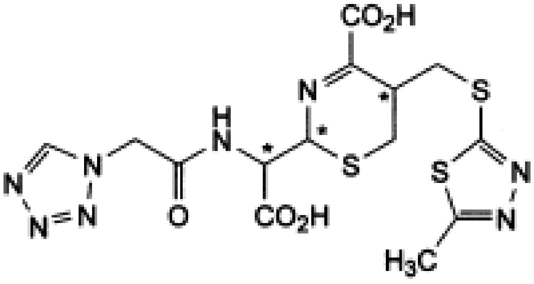
E. 5-methyl-1,3,4-thiadiazol-2-thiol (MMTD),



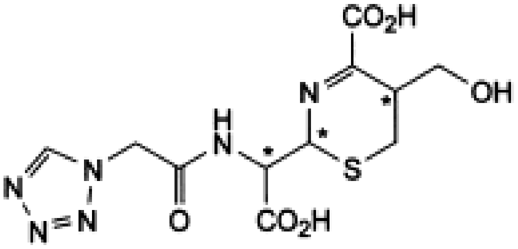
G. (5a*R*,6*R*)-6-[(1*H*-tetrazol-1-ylacetyl)amino]-5a,6-dihydro-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1, 3]thiazine-1,7(4*H*)-dione,



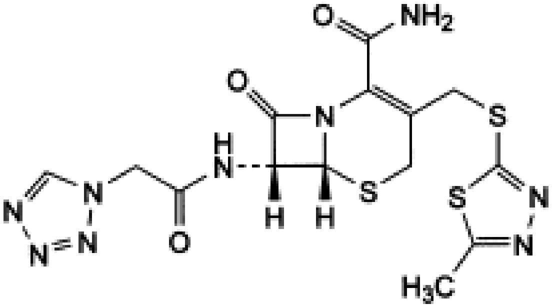
H. (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA),



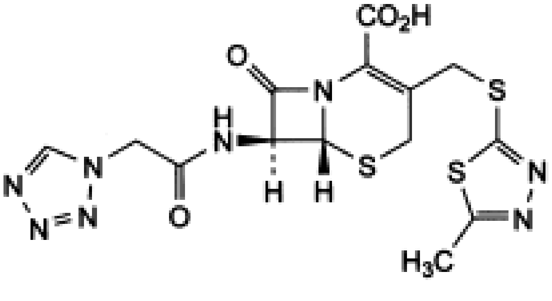
I. 2-[carboxy[(1*H*-tetrazol-1-ylacetyl)amino]methyl]-5-[[(5-methyl-1,3,4-thiadiazol-2-yl) sulphanyl]methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid (cefazoloic acid),



J. 2-[carboxy[(1*H*-tetrazol-1-ylacetyl)amino]methyl]-5-(hydroxymethyl)-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid (hydrolysed cefazoloic acid),



K. (6*R*,7*R*)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)sulphanyl]methyl]-8-oxo-7-[(1*H*-tetrazol-1-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide (cefazolinamide),

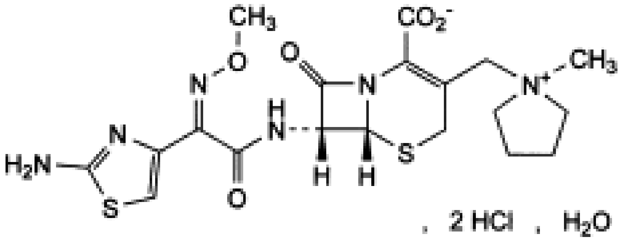


L. (6*R*,7*S*)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)sulphanyl]methyl]-8-oxo-7-[(1*H*-tetrazol-1-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Cefepime Hydrochloride Monohydrate

General Notices

(Cefepime Dihydrochloride Monohydrate, Ph Eur monograph 2126)



$C_{19}H_{24}N_6O_5S_2,2HCl,H_2O$ 571.5 *123171-59-5*

Action and use

Cephalosporin antibacterial.

Ph Eur

DEFINITION

(6*R*,7*R*)-7-[[(2*Z*)-(2-Aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[(1-methylpyrrolidinio) methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate dihydrochloride monohydrate. Semi-synthetic product derived from a fermentation product.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefepime dihydrochloride monohydrate CRS .*

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_3 *(2.2.2, Method II)*.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 40 to + 45 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Impurity G

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use.*

Test solution Dissolve 0.100 g of the substance to be examined in *0.01 M nitric acid* and dilute to 10.0 ml with the same acid.

Reference solution (a) Dilute 0.250 g of *N-methylpyrrolidine R* (impurity G) to 100.0 ml with *water R* . Dilute 2.0 ml of this solution to 100.0 ml with *0.01 M nitric acid* .

Reference solution (b) Dilute 0.250 g of *pyrrolidine R* to 100 ml with *0.01 M nitric acid* . Dilute 2 ml of the solution to 100 ml with *0.01 M nitric acid* . Mix 5 ml of this solution with 5 ml of reference solution (a).

Column:

— *size:* *l* = 0.05 m, Ø = 4.6 mm;

— *stationary phase*: *strong cation-exchange resin R* (5 µm).

Mobile phase Mix 1 volume of *acetonitrile R* and 100 volumes of *0.01 M nitric acid* ; filter through a 0.2 µm filter.

Flow rate 1 ml/min.

Detection Conductivity detector.

Injection 100 µl.

Run time 1.1 times the retention time of cefepime (retention time = about 50 min, eluting as a broadened peak).

System suitability:

— *symmetry factor* : maximum 2.5 for the peak due to impurity G in the chromatogram obtained with reference solution (a);

— *repeatability*: maximum relative standard deviation of 5.0 per cent after 6 injections of reference solution (a);

— *peak-to-valley ratio* : minimum 3 between the peaks due to pyrrolidine and impurity G in the chromatogram obtained with reference solution (b).

Calculate the percentage content of impurity G in the test solution using reference solution (a) .

Limit:
— *impurity G*: maximum 0.5 per cent.

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use or keep refrigerated at 4-8 ‘C for not more than 12 h.*

Test solution Dissolve 70.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A. Sonicate for 30 s and stir for about 5 min.

Reference solution (a) Dissolve 70.0 mg of *cefepime dihydrochloride monohydrate CRS* in mobile phase A and dilute to 50.0 ml with mobile phase A. Sonicate for 30 s and stir for about 5 min.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with mobile phase A. Dilute 2.0 ml of this solution to 100.0 ml with mobile phase A.

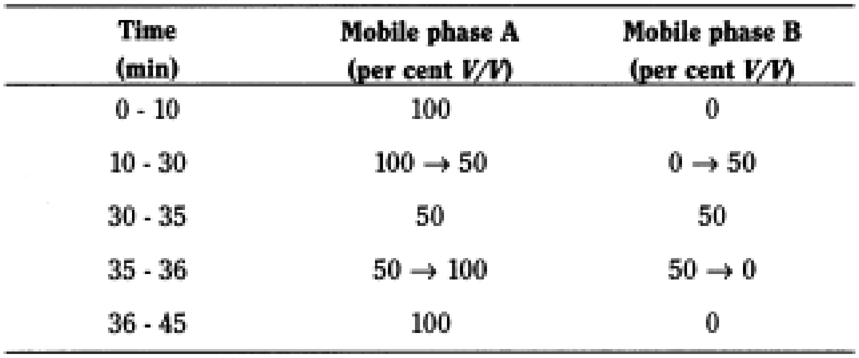
Reference solution (c) Dissolve 7 mg of *cefepime dihydrochloride monohydrate for system suitability CRS* (containing impurities A, B, E and F) in mobile phase A and dilute to 5 ml with mobile phase A.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: mix 10 volumes of *acetonitrile R* and 90 volumes of a 0.68 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 5.0 with *0.5 M potassium hydroxide* ;

— *mobile phase B*: mix equal volumes of *acetonitrile R* and a 0.68 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 5.0 with *0.5 M potassium hydroxide* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 30	100 → 50	0 → 50
30 - 35	50	50
35 - 36	50 → 100	50 → 0
36 - 45	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *cefepime dihydrochloride monohydrate for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, E and F.

Relative retention With reference to cefepime (retention time = about 7 min): impurity E = about 0.4; impurity F = about 0.8; impurity A = about 2.5; impurity B = about 4.1.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to impurity F and cefepime.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.4; impurity B = 1.4; impurity E = 1.8;

— *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities B, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity E*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water ***(2.5.12)***

3.0 per cent to 4.5 per cent, determined on 0.400 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.04 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Mobile phase A.

Injection Test solution and reference solution (a).

Run time 1.4 times the retention time of cefepime.

Calculate the percentage content of $C_{19}H_{26}Cl_2N_6O_5S_2$ from the declared content of *cefepime dihydrochloride monohydrate CRS* .

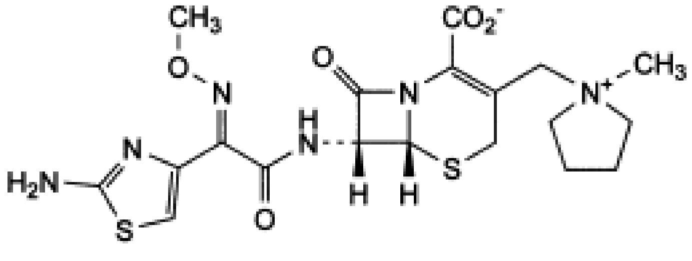
STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

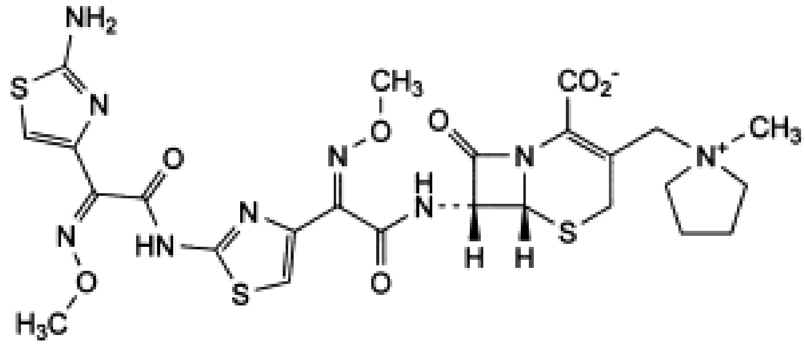
IMPURITIES

Specified impurities *A, B, E, F, G.*

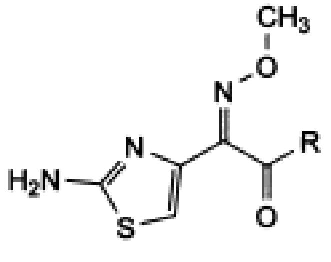
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C, D*.



A. (6*R*,7*R*)-7-[[(2*E*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[(1-methylpyrrolidinio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (*anti*-cefepime),

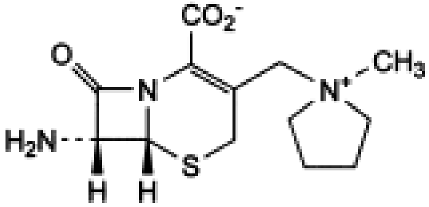


B. (6*R*,7*R*)-7-[[(2*Z*)-[2-[[(2*Z*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]thiazol-4-yl](methoxyimino)acetyl]amino]-3-[(1-methylpyrrolidinio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate,

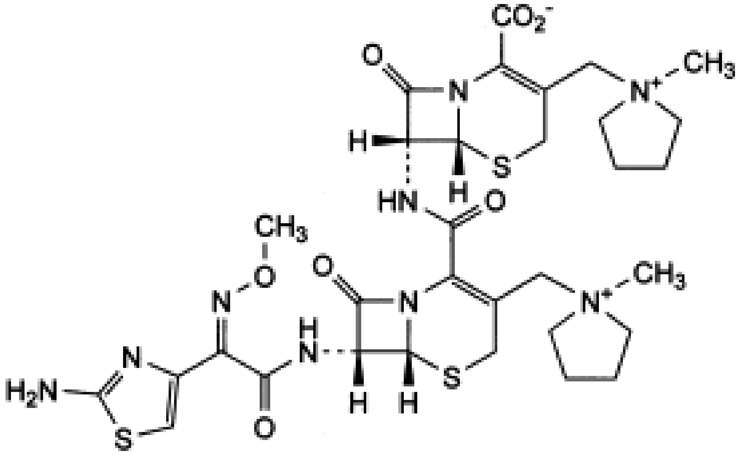


C. R = NH-CH_2-CHO: (2*Z*)-2-(2-aminothiazol-4-yl)-*N*-(formylmethyl)-2-(methoxyimino) acetamide,

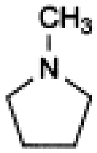
D. R = OH: (2*Z*)-(2-aminothiazol-4-yl)(methoxyimino)acetic acid,



E. (6*R*,7*R*)-7-amino-3-[(1-methylpyrrolidinio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate,



F. (6*R*,7*R*)-7-[[[(6*R*,7*R*)-7-[[(2*Z*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[(1-methylpyrrolidinio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-2-yl]carbonyl]amino]-3-[(1-methylpyrrolidinio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate,



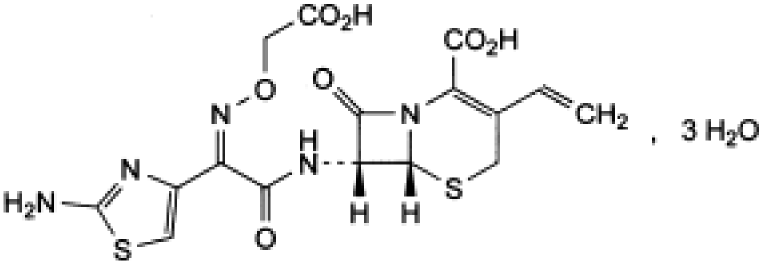
G. *N*-methylpyrrolidine.

Ph Eur

Cefixime

General Notices

(Ph Eur monograph 1188



$C_{16}H_{15}N_5O_7S_2,3H_2O$ 507.5 *79350-37-1*

Action and use

Cephalosporin antibacterial.

Ph Eur

DEFINITION

(6*R*,7*R*)-7-[[(*Z*)-2-(2-Aminothiazol-4-yl)-2-[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trihydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic powder.

Solubility

Slightly soluble in water, soluble in methanol, sparingly soluble in anhydrous ethanol, practically insoluble in ethyl acetate.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefixime CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R* , evaporate to dryness and record new spectra using the residues.

TESTS

pH *(2.2.3)*

2.6 to 4.1.

Suspend 0.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *cefixime CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 10 mg of *cefixime CRS* in 10 ml of *water R* . Heat on a water-bath for 45 min and cool (in situ preparation of impurity D). Inject immediately.

Column:

— *size*: *l* = 0.125 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 250 volumes of *acetonitrile R* and 750 volumes of a tetrabutylammonium hydroxide solution prepared as follows: dissolve 8.2 g of *tetrabutylammonium hydroxide R* in *water R* and dilute to 800 ml with the same solvent; adjust to pH 6.5 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl of the test solution and reference solutions (b) and (c).

Run time 3 times the retention time of cefixime.

System suitability Reference solution (c):

— *resolution*: minimum 2.0 between the peaks due to cefixime and impurity D; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Ethanol ***(2.4.24)***

Head-space gas chromatography *(2.2.28)* : use the standard additions method.

Sample solution Dissolve 0.250 g of the substance to be examined in a mixture of 1 volume of *dimethylacetamide R* and 4 volumes of *water R* and dilute to 25.0 ml with the same mixture of solvents.

Limit:
— *ethanol* : maximum 1.0 per cent *m/m*.

Water ***(2.5.12)***

9.0 per cent to 12.0 per cent, determined on 0.200 g.

Sulphated ash ***(2.4.14)***

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection The test solution and reference solution (a).

System suitability Reference solution (a):

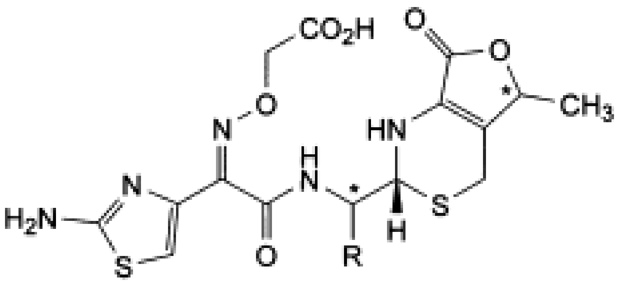
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{16}H_{15}N_5O_7S_2$ from the declared content of *cefixime CRS* .

STORAGE

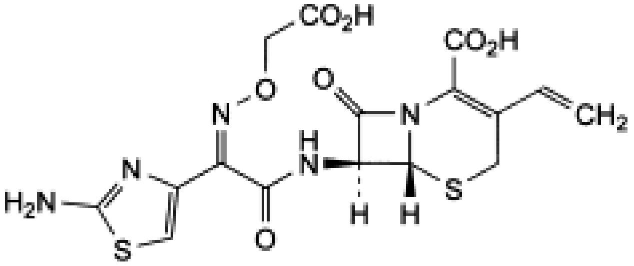
In an airtight container , protected from light.

IMPURITIES

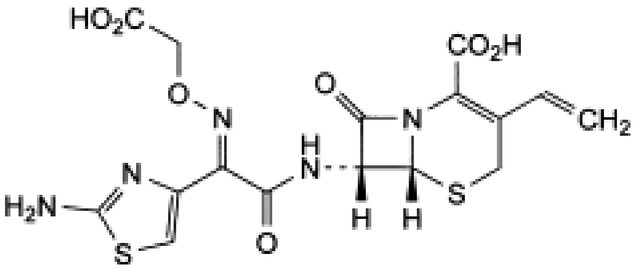


A. R = CO_2H: 2-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(carboxymethoxy)imino]acetyl]amino]-2-[(2R)-5-methyl-7-oxo-1,2,5,7-tetrahydro-4*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid,

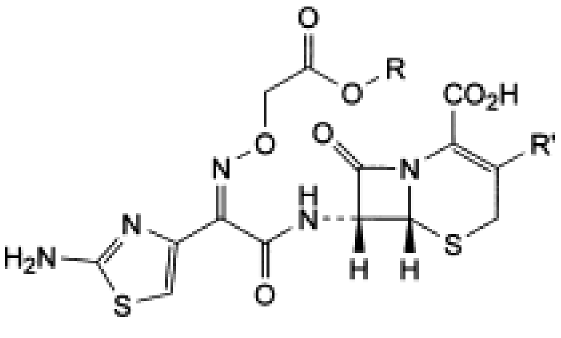
B. R = H: 2-[[[(*Z*)-1-(2-aminothiazol-4-yl)-2-[[[(2*R*,5*RS*)-5-methyl-7-oxo-1,2,5,7-tetrahydro-4*H*-furo[3,4-*d*][1,3]thiazin-2-yl]methyl]amino]-2-oxoethylidene]amino]oxy]acetic acid,



C. (6*R*,7*S*)-7-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (cefixime 7-epimer),



D. (6*R*,7*R*)-7-[[(*E*)-2-(2-aminothiazol-4-yl)-2-[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (cefixime *E*-isomer),



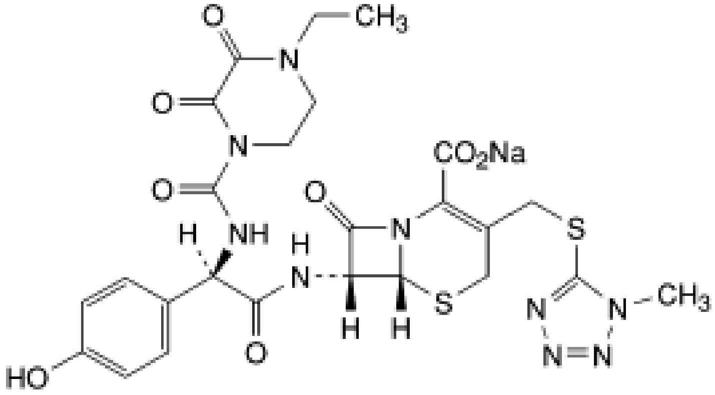
E. R = H, R′ = CH_3: (6*R*,7*R*)-7-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(carboxymethoxy)imino]acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

F. R = C_2H_5, R′ = $CH=CH_2$: (6*R*,7*R*)-7-[[(Z)-2-(2-aminothiazol-4-yl)-2-[(2-ethoxy-2-oxoethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Cefoperazone Sodium

General Notices

(Ph Eur monograph 1404)



$C_{25}H_{26}N_9NaO_8S_2$ 668 *62893-19-0*

Action and use

Cephalosporin antibacterial.

Ph Eur

DEFINITION

Sodium (6*R*,7*R*)-7-[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, hygroscopic powder.

Solubility

Freely soluble in water, soluble in methanol, slightly soluble in ethanol (96 per cent).

If crystalline, it shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Dissolve the substance to be examined in *methanol R* and evaporate to dryness. Examine the residue.

Comparison *Ph. Eur. reference spectrum of cefoperazone sodium.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (a) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and its absorbance *(2.2.25)* at 430 nm is not greater than 0.15.

Dissolve 2.5 g in *water R* and dilute to 25.0 ml with the same solvent.

pH *(2.2.3)*

4.5 to 6.5.

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use*.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 250.0 ml with the mobile phase.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *cefoperazone dihydrate CRS* in the mobile phase and dilute to 250.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 884 volumes of *water R*, 110 volumes of *acetonitrile R*, 3.5 volumes of a 60 g/l solution of *acetic acid R* and 2.5 volumes of a triethylammonium acetate solution prepared as follows: dilute 14 ml of *triethylamine R* and 5.7 ml of *glacial acetic acid R* to 100 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of test solution (b) and reference solutions (a) and (b).

Run time 2.5 times the retention time of cefoperazone.

Retention time Cefoperazone = about 15 min.

System suitability Reference solution (a):

— *number of theoretical plates*: minimum 5000, calculated for the principal peak; if necessary, adjust the content of *acetonitrile R* in the mobile phase;

— *symmetry factor*: maximum 1.6 for the principal peak; if necessary, adjust the content of *acetonitrile R* in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *total*: 4.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.5 per cent);

— *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

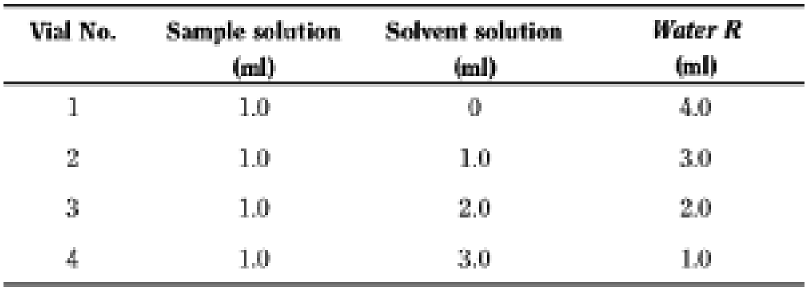
Acetone ***(2.4.24, System B)***

Maximum 2.0 per cent.

Sample solution Dissolve 0.500 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Solvent solution Dissolve 0.350 g of *acetone R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water R*.

Prepare each of 4 injection vials as shown in the table below:



Vial No.	Sample solution (ml)	Solvent solution (ml)	*Water R* (ml)
1	1.0	0	4.0
2	1.0	1.0	3.0
3	1.0	2.0	2.0
4	1.0	3.0	1.0

Static head-space conditions which may be used:

— *equilibration time*: 15 min;

— *transfer-line temperature*: 110 °C.

Temperature:

— *Column*: 40 °C for 10 min.

Heavy metals ***(2.4.8)***

Maximum 5 ppm.

2.0 g complies with test C. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 5.0 per cent, determined on 0.200 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.20 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

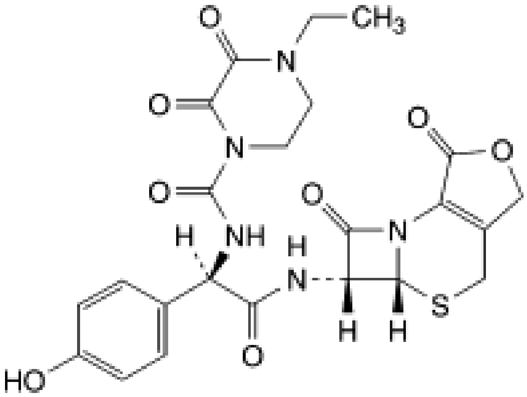
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of cefoperazone sodium by multiplying the percentage content of cefoperazone by 1.034.

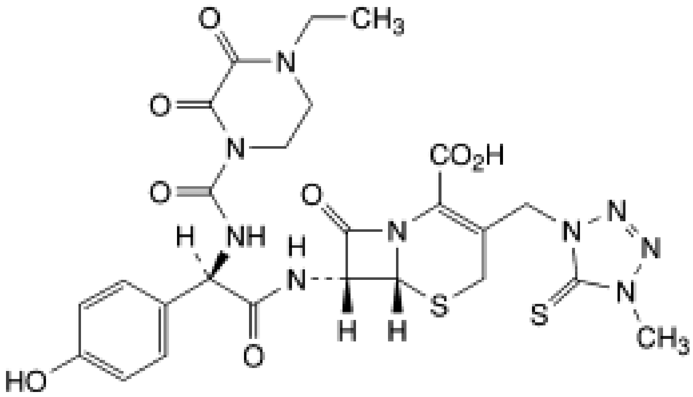
STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

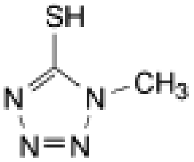
IMPURITIES



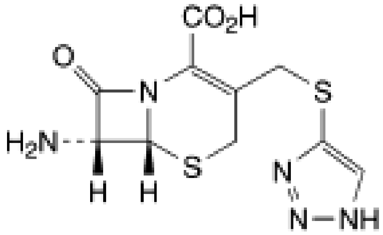
A. (5a*R*,6*R*)-6-[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]-5a,6-dihydro-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione,



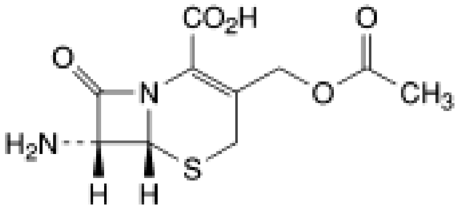
B. (6*R*,7*R*)-7-[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]-3-[(4-methyl-5-thioxo-4,5-dihydro-1*H*-tetrazol-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,



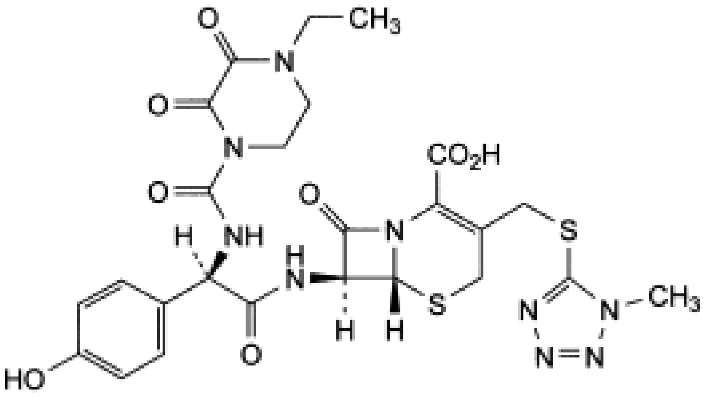
C. 1-methyl-1*H*-tetrazole-5-thiol,



D. (*6R,*7*R*)-7-amino-8-oxo-3-[(1*H*-1,2,3-triazol-4-ylsulphanyl)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-TACA),



E. (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA),



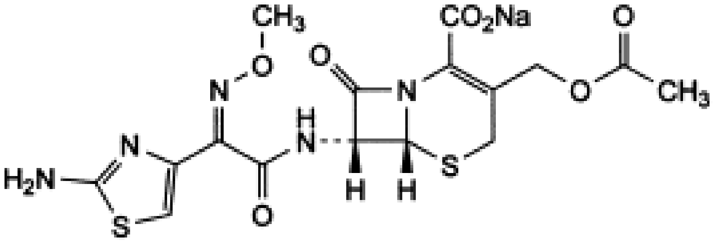
F. (6*R*,7*S*)-7-[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazine-1-yl)carbonyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Ph Eur

Cefotaxime Sodium

General Notices

(Ph Eur monograph 0989)



$C_{16}H_{16}N_5NaO_7S_2$ 477.4 *64485-93-4*

Action and use

Cephalosporin antibacterial.

Preparation

Cefotaxime Injection

Ph Eur

DEFINITION

Sodium (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[[(2*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow powder, hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefotaxime sodium CRS.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* . Add 1 ml of *glacial acetic acid R* to 10 ml of solution S. The solution, examined immediately, is clear.

pH *(2.2.3)*

4.5 to 6.5 for solution S.

Specific optical rotation *(2.2.7)*

+ 58.0 to + 64.0 (anhydrous substance).

Dissolve 0.100 g in *water R* and dilute to 10.0 ml with the same solvent.

Absorbance *(2.2.25)*

Maximum 0.40 at 430 nm for solution S.

Specific absorbance *(2.2.25)*

360 to 390, determined at the absorption maximum at 235 nm (anhydrous substance).

Dissolve 20.0 mg in *water R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *water R* .

Related substances

Liquid chromatography *(2.2.29)*

Prepare the solutions immediately before use.

Solution A Mobile phase B, mobile phase A (14:86 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in solution A and dilute to 50.0 ml with the same solution.

Reference solution (a) Dissolve 8.0 mg of *cefotaxime acid CRS* in solution A and dilute to 10.0 ml with the same solution.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with solution A.

Reference solution (c) Add 1.0 ml of *dilute hydrochloric acid R* to 4.0 ml of the test solution. Heat the solution at 40 °C for 2 h. Add 5.0 ml of *buffer solution pH 6.6 R* and 1.0 ml of *dilute sodium hydroxide solution R .*

Reference solution (d) Dissolve 4 mg of *cefotaxime for peak identification CRS* (containing impurities A, B, C, E and F) in 5 ml of solution A.

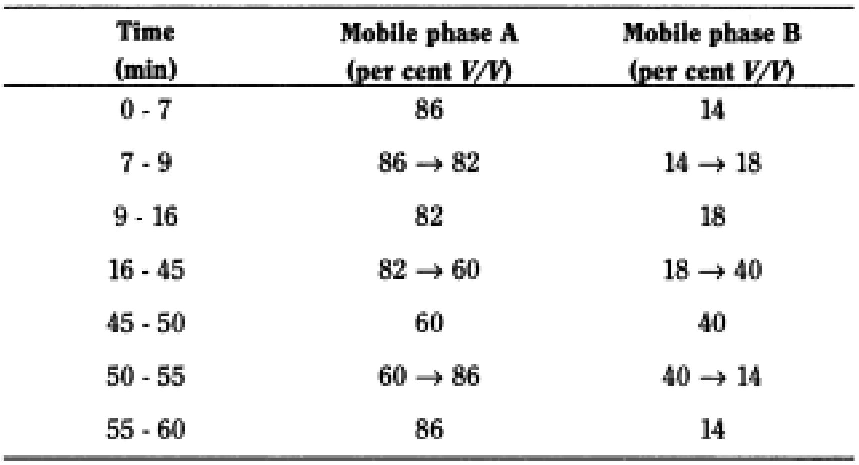
Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: 7.1 g/l solution of *disodium hydrogen phosphate R* adjusted to pH 6.25 using *phosphoric acid R;*

— *mobile phase B*: *methanol R;*



Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 7	86	14
7 - 9	86 → 82	14 → 18
9 - 16	82	18
16 - 45	82 → 60	18 → 40
45 - 50	60	40
50 - 55	60 → 86	40 → 14
55 - 60	86	14

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µl of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *cefotaxime for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, B, C, E and F.

Relative retention With reference to cefotaxime (retention time = about 13 min): impurity B = about 0.3; impurity A = about 0.5; impurity E = about 0.6; impurity C = about 1.9; impurity D = about 2.3; impurity F = about 2.4; impurity G = about 3.1.

System suitability Reference solution (c):

— *resolution*: minimum 3.5 between the peaks due to impurity E and cefotaxime;

— *symmetry factor* : maximum 2.0 for the peak due to cefotaxime.

Limits:
— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol ***(2.4.24, System A)***

Maximum 1.0 per cent.

N,N**-Dimethylaniline** ***(2.4.26, Method B)***

Maximum 20 ppm.

2-Ethylhexanoic acid ***(2.4.28)***

Maximum 0.5 per cent *m/m*.

Water ***(2.5.12)***

Maximum 3.0 per cent, determined on 0.300 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.05 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{16}H_{16}N_5NaO_7S_2$ by multiplying the percentage content of cefotaxime by 1.048.

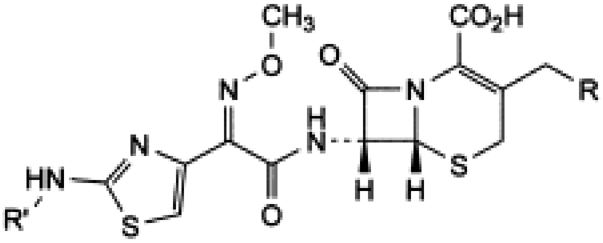
STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities A, B, C, D, E, F.

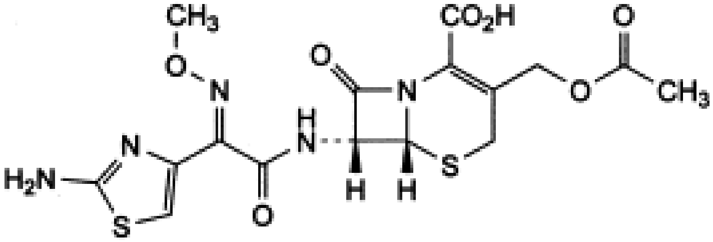
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): G.*



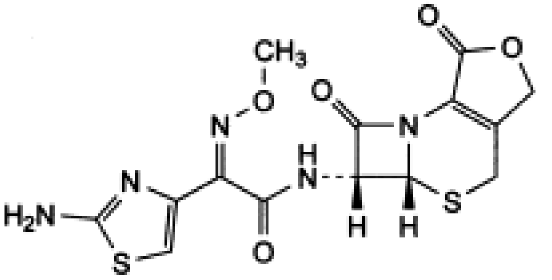
A. R = R′ = H: (6*R*,7*R*)-7-[[(2*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (deacetoxycefotaxime),

B. R = OH, R′ = H: (6*R*,7*R*)-7-[[(2Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (deacetylcefotaxime),

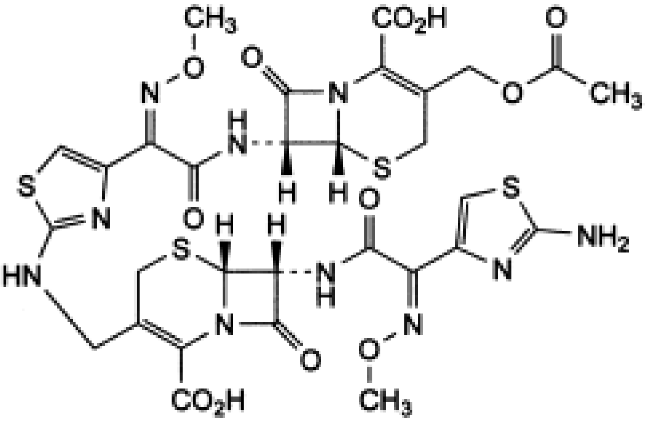
C. R = O-CO-CH_3, R′ = CHO: (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[[(2*Z*)-2-[2-(formylamino) thiazol-4-yl]-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (*N*-formylcefotaxime),



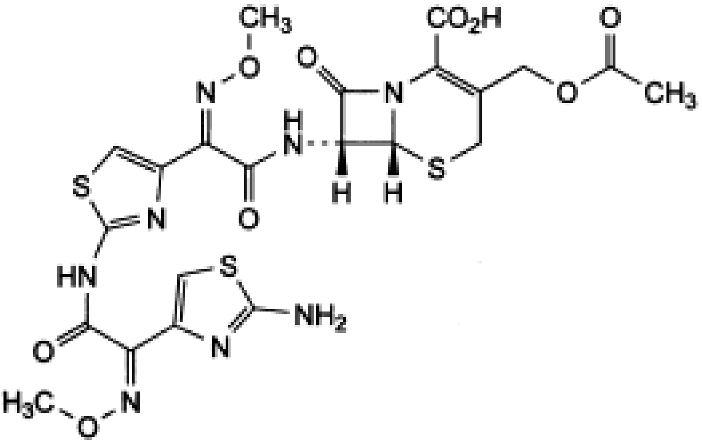
D. (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[[(2E)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl] amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (*E*-cefotaxime),



E. (5a*R*,6*R*)-6-[[(2*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-5a,6-dihydro-3*H*, 7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione (deacetylcefotaxime lactone),



F. (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[[(2*Z*)-2-[2-[[[(6*R*,7*R*)-7-[[(2*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-2-yl]methyl]amino]thiazol-4-yl]-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (cefotaxime dimer),



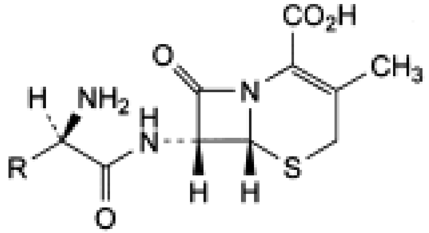
G. (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[[(2*Z*)-2-[2-[[(2*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]thiazol-4-yl]-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (ATA cefotaxime).

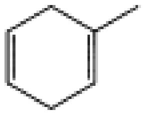
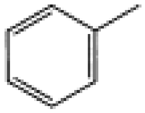
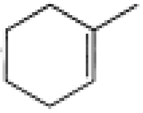
Ph Eur

Cefradine

General Notices

(Ph Eur monograph 0814)



Compound	R	Mol. Formula	M_r
cefradine		$C_{16}H_{19}N_3O_4S$	349.4
cefalexin		$C_{16}H_{17}N_3O_4S$	347.4
4',5'-dihydrocefradine		$C_{16}H_{21}N_3O_4S$	351.4

Action and use

Cephalosporin antibacterial.

Preparations

Cefradine Capsules

Cefradine Oral Suspension

Ph Eur

DEFINITION

Main component

(6*R*,7*R*)-7-[[(2*R*)-amino(cyclohexa-1,4-dienyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-

azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (cefradine).

Semi-synthetic product derived from a fermentation product.

Content:

— *cefradine:* minimum 90.0 per cent (anhydrous substance),

— *cefalexin*: maximum 5.0 per cent (anhydrous substance),

— *4′ ,5′ -dihydrocefradine*: maximum 2.0 per cent (anhydrous substance).

— *sum of the percentage contents of cefradine, cefalexin and 4′ ,5′ -dihydrocefradine*: 96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, hygroscopic powder.

Solubility

Sparingly soluble in water, practically insoluble in ethanol 96 per cent and in hexane.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison cefradine CRS.

If the spectra obtained in the solid state show differences, dissolve 30 mg of the substance to be examined and of the reference substance separately in 10 ml of *methanol R,* evaporate to dryness at 40 °C at a pressure less than 2 kPa and record new spectra using the residues.

TESTS

Solution S

Dissolve 2.50 g in *sodium carbonate solution R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*). Allow solution S to stand for 5 min. The absorbance of solution S measured at 450 nm (*2.2.25*) is not greater than 0.60.

pH *(2.2.3)*

3.5 to 6.0.

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 80.0 to + 90.0 (anhydrous substance).

Dissolve 0.250 g in *acetate buffer solution pH 4.6 R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.300 g of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a) Dissolve 3.0 mg of *cyclohexa-1,4-dienylglycine CRS* (impurity B) in mobile phase A and dilute to 100.0 ml with mobile phase A.

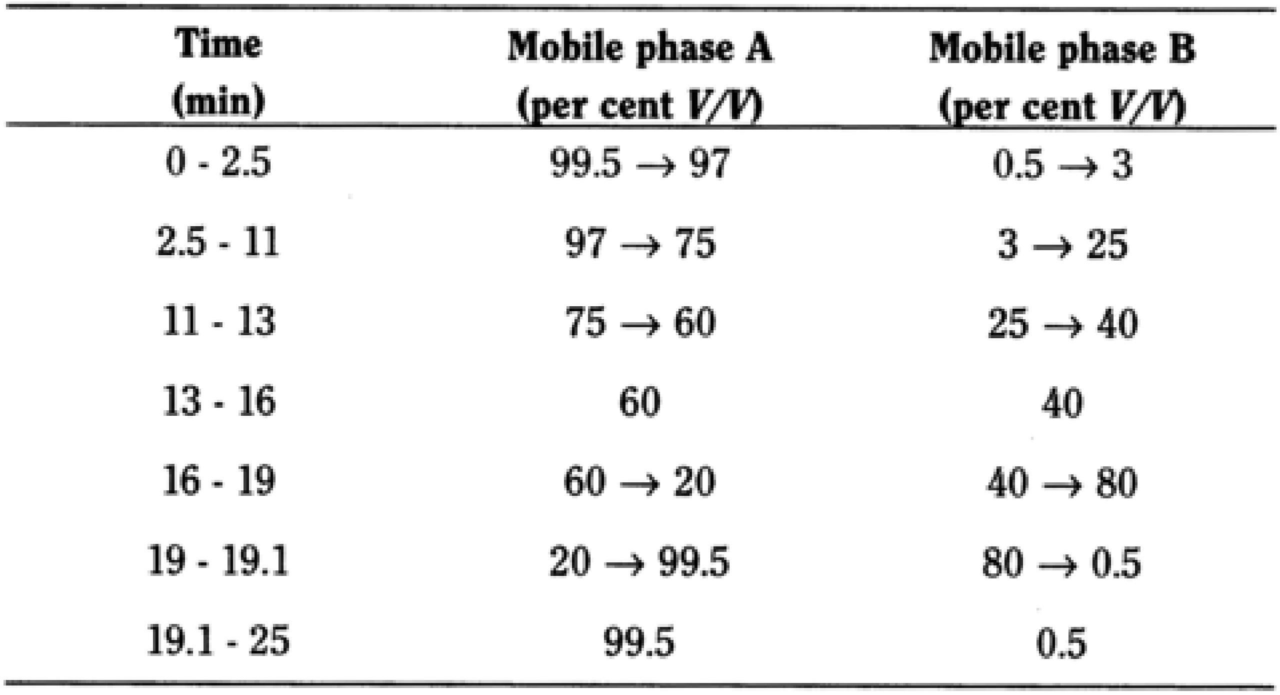
Reference solution (b) Dissolve 3 mg of the substance to be examined and 3 mg of *cefalexin CRS* in mobile phase A and dilute to 25 ml with mobile phase A.

Reference solution (c) Dilute 1.0 ml of test solution to 100.0 ml with mobile phase A.

Reference solution (d) Dissolve the contents of a vial of *cefradine for peak identification CRS* (containing impurities A, C, D, E and G) in 1 ml of *water for chromatography R* .

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm,
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: 2.72 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *dilute phosphoric acid R;*
- *mobile phase B: methanol R2* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2.5	99.5 → 97	0.5 → 3
2.5 - 11	97 → 75	3 → 25
11 - 13	75 → 60	25 → 40
13 - 16	60	40
16 - 19	60 → 20	40 → 80
19 - 19.1	20 → 99.5	80 → 0.5
19.1 - 25	99.5	0.5

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 25 µl.

Identification of impurities Use the chromatogram obtained with reference solution (d) and the chromatogram supplied with *cefradine for peak identification CRS* to identify the peaks due to impurities A, C, D, E and G.

Relative retention With reference to cefradine (retention time = about 15 min): impurity A = about 0.27; impurity B = about 0.32; impurity C = about 0.53; impurity D = about 0.63; impurity E = about 0.80; impurity F = about 0.92; cefalexin = about 0.95; 4′,5′-dihydrocefradine = about 1.06; impurity G = about 1.32.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to cefalexin and cefradine.

Limits:

— *impurity B*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *impurities A, C, D, E, F, G*: for each impurity, not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.25 per cent);

— *any other impurity*: for each impurity, not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peaks due to cefalexin and 4′,5′-dihydrocefradine.

N,N*-Dimethylaniline *(2.4.26, Method B)

Maximum 20 ppm.

Water *(2.5.12)*

Maximum 6.0 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in *phosphate buffer solution pH 5.0* R and dilute to 100.0 ml with the same solution.

Reference solution (a) Dissolve 50.0 mg of *cefradine CRS* (containing 4′,5′-dihydrocefradine) in *phosphate buffer solution pH 5.0 R* and dilute to 100.0 ml with the same solution.

Reference solution (b) Dissolve 5.0 mg of *cefalexin CRS* in *phosphate buffer solution pH 5.0 R* and dilute to 100.0 ml with the same solution.

Reference solution (c) Dilute 1 ml of reference solution (a) to 10 ml with *phosphate buffer solution pH 5.0 R*. Mix 5 ml of this solution with 5 ml of reference solution (b).

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R (5 µm).*

Mobile phase *methanol R, phosphate buffer solution pH 5.0 R* (25:75 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 5 µl.

Run time Twice the retention time of cefradine.

Relative retention With reference to cefradine (retention time = about 3 min): cefalexin = about 0.7; 4′,5′-dihydrocefradine = about 1.5.

System suitability Reference solution (c):

— *resolution*: minimum 4.0 between the peaks due to cefalexin and cefradine.

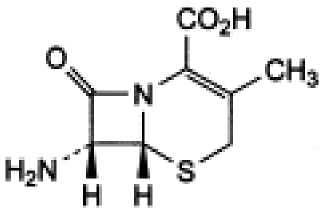
Calculate the percentage content of cefradine using the chromatogram obtained with reference solution (a) and the declared content of *cefradine CRS*. Calculate the percentage content of cefalexin using the chromatogram obtained with reference solution (b) and the declared content of *cefalexin CRS*. Calculate the percentage content of 4′,5′-dihydrocefradine using the chromatogram obtained with reference solution (b) and multiplying the area of the peak due to 4′,5′-dihydrocefradine by a correction factor of 1.6.

STORAGE

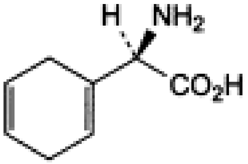
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES

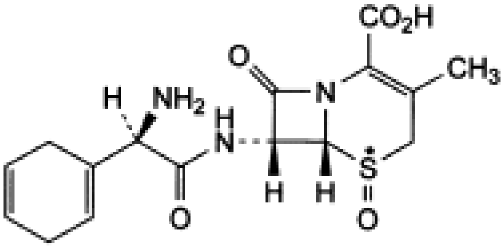
Specified impurities *A, B, C, D, E, F, G.*



A. (6*R*,7*R*)-7-amino-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-aminodeacetoxycephalosporanic acid, 7-ADCA),

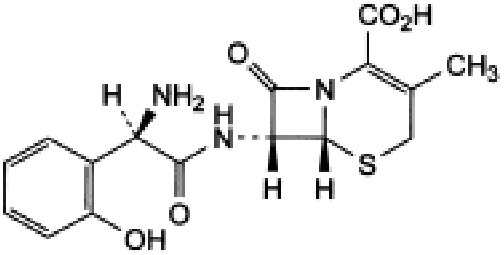


B. (2*R*)-amino(cyclohexa-1,4-dienyl)acetic acid (D-dihydrophenylglycine),

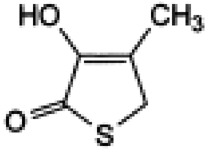


C. (6*R*,7*R*)-7-[[(2*R*)-amino(cyclohexa-1,4-dienyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide (isomer 1),

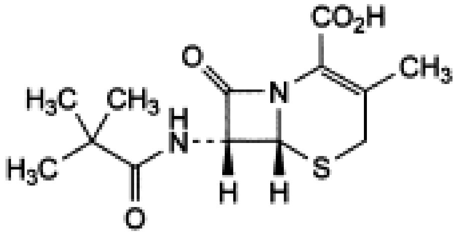
D. (6*R*,7*R*)-7-[[(2*R*)-amino(cyclohexa-1,4-dienyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide (isomer 2),



E. ((6*R*,7*R*)-7-[[(2*R*)-amino(2-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,



F. 3-hydroxy-4-methylthiophen-2(5*H*)-one,



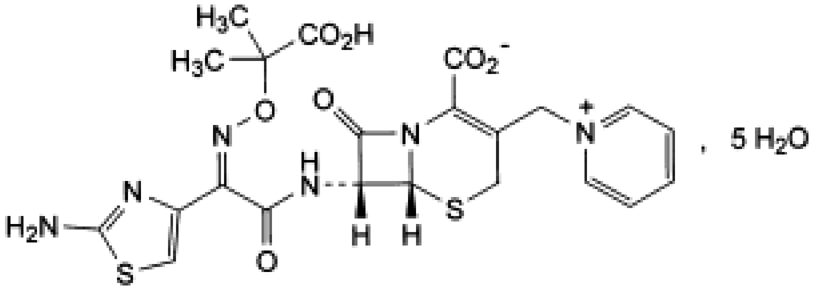
G. (6*R*,7*R*)-7-[(2,2-dimethylpropanoyl)amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ADCA pivalamide).

Ph Eur

Ceftazidime

General Notices

(Ph Eur monograph 1405)



$C_{22}H_{22}N_6O_7S_2,5H_2O$ 637 *72558-82-8*

Action and use

Cephalosporin antibacterial.

Ph Eur

DEFINITION

Ceftazidime is (6*R*,7*R*)-7-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-8-oxo-3-[(1-pyridinio)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate pentahydrate. It contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{22}H_{22}N_6O_7S_2$, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water and in methanol, practically insoluble in acetone and in alcohol. It dissolves in acid and alkali solutions.

IDENTIFICATION

Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *ceftazidime CRS* .

TESTS

Solution S

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 3.0 to 4.0.

Related substances

A. Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel* F_{254} *plate R*.

Test solution Dissolve 0.100 g of the substance to be examined in a 36 g/l solution of *disodium hydrogen phosphate R* and dilute to 2.0 ml with the same solution.

Reference solution Dilute 1 ml of the test solution to 200 ml with a 36 g/l solution of *disodium hydrogen phosphate R*.

Apply to the plate 2 µl of each solution. Develop over a path of 15 cm using a mixture of 6 volumes of *butanol R*, 26 volumes of *sodium acetate buffer solution pH 4.5 R*, 32 volumes of *butyl acetate R* and 32 volumes of *glacial acetic acid R*. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any spot with an R_F value greater than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

B. Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *ceftazidime impurity A CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *ceftazidime impurity A CRS* and 5 mg of *ceftazidime CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.3 ml/min a mixture of 7 volumes of *acetonitrile R* and 93 volumes of a 22.6 g/l solution of *ammonium dihydrogen phosphate R,* adjusted to pH 3.9 with a 10 per cent *V/V* solution of *phosphoric acid R*,

— as detector a spectrophotometer set at 255 nm,

maintaining the temperature of the column at 35 °C.

Inject 20 µl of reference solution (b). Adjust the sensitivity of the system so that the heights of the 2 peaks in the chromatogram obtained are at least 50 per cent of the full scale of the recorder. The test is not valid unless in the chromatogram obtained, the resolution between the peaks corresponding to ceftazidime and impurity A is at least 5.9. Inject 20 µl of the test solution and 20 µl of reference solution (a). Continue the chromatography of the test solution for 3 times the retention time of ceftazidime. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Impurity F

Not more than 500 ppm, determined by liquid chromatography *(2.2.29)*

Prepare the solutions immediately before use.

Test solution Dissolve 0.500 g of the substance to be examined in a 10 per cent *V/V* solution of *phosphate buffer solution pH 7.0 R4* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 1.00 g of *pyridine R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 200.0 ml with *water R.* To 1.0 ml of the solution, add 10 ml of *phosphate buffer solution pH 7.0 R4* and dilute to 100.0 ml with *water R* .

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with a 10 per cent *V/V* solution of *phosphate buffer solution pH 7.0 R4*. To 1.0 ml of the solution add 20 ml of reference solution (a) and dilute to 200 ml with a 10 per cent *V/V* solution of *phosphate buffer solution pH 7.0 R4*.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture of 8 volumes of a 28.8 g/l solution of *ammonium dihydrogen phosphate R* previously adjusted to pH 7.0 with *ammonia R* , 24 volumes of *acetonitrile R* and 68 volumes of *water R* ,

— as detector a spectrophotometer set at 255 nm,

Inject 20 µl of reference solution (b). The test is not valid unless in the chromatogram obtained, the resolution between the peaks due to ceftazidime and to impurity F is at least 7.0.

Inject 20 µl of reference solution (a). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full scale of the recorder. Inject reference solution (a) 6 times. The test is not valid unless the relative standard deviation of the area of the principal peak is at most 1.0 per cent. Inject alternately 20 µl of the test solution and 20 µl of reference solution (a).

Water ***(2.5.12)***

13.0 per cent to 15.0 per cent, determined on 0.200 g by the semi-micro determination of water.

Bacterial endotoxins ***(2.6.14)***

Less than 0.10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Examine by liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *ceftazidime CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *ceftazidime impurity A CRS* in 5.0 ml of reference solution (a).

The chromatographic procedure may be carried out using:

— a column 0.15 m long and 4.6 mm in internal diameter packed with *hexylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 2 ml/min a mixture prepared as follows: dissolve 4.26 g of *disodium hydrogen phosphate R* and 2.73 g of *potassium dihydrogen phosphate R* in 980 ml of *water R* , then add 20 ml of *acetonitrile R* ,

— as detector a spectrophotometer set at 245 nm.

Inject 20 µl of reference solution (b). Adjust the sensitivity of the system so that the heights of the 2 principal peaks in the chromatogram obtained are at least 50 per cent of the full scale of the recorder. The test is not valid unless, in the chromatogram obtained, the resolution between the peaks corresponding to ceftazidime and impurity A is at least 1.0. Inject alternately the test solution and reference solution (a). Calculate the percentage content of ceftazidime.

STORAGE

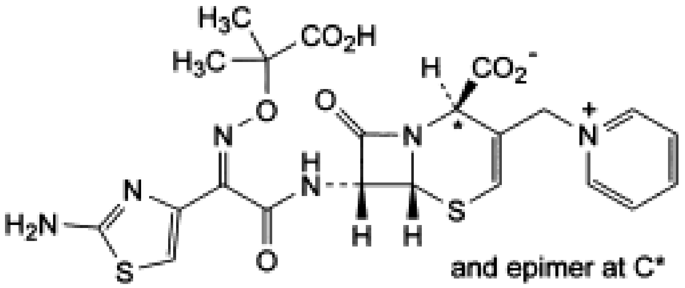
Store in an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

By liquid chromatography (related substances test) *A, B, C.*

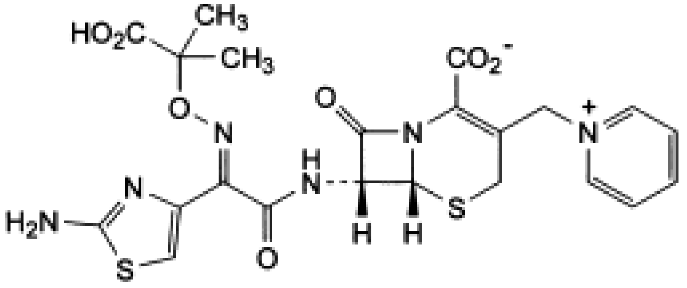
By thin-layer chromatography (related substances test) *D, E.*

By liquid chromatography (impurity F test) *F.*

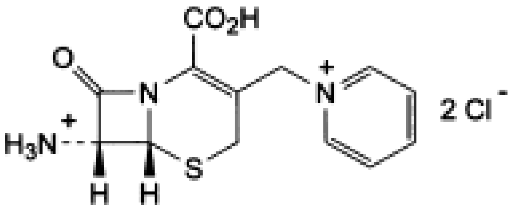


A. (2*RS*,6*R*,7*R*)-7-[[(*Z*)-2-(2-aminothiazol-4-yl)-2-[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-8-oxo-3-[(1-pyridinio)methyl]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (Δ-2-

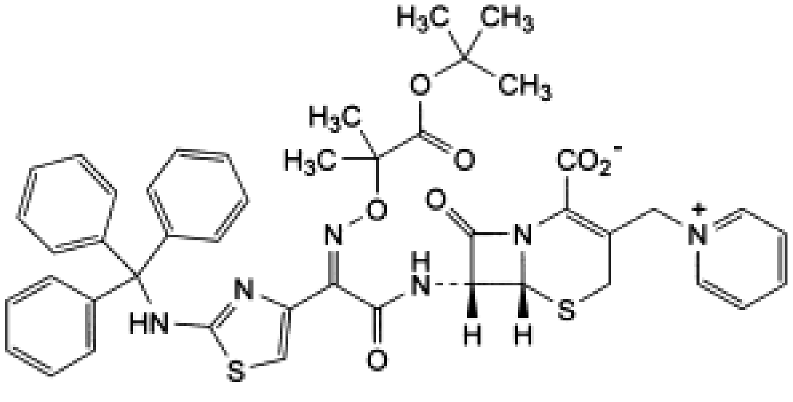
ceftazidime),



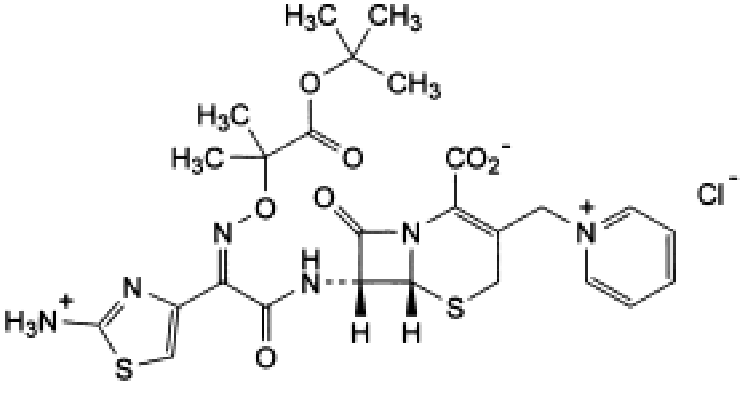
B. (6*R*,7*R*)-7-[[(*E*)-2-(2-aminothiazol-4-yl)-2-[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-8-oxo-3-[(1-pyridinio)methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate,



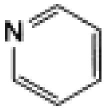
C. (6*R*,7*R*)-2-carboxy-8-oxo-3-(pyridiniomethyl)-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-aminium dichloride,



D. (6*R*,7*R*)-7-[[(*Z*)-2-[[2-(1,1-dimethylethoxy)-1,1-dimethyl-2-oxoethoxy]imino]-2-[2-[(triphenylmethyl)amino]thiazol-4-yl]acetyl]amino]-8-oxo-3-(pyridiniomethyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate,



E. (6*R*,7*R*)-7-[[(*Z*)-2-(2-ammoniothiazol-4-yl)-2-[[2-(1,1-dimethylethoxy)-1,1-dimethyl-2-oxoethoxy]imino]acetyl]amino]-8-oxo-3-(pyridiniomethyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate chloride,



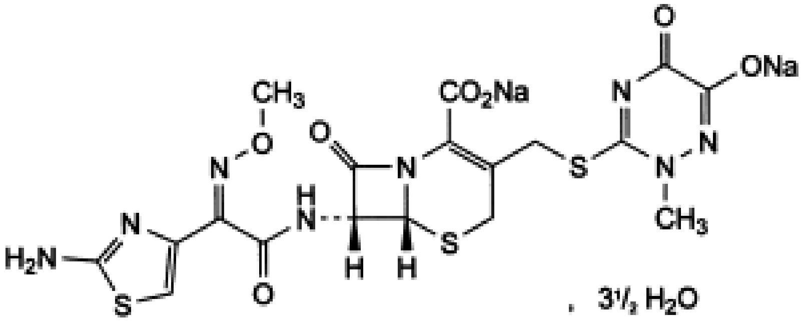
F. pyridine.

Ph Eur

Ceftriaxone Sodium

General Notices

(Ph Eur monograph 0991)



$C_{18}H_{16}N_8Na_2O_7S_3,3½H_2O$ 662 *104376-79-6*

Action and use

Cephalosporin antibacterial.

Preparation

Ceftriaxone Injection

Ph Eur

DEFINITION

Disodium (6*R*,7*R*)-7-[[(2Z)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[[(2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 3.5 hydrate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Almost white or yellowish, slightly hygroscopic, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in methanol, very slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *ceftriaxone sodium CRS.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 2.40 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 or BY_5 *(2.2.2)* .

Dilute 2 ml of solution S to 20 ml with *water R* .

pH *(2.2.3)*

6.0 to 8.0 for solution S.

Specific optical rotation *(2.2.7)*

- 155 to - 170 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 30.0 mg of *ceftriaxone sodium CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *ceftriaxone sodium CRS* and 5.0 mg of *ceftriaxone impurity A CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 2.0 g of *tetradecylammonium bromide R* and 2.0 g of *tetraheptylammonium bromide R* in a mixture of 440 ml of *water R*, 55 ml of *0.067 M phosphate buffer solution pH 7.0 R*, 5.0 ml of citrate buffer solution pH 5.0 prepared by dissolving 20.17 g of *citric acid R* in 800 ml of *water R*, adjusting to pH 5.0 with *strong sodium hydroxide solution R* and diluting to 1000.0 ml with *water R,* and 500 ml of *acetonitrile R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of ceftriaxone.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to ceftriaxone and impurity A.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

N*,*N*-Dimethylaniline** ***(2.4.26, Method B)

Maximum 20 ppm.

2-Ethylhexanoic acid ***(2.4.28)***

Maximum 0.8 per cent m/m.

Water ***(2.5.12)***

8.0 per cent to 11.0 per cent, determined on 0.100 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.08 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

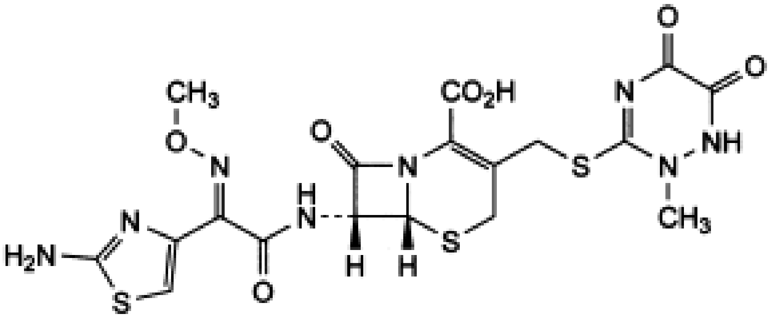
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{18}H_{16}N_8Na_2O_7S_3$ from the declared content of *ceftriaxone sodium CRS*.

STORAGE

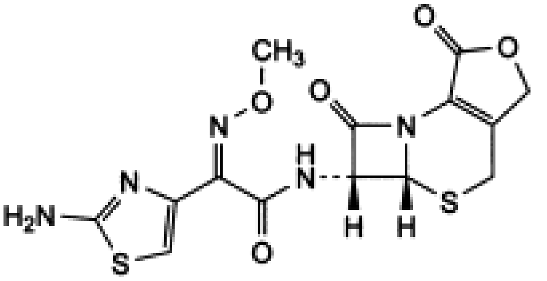
In an airtight container protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES



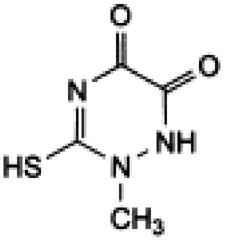
CH3
O
N
N
H2N
S
O
H
N
H
H
O
N
S
CO2H
S
N
O
O
NH
N
CH3

A. (6*R*,7*R*)-7-[[(2*E*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid ((*E*)-isomer),



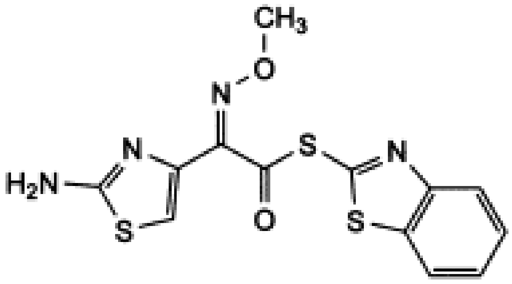
CH3
O
N
N
H2N
S
O
H
N
H
H
O
N
S
O
O

B. (5a*R*,6*R*)-6-[[(2*Z*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-5a,6-dihydro-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione,

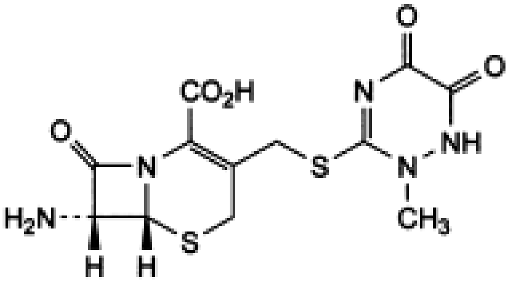


O
O
N
NH
HS
N
CH3

C. 2-methyl-3-sulphanyl-1,2-dihydro-1,2,4-triazine-5,6-dione,



D. *S*-benzothiazol-2-yl (2*Z*)-(2-aminothiazol-4-yl)(methoxyimino)thioacetate,



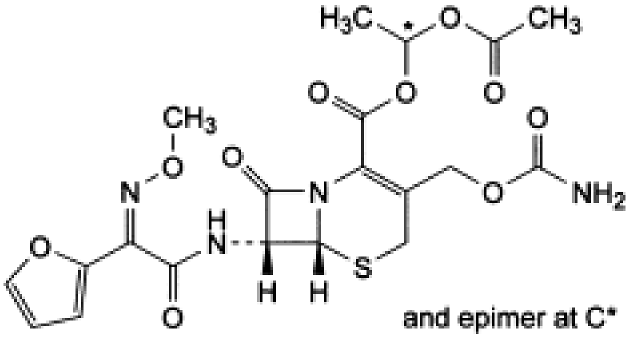
E. (6*R*,7*R*)-7-amino-3-[[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Ph Eur

Cefuroxime Axetil

General Notices

(Ph Eur monograph 1300)



$C_{20}H_{22}N_4O_{10}S$ 510.5 *64544-07-6*

Action and use

Cephalosporin antibacterial.

Preparation

Cefuroxime Axetil Tablets

Ph Eur

DEFINITION

Mixture of the 2 diastereoisomers of (1*RS*)-1-(acetyloxy)ethyl (6*R*,7*R*)-3-[(carbamoyloxy) methyl]-7-[[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, soluble in acetone, in ethyl acetate and in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefuroxime axetil CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the peaks due to cefuroxime axetil diastereoisomers A and B in the chromatogram obtained with reference solution (d).

TESTS

Related substances

Liquid chromatography *(2.2.29)* : use the normalisation procedure. *Prepare the test solution and reference solution (d) immediately before use.*

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) In order to prepare *in situ* impurity A, heat 5 ml of the test solution at 60 °C for 1 h.

Reference solution (c) In order to prepare *in situ* impurity B, expose 5 ml of the test solution to ultraviolet light at 254 nm for 24 h.

Reference solution (d) Dissolve 10.0 mg of *cefuroxime axetil CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: trimethylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, 23 g/l solution of ammonium dihydrogen phosphate R* (38:62 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 278 nm.

Injection 20 µl of the test solution and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the pair of peaks due to impurity A and use the chromatogram obtained with reference solution (c) to identify the pair of peaks due to impurity B.

Relative retention With reference to cefuroxime axetil diastereoisomer A: cefuroxime axetil diastereoisomer B = about 0.9, impurity A = about 1.2; impurity B = 1.7 and 2.1.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to cefuroxime axetil diastereoisomer A and impurity A.

Limits:

— *impurity A*: maximum 1.5 per cent for the sum of the pair of peaks;

— *impurity B*: maximum 1.0 per cent for the sum of the pair of peaks;

— *impurity E*: maximum 0.5 per cent;

— *any other impurity*: for each impurity, maximum 0.5 per cent;

— *total*: maximum 3.0 per cent;

— *disregard limit*: 0.05 times the area of the 2 principal peaks in the chromatogram obtained with reference solution (a) (0.05 per cent).

Diastereoisomer ratio

Liquid chromatography *(2.2.29)* as described in the test for related substances.

Limit Test solution:

— the ratio of the area of the peak due to cefuroxime axetil diastereoisomer A to the sum of the areas of the peaks due to cefuroxime axetil diastereoisomers A and B is between 0.48 and 0.55.

Acetone ***(2.4.24)***

Maximum 1.1 per cent.

Water ***(2.5.12)***

Maximum 1.5 per cent, determined on 0.400 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (d).

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to cefuroxime axetil diastereoisomers A and B;

— *repeatability*: maximum relative standard deviation of 2.0 per cent for the sum of the peaks due to cefuroxime axetil diastereoisomers A and B after 6 injections.

Calculate the percentage content of $C_{20}H_{22}N_4O_{10}S$ from the sum of the areas of the 2 diastereoisomer peaks and the declared content of $C_{20}H_{22}N_4O_{10}S$ in *cefuroxime axetil CRS.*

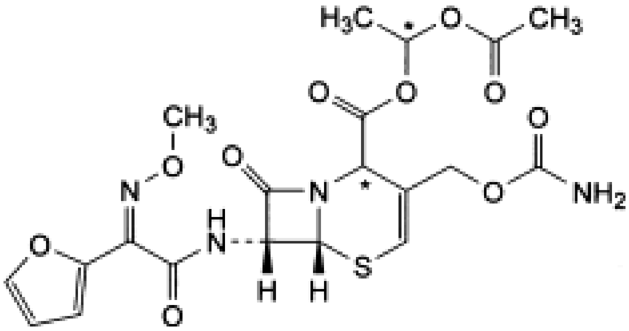
STORAGE

In an airtight container , protected from light.

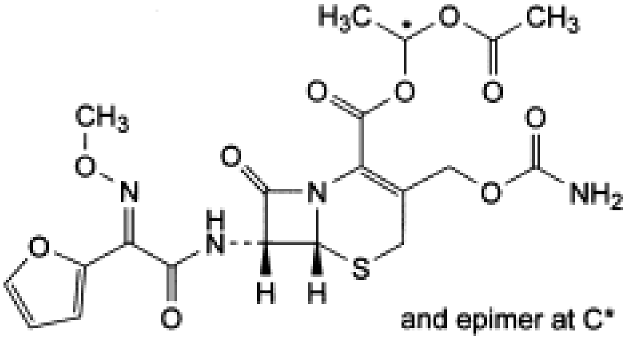
IMPURITIES

Specified impurities   A, B, E.

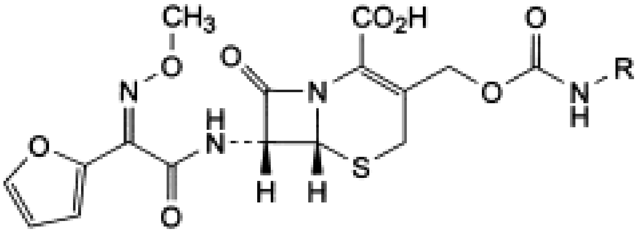
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : C, D.*



A. 1-(acetyloxy)ethyl (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-[[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (Δ^3-isomers),

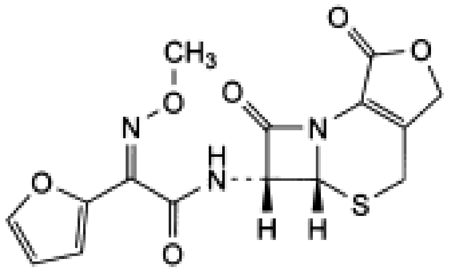


B. (1*RS*)-1-(acetyloxy)ethyl (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-[[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ((*E*)-isomers),



C. R = CO-CCl_3: (6*R*,7*R*)-7-[[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-3-[[[(trichloroacetyl)carbamoyl]oxy]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

D. R = H: cefuroxime.



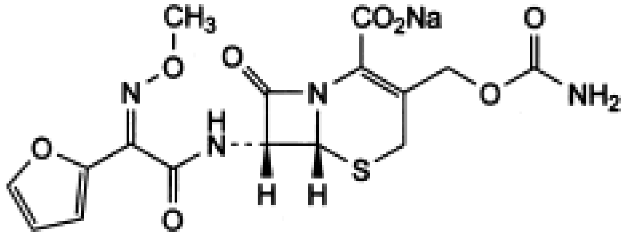
E. (5a*R*,6*R*)-6-[[(2*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-5a,6-dihydro-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione (descarbamoylcefuroxime lactone).

Ph Eur

Cefuroxime Sodium

General Notices

(Ph Eur monograph 0992)



$C_{16}H_{15}N_4NaO_8S$ 446.4 *56238-63-2*

Action and use

Cephalosporin antibacterial.

Preparation

Cefuroxime Injection

Ph Eur

DEFINITION

Sodium (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-[[(*Z*)-(furan-2-yl)(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cefuroxime sodium CRS.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* . The absorbance *(2.2.25)* of solution S measured at 450 nm is not greater than 0.25.

pH *(2.2.3)*

5.5 to 8.5.

Dilute 2 ml of solution S to 20 ml with *carbon dioxide-free water R* .

Specific optical rotation *(2.2.7)*

+ 59 to + 66 (anhydrous substance).

Dissolve 0.500 g in *acetate buffer solution pH 4.6 R* and dilute to 25.0 ml with the same buffer solution.

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use or keep at 2-8 °C.*

Test solution (a) Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with *water R.*

Reference solution (a) Dissolve 25.0 mg of *cefuroxime sodium CRS* in *water R* and dilute to 25.0 ml with the same solvent. Dilute 5.0 ml to 50.0 ml with *water R* .

Reference solution (b) Place 20 ml of reference solution (a) in a water-bath at 80 °C for 15 min. Cool and inject immediately.

Reference solution (c) Dilute 1.0 ml of test solution (a) to 100.0 ml with *water R.*

Column:
- *size*: *l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase: hexylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 1 volume of *acetonitrile R* and 99 volumes of an acetate buffer solution pH 3.4, prepared by dissolving 6.01 g of *glacial acetic acid R* and 0.68 g of *sodium acetate R* in *water R* and diluting to 1000 ml with the same solvent.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µl loop injector; inject test solution (a) and reference solutions (b) and (c).

Run time 4 times the retention time of cefuroxime.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to cefuroxime and impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

N*,*N*-Dimethylaniline *(2.4.26, Method B)

Maximum 20 ppm.

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.5 per cent *m/m*.

Water *(2.5.12)*

Maximum 3.5 per cent, determined on 0.400 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

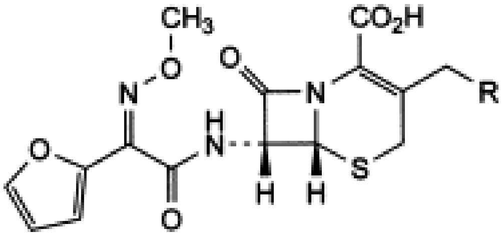
Injection Test solution (b) and reference solution (a).

Calculate the percentage content of cefuroxime sodium.

STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

IMPURITIES

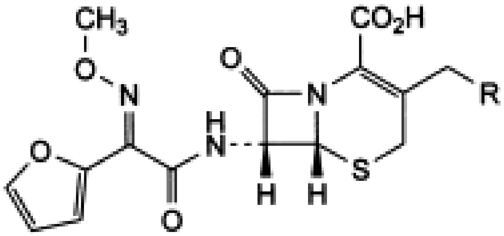


A. R = OH: (6*R*,7*R*)-7-[[(*Z*)-(furan-2-yl)(methoxyimino)acetyl]amino]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (descarbamoylcefuroxime),

B. R = O-CO-CH_3: (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[[(*Z*)-(furan-2-yl)(methoxyimino)acetyl] amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

C. R = H: (6*R*,7*R*)-7-[[(*Z*)-(furan-2-yl)(methoxyimino)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

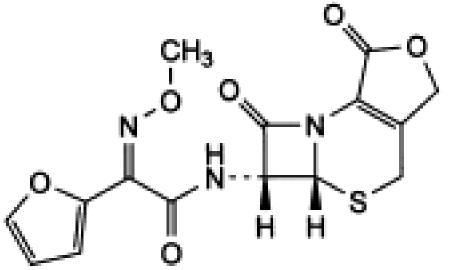
D. R = O-CO-NH-CO-CCl_3: (6*R*,7*R*)-7-[[(*Z*)-(furan-2-yl)(methoxyimino)acetyl]amino]-8-oxo-3-[[[(trichloroacetyl)carbamoyl]oxy]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,



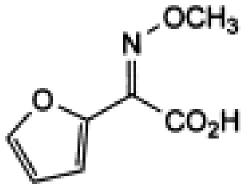
E. R = O-CO-NH_2: (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-[[(*E*)-(furan-2-yl)(methoxyimino) acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (*trans*-cefuroxime),

F. R = OH: (6*R*,7*R*)-7-[[(*E*)-(furan-2-yl)(methoxyimino)acetyl]amino]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

G. R = O-CO-CH_3: (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[[(*E*)-(furan-2-yl)(methoxyimino)acetyl] amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,



H. (5a*R*,6*R*)-6-[[(*Z*)-(furan-2-yl)(methoxyimino)acetyl]amino]-5a,6-dihydro-3*H*,7*H*-azeto[2,1-*b*]furo[3,4-*d*][1,3]thiazine-1,7(4*H*)-dione,



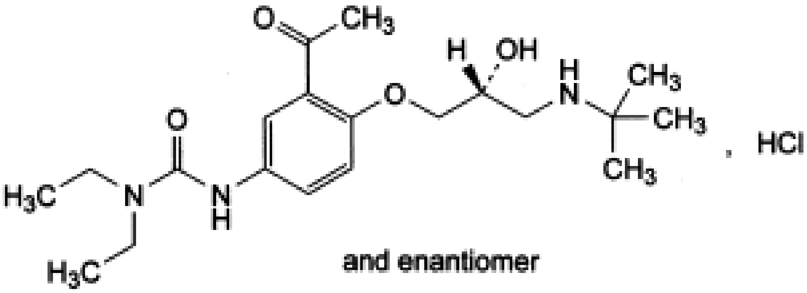
I. (*Z*)-(furan-2-yl)(methoxyimino)acetic acid.

Ph Eur

Celiprolol Hydrochloride

General Notices

(Ph Eur monograph 1632)



$C_{20}H_{33}N_3O_4$,HCl *416.0* *57470-78-7*

Action and use

Beta-adrenoceptor antagonist.

Preparation

Celiprolol Hydrochloride Tablets

Ph Eur

DEFINITION

3-[3-Acetyl-4-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]phenyl]-1,1-diethylurea hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or very slightly yellow, crystalline powder.

Solubility

Freely soluble in water and in methanol, soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison celiprolol hydrochloride CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R* , evaporate to dryness and record new spectra using the residues.

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 1.0 g in *water R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*) *. Prepare the solutions immediately before use.*

Test solution Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 20.0 ml with mobile phase A.

Reference solution (a) Dissolve 2 mg of the substance to be examined and 2 mg of *acebutolol hydrochloride R* in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (b) Dissolve 10 mg of the substance to be examined in 2 ml of mobile phase A and allow to stand for 24 h (for identification of impurity A).

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (d) Dissolve 10 mg of *celiprolol for peak identification CRS* in mobile phase A and dilute to 2 ml with mobile phase A.

Reference solution (e) This solution is only prepared if required (see below) and is used to determine the identity of impurity I which co-elutes with impurity H (the 2 impurities originate from different routes of synthesis). Dissolve the contents of a vial of *celiprolol impurity I CRS* in mobile phase A and dilute to 2.0 ml with mobile phase A.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: mix 91 ml of *tetrahydrofuran R,* 63 ml of *acetonitrile R1* , 0.6 ml of

pentafluoropropanoic acid R and 0.2 ml of *trifluoroacetic acid R* ; dilute to 1000 ml with water R;

— *mobile phase B: acetonitrile R1* ;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	100 → 80	0 → 20
50 - 51	80 → 100	20 → 0
51 - 65	100	0

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 232 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *celiprolol for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B, E and F.

Relative retention With reference to celiprolol (retention time = about 10 min): impurity A = about 0.3; impurity D = about 0.7; impurity G = about 1.2; impurity B = about 1.4; impurity F = about 1.6; impurity C = about 2.2; impurity H or I = about 2.5; impurity E = about 3.9.

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to celiprolol and acebutolol.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 4.0; impurity B = 1.5; impurity E = 2.3; impurity F = 0.5; impurity I = 1.7;

— *any impurity*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent), and not more than 1 such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *total:* not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— if any of the above limits are exceeded and if a peak occurs with a relative retention of about 2.5 (impurity H or I), the identity of this peak has to be clarified by use of a UV spectrum recorded with a diode array detector; if this spectrum is different from the one obtained with reference solution (e), no correction factor is applied;

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.350 g under an atmosphere of nitrogen in 50 ml of *ethanol (96 per cent) R* and add 1.0 ml of *0.1 M hydrochloric acid* . Carry out a potentiometric titration *(2.2.20)* , using *0.1 M sodium hydroxide* . Read the volume added between the 2 points of inflexion.

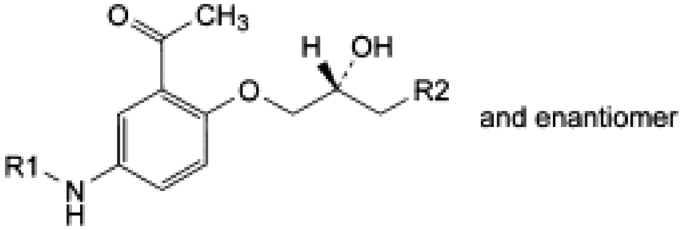
1 ml of *0.1 M sodium hydroxide* is equivalent to 41.60 mg of $C_{20}H_{34}ClN_3O_4$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I.

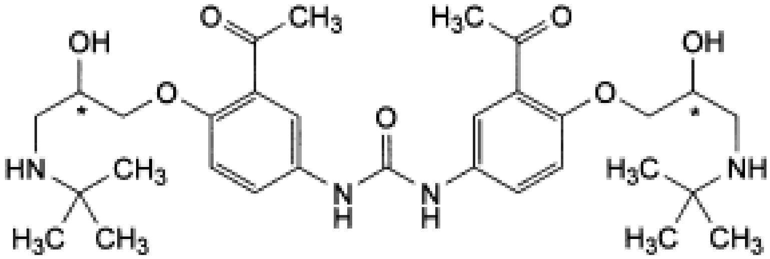


A. R1 = H, R2 = NH-C(CH_3)$_3$: 1-[5-amino-2-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]phenyl]ethanone,

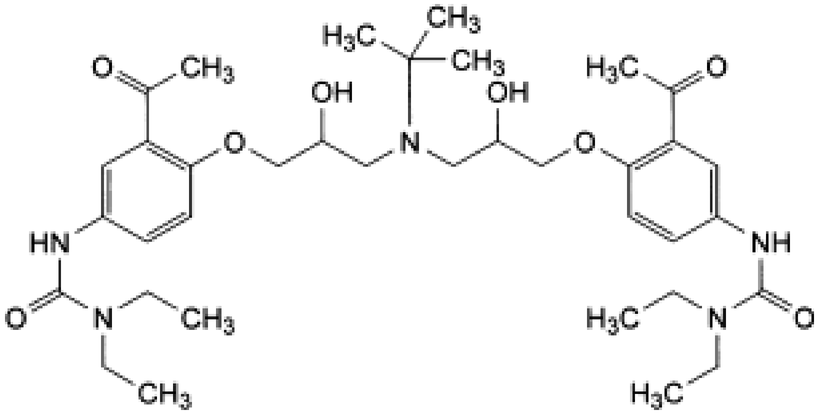
C. R1 = CO-NH-C(CH_3)$_3$, R2 = NH-C(CH_3)$_3$: 1-[3-acetyl-4-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]phenyl]-3-(1,1-dimethylethyl)urea,

D. R1 = CO-N(C_2H_5)$_2$, R2 = N(C_2H_5)2: 3-[3-acetyl-4-[(2*RS*)-3-(diethylamino)-2-hydroxypropoxy]phenyl]-1,1-diethylurea,

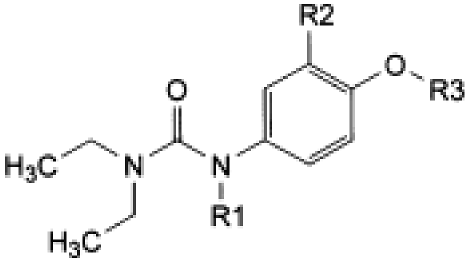
H. R1 = CO-N(C_2H_5)2, R2 = Br: 3-[3-acetyl-4-[(2*RS*)-3-bromo-2-hydroxypropoxy]phenyl]-1,1-diethylurea (bromhydrin compound),



B. 1,3-bis[3-acetyl-4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]phenyl]urea,

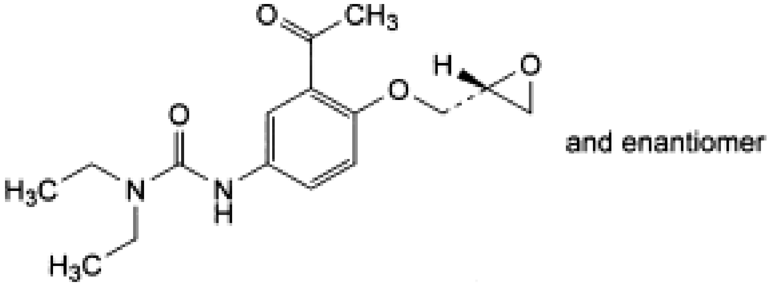


E. 1,1′-[[(1,1-dimethylethyl)imino]bis[(2-hydroxypropane-1,3-diyl)oxy(3-acetyl-1,4-phenylene)]]bis(3,3-diethylurea),



F. R1 = R3 = H, R2 = CO-CH_3: 3-(3-acetyl-4-hydroxyphenyl)-1,1-diethylurea,

I. R1 = CO-CH_3, R2 = H, R3 = C_2H_5: 1-acetyl-1-(4-ethoxyphenyl)-3,3-diethylurea,



G. 3-[3-acetyl-4-[[(*RS*)-oxiranyl]methoxy]phenyl]-1,1-diethylurea.

Ph Eur

Cellacefate

General Notices

(Cellulose Acetate Phthalate, Ph Eur monograph 0314)

Action and use

Enteric coating in pharmaceutical products.

Ph Eur

DEFINITION

Partly *O*-acetylated and *O*-phthalylated cellulose.

Content:

— phthaloyl groups ($C_8H_5O_3$, relative mass of the group 149.1): 30.0 per cent to 36.0 per cent (anhydrous and acid-free substance).

— acetyl groups (C_2H_3O, relative mass of the group 43.05): 21.5 per cent to 26.0 per cent (anhydrous and acid-free substance).

CHARACTERS

Appearance

White or almost white, free-flowing powder or colourless flakes, hygroscopic.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in diethylene glycol, practically insoluble in ethanol and in methylene chloride. It dissolves in dilute solutions of alkalis.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of cellulose acetate phthalate.*

TESTS

Viscosity *(2.2.9)*

45 mPa·s to 90 mPa·s for the apparent viscosity determined at 25 °C.

Dissolve 15 g, calculated with reference to the anhydrous substance, in 85 g of a mixture of 1 part of *water R* and 249 parts of *acetone R* .

Free acid

Maximum 3.0 per cent, calculated as phthalic acid (anhydrous substance).

Shake 3.0 g for 2 h with 100 ml of a 50 per cent *V/V* solution of *methanol R* and filter. Wash the flask and the filter with 2 quantities, each of 10 ml, of a mixture of a 50 per cent *V/V* solution of *methanol R.* Combine the filtrate and washings, add *phenolphthalein solution R* and titrate with *0.1 M sodium hydroxide* until a faint pink colour is obtained. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 8.3 mg of free acid, calculated as phthalic acid.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R* .

Water *(2.5.12)*

Maximum 5.0 per cent, determined on 0.500 g.

Carry out the test using a mixture of 2 volumes of *methylene chloride R* and 3 volumes of *ethanol R.*

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Phthaloyl groups

Dissolve 1.000 g in 50 ml of a mixture of 2 volumes of *acetone R* and 3 volumes of *alcohol R* . Add 0.1 ml of *phenolphthalein solution R* and titrate with *0.1 M sodium hydroxide* until a faint pink colour is obtained. Carry out a blank titration.

Calculate the percentage content of phthaloyl groups (*P*) from the expression:

$$\frac{14\,900n}{(100-a)(100-S)m} - \frac{179.5S}{(100-S)}$$

a = percentage content of water,

m = mass of the substance to be examined, in grams,

n = number of millilitres of 0.1 M sodium hydroxide used,

S = percentage content of free acid.

Acetyl groups

To 0.100 g add 25.0 ml of *0.1 M sodium hydroxide* and heat on a water-bath under a reflux condenser for 30 min. Cool, add 0.1 ml of *phenolphthalein solution R* and titrate with *0.1 M hydrochloric acid* until the colour is discharged. Carry out a blank titration.

Calculate the percentage content of acetyl groups from the expression:

$$\left[\frac{4300\,(n_2 - n_1)}{(100 - a)\,(100 - S)\,m} - \frac{51.8S}{(100 - S)}\right] - 0.578P$$

a = percentage content of water,

m = mass of the substance to be examined, in grams,

n_1 = number of millilitres of 0.1 M hydrochloric acid used in the test,

n_2 = number of millilitres of 0.1 M hydrochloric acid used in the blank titration,

P = percentage content of phthaloyl groups,

S = percentage content of free acid (see Tests).

STORAGE

In an airtight container.

Ph Eur

Cellulose Acetate Butyrate

General Notices

(Ph Eur monograph 1406)

Action and use

Excipient.

Ph Eur

DEFINITION

Partly or completely *O*-acetylated and *O*-butyrated cellulose.

Content:

- *acetyl groups* (C_2H_3O): 2.0 per cent to 30.0 per cent (dried substance); 90.0 per cent to 110.0 per cent of that stated on the label (dried substance);
- *butyryl groups* (C_4H_7O): 16.0 per cent to 53.0 per cent (dried substance); 90.0 per cent to 110.0 per cent of that stated on the label (dried substance).

CHARACTERS

Appearance

White, yellowish-white or greyish-white powder or granules, slightly hygroscopic.

Solubility

Practically insoluble in water, soluble in acetone, in formic acid and in a mixture of equal volumes of methanol and methylene chloride, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison Ph. Eur. reference spectrum of cellulose acetate butyrate.

The intensity of the bands may vary according to the degree of substitution.

B. It complies with the limits of the assay.

TESTS

Acidity

To 5.00 g in a 250 ml conical flask, add 150 ml of *carbon dioxide-free water R,* insert the stopper, swirl the suspension gently and allow to stand for 3 h. Filter, wash the flask and the filter with *carbon dioxide-free water R* . Combine the filtrate and washings. Add 0.1 ml of *phenolphthalein solution R1* . Not more than 3.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Total ash ***(2.4.16)***

Maximum 0.1 per cent.

ASSAY

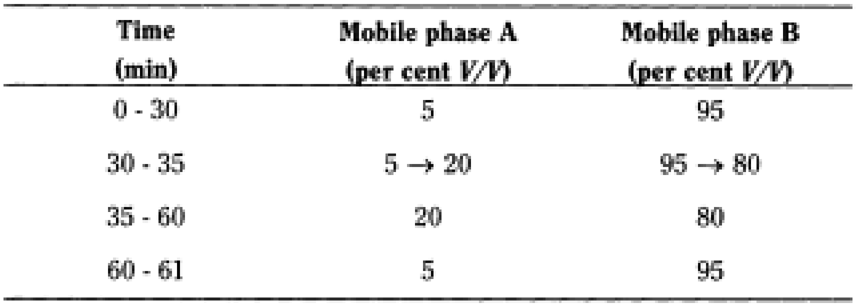
Liquid chromatography *(2.2.29).*

Test solution To 1.000 g of the substance to be examined in a 500 ml conical flask, add 100 ml of *acetone R* and 10 ml of *water R* . Close the flask and stir with a magnetic stirrer until dissolution is complete. Add 30.0 ml of *1 M sodium hydroxide* with constant stirring. Close the flask and stir with a magnetic stirrer for 30 min. Add 100 ml of hot *water R* at 80 °C, washing down the sides of the flask and stir for 2 min. Cool, centrifuge or filter the suspension and wash the residue with *water R* . Combine the filtrate and washings, adjust to pH 3 (*2.2.3*) with *dilute phosphoric acid R* and dilute to 500.0 ml with *water R* .

Reference solution Dissolve 0.200 g of *glacial acetic acid R* and 0.400 g of *butyric acid R* in *water R* , adjust to pH 3 *(2.2.3)* with *dilute phosphoric acid R* and dilute to 500.0 ml with *water R* .

Column:
- *dimensions:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A: methanol R;*
- *mobile phase B: phosphate buffer solution pH 3.0 R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	5	95
30 - 35	5 → 20	95 → 80
35 - 60	20	80
60 - 61	5	95

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Calculate the percentage content of acetic acid and butyric acid using the chromatograms obtained with the 2 solutions. To calculate the percentage content of acetyl (C_2H_3O) and of butyryl (C_4H_7O) groups, multiply the percentage content of acetic acid and butyric acid by 0.717 and 0.807, respectively.

STORAGE

In an airtight container.

LABELLING

The label states the nominal percentage content of acetyl and butyryl groups.

Ph Eur

Cellulose Acetate

General Notices

(Ph Eur monograph 0887)

Action and use

Excipient.

Ph Eur

DEFINITION

Partly or completely *O*-acetylated cellulose.

Content

29.0 per cent to 44.8 per cent of acetyl groups (C_2H_3O) (dried substance) and 90.0 per cent to 110.0 per cent of the acetyl content stated on the label (dried substance).

CHARACTERS

Appearance

White, yellowish-white or greyish-white powder or granules, hygroscopic.

Solubility

Practically insoluble in water, soluble in acetone, in formic acid and in a mixture of equal volumes of methanol and methylene chloride, practically insoluble in alcohol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison Ph. Eur. reference spectrum of cellulose acetate.

TESTS

Free acid

Maximum 0.1 per cent, calculated as acetic acid (dried substance).

To 5.00 g in a 250 ml conical flask, add 150 ml of *carbon dioxide-free water R* , insert the stopper, swirl the suspension gently and allow to stand for 3 h. Filter, wash the flask and the filter with *carbon dioxide-free water R* . Combine the filtrate and washings. Add 0.1 ml of *phenolphthalein solution R1* . Titrate with *0.01 M sodium hydroxide* until a faint pink colour is obtained.

1 ml of *0.01 M sodium hydroxide* is equivalent to 0.6005 mg of free acid, calculated as acetic acid.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram of which not more than 10^2 fungi per gram determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13).*

ASSAY

Cellulose acetate containing not more than 42.0 per cent of acetyl groups

To 2.000 g in a 500 ml conical flask add 100 ml of *acetone R* and 10 ml of *water R* . Close the flask and stir with a magnetic stirrer until dissolution is complete. Add 30.0 ml of *1 M sodium hydroxide* with constant stirring. Close the flask and stir with a magnetic stirrer for 30 min. Add 100 ml of *water R* at 80 °C, washing down the sides of the flask, stir for 2 min and cool to room temperature. Titrate with *0.5 M sulphuric acid ,* using 0.1 ml of *phenolphthalein solution R* as indicator. Carry out a blank titration.

Calculate the percentage content of acetyl groups from the expression:

$$\frac{4.305\,(n_2 - n_1)}{(100 - d) \times m} \times 100$$

d = loss on drying as a percentage,

m = mass of the substance to be examined, in grams,

n_1 = number of millilitres of *0.5 M sulphuric acid* used in the test,

n_2 = number of millilitres of *0.5 M sulphuric acid* used in the blank titration.

Cellulose acetate containing more than 42.0 per cent of acetyl groups

To 2.000 g in a 500 ml conical flask add 30 ml of *dimethyl sulphoxide R* and 100 ml of *acetone R.* Close the flask and stir with a magnetic stirrer for 16 h. Add 30.0 ml of *1 M sodium hydroxide* with constant stirring. Close the flask and stir with a magnetic stirrer for 6 min. Allow to stand without stirring for 60 min. Add 100 ml of *water R* at 80 °C, washing down the sides of the flask, stir for 2 min and cool to room temperature. Titrate with *0.5 M hydrochloric acid* , using 0.1 ml of *phenolphthalein solution R* . Add 0.5 ml *of 0.5 M hydrochloric acid* in excess, stir for 5 min and allow to stand for 30 min. Titrate with *0.5 M sodium hydroxide* , until a persistent pink colour is obtained, stirring with a magnetic stirrer. Calculate the net number of millimoles of *0.5 M sodium hydroxide* consumed, taking the mean of 2 blank titrations into consideration.

Calculate the percentage content of acetyl groups from the expression:

$$\frac{4.305 \times n}{(100 - d) \times m} \times 100$$

d = loss on drying as a percentage,

m = mass of the substance to be examined, in grams,

n = net number of millimoles of *0.5 M sodium hydroxide* ,

STORAGE

In an airtight container .

LABELLING

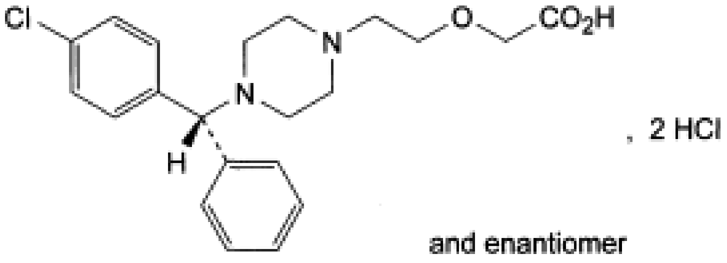
The label states the nominal percentage content of acetyl groups.

Ph Eur

Cetirizine Hydrochloride

General Notices

(Cetirizine Dihydrochloride, Ph Eur monograph 1084)



$C_{21}H_{25}ClN_2O_3,2HCl$ 461.8 *83881-52-1*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

(*RS*)-2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid dihydrochloride.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, practically insoluble in acetone and in methylene chloride.

IDENTIFICATION

First identification B, D.

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 20.0 mg in 50 ml of a 10.3 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of this solution to 100.0 ml with a 10.3 g/l solution of *hydrochloric acid R* .

Spectral range 210-350 nm.

Absorption maximum At 231 nm.

Specific absorbance at the absorption maximum 359 to 381.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *cetirizine dihydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *cetirizine dihydrochloride CRS* in *water R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *chlorphenamine maleate CRS* in *water R* and dilute to 5 ml with the same solvent. To 1 ml of the solution add 1 ml of reference solution (a).

Plate *TLC silica gel GF$_{254}$ plate R.*

Mobile phase *ammonia R, methanol R, methylene chloride R* (1:10:90 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In a current of cold air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram obtained shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

1.2 to 1.8 for solution S.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 2 mg of *cetirizine dihydrochloride CRS* and 2 mg of *cetirizine impurity A CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 2.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase *dilute sulphuric acid R, water R, acetonitrile R* (0.4:6.6:93 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 3 times the retention time of cetirizine.

System suitability Reference solution (a):
- *resolution*: minimum 3 between the peaks due to cetirizine and impurity A;
- *symmetry factors*: maximum 2.0.

Limits:
- *impurities A, B, C, D, E, F*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 70 ml of a mixture of 30 volumes of *water R* and 70 volumes of *acetone R* . Titrate with *0.1 M sodium hydroxide* to the 2nd point of inflexion. Determine the end-point potentiometrically *(2.2.20)* . Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 15.39 mg of $C_{21}H_{27}C_{l3}N_2O_3$.

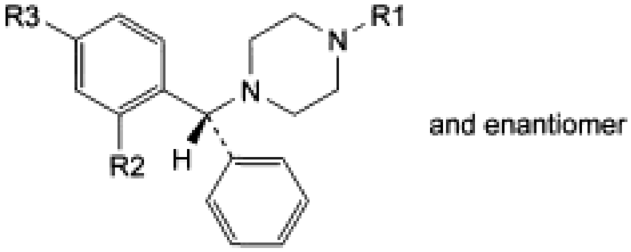
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): G*.



A. R1 = R2 = H, R3 = Cl: (*RS*)-1-[(4-chlorophenyl)phenylmethyl]piperazine,

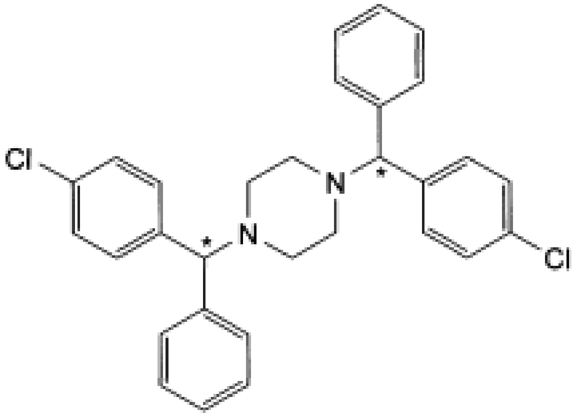
B. R1 = CH_2-CO_2H, R2 = H, R3 = Cl: (*RS*)-2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl] acetic acid,

C. R1 = CH_2-CH_2-O-CH_2-CO_2H, R2 = Cl, R3 = H: (*RS*)-2-[2-[4-[(2-chlorophenyl) phenylmethyl]piperazin-1-yl]ethoxy]acetic acid,

E. R1 = CH_2-[CH_2-O-CH_2]$_2$-CO_2H, R2 = H, R3 = Cl: (*RS*)-2-[2-[2-[4-[(4-chlorophenyl) phenylmethyl]piperazin-1-yl]ethoxy]ethoxy]acetic acid (ethoxycetirizine),

F. R1 = CH_2-CH_2-O-CH_2-CO_2H, R2 = R3 = H: [2-[4-(diphenylmethyl)piperazin-1-yl]ethoxy] acetic acid,

G. R1 = CH_2-CH_2-OH, R2 = H, R3 = Cl: 2-[4-[(*RS*)-(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol,



D. 1,4-bis[(4-chlorophenyl)phenylmethyl]piperazine.

Ph Eur

Cetostearyl Alcohol

General Notices

(Ph Eur monograph 0702)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of solid aliphatic alcohols, mainly octadecan-1-ol (stearyl alcohol; $C_{18}H_{38}O$; M_r 270.5) and hexadecan-1-ol (cetyl alcohol; $C_{16}H_{34}O$; M_r 242.4), of animal or vegetable origin.

Content:

— *stearyl alcohol* : minimum 40.0 per cent,

— *sum of the contents of stearyl alcohol and cetyl alcohol* : minimum 90.0 per cent.

CHARACTERS

Appearance

White or pale yellow, wax-like mass, plates, flakes or granules.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) and in light petroleum. When melted, it is miscible with fatty oils, with liquid paraffin and with melted wool fat.

IDENTIFICATION

Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the principal peaks in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_6

(2.2.2, Method II).

Dissolve 0.50 g in 20 ml of boiling *ethanol (96 per cent) R*. Allow to cool.

Melting point ***(2.2.14)***

49 °C to 56 °C.

Acid value ***(2.5.1)***

Maximum 1.0.

Hydroxyl value ***(2.5.3, Method A)***

208 to 228.

Iodine value ***(2.5.4, Method A)***

Maximum 2.0.

Dissolve 2.00 g in *methylene chloride R* and dilute to 25 ml with the same solvent.

Saponification value ***(2.5.6)***

Maximum 2.0.

ASSAY

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution Dissolve 0.100 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 60 mg of *cetyl alcohol CRS* and 40 mg of *stearyl alcohol CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *ethanol (96 per cent) R*.

Column:

— *size*: *l* = 30 m, Ø = 0.32 mm,

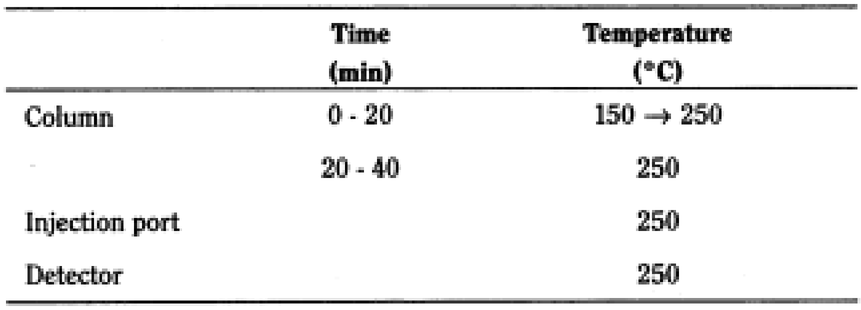
— *stationary phase*: *poly(dimethyl)siloxane R* (1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 20	150 → 250
	20 - 40	250
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution:

— *resolution*: minimum 5.0 between the peaks due to cetyl alcohol and stearyl alcohol.

Calculate the percentage contents of $C_{16}H_{34}O$ and $C_{18}H_{38}O$.

Ph Eur

Cetostearyl Isononanoate

General Notices

(Ph Eur monograph 1085)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of esters of cetostearyl alcohol with isononanoic acid, mainly 3,5,5-trimethylhexanoic acid.

CHARACTERS

Appearance

Clear, colourless or slightly yellowish liquid.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) and in light petroleum, miscible with fatty oils and with liquid paraffins.

Viscosity

15 mPa·s to 30 mPa·s.

Relative density

0.85 to 0.86.

Refractive index

1.44 to 1.45.

IDENTIFICATION

A. On cooling, turbidity occurs below 15 °C.

B. Saponification value (see Tests).

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   Ph. Eur. reference spectrum of cetostearyl isononanoate.

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method I)*.

Acid value ***(2.5.1)***

Maximum 1.0, determined on 5.0 g.

Hydroxyl value ***(2.5.3, Method A)***

Maximum 5.0.

Iodine value ***(2.5.4, Method A)***

Maximum 1.0.

Saponification value ***(2.5.6)***

135 to 148, determined on 1.0 g.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 0.2 per cent, determined on 10.0 g.

Total ash ***(2.4.16)***

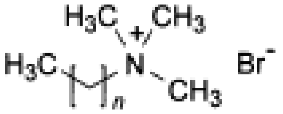
Maximum 0.2 per cent, determined on 2.0 g.

Ph Eur

Cetrimide

General Notices

(Ph Eur monograph 0378)



Action and use

Antiseptic.

Preparations

Cetrimide Cream

Cetrimide Emulsifying Ointment

Ph Eur

DEFINITION

Cetrimide consists of trimethyltetradecylammonium bromide and may contain smaller amounts of dodecyl- and hexadecyl-trimethylammonium bromides.

Content

96.0 per cent to 101.0 per cent of alkyltrimethylammonium bromides, calculated as $C_{17}H_{38}BrN$ (M_r 336.4) (dried substance).

CHARACTERS

Appearance

White or almost white, voluminous, free-flowing powder.

Solubility

Freely soluble in water and in alcohol.

IDENTIFICATION

A. Dissolve 0.25 g in *alcohol R* and dilute to 25.0 ml with the same solvent. At wavelengths from 260 nm to 280 nm, the absorbance *(2.2.25)* of the solution has a maximum of 0.05.

B. Dissolve about 5 mg in 5 ml of *buffer solution pH 8.0 R*. Add about 10 mg of *potassium ferricyanide R*. A yellow precipitate is formed. Prepare a blank in the same manner but omitting the substance to be examined: a yellow solution is observed but no precipitate is formed.

C. Solution S (see Tests) froths copiously when shaken.

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 0.10 g of *trimethyltetradecylammonium bromide CRS* in *water R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel F_{254} silanised plate R.*

Mobile phase *acetone R*, 270 g/l solution of *sodium acetate R, methanol R* (20:35:45 *V/V/V*).

Application 1 µl.

Development Over a path of 12 cm.

Drying In a current of hot air.

Detection Allow to cool; expose the plate to iodine vapour and examine in daylight.

Result The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

E. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 50 ml of solution S add 0.1 ml of *bromocresol purple solution R*. Not more than 0.1 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Amines and amine salts

Dissolve 5.0 g in 30 ml of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and add 100 ml of *2-propanol R*. Pass a stream of *nitrogen R* slowly through the solution. Gradually add 15.0 ml of *0.1 M tetrabutylammonium hydroxide* and record the potentiometric titration curve *(2.2.20)*. If the curve shows 2 points of inflexion, the volume of titrant added between the 2 points is not greater than 2.0 ml.

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Dissolve 2.000 g in *water R* and dilute to 100.0 ml with the same solvent. Transfer 25.0 ml of the solution to a separating funnel, add 25 ml of *chloroform R,* 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 50 g/l solution of *potassium iodide R*. Shake, allow to separate and discard the chloroform layer. Shake the aqueous layer with 3 quantities, each of 10 ml, of *chloroform R* and discard the chloroform layers. Add 40 ml of *hydrochloric acid R*, allow to cool and titrate with *0.05 M potassium iodate* until the deep brown colour is almost discharged. Add 2 ml of *chloroform R* and continue the titration, shaking vigorously, until the colour of the chloroform layer no longer changes. Carry out a blank titration on a mixture of 10.0 ml of the freshly prepared 50 g/l solution of *potassium iodide R*, 20 ml of *water R* and 40 ml of *hydrochloric acid R.*

1 ml of *0.05 M potassium iodate* is equivalent to 33.64 mg of $C_{17}H_{38}B_rN$.

Ph Eur

Cetyl Alcohol

General Notices

(Ph Eur monograph 0540)

36653-82-4

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of solid alcohols, mainly hexadecan-1-ol ($C_{16}H_{34}O$; M_r 242.4), of animal or vegetable origin.

Content

Minimum 95.0 per cent of $C_{16}H_{34}O$.

CHARACTERS

Appearance

White or almost white, unctuous mass, powder, flakes or granules.

Solubility

Practically insoluble in water, freely soluble or sparingly soluble in *ethanol (96 per cent)*. When melted, it is miscible with vegetable and animal oils, with liquid paraffin and with melted wool fat.

IDENTIFICATION

Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

Dissolve 0.50 g in 20 ml of boiling *ethanol (96 per cent) R*. Allow to cool.

Melting point *(2.2.14)*

46 °C to 52 °C.

Acid value ***(2.5.1)***

Maximum 1.0.

Hydroxyl value ***(2.5.3, Method A)***

218 to 238.

Iodine value ***(2.5.4, Method A*)**

Maximum 2.0.

Dissolve 2.00 g in *methylene chloride R* and dilute to 25 ml with the same solvent.

Saponification value ***(2.5.6)***

Maximum 2.0.

ASSAY

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.100 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *cetyl alcohol CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 50 mg of *stearyl alcohol R* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution (c) Mix 1 ml of reference solution (a) and 1 ml of reference solution (b) and dilute to 10 ml with *ethanol (96 per cent) R*.

Column:

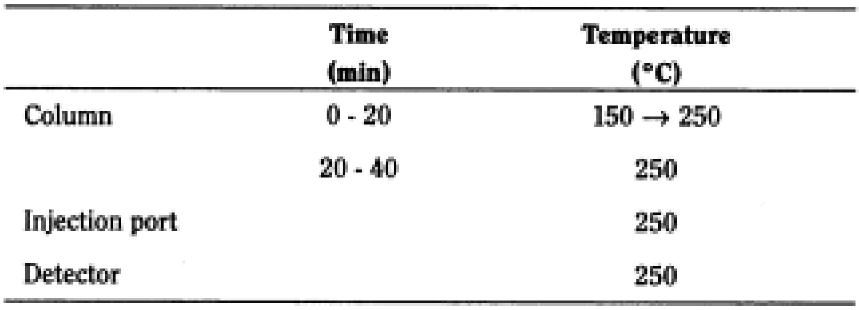
- *size*: *l* = 30 m, Ø = 0.32 mm,
- *stationary phase*: *poly(dimethyl)siloxane R* (1 µm).

Carrier gas helium for chromatography R.

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 20	150 → 250
	20 - 40	250
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl of the test solution and reference solutions (a) and (c).

System suitability Reference solution (c):

— *resolution*: minimum 5.0 between the peaks due to cetyl alcohol and stearyl alcohol.

Calculate the percentage content of $C_{16}H_{34}O$.

Ph Eur

Cetyl Palmitate

General Notices

(Ph Eur monograph 1906)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of C_{14}-C_{18} esters of lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic (octadecanoic) acids ('Cetyl esters wax').

Content (expressed as hexadecyl hexadecanoate)

10.0 per cent to 20.0 per cent for Cetyl palmitate 15, 60.0 per cent to 70.0 per cent for Cetyl palmitate 65 and minimum 90.0 per cent for Cetyl palmitate 95.

CHARACTERS

Appearance

White or almost white, waxy plates, flakes or powder.

Solubility

Practically insoluble in water, soluble in boiling anhydrous ethanol and in methylene chloride, slightly soluble in light petroleum, practically insoluble in anhydrous ethanol.

mp

About 45 °C for Cetyl palmitate 15 and Cetyl palmitate 65 and about 52 °C for Cetyl palmitate 95.

IDENTIFICATION

A. It complies with the limits of the assay and the chromatogram obtained with the test solution shows the typical main peak(s).

B. Saponification value (see Tests).

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 4.0 g in *methylene chloride R* and dilute to 20 ml with the same solvent.

Acid value ***(2.5.1)***

Maximum 4.0.

Dissolve 10.0 g in 50 ml of the solvent mixture described by heating under reflux on a water-bath for 5 min.

Hydroxyl value ***(2.5.3, Method A)***

Maximum 20.0.

Iodine value ***(2.5.4, Method A)*****:**

Maximum 2.0.

Saponification value ***(2.5.6)***

105 to 120.

Heat under reflux for 2 h.

Alkaline impurities

Dissolve 2.0 g 'with gentle heating' in a mixture of 1.5 ml of *ethanol (96 per cent) R* and 3 ml of *toluene R*. Add 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96 per cent) R*. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to yellow.

Nickel ***(2.4.31)***

Maximum 1 ppm.

Water ***(2.5.12)***

Maximum 0.3 per cent, determined on 1.0 g using a mixture of equal volumes of *anhydrous methanol R* and *methylene chloride R* as solvent.

Total ash ***(2.4.16)***

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 20.0 mg of the substance to be examined in *hexane R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *cetyl palmitate 95 CRS* in *hexane R* and dilute to 20.0 ml with the same solvent.

Reference solution (b) Dissolve 20.0 mg of *cetyl palmitate 15 CR*S in *hexane R* and dilute to 20.0 ml with the same solvent.

Column:

— *material*: stainless steel;

— *size*: *l* = 10 m, Ø = 0.53 mm;

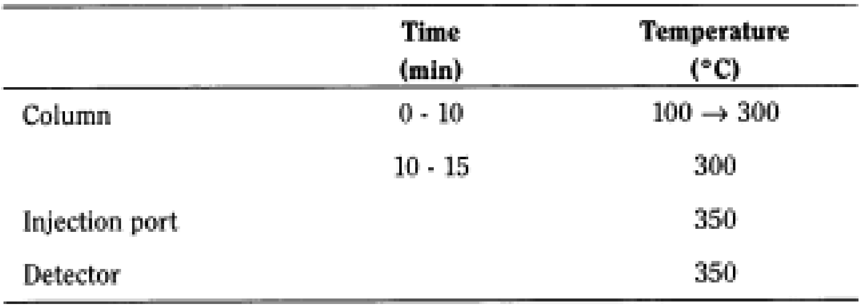
— *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 2.65 µm).

Carrier gas *helium for chromatography R.*

Flow rate 6.5 ml/min.

Split ratio 1:10.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	100 → 300
	10 - 15	300
Injection port		350
Detector		350

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to cetyl palmitate (retention time = about 9 min): cetyl alcohol = about 0.3; palmitic acid = about 0.4; lauric ester = about 0.8; myristic ester = about 0.9; stearic ester = about 1.1.

System suitability Reference solution (b):

— *resolution*: minimum of 1.5 between the peaks due to cetyl palmitate and cetyl stearate.

STORAGE

At a temperature not exceeding 25 °C.

LABELLING

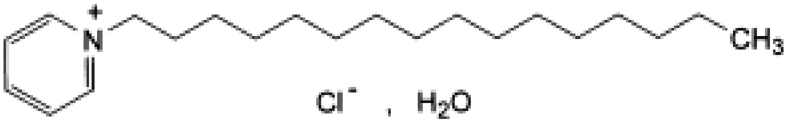
The label states the type of cetyl palmitate.

Ph Eur

Cetylpyridinium Chloride

General Notices

(Ph Eur monograph 0379)



$C_{21}H_{38}ClN,H_2O$ 358.0 *6004-24-6*

Action and use

Antiseptic.

Ph Eur

DEFINITION

Cetylpyridinium chloride contains not less than 96.0 per cent and not more than the equivalent of 101.0 per cent of 1-hexadecylpyridinium chloride, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white powder, slightly soapy to the touch, soluble in water and in alcohol. An aqueous solution froths copiously when shaken.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 0.10 g in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. Examined between 240 nm and 300 nm *(2.2.25)*, the solution shows an absorption maximum at 259 nm and 2 shoulders at about 254 nm and at about 265 nm. The specific absorbance at the maximum is 126 to 134, calculated with reference to the anhydrous substance.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cetylpyridinium chloride CRS*. Examine the substances in the solid state.

C. To 5 ml of *dilute sodium hydroxide solution R* add 0.1 ml of *bromophenol blue solution R1* and 5 ml of *chloroform R* and shake. The chloroform layer is colourless. Add 0.1 ml of solution S (see Tests) and shake. The chloroform layer becomes blue.

D. Solution S gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and is colourless *(2.2.2, Method II)*.

Acidity

To 50 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 2.5 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Amines and amine salts

Dissolve 5.0 g with heating in 20 ml of a mixture of 3 volumes of *1 M hydrochloric acid* and 97 volumes of *methanol R* and add 100 ml of *2-propanol R*. Pass a stream of *nitrogen R* slowly through the solution. Gradually add 12.0 ml of *0.1 M tetrabutylammonium hydroxide* and record the potentiometric titration curve *(2.2.20)*. If the curve shows 2 points of inflexion, the volume of titrant added between the two points is not greater than 5.0 ml. If the curve shows no point of inflexion, the substance to be examined does not comply with the test. If the curve shows one point of inflexion, repeat the test but add 3.0 ml of a 25.0 g/l solution of *dimethyldecylamine R* in *2-propanol R* before the titration. If the titration curve after the addition of 12.0 ml of the titrant shows only one point of inflexion, the substance to be examined does not comply with the test.

Water *(2.5.12)*

4.5 per cent to 5.5 per cent, determined on 0.300 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 2.00 g in *water R* and dilute to 100.0 ml with the same solvent. Transfer 25.0 ml of the solution to a separating funnel, add 25 ml of *chloroform R*, 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 50 g/l solution of *potassium iodide R*. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous layer with three quantities, each of 10 ml, of *chloroform R* and discard the chloroform layers. To the aqueous layer add 40 ml of *hydrochloric acid R,* allow to cool and titrate with *0.05 M potassium iodate* until the deep-brown colour is almost discharged. Add 2 ml of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 10.0 ml of the freshly prepared 50 g/l solution of *potassium iodide R*, 20 ml of *water R* and 40 ml of *hydrochloric acid R*.

1 ml of *0.05 M potassium iodate* is equivalent to 34.0 mg of $C_{21}H_{38}ClN$.

Ph Eur

Chalk

General Notices

Prepared Chalk
$CaCO_3$ 100.1

Action and use

Antacid.

DEFINITION

Chalk is a native form of calcium carbonate freed from most of its impurities by elutriation and dried. It contains not less than 97.0% and not more than 100.5% of $CaCO_3$, calculated with reference to the dried substance.

CHARACTERISTICS

Chalk absorbs water readily.

Practically insoluble in *water*; slightly soluble in *water* containing carbon dioxide.

Macroscopical White or greyish white, small friable masses, usually conical in form, or in powder; amorphous; earthy; soft to the touch.

Microscopical Consists of the calcareous shells and detritus of various foraminifera; the calcareous shells vary from about 35 to 100 µm in breadth and from about 50 to 180 µm in length; among the detritus are numerous small rings and discs about 5 to 10 µm in diameter.

IDENTIFICATION

A. A solution in 6M *acetic acid* yields reaction C characteristic of *calcium salts*, Appendix VI.

B. Yields reaction A characteristic of *carbonates*, Appendix VI.

TESTS

Acidity or alkalinity

1 g, boiled with 50 ml of *water* and filtered, yields a filtrate which is neutral to *bromothymol blue solution R3* or requires not more than 0.05 ml of 0.1M *hydrochloric acid VS* to make it so.

Aluminium, iron, phosphate and matter insoluble in hydrochloric acid

Dissolve 2 g in a mixture of 5 ml of *hydrochloric acid* and 75 ml of *water*, boil to remove carbon dioxide and make alkaline with 5M *ammonia* using *methyl red solution* as indicator. Boil for 1 minute, filter and wash the precipitate with a hot 2% w/v solution of *ammonium chloride*. Dissolve the precipitate as completely as possible by passing 20 ml of hot 2M *hydrochloric acid* through the filter and wash the filter with sufficient hot *water* to adjust the volume of the solution to 50 ml. Boil the solution and make alkaline with 5M *ammonia* using *methyl red solution* as indicator. Boil for 1 minute, filter through the same filter, wash the precipitate with a hot 2% w/v solution of *ammonium nitrate*, dry and ignite at a temperature not lower than 1000°. The residue weighs not more than 40 mg.

Arsenic

Dissolve 0.5 g in 5 ml of *brominated hydrochloric acid* and dilute to 50 ml with *water*. 25 ml of the resulting solution complies with the *limit test for arsenic*, Appendix VII (4 ppm).

Heavy metals

Dissolve 1.0 g in 10 ml of 2M *hydrochloric acid*, add 0.1 ml of *nitric acid* and boil to remove carbon dioxide. Cool, make alkaline with 5M *ammonia*, filter and wash the precipitate with *water*. Pass 5 ml of hot 2M *hydrochloric acid* through the filter, cool the filtrate, add 0.5 g of *ammonium thiocyanate* and extract with two 5 ml quantities of a mixture of equal volumes of *isoamyl alcohol* and *ether*. To the aqueous layer add 0.5 g of *citric acid* and dilute to 20 ml with *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard (40 ppm).

Chloride

Dissolve 0.3 g in 2 ml of *nitric acid* and 10 ml of *water*, filter and dilute the filtrate to 30 ml with *water*. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Sulphate

Dissolve 0.25 g in 5.5 ml of 2M *hydrochloric acid*, dilute to 30 ml with *water* and filter. 15 ml of the resulting solution complies with the *limit test for sulphates*, Appendix VII (0.12%).

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

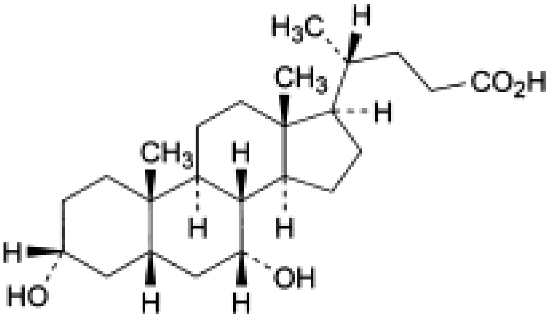
ASSAY

To 2 g in 100 ml of *water* add 50 ml of 1M *hydrochloric acid VS*, boil to remove carbon dioxide, cool and titrate the excess of acid with 1M *sodium hydroxide VS* using *methyl orange solution* as indicator. Each ml of 1M *hydrochloric acid VS* is equivalent to 50.04 mg of $CaCO_3$.

Chenodeoxycholic Acid

General Notices

(Ph Eur monograph 1189)



$C_{24}H_{40}O_4$ 392.6 *474-25-9*

Action and use

Bile acid; treatment of gallstones.

Ph Eur

DEFINITION

Chenodeoxycholic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3α,7α-dihydroxy-5β-cholan-24-oic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, very slightly soluble in water, freely soluble in alcohol, soluble in acetone, slightly soluble in methylene chloride.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *chenodeoxycholic acid CRS*. Examine the substances prepared as discs using *potassium bromide R*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 1 ml of *sulphuric acid R*. Add 0.1 ml of *formaldehyde solution R* and allow to stand for 5 min. Add 5 ml of *water R.* The suspension obtained is greenish-blue.

TESTS

Specific optical rotation *(2.2.7)*

Dissolve 0.500 g in *methanol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 11.0 to + 13.0, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 0.40 g of the substance to be examined in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R*.

Reference solution (a) Dissolve 40 mg of *chenodeoxycholic acid CR*S in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 20 mg of *lithocholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents. Dilute 2 ml of the solution to 100 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R.*

Reference solution (c) Dissolve 20 mg of *ursodeoxycholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 50 ml with the same mixture of solvents.

Reference solution (d) Dissolve 20 mg of *cholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (e) Dilute 0.5 ml of test solution (a) to 20 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R*. Dilute 1 ml of the solution to 10 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R.*

Reference solution (f) Dissolve 10 mg of *chenodeoxycholic acid CRS* in reference solution (c) and dilute to 25 ml with the same solution.

Apply separately to the plate 5 µl of each solution. Develop in an unsaturated tank over a path of 15 cm using a mixture of 1 volume of *glacial acetic acid R*, 30 volumes of *acetone R* and 60 volumes of *methylene chloride R*. Dry the plate at 120 °C for 10 min. Spray the plate immediately with a 47.6 g/l solution of *phosphomolybdic acid R* in a mixture of 1 volume of *sulphuric acid R* and 20 volumes of *glacial acetic acid R* and heat again at 120 °C until blue spots appear on a lighter background. In the chromatogram obtained with test solution (a): any spot corresponding to lithocholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent); any spot corresponding to ursodeoxycholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (1 per cent); any spot corresponding to cholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (d) (0.5 per cent); any spot apart from the principal spot and any spots corresponding to lithocholic acid, ursodeoxycholic acid and cholic acid, is not more intense than the principal spot in the chromatogram obtained with reference solution (e) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (f) shows two clearly separated principal spots.

Heavy metals ***(2.4.8)***

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

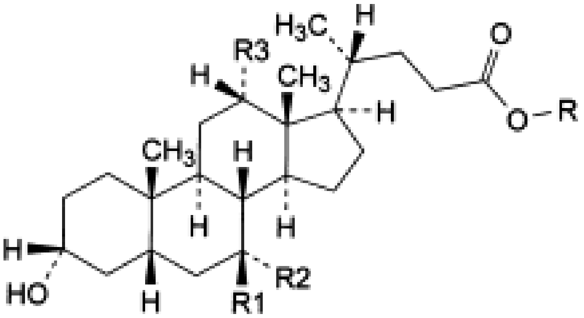
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 50 ml of *alcohol R,* previously neutralised to 0.2 ml of *phenolphthalein solution R*. Add 50 ml of *water* R and titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 39.26 mg of $C_{24}H_{40}O4$.

IMPURITIES



A. R = H, R1 = OH, R2 = H, R3 = H: ursodeoxycholic acid,

B. R = H, R1 = H, R2 = OH, R3 = OH: 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (cholic acid),

C. R = H, R1 = H, R2 = H, R3 = H: 3α-hydroxy-5β-cholan-24-oic acid (lithocholic acid),

D. R = H, R1 = OH, R2 = H, R3 = OH: 3α,7β,12α-trihydroxy-5β-cholan-24-oic acid (ursocholic acid),

E. R = H, R1 = H, R2 = H, R3 = OH: 3α,12α-dihydroxy-5β-cholan-24-oic acid (deoxycholic acid),

F. R = H, R1+R2 = = O, R3 = H: 3α-hydroxy-7-oxo-5β-cholan-24-oic acid,

G. R = CH_3, R1 = OH, R2 = H, R3 = H: methyl 3α,7β-dihydroxy-5β-cholan-24-oate.

Ph Eur

Chitosan Hydrochloride

General Notices

(Ph Eur monograph 1774)

Ph Eur

DEFINITION

Chitosan hydrochloride is the chloride salt of an unbranched binary heteropolysaccharide consisting of the two units *N*-acetyl-D-glucosamine and D-glucosamine, obtained by partial deacetylation of chitin normally leading to a degree of deacetylation of 70.0 per cent to 95.0 per cent. Chitin is extracted from the shells of shrimp and crab.

PRODUCTION

The animals from which chitosan hydrochloride is derived must fulfil the requirements for the health of animals suitable for human consumption to the satisfaction of the competent authority. It must have been shown to what extent the method of production allows inactivation or removal of any contamination by viruses or other infectious agents.

CHARACTERS

Appearance

White or almost white fine powder.

Solubility

Sparingly soluble in water, practically insoluble in ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *chitosan hydrochloride CRS.*

B. It gives reaction (a) of chlorides *(2.3.1)*.

C. Dilute 50 ml of solution S (see Tests) to 250 ml with a 25 per cent *V/V* solution of *ammonia R*. A voluminous gelatinous mass is formed.

D. To 10 ml of solution S add 90 ml of *acetone R*. A voluminous gelatinous mass is formed.

TESTS

Solution S

Dissolve 1.0 g in 100 ml of *water R* and stir vigorously for 20 min with a mechanical stirrer.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Matter insoluble in water

Maximum 0.5 per cent.

Add 2.00 g to 400.0 ml of *water R* while stirring until no further dissolution takes place. Transfer the solution to a 2 litre beaker, add 200 ml of *water R*. Boil the solution gently for 2 h, covering the beaker during the operation. Filter through a sintered-glass filter (40) *(2.1.2)* wash the residue with water and dry to constant weight in an oven at 100-105 °C. The residue weighs a maximum of 10 mg.

pH ***(2.2.3)***

4.0 to 6.0 for solution S.

Viscosity ***(2.2.10)***

80 per cent to 120 per cent of the value stated on the label, determined on solution S.

Determine the viscosity using a rotating viscometer at 20 °C with a spindle rotating at 20 r/min, using a suitable spindle for the range of the expected viscosity.

Degree of deacetylation

Test solution Dissolve 0.250 g in *water R* and dilute to 50.0 ml with the same solvent stirring vigorously. Dilute 1.0 ml of the solution to 100.0 ml with *water R*. Measure the absorbance *(2.2.25)* from 200 nm to 205 nm as the first derivative of the absorbance curve. Determine the pH of the solution.

Reference solutions Prepare solutions of 1.0 µg/ml, 5.0 µg/ml, 15.0 µg/ml and 35.0 µg/ml of *N-acetylglucosamine R* in *water R*. Measure the absorbance *(2.2.25)* from 200 nm to 205 nm of each solution as first derivative of the absorption curve. Make a standard curve by plotting the first derivative at 202 nm as a function of the concentration of *N*-acetylglucosamine, and calculate the slope of the curve by least squares linear regression. Use the standard curve to determine the equivalent amount of *N*-acetylglucosamine for the substance to be examined.

Calculate the degree of deacetylation (molar) using the expression:

$$\frac{100 \times M_1 \times (C_1 - C_2)}{(M_1 \times C_1) - [(M_1 - M_3) \times C_2]}$$

C_1 = concentration of chitosan hydrochloride in the test solution in micrograms per millilitre,

C_2 = concentration of *N*-acetylglucosamine in the test solution, as determined from the standard curve prepared using the reference solution in micrograms per millilitre,

M_1 = 203 (relative molecular mass of *N*-acetylglucosamine unit ($C_8H_{13}NO_5$) in polymer),

M_3 = relative molecular mass of chitosan hydrochloride.

M_3 is calculated from the pH in solution, assuming a pKa value of 6.8, using the following expressions:

$$M_3 = f \times M_2 + (1 - f) \times (M_2 + 36.5)$$

$$f = \frac{p}{1 + p}$$

$$p = 10^{(\text{pH} - \text{pKa})}$$

M_2 = 161 (relative molecular mass of deacetylated unit (glucosamine) ($C_6H_{11}NO_4$) in polymer).

Chlorides

10.0 per cent to 20.0 per cent.

Introduce 0.200 g into a 250 ml borosilicate flask fitted with a reflux condenser. Add 40 ml of a mixture of 1 volume of *nitric acid R* and 2 volumes of *water R*. Boil gently under a reflux condenser for 5 min. Cool and add 25 ml of *water R* through the condenser. Add 16.0 ml of *0.1 M silver nitrate*, shake vigorously and titrate with *0.1 M ammonium thiocyanate*, using 1 ml of ferric *ammonium sulphate solution R2* as indicator, and shaking vigorously towards the end-point. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 3.55 mg of Cl.

Heavy metals *(2.4.8)*

Maximum 40 ppm.

1.0 g complies with limit test F. Prepare the standard using 4 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 10 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 1.0 per cent, determined on 1.0 g.

STORAGE

At a temperature of 2-8 °C, protected from moisture and light.

LABELLING

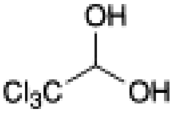
The label states the nominal viscosity in millipascal seconds for a 10 g/l solution in *water R.*

Ph Eur

Chloral Hydrate

General Notices

(Ph Eur monograph 0265)



$C_2H_3Cl_3O_2$ 165.4 *302-17-0*

Action and use

Hypnotic.

Ph Eur

DEFINITION

2,2,2-Trichloroethane-1,1-diol.

Content

98.5 per cent to 101.0 per cent.

CHARACTERS

Appearance

Colourless, transparent crystals.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. To 10 ml of solution S (see Tests) add 2 ml of *dilute sodium hydroxide solution R*. The mixture becomes cloudy and, when heated, gives off an odour of chloroform.

B. To 1 ml of solution S add 2 ml of *sodium sulphide solution R*. A yellow colour develops which quickly becomes reddish-brown. On standing for a short time, a red precipitate may be formed.

TESTS

Solution S

Dissolve 3.0 g in *carbon dioxide-free water R* and dilute to 30 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

3.5 to 5.5 for solution S.

Chloral alcoholate

Warm 1.0 g with 10 ml of *dilute sodium hydroxide solution R*, filter the supernatant solution and add *0.05 M iodine* dropwise until a yellow colour is obtained. Allow to stand for 1 h. No precipitate is formed.

Chlorides *(2.4.4)*

Maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

10 ml of solution S diluted to 20 ml with *water R* complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Non-volatile residue

Maximum 0.1 per cent.

Evaporate 2.000 g on a water-bath. The residue weighs a maximum of 2 mg.

ASSAY

Dissolve 4.000 g in 10 ml of *water R* and add 40.0 ml of *1 M sodium hydroxide*. Allow to stand for exactly 2 min and titrate with *0.5 M sulphuric acid*, using 0.1 ml of *phenolphthalein solution R* as indicator. Titrate the neutralised solution with *0.1 M silver nitrate,* using 0.2 ml of *potassium chromate solution R* as indicator. Calculate the number of millilitres of *1 M sodium hydroxide* used by deducting from the volume of *1 M sodium hydroxide*, added at the beginning of the titration, the volume *of 0.5 M sulphuric acid* used in the 1st titration and two-fifteenths of the volume *of 0.1 M silver nitrate* used in the 2nd titration.

1 ml of *1 M sodium hydroxide* is equivalent to 0.1654 g of $C_2H_3C_{l3}O_2$.

STORAGE

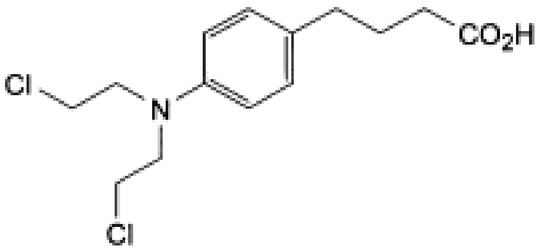
In an airtight container.

Ph Eur

Chlorambucil

General Notices

(Ph Eur monograph 0137)



$C_{14}H_{19}Cl_2NO_2$ 304.2 *305-03-3*

Action and use

Cytotoxic alkylating agent.

Preparation

Chlorambucil Tablets

Ph Eur

DEFINITION

Chlorambucil contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 4-4-[di(2-chloroethyl)amino]phenylbutyric acid, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone and in alcohol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 64 °C to 67 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the spectrum obtained with *chlorambucil CRS*.

C. To 0.4 g add 10 ml of *dilute hydrochloric acid R*, mix and allow to stand for 30 min, shaking from time to time. Filter and wash the precipitate with 2 quantities, each of 10 ml, of *water R*. To 10 ml of the combined filtrate and washings add 0.5 ml of *potassium tetraiodomercurate solution R*. A pale-brown precipitate is formed. To another 10 ml of the combined filtrate and washings add 0.2 ml of *potassium permanganate solution R*. The colour of the latter is discharged immediately.

D. Dissolve 50 mg in 5 ml of *acetone R* and dilute to 10 ml with *water R*. Add 0.05 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2*. No opalescence is produced immediately. Heat the solution on a water-bath; an opalescence develops.

TESTS

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance. *Carry out all operations as rapidly as possible protected from light. Prepare the solutions immediately before use*.

Test solution Dissolve 0.2 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 50 ml with *acetone R*.

Reference solution (b) Dilute 25 ml of reference solution (a) to 100 ml with *acetone R.*

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 20 volumes of *methyl ethyl ketone R*, 20 volumes of *heptane R*, 25 volumes of *methanol R* and 40 volumes of *toluene R*. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (2.0 per cent) and at most 1 such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Water *(2.5.12)*

Not more than 0.5 per cent, determined on 1.000 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 10 ml of *acetone R* and add 10 ml of *water R.* Titrate with *0.1 M sodium hydroxide*, using 0.1 ml of *phenolphthalein solution R* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 30.42 mg of $C_{14}H_{19}Cl_2NO_2$.

STORAGE

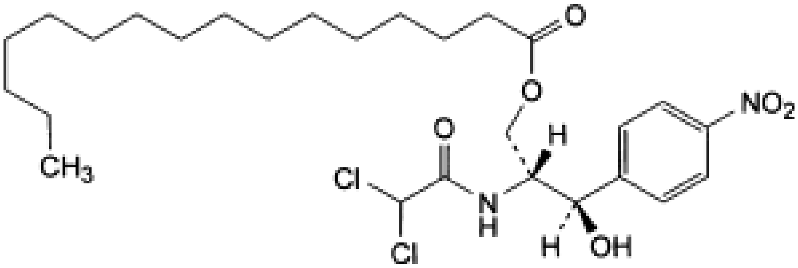
Store protected from light.

Ph Eur

Chloramphenicol Palmitate

General Notices

(Ph Eur monograph 0473)



$C_{27}H_{42}Cl_2N_2O_6$ 561.6 *530-43-8*

Action and use

Antibacterial.

Ph Eur

DEFINITION

Chloramphenicol palmitate contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (2*R*,3*R*)-2-[(dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl) propyl hexadecanoate, calculated with reference to the dried substance.

Semi-synthetic product derived from a fermentation product.

CHARACTERS

A white or almost white, fine, unctuous powder, practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol (96 per cent), very slightly soluble in hexane.

It melts at 87 °C to 95 °C.

It shows polymorphism (*5.9*). The thermodynamically stable form has low bioavailability following oral administration.

IDENTIFICATION

A. Examine by thin-layer chromatography *(2.2.27)*, using *TLC silanised silica gel plate R*.

Test solution Dissolve 50 mg of the substance to be examined in a mixture of 1 ml of *1 M sodium hydroxide* and 5 ml of *acetone R* and allow to stand for 30 min. Add 1.1 ml of *1 M hydrochloric acid* and 3 ml of *acetone R.*

Reference solution (a) Dissolve 10 mg of *chloramphenicol CRS* in *acetone R* and dilute to 5

ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *palmitic acid R* in *acetone R* and dilute to 5 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of the substance to be examined in *acetone R* and dilute to 5 ml with the same solvent.

Apply to the plate 4 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of a 100 g/l solution of *ammonium acetate R* and 70 volumes of *ethanol (96 per cent) R*. Allow the plate to dry in air and spray with a solution containing 0.2 g/l of *dichlorofluorescein R* and 0.1 g/l of *rhodamine B R* in *ethanol (96 per cent) R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution shows 3 spots corresponding in position to the principal spots in the chromatograms obtained with reference solutions (a), (b) and (c).

B. Dissolve 0.2 g in 2 ml of *pyridine R,* add 2 ml of a 100 g/l solution of *potassium hydroxide R* and heat on a water-bath. A red colour is produced.

C. Dissolve about 10 mg in 5 ml of *ethanol (96 per cent) R* and add 4.5 ml of *dilute sulphuric acid R* and 50 mg of *zinc powder R*. Allow to stand for 10 min and if necessary decant the supernatant liquid or filter. Cool the solution in iced water and add 0.5 ml of *sodium nitrite solution R.* Allow to stand for 2 min and add 1 g of *urea R*, 2 ml of *strong sodium hydroxide solution R* and 1 ml of *β-naphthol solution R*. A red colour develops.

TESTS

Acidity

Dissolve 1.0 g in 5 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *ether R,* warming to 35 °C. Add 0.2 ml of *phenolphthalein solution R.* Not more than 0.4 ml of *0.1 M sodium hydroxide* is required to produce a pink colour persisting for 30 s.

Specific optical rotation *(2.2.7)*

Dissolve 1.25 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 22.5 to + 25.5.

Free chloramphenicol

Maximum 450 ppm. Dissolve 1.0 g, with gentle heating, in 80 ml of *xylene R*. Cool and shake with 3 quantities, each of 15 ml, of *water R*. Dilute the combined aqueous extracts to 50 ml with *water R* and shake with 10 ml of *toluene R*. Allow to separate and discard the toluene layer. Centrifuge a portion of the aqueous layer and measure the absorbance (A) (*2.2.25*) at the maximum at 278 nm using as the compensation liquid a blank solution having an absorbance not greater than 0.05.

Calculate the content of free chloramphenicol in parts per million from the expression:

$$\frac{A \times 10^4}{5.96}$$

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *chloramphenicol palmitate isomer CRS* in *acetone R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *acetone R*.

Reference solution (b) Dissolve 20 mg of *chloramphenicol dipalmitate C*RS in *acetone R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *acetone R*.

Reference solution (c) Dissolve 5 mg of *chloramphenicol CRS* in *acetone R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *acetone R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R*, 40 volumes of *chloroform R* and 50 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spots due to chloramphenicol palmitate isomer and chloramphenicol dipalmitate are not more intense than the corresponding spots in the chromatograms obtained with reference solutions (a) and (b) respectively (2.0 per cent) and any spot, apart from the principal spot and the spots due to chloramphenicol palmitate isomer and chloramphenicol dipalmitate, is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by heating at 80 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

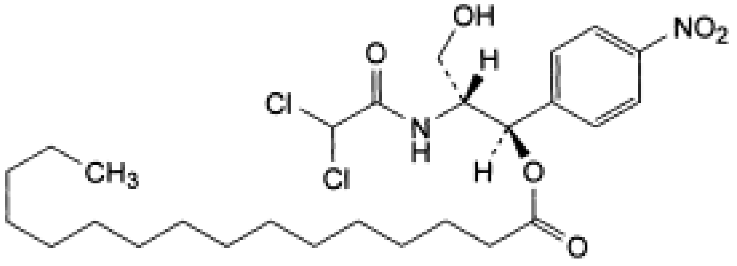
Dissolve 90.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 250.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* of the solution at the maximum at 271 nm.

Calculate the content of $C_{27}H_{42}Cl_2N_2O_6$taking the specific absorbance to be 178.

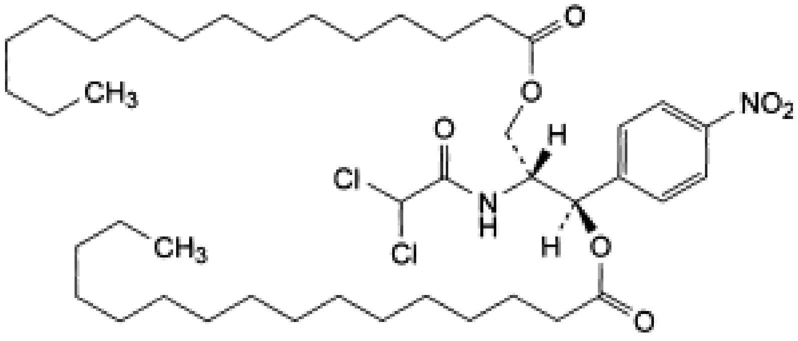
STORAGE

Protected from light.

IMPURITIES



A. (1*R*,2*R*)-2-[(dichloroacetyl)amino]-3-hydroxy-1-(4-nitrophenyl)propyl hexadecanoate (chloramphenicol palmitate isomer),


O
CH3
O
NO2
O
H
Cl
N
H
CH3
Cl
H
O
O

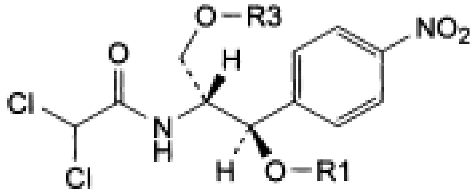
B. (1*R*,2*R*)-2-[(dichloroacetyl)amino]-1-(4-nitrophenyl)propane-1,3-diyl bishexadecanoate (chloramphenicol dipalmitate).

Ph Eur

Chloramphenicol Sodium Succinate

General Notices

(Ph Eur monograph 0709)



1 isomer : R1 = $CO\text{-}CH_2\text{-}CH_2\text{-}CO_2Na$, R3 = H

3 isomer : R1 = H, R3 = $CO\text{-}CH_2\text{-}CH_2\text{-}CO_2Na$

$C_{15}H_{15}Cl_2N_2NaO_8$ 445.2 *982-57-0*

Action and use

Antibacterial.

Preparation

Chloramphenicol Sodium Succinate Injection

Ph Eur

DEFINITION

Mixture in variable proportions of sodium (2*R*,3*R*)-2-[(dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl butanedioate (3 isomer) and of sodium (1*R*,2*R*)-2-[(dichloroacetyl)amino]-3-hydroxy-1-(4-nitrophenyl)propyl butanedioate (1 isomer).

Semi-synthetic product derived from a fermentation product.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish-white powder, hygroscopic.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in 2 ml of *acetone R*.

Reference solution (a) Dissolve 20 mg of *chloramphenicol sodium succinate CRS* in 2 ml of *acetone R*.

Reference solution (b) Dissolve 20 mg of *chloramphenicol CRS* in 2 ml of *acetone R.*

Plate TLC silica gel GF_{254} plate R.

Mobile phase *dilute acetic acid R, methanol R, chloroform R* (1:14:85 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The 2 principal spots in the chromatogram obtained with the test solution are similar in position and size to the 2 principal spots in the chromatogram obtained with reference solution (a); their positions are different from that of the principal spot in the chromatogram obtained with reference solution (b).

B. Dissolve about 10 mg in 1 ml of *ethanol (50 per cent V/V) R,* add 3 ml of a 10 g/l solution of *calcium chloride R* and 50 mg of *zinc powder R* and heat on a water-bath for 10 min. Filter the hot solution and allow to cool. Add 0.1 ml of *benzoyl chloride R* and shake for 1 min. Add 0.5 ml of *ferric chloride solution R1* and 2 ml of *chloroform R* and shake. The upper layer is light violet-red or purple.

C. Dissolve 50 mg in 1 ml of *pyridine R*. Add 0.5 ml of *dilute sodium hydroxide solution R* and 1.5 ml of *water R.* Heat in a water-bath for 3 min. A red colour develops. Add 2 ml of *nitric acid R* and cool under running water. Add 1 ml of *0.1 M silver nitrate*. A white precipitate is formed slowly.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

pH *(2.2.3)*

6.4 to 7.0.

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 5.0 to + 8.0 (anhydrous substance).

Dissolve 0.50 g in *water R* and dilute to 10.0 ml with the same solvent.

Chloramphenicol and chloramphenicol disodium disuccinate

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *chloramphenicol CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase (solution A). Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *chloramphenicol disodium disuccinate CR*S in the mobile phase and dilute to 100.0 ml with the mobile phase (solution B). Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 25 mg of the substance to be examined in the mobile phase, add 5 ml of solution A and 5 ml of solution B and dilute to 100 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 20 g/l solution of *phosphoric acid R, methanol R, water R* (5:40:55 *V/V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µl.

System suitability Reference solution (c):

— the 2 peaks corresponding to those in the chromatograms obtained with reference solutions (a) and (b) are clearly separated from the peaks corresponding to the 2 principal peaks in the chromatogram obtained with the test solution; if necessary, adjust the methanol content of the mobile phase.

Limits:

— *chloramphenicol*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *chloramphenicol disodium disuccinate*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Water ***(2.5.12)***

Maximum 2.0 per cent, determined on 0.500 g.

Pyrogens ***(2.6.8)***

If intended for use in the manufacture of a parenteral dosage form without a further appropriate procedure for removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 2.5 ml of a solution in *water for injections R* containing 2 mg of the substance to be examined per millilitre.

ASSAY

Dissolve 0.200 g in *water R* and dilute to 500.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 276 nm.

Calculate the content of $C_{15}H_{15}Cl_2N_2NaO_8$, taking the specific absorbance to be 220.

STORAGE

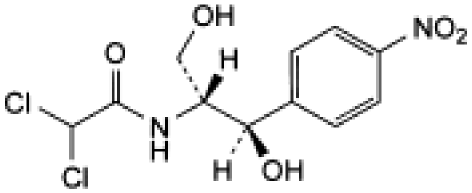
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container, protected from light.

Ph Eur

Chloramphenicol

General Notices

(Ph Eur monograph 0071)



$C_{11}H_{12}Cl_2N_2O_5$ 323.1 *56-75-7*

Action and use

Antibacterial.

Preparations

Chloramphenicol Capsules

Chloramphenicol Ear Drops

Chloramphenicol Eye Drops

Chloramphenicol Eye Ointment

Ph Eur

DEFINITION

Chloramphenicol is 2,2-dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl]acetamide, produced by the growth of certain strains of *Streptomyces venezuelae* in a suitable medium. It is normally prepared by synthesis. It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_{11}H_{12}C_{l2}N_2O_5$, calculated with reference to the dried substance.

CHARACTERS

A white, greyish-white or yellowish-white, fine, crystalline powder or fine crystals, needles or elongated plates, slightly soluble in water, freely soluble in alcohol and in propylene glycol.

A solution in ethanol is dextrorotatory and a solution in ethyl acetate is laevorotatory.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D, E.*

A. Melting point (*2.2.14*): 149 °C to 153 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *chloramphenicol CRS.*

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with 1 µl of the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 1 ml of *alcohol (50 per cent V/V) R*, add 3 ml of a 10 g/l solution of *calcium chloride R* and 50 mg of *zinc powder R and* heat on a water-bath for 10 min. Filter the hot solution and allow to cool. Add 0.1 ml of *benzoyl chloride R* and shake for 1 min. Add 0.5 ml of *ferric chloride solution R1* and 2 ml of *chloroform R* and shake. The aqueous layer is coloured light violet-red to purple.

E. To 50 mg in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Acidity or alkalinity

To 0.1 g add 20 ml of *carbon dioxide-free water R*, shake and add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.1 ml of *0.02 M hydrochloric acid* or *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation *(2.2.7)*

Dissolve 1.50 g in *ethanol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 18.5 to + 20.5.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 0.10 g of *chloramphenicol CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of reference solution (a) to 100 ml with *acetone R*.

Apply separately to the plate 1 µl and 20 µl of the test solution, 1 µl of reference solution (a) and 20 µl of reference solution (b). Develop over a path of 15 cm using a mixture of 1 volume of *water R*, 10 volumes of *methanol R* and 90 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with 20 µl of the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides *(2.4.4)*

To 1.00 g add 20 ml of *water R* and 10 ml of *nitric acid R* and shake for 5 min. Filter through a filter paper previously washed by filtering 5 ml portions of *water R* until 5 ml of filtrate no longer becomes opalescent on addition of 0.1 ml of *nitric acid R* and 0.1 ml of *silver nitrate solution R1*. 15 ml of the filtrate complies with the limit test for chlorides (100 ppm).

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 2.0 g.

Pyrogens ***(2.6.8)***

If intended for use in the manufacture of a parenteral dosage form without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 2.5 ml of a solution containing per millilitre 2 mg of the substance to be examined.

ASSAY

Dissolve 0.100 g in *water R* and dilute to 500.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water R*. Measure the absorbance *(2.2.25)* at the maximum at 278 nm.

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking the specific absorbance to be 297.

STORAGE

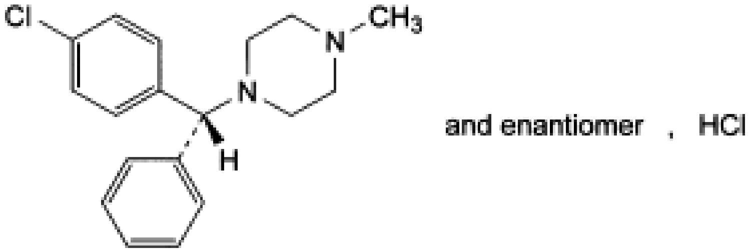
Store protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

Ph Eur

Chlorcyclizine Hydrochloride

General Notices

(Ph Eur monograph 1086)



$C_{18}H_{21}ClN_2,HCl$ 337.3 *1620-21-9*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

Chlorcyclizine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (*RS*)-1-[(4-chlorophenyl)phenylmethyl]-4-methylpiperazine hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A.white or almost white, crystalline powder, freely soluble in water and in methylene chloride, soluble in alcohol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 10.0 mg in a 5 g/l solution of *sulphuric acid R* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with a 5 g/l solution of *sulphuric acid R*. Examined between 215 nm and 300 nm *(2.2.25)*, the solution shows an absorption maximum at 231 nm. The specific absorbance at the maximum is 475 to 525, calculated with reference to the dried substance.

B. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the spectrum obtained with *chlorcyclizine hydrochloride CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances (see Tests). The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 0.5 g in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. The pH of the solution is 5.0 to 6.0.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a plate coated with a suitable silica gel.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 5 ml of test solution (a) to 100 ml with *methanol R*.

Reference solution (a) Dissolve 10 mg of *chlorcyclizine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *methylpiperazine R* in *methanol R* and dilute to 50 ml with the same solvent.

Reference solution (c) Dilute 1 ml of test solution (b) to 25 ml with *methanol R*.

Reference solution (d) Dissolve 10 mg of *hydroxyzine hydrochloride CRS* and 10 mg of *chlorcyclizine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 10 µl of each solution and develop over a path of 15 cm using a mixture of 2 volumes of *concentrated ammonia R*, 13 volumes of *methanol R* and 85 volumes of *methylene chloride R*. Allow the plate to dry in air and expose it to iodine vapour for 10 min. In the chromatogram obtained with test solution (a): any spot corresponding to methylpiperazine is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent); any spot, apart from the principal spot and any spot corresponding to methylpiperazine, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 130 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 1 ml of *0.1 M hydrochloric acid* and 50 ml of *methanol R*.

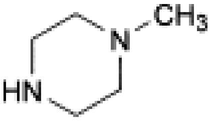
Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 33.73 mg of $C_{18}H_{22}Cl_2N_2$.

STORAGE

Store protected from light.

IMPURITIES



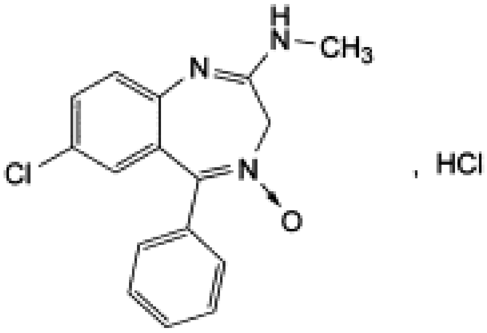
A. *N*-methylpiperazine.

Ph Eur

Chlordiazepoxide Hydrochloride

General Notices

(Ph Eur monograph 0474)



$C_{16}H_{14}ClN_3O,HCl$ 336.2 *438-41-5*

Action and use

Benzodiazepine.

Ph Eur

DEFINITION

7-Chloro-*N*-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-amine 4-oxide hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Soluble in water, sparingly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *chlordiazepoxide hydrochloride CRS*.

If the spectra obtained in the solid state show differences, dissolve 100 mg in 9 ml of *water R* and add 1 ml of *dilute sodium hydroxide solution R.* Extract with 10 ml of *methylene chloride R* in a separating funnel. Evaporate the organic layer and dry the residue obtained at 100-105 °C. Proceed in the same way with the reference substance. Record new spectra using the residues.

B. Dissolve 50 mg in 5 ml of *water R*, add 1 ml of *dilute ammonia R1*, mix, allow to stand for 5 min and filter. Acidify the filtrate with *dilute nitric acid R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution GY_6 *(2.2.2, Method II)*.

Dissolve 2.5 g in *water R* and dilute to 25 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the following operations protected from bright light and prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *chlordiazepoxide impurity A CRS* in the mobile phase, add 25.0 ml of the test solution and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 4.0 mg of *aminochlorobenzophenone R* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water R* (50:50 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 6 times the retention time of chlordiazepoxide.

Relative retention With reference to chlordiazepoxide (retention time = about 3.6 min): impurity A = about 0.7; impurity B = about 2.3; impurity C = about 3.9.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to impurity A and chlordiazepoxide.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit:* 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *water R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*.

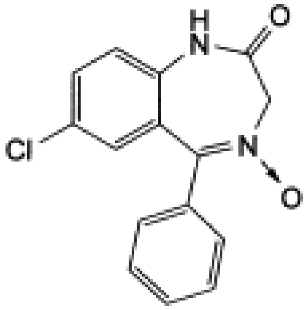
1 ml of *0.1 M silver nitrate* is equivalent to 33.62 mg of $C_{16}H_{15}Cl_2N_3O$.

STORAGE

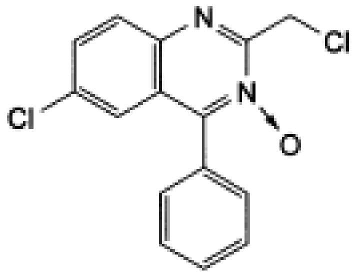
Protected from light.

IMPURITIES

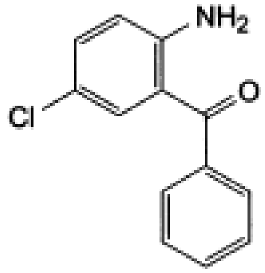
Specified impurities *A, B, C.*



A. 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide,



B. 6-chloro-2-(chloromethyl)-4-phenylquinazoline 3-oxide,



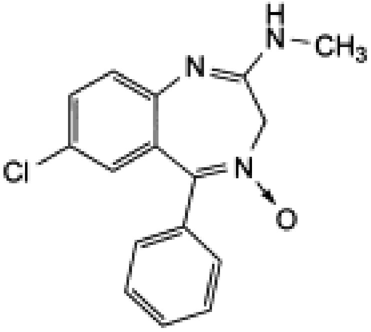
C. (2-amino-5-chlorophenyl)phenylmethanone (aminochlorobenzophenone).

Ph Eur

Chlordiazepoxide

General Notices

(Ph Eur monograph 0656)



$C_{16}H_{14}ClN_3O$ 299.8 *58-25-3*

Action and use

Benzodiazepine.

Ph Eur

DEFINITION

7-Chloro-*N*-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-amine 4-oxide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Almost white or light yellow, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison chlordiazepoxide CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from bright light and prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *chlordiazepoxide impurity A CRS* in the mobile phase, add 25.0 ml of the test solution and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 4.0 mg of *aminochlorobenzophenone R* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water R* (50:50 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 6 times the retention time of chlordiazepoxide.

Relative retention With reference to chlordiazepoxide (retention time = about 3.6 min): impurity A = about 0.7; impurity B = about 2.3; impurity C = about 3.9.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to impurity A and chlordiazepoxide.

Limits:
— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g, with heating if necessary, in 80 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

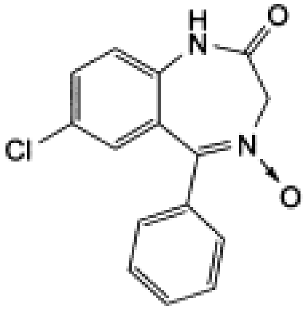
1 ml of *0.1 M perchloric acid* is equivalent to 29.98 mg of $C_{16}H_{14}ClN_3O$.

STORAGE

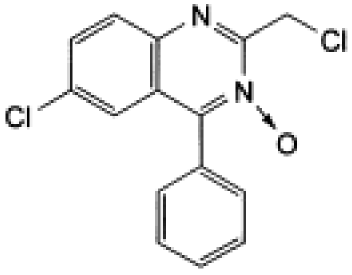
Protected from light.

IMPURITIES

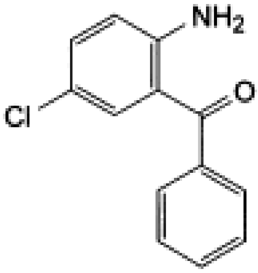
Specified impurities *A, B, C.*



A. 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide,



B. 6-chloro-2-(chloromethyl)-4-phenylquinazoline 3-oxide,



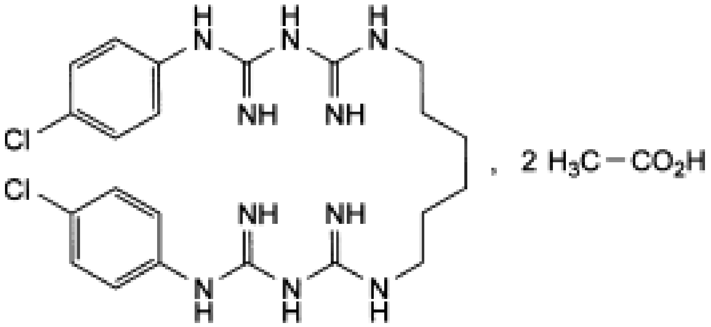
C. (2-amino-5-chlorophenyl)phenylmethanone (aminochlorobenzophenone).

Ph Eur

Chlorhexidine Acetate

General Notices

(Chlorhexidine Diacetate, Ph Eur monograph 0657)



$C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$ 625.6 *56-95-1*

Action and use

Antiseptic.

Preparation

Chlorhexidine Irrigation Solution

Ph Eur

DEFINITION

1,1′-(Hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] diacetate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, microcrystalline powder.

Solubility

Sparingly soluble in water, soluble in ethanol (96 per cent), slightly soluble in glycerol and in propylene glycol.

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *chlorhexidine diacetate CRS.*

B. Dissolve about 5 mg in 5 ml of a warm 10 g/l solution of *cetrimide R* and add 1 ml of *strong sodium hydroxide solution R* and 1 ml of *bromine water R*. A deep red colour is produced.

C. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of *hydrochloric acid R* and *water R*. Add 40 ml of *water R*, filter if necessary and cool in iced water. Make alkaline to *titan yellow paper R* by adding dropwise, and with stirring, *strong sodium hydroxide solution R* and add 1 ml in excess. Filter, wash the precipitate with *water R* until the washings are free from alkali and recrystallise from *ethanol (70 per cent V/V) R*. Dry at 100-105 °C. The residue melts (*2.2.14*) at 132 °C to 136 °C.

D. It gives reaction (a) of acetates (*2.3.1*).

TESTS

Chloroaniline

Maximum 500 ppm.

Dissolve 0.20 g in 25 ml of *water R* with shaking if necessary. Add 1 ml of *hydrochloric acid R* and dilute to 30 ml with *water R*. Add rapidly and with thorough mixing after each addition: 2.5 ml of *dilute hydrochloric acid R*, 0.35 ml of *sodium nitrite solution R*, 2 ml of a 50 g/l solution of *ammonium sulphamate R*, 5 ml of a 1.0 g/l solution of *naphthylethylenediamine dihydrochloride R* and 1 ml of *ethanol (96 per cent) R*, dilute to 50.0 ml with *water R* and allow to stand for 30 min. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner, using a mixture of 10.0 ml of a 0.010 g/l solution of *chloroaniline R* in *dilute hydrochloric acid R* and 20 ml of *dilute hydrochloric acid R* instead of the solution of the substance to be examined.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (a) Dissolve 15 mg of *chlorhexidine for performance test CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 2.5 ml of the test solution to 100 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10 ml with the mobile phase. Dilute 1.0 ml of this solution to 10 ml with the mobile phase.

Column:

— *size*: *l* = 0.2 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Solution of 2.0 g of sodium *octanesulphonate R* in a mixture of 120 ml of *glacial acetic acid R,* 270 ml of *water R* and 730 ml of *methanol R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for at least 1 h.

Injection 10 µl.

Run time 6 times the retention time of chlorhexidine.

System suitability Reference solution (a):

— the chromatogram obtained is similar to the chromatogram supplied with *chlorhexidine for performance test CRS* in that the peaks due to impurity A and impurity B precede that due to chlorhexidine; if necessary, adjust the concentration of acetic acid in the mobile phase (increasing the concentration decreases the retention times).

Limits:

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak with a relative retention time with reference to chlorhexidine of 0.25 or less.

Loss on drying *(2.2.32)*

Maximum 3.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

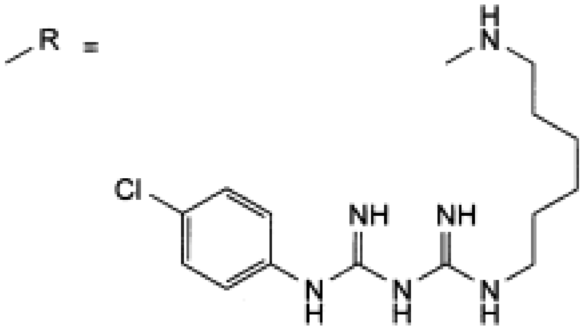
Maximum 0.15 per cent, determined on 1.0 g.

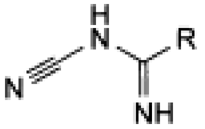
ASSAY

Dissolve 0.140 g in 100 ml of *anhydrous acetic acid R* and titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically *(2.2.20)*.

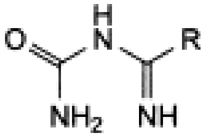
1 ml of *0.1 M perchloric acid* is equivalent to 15.64 mg of $C_{26}H_{38}Cl_2N_{10}O_4$.

IMPURITIES

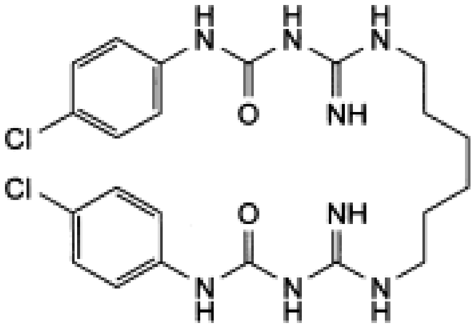




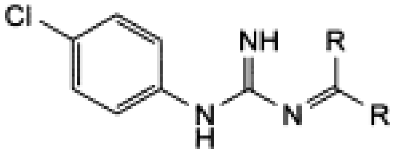
A. 1-(4-chlorophenyl)-5-[6-(3-cyanoguanidino)hexyl]biguanide,



B. [[[6-[5-(4-chlorophenyl)guanidino]hexyl]amino]iminomethyl]urea,



C 1,1′-[hexane-1,6-diylbis[imino(iminocarbonyl)]]bis[3-(4-chlorophenyl)urea],



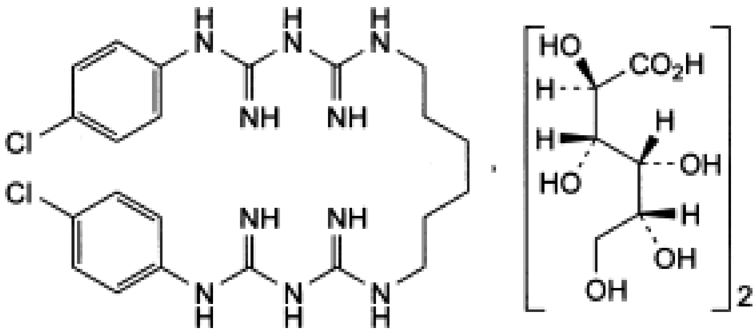
D. 1,1′-[[[[[(4-chlorophenyl)amino]iminomethyl]imino]methylene]bis[imino(hexane-1,6-diyl)]] bis[5-(4-chlorophenyl)biguanide].

Ph Eur

Chlorhexidine Gluconate Solution

General Notices

(Chlorhexidine Digluconate Solution, Ph Eur monograph 0658)



$C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ 898 *18472-51-0*

Action and use

Antiseptic.

Preparations

Chlorhexidine Gluconate Gel

Chlorhexidine Irrigation Solution

Chlorhexidine Mouthwash

Lidocaine and Chlorhexidine Gel

Ph Eur

DEFINITION

Aqueous solution of 1,1′-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] di-D-gluconate.

Content

190 g/l to 210 g/l.

CHARACTERS

Appearance

Almost colourless or pale-yellowish liquid.

Solubility

Miscible with water, with not more than 3 parts of acetone and with not more than 5 parts of ethanol (96 per cent).

IDENTIFICATION

First identification A, B.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation To 1 ml add 40 ml of *water R*, cool in iced water, make alkaline to *titan yellow paper R* by adding dropwise, and with stirring, *strong sodium hydroxide solution R* and add 1 ml in excess. Filter, wash the precipitate with *water R* until the washings are free from alkali and recrystallise from *ethanol (70 per cent V/V) R*. Dry at 100-105 °C. Examine the residue.

Comparison *chlorhexidine CRS*.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dilute 10.0 ml of the preparation to be examined to 50 ml with *water R*.

Reference solution Dissolve 25 mg of *calcium gluconate CRS* in 1 ml of *water R*.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, ethyl acetate R, water R, ethanol (96 per cent) R* (10:10:30:50 V/V/V/V).

Application 5 µl.

Development Over a path of 10 cm.

Drying At 100 °C for 20 min and allow to cool.

Detection Spray with a 50 g/l solution of *potassium dichromate R* in a 40 per cent *m/m* solution of *sulphuric acid R.*

Results After 5 min, the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 1 ml add 40 ml of *water R*, cool in iced water, make alkaline to *titan yellow paper R* by adding dropwise, and with stirring, *strong sodium hydroxide solution R* and add 1 ml in excess. Filter, wash the precipitate with *water R* until the washings are free from alkali and recrystallise from *ethanol (70 per cent V/V) R.* Dry at 100-105 °C. The residue melts *(2.2.14)* at 132 °C to 136 °C.

D. To 0.05 ml add 5 ml of a 10 g/l solution of *cetrimide R*, 1 ml of *strong sodium hydroxide solution R* and 1 ml of *bromine water R;* a deep red colour is produced.

TESTS

Relative density *(2.2.5)*

1.06 to 1.07.

pH *(2.2.3)*

5.5 to 7.0.

Dilute 5.0 ml to 100 ml with *carbon dioxide-free water R*.

Chloroaniline

Maximum 0.25 per cent, calculated with reference to chlorhexidine digluconate at a nominal concentration of 200 g/l.

Dilute 2.0 ml to 100 ml with *water R*. To 10 ml of this solution add 2.5 ml of *dilute hydrochloric acid R* and dilute to 20 ml with *water R*. Add rapidly and with thorough mixing after each addition: 0.35 ml of *sodium nitrite solution R*, 2 ml of a 50 g/l solution of *ammonium sulphamate R,* 5 ml of a 1 g/l solution of *naphthyle thylenediamine dihydrochloride R*, 1 ml of *ethanol (96 per cent) R*; dilute to 50.0 ml with *water R* and allow to stand for 30 min. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 10.0 ml of a 0.010 g/l solution of *chloroaniline R* in *dilute hydrochloric acid R* and 10 ml of *water R* instead of the dilution of the preparation to be examined.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dilute 5.0 ml of the preparation to be examined to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 15 mg of *chlorhexidine for performance test CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 3.0 ml of the test solution to 100 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 50 ml with the mobile phase.

Column:
- size: *l* = 0.2 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase solution of 2.0 g of *sodium octanesulphonate R* in a mixture of 120 ml of *glacial acetic acid R*, 270 ml of *water R* and 730 ml of *methanol R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for at least 1 hour.

Injection 10 µl.

Run time 6 times the retention time of chlorhexidine.

System suitability Reference solution (a):

- the chromatogram obtained is similar to the chromatogram supplied with *chlorhexidine for performance test CRS* in that the peaks due to impurity A and impurity B precede that due to chlorhexidine; if necessary, adjust the concentration of acetic acid in the mobile phase (increasing the concentration decreases the retention times).

Limits:
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.06 per cent); disregard any peak with a relative retention time with reference to the principal peak of 0.25 or less.

ASSAY

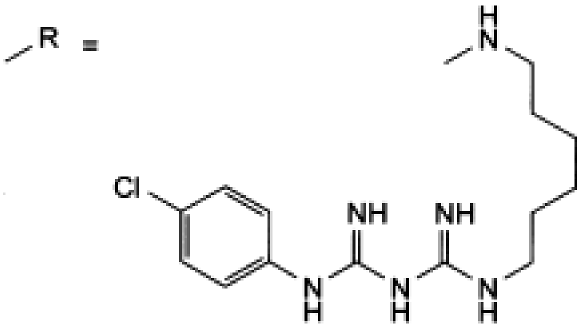
Determine the density *(2.2.5)* of the preparation to be examined. Transfer 1.00 g to a 250 ml beaker and add 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically *(2.2.20).*

1 ml of *0.1 M perchloric acid* is equivalent to 22.44 mg of $C_{34}H_{54}Cl_2N_{10}O_{14}$.

STORAGE

Protected from light.

IMPURITIES


R =
H
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Cl
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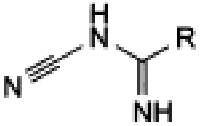
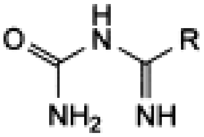
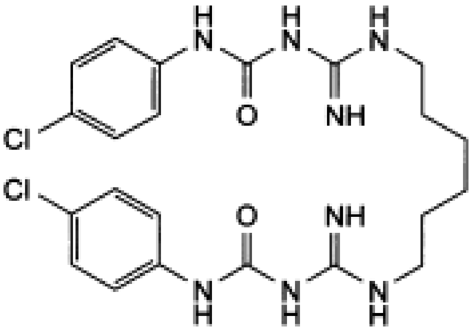
H



A. 1-(4-chlorophenyl)-5-[6-(3-cyanoguanidino)hexyl]biguanide,



B. [[[6-[5-(4-chlorophenyl)guanidino]hexyl]amino]iminomethyl]urea,


H
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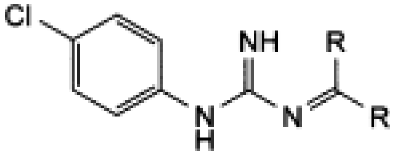
C. 1,1′-[hexane-1,6-diylbis[imino(iminocarbonyl)]]bis[3-(4-chlorophenyl)urea],



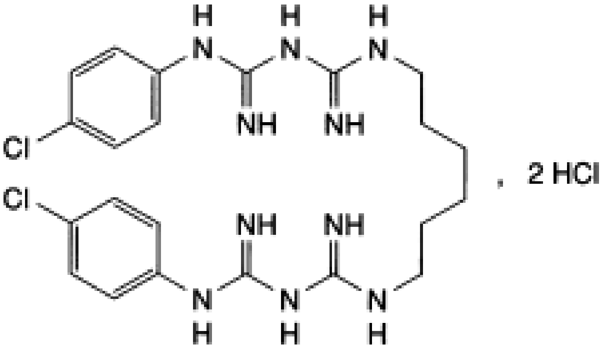
D. 1,1′-[[[[[(4-chlorophenyl)amino]iminomethyl]imino]methylene]bis[imino(hexane-1,6-diyl)]] bis[5-(4-chlorophenyl)biguanide].

Ph Eur

Chlorhexidine Hydrochloride

General Notices

(Chlorhexidine Dihydrochloride, Ph Eur monograph 0659)



$C_{22}H_{30}Cl_2N_{10}$,2HCl 578.4 *3697-42-5*

Action and use

Antiseptic.

Ph Eur

DEFINITION

1,1'-(Hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] dihydrochloride.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water and in propylene glycol, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *chlorhexidine dihydrochloride CRS.*

B. Dissolve about 5 mg in 5 ml of a warm 10 g/l solution of *cetrimide R* and add 1 ml of *strong sodium hydroxide solution R* and 1 ml of *bromine water R*. A dark red colour is produced.

C. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of *hydrochloric acid R* and *water R*. Add 40 ml of *water R*, filter if necessary and cool in iced water. Make alkaline to *titan yellow paper R* by adding dropwise, and with stirring, *strong sodium hydroxide solution R* and add 1 ml in excess. Filter, wash the precipitate with *water R* until the washings are free from alkali and recrystallise from *ethanol (70 per cent V/V) R*. Dry at 100-105 °C. The residue melts *(2.2.14)* at 132 °C to 136 °C.

D. It gives reaction (a) of chlorides *(2.3.1).*

TESTS

Chloroaniline

Maximum 500 ppm.

To 0.20 g add 1 ml of *hydrochloric acid R*, shake for about 30 s, dilute to 30 ml with *water R* and shake until a clear solution is obtained. Add rapidly and with thorough mixing after each addition: 2.5 ml of *dilute hydrochloric acid R*, 0.35 ml of *sodium nitrite solution R*, 2 ml of a 50 g/l solution of *ammonium sulphamate R*, 5 ml of a 1.0 g/l solution of *naphthylethylenediamine dihydrochloride R* and 1 ml of *ethanol (96 per cent) R;* dilute to 50.0 ml with *water R* and allow to stand for 30 min. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 10.0 ml of a 0.010 g/l solution of *chloroaniline R* in *dilute hydrochloric acid R* and 20 ml of *dilute hydrochloric acid R* instead of the solution of the substance to be examined.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (a) Dissolve 15 mg of *chlorhexidine for performance test CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 2.5 ml of the test solution to 100 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10 ml with the mobile phase. Dilute 1.0 ml of this solution to 10 ml with the mobile phase.

Column:

— size: *l* = 0.2 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Solution of 2.0 g of *sodium octanesulphonate R* in a mixture of 120 ml of *glacial acetic acid R*, 270 ml of *water R* and 730 ml of *methanol R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for at least 1 h.

Injection 10 µl.

Run time 6 times the retention time of chlorhexidine.

System suitability Reference solution (a):

— the chromatogram obtained is similar to the chromatogram supplied with *chlorhexidine for performance test CRS* in that the peaks due to impurity A and impurity B precede that due to chlorhexidine; if necessary, adjust the concentration of acetic acid in the mobile phase (increasing the concentration decreases the retention times).

Limits:

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak with a relative retention time with reference to chlorhexidine of 0.25 or less.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

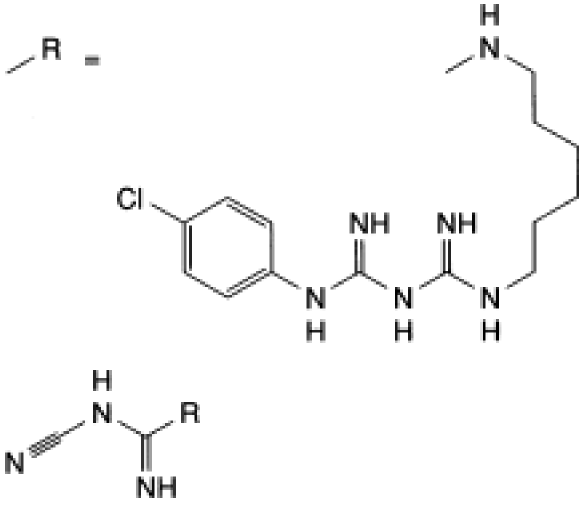
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

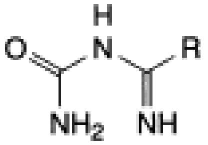
Dissolve 100.0 mg in 5 ml of *anhydrous formic acid R* and add 70 ml of *acetic anhydride R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 14.46 mg of $C_{22}H_{32}Cl_4N_{10}$.

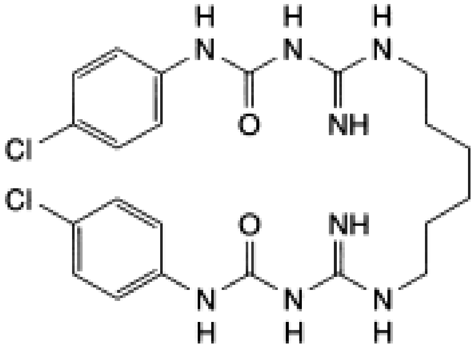
IMPURITIES



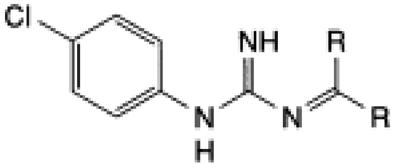
A. 1-(4-chlorophenyl)-5-[6-(3-cyanoguanidino)hexyl]biguanide,



B. [[[6-[5-(4-chlorophenyl)guanidino]hexyl]amino]iminomethyl]urea,



C. 1,1′-[hexane-1,6-diylbis[imino(iminocarbonyl)]]bis[3-(4-chlorophenyl)urea],



D. 1,1′-[[[[[(4-chlorophenyl)amino]iminomethyl]imino]methylene]bis[imino(hexane-1,6-diyl)]]
bis[5-(4-chlorophenyl)biguanide].

Ph Eur

Chlorinated Lime

General Notices

Action and use

Disinfectant.

DEFINITION

Chlorinated Lime contains not less than 30.0% w/w of available chlorine, Cl.

CHARACTERISTICS

A dull white powder.

Partly soluble in *water* and in *ethanol (96%)*.

IDENTIFICATION

A. Evolves chlorine copiously on the addition of 2M *hydrochloric acid*.

B. When shaken with *water* and filtered, the filtrate yields reaction C characteristic of *calcium salts* and reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

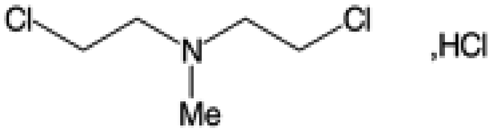
Triturate 4 g with successive small quantities of *water*, dilute to 1000 ml with *water* and shake thoroughly. Mix 100 ml of the resulting suspension with a solution containing 3 g of *potassium iodide* in 100 ml of *water*, acidify with 5 ml of 6M *acetic acid* and titrate the liberated iodine with 0.1M *sodium thiosulphate VS*. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 3.545 mg of available chlorine, Cl.

STORAGE

On exposure to air Chlorinated Lime becomes moist and gradually decomposes, carbon dioxide being absorbed and chlorine evolved.

Chlormethine Hydrochloride

General Notices



$C_5H_{11}Cl_2N,HCl$ 192.5   *55-86-7*

Action and use

Cytotoxic alkylating agent.

Preparation

Chlormethine Injection

DEFINITION

Chlormethine Hydrochloride is bis(2-chloroethyl)methylamine hydrochloride. It contains not less than 98.0% and not more than 101.0% of $C_5H_{11}Cl_2N,HCl$.

CHARACTERISTICS

A white or almost white crystalline powder or mass; hygroscopic; vesicant.

Very soluble in *water*.

IDENTIFICATION

A. Dissolve 50 mg in 5 ml of *water* and add 1 ml of 5M *sodium hydroxide*. Oily globules are produced which dissolve on warming.

B. Dissolve 50 mg in 5 ml of *water* and add 0.02 ml of *potassium tetraiodomercurate solution*. A cream precipitate is produced.

C. *Melting point*, about 108°, Appendix V A.

ASSAY

To 0.2 g add 15 ml of 1M *ethanolic potassium hydroxide* and 15 ml of *water* and boil under a reflux condenser for 2 hours. Evaporate the solution to half its volume on a water bath, dilute to 150 ml with *water*, add 3 ml of *nitric acid* and 50 ml of 0.1M *silver nitrate VS*, shake vigorously and filter. Wash the residue with *water* and titrate the excess of silver nitrate in the combined filtrate and washings with 0.1M *ammonium thiocyanate VS* using 1 ml of *ammonium iron(III) sulphate solution R2* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 6.418 mg of $C_5H_{11}Cl_2N,HCl$.

STORAGE

Chlormethine Hydrochloride should be stored at a temperature of 8° to 15°.

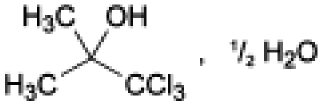
LABELLING

The label states that the contents of the container are strongly vesicant.

Chlorobutanol

General Notices

(Chlorobutanol Hemihydrate, Ph Eur monograph 0383)



$C_4H_7Cl_3O,½H_2O$ 186.5   *6001-64-5*

Action and use

Disinfectant preservative.

Ph Eur

DEFINITION

1,1,1-Trichloro-2-methylpropan-2-ol hemihydrate.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white crystalline powder or colourless crystals, sublimes readily.

Solubility

Slightly soluble in water, very soluble in ethanol (96 per cent), soluble in glycerol (85 per cent).

mp: about 78 °C (without previous drying).

IDENTIFICATION

A. Add about 20 mg to a mixture of 1 ml of *pyridine R* and 2 ml of *strong sodium hydroxide solution R.* Heat in a water-bath and shake. Allow to stand. The pyridine layer becomes red.

B. Add about 20 mg to 5 ml of *ammoniacal silver nitrate solution R* and warm slightly. A black precipitate is formed.

C. To about 20 mg add 3 ml of *1 M sodium hydroxide* and shake to dissolve. Add 5 ml of *water R* and then, slowly, 2 ml of *iodinated potassium iodide solution R*. A yellowish precipitate is formed.

D. Water (see Tests).

TESTS

Solution S

Dissolve 5 g in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Acidity

To 4 ml of solution S add 15 ml of *ethanol (96 per cent) R* and 0.1 ml of *bromothymol blue solution R1*. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Chlorides *(2.4.4)*

Maximum 100 ppm.

To 1 ml of solution S add 4 ml of *ethanol (96 per cent) R* and dilute to 15 ml with *water R*. When preparing the standard, replace the 5 ml of *water R* by 5 ml of *ethanol (96 per cent) R*.

Water *(2.5.12)*

4.5 per cent to 5.5 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 20 ml of *ethanol (96 per cent) R*. Add 10 ml of *dilute sodium hydroxide solution R*, heat in a water-bath for 5 min and cool. Add 20 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R* and shake vigorously. Add 2 ml of *ferric ammonium sulphate solution R2* and titrate with *0.1 M ammonium thiocyanate* until an orange colour is obtained.

1 ml of *0.1 M silver nitrate* is equivalent to 5.92 mg of $C_4H_7Cl_3O$.

STORAGE

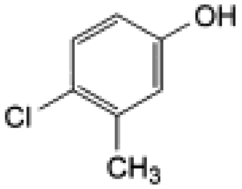
In an airtight container .

Ph Eur

Chlorocresol

General Notices

(Ph Eur monograph 0384)



C_7H_7ClO 142.6 *59-50-7*

Action and use

Antiseptic; antimicrobial preservative.

Ph Eur

DEFINITION

4-Chloro-3-methylphenol.

Content

98.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or compacted crystalline masses supplied as pellets or colourless or white crystals.

Solubility

Slightly soluble in water, very soluble in ethanol (96 per cent), freely soluble in fatty oils. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

A. Melting point *(2.2.14)*: 64 °C to 67 °C.

B. To 0.1 g add 0.2 ml of *benzoyl chloride R* and 0.5 ml of *dilute sodium hydroxide solution R*. Shake vigorously until a white, crystalline precipitate is formed. Add 5 ml of *water R* and filter. The precipitate, recrystallised from 5 ml of *methanol R* and dried at 70 °C, melts (*2.2.14*) at 85 °C to 88 °C.

C. To 5 ml of solution S (see Tests) add 0.1 ml of *ferric chloride solution R1*. A bluish colour is produced.

TESTS

Solution S

To 3.0 g, finely powdered, add 60 ml of *carbon dioxide-free water R*, shake for 2 min and filter.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Dissolve 1.25 g in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.

Acidity

To 10 ml of solution S add 0.1 ml of *methyl red solution R*. The solution is orange or red. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 1.0 g of the substance to be examined in *acetone R* and dilute to 100 ml with the same solvent.

Column:

— *material*: glass;

— *size*: *l* = 1.80 m, Ø = 3-4 mm;

— *stationary phase*: *silanised diatomaceous earth for gas chromatography R* impregnated with 3-5 per cent *m/m* of *polymethylphenylsiloxane R*.

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:

— *column*: 125 °C;

— *injection port*: 210 °C;

— *detector*: 230 °C.

Detection Flame ionisation.

Run time 3 times the retention time of chlorocresol.

Retention time Chlorocresol = about 8 min.

Limits:

— *total*: maximum 1 per cent;

— *disregard limit*: disregard the peak due to the solvent.

Non-volatile matter

Maximum 0.1 per cent.

Evaporate 2.0 g to dryness on a water-bath and dry the residue at 100-105 °C. The residue weighs not more than 2 mg.

ASSAY

In a ground-glass-stoppered flask, dissolve 70.0 mg in 30 ml of *glacial acetic acid R*. Add 25.0 ml of *0.0167 M potassium bromate*, 20 ml of a 150 g/l solution of *potassium bromide R* and 10 ml of *hydrochloric acid R*. Allow to stand protected from light for 15 min. Add 1 g of *potassium iodide R* and 100 ml of *water R.* Titrate with *0.1 M sodium thiosulphate*, shaking vigorously and using *1 ml of starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of *0.0167 M potassium bromate* is equivalent to 3.565 mg of C_7H_7ClO.

STORAGE

Protected from light.

Ph Eur

Chloroform

General Notices

$CHCl_3$ 119.4 *67-66-3*

Action and use

General anaesthetic; antimicrobial preservative.

Preparations

Chloroform Spirit

Chloroform Water

Double-strength Chloroform Water

DEFINITION

Chloroform is trichloromethane to which either 1.0 to 2.0% of ethanol or 50 mg per litre of amylene has been added.

CHARACTERISTICS

A colourless, volatile liquid.

Slightly soluble in *water*; miscible with *absolute ethanol* , with *ether*, with fixed and volatile oils and with most organic solvents.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, of the substance being examined after washing with *water* and drying with *anhydrous sodium sulphate* is concordant with the *reference spectrum* of chloroform *(RS 053)*.

TESTS

Distillation range

Not more than 5.0% v/v distils below 60° and the remainder distils at 60° to 62°, Appendix V C.

Weight per ml

1.474 to 1.479 g, Appendix V G.

Acidity or alkalinity

Shake 10 ml with 20 ml of freshly boiled and cooled *water* for 3 minutes and allow to separate. To 5 ml of the aqueous layer add 0.1 ml of neutral *litmus solution*. The colour produced is the same as that produced on adding 0.1 ml of the neutral litmus solution to 5 ml of freshly boiled and cooled *water*.

Chloride

To 5 ml of the aqueous layer obtained in the test for Acidity or alkalinity add 5 ml of *water* and 0.2 ml of *silver nitrate solution*. The solution is *clear*, Appendix IV A.

Free chlorine

To 10 ml of the aqueous layer obtained in the test for Acidity or alkalinity add 1 ml of a 5.0% w/v solution of *zinc iodide* and 0.1 ml of *starch mucilage*. No blue colour is produced.

Aldehyde

Shake 5 ml with 5 ml of *water* and 0.2 ml of *alkaline potassium tetraiodomercurate solution* in a glass-stoppered flask and allow to stand in the dark for 15 minutes. Not more than a pale yellow colour is produced.

Foreign chlorine compounds

Shake 20 ml for 5 minutes with 10 ml of *sulphuric acid* in a glass-stoppered flask previously rinsed with *sulphuric acid*, allow to stand in the dark for 30 minutes and discard the acid layer. Shake 15 ml of the chloroform layer with 30 ml of *water* in a glass-stoppered flask for 3 minutes and allow to separate. To the aqueous layer add 0.2 ml of *silver nitrate solution* and allow to stand in the dark for 5 minutes. No opalescence is produced.

Related substances

Carry out the method for *gas chromatography*, Appendix III B, injecting 0.1 µl of each of the following solutions. Solution (1) contains 0.2% v/v of *carbon tetrachloride*, 0.2% v/v of *1,1,1-trichloroethane* (internal standard), 0.2% v/v of *dichloromethane*, 0.2% v/v of *ethanol*, 0.5% v/v of *bromochloromethane* and 0.2% v/v of the substance being examined in *propan-1-ol*. Solution (2) is the substance being examined. Solution (3) contains 0.2% v/v of the internal standard in the substance being examined. Solution (4) is *propan-1-ol*.

The chromatographic procedure may be carried out using a glass column (4 m × 3.0 mm) packed with *acid-washed kieselguhr* (60 to 100 mesh) coated with 15% w/w of *di-2-cyanoethyl ether* and maintained at 40° with both the inlet port and the detector at 100° and a flow rate of 30 ml per minute for the carrier gas.

The test is not valid unless the *column efficiency*, determined using the chloroform peak in the chromatogram obtained with solution (1), is greater than 700 plates per metre and the total number of plates is greater than 2,500.

In the chromatogram obtained with solution (1) the peaks, in order of emergence, are due to carbon tetrachloride, 1,1,1-trichloroethane, dichloromethane, chloroform, ethanol, bromochloromethane and propan-1-ol (solvent).

Using the chromatogram obtained with solution (4), make any corrections due to the contribution of *secondary peaks* from the solvent to the peaks in the chromatogram obtained with solution (1).

In the chromatogram obtained with solution (3), the ratio of the areas of any peaks due to carbon tetrachloride, dichloromethane and bromochloromethane to the area of the peak due to the internal standard is not greater than the corresponding ratios in the chromatogram obtained with solution (1) and the ratio of the area of any other *secondary peak* that elutes prior to the solvent peak, except for the peak corresponding to ethanol, to the area of the peak due to the internal standard is not greater than the ratio of the area of the peak due to chloroform to the area of the peak due to the internal standard in the chromatogram obtained with solution (1).

Calculate the percentage content of each of the specified impurities and also calculate the percentage content of each of any other impurities assuming the same response per unit volume as for chloroform. The total content of all impurities is not more than 1.0% v/v.

Ethanol

Carry out the following test for Chloroform that contains ethanol. Carry out the method for *gas chromatography*, Appendix III B, injecting 0.1 µl of each of the following solutions. Solution (1) contains 1.0% v/v of *absolute ethanol* and 1.0% v/v of *propan-1-ol* (internal standard) in *water*. Solution (2) is the substance being examined. Solution (3) contains 1.0% v/v of the internal standard in the substance being examined.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless the height of the trough separating the ethanol peak from the chloroform peak in the chromatogram obtained with solution (2) is less than 15% of the height of the ethanol peak.

Calculate the percentage content of ethanol from the areas of the peaks due to ethanol and the internal standard in the chromatograms obtained with solutions (1) and (3).

Non-volatile matter

25 ml, when evaporated to dryness and dried at 105°, leaves not more than 1 mg of residue.

STORAGE

Chloroform should be kept in a well-closed container with a glass stopper or other suitable closure and protected from light.

LABELLING

The label states whether it contains ethanol or amylene.

IMPURITIES

A. carbon tetrachloride,

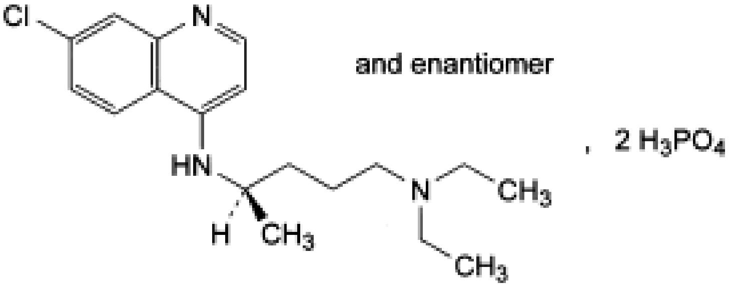
B. dichloromethane,

C. bromochloromethane

Chloroquine Phosphate

General Notices

(Ph Eur monograph 0544)



$C_{18}H_{26}ClN_3,2H_3PO_4$ *515.9* *50-63-5*

Action and use

Antiprotozoal (malaria).

Preparation

Chloroquine Phosphate Tablets

Ph Eur

DEFINITION

Chloroquine phosphate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of *N*^{4}-(7-chloroquinolin-4-yl)-*N*^{1},*N*^{1}-diethylpentane-1,4-diamine bis (dihydrogen phosphate), calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, hygroscopic, freely soluble in water, very slightly soluble in alcohol and in methanol.

It exists in 2 forms, one of which melts at about 195 °C and the other at about 218 °C.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Dissolve 0.100 g in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *water R.* Examined between 210 nm and 370 nm *(2.2.25)*, the solution shows absorption maxima at 220 nm, 235 nm, 256 nm, 329 nm and 342 nm. The specific absorbances at the maxima are respectively 600 to 660, 350 to 390, 300 to 330, 325 to 355 and 360 to 390.

B. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the spectrum obtained with the base isolated from *chloroquine sulphate CRS*. Record the spectra using solutions prepared as follows: dissolve separately 0.1 g of the substance to be examined and 80 mg of the reference substance in 10 ml of *water R*, add 2 ml of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 20 ml, of *methylene chloride R*; combine the organic layers, wash with *water R*, dry over *anhydrous sodium sulphate R*, evaporate to dryness and dissolve the residues separately, each in 2 ml of *methylene chloride R.*

C. Dissolve 25 mg in 20 ml of *water R* and add 8 ml of *picric acid solution R1*. The precipitate, washed with *water R*, with *alcohol R* and finally with *methylene chloride R*, melts *(2.2.14)* at 206-209 °C.

D. Dissolve 0.1 g in 10 ml of *water R*, add 2 ml of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 20 ml, of *methylene chloride R*. The aqueous layer, acidified by the addition of *nitric acid R*, gives reaction (b) of phosphates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 or GY_5 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 3.8 to 4.3.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using silica gel GF_{254} R as the coating substance.

Test solution Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *water R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *water R*.

Apply to the plate 2 µl of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *diethylamine R*, 40 volumes of *cyclohexane R* and 50 volumes of *chloroform R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

Dissolve 2.0 gin 10 ml of *water R*. Add 5 ml of *concentrated ammonia R* and shake with 40 ml of *methylene chloride R*. Filter the aqueous layer and neutralise the filtrate with *glacial acetic* *acid R*. Heat on a water-bath to eliminate methylene chloride, allow to cool and dilute to 20.0 ml with *water R*. 12 ml of this solution complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.200 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 25.79 mg of $C_{18}H_{32}ClN_3O_8P_2$.

STORAGE

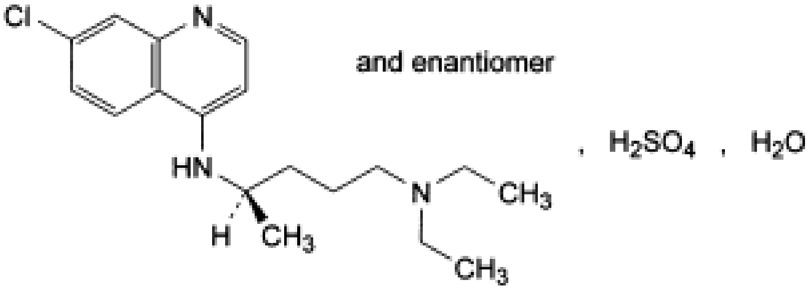
In an airtight container, protected from light.

Ph Eur

Chloroquine Sulphate

General Notices

(Ph Eur monograph 0545)



$C_{18}H_{26}ClN_3,H_2SO_4,H_2O$ 436.0 *132-73-0*

Action and use

Antiprotozoal (malaria).

Preparations

Chloroquine Sulphate Injection

Chloroquine Sulphate Tablets

Ph Eur

DEFINITION

Chloroquine sulphate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of *N*^{4}-(7-chloroquinolin-4-yl)-*N*^{1},*N*^{1}-diethylpentane-1,4-diamine sulphate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water and in methanol, very slightly soluble in ethanol (96 per cent).

It melts at about 208 °C (instantaneous method).

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 0.100 g in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *water R.* Examined between 210 nm and 370 nm *(2.2.25)*, the solution shows absorption maxima at 220 nm, 235 nm, 256 nm, 329 nm and 342 nm. The specific absorbances at the maxima are respectively 730 to 810, 430 to 470, 370 to 410, 400 to 440 and 430 to 470.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with the base isolated from *chloroquine sulphate CRS*. Record the spectra using solutions prepared as follows: dissolve separately 0.1 g of the substance to be examined and of the reference substance in 10 ml of *water R*, add 2 ml of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 20 ml, of *methylene chloride R;* combine the organic layers, wash with *water R*, dry over *anhydrous sodium sulphate R*, evaporate to dryness and dissolve the residues separately each in 2 ml of *methylene chloride R*.

C. Dissolve 25 mg in 20 ml of *water R* and add 8 ml of *picric acid solution R1*. The precipitate, washed with *water R*, with *ethanol (96 per cent) R* and finally with *ether R,* melts (*2.2.14)* at 206 °C to 209 °C.

D. It gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 or GY_5 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 4.0 to 5.0.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *water R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *water R.*

Apply separately to the plate 2 µl of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *diethylamine R*, 40 volumes of *cyclohexane R* and 50 volumes of *methylene chloride R.* Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

Dissolve 2.0 g in 10 ml of *water R*. Add 5 ml of *concentrated ammonia R* and shake with 40 ml of *ether R*. Filter the aqueous layer and neutralise the filtrate with *glacial acetic acid R*. Heat on a water-bath to eliminate ether, allow to cool and dilute to 20.0 ml with *water R*. 12 ml of this solution complies with test A (20 ppm). Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Water ***(2.5.12)***

3.0 per cent to 5.0 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 41.8 mg of $C_{18}H_{28}ClN_3O_4S$.

STORAGE

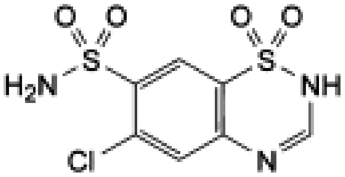
Store in an airtight container, protected from light.

Ph Eur

Chlorothiazide

General Notices

(Ph Eur monograph 0385)



$C_7H_6ClN_3O_4S_2$ 295.7 *58-94-6*

Action and use

Diuretic.

Ph Eur

DEFINITION

Chlorothiazide contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 6-chloro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, sparingly soluble in acetone, slightly soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D.*

A. Dissolve 80.0 mg in 100 ml of *0.1 M sodium hydroxide* and dilute to 1000.0 ml with *water R.* Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M sodium hydroxide*. Examined between 220 nm and 320 nm *(2.2.25)*, the solution shows 2 absorption maxima, at 225 nm and 292 nm, and a shoulder at about 310 nm. The specific absorbances at the maxima are 725 to 800 and 425 to 455, respectively.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *chlorothiazide CRS*.

C. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 25 mg of the substance to be examined in *acetone R* and dilute to 5

ml with the same solvent.

Reference solution Dissolve 25 mg of *chlorothiazide CRS* in *acetone R* and dilute to 5 ml with the same solvent.

Apply to the plate 2 µl of each solution. Develop over a path of 10 cm using *ethyl acetate R*. Dry the plate in a current of air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 0.1 g add a pellet of *sodium hydroxide R* and heat strongly. Gas is evolved which turns *red litmus paper R* blue. After cooling, take up the residue with 10 ml of *dilute hydrochloric acid R*. Gas is evolved which turns lead *acetate paper R* black.

TESTS

Solution S

To 1.0 g of the powdered substance to be examined add 50 ml of *water R*, shake for 2 min and filter.

Acidity or alkalinity

To 10 ml of solution S add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution R*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution Dissolve 25 mg of the substance to be examined in *acetone R* and dilute to 5 ml with the same solvent.

Reference solution Dilute 1 ml of the test solution to 100 ml with *acetone R.*

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 15 volumes of *2-propanol R* and 85 volumes of *ethyl acetate R*. Dry the plate in a current of air until the solvents have evaporated (about 10 min) and spray with a mixture of equal volumes of *alcoholic solution of sulphuric acid R* and *alcohol R*; use about 10 ml for a plate 200 mm square and spray in small portions, allowing the solvent to evaporate each time to avoid excessive wetting. Heat at 100-105 °C for 30 min and immediately place the plate above, but not in contact with, 10 ml of a saturated solution of *sodium nitrite R* in a glass tank. Carefully add 0.5 ml of *sulphuric acid R* to the sodium nitrite solution, close the tank, and allow to stand for 15 min. Remove the plate, heat in a ventilated oven at 40 °C for 15 min and spray with 3 quantities, each of 5 ml, of a freshly prepared 5 g/l solution of *naphthylethylenediamine dihydrochloride R* in *alcohol R.* Examine the plate by transmitted light. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Chlorides *(2.4.4)*

15 ml of solution S complies with the limit test for chlorides (160 ppm).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of

lead standard solution (10 ppm Pb) R.

Loss on drying ***(2.2.32)***

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

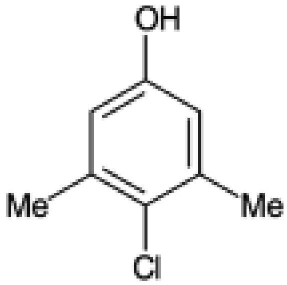
Dissolve 0.250 g in 50 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide* in 2-propanol determining the end-point potentiometrically *(2.2.20)* at the first point of inflexion. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* in 2-propanol is equivalent to 29.57 mg of $C_7H_6ClN_3O_4S_2$.

Ph Eur

Chloroxylenol

General Notices



C_8H_9ClO 156.6 *88-04-0*

Action and use

Antiseptic.

Preparation

Chloroxylenol Solution

DEFINITION

Chloroxylenol is 4-chloro-3,5-xylenol. It contains not less than 98.0% and not more than 103.0% of C_8H_9ClO.

CHARACTERISTICS

White or cream crystals or crystalline powder. It is volatile in steam.

Very slightly soluble in *water*; freely soluble in *ethanol (96%)*; soluble in *ether*, in terpenes and in fixed oils. It dissolves in solutions of the alkali hydroxides.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of chloroxylenol *(RS 055)*.

B. Dissolve 0.1 g in 5 ml of *chloroform* and add 0.5 ml of a filtered 1% w/v solution of *anhydrous iron(III) chloride* in *chloroform* and 0.1 ml of *pyridine*. A blue colour is produced.

C. To 5 ml of a saturated solution in *water* add 0.5 ml of *iron(III) chloride solution R1*. No blue colour is produced.

D. Mix 50 mg with 0.5 g of *anhydrous sodium carbonate* and ignite strongly, cool, boil the residue with 5 ml of *water*, acidify with *nitric acid*, filter and add *silver nitrate solution*. A white precipitate is produced.

TESTS

Melting point

114° to 116°, Appendix V A.

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using solutions in *chloroform* containing (1) 2.0% w/v of the substance being examined and (2) 2.0% w/v of the substance being examined and 0.040% w/v of *4-chloro*-o-*cresol* (internal standard).

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed diatomaceous support* (80 to 100 mesh) coated with 3% w/w of polyethylene glycol (Carbowax 20M is suitable) and maintained at 160°.

In the chromatogram obtained with solution (2) the sum of the areas of any *secondary peaks* is not greater than the area of the peak due to the internal standard.

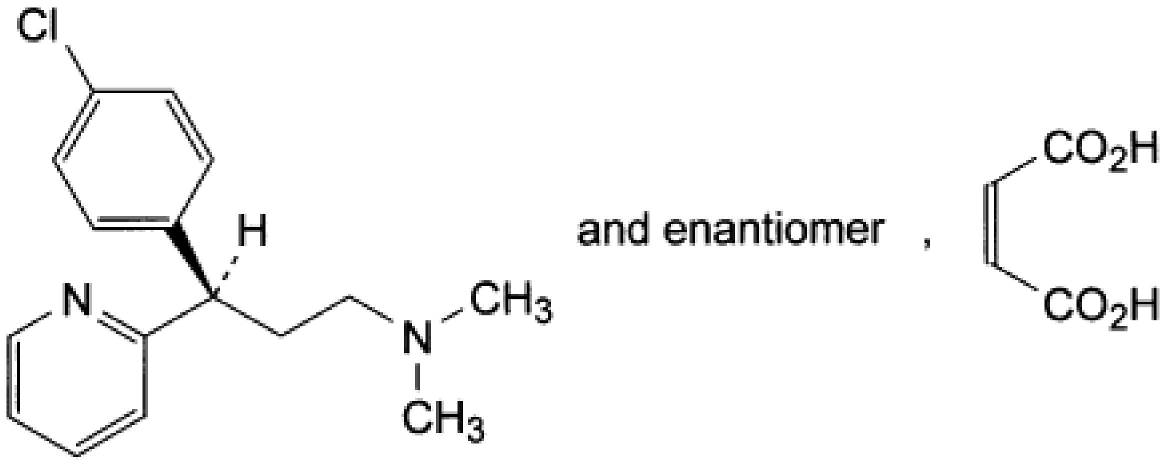
ASSAY

Dissolve 70 mg in 30 ml of *glacial acetic acid*, add 25 ml of 0.0167M *potassium bromate VS*, 20 ml of a 15% w/v solution of *potassium bromide* and 10 ml of *hydrochloric acid*, stopper the flask and allow to stand protected from light for 15 minutes. Add 1 g of *potassium iodide* and 100 ml of *water* and titrate with 0.1M *sodium thiosulphate VS*, shaking vigorously and using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of potassium bromate required. Each ml of 0.0167M *potassium bromate VS* is equivalent to 3.915 mg of C_8H_9ClO.

Chlorphenamine Maleate

General Notices

(Ph Eur monograph 0386)



$C_{16}H_{19}ClN_2,C_4H_4O_4$ 390.9   *113-92-8*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparations

Chlorphenamine Injection

Chlorphenamine Oral Solution

Chlorphenamine Tablets

Ph Eur

DEFINITION

(3*RS*)-3-(4-Chlorophenyl)-*N*,*N*-dimethyl-3-(pyridin-2-yl)propan-1-amine hydrogen (*Z*)-butenedioate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. Melting point (*2.2.14*): 130 °C to 135 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison chlorphenamine maleate CRS.

C. Optical rotation (see Tests).

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 0.5 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 5 mg of *chlorphenamine impurity C CRS* in 5 ml of the test solution and dilute to 50.0 ml with the mobile phase. Dilute 2 ml of this solution to 20 ml with the mobile phase.

Reference solution (d) Dissolve 5 mg of *2,2′-dipyridylamine R* (impurity B) in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (e) Dissolve the contents of a vial of *chlorphenamine impurity A CRS* in 2 ml of the test solution. Sonicate for 5 min.

Column:

— *size:* *l* = 0.30 m, Ø = 3.9 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a 8.57 g/l solution of *ammonium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R.*

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl.

Run time 3.5 times the retention time of chlorphenamine.

Relative retention With reference to chlorphenamine (retention time = about 11 min): maleic acid = about 0.2; impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.9; impurity D = about 3.0.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to impurity C and chlorphenamine.

Limits:

— *correction factors:* for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.5; impurity B = 1.4;

— *impurity A*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities B, C, D*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peaks due to the blank and maleic acid.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml *of lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

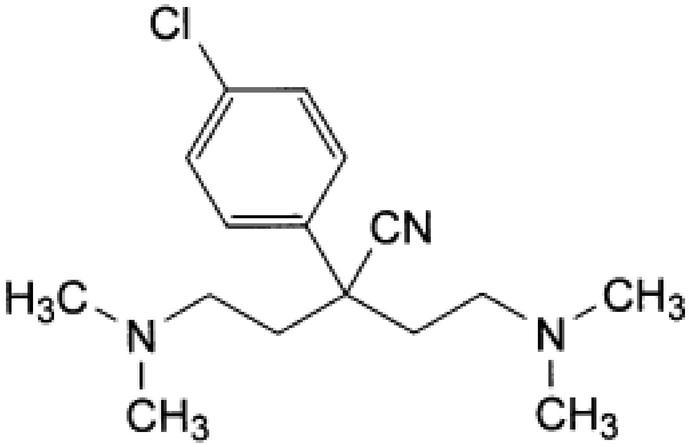
1 ml of *0.1 M perchloric acid* is equivalent to 19.54 mg of $C_{20}H_{23}ClN_2O_4$.

STORAGE

Protected from light.

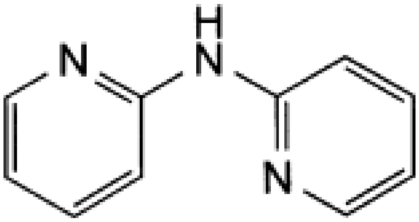
IMPURITIES

Specified impurities   A, B, C, D.

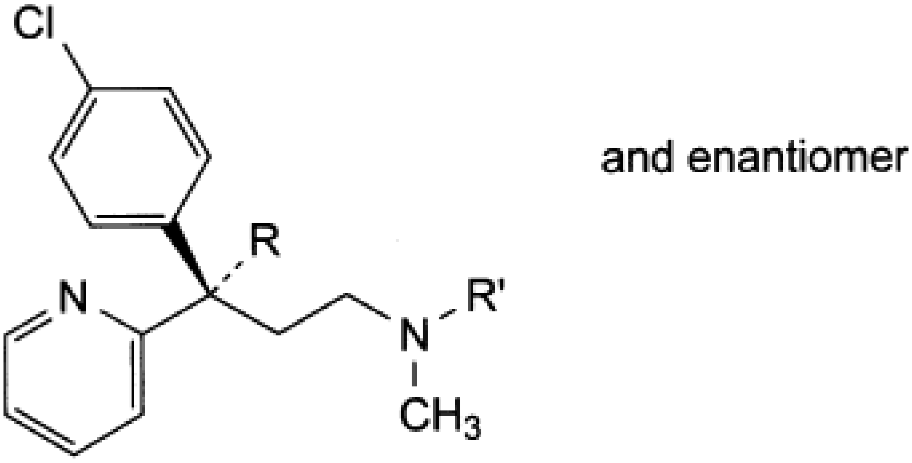


Cl
CN
H_3C
N
CH_3
CH_3
N
CH_3

A. 2-(4-chlorophenyl)-4-(dimethylamino)-2-[2-(dimethylamino)ethyl]butanenitrile,



B. *N*-(pyridin-2-yl)pyridin-2-amine (2,2′-dipyridylamine),



Cl
R
N
N
R'
CH_3
and enantiomer

C. R = R′ = H: (3*RS*)-3-(4-chlorophenyl)-*N*-methyl-3-(pyridin-2-yl)propan-1-amine,

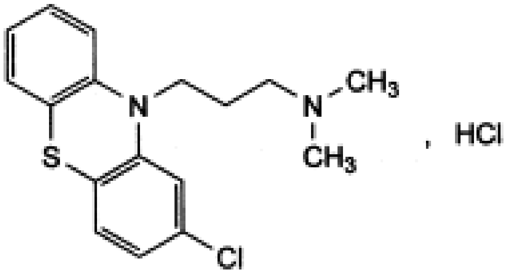
D. R = CN, R′ = CH_3: (2*RS*)-2-(4-chlorophenyl)-4-(dimethylamino)-2-(pyridin-2-yl) butanenitrile.

Ph Eur

Chlorpromazine Hydrochloride

General Notices

(Ph Eur monograph 0475)



$C_{17}H_{19}ClN_2S,HCl$ 355.3 *69-09-0*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparations

Chlorpromazine Injection

Chlorpromazine Oral Solution

Chlorpromazine Tablets

Ph Eur

DEFINITION

3-(2-Chloro-10*H*-phenothiazin-10-yl)-*N*,*N*-dimethylpropan-1-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

It decomposes on exposure to air and light.

mp

About 196 °C.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25). Prepare the solutions protected from bright light and measure the absorbances immediately*.

Test solution Dissolve 50.0 mg in a 10.3 g/l solution of *hydrochloric acid R* and dilute to 500.0 ml with the same solution. Dilute 5.0 ml of the solution to 100.0 ml with a 10.3 g/l solution of *hydrochloric acid R.*

Spectral range 230-340 nm.

Absorption maximum At 254 nm and 306 nm.

Specific absorbance at the absorption maximum
— at 254 nm: 890 to 960.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *chlorpromazine hydrochloride CRS.*

C. Identification test for phenothiazines by thin-layer chromatography *(2.3.3):* use *chlorpromazine hydrochloride CRS* to prepare the reference solution.

D. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

pH *(2.2.3)*

3.5 to 4.5. *Carry out the test protected from light and use freshly prepared solutions.*

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light and use freshly prepared solutions.*

Test solution Dissolve 40 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 4 mg of *chlorpromazine impurity D CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. To 1 ml of this solution add 1 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 4.0 mg of *chlorpromazine impurity A CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (d) Dissolve 4 mg of *promazine hydrochloride CRS* (impurity C) and 4.0 mg of *chlorpromazine impurity E CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm,

— *stationary phase: base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 0.2 volumes of *thiodiethylene glycol R* with 50 volumes of *acetonitrile R* and 50 volumes of a 0.5 per cent *V/V* solution of *trifluoroacetic acid R* previously adjusted to pH 5.3 with *tetramethylethylenediamine R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 4 times the retention time of chlorpromazine.

Relative retention

With reference to chlorpromazine (retention time = about 8 min): impurity A = about 0.4; impurity B = about 0.5; impurity C = about 0.7; impurity D = about 0.9; impurity E = about 3.4.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to impurity D and chlorpromazine.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *impurities B, C, D*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity E*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.1.0 g complies with test C. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 ml of *0.1 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

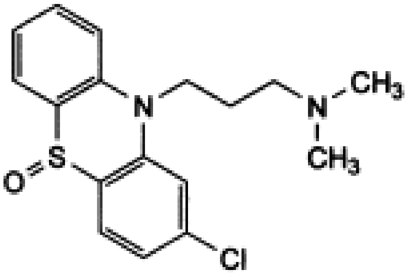
1 ml of 0*.1 M sodium hydroxide* is equivalent to 35.53 mg of $C_{17}H_{20}Cl_2N_2S$.

STORAGE

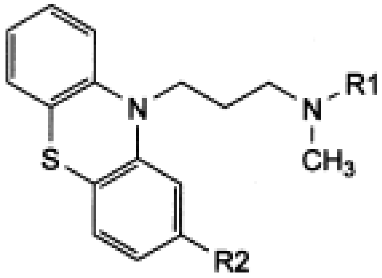
In an airtight container, protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*



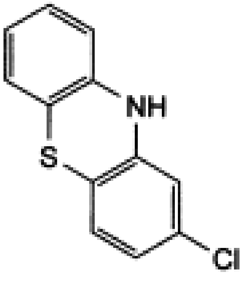
A. 3-(2-chloro-10*H*-phenothiazin-10-yl)-*N,N*-dimethylpropan-1-amine *S*-oxide (chlorpromazine sulphoxide),



B. R1 = $[CH_2]_3$-$N(CH_3)_2$, R2 = Cl: *N*-[3-(2-chloro-10*H*-phenothiazin-10-yl)propyl]-*N,N′,N′*-trimethylpropane-1,2-diamine,

C. R1 = CH_3, R2 = H: promazine,

D. R1 = H, R2 = Cl: 3-(2-chloro-10*H*-phenothiazin-10-yl)-*N*-methylpropan-1-amine (desmethylchlorpromazine),

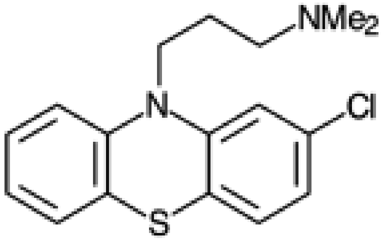


E. 2-chloro-10*H*-phenothiazine.

Ph Eur

Chlorpromazine

General Notices



$C_{17}H_{19}ClN_2S$ 318.9 *50-53-3*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Chlorpromazine Suppositories

DEFINITION

Chlorpromazine is [3-(2-chlorophenothiazin-10-yl)propyl]-dimethylamine. It contains not less than 99.0% and not more than 101.0% of $C_{17}H_{19}ClN_2S$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or creamy white powder or waxy solid.

Practically insoluble in *water*; freely soluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of chlorpromazine *(RS 056)*.

B. Complies with the test for *identification of phenothiazines*, Appendix III A, using *chlorpromazine hydrochloride EPCRS* to prepare solution (2).

TESTS

Melting point

56° to 58°, Appendix V A.

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A*.

Loss on drying

When dried to constant weight over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.8 g in 300 ml of *acetone* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 31.89 mg of $C_{17}H_{19}ClN_2S$.

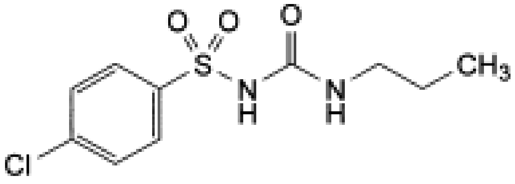
STORAGE

Chlorpromazine should be protected from light.

Chlorpropamide

General Notices

(Ph Eur monograph 1087)



$C_{10}H_{13}ClN_2O_3S$ 276.7 *94-20-2*

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

Preparation

Chlorpropamide Tablets

Ph Eur

DEFINITION

Chlorpropamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 1-[(4-chlorophenyl)sulphonyl]-3-propylurea, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone and in methylene chloride, soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification C, D.

Second identification A, B, D.

A. Melting point *(2.2.14)*: 126 °C to 130 °C.

B. Dissolve 0.10 g in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *0.01 M hydrochloric acid*. Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M hydrochloric acid*. Examined between 220 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 232 nm. The specific absorption at the maximum is 570 to 630.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *chlorpropamide CRS*. Examine the substances prepared as discs. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance in *methylene chloride R*, evaporate to dryness and record the new spectra using the residues.

D. Heat 0.1 g with 2 g of *anhydrous sodium carbonate R* until a dull red colour appears for 10 min. Allow to cool, extract the residue with about 5 ml of *water R*, dilute to 10 ml with *water R* and filter. The solution gives the reaction (a) of chloride *(2.3.1)*.

TESTS

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable *silica gel* as the coating substance.

Test solution Dissolve 0.50 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 15 mg of *4-chlorobenzenesulphonamide R* (chlorpropamide impurity A) in *acetone R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dissolve 15 mg of *chlorpropamide impurity B CRS* in *acetone R* and dilute to 100 ml with the same solvent.

Reference solution (c) Dilute 0.3 ml of the test solution to 100 ml with *acetone R*.

Reference solution (d) Dilute 5 ml of reference solution (c) to 15 ml with *acetone R*.

Reference solution (e) Dissolve 0.10 g of the substance to be examined, 5 mg of *4-chlorobenzenesulphonamide R* and 5 mg of *chlorpropamide impurity B CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 11.5 volumes of *concentrated ammonia R,* 30 volumes of *cyclohexane R*, 50 volumes of *methanol R* and 100 volumes of *methylene chloride R*. Allow the plate to dry in a current of cold air, heat at 110 °C for 10 min. At the bottom of a chromatographic tank, place an evaporating dish containing a mixture of 1 volume of *hydrochloric acid R*, 1 volume of *water R* and 2 volumes of a 50 g/l solution of *potassium permanganate R*, close the tank and allow to stand for 15 min. Place the dried hot plate in the tank and close the tank. Leave the plate in contact with the chlorine vapour for 2 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide* and *starch solution R*. Spray with *potassium iodide* and *starch solution R.* In the chromatogram obtained with the test solution: any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.3 per cent); any spot corresponding to impurity B is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent); any spot, apart from the principal spot and any spot corresponding to impurity A and B, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.3 per cent); not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (d) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows three clearly separated spots with approximate R_F values of 0.4, 0.6 and 0.9 corresponding to chlorpropamide, impurity A and impurity B respectively.

Heavy metals *(2.4.8)*

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R* and dilute to 20 ml with the same mixture of solvents. 12 ml of solution complies with limit test B for heavy metals (20 ppm). Prepare the standard using lead standard solution (2 ppm Pb) prepared by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 100 °C to 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

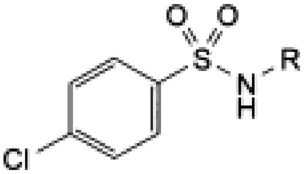
Dissolve 0.250 g in 50 ml of *alcohol R* previously neutralised using *phenolphthalein solution R1* as indicator and add 25 ml of *water R*. Titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 27.67 mg of $C_{10}H_{13}ClN_2O_3S$.

STORAGE

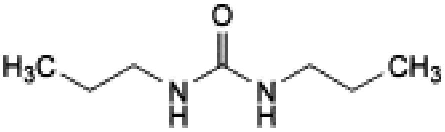
Store protected from light.

IMPURITIES



A. R = H: 4-chlorobenzenesulphonamide,

C. R = $CO-NH_2$: [(4-chlorophenyl)sulphonyl]urea.



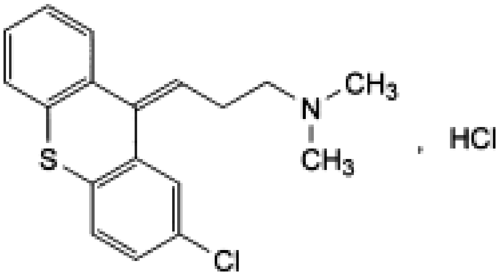
B. 1,3-dipropylurea,

Ph Eur

Chlorprothixene Hydrochloride

General Notices

(Ph Eur monograph 0815)



$C_{18}H_{18}ClNS,HCl$ 352.3 *6469-93-8*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

(*Z*)-3-(2-Chloro-9*H*-thioxanthen-9-ylidene)-*N*,*N*-dimethylpropan-1-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water and in alcohol, slightly soluble in methylene chloride.

mp

About 220 °C.

IDENTIFICATION

First identification A, E.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 0.25 g in 10 ml of *water R*. Add 1 ml of *dilute sodium hydroxide solution R.* Shake with 20 ml of *methylene chloride R*. Separate the organic layer and wash with 5 ml of *water R*. Evaporate the organic layer to dryness and dry the residue at 40-50 °C. Examine the residues prepared as discs.

Comparison *chlorprothixene hydrochloride CRS*.

B. Dissolve 0.2 g in a mixture of 5 ml of *dioxan R* and 5 ml of a 1.5 g/l solution of *sodium nitrite R*. Add 0.8 ml of *nitric acid R*. After 10 min add the solution to 20 ml of *water R*. 1 h later filter the precipate formed. The filtrate is used immediately for identification test C. Dissolve the precipitate by warming in about 15 ml of *alcohol R* and add the solution to 10 ml of *water R*. Filter and dry the precipitate at 100-105 °C for 2 h. The melting point (*2.2.14*) is 152 °C to 154 °C.

C. To 1 ml of the filtrate obtained in identification test B, add 0.2 ml of a suspension of 50 mg of *fast red B salt R* in 1 ml of *alcohol R*. Add 1 ml of *0.5 M alcoholic potassium hydroxide*. A dark red colour is produced. Carry out a blank test.

D. Dissolve about 20 mg in 2 ml of *nitric acid R*. A red colour is produced. Add 5 ml of *water R* and examine in ultraviolet light at 365 nm. The solution shows green fluorescence.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.4 to 5.2 for solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from bright light.*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *chlorprothixene hydrochloride CRS* (with a defined content of *E*-isomer) in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 3.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.12 m, Ø = 4.0 mm,

— *stationary phase:* base-deactivated *octadecylsilyl silica gel for chromatography R* (3 µm or 5 µm).

Mobile phase Solution containing 6.0 g/l of *potassium dihydrogen phosphate R*, 2.9 g/l of *sodium laurilsulfate R* and 9 g/l of *tetrabutylammonium bromide R* in a mixture of 50 volumes of *methanol R*, 400 volumes of *acetonitrile R* and 550 volumes of *distilled water R.*

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration For about 30 min with the mobile phase.

Injection 20 µl.

Run time Twice the retention time of chlorprothixene.

Relative retention With reference to chlorprothixene: impurity E = about 1.55.

System suitability Reference solution (a):

— *retention time*: chlorprothixene = about 10 min,

— *relative retention* with reference to chlorprothixene: *E*-isomer = about 1.35.

Limits:

— *E-isomer*: not more than 2.0 per cent, calculated from the area of the corresponding peak in the chromatogram obtained with reference solution (a) and taking into account the assigned content of this isomer in *chlorprothixene hydrochloride CRS,*

— *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent taking into account a response factor of 3),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *total* of any other impurity: not more than 2.33 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test F. Prepare the standard using 2 ml *of lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R.* Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

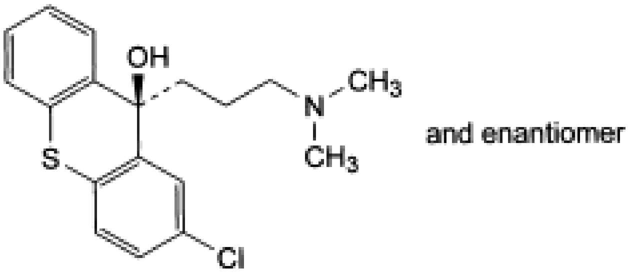
1 ml of *0.1 M sodium hydroxide* is equivalent to 35.23 mg of $C_{18}H_{19}Cl_2NS$.

STORAGE

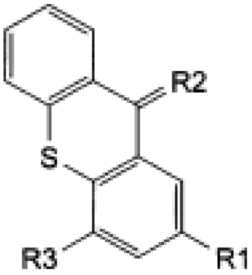
Protected from light.

IMPURITIES

Specified impurities   A, B, C, D, E, F.



A. (*RS*)-2-chloro-9-[3-(dimethylamino)propyl]-9*H*-thioxanthen-9-ol,

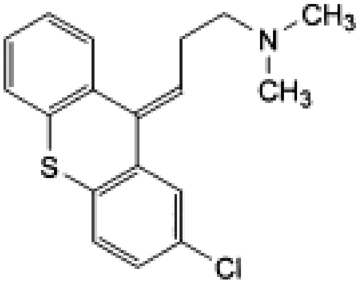


B. R1 = H, R2 = CH-CH_2-CH_2-N(CH_3)$_2$, R3 = H: *N,N*-dimethyl-3-(9*H*-thioxanthen-9-ylidene) propan-1-amine,

C R1 = Cl, R2 = CH-CH_2-CH_2-NH-CH_3, R3 = H: (*Z*)-3-(2-chloro-9*H*-thioxanthen-9-ylidene)-*N*-methylpropan-1-amine,

D. R1 = H, R2 = CH-CH_2-CH_2-N(CH_3)$_2$, R3 = Cl: (*Z*)-3-(4-chloro-9*H*-thioxanthen-9-ylidene) -*N*,*N*-dimethylpropan-1-amine,

E. R1 = Cl, R2 = O, R3 = H: 2-chloro-9*H*-thioxanthen-9-one,



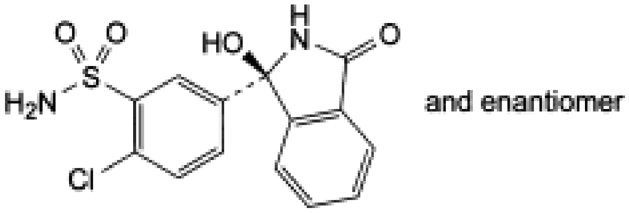
F. (*E*)-3-(2-chloro-9*H*-thioxanthen-9-ylidene)-*N,N*-dimethylpropan-1-amine (*E*-isomer).

Ph Eur

Chlortalidone

General Notices

(Ph Eur monograph 0546)



$C_{14}H_{11}ClN_2O_4S$ 338.8 *77-36-1*

Action and use

Thiazide-like diuretic.

Preparations

Chlortalidone Tablets

Co-tenidone Tablets

Ph Eur

DEFINITION

2-Chloro-5-[(1*RS*)-1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl]benzenesulphonamide.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Very slightly soluble in water, soluble in acetone and in methanol, practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*)

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison chlortalidone CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Acidity

Dissolve 1.0 g with heating in a mixture of 25 ml of *acetone R* and 25 ml of *carbon dioxide-free water R.* Cool. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20).* Not more than 0.75 ml of *0.1 M sodium hydroxide* is required.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture Mix 2 volumes of a 2 g/l solution of *sodium hydroxyde R*, 48 volumes of mobile phase B and 50 volumes of mobile phase A.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Test solution (b) Dilute 10.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *chlortalidone for peak identification CRS* (containing impurities B, G and J) in 1 ml of the solvent mixture.

Reference solution (c) Dissolve 50.0 mg of *chlortalidone CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 10.0 ml of this solution to 100.0 ml with the solvent mixture.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

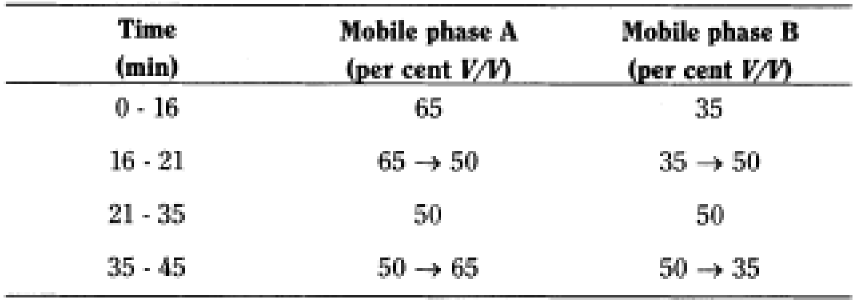
— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: dissolve 1.32 g of *ammonium phosphate R* in about 900 ml of *water R* and adjust to pH 5.5 with *dilute phosphoric acid R*; dilute to 1000 ml with *water R*;

— *mobile phase B*: *methanol R2*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 16	65	35
16 - 21	65 → 50	35 → 50
21 - 35	50	50
35 - 45	50 → 65	50 → 35

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram obtained with reference solution (b) and the chromatogram supplied with *chlortalidone for peak identification CRS* to identify the peaks due to impurities B, G and J.

Relative retention With reference to chlortalidone (retention time = about 7 min): impurity B = about 0.7; impurity J = about 0.9; impurity G = about 6.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity J and chlortalidone.

Limits:

— *impurity B*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

— *impurity J*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— impurity G: not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 12 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides *(2.4.4)*

Maximum 350 ppm.

Triturate 0.3 g finely, add 30 ml of *water R*, shake for 5 min and filter. 15 ml of the filtrate complies with the test. Prepare the standard using 10 ml of *chloride standard solution (5 ppm Cl) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

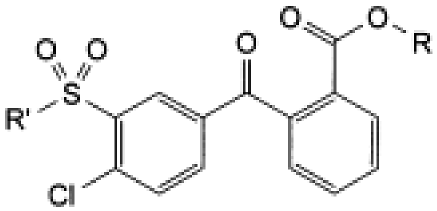
Injection 20 µl of test solution (b) and reference solution (c).

Calculate the percentage content of $C_{14}H_{11}ClN_2O_4S$ from the declared content of *chlortalidone CRS*.

IMPURITIES

Specified impurities B, G, J.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, C, D, E, F, H, I.*

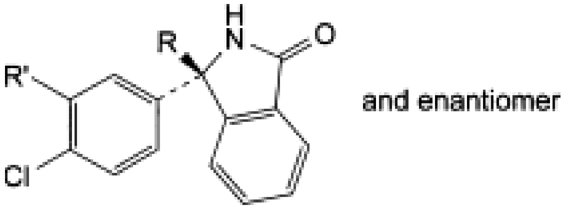


A. R = H, R′ = OH: 2-(4-chloro-3-sulphobenzoyl)benzoic acid,

B. R = H, R′ = NH_2: 2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid,

C. R = C_2H_5, R′ = NH_2: ethyl 2-(4-chloro-3-sulphamoylbenzoyl)benzoate,

I. R = $CH(CH_3)_2$, R′ = NH_2: 1-methylethyl 2-(4-chloro-3-sulphamoylbenzoyl)benzoate,

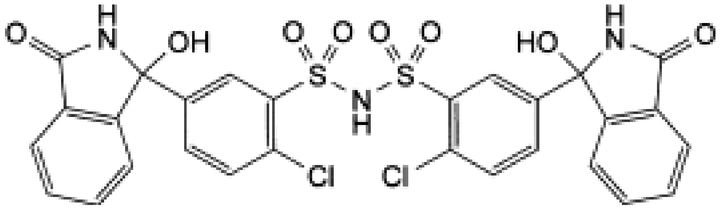


D. R = OC_2H_5, R′ = SO_2-NH_2: 2-chloro-5-[(1*RS*)-1-ethoxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl] benzenesulphonamide,

E. R = H, R′ = SO_2-NH_2: 2-chloro-5-[(1*RS*)-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl] benzenesulphonamide,

G. R = OH, R′ = Cl: (3*RS*)-3-(3,4-dichlorophenyl)-3-hydroxy-2,3-dihydro-1*H*-isoindol-1-one,

H. R = $OCH(CH_3)_2$, R′ = SO_2-NH_2: 2-chloro-5-[(1*RS*)-1-(1-methylethoxy)-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl]benzenesulphonamide,



F. bis[2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)benzenesulphonyl]amine,

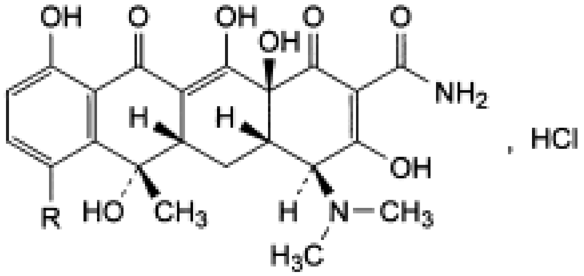
J. impurity of unknown structure with a relative retention of about 0.9.

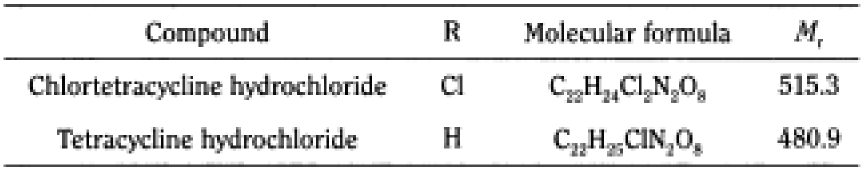
Ph Eur

Chlortetracycline Hydrochloride

General Notices

(Ph Eur monograph 0173)





Compound	R	Molecular formula	M_r
Chlortetracycline hydrochloride	Cl	$C_{22}H_{24}Cl_2N_2O_8$	515.3
Tetracycline hydrochloride	H	$C_{22}H_{25}ClN_2O_8$	480.9

Action and use

Tetracycline antibacterial.

Ph Eur

DEFINITION

Mixture of antibiotics, the main component being the hydrochloride of (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (chlortetracycline hydrochloride), a substance produced by the growth of certain strains of *Streptomyces aureofaciens* or obtained by any other means.

Content:

— $C_{22}H_{24}Cl_2N_2O_8$: minimum 89.5 per cent (anhydrous substance),

— $C_{22}H_{25}ClN_2O_8$: maximum 8.0 per cent (anhydrous substance),

— 94.5 per cent to 102.0 per cent for the sum of the contents of chlortetracycline hydrochloride and tetracycline hydrochloride (anhydrous substance).

CHARACTERS

Appearance

Yellow powder.

Solubility

Slightly soluble in water and in alcohol. It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *chlortetracycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *chlortetracycline hydrochloride CRS*, 5 mg of *doxycycline R* and 5 mg of *demeclocycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A deep blue colour develops which becomes bluish-green. Add the solution to 2.5 ml of *water R*. The colour becomes brownish.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

2.3 to 3.3.

Dissolve 0.1 g in 10 ml of *carbon dioxide-free water R*, heating slightly.

Specific optical rotation *(2.2.7)*

- 235 to - 250 (anhydrous substance).

Dissolve 0.125 g in *water R* and dilute to 50.0 ml with the same solvent.

Absorbance *(2.2.25)*

Maximum 0.40 at 460 nm.

Dissolve 0.125 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 25.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 25.0 mg of *chlortetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 10.0 mg of *4-epichlortetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c) Dissolve 20.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (d) Mix 5.0 ml of reference solution (a) and 10.0 ml of reference solution (b) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (e) Mix 5.0 ml of reference solution (b) and 5.0 ml of reference solution (c) and dilute to 50.0 ml with *0.01 M hydrochloric acid*.

Reference solution (f) Dilute 1.0 ml of reference solution (c) to 20.0 ml with *0.01 M hydrochloric acid*. Dilute 5.0 ml of this solution to 200.0 ml with *0.01 M hydrochloric acid*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 35 °C.

Mobile phase To 500 ml of *water R*, add 50 ml of *perchloric acid solution R*, shake and add 450 ml of *dimethyl sulphoxide R,*

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl; inject the test solution and reference solutions (d), (e) and (f).

System suitability Reference solution (d):

— *resolution*: minimum 2.0 between the peaks due to impurity A and to chlortetracycline; if necessary, adjust the dimethyl sulphoxide content in the mobile phase,

— *symmetry factor*: maximum 1.3 for the peak due to chlortetracycline.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (4.0 per cent),

— *total of other impurities eluting between the solvent peak and the peak corresponding to chlortetracycline:* not more than 0.25 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (1.0 per cent),

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (f) (0.1 per cent).

Heavy metals *(2.4.8)*

Maximum 50 ppm.

0.5 g complies with limit test C. Prepare the standard using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 1 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29*) as described in the test for related substances with the following modification.

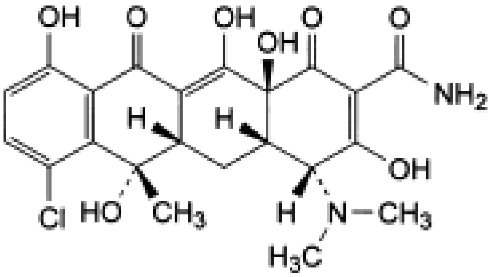
Injection Test solution and reference solutions (a) and (e).

Calculate the percentage content of $C_{22}H_{24}Cl_2N_2O_8$ using the chromatogram obtained with reference solution (a). Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$ using the chromatogram obtained with reference solution (e).

STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

IMPURITIES



A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epichlortetracycline),

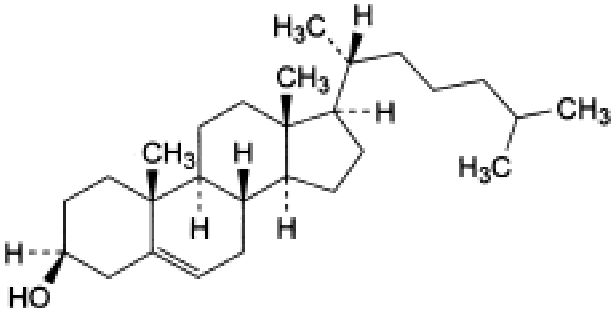
B. demeclocycline.

Ph Eur

Cholesterol

General Notices

(Ph Eur monograph 0993)



$C_{27}H_{46}O$ 386.7 *57-88-5*

Action and use

Excipient.

Ph Eur

DEFINITION

Cholest-5-en-3β-ol.

Content:

— *cholesterol*: minimum 95.0 per cent (dried substance);

— *total sterols*: 97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in acetone and in ethanol (96 per cent).

It is sensitive to light.

IDENTIFICATION

A. Melting point *(2.2.14):* 147 °C to 150 °C.

B. Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 10 mg of the substance to be examined in ethylene chloride R and dilute to 5 ml with the same solvent.

Reference solution Dissolve 10 mg of *cholesterol CRS* in *ethylene chloride R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *ethyl acetate R, toluene R* (33:66 *V/V*).

Application 20 µl.

Development Immediately, protected from light, over a path of 15 cm.

Drying In air.

Detection Spray 3 times with *antimony trichloride solution R*; examine within 3-4 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 5 mg in 2 ml of *methylene chloride R*. Add 1 ml of *acetic anhydride R,* 0.01 ml of *sulphuric acid R* and shake. A pink colour is produced which rapidly changes to red, then to blue and finally to brilliant green.

TESTS

Solubility in ethanol (96 per cent)

In a stoppered flask, dissolve 0.5 g in 50 ml of *ethanol (96 per cent) R* at 50 °C. Allow to stand for 2 h. No deposit or turbidity is formed.

Acidity

Dissolve 1.0 g in 10 ml of *ether R*, add 10.0 ml of *0.1 M sodium hydroxide* and shake for about 1 min. Heat gently to eliminate ether and then boil for 5 min. Cool, add 10 ml of *water R* and *0.1 ml of phenolphthalein solution R* as indicator and titrate with *0.1 M hydrochloric acid* until the pink colour just disappears, stirring the solution vigorously throughout the titration. Carry out a blank titration. The difference between the volumes of *0.1 M hydrochloric acid* required to change the colour of the indicator in the blank and in the test is not more than 0.3 ml.

Loss on drying *(2.2.32)*

Maximum 0.3 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.100 g of *pregnenolone isobutyrate CRS* in *heptane R* and dilute to 100.0 ml with the same solvent.

Test solution Dissolve 25.0 mg of the substance to be examined in the internal standard solution and dilute to 25.0 ml with the same solution.

Reference solution Dissolve 25.0 mg of *cholesterol CRS* in the internal standard solution and dilute to 25.0 ml with the same solution.

Column:
- *material*: fused silica;
- *size:* l = 30 m, Ø = 0.25 mm;
- *stationary phase: poly(dimethyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 2 ml/min.

Split ratio 1:25.

Temperature:
- *column*: 275 °C;
- *injection port*: 285 °C;
- *detector*: 300 °C.

Detection Flame ionisation.

Injection 1.0 µl.

System suitability Reference solution:

- *resolution*: minimum 10.0 between the peaks due to pregnenolone isobutyrate and cholesterol.

Calculate the percentage content of cholesterol from the declared content in *cholesterol CRS.* Calculate the percentage content of total sterols by adding together the contents of cholesterol and other substances with a retention time less than or equal to 1.5 times the retention time of cholesterol. Disregard the peaks due to the internal standard and the solvent.

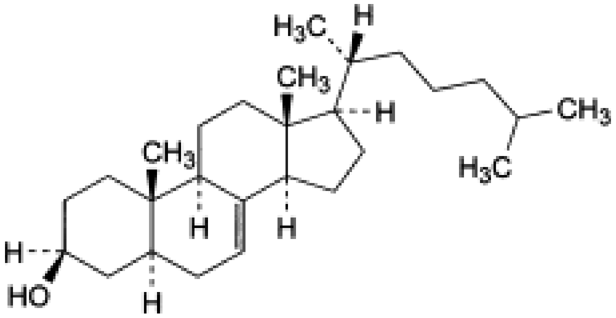
STORAGE

Protected from light.

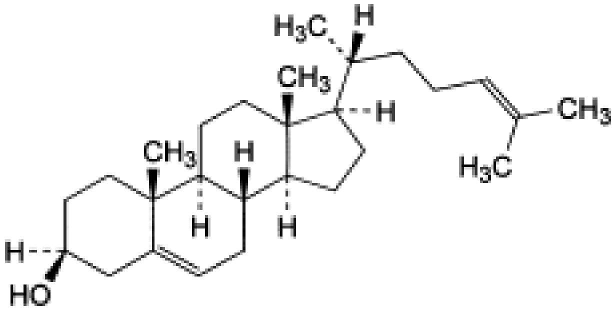
LABELLING

The label states the source material for the production of cholesterol (for example bovine brain and spinal cord, wool fat or chicken eggs).

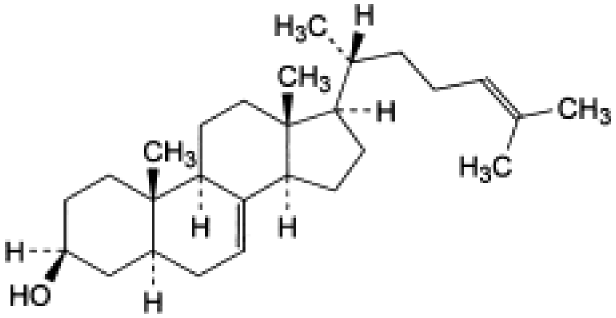
IMPURITIES



A. 5α-cholest-7-en-3β-ol (lathosterol),



B. cholesta-5,24-dien-3β-ol (desmosterol),



C. 5α-cholesta-7,24-dien-3β-ol.

Ph Eur

Choline Salicylate Solution

General Notices

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Choline Salicylate Ear Drops

Choline Salicylate Oromucosal Gel

Ph Eur

DEFINITION

Choline Salicylate Solution is an aqueous solution of choline salicylate. It contains not less than 47.5% w/v and not more than 52.5% w/v of choline salicylate, $C_{12}H_{19}NO_4$. It may contain a suitable antimicrobial preservative.

CHARACTERISTICS

A clear, colourless liquid.

IDENTIFICATION

A. Mix 0.5 ml with 10 ml of *methanol*, dry with *anhydrous sodium sulphate*, filter and evaporate the *methanol* to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of choline salicylate *(RS 059)*.

B. Dilute 5 ml to 25 ml with *water*. The resulting solution yields the reactions characteristic of *salicylates*, Appendix VI.

TESTS

Acidity

Dilute 4 ml to 20 ml with *water* and add 0.1 ml of *phenol red solution*. The solution is yellow and not more than 0.4 ml of 0.1M *sodium hydroxide VS* is required to change the colour of the solution to reddish violet.

Clarity and colour of solution

Dilute 1 volume of the solution to 5 volumes with *water*. The resulting solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Weight per ml

1.070 to 1.110 g, Appendix V G.

Chloride

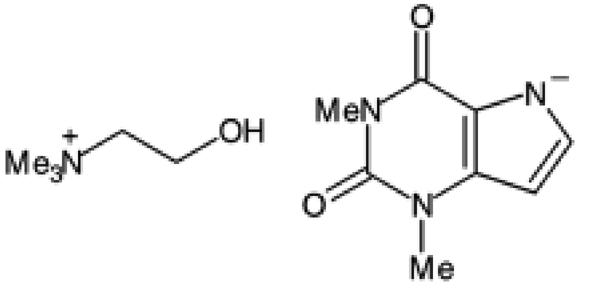
Mix 0.2 ml with 10 ml of *water* and add carefully, with mixing, 0.1 ml of a mixture of 10 volumes of *silver nitrate solution* and 1 volume of *nitric acid*. The resulting solution is not more opalescent than a standard prepared by treating 10 ml of a 0.00164% w/v solution of *sodium chloride* in the same manner beginning at the words 'add carefully …' (0.1%).

ASSAY

To 1 g add 50 ml of *1,4-dioxan* and 5 ml of *acetic anhydride* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 ml of *methyl orange-xylene cyanol FF solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 24.13 mg of $C_{12}H_{19}NO_4$. Use the *weight per ml* to calculate the percentage of $C_{12}H_{19}NO_4$, weight in volume.

Choline Theophyllinate

General Notices



$C_{12}H_{21}N_5O_3$ 283.3 *4499-40-5*

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

Preparation

Choline Theophyllinate Tablets

DEFINITION

Choline Theophyllinate is choline 1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7*H*-purin-7-ide. It contains not less than 41.9% and not more than 43.6% of choline, $C_5H_{15}NO_2$, and not less than 61.7% and not more than 65.5% of theophylline, $C_7H_9N_4O_2$, each calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder. It melts between 187° and 192°, Appendix V A.

Very soluble in *water*; soluble in *ethanol (96%)*; very slightly soluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.01M *sodium hydroxide* exhibits a maximum only at 275 nm. The *absorbance* at 275 nm is about 0.83.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of choline theophyllinate *(RS 060)*.

TESTS

Clarity and colour of solution

50 ml of a 10% w/v solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution GY_4*, Appendix IV B, Method I.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel HF_{254}* as the coating substance and a mixture of 95 volumes of *chloroform* and 5 volumes of *ethanol (96%)* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions of the substance being examined in *ethanol (96%)* containing (1) 1.0% w/v and (2) 0.010% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (254 nm). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

For choline

Dissolve 0.6 g in 50 ml of *water* and titrate with 0.05M *sulphuric acid VS*, using *methyl red mixed solution* as indicator, until a violet end point is obtained. Each ml of 0.05M *sulphuric acid VS* is equivalent to 12.12 mg of choline, $C_5H_{15}NO_2$.

For theophylline

To the solution obtained in the Assay for choline, add 25 ml of 0.1M *silver nitrate VS* and warm on a water bath for 15 minutes. Cool in ice for 30 minutes, filter and wash the residue with three 10 ml quantities of *water*. Titrate the combined filtrate and washings with 0.1M *sodium hydroxide VS*. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 18.02 mg of theophylline, $C_7H_8N_4O_2$.

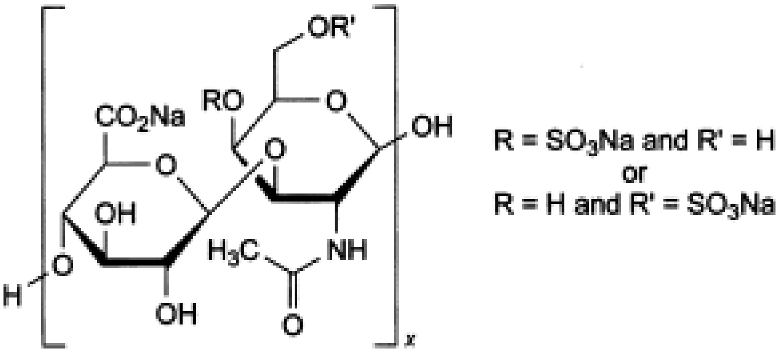
STORAGE

Choline Theophyllinate should be protected from light.

Chondroitin Sulphate Sodium

General Notices

(Ph Eur monograph 2064)



$H_2O(C_{14}H_{19}NNa_2O_{14}S)_x$ *9082-07-9*

Action and use

Acid mucopolysaccharide; treatment of osteoarthritis.

Ph Eur

DEFINITION

Natural copolymer based mainly on the 2 disaccharides: [4)-(β-D-glucopyranosyluronic acid)-(1→3)-[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl 4-sulphate]-(1→] and [4)-(β-D-glucopyranosyluronic acid)-(1→3)-[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl 6-sulphate]-(1→], sodium salt. On complete hydrolysis it liberates D-galactosamine, D-glucuronic acid, acetic acid and sulphuric acid. It is obtained from cartilage of both terrestrial and marine origins. Depending on the animal species of origin, it shows different proportions of 4-sulphate and 6-sulphate groups.

Content

95 per cent to 105 per cent (dried substance).

PRODUCTION

The animals from which chondroitin sulphate sodium is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation *discs of potassium bromide R*.

Comparison For chondroitin sulphate sodium of terrestrial origin use *chondroitin sulphate sodium CRS* and for chondroitin sulphate sodium of marine origine use *chondroitin sulphate sodium (marine) CRS.*

B. Solution S1 (see Tests) gives reaction (b) of sodium *(2.3.1)*.

C. Examine the electropherograms obtained in the test for related substances.

Results The principal band in the electropherogram obtained with the test solution is similar in position to the principal band in the electropherogram obtained with reference solution (a).

TESTS

Solution S1

Dissolve 2.500 g in 50.0 ml of *carbon dioxide-free water R*.

Solution S2

Dilute 1.0 ml of solution S1 to 10.0 ml with *water R*.

pH *(2.2.3)*

5.5 to 7.5 for solution S1.

Specific optical rotation *(2.2.7)*

- 20 to - 30 (terrestrial origin) or - 12 to - 19 (marine origin) (dried substance), determined on solution S1.

Intrinsic viscosity

0.01 m^3/kg to 0.15 m^3/kg.

Test solution (a) Weigh 5.000 g (m_{0p}) of the substance to be examined and add about 80 ml of an 11.7 g/l solution of *sodium chloride R* at room temperature. Dissolve by shaking at room temperature for 30 min. Dilute to 100.0 ml with an 11.7 g/l solution of *sodium chloride R*. Filter through a 0.45 µm filter membrane and discard the first 10 ml. The concentration of test solution (a) is only indicative and must be adjusted after an initial measurement of the viscosity of test solution (a).

Test solution (b) To 15.0 ml of test solution (a) add 5.0 ml of an 11.7 g/l solution of *sodium chloride R.*

Test solution (c) To 10.0 ml of test solution (a) add 10.0 ml of an 11.7 g/l solution of *sodium chloride R*.

Test solution (d) To 5.0 ml of test solution (a) add 15.0 ml of an 11.7 g/l solution of *sodium chloride R.*

Determine the flow-time *(2.2.9)* for an 11.7 g/l solution of *sodium chloride R* (t_0) and the flow times for the 4 test solutions (t_1, t_2, t_3 and t_4), at 25.00 ± 0.03 °C. Use an appropriate suspended level viscometer (specifications: viscometer constant = about 0.005 mm^2/s^2, kinematic viscosity range = 1-5 mm^2/s, internal diameter of tube R = 0.53 mm, volume of bulb C = 5.6 ml, internal diameter of tube N = 2.8-3.2 mm) with a funnel-shaped lower capillary end. Use the same viscometer for all measurements; measure all outflow times in triplicate. The test is not valid unless the results do not differ by more than 0.35 per cent from the mean and if the flow time t_1 is not less than $1.6 \times t_0$ and not more than $1.8 \times t_0$. If this is not the case, adjust the concentration of test solution (a) and repeat the procedure.

Calculation of the relative viscosities Since the densities of the chondroitin sulphate solutions and of the solvent are almost equal, the relative viscosities η_{ri} (being η_{r1}, η_{r2}, η_{r3} and η_{r4}) can be calculated from the ratio of the flow times for the respective solutions t_i (being t_1, t_2, t_3 and t_4) to the flow time of the solvent t_0, but taking into account the kinetic energy correction factor for the capillary (B = 30 800 s^3), as shown below:

$$\frac{t_i - \frac{B}{t_i^2}}{t_0 - \frac{B}{t_0^2}}$$

Calculation of the concentrations Calculate the concentration c_1 (expressed in kg/m^3) of chondroitin sulphate sodium in test solution (a) using the following expression:

$$m_{0p} \times \frac{x}{100} \times \frac{100 - h}{100} \times 10$$

x = percentage content of chondroitin sulphate sodium as determined in the assay,

h = loss on drying as a percentage.

Calculate the concentration c_2 (expressed in kg/m^3) of chondroitin sulphate sodium in test solution (b) using the following expression:

$$c_1 \times 0.75$$

Calculate the concentration c_3 (expressed in kg/m^3) of chondroitin sulphate sodium in test solution (c) using the following expression:

$$c_1 \times 0.50$$

Calculate the concentration c_4 (expressed in kg/m^3) of chondroitin sulphate sodium in test solution (d) using the following expression:

$$c_1 \times 0.25$$

Calculation of the intrinsic viscosity The specific viscosity η_{s_i} of the test solution (being η_{s1}, η_{s2}, η_{s3} and η_{s4}) is calculated from the relative viscosities η_{ri} (being η_{r1}, η_{r2}, η_{r3} and η_{r4}) according to the following expression:

$$n_{r_i} \times 1$$

The intrinsic viscosity $[\eta]$, defined as

$$[\eta] = \lim_{c \to 0} \left(\frac{\eta_s}{c} \right)$$

is calculated by linear least-squares regression analysis using the following equation:

$$\frac{\eta_{si}}{c_i} = c_i \times k_H + [\eta]$$

c_i = concentration of the substance to be examined expressed in kg/m^3;

k_H = Huggins' constant.

Related substances

Electrophoresis *(2.2.31)*.

Buffer solution A (0.1 M barium acetate pH 5.0). Dissolve 25.54 g of *barium acetate R* in 900 ml of *water R*. Adjust to pH 5.0 with *glacial acetic acid R* and dilute to 1000.0 ml with *water R*.

Buffer solution B (1 M barium acetate pH 5.0). Dissolve 255.43 g of *barium acetate R* in 900 ml of *water R.* Adjust to pH 5.0 with *glacial acetic acid R* and dilute to 1000.0 ml with *water R*.

Staining solution Dissolve 1.0 g of *toluidine blue R* and 2.0 g of *sodium chloride R* in 1000 ml of *0.01 M hydrochloric acid*. Filter.

Test solution Prepare a 30 mg/ml solution of the substance to be examined in *water R.*

Reference solution (a) Prepare a 30 mg/ml solution of *chondroitin sulphate sodium CRS* in *water R*.

Reference solution (b) Dilute 2.0 ml of reference solution (a) to 100.0 ml with *water R.*

Reference solution (c) Mix equal volumes of reference solution (b) and *water R*.

Procedure Allow the electrophoresis support to cool the plate to 10 °C. Pre-equilibrate the agarose gel for 1 min in buffer solution A. Remove excess liquid by careful decanting. Dry the gel for approximately 5 min. Place 400 ml of buffer solution B into each of the containers of the electrophoresis equipment. Transfer 1 µl of each solution to the slots of the agarose gel. Pipette a few millilitres of a 50 per cent *V/V* solution of *glycerol R* onto the cooled plate of the electrophoresis equipment and place the gel in the middle of the ceramic plate. Place a wick, saturated with buffer solution B, at the positive and negative sides of the agarose gel. Ensure that there is good contact between the electrophoresis buffer and the agarose gel. Perform the electrophoresis under the following conditions: 75 mA/gel, resulting in a voltage of 100-150 V (maximum 300-400 V) for a gel of about 12 cm × 10 cm. Carry out the electrophoresis for 12 min. Place the gel in a mixture consisting of 10 volumes of *anhydrous ethanol R* and 90 volumes of buffer solution A for 2 min. Carry out the electrophoresis for 20 min. Place the gel in a mixture consisting of 30 volumes of *anhydrous ethanol R* and 70 volumes of buffer solution A for 2 min. Carry out the electrophoresis for 20 min. Stain the gel in the staining solution for 10 min. Destain the gel for 15 min under running tap water followed by 10-15 min with *water R* until the band in the electropherogram obtained with reference solution (c) is visible. Allow the gel to dry.

System suitability:

— the electropherogram obtained with reference solution (c) shows a visible band;

— the band in the electropherogram obtained with reference solution (b) is clearly visible and similar in position to the band in the electropherogram obtained with reference solution (a).

Results Any secondary band in the electropherogram obtained with the test solution is not more intense than the band in the electropherogram obtained with reference solution (b) (2 per cent).

Protein ***(2.5.33, Method 2)***

Maximum 3.0 per cent (dried substance).

Test solution Dilute 1.0 ml of solution S1 to 50.0 ml with *0.1 M sodium hydroxide*.

Reference solutions Dissolve about 0.100 g of *bovine albumin R*, accurately weighed, in *0.1 M sodium hydroxide* and dilute to 50.0 ml with the same solvent. Carry out all additional dilutions using *0.1 M sodium hydroxide.*

Chlorides ***(2.4.4)***

Maximum 0.5 per cent.

Dilute 1 ml of solution S2 to 15 ml with *water R*. Do not add diluted nitric acid. Prepare the standard using 5 ml of *chloride standard solution (5 ppm Cl*) and 10 ml of *water R*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 12.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram. It complies with the tests for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* and Enterobacteria and certain other gram-negative bacteria *(2.6.13)*.

ASSAY

Test solution (a) Weigh 0.100 g (m_1) of the substance to be examined, dissolve in *water R* and dilute to 100.0 ml with the same solvent.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with *water R*.

Reference solution (a) Weigh 0.100 g (m_0) of *chondroitin sulphate sodium CRS*, previously dried as described in the test for loss on drying, dissolve in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water R.*

Titrant solution (a) Weigh 4.000 g of *cetylpyridinium chloride monohydrate R* and dilute to 1000 ml with *water R*.

Titrant solution (b) Weigh 1.000 g of *cetylpyridinium chloride monohydrate* R and dilute to 1000 ml with *water R*.

Perform either visual or photometric titration as follows:

Visual titration Titrate 40.0 ml of reference solution (a) and 40.0 ml of test solution (a) with titrant solution (a). The solution becomes turbid. At the end point, the liquid appears clear, with an almost-white precipitate in suspension. The precipitate is more apparent if 0.1 ml of a 1 per cent solution of *methylene blue R* is added before starting the titration. The precipitated particles are more apparent against the blue background.

Photometric titration Titrate 50.0 ml of reference solution (b) and 50.0 ml of test solution (b) with titrant solution (b). To determine the end point, use a suitable autotitrator equipped with a phototrode at a suitable wavelength (none is critical) in the visible range.

Calculate the percentage content of chondroitin sulphate sodium using the following expression:

$$\frac{v_1 \times m_0}{v_0 \times m_1} \times \frac{100}{100 - h} \times Z$$

v_0 = volume of appropriate titrant solution when titrating the appropriate reference solution, in millilitres,

v_1 = volume of appropriate titrant solution when titrating the appropriate test solution, in millilitres,

h = loss on drying of the substance to be examined, as a percentage,

Z = percentage content of $H_2O(C_{14}H_{19}NNa_2O_{14}S)_x$ in *chondroitin sulphate sodium CRS.*

STORAGE

In an airtight container, protected from light.

LABELLING

The label states the origin of the substance (marine or terrestrial).

Ph Eur

Chorionic Gonadotrophin

General Notices

(Ph Eur monograph 0498)

Action and use

Gonadotrophic hormone.

Preparation

Chorionic Gonadotrophin Injection

Ph Eur

DEFINITION

Chorionic gonadotrophin is a dry preparation of placental glycoproteins which have luteinising activity. The potency is not less than 2500 IU/mg.

PRODUCTION

Chorionic gonadotrophin is extracted from the urine of pregnant women using a suitable fractionation procedure. It is either dried under reduced pressure or freeze-dried.

CHARACTERS

Appearance

White to yellowish-white, amorphous powder.

Solubility

Soluble in water.

IDENTIFICATION

When administered to immature rats as prescribed in the assay, it causes an increase in the mass of the seminal vesicles and of the prostate gland.

TESTS

Water *(2.5.32)*

Maximum 5.0 per cent.

Bacterial endotoxins *(2.6.14)*

Less than 0.02 IU per IU of chorionic gonadotrophin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

The potency of chorionic gonadotrophin is estimated by comparing under given conditions its effect of increasing the mass of the seminal vesicles (or the prostate gland) of immature rats with the same effect of the International Standard of chorionic gonadotrophin or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the International Standard, which consists of a mixture of a freeze-dried extract of chorionic gonadotrophin from the urine of pregnant women with lactose. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Use immature male rats of the same strain, 19 to 28 days old, differing in age by not more than 3 days and having body masses such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to 6 equal groups of at least 5 animals. If sets of 6 litter mates are available, assign one litter mate from each set to each group and mark according to litter.

Choose 3 doses of the reference preparation and 3 doses of the preparation to be examined such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximal response in all the rats. Use doses in geometric progression and as an initial approximation total doses of 4 IU, 8 IU and 16 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation to be examined and of the reference preparation corresponding to the daily doses to be used in sufficient *phosphate-albumin buffered saline pH 7.2 R* such that the daily dose is administered in a volume of about 0.5 ml. Add a suitable antimicrobial preservative such as 4 g/l of phenol or 0.02 g/l of thiomersal. Store the solutions at 5 ± 3 °C.

Inject subcutaneously into each rat the daily dose allocated to its group, on 4 consecutive days at the same time each day. On the fifth day, about 24 h after the last injection, euthanise the rats and remove the seminal vesicles. Remove any extraneous fluid and tissue and weigh the vesicles immediately. Calculate the results by the usual statistical methods, using the mass of the vesicles as the response. (The precision of the assay may be improved by a suitable correction of the organ mass with reference to the body mass of the animal from which it was taken; an analysis of covariance may be used).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) of the estimated potency are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE

In an airtight, tamper-proof container, protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

— the number of International Units per container,

— the potency in International Units per milligram.

Ph Eur

Chymotrypsin

General Notices

(Ph Eur monograph 0476)

Action and use

Proteolytic enzyme.

Ph Eur

DEFINITION

Chymotrypsin is a proteolytic enzyme obtained by the activation of chrymotrypsinogen extracted from the pancreas of beef *(Bos taurus* L.*)*. It has an activity of not less than 5.0 microkatals per milligram. In solution it has maximal enzymic activity at about pH 8; the activity is reversibly inhibited at pH 3, at which pH it is most stable.

PRODUCTION

The animals from which chymotrypsin is derived must fulfil the requirements for the health of animals suitable for human consumption. Furthermore, the tissues used shall not include any specified risk material as defined by any relevant international or, where appropriate, national legislation.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine *(2.6.10)*

Not more than 1 µg (calculated as histamine base) per 5 microkatals of chymotrypsin activity. Before carrying out the test, heat the solution of the substance to be examined on a water-bath for 30 min.

CHARACTERS

A white or almost white, crystalline or amorphous powder, sparingly soluble in water. The amorphous form is hygroscopic.

IDENTIFICATION

A. Dilute 1 ml of solution S (see Tests) to 10 ml with *water R*. In a depression in a white spot plate, mix 0.05 ml of this solution with 0.2 ml of substrate solution. A purple colour develops.

Substrate solution To 24.0 mg of *acetyltyrosine ethyl ester R* add 0.2 ml of *ethanol (96 per cent) R*, and swirl until solution is effected. Add 2.0 ml of *0.067 M phosphate buffer solution pH 7.0 R* and 1 ml of *methyl red mixed solution R* and dilute to 10.0 ml with *water R*.

B. Dilute 0.5 ml of solution S to 5 ml with *water R*. Add 0.10 ml of a 20 g/l solution of *tosylphenylalanylchloromethane R* in *ethanol (96 per cent) R*. Adjust to pH 7.0 and shake for 2 h. In a depression in a white spot plate, mix 0.05 ml of this solution with 0.2 ml of the substrate solution (see Identification test A). No colour develops within 3 min of mixing.

TESTS

Solution S

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)*.

pH *(2.2.3)*

The pH of solution S is 3.0 to 5.0.

Absorbance *(2.2.25)*

Dissolve 30.0 mg in *0.001 M hydrochloric acid* and dilute to 100.0 ml with the same acid. The solution shows an absorption maximum at 281 nm and a minimum at 250 nm. The specific absorbance at the absorption maximum is 18.5 to 22.5 and at the absorption minimum is not greater than 8.

Trypsin

Transfer to a depression in a white spot plate 0.05 ml of *tris(hydroxymethyl)aminomethane buffer solution pH 8.1 R* and 0.1 ml of solution S. Add 0.2 ml of substrate solution (test solution). At the same time and in the same manner, prepare a reference solution using the substance to be examined to which not more than 1 per cent *m/m* of *trypsin BRP* has been added. Start a timer. No colour appears in the test solution within 3-5 min after the addition of the substrate solution. A purple colour is produced in the control solution.

Substrate solution To 98.5 mg of *tosylarginine methyl ester hydrochloride R*, suitable for assaying trypsin, add 5 ml of *tris(hydroxymethyl)aminomethane buffer solution pH 8.1 R* and swirl to dissolve. Add 2.5 ml of *methyl red mixed solution R* and dilute to 25.0 ml with *water R.*

Loss on drying *(2.2.32)*

Not more than 5.0 per cent, determined on 0.100 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 2 h.

ASSAY

The activity of chymotrypsin is determined by comparing the rate at which it hydrolyses *acetyltyrosine ethyl ester R* with the rate at which *chymotrypsin BRP* hydrolyses the same substrate under the same conditions.

Apparatus Use a reaction vessel of about 30 ml capacity provided with:

— a device that will maintain a temperature of 25.0 ± 0.1 °C;

— a stirring device, for example a magnetic stirrer;

— a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration apparatus may be used. For the latter the burette is graduated in 0.005 ml and the pH meter is provided with a wide scale and glass-calomel or glass-silver-silver chloride electrodes.

Test solution Dissolve 25.0 mg of the substance to be examined in *0.001 M hydrochloric acid* and dilute to 250.0 ml with the same acid.

Reference solution Dissolve 25.0 mg of *chymotrypsin BRP* in *0.001 M hydrochloric acid* and dilute to 250.0 ml with the same acid.

Store the solutions at 0-5 °C. Warm 1 ml of each solution to about 25 °C over 15 min and use 50 µl of each solution (corresponding to about 25 nanokatals) for each titration. Carry out the titration in an atmosphere of nitrogen. Transfer 10.0 ml of *0.01 M calcium chloride solution R* to the reaction vessel and, while stirring, add 0.35 ml of *0.2 M acetyltyrosine ethyl ester solution R.* When the temperature is steady at 25.0 ± 0.1 °C (after about 5 min) adjust the pH to exactly 8.0 with *0.02 M sodium hydroxide*. Add 50 µl of the test solution (equivalent to about 5 µg of the substance to be examined) and start a timer. Maintain the pH at 8.0 by the addition of *0.02 M sodium hydroxide*, noting the volume added every 30 s. Calculate the volume of *0.02 M sodium hydroxide* used per second between 30 s and 210 s. Carry out a titration in the same manner using the reference solution and calculate the volume of *0.02 M sodium hydroxide* used per second.

Calculate the activity in microkatals per milligram using the following expression:

$$\frac{m' \times V}{m \times V'} \times A$$

m = mass of the substance to be examined, in milligrams,

m' = mass of *chymotrypsin BRP*, in milligrams,

V = volume of *0.02 M sodium hydroxide* used per second by the test solution,

V' = volume of *0.02 M sodium hydroxide* used per second by the reference solution,

A = activity of *chymotrypsin BRP*, in microkatals per milligram.

STORAGE

In an airtight container at 2 °C to 8 °C, protected from light.

LABELLING

The label states:

— the quantity of chymotrypsin and the total activity in microkatals per container;

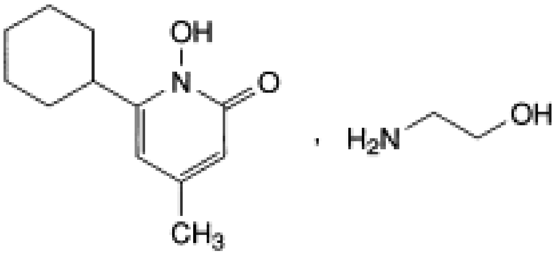
— for the amorphous substance, that it is hygroscopic.

Ph Eur

Ciclopirox Olamine

General Notices

(Ph Eur monograph 1302)



$C_{12}H_{17}NO_2.C_2H_7NO$ 268.4 *41621-49-2*

Action and use

Antifungal.

Ph Eur

DEFINITION

6-Cyclohexyl-1-hydroxy-4-methylpyridin-2(1*H*)-one and 2-aminoethanol.

Content:

- ciclopirox ($C_{12}H_{17}NO_2$; M_r 207.3): 76.0 per cent to 78.5 per cent (dried substance),
- 2-aminoethanol (C_2H_7NO; M_r 61.1): 22.2 per cent to 23.3 per cent (dried substance).

CHARACTERS

Appearance

White or pale yellow, crystalline powder.

Solubility

Sparingly soluble in water, very soluble in alcohol and in methylene chloride, slightly soluble in ethyl acetate, practically insoluble in cyclohexane.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification A.

Second identification B.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ciclopirox olamine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethyl acetate R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in *methanol* R and dilute to 10 ml with the same solvent.

Reference solution Dissolve 25 mg of *ciclopirox olamine CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Before use wash 2 plates by allowing a mixture of 10 volumes of *concentrated ammonia R*, 15 volumes of *water R* and 75 volumes of *ethanol R* to migrate until the solvent front has reached the top of the plate. Allow the plates to dry in air for 5 min.

Mobile phase *concentrated ammonia R, water R, ethanol R* (10:15:75 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air for 10 min.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

Detection B Spray one plate with *ferric chloride solution R3.*

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

Detection C Spray the second plate with *ninhydrin solution R*. Heat at 110 °C until the spots appear.

Results C The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 2.0 g in *methanol R* and dilute to 20 ml with the same solvent.

pH *(2.2.3)*

8.0 to 9.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the operations avoiding exposure to actinic light. All materials which are in direct contact with the substance to be examined, such as column materials, reagents, solvents, etc. should contain only small amounts of extractable metal cations.*

Test solution Dissolve 40.0 mg of the substance to be examined (corresponding to about 30 mg of ciclopirox) in a mixture of 20 µl of *anhydrous acetic acid R*, 2 ml of *acetonitrile R,* and 15 ml of the mobile phase. If necessary, use an ultrasonic bath. Dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 15.0 mg of *ciclopirox impurity A CRS* and 15.0 mg of *ciclopirox impurity B CRS* in a mixture of 1 ml of *acetonitrile R* and 7 ml of the mobile phase. Dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 200.0 ml with a mixture of 1 volume of *acetonitrile R* and 9 volumes of the mobile phase.

Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10.0 ml with a mixture of 1 volume of *acetonitrile R* and 9 volumes of the mobile phase.

Reference solution (d) Mix 5 ml of reference solution (a) with 5 ml of the test solution.

Column:

— *size*: *l* = 80 mm, Ø = 4 mm,

— *stationary phase*: *nitrile silica gel for chromatography R* (5 µm).

In order to ensure desorption of interfering metal ions, a new column is to be rinsed with the rinsing solution over a period of at least 15 h and then with the mobile phase for at least 5 h at a flow rate of 0.2 ml/min.

Rinsing solution A mixture of 1 volume of *anhydrous acetic acid R*, 1 volume of *acetylacetone R*, 500 volumes of *acetonitrile R* and 500 volumes of *water R*.

Mobile phase A mixture of 0.1 volumes of *anhydrous acetic acid R*, 230 volumes of *acetonitrile R* and 770 volumes of a 0.96 g/l solution of *sodium edetate R*. If the retention time of the principal peak in the chromatogram obtained with the test solution is not between 8 min and 11 min adjust the ratio of the 0.96 g/l solution of sodium edetate to acetonitrile accordingly.

Flow rate 0.7 ml/min.

Detection Variable wavelength spectrophotometer capable of operating at 220 nm and 298 nm.

Injection 10 µl; inject the test solution and reference solutions (b), (c) and (d).

Run time 2.5 times the retention time of ciclopirox.

Relative retention With reference to ciclopirox: impurity A = about 0.5; impurity C = about 0.9; impurity B = about 1.3.

System suitability:

— *resolution*: minimum of 2.0 between the peaks corresponding to impurity B and ciclopirox in the chromatogram obtained with reference solution (d),

— *signal-to-noise ratio*: minimum of 10 for the peak corresponding to impurity B in the chromatogram obtained with reference solution (c) at 298 nm,

— *symmetry factor*: 0.8 to 2.0 for the principal peak in the chromatogram obtained with the test solution.

Limits:

— *impurity A at 220 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) at the same wavelength (0.5 per cent),

— *any impurity at 298 nm:* not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) at the same wavelength (0.5 per cent),

— *total at 298 nm apart from impurity B*: not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *disregard limit at 298 nm*: area of the peak due to impurity B in the chromatogram obtained with reference solution (c) at the same wavelength (0.1 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 1.5 per cent, determined on 1.000 g by drying under high vacuum.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

2-Aminoethanol

Dissolve 0.250 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 6.108 mg of C_2H_7NO.

Ciclopirox

Dissolve 0.200 g in 2 ml of *methanol R*. Add 38 ml of *water R*, swirl and titrate immediately with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

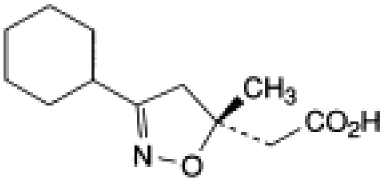
Use *0.1 M sodium hydroxide*, the titre of which has been determined under the conditions prescribed above using 0.100 g of *benzoic acid RV*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 20.73 mg of $C_{12}H_{17}NO_2$.

STORAGE

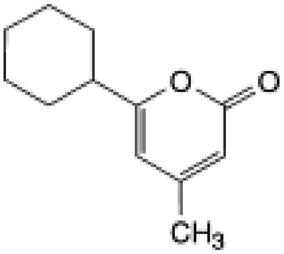
Protected from light.

IMPURITIES

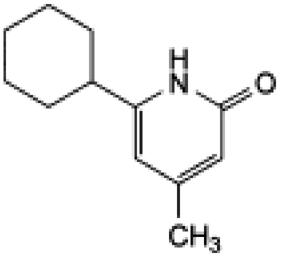


and enantiomer

A. (*RS*)-2-(3-cyclohexyl-5-methyl-4,5-dihydroisoxazol-5-yl)acetic acid,



B. 6-cyclohexyl-4-methyl-2*H*-pyran-2-one,



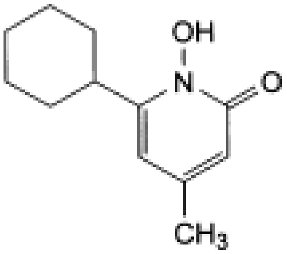
C. 6-cyclohexyl-4-methylpyridin-2(1*H*)-one.

Ph Eur

Ciclopirox

General Notices

(Ph Eur monograph 1407)



$C_{12}H_{17}NO_2$ 207.3 *29342-05-0*

Action and use

Antifungal.

Ph Eur

DEFINITION

6-Cyclohexyl-1-hydroxy-4-methylpyridin-2(1*H*)-one.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Melting point *(2.2.14)*: 140 °C to 145 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ciclopirox CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *ciclopirox CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R*.

Pretreatment Before use predevelop with the mobile phase until the solvent front has migrated to the top of the plate. Allow to dry in air for 5 min.

Mobile phase *concentrated ammonia R, water R, ethanol (96 per cent) R* (10:15:75 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air for 10 min.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

Detection B Spray with a 20 g/l solution of *ferric chloride R* in *anhydrous ethanol R*.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Dissolve 2.0 g in *methanol R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the operations avoiding exposure to actinic light. All materials which are in direct contact with the substance to be examined like column materials, reagents, solvents and others should contain only very low amounts of extractable metal cations.*

Solvent mixture *acetonitrile R*, mobile phase (1:9 *V/V*).

Test solution Dissolve 30.0 mg of the substance to be examined in 15 ml of the solvent mixture. If necessary, use an ultrasonic bath. Dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 15.0 mg of *ciclopirox impurity A CRS* and 15.0 mg of *ciclopirox impurity B CRS* in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 200.0 ml with the solvent mixture.

Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10.0 ml with the solvent mixture.

Reference solution (d) Mix 5 ml of reference solution (a) with 5 ml of the test solution.

Column:

- *size*: *l* = 0.08 m, Ø = 4 mm;
- *stationary phase*: *nitrile silica gel for chromatography R2* (5 µm).

In order to ensure desorption of disruptive metal ions, every new column is to be rinsed with the rinsing solution over a period of not less than 15 h and then with the mobile phase for not less than 5 h at a flow rate of 0.2 ml/min.

Rinsing solution *glacial acetic acid R, acetylacetone R, acetonitrile R, water R* (1:1:500:500 *V/V/V/V*).

Mobile phase Mix 0.1 ml of *glacial acetic acid R*, 230 ml of *acetonitrile R* and 770 ml of a 0.96 g/l solution of *sodium edetate R.*

Flow rate 0.7 ml/min.

Detection Spectrophotometer at 220 nm and at 298 nm.

Injection 10 µl of the test solution and reference solutions (b), (c) and (d); inject the solvent mixture as a blank.

Run time 2.5 times the retention time of ciclopirox.

Retention time Ciclopirox = 8 min to 11 min; if necessary adjust the ratio of the 0.96 g/l solution of sodium edetate to acetonitrile in the mobile phase.

Relative retention With reference to ciclopirox: impurity A = about 0.5; impurity C = about 0.9; impurity B = about 1.3.

System suitability:

- *resolution*: minimum 2.0 between the peaks due to ciclopirox and impurity B in the chromatogram obtained with reference solution (d);
- *signal-to-noise ratio*: minimum 3 for the peak due to impurity B in the chromatogram obtained with reference solution (c) at 298 nm;
- *symmetry factor*: 0.8 to 2.0 for the principal peak in the chromatogram obtained with the test solution.

Limits:

- *impurity A at 220 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurities B, C at 298 nm*: for each impurity, not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *sum of impurities other than B at 298 nm*: not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit at 298 nm*: 0.5 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 1.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C over *diphosphorus pentoxide R.*

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 20 ml of *methanol R*. Add 20 ml of *water R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

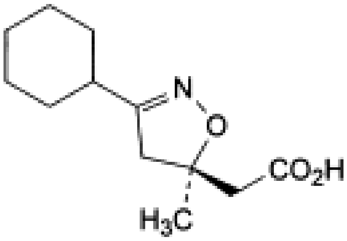
1 ml of *0.1 M sodium hydroxide* is equivalent to 20.73 mg of $C_{12}H_{17}NO_2$.

STORAGE

Protected from light.

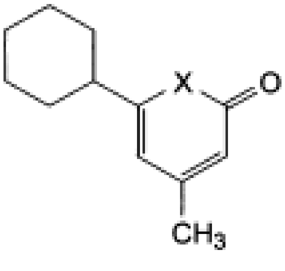
IMPURITIES

Specified impurities *A, B, C.*



and enantiomer

A. (*RS*)-2-(3-cyclohexyl-5-methyl-4,5-dihydroisoxazol-5-yl)acetic acid,



B. X = O: 6-cyclohexyl-4-methyl-2*H*-pyran-2-one,

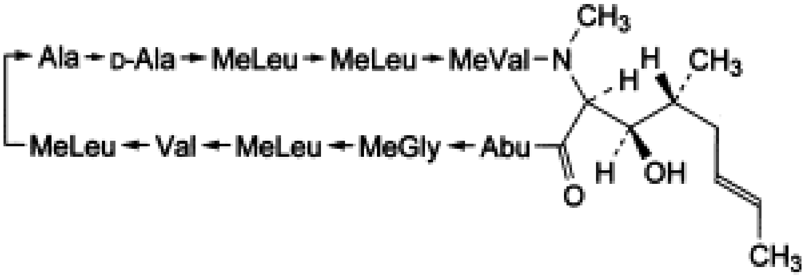
C. X = NH: 6-cyclohexyl-4-methylpyridin-2(1*H*)-one.

Ph Eur

Ciclosporin

General Notices

(Ph Eur monograph 0994)



$C_{62}H_{111}N_{11}O_{12}$ *1203* *59865-13-3*

Action and use

Calcineurin inhibitor; immunosuppressant.

Ph Eur

DEFINITION

Cyclo[[(2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-(methylamino)oct-6-enoyl]-L-2-aminobutanoyl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl].

Substance produced by *Beauveria nivea* (*Tolypocladium inflatum Gams*) or obtained by any other means.

Content

98.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ciclosporin CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5, BY_5 or R_7 *(2.2.2, Method II).*

Dissolve 1.5 g in *anhydrous ethanol R* and dilute to 15 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 185 to - 193 (dried substance).

Dissolve 0.125 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution Dissolve 30.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (a) Dissolve 30.0 mg of *ciclosporin CRS* in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (b) Dilute 2.0 ml of reference solution (a) to 200.0 ml with the solvent mixture.

Reference solution (c) Dissolve the contents of a vial of *ciclosporin for system suitability CRS* in 5.0 ml of the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3-5 µm);

— *temperature*: 80 °C.

The column is connected to the injection port by a steel capillary tube about 1 m long, having an internal diameter of 0.25 mm and maintained at 80 °C.

Mobile phase *phosphoric acid R, 1,1-dimethylethyl methyl ether R, acetonitrile R, water R* (1:50:430:520 *V/V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time 1.7 times the retention time of ciclosporin.

System suitability Reference solution (c):

— *retention time*: ciclosporin = 25 min to 30 min; if necessary, adjust the ratio of acetonitrile to water in the mobile phase;

— *peak-to-valley ratio*: minimum 1.4, where H_p = height above the baseline of the peak due to ciclosporin U and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ciclosporin; if necessary, adjust the ratio of 1,1-dimethylethyl methyl ether to acetonitrile in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

The residue obtained in the test for loss on drying complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.000 g at 60 °C at a pressure not exceeding 15 Pa for 3 h.

Bacterial endotoxins *(2.6.14)*

Less than 0.84 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins. Dissolve 50 mg of the substance to be examined in a mixture of 280 mg of *ethanol (96 per cent) R* and 650 mg of *polyoxyethylated castor oil R* and dilute to the required concentration using water for BET.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

System suitability Reference solutions (a):

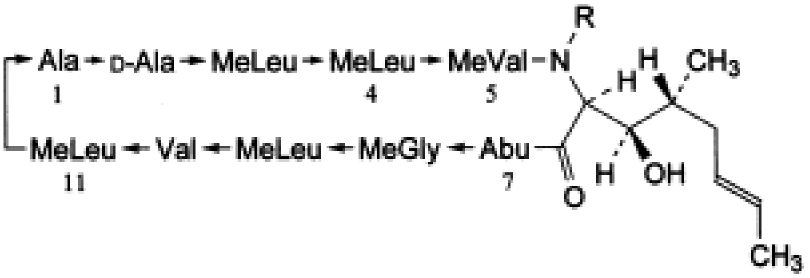
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{62}H_{111}N_{11}O_{12}$ from the declared content of *ciclosporin CRS.*

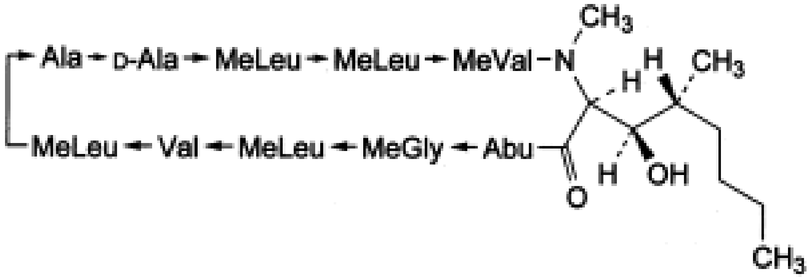
STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES



A. different ciclosporins [difference with ciclosporin (R = CH_3: ciclosporin A)]: ciclosporin B [7-L-Ala]; ciclosporin C [7-L-Thr]; ciclosporin D [7-L-Val]; ciclosporin E [5-L-Val]; ciclosporin G [7-(L-2-aminopentanoyl)]; ciclosporin H [5-D-MeVal]; ciclosporin L [R = H]; ciclosporin T [4-L-Leu]; ciclosporin U [11-L-Leu]; ciclosporin V [1-L-Abu],



B. [6-[(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)octanoic acid]]ciclosporin A,

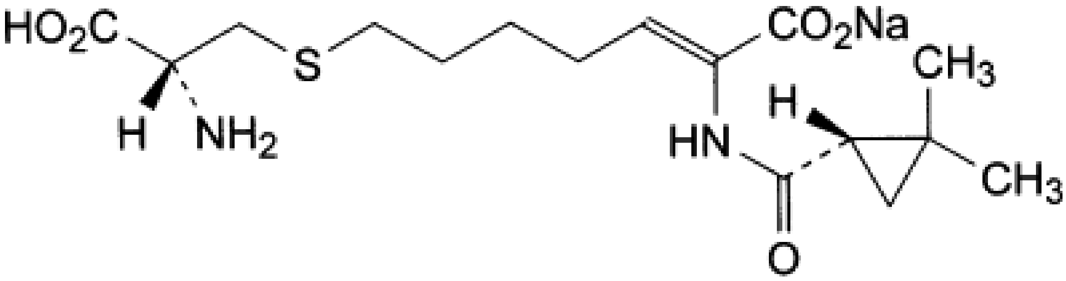
C. isociclosporin A.

Ph Eur

Cilastatin Sodium

General Notices

(Ph Eur monograph 1408)



$C_{16}H_{25}N_2NaO_5S$ 380.4 *81129-83-1*

Action and use

Dehydropeptidase-I inhibitor; inhibition of the renal metabolism of imipenem.

Ph Eur

DEFINITION

Sodium (*Z*)-7-[[(*R*)-2-amino-2-carboxyethyl]sulphanyl]-2-[[[(1*S*)-2,2-dimethylcyclopropyl]carbonyl]amino]hept-2-enoate.

Content

98.0 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or light yellow amorphous, hygroscopic powder.

Solubility

Very soluble in water and in methanol, slightly soluble in anhydrous ethanol, very slightly soluble in dimethyl sulphoxide, practically insoluble in acetone and in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *cilastatin sodium CRS.*

C. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)

6.5 to 7.5 for solution S.

Specific optical rotation (*2.2.7*)

+ 41.5 to + 44.5 (anhydrous substance).

Dissolve 0.250 g in a mixture of 1 volume of *hydrochloric acid R* and 120 volumes of *methanol R*, then dilute to 25.0 ml with the same mixture of solvents.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 32.0 mg of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with *water R*. Dilute 5.0 ml of this solution to 100.0 ml with *water R.*

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *water R.* Dilute 2.0 ml of this solution to 20.0 ml with *water R.*

Reference solution (c) Dissolve 16 mg of the substance to be examined in *dilute hydrogen peroxide solution R* and dilute to 10.0 ml with the same solution. Allow to stand for 30 min. Dilute 1 ml of this solution to 100 ml with *water R*.

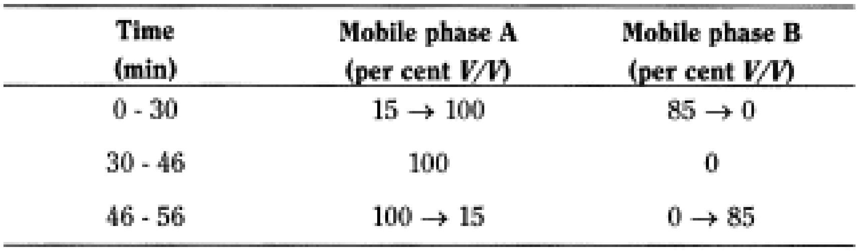
Reference solution (d) Dissolve 32 mg of *mesityl oxide R* (impurity D) in 100 ml of *water R*. Dilute 1 ml of this solution to 50 ml with *water R*.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 50 °C.

Mobile phase:

- *mobile phase A*: mix 300 volumes of *acetonitrile R1* and 700 volumes of a 0.1 per cent *V/V* solution of *phosphoric acid R* in *water R;*
- *mobile phase B*: 0.1 per cent *V/V* solution of *phosphoric acid R* in *water R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	15 → 100	85 → 0
30 - 46	100	0
46 - 56	100 → 15	0 → 85

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

System suitability:

— the chromatogram obtained with reference solution (c) shows 3 principal peaks: the first 2 peaks (impurity A) may elute without being completely resolved;

— *mass distribution ratio*: minimum 10 for the peak due to cilastatin (3^{rd} peak) in the chromatogram obtained with reference solution (c);

— *signal-to-noise ratio*: minimum 5.0 for the principal peak in the chromatogram obtained with reference solution (a).

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard any peak corresponding to the peak due to impurity D in the chromatogram obtained with reference solution (d).

Impurity D, acetone and methanol

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 0.5 ml of *propanol R* in *water R* and dilute to 1000 ml with the same solvent.

Test solution Dissolve 0.200 g of the substance to be examined in *water R*, add 2.0 ml of the internal standard solution and dilute to 10.0 ml with *water R.*

Reference solution Dissolve 2.0 ml of *acetone R*, 0.5 ml of *methanol R* and 0.5 ml of *mesityl oxide R* (impurity D) in *water R* and dilute to 1000 ml with the same solvent. To 2.0 ml of this solution add 2.0 ml of the internal standard solution and dilute to 10.0 ml with *water R.* This solution contains 316 µg of acetone, 79 µg of methanol and 86 µg of impurity D per millilitre.

Column:

— *material*: fused silica;

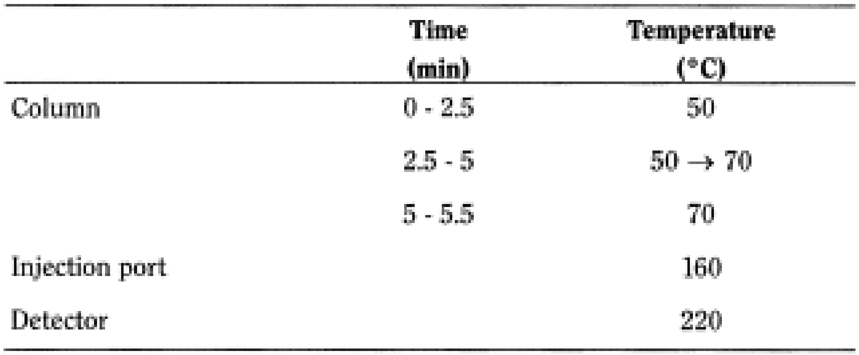
— *size: l* = 30 m, Ø = 0.53 mm;

— *stationary phase*: *macrogol 20 000 R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R.*

Flow rate 9 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2.5	50
	2.5 - 5	50 → 70
	5 - 5.5	70
Injection port		160
Detector		220

Detection Flame ionisation.

Injection 1 µl.

Calculate the percentage contents of acetone, methanol and impurity D using the following expression:

$$\left(\frac{C}{W}\right) \times \left(\frac{R_u}{R_s}\right)$$

C = concentration of the solvent in the reference solution, in µg/ml

W = quantity of cilastatin sodium in the test solution, in milligrams

R_u = ratio of the area of the solvent peak to the area of the propanol peak in the chromatogram obtained with the test solution

R_s = ratio of the area of the solvent peak to the area of the propanol peak in the chromatogram obtained with the reference solution.

Limits:

- *acetone*: maximum 1.0 per cent *m/m*;
- *methanol*: maximum 0.5 per cent *m/m*;
- *impurity D*: maximum 0.4 per cent *m/m.*

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2.0 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 2.0 per cent, determined on 0.50 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.17 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.300 g in 30 ml of *methanol R* and add 5 ml of *water R*. Add *0.1 M hydrochloric acid* to a pH of about 3.0. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. 3 jumps of potential are observed. Titrate to the 3rd equivalence point.

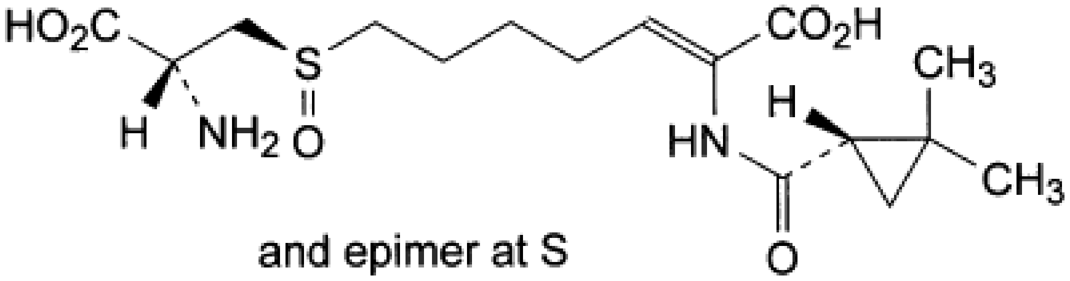
1 ml of *0.1 M sodium hydroxide* is equivalent to 19.02 mg of $C_{16}H_{25}N_2NaO_5S$.

STORAGE

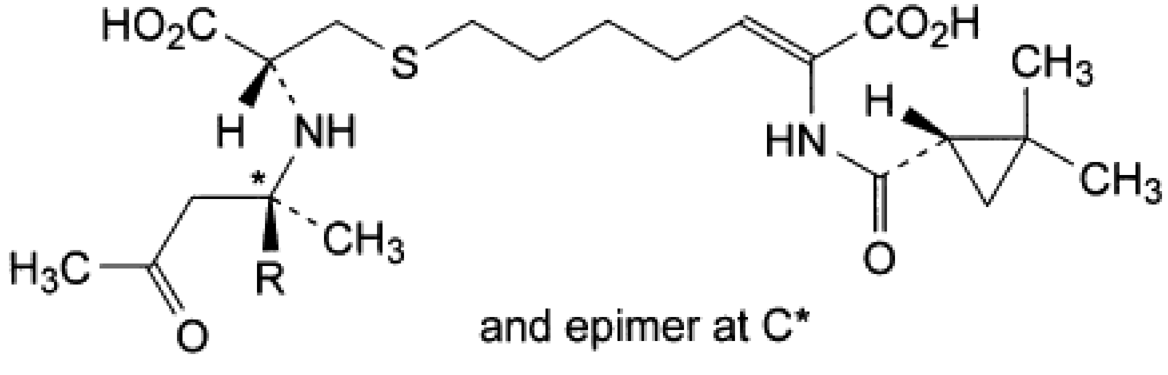
In an airtight container, at a temperature not exceeding 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities A, B, C, D.

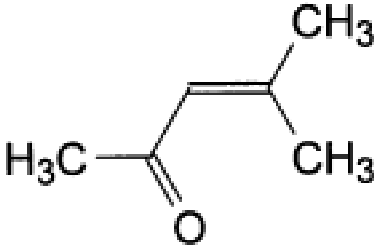


A. (*Z*)-7-[(*RS*)-[(*R*)-2-amino-2-carboxyethyl]sulphinyl]-2-[[[(1*S*)-2,2-dimethylcyclopropyl] carbonyl]amino]hept-2-enoic acid,



B. R = H: (*Z*)-7-[[(*R*)-2-[[(1*RS*)-1-methyl-3-oxobutyl]amino]2-carboxyethyl]sulphanyl]-2-[[[(1*S*)-2,2-dimethylcyclopropyl]carbonyl]amino]hept-2-enoic acid,

C. R = CH_3: (*Z*)-7-[[(*R*)-2-[(1,1-dimethyl-3-oxobutyl)amino]2-carboxyethyl]sulphanyl]-2-[[[(1*S*)-2,2-dimethylcyclopropyl]carbonyl]amino]hept-2-enoic acid,



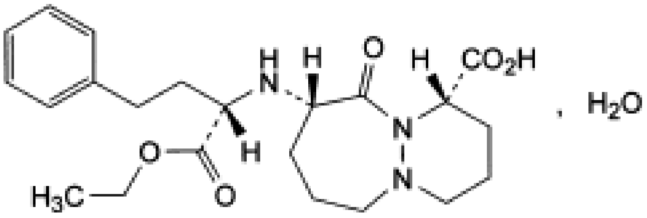
D. 4-methylpent-3-en-2-one (mesityl oxide).

Ph Eur

Cilazapril

General Notices

(Ph Eur monograph 1499)



$C_{22}H_{31}N_3O_5,H_2O$ 435.5   *92077-78-6*

Action and use

Angiotensin converting enzyme inhibitor.

Ph Eur

DEFINITION

(1*S*,9*S*)-9-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxooctahydro-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylic acid monohydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in methanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison   cilazapril CRS.

B. Specific optical rotation (see Tests).

TESTS

Specific optical rotation *(2.2.7)*

- 383 to - 399 (anhydrous substance).

Dissolve 0.200 g in *0.067 M phosphate buffer solution pH 7.0 R*, with the aid of ultrasound if necessary, and dilute to 50.0 ml with the same buffer solution. Carry out the determination at 365 nm.

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *cilazapril impurity A CRS in methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *cilazapril impurity A CRS* and 5 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase glacial acetic acid R, water R, hexane R, methanol R, ethyl acetate R (5:5:15:15:60 *V/V/V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In a current of cold air for 10 min.

Detection Spray with a freshly prepared mixture of 1 volume of *potassium iodobismuthate solution R* and 10 volumes of *dilute acetic acid R* and then with *dilute hydrogen peroxide solution R*.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *cilazapril impurity D CRS* in the test solution and dilute to 10.0 ml with the test solution.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 10 volumes of *triethylamine R* and 750 *volumes of water R*, adjust to pH 2.30 with *phosphoric acid R,* and add 200 volumes of *tetrahydrofuran R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl.

Run time Twice the retention time of cilazapril; when impurity A is present, it may be necessary to continue the chromatography until it is eluted.

Relative retention With reference to cilazapril: impurity B = about 0.6; impurity D = about 0.9; impurity C = about 1.6; impurity A = 4 to 5.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to impurity D and cilazapril;

— *symmetry factor*: maximum 3.0 for the peak due to cilazapril.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity D*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity C*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak due to impurity A.

Water *(2.5.12)*

3.5 per cent to 5.0 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 10 ml of *anhydrous ethanol R* and add 50 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

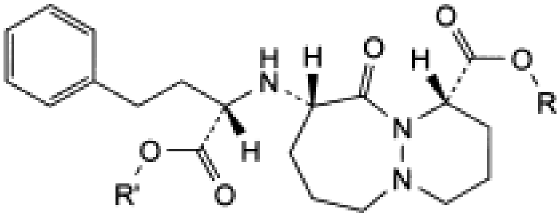
1 ml of *0.1 M sodium hydroxide* is equivalent to 41.75 mg of $C_{22}H_{31}N_3O_5$.

STORAGE

Protected from light.

IMPURITIES

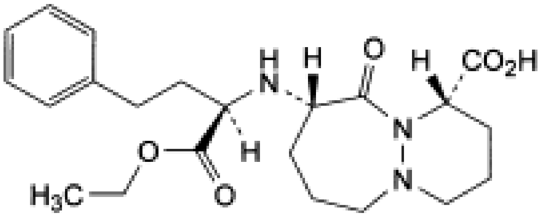
Specified impurities *A, B, C, D.*



A. R = $C(CH_3)_3$, R′ = C_2H_5: 1,1-dimethylethyl (1*S*,9*S*)-9-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxooctahydro-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylate,

B. R = R′ = H: (1*S*,9*S*)-9-[[(*S*)-1-carboxy-3-phenylpropyl]amino]-10-oxooctahydro-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylic acid,

C. R = R′ = C_2H_5: ethyl (1*S*,9*S*)-9-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxooctahydro-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylate,



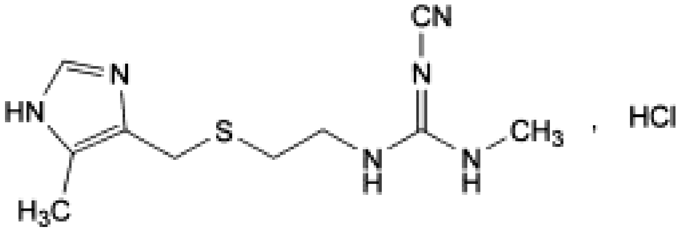
D (1*S*,9*S*)-9-[[(*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxooctahydro-6*H*-pyridazino-[1,2-*a*][1,2]diazepine-1-carboxylic acid.

Ph Eur

Cimetidine Hydrochloride

General Notices

(Ph Eur monograph 1500)



$C_{10}H_{16}N_6S,HCl$ 288.8 *70059-30-2*

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

Ph Eur

DEFINITION

Cimetidine hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of 2-cyano-1-methyl-3-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl] sulphanyl]ethyl]guanidine hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water, sparingly soluble in ethanol.

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. Dissolve 70 mg in *0.2 M sulphuric acid* and dilute to 100.0 ml with the same acid. Dilute 2.0 ml of the solution to 100.0 ml with *0.2 M sulphuric acid*. Measure the absorbance *(2.2.25)* at the absorption maximum at 218 nm. The specific absorbance at the maximum is 650 to 705.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cimetidine hydrochloride CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (d).

D. Dissolve about 1 mg in a mixture of 1 ml of *ethanol R* and 5 ml of a freshly prepared 20 g/l solution of *citric acid R* in *acetic anhydride R.* Heat on a water-bath for 10 min to 15 min. A reddish-violet colour develops.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 3.0 g in 12 ml of *1 M hydrochloric acid* and dilute to 20 ml with *water R*. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

pH *(2.2.3)*

Dissolve 100 mg in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent. The pH of the solution is 4.0 to 5.0.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a TLC *silica gel* GF_{254} *plate R*.

Test solution (a) Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dilute 2 ml of test solution (b) to 100 ml with *methanol R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *methanol R*.

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with *methanol R*.

Reference solution (d) Dissolve 10 mg of *cimetidine hydrochloride CRS* in 2 ml of *methanol R*.

A. Apply to the plate 4 µl of each solution. Allow the plate to stand for 15 min in a chromatographic tank saturated with vapour from the mobile phase, which consists of a mixture of 15 volumes of *concentrated ammonia R*, 20 volumes of *methanol R* and 65 volumes of *ethyl acetate R*, and develop immediately over a path of 15 cm using the same mixture of solvents. Dry the plate in a stream of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.2 per cent) and at most two such spots are more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

B. Apply to the plate 4 µl of each solution. Develop over a path of 15 cm using a mixture of 8 volumes of *concentrated ammonia R*, 8 volumes of *methanol R* and 84 volumes of *ethyl acetate R*. Dry the plate in a stream of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.2 per cent) and at most two such spots are more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

Heavy metals ***(2.4.8)***

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

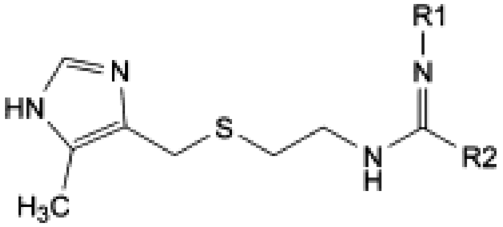
Dissolve 0.200 g of the substance to be examined in a mixture of 5 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 28.88 mg of $C_{10}H_{17}ClN_6S$.

STORAGE

Store in an airtight container, protected from light.

IMPURITIES

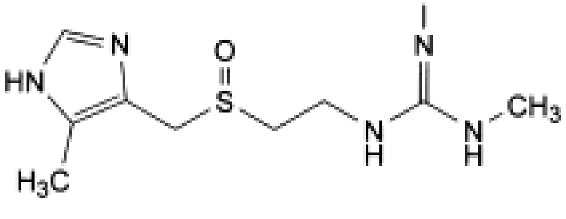


A. R1 = CN, R2 = SCH_3: 3-cyano-2-methyl-1-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]sulphanyl]ethyl]isothiourea,

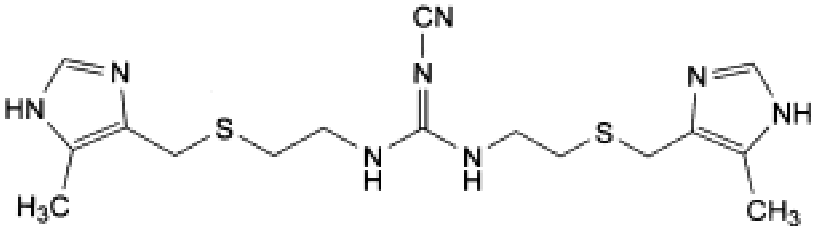
B. R1 = CN, R2 = OCH_3: 3-cyano-2-methyl-1-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]sulphanyl]ethyl]isourea,

C. R1 = $CONH_2$, R2 = $NHCH_3$: 1-[(methylamino)[[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]sulphanyl]ethyl]amino]methylene]urea,

D. R1 = H, R2 = $NHCH_3$: 1-methyl-3-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]sulphanyl]ethyl]guanidine,



E. 2-cyano-1-methyl-3-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]sulphinyl]ethyl]guanidine,



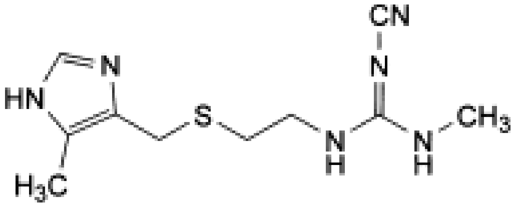
F. 2-cyano-1,3-bis[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]-sulphanyl]ethyl]guanidine.

Ph Eur

Cimetidine

General Notices

(Ph Eur monograph 0756)



$C_{10}H_{16}N_6S$ 252.3 *51481-61-9*

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

Preparations

Cimetidine Injection

Cimetidine Oral Solution

Cimetidine Oral Suspension

Cimetidine Tablets

Ph Eur

DEFINITION

Cimetidine contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of 2-cyano-1-methyl-3-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]sulphanyl]ethyl]guanidine, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, slightly soluble in water, soluble in alcohol, practically insoluble in methylene chloride. It dissolves in dilute mineral acids.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 139 °C to 144 °C. If necessary, dissolve the substance to be examined in *2-propanol R*, evaporate to dryness and determine the melting point again.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cimetidine CRS*. If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *2-propanol R*, evaporate to dryness and record the spectra again.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (d).

D. Dissolve about 1 mg in a mixture of 1 ml of *ethanol R* and 5 ml of a freshly prepared 20 g/l solution of *citric acid R* in *acetic anhydride R*. Heat in a water-bath for 10 min to 15 min. A reddish-violet colour develops.

TESTS

Appearance of solution

Dissolve 3.0 g in 12 ml of *1 M hydrochloric acid* and dilute to 20 ml with *water R*. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R.*

Reference solution (a) Dilute 1 ml of test solution (a) to 100 ml with *methanol R.* Dilute 20 ml of this solution to 100 ml with *methanol R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *methanol R.*

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with *methanol R.*

Reference solution (d) Dissolve 10 mg of *cimetidine CRS* in 2 ml of *methanol R*.

A. Apply separately to the plate 4 µl of each solution. Allow the plate to stand for 15 min in the chromatographic tank saturated with vapour from the mobile phase which consists of a mixture of 15 volumes of *concentrated ammonia R*, 20 volumes of *methanol R* and 65 volumes of *ethyl acetate R* and develop immediately over a path of 15 cm using the same mixture of solvents. Dry the plate in a stream of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.2 per cent) and not more than two such spots are more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

B. Apply separately to the plate 4 µl of each solution. Develop over a path of 15 cm using a mixture of 8 volumes of *concentrated ammonia* R, 8 volumes of *methanol R* and 84 volumes of *ethyl acetate R*. Dry the plate in a stream of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution (a), apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.2 per cent) and not more than two such spots are more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

Heavy metals ***(2.4.8)***

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 25.23 mg of $C_{10}H_{16}N_6S$.

STORAGE

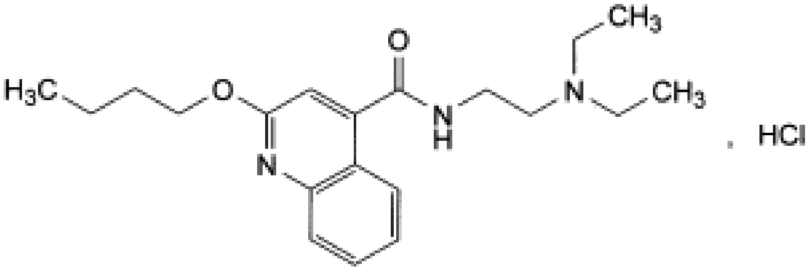
Store in an airtight container, protected from light.

Ph Eur

Cinchocaine Hydrochloride

General Notices

(Ph Eur monograph 1088)



$C_{20}H_{29}N_3O_2$, HCl   379.9   *61-12-1*

Action and use

Local anaesthetic.

Ph Eur

DEFINITION

Cinchocaine hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 2-butoxy-*N*-[2-(diethylamino)ethyl]quinoline-4-carboxamide hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, hygroscopic, very soluble in water, freely soluble in acetone, in alcohol and in methylene chloride. It agglomerates very easily.

IDENTIFICATION

First identification   *B, E.*

Second identification   *A, C, D, E.*

A. Dissolve 60.0 mg in *1 M hydrochloric acid* and dilute to 100 ml with the same acid. Dilute 2 ml of the solution to 100 ml with *1 M hydrochloric a*cid. Examined between 220 nm and 350 nm *(2.2.25*), the solution shows two absorption maxima, at 246 nm and 319 nm. The ratio of the absorbance measured at 246 nm to that measured at 319 nm is 2.7 to 3.0.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *cinchocaine hydrochloride CRS*. Examine the substances prepared as discs using *potassium chloride R*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.5 g in 5 ml of *water R*. Add 1 ml of *dilute ammonia R2*. A white precipitate is formed. Filter, wash the precipitate with five quantities, each of 10 ml, of *water R* and dry in a desiccator. It melts at 64 °C to 66 °C *(2.2.14).*

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R*, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH ***(2.2.3)***

Dilute 10 ml of solution S to 50 ml with *carbon dioxide-free water R*. The pH of the solution is 5.0 to 6.0.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R.*

Reference solution (a) Dissolve 20 mg of *cinchocaine hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 20 ml with *methanol R*.

Reference solution (c) Dilute 1 ml of test solution (b) to 50 ml with *methanol R*.

Reference solution (d) Dissolve 20 mg of *benzocaine CRS* in *methanol R* and dilute to 5 ml with the same solvent. Dilute 1 ml of the solution and 1 ml of reference solution (a) to 20 ml with *methanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *ammonia R*, 5 volumes of *methanol R*, 30 volumes of *acetone R* and 50 volumes of *toluene R*. Dry the plate in a current of warm air for 15 min. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots.

Heavy metals ***(2.4.8)***

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 2.0 per cent, determined on 0.500 g by drying *in vacuo* at 60 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

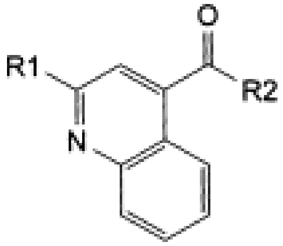
Dissolve 0.300 g in a mixture of 15.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 37.99 mg of $C_{20}H_{30}ClN_3O_2$.

STORAGE

Store in an airtight container, protected from light.

IMPURITIES



A R1 = Cl, R2 = NH-$[CH_2]_2$-$N(C_2H_5)_2$: 2-chloro-*N*-[2-(diethylamino)ethyl]quinoline-4-carboxamide,

B. R1 = R2 = OH: 2-hydroxyquinoline-4-carboxylic acid,

C. R1 = OH, R2 = NH-$[CH_2]_2$-$N(C_2H_5)_2$: *N*-[2-(diethylamino)ethyl]-2-hydroxyquinoline-4-carboxamide,

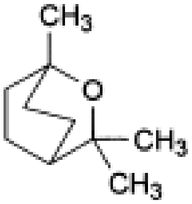
D. R1 = O-$[CH_2]_3$-CH_3, R2 = OH: 2-butoxyquinoline-4-carboxylic acid.

Ph Eur

Cineole

General Notices

(Ph Eur monograph 1973)



$C_{10}H_{18}O$ 154.3 *470-82-6*

Ph Eur

DEFINITION

1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane.

CHARACTERS

Appearance

Clear colourless liquid.

Solubility

Practically insoluble in water, miscible with alcohol and with methylene chloride.

It solidifies at about 0.5 °C.

IDENTIFICATION

A. It complies with the test for refractive index (see Tests).

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dilute 1 ml of solution S (see Tests) to 25 ml with *alcohol R*.

Reference solution Mix 80 mg of *cineole CRS* with *alcohol R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase ethyl acetate R, toluene R (10:90 *V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Drying In a current of cold air.

Detection Spray with *anisaldehyde solution R*, heat at 100-105 °C for 5 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 0.1 ml add 4 ml of *sulphuric acid R*. An orange-red colour develops. Add 0.2 ml of *formaldehyde solution R*. The colour changes to deep brown.

TESTS

Solution S

Dilute 2.00 g to 10.0 ml with *alcohol R*.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method I)*.

Chiral impurities

The optical rotation *(2.2.7)* of solution S is - 0.10° to + 0.10°.

Refractive index *(2.2.6)*

1.456 to 1.460.

Related substances

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 1.0 g of *camphor R* in *heptane R* and dilute to 200 ml with the same solvent.

Test solution (a) Dissolve 2.5 g of the substance to be examined in *heptane R* and dilute to 25.0 ml with the same solvent.

Test solution (b) Dissolve 2.5 g of the substance to be examined in *heptane R*, add 5.0 ml of the internal standard solution and dilute to 25.0 ml with *heptane R*.

Reference solution (a) To 2.0 ml of test solution (a) add 20.0 ml of the internal standard solution and dilute to 100.0 ml with *heptane R*.

Reference solution (b) Dissolve 50 mg of *1,4-cineole R* and 50 mg of the substance to be examined in *heptane R* and dilute to 50.0 ml with the same solvent.

Column:

— *size*: *l* = 30 m, Ø = 0.25 mm,

— *stationary phase: macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Linear velocity 45 cm/s.

Split-ratio 1:70.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	50
	10 - 35	50 → 100
	35 - 45	100 → 200
	45 - 55	200
Injection port		220
Detector		250

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

— *resolution*: minimum 10 between the peaks due to impurity A and to cineole.

Limits:

— *total:* calculate the ratio (*R*) of the area of the peak due to cineole to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (a); from the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to internal standard: this ratio is not greater than *R* (2 per cent),

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Residue on evaporation

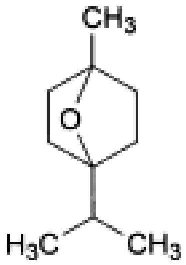
Maximum 0.1 per cent.

To 2.0 g add 5 ml of *water R*, evaporate to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs a maximum of 2 mg.

STORAGE

In an airtight container , protected from light.

IMPURITIES

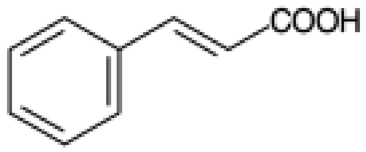


A. 1-methyl-4-(1-methylethyl)-7-oxabicyclo[2.2.1]heptane (1,4-cineole).

Ph Eur

Cinnamic Acid

General Notices



$C_9H_8O_2$ 148.2 *621-82-9*

Action and use

Antimicrobial preservative; excipient.

DEFINITION

Cinnamic Acid is (*E*)-3-phenylprop-2-enoic acid. It contains not less than 99.0% and not more than 100.5% of $C_9H_8O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

Colourless crystals.

Very slightly soluble in *water*; freely soluble in *ethanol (96%)*; soluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cinnamic acid *(RS 062)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.0010% w/v solution in 0.1M *sodium hydroxide* exhibits a maximum only at 267 nm. The *absorbance* at 267 nm is about 1.4.

TESTS

Melting point

132° to 134°, Appendix V A.

Ethanol-insoluble matter

A 10% w/v solution in *ethanol (96%)* is *clear*, Appendix IV A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 90 volumes of *toluene* and 10 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions of the substance being examined in *methanol* containing (1) 5.0% w/v and (2) 0.025% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (5%).

Loss on drying

When dried to constant weight at 60° at a pressure not exceeding 0.7 kPa, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

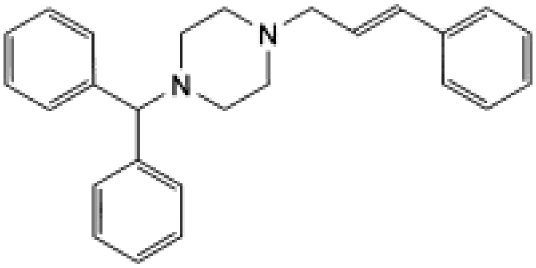
ASSAY

Dissolve 0.5 g in 15 ml of *ethanol (96%)* previously neutralised to *phenol red solution* and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 14.82 mg of $C_9H_8O_2$.

Cinnarizine

General Notices

(Ph Eur monograph 0816)



$C_{26}H_{28}N_2$ 368.5 *298-57-7*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

(*E*)-1-(Diphenylmethyl)-4-(3-phenylprop-2-enyl)piperazine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, slightly soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point *(2.2.14)*

118 °C to 122 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *cinnarizine CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *cinnarizine CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *cinnarizine CRS* and 10 mg of *flunarizine dihydrochloride CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R*.

Mobile phase *1 M sodium chloride*, *methanol R*, *acetone R* (20:30:50 *V/V/V*).

Application 5 µl.

Development In an unsaturated tank, over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.2 g of *anhydrous citric acid R* in 10 ml of *acetic anhydride R* in a water-bath at 80 °C and maintain the temperature of the water-bath at 80 °C for 10 min. Add about 20 mg of the substance to be examined. A purple colour develops.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 0.5 g in *methylene chloride R* and dilute to 20 ml with the same solvent.

Acidity or alkalinity

Suspend 0.5 g in 15 ml of *water R.* Boil for 2 min. Cool and filter. Dilute the filtrate to 20 ml with *carbon dioxide-free water R*. To 10 ml of this solution add 0.1 ml of *phenolphthalein solution R* and 0.25 ml of *0.01 M sodium hydroxide*. The solution is pink. To 10 ml of the solution add 0.1 ml of *methyl red solution R* and 0.25 ml of *0.01 M hydrochloric acid*. The solution is red.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 12.5 mg of *cinnarizine CRS* and 15.0 mg of *flunarizine dihydrochloride CRS* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 20.0 ml with *methanol R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol R*.

Column:

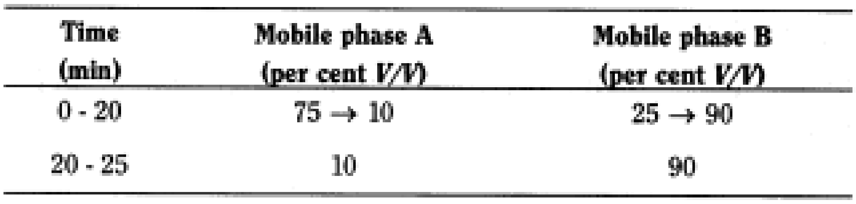
— *size*: *l* = 0.1 m, Ø = 4.0 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 10 g/l solution of *ammonium acetate R*;

— *mobile phase B*: 0.2 per cent *V/V* solution of *glacial acetic acid R* in *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	75 → 10	25 → 90
20 - 25	10	90

If necessary, adjust the concentration of glacial acetic acid in mobile phase B to obtain a horizontal baseline in the chromatogram.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Equilibration With the mobile phase at the initial composition for at least 30 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Cinnarizine = about 11 min; flunarizine = about 11.5 min.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to cinnarizine and flunarizine; if necessary, adjust the time programme for the gradient elution.

Limits:

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*. Add *dilute hydrochloric acid R* until dissolution is complete. Dilute to 20 ml with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*. 12 ml of the solution complies with test B. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb*) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 60 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *ethyl methyl ketone R*. Titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution R* as indicator.

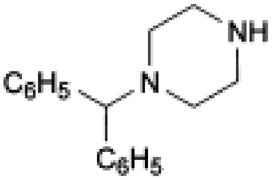
1 ml of *0.1 M perchloric acid* is equivalent to 18.43 mg of $C_{26}H_{28}N_2$.

STORAGE

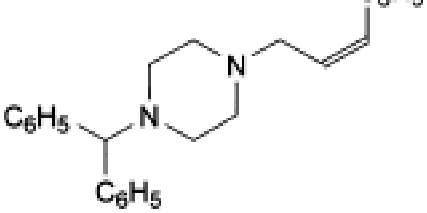
Protected from light.

IMPURITIES

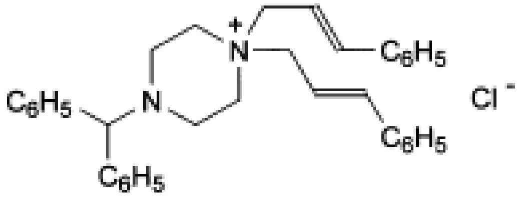
Specified impurities A, B, C, D, E.



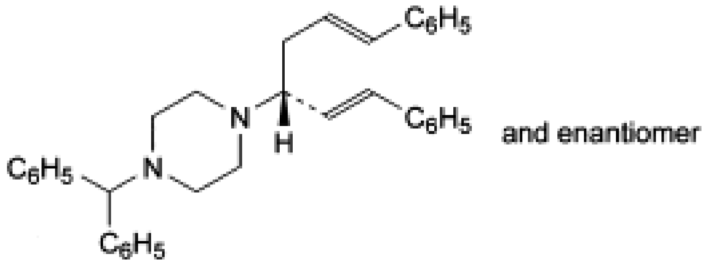
A. 1-(diphenylmethyl)piperazine,



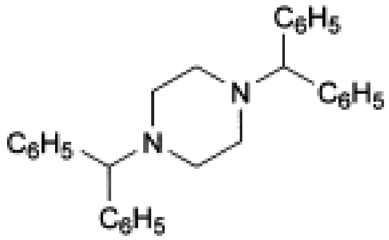
B. (*Z*)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl)piperazine,



C. (4-(diphenylmethyl)-1,1-bis[(*E*)-3-phenylprop-2-enyl]piperazinium chloride,



D. 1-(diphenylmethyl)-4-[(1*RS*,3*E*)-4-phenyl-1-[(*E*)-2-phenylethenyl]but-3-enyl]piperazine,



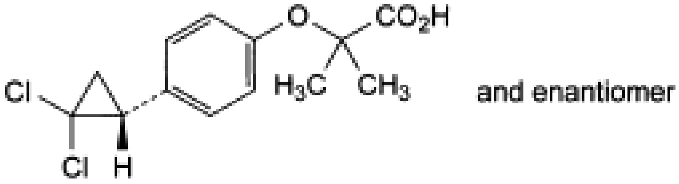
E. 1,4-bis(diphenylmethyl)piperazine.

Ph Eur

Ciprofibrate

General Notices

(Ph Eur monograph 2013)



$C_{13}H_{14}Cl_2O_3$ 289.2 *52214-84-3*

Action and use

Fibrate; lipid-regulating drug.

Ph Eur

DEFINITION

2-[4-[(1*RS*)-2,2-Dichlorocyclopropyl]phenoxy]-2-methylpropanoic acid.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol, soluble in toluene.

mp

About 115 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison ciprofibrate CRS.

TESTS

Appearance of solution

The solution is clear *(2.2.1*) and not more intensely coloured than reference solution BY$_4$ *(2.2.2, Method II*).

Dissolve 1.0 g in *anhydrous ethanol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.125 g of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 50 ml with the same mixture of solvents.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*.

Reference solution (b) Dissolve the contents of a vial of *ciprofibrate for system suitability CRS* in 2.0 ml of a mixture of equal volumes of *acetonitrile R* and *water R*.

Column:

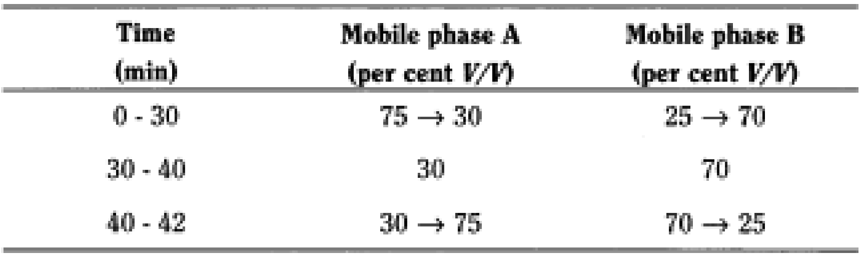
— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: 1.36 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 2.2 with *phosphoric acid R,*

— *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	75 → 30	25 → 70
30 - 40	30	70
40 - 42	30 → 75	70 → 25

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *ciprofibrate for system suitability CRS* to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to ciprofibrate (retention time = about 18 min): impurity A = about 0.7; impurity B = about 0.8; impurity C = about 0.95; impurity D = about 1.3; impurity E = about 1.5.

System suitability Reference solution (b):

— *resolution*: baseline separation between the peaks due to impurity C and ciprofibrate.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 2.3,

— *impurities A, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *impurity E*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent),

— *any other impurity:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total of other impurities*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides *(2.4.4)*

Maximum 350 ppm.

To 0.190 g add 20 ml of *water* R and treat in an ultrasonic bath for 8 min. Filter. 15 ml of the filtrate complies with the test.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 20 ml of *water R* and 40 ml of *anhydrous ethanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

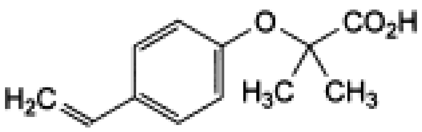
1 ml of *0.1 M sodium hydroxide* is equivalent to 28.92 mg of $C_{13}H_{14}Cl_2O_3$.

STORAGE

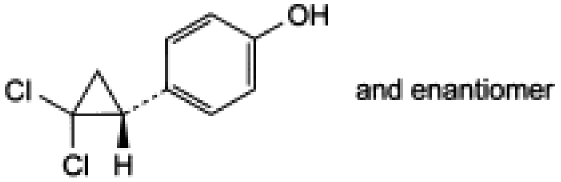
In an airtight container , protected from light.

IMPURITIES

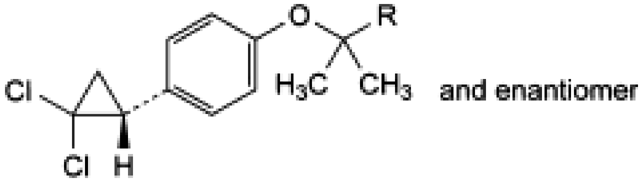
Specified impurities A, B, C, D, E.



A. 2-(4-ethenylphenoxy)-2-methylpropanoic acid,



B. 4-[(1*RS*)-2,2-dichlorocyclopropyl]phenol,



C. R = CH_2OH: 2-[4-[(1*RS*)-2,2-dichlorocyclopropyl]phenoxy]-2-methylpropan-1-ol,

D. R = CO-OCH_3: methyl 2-[4-[(1*RS*)-2,2-dichlorocyclopropyl]phenoxy]-2-methylpropanoate,

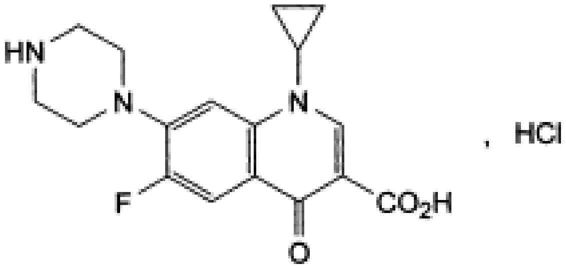
E. R = CO-OC_2H_5: ethyl 2-[4-[(1*RS*)-2,2-dichlorocyclopropyl]phenoxy]-2-methylpropanoate.

Ph Eur

Ciprofloxacin Hydrochloride

General Notices

(Ph Eur monograph 0888)



$C_{17}H_{18}FN_3O_3,HCl$ 367.8 *86393-32-0*

Action and use

Fluoroquinolone antibacterial.

Preparation

Ciprofloxacin Tablets

Ph Eur

DEFINITION

1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Pale yellow, crystalline powder, slightly hygroscopic.

Solubility

Soluble in water, slightly soluble in methanol, very slightly soluble in ethanol, practically insoluble in acetone, in ethyl acetate and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *ciprofloxacin hydrochloride CRS.*

B. 0.1 g gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution GY_5 *(2.2.2, Method II)*.

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R.*

pH *(2.2.3)*

3.5 to 4.5 for solution S.

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 10 mg of *ciprofloxacin impurity A CRS* in a mixture of 0.1 ml of *dilute ammonia R1* and 90 ml of *water R* and dilute to 100 ml with *water R.* Dilute 2 ml of the solution to 10 ml with *water R.*

Plate *TLC silica gel* F_{254} *plate R.*

Application 5 µl.

At the bottom of a chromatographic tank, place an evaporating dish containing 50 ml of *concentrated ammonia R*. Expose the plate to the ammonia vapour for 15 min in the closed tank. Withdraw the plate, transfer to a second chromatographic tank and proceed with development.

Mobile phase *acetonitrile R, concentrated ammonia R, methanol R, methylene chloride R* (10:20:40:40 *V/V/V/V*).

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Limit:

- *impurity A*: any spot corresponding to impurity A is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.2 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *ciprofloxacin hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *ciprofloxacin hydrochloride for peak identification CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 13 volumes of *acetonitrile R* and 87 volumes of a 2.45 g/l solution of *phosphoric acid R*, previously adjusted to pH 3.0 with *triethylamine R.*

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 278 nm.

Injection 50 µl.

Run time Twice the retention time of ciprofloxacin.

Relative retention With reference to ciprofloxacin (retention time = about 9 min): impurity E = about 0.4; impurity F = about 0.5; impurity B = about 0.6; impurity C = about 0.7; impurity D = about 1.2.

System suitability Reference solution (b):

— *resolution*: minimum 1.3 between the peaks due to impurity B and impurity C.

Limits:
— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.7; impurity C = 0.6; impurity D = 1.4; impurity E = 6.7; use the chromatogram obtained with reference solution (b) and the type chromatogram supplied with the CRS to identify the corresponding peaks;

— *impurities B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *any other impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 0.25 g in *water R* and dilute to 30 ml with the same solvent. Carry out the prefiltration. The filtrate complies with limit test E. Prepare the standard using 5 ml of *lead* *standard solution (1 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 6.7 per cent, determined on 0.200 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection 10 µl; inject the test solution and reference solution (a).

Calculate the percentage content of $C_{17}H_{19}ClFN_3O_3$.

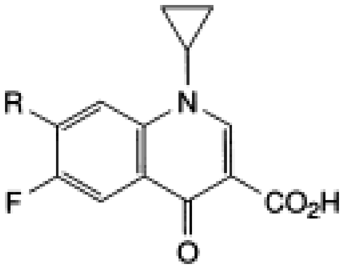
STORAGE

In an airtight container, protected from light.

IMPURITIES

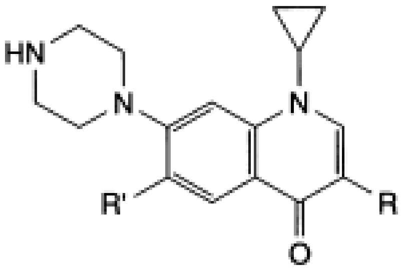
Specified impurities A, B, C, D, E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : F.*



A. R = Cl: 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (fluoroquinolonic acid),

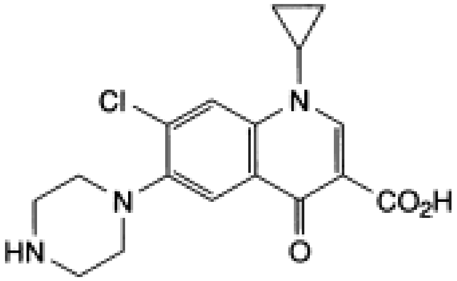
C. R = NH-$[CH_2]_2$-NH_2: 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (ethylenediamine compound),



B. R = CO_2H, R′ = H: 1-cyclopropyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (desfluoro compound),

E. R = H, R′ = F: 1-cyclopropyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1*H*)-one (decarboxylated compound),

F. R = CO_2H, R′ = OH: 1-cyclopropyl-6-hydroxy-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid,



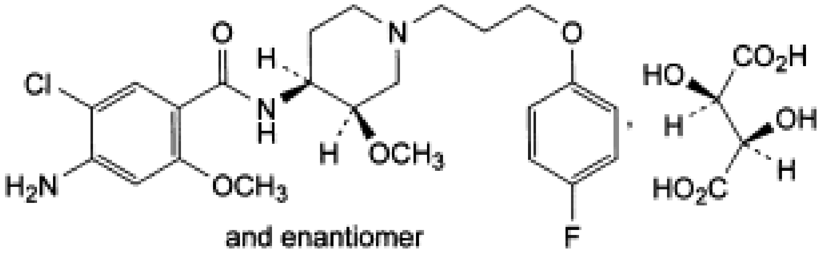
D. 7-chloro-1-cyclopropyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

Ph Eur

Cisapride Tartrate

General Notices

(Ph Eur monograph 1503)



$C_{23}H_{29}ClFN_3O_6,C_4H_6O_6$ 616.0

Action and use

Enterokinetic agent.

Ph Eur

DEFINITION

4-Amino-5-chloro-*N*-[(3*RS*,4*SR*)-1-[3-(4-fluorophenoxy)propyl]-3-methoxypiperidin-4-yl]-2-methoxybenzamide (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in dimethylformamide, slightly soluble in methanol, very slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *cisapride tartrate CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R,* evaporate to dryness in a current of air and record new spectra using the residues.

B. Specific optical rotation (see Tests).

TESTS

Specific optical rotation *(2.2.7)*

+ 5.0 to + 10.0 (dried substance).

Dissolve 0.100 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *water R, methanol R* (10:90 *V/V*).

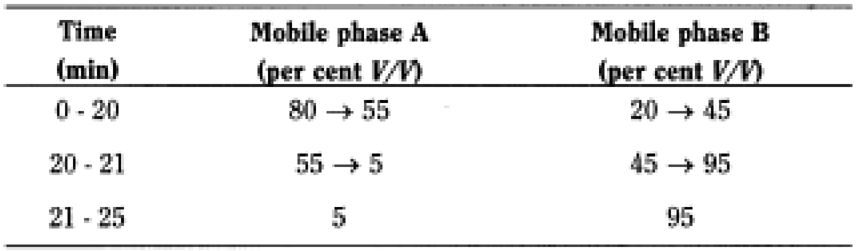
Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture, with gentle warming if necessary, and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 5.0 mg of *cisapride tartrate CRS* and 30.0 mg of *haloperidol CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 20.0 ml with the solvent mixture.

Column:
- *size: l* = 0.1 m, Ø = 4.0 mm;
- *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: 20 g/l solution of *tetrabutylammonium hydrogen sulphate R;*
- *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	80 → 55	20 → 45
20 - 21	55 → 5	45 → 95
21 - 25	5	95

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µl; inject the solvent mixture as a blank.

Retention time Cisapride = about 15 min; haloperidol = about 16 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to cisapride and haloperidol; if necessary, adjust the concentration of methanol in the mobile phase or adjust the time programme for the linear gradient.

Limits:

— *impurities A, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.500 g in 70 ml of *anhydrous acetic acid R* and titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 61.60 mg of $C_{27}H_{35}ClFN_3O_{10}$.

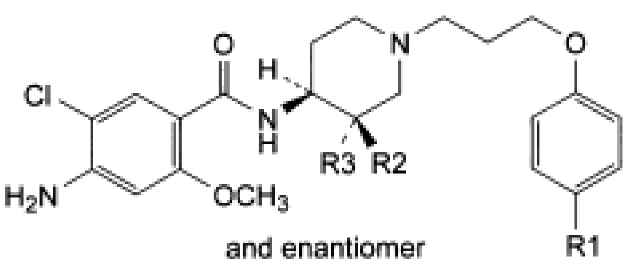
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, C.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use : B, D, E.*

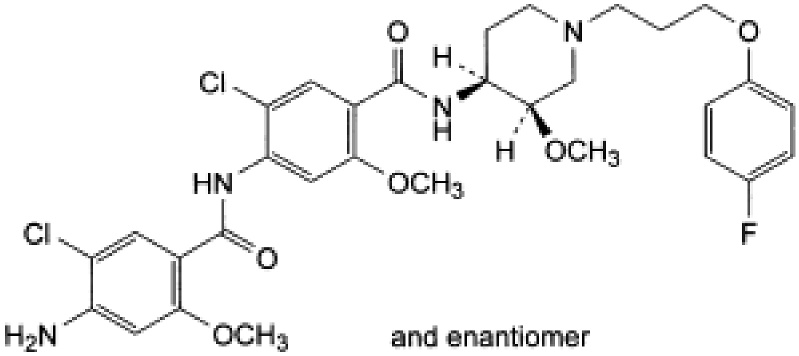


A. R1 = R3 = H, R2 = OCH_3: 4-amino-5-chloro-2-methoxy-*N*-[(3*RS*,4*SR*)-3-methoxy-1-(3-phenoxypropyl)piperidin-4-yl]benzamide,

B. R1 = F, R2 = R3 = H: 4-amino-5-chloro-*N*-[1-[3-(4-fluorophenoxy)propyl]piperidin-4-yl]-2-methoxybenzamide,

C. R1 = F, R2 = H, R3 = OCH_3: 4-amino-5-chloro-*N*-[(3*RS*,4*RS*)-1-[3-(4-fluorophenoxy)propyl]-3-methoxypiperidin-4-yl]-2-methoxybenzamide,

E. R1 = F, R2 = OH, R3 = H: 4-amino-5-chloro-*N*-[(3*RS*,4*SR*)-1-[3-(4-fluorophenoxy)propyl]-3-hydroxypiperidin-4-yl]-2-methoxybenzamide,



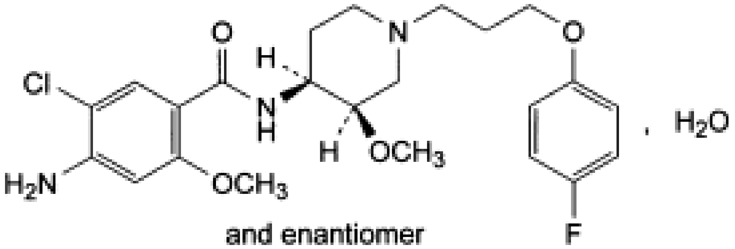
D. 4-[(4-amino-5-chloro-2-methoxybenzoyl)amino]-5-chloro-*N*-[(3*RS*,4*SR*)-1-[3-(4-fluorophenoxy)propyl]-3-methoxypiperidin-4-yl]-2-methoxybenzamide.

Ph Eur

Cisapride

General Notices

(Cisapride Monohydrate, Ph Eur monograph 0995)



$C_{23}H_{29}ClFN_3O_4, H_2O$ 484.0 *81098-60-4*

(anhydrous)

Action and use

Enterokinetic agent.

Ph Eur

DEFINITION

4-Amino-5-chloro-*N*-[(3*RS*,4*SR*)-1-[3-(4-fluorophenoxy)propyl]-3-methoxypiperidin-4-yl]-2-methoxybenzamide monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide, soluble in methylene chloride, sparingly soluble in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *cisapride monohydrate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R*, evaporate to dryness in a current of air and record new spectra using the residues.

TESTS

Solution S

Dissolve 0.20 g in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II).*

Optical rotation *(2.2.7)*

- 0.1 ° to + 0.1 °, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *cisapride monohydrate CRS* and 40.0 mg of *haloperidol CRS* in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R.*

Column:

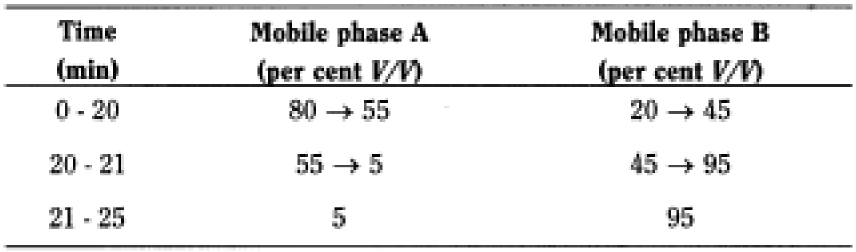
— *size:* *l* = 0.1 m, Ø = 4.0 mm;

— *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 20 g/l solution *of tetrabutylammonium hydrogen sulphate R*;

— *mobile phase B: methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	80 → 55	20 → 45
20 - 21	55 → 5	45 → 95
21 - 25	5	95

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µl; inject *methanol R* as a blank.

Retention time cisapride = about 15 min; haloperidol = about 16 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to cisapride and haloperidol; if necessary, adjust the concentration of methanol in the mobile phase or adjust the time programme for the linear gradient.

Limits:

— *impurities A, B, C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

3.4 per cent to 4.0 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.350 g in 70 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R* and titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 46.60 mg of $C_{23}H_{29}ClFN_3O_4$.

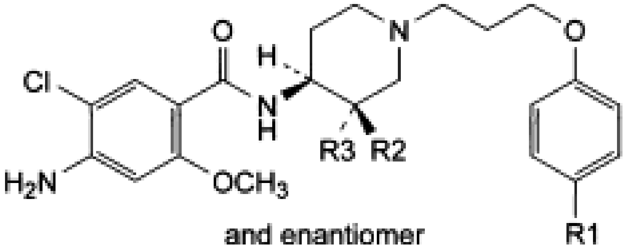
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : D.*

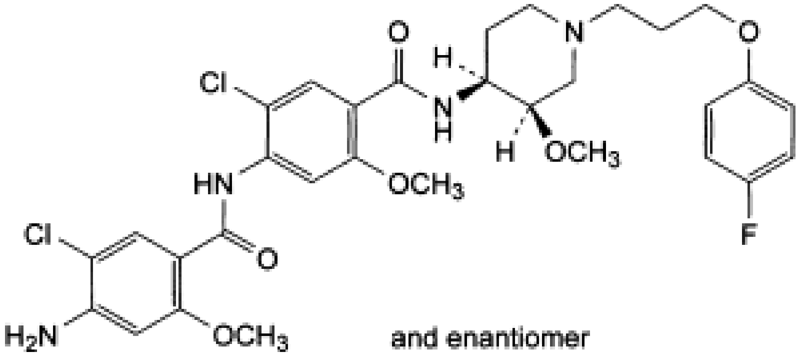


A. R1 = R3 = H, R2 = OCH_3: 4-amino-5-chloro-2-methoxy-*N*-[(3*RS*,4*SR*)-3-methoxy-1-(3-phenoxypropyl)piperidin-4-yl]benzamide,

B. R1 = F, R2 = R3 = H: 4-amino-5-chloro-*N*-[1-[3-(4-fluorophenoxy)propyl]piperidin-4-yl]-2-methoxybenzamide,

C. R1 = F, R2 = H, R3 = OCH_3: 4-amino-5-chloro-*N*-[(3*RS*,4*RS*)-1-[3-(4-fluorophenoxy) propyl]-3-methoxypiperidin-4-yl-2-methoxybenzamide,

E. R1 = F, R2 = OH, R3 = H: 4-amino-5-chloro-*N*-[(3*RS*,4*SR*)-1-[3-(4-fluorophenoxy)propyl]-3-hydroxypiperidin-4-yl]-2-methoxybenzamide,



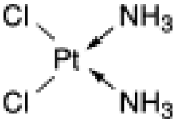
D. 4-[(4-amino-5-chloro-2-methoxybenzoyl)amino]-5-chloro-*N*-[(3*RS*,4*SR*)-1-[3-(4-fluorophenoxy)propyl]-3-methoxypiperidin-4-yl]-2-methoxybenzamide.

Ph Eur

Cisplatin

General Notices

(Ph Eur monograph 0599)



$H_6Cl_2N_2Pt$ 300.0 *15663-27-1*

Action and use

Platinum-containing cytotoxic.

Preparation

Cisplatin Injection

Ph Eur

DEFINITION

Cisplatin contains not less than 97.0 per cent and not more than the equivalent of 102.0 per cent of *cis*-diamminedi-chloroplatinum (II).

CHARACTERS

A yellow powder or yellow or orange-yellow crystals, slightly soluble in water, sparingly soluble in dimethylformamide, practically insoluble in alcohol.

It decomposes with blackening at about 270 °C.

Carry out identification test B, the tests (except that for silver) and the assay protected from light.

IDENTIFICATION

First identification *A, B.*

Second identification *B, C.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cisplatin CRS*. Examine the substances prepared as discs in *potassium bromide R*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (a) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Add 50 mg to 2 ml of *dilute sodium hydroxide solution R* in a glass dish. Evaporate to dryness. Dissolve the residue in a mixture of 0.5 ml of *nitric acid R* and 1.5 ml of *hydrochloric acid R.* Evaporate to dryness. The residue is orange. Dissolve the residue in 0.5 ml of *water R* and add 0.5 ml of *ammonium chloride solution R*. A yellow, crystalline precipitate is formed.

TESTS

Solution S1

Dissolve 25 mg in a 9 g/l solution of *sodium chloride R* prepared with *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Solution S2

Dissolve 0.20 g in *dimethylformamide R* and dilute to 10 ml with the same solvent.

Appearance of solution S1

Solution S1 is clear *(2.2.1)* and not more intensely coloured than reference solution GY_5 *(2.2.2, Method II)*.

Appearance of solution S2

Solution S2 is clear *(2.2.1)*.

pH *(2.2.3)*

The pH of solution S1, measured immediately after preparation, is 4.5 to 6.0.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *cellulose for chromatography R1* as the coating substance. Activate the plate by heating at 150 °C for 1 h.

Test solution (a) Dilute 1 ml of solution S2 to 10 ml with *dimethylformamide R*.

Test solution (b). Use solution S2.

Reference solution (a) Dissolve 10 mg of *cisplatin CRS* in 5 ml of *dimethylformamide R*.

Reference solution (b) Dilute 1 ml of solution S2 to 50 ml with *dimethylformamide R.*

Apply separately to the plate 2.5 µl of test solution (a), 2.5 µl of reference solution (a), 5 µl of test solution (b) and 5 µl of reference solution (b). Develop over a path of 15 cm using a mixture of 10 volumes of *acetone R* and 90 volumes of *dimethylformamide R*. Allow the plate to dry in air and spray with a 50 g/l solution of *stannous chloride R* in a mixture of equal volumes of *dilute hydrochloric acid R* and *water R*. After 1 h, the chromatogram obtained with test solution (b) shows no spot with an R_F value less than that of the principal spot and any spot with an R_F value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

Silver

Not more than 2.5×10^2 ppm of Ag, determined by atomic absorption spectrometry *(2.2.23, Method I).*

Test solution Dissolve 0.100 g of the substance to be examined in 15 ml of *nitric acid R*, heating to 80 °C. Cool and dilute to 25.0 ml with *water R*.

Reference solutions To suitable volumes (10 ml to 30 ml) of *silver standard solution (5 ppm Ag) R* add 50 ml of *nitric acid R* and dilute to 100.0 ml with *water R.*

Measure the absorbance at 328 nm using a silver hollow-cathode lamp as source of radiation, a fuel-lean air-acetylene flame and, preferably, a spectral slit width of 0.5 nm. Carry out a blank determination.

ASSAY

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in a 9 g/l solution of *sodium chloride R* and dilute to 100.0 ml with the same solvent.

Reference solution Dissolve 50.0 mg of *cisplatin CRS* in a 9 g/l solution of *sodium chloride R* and dilute to 100.0 ml with the same solvent.

The chromatographic procedure may be carried out using:

- — a column 0.25 m long and 4.6 mm in internal diameter packed with *strong-anion-exchange silica gel for chromatography R* (10 µm),
- — as mobile phase at a flow rate of 1.2 ml/min a mixture of 10 volumes of a 9 g/l solution of *sodium chloride R* and 90 volumes of *methanol R*,
- — as detector a spectrophotometer set at 220 nm.

Use a sample loop. Inject separately 20 µl of the test solution and 20 µl of the reference solution.

STORAGE

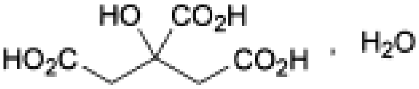
Store in an airtight container, protected from light.

Ph Eur

Citric Acid Monohydrate

General Notices

(Ph Eur monograph 0456)



$C_6H_8O_7,H_2O$ 210.1 *5949-29-1*

Ph Eur

DEFINITION

2-Hydroxypropane-1,2,3-tricarboxylic acid monohydrate.

Content

99.5 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, colourless crystals or granules, efflorescent.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Dissolve 1 g in 10 ml of *water R*. The solution is strongly acidic *(2.2.4)*.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dry the substance to be examined and the reference substance at 100-105 °C for 2 h.

Comparison *citric acid monohydrate CRS*.

C. Add about 5 mg to a mixture of 1 ml of *acetic anhydride R* and 3 ml of *pyridine R*. A red colour develops.

D. Dissolve 0.5 g in 5 ml of *water R*, neutralise using *1 M sodium hydroxide* (about 7 ml), add 10 ml of *calcium chloride solution R* and heat to boiling. A white precipitate is formed.

E. Water (see Tests).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7, BY_7 or GY_7 (*2.2.2, Method II)*.

Dissolve 2.0 g in *water R* and dilute to 10 ml with the same solvent.

Readily carbonisable substances

To 1.0 g in a cleaned test tube add 10 ml of *sulphuric acid R* and immediately heat the mixture in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly immediately afterwards. The solution is not more intensely coloured than a mixture of 1 ml of red primary solution and 9 ml of yellow primary solution *(2.2.2, Method I)*.

Oxalic acid

Maximum 360 ppm, calculated as anhydrous oxalic acid.

Dissolve 0.80 g in 4 ml of *water R*. Add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules. Boil for 1 min. Allow to stand for 2 min. Transfer the supernatant liquid to a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of a 50 g/l solution of *potassium ferricyanide R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 4 ml of a 0.1 g/l solution of *oxalic acid R*.

Sulphates *(2.4.13)*

Maximum 150 ppm.

Dissolve 2.0 g in distilled *water R* and dilute to 30 ml with the same solvent.

Aluminium *(2.4.17)*

Maximum 0.2 ppm, if intended for use in the manufacture of dialysis solutions.

Prescribed solution Dissolve 20 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R.*

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 5.0 g in several portions in 39 ml of *dilute sodium hydroxide solution R* and dilute to 50 ml with *distilled water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

7.5 per cent to 9.0 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.550 g in 50 ml of *water R*. Titrate with *1 M sodium hydroxide*, using 0.5 ml of *phenolphthalein solution R* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 64.03 mg of $C_6H_8O_7$.

STORAGE

In an airtight container.

LABELLING

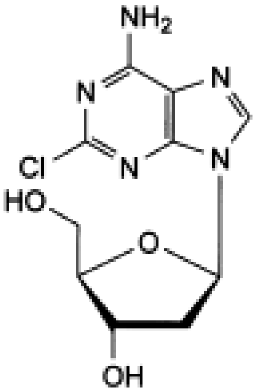
The label states, where applicable, that the substance is intended for use in the manufacture of dialysis solutions.

Ph Eur

Cladribine

General Notices

(Ph Eur monograph 2174)



$C_{10}H_{12}ClN_5O_3$ 285.7 *4291-63-8*

Action and use

Purine analogue; cytostatic.

Ph Eur

DEFINITION

2-Chloro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-9*H*-purin-6-amine.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, soluble in dimethyl sulphoxide, slightly soluble in methanol, practically insoluble in acetonitrile.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *cladribine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined in the minimum volume of *methanol R* and evaporate to dryness. Dry the precipitate at 100 °C for 2 h and record a new spectrum using the residue.

TESTS

Solution S

Disperse 0.15 g in *carbon dioxide-free water R*, dilute to 50 ml with the same solvent and sonicate until dissolution is complete.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

7.0 to 8.1 for solution S.

Specific optical rotation *(2.2.7)*

- 21.0 to - 27.0 (anhydrous substance).

Dissolve 0.25 g in *dimethyl sulphoxide R* and dilute to 25.0 ml with the same solvent.

Impurity E

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 40.0 mg of the substance to be examined in *dimethylformamide R* and dilute to 2.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *2-deoxy-D-ribose R* (impurity E) in *dimethylformamide R* and dilute to 25.0 ml with the same solvent. Dilute 3.0 ml of this solution to 10.0 ml with *dimethylformamide R*.

Reference solution (b) Dissolve 10.0 mg of *2-deoxy-D-ribose R* (impurity E) in *dimethylformamide R* and dilute to 5.0 ml with the same solvent. Mix 9 volumes of this solution with 1 volume of the test solution.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *Concentrated ammonia R, ethanol (96 per cent) R, ethyl acetate R* (20:40:40 *V/V/V).*

Application 5 µl as bands of 10 mm; thoroughly dry the starting points in a current of warm air.

Development Over 2/3 of the plate.

Drying In air, then heat at 45 °C for 10 min.

Detection Spray with a solution containing 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*; heat at 110 °C for 20 min or until the spots appear.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity E*: any spot due to impurity E is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.3 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile R, water R* (10:90 *V/V*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Test solution (b) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *cladribine CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with the solvent mixture.

Reference solution (d) Dissolve 1.0 mg of *cladribine impurity C CRS* in reference solution (b) and dilute to 25.0 ml with the same solution.

Reference solution (e) Dilute 5.0 ml of reference solution (c) to 10.0 ml with the solvent mixture.

Reference solution (f) Dissolve 3 mg of *cladribine for peak identification CRS* (containing impurities A, B, C and D) in 2 ml of the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

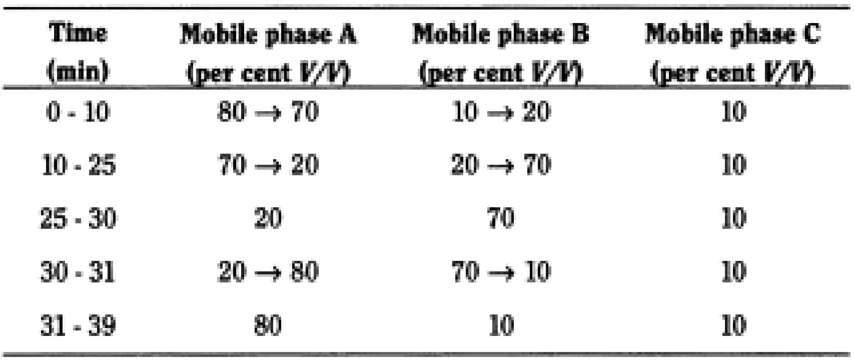
— *stationary phase: base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A: water for chromatography R*;

— *mobile phase B: acetonitrile for chromatography R*;

— *mobile phase C:* 50 g/l solution of *phosphoric acid R* in *water for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 10	80 → 70	10 → 20	10
10 - 25	70 → 20	20 → 70	10
25 - 30	20	70	10
30 - 31	20 → 80	70 → 10	10
31 - 39	80	10	10

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 252 nm.

Injection 20 µl of test solution (a) and reference solutions (c), (d), (e) and (f).

Identification of impurities Use the chromatogram supplied with *cladribine for peak identification CRS* to identify the peaks due to impurities A, B, C and D.

Relative retention With reference to cladribine (retention time = about 10 min): impurity A = about 0.33; impurity B = about 0.44; impurity C = about 0.73; impurity D = about 0.92.

System suitability Reference solution (d):

— *resolution*: minimum 4.5 between the peaks due to impurity C and cladribine.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.7; impurity C = 0.8;

— *impurities A, C*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *impurities B, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Water *(2.5.32)*

Maximum 0.5 per cent, determined on 0.100 g.

Bacterial endotoxins *(2.6.14)*

Less than 3 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{10}H_{12}ClN_5O_3$ from the declared content of *cladribine CRS.*

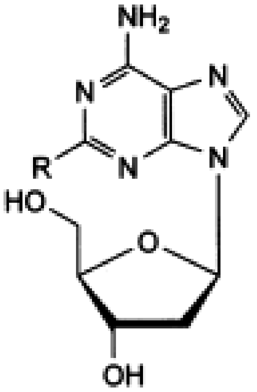
STORAGE

Protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

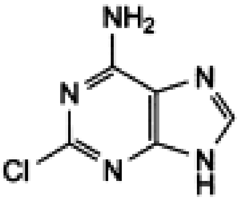
Specified impurities *A, B, C, D, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : F, G.*

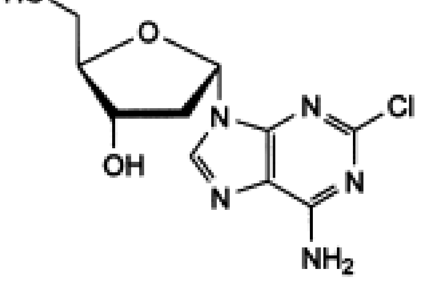


A. R = NH_2: 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-9*H*-purin-2,6-diamine,

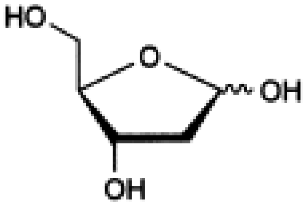
B. R = OCH_3: 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-methoxy-9*H*-purin-6-amine,



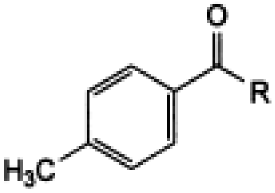
C. 2-chloro-7*H*-purin-6-amine (2-chloroadenine),



D. 2-chloro-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)-9*H*-purin-6-amine,



E. 2-deoxy-D-*erythro*-pentofuranose (2-deoxy-D-ribose),



F. R = NH_2: 4-methylbenzamide,

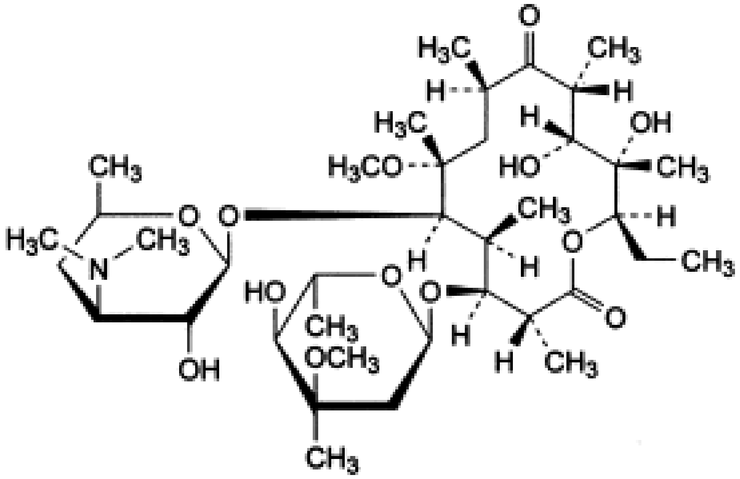
G. R = OCH_3: methyl 4-methylbenzoate.

Ph Eur

Clarithromycin

General Notices

(Ph Eur monograph 1651)


O
H3C
CH3
H
H
H3C
H
OH
CH3
H3CO
HO
CH3
CH3
O
O
H
H3C
CH3
O
N
H
O
CH3
H
HO
O
O
CH3
OCH3
H
O
OH
H
CH3
CH3

$C_{38}H_{69}NO_{13}$ 748 *81103-11-9*

Action and use

Macrolide antibacterial.

Preparations

Clarithromycin Tablets

Prolonged-release Clarithromycin Tablets

Ph Eur

DEFINITION

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (6-*O*-methylerythromycin A).

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone and in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison clarithromycin CRS.

TESTS

Solution S

Dissolve 0.500 g in *methylene chloride R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear or not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

- 94 to - 102 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 75.0 mg of the substance to be examined in 25 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R.*

Reference solution (a) Dissolve 75.0 mg of *clarithromycin CRS* in 25 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 100.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (d) Dissolve 15.0 mg of *clarithromycin for peak identification CRS* in 5.0 ml of *acetonitrile R1* and dilute to 10.0 ml with *water R.*

Blank solution Dilute 25.0 ml of *acetonitrile R1* to 50.0 ml with *water R* and mix.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm,

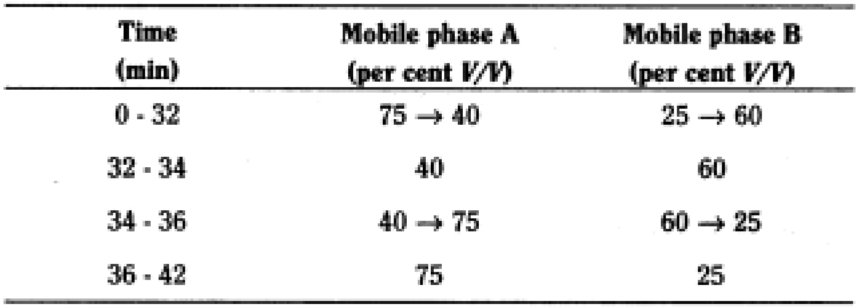
— *stationary phase: octadecylsilyl silica gel for chromatography R* (3.5 µm),

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: a 4.76 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 4.4 with *dilute phosphoric acid R* or a 45 g/l solution of *potassium hydroxide R,* filtered through a C18 filtration kit,

— *mobile phase B: acetonitrile R1*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 32	75 → 40	25 → 60
32 - 34	40	60
34 - 36	40 → 75	60 → 25
36 - 42	75	25

Flow rate 1.1 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µl of the blank solution, the test solution and reference solutions (b), (c) and (d).

Relative retention r (not r_G) with reference to clarithromycin (retention time = about 11 min): impurity I = about 0.38; impurity A = about 0.42; impurity J = about 0.63; impurity L = about 0.74; impurity B = about 0.79; impurity M = about 0.81; impurity C = about 0.89; impurity D = about 0.96; impurity N = about 1.15; impurity E = about 1.27; impurity F = about 1.33; impurity P = about 1.35; impurity O = about 1.41; impurity K = about 1.59; impurity G = about 1.72; impurity H = about 1.82.

System suitability:

— *symmetry factor*: maximum 1.7 for the peak due to clarithromycin in the chromatogram obtained with reference solution (b),

— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to clarithromycin in the chromatogram obtained with reference solution (d).

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity G = 0.27; impurity H = 0.15; use the chromatogram supplied with *clarithromycin for peak identification CRS* to identify the peaks;

— *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent), and not more than 4 such peaks have an area greater than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.4 per cent);

— *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3.5 per cent);

— *disregard limit:* 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent); disregard the peaks eluting before impurity I and after impurity H.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *dioxan R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *dioxan R.*

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 0.5 g.

ASSAY

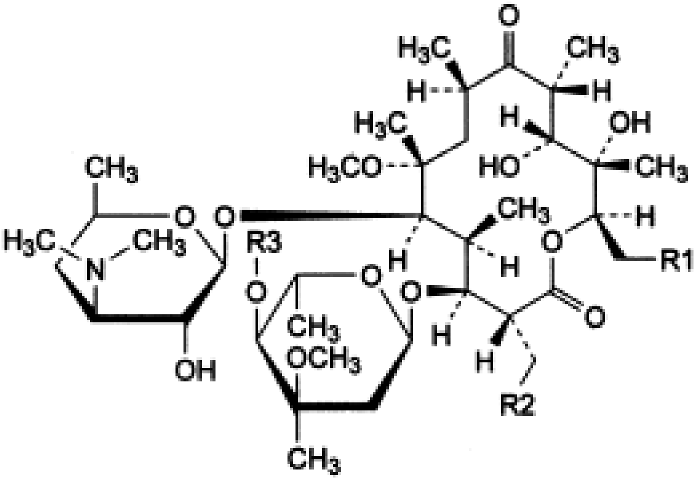
Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{38}H_{69}NO_{13}$.

IMPURITIES

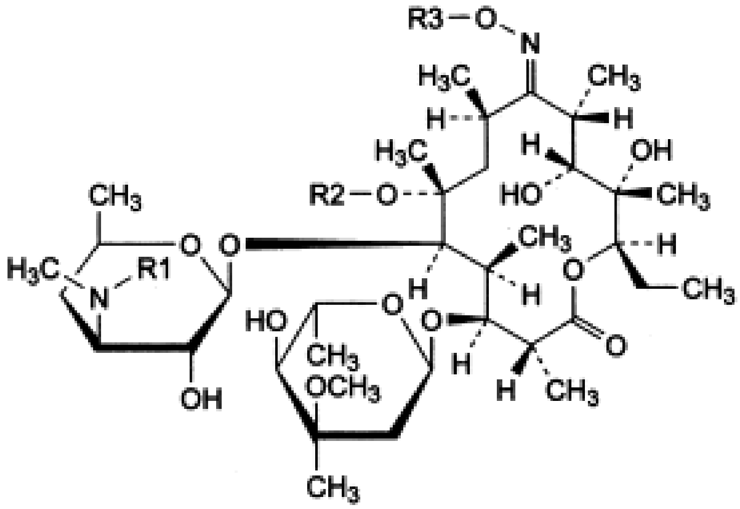
Specified impurities *A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P.*



A. R1 = CH_3, R2 = OH, R3 = H: 2-demethyl-2-(hydroxymethyl)-6-*O*-methylerythromycin A (clarithromycin F),

B. R1 = R2 = R3 = H: 6-*O*-methyl-15-norerythromycin A,

P. R1 = R3 = CH_3, R2 = H: 4′,6-di-*O*-methylerythromycin A,

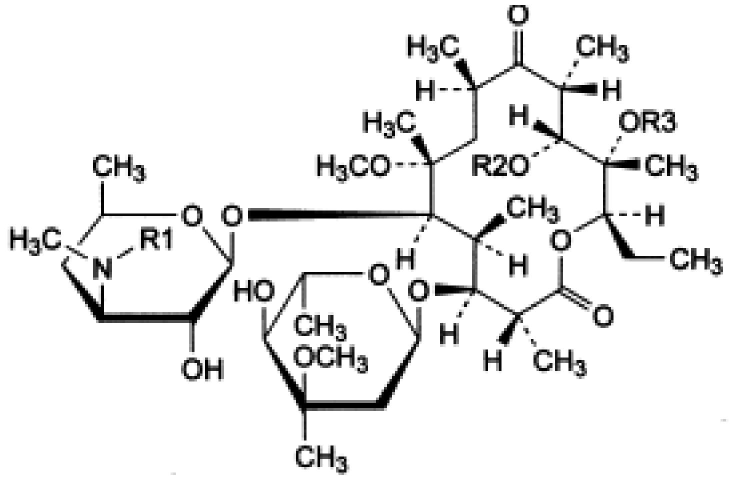

R3—O
N
H$_3$C
CH$_3$
H
H
H$_3$C
H
OH
CH$_3$
R2—O
HO
CH$_3$
O
O
CH$_3$
H$_3$C
R1
N
H
H
O
CH$_3$
HO
O
O
CH$_3$
H
OH
OCH$_3$
H
CH$_3$
O
CH$_3$

C. R1 = R2 = CH_3, R3 = H: 6-*O*-methylerythromycin A (*E*)-9-oxime,

G. R1 = R2 = R3 = CH_3: 6-*O*-methylerythromycin A (*E*)-9-(*O*-methyloxime),

J. R1 = CH_3, R2 = R3 = H: erythromycin A (*E*)-9-oxime,

M. R1 = R3 = H, R2 = CH_3: 3″-*N*-demethyl-6-*O*-methylerythromycin A (*E*)-9-oxime,

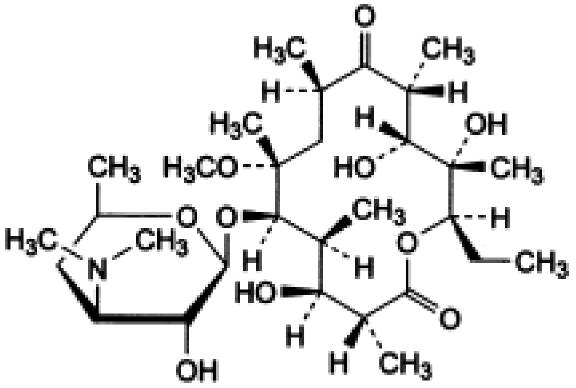

O
H$_3$C
CH$_3$
H
H
H$_3$C
H
OR3
H$_3$CO
R2O
CH$_3$
CH$_3$
O
O
H$_3$C
R1
N
CH$_3$
H
H
O
CH$_3$
HO
O
O
CH$_3$
H
OH
OCH$_3$
H
CH$_3$
O
CH$_3$

D. R1 = R2 = R3 = H: 3″-*N*-demethyl-6-*O*-methylerythromycin A,

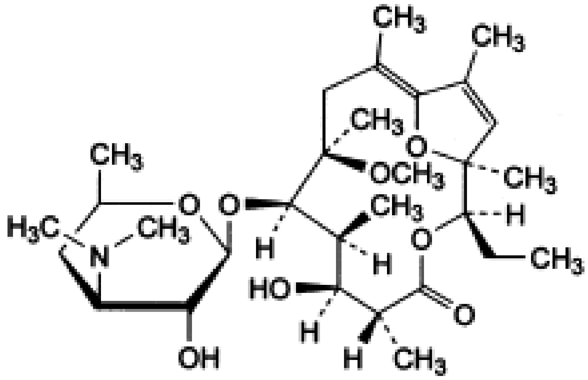
E. R1 = R2 = CH_3, R3 = H: 6,11-di-*O*-methylerythromycin A,

F. R1 = R3 = CH_3, R2 = H: 6,12-di-*O*-methylerythromycin A,

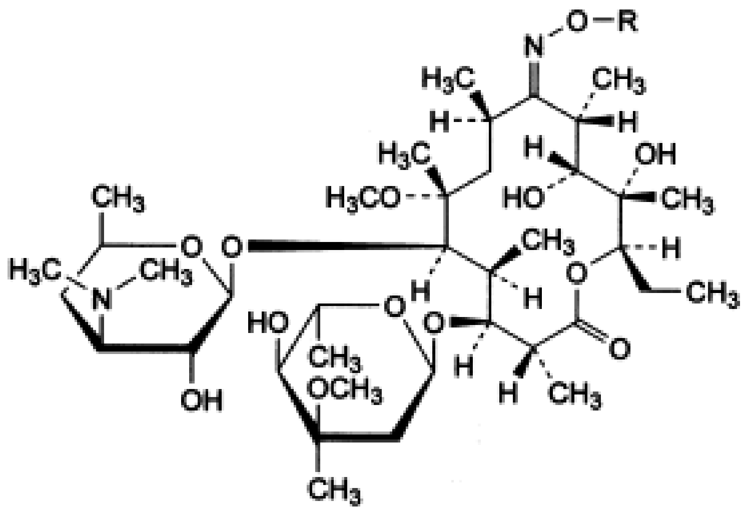
H. R1 = CHO, R2 = R3 = H: 3″-*N*-demethyl-3′-*N*-formyl-6-*O*-methylerythromycin A,



I. 3-*O*-decladinosyl-6-*O*-methylerythromycin A,

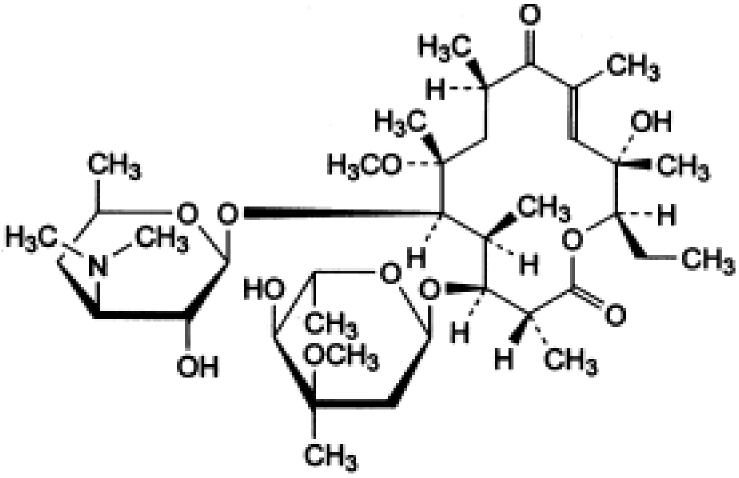


K. (1*S*,2*R*,5*R*,6*S*,7*S*,8*R*,9*R*,11*Z*)-2-ethyl-6-hydroxy-9-methoxy-1,5,7,9,11,13-hexamethyl-8-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-4-one (3-*O*-decladinosyl-8,9:10,11-dianhydro-6-*O*-methylerythromycin A-9,12-hemiketal,



L. R = H: 6-*O*-methylerythromycin A (*Z*)-9-oxime,

O. R = CH_3: 6-*O*-methylerythromycin A (*Z*)-9-(*O*-methyloxime),



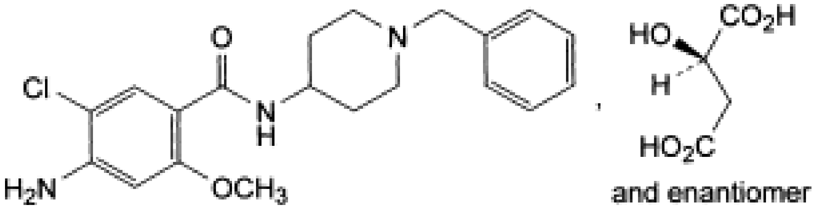
N. (10*E*)-10,11-didehydro-11-deoxy-6-*C*-methylerythromycin A.

Ph Eur

Clebopride Malate

General Notices

(Ph Eur monograph 1303)



$C_{20}H_{24}ClN_3O_2,C_4H_6O_5$ 508.0 *57645-91-7*

Action and use

Dopamine receptor antagonist; antiprotozoal (veterinary).

Ph Eur

DEFINITION

4-Amino-*N*-(1-benzylpiperidin-4-yl)-5-chloro-2-methoxybenzamide acid (*RS*)-2-hydroxybutanedioate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water and in methanol, slightly soluble in anhydrous ethanol, practically insoluble in methylene chloride.

mp: about 164 °C, with decomposition.

IDENTIFICATION

First identification B, C.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 20.0 mg in *water R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water R.*

Spectral range 230-350 nm.

Absorption maxima At 270 nm and 307 nm.

Specific absorbances at the absorption maxima:

— at 270 nm: 252 to 278;

— at 307 nm: 204 to 226.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *clebopride malate CRS*.

C. Dissolve 20 mg in 1 ml of *sulphuric acid R*, add 1 ml of β*-naphthol solution R1* and mix. The solution examined in daylight is yellow with blue fluorescence.

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *clebopride malate CRS* in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *clebopride malate CRS* and 5 mg of *metoclopramide hydrochloride CRS* in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *concentrated ammonia R, acetone R, methanol R, toluene R* (2:14:14:70 *V/V/V/V*).

Application 5 µl, as bands of 10 mm by 3 mm.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated bands.

Results The principal band in the chromatogram obtained with the test solution is similar in position and size to the principal band in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S, examined immediately after preparation, is clear *(2.2.1)* and colourless *(2.2.2,* *Method I)*.

pH ***(2.2.3)***

3.8 to 4.2 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *clebopride malate CRS* and 10.0 mg of *metoclopramide hydrochloride CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.12 m, Ø = 4.0 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *acetonitrile R1* and 80 volumes of a 1 g/l solution of *sodium heptanesulphonate R* adjusted to pH 2.5 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 215 nm.

Equilibration With the mobile phase for 30 min.

Injection 20 µl.

Run time Twice the retention time of clebopride.

System suitability Reference solution (b):

— *retention time*: clebopride (2nd peak) = about 15 min;

— *relative retention* with reference to clebopride: metoclopramide (1st peak) = about 0.45.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent); disregard the 2 peaks eluting within the first 2 min.

Chlorides

Maximum 100 ppm.

Prepare the solutions at the same time.

Test solution Dissolve 0.530 g in 20.0 ml of *anhydrous acetic acid R*, add 6 ml of *dilute nitric acid R* and dilute to 50.0 ml with *water R*.

Reference solution To 1.5 ml of *0.001 M hydrochloric acid*, add 20.0 ml of *anhydrous acetic acid R* and 6 ml of *dilute nitric acid R* and dilute to 50.0 ml with *water R*.

Transfer both recently prepared solutions to separate test-tubes. Add to each tube 1 ml of *silver nitrate solution R2*. Allow to stand for 5 min protected from light. Examine the tubes laterally against a black background. Any opalescence in the test solution is not more intense than that in the reference solution.

Sulphates

Maximum 100 ppm.

Prepare the solutions at the same time.

Test solution Dissolve 3.00 g in 20.0 ml of *glacial acetic acid R*, heating gently if necessary. Allow to cool and dilute to 50.0 ml with *water R*.

Reference solution To 9 ml of *sulphate standard solution (10 ppm SO_4) R1*, add 6 ml of *glacial acetic acid R*.

Into 2 test tubes introduce 1.5 ml of *sulphate standard solution (10 ppm SO_4) R1* and add 1 ml of a 250 g/l solution of *barium chloride R*. Shake and allow to stand for 1 min. To one of the tubes add 15 ml of the test solution and to the other add 15 ml of the reference solution. After 5 min, any opalescence in the tube containing the test solution is not more intense than that in the tube containing the reference solution.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

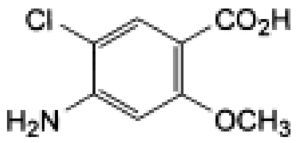
Dissolve 0.400 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 50.80 mg of $C_{24}H_{30}ClN_3O_7$.

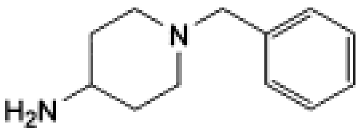
STORAGE

Protected from light.

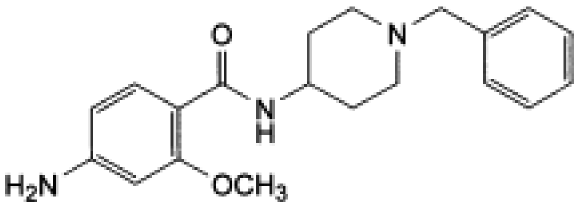
IMPURITIES



A. 4-amino-5-chloro-2-methoxybenzoic acid,



B. 1-benzylpiperidin-4-amine,


O
N
H
N
H2N
OCH3

C. 4-amino-*N*-(1-benzylpiperidin-4-yl)-2-methoxybenzamide.

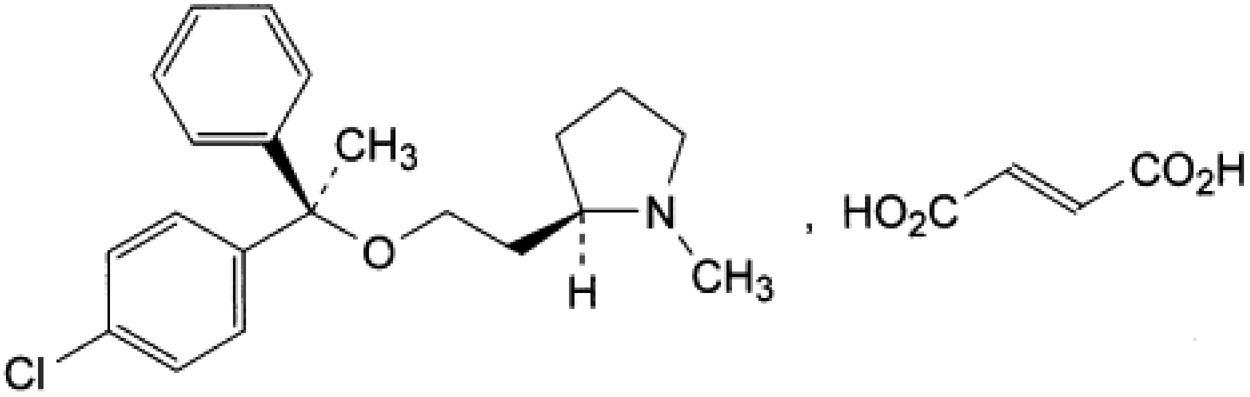
Ph Eur

Clemastine Fumarate

General Notices

Clemastine Hydrogen Fumarate

(Ph Eur monograph 1190)



$C_{21}H_{26}ClNO,C_4H_4O_4$ 460.0 *14976-57-9*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparations

Clemastine Oral Solution

Clemastine Tablets

Ph Eur

DEFINITION

(2*R*)-2-[2-[(*R*)-1-(4-Chlorophenyl)-1-phenylethoxy]ethyl]-1-methylpyrrolidine (*E*)-butenedioate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, sparingly soluble in ethanol (70 per cent *V/V*), slightly soluble in ethanol (50 per cent *V/V*) and in methanol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *clemastine fumarate CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 2 ml with the same solvent.

Reference solution Dissolve 50 mg of *fumaric acid CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *water R*, *anhydrous formic acid R*, *di-isopropyl ether R* (5:25:70 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying At 100-105 °C for 30 min and allow to cool.

Detection Spray with a 16 g/l solution of *potassium permanganate R* and examine in daylight.

Results The spot with the highest R_F value in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 0.500 g in *methanol R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

pH (*2.2.3*)

3.2 to 4.2.

Suspend 1.0 g in 10 ml of *carbon dioxide-free water R.*

Specific optical rotation (*2.2.7*)

+ 15.0 to + 18.0 (dried substance), determined on solution S.

Related substances

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol R*.

Reference solution (a) Dissolve 20.0 mg of *clemastine fumarate CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.5 ml of test solution (b) to 50.0 ml with *methanol R*.

Reference solution (c) Dilute 0.5 ml of test solution (b) to 50.0 ml with *methanol R.*

Reference solution (d) Dissolve 10.0 mg of *diphenhydramine hydrochloride CRS* in 5.0 ml of reference solution (a).

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R*, *methanol R*, *tetrahydrofuran R* (1:20:80 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of cold air for 5 min.

Detection Spray with a freshly prepared mixture of 1 volume of *potassium iodobismuthate solution R* and 10 volumes of *dilute acetic acid R* and then with *dilute hydrogen peroxide solution R;* cover the plate immediately with a glass plate of the same size and examine the chromatograms after 2 min.

System suitability Reference solution (d):

— the chromatogram shows 2 clearly separated spots.

Limits Test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.3 per cent) and at most 4 such spots are more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *disregard limit*: disregard any spot remaining at the starting point (fumaric acid).

Impurity C

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1*, 10 g/l solution of *ammonium dihydrogen phosphate R* (25:75 *V/V*).

Test solution Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 100 ml with the solvent mixture.

Reference solution (a) Dissolve 6 mg of *1-(4-chlorophenyl)-1-phenylethanol CRS* (impurity C) in the solvent mixture and dilute to 100 ml with the solvent mixture.

Reference solution (b) Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Reference solution (c) Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 100 ml with the solvent mixture. To 1 ml of this solution add 1 ml of reference solution (a) and dilute to 100 ml with the solvent mixture.

Column:

— *size:* *l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *phosphoric acid R*, *acetonitrile R1*, 10 g/l solution of *ammonium dihydrogen phosphate R* (0.1:45:55 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 100 µl.

System suitability Reference solution (c):

— *resolution*: minimum 2.2 between the peaks due to clemastine and impurity C.

Limit:

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 6 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

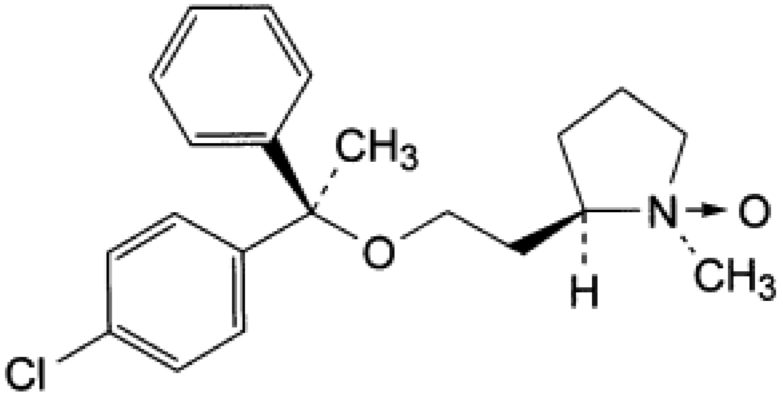
Dissolve 0.350 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 46.00 mg of $C_{25}H_{30}ClNO_5$.

IMPURITIES

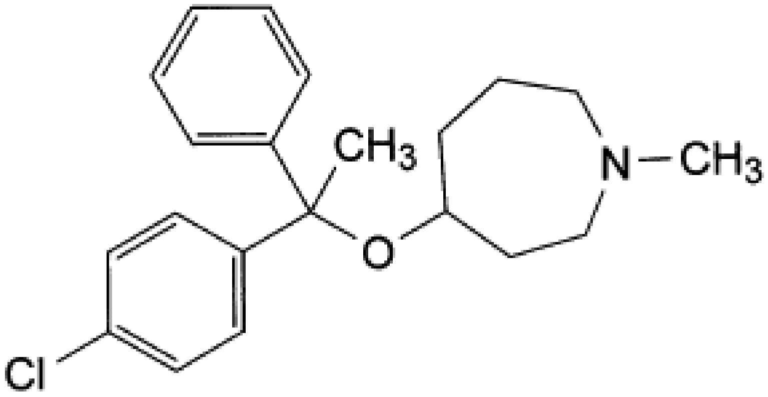
Specified impurities A, B, C.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D.*

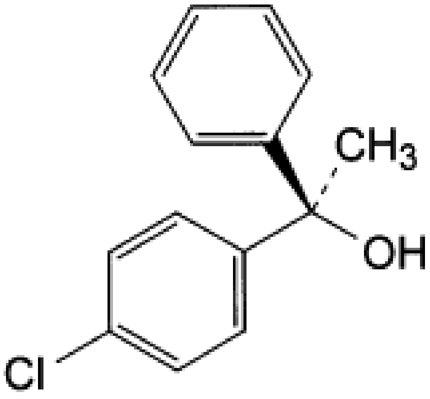


and epimer at N

A. (1*RS*,2*R*)-2-[2-[(*R*)-1-(4-chlorophenyl)-1-phenylethoxy]ethyl]-1-methylpyrrolidine 1-oxide,

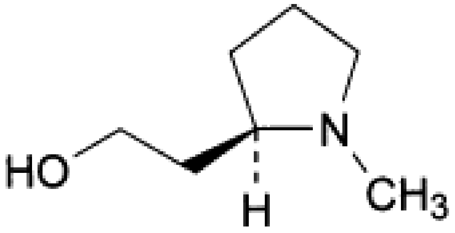


B. 4-[1-(4-chlorophenyl)-1-phenylethoxy]-1-methylazepane,



and enantiomer

C. (*RS*)-1-(4-chlorophenyl)-1-phenylethanol,



and enantiomer

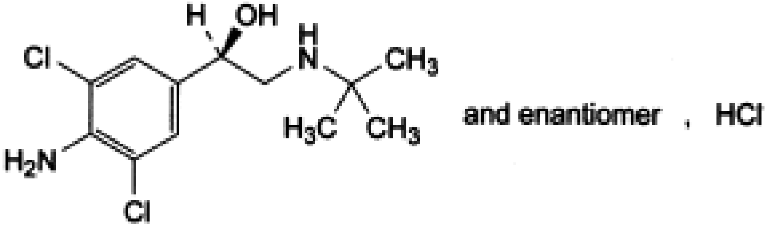
D. 2-[(2*RS*)-1-methylpyrrolidin-2-yl]ethanol.

Ph Eur

Clenbuterol Hydrochloride

General Notices

(Ph Eur monograph 1409)



$C_{12}H_{18}Cl_2N_2O,HCl$ 313.7 *21898-19-1*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Ph Eur

DEFINITION

(1*RS*)-1-(4-Amino-3,5-dichlorophenyl)-2-[(1,1-dimethylethyl)amino]ethanol hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water and in ethanol (96 per cent), slightly soluble in acetone.

mp

About 173 °C, with decomposition.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *clenbuterol hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 10 ml of *methanol R*.

Reference solution Dissolve 10 mg of *clenbuterol hydrochloride CRS* in 10 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ammonia R*, *anhydrous ethanol R, toluene R* (0.15:10:15 *V/V/V*).

Application 10 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 10 g/l solution of *sodium nitrite R* in *1 M hydrochloric acid* and dip after 10 min in a 4 g/l solution of *naphthylethylenediamine dihydrochloride R* in *methanol R*. Allow to dry in air.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in 10 ml of *carbon dioxide-free water R*.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II).*

pH *(2.2.3)*

5.0 to 7.0 for solution S.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 0.30 g in *water R* and dilute to 10.0 ml with the same solvent. Filter if necessary.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Disperse 100.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 0.1 ml of the test solution to 100.0 ml with *water R.*

Reference solution (b) Dissolve 5 mg of *clenbuterol impurity B CRS* in 10 ml of the mobile phase, add 2.5 ml of the test solution and dilute to 25.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.125 m, Ø = 4 mm,
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 40 °C.

Mobile phase Mix 200 volumes of *acetonitrile R*, 200 volumes of *methanol R* and 600 volumes of a solution prepared as follows: dissolve 3.0 g of *sodium decanesulphonate R* and 5.0 g of *potassium dihydrogen phosphate R* in 900 ml of *water R*, adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R*.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µl.

Run time 1.5 times the retention time of clenbuterol .

Retention time Clenbuterol = about 29 min.

System suitability Reference solution (b):

- *resolution*: minimum 4.0 between the peaks due to impurity B and clenbuterol.

Limits:

- *impurities A, B, C, D, E,* F: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

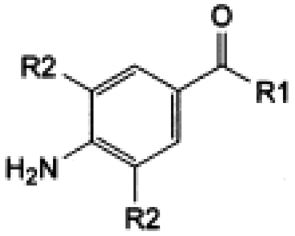
ASSAY

Dissolve 0.250 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.37 mg of $C_{12}H_{19}C_{l3}N_2O$.

IMPURITIES

Specified impurities *A, B, C, D, E, F.*



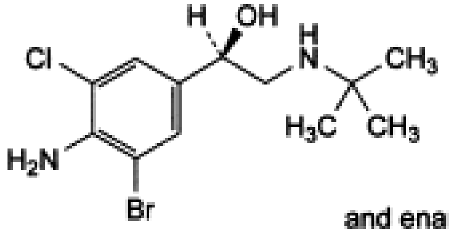
A. R1 = H, R2 = Cl: 4-amino-3,5-dichlorobenzaldehyde,

B. R1 = CH_2-NH-C$(CH_3)_3$, R2 = Cl: 1-(4-amino-3,5-dichlorophenyl)-2-[(1,1-dimethylethyl)amino]ethanone,

C. R1 = CH_3, R2 = Cl: 1-(4-amino-3,5-dichlorophenyl)ethanone,

D. R1 = CH_3, R2 = H: 1-(4-aminophenyl)ethanone,

E. R1 = CH_2Br, R2 = Cl: 1-(4-amino-3,5-dichlorophenyl)-2-bromoethanone,



and enantiomer

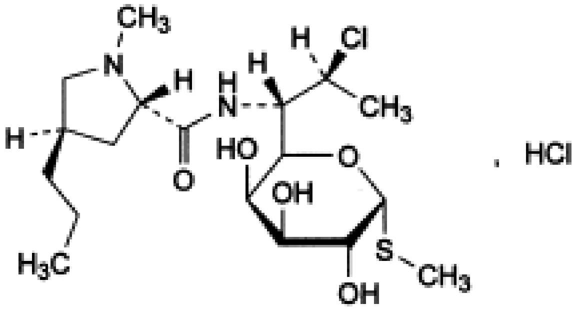
F. (1*RS*)-1-(4-amino-3-bromo-5-chlorophenyl)-2-[(1,1-dimethylethyl)amino]ethanol.

Ph Eur

Clindamycin Hydrochloride

General Notices

(Ph Eur monograph 0582)



$C_{18}H_{33}ClN_2O_5S,HCl$ 461.5 *21462-39-5*

Action and use

Lincosamide antibacterial.

Preparation

Clindamycin Capsules

Ph Eur

DEFINITION

Methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside hydrochloride. It contains a variable quantity of water.

Semi-synthetic product derived from a fermentation product.

Content

91.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *clindamycin hydrochloride CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *clindamycin hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *clindamycin hydrochloride CRS* and 10 mg of *lincomycin hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase Mix 19 volumes of *2-propanol R*, 38 volumes of a 150 g/l solution of *ammonium acetate R* adjusted to pH 9.6 with *ammonia R,* and 43 volumes of *ethyl acetate R.*

Application 5 µl.

Development Over a path of 15 cm using the upper layer of the mobile phase.

Drying In air.

Detection Spray with a 1 g/l solution of *potassium permanganate R.*

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 2 ml of *dilute hydrochloric acid R* and heat on a water-bath for 3 min. Add 3 ml of *sodium carbonate solution R* and 1 ml of a 20 g/l solution of *sodium nitroprusside R.* A violet-red colour develops.

D. Dissolve 0.1 g in *water R* and dilute to 10 ml with the same solvent. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

3.0 to 5.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 135 to + 150 (anhydrous substance).

Dissolve 1.000 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *clindamycin hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 45 volumes of *acetonitrile R* and 55 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 7.5 with a 250 g/l solution of *potassium hydroxide R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Run time Twice the retention time of clindamycin.

System suitability Reference solution (a):

- *Relative retention* with reference to clindamycin (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.65; impurity C = about 0.8.

Limits:

- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
- *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent),
- *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
- *total:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent),
- *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

3.0 per cent to 6.0 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection 20 µl of the test solution and reference solution (a).

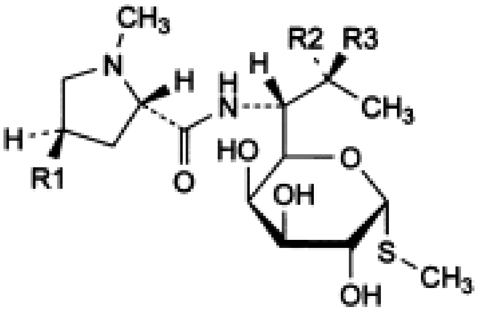
System suitability:

— *repeatability*: maximum relative standard deviation of 0.85 per cent after 6 injections of reference solution (a).

STORAGE

In an airtight container .

IMPURITIES



A. R1 = CH_2-CH_2-CH_3, R2 = OH, R3 = H: methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (lincomycin),

B. R1 = C_2H_5, R2 = H, R3 = Cl: methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-4-ethyl-1-methylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside (clindamycin B),

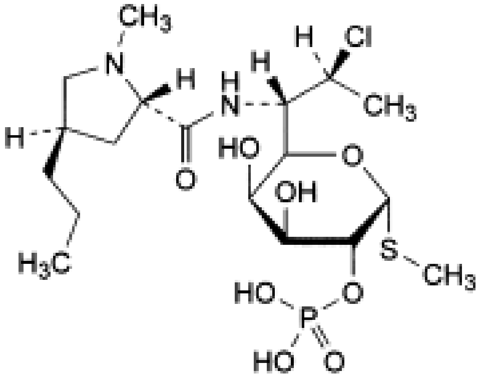
C. R1 = CH_2-CH_2-CH_3, R2 = Cl, R3 = H: methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (7-epiclindamycin).

Ph Eur

Clindamycin Phosphate

General Notices

(Ph Eur monograph 0996)



$C_{18}H_{34}ClN_2O_8PS$ 505.0 *24729-96-2*

Action and use

Lincosamide antibacterial.

Preparation

Clindamycin Injection

Ph Eur

DEFINITION

Methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-(dihydrogen phosphate).

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

In 2 separate tubes place 50 mg of the substance to be examined and 50 mg of *clindamycin phosphate CRS.* Add 0.2 ml of *water R* and heat until completely dissolved. Evaporate to dryness under reduced pressure and dry the residues at 100-105 °C for 2 h.

Comparison *clindamycin phosphate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *clindamycin phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *lincomycin hydrochloride CRS* in 5 ml of reference solution (a).

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over a path of 12 cm.

Drying At 100-105 °C for 30 min.

Detection Spray with a 1 g/l solution of *potassium permanganate R*.

System suitability Reference solution (b):

— the chromatogram shows 2 principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 2 ml of *dilute hydrochloric acid R* and heat in a water-bath for 3 min. Add 4 ml of *sodium carbonate solution R* and 1 ml of a 20 g/l solution of *sodium nitroprusside R*. Prepare a standard in the same manner using *clindamycin phosphate CRS*. The colour of the test solution corresponds to that of the standard.

D. Boil 0.1 g under a reflux condenser with a mixture of 5 ml of *strong sodium hydroxide solution R* and 5 ml of *water R* for 90 min. Cool and add 5 ml of *nitric acid R*. Extract with 3 quantities, each of 15 ml, of *methylene chloride R* and discard the extracts. Filter the upper layer through a paper filter. The filtrate gives reaction (b) of phosphates *(2.3.1)*.

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R*. Heat gently if necessary. Cool and dilute to 25.0 ml with *carbon dioxide-free water R*.

Appearance of the solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

3.5 to 4.5.

Dilute 5.0 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7)*

+ 115 to + 130 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 75.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 75.0 mg of *clindamycin phosphate CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *lincomycin hydrochloride CRS* (impurity A) and 15.0 mg of *clindamycin hydrochloride CRS* (impurity E) in 5.0 ml of reference solution (a), then dilute to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Mix 200 ml of *acetonitrile R1* and 800 ml of a 13.6 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.5 with *phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time The retention time of impurity E.

System suitability Reference solution (b):

— *resolution*: minimum 6.0 between the peaks due to clindamycin phosphate (2nd peak) and impurity E (3rd peak); if necessary, adjust the concentration of acetonitrile in the mobile phase;

- *symmetry factor*: maximum 1.5 for the peak due to clindamycin phosphate;
- the peak due to impurity A (1st peak) is clearly separated from the peak due to the solvent.

Limits:

- *any impurity*: for each impurity, not more than 2.5 times the area of the peak due to clindamycin phosphate in the chromatogram obtained with reference solution (c) (2.5 per cent);
- *total*: not more than 4 times the area of the peak due to clindamycin phosphate in the chromatogram obtained with reference solution (c) (4.0 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Water *(2.5.12)*

Maximum 6.0 per cent, determined on 0.250 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.6 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection The test solution and reference solution (a).

System suitability Reference solution (a):

- *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections; if necessary, adjust the integrator parameters.

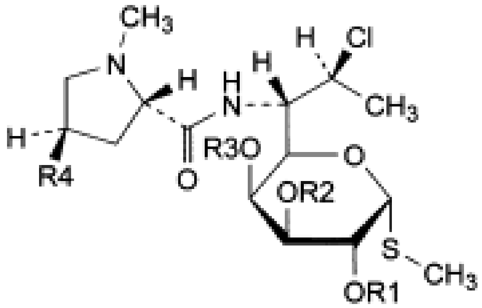
Calculate the percentage content of $C_{18}H_{34}ClN_2O_8PS$ from the declared content of *clindamycin phosphate CRS*.

STORAGE

In an airtight container, at a temperature not exceeding 30 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

A. lincomycin,



B. R1 = PO_3H_2, R2 = R3 = H, R4 = C_2H_5: clindamycin B 2-(dihydrogen phosphate),

C. R1 = R3 = H, R2 = PO_3H_2, R4 = C_3H_7: clindamycin 3-(dihydrogen phosphate),

D. R1 = R2 = H, R3 = PO_3H_2, R4 = C_3H_7: clindamycin 4-(dihydrogen phosphate),

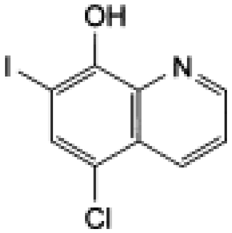
E. R1 = R2 = R3 = H, R4 = C_3H_7: clindamycin.

Ph Eur

Clioquinol

General Notices

(Ph Eur monograph 2111)



C_9H_5ClINO 305.5 *130-26-7*

Action and use

Antibacterial; antiprotozoal.

Preparations

Betamethasone and Clioquinol Cream

Betamethasone and Clioquinol Ointment

Hydrocortisone and Clioquinol Cream

Hydrocortisone and Clioquinol Ointment

Ph Eur

DEFINITION

5-Chloro-7-iodoquinolin-8-ol.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Almost white, light yellow, brownish-yellow or yellowish-grey powder.

Solubility

Practically insoluble in water, sparingly soluble in methylene chloride, very slightly soluble or slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Dissolve 40.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml to 100.0 ml with *methanol R* (solution A). Examined between 280 nm and 350 nm *(2.2.25)*, solution A shows an absorption maximum at 321 nm. Dilute 10.0 ml of solution A to 100.0 ml with *methanol R* (solution B). Examined between 230 nm and 280 nm, solution B shows an absorption maximum at 255 nm. The specific absorbance at this absorption maximum is 1530 to 1660.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *clioquinol CRS.*

C. When heated, violet fumes are produced.

D. Dissolve about 1 mg in 5 ml of *ethanol (96 per cent) R.* Add 0.05 ml of *ferric chloride solution R1*. A dark green colour develops.

TESTS

Acidity or alkalinity

Shake 0.5 g with 10 ml of *carbon dioxide-free water R* and filter. To the filtrate add 0.2 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent, heating gently if necessary. Dilute 10.0 ml of the solution to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *5-chloroquinolin-8-ol R*, 10.0 mg of *5,7-dichloroquinolin-8-ol R*, 5 mg of the substance to be examined and 10.0 mg of *5,7-diiodoquinolin-8-ol R* in *methanol R*, heating gently if necessary and dilute to 20.0 ml with the same solvent. Dilute 4.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 3.9 mm,

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.50 g of *sodium edetate R* in 350 ml of *water R*, add 4.0 ml of *hexylamine R* and mix. Adjust to pH 3.0 with *phosphoric acid R*. Add 600 ml of *methanol R* and dilute to 1000 ml with *water R*.

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 4 times the retention time of clioquinol.

Relative retention With reference to clioquinol (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.7; impurity C = about 1.3.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to clioquinol and impurity C.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (2.0 per cent),

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *any other impurity*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *total of the nominal contents of impurities A, B, C and any other impurities*: maximum 3.0 per cent,

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Halides

Maximum 140 ppm, expressed as chlorides.

Shake 0.5 g with 25 ml of *water R* for 1 min and filter. To the filtrate add 0.5 ml of *dilute nitric acid R* and 0.5 ml of *silver nitrate solution R2*. Allow to stand for 5 min. Any opalescence is not more intense than that in a standard prepared at the same time by adding 0.5 ml of *silver nitrate solution R2* to 25 ml of *water R* containing 0.2 ml of *0.01 M hydrochloric acid* and 0.5 ml of *dilute nitric acid R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 24 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 20 ml of *acetic anhydride R* and add 30 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

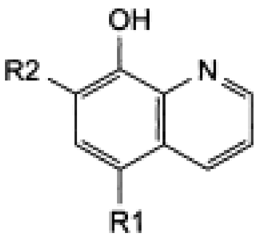
1 ml of *0.1 M perchloric acid* is equivalent to 30.55 mg of total quinolines, calculated as clioquinol.

STORAGE

Protected from light.

IMPURITIES

Specified impurities   A, B, C.



A. R1 = Cl, R2 = H: 5-chloroquinolin-8-ol,

B. R1 = R2 = Cl: 5,7-dichloroquinolin-8-ol,

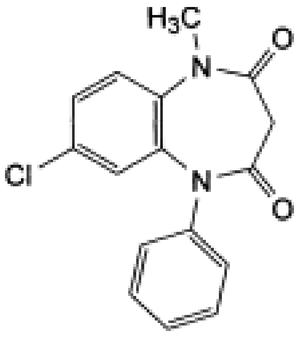
C. R1 = R2 = I: 5,7-diiodoquinolin-8-ol.

Ph Eur

Clobazam

General Notices

(Ph Eur monograph 1974)



$C_{16}H_{13}ClN_2O_2$ *300.7* *22316-47-8*

Action and use

Benzodiazepine.

Preparation

Clobazam Capsules

Ph Eur

DEFINITION

7-Chloro-1-methyl-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in methylene chloride, sparingly soluble in alcohol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of clobazam.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *clobazam impurity A CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *chlordiazepoxide CRS* and 5 mg of *clonazepam CRS* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 1 ml of the solution to 100 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm,
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water R* (40:60 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 5 times the retention time of clobazam.

Retention time clobazam = about 15 min.

System suitability Reference solution (b):

- *resolution*: minimum 1.3 between the peaks due to chlordiazepoxide and clonazepam.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- *any other impurity*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),
- *total of other impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent),
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

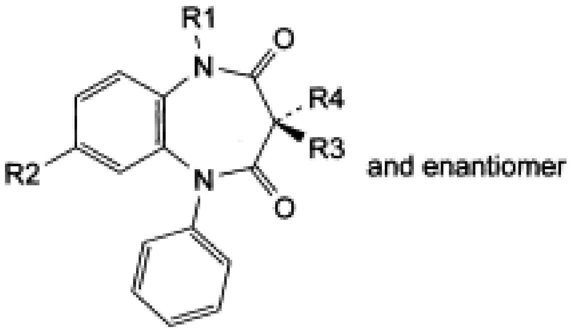
Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 50.0 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 250.0 ml with *alcohol R*. Measure the absorbance *(2.2.25)* at the maximum at 232 nm.

Calculate the content of $C_{16}H_{13}ClN_2O_2$ taking the specific absorbance to be 1380.

IMPURITIES

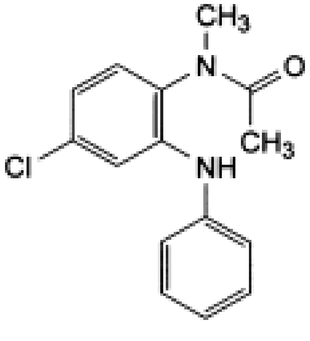


A. R1 = R3 = R4 = H, R2 = Cl: 7-chloro-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione,

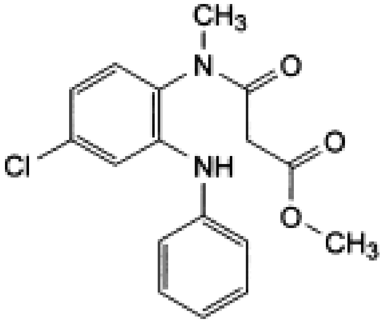
B. R1 = CH_3, R2 = R3 = R4 = H: 1-methyl-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione,

C. R1 = R3 = CH_3, R2 = Cl, R4 = H: (3*RS*)-7-chloro-1,3-dimethyl-5-phenyl-1,5-dihydro-3*H*-1, 5-benzodiazepine-2,4-dione,

D. R1 = R3 = R4 = CH_3, R2 = Cl: 7-chloro-1,3,3-trimethyl-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione,



E. *N*-[4-chloro-2-(phenylamino)phenyl]-*N*-methylacetamide,



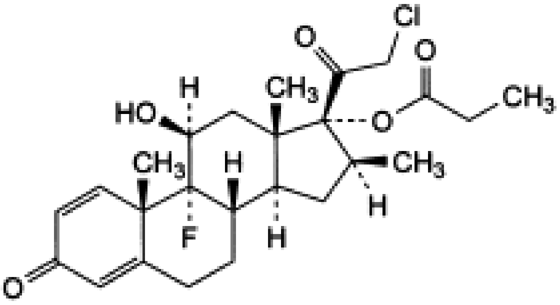
F. methyl 3-[[4-chloro-2-(phenylamino)phenyl]methylamino]-3-oxopropanoate.

Ph Eur

Clobetasol Propionate

General Notices

(Ph Eur monograph 2127)



$C_{25}H_{32}ClFO_5$ 467.0 *25122-46-7*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

21-Chloro-9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison clobetasol propionate CRS.

TESTS

Specific optical rotation *(2.2.7)*

+ 112 to + 118 (dried substance).

Dissolve 0.500 g in *acetone R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Test solution (b) Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *clobetasol propionate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *clobetasol impurity J CRS* in 2.0 ml of the mobile phase. To 0.5 ml of this solution add 0.5 ml of test solution (b) and dilute to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *clobetasol for peak identification CRS* (containing impurities A, B, C, D, E, L and M) in 2 ml of the mobile phase.

Reference solution (d) Dilute 1.0 ml of test solution (a) to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: spherical octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase Mix 10 volumes of *methanol R*, 42.5 volumes of a 7.85 g/l solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 5.5 with a 100 g/l solution of *sodium hydroxide R* and 47.5 volumes of *acetonitrile R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl of test solution (a) and reference solutions (b), (c) and (d).

Run time 3 times the retention time of clobetasol propionate.

Identification of impurities Use the chromatogram supplied with *clobetasol for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D, E, L and M.

Relative retention With reference to clobetasol propionate (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.6; impurity C = about 0.9; impurity J = about 1.1; impurity D = about 1.2; impurity L = about 1.3; impurity M = about 1.6; impurity E = about 2.1.

System suitability:

- *resolution*: minimum 2.0 between the peaks due to clobetasol propionate and impurity J in the chromatogram obtained with reference solution (b);

— the chromatogram obtained with reference solution (c) is similar to the chromatogram supplied with *clobetasol for peak identification CRS*.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.6; impurity C = 1.5;

— *impurity E*: not more than 1.4 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.7 per cent);

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *impurities B, C*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *impurities A, L, M*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.10 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{25}H_{32}ClFO_5$ using the chromatogram obtained with reference solution (a) and the declared content of *clobetasol propionate CRS*.

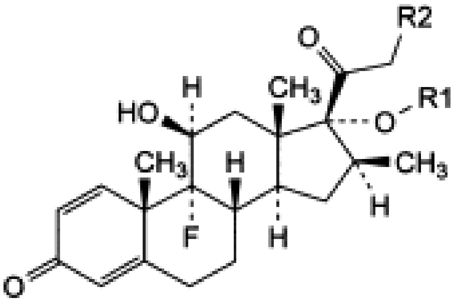
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, L, M.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : F, G, H, I, J, K.*



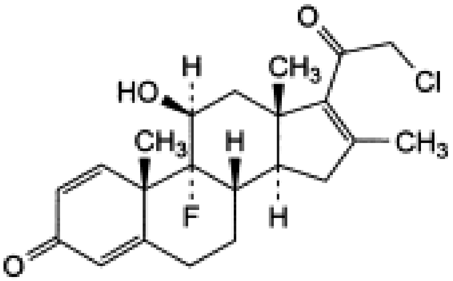
A. R1 = CO-C_2H_5, R2 = OH: 9-fluoro-11β,21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate (betamethasone 17-propionate),

G. R1 = H, R2 = Cl: 21-chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione (clobetasol),

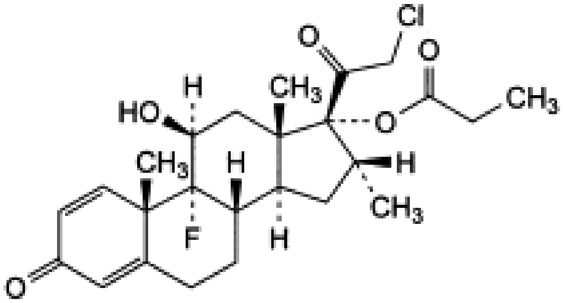
H. R1 = CO-C_2H_5, R2 = H: 9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,

I. R1 = CO-C_2H_5, R2 = O-SO_2-CH_3: 9-fluoro-11β-hydroxy-16β-methyl-21-[(methylsulphonyl) oxy]-3,20-dioxopregna-1,4-dien-17-yl propanoate,

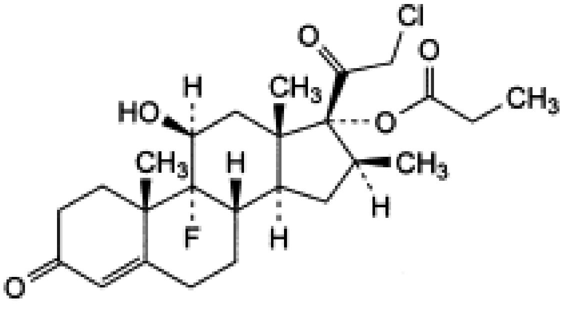
K. R1 = H, R2 = O-CO-C_2H_5: 9-fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl propanoate (betamethasone 21-propionate),



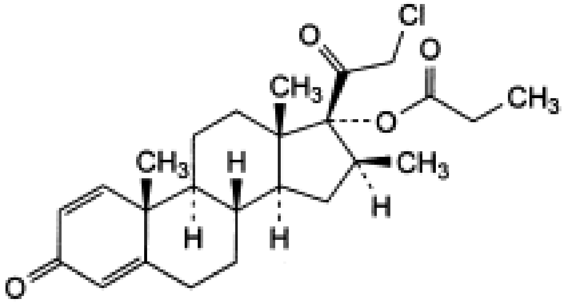
B. 21-chloro-9-fluoro-11β-hydroxy-16-methylpregna-1,4,16-triene-3,20-dione,



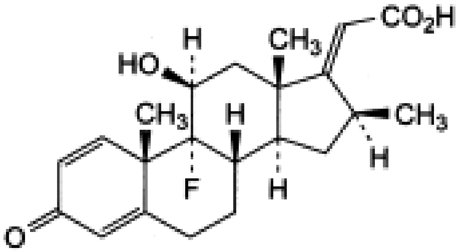
C. q21-chloro-9-fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,



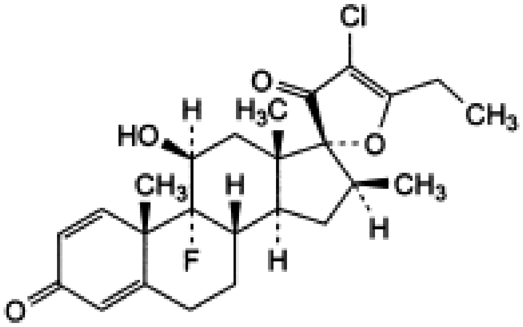
D. 21-chloro-9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregn-4-en-17-yl propanoate (1,2-dihydroclobetasol 17-propionate),



E. 21-chloro-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,



F. 9-fluoro-11β-hydroxy-16β-methyl-3-oxopregna-1,4,17(20)-trien-21-oic acid,



J. (17*R*)-4′-chloro-5′-ethyl-9-fluoro-11β-hydroxy-16β-methylspiro[androsta-1,4-diene-17,2′(3′*H*)-furan]-3,3′-dione (17α-spiro compound),

L. unknown structure,

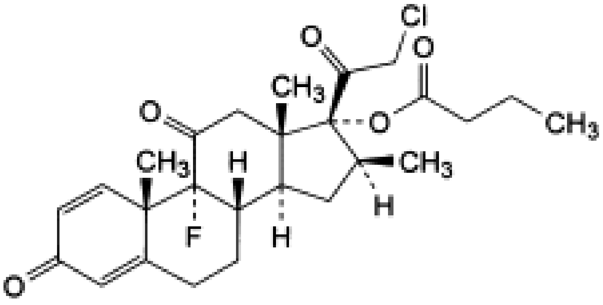
M. unknown structure.

Ph Eur

Clobetasone Butyrate

General Notices

(Ph Eur monograph 1090)



$C_{26}H_{32}ClFO_5$ 479.0 *25122-57-0*

Action and use

Glucocorticoid.

Preparations

Clobetasone Cream

Clobetasone Ointment

Ph Eur

DEFINITION

Clobetasone butyrate contains not less than 97.0 per cent and not more than the equivalent of 102.0 per cent of 21-chloro-9-fluoro-16β-methyl-3,11,20-trioxopregna-1,4-dien-17-yl butanoate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, practically insoluble in water, freely soluble in acetone and in methylene chloride, slightly soluble in alcohol.

It melts at about 178 °C.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *clobetasone butyrate CRS*. Examine the substances prepared as discs.

B. Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dissolve 10 mg of *clobetasone butyrate CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *clobetasol propionate R* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents. Dilute 5 ml of this solution to 10 ml with reference solution (a).

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of equal volumes of *cyclohexane R* and *methyl acetate R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The colour of the test solution is yellow and that of the blank is red.

TESTS

Specific optical rotation *(2.2.7)*

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 127 to + 133, calculated with reference to the dried substance.

Related substances

Prepare the solutions immediately before use.

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in 5.0 ml of *ethanol R* and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 2 mg of *clobetasone butyrate CRS* and 1.5 mg of *clobetasol propionate R* in 5 ml of *ethanol R* and dilute to 100 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.
The chromatographic procedure may be carried out using:

— a stainless steel column 0.20 m long and 4.6 mm in internal diameter, packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1 ml/min a mixture of 45 volumes of *ethanol R* and 55 volumes of *water R,*

— as detector a spectrophotometer set at 241 nm,

Maintaining the temperature of the column at 60 °C. Inject 20 µl of reference solution (a).

When using a recorder, adjust the sensitivity of the system so that the height of the two principal peaks are at least 50 per cent of the full scale of the recorder. The test is not valid unless the resolution between the first peak (clobetasol propionate) and the second peak (clobetasone butyrate) is at least five.

Inject 20 µl of the test solution and 20 µl of reference solution (b). Continue the chromatography for 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

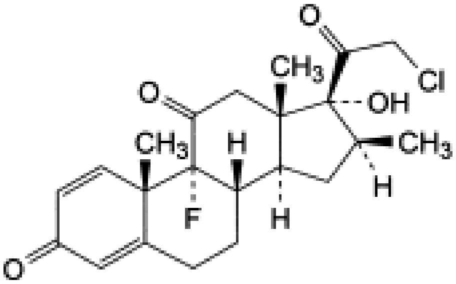
Dissolve 20.0 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *alcohol R*. Measure the absorbance *(2.2.25)* at the maximum at 235 nm.

Calculate the content of $C_{26}H_{32}ClFO_5$, taking the specific absorbance to be 327.

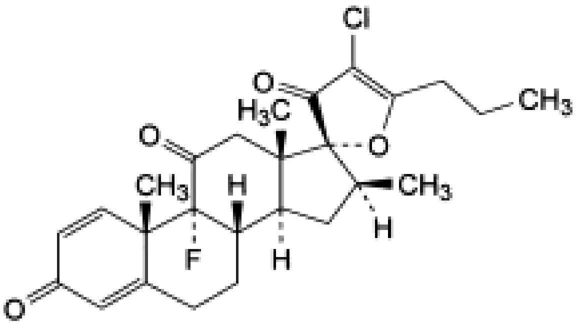
STORAGE

Store protected from light.

IMPURITIES



A. clobetasone,



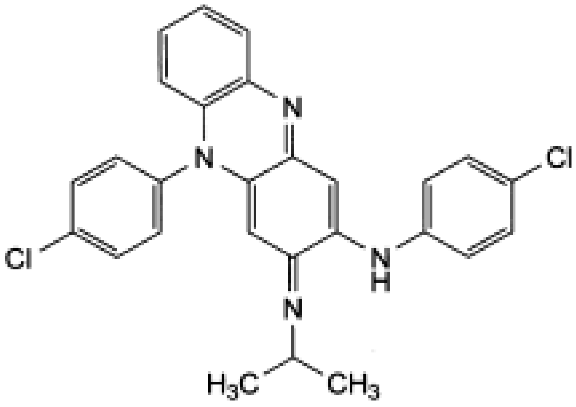
B. (17*R*)-4′-chloro-9-fluoro-16β-methyl-5′-propylspiro[androsta-1,4-diene-17,2′(3′*H*)furan]-3,3′,11-trione.

Ph Eur

Clofazimine

General Notices

(Ph Eur monograph 2054)



$C_{27}H_{22}Cl_2N_4$ 473.4 *2030-63-9*

Action and use

Antileprosy drug.

Preparation

Clofazimine Capsules

Ph Eur

DEFINITION

N,5-Bis(4-chlorophenyl)-3-[(1-methylethyl)imino]-3,5-dihydrophenazin-2-amine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Reddish-brown, fine powder.

Solubility

Practically insoluble in water, soluble in methylene chloride, very slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *clofazimine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R,* evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *clofazimine CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *propanol R, methylene chloride R* (6:85 *V/V*).

Application 5 µl.

First development Over 2/3 of the plate.

Drying Horizontally in air for 5 min.

Second development Over 2/3 of the plate.

Drying In air for 5 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 2 mg in 3 ml of *acetone R* and add 0.1 ml of *hydrochloric acid R*. An intense violet colour is produced. Add 0.5 ml of a 200 g/l solution of *sodium hydroxide R*; the colour changes to orange-red.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *clofazimine for system suitability CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 2.25 g of *sodium laurilsulfate R*, 0.85 g of *tetrabutylammonium hydrogen sulphate* R and 0.885 g of *disodium hydrogen phosphate R* in *water R*. Adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 500 ml with *water R*. Mix 35 volumes of this solution and 65 volumes of *acetonitrile R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Run time 3 times the retention time of clofazimine.

Identification of impurities Use the chromatogram supplied with *clofazimine for system suitability CRS* to identify the peak due to impurity B.

Relative retention With reference to clofazimine (retention time = about 15 min): impurity A = about 0.7; impurity B = about 0.8.

System suitability Reference solution (b):

- *resolution*: baseline separation between the peaks due to impurity B and clofazimine.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *total:* not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

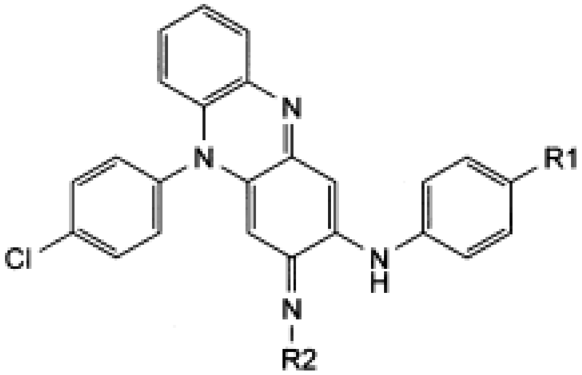
ASSAY

Dissolve 0.400 g in 5 ml of *methylene chloride R* and add 20 ml of *acetone R* and 5 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 47.34 mg of $C_{27}H_{22}Cl_2N_4$.

IMPURITIES

Specified impurities A, B.



A. R1 = Cl, R2 = H: *N*,5-bis(4-chlorophenyl)-3-imino-3,5-dihydrophenazin-2-amine,

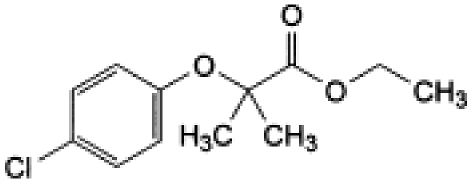
B. R1 = H, R2 = $CH(CH_3)_2$: 5-(4-chlorophenyl)-3-[(1-methylethyl)imino]-*N*-phenyl-3,5-dihydrophenazin-2-amine.

Ph Eur

Clofibrate

General Notices

(Ph Eur monograph 0318)



$C_{12}H_{15}ClO_3$ 242.7 *637-07-0*

Action and use

Fibrate; lipid-regulating drug.

Preparation

Clofibrate Capsules

Ph Eur

DEFINITION

Ethyl 2-(4-chlorophenoxy)-2-methylpropionate.

CHARACTERS

Appearance

Clear, almost colourless liquid.

Solubility

Very slightly soluble in water, miscible with ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *clofibrate CRS.*

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution (a) Dissolve 0.10 g in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *methanol R*.

Test solution (b) Dilute 10.0 ml of test solution (a) to 100.0 ml with *methanol R*.

Spectral range 250-350 nm for test solution (a); 220-250 nm for test solution (b).

Absorption maxima At 280 nm and 288 nm for test solution (a); at 226 nm for test solution (b).

Specific absorbances at the absorption maxima:

- — at 226 nm: about 460 for test solution (b);
- — at 280 nm: about 44 for test solution (a);
- — at 288 nm: about 31 for test solution (a).

TESTS

Relative density *(2.2.5)*

1.138 to 1.147.

Refractive index *(2.2.6)*

1.500 to 1.505.

Acidity

To 1.0 g add 10 ml of *anhydrous ethanol R* and 0.1 ml of *phenol red solution R*. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Volatile related substances

Gas chromatography (*2.2.28*).

Test solution To 10.0 g of the substance to be examined add a mixture of 10 ml of *dilute sodium hydroxide solution R* and 10 ml of *water R*. Shake, separate the lower (organic) layer, wash with 5 ml of *water R* and add the washings to the aqueous layer. Dry the organic layer with *anhydrous sodium sulphate R* and use as the test solution. Reserve the aqueous layer for the test for 4-chlorophenol.

Reference solution (a) Dissolve 0.12 g of the substance to be examined in *chloroform R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *chloroform R.*

Reference solution (b) Dissolve 0.12 g of *methyl 2-(4-chlorophenoxy)-2-methylpropionate CRS* in the substance to be examined and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with the substance to be examined. Dilute 1.0 ml of this solution to 10.0 ml with the substance to be examined.

Column:

- — *size*: *l* = 1.5 m, Ø = 4 mm;
- — *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (250-420 µm) impregnated with 30 per cent *m/m* of *poly(dimethyl)siloxane R*; or *silanised diatomaceous earth for gas chromatography R* (150-180 µm) impregnated with 10 per cent *m/m* of *poly (dimethyl)siloxane R*;
- — *temperature*: 185 °C.

Carrier gas *nitrogen for chromatography R.*

Detection Flame ionisation.

Injection 2 µl.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 4, where H_p = height above the baseline of the peak due to methyl 2-(4-chlorophenoxy)-2-methylpropionate and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to clofibrate.

Limit:

— *total*: not more than 10 times the area of the peak due to clofibrate in the chromatogram obtained with reference solution (a) (0.1 per cent).

4-Chlorophenol

Gas chromatography *(2.2.28)* as described in the test for volatile related substances with the following modifications.

Test solution Shake the aqueous layer reserved in the test for volatile related substances with 2 quantities, each of 5 ml, of *chloroform R* and discard the organic layers. Acidify the aqueous layer by the dropwise addition of *hydrochloric acid R.* Shake with 3 quantities, each of 3 ml, of *chloroform R*. Combine the organic layers and dilute to 10.0 ml with *chloroform R.*

Reference solution Dissolve 0.25 g of *chlorophenol R* in *chloroform R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *chloroform R*.

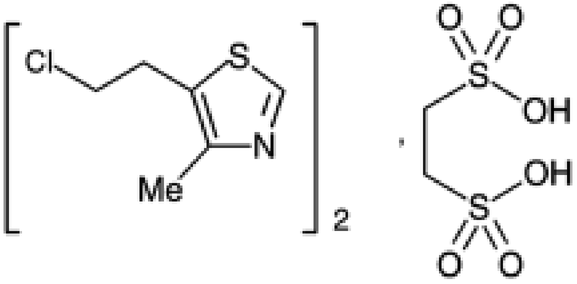
Limit:

— 4-chlorophenol: not more than the area of the peak due to 4-chlorophenol in the chromatogram obtained with the reference solution (25 ppm).

Ph Eur

Clomethiazole Edisilate

General Notices



$(C_6H_8ClNS)_2,C_2H_6O_6S_2$ 513.5   *1867-58-9*

Action and use

Hypnotic.

Preparations

Clomethiazole Intravenous Infusion

Clomethiazole Oral Solution

DEFINITION

Clomethiazole Edisilate is 5-(2-chloroethyl)-4-methylthiazole ethanedisulphonate. It contains not less than 99.0% and not more than 101.0% of $(C_6H_8ClNS)_2,C_2H_6O_6S_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Freely soluble in *water*; soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. *Melting point*, about 128°, Appendix V A.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.005% w/v solution in 0.1M *hydrochloric acid* exhibits a maximum only at 257 nm. The *absorbance* at the maximum is about 0.92.

C. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of clomethiazole edisilate *(RS 052)*.

D. Mix 0.1 g with 0.2 g of powdered *sodium hydroxide*, heat to fusion and continue heating for a further few seconds. Cool, add 0.5 ml of *water* and a slight excess of 2M *hydrochloric acid* and warm. Fumes are evolved which turn moistened *starch iodate paper* blue (distinction from clomethiazole).

TESTS

Calcium

10 ml of a 10.0% w/v solution diluted to 15 ml with *water* complies with the *limit test for calcium*, Appendix VII (100 ppm).

Heavy metals

Moisten the residue obtained in the test for Sulphated ash with 2 ml of *hydrochloric acid* and evaporate to dryness. Dissolve the residue in *water* and add sufficient *water* to produce 20 ml. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (20 ppm).

Chloride

10 ml of a 10% w/v solution diluted to 15 ml with *water* complies with the *limit test for chlorides*, Appendix VII (50 ppm).

Sulphate

10 ml of a 1.0% w/v solution diluted to 15 ml with *distilled water* complies with the *limit test for sulphates*, Appendix VII (0.15%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.30% w/v of the substance being examined in the mobile phase. For solution (2) dilute 1 volume of solution (1) to 1000 volumes with the mobile phase. For solution (3) dilute 1 volume of a 0.030% w/v solution of *4-methyl-5-vinylthiazole edisilate BPCRS* in *methanol* (solution A) to 50 volumes with the mobile phase. For solution (4) dilute 1 volume of a 0.020% w/v solution of *5-(2-chloroethyl)-4-methyl-3-[2-(4-methylthiazol-5-yl)ethyl]-thiazolium chloride BPCRS* (quaternary dimer) in *methanol* (solution B) to 50 volumes with the mobile phase. For solution (5) dilute 1 volume of a 0.020% w/v solution of *4-methyl-5-(2-hydroxyethyl)thiazole BPCRS* in *methanol* (solution C) to 50 volumes with the mobile phase. For solution (6) add 1 ml each of solutions A, B and C to 0.15 g of the substance being examined and dilute to 50 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute, a mixture of 70 volumes of a solution containing 0.13% w/v of *sodium hexanesulphonate* and 2.7% w/v of *tetramethylammonium hydrogen sulphate*, adjusted to pH 2.0 with 5M *sodium hydroxide*, and 30 volumes of *methanol* and (c) a detection wavelength of 257 nm.

The test is not valid unless in the chromatogram obtained with solution (6) baseline separation is achieved between the peaks due to the three specified impurities and also between the principal peak and the two adjacent specified impurity peaks.

Calculate the content of each of the three specified impurities in the substance being examined with reference to clomethiazole base (1 mg of clomethiazole edisilate is equivalent to 0.629 mg of base) expressing the content of 4-methyl-5-vinylthiazole as the base (1 mg of 4-methyl-5-vinylthiazole edisilate is equivalent to 0.568 mg of base). The total content of the three specified impurities is not greater than 0.5%. In the chromatogram obtained with solution (1) the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2).

Loss on drying

When dried at 50° at a pressure not exceeding 0.7 kPa for 6 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

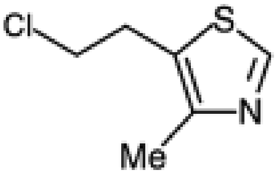
Not more than 0.3%, Appendix IX A. Use 1 g.

ASSAY

Dissolve 0.4 g in 50 ml of *water* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 25.67 mg of $(C_6H_8ClNS)_2,C_2H_6O_6S_2$.

Clomethiazole

General Notices



C_6H_8ClNS 161.6 *533-45-9*

Action and use

Hypnotic.

Preparation

Clomethiazole Capsules

DEFINITION

Clomethiazole is 5-(2-chloroethyl)-4-methyl-thiazole. It contains not less than 98.0% and not more than 101.0% of C_6H_8ClNS.

CHARACTERISTICS

A colourless to slightly yellowish brown liquid.

Slightly soluble in *water*; miscible with *ethanol (96%)* and with *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.004% w/v solution in 0.1M *hydrochloric acid* exhibits a maximum only at 257 nm. The *absorbance* at the maximum is about 1.1.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of clomethiazole *(RS 051)*.

C. Mix 0.1 g with 0.2 g of powdered *sodium hydroxide*, heat to fusion and continue heating for a further few seconds. Cool, add 0.5 ml of *water* and a slight excess of 2M *hydrochloric acid* and warm. Any fumes evolved do not turn moistened *starch iodate paper* blue (distinction from clomethiazole edisilate).

TESTS

Acidity or alkalinity

pH of a 0.5% w/v solution, 5.5 to 7.0, Appendix V L.

Heavy metals

Moisten the residue obtained in the test for Sulphated ash with 2 ml of *hydrochloric acid* and evaporate to dryness. Dissolve the residue in *water* and add sufficient *water* to produce 20 ml. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (20 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.20% w/v of the substance being examined in the mobile phase. For solution (2) dilute 1 volume of solution (1) to 1000 volumes with the mobile phase. For solution (3) dilute 1 volume of a 0.030% w/v solution of *4-methyl-5-vinylthiazole edisilate BPCRS* in *methanol* (solution A) to 50 volumes with the mobile phase. For solution (4) dilute 1 volume of a 0.020% w/v solution of *5-(2-chloroethyl)-4-methyl-3-[2-(4-methylthiazol-5-yl)ethyl]-thiazolium chloride BPCRS* (quaternary dimer) in *methanol* (solution B) to 50 volumes with the mobile phase. For solution (5) dilute 1 volume of a 0.020% w/v solution of *4-methyl-5-(2-hydroxyethyl)thiazole BPCRS* in *methanol* (solution C) to 50 volumes with the mobile phase. For solution (6) add 1 ml each of solutions A, B and C to 0.10 g of the substance being examined and dilute to 50 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute, a mixture of 70 volumes of a solution containing 0.13% w/v of *sodium hexanesulphonate* and 2.7% w/v of *tetramethylammonium hydrogen sulphate*, adjusted to pH 2.0 with 5M *sodium hydroxide*, and 30 volumes of *methanol* and (c) a detection wavelength of 257 nm.

The test is not valid unless in the chromatogram obtained with solution (6) baseline separation is achieved between the peaks due to the three specified impurities and also between the principal peak and the two adjacent specified impurity peaks.

Calculate the content of each of the three specified impurities in the substance being examined expressing the content of 4-methyl-5-vinylthiazole as the base (1 mg of 4-methyl-5-vinylthiazole edisilate is equivalent to 0.568 mg of base). The total content of the three specified impurities is not greater than 0.5%. In the chromatogram obtained with solution (1) the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2).

Sulphated ash

Not more than 0.1%, Appendix IX A. Use 1 g.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.3 g and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 16.16 mg of C_6H_8ClNS.

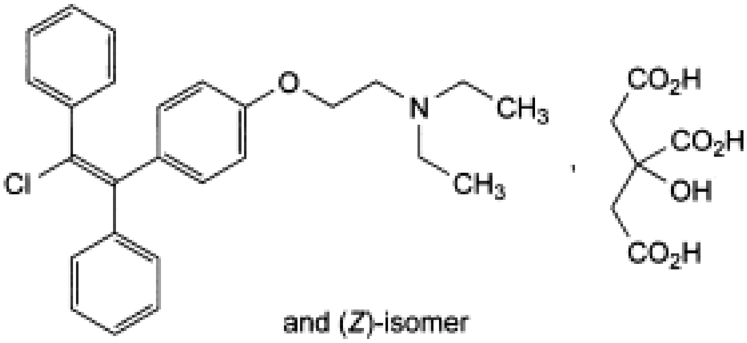
STORAGE

Clomethiazole should be stored at a temperature of 2° to 8°.

Clomifene Citrate

General Notices

(Ph Eur monograph 0997)



$C_{26}H_{28}ClNO,C_6H_8O_7$ 598.1 *50-41-9*

Action and use

Estrogen receptor modulator.

Preparation

Clomifene Tablets

Ph Eur

DEFINITION

Mixture of the (*E*)- and (*Z*)-isomers of 2-[4-(2-chloro-1,2diphenylethenyl)phenoxy]-*N*,*N*-diethylethanamine dihydrogen citrate.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or pale yellow, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *clomifene citrate CRS*.

B. Dissolve about 5 mg in 5 ml of a mixture of 1 volume of *acetic anhydride R* and 5 volumes of *pyridine R*, then heat in a water-bath. A deep red colour is produced.

TESTS

Prepare the solutions protected from light in brown-glass vessels. Ensure minimum exposure of the solutions to daylight until they are required for chromatography.

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 12.5 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 12.5 mg of *clomifene citrate for performance test CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *butylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 400 ml of *acetonitrile R* with 600 ml of *water R* and add 8.0 ml of *diethylamine R*; adjust to pH 6.2 with about 1-2 ml of *phosphoric acid R*, taking care to reduce progressively the volume of each addition as the required pH is approached.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 233 nm.

Equilibration With the mobile phase for about 1 h.

Injection 10 µl.

Run time 4 times the retention time of clomifene.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 15, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to clomifene; if necessary, adjust the concentration of acetonitrile in the mobile phase;

— the chromatogram obtained is similar to the chromatogram supplied with *clomifene citrate for performance test CRS*.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *impurities B, C, D, E, F, G, H*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak with a retention time relative to the clomifene peak of 0.2 or less.

(*Z*)-isomer

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25 mg of the substance to be examined in 25 ml of *0.1 M hydrochloric acid*, add 5 ml of *1 M sodium hydroxide* and shake with 3 quantities, each of 25 ml, of *ethanol-free chloroform R*. Wash the combined extracts with 10 ml of *water R,* dry over *anhydrous sodium sulphate R* and dilute to 100 ml with *ethanol-free chloroform R*. To 20 ml of this solution add 0.1 ml of *triethylamine R* and dilute to 100 ml with *hexane R*.

Reference solution Dissolve 25 mg of *clomifene citrate CRS* in 25 ml of *0.1 M hydrochloric acid*, add 5 ml of *1 M sodium hydroxide* and shake with 3 quantities, each of 25 ml, of *ethanol-free chloroform R*. Wash the combined extracts with 10 ml of *water R*, dry over *anhydrous sodium sulphate R* and dilute to 100 ml with *ethanol-free chloroform R*. To 20 ml of this solution add 0.1 ml of *triethylamine R* and dilute to 100 ml with *hexane R.*

Column:
— *size*: *l* = 0.3 m, Ø = 4 mm;

— *stationary phase: silica gel for chromatography R* (10 µm).

Mobile phase *triethylamine R*, *ethanol-free chloroform R, hexane R* (1:200:800 *V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 302 nm.

Equilibration With the mobile phase for about 2 h.

Injection 50 µl.

Identification of peaks The chromatogram obtained with the reference solution shows a peak due to the (*E*)-isomer just before a peak due to the (*Z*)-isomer.

System suitability Reference solution:

— *resolution*: minimum 1.0 between the peaks due to the (*E*)- and (*Z*)-isomers; if necessary, adjust the relative proportions of ethanol-free chloroform and hexane in the mobile phase.

Measure the area of the peak due to the (*Z*)-isomer in the chromatograms obtained with the test solution and the reference solution. Calculate the content of the (*Z*)-isomer, as a percentage of the total clomifene citrate present, from the declared content of *clomifene citrate CRS.*

Limit:
— *(Z)-isomer*: 30.0 per cent to 50.0 per cent.

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 1.000 g.

ASSAY

Dissolve 0.500 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

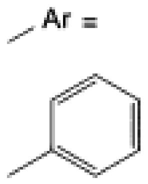
1 ml of *0.1 M perchloric acid* is equivalent to 59.81 mg of $C_{32}H_{36}ClNO_8$.

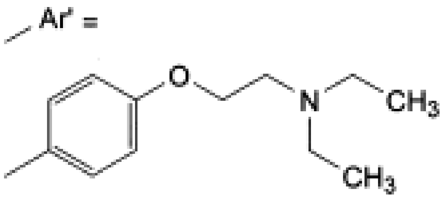
STORAGE

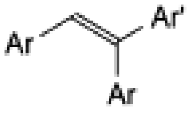
Protected from light.

IMPURITIES

Specified impurities   A, B, C, D, E, F, G, H.

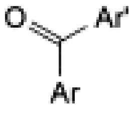




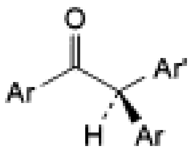


and (*Z*)-isomer

A. 2-[4-(1,2-diphenylethenyl)phenoxy]-*N,N*-diethylethanamine,

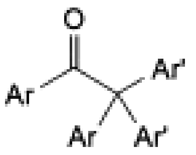


B. [4-[2-(diethylamino)ethoxy]phenyl]phenylmethanone,

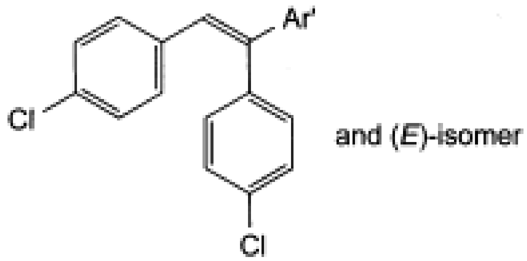


and enantiomer

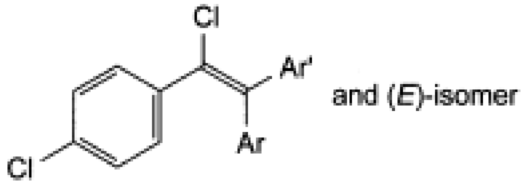
C. (2*RS*)-2-[4-[2-(diethylamino)ethoxy]phenyl]-1,2-diphenylethanone,



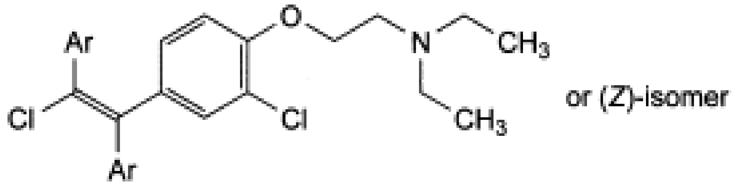
D. 2,2-bis[4-[2-(diethylamino)ethoxy]phenyl]-1,2-diphenylethanone,



E. 2-[4-[1,2-bis(4-chlorophenyl)ethenyl]phenoxy]-*N,N*-diethylethanamine,



F. 2-[4-[2-chloro-2-(4-chlorophenyl)-1-phenylethenyl]phenoxy]-*N,N*-diethylethanamine,



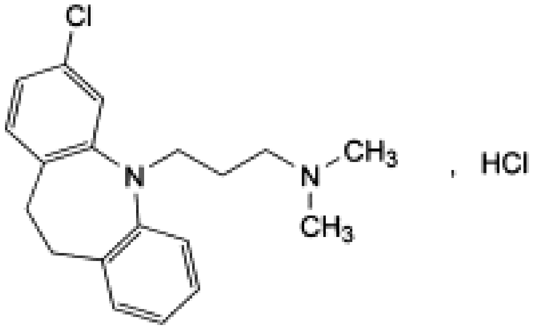
GH. 2-[2-chloro-4-(2-chloro-1,2-diphenylethenyl)phenoxy]-*N,N*-diethylethanamine (G. higher-melting-point isomer; *H*. lower-melting-point isomer).

Ph Eur

Clomipramine Hydrochloride

General Notices

(Ph Eur monograph 0889)



$C_{19}H_{23}ClN_2,HCl$ 351.3 *17321-77-6*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Clomipramine Capsules

Ph Eur

DEFINITION

3-(3-Chloro-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder, slightly hygroscopic.

Solubility

Freely soluble in water and in methylene chloride, soluble in alcohol.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Melting point *(2.2.14)*: 191 °C to 195 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*. The transmittance at about 2000 cm^{-1} (5 µm) is at least 65 per cent without compensation.

Comparison *clomipramine hydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use and protected from light.*

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *clomipramine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, acetone R*, *ethyl acetate R* (5:25:75 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 5 g/l solution of *potassium dichromate R* in a 20 per cent *V/V* solution of *sulphuric acid R*. Examine immediately.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 5 mg in 2 ml of *nitric acid R*. An intense blue colour develops.

E. Dissolve about 50 mg in 5 ml of *water R* and add 1 ml of *dilute ammonia R1*. Mix, allow to stand for 5 min and filter. Acidify the filtrate with *dilute nitric acid R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method I)*.

pH *(2.2.3)*

3.5 to 5.0 for solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protected from light.*

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Reference solution (a) Dissolve 22.6 mg of *imipramine hydrochloride CRS*, 4.0 mg of *clomipramine impurity C CRS*, 4.0 mg of *clomipramine impurity D CRS* and 2.0 mg of *clomipramine impurity F CRS* in a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A and dilute to 100.0 ml with the same mixture of mobile phases. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A.

Reference solution (c) Dissolve 10.0 mg of *clomipramine hydrochloride CRS* and 3.0 mg of *clomipramine impurity C CRS* in a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A and dilute to 20.0 ml with the same mixture of mobile phases. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A.

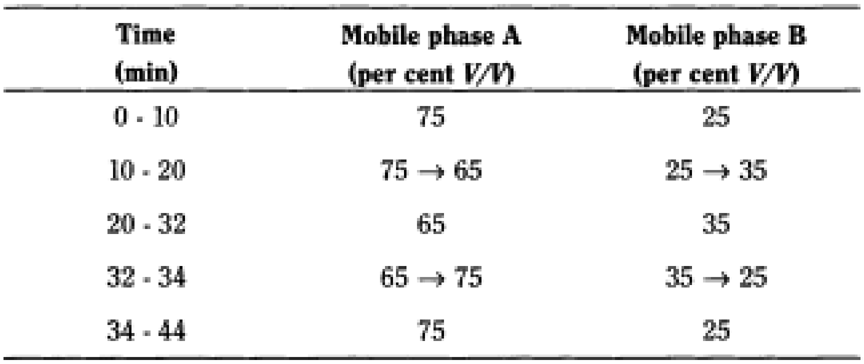
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: cyanopropylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: dissolve 1.2 g of *sodium dihydrogen phosphate R* in *water R*, add 1.1 ml of *nonylamine R,* adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 ml with *water R*,

— *mobile phase B*: *acetonitrile R*.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	75	25
10 - 20	75 → 65	25 → 35
20 - 32	65	35
32 - 34	65 → 75	35 → 25
34 - 44	75	25

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Relative retentions With reference to clomipramine (retention time = about 8 min): impurity A = about 0.5; impurity B = about 0.7; impurity C = about 0.9; impurity D = about 1.7; impurity E =

about 2.5; impurity F = about 3.4; impurity G = about 4.3.

System suitability Reference solution (c):

- *resolution*: minimum 3.0 between the peaks due to clomipramine and to impurity C.

Limits:

- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.0 per cent),
- *impurity C, D*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
- *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *any other impurity*: not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *total of other impurities*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
- *disregard limit*: 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

2.0 g complies with limit test C. Prepare the standard using 4 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

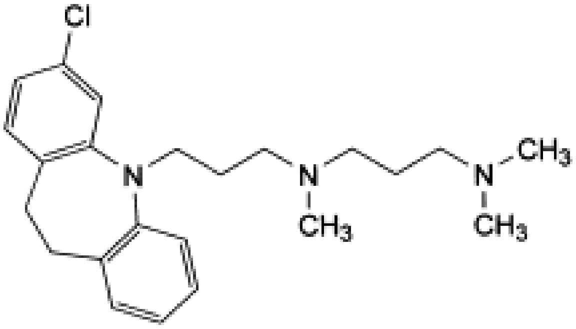
Dissolve 0.250 g in 50 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 35.13 mg of $C_{19}H_{24}Cl_2N_2$.

STORAGE

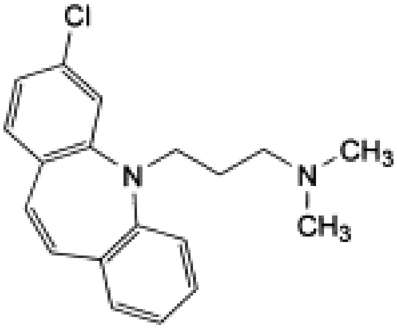
In an airtight container, protected from light.

IMPURITIES

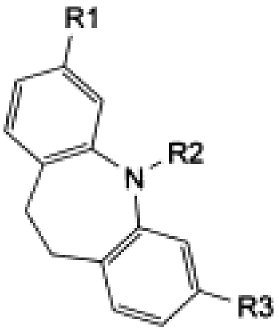


A. *N*-[3-(3-chloro-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)propyl]-*N,N′,N′*-trimethylpropane-1,3-diamine,

B. imipramine,



C. 3-(3-chloro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine,



D. R1 = R3 = Cl, R2 = CH_2-CH_2-CH_2-N$(CH_3)_2$: 3-(3,7-dichloro-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine,

E. R1 = R2 = R3 = H: 10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (iminodibenzyl),

F. R1 = Cl, R2 = R3 = H: 3-chloro-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine,

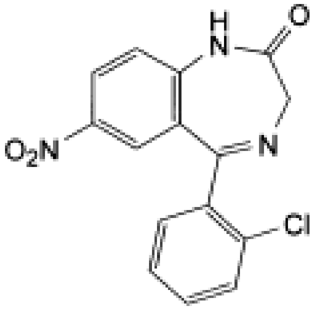
G. R1 = Cl, R2 = CH_2-CH=CH_2, R3 = H: 3-chloro-5-(prop-2-enyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine.

Ph Eur

Clonazepam

General Notices

(Ph Eur monograph 0890)



$C_{15}H_{10}ClN_3O_3$ 315.7 *1622-61-3*

Action and use

Benzodiazepine.

Preparation

Clonazepam Injection

Ph Eur

DEFINITION

5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Slightly yellowish, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in alcohol and in methanol.

mp

About 239 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   Ph. Eur. reference spectrum of clonazepam.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture   tetrahydrofuran R, methanol R, water R (10:42:48 *V/V/V*).

Test solution   Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 20.0 ml with the same solvent. Dilute 1.0 ml to 10.0 ml with the solvent mixture.

Reference solution (a)   Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b)   Dissolve 5 mg of the substance to be examined and 5 mg of *flunitrazepam R* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (c)   Dissolve 1.0 mg of *clonazepam impurity B CRS* in the solvent mixture and dilute to 20.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Column:
- — *size*: *l* = 0.15 m, Ø = 4.6 mm,
- — *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase   Mix 10 volumes of *tetrahydrofuran R*, 42 volumes of *methanol R* and 48 volumes of a 6.6 g/l solution of *ammonium phosphate R* previously adjusted to pH 8.0 with a 40 g/l solution of *sodium hydroxide R* or *dilute phosphoric acid R*.

Flow rate   1.0 ml/min.

Detection   Spectrophotometer at 254 nm.

Injection   10 µl.

Run time   3 times the retention time of clonazepam.

Relative retention   With reference to clonazepam (retention time = about 7 min): impurity B = about 2.1; impurity A = about 2.4.

System suitability   Reference solution (b):

- — *resolution*: minimum 1.8 between the peaks due to flunitrazepam and to clonazepam.

Limits:
- — *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- — *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent)
- — *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.275 g in 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

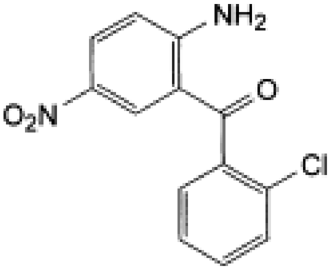
1 ml of *0.1 M perchloric acid* is equivalent to 31.57 mg of $C_{15}H_{10}ClN_3O_3$.

STORAGE

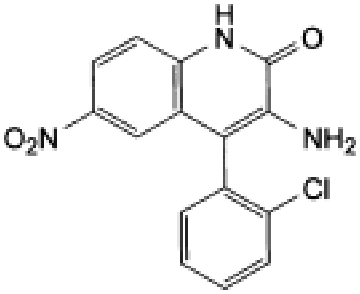
Protected from light.

IMPURITIES

Specified impurities *A, B.*



A. (2-amino-5-nitrophenyl)(2-chlorophenyl)methanone,



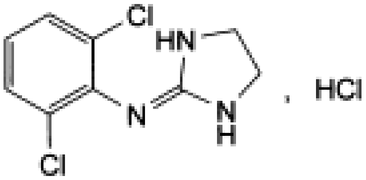
B. 3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2(1*H*)-one.

Ph Eur

Clonidine Hydrochloride

General Notices

(Ph Eur monograph 0477)



$C_9H_9Cl_2N_3,HCl$ 266.6 *4205-91-8*

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

Preparations

Clonidine Injection

Clonidine Tablets

Ph Eur

DEFINITION

2,6-Dichloro-*N*-(imidazolidin-2-ylidene)aniline hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water and in anhydrous ethanol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 30.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid.

Spectral range 245-350 nm.

Absorption maxima At 272 nm and 279 nm.

Point of inflexion At 265 nm.

Specific absorbance at the absorption maxima:

— at 272 nm: about 18;

— at 279 nm: about 16.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *clonidine hydrochloride CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 5 mg of *clonidine hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *glacial acetic acid R, butanol R, water R* (10:40:50 *V/V/V*); allow to separate, filter the upper layer and use the filtrate.

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *potassium iodobismuthate solution R2*. Allow to dry in air for 1 h. Spray again with *potassium iodobismuthate solution R2* and then immediately spray with a 50 g/l solution of *sodium nitrite R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II).*

pH *(2.2.3)*

4.0 to 5.0 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

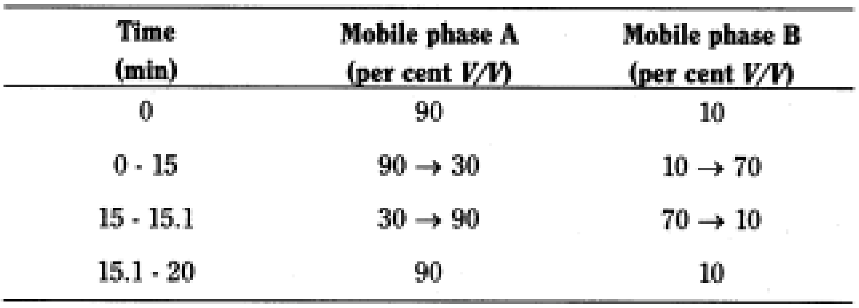
Test solution Dissolve 50 mg of the substance to be examined in mobile phase A and dilute to 50 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 5 mg of *clonidine impurity B CRS* in mobile phase A and dilute to 5 ml with mobile phase A. To 1 ml of this solution, add 1 ml of the test solution and dilute to 10 ml with mobile phase A.

Column:
- *size*: *l* = 0.15 m, Ø = 3.0 mm;
- *stationary phase: propylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: dissolve 4 g of *potassium dihydrogen phosphate R* in 1000 ml of *water for chromatography R*, and adjust to pH 4.0 with *phosphoric acid R*;
- *mobile phase B*: *mobile phase A, acetonitrile R1* (25:75 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0	90	10
0 - 15	90 → 30	10 → 70
15 - 15.1	30 → 90	70 → 10
15.1 - 20	90	10

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 5 µl.

System suitability Reference solution (b):

- *resolution*: minimum 5 between the peaks due to clonidine and impurity B.

Limits:
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

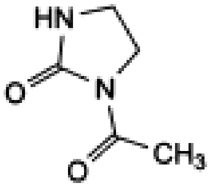
ASSAY

Dissolve 0.200 g in 70 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M ethanolic sodium hydroxide determining* the end-point potentiometrically *(2.2.20)*.

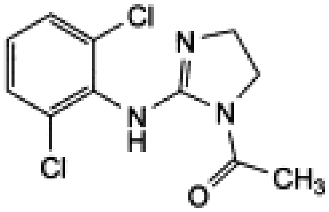
1 ml of *0.1 M sodium hydroxide* is equivalent to 26.66 mg of $C_9H_{10}Cl_3N_3$.

IMPURITIES

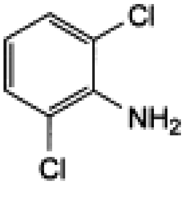
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : A, B, C.*



A. 1-acetylimidazolidin-2-one,



B. 1-acetyl-2-[(2,6-dichlorophenyl)amino]-4,5-dihydro-1*H*-imidazole,



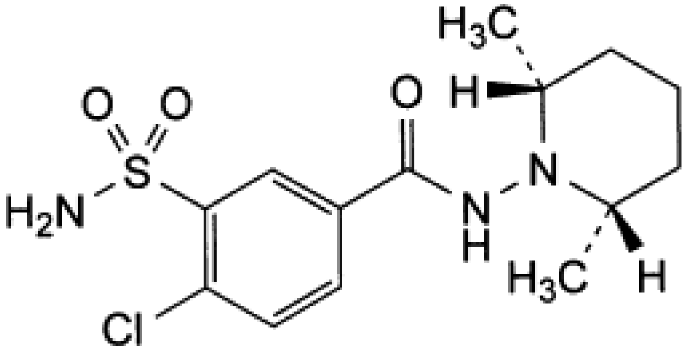
C. 2,6-dichloroaniline.

Ph Eur

Clopamide

General Notices

(Ph Eur monograph 1747)



$C_{14}H_{20}ClN_3O_3S$ 345.8 *636-54-4*

Action and use

Thiazide-like diuretic.

Ph Eur

DEFINITION

4-Chloro-*N*-[(2*RS*,6*SR*)-2,6-dimethylpiperidin-1-yl]-3-sulfamoylbenzamide.

Content

99.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

The production method is evaluated to determine the potential for formation of an *N*-nitroso compound (*cis*-2,6-dimethyl-1-nitrosopiperidine). Where necessary, the production method is validated to demonstrate that the *N*-nitroso compound is absent in the final product.

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Slightly soluble in water and in anhydrous ethanol, sparingly soluble in methanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison clopamide CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R,* evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 100 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *clopamide for system suitability CRS* (containing impurities B, C and H) in 1.0 ml of *methanol R*.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 2.0 ml of this solution to 40.0 ml with *methanol R.*

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);

Mobile phase:

— *mobile phase A*: dissolve 1.0 g of *ammonium acetate R* in 950 ml of *water R*, adjust to pH 2.0 with *phosphoric acid R* and dilute to 1000 ml with *water R;*

— *mobile phase B*: *acetonitrile R*;

— *mobile phase C*: *water R*, *tetrahydrofuran for chromatography R* (20:80 *V/V*); this mobile phase allows adequate rinsing of the system;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 35	95 → 75	5 → 25	0
35 - 45	75 → 35	25 → 65	0
45 - 50	35 → 30	65 → 0	0 → 70
50 - 60	30	0	70
60 - 63	30 → 95	0 → 5	70 → 0
63 - 73	95	5	0

Flow rate 0.4 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *clopamide for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, C and H.

Relative retention With reference to clopamide (retention time = about 33 min): impurity C = about 0.8; impurity H = about 1.2; impurity B = about 1.4.

System suitability Reference solution (a):

— *resolution*: minimum 3 between the peaks due to impurity C and clopamide.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.5; impurity H = 0.4;

— *impurities B, C, H*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 0.25 g in a mixture of 20 volumes of *acetone R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 20 ml of the solution complies with modified test B. Prepare the reference solution by diluting 0.5 ml of *lead standard solution (10 ppm Pb) R* to 20 ml with a mixture of 20 volumes of *acetone R* and 85 volumes of *methanol R*. Prepare the blank solution by using 20 ml of a mixture of 20 volumes of *acetone R* and 85 volumes of *methanol R.*

Filter the solutions through a membrane filter (0.45 µm) to evaluate the result.

Loss on drying *(2.2.32)*

Maximum 2.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.280 g in 70 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20).*

1 ml of *0.1 M perchloric acid* is equivalent to 34.58 mg of $C_{14}H_{20}ClN_3O_3S$.

STORAGE

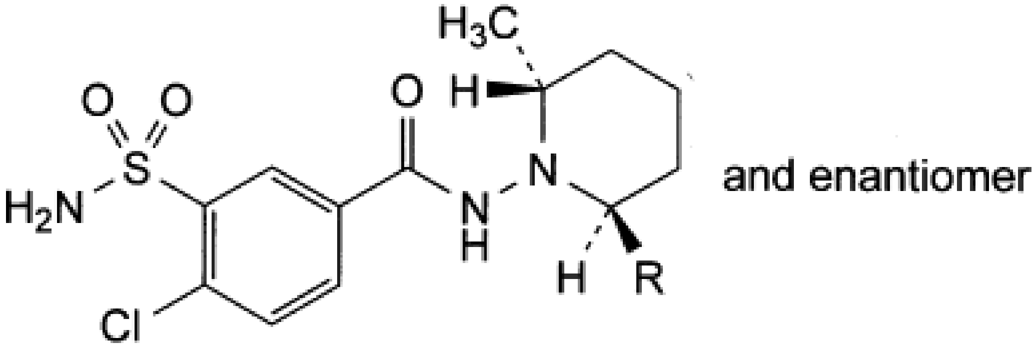
In an airtight container, protected from light.

IMPURITIES

Specified impurities *B, C, H.*

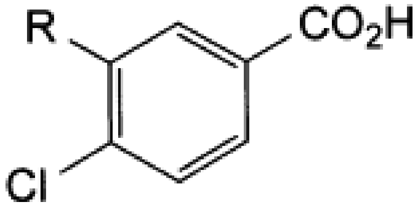
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*):

A, G.



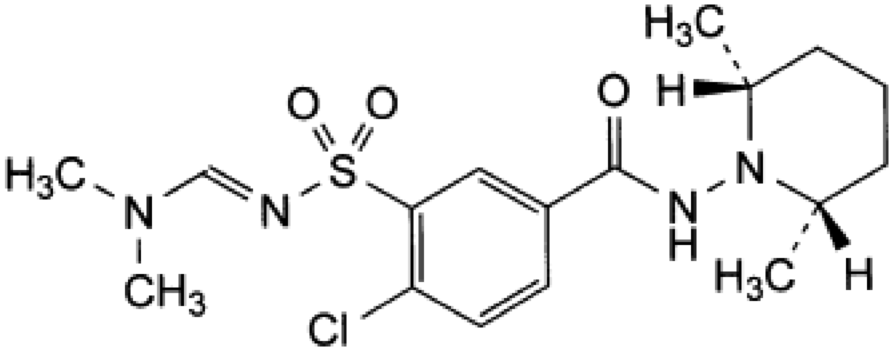
A. R = CH_3: 4-chloro-*N*-[(2*RS*,6*RS*)-2,6-dimethylpiperidin-1-yl]-3-sulfamoylbenzamide (*trans*-clopamide),

G. R = H: 4-chloro-*N*-[(2*RS*)-2-methylpiperidin-1-yl]-3-sulfamoylbenzamide,



B. R = H: 4-chlorobenzoic acid,

C. R = SO_2-NH_2: 4-chloro-3-sulfamoylbenzoic acid,


H_3C
H
O
O
O
H_3C
N
N
S
N
H
N
CH_3
Cl
H_3C
H

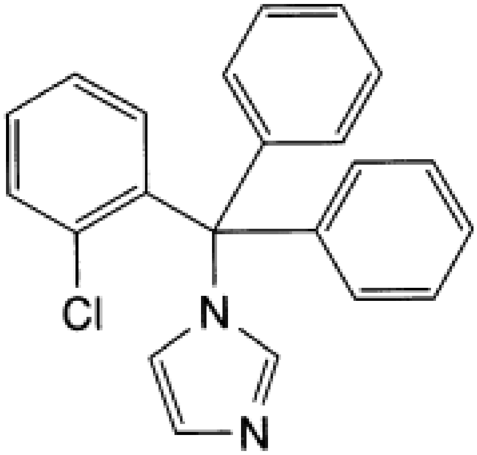
H. 4-chloro-3-[(*E*)-[(dimethylamino)methylene]sulfamoyl]-*N*-[(2*RS*,6*SR*)-2,6-dimethylpiperidin-1-yl]benzamide.

Ph Eur

Clotrimazole

General Notices

(Ph Eur monograph 0757)



$C_{22}H_{17}ClN_2$ 344.8 *23593-75-1*

Action and use

Antifungal.

Preparations

Clotrimazole Cream

Clotrimazole Pessaries

Ph Eur

DEFINITION

1-[(2-Chlorophenyl)diphenylmethyl]-1*H*-imidazole.

Content

98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or pale yellow, crystalline powder.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Melting point (*2.2.14*): 141 °C to 145 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *clotrimazole CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 50 mg of *clotrimazole CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *concentrated ammonia R1*, *propanol R*, *toluene R* (0.5:10:90 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *acetonitrile R1* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R1*. Dilute 1.0 ml of this solution to 10.0 ml with *acetonitrile R1.*

Reference solution (b) Dissolve the contents of a vial of *clotrimazole for peak identification CRS* (containing impurities A, B and F) in 1.0 ml of *acetonitrile R1.*

Reference solution (c) Dissolve 5.0 mg of *imidazole CRS* (impurity D) and 5.0 mg of *clotrimazole impurity E CRS* in *acetonitrile R1* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 25.0 ml with *acetonitrile R1.*

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

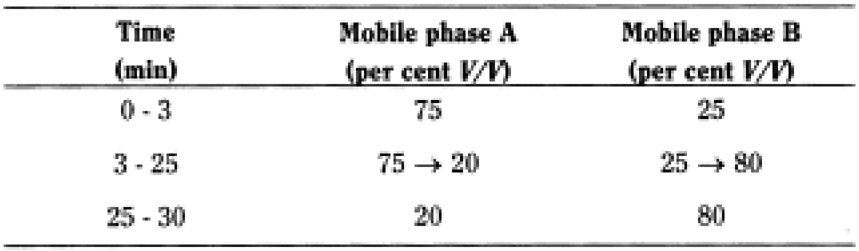
— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: dissolve 1.0 g of *potassium dihydrogen phosphate R* and 0.5 g of *tetrabutylammonium hydrogen sulphate R1* in *water R* and dilute to 1000 ml with the same solvent;

— *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	75	25
3 - 25	75 → 20	25 → 80
25 - 30	20	80

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Relative retention With reference to clotrimazole (retention time = about 12 min): impurity D = about 0.1; impurity F = about 0.9; impurity B = about 1.1; impurity E = about 1.5; impurity A = about 1.8.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity F and clotrimazole;

— the chromatogram obtained is similar to the chromatogram supplied with *clotrimazole for peak identification CRS.*

Limits:

— *impurities A, B*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities D, E*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurity F:* not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 80 ml of *anhydrous acetic acid R*. Using 0.3 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 34.48 mg of $C_{22}H_{17}ClN_2$.

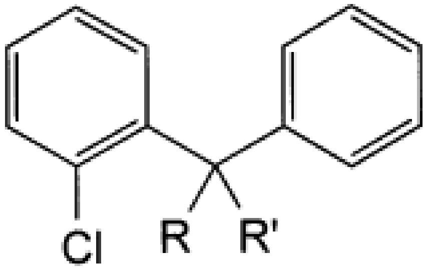
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, D, E, F.*

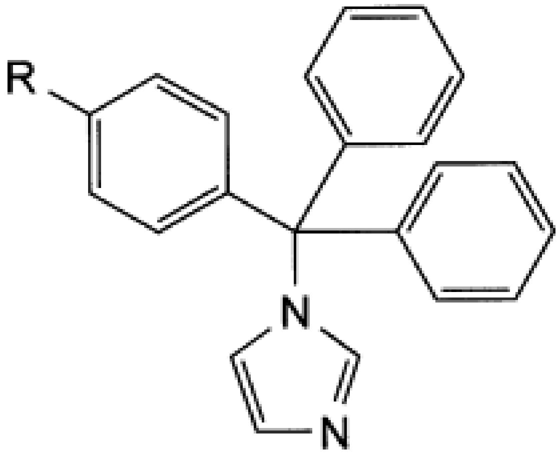
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C.*



A. R = OH, R′ = C_6H_5: (2-chlorophenyl)diphenylmethanol,

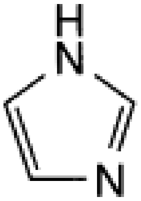
C. R = Cl, R′ = C_6H_5: 1-chloro-2-(chlorodiphenylmethyl)benzene,

E. R + R′ = O: (2-chlorophenyl)phenylmethanone (2-chlorobenzophenone),



B. R = Cl: 1-[(4-chlorophenyl)diphenylmethyl]-1*H*-imidazole,

F. R = H: 1-(triphenylmethyl)-1*H*-imidazole (deschloroclotrimazole),



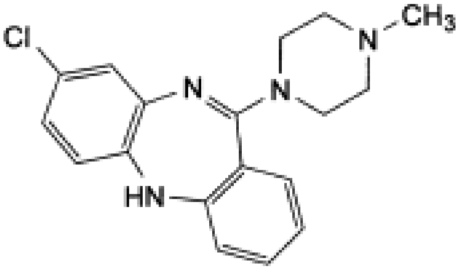
D. imidazole.

Ph Eur

Clozapine

General Notices

(Ph Eur monograph 1191)



$C_{18}H_{19}ClN_4$ 326.8 *5786-21-0*

Action and use

Dopamine D_4 receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

8-Chloro-11-(4-methylpiperazin-1-yl)-5*H*-dibenzo[*b,e*][1,4]diazepine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in ethanol (96 per cent). It dissolves in dilute acetic acid.

IDENTIFICATION

A. Melting point *(2.2.14)*. 182 °C to 186 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison clozapine CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *water R, methanol R2* (20:80 *V/V*).

Solution A Dissolve 2.04 g of *potassium dihydrogen phosphate R* in 1000 ml of *water R* and adjust to pH 2.4 ± 0.05 with *dilute phosphoric acid R*.

Test solution Dissolve 75 mg of the substance to be examined in 80 ml of *methanol R2* and dilute to 100 ml with *water R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

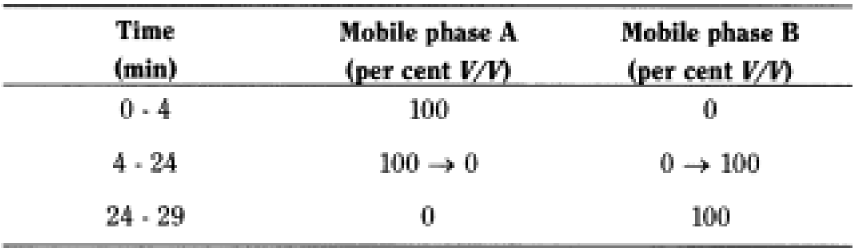
Reference solution (b) Dissolve the contents of a vial of *clozapine for peak identification CRS* (containing impurities A, B, C and D) in 1.0 ml of the solvent mixture.

Column:

- *size*: *l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A: acetonitrile for chromatography R, methanol R2*, solution A (1:1:8 *V/V/V*) ;
- *mobile phase B: acetonitrile for chromatography R, methanol R2*, solution A (4:4:2 *V/V/V*) ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 24	100 → 0	0 → 100
24 - 29	0	100

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 257 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *clozapine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and D.

Relative retention With reference to clozapine (retention time = about 11 min): impurity C = about 0.9; impurity D = about 1.1; impurity A = about 1.6; impurity B = about 1.7.

System suitability Reference solution (b):

- *resolution*: minimum 2.5 between the peaks due to impurity C and clozapine;
- the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *clozapine for peak identification CRS*.

Limits:

- — *correction factor*: for the calculation of content, multiply the peak area of impurity D by 2.7;
- — *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- — *impurities B, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

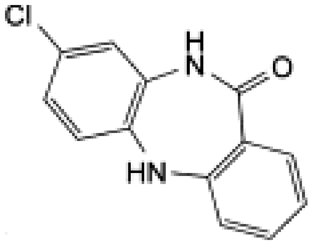
ASSAY

Dissolve 0.100 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

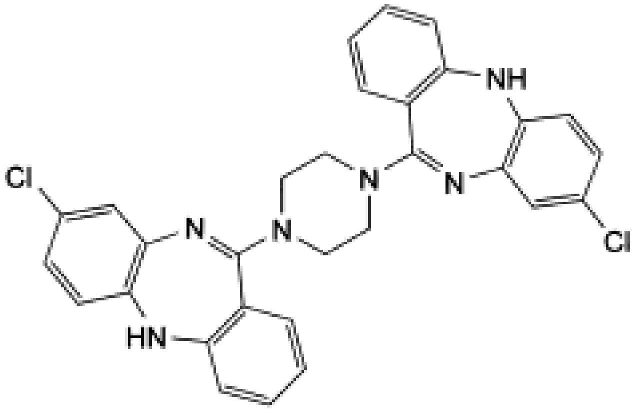
1 ml of *0.1 M perchloric acid* is equivalent to 16.34 mg of $C_{18}H_{19}ClN_4$.

IMPURITIES

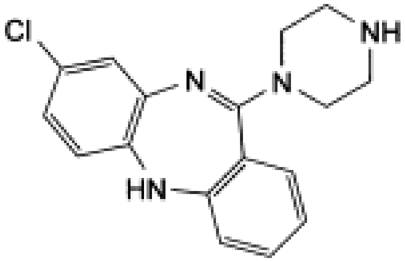
Specified impurities A, B, C, D.



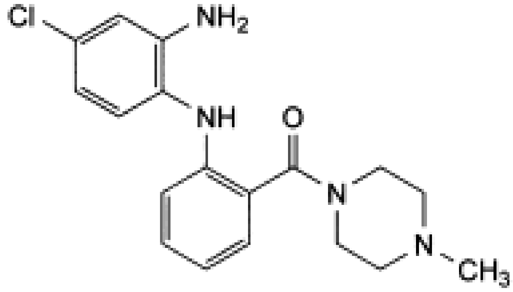
A. 8-chloro-5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one,



B. 11,11′-(piperazine-1,4-diyl)bis(8-chloro-5*H*-dibenzo[*b*,*e*][1,4]diazepine),



C 8-chloro-11-(piperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine,



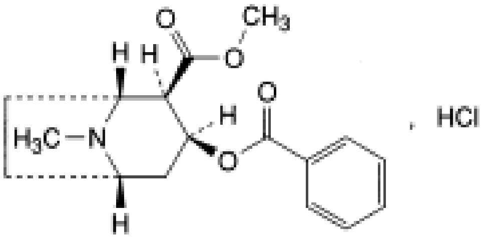
D. 1-[2-[(2-amino-4-chlorophenyl)amino]benzoyl]-4-methylpiperazine.

Ph Eur

Cocaine Hydrochloride

General Notices

(Ph Eur monograph 0073)



$C_{17}H_{21}NO_4,HCl$ 339.8   *53-21-4*

Action and use

Local anaesthetic.

Preparations

Adrenaline and Cocaine Intranasal Solution

Cocaine Eye Drops

Cocaine Paste

Ph Eur

DEFINITION

Methyl (1*R*,2*R*,3*S*,5*S*)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, freely soluble in alcohol, slightly soluble in methylene chloride.

mp

About 197 °C, with decomposition.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D, E.*

A. Dissolve 20.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of the solution to 50.0 ml with *0.01 M hydrochloric acid*. Examined between 220 nm and 350 nm *(2.2.25)*, the solution shows 2 absorption maxima, at 233 nm and 273 nm. The specific absorbance at 233 nm is 378 to 402.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of cocaine hydrochloride.*

C. Dissolve 0.1 g in 5 ml of *water R* and add 1 ml of *dilute ammonia R2*. A white precipitate is formed. Initiate crystallisation by scratching the wall of the tube with a glass rod. The crystals, washed with *water R* and dried *in vacuo*, melt *(2.2.14)* at 96 °C to 99 °C.

D. It gives reaction (a) of chlorides *(2.3.1)*.

E. It gives the reaction of alkaloids *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity

To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.2 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation *(2.2.7)*

- 70 to - 73 (dried substance).

Dissolve 0.50 g in *water R* and dilute to 20.0 ml with the same solvent.

Readily carbonisable substances

To 0.2 g add 2 ml of *sulphuric acid R*. After 15 min, the solution is not more intensely coloured than reference solution BY_5 *(2.2.2, Method I)*.

Related substances

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 25 mg of the substance to be examined in *0.01 M sodium hydroxide* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *0.01 M sodium hydroxide*. Allow the solution to stand for 15 min.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 335 m^2/g, a pore size of 10 nm and a carbon loading of 19.1 per cent,

— *temperature*: 35 °C.

Mobile phase *triethylamine R, tetrahydrofuran R, acetonitrile R, water R* (0.5:100:430:479.5 *V/V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 216 nm.

Injection 20 µl.

Relative retention With reference to cocaine (retention time = about 7.4 min): degradation product = about 0.7.

System suitability Reference solution (b):

— *resolution*: minimum of 5 between the peaks due to cocaine and to the degradation product.

Limits:

— *any impurity eluting after the principal peak*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on the residue from the test for loss on drying.

ASSAY

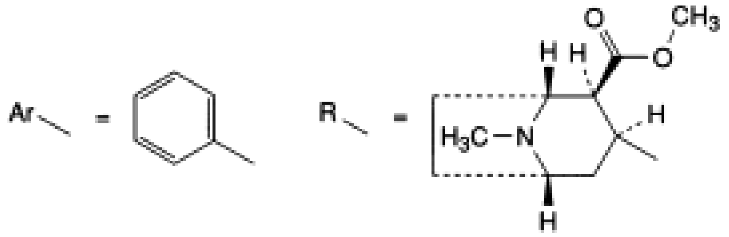
Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

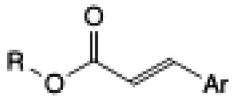
1 ml of *0.1 M sodium hydroxide* is equivalent to 33.98 mg of $C_{17}H_{22}ClNO_4$.

STORAGE

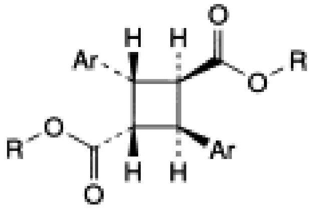
Protected from light.

IMPURITIES

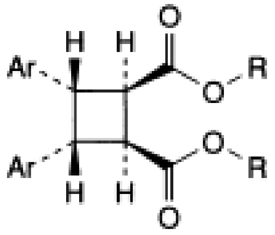

Ar
=
R
=
H₃C—N
H
H
H
O
CH₃
O
H
H



A. methyl (1*R*,2*R*,3*S*,5*S*)-8-methyl-3-[[(*E*)-3-phenylpropenoyl]oxy]-8-azabicyclo[3.2.1]octane-2-carboxylate (cinnamoylcocaine),


H
H
O
Ar
O
R
R
O
Ar
O
H
H

B. bis[(1*R*,2*R*,3*S*,5*S*)-2-(methoxycarbonyl)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl] (1*r*,2*c*,3*t*,4*t*)-2,4-diphenylcyclobutane-1,3-dicarboxylate (α-truxilline),

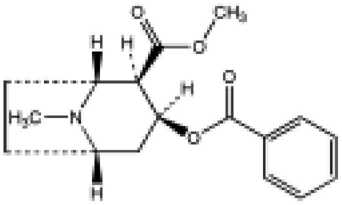

O
H
H
Ar
O
R
O
Ar
R
H
H
O

C. bis[(1*R*,2*R*,3*S*,5*S*)-2-(methoxycarbonyl)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl] (1*r*,2*c*,3*t*,4*t*)-3,4-diphenylcyclobutane-1,2-dicarboxylate (β-truxilline).

Ph Eur

Cocaine

General Notices



$C_{17}H_{21}NO_4$ 303.4 *50-36-2*

Action and use

Local anaesthetic.

DEFINITION

Cocaine is methyl (1*R*,2*R*,3*S*,5*S*)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate and may be obtained from the leaves of *Erythroxylum coca* Lam. and other species of *Erythroxylum* or by synthesis. It contains not less than 98.0% and not more than 101.0% of $C_{17}H_{21}NO_4$, calculated with reference to the dried substance.

CHARACTERISTICS

Colourless crystals or a white, crystalline powder. Slightly volatile.

Practically insoluble in *water*; freely soluble in *ethanol (96%)* and in *ether*; soluble in arachis oil; slightly soluble in *liquid paraffin*.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cocaine *(RS 071)*.

TESTS

Melting point

96° to 98°, Appendix V A.

Specific optical rotation

In a 2.4 % w/v solution in 0.1M *hydrochloric acid*, -79 to -81, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution (1) contains 0.05% w/v of the substance being examined in the mobile phase. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase, dilute 5.0 ml of this solution to 100.0 ml with the mobile phase. For solution (3) dissolve 25 mg of the substance being examined in 0.01M *sodium hydroxide* and dilute to 100.0 ml with the same solvent. Allow the solution to stand for 15 minutes.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry is suitable) and maintained at 35°, (b) as the mobile phase with a flow rate of 1 ml per minute a solution containing 1 volume of *triethylamine,* 200 volumes of *tetrahydrofuran,* 860 volumes of *acetonitrile* and 959 volumes of *water* and (c) a detection wavelength of 216 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cocaine (retention time, about 7 minutes) and the degradation product is at least 5.0.

In the chromatogram obtained with solution (1) the area of any peak eluting after the principal peak is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0. 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05 %).

Loss on drying

When dried to constant weight at 80°, loses not more than 0.5% of its weight, Appendix IX D. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

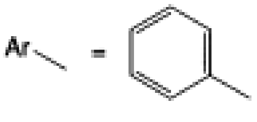
ASSAY

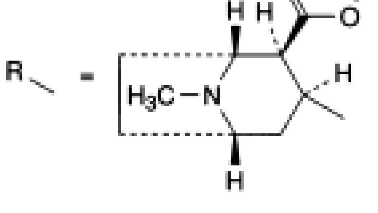
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.7 g dissolved in 50 ml of *1,4-dioxan* and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 30.34 mg of $C_{17}H_{21}NO_4$.

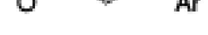
STORAGE

Cocaine should be stored protected from light.

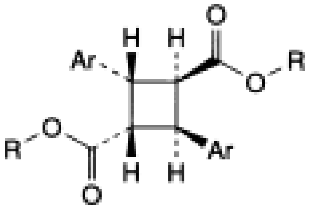
IMPURITIES



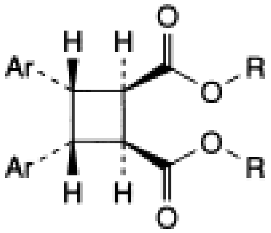




A. methyl (1*R*,2*R*,3*S*,5*S*)-8-methyl-3-[[(*E*)-3-phenylpropenoyl]oxy]-8-azabicyclo[3.2.1]octane-2-carboxylate (cinnamoylcocaine),



B. bis[(1*R*,2*R*,3*S*,5*S*)-2-(methoxycarbonyl)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl] (1*r*,2*c*,3*t*,4*t*)-2,4-diphenylcyclobutane-1,3-dicarboxylate (α-truxilline),



C. bis[(1*R*,2*R*,3*S*,5*S*)-2-(methoxycarbonyl)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl] (1*r*,2*c*,3*t*,4*t*)-3,4-diphenylcyclobutane-1,2-dicarboxylate (β-truxilline).

Coconut Oil

General Notices

(Refined Coconut Oil, Ph Eur monograph 1410)

8001-31-8

Action and use

Excipient.

Ph Eur

DEFINITION

Fatty oil obtained from the dried, solid part of the endosperm of *Cocos nucifera* L., then refined.

CHARACTERS

Appearance

White or almost white, unctuous mass.

Solubility

Practically insoluble in water, freely soluble in methylene chloride and in light petroleum (bp: 65-70 °C), very slightly soluble in ethanol (96 per cent).

Refractive index

About 1.449, determined at 40 °C.

IDENTIFICATION

A. Melting point (see Tests).

B. Composition of fatty acids (see Tests).

TESTS

Melting point *(2.2.14)*

23 °C to 26 °C.

Acid value *(2.5.1)*

Maximum 0.5, determined on 20.0 g.

Peroxide value *(2.5.5, Method A)*

Maximum 5.0.

Unsaponifiable matter ***(2.5.7)***

Maximum 1.0 per cent, determined on 5.0 g.

Alkaline impurities in fatty oils ***(2.4.19)***

It complies with the test.

Composition of fatty acids ***(2.4.22, Method B)***

Refined coconut oil is melted under gentle heating to a homogeneous liquid prior to sampling.

Reference solution Dissolve 15.0 mg of *tricaproin CRS*, 80.0 mg of *tristearin CRS*, 0.150 g of *tricaprin CRS*, 0.200 g of *tricaprylin CRS*, 0.450 g of *trimyristin CRS* and 1.25 g of *trilaurin CRS* in a mixture of 2 volumes of *methylene chloride R* and 8 volumes of *heptane R*, then dilute to 50 ml with the same mixture of solvents heating at 45-50 °C. Transfer 2 ml of this mixture to a 10 ml centrifuge tube with a screw cap and evaporate the solvent in a current of *nitrogen R*. Dissolve with 1 ml of *heptane R* and 1 ml of *dimethyl carbonate R* and mix vigorously under gentle heating (50-60 °C). Add, while still warm, 1 ml of a 12 g/l solution of *sodium R* in *anhydrous methanol R*, prepared with the necessary precautions, and mix vigorously for about 5 min. Add 3 ml of *distilled water R* and mix vigorously for about 30 s. Centrifuge for 15 min at 1500 *g*. Inject 1 µl of the organic phase.

Calculate the percentage content of each fatty acid using the following expression:

$$\frac{A_{x,s,c}}{\sum A_{x,s,c}} \times 100 \text{ per cent } m/m$$

$A_{x,s,c}$ is the corrected peak area of each fatty acid in the test solution:

$$A_{x,s,c} = A_{x,s} \times R_c$$

R_c is the relative correction factor:

$$R_c = \frac{m_{x,r} \times A_{1,r}}{A_{x,r} \times m_{1,r}}$$

for the peaks due to caproic, caprylic, capric, lauric and myristic acid methyl esters.

$m_{x,r}$ = mass of tricaproin, tricaprylin, tricaprin, trilaurin or trimyristin in the reference solution, in milligrams;

$m_{1,r}$ = mass of tristearin in the reference solution, in milligrams:

$A_{x,r}$ = area of the peaks due to caproic, caprylic, capric, lauric and myristic acid methyl esters in the reference solution;

$A_{1,r}$ = area of the peak due to stearic acid methyl ester in the reference solution;

$A_{x,s}$ = area of the peaks due to any specified or unspecified fatty acid methyl esters;

R_c = 1 for the peaks due to each of the remaining specified fatty acid methyl esters or any unspecified fatty acid methyl ester.

Composition of the fatty-acid fraction of the oil:

— *caproic acid* (*R_{Rt} 0.11*): maximum 1.5 per cent;

— *caprylic acid* (*R_{Rt} 0.23*): 5.0 per cent to 11.0 per cent;

— *capric acid* (*R_{Rt} 0.56*): 4.0 per cent to 9.0 per cent;

— *lauric acid* (*R_{Rt} 0.75*): 40.0 per cent to 50.0 per cent;

— *myristic acid* (*R_{Rt} 0.85*): 15.0 per cent to 20.0 per cent;

— *palmitic acid* (*R_{Rt} 0.93*): 7.0 per cent to 12.0 per cent;

— *stearic acid* (*R_{Rt} 1.00*): 1.5 per cent to 5.0 per cent;

— *oleic acid and isomers* (*R_{Rt} 1.01*): 4.0 per cent to 10.0 per cent;

— *linoleic acid* (*R_{Rt} 1.03*): 1.0 per cent to 3.0 per cent;

— *linolenic acid* (*R_{Rt} 1.06*): maximum 0.2 per cent;

— *arachidic acid* (*R_{Rt} 1.10*): maximum 0.2 per cent;

— *eicosenoic acid* (*R_{Rt} 1.11*): maximum 0.2 per cent.

STORAGE

In a well-filled container, protected from light.

Ph Eur

Cocoyl Caprylocaprate

General Notices

(Ph Eur monograph 1411)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of esters of saturated C_{12} - C_{18} alcohols with caprylic (octanoic) and capric (decanoic) acids obtained by the reaction of these acids with vegetable saturated fatty alcohols.

CHARACTERS

Appearance

Slightly yellowish liquid.

Solubility

Practically insoluble in water, miscible with ethanol (96 per cent) and with liquid paraffin.

Relative density

About 0.86.

Refractive index

About 1.445.

Viscosity

About 11 mPa·s.

IDENTIFICATION

A. Freezing point *(2.2.18):* Maximum 15 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison cocoyl caprylocaprate CRS.

C. Composition of fatty acids and fatty alcohols (see Tests).

TESTS

Appearance

The substance to be examined is not more intensely coloured than reference solution Y_5 *(2.2.2, Method I)*.

Acid value ***(2.5.1)***

Maximum 0.5, determined on 5.00 g.

Hydroxyl value ***(2.5.3, Method A)***

Maximum 5.0.

Iodine value ***(2.5.4, Method A)***

Maximum 1.0.

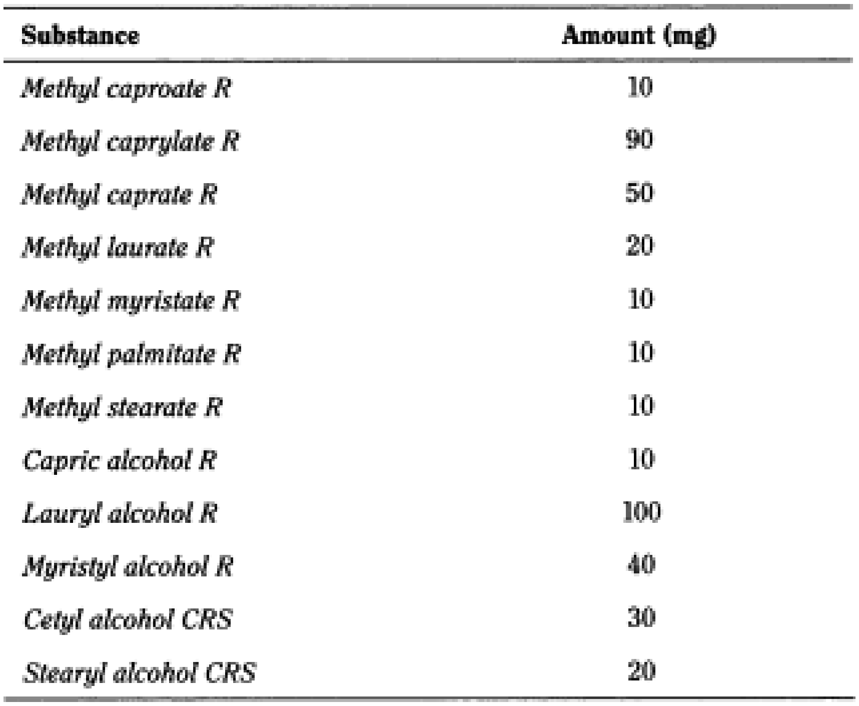
Saponification value ***(2.5.6)***

160 to 173.

Composition of fatty acids and fatty alcohols ***(2.4.22, Method C)***

Use the chromatogram obtained with the following reference solution for identification of the peaks due to the fatty alcohols.

Reference solution Dissolve the amounts of the substances listed in the following table in 10 ml of *heptane R*.



Substance	Amount (mg)
Methyl caproate R	10
Methyl caprylate R	90
Methyl caprate R	50
Methyl laurate R	20
Methyl myristate R	10
Methyl palmitate R	10
Methyl stearate R	10
Capric alcohol R	10
Lauryl alcohol R	100
Myristyl alcohol R	40
Cetyl alcohol CRS	30
Stearyl alcohol CRS	20

Consider the sum of the areas of the peaks due to the fatty acids listed below to be equal to 100 and the sum of the areas of the peaks due to the fatty alcohols listed below to be equal to 100.

Composition of the fatty acid fraction of the substance:

- — *caproic acid*: maximum 2.0 per cent,
- — *caprylic acid*: 50.0 per cent to 80.0 per cent,

— *capric acid*: 20.0 per cent to 50.0 per cent,

— *lauric acid*: maximum 3.0 per cent,

— *myristic acid*: maximum 2.0 per cent.

Composition of the fatty alcohol fraction of the substance:

— *capric alcohol*: maximum 3.0 per cent,

— *lauryl alcohol*: 48.0 per cent to 63.0 per cent,

— *myristyl alcohol*: 18.0 per cent to 27.0 per cent,

— *cetyl alcohol*: 6.0 per cent to 13.0 per cent,

— *stearyl alcohol*: 9.0 per cent to 16.0 per cent.

Water ***(2.5.12)***

Maximum 0.1 per cent, determined on 5.00 g.

Total ash ***(2.4.16)***

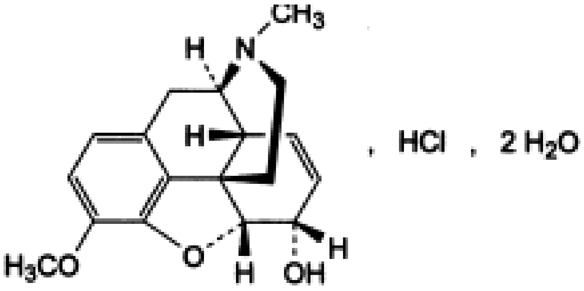
Maximum 0.1 per cent, determined on 1.0 g.

Ph Eur

Codeine Hydrochloride

General Notices

(Codeine Hydrochloride Dihydrate, Ph Eur monograph 1412)



$C_{18}H_{21}NO_{3,}HCl,2H_2O$ 371.9   *1422-07-7*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol hydrochloride dihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or small, colourless crystals.

Solubility

Soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in cyclohexane.

IDENTIFICATION

First identification   *A, D.*

Second identification   *B, C, D, E.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of codeine hydrochloride dihydrate.*

B. To 5 ml of solution S (see Tests) add 1 ml of a mixture of equal volumes of *strong sodium hydroxide solution R* and *water R* and initiate crystallisation, if necessary, by scratching the wall of the tube with a glass rod and cooling in iced water. Wash the precipitate with *water R* and dry at 100-105 °C.It melts *(2.2.15)* at 155 °C to 159 °C.

C. To about 10 mg add 1 ml of *sulphuric acid R* and 0.05 ml of *ferric chloride solution R2* and heat on a water-bath. A blue colour develops. Add 0.05 ml of *nitric acid R*. The colour changes to red.

D. Solution S gives reaction (a) of chlorides *(2.3.1).*

E. It gives the reaction of alkaloids *(2.3.1).*

TESTS

Solution S

Dissolve 2.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II).*

Acidity or alkalinity

To 5 ml of solution S add 5 ml of *carbon dioxide-free water R*. Add 0.05 ml of *methyl red solution R* and 0.2 ml of *0.02 M hydrochloric acid*; the solution is red. Add 0.4 ml of *0.02 M sodium hydroxide*; the solution becomes yellow.

Specific optical rotation *(2.2.7)*

- 117 to - 121 (anhydrous substance).

Dilute 5.0 ml of solution S to 10.0 ml with *water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined and 0.100 g of *sodium octanesulphonate R* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *codeine impurity A CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (d) To 0.25 ml of the test solution add 2.5 ml of reference solution (a).

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.08 g of *sodium octanesulphonate R* in a mixture of 20 ml of *glacial acetic acid R* and 250 ml of *acetonitrile R* and dilute to 1000 ml with *water R.*

Flow rate 2 ml/min.

Detection Spectrophotometer at 245 nm.

Injection 10 µl.

Run time 10 times the retention time of codeine.

Relative retention With reference to codeine (retention time = about 6 min): impurity B = about 0.6; impurity E = about 0.7; impurity A = about 2.0; impurity C = about 2.3; impurity D = about 3.6.

System suitability Reference solution (d):

— *resolution*: minimum 3 between the peaks due to codeine and impurity A.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.25,

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *impurities B, C, D, E*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *sum of impurities other than A*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulphates *(2.4.13)*

Maximum 0.1 per cent.

Dilute 5 ml of solution S to 20 ml with *distilled water R*. 15 ml of the solution complies with the limit test for sulphates.

Water *(2.5.12)*

8.0 per cent to 10.5 per cent, determined on 0.250 g.

ASSAY

Dissolve 0.300 g in a mixture of 5 ml of *0.01 M hydrochloric acid R* and 30 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 33.59 mg of $C_{18}H_{22}ClNO_3$.

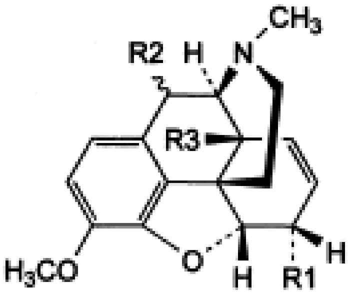
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : F, G.*

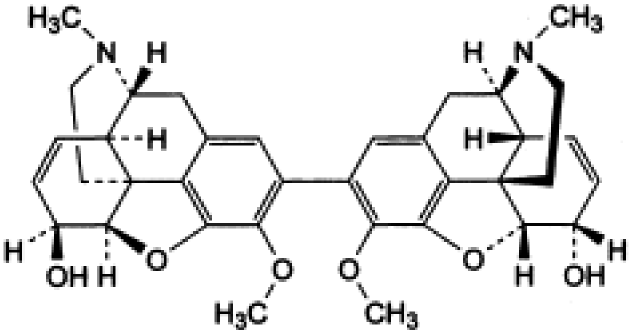


A. R1 = OCH_3, R2 = R3 = H: 7,8-didehydro-4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan (methylcodeine),

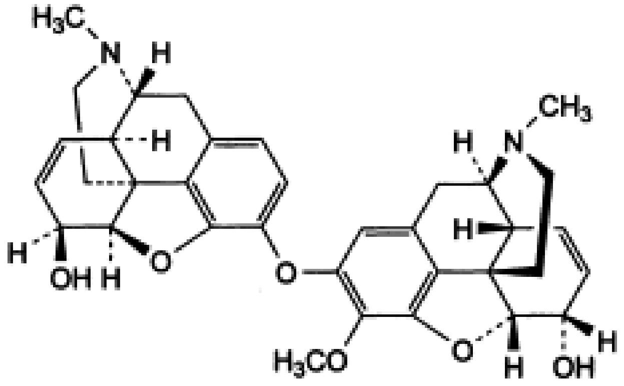
E. R1 = R2 = OH, R3 = H: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,10-diol,

F. R1 = R3 = OH, R2 = H: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,14-diol,

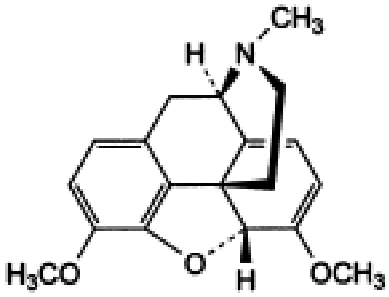
B. morphine,



C. 7,7′,8,8′-tetradehydro-4,5α:4′,5′α-diepoxy-3,3′-dimethoxy-17,17′-dimethyl-2,2′-bimorphinanyl-6α,6′α-diol (codeine dimer),



D. 7,8-didehydro-2-[(7,8-didehydro-4,5α-epoxy-6α-hydroxy-17-methylmorphinan-3-yl)oxy]-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol (3-*O*-(codein-2-yl)morphine),



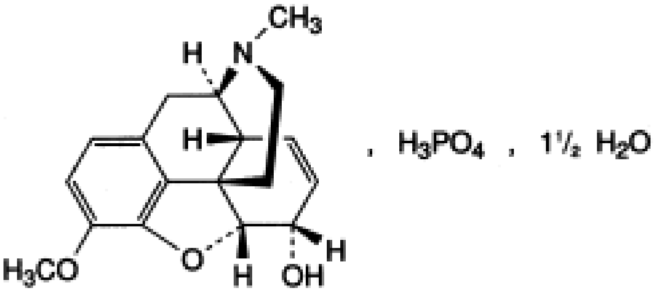
G. 6,7,8,14-tetradehydro-4,5α-epoxy-3,6-dimethoxy-17-methylmorphinan (thebaine).

Ph Eur

Codeine Phosphate Sesquihydrate

General Notices

(Ph Eur monograph 0075)



$C_{18}H_{21}NO_3,H_3PO_4,1\frac{1}{2}H_2O$ 424.4 *5913-76-8*

Action and use

Opioid receptor agonist; analgesic.

Preparations

Codeine Linctus

Paediatric Codeine Linctus

Codeine Phosphate Oral Solution

Codeine Phosphate Tablets

Ph Eur

DEFINITION

7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol phosphate sesquihydrate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or small, colourless crystals.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, E, F.*

Second identification *A, C, D, E, F, G.*

A. Dilute 1.0 ml of solution S (see Tests) to 100.0 ml with *water R*. To 25.0 ml of this solution add 25 ml of *water R* then 10 ml of *1 M sodium hydroxide* and dilute to 100.0 ml with *water R*. Examined between 250 nm and 350 nm *(2.2.25)*, the solution shows only 1 absorption maximum, at 284 nm. The specific absorbance at the absorption maximum is about 38 (dried substance).

B. Infrared absorption spectrophotometry *(2.2.24).*

Preparation Dissolve 0.20 g in 4 ml of *water R*. Add 1 ml of a mixture of equal volumes of *strong sodium hydroxide solution R* and *water R* and initiate crystallisation, if necessary, by scratching the wall of the tube with a glass rod and cooling in iced water. Wash the precipitate with *water R* and dry at 100-105 °C. Examine the dried precipitate prepared as discs using *potassium bromide R*.

Comparison *Ph. Eur. reference spectrum of codeine.*

C. Dissolve 0.20 g in 4 ml of *water R*. Add 1 ml of a mixture of equal volumes of *strong sodium hydroxide solution R* and *water R* and initiate crystallisation, if necessary, by scratching the wall of the tube with a glass rod and cooling in iced water. The precipitate, washed with *water R* and dried at 100-105 °C, melts *(2.2.14)* at 155 °C to 159 °C.

D. To about 10 mg add 1 ml of *sulphuric acid R* and 0.05 ml of *ferric chloride solution R2* and heat on a water-bath. A blue colour develops. Add 0.05 ml of *nitric acid R*. The colour changes to red.

E. It complies with the test for loss on drying (see Tests).

F. Solution S gives reaction (a) of phosphates *(2.3.1)*.

G. It gives the reaction of alkaloids *(2.3.1)*.

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 25.0 ml with the same solvent.

pH *(2.2.3)*

4.0 to 5.0 for solution S.

Specific optical rotation *(2.2.7)*

- 98 to - 102 (dried substance).

Dilute 5.0 ml of solution S to 10.0 ml with *water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined and 0.100 g of *sodium octanesulphonate R* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *codeine impurity A CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (d) To 0.25 ml of the test solution add 2.5 ml of reference solution (a).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.08 g of *sodium octanesulphonate R* in a mixture of 20 ml of *glacial acetic acid R* and 250 ml of *acetonitrile R* and dilute to 1000 ml with *water R*.

Flow rate 2 ml/min.

Detection Spectrophotometer at 245 nm.

Injection 10 µl.

Run time 10 times the retention time of codeine.

Relative retention With reference to codeine (retention time = about 6 min): impurity B = about 0.6; impurity E = about 0.7; impurity A = about 2.0; impurity C = about 2.3; impurity D = about 3.6.

System suitability Reference solution (d):

— *resolution*: minimum 3 between the peaks due to codeine and impurity A.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.25,

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *impurities B, C, D, E*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *sum of impurities other than A*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulphates *(2.4.13)*

Maximum 0.1 per cent.

Dilute 5 ml of solution S to 20 ml with *distilled water R*. 15 ml of the solution complies with the limit test for sulphates.

Loss on drying *(2.2.32)*

5.0 per cent to 7.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.350 g in a mixture of 10 ml of *anhydrous acetic acid R* and 20 ml of *dioxan R*. Titrate with *0.1 M perchloric acid* using 0.05 ml of *crystal violet solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 39.74 mg of $C_{18}H_{24}NO_7P$.

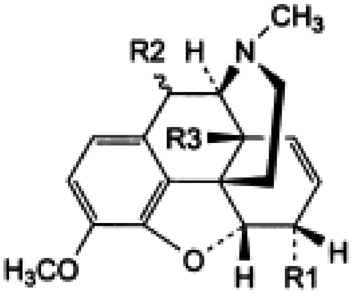
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : F, G.*

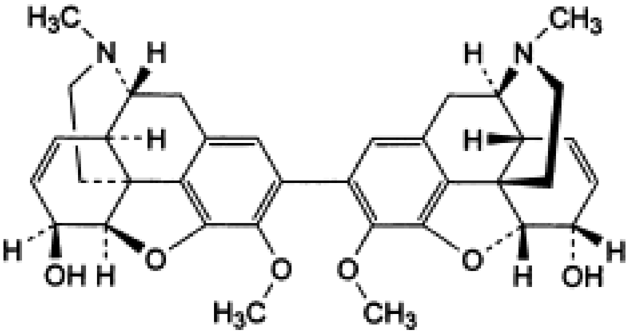


A. R1 = OCH_3, R2 = R3 = H: 7,8-didehydro-4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan (methylcodeine),

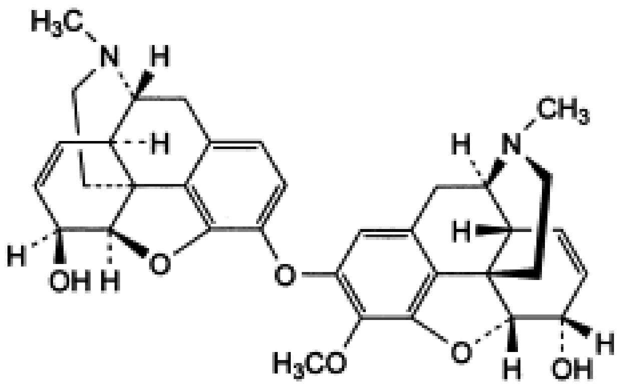
E. R1 = R2 = OH, R3 = H: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,10-diol,

F. R1 = R3 = OH, R2 = H: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,14-diol,

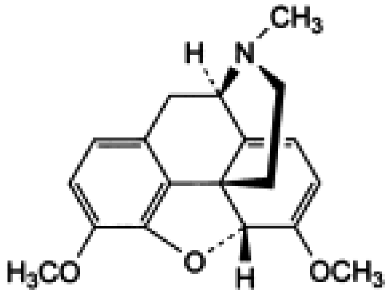
B. morphine,



C. 7,7′,8,8′-tetradehydro-4,5α:4′,5′α-diepoxy-3,3′-dimethoxy-17,17′-dimethyl-2,2′-bimorphinanyl-6α,6′α-diol (codeine dimer),


H3C
N
H
H
H
OH H
O
O
CH3
H
N
H
H3CO
O
H
OH
H

D. 7,8-didehydro-2-[(7,8-didehydro-4,5α-epoxy-6α-hydroxy-17-methylmorphinan-3-yl)oxy]-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol (3-*O*-(codein-2-yl)morphine),


CH3
H
N
H3CO
O
H
OCH3

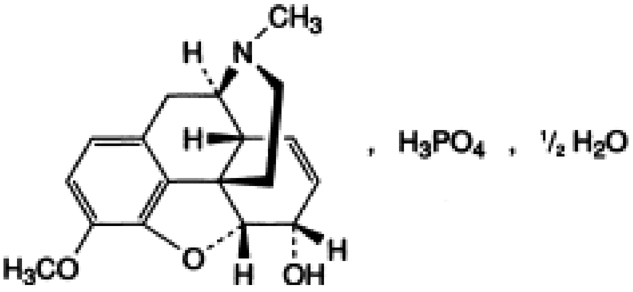
G. 6,7,8,14-tetradehydro-4,5α-epoxy-3,6-dimethoxy-17-methylmorphinan (thebaine).

Ph Eur

Codeine Phosphate

General Notices

(Codeine Phosphate Hemihydrate, Ph Eur monograph 0074)



$C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ 406.4 *41444-62-6*

Action and use

Opioid receptor agonist; analgesic.

Preparations

Co-codamol Capsules

Co-codamol Tablets

Effervescent Co-codamol Tablets

Co-codaprin Tablets

Dispersible Co-codaprin Tablets

Codeine Linctus

Paediatric Codeine Linctus

Codeine Phosphate Injection

Codeine Phosphate Oral Solution

Codeine Phosphate Tablets

Paracetamol, Codeine Phosphate and Caffeine Capsules

Paracetamol, Codeine Phosphate and Caffeine Tablets

Ph Eur

DEFINITION

7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol phosphate hemihydrate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or small, colourless crystals.

Solubility

Freely soluble in water, slightly soluble or very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, E, F.*

Second identification *A, C, D, E, F, G.*

A. Dilute 1.0 ml of solution S (see Tests) to 100.0 ml with *water R*. To 25.0 ml of this solution add 25 ml of *water R* then 10 ml of *1 M sodium hydroxide* and dilute to 100.0 ml with *water R*. Examined between 250 nm and 350 nm *(2.2.25)*, the solution shows only 1 absorption maximum, at 284 nm. The specific absorbance at the absorption maximum is about 38 (dried substance).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 0.20 g in 4 ml of *water R*. Add 1 ml of a mixture of equal volumes of *strong sodium hydroxide solution R* and *water R* and initiate crystallisation, if necessary, by scratching the wall of the tube with a glass rod and cooling in iced water. Wash the precipitate with *water R* and dry at 100-105 °C. Examine the dried precipitate prepared as discs using *potassium bromide R.*

Comparison *Ph. Eur. reference spectrum of codeine.*

C. Dissolve 0.20 g in 4 ml of *water R.* Add 1 ml of a mixture of equal volumes of *strong sodium hydroxide solution R* and *water R* and initiate crystallisation, if necessary, by scratching the wall of the tube with a glass rod and cooling in iced water. The precipitate, washed with *water R* and dried at 100-105 °C, melts *(2.2.14)* at 155 °C to 159 °C.

D. To about 10 mg add 1 ml of *sulphuric acid R* and 0.05 ml of *ferric chloride solution R2* and heat on a water-bath. A blue colour develops. Add 0.05 ml of *nitric acid R*. The colour changes to red.

E. It complies with the test for loss on drying (see Tests).

F. Solution S gives reaction (a) of phosphates *(2.3.1)*.

G. It gives the reaction of alkaloids *(2.3.1)*.

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 25.0 ml with the same solvent.

pH *(2.2.3)*

4.0 to 5.0 for solution S.

Specific optical rotation ***(2.2.7)***

- 98 to - 102 (dried substance).

Dilute 5.0 ml of solution S to 10.0 ml with *water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined and 0.100 g of *sodium octanesulphonate R* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *codeine impurity A CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (d) To 0.25 ml of the test solution add 2.5 ml of reference solution (a).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.08 g of *sodium octanesulphonate R* in a mixture of 20 ml of *glacial acetic acid R* and 250 ml of *acetonitrile R* and dilute to 1000 ml with *water R.*

Flow rate 2 ml/min.

Detection Spectrophotometer at 245 nm.

Injection 10 µl.

Run time 10 times the retention time of codeine.

Relative retention With reference to codeine (retention time = about 6 min): impurity B = about 0.6; impurity E = about 0.7; impurity A = about 2.0; impurity C = about 2.3; impurity D = about 3.6.

System suitability Reference solution (d):

— *resolution*: minimum 3 between the peaks due to codeine and impurity A.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.25,

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *impurities B, C, D, E*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *sum of impurities other than A*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulphates ***(2.4.13)***

Maximum 0.1 per cent.

Dilute 5 ml of solution S to 20 ml with *distilled water R*. 15 ml of the solution complies with the limit test for sulphates.

Loss on drying ***(2.2.32)***

1.5 per cent to 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.350 g in a mixture of 10 ml of *anhydrous acetic acid R* and 20 ml of *dioxan R*. Titrate with *0.1 M perchloric acid* using 0.05 ml of *crystal violet solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 39.74 mg of $C_{18}H_{24}NO_7P$.

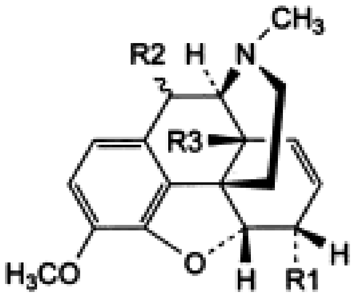
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : F, G.*

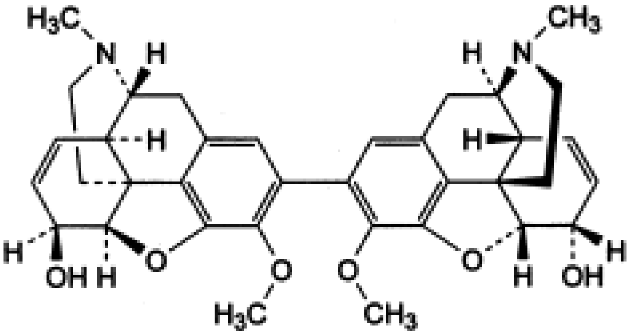


A. R1 = OCH_3, R2 = R3 = H: 7,8-didehydro-4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan (methylcodeine),

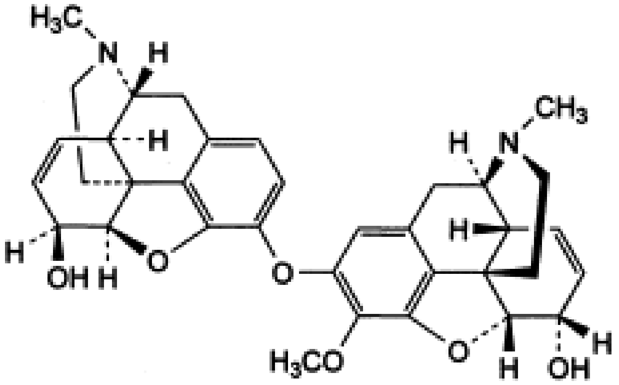
E. R1 = R2 = OH, R3 = H: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,10-diol,

F. R1 = R3 = OH, R2 = H: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,14-diol,

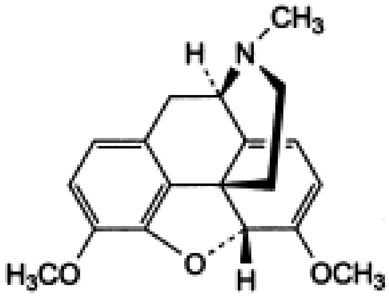
B. morphine,



C. 7,7′,8,8′-tetradehydro-4,5α:4′,5′α-diepoxy-3,3′-dimethoxy-17,17′-dimethyl-2,2′-bimorphinanyl-6α,6′α-diol (codeine dimer),



D. 7,8-didehydro-2-[(7,8-didehydro-4,5α-epoxy-6α-hydroxy-17-methylmorphinan-3-yl)oxy]-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol (3-*O*-(codein-2-yl)morphine),



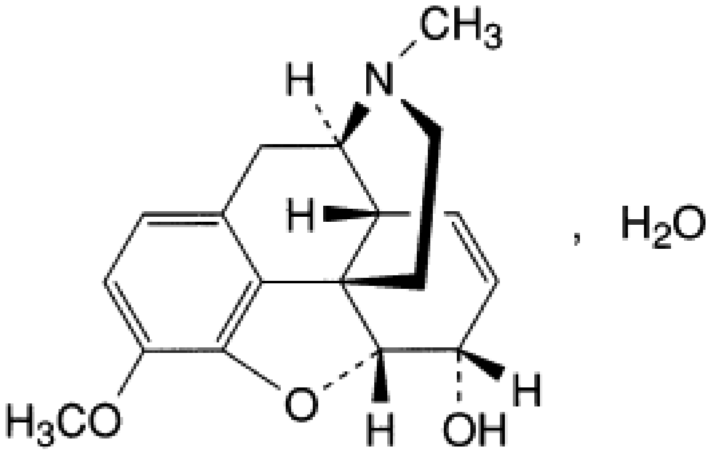
G. 6,7,8,14-tetradehydro-4,5α-epoxy-3,6-dimethoxy-17-methylmorphinan (thebaine).

Ph Eur

Codeine

General Notices

(Ph Eur monograph 0076)



$C_{18}H_{21}NO_3,H_2O$ 317.4 *76-57-3*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Soluble in boiling water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, C.

Second identification A, B, D, E.

A. Melting point (*2.2.14*): 155 °C to 159 °C.

B. To 2.0 ml of solution S (see Tests) add 50 ml of *water R* then 10 ml of *1 M sodium hydroxide* and dilute to 100.0 ml with *water R*. Examined between 250 nm and 350 nm (*2.2.25*), the solution shows only 1 absorption maximum, at 284 nm. The specific absorbance at the absorption maximum is about 50 (dried substance).

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dried substance prepared as a disc of *potassium bromide R.*

Comparison *codeine CRS.*

D. To about 10 mg add 1 ml of *sulphuric acid R* and 0.05 ml of *ferric chloride solution R2* and heat on a water-bath. A blue colour develops. Add 0.05 ml of *nitric acid R.* The colour changes to red.

E. It gives the reaction of alkaloids (*2.3.1*).

TESTS

Solution S

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

- 142 to - 146 (dried substance).

Dissolve 0.50 g in *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined and 0.100 g of *sodium octanesulphonate R* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *codeine impurity A CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (d) To 0.25 ml of the test solution, add 2.5 ml of reference solution (a).

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.08 g of *sodium octanesulphonate R* in a mixture of 20 ml of *glacial acetic acid R* and 250 ml of *acetonitrile R* and dilute to 1000 ml with *water R.*

Flow rate 2 ml/min.

Detection Spectrophotometer at 245 nm.

Injection 10 µl.

Run time 10 times the retention time of codeine.

Relative retention With reference to codeine (retention time = about 6 min): impurity B = about 0.6; impurity E = about 0.7; impurity A = about 2.0; impurity C = about 2.3; impurity D = about 3.6.

System suitability Reference solution (d):

— *resolution*: minimum 3 between the peaks due to codeine and impurity A.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.25;

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurities B, C, D, E*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *sum of impurities other than A*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)

4.0 per cent to 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 10 ml of *anhydrous acetic acid R*. Add 20 ml of *dioxan R*. Titrate with *0.1 M perchloric acid*, using 0.05 ml of *crystal violet solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 29.94 mg of $C_{18}H_{21}NO_3$.

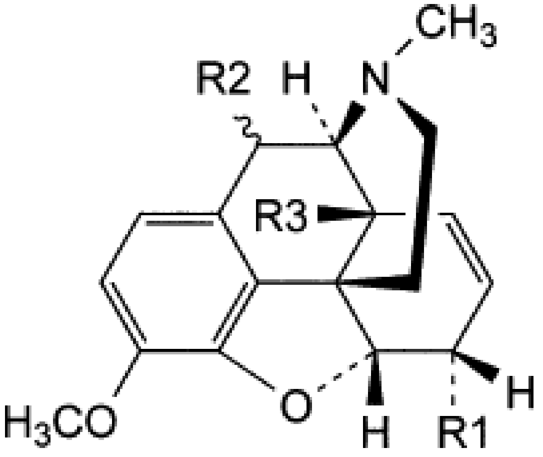
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F, G.*

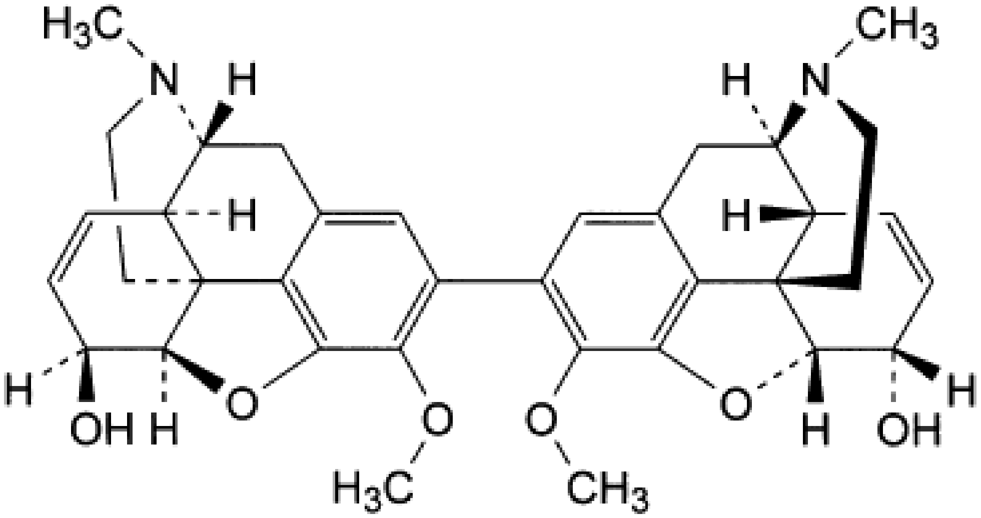


A. R1 = OCH_3, R2 = R3 = H: 7,8-didehydro-4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan (methylcodeine),

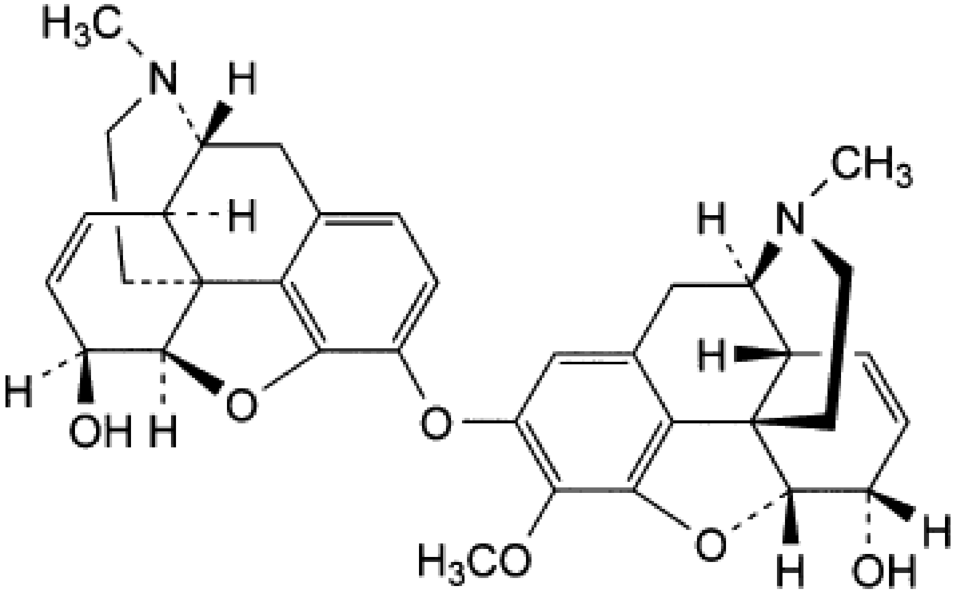
E. R1 = R2 = OH, R3 = H: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,10-diol,

F. R1 = R3 = OH, R2 = H: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,14-diol,

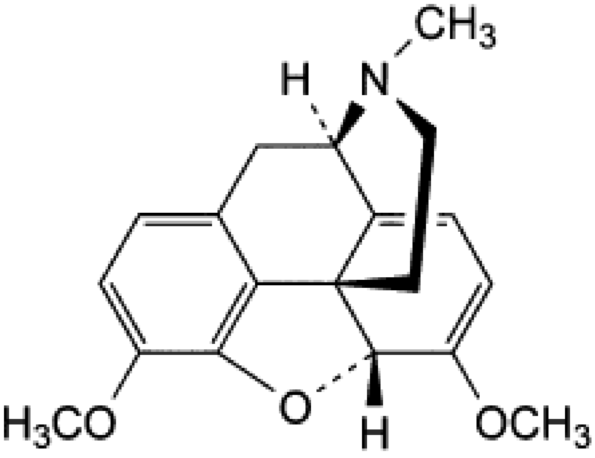
B. morphine,



C. 7,7′,8,8′-tetradehydro-4,5α:4′,5′α-diepoxy-3,3′-dimethoxy-17,17′-dimethyl-2,2′-bimorphinanyl-6α,6′α-diol (codeine dimer),



D. 7,8-didehydro-2-[(7,8-didehydro-4,5α-epoxy-6α-hydroxy-17-methylmorphinan-3-yl)oxy]-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol (3-*O*-(codein-2-yl)morphine),



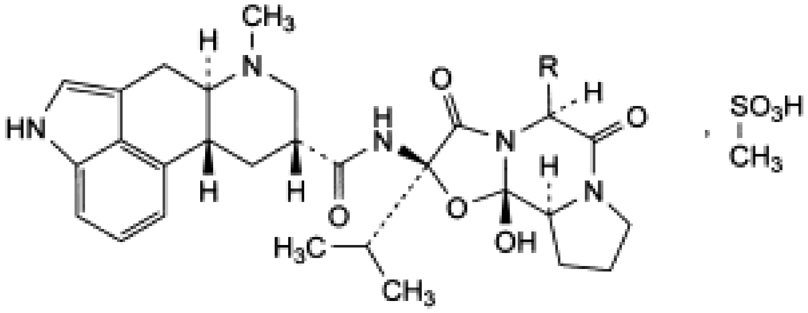
G. 6,7,8,14-tetradehydro-4,5α-epoxy-3,6-dimethoxy-17-methylmorphinan (thebaine).

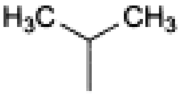
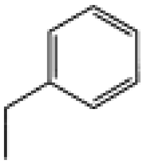
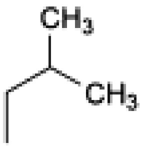
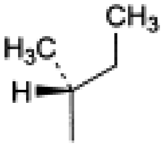
Ph Eur

Codergocrine Mesilate

General Notices

(Ph Eur monograph 2060)



Name	Mol. Formula	M_r	R
dihydroergocornine mesilate	$C_{32}H_{45}N_5O_8S$	660	
dihydroergocristine mesilate	$C_{36}H_{45}N_5O_8S$	708	
α-dihydroergocryptine mesilate	$C_{33}H_{47}N_5O_8S$	674	
β-dihydroergocryptine mesilate	$C_{33}H_{47}N_5O_8S$	674	

Action and use

Vasodilator.

Preparation

Codergocrine Tablets

Ph Eur

DEFINITION

A mixture of:

— (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2,5-bis(1-methylethyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-a]pyrrolo[2,1-c]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide methanesulphonate (dihydroergocornine mesilate);

— (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-(1-methylethyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide methanesulphonate (dihydroergocristine mesilate);

— (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2-(1-methylethyl)-5-(2-methylpropyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide methanesulphonate (α-dihydroergocryptine mesilate);

— (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2-(1-methylethyl)-5-[(1*RS*)-1-methylpropyl]-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide methanesulphonate (β-dihydroergocryptine mesilate or epicriptine mesilate).

Content

98.0 per cent to 102.0 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

White or yellowish powder.

Solubility

Sparingly soluble in water, sparingly soluble to soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dissolve 0.20 g of *methanesulphonic acid R* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Plate *TLC silica gel plate R*.

Mobile phase *water R, concentrated ammonia R, butanol R, acetone R* (5:10:20:65 *V/V/V/V*)
.

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of cold air for not more than 1 min.

Detection Spray with a 1 g/l solution of *bromocresol purple R* in *methanol R*, adjusted to a violet-red colour with 0.05 ml of *dilute ammonia R1*.

Drying In a current of hot air at 100 °C.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the test for composition.

Results The 4 principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the 4 principal peaks in the chromatogram obtained with the reference solution.

TESTS

pH *(2.2.3)*

4.2 to 5.2.

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Composition

Liquid chromatography *(2.2.29)*. Use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in a mixture of 1 volume of *anhydrous ethanol R* and 2 volumes of a 10 g/l solution of *tartaric acid R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 20 mg of *codergocrine mesilate CRS* in a mixture of 1 volume of *anhydrous ethanol R* and 2 volumes of a 10 g/l solution of *tartaric acid R* and dilute to 10 ml with the same mixture of solvents.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *triethylamine R, acetonitrile R, water R* (2.5:25:75 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Run time 20 min.

Elution order Dihydroergocornine, α-dihydroergocryptine, dihydroergocristine, β-dihydroergocryptine.

System suitability Test solution:

- *resolution*: minimum 3 between any 2 consecutive principal peaks.

Composition:

- *dihydroergocornine*: 30.0 per cent to 35.0 per cent,
- α-*dihydroergocryptine*: 20.0 per cent to 25.0 per cent,
- *dihydroergocristine*: 30.0 per cent to 35.0 per cent,
- β-*dihydroergocryptine*: 10.0 per cent to 13.0 per cent,
- *disregard limit*: 1.0 per cent.

Related substances

Thin-layer chromatography *(2.2.27)*. *Perform the test as rapidly as possible and protected from direct light. Prepare the test solution last and immediately before application on the plate.*

Test solution Dissolve 0.40 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 40 mg of *dihydroergocristine mesilate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10.0 ml with the same mixture of solvents. Dilute 3.0 ml of the solution to 50.0 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (b) To 2.0 ml of reference solution (a), add 1.0 ml of a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (c) To 1.0 ml of reference solution (a), add 2.0 ml of a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (d) To 1.0 ml of reference solution (a), add 5.0 ml of a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R, methanol R, ethyl acetate R, methylene chloride R* (1:3:50:50 *V/V/V/V*).

Application 10 µl.

Drying In the dark for 2 min after the application of the last solution.

First development In an unsaturated tank, over 2/3 of the plate.

Drying In a current of cold air for not more than 1 min.

Second development In an unsaturated tank, over 2/3 of the plate; use freshly prepared mobile phase.

Drying In a current of cold air for not more than 1 min.

Detection Spray thoroughly with *dimethylaminobenzaldehyde solution R7* and dry in a current of hot air until the spot in the chromatogram obtained with reference solution (d) is clearly visible.

System suitability Test solution:

- the chromatogram shows at least 3 separated secondary spots.

Limits:

- *any impurity*: any spots, apart from the principal spot, are not more intense than the spot in the chromatogram obtained with reference solution (a) (0.3 per cent); not more than 4 such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent) and 2 of these may be more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 0.500 g by drying at 120 °C under high vacuum.

ASSAY

Dissolve 0.500 g in 60 ml of *pyridine R*. Pass a stream of *nitrogen R* over the surface of the solution and titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 68.04 mg of codergocrine mesilate (average M_r = 680).

STORAGE

Protected from light.

Ph Eur

Cod-liver Oil (Type A)

General Notices

(Ph Eur monograph 1192)

Action and use

Source of vitamins A and D.

Each IU of vitamin D_3 is equivalent to 0.025 µg of colecalciferol.

Ph Eur

DEFINITION

Purified fatty oil obtained from the fresh livers of *Gadus morhua* L. and other species of *Gadidae*, solid substances being removed by cooling and filtering. A suitable antioxidant may be added.

Content

600 IU (180 µg) to 2500 IU (750 µg) of vitamin A per gram and 60 IU (1.5 µg) to 250 IU (6.25 µg) of vitamin D_3 per gram.

CHARACTERS

Appearance

Clear, yellowish, viscous liquid.

Solubility

Practically insoluble in water, slightly soluble in alcohol, miscible with light petroleum.

IDENTIFICATION

First identification A, B, C.

Second identification C, D.

A. In the assay for vitamin A using method A, the test solution shows an absorption maximum *(2.2.25)* at 325 ± 2 nm. In the assay for vitamin A using method B, the chromatogram obtained with the test solution shows a peak corresponding to the peak of all-*trans*-retinol in the chromatogram obtained with the reference solution.

B. In the assay for vitamin D_3, the chromatogram obtained with test solution (a) shows a peak corresponding to the peak of cholecalciferol in the chromatogram obtained with reference solution (b).

C. It complies with the test for composition of fatty acids (see Tests).

D. To 0.1 g add 0.5 ml of *methylene chloride R* and 1 ml of *antimony trichloride solution R*.

Mix. A deep blue colour develops in about 10 s.

TESTS

Colour

Not more intensely coloured than a reference solution prepared as follows: to 3.0 ml of red primary solution add 25.0 ml of yellow primary solution and dilute to 50.0 ml with a 10 g/l solution of *hydrochloric acid R (2.2.2, Method II)*.

Relative density *(2.2.5)*

0.917 to 0.930.

Refractive index *(2.2.6)*

1.477 to 1.484.

Acid value *(2.5.1)*

Maximum 2.0.

Anisidine value *(2.5.36)*

Maximum 30.0.

Iodine value *(2.5.4, Method B)*

150 to 180.

Use *starch solution R2*.

Peroxide value *(2.5.5, Method B)*

Maximum 10.0.

Unsaponifiable matter *(2.5.7)*

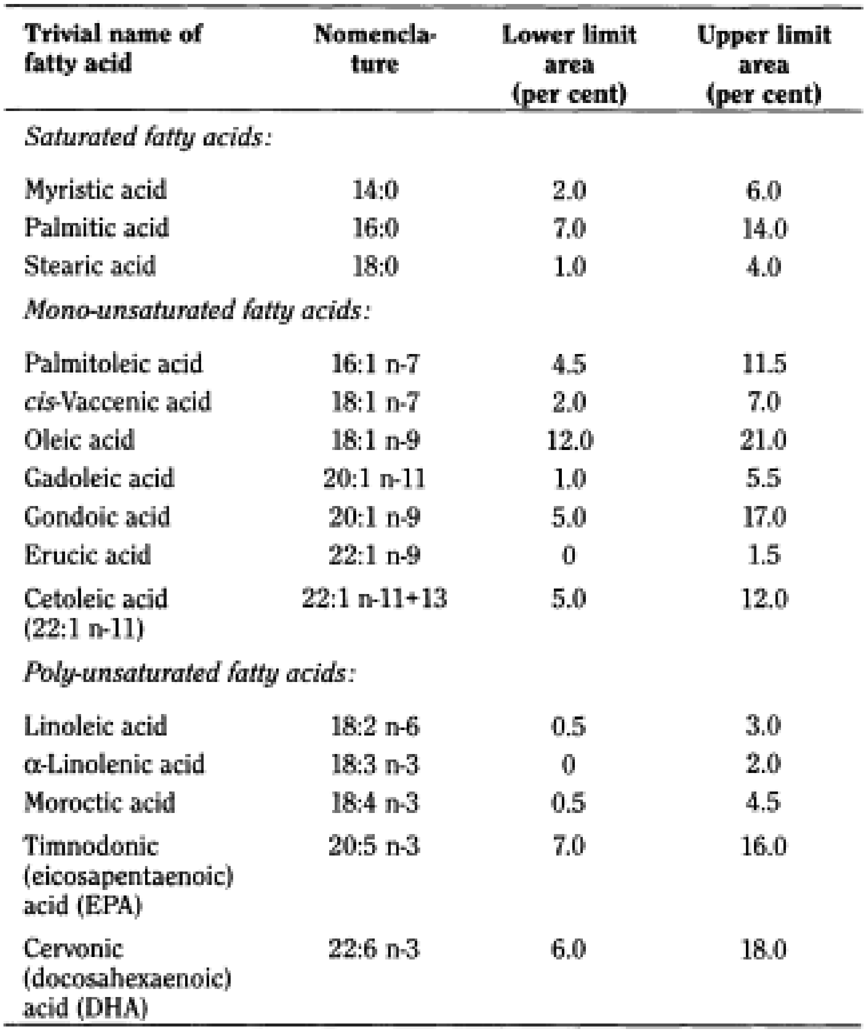
Maximum 1.5 per cent, determined on 2.0 g, and extracting with 3 quantities, each of 50 ml, of *peroxide-free ether R*.

Stearin

10 ml remains clear after cooling in iced water for 3 h.

Composition of fatty acids

Gas chromatography *(2.2.28)*.



Trivial name of fatty acid	Nomenclature	Lower limit area (per cent)	Upper limit area (per cent)
Saturated fatty acids:			
Myristic acid	14:0	2.0	6.0
Palmitic acid	16:0	7.0	14.0
Stearic acid	18:0	1.0	4.0
Mono-unsaturated fatty acids:			
Palmitoleic acid	16:1 n-7	4.5	11.5
cis-Vaccenic acid	18:1 n-7	2.0	7.0
Oleic acid	18:1 n-9	12.0	21.0
Gadoleic acid	20:1 n-11	1.0	5.5
Gondoic acid	20:1 n-9	5.0	17.0
Erucic acid	22:1 n-9	0	1.5
Cetoleic acid (22:1 n-11)	22:1 n-11+13	5.0	12.0
Poly-unsaturated fatty acids:			
Linoleic acid	18:2 n-6	0.5	3.0
α-Linolenic acid	18:3 n-3	0	2.0
Moroctic acid	18:4 n-3	0.5	4.5
Timnodonic (eicosapentaenoic) acid (EPA)	20:5 n-3	7.0	16.0
Cervonic (docosahexaenoic) acid (DHA)	22:6 n-3	6.0	18.0

Test solution Introduce about 0.45 g of the substance to be examined into a 10 ml volumetric flask, dissolve in *hexane R* containing 50 mg of *butylhydroxytoluene R* per litre and dilute to 10.0 ml with the same solvent. Transfer 2.0 ml of the solution into a quartz tube and evaporate the solvent with a gentle current of *nitrogen R.* Add 1.5 ml of a 20 g/l solution of *sodium hydroxide R* in *methanol R,* cover with *nitrogen R*, cap tightly with a polytetrafluoroethylene lined cap, mix and heat in a water-bath for 7 min. Cool, add 2 ml of *boron trichloride-methanol solution R*, cover with *nitrogen R*, cap tightly, mix and heat in a water-bath for 30 min. Cool to 40-50 °C, add 1 ml of *trimethylpentane R*, cap and vortex or shake vigorously for at least 30 s. Immediately add 5 ml of *saturated sodium chloride solution R*, cover with *nitrogen R*, cap and vortex or shake thoroughly for at least 15 s. Allow the upper layer to become clear and transfer to a separate tube. Shake the methanol layer once more with 1 ml of *trimethylpentane R* and combine the trimethylpentane extracts. Wash the combined extracts with 2 quantities, each of 1 ml, of *water R* and dry over *anhydrous sodium sulphate R*. Prepare 2 solutions for each sample.

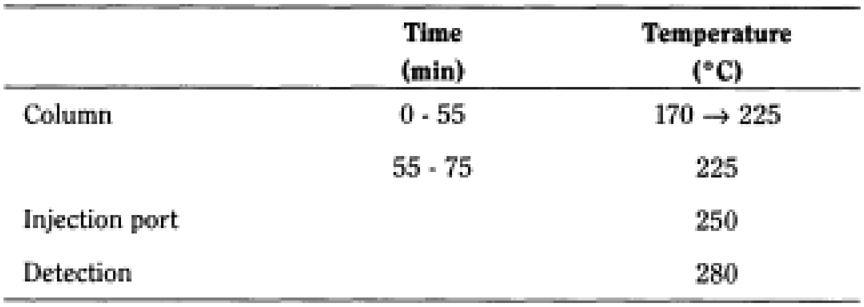
Column:

- *material*: fused silica,
- *size: l* = 30 m, Ø = 0.25 mm,
- *stationary phase: macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *hydrogen for chromatography R* or *helium for chromatography R,* where oxygen scrubber is applied.

Split ratio 1:200.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 55	170 → 225
	55 - 75	225
Injection port		250
Detection		280

Detection Flame ionisation.

Injection 1 µl, twice.

System suitability:

— the 15 fatty acids to be tested are satisfactorily identified from the chromatogram shown in Figure 1192.-1,

— injection of a mixture of equal amounts of *methyl palmitate R, methyl stearate R, methyl arachidate R* and *methyl behenate R* give area percentages of 24.4, 24.8, 25.2 and 25.6 (± 0.5 per cent), respectively,

— *resolution*: minimum of 1.3 between the peaks due to methyl oleate and methyl *cis*-vaccenate; the resolution between the pair due to methyl gadoleate and methyl gondoate is sufficient for purposes of identification and area measurement.

Calculate the area per cent for each fatty acid methyl ester from the expression:

$$\frac{A_x}{A_t} \times 100$$

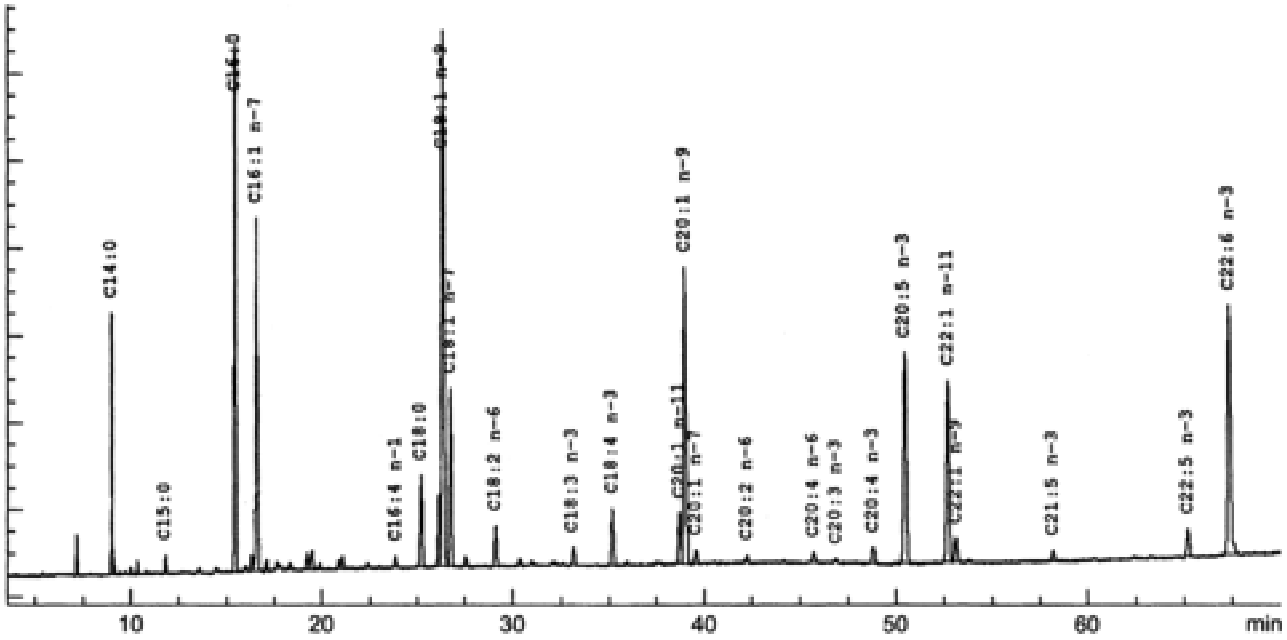


Figure 1192.-1. – *Chromatogram for the test for composition of fatty acids of cod-liver oil (type A)*

A_x = peak area of fatty acid,

A_t = sum of the peak areas (up to C22:6 n-3).

The calculation is not valid unless:

— the total area is based only on peaks due to solely fatty acids methyl esters,

— the number of fatty acid methyl ester peaks exceeding 0.05 per cent of the total area is at least 24,

— the 24 largest peaks of the methyl esters account for more than 90 per cent of the total area. (These correspond to, in common elution order: 14:0, 15:0, 16:0, 16:1 n-7, 16:4 n-1, 18:0, 18:1 n-9, 18:1 n-7, 18:2 n-6, 18:3 n-3, 18:4 n-3, 20:1 n-11, 20:1 n-9, 20:1 n-7, 20:2 n-6, 20:4 n-6, 20:3 n-3, 20:4 n-3, 20:5 n-3, 22:1 n-11, 22:1 n-9, 21:5 n-3, 22:5 n-3, 22:6 n-3).

ASSAY

Vitamin A

Carry out the test as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (for example, copper and iron) and acids.

Use method A. If method A is found not to be valid, use method B.

Method a

Ultraviolet absorption spectrophotometry *(2.2.25)*.

Test solution To 1.00 g in a round-bottomed flask, add 3 ml of a freshly prepared 50 per cent *m/m* solution of *potassium hydroxide R* and 30 ml of *ethanol R*. Boil under reflux in a current of *nitrogen R* for 30 min. Cool rapidly and add 30 ml of *water R*. Extract with 50 ml of *ether R*.

Repeat the extraction 3 times and discard the lower layer after complete separation. Wash the combined upper layers with 4 quantities, each of 50 ml, of *water R* and evaporate to dryness under a gentle current of *nitrogen R* at a temperature not exceeding 30 °C or in a rotary evaporator at a temperature not exceeding 30 °C under reduced pressure (water ejector). Dissolve the residue in sufficient *2-propanol R1* to give an expected concentration of vitamin A equivalent to 10-15 IU/ml.

Measure the absorbances of the solution at 300 nm, 310 nm, 325 nm and 334 nm and at the wavelength of maximum absorption with a suitable spectrophotometer in 1 cm specially matched cells, using *2-propanol R1* as the compensation liquid.

Calculate the content of vitamin A, as all-*trans*-retinol, in International Units per gram from the expression:

$$A_{325} \times \frac{1830}{100m} \times V$$

A_{325} = absorbance at 325 nm,

m = mass of the substance to be examined, in grams,

V = total volume of solution containing 10-15 IU of vitamin A per millilitre,

1830 = conversion factor for the specific absorbance of all-*trans*-retinol, in International Units.

The above expression can be used only if A_{325} has a value of not greater than $A_{325,corr}/0.970$ where $A_{325,corr}$ is the corrected absorbance at 325 nm and is given by the equation:

$$A_{325,\,corr} = 6.815A_{325} - 2.555A_{310} - 4.260A_{334}$$

A designates the absorbance at the wavelength indicated by the subscript.

If A_{325} has a value greater than $A_{325,corr}/0.970$, calculate the content of vitamin A from the following expression:

$$A_{325,\,corr} \times \frac{1830}{100m} \times V$$

The assay is not valid unless:

— the wavelength of maximum absorption lies between 323 nm and 327 nm,

— the absorbance at 300 nm relative to that at 325 nm is at most 0.73.

Method b

Liquid chromatography *(2.2.29)*.

Test solution To 2.00 g in a round-bottomed flask, add 5 ml of a freshly prepared 100 g/l solution of *ascorbic acid R* and 10 ml of a freshly prepared 800 g/l solution of *potassium* *hydroxide R* and 100 ml of *ethanol R*. Boil under a reflux condenser on a water-bath for 15 min. Add 100 ml of a 10 g/l solution of *sodium chloride R* and cool. Transfer the solution to a 500 ml separating funnel rinsing the round-bottomed flask with about 75 ml of a 10 g/l solution of *sodium chloride R* and then with 150 ml of a mixture of equal volumes of *light petroleum R1* and *ether R*. Shake for 1 min. When the layers have separated completely, discard the lower layer and wash the upper layer, first with 50 ml of a 30 g/l solution of *potassium hydroxide R* in a 10 per cent *V/V* solution of *ethanol R* and then with 3 quantities, each of 50 ml, of a 10 g/l solution of *sodium chloride R*. Filter the upper layer through 5 g of *anhydrous sodium sulphate R* on a fast filter paper into a 250 ml flask suitable for a rotary evaporator. Wash the funnel with 10 ml of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30 °C under reduced pressure (water ejector) and fill with *nitrogen R* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen R* at a temperature not exceeding 30 °C. Dissolve the residue in *2-propanol R*, transfer to a 25 ml volumetric flask and dilute to 25 ml with *2-propanol R*. Gentle heating in an ultrasonic bath may be required. (A large fraction of the white residue is cholesterol, constituting approximately 50 per cent of the unsaponifiable matter of cod-liver oil).

Reference solution (a) Prepare a solution of *retinol acetate CRS in 2-propanol R1* so that 1 ml contains about 1000 IU of all-*trans*-retinol.

The exact concentration of reference solution (a) is assessed by ultraviolet absorption spectrophotometry *(2.2.25).* Dilute reference solution (a) with *2-propanol R1* to a presumed concentration of 10-15 IU/ml and measure the absorbance at 326 nm in matched 1 cm cells using *2-propanol R1* as the compensation liquid.

Calculate the content of vitamin A in International Units per millilitre of reference solution (a) from the following expression, taking into account the assigned content of *retinol acetate CRS:*

$$A_{326} \times \frac{1900 \times V_2}{100 \times V_1}$$

A_{326} = absorbance at 326 nm,

V_1 = volume of reference solution (a) used,

V_2 = volume of the diluted solution,

1900 = conversion factor for the specific absorbance of *retinol acetate CRS*, in International Units.

Reference solution (b) Proceed as described for the test solution but using 2.00 ml of reference solution (a) in place of the substance to be examined.

The exact concentration of reference solution (b) is assessed by ultraviolet absorption spectrophotometry *(2.2.25)*. Dilute reference solution (b) with *2-propanol R1* to a presumed concentration of 10-15 IU/ml of all-*trans*-retinol and measure the absorbance at 325 nm in matched 1 cm cells using *2-propanol R1* as the compensation liquid.

Calculate the content of all-*trans*-retinol in International Units per millilitre of reference solution (b) from the expression:

$$A_{325} \times \frac{1830 \times V_3}{100 \times V_4}$$

A_{325} = absorbance at 325 nm,

V_3 = volume of the diluted solution,

V_4 = volume of reference solution (b) used,

1830 = conversion factor for the specific absorbance of all-*trans*-retinol, in International Units.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (film thickness 5-10 µm).

Mobile phase *water R, methanol R* (3:97 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 325 nm.

Injection 10 µl; inject in triplicate the test solution and reference solution (b).

Retention time All-*trans*-retinol = 5 ± 1 min.

System suitability:

— the chromatogram obtained with the test solution shows a peak due to that of all-*trans*-retinol in the chromatogram obtained with reference solution (b),

— when using the method of standard additions to the test solution there is greater than 95 per cent recovery of the added *retinol acetate CRS*,

— the recovery of all-*trans*-retinol in reference solution (b) as assessed by direct absorption spectrophotometry is greater than 95 per cent.

Calculate the content of vitamin A using the following expression:

$$A_1 \times \frac{C \times V}{A_2} \times \frac{1}{m}$$

A_1 = area of the peak due to all-*trans*-retinol in the chromatogram obtained with the test solution,

A_2 = area of the peak due to all-*trans*-retinol in the chromatogram obtained with reference solution (b),

C = concentration of *retinol acetate CRS* in reference solution (a) as assessed prior to the saponification, in International Units per millilitre (= 1000 IU/ml),

V = volume of reference solution (a) treated (2.00 ml), *m* = mass of the substance to be examined in the test solution (2.00 g).

Vitamin D$_3$

Liquid chromatography *(2.2.29)*. *Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air.*

Internal standard solution Dissolve 0.50 mg of *ergocalciferol CRS* in 100 ml of *ethanol R*.

Test solution (a) To 4.00 g in a round-bottomed flask, add 5 ml of a freshly prepared 100 g/l solution of *ascorbic acid R*, 10 ml of a freshly prepared 800 g/l solution of *potassium hydroxide R* and 100 ml of *ethanol R*. Boil under a reflux condenser on a water-bath for 30 min. Add 100 ml of a 10 g/l solution of *sodium chloride R* and cool the solution to room temperature. Transfer the solution to a 500 ml separating funnel rinsing the round-bottomed flask with about 75 ml of a 10 g/l solution of *sodium chloride R* and then with 150 ml of a mixture of equal volumes of *light petroleum R1* and *ether R*. Shake for 1 min. When the layers have separated completely, discard the lower layer and wash the upper layer, first with 50 ml of a 30 g/l solution of *potassium hydroxide R* in a 10 per cent *V/V* solution of *ethanol R*, and then with 3 quantities, each of 50 ml, of a 10 g/l solution of *sodium chloride R*. Filter the upper layer through 5 g of *anhydrous sodium sulphate R* on a fast filter paper into a 250 ml flask suitable for a rotary evaporator. Wash the funnel with 10 ml of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30 °C under reduced pressure (water ejector) and fill with *nitrogen R* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen R* at a temperature not exceeding 30 °C. Dissolve the residue in 1.5 ml of the mobile phase described under Purification. Gentle heating in an ultrasonic bath may be required. (A large fraction of the white residue is cholesterol, constituting approximately 50 per cent *m/m* of the unsaponifiable matter of cod-liver oil).

Test solution (b) To 4.00 g add 2.0 ml of the internal standard solution and proceed as described for test solution (a).

Reference solution (a) Dissolve 0.50 mg of *cholecalciferol CRS* in 100.0 ml of *ethanol R.*

Reference solution (b) In a round-bottomed flask, add 2.0 ml of reference solution (a) and 2.0 ml of the internal standard solution and proceed as described for test solution (a).

Purification

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *nitrile silica gel for chromatography R* (film thickness 10 µm).

Mobile phase *isoamyl alcohol R, hexane R* (1.6:98.4 *V/V*).

Flow rate 1.1 ml/min.

Detection Spectrophotometer at 265 nm.

Inject 350 µl of reference solution (b). Collect the eluate from 2 min before until 2 min after the retention time of cholecalciferol, in a ground-glass-stoppered tube containing 1 ml of a 1 g/l solution of *butylhydroxytoluene R* in *hexane R*. Repeat the procedure with test solutions (a) and (b). Evaporate the eluates obtained from reference solution (b) and from test solutions (a) and (b), separately, to dryness at a temperature not exceeding 30 °C under a gentle current of *nitrogen R*. Dissolve each residue in 1.5 ml of *acetonitrile R*.

Determination

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm,
- *stationary phase: octadecylsilyl silica gel for chromatography R* (film thickness 5 µm).

Mobile phase *phosphoric acid R, 96 per cent V/V solution of acetonitrile R* (0.2:99.8 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 2 quantities not exceeding 200 µl of each of the 3 solutions obtained under Purification.

System suitability:

- *resolution*: minimum 1.4 between the peaks corresponding to ergocalciferol and cholecalciferol in the chromatogram obtained with reference solution (b),
- when using the method of standard additions to test solution (a) there is greater than 95 per cent recovery of the added *cholecalciferol CRS* when due consideration has been given to correction by the internal standard.

Calculate the content of vitamin D_3 in International Units per gram using the following expression, taking into account the assigned content of *cholecalciferol CRS*:

$$\frac{A_2}{A_6} \times \frac{A_3}{A_4 - \left[\frac{A_5}{A_1}\right] \times A_2} \times \frac{m_2}{m_1} \times \frac{V_2}{V_1} \times 40$$

m_1 = mass of the sample in test solution (b) in grams,

m_2 = total mass of *cholecalciferol CRS* used for the preparation of reference solution (a) in micrograms (500 µg),

A_1 = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with test solution (a),

A_2 = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with test solution (b),

A_3 = area (or height) of the peak due to ergocalciferol in the chromatogram obtained with reference solution (b),

A_4 = area (or height) of the peak due to ergocalciferol in the chromatogram obtained with test solution (b),

A_5 = area (or height) of a possible peak in the chromatogram obtained with test solution (a) with the same retention time as the peak co-eluting with ergocalciferol in test solution (b),

A_6 = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (b),

V_1 = total volume of reference solution (a) (100 ml),

V_2 = volume of reference solution (a) used for preparing reference solution (b) (2.0 ml).

STORAGE

In an airtight and well-filled container, protected from light. If no antioxidant is added, store under an inert gas.

Once the container has been opened, its contents are used as soon as possible and any part of the contents not used at once is protected by an atmosphere of inert gas.

LABELLING

The label states:

- — the number of International Units of vitamin A,
- — the number of International Units of vitamin D_3.

Ph Eur

Cod-liver Oil (Type B)

General Notices

(Ph Eur monograph 1193)

Action and use

Source of vitamins A and D.

Each IU of vitamin D_3 is equivalent to 0.025 µg of colecalciferol.

Ph Eur

DEFINITION

Purified fatty oil obtained from the fresh livers of *Gadus morhua* L. and other species of *Gadidae*, solid substances being removed by cooling and filtering. A suitable antioxidant may be added.

Content

600 IU (180 µg) to 2500 IU (750 µg) of vitamin A per gram and 60 IU (1.5 µg) to 250 IU (6.25 µg) of vitamin D_3 per gram.

CHARACTERS

Appearance

Clear, yellowish, viscous liquid.

Solubility

Practically insoluble in water, slightly soluble in alcohol, miscible with light petroleum.

IDENTIFICATION

First identification A, B, C.

Second identification C, D.

A. In the assay for vitamin A using method A, the test solution shows an absorption maximum *(2.2.25)* at 325 ± 2 nm. In the assay for vitamin A using method B, the chromatogram obtained with the test solution shows a peak corresponding to the peak of all-*trans*-retinol in the chromatogram obtained with the reference solution.

B. In the assay for vitamin D_3, the chromatogram obtained with test solution (a) shows a peak corresponding to the peak of cholecalciferol in the chromatogram obtained with reference solution (b).

C. It complies with the test for composition of fatty acids (see Tests).

D. To 0.1 g add 0.5 ml of *methylene chloride R* and 1 ml of *antimony trichloride solution R.*

Mix. A deep blue colour develops in about 10 s.

TESTS

Colour

Not more intensely coloured than a reference solution prepared as follows: to 3.0 ml of red primary solution add 25.0 ml of yellow primary solution and dilute to 50.0 ml with a 10 g/l solution of *hydrochloric acid R (2.2.2, Method II)*.

Relative density *(2.2.5)*

0.917 to 0.930.

Refractive index *(2.2.6)*

1.477 to 1.484.

Acid value *(2.5.1)*

Maximum 2.0.

Iodine value *(2.5.4, Method B)*

150 to 180.

Use *starch solution R2*.

Peroxide value *(2.5.5, Method B)*

Maximum 10.0.

Unsaponifiable matter *(2.5.7)*

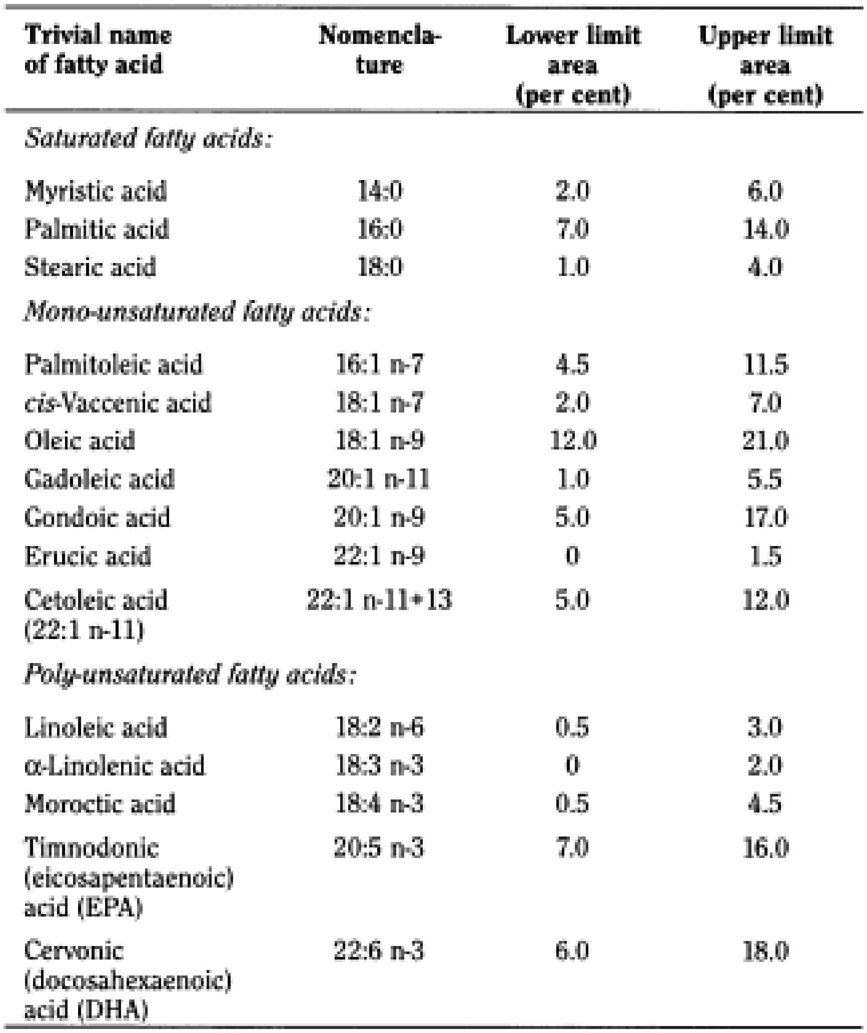
Maximum 1.5 per cent, determined on 2.0 g and extracting with 3 quantities, each of 50 ml, of *peroxide-free ether R*.

Stearin

10 ml remains clear after cooling in iced water for 3 h.

Composition of fatty acids

Gas chromatography *(2.2.28)*.



Trivial name of fatty acid	Nomenclature	Lower limit area (per cent)	Upper limit area (per cent)
Saturated fatty acids:			
Myristic acid	14:0	2.0	6.0
Palmitic acid	16:0	7.0	14.0
Stearic acid	18:0	1.0	4.0
Mono-unsaturated fatty acids:			
Palmitoleic acid	16:1 n-7	4.5	11.5
cis-Vaccenic acid	18:1 n-7	2.0	7.0
Oleic acid	18:1 n-9	12.0	21.0
Gadoleic acid	20:1 n-11	1.0	5.5
Gondoic acid	20:1 n-9	5.0	17.0
Erucic acid	22:1 n-9	0	1.5
Cetoleic acid (22:1 n-11)	22:1 n-11+13	5.0	12.0
Poly-unsaturated fatty acids:			
Linoleic acid	18:2 n-6	0.5	3.0
α-Linolenic acid	18:3 n-3	0	2.0
Moroctic acid	18:4 n-3	0.5	4.5
Timnodonic (eicosapentaenoic) acid (EPA)	20:5 n-3	7.0	16.0
Cervonic (docosahexaenoic) acid (DHA)	22:6 n-3	6.0	18.0

Test solution Introduce about 0.45 g of the substance to be examined into a 10 ml volumetric flask, dissolve in *hexane R* containing 50 mg *of butylhydroxytoluene R* per litre and dilute to 10.0 ml with the same solvent. Transfer 2.0 ml of the solution into a quartz tube and evaporate the solvent with a gentle current of *nitrogen R*. Add 1.5 ml of a 20 g/l solution of *sodium hydroxide R* in *methanol R*, cover with *nitrogen R*, cap tightly with a polytetrafluoroethylene lined cap, mix and heat in a water-bath for 7 min. Cool, add 2 ml of *boron trichloride-methanol solution R,* cover with *nitrogen R,* cap tightly, mix and heat in a water-bath for 30 min. Cool to 40-50 °C, add 1 ml of *trimethylpentane* R, cap and vortex or shake vigorously for at least 30 s. Immediately add 5 ml of *saturated sodium chloride solution R,* cover with *nitrogen R*, cap and vortex or shake thoroughly for at least 15 s. Allow the upper layer to become clear and transfer to a separate tube. Shake the methanol layer once more with 1 ml of *trimethylpentane R* and combine the trimethylpentane extracts. Wash the combined extracts with 2 quantities, each of 1 ml, of *water R* and dry over *anhydrous sodium sulphate R*. Prepare 2 solutions for each sample.

Column:

— *material*: fused silica,

— *size*: *l* = 30 m, Ø = 0.25 mm,

— *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *hydrogen for chromatography R* or *helium for chromatography R*, where oxygen scrubber is applied.

Split ratio 1:200.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 55	170 → 225
	55 - 75	225
Injection port		250
Detector		280

Detection Flame ionisation.

Injection 1 µl, twice.

System suitability:

— the 15 fatty acids to be tested are satisfactorily identified from the chromatogram shown in Figure 1193.-1.

— injection of a mixture of equal amounts of *methyl palmitate R, methyl stearate R, methyl arachidate R, and methyl behenate R* give area percentages of 24.4, 24.8, 25.2 and 25.6 (± 0.5 per cent), respectively,

— *resolution*: minimum of 1.3 between the peaks due to methyl oleate and methyl *cis*-vaccenate; the resolution between the pair due to methyl gadoleate and methyl gondoate is sufficient for purposes of identification and area measurement.

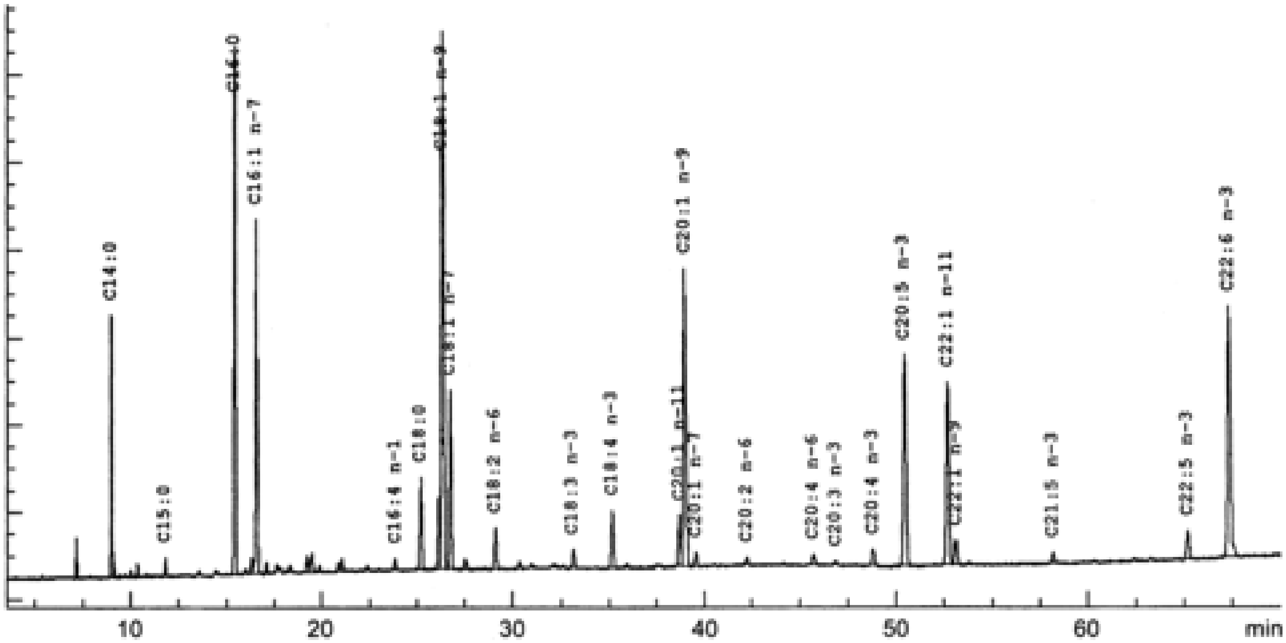


Figure 1193.-1. – *Chromatogram for the test for composition of fatty acids of cod-liver oil (type B)*

Calculate the area per cent for each fatty acid methyl ester from the expression:

$$\frac{A_x}{A_t} \times 100$$

A_x = peak area of fatty acid,

A_t = sum of the peak areas (up to C22:6 n-3.

The calculation is not valid unless:

— the total area is based only on peaks due to solely fatty acids methyl esters,

— the number of fatty acid methyl ester peaks exceeding 0.05 per cent of the total area is at least 24,

— the 24 largest peaks of the methyl esters account for more than 90 per cent of the total area. (These correspond to, in common elution order: 14:0, 15:0, 16:0, 16:1 n-7, 16:4 n-1, 18:0, 18:1 n-9, 18:1 n-7, 18:2 n-6, 18:3 n-3, 18:4 n-3, 20:1 n-11, 20:1 n-9, 20:1 n-7, 20:2 n-6, 20:4 n-6, 20:3 n-3, 20:4 n-3, 20:5 n-3, 22:1 n-11, 22:1 n-9, 21:5 n-3, 22:5 n-3, 22:6 n-3).

ASSAY

Vitamin A

Carry out the test as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (for example, copper and iron) and acids.

Use method A. If method A is found not to be valid, use method B.

Method A

Ultraviolet absorption spectrophotometry *(2.2.25)*.

Test solution To 1.00 g in a round-bottomed flask, add 3 ml of a freshly prepared 50 per cent *m/m* solution of *potassium hydroxide R* and 30 ml of *ethanol R*. Boil under reflux in a current of *nitrogen R* for 30 min. Cool rapidly and add 30 ml of *water R*. Extract with 50 ml of *ether R*. Repeat the extraction 3 times and discard the lower layer after complete separation. Wash the combined upper layers with 4 quantities, each of 50 ml, of *water R* and evaporate to dryness under a gentle current of *nitrogen R* at a temperature not exceeding 30 °C or in a rotary evaporator at a temperature not exceeding 30 °C under reduced pressure (water ejector). Dissolve the residue in sufficient *2-propanol R1* to give an expected concentration of vitamin A equivalent to 10-15 IU/ml.

Measure the absorbances of the solution at 300 nm, 310 nm, 325 nm and 334 nm and at the wavelength of maximum absorption with a suitable spectrophotometer in 1 cm specially matched cells, using *2-propanol R1* as the compensation liquid.

Calculate the content of vitamin A, as all-*trans*-retinol, in International Units per gram from the expression:

$$A_{325} \times \frac{1830}{100m} \times V$$

A_{325} = absorbance at 325 nm,

m = mass of the substance to be examined, in grams,

V = total volume of solution containing 10-15 IU of vitamin A per millilitre,

1830 = conversion factor for the specific absorbance of all-trans-retinol, in International Units.

The above expression can be used only if A_{325} has a value of not greater than $A_{325,\mathrm{corr}}/0.970$ where $A_{325,\mathrm{corr}}$ is the corrected absorbance at 325 nm and is given by the equation:

$$A_{325,\,\mathrm{corr}} = 6.815A_{325} - 2.555A_{310} - 4.260A_{334}$$

A designates the absorbance at the wavelength indicated by the subscript.

If A_{325} has a value greater than $A_{325,\mathrm{corr}}/0.970$, calculate the content of vitamin A from the expression:

$$A_{325,\,\mathrm{corr}} \times \frac{1830}{100m} \times V$$

The assay is not valid unless:

- the wavelength of maximum absorption lies between 323 nm and 327 nm,
- the absorbance at 300 nm relative to that at 325 nm is at most 0.73.

Method B

Liquid chromatography *(2.2.29)*.

Test solution To 2.00 g in a round-bottomed flask, add 5 ml of a freshly prepared 100 g/l solution of *ascorbic acid R* and 10 ml of a freshly prepared 800 g/l solution of *potassium hydroxide R* and 100 ml of *ethanol R.* Boil under a reflux condenser on a water-bath for 15 min. Add 100 ml of a 10 g/l solution of *sodium chloride R* and cool. Transfer the solution to a 500 ml separating funnel rinsing the round-bottomed flask with about 75 ml of a 10 g/l solution of *sodium chloride R* and then with 150 ml of a mixture of equal volumes of *light petroleum R1* and *ether R*. Shake for 1 min. When the layers have separated completely, discard the lower layer and wash the upper layer, first with 50 ml of a 30 g/l solution of *potassium hydroxide R* in a 10 per cent *V/V* solution of *ethanol R* and then with 3 quantities, each of 50 ml, of a 10 g/l solution of *sodium chloride R*. Filter the upper layer through 5 g of *anhydrous sodium sulphate R* on a fast filter paper into a 250 ml flask suitable for a rotary evaporator. Wash the funnel with 10 ml of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30 °C under reduced pressure (water ejector) and fill with *nitrogen R* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen R* at a temperature not exceeding 30 °C. Dissolve the residue in *2-propanol R,* transfer to a 25 ml volumetric flask and dilute to 25 ml with *2-propanol R*. Gentle heating in an ultrasonic bath may be required. (A large fraction of the white residue is cholesterol, constituting approximately 50 per cent of the unsaponifiable matter of cod-liver oil).

Reference solution (a) Prepare a solution of *retinol acetate CRS* in *2-propanol R1* so that 1 ml contains about 1000 IU of all-*trans*-retinol.

The exact concentration of reference solution (a) is assessed by ultraviolet absorption spectrophotometry *(2.2.25)*. Dilute reference solution (a) with *2-propanol R1* to a presumed concentration of 10-15 IU/ml and measure the absorbance at 326 nm in matched 1 cm cells using *2-propanol R1* as the compensation liquid.

Calculate the content of vitamin A in International Units per millilitre of reference solution (a) using the following expression, taking into account the assigned content of *retinol acetate CRS:*

$$A_{326} \times \frac{1900 \times V_2}{100 \times V_1}$$

A_{326} = absorbance at 326 nm,

V_1 = volume of reference solution (a) used,

V_2 = volume of the diluted solution,

1900 = conversion factor for the specific absorbance of *retinol acetate CRS*, in International Units.

Reference solution (b) Proceed as described for the test solution but using 2.00 ml of reference solution (a) in place of the substance to be examined.

The exact concentration of reference solution (b) is assessed by ultraviolet absorption spectrophotometry *(2.2.25)*. Dilute reference solution (b) with *2-propanol R1* to a presumed concentration of 10-15 IU/ml of all-*trans*-retinol and measure the absorbance at 325 nm in matched 1 cm cells using *2-propanol R1* as the compensation liquid.

Calculate the content of all-*trans*-retinol in International Units per millilitre of reference solution (b) from the expression:

$$A_{325} \times \frac{1830 \times V_3}{100 \times V_4}$$

A_{325} = absorbance at 325 nm,

V_3 = volume of the diluted solution,

V_4 = volume of reference solution (b) used,

1830 = conversion factor for the specific absorbance of all-*trans*-retinol, in International Unit.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (film thickness 5-10 µm).

Mobile phase *water R, methanol R* (3:97 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 325 nm.

Injection 10 µl; inject in triplicate the test solution and reference solution (b).

Retention time All-*trans*-retinol = 5 ± 1 min.

System suitability:

— the chromatogram obtained with the test solution shows a peak due to that of all-*trans*-retinol in the chromatogram obtained with reference solution (b),

— when using the method of standard additions to the test solution there is greater than 95 per cent recovery of the added *retinol acetate CRS*,

— the recovery of all-*trans*-retinol in reference solution (b) as assessed by direct absorption spectrophotometry is greater than 95 per cent.

Calculate the content of vitamin A using the following expression:

$$A_1 \times \frac{C \times V}{A_2} \times \frac{1}{m}$$

A_1 = area of the peak due to all-*trans*-retinol in the chromatogram obtained with the test solution,

A_2 = area of the peak due to all-*trans*-retinol in the chromatogram obtained with reference solution (b),

C = concentration of *retinol acetate CRS* in reference solution (a) as assessed prior to the saponification, in International Units per millilitre (= 1000 IU/ml),

V = volume of reference solution (a) treated (2.00 ml),

m = mass of the substance to be examined in the test solution (2.00 g).

Vitamin D_3

Liquid chromatography *(2.2.29). Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air.*

Internal standard solution Dissolve 0.50 mg of *ergocalciferol CRS* in 100 ml of *ethanol R*.

Test solution (a) To 4.00 g in a round-bottomed flask, add 5 ml of a freshly prepared 100 g/l solution of *ascorbic acid R*, 10 ml of a freshly prepared 800 g/l solution of *potassium hydroxide R* and 100 ml of *ethanol R*. Boil under a reflux condenser on a water-bath for 30 min. Add 100 ml of a 10 g/l solution of *sodium chloride R* and cool the solution to room temperature. Transfer the solution to a 500 ml separating funnel rinsing the round-bottomed flask with about 75 ml of a 10 g/l solution of *sodium chloride R* and then with 150 ml of a mixture of equal volumes of *light petroleum R1* and *ether R*. Shake for 1 min. When the layers have separated completely, discard the lower layer and wash the upper layer, first with 50 ml of a 30 g/l solution of *potassium hydroxide R* in a 10 per cent *V/V* solution of *ethanol R*, and then with 3 quantities, each of 50 ml, of a 10 g/l solution of *sodium chloride R*. Filter the upper layer through 5 g of *anhydrous sodium sulphate R* on a fast filter paper into a 250 ml flask suitable for a rotary evaporator. Wash the funnel with 10 ml of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30 °C under reduced pressure (water ejector) and fill with *nitrogen R* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen R* at a temperature not exceeding 30 °C. Dissolve the residue in 1.5 ml of the mobile phase described under Purification. Gentle heating in an ultrasonic bath may be required. (A large fraction of the white residue is cholesterol, constituting approximately 50 per cent *m/m* of the unsaponifiable matter of cod-liver oil).

Test solution (b) To 4.00 g add 2.0 ml of the internal standard solution and proceed as described for test solution (a).

Reference solution (a) Dissolve 0.50 mg of *cholecalciferol CRS* in 100.0 ml of *ethanol R*.

Reference solution (b) In a round-bottomed flask, add 2.0 ml of reference solution (a) and 2.0 ml of the internal standard solution and proceed as described for test solution (a).

Purification

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: nitrile silica gel for chromatography R* (film thickness 10 µm).

Mobile phase *isoamyl alcohol R, hexane R* (1.6:98.4 *V/V*).

Flow rate 1.1 ml/min.

Detection Spectrophotometer at 265 nm.

Inject 350 µl of reference solution (b). Collect the eluate from 2 min before until 2 min after the retention time of cholecalciferol, in a ground-glass-stoppered tube containing 1 ml of a 1 g/l solution of *butylhydroxytoluene R* in *hexane R*. Repeat the procedure with test solutions (a) and (b). Evaporate the eluates obtained from reference solution (b) and from test solutions (a) and (b), separately, to dryness at a temperature not exceeding 30 °C under a gentle current of *nitrogen R*. Dissolve each residue in 1.5 ml of *acetonitrile R*.

Determination

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (film thickness 5 µm).

Mobile phase *phosphoric acid R, a 96 per cent V/V solution of acetonitrile R* (0.2:99.8 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 2 quantities not exceeding 200 µl of each of the 3 solutions obtained under Purification.

System suitability:

— *resolution*: minimum 1.4 between the peaks due to ergocalciferol and cholecalciferol in the chromatogram obtained with reference solution (b),

— when using the method of standard additions to test solution (a) there is greater than 95 per cent recovery of the added *cholecalciferol CRS* when due consideration has been given to correction by the internal standard.

Calculate the content of vitamin D_3 in International Units per gram using the following expression, taking into account the assigned content of *cholecalciferol CRS*:

$$\frac{A_2}{A_6} \times \frac{A_3}{A_4 - \left[\frac{A_5}{A_1}\right] \times A_2} \times \frac{m_2}{m_1} \times \frac{V_2}{V_1} \times 40$$

m_1 = mass of the sample in test solution (b) in grams,

m_2 = total mass of *cholecalciferol CRS* used for the preparation of reference solution (a) in micrograms (500 µg),

A_1 = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with test solution (a),

A_2 = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with test solution (b),

A_3 = area (or height) of the peak due to ergocalciferol in the chromatogram obtained with reference solution (b),

A_4 = area (or height) of the peak due to ergocalciferol in the chromatogram obtained with test solution (b),

A_5 = area (or height) of a possible peak in the chromatogram obtained with test solution (a) with the same retention time as the peak co-eluting with ergocalciferol in test solution (b),

A_6 = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (b),

V_1 = total volume of reference solution (a) (100 ml),

V_2 = volume of reference solution (a) used for preparing reference solution (b) (2.0 ml).

STORAGE

In an airtight and well-filled container, protected from light. If no antioxidant is added, store under an inert gas.

Once the container has been opened, its contents are used as soon as possible and any part of the contents not used at once is protected by an atmosphere of inert gas.

LABELLING

The label states:

— the number of International Units of vitamin A,

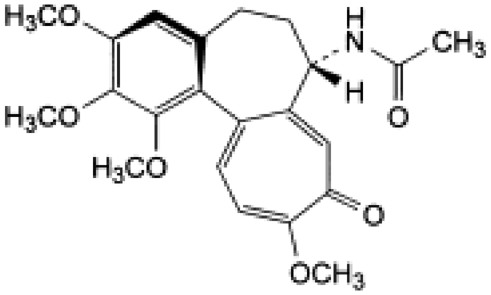
— the number of International Units of vitamin D_3.

Ph Eur

Colchicine

General Notices

(Ph Eur monograph 0758)



$C_{22}H_{25}NO_6$ 399.4 *64-86-8*

Action and use

Used in treatment of gout.

Preparation

Colchicine Tablets

Ph Eur

DEFINITION

(-)-*N*-[(7*S*,12a*S*)-1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl] acetamide.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellowish-white, amorphous or crystalline powder.

Solubility

Very soluble in water, rapidly recrystallising from concentrated solutions as the sesquihydrate, freely soluble in alcohol, practically insoluble in cyclohexane.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Dissolve 5 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with *alcohol R*. Examined between 230 nm and 400 nm *(2.2.25)*, the solution shows 2 absorption maxima, at 243 nm and 350 nm. The ratio of the absorbance measured at 243 nm to that measured at 350 nm is 1.7 to 1.9.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *colchicine CRS.*

C. To 0.5 ml of solution S (see Tests) add 0.5 ml of *dilute hydrochloric acid R* and 0.15 ml of *ferric chloride solution R1*. The solution is yellow and becomes dark green on boiling for 30 s. Cool, add 2 ml of *methylene chloride R* and shake. The organic layer is greenish-yellow.

D. Dissolve about 30 mg in 1 ml of *alcohol R* and add 0.15 ml of *ferric chloride solution R1*. A brownish-red colour develops.

TESTS

Solution S

Dissolve 0.10 g in *water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution GY_3 *(2.2.2, Method II).*

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Either the solution does not change colour or it becomes green. Not more than 0.1 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Specific optical rotation *(2.2.7)*

- 235 to - 250 (anhydrous substance).

Dissolve 50.0 mg in *alcohol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and water R and dilute to 20.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 20.0 mg of *colchicine for system suitability CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 20.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution (c) Dilute 1 ml of reference solution (b) to 20.0 ml with a mixture of equal volumes of *methanol R* and *water R*.

Column:

- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase Mix 450 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* and 530 volumes of *methanol R*. After cooling to room temperature, adjust the volume to 1000 ml with *methanol R.* Adjust the apparent pH to 5.5 with *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 3 times the retention time of colchicine.

Relative retention With reference to colchicine (retention time = about 7 min): impurity D = about 0.4; impurity E = about 0.7; impurity B = about 0.8; impurity A = about 0.94; impurity C = about 1.2.

System suitability Reference solution (a):

Peak-to-valley ratio Minimum 2, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to colchicine.

Limits:

- *impurity A*: not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.5 per cent);
- *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- *total:* not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent);
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Colchiceine

Maximum 0.2 per cent.

Dissolve 50 mg in *water R* and dilute to 5 ml with the same solvent. Add 0.1 ml of *ferric chloride solution R1*. The solution is not more intensely coloured than a mixture of 1 ml of red primary solution, 2 ml of yellow primary solution and 2 ml of blue primary solution *(2.2.2, Method II).*

Chloroform *(2.4.24)*

Maximum 500 ppm.

Ethyl acetate *(2.4.24)*

Maximum 6.0 per cent *m/m*.

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 0.5 g.

ASSAY

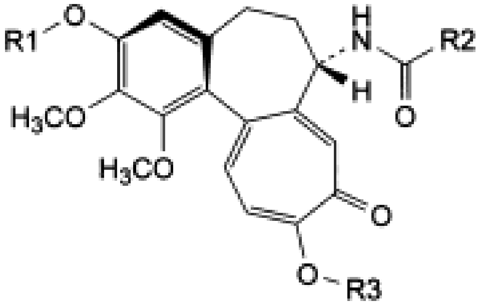
Dissolve 0.250 g with gentle heating in a mixture of 10 ml of *acetic anhydride R* and 20 ml of *toluene R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of 0*.1 M perchloric acid* is equivalent to 39.94 mg of $C_{22}H_{25}NO_6$.

STORAGE

Protected from light.

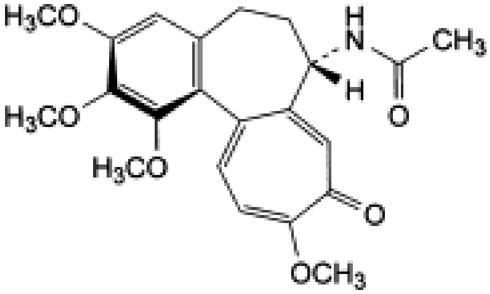
IMPURITIES



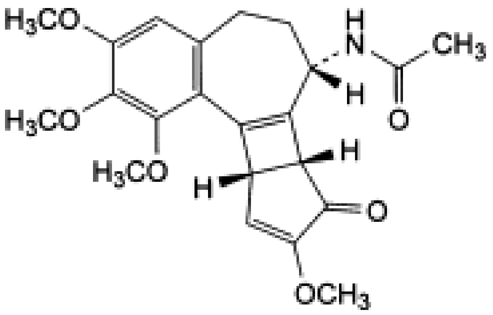
A. R1 = R3 = CH_3, R2 = H: *N*-[(7*S*,12a*S*)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]formamide (*N*-deacetyl-*N*-formylcolchicine),

E. R1 = H, R2 = R3 = CH_3: *N*-[(7*S*,12a*S*)-3-hydroxy-1,2,10-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide (3-*C*-demethylcolchicine),

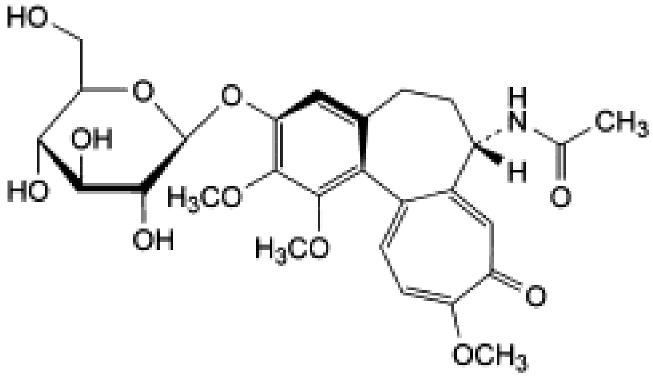
F. R1 = R2 = CH_3, R3 = H: *N*-[(7*S*,12a*S*)-10-hydroxy-1,2,3-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchiceine),



B. (-)-*N*-[(7*S*,12a*R*)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl] acetamide (conformationnal isomer),



C. *N*-[(7*S*,7b*R*,10a*S*)-1,2,3,9-tetramethoxy-8-oxo-5,6,7,7b,8,10a-hexahydrobenzo[*a*]cyclopenta[3,4]cyclobuta[1,2-c]cyclohepten-7-yl]acetamide (β-lumicolchicine),



D. *N*-[(7*S*,12a*S*)-3-(β-D-glucopyranosyloxy)-1,2,10-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchicoside).

Ph Eur

Colecalciferol Concentrate (Oily Form)

General Notices

(Cholecalciferol Concentrate (Oily Form), Ph Eur monograph 0575)

Action and use

Vitamin D analogue (Vitamin D_3).

Ph Eur

DEFINITION

Solution of *Cholecalciferol (0072)* in a suitable vegetable fatty oil, authorised by the competent authority.

Content

90.0 per cent to 110.0 per cent of the cholecalciferol content stated on the label, which is not less than 500 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance

Clear, yellow liquid.

Solubility

Practically insoluble in water, slightly soluble in anhydrous ethanol, miscible with solvents of fats.

Partial solidification may occur, depending on the temperature.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B.*

A. Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use*.

Test solution Dissolve an amount of the preparation to be examined corresponding to 400 000 IU in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 4 ml with the same solution.

Reference solution (a) Dissolve 10 mg of *cholecalciferol CRS* in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 4 ml with the same solution.

Reference solution (b) Dissolve 10 mg of *ergocalciferol CRS* in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 4 ml with the same solution.

Plate *TLC silica gel G plate R*.

Mobile phase 0.1 g/l solution of *butylhydroxytoluene R* in a mixture of equal volumes of *cyclohexane* R and *peroxide-free ether R.*

Application 20 µl.

Development Immediately, protected from light, over a path of 15 cm.

Drying In air.

Detection Spray with *sulphuric acid R*.

Results The chromatogram obtained with the test solution shows immediately a bright yellow principal spot which rapidly becomes orange-brown, then gradually greenish-grey, remaining so for 10 min. This spot is similar in position, colour and size to the spot in the chromatogram obtained with reference solution (a). The chromatogram obtained with reference solution (b) shows immediately at the same level an orange principal spot which gradually becomes reddish-brown and remains so for 10 min.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Prepare a solution in *cyclohexane R* containing the equivalent of about 400 IU/ml.

Spectral range 250-300 nm.

Absorption maximum At 267 nm.

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Acid value *(2.5.1)*

Maximum 2.0.

Dissolve 5.0 g in 25 ml of the prescribed mixture of solvents.

Peroxide value *(2.5.5, Method A)*

Maximum 20.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air.

Liquid chromatography *(2.2.29)*.

Test solution Dissolve a quantity of the preparation to be examined, weighed with an accuracy of 0.1 per cent, equivalent to about 400 000 IU, in 10.0 ml of *toluene R* and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *cholecalciferol CRS* without heating in 10.0 ml of *toluene R* and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of *cholecalciferol for performance test CRS* to 5.0 ml with the mobile phase. Heat in a water-bath at 90 °C under a reflux condenser for 45 min and cool.

Reference solution (c) Dissolve 0.10 g of *cholecalciferol CRS* without heating in *toluene R* and dilute to 100.0 ml with the same solvent.

Reference solution (d) Dilute 5.0 ml of reference solution (c) to 50.0 ml with the mobile phase. Keep the solution in iced water.

Reference solution (e) Place 5.0 ml of reference solution (c) in a volumetric flask, add about 10 mg of *butylhydroxytoluene R* and displace air from the flask with *nitrogen R*. Heat in a water-bath at 90 °C under a reflux condenser protected from light and under *nitrogen R* for 45 min. Cool and dilute to 50.0 ml with the mobile phase.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase *pentanol R, hexane R* (3:997 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection The chosen volume of each solution (the same volume for reference solution (a) and for the test solution); automatic injection device or sample loop recommended.

Relative retention With reference to cholecalciferol: pre-cholecalciferol = about 0.4; *trans*-cholecalciferol = about 0.5.

System suitability Reference solution (b):

— *resolution:* minimum 1.0 between the peaks due to pre-cholecalciferol and *trans*-cholecalciferol; if necessary adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution;

— *repeatability*: maximum relative standard deviation of 1.0 per cent for the peak due to cholecalciferol after 6 injections.

Calculate the conversion factor (f) using the following expression:

$$\frac{K - L}{M}$$

K = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (d),

L = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (e),

M = area (or height) of the peak due to pre-cholecalciferol in the chromatogram obtained with reference solution (e).

The value of f determined in duplicate on different days may be used during the entire procedure.

Calculate the content of cholecalciferol in International Units per gram using the following expression:

$$\frac{m'}{V'} \times \frac{V}{m} \times \frac{S_D + (f \times S_p)}{S'_D} \times 40\,000 \times 1000$$

m = mass of the preparation to be examined in the test solution, in milligrams,

m' = mass of *cholecalciferol CRS* in reference solution (a), in milligrams,

V = volume of the test solution (100 ml),

V' = volume of reference solution (a) (100 ml),

S_D = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with the test solution,

S'_D = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (a),

S_p = area (or height) of the peak due to pre-cholecalciferol in the chromatogram obtained with the test solution,

f = conversion factor.

STORAGE

In an airtight, well-filled container, protected from light. The contents of an opened container are to be used as soon as possible; any unused part is to be protected by an atmosphere of nitrogen.

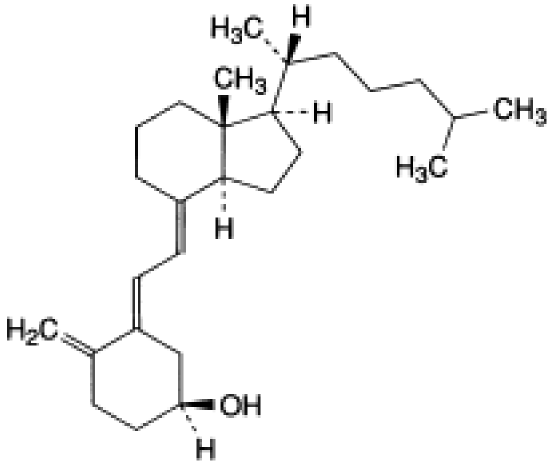
LABELLING

The label states:

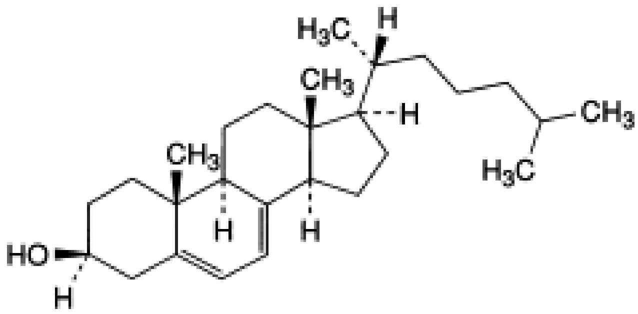
— the number of International Units per gram;

— the method of restoring the solution if partial solidification occurs.

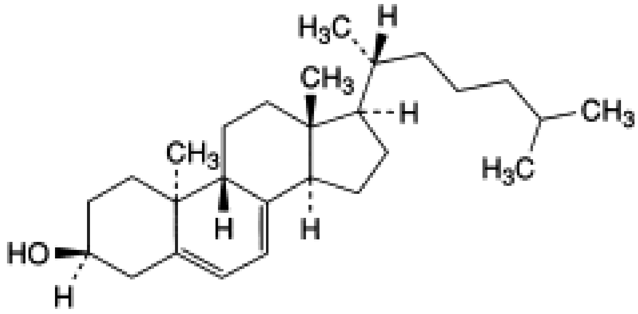
IMPURITIES



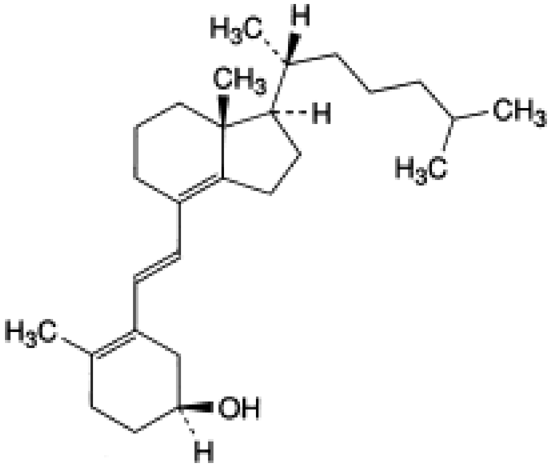
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



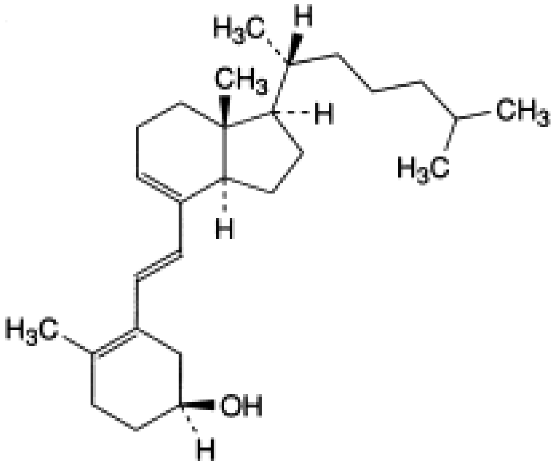
B. cholesta-5,7-dien-3β-ol (7,8-di dehydrocholesterol, provitamin D_3),



C. (9β,10α)-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol$_3$),



E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol$_3$).

Ph Eur

Colecalciferol Concentrate (Powder Form)

General Notices

(Cholecalciferol Concentrate (Powder Form), Ph Eur monograph 0574)

Action and use

Vitamin D analogue (Vitamin D_3).

Ph Eur

DEFINITION

Powder concentrate obtained by dispersing an oily solution of *Cholecalciferol* (0072) in an appropriate matrix, which is usually based on a combination of gelatin and carbohydrates of suitable quality, authorised by the competent authority.

Content

90.0 per cent to 110.0 per cent of the cholecalciferol content stated on the label, which is not less than 100 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance

White or yellowish-white, small particles.

Solubility

Practically insoluble, swells, or forms a dispersion in water, depending on the formulation.

IDENTIFICATION

First identification A, C.

Second identification A, B.

A. Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use.*

Test solution Place 10.0 ml of the test solution prepared for the assay in a suitable flask and evaporate to dryness under reduced pressure by swirling in a water-bath at 40 °C. Cool under running water and restore atmospheric pressure with *nitrogen R*. Dissolve the residue immediately in 0.4 ml of *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R*.

Reference solution (a) Dissolve 10 mg of *cholecalciferol CRS* in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 4 ml with the same solution.

Reference solution (b) Dissolve 10 mg of *ergocalciferol CRS* in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 4 ml with the same solution.

Plate *TLC silica gel G plate R*.

Mobile phase a 0.1 g/l solution of *butylhydroxytoluene R* in a mixture of equal volumes of *cyclohexane R* and *peroxide-free ether R*.

Application 20 µl.

Development Immediately, protected from light, over a path of 15 cm.

Drying In air.

Detection Spray with *sulphuric acid R*.

Results The chromatogram obtained with the test solution shows immediately a bright yellow principal spot, which rapidly becomes orange-brown, then gradually greenish-grey, remaining so for 10 min. This spot is similar in position, colour and size to the spot in the chromatogram obtained with reference solution (a). The chromatogram obtained with reference solution (b) shows immediately at the same level an orange principal spot, which gradually becomes reddish-brown and remains so for 10 min.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Place 5.0 ml of the test solution prepared for the assay in a suitable flask and evaporate to dryness under reduced pressure by swirling in a water-bath at 40 °C. Cool under running water and restore atmospheric pressure with *nitrogen R*. Dissolve the residue immediately in 50.0 ml of *cyclohexane R.*

Spectral range 250-300 nm.

Absorption maximum At 265 nm.

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air.

Liquid chromatography (2.2.29).

Test solution Introduce into a saponification flask a quantity of the preparation to be examined, weighed with an accuracy of 0.1 per cent, equivalent to about 100 000 IU. Add 5 ml of *water R*, 20 ml of *anhydrous ethanol R*, 1 ml of *sodium ascorbate solution R* and 3 ml of a freshly prepared 50 per cent *m/m* solution of *potassium hydroxide R*. Heat in a water-bath under a reflux condenser for 30 min. Cool rapidly under running water. Transfer the liquid to a separating funnel with the aid of 2 quantities, each of 15 ml, of *water R*, 1 quantity of 10 ml of *ethanol (96 per cent) R* and 2 quantities, each of 50 ml, of *pentane R*. Shake vigorously for 30 s. Allow to stand until the 2 layers are clear. Transfer the lower aqueous-alcoholic layer to a 2nd separating funnel and shake with a mixture of 10 ml of *ethanol (96 per cent) R* and 50 ml of *pentane R*. After separation, transfer the aqueous-alcoholic layer to a 3rd separating funnel and the pentane layer to the 1st separating funnel, washing the 2nd separating funnel with 2 quantities, each of 10 ml, of *pentane R* and adding the washings to the 1st separating funnel.

Shake the aqueous-alcoholic layer with 50 ml of *pentane R* and add the pentane layer to the 1st funnel. Wash the pentane layer with 2 quantities, each of 50 ml, of a freshly prepared 30 g/l solution of *potassium hydroxide R* in *ethanol (10 per cent V/V) R*, shaking vigorously, then wash with successive quantities, each of 50 ml, of *water R* until the washings are neutral to phenolphthalein. Transfer the washed pentane extract to a ground-glass-stoppered flask. Evaporate the contents of the flask to dryness under reduced pressure by swirling in a water-bath at 40 °C. Cool under running water and restore atmospheric pressure with *nitrogen R*. Dissolve the residue immediately in 5.0 ml of *toluene R* and add 20.0 ml of the mobile phase to obtain a solution containing about 4000 IU/ml.

Reference solution (a) Dissolve 10.0 mg of *cholecalciferol CRS*, without heating, in 10.0 ml of *toluene R* and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of *cholecalciferol for performance test CRS* to 5.0 ml with the mobile phase. Heat in a water-bath at 90 °C under a reflux condenser for 45 min and cool.

Reference solution (c) Dissolve 0.10 g of *cholecalciferol CRS*, without heating, in *toluene R* and dilute to 100.0 ml with the same solvent.

Reference solution (d) Dilute 5.0 ml of reference solution (c) to 50.0 ml with the mobile phase. Keep the solution in iced water.

Reference solution (e) Place 5.0 ml of reference solution (c) in a volumetric flask, add about 10 mg of *butylhydroxytoluene R* and displace the air from the flask with *nitrogen R*. Heat in a water-bath at 90 °C under a reflux condenser, protected from light and under *nitrogen R*, for 45 min. Cool and dilute to 50.0 ml with the mobile phase.

Column:

— *size*: $l = 0.25$ m, Ø = 4.6 mm;

— *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase *pentanol R, hexane R* (3:997 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection The chosen volume of each solution (the same volume for reference solution (a) and for the test solution); automatic injection device or sample loop recommended.

Relative retention With reference to cholecalciferol: pre-cholecalciferol = about 0.4; *trans*-cholecalciferol = about 0.5.

System suitability Reference solution (b):

— *resolution*: minimum 1.0 between the peaks due to pre-cholecalciferol and *trans*-cholecalciferol; if necessary, adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution;

— *repeatability*: maximum relative standard deviation of 1.0 per cent for the peak due to cholecalciferol after 6 injections.

Calculate the conversion factor (f) using the following expression:

$$\frac{K - L}{M}$$

K = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (d),

L = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (e),

M = area (or height) of the peak due to pre-cholecalciferol in the chromatogram obtained with reference solution (e).

The value of f determined in duplicate on different days may be used during the entire procedure.

Calculate the content of cholecalciferol in International Units per gram using the following expression:

$$\frac{m'}{V'} \times \frac{V}{m} \times \frac{S_D + (f \times S_p)}{S'_D} \times 40\,000 \times 1000$$

m = mass of the preparation to be examined in the test solution, in milligrams,

m' = mass of *cholecalciferol CRS* in reference solution (a), in milligrams,

V = volume of the test solution (25 ml),

V' = volume of reference solution (a) (100 ml),

S_D = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with the test solution,

S'_D = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (a),

S_p = area (or height) of the peak due to pre-cholecalciferol in the chromatogram obtained with the test solution,

f = conversion factor.

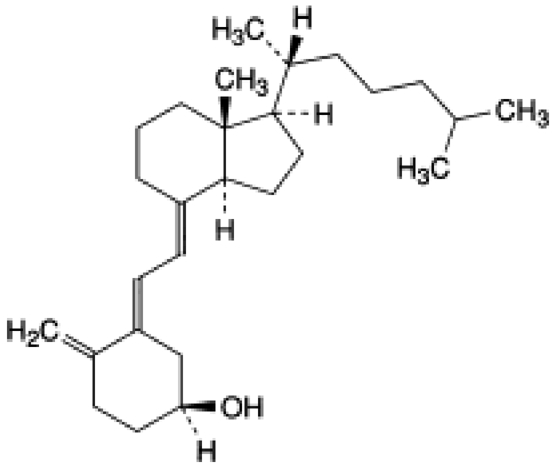
STORAGE

In an airtight, well-filled container, protected from light. The contents of an opened container are to be used as soon as possible; any unused part is to be protected by an atmosphere of nitrogen.

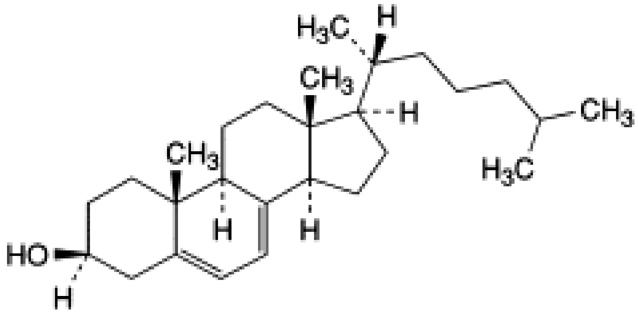
LABELLING

The label states the number of International Units per gram.

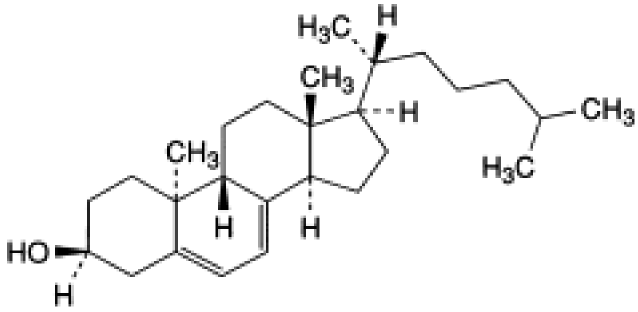
IMPURITIES



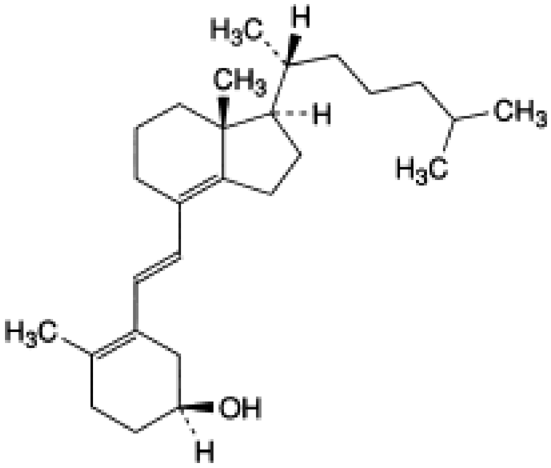
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



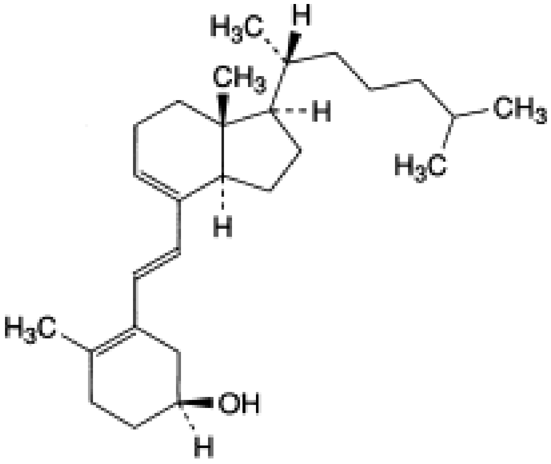
B. cholesta-5,7-dien-3β-ol (7,8-di dehydrocholesterol, provitamin D_3),



C. (9β,10α)-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol$_3$),



E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol$_3$).

Ph Eur

Colecalciferol Concentrate (Water-dispersible Form)

General Notices

(Cholecalciferol Concentrate (Water-Dispersible Form), Ph Eur monograph 0598)

Action and use

Vitamin D analogue (Vitamin D_3).

Ph Eur

DEFINITION

Solution of *Cholecalciferol (0072)* in a suitable vegetable fatty oil, authorised by the competent authority, to which suitable solubilisers have been added.

Content

90.0 per cent to 115.0 per cent of the cholecalciferol content stated on the label, which is not less than 100 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance

Slightly yellowish liquid of variable opalescence and viscosity.

Highly concentrated solutions may become cloudy at low temperatures or form a gel at room temperature.

IDENTIFICATION

First identification A, C, D.

Second identification A, B, D.

A. Thin-layer chromatography *(2.2.27). Prepare the solutions immediately before use.*

Test solution Place 10.0 ml of the test solution prepared for the assay in a suitable flask and evaporate to dryness under reduced pressure by swirling in a water-bath at 40 °C. Cool under running water and restore atmospheric pressure with *nitrogen R*. Dissolve the residue immediately in 0.4 ml of *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R.*

Reference solution (a) Dissolve 10 mg of *cholecalciferol CRS* in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 4 ml with the same solution.

Reference solution (b) Dissolve 10 mg of *ergocalciferol CRS* in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 4 ml with the same solution.

Plate TLC silica gel G plate R.

Mobile phase A 0.1 g/l solution of *butylhydroxytoluene R* in a mixture of equal volumes of *cyclohexane R* and *peroxide-free ether R*.

Application 20 µl.

Development Immediately, protected from light, over a path of 15 cm.

Drying In air.

Detection Spray with *sulphuric acid R*.

Results The chromatogram obtained with the test solution shows immediately a bright yellow principal spot, which rapidly becomes orange-brown, then gradually greenish-grey, remaining so for 10 min. This spot is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The chromatogram obtained with reference solution (b) shows immediately at the same level an orange principal spot, which gradually becomes reddish-brown and remains so for 10 min.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Place 5.0 ml of the test solution prepared for the assay in a suitable flask and evaporate to dryness under reduced pressure by swirling in a water-bath at 40 °C. Cool under running water and restore atmospheric pressure with *nitrogen R*. Dissolve the residue immediately in 50.0 ml of *cyclohexane R*.

Spectral range 250-300 nm.

Absorption maximum At 265 nm.

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

D. Mix about 1 g with 10 ml of *water R* previously warmed to 50 °C, and cool to 20 °C. Immediately after cooling, a uniform, slightly opalescent and slightly yellow dispersion is obtained.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air.

Liquid chromatography *(2.2.29)*.

Test solution Introduce into a saponification flask a quantity of the preparation to be examined, weighed with an accuracy of 0.1 per cent, equivalent to about 100 000 IU. Add 5 ml of *water R*, 20 ml of *anhydrous ethanol R*, 1 ml of *sodium ascorbate solution R* and 3 ml of a freshly prepared 50 per cent *m/m* solution of *potassium hydroxide R*. Heat in a water-bath under a reflux condenser for 30 min. Cool rapidly under running water. Transfer the liquid to a separating funnel with the aid of 2 quantities, each of 15 ml, of *water R*, 1 quantity of 10 ml of *ethanol (96 per cent) R* and 2 quantities, each of 50 ml, of *pentane R.* Shake vigorously for 30 s. Allow to stand until the 2 layers are clear. Transfer the aqueous-alcoholic layer to a 2nd separating funnel and shake with a mixture of 10 ml of *ethanol (96 per cent) R* and 50 ml of *pentane R*. After separation, transfer the aqueous-alcoholic layer to a 3rd separating funnel and the pentane layer to the 1st separating funnel, washing the 2nd separating funnel with 2 quantities, each of 10 ml, of *pentane R* and adding the washings to the 1st separating funnel. Shake the aqueous-alcoholic layer with 50 ml of *pentane R* and add the pentane layer to the 1st funnel. Wash the pentane layer with 2 quantities, each of 50 ml, of a freshly prepared 30 g/l solution of *potassium hydroxide R* in *ethanol (10 per cent V/V) R*, shaking vigorously, and then wash with successive quantities, each of 50 ml, of *water R* until the washings are neutral to phenolphthalein. Transfer the washed pentane extract to a ground-glass-stoppered flask. Evaporate the contents of the flask to dryness under reduced pressure by swirling in a water-bath at 40 °C. Cool under running water and restore atmospheric pressure with *nitrogen R*. Dissolve the residue immediately in 5.0 ml of *toluene R* and add 20.0 ml of the mobile phase to obtain a solution containing about 4000 IU/ml.

Reference solution (a) Dissolve 10.0 mg of *cholecalciferol CRS*, without heating, in 10.0 ml of *toluene R* and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of *cholecalciferol for performance test CRS* to 5.0 ml with the mobile phase. Heat in a water-bath at 90 °C under a reflux condenser for 45 min and cool.

Reference solution (c) Dissolve 0.10 g of *cholecalciferol CRS*, without heating, in *toluene R* and dilute to 100.0 ml with the same solvent.

Reference solution (d) Dilute 5.0 ml of reference solution (c) to 50.0 ml with the mobile phase. Keep the solution in iced water.

Reference solution (e) Place 5.0 ml of reference solution (c) in a volumetric flask, add about 10 mg of *butylhydroxytoluene R* and displace the air from the flask with *nitrogen R*. Heat in a water-bath at 90 °C under a reflux condenser, protected from light and under *nitrogen R*, for 45 min. Cool and dilute to 50.0 ml with the mobile phase.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: silica gel for chromatography R (5 µm).*

Mobile phase *pentanol R, hexane R* (3:997 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection The chosen volume of each solution (the same volume for reference solution (a) and for the test solution); automatic injection device or sample loop recommended.

Relative retention With reference to cholecalciferol: pre-cholecalciferol = about 0.4; *trans*-cholecalciferol = about 0.5.

System suitability Reference solution (b):

— *resolution*: minimum 1.0 between the peaks due to pre-cholecalciferol and *trans*-cholecalciferol; if necessary, adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution;

— *repeatability*: maximum relative standard deviation of 1.0 per cent for the peak due to cholecalciferol after 6 injections.

Calculate the conversion factor (f) using the following expression:

$$\frac{K - L}{M}$$

K = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (d),

L = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (e),

M = area (or height) of the peak due to pre-cholecalciferol in the chromatogram obtained with reference solution (e).

The value of f determined in duplicate on different days may be used during the entire procedure.

Calculate the content of cholecalciferol in International Units per gram using the following expression:

$$\frac{m'}{V'} \times \frac{V}{m} \times \frac{S_D + (f \times S_p)}{S'_D} \times 40\,000 \times 1000$$

m = mass of the preparation to be examined in the test solution, in milligrams

m' = mass of *cholecalciferol CRS* in reference solution (a), in milligrams

V = volume of the test solution (25 ml)

V' = volume of reference solution (a) (100 ml);

S_D = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with the test solution

S'_D = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (a)

S_p = area (or height) of the peak due to pre-cholecalciferol in the chromatogram obtained with the test solution

f = conversion factor

STORAGE

In an airtight, well-filled container, protected from light, at the temperature stated on the label.

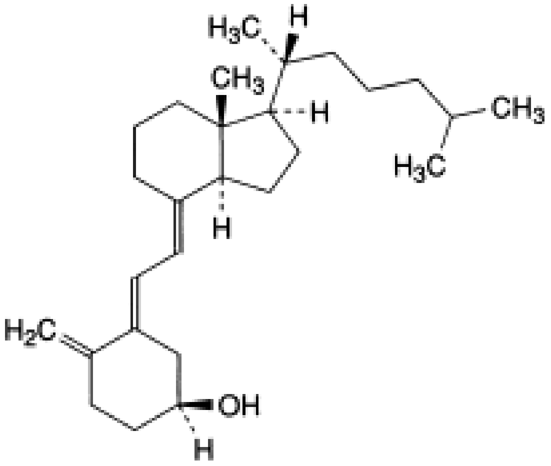
The contents of an opened container are to be used as soon as possible; any unused part is to be protected by an atmosphere of inert gas.

LABELLING

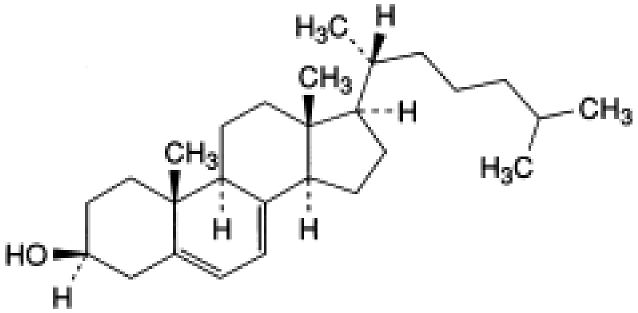
The label states:

- — the number of International Units per gram;
- — the storage temperature.

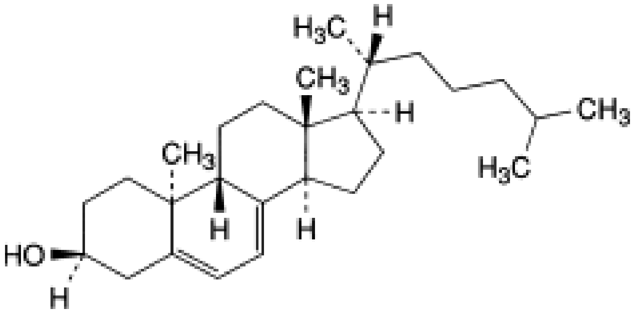
IMPURITIES



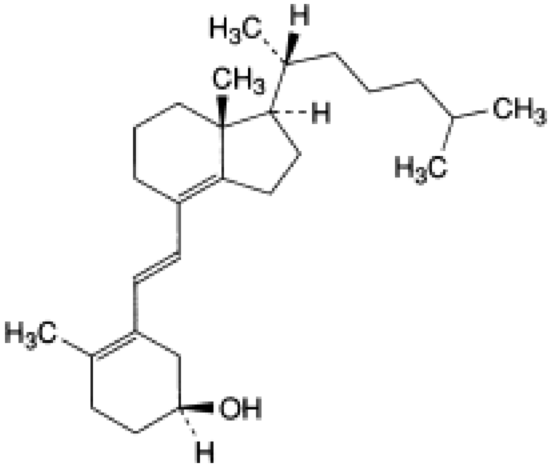
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



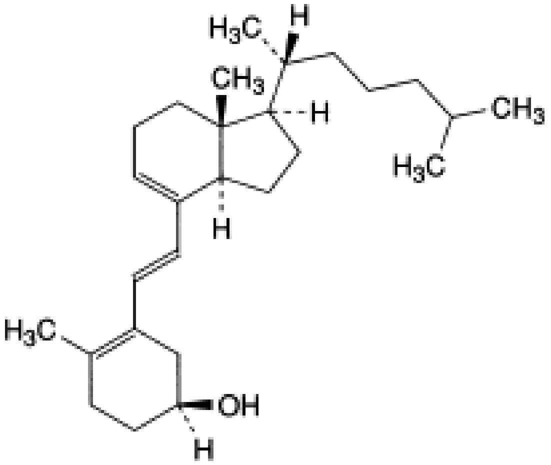
B. cholesta-5,7-dien-3β-ol (7,8-di dehydrocholesterol, provitamin D_3),



C. (9β,10α)-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol$_3$),



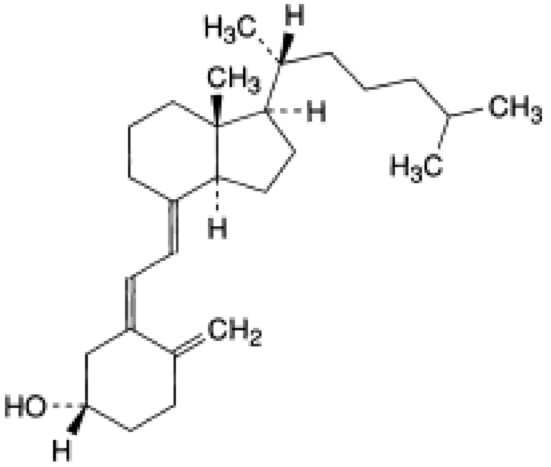
E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol$_3$).

Ph Eur

Colecalciferol

General Notices

(Cholecalciferol, Ph Eur monograph 0072)



$C_{27}H_{44}O$ 384.6 *67-97-0*

Action and use

Vitamin D3 analogue.

Preparations

Calcium and Colecalciferol Tablets

Colecalciferol Injection

Colecalciferol Tablets

Paediatric Vitamins A, C and D Oral Drops

When cholecalciferol or vitamin D3 is prescribed or demanded, Colecalciferol shall be dispensed or supplied. When calciferol or vitamin D is prescribed or demanded, Colecalciferol or Ergocalciferol shall be dispensed or supplied.

Ph Eur

DEFINITION

(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3β-ol.

Content

97.0 per cent to 102.0 per cent.

1 mg of cholecalciferol is equivalent to 40 000 IU of antirachitic activity (vitamin D) in rats.

CHARACTERS

Appearance

White or almost white crystals.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in trimethylpentane and in fatty oils.

It is sensitive to air, heat and light. Solutions in solvents without an antioxidant are unstable and are to be used immediately.

A reversible isomerisation to pre-cholecalciferol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *cholecalciferol CRS*.

TESTS

Specific optical rotation *(2.2.7)*

+ 105 to + 112, determined within 30 min of preparing the solution.

Dissolve 0.200 g rapidly in *aldehyde-free alcohol R* without heating and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*

Prepare the solutions immediately before use, avoiding exposure to actinic light and air.

Test solution Dissolve 10.0 mg of the substance to be examined in *trimethylpentane R* without heating and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *cholecalciferol CRS* in *trimethylpentane R* without heating and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of *cholecalciferol for system suitability CRS* (containing impurity A) to 5.0 ml with the mobile phase. Heat in a water-bath at 90 °C under a reflux condenser for 45 min and cool (formation of pre-cholecalciferol).

Reference solution (c) Dilute 10.0 ml of reference solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase *pentanol R, hexane R* (3:997 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 5 µl of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of cholecalciferol.

Relative retention With reference to cholecalciferol (retention time = about 19 min): pre-cholecalciferol = about 0.5; impurity A = about 0.6.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to pre-cholecalciferol and impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total:* not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peak due to pre-cholecalciferol.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances, with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of cholecalciferol ($C_{27}H_{44}O$) from the declared content of *cholecalciferol CRS*.

STORAGE

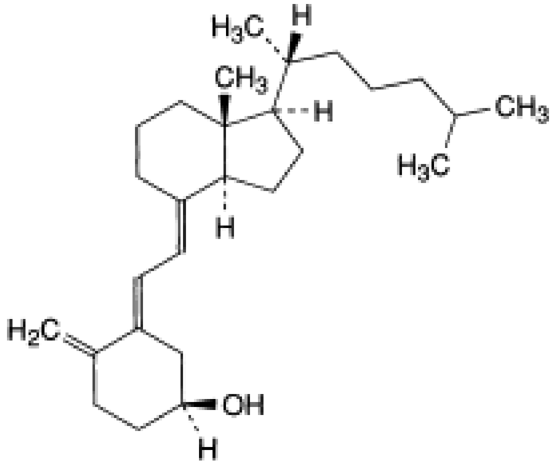
In an airtight container , under nitrogen, protected from light, at a temperature of 2 °C to 8 °C.

The contents of an opened container are to be used immediately.

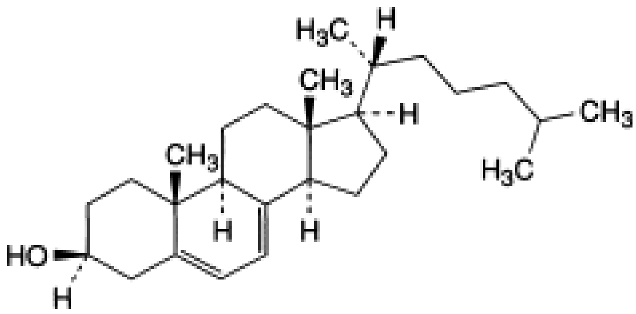
IMPURITIES

Specified impurities A.

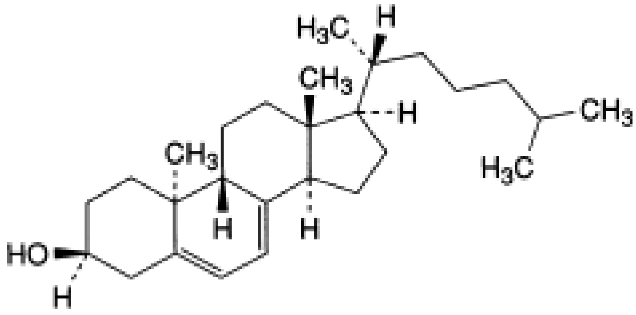
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E.*


H$_3$C
H
CH$_3$
H
CH$_3$
H$_3$C
H
H$_2$C
OH
H

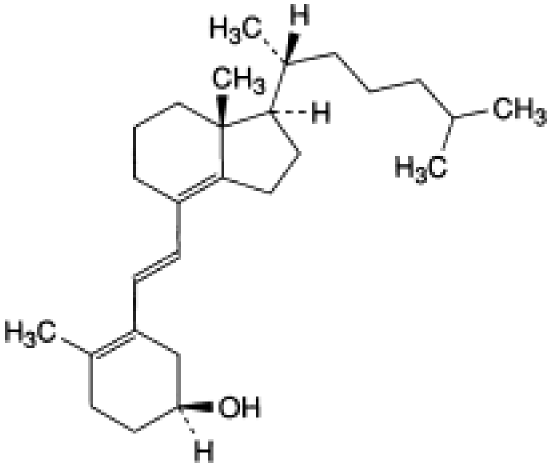
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),


H$_3$C
H
CH$_3$
H
CH$_3$
CH$_3$
H$_3$C
H
H
HO
H

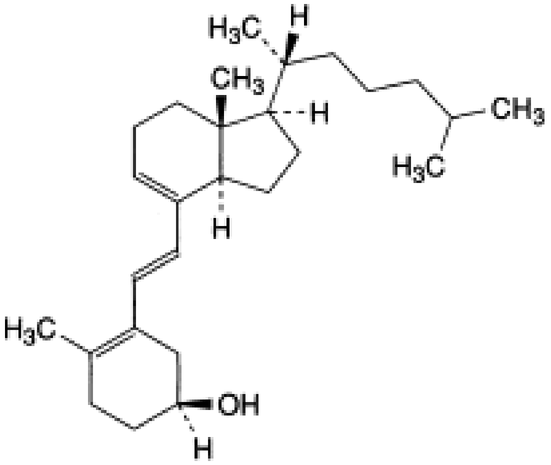
B. cholesta-5,7-dien-3β-ol (7,8-didehydrocholesterol, provitamin D_3),


H$_3$C
H
CH$_3$
H
CH$_3$
CH$_3$
H$_3$C
H
H
HO
H

C. 9β,10α-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol$_3$),



E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol$_3$).

Ph Eur

Colestipol Hydrochloride

General Notices

37296-80-3

Action and use

Lipid-regulating drug.

Preparation

Colestipol Granules

DEFINITION

Colestipol Hydrochloride is a co-polymer of diethylenetriamine and 1-chloro-2,3-epoxypropane. Each g binds not less than 1.1 mEq and not more than 1.7 mEq of sodium cholate, determined in the test for Cholate binding capacity and calculated with reference to the dried material.

CHARACTERISTICS

Yellow to orange beads; hygroscopic.

Practically insoluble in *ethanol (96%)* and in *dichloromethane*; swells but does not dissolve in *water* and dilute aqueous solutions of acids and alkalis.

IDENTIFICATION

Carry out the method for *gas chromatography*, Appendix III B, using a suitable gas chromatograph fitted with a pyrolysis unit.

To prepare the sample, mix 1 part of n-*eicosane* and 4 parts of the substance being examined and grind the mixture in a mortar with *chloroform* until the substance being examined is uniformly coated with the *n*-eicosane. Prepare the standard in the same manner but adding 4 parts of *colestipol hydrochloride BPCRS* in place of the substance being examined. Load the sample and the standard separately into the pyrolysis unit.

The chromatographic procedure may be carried out using a glass column (1.8 m × 3 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) (Chromosorb W is suitable) coated with 0.25% w/w of *potassium hydroxide* and 5% w/w of *polyethylene glycol 20,000* (Carbowax 20M is suitable) maintained at about 85° with the detector at about 270°. The pyrolysis unit is capable of attaining a temperature of about 1000° when fitted with a platinum ribbon probe and using *helium* as the carrier gas with a flow rate of 60 ml per minute. Operate the unit in accordance with the manufacturer's instructions to obtain a pyrogram for *colestipol hydrochloride BPCRS* that is similar to that supplied with the reference material.

The pyrogram obtained with the substance being examined is concordant with that obtained with *colestipol hydrochloride BPCRS*.

TESTS

Acidity or alkalinity

Shake a 10% w/w suspension in a stoppered vial at approximately 10-minute intervals for 1 hour and centrifuge. Transfer a portion of the clear supernatant liquid to a suitable container and record the pH as soon as the reading has stabilised. The pH is 6.0 to 7.5, Appendix V L.

Water-soluble substances

Place 5 g in a glass-stoppered, 125 ml conical flask, add 80 ml of *water*, close the flask and shake in a water bath at 36° to 38° for 72 hours. Filter the contents of the flask through a fine-porosity, sintered glass funnel or woven glass-fibre filter, collecting the filtrate in a tared 125 ml conical flask. Rinse any residual contents in the flask with two 5 ml quantities of *water*, filter the washings and combine the filtrates from the washings with the filtrate obtained previously. Evaporate the filtrate to dryness, using filtered air or nitrogen, if necessary, to aid in the evaporation. Dry the residue at 75° at a pressure of not more than 2 kPa for 1 hour, allow to cool in a desiccator and weigh. Repeat the procedure at the same time without the substance being examined beginning at the words 'add 80 ml of *water* …'. The difference in the weights of the residues is not more than 25 mg (0.5%).

Heavy metals

Transfer 1.0 g to a suitable crucible, wet with *sulphuric acid* and carefully ignite at a low temperature until thoroughly charred. To the carbonised substance add 2 ml of *nitric acid* and 0.25 ml of *sulphuric acid* and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely removed and cool. Add 4 ml of 6M *hydrochloric acid*, cover, heat on a water bath for 15 minutes, uncover and slowly evaporate to dryness. Dissolve the residue using two 5 ml quantities of 2M *hydrochloric acid*. Add 0.1 ml of *phenolphthalein solution* and 13.5M *ammonia* drop wise until a pink colour is produced. Cool, add *glacial acetic acid* until the solution is decolourised and add a further 0.5 ml. Filter if necessary and dilute the solution to 20 ml with *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII (20 ppm). Prepare the standard using a mixture of 2 ml of the test solution obtained above and 10 ml of *lead standard solution (2 ppm Pb)*.

Loss on drying

When dried at 75° at a pressure of not more than 2 kPa for 16 hours, loses not more than 1.0% of its weight.

Sulphated ash

Not more than 0.3%, Appendix IX A.

Chloride content

Not less than 6.5% and not more than 9.0% calculated with reference to the dried substance. Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 10 ml of 0.05M *sodium hydroxide* as the absorbing liquid. When the process is complete shake the flask vigorously, allow to stand with frequent shaking for about 40 minutes or until no cloudiness is observed; add 20 ml of *ethanol (96%)* and 0.2 ml of *nitric acid*. Titrate the resulting solution with 0.05M *silver nitrate VS,* determining the end point potentiometrically using a silver-silver chloride electrode and a glass reference electrode (V ml). Repeat the procedure without the substance being examined adding 10 ml of 0.0075M *sodium chloride* to the solution in the flask (V_1 ml). Add 10 ml of 0.0075M *sodium chloride* to a flask containing a mixture of 10 ml of *water* and 20 ml of *ethanol (96%),* add 0.2 ml of *nitric acid* and titrate with

0.05M *silver nitrate VS*, determining the end point potentiometrically using a silver-silver chloride electrode and a glass reference electrode (V_2 ml). Determine the volume of 0.05M *silver nitrate VS* required by the substance being examined using the following expression:

$V-(V_1-V_2)$

Each ml of 0.05M *silver nitrate VS* is equivalent to 1.773 mg of Cl.

Water absorption capacity

Each g of Colestipol Hydrochloride absorbs not less than 3.3 g and not more than 5.3 g of *water* when determined in the following manner. Transfer 5 g to a dry, plastic container and add 80 g of *water*. Cover the container and allow the resulting suspension to equilibrate for 72 hours. Filter the resulting slurry through a medium-porosity fritted-glass funnel (KIMAX 60 ml-40M is suitable) at a pressure of 2kPa; collect the filtrate in a tared, plastic container, disconnecting the vacuum 2 minutes after collection of the last portion of the filtrate. Immediately weigh the container and the filtrate and determine the weight, in g, of the filtrate. Calculate the weight of water absorbed per g from the difference between the weight of the filtrate and the original weight of *water* used in the test.

Cholate binding capacity

Prepare a solution containing 1.0% w/v of *sodium cholate* and 0.9% w/v of *sodium chloride* and adjust to pH 6.4 by the drop wise addition of *hydrochloric acid* (solution A). Transfer 1 g (*m* g) of the substance being examined to a ground-glass-stoppered flask, add 100 ml of freshly prepared solution A and shake vigorously for 90 minutes with the flask positioned horizontally. Remove the flask from the shaker and allow the contents to settle for 5 minutes. Adjust the pH of a 20 ml aliquot of the supernatant liquid to 10.5 by the drop wise addition of 1M *sodium hydroxide* and titrate potentiometrically with 0.1M *hydrochloric acid* VS to the second inflection point of the pH curve. Determine the volume of titrant added between the inflection points. Carry out a blank titration on 20 ml of freshly prepared solution A. The difference between the titrations represents the amount of hydrochloric acid required (V ml). Calculate the cholate binding capacity of the substance being examined in milliequivalents from the expression $0.5V/m$.

Colestipol exchange capacity limit

Not less than 9.0 mEq per g and not more than 11.0 mEq per g determined in the following manner. Transfer not less than 2 g and 100 ml of 1M *sodium hydroxide* to a stoppered flask and shake for 4 hours. Filter the suspension through a coarse-porosity sintered-glass funnel and wash the resin with 500 ml of *water*. Transfer the resin to a 1000 ml beaker, add 200 ml of *water* and allow to stand for 10 minutes. Filter the suspension, check the pH of the filtrate and repeat the washing procedure with 200 ml quantities of *water* until the pH of the filtrate is less than 8 [5 litres may be required]. Dry the resin and funnel at 60^{c} at a pressure of 2 kPa for at least 16 hours, breaking up any aggregates with a spatula and store in a dessicator.

Place 1 g (*w* g) of the free base resin prepared above and add 100 ml of 0.2M *hydrochloric acid VS* in a stoppered flask and shake for not less than 2.5 hours. Filter a portion of the suspension through glass wool. Titrate 8 ml of the filtrate with 0.2M *sodium hydroxide VS*, determining the end point potentiometrically (*a* ml). Carry out a blank titration on 5 ml of the 0.2M *hydrochloric acid VS* used to equilibrate the free-base resin diluted with 5 ml of *water* (*b* ml). Calculate the exchange capacity in milliequivalents per g from the expression:

$(20/w)(b/5-a/8)$

STORAGE

Colestipol Hydrochloride should be kept in an airtight container.

Colestyramine

General Notices

(Ph Eur monograph 1775)

11041-12-6

Action and use

Lipid-regulating drug.

Preparation

Colestyramine Oral Powder

Ph Eur

DEFINITION

Strongly basic anion-exchange resin in chloride form, consisting of styrene-divinylbenzene copolymer with quaternary ammonium groups.

Nominal exchange capacity

1.8 g to 2.2 g of sodium glycocholate per gram (dried substance).

CHARACTERS

Appearance

White or almost white, fine powder, hygroscopic.

Solubility

Insoluble in water, in methylene chloride and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison colestyramine CRS.

B. Chloride (see Tests).

TESTS

pH *(2.2.3)*

4.0 to 6.0.

Suspend 0.100 g in 10 ml of *water R* and allow to stand for 10 min.

Dialysable quaternary amines

Maximum 500 ppm, expressed as benzyltrimethylammonium chloride.

Test solution Place a 25 cm piece of cellulose dialysis tubing having a molecular weight cut-off of 12 000-14 000 and an inflated diameter of 3-6 cm (flat width of 5-9 cm) in *water R* to hydrate until pliable, appropriately sealing one end. Introduce 2.0 g of the substance to be examined into the tube and add 10 ml of *water R*. Seal the tube and completely immerse it in 100 ml of *water R* in a suitable vessel and stir the liquid for 16 h to effect dialysis. Use the dialysate as test solution.

Reference solution Prepare the reference solution in a similar manner but using 10 ml of a freshly prepared 0.1 g/l solution of *benzyltrimethylammonium chloride R* instead of the substance to be examined.

Transfer 5.0 ml of the test solution to a separating funnel and add 5 ml of a 3.8 g/l solution of *disodium tetraborate R*, 1 ml of a solution containing 1.5 g/l of *bromothymol blue R* and 4.05 g/l of *sodium carbonate R* and 10 ml of *chloroform R*. Shake the mixture vigourously for 1 min, allow the phases to separate and transfer the clear organic layer to a 25 ml volumetric flask. Repeat the extraction with a further 10 ml of *chloroform R*, combine the organic layers and dilute to 25 ml with *chloroform R*. Measure the absorbance *(2.2.25)* of the solution at the absorption maximum at 420 nm, using as compensation liquid a solution prepared in the same manner but using 5.0 ml of *water R* instead of the test solution.

Repeat the operation using 5.0 ml of the reference solution.

The absorbance obtained with the test solution is not greater than that obtained with the reference solution.

Impurity A

Liquid chromatography *(2.2.29)*.

Test solution Shake 5.0 g with 10 ml of *acetone R* for 30 min. Centrifuge and use the supernatant liquid.

Reference solution (a) Dissolve 5 mg of *styrene R* in *acetone R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *acetone R*.

Reference solution (b) Dissolve 0.35 ml of *styrene R* in *acetone R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *acetone R*.

Reference solution (c) Dissolve 0.35 ml of *toluene R* in *acetone R* and dilute to 100.0 ml with the same solvent.

Reference solution (d) Mix 1.0 ml of reference solution (b) and 1.0 ml of reference solution (c) with *acetone R* and dilute to 100.0 ml with the same solvent.

Column:

- *size*: *l* = 0.30 m, Ø = 3.9 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm) with a specific surface area of 330 m2/g and a pore size of 12.5 nm.

Mobile phase *acetonitrile R*, *water R* (50:50 *V/V*).

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of test solution and reference solutions (a) and (d).

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to impurity A and toluene.

Limit:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 ppm).

Chloride

13.0 per cent to 17.0 per cent (dried substance).

To 0.2 g add 100 ml of *water R* and 50 mg of *potassium nitrate R*. Add, with stirring, 2 ml of *nitric acid R* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M silver nitrate* is equivalent to 3.55 mg of Cl.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 12 per cent, determined on 1.000 g by drying in an oven at 70 °C over *diphosphorus pentoxide R* at a pressure not exceeding 7 kPa for 16 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Exchange capacity

Liquid chromatography *(2.2.29)*.

Solution A Dissolve 1.500 g of *sodium glycocholate R* in a solution containing 4 g/l of *potassium dihydrogen phosphate R* and 12 g/l of *dipotassium hydrogen phosphate R* and dilute to 100.0 ml with the same solution.

Test solution Add 20.0 ml of solution A to a quantity of the substance to be examined equivalent to about 0.100 g of the dried substance. Shake mechanically for 2 h and centrifuge for 15 min. Dilute 5.0 ml of the supernatant liquid to 50.0 ml with *water R*.

Reference solution (a) Dilute 4.0 ml of solution A to 100.0 ml with *water R*.

Reference solution (b) Dissolve 60 mg of *sodium glycocholate R* and 30 mg of *sodium taurodeoxycholate R* in *water R* and dilute to 100 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *water R*.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 10.9 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 50 µl.

Run time Twice the retention time of glycocholate.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to glycocholate and taurodeoxycholate.

Calculate the nominal exchange capacity using the following expression:

$$\frac{(2.5\,A_1 - A_2) \times m_1 \times 1.2}{12.5 \times A_1 \times m_2}$$

A_1 = area of the peak due to glycocholate in the chromatogram obtained with reference solution (a),

A_2 = area of the peak due to glycocholate in the chromatogram obtained with the test solution,

m_1 = mass, in milligrams, of *sodium glycocholate R* used in the preparation of solution A,

m_2 = mass, in milligrams, of the dried substance to be examined used in the preparation of the test solution,

1.2 = correction factor to convert the true exchange capacity to the conventionally used nominal exchange capacity.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities *A.*

A. styrene.

Ph Eur

Colistimethate Sodium

General Notices

(Ph Eur monograph 0319)

Action and use

Antibacterial.

Preparation

Colistimethate Injection

Ph Eur

DEFINITION

Colistimethate sodium is prepared from colistin by the action of formaldehyde and sodium hydrogen sulphite.

Semi-synthetic product derived from a fermentation product.

Content

Minimum 11 500 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *hydrochloric acid R* and *water R*. Heat at 135 °C in a sealed tube for 5 h. Evaporate to dryness on a water-bath and continue the heating until the hydrochloric acid has evaporated. Dissolve the residue in 0.5 ml of *water R*.

Reference solution (a) Dissolve 20 mg of *leucine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *threonine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 20 mg of *phenylalanine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (d) Dissolve 20 mg of *serine R* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Carry out the following procedures protected from light.

Mobile phase *water R*, *phenol R* (25:75 *V/V*).

Application 5 µl as bands of 10 mm, then place the plate in the chromatographic tank so that it is not in contact with the mobile phase, and allow it to become impregnated with the vapour of the mobile phase for at least 12 h.

Development Over a path of 12 cm using the same mobile phase.

Drying At 100-105 °C.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The chromatogram obtained with the test solution shows zones corresponding to those in the chromatograms obtained with reference solutions (a) and (b), but shows no zones corresponding to those in the chromatograms obtained with reference solutions (c) and (d); the chromatogram obtained with the test solution also shows a zone with a very low R_F value (2,4-diaminobutyric acid).

B. Dissolve about 5 mg in 3 ml of *water R*. Add 3 ml of *dilute sodium hydroxide solution R*. Shake and add 0.5 ml of a 10 g/l solution of *copper sulphate R*. A violet colour is produced.

C. Dissolve about 50 mg in 1 ml of *1 M hydrochloric acid* and add 0.5 ml of *0.01 M iodine*. The solution is decolourised and gives reaction (a) of sulphates *(2.3.1)*.

D. It gives reaction (b) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)*.

Dissolve 0.16 g in 10 ml of *water R*.

pH *(2.2.3)*

6.5 to 8.5.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. Measure after 30 min.

Specific optical rotation *(2.2.7)*

- 46 to - 51 (dried substance).

Dissolve 1.25 g in *water R* and dilute to 25.0 ml with the same solvent.

Free colistin

Dissolve 80 mg in 3 ml of *water R*. Add 0.1 ml of a 100 g/l solution of *silicotungstic acid R*; 10-20 s after addition of the reagent, the solution is not more opalescent than reference suspension II *(2.2.1)*.

Total sulphite

Work in a fume cupboard. Dissolve 0.100 g in 50 ml of *water R* and add 5 ml of a 100 g/l solution of *sodium hydroxide R* and 0.3 g of *potassium cyanide R*. Boil gently for 3 min and then cool. Neutralise with *0.5 M sulphuric acid* using 0.2 ml of *methyl orange solution R* as indicator. Add an excess of *0.5 ml of the acid* and 0.2 g of *potassium iodide R*. Titrate with *0.05 M iodine* using 1 ml of *starch solution R* as indicator. The volume of *0.05 M iodine* used in the titration is 5.5 ml to 7.0 ml.

Loss on drying ***(2.2.32)***

Maximum 5.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash ***(2.4.14)***

16 per cent to 21 per cent, determined on 0.50 g.

Pyrogens ***(2.6.8)***

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of pyrogens, it complies with the test. Inject, per kilogram of the rabbit's mass, 1 ml of a solution in *water for injections R* containing 2.5 mg of the substance to be examined per millilitre.

ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)*.

STORAGE

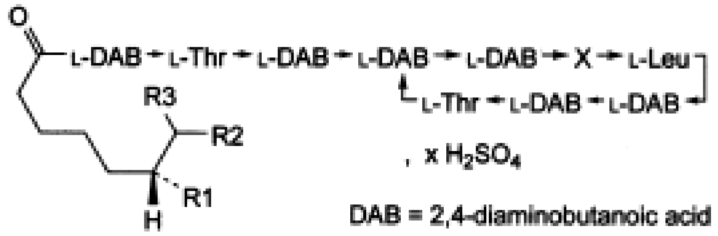
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

Ph Eur

Colistin Sulphate

General Notices

(Ph Eur monograph 0320)



DAB = 2,4-diaminobutanoic acid

polymyxin	X	R1	R2	R3	Mol. Formula	M_r
E1	D-Leu	CH_3	CH_3	H	$C_{53}H_{100}N_{16}O_{13}$	1170
E2	D-Leu	CH_3	H	H	$C_{52}H_{98}N_{16}O_{13}$	1155
E3	D-Leu	H	CH_3	H	$C_{52}H_{98}N_{16}O_{13}$	1155
E1-I	D-Ile	CH_3	CH_3	H	$C_{53}H_{100}N_{16}O_{13}$	1170
E1-7MOA	D-Leu	H	CH_3	CH_3	$C_{53}H_{100}N_{16}O_{13}$	1170

1264-72-8

Action and use

Antibacterial.

Preparation

Colistin Tablets

Ph Eur

DEFINITION

A mixture of the sulphates of polypeptides produced by certain strains of *Bacillus polymyxa* var. *colistinus* or obtained by any other means.

Content:

- *sum of polymyxins E1, E2, E3, E1-I and E1-7MOA*: minimum 77.0 per cent (dried substance);
- *polymyxin E1-I*: maximum 10.0 per cent (dried substance);
- *polymyxin E1-7MOA*: maximum 10.0 per cent (dried substance);
- *polymyxin E3*: maximum 10.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *hydrochloric acid R* and *water R*. Heat at 135 °C in a sealed tube for 5 h. Evaporate to dryness on a water-bath and continue the heating until moistened *blue litmus paper R* does not turn red. Dissolve the residue in 0.5 ml of *water R*.

Reference solution (a) Dissolve 20 mg of *leucine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *threonine* R in *water R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 20 mg of *phenylalanine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (d) Dissolve 20 mg of *serine R* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Carry out the following procedures protected from light.

Mobile phase *water R, phenol R* (25:75 *V/V*).

Application 5 µl as 10 mm bands, then place the plate in the chromatographic tank so that it is not in contact with the mobile phase, and allow it to become impregnated with the vapour of the mobile phase for at least 12 h.

Development Over a path of 12 cm.

Drying At 100-105 °C.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The chromatogram obtained with the test solution shows zones corresponding to those in the chromatograms obtained with reference solutions (a) and (b), but shows no zones corresponding to those in the chromatograms obtained with reference solutions (c) and (d); the chromatogram obtained with the test solution also shows a zone with a very low R_F value (2,4-diaminobutyric acid).

B. Examine the chromatograms obtained in the assay.

Results The peaks due to polymyxin E1 and polymyxin E2 in the chromatogram obtained

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with the test solution are similar in retention time to the corresponding peaks in the chromatogram obtained with reference solution (a).

C. Dissolve about 5 mg in 3 ml of *water R*. Add 3 ml of *dilute sodium hydroxide solution R*. Shake and add 0.5 ml of a 10 g/l solution of *copper sulphate R*. A violet colour is produced.

D. Dissolve about 50 mg in 1 ml of *1 M hydrochloric acid* and add 0.5 ml of *0.01 M iodine.* The solution remains coloured.

E. It gives reaction (a) of sulphates *(2.3.1)*.

TESTS

pH *(2.2.3)*

4.0 to 6.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 63 to - 73 (dried substance).

Dissolve 1.25 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29);* use the normalisation procedure.

Test solution Dissolve 25.0 mg of the substance to be examined in 40 ml of *water R* and dilute to 50.0 ml with *acetonitrile R*.

Reference solution (a) Dissolve 25.0 mg of *colistin sulphate CRS* in 40 ml of *water R* and dilute to 50.0 ml with *acetonitrile R*.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 22 volumes of *acetonitrile R* and 78 volumes of a solution prepared as follows: dissolve 4.46 g of *anhydrous sodium sulphate R* in 900 ml of *water R*, adjust to pH 2.4 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 1.5 times the retention time of polymyxin E1.

Relative retention With reference to polymyxin E1 (retention time = about 16 min): polymyxin E2 = about 0.45; polymyxin E3 = about 0.5; polymyxin E1-I = about 0.8; polymyxin E1-7MOA = about 1.1.

System suitability Reference solution (a):

— *resolution*: minimum 8.0 between the peaks due to polymyxin E2 and polymyxin E1, minimum 6.0 between the peaks due to polymyxin E2 and polymyxin E1-I, minimum 2.5 between the peaks due to polymyxin E1-I and polymyxin E1, minimum 1.5 between the peaks due to polymyxin E1 and polymyxin E1-7MOA;

— the chromatogram obtained is similar to the chromatogram supplied with *colistin sulphate CRS*.

Limits:

— *any impurity*: maximum 4.0 per cent;

— *total*: maximum 23.0 per cent;

— *disregard limit*: the area of the peak due to polymyxin E1 in the chromatogram obtained with reference solution (b); disregard the peaks due to polymyxins E2, E3, E1-I, E1 and E1-7MOA.

Sulphate

16.0 per cent to 18.0 per cent (dried substance).

Dissolve 0.250 g in 100 ml of *water R* and adjust to pH 11 with *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R.* Titrate with *0.1 M sodium edetate*, adding 50 ml of *ethanol (96 per cent) R* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of SO4.

Loss on drying *(2.2.32)*

Maximum 3.5 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 1.0 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of polymyxin E3, of polymyxin E1-I, of polymyxin E1-7MOA, and of the sum of polymyxins E1, E2, E3, E1-I and E1-7MOA, using the following expression:

$$C_{Ei} = \frac{A_{Ei} \times m_2 \times D_{Ei}}{m_1 \times B_{Ei}}$$

C_{Ei} = percentage content of polymyxin E*i,*

A_{Ei} = area of the peak due to polymyxin E*i* in the chromatogram obtained with the test solution,

m_1 = mass in milligrams of the substance to be examined (dried substance) in the test solution,

B_{E_i} = area of the peak due to polymyxin E*i* in the chromatogram obtained with reference solution (a),

m_2 = mass in milligrams of *colistin sulphate CRS* in reference solution (a),

D_{E_i} = declared percentage content for polymyxin E*i* in *colistin sulphate CRS.*

STORAGE

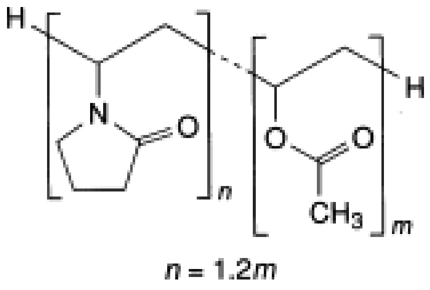
In an airtight container , protected from light.

Ph Eur

Copovidone

General Notices

(Ph Eur monograph 0891)



$C_6H_9NO)_n + (C_4H_6O_2)_m$ $(111.1)_n + (86.1)_m$

Action and use

Excipient in pharmaceutical products.

Ph Eur

DEFINITION

Copovidone is a copolymer of 1-ethenylpyrrolidin-2-one and ethenyl acetate in the mass proportion 3:2.

Content:

- — nitrogen (N; A_r 14.01): 7.0 per cent to 8.0 per cent (dried substance);
- — ethenyl acetate $C_4H_6O_2$; M_r 86.10): 35.3 per cent to 42.0 per cent (dried substance).

K-value

90.0 per cent to 110.0 per cent of the value stated on the label.

CHARACTERS

Aspect

White or yellowish-white powder or flakes, hygroscopic.

Solubility

Freely soluble in water, in alcohol and in methylene chloride.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of copovidone.*

B. To 1 ml of solution S (see Tests) add 5 ml of *water R* and 0.2 ml of *0.05 M iodine*. A red colour appears.

C. Dissolve 0.7 g of *hydroxylamine hydrochloride R* in 10 ml of *methanol R*, add 20 ml of a 40 g/l solution of *sodium hydroxide R* and filter if necessary. To 5 ml of the solution add 0.1 g of the substance to be examined and boil for 2 min. Transfer 50 µl to a filter paper and add 0.1 ml of a mixture of equal volumes of *ferric chloride solution R1* and *hydrochloric acid R*. A violet colour appears.

TESTS

Solution S

Dissolve 10 g in *water R* and dilute to 100 ml with the same solvent. Add the substance to be examined to the *water R* in small portions with constant stirring.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* and not more intensely coloured than reference solution B_5, R_5 or BY_5 *(2.2.2, Method II).*

Aldehydes

Maximum 500 ppm, expressed as acetaldehyde.

Test solution Dissolve 1.0 g of the substance to be examined in *phosphate buffer solution pH 9.0 R* and dilute to 100.0 ml with the same solvent. Stopper the flask and heat at 60 °C for 1 h. Allow to cool.

Reference solution Dissolve 0.140 g of *acetaldehyde ammonia trimer trihydrate R* in *water R* and dilute to 200.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *phosphate buffer solution pH 9.0 R.*

Into 3 identical spectrophotometric cells with a path length of 1 cm, introduce separately 0.5 ml of the test solution, 0.5 ml of the reference solution and 0.5 ml of *water R* (blank). To each cell, add 2.5 ml of *phosphate buffer solution pH 9.0 R* and 0.2 ml of *nicotinamide-adenine dinucleotide solution R*. Mix and stopper tightly. Allow to stand at 22 ± 2 °C for 2-3 min and measure the absorbance *(2.2.25)* of each solution at 340 nm, using *water R* as the compensation liquid. To each cell, add 0.05 ml of *aldehyde dehydrogenase solution R,* mix and stopper tightly. Allow to stand at 22 ± 2 °C for 5 min. Measure the absorbance of each solution at 340 nm using *water R* as compensation liquid. Determine the content of aldehydes using the expression:

$$\frac{(A_{t2} - A_{t1}) - (A_{b2} - A_{b1})}{(A_{s2} - A_{s1}) - (A_{b2} - A_{b1})} \times \frac{100\,000 \times C}{m}$$

A_{t1} = absorbance of the test solution before the addition of aldehyde dehydrogenase,

A_{t2} = absorbance of the test solution after the addition of aldehyde dehydrogenase,

A_{s1} = absorbance of the reference solution before the addition of aldehyde dehydrogenase,

A_{s2} = absorbance of the reference solution after the addition of aldehyde dehydrogenase,

A_{b1} = absorbance of the blank before the addition of aldehyde dehydrogenase,

A_{b2} = absorbance of the blank after the addition of aldehyde dehydrogenase,

m = mass of povidone, in grams, calculated with reference to the dried substance,

C = concentration (mg/ml), of acetaldehyde in the reference solution, calculated from the weight of the acetaldehyde ammonia trimer trihydrate with the factor 0.72.

Peroxides

Maximum 400 ppm, expressed as H_2O_2.

Dilute 10 ml of solution S to 25 ml with *water R*. Add 2 ml of *titanium trichloride-sulphuric acid reagent R* and allow to stand for 30 min. The absorbance *(2.2.25)* of the solution, measured at 405 nm using a mixture of 25 ml of a 40 g/l solution of the substance to be examined and 2 ml of a 13 per cent *V/V* solution of *sulphuric acid R* as the compensation liquid, is not greater than 0.35.

Hydrazine

Thin-layer chromatography *(2.2.27)*. *Use freshly prepared solutions*.

Test solution To 25 ml of solution S add 0.5 ml of a 50 g/l solution of *salicylaldehyde R* in *methanol R*, mix and heat in a water-bath at 60 °C for 15 min. Allow to cool, add 2.0 ml of *xylene R*, shake for 2 min and centrifuge. Use the clear supernatant layer.

Reference solution Dissolve 9 mg of *salicylaldehyde azine R* in *xylene R* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *xylene R*.

Plate *TLC silanised silica gel plate R*.

Mobile phase *water R, methanol R* (20:80 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Limit:

— *hydrazine*: any spot corresponding to salicylaldehyde azine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1 ppm).

Monomers

Maximum 0.1 per cent.

Dissolve 10.0 g in 30 ml of *methanol R* and add slowly 20.0 ml of *iodine bromide solution R*. Allow to stand for 30 min protected from light with repeated shaking. Add 10 ml of a 100 g/l solution of *potassium iodide R* and titrate with *0.1 M sodium thiosulphate* until a yellow colour is obtained. Continue titration dropwise until the solution becomes colourless. Carry out a blank titration. Not more than 1.8 ml of *0.1 M sodium thiosulphate* is used.

Impurity A

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 100 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution Dissolve 100 mg of *2-pyrrolidone R* in *water R* and dilute to 100 ml with the same solvent. Dilute 1.0 ml to 100.0 ml with *water R*.

Precolumn:

- *size: l* = 0.025 m, Ø = 4 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography* R (5 µm).

Column:

- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase: spherical aminohexadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase *water R*, adjusted to pH 2.4 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 205 nm. A detector is placed between the precolumn and the analytical column. A second detector is placed after the analytical column.

Injection 10 µl. When impurity A has left the precolumn (after about 1.2 min) switch the flow directly from the pump to the analytical column. Before the next chromatogram is run, wash the precolumn by reversed flow.

Limit:

- *impurity A:* not more than the area of the principal peak obtained with the reference solution (0.5 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Viscosity, expressed as *K*-value

Dilute 5.0 ml of solution S to 50.0 ml with *water R*. Allow to stand for 1 h and determine the viscosity *(2.2.9)* of the solution at 25 ± 0.1 °C using viscometer No. 1 with a minimum flow time of 100 s. Calculate the *K*-value from the expression:

$$\frac{1.5 \log\eta - 1}{0.15 + 0.003c} + \frac{\sqrt{300c \log\eta + (c + 1.5c \log\eta)^2}}{0.15c + 0.003c^2}$$

c = percentage concentration (g/100 ml) of the substance to be examined, calculated with reference to the dried substance,

η = viscosity of the solution relative to that of water.

ASSAY

Ethenyl acetate

Determine the saponification value *(2.5.6)* on 2.00 g of the substance to be examined. Multiply the result obtained by 0.1534 to obtain the percentage content of the ethenyl acetate component.

Nitrogen

Carry out the determination of nitrogen *(2.5.9)* using 30.0 mg of the substance to be examined and 1 g of a mixture of 3 parts of *copper sulphate R* and 997 parts of *dipotassium sulphate R*, heating until a clear, light green solution is obtained and then for a further 45 min.

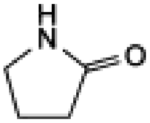
STORAGE

In an airtight container.

LABELLING

The label states the *K*-value.

IMPURITIES



A. pyrrolidin-2-one (2-pyrrolidone).

Ph Eur

Copper Sulphate Pentahydrate

General Notices

Copper Sulphate

(Ph Eur monograph 0894)

$CuSO_4,5H_2O$ 249.7 *7758-99-8*

Action and use

Used in treatment of copper deficiency.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

Blue, crystalline powder or transparent, blue crystals.

Solubility

Freely soluble in water, soluble in methanol, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Add several drops of *dilute ammonia R2* to 1 ml of solution S (see Tests). A blue precipitate is formed. On further addition of *dilute ammonia R2* the precipitate dissolves and a dark blue colour is produced.

B. Loss on drying (see Tests).

C. Dilute 1 ml of solution S to 5 ml with *water R.* The solution gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 5 g in *water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

Chlorides *(2.4.4)*

Maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R.*

Iron

Maximum 1.00 × 102 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 0.5 g in 10 ml of *water R*, add 2.5 ml of *lead-free nitric acid R* and dilute to 25.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, adding 2.5 ml of *lead-free nitric acid R* and diluting to 25.0 ml with *water R.*

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Copper may form explosive acetylides with acetylene. Therefore, clean the burner thoroughly before any residues become dry.

Lead

Maximum 50.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 2.5 g in 10 ml of *water R,* add 2.5 ml of *lead-free nitric acid R* and dilute to 25.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *lead standard solution (100 ppm Pb) R*, adding 2.5 ml of *lead-free nitric acid R* and diluting to 25.0 ml with *water R*.

Source Lead hollow-cathode lamp.

Wavelength 217.0 nm.

Atomisation device Air-acetylene flame.

Copper may form explosive acetylides with acetylene. Therefore, clean the burner thoroughly before any residues become dry.

Loss on drying *(2.2.32)*

35.0 per cent to 36.5 per cent, determined on 0.500 g by drying in an oven at 250 ± 10 °C.

ASSAY

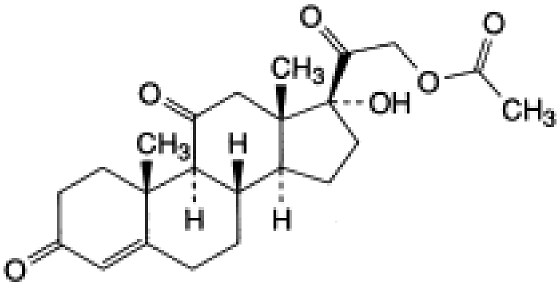
Dissolve 0.200 g in 50 ml of *water R*. Add 2 ml of *sulphuric acid R* and 3 g of *potassium iodide R*. Titrate with *0.1 M sodium thiosulphate*, adding 1 ml of *starch solution R* towards the end of the titration.

1 ml *0.1 M sodium thiosulphate* is equivalent to 24.97 mg of $CuSO_4,5H_2O$.

Cortisone Acetate

General Notices

(Ph Eur monograph 0321)



$C_{23}H_{30}O_6$ 402.5 *50-04-4*

Action and use

Corticosteroid.

Preparation

Cortisone Tablets

Ph Eur

DEFINITION

17-Hydroxy-3,11,20-trioxopregn-4-en-21-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in dioxan, sparingly soluble in acetone, slightly soluble in ethanol (96 per cent) and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification   *A, B.*

Second identification   *C, D, E.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison   *cortisone acetate CRS.*

If the spectra obtained in the solid state show differences, record new spectra using 50 g/l solutions in *methylene chloride R* in a 0.2 mm cell.

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture   *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution   Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a)   Dissolve 20 mg of *cortisone acetate CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b)   Dissolve 10 mg of *hydrocortisone acetate R* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate   *TLC silica gel F_{254} plate R*.

Mobile phase   Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application   5 µl.

Development   Over a path of 15 cm.

Drying   In air.

Detection A   Examine in ultraviolet light at 254 nm.

Results A   The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B   Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B   The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability   Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

C. Thin-layer chromatography (*2.2.27*).

Test solution (a)   Dissolve 25 mg of the substance to be examined in *methanol R* with gentle heating and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of this solution to 10 ml with *methylene chloride R.*

Test solution (b)   Transfer 2 ml of solution A to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C protected from light for 2.5 h. Allow to cool.

Reference solution (a) Dissolve 25 mg of *cortisone acetate CRS* in *methanol R* with gentle heating and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of this solution to 10 ml with *methylene chloride R.*

Reference solution (b) Transfer 2 ml of solution B to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C protected from light for 2.5 h. Allow to cool.

Plate *TLC silica gel F254 plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulphuric acid R* and heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_F value distinctly lower than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a faint yellow colour develops. Add this solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

E. About 10 mg gives the reaction of acetyl *(2.3.1)*.

TESTS

Specific optical rotation *(2.2.7)*

+ 211 to + 220 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *cortisone acetate CRS* and 2 mg of *hydrocortisone acetate CRS* (impurity A) in *acetonitrile R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 400 ml of *acetonitrile R* with 550 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl; inject *acetonitrile R* as a blank.

Run time Twice the retention time of cortisone acetate.

Retention time Impurity A = about 10 min; cortisone acetate = about 12 min.

System suitability Reference solution (a):

— *resolution*: minimum 4.2 between the peaks due to impurity A and cortisone acetate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 237 nm.

Calculate the content of $C_{23}H_{30}O_6$ taking the specific absorbance to be 395.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*

A. hydrocortisone acetate.

Cresol

General Notices

Action and use

Antiseptic; antimicrobial preservative.

DEFINITION

Cresol is a mixture of cresols and other phenols obtained from coal tar.

CHARACTERISTICS

An almost colourless to pale brownish yellow liquid.

Almost completely soluble in 50 volumes of *water*; freely soluble in *ethanol (96%)*, in *ether* and in fixed and volatile oils.

IDENTIFICATION

Shake 0.5 ml with 300 ml of *water* and filter. The filtrate complies with the following tests.

A. Add *iron(III) chloride solution R1*. A transient blue colour is produced.

B. Add *bromine water*. A pale yellow flocculent precipitate is produced.

TESTS

Acidity

A 2.0% w/v solution is neutral to *bromocresol purple solution*.

Distillation range

Not more than 2% v/v distils below 188° and not less than 80% v/v distils between 195° and 205°, Appendix V C.

Weight per ml

1.029 to 1.044 g, Appendix V G.

Hydrocarbons

Place 50 ml in a 500 ml round-bottomed flask, add 150 ml of 5M *sodium hydroxide* and 30 ml of *water* and mix thoroughly. Connect the flask to a splash-bulb and air-condenser about 60 cm long, with the end of the air-condenser fitting closely into the neck of a 250 ml pear-shaped separating funnel and passing well into the separating funnel, which has a cylindrical graduated portion above the stopcock. Fill the graduated portion of the separating funnel with *water*. Distil rapidly until 75 ml of distillate has been collected, cooling the separating funnel in running water if necessary. Allow the separating funnel to stand in a vertical position until separation is complete and draw off the aqueous liquid into a titration flask for use in the test for Volatile bases.

Allow the separating funnel to stand for a few minutes, measure the volume of hydrocarbon oil in the graduated portion and warm, if necessary, to keep the oil in the liquid state. Subtract the volume of volatile bases in the hydrocarbon oil, as determined in the following test. Not more than 0.15% v/v of hydrocarbon oil is present.

Volatile bases

To the aqueous liquid reserved in the test for Hydrocarbons add any aqueous liquid still remaining in the separating funnel and neutralise, if necessary, with 0.1M *hydrochloric acid* using *phenolphthalein solution R1* as indicator. Titrate with 1M *hydrochloric acid VS* using *methyl orange solution* as indicator. Wash the oil from the separating funnel into the titration flask with *water* and again titrate with 1M *hydrochloric acid VS*. From the volume of additional 1M *hydrochloric acid VS*, calculate the proportion of volatile bases in the hydrocarbon oil. From the total volume of 1M *hydrochloric acid VS* used in both titrations calculate the volume of volatile bases in the substance being examined. Each ml of 1M *hydrochloric acid VS* is equivalent to 0.080 ml of volatile bases. Not more than 0.15% v/v of volatile bases is present.

Hydrocarbons and volatile bases

The sum of the contents of hydrocarbon oil and volatile bases, as determined in the tests for Hydrocarbons and for Volatile bases, does not exceed 0.25% v/v.

Sulphur compounds

Place 20 ml in a small conical flask and over the mouth of the flask fix a piece of filter paper moistened with a 10% w/v solution of *lead(II) acetate*. Heat the flask on a water bath for 5 minutes. Not more than a light yellow colour is produced on the filter paper.

Non-volatile matter

When evaporated on a water bath and dried at 105°, leaves not more than 0.1% w/v of residue.

STORAGE

Cresol should be protected from light. It darkens with age or on exposure to light.

Croscarmellose Sodium

General Notices

(Ph Eur monograph 0985)

Action and use

Excipient in pharmaceutical products.

Ph Eur

DEFINITION

Cross-linked sodium carboxymethylcellulose.

Sodium salt of a cross-linked, partly *O*-carboxymethylated cellulose.

CHARACTERS

Appeareance

White or greyish-white powder.

Solubility

Practically insoluble in acetone, in anhydrous ethanol and in toluene.

IDENTIFICATION

A. Mix 1 g with 100 ml of a solution containing 4 ppm of *methylene blue R*, stir the mixture and allow it to settle. The substance to be examined absorbs the methylene blue and settles as a blue, fibrous mass.

B. Mix 1 g with 50 ml of *water R*. Transfer 1 ml of the mixture to a small test-tube and add 1 ml of *water R* and 0.05 ml of a freshly prepared 40 g/l solution of α-*naphthol R* in *methanol R*. Incline the test-tube and carefully add 2 ml of *sulphuric acid R* down the side so that it forms a lower layer. A reddish-violet colour develops at the interface.

C. The solution prepared from the sulphated ash in the test for heavy metals (see Tests) gives reaction (a) of sodium *(2.3.1)*.

TESTS

pH *(2.2.3)*

5.0 to 7.0 for the suspension.

Shake 1 g with 100 ml of *carbon dioxide-free water R* for 5 min.

Sodium chloride and sodium glycollate

Maximum 0.5 per cent (dried substance) for the sum of the percentage contents of sodium chloride and sodium glycollate.

Sodium chloride Place 5.00 g in a 250 ml conical flask, add 50 ml of *water R* and 5 ml of *strong hydrogen peroxide solution R* and heat on a water-bath for 20 min, stirring occasionally to ensure total hydration. Cool, add 100 ml of *water R* and 10 ml of *nitric acid R*. Titrate with *0.05 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)* using a silver indicator electrode and a double-junction reference electrode containing a 100 g/l solution of *potassium nitrate R* in the outer jacket and a standard filling solution in the inner jacket, and stirring constantly.

1 ml of *0.05 M silver nitrate* is equivalent to 2.922 mg of NaCl.

Sodium glycollate Place a quantity of the substance to be examined equivalent to 0.500 g of the dried substance in a 100 ml beaker. Add 5 ml of *glacial acetic acid R* and 5 ml of *water R* and stir to ensure total hydration (about 15 min). Add 50 ml of *acetone R* and 1 g of *sodium chloride R*. Stir for several minutes to ensure complete precipitation of the carboxymethylcellulose. Filter through a fast filter paper impregnated with *acetone R* into a volumetric flask, rinse the beaker and the filter with 30 ml of *acetone R* and dilute the filtrate to 100.0 ml with the same solvent. Allow to stand for 24 h without shaking. Use the clear supernatant to prepare the test solution.

Prepare the reference solutions as follows: in a 100 ml volumetric flask, dissolve 0.100 g of *glycollic acid R*, previously dried *in vacuo* over *diphosphorus pentoxide R*, in *water R* and dilute to 100.0 ml with the same solvent; use the solution within 30 days; transfer 1.0 ml, 2.0 ml, 3.0 ml and 4.0 ml of the solution to separate volumetric flasks, dilute the contents of each flask to 5.0 ml with *water R*, add 5 ml of *glacial acetic acid R*, dilute to 100.0 ml with *acetone R* and mix.

Transfer 2.0 ml of the test solution and 2.0 ml of each of the reference solutions to separate 25 ml volumetric flasks. Heat the uncovered flasks for 20 min on a water-bath to eliminate acetone. Allow to cool and add 5.0 ml of *2,7-dihydroxynaphthalene solution R* to each flask. Mix, add a further 15.0 ml of *2,7-dihydroxynaphthalene solution R* and mix again. Close the flasks with aluminium foil and heat on a water-bath for 20 min. Cool and dilute to 25.0 ml with *sulphuric acid R*.

Measure the absorbance *(2.2.25)* of each solution at 540 nm. Prepare a blank using 2.0 ml of a solution containing 5 per cent *V/V* each of *glacial acetic acid R* and *water R* in *acetone R*. Prepare a standard curve using the absorbances obtained with the reference solutions. From the standard curve and the absorbance of the test solution, determine the mass (a) of glycollic acid in the substance to be examined, in milligrams, and calculate the content of sodium glycollate using the following expression:

$$\frac{10 \times 1.29 \times a}{(100 - b)\,m}$$

1.29 = the factor converting glycollic acid to sodium glycollate,

b = loss on drying as a percentage,

m = mass of the substance to be examined, in grams.

Water-soluble substances

Maximum 10.0 per cent.

Disperse 10.00 g in 800.0 ml of *water R* and stir for 1 min every 10 min during the first 30 min. Allow to stand for 1 h and centrifuge if necessary. Decant 200.0 ml of the supernatant liquid onto a fast filter paper in a vacuum filtration funnel, apply vacuum and collect 150.0 ml of the filtrate. Evaporate to dryness and dry the residue at 100-105 °C for 4 h.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

To the residue obtained in the determination of the sulphated ash add 1 ml of *hydrochloric acid R* and evaporate on a water-bath. Take up the residue in 20 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 6 h.

Sulphated ash*(2.4.14)*

14.0 per cent to 28.0 per cent (dried substance), determined on 1.0 g, using a mixture of equal volumes of *sulphuric acid R* and *water R*.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the test for *Escherichia coli (2.6.13)*.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for croscarmellose sodium used as disintegrant.

Settling volume

Place 75 ml of *water R* in a 100 ml graduated cylinder and add 1.5 g of the substance to be examined in 0.5 g portions, shaking vigorously after each addition. Dilute to 100.0 ml with *water R* and shake again until the substance is homogeneously distributed. Allow to stand for 4 h. The settling volume is between 10.0 ml and 30.0 ml.

Degree of substitution

0.60 to 0.85 (dried substance).

Place 1.000 g in a 500 ml conical flask, add 300 ml of a 100 g/l solution of *sodium chloride R* and 25.0 ml of *0.1 M sodium hydroxide*, stopper the flask and allow to stand for 5 min, shaking occasionally. Add 0.05 ml of *m-cresol purple solution R* and about 15 ml of *0.1 M hydrochloric acid* from a burette. Insert the stopper and shake. If the solution is violet, add *0.1 M hydrochloric acid* in 1 ml portions until the solution becomes yellow, shaking after each addition. Titrate with *0.1 M sodium hydroxide* until the colour turns to violet.

Calculate the number of milliequivalents (*M*) of base required to neutralise the equivalent of 1 g of dried substance.

Calculate the degree of acid carboxymethyl substitution (*A*) using the following expression:

$$\frac{1150M}{(7102 - 412M - 80C)}$$

C = sulphated ash as a percentage.

Calculate the degree of sodium carboxymethyl substitution (*S*) using the following expression:

$$\frac{(162 + 58A)\,C}{(7102 - 80C)}$$

The degree of substitution is the sum of *A* and *S*.

Particle size distribution

(2.9.31 or 2.9.38).

Hausner ratio

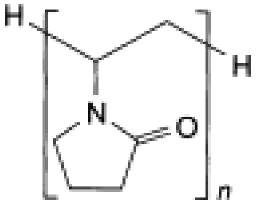
(2.9.36).

Ph Eur

Crospovidone

General Notices

(Ph Eur monograph 0892)



$(C_6H_9NO)_n$ $(111.1)_n$ *9003-39-8*

Action and use

Excipient in pharmaceutical products.

Ph Eur

DEFINITION

Crospovidone is a cross-linked homopolymer of 1-ethenylpyrrolidin-2-one. It is available in different degrees of powder fineness (type A and type B).

Content

11.0 per cent to 12.8 per cent of nitrogen (N; A_r 14.01) (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder or flakes, hygroscopic.

Solubility

Practically insoluble in water, in alcohol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of crospovidone.

B. Suspend 1 g in 10 ml of *water R*, add 0.1 ml of *0.05 M iodine* and shake for 30 s. Add 1 ml of *starch solution R* and shake. No blue colour develops within 30 s.

C. To 10 ml of *water R*, add 0.1 g and shake. A suspension is formed and no clear solution is obtained within 15 min.

D. Weigh a suitable quantity of the substance to be examined (for example 10 mg to 100 mg) and suspend it in 10.0 ml of *water R*, adding a wetting agent. Observe under a microscope at a suitable magnification using a calibrated ocular micrometer. If the majority of particles are in the range 50 µm to 300 µm, the product is classified as type A. If almost all the particles are below 50 µm, the product is classified as type B.

TESTS

Peroxides

Type A: maximum 400 ppm expressed as H_2O_2; type B: maximum 1000 ppm expressed as H_2O_2.

Suspend 2.0 g in 50 ml of *water R*. To 25 ml of this suspension add 2 ml of *titanium trichloride-sulphuric acid reagent R*. Allow to stand for 30 min and filter. The absorbance (*2.2.25*) of the filtrate, measured at 405 nm using a mixture of 25 ml of a filtered 40 g/l suspension of the substance to be examined and 2 ml of a 13 per cent *V/V* solution of *sulphuric acid R* as the compensation liquid has a maximum of 0.35.

For type B use 10 ml of the suspension diluted to 25 ml with *water R* for the test.

Water-soluble substances

Maximum 1.0 per cent.

Transfer 25.0 g to a 400 ml beaker, add 200 ml of *water R* and stir for 1 h using a magnetic stirrer. Transfer the suspension to a 250.0 ml volumetric flask, rinsing with *water R*, and dilute to volume with the same solvent. Allow the bulk of the solids to settle. Filter about 100 ml of the almost clear supernatant liquid through a 0.45 µm membrane filter, protected by superimposing a 3 µm membrane filter. While filtering, stir the liquid above the filter manually or by means of a mechanical stirrer, taking care not to damage the filter. Transfer 50.0 ml of the clear filtrate to a tared 100 ml beaker, evaporate to dryness and dry at 105-110 °C for 3 h. The residue weighs a maximum of 50 mg.

Impurity A

Liquid chromatography (2.2.29).

Test solution Suspend 1.250 g in 50.0 ml of *methanol R* and shake for 60 min. Leave the bulk to settle and filter through a 0.2 µm filter.

Reference solution (a) Dissolve 50 mg of *1-vinylpyrrolidin-2-one R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *1-vinylpyrrolidin-2-one R* and 0.50 g of *vinyl acetate R* in *methanol R* and dilute to 100 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Precolumn:
— *size*: *l* = 0.025 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *acetonitrile R, water R* (10:90 *V/V*).

Flow rate Adjusted so that the retention time of the peak corresponding to impurity A is about 10 min.

Detection Spectrophotometer at 235 nm.

Injection 50 µl. After each injection of the test solution, wash the precolumn by passing the mobile phase backward, at the same flow rate as applied in the test, for 30 min.

System suitability:

— *resolution*: minimum of 2.0 between the peaks corresponding to impurity A and to vinyl acetate in the chromatogram obtained with reference solution (b);

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 5 injections of reference solution (a).

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (10 ppm).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Place 100.0 mg of the substance to be examined (*m* mg) in a combustion flask, add 5 g of a mixture of 1 g of *copper sulphate R*, 1 g of *titanium dioxide R* and 33 g of *dipotassium sulphate R,* and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of *water R*. Add 7 ml of *sulphuric acid R*, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulphuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulphuric acid in the neck of the flask; precautions are to be taken to prevent the upper part of the flask from becoming overheated. Continue the heating for 45 min. Cool, dissolve the solid material by cautiously adding to the mixture 20 ml of *water R*, cool again and place in a steam-distillation apparatus. Add 30 ml of *strong sodium hydroxide solution R* through the funnel, rinse the funnel cautiously with 10 ml of *water R* and distil immediately by passing steam through the mixture. Collect 80-100 ml of distillate in a mixture of 30 ml of a 40 g/l solution of *boric acid R* and 0.05 ml of *bromocresol green-methyl red solution R* and enough *water R* to cover the tip of the condenser. Towards the end of the distillation lower the receiver so that the tip of the condenser is above the surface of the acid solution and rinse the end part of the condenser with a small quantity of *water R*. Titrate the distillate with *0.025 M sulphuric acid* until the colour of the solution changes from green through pale greyish-blue to pale greyish-red-purple (n_1 ml of *0.025 M sulphuric acid*).

Repeat the test using about 100 mg of *glucose R* in place of the substance to be examined (n_2 ml of *0.025 M sulphuric acid*).

$$\text{Percentage content of nitrogen} = \frac{0.7004\,(n_1 - n_2)}{m} \times 100$$

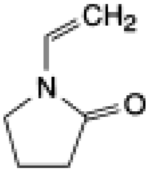
STORAGE

In an airtight container.

LABELLING

The label states the type (type A or type B).

IMPURITIES



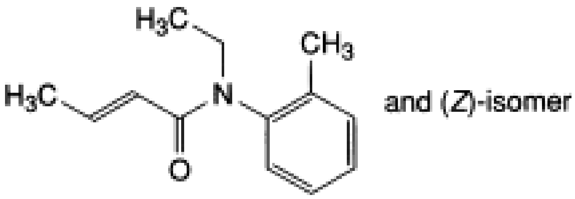
1-ethenylpyrrolidin-2-one (1-vinylpyrrolidin-2-one).

Ph Eur

Crotamiton

General Notices

(Ph Eur monograph 1194)



$C_{13}H_{17}NO$ 203.3   *483-63-6*

Action and use

Acaricide.

Preparations

Crotamiton Cream

Crotamiton Lotion

Ph Eur

DEFINITION

N-Ethyl-*N*-(2-methylphenyl)but-2-enamide.

Content:

- *sum of the E- and Z-isomers*: 96.0 per cent to 102.0 per cent;
- *Z-isomer*: maximum 15.0 per cent.

CHARACTERS

Appearance

Colourless or pale yellow, oily liquid.

Solubility

Slightly soluble in water, miscible with ethanol (96 per cent).

At low temperatures it may partly or completely solidify.

IDENTIFICATION

First identification   *B.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 25.0 mg in *cyclohexane R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *cyclohexane R.*

Spectral range 220-300 nm.

Absorption maximum At 242 nm.

Specific absorbance at the absorption maximum 300 to 330.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *crotamiton CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution.

Dissolve 25 mg of the substance to be examined in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 25 mg of *crotamiton CRS* in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase Shake 98 volumes of *methylene chloride R* with 2 volumes of *concentrated ammonia R*, dry over *anhydrous sodium sulphate R*, filter and mix 97 volumes of the filtrate with 3 volumes of *2-propanol R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 10 ml of a saturated solution add a few drops of a 3 g/l solution of *potassium permanganate R*. A brown colour is produced and a brown precipitate is formed on standing.

TESTS

Relative density *(2.2.5)*

1.006 to 1.011.

Refractive index *(2.2.6)*

1.540 to 1.542.

Free amines

Maximum 500 ppm, expressed as ethylaminotoluene.

Dissolve 5.00 g in 16 ml of *methylene chloride R* and add 4.0 ml of *glacial acetic acid R*. Add 0.1 ml of *metanil yellow solution R* and 1.0 ml of *0.02 M perchloric acid*. The solution is red-violet.

Chlorides

Maximum 100 ppm.

Boil 5.0 g under a reflux condenser for 1 h with 25 ml of *ethanol (96 per cent) R* and 5 ml of a 200 g/l solution of *sodium hydroxide R*. Cool, add 5 ml of *water R* and shake with 25 ml of *ether R*. Dilute the lower layer to 20 ml with *water R;* add 5 ml of *nitric acid* R, dilute to 50 ml with *water R* and add 1 ml of a freshly prepared 50 g/l solution of *silver nitrate R.* Any opalescence in the solution is not more intense than that in a mixture of 1 ml of a freshly prepared 50 g/l solution of *silver nitrate R* and a solution prepared by diluting 5 ml of a 200 g/l solution of *sodium hydroxide R* to 20 ml with *water R* and adding 1.5 ml of *0.01 M hydrochloric acid*, 5 ml of *nitric acid R* and diluting to 50 ml with water R.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b) Dilute 1.0 ml of test solution (a) to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *crotamiton CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 15.0 mg of *crotamiton impurity A CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (d) Dissolve 15 mg of *crotamiton impurity A CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with test solution (a).

Column:

- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: silica gel for *chromatography R* (5 µm).

Mobile phase *tetrahydrofuran R*, *cyclohexane R* (8:92 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 242 nm.

Injection 20 µl of test solution (a) and reference solutions (b), (c) and (d).

Run time 2.5 times the retention time of the *E*-isomer.

Relative retention With reference to the *E*-isomer: *Z*-isomer = about 0.5; impurity A = about 0.8.

System suitability Reference solution (d):

- *resolution*: minimum 4.5 between the peaks due to impurity A and the *E*-isomer.

Limits:

- — *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (3 per cent);
- — *sum of impurities other than A*: not more than the sum of the areas of the peaks due to the *Z*- and *E*-isomers in the chromatogram obtained with reference solution (c) (1 per cent);
- — *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (c); disregard any peak due to the *Z*-isomer.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

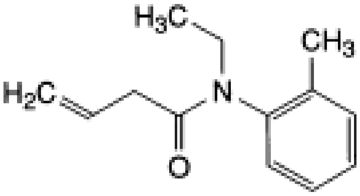
Calculate the percentage content of $C_{13}H_{17}NO$ from the sum of the areas of the peaks due to the *Z*- and *E*-isomers in the chromatograms obtained. Calculate the content of the *Z*-isomer, as a percentage of the total content of the *E*- and *Z*-isomers, from the chromatogram obtained with test solution (b).

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*



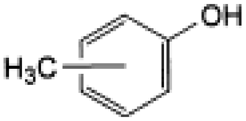
N-ethyl-*N*-(2-methylphenyl)but-3-enamide.

Ph Eur

Crude Cresol

General Notices

(Ph Eur monograph 1628)



C_7H_8O 108.1

Action and use

Antiseptic.

Ph Eur

DEFINITION

Mixture of 2-, 3- and 4-methylphenol.

CHARACTERS

Appearance

Colourless or pale brown liquid.

Solubility

Sparingly soluble in water, miscible with alcohol and with methylene chloride.

IDENTIFICATION

A. To 0.5 ml add 300 ml of *water R*, mix and filter. To 10 ml of the filtrate add 1 ml of *ferric chloride solution R1*. A blue colour is produced.

B. To 10 ml of the filtrate obtained in identification test A, add 1 ml of *bromine water R*. A pale yellow flocculent precipitate is produced.

C. It complies with the test for relative density (see Tests).

TESTS

Solution S

To 2.5 g of the substance to be examined add 50 ml of *water R*, shake for 1 min and filter through a moistened filter.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.3 ml of *0.01 M hydrochloric acid*. The solution is red.

Relative density *(2.2.5)*

1.029 to 1.044.

Distillation range *(2.2.11)*

A maximum of 2.0 per cent *V/V* distils below 188 °C and a minimum of 80 per cent *V/V* distils between 195 °C and 205 °C.

Sulphur compounds

Place 20 ml in a small conical flask. Over the mouth of the flask fix a piece of filter paper moistened with *lead acetate solution R*. Heat on a water-bath for 5 min. Not more than a light yellow colour is produced on the filter paper.

Residue on evaporation

Maximum 0.1 per cent.

Evaporate 2.0 g to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs not more than 2 mg.

STORAGE

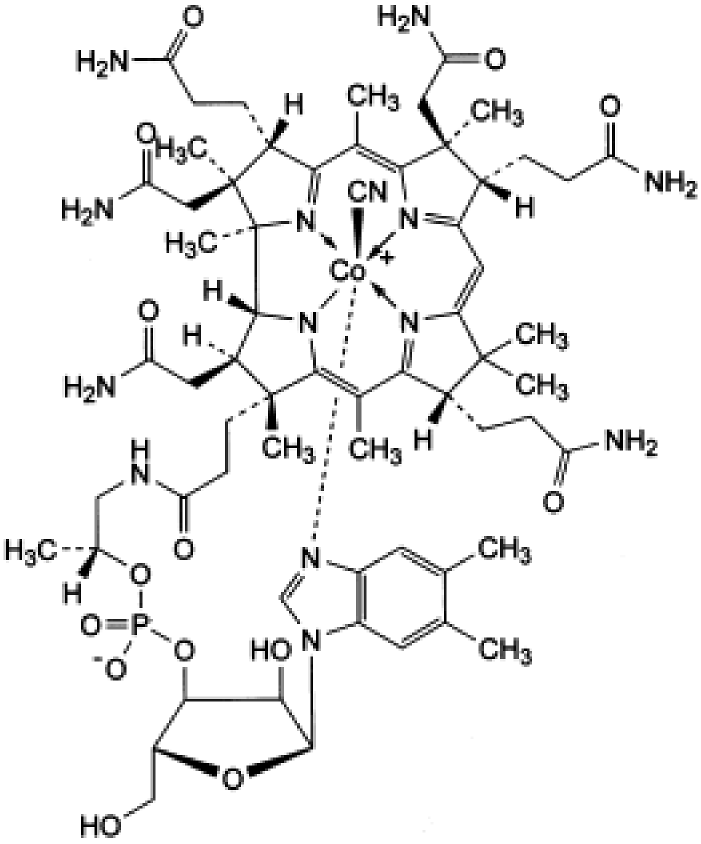
Protected from light.

Ph Eur

Cyanocobalamin

General Notices

(Ph Eur monograph 0547)



$C_{63}H_{88}CoN_{14}O_{14}P$ 1355 *68-19-9*

Action and use

Vitamin B_{12} analogue.

Preparation

Cyanocobalamin Tablets

Ph Eur

DEFINITION

α-(5,6-Dimethylbenzimidazol-1-yl)cobamide cyanide.

Content

96.0 per cent to 102.0 per cent (dried substance).

This monograph applies to cyanocobalamin produced by fermentation.

CHARACTERS

Appearance

Dark red, crystalline powder or dark red crystals.

Solubility

Sparingly soluble in water and in ethanol (96 per cent), practically insoluble in acetone.

The anhydrous substance is very hygroscopic.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 2.5 mg in *water R* and dilute to 100.0 ml with the same solvent.

Spectral range 260-610 nm.

Absorption maxima At 278 nm, 361 nm and from 547 nm to 559 nm.

Absorbance ratio
— $A_{361} / A_{547\text{-}559}$ = 3.15 to 3.45;

— A_{361} / A_{278} = 1.70 to 1.90.

B. Thin-layer chromatography *(2.2.27)*. *Carry out the test protected from light.*

Test solution Dissolve 2 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R*.

Reference solution Dissolve 2 mg of *cyanocobalamin CRS* in 1 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R*.

Plate *TLC silica gel G plate R*.

Mobile phase *dilute ammonia R1, methanol R, methylene chloride R* (9:30:45 *V/V/V*).

Application 10 µl.

Development In an unsaturated tank, over a path of 12 cm.

Drying In air.

Detection Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase. *Use within 1 h*.

Reference solution (a) Dilute 3.0 ml of the test solution to 100.0 ml with the mobile phase. *Use within 1 h.*

Reference solution (b) Dilute 5.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase. *Use within 1 h.*

Reference solution (c) Dissolve 25 mg of the substance to be examined in 10 ml of *water R*, warming if necessary. Allow to cool and add 5 ml of a 1.0 g/l solution of *chloramine R* and 0.5 ml of *0.05 M hydrochloric acid*, then dilute to 25 ml with *water R*. Shake and allow to stand for 5 min. Dilute 1 ml of this solution to 10 ml with the mobile phase and inject immediately.

Column:

— *size:* l = 0.25 m, Ø = 4 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 26.5 volumes of *methanol R* and 73.5 volumes of a 10 g/l solution of *disodium hydrogen phosphate R* adjusted to pH 3.5 with *phosphoric acid R* and use within 2 days.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 361 nm.

Injection 20 µl.

Run time 3 times the retention time of cyanocobalamin.

System suitability:

— the chromatogram obtained with reference solution (c) shows 2 principal peaks;

— *resolution*: minimum 2.5 between the 2 principal peaks in the chromatogram obtained with reference solution (c);

— *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (3 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 20.00 mg by drying *in vacuo* at 105 °C for 2 h.

ASSAY

Dissolve 25.00 mg in *water R* and dilute to 1000.0 ml with the same solvent. Measure the absorbance *(2.2.25)* at the absorption maximum at 361 nm.

Calculate the content of $C_{63}H_{88}CoN_{14}O_{14}P$ taking the specific absorbance to be 207.

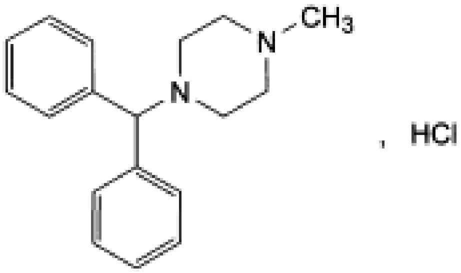
STORAGE

In an airtight container, protected from light.

Cyclizine Hydrochloride

General Notices

(Ph Eur monograph 1092)



$C_{18}H_{22}N_2,HCl$ 302.8 *305-25-3*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparations

Cyclizine Injection

Cyclizine Tablets

Dipipanone and Cyclizine Tablets

Ph Eur

DEFINITION

1-(Diphenylmethyl)-4-methylpiperazine hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution (a) Dissolve 20.0 mg in a 5 g/l solution of *sulphuric acid R* and dilute to 100.0 ml with the same acid solution.

Test solution (b) Dilute 10.0 ml of test solution (a) to 100.0 ml with a 5 g/l solution of *sulphuric acid R*.

Spectral range 240-350 nm for test solution (a); 210-240 nm for test solution (b).

Resolution (2.2.25) Minimum 1.7.

Absorption maxima At 258 nm and 262 nm for test solution (a); at 225 nm for test solution (b).

Absorbance ratio A_{262}/A_{258} = 1.0 to 1.1.

Specific absorbance at the absorption maximum at 225 nm 370 to 410 for test solution (b).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *cyclizine hydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *cyclizine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (2:13:85 *V/V/V*).

Application 20 µl.

Development Over 2/3 of the plate.

Drying In air for 30 min.

Detection Expose to iodine vapour for 10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 0.5 g in 10 ml of *ethanol (60 per cent) R*, heating if necessary. Cool in iced water. Add 1 ml of *dilute sodium hydroxide solution R* and 10 ml of *water R*. Filter, wash the precipitate with *water R* and dry at 60 °C at a pressure not exceeding 0.7 kPa for 2 h. The melting point *(2.2.14)* is 105 °C to 108 °C.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

4.5 to 5.5.

Dissolve 0.5 g in a mixture of 40 volumes of *ethanol (96 per cent) R* and 60 volumes of *carbon dioxide-free water R* and dilute to 25 ml with the same mixture of solvents.

Related substances

Gas chromatography *(2.2.28)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 0.250 g of the substance to be examined in 4.0 ml of *methanol R* and dilute to 5.0 ml with *1 M sodium hydroxide*.

Reference solution (a) Dissolve 25 mg of the substance to be examined in 10.0 ml of *methanol R*. Dilute 1.0 ml of this solution to 50.0 ml with *methanol R*.

Reference solution (b) Dissolve 5 mg of the substance to be examined, 5.0 mg of *cyclizine impurity A CRS* and 5.0 mg of *cyclizine impurity B CRS* in *methanol R* and dilute to 20.0 ml with the same solvent.

Column:

— *material*: fused silica;

— *size:* l = 25 m, Ø = 0.33 mm;

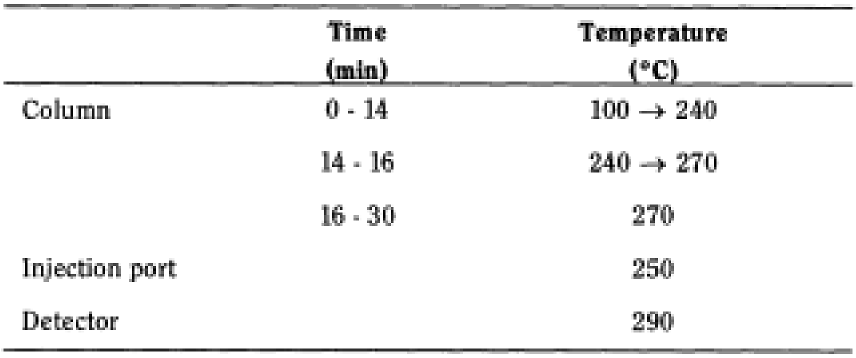
— *stationary phase: poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.50 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.0 ml/min.

Split ratio 1:25.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 14	100 → 240
	14 - 16	240 → 270
	16 - 30	270
Injection port		250
Detector		290

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to cyclizine (retention time = about 15 min): impurity A = about 0.2; impurity B = about 0.7.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 50, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to methanol.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 130 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.120 g in 15 ml of *anhydrous formic acid R* and add 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

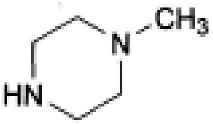
1 ml of *0.1 M perchloric acid* is equivalent to 15.14 mg of $C_{18}H_{23}ClN_2$.

STORAGE

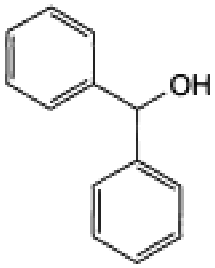
Protected from light.

IMPURITIES

Specified impurities A, B.



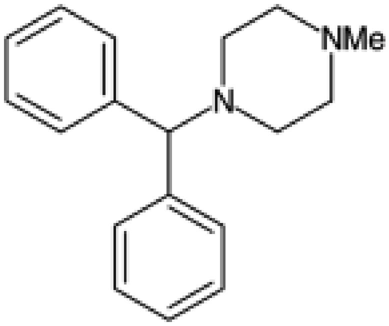
A. 1-methylpiperazine,



B. diphenylmethanol (benzhydrol).

Cyclizine

General Notices



$C_{18}H_{22}N_2$ 266.4 *82-92-8*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Cyclizine Injection

DEFINITION

Cyclizine is 1-benzhydryl-4-methylpiperazine. It contains not less than 98.5% and not more than 101.0% of $C_{18}H_{22}N_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or creamy white, crystalline powder.

Practically insoluble in *water*. It dissolves in most organic solvents and in dilute acids.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cyclizine *(RS 075)*.

B. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a freshly prepared 0.002% w/v solution in 0.05M *sulphuric acid* exhibits a maximum at 227 nm with a series of ill-defined maxima between 258 and 272 nm. The *absorbance* at 227 nm is about 0.87.

C. *Melting point*, about 107°, Appendix V A.

TESTS

Alkalinity

Shake 1 g with 25 ml of *carbon dioxide-free water* for 5 minutes and filter. The pH of the filtrate is 7.6 to 8.6, Appendix V L.

Clarity of solution

A 1.0% w/v solution in *ether* and a 1.0% w/v solution in 2M *hydrochloric acid* are *clear*, Appendix IV A.

Chloride

Dissolve 0.20 g in 2 ml of *methanol* and dilute to 30 ml with 2M *nitric acid*. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (500 ppm).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and as the mobile phase the lower layer obtained after shaking together a mixture of 2 volumes of 13.5M *ammonia*, 8 volumes of *methanol* and 90 volumes of *dichloromethane* and allowing the layers to separate. Apply separately to the plate 20 µl of each of four freshly prepared solutions in *methanol* containing (1) 2.0% w/v of the substance being examined, (2) 0.010% w/v of the substance being examined, (3) 0.010% w/v of N-*methylpiperazine* and (4) 0.10% w/v of each of the substance being examined and *hydroxyzine hydrochloride EPCRS*. After removal of the plate, allow it to dry in air and expose to iodine vapour for 10 minutes. Any spot corresponding to N-*methylpiperazine* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

Loss on drying

When dried to constant weight at 80°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

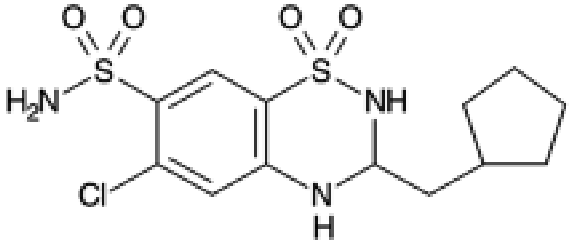
Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.1 g and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 13.32 mg of $C_{18}H_{22}N_2$.

Cyclopenthiazide

General Notices



$C_{13}H_{18}ClN_3O_4S_2$ 379.9 *742-20-1*

Action and use

Thiazide-diuretic.

Preparation

Cyclopenthiazide Tablets

DEFINITION

Cyclopenthiazide is 6-chloro-3-cyclopentylmethyl-3,4-dihydro-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide. It contains not less than 98.0% and not more than 102.0% of $C_{13}H_{18}ClN_3O_4S_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white powder.

Practically insoluble in *water*; soluble in *acetone* and in *ethanol (96%)*; very slightly soluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cyclopenthiazide *(RS 077)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.01M *sodium hydroxide* exhibits two maxima, at 273 nm and 320 nm. The *absorbance* at 273 nm is about 0.88 and at 320 nm is about 0.12.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in *acetone* containing (1) 0.1% w/v of the substance being examined and (2) 0.1% w/v of *cyclopenthiazide BPCRS*. After removal of the plate, dry it in a current of air, examine under *ultraviolet light (254 nm)* and then reveal the spots by

Method I. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions of the substance being examined in *acetone* containing (1) 0.50% w/v and (2) 0.0050% w/v. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

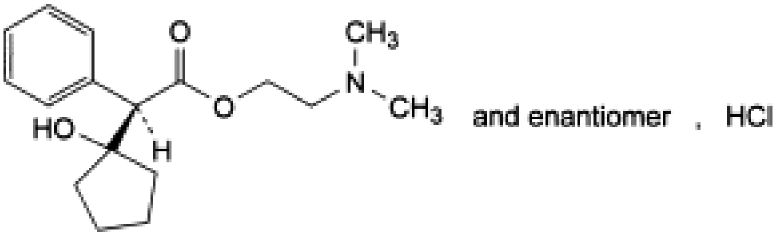
ASSAY

Dissolve 0.5 g in 50 ml of *butylamine* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and *magneson solution* as indicator; titrate to a pure blue end point. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 18.99 mg of $C_{13}H_{18}ClN_3O_4S_2$.

Cyclopentolate Hydrochloride

General Notices

(Ph Eur monograph 1093)



$C_{17}H_{25}NO_3,HCl$ 327.9 *5870-29-1*

Action and use

Anticholinergic.

Preparation

Cyclopentolate Eye Drops

Ph Eur

DEFINITION

Cyclopentolate hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of 2-(dimethylamino)ethyl (*RS*)-2-(1-hydroxycyclopentyl)-2-phenylacetate hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Melting point *(2.2.14)*

135 °C to 141 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cyclopentolate hydrochloride CRS*. Examine the substances as discs prepared using *potassium chloride R*. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separ-ately in *alcohol R*, evaporate to dryness and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with reference solution (b).

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. The pH of the solution is 4.5 to 5.5.

Related substances

Examine by thin-layer chromatography *(2.2.27*), using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *alcohol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 20 ml with *alcohol R*.

Reference solution (a) Dilute 1 ml of test solution (a) to 200 ml with *alcohol R*.

Reference solution (b) Dissolve 10 mg of *cyclopentolate hydrochloride CRS* in *alcohol R* and dilute to 5 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *concentrated ammonia R,* 15 volumes of *water R*, 30 volumes of *butyl acetate R* and 50 volumes of *2-propanol R*. Allow the plate to dry in air. Spray with *analcoholic solution of sulphuric acid R* and heat the plate at 120 °C for 30 min. Examine in ultraviolet light at 365 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 1.0 ml of *0.1 M hydrochloric acid* and 50 ml of *alcohol R.* Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

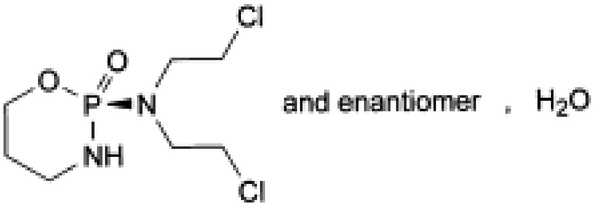
1 ml of *0.1 M sodium hydroxide* is equivalent to 32.79 mg of $C_{17}H_{26}ClNO_3$.

Ph Eur

Cyclophosphamide

General Notices

(Ph Eur monograph 0711)



$C_7H_{15}Cl_2N_2O_2P,H_2O$ 279.1 *6055-19-2*

Action and use

Cytotoxic alkylating agent.

Preparations

Cyclophosphamide Injection

Cyclophosphamide Tablets

Ph Eur

DEFINITION

Cyclophosphamide contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (2*RS*)-*N*,*N*-bis(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Determine the melting point *(2.2.14)* of the substance to be examined. Mix equal parts of the substance to be examined and *cyclophosphamide CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 51 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cyclophosphamide CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g in 10 ml of *water R* and add 5 ml of *silver nitrate solution R1*; the solution remains clear. Boil, a white precipitate is formed which dissolves in *concentrated ammonia R* and is reprecipitated on the addition of *dilute nitric acid R*.

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 4.0 to 6.0, determined immediately after preparation of the solution.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *alcohol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *alcohol R*.

Reference solution (a) Dissolve 10 mg of *cyclophosphamide CRS* in *alcohol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 0.1 ml of test solution (a) to 10 ml with *alcohol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 2 volumes of *anhydrous formic acid R*, 4 volumes of *acetone R*, 12 volumes of *water R* and 80 volumes of *methyl ethyl ketone R*. Dry the plate in a current of warm air and heat at 110 °C for 10 min. At the bottom of a chromatography tank, place an evaporating dish containing a 50 g/l solution of *potassium permanganate R* and add an equal volume of *hydrochloric acid R.* Place the plate whilst still hot in the tank and close the tank. Leave the plate in contact with the chlorine gas for 2 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the points of application gives at most a very faint blue colour with a drop of *potassium iodide and starch solution R*. Avoid prolonged exposure to cold air. Spray with *potassium iodide and starch solution R* and allow to stand for 5 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent). Disregard any spot remaining at the starting-point.

Chlorides *(2.4.4)*

Dissolve 0.15 g in *water R* and dilute to 15 ml with the same solvent. The freshly prepared solution complies with the limit test for chlorides (330 ppm).

Phosphates *(2.4.11)*

Dissolve 0.10 g in *water R* and dilute to 100 ml with the same solvent. The solution complies with the limit test for phosphates (100 ppm).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

6.0 per cent to 7.0 per cent, determined on 0.300 g by the semi-micro determination of water.

ASSAY

Dissolve 0.100 g in 50 ml of a 1 g/l solution of *sodium hydroxide R* in *ethylene glycol R* and boil under a reflux condenser for 30 min. Allow to cool and rinse the condenser with 25 ml of *water R*. Add 75 ml of *2-propanol R*, 15 ml of *dilute nitric acid R*, 10.0 ml of *0.1 M silver nitrate* and 2.0 ml of *ferric ammonium sulfate solution R2* and titrate with 0.*1 M ammonium thiocyanate.*

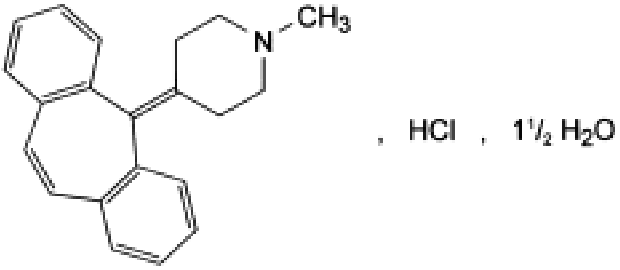
1 ml of *0.1 M silver nitrate* is equivalent to 13.05 mg of $C_7H_{15}Cl_2N_2O_2P$.

Ph Eur

Cyproheptadine Hydrochloride

General Notices

(Ph Eur monograph 0817)



$C_{21}H_{21}N,HCl,1½H_2O$ 350.9 *969-33-5*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Cyproheptadine Tablets

Ph Eur

DEFINITION

Cyproheptadine hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 4-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride, calculated with reference to the anhydrous substance.

CHARACTERS

A white or slightly yellow, crystalline powder, slightly soluble in water, freely soluble in methanol, sparingly soluble in alcohol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 50.0 mg in *alcohol R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *alcohol R*. Examined between 230 nm and 320 nm *(2.2.25)*, the solution shows an absorption maximum at 286 nm. The specific absorbance at the maximum is 335 to 365, calculated with reference to the anhydrous substance.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cyproheptadine hydrochloride CRS*. Examine the substances as mulls in *liquid paraffin R.*

C. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 25 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *cyproheptadine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *imipramine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent. Dilute 1 ml to 2 ml with reference solution (a).

Apply to the plate 2 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *diethylamine R*, 20 volumes of *ether R* and 75 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated principal spots.

D. A saturated solution gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Acidity

Dissolve 0.10 g in *water R* and dilute to 25 ml with the same solvent. Add 0.1 ml of *methyl red solution R*. Not more than 0.15 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution Dissolve 50 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (a) Dissolve 10 mg of *dibenzocycloheptene CRS* (5*H*-dibenzo[*a*,*d*]cycloheptene) in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 50 ml with the same mixture of solvents. Dilute 1 ml to 10 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (b) Dilute 1 ml of the test solution to 100 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*. Dilute 1 ml to 10 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R* and 90 volumes of *methylene chloride R*. Allow the plate to dry in air and spray with *alcoholic solution of sulphuric acid R*. Heat at 110 °C for 30 min and examine in ultraviolet light at 365 nm while hot. In the chromatogram obtained with the test solution: any spot corresponding to dibenzocycloheptene is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent); any spot apart from the principal spot and any spot corresponding to dibenzocycloheptene is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Water ***(2.5.12)***

7.0 per cent to 9.0 per cent, determined on 0.200 g.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

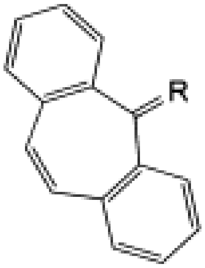
Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 32.39 mg of $C_{21}H_{22}ClN$.

STORAGE

Store protected from light.

IMPURITIES



A. R = H_2: dibenzo[*a*,*d*]cycloheptene,

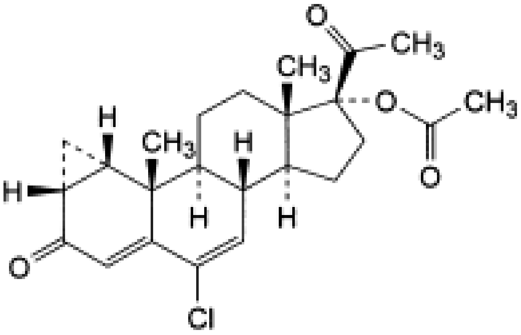
B. R = O: 5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (dibenzosuberone).

Ph Eur

Cyproterone Acetate

General Notices

(Ph Eur monograph 1094)



$C_{24}H_{29}ClO_4$ 416.9 *427-51-0*

Action and use

Anti-estrogen.

Preparation

Cyproterone Tablets

Ph Eur

DEFINITION

6-Chloro-3,20-dioxo-1β,2β-dihydro-3′*H*-cyclopropa[1,2]pregna-1,4,6-trien-17-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, freely soluble in acetone, soluble in methanol, sparingly soluble in anhydrous ethanol.

mp: about 210 °C.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *cyproterone acetate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *cyproterone acetate CRS* in *methylene chloride R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *cyclohexane R, ethyl acetate R* (50:50 *V/V*).

Application 5 µl.

Development Twice over a path of 15 cm; dry the plate in air between the 2 developments.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 1 mg add 2 ml of *sulphuric acid R* and heat on a water-bath for 2 min. A red colour develops. Cool. Add this solution cautiously to 4 ml of *water R* and shake. The solution becomes violet.

D. Incinerate about 30 mg with 0.3 g of *anhydrous sodium carbonate R* over a naked flame for about 10 min. Cool and dissolve the residue in 5 ml of *dilute nitric acid R*. Filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

E. It gives the reaction of acetyl *(2.3.1).*

TESTS

Specific optical rotation *(2.2.7)*

+ 152 to + 157 (dried substance).

Dissolve 0.25 g in *acetone R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*.

Reference solution (b) Dissolve 5 mg of *medroxyprogesterone acetate CRS* in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with reference solution (a).

Column:

— *size*: *l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase *acetonitrile R, water R* (40:60 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time Twice the retention time of cyproterone acetate.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to cyproterone acetate and medroxyprogesterone acetate.

Limits:

— *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying at 80 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

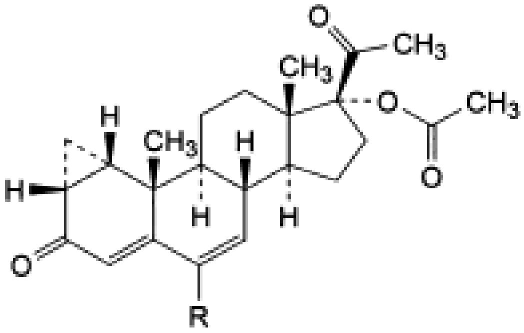
Dissolve 50.0 mg in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *methanol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 282 nm.

Calculate the content of $C_{24}H_{29}ClO_4$ taking the specific absorbance to be 414.

STORAGE

Protected from light.

IMPURITIES



A. R = H: 3,20-dioxo-1β,2β-dihydro-3′*H*-cyclopropa[1,2]pregna-1,4,6-trien-17-yl acetate,

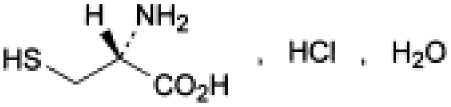
B. R = OCH_3: 6-methoxy-3,20-dioxo-1β,2β-dihydro-3′*H*-cyclopropa[1,2]pregna-1,4,6-trien-17-yl acetate.

Ph Eur

Cysteine Hydrochloride

General Notices

(Cysteine Hydrochloride Monohydrate, Ph Eur monograph 0895)



$C_3H_7NO_2S,HCl,H_2O$ 175.6 *7048-04-6*

Action and use

Amino acid.

Ph Eur

DEFINITION

Cysteine hydrochloride monohydrate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*R*)-2-amino-3-sulfanylpropanoic acid hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cysteine hydrochloride monohydrate CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour, and size to the principal spot in the chromatogram obtained with reference solution (b).

D. Dissolve about 5 mg in 1 ml of *dilute sodium hydroxide solution R*. Add 1 ml of a 30 g/l solution of *sodium nitroprusside R*. An intense violet colour develops which becomes brownish-red and then orange. Add 1 ml of *hydrochloric acid R*. The solution becomes green.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Dilute 10 ml of solution S to 20 ml with *water R*. The solution is clear (*2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation (*2.2.7)*

Dissolve 2.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 5.5 to + 7.0, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R.*

Test solution (a) Dissolve 0.20 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent. To 5 ml of the solution add 5 ml of a 40 g/l solution of *N-ethylmaleimide R* in *alcohol R*. Allow to stand for 5 min.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 20 mg of *cysteine hydrochloride monohydrate CRS* in *water R* and dilute to 10 ml with the same solvent. Add 10 ml of a 40 g/l solution of *N-ethylmaleimide R* in *alcohol R*. Allow to stand for 5 min.

Reference solution (b) Dilute 2 ml of reference solution (a) to 10 ml with *water R*.

Reference solution (c) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (d) Dissolve 10 mg of *tyrosine CRS* in 10 ml of reference solution (a) and dilute to 25 ml with *water R.*

Apply separately to the plate 5 µl of each test solution and reference solutions (b), (c), and (d) . Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R,* 20 volumes of *water R* and 60 volumes of *butanol R*. Dry the plate at 80 °C for 30 min. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows 2 clearly separated principal spots.

Sulphates *(2.4.13)*

Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 0.50 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (20 ppm).

Heavy metals ***(2.4.8)***

Dissolve 2.0 g in *water R*. Adjust to pH 3 to 4 with *concentrated ammonia R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

8.0 per cent to 12.0 per cent, determined on 1.000 g by drying at a pressure not exceeding 0.7 kPa for 24 h.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

In a ground-glass stoppered flask dissolve 0.300 g of the substance to be examined and 4 g of *potassium iodide R* in 20 ml of *water R*. Cool the solution in iced water and add 3 ml of *hydrochloric acid R1* and 25.0 ml of *0.05 M iodine*. Stopper the flask and allow to stand in the dark for 20 min. Titrate with *0.1 M sodium thiosulphate* using 3 ml of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 15.76 mg of $C_3H_8ClNO_2S$.

STORAGE

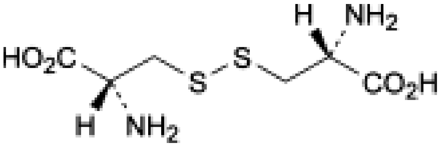
Store protected from light.

Ph Eur

Cystine

General Notices

(Ph Eur monograph 0998)



$C_6H_{12}N_2O_4S_2$ 240.3 *56-89-3*

Action and use

Amino acid.

Ph Eur

DEFINITION

Cystine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 3,3′-disulfanediylbis[(2*R*)-2-aminopropanoic acid], calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water and in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cystine CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 g carefully add 1 ml of *strong hydrogen peroxide solution R* and 0.1 ml of *ferric chloride solution R1*. Allow to cool. Add 1 ml of *dilute hydrochloric acid R* and 5 ml of *water R*. Add 1 ml of *barium chloride solution R1*. Turbidity or a white precipitate develops within 3 min.

TESTS

Appearance of solution

Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 10 ml with the same acid. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 0.50 g in *1 M hydrochloric acid* and dilute to 25.0 ml with the same acid. The specific optical rotation is - 218 to - 224, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R.*

Test solution (a) Dissolve 0.10 g of the substance to be examined in *1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *cystine CRS* in 1 ml of *1 M hydrochloric acid* and dilute to 50 ml with *water R.*

Reference solution (b). Dilute 2 ml of test solution (b) to 20 ml with *water R.*

Reference solution (c) Dissolve 10 mg of *cystine CRS* and 10 mg of *arginine hydrochloride CRS* in 1 ml of *1 M hydrochloric acid* and dilute to 25 ml with *water R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of *concentrated ammonia R* and 70 volumes of *2-propanol R*. Allow the plate to dry in air. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

Dissolve 0.25 g in 5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution, without further addition of nitric acid, complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dissolve 0.5 g in 5 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

0.10 g complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.2 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals ***(2.4.8)***

2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

In a flask with a ground-glass stopper, dissolve 0.100 g in a mixture of 2 ml of *dilute sodium hydroxide solution R* and 10 ml of *water R.* Add 10 ml of a 200 g/l solution of *potassium bromide R*, 50.0 ml of *0.0167 M potassium bromate* and 15 ml of *dilute hydrochloric acid R*. Stopper the flask and cool in iced water. Allow to stand in the dark for 10 min. Add 1.5 g of *potassium iodide R*. After 1 min, titrate with *0.1 M sodium thiosulphate*, using 2 ml of *starch solution R*, added towards the end-point, as indicator. Carry out a blank titration.

1 ml of *0.0167 M potassium bromate* is equivalent to 2.403 mg of $C_6H_{12}N_2O_4S_2$.

STORAGE

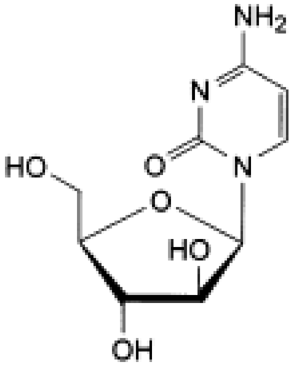
Store protected from light.

Ph Eur

Cytarabine

General Notices

(Ph Eur monograph 0760)



$C_9H_{13}N_3O_5$ 243.2 *147-94-4*

Action and use

Pyrimidine analogue, cytotoxic.

Preparation

Cytarabine Injection

Ph Eur

DEFINITION

Cytarabine contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of 4-amino-1-β-D-arabinofuranosylpyrimidin-2(1*H*)-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water, very slightly soluble in alcohol and in methylene chloride.

It melts at about 215 °C.

IDENTIFICATION

A. Dissolve 20.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 281 nm. The specific absorbance at the maximum is 540 to 570.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *cytarabine CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

Dissolve 1.0 g in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 154 to + 160, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.25 g of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 2 ml of test solution (a) to 50 ml with *water R.*

Reference solution (a) Dissolve 10 mg of *cytarabine CRS* in *water R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100 ml with *water R*.

Reference solution (c) Dissolve 20 mg of *uridine R* and 20 mg of *uracil arabinoside CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 15 volumes of *water R*, 20 volumes of *acetone R* and 65 volumes of *methyl ethyl ketone R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 0.250 g by drying over *diphosphorus pentoxide R* at 60 °C at a pressure of 0.2 kPa to 0.7 kPa for 3 h.

Sulphated ash *(2.4.14)*

Not more than 0.5 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 60 ml of *anhydrous acetic acid R*, warming if necessary. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

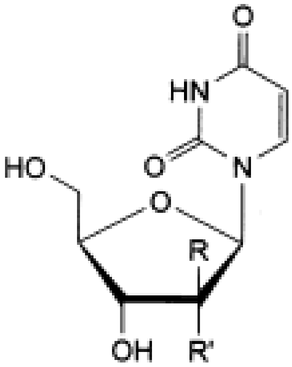
---

1 ml of *0.1 M perchloric acid* is equivalent to 24.32 mg of $C_9H_{13}N_3O_5$.

STORAGE

Store in an airtight container , protected from light.

IMPURITIES



A. R = OH, R′ = H: 1-β-D-arabinofuranosylpyrimidine-2,4(1*H*,3*H*)-dione (uracil arabonoside),

B. R = H, R′ = OH: 1-β-D-ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione (uridine).

Ph Eur

Dispersible Cellulose

General Notices

Action and use

Pharmaceutical excipient.

DEFINITION

Dispersible Cellulose is a colloid-forming, attrited mixture of Microcrystalline Cellulose and Carmellose Sodium.

Content of carmellose sodium

75.0 to 125.0% w/w of the stated amount.

CHARACTERISTICS

A white or off-white, coarse or fine powder.

Disperses in *water* producing a white, opaque dispersion or gel; practically insoluble in organic solvents and in dilute acids.

IDENTIFICATION

A. Mix 6 g with 300 ml of *water* stirring at 18,000 revolutions per minute for 5 minutes. A white, opaque dispersion is obtained which does not produce a supernatant liquid.

B. Add several drops of the dispersion obtained in test A to a 10% w/v solution of *aluminium chloride*. Each drop forms a white, opaque globule which does not disperse on standing.

C. Add 2 ml of *iodinated potassium iodide solution* to the dispersion obtained in test A. No blue or purplish colour is produced.

D. The solution obtained in the test for Heavy metals yields the reactions characteristic of *sodium salts* , Appendix VI, except that in test A the white precipitate produced may not be dense.

TESTS

Acidity or alkalinity

pH of the dispersion obtained in the test for Apparent viscosity, 6.0 to 8.0, Appendix V L.

Solubility

Add 50 mg to 10 ml of *ammoniacal solution of copper tetrammine* and shake. It dissolves completely leaving no residue.

Apparent viscosity

60 to 140% of the declared value when determined by the following method. Calculate the quantity (*x* g) needed to prepare exactly 600 g of a dispersion of the stated percentage w/w, with reference to the dried substance. To (600-*x*) g of *water* at 23° to 25° contained in a 1000 ml high-speed blender bowl add *x* g of the substance being examined, stirring at reduced speed, taking care to avoid contacting the sides of the bowl with the powder. Continue stirring at low speed for 15 seconds after the addition and then stir at 18,000 revolutions per minute for exactly 2 minutes. Immerse the appropriate spindle of a rotational viscometer, switch on after 30 seconds and after a further 30 seconds determine the *viscosity*, Appendix V H, Method III, using a speed of 20 revolutions per minute (2.09 radians per second).

Heavy metals

To the residue obtained in the test for Sulphated ash add 1 ml of *hydrochloric acid*, evaporate to dryness on a water bath and dissolve the residue in 20 ml of *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm) Pb* to prepare the standard (10 ppm).

Loss on drying

When dried to constant weight at 105°, loses not more than 8.0% of its weight. Use 1 g.

Sulphated ash

Not more than 5.0%, Appendix IX A. Use 2 g.

ASSAY

Heat 2 g with 75 ml of *anhydrous acetic acid* under a reflux condenser for 2 hours, cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 29.6 mg of carmellose sodium.

STORAGE

Dispersible Cellulose should be stored at a temperature of 8° to 15°.

LABELLING

The label states (1) the percentage w/w of Carmellose Sodium; (2) the viscosity of a dispersion in water of a stated percentage w/w of Carmellose Sodium.

Dried Calcium Sulphate

General Notices

Exsiccated Calcium Sulphate; Plaster of Paris

$CaSO_4,½H_2O$ 145.1 *26499-65-0*

DEFINITION

Dried Calcium Sulphate is prepared by heating powdered gypsum, $CaSO_4,2H_2O$, at about 150° in a controlled manner such that it is substantially converted into the hemihydrate, $CaSO_4,½H_2O$, with minimum production of the anhydrous phases of calcium sulphate. It may contain suitable setting accelerators or decelerators.

CHARACTERISTICS

A white or almost white powder; hygroscopic.

Slightly soluble in *water*; more soluble in dilute mineral acids; practically insoluble in *ethanol (96%)* .

IDENTIFICATION

Yields the reactions characteristic of *calcium salts* and of *sulphates* , Appendix VI.

TESTS

Setting properties

20 g mixed with 10 ml of *water* at 15° to 20° in a cylindrical mould about 2.4 cm in diameter sets in 4 to 11 minutes. The mass thus produced, after standing for 3 hours, possesses sufficient hardness to resist pressure of the fingers at the edges, which retain their sharpness of outline and do not crumble.

Loss on ignition

When ignited to constant weight at red heat, loses 4.5% to 8.0% of its weight. Use 1 g.

Emulsifying Cetostearyl Alcohol (Type A)

General Notices

(Ph Eur monograph 0801)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of cetostearyl alcohol and sodium cetostearyl sulphate. A suitable buffer may be added.

Content:

— *cetostearyl alcohol*: minimum 80.0 per cent (anhydrous substance);

— *sodium cetostearyl sulphate*: minimum 7.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or pale yellow, waxy mass, plates, flakes or granules.

Solubility

Soluble in hot water giving an opalescent solution, practically insoluble in cold water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, C, D.*

Second identification *A, C.*

A. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.1 g of the substance to be examined in 10 ml of *trimethylpentane R*, heating on a water-bath. Shake with 2 ml of *ethanol (70 per cent V/V) R* and allow to separate. Use the lower layer as test solution (b). Dilute 1 ml of the upper layer to 8 ml with *trimethylpentane R*.

Test solution (b) Use the lower layer obtained in the preparation of test solution (a).

Reference solution (a) Dissolve 24 mg of *cetyl alcohol CRS* and 16 mg of *stearyl alcohol CRS* in 10 ml of *trimethylpentane R*.

Reference solution (b) Dissolve 20 mg of *sodium cetostearyl sulphate R* in 10 ml of *ethanol (70 per cent V/V) R*, heating on a water-bath.

Plate *TLC silanised silica gel plate R.*

Mobile phase *water R, acetone R, methanol R* (20:40:40 *V/V/V*).

Application 2 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with a 50 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R*; heat at 120 °C until spots appear (about 3 h).

Results:

— the 2 principal spots in the chromatogram obtained with test solution (a) are similar in position and colour to the principal spots in the chromatogram obtained with reference solution (a);

— 2 of the spots in the chromatogram obtained with test solution (b) are similar in position and colour to the principal spots in the chromatogram obtained with reference solution (b).

B. Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with test solution (b) are similar in retention time to the 2 principal peaks in the chromatogram obtained with the reference solution.

C. It gives a yellow colour to a non-luminous flame.

D. To 0.3 g add 20 ml of *anhydrous ethanol R* and heat to boiling on a water-bath with shaking. Filter the mixture immediately, evaporate to dryness and take up the residue in 7 ml of *water R*. To 1 ml of the solution add 0.1 ml of a 1 g/l solution of *methylene blue R*, 2 ml of *dilute sulphuric acid R* and 2 ml of *methylene chloride R* and shake. A blue colour develops in the lower layer.

TESTS

Acid value *(2.5.1)*

Maximum 2.0.

Iodine value *(2.5.4, Method A)*

Maximum 3.0.

Dissolve 2.00 g in 25 ml of *methylene chloride R*.

Saponification value *(2.5.6)*

Maximum 2.0.

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 2.50 g.

ASSAY

Cetostearyl alcohol

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.60 g of *heptadecanol CRS* in *anhydrous ethanol R* and dilute to 150 ml with the same solvent.

Test solution (a) Dissolve 0.300 g of the substance to be examined in 50 ml of the internal standard solution, add 50 ml of *water R* and shake with 4 quantities, each of 25 ml, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the organic layers. Wash with 2 quantities, each of 30 ml, of *water R*, dry over *anhydrous sodium sulphate R* and filter.

Test solution (b) Dissolve 0.300 g of the substance to be examined in 50 ml of *anhydrous ethanol R*, add 50 ml of *water R* and shake with 4 quantities, each of 25 ml, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the organic layers. Wash with 2 quantities, each of 30 ml, of *water R*, dry over *anhydrous sodium sulphate R* and filter.

Reference solution Dissolve 50 mg of *cetyl alcohol CRS* and 50 mg of *stearyl alcohol CRS* in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

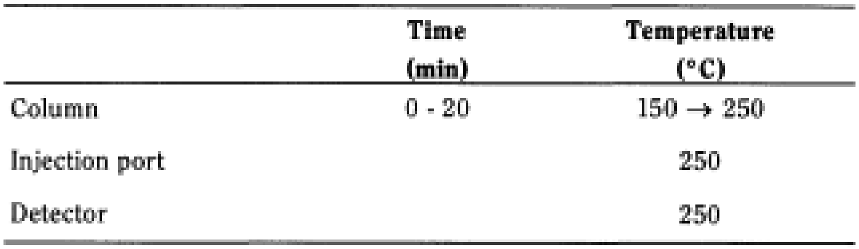
Column:
- — *material*: fused silica;
- — *size: l* = 25 m, Ø = 0.25 mm;
- — *stationary phase: poly(dimethyl)siloxane R*.

Carrier gas *nitrogen for chromatography R.*

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 20	150 → 250
Injection port		250
Detector		250

Detection Flame ionisation.

Elution order Cetyl alcohol, heptadecanol, stearyl alcohol.

Inject 1 µl of test solution (a) and 1 µl of test solution (b). If the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak due to the internal standard in the chromatogram obtained with test solution (a), calculate the ratio *r* using the following expression:

$$\frac{S_{ci}}{S_i}$$

S_{ci} = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (b);

S_i = area of the peak with the same retention time as the peak due to the internal standard in the chromatogram obtained with test solution (a).

If *r* is less than 300, calculate the corrected area $S_{Ha(corr)}$ of the peak due to the internal standard in the chromatogram obtained with test solution (a) using the following expression:

$$S'_{Ha} - \frac{S_i \times S_c}{S_{ci}}$$

S'_{Ha} = area of the peak due to the internal standard in the chromatogram obtained with test solution (a);

S_c = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (a).

Inject, under the same conditions, equal volumes of the reference solution and of test solution (a). Identify the peaks in the chromatogram obtained with test solution (a) by comparing their retention times with those of the peaks in the chromatogram obtained with the reference solution and determine the area of each peak.

Calculate the percentage content of cetyl alcohol using the following expression:

$$S_A \frac{100 \times m_H}{S_{Ha(corr)} \times m}$$

S_A = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (a);

m_H = mass of the internal standard in test solution (a), in milligrams;

$S_{Ha(corr)}$ = corrected area of the peak due to the internal standard in the chromatogram obtained with test solution (a);

m = mass of the substance to be examined in test solution (a), in milligrams.

Calculate the percentage content of stearyl alcohol using the following expression:

$$S_B \frac{100 \times m_H}{S_{Ha(corr)} \times m}$$

S_B = area of the peak due to stearyl alcohol in the chromatogram obtained with test solution (a).

The percentage content of cetostearyl alcohol corresponds to the sum of the percentage content of cetyl alcohol and of stearyl alcohol.

Sodium cetostearyl sulphate

Disperse 0.300 g in 25 ml of *methylene chloride R*. Add 50 ml of *water R* and 10 ml of *dimidium bromide-sulphan blue mixed solution R*. Titrate with *0.004 M benzethonium chloride*, using sonication, heating and allowing the layers to separate before each addition, until the colour of the lower layer changes from pink to grey.

1 ml of *0.004 M benzethonium chloride* is equivalent to 1.434 mg of sodium cetostearyl sulphate.

LABELLING

The label states, where applicable, the name and concentration of any added buffer.

Ph Eur

Emulsifying Cetostearyl Alcohol (Type B)

General Notices

(Ph Eur monograph 0802)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of cetostearyl alcohol and sodium laurilsulfate. A suitable buffer may be added.

Content:

- *cetostearyl alcohol*: minimum 80.0 per cent (anhydrous substance);
- *sodium laurilsulfate*: minimum 7.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or pale yellow, waxy mass, plates, flakes or granules.

Solubility

Soluble in hot water giving an opalescent solution, practically insoluble in cold water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, C, D.*

Second identification *A, C.*

A. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.1 g of the substance to be examined in 10 ml of *trimethylpentane R*, heating on a water-bath. Shake with 2 ml of *ethanol (70 per cent V/V) R* and allow to separate. Use the lower layer as test solution (b). Dilute 1 ml of the upper layer to 8 ml with *trimethylpentane R*.

Test solution (b) Use the lower layer obtained in the preparation of test solution (a).

Reference solution (a) Dissolve 24 mg of *cetyl alcohol CRS* and 16 mg of *stearyl alcohol CRS* in 10 ml of *trimethylpentane R*.

Reference solution (b) Dissolve 20 mg of *sodium laurilsulfate CRS* in 10 ml of *ethanol (70* *per cent V/V) R*, heating on a water-bath.

Plate *TLC silanised silica gel plate R.*

Mobile phase *water R, acetone R, methanol R* (20:40:40 *V/V/V*).

Application 2 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with a 50 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R*; heat at 120 °C until spots appear (about 3 h).

Results:

— the 2 principal spots in the chromatogram obtained with test solution (a) are similar in position and colour to the principal spots in the chromatogram obtained with reference solution (a);

— 1 of the spots in the chromatogram obtained with test solution (b) is similar in position and colour to the principal spot in the chromatogram obtained with reference solution (b).

B. Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with test solution (b) are similar in retention time to the 2 principal peaks in the chromatogram obtained with the reference solution.

C. It gives a yellow colour to a non-luminous flame.

D. To 0.3 g add 20 ml of *anhydrous ethanol R* and heat to boiling on a water-bath with shaking. Filter the mixture immediately, evaporate to dryness and take up the residue in 7 ml of *water R*. To 1 ml of the solution add 0.1 ml of a 1 g/l solution of *methylene blue R*, 2 ml of *dilute sulphuric acid R* and 2 ml of *methylene chloride R* and shake. A blue colour develops in the lower layer.

TESTS

Acid value *(2.5.1)*

Maximum 2.0.

Iodine value *(2.5.4, Method A)*

Maximum 3.0.

Dissolve 2.00 g in 25 ml of *methylene chloride R*.

Saponification value *(2.5.6)*

Maximum 2.0.

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 2.50 g.

ASSAY

Cetostearyl alcohol

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.60 g of *heptadecanol CRS* in *anhydrous ethanol R* and dilute to 150 ml with the same solvent.

Test solution (a) Dissolve 0.300 g of the substance to be examined in 50 ml of the internal standard solution, add 50 ml of *water R* and shake with 4 quantities, each of 25 ml, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the organic layers. Wash with 2 quantities, each of 30 ml, of *water R*, dry over *anhydrous sodium sulphate R* and filter.

Test solution (b) Dissolve 0.300 g of the substance to be examined in 50 ml of *anhydrous ethanol R*, add 50 ml of *water R* and shake with 4 quantities, each of 25 ml, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the organic layers. Wash with 2 quantities, each of 30 ml, of *water R*, dry over *anhydrous sodium sulphate R* and filter.

Reference solution Dissolve 50 mg of *cetyl alcohol CRS* and 50 mg of *stearyl alcohol CRS* in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Column:

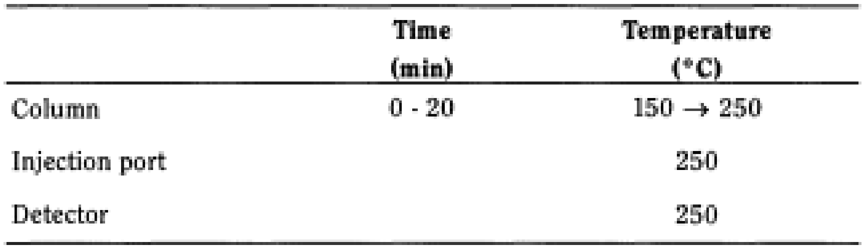
- *material*: fused silica;
- *size: l* = 25 m, Ø = 0.25 mm;
- *stationary phase: poly(dimethyl)siloxane R*.

Carrier gas *nitrogen for chromatography R.*

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 20	150 → 250
Injection port		250
Detector		250

Detection Flame ionisation.

Elution order Cetyl alcohol, heptadecanol, stearyl alcohol.

Inject 1 µl of test solution (a) and 1 µl of test solution (b). If the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak due to the internal standard in the chromatogram obtained with test solution (a), calculate the ratio *r* using the following expression:

$$\frac{S_{ci}}{S_i}$$

S_{ci} = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (b);

S_i = area of the peak with the same retention time as the peak due to the internal standard in the chromatogram obtained with test solution (a).

If r is less than 300, calculate the corrected area $S_{Ha(corr)}$ of the peak due to the internal standard in the chromatogram obtained with test solution (a) using the following expression:

$$S'_{Ha} - \frac{S_i \times S_c}{S_{ci}}$$

S'_{Ha} = area of the peak due to the internal standard in the chromatogram obtained with test solution (a);

S_c = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (a).

Inject, under the same conditions, equal volumes of the reference solution and of test solution (a). Identify the peaks in the chromatogram obtained with test solution (a) by comparing their retention times with those of the peaks in the chromatogram obtained with the reference solution and determine the area of each peak.

Calculate the percentage content of cetyl alcohol using the following expression:

$$S_A \frac{100 \times m_H}{S_{Ha(corr)} \times m}$$

S_A = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (a);

m_H = mass of the internal standard in test solution (a), in milligrams;

$S_{Ha(corr)}$ = corrected area of the peak due to the internal standard in the chromatogram obtained with test solution (a);

m = mass of the substance to be examined in test solution (a), in milligrams.

Calculate the percentage content of stearyl alcohol using the following expression:

$$S_B \frac{100 \times m_H}{S_{Ha(corr)} \times m}$$

S_B = area of the peak due to stearyl alcohol in the chromatogram obtained with test solution (a).

The percentage content of cetostearyl alcohol corresponds to the sum of the percentage content of cetyl alcohol and of stearyl alcohol.

Sodium laurilsulfate

Disperse 0.300 g in 25 ml of *methylene chloride R*. Add 50 ml of *water R* and 10 ml of *dimidium bromide-sulphan blue mixed solution R*. Titrate with *0.004 M benzethonium chloride*, using sonication, heating, and allowing the layers to separate before each addition, until the colour of the lower layer changes from pink to grey.

1 ml of *0.004 M benzethonium chloride* is equivalent to 1.154 mg of sodium laurilsulfate.

LABELLING

The label states, where applicable, the name and concentration of any added buffer.

Ph Eur

Hydrogenated Castor Oil

General Notices

(Ph Eur monograph 1497)

Action and use

Excipient.

Ph Eur

DEFINITION

Fatty oil obtained by hydrogenation of *Virgin Castor oil (0051*). It consists mainly of the triglyceride of 12-hydroxystearic (12-hydroxyoctadecanoic) acid.

CHARACTERS

Appearance

Fine, almost white or pale yellow powder or almost white or pale yellow masses or flakes.

Solubility

Practically insoluble in water, slightly soluble in methylene chloride, very slightly soluble in anhydrous ethanol, practically insoluble in light petroleum.

IDENTIFICATION

A. Melting point *(2.2.14)* : 83 °C to 88 °C.

B. Hydroxyl value (see Tests).

C. Composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 4.0, determined on 10.0 g dissolved in 75 ml of hot *ethanol (96) per cent R*.

Hydroxyl value *(2.5.3, Method A)*

145 to 165, determined on a warm solution.

Iodine value *(2.5.4, Method A)*

Maximum 5.0.

Alkaline impurities

Dissolve 1.0 g by gentle heating in a mixture of 1.5 ml of *ethanol (96) per cent R* and 3 ml of *toluene R* . Add 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96) per cent R.* Not more than 0.2 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Composition of fatty acids ***(2.4.22)***

Use the mixture of calibrating substances in Table 2.4.22.-3.

Test solution Introduce 75 mg of the substance to be examined into a 10 ml centrifuge tube with a screw cap. Dissolve in 2 ml of 1,*1-dimethylethyl methyl ether R1* by shaking and heat gently (50-60 °C). Add, when still warm, 1 ml of a 12 g/l solution of *sodium R* in *anhydrous methanol R,* prepared with the necessary precautions, and mix vigorously for at least 5 min. Add 5 ml of *distilled water R* and mix vigorously for about 30 s. Centrifuge for 15 min at 1500 g. Use the upper layer.

Reference solution Dissolve 50 mg of *methyl 12-hydroxystearate CRS* and 50 mg of *methyl stearate CRS* in 10.0 ml of 1,*1-dimethylethyl methyl ether R1.*

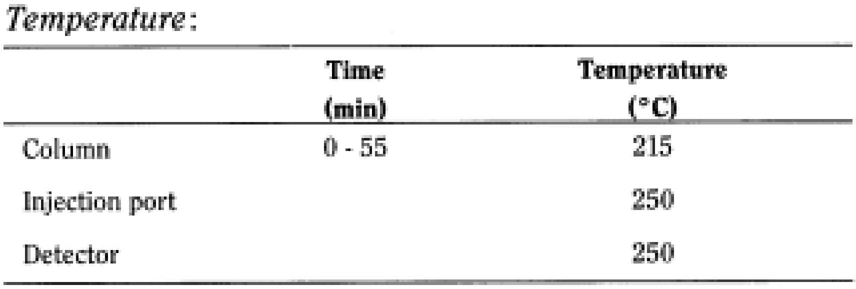
Column:

- *material*: fused silica;
- *size*: l = 30 m; Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 0.9 ml/min.

Split ratio 1:100.



Temperature:

	Time (min)	Temperature (°C)
Column	0 - 55	215
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Calculate the fraction of each fatty-acid using the following expression:

$$A_{x,s,c} / \sum A_{x,s,c} \times 100 \text{ per cent } m/m$$

$A_{x,s,c}$ = corrected peak area of the fatty acid in the test solution

$$A_{x,s,c} = A_{x,s} \times R_c$$

R_c = relative correction factor for the peak due to methyl 12-hydroxystearate

$$R_c = \frac{m_{1,r} \times A_{2,r}}{A_{1,r} \times m_{2,r}}$$

R_c = 1 for peaks corresponding to each of the other specified fatty acids or any unspecified fatty acid

$m_{1,r}$ = mass of methyl 12-hydroxystearate in the reference solution

$m_{2,r}$ = mass of methyl stearate in the reference solution

$A_{1,r}$ = area of any peak due to methyl 12-hydroxystearate in the chromatogram obtained with the reference solution

$A_{2,r}$ = area of any peak due to methyl stearate in the chromatogram obtained with the reference solution

$A_{x,s}$ = area of the peaks due to any specified or unspecified fatty acid methyl esters

Composition of the fatty acid fraction of the oil:

— *palmitic acid* : not more than 2.0 per cent;

— *stearic acid* : 7.0 per cent to 14.0 per cent;

— *arachidic acid*: not more than 1.0 per cent;

— *12-oxostearic acid*: not more than 5.0 per cent;

— *12-hydroxystearic acid* : 78.0 per cent to 91.0 per cent;

— *any other fatty acid*: not more than 3.0 per cent.

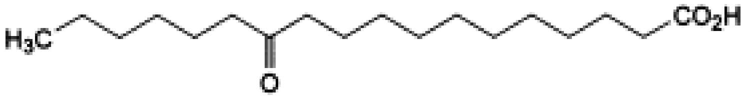
Nickel ***(2.4.31):***

Maximum 1 ppm.

STORAGE

In a well-filled container.

IMPURITIES



A. 12-oxostearic acid.

Ph Eur

Hydrogenated Cottonseed Oil

General Notices

(Ph Eur monograph 1305)

Ph Eur

DEFINITION

Product obtained by refining and hydrogenation of oil obtained from seeds of cultivated plants of various varieties of *Gossypium hirsutum* L. or of other species of *Gossypium*. The product consists mainly of triglycerides of palmitic and stearic acids.

CHARACTERS

Appearance

White or almost white mass or powder which melts to a clear, pale yellow liquid when heated.

Solubility

Practically insoluble in water, freely soluble in methylene chloride and in toluene, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Melting point (see Tests).

B. Composition of fatty acids (see Tests).

TESTS

Melting point *(2.2.14)*

57 °C to 70 °C.

Acid value *(2.5.1)*

Maximum 0.5.

Dissolve 10.0 g in 50 ml of a hot mixture of equal volumes of *ethanol (96 per cent) R* and *toluene R*, previously neutralised with *0.1 M potassium hydroxide* using 0.5 ml of *phenolphthalein solution R1* as indicator. Titrate the solution immediately while still hot.

Peroxide value *(2.5.5, Method A)*

Maximum 5.0.

Unsaponifiable matter *(2.5.7)*

Maximum 1.0 per cent, determined on 5.0 g.

Alkaline impurities

Dissolve by gentle heating 2.0 g in a mixture of 1.5 ml of *ethanol (96 per cent) R* and 3 ml of *toluene R*. Add 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96 per cent) R*. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour to yellow.

Composition of fatty acids *(2.4.22, Method A)*

Use the mixture of calibrating substances in Table 2.4.22.-3.

Column:
- *material*: fused silica;
- *size*: *l* = 25 m, Ø = 0.25 mm;
- *stationary phase*: *poly(cyanopropyl)siloxane R* (film thickness 0.2 µm).

Carrier gas *helium for chromatography R.*

Flow rate 0.65 ml/min.

Split ratio 1:100.

Temperature:
- *column*: 180 °C for 35 min;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Composition of the fatty-acid fraction of the oil:
- *saturated fatty acids of chain length less than C_{14}*: maximum 0.2 per cent;
- *myristic acid*: maximum 1.0 per cent;
- *palmitic acid*: 19.0 per cent to 26.0 per cent;
- *stearic acid*: 68.0 per cent to 80.0 per cent;
- *oleic acid and isomers*: maximum 4.0 per cent;
- *linoleic acid and isomers*: maximum 1.0 per cent;
- *arachidic acid*: maximum 1.0 per cent;
- *behenic acid*: maximum 1.0 per cent;
- *lignoceric acid*: maximum 0.5 per cent.

Nickel

Maximum 1.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Introduce 5.0 g into a platinum or silica crucible tared after ignition. Cautiously heat and introduce into the substance a wick formed from twisted ashless filter paper. Ignite the wick. When the substance ignites, stop heating. After combustion, ignite in a muffle furnace at about 600 ± 50 °C. Continue the incineration until white ash is obtained. After cooling, take up the residue with 2 quantities, each of 2 ml, of *dilute hydrochloric acid R* and transfer into a 25 ml graduated flask. Add 0.3 ml of *nitric acid R* and dilute to 25.0 ml with *distilled water R*.

Reference solutions Prepare 3 reference solutions by adding 1.0 ml, 2.0 ml and 4.0 ml of *nickel standard solution (0.2 ppm Ni) R* to 2.0 ml portions of the test solution, diluting to 10.0 ml with *distilled water R*.

Source Nickel hollow-cathode lamp.

Wavelength 232 nm.

Atomisation device Graphite furnace.

Carrier gas *argon R.*

STORAGE

Protected from light.

Ph Eur

Hydrogenated Polyoxyl Castor Oil

General Notices

(Macrogolglycerol Hydroxystearate, Ph Eur monograph 1083)

Action and use

Excipient.

Ph Eur

DEFINITION

Contains mainly trihydroxystearyl glycerol ethoxylated with 7 to 60 molecules of ethylene oxide (nominal value), with small amounts of macrogol hydroxystearate and of the corresponding free glycols. It results from the reaction of hydrogenated castor oil with ethylene oxide.

CHARACTERS

Appearance:

— if less than 10 units of ethylene oxide per molecule: yellowish, turbid, viscous liquid;

— if more than 20 units of ethylene oxide per molecule: white or yellowish semi-liquid or pasty mass.

Solubility:

— if less than 10 units of ethylene oxide per molecule: practically insoluble in water, soluble in acetone, dispersible in ethanol (96 per cent);

— if more than 20 units of ethylene oxide per molecule: freely soluble in water, in acetone and in ethanol (96 per cent), practically insoluble in light petroleum.

IDENTIFICATION

A. It complies with the test for iodine value (see Tests).

B. It complies with the test for saponification value (see Tests).

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1 g of the substance to be examined, add 100 ml of a 100 g/l solution of *potassium hydroxide R* and boil under a reflux condenser for 30 min. Allow to cool. Acidify the solution with 20 ml of *hydrochloric acid R.* Shake the mixture with 50 ml of *ether R* and allow to stand until separation of the layers is obtained. Transfer the clear upper layer to a suitable tube, add 5 g of *anhydrous sodium sulphate R*, close the tube and allow to stand for 30 min. Filter and evaporate the filtrate to dryness on a water-bath. Dissolve 50 mg of the residue in 25 ml of *ether R.*

Reference solution Dissolve 50 mg of *12-hydroxystearic acid R* in *methylene chloride R* and dilute to 25 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *methylene chloride R, glacial acetic acid R, acetone R* (10:40:50 *V/V/V*).

Application 2 µl.

Development Over a path of 8 cm.

Drying In a current of cold air.

Detection Spray with a 80 g/l solution of *phosphomolybdic acid R* in *2-propanol R* and heat at 120 °C for about 1-2 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with the reference solution.

D. Place about 2 g in a test-tube and add 0.2 ml of *sulphuric acid R*. Close the tube using a stopper fitted with a glass tube bent twice at right angles. Heat the tube until white fumes appear. Collect the fumes in 1 ml of *mercuric chloride solution R*. A white precipitate is formed and the fumes turn a filter paper impregnated with alkaline *potassium tetraiodomercurate solution R* black.

TESTS

Solution S

Dissolve 5.0 g of macrogolglycerol hydroxystearate with less than 40 units of ethylene oxide per molecule in a mixture of 50 volumes of *acetone R* and 50 volumes of *anhydrous ethanol R* and dilute to 50 ml with the same mixture of solvents.

Dissolve 5.0 g of macrogolglycerol hydroxystearate with 40 units or more of ethylene oxide per molecule in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Alkalinity

To 2 ml of solution S add 0.5 ml of *bromothymol blue solution R1*. The solution is not blue.

Acid value *(2.5.1)*

Maximum 2.0, determined on 5.0 g.

Hydroxyl value *(2.5.3, Method A)*

See Table 1083.-1.

Iodine value ***(2.5.4)***

Maximum 5.0.

Saponification value ***(2.5.6)***

See Table 1083.-1.

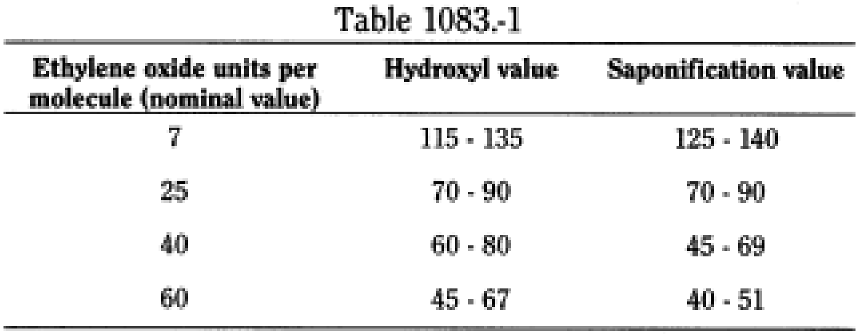


Table 1083.-1

Ethylene oxide units per molecule (nominal value)	Hydroxyl value	Saponification value
7	115 - 135	125 - 140
25	70 - 90	70 - 90
40	60 - 80	45 - 69
60	45 - 67	40 - 51

Residual ethylene oxide and dioxan ***(2.4.25)***

Maximum 1 ppm of residual ethylene oxide and 10 ppm of residual dioxan.

Heavy metals ***(2.4.8)*** **.**

Substances soluble in acetone/anhydrous ethanol Maximum 10 ppm.

12 ml of solution S complies with limit test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of equal volumes of *acetone R* and *anhydrous ethanol R* .

Substances soluble in water Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water ***(2.5.12*****)**

Maximum 3.0 per cent, determined on 2.000 g.

Total ash ***(2.4.16)***

Maximum 0.3 per cent, determined on 2.0 g.

LABELLING

The label states the number of ethylene oxide units per molecule (nominal value).

Ph Eur

Low-substituted Carmellose Sodium

General Notices

(Ph Eur monograph 1186)

9050-32-4

Action and use

Excipient in pharmaceutical products; bulk laxative.

Ph Eur

DEFINITION

Low-substituted sodium carboxymethylcellulose. Sodium salt of a partly *O*-(carboxymethylated) cellulose.

Content

2.0 per cent to 4.5 per cent of sodium (Na) (dried substance).

CHARACTERS

Appearance

White or almost white powder or short fibres.

Solubility

Practically insoluble in acetone, in anhydrous ethanol and in toluene. It swells in water to form a gel.

IDENTIFICATION

A. Shake 1 g with 100 ml of a 100 g/l solution of *sodium hydroxide R* . A suspension is produced.

B. Shake 1 g with 50 ml of *water R* . Transfer 1 ml of the mixture to a test tube, add 1 ml of *water R* and 0.05 ml of a freshly prepared 40 g/l solution of *α-naphthol R* in *methanol R* . Incline the test tube and add carefully 2 ml of *sulphuric acid R* down the side so that it forms a lower layer. A reddish-purple colour develops at the interface.

C. Sulphated ash *(2.4.14)* (see Tests).

D. The solution prepared for the test for heavy metals gives reaction (a) of sodium *(2.3.1)* .

TESTS

pH *(2.2.3)*

6.0 to 8.5.

Shake 1 g with 100 ml of *carbon dioxide-free water R* for 5 min. Centrifuge.

Sodium chloride and sodium glycollate

Maximum 0.5 per cent (dried substance) for the sum of the percentage contents.

Sodium chloride Place 5.00 g in a 250 ml conical flask, add 50 ml of *water R* and 5 ml of *strong hydrogen peroxide solution R* and heat on a water bath for 20 min, stirring occasionally to ensure total hydration. Cool, add 100 ml of *water R* and 10 ml of *nitric acid R* . Titrate with *0.05 M silver nitrate* determining the end-point potentiometrically *(2.2.20)* using a silver-based indicator electrode and a double-junction reference electrode containing a 100 g/l solution of *potassium nitrate R* in the outer jacket and a standard filling solution in the inner jacket.

1 ml of *0.05 M silver nitrate* is equivalent to 2.922 mg of NaCl.

Sodium glycollate Place a quantity of the substance to be examined equivalent to 0.500 g of the dried substance in a beaker. Add 5 ml of *glacial acetic acid R* and 5 ml of *water R* and stir to ensure total hydration (about 30 min). Add 80 ml of *acetone R* and 2 g of *sodium chloride R* . Stir for several minutes to ensure complete precipitation of the carboxymethylcellulose. Filter through a fast filter paper impregnated with *acetone R* into a volumetric flask, rinse the beaker and filter with *acetone R* and dilute the filtrate to 100.0 ml with the same solvent. Allow to stand for 24 h without shaking. Use the clear supernatant as the test solution.

Prepare the reference solutions as follows: in a 100 ml volumetric flask, dissolve 0.100 g of *glycollic acid R*, previously dried *in vacuo* over *diphosphorus pentoxide R* , in *water R* and dilute to 100.0 ml with the same solvent. Transfer 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml of the solution to separate volumetric flasks; dilute the contents of each flask to 5.0 ml with *water R,* add 5 ml of *glacial acetic acid R* , dilute to 100.0 ml with *acetone R* and mix.

Transfer 2.0 ml of the test solution and 2.0 ml of each of the reference solutions to separate 25 ml volumetric flasks. Heat the uncovered flasks in a water-bath to eliminate the acetone. Allow to cool and add 5.0 ml of *2,7-dihydroxynaphthalene solution R* to each flask. Mix, add a further 15.0 ml of *2,7-dihydroxynaphthalene solution R* and mix again. Close the flasks with aluminium foil and heat in a water-bath for 20 min. Cool and dilute to 25.0 ml with *sulphuric acid R* .

Measure the absorbance *(2.2.25)* of each solution at 540 nm. Prepare a blank using 2.0 ml of a solution containing 5 per cent *V/V* each of *glacial acetic acid R* and *water R* in *acetone R.* Prepare a standard curve using the absorbances obtained with the reference solutions. From the standard curve and the absorbance of the test solution, determine the mass a, in milligrams, of glycollic acid in the substance to be examined and calculate the content of sodium glycollate from the following expression:

$$\frac{10 \times 1.29 \times a}{(100 - b)\, m}$$

1.29 = the factor converting glycollic acid to sodium glycollate

b = the loss on drying as a percentage

m = the mass of the substance to be examined, in grams

Water-soluble substances

Maximum 70.0 per cent.

Disperse 5.00 g in 400.0 ml of *water R* and stir for 1 min every 10 min during the first 30 min. Allow to stand for 1 h and centrifuge, if necessary. Decant 100.0 ml of the supernatant liquid onto a fast filter paper in a vacuum filtration funnel, apply vacuum and collect 75.0 ml of the filtrate. Evaporate to dryness and dry the residue at 100-105 °C for 4 h.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

To the residue obtained in the determination of the sulphated ash add 1 ml of *hydrochloric acid R* and evaporate on a water-bath. Take up the residue in 20 ml of *water R (*this solution is used for identification test D). 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R* .

Loss on drying ***(2.2.32)***

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

6.5 per cent to 13.5 per cent (dried substance), corresponding to a content of 2.0 per cent to 4.5 per cent of Na.

Use 1.0 g with a mixture of equal volumes of *sulphuric acid R* and *water R* .

FUNCTIONALITY-RELATED CHARCTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for low-substituted carmellose sodium used as disintegrant.

Settling volume

15.0 ml to 35.0 ml.

In a 100 ml graduated cylinder, place 20 ml of *2-propanol R*, add 5.0 g of the substance to be examined and shake vigorously. Dilute to 30 ml with *2-propanol R* then to 50 ml with *water R* and shake vigorously. Within 15 min, repeat the shaking 3 times. Allow to stand for 4 h and determine the volume of the settled mass.

Ph Eur

Microcrystalline Cellulose and Carmellose Sodium

General Notices

(Ph Eur monograph 2050)

Action and use

Excipient.

Ph Eur

DEFINITION

Colloid-forming, powdered mixture of *Microcrystalline Cellulose (0316)* with 5 per cent to 22 per cent of *Carmellose sodium (0472)*.

Content

75.0 per cent to 125.0 per cent of the nominal amount of carmellose sodium (dried substance).

CHARACTERS

Appearance

White or off-white, coarse or fine powder.

Solubility

Dispersible in water producing a white, opaque colloidal dispersion; practically insoluble in organic solvents and in dilute acids.

IDENTIFICATION

A. Mix 6 g with 300 ml of *water R* and stir at 18 000 r/min for 5 min. A white opaque dispersion is obtained which does not produce a supernatant liquid.

B. Add several drops of the dispersion obtained in identification A to a 10 per cent *V/V* solution of *aluminium chloride R*. Each drop forms a white, opaque globule which does not disperse on standing.

C. Add 2 ml of *iodinated potassium iodide solution R* to the dispersion obtained in test A. No blue or purplish colour is produced.

D. It complies with the limits of the assay.

TESTS

Solubility

Add 50 mg to 10 ml of *ammoniacal solution of copper tetrammine R* and shake. It dissolves completely leaving no residue.

pH *(2.2.3)*

6.0 to 8.0 for the dispersion obtained in identification A.

Loss on drying *(2.2.32)*

Maximum 8.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 7.4 per cent, determined on 2.0 g.

ASSAY

Heat 2.00 g with 75 ml of *anhydrous acetic acid R* under a reflux condenser for 2 h, cool and titrate with *0.1 M perchloric acid*, determining the end point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 29.6 mg of carmellose sodium.

LABELLING

The label states the nominal percentage *m/m* of carmellose sodium.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for microcrystalline cellulose and carmellose sodium used as a suspending agent.

Apparent viscosity *(2.2.10)*

60 per cent to 140 per cent of the nominal value.

Calculate the quantity (x g) needed to prepare exactly 600 g of a dispersion of the stated percentage *m/m* (dried substance). To (600 - x) g of *water R* at 23-25 °C contained in a 1000 ml high-speed blender bowl add x g of the substance to be examined and stir at reduced speed, taking care to avoid contacting the sides of the bowl with the powder. Continue stirring at low speed for 15 s after the addition of the powder and then stir at 18 000 r/min for exactly 2 min.

Determine the viscosity with a suitable relative rotational viscometer under the following conditions:

- — spindle: as appropriate;
- — speed: 20 r/min.

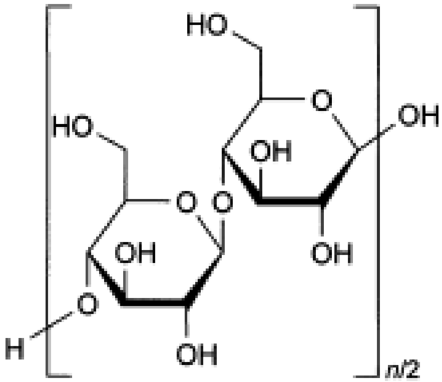
Immerse the spindle into the suspension immediately after preparation, switch on the rotation spindle after 30 s, after a further 30 s take scale readings and calculate the viscosity according to the viscometer manual.

Ph Eur

Microcrystalline Cellulose

General Notices

(Ph Eur monograph 0316)



$C_{6n}H_{10n+2}O_{5n+1}$ *9004-34-6*

Action and use

Excipient.

Ph Eur

DEFINITION

Purified, partly depolymerised cellulose prepared by treating alpha-cellulose, obtained as a pulp from fibrous plant material, with mineral acids.

CHARACTERS

Appearance

White or almost white, fine or granular powder.

Solubility

Practically insoluble in water, in acetone, in anhydrous ethanol, in toluene, in dilute acids and in a 50 g/l solution of sodium hydroxide.

IDENTIFICATION

A. Place about 10 mg on a watch-glass and disperse in 2 ml of *iodinated zinc chloride solution R*. The substance becomes violet-blue.

B. The degree of polymerisation is not more than 350.

Transfer 1.300 g to a 125 ml conical flask. Add 25.0 ml of *water R* and 25.0 ml of *cupriethylenediamine hydroxide solution R*. Immediately purge the solution with *nitrogen R*, insert the stopper and shake until completely dissolved. Transfer an appropriate volume of the solution to a suitable capillary viscometer *(2.2.9)*. Equilibrate the solution at 25 ± 0.1 °C for at least 5 min. Record the flow time (t_1) in seconds between the 2 marks on the viscometer. Calculate the kinematic viscosity (ν_1) of the solution using the following expression:

$$t_1 (k_1)$$

where k_1 is the viscometer constant.

Dilute a suitable volume of *cupriethylenediamine hydroxide solution R* with an equal volume of *water R* and measure the flow time (t_2) using a suitable capillary viscometer. Calculate the kinematic viscosity (ν_2) of the solvent using the following expression:

$$t_2 (k_2)$$

where k_2 is the viscometer constant.

Determine the relative viscosity (η_{rel}) of the substance to be examined using the following expression:

$$v_1 / v_2$$

Determine the intrinsic viscosity ($[\eta]_c$) by interpolation, using the intrinsic viscosity table (Table 0316.-1).

Calculate the degree of polymerisation (P) using the following expression:

$$\frac{95\,[\eta]_c}{m\,[(100 - b)/100]}$$

where m is the mass in grams of the substance to be examined and b is the loss on drying as a percentage.

TESTS

Solubility

Dissolve 50 mg in 10 ml of *ammoniacal solution of copper tetrammine R*. It dissolves completely, leaving no residue.

pH *(2.2.3)*

5.0 to 7.5 for the supernatant liquid.

Shake 5 g with 40 ml of *carbon dioxide-free water R* for 20 min and centrifuge.

Conductivity *(2.2.38)*

The conductivity of the test solution does not exceed the conductivity of the water by more than 75 µS·cm^{-1}.

Use as test solution the supernatant liquid obtained in the test for pH. Measure the conductivity of the supernatant liquid after a stable reading has been obtained and measure the conductivity of the water used to prepare the test solution.

Ether-soluble substances

Maximum 0.05 per cent (5 mg) for the difference between the weight of the residue and the weight obtained from a blank determination.

Place 10.0 g in a chromatography column about 20 mm in internal diameter and pass 50 ml of *peroxide-free ether R* through the column. Evaporate the eluate to dryness. Dry the residue at 105 °C for 30 min, allow to cool in a dessicator and weigh. Carry out a blank determination.

Water-soluble substances

Maximum 0.25 per cent (12.5 mg) for the difference between the mass of the residue and the mass obtained from a blank determination.

Shake 5.0 g with 80 ml of *water R* for 10 min. Filter through a filter paper with the aid of vacuum into a tared flask. Evaporate to dryness on a water-bath avoiding charring. Dry at 105 °C for 1 h and weigh. Carry out a blank determination.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R* .

Loss on drying *(2.2.32)*

Maximum 7.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram and with a limit for fungi of 10^2 per gram, determined by plate count. It complies with the tests for *Escherichia coli* , for *Pseudomonas aeruginosa* , for *Staphylococcus aureus* and for

Salmonella (2.6.13) .

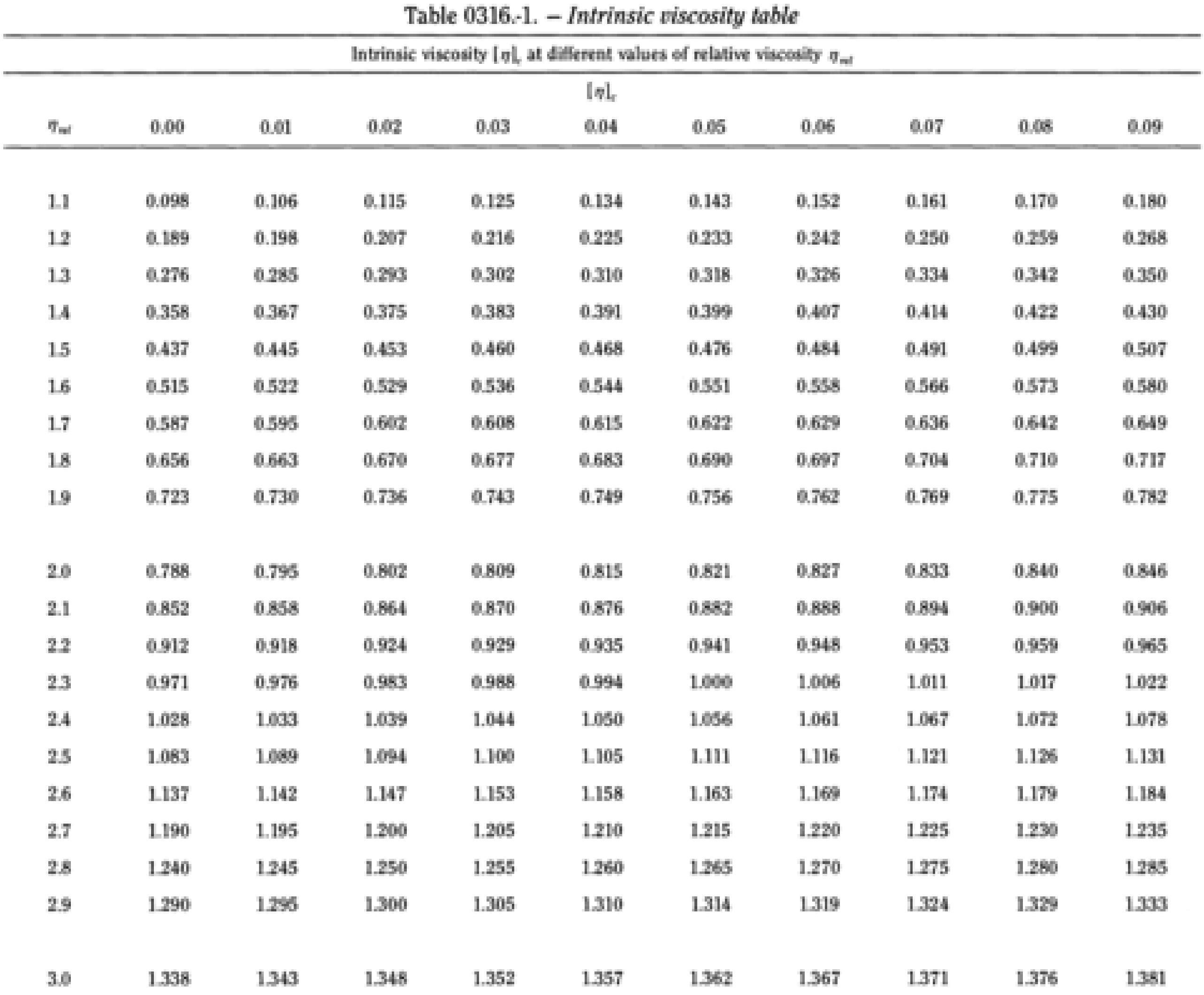


Table 0316.-1. – *Intrinsic viscosity table*

Intrinsic viscosity $[\eta]_c$ at different values of relative viscosity η_{rel}

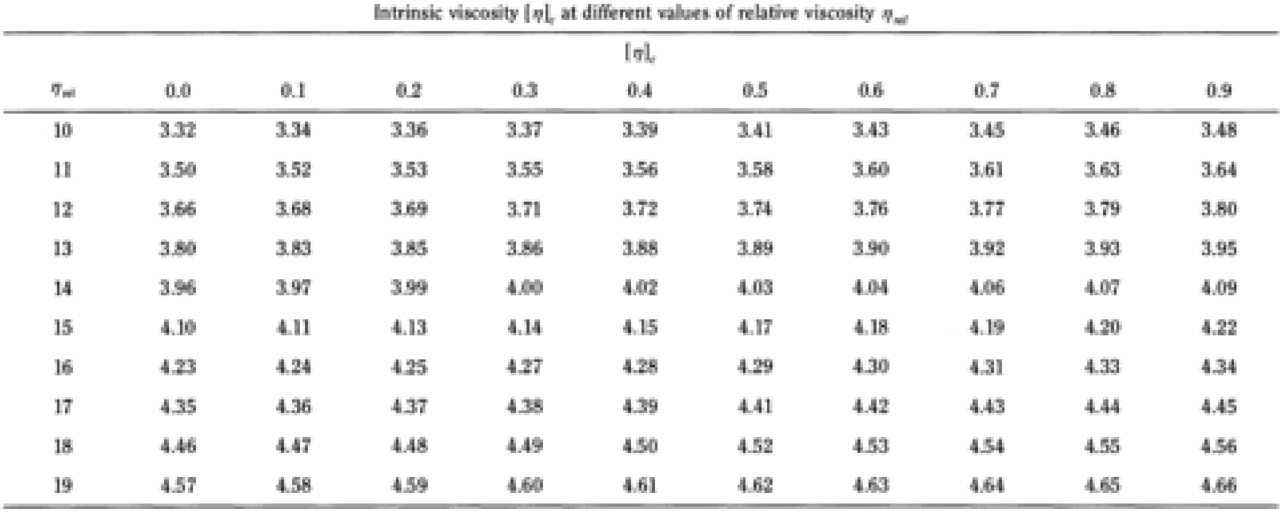
	$[\eta]_c$									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
1.1	0.098	0.106	0.115	0.125	0.134	0.143	0.152	0.161	0.170	0.180
1.2	0.189	0.198	0.207	0.216	0.225	0.233	0.242	0.250	0.259	0.268
1.3	0.276	0.285	0.293	0.302	0.310	0.318	0.326	0.334	0.342	0.350
1.4	0.358	0.367	0.375	0.383	0.391	0.399	0.407	0.414	0.422	0.430
1.5	0.437	0.445	0.453	0.460	0.468	0.476	0.484	0.491	0.499	0.507
1.6	0.515	0.522	0.529	0.536	0.544	0.551	0.558	0.566	0.573	0.580
1.7	0.587	0.595	0.602	0.608	0.615	0.622	0.629	0.636	0.642	0.649
1.8	0.656	0.663	0.670	0.677	0.683	0.690	0.697	0.704	0.710	0.717
1.9	0.723	0.730	0.736	0.743	0.749	0.756	0.762	0.769	0.775	0.782
2.0	0.788	0.795	0.802	0.809	0.815	0.821	0.827	0.833	0.840	0.846
2.1	0.852	0.858	0.864	0.870	0.876	0.882	0.888	0.894	0.900	0.906
2.2	0.912	0.918	0.924	0.929	0.935	0.941	0.948	0.953	0.959	0.965
2.3	0.971	0.976	0.983	0.988	0.994	1.000	1.006	1.011	1.017	1.022
2.4	1.028	1.033	1.039	1.044	1.050	1.056	1.061	1.067	1.072	1.078
2.5	1.083	1.089	1.094	1.100	1.105	1.111	1.116	1.121	1.126	1.131
2.6	1.137	1.142	1.147	1.153	1.158	1.163	1.169	1.174	1.179	1.184
2.7	1.190	1.195	1.200	1.205	1.210	1.215	1.220	1.225	1.230	1.235
2.8	1.240	1.245	1.250	1.255	1.260	1.265	1.270	1.275	1.280	1.285
2.9	1.290	1.295	1.300	1.305	1.310	1.314	1.319	1.324	1.329	1.333
3.0	1.338	1.343	1.348	1.352	1.357	1.362	1.367	1.371	1.376	1.381

Intrinsic viscosity $[\eta]_c$ at different values of relative viscosity η_{rel}

					$[\eta]_c$					
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
3.1	1.386	1.390	1.395	1.400	1.405	1.409	1.414	1.418	1.423	1.427
3.2	1.432	1.436	1.441	1.446	1.450	1.455	1.459	1.464	1.468	1.473
3.3	1.477	1.482	1.486	1.491	1.496	1.500	1.504	1.508	1.513	1.517
3.4	1.521	1.525	1.529	1.533	1.537	1.542	1.546	1.550	1.554	1.558
3.5	1.562	1.566	1.570	1.575	1.579	1.583	1.587	1.591	1.595	1.600
3.6	1.604	1.608	1.612	1.617	1.621	1.625	1.629	1.633	1.637	1.642
3.7	1.646	1.650	1.654	1.658	1.662	1.666	1.671	1.675	1.679	1.683
3.8	1.687	1.691	1.695	1.700	1.704	1.708	1.712	1.715	1.719	1.723
3.9	1.727	1.731	1.735	1.739	1.742	1.746	1.750	1.754	1.758	1.762
4.0	1.765	1.769	1.773	1.777	1.781	1.785	1.789	1.792	1.796	1.800
4.1	1.804	1.808	1.811	1.815	1.819	1.822	1.826	1.830	1.833	1.837
4.2	1.841	1.845	1.848	1.852	1.856	1.859	1.863	1.867	1.870	1.874
4.3	1.878	1.882	1.885	1.889	1.893	1.896	1.900	1.904	1.907	1.911
4.4	1.914	1.918	1.921	1.925	1.929	1.932	1.936	1.939	1.943	1.946
4.5	1.950	1.954	1.957	1.961	1.964	1.968	1.971	1.975	1.979	1.982
4.6	1.986	1.989	1.993	1.996	2.000	2.003	2.007	2.010	2.013	2.017
4.7	2.020	2.023	2.027	2.030	2.033	2.037	2.040	2.043	2.047	2.050
4.8	2.053	2.057	2.060	2.063	2.067	2.070	2.073	2.077	2.080	2.083
4.9	2.087	2.090	2.093	2.097	2.100	2.103	2.107	2.110	2.113	2.116
5.0	2.119	2.122	2.125	2.129	2.132	2.135	2.139	2.142	2.145	2.148
5.1	2.151	2.154	2.158	2.160	2.164	2.167	2.170	2.173	2.176	2.180
5.2	2.183	2.186	2.190	2.192	2.195	2.197	2.200	2.203	2.206	2.209
5.3	2.212	2.215	2.218	2.221	2.224	2.227	2.230	2.233	2.236	2.240
5.4	2.243	2.246	2.249	2.252	2.255	2.258	2.261	2.264	2.267	2.270
5.5	2.273	2.276	2.279	2.282	2.285	2.288	2.291	2.294	2.297	2.300
5.6	2.303	2.306	2.309	2.312	2.315	2.318	2.320	2.324	2.326	2.329
5.7	2.332	2.335	2.338	2.341	2.344	2.347	2.350	2.353	2.355	2.358
5.8	2.361	2.364	2.367	2.370	2.373	2.376	2.379	2.382	2.384	2.387
5.9	2.390	2.393	2.396	2.400	2.403	2.405	2.408	2.411	2.414	2.417
6.0	2.419	2.422	2.425	2.428	2.431	2.433	2.436	2.439	2.442	2.444
6.1	2.447	2.450	2.453	2.456	2.458	2.461	2.464	2.467	2.470	2.472
6.2	2.475	2.478	2.481	2.483	2.486	2.489	2.492	2.494	2.497	2.500
6.3	2.503	2.505	2.508	2.511	2.513	2.516	2.518	2.521	2.524	2.526
6.4	2.529	2.532	2.534	2.537	2.540	2.542	2.545	2.547	2.550	2.553
6.5	2.555	2.558	2.561	2.563	2.566	2.568	2.571	2.574	2.576	2.579
6.6	2.581	2.584	2.587	2.590	2.592	2.595	2.597	2.600	2.603	2.605
6.7	2.608	2.610	2.613	2.615	2.618	2.620	2.623	2.625	2.627	2.630
6.8	2.633	2.635	2.637	2.640	2.643	2.645	2.648	2.650	2.653	2.655
6.9	2.658	2.660	2.663	2.665	2.668	2.670	2.673	2.675	2.678	2.680
7.0	2.683	2.685	2.687	2.690	2.693	2.695	2.698	2.700	2.702	2.705
7.1	2.707	2.710	2.712	2.714	2.717	2.719	2.721	2.724	2.726	2.729

Intrinsic viscosity $[\eta]_r$ at different values of relative viscosity η_{rel}

	$[\eta]_r$									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
7.2	2.731	2.733	2.736	2.738	2.740	2.743	2.745	2.748	2.750	2.752
7.3	2.755	2.757	2.760	2.762	2.764	2.767	2.769	2.771	2.774	2.776
7.4	2.779	2.781	2.783	2.786	2.788	2.790	2.793	2.795	2.798	2.800
7.5	2.802	2.805	2.807	2.809	2.812	2.814	2.816	2.819	2.821	2.823
7.6	2.826	2.828	2.830	2.833	2.835	2.837	2.840	2.842	2.844	2.847
7.7	2.849	2.851	2.854	2.856	2.858	2.860	2.863	2.865	2.868	2.870
7.8	2.873	2.875	2.877	2.879	2.881	2.884	2.887	2.889	2.891	2.893
7.9	2.895	2.898	2.900	2.902	2.905	2.907	2.909	2.911	2.913	2.915
8.0	2.918	2.920	2.922	2.924	2.926	2.928	2.931	2.933	2.935	2.937
8.1	2.939	2.942	2.944	2.946	2.948	2.950	2.952	2.955	2.957	2.959
8.2	2.961	2.963	2.966	2.968	2.970	2.972	2.974	2.976	2.979	2.981
8.3	2.983	2.985	2.987	2.990	2.992	2.994	2.996	2.998	3.000	3.002
8.4	3.004	3.006	3.008	3.010	3.012	3.015	3.017	3.019	3.021	3.023
8.5	3.025	3.027	3.029	3.031	3.033	3.035	3.037	3.040	3.042	3.044
8.6	3.046	3.048	3.050	3.052	3.054	3.056	3.058	3.060	3.062	3.064
8.7	3.067	3.069	3.071	3.073	3.075	3.077	3.079	3.081	3.083	3.085
8.8	3.087	3.089	3.092	3.094	3.096	3.098	3.100	3.102	3.104	3.106
8.9	3.108	3.110	3.112	3.114	3.116	3.118	3.120	3.122	3.124	3.126
9.0	3.128	3.130	3.132	3.134	3.136	3.138	3.140	3.142	3.144	3.146
9.1	3.148	3.150	3.152	3.154	3.156	3.158	3.160	3.162	3.164	3.166
9.2	3.168	3.170	3.172	3.174	3.176	3.178	3.180	3.182	3.184	3.186
9.3	3.188	3.190	3.192	3.194	3.196	3.198	3.200	3.202	3.204	3.206
9.4	3.208	3.210	3.212	3.214	3.215	3.217	3.219	3.221	3.223	3.225
9.5	3.227	3.229	3.231	3.233	3.235	3.237	3.239	3.241	3.242	3.244
9.6	3.246	3.248	3.250	3.252	3.254	3.256	3.258	3.260	3.262	3.264
9.7	3.266	3.268	3.269	3.271	3.273	3.275	3.277	3.279	3.281	3.283
9.8	3.285	3.287	3.289	3.291	3.293	3.295	3.297	3.298	3.300	3.302
9.9	3.304	3.305	3.307	3.309	3.311	3.313	3.316	3.318	3.320	3.321



Intrinsic viscosity $[\eta]_c$ at different values of relative viscosity η_{rel}

					$[\eta]_c$					
η_{rel}	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
10	3.32	3.34	3.36	3.37	3.39	3.41	3.43	3.45	3.46	3.48
11	3.50	3.52	3.53	3.55	3.56	3.58	3.60	3.61	3.63	3.64
12	3.66	3.68	3.69	3.71	3.72	3.74	3.76	3.77	3.79	3.80
13	3.80	3.83	3.85	3.86	3.88	3.89	3.90	3.92	3.93	3.95
14	3.96	3.97	3.99	4.00	4.02	4.03	4.04	4.06	4.07	4.09
15	4.10	4.11	4.13	4.14	4.15	4.17	4.18	4.19	4.20	4.22
16	4.23	4.24	4.25	4.27	4.28	4.29	4.30	4.31	4.33	4.34
17	4.35	4.36	4.37	4.38	4.39	4.41	4.42	4.43	4.44	4.45
18	4.46	4.47	4.48	4.49	4.50	4.52	4.53	4.54	4.55	4.56
19	4.57	4.58	4.59	4.60	4.61	4.62	4.63	4.64	4.65	4.66

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for microcrystalline cellulose used as binder, diluent or disintegrant.

Particle-size distribution *(2.9.31 or 2.9.38)*.

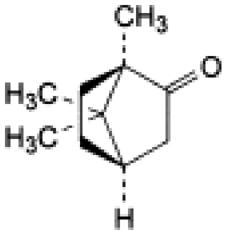
Powder flow *(2.9.36)* .

Ph Eur

Natural Camphor

General Notices

(D-Camphor, Ph Eur monograph 1400)



$C_{10}H_{16}O$ 152.2 *464-49-3*

Ph Eur

DEFINITION

(1*R*,4*R*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one.

CHARACTERS

Appearance

White or almost white, crystalline powder or friable, crystalline masses.

Highly volatile even at room temperature.

Solubility

Slightly soluble in water, very soluble in alcohol and in light petroleum, freely soluble in fatty oils, very slightly soluble in glycerol.

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Specific optical rotation (see Tests).

B. Melting point *(2.2.14)* : 175 °C to 179 °C.

C. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *racemic camphor CRS.*

D. Dissolve 1.0 g in 30 ml of *methanol* R. Add 1.0 g of *hydroxylamine hydrochloride R* and 1.0 g of *anhydrous sodium acetate R* . Boil under a reflux condenser for 2 h. Allow to cool and add 100 ml of *water R* . Filter, wash the precipitate obtained with 10 ml of *water R* and recrystallise from 10 ml of a mixture of 4 volumes of *alcohol R* and 6 volumes of *water R* . The crystals, dried *in vacuo*, melt *(2.2.14)* at 118 °C to 121 °C.

TESTS

Carry out the weighings and dissolution rapidly.

Solution S

Dissolve 2.50 g in 10 ml of *alcohol R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R1* . The solution is colourless. Not more than 0.2 ml *of 0.1 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation *(2.2.7)*

+ 40.0 to + 43.0, determined on solution S.

Related substances

Gas chromatography *(2.2.28)* .

Test solution Dissolve 2.50 g of the substance to be examined in *heptane R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *heptane R* .

Reference solution (b) Dilute 10.0 ml of reference solution (a) to 20.0 ml with *heptane R* .

Reference solution (c) Dissolve 0.50 g of *borneol R* in *heptane R* and dilute to 25.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *heptane R* .

Reference solution (d) Dissolve 50 mg of *linalol R* and 50 mg of *bornyl acetate R* in *heptane R* and dilute to 100.0 ml with the same solvent.

Column:

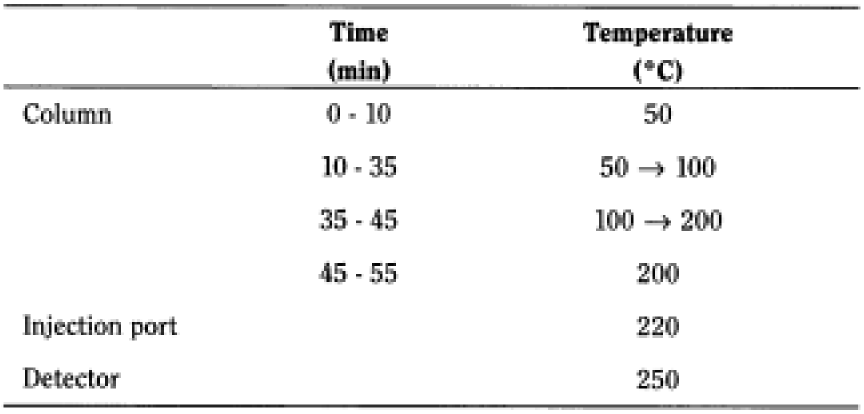
- *size:* *l* = 30 m, Ø = 0.25 mm,
- *stationary phase: macrogol 20 000 R* (0.25 µm).

Carrier gas *helium for chromatography R.*

Split ratio 1:70.

Flow rate 45 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	50
	10 - 35	50 → 100
	35 - 45	100 → 200
	45 - 55	200
Injection port		220
Detector		250

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (d):

— *resolution*: minimum 3.0 between the peaks due to bornyl acetate and to linalol.

Limits:

— *borneol*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent),

— *any other impurity*: not more than half of the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *total* of other impurities: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Halogens

Maximum 100 ppm.

Dissolve 1.0 g in 10 ml of *2-propanol R* in a distillation flask. Add 1.5 ml of *dilute sodium hydroxide solution R* and 50 mg of *nickel-aluminium alloy R*. Heat on a water-bath until the *2-propanol R* has evaporated. Allow to cool and add 5 ml of *water R*. Mix and filter through a wet filter previously washed with *water R* until free from chlorides. Dilute the filtrate to 10.0 ml with *water R*. To 5.0 ml of the solution, add *nitric acid R* dropwise until the precipitate which forms is redissolved and dilute to 15 ml with *water R*. The solution complies with the limit test for chlorides *(2.4.4)*.

Residue on evaporation ***(2.8.9)***

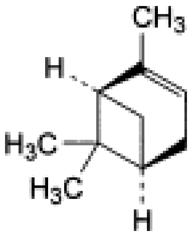
Maximum 0.05 per cent.

Evaporate 2.0 g on a water-bath and dry in an oven at 100-105 °C for 1 h. The residue weighs a maximum of 1 mg.

Water

Dissolve 1 g in 10 ml of *light petroleum R* . The solution is clear *(2.2.1)* .

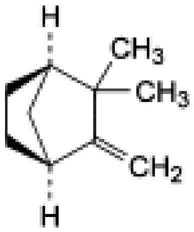
IMPURITIES



CH3
H
H3C
H3C
H

and enantiomer

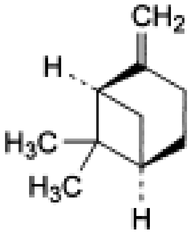
A. 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (α-pinene),



H
CH3
CH3
CH2
H

and enantiomer

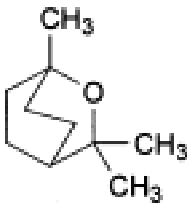
B. 2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane (camphene),



CH2
H
H3C
H3C
H

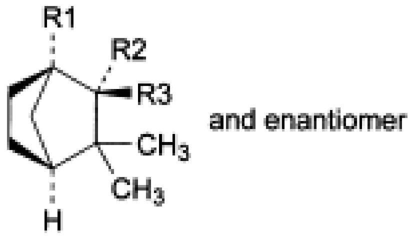
and enantiomer

C. 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (β-pinene),



CH3
O
CH3
CH3

D. 3,3-dimethyl-2-oxabicyclo[2.2.2]octane (cineole),



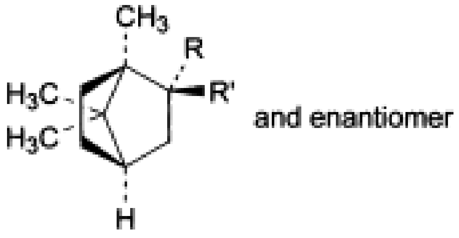
and enantiomer

E. R1 = CH_3, R2 + R3 = O: 1,3,3-trimethylbicyclo[2.2.1]heptan-2-one (fenchone),

F. R1 = CH_3, R2 = OH, R3 = H: *exo*-1,3,3-trimethylbicyclo[2.2.1]heptan-2-ol (fenchol),

G. R1 = H, R2 = OH, R3 = CH_3: *exo*-2,3,3-trimethylbicyclo[2.2.1]heptan-2-ol (camphene hydrate),

H. R1 = H, R2 = CH_3, R3 = OH: *endo*-2,3,3-trimethylbicyclo[2.2.1]heptan-2-ol (methylcamphenilol),



and enantiomer

I. R = OH, R′ = H: *exo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (*exo*-borneol),

J. R = H, R′ = OH: *endo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (*endo*-borneol).

Ph Eur

Polyoxyl Castor Oil

General Notices

(Macrogolglycerol Ricinoleate, Ph Eur monograph 1082)

Action and use

Excipient.

Ph Eur

DEFINITION

Contains mainly ricinoleyl glycerol ethoxylated with 30-50 molecules of ethylene oxide (nominal value), with small amounts of macrogol ricinoleate and of the corresponding free glycols. It results from the reaction of castor oil with ethylene oxide.

CHARACTERS

Appearance

Clear, yellow viscous liquid or semi-solid.

Solubility

Freely soluble in water, very soluble in methylene chloride, freely soluble in ethanol (96 per cent).

Relative density

About 1.05.

Viscosity

500 mPa·s to 800 mPa·s at 25 °C.

IDENTIFICATION

A. Iodine value (see Tests).

B. Saponification value (see Tests).

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1 g of the substance to be examined add 100 ml of a 100 g/l solution of *potassium hydroxide R* and boil under a reflux condenser for 30 min. Allow to cool. Acidify the solution with 20 ml of *hydrochloric acid R*. Shake the mixture with 50 ml of *ether R* and allow to stand until separation of the layers is obtained. Transfer the clear upper layer to a suitable tube, add 5 g of *anhydrous sodium sulphate R*, close the tube and allow to stand for 30 min. Filter and evaporate the filtrate to dryness on a water-bath. Dissolve 50 mg of the residue in 25 ml of *ether R*.

Reference solution Dissolve 50 mg of *ricinoleic acid R* in *methylene chloride R* and dilute to 25 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *methylene chloride R*, *glacial acetic acid R*, *acetone R* (10:40:50 *V/V/V*).

Application 2 µl.

Development Over a path of 8 cm.

Drying In a current of cold air.

Detection Spray with an 80 g/l solution of *phosphomolybdic acid R* in *2-propanol R* and heat at 120 °C for 1-2 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with the reference solution.

D. Place about 2 g of the substance to be examined in a test-tube and add 0.2 ml of *sulphuric acid R*. Close the tube using a stopper fitted with a glass tube bent twice at right angles. Heat the tube until white fumes appear. Collect the fumes in 1 ml of *mercuric chloride solution R*. A white precipitate is formed and the fumes turn a filter paper impregnated with *alkaline potassium tetraiodomercurate solution R* black.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*. If intended for use in the manufacture of parenteral dosage forms, solution S is not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Alkalinity

Dissolve 2.0 g in a hot mixture of 10 ml of *water R* and 10 ml of *ethanol (96 per cent) R*. Add *0.1 ml of bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value *(2.5.1)*

Maximum 2.0, determined on 5.0 g.

Hydroxyl value *(2.5.3, Method A)*

Iodine value *(2.5.4)*

25 to 35.

Saponification value *(2.5.6)* .

See table 1082.-1.

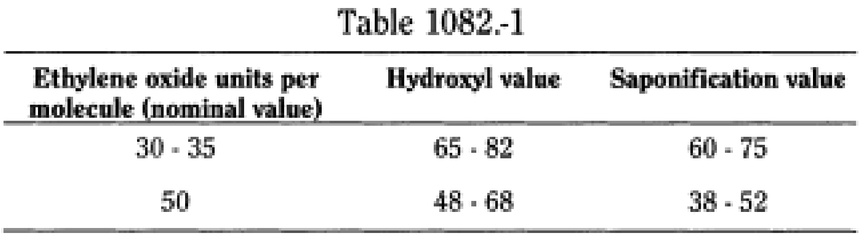


Table 1082.-1

Ethylene oxide units per molecule (nominal value)	Hydroxyl value	Saponification value
30 - 35	65 - 82	60 - 75
50	48 - 68	38 - 52

Residual ethylene oxide and dioxan *(2.4.25)*

Maximum 1 ppm of residual ethylene oxide and 10 ppm of residual dioxan.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S, filtered if necessary, complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R* .

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 2.000 g.

Total ash *(2.4.16)*

Maximum 0.3 per cent, determined on 2.0 g.

STORAGE

Protected from light.

LABELLING

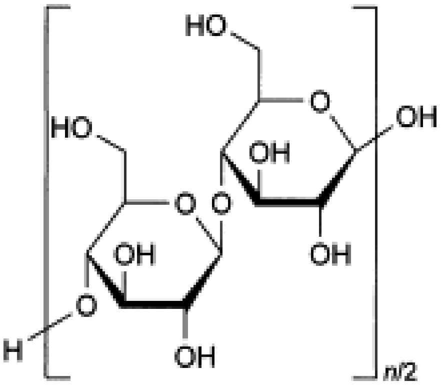
The label states:
- the amount of ethylene oxide reacted with castor oil (nominal value),
- where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Powdered Cellulose

General Notices

(Ph Eur monograph 0315)



$C_{6n}H_{10n+2}O_{5n+1}$

Action and use

Excipient.

Ph Eur

DEFINITION

Purified, mechanically disintegrated cellulose prepared by processing alpha-cellulose obtained as a pulp from fibrous plant material.

CHARACTERS

Appearance

White or almost white, fine or granular powder.

Solubility

Practically insoluble in water, slightly soluble in a 50 g/l solution of sodium hydroxide, practically insoluble in acetone, in anhydrous ethanol, in toluene, in dilute acids and in most organic solvents.

IDENTIFICATION

A. Place about 10 mg on a watch-glass and disperse in 2 ml of *iodinated zinc chloride solution R* . The substance becomes violet-blue.

B. The degree of polymerisation is greater than 440.

Transfer 0.250 g to a 125 ml conical flask. Add 25.0 ml of *water R* and 25.0 ml of *cupriethylenediamine hydroxide solution R* . Immediately purge the solution with *nitrogen R* , insert the stopper and shake until completely dissolved. Transfer an appropriate volume of the solution to a suitable capillary viscometer *(2.2.9)* . Equilibrate the solution at 25 ± 0.1 °C for at least 5 min. Record the flow time (t_1) in seconds between the 2 marks on the viscometer. Calculate the kinematic viscosity (ν_1) of the solution using the following expression:

$$t_1 (k_1)$$

where k_1 is the viscometer constant.

Dilute a suitable volume of *cupriethylenediamine hydroxide solution R* with an equal volume of *water R* and measure the flow time (t_2) using a suitable capillary viscometer. Calculate the kinematic viscosity (ν_2) of the solvent using the following expression:

$$t_2 (k_2)$$

where k_2 is the viscometer constant.

Determine the relative viscosity (η_{rel}) of the substance to be examined using the following expression:

$$\nu_1 / \nu_2$$

Determine the intrinsic viscosity ($[\eta]_c$) by interpolation, using the intrinsic viscosity table (Table 0315.-1).

Calculate the degree of polymerisation (P) using the following expression:

$$\frac{95\,[\eta]_c}{m\,[(100 - b)/100]}$$

where m is the mass in grams of the substance to be examined and b is the loss on drying as a percentage.

TESTS

Solubility

Dissolve 50 mg in 10 ml of *ammoniacal solution of copper tetrammine R*. It dissolves completely, leaving no residue.

pH *(2.2.3)*

5.0 to 7.5 for the supernatant liquid.

Mix 10 g with 90 ml of *carbon dioxide-free water R* and allow to stand with occasional stirring for 1 h.

Ether-soluble substances

Maximum 0.15 per cent (15 mg) for the difference between the mass of the residue and the mass obtained from a blank determination.

Place 10.0 g in a chromatography column about 20 mm in internal diameter and pass 50 ml of *peroxide-free ether R* through the column. Evaporate the eluate to dryness in a previously dried and tared evaporating dish, with the aid of a current of air in a fume hood. After all the ether has evaporated, dry the residue at 105 °C for 30 min, allow to cool in a dessiccator and weigh. Carry out a blank determination.

Water-soluble substances

Maximum 1.5 per cent (15.0 mg) for the difference between the mass of the residue and the mass obtained from a blank determination.

Shake 6.0 g with 90 ml of *carbon dioxide-free water R* for 10 min. Filter with the aid of vacuum into a tared flask. Discard the first 10 ml of the filtrate and pass the filtrate through the same filter a second time, if necessary, to obtain a clear filtrate. Evaporate a 15.0 ml portion of the filtrate to dryness in a tared evaporating dish without charring. Dry at 105 °C for 1 h, allow to cool in a desiccator and weigh. Carry out a blank determination.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 6.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.3 per cent, determined on 1.0 g (dried substance).

Microbial contamination

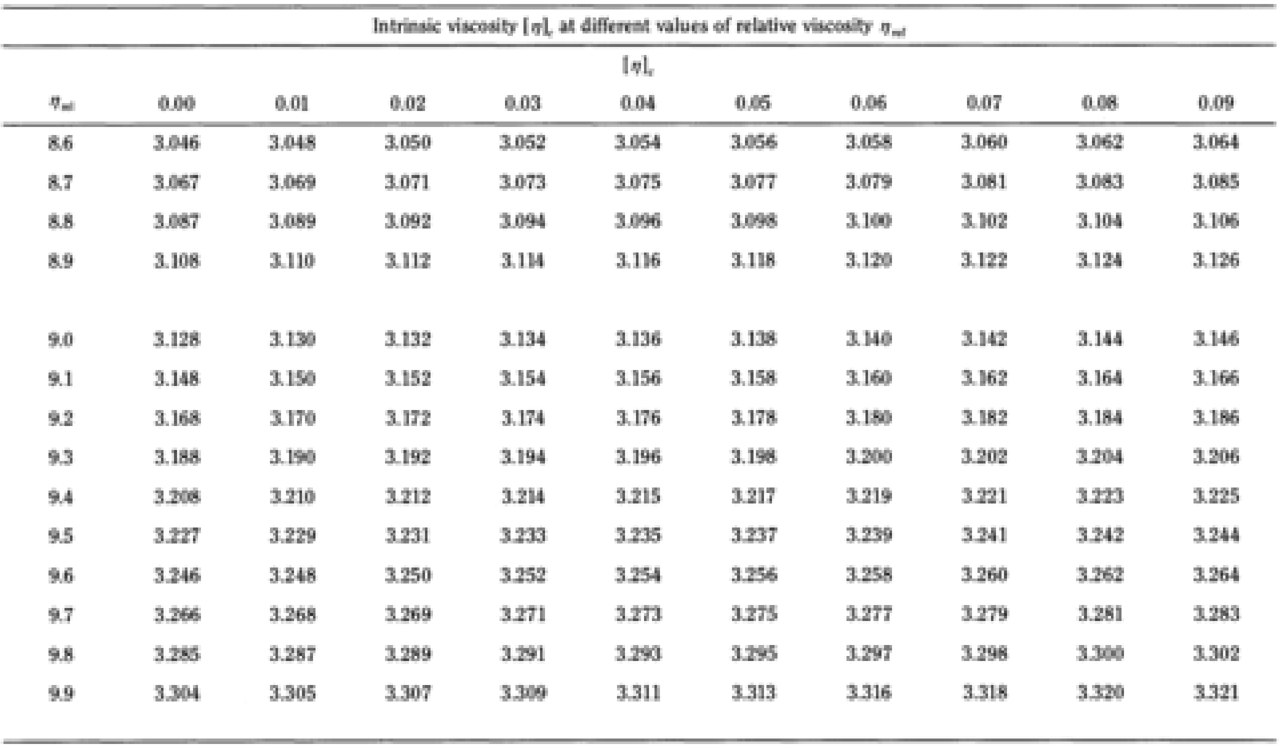
Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram and with a limit for fungi of 10^2 per gram, determined by plate count. It complies with the tests for *Escherichia coli*, for *Pseudomonas aeruginosa*, for *Staphylococcus aureus* and for *Salmonella (2.6.13)*.

Table 0315.-1. – *Intrinsic viscosity table*

Intrinsic viscosity [η], at different values of relative viscosity η_{rel}										
	[η],									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
1.1	0.098	0.106	0.115	0.125	0.134	0.143	0.152	0.161	0.170	0.180
1.2	0.189	0.198	0.207	0.216	0.225	0.233	0.242	0.250	0.259	0.268
1.3	0.276	0.285	0.293	0.302	0.310	0.318	0.326	0.334	0.342	0.350
1.4	0.358	0.367	0.375	0.383	0.391	0.399	0.407	0.414	0.422	0.430
1.5	0.437	0.445	0.453	0.460	0.468	0.476	0.484	0.491	0.499	0.507
1.6	0.515	0.522	0.529	0.536	0.544	0.551	0.558	0.566	0.573	0.580
1.7	0.587	0.595	0.602	0.608	0.615	0.622	0.629	0.636	0.642	0.649
1.8	0.656	0.663	0.670	0.677	0.683	0.690	0.697	0.704	0.710	0.717
1.9	0.723	0.730	0.736	0.743	0.749	0.756	0.762	0.769	0.775	0.782
2.0	0.788	0.795	0.802	0.809	0.815	0.821	0.827	0.833	0.840	0.846
2.1	0.852	0.858	0.864	0.870	0.876	0.882	0.888	0.894	0.900	0.906
2.2	0.912	0.918	0.924	0.929	0.935	0.941	0.948	0.953	0.959	0.965
2.3	0.971	0.976	0.983	0.988	0.994	1.000	1.006	1.011	1.017	1.022
2.4	1.028	1.033	1.039	1.044	1.050	1.056	1.061	1.067	1.072	1.078
2.5	1.083	1.089	1.094	1.100	1.105	1.111	1.116	1.121	1.126	1.131
2.6	1.137	1.142	1.147	1.153	1.158	1.163	1.169	1.174	1.179	1.184
2.7	1.190	1.195	1.200	1.205	1.210	1.215	1.220	1.225	1.230	1.235
2.8	1.240	1.245	1.250	1.255	1.260	1.265	1.270	1.275	1.280	1.285
2.9	1.290	1.295	1.300	1.305	1.310	1.314	1.319	1.324	1.329	1.333
3.0	1.338	1.343	1.348	1.352	1.357	1.362	1.367	1.371	1.376	1.381
3.1	1.386	1.390	1.395	1.400	1.405	1.409	1.414	1.418	1.423	1.427
3.2	1.432	1.436	1.441	1.446	1.450	1.455	1.459	1.464	1.468	1.473
3.3	1.477	1.482	1.486	1.491	1.496	1.500	1.504	1.508	1.513	1.517
3.4	1.521	1.525	1.529	1.533	1.537	1.542	1.546	1.550	1.554	1.558
3.5	1.562	1.566	1.570	1.575	1.579	1.583	1.587	1.591	1.595	1.600
3.6	1.604	1.608	1.612	1.617	1.621	1.625	1.629	1.633	1.637	1.642
3.7	1.646	1.650	1.654	1.658	1.662	1.666	1.671	1.675	1.679	1.683
3.8	1.687	1.691	1.695	1.700	1.704	1.708	1.712	1.715	1.719	1.723
3.9	1.727	1.731	1.735	1.739	1.742	1.746	1.750	1.754	1.758	1.762
4.0	1.765	1.769	1.773	1.777	1.781	1.785	1.789	1.792	1.796	1.800
4.1	1.804	1.808	1.811	1.815	1.819	1.822	1.826	1.830	1.833	1.837
4.2	1.841	1.845	1.848	1.852	1.856	1.859	1.863	1.867	1.870	1.874
4.3	1.878	1.882	1.885	1.889	1.893	1.896	1.900	1.904	1.907	1.911
4.4	1.914	1.918	1.921	1.925	1.929	1.932	1.936	1.939	1.943	1.946

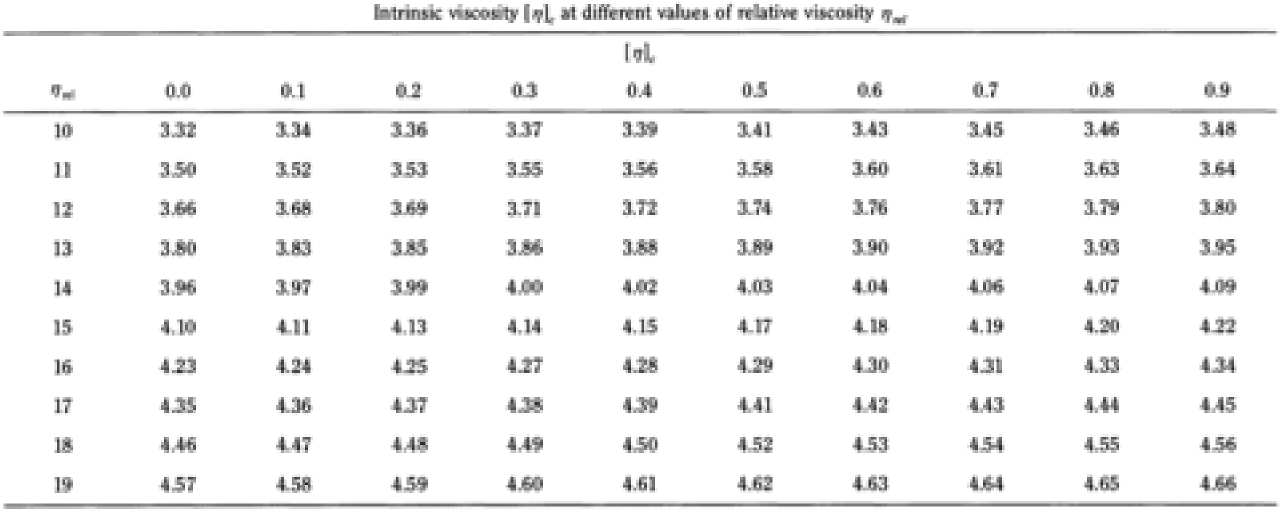
Intrinsic viscosity $[\eta]_r$ at different values of relative viscosity η_{rel}

					$[\eta]_r$					
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
4.5	1.950	1.954	1.957	1.961	1.964	1.968	1.971	1.975	1.979	1.982
4.6	1.986	1.989	1.993	1.996	2.000	2.003	2.007	2.010	2.013	2.017
4.7	2.020	2.023	2.027	2.030	2.033	2.037	2.040	2.043	2.047	2.050
4.8	2.053	2.057	2.060	2.063	2.067	2.070	2.073	2.077	2.080	2.083
4.9	2.087	2.090	2.093	2.097	2.100	2.103	2.107	2.110	2.113	2.116
5.0	2.119	2.122	2.125	2.129	2.132	2.135	2.139	2.142	2.145	2.148
5.1	2.151	2.154	2.158	2.160	2.164	2.167	2.170	2.173	2.176	2.180
5.2	2.183	2.186	2.190	2.192	2.195	2.197	2.200	2.203	2.206	2.209
5.3	2.212	2.215	2.218	2.221	2.224	2.227	2.230	2.233	2.236	2.240
5.4	2.243	2.246	2.249	2.252	2.255	2.258	2.261	2.264	2.267	2.270
5.5	2.273	2.276	2.279	2.282	2.285	2.288	2.291	2.294	2.297	2.300
5.6	2.303	2.306	2.309	2.312	2.315	2.318	2.320	2.324	2.326	2.329
5.7	2.332	2.335	2.338	2.341	2.344	2.347	2.350	2.353	2.355	2.358
5.8	2.361	2.364	2.367	2.370	2.373	2.376	2.379	2.382	2.384	2.387
5.9	2.390	2.393	2.396	2.400	2.403	2.405	2.408	2.411	2.414	2.417
6.0	2.419	2.422	2.425	2.428	2.431	2.433	2.436	2.439	2.442	2.444
6.1	2.447	2.450	2.453	2.456	2.458	2.461	2.464	2.467	2.470	2.472
6.2	2.475	2.478	2.481	2.483	2.486	2.489	2.492	2.494	2.497	2.500
6.3	2.503	2.505	2.508	2.511	2.513	2.516	2.518	2.521	2.524	2.526
6.4	2.529	2.532	2.534	2.537	2.540	2.542	2.545	2.547	2.550	2.553
6.5	2.555	2.558	2.561	2.563	2.566	2.568	2.571	2.574	2.576	2.579
6.6	2.581	2.584	2.587	2.590	2.592	2.595	2.597	2.600	2.603	2.605
6.7	2.608	2.610	2.613	2.615	2.618	2.620	2.623	2.625	2.627	2.630
6.8	2.633	2.635	2.637	2.640	2.643	2.645	2.648	2.650	2.653	2.655
6.9	2.658	2.660	2.663	2.665	2.668	2.670	2.673	2.675	2.678	2.680
7.0	2.683	2.685	2.687	2.690	2.693	2.695	2.698	2.700	2.702	2.705
7.1	2.707	2.710	2.712	2.714	2.717	2.719	2.721	2.724	2.726	2.729
7.2	2.731	2.733	2.736	2.738	2.740	2.743	2.745	2.748	2.750	2.752
7.3	2.755	2.757	2.760	2.762	2.764	2.767	2.769	2.771	2.774	2.776
7.4	2.779	2.781	2.783	2.786	2.788	2.790	2.793	2.795	2.798	2.800
7.5	2.802	2.805	2.807	2.809	2.812	2.814	2.816	2.819	2.821	2.823
7.6	2.826	2.828	2.830	2.833	2.835	2.837	2.840	2.842	2.844	2.847
7.7	2.849	2.851	2.854	2.856	2.858	2.860	2.863	2.865	2.868	2.870
7.8	2.873	2.875	2.877	2.879	2.881	2.884	2.887	2.889	2.891	2.893
7.9	2.895	2.898	2.900	2.902	2.905	2.907	2.909	2.911	2.913	2.915
8.0	2.918	2.920	2.922	2.924	2.926	2.928	2.931	2.933	2.935	2.937
8.1	2.939	2.942	2.944	2.946	2.948	2.950	2.952	2.955	2.957	2.959
8.2	2.961	2.963	2.966	2.968	2.970	2.972	2.974	2.976	2.979	2.981
8.3	2.983	2.985	2.987	2.990	2.992	2.994	2.996	2.998	3.000	3.002
8.4	3.004	3.006	3.008	3.010	3.012	3.015	3.017	3.019	3.021	3.023
8.5	3.025	3.027	3.029	3.031	3.033	3.035	3.037	3.040	3.042	3.044



Intrinsic viscosity $[\eta]_c$ at different values of relative viscosity η_{rel}

	$[\eta]_c$									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
8.6	3.046	3.048	3.050	3.052	3.054	3.056	3.058	3.060	3.062	3.064
8.7	3.067	3.069	3.071	3.073	3.075	3.077	3.079	3.081	3.083	3.085
8.8	3.087	3.089	3.092	3.094	3.096	3.098	3.100	3.102	3.104	3.106
8.9	3.108	3.110	3.112	3.114	3.116	3.118	3.120	3.122	3.124	3.126
9.0	3.128	3.130	3.132	3.134	3.136	3.138	3.140	3.142	3.144	3.146
9.1	3.148	3.150	3.152	3.154	3.156	3.158	3.160	3.162	3.164	3.166
9.2	3.168	3.170	3.172	3.174	3.176	3.178	3.180	3.182	3.184	3.186
9.3	3.188	3.190	3.192	3.194	3.196	3.198	3.200	3.202	3.204	3.206
9.4	3.208	3.210	3.212	3.214	3.215	3.217	3.219	3.221	3.223	3.225
9.5	3.227	3.229	3.231	3.233	3.235	3.237	3.239	3.241	3.242	3.244
9.6	3.246	3.248	3.250	3.252	3.254	3.256	3.258	3.260	3.262	3.264
9.7	3.266	3.268	3.269	3.271	3.273	3.275	3.277	3.279	3.281	3.283
9.8	3.285	3.287	3.289	3.291	3.293	3.295	3.297	3.298	3.300	3.302
9.9	3.304	3.305	3.307	3.309	3.311	3.313	3.316	3.318	3.320	3.321



Intrinsic viscosity $[\eta]_c$ at different values of relative viscosity η_{rel}

	$[\eta]_c$									
η_{rel}	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
10	3.32	3.34	3.36	3.37	3.39	3.41	3.43	3.45	3.46	3.48
11	3.50	3.52	3.53	3.55	3.56	3.58	3.60	3.61	3.63	3.64
12	3.66	3.68	3.69	3.71	3.72	3.74	3.76	3.77	3.79	3.80
13	3.80	3.83	3.85	3.86	3.88	3.89	3.90	3.92	3.93	3.95
14	3.96	3.97	3.99	4.00	4.02	4.03	4.04	4.06	4.07	4.09
15	4.10	4.11	4.13	4.14	4.15	4.17	4.18	4.19	4.20	4.22
16	4.23	4.24	4.25	4.27	4.28	4.29	4.30	4.31	4.33	4.34
17	4.35	4.36	4.37	4.38	4.39	4.41	4.42	4.43	4.44	4.45
18	4.46	4.47	4.48	4.49	4.50	4.52	4.53	4.54	4.55	4.56
19	4.57	4.58	4.59	4.60	4.61	4.62	4.63	4.64	4.65	4.66

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited they are recognised as being suitable for the purpose but other *methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.*

The following characteristics may be relevant for powdered cellulose used as diluent or disintegrant.

Particle-size distribution ***(2.9.31 or 2.9.38)*****.**

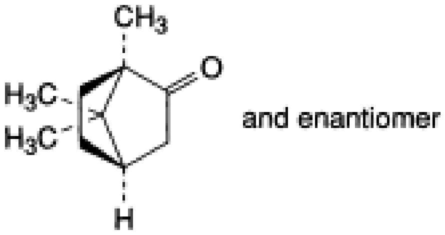
Powder flow ***(2.9.36)*** **.**

Ph Eur

Racemic Camphor

General Notices

(Ph Eur monograph 0655)



$C_{10}H_{16}O$ 152.2 *76-22-2*

Action and use

Counter-irritant.

Preparations

Camphorated Opium Tincture

Concentrated Camphorated Opium Tincture

Concentrated Camphor Water

Ph Eur

DEFINITION

(1*RS*,4*RS*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one.

CHARACTERS

Appearance

White or almost white, crystalline powder or friable, crystalline masses, highly volatile even at room temperature.

Solubility

Slightly soluble in water, very soluble in ethanol (96 per cent) and in light petroleum, freely soluble in fatty oils, very slightly soluble in glycerol.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D.*

A. Optical rotation (see Tests).

B. Melting point *(2.2.14)* : 172 °C to 180 °C.

C. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Mulls in *liquid paraffin R.*

Comparison *racemic camphor CRS.*

D. Dissolve 1.0 g in 30 ml of *methanol R.* Add 1.0 g of *hydroxylamine hydrochloride R* and 1.0 g of *anhydrous sodium acetate R* . Boil under a reflux condenser for 2 h. Allow to cool and add 100 ml of *water R* . A precipitate is formed. Filter, wash with 10 ml of *water R* and recrystallize from 10 ml of a mixture of 4 volumes of *ethanol (96 per cent) R* and 6 volumes of *water R.* The crystals, dried *in vacuo*, melt *(2.2.14)* at 118 °C to 121 °C.

TESTS

Carry out the weighings rapidly.

Solution S

Dissolve 2.50 g in 10 ml of *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Dissolve 1.0 g in 10 ml of *ethanol (96 per cent) R* and add 0.1 ml of *phenolphthalein solution R1* . The solution is colourless. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Optical rotation *(2.2.7)*

- 0.15 ° to + 0.15 °, determined on solution S.

Related substances

Gas chromatography *(2.2.28)* .

Test solution Dissolve 50 mg of the substance to be examined in *hexane R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of the substance to be examined and 50 mg of *bornyl acetate R* in *hexane R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with *hexane R* .

Column:
- *size*: *l* = 2 m, Ø = 2 mm;
- *stationary phase: diatomaceous earth for gas chromatography* R impregnated with 10 per cent m/m of *macrogol 20 000 R.*

Carrier gas *nitrogen for chromatography R* .

Flow rate 30 ml/min.

Temperature:
- *column*: 130 °C;
- *injection port and detector*: 200 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 3 times the retention time of camphor.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to camphor and bornyl acetate in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio* : minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:
- *any impurity*: for each impurity, not more than 2 per cent of the area of the principal peak;
- *total*: not more than 4 per cent of the area of the principal peak;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b).

Halogens

Maximum 100 ppm.

Dissolve 1.0 g in 10 ml of *2-propanol R* in a distillation flask. Add 1.5 ml of *dilute sodium hydroxide solution R* and 50 mg of *nickel-aluminium alloy R* . Heat on a water-bath until the *2-propanol R* has evaporated. Allow to cool and add 5 ml of *water R* . Mix and filter through a wet filter previously washed with *water R* until free from chlorides. Dilute the filtrate to 10.0 ml with *water R* . To 5.0 ml of this solution, add *nitric acid R* dropwise until the precipitate which forms is redissolved and dilute to 15 ml with *water R.* The solution complies with the limit test for chlorides *(2.4.4)* .

Water

Dissolve 1 g in 10 ml of *light petroleum R* . The solution is clear *(2.2.1)* .

Residue on evaporation

Maximum 0.05 per cent.

Evaporate 2.0 g on a water-bath and dry at 100-105 °C for 1 h. The residue weighs not more than 1 mg.

Refined Castor Oil

General Notices

(Ph Eur monograph 2367)

Ph Eur

DEFINITION

Fatty oil obtained from the seeds of *Ricinus communis* L. by cold expression. It is then refined. A suitable antioxidant may be added.

PRODUCTION

During the expression step, the temperature of the oil must not exceed 50 °C.

CHARACTERS

Appearance

Clear, almost colourless or slightly yellow, viscous, hygroscopic liquid.

Solubility

Slightly soluble in light petroleum, miscible with ethanol (96 per cent) and with glacial acetic acid.

Relative density

About 0.958.

Refractive index

About 1.479.

Viscosity

About 1000 mPa·s.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. A mixture of 2 ml of the substance to be examined and 8 ml of *ethanol (96 per cent) R* is clear *(2.2.1)* .

B. Composition of fatty acids (see Tests).

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured *(2.2.2, Method II)* than 20 ml of a mixture of 0.25 ml of blue primary solution, 0.25 ml of red primary solution, 0.8 ml of yellow primary solution, and 18.7 ml of a solution prepared by diluting 4.0 ml of *hydrochloric acid R1* to 100.0 ml with *water R* .

Optical rotation *(2.2.7)*

+ 3.5° to + 6.0°.

Specific absorbance *(2.2.25)*

Maximum 1.5, determined in *ethanol (96 per cent) R* at the absorption maximum between 268 nm and 270 nm.

Acid value *(2.5.1)*

Maximum 0.8.

Dissolve 5.00 g in 25 ml of the prescribed mixture of solvents.

Hydroxyl value *(2.5.3, Method A)*

Minimum 150.

Peroxide value *(2.5.5, Method A)*

Maximum 5.0.

Unsaponifiable matter *(2.5.7)*

Maximum 0.8 per cent, determined on 5.0 g.

Oil obtained by extraction and adulteration.

In a ground-glass-stoppered tube about 125 mm long and 18 mm in internal diameter, thoroughly mix 3 ml of the substance to be examined with 3 ml of *carbon disulphide R* . Shake for 3 min with 1 ml of *sulphuric acid R* . The mixture is less intensely coloured than a freshly prepared mixture of 3.2 ml of *ferric chloride solution R1* , 2.3 ml of *water R* and 0.5 ml of *dilute ammonia R1* .

Composition of fatty acids

Gas chromatography *(2.4.22)* with the following modifications.

Use the mixture of calibrating substances in Table 2.4.22.-3.

Test solution Introduce 75 mg of the substance to be examined into a 10 ml centrifuge tube with a screw cap. Dissolve in 2 ml of *1,1-dimethylethyl methyl ether R1* with shaking and heat gently (50-60 °C). To the still-warm solution, add 1 ml of a 12 g/l solution of *sodium R* in *anhydrous methanol R* , prepared with the necessary precautions, and shake vigorously for at least 5 min. Add 5 ml of *distilled water R* and shake vigorously for about 30 s. Centrifuge for 15 min at 1500 *g*. Use the upper layer.

Reference solution Dissolve 50 mg of *methyl ricinoleate CRS* and 50 mg of *methyl stearate CRS* in 10.0 ml of *1,1-dimethylethyl methyl ether R1* .

Column:

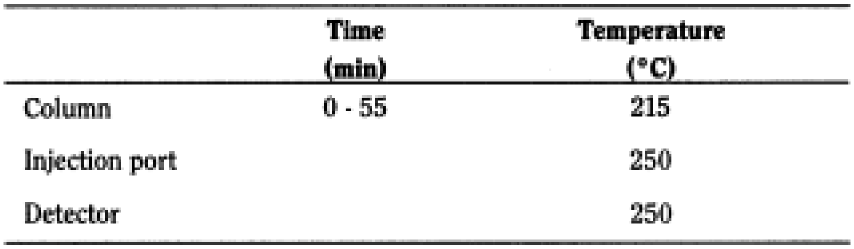
- *material*: fused silica;
- *size:* l = 30 m, Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 0.9 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 55	215
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Calculate the percentage content of each fatty acid by the normalisation procedure.

Correct the area of the peak due to methyl ricinoleate, multiplying by a factor (*R*) calculated using the following expression:

$$\frac{m_1 \times A_2}{A_1 \times m_2}$$

m_1 = mass of methyl ricinoleate in the reference solution;

m_2 = mass of methyl stearate in the reference solution;

A_1 = area of the peak due to methyl ricinoleate in the chromatogram obtained with the reference solution;

A_2 = area of the peak due to methyl stearate in the chromatogram obtained with the reference solution

Composition of the fatty-acid fraction of the oil:

- *palmitic acid* : maximum 2.0 per cent;

— *stearic acid* : maximum 2.5 per cent;

— *oleic acid and isomers*: 2.5 per cent to 6.0 per cent;

— *linoleic acid* : 2.5 per cent to 7.0 per cent;

— *linolenic acid* : maximum 1.0 per cent;

— *eicosenoic acid*: maximum 1.0 per cent;

— *ricinoleic acid* : 85.0 per cent to 92.0 per cent;

— *any other fatty acid*: maximum 1.0 per cent.

Water ***(2.5.32)***

Maximum 0.2 per cent, determined on 5.00 g, if intended for use in the manufacture of parenteral dosage forms.

STORAGE

In an airtight, well-filled container, protected from light.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Strong Cetrimide Solution

General Notices

Action and use

Antiseptic.

Preparation

Cetrimide Solution

DEFINITION

Strong Cetrimide Solution is an aqueous solution of cetrimide. It contains 20 to 40% w/v of cetrimide, calculated as $C_{17}H_{38}BrN$. It contains Ethanol (96 per cent) or Isopropyl Alcohol or both. It may be perfumed and may contain colouring matter.

PRODUCTION

In making Strong Cetrimide Solution, Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit, provided that the law and the statutory regulations governing the use of Industrial Methylated Spirit are observed.

Content of cetrimide $C_{17}H_{38}BrN$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume of the solution containing 0.1 g of cetrimide to 5 ml with *water* and add 2 ml of a 5% w/v solution of *potassium hexacyanoferrate(III)*. A yellow precipitate is produced.

B. Shake together 5 ml of *water*, 1 ml of 2M *sulphuric acid* , 2 ml of *chloroform* and 0.05 ml of *methyl orange solution*; the chloroform layer is colourless. Add 0.1 ml of the solution being examined and shake; a yellow colour is produced slowly in the chloroform layer.

C. Yields reaction A characteristic of *bromides*, Appendix VI.

TESTS

Acidity or alkalinity

Dilute a volume of the solution containing 10 g of cetrimide to 100 ml and add 0.1 ml of *bromocresol purple solution*. Not more than 1.0 ml of either 0.1M *hydrochloric acid VS* or 0.1M *sodium hydroxide VS* is required to change the colour of the solution.

Miscibility with ethanol

Mix a volume of the solution containing 1.6 g of cetrimide with a mixture of 2 ml of *water* and 16 ml of *ethanol (96%)*. The solution remains *clear*, Appendix IV A.

Neutral substances

To a volume of the solution containing 10 g of cetrimide add 25 ml of *ethanol (50%)*, acidify to *bromophenol blue solution* by the drop wise addition of *hydrochloric acid* and add 0.05 ml in excess. Transfer quantitatively to the extraction compartment of an apparatus designed for continuous liquid-liquid extraction by fluids of a lesser density than water, washing out the beaker with 10 ml *ethanol (50%)* and adding the washings to the bulk of the solution in the extractor. Add sufficient *ethanol (50%)*, if necessary, to half-fill the extraction chamber to the level of the overflow limb. Add sufficient *purified hexane* to fill the extraction chamber, secure an overflow volume of about 30 ml in the ebullition flask and heat using an electrically heated mantle. Ensure that a continuous flow of hexane through the aqueous ethanol layer is observed and continue the extraction for 16 hours. Transfer the hexane extract to a separating funnel, washing out the flask with 10 ml of *purified hexane*. Shake the combined extract and washings with 25 ml of *ethanol (50%)* and discard the aqueous ethanol layer. Filter the hexane layer through a dry filter paper (Whatman No. 1 is suitable) into a tared flask and remove the solvent using a rotary evaporator at 40° and then at room temperature at a pressure not exceeding 0.7 kPa for 2 hours. The residue weighs not more than 0.4 g.

Non-quaternised amines

To a volume of the solution containing 10 g of cetrimide add a mixture of 100 ml of *propan-2-ol*, 0.1 ml of *hydrochloric acid* and 20 ml of *methanol*. Titrate with 0.1M *tetrabutylammonium hydroxide VS* passing a slow current of *nitrogen* through the solution and determining the end point potentiometrically using a platinum-glass electrode system. Inflections in the titration curve indicate (A) neutralisation of excess hydrochloric acid and (B) neutralisation of non-quaternised amine salts. The difference between the volumes corresponding to A and B is not more than 10 ml (2.4%, calculated as $C_{16}H_{35}N$).

Ethanol; Isopropyl alcohol

Carry out one or both of the following methods according to the declared alcohol content of the solution being examined.

Ethanol Not more than 10.0% v/v, by the method for the *determination of ethanol*, Appendix VIII F. Use on-column injection and do not heat the injection port.

Isopropyl alcohol Not more than 10.0% v/v, by the method for the *determination of ethanol*, Appendix VIII F, with the following modifications. For solution (1) use a solution containing 5.0% v/v of *propan-2-ol* and 5.0% v/v of *propan-1-ol* (internal standard). For solution (2) use the solution being examined, diluted with *water*, if necessary, to contain about 5.0% v/v of isopropyl alcohol. Maintain the column temperature at 170°, use on-column injection and do not heat the injection port.

ASSAY

Dilute a volume containing 4 g of cetrimide with sufficient *water* to produce 100 ml. Transfer 25 ml of the solution to a separating funnel and add 25 ml of *chloroform*, 10 ml of 0.1M *sodium hydroxide* and 10 ml of a freshly prepared 8.0% w/v solution of *potassium iodide*. Shake well, allow to separate and discard the chloroform layer. Wash the aqueous layer with three 10 ml quantities of *chloroform* and discard the washings. Add 40 ml of *hydrochloric acid*, cool and titrate with 0.05M *potassium iodate VS* until the deep brown colour is almost discharged. Add 2 ml of *chloroform* and continue the titration, with shaking, until the chloroform layer becomes colourless. Carry out a blank titration on a mixture of 10 ml of the freshly prepared potassium iodide solution, 20 ml of *water* and 40 ml of *hydrochloric acid*. The difference between the titrations represents the amount of potassium iodate required. Each ml of 0.05M *potassium iodate VS* is equivalent to 33.64 mg of $C_{17}H_{38}BrN$.

STORAGE

Strong Cetrimide Solution should be stored at a temperature above 15°.

LABELLING

The label states whether Ethanol, Isopropyl Alcohol or both are present and the percentage of cetrimide, weight in volume.

Virgin Castor Oil

General Notices

Castor Oil

(Ph Eur monograph 0051)

Action and use

Stimulant laxative; emollient.

Preparation

Zinc and Castor Oil Ointment

Ph Eur

DEFINITION

Fatty oil obtained by cold expression from the seeds of *Ricinus communis L*. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, almost colourless or slightly yellow, viscous, hygroscopic liquid.

Solubility

Slightly soluble in light petroleum, miscible with alcohol and with glacial acetic acid.

Relative density

About 0.958.

Refractive index

About 1.479.

IDENTIFICATION

First identification D.

Second identification A, B, C.

A. It complies with the test for optical rotation (see Tests).

B. It complies with the test for hydroxyl value (see Tests).

C. Iodine value *(2.5.4)* : 82 to 90.

D. It complies with the test for composition of fatty acids (see Tests).

TESTS

Optical rotation *(2.2.7)*

+ 3.5° to + 6.0°.

Specific absorbance *(2.2.25)*

Maximum 1.0, determined at the absorption maximum at 269 nm ± 1 nm.

To 1.0 g add *alcohol R* and dilute to 100.0 ml with the same solvent.

Acid value *(2.5.1)*

Maximum 2.0.

Dissolve 5.0 g in 25 ml of the prescribed mixture of solvents.

Hydroxyl value *(2.5.3, Method A)*

Minimum 150.

Peroxide value *(2.5.5)*

Maximum 10.0.

Unsaponifiable matter *(2.5.7)*

Maximum 0.8 per cent, determined on 5.0 g.

Composition of fatty acids

Gas chromatography *(2.4.22)* with the following modifications.

Use the mixture of calibrating substances in Table 2.4.22.-3.

Test solution Introduce 75 mg of the substance to be examined into a 10 ml centrifuge tube with a screw cap. Dissolve in 2 ml of 1,*1-dimethylethyl methyl ether R1* with shaking and heat gently (50-60 °C). Add, when still warm, 1 ml of a 12 g/l solution of *sodium R* in *anhydrous methanol R*, prepared with the necessary precautions, and mix vigorously for at least 5 min. Add 5 ml of *distilled water R* and mix vigorously for about 30 s. Centrifuge for 15 min at 1500 *g*. Use the upper layer.

Reference solution Dissolve 50 mg of *methyl ricinoleate CRS* and 50 mg of *methyl stearate CRS* in 10.0 ml of *1,1-dimethylethyl methyl ether R1.*

Column:

- *material*: fused silica,
- *size*: *l* = 30 m, Ø = 0.25 mm,

— *stationary phase: macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 0.9 ml/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 55	215
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Calculate the percentage content of each fatty acid by normalisation.

Correct the area of the peak due to methyl ricinoleate, multiplying by a factor *R* calculated using the following expression:

$$R = \frac{m_1 \times A_2}{A_1 \times m_2}$$

m_1 = mass of methyl ricinoleate in the reference solution

m_2 = mass of methyl stearate in the reference solution

A_1 = area of the peak due to methyl ricinoleate in the chromatogram obtained with the reference solution

A_2 = area of the peak due to methyl stearate in the chromatogram obtained with the reference solution

Composition of the fatty-acid fraction of the oil:

— *palmitic acid* : maximum 2.0 per cent,

— *stearic acid* : maximum 2.5 per cent,

— *oleic acid and isomers* (C18:1 equivalent chain length on macrogol 20 000: 18.3): 2.5 per cent to 6.0 per cent,

— *linoleic acid* (C18:2 equivalent chain length on macrogol 20 000: 18.8): 2.5 per cent to 7.0 per cent,

— *linolenic acid* (C18:3 equivalent chain length on macrogol 20 000: 19.2): maximum 1.0 per cent,

— *eicosenoic acid* (C20:1 equivalent chain length on macrogol 20 000: 20.2): maximum 1.0 per cent,

— *ricinoleic acid* (equivalent chain length on macrogol 20 000: 23.9): 85.0 per cent to 92.0 per cent,

— *any other fatty acid*: maximum 1.0 per cent.

Water *(2.5.12)*

Maximum 0.3 per cent, determined on 5.0 g.

STORAGE

In an airtight, well-filled container, protected from light.

Ph Eur

Anhydrous Disodium Hydrogen Phosphate

General Notices

(Anhydrous Disodium Phosphate, Ph Eur monograph 1509)

Na_2HPO_4 142.0 *7558-79-4*

Action and use

Excipient.

Preparation

Phosphates Enema

Ph Eur

DEFINITION

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearances

White or almost white powder, hygroscopic.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly alkaline *(2.2.4)*.

B. Loss on drying (see Tests).

C. Solution S gives reaction (b) of phosphates *(2.3.1)*.

D. Solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Reducing substances

To 10 ml of solution S add 5 ml of *dilute sulphuric acid R* and 0.25 ml of *0.02 M potassium permanganate* and heat on a water-bath for 5 min. The solution retains a slight red colour.

Monosodium phosphate

Maximum 2.5 per cent.

From the volume of *1 M hydrochloric acid* (25 ml) and of *1 M sodium hydroxide* (n_1 ml and n_2 ml) used in the assay, calculate the following ratio:

$$\frac{n_2 - 25}{25 - n_1}$$

This ratio is not greater than 0.025.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *dilute nitric acid R*.

Sulphates *(2.4.13)*

Maximum 500 ppm.

To 6 ml of solution S add 2 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*.

Arsenic *(2.4.2, Method A)*

Maximum 2 ppm, determined on solution S.

Iron *(2.4.9)*

Maximum 20 ppm, determined on solution S.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using 5 ml of *lead standard solution (1 ppm Pb) R* and 5 ml of *water R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

ASSAY

Dissolve 1.600 g (m) in 25.0 ml of *carbon dioxide-free water R* and add 25.0 ml of *1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)* using *1 M sodium hydroxide*. Read the volume added at the 1st inflexion point (n_1 ml). Continue the titration to the 2nd inflexion point (total volume of *1 M sodium hydroxide* required, n_2 ml).

Calculate the percentage content of Na_2HPO_4 from the following expression:

$$\frac{1420\,(25 - n_1)}{m\,(100 - d)}$$

d = percentage loss on drying.

STORAGE

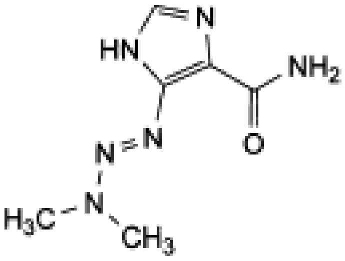
In an airtight container .

Ph Eur

Dacarbazine

General Notices

(Ph Eur monograph 1691)



$C_6H_{10}N_6O$ 182.2 *4342-03-4*

Action and use

Cytotoxic alkylating agent

Ph Eur

DEFINITION

5-[(1*E*)-3,3-Dimethyltriaz-1-enyl]-1*H*-imidazole-4-carboxamide.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder.

Solubility

Slightly soluble in water and in anhydrous ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 15.0 mg in 100.0 ml of *0.1 M hydrochloric acid*. Dilute 5.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*.

Spectral range 200-400 nm.

Absorption maximum At 323 nm.

Shoulder At 275 nm.

Specific absorbance at the absorption maximum 1024 to 1131.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dacarbazine CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 2.0 mg of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution Dissolve 2.0 mg of *dacarbazine CRS* in *methanol R* and dilute to 5.0 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (1:2:5 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Dissolve 0.25 g in a 210 g/l solution of *citric acid R* and dilute to 25.0 ml with the same solution.

Related substances

A. Liquid chromatography *(2.2.29)*. Use freshly prepared solutions and protect them from light.

Test solution Dissolve 50.0 mg of the substance to be examined and 75 mg of *citric acid R* in *distilled water R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *dacarbazine impurity A CRS* in *distilled water R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 25.0 ml with *distilled water R*.

Reference solution (b) Dissolve 5.0 mg of *dacarbazine impurity B CRS* in *distilled water R*, add 0.5 ml of the test solution and dilute to 10.0 ml with *distilled water R*. Dilute 1.0 ml of this solution to 50.0 ml with *distilled water R*.

Column:

— *size:* *l* = 0.25 m, Ø = 4.5 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 15.63 g/l solution of *glacial acetic acid R* containing 2.33 g/l of *sodium dioctyl sulfosuccinate R*. As the mobile phase contains sodium dioctyl sulfosuccinate, it must be freshly prepared every day, and the column must be flushed with a mixture of equal volumes of *methanol R* and *water R*, after all tests have been completed or at the end of the day, for at least 2 h.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 25 µl of the test solution and reference solution (a).

Run time 3 times the retention time of impurity A.

Retention time Impurity A = about 3 min.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities eluting after impurity A*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent).

B. Liquid chromatography *(2.2.29)* as described in related substances test A with the following modifications.

Mobile phase Mix 45 volumes of a 15.63 g/l solution of *glacial acetic acid R* containing 2.33 g/l of *sodium dioctyl sulfosuccinate R* with 55 volumes of *methanol R*.

Injection 10 µl of the test solution and reference solution (b).

Run time Twice the retention time of dacarbazine.

Relative retention With reference to dacarbazine (retention time = about 12 min): impurity B = about 0.7.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity B and dacarbazine.

Limits:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to dacarbazine in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 5 times the area of the peak due to dacarbazine in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the peak due to dacarbazine in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity D

Head-space gas chromatography *(2.2.28)*.

Test solution Introduce 0.200 g of the substance to be examined into a 20 ml vial and firmly attach the septum and cap. Using a 10 µl syringe, inject 5 µl of *water R* into the vial.

Reference solution (a) Dilute 2.5 ml of *dimethylamine solution R* (impurity D) to 100.0 ml with *water R* (solution A). Firmly attach the septum and cap to a 20 ml vial. Using a 10 µl syringe, inject 10 µl of solution A into the vial.

Reference solution (b) Firmly attach the septum and cap to a 20 ml vial. Using a 10 µl syringe, inject 10 µl of solution A and 10 µl of a 10 g/l solution of *triethylamine R* into the vial.

Column:

— *material*: fused silica;

— *size*: *l* = 30.0 m, Ø = 0.53 mm;

— *stationary phase*: *base-deactivated polyethyleneglycol R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R.*

Flow rate 13 ml/min.

Split ratio 1:1.

Static head-space conditions that may be used:

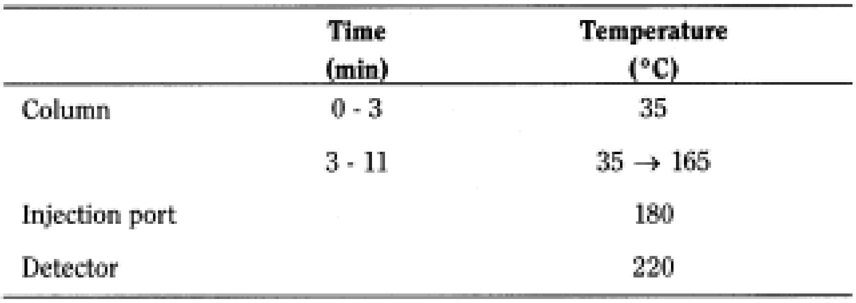
— *equilibration temperature*: 60 °C;

— *equilibration time*: 10 min;

— *transfer-line temperature*: 90 °C;

— *pressurisation time*: 30 s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 3	35
	3 - 11	35 → 165
Injection port		180
Detector		220

Detection Flame ionisation.

Injection 1 ml.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to impurity D and triethylamine.

Limit:

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 18.22 mg of $C_6H_{10}N_6O$.

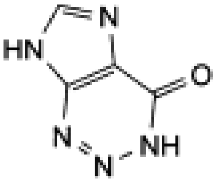
STORAGE

At a temperature of 2 °C to 8 °C, protected from light.

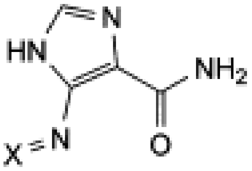
IMPURITIES

Specified impurities *A, B, D.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: C.

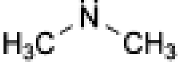


A. 3,7-dihydro-4*H*-imidazo[4,5-*d*]-1,2,3-triazin-4-one (2-azahypoxanthine),



B. X = H_2: 5-amino-1*H*-imidazole-4-carboxamide,

C. X = NH: 5-diazenyl-1*H*-imidazole-4-carboxamide,



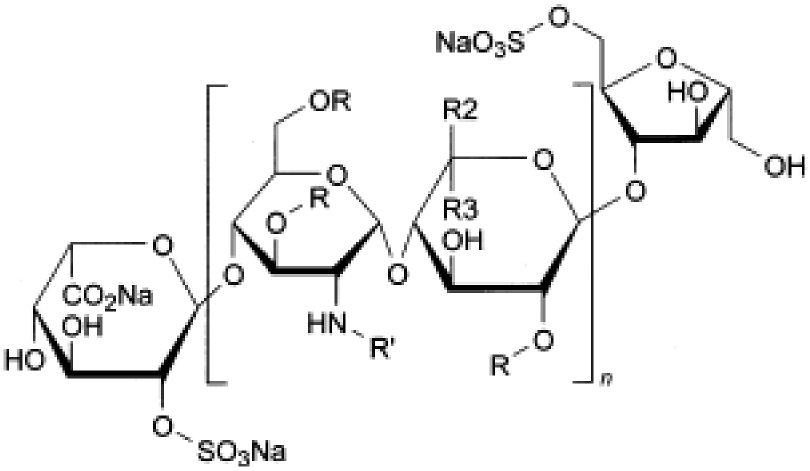
D. *N*-methylmethanamine.

Ph Eur

Dalteparin Sodium

General Notices

(Ph Eur monograph 1195)



Action and use

Low molecular weight heparin.

Ph Eur

DEFINITION

Dalteparin sodium is the sodium salt of a low-molecular-mass heparin that is obtained by nitrous acid depolymerisation of heparin from porcine intestinal mucosa. The majority of the components have a 2-*O*-sulpho-α-L-idopyranosuronic acid structure at the non-reducing end and a 6-*O*-sulpho-2,5-anhydro-D-mannitol structure at the reducing end of their chain.

Dalteparin sodium complies with the monograph Low-molecular-mass heparins (0828) with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 5600 and 6400, with a characteristic value of about 6000.

The degree of sulphatation is 2.0 to 2.5 per disaccharide unit.

The potency is not less than 110 IU and not more than 210 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The anti-factor IIa activity is not less than 35 IU/mg and not more than 100 IU/mg, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.9 and 3.2.

PRODUCTION

Dalteparin sodium is produced by a validated manufacturing and purification procedure under conditions designed to minimise the presence of N-NO groups.

The manufacturing procedure must have been shown to reduce any contamination by N-NO groups to approved limits using an appropriate, validated quantification method.

IDENTIFICATION

Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *dalteparin sodium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. The following requirements apply.

The mass-average relative molecular mass ranges between 5600 and 6400. The mass percentage of chains lower than 3000 is not more than 13.0 per cent. The mass percentage of chains higher than 8000 ranges between 15.0 per cent and 25.0 per cent.

TESTS

Appearance of solution

Dissolve 1 g in 10 ml of *water R*. The solution is clear *(2.2.1)* and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Nitrite

Not more than 5 ppm. Examine by liquid chromatography *(2.2.29)*. *Rinse all volumetric flasks at least three times with water R before the preparation of the solutions.*

Test solution Dissolve 80.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent. Allow to stand for at least 30 min.

Reference solution (a) Dissolve 60.0 mg of *sodium nitrite R* in *water R* and dilute to 1000.0 ml with the same solvent.

For the preparation of reference solution (b), use a pipette previously rinsed with reference solution (a).

Reference solution (b)

Dilute 1.00 ml of reference solution (a) to 50.0 ml with *water R*.

Before preparing reference solutions (c), (d) and (e), rinse all pipettes with reference solution (b).

Reference solution (c) Dilute 1.00 ml of reference solution (b) to 100.0 ml with *water R* (corresponding to 1 ppm of nitrite in the test sample).

Reference solution (d) Dilute 3.00 ml of reference solution (b) to 100.0 ml with *water R* (corresponding to 3 ppm of nitrite in the test sample).

Reference solution (e) Dilute 5.00 ml of reference solution (b) to 100.0 ml with *water R* (corresponding to 5 ppm of nitrite in the test sample).

The chromatographic procedure may be carried out using:

— a column 0.125 m long and 4.3 mm in internal diameter packed with a strong anion-exchange resin;

— as mobile phase at a flow rate of 1.0 ml/min a solution consisting of 13.61 g of *sodium acetate R* dissolved in *water R*, adjusted to pH 4.3 with *phosphoric acid R* and diluted to 1000 ml with *water R*;

— as detector an appropriate electrochemical device with the following characteristics and settings: a suitable working electrode, a detector potential of + 1.00 V versus Ag/AgCl reference electrode and a detector sensitivity of 0.1 µA full scale.

Inject 100 µl of reference solution (d). When the chromatograms are recorded in the prescribed conditions, the retention time for nitrite is 3.3 to 4.0 min. The test is not valid unless:

— the number of theoretical plates calculated for the nitrite peak is at least 7000 per metre per column (dalteparin sodium will block the binding sites of the stationary phase, which will cause shorter retention times and lower separation efficiency for the analyte; the initial performance of the column may be partially restored using a 58 g/l solution of *sodium chloride R* at a flow rate of 1.0 ml/min for 1 h; after regeneration the column is rinsed with 200 ml to 400 ml of *water R*);

— the symmetry factor for the nitrite peak is less than 3;

— the relative standard deviation of the peak area for nitrite obtained from 6 injections is less than 3.0 per cent.

Inject 100 µl each of reference solutions (c) and (e). The test is not valid unless:

— the correlation factor for a linear relationship between concentration and response for reference solutions (c), (d) and (e) is at least 0.995;

— the signal-to-noise ratio for reference solution (c) is not less than 5 (if the noise level is too high, electrode recalibration is recommended);

— a blank injection of *water R* does not give rise to spurious peaks.

Inject 100 µl of the test solution. Calculate the content of nitrite from the peak areas in the chromatogram obtained with reference solutions (c), (d) and (e).

Boron

Not more than 1 ppm, determined by inductively coupled plasma atomic emission spectroscopy.

Boron is determined by measurement of the emission from an inductively coupled plasma (ICP) at a wavelength specific to boron. The emission line at 249.733 nm is used. Use an appropriate apparatus, whose settings have been optimised as directed by the manufacturer.

Test solution Dissolve 0.2500 g of the substance to be examined in about 2 ml of *water for chromatography R*, add 100 µl of *nitric acid R* and dilute to 10.00 ml with the same solvent.

Reference solution (a) Prepare a 1 per cent V/V solution of *nitric acid R* in *water for chromatography R* (blank).

Reference solution (b) Prepare a 11.4 µg/ml solution of *boric acid R* in a 1 per cent *V/V* solution of *nitric acid R* in *water for chromatography R* (STD_{cal}).

Reference solution (c) Dissolve 0.2500 g of a reference dalteparin sodium with no detectable boron in about 2 ml of *water for chromatography R*, add 100 µl of *nitric acid R* and dilute to 10.00 ml with the same solvent (STD_0).

Reference solution (d) Dissolve 0.2500 g of a reference dalteparin sodium with no boron detected in about 2 ml of a 1 per cent *V/V* solution of *nitric acid R* in *water for chromatography*

R, add 10 µl of a 5.7 mg/ml solution of *boric acid R* and dilute to 10.00 ml with the same solvent (STD_1). This solution contains 1 µg/ml of boron.

Calculate the content of boron in the substance to be examined, using the following correction factor:

$$f = \frac{(STD_1 - STD_0) \times 2}{(STD_{cal} - blank)}$$

Loss on drying ***(2.2.32)***

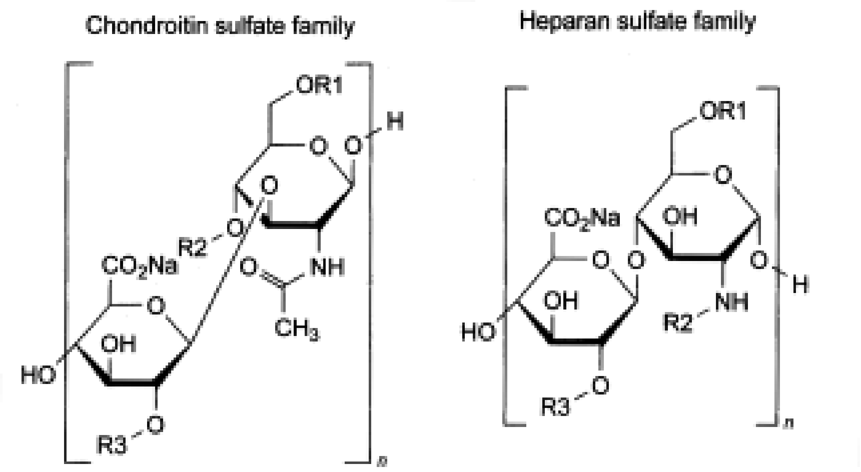
Not more than 5.0 per cent, determined on 1.000 g by drying in an oven at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Ph Eur

Danaparoid Sodium

General Notices

(Ph Eur monograph 2090)



ΔDi	R1	R2	R3
-OS	H	H	H
-6S	SO_3Na	H	H
-4S	H	SO_3Na	H
-US	H	H	SO_3Na
-(U,6)S	SO_3Na	H	SO_3Na
-(U,4)S	H	SO_3Na	SO_3Na
-(4,6)S	SO_3Na	SO_3Na	H
-(U,4,6)S	SO_3Na	SO_3Na	SO_3Na

ΔDiHS	R1	R2	R3
-OS	H	Ac	H
-6S	SO_3Na	Ac	H
-NS	H	SO_3Na	H
-US	H	Ac	SO_3Na
-(U,N)S	H	SO_3Na	SO_3Na
-(6,N)S	SO_3Na	SO_3Na	H
-(U,N,6)S	SO_3Na	SO_3Na	SO_3Na

83513-48-8

Action and use

Heparinoid; prevention of deep vein thrombosis.

Ph Eur

DEFINITION

Preparation containing the sodium salts of a mixture of sulphated glycosaminoglycans present in porcine tissues. Its major constituents are heparan sulphate and dermatan sulphate. On complete hydrolysis it liberates D-glucosamine, D-galactosamine, D-glucuronic acid, L-iduronic acid, acetic acid and sulphuric acid. It has the characteristic property of enhancing the inactivation of activated factor X (factor Xa) by antithrombin. It has a negligible effect on the inactivation rate of thrombin by antithrombin.

Potency

11.0 to 17.0 anti-factor Xa units per milligram (dried substance).

PRODUCTION

Danaparoid sodium is prepared from the intestinal mucosa of pigs. It is prepared using a process that ensures that the relative proportion of active sulphated glycosaminoglycans is consistent. It is produced by methods of manufacturing designed to minimise or eliminate endotoxins and hypotensive substances.

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water.

IDENTIFICATION

A. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay and Tests respectively, is not less than 22.

B. It complies with the test for molecular mass distribution (see Tests): the mass-average relative molecular mass ranges between 4000 and 7000.

TESTS

pH *(2.2.3)*

5.5 to 7.0.

Dissolve 0.5 g of the dried substance to be examined in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Anti-factor IIa activity

Maximum 0.5 units per milligram (dried substance).

Test solutions Prepare 2 independent series of dilutions in geometric progression of the substance to be examined in *phosphate buffer solution pH 6.5 R* and in the concentration range of 0.0005 to 0.005 units of anti-factor IIa activity per millilitre.

Reference solutions Prepare 2 independent series of dilutions in geometric progression of *danaparoid sodium CRS* in *phosphate buffer solution pH 6.5 R* and in the concentration range of 0.0005 to 0.005 units of anti-factor IIa activity per millilitre.

Transfer 50 µl of each solution into the wells of a 96-well microtitre plate. To each well add 50 µl of *antithrombin III solution R3* and 50 µl of *human thrombin solution R1*. Shake the microtitre plate but do not allow bubbles to form. Incubate for 75 min. To each well add 50 µl of *chromogenic substrate R4*. Shake the microtitre plate. Measure the absorbances at 405 nm *(2.2.25)* using a suitable reading device, exactly 4 min after the addition of the chromogenic substrate. The reaction may be stopped using 75 µl of a 20 per cent *V/V* solution of *glacial acetic acid R*. Determine the blank amidolytic activity in a similar manner, using *phosphate* *buffer solution pH 6.5 R* as the blank solution (minimum 10 blanks per microtitre plate). Calculate the activity of the substance to be examined in units of anti-factor IIa activity per milligram using a suitable statistical method, for example the parallel-line assay.

Chondroitin sulphate and dermatan sulphate

Chondroitin sulphate: maximum 8.5 per cent (dried substance); dermatan sulphate: 8.0 per cent to 16.0 per cent (dried substance).

Determine by selective enzymatic degradation.

Test solutions Dry the substance to be examined at 60 °C over *diphosphorus pentoxide R* at a pressure of about 670 Pa for 3 h. Dissolve 0.200 g of the dried substance in 10.0 ml of *water R*. Dilute this solution as necessary to obtain 3 test solutions containing 20 mg/ml, 10 mg/ml and 5 mg/ml of the dried substance to be examined in *water R*.

Chondroitin sulphate reference solutions Dry *chondroitin sulphate CRS* over *diphosphorus pentoxide R* at room temperature at a pressure of about 670 Pa for 16 h. Prepare solutions containing 1 mg/ml, 2 mg/ml and 3 mg/ml of dried *chondroitin sulphate CRS* in *water R*.

Dermatan sulphate reference solutions Dry *dermatan sulphate CRS* over *diphosphorus pentoxide R* at room temperature at a pressure of about 670 Pa for 16 h. Prepare solutions containing 1 mg/ml, 2 mg/ml and 3 mg/ml of dried *dermatan sulphate CRS* in *water R*.

Chondroitinase ABC solution Dissolve *chondroitinase ABC R* in *tris-sodium acetate-sodium chloride buffer solution pH 8.0 R* to obtain an activity of 0.5-1.0 units per millilitre.

Chondroitinase AC solution Dissolve *chondroitinase AC R* in *tris-sodium acetate-sodium chloride buffer solution pH 7.4 R* to obtain an activity of 1.0-2.0 units per millilitre.

Procedure:

— *Degradation with chondroitinase ABC*: label 2 sets of 10 tubes in triplicate: T1, T2 and T3 for the test solutions; SD1, SD2 and SD3 for the dermatan sulphate reference solutions; SC1, SC2 and SC3 for the chondroitin sulphate reference solutions; and B for the blank (*water R*). To each tube add 1.25 ml of *tris-sodium acetate buffer solution pH 8.0 R* and 150 µl of the test solutions, dermatan sulphate reference solutions, chondroitin sulphate reference solutions or *water R*. To each tube in 1 set of tubes add 75 µl of chondroitinase ABC solution. To determine the blank response level, add 75 µl *of tris-sodium acetate-sodium chloride buffer solution pH 8.0 R* to each tube in the other set of tubes. Mix the contents of the tubes using a vortex mixer, cover with appropriate stoppers and incubate at 37 °C for at least 24 h.

— *Degradation with chondroitinase AC*: label 7 tubes in triplicate: T1, T2 and T3 for the test solutions; SC1, SC2 and SC3 for the chondroitin sulphate reference solutions; and B for the blank (*water R*). To each tube add 1.25 ml of *tris-sodium acetate buffer solution pH 7.4 R* and 150 µl of the test solutions, chondroitin sulphate reference solutions or *water R*. Add 75 µl of chondroitinase AC solution to each tube. Mix the contents of the tubes using a vortex mixer, cover with appropriate stoppers and incubate at 37 °C for at least 24 h. After the incubation period mix the contents of the tubes using a vortex mixer and dilute to 12 times with *water R*. Measure the absorbances *(2.2.25)* of the diluted solutions at 234 nm against *water R* using a suitable spectrophotometer.

Calculation Calculate the mean blank absorbance of each reference solution, i.e. the mean of the absorbances of the reference solutions to which no chondroitinase ABC has been added. Subtract the mean blank absorbance value from the individual absorbance of each reference solution. Calculate linear regression curves for the 2 chondroitin sulphate reference and the dermatan sulphate reference by plotting the blank-corrected absorbances against the concentrations.

Calculate the average percentage content of dermatan sulphate in the test solutions of all tested concentrations using the following expression:

$$\frac{A_2 - A_1 - \dfrac{(A_3 - A_1 - I_1) \times B_2}{B_1} - I_2 - I_3}{B_3 \times C} \times 100$$

A_1 = blank absorbance of the test solution;

A_2 = absorbance of the test solution with chondroitinase ABC;

A_3 = absorbance of the test solution with chondroitinase AC;

B_1 = gradient of the curve obtained with the chondroitin sulphate reference solutions with chondroitinase AC;

B_2 = = gradient of the curve obtained with the chondroitin sulphate reference solutions with chondroitinase ABC;

B_3 = gradient of the curve obtained with the dermatan sulphate reference solutions with chondroitinase ABC;

C = concentration of the test solution, in milligrams per millilitre;

I_1 = y-intercept of the curve obtained with the chondroitin sulphate reference solutions with chondroitinase AC;

I_2 = y-intercept of the curve obtained with the chondroitin sulphate reference solutions with chondroitinase ABC;

I_3 = y-intercept of the curve obtained with the dermatan sulphate reference solutions with chondroitinase ABC.

Calculate the average percentage content of chondroitin sulphate in the test solutions for all tested concentrations using the following expression:

$$\frac{(A_3 - A_1 - I_1) \times 100}{B_1 \times C}$$

Molecular mass distribution

Size-exclusion chromatography *(2.2.30)*.

Test solution Dissolve 10 mg of the substance to be examined in 2 ml of the mobile phase.

Reference solution Dissolve 10 mg of *danaparoid sodium CRS* in 2 ml of the mobile phase.

Column:

— *size:* *l* = 0.60 m, Ø = 7.5 mm;

— *stationary phase*: *hydrophilic silica gel for chromatography R* (10 µm) with a fractionation range for proteins with a relative molecular mass of approximately 5000-100 000;

— *temperature*: 30 °C.

Mobile phase 28.4 g/l solution of *anhydrous sodium sulphate R* adjusted to pH 5.0 with *dilute sulphuric acid R*.

Flow rate 0.9 ml/min ± 2 per cent.

Detection Spectrophotometer at 210 nm.

Injection 100 µl.

Run time For a period of time ensuring complete elution of sample and solvent peaks (about 40 min).

System suitability Inject the reference solution twice.

The difference between the retention times corresponding to the maxima of the peaks is not more than 5 s.

Calibration Calibration is achieved by taking the relevant part of the chromatogram obtained with the reference solution, i.e. excluding the sharp peak at the end of the chromatogram, and matching the chromatogram obtained with the test solution with the calibration table obtained with the reference solution. From the calibration curve obtained, determine the molecular mass distribution of the sample. A calibration table is supplied with *danaparoid sodium CRS*.

Limits:

— *chains with a relative molecular mass less than 2000*: maximum 13 per cent;

— *chains with a relative molecular mass less than 4000*: maximum 39 per cent;

— *chains with a relative molecular mass between 4000 and 8000*: minimum 50 per cent;

— *chains with a relative molecular mass higher than 8000*: maximum 19 per cent;

— *chains with a relative molecular mass higher than 10 000*: maximum 11 per cent.

Nitrogen *(2.5.9)*

2.4 per cent to 3.0 per cent (dried substance).

Nucleic acids

Maximum 0.5 per cent (dried substance).

Test solution Weigh about 50 mg of the dried substance to be examined into a centrifuge tube and dissolve in 200 µl of *water R*.

Reference solution Dissolve about 50 mg of *ribonucleic acid CRS* in 5 ml of *0.1 M sodium hydroxide* and dilute to 20.0 ml with *water R*. Transfer 200 µl of the solution into a centrifuge tube.

Add 4.0 ml of a 50 g/l solution of *trichloroacetic acid R* to each tube and mix. Place all tubes in boiling water for 30 min. Allow to cool to room temperature. Add again 4.0 ml of a 50 g/l solution of *trichloroacetic acid R* to each tube and mix. If any of the test solutions is not clear, sonicate all the tubes in an ultrasonic bath for 10 min and centrifuge at 1500 g for 15 min. Dilute 1.0 ml of the clear supernatant to 4.0 ml with *water R*. Measure the absorbances of the diluted reference and test solutions at 265 nm *(2.2.25)* against a blank solution prepared in the same manner, and calculate the percentage nucleic acid content of the sample.

Total protein *(2.5.33, Method 2)*

Maximum 0.5 per cent.

Dissolve the substance to be examined in *water R*. Use *bovine albumin R* as the reference substance.

Sodium

9.0 per cent to 11.0 per cent (dried substance).

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 0.125 g of the substance to be examined in 100.0 ml of a 1.27 mg/ml solution of *caesium chloride R* in *0.1 M hydrochloric acid*.

Reference solutions
Prepare reference solutions containing 50 ppm, 100 ppm and 150 ppm of Na by diluting *sodium standard solution (1000 ppm Na) R* with a 1.27 mg/ml solution of *caesium chloride R* in *0.1 M hydrochloric acid*.

Source Sodium hollow-cathode lamp.

Wavelength 330.3 nm.

Atomisation device Air-acetylene flame.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 0.500 g by drying in an oven at 60 °C over *diphosphorus pentoxide R* at a pressure of 670 Pa for 3 h.

Bacterial endotoxins *(2.6.14)*

Less than 0.02 IU per unit of anti-factor Xa activity, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

The anticoagulant activity of danaparoid sodium is determined *in vitro* by an assay which determines its ability to accelerate the inhibition of factor Xa by antithrombin III (anti-factor Xa assay).

Test solutions Prepare 2 independent series of dilutions in geometric progression of the substance to be examined in *tris(hydroxymethyl)aminomethane EDTA buffer solution pH 8.4 R* and in the concentration range of 0.1 to 0.32 units of anti-factor Xa activity per millilitre.

Reference solutions Prepare 2 independent series of dilutions in geometric progression of *danaparoid sodium CRS* in *tris(hydroxymethyl)aminomethane EDTA buffer solution pH 8.4 R* and in the concentration range of 0.08 to 0.35 units of anti-factor Xa activity per millilitre.

Transfer 40 µl of each solution into the wells of a 96-well microtitre plate. Add 40 µl of *antithrombin III solution R4* to each well and shake the microtitre plate but do not allow bubbles to form. Add 40 µl of *bovine factor Xa solution R1* to each well. Exactly 2 min after the addition of the factor Xa solution, add 80 µl of *chromogenic substrate R5*. Measure the absorbance at 405 nm *(2.2.25)* using a suitable reading device, exactly 4 min after the addition of the factor Xa solution. The reaction may be stopped using 75 µl of a 20 per cent *V/*

V solution of *glacial acetic acid R*. Determine the blank amidolytic activity in the same manner, using *tris(hydroxymethyl)aminomethane EDTA buffer solution pH 8.4 R* as the blank (minimum 8 blanks per microtitre plate). Calculate the potency of the substance to be examined in units of anti-factor Xa activity per milligram using a suitable statistical method, for example the parallel-line assay.

STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

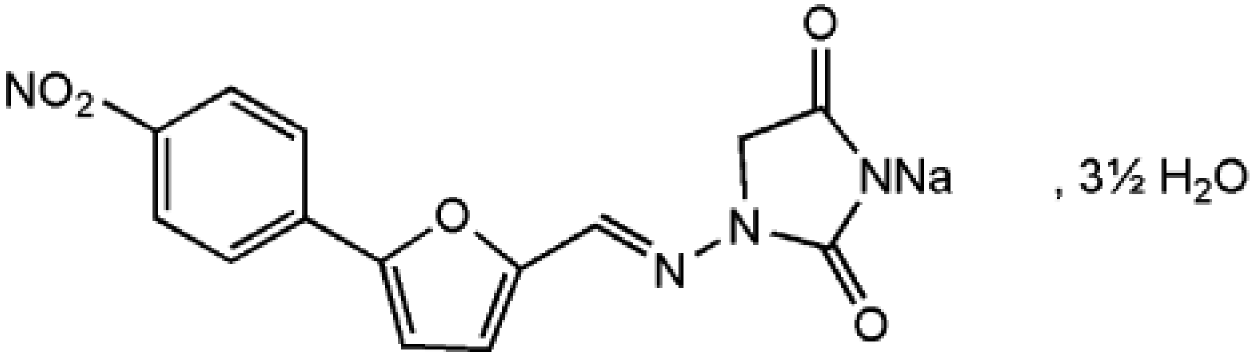
LABELLING

The label states the number of units of anti-factor Xa activity per milligram.

Ph Eur

Dantrolene Sodium

General Notices



$C_{14}H_9N_4NaO_5,3½H_2O$ 399.3 *24868-20-0*

Action and use

Skeletal muscle relaxant.

Preparation

Dantrolene Oral Suspension

DEFINITION

Dantrolene Sodium is 1-(5-*p*-nitrophenylfurfurylideneamino)hydantoin sodium. It contains not less than 98.0% and not more than 102.0% of $C_{14}H_9N_4NaO_5$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A yellowish-orange to orange crystalline powder.

Very slightly soluble in *water*; slightly soluble in *ethanol (96%)*; sparingly soluble in *methanol*; practically insoluble in *acetone*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of dantrolene sodium (*RS 422*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. To 0.1 g of the substance being examined add 20 ml of *water* and 2 drops of *acetic acid*, shake well and filter. The filtrate yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

Shake 0.7 g in 10 ml of *water* for 5 minutes and centrifuge. To 5 ml of the supernatant add 45 ml of *water* and 3 drops of *phenolphthalein solution R1* and 0.1 ml of 0.1M *hydrochloric acid VS*. A red colour is not produced.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve 50 mg of the substance being examined in 20 ml of *tetrahydrofuran* and 2 ml of *glacial acetic acid* and dilute with sufficient *absolute ethanol* to produce 100 ml.

(2) Dilute 1 ml of solution (1) to 100 ml with *absolute ethanol*.

(3) Dissolve 5 mg of *dantrolene sodium BPCRS* and 0.1 g of *theophylline BPCRS* in 20 ml of *tetrahydrofuran* and 2 ml of *glacial acetic acid* and dilute with sufficient *absolute ethanol* to produce 100 ml. Further dilute 10 ml of this solution to 100 ml with *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Zorbax Sil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Adjust the flow rate of the mobile phase so that the retention time of the peak corresponding to Dantrolene Sodium is about 8 minutes.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 300 nm.

(f) Inject 10 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak.

MOBILE PHASE

9 volumes of *absolute ethanol*, 10 volumes of *glacial acetic acid* and 90 volumes of *hexane*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to theophylline and dantrolene is at least 6.

LIMITS

In the chromatogram obtained with solution (1):

the total area of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Heavy metals

1.0 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (20 ppm).

Water

14.5 to 17.0% w/w, Appendix IX C. Use 0.2 g

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve 60 mg of the substance being examined in 50 ml of *dimethylformamide* and dilute 1 volume of the resulting solution to 100 volumes with the mobile phase.

(2) Dilute 1 volume of a 0.12% w/v solution of *dantrolene sodium BPCRS* in *dimethylformamide* to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with spherical particles of silica, 5 µm in diameter, the surface of which has been modified with chemically-bonded nitrile groups (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 262 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

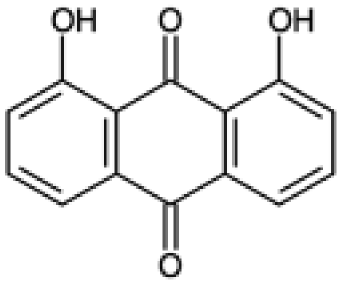
15 volumes of *acetonitrile* and 85 volumes of a phosphate buffer pH 6.8 prepared by dissolving 11.88 g of *disodium hydrogen orthophosphate* and 9.08 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_9N_4NaO_5$ in the substance being examined using the declared content of $C_{14}H_9N_4NaO_5$ in *dantrolene sodium BPCRS*.

Dantron

General Notices



$C_{14}H_8O_4$ 240.2 *117-10-2*

Action and use

Anthraquinone stimulant laxative.

Preparation

Co-danthrusate Capsules

DEFINITION

Dantron is mainly 1,8-dihydroxyanthraquinone. It contains not less than 98.0% and not more than 102.0% of total phenols, calculated as $C_{14}H_8O_4$ and with reference to the dried substance.

CHARACTERISTICS

An orange, crystalline powder.

Practically insoluble in *water*; slightly soluble in *ether*; very slightly soluble in *ethanol (96%)*. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of dantron *(RS 083)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in *dichloromethane* exhibits maxima at 255 nm and 285 nm and a less well-defined maximum at 275 nm. The *absorbance* at the maximum at 255 nm is about 0.82 and at the maximum at 285 nm is about 0.48, each calculated with reference to the dried substance.

C. Dissolve 5 mg in 5 ml of 1M *sodium hydroxide*. A clear red solution is produced immediately.

TESTS

Mercury

To 0.50 g in a Kjeldahl flask add 2.5 ml of *nitric acid* and allow to stand until the initial vigorous reaction has subsided. Add 2.5 ml of *sulphuric acid* and heat until dense white fumes are evolved. Cool, add 2.5 ml of *nitric acid* and heat until fumes are again evolved. Repeat the procedure with a further 2.5 ml of *nitric acid*, cool, add 50 ml of *water*, boil the solution until the volume has been reduced to about 25 ml and cool. Transfer to a separating funnel using *water*, dilute to about 50 ml with *water* and add 50 ml of 0.5M *sulphuric acid*. Add 100 ml of *water*, 2 g of *hydroxylamine hydrochloride*, 1 ml of 0.05M *disodium edetate*, 1 ml of *glacial acetic acid* and 5 ml of *dichloromethane*, shake, allow to separate and discard the dichloromethane layer. Titrate the aqueous layer with a 0.0008% w/v solution of *dithizone* in *dichloromethane*, shaking vigorously after each addition, allowing the layers to separate and discarding the dichloromethane layer, until the dichloromethane layer remains green. Repeat the operation using a solution prepared by diluting 1 ml of *mercury standard solution (5 ppm Hg)* to 100 ml with 0.5M *sulphuric acid* and beginning at the words 'Add 100 ml of *water* …'. The volume of the dithizone solution required by the substance being examined does not exceed that required by the mercury standard solution.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve 50 mg of the substance being examined in 20 ml of *tetrahydrofuran* and dilute to 100 ml with the mobile phase.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase.

(3) Dissolve 50 mg of *dantron impurity standard BPCRS* in 20 ml of *tetrahydrofuran* and dilute to 100 ml with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use an isocratic system using the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µl of each solution.

(g) Allow the chromatography to proceed for 1.5 times the retention time of the principal peak.

MOBILE PHASE

A mixture of 2.5 volumes of *glacial acetic acid*, 40 volumes of *tetrahydrofuran* and 60 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

— a peak due to 1-hydroxyanthraquinone appears immediately before the principal peak, as indicated in the reference chromatogram supplied with *dantron impurity standard BPCRS*;

— the height of the trough separating the two peaks is not greater than one third of the height of the peak due to 1-hydroxyanthraquinone.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to 1-hydroxyanthraquinone is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.3% taking into account the correction factor of the impurity);

— the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

— disregard any peak with a retention time less than one third of that of the principal peak.

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

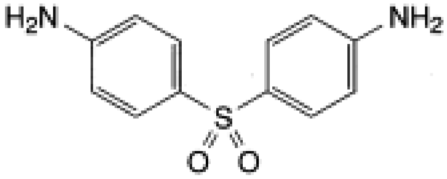
ASSAY

Dissolve 0.2 g in 50 ml of *anhydrous pyridine* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 24.02 mg of total phenols, calculated as $C_{14}H_8O_4$.

Dapsone

General Notices

(Ph Eur monograph 0077)



$C_{12}H_{12}N_2O_2S$ 248.3 *80-08-0*

Action and use

Folic acid synthesis inhibitor; treatment of leprosy.

Preparation

Dapsone Tablets

Ph Eur

DEFINITION

Dapsone contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4,4′-sulphonyldianiline, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish-white, crystalline powder, very slightly soluble in water, freely soluble in acetone, sparingly soluble in alcohol. It dissolves freely in dilute mineral acids.

IDENTIFICATION

A. Melting point *(2.2.14)*: 175 °C to 181 °C.

B. Dissolve 50.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *methanol R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows 2 absorption maxima, at 260 nm and 295 nm. The specific absorbances at these maxima are 700 to 760 and 1150 to 1250, respectively.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 10 mg of *dapsone CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 10 ml with *methanol R*.

Reference solution (c) Dilute 2 ml of reference solution (b) to 10 ml with *methanol R*.

Apply separately to the plate 1 µl of test solution (b), 1 µl of reference solution (a), 10 µl of test solution (a), 10 µl of reference solution (b) and 10 µl of reference solution (c). Develop in an unsaturated tank over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 6 volumes of *methanol R*, 20 volumes of *ethyl acetate R* and 20 volumes of *heptane R*. Allow the plate to dry in air. Spray the plate with a 1 g/l solution of *4-dimethylaminocinnamaldehyde R* in a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *alcohol R*. Examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than 2 such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Loss on drying *(2.2.32)*

Not more than 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 50 ml of *dilute hydrochloric acid R*. Carry out the determination of primary aromatic amino-nitrogen *(2.5.8)*.

1 ml of *0.1 M sodium nitrite* is equivalent to 12.42 mg of $C_{12}H_{12}N_2O_2S$.

STORAGE

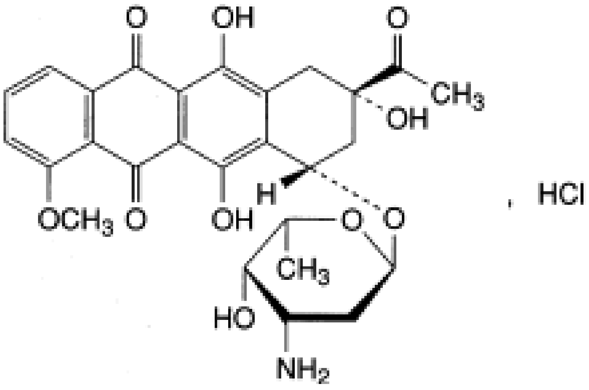
Store protected from light.

Ph Eur

Daunorubicin Hydrochloride

General Notices

(Ph Eur monograph 0662)



$C_{27}H_{30}ClNO_{10}$ 564.0 *23541-50-6*

Action and use

Cytostatic; anthracycline antibacterial.

Ph Eur

DEFINITION

(8*S*,10*S*)-8-Acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride.

Substance produced by certain strains of *Streptomyces coeruleorubidus* or of *Streptomyces peucetius* or obtained by any other means.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise the presence of histamine.

CHARACTERS

Appearance

Crystalline, orange-red powder, hygroscopic.

Solubility

Freely soluble in water and in methanol, slightly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison daunorubicin hydrochloride CRS.

B. Dissolve about 10 mg in 0.5 ml of *nitric acid R*, add 0.5 ml of *water R* and heat over a flame for 2 min. Allow to cool and add 0.5 ml of *silver nitrate solution R1*. A white precipitate is formed.

TESTS

pH *(2.2.3)*

4.5 to 6.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *daunorubicin hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *doxorubicin hydrochloride CRS* and 10 mg of *epirubicin hydrochloride CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *daunorubicinone CRS* and 5.0 mg of *doxorubicin hydrochloride CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 200.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.0 mm,

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mixture of equal volumes of *acetonitrile R* and a solution containing 2.88 g/l of *sodium laurilsulfate R* and 2.25 g/l of *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 5 µl; inject the test solution and reference solutions (b), (c) and (d).

Run time Twice the retention time of daunorubicin.

Relative retention With reference to daunorubicin (retention time = about 15 min): impurity A = about 0.4; impurity D = about 0.5; epirubicin = about 0.6; impurity B = about 0.7.

System suitability Reference solution (b):

— *resolution*: minimum of 2.0 between the peaks due to impurity D and epirubicin.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (d) (1.5 per cent),

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent),

— *total of other impurities*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (2.5 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Butanol *(2.4.24, System B)*

Maximum 1.0 per cent.

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 0.100 g.

Bacterial endotoxins *(2.6.14)*

Less than 4.3 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

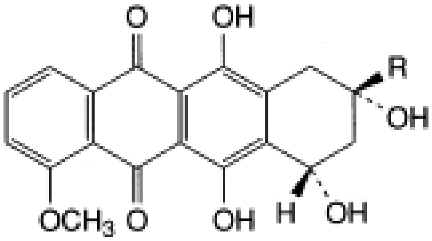
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{27}H_{30}ClNO_{10}$.

STORAGE

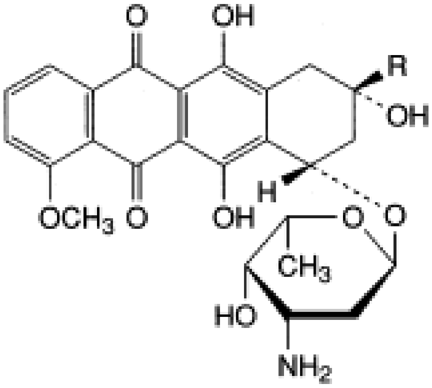
In an airtight container , protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

IMPURITIES



A. R = $CO-CH_3$: (8*S*,10*S*)-8-acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicin aglycone, daunorubicinone),

E. R = $CHOH-CH_3$: (8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-[(1*RS*)-1-hydroxyethyl]-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (13-dihydrodaunorubicinone),



B. R = $CHOH-CH_3$: (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-[(1*RS*)-1-hydroxyethyl]-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicinol),

C. R = $CH_2-CO-CH_3$: (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-8-(2-oxopropyl)-7,8,9,10-tetrahydrotetracene-5,12-dione (feudomycin B),

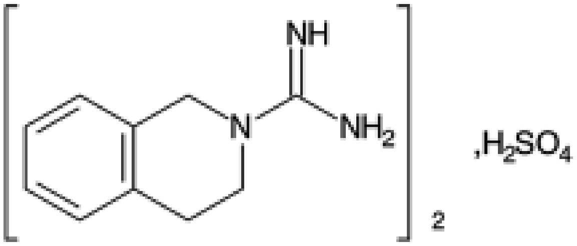
D. R = $CO-CH_2-OH$: doxorubicin,

F. R = $CO-CH_2-CH_3$: (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-8-propanoyl-7,8,9,10-tetrahydrotetracene-5,12-dione (8-ethyldaunorubicin).

Ph Eur

Debrisoquine Sulphate

General Notices



$(C_{10}H_{13}N_3)_2,H_2SO_4$ 448.5 *581-88-4*

Action and use

Adrenergic neuron blocker.

DEFINITION

Debrisoquine Sulphate is 1,2,3,4-tetrahydroisoquinoline-2-carboxamidine sulphate. It contains not less than 99.0% and not more than 101.0% of $(C_{10}H_{13}N_3)_2,H_2SO_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Sparingly soluble in *water*; very slightly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.05% w/v solution in 0.05M *sulphuric acid* exhibits two maxima, at 262 nm and 270 nm. The *absorbance* at the maximum at 262 nm is about 0.69 and at the maximum at 270 nm is about 0.51.

B. Carry out the method described under Related substances, applying separately to the plate 10 µl of each of the following solutions in *water*. Solution (1) contains 0.25% w/v of the substance being examined. Solution (2) contains 0.25% w/v of *debrisoquine sulphate BPCRS*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

C. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH of a 3% w/v solution, 5.3 to 6.8, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 60 volumes of *butan-1-ol*, 25 volumes of *water* and 15 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in *water* containing (1) 2.0% w/v of the substance being examined and (2) 0.010% w/v of *debrisoquine sulphate BPCRS*. After removal of the plate, allow it to dry in air and spray with a solution prepared by adding 1 ml of *sulphuric acid* to 40 ml of a freshly prepared mixture of equal volumes of a 0.135% w/v solution of *chloroplatinic(IV) acid* and a 1.1% w/v solution of *potassium iodide*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 1 g and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 44.85 mg of $(C_{10}H_{13}N_3)_2,H_2SO_4$.

STORAGE

Debrisoquine Sulphate should be protected from light.

Decyl Oleate

General Notices

(Ph Eur monograph 1307)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture consisting of decyl esters of fatty acids, mainly oleic (*cis*-9-octadecenoic) acid.

A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, pale yellow or colourless liquid.

Solubility

Practically insoluble in water, miscible with ethanol (96 per cent), with methylene chloride and with light petroleum (40-60 °C).

IDENTIFICATION

A. Relative density (see Tests).

B. Saponification value (see Tests).

C. Oleic acid (see Tests).

TESTS

Relative density *(2.2.5)*

0.860 to 0.870.

Acid value *(2.5.1)*

Maximum 1.0, determined on 10.0 g.

Iodine value *(2.5.4, Method A)*

55 to 70.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0.

Saponification value ***(2.5.6)***

130 to 140, determined on 2.0 g.

Oleic acid ***(2.4.22, Method A)***

Minimum 60.0 per cent in the fatty acid fraction of the substance.

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.00 g.

Total ash ***(2.4.16)***

Maximum 0.1 per cent, determined on 2.0 g.

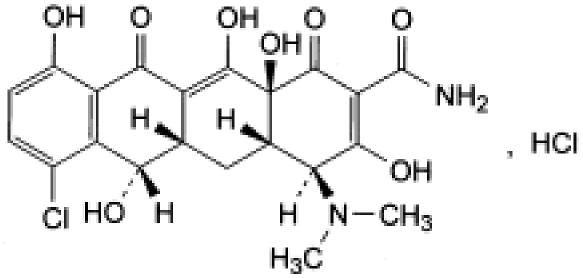
STORAGE

Protected from light.

Demeclocycline Hydrochloride

General Notices

(Ph Eur monograph 0176)



$C_{21}H_{21}ClN_2O_8,HCl$ 501.3 *64-73-3*

Action and use

Tetracycline antibacterial.

Preparation

Demeclocycline Capsules

Ph Eur

DEFINITION

(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-Chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by certain strains of *Streptomyces aureofaciens* or obtained by any other means.

Content

89.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow powder.

Solubility

Soluble or sparingly soluble in water, slightly soluble in alcohol, very slightly soluble in acetone. It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *demeclocycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *demeclocycline hydrochloride CRS*, 5 mg of *chlortetracycline hydrochloride R* and 5 mg *of tetracycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A violet colour develops. Add the solution to 2.5 ml of *water R*. The colour becomes yellow.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

2.0 to 3.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 248 to - 263 (anhydrous substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Specific absorbance *(2.2.25)*

340 to 370 determined at the maximum at 385 nm (anhydrous substance).

Dissolve 10.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. To 10.0 ml of the solution add 12 ml of *dilute sodium hydroxide solution R* and dilute to 100.0 ml with *water R*.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 25.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 25.0 mg of *demeclocycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 5.0 mg of *4-epidemeclocycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c) Mix 1.0 ml of reference solution (a) and 5.0 ml of reference solution (b) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (d) Dilute 5.0 ml of reference solution (a) to 100.0 ml with *0.01 M hydrochloric acid*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm),

— *temperature*: 60 °C,

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R*; add 100 ml of a 35 g/l solution *of dipotassium hydrogen phosphate R* adjusted to pH 9.0 with *dilute phosphoric acid R*, 150 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R*; dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl; inject the test solution and reference solutions (c) and (d).

System suitability Reference solution (c):

— *resolution*: minimum of 2.8 between the peaks due to impurity B (1st peak) and demeclocycline (2nd peak); if necessary, adjust the 2-methyl-2-propanol content of the mobile phase or lower the pH of the mobile phase,

— *symmetry factor*: maximum 1.25 for the peak due to demeclocycline.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (5.0 per cent), and not more than 1 such peak has an area greater than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (d) (4.0 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (10.0 per cent),

— *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Heavy metals *(2.4.8)*

Maximum 50 ppm.

0.5 g complies with limit test C. Prepare the standard using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 3.0 per cent, determined on 1.000 g.

Sulphated ash ***(2.4.14)***

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

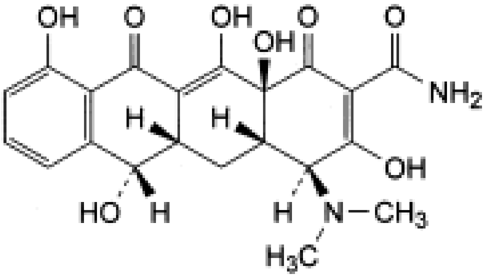
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{21}H_{22}Cl_2N_2O_8$.

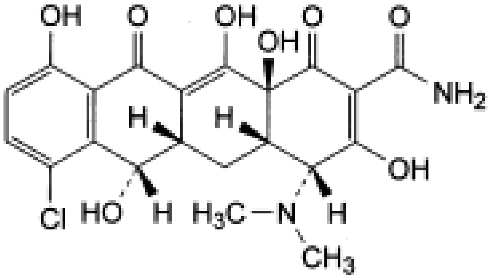
STORAGE

Protected from light.

IMPURITIES



A. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (demethyltetracycline),



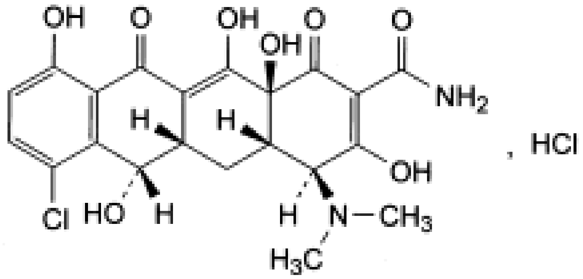
B. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidemeclocycline).

Ph Eur

Demeclocycline Hydrochloride

General Notices

(Ph Eur monograph 0176)



$C_{21}H_{21}ClN_2O_8,HCl$ 501.3 *64-73-3*

Action and use

Tetracycline antibacterial.

Preparation

Demeclocycline Capsules

Ph Eur

DEFINITION

(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-Chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by certain strains of *Streptomyces aureofaciens* or obtained by any other means.

Content

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CHARACTERS

Appearance

Yellow powder.

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IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *demeclocycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *demeclocycline hydrochloride CRS*, 5 mg of *chlortetracycline hydrochloride R* and 5 mg *of tetracycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A violet colour develops. Add the solution to 2.5 ml of *water R*. The colour becomes yellow.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

2.0 to 3.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 248 to - 263 (anhydrous substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Specific absorbance *(2.2.25)*

340 to 370 determined at the maximum at 385 nm (anhydrous substance).

Dissolve 10.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. To 10.0 ml of the solution add 12 ml of *dilute sodium hydroxide solution R* and dilute to 100.0 ml with *water R*.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 25.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 25.0 mg of *demeclocycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 5.0 mg of *4-epidemeclocycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c) Mix 1.0 ml of reference solution (a) and 5.0 ml of reference solution (b) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (d) Dilute 5.0 ml of reference solution (a) to 100.0 ml with *0.01 M hydrochloric acid*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm),

— *temperature*: 60 °C,

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R*; add 100 ml of a 35 g/l solution *of dipotassium hydrogen phosphate R* adjusted to pH 9.0 with *dilute phosphoric acid R*, 150 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R*; dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl; inject the test solution and reference solutions (c) and (d).

System suitability Reference solution (c):

— *resolution*: minimum of 2.8 between the peaks due to impurity B (1st peak) and demeclocycline (2nd peak); if necessary, adjust the 2-methyl-2-propanol content of the mobile phase or lower the pH of the mobile phase,

— *symmetry factor*: maximum 1.25 for the peak due to demeclocycline.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (5.0 per cent), and not more than 1 such peak has an area greater than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (d) (4.0 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (10.0 per cent),

— *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Heavy metals *(2.4.8)*

Maximum 50 ppm.

0.5 g complies with limit test C. Prepare the standard using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

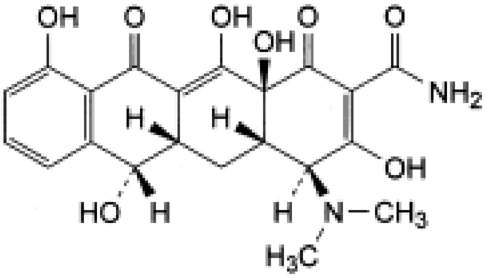
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{21}H_{22}Cl_2N_2O_8$.

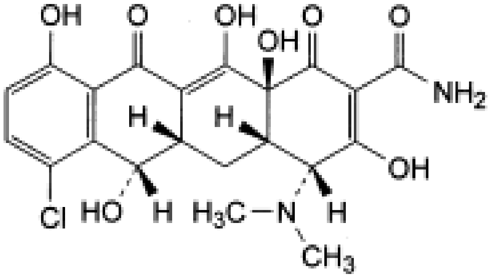
STORAGE

Protected from light.

IMPURITIES



A. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (demethyltetracycline),



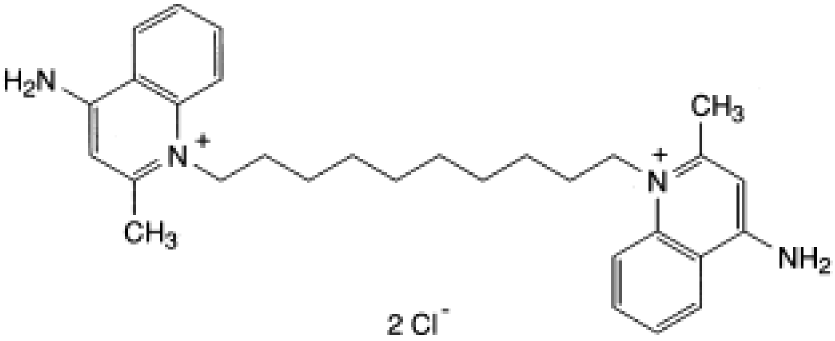
B. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidemeclocycline).

Ph Eur

Dequalinium Chloride

General Notices

(Ph Eur monograph 1413)



$C_{30}H_{40}Cl_2N_4$ 527.6 *522-51-0*

Action and use

Antiseptic.

Ph Eur

DEFINITION

Dequalinium chloride contains not less than 95.0 per cent and not more than the equivalent of 101.0 per cent of 1,1′-(decane-1,10-diyl)bis(4-amino-2-methylquinolinium) dichloride, calculated with reference to the dried substance.

CHARACTERS

A white or yellowish-white powder, hygroscopic, slightly soluble in water and in alcohol.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Dissolve about 10 mg in *water R* and dilute to 100 ml with the same solvent. Dilute 10 ml of the solution to 100 ml with *water R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows 2 absorption maxima, at 240 nm and 326 nm and a shoulder at 336 nm. The ratio of the absorbance measured at the maximum at 240 nm to that measured at the maximum at 326 nm is 1.56 to 1.80 and the ratio of the absorbance measured at the maximum at 326 nm to that measured at the shoulder at 336 nm is 1.12 to 1.30.

B. Examine by infrared absorption spectrophotometry between 600 cm^{-1} and 2000 cm^{-1}

(2.2.24), comparing with the spectrum obtained with *dequalinium chloride CRS*.

C. To 5 ml of solution S (see Tests) add 5 ml of *potassium ferricyanide solution R*. A yellow precipitate is formed.

D. To 10 ml of solution S add 1 ml of *dilute nitric acid R*. A white precipitate is formed. Filter and reserve the filtrate for identification test E.

E. The filtrate from identification test D gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.2 g in 90 ml of *carbon dioxide-free water R*, heating if necessary, and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 5 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *dequalinium chloride for performance test CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *dequalinium chloride CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml to 50.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with an *end-capped octadecylsilyl silica gel for chromatography R*,

— as mobile phase at a flow rate of 1.5 ml/min the following solution: dissolve 2 g of *sodium hexanesulphonate R* in 300 ml of *water R*. Adjust to pH 4.0 with *acetic acid R* and add 700 ml of *methanol R*,

— as detector a spectrophotometer set at 240 nm.

Adjust the sensitivity of the system so that the height of the peak due to impurity B in the chromatogram obtained with 10 µl of reference solution (a) is at least 25 per cent of the full scale of the recorder. Measure the height *(A)* above the baseline of the peak due to impurity B and the height *(B)* above the baseline at the lowest point of the curve separating this peak from the peak due to dequalinium chloride. The test is not valid unless *A* is greater than twice *B*. If necessary, adjust the concentration of methanol in the mobile phase.

Inject 10 µl of the test solution and 10 µl of reference solution (b). Continue the chromatography of the test solution for 5 times the retention time of the peak due to dequalinium chloride. In the chromatogram obtained with the test solution: the area of any peak due to impurity A is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent); and the sum of the areas of all the peaks, apart from the principal peak, is not greater than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (10 per cent). Disregard any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Readily carbonisable substances

Dissolve 20 mg in 2 ml of *sulphuric acid R*. After 5 min the solution is not more intensely coloured than reference solution BY_4 *(2.2.2, Method I)*.

Loss on drying *(2.2.32)*

Not more than 7.0 per cent, determined on 1.000 g by drying at 105 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

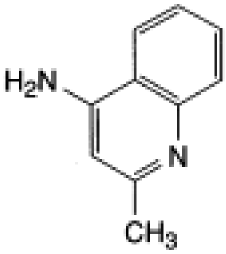
Dissolve 0.200 g in 5 ml of *anhydrous formic acid R* and add 50 ml *of acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 26.38 mg of $C_{30}H_{40}Cl_2N_4$.

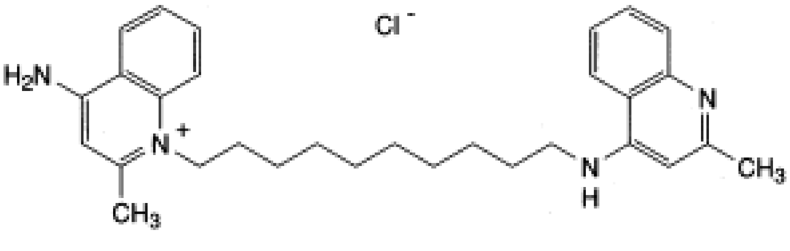
STORAGE

In an airtight container.

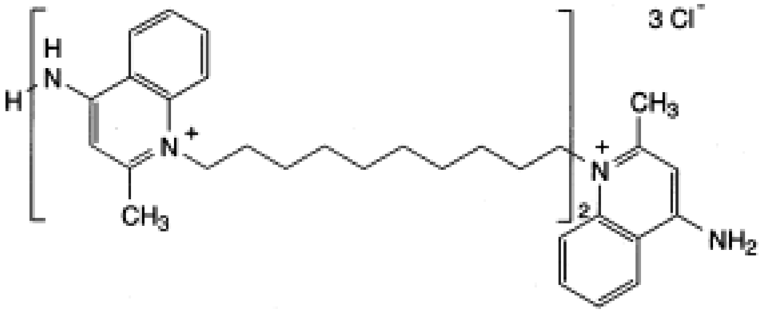
IMPURITIES



A. 2-methylquinolin-4-amine,



B. 4-amino-1-[10-[(2-methylquinolin-4-yl)amino]decyl]-2-methylquinolinium chloride,



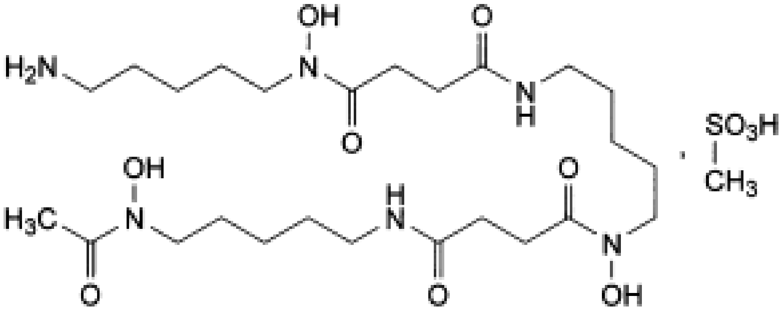
C. 1-[10-(4-amino-2-methylquinolinio)decyl]-4-[[10-(4-amino-2-methylquinolinio)decyl]amino] -2-methylquinolinium trichloride.

Ph Eur

Desferrioxamine Mesilate

General Notices

(Deferoxamine Mesilate, Ph Eur monograph 0896)



$C_{25}H_{48}N_6O_8,CH_4SO_3$ *657* 138-14-7

Action and use

Chelating agent (iron).

Preparation

Desferrioxamine Injection

Ph Eur

DEFINITION

N′-[5-[[4-[[5-(Acetylhydroxyamino)pentyl]amino]-4-oxobutanoyl]hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxybutanediamide methanesulfonate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *deferoxamine mesilate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *ethanol (96 per cent) R*, evaporate to dryness and record new spectra using the residues.

B. Dissolve about 5 mg in 5 ml of *water R*. Add 2 ml of a 5 g/l solution of *trisodium phosphate dodecahydrate R* and 0.5 ml of a 25 g/l solution of *sodium naphthoquinonesulphonate R*. A brownish-black colour develops.

C. Solution A obtained in the Assay is brownish-red. To 10 ml of solution A add 3 ml of *ether R* and shake. The organic layer is colourless. To 10 ml of solution A add 3 ml of *benzyl alcohol R* and shake. The organic layer is brownish-red.

D. Dissolve 0.1 g in 5 ml of *dilute hydrochloric acid R*. Add 1 ml of *barium chloride solution R2*. The solution is clear. In a porcelain crucible, mix 0.1 g with 1 g of *anhydrous sodium carbonate R*, heat and ignite over a naked flame. Allow to cool. Dissolve the residue in 10 ml of *water R*, heating if necessary, and filter. The filtrate gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

pH *(2.2.3)*

3.7 to 5.5 for freshly prepared solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use, protected from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *deferoxamine mesilate CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 25.0 ml with the mobile phase.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Dissolve 1.32 g of *ammonium phosphate R* and 0.37 g of *sodium edetate R* in 950 ml of *water R*; adjust to pH 2.8 with *phosphoric acid R* (about 3-4 ml) and add 55 ml of *tetrahydrofuran R*.

Flow rate 2 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 3 times the retention time of deferoxamine.

System suitability Reference solution (a):

- *resolution*: minimum 1.0 between the peak with a relative retention time of about 0.8 and the principal peak.

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent);
- *total*: not more than 1.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (7.0 per cent);
- *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.08 per cent).

Chlorides *(2.4.4)*

Maximum 330 ppm.

Dilute 2 ml of solution S to 20 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 400 ppm.

Dilute 5 ml of solution S to 20 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.025 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.500 g in 25 ml of *water R*. Add 4 ml of *0.05 M sulphuric acid*. Titrate with *0.1 M ferric ammonium sulphate*. Towards the end of the titration, titrate uniformly and at a rate of about 0.2 ml/min. Determine the end-point potentiometrically *(2.2.20)* using a platinum indicator electrode and a calomel reference electrode. Retain the titrated solution (solution A) for identification test C.

1 ml of *0.1 M ferric ammonium sulphate* is equivalent to 65.68 mg of $C_{26}H_{52}N_6O_{11}S$.

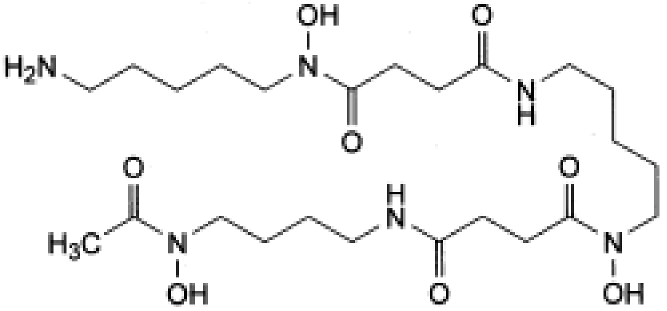
STORAGE

Protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities *A.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *B*.



A. *N′*-[5-[[4-[[4-(acetylhydroxyamino)butyl]amino]-4-oxobutanoyl]hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxybutanediamide (desferrioxamine A_1),

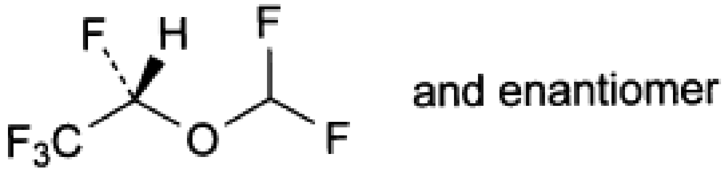
B. other desferrioxamines.

Ph Eur

Desflurane

General Notices

(Ph Eur monograph 1666)



$C_3H_2F_6O$ 168.0 *57041-67-5*

Ph Eur

DEFINITION

(2*RS*)-2-(Difluoromethoxy)-1,1,1,2-tetrafluoroethane.

CHARACTERS

Appearance

Clear, colourless, mobile, heavy liquid.

Solubility

Practically insoluble in water, miscible with anhydrous ethanol.

Relative density

1.47, determined at 15 °C.

bp

About 22 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Examine the substance in the gaseous state.

Comparison Ph. Eur. reference spectrum of desflurane.

TESTS

The substance to be examined must be cooled to a temperature below 10 °C and the tests *must be carried out at a temperature below 20 °C.*

Acidity or alkalinity

To 20 ml add 20 ml of *carbon dioxide-free water R*, shake for 3 min and allow to stand. Collect the upper layer and add 0.2 ml of *bromocresol purple solution R*. Not more than 0.1 ml of *0.01 M sodium hydroxide* or 0.1 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Related substances

Gas chromatography *(2.2.28)*.

Test solution The substance to be examined.

Reference solution (a) Introduce 25 ml of the substance to be examined into a 50 ml flask fitted with a septum, and add 0.50 ml of *desflurane impurity A CRS* and 1.0 ml of *isoflurane CRS* (impurity B). Add 50 µl of *acetone R* (impurity H), 10 µl of *chloroform R* (impurity F) and 50 µl of *methylene chloride R* (impurity E) to the solution, using an airtight syringe, and dilute to 50.0 ml with the substance to be examined. Dilute 5.0 ml of this solution to 50.0 ml with the substance to be examined. Store at a temperature below 10 °C.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the substance to be examined. Store at a temperature below 10 °C.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 25.0 ml with the substance to be examined. Store at a temperature below 10 °C.

Column:

- — *material*: fused silica;
- — *size:* *l* = 105 m, Ø = 0.32 mm;
- — *stationary phase*: *poly[methyl(trifluoropropylmethyl)siloxane] R* (film thickness 1.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.0 ml/min.

Split ratio 1:25.

Temperature:

- — *column*: 30 °C;
- — *injection port*: 150 °C;
- — *detector*: 200 °C.

Detection Flame ionisation.

Injection 2.0 µl.

Run time 35 min.

Relative retention With reference to desflurane (retention time = about 11.5 min): impurity C = about 1.06; impurity D = about 1.09; impurity A = about 1.14; impurity G = about 1.39; impurity E = about 1.5; impurity B = about 1.7; impurity F = about 2.2; impurity H = about 2.6.

System suitability Reference solution (a):

- — *number of theoretical plates*: minimum 20 000, calculated for the peak due to impurity A;
- — *symmetry factor*: maximum 2.0 for the peak due to impurity B.

Limits:

— *impurity B*: not more than the difference between the area of the corresponding peak in the chromatogram obtained with reference solution (a) and the area of the corresponding peak in the chromatogram obtained with the test solution (0.2 per cent *V/V*);

— *impurity A*: not more than the difference between the area of the corresponding peak in the chromatogram obtained with reference solution (a) and the area of the corresponding peak in the chromatogram obtained with the test solution (0.1 per cent *V/V*);

— *impurities C, D, G*: for each impurity, not more than the difference between the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) and the area of the peak due to impurity A in the chromatogram obtained with the test solution (0.01 per cent *V/V*);

— *impurities E, H*: for each impurity, not more than the difference between the area of the corresponding peak in the chromatogram obtained with reference solution (a) and the area of the corresponding peak in the chromatogram obtained with the test solution (0.01 per cent *V/V*);

— *impurity F*: not more than the difference between the area of the corresponding peak in the chromatogram obtained with reference solution (a) and the area of the corresponding peak in the chromatogram obtained with the test solution (0.002 per cent *V/V*);

— *unspecified impurities*: for each impurity, not more than 0.5 times the difference between the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) and the area of the peak due to impurity A in the chromatogram obtained with the test solution (0.005 per cent *V/V*);

— *sum of impurities other than A, B, C, D, E, F, G and H*: not more than the difference between the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) and the area of the peak due to impurity A in the chromatogram obtained with the test solution (0.01 per cent *V/V*);

— *disregard limit*: the difference between the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) and the area of the peak due to impurity A in the chromatogram obtained with the test solution (0.002 per cent *V/V*).

Fluorides

Maximum 10.0 ppm.

Potentiometry *(2.2.36, Method I)*.

Test solution To 10.0 ml in a separating funnel, add 10 ml of a mixture of 30.0 ml of *dilute ammonia R2* and 70.0 ml of *distilled water R.* Shake for 1 min and collect the upper layer. Repeat this extraction procedure twice, collecting the upper layer each time. Adjust the combined upper layers to pH 5.2 with *dilute hydrochloric acid R*. Add 5.0 ml of *fluoride standard solution (1 ppm F) R* and dilute to 50.0 ml with *distilled water R*. To 20.0 ml of this solution add 20.0 ml of *total-ionic-strength-adjustment buffer R* and dilute to 50.0 ml with *distilled water R.*

Reference solutions To each of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml of *fluoride standard solution (10 ppm F) R* add 20.0 ml of *total-ionic-strength-adjustment buffer R* and dilute to 50.0 ml with *distilled water R.*

Indicator electrode Fluoride selective.

Reference electrode Silver-silver chloride.

Carry out the measurements on 20 ml of each solution. Calculate the concentration of fluorides using the calibration curve, taking into account the addition of fluoride to the test solution.

Antimony

Maximum 3.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Solvent mixture *hydrochloric acid R*, *nitric acid R* (50:50 *V/V*).

Test solution Transfer 10 g, cooled to below 10 °C, to a tared flask containing 20 ml of *water R* cooled to below 5 °C. Add 1 ml of the solvent mixture and leave at room temperature until the desflurane has evaporated completely. Subsequently, reduce the volume to about 8 ml on a hot plate. Cool to room temperature and transfer to a volumetric flask. Add 1 ml of the solvent mixture and adjust to 10.0 ml with *water R.*

Reference solutions To each of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml of *antimony standard solution (100 ppm Sb) R* add 20 ml of the solvent mixture and dilute to 100.0 ml with *water R.*

Source Antimony hollow-cathode lamp using a transmission band of 0.2 nm and a 75 per cent lamp current.

Wavelength 217.6 nm.

Atomisation device Air-acetylene flame.

Non-volatile matter

Maximum 100 mg/l.

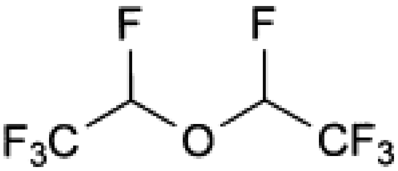
Evaporate 20.0 ml to dryness with the aid of a stream of *nitrogen R*. The residue weighs not more than 2.0 mg.

STORAGE

In a glass bottle fitted with a polyethylene-lined cap. Before opening the bottle, cool the contents to below 10 °C.

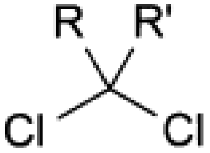
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H.*



A. 1,1′-oxybis(1,2,2,2-tetrafluoroethane),

B. isoflurane,

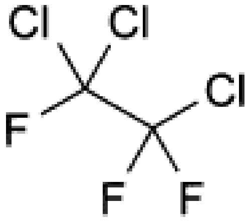


C. R = H, R′ = F: dichlorofluoromethane,

D. R = Cl, R′ = F: trichlorofluoromethane,

E. R = R′ = H: dichloromethane (methylene chloride),

F. R = H, R′ = Cl: trichloromethane (chloroform),



G. 1,1,2-trichloro-1,2,2-trifluoroethane,

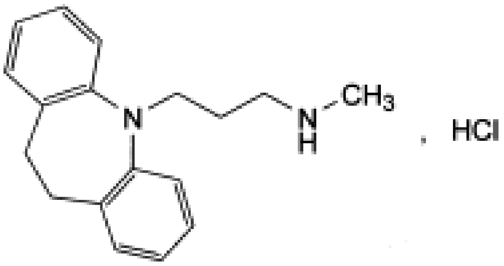
H. acetone.

Ph Eur

Desipramine Hydrochloride

General Notices

(Ph Eur monograph 0481)



$C_{18}H_{22}N_2$,HCl 302.8 *58-28-6*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Desipramine Tablets

Ph Eur

DEFINITION

Desipramine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3-(10,11-dihydro-5*H*-dibenzo[*b,i*]azepin-5-yl)-*N*-methylpropan-1-amine hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, soluble in water and in alcohol.

It melts at about 214 °C.

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. Dissolve 40.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of the solution to 100.0 ml with *0.01 M hydrochloric acid*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 251 nm and a shoulder at 270 nm. The specific absorbance at the maximum is 255 to 285.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *desipramine hydrochloride CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 50 mg in 3 ml of *water R* and add 0.05 ml of a 25 g/l solution of *quinhydrone R* in *methanol R*. An intense pink colour develops within about 15 min.

E. To 0.5 ml of solution S (see Tests) add 1.5 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R*, warming to not more than 30 °C if necessary, and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S, examined immediately after preparation, is not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* and 0.3 ml of *0.01 M sodium hydroxide*. The solution is yellow. Not more than 0.5 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances

Carry out the test protected from bright light. Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in a mixture of equal volumes of *ethanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents. *Prepare immediately before use*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of equal volumes of *ethanol R* and *methylene chloride R*.

Reference solution (a) Dissolve 25 mg of *desipramine hydrochloride CRS* in a mixture of equal volumes of *ethanol R* and *methylene chloride R* and dilute to 25 ml with the same mixture of solvents. *Prepare immediately before use*.

Reference solution (b) Dilute 1 ml of reference solution (a) to 50 ml with a mixture of equal volumes of *ethanol R* and *methylene chloride R*.

Apply to the plate 5 µl of each solution. Develop over a path of 7 cm using a mixture of 1 volume of *water R*, 10 volumes of *anhydrous acetic acid R* and 10 volumes of *toluene R*. Dry the plate in a current of air for 10 min, spray with a 5 g/l solution of *potassium dichromate R* in a mixture of 1 volume of *sulphuric acid R* and 4 volumes of *water R* and examine immediately. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Heavy metals *(2.4.8)*

2.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 4 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.2500 g in a mixture of 5 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 30.28 mg of $C_{18}H_{23}ClN_2$.

STORAGE

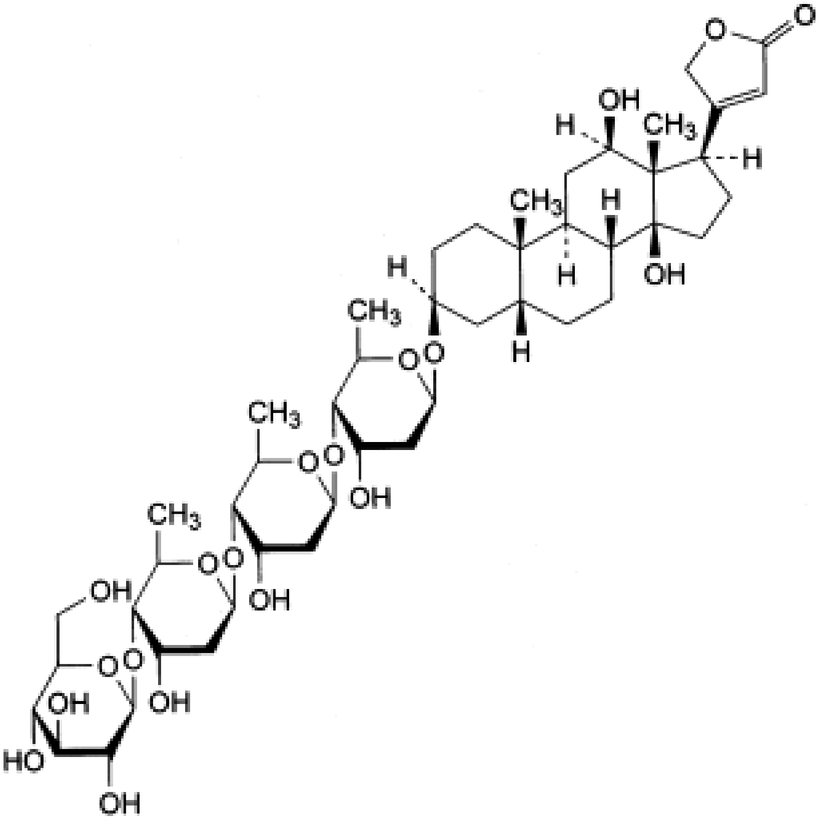
Store protected from light.

Ph Eur

Deslanoside

General Notices

(Ph Eur monograph 0482)



$C_{47}H_{74}O_{19}$ 943.1 *17598-65-1*

Action and use

Na / K-ATPase inhibitor; cardiac glycoside.

Ph Eur

DEFINITION

Deslanoside contains not less than 95.0 per cent and not more than the equivalent of 105.0 per cent of 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β,14β-card-20(22)-enolide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline or finely crystalline powder, hygroscopic, practically insoluble in water, very slightly soluble in alcohol. In an atmosphere of low relative humidity, it loses water.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *deslanoside CRS*. When comparing the spectra, special attention is given to the absence of a distinct absorption maximum at about 1260 cm^{-1} and to the intensity of the absorption maximum at about 1740 cm^{-1}. Examine the substances in discs prepared by dissolving 1 mg of the substance to be examined or 1 mg of the reference substance in 0.3 ml of *methanol R* and triturating with about 0.4 g of dry, finely powdered *potassium bromide R* until the mixture is uniform and completely dry.

B. Examine the chromatograms obtained in the test for related substances. The principal band in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal band in the chromatogram obtained with reference solution (a).

C. Suspend about 0.5 mg in 0.2 ml of *alcohol (60 per cent V/V) R*. Add 0.1 ml of *dinitrobenzoic acid solution R* and 0.1 ml of *dilute sodium hydroxide solution R*. A violet colour develops.

D. Dissolve about 5 mg in 5 ml of *glacial acetic acid R* and add 0.05 ml of *ferric chloride solution R1*. Cautiously add 2 ml of *sulphuric acid R*, avoiding mixing the two liquids. Allow to stand; a brown but not reddish ring develops at the interface and a greenish-yellow, then bluish-green colour diffuses from it to the upper layer.

TESTS

Solution S

Dissolve 0.20 g in a mixture of equal volumes of *chloroform R* and *methanol R* and dilute to 10 ml with the same mixture of solvents.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 0.200 g in *anhydrous pyridine R* and dilute to 10.0 ml with the same solvent. The specific optical rotation is + 6.5 to + 8.5, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Use solution S.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of equal volumes of *chloroform R* and *methanol R*.

Reference solution (a) Dissolve 20 mg of *deslanoside CRS* in a mixture of equal volumes of *chloroform R* and *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dilute 2.5 ml of reference solution (a) to 10 ml with a mixture of equal volumes of *chloroform R* and *methanol R*.

Reference solution (c) Dilute 1 ml of reference solution (a) to 10 ml with a mixture of equal volumes of *chloroform R* and *methanol R*.

Apply separately to the plate as 10 mm bands 5 µl of each solution. Develop immediately over a path of 15 cm using a mixture of 3 volumes of *water R*, 36 volumes of *methanol R* and 130 volumes of *methylene chloride R*. Dry the plate in a current of warm air, spray with a mixture of 5 volumes of *sulphuric acid R* and 95 volumes of *alcohol R* and heat at 140 °C for 15 min. Examine in daylight. In the chromatogram obtained with test solution (a), any band, apart from the principal band, is not more intense than the band in the chromatogram obtained with reference solution (b) (2.5 per cent) and at most two such bands are more intense than the band in the chromatogram obtained with reference solution (c) (1.0 per cent).

Loss on drying *(2.2.32)*

Not more than 5.0 per cent, determined on 0.500 g by drying *in vacuo* at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 50.0 mg in *alcohol R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *alcohol R*. Prepare a reference solution in the same manner, using 50.0 mg of *deslanoside CRS* (undried). To 5.0 ml of each solution add 3.0 ml of *alkaline sodium picrate solution R* and allow to stand protected from bright light in a water-bath at 20 ± 1 °C for 40 min. Measure the absorbance *(2.2.25)* of each solution at the maximum at 484 nm, using as the compensation liquid a mixture of 3.0 ml of *alkaline sodium picrate solution R* and 5.0 ml of *alcohol R* prepared at the same time.

Calculate the content of $C_{47}H_{74}O_{19}$ from the absorbances measured and the concentrations of the solutions.

STORAGE

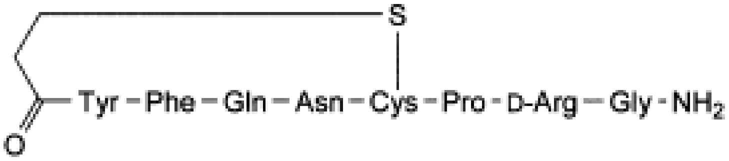
Store in an airtight, glass container, protected from light, at a temperature below 10 °C.

Ph Eur

Desmopressin

General Notices

(Ph Eur monograph 0712)



$C_{46}H_{64}N_{14}O_{12}S_2$ 1069 *16679-58-6*

Action and use

Vasopressin analogue; treatment of diabetes insipidus; nocturnal enuresis; haemophilia; von Willebrand's disease.

Preparations

Desmopressin Tablets

Desmopressin Injection

Desmopressin Intranasal Solution

Ph Eur

DEFINITION

(3-Sulphanylpropanoyl)-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-D-arginylglycinamide cyclic (1→6)-disulfide.

Synthetic cyclic nonapeptide, available as an acetate.

Content

95.0 per cent to 105.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance

White or almost white, fluffy powder.

Solubility

Soluble in water, in ethanol (96 per cent) and in glacial acetic acid.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis *(2.2.56)*. For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, arginine and phenylalanine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; arginine: 0.90 to 1.10; phenylalanine: 0.90 to 1.10; tyrosine: 0.70 to 1.05; half-cystine: 0.30 to 1.05. Lysine, isoleucine and leucine are absent; not more than traces of other amino acids are present.

TESTS

Specific optical rotation *(2.2.7)*

- 72 to - 82 (anhydrous and acetic acid-free substance).

Dissolve 10.0 mg in a 1 per cent *V/V* solution of *glacial acetic acid R* and dilute to 5.0 ml with the same acid.

Related substances

Liquid chromatography *(2.2.29)*: use the normalisation procedure.

Test solution Dissolve 1.0 mg of the substance to be examined in 2.0 ml of *water R*.

Resolution solution Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture CRS* in 500 µl of *water R*.

Column:

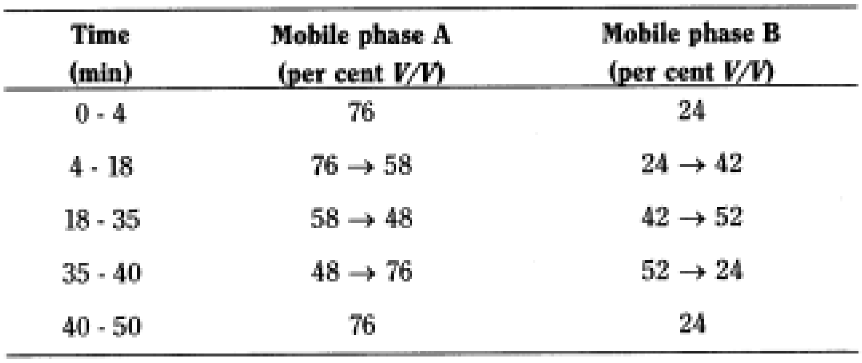
— *size: l* = 0.12 m, Ø = 4.0 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *0.067 M phosphate buffer solution pH 7.0 R*; filter and degas;

— *mobile phase B*: *acetonitrile for chromatography R*, mobile phase A (50:50 *V/V*); filter and degas.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	76	24
4 - 18	76 → 58	24 → 42
18 - 35	58 → 48	42 → 52
35 - 40	48 → 76	52 → 24
40 - 50	76	24

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µl.

Retention time Desmopressin = about 16 min; oxytocin = about 17 min.

System suitability Resolution solution:

— *resolution*: minimum 1.5 between the peaks due to desmopressin and oxytoxin.

Limits:

— *any impurity*: maximum 0.5 per cent;

— *total*: maximum 1.5 per cent;

— *disregard limit*: 0.05 per cent.

Acetic acid ***(2.5.34)***

3.0 per cent to 8.0 per cent.

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Water ***(2.5.32)***

Maximum 6.0 per cent, determined on 20.0 mg.

Bacterial endotoxins ***(2.6.14)***

Less than 500 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Reference solution Dissolve the contents of a vial of *desmopressin CRS* in *water R* to obtain a concentration of 0.5 mg/ml.

Mobile phase Mobile phase B, mobile phase A (40:60 *V/V*).

Flow rate 2.0 ml/min.

Retention time Desmopressin = about 5 min.

Calculate the content of desmopressin ($C_{46}H_{64}N_{14}O_{12}S_2$) from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin CRS*.

STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

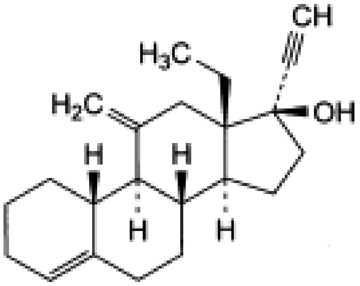
The label states the mass of peptide per container.

Ph Eur

Desogestrel

General Notices

(Ph Eur monograph 1717)



$C_{22}H_{30}O$ 310.5 *54024-22-5*

Action and use

Progestogen.

Preparation

Desogestrel Tablets

Ph Eur

DEFINITION

13-Ethyl-11-methylidene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in methanol, freely soluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *desogestrel CRS.*

B. Specific optical rotation (see Tests).

TESTS

Specific optical rotation *(2.2.7)*

+ 53 to + 57 (dried substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in 25 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*.

Reference solution (a) Dissolve 4 mg of *desogestrel for system suitability CRS* (containing impurities A, B, C and D) in 5 ml of *acetonitrile R1* and dilute to 10.0 ml with *water R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (d) Dissolve 20.0 mg of *desogestrel CRS* in 25 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: sterically protected *octadecylsilyl silica gel for chromatography R* (5 µm)
,

— *temperature*: 50 °C.

Mobile phase *Water R*, *acetonitrile R1* (27:73 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 15 µl of the test solution and reference solutions (a), (b) and (c).

Run time 2.5 times the retention time of desogestrel.

Identification of impurities Use the chromatogram supplied with *desogestrel for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C and D.

Relative retention With reference to desogestrel (retention time = about 22 min): impurity E = about 0.2; impurity D = about 0.25; impurity B = about 0.7; impurity A = about 0.95; impurity C = about 1.05.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to desogestrel.

Limits:

— *correction factors*: for the calculation of content, multiply the peak area of the following impurities by the corresponding correction factor: impurity A = 1.8, impurity D = 1.5;

— *impurities A, B, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at a pressure not exceeding 2 kPa.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

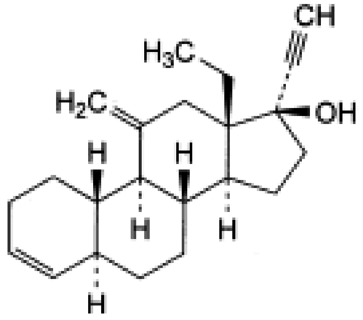
Injection Test solution and reference solution (d).

Calculate the percentage content of $C_{22}H_{30}O$ from the areas of the peaks and the declared content of *desogestrel CRS*.

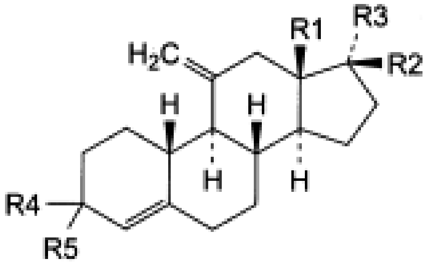
IMPURITIES

Specified impurities A, B, C, D.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See *also 5.10. Control of impurities in substances for pharmaceutical use*): *E*.



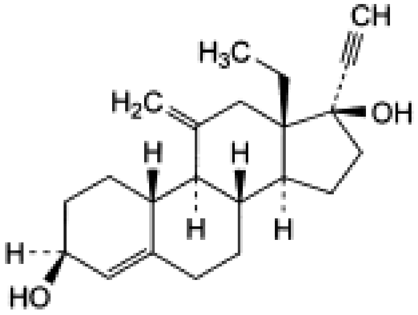
A. 13-ethyl-11-methylidene-18,19-dinor-5α,17α-pregn-3-en-20-yn-17-ol (desogestrel Δ^3-isomer),



B. R1 = CH_3, R2 = OH, R3 = C≡CH, R4 = R5 = H: 11-methylidene-19-nor-17α-pregn-4-en-20-yn-17-ol,

C. R1 = C_2H_5, R2 + R3 = O, R4 = R5 = H: 13-ethyl-11-methylidenegon-4-en-17-one,

D. R1 = C_2H_5, R2 = OH, R3 = C≡CH, R4 + R5 = O: 13-ethyl-17-hydroxy-11-methylidene-18, 19-dinor-17α-pregn-4-en-20-yn-3-one,



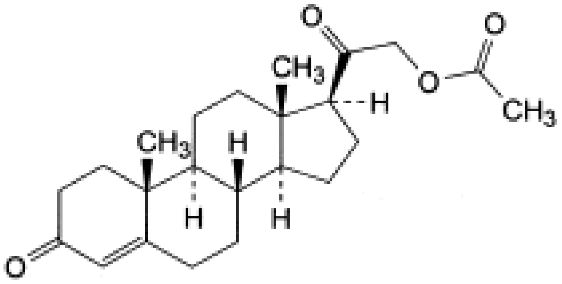
E. 13-ethyl-11-methylidene-18,19-dinor-17α-pregn-4-en-20-yne-3β,17-diol.

Ph Eur

Desoxycortone Acetate

General Notices

(Ph Eur monograph 0322)



$C_{23}H_{32}O_4$ 372.5 *56-47-3*

Action and use

Mineralocorticoid.

Ph Eur

DEFINITION

3,20-Dioxopregn-4-en-21-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, sparingly soluble in ethanol (96 per cent), slightly soluble in propylene glycol and in fatty oils.

IDENTIFICATION

First identification B, C.

Second identification A, C, D, E.

A. Melting point *(2.2.14)*: 157 °C to 161 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *sesoxycortone acetate CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *desoxycortone acetate CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *cortisone acetate R* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*, heat at 120 °C for 10 min or until the spots appear, and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— The chromatogram shows 2 clearly separated spots.

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a yellow colour develops. Add this solution to 2 ml of *water R* and mix. The resulting solution is dichroic, showing an intense blue colour by transparency, and red fluorescence that is particularly intense in ultraviolet light at 365 nm.

E. About 10 mg gives the reaction of acetyl *(2.3.1)*.

TESTS

Specific optical rotation *(2.2.7)*

+ 171 to + 179 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2 mg of *desoxycortone acetate CRS* and 2 mg of *betamethasone 17-valerate CRS* in the mobile phase and dilute to 200.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 350 ml of *water R* with 600 ml of *acetonitrile R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time 3 times the retention time of desoxycortone acetate.

Retention time Betamethasone 17-valerate = about 7.5 min; desoxycortone acetate = about 9.5 min.

System suitability Reference solution (a):

- *resolution*: minimum 4.5 between the peaks due to betamethasone 17-valerate and desoxycortone acetate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 240 nm.

Calculate the content of $C_{23}H_{32}O_4$ taking the specific absorbance to be 450.

STORAGE

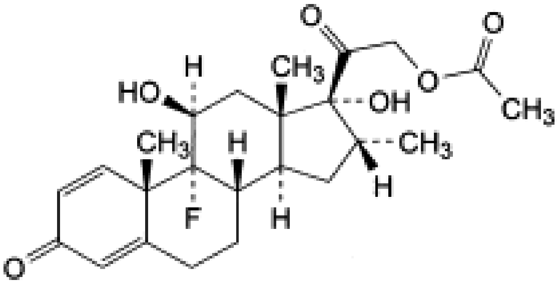
Protected from light.

Ph Eur

Dexamethasone Acetate

General Notices

(Ph Eur monograph 0548)



$C_{24}H_{31}FO_6$ 434.5 *55812-90-3*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

9-Fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent), slightly soluble in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D, E, F.*

A. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* measured at the absorption maximum at 419 nm is not less than 0.35.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dexamethasone acetate CRS*.

If the spectra obtained in the solid state show differences, record new spectra using saturated solutions (about 30 g/l) in *chloroform R* in a 0.2 mm cell.

C. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *dexamethasone acetate CRS* in the solvent and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *cortisone acetate R* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a faint reddish-brown colour develops. Add this solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

E. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

F. About 10 mg gives the reaction of acetyl *(2.3.1)*.

TESTS

Specific optical rotation *(2.2.7*

+ 84 to + 90 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in about 4 ml of *acetonitrile R* and dilute to 10.0 ml with *water R*.

Reference solution (a) Dissolve 2 mg of *dexamethasone acetate CRS* and 2 mg of *betamethasone acetate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 380 ml of *acetonitrile R* with 550 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time 1.5 times the retention time of dexamethasone acetate.

Retention time betamethasone acetate = about 19 min; dexamethasone acetate = about 22 min.

System suitability Reference solution (a):

— *resolution*: minimum 3.3 between the peaks due to betamethasone acetate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than 0.5 times the area of the principal in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than the area of the principal in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 0.500 g by drying *in vacuo* in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 238.5 nm.

Calculate the content of $C_{24}H_{31}FO_6$ taking the specific absorbance to be 357.

STORAGE

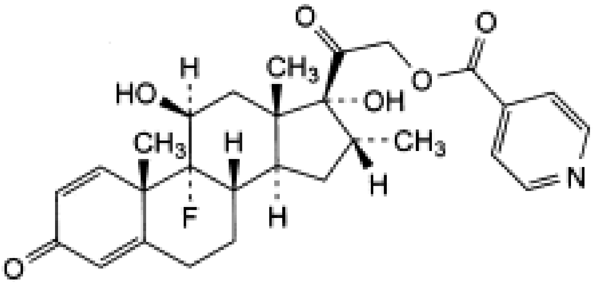
Protected from light.

Ph Eur

Dexamethasone Isonicotinate

General Notices

(Ph Eur monograph 2237)



$C_{28}H_{32}FNO_6$ 497.6 *2265-67-7*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

9-Fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl pyridine-4-carboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in anhydrous ethanol and in acetone.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison dexamethasone isonicotinate CRS.

TESTS

Specific optical rotation *(2.2.7)*

+ 142 to + 146 (dried substance).

Suspend 0.200 g in 4.0 ml of *ethyl acetate R* and dilute to 20.0 ml with *ethanol (96 per cent) R*. Treat in an ultrasonic bath until a clear solution is obtained.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare solutions immediately before use.*

Test solution Suspend 50.0 mg in 7 ml of *acetonitrile R* and dilute to 10.0 ml with *water R*. Treat in an ultrasonic bath until a clear solution is obtained.

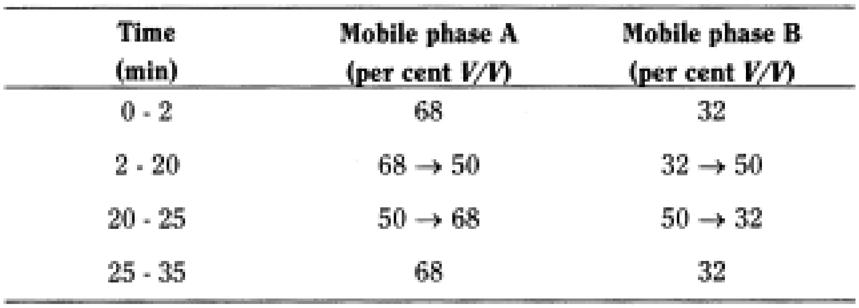
Reference solution (a) Suspend 5.0 mg of *dexamethasone CRS* and 5.0 mg of *dexamethasone acetate CRS* in 70 ml of *acetonitrile R*, add 1.0 ml of the test solution and dilute to 100.0 ml with *water R*. Treat in an ultrasonic bath until a clear solution is obtained.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with *water R*.

Reference solution (c) Suspend 5 mg of *dexamethasone isonicotinate for impurity C identification CRS* in 0.7 ml of *acetonitrile R* and dilute to 1 ml with *water R*. Treat in an ultrasonic bath until a clear solution is obtained.

Column:
- *size: l* = 0.125 m, Ø = 4.0 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A: water R,*
- *mobile phase B: acetonitrile R,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	68	32
2 - 20	68 → 50	32 → 50
20 - 25	50 → 68	50 → 32
25 - 35	68	32

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *dexamethasone isonicotinate for impurity C identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity C.

Relative retention With reference to dexamethasone isonicotinate (retention time = about 12 min): impurity A = about 0.4; impurity C = about 0.6; impurity B = about 0.8.

System suitability Reference solution (a):

- — *resolution*: minimum 5.0 between the peaks due to impurity B and dexamethasone isonicotinate.

Limits:

- — *impurity A*: not more than 5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- — *impurity B*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent),
- — *impurity C*: not more than 3 times the area of the peak due to dexamethasone isonicotinate in the chromatogram obtained with reference solution (b) (0.3 per cent),
- — *unspecified impurities*: for each impurity, not more than the area of the peak due to dexamethasone isonicotinate in the chromatogram obtained with reference solution (b) (0.1 per cent),
- — *total*: not more than 8 times the area of the peak due to dexamethasone isonicotinate in the chromatogram obtained with reference solution (b) (0.8 per cent),
- — *disregard limit*: 0.5 times the area of the peak due to dexamethasone isonicotinate in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 102 °C under high vacuum for 4 h.

ASSAY

Dissolve 0.400 g in a mixture of 5 ml of *anhydrous formic acid R* and 50 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

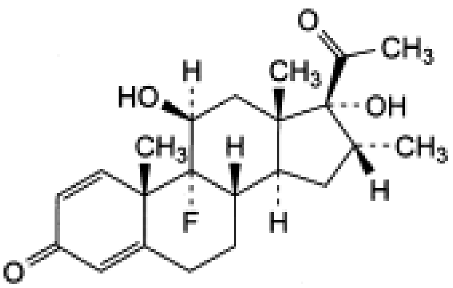
1 ml of *0.1 M perchloric acid* is equivalent to 49.76 mg of $C_{28}H_{32}FNO_6$.

IMPURITIES

Specified impurities A, B, C.

A. dexamethasone,

B. dexamethasone acetate,

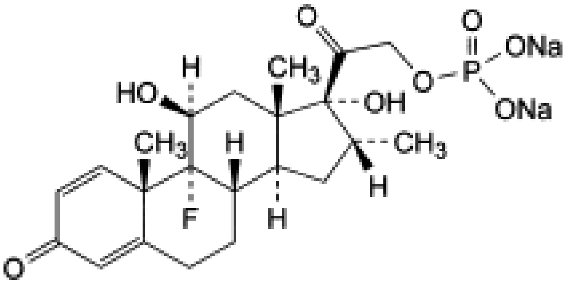


C. 9-fluoro-11,17-dihydroxy-16-methylpregna-1,4-diene-3,20-dione (21-deoxydexamethasone).

Dexamethasone Sodium Phosphate

General Notices

(Ph Eur monograph 0549)



$C_{22}H_{28}FNa_2O_8P$ 516.4 *2392-39-4*

Action and use

Glucocorticoid.

Preparation

Dexamethasone Sodium Phosphate Eye Drops

Dexamethasone Sodium Phosphate Injection

Ph Eur

DEFINITION

9-Fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl disodium phosphate.

Content

97.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, very hygroscopic powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D, E, F.*

A. Dissolve 10.0 mg in 5 ml of *water R* and dilute to 100.0 ml with *anhydrous ethanol R*. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* measured at the absorption maximum at 419 nm is at least 0.20.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dexamethasone sodium phosphate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *dexamethasone sodium phosphate CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *prednisolone sodium phosphate CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *Glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a faint yellowish-brown colour develops. Add this solution to 10 ml of *water R* and mix. The colour fades and a clear solution remains.

E. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

F. To 40 mg add 2 ml of *sulphuric acid R* and heat gently until white fumes are evolved, add *nitric acid R* dropwise, continue the heating until the solution is almost colourless and cool. Add 2 ml of *water R*, heat until white fumes are again evolved, cool, add 10 ml of *water R* and neutralise to *red litmus paper R* with *dilute ammonia R1*. The solution gives reaction (a) of sodium *(2.3.1)* and reaction (b) of phosphates *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

7.5 to 9.5.

Dilute 1 ml of solution S to 5 ml with *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7)*

+ 75 to + 83 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2 mg of *dexamethasone sodium phosphate CRS* and 2 mg of *betamethasone sodium phosphate CRS* in the mobile phase, then dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 250 ml conical flask, weigh 1.360 g of *potassium dihydrogen phosphate R* and 0.600 g of *hexylamine R*, mix and allow to stand for 10 min and then dissolve in 182.5 ml of *water R*; add 67.5 ml of *acetonitrile R*, mix and filter (0.45 µm).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 45 min.

Injection 20 µl.

Run time Twice the retention time of dexamethasone sodium phosphate.

Retention time impurity B = about 12.5 min; dexamethasone sodium phosphate = about 14 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.2 between the peaks due to impurity B and dexamethasone sodium phosphate; if necessary, adjust slightly the concentration of acetonitrile or increase the concentration of water in the mobile phase.

Limits:

— *impurities A, B*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Inorganic phosphates

Maximum 1 per cent.

Dissolve 50 mg in *water R* and dilute to 100 ml with the same solvent. To 10 ml of this solution add 5 ml of *molybdovanadic reagent R*, mix and allow to stand for 5 min. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 10 ml of *phosphate standard solution (5 ppm PO4) R*.

Ethanol

Gas chromatography *(2.2.28)*.

Internal standard solution Dilute 1.0 ml of *propanol R* to 100.0 ml with *water R*.

Test solution Dissolve 0.50 g of the substance to be examined in 5.0 ml of the internal standard solution and dilute to 10.0 ml with *water R*.

Reference solution Dilute 1.0 g of *anhydrous ethanol R* to 100.0 ml with *water R*. To 2.0 ml of this solution add 5.0 ml of the internal standard solution and dilute to 10.0 ml with *water R*.

Column:

— *size: l* = 1 m, Ø = 3.2 mm;

— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R1* (150-180 µm).

Carrier gas *Nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:

— *column*: 150 °C;

— *injection port*: 250 °C;

— *detector*: 280 °C.

Detection Flame ionisation.

Injection 2 µl.

Limit:
— *ethanol* : maximum 3.0 per cent *m/m*.

Ethanol and water

Maximum 13.0 per cent *m/m* for the sum of the percentage contents.

Determine the water content using 0.200 g *(2.5.12)*. Add the percentage content of water and the percentage content of ethanol obtained in the test for ethanol.

ASSAY

Dissolve 0.100 g in *water R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 500.0 ml with *water R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 241.5 nm.

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ taking the specific absorbance to be 303.

STORAGE

In an airtight container , protected from light.

IMPURITIES

Specified impurities A, B.

A. dexamethasone,

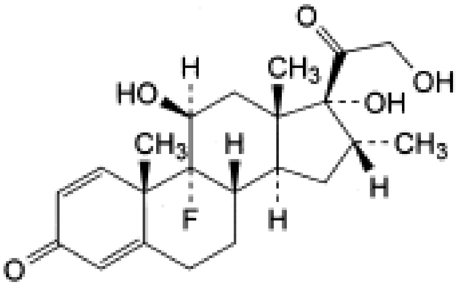
B. betamethasone sodium phosphate.

Ph Eur

Dexamethasone

General Notices

(Ph Eur monograph 0388)



$C_{22}H_{29}FO_5$ 392.5   *50-02-2*

Action and use

Glucocorticoid.

Preparations

Dexamethasone Eye Drops

Dexamethasone and Neomycin Ear Spray

Dexamethasone Tablets

Ph Eur

DEFINITION

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in anhydrous ethanol, slightly soluble in methylene chloride.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D, E.*

A. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Place 2.0 ml of this solution in a stoppered test tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* measured at the absorption maximum at 419 nm is not less than 0.4.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dexamethasone CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *dexamethasone CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *betamethasone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *Butanol R* saturated with *water R*, *toluene R, ether R* (5:10:85 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a faint reddish-brown colour develops. Add this solution to 10 ml of *water R* and mix. The colour is discharged.

E. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Specific optical rotation *(2.2.7)*

+ 75 to + 80 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Place 25.0 mg of the substance to be examined in a 10.0 ml volumetric flask, add 1.5 ml of *acetonitrile R* and then 5 ml of mobile phase A. Mix with the aid of an ultrasonic bath until complete dissolution, and dilute to 10.0 ml with mobile phase A.

Reference solution (a) Dissolve 2 mg of *dexamethasone CRS* and 2 mg of *methylprednisolone CRS* in mobile phase A, then dilute to 100.0 ml with mobile phase A.

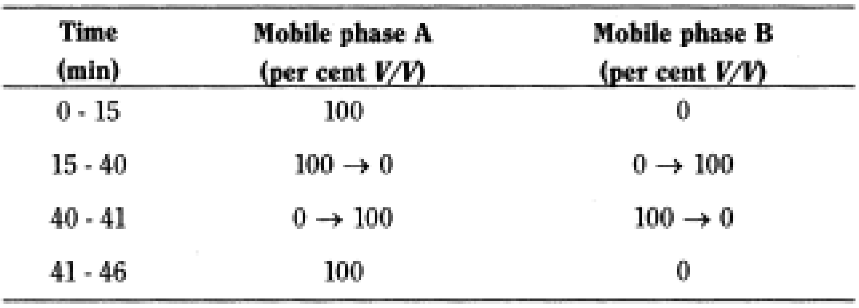
Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Column:

- size: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:

- *mobile phase A*: in a 1000 ml volumetric flask, mix 250 ml of *acetonitrile R* with 700 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 40	100 → 0	0 → 100
40 - 41	0 → 100	100 → 0
41 - 46	100	0

Flow rate 2.5 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With mobile phase B for at least 30 min and then with mobile phase A for 5 min; for subsequent chromatograms, use the conditions described from 40.0 min to 46.0 min.

Injection 20 µl; inject mobile phase A as a blank.

Retention time Methylprednisolone = about 11.5 min; dexamethasone = about 13 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.8 between the peaks due to methylprednisolone and dexamethasone; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Limits:

— *any impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 238.5 nm.

Calculate the content of $C_{22}H_{29}FO_5$ taking the specific absorbance to be 394.

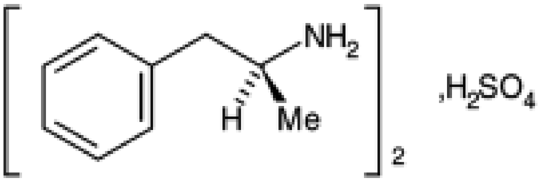
STORAGE

Protected from light.

Ph Eur

Dexamfetamine Sulphate

General Notices



$(C_9H_{13}N)_2,H_2SO_4$ 368.5 *51-63-8*

Action and use

Amfetamine.

Preparation

Dexamfetamine Tablets

DEFINITION

Dexamfetamine Sulphate is (*S*)-α-methylphenethylamine sulphate. It contains not less than 99.0% and not more than 100.5% of $(C_9H_{13}N)_2,H_2SO_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Freely soluble in *water*; slightly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. Dissolve 1 g in 50 ml of *water*, add 10 ml of 5M *sodium hydroxide* and 0.5 ml of *benzoyl chloride* and shake. Repeat the addition of *benzoyl chloride* in 0.5 ml quantities until no further precipitate is produced. The *melting point* of the precipitate, after recrystallising twice from *ethanol (50%)*, is about 157°, Appendix V A.

B. Dissolve 2 mg in 4 ml of *water*, add 1 ml of 1M *hydrochloric acid*, 2 ml of *diazotised nitroaniline solution*, 4 ml of 1M *sodium hydroxide* and 2 ml of *butan-1-ol*, shake and allow to separate. A red colour is produced in the butanol layer (distinction from methylamfetamine).

C. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity or alkalinity

Dissolve 0.5 g in 10 ml of *water* and titrate with 0.01M *hydrochloric acid VS* or 0.01M *sodium* *hydroxide VS* using *methyl red solution* as indicator. Not more than 0.1 ml of 0.01M *hydrochloric acid VS* or 0.01M *sodium hydroxide VS* is required to change the colour of the solution.

Specific optical rotation

In an 8.0% w/v solution, +19.5 to +22.0, calculated with reference to the dried substance, Appendix V F.

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

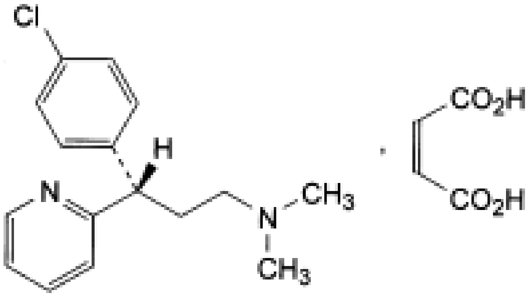
ASSAY

Dissolve 0.4 g in 120 ml of *water*, add 2 ml of 5M *sodium hydroxide* and distil into 50 ml of 0.1M *hydrochloric acid VS*, continuing the distillation until only 5 ml of liquid is left in the distillation flask. Titrate the excess of acid with 0.1M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 18.42 mg of $(C_9H_{13}N)_2,H_2SO_4$.

Dexchlorpheniramine Maleate

General Notices

(Ph Eur monograph 1196)



$C_{16}H_{19}ClN_2,C_4H_4O_4$ 390.9 *2438-32-6*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

(3*S*)-3-(4-Chlorophenyl)-*N,N*-dimethyl-3-(pyridin-2-yl)propan-1-amine (*Z*)-butenedioate.

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), in methanol and in methylene chloride.

IDENTIFICATION

First identification: A, C, E.

Second identification A, B, D, E.

A. Specific optical rotation (see Tests).

B. Melting point *(2.2.14)*: 110 °C to 115 °C.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *dexchlorpheniramine maleate CRS.*

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution Dissolve 56 mg of *maleic acid R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *water R, anhydrous formic acid R, methanol R, di-isopropyl ether R* (3:7:20:70 *V/V/V/V*).

Application 5 µl.

Development Over a path of 12 cm.

Drying In a current of air for a few minutes.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution shows 2 clearly separated spots. The upper spot is similar in position and size to the spot in the chromatogram obtained with the reference solution.

E. To 0.15 g in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 10 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 5.5.

Dissolve 0.20 g in 20 ml of *water R*.

Specific optical rotation *(2.2.7)*

+ 22 to + 23 (dried substance), determined on solution S.

Related substances

Gas chromatography *(2.2.28)*.

Test solution Dissolve 10.0 mg of the substance to be examined in 1.0 ml of *methylene chloride R*.

Reference solution Dissolve 5.0 mg of *brompheniramine maleate CRS* in 0.5 ml of *methylene chloride R* and add 0.5 ml of the test solution. Dilute 0.5 ml of this solution to 50.0 ml with *methylene chloride R*.

Column:

— *material*: glass;

— size: *l* = 2.3 m, Ø = 2 mm;

— *stationary phase*: acid- and base- washed *silanised diatomaceous earth for gas chromatography R* (135-175 µm) impregnated with 3 per cent *m/m* of a mixture of 50 per cent of poly(dimethyl)siloxane and 50 per cent of poly(diphenyl)siloxane.

Carrier gas *Nitrogen for chromatography R*.

Flow rate 20 ml/min.

Temperature:

— *column*: 205 °C;

— *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 2.5 times the retention time of dexchlorpheniramine.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to dexchlorpheniramine and brompheniramine.

Limits:

— *impurities A, B*: for each impurity, not more than 0.8 times the area of the peak due to dexchlorpheniramine in the chromatogram obtained with the reference solution (0.4 per cent);

— *total*: not more than twice the area of the peak due to dexchlorpheniramine in the chromatogram obtained with the reference solution (1 per cent).

Enantiomeric purity

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in 3 ml of *water R*. Add a few drops of *concentrated ammonia R* until an alkaline reaction is produced. Shake with 5 ml of *methylene chloride R*. Separate the layers. Evaporate the lower, methylene chloride layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *dexchlorpheniramine maleate CRS* in 3 ml of *water R*. Add a few drops of *concentrated ammonia R* until an alkaline reaction is produced. Shake with 5 ml of *methylene chloride R*. Separate the layers. Evaporate the lower, methylene chloride layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dissolve 10.0 mg of *chlorphenamine maleate CRS* in 3 ml of *water R*. Add a few drops of *concentrated ammonia R* until an alkaline reaction is produced. Shake with 5 ml of *methylene chloride R*. Separate the layers. Evaporate the lower, methylene chloride layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of the test solution to 50 ml with *2-propanol R*.

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *amylose derivative of silica gel for chromatography R*.

Mobile phase *diethylamine R, 2-propanol R, hexane R* (3:20:980 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Under these conditions the peak of the (*S*)-isomer appears first.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to the (*R*)-enantiomer and to the (*S*)-enantiomer in the chromatogram obtained with reference solution (b);
- the retention times of the principal peaks in the chromatograms obtained with the test solution and reference solution (a) are identical ((*S*)-enantiomer).

Limits:
- *(R)-enantiomer*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2 per cent);
- *any other impurity*: for each impurity, not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 65 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

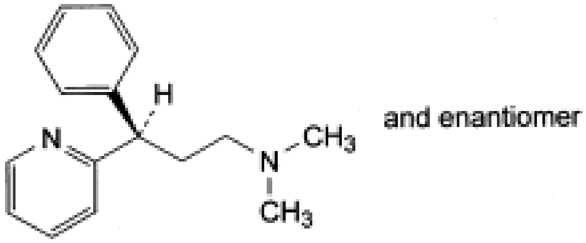
1 ml of *0.1 M perchloric acid* is equivalent to 19.54 mg of $C_{20}H_{23}ClN_2O_4$.

STORAGE

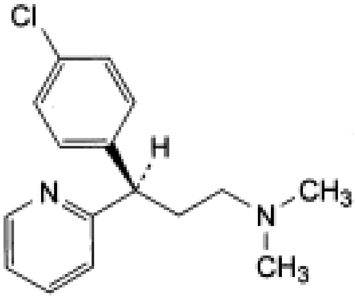
Protected from light.

IMPURITIES

Specified impurities *A, B.*



A. (3*RS*)-*N,N*-dimethyl-3-phenyl-3-(pyridin-2-yl)propan-1-amine,



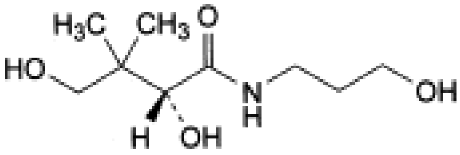
B. (3*R*)-3-(4-chlorophenyl)-*N,N*-dimethyl-3-(pyridin-2-yl)propan-1-amine.

Ph Eur

Dexpanthenol

General Notices

(Ph Eur monograph 0761)



$C_9H_{19}NO_4$ 205.3 *81-13-0*

Action and use

Vitamin B_5 analogue.

Ph Eur

DEFINITION

Dexpanthenol contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of (2*R*)-2,4-dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethylbutanamide, calculated with reference to the anhydrous substance.

CHARACTERS

A colourless or slightly yellowish, viscous hygroscopic liquid, or a white or almost white, crystalline powder, very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *dexpanthenol CRS*. Examine the substances using discs prepared as follows: dissolve the substance to be examined and the reference substance separately in 1.0 ml of *anhydrous ethanol R* to obtain a concentration of 5 mg/ml. Place dropwise 0.5 ml of this solution on a disc of *potassium bromide R*. Dry the disc at 100-105 °C for 15 min.

C. Examine the chromatograms obtained in the test for 3-aminopropanol. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 1 ml of solution S (see Tests) add 1 ml of *dilute sodium hydroxide solution R* and 0.1 ml of *copper sulphate solution R*. A blue colour develops.

TESTS

Solution S

Dissolve 2.500 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is not greater than 10.5.

Specific optical rotation *(2.2.7)*

The specific optical rotation is + 29.0 to + 32.0, determined on solution S and calculated with reference to the anhydrous substance.

3-Aminopropanol

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.25 g of the substance to be examined in *anhydrous ethanol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *anhydrous ethanol R*.

Reference solution (a) Dissolve the contents of a vial of *dexpanthenol CRS* in 1.0 ml of *anhydrous ethanol R* to obtain a concentration of 5 mg/ml.

Reference solution (b) Dissolve 25 mg of *3-aminopropanol R* in *anhydrous ethanol R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *concentrated ammonia R*, 25 volumes of *methanol R* and 55 volumes of *butanol R*. Allow the plate to dry in air, spray with a 100 g/l solution of *trichloroacetic acid R* in *methanol R* and heat at 150 °C for 10 min. Spray with a 1 g/l solution of *ninhydrin R* in *methanol R* and heat at 120 °C until a colour appears. Any spot due to 3-aminopropanol in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

Not more than 1.0 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

To 0.400 g add 50.0 ml of *0.1 M perchloric acid*. Boil under a reflux condenser for 5 h protected from humidity. Allow to cool. Add 50 ml of *dioxan R* by rinsing the condenser, protected from humidity. Add 0.2 ml of *naphtholbenzein solution R* and titrate with *0.1 M potassium hydrogen phthalate* until the colour changes from green to yellow. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 20.53 mg of $C_9H_{19}NO_4$.

STORAGE

In an airtight container.

Ph Eur

Dextran 1 for Injection

General Notices

(Ph Eur monograph 1506)

Action and use

Plasma substitute.

Ph Eur

DEFINITION

Low molecular weight fraction of dextran, consisting of a mixture of isomaltooligosaccharides.

Average relative molecular mass: about 1000.

PRODUCTION

It is obtained by hydrolysis and fractionation of dextrans produced by fermentation of sucrose using *Leuconostoc mesenteroides* strain NRRL B-512 = CIP 78.59 or substrains thereof (for example *L. mesenteroides* B-512 F = NCTC 10817).

It is prepared in conditions designed to minimise the risk of microbial contamination.

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Very soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 3.000 g in *water R*, heat on a water-bath and dilute to 100.0 ml with the same solvent. The specific optical rotation *(2.2.7)* is + 148 to + 164, calculated with reference to the dried substance. Dry an aliquot of the solution first on a water-bath and then to constant weight *in vacuo* at 70 °C. Calculate the dextran content after correction for the content of sodium chloride.

B. Infrared absorption spectrophotometry *(2.2.24).*

Preparation To 1-2 mg add 1 or a few drops of *water R*. Grind in an agate mortar for 1-2 min. Add about 300 mg of *potassium bromide R* and mix to a slurry but do not grind. Dry *in vacuo* at 40 °C for 15 min. Crush the residue. If it is not dry, dry for another 15 min. Prepare a disc using *potassium bromide R*.

Comparison Repeat the operations using *dextran 1 CRS*.

Blank Run the infrared spectrum with a blank disc using *potassium bromide R* in the reference beam.

C. Molecular-mass distribution (see Tests).

TESTS

Solution S

Dissolve 7.5 g in *carbon dioxide-free water R*, heat on a water-bath and dilute to 50 ml with the same solvent.

Absorbance *(2.2.25)*

Maximum 0.12, determined at 375 nm on solution S.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Add 0.2 ml of *0.01 M sodium hydroxide*. The solution is pink. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.1 ml of *methyl red solution R*. The solution is red or orange.

Nitrogen-containing substances

Maximum 110 ppm of N.

Carry out the determination of nitrogen by sulphuric acid digestion *(2.5.9)*, using 0.200 g and heating for 2 h. Collect the distillate in a mixture of 0.5 ml of *bromocresol green solution R*, 0.5 ml of *methyl red solution R* and 20 ml of *water R*. Titrate with *0.01 M hydrochloric acid*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Sodium chloride

Maximum 1.5 per cent.

Accurately weigh 3-5 g and dissolve in 100 ml of *water R*. Add 0.3 ml of *potassium chromate solution R* and titrate with *0.1 M silver nitrate* until the yellowish-white colour changes to reddish-brown.

1 ml of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Molecular-mass distribution

Size-exclusion chromatography *(2.2.30)*.

Test solution Dissolve 6.0-6.5 mg of the substance to be examined in 1.0 ml of the mobile phase.

Reference solution (a) Dissolve 6.0-6.5 mg of *dextran 1 CRS* in 1.0 ml of the mobile phase.

Reference solution (b) Dissolve the content of an ampoule of *isomaltooligosaccharide CRS* in 1 ml of the mobile phase, and mix. This corresponds to approximately 45 µg of isomaltotriose (3 glucose units), approximately 45 µg of isomaltononaose (9 glucose units), and approximately 60 µg of sodium chloride per 100 µl.

Column 2 columns coupled in series:

— *size:* l = 0.30 m, Ø = 10 mm,

— *stationary phase*: dextran covalently bound to highly cross-linked porous agarose beads, allowing resolution of oligosaccharides in the molecular mass range of 180 to 3000,

— *temperature*: 20-25 °C.

Mobile phase 2.92 g/l solution of *sodium chloride R*.

Flow rate 0.07-0.08 ml/min maintained constant to ± 1 per cent.

Detection Differential refractometer.

Injection 100 µl.

Identification of peaks Use the chromatogram obtained with reference solution (b) to identify the peaks due to isomaltotriose, isomaltononaose and sodium chloride.

Determine the peak areas. Disregard any peak due to sodium chloride. Calculate the average relative molecular mass M_w and the amount of the fraction with less than 3 and more than 9 glucose units, of *dextran 1 CRS* and of the substance to be examined, using the following expression:

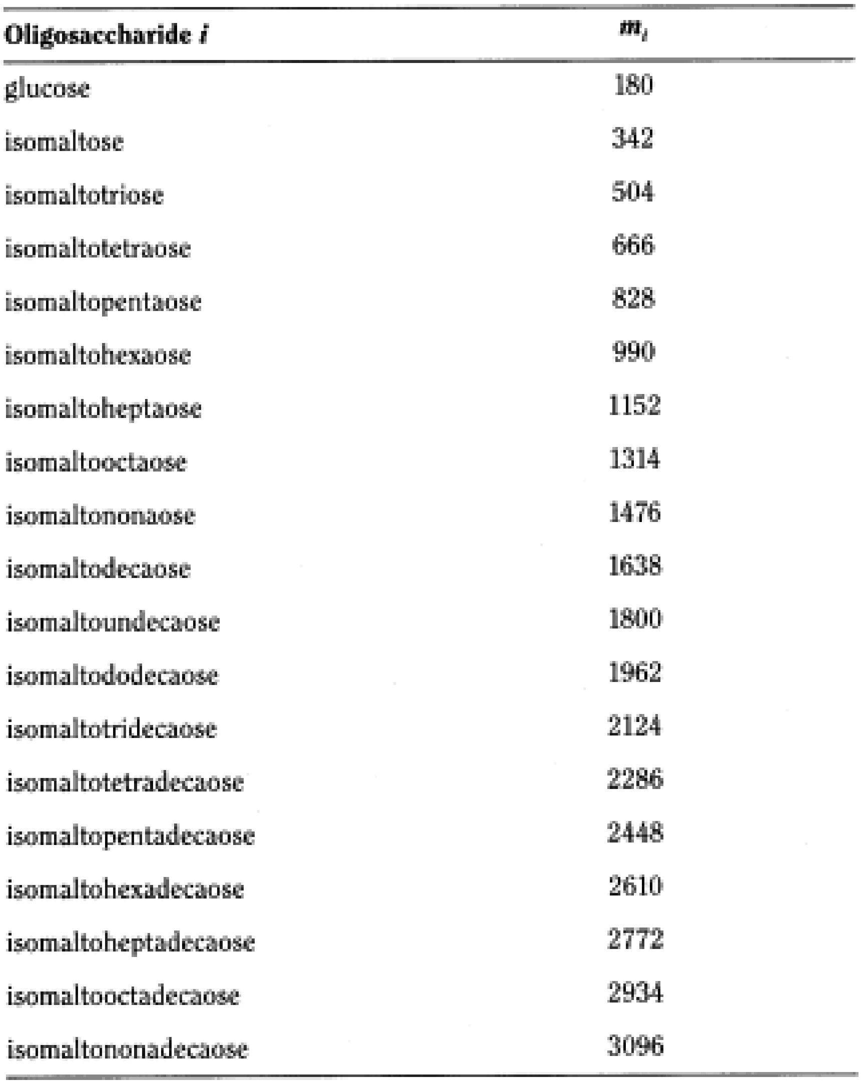
$$M_w = \sum w_i \times m_i$$

M_w = average molecular mass of the dextran,

m_i = molecular mass of oligosaccharide *i*,

w_i = weight proportion of oligosaccharide *i*.

Use the following m_i values for the calculation:



Oligosaccharide i	m_i
glucose	180
isomaltose	342
isomaltotriose	504
isomaltotetraose	666
isomaltopentaose	828
isomaltohexaose	990
isomaltoheptaose	1152
isomaltooctaose	1314
isomaltononaose	1476
isomaltodecaose	1638
isomaltoundecaose	1800
isomaltododecaose	1962
isomaltotridecaose	2124
isomaltotetradecaose	2286
isomaltopentadecaose	2448
isomaltohexadecaose	2610
isomaltoheptadecaose	2772
isomaltooctadecaose	2934
isomaltononadecaose	3096

System suitability The values obtained for *dextran 1 CRS* are within the values stated on the label.

Limits:

- — *average molecular mass (M_w)*: 850 to 1150,
- — *fraction with less than 3 glucose units*: less than 15 per cent,
- — *fraction with more than 9 glucose units*: less than 20 per cent .

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dilute 20 ml of solution S to 30 ml with *water R*. 12 ml of solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 5.000 g by drying in an oven at 105 °C for 5 h.

Bacterial endotoxins *(2.6.14)*

Less than 25 IU/g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^2 micro-organisms per gram, determined by plate-count. It complies with the test for *Escherichia coli (2.6.13)*.

Ph Eur

Dextran 40 for Injection

General Notices

(Ph Eur monograph 0999)

Action and use

Plasma substitute.

Preparation

Dextran 40 Intravenous Infusion

Ph Eur

DEFINITION

Mixture of polysaccharides, principally of the α-1,6-glucan type.

Average relative molecular mass: about 40 000.

PRODUCTION

It is obtained by hydrolysis and fractionation of dextrans produced by fermentation of sucrose using *Leuconostoc mesenteroides* strain NRRL B-512 = CIP 78.59 or substrains thereof (for example *L. mesenteroides* B-512F = NCTC 10817).

It is prepared in conditions designed to minimise the risk of microbial contamination.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: + 195 to + 201 (dried substance).

Dissolve 1.0 g in *water R*, heating on a water-bath, and dilute to 50.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   dextran CRS.

C. Molecular-mass distribution (see Tests).

TESTS

Solution S

Dissolve 5.0 g in *distilled water R*, heating on a water-bath, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution remains colourless. Add 0.2 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.1 ml of *methyl red solution R*. The solution is red or orange.

Nitrogen-containing substances

Maximum 110 ppm N.

Carry out the determination of nitrogen by sulphuric acid digestion *(2.5.9)*, using 0.200 g and heating for 2 h. Collect the distillate in a mixture of 0.5 ml of *bromocresol green solution R*, 0.5 ml of *methyl red solution R* and 20 ml of *water R*. Titrate with *0.01 M hydrochloric acid*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Residual solvents

Gas chromatography *(2.2.28)*.

Internal standard   *propanol R.*

Test solution   Dissolve 5 g of the substance to be examined in 100 ml of *water R* and distil. Collect the first 45 ml of the distillate, add 1 ml of a 25 g/l solution of *propanol R* and dilute to 50 ml *with water R*.

Reference solution   Mix 0.5 ml of a 25 g/l solution of *anhydrous ethanol R*, 0.5 ml of a 25 g/l solution of *propanol R* and 0.5 ml of a 2.5 g/l solution of *methanol R* and dilute to 25.0 ml with *water R*.

Column:
- *material*: stainless steel,
- *size: l* = 1.8 m, Ø = 2 mm,
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (125-150 µm).

Carrier gas   *nitrogen for chromatography R.*

Flow rate   25 ml/min.

Temperature:
- *column*: 190 °C,
- *injection port*: 240 °C,
- *detector*: 210 °C.

Detection   Flame ionisation.

Injection The chosen volume of each solution.

Limits:

- *ethanol*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent),
- *methanol*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent),
- *sum of solvents other than ethanol, methanol and propanol*: not more than the area of the peak due to the internal standard (0.5 per cent, calculated as propanol).

Molecular-mass distribution *(2.2.39)*

The average molecular mass (M_w) is 35 000 to 45 000. The average molecular mass of the 10 per cent high fraction is not greater than 110 000. The average molecular mass of the 10 per cent low fraction is not less than 7000.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 7.0 per cent, determined on 0.200 g by heating in an oven at 105 ± 2 °C for 5 h.

Sulphated ash *(2.4.14)*

Maximum 0.3 per cent, determined on 0.50 g.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^2 micro-organisms per gram, determined by plate-count. It complies with the test for *Escherichia coli (2.6.13)*.

Ph Eur

Dextran 60 for Injection

General Notices

(Ph Eur monograph 1000)

Action and use

Plasma substitute.

Ph Eur

DEFINITION

Mixture of polysaccharides, principally of the α-1,6-glucan type.

Average relative molecular mass: about 60 000.

PRODUCTION

It is obtained by hydrolysis and fractionation of dextrans produced by fermentation of sucrose using *Leuconostoc mesenteroides* strain NRRL B-512 = CIP 78.59 or substrains thereof (for example *L. mesenteroides* B-512F = NCTC 10817).

It is prepared in conditions designed to minimise the risk of microbial contamination.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: + 195 to + 201 (dried substance).

Dissolve 1.0 g in *water R*, heating on a water-bath, and dilute to 50.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison dextran CRS.

C. Molecular-mass distribution (see Tests).

TESTS

Solution S

Dissolve 5.0 g in *distilled water R*, heating on a water-bath, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution remains colourless. Add 0.2 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.1 ml of *methyl red solution R*. The solution is red or orange.

Nitrogen-containing substances

Maximum 110 ppm N.

Carry out the determination of nitrogen by sulphuric acid digestion *(2.5.9)*, using 0.200 g and heating for 2 h. Collect the distillate in a mixture of 0.5 ml of *bromocresol green solution R*, 0.5 ml of *methyl red solution R* and 20 ml of *water R*. Titrate with *0.01 M hydrochloric acid*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Residual solvents

Gas chromatography *(2.2.28)*.

Internal standard *propanol R.*

Test solution Dissolve 5 g of the substance to be examined in 100 ml of *water R* and distil. Collect the first 45 ml of the distillate, add 1 ml of a 25 g/l solution of *propanol R* and dilute to 50 ml *with water R*.

Reference solution Mix 0.5 ml of a 25 g/l solution of *anhydrous ethanol R*, 0.5 ml of a 25 g/l solution of *propanol R* and 0.5 ml of a 2.5 g/l solution of *methanol R* and dilute to 25.0 ml with *water R*.

Column:

— *material*: stainless steel,

— *size:* l = 1.8 m, Ø = 2 mm,

— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (125-150 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 25 ml/min.

Temperature:

— *column*: 190 °C,

— *injection port*: 240 °C,

— *detector*: 210 °C.

Detection Flame ionisation.

Injection The chosen volume of each solution.

Limits:

— *ethanol*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent),

— *methanol*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent),

— *sum of solvents other than ethanol, methanol and propanol*: not more than the area of the peak due to the internal standard (0.5 per cent, calculated as propanol).

Molecular-mass distribution *(2.2.39)*

The average molecular mass (M_w) is 54 000 to 66 000. The average molecular mass of the 10 per cent high fraction is not greater than 180 000. The average molecular mass of the 10 per cent low fraction is not less than 14 000.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 7.0 per cent, determined on 0.200 g by heating in an oven at 105 ± 2 °C for 5 h.

Sulphated ash *(2.4.14)*

Maximum 0.3 per cent, determined on 0.50 g.

Bacterial endotoxins *(2.6.14)*

Less than 16 IU/g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^2 micro-organisms per gram, determined by plate-count. It complies with the test for *Escherichia coli (2.6.13)*.

Ph Eur

Dextran 70 for Injection

General Notices

(Ph Eur monograph 1001)

Action and use

Plasma substitute.

Preparation

Dextran 70 Intravenous Infusion

Ph Eur

DEFINITION

Mixture of polysaccharides, principally of the α-1,6-glucan type.

Average relative molecular mass: about 70 000.

PRODUCTION

It is obtained by hydrolysis and fractionation of dextrans produced by fermentation of sucrose using *Leuconostoc mesenteroides* strain NRRL B-512 = CIP 78.59 or substrains thereof (for example *L. mesenteroides* B-512F = NCTC 10817).

It is prepared in conditions designed to minimise the risk of microbial contamination.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: + 195 to + 201 (dried substance).

Dissolve 1.0 g in *water R*, heating on a water-bath, and dilute to 50.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison dextran CRS.

C. Molecular-mass distribution (see Tests).

TESTS

Solution S

Dissolve 5.0 g in *distilled water R*, heating on a water-bath, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution remains colourless. Add 0.2 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.1 ml of *methyl red solution R*. The solution is red or orange.

Nitrogen-containing substances

Maximum 110 ppm N.

Carry out the determination of nitrogen by sulphuric acid digestion *(2.5.9)*, using 0.200 g and heating for 2 h. Collect the distillate in a mixture of 0.5 ml of *bromocresol green solution R*, 0.5 ml of *methyl red solution R* and 20 ml of *water R*. Titrate with *0.01 M hydrochloric acid*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Residual solvents

Gas chromatography *(2.2.28)*.

Internal standard   *propanol R.*

Test solution   Dissolve 5 g of the substance to be examined in 100 ml of *water R* and distil. Collect the first 45 ml of the distillate, add 1 ml of a 25 g/l solution of *propanol R* and dilute to 50 ml with *water R*.

Reference solution   Mix 0.5 ml of a 25 g/l solution of *anhydrous ethanol R*, 0.5 ml of a 25 g/l solution of *propanol R* and 0.5 ml of a 2.5 g/l solution of *methanol R* and dilute to 25.0 ml with *water R*.

Column:
- *material*: stainless steel,
- size: *l* = 1.8 m, Ø = 2 mm,
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (125-150 µm).

Carrier gas   *nitrogen for chromatography R.*

Flow rate   25 ml/min.

Temperature:
- *column*: 190 °C,
- *injection port*: 240 °C,
- *detector*: 210 °C.

Detection   Flame ionisation.

Injection The chosen volume of each solution.

Limits:

- *ethanol*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent),
- *methanol*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent),
- *sum of solvents other than ethanol, methanol and propanol*: not more than the area of the peak due to the internal standard (0.5 per cent, calculated as propanol).

Molecular-mass distribution *(2.2.39)*

The average molecular mass (M_w) is 64 000 to 76 000. The average molecular mass of the 10 per cent high fraction is not greater than 185 000. The average molecular mass of the 10 per cent low fraction is not less than 15 000.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 7.0 per cent, determined on 0.200 g by heating in an oven at 105 ± 2 °C for 5 h.

Sulphated ash *(2.4.14)*

Maximum 0.3 per cent, determined on 0.50 g.

Bacterial endotoxins *(2.6.14)*

Less than 16 IU/g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^2 micro-organisms per gram, determined by plate-count. It complies with the test for *Escherichia coli (2.6.13)*.

Ph Eur

Dextranomer

General Notices

(Ph Eur monograph 2238)

56087-11-7

Action and use

Fluid absorber; treatment of burns, wounds and skin ulcers; preparation for skin grafting.

Ph Eur

DEFINITION

Three-dimensional network made of dextran chains *O,O′*-cross-linked with 2-hydroxypropane-1,3-diyl bridges and *O*-substituted with 2,3-dihydroxypropyl and 2-hydroxy-1-(hydroxymethyl) ethyl groups.

CHARACTERS

Appearance

White or almost white, spherical beads.

Solubility

Practically insoluble in water. It swells in water and electrolyte solutions.

PRODUCTION

The absorption capacity is determined using a 9.0 g/l solution of *sodium chloride R* containing 20 µl/l of *polysorbate 20 R* or another suitable solution, with a suitable, validated method.

The particle size is controlled to a minimum of 80 per cent of the number of dry beads within 100 µm to 300 µm and a maximum of 7 per cent of their number below 100 µm using a suitable, validated method.

IDENTIFICATION

A. The substance to be examined is practically insoluble in *water R*. It swells in *water R*.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Grind the substance to be examined in *acetone R*. Evaporate the solvent at room temperature and use the residue.

Comparison *dextranomer CRS.*

TESTS

pH ***(2.2.3)***

5.3 to 7.5.

Introduce 0.50 g to 30 ml of a freshly prepared 74.6 g/l solution of *potassium chloride R*. Allow to stand for 2 min. Determine the pH on the mucilage obtained.

Boron

Maximum 30 ppm.

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) *(2.2.57)*.

Test solution Introduce 3.0 g into a platinum dish, moisten with 5 ml of a 32.1 g/l solution of *magnesium nitrate R* in a mixture of equal volumes of *ethanol (96 per cent) R* and *distilled water R*. Evaporate to dryness on a water-bath. Ignite at 550 °C for 5 h. Take up the residue with 5 ml of *6 M hydrochloric acid R* and transfer to a 50 ml volumetric flask. Add about 20 ml of *distilled water R* and allow to digest for 1 h on a water-bath. Allow to cool and dilute to 50.0 ml with *distilled water R*.

Standard solutions Prepare the standard solutions using a solution of *boric acid R* containing 10 ppm of boron. Proceed as described for the test solution.

Wavelength 249.773 nm.

Heavy metals ***(2.4.8)***

Maximum 30 ppm.

1.0 g complies with test F. Prepare the reference solution using 3 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 10.0 per cent.

To 1.000 g, add *distilled water R* dropwise until the sample has completely swollen. Dry in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.4 per cent, determined on 1.0 g.

Microbial contamination

Total aerobic microbial count (TAMC) *(2.6.12)* not more than 10^2 CFU per gram, determined using the pour-plate method.

Ph Eur

Dextrin

General Notices

(Ph Eur monograph 1507)

Action and use

Excipient.

Ph Eur

DEFINITION

Maize, potato or cassava starch partly hydrolysed and modified by heating with or without the presence of acids, alkalis or pH control agents.

CHARACTERS

Appearance

White or almost white, free-flowing powder.

Solubility

Very soluble in boiling water forming a mucilaginous solution, slowly soluble in cold water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Suspend 1 g in 50 ml of *water R*, boil for 1 min and cool. To 1 ml of the solution add 0.05 ml of *iodine solution R1*. A dark blue or reddish-brown colour is produced which disappears on heating.

B. Centrifuge 5 ml of the mucilage obtained in identification test A. To the upper layer add 2 ml of *dilute sodium hydroxide solution R* and, dropwise with shaking, 0.5 ml of *copper sulphate solution R* and boil. A red precipitate is produced.

C. It is very soluble in boiling *water R*, forming a mucilaginous solution.

TESTS

pH *(2.2.3)*:

2.0 to 8.0.

Disperse 5.0 g in 100 ml of *carbon dioxide-free water R*.

Chlorides

Maximum 0.2 per cent.

Dissolve 2.5 g in 50 ml of boiling *water R*, dilute to 100 ml with *water R* and filter. Dilute 1 ml of the filtrate to 15 ml, add 1 ml of *dilute nitric acid R*, pour the mixture as a single addition into 1 ml of *silver nitrate solution R2* and allow to stand for 5 min protected from light. When viewed transversely against a black background any opalescence produced is not more intense than that obtained by treating a mixture of 10 ml of *chloride standard solution (5 ppm Cl) R* and 5 ml of *water R*, prepared in the same manner.

Reducing sugars

Maximum 10 per cent, calculated as glucose $C_6H_{12}O_6$.

To a quantity of dextrin equivalent to 2.0 g (dried substance) add 100 ml of *water R*, shake for 30 min, dilute to 200.0 ml with *water R* and filter. To 10.0 ml of alkaline *cupri-tartaric solution R* add 20.0 ml of the filtrate, mix, and heat on a hot plate adjusted to bring the solution to boil within 3 min. Boil for 2 min, and cool immediately. Add 5 ml of a 300 g/l solution of *potassium iodide R* and 10 ml of *1 M sulphuric acid*, mix, and titrate immediately with *0.1 M sodium thiosulphate*, using *starch solution R*, added towards the end of the titration, as indicator. Repeat the procedure beginning with "To 10.0 ml of...", using, in place of the filtrate, 20.0 ml of a 1 g/l solution of *glucose R*, accurately prepared. Perform a blank titration. (V_B - V_U) is not greater than (V_B - V_S), in which V_B, V_U and V_S are the number of millilitres of *0.1 M sodium thiosulphate* consumed in the titrations of the blank, the dextrin and the glucose, respectively.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 13.0 per cent, determined on 1.000 g by drying at 130-135 °C for 90 min.

Sulphated ash *(2.4.14)*

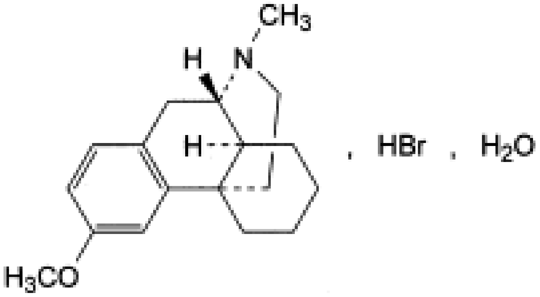
Maximum 0.5 per cent, determined on 1.0 g.

Ph Eur

Dextromethorphan Hydrobromide

General Notices

(Ph Eur monograph 0020)



$C_{18}H_{26}BrNO,H_2O$ *370.3* *6700-34-1*

Action and use

Opioid receptor agonist; cough suppressant.

Ph Eur

DEFINITION

ent-3-Methoxy-17-methylmorphinan hydrobromide monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Almost white, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in alcohol.

mp

About 125 °C, with decomposition.

IDENTIFICATION

First identification A, B, D.

Second identification *A, C, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *dextromethorphan hydrobromide CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 25 mg of *dextromethorphan hydrobromide CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R, methylene chloride R, methanol R, ethyl acetate R, toluene R* (2:10:13:20:55 *V/V/V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *potassium iodobismuthate solution R2*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *alcohol R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Dissolve 0.4 g in *carbon dioxide-free water R* with gentle heating, cool and dilute to 20 ml with the same solvent. Add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Specific optical rotation *(2.2.7)*

+ 28 to + 30 (anhydrous substance).

Dissolve 0.200 g in *0.1 M hydrochloric acid* and dilute to 10.0 ml with the same acid.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2 mg of *dextromethorphan impurity A CRS* in 2 ml of the test solution and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Column:

— size: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.11 g of *docusate sodium R* in a mixture of 400 ml of *water R* and 600 ml of *acetonitrile R*. Add 0.56 g of *ammonium nitrate R*. Adjust to apparent pH 2.0 with *glacial acetic acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Run time Twice the retention time of dextromethorphan.

Relative retention With reference to dextromethorphan (retention time = about 21.9 min): impurity B = about 0.44; impurity C = about 0.85; impurity D = about 0.90; impurity A = about 1.13.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurity A and dextromethorphan.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.2,

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), and not more than 1 such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

***N,N*-Dimethylaniline**

Maximum 10 ppm.

Dissolve 0.5 g with heating in 20 ml of *water R*. Allow to cool, add 2 ml of *dilute acetic acid R* and 1 ml of a 10 g/l solution of *sodium nitrite R* and dilute to 25 ml with *water R*. The solution is not more intensely coloured than a reference solution prepared at the same time in the same manner using 20 ml of a 0.25 mg/l solution of *dimethylaniline R*.

Water *(2.5.12)*

4.0 per cent to 5.5 per cent, determined on 0.200 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

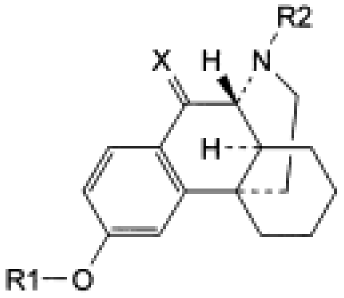
Dissolve 0.300 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 20 ml of *alcohol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 35.23 mg of $C_{18}H_{26}BrNO$.

STORAGE

Protected from light.

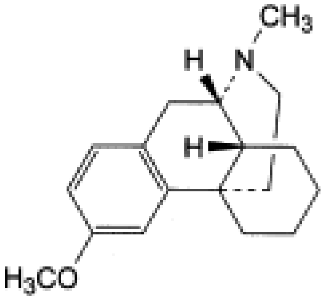
IMPURITIES



A. R1 = CH_3, R2 = H, X = H_2: *ent*-3-methoxymorphinan,

B. R1 = H, R2 = CH_3, X = H_2: *ent*-17-methylmorphinan-3-ol,

C. R1 = R2 = CH_3, X = O: *ent*-3-methoxy-17-methylmorphinan-10-one,



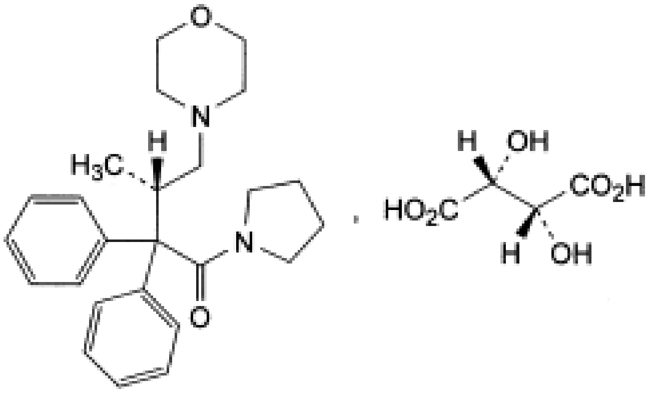
D. *ent*-(14*S*)-3-methoxy-17-methylmorphinan.

Ph Eur

Dextromoramide Tartrate

General Notices

(Ph Eur monograph 0021)



$C_{25}H_{32}N_2O_2,C_4H_6O_6$ 542.6 *2922-44-3*

Action and use

Opioid receptor agonist; analgesic.

Preparation

Dextromoramide Tablets

Ph Eur

DEFINITION

Dextromoramide tartrate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 1-[(3*S*)-3-methyl-4-(morpholin-4-yl)-2,2-diphenylbutanoyl] pyrrolidine hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, amorphous or crystalline powder, soluble in water, sparingly soluble in alcohol.

It melts at about 190 °C, with slight decomposition.

IDENTIFICATION

A. Dissolve 75 mg in *1 M hydrochloric* acid and dilute to 100.0 ml with the same acid. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows 3 absorption maxima, at 254 nm, 259 nm and 264 nm. The specific absorbances at the maxima are about 6.9, 7.7 and 6.5, respectively.

B. Dissolve about 50 mg in *water R* and dilute to 10 ml with the same solvent. To 2 ml of the solution add 3 ml of *ammoniacal silver nitrate solution R* and heat on a water-bath. A grey or black precipitate is formed.

C. It gives reaction (b) of tartrates *(2.3.1).*

TESTS

pH *(2.2.3)*

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. The pH of the solution is 3.0 to 4.0.

Specific optical rotation *(2.2.7)*

Dissolve 0.50 g in *0.1 M hydrochloric acid* and dilute to 10.0 ml with the same acid. The specific optical rotation is + 21 to + 23.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 1 ml of the test solution to 100 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using *methanol R*. Allow the plate to dry in air and spray with *dilute potassium iodobismuthate solution R*. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.05 M perchloric acid* using 0.15 ml of *naphtholbenzein solution R* as indicator.

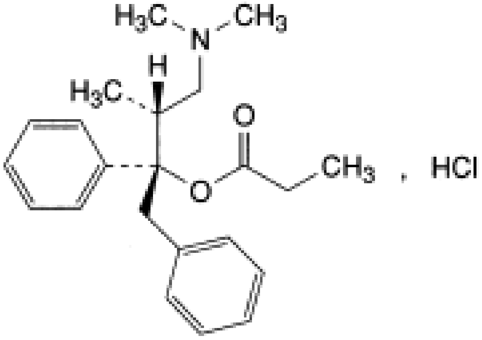
1 ml of *0.05 M perchloric acid* is equivalent to 27.13 mg of $C_{29}H_{38}N_2O_8$.

Ph Eur

Dextropropoxyphene Hydrochloride

General Notices

(Ph Eur monograph 0713)



$C_{22}H_{29}NO_2$,HCl *375.9* *1639-60-7*

Action and use

Opioid receptor agonist; analgesic.

Preparation

Co-proxamol Tablets

Ph Eur

DEFINITION

Dextropropoxyphene hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (1*S*,2*R*)-1-benzyl-3-(dimethylamino)-2-methyl-1-phenylpropyl propanoate hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very soluble in water, freely soluble in alcohol.

It melts at about 165 °C.

IDENTIFICATION

First identification *A, C, D.*

Second identification *A, B, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Dissolve 50.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid.

Examined between 220 nm and 360 nm *(2.2.25)*, the solution shows 3 absorption maxima, at 252 nm, 257 nm and 263 nm and 2 shoulders, at 240 nm and 246 nm. The ratio of the absorbance at the maximum at 257 nm to that at the maximum at 252 nm is 1.22 to 1.28. The ratio of the absorbance at the maximum at 257 nm to that at the maximum at 263 nm is 1.29 to 1.35. The test is not valid unless, in the test for resolution *(2.2.25)*, the ratio of the absorbances is at least 1.5.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the *Ph. Eur. reference spectrum of dextropropoxyphene hydrochloride*.

D. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.5 g in *carbon dioxide-free water R* and dilute to 30 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Dilute 10 ml of solution S to 25 ml with *carbon dioxide-free water R*. To 10 ml of this solution, add 0.1 ml of *methyl red solution R* and 0.2 ml *of 0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Specific optical rotation *(2.2.7)*

Dissolve 0.100 g in *water R* and dilute to 10.0 ml with the same solvent. The specific optical rotation is + 52 to + 57.

Related substances

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 0.50 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 50.0 mg of the substance to be examined in 2.5 ml of *2 M alcoholic potassium hydroxide*. Add 2.5 ml of *water R* and boil under a reflux condenser for 30 min. Add 2.5 ml of *dilute hydrochloric acid R* and dilute to 50 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a column 0.125 m long and 4.6 mm in internal diameter packed with *silica gel for chromatography R* (5 µm),

— a guard column packed with a suitable silica gel, equilibrated with the mobile phase and placed between the column and the injection device,

— as mobile phase at a flow rate of 1.0 ml/min a mixture of 50 volumes of *0.2 M phosphate buffer solution pH 7.5 R*, 84 volumes of *tetrahydrofuran R*, 350 volumes of *methanol R* and 516 volumes of *water R*, containing 0.9 g/l of *cetyltrimethylammonium bromide R*,

— as detector a spectrophotometer set at 220 nm,

— a loop injector.

Equilibrate the chromatographic system by passage of the mobile phase for 16 h (the mobile phase may be recirculated after 6 h).

Inject 20 µl of each solution and record the chromatograms for twice the retention time of the principal peak. The test is not valid unless:

— the chromatogram obtained with reference solution (a) shows a peak with a signal-to-noise ratio of at least 5,

— the chromatogram obtained with reference solution (b) shows 2 peaks with a resolution of not less than 2.0.

In the chromatogram obtained with the test solution, the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals *(2.4.8)*

12 ml of solution S complies with limit test A (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

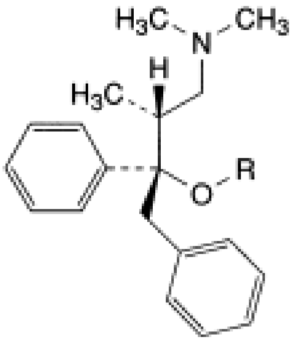
Dissolve 0.270 g in 60 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 37.59 mg of $C_{22}H_{30}ClNO_2$.

STORAGE

Protected from light.

IMPURITIES

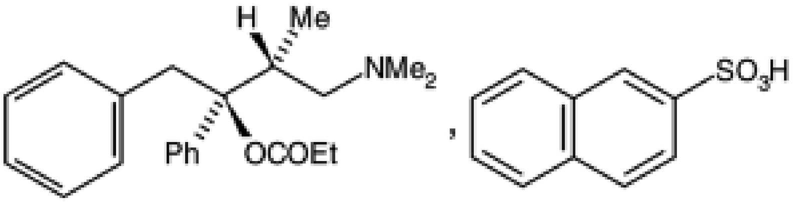


A. R = H: (2*S*,3*R*)-4-(dimethylamino)-1,2-diphenyl-3-methyl-butan-2-ol,

B. R = CO-CH_3: (*1S*,2*R*)-1-benzyl-3-(dimethylamino)-2-methyl-1-phenylpropyl acetate.

Dextropropoxyphene Napsilate

General Notices



$C_{22}H_{29}NO_2,C_{10}H_8O_3S,H_2O$ 565.8 *26570-10-5*

Action and use

Opioid receptor agonist; analgesic.

Preparation

Dextropropoxyphene Capsules

DEFINITION

Dextropropoxyphene Napsilate is (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1phenylpropyl propionate naphthalene-2-sulphonate monohydrate. It contains not less than 98.0% and not more than 101.0% of $C_{22}H_{29}NO_2,C_{10}H_8O_3S$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white powder. It exhibits polymorphism.

Practically insoluble in *water*; soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of dextropropoxyphene napsilate *(RS 092)*. If the spectra are not concordant, dissolve a sufficient quantity in the minimum volume of *dichloromethane IR*, evaporate to dryness, dry the residue at 105° for 1 hour and prepare a new spectrum.

B. Dissolve 25 mg in 5 ml of *dichloromethane*, evaporate 0.05 ml of the solution in a porcelain dish and streak the spot with *sulphuric acid* containing 5% v/v of *formaldehyde solution*. A purple colour is produced.

C. Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 5 ml of 1.25M *sodium hydroxide* as the absorbing liquid. When the process is complete, dilute the liquid to 25 ml with *water*. To 5 ml of the solution so obtained add 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of 1M *hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*. The solution becomes turbid.

TESTS

Specific optical rotation

In a 5% w/v solution in *ethanol (96%)*, +26 to +31, calculated with reference to the anhydrous substance, Appendix V F.

Related substances

Dissolve 10 mg of *triphenylamine* (internal standard) in sufficient *dichloromethane* to produce 50 ml (solution A). Carry out the method for *gas chromatography,* Appendix III B, using the following solutions. For solution (1) add 5 ml of solution A, 10 ml of *water*, 2 ml of I.25M *sodium hydroxide* and 15 ml of *dichloromethane* to 5 ml of a solution in *dichloromethane* containing 0.022% w/v of *(1S,2R)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate BPCRS* and 0.020% w/v of *4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride BPCRS* and shake. Extract the aqueous layer with two 20 ml quantities of *dichloromethane.* Shake the combined dichloromethane extracts with 5 g of *anhydrous sodium sulphate,* filter and evaporate to dryness at a temperature not exceeding 40° using a rotary evaporator. Dissolve the residue in 10 ml of *dichloromethane.* For solution (2) dissolve 0.3 g of the substance being examined in 5 ml of *dichloromethane,* add 10 ml of *water*, 2 ml of 1.25M *sodium hydroxide* and 15 ml of *dichloromethane* and shake. Extract the aqueous layer with two 20 ml quantities of *dichloromethane.* Shake the combined dichloromethane extracts with 5 g of *anhydrous sodium sulphate,* filter and evaporate to dryness at a temperature not exceeding 40° using a rotary evaporator. Dissolve the residue in 10 ml of *dichloromethane.* Prepare solution (3) in the same manner as solution (2) but add 5 ml of solution A to the initial solution of the substance being examined.

The chromatographic procedure may be carried out using a glass column (60 cm × 3 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of dimethyl silicone fluid (OV-101 is suitable) and maintained at 160° with an inlet port temperature of 150° and using 60 ml per minute as the flow rate of the carrier gas.

The peaks, other than the solvent peak, in the chromato-gram obtained with solution (1) are due, in order of emergence, to (a) the internal standard, (b) (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate and (c) 4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride. In the chromatogram obtained with solution (3) the ratio of the area of any peak corresponding to (b) to that of the peak due to (a) and the ratio of the area of any peak corresponding to (c) to that of the peak due to (a) are not greater than the corresponding ratios in the chromato-gram obtained with solution (1) (0.67% each).

Sulphated ash

Not more than 0.1%, Appendix IX A.

Water

3.0 to 5.0% w/w, Appendix IX C. Use 0.5 g.

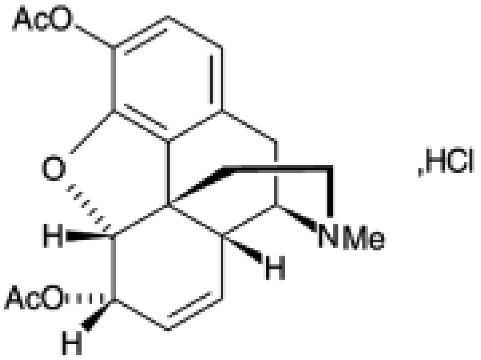
ASSAY

To 0.75 g add 50 ml of *water*, swirl to disperse, add 5 ml of 5M *sodium hydroxide* and extract with five 25 ml quantities of *dichloromethane*, washing each extract with the same 20 ml of *water*. Dry the combined extracts with *anhydrous sodium sulphate*, evaporate to about 3 ml on a water bath in a current of air and allow to evaporate to dryness at room temperature. Carry out Method I for *non-aqueous titration* on the residue, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid* VS is equivalent to

54.78 mg of $C_{22}H_{29}NO_2,C_{10}H_8O_3S$.

Diamorphine Hydrochloride

General Notices



$C_{21}H_{23}NO_5,HCl,H_2O$ 423.9 *1502-95-0*

Action and use

Opioid receptor agonist; analgesic.

Preparation

Diamorphine Injection

DEFINITION

Diamorphine Hydrochloride is 4,5-epoxy-17-methylmorphinan-3,6-diyl diacetate hydrochloride monohydrate. It contains not less than 98.0% and not more than 102.0% of $C_{21}H_{23}NO_5,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Freely soluble in *water*; soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. Dissolve a sufficient quantity in the minimum volume of *dichloromethane* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of diamorphine hydrochloride *(RS 093)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

Dissolve 0.2 g in 10 ml of *carbon dioxide-free water* and titrate with 0.02M *sodium hydroxide VS* using *methyl red solution* as indicator. Not more than 0.2 ml of 0.02M *sodium hydroxide VS* is required.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.5% w/v of the substance being examined in *water*. For solution (2) dilute 1 volume of solution (1) to 50 volumes with *water*. For solution (3) use a freshly prepared solution containing 0.1% w/v of the substance being examined in 0.01M *sodium hydroxide*. For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *base-deactivated octylsilyl silica gel for chromatography,* (5 µm) (Lichrospher RP-select B is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a solution containing 0.11 % w/v of *sodium octanesulphonate* in 10 volumes of *glacial acetic acid*, 10 volumes of *methanol*, 115 volumes of *acetonitrile* and 365 volumes of *water* and (c) a detection wavelength of 283 nm.

The chromatogram obtained with solution (3) exhibits two *secondary peaks* with retention times relative to the principal peak of about 0.23 (morphine) and 0.43 (6-*O*-acetyl-morphine). The test is not valid unless the *resolution factor* between the peaks due to morphine and 6-*O*-acetyl-morphine is at least 2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 6-*O*-acetylmorphine is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%) and the sum of the areas of any other *secondary peaks* is not greater than 0.25 times the area of the peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.05 times the area of the peak in the chromatogram obtained with solution (2) (0.1%).

Loss on drying

When dried to constant weight at 105°, loses 3.0 to 4.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

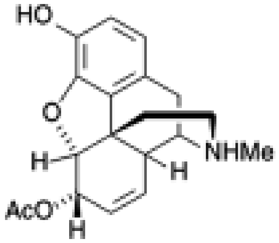
Dissolve 0.40 g in 50 ml of *ethanol (96%)* and add 5.0 ml of 0.01M *hydrochloric acid VS*. Titrate with 0.1M *sodium hydroxide VS* , determining the end point potentiometrically. Measure the volume of titrant required between the two points of inflection. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 40.59 mg of $C_{21}H_{23}NO_5$,HCl.

STORAGE

Diamorphine Hydrochloride should be protected from light.

IMPURITIES

The impurities listed by the requirements of this monograph include:

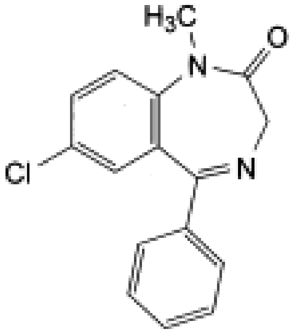

HO
O
H
AcO
H
H
NHMe

A. 6-*O*-acetylmorphine.

Diazepam

General Notices

(Ph Eur monograph 0022)



$C_{16}H_{13}ClN_2O$ 284.7 *439-14-5*

Action and use

Benzodiazepine.

Preparations

Diazepam Injection

Diazepam Oral Solution

Diazepam Rectal Solution

Diazepam Tablets

Ph Eur

DEFINITION

7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison diazepam CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions protected from bright light*.

Test solution Dissolve 25.0 mg of the substance to be examined in 0.5 ml of *acetonitrile R* and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *diazepam for system suitability CRS* (containing impurities A, B and E) in 1.0 ml of the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 22 volumes of *acetonitrile R*, 34 volumes of *methanol R* and 44 volumes of a 3.4 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 5.0 with *dilute sodium hydroxide solution R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time About 4 times the retention time of diazepam.

Identification of impurities Use the chromatogram supplied with *diazepam for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and E.

Relative retention With reference to diazepam (retention time = about 9 min): impurity E = about 0.7; impurity A = about 0.8; impurity B = about 1.3.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to impurities E and A and minimum 6.0 between the peaks due to impurity A and diazepam.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.3; impurity E = 1.3;

— *impurities A, B, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in

the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

2.0 g complies with test C. Prepare the reference solution using 4 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 28.47 mg of $C_{16}H_{13}ClN_2O$.

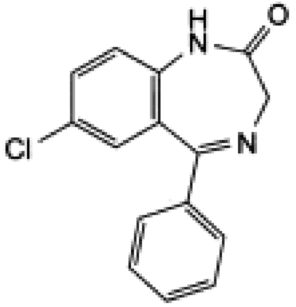
STORAGE

Protected from light.

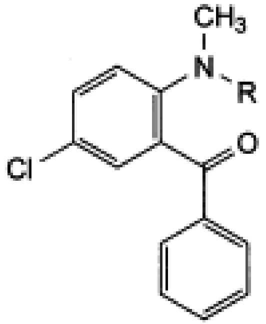
IMPURITIES

Specified impurities *A, B, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: C, D, F.

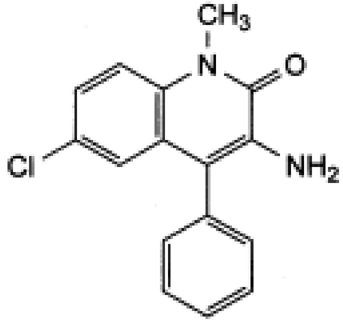


A. 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (nordazepam),

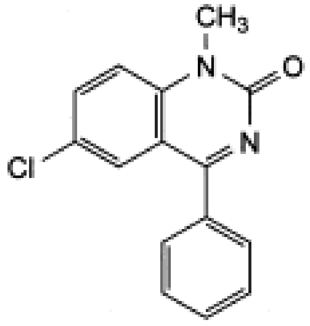


B. R = $CO\text{-}CH_2\text{-}Cl$: 2-chloro-*N*-(4-chloro-2-benzoylphenyl)-*N*-methylacetamide,

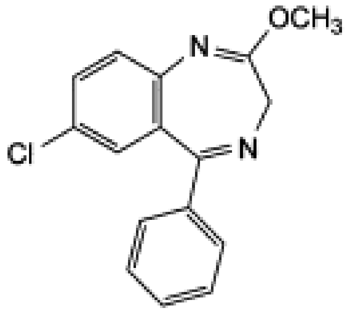
D. R = H: [5-chloro-2-(methylamino)phenyl]phenylmethanone,



C. 3-amino-6-chloro-1-methyl-4-phenylquinolin-2(1*H*)-one,



E. 6-chloro-1-methyl-4-phenylquinazolin-2(1*H*)-one,

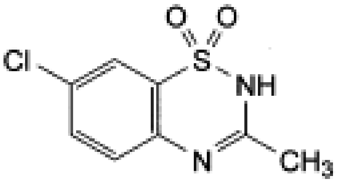


F. 7-chloro-2-methoxy-5-phenyl-3*H*-1,4-benzodiazepine.

Diazoxide

General Notices

(Ph Eur monograph 0550)



$C_8H_7ClN_2O_2S$ 230.7 *364-98-7*

Action and use

Vasodilator; Treatment of hypertension.

Preparations

Diazoxide Injection

Diazoxide Tablets

Ph Eur

DEFINITION

Diazoxide contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, fine or crystalline powder, practically insoluble in water, freely soluble in dimethylformamide, slightly soluble in alcohol. It is very soluble in dilute solutions of the alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Dissolve 50.0 mg in 5 ml of *1 M sodium hydroxide* and dilute to 50.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *0.1 M sodium hydroxide*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 280 nm and a shoulder at 304 nm. The specific absorbance at the maximum is 570 to 610.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *diazoxide CRS*. Examine the substances prepared as discs using *potassium bromide R*.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

D. Dissolve about 20 mg in a mixture of 5 ml of *hydrochloric acid R* and 10 ml of *water R*. Add 0.1 g of *zinc powder R*. Boil for 5 min, cool and filter. To the filtrate add 2 ml of a 1 g/l solution of *sodium nitrite R* and mix. Allow to stand for 1 min and add 1 ml of a 5 g/l solution of *naphthylethylenediamine dihydrochloride R*. A red or violet-red colour develops.

TESTS

Appearance of solution

Dissolve 0.4 g in 2 ml of *1 M sodium hydroxide* and dilute to 20 ml with *water R*. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Acidity or alkalinity

To 0.5 g of the powdered substance to be examined add 30 ml of *carbon dioxide-free water R*, shake for 2 min and filter. To 10 ml of the filtrate add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution R*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.1 g of the substance to be examined in a mixture of 0.5 ml of *1 M sodium hydroxide* and 1 ml of *methanol R* and dilute to 5 ml with *methanol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 5 ml with a mixture of 1 volume of *1 M sodium hydroxide* and 9 volumes of *methanol R*.

Reference solution (a) Dilute 0.5 ml of test solution (a) to 100 ml with a mixture of 1 volume of *1 M sodium hydroxide* and 9 volumes of *methanol R*.

Reference solution (b) Dissolve 20 mg of *diazoxide CRS* in a mixture of 0.5 ml of *1 M sodium hydroxide* and 1 ml of *methanol R* and dilute to 5 ml with *methanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 7 volumes of *concentrated ammonia R*, 25 volumes of *methanol R* and 68 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g with gentle heating in 50 ml of a mixture of 1 volume of *water R* and 2 volumes of *dimethylformamide R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

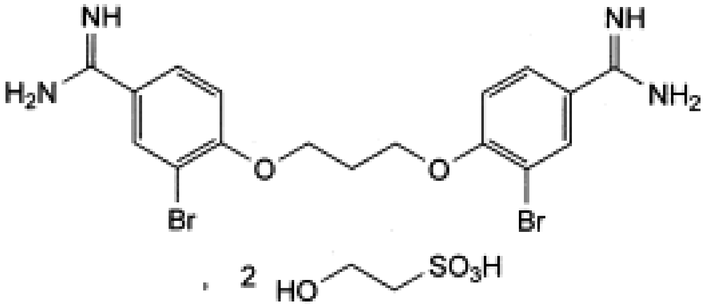
1 ml of *0.1 M sodium hydroxide* is equivalent to 23.07 mg of $C_8H_7ClN_2O_2S$.

Ph Eur

Dibrompropamidine Isetionate

General Notices

(Dibrompropamidine Diisetionate, Ph Eur monograph 2300)



$C_{17}H_{18}Br_2N_4O_2,2C_2H_6O_4S$ 722 *614-87-9*

Action and use

Antiseptic.

Ph Eur

DEFINITION

3,3′-Dibromo-4,4′-(propane-1,3-diylbisoxy)dibenzimidamide bis(2-hydroxyethanesulphonate).

Content

99.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl 2-hydroxyethanesulphonates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl 2-hydroxyethanesulphonates are not detectable in the final product.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble or soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dibrompropamidine diisetionate CRS.*

B. Mix 0.1 g with 0.5 g of *anhydrous sodium carbonate R*, ignite and take up the residue with 20 ml of *water R*. Filter and neutralise the filtrate to *blue litmus paper R* with *nitric acid R*. The filtrate gives reaction (a) of bromides *(2.3.1)*.

TESTS

pH *(2.2.3)*

5.0 to 6.0.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *anhydrous formic acid R, methanol R, ethyl acetate R* (0.01:8:12 *V/V/V*).

Test solution To 8 ml of *methanol R* add 20.0 mg of the substance to be examined and dissolve with the aid of an ultrasonic bath. Add 11 ml of *ethyl acetate R* then 10 µl of *anhydrous formic acid R* and mix. Dilute to 20.0 ml with *ethyl acetate R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *dibrompropamidine for system suitability CRS* (containing impurities A and B) in 4 ml of *methanol R* using an ultrasonic bath. Add 5 ml of *ethyl acetate R* then 5 µl of *anhydrous formic acid R* and mix. Dilute to 10.0 ml with *ethyl acetate R*.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *strong cation-exchange silica gel for chromatography R* (5 µm).

Mobile phase Mix 4 volumes of a 25 g/l solution of *ammonium formate R* in *methanol R* and 6 volumes of *ethyl acetate R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 40 µl.

Run time 1.5 times the retention time of dibrompropamidine.

Identification of impurities Use the chromatogram supplied with *dibrompropamidine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to dibrompropamidine (retention time = about 20 min): impurity A = about 0.4; impurity B = about 1.1.

System suitability   Reference solution (b):

— *peak-to-valley* ratio: minimum 1.5, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to dibrompropamidine.

Limits:

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

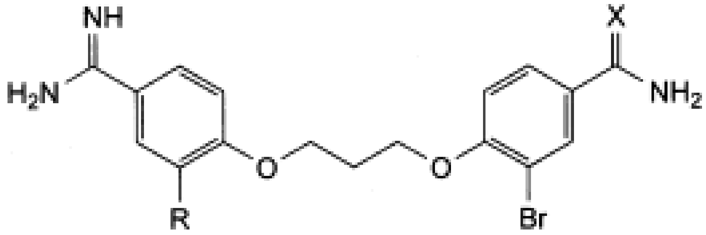
ASSAY

Dissolve 0.250 g in 50 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide* under a current of *nitrogen R*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 36.12 mg of $C_{21}H_{30}Br_2N_4O_{10}S_2$.

IMPURITIES

Specified impurities   A, B.



A. R = Br, X = O: 3-bromo-4-[3-(2-bromo-4-carbamimidoylphenoxy)propoxy]benzamide,

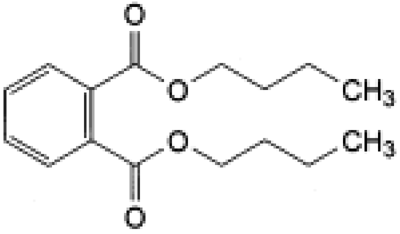
B. R = H, X = NH: 3-bromo-4-[3-(4-carbamimidoylphenoxy)propoxy]benzimidamide.

Ph Eur

Dibutyl Phthalate

General Notices

(Ph Eur monograph 0762)



$C_{16}H_{22}O_4$ 278.3 *84-74-2*

Action and use

Insect repellent.

Ph Eur

DEFINITION

Dibutyl benzene-1,2-dicarboxylate.

Content

99.0 per cent *m/m* to 101.0 per cent *m/m*.

CHARACTERS

Appearance

Clear, oily liquid, colourless or very slightly yellow.

Solubility

Practically insoluble in water, miscible with ethanol (96 per cent).

IDENTIFICATION

First identification B, C.

Second identification A, D, E.

A. Relative density *(2.2.5)*: 1.043 to 1.048.

B. Refractive index *(2.2.6)*: 1.490 to 1.495.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dibutyl phthalate CRS.*

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *ether R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *dibutyl phthalate CRS* in *ether R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *heptane R, ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

E. To about 0.1 ml add 0.25 ml of *sulphuric acid R* and 50 mg of *resorcinol R*. Heat in a water-bath for 5 min. Allow to cool. Add 10 ml of *water R* and 1 ml of *strong sodium hydroxide solution R*. The solution becomes yellow or brownish-yellow and shows a green fluorescence.

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity

Dissolve 20.0 g in 50 ml of *ethanol (96 per cent) R* previously neutralised to *phenolphthalein solution R1*. Add 0.2 ml of *phenolphthalein solution R1*. Not more than 0.50 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 60 mg of *bibenzyl R* in *methylene chloride R* and dilute to 20 ml with the same solvent.

Test solution (a) Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Test solution (b) Dissolve 1.0 g of the substance to be examined in *methylene chloride R*, add 2.0 ml of the internal standard solution and dilute to 20.0 ml with *methylene chloride R*.

Reference solution To 1.0 ml of test solution (a) add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *methylene chloride R*.

Column:
- *material*: glass;
- *size: l* = 1.5 m, Ø = 4 mm;
- *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (150-180 µm) impregnated with 3 per cent m/m of *polymethylphenylsiloxane R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 ml/min.

Temperature:
- *column*: 190 °C;
- *injection port and detector*: 225 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 3 times the retention time of dibutyl phthalate.

Elution order Bibenzyl, dibutyl phthalate.

Retention time Dibutyl phthalate = about 12 min.

System suitability:
- *resolution*: minimum 12 between the peaks due to bibenzyl and dibutyl phthalate in the chromatogram obtained with the reference solution;
- in the chromatogram obtained with test solution (a), there is no peak with the same retention time as the internal standard.

Limit:
- *total*: calculate the ratio (*R*) of the area of the peak due to dibutyl phthalate to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (1.0 per cent).

Water *(2.5.12)*

Maximum 0.2 per cent, determined on 10.00 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Introduce 0.750 g into a 250 ml borosilicate glass flask. Add 25.0 ml of *0.5 M alcoholic potassium hydroxide* and a few glass beads. Heat in a water-bath under a reflux condenser for 1 h. Add 1 ml of *phenolphthalein solution R1* and titrate immediately with *0.5 M hydrochloric acid* until the colour changes from red to colourless. Carry out a blank titration. Calculate the volume of potassium hydroxide used in the saponification.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 69.59 mg of $C_{16}H_{22}O_4$.

STORAGE

In an airtight container.

Ph Eur

Dichloromethane

General Notices

(Methylene Chloride, Ph Eur monograph 0932)

CH_2Cl_2 84.9 *75-09-02*

Action and use

Excipient.

Ph Eur

DEFINITION

Dichloromethane.

It may contain maximum 2.0 per cent *V/V* of ethanol and/or maximum 0.03 per cent *V/V* of 2-methylbut-2-ene as stabiliser.

CHARACTERS

Appearance

Clear, colourless, volatile liquid.

Solubility

Sparingly soluble in water, miscible with ethanol (96 per cent).

IDENTIFICATION

First identification B, C.

Second identification A, D, E.

A. Relative density (see Tests).

B. Refractive index (see Tests).

C. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

D. Heat 2 ml with 2 g of *potassium hydroxide R* and 20 ml of *ethanol (96 per cent) R* under a reflux condenser for 30 min. Allow to cool. Add 15 ml of *dilute sulphuric acid R* and filter. To 1 ml of the filtrate add 1 ml of a 15 g/l solution of *chromotropic acid, sodium salt R*, 2 ml of *water R* and 8 ml of *sulphuric acid R*. A violet colour is produced.

E. 2 ml of the filtrate obtained in identification test D gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance

It is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity

To 50 ml of *methanol R* previously neutralised to 0.1 ml of *bromothymol blue solution R1*, add 50 g of the substance to be examined. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Relative density *(2.2.5)*

1.320 to 1.332.

Refractive index *(2.2.6)*

1.423 to 1.425.

Related substances

Gas chromatography *(2.2.28)*.

Test solution (a) The substance to be examined.

Test solution (b) Dilute 0.5 ml of test solution (a) to 100.0 ml with *water R*.

Reference solution (a) Dilute 0.5 ml of *methylene chloride CRS* to 100.0 ml with *water R*.

Reference solution (b) Dilute 2.0 ml of test solution (b) to 10.0 ml with *water R*.

Reference solution (c) To 20.0 ml of *anhydrous ethanol R* (impurity C), add 0.3 ml of *2-methylbut-2-ene R* (impurity E) and dilute to 100.0 ml with test solution (a). Dilute 1.0 ml of this solution to 10.0 ml with test solution (a).

Reference solution (d) Dilute 0.1 ml of *methanol R* (impurity D) and 0.1 ml of *methylene chloride CRS* to 100.0 ml with *water R*.

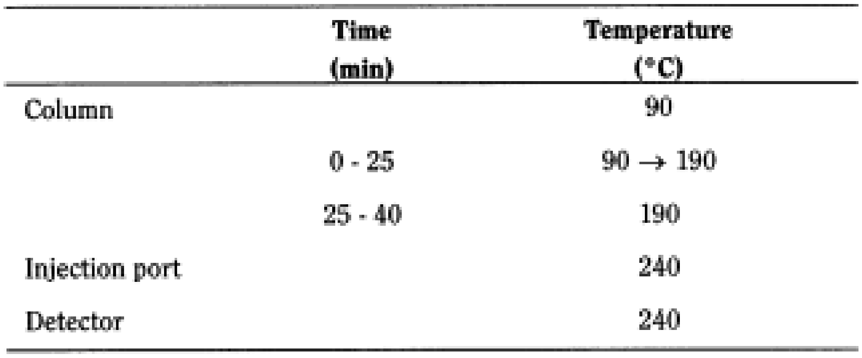
Column:

- *material*: glass;
- *size:* l = 2 m, Ø = 2 mm;
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (136-173 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column		90
	0 - 25	90 → 190
	25 - 40	190
Injection port		240
Detector		240

Detection Flame ionisation.

Injection 1 µl of reference solution (d) and 2 µl of the test solution and reference solutions (a) , (b) and (c).

System suitability:

— *resolution*: minimum 3.0 between the peaks due to impurity D and methylene chloride in the chromatogram obtained with reference solution (d);

— inject twice 2 µl of reference solution (c), if the peaks obtained show an area difference greater than 1.0 per cent, verify the repeatability by making 4 separate injections of reference solution (c); the test is not valid unless the maximum relative standard deviation of the peak area is 5.0 per cent.

Limits Test solution (a):

— *impurity C*: not more than the difference between the area of the peak due to impurity C in the chromatogram obtained with reference solution (c) and that of the peak due to impurity C in the chromatogram obtained with test solution (a) (2.0 per cent);

— *impurity E*: not more than the difference between the area of the peak due to impurity E in the chromatogram obtained with reference solution (c) and that of the peak due to impurity E in the chromatogram obtained with test solution (a) (0.03 per cent);

— *sum of impurities other than C and E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Free chlorine

Place 5 ml in a ground-glass-stoppered tube. Add 5 ml of a 100 g/l solution of *potassium iodide R* and 0.2 g of *soluble starch R*. Shake for 30 s and allow to stand for 5 min. No blue colour develops.

Heavy metals *(2.4.8)*

Maximum 1 ppm.

Evaporate 25.0 g to dryness on a water-bath. Allow to cool. Add 1 ml of *hydrochloric acid R* and evaporate again. Dissolve the residue in 1 ml of *acetic acid R* and dilute to 25 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R.*

Residue on evaporation

Maximum 20 ppm.

Evaporate 50.0 g to dryness on a water-bath and dry at 100-105 °C for 30 min. The residue weighs a maximum of 1 mg.

Water ***(2.5.12)***

Maximum 0.05 per cent *m/m*, determined on 10.00 g.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities *C, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *A, B, D.*

A. carbon tetrachloride,

B. chloroform,

C. ethanol,

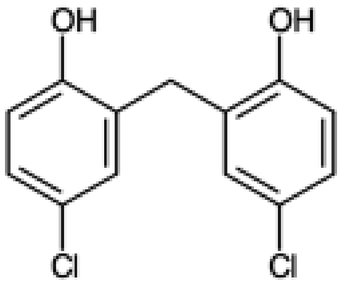
D. methanol,

E. 2-methylbut-2-ene.

Ph Eur

Dichlorophen

General Notices



$C_{13}H_{10}Cl_2O_2$ 269.1 *97-23-4*

Action and use

Antihelminthic.

Preparation

Dichlorophen Tablets

DEFINITION

Dichlorophen is 4,4′-dichloro-2,2′-methylenediphenol. It contains not less than 97.0% and not more than 101.0% of $C_{13}H_{10}Cl_2O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or not more than slightly cream powder.

Practically insoluble in *water*; very soluble in *ether*; freely soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a 0.002% w/v solution in 0.1M *sodium hydroxide* exhibits two maxima, at 245 nm and 304 nm. The *absorbances* at the maxima are about 1.3 and about 0.54, respectively.

B. Dissolve 0.2 g in a mixture of 5 ml of *water* and 5 ml of 5M *sodium hydroxide*, cool in ice and add a solution prepared by mixing 1 ml of *sodium nitrite solution* with a cold solution containing 0.15 ml of *aniline* in a mixture of 4 ml of *water* and 1 ml of *hydrochloric acid*. A reddish brown precipitate is produced.

C. Fuse 0.5 g with 2 g of *anhydrous sodium carbonate*, cool, extract the residue with *water* and filter. The filtrate yields reaction A characteristic of *chlorides*, Appendix VI.

D. *Melting point*, about 175°, Appendix V A.

TESTS

Chloride

Dissolve 1.0 g in 2 ml of *ethanol (96%)*, dilute to 100 ml with *water*, allow to stand for 5 minutes and filter through a slow filter paper (Whatman No. 42 is suitable). 15 ml of the filtrate complies with the *limit test for chlorides*, Appendix VII (350 ppm).

Sulphate

Shake 0.8 g with 16 ml of *water* for 2 minutes, filter and dilute 5 ml of the filtrate to 15 ml with *water*. The solution complies with the *limit test for sulphates*, Appendix VII (600 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using three solutions in the mobile phase containing (1) 1.0% w/v of *dichlorophen impurity standard BPCRS*, (2) 1.0% w/v of the substance being examined and (3) 0.0010% w/v of *4-chlorophenol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 25 volumes of *water* and 1 volume of *glacial acetic acid* and sufficient *methanol* to produce a chromatogram with solution (1) closely resembling the reference chromatogram supplied with the impurity standard (75 volumes of *methanol* is usually suitable) and (c) a detection wavelength of 280 nm. Record the chromatograms until all of the peaks named on the reference chromatogram have emerged.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 4-chlorophenol is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). The content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-α,α′-diyl)diphenol in the substance being examined does not exceed 8.0% w/w and the sum of the contents of any other impurities, excluding 4-chlorophenol, is not greater than 2.0% w/w calculated using the declared content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-α,α′-diyl)diphenol in *dichlorophen impurity standard BPCRS*.

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

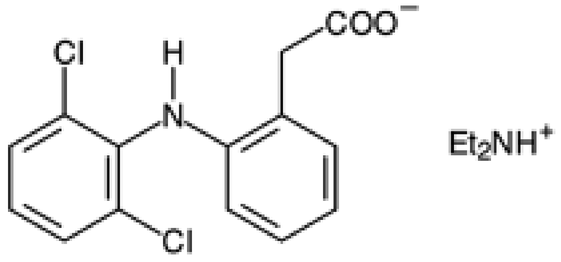
Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.5 g in 20 ml of *propan-2-ol* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 26.91 mg of $C_{13}H_{10}Cl_2O_2$.

Diclofenac Diethylamine

General Notices



$C_{18}H_{22}Cl_2N_2O_2$ 369.29 *78213-16-8*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparation

Diclofenac Gel

DEFINITION

Diclofenac Diethylamine is diethylammonium 2-[(2,6-dichloroanilino)phenyl]acetate. It contains not less than 99.0% and not more than 101.0% of $C_{18}H_{22}Cl_2N_2O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to light beige, crystalline powder.

Sparingly soluble in *water* and in *acetone*; freely soluble in *ethanol (96%)* and in *methanol*; practically insoluble in 1M *sodium hydroxide*.

It melts at about 154°, with decomposition.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of diclofenac diethylamine *(RS 371)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Macherey Nagel SIL G-25 HR or silica gel $60F_{254}$ HPTLC plates are suitable) and a mixture of 1 volume of *hydrochloric acid*, 1 volume of *water*, 6 volumes of *glacial acetic acid* and 11 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 2 µl of each of two solutions in *methanol* containing (1) 5.0% w/v of the substance being examined, and (2) 5.0% w/v of *diclofenac diethylamine BPCRS*. After removal of the plate, dry it in a stream of warm air for 10 minutes. Spray with *ninhydrin solution* and heat at 110° for 15 minutes. The two principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to the corresponding spots in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH of a 1% w/v solution in *ethanol (10%)*, 6.4 to 8.4, Appendix V L.

Clarity and colour of solution

A 5% w/v solution in *methanol* is *clear*, Appendix IV A. The *absorbance* of the solution measured at 440 nm is not greater than 0.05, Appendix II B.

Heavy metals

2 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.10% w/v of the substance being examined in the mobile phase. For solution (2) dilute 2 volumes of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 10 volumes with the same solvent. For solution (3) dissolve 1 mg of *diclofenac impurity A EPCRS* in the mobile phase, add 1 ml of solution (1) and dilute to 200 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (end-capped Zorbax C8 is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute a mixture of 34 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 2.5 and 66 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Inject 20 µl of solution (3). When the chromatograms are recorded in the prescribed conditions, the retention times are about 25 minutes for diclofenac and about 12 minutes for diclofenac impurity A. Adjust the sensitivity of the system so that the height of the peaks in the chromatogram obtained with solution (3) is not less than 50% of the full scale of the recorder. Continue the chromatography for 1.5 times the retention time of diclofenac. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

Inject 20 µl of solution (1) and 20 µl of solution (2). In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Loss on drying

When dried at a pressure not exceeding 1 kPa for 24 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A, Method II. Use 1 g.

ASSAY

Dissolve 0.5 g in 30 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 36.93 mg of $C_{18}H_{22}Cl_2N_2O_2$.

STORAGE

Diclofenac Diethylamine should be kept in an airtight container and protected from light.

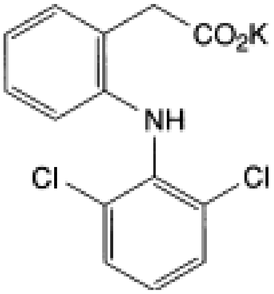
IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Diclofenac Sodium.

Diclofenac Potassium

General Notices

(Ph Eur monograph 1508)



$C_{14}H_{10}Cl_2KNO_2$ 334.2 *15307-81-0*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

Potassium [2-[(2,6-dichlorophenyl)amino]phenyl]acetate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, slightly hygroscopic, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent), slightly soluble in acetone.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *diclofenac potassium CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *diclofenac potassium CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *indometacin R* in reference solution (a) and dilute to 2 ml with the same solution.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *concentrated ammonia R, methanol R, ethyl acetate R* (10:10:80 *V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 10 ml of *ethanol (96 per cent) R*. To 1 ml of this solution add 0.2 ml of a mixture, prepared immediately before use, of equal volumes of a 6 g/l solution of *potassium ferricyanide R* and a 9 g/l solution of *ferric chloride R*. Allow to stand protected from light for 5 min. Add 3 ml of a 10 g/l solution of *hydrochloric acid R*. Allow to stand protected from light for 15 min. A blue colour develops and a precipitate is formed.

D. Suspend 0.5 g in 10 ml of *water R*. Stir and add *water R* until the substance is dissolved. Add 2 ml of *hydrochloric acid R1*, stir for 1 h and filter with the aid of vacuum. Neutralise with *sodium hydroxide solution R*. The solution gives reaction (b) of potassium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and its absorbance *(2.2.25)* at 440 nm is not greater than 0.05.

Dissolve 1.25 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute

1.0 ml of this solution to 10.0 ml with *methanol R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with *methanol R*. In 1.0 ml of this solution dissolve the contents of a vial of *diclofenac impurity A CRS*.

Column:
- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 34 volumes of a solution containing 0.5 g/l of *phosphoric acid R* and 0.8 g/l of *sodium dihydrogen phosphate R*, adjusted to pH 2.5 with *phosphoric acid R*, and 66 volumes of *methanol R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 1.5 times the retention time of diclofenac.

Retention time Impurity A = about 12 min; diclofenac = about 25 min.

System suitability Reference solution (b):

- *resolution*: minimum 6.5 between the peaks due to impurity A and diclofenac.

Limits:
- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Use a quartz crucible. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.250 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

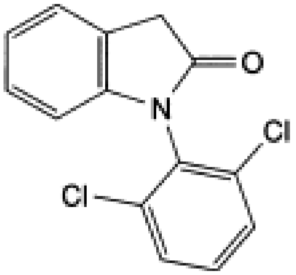
1 ml of *0.1 M perchloric acid* is equivalent to 33.42 mg of $C_{14}H_{10}Cl_2KNO_2$.

STORAGE

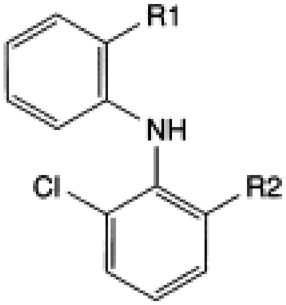
In an airtight container, protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*



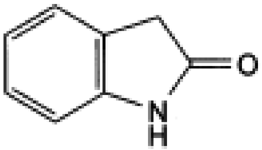
A. 1-(2,6-dichlorophenyl)-1,3-dihydro-2*H*-indol-2-one,



B. R1 = CHO, R2 = Cl: 2-[(2,6-dichlorophenyl)amino]benzaldehyde,

C. R1 = CH_2OH, R2 = Cl: [2-[(2,6-dichlorophenyl)amino]phenyl]methanol,

D. R1 = CH_2-CO_2H, R2 = Br: 2-[2-[(2-bromo-6-chlorophenyl)amino]phenyl]acetic acid,



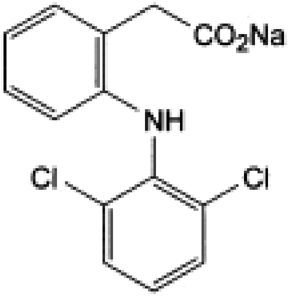
E. 1,3-dihydro-2*H*-indol-2-one.

Ph Eur

Diclofenac Sodium

General Notices

(Ph Eur monograph 1002)



$C_{14}H_{10}Cl_2NNaO_2$ 318.1 *15307-79-6*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Prolonged-release Diclofenac Capsules

Gastro-resistant Diclofenac Tablets

Prolonged-release Diclofenac Tablets

Ph Eur

DEFINITION

Sodium 2-[(2,6-dichlorophenyl)amino]phenyl]acetate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, slightly hygroscopic, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent), slightly soluble in acetone.

mp: about 280 °C, with decomposition.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison diclofenac sodium CRS.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *diclofenac sodium CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *indometacin R* in reference solution (a) and dilute to 2 ml with the same solution.

Plate TLC silica gel GF_{254} *plate R*.

Mobile phase concentrated ammonia R, methanol R, ethyl acetate R (10:10:80 *V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 10 ml of *ethanol (96 per cent) R*. To 1 ml of this solution add 0.2 ml of a mixture, prepared immediately before use, of equal volumes of a 6 g/l solution of *potassium ferricyanide R* and a 9 g/l solution of *ferric chloride R*. Allow to stand protected from light for 5 min. Add 3 ml of a 10 g/l solution of *hydrochloric acid R*. Allow to stand, protected from light, for 15 min. A blue colour develops and a precipitate is formed.

D. Dissolve 60 mg in 0.5 ml of *methanol R* and add 0.5 ml of *water R*. The solution gives reaction (b) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and its absorbance *(2.2.25)* at 440 nm is not greater than 0.05.

Dissolve 1.25 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with *methanol R*. In 1.0 ml of this solution dissolve the contenst of a vial of *diclofenac impurity A CRS*.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 34 volumes of a solution containing 0.5 g/l of *phosphoric acid R* and 0.8 g/l of *sodium dihydrogen phosphate R*, adjusted to pH 2.5 with *phosphoric acid R*, and 66 volumes of *methanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 1.5 times the retention time of diclofenac.

Retention times impurity A = about 12 min; diclofenac = about 25 min.

System suitability Reference solution (b):

- *resolution*: minimum 6.5 between the peaks due to impurity A and diclofenac.

Limits:

- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Use a quartz crucible. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.250 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

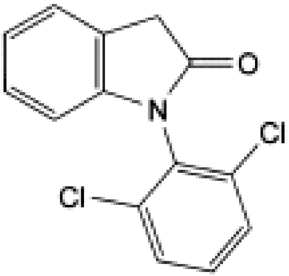
1 ml of *0.1 M perchloric acid* is equivalent to 31.81 mg of $C_{14}H_{10}Cl_2NNaO_2$.

STORAGE

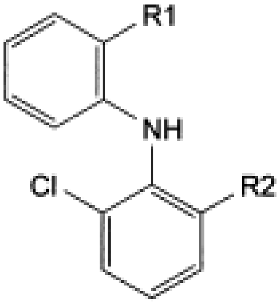
In an airtight container , protected from light.

IMPURITIES

Specified impurities   A, B, C, D, E.



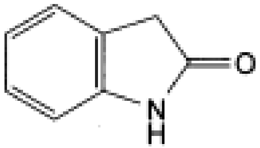
A. 1-(2,6-dichlorophenyl)-1,3-dihydro-2*H*-indol-2-one,



B. R1 = CHO, R2 = Cl: 2-[(2,6-dichlorophenyl)amino]benzaldehyde,

C. R1 = CH_2OH, R2 = Cl: [2-[(2,6-dichlorophenyl)amino]phenyl]methanol,

D. R1 = CH_2-CO_2H, R2 = Br: 2-[2-[(2-bromo-6-chlorophenyl)amino]phenyl]acetic acid,



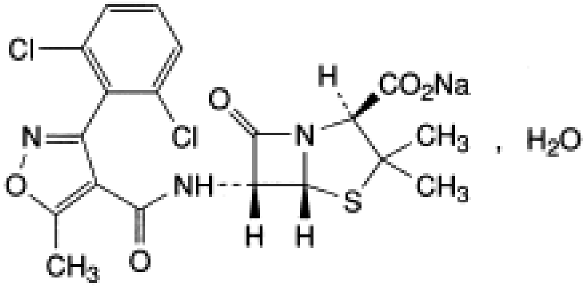
E. 1,3-dihydro-2*H*-indol-2-one.

Ph Eur

Dicloxacillin Sodium

General Notices

(Ph Eur monograph 0663)



$C_{19}H_{16}Cl_2N_3NaO_5S,H_2O$ 510.3 *13412-64-1*

Action and use

Penicillin antibacterial.

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-6-[[[3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *dicloxacillin sodium CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *water R*.

Reference solution (a) Dissolve 25 mg of *dicloxacillin sodium CRS* in 5 ml of *water R*.

Reference solution (b) Dissolve 25 mg of *cloxacillin sodium CRS*, 25 mg of *dicloxacillin sodium CRS* and 25 mg of *flucloxacillin sodium CRS* in 5 ml of *water R*.

Plate *TLC silanised silica gel plate R*.

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate* R adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is slightly greenish-yellow. Place the test-tube in a water-bath for 1 min; a yellow colour develops.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 430 nm is not greater than 0.04.

pH *(2.2.3)*

5.0 to 7.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 128 to + 143 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29).*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *dicloxacillin sodium CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of test solution (b) to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 5 mg of *flucloxacillin sodium CRS* and 5 mg of *dicloxacillin sodium CRS* in the mobile phase, then dilute to 50.0 ml with the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of *acetonitrile R* and 75 volumes of a 2.7 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 5.0 with *dilute sodium hydroxide solution R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl of test solution (a) and reference solutions (b) and (c).

Run time 5 times the retention time of dicloxacillin.

Retention time Dicloxacillin = about 10 min.

System suitability Reference solution (c):

- *resolution*: minimum 2.5 between the peaks due to flucloxacillin (1st peak) and dicloxacillin (2nd peak).

Limits:
- *any impurity:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- *total:* not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent);
- *disregard limit:* 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

N,N*-Dimethylaniline *(2.4.26, Method B)

Maximum 20 ppm.

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.8 per cent *m/m*.

Water *(2.5.12)*

3.0 per cent to 4.5 per cent, determined on 0.300 g.

Pyrogens ***(2.6.8)***

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 ml of a solution in *water for injections R* containing 20 mg of the substance to be examined per millilitre.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

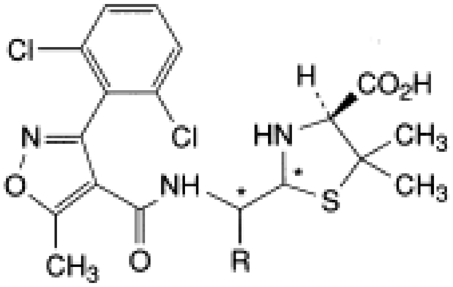
System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

STORAGE

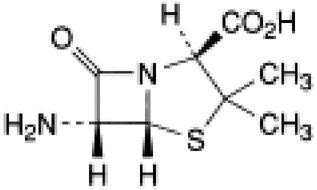
In an airtight container, at a temperature not exceeding 25 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

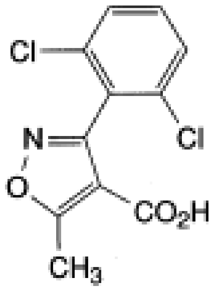


A. R = CO_2H: (4*S*)-2-[carboxy[[[3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino] methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of dicloxacillin),

B. R = H: (2*RS*,4*S*)-2-[[[[3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]methyl] -5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of dicloxacillin),



C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



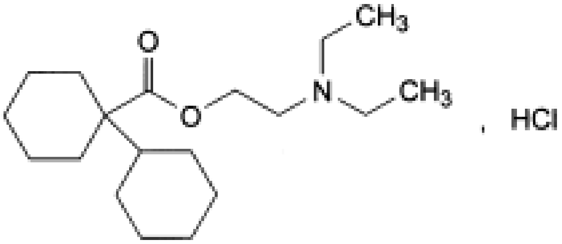
D. 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxylic acid.

Ph Eur

Dicycloverine Hydrochloride

General Notices

(Ph Eur monograph 1197)



$C_{19}H_{35}NO_2$,HCl 346.0 *67-92-5*

Action and use

Anticholinergic.

Preparations

Dicycloverine Oral Solution

Dicycloverine Tablets

Ph Eur

DEFINITION

Dicycloverine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 2-(diethylamino)ethyl bicyclohexyl-1-carboxylate hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, soluble in water, freely soluble in alcohol and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *dicycloverine hydrochloride CRS*. Examine the substances prepared as discs using *potassium chloride R*. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (b).

C. To 3 ml of a 1.0 g/l solution of *sodium laurilsulfate R* add 5 ml of *methylene chloride R* and 0.05 ml of a 2.5 g/l solution of *methylene blue R*, mix gently and allow to stand; the lower layer is blue. Add 2 ml of a 20 g/l solution of the substance to be examined, mix gently and allow to stand; the upper layer is blue and the lower layer is colourless.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

Dissolve 0.5 g in *water R* and dilute to 50 ml with the same solvent. The pH of the solution is 5.0 to 5.5.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 0.25 g to the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *methanol R*.

Reference solution (a) Dilute 1 ml of test solution (b) to 10 ml with *methanol R*.

Reference solution (b) Dissolve 10 mg of *dicycloverine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 5 mg of *tropicamide CRS* in reference solution (b) and dilute to 5 ml with the same solution.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *concentrated ammonia R*, 10 volumes of *ethyl acetate R*, 10 volumes of water R and 75 volumes of *propanol R*. Dry the plate in a current of warm air. Spray with *dilute potassium iodobismuthate solution R*. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

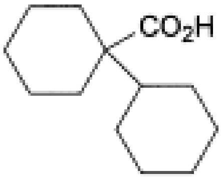
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 34.60 mg of $C_{19}H_{36}ClNO_2$.

IMPURITIES



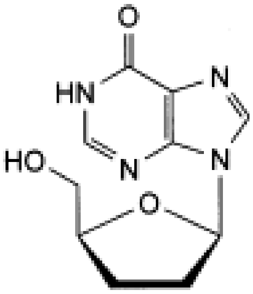
A. bicyclohexyl-1-carboxylic acid.

Ph Eur

Didanosine

General Notices

(Ph Eur monograph 2200)



$C_{10}H_{12}N_4O_3$ 236.2 *69655-05-6*

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

9-(2,3-Dideoxy-β-D-*glycero*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′,3′-dideoxyinosine) .

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in dimethyl sulfoxide, slightly soluble in methanol and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison didanosine CRS.

B. Specific optical rotation *(2.2.7)*: - 24.2 to - 28.2 (anhydrous substance).

Dissolve 0.100 g in *water R* and dilute to 10.0 ml with the same solvent.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Solvent mixture Mix 8 volumes of mobile phase B and 92 volumes of mobile phase A.

Test solution Dissolve 25.0 mg of the substance to be examined in 50.0 ml of the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 5.0 mg of *didanosine impurity A CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml to 20.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *didanosine for system suitability CRS* (containing impurities A to F) in the solvent mixture and dilute to 10 ml with the solvent mixture.

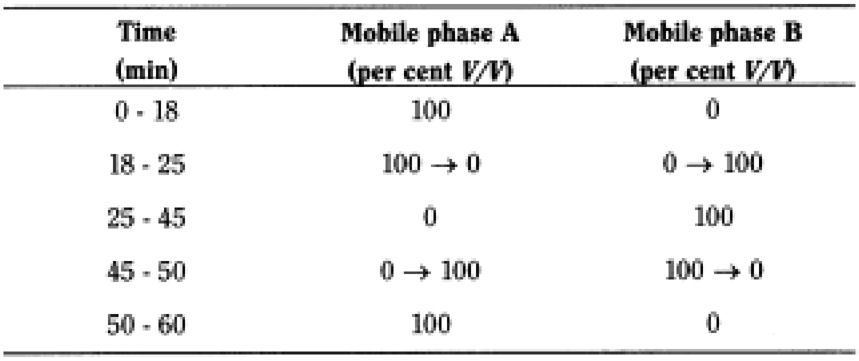
Reference solution (d) Dissolve 5 mg of *didanosine impurity G CRS* in the solvent mixture and dilute to 100 ml with the solvent mixture. Dilute 1 ml to 20 ml with the solvent mixture.

Column:

- size: *l* = .25 m, Ø = 4.6 mm,
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: mix 8 volumes of *methanol R* and 92 volumes of a 3.86 g/l solution of *ammonium acetate R* adjusted to pH 8.0 with *concentrated ammonia R*,
- *mobile phase B*: mix 30 volumes of *methanol R* and 70 volumes of a 3.86 g/l solution of *ammonium acetate R* adjusted to pH 8.0 with *concentrated ammonia R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 18	100	0
18 - 25	100 → 0	0 → 100
25 - 45	0	100
45 - 50	0 → 100	100 → 0
50 - 60	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *didanosine for system* *suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A to F and use the chromatogram obtained with reference solution (d) to identify the peak due to impurity G.

Relative retention With reference to didanosine (retention time = about 13-15 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.44; impurity D = about 0.48; impurity E = about 0.5; impurity F = about 0.8; impurity I = about 1.4; impurity G = about 1.6; impurity H = about 2.0.

System suitability Reference solution (c):

— *resolution*: minimum 2.5 between the peaks due to impurity C and impurity D.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *impurities B, C, D, E, F, G*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

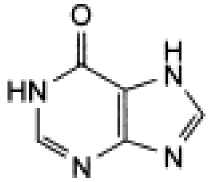
1 ml of *0.1 M perchloric acid* is equivalent to 23.62 mg of $C_{10}H_{12}N_4O_3$.

IMPURITIES

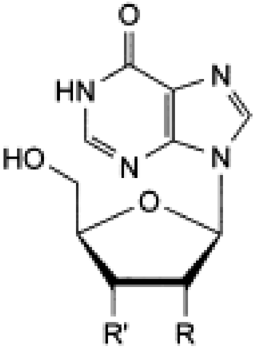
Specified impurities A, B, C, D, E, F, G.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances*

for pharmaceutical use): H, I.



A. 1,7-dihydro-6*H*-purin-6-one (hypoxanthine),

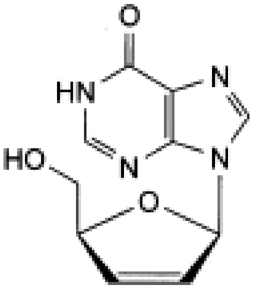


B. R = R′ = OH: 9-β-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (inosine),

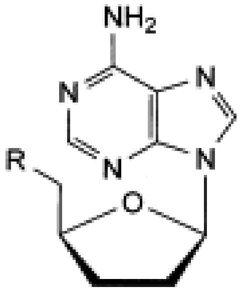
C. R = H, R′ = OH: 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,9dihydro-6*H*-purin-6-one (2′-deoxyinosine),

D. R = OH, R′ = H: 9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-1,9dihydro-6*H*-purin-6-one (3′-deoxyinosine),

E. R + R′ = O: 9-(2,3-anhydro-β-D-ribofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′,3′-anhydroinosine),

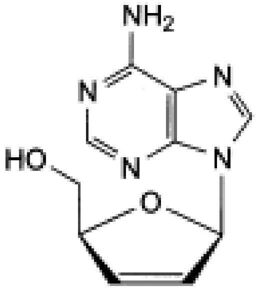


F. 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′,3′-dideoxy-2′,3′-didehydroinosine),



G. R = OH: 9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine (2′,3′-dideoxyadenosine),

H. R = H: 9-(2,3,5-trideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine (2′,3′,5′-trideoxyadenosine),



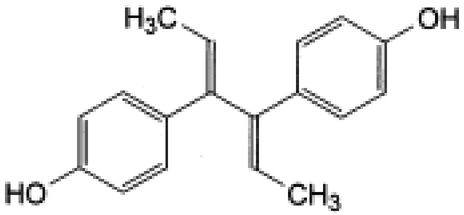
I. 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-9*H*-purin-6-amine (2′,3′-dideoxy-2′,3′-didehydroadenosine).

Ph Eur

Dienestrol

General Notices

(Ph Eur monograph 0483)



$C_{18}H_{18}O_2$ 266.3 *84-17-3*

Action and use

Estrogen.

Ph Eur

DEFINITION

Dienestrol contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of (*E,E*)-4,4′-(1,2-diethylidene-ethylene)diphenol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone and in alcohol. It dissolves in dilute solutions of the alkali hydroxides.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *dienestrol CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 1 mg in 5 ml of *glacial acetic acid R*, add 1 ml of a 1 per cent *V/V* solution of *bromine R* in *glacial acetic acid R* and heat in a water-bath for 2 min. Place 0.5 ml of this solution in a dry test-tube, add 0.5 ml of *ethanol R*, mix and add 10 ml of *water R*. A reddish-violet colour is produced. Add 5 ml of *chloroform R*, shake vigorously and allow to separate. The chloroform layer is red and the aqueous layer is almost colourless.

D. Dissolve about 0.5 mg in 0.2 ml of *glacial acetic acid R*, add 1 ml of *phosphoric acid R* and heat on a water-bath for 3 min. A reddish-violet colour is produced.

TESTS

Melting range

Determined by the capillary method *(2.2.14)*, the melting point is 227 °C to 234 °C. The temperature interval between the formation of a definite meniscus in the melt and the disappearance of the last particle does not exceed 3 °C.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g of the substance to be examined in 2 ml of *alcohol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 20 ml with *alcohol R*.

Reference solution (a) Dissolve 25 mg of *dienestrol CRS* in *alcohol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with *alcohol R*.

Reference solution (c) Dissolve 10 mg of *diethylstilbestrol CRS* in 2 ml of *alcohol R*. To 1 ml of this solution add 1 ml of reference solution (a).

Apply separately to the plate 1 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *diethylamine R* and 90 volumes of *toluene R*. Allow the plate to dry in air, spray with *alcoholic solution of sulphuric acid R* and heat at 120 °C for 10 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows at least two clearly separated spots having approximately the same intensity.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 25.0 mg in *ethanol R* and dilute to 100.0 ml with the same solvent. To 5.0 ml of this solution add 10 ml of *ethanol R* and dilute to 250.0 ml with *0.1 M sodium hydroxide*. Prepare a reference solution in the same manner using 25.0 mg of *dienestrol CRS*. Measure the absorbance *(2.2.25)* of the solutions at the maximum at 245 nm.

Calculate the content of $C_{18}H_{18}O_2$ from the measured absorbances and the concentrations of the solutions.

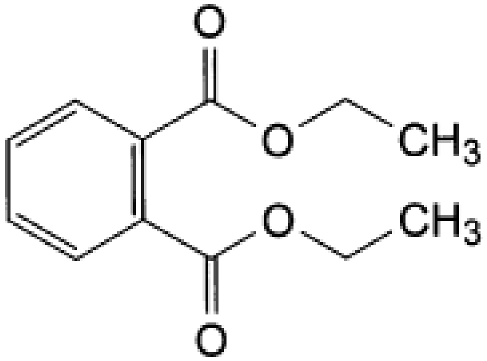
STORAGE

Store protected from light.

Diethyl Phthalate

General Notices

(Ph Eur monograph 0897)



$C_{12}H_{14}O_4$ 222.2 *84-66-2*

Action and use

Excipient.

Ph Eur

DEFINITION

Diethyl benzene-1,2-dicarboxylate.

Content

99.0 per cent *m/m* to 101.0 per cent *m/m*.

CHARACTERS

Appearance

Clear, colourless or very slightly yellow, oily liquid.

Solubility

Practically insoluble in water, miscible with ethanol (96 per cent).

IDENTIFICATION

First identification *B, C.*

Second identification *A, D, E.*

A. Relative density (*2.2.5*): 1.117 to 1.121.

B. Refractive index (*2.2.6*): 1.500 to 1.505.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Thin films.

Comparison *diethyl phthalate CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *ether R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *diethyl phthalate CRS* in *ether R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *heptane R*, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

E. To about 0.1 ml add 0.25 ml of *sulphuric acid R* and 50 mg of *resorcinol R*. Heat on a water-bath for 5 min. Allow to cool. Add 10 ml of *water R* and 1 ml of *strong sodium hydroxide solution R*. The solution becomes yellow or brownish-yellow and shows green fluorescence.

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity

Dissolve 20.0 g in 50 ml of *ethanol (96 per cent) R* previously neutralised to *phenolphthalein solution R1*. Add 0.2 ml of *phenolphthalein solution R1*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 60 mg of *naphthalene R* in *methylene chloride R* and dilute to 20 ml with the same solvent.

Test solution (a) Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Test solution (b) Dissolve 1.0 g of the substance to be examined in *methylene chloride R*, add 2.0 ml of the internal standard solution and dilute to 20.0 ml with *methylene chloride R.*

Reference solution To 1.0 ml of test solution (a) add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *methylene chloride R.*

Column:
— *material*: glass;

— *size:* *l* = 2 m, Ø = 2 mm;

— *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (150-180 µm) impregnated with 3 per cent *m/m* of *polymethylphenylsiloxane R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 ml/min.

Temperature:
— *column*: 150 °C;

— *injection port and detector:* 225 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 3 times the retention time of diethyl phthalate.

Elution order Naphthalene, diethyl phtalate.

System suitability:
— *resolution*: minimum 10 between the peaks due to naphthalene and diethyl phthalate in the chromatogram obtained with the reference solution;

— in the chromatogram obtained with test solution (a), there is no peak with the same retention time as the internal standard.

Limit:
— *total*: calculate the ratio (*R*) of the area of the peak due to diethyl phthalate to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (1.0 per cent).

Water (*2.5.12*)

Maximum 0.2 per cent, determined on 10.0 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Introduce 0.750 g into a 250 ml borosilicate glass flask. Add 25.0 ml of *0.5 M alcoholic potassium hydroxide* and a few glass beads. Boil in a water-bath under a reflux condenser for 1 h. Add 1 ml of *phenolphthalein solution R1* and titrate immediately with *0.5 M hydrochloric* acid. Carry out a blank titration. Calculate the volume of *0.5 M alcoholic potassium hydroxide* used in the saponification.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 55.56 mg of $C_{12}H_{14}O_4$.

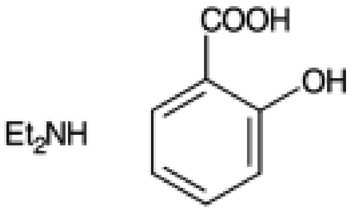
STORAGE

In an airtight container .

Ph Eur

Diethylamine Salicylate

General Notices



$C_{11}H_{17}NO_3$ 211.3 *4419-92-5*

Action and use

Counter-irritant.

Preparation

Diethylamine Salicylate Cream

DEFINITION

Diethylamine Salicylate contains not less than 99.0% and not more than 101.0% of $C_{11}H_{17}NO_3$.

CHARACTERISTICS

White or almost white crystals.

Very soluble in *water*; freely soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of diethylamine salicylate *(RS 099)*.

B. To 0.2 g add 5 ml of 1M *sodium hydroxide*, heat to boiling point, cool and acidify with 2M *hydrochloric acid*; a white precipitate is produced. The *melting point* of the precipitate, after recrystallisation from *water* and drying at 105°, is about 160°, Appendix V A.

TESTS

Acidity

Dissolve 2 g in 25 ml of *water* and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Not more than 0.2 ml of 0.1M *sodium hydroxide VS* is required to change the colour of the solution.

Clarity and colour of solution

A 50% w/v solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* BY_5, Appendix IV B, Method II.

Melting point

100° to 102°, Appendix V A.

Heavy metals

12 ml of a 10.0% w/v solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (10 ppm).

Sulphate

0.6 g complies with the *limit test for sulphates*, Appendix VII (250 ppm).

Loss on drying

When dried at 60° for 3 hours, loses not more than 0.1% of its weight. Use 1 g.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.4 g and *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 21.13 mg of $C_{11}H_{17}NO_3$.

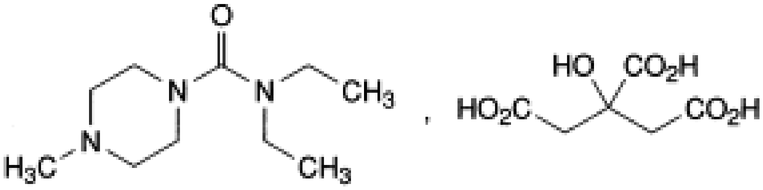
STORAGE

Diethylamine Salicylate should be protected from light. It should not be allowed to come into contact with iron or iron salts.

Diethylcarbamazine Citrate

General Notices

(Ph Eur monograph 0271)



$C_{10}H_{21}N_3O,C_6H_8O_7$ 391.4 *1642-54-2*

Action and use

Antihelminthic.

Preparation

Diethylcarbamazine Tablets

Ph Eur

DEFINITION

N,N-Diethyl-4-methylpiperazine-1-carboxamide dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, slightly hygroscopic.

Solubility

Very soluble in water, soluble in ethanol (96 per cent), practically insoluble in acetone.

mp: about 138 °C, with decomposition.

IDENTIFICATION

First identification A, C.

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *diethylcarbamazine citrate CRS.*

B. Examine the chromatograms obtained in the test for impurities A and B.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 5 ml of *water R*. The solution gives the reaction of citrates *(2.3.1)*.

TESTS

Solution S

Shake 2.5 g with *water R* until dissolved and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Impurities A and B

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.5 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 0.1 g of *diethylcarbamazine citrate CRS* in *methanol R* and dilute to 2.0 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *methylpiperazine R* (impurity A) in *methanol R* and dilute to 100 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of *dimethylpiperazine R* (impurity B) in *methanol R* and dilute to 100 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, methyl ethyl ketone R, methanol R* (5:30:65 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying At 100-105 °C.

Detection Expose to iodine vapour for 30 min.

Retardation factors Impurity A = about 0.2; impurity B = about 0.5.

Limits:

- *impurity A:* any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurity B*: any spot due to impurity B is not more intense than the corresponding spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Solution A Dissolve 31.2 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000 ml with the same solvent.

Test solution (a) Suspend 0.30 g of the substance to be examined in solution A and dilute to 100 ml with solution A. Filter or centrifuge and use the clear filtrate or supernatant.

Test solution (b) Dissolve 10.0 mg of the substance to be examined in solution A and dilute to 100.0 ml with solution A.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with solution A. Dilute 1.0 ml of this solution to 10.0 ml with solution A.

Reference solution (b) Dissolve 10 mg of *citric acid R* in solution A and dilute to 10 ml with solution A.

Reference solution (c) To 3 ml of test solution (a) add 0.5 ml of *strong hydrogen peroxide solution R* and maintain at 80 °C for 3 h. Dilute to 100 ml with solution A.

Reference solution (d) Dissolve 5.0 mg of *diethylcarbamazine citrate CRS* in solution A and dilute to 50.0 ml with solution A.

Column:

- *size:* *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 100 volumes of *methanol R2* and 900 volumes of a 10 g/l solution of *potassium dihydrogen phosphate R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (a) and reference solutions (a), (b) and (c).

Run time Twice the retention time of diethylcarbamazine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to the citrate.

Relative retention With reference to diethylcarbamazine (retention time = about 7 min): citrate = about 0.2; degradation product = about 1.6.

System suitability Reference solution (c):

- *resolution*: minimum 5 between the peaks due to diethylcarbamazine and the degradation product.

Limits:

- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to the citrate.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using 10 ml *of lead standard solution (2 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection 20 µl of test solution (b) and reference solution (d).

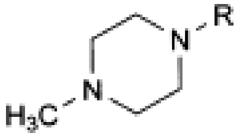
Calculate the percentage content of $C_{16}H_{29}N_3O_8$ from the declared content of *diethylcarbamazine citrate CRS*.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities *A, B.*



A. R = H: 1-methylpiperazine,

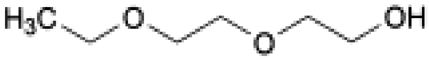
B. R = CH_3: 1,4-dimethylpiperazine.

Ph Eur

Diethylene Glycol Monoethyl Ether

General Notices

(Ph Eur monograph 1198)



$C_6H_{14}O_3$ 134.2 *111-90-0*

Action and use

Excipient.

Ph Eur

DEFINITION

2-(2-Ethoxyethoxy)ethanol, produced by condensation of ethylene oxide and alcohol, followed by distillation.

CHARACTERS

Appearance

Clear, colourless, hygroscopic liquid.

Solubility

Miscible with water, with acetone and with alcohol, miscible in certain proportions with vegetable oils, not miscible with mineral oils.

Relative density

About 0.991.

IDENTIFICATION

A. Refractive index *(2.2.6)*: 1.426 to 1.428.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of diethylene glycol monoethyl ether.

TESTS

Acid value *(2.5.1)*

Maximum 0.1.

Mix 30.0 ml with 30 ml of *alcohol R* previously neutralised with *0.1 M potassium hydroxide* using *phenolphthalein solution R* as indicator. Titrate with *0.01 M alcoholic potassium hydroxide*.

Peroxide value ***(2.5.5)***

Maximum 8.0, determined on 2.00 g.

Related substances

Gas chromatography *(2.2.28)*.

Internal standard solution Dilute 1.00 g of *decane R* to 100.0 ml with *methanol R*.

Test solution To 5.00 g of the substance to be examined, add 0.1 ml of the internal standard solution and dilute to 10.0 ml with *methanol R*.

Reference solution (a) Dilute 25.0 mg *of ethylene glycol monomethyl ether R*, 80.0 mg of *ethylene glycol monoethyl ether R*, 0.310 g of *ethylene glycol R* and 0.125 g of *diethylene glycol R* to 100.0 ml with *methanol R*. To 1.0 ml of this solution add 0.1 ml of the internal standard solution and dilute to 10.0 ml with *methanol R*.

Reference solution (b) Dilute 25.0 mg of *ethylene glycol monoethyl ether R* and 25.0 mg of *ethylene glycol R* to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 5.0 ml with *methanol R*.

Reference solution (c) Dilute 1.00 g of the substance to be examined to 100.0 ml with *methanol R*. To 1.0 ml of this solution add 0.1 ml of the internal standard solution and dilute to 10.0 ml with *methanol R*.

Column:

— *material*: fused silica,

— *size: l* = 30 m, Ø = 0.32 mm,

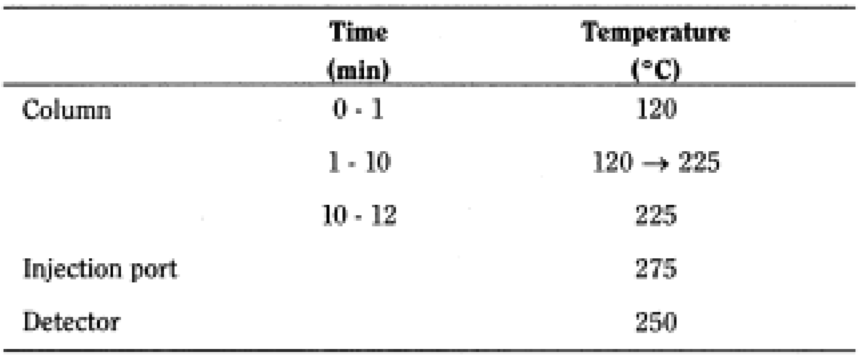
— *stationary phase*: *poly(cyanopropyl)(7)(phenyl)(7)methyl(86)siloxane R* (film thickness 1 µm).

Carrier gas *nitrogen for chromatography R* or *helium for chromatography R*.

Flow rate 2.0 ml/min.

Split ratio 1:80.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	120
	1 - 10	120 → 225
	10 - 12	225
Injection port		275
Detector		250

Detection Flame ionisation.

Injection 0.5 µl.

Relative retentions With reference to diethylene glycol monoethyl ether (retention time = about 4 min): ethylene glycol monomethyl ether = about 0.4; ethylene glycol monoethyl ether = about 0.5; ethylene glycol = about 0.55; diethylene glycol = about 1.1.

System suitability:

— *resolution*: minimum 3.0 between the peaks due to ethylene glycol monoethyl ether and to ethylene glycol in the chromatogram obtained with reference solution (b),

— *signal-to-noise ratio*: minimum 3.0 for the peak due to ethylene glycol monomethyl ether in the chromatogram obtained with reference solution (a),

Limits (take into account the impurity/internal standard peak area ratio):

— *ethylene glycol monomethyl ether*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (50 ppm),

— *ethylene glycol monoethyl ether*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (160 ppm),

— *ethylene glycol*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (620 ppm),

— *diethylene glycol*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (250 ppm),

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent).

Ethylene oxide

Head-space gas chromatography *(2.2.28)*.

Test solution To 1.00 g of the substance to be examined in a vial, add 50 µl of *water R*.

Reference solution To 1.00 g of the substance to be examined in a vial, add 50 µl of *ethylene oxide solution R4* and close tightly.

Column:

— *material*: fused silica,

— *size:* l = 30 m, Ø = 0.32 mm,

— *stationary phase*: p*oly(cyanopropyl)(7)(phenyl)(7)methyl(86)siloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.1 ml/min.

Static head-space conditions which may be used:

— *equilibration temperature*: 80 °C,

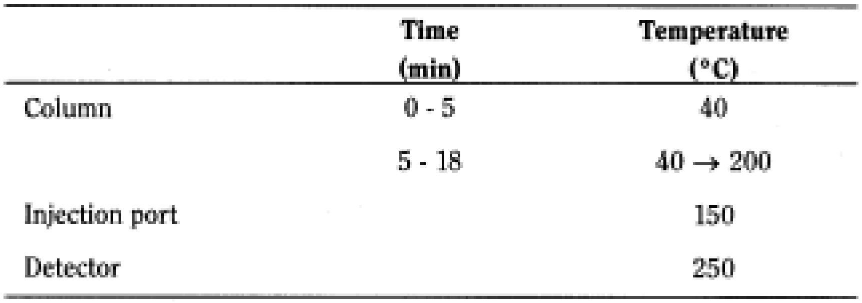
— *equilibration time*: 45 min,

— *transfer line temperature*: 110 °C,

— *pressurisation time*: 2 min,

— *injection time*: 12 s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	40
	5 - 18	40 → 200
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 1.0 ml.

The peak due to ethylene oxide is identified by injecting solutions of ethylene oxide of increasing concentration.

Determine the content of ethylene oxide (ppm) in the substance to be examined using the following expression:

$$\frac{S_T \times C}{(S_S \times M_T) - (S_T \times M_S)}$$

S_T = area of the peak corresponding to ethylene oxide in the chromatogram obtained with the test solution,

S_S = area of the peak corresponding to ethylene oxide in the chromatogram obtained with the reference solution,

M_T = mass of the substance to be examined in the test solution, in grams,

M_S = mass of the substance to be examined in the reference solution, in grams,

C = mass of added ethylene oxide in the reference solution, in micrograms.

Limit:
— *ethylene oxide*: maximum 1 ppm.

Water *(2.5.12)*

Maximum 0.1 per cent, determined on 10.0 g.

STORAGE

Under an inert gas, in an airtight container.

LABELLING

The label states that the substance is stored under an inert gas.

Diethylene Glycol Palmitostearate

General Notices

(Ph Eur monograph 1415)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of diethylene glycol mono- and diesters of stearic (octadecanoic) and palmitic (hexadecanoic) acids.

It is produced by esterification of diethylene glycol and stearic acid 50 (see *Stearic acid (1474)*) of vegetable or animal origin.

Content:

- *monoesters*: 45.0 per cent to 60.0 per cent;
- *diesters*: 35.0 per cent to 55.0 per cent.

CHARACTERS

Appearance

White or almost white, waxy solid.

Solubility

Practically insoluble in water, soluble in acetone and in hot ethanol (96 per cent).

IDENTIFICATION

A. Melting point (see Tests).

B. Composition of fatty acids (see Tests).

C. It complies with the limit of the assay (monoesters content).

TESTS

Melting point *(2.2.15)*

43 °C to 50 °C.

Acid value *(2.5.1)*

Maximum 4.0.

Iodine value *(2.5.4, Method A)*

Maximum 3.0.

Saponification value *(2.5.6)*

155 to 180, determined on 2.0 g.

Composition of fatty acids *(2.4.22, Method A)*

Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty acid fraction of the substance:

- *stearic acid*: 40.0 per cent to 60.0 per cent;
- *sum of contents of palmitic acid and stearic acid*: minimum 90.0 per cent.

Free diethylene glycol

Maximum 2.5 per cent, determined as described in the assay.

Total ash *(2.4.16)*

Maximum 0.1 per cent.

ASSAY

Size-exclusion chromatography *(2.2.30)*.

Test solution Into a 15 ml flask, weigh 0.200 g (m). Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Heat gently, if necessary. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 ml flasks, weigh, 2.5 mg, 5.0 mg, 10.0 mg and 20.0 mg respectively of *diethylene glycol R*. Add 5.0 ml of *tetrahydrofuran R*. Weigh the flasks again and calculate the concentration of diethylene glycol in milligrams per gram for each reference solution.

Column:

- size: l = 0.6 m, Ø = 7 mm,
- *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R.*

Flow rate 1 ml/min.

Detection Differential refractometer.

Injection 40 µl.

Relative retention With reference to diethylene glycol: diesters = about 0.78; monoesters = about 0.84.

Calculations:

- *free diethylene glycol*: from the calibration curve obtained with the reference solutions, determine the concentration (*C*) of diethylene glycol in milligrams per gram in the test solution and calculate the percentage content of free diethylene glycol in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\frac{A}{A + B} \times (100 - D)$$

A = area of the peak due to the monoesters,

B = area of the peak due to the diesters,

D = percentage content of free diethylene glycol + percentage content of free fatty acids.

Calculate the percentage content of free fatty acids using the following expression:

$$\frac{I_A \times 270}{561.1}$$

I_A = acid value.

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{B}{A + B} \times (100 - D)$$

STORAGE

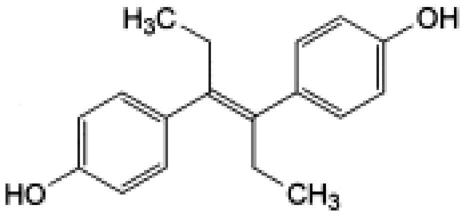
Protected from light.

Ph Eur

Diethylstilbestrol

General Notices

(Ph Eur monograph 0484)



$C_{18}H_{20}O_2$ 268.4 *56-53-1*

Action and use

Estrogen.

Preparations

Diethylstilbestrol Pessaries

Diethylstilbestrol Tablets

Ph Eur

DEFINITION

Diethylstilbestrol contains not less than 97.0 per cent and not more than the equivalent of 101.0 per cent of (*E*)-4,4′-(1,2-diethylethene-1,2-diyl)diphenol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in alcohol. It dissolves in solutions of the alkali hydroxides.

It melts at about 172 °C.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Examined between 230 nm and 450 nm *(2.2.25)*, the irradiated solution of the substance to be examined prepared as prescribed in the assay shows two absorption maxima, at 292 nm and 418 nm.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *diethylstilbestrol CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for mono-and dimethyl ethers. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 0.5 mg in 0.2 ml of *glacial acetic acid R*, add 1 ml of *phosphoric acid R* and heat on a water-bath for 3 min. A deep-yellow colour develops.

TESTS

4,4′-Dihydroxystilbene and related ethers

Dissolve 0.100 g in *ethanol R* and dilute to 10.0 ml with the same solvent. The absorbance *(2.2.25)* of the solution measured at 325 nm is not greater than 0.50.

Mono- and dimethyl ethers

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g of the substance to be examined in 2 ml of *alcohol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 20 ml with *alcohol R*.

Reference solution (a) Dissolve 10 mg of *diethylstilbestrol CRS* in 2 ml of *alcohol R*.

Reference solution (b) Dissolve 5 mg of *diethylstilbestrol monomethyl ether CRS* in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 5 mg of *diethylstilbestrol dimethyl ether CRS* in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution (d) Dissolve 10 mg of *dienestrol CRS* in 2 ml of *alcohol R*. To 1 ml of this solution add 1 ml of reference solution (a).

Apply to the plate 1 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *diethylamine R* and 90 volumes of *toluene R*. Allow the plate to dry in air, spray with *alcoholic solution of sulphuric acid R* and heat at 120 °C for 10 min. In the chromatogram obtained with test solution (a), any spots corresponding to diethylstilbestrol monomethyl ether and diethylstilbestrol dimethyl ether are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively (0.5 per cent). Diethylstilbestrol gives one or sometimes two spots. The test is not valid unless the chromatogram obtained with reference solution (d) shows at least two clearly separated spots having approximately the same intensity.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 20.0 mg in *ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *ethanol R*. To 25.0 ml of the resulting solution add 25.0 ml of a solution of 1 g of *dipotassium hydrogen phosphate R* in 55 ml of *water R*. Prepare in the same manner a reference solution using 20.0 mg of *diethylstilbestrol CRS*. Transfer an equal volume of each solution to separate 1 cm quartz cells and close the cells; place the two cells about 5 cm from a low-pressure, short-wave 2 W to 20 W mercury lamp and irradiate for about 5 min. Measure the absorbance *(2.2.25)* of the irradiated solutions at the maximum at 418 nm, using *water R* as the compensation liquid. Continue the irradiation for successive periods of 3 min to 15 min, depending on the power of the lamp, and repeat the measurement of the absorbances at 418 nm until the maximum absorbance (about 0.7) is obtained. If necessary, adjust the geometry of the irradiation apparatus to obtain a maximum, reproducible absorbance at 418 nm.

Calculate the content of $C_{18}H_{20}O_2$ from the measured absorbances and the concentrations of the solutions.

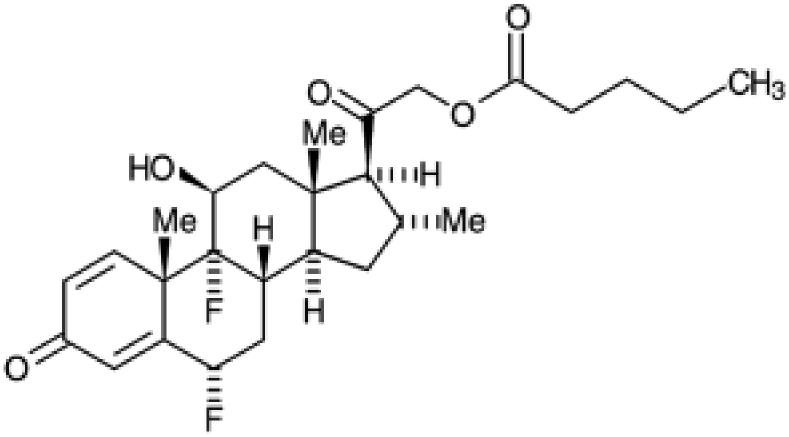
STORAGE

Store protected from light.

Ph Eur

Diflucortolone Valerate

General Notices



$C_{27}H_{36}F_2O_5$ 478.6 *59198-70-8*

Action and use

Glucocorticoid.

Preparations

Diflucortolone Cream

Diflucortolone Oily Cream

Diflucortolone Ointment

DEFINITION

Diflucortolone Valerate is 6α,9α-difluoro-3,20-dioxo-11β-hydroxy-16α-methylpregna-1,4-dien-21-yl valerate. It contains not less than 97.0% and not more than 102.0% of $C_{27}H_{36}F_2O_5$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to creamy white, crystalline powder.

Practically insoluble in *water*; freely soluble in *dichloromethane* and in *1,4-dioxan*; slightly soluble in *methanol*; sparingly soluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 350 nm of a 0.002% w/v solution in *methanol* exhibits a maximum only at 238 nm. The *absorbance* at the maximum at 238 nm is about 0.69.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of diflucortolone valerate *(RS 100)*.

TESTS

Specific optical rotation

In a 1% w/v solution in *1,4-dioxan*, +98 to +103, Appendix V F, calculated with reference to the dried substance.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µl of each of the following solutions in a mixture of 25 volumes of *water* and 75 volumes of *acetonitrile*. Solution (1) contains 0.060% w/v of the substance being examined. For solution (2) dilute 1 volume of solution (1) to 100 volumes. Solution (3) contains 0.060% w/v of *diflucortolone valerate impurity standard BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of equal volumes of *acetonitrile* and *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 238 nm. If necessary the composition of the mobile phase may be altered so that the chromatogram obtained with solution (3) shows similar resolution to the reference chromatogram supplied with *diflucortolone valerate impurity standard BPCRS*.

Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following three solutions in a mixture of 25 volumes of *water* and 75 volumes of *acetonitrile* containing (1) 0.03% w/v of the substance being examined, (2) 0.03% w/v of *diflucortolone valerate BPCRS* and (3) 0.03% w/v of *diflucortolone valerate impurity standard BPCRS*.

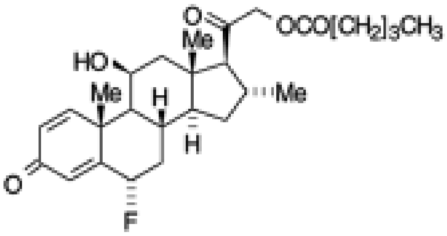
The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{27}H_{36}F_2O_5$ from the declared content of $C_{27}H_{36}F_2O_5$ in *diflucortolone valerate BPCRS*.

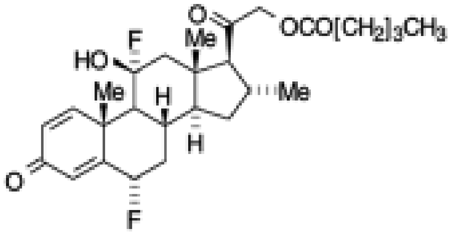
STORAGE

Diflucortolone Valerate should be protected from light.

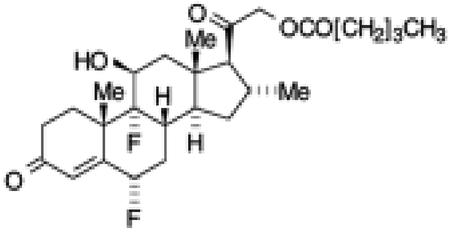
IMPURITIES



A. 6α-fluoro-3,20-dioxo-11β-hydroxy-16α-methylpregna-1,4-dien-21-yl valerate



B. 6α,12α-difluoro-3,20-dioxo-11β-hydroxy-16α-methylpregna-1,4-dien-21-yl valerate

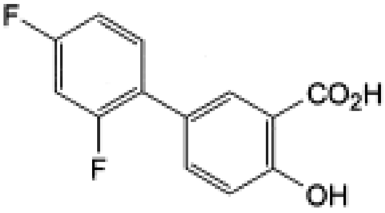


C. 6α,9α-difluoro-3,20-dioxo-11β-hydroxy-16α-methylpregna-1,4-dien-21-yl valerate

Diflunisal

General Notices

(Ph Eur monograph 0818)



$C_{13}H_8F_2O_3$ 250.2 *22494-42-4*

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

Preparation

Diflunisal Tablets

Ph Eur

DEFINITION

2′,4′-Difluoro-4-hydroxybiphenyl-3-carboxylic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 10 mg in a 0.3 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* and dilute to 100.0 ml with the same solution. Dilute 2.0 ml of this solution to 10.0 ml with a 0.3 per cent *V/V* solution of *hydrochloric acid R* in *methanol R*.

Spectral range 230-350 nm.

Absorption maxima At 251 nm and 315 nm.

Absorbance ratio A_{251} / A_{315} = 4.2 to 4.6.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *diflunisal CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *ethanol (96 per cent) R*, evaporate to dryness and record new spectra using the residues.

C. Dissolve about 2 mg in 10 ml of *ethanol (96 per cent) R* and add 0.1 ml of *ferric chloride solution R1*. A violet-red colour is produced.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Dissolve 0.5 g in *ethanol (96 per cent) R* and dilute to 50 ml with the same solvent.

Related substances

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 30 mg of *biphenyl-4-ol R* (impurity A) in *methanol R* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *methanol R*.

Reference solution (b) Dissolve 20 mg of *biphenyl-4-ol R* (impurity A) in *methanol R*, add 1 ml of the test solution and dilute to 10 ml with *methanol R*.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *glacial acetic acid R, acetone R, methylene chloride R* (10:20:70 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

- the chromatogram shows 2 clearly separated principal spots.

Limit:

- *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.15 per cent).

B. Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the reference solution and dilute to 10.0 ml with the reference solution.

Reference solution Dissolve 55.0 mg of *fluoranthene R* in a mixture of 1 volume of *water R* and 4 volumes of *acetonitrile R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 1.0 ml of this solution to 100.0 ml with a mixture of 1 volume of *water R* and 4 volumes of *acetonitrile R*.

Column:

- *size:* *l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase *glacial acetic acid R, methanol R, water R, acetonitrile R* (2:25:55:70 *V/V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 3 times the retention time of fluoranthene.

Limits:

- *sum of the impurities with a retention time greater than that of fluoroanthene*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.005 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Use a platinum crucible. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.3 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

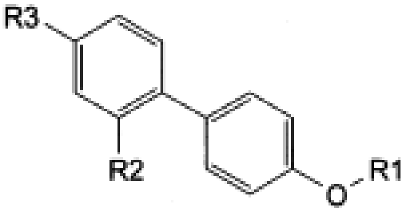
Dissolve 0.200 g in 40 ml of *methanol R*. Add 5 ml of *water R* and 0.2 ml of *phenol red solution R*. Titrate with *0.1 M sodium hydroxide* until the colour changes from yellow to reddish-violet.

1 ml of *0.1 M sodium hydroxide* is equivalent to 25.02 mg of $C_{13}H_8F_2O_3$.

STORAGE

Protected from light.

IMPURITIES



A. R1 = R2 = R3 = H: biphenyl-4-ol,

B. R1 = H, R2 = R3 = F: 2′,4′-difluorobiphenyl-4-ol,

C. R1 = CO-CH_3, R2 = R3 = F: 2′,4′-difluorobiphenyl-4-yl acetate,

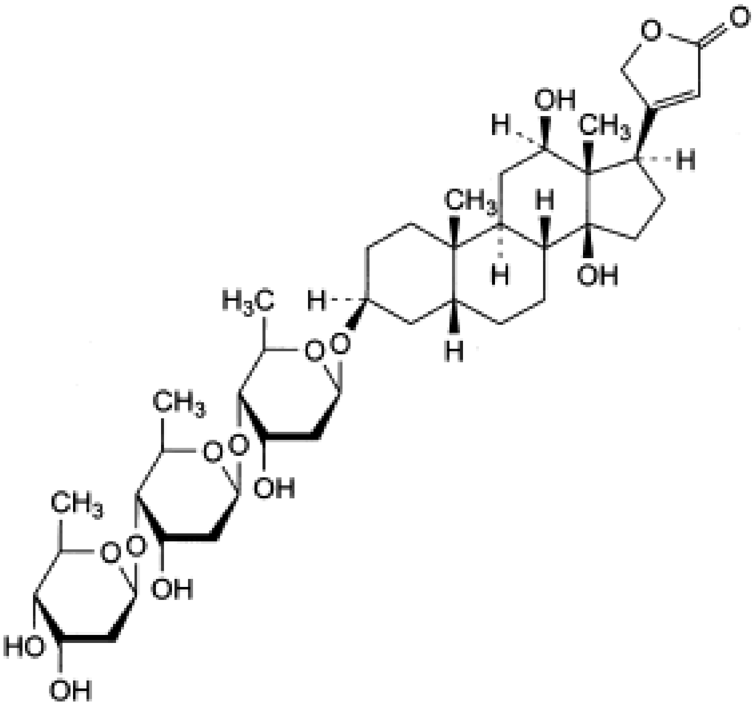
D. condensation products.

Ph Eur

Digoxin

General Notices

(Ph Eur monograph 0079)



$C_{41}H_{64}O_{14}$ 781 *20830-75-5*

Action and use

Na/K-ATPase inhibitor; cardiac glycoside.

Preparations

Digoxin Injection

Paediatric Digoxin Injection

Paediatric Digoxin Oral Solution

Digoxin Tablets

Ph Eur

DEFINITION

3β-[(2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-

2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide.

Content

96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder, or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in a mixture of equal volumes of methanol and methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *digoxin CRS.*

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method I)*.

Dissolve 50 mg in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Specific optical rotation *(2.2.7)*

+ 13.9 to + 15.9 (dried substance).

Dissolve 0.50 g in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 25.0 ml with the same mixture of solvents.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in 100.0 ml of *methanol R*.

Reference solution (a) Dissolve 10.0 mg of *digoxin CRS* in *methanol R* and dilute to 20.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*.

Reference solution (c) Dissolve 2.5 mg of *digoxigenin CRS* (impurity C) in *methanol R* and dilute to 5.0 ml with the same solvent. Dilute 1.0 ml of the solution to 50.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Reference solution (d) Dissolve 50.0 mg of *lanatoside C R* (impurity H) in *methanol R* and dilute to 100.0 ml with the same solvent. To 1.0 ml of this solution, add 1.0 ml of the test solution and dilute to 20.0 ml with *methanol R*.

Reference solution (e) Dissolve 5.0 mg of *digoxin for peak identification CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Column:
- *size*: *l* = 15 m, Ø = 3.9 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A: acetonitrile R, water R* (10:90 *V/V*);
- *mobile phase B: water R, acetonitrile R* (10:90 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	78	22
5 - 15	78 → 30	22 → 70
15 - 16	30 → 78	70 → 22
16 - 30	78	22

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl of the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *digoxin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities A, B, C, E, F, G, K.

Relative retention With reference to digoxin (retention time = about 4.3 min): impurity C = about 0.3; impurity E = about 0.5; impurity F = about 0.6; impurity G = about 0.8; impurity L = about 1.4; impurity K = about 1.6; impurity B = about 2.2; impurity A = about 2.6.

System suitability Reference solution (d):
- *resolution*: minimum 1.5 between the peaks due to impurity H and digoxin.

Limits:
- *impurities E, K*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurity L*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *impurity G*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);
- *impurities A, B*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurity F*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);
- *impurity C*: not more than 5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *sum of impurities other than A, B, C, E, F, G, K, L*: not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);

— *total*: not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

The thresholds indicated under Related Substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven *in vacuo*.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{41}H_{64}O_{14}$ from the declared content of *digoxin CRS*.

STORAGE

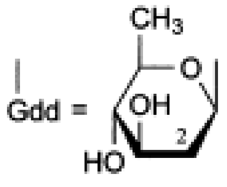
Protected from light.

IMPURITIES

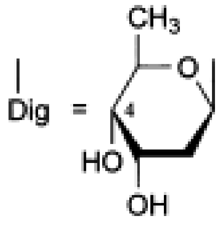
Specified impurities

A, B, C, E, F, G, K, L.

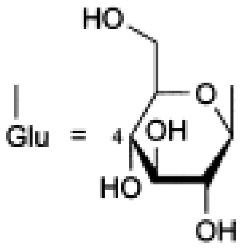
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D, H, I, J.*



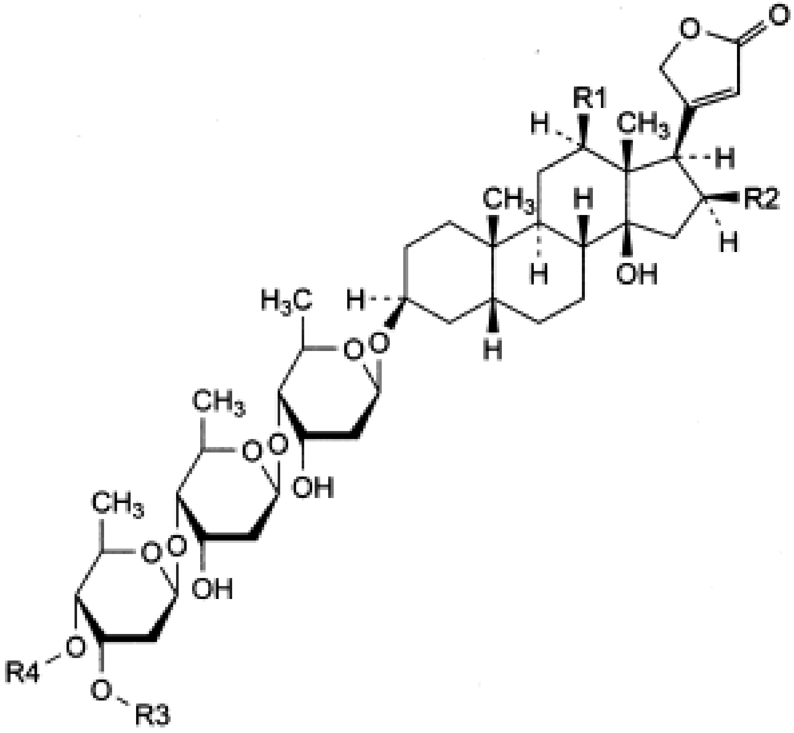
2,6-dideoxy-
β-D-glucopyranosyl



β-D-digitoxosyl



β-D-glucopyranosyl



A. R1 = R2 = R3 = R4 = H: digitoxin,

B. R1 = R3 = R4 = H, R2 = OH: 3β-[(2,6-dideoxy-β-D -*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14,16β-dihydroxy-5β-card-20(22)-enolide (gitoxin),

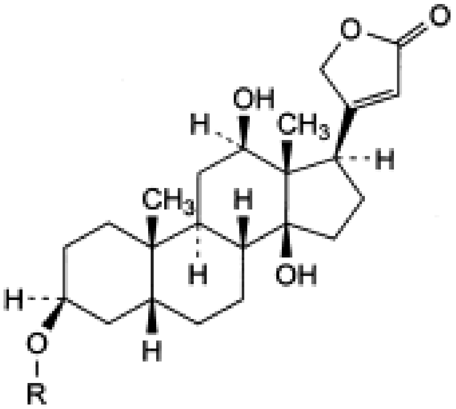
E. R1 = R2 = OH, R3 = R4 = H: 3β-[(2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]- 12β,14, 16β-trihydroxy-5β-card-20(22)-enolide (diginatin),

H. R1 = OH, R2 = H, R3 = CO-CH_3, R4 = Glu: 3β-[(β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-2,6-dideoxy-β-D -*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (lanatoside C),

I. R1 = OH, R2 = R4 = H, R3 = CO-CH_3: 3β-[(3-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (α-acetyldigoxin),

J. R1 = OH, R2 = R3 = H, R4 = CO-CH_3: 3β-[(4-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (β-acetyldigoxin),

K. R1 = OH, R2 = R3 = H, R4 = Dig: 3β-[(2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (digoxigenin tetrakisdigitoxoside),



C. R = H: 3β,12β,14-trihydroxy-5β-card-20(22)-enolide (digoxigenin),

D. R = Dig: 3β-(2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy)-12β,14-dihydroxy-5β-card-20(22)-enolide (digoxigenin monodigitoxoside),

F. R = Dig-(1→4)-Dig: 3β-[(2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (digoxigenin bisdigitoxoside),

G. R = Gdd-(1→4)-Dig-(1→4)-Dig: 3β-[(2,6-dideoxy-β-D-*arabino*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide (neodigoxin),

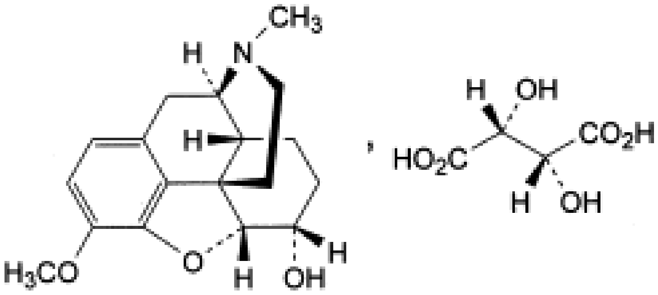
L. unknown structure.

Ph Eur

Dihydrocodeine Tartrate

General Notices

(Dihydrocodeine Hydrogen Tartrate, Ph Eur monograph 1776)



$C_{18}H_{23}NO_3,C_4H_6O_6$ 451.5 *5965-13-9*

Action and use

Opioid receptor agonist; analgesic.

Preparations

Co-dydramol Tablets

Dihydrocodeine Injection

Dihydrocodeine Oral Solution

Dihydrocodeine Tablets

Ph Eur

DEFINITION

4,5α-Epoxy-3-methoxy-17-methylmorphinan-6α-ol hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in alcohol, practically insoluble in cyclohexane.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of dihydrocodeine hydrogen tartrate.*

B. To about 0.1 g add 1 ml of *sulphuric acid R* and 0.05 ml of *ferric chloride solution R1* and heat on a water-bath. A brownish-yellow colour develops. Add 0.05 ml of *dilute nitric acid R*. The colour does not become red.

C. To 1 ml of solution S (see Tests) add 5 ml of *picric acid solution R*. Heat on a water-bath until a clear solution is obtained. Allow to cool. A precipitate is formed. Filter, wash with 5 ml of *water R* and dry at 100-105 °C. The crystals melt *(2.2.14)* at 220 °C to 223 °C.

D. It gives reaction (b) of tartrates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

pH *(2.2.3)*

3.2 to 4.2 for solution S.

Specific optical rotation *(2.2.7)*

- 70.5 to - 73.5 (anhydrous substance).

Dilute 10.0 ml of solution S to 20.0 ml with *water R.*

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *codeine phosphate R* in 2.0 ml of the test solution and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 200 ml with the mobile phase.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase To 1.0 g of *sodium heptanesulphonate R*, add 10.0 ml of *glacial acetic acid R* and 4.0 ml of a solution of 5.0 ml of *triethylamine R* diluted to 25.0 ml with a mixture of equal volumes of *water R* and *acetonitrile R*. Add 170 ml of *acetonitrile R* and dilute to 1000 ml with *water R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 284 nm.

Injection 20 µl.

Run time 5 times the retention time of dihydrocodeine.

Retention time Dihydrocodeine = about 14 min.

System suitability
Reference solution (a):

— *resolution*: minimum of 2 between the peaks due to dihydrocodeine and to impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *any other peak*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent); disregard any peak due to tartaric acid (relative retention with reference to dihydrocodeine = about 0.25),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

Maximum 0.7 per cent, determined on 1.00 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 45.15 mg of $C_{22}H_{29}NO_9$.

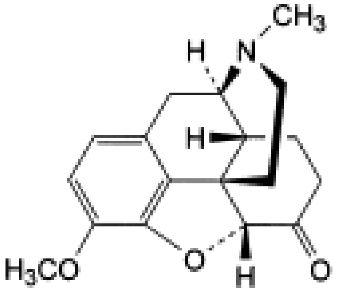
STORAGE

Protected from light.

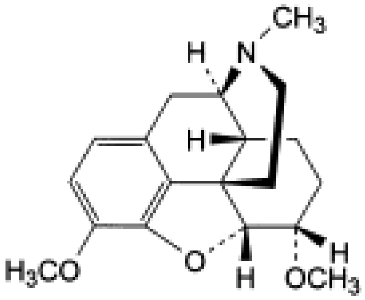
IMPURITIES

A. codeine,

B. morphine,



C. 4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one (hydrocodone),



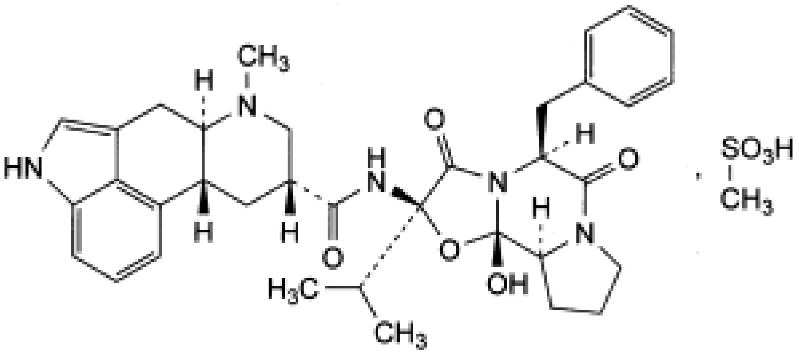
D. 4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan (tetrahydrothebaine).

Ph Eur

Dihydroergocristine Mesilate

General Notices

(Ph Eur monograph 1416)



$C_{36}H_{45}N_5O_8S$ 708 *24730-10-7*

Action and use

Vasodilator.

Ph Eur

DEFINITION

(6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-Benzyl-10b-hydroxy-2-(1-methylethyl)-3,6-dioxo-octahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-c]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide methanesulphonate.

Content

98.0 per cent to 102.0 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

White or almost white, fine crystalline powder.

Solubility

Slightly soluble in water, soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *dihydroergocristine mesilate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.10 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dissolve 0.10 g of *dihydroergocristine mesilate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *concentrated ammonia R, dimethylformamide R, ether R* (2:15:85 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate protected from light.

Drying In a current of cold air for 5 min.

Detection Spray with *dimethylaminobenzaldehyde solution R7* and dry in a current of hot air for 2 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dissolve 0.20 g of *methanesulphonic acid R* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents. Dilute 1 ml of the solution to 10 ml with a mixture of 1 volume of *methanol* R and 9 volumes of *methylene chloride R*.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *water R, concentrated ammonia R, butanol R, acetone R* (5:10:20:65 *V/V/V/V*).

Application 10 µl.

Development Over a path of 10 cm protected from light.

Drying In a current of cold air for not more than 1 min.

Detection Spray with a 1 g/l solution of *bromocresol purple R* in *methanol R*, adjusting the colour to violet-red with one drop of *dilute ammonia R1* and dry the plate in a current of hot air at 100 °C.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear (2.2.1) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Dissolve 0.50 g in *methanol R* and dilute to 25.0 ml with the same solvent.

pH *(2.2.3)*

4.0 to 5.0.

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 37 to - 43 (dried substance).

Dissolve 0.250 g in *anhydrous pyridine R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test and preparation of the solutions protected from bright light.*

Test solution Dissolve 75.0 mg of the substance to be examined in 10 ml of *acetonitrile R*. Add 10 ml of a 1.0 g/l solution of *phosphoric acid R* and dilute to 50.0 ml with *water R*.

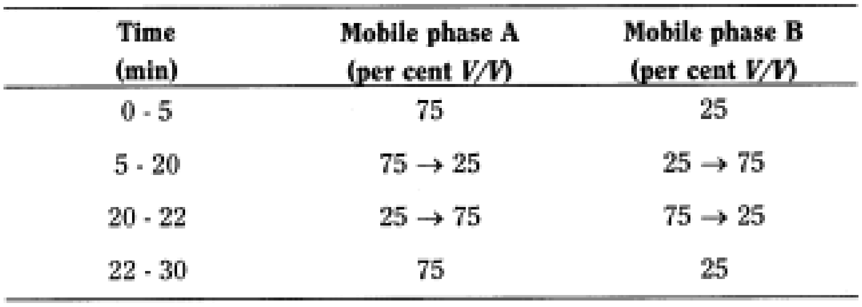
Reference solution Dissolve 20.0 mg of *codergocrine mesilate CRS* in 10 ml of *acetonitrile R*. Add 10 ml of a 1.0 g/l solution of *phosphoric acid R* and dilute to 50.0 ml with *water R*. Dilute 6.0 ml of the solution to 50.0 ml with a mixture of 20 volumes of *acetonitrile R*, 20 volumes of a 1.0 g/l solution of *phosphoric acid R* and 60 volumes of *water R*.

Column:

- size: l = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase:

- *mobile phase A*: mix 100 volumes of *acetonitrile R* with 900 volumes of *water R* and add 10 volumes of *triethylamine R*,
- *mobile phase B*: mix 100 volumes of *water R* with 900 volumes of *acetonitrile R* and add 10 volumes of *triethylamine R*.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	75	25
5 - 20	75 → 25	25 → 75
20 - 22	25 → 75	75 → 25
22 - 30	75	25

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µl.

Relative retention With reference to dihydroergocristine (retention time = 13.7 min): impurity F = about 0.8; impurity H = about 0.9; impurity I = about 1.02.

System suitability Reference solution:

— the chromatogram shows 4 peaks,

— *resolution*: minimum 1 between the peaks corresponding to dihydroergocristine and impurity I.

Limits:

— *any impurity*: not more than the area of the peak corresponding to dihydroergocristine in the chromatogram obtained with the reference solution (1 per cent),

— *total*: not more than twice the area of the peak corresponding to dihydroergocristine in the chromatogram obtained with the reference solution (2 per cent),

— *disregard limit*: 0.1 times the area of the peak corresponding to dihydroergocristine in the chromatogram obtained with the reference solution (0.1 per cent).

Loss on drying *(2.2.32)*

Maximum 3.0 per cent, determined on 0.500 g by drying under high vacuum at 80 °C.

ASSAY

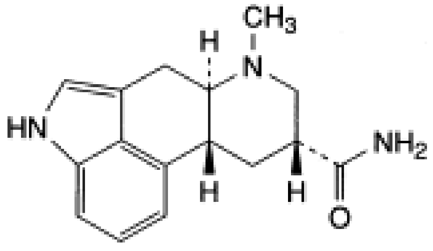
Dissolve 0.300 g in 60 ml of *pyridine R*. Pass a stream of *nitrogen R* over the surface of the solution and titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Note the volume used at the second point of inflexion.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 35.39 mg of $C_{36}H_{45}N_5O_8S$.

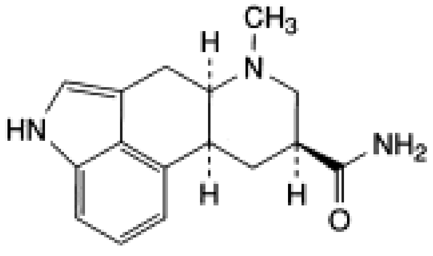
STORAGE

Store protected from light.

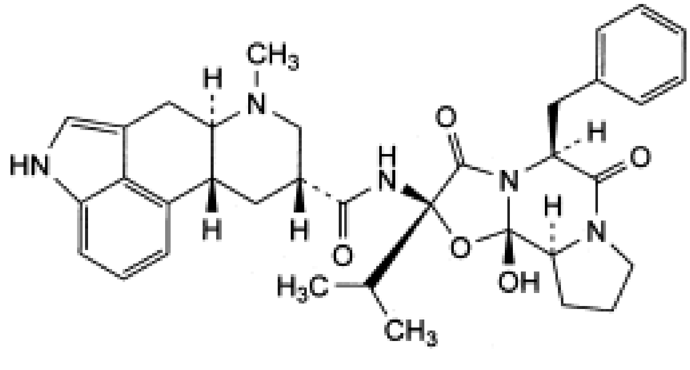
IMPURITIES



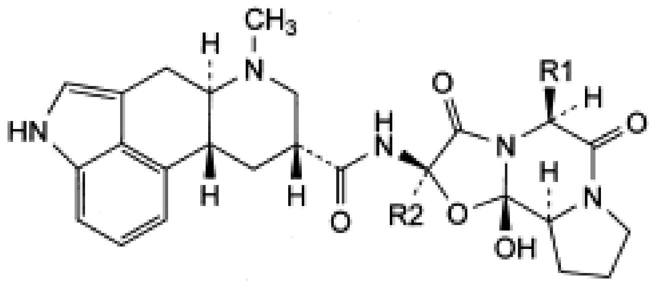
A. (6a*R*,9*R*,10a*R*)-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (6-methylergoline-8β-carboxamide),



B. (6a*R*,9*S*,10a*S*)-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (6-methylisoergoline-8α-carboxamide),



C. (6a*R*,9*R*,10a*R*)-N-[(2*S*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-(1-methylethyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (2′-epidihydroergocristine),



D. R1 = $CH(CH_3)_2$, R2 = CH_3: (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10bS)-10b-hydroxy-2-methyl-5-(1-methylethyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-

4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (dihydroergosine),

E. R1 = CH_2-C_6H_5, R2 = CH_3: (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-c]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline- 9-carboxamide (dihydroergotamine),

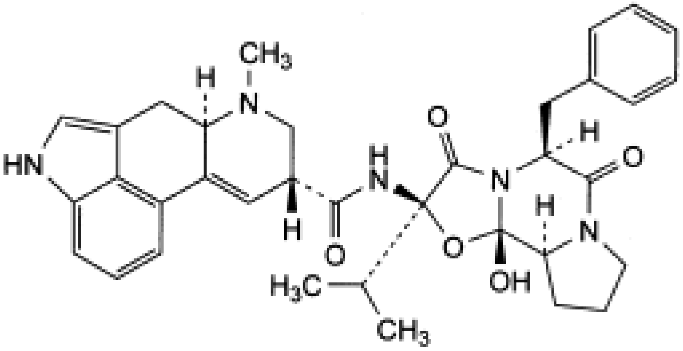
F. R1 = R2 = $CH(CH_3)_2$: (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)10b-hydroxy-2,5-bis(1-methylethyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (dihydroergocornine),

G. R1 = CH_2-C_6H_5, R2 = CH_2-CH_3: (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-2-ethyl-10b-hydroxy-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (dihydroergostine),

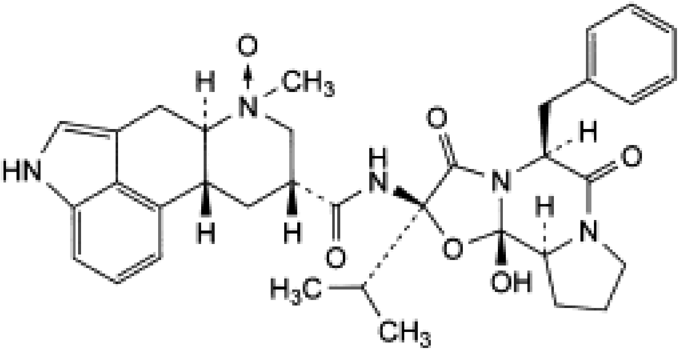
H. R1 = CH_2-$CH(CH_3)_2$, R2 = $CH(CH_3)_2$: (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2-(1-methylethyl)-5-(2-methylpropyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-a]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (α-dihydroergocryptine),

I. R1 = $C^*H(CH_3)$-CH_2-CH_3, R2 = $CH(CH_3)_2$: (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-10b-hydroxy-2-(1-methylethyl)-5-[(1*RS*-1-methylpropyl]-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (β-dihydroergocryptine or epicriptine),

J. R1 = CH_2-C_6H_5, R2 = $C^*H(CH_3)$-CH_2-CH_3: (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-[(1*RS*)-1-methylpropyl]-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (dihydroergosedmine),



K. (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-(1-methylethyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (ergocristine),



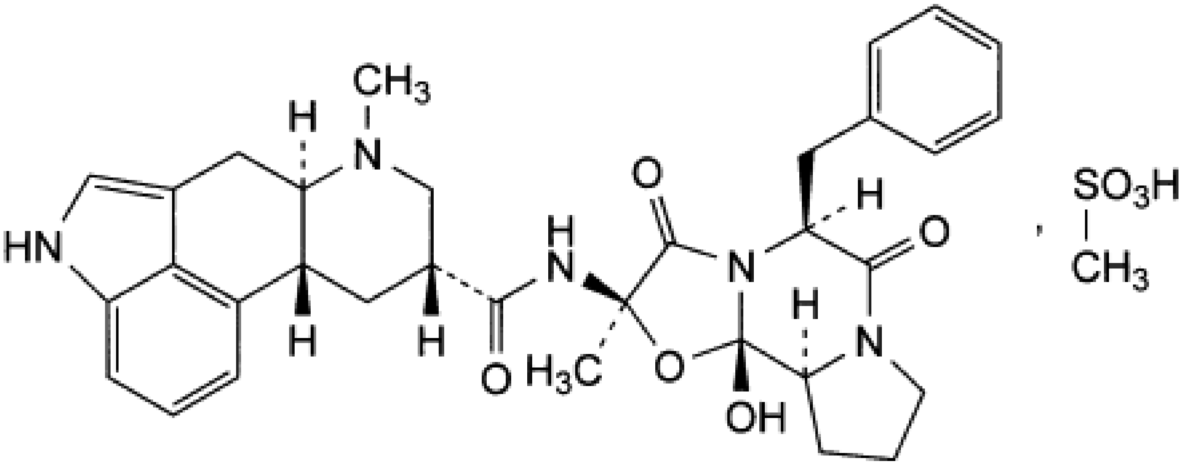
L. (6a*R*,7*RS*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-(1-methylethyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide 7-oxide (dihydroergocristine 6-oxide).

Ph Eur

Dihydroergotamine Mesilate

General Notices

(Ph Eur monograph 0551)



$C_{33}H_{37}N_5O_5,CH_4SO_3$ 680 *6190-39-2*

Action and use

Vasodilator.

Ph Eur

DEFINITION

(6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-Benzyl-10b-hydroxy-2-methyl-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide methanesulphonate.

Content

98.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent) .

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 5.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent.

Spectral range 250-350 nm.

Absorption maxima At 281 nm and 291 nm.

Shoulder At 275 nm.

Absorbance Negligible above 320 nm.

Specific absorbance at the absorption maximum at 281 nm 95 to 105 (dried substance).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *dihydroergotamine mesilate CRS.*

C. Thin-layer chromatography (*2.2.27*). *Prepare the reference solution and the test solution immediately before use.*

Solvent mixture *methanol R*, *methylene chloride R* (10:90 *V/V*).

Test solution Dissolve 5 mg of the substance to be examined in the solvent mixture and dilute to 2.5 ml with the solvent mixture.

Reference solution Dissolve 5 mg of *dihydroergotamine mesilate CRS* in the solvent mixture and dilute to 2.5 ml with the solvent mixture.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R*, *methanol R*, *ethyl acetate R*, *methylene chloride R* (1:6:50:50 *V/V/V/V*).

Application 5 µl.

Development Protected from light, over a path of 15 cm; dry in a current of cold air for not longer than 1 min and repeat the development protected from light over a path of 15 cm using a freshly prepared amount of the mobile phase.

Drying In a current of cold air.

Detection Spray abundantly with *dimethylaminobenzaldehyde solution R7* and dry in a current of hot air for about 2 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 0.1 g of the substance to be examined, add 5 ml of *dilute hydrochloric acid R* and shake for about 5 min. Filter, then add 1 ml of *barium chloride solution R1*. The filtrate remains clear. Mix 0.1 g of the substance to be examined with 0.4 g of powdered *sodium hydroxide R*, heat to fusion and continue to heat for 1 min. Cool, add 5 ml of *water R*, boil and filter. Acidify the filtrate with *hydrochloric acid R1* and filter again. The filtrate gives reaction (a) of sulphates (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 or BY_7 (*2.2.2, Method II*).

Dissolve 0.10 g in a mixture of 0.1 ml of a 70 g/l solution of *methanesulphonic acid R* and 50 ml of *water R.*

pH (*2.2.3*)

4.4 to 5.4.

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Specific optical rotation (*2.2.7*)

- 42 to - 47 (dried substance).

Dissolve 0.250 g in *anhydrous pyridine R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *acetonitrile R*, *water R* (50:50 *V/V*).

Test solution Dissolve 70 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dissolve 7 mg of the substance to be examined and 6.8 mg of *ergotamine tartrate CRS* (impurity A) (equivalent to 7 mg of ergotamine mesilate) in the solvent mixture and dilute to 100 ml with the solvent mixture. Dilute 5 ml of this solution to 10 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *dihydroergotamine for peak identification CRS* (containing impurities A, B, C and D) in the solvent mixture, add 100 µl of *dilute sulphuric acid R* and dilute to 5 ml with the solvent mixture.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 25 °C.

Mobile phase:

— *mobile phase A*: 3 g/l solution of *sodium heptanesulphonate monohydrate R* adjusted to pH 2.0 with *phosphoric acid R;*

— *mobile phase B*: mobile phase A, *acetonitrile for chromatography R* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	58 → 40	42 → 60

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 5 µl.

Identification of impurities Use the chromatogram supplied with *dihydroergotamine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C and D.

Relative retention With reference to dihydroergotamine (retention time = about 6.5 min): impurity D = about 0.7; impurity C = about 0.86; impurity A = about 0.95; impurity B = about 1.2.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity A and dihydroergotamine.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.3; impurity C = 1.3;

— *impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities A and D*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 4.0 per cent, determined on 0.500 g by drying at 105 °C at a pressure not exceeding 0.1 kPa for 5 h.

ASSAY

Dissolve 0.500 g in a mixture of 10 ml of *anhydrous acetic acid R* and 70 ml of *acetic anhydride R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 68.00 mg of $C_{34}H_{41}N_5O_8S$.

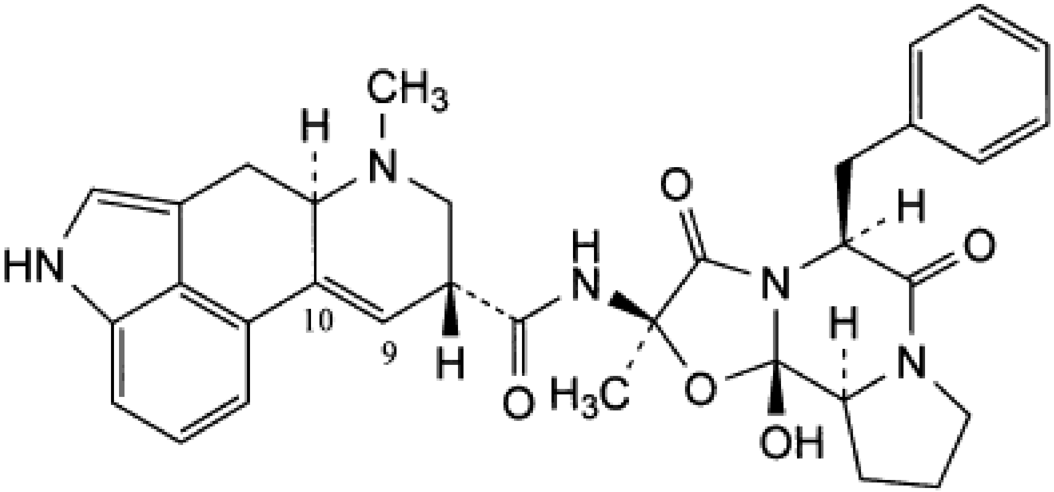
STORAGE

Protected from light.

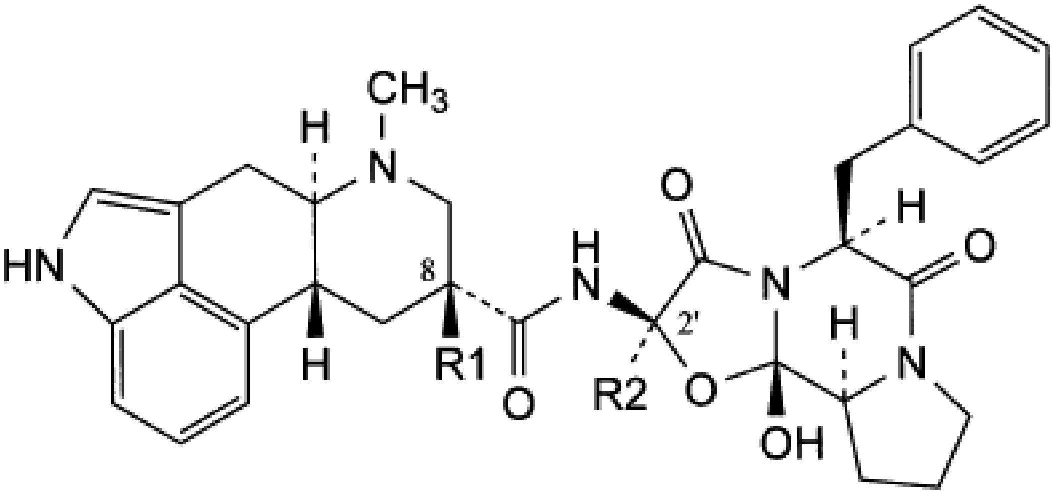
IMPURITIES

Specified impurities   *A, B, C, D.*

Other detectable impurities   (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E*.

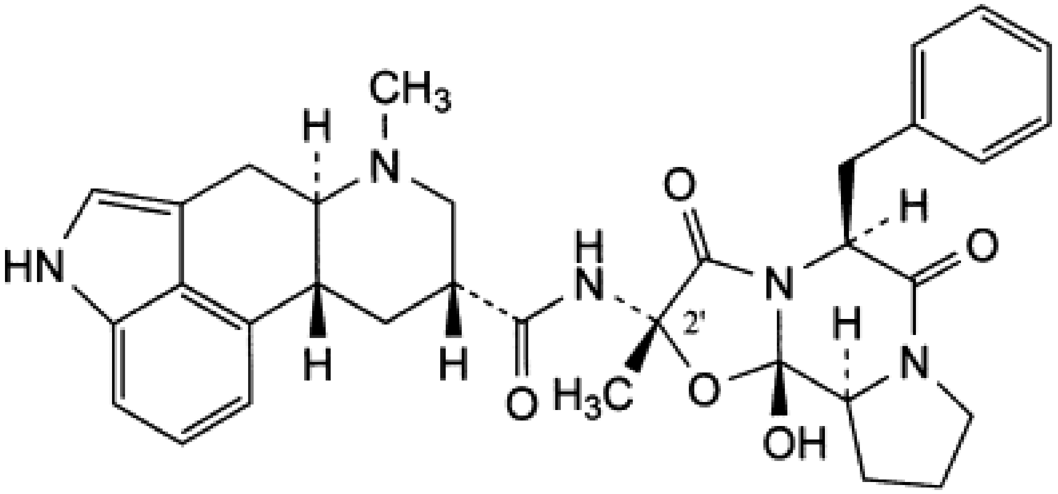


A. (6a*R*,9*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*] quinoline-9-carboxamide (ergotamine),



B. R1 = H, R2 = C_2H_5: (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-2-ethyl-10b-hydroxy-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide (9,10-dihydroergostine),

C. R1 = OH, R2 = CH_3: (6a*R*,9*S*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-9-hydroxy-7-methyl-4,6,6a,7,8,9,10,10aoctahydroindolo[4,3-*fg*]quinoline-9-carboxamide (8-hydroxy-9,10-dihydroergotamine),



D. (6a*R*,9*R*,10a*R*)-*N*-[(2*S*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10aoctahydroindolo[4,3-*fg*]quinoline-9-carboxamide (2′-epi-9,10-dihydroergotamine),

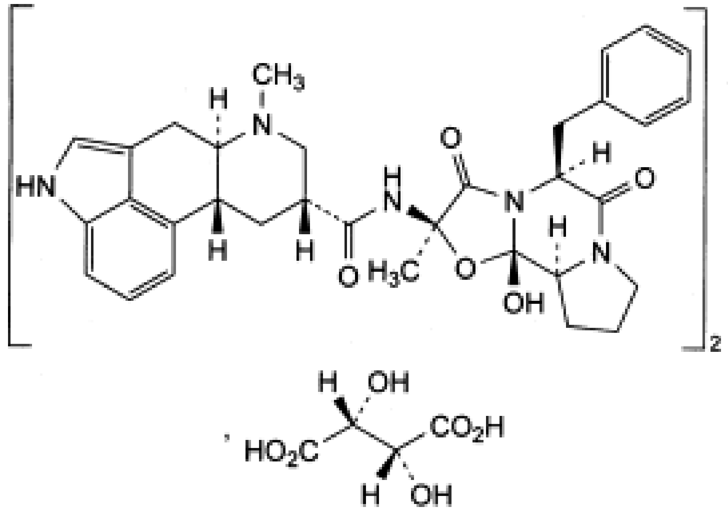
E. dihydroergocristine.

Ph Eur

Dihydroergotamine Tartrate

General Notices

(Ph Eur monograph 0600)



$(C_{33}H_{37}N_5O_5)_2,C_4H_6O_6$ 1317 *5989-77-5*

Action and use

Vasodilator.

Ph Eur

DEFINITION

Bis[(6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxooctahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide] (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very slightly soluble in water, sparingly soluble in alcohol.

IDENTIFICATION

First identification B, C.

Second identification A, C, D.

A. Dissolve 5.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Examined between 250 nm and 350 nm *(2.2.25)*, the solution shows 2 absorption maxima, at 281 nm and 291 nm, and a shoulder at 275 nm. Above 320 nm the absorbance is negligible. The specific absorbance at the maximum at 281 nm is 95 to 115 (dried substance).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *dihydroergotamine tartrate CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Suspend about 15 mg in 1 ml of *water R*. 0.1 ml of the suspension gives reaction (b) of tartrates (*2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 or BY_7 *(2.2.2, Method II)*.

Dissolve 0.1 g in *alcohol (85 per cent V/V) R* warming carefully in a water-bath at 40 °C and dilute to 50 ml with the same solvent.

pH *(2.2.3)*

4.0 to 5.5 for the clear supernatant.

Suspend 50 mg in 50 ml of *carbon dioxide-free water R* and shake for 10 min. Allow to stand.

Specific optical rotation *(2.2.7)*

- 52 to - 57 (dried substance).

Dissolve 0.250 g in *anhydrous pyridine R* and dilute to 25.0 ml with the same solvent.

Related substances

Thin-layer chromatography *(2.2.27)*. *Prepare the reference solutions and the test solutions immediately before use and in the order indicated.*

Reference solution (a) Dissolve 20 mg of *dihydroergotamine tartrate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dilute 2.5 ml of reference solution (a) to 50 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R*.

Reference solution (c) Dilute 2 ml of reference solution (b) to 5 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R* and dilute to 5 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R*.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, methanol R, ethyl acetate R, methylene chloride R* (1:6:50:50 *V/V/V/V*).

Application 5 µl.

Development Protected from light over a path of 15 cm. Dry the plate in a current of cold air for not longer than 1 min. Repeat the development protected from light over a path of 15 cm using a freshly prepared amount of the mobile phase.

Drying In a current of cold air.

Detection Spray the plate abundantly with *dimethylaminobenzaldehyde solution R7* and dry in a current of hot air for about 2 min.

Limits In the chromatogram obtained with test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than 2 such spots are more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 0.200 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.05 M perchloric acid* is equivalent to 32.93 mg of $C_{70}H_{80}N_{10}O_{16}$.

STORAGE

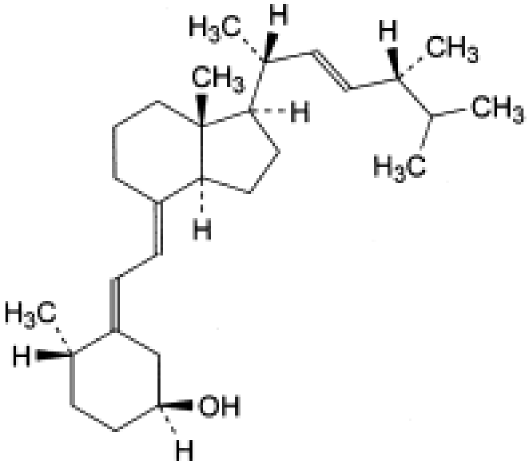
Protected from light.

Ph Eur

Dihydrotachysterol

General Notices

(Ph Eur monograph 2014)



$C_{28}H_{46}O$ 398.7 *67-96-9*

Vitamin D analogue.

Ph Eur

DEFINITION

(5*E*,7*E*,22*E*)-9,10-Seco-10α-ergosta-5,7,22-trien-3β-ol.

Content

97.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

Colourless crystals or white or almost white crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and hexane, sparingly soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   dihydrotachysterol CRS.

If the spectra obtained in the solid state show differences, record new spectra using the residues after recrystallisation from *methanol R*.

TESTS

Specific optical rotation *(2.2.7)*

+ 99 to + 103.

Dissolve 0.500 g in *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution   Dissolve 10.00 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution (a)   Dissolve 1.0 mg of *dihydrotachysterol for system suitability CRS* (containing impurities A, B and C) in *acetonitrile R* and dilute to 5.0 ml with the same solvent.

Reference solution (b)   Dissolve 10.00 mg of *dihydrotachysterol CRS* in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution (c)   Dilute 5.0 ml of the test solution to 100.0 ml with *acetonitrile R*. Dilute 5.0 ml of this solution to 50.0 ml with *acetonitrile R*.

Column:

- size: *l* = 0.25 m, Ø = 3.0 mm,
- *stationary phase*: spherical trifunctional *end-capped octadecylsilyl silica gel for chromatography R* (4 µm),
- *temperature*: 40 °C.

Mobile phase   *decanol R, water for chromatography R, acetonitrile for chromatography R* (1:25:1000 *V/V/V*).

Flow rate   0.5 ml/min.

Detection   Variable-wavelength spectrophotometer capable of operating at 251 nm and at 203 nm.

Injection   5 µl of the test solution and reference solutions (a) and (c).

Run time   Twice the retention time of dihydrotachysterol.

Identification of impurities   Reference solution (a):

- use the chromatogram obtained at 203 nm and the chromatogram obtained at 203 nm supplied with *dihydrotachysterol for system suitability CRS* to identify the peak due to impurity A,
- use the chromatogram obtained at 251 nm and the chromatogram obtained at 251 nm supplied with *dihydrotachysterol for system suitability CRS* to identify the peak due to

impurities B and C.

Relative retention With reference to dihydrotachysterol (retention time = about 15 min); impurity B = about 0.9; impurity C = about 1.2; impurity A (not visible at 251 nm, detected at 203 nm) = about 1.2.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum of 4, where H_p = height above the baseline of the peak due to impurity B, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to dihydrotachysterol in the chromatogram obtained at 251 nm.

Examine the chromatogram obtained at 203 nm for impurity A and the chromatogram obtained at 251 nm for the impurities other than A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *impurities B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *total (including A)*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) at 251 nm (1.0 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water *(2.5.32)*

Maximum 0.1 per cent, determined on 40.0 mg.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 251 nm.

Injection Test solution and reference solution (b).

Calculate the percentage content of $C_{28}H_{46}O$ using the chromatograms obtained with the test solution and reference solution (b) and the declared content of *dihydrotachysterol CRS*.

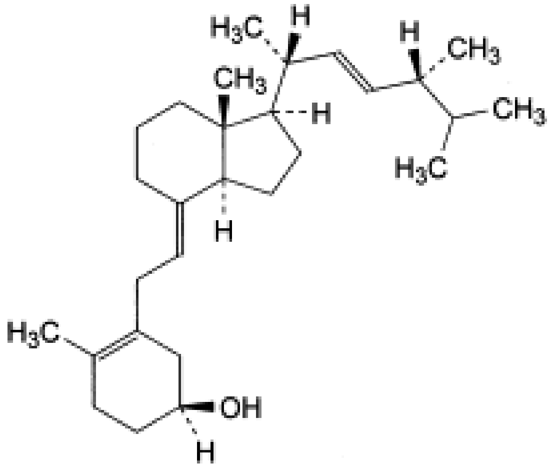
STORAGE

Under an inert gas, in an airtight container, at a temperature of 2 °C to 8 °C.

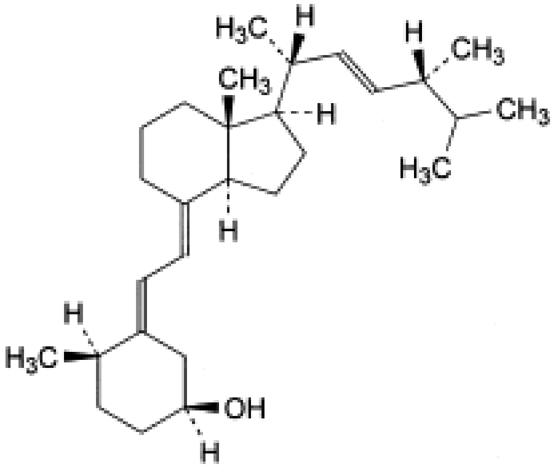
The contents of an opened container are to be used immediately.

IMPURITIES

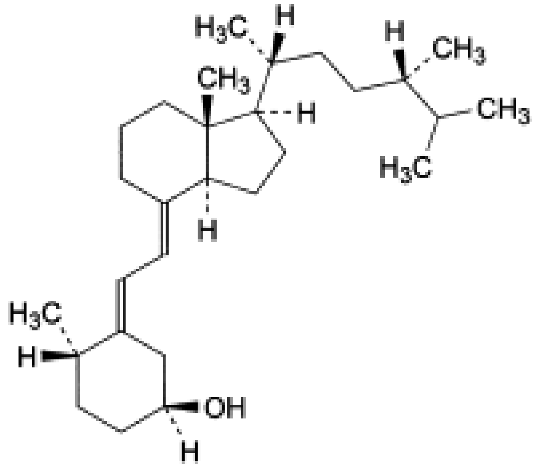
Specified impurities A, B, C.



A. (7*E*,22*E*)-9,10-secoergosta-5(10),7,22-trien-3β-ol (dihydrovitamin D_2-I),



B. (5*E*,7*E*,22*E*)-9,10-secoergosta-5,7,22-trien-3β-ol (dihydrovitamin D_2-IV),

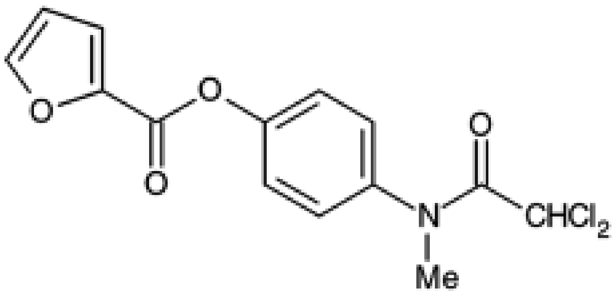


C. (5*E*,7*E*)-9,10-seco-10α-ergosta-5,7-dien-3β-ol (dihydrotachysterol$_4$).

Ph Eur

Diloxanide Furoate

General Notices



$C_{14}H_{11}Cl_2NO_4$ 328.2 *3736-81-0*

Action and use

Antiprotozoal.

Preparation

Diloxanide Tablets

DEFINITION

Diloxanide Furoate is 4-(*N*-methyl-2,2-dichloroacetamido)phenyl 2-furoate. It contains not less than 98.0% and not more than 102.0% of $C_{14}H_{11}Cl_2NO_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Very slightly soluble in *water*; slightly soluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of diloxanide furoate *(RS 103)*.

B. The *light absorption*, Appendix II B, in the range 240 to 350 nm of a 0.0014% w/v solution in *ethanol (96%)* exhibits a maximum only at 258 nm. The *absorbance* at the maximum is about 0.98.

C. Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 10 ml of 1M *sodium hydroxide* as the absorbing liquid. When the process is complete, acidify the liquid with *nitric acid* and add *silver nitrate solution*. A white precipitate is produced.

TESTS

Melting point

114° to 116°, Appendix V A.

Free acidity

Shake 3 g with 50 ml of *water*, filter and wash the residue with three 20 ml quantities of *water*. Titrate the combined filtrate and washings with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 1.3 ml is required.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* HF_{254} as the coating substance and a mixture of 96 volumes of *dichloromethane* and 4 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions of the substance being examined in *dichloromethane* containing (1) 10.0% w/v and (2) 0.025% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.3 g in 50 ml of *anhydrous pyridine* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 32.82 mg of $C_{14}H_{11}Cl_2NO_4$.

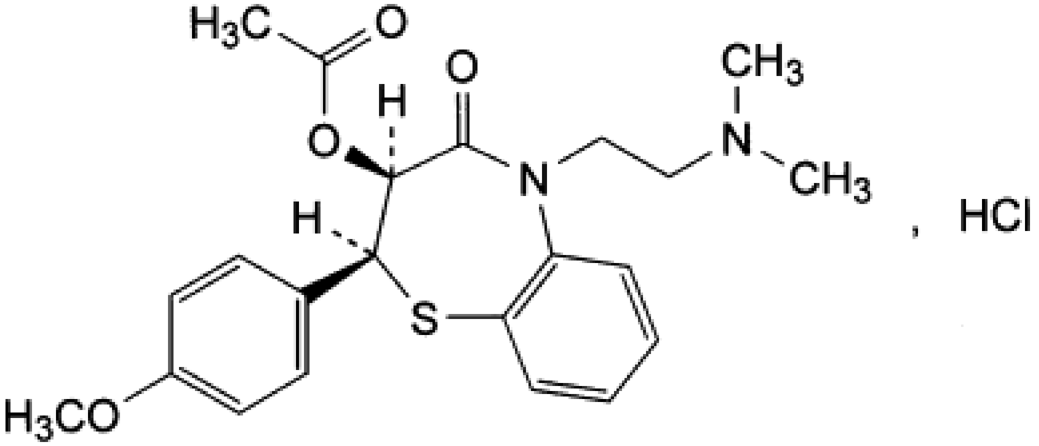
STORAGE

Diloxanide Furoate should be protected from light.

Diltiazem Hydrochloride

General Notices

(Ph Eur monograph 1004)



$C_{22}H_{26}N_2O_4S,HCl$ 451.0 *33286-22-5*

Action and use

Calcium channel blocker.

Ph Eur

DEFINITION

Hydrochloride of (2*S*,3*S*)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, in methanol and in methylene chloride, slightly soluble in anhydrous ethanol.

mp

About 213 °C, with decomposition.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *diltiazem hydrochloride CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.10 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.10 g of *diltiazem hydrochloride CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetic acid R*, *water R*, *methylene chloride R*, *anhydrous ethanol R* (1:3:10:12 *V/V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 50 mg in 5 ml of *water R*. Add 1 ml of *ammonium reineckate solution R*. A pink precipitate is produced.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.00 g in *carbon-dioxide free water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.3 to 5.3.

Dilute 2.0 ml of solution S to 10.0 ml with *carbon dioxide-free water R.*

Specific optical rotation *(2.2.7)*

+ 115 to + 120 (dried substance).

Dilute 5.0 ml of solution S to 25.0 ml with *water R.*

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 200.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *diltiazem hydrochloride CRS* in the mobile phase and dilute to 200.0 ml with the mobile phase. Dilute 1.2 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *diltiazem impurity A CRS* in 1.0 ml of reference solution (a).

Reference solution (c) Dilute 0.3 ml of the test solution to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Mix 5 volumes of *anhydrous ethanol R,* 25 volumes of *acetonitrile R* and 70 volumes of a solution containing 6.8 g/l of *potassium dihydrogen phosphate R* and 0.1 ml/l of *N,N-dimethyloctylamine R,* adjusted to pH 4.5 with *dilute phosphoric acid R.*

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time 5 times the retention time of diltiazem.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to impurity A and diltiazem; if necessary, adjust the concentration of *N*,*N*-dimethyloctylamine in the mobile phase;

— *symmetry factor*: maximum 2.0 for the peaks due to impurity A and diltiazem; if necessary, adjust the concentration of *N*,*N*-dimethyloctylamine in the mobile phase.

Limits:

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (c).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20.0 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

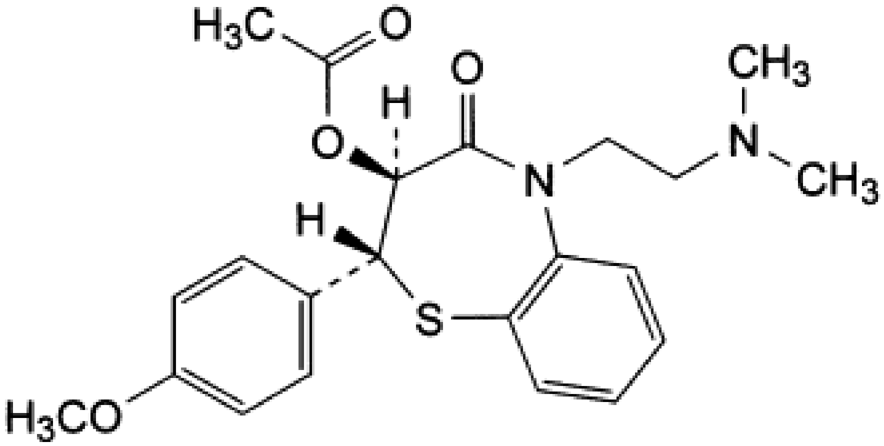
Dissolve 0.400 g in a mixture of 2 ml of *anhydrous formic acid R* and 60 ml of *acetic anhydride R* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 45.1 mg of $C_{22}H_{27}ClN_2O_4S$.

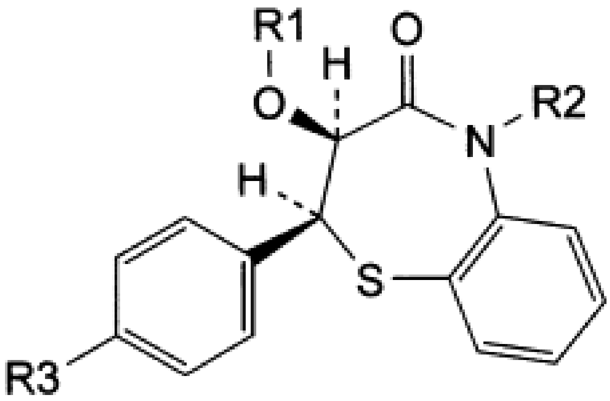
STORAGE

In an airtight container, protected from light.

IMPURITIES



A. (2*R*,3*S*)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate,



B. R1 = CO-CH_3, R2 = H, R3 = OCH_3: (2*S*,3*S*)-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate,

C. R1 = CO-CH_3, R2 = CH_2-CH_2-N$(CH_3)_2$, R3 = OH: (2*S*,3*S*)-5-[2-(dimethylamino)ethyl]-2-(4-

hydroxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate,

D. R1 = CO-CH_3, R2 = CH_2-CH_2-NH-CH_3, R3 = OCH_3: (2*S*,3*S*)-2-(4-methoxyphenyl)-5-[2-(methylamino)ethyl]-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate,

E. R1 = R2 = H, R3 = OCH_3: (2*S*,3*S*)-3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one,

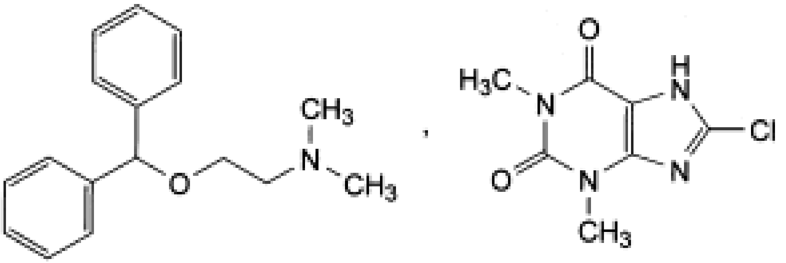
F. R1 = H, R2 = CH_2-CH_2-N$(CH_3)_2$, R3 = OCH_3: (2*S*,3*S*)-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one.

Ph Eur

Dimenhydrinate

General Notices

(Ph Eur monograph 0601)



$C_{17}H_{21}NO,C_7H_7ClN_4O_2$ 470.0 *523-87-5*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Dimenhydrinate Tablets

Ph Eur

DEFINITION

Dimenhydrinate contains not less than 53.0 per cent and not more than 55.5 per cent of diphenhydramine [2-(diphenylmethoxy)-*N,N*-dimethylethylamine, $C_{17}H_{21}NO$, M_r 255.4] and not less than 44.0 per cent and not more than 46.5 per cent of 8-chlorotheophylline (8-chloro-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione, $C_7H_7ClN_4O_2$, M_r 214.6), both calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, slightly soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Melting point *(2.2.14):* 102 °C to 106 °C.

B. Dissolve 0.1 g in a mixture of 3 ml of *water R* and 3 ml of *alcohol R*, add 6 ml of *water R* and 1 ml of *dilute hydrochloric acid R* and cool in iced water for 30 min, scratching the wall of the tube with a glass rod if necessary to initiate crystallisation. Dissolve about 10 mg of the precipitate obtained in 1 ml of *hydrochloric acid R*, add 0.1 g of *potassium chlorate R* and evaporate to dryness in a porcelain dish. A reddish residue is obtained which becomes violet-red when exposed to ammonia vapour.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *dimenhydrinate CRS*.

D. Dissolve 0.2 g in 10 ml of *alcohol R*. Add 10 ml of *picric acid solution R* and initiate crystallisation by scratching the wall of the tube with a glass rod. The precipitate, washed with *water R* and dried at 100 °C to 105 °C, melts *(2.2.14)* at 130 °C to 134 °C.

TESTS

Appearance of solution

Dissolve 1.0 g in *alcohol R* and dilute to 20 ml with the same solvent. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

To 0.4 g add 20 ml of *carbon dioxide-free water R*, shake for 2 min and filter. The pH of the filtrate is 7.1 to 7.6.

Theophylline and substances related to diphenhydramine

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.40 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *theophylline R* in *methylene chloride R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dilute 5 ml of the test solution to 100 ml with *methylene chloride R*. Dilute 10 ml of this solution to 100 ml with *methylene chloride R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 9 volumes of *methanol R* and 90 volumes of *methylene chloride R*. Dry the plate in a current of cold air and examine in ultraviolet light at 254 nm. Any spot corresponding to theophylline in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent). Spray with *potassium iodobismuthate solution R*. Allow the plate to dry in air and spray with *dilute hydrogen peroxide solution R*. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Disregard any spot extending from the starting point to an R_F of about 0.1.

Heavy metals *(2.4.8)*

Dissolve 2.5 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R* and dilute to 25 ml with the same mixture of solvents. The solution complies with limit test B for heavy metals (20 ppm). Prepare the standard using lead standard solution (2 ppm Pb) prepared by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying *in vacuo*.

Sulphated ash ***(2.4.14)***

Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

Diphenhydramine

Dissolve 0.200 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 25.54 mg of $C_{17}H_{21}NO$.

8-Chlorotheophylline

To 0.800 g add 50 ml of *water R*, 3 ml of *dilute ammonia R1* and 0.6 g of *ammonium nitrate R* and heat on a water-bath for 5 min. Add 25.0 ml of *0.1 M silver nitrate* and continue heating on a water-bath for 15 min with frequent swirling. Cool, add 25 ml of *dilute nitric acid R* and dilute to 250.0 ml with *water R*. Filter and discard the first 25 ml of the filtrate. Using 5 ml of *ferric ammonium sulphate solution R2* as indicator, titrate 100.0 ml of the filtrate with *0.1 M ammonium thiocyanate* until a yellowish-brown colour is obtained.

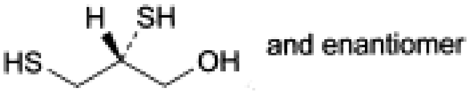
1 ml of *0.1 M silver nitrate* is equivalent to 21.46 mg of $C_7H_7ClN_4O_2$.

Ph Eur

Dimercaprol

General Notices

(Ph Eur monograph 0389)



$C_3H_8OS_2$ 124.2 *59-52-9*

Action and use

Chelating agent for use in heavy metal poisoning.

Preparation

Dimercaprol Injection

When B.A.L. is prescribed or demanded, Dimercaprol shall be dispensed or supplied.

Ph Eur

DEFINITION

(2*RS*)-2,3-Disulfanylpropan-1-ol.

Content

98.5 per cent to 101.5 per cent.

CHARACTERS

Appearance

Clear, colourless or slightly yellow liquid.

Solubility

Soluble in water and in arachis oil, miscible with ethanol (96 per cent) and with benzyl benzoate.

IDENTIFICATION

A. Dissolve 0.05 ml in 2 ml of *water R*. Add 1 ml of *0.05 M iodine*. The colour of the iodine is discharged immediately.

B. Dissolve 0.1 ml in 5 ml of *water R* and add 2 ml of *copper sulphate solution R*. A bluish-black precipitate is formed which quickly becomes dark grey.

C. In a ground-glass-stoppered tube, suspend 0.6 g of *sodium bismuthate R*, previously heated to 200 °C for 2 h, in a mixture of 2.8 ml of *dilute phosphoric acid R* and 6 ml of *water R*. Add 0.2 ml of the substance to be examined, mix and allow to stand for 10 min with frequent shaking. To 1 ml of the supernatant liquid add 5 ml of a 4 g/l solution of *chromotropic acid, sodium salt R* in *sulphuric acid R* and mix. Heat in a water-bath for 15 min. A violet-red colour develops.

TESTS

Appearance

It is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 or BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. Add 0.25 ml of *bromocresol green solution R* and 0.3 ml of *0.01 M hydrochloric acid*. The solution is yellow. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Refractive index *(2.2.6)*

1.568 to 1.574.

Halides

To 2.0 g add 25 ml of *alcoholic potassium hydroxide solution R* and boil under a reflux condenser for 2 h. Eliminate the ethanol by evaporation in a stream of hot air. Add 20 ml of *water R* and cool. Add 40 ml of *water R* and 10 ml of *strong hydrogen peroxide solution R*, boil gently for 10 min, cool and filter rapidly. Add 10 ml of *dilute nitric acid R* and 5.0 ml of *0.1 M silver nitrate*. Using 2 ml of *ferric ammonium sulphate solution R2* as indicator, titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained. Carry out a blank titration. The difference between the titration volumes is not greater than 1.0 ml.

ASSAY

Dissolve 0.100 g in 40 ml of *methanol R*. Add 20 ml of *0.1 M hydrochloric acid* and 50.0 ml of *0.05 M iodine*. Allow to stand for 10 min and titrate with *0.1 M sodium thiosulphate*. Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 6.21 mg of $C_3H_8OS_2$.

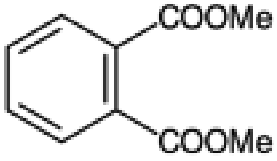
STORAGE

In a well-filled, airtight container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Dimethyl Phthalate

General Notices



$C_{10}H_{10}O_4$ 194.2 *131-11-3*

Action and use

Insect repellent.

DEFINITION

Dimethyl Phthalate contains not less than 99.0% and not more than 100.5% w/w of $C_{10}H_{10}O_4$.

CHARACTERISTICS

A colourless or faintly coloured liquid.

Slightly soluble in *water*; miscible with *ethanol (96%)*, with *ether* and with most organic solvents.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of dimethyl phthalate *(RS 105)*.

B. Gently boil 1 g with 5 ml of 2M *methanolic potassium hydroxide* for 10 minutes, add 5 ml of *water*, evaporate the mixture to half its volume and cool. Add 1 ml of *hydrochloric acid*, filter, melt the dried precipitate in a small tube, add 0.5 g of *resorcinol* and 0.05 ml of *chloroform* and heat to about 180° for 3 minutes. Cool, add 1 ml of 5M *sodium hydroxide* and pour into *water*. An intense yellowish green fluorescence is produced.

TESTS

Acidity

Mix 20 ml with 50 ml of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1*. Not more than 0.1 ml of 0.1M *sodium hydroxide VS* is required to neutralise the solution using *phenolphthalein solution R1* as indicator.

Refractive index

1.515 to 1.517, Appendix V E.

Weight per ml

1.186 to 1.192 g, Appendix V G.

Related substances

Prepare a 0.075% w/v solution of *phenyl benzoate* (internal standard) in *chloroform* (solution A). Carry out the method for *gas chromatography*, Appendix III B, using solutions of the substance being examined containing (1) 0.10% w/v in solution A, (2) 5.0% w/v in *chloroform* and (3) 5.0% w/v in solution A.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 145°.

In the chromatogram obtained with solution (3) the ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard is not greater than the ratio of the area of the peak due to dimethyl phthalate to the area of the peak due to the internal standard in the chromatogram obtained with solution (1).

Sulphated ash

Not more than 0.1% w/w, Appendix IX A.

Water

Not more than 0.1% w/w, Appendix IX C. Use 20 g.

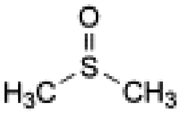
ASSAY

In a borosilicate glass flask dissolve 1.5 g of the substance being examined in 5 ml of carbon dioxide-free ethanol prepared by boiling *ethanol (96%)* thoroughly and neutralising to *phenolphthalein solution R1*. Neutralise the free acid in the solution with 0.1M *ethanolic potassium hydroxide VS* using 0.2 ml of *phenolphthalein solution R1* as indicator. Add 50 ml of 0.5M *ethanolic potassium hydroxide* VS and boil under a reflux condenser on a water bath for 1 hour. Add 20 ml of *water* and titrate the excess of alkali with 0.5M *hydrochloric acid VS* using a further 0.2 ml of *phenolphthalein solution R1* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the alkali required to saponify the esters. Each ml of 0.5M *ethanolic potassium hydroxide VS* is equivalent to 48.55 mg of $C_{10}H_{10}O_4$.

Dimethyl Sulfoxide

General Notices

(Ph Eur monograph 0763)



C_2H_6OS 78.1 *67-68-5*

Action and use

Pharmaceutical solvent; excipient.

When dimethyl sulphoxide is demanded, Dimethyl Sulfoxide shall be supplied.

Ph Eur

DEFINITION

Sulphinylbismethane.

CHARACTERS

Appearance

Colourless liquid or colourless crystals, hygroscopic.

Solubility

Miscible with water and with ethanol (96 per cent).

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Relative density (see Tests).

B. Refractive index (see Tests).

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison dimethyl sulfoxide CRS.

D. Dissolve 50 mg of *nickel chloride R* in 5 ml of the substance to be examined. The solution is greenish-yellow. Heat in a water-bath at 50 °C. The colour changes to green or bluish-green. Cool. The colour changes to greenish-yellow.

TESTS

Acidity

Dissolve 50.0 g in 100 ml of *carbon dioxide-free water R*. Add 0.1 ml of *phenolphthalein solution R1*. Not more than 5.0 ml of *0.01 M sodium hydroxide* is required to produce a pink colour.

Relative density *(2.2.5)*

1.100 to 1.104.

Refractive index *(2.2.6)*

1.478 to 1.479.

Freezing point *(2.2.18)*

Minimum 18.3 °C.

Absorbance *(2.2.25)*

Purge with *nitrogen R* for 15 min. The absorbance, measured using *water R* as the compensation liquid, is not more than 0.30 at 275 nm and not more than 0.20 at both 285 nm and 295 nm. Examined between 270 nm and 350 nm, the substance to be examined shows no absorption maximum.

Related substances

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.125 g of *bibenzyl R* in *acetone R* and dilute to 50 ml with the same solvent.

Test solution (a) Dissolve 5.0 g of the substance to be examined in *acetone R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 5.0 g of the substance to be examined in *acetone R*, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *acetone R*.

Reference solution Dissolve 50.0 mg of the substance to be examined and 50 mg of *dimethyl sulphone R* in *acetone R*, add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *acetone R*.

Column:
- *material*: glass;
- *size: l* = 1.5 m, Ø = 4 mm;
- *stationary phase*: *diatomaceous earth for gas chromatography R* (125-180 µm) impregnated with 10 per cent m/m of *polyethyleneglycol adipate R*.

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:
- *column*: 165 °C;
- *injection port and detector*: 190 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 4 times the retention time of dimethyl sulfoxide.

Elution order Dimethyl sulfoxide, dimethyl sulphone, bibenzyl.

Retention time Dimethyl sulfoxide = about 5 min.

System suitability:

— *resolution*: minimum 3 between the peaks due to dimethyl sulfoxide and dimethyl sulphone in the chromatogram obtained with the reference solution;

— in the chromatogram obtained with test solution (a) there is no peak with the same retention time as the internal standard.

Limit:

— *total*: calculate the ratio *R* of the area of the peak due to dimethyl sulfoxide to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard to the area of the peak due to the internal standard: this ratio is not greater than *R* (0.1 per cent).

Water *(2.5.12)*

Maximum 0.2 per cent, determined on 10.0 g.

STORAGE

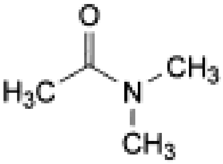
In an airtight, glass container, protected from light.

Ph Eur

Dimethylacetamide

General Notices

(Ph Eur monograph 1667)



C_4H_9NO 87.1 *127-19-5*

Action and use

Excipient.

Ph Eur

DEFINITION

N,N-Dimethylacetamide.

CHARACTERS

Appearance

Clear, colourless, slightly hygroscopic liquid.

Solubility

Miscible with water, with ethanol (96 per cent), and with most common organic solvents.

bp: about 165 °C.

IDENTIFICATION

First identification *C.*

Second identification *A, B, D.*

A. Relative density *(2.2.5)*: 0.941 to 0.944.

B. Refractive index *(2.2.6)*: 1.435 to 1.439.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Films.

Comparison *Ph. Eur. reference spectrum of dimethylacetamide.*

D. Dilute 50 mg with 1 ml of *methanol R*. Add 1 ml of a 15 g/l solution of *hydroxylamine hydrochloride R* and mix. Add 1 ml of *dilute sodium hydroxide solution R*, mix and allow to stand for 30 min. Add 1 ml of *dilute hydrochloric acid R* and add 1 ml of a 100 g/l solution of *ferric chloride R* in *0.1 M hydrochloric acid*. A reddish-brown colour develops, reaching a maximum intensity after about 5 min.

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Acidity

Dilute 50 ml with 50 ml of *water R* previously adjusted with *0.02 M potassium hydroxide* or *0.02 M hydrochloric acid* to a bluish-green colour, using 0.5 ml of *bromothymol blue solution R1* as indicator. Not more than 5.0 ml of *0.02 M potassium hydroxide* is required to restore the initial (bluish-green) colour.

Alkalinity

To 50 ml add 50 ml of *water R* previously adjusted with *0.02 M potassium hydroxide* or *0.02 M hydrochloric acid* to a yellow colour, using 0.5 ml of *bromothymol blue solution R1* as indicator. Not more than 0.5 ml of *0.02 M hydrochloric acid* is required to restore the initial (yellow) colour.

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution The substance to be examined.

Reference solution (a) Dilute a mixture of 1 ml of the substance to be examined and 1 ml of *dimethylformamide R* to 20 ml with *methylene chloride R*.

Reference solution (b) Dilute 1 ml of the substance to be examined to 20.0 ml with *methylene chloride R*. Dilute 0.1 ml of the solution to 10.0 ml with *methylene chloride R*.

Column:

— *material*: fused silica,

— *size: l* = 30 m, Ø = 0.32 mm,

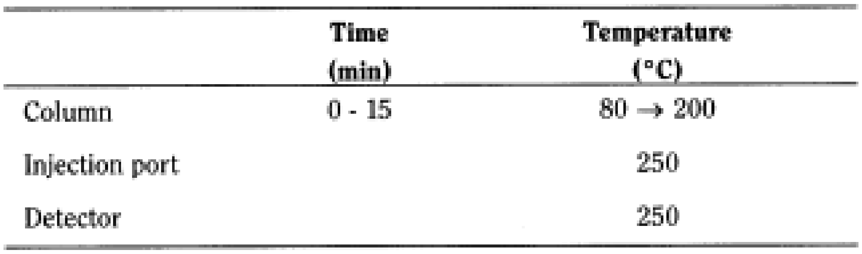
— *stationary phase*: *macrogol 20 000 R* (film thickness 1 µm).

Carrier gas *nitrogen for chromatography R.*

Linear velocity 30 cm/s.

Split ratio 1:20.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 15	80 → 200
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 0.5 µl.

System suitability:

- *resolution*: minimum 5.0 between the peaks due to dimethylacetamide and impurity B in the chromatogram obtained with reference solution (a),
- *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

- *any impurity*: maximum 0.1 per cent,
- *total*: maximum 0.3 per cent,
- *disregard limit*: the area of the peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dilute 4.0 g to 20.0 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Non-volatile matter

Maximum 20 ppm.

Evaporate 50 g to dryness using a rotary evaporator at a pressure not exceeding 1 kPa and on a water-bath. Dry the residue in an oven at 170-175 °C. The residue weighs not more than 1 mg.

Water *(2.5.32)*

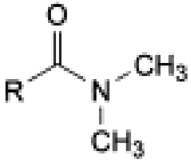
Maximum 0.1 per cent, determined on 0.100 g.

STORAGE

In an airtight container , protected from light.

IMPURITIES

A. acetic acid,



B. R = H: *N,N*-dimethylformamide,

C. R = C_2H_5: *N,N*-dimethylpropanamide,

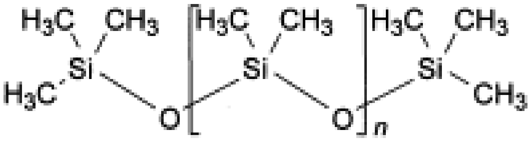
D. R = CH_2-CH_2-CH_3: *N,N*-dimethylbutanamide.

Ph Eur

Dimeticone

General Notices

(Ph Eur monograph 0138)



9006-75-9

Action and use

Antifoaming agent; water repellent.

When dimethicone is demanded, Dimeticone shall be supplied.

Ph Eur

DEFINITION

Poly(dimethylsiloxane) obtained by hydrolysis and polycondensation of dichlorodimethylsilane and chlorotrimethylsilane. Different grades of dimeticone exist which are distinguished by a number indicating the nominal kinematic viscosity placed after the name.

Their degree of polymerisation (n = 20 to 400) is such that their kinematic viscosities are nominally between 20 $mm^2 \cdot s^{-1}$ and 1300 $mm^2 \cdot s^{-1}$.

Dimeticones with a nominal viscosity of 50 $mm^2 \cdot s^{-1}$ *or lower are intended for external use only.*

CHARACTERS

Appearance

Clear, colourless liquid of various viscosities.

Solubility

Practically insoluble in water, very slightly soluble or practically insoluble in anhydrous ethanol, miscible with ethyl acetate, with methyl ethyl ketone and with toluene.

IDENTIFICATION

A. It is identified by its kinematic viscosity at 25 °C (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   dimeticone CRS.

The region of the spectrum from 850 cm^{-1} to 750 cm^{-1} is not taken into account.

C. Heat 0.5 g in a test-tube over a small flame until white fumes begin to appear. Invert the tube over a 2nd tube containing 1 ml of a 1 g/l solution of *chromotropic acid*, *sodium salt R* in *sulphuric acid R* so that the fumes reach the solution. Shake the 2nd tube for about 10 s and heat on a water-bath for 5 min. The solution is violet.

D. In a platinum crucible, prepare the sulphated ash *(2.4.14)* using 50 mg. The residue is a white powder that gives the reaction of silicates *(2.3.1)*.

TESTS

Acidity

To 2.0 g add 25 ml of a mixture of equal volumes of *anhydrous ethanol R* and *ether R*, previously neutralised to 0.2 ml of *bromothymol blue solution R1* and shake. Not more than 0.15 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to blue.

Viscosity *(2.2.9)*

90 per cent to 110 per cent of the nominal kinematic viscosity stated on the label, determined at 25 °C.

Mineral oils

Place 2 g in a test-tube and examine in ultraviolet light at 365 nm. The fluorescence is not more intense than that of a solution containing 0.1 ppm of *quinine sulphate R* in *0.005 M sulphuric acid* examined in the same conditions.

Phenylated compounds

Dissolve 5.0 g with shaking in 10 ml of *cyclohexane R*. At wavelengths from 250 nm to 270 nm, the absorbance *(2.2.25)* of the solution is not greater than 0.2.

Heavy metals

Maximum 5 ppm.

Mix 1.0 g with *methylene chloride R* and dilute to 20 ml with the same solvent. Add 1.0 ml of a freshly prepared 0.02 g/l solution of *dithizone R* in *methylene chloride R*, 0.5 ml of *water R* and 0.5 ml of a mixture of 1 volume of *dilute ammonia R2* and 9 volumes of a 2 g/l solution of *hydroxylamine hydrochloride R*. At the same time, prepare a reference solution as follows: to 20 ml of *methylene chloride R* add 1.0 ml of a freshly prepared 0.02 g/l solution of *dithizone R* in *methylene chloride R*, 0.5 ml of *lead standard solution (10 ppm Pb) R* and 0.5 ml of a mixture of 1 volume of *dilute ammonia R2* and 9 volumes of a 2 g/l solution of *hydroxylamine hydrochloride R*. Immediately shake each solution vigorously for 1 min. Any red colour in the test solution is not more intense than that in the reference solution.

Volatile matter

Maximum 0.3 per cent, for dimeticones with a nominal viscosity greater than 50 $mm^2 \cdot s^{-1}$, determined on 1.00 g by heating in an oven at 150 °C for 2 h. Carry out the test using a dish 60 mm in diameter and 10 mm deep.

LABELLING

The label states:

— the nominal kinematic viscosity by a number placed after the name of the product,

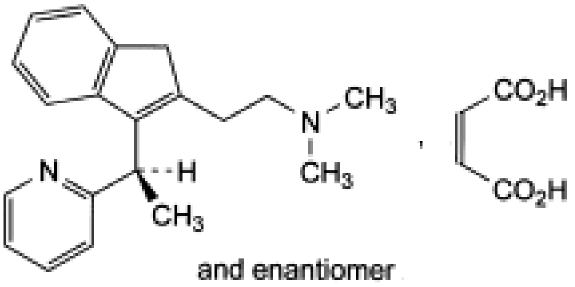
— where applicable, that the product is intended for external use.

Ph Eur

Dimetindene Maleate

General Notices

(Ph Eur monograph 1417)



$C_{24}H_{28}N_2O_4$ 408.5 *3614-69-5*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

N,N-Dimethyl-2-[3-[(*RS*)-1-(pyridin-2-yl)ethyl]-1*H*-inden-2-yl]ethanamine (*Z*)-butenedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison dimetindene maleate CRS.

TESTS

Solution S

Dissolve 0.20 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than Y_6 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*:

- 0.10° to + 0.10°, determined on solution S.

Related substances

Gas chromatography *(2.2.28)*.

Solvent mixture *acetone R, methylene chloride R* (50:50 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution (a) Dilute 1 ml of the test solution to 100.0 ml with the solvent mixture.

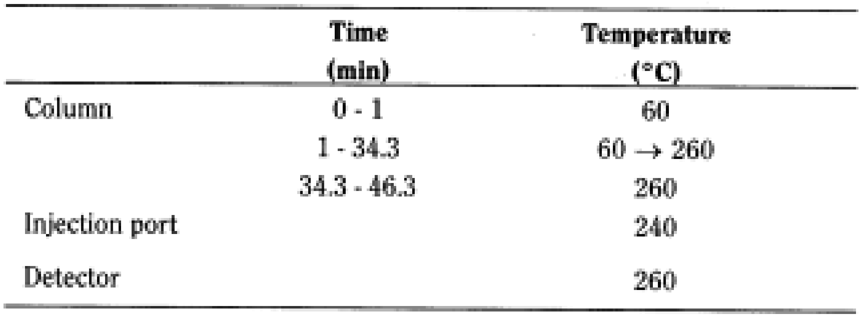
Reference solution (b) Dissolve 5.0 mg of *2-ethylpyridine R* (impurity A) in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 10.0 ml of this solution to 100.0 ml with the solvent mixture.

Column:
- *material*: fused silica;
- *size:* *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *polymethylphenylsiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Linear velocity About 30 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	60
	1 - 34.3	60 → 260
	34.3 - 46.3	260
Injection port		240
Detector		260

Detection Flame ionisation.

Injection 2 µl; inject via a split injector with a split flow of 30 ml/min.

Run time 1.3 times the retention time of dimetindene.

Elution order Impurity A and maleic acid appear during the first 8 min.

System suitability Reference solution (a):

— *symmetry factor*: maximum 1.3 for the principal peak.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurities B, C, D, E, F, G, H, I*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *sum of impurities other than A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to maleic acid.

Loss on drying ***(2.2.32)***

Maximum 0.1 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 80 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

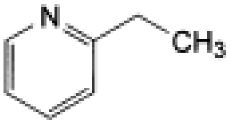
1 ml of *0.1 M perchloric acid* is equivalent to 20.43 mg of $C_{24}H_{28}N_2O_4$.

STORAGE

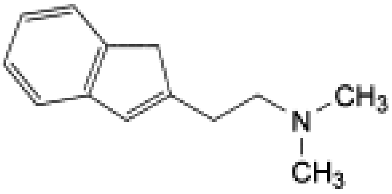
Protected from light.

IMPURITIES

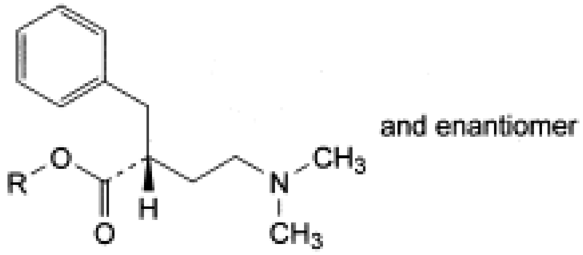
Specified impurities *A, B, C, D, E, F, G, H, I.*



A. 2-ethylpyridine,

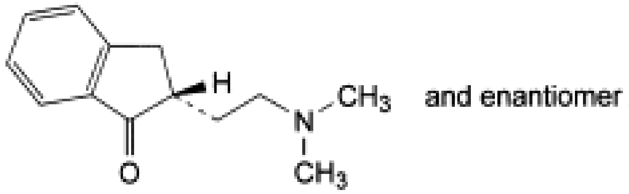


B. 2-(1*H*-inden-2-yl)-*N,N*-dimethylethanamine,

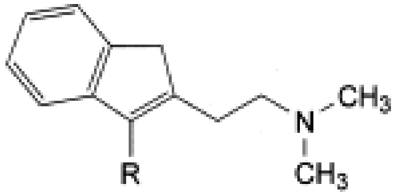

and enantiomer

C. R = C_2H_5: ethyl (2*RS*)-2-benzyl-4-(dimethylamino)butanoate,

D. R = H: (2*RS*)-2-benzyl-4-(dimethylamino)butanoic acid,

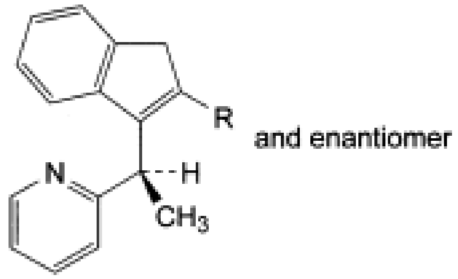

and enantiomer

E. (2*RS*)-2-[2-(dimethylamino)ethyl]indan-1-one,



F. R = $[CH_2]_3$-CH_3: 2-(3-butyl-1*H*-inden-2-yl)-*N,N*-dimethylethanamine,

G. R = C_6H_5: *N,N*-dimethyl-2-(3-phenyl-1*H*-inden-2-yl)ethanamine,


and enantiomer

H. R = CH = CH_2: 2-[(1*RS*)-1-(2-ethenyl-1*H*-inden-3-yl)ethyl]pyridine,

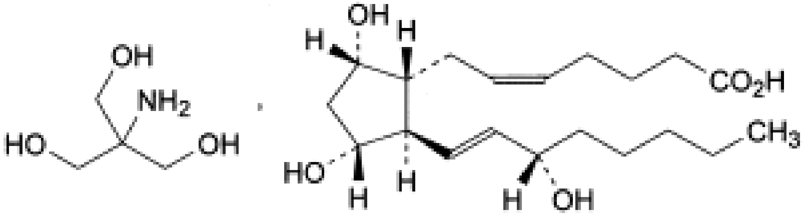
I. R = CH_2-CH_2-NH-CH_3: *N*-methyl-2-[3-[(1*RS*)-1-(pyridin-2-yl)ethyl]-1*H*-inden-2-yl] ethanamine.

Ph Eur

Dinoprost Trometamol

General Notices

(Ph Eur monograph 1312)



$C_{20}H_{34}O_5,C_4H_{11}NO_3$ 475.6 *38562-01-5*

Action and use

Prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$); inducer of uterine muscle contraction.

Ph Eur

DEFINITION

Trometamol (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]cyclopentyl] hept-5-enoate ($PGF_{2\alpha}$).

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in acetonitrile.

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: + 19 to + 26 (anhydrous substance).

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dinoprost trometamol CRS.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile R, water R* (23:77 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) *Degradation of dinoprost trometamol to impurity B.* Dissolve 1 mg of the substance to be examined in 1 ml of the mobile phase and heat the solution on a water-bath at 85 °C for 5 min and cool.

Reference solution (b) Dilute 2.0 ml of the test solution to 20.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 20.0 ml with the solvent mixture.

Column:

- *size:* *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Dissolve 2.44 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000 ml with *water R*; adjust to pH 2.5 with *phosphoric acid R* (about 0.6 ml); mix 770 ml of this solution with 230 ml of *acetonitrile R1*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 200 nm.

Injection 20 µl.

Run time 2.5 times the retention time of the principal peak (to elute degradation products formed during heating) for reference solution (a) and 10 min after the elution of dinoprost for the test solution and reference solution (b).

Retention time Impurity B = about 55 min; impurity A = about 60 min; dinoprost = about 66 min.

System suitability Reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to impurities B and A and minimum 2.0 between the peaks due to impurity A and dinoprost; if necessary, adjust the composition of the mobile phase by increasing the concentration of acetonitrile to decrease the retention times;
- *symmetry factor*: maximum 1.2 for the peaks due to impurities A and B.

Limits:

- *impurity A*: not more than twice the area of the principal peak obtained with reference solution (b) (2 per cent);
- *impurities B, C, D*: for each impurity, not more than 1.5 times the area of the principal peak obtained with reference solution (b) (1.5 per cent) and not more than one such peak has an area greater than 0.5 times the area of the principal peak obtained with reference solution (b) (0.5 per cent);

— *sum of impurities other than A*: not more than twice the area of the principal peak obtained with reference solution (b) (2 per cent);

— *disregard limit*: 0.05 times the area of the principal peak obtained with reference solution (b) (0.05 per cent); disregard any peak due to trometamol (retention time = about 1.5 min).

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile R, water R* (23:77 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution Dissolve 10.0 mg of *dinoprost trometamol CRS* in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Column:

— *size: l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: octadecylsilyl *silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Dissolve 2.44 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000 ml with *water R*; adjust to pH 2.5 with *phosphoric acid R* (about 0.6 ml); mix 730 ml of this solution with 270 ml of *acetonitrile R1*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 200 nm.

Injection 20 µl.

Retention time Dinoprost = about 23 min.

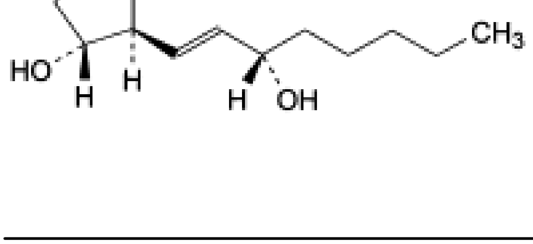
System suitability Reference solution:

— *repeatability*: maximum relative standard deviation of 2.0 per cent for the peak due to dinoprost after 6 injections.

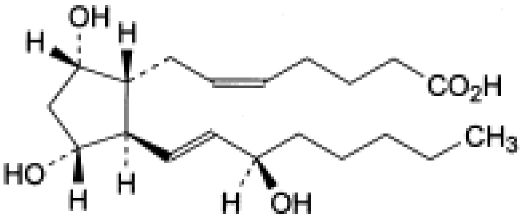
Calculate the percentage of dinoprost trometamol from the declared content of *dinoprost trometamol CRS*.

IMPURITIES

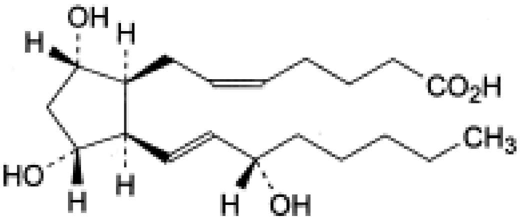
Specified impurities *A, B, C, D.*



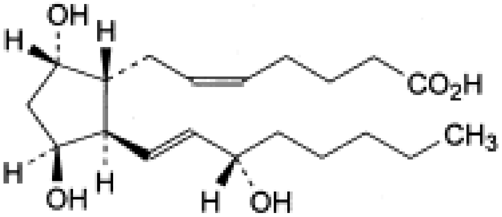
A. (*E*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid ((5*E*)-PGF$_{2\alpha}$; 5,6-*trans*-PGF$_{2\alpha}$),


OH
H
H
CO2H
CH3
HO
H
H
H
OH

B. (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*)-(3*R*)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid ((15*R*)-PGF$_{2\alpha}$;15-epiPGF$_{2\alpha}$),


OH
H
H
CO2H
CH3
HO
H
H
H
OH

C. (*Z*)-7-[(1*S*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid ((8*S*)-PGF$_{2\alpha}$; 8-epiPGF$_{2\alpha}$),


OH
H
H
CO2H
CH3
H
OH
H
H
OH

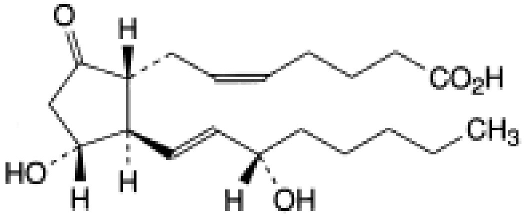
D. (*Z*)-7-[(1*R*,2*R*,3*S*,5*S*)-3,5-dihydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid (11β-PGF$_{2\alpha}$; 11-epiPGF$_{2\alpha}$).

Ph Eur

Dinoprostone

General Notices

(Ph Eur monograph 1311)



$C_{20}H_{32}O_5$ 352.5 *363-24-6*

Action and use

Prostaglandin E_2(PGE_2); inducer of uterine muscle contraction.

Ph Eur

DEFINITION

(*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (PGE_2).

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, very soluble in methanol, freely soluble in alcohol.

The substance degrades at room temperature.

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: - 82 to - 90 (anhydrous substance).

Immediately before use, dissolve 50.0 mg in *alcohol R* and dilute to 10.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dinoprostone CRS.*

TESTS

Prepare the solutions immediately before use.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 10.0 mg of the substance to be examined in a 58 per cent *V/V* solution of *methanol R2* and dilute to 2.0 ml with the same solvent.

Test solution (b) Dissolve 20.0 mg of the substance to be examined in a 58 per cent *V/V* solution of *methanol R2* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dissolve 1 mg of *dinoprostone CRS* and 1 mg of *dinoprostone impurity C CRS* in a 58 per cent *V/V* solution of *methanol R2* and dilute to 10.0 ml with the same solvent. Dilute 4.0 ml of the solution to 10.0 ml with a 58 per cent *V/V* solution of *methanol R2*.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 10.0 ml with a 58 per cent *V/V* solution of *methanol R2*. Dilute 1.0 ml of the solution to 10.0 ml with a 58 per cent *V/V* solution of *methanol R2*.

Reference solution (c) In order to prepare *in situ* the degradation compounds (impurity D and impurity E), dissolve 1 mg of the substance to be examined in 100 µl of *1 M sodium hydroxide* (the solution becomes brownish-red), wait 4 min, add 150 µl of *1 M acetic acid* (yellowish-white opalescent solution) and dilute to 5.0 ml with a 58 per cent *V/V* solution of *methanol R2*.

Reference solution (d) Dissolve 20 mg of *dinoprostone CRS* in a 58 per cent *V/V* solution of *methanol R2* and dilute to 20.0 ml with the same solvent.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R*,
- *temperature*: 30 °C.

Mobile phase Mix 42 volumes of a 0.2 per cent *V/V* solution of *acetic acid R* and 58 volumes of *methanol R2*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl; inject test solution (a) and reference solutions (a), (b) and (c).

Relative retention With reference to dinoprostone (retention time = about 18 min): impurity C = about 1.2; impurity D = about 1.8; impurity E = about 2.0.

System suitability Reference solution (a):

- *resolution*: minimum of 3.8 between the peaks due to dinoprostone and to impurity C. If necessary adjust the concentration of the acetic acid solution and/or methanol (increase the concentration of the acetic acid solution to increase the retention time for dinoprostone and impurity C and increase the concentration of methanol to decrease the retention time for both compounds).

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.2; impurity E = 0.7,

— *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent),

— *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),

— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *total of other impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

If any peak with a relative retention to dinoprostone of about 0.8 is greater than 0.5 per cent or if the total of other impurities is greater than 1.0 per cent, record the chromatogram of test solution (a) with a detector set at 230 nm. If the area of the peak at 230 nm is twice the area of the peak at 210 nm, multiply the area at 210 nm by 0.2 (correction factor for impurity F).

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 0.50 g.

ASSAY

Prepare the solutions immediately before use.

Liquid chromatography *(2.2.29)* as described in the test for related substances.

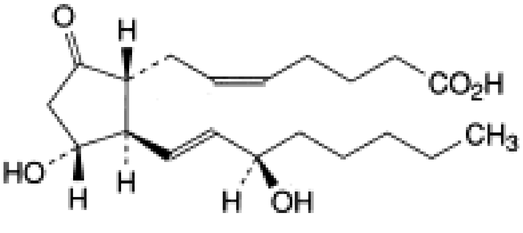
Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{20}H_{32}O_5$.

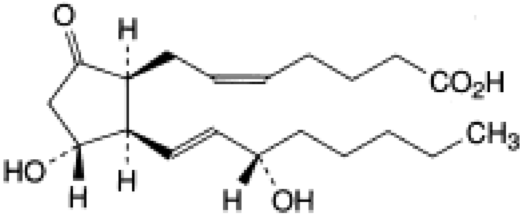
STORAGE

At a temperature not exceeding - 15 °C.

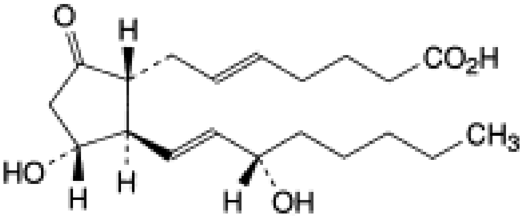
IMPURITIES



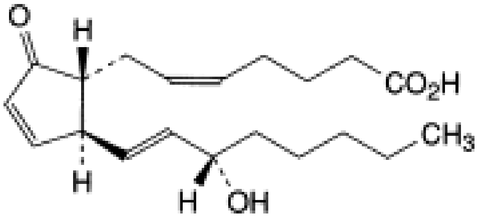
A. (*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3R)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (15-epiPGE$_2$; (15*R*)-PGE$_2$),



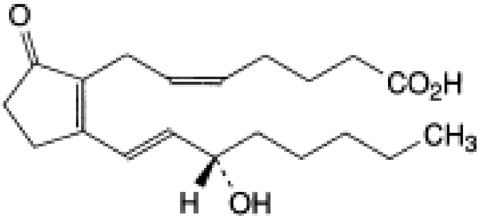
B. (*Z*)-7-[(1*S*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (8-epiPGE$_2$; (8*S*)-PGE$_2$),



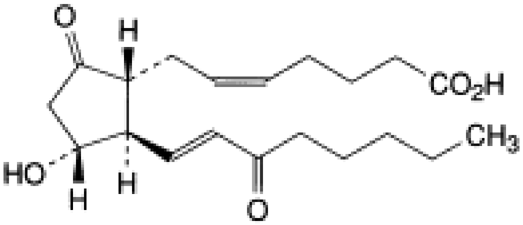
C. (*E*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (5-*trans*-PGE$_2$; (5*E*)-PGE$_2$),



D. (*Z*)-7-[(1*R*,2*S*)-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-3-enyl]hept-5-enoic acid (PGA$_2$),



E. (*Z*)-7-[2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-1-enyl]hept-5-enoic acid (PGB$_2$),



F. (*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-3-oxo-oct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid

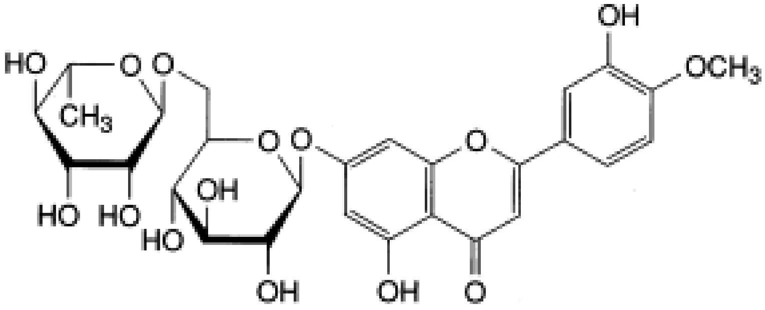
(15-oxo-PGE_2; 15-keto-PGE_2).

Ph Eur

Diosmin

General Notices

(Ph Eur monograph 1611)



$C_{28}H_{32}O_{15}$ 609 *520-27-4*

Action and use

Chronic venous insufficiency (flavonoid).

Ph Eur

DEFINITION

7-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one.

Substance obtained through iodine-assisted oxidation of (2*S*)-7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (hesperidin) of natural origin.

Content

90.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Greyish-yellow or light yellow hygroscopic powder.

Solubility

Practically insoluble in water, soluble in dimethyl sulphoxide, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison diosmin CRS.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Iodine

Maximum 0.1 per cent.

Determine the total content of iodine by potentiometry, using an iodide-selective electrode *(2.2.36)*, after oxygen combustion *(2.5.10)*.

Test solution Wrap 0.100 g of the substance to be examined in a piece of filter paper and place it in a sample carrier. Introduce into the flask 50 ml of a 0.2 g/l solution of *hydrazine R*. Flush the flask with oxygen for 10 min. Ignite the filter paper. Stir the contents of the flask immediately after the end of the combustion to dissolve completely the combustion products. Continue stirring for 1 h.

Reference solution Dilute 2.0 ml of a 16.6 g/l solution of *potassium iodide R* to 100.0 ml with *water R*. Dilute 10.0 ml of the solution to 100.0 ml with *water R*.

Introduce into a beaker 30 ml of a 200 g/l solution of *potassium nitrate R* in *0.1 M nitric acid*. Immerse the electrodes and stir for 10 min. The potential of the solution (nT_1) must remain stable. Add 1 ml of the test solution and measure the potential (nT_2).

Introduce into a beaker 30 ml of a 200 g/l solution of *potassium nitrate R* in *0.1 M nitric acid*. Immerse the electrodes and stir for 10 min. The potential of the solution must remain stable (nR_1). Add 80 µl of the reference solution and measure the potential (nR_2).

The absolute value $|nT_2 - nT_1|$ is not higher than the absolute value $|nR_2 - nR_1|$.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in *dimethyl sulphoxide R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dissolve 25.0 mg of *diosmin CRS* in *dimethyl sulphoxide R* and dilute to 25.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 100.0 ml with *dimethyl sulphoxide R*.

Reference solution (c) Dissolve 5.0 mg of *diosmin for system suitability CRS* in *dimethyl sulphoxide R* and dilute to 5.0 ml with the same solvent.

Column:

— *size:* l = 0.10 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm),

— *temperature*: 40 °C.

Mobile phase *acetonitrile R, glacial acetic acid R, methanol R, water R* (2:6:28:66 *V/V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µl loop injector; inject the test solution and reference solutions (b) and (c).

Run time 6 times the retention time of diosmin.

Relative retention With reference to diosmin (retention time = about 4.6 min): impurity A = about 0.5, impurity B = about 0.6, impurity C = about 0.8, impurity D = about 2.2, impurity E = about 2.6, impurity F = about 4.5.

System suitability Reference solution (c):

— *resolution*: minimum of 2.5 between the peaks due to impurities B and C.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.38; impurity F = 0.61,

— *impurity A*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent),

— *impurity C*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),

— *impurity E*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),

— *impurity F*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),

— *any other impurity*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),

— *total of other impurities and impurity A*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (10 per cent),

— *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

2.0 g complies with limit test C. Prepare the standard using 4.0 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

Maximum 6.0 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

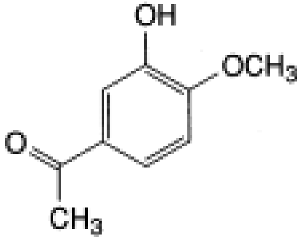
Liquid chromatography *(2.2.29)*, as described in the test for related substances.

Injection Test solution and reference solution (a).

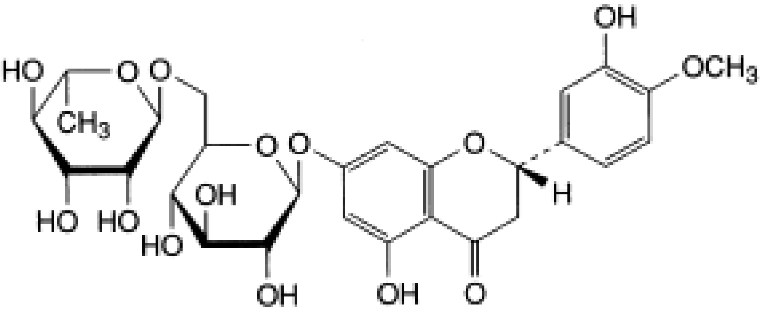
STORAGE

In an airtight container .

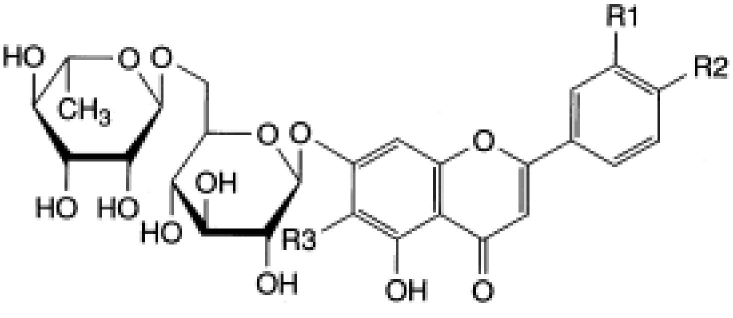
IMPURITIES


OH
OCH3
O
CH3

A. 1-(3-hydroxy-4-methoxyphenyl)ethanone (acetoisovanillone),


OH
OCH3
HO
O
O
CH3
O
O
O
H
OH
HO
HO
HO
OH
OH
O

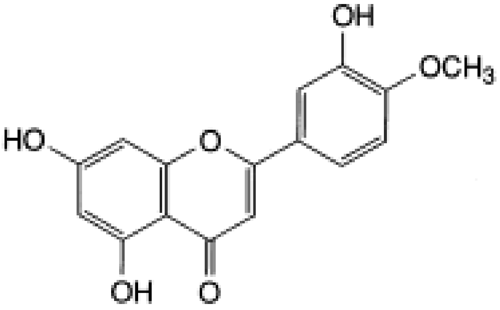
B. (2*S*)-7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (hesperidin),


R1
R2
HO
O
O
CH3
O
O
O
OH
HO
HO
HO
R3
OH
OH
O

C. R1 = R3 = H, R2 = OH: 7-[[6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (isorhoifolin),

D. R1 = OH, R2 = OCH_3, R3 = I: 7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl] oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-6-iodo-4*H*-1-benzopyran-4-one (6-iododiosmin),

E. R1 = R3 = H, R2 = OCH_3: 7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one (linarin),



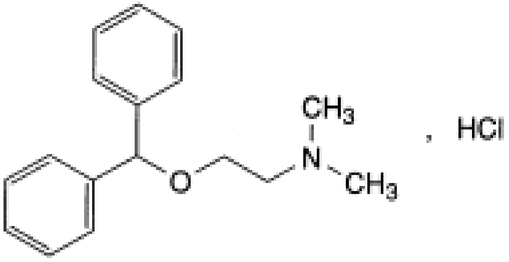
F. 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (diosmetin).

Ph Eur

Diphenhydramine Hydrochloride

General Notices

(Ph Eur monograph 0023)



$C_{17}H_{21}NO,HCl$ 291.8 *147-24-0*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Diphenhydramine Oral Solution

Ph Eur

DEFINITION

2-(Diphenylmethoxy)-*N,N*-dimethylethanamine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification C, D.

Second identification A, B, D.

A. Melting point *(2.2.14)*: 168 °C to 172 °C.

B. Dissolve 50 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Examined between 230 nm and 350 nm, the solution shows 3 absorption maxima *(2.2.25)*, at 253 nm, 258 nm and 264 nm. The ratio of the absorbance measured at the maximum at 258 nm to that measured at the maximum at 253 nm is 1.1 to 1.3. The ratio of the absorbance measured at the maximum at 258 nm to that measured at the maximum at 264 nm is 1.2 to 1.4.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *diphenhydramine hydrochloride CRS.*

D. It gives the reactions of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S and a fivefold dilution of solution S are clear *(2.2.1)*. Solution S is not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.15 ml of *methyl red solution R* and 0.25 ml of *0.01 M hydrochloric acid*. The solution is pink. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 70 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *diphenhydramine impurity A CRS* and 5 mg of *diphenylmethanol R* in the mobile phase and dilute to 10.0 ml with the mobile phase. To 2.0 ml of this solution add 1.5 ml of the test solution and dilute to 10.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: base*-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 5.4 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 using *phosphoric acid R*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Run time 7 times the retention time of diphenhydramine.

Relative retention With reference to diphenhydramine (retention time = about 6 min): impurity A = about 0.9; impurity B = about 1.5; impurity C = about 1.8; impurity D = about 2.6; impurity E = about 5.1.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to diphenhydramine and to impurity A.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 0.7,

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *any other impurity*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

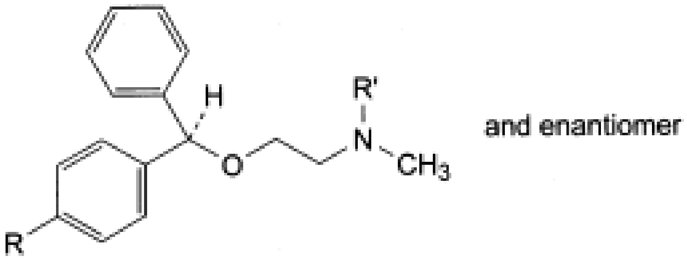
1 ml of *0.1 M sodium hydroxide* is equivalent to 29.18 mg of $C_{17}H_{22}ClNO$.

STORAGE

Protected from light.

IMPURITIES

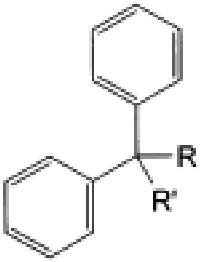
Specified impurities A, B, C, D, E.



A. R = R′ = H: 2-(diphenylmethoxy)-*N*-methylethanamine,

B. R = R′ = CH_3: 2-[(*RS*)-(4-methylphenyl)phenylmethoxy]-*N,N*-dimethylethanamine,

C. R = Br, R′ = CH_3: 2-[(*RS*)-(4-bromophenyl)phenylmethoxy]-*N,N*-dimethylethanamine,



D. R = OH, R′ = H: diphenylmethanol (benzhydrol),

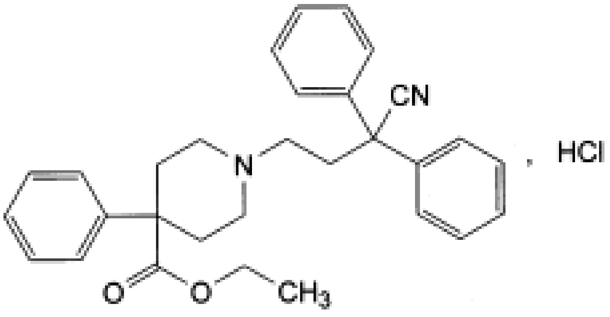
E. R + R′ = O: diphenylmethanone (benzophenone).

Ph Eur

Diphenoxylate Hydrochloride

General Notices

(Ph Eur monograph 0819)



$C_{30}H_{32}N_2O_2,HCl$ 489.1 *3810-80-8*

Action and use

Opioid receptor agonist; treatment of diarrhoea.

Ph Eur

DEFINITION

Diphenoxylate hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylate hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, freely soluble in methylene chloride, sparingly soluble in alcohol.

It melts at about 220 °C, with decomposition.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the *Ph. Eur. reference spectrum of diphenoxylate hydrochloride*.

B. Dissolve about 30 mg in 5 ml of *methanol R*. Add 0.25 ml of *nitric acid R* and 0.4 ml of *silver nitrate solution R1*. Shake and allow to stand. A curdled precipitate is formed. Centrifuge and rinse the precipitate with three quantities, each of 2 ml, of *methanol R*. Carry out this operation rapidly and protected from bright light. Suspend the precipitate in 2 ml of *water R* and add 1.5 ml of *ammonia R*. The precipitate dissolves easily.

TESTS

Appearance of solution

Dissolve 1.0 g in *methylene chloride R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a plate coated with a suitable octadecylsilyl silica gel (5 µm) with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution Dissolve 1.0 g in a mixture of 1 volume of *methanol R* and 2 volumes of *methylene chloride R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (a) Dilute 0.5 ml of the test solution to 100 ml with a mixture of 1 volume of *methanol R* and 2 volumes of *methylene chloride R*.

Reference solution (b) Dissolve 0.50 g of the substance to be examined in 25 ml of a 15 g/l solution of *potassium hydroxide R* in *methanol R* and add 1 ml of *water R*. Heat on a water-bath under a reflux condenser for 4 h. Cool and add 25 ml of 0.5 M *hydrochloric acid*. Shake with 100 ml of *methylene chloride R*. Evaporate the lower layer to dryness on a water-bath. Dissolve the residue in 10 ml of a mixture of 1 volume of *methanol R* and 2 volumes of *methylene chloride R*, add 10 ml of the test solution and dilute to 25 ml with a mixture of 1 volume of *methanol R* and 2 volumes of *methylene chloride R*.

Apply separately to a plate (100 mm square) 1 µl of each solution. Develop in an unsaturated tank over a path of 7 cm using a mixture of 10 volumes of *methanol R*, 30 volumes of a 59 g/l solution of *sodium chloride R* and 60 volumes of *dioxan R*. Allow the plate to dry in an oven at 160 °C for 15 min and place the hot plate in a closed tank containing about 20 ml of *fuming nitric acid R* for 30 min. Remove the plate and heat it again at 160 °C for 15 min. Allow to cool and examine immediately in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 40 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M ethanolic sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 48.91 mg of $C_{30}H_{33}ClN_2O_2$.

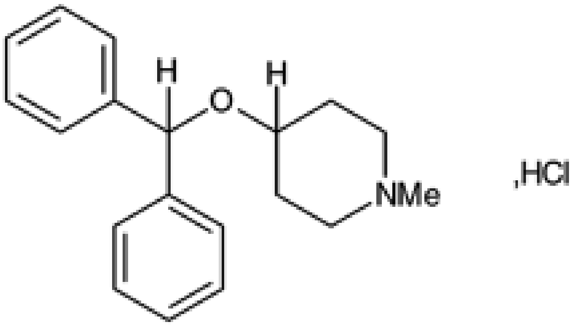
STORAGE

Store protected from light.

Ph Eur

Diphenylpyraline Hydrochloride

General Notices



$C_{19}H_{23}NO,HCl$ 317.9 *132-18-3*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Diphenylpyraline Hydrochloride is 4-benzhydryloxy-1-methylpiperidine hydrochloride. It contains not less than 98.0% and not more than 101.0% of $C_{19}H_{23}NO,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder; odourless or almost odourless.

Freely soluble in *water* and in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of diphenylpyraline hydrochloride *(RS 106)*.

B. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Dissolve 45 mg of *bibenzyl* (internal standard) in sufficient *dichloromethane* to produce 100 ml.

(2) Dissolve 0.20 g of the substance being examined in 20 ml of *water*, make the solution

alkaline with 5M *ammonia* and extract with three 25-ml quantities of *dichloromethane*. Shake the combined extracts with 10 g of *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness at about 30° and dissolve the residue in 2 ml of *dichloromethane*.

(3) Prepare solution (3) in the same manner as solution (2) but dissolve the residue in 2 ml of solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable).

(b) Use *nitrogen* as the carrier gas.

(c) Use an oven temperature of 165°. Allow the chromatography to proceed for 3 times the retention time of bibenzyl.

(d) Increase the oven temperature to 240° to elute the diphenylpyraline from the column.

LIMITS

In the chromatogram obtained with solution (3):

the sum of the areas of any *secondary peaks* is not greater than the area of the peak due to the internal standard.

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

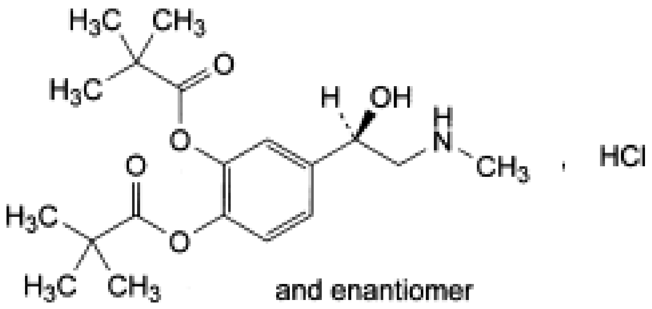
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.2 g, adding 5 ml of *mercury(II) acetate solution* and determining the end-point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 31.79 mg of $C_{19}H_{23}NO,HCl$.

Dipivefrine Hydrochloride

General Notices

(Ph Eur monograph 1719)



$C_{19}H_{29}NO_5,HCl$ 387.9 *64019-93-8*

Action and use

Adrenaline prodrug; treatment of glaucoma.

Preparation

Dipivefrine Eye Drops

Ph Eur

DEFINITION

Hydrochloride of 4-[(1*RS*)-1-hydroxy-2-(methylamino)ethyl]-1,2-phenylene bis(2,2-dimethylpropanoate).

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very soluble in methanol, freely soluble in ethanol (96 per cent) and in methylene chloride.

mp

About 160 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison dipivefrine hydrochloride CRS.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Impurities A and B

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 10.0 ml with the same acid.

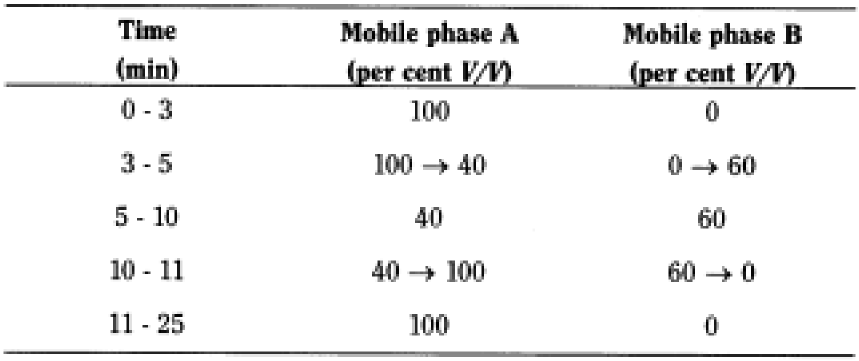
Reference solution Dissolve 10.0 mg of *adrenaline R* and 10.0 mg of *adrenalone hydrochloride R* in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 1.0 ml of this solution to 10.0 ml with *0.01 M hydrochloric acid*. *Protect this solution from light*.

Column:

- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).

Mobile phase:

- *mobile phase A*: 0.1 per cent *V/V* solution of *anhydrous formic acid R*;
- *mobile phase B*: *methanol R2, acetonitrile R* (40:60 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 5	100 → 40	0 → 60
5 - 10	40	60
10 - 11	40 → 100	60 → 0
11 - 25	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 10 µl.

Retention times Impurity A = about 2.2 min; impurity B = about 3.2 min.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the peaks due to impurity A and impurity B.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture Mix 40 volumes of *methanol R2* and 60 volumes of *acetonitrile R*. Mix 55 volumes of this mixture and 45 volumes of *0.01 M hydrochloric acid*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *dipivefrine for system suitability CRS* (containing impurities C, D and E) in the solvent mixture and dilute to 2.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *dipivefrine hydrochloride CRS* in the solvent mixture and dilute to 2.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 25.0 ml with the solvent mixture.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).

Mobile phase Mix 45 volumes of a 2.7 g/l solution of *concentrated ammonia R* adjusted to pH 10.0 with *dilute acetic acid R* and 55 volumes of a mixture of 40 volumes of *methanol R2* and 60 volumes of *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 10 µl.

Run time 2.5 times the retention time of dipivefrine.

Relative retention With reference to dipivefrine (retention time = about 7 min): impurities C and D = about 0.4; impurity E = about 1.3; impurity F = about 2.0.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to dipivefrine and impurity E.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurities C and D = 0.5; impurity E = 0.06;

— *sum of impurities C and D*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities E, F*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak with a mass distribution ratio less than 0.5.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 6 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection 20 µl of reference solutions (a) and (c).

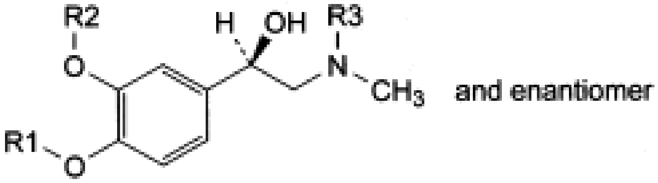
System suitability Reference solution (c):

— *symmetry factor*: maximum 2.0 for the peak due to dipivefrine.

Calculate the percentage content of $C_{19}H_{30}ClNO_5$ using the chromatograms obtained with reference solutions (a) and (c) and the declared content of *dipivefrine hydrochloride CRS*.

IMPURITIES

Specified impurities A, B, C, D, E, F.

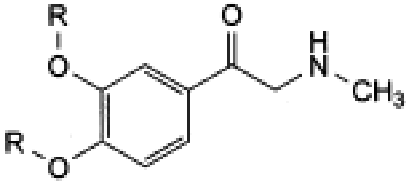


A. R1 = R2 = R3 = H: 4-[(1*RS*)-1-hydroxy-2-(methylamino)ethyl]benzene-1,2-diol ((±)-adrenaline),

C. R1 = R3 = H, R2 = CO-C$(CH_3)_3$: 2-hydroxy-5-[(1*RS*)-1-hydroxy-2-(methylamino)ethyl] phenyl 2,2-dimethylpropanoate,

D. R1 = CO-C$(CH_3)_3$, R2 = R3 = H: 2-hydroxy-4-[(1*RS*)-1-hydroxy-2-(methylamino)ethyl] phenyl 2,2-dimethylpropanoate,

F. R1 = R2 = CO-C$(CH_3)_3$, R3 = C_2H_5: 4-[(1*RS*)-2-(ethylmethylamino)-1-hydroxyethyl]-1,2-phenylene bis(2,2-dimethylpropanoate),



B. R = H: 1-(3,4-dihydroxyphenyl)-2-(methylamino)ethanone (adrenalone),

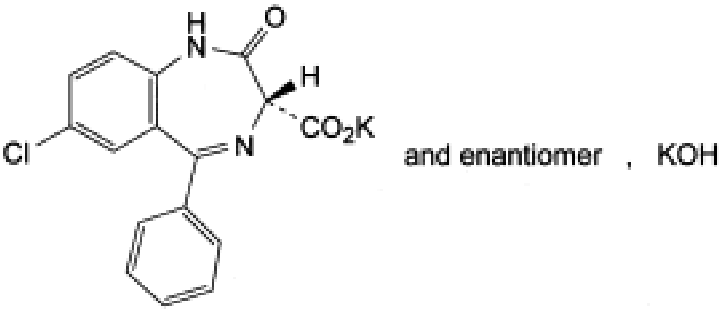
E. R = CO-C$(CH_3)_3$: 4-[(methylamino)acetyl]-1,2-phenylene bis(2,2-dimethylpropanoate) (adrenalone dipivalate ester).

Ph Eur

Dipotassium Clorazepate

General Notices

Potassium Clorazepate
(Ph Eur monograph 0898)



$C_{16}H_{11}ClK_2N_2O_4$ 408.9 *57109-90-7*

Action and use

Hypnotic; anxiolytic.

Ph Eur

DEFINITION

Potassium (3*RS*)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepine-3-carboxylate compound with potassium hydroxide (1:1).

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or light yellow, crystalline powder.

Solubility

Freely soluble to very soluble in water, very slightly soluble in alcohol, practically insoluble in methylene chloride.

Solutions in water and in alcohol are unstable and are to be used immediately.

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. Dissolve 10.0 mg in a 0.3 g/l solution of *potassium carbonate R* and dilute to 100.0 ml with the same solution (solution A). Dilute 10.0 ml of solution A to 100.0 ml with a 0.3 g/l solution of *potassium carbonate R* (solution B). Examined between 280 nm and 350 nm *(2.2.25)*, solution A shows a broad absorption maximum at about 315 nm. The specific absorbance at the absorption maximum at 315 nm is 49 to 56. Examined between 220 nm and 280 nm *(2.2.25)*, solution B shows an absorption maximum at 230 nm. The specific absorbance at the absorption maximum at 230 nm is 800 to 870.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison Ph. Eur. reference spectrum of dipotassium clorazepate.

C. Dissolve about 20 mg in 2 ml of *sulphuric acid R*. Observed in ultraviolet light at 365 nm, the solution shows yellow fluorescence.

D. Dissolve 0.5 g in 5 ml of *water R*. Add 0.1 ml of *thymol blue solution R*. The solution is violet-blue.

E. Place 1.0 g in a crucible and add 2 ml of *dilute sulphuric acid R*. Heat at first on a water-bath, then ignite until all black particles have disappeared. Allow to cool. Take up the residue with *water R* and dilute to 20 ml with the same solvent. The solution gives reaction (b) of potassium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution GY_5 *(2.2.2, Method II)*.

Rapidly dissolve 2.0 g with shaking in *water R* and dilute to 20.0 ml with the same solvent. Observe immediately.

Related substances

Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use and carry out the test protected from light*.

Test solution Dissolve 0.20 g of the substance to be examined in *water R* and dilute to 5.0 ml with the same solvent. Shake immediately with 2 quantities, each of 5.0 ml, of *methylene chloride R*. Combine the organic layers and dilute to 10.0 ml with *methylene chloride R*.

Reference solution (a) Dissolve 10 mg of *aminochlorobenzophenone R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with *methylene chloride R*.

Reference solution (b) Dissolve 5 mg of *nordazepam CRS* in *methylene chloride R* and dilute to 25.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with *methylene chloride R*.

Reference solution (c) Dilute 10.0 ml of reference solution (b) to 20.0 ml with *methylene chloride R*.

Reference solution (d) Dissolve 5 mg of *nordazepam CRS* and 5 mg of *nitrazepam CRS* in *methylene chloride R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetone* R, *methylene chloride R* (15:85 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (d) shows 2 clearly separated spots.

Limits A:

— *impurity B*: any spot due to impurity B is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *any other impurity*: any spot, apart from any spot due to impurity B, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

Detection B Spray with a freshly prepared 10 g/l solution of *sodium nitrite R* in *dilute hydrochloric acid R*. Dry in a current of warm air and spray with a 4 g/l solution of *naphthylethylenediamine dihydrochloride R* in *alcohol R*.

Limits B:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

ASSAY

Dissolve 0.130 g in 10 ml of *anhydrous acetic acid R*. Add 30 ml of *methylene chloride R*. Titrate with *0.1 M perchloric acid*, determining the 2 points of inflexion by potentiometry *(2.2.20)*.

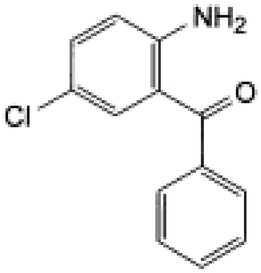
At the 2nd point of inflexion, 1 ml of *0.1 M perchloric acid* is equivalent to 13.63 mg of $C_{16}H_{11}ClK_2N_2O_4$.

STORAGE

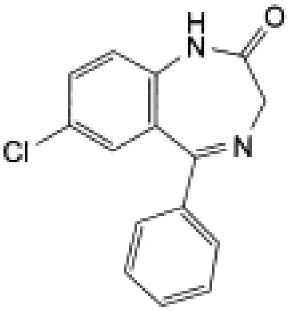
In an airtight container, protected from light.

IMPURITIES

Specified impurities *A, B.*



A. (2-amino-5-chlorophenyl)phenylmethanone (aminochlorobenzophenone),



B. 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (nordazepam).

Ph Eur

Dipotassium Hydrogen Phosphate

General Notices

(Dipotassium Phosphate, Ph Eur monograph 1003)

K_2HPO_4 174.2 *7758-11-4*

Action and use

Excipient.

Preparation

Dipotassium Hydrogen Phosphate Injection

Ph Eur

DEFINITION

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or colourless crystals, very hygroscopic.

Solubility

Very soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly alkaline *(2.2.4)*.

B. Solution S gives reaction (b) of phosphates *(2.3.1)*.

C. Solution S gives reaction (a) of potassium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Reducing substances

To 5 ml of solution S add 5 ml of *dilute sulphuric acid R* and 0.25 ml of *0.02 M potassium permanganate* and heat on a water-bath for 5 min. The solution remains faintly pink.

Monopotassium phosphate

Maximum 2.5 per cent.

From the volume of *1 M hydrochloric acid* (10.0 ml) and of *1 M sodium hydrox*ide (n_1 ml and n_2 ml) used in the assay, calculate the following ratio:

$$\frac{n_2 - 10}{10 - n_1}$$

This ratio is not greater than 0.025.

Chlorides ***(2.4.4)***

Maximum 200 ppm.

To 2.5 ml of solution S add 10 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 0.1 per cent.

To 1.5 ml of solution S add 2 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*.

Arsenic ***(2.4.2, Method A)***

Maximum 2 ppm, determined on 5 ml of solution S.

Iron ***(2.4.9)***

Maximum 10 ppm, determined on solution S.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 2.0 g in 8 ml of *water R*. Acidify with about 6 ml of *dilute hydrochloric acid R* (pH 3-4) and dilute to 20 ml with *water R*. 12 ml of this solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Sodium

Maximum 0.10 per cent, if intended for use in the manufacture of parenteral dosage forms.

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589 nm.

Loss on drying ***(2.2.32)***

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 125-130 °C.

Bacterial endotoxins ***(2.6.14)***

Less than 1.1 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.800 g (m) in 40 ml of *carbon dioxide-free water R* and add 10.0 ml of *1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)* using *1 M sodium hydroxide*. Read the volume added at the 1st inflexion point (n_1 ml). Continue the titration to the 2nd inflexion point (total volume of *1 M sodium hydroxide* required, n_2 ml).

Calculate the percentage content of K_2HPO_4 from the following expression:

$$\frac{1742\,(10 - n_1)}{m\,(100 - d)}$$

d = percentage loss on drying.

STORAGE

In an airtight container.

LABELLING

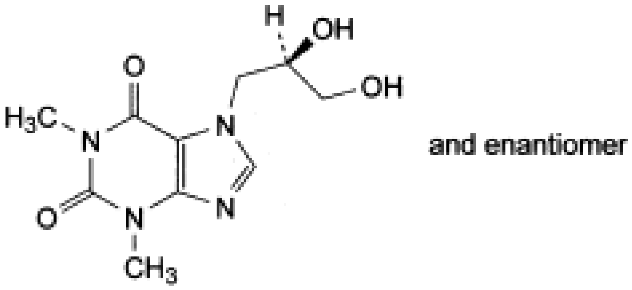
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Diprophylline

General Notices

(Ph Eur monograph 0486)



$C_{10}H_{14}N_4O_4$ 254.2 *479-18-5*

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

Ph Eur

DEFINITION

Diprophylline contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7-[(2*RS*)-2,3-dihydroxypropyl]-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D.*

A. Melting point *(2.2.14):* 160 °C to 165 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *diprophylline CRS*. Examine the substances as discs prepared using 0.5 mg to 1 mg of the substance to be examined in 0.3 g of *potassium bromide R*.

C. Dissolve 1 g in 5 ml of *acetic anhydride R* and boil under a reflux condenser for 15 min. Allow to cool and add 100 ml of a mixture of 20 volumes of *ether R* and 80 volumes of *light petroleum R*. Cool in iced water for at least 20 min, shaking from time to time. Filter, wash the precipitate with a mixture of 20 volumes of *ether R* and 80 volumes of *light petroleum R*, recrystallise from *alcohol R* and dry *in vacuo*. The crystals melt *(2.2.14)* at 142 °C to 148 °C.

D. It gives the reaction of xanthines *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1*) and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.25 ml of *bromothymol blue solution R1*. The solution is yellow or green. Not more than 0.4 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* HF_{254} *R* as the coating substance.

Test solution Dissolve 0.3 g of the substance to be examined in a mixture of 20 volumes of *water R* and 30 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents. *Prepare immediately before use.*

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *methanol R*.

Reference solution (b) Dilute 0.2 ml of the test solution to 100 ml with *methanol R*.

Reference solution (c) Dissolve 10 mg of *theophylline R* in *methanol R*, add 0.3 ml of the test solution and dilute to 10 ml with *methanol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 10 volumes of *ethanol R* and 90 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

Dilute 2.5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (400 ppm).

Heavy metals *(2.4.8)*

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.200 g in 3.0 ml of *anhydrous formic acid R* and add 50.0 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 25.42 mg of $C_{10}H_{14}N_4O_4$.

STORAGE

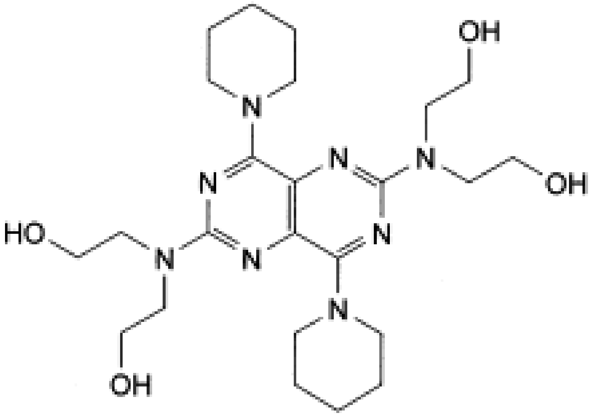
Store protected from light.

Ph Eur

Dipyridamole

General Notices

(Ph Eur monograph 1199)



$C_{24}H_{40}N_8O_4$ 504.6 *58-32-2*

Action and use

Adenosine reuptake inhibitor; inhibitor of platelet aggregation.

Preparation

Dipyridamole Tablets

Ph Eur

DEFINITION

2,2′,2″,2‴-[[4,8-Di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,6-diyl]dinitrilo]tetraethanol.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Bright yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in anhydrous ethanol. It dissolves in dilute mineral acids.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *dipyridamole CRS.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*

Prepare the solutions immediately before use.

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 50 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

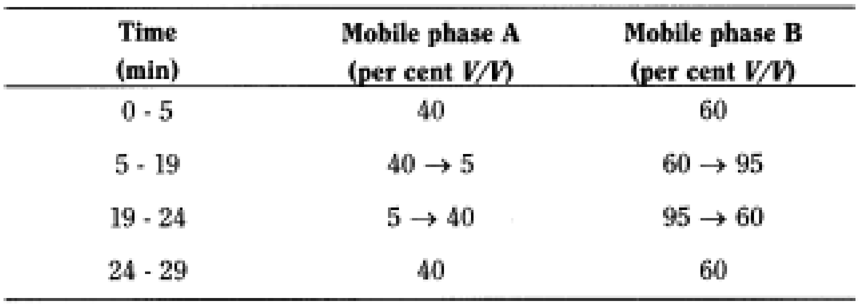
Reference solution (b) Dissolve the contents of a vial of *dipyridamole for peak identification CRS* (containing impurities A, B, C, D, E and F) in 1 ml of *methanol R*.

Column:

- *size*: *l* = 0.10 m, Ø = 4.0 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:

- *mobile phase A*: dissolve 1.0 g of *potassium dihydrogen phosphate R* in 900 ml of *water R*, adjust to pH 7.0 with *0.5 M sodium hydroxide* and dilute to 1000 ml with *water R*;
- *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	40	60
5 - 19	40 → 5	60 → 95
19 - 24	5 → 40	95 → 60
24 - 29	40	60

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 295 nm.

Injection 5 µl.

Identification of impurities Use the chromatogram supplied with *dipyridamole for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D, E and F.

Relative retention With reference to dipyridamole (retention time = about 8 min): impurity B = about 0.2; impurity F = about 0.3; impurity D = about 0.9; impurity E = about 1.3; impurity C = about 1.6; impurity A = about 2.2.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity D and dipyridamole;

— *peak-to-valley ratio*: minimum 4, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 1.7;

— *impurities A, B, C*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurities D, E*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides *(2.4.4)*

Maximum 200 ppm.

To 0.250 g add 10 ml of *water R* and shake vigorously. Filter, rinse the filter with 5 ml of *water R* and dilute to 15 ml with *water R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 70 ml of *methanol R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 50.46 mg of $C_{24}H_{40}N_8O_4$.

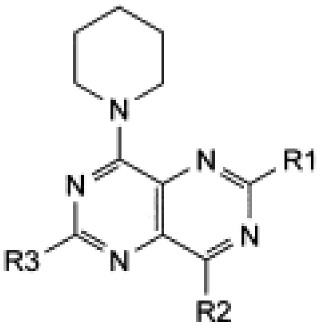
STORAGE

Protected from light.

IMPURITIES

Specified impurities   A, B, C, D, E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F, G*.



A. R1 = $N(CH_2\text{-}CH_2\text{-}OH)_2$, R2 = R3 = NC_5H_{10}: 2,2′-[[4,6,8-tri(piperidin-1-yl)pyrimido[5,4-*d*] pyrimidin-2-yl]nitrilo]diethanol,

B. R1 = R2 = R3 = $N(CH_2\text{-}CH_2\text{-}OH)_2$: 2,2′,2″,2‴,2⁗,2′′′′′[[8-(piperidin-1-yl)pyrimido[5,4-*d*] pyrimidine-2,4,6-triyl]trinitrilo]hexaethanol,

C. R1 = $N(CH_2\text{-}CH_2\text{-}OH)_2$, R2 = NC_5H_{10}, R3 = Cl: 2,2′-[[6-chloro-4,8-di(piperidin-1-yl)pyrimido [5,4-*d*]pyrimidin-2-yl]nitrilo]diethanol,

D. R1 = $N(CH_2\text{-}CH_2\text{-}OH)_2$, R2 = NC_5H_{10}, R3 = $NH\text{-}CH_2\text{-}CH_2\text{-}OH$: 2,2′-[[6-[(2-hydroxyethyl) amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]nitrilo]diethanol,

E. R1 = R2 = $N(CH_2\text{-}CH_2\text{-}OH)_2$, R3 = NC_5H_{10}: 2,2′,2″,2‴-[[6,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4-diyl]dinitrilo]tetraethanol,

F. R1 = R3 = $N(CH_2\text{-}CH_2\text{-}OH)_2$, R2 = $NH\text{-}CH_2\text{-}CH_2\text{-}OH$: 2,2′,2″,2‴-[[4-[(2-hydroxyethyl) amino]-8-(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,6-diyl]dinitrilo]tetraethanol,

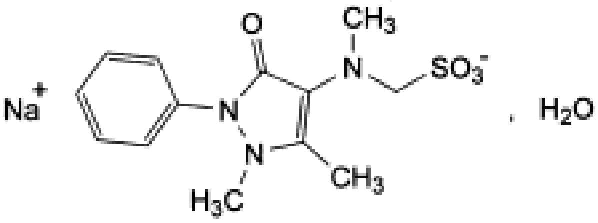
G. R1 = R3 = Cl, R2 = NC_5H_{10}: 2,6-dichloro-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine.

Ph Eur

Dipyrone

General Notices

(Metamizole Sodium, Ph Eur monograph 1346)



$C_{13}H_{16}N_3NaO_4S,H_2O$ 351.4 *5907-38-0*

Action and use

Analgesic.

Ph Eur

DEFINITION

Sodium [(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-*N*-methylamino] methanesulphonate monohydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison metamizole sodium CRS.

B. Dissolve 50 mg in 1 ml of *strong hydrogen peroxide solution R*. A blue colour is produced which fades rapidly and turns to intense red in a few minutes.

C. Place 0.10 g in a test tube, add some glass beads and dissolve the substance in 1.5 ml of *water R*. Add 1.5 ml of *dilute hydrochloric acid R* and place a filter paper wetted with a solution of 20 mg of *potassium iodate R* in 2 ml of *starch solution R* at the open end of the test tube. Heat gently, the evolving vapour of sulphur dioxide colours the filter paper blue. After heating gently for 1 min take a glass rod with a drop of a 10 g/l solution of *chromotropic acid, sodium salt R* in *sulphuric acid R* and place in the opening of the tube. Within 10 min, a blue-violet colour develops in the drop of the reagent.

D. 0.5 ml of solution S (see Tests) gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 40 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and immediately after preparation, not more intensely coloured than reference solution BY_6 *(2.2.2, Method I)*.

Acidity or alkalinity

To 5 ml of solution S, add 0.1 ml of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.1 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *metamizole impurity A CRS* in *methanol R* and dilute to 20.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 20.0 ml with *methanol R*.

Reference solution (c) Dissolve 40 mg of *metamizole sodium CRS* in *methanol* R and dilute to 20.0 ml with the same solvent.

Reference solution (d) In order to prepare impurity C *in situ*, boil 10 ml of reference solution (c) under reflux for 10 min. Allow to cool to room temperature and dilute to 20.0 ml with *methanol R*.

Reference solution (e) To 6 ml of reference solution (a) add 1 ml of reference solution (c).

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 28 volumes of *methanol R* and 72 volumes of a buffer solution prepared as follows : mix 1000 volumes of a 6.0 g/l solution of *sodium dihydrogen phosphate R* and 1 volume of *triethylamine R*, then adjust to pH 7.0 with *strong sodium hydroxide solution R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl of the test solution and reference solutions (b), (d) and (e).

Run time 3.5 times the retention time of metamizole.

Elution order Impurity A, metamizole, impurity B, impurity C, impurity D.

System suitability Reference solution (e):

— *resolution*: minimum 2.5 between the peaks due to impurity A and metamizole.

Limits:

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities A, B, D*: for each impurity, not more 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.025 per cent).

Sulphates *(2.4.13)*

Maximum 0.1 per cent.

Dissolve 0.150 g in *distilled water R* and dilute to 15 ml with the same solvent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the freshly prepared solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying *(2.2.32)*

4.9 per cent to 5.3 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.200 g in 10 ml of *0.01 M hydrochloric acid* previously cooled in iced water and titrate immediately, dropwise, with *0.05 M iodine*. Before each addition of *0.05 M iodine* dissolve the precipitate by swirling. At the end of the titration add 2 ml of *starch solution R* and titrate until the blue colour of the solution persists for at least 2 min. The temperature of the solution during the titration must not exceed 10 °C.

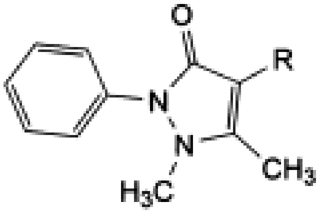
1 ml of *0.05 M iodine* is equivalent to 16.67 mg of $C_{13}H_{16}N_3NaO_4S$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D.*



A. R = NHCHO: 4-formylamino-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one,

B. R = NH_2: 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one,

C. R = $NHCH_3$: 4-methylamino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one,

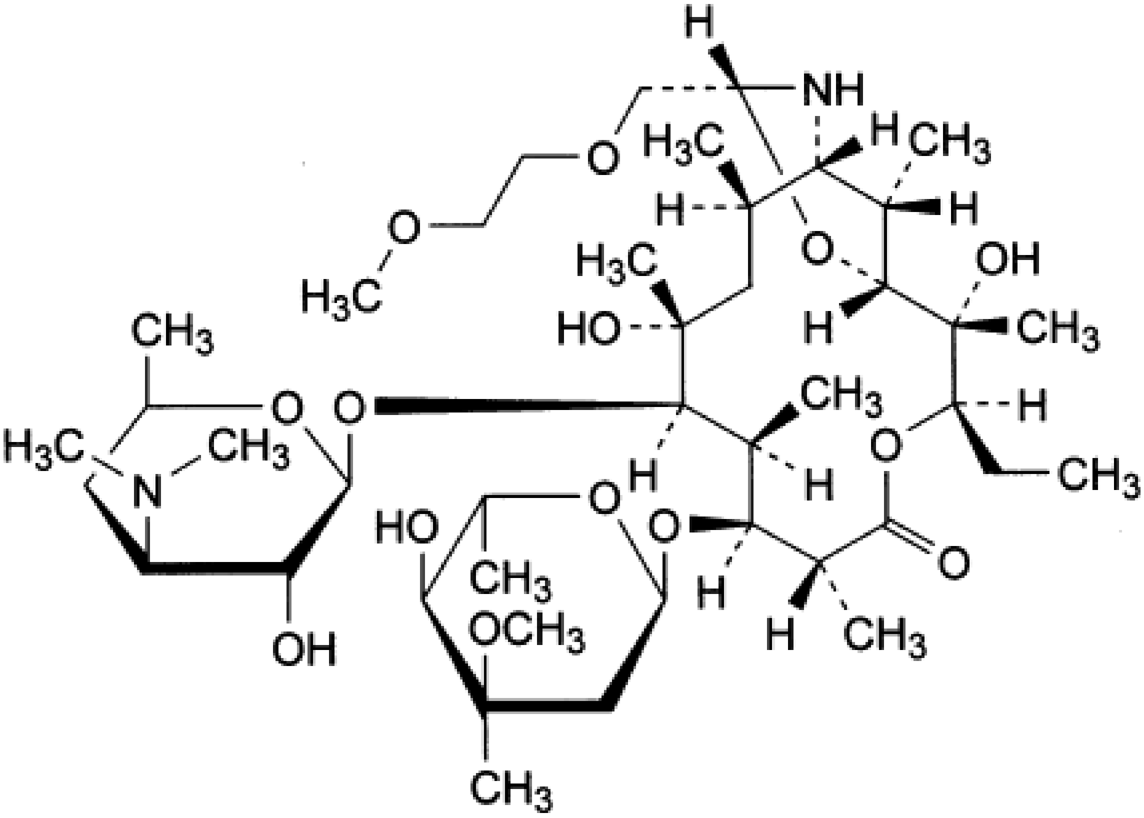
D. R = $N(CH_3)_2$: 4-dimethylamino-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one.

Ph Eur

Dirithromycin

General Notices

(Ph Eur monograph 1313)



$C_{42}H_{78}N_2O_{14}$ 835 *62013-04-1*

Action and use

Macrolide antibacterial.

Ph Eur

DEFINITION

(1*R*,2*S*,3*R*,6*R*,7*S*,8*S*,9*R*,10*R*,12*R*,13*S*,15*R*,17*S*)-9-[[3-(Dimethylamino)-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-3-ethyl-2,10-dihydroxy-15-[(2-methoxyethoxy)methyl]-2, 6,8,10,12,17-hexamethyl-7-[(3-*C*-methyl-3-*O*-methyl-2,6dideoxy-α-L-*ribo*-hexopyranosyl)oxy]-4,16-dioxa-14azabicyclo[11.3.1]heptadecan-5-one (or (9*S*)-9,11-[imino[(1*R*)-2-(2-methoxyethoxy)ethylidene]oxy]-9-deoxo-11-deoxyerythromycin).

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent for the sum of the percentage contents of $C_{42}H_{78}N_2O_{14}$ and dirithromycin 15*S*-epimer (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, very soluble in methanol and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison

dirithromycin CRS.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (a) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29).*

Solvent mixture *methanol R*, *acetonitrile R1* (30:70 *V/V*).

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Test solution (b) Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *dirithromycin CRS* in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Reference solution (c) Dissolve 20 mg of *dirithromycin CRS* in the mobile phase and dilute to 10 ml with the mobile phase. Allow to stand for 24 h before use.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 9 volumes of *water R*, 19 volumes of *methanol R*, 28 volumes of a solution containing 1.9 g/l of *potassium dihydrogen phosphate R* and 9.1 g/l of *dipotassium hydrogen phosphate R* adjusted to pH 7.5 if necessary with a 100 g/l solution of *potassium hydroxide R,* and 44 volumes of *acetonitrile R1*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µl of test solution (b) and reference solutions (b) and (c).

Run time 3 times the retention time of dirithromycin.

Relative retention With reference to dirithromycin: impurity A = about 0.7; 15*S*-epimer = about 1.1.

System suitability Reference solution (c):

— *resolution*: minimum 2.0 between the peaks due to dirithromycin and its 15*S*-epimer; if necessary, adjust the concentration of the organic modifiers in the mobile phase.

Limits:

— *impurity A*: not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: disregard the peak due to the 15*S*-epimer.

Dirithromycin 15*S*-epimer

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (b).

System suitability Reference solution (b):

— *repeatability*: maximum relative standard deviation of 5.0 per cent after 6 injections.

Limit:

— *15S-epimer*: maximum 1.5 per cent.

Acetonitrile (*2.4.24, System A*)

Maximum 0.1 per cent.

Prepare the solutions using *dimethylformamide R* instead of *water R.*

Sample solution Dissolve 0.200 g of the substance to be examined in *dimethylformamide R* and dilute to 20.0 ml with the same solvent.

Static head-space injection conditions that may be used:

— *equilibration temperature*: 120 °C;

— *equilibration time*: 60 min;

— *transfer-line temperature*: 125 °C.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dissolve 1.0 g in 20 ml of a mixture of equal volumes of *methanol R* and *water R*. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of equal volumes of *methanol R* and *water R*.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (a) and reference solution (a).

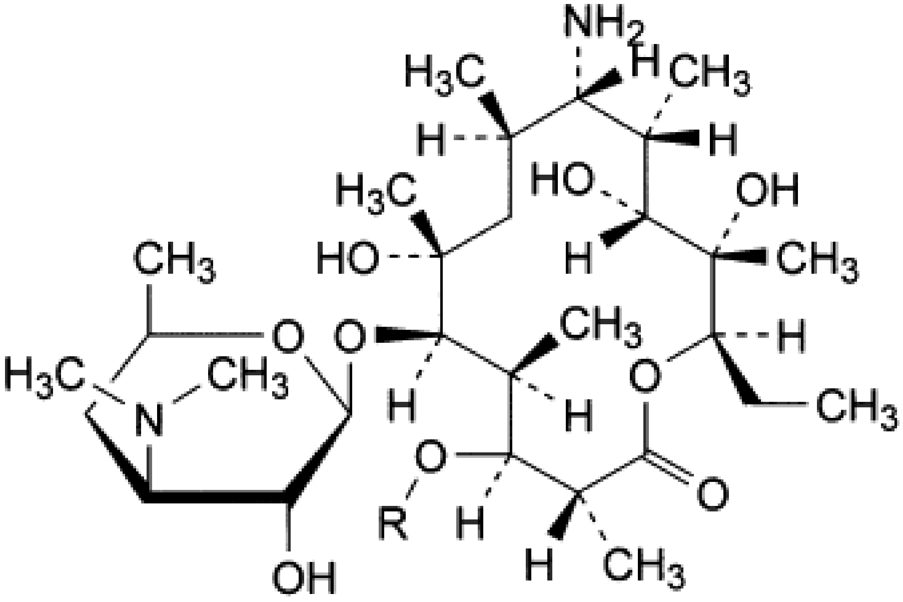
System suitability Reference solution (a):

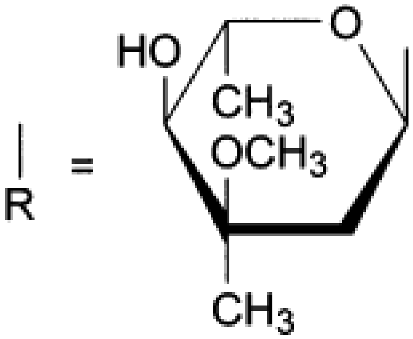
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

IMPURITIES

Specified impurities A.

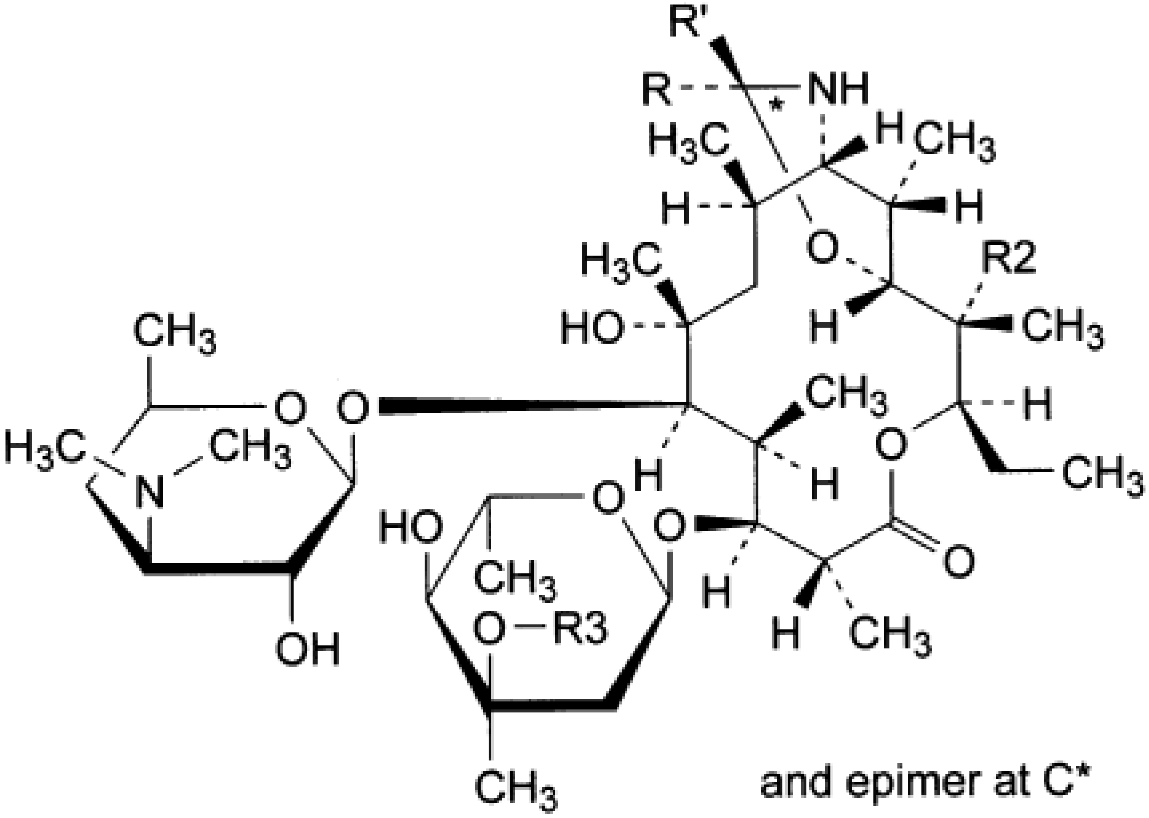
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E.*





A. (9*S*)-9-amino-9-deoxoerythromycin,

B. R = H: (9*S*)-9-amino-3-de(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)-9-deoxoerythromycin,


R'
R
NH
*
H
H$_3$C
CH$_3$
H
H
O
R2
H$_3$C
HO
H
CH$_3$
CH$_3$
O
O
O
H$_3$C
N
CH$_3$
CH$_3$
H
H
O
H
HO
O
O
CH$_3$
OH
O—R3
H
H
CH$_3$
O
CH$_3$
CH$_3$

and epimer at C*

C. R = CH_2-*O*-CH_2-CH_2-*O*-CH_3, R′ = H, R2 = H, R3 = CH_3: (9*S*)-9,11-[imino[(1*RS*)-2-(2-methoxyethoxy)ethylidene]oxy]-9-deoxo-11,12-dideoxyerythromycin (dirithromycin B),

D. R = CH_2-*O*-CH_2-CH_2-*O*-CH_3, R′ = H, R2 = OH, R3 = H: (9*S*)-9,11-[imino[(1*RS*)-2-(2-methoxyethoxy)ethylidene]oxy]-3′-*C*-demethyl-9-deoxo-11-deoxyerythromycin (dirithromycin C),

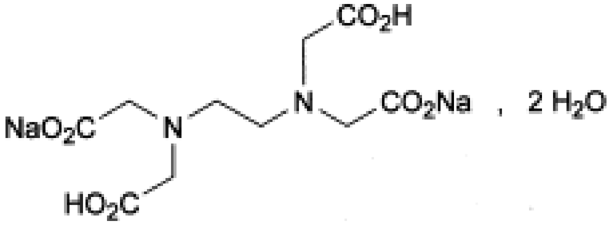
E. R = CH_3, R′ = CH_3, R2 = OH, R3 = CH_3: 9,11-[imino(1-methylethylidene)oxy]-9-deoxo-11-deoxyerythromycin.

Ph Eur

Disodium Edetate

General Notices

(Ph Eur monograph 0232)



$C_{10}H_{14}N_2Na_2O_8,2H_2O$ 372.2 6381-92-6

Action and use

Chelating agent.

Preparation

Trisodium Edetate Intravenous Infusion

Ph Eur

DEFINITION

Disodium dihydrogen (ethylenedinitrilo)tetraacetate dihydrate.

Content

98.5 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *disodium edetate CRS.*

B. Dissolve 2 g in 25 ml of *water R*, add 6 ml of *lead nitrate solution R*, shake and add 3 ml of *potassium iodide solution R*. No yellow precipitate is formed. Make alkaline to *red litmus paper R* by the addition of *dilute ammonia R2*. Add 3 ml of *ammonium oxalate solution R*. No precipitate is formed.

C. Dissolve 0.5 g in 10 ml of *water R* and add 0.5 ml of *calcium chloride solution R*. Make alkaline to *red litmus paper R* by the addition of *dilute ammonia R2* and add 3 ml of *ammonium oxalate solution R.* No precipitate is formed.

D. It gives the reactions of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.0 to 5.5 for solution S.

Impurity A

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light.*

Solvent mixture Dissolve 10.0 g of *ferric sulphate pentahydrate R* in 20 ml of *0.5 M sulphuric acid* and add 780 ml of *water R*. Adjust to pH 2.0 with *1 M sodium hydroxide* and dilute to 1000 ml with *water R*.

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution Dissolve 40.0 mg of *nitrilotriacetic acid R* in the solvent mixture and dilute to 100.0 ml with the solvent mixture. To 1.0 ml of the solution add 0.1 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.10 m, Ø = 4.6 mm,

— *stationary phase*: *spherical graphitised carbon for chromatography R1* (5 µm) with a specific surface area of 120 m^2/g and a pore size of 25 nm.

Mobile phase Dissolve 50.0 mg of *ferric sulphate pentahydrate R* in 50 ml of *0.5 M sulphuric acid* and add 750 ml of *water R*. Adjust to pH 1.5 with *0.5 M sulphuric acid* or *1 M sodium hydroxide*, add 20 ml of *ethylene glycol R* and dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µl; filter the solutions and inject immediately.

Run time 4 times the retention time of the iron complex of impurity A.

Retention times Iron complex of impurity A = about 5 min; iron complex of edetic acid = about 10 min.

System suitability Reference solution:

- *resolution*: minimum 7 between the peaks due to the iron complex of impurity A and the iron complex of edetic acid,
- *signal-to-noise ratio*: minimum 50 for the peak due to impurity A.

Limit:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Iron ***(2.4.9)***

Maximum 80 ppm.

Dilute 2.5 ml of solution S to 10 ml with *water R*. Add 0.25 g of *calcium chloride R* to the test solution and the standard before the addition of the *thioglycollic acid R*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

ASSAY

Dissolve 0.300 g in *water R* and dilute to 300 ml with the same solvent. Add 2 g of *hexamethylenetetramine R* and 2 ml of *dilute hydrochloric acid R*. Titrate with *0.1 M lead nitrate*, using about 50 mg of *xylenol orange triturate R* as indicator.

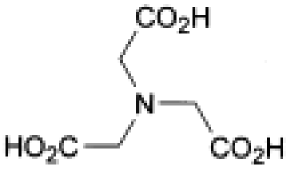
1 ml of *0.1 M lead nitrate* is equivalent to 37.22 mg of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*



A. nitrilotriacetic acid.

Ph Eur

Disodium Hydrogen Phosphate Dihydrate

General Notices

Sodium Phosphate Dihydrate

(Disodium Phosphate Dihydrate, Ph Eur monograph 0602)

$Na_2HPO_4,2H_2O$ 178.0 *10028-24-7*

Action and use

Excipient.

Preparation

Phosphates Enema

Ph Eur

DEFINITION

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or colourless crystals.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly alkaline *(2.2.4)*.

B. Loss on drying (see Tests).

C. Solution S gives reaction (b) of phosphates *(2.3.1)*.

D. Solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Reducing substances

To 5 ml of solution S add 5 ml of *dilute sulphuric acid R* and 0.25 ml of *0.02 M potassium permanganate* and heat on a water-bath for 5 min. The solution retains a slight red colour.

Monosodium phosphate

Maximum 2.5 per cent.

From the volume of *1 M hydrochloric acid* (25 ml) and of *1 M sodium hydroxide* (n_1 ml and n_2 ml) used in the assay, calculate the following ratio:

$$\frac{n_2 - 25}{25 - n_1}$$

This ratio is not greater than 0.025.

Chlorides *(2.4.4)*

Maximum 400 ppm.

To 2.5 ml of solution S add 10 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 0.1 per cent.

To 3 ml of solution S add 2 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*.

Arsenic *(2.4.2, Method A)*

Maximum 4 ppm, determined on 5 ml of solution S.

Iron *(2.4.9)*

Maximum 40 ppm

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

19.5 per cent to 21.0 per cent, determined on 1.000 g by drying in an oven at 130 °C.

ASSAY

Dissolve 2.000 g (*m*) in 50 ml of *water R* and add 25.0 ml of *1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)* using *1 M sodium hydroxide*. Read the volume added at the 1st inflexion point (n_1 ml). Continue the titration to the 2nd inflexion point (total volume of *1 M* *sodium hydroxide required*, n_2 ml).

Calculate the percentage content of Na_2HPO_4 from the following expression:

$$\frac{1420\,(25 - n_1)}{m\,(100 - d)}$$

d = percentage loss on drying.

Ph Eur

Disodium Hydrogen Phosphate Dodecahydrate

General Notices

Disodium Hydrogen Phosphate, Sodium Phosphate

(Disodium Phosphate Dodecahydrate, Ph Eur monograph 0118)

$Na_2HPO_4,12H_2O$ 358.1 *10039-32-4*

Preparation

Phosphates Enema

Ph Eur

DEFINITION

Content

98.5 per cent to 102.5 per cent.

CHARACTERS

Appearance

Colourless, transparent crystals, very efflorescent.

Solubility

Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly alkaline (*2.2.4*).

B. Water (see Tests).

C. Solution S gives reaction (b) of phosphates (*2.3.1*).

D. Solution S gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Reducing substances

To 5 ml of solution S add 5 ml of *dilute sulphuric acid R* and 0.25 ml of *0.02 M potassium permanganate* and heat on a water-bath for 5 min. The solution retains a slight red colour.

Monosodium phosphate

Maximum 2.5 per cent.

From the volume of *1 M hydrochloric acid* (25 ml) and of *1 M sodium hydroxide* (n_1 ml and n_2 ml) used in the assay, calculate the following ratio:

$$\frac{n_2 - 25}{25 - n_1}$$

This ratio is not greater than 0.025.

Chlorides (*2.4.4*)

Maximum 200 ppm.

To 2.5 ml of solution S add 10 ml of *dilute nitric acid R* and dilute to 15 ml with *water R.*

Sulphates (*2.4.13*)

Maximum 500 ppm.

To 3 ml of solution S add 2 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R.*

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 5 ml of solution S.

Iron (*2.4.9*)

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R.*

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

57.0 per cent to 61.0 per cent, determined on 50.0 mg. Use a mixture of 10 volumes of *anhydrous methanol R* and 40 volumes of *formamide R1* as solvent.

ASSAY

Dissolve 4.00 g (*m*) in 25 ml of *water R* and add 25.0 ml of *1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*) using *1 M sodium hydroxide*. Read the volume added at the 1st inflexion point (n_1 ml). Continue the titration to the 2nd inflexion point (total volume of *1 M sodium hydroxide* required, n_2 ml).

Calculate the percentage content of $Na_2HPO_4,12H_2O$ from the following expression:

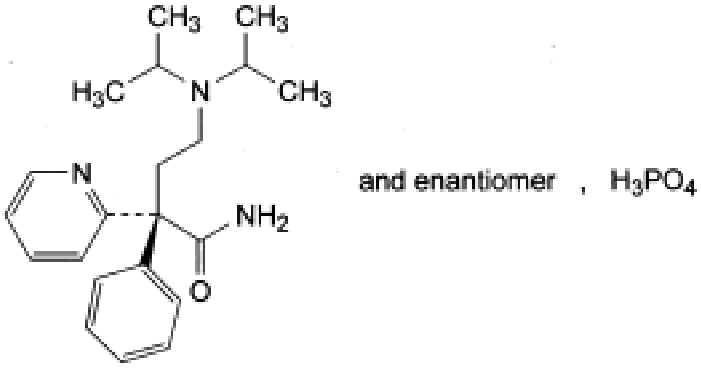
$$\frac{3581\,(25 - n_1)}{m \times 100}$$

Ph Eur

Disopyramide Phosphate

General Notices

(Ph Eur monograph 1005)



$C_{21}H_{29}N_3O,H_3PO_4$ 437.5 *22059-60-5*

Action and use

Antiarrhythmic.

Preparation

Disopyramide Phosphate Capsules

Ph Eur

DEFINITION

Disopyramide phosphate contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (2*RS*)-4-[bis(1-methylethyl)amino]-2-phenyl-2-(pyridin-2-yl) butanamide dihydrogen phosphate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, soluble in water, sparingly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Dissolve 50.0 mg in a 5 g/l solution of *sulphuric acid R* in *methanol R* and dilute to 100.0 ml with the same solution. Dilute 5.0 ml of this solution to 50.0 ml with a 5 g/l solution of *sulphuric acid R* in *methanol R*. Examined between 240 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 269 nm and a shoulder at 263 nm. The specific absorbance at the maximum is 147 to 163.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *disopyramide phosphate CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Spray with *dilute potassium iodobismuthate solution R*. Examine in daylight. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Solution S (see Tests) gives reaction (a) of phosphates *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 4.0 to 5.0.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.25 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 25 mg of *disopyramide phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 20 ml with *methanol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 30 volumes of *acetone R* and 30 volumes of *cyclohexane R*. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

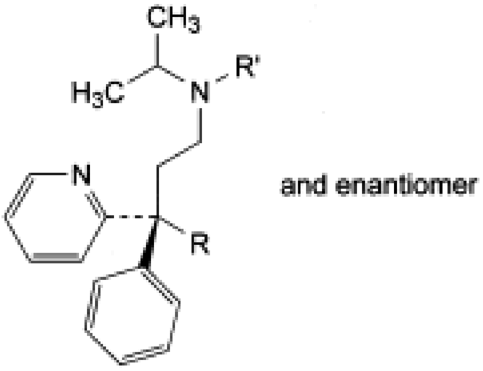
Dissolve 0.180 g in 30 ml of *anhydrous acetic acid R*. Add 0.2 ml of *naphtholbenzein solution R*. Titrate with *0.1 M perchloric acid* until the colour changes from yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 21.88 mg of $C_{21}H_{32}N_3O_5P$.

STORAGE

Store protected from light.

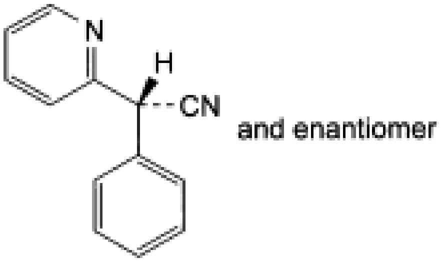
IMPURITIES



A. R = CN, R' = $CH(CH_3)_2$: (2*RS*)-4-[bis(1-methylethyl)amino]-2-phenyl-2-(pyridin-2-yl) butanenitrile (di-isopyronitrile),

B. R = H, R' = $CH(CH_3)_2$: (3*RS*)-*N,N*-bis(1-methylethyl)-3-phenyl-3-(pyridin-2-yl)propan-1-amine,

C. R = $CO-NH_2$, R′ = H: (2*RS*)-4-[(1-methylethyl)amino]-2-phenyl-2-(pyridin-2-yl)butanamide,



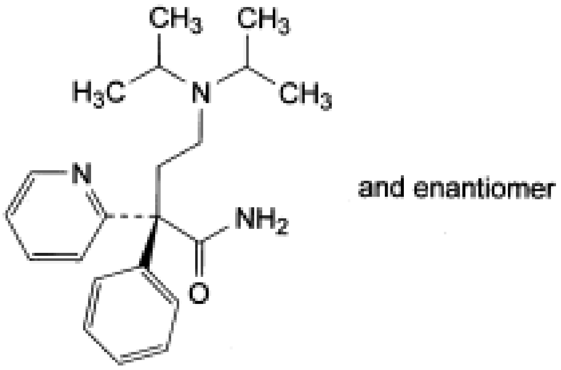
D. (*RS*)-phenyl(pyridin-2-yl)acetonitrile (pyronitrile).

Ph Eur

Disopyramide

General Notices

(Ph Eur monograph 1006)



$C_{21}H_{29}N_3O$ 339.5 *3737-09-5*

Action and use

Class I antiarrhythmic.

Preparation

Disopyramide Capsules

Ph Eur

DEFINITION

Disopyramide contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of (2*RS*)-4-[bis(1-methylethyl)amino]-2-phenyl-2-(pyridin-2-yl)butanamide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, slightly soluble in water, freely soluble in methylene chloride, soluble in alcohol.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Dissolve 40.0 mg in a 5 g/l solution of *sulphuric acid R* in *methanol R* and dilute to 100.0 ml with the same solution. Dilute 5.0 ml of this solution to 50.0 ml with a 5 g/l solution of *sulphuric acid R* in *methanol R*. Examined between 240 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 269 nm and a shoulder at 263 nm. The specific absorbance at the maximum is 190 to 210.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *disopyramide CRS*. Examine the substances as discs prepared by placing 50 µl of a 50 g/l solution in *methylene chloride R* on a disc of *potassium bromide R*. Dry the discs at 60 °C for 1 h before use.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Spray with *dilute potassium iodobismuthate solution R*. Examine in daylight. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 20 mg of *disopyramide CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of test solution (b) to 20 ml with *methanol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 30 volumes of *acetone R* and 30 volumes of *cyclohexane R*. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Heavy metals *(2.4.8)*

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying at 80 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 2 h.

Sulphated ash *(2.4.14)*

Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

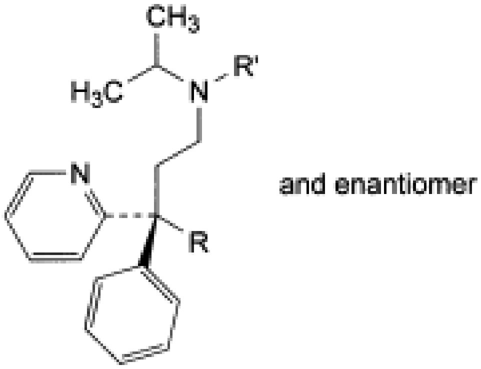
Dissolve 0.130 g in 30 ml of *anhydrous acetic acid R*. Add 0.2 ml of *naphtholbenzein solution R*. Titrate with *0.1 M perchloric acid* until the colour changes from yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 16.97 mg of $C_{21}H_{29}N_3O$.

STORAGE

Store protected from light.

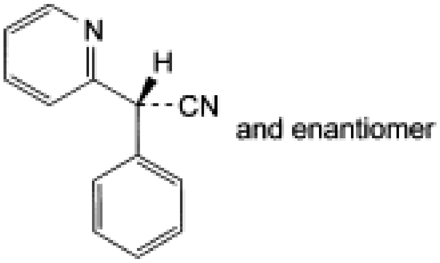
IMPURITIES


CH3
H3C
N
R'
N
R
and enantiomer

A. R = CN, R′ = $CH(CH_3)_2$: (2*RS*)-4-[bis(1-methylethyl)amino]-2-phenyl-2-(pyridin-2-yl) butanenitrile (di-isopyronitrile),

B. R = H, R′ = $CH(CH_3)_2$: (3*RS*)-*N,N*-bis(1-methylethyl)-3-phenyl-3-(pyridin-2-yl)propan-1-amine,

C. R = $CO-NH_2$, R′ = H: (2*RS*)-4-[(1-methylethyl)amino]-2-phenyl-2-(pyridin-2-yl)butanamide,


N
H
CN
and enantiomer

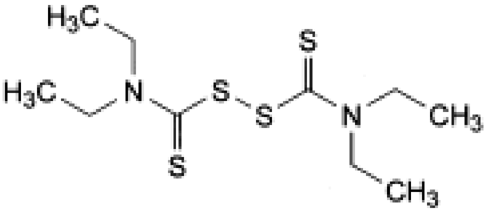
D. (*RS*)-phenyl(pyridin-2-yl)acetonitrile (pyronitrile).

Ph Eur

Disulfiram

General Notices

(Ph Eur monograph 0603)



$C_{10}H_{20}N_2S_4$ 296.5 *97-77-8*

Action and use

Aldehyde dehyrogenase inhibitor; treatment of alcoholism.

Preparation

Disulfiram Tablets

Ph Eur

DEFINITION

Disulfiram contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of tetraethyldisulfanedicarbothioamide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in alcohol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)* 70 °C to 73 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *disulfiram CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 10 ml of *methanol R*. Add 2 ml of a 0.5 g/l solution of *cupric chloride R* in *methanol R*. A yellow colour develops which becomes greenish-yellow.

TESTS

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *ethyl acetate R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *ethyl acetate R*.

Reference solution (a) Dissolve 10 mg of *disulfiram CRS* in *ethyl acetate R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 20 ml with *ethyl acetate R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of *butyl acetate R* and 70 volumes of *hexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Diethyldithiocarbamate

Dissolve 0.20 g in 10 ml of *peroxide-free ether R*, add 5 ml of *buffer solution pH 8.0 R* and shake vigorously. Discard the upper layer and wash the lower layer with 10 ml of *peroxide-free ether R*. Add to the lower layer 0.2 ml of a 4 g/l solution of *copper sulphate R* and 5 ml of *cyclohexane R*. Shake. Any yellow colour in the upper layer is not more intense than that of a standard prepared at the same time using 0.2 ml of a freshly prepared 0.15 g/l solution of *sodium diethyldithiocarbamate R* (150 ppm).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 50 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

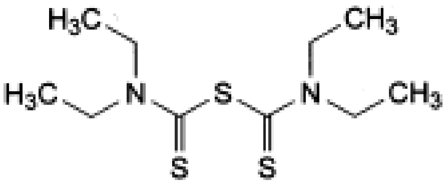
Dissolve 0.450 g in 80 ml of *acetone R* and add 20 ml of a 20 g/l solution of *potassium nitrate R*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20)*, using a silver electrode and a silver-silver chloride double-junction electrode saturated with potassium nitrate.

1 ml of *0.1 M silver nitrate* is equivalent to 59.30 mg of $C_{10}H_{20}N_2S_4$.

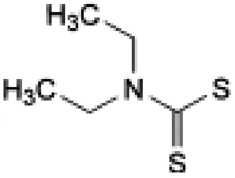
STORAGE

Store protected from light.

IMPURITIES



A. diethylthiocarbamic thioanhydride (sulfiram),



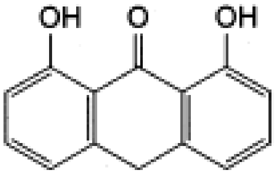
B. diethyldithiocarbamate.

Ph Eur

Dithranol

General Notices

(Ph Eur monograph 1007)



$C_{14}H_{10}O_3$ 226.2 1143-38-0

Action and use

Coal tar extract; treatment of psoriasis.

Preparations

Dithranol Cream

Dithranol Ointment

Dithranol Paste

Ph Eur

DEFINITION

1,8-Dihydroxyanthracen-9(10*H*)-one.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow or brownish-yellow, crystalline powder.

Solubility

Practically insoluble in water, soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

Carry out all tests protected from bright light and use freshly prepared solutions.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)*: 178 °C to 182 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *dithranol CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *dithranol CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve about 5 mg of *dantron R* in 5 ml of reference solution (a).

Plate *TLC silica gel plate R.*

Mobile phase *hexane R, methylene chloride R* (50:50 *V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Place the plate in a tank saturated with ammonia vapour until the spots appear. Examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 5 mg add 0.1 g of *anhydrous sodium acetate R* and 1 ml of *acetic anhydride R*. Boil for 30 s. Add 20 ml of *ethanol (96 per cent) R*. Examined in ultraviolet light at 365 nm, the solution shows a blue fluorescence.

TESTS

Related substances

A. Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.200 g of the substance to be examined in 20 ml of *methylene chloride R*, add 1.0 ml of *glacial acetic acid R* and dilute to 100.0 ml with *hexane R*.

Reference solution Dissolve 5.0 mg of *anthrone R* (impurity A), 5.0 mg of *dantron R* (impurity B), 5.0 mg of *dithranol impurity C CRS* and 5.0 mg of *dithranol CRS* in *methylene chloride R* and dilute to 5.0 ml with the same solvent. To 1.0 ml of this solution, add 19.0 ml of *methylene chloride R* and 1.0 ml of *glacial acetic acid R*, and dilute to 50.0 ml with *hexane R*.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, methylene chloride R, hexane R* (1:5:82 *V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µl.

Run time 1.5 times the retention time of impurity C.

Elution order dithranol, impurity B, impurity A, impurity C.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the peaks due to dithranol and impurity B.

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (1 per cent).

B. Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in 5 ml of *tetrahydrofuran R* and dilute to 25.0 ml with the mobile phase.

Reference solution Dissolve 5.0 mg of *dithranol impurity D CRS* and 5.0 mg of *dithranol CRS* in 5 ml of *tetrahydrofuran R* and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.20 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, tetrahydrofuran R, water R* (2.5:40:60 *V/V/V*).

Flow rate 0.9 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 3 times the retention time of dithranol.

System suitability Reference solution:

— *resolution*: minimum 2.5 between the peaks due to impurity D and dithranol.

Limit:

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (2.5 per cent).

Total (tests A + B) Maximum 3.0 per cent for the sum of the contents of all impurities.

Chlorides *(2.4.4)*

Maximum 100 ppm.

Shake 1.0 g with 20 ml of *water R* for 1 min and filter. Dilute 10 ml of the filtrate to 15 ml with *water R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *anhydrous pyridine R*. Titrate with *0.1 M tetrabutylammonium hydroxide* under *nitrogen R*. Determine the end-point potentiometrically *(2.2.20)*, using a glass indicator electrode and a calomel reference electrode containing, as the electrolyte, a saturated solution of *potassium chloride R* in *methanol R*.

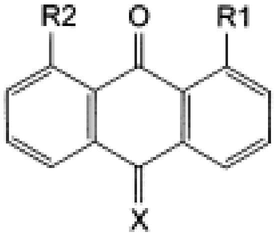
1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 22.62 mg of $C_{14}H_{10}O_3$.

STORAGE

Protected from light.

IMPURITIES

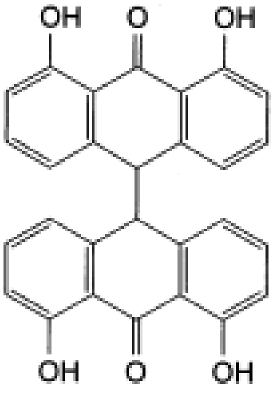
Specified impurities A, B, C, D.



A. R1 = R2 = H, X = H_2: anthracen-9(10*H*)-one (anthrone),

B. R1 = R2 = OH, X = O: 1,8-dihydroxyanthracene-9,10-dione (dantron),

D. R1 = OH, R2 = H, X = H_2: 1-hydroxyanthracen-9(10*H*)-one,



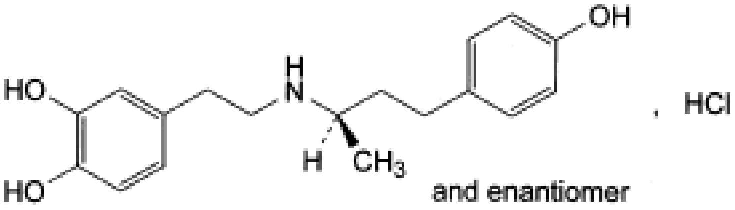
C. 4,4′,5,5′-tetrahydroxy-9,9′-bianthracenyl-10,10′(9*H*,9′*H*)-dione.

Ph Eur

Dobutamine Hydrochloride

General Notices

(Ph Eur monograph 1200)



$C_{18}H_{23}NO_3,HCl$ 337.9 *49745-95-1*

Action and use

$Beta_1$-adrenoceptor agonist.

Preparation

Dobutamine Intravenous Infusion

Ph Eur

DEFINITION

(*RS*)-4-[2-[[3-(4-Hydroxyphenyl)-1-methylpropyl]amino]ethyl]benzene-1,2-diol hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, soluble in methanol, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification C, E.

Second identification A, B, D, E.

A. Melting point *(2.2.14)* 189 °C to 192 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 20.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *methanol R*.

Spectral range 220-300 nm.

Absorption maxima At 223 nm and 281 nm.

Absorbance ratio A_{281} / A_{223} = 0.34 to 0.36.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *dobutamine hydrochloride CRS.*

D. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *glacial acetic acid R, methanol R* (50:50 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 10.0 mg of *dobutamine hydrochloride CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 5.0 mg of *dopamine hydrochloride CRS* in 5 ml of the test solution.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, glacial acetic acid R, ether R, butanol R* (5:15:30:45 *V/V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 1 g/l solution of *potassium permanganate R*.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

E. It gives reaction (a) of chlorides *(2.3.1)* using a mixture of equal volumes of *methanol R* and *water R*.

TESTS

Acidity or alkalinity

Dissolve 0.1 g in *water R* with gentle heating and dilute to 10 ml with the same solvent. Add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Optical rotation *(2.2.7)*

- 0.05° to + 0.05°.

Dissolve 0.50 g in *methanol R* and dilute to 10.0 ml with the same solvent.

Absorbance *(2.2.25)*

Maximum 0.04 at 480 nm.

Dissolve 0.5 g in a mixture of equal volumes of *methanol R* and of *water R* with heating, if necessary, at 30-35 °C and dilute to 25 ml with the same mixture of solvents. Cool quickly. Examine immediately.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *mobile phase B, mobile phase A* (35:65 *V/V*).

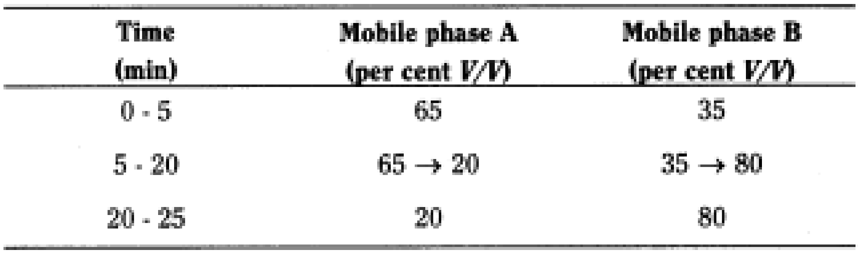
Test solution Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dilute 4.0 ml of the test solution to 100.0 ml with a 0.05 g/l solution of *anisaldehyde R* in the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:
- *size:* *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: dissolve 2.60 g of *sodium octanesulphonate R* in 1000 ml of *water R*, add 3 ml of *triethylamine R* and adjust to pH 2.5 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R, methanol R* (18:82 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	65	35
5 - 20	65 → 20	35 → 80
20 - 25	20	80

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

System suitability Reference solution (a):

- *resolution*: minimum 4.0 between the peaks due to dobutamine and anisaldehyde.

Limits:
- *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the

chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Mximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.250 g in 10 ml of *anhydrous formic acid R*. Add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 33.79 mg of $C_{18}H_{24}ClNO_3$.

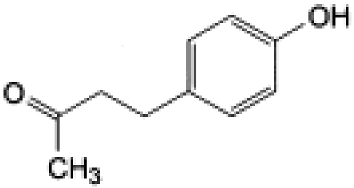
STORAGE

Protected from light.

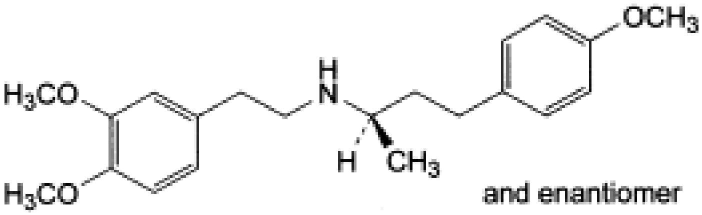
IMPURITIES

Specified impurities A, B, C.

A. dopamine,



B. 4-(4-hydroxyphenyl)butan-2-one,



C. (2*RS*)-*N*-[2-(3,4-dimethoxyphenyl)ethyl]-4-(4-methoxyphenyl)butan-2-amine.

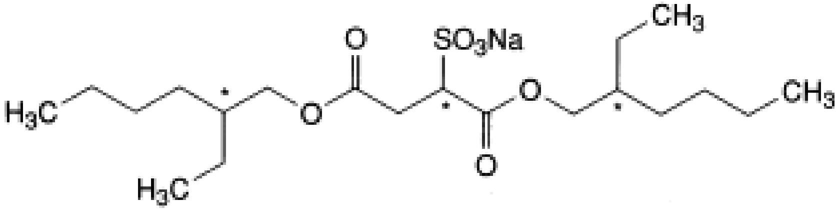
Ph Eur

Docusate Sodium

General Notices

Dioctyl Sodium Sulphosuccinate

(Ph Eur monograph 1418)



$C_{20}H_{37}NaO_7S$ 444.6 *577-11-7*

Action and use

Stimulant laxative; faecal softener.

Preparations

Co-danthrusate Capsules

Docusate Capsules

Compound Docusate Enema

Docusate Oral Solution

Paediatric Docusate Oral Solution

Ph Eur

DEFINITION

Sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulphonate.

Content

98.0 to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, waxy masses or flakes, hygroscopic.

Solubility

Sparingly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Place about 3 mg of the substance to be examined on a sodium chloride plate, add 0.05 ml of *acetone R* and immediately cover with another sodium chloride plate. Rub the plates together to dissolve the substance to be examined, slide the plates apart and allow the acetone to evaporate.

Comparison Ph. Eur. reference spectrum of docusate sodium.

B. In a crucible, ignite 0.75 g in the presence of *dilute sulphuric acid R*, until an almost white residue is obtained. Allow to cool and take up the residue with 5 ml of *water R*. Filter. 2 ml of the filtrate gives reaction (a) of sodium *(2.3.1).*

TESTS

Alkalinity

Dissolve 1.0 g in 100 ml of a mixture of equal volumes of *methanol R* and *water R*, previously neutralised to *methyl red solution R*. Add 0.1 ml of *methyl red solution R*. Not more than 0.2 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to red.

Related non-ionic substances

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 10 mg of *methyl behenate R* in *hexane R* and dilute to 50 ml with the same solvent.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 2.0 ml of the internal standard solution and dilute to 5.0 ml with *hexane R*. Pass the solution, at a rate of about 1.5 ml/min, through a column 10 mm in internal diameter, packed with 5 g of *basic aluminium oxide R* and previously washed with 25 ml of *hexane R*. Elute with 5 ml of *hexane R* and discard the eluate. Elute with 20 ml of a mixture of equal volumes of *ether R* and *hexane R*. Evaporate the eluate to dryness and dissolve the residue in 2.0 ml of *hexane R*.

Test solution (b) Prepare as described for test solution (a) but dissolving 0.10 g of the substance to be examined in *hexane R*, diluting to 5.0 ml with the same solvent, and using a new column.

Reference solution Dilute 2.0 ml of the internal standard solution to 5.0 ml with *hexane R*.

Column:

- *material*: glass,
- *size: l* = 2 m, Ø = 2 mm,
- *stationary phase*: silanised *diatomaceous earth for gas chromatography R* impregnated with 3 per cent m/m of *polymethylphenylsiloxane R*.

Carrier gas nitrogen for chromatography R.

Flow rate 30 ml/min.

Temperature:

- *column*: 230 °C,

— *injection port and detector*: 280 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 2.5 times the retention time of the internal standard.

System suitability There is no peak with the same retention time as the internal standard in the chromatogram obtained with test solution (b).

Limits Test solution (a):

— *any impurity*: for each impurity, not more than the area of the peak due to the internal standard (0.4 per cent).

Chlorides

Maximum 350 ppm.

Dissolve 5.0 g in 50 ml of *ethanol (50 per cent V/V) R* and add 0.1 ml of *potassium dichromate solution R*. Not more than 0.5 ml of *0.1 M silver nitrate* is required to change the colour of the indicator from yellow to orange.

Sodium sulphate

Maximum 2 per cent.

Dissolve 0.25 g in 40 ml of a mixture of 20 volumes of *water R* and 80 volumes of *2-propanol R*. Adjust to pH between 2.5 and 4.0 using *perchloric acid solution R*. Add 0.4 ml of *naphtharson solution R* and 0.1 ml of a 0.125 g/l solution of *methylene blue R*. Not more than 1.5 ml of *0.025 M barium perchlorate* is required to change the colour of the indicator from yellowish-green to yellowish-pink.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 4.0 g in *ethanol (80 per cent V/V) R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with *ethanol (80 per cent V/V) R*.

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 0.250 g.

ASSAY

To 1.000 g in a 250 ml conical flask fitted with a reflux condenser add 25.0 ml of *0.5 M alcoholic potassium hydroxide* and heat on a water-bath under reflux for 45 min. Allow to cool. Add 0.25 ml of *phenolphthalein solution R1* and titrate with *0.5 M hydrochloric acid* until the red colour disappears. Carry out a blank titration.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 0.1112 g of $C_{20}H_{37}NaO_7S$.

STORAGE

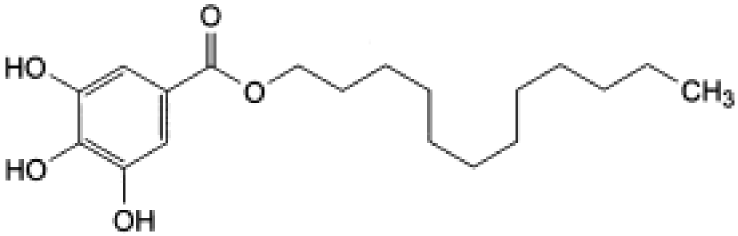
In an airtight container.

Ph Eur

Dodecyl Gallate

General Notices

(Ph Eur monograph 2078)



$C_{19}H_{30}O_5$ 338.4 *1166-52-5*

Action and use

Antioxidant.

Ph Eur

DEFINITION

Dodecyl 3,4,5-trihydroxybenzoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble or practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION

A. Melting point *(2.2.14)*.

Determine the melting point of the substance to be examined. Mix equal parts of the substance to be examined and *dodecyl gallate CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 96 °C) is not greater than 2 °C.

B. Examine the chromatograms obtained in the test for impurity A.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 20 ml with *acetone R*.

Reference solution (a) Dissolve 10 mg of *dodecyl gallate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *gallic acid R* in *acetone R* and dilute to 20 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10 ml with *acetone R*.

Reference solution (d) Dilute 1.0 ml of reference solution (b) to 5 ml with test solution (a).

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R, ethyl formate R, toluene R* (10:40:50 *V/V/V*).

Application 5 µl of test solutions (a) and (b) and reference solutions (a), (c) and (d).

Development Over 2/3 of the plate.

Drying In air for 10 min.

Detection Spray with a mixture of 1 volume of *ferric chloride solution R1* and 9 volumes of *ethanol (96 per cent) R*.

System suitability Reference solution (d):

— the chromatogram shows 2 clearly separated principal spots.

Limit Test solution (a):

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Chlorides *(2.4.4)*

Maximum 100 ppm.

To 1.65 g add 50 ml of *water R*. Shake for 5 min. Filter. 15 ml of the filtrate complies with the test.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 70 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in *methanol R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 200.0 ml with *methanol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 275 nm.

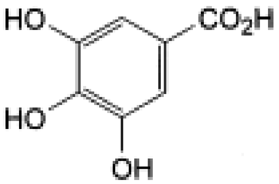
Calculate the content of $C_{19}H_{30}O_5$ taking the specific absorbance to be 321.

STORAGE

In a non-metallic container, protected from light.

IMPURITIES

Specified impurities A.

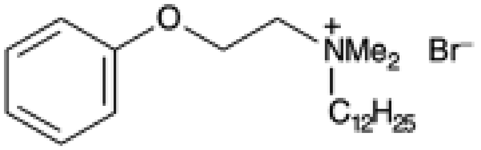


A. 3,4,5-trihydroxybenzoic acid (gallic acid).

Ph Eur

Domiphen Bromide

General Notices



$C_{22}H_{40}BrNO$ 414.5 *538-71-6*

Action and use

Antiseptic.

DEFINITION

Domiphen Bromide consists chiefly of dodecyldimethyl-2-phenoxyethylammonium bromide. It contains not less than 97.0% and not more than 100.5% of $C_{22}H_{40}BrNO$, calculated with reference to the dried substance.

CHARACTERISTICS

Colourless or faintly yellow, crystalline flakes.

Freely soluble in *water* and in *ethanol (96%)*; soluble in *acetone*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of domiphen bromide (*RS 383*).

B. Dissolve 10 mg in 10 ml of *water* and add 0.1 ml of a 0.5% w/v solution of *eosin* and 100 ml of *water*. An intense pink colour is produced.

C. Yields the reactions characteristic of *bromides*, Appendix VI.

Melting point, 106° to 116°, Appendix V A.

TESTS

Acidity or alkalinity

Add 0.5 ml of *bromothymol blue solution R3* to each of 10 ml of *phosphate buffer pH 6.4* (solution A) and 10 ml of *phosphate buffer pH 7.6* (solution B). Dissolve 0.10 g in 10 ml of *carbon dioxide-free water* and add 0.5 ml of *bromothymol blue solution R3*. The resulting solution is not more yellow than solution A and not more blue than solution B.

Clarity and colour of solution

Dissolve 1.0 g in 10 ml of *carbon dioxide-free water*. The solution is not more opalescent than *reference suspension II*, Appendix IV A, and not more intensely coloured than *reference solution Y_7*, Appendix IV B, Method I.

Non-quaternary amines

Carry out the Assay described below using a further 25 ml of the original solution and 10 ml of 0.1M *hydrochloric acid* in place of the 0.1M *sodium hydroxide*. The difference between the volume of 0.05M *potassium iodate VS* required in this titration and that required in the Assay is not more than 0.5 ml for each g of substance taken.

Loss on drying

When dried to constant weight at 70° at a pressure not exceeding 0.7 kPa, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

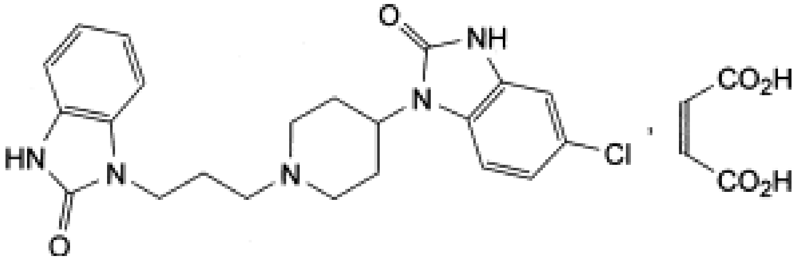
ASSAY

Dissolve 2 g in sufficient *water* to produce 100 ml. Transfer 25 ml to a separating funnel and add 25 ml of *chloroform*, 10 ml of 0.1M *sodium hydroxide* and 10 ml of a freshly prepared 5% w/v solution of *potassium iodide*. Shake well, allow to separate and discard the chloroform layer. Wash the aqueous layer with three 10 ml quantities of *chloroform* and discard the chloroform solutions. Add 40 ml of *hydrochloric acid*, allow to cool and titrate with 0.05M *potassium iodate VS* until the deep brown colour is discharged. Add 2 ml of *chloroform* and continue the titration, shaking vigorously, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 10 ml of the freshly prepared potassium iodide solution, 20 ml of *water* and 40 ml of *hydrochloric acid*. The difference between the titrations represents the amount of potassium iodate required. Each ml of 0.05M *potassium iodate VS* is equivalent to 41.45 mg of $C_{22}H_{40}BrNO$.

Domperidone Maleate

General Notices

(Ph Eur monograph 1008)



$C_{22}H_{24}ClN_5O_2,C_4H_4O_4$ 542.0 *99497-03-7*

Action and use

Peripheral dopamine receptor antagonist; antiemetic.

Preparation

Domperidone Tablets

Ph Eur

DEFINITION

5-Chloro-1-[1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one hydrogen (*Z*)-butenedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, sparingly soluble in dimethylformamide, slightly soluble in methanol, very slightly soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *domperidone maleate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *2-propanol R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *domperidone maleate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *domperidone maleate CRS* and 20 mg of *droperidol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *ammonium acetate solution R, dioxan R, methanol R* (20:40:40 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear. Examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Triturate 0.1 g with a mixture of 1 ml of *strong sodium hydroxide solution R* and 3 ml of *water R*. Shake with 3 quantities, each of 5 ml, of *ether R*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min. No colour develops. To the remainder of the aqueous layer add 2 ml of *bromine solution R*. Heat on a water-bath for 15 min and then heat to boiling. Cool. To 0.1 ml of this solution add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min. A violet colour develops.

TESTS

Appearance of solution

The solution is clear (*2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 0.20 g in *dimethylformamide R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*

Prepare the solutions immediately before use.

Test solution Dissolve 0.10 g of the substance to be examined in *dimethylformamide R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *domperidone maleate CRS* and 15.0 mg of *droperidol CRS* in *dimethylformamide R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide R*. Dilute 5.0 ml of this solution to 20.0 ml with *dimethylformamide R*.

Column:

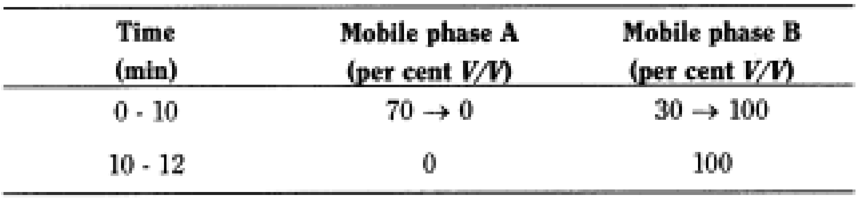
— *size*: *l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 5 g/l solution of *ammonium acetate R*;

— *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	70 → 0	30 → 100
10 - 12	0	100

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Equilibration With *methanol R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *dimethylformamide R* as a blank.

Retention time Domperidone = about 6.5 min; droperidol = about 7 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to domperidone and droperidol; if necessary, adjust the concentration of methanol in the mobile phase or adjust the time programme for the linear gradient.

Limits:

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *anhydrous acetic acid R*. Using 0.2 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from orange-yellow to green.

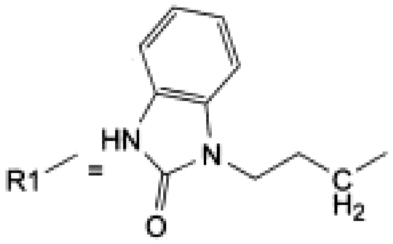
1 ml of *0.1 M perchloric acid* is equivalent to 54.20 mg of $C_{26}H_{28}ClN_5O_2$.

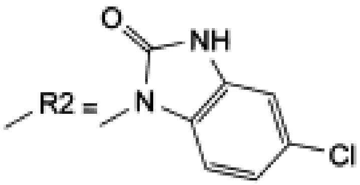
STORAGE

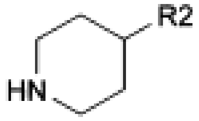
Protected from light.

IMPURITIES

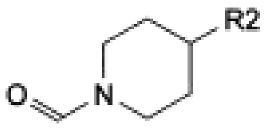
Specified impurities   A, B, C, D, E, F.



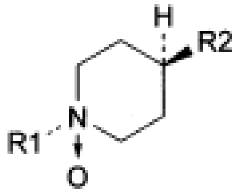




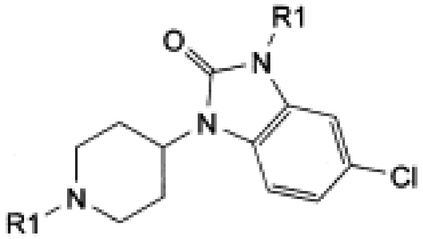
A. 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one,



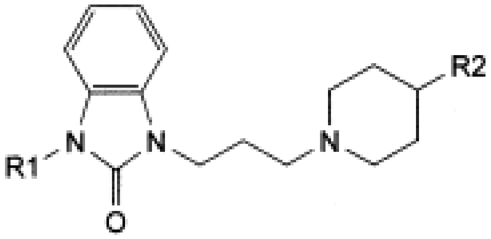
B. 4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)-1-formylpiperidine,



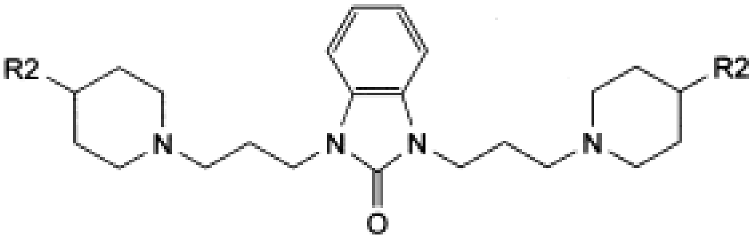
C. *cis*-4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)-1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidine 1-oxide,



D. 5-chloro-3-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]-1-[1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



E. 1-[3-[4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl]propyl]-3-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]-1,3-dihydro-2*H*-benzimidazol-2-one,



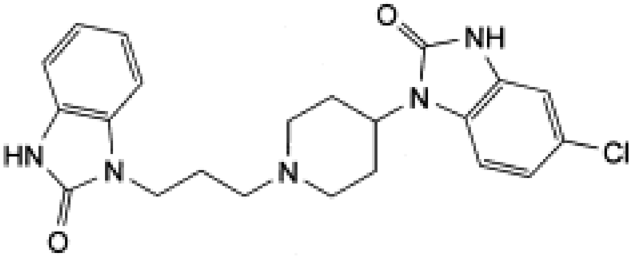
F. 1,3-bis[3-[4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl]propyl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Ph Eur

Domperidone

General Notices

(Ph Eur monograph 1009)



$C_{22}H_{24}ClN_5O_2$ 425.9 *57808-66-9*

Action and use

Peripheral dopamine receptor antagonist; antiemetic.

Ph Eur

DEFINITION

5-Chloro-1-[1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in dimethylformamide, slightly soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)*: 244 °C to 248 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *domperidone CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *domperidone CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *domperidone CRS* and 20 mg of *droperidol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *ammonium acetate solution R, dioxan R, methanol R* (20:40:40 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear. Examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives the reaction of non-nitrogen substituted barbiturates *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear (*2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 0.20 g in *dimethylformamide R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*

Prepare the solutions immediately before use.

Test solution Dissolve 0.10 g of the substance to be examined in *dimethylformamide R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *domperidone CRS* and 15.0 mg of *droperidol CRS* in *dimethylformamide R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide R*. Dilute 5.0 ml of this solution to 20.0 ml with *dimethylformamide R*.

Column:

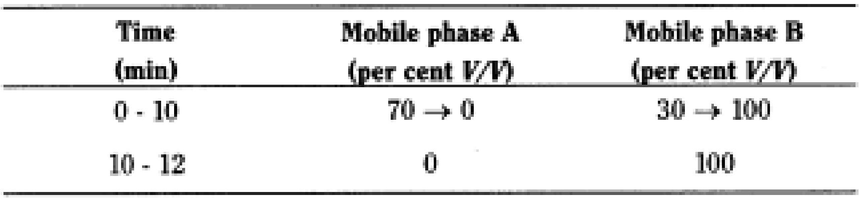
— *size: l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 5 g/l solution of *ammonium acetate R*;

— *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	70 → 0	30 → 100
10 - 12	0	100

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Equilibration With *methanol R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *dimethylformamide R* as a blank.

Retention time Domperidone = about 6.5 min; droperidol = about 7 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to domperidone and droperidol; if necessary, adjust the concentration of methanol in the mobile phase or adjust the time programme for the linear gradient.

Limits:

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid* until the colour changes from orange-yellow to green using 0.2 ml of *naphtholbenzein solution R* as indicator.

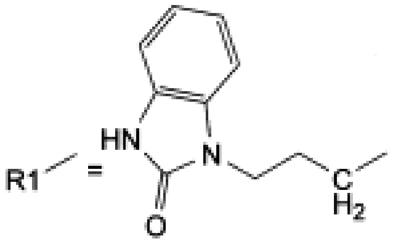
1 ml of *0.1 M perchloric acid* is equivalent to 42.59 mg of $C_{22}H_{24}ClN_5O_2$.

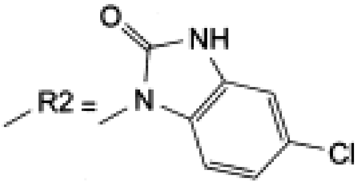
STORAGE

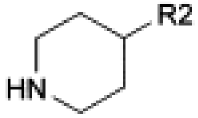
Protected from light.

IMPURITIES

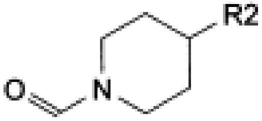
Specified impurities A, B, C, D, E, F.



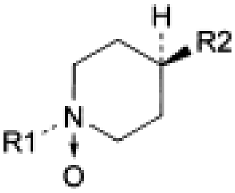




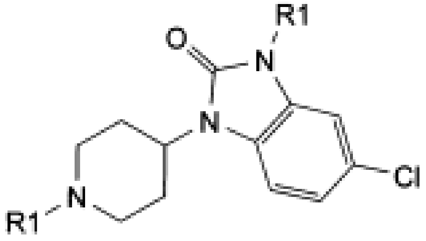
A. 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one,



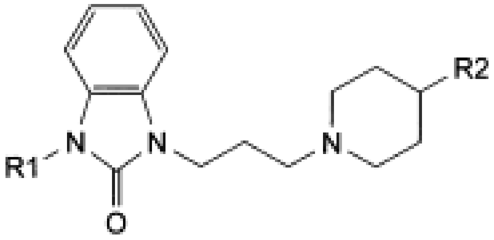
B. 4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)-1-formylpiperidine,



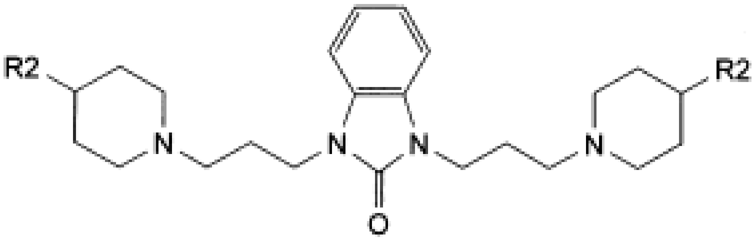
C. *cis*-4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)-1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidine 1-oxide,



D. 5-chloro-3-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]-1-[1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



E. 1-[3-[4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl]propyl]-3-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]-1,3-dihydro-2*H*-benzimidazol-2-one,



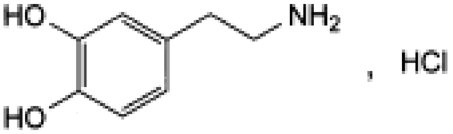
F. 1,3-bis[3-[4-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl]propyl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Ph Eur

Dopamine Hydrochloride

General Notices

(Ph Eur monograph 0664)



$C_8H_{11}NO_2,HCl$ 189.6   *62-31-7*

Action and use

Dopamine receptor antagonist; $beta_1$-adrenoceptor agonist; alpha-adrenoceptor agonist.

Preparation

Dopamine Intravenous Infusion

Ph Eur

DEFINITION

4-(2-Aminoethyl)benzene-1,2-diol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent), sparingly soluble in acetone and in methylene chloride.

IDENTIFICATION

First identification   B, E.

Second identification   A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 40.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*.

Spectral range 230-350 nm.

Absorption maximum At 280 nm.

Specific absorbance at the absorption maximum 136 to 150.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison dopamine hydrochloride CRS.

C. Dissolve about 5 mg in a mixture of 5 ml of *1 M hydrochloric acid* and 5 ml of *water R*. Add 0.1 ml of *sodium nitrite solution R* containing 100 g/l of *ammonium molybdate R*. A yellow colour develops which becomes red on the addition of *strong sodium hydroxide solution R*.

D. Dissolve about 2 mg in 2 ml of *water R* and add 0.2 ml of *ferric chloride solution R2*. A green colour develops which changes to bluish-violet on the addition of 0.1 g of *hexamethylenetetramine R*.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 or Y_6 *(2.2.2, Method II)*.

Dissolve 0.4 g in *water R* and dilute to 10 ml with the same solvent.

Acidity or alkalinity

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. Add 0.1 ml of *methyl red solution R* and 0.75 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 1.5 ml of *0.01 M hydrochloric acid*. The solution is red.

Related substances

Liquid chromatography *(2.2.29)*. *Protect the solutions from light.*

Buffer solution Dissolve 21 g of *citric acid R* in 200 ml of *1 M sodium hydroxide* and dilute to 1000 ml with *water R*. To 600 ml of this solution add 400 ml of *0.1 M hydrochloric acid*.

Test solution Dissolve 50 mg of the substance to be examined in mobile phase A and dilute to 25 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 10 mg of *3-O-methyldopamine hydrochloride R* (impurity B) and 10 mg of *4-O-methyldopamine hydrochloride R* (impurity A) in mobile phase A and dilute to 100 ml with mobile phase A. Dilute 6 ml of this solution to 25 ml with mobile phase A.

Column:

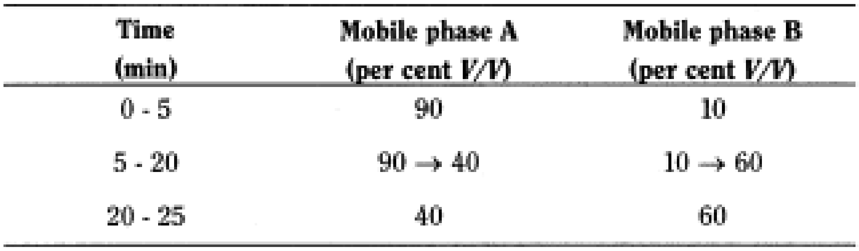
— *size:* l = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *spherical end-capped octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:

— *mobile phase A*: dissolve 1.08 g of *sodium octanesulphonate R* in 880 ml of the buffer solution and add 50 ml of *methanol R* and 70 ml of *acetonitrile R*;

— *mobile phase B*: dissolve 1.08 g of *sodium octanesulphonate R* in 700 ml of the buffer solution and add 100 ml of *methanol R* and 200 ml of *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 20	90 → 40	10 → 60
20 - 25	40	60

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µl.

Retention time Dopamine = about 5 min.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to impurities B and A.

Limits:

— *unspecificied impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached.

Dissolve 0.150 g in 10 ml of *anhydrous formic acid R*. Add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 18.96 mg of $C_8H_{12}ClNO_2$.

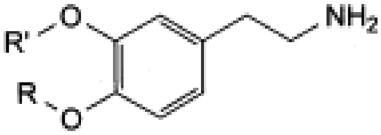
STORAGE

In an airtight container, under nitrogen, protected from light.

IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*

A, B, C.



A. R = CH_3, R′ = H: 5-(2-aminoethyl)-2-methoxyphenol (4-*O*-methyldopamine),

B. R = H, R′ = CH_3: 4-(2-aminoethyl)-2-methoxyphenol (3-*O*-methyldopamine),

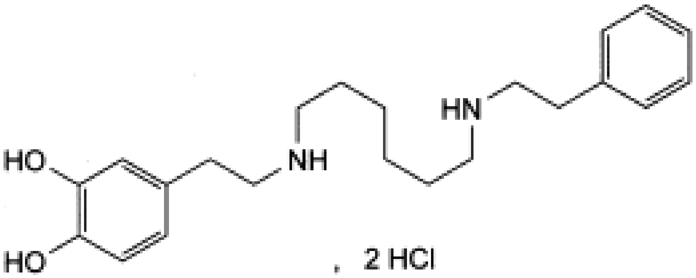
C. R = R′ = CH_3: 2-(3,4-dimethoxyphenyl)ethanamine.

Ph Eur

Dopexamine Hydrochloride

General Notices

(Dopexamine Dihydrochloride, Ph Eur monograph 1748)



$C_{22}H_{32}N_2O_2,2HCl$ 429.4 *86484-91-5*

Ph Eur

DEFINITION

4-[2-[[6-[(2-Phenylethyl)amino]hexyl]amino]ethyl]benzene1,2-diol dihydrochloride.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, sparingly soluble in ethanol (96 per cent) and in methanol, practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison dopexamine dihydrochloride CRS.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 0.10 g in *0.1 M hydrochloric acid* and dilute to 10 ml with the same acid.

pH *(2.2.3)*

3.7 to 5.7.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A.

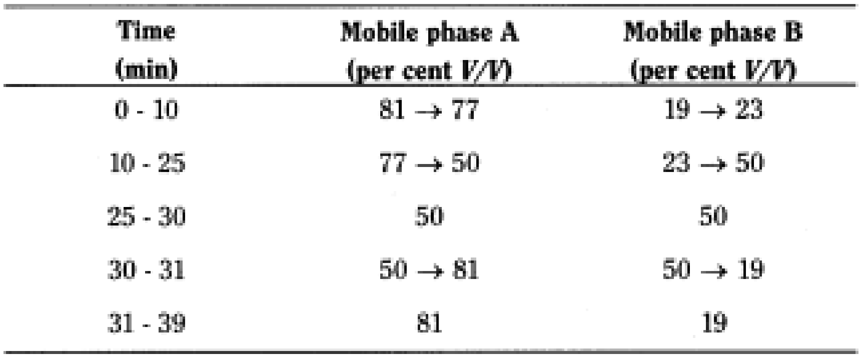
Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *dopexamine impurity B CRS* in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (c) Dissolve 5 mg of *dopexamine impurity F CRS* in mobile phase A and dilute to 100 ml with mobile phase A.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: mix 5 volumes of *buffer solution pH 2.5 R* and 95 volumes of *water R*;
- *mobile phase B*: mix 5 volumes of *buffer solution pH 2.5 R* and 95 volumes of a 60 per cent *V/V* solution of *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	81 → 77	19 → 23
10 - 25	77 → 50	23 → 50
25 - 30	50	50
30 - 31	50 → 81	50 → 19
31 - 39	81	19

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Preconditioning of the column Rinse for 5 min with a mixture of 19 volumes of mobile phase B and 81 volumes of mobile phase A.

Injection 20 µl.

Relative retention With reference to dopexamine (retention time = about 5 min): impurity A = about 0.5; impurity B = about 2.0; impurity C = about 2.3; impurity D = about 2.8; impurity E = about 2.9; impurity F = about 3.0; impurity I = about 3.6; impurity J = about 5.0; impurity K = about 5.9.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to dopexamine and impurity B.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.4; impurity F = 0.7;

— *impurities A, B, C, D, E, F, I, K*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity J

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Detection Spectrophotometer at 210 nm.

Limit:

— *impurity J*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals *(2.4.8*

Maximum 10 ppm.

Dissolve 0.50 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (0.25 ppm Pb) R*. For the evaluation of the results, filter the solutions through a membrane filter (0.45 µm).

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/mg.

ASSAY

Carry out the titration immediately after preparation of the test solution. In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.150 g in 10 ml of *anhydrous formic acid R*. Add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 21.47 mg of $C_{22}H_{34}Cl_2N_2O_2$.

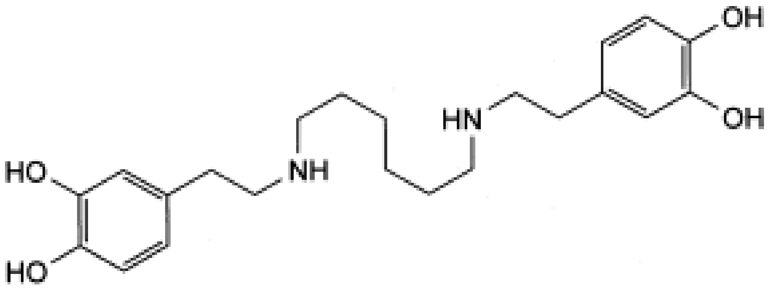
STORAGE

Protected from light.

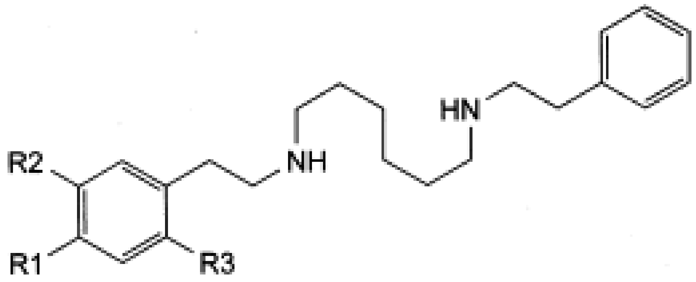
IMPURITIES

Specified impurities A, B, C, D, E, F, I, J, K.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *G, H*.



A. 4,4′-[hexane-1,6-diylbis(iminoethylene)]dibenzene-1,2-diol,



B. R1 = OH, R2 = OCH_3, R3 = H: 2-methoxy-4-[2-[[6-[(2-phenylethyl)amino]hexyl]amino]ethyl]phenol,

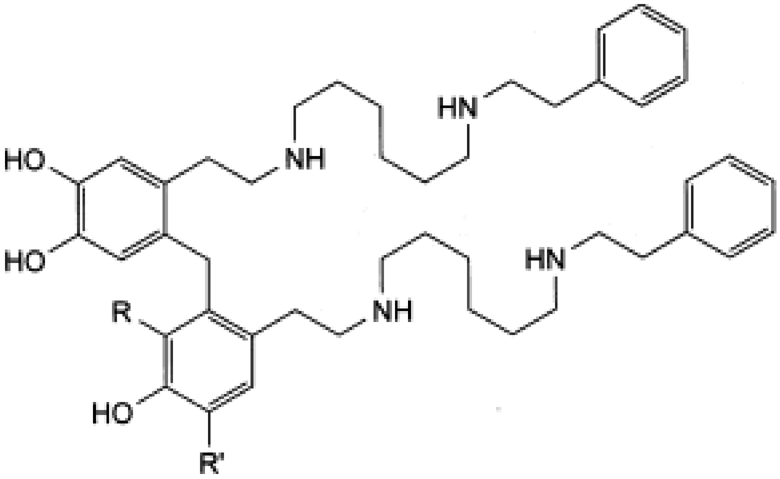
C. R1 = OCH_3, R2 = OH, R3 = H: 2-methoxy-5-[2-[[6-[(2-phenylethyl)amino]hexyl]amino]ethyl]phenol,

F. R1 = R2 = OH, R3 = Cl: 4-chloro-5-[2-[[6-[(2-phenylethyl)amino]hexyl]amino]ethyl]benzene-1,2-diol,

H. R1 = R2 = OCH_3, R3 = H: *N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*′-(2-phenylethyl)hexane-1,6-

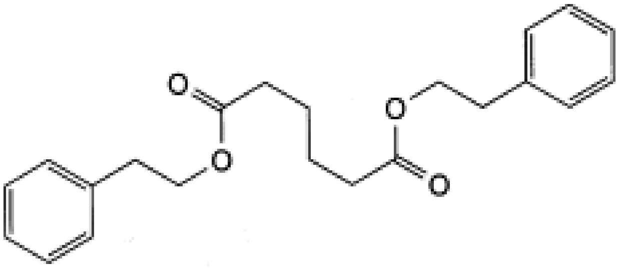
diamine,

J. R1 = R2 = R3 = H: *N,N′*-bis(2-phenylethyl)hexane-1,6-diamine,

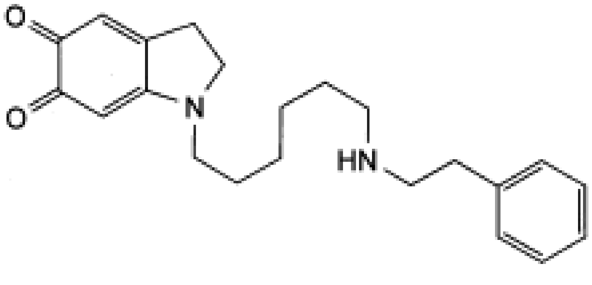


D. R = H, R′ = OH: 4,4′-methylenebis[5-[2-[[6-[(2-phenylethyl)amino]hexyl]amino]ethyl] benzene-1,2-diol],

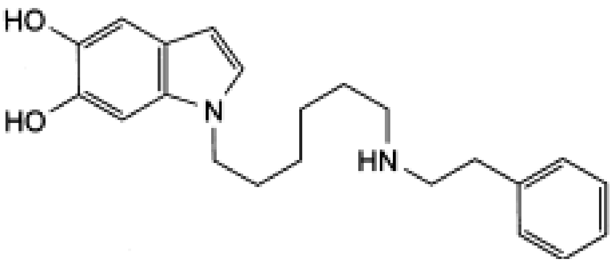
E. R = OH, R′ = H: 3-[4,5-dihydroxy-2-[2-[[6-[(2-phenylethyl)amino]hexyl]amino]ethyl]benzyl]-4-[2-[[6-[(2-phenylethyl)amino]hexyl]amino]ethyl]benzene-1,2-diol,



G. bis(2-phenylethyl) hexanedioate,



I. 1-[6-[(2-phenylethyl)amino]hexyl]-2,3-dihydro-1*H*-indole5,6-dione (dopexamine aminochrome),



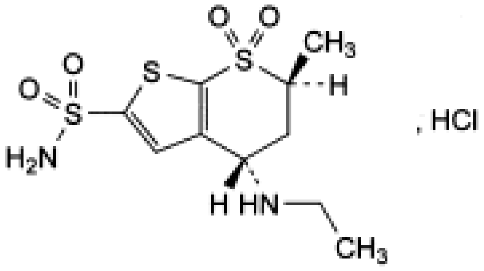
K. 1-[6-[(2-phenylethyl)amino]hexyl]-1*H*-indole-5,6-diol.

Ph Eur

Dorzolamide Hydrochloride

General Notices

(Ph Eur monograph 2359)



$C_{10}H_{16}N_2O_4S_3$,HCl 360.9 *130693-82-2*

Action and use

Carbonic anhydrase inhibitor; treatment of glaucoma and ocular hypertension.

Ph Eur

DEFINITION

(4*S*,6*S*)-4-(Ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulphonamide 7,7-dioxide hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, slightly soluble in methanol, very slightly soluble in anhydrous ethanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison dorzolamide hydrochloride CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. It complies with the test for impurity A (see Tests).

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Impurity A

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile R, glacial acetic acid R, 1,1-dimethylethyl methyl ether R* (3:10:87 *V/V/V*).

Test solution In a centrifuge tube, dissolve 20.0 mg of the substance to be examined in 4 ml of *dilute ammonia R4*, add 4 ml of *ethyl acetate R*, and mix. Separate the organic layer and transfer it to a separate centrifuge tube. Add 4 ml of *ethyl acetate R* to the aqueous layer, mix, separate the organic layer, and combine it with the 1st extract. Evaporate the combined organic layers to dryness in a water-bath at 50 °C under a stream of *nitrogen R*. Dissolve the residue in 3 ml of *acetonitrile R*, add 0.06 ml of *(S)-(-)-α-methylbenzyl isocyanate R*, and heat in a water-bath at 50 °C for 5 min. Evaporate to dryness in a water-bath at 50 °C under a stream of *nitrogen R*. Dissolve the residue in 10 ml of the solvent mixture.

Reference solution In a centrifuge tube, dissolve 18.0 mg of *dorzolamide hydrochloride CRS* and 2.0 mg of *dorzolamide impurity A CRS* in 4 ml of *dilute ammonia R4*, and proceed as indicated for the test solution beginning with "add 4 ml of *ethyl acetate R*, and mix".

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase *water R, acetonitrile R, heptane R, 1,1-dimethylethyl methyl ether R* (0.2:2:35:63 *V/V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 3 times the retention time of dorzolamide.

Relative retention With reference to dorzolamide (retention time = about 10 min): impurity A = about 1.4.

System suitability Reference solution:

— *resolution*: minimum 4.0 between the peaks due to dorzolamide and impurity A.

Calculate the percentage content of impurity A using the following expression:

$$\frac{A}{A+B} \times 100$$

A = area of the peak due to impurity A in the chromatogram obtained with the test solution;

B = area of the peak due to dorzolamide in the chromatogram obtained with the test solution.

Limit:

— *impurity A*: maximum 0.5 per cent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 30.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a) Dissolve 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 2 mg of *dorzolamide for system suitability CRS* (containing impurity C) in 2 ml of mobile phase A.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

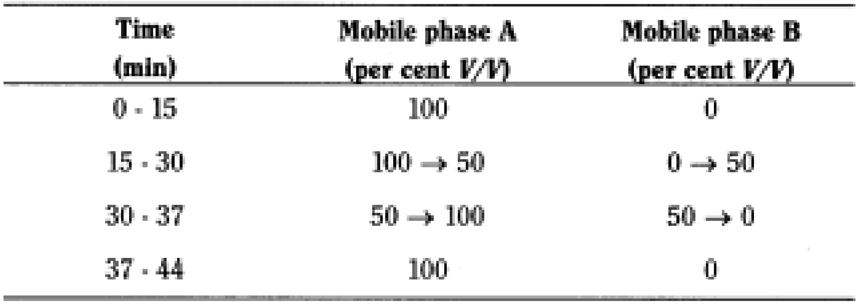
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 35 °C.

Mobile phase:

— mobile phase A: mix 65 ml of *acetonitrile R* and 935 ml of a 3.7 g/l solution of *potassium dihydrogen phosphate R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 30	100 → 50	0 → 50
30 - 37	50 → 100	50 → 0
37 - 44	100	0

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *dorzolamide for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention With reference to dorzolamide (retention time = about 11 min): Impurity C

= about 0.9.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity C and dorzolamide.

Limits:

— *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

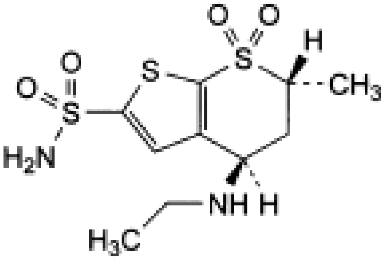
Dissolve 0.150 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*, using sonication if necessary. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 1st and the 3rd points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 18.05 mg of $C_{10}H_{17}N_2O_4S_3Cl$.

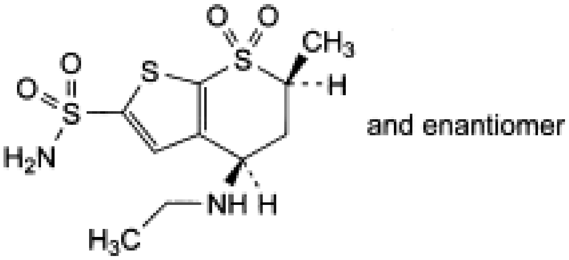
IMPURITIES

Specified impurities *A, C.*

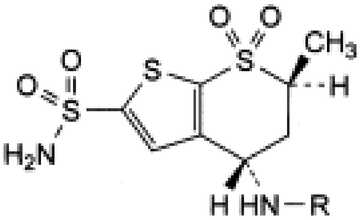
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *B, D*.



A. (4*R*,6*R*)-4-(ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulphonamide 7,7-dioxide,



B. (4*RS*,6*SR*)-4-(ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulphonamide 7,7-dioxide,



C. R = CH_2-CH_2-$B(OH)_2$: [2-[[(4*S*,6*S*)-6-methyl-7,7-dioxo-2-sulphamoyl-4,5,6,7-tetrahydro-7λ^6-thieno[2,3-*b*]thiopyran-4-yl]amino]ethyl]boronic acid,

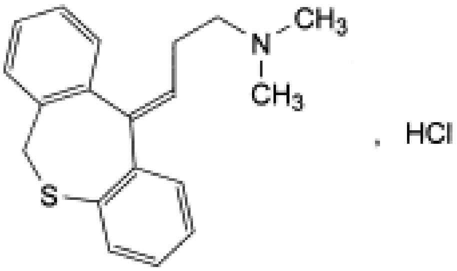
D. R = H: (4*S*,6*S*)-4-amino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulphonamide 7,7-dioxide.

Ph Eur

Dosulepin Hydrochloride

General Notices

(Ph Eur monograph 1314)



$C_{19}H_{21}NS,HCl$ *331.9* *1897-15-4*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparations

Dosulepin Capsules

Dosulepin Tablets

Ph Eur

DEFINITION

(*E*)-3-(Dibenzo[*b,e*]thiepin-11(6*H*)-ylidene)-*N,N*-dimethylpropan-1-amine hydrochloride.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or faintly yellow, crystalline powder.

Solubility

Freely soluble in water, in alcohol and in methylene chloride.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 25.0 mg in a 1 g/l solution of *hydrochloric acid R* in *methanol R* and dilute to 100.0 ml with the same solution. Dilute 2.0 ml to 50.0 ml with a 1 g/l solution of *hydrochloric acid R* in *methanol R*. Examined between 220 nm and 350 nm *(2.2.25)*, the solution shows 2 absorption maxima at 231 nm and 306 nm and a shoulder at about 260 nm. The specific absorbance at the maximum at 231 nm is 660 to 730.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *dosulepin hydrochloride CRS.*

C. Dissolve about 1 mg in 5 ml of *sulphuric acid R*. A dark red colour is produced.

D. It gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II)*.

Dissolve 1 g in *water R* and dilute to 20 ml with the same solvent.

pH *(2.2.3)*

4.2 to 5.2.

Dissolve 1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Impurity E and related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in 5 ml of *methanol R* and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 12.5 mg of *dosulepin impurity A CRS* in 5 ml of *methanol R* and dilute to 50.0 ml with the mobile phase. Dilute 0.5 ml to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *dosulepin hydrochloride CRS* in 5 ml of *methanol R* and dilute to 20.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *nitrile silica gel for chromatography R1* (5 µm);
- *temperature*: 35 °C.

Mobile phase 0.83 per cent *V/V* solution of *perchloric acid R, propanol R, methanol R, water R* (1:10:30:60 *V/V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 229 nm.

Injection 5 µl.

Run time 2.5 times the retention time of dosulepin (*E*-isomer).

Relative retention With reference to dosulepin (*E*-isomer; retention time = about 25 min): impurity E = about 0.9.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 4, where H_r = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to dosulepin (E-isomer).

Limits:

— *impurity E*: not more than 5 per cent of the sum of the areas of the peak due to impurity E and the principal peak in the chromatogram obtained with the test solution;

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *any other impurity*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total of other impurities and impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

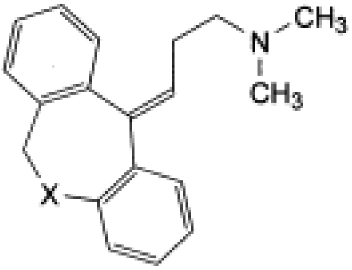
Dissolve 0.250 g in a mixture of 5 ml of *anhydrous acetic acid R* and 35 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 33.19 mg of $C_{19}H_{22}ClNS$.

STORAGE

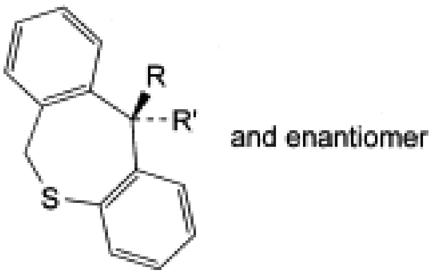
Protected from light.

IMPURITIES



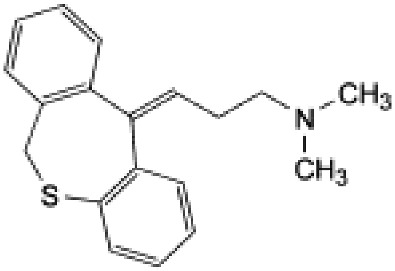
A. X = SO: (*E*)-3-(5-oxo-5λ^4-dibenzo[*b,e*]thiepin-11(6*H*)-ylidene)-*N,N*-dimethylpropan-1-amine,

D. X = SO_2: (*E*)-3-(5,5-dioxo-5λ^6-dibenzo[*b,e*]thiepin-11(6*H*)-ylidene)-*N,N*-dimethylpropan-1-amine,


R
R'
and enantiomer
S

B. R + R′ = O: dibenzo[*b,e*]thiepin-11(6*H*)-one,

C. R = OH, R′ = $[CH_2]_3$-$N(CH_3)_2$: (11*RS*)-11-[3-(dimethylamino)propyl]-6,11-dihydrodibenzo [*b,e*]thiepin-11-ol,



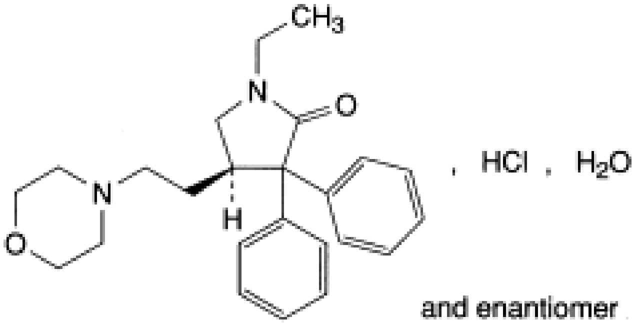
E. (*Z*)-3-(dibenzo[*b,e*]thiepin-11(6*H*)-ylidene)-*N,N*-dimethylpropan-1-amine.

Ph Eur

Doxapram Hydrochloride

General Notices

(Ph Eur monograph 1201)



$C_{24}H_{30}N_2O_2,HCl,H_2O$ *433.0* *7081-53-0*

Action and use

Respiratory stimulant.

Preparation

Doxapram Injection

Ph Eur

DEFINITION

(4*RS*)-1-Ethyl-4-[2-(morpholin-4-yl)ethyl]-3,3-diphenylpyrrolidin-2-one hydrochloride.

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, in alcohol and in methylene chloride.

IDENTIFICATION

First identification *A, C.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *doxapram hydrochloride CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *doxapram hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase Solution of *ammonia R* containing 17 g/l of NH_3, 2-*propanol R, 2-methylpropanol R* (10:10:80 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *dilute potassium iodobismuthate solution R* and examine immediately.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.500 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dilute 10 ml of solution S to 25 ml with *water R*.

pH *(2.2.3)*

3.5 to 5.0.

Dilute 5 ml of solution S to 25 ml with *carbon dioxide-free water R*.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 5.0 ml with the mobile phase.

Reference solution (c) Dissolve 5 mg of *doxapram impurity B CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase. To 1.0 ml of the solution, add 1.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a carbon loading of 14 per cent, a specific surface area of 350 m_2/g and a pore size of 10 nm.

Mobile phase Mix 50 volumes of *acetonitrile R* and 50 volumes of a 0.82 g/l solution of *sodium acetate R* adjusted to pH 4.5 with *glacial acetic acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl.

Run time 4 times the retention time of doxapram.

Retention time Doxapram = about 6 min.

System suitability Reference solution (c):
- *resolution*: minimum of 3.0 between the peaks corresponding to doxapram and to impurity B.

Limits:
- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *methanol R*.

Loss on drying *(2.2.32)*

3.0 per cent to 4.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

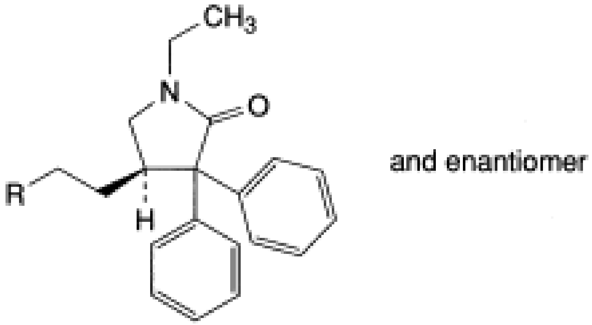
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 10 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)* using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 41.50 mg of $C_{24}H_{31}ClN_2O_2$.

IMPURITIES



A. R = Cl: (4*RS*)-4-(2-chloroethyl)-1-ethyl-3,3-diphenylpyrrolidin-2-one,

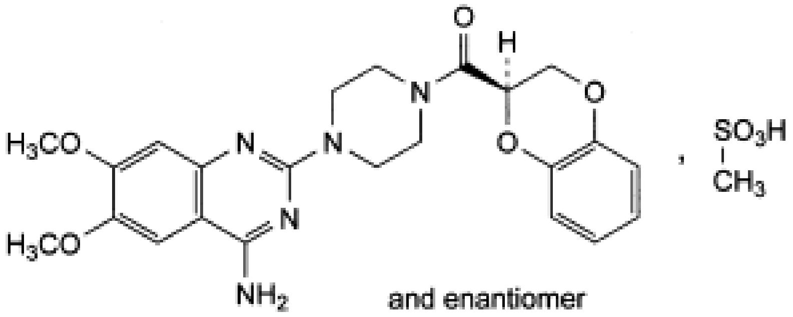
B. R = NH-CH_2-CH_2-OH: (4*RS*)-1-ethyl-4-[2-[(2-hydroxyethyl)amino]ethyl]-3,3-diphenylpyrrolidin-2-one.

Ph Eur

Doxazosin Mesilate

General Notices

(Ph Eur monograph 2125)



$C_{23}H_{25}N_5O_5,CH_3SO_3H$ 547.6 *77883-43-3*

Action and use

Alpha$_1$-adrenoceptor antagonist.

Ph Eur

DEFINITION

1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-4-[(2*RS*)-2,3-dihydro-1,4-benzodioxin-2-ylcarbonyl] piperazine methanesulphonate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Slightly soluble in water, soluble in a mixture of 15 volumes of water and 35 volumes of tetrahydrofuran, slightly soluble in methanol, practically insoluble in acetone.

It shows polymorphism *(5.9)*, some forms may be hygroscopic.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison doxazosin mesilate CRS.

If the spectra obtained in the solid state show differences, mix 1 part of the substance to be examined and 1 part of the reference substance separately with 10 parts of *anhydrous ethanol R* and heat to boiling. Continue heating the suspension under a reflux condenser for about 3 h. Cool and filter. Record new spectra using the previously dried residues on the filters.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Dissolve 1.0 g in a mixture of 15 ml of *water R* and 35 ml of *tetrahydrofuran R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in 5 ml of mobile phase B, adding *water R*, and dilute to 50.0 ml with *water R*.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with *water R*. Dilute 2.0 ml of this solution to 100.0 ml with *water R*.

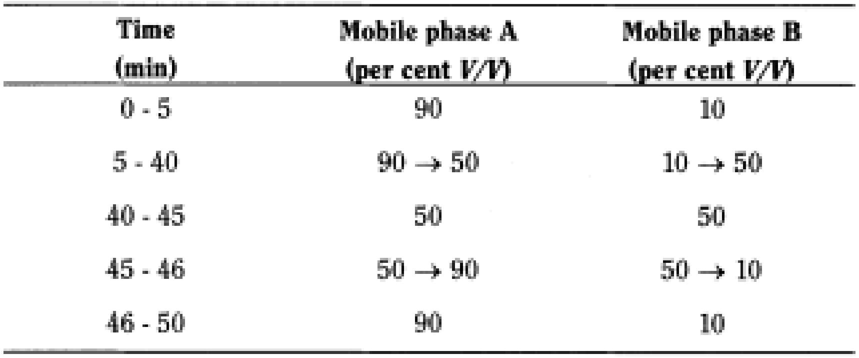
Reference solution (b) Dissolve 5 mg of *doxazosin impurity D CRS* and 5 mg of *doxazosin impurity F CRS* in 5 ml of mobile phase B, adding *water R*, and dilute to 50.0 ml with *water R*. Dilute 10.0 ml of this solution to 50.0 ml with *water R*.

Reference solution (c) Dilute 5.0 ml of reference solution (a) to 10.0 ml with *water R*.

Reference solution (d) Dissolve 25.0 mg of *doxazosin mesilate CRS* in 5 ml of mobile phase B, adding *water R*, and dilute to 50.0 ml with *water R*.

Column:
- *size:* l = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: 10 g/l solution of *phosphoric acid R*;
- *mobile phase B*: 10 g/l solution of *phosphoric acid R* in *acetonitrile R1*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 5	90	10
5 - 40	90 → 50	10 → 50
40 - 45	50	50
45 - 46	50 → 90	50 → 10
46 - 50	90	10

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl of the test solution and reference solutions (a), (b) and (c).

Relative retention With reference to doxazosin (retention time = about 30 min): impurity D = about 0.5; impurity F = about 0.6.

System suitability Reference solution (b):

— *resolution*: minimum 4.5 between the peaks due to impurities D and F.

Limits:

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water *(2.5.12)*

Maximum 1.5 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (d).

Calculate the percentage content of $C_{24}H_{29}N_5O_8S$ using the chromatogram obtained with reference solution (d) and the declared content of *doxazosin mesilate CRS*.

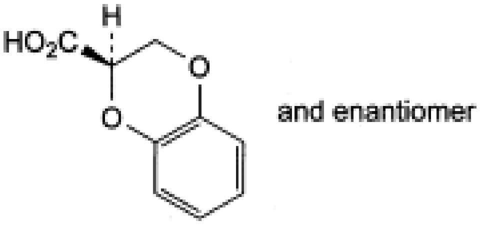
STORAGE

In an airtight container .

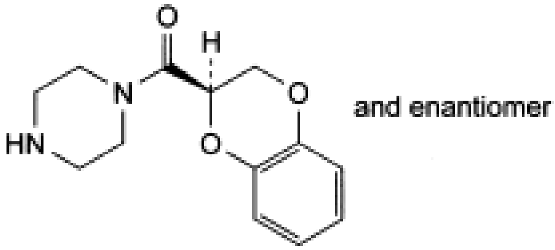
IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*

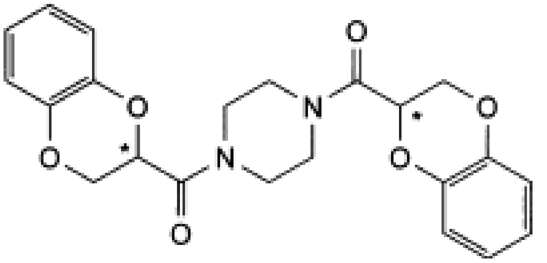
A, B, C, D, E, F, G, H.



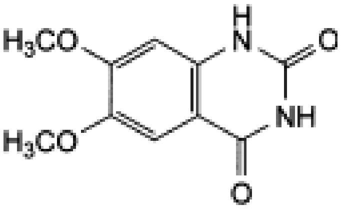
A. (2*RS*)-2,3-dihydro-1,4-benzodioxine-2-carboxylic acid,



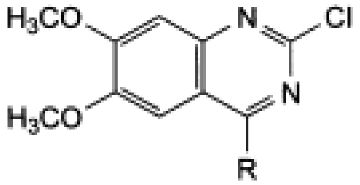
B. 1-[(2*RS*)-2,3-dihydro-1,4-benzodioxin-2-ylcarbonyl]piperazine,



C. 1,4-bis(2,3-dihydro-1,4-benzodioxin-2-ylcarbonyl)piperazine,

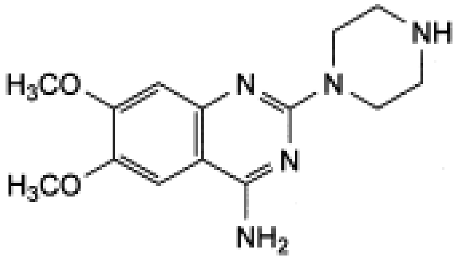


D. 6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione,

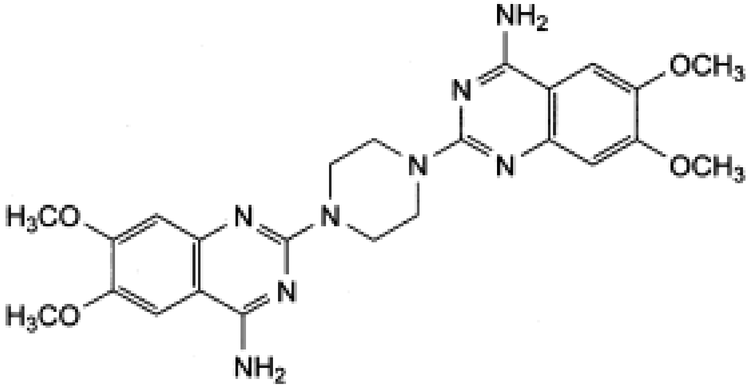


E. R = Cl: 2,4-dichloro-6,7-dimethoxyquinazoline,

F. R = NH_2: 2-chloro-6,7-dimethoxyquinazolin-4-amine,



G. 6,7-dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine,



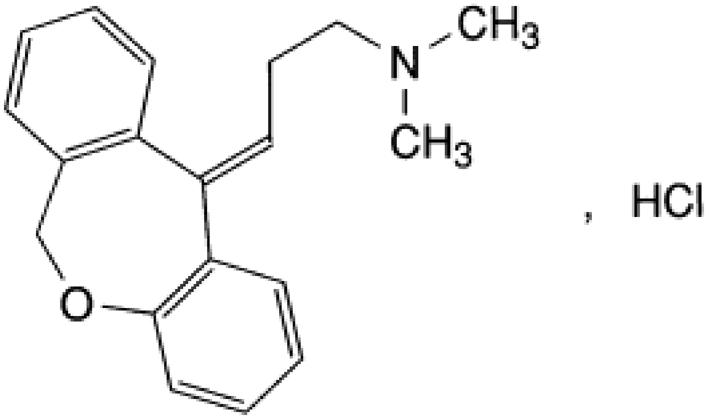
H. 2,2′-(piperazine-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine).

Ph Eur

Doxepin Hydrochloride

General Notices

(Ph Eur monograph 1096)



$C_{19}H_{21}NO,HCl$ 315.8 *1229-29-4*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Doxepin Capsules

Ph Eur

DEFINITION

(*E*)-3-(Dibenzo[*b,e*]oxepin-11(6*H*)-ylidene)-*N*,*N*-dimethylpropan-1-amine hydrochloride.

Content

98.0 per cent to 101.0 per cent of $C_{19}H_{22}ClNO$ (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *C, E.*

Second identification *A, B, D, E.*

A. Melting point *(2.2.14)*: 185 °C to 191 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in a 1 g/l solution of *hydrochloric acid R* in *methanol R* and dilute to 100.0 ml with the same acid solution. Dilute 5.0 ml to 50.0 ml with a 1 g/l solution of *hydrochloric acid R* in *methanol R*.

Spectral range 230-350 nm.

Absorption maximum At 297 nm.

Specific absorbance at the absorption maximum 128 to 142.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *doxepin hydrochloride CRS.*

D. Dissolve about 5 mg in 2 ml of *sulphuric acid R*. A dark red colour is produced.

E. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.5 g in *carbon dioxide-free water R* and dilute to 30 ml with the same solvent.

Appearance of solution

Dilute 10 ml of solution S to 25 ml with *water R*. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity

To 10 ml of solution S add 0.1 ml of *methyl red solution R*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect them from light.*

Phosphate buffer solution Dissolve 1.42 g of *anhydrous disodium hydrogen phosphate R* in *water R*, adjust to pH 7.7 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R.*

Solvent mixture Mix 1 volume of *1 M sodium hydroxide* and 250 volumes of the mobile phase.

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *doxepin for system suitability CRS* (containing impurities A, B and C) in 1.0 ml of mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R1*, phosphate buffer solution, *methanol R1* (20:30:50 *V/V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 1.5 times the retention time of doxepin.

Identification of impurities Use the chromatogram supplied with *doxepin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to doxepin (retention time = about 18 min): impurity A = about 0.5; impurity C = about 0.6; impurity B = about 0.7; the peak due to doxepin might show a shoulder caused by the (*Z*)-isomer (impurity D).

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurities A and C, and minimum 1.5 between the peaks due to impurities C and B;

— the chromatogram obtained is similar to the chromatogram supplied with *doxepin for system suitability CRS.*

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 1.7;

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

(*Z*)-Isomer

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
— *size: l* = 0.12 m, Ø = 4 mm;

— *stationary phase*: spherical *octylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 220 m^2/g and a pore size of 80 nm;

— *temperature*: 50 °C.

Mobile phase Mix 30 volumes of *methanol R* and 70 volumes of a 30 g/l solution of *sodium dihydrogen phosphate R* previously adjusted to pH 2.5 with *phosphoric acid R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

System suitability:
— *resolution*: minimum 1.5 between the peaks due to the (*E*)-isomer (1st peak) and to the (*Z*)-isomer (2nd peak).

Results:
— calculate the ratio of the area of the peak due to the *(E)*-isomer to the area of the peak due to the *(Z)*-isomer: this ratio is 4.4 to 6.7 (13.0 per cent to 18.5 per cent of the (*Z*)-isomer) .

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5 ml of *anhydrous acetic acid R* and 35 ml of *acetic anhydride R*. Using 0.2 ml of *crystal violet solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from blue to green.

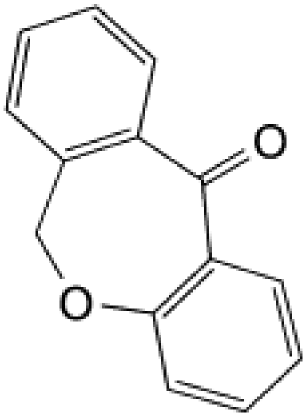
1 ml of *0.1 M perchloric acid* is equivalent to 31.58 mg of $C_{19}H_{22}ClNO$.

STORAGE

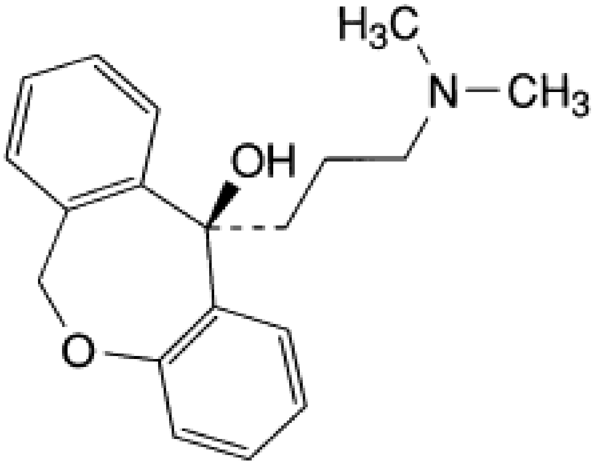
Protected from light.

IMPURITIES

Specified impurities A, B, C, D.

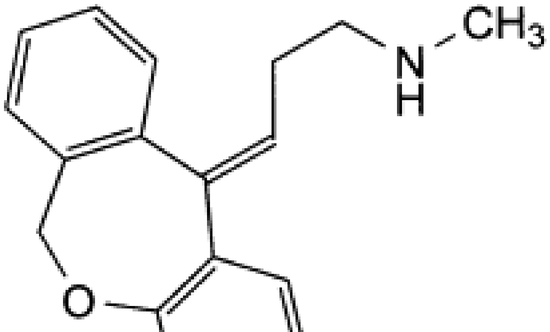


A. dibenzo[*b*,*e*]oxepin-11(6*H*)-one (doxepinone),

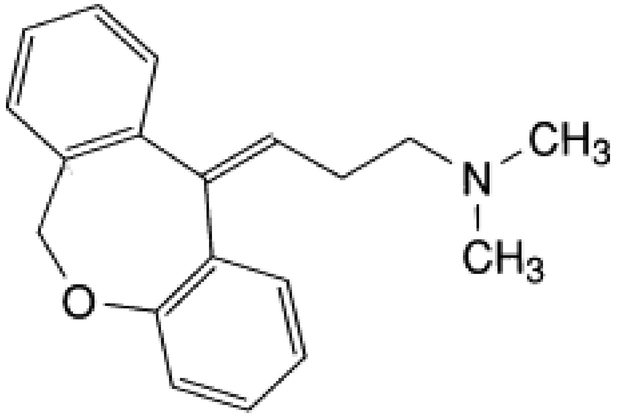


and enantiomer

B. (11*RS*)-11-[3-(dimethylamino)propyl]-6,11-dihydrodibenzo[*b*,*e*]oxepin-11-ol (doxepinol),



C. (*E*)-3-(dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)-*N*-methylpropan-1-amine (desmethyldoxepin),



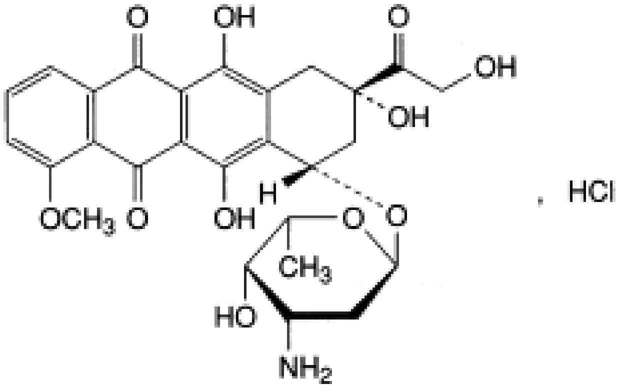
D. (*Z*)-3-(dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)-*N*,*N*-dimethylpropan-1-amine.

Ph Eur

Doxorubicin Hydrochloride

General Notices

(Ph Eur monograph 0714)



$C_{27}H_{29}NO_{11},HCl$ 580.0 *25316-40-9*

Action and use

Anthracycline antibiotic; cytotoxic.

Preparation

Doxorubicin Injection

Ph Eur

DEFINITION

(8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride.

Substance produced by certain strains of *Streptomyces coeruleorubidus* or *Streptomyces peucetius* or obtained by any other means.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Orange-red, crystalline powder, hygroscopic.

SOLUBILITY

Soluble in water, slightly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *doxorubicin hydrochloride CRS.*

B. Dissolve about 10 mg in 0.5 ml of *nitric acid R*, add 0.5 ml of *water R* and heat over a flame for 2 min. Allow to cool and add 0.5 ml of *silver nitrate solution R1*. A white precipitate is formed.

TESTS

pH *(2.2.3)*

4.0 to 5.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b) Dilute 10.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *doxorubicin hydrochloride CRS* and 10 mg of *epirubicin hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *doxorubicin hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.0 mm,

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix equal volumes of *acetonitrile R* and a solution containing 2.88 g/l of *sodium laurilsulfate R* and 2.25 g/l of *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 5 µl; inject test solution (a) and reference solutions (a) and (b).

Run time 3.5 times the retention time of doxorubicin.

Retention time Doxorubicin = about 8 min.

System suitability Reference solution (a):

— *resolution*: minimum of 2.0 between the peaks due to doxorubicin and to epirubicin.

Limits:

— *any impurity*: not more than the area of the peak corresponding to doxorubicin in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *disregard limit*: 0.1 times the area of the peak corresponding to doxorubicin in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol *(2.4.24, System B)*

Maximum 1.0 per cent.

Water *(2.5.12)*

Maximum 4.0 per cent, determined on 0.100 g.

Bacterial endotoxins *(2.6.14)*

Less than 2.2 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

Injection Test solution (b) and reference solution (c).

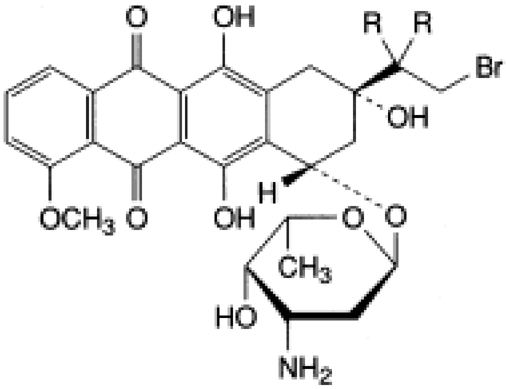
Calculate the percentage content of $C_{27}H_{30}ClNO_{11}$.

STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

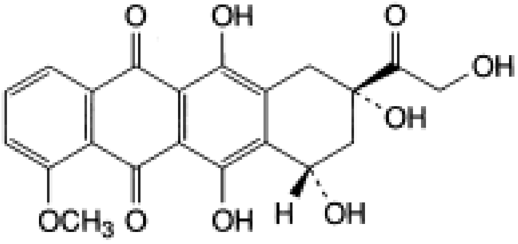
IMPURITIES

A. daunorubicin,



B. R = OCH_3: (8*S*,10*S*)-10[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-(2-bromo-1,1-dimethoxyethyl)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione,

C. R + R = O: (8*S*,10*S*)-10[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-(bromoacetyl)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione,



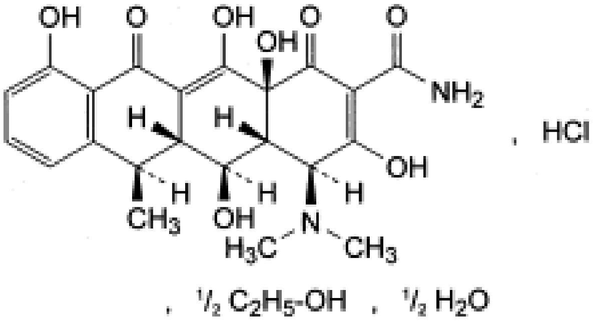
D. (8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (doxorubicin aglycone, doxorubicinone).

Ph Eur

Doxycycline Hyclate

General Notices

(Ph Eur monograph 0272)



$C_{22}H_{24}N_2O_8,HC1,\frac{1}{2}C_2H_6O,\frac{1}{2}H_2O$ 512.9 *24390-14-5*

Action and use

Tetracycline antibacterial.

Preparation

Doxycycline Capsules

Ph Eur

DEFINITION

Hydrochloride hemiethanol hemihydrate of (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

Substance obtained from oxytetracycline or metacycline or by any other means.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent of $C_{22}H_{25}ClN_2O_8$ (anhydrous substance).

CHARACTERS

Appearance

Yellow, crystalline powder, hygroscopic.

Solubility

Freely soluble in water and in methanol, sparingly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A yellow colour develops.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

2.0 to 3.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 105 to - 120 (anhydrous substance).

Dissolve 0.250 g in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 25.0 ml with the same mixture of solvents. Carry out the measurement within 5 min of preparing the solution.

Specific absorbance *(2.2.25)*

300 to 335, determined at the absorption maximum at 349 nm (anhydrous substance).

Dissolve 25.0 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 25.0 ml with the same mixture of solvents. Dilute 1.0 ml of the solution to 100.0 ml with a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R*. Carry out the measurement within 1 h of preparing the solution.

Light-absorbing impurities

The absorbance *(2.2.25)*, determined at 490 nm is not greater than 0.07 (anhydrous and ethanol-free substance).

Dissolve 0.10 g in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 10.0 ml with the same mixture of solvents. Carry out the measurement within 1 h of preparing the solution.

Related substances

Liquid chromatography *(2.2.29). Prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 20.0 mg of *doxycycline hyclate CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 20.0 mg of *6-epidoxycycline hydrochloride CRS* in *0.01 M* *hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c) Dissolve 20.0 mg of *metacycline hydrochloride CRS* in 0*.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (d) Mix 4.0 ml of reference solution (a), 1.5 ml of reference solution (b) and 1.0 ml of reference solution (c) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (e) Mix 2.0 ml of reference solution (b) and 2.0 ml of reference solution (c) and dilute to 100.0 ml with *0.01 M hydrochloric acid*.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: styrene-divinylbenzene copolymer R (8 µm),

— *temperature*: 60 °C.

Mobile phase Weigh 60.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R*; add 400 ml of *buffer solution pH 8.0 R*, 50 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 ml with *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (d) and (e).

Relative retention With reference to doxycycline: impurity E = about 0.2; impurity D = about 0.3; impurity C = about 0.5; impurity F = about 1.2.

System suitability Reference solution (d):

— *resolution*: minimum 1.25 between the peaks due to impurity B (1st peak) and impurity A (2nd peak) and minimum 2.0 between the peaks due to impurity A and doxycycline (3rd peak) ; if necessary, adjust the 2-methyl-2-propanol content in the mobile phase;

— *symmetry factor*: maximum 1.25 for the peak due to doxycycline.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per cent),

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per cent),

— *impurities C, D, E, F*: for each impurity, not more than 0.25 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.5 per cent),

— *any other impurity*: for each impurity, not more than 0.25 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.5 per cent),

— *disregard limit*: 0.05 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.1 per cent).

Ethanol

Gas chromatography *(2.2.28)*.

Internal standard solution Dilute 0.50 ml of *propanol R* to 1000.0 ml with *water R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 0.10 g of the substance to be examined in the internal standard solution and dilute to 10.0 ml with the same solution.

Reference solution Dilute 0.50 ml of *ethanol R* to 100.0 ml with the internal standard solution. Dilute 1.0 ml of this solution to 10.0 ml with the internal standard solution.

Column:
- *size:* l = 1.5 m, Ø = 4.0 mm,
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (150-180 µm).

Carrier gas *nitrogen for chromatography R.*

Temperature:
- *column*: 135 °C,
- *injection port and detector*: 150 °C.

Detection Flame ionisation.

Calculate the content of ethanol taking the density *(2.2.5)* at 20 °C to be 0.790 g/ml.

Limit:
- *ethanol* : 4.3 per cent to 6.0 per cent.

Heavy metals *(2.4.8)*

Maximum 50 ppm.

0.5 g complies with limit test C. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

1.4 per cent to 2.8 per cent, determined on 1.20 g.

Sulphated ash *(2.4.14)*

Maximum 0.4 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 1.14 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

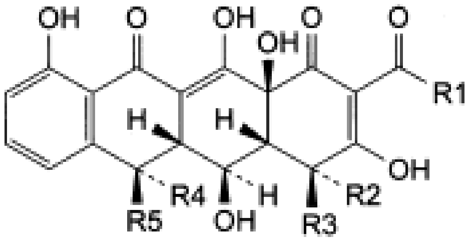
Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$ (M_r = 480.9).

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities A, B, C, D, E, F.



A. R1 = NH_2, R2 = R5 = H, R3 = $N(CH_3)_2$, R4 = CH_3: (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (6-epidoxycycline),

B. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$, R4 + R5 = CH_2: (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (metacycline),

C. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = R4 = H, R5 = CH_3: (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidoxycycline),

D. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = R5 = H, R4 = CH_3: (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epi-6-epidoxycycline),

E. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$, R4 = OH, R5 = CH_3: oxytetracycline,

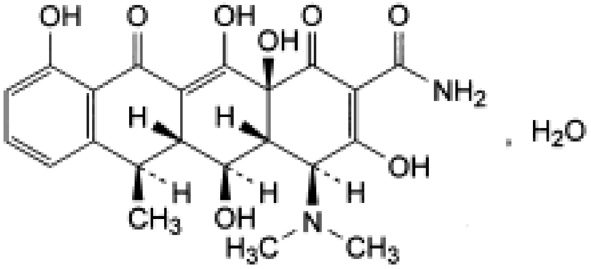
F. R1 = CH_3, R2 = R4 = H, R3 = $N(CH_3)_2$, R5 = CH_3: (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-2-acetyl-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyldoxycycline).

Ph Eur

Doxycycline Monohydrate

General Notices

(Ph Eur monograph 0820)



$C_{22}H_{24}N_2O_8,H_2O$ 462.5 *17086-28-1*

Action and use

Tetracycline antibacterial.

Preparation

Dispersible Doxycycline Tablets

Ph Eur

DEFINITION

(4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrate.

Substance obtained from oxytetracycline or metacycline or by any other means.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Very slightly soluble in water and in alcohol. It dissolves in dilute solutions of mineral acids and in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A yellow colour develops.

C. Dissolve 25 mg in a mixture of 0.2 ml of *dilute nitric acid R* and 1.8 ml of *water R*. The solution does not give reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

5.0 to 6.5.

Suspend 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 113 to - 130 (anhydrous substance).

Dissolve 0.250 g in a mixture of 0.5 volumes of *hydrochloric acid R* and 99.5 volumes of *methanol R* and dilute to 25.0 ml with the same mixture of solvents. Carry out the measurement within 5 min of preparing the solution.

Specific absorbance *(2.2.25)*

325 to 363 determined at the maximum at 349 nm (anhydrous substance).

Dissolve 25.0 mg in a mixture of 0.5 volumes of *hydrochloric acid R* and 99.5 volumes of *methanol R* and dilute to 50.0 ml with the same mixture of solvents. Dilute 2.0 ml of the solution to 100.0 ml with a mixture of 0.5 volumes of *1 M hydrochloric acid* and 99.5 volumes of *methanol R*. Carry out the measurement within 1 h of preparing the solution.

Light-absorbing impurities

The absorbance *(2.2.25)* determined at 490 nm has a maximum of 0.07 (anhydrous substance).

Dissolve 0.10 g in a mixture of 0.5 volumes of *hydrochloric acid R* and 99.5 volumes of *methanol R* and dilute to 10.0 ml with the same mixture of solvents. Carry out the measurement within 1 h of preparing the solution.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 20.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 20.0 mg of *doxycycline hyclate CRS* in 0*.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 20.0 mg of *6-epidoxycycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c) Dissolve 20.0 mg of *metacycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (d) Mix 4.0 ml of reference solution (a), 1.5 ml of reference solution (b) and 1.0 ml of reference solution (c) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (e) Mix 2.0 ml of reference solution (b) and 2.0 ml of reference solution (c) and dilute to 100.0 ml with *0.01 M hydrochloric acid*.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm),

— *temperature*: 60 °C.

Mobile phase Weigh 60.0 g of *2-methyl-2-propanol R* and transfer into a 1000 ml volumetric flask with the aid of 200 ml of *water R*; add 400 ml of *buffer solution pH 8.0 R*, 50 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 ml with *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl; inject the test solution and reference solutions (d) and (e).

Relative retention With reference to doxycycline: impurity E = about 0.2; impurity D = about 0.3; impurity C = about 0.5; impurity F = about 1.2.

System suitability Reference solution (d):

— *resolution*: minimum 1.25 between the peaks due to impurity B (1st peak) and impurity A (2nd peak) and minimum 2.0 between the peaks due to impurity A and doxycycline (3rd peak) ; if necessary, adjust the 2-methyl-2-propanol content in the mobile phase,

— *symmetry factor*: maximum 1.25 for the peak due to doxycycline.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per cent),

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per cent),

— *any other impurity*: not more than 0.25 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.5 per cent),

— *disregard limit*: 0.05 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.1 per cent).

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

0.5 g complies with limit test C. Prepare the standard using 2.5 ml of *lead standard solution (10 ppm Pb) R*.

Water ***(2.5.12)***

3.6 per cent to 4.6 per cent, determined on 0.200 g.

Sulphated ash *(2.4.14)*

Maximum 0.4 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

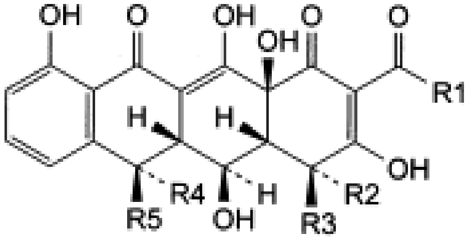
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{24}N_2O_8$.

STORAGE

Protected from light.

IMPURITIES



A. R1 = NH_2, R2 = R5 = H, R3 = $N(CH_3)_2$, R4 = CH_3: (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (6-epidoxycycline),

B. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$, R4 + R5 = CH_2: (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (metacycline),

C. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = R4 = H, R5 = CH_3: (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidoxycycline),

D. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = R5 = H, R4 = CH_3: (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epi-6-epidoxycycline),

E. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$, R4 = OH, R5 = CH_3: oxytetracycline,

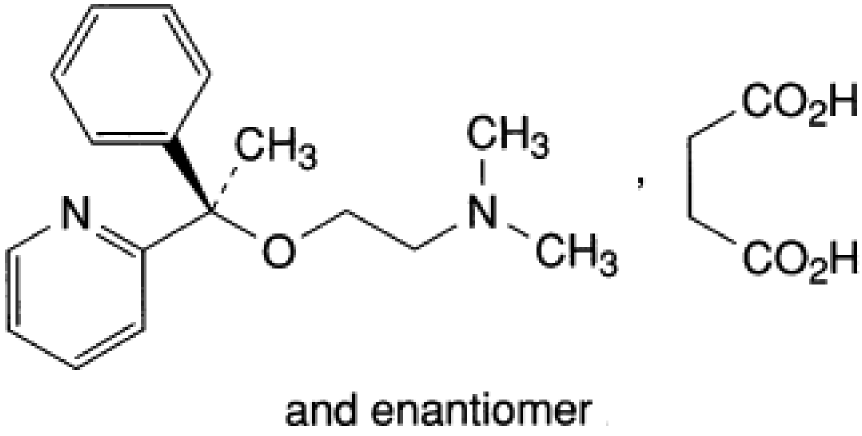
F. R1 = CH_3, R2 = R4 = H, R3 = $N(CH_3)_2$, R5 = CH_3: (4*S*,4a*R,*5*S*,5a*R*,6*R*,12a*S*)-2-acetyl-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1, 11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyldoxycycline).

Ph Eur

Doxylamine Succinate

General Notices

(Doxylamine Hydrogen Succinate, Ph Eur monograph 1589)



$C_{17}H_{22}N_2O,C_4H_6O_4$ 388.5 *562-10-7*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

N,*N*-dimethyl-2-[(1*RS*)-1-phenyl-1-(pyridin-2-yl)ethoxy(ethanamine hydrogen butanedioate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

A white or almost white powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *C.*

Second identification *A, B.*

A. Melting point (*2.2.14*): 103 °C to 108 °C.

B. Dissolve 0.200 g in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 262 nm. The specific absorbance at the maximum is 229 to 243 (anhydrous substance).

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of doxylamine hydrogen succinate.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.4 g of the substance to be examined in *water R* and dilute to 20 ml with the same solvent.

Optical rotation (*2.2.7*)

- 0.10° to + 0.10°.

Dissolve 2.50 g of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.650 g of the substance to be examined in 20 ml of *0.1 M hydrochloric acid*. Add 3 ml of a 100 g/l solution of *sodium hydroxide R* and extract with 3 quantities, each of 25 ml, of *methylene chloride R*. Combine the methylene chloride extracts and filter using hydrophobic phase-separation filter paper. Rinse the filter with 10 ml of *methylene chloride R* and combine the rinsings with the methylene chloride extracts. Evaporate the solvent under reduced pressure at a temperature not exceeding 40 °C. Dissolve the residue in 20.0 ml of *anhydrous ethanol R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with *anhydrous ethanol R.*

Reference solution (b) Dissolve 4 mg of *doxylamine impurity A CRS* and 4 mg of *2-benzoylpyridine R* in *anhydrous ethanol R* and dilute to 40 ml with the same solvent.

Column:

— *material*: fused silica;

— *size*: *l* = 30 m, Ø = 0.53 mm;

— *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 1.5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 7 ml/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 12	160 → 220
	12 - 27	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurities A and D.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 2.00 g.

Sulphated ash (*2.4.14*)

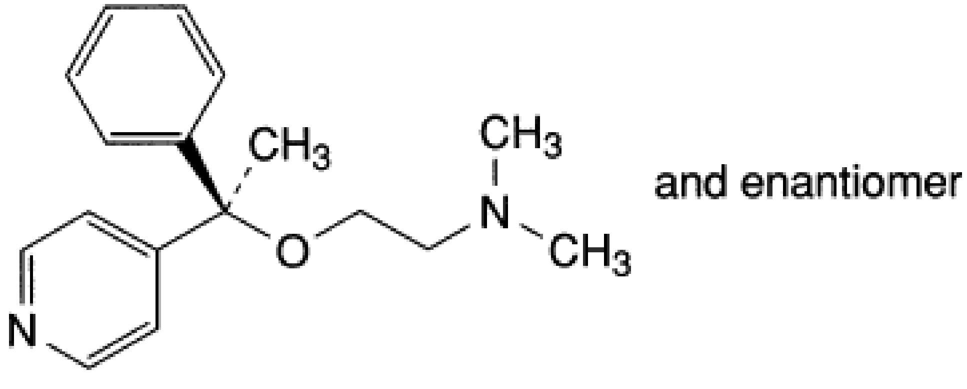
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

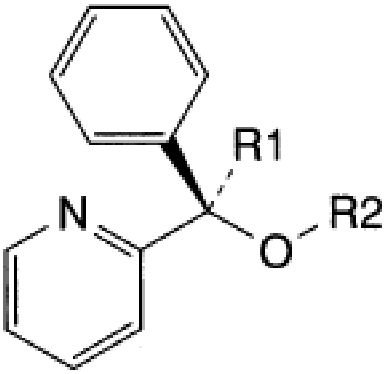
Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 19.43 mg of $C_{21}H_{28}N_2O_5$.

IMPURITIES



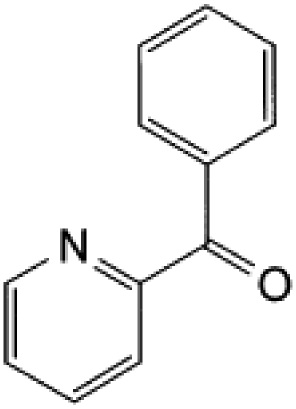
A. *N*,*N*-dimethyl-2-[1(*RS*)-1-phenyl-1-(pyridin-4-yl)ethoxy]ethanamine,



and enantiomer

B. R1 = CH_3, R2 = H: (1*RS*)-1-phenyl-1-(pyridin-2-yl)ethanol,

C. R1 = H, R2 = CH_2-CH_2-N(CH_3)$_2$: *N*,*N*-dimethyl-2-[(*RS*)-1-phenyl(pyridin-2-yl)methoxy] ethanamine,



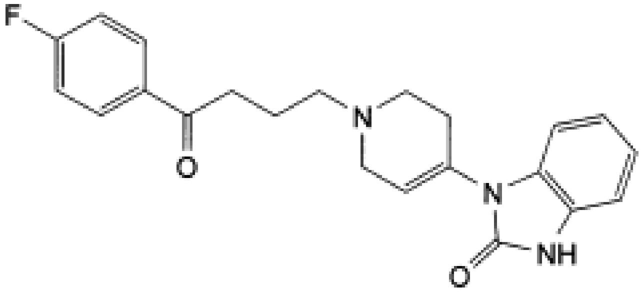
D. phenyl(pyridin-2-yl)methanone (2-benzoylpyridine).

Ph Eur

Droperidol

General Notices

(Ph Eur monograph 1010)



$C_{22}H_{22}FN_3O_2$ 379.4 *548-73-2*

Action and use

Dopamine receptor antagonist; $beta_1$-adrenoceptor agonist; alpha-adrenoceptor agonist; neuroleptic.

Preparations

Droperidol Injection

Droperidol Tablets

Ph Eur

DEFINITION

1-[1-[4-(4-Fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide and in methylene chloride, sparingly soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *droperidol CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a) Dissolve 30 mg of *droperidol CRS* in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b) Dissolve 30 mg of *droperidol CRS* and 30 mg of *benperidol CRS* in the mobile phase, then dilute to 10 ml with the mobile phase.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *acetone R, methanol R* (1:9 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 5 ml of *anhydrous ethanol R*. Add 0.5 ml of *dinitrobenzene solution R* and 0.5 ml of *2 M alcoholic potassium hydroxide R*. A violet colour is produced and becomes brownish-red after 20 min.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Dissolve 0.20 g in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

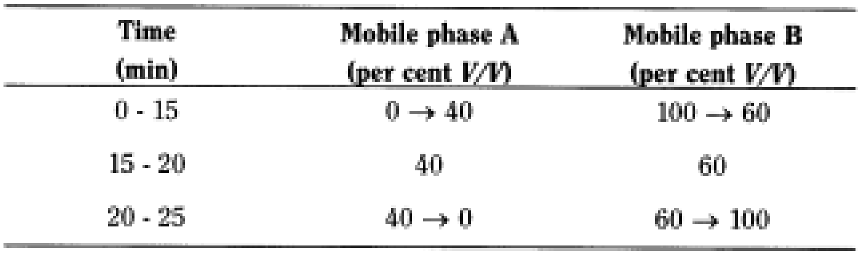
Test solution Dissolve 0.10 g of the substance to be examined in *dimethylformamide R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *droperidol CRS* and 2.5 mg of *benperidol CRS* in *dimethylformamide R*, then dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide R*. Dilute 5.0 ml of this solution to 20.0 ml with *dimethylformamide R.*

Column:
- *size: l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: *acetonitrile R*;
- *mobile phase B*: 10 g/l solution of *tetrabutylammonium hydrogen sulphate R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	0 → 40	100 → 60
15 - 20	40	60
20 - 25	40 → 0	60 → 100

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µl; inject *dimethylformamide R* as a blank.

Retention time Benperidol = about 6.5 min; droperidol = about 7 min.

System suitability Reference solution (a):

- *resolution*: minimum 2.0 between the peaks due to benperidol and droperidol; if necessary, adjust the final concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient.

Limits:
- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Using 0.2 ml of *naphtholbenzein solution R*, titrate with *0.1 M perchloric acid* until the colour changes from orange-yellow to green.

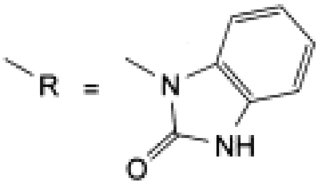
1 ml of *0.1 M perchloric acid* is equivalent to 37.94 mg of $C_{22}H_{22}FN_3O_2$.

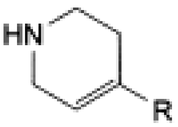
STORAGE

Protected from light.

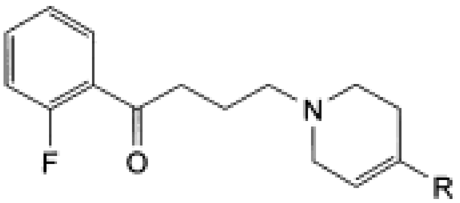
IMPURITIES

Specified impurities A, B, C, D, E.

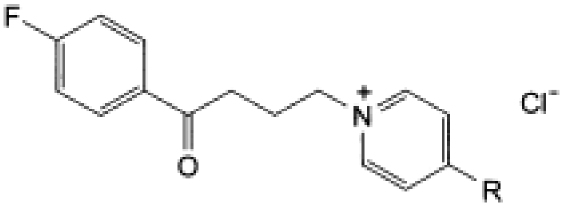




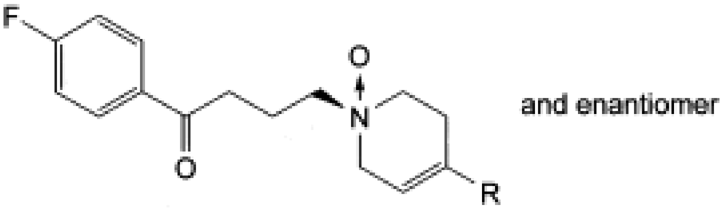
A. 1-(1,2,3,6-tetrahydropyridin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one,



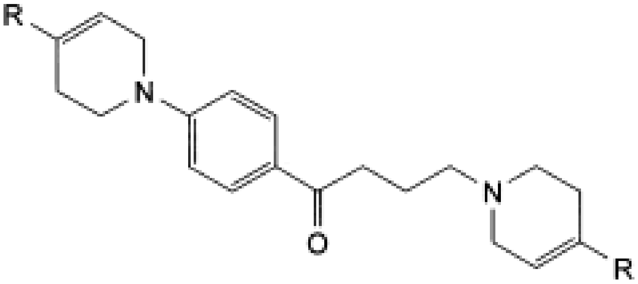
B. 1-[1-[4-(2-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



C. 1-[4-(4-fluorophenyl)-4-oxobutyl]-4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)pyridinium chloride,



D. (1*RS*)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)-1,2,3, 6-tetrahydropyridine 1-oxide,

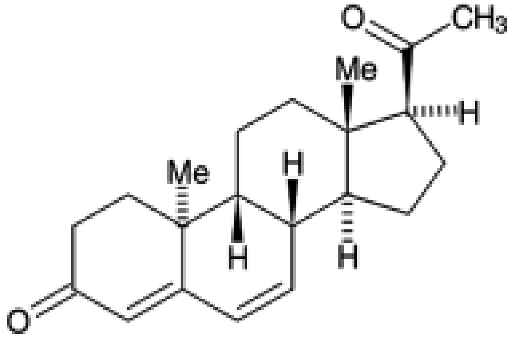


E. 1-[1-[4-[4-[4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)-3,6-dihydropyridin-1(2*H*)-yl]-1-oxobutyl]phenyl]-1,2,3,6-tetrahydropyridin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Ph Eur

Dydrogesterone

General Notices



$C_{21}H_{28}O_2$ 312.5 *152-62-5*

Action and use

Progestrogen.

Preparation

Dydrogesterone Tablets

DEFINITION

Dydrogesterone is 9β,10α-pregna-4,6-diene-3,20-dione. It contains not less than 97.0% and not more than 103.0% of $C_{21}H_{28}O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Practically insoluble in *water*; soluble in *acetone*; sparingly soluble in *ethanol (96%)* and in *methanol*; slightly soluble in *ether* and in fixed oils.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of dydrogesterone *(RS 118)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase E*.

TESTS

Light absorption

The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 286 nm. The ratio of the *absorbance* at 240 nm to that at the maximum at 286 nm is not more than 0.12.

Melting point

167° to 171°, Appendix V A.

Specific optical rotation

In a 1% w/v solution in *1,4-dioxan*, –446 to –464, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using three solutions in *chloroform* containing (1) 0.040% w/v of the substance being examined and 0.020% w/v of *1,2,3,4-tetraphenylcyclopenta-1,3-diene* (internal standard), (2) 2.0% w/v of the substance being examined and (3) 2.0% w/v of the substance being examined and 0.020% w/v of the internal standard.

The chromatographic procedure may be carried out using a glass column (1.5 m × 2.5 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of *cyanopropyl methyl silicone fluid* (SP-2300 is suitable) and maintained at 240°.

In the chromatogram obtained with solution (3) the ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard is not greater than the ratio of the area of the peak due to the substance being examined to the area of the peak due to the internal standard in the chromatogram obtained with solution (1).

Loss on drying

When dried at 105° for 3 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 40 mg in sufficient *methanol* to produce 200 ml, dilute 5 ml to 100 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 286 nm, Appendix II B. Calculate the content of $C_{21}H_{28}O_2$ taking 845 as the value of A(1%, 1 cm) at the maximum at 286 nm.

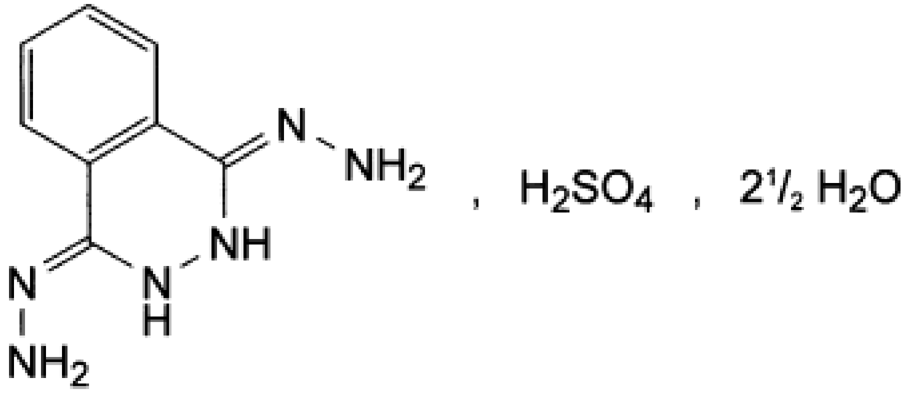
STORAGE

Dydrogesterone should be protected from light.

Hydrated Dihydralazine Sulphate

General Notices

(Ph Eur monograph 1310)



$C_8H_{10}N_6,H_2SO_4,2½H_2O$ 333.3 *7327-87-9 (anhydrous)*

Action and use

Vasodilator.

Ph Eur

DEFINITION

(Phthalazine-1,4(2*H*,3*H*)-diylidene)dihydrazine sulphate 2.5-hydrate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Slightly soluble in water, practically insoluble in anhydrous ethanol. It dissolves in dilute mineral acids.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of dihydralazine sulphate hydrated.*

B. Dissolve about 50 mg in 5 ml of *dilute hydrochloric acid R*. The solution gives reaction (a) of sulphates (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 0.20 g in *dilute nitric acid R* and dilute to 10 ml with the same acid.

Related substances

Liquid chromatography (*2.2.29*)

Prepare the solutions immediately before use.

Test solution Dissolve 50.0 mg of the substance to be examined in a 6 g/l solution of *glacial acetic acid R* and dilute to 50.0 ml with the same solution.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase containing 0.5 g/l of *sodium edetate R*. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase containing 0.5 g/l of *sodium edetate R.*

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase containing 0.5 g/l of *sodium edetate R.*

Reference solution (c) Dissolve 5 mg of *dihydralazine for system suitability CRS* in a 6 g/l solution of *glacial acetic acid R* and dilute to 5.0 ml with the same solution.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *nitrile silica gel for chromatography R* (5 µm).

Mobile phase Mix 22 volumes of *acetonitrile R1* and 78 volumes of a solution containing 1.44 g/l of *sodium laurilsulfate R* and 0.75 g/l of *tetrabutylammonium bromide R*, then adjust to pH 3.0 with *0.05 M sulphuric acid*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time Twice the retention time of dihydralazine.

Relative retention With reference to dihydralazine: impurity A = about 0.8.

System suitability Reference solution (c):

— the peaks due to impurity A and dihydralazine are baseline separated as in the chromatogram supplied with *dihydralazine for system suitability CRS.*

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.1 per cent);

- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *sum of impurities other than A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Impurity B

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 40.0 mg of *hydrazine sulphate R* (impurity B) in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 25.0 ml with *water R*. To 0.50 ml of this solution, add 0.200 g of the substance to be examined and dissolve in 6 ml of *dilute hydrochloric acid R,* then dilute to 10.0 ml with *water R*. In a centrifuge tube with a ground-glass stopper, place immediately 0.50 ml of this solution and 2.0 ml of a 60 g/l solution of *benzaldehyde R* in a mixture of equal volumes of *methanol R* and *water R*. Shake for 90 s. Add 1.0 ml of *water R* and 5.0 ml of *heptane R*. Shake for 1 min and centrifuge. Use the upper layer.

Reference solution Dissolve 40.0 mg of *hydrazine sulphate R* (impurity B) in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 25.0 ml with *water R*. To 0.50 ml of this solution, add 6 ml of *dilute hydrochloric acid R* and dilute to 10.0 ml with *water R*. In a centrifuge tube with a ground-glass stopper, place 0.50 ml of this solution and 2.0 ml of a 60 g/l solution of *benzaldehyde R* in a mixture of equal volumes of *methanol R* and *water R.* Shake for 90 s. Add 1.0 ml of *water R* and 5.0 ml of *heptane R.* Shake for 1 min and centrifuge. Use the upper layer.

Blank solution Prepare in the same manner as for the reference solution but replacing the 0.50 ml of hydrazine sulphate solution by 0.50 ml of *water R*.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 0.3 g/l solution of *sodium edetate R*, *acetonitrile R* (30:70 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 305 nm.

Injection 20 µl.

Relative retention With reference to benzaldehyde: benzaldehyde azine (benzalazine) corresponding to impurity B = about 1.8.

Limit:

- *impurity B*: the area of the peak due to benzaldehyde azine is not greater than twice the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm).

Iron (*2.4.9*)

Maximum 20 ppm.

To the residue obtained in the test for sulphated ash add 0.2 ml of *sulphuric acid R* and heat carefully until the acid is almost completely eliminated. Allow to cool and dissolve the residue with heating in 5.5 ml of *hydrochloric acid R1*. Filter the hot solution through a filter previously washed 3 times with *dilute hydrochloric acid R*. Wash the crucible and the filter with 5 ml of *water R*. Combine the filtrate and the washings and neutralise with about 3.5 ml of *strong sodium hydroxide solution R*. Adjust to pH 3-4 with *acetic acid R* and dilute to 20 ml with *water R*. Prepare the standard with 5 ml of *iron standard solution (2 ppm Fe) R* and 5 ml of *water R.*

Loss on drying (*2.2.32*)

13.0 per cent to 15.0 per cent, determined on 1.000 g by drying in an oven at 50 °C at a pressure not exceeding 0.7 kPa for 5 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

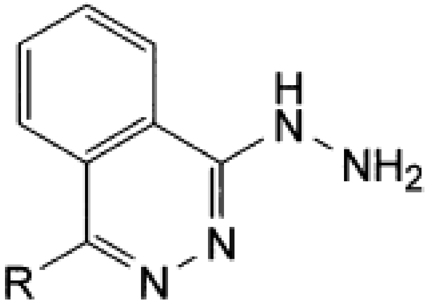
ASSAY

Dissolve 60.0 mg in 25 ml of *water R*. Add 35 ml of *hydrochloric acid R* and titrate slowly with *0.05 M potassium iodate*, determining the end-point potentiometrically (*2.2.20*), using a calomel reference electrode and a platinum indicator electrode.

1 ml of *0.05 M potassium iodate* is equivalent to 7.208 mg of $C_8H_{12}N_6O_4S$.

IMPURITIES

Specified impurities A, B, C.



A. R = NH_2: 4-hydrazinophthalazin-1-amine,

C. R = H: (phthalazin-1-yl)hydrazine (hydralazine),

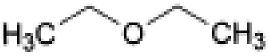
B. H_2N-NH_2: hydrazine.

Ph Eur

Anaesthetic Ether

General Notices

(Ph Eur monograph 0367)



$C_4H_{10}O$ 74.1 *60-29-7*

Ph Eur

DEFINITION

Diethyl ether.

It may contain a suitable non-volatile antioxidant at an appropriate concentration.

CHARACTERS

Appearance

Clear, colourless liquid, volatile, very mobile.

Solubility

Soluble in 15 parts of water, miscible with ethanol (96 per cent) and with fatty oils.

It is highly flammable.

IDENTIFICATION

A. Relative density (see Tests).

B. Distillation range (see Tests).

TESTS

Acidity

To 20 ml of *ethanol (96 per cent) R* add 0.25 ml of *bromothymol blue solution R1* and, dropwise, *0.02 M sodium hydroxide* until a blue colour persists for 30 s. Add 25 ml of the substance to be examined, shake and add, dropwise, *0.02 M sodium hydroxide* until the blue colour reappears and persists for 30 s. Not more than 0.4 ml of *0.02 M sodium hydroxide* is required.

Relative density *(2.2.5)*

0.714 to 0.716.

Distillation range *(2.2.11)*

Do not distil if the substance to be examined does not comply with the test for peroxides. It distils completely between 34.0 °C and 35.0 °C. Carry out the test using a suitable heating device and taking care to avoid directly heating the flask above the level of the liquid.

Acetone and aldehydes

To 10.0 ml in a ground-glass-stoppered cylinder add 1 ml of *alkaline potassium tetra-iodomercurate solution R* and shake for 10 s. Allow to stand for 5 min, protected from light. The lower layer shows only a slight opalescence.

If the substance to be examined does not comply with the test, distil 40 ml, *after ensuring that the substance to be examined complies with the test for peroxides*, until only 5 ml remains. Collect the distillate in a receiver cooled in a bath of iced water and repeat the test described above using 10.0 ml of the distillate.

Peroxides

Place 8 ml of *potassium iodide and starch solution R* in a 12 ml ground-glass-stoppered cylinder about 15 mm in diameter. Fill completely with the substance to be examined, shake vigorously and allow to stand protected from light for 30 min. No colour develops.

Non-volatile matter

Maximum 20 mg/l.

After ensuring that the substance to be examined complies with the test for peroxides, evaporate 50 ml to dryness on a water-bath and dry the residue in an oven at 100-105 °C. The residue weighs a maximum of 1 mg.

Substances with a foreign odour

Moisten a disc of filter paper 80 mm in diameter with 5 ml of the substance to be examined and allow to evaporate. No foreign odour is perceptible immediately after the evaporation.

Water *(2.5.12)*

Maximum 2 g/l, determined on 20 ml.

STORAGE

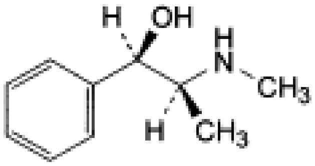
In an airtight container , protected from light, at a temperature of 8 °C to 15 °C. The contents of a partly filled container may deteriorate rapidly.

Ph Eur

Anhydrous Ephedrine

General Notices

(Ephedrine, Anhydrous, Ph Eur monograph 0488)



$C_{10}H_{15}NO$ 165.2   *299-42-3*

Action and use

Adrenoceptor agonist.

Ph Eur

DEFINITION

Anhydrous ephedrine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (1*R*,2*S*)-2-methylamino-1-phenylpropan-1-ol, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, soluble in water, very soluble in alcohol.

It melts at about 36 °C.

IDENTIFICATION

First identification   B, D.

Second identification   A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with the base isolated from *ephedrine hydrochloride CRS*. Examine the substances in discs prepared as follows: dissolve 40 mg of the substance to be examined in 1 ml of *water R*, add 1 ml of *dilute sodium hydroxide solution R* and 4 ml of *chloroform R* and shake; dry the organic layer over 0.2 g of *anhydrous sodium sulphate R*; prepare a blank disc using about 0.3 g of *potassium bromide R*; apply dropwise to the disc 0.1 ml of the organic layer, allowing the solvent to evaporate between applications; dry the disc at 50 °C for 2 min. Repeat the operations using 50 mg of *ephedrine hydrochloride CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 1 ml of *water R*. Add 0.2 ml of *strong sodium hydroxide solution R* and 0.2 ml of *copper sulphate solution R*. A violet colour is produced. Add 2 ml of *ether R* and shake. The ether layer is purple and the aqueous layer blue.

E. It complies with the test for water (see Tests).

TESTS

Appearance of solution

Dissolve 0.25 g in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 2.25 g in 15 ml of *dilute hydrochloric acid R* and dilute to 50.0 ml with *water R*. The specific optical rotation is - 41 to - 43, calculated with reference to the anhydrous substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 25 mg of *ephedrine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 200 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *chloroform R*, 15 volumes of *concentrated ammonia R* and 80 volumes of *2-propanol R*. Allow the plate to dry in air and spray with *ninhydrin solution R*. Heat at 110 °C for 5 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Disregard any spot of lighter colour than the background.

Chlorides

Dissolve 0.17 g in 10 ml of *water R*. Add 5 ml of *dilute nitric acid R* and 0.5 ml of *silver nitrate solution R1*. Allow to stand for 2 min, protected from bright light. Any opalescence in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of *chloride standard solution (5 ppm Cl) R*, 5 ml of *dilute nitric acid R* and 0.5 ml of *silver nitrate solution R1* (290 ppm).

Water *(2.5.12)*

Not more than 0.5 per cent, determined on 2.000 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 5 ml of *alcohol R* and add 20.0 ml of *0.1 M hydrochloric acid*. Using 0.05 ml of *methyl red solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a yellow colour is obtained.

1 ml of *0.1 M hydrochloric acid* is equivalent to 16.52 mg of $C_{10}H_{15}NO$.

STORAGE

Store protected from light.

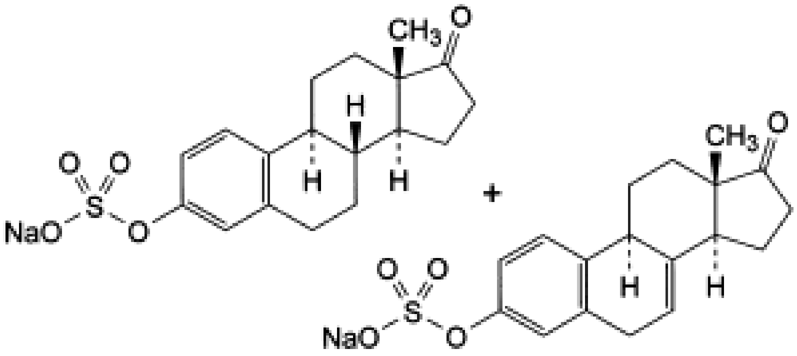
Ph Eur

Conjugated Estrogens

General Notices

Conjugated Oestrogens

(Ph Eur monograph 1512)



$C_{18}H_{21}O_5NaS + C_{18}H_{19}O_5NaS$ 372.4+ 370.4

Action and use

Estrogen.

Ph Eur

DEFINITION

Mixture of various conjugated forms of estrogens obtained from the urine of pregnant mares or by synthesis, dispersed in a suitable powdered diluent.

The 2 principal components are 17-oxoestra-1,3,5(10)-trien-3-yl sodium sulphate (sodium estrone sulphate) and 17-oxoestra-1,3,5(10),7-tetraen-3-yl sodium sulphate (sodium equilin sulphate). Concomitants are sodium 17α-estradiol sulphate, sodium 17α-dihydroequilin sulphate and sodium 17β-dihydroequilin sulphate.

Content (percentages related to the labelled content)

— *sodium estrone sulphate*: 52.5 per cent to 61.5 per cent;

— *sodium equilin sulphate*: 22.5 per cent to 30.5 per cent;

— *sodium 17α-estradiol sulphate*: 2.5 per cent to 9.5 per cent;

— *sodium 17α-dihydroequilin sulphate*: 13.5 per cent to 19.5 per cent;

— *sodium 17β-dihydroequilin sulphate*: 0.5 per cent to 4.0 per cent;

— *sum of sodium estrone sulphate and sodium equilin sulphate*: 79.5 per cent to 88.0 per cent.

CHARACTERS

Appearance

Almost white or brownish, amorphous powder.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The 2 principal peaks due to estrone and equilin in the chromatogram obtained with test solution (a) are similar in retention time and size to the 2 principal peaks in the chromatogram obtained with reference solution (a).

B. Examine the chromatogram obtained in the test for chromatographic profile.

Results The chromatogram obtained with test solution (b) exhibits additional peaks due to 17α-estradiol, 17α-dihydroequilin and 17β-dihydroequilin, at relative retentions with reference to 3-*O*-methylestrone (internal standard) of about 0.24, 0.30 and 0.35 respectively.

TESTS

Chromatographic profile

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 8 mg of *3-O-methylestrone R* in 10.0 ml of *anhydrous ethanol R*. Dilute 2.0 ml of this solution to 10.0 ml with *anhydrous ethanol R*.

Acetate buffer solution pH 5.2 Dissolve 10 g of *sodium acetate R* in 100 ml of *water R* and add 10 ml of *dilute acetic acid R*. Dilute to 500 ml with *water R* and adjust to pH 5.2 ± 0.1.

Test solution (a) Considering the labelled content, transfer an accurately weighed quantity corresponding to about 2 mg of conjugated estrogens to a 50 ml centrifuge tube containing 15 ml of the acetate buffer solution pH 5.2 and 1 g of *barium chloride R*. Cap the tube tightly and shake for 30 min. If necessary, adjust to pH 5.0 ± 0.5 with *acetic acid R* or a 120 g/l solution of *sodium acetate R*. Sonicate for 30 s, then shake for 30 min. Add a suitable sulphatase preparation equivalent to 2500 units and shake mechanically for 10 min in a water-bath at 50 ± 1 °C. Swirl the tube by hand, then shake mechanically for 10 min in the water-bath. Allow to cool. Add 15.0 ml of *ethylene chloride R* to the mixture, immediately cap the tube tightly and shake for 15 min. Centrifuge for 10 min or until the lower layer is clear. Draw out the organic layer to a screw-cap tube, add 5 g of *anhydrous sodium sulphate R* and shake. Allow the solution to stand until clear. Protect the solution from any loss due to evaporation. Transfer 3.0 ml of the clear solution to a suitable centrifuge tube fitted with a screw cap. Add 1.0 ml of the internal standard solution. Evaporate the mixture to dryness with the aid of a stream of *nitrogen R*, maintaining the temperature below 50 °C. To the dry residue add 15 µl of *anhydrous pyridine R* and 65 µl of *N,O-bis(trimethylsilyl)trifluoroacetamide R* containing 1 per cent of *chlorotrimethylsilane R*. Immediately cap the tube tightly, mix thoroughly and allow to stand for 15 min. Add 0.5 ml of *toluene R* and mix mechanically.

Test solution (b) Prepare as described in test solution (a), but do not add the sulphatase and use 6.0 ml of the upper layer instead of 3.0 ml. Prepare a blank in the same manner.

Reference solution (a) Dissolve separately 8 mg of *estrone CRS*, 7 mg of *equilin CRS* and 5 mg of *17α-dihydroequilin CRS* in 10.0 ml of *anhydrous ethanol R*. Dilute together 2.0 ml, 1.0 ml and 1.0 ml respectively of these solutions to 10.0 ml with *anhydrous ethanol R*. Transfer 1.0 ml of this solution and 1.0 ml of the internal standard solution to a centrifuge tube fitted with a screw cap. Evaporate the mixture to dryness with the aid of a stream of *nitrogen R*, maintaining the temperature below 50 °C. To the dry residue add 15 µl of *anhydrous pyridine R* and 65 µl of *N,O-bis(trimethylsilyl)trifluoroacetamide R* containing 1 per cent of *chlorotrimethylsilane R*. Immediately cap the tube tightly, mix and allow to stand for 15 min. Add 0.5 ml of *toluene R*.

Reference solution (b) Prepare as described in reference solution (a), but dilute tenfold with *anhydrous ethanol R* before adding the internal standard.

Column:

— *material*: fused silica;

— *size:* *l* = 15 m, Ø = 0.25 mm;

— *stationary phase*: *poly[(cyanopropyl)(methyl)][(phenyl)(methyl)]siloxane R* (film thickness 0.25 µm).

Carrier gas *hydrogen for chromatography R.*

Flow rate 2 ml/min.

Split ratio 1:20 to 1:30.

Temperature:

— *column*: 220 °C;

— *injection port and detector*: 260 °C.

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to 3-*O*-methylestrone: 17α-dihydroequilin = about 0.30; estrone = about 0.80; equilin = about 0.87.

System suitability Reference solution (a):

— *resolution*: minimum 1.2 between the peaks due to estrone and equilin; if necessary, adjust the temperature and the flow rate of the carrier gas.

In the chromatogram obtained with reference solution (a), measure the areas of the peaks due to 17α-dihydroequilin, estrone and 3-*O*-methylestrone.

In the chromatogram obtained with test solution (a), locate the peaks with relative retentions with reference to 3-*O*-methylestrone of 1 and about 0.24, 0.29, 0.30, 0.35, 0.56, 0.64, 0.90 and 1.3 and measure their areas.

Calculate the percentage content of the components occurring as sodium sulphate salts using expression (1) below.

In the chromatogram obtained with reference solution (b), measure the areas of the peaks due to estrone and 3-*O*-methylestrone.

In the chromatogram obtained with test solution (b), locate the peaks with relative retentions with reference to 3-*O*-methylestrone of about 0.30, 0.80 and 0.87 and measure the sum of the areas.

Calculate the percentage content of 17α-dihydroequilin, estrone and equilin occurring as free steroids using expression (2) below.

$$\frac{S'_A \times S_I \times m_R \times 137.8 \times 1000}{S_R \times S'_I \times m \times LC}$$

$$\frac{S'_{FS} \times S_I \times m_E \times 100 \times 1000}{S_E \times S'_I \times m \times LC}$$

S_I = area of the peak due to the internal standard in the chromatogram obtained with the corresponding reference solution;

S'_I = area of the peak due to the internal standard in the chromatogram obtained with the corresponding test solution;

S_R = area of the peak due to the reference substance (Table 1512.-1) in the chromatogram obtained with the corresponding reference solution;

S'_A = area of the peak due to the analyte in the chromatogram obtained with the corresponding test solution;

m_R = mass of the reference substance (Table 1512.-1) in the corresponding reference solution, in milligrams;

m = mass of the substance to be examined in the corresponding test solution, in milligrams;

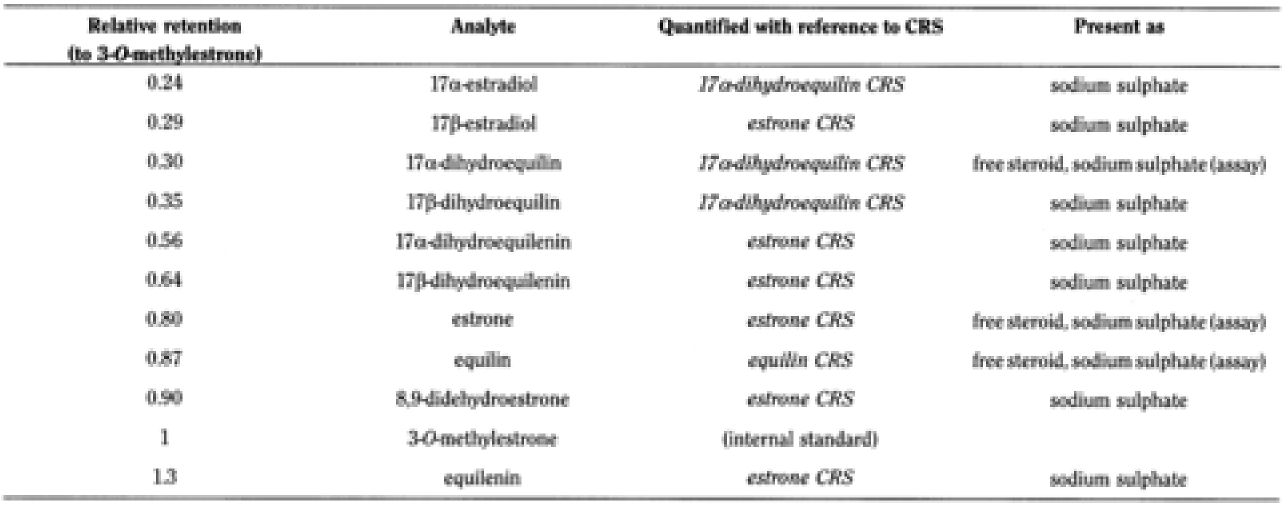
S'_{FS} = sum of the areas of the peaks due to 17α-dihydroequilin, estrone and equilin in the chromatogram obtained with the corresponding test solution;

S_E = area of the peak due to *estrone CRS* in the chromatogram obtained with the corresponding reference solution;

m_E = mass of *estrone CRS* in the corresponding reference solution, in milligrams;

L_C = labelled content, in milligrams per gram.

Table 1512.-1



Relative retention (to 3-*O*-methylestrone)	Analyte	Quantified with reference to CRS	Present as
0.24	17α-estradiol	*17α-dihydroequilin CRS*	sodium sulphate
0.29	17β-estradiol	*estrone CRS*	sodium sulphate
0.30	17α-dihydroequilin	*17α-dihydroequilin CRS*	free steroid, sodium sulphate (assay)
0.35	17β-dihydroequilin	*17α-dihydroequilin CRS*	sodium sulphate
0.56	17α-dihydroequilenin	*estrone CRS*	sodium sulphate
0.64	17β-dihydroequilenin	*estrone CRS*	sodium sulphate
0.80	estrone	*estrone CRS*	free steroid, sodium sulphate (assay)
0.87	equilin	*equilin CRS*	free steroid, sodium sulphate (assay)
0.90	8,9-didehydroestrone	*estrone CRS*	sodium sulphate
1	3-*O*-methylestrone	(internal standard)	
1.3	equilenin	*estrone CRS*	sodium sulphate

The percentages are within the following ranges:

— *sodium 17α-estradiol sulphate*: 2.5 per cent to 9.5 per cent;

— *sodium 17α-dihydroequilin sulphate*: 13.5 per cent to 19.5 per cent;

— *sodium 17β-dihydroequilin sulphate*: 0.5 per cent to 4.0 per cent;

— *sodium 17β-estradiol sulphate*: maximum 2.25 per cent;

— *sodium 17α-dihydroequilenin sulphate*: maximum 3.25 per cent;

— *sodium 17β-dihydroequilenin sulphate*: maximum 2.75 per cent;

— *sodium 8,9-didehydroestrone sulphate*: maximum 6.25 per cent;

— *sodium equilenin sulphate*: maximum 5.5 per cent;

— *sum of estrone, equilin and 17α-dihydroequilin*: maximum 1.3 per cent.

ASSAY

Gas chromatography *(2.2.28)* as described in the test for chromatographic profile with the following modifications.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 2.0 per cent for the ratio of the area of the peak due to estrone to that due to the internal standard after at least 6 injections.

In the chromatogram obtained with reference solution (a), measure the areas of the peaks due to estrone or equilin and 3-*O*-methylestrone. In the chromatogram obtained with test solution (a), measure the areas of the peaks due to estrone, equilin and 3-*O*-methylestrone.

Calculate the percentage content of sodium estrone sulphate and sodium equilin sulphate using expression (1).

LABELLING

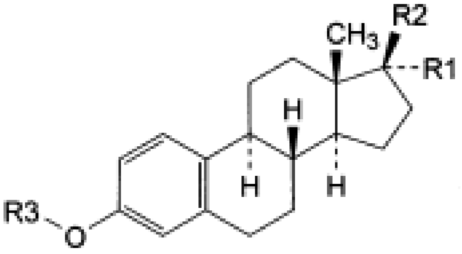
The label states:

— the name of the substance;

— the content of the substance;

— the nature of the diluent.

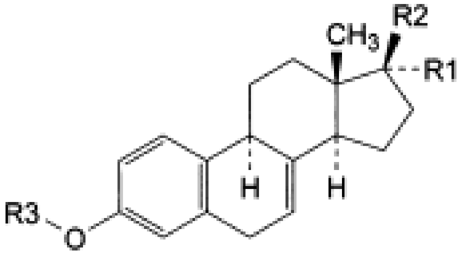
IMPURITIES AND CONCOMITANTS



A. R1 = OH, R2 = H, R3 = SO_3Na: 17α-hydroxyestra-1,3,5(10)-trien-3-yl sodium sulphate (sodium 17α-estradiol sulphate),

D. R1 = H, R2 = OH, R3 = SO_3Na: 17β-hydroxyestra-1,3,5(10)-trien-3-yl sodium sulphate (sodium 17β-estradiol sulphate),

I. R1 + R2 = O, R3 = H: 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),

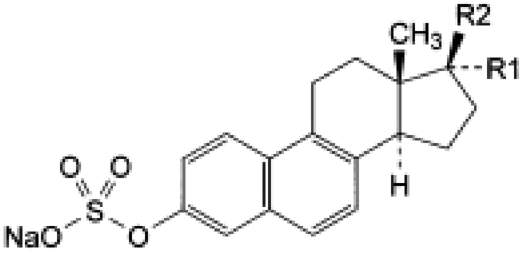


B. R1 = OH, R2 = H, R3 = SO_3Na: 17α-hydroxyestra-1,3,5(10),7-tetraen-3-yl sodium sulphate (sodium 17α-dihydroequilin sulphate),

C. R1 = H, R2 = OH, R3 = SO_3Na: 17β-hydroxyestra-1,3,5(10),7-tetraen-3-yl sodium sulphate (sodium 17β-dihydroequilin sulphate),

J. R1 + R2 = O, R3 = H: 3-hydroxyestra-1,3,5(10),7-tetraen-17-one (equilin),

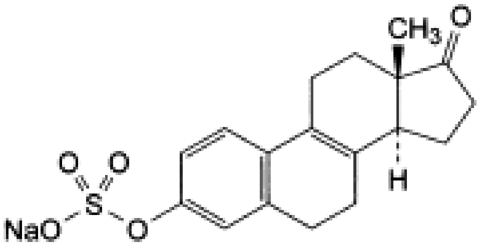
K. R1 = OH, R2 = R3 = H: estra-1,3,5(10),7-tetraene-3,17α-diol (17α-dihydroequilin),



E. R1 = OH, R2 = H: 17α-hydroxyestra-1,3,5(10),6,8-pentaen-3-yl sodium sulphate (sodium 17α-dihydroequilenin sulphate),

F. R1 = H, R2 = OH: 17β-hydroxyestra-1,3,5(10),6,8-pentaen-3-yl sodium sulphate (sodium 17β-dihydroequilenin sulphate),

H. R1 + R2 = O: 17-oxoestra-1,3,5(10),6,8-pentaen-3-yl sodium sulphate (sodium equilenin sulphate),



G. 17-oxoestra-1,3,5(10),8-tetraen-3-yl sodium sulphate (sodium 8,9-didehydroestrone sulphate).

Ph Eur

Dilute Ethanols

General Notices

DEFINITION

The official Dilute Ethanols contain 90, 80, 70, 60, 50, 45, 25 and 20% v/v respectively of ethanol. They may be prepared as described below, the final adjustment of volume being made at the same temperature, 20°, as that at which the Ethanol (96 per cent) is measured.

NOTE *On mixing ethanol and water, contraction of volume and rise of temperature occur*.

TESTS

Acidity or alkalinity; Appearance; Volatile impurities; Residue on evaporation

Comply with the requirements stated under Ethanol (96 per cent).

ETHANOL (90 PER CENT)

Alcohol (90 per cent); Rectified Spirit

Dilute 934 ml of Ethanol (96 per cent) to 1000 ml with Purified Water.

Content of ethanol

89.6 to 90.5% v/v.

Apparent density

826.4 to 829.4 kg m^{-3}, Appendix V G.

ETHANOL (80 PER CENT)

Alcohol (80 per cent)

Dilute 831 ml of Ethanol (96 per cent) to 1000 ml with Purified Water.

Content of ethanol

79.5 to 80.3% v/v.

Apparent density

857.4 to 859.6 kg m^{-3}, Appendix V G.

ETHANOL (70 PER CENT)

Alcohol (70 per cent)

Dilute 727 ml of Ethanol (96 per cent) to 1000 ml with Purified Water.

Content of ethanol

69.5 to 70.4% v/v.

Apparent density

883.5 to 885.8 kg m^{-3}, Appendix V G.

ETHANOL (60 PER CENT)

Alcohol (60 per cent)

Dilute 623 ml of Ethanol (96 per cent) to 1000 ml with Purified Water.

Content of ethanol

59.7 to 60.2% v/v.

Apparent density

907.6 to 908.7 kg m^{-3}, Appendix V G.

ETHANOL (50 PER CENT)

Alcohol (50 per cent)

Dilute 519 ml of Ethanol (96 per cent) to 1000 ml with Purified Water.

Content of ethanol

49.6 to 50.2% v/v.

Apparent density

928.6 to 929.8 kg m^{-3}, Appendix V G.

ETHANOL (45 PER CENT)

Alcohol (45 per cent)

Dilute 468 ml of Ethanol (96 per cent) to 1000 ml with Purified Water.

Content of ethanol

44.7 to 45.3% v/v.

Apparent density

938.0 to 939.0 kg m^{-3}, Appendix V G.

ETHANOL (25 PER CENT)

Alcohol (25 per cent)

Dilute 259 ml of Ethanol (96 per cent) to 1000 ml with Purified Water.

Content of ethanol

24.6 to 25.4% v/v.

Apparent density

966.6 to 967.5 kg m^{-3}, Appendix V G.

ETHANOL (20 PER CENT)

Alcohol (20 per cent)

Dilute 207 ml of Ethanol (96 per cent) to 1000 ml with Purified Water.

Content of ethanol

19.5 to 20.5% v/v.

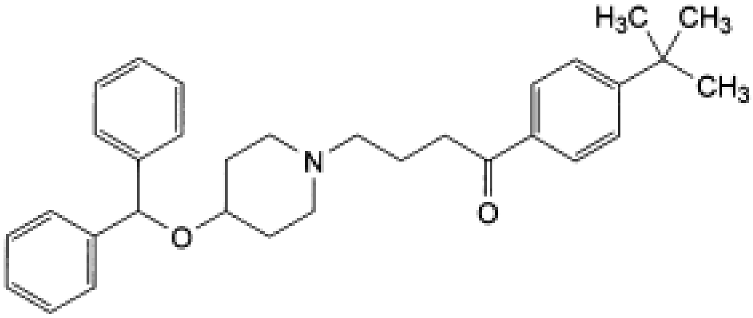
Apparent density

972.0 to 973.1 kg m^{-3}, Appendix V G.

Ebastine

General Notices

(Ph Eur monograph 2015)



$C_{32}H_{39}NO_2$ 469.7 *90729-43-4*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

1-[4-(1,1-Dimethylethyl)phenyl]-4-[4-(diphenylmethoxy)piperidin-1-yl]butan-1-one.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, sparingly soluble in methanol.

mp

About 86 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of ebastine.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Keep the solutions protected from light*.

Solution A. Mix 65 volumes of *acetonitrile R* and 35 volumes of a 1.1 g/l solution of *phosphoric acid R* adjusted to pH 5.0 with a 40 g/l solution of *sodium hydroxide R*.

Test solution Dissolve 0.125 g of the substance to be examined in solution A and dilute to 50.0 ml with the same solution.

Reference solution (a) Dissolve 5.0 mg of *ebastine impurity C CRS* and 5.0 mg of *ebastine impurity D CRS* in solution A and dilute to 20.0 ml with the same solution. Dilute 1.0 ml of the solution to 100.0 ml with solution A.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with solution A. Dilute 1.0 ml of this solution to 10.0 ml with solution A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *nitrile silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 1.1 g/l solution of *phosphoric acid R* adjusted to pH 5.0 with a 40 g/l solution of *sodium hydroxide R*. Adjust the percentage of acetonitrile to between 30 per cent *V/V* and 40 per cent *V/V* so that the retention time of ebastine is about 110 min.

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Run time 1.4 times the retention time of ebastine.

Relative retention With reference to ebastine: impurity A = about 0.04; impurity B = about 0.05; impurity D = about 0.20; impurity C = about 0.22; impurity F = about 0.42; impurity G = about 0.57; impurity E = about 1.14.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to impurity D and impurity C.

Limits:

— *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphates ***(2.4.13)***

Maximum 100 ppm.

Suspend 2.5 g in 25 ml of *dilute nitric acid R*. Boil under a reflux condenser for 10 min. Cool and filter. 15 ml of the filtrate complies with the limit test for sulphates.

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

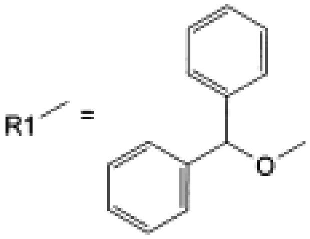
Dissolve 0.350 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

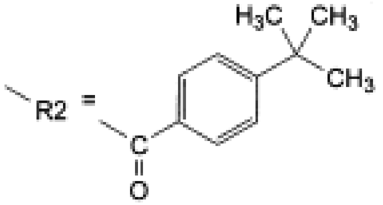
1 ml of *0.1 M perchloric acid* is equivalent to 46.97 mg of $C_{32}H_{39}NO_2$.

STORAGE

Protected from light.

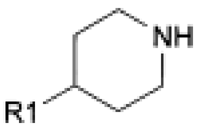
IMPURITIES



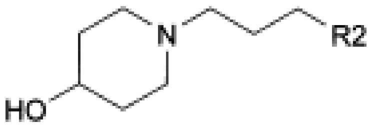


A. R1–H: diphenylmethanol (benzhydrol),

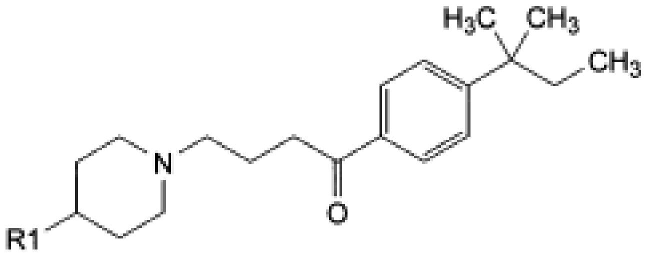
B. R2–CH_3: 1-[4-(1,1-dimethylethyl)phenyl]ethanone,



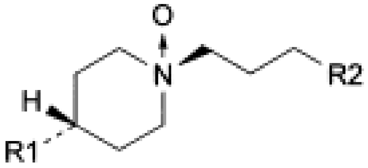
C. 4-(diphenylmethoxy)piperidine,



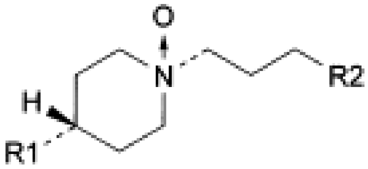
D. 1-[4-(1,1-dimethylethyl)phenyl]-4-(4-hydroxypiperidin-1-yl)butan-1-one,


H₃C
CH₃
CH₃
N
O
R1

E. 1-[4-(1,1-dimethylpropyl)phenyl]-4-[4-(diphenylmethoxy)piperidin-1-yl]butan-1-one,



F. 1-[4-(1,1-dimethylethyl)phenyl]-4-[*cis*-4-(diphenylmethoxy)-1-oxidopiperidin-1-yl]butan-1-one,



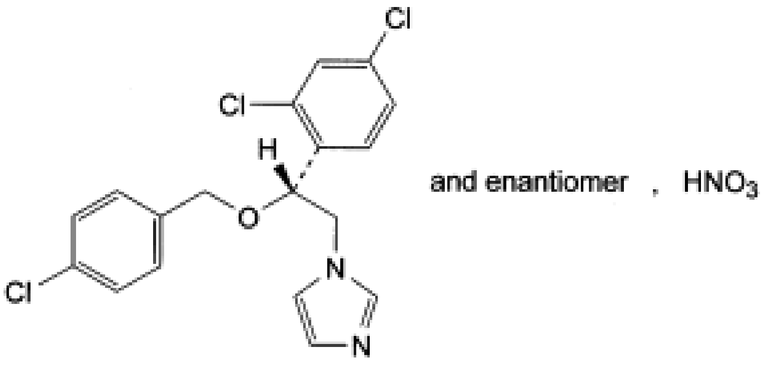
G. 1-[4-(1,1-dimethylethyl)phenyl]-4-[*trans*-4-(diphenylmethoxy)-1-oxidopiperidin-1-yl]butan-1-one.

Ph Eur

Econazole Nitrate

General Notices

(Ph Eur monograph 0665)



$C_{18}H_{15}Cl_3N_2O,HNO_3$ 444.7 *68797-31-9*

Action and use

Antifungal.

Preparations

Econazole Cream

Econazole Pessaries

Ph Eur

DEFINITION

1-[(2*RS*)-2-[(4-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Very slightly soluble in water, soluble in methanol, sparingly soluble in methylene chloride, slightly soluble in alcohol.

mp

About 165 °C, with decomposition.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   Ph. Eur. reference spectrum of econazole nitrate.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

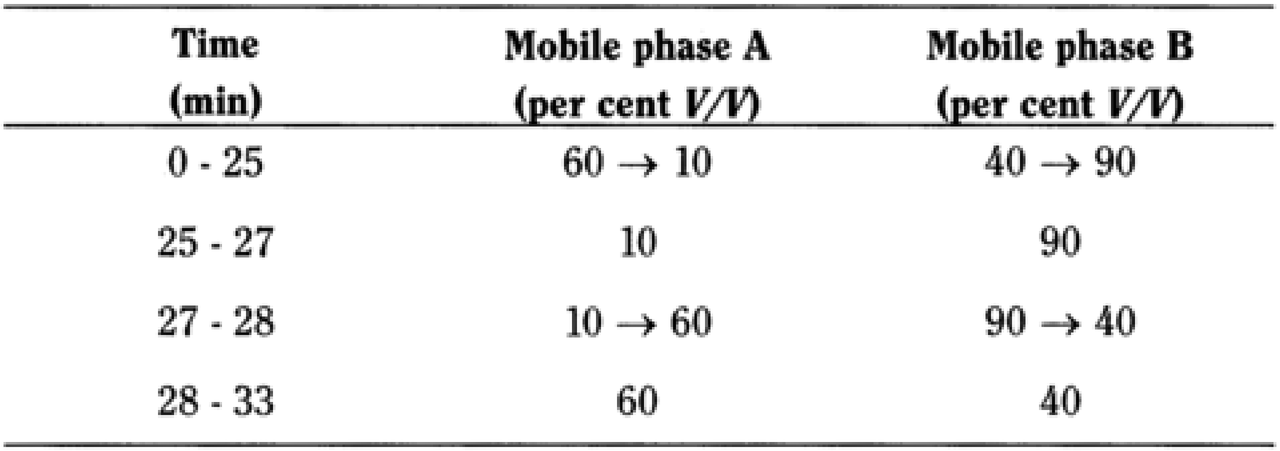
Test solution   Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a)   Dissolve 10 mg of *econazole for system suitability CRS* in *methanol R* and dilute to 1.0 ml with the same solvent.

Reference solution (b)   Dilute 1.0 ml of the test solution to 20.0 ml with *methanol R*. Dilute 1.0 ml of the solution to 25.0 ml with *methanol R*.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm,
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm),
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: mix 20 volumes of *methanol R* and 80 volumes of a 0.77 g/l solution of *ammonium acetate R*,
- *mobile phase B*: *methanol R, acetonitrile R* (40:60 *V/V*),



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	60 → 10	40 → 90
25 - 27	10	90
27 - 28	10 → 60	90 → 40
28 - 33	60	40

Flow rate   1.5 ml/min.

Detection   Spectrophotometer at 225 nm.

Injection   10 µl.

Relative retention With reference to econazole (retention time = about 15 min): impurity A = about 0.2; impurity B = about 0.6; impurity C = about 1.1.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum of 1.5, where H_p = height above the baseline of the peak due to impurity C, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to econazole.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.4,

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to the nitrate ion at the beginning of the chromatogram.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

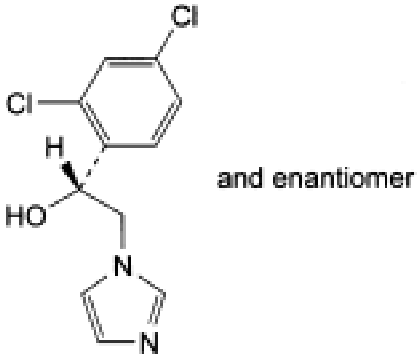
1 ml of *0.1 M perchloric acid* is equivalent to 44.47 mg of $C_{18}H_{16}Cl_3N_3O_4$.

STORAGE

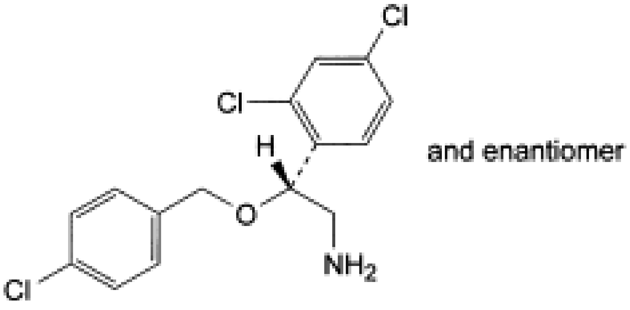
Protected from light.

IMPURITIES

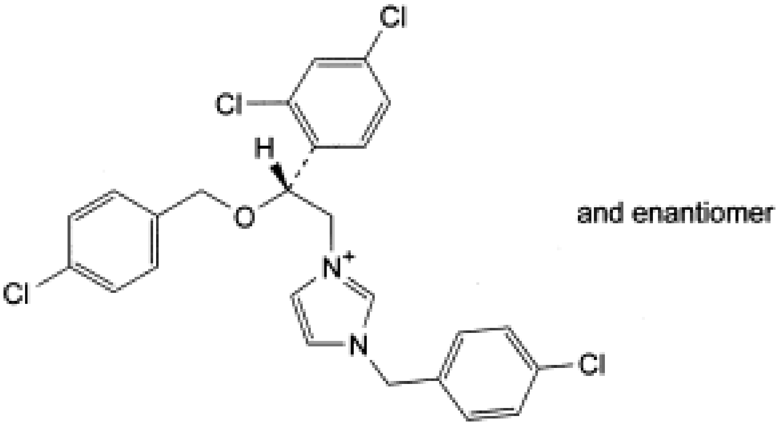
Specified impurities *A, B, C.*



A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,


Cl
Cl
H
O
NH2
Cl
and enantiomer

B. (2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethanamine,


Cl
Cl
H
O
Cl
N+
N
Cl
and enantiomer

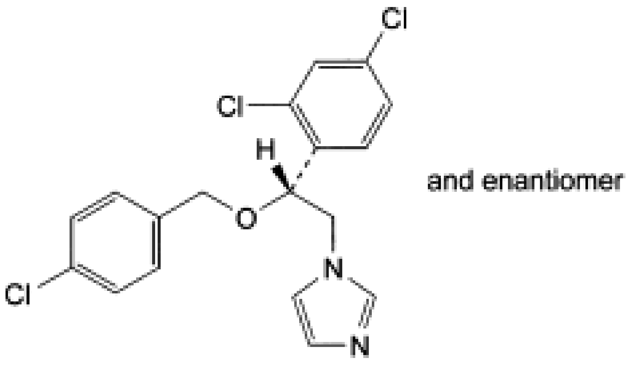
C. 1-(4-chlorobenzyl)-3-[(2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl] imidazolium.

Ph Eur

Econazole

General Notices

(Ph Eur monograph 2049)



$C_{18}H_{15}Cl_3N_2O$ 381.7 *27220-47-9*

Action and use

Antifungal.

Ph Eur

DEFINITION

1-[(2*RS*)-2-[(4-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, very soluble in alcohol and in methylene chloride.

IDENTIFICATION

A. Melting point *(2.2.14)*: 88 °C to 92 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of econazole.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *econazole for system suitability CRS* in *methanol R* and dilute to 1.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 25.0 ml with *methanol R*.

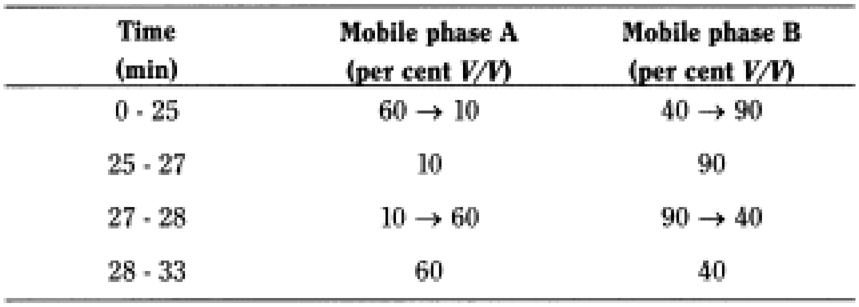
Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm),

— *temperature*: 35 °C.

Mobile phase:
— *mobile phase A*: mix 20 volumes of *methanol R* and 80 volumes of a 0.77 g/l solution of *ammonium acetate R*,

— *mobile phase B*: *methanol R, acetonitrile R* (40:60 *V/V*),



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	60 → 10	40 → 90
25 - 27	10	90
27 - 28	10 → 60	90 → 40
28 - 33	60	40

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl.

Relative retention With reference to econazole (retention time = about 15 min): impurity A = about 0.2; impurity B = about 0.6; impurity C = about 1.1.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to econazole.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.4,

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 75 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

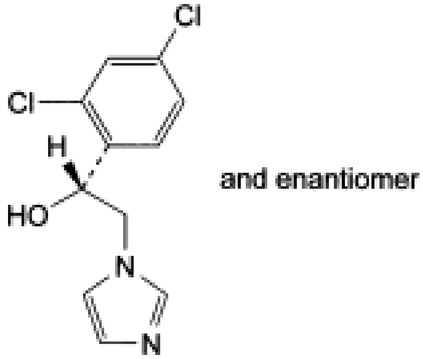
1 ml of *0.1 M perchloric acid* is equivalent to 38.17 mg of $C_{18}H_{15}Cl_3N_2O$.

STORAGE

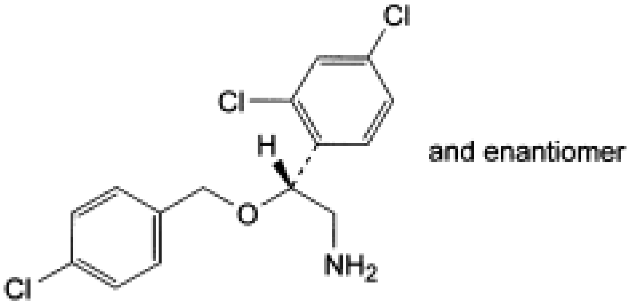
Protected from light.

IMPURITIES

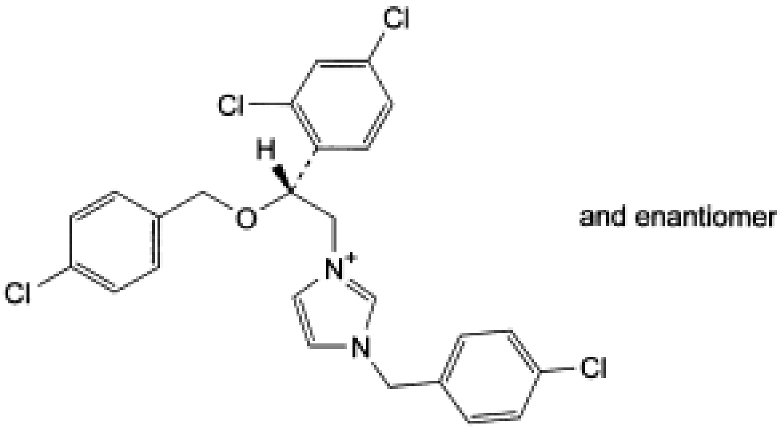
Specified impurities
A, B, C.



A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,



B. (2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethanamine,



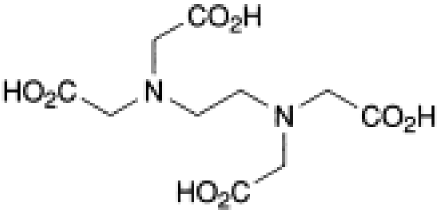
C. 1-(4-chlorobenzyl)-3-[(2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl] imidazolium.

Ph Eur

Edetic Acid

General Notices

(Ph Eur monograph 1612)



$C_{10}H_{16}N_2O_8$ 292.2 *60-00-4*

Action and use

Chelating agent.

Ph Eur

DEFINITION

(Ethylenedinitrilo)tetraacetic acid.

Content

98.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs, after drying the substance to be examined in an oven at 100-105 °C for 2 h.

Comparison *sodium edetate R*, treated as follows: dissolve 0.25 g of *sodium edetate R* in 5 ml of *water R*, add 1.0 ml of *dilute hydrochloric acid R*. Filter, wash the residue with 2 quantities, each of 5 ml, of *water R* and dry the residue in an oven at 100-105 °C for 2 h.

B. To 5 ml of *water R* add 0.1 ml of *ammonium thiocyanate solution R* and 0.1 ml of *ferric chloride solution R1* and mix. The solution is red. Add 0.5 ml of solution S (see Tests). The solution becomes yellowish.

C. To 10 ml of solution S add 0.5 ml of *calcium chloride solution R*. Make alkaline to *red litmus paper R* by the addition of *dilute ammonia R2* and add 3 ml of *ammonium oxalate solution R*. No precipitate is formed.

TESTS

Solution S

Dissolve 5.0 g in 20 ml of *dilute sodium hydroxide solution R* and dilute to 100 ml with *water R*.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Impurity A

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light.*

Solvent mixture Dissolve 10.0 g of *ferric sulphate pentahydrate R* in 20 ml of *0.5 M sulphuric acid* and add 780 ml of *water R*. Adjust to pH 2.0 with *1 M sodium hydroxide* and dilute to 1000 ml with *water R*.

Test solution Dissolve 0.100 g of the substance to be examined in 1.0 ml of *1 M sodium hydroxide* and dilute to 25.0 ml with the solvent mixture.

Reference solution Dissolve 40.0 mg of *nitrilotriacetic acid R* in the solvent mixture and dilute to 100.0 ml with the solvent mixture. To 1.0 ml of the solution add 0.1 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Column:

- *size*: l = 0.10 m, Ø = 4.6 mm,
- *stationary phase*: spherical *graphitised carbon for chromatography R1* (5 µm) with a specific surface area of 120 m^2/g and a pore size of 25 nm.

Mobile phase Dissolve 50.0 mg of *ferric sulphate pentahydrate R* in 50 ml of *0.5 M sulphuric acid* and add 750 ml of *water R*. Adjust to pH 1.5 with *0.5 M sulphuric acid* or *1 M sodium hydroxide*, add 20 ml of *ethylene glycol R* and dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µl; filter the solutions and inject immediately.

Run time 4 times the retention time of the iron complex of impurity A.

Retention time Iron complex of impurity A = about 5 min; iron complex of edetic acid = about 10 min.

System suitability Reference solution:

— *resolution*: minimum 7 between the peaks due to the iron complex of impurity A and the iron complex of edetic acid,

— *signal-to-noise ratio*: minimum 50 for the peak due to impurity A.

Limit:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Chlorides *(2.4.4)*

Maximum 200 ppm.

To 10 ml of solution S add 8 ml of *nitric acid R* and stir for 10 min. A precipitate is formed. Filter and wash the filter with *water R*. Collect the filtrate and the washings and dilute to 20 ml with *water R*. Dilute 10 ml of this solution to 15 ml with *water R*.

Iron *(2.4.9)*

Maximum 80 ppm.

Dilute 2.5 ml of solution S to 10 ml with *water R* and add 0.25 g of *calcium chloride R* before adding the *thioglycollic acid R*. Allow to stand for 5 min. Also add 0.25 g of *calcium chloride* R to the standard.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 2.0 ml of *dilute sodium hydroxide solution R* and dilute to 300 ml with water R. Add 2 g of *hexamethylenetetramine R* and 2 ml of *dilute hydrochloric acid R*. Titrate with *0.1 M zinc sulphate* using about 50 mg of *xylenol orange triturate R* as indicator.

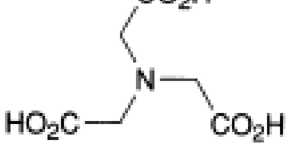
1 ml of *0.1 M zinc sulphate* corresponds to 29.22 mg of $C_{10}H_{16}N_2O_8$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*



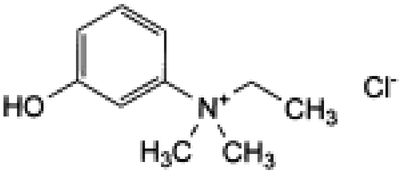
A. nitrilotriacetic acid.

Ph Eur

Edrophonium Chloride

General Notices

(Ph Eur monograph 2106)



$C_{10}H_{16}ClNO$ 201.7*1* *16-38-1*

Action and use

Cholinesterase inhibitor.

Preparation

Edrophonium Injection

Ph Eur

DEFINITION

N-Ethyl-3-hydroxy-*N,N*-dimethylanilinium chloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison edrophonium chloride CRS.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless (*2.2.2, Method II)*.

Dissolve 0.5 g in *water R* and dilute to 25 ml with the same solvent.

pH *(2.2.3)*

4.0 to 5.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *3-dimethylaminophenol R* in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Mix 1.0 ml of the test solution and 1.0 ml of reference solution (a) and dilute to 100.0 ml with *water R*. Dilute 10.0 ml of this solution to 100.0 ml with *water R*.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *styrene-divinylbenzene copolymer R* (8-10 µm).

Mobile phase Mix 10 volumes of *acetonitrile R* and 90 volumes of a 7.7 g/l solution of *tetramethylammonium bromide R* previously adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 281 nm.

Injection 20 µl.

Run time Twice the retention time of edrophonium.

Relative retention With reference to edrophonium (retention time = about 3.8 min): impurity A = about 1.3.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to edrophonium and impurity A.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *any other impurity*: for each impurity, not more than the area of the peak due to edrophonium in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *total*: not more than 5 times the area of the peak due to edrophonium in the chromatogram obtained with reference solution (b) (0.5 per cent),
- *disregard limit*: 0.5 times the area of the peak due to edrophonium in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 24 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 8.3 IU/mg.

ASSAY

Dissolve 0.150 g in 60 ml of a mixture of equal volumes of *acetic anhydride R* and *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

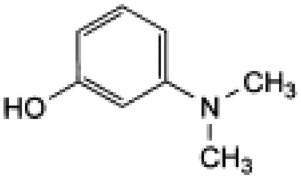
1 ml of *0.1 M perchloric acid* is equivalent to 20.17 mg of $C_{10}H_{16}ClNO$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*



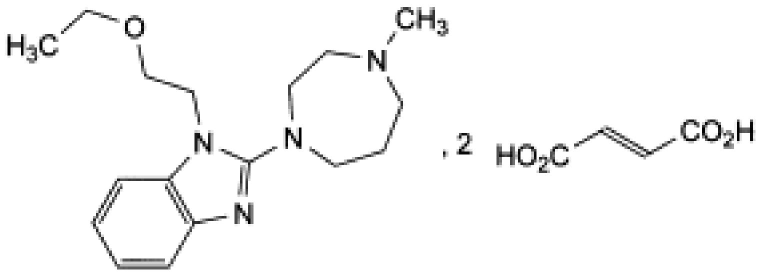
A. 3-(dimethylamino)phenol.

Ph Eur

Emedastine Fumarate

General Notices

(Emedastine Difumarate, Ph Eur monograph 2242)



$C_{25}H_{34}N_4O_9$ 534.6 *87233-62-3*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

1-(2-Ethoxyethyl)-2-(4-methylhexahydro-1*H*-1,4-diazepin-1-yl)-1*H*-benzimidazole bis [hydrogen (2*E*)-butenedioate].

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish powder.

Solubility

Soluble in water, sparingly soluble in anhydrous ethanol, very slightly soluble in acetone.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison emedastine difumarate CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Dissolve 2.50 g in *water R* and dilute to 50 ml with the same solvent.

pH *(2.2.3)*

3.0 to 4.5.

Dissolve 0.20 g in 100 ml of *carbon dioxide-free water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *emedastine impurity E CRS* in the mobile phase and dilute to 25 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of the substance to be examined in the mobile phase. Add 0.5 ml of reference solution (a) and dilute to 10 ml with the mobile phase.

Reference solution (c) Dilute 5.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.9 g of *disodium hydrogen phosphate R* and 2.5 g of *sodium dodecyl sulphate R* in *water R* and dilute to 1000.0 ml with the same solvent. Adjust to pH 2.4 with *phosphoric acid R*. Mix 550 volumes of this solution with 450 volumes of *acetonitrile R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µl of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of emedastine.

Relative retention With reference to emedastine (retention time = about 18 min): fumaric acid = about 0.1; impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.5; impurity D = about 0.7; impurity E = about 0.9; impurity F = about 1.4.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 4, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to emedastine.

Limits:

- *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peak due to fumaric acid.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

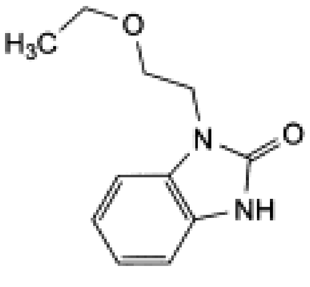
1 ml of *0.1 M perchloric acid* is equivalent to 26.73 mg of $C_{25}H_{34}N_4O_9$.

STORAGE

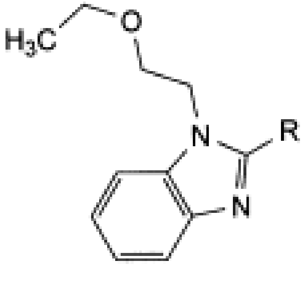
Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F.

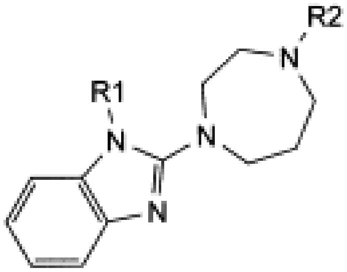


A. 1-(2-ethoxyethyl)-1,3-dihydro-2*H*-benzimidazol-2-one,



B. R = Cl: 2-chloro-1-(2-ethoxyethyl)-1*H*-benzimidazole,

F. R = NH-[CH_2]$_3$-NH-CH_3: *N*-[1-(2-ethoxyethyl)-1*H*-benzimidazol-2-yl]-*N′*-methylpropane-1, 3-diamine,



C. R1 = CH_2-CH_2OH, R2 = CH_3: 2-[2-(4-methylhexahydro-1*H*-1,4-diazepin-1-yl)-1*H*-benzimidazol-1-yl]ethanol,

D. R1 = CH=CH_2, R2 = CH_3: 1-ethenyl-2-(4-methylhexahydro-1*H*-1,4-diazepin-1-yl)-1*H*-benzimidazole,

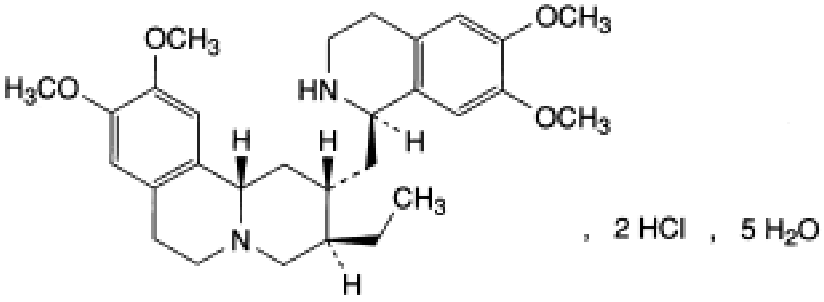
E. R1 = CH_2-CH_2-O-C_2H_5, R2 = H: 1-(2-ethoxyethyl)-2-(hexahydro-1*H*-1,4-diazepin-1-yl)-1*H*-benzimidazole.

Ph Eur

Emetine Hydrochloride Pentahydrate

General Notices

(Ph Eur monograph 0081)



$C_{29}H_{40}N_2O_4,2HCl,5H_2O$ 643.6 *316-42-7*
(anhydrous)

Action and use

Antiprotozoal.

Ph Eur

DEFINITION

Emetine hydrochloride pentahydrate contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (2*S*,3*R*,11b*S*)-2-[[(1*R*)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl]methyl]-3-ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-benzo[a]quinolizine dihydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, freely soluble in water and in alcohol.

IDENTIFICATION

First identification A, E.

Second identification B, C, D, E.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *emetine hydrochloride CRS*.

B. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence and size to the spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 2 ml of *dilute hydrogen peroxide solution R*, add 1 ml of *hydrochloric acid R* and heat. An orange colour develops.

D. Sprinkle about 5 mg on the surface of 1 ml of *sulphomolybdic reagent R2*. A bright-green colour develops.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 or BY_5 (*2.2.2, Method II)*.

pH *(2.2.3)*

Dilute 4 ml of solution S to 10 ml with *carbon dioxide-free water R*. The pH of the solution is 4.0 to 6.0.

Specific optical rotation *(2.2.7)*

Dissolve in *water R* a quantity of the substance to be examined corresponding to 1.250 g of dried substance and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 16 to + 19, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel G plate R*. *Prepare the solutions immediately before use.*

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *emetine hydrochloride CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *isoemetine hydrobromide CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 ml with the same solvent. Dilute 5 ml of this solution to 50 ml with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (c) Dissolve 10 mg of *cephaeline hydrochloride CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 ml with the same solvent. Dilute 5 ml of this solution to 50 ml with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (d) Dilute 1 ml of reference solution (a) to 100 ml with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (e) To 1 ml of reference solution (a) add 1 ml of reference solution (b) and 1 ml of reference solution (c).

Apply to the plate 10 µl of the test solution and each of reference solutions (a), (b), (c) and (d) and 30 µl of reference solution (e). Develop over a path of 15 cm using a mixture of 0.5

volumes of *diethylamine R*, 2 volumes of *water R*, 5 volumes of *methanol R*, 20 volumes of *ethylene glycol monomethyl ether R* and 100 volumes of *chloroform R*. Allow the plate to dry in air until the solvent has evaporated. Spray in a well ventilated fume-cupboard with *chloroformic solution of iodine R* and heat at 60 °C for 15 min. Examine in ultraviolet light at 365 nm. In the chromatogram obtained with the test solution, any spots corresponding to isoemetine and cephaeline are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively (2.0 per cent); any spot, apart from the principal spot and the spots corresponding to isoemetine and cephaeline, is not more intense than the spot in the chromatogram obtained with reference solution (d) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows three clearly separated spots.

Loss on drying *(2.2.32)*

11.0 per cent to 15.0 per cent, determined on 1.00 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 27.68 mg of $C_{29}H_{42}Cl_2N_2O_4$.

STORAGE

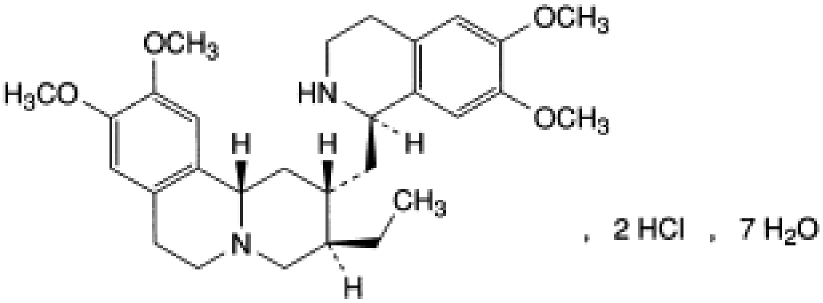
Store protected from light.

Ph Eur

Emetine Hydrochloride

General Notices

(Emetine Hydrochloride Heptahydrate, Ph Eur monograph 0080)



$C_{29}H_{40}N_2O_4,2HCl,7H_2O$ 679.7 *316-42-7*
(anhydrous)

Action and use

Antiprotozoal.

Ph Eur

DEFINITION

Emetine hydrochloride heptahydrate contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (2*S*,3*R*,11b*S*)-2-[[(1*R*)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl]methyl]-3-ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-benzo[*a*]quinolizine dihydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, freely soluble in water and in alcohol.

IDENTIFICATION

First identification A, E.

Second identification B, C, D, E.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *emetine hydrochloride CRS*.

B. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence and size to the spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 2 ml of *dilute hydrogen peroxide solution R*, add 1 ml of *hydrochloric acid R* and heat. An orange colour develops.

D. Sprinkle about 5 mg on the surface of 1 ml of *sulphomolybdic reagent R2*. A bright-green colour develops.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 or BY_5 *(2.2.2, Method II)*.

pH *(2.2.3)*

Dilute 4 ml of solution S to 10 ml with *carbon dioxide-free water R*. The pH of the solution is 4.0 to 6.0.

Specific optical rotation *(2.2.7)*

Dissolve in *water R* a quantity of the substance to be examined corresponding to 1.250 g of dried substance and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 16 to + 19, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel G plate R*. *Prepare the solutions immediately before use.*

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *emetine hydrochloride CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *isoemetine hydrobromide CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 ml with the same solvent. Dilute 5 ml of this solution to 50 ml with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (c) Dissolve 10 mg of *cephaeline hydrochloride CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 ml with the same solvent. Dilute 5 ml of this solution to 50 ml with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (d) Dilute 1 ml of reference solution (a) to 100 ml with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (e) To 1 ml of reference solution (a) add 1 ml of reference solution (b) and 1 ml of reference solution (c).

Apply to the plate 10 µl of the test solution and each of reference solutions (a), (b), (c) and (d) and 30 µl of reference solution (e). Develop over a path of 15 cm using a mixture of 0.5

volumes of *diethylamine R*, 2 volumes of *water R*, 5 volumes of *methanol R*, 20 volumes of *ethylene glycol monomethyl ether R* and 100 volumes of *chloroform R*. Allow the plate to dry in air until the solvent has evaporated. Spray in a well ventilated fume-cupboard with *chloroformic solution of iodine R* and heat at 60 °C for 15 min. Examine in ultraviolet light at 365 nm. In the chromatogram obtained with the test solution, any spots corresponding to isoemetine and cephaeline are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively (2.0 per cent); any spot, apart from the principal spot and the spots corresponding to isoemetine and cephaeline, is not more intense than the spot in the chromatogram obtained with reference solution (d) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows three clearly separated spots.

Loss on drying *(2.2.32)*

15.0 per cent to 19.0 per cent, determined on 1.00 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 27.68 mg of $C_{29}H_{42}Cl_2N_2O_4$.

STORAGE

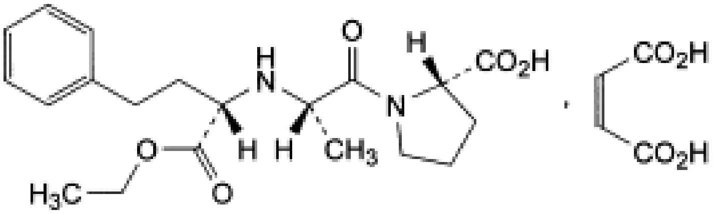
Store protected from light.

Ph Eur

Enalapril Maleate

General Notices

(Ph Eur monograph 1420)



$C_{20}H_{28}N_2O_5,C_4H_4O_4$ 492.5 *76095-16-4*

Action and use

Angiotensin converting enzyme inhibitor.

Ph Eur

DEFINITION

(2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid (*Z*)-butenedioate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in methanol, practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

mp

About 144 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *enalapril maleate CRS.*

TESTS

Solution S

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless (*2.2.2, Method II)*.

pH *(2.2.3)*

2.4 to 2.9 for solution S.

Specific optical rotation *(2.2.7)*

- 48 to - 51 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Buffer solution A Dissolve 2.8 g of *sodium dihydrogen phosphate monohydrate R* in 950 ml of *water R*. Adjust to pH 2.5 with *phosphoric acid R* and dilute to 1000 ml with *water R*.

Buffer solution B Dissolve 2.8 g of *sodium dihydrogen phosphate monohydrate R* in 950 ml of *water R*. Adjust to pH 6.8 with *strong sodium hydroxide solution R* and dilute to 1000 ml with *water R*.

Dissolution mixture Mix 50 ml of *acetonitrile R1* and 950 ml of buffer solution A.

Test solution Dissolve 30.0 mg of the substance to be examined in the dissolution mixture and dilute to 100.0 ml with the dissolution mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the dissolution mixture.

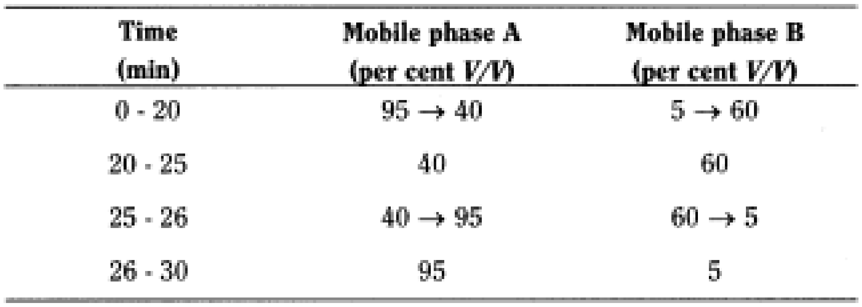
Reference solution (b) Dissolve 3.0 mg of *enalapril for system suitability CRS* in the dissolution mixture and dilute to 10.0 ml with the dissolution mixture.

Column:

- — *size: l* = 0.15 m, Ø = 4.1 mm;
- — *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm);
- — *temperature*: 70 °C.

Mobile phase:

- — *mobile phase A*: mix 50 ml of *acetonitrile R1* and 950 ml of buffer solution B;
- — *mobile phase B*: mix 340 ml of buffer solution B and 660 ml of *acetonitrile R1*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 20	95 → 40	5 → 60
20 - 25	40	60
25 - 26	40 → 95	60 → 5
26 - 30	95	5

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 50 µl.

Retention time Enalapril = about 11 min; impurity A = about 12 min.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 10, where H_r = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to enalapril.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *impurities B, C, D, E, H*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to maleic acid.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 30 ml with the same solvent. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20)*. Titrate to the 2nd point of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 16.42 mg of $C_{24}H_{32}N_2O_9$.

STORAGE

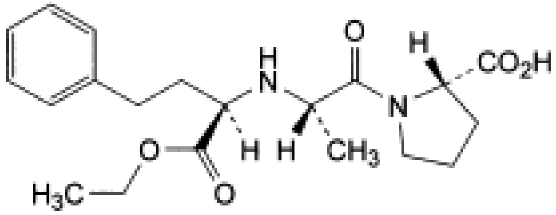
Protected from light.

IMPURITIES

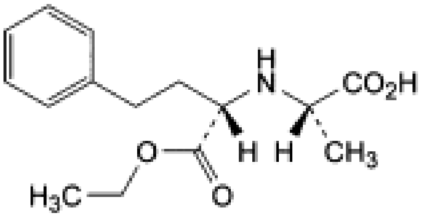
Specified impurities *A, B, C, D, E, H.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*

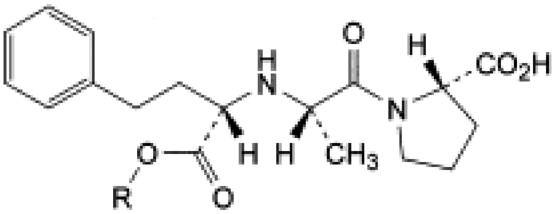
F, G, I.



A. (2*S*)-1-[(2*S*)-2-[[(1*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,



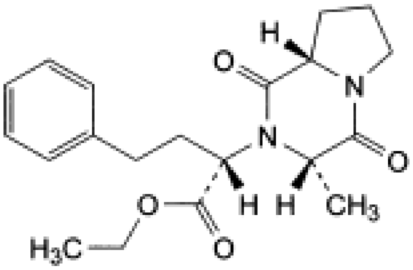
B. (2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoic acid,



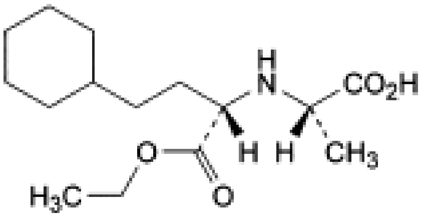
C. R = H: (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,

E. R = CH_2-CH_2-C_6H_5: (2*S*)-1-[(2*S*)-2-[[(1*S*)-3-phenyl-1-[(2phenylethoxy)carbonyl]propyl] amino]propanoyl]pyrrolidine-2-carboxylic acid,

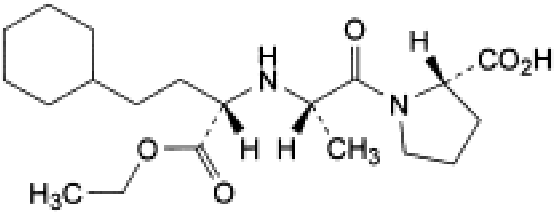
F. R = C_4H_9: (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(butoxycarbonyl)-3-phenylpropyl]amino]propanoyl] pyrrolidine-2-carboxylic acid,



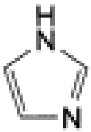
D. ethyl (2*S*)-2-[(3*S*,8a*S*)-3-methyl-1,4-dioxo-octahydropyrrolo[1,2-*a*]pyrazin-2-yl]-4-phenylbutanoate,



G (2*S*)-2-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)propyl]amino]propanoic acid,



H. (2*S*)-1-[(2*S*)-2-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)propyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,



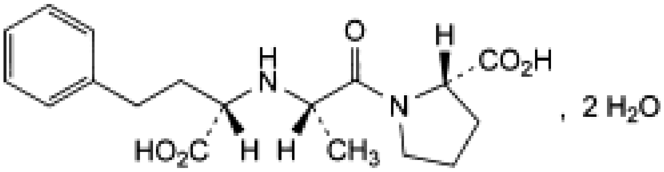
I. 1*H*-imidazole.

Ph Eur

Enalaprilat Dihydrate

General Notices

(Ph Eur monograph 1749)



$C_{18}H_{24}N_2O_5,2H_2O$ 384.4 *76420-72-9*
(anhydrous)

Action and use

Angiotensin converting enzyme inhibitor.

Ph Eur

DEFINITION

(2*S*)-1-[(2*S*)-2-[[(1*S*)-1-Carboxy-3-phenylpropyl]amino]propanoyl)pyrrolidine-2-carboxylic acid dihydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Very slightly soluble or slightly soluble in water, sparingly soluble in methanol, practically insoluble in acetonitrile.

It shows pseudopolymorphism *(5.9)*.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Mulls in *liquid paraffin R*.

Comparison *enalaprilat dihydrate CRS.*

If the spectra obtained show differences, expose the substance to be examined and the reference substance to a 98 per cent relative humidity for 3 days using a chamber conditioned with a saturated solution of *calcium sulphate R*. Record new spectra.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.10 g in *water R* and dilute to 100.0 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 53.0 to - 56.0 (anhydrous substance).

Dissolve 0.200 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Use freshly prepared solutions*.

Buffer solution Dissolve 1.36 g of *potassium dihydrogen phosphate R* in 950 ml of *water R*. Adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 ml with *water R*.

Solvent mixture Buffer solution, *acetonitrile R1, methanol R1* (1:2:2 *V/V/V*).

Dissolution mixture Solvent mixture, buffer solution (8:92 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in 2.5 ml of *methanol R1* and dilute to 25.0 ml with the dissolution mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the dissolution mixture. Dilute 5.0 ml of this solution to 10.0 ml with the dissolution mixture.

Reference solution (b) Dissolve 5 mg of *enalaprilat for system suitability CRS* (containing impurity C) in 0.5 ml of *methanol R1* and dilute to 5 ml with the dissolution mixture.

Reference solution (c) Dissolve the contents of a vial of *enalaprilat impurity G CRS* in 1 ml of the test solution.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

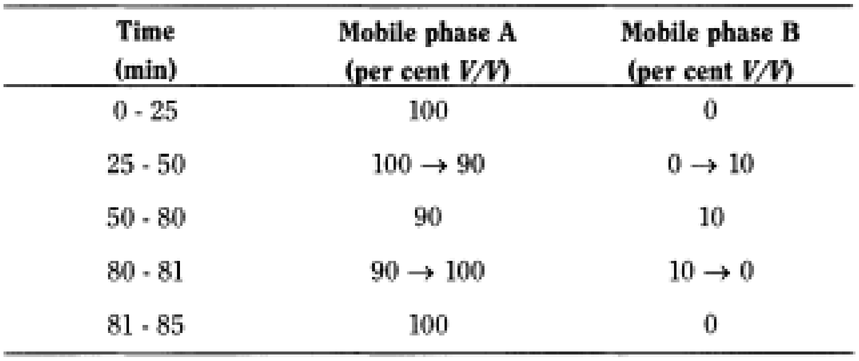
— *stationary phase*: end-*capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 70 °C.

Mobile phase:

— *mobile phase A*: solvent mixture, buffer solution (10:90 *V/V*);

— *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 50	100 → 90	0 → 10
50 - 80	90	10
80 - 81	90 → 100	10 → 0
81 - 85	100	0

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *enalaprilat for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity G.

Relative retention With reference to enalaprilat (retention time = about 21 min): impurity C = about 1.2; impurity G = about 2.9.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to enalaprilat.

Limits:

— *impurities C, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test G. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

7.0 per cent to 11.0 per cent, determined on 0.100 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.1 IU/mg.

ASSAY

Dissolve 0.300 g in *glacial acetic acid R* and dilute to 50 ml with the same solvent. Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 34.84 mg of $C_{18}H_{24}N_2O_5$.

STORAGE

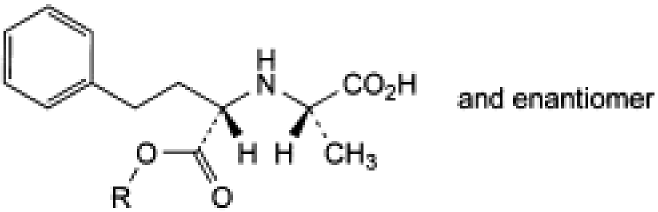
In an airtight container .

IMPURITIES

Specified impurities   C, G.

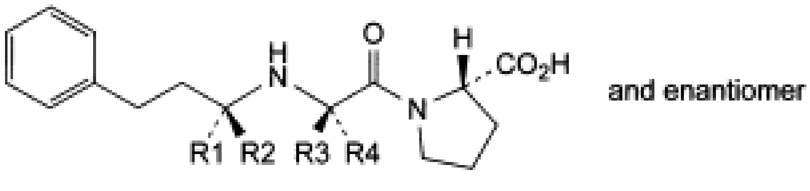
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*

A, B, D, E, F.



A. R = H: (2*SR*)-2-[[(1*SR*)-1-carboxyethyl]amino]-4-phenylbutanoic acid,

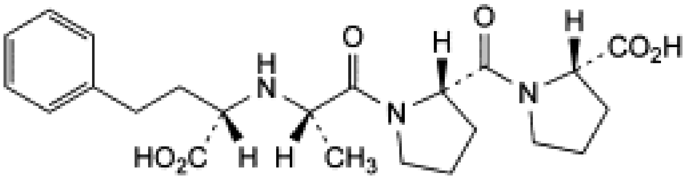
F. R = C_2H_5: (2*SR*)-2-[[(1*SR*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoic acid,



B. R1 = R4 = H, R2 = CO_2H, R3 = CH_3: (2*SR*)-1-[(2*RS*)-2-[[(1*RS*)-1-carboxy-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,

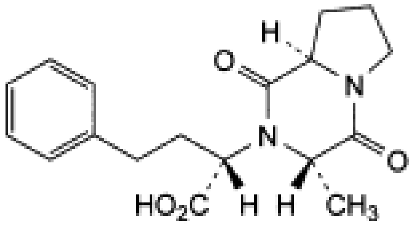
C. R1 = R3 = H, R2 = CO_2H, R4 = CH_3: (2*SR*)-1-[(2*SR*)-2-[[(1*RS*)-1-carboxy-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,

D. R1 = CO_2H, R2 = R4 = H, R3 = CH_3: (2*SR*)-1-[(2*RS*)-2-[[(1*SR*)-1-carboxy-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,



and enantiomer

E. (2*SR*)-1-[[(2*SR*)-1-[(2*SR*)-2-[[(1*SR*)-1-carboxy-3-phenylpropyl]amino]propanoyl]pyrrolidin-2-yl]carbonyl]pyrrolidine-2-carboxylic acid,



and enantiomer

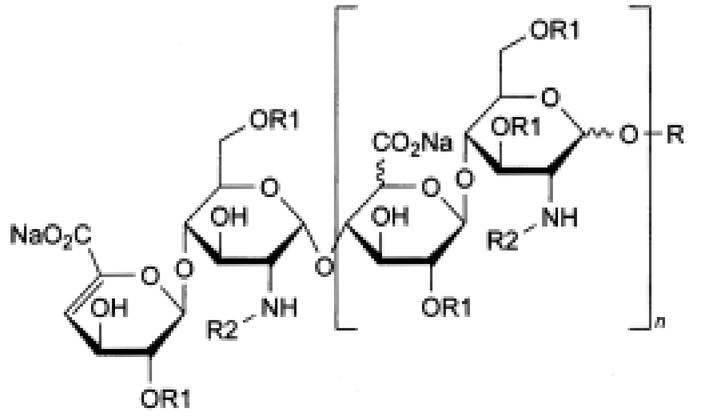
G. (2*SR*)-2-[(3*SR*,8a*RS*)-3-methyl-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid.

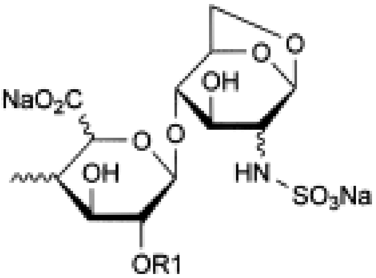
Ph Eur

Enoxaparin Sodium

General Notices

(Ph Eur monograph 1097)



	Structure at the 'reducing end'	
	1,6-anhydro	non 1,6-anhydro
n	0 to 20	1 to 21
—R		—H

R1 = H or SO_3Na R2 = SO_3Na or $CO\text{-}CH_3$

Action and use

Low molecular weight heparin.

Ph Eur

DEFINITION

Enoxaparin sodium is the sodium salt of a low-molecular-mass heparin that is obtained by alkaline depolymerisation of the benzyl ester derivative of heparin from porcine intestinal mucosa. Enoxaparin consists of a complex set of oligosaccharides that have not yet been completely characterised. Based on current knowledge, the majority of the components have a 4-enopyranose uronate structure at the non-reducing end of their chain. 15 per cent to 25 per cent of the components have a 1,6-anhydro structure at the reducing end of their chain.

Enoxaparin sodium complies with the monograph Low-molecular-mass heparins (0828) with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 3800 and 5000, with a characteristic value of about 4500.

The degree of sulphatation is about 2 per disaccharide unit.

The potency is not less than 90 IU and not more than 125 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The anti-factor IIa activity is not less than 20.0 IU and not more than 35.0 IU per milligram, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 3.3 and 5.3.

PRODUCTION

Enoxaparin is produced by alkaline depolymerisation of benzyl ester derivatives of heparin from porcine intestinal mucosa under conditions that yield a product complying with the structural requirements stated under Definition.

IDENTIFICATION

Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *enoxaparin sodium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. The following requirements apply.

The mass-average relative molecular mass ranges between 3800 and 5000. The mass percentage of chains lower than 2000 ranges between 12.0 per cent and 20.0 per cent. The mass percentage of chains between 2000 and 8000 ranges between 68.0 per cent and 82.0 per cent.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II)*.

Dissolve 1.0 g in 10 ml of *water R*.

pH *(2.2.3)*

6.2 to 7.7.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Specific absorbance *(2.2.25)*

14.0 to 20.0 (dried substance), determined at 231 nm.

Dissolve 50.0 mg in 100 ml of *0.01 M hydrochloric acid*.

Benzyl alcohol

Liquid chromatography *(2.2.29)*.

Internal standard solution 1 g/l solution of *3,4-dimethylphenol R* in *methanol R*.

Test solution Dissolve about 0.500 g of the substance to be examined in 5.0 ml of *1 M sodium hydroxide*. Allow to stand for 1 h. Add 1.0 ml of *glacial acetic acid R* and 1.0 ml of the internal standard solution and dilute to 10.0 ml with *water R*.

Reference solution Prepare a 0.25 g/l solution of *benzyl alcohol R* in *water R*. Mix 0.50 ml of this solution with 1.0 ml of the internal standard solution and dilute to 10.0 ml with *water R*.

Precolumn:
- — *size: l* = 0.02 m, Ø = 4.6 mm;
- — *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Column:
- — *size: l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, acetonitrile R, water R* (5:15:80 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 256 nm.

From the chromatogram obtained with the reference solution, calculate the ratio (R_1) of the height of the peak due to benzyl alcohol to the height of the peak due to the internal standard. From the chromatogram obtained with the test solution, calculate the ratio (R_2) of the height of the peak due to benzyl alcohol to the height of the peak due to the internal standard.

Calculate the percentage content (*m/m*) of benzyl alcohol using the following expression:

$$\frac{0.0125 \times R_2}{m \times R_1}$$

m = mass of the substance to be examined, in grams.

Limit:
- — *benzyl alcohol*: maximum 0.1 per cent *m/m*.

Sodium *(2.2.23, Method I)*

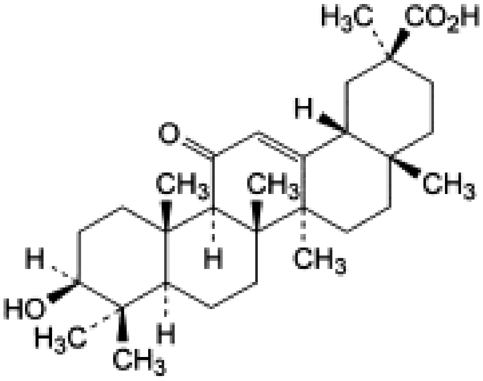
11.3 per cent to 13.5 per cent (dried substance).

Ph Eur

Enoxolone

General Notices

(Ph Eur monograph 1511)



$C_{30}H_{46}O_4$ 470.7 *471-53-4*

Action and use

Treatment of benign peptic ulcer disease.

Ph Eur

DEFINITION

(20β)-3β-Hydroxy-11-oxo-olean-12-en-29-oic acid.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Practically insoluble in water, soluble in ethanol, sparingly soluble in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification *A.*

Second identification   *B, C.*

A. Examine by infrared absorption spectrophotometry *(2.2.24).*

Comparison   *enoxolone CRS.*

If the spectra obtained in the solid state show differences, dissolve 0.2 g of the substance to be examined and 0.2 g of the reference substance separately in 6 ml of *ethanol R*. Boil under a reflux condenser for 1 h and add 6 ml of *water R*. A precipitate is formed. Cool to about 10 °C and filter with the aid of vacuum. Wash the precipitate with 10 ml of *alcohol R*, dry in an oven at 80 °C and record new spectra.

B. Thin-layer chromatography *(2.2.27)*.

Test solution   Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution   Dissolve 10 mg of *enoxolone CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Plate   *TLC silica gel plate R.*

Mobile phase   *glacial acetic acid R, acetone R, methylene chloride R* (5:10:90 *V/V/V*).

Application   5 µl.

Development   Over 2/3 of the plate.

Drying   In air for 5 min.

Detection   Spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min.

Results   The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 50 mg in 10 ml of *methylene chloride R*. To 2 ml of this solution, add 1 ml of *acetic anhydride R* and 0.3 ml of *sulphuric acid R*. A pink colour is produced.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II)*.

Dissolve 0.1 g in *ethanol R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 145 to + 154 (dried substance).

Dissolve 0.50 g in *dioxan R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution   Dissolve 0.10 g of the substance to be examined in the mobile phaseand dilute to 100.0 ml with the mobile phase.

Reference solution (a)   Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 0.1 g of *18α-glycyrrhetinic acid R* in *tetrahydrofuran R* and dilute to 100.0 ml with the same solvent. To 2.0 ml of the solution, add 2.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 30 °C.

Mobile phase Mix 430 volumes of *tetrahydrofuran R* and 570 volumes of a 1.36 g/l solution of *sodium acetate R* adjusted to pH 4.8 with *glacial acetic acid R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 250 nm.

Injection 20 µl loop injector; inject the test solution and the reference solutions.

Run time 4 times the retention time of enoxolone.

System suitability:
- *resolution*: minimum of 2.0 between the peaks due to enoxolone and to 18α-glycyrrhetinic acid in the chromatogram obtained with reference solution (c).

Limits:
- *any impurity*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent),
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent),
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test F. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

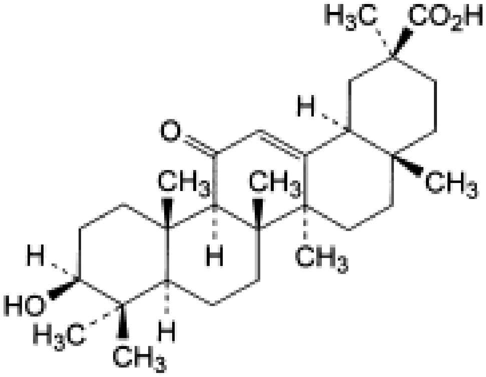
Dissolve 0.330 g in 40 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 47.07 mg of $C_{30}H_{46}O_4$.

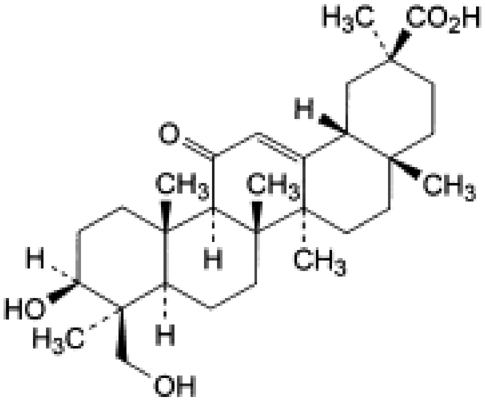
STORAGE

Protected from light.

IMPURITIES



A. (20β)-3β-hydroxy-11-oxo-18α-olean-12-en-29-oic acid,



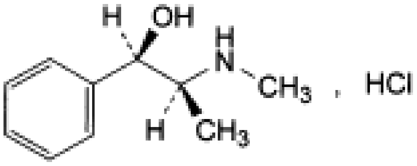
B. (4β,20β)-3β,23-dihydroxy-11-oxo-olean-12-en-29-oic acid.

Ph Eur

Ephedrine Hydrochloride

General Notices

(Ph Eur monograph 0487)



$C_{10}H_{15}NO,HCl$ 201.7 *50-98-6*

Action and use

Adrenoceptor agonist.

Preparations

Ephedrine Elixir

Ephedrine Nasal Drops

Ephedrine Hydrochloride Tablets

Ph Eur

DEFINITION

(1*R*,2*S*)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

mp

About 219 °C.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ephedrine hydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *ephedrine hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *methylene chloride R, concentrated ammonia R, 2-propanol R* (5:15:80 *V/V/V*) .

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R*; heat at 110 °C for 5 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 0.1 ml of solution S (see Tests) add 1 ml of *water R*, 0.2 ml of *copper sulphate solution R* and 1 ml of *strong sodium hydroxide solution R*. A violet colour is produced. Add 2 ml of *methylene chloride R* and shake. The lower (organic) layer is dark grey and the upper (aqueous) layer is blue.

E. To 5 ml of solution S (see Tests) add 5 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 5.00 g in distilled *water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Specific optical rotation *(2.2.7)*

- 33.5 to - 35.5 (dried substance).

Dilute 12.5 ml of solution S to 25.0 ml with *water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 75 mg of the substance to be examined in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *pseudoephedrine hydrochloride CRS* in the mobile phase and dilute to 50 ml with the mobile phase.

Column:
- — *size: l* = 0.15 m, Ø = 4.6 mm,
- — *stationary phase*: spherical *phenylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Mix 6 volumes of *methanol R* and 94 volumes of a 11.6 g/l solution of *ammonium acetate R* adjusted to pH 4.0 with *glacial acetic acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 257 nm.

Injection 20 µl.

Run time 2.5 times the retention time of ephedrine.

Relative retention With reference to ephedrine (retention time = about 8 min): impurity B = about 1.1; impurity A = about 1.4.

System suitability Reference solution (b):

- — *resolution*: minimum 2.0 between the peaks due to ephedrine and impurity B.

Limits:
- — *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.4,
- — *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
- — *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- — *sum of impurities other than A*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- — *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphates *(2.4.13)*

Maximum 100 ppm, determined on solution S.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 20.17 mg of $C_{10}H_{16}ClNO$.

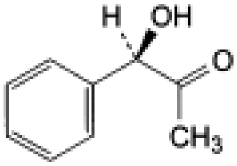
STORAGE

Protected from light.

IMPURITIES

Specified impurities A.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : B.*



A. (-)-(1*R*)-1-hydroxy-1-phenylpropan-2-one,

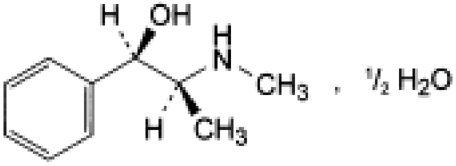
B. pseudoephedrine.

Ph Eur

Ephedrine

General Notices

(Ephedrine Hemihydrate, Ph Eur monograph 0489)



$C_{10}H_{15}NO,½H_2O$ 174.2 *50906-05-3*

Action and use

Adrenoceptor agonist.

Ph Eur

DEFINITION

Ephedrine hemihydrate contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (1*R*,2*S*)-2-(methylamino)-1-phenylpropan-1-ol, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, soluble in water, very soluble in alcohol.

It melts at about 42 °C, determined without previous drying.

IDENTIFICATION

First identification B, D.

Second identification A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with the base isolated from *ephedrine hydrochloride CRS*. Examine the substances in discs prepared as follows: dissolve 40 mg of the substance to be examined in 1 ml of *water R*, add 1 ml of *dilute sodium hydroxide solution R* and 4 ml of *chloroform R* and shake; dry the organic layer over 0.2 g of *anhydrous sodium sulphate R*; prepare a blank disc using about 0.3 g of *potassium bromide R*; apply dropwise to the disc 0.1 ml of the organic layer, allowing the solvent to evaporate between applications; dry the disc at 50 °C for 2 min. Repeat the operations using 50 mg of *ephedrine hydrochloride CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 1 ml of *water R*. Add 0.2 ml of *strong sodium hydroxide solution R* and 0.2 ml of *copper sulphate solution R*. A violet colour is produced. Add 2 ml of *ether R* and shake. The ether layer is purple and the aqueous layer blue.

E. It complies with the test for water (see Tests).

TESTS

Appearance of solution

Dissolve 0.25 g in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation (*2.2.7)*

Dissolve 2.25 g in 15 ml of *dilute hydrochloric acid R* and dilute to 50.0 ml with *water R*. The specific optical rotation is - 41 to - 43, calculated with reference to the anhydrous substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 25 mg of *ephedrine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 200 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *chloroform R*, 15 volumes of *concentrated ammonia R* and 80 volumes of *2-propanol R*. Allow the plate to dry in air and spray with *ninhydrin solution R*. Heat at 110 °C for 5 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Disregard any spot of lighter colour than the background.

Chlorides

Dissolve 0.18 g in 10 ml of *water R*. Add 5 ml of *dilute nitric acid R* and 0.5 ml of *silver nitrate solution R1*. Allow to stand for 2 min, protected from bright light. Any opalescence in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of *chloride standard solution (5 ppm Cl) R*, 5 ml of *dilute nitric acid R* and 0.5 ml of *silver nitrate solution R1* (280 ppm).

Water *(2.5.12)*

4.5 per cent to 5.5 per cent, determined on 0.300 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 5 ml of *alcohol R* and add 20.0 ml of *0.1 M hydrochloric acid*. Using 0.05 ml of *methyl red solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a yellow colour is obtained.

1 ml of *0.1 M hydrochloric acid* is equivalent to 16.52 mg of $C_{10}H_{15}NO$.

STORAGE

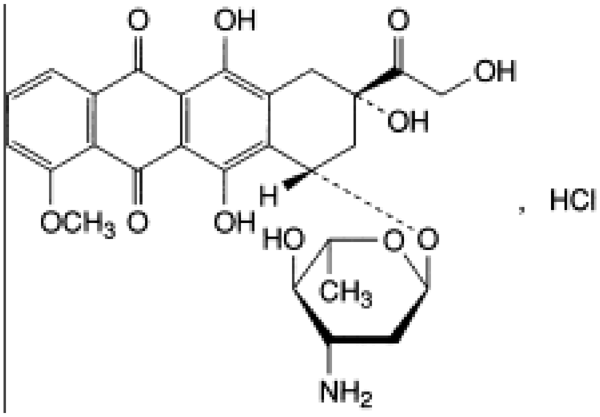
Store protected from light.

Ph Eur

Epirubicin Hydrochloride

General Notices

(Ph Eur monograph 1590)



$C_{27}H_{29}NO_{11},HCl$ 580   *56390-09-1*

Action and use

Cytotoxic.

Ph Eur

DEFINITION

(8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-arabino-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride.

Substance obtained by chemical transformation of a substance produced by certain strains of *Streptomyces peucetius*.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Orange-red powder.

Solubility

Soluble in water and in methanol, slightly soluble in anhydrous ethanol, practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *epirubicin hydrochloride CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 0.5 ml of *nitric acid R*, add 0.5 ml of *water R* and heat over a flame for 2 min. Allow to cool and add 0.5 ml of *silver nitrate solution R1*. A white precipitate is formed.

TESTS

pH *(2.2.3)*

4.0 to 5.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Allow the solutions to stand for 3 h before use.*

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *epirubicin hydrochloride CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *epirubicin hydrochloride CRS* and 10 mg of *doxorubicin hydrochloride CRS* in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (c) Dissolve 10 mg of *doxorubicin hydrochloride CRS* in a mixture of 5 ml of *water R* and 5 ml of *phosphoric acid R*. Allow to stand for 30 min. Adjust to pH 2.6 with an 80 g/l solution of *sodium hydroxide R*. Add 15 ml of *acetonitrile R* and 10 ml of *methanol R*. Mix.

Reference solution (d) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *trimethylsilyl silica gel for chromatography R* (6 µm);
- *temperature*: 35 °C.

Mobile phase Mix 17 volumes of *methanol R*, 29 volumes of *acetonitrile R* and 54 volumes of a solution containing 3.7 g/l of *sodium laurilsulfate R* and 2.8 per cent *V/V* of *dilute phosphoric acid R*.

Flow rate 2.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl of the test solution and reference solutions (b), (c) and (d).

Run time 3.5 times the retention time of epirubicin.

Identification of impurities Use the 2nd most abundant peak present in the chromatogram obtained with reference solution (c) to identify impurity A.

Relative retention With reference to epirubicin (retention time = about 9.5 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.8; impurity E = about 1.1; impurity D = about 1.5; impurity F = about 1.7; impurity G = about 2.1.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity C and epirubicin.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.7;

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Acetone *(2.4.24)*

Maximum 1.5 per cent.

Water *(2.5.12)*

Maximum 4.0 per cent, determined on 0.100 g.

Bacterial endotoxins *(2.6.14)*

Less than 1.1 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

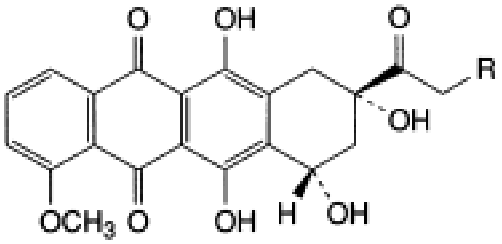
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{27}H_{30}ClNO_{11}$.

STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

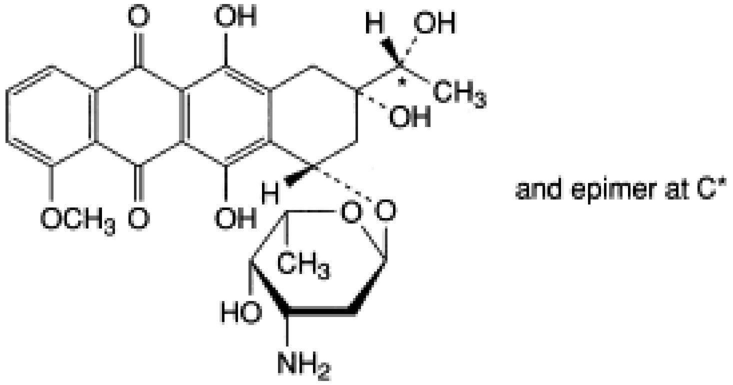


A. R = OH: (8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (doxorubicinone),

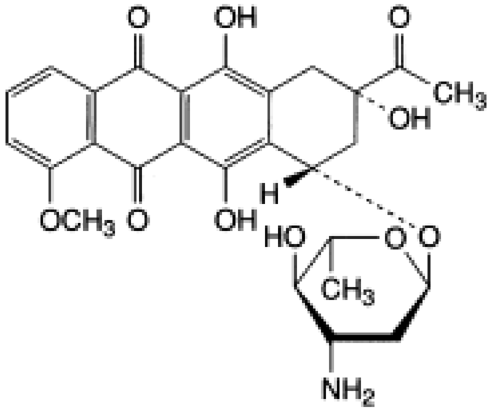
B. R = H: (8*S*,10*S*)-8-acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicinone),

C. doxorubicin,

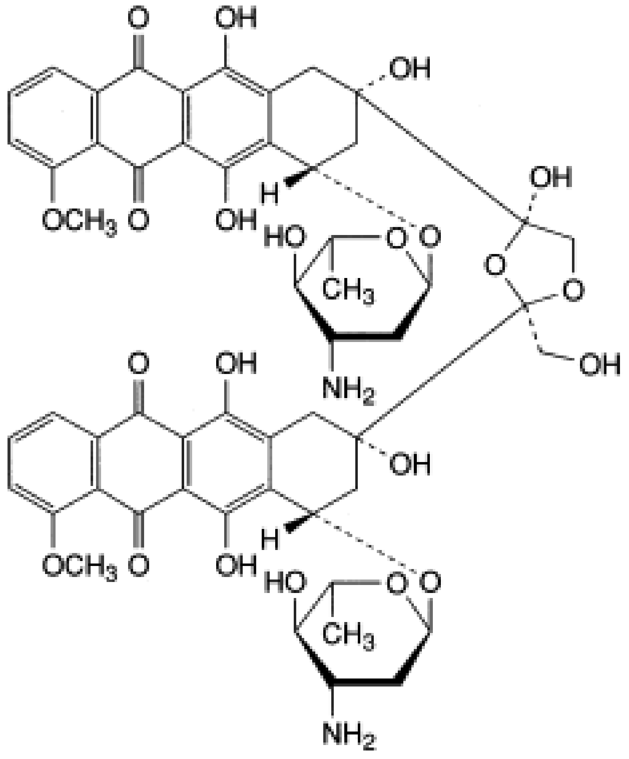
D. daunorubicin,



E. (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-[(1*RS*)-1-hydroxyethyl]-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (dihydrodaunorubicin),



F. (8*S*,10*S*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (epi-daunorubicin),



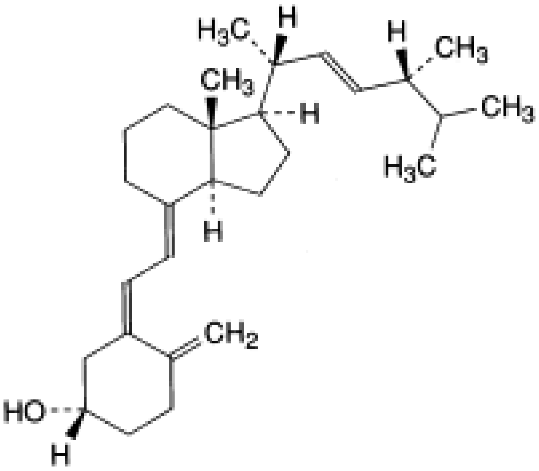
G. 8,8′-[(2*R*,4*R*)-4-hydroxy-2-(hydroxymethyl)-1,3-dioxolan-2,4-diyl]bis[(8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione] (epirubicin dimer).

Ph Eur

Ergocalciferol

General Notices

(Ph Eur monograph 0082)



$C_{28}H_{44}O$ 396.7 *50-14-6*

Action and use

Vitamin D analogue (Vitamin D_2).

Preparations

Calcium and Ergocalciferol Tablets

Ergocalciferol Injection

Ergocalciferol Tablets

When vitamin D_2 is prescribed or demanded, Ergocalciferol shall be dispensed or supplied. When calciferol or vitamin D is prescribed or demanded, Ergocalciferol or Colecalciferol shall be dispensed or supplied.

Ph Eur

DEFINITION

Ergocalciferol contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of (5*Z*,7*E*,22*E*)-9,10-secoergosta-5,7,10(19),22-tetraen-3β-ol.

1 mg of ergocalciferol is equivalent to 40 000 IU of antirachitic activity (vitamin D) in rats.

CHARACTERS

A white or slightly yellowish, crystalline powder or white or almost white crystals, practically insoluble in water, freely soluble in alcohol, soluble in fatty oils. It is sensitive to air, heat and light. Solutions in volatile solvents are unstable and are to be used immediately.

A reversible isomerisation to pre-ergocalciferol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *ergocalciferol CRS*. Examine the substances prepared as discs.

TESTS

Specific optical rotation *(2.2.7)*

Dissolve 0.200 g rapidly and without heating in *aldehyde-free alcohol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation, determined within 30 min of preparing the solution, is + 103 to + 107.

Reducing substances

Dissolve 0.1 g in *aldehyde-free alcohol R* and dilute to 10.0 ml with the same solvent. Add 0.5 ml of a 5 g/l solution of *tetrazolium blue R* in *aldehyde-free alcohol R* and 0.5 ml of *dilute tetramethylammonium hydroxide solution R*. Allow to stand for exactly 5 min and add 1.0 ml of *glacial acetic acid R*. Prepare a reference solution at the same time and in the same manner using 10.0 ml of a solution containing 0.2 µg/ml of *hydroquinone R* in aldehyde*-free alcohol R*. Measure the absorbance *(2.2.25)* of the two solutions at 525 nm using as the compensation liquid 10.0 ml of *aldehyde-free alcohol R* treated in the same manner. The absorbance of the test solution is not greater than that of the reference solution (20 ppm).

Ergosterol

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel G plate R*.

Test solution Dissolve 0.25 g of the substance to be examined in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 5 ml with the same solvent. Prepare immediately before use.

Reference solution (a) Dissolve 0.10 g of *ergocalciferol CRS* in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 2 ml with the same solvent. Prepare immediately before use.

Reference solution (b) Dissolve 5 mg of *ergosterol CRS* in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 50 ml with the same solvent. Prepare immediately before use.

Reference solution (c) Mix equal volumes of reference solution (a) and reference solution (b) . Prepare immediately before use.

Apply to the plate 10 µl of the test solution, 10 µl of reference solution (a), 10 µl of reference solution (b) and 20 µl of reference solution (c). Develop immediately, protected from light, over a path of 15 cm using a mixture of equal volumes of *cyclohexane R* and *peroxide-free ether R*, the mixture containing 0.1 g/l of *butylhydroxytoluene R*. Allow the plate to dry in air and spray three times with *antimony trichloride solution R1*. Examine the chromatograms for 3 min to 4 min after spraying. The principal spot in the chromatogram obtained with the test solution is initially orange-yellow and then becomes brown. In the chromatogram obtained with the test solution, any slowly appearing violet spot (corresponding to ergosterol) immediately below the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). There is no spot in the chromatogram obtained with the test solution that does not correspond to one of the spots in the chromatograms obtained with reference solutions (a) and (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

ASSAY

Carry out the operations as rapidly as possible, avoiding exposure to actinic light and air.

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined without heating in 10.0 ml of *toluene R* and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *ergocalciferol CRS* without heating in 10.0 ml of *toluene R* and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of *cholecalciferol for performance test CRS* to 5.0 ml with the mobile phase. Heat in a water-bath at 90 °C under a reflux condenser for 45 min and cool.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with a suitable silica gel (5 µm),

— as mobile phase at a flow rate of 2 ml/min a mixture of 3 volumes of *pentanol R* and 997 volumes of *hexane R*,

— as detector a spectrophotometer set at 254 nm.

An automatic injection device or a sample loop is recommended. Inject a suitable volume of reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder. Inject reference solution (b) 6 times. When the chromatograms are recorded in the prescribed conditions, the approximate relative retention times with reference to cholecalciferol are 0.4 for pre-cholecalciferol and 0.5 for *trans*-cholecalciferol. The relative standard deviation of the response for cholecalciferol is not greater than 1 per cent and the resolution between the peaks corresponding to pre-cholecalciferol and *trans*-cholecalciferol is not less than 1.0. If necessary adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution.

Inject a suitable volume of reference solution (a). Adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder. Inject the same volume of the test solution and record the chromatogram in the same manner.

Calculate the percentage content of ergocalciferol from the expression:

$$\frac{m'}{m} \times \frac{S_D}{S'_D} \times 100$$

m = mass of the substance to be examined in the test solution, in milligrams,

m' = mass of *ergocalciferol CRS* in reference solution (a), in milligrams,

S_D = area (or height) of the peak due to ergocalciferol in the chromatogram obtained with the test solution,

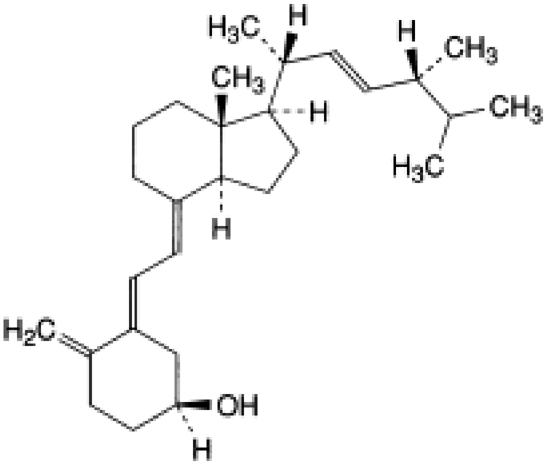
S'_D = area (or height) of the peak due to ergocalciferol in the chromatogram obtained with reference solution (a).

STORAGE

Store in an airtight container, under nitrogen, protected from light, at a temperature between 2 °C and 8 °C.

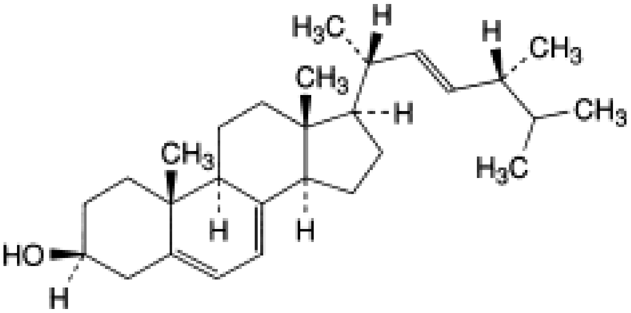
The contents of an opened container are to be used immediately.

IMPURITIES



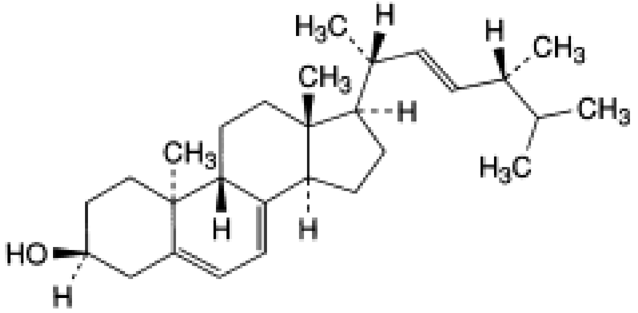
H3C
H
H
CH3
CH3
H
CH3
H3C
H
H2C
OH
H

A. (5*E*,7*E*,22*E*)-9,10-secoergosta-5,7,10(19),22-tetraen-3β-ol (*trans*-vitamin D_2),

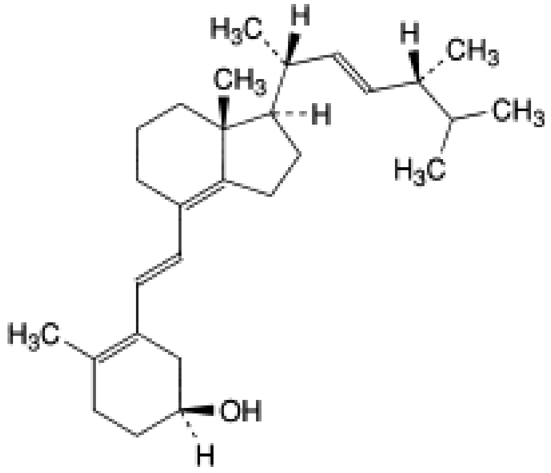


H3C
H
H
CH3
CH3
H
CH3
CH3
H3C
H
H
HO
H

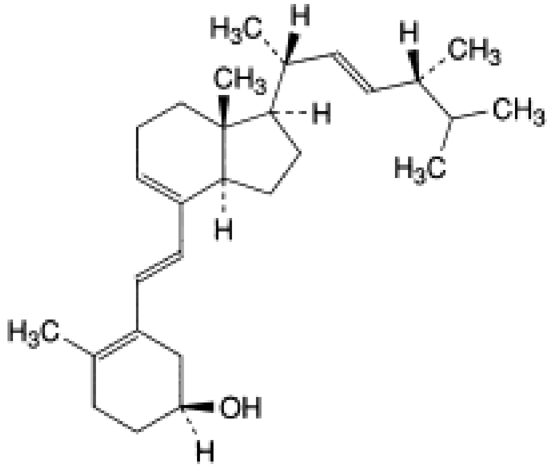
B. (22*E*)-ergosta-5,7,22-trien-3β-ol (ergosterol),



C. (9β,10α,22*E*)-ergosta-5,7,22-trien-3β-ol (lumisterol$_2$),



D. (6*E*,22*E*)-9,10-secoergosta-5(10),6,8(14),22-tetraen-3β-ol (iso-tachysterol$_2$),



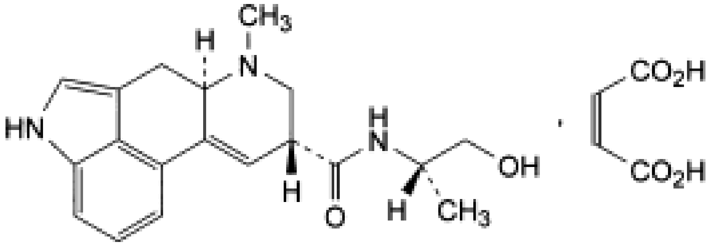
E. (6*E*,22*E*)-9,10-secoergosta-5(10),6,8,22-tetraen-3β-ol (tachysterol$_2$).

Ph Eur

Ergometrine Maleate

General Notices

(Ph Eur monograph 0223)



$C_{19}H_{23}N_3O_2,C_4H_4O_4$ 441.5 *129-51-1*

Action and use

Oxytocic.

Preparations

Ergometrine Injection

Ergometrine and Oxytocin Injection

Ergometrine Tablets

Ph Eur

DEFINITION

Ergometrine maleate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of (6a*R*,9*R*)-*N*-[(*S*)-2-hydroxy-1-methylethyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (*Z*)-butenedioate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white or slightly coloured, crystalline powder, sparingly soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification B, C.

Second identification A, C, D, E.

A. Dissolve 30 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M hydrochloric acid*. Examined between

250 nm and 360 nm *(2.2.25)*, the solution shows an absorption maximum at 311 nm and a minimum at 265 nm to 272 nm. The specific absorbance at the maximum is 175 to 195.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *ergometrine maleate CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 ml of solution S (see Tests) add 1 ml of *glacial acetic acid R*, 0.05 ml of *ferric chloride solution R1* and 1 ml of *phosphoric acid R* and heat in a water-bath at 80 °C. After about 10 min, a blue or violet colour develops which becomes more intense on standing.

E. Dissolve 0.1 g in a mixture of 0.5 ml of *dilute sulphuric acid R* and 2.5 ml of *water R*. Add 5 ml of *ether R* and 1 ml of *strong sodium hydroxide solution R* and shake. Separate the aqueous layer and shake with two quantities, each of 5 ml, of *ether R*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min. No colour develops. To the rest of the aqueous layer add 1 ml of *bromine water R*. Heat on a water-bath for 10 min, then heat to boiling and cool. To 0.2 ml of this solution add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min. A pinkish-violet colour develops.

TESTS

Solution S

Dissolve 0.100 g, without heating and protected from light, in 9 ml of *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 or BY_5 *(2.2.2, Method II)*.

pH (*2.2.3)*

The pH of solution S is 3.6 to 4.4.

Specific optical rotation *(2.2.7)*

+ 50 to + 56, determined on solution S and calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance. *Carry out all operations as rapidly as possible, protected from light. Prepare the test and reference solutions immediately before use*.

Test solution (a) Dissolve 50 mg of the substance to be examined in a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol (80 per cent V/V) R* and dilute to 5.0 ml with the same mixture of solvents.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol (80 per cent V/V) R*.

Reference solution (a) Dissolve 10 mg of *ergometrine maleate CRS* in a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol (80 per cent V/V) R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol (80 per cent V/V) R*.

Reference solution (c) To 2.0 ml of reference solution (b) add 2.0 ml of a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol (80 per cent V/V) R*.

Apply separately to the plate 5 µl of each solution. Develop immediately over a path of 14 cm using a mixture of 3 volumes of *water R*, 25 volumes of *methanol R* and 75 volumes of *chloroform R*. Dry the plate in a current of cold air and spray with *dimethylaminobenzaldehyde solution R7*. Dry the plate in a current of warm air for about 2 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (1.0 per cent) and at most one such spot is more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 2.0 per cent, determined on 0.20 g by drying over *diphosphorus pentoxide R* at 80 °C at a pressure not exceeding 2.7 kPa for 2 h.

ASSAY

Dissolve 0.150 g in 40 ml of *anhydrous acetic acid R*. Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.05 M perchloric acid* is equivalent to 22.07 mg of $C_{23}H_{27}N_3O_6$.

STORAGE

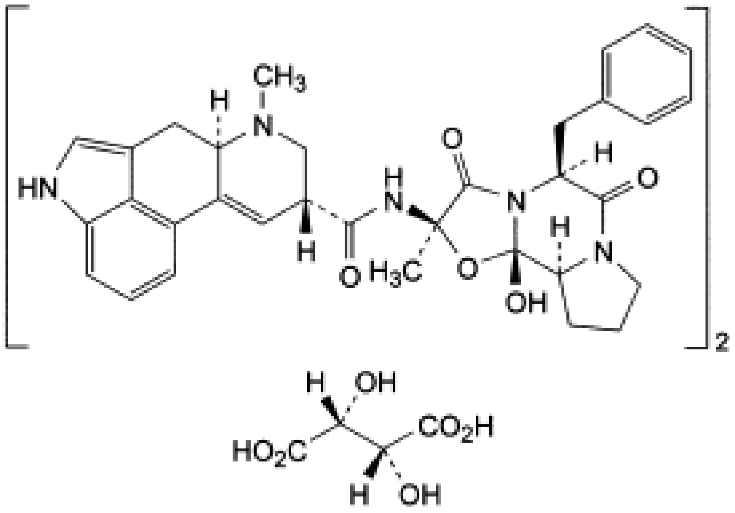
Store in an airtight, glass container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Ergotamine Tartrate

General Notices

(Ph Eur monograph 0224)



$(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ 1313 *379-79-3*

Action and use

Oxytocic.

Preparation

Ergotamine Sublingual Tablets

Ph Eur

DEFINITION

Ergotamine tartrate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of bis[(6a*R*,9*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxo-octahydro-8*H*-oxazolo[3,2-*a*]pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide] tartrate, calculated with reference to the dried substance. It may contain two molecules of methanol of crystallisation.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, slightly hygroscopic, slightly soluble in alcohol. Aqueous solutions slowly become cloudy owing to hydrolysis; this may be prevented by the addition of tartaric acid.

IDENTIFICATION

First identification B, C.

Second identification A, C, D, E.

A. Dissolve 50 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M hydrochloric acid*. Examined between 250 nm and 360 nm *(2.2.25)*, the solution shows an absorption maximum at 311 nm to 321 nm and a minimum at 265 nm to 275 nm. The specific absorbance at the maximum is 118 to 128, calculated with reference to the dried substance.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *ergotamine tartrate CRS*. Examine the substances as discs prepared as follows: triturate the substance to be examined and the reference substance separately with 0.2 ml of *methanol R* and then with *potassium bromide R* as prescribed in the general method.

C. Examine for not more than 1 min in ultraviolet light at 365 nm the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and fluorescence to the principal spot in the chromatogram obtained with reference solution (a). After spraying with *dimethylaminobenzaldehyde solution R7*, examine in daylight. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 ml of solution S (see Tests) add 1 ml of *glacial acetic acid R*, 0.05 ml of *ferric chloride solution R1* and 1 ml of *phosphoric acid R* and heat in a water-bath at 80 °C. After about 10 min, a blue or violet colour develops which becomes more intense on standing.

E. Dissolve about 10 mg in 1.0 ml of *0.1 M sodium hydroxide*. Transfer to a separating funnel and shake with 5 ml of *methylene chloride R*. Discard the organic layer. Neutralise the aqueous layer with a few drops of *dilute hydrochloric acid R*. 0.1 ml of this solution gives reaction (b) of tartrates *(2.3.1)*. Pour the reaction mixture into 1 ml of *water R* to observe the colour change to red or brownish-red.

TESTS

Carry out all operations as rapidly as possible, protected from light.

Solution S

Triturate 30 mg finely with about 15 mg of *tartaric acid R* and dissolve with shaking in 6 ml of *water R*.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

Shake 10 mg, finely powdered, with 4 ml of *carbon dioxide-free water R*. The pH of the suspension is 4.0 to 5.5.

Specific optical rotation *(2.2.7)*

Dissolve 0.40 g in 40 ml of a 10 g/l solution of *tartaric acid R*. Add 0.5 g of *sodium hydrogen carbonate R* cautiously in several portions and mix thoroughly. Shake with four quantities, each of 10 ml, of *chloroform R* previously washed with five quantities of *water R*, each of 50 ml per 100 ml of *chloroform R*. Combine the organic layers. Filter through a small filter moistened with *chloroform R* previously washed as described above. Dilute the filtrate to 50.0 ml with *chloroform R* previously washed as described above. Measure the angle of rotation.

Determine the amount of ergotamine base in the chloroformic solution as follows: to 25.0 ml of the solution add 50 ml of *anhydrous acetic acid R* and titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.05 M perchloric acid* is equivalent to 29.08 mg of $C_{33}H_{35}N_5O_5$.

The specific optical rotation is - 154 to - 165, calculated from the angle of rotation and the concentration of ergotamine base.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel G plate R*. *Prepare the reference solutions and the test solutions immediately before use and in the order indicated below.*

Reference solution (a) Dissolve 10 mg of *ergotamine tartrate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 7.5 ml of reference solution (a) to 50.0 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (c) To 2.0 ml of reference solution (b) add 4.0 ml of a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Test solution (a) Dissolve 50 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5.0 ml with the same mixture of solvents.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Apply immediately to the plate 5 µl of each reference solution and then 5 µl of each test solution. Expose the starting points immediately to ammonia vapour and for exactly 20 s by moving the starting line from side to side above a beaker 55 mm high and 45 mm in diameter containing about 20 ml of *concentrated ammonia R*. Dry the starting line in a current of cold air for exactly 20 s. Develop immediately over a path of 17 cm using a mixture of 5 volumes of *ethanol R*, 10 volumes of *methylene chloride R*, 15 volumes of *dimethylformamide R* and 70 volumes of *ether R*. Dry the plate in a current of cold air for about 2 min. Examine for not more than 1 min in ultraviolet light at 365 nm for the identification. Spray the plate abundantly with *dimethylaminobenzaldehyde solution R7* and dry in a current of warm air for about 2 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (1.5 per cent) and at most one such spot is more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 6.0 per cent, determined on 0.100 g by drying *in vacuo* at 95 °C for 6 h.

ASSAY

Dissolve 0.200 g in 40 ml of *anhydrous acetic acid R*. Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.05 M perchloric acid* is equivalent to 32.84 mg of $C_{70}H_{76}N_{10}O_{16}$.

STORAGE

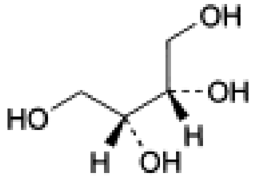
Store in an airtight, glass container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Erythritol

General Notices

(Ph Eur monograph 1803)



$C_4H_{10}O_4$ 122.1 *149-32-6*

Ph Eur

DEFINITION

(2*R*,3*S*)-Butane1,2,3,4-tetrol (*meso*-erythritol).

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or free-flowing granules.

Solubility

Freely soluble in water, very slightly soluble in alcohol.

IDENTIFICATION

A. Melting point *(2.2.14)*: 119 °C to 122 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison erythritol CRS.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless (*2.2.2, Method II)*.

Dissolve 5.0 g in *water R* and dilute to 50 ml with the same solvent.

Conductivity ***(2.2.38)***

Maximum 20 µS·cm^{-1}.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent. Measure the conductivity of the solution, while gently stirring with a magnetic stirrer.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 0.50 g of *erythritol CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with *water R*.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 100.0 ml with *water R*.

Reference solution (d) Dissolve 1.0 g of *erythritol R* and 1.0 g of *glycerol R* in *water R* and dilute to 20.0 ml with the same solvent.

Column:
- *size:* l = 0.3 m, Ø = 7.8 mm,
- *stationary phase: cation-exchange resin R* (9 µm),
- *temperature:* 70 °C.

Mobile phase 0.01 per cent *V/V* solution of *sulphuric acid R*.

Flow rate 0.8 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl; inject the test solution and reference solutions (b), (c) and (d).

Run time 3 times the retention time of erythritol.

Relative retention With reference to erythritol (retention time = about 11 min): impurity A = about 0.77; impurity B = about 0.90; impurity C = about 0.94; impurity D = about 1.10.

System suitability Reference solution (d):

- *resolution*: minimum 2 between the peaks due to erythritol and impurity D.

Limits:
- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Lead ***(2.4.10)***

Maximum 0.5 ppm.

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 1.00 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*. If intended for use in the manufacture of parenteral dosage forms, the total viable aerobic count *(2.6.12)* is not more than 10^2 bacteria and 10^2 fungi per gram, determined by plate count.

Bacterial endotoxins ***(2.6.14)***

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins:

— less than 4 IU/g for parenteral dosage forms having a concentration of 100 g/l or less of erythritol,

— less than 2.5 IU/g for parenteral dosage forms having a concentration of more than 100 g/l of erythritol.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of erythritol using the chromatogram obtained with reference solution (a) and the declared content of *erythritol CRS*.

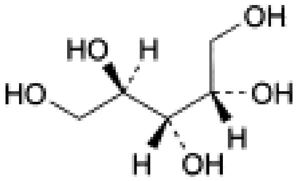
LABELLING

The label states where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES

A. maltitol,

B. sorbitol,



C. (2*R*,3*s*,4*S*)-pentane-1,2,3,4,5-pentol (*meso*-ribitol),

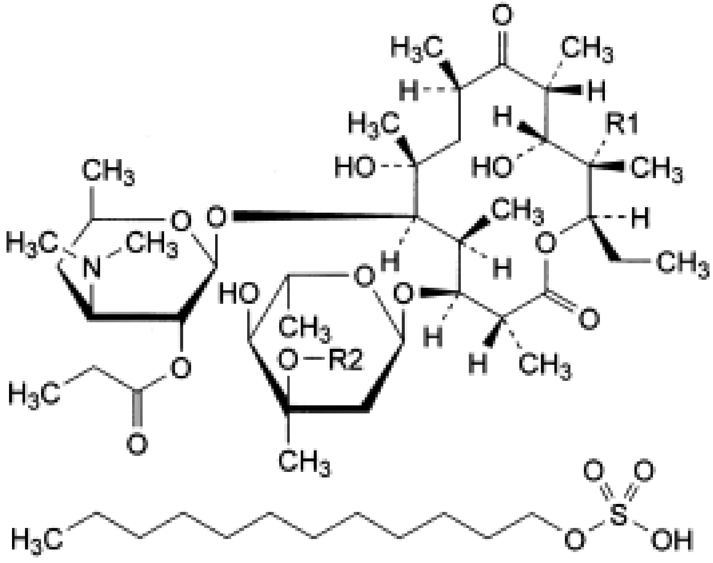
D. glycerol.

Ph Eur

Erythromycin Estolate

General Notices

(Ph Eur monograph 0552)



Erythromycin (estolate)	Mol. Formula	M_r	R1	R2
A	$C_{52}H_{97}NO_{18}S$	1056	OH	CH_3
B	$C_{52}H_{97}NO_{17}S$	1040	H	CH_3
C	$C_{51}H_{95}NO_{18}S$	1042	OH	H

$C_{40}H_{71}NO_{14},C_{12}H_{26}O_4S$ 1056 *3521-62-8*

Action and use

Macrolide antibacterial.

Preparation

Erythromycin Estolate Capsules

Ph Eur

DEFINITION

Main component

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-Dideoxy-3-*C*-methyl-3-O-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-2-*O*-propionyl-β-D-*xylo*-hexopyranosyl]oxy] oxacyclotetradecane-2,10-dione dodecyl sulphate (erythromycin A 2″-propionate dodecyl sulphate).

Semi-synthetic product derived from a fermentation product.

Content:

- *erythromycin estolate*: 86.0 per cent to 102.0 per cent (anhydrous substance),
- *erythromycin B*: maximum 5.0 per cent (anhydrous substance),
- *erythromycin C*: maximum 5.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in acetone. It is practically insoluble in dilute hydrochloric acid.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *erythromycin estolate CRS.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Hydrolysis solution A 20 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *phosphoric acid R*.

Test solution Dissolve 0.150 g of the substance to be examined in 25 ml of *methanol R*. Add 20 ml of the hydrolysis solution, mix and allow to stand at room temperature for at least 12 h. Dilute to 50.0 ml with the hydrolysis solution.

Reference solution (a) Dissolve 40.0 mg of *erythromycin A CRS* in 10 ml of *methanol R* and dilute to 20.0 ml with the hydrolysis solution.

Reference solution (b) Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in 50.0 ml of *methanol R*. Add 5.0 ml of reference solution (a) and dilute to 100.0 ml with the hydrolysis solution.

Reference solution (c) Dissolve 2 mg of *N-demethylerythromycin A CRS* in 20 ml of reference solution (b).

Reference solution (d) Dilute 3.0 ml of reference solution (a) to 100.0 ml with a mixture of equal volumes of *methanol R* and the hydrolysis solution.

Reference solution (e) Dissolve 40 mg of *erythromycin A CRS*, previously heated at 130 °C for 3 h, in 10 ml of *methanol R* and dilute to 20 ml with the hydrolysis solution (in situ preparation of impurities E and F).

Reference solution (f) Dissolve 2 mg of *erythromycin A CRS* in 10 ml of *0.01 M hydrochloric acid*. Allow to stand at room temperature for 30 min. Dilute to 20 ml with the hydrolysis solution (*in situ* preparation of impurity D).

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm,
- *temperature*: 70 °C using a water-bath for the column and at least one third of the tubing preceding the column.

Mobile phase To 50 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *dilute phosphoric acid R*, add 400 ml of *water R*, 165 ml of *2-methyl-2-propanol R* and 30 ml of *acetonitrile R*, and dilute to 1000 ml with *water R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 200 µl of the test solution and reference solutions (c), (d), (e) and (f).

Run time 5 times the retention time of erythromycin A; begin integration after the hydrolysis peak.

Identification of impurities Use the chromatogram obtained with reference solution (e) to identify the peaks due to impurities E and F.

Relative retention With reference to erythromycin A (retention time = about 15 min): hydrolysis peak = less than 0.3; impurity A = about 0.3; impurity B = about 0.45; erythromycin C = about 0.5; impurity C = about 0.9; impurity G = about 1.3; impurity D = about 1.4; impurity F = about 1.5; erythromycin B = about 1.8; impurity E = about 4.3.

System suitability Reference solution (c):

- *resolution*: minimum 0.8 between the peaks due to impurity B and erythromycin C and minimum 5.5 between the peaks due to impurity B and erythromycin A.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.09; impurity F = 0.15; impurity G = 0.14;
- *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (3.0 per cent);
- *any other impurity*: for each impurity, not more than 0.067 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent);
- *total*: not more than 1.67 times the area of the principal peak in the chromatogram obtained with reference solution (d) (5.0 per cent);
- *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.06 per cent).

Free erythromycin

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 0.250 g of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution Dissolve 75.0 mg of *erythromycin A CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with *acetonitrile R*.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 30 °C.

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a solution containing 3.4 g/l of *potassium dihydrogen phosphate R* and 2.75 ml/l *of triethylamine R*, adjusted to pH 3.0 with *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 195 nm.

Injection 20 µl.

Run time Twice the retention time of erythromycin A for the reference solution and 4.5 times the retention time of the 1st peak of erythromycin propionate for the test solution.

Retention time Erythromycin A = about 5 min; 1st peak of erythromycin propionate = about 10 min.

Limit:

- *free erythromycin*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (6.0 per cent).

Dodecyl sulphate

23.0 per cent to 25.5 per cent of $C_{12}H_{26}O_4S$ (anhydrous substance).

Dissolve 0.500 g in 25 ml of *dimethylformamide R*. Titrate with *0.1 M sodium methoxide* using 0.05 ml of a 3 g/l solution of *thymol blue R* in *methanol R* as indicator.

1 ml of *0.1 M sodium methoxide* is equivalent to 26.64 mg of $C_{12}H_{26}O_4S$.

Water *(2.5.12)*

Maximum 4.0 per cent, determined on 0.300 g.

Use a 100 g/l solution of *imidazole R* in *anhydrous methanol R* as the solvent.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 0.5 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection
Test solution and reference solutions (a) and (b).

System suitability:

- *repeatability*: maximum relative standard deviation of 1.2 per cent after 6 injections of reference solution (a).

Calculate the percentage content of erythromycin A using the chromatogram obtained with reference solution (a). Express the result as erythromycin A estolate by multiplying the percentage content of erythromycin A by 1.4387.

Calculate the percentage contents of erythromycin B and erythromycin C using the chromatogram obtained with reference solution (b). Express the result as erythromycin B estolate and as erythromycin C estolate by multiplying by 1.4387.

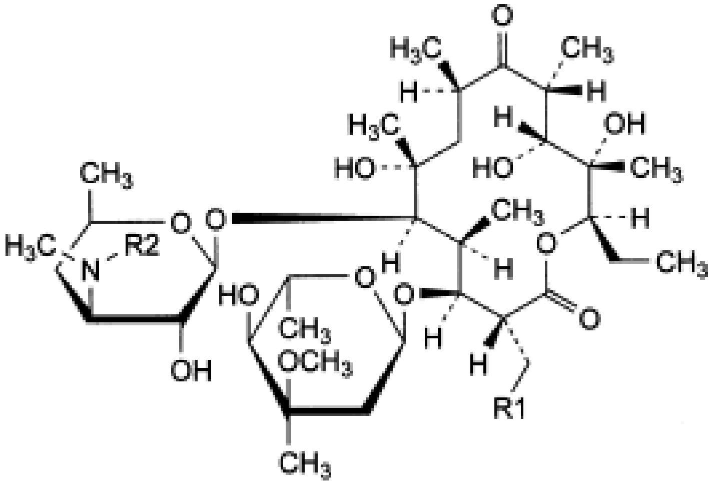
For the calculation of content of erythromycin estolate use the sum of erythromycins A, B and C expressed as estolate as described above.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F, G.*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H3C
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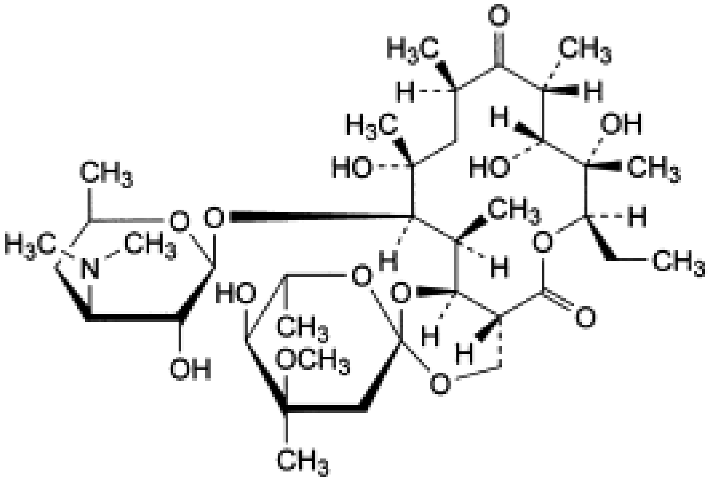
H
H
H3C
H
OH
HO
HO
CH3
CH3
CH3
O
O
H
H3C
R2
N
O
CH3
H
H
HO
O
O
O
CH3
CH3
OH
OCH3
H
O
H
R1
CH3

A. R1 = OH, R2 = CH_3: erythromycin F,

B. R1 = R2 = H: *N*-demethylerythromycin A,

G. R1 = H, R2 = CO-C_2H_5: *N*-demethyl-*N*-propanoylerythromycin A,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OH
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CH3
CH3
CH3
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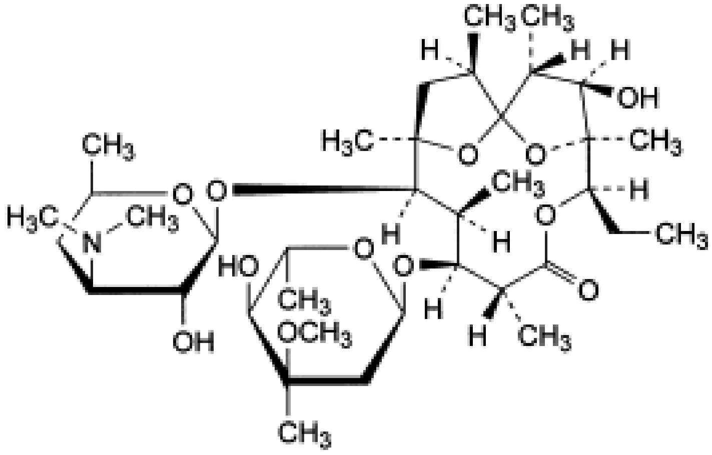
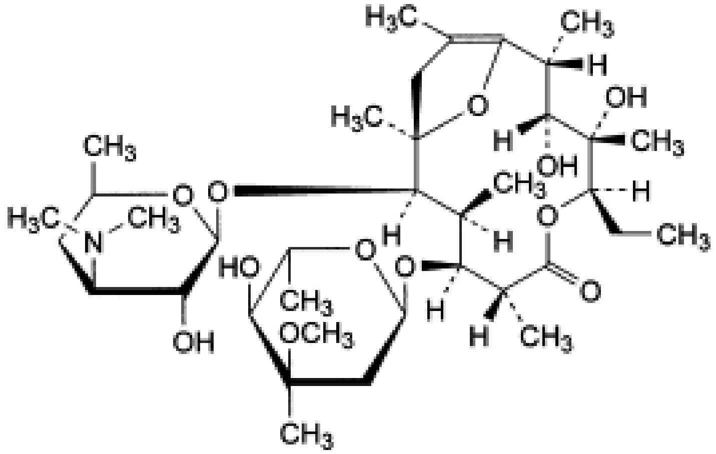
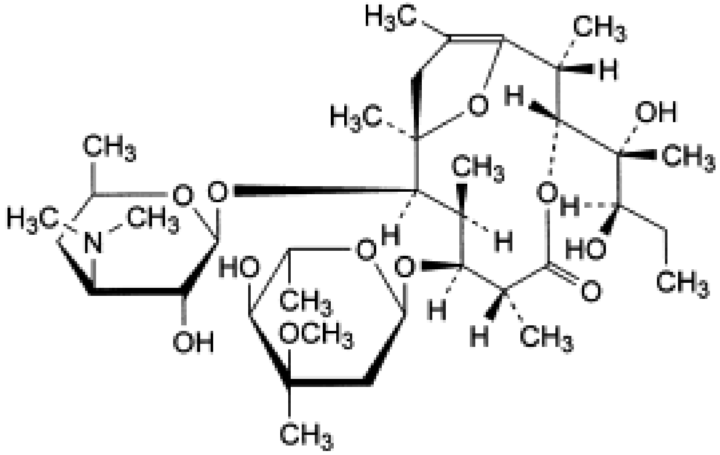
C. erythromycin E,



D. anhydroerythromycin A,



E. erythromycin A enol ether,

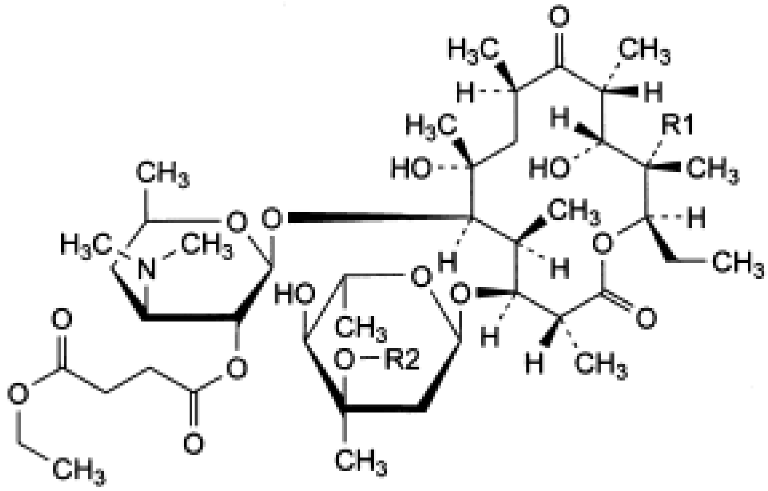


F. pseudoerythromycin A enol ether.

Erythromycin Ethyl Succinate

General Notices

(Erythromycin Ethylsuccinate, Ph Eur monograph 0274)


O
H₃C
CH₃
H
H
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R1
H₃C
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HO
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H₃C
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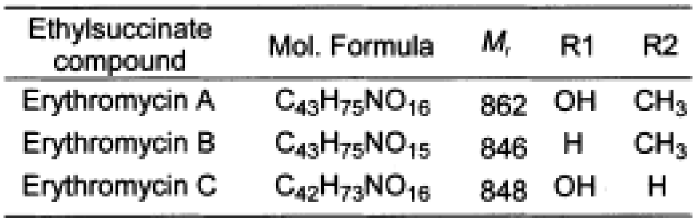


Ethylsuccinate compound	Mol. Formula	M_r	R1	R2
Erythromycin A	$C_{43}H_{75}NO_{16}$	862	OH	CH_3
Erythromycin B	$C_{43}H_{75}NO_{15}$	846	H	CH_3
Erythromycin C	$C_{42}H_{73}NO_{16}$	848	OH	H

41342-53-4

Action and use

Macrolide antibacterial.

Preparations

Erythromycin Ethyl Succinate Oral Suspension

Erythromycin Ethyl Succinate Tablets

Ph Eur

DEFINITION

Main component

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-

hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-2-*O*-(4-ethoxy-4-oxobutanoyl)-β-D-*xylo*-hexopyranosyl]oxy] oxacyclotetradecane-2,10-dione (erythromycin A ethylsuccinate).

Semi-synthetic product derived from a fermentation product.

Content:

— sum of erythromycin A, erythromycin B and erythromycin C: minimum 78.0 per cent (anhydrous substance),

— erythromycin B: maximum 5.0 per cent (anhydrous substance),

— erythromycin C: maximum 5.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, hygroscopic.

Solubility

Practically insoluble in water, freely soluble in acetone, in ethanol and in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   erythromycin ethylsuccinate CRS.

TESTS

Specific optical rotation *(2.2.7)*

- 70 to - 82 (anhydrous substance).

Dissolve 0.100 g in *acetone R* and dilute to 10.0 ml with the same solvent. Measure the angle of rotation at least 30 min after preparing the solution.

Related substances

Liquid chromatography *(2.2.29)*.

Hydrolysis solution   A 20 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *phosphoric acid R*.

Test solution   Dissolve 0.115 g of the substance to be examined in 25 ml of *methanol R*. Add 20 ml of the hydrolysis solution, mix and allow to stand at room temperature for at least 12 h. Dilute to 50.0 ml with the hydrolysis solution.

Reference solution (a)   Dissolve 40.0 mg of *erythromycin A CRS* in 10 ml of *methanol R* and dilute to 20.0 ml with the hydrolysis solution.

Reference solution (b)   Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in 50 ml of *methanol R*. Add 5.0 ml of reference solution (a) and dilute to 100.0 ml with the hydrolysis solution.

Reference solution (c)   Dissolve 2 mg of *N-demethylerythromycin A CRS* in 20 ml of reference solution (b).

Reference solution (d) Dilute 3.0 ml of reference solution (a) to 100.0 ml with a mixture of equal volumes of *methanol R* and the hydrolysis solution.

Reference solution (e) Dissolve 40 mg of *erythromycin A CRS*, previously heated at 130 °C for 3 h, in 10 ml of *methanol R* and dilute to 20 ml with the hydrolysis solution.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: styrene-*divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm,

— *temperature*: 70 °C using a water-bath for the column and at least one third of the tubing preceding the column.

Mobile phase To 50 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *dilute phosphoric acid R*, add 400 ml of *water R*, 165 ml of *2-methyl-2-propanol R* and 30 ml of *acetonitrile R*, and dilute to 1000 ml with *water R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 200 µl; inject the test solution and reference solutions (a), (c), (d) and (e).

Run time 5 times the retention time of erythromycin A; begin integration after the hydrolysis peak.

Relative retention With reference to erythromycin A (retention time = about 15 min): hydrolysis peak = less than 0.3; impurity B = about 0.45; erythromycin C = about 0.5; impurity C = about 0.9; impurity G = about 1.3; impurity D = about 1.4; impurity F = about 1.5; erythromycin B = about 1.8; impurity E = about 4.3.

System suitability Reference solution (c):

— *resolution*: minimum 0.8 between the peaks due to impurity B and to erythromycin C and minimum 5.5 between the peaks due to impurity B and to erythromycin A.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.09; impurity F = 0.15; impurity G = 0.14; use the chromatogram obtained with reference solution (e) to identify the peaks due to impurities E and F.

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (3.0 per cent).

— *total*: not more than 1.67 times the area of the principal peak in the chromatogram obtained with reference solution (d) (5.0 per cent).

— *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.06 per cent).

Free erythromycin

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.250 g of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution Dissolve 75.0 mg of *erythromycin A CRS* in *acetonitrile R* and dilute to

50.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with *acetonitrile R*.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a solution containing 3.4 g/l of *potassium dihydrogen phosphate R* and 2.0 g/l of *triethylamine R*, adjusted to pH 3.0 with *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 195 nm.

Injection 20 µl.

Run time Twice the retention time of erythromycin A (retention time = about 8 min) for the reference solution and twice the retention time of erythromycin ethylsuccinate (retention time = about 24 min) for the test solution.

Limit:

- *free erythromycin*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (6.0 per cent).

Water ***(2.5.12)***

Maximum 3.0 per cent, determined on 0.30 g.

Use a 100 g/l solution of *imidazole R* in *anhydrous methanol R* as the solvent.

Sulphated ash ***(2.4.14)***

Maximum 0.3 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

Injection Inject the test solution and reference solutions (a) and (b).

System suitability Reference solution (a):

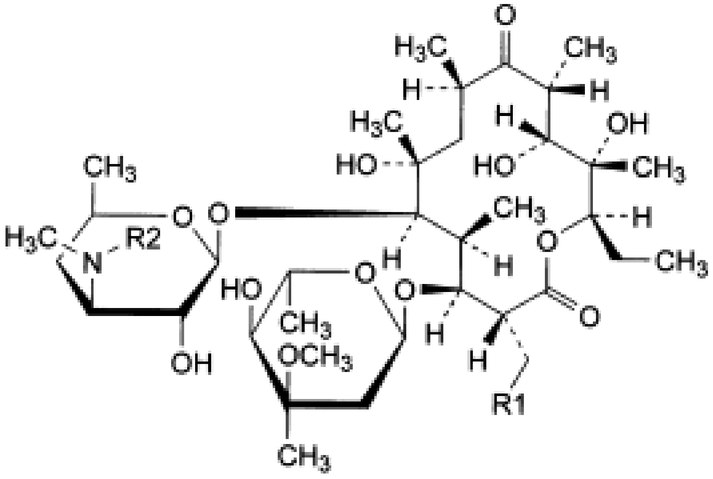
- *relative standard deviation*: maximum 1.2 per cent for 6 replicate injections.

Calculate the percentage content of erythromycin A using the chromatogram obtained with reference solution (a). Calculate the percentage contents of erythromycin B and erythromycin C using the chromatogram obtained with reference solution (b).

STORAGE

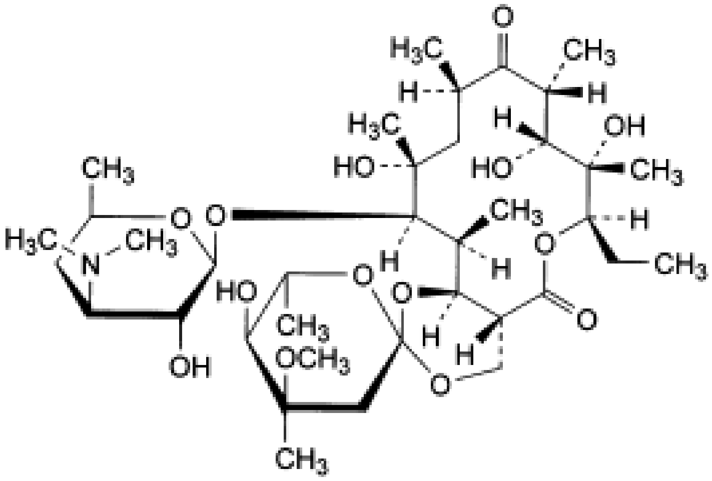
In an airtight container , protected from light.

IMPURITIES

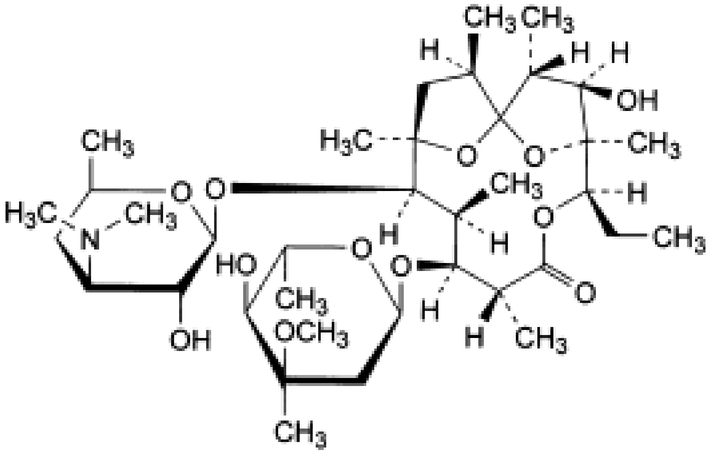


A. R1 = OH, R2 = CH_3: erythromycin F,

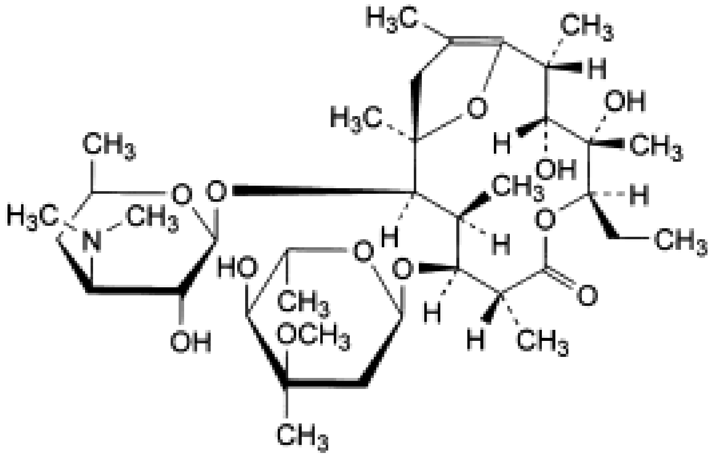
B. R1 = R2 = H: *N*-demethylerythromycin A,



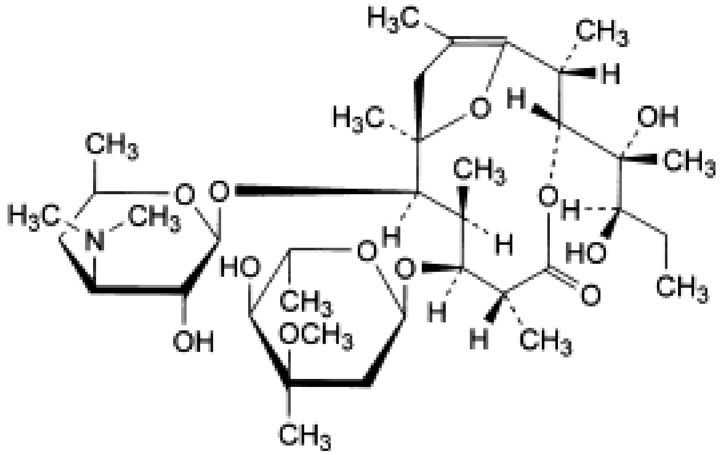
C. erythromycin E,



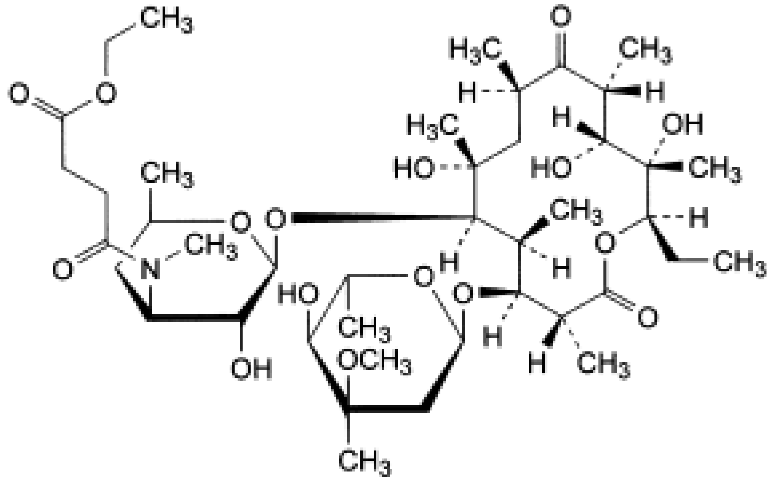
D. anhydroerythomycin A,



E. erythromycin A enol ether,



F. pseudoerythromycin A enol ether,



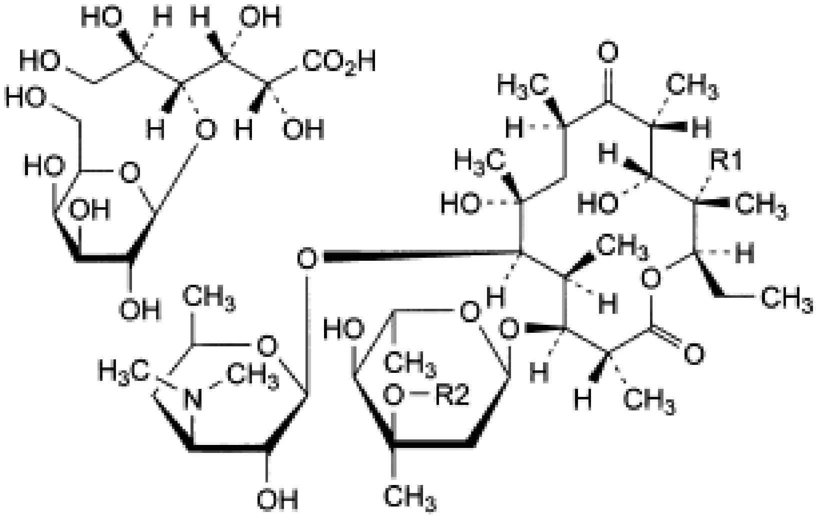
G. erythromycin *N*-ethylsuccinate.

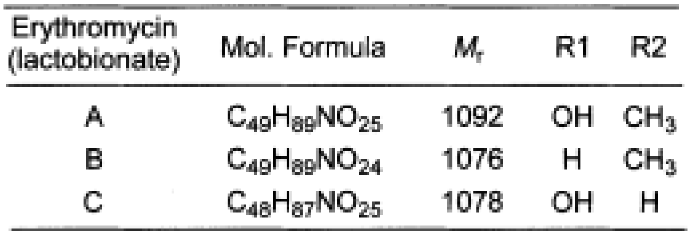
Ph Eur

Erythromycin Lactobionate

General Notices

(Ph Eur monograph 1098)





Erythromycin (lactobionate)	Mol. Formula	M_r	R1	R2
A	$C_{49}H_{89}NO_{25}$	1092	OH	CH_3
B	$C_{49}H_{89}NO_{24}$	1076	H	CH_3
C	$C_{48}H_{87}NO_{25}$	1078	OH	H

3847-29-8

Action and use

Macrolide antibacterial.

Preparation

Erythromycin Lactobionate Intravenous Infusion

Ph Eur

DEFINITION

Main component

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-

(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione 4-*O*-β-D-galactopyranosyl-D-gluconate (erythromycin A lactobionate).

Salt of a product obtained by fermentation using a strain of *Streptomyces erythreus*.

Content:

— *sum of erythromycin A lactobionate, erythromycin B lactobionate and erythromycin C lactobionate*: 93.0 per cent to 102.0 per cent (anhydrous substance);

— *erythromycin B lactobionate*: maximum 5.0 per cent (anhydrous substance);

— *erythromycin C lactobionate*: maximum 5.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow hygroscopic, powder.

Solubility

Soluble in water, freely soluble in anhydrous ethanol and in methanol, very slightly soluble in acetone and in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *erythromycin A CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *lactobionic acid R* in *water R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase glacial acetic acid R, water R, methanol R (3:10:90 *V/V/V*).

Application 5 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with a 5 g/l solution of *potassium permanganate R* in *1 M sodium hydroxide* and heat at 110 °C for 5 min.

Results The 2 spots in the chromatogram obtained with the test solution are similar in position, colour and size, one to the principal spot in the chromatogram obtained with reference solution (a) and the other to the principal spot in the chromatogram obtained with reference solution (b).

B. To about 5 mg add 5 ml of a 0.2 g/l solution of *xanthydrol R* in a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *acetic acid R*. A red colour develops.

C. Dissolve about 10 mg in 5 ml of *hydrochloric acid R1*. A yellowish-green colour develops.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in 20 ml of *water R*.

pH *(2.2.3)*

6.5 to 7.5.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*

The test solution and the reference solutions can be used within 24 h if stored at 2-8 °C.

Solvent mixture *methanol R*, *phosphate buffer solution pH 7.0 R* (25:75 *V/V*).

Test solution Dissolve 60.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 40.0 mg of *erythromycin A CRS* in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *N-demethylerythromycin A CRS* (impurity B) in reference solution (b). Add 1.0 ml of reference solution (a) and dilute to 25 ml with reference solution (b).

Reference solution (d) Dilute 3.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Reference solution (e) Dissolve 40 mg of *erythromycin A CRS*, previously heated at 130 °C for 4 h, in the solvent mixture and dilute to 10 ml with the solvent mixture (in situ preparation of impurities E and F).

Reference solution (f) Dissolve 2 mg of *erythromycin A CRS* in 5 ml of *0.01 M hydrochloric acid*. Allow to stand at room temperature for 30 min. Dilute to 10 ml with the solvent mixture (in situ preparation of impurity D).

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm;
- *temperature*: 70 °C using a water-bath for the column and at least 1/3 of the tubing preceding the column.

Mobile phase To 50 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 9.0 with *dilute phosphoric acid R*, add 400 ml of *water R*, 165 ml of *2-methyl-2-propanol R* and 30 ml of *acetonitrile R1*, and dilute to 1000 ml with *water R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 100 µl of the test solution and reference solutions (a), (c), (d), (e) and (f).

Run time 5 times the retention time of erythromycin A.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B, with reference solution (e) to identify the peaks due to impurities E and F, and with reference solution (f) to identify the peak due to impurity D.

Relative retention With reference to erythromycin A (retention time = about 15 min): impurity A = about 0.3; impurity B = about 0.45; erythromycin C = about 0.5; impurity C = about 0.9; impurity D = about 1.4; impurity F = about 1.5; erythromycin B = about 1.8; impurity E = about 4.3.

System suitability Reference solution (c):

— *resolution*: minimum 0.8 between the peaks due to impurity B and erythromycin C and minimum 5.5 between the peaks due to impurity B and erythromycin A. If necessary adjust the concentration of 2-methyl-2-propanol in the mobile phase or reduce the flow rate to 1.5 ml/min or 1.0 ml/min.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.09; impurity F = 0.15;

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (3.0 per cent);

— *any other impurity*: for each impurity, not more than 0.067 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (6.0 per cent);

— *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.06 per cent).

Free lactobionic acid

Mximum 1.0 per cent of $C_{12}H_{22}O_{12}$ (anhydrous substance).

Dissolve 0.400 g in 50 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Calculate the volume of *0.1 M sodium hydroxide* required per gram of the substance to be examined (n_1 ml). Dissolve 0.500 g in 40 ml of *anhydrous acetic acid R* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Calculate the volume of *0.1 M perchloric acid* required per gram of the substance to be examined (n_2 ml).

Calculate the percentage content of $C_{12}H_{22}O_{12}$ using the following expression:

$$3.580\,(n_1 - n_2)$$

Water *(2.5.12)*

Maximum 5.0 per cent, determined on 0.200 g.

Use a 100 g/l solution of *imidazole R* in *anhydrous methanol R* as the solvent.

Sulphated ash ***(2.4.14)***

Maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.35 IU/mg of erythromycin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solutions (a) and (b).

System suitability:
- — *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (a).

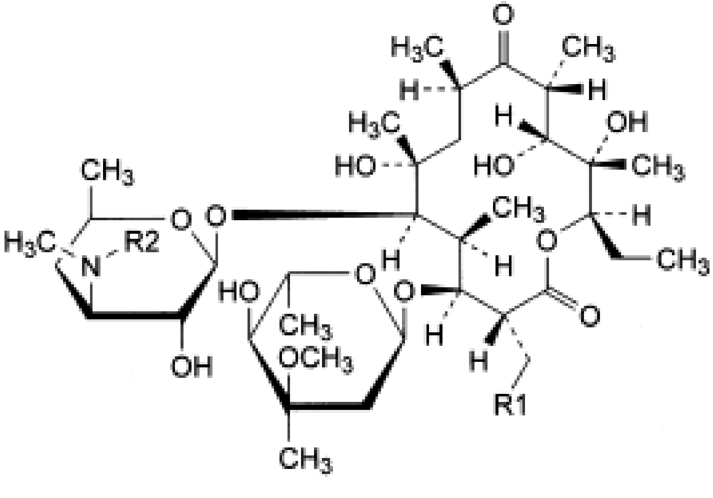
Calculate the percentage content of erythromycin A using the chromatogram obtained with reference solution (a). Express the result as erythromycin A lactobionate by multiplying the percentage content of erythromycin A by 1.4877. Calculate the percentage contents of erythromycin B and erythromycin C using the chromatogram obtained with reference solution (b). Express the result as erythromycin B lactobionate and as erythromycin C lactobionate by multiplying by 1.4877.

STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

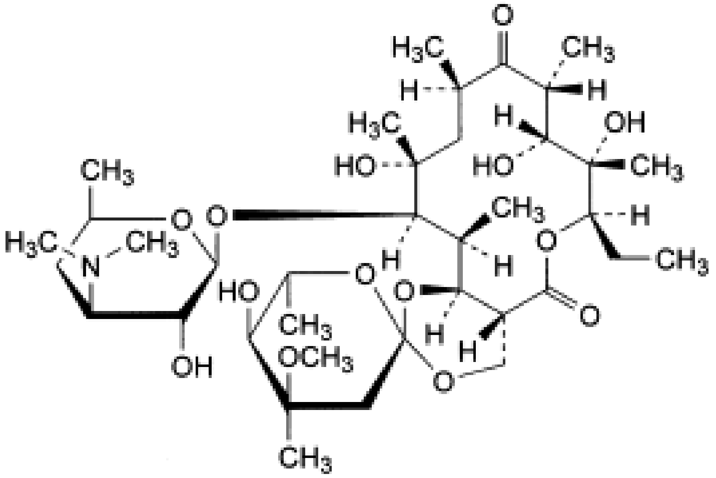
IMPURITIES

Specified impurities *A, B, C, D, E, F.*

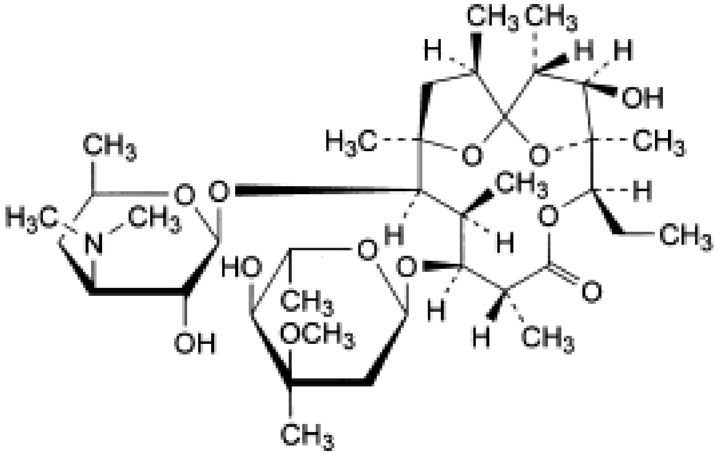


A. R1 = OH, R2 = CH_3: erythromycin F,

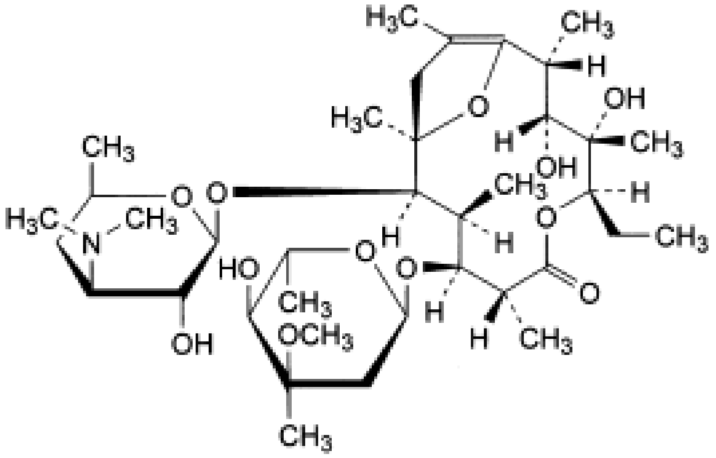
B. R1 = R2 = H: *N*-demethylerythromycin A,



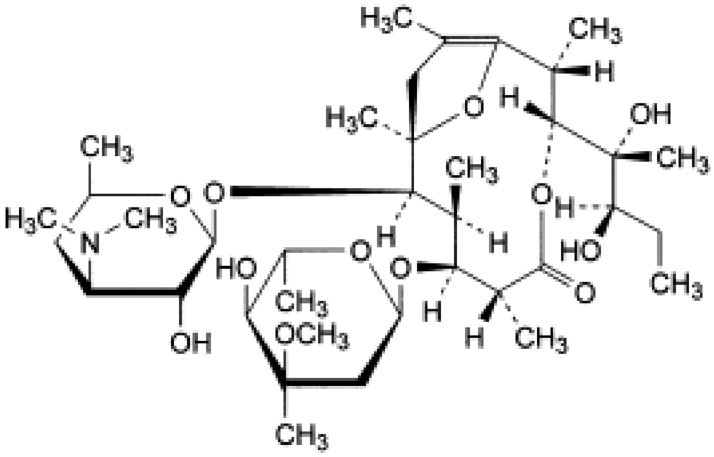
C. erythromycin E,



D. anhydroerythromycin A,



E. erythromycin A enol ether,



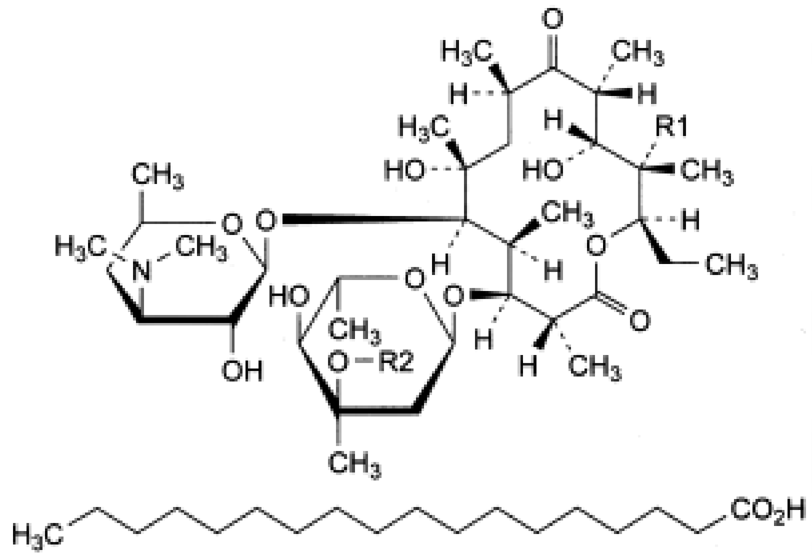
F pseudoerythromycin A enol ether.

Ph Eur

Erythromycin Stearate

General Notices

(Ph Eur monograph 0490)



H3C
O
CH3
H
H
H3C
H
R1
HO
HO
CH3
CH3
O
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CH3
H
H3C
N
CH3
O
H
H
O
CH3
HO
O
O
CH3
H
OH
O−R2
H
CH3
CH3
H3C
CO2H

Erythromycin	Mol. Formula	R1	R2
A	$C_{55}H_{103}NO_{15}$	OH	CH_3
B	$C_{55}H_{103}NO_{14}$	H	CH_3
C	$C_{54}H_{101}NO_{15}$	OH	H

1018 *1643-22-1*

Action and use

Macrolide antibacterial.

Preparation

Erythromycin Stearate Tablets

Ph Eur

DEFINITION

A mixture of the stearates of erythromycin and stearic acid. The main component is the octadecanoate of (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-

dione (erythromycin A stearate).

Fermentation product.

Content:

- sum of the contents of erythromycin A, erythromycin B and erythromycin C: minimum 60.5 per cent (anhydrous substance),
- erythromycin B: maximum 5.0 per cent,
- erythromycin C: maximum 5.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone and in methanol.

Solutions may be opalescent.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *erythromycin stearate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 28 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *erythromycin A CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *stearic acid R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase Mix 4 volumes of *2-propanol R*, 8 volumes of a 150 g/l solution of *ammonium acetate R* previously adjusted to pH 9.6 with *ammonia R* and 9 volumes of *ethyl acetate R*. Allow to settle and use the upper layer.

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection A Spray with a solution containing 0.2 g/l of *dichlorofluorescein* R and 0.1 g/l of *rhodamine B R* in *alcohol R*. Maintain the plate for a few seconds in the vapour above a water-bath. Examine in ultraviolet light at 365 nm.

Results A The chromatogram obtained with the test solution shows 2 spots, one of which corresponds in position to the principal spot in the chromatogram obtained with reference solution (a) and the other to the principal spot in the chromatogram obtained with reference solution (b).

Detection B Spray the plate with *anisaldehyde solution R1*. Heat at 110 °C for 5 min and examine in daylight.

Results B The spot in the chromatogram obtained with the test solution corresponds in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Free stearic acid

Maximum 14.0 per cent (anhydrous substance) of $C_{18}H_{36}O_2$.

Dissolve 0.400 g in 50 ml of *methanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Calculate the volume of *0.1 M sodium hydroxide* required per gram of the substance to be examined (n_1 ml). Dissolve 0.500 g in 30 ml of *methylene chloride R*. If the solution is opalescent, filter and shake the residue with 3 quantities, each of 25 ml, of *methylene chloride R*. Filter, if necessary, and rinse the filter with *methylene chloride R*. Reduce the volume of the combined filtrate and rinsings to 30 ml by evaporation on a water-bath. Add 50 ml of *glacial acetic acid R* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Calculate the volume of *0.1 M perchloric acid* required per gram of the substance to be examined (n_2 ml).

Calculate the percentage content of $C_{18}H_{36}O_2$ from the expression:

$$2.845\,(n_1 - n_2) \times \frac{100}{100 - h}$$

h = percentage water content.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 55.0 mg of the substance to be examined in 5.0 ml of *methanol R* and dilute to 10.0 ml with *buffer solution pH 8.0 R*1. Centrifuge and use the clear solution.

Reference solution (a) Dissolve 40.0 mg of *erythromycin A CRS* in 5.0 ml of *methanol R* and dilute to 10.0 ml with *buffer solution pH 8.0 R1*.

Reference solution (b) Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in 25.0 ml of *methanol R* and dilute to 50.0 ml with *buffer solution pH 8.0 R1*.

Reference solution (c) Dissolve 5 mg of *N-demethylerythromycin A CRS* in reference solution (b). Add 1.0 ml of reference solution (a) and dilute to 25 ml with reference solution (b) .

Reference solution (d) Dilute 3.0 ml of reference solution (a) to 100.0 ml with a mixture of equal volumes of *methanol R* and *buffer solution pH 8.0 R1*.

Reference solution (e) Transfer 40 mg of *erythromycin A CRS* to a glass vial and spread evenly such that it forms a layer not more than about 1 mm thick. Heat at 130 °C for 4 h.

Allow to cool and dissolve in a mixture of 1 volume of *methanol R* and 3 volumes of *buffer solution pH 8.0 R1* and dilute to 10 ml with the same mixture of solvents.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm,

— *temperature*: 70 °C using a water-bath for the column and at least one third of the tubing preceding the column.

Mobile phase To 50 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 9.0 ± 0.05 with *dilute phosphoric* acid R, add 400 ml of *water R*, 165 ml of *2-methyl-2-propanol R* and 30 ml of *acetonitrile R*, and dilute to 1000 ml with *water R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 100 µl; inject the test solution and reference solutions (c), (d) and (e).

Run time 5 times the retention time of erythromycin A.

Relative retention With reference to erythromycin A (retention time = about 15 min): impurity A = about 0.3; impurity B = about 0.45; erythromycin C = about 0.5; impurity C = about 0.9; impurity D = about 1.4; impurity F = about 1.5; erythromycin B = about 1.8; impurity E = about 4.3.

System suitability Reference solution (c):

— *resolution*: minimum 0.8 between the peaks due to impurity B and to erythromycin C and minimum 5.5 between the peaks due to impurity B and to erythromycin A. If necessary, adjust the concentration of 2-methyl-2-propanol in the mobile phase or reduce the flow rate to 1.5 ml/min or 1.0 ml/min.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities (use the chromatogram obtained with reference solution (e) to identify them) by the corresponding correction factor: impurity E = 0.09; impurity F = 0.15,

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (3 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (6 per cent),

— *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.06 per cent); disregard the peaks due to erythromycin B and to erythromycin C.

Water *(2.5.12)*

Maximum 4.0 per cent, determined on 0.300 g.

Use a 100 g/l solution of *imidazole R* in *anhydrous methanol R* as the solvent.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

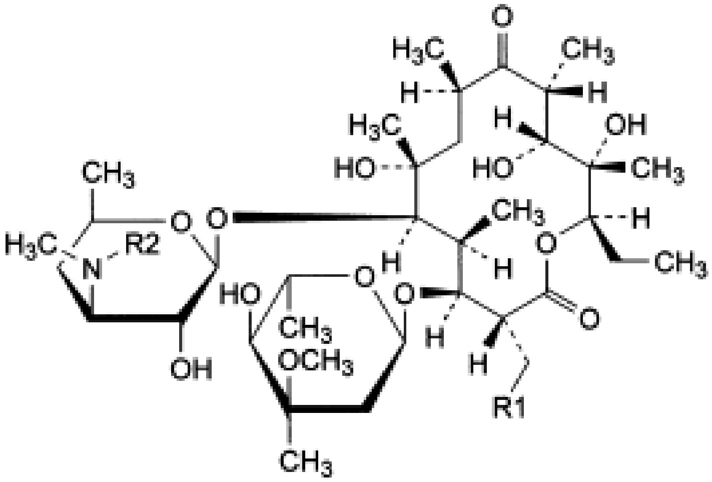
Injection Test solution and reference solutions (a) and (b).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.2 per cent for 6 replicate injections.

Calculate the percentage content of erythromycin A using the chromatogram obtained with reference solution (a). Calculate the percentage contents of erythromycin B and erythromycin C using the chromatogram obtained with reference solution (b).

IMPURITIES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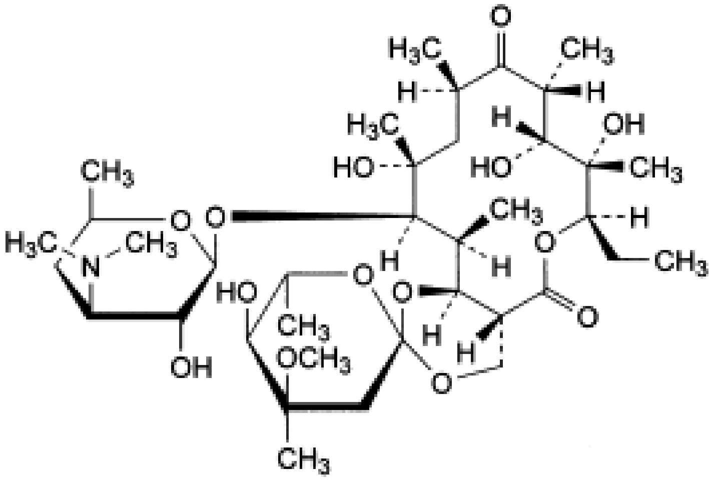
H
H3C
H
OH
HO
HO
CH3
CH3
CH3
O
O
CH3
O
H
H3C
R2
N
H
H
CH3
HO
O
O
O
CH3
H
OCH3
OH
H
R1
CH3

A. R1 = OH, R2 = CH_3: erythromycin F,

B. R1 = R2 = H: *N*-demethylerythromycin A,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CH3
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H3C
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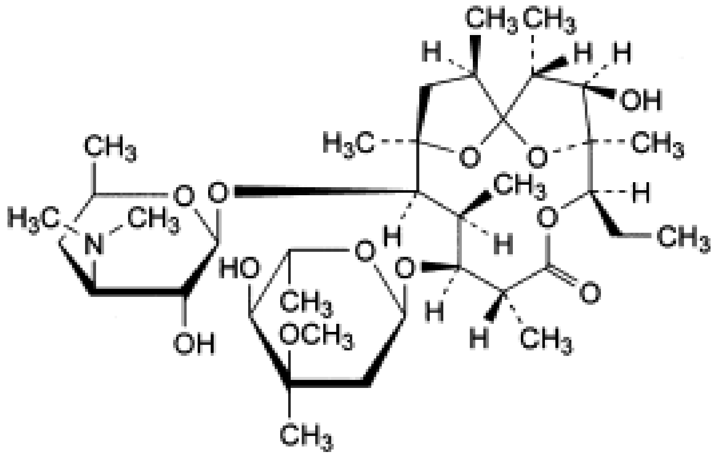
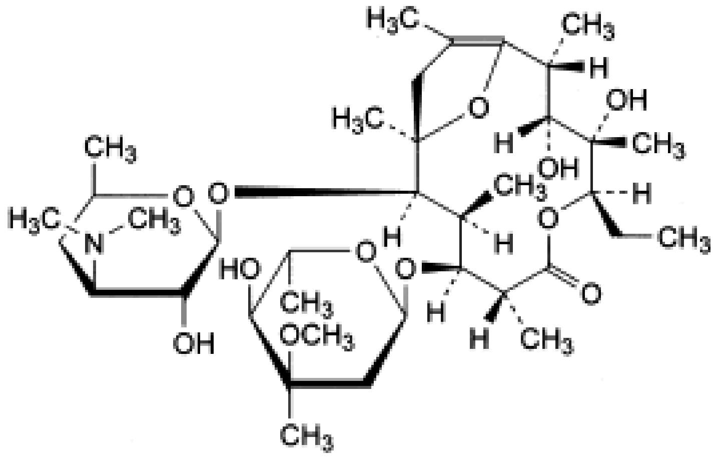
O
CH3

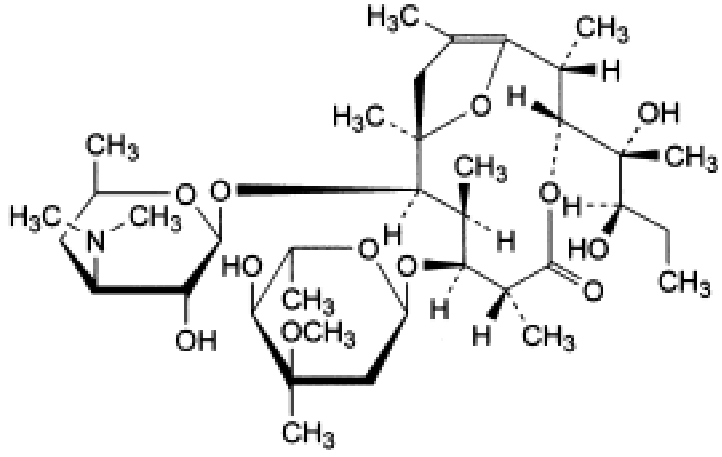
C. erythromycin E,



D. anhydroerythromycin A,



E. erythromycin A enol ether,



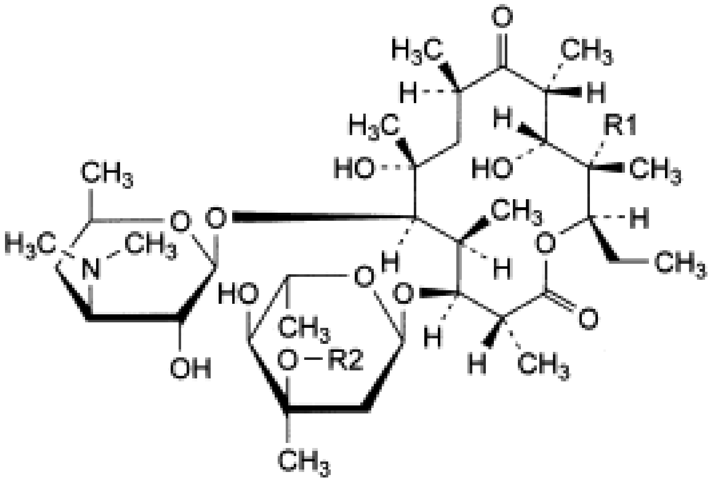
F. pseudoerythromycin A enol ether.

Ph Eur

Erythromycin

General Notices

(Ph Eur monograph 0179)



Erythromycin	Mol. Formula	M_r	R1	R2
A	$C_{37}H_{67}NO_{13}$	734	OH	CH_3
B	$C_{37}H_{67}NO_{12}$	718	H	CH_3
C	$C_{36}H_{65}NO_{13}$	720	OH	H

114-07-8

Action and use

Macrolide antibacterial.

Preparations

Gastro-resistant Erythromycin Tablets

Erythromycin and Zinc Acetate Lotion

Ph Eur

DEFINITION

Mixture of macrolide antibiotics produced by a strain of *Streptomyces erythreus*, the main component being (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-O-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[(3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl)-oxy]oxacyclotetradecane-2,10-dione (erythromycin A).

Content:

- — sum of the contents of erythromycin A, erythromycin B and erythromycin C: 93.0 per cent to 102.0 per cent (anhydrous substance),
- — erythromycin B: maximum 5.0 per cent,
- — erythromycin C: maximum 5.0 per cent.

CHARACTERS

Appearance

White or slightly yellow powder or colourless or slightly yellow crystals, slightly hygroscopic.

Solubility

Slightly soluble in water (the solubility decreases as the temperature rises), freely soluble in alcohol, soluble in methanol.

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *erythromycin A CRS.*

Disregard any band in the region from 1980 cm^{-1} to 2050 cm^{-1}.

If the spectra obtained show differences, dissolve 50 mg of the substance to be examined and of the reference substance separately in 1.0 ml of *methylene chloride R*, dry at 60 °C at a pressure not exceeding 670 Pa for 3 h and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *erythromycin A CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *spiramycin CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase Mix 4 volumes of *2-propanol R*, 8 volumes of a 150 g/l solution of *ammonium acetate R* previously adjusted to pH 9.6 with *ammonia R* and 9 volumes of *ethyl acetate R*. Allow to settle and use the upper layer.

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *anisaldehyde solution R1* and heat at 110 °C for 5 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) and its position and colour are different from those of the spots in the chromatogram obtained with reference solution (b).

C. To about 5 mg add 5 ml of a 0.2 g/l solution of *xanthydrol R* in a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *acetic acid R* and heat on a water-bath. A red colour develops.

D. Dissolve about 10 mg in 5 ml of *hydrochloric acid R1* and allow to stand for 10-20 min. A yellow colour develops.

TESTS

Specific optical rotation *(2.2.7)*

- 71 to - 78 (anhydrous substance).

Dissolve 1.00 g in *ethanol R* and dilute to 50.0 ml with the same solvent. The specific optical rotation is determined at least 30 min after preparing the solution.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 40.0 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 40.0 mg of *erythromycin A CRS* in a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (c) Dissolve 5 mg of *N-demethylerythromycin A CRS* in reference solution (b). Add 1.0 ml of reference solution (a) and dilute to 25 ml with reference solution (b) .

Reference solution (d) Dilute 3.0 ml of reference solution (a) to 100.0 ml with a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1*.

Reference solution (e) Transfer 40 mg of *erythromycin A CRS* to a glass vial and spread evenly such that it forms a layer not more than about 1 mm thick. Heat at 130 °C for 4 h. Allow to cool and dissolve in a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1* and dilute to 10 ml with the same mixture of solvents.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm,

— *temperature*: 70 °C using a water-bath for the column and at least one-third of the tubing preceding the column.

Mobile phase To 50 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 9.0 ± 0.05 with *dilute phosphoric acid R*, add 400 ml of *water R*, 165 ml of *2-methyl-2-propanol R* and 30 ml of *acetonitrile R*, and dilute to 1000 ml with *water R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 100 µl; inject the test solution and reference solutions (c), (d) and (e).

Run time 5 times the retention time of erythromycin A.

Relative retention With reference to erythromycin A (retention time = about 15 min): impurity A = about 0.3; impurity B = about 0.45; erythromycin C = about 0.5; impurity C = about 0.9; impurity D = about 1.4; impurity F = about 1.5; erythromycin B = about 1.8; impurity E = about 4.3.

System suitability Reference solution (c):

— *resolution*: minimum 0.8 between the peaks due to impurity B and erythromycin C and minimum 5.5 between the peaks due to impurity B and erythromycin A. If necessary, adjust the concentration of 2-methyl-2-propanol in the mobile phase or reduce the flow rate to 1.5 ml or 1.0 ml/min.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities (use the chromatogram obtained with reference solution (e) to identify them) by the corresponding correction factor: impurity E = 0.09; impurity F = 0.15,

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (3.0 per cent),

— *total*: not more than 2.3 times the area of the principal peak in the chromatogram obtained with reference solution (d) (7.0 per cent),

— *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.06 per cent); disregard the peaks due to erythromycin B and erythromycin C.

Thiocyanate

Maximum 0.3 per cent.

Prepare the solutions immediately before use and protect from actinic light.

Compensation liquid Dilute 1.0 ml of a 90 g/l solution of *ferric chloride R* to 50.0 ml with *methanol R*.

Test solution Dissolve 0.100 g (m g) of the substance to be examined in 20 ml of *methanol R*, add 1.0 ml of a 90 g/l solution of *ferric chloride R* and dilute to 50.0 ml with *methanol R*.

Prepare 2 independent reference solutions.

Reference solution Dissolve 0.100 g of *potassium thiocyanate R*, previously dried at 105 °C for 1 h, in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml to 50.0 ml with *methanol R*. To 5.0 ml of this solution, add 1.0 ml of a 90 g/l solution of *ferric chloride R* and dilute to 50.0 ml with *methanol R*.

Measure the absorbances *(2.2.25)* of each reference solution (A_1, A_2) and of the test solution (A) at the maximum (about 492 nm).

Suitability value:

$$S = \frac{m_2 \times A_1}{m_1 \times A_2}$$

m_1, m_2 = mass of the potassium thiocyanate used to prepare the respective reference solutions, in grams.

The test is not valid unless S is not less than 0.985 and not more than 1.015.

Calculate the percentage content of thiocyanate from the expression:

$$\frac{A \times 58.08 \times 0.5}{m \times 97.18} \times \left(\frac{m_1}{A_1} + \frac{m_2}{A_2}\right)$$

58.08 = relative molecular mass of the thiocyanate moiety,

97.18 = relative molecular mass of potassium thiocyanate.

Water *(2.5.12)*

Maximum 6.5 per cent, determined on 0.200 g.

Use a 100 g/l solution of *imidazole R* in *anhydrous methanol R* as the solvent.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solutions (a) and (b).

System suitability Reference solution (a):

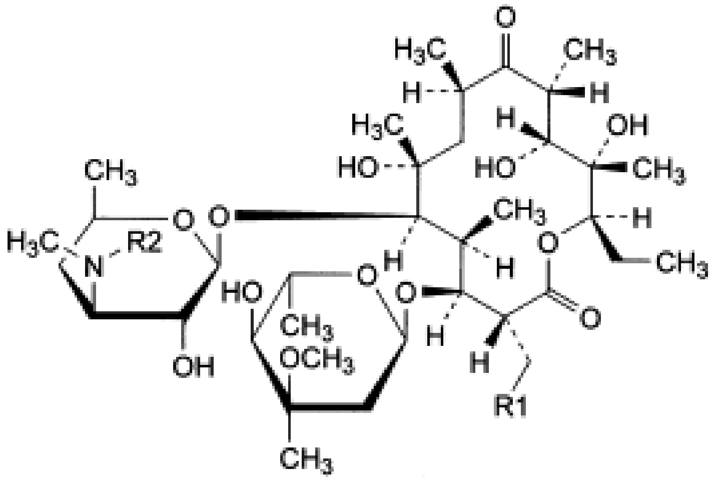
— *repeatability*: maximum relative standard deviation of 1.2 per cent for 6 replicate injections.

Calculate the percentage content of erythromycin A using the chromatogram obtained with reference solution (a). Calculate the percentage contents of erythromycin B and erythromycin C using the chromatogram obtained with reference solution (b).

STORAGE

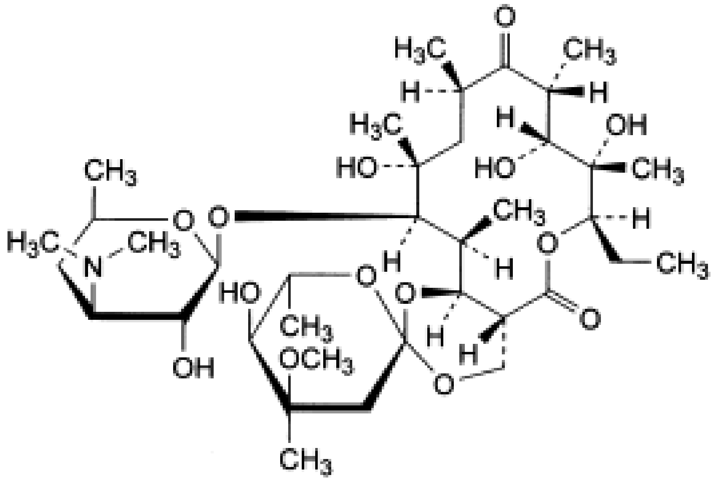
Protected from light.

IMPURITIES

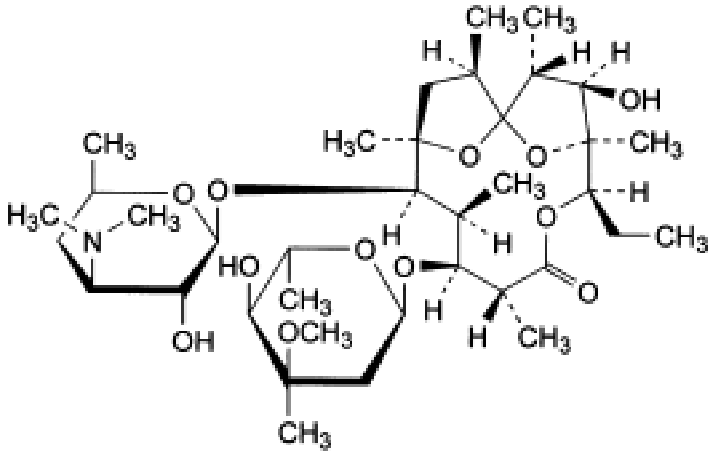


A. R1 = OH, R2 = CH_3: erythromycin F,

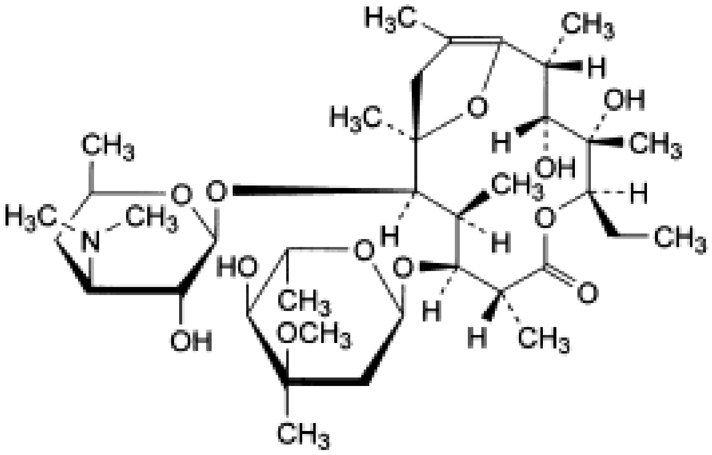
B. R1 = R2 = H: *N*-demethylerythromycin A,



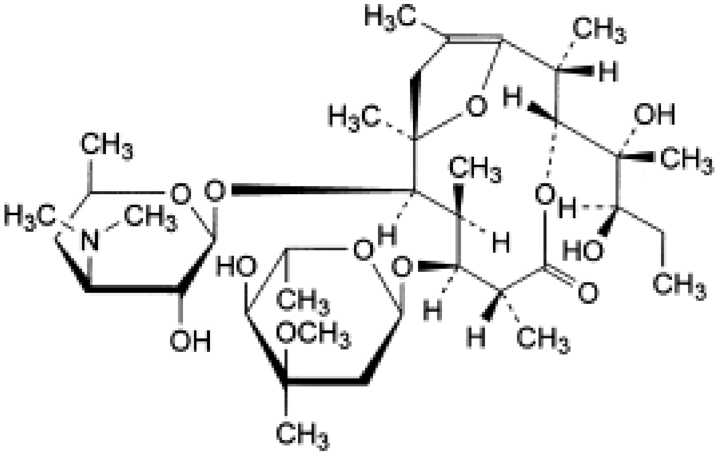
C. erythromycin E,



D. anhydroerythromycin A,



E. erythromycin A enol ether,



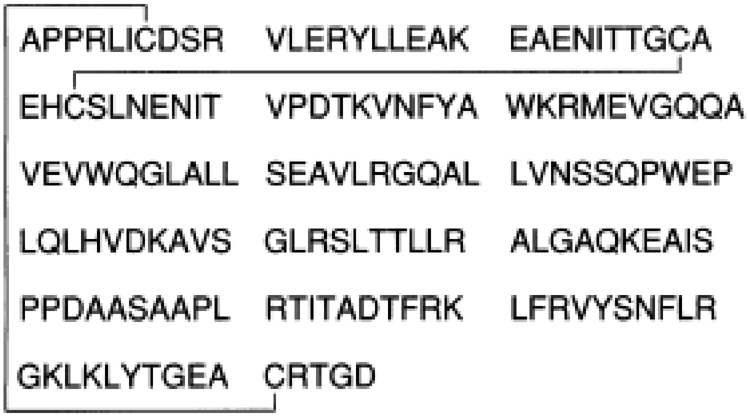
F. pseudoerythromycin A enol ether.

Ph Eur

Erythropoietin Concentrated Solution

General Notices

(Ph Eur monograph 1316)


APPRLICDSR VLERYLLEAK EAENITTGCA
EHCSLNENIT VPDTKVNFYA WKRMEVGQQA
VEVWQGLALL SEAVLRGQAL LVNSSQPWEP
LQLHVDKAVS GLRSLTTLLR ALGAQKEAIS
PPDAASAAPL RTITADTFRK LFRVYSNFLR
GKLKLYTGEA CRTGD

Molecular weight 30,600 (approx)

The label states (1) the type of erythropoeitin using the appropriate International Nonproprietary Name (Epoetin Alfa, Epoetin Beta, etc) and (2) the approved code in lower case letters indicative of the method of production.

Action and use

Erythropoietin analogue.

Ph Eur

DEFINITION

Erythropoietin concentrated solution is a solution containing a family of closely-related glycoproteins which are indistinguishable from the naturally occurring human erythropoietin (urinary erythropoietin) in terms of amino acid sequence (165 amino acids) and average glycosylation pattern, at a concentration of 0.5-10 mg/ml. It may also contain buffer salts and other excipients. It has a potency of not less than 100 000 IU/mg of active substance determined using the conditions described under Assay and in the test for protein.

PRODUCTION

Erythropoietin is produced in rodent cells *in vitro* by a method based on recombinant DNA technology.

Prior to batch release, the following tests are carried out on each batch of the final product, unless exemption has been granted by the competent authority.

Host cell-derived proteins

The limit is approved by the competent authority.

Host cell- and vector-derived DNA

The limit is approved by the competent authority.

CHARACTERS

Appearance

Clear or slightly turbid, colourless solution.

IDENTIFICATION

A. It gives the appropriate response when examined using the conditions described under Assay.

B. Capillary zone electrophoresis *(2.2.47)*.

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of 1 mg/ml. Desalt 0.25 ml of the solution by passage through a micro-concentrator cartridge provided with a membrane with a molecular mass cut-off of not more than 10 000 Da. Add 0.2 ml of *water R* to the sample and desalt again. Repeat the desalting procedure once more. Dilute the sample with *water R*, determine its protein concentration as described under Tests and adjust to a concentration of approximately 1 mg/ml with *water R*.

Reference solution Dissolve the contents of a vial of *erythropoietin BRP* in 0.25 ml of *water R*. Proceed with desalting as described for the test solution.

Capillary:

— *material*: uncoated fused silica,

— *size*: effective length = about 100 cm, Ø = 50 µm,

Temperature 35 °C.

CZE buffer concentrate (*0.1 M sodium chloride, 0.1 M tricine, 0.1 M sodium acetate*). Dissolve 0.584 g of *sodium chloride R*, 1.792 g of *tricine R* and 0.820 g of *anhydrous sodium acetate R* in *water R* and dilute to 100.0 ml with the same solvent.

1 M putrescine solution. Dissolve 0.882 g of *putrescine R* in 10 ml of *water R*. Distribute in 0.5 ml aliquots.

CZE buffer (*0.01 M tricine, 0.01 M sodium chloride, 0.01 M sodium acetate, 7 M urea, 2.5 mM putrescine*). Dissolve 21.0 g of *urea R* in 25 ml of *water R* by warming in a water-bath at 30 °C. Add 5.0 ml of CZE buffer concentrate and 125 µl of 1 M putrescine solution. Dilute to 50.0 ml with *water R*. Using *dilute acetic acid R*, adjust to pH 5.55 at 30 °C and filter through a 0.45 µm membrane filter.

Detection Spectrophotometer at 214 nm.

Set the autosampler to store the samples at 4 °C during analysis.

Preconditioning of the capillary Rinse the capillary for 60 min with *0.1 M sodium hydroxide* filtered through a 0.45 µm membrane filter and for 60 min with CZE buffer. Apply voltage for 12 h (20 kV).

Between-run rinsing Rinse the capillary for 10 min with *water R*, for 5 min with *0.1 M sodium hydroxide* filtered through a 0.45 µm membrane filter and for 10 min with CZE buffer.

Injection Under pressure or vacuum.

Migration Apply a field strength of 143 V/cm (15.4 kV for capillaries of 107 cm total length) for 80 min, using CZE buffer as the electrolyte in both buffer reservoirs.

System suitability In the electropherogram obtained with the reference solution, a pattern of well separated peaks corresponding to the peaks in the electropherogram of erythropoietin supplied with *erythropoietin BRP* is seen, and the largest peak is at least 50 times greater than the baseline noise. If necessary, adjust the sample load to give peaks of sufficient height. Identify the peaks due to isoforms 1 to 8. Isoform 1 may not be visible. The peak due to isoform 8 is detected and the resolution between the peaks due to isoforms 5 and 6 is not less than 1. Repeat the separation at least 3 times. The baseline is stable, showing little drift, and the distribution of peaks is qualitatively and quantitatively similar to the distribution of peaks in the electropherogram of erythropoietin supplied with *erythropoietin BRP*. The relative standard deviation of the migration time of the peak due to isoform 2 is less than 2 per cent.

Limits Identify the peaks due to isoforms 1 to 8 in the electropherogram obtained with the test solution by comparison with the electropherogram obtained with the reference solution. Calculate the percentage content of each isoform from the corresponding peak area. The percentages are within the following ranges:

Isoform	Content (per cent)
1	0 - 15
2	0 - 15
3	1 - 20
4	10 - 35
5	15 - 40
6	10 - 35
7	5 - 25
8	0 - 15

C. Polyacrylamide gel electrophoresis and immunoblotting.

(a) Polyacrylamide gel electrophoresis *(2.2.31)*

Gel dimensions 0.75 mm thick, about 16 cm square.

Resolving gel 12 per cent acrylamide.

Sample buffer *SDS-PAGE sample buffer (concentrated) R*.

Test solution (a) Dilute the preparation to be examined in *water R* to obtain a concentration of 1.0 mg/ml. To 1 volume of this solution add 1 volume of sample buffer.

Test solution (b) Dilute the preparation to be examined in *water R* to obtain a concentration of 0.1 mg/ml. To 1 volume of this solution add 1 volume of sample buffer.

Reference solution (a) Dissolve the contents of a vial of *erythropoietin BRP* in 0.25 ml of *water R*. To 1 volume of this solution add 1 volume of sample buffer.

Reference solution (b) Dissolve the contents of a vial of *erythropoietin BRP* in *water R* and dilute with the same solvent to obtain a concentration of 0.1 mg/ml. To 1 volume of this solution add 1 volume of sample buffer.

Reference solution (c) A solution of molecular mass markers suitable for calibrating SDS-polyacrylamide gels in the range of 10-70 kDa.

Reference solution (d) A solution of pre-stained molecular mass markers suitable for calibrating SDS-polyacrylamide gels in the range of 10-70 kDa and suitable for the electrotransfer to an appropriate membrane.

Sample treatment Boil for 2 min.

Application 20 µl, in the following order: reference solution (c), reference solution (a), test solution (a), empty well, reference solution (b), test solution (b), reference solution (d).

At the end of the separation, remove the gel-cassette from the apparatus and cut the gel into 2 parts: the first part containing reference solution (c), reference solution (a) and test solution (a); the second part containing reference solution (b), test solution (b) and reference solution (d).

Detection By Coomassie staining on the first part of the gel.

System suitability Reference solution (c):

— the validation criteria are met.

Results The electropherogram obtained with test solution (a) shows a single diffuse band corresponding in position and intensity to the single band seen in the electropherogram obtained with reference solution (a).

(b) Immunoblotting

Transfer the second part of the gel onto a membrane suitable for the immobilisation of proteins, using commercially available electrotransfer equipment and following the manufacturer's instructions. After electrotransfer, incubate the membrane in a neutral isotonic buffer containing a suitable blocking agent (for example, 50 g/l of dried milk or 10 per cent *V/V* foetal calf serum), for 1-2 h, followed by incubation for 1-14 h in the same blocking solution with a suitable dilution of either a polyclonal or monoclonal anti-erythropoietin antibody. Detect erythropoietin-bound antibody using a suitable enzyme- or radiolabelled antibody (for example, an alkaline phosphatase-conjugated second antibody). The precise details of blocking agents, concentrations and incubation times should be optimised using the principles set out in *Immunochemical methods (2.7.1)*.

System suitability In the electropherogram obtained with reference solution (d), the molecular mass markers are resolved on the membrane into discrete bands, with a linear relationship between distance migrated and logarithm_{10} of the molecular mass.

Results The electropherogram obtained with test solution (b) shows a single broad band corresponding in position and intensity to the single band seen in the electropherogram obtained with reference solution (b).

D. Peptide mapping *(2.2.55)*

Liquid chromatography *(2.2.29)*.

Test solution Dilute the preparation to be examined in *tris-acetate buffer solution pH 8.5 R* to a concentration of 1.0 mg/ml. Equilibrate the solution in *tris-acetate buffer solution pH 8.5 R* using a suitable procedure (dialysis against *tris-acetate buffer solution pH 8.5 R*, or membrane filtration using the procedure described under Identification B, but reconstituting the desalted sample with *tris-acetate buffer solution pH 8.5 R*, are suitable). Transfer the dialysed solution to a polypropylene centrifuge tube. Freshly prepare a solution of *trypsin for peptide mapping R* at a concentration of 1 mg/ml in *water R*, and add 5 µl to 0.25 ml of the dialysed solution. Cap the tube and place in a water-bath at 37 °C for 18 h. Remove the sample from the water-bath and stop the reaction immediately by freezing.

Reference solution Dissolve the contents of a vial of *erythropoietin BRP* in 0.25 ml of *water R*. Prepare as for the test solution, ensuring that all procedures are carried out simultaneously, and under identical conditions.

Column:

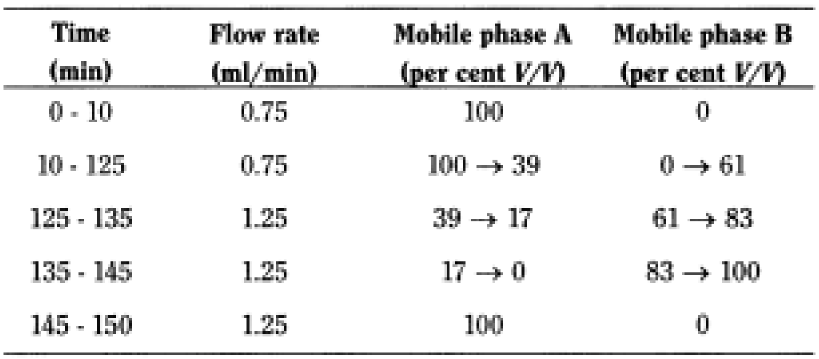
— *size: l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *butylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase:

— *mobile phase A*: 0.06 per cent *V/V* solution of *trifluoroacetic acid R*,

— *mobile phase B*: to 100 ml of *water R* add 0.6 ml of *trifluoroacetic acid R* and dilute to 1000 ml with *acetonitrile for chromatography R*,



Time (min)	Flow rate (ml/min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	0.75	100	0
10 - 125	0.75	100 → 39	0 → 61
125 - 135	1.25	39 → 17	61 → 83
135 - 145	1.25	17 → 0	83 → 100
145 - 150	1.25	100	0

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.

Injection 50 µl.

System suitability The chromatograms obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of erythropoietin digest supplied with *erythropoietin BRP*.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

E. *N*-terminal sequence analysis.

The first 15 amino acids are: Ala - Pro - Pro - Arg - Leu - Ile - (no recovered peak) - Asp - Ser - Arg - Val - Leu - Glu - Arg - Tyr.

Perform the Edman degradation using an automated solid-phase sequencer, operated in accordance with the manufacturer's instructions.

Desalt the equivalent of 50 µg of erythropoietin. For example, dilute a volume of the preparation to be examined equivalent to 50 µg of the active substance in 1 ml of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R*. Pre-wash a C18 reverse-phase sample preparation cartridge according to the instructions supplied and equilibrate the cartridge in a 0.1 per cent *V/V* solution of *trifluoroacetic acid R*. Apply the sample to the cartridge, and wash successively with a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* containing 0 per cent, 10 per cent and 50 per cent *V/V* of *acetonitrile R* according to the manufacturer's instructions. Lyophilise the 50 per cent *V/V acetonitrile R* eluate.

Redissolve the desalted sample in 50 µl of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* and couple to a sequencing cartridge using the protocol provided by the manufacturer. Run 15 sequencing cycles, using the reaction conditions for proline when running the 2nd and 3rd cycles.

Identify the phenylthiohydantoin (PTH)-amino acids released at each sequencing cycle by reverse-phase liquid chromatography. The procedure may be carried out using the column and reagents recommended by the manufacturer of the sequencing equipment for the separation of PTH-amino-acids.

The separation procedure is calibrated using:

- — the mixture of PTH-amino acids provided by the manufacturer of the sequencer, with the gradient conditions adjusted as indicated to achieve optimum resolution of all amino acids,
- — a sample obtained from a blank sequencing cycle obtained as recommended by the equipment manufacturer.

TESTS

Protein *(2.5.33, Method I)*

80 per cent to 120 per cent of the stated concentration.

Test solution Dilute the preparation to be examined in a 4 g/l solution of *ammonium hydrogen carbonate R*.

Record the absorbance spectrum between 250 nm and 400 nm. Measure the value at the absorbance maximum (276-280 nm), after correction for any light scattering, measured up to 400 nm. Calculate the concentration of erythropoietin taking the specific absorbance to be 7.43.

Dimers and related substances of higher molecular mass

Size-exclusion chromatography *(2.2.30)*.

Test solution Dilute the preparation to be examined in the mobile phase to obtain a concentration of 0.2 mg/ml.

Reference solution To 0.02 ml of the test solution add 0.98 ml of the mobile phase.

Column:

- — *size:* *l* = 0.6 m, Ø = 7.5 mm,
- — *stationary phase*: *hydrophilic silica gel for chromatography R*, of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 20 000 to 200 000.

Mobile phase Dissolve 1.15 g of *anhydrous disodium hydrogen phosphate R*, 0.2 g of *potassium dihydrogen phosphate R* and 23.4 g of *sodium chloride R* in 1 litre of *water R* (1.5 mM potassium dihydrogen phosphate, 8.1 mM disodium hydrogen phosphate, 0.4 M sodium chloride, pH 7.4); adjust to pH 7.4 if necessary.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µl.

Run time Minimum 1 h.

System suitability The area of the principal peak in the chromatogram obtained with the reference solution is 1.5 per cent to 2.5 per cent of the area of the principal peak in the chromatogram obtained with the test solution.

Limits:

— *total of any peaks eluted before the principal peak*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (2 per cent).

Sialic acids

Minimum 10 mol of sialic acids (calculated as *N*-acetylneuraminic acid) per mole of erythropoietin.

Test solution (a) Dilute the preparation to be examined in the mobile phase used in the test for dimers and related substances of higher molecular mass to obtain a concentration of 0.3 mg/ml.

Test solution (b) To 0.5 ml of test solution (a) add 0.5 ml of the mobile phase used in the test for dimers and related substances of higher molecular mass.

Reference solution (a) Dissolve a suitable amount of *N-acetylneuraminic acid R* in *water R* to obtain a concentration of 0.1 mg/ml.

Reference solution (b) To 0.8 ml of reference solution (a) add 0.2 ml of *water R*.

Reference solution (c) To 0.6 ml of reference solution (a) add 0.4 ml of *water R*.

Reference solution (d) To 0.4 ml of reference solution (a) add 0.6 ml of *water R*.

Reference solution (e) To 0.2 ml of reference solution (a) add 0.8 ml of *water R*.

Reference solution (f) Use *water R*.

Carry out the test in triplicate. Transfer 100 µl of each of the test and reference solutions to 10 ml glass test tubes. To each tube add 1.0 ml of *resorcinol reagent R*. Stopper the tubes and incubate at 100 °C for 30 min. Cool on ice. To each tube, add 2.0 ml of a mixture of 12 volumes of *butanol R* and 48 volumes of *butyl acetate R*. Mix vigorously, and allow the 2 phases to separate. Ensuring that the upper phase is completely clear, remove the upper phase, taking care to exclude completely any of the lower phase. Measure the absorbance *(2.2.25)* of all samples at 580 nm. Using the calibration curve generated by the reference solutions, determine the content of sialic acids in test solutions (a) and (b) and calculate the mean. Calculate the number of moles of sialic acids per mole of erythropoietin assuming that the relative molecular mass of erythropoietin is 30 600 and that the relative molecular mass of *N*-acetylneuraminic acid is 309.

System suitability:

— the individual replicates agree to within ± 10 per cent of each other,

— the value obtained from reference solution (a) is between 1.5 and 3.3 times that obtained with test solution (a).

Bacterial endotoxins *(2.6.14)*

Less than 20 IU in the volume that contains 100 000 IU of erythropoietin.

ASSAY

The activity of the preparation is compared with that of *erythropoietin BRP* and expressed in International Units (IU).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits of the estimated potency ($P = 0.95$) are not less than 64 per cent and not more than 156 per cent of the stated potency.

Carry out the determination of potency by Method A or B.

A. In polycythaemic mice

The activity of the preparation is estimated by examining, under given conditions, its effect in stimulating the incorporation of ^{59}Fe into circulating red blood cells of mice made polycythaemic by exposure to reduced atmospheric pressure.

The following schedule, using treatment in a hypobaric chamber, has been found to be suitable.

Induce polycythaemia in female mice of the same strain, weighing 16-18 g. Place the mice in a hypoxic chamber and reduce the pressure to 0.6 atmospheres. After 3 days at 0.6 atmospheres, further reduce the pressure to 0.4-0.5 atmospheres and maintain the animals at this pressure for a further 11 days (the partial vacuum is interrupted daily for a maximum of 1 h at about 11:00 a.m., in order to clean the cages and feed the animals). At the end of the specified period, return the mice to normal atmospheric conditions. Randomly distribute the mice into cages, each containing 6 animals, and mark them.

Test solution (a) Dilute the substance to be examined in *phosphate-albumin buffered saline pH 7.2 R1* to obtain a concentration of 0.2 IU/ml.

Test solution (b) Mix equal volumes of test solution (a) and *phosphate-albumin buffered saline pH 7.2 R1*.

Test solution (c) Mix equal volumes of test solution (b) and *phosphate-albumin buffered saline pH 7.2 R1*.

Reference solution (a) Dissolve *erythropoietin BRP* in *phosphate-albumin buffered saline pH 7.2 R1* to obtain a concentration of 0.2 IU/ml.

Reference solution (b) Mix equal volumes of reference solution (a) and *phosphate-albumin buffered saline pH 7.2 R1*.

Reference solution (c) Mix equal volumes of reference solution (b) and *phosphate-albumin buffered saline pH 7.2 R1*.

Radiolabelled ferric [^{59}Fe] chloride solution, concentrated Use a commercially available solution of [^{59}Fe]ferric chloride (approximate specific activity: 100-1000 MBq/mg of Fe).

Radiolabelled [^{59}Fe]ferric chloride solution Dilute the concentrated radiolabelled [^{59}Fe]ferric chloride solution in *sodium citrate buffer solution pH 7.8 R* to obtain a solution with an activity of 3.7×10^4 Bq/ml.

The concentrations of the test solutions and reference solutions may need to be modified, based on the response range of the animals used.

3 days after returning the animals to atmospheric pressure, inject each animal subcutaneously with 0.2 ml of one of the solutions. The 6 animals in each cage must each receive one of the 6 different treatments (3 test solutions and 3 reference solutions), and the

order of injection must be separately randomised for each cage. A minimum of 8 cages is recommended. 2 days after injection of the test or reference solution, inject each animal intraperitoneally with 0.2 ml of radiolabelled [^{59}Fe]ferric chloride solution. The order of the injections must be the same as that of the erythropoietin injections, and the time interval between administration of the erythropoietin and the radiolabelled ferric chloride solution must be the same for each animal. After a further 48 h, anaesthetise each animal by injection of a suitable anaesthetic, record body weights and withdraw blood samples (0.65 ml) into haematocrit capillaries from the bifurcation of the aorta. After determining the packed cell volume for each sample, measure the radioactivity.

Calculate the response (percentage of iron-59 in total circulating blood) for each mouse using the expression:

$$\frac{A_s \times M \times 7.5}{A_t \times V_s}$$

A_s = radioactivity in the sample,

A_t = total radioactivity injected,

7.5 = total blood volume as per cent body weight,

M = body weight, in grams,

V_s = sample volume.

Calculate the potency by the usual statistical methods for a parallel line assay. Eliminate from the calculation any animal where the packed cell volume is less than 54 per cent, or where the body weight is more than 24 g.

B. In normocythaemic mice

The assay is based on the measurement of stimulation of reticulocyte production in normocythaemic mice.

The assay may be carried out using the following procedure:

Test solution (a) Dilute the preparation to be examined in *phosphate-albumin buffered saline pH 7.2 R1* to obtain a concentration of 80 IU/ml.

Test solution (b) Mix equal volumes of test solution (a) and *phosphate-albumin buffered saline pH 7.2 R1*.

Test solution (c) Mix equal volumes of test solution (b) and *phosphate-albumin buffered saline pH 7.2 R1*.

Reference solution (a) Dissolve *erythropoietin BRP* in *phosphate-albumin buffered saline pH 7.2 R1* to obtain a concentration of 80 IU/ml.

Reference solution (b) Mix equal volumes of reference solution (a) and *phosphate-albumin buffered saline pH 7.2 R1*.

Reference solution (c) Mix equal volumes of reference solution (b) and *phosphate-albumin buffered saline pH 7.2 R1.*

The exact concentrations of the test solutions and reference solutions may need to be modified, based on the response range of the animals used.

At the beginning of the assay procedure, randomly distribute mice of a suitable age and strain (8-week old B6D2F1 mice are suitable) into 6 cages. A minimum of 8 mice per cage is recommended. Inject each animal subcutaneously with 0.5 ml of the appropriate treatment (one solution per cage) and put the animal in a new cage. Combine the mice in such a way that each cage housing the treated mice contains one mouse out of the 6 different treatments (3 test solutions and 3 reference solutions, 6 mice per cage). 4 days after the injections, collect blood samples from the animals and determine the number of reticulocytes using a suitable procedure.

The following method may be employed:

The volume of blood, dilution procedure and fluorescent reagent may need to be modified to ensure maximum development and stability of fluorescence.

Colorant solution, concentrated Use a solution of thiazole orange suitable for the determination of reticulocytes. Prepare at a concentration twice that necessary for the analysis.

Proceed with the following dilution steps. Dilute whole blood 500-fold in the buffer used to prepare the colorant solution. Dilute this solution 2-fold in the concentrated colorant solution. After staining for 3-10 min, determine the reticulocyte count microfluorometrically in a flow cytometer. The percentage of reticulocytes is determined using a biparametric histogram: number of cells/red fluorescence (620 nm).

Calculate the potency by the usual statistical methods for a parallel line assay.

STORAGE

In an airtight container at a temperature below - 20 °C. Avoid repeated freezing and thawing.

LABELLING

The label states:

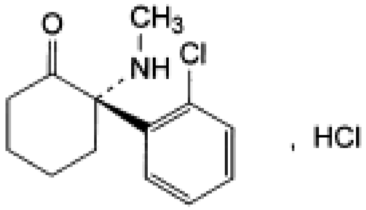
- — the erythropoietin content in milligrams per millilitre,
- — the activity in International Units per millilitre,
- — the name and the concentration of any other excipients.

Ph Eur

Esketamine Hydrochloride

General Notices

(Ph Eur monograph 1742)



$C_{13}H_{16}ClNO,HCl$ 274.2 *33795-24-3*

Action and use

General anaesthetic.

Ph Eur

DEFINITION

(2*S*)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride.

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, soluble in alcohol.

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: + 85.0 to + 95.0.

Dilute 12.5 ml of solution S (see Tests) to 40.0 ml with *water R*.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of esketamine hydrochloride.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 8.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

3.5 to 4.5.

Dilute 12.5 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Impurity D

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *esketamine impurity D CRS* in *water R*, add 20 ml of the test solution and dilute to 50 ml with *water R*. Dilute 10 ml of this solution to 100 ml with *water R*.

Reference solution (b) Dilute 5.0 ml of the test solution to 25.0 ml with *water R*. Dilute 5.0 ml of this solution to 50.0 ml with *water R*.

Reference solution (c) Dilute 2.5 ml of reference solution (b) to 10.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Precolumn:

— *size:* l = 0.01 m, Ø = 3.0 mm,

— *stationary phase*: *silica gel AGP for chiral chromatography R* (5 µm),

— *temperature*: 30 °C.

Column:

— *size:* l = 0.125 m, Ø = 4.6 mm,

— *stationary phase*: *silica gel AGP for chiral chromatography R* (5 µm),

— *temperature*: 30 °C.

Mobile phase Mix 16 volumes of *methanol R* and 84 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with *potassium hydroxide R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 20 min.

Relative retention With reference to esketamine (retention time = about 10 min): impurity D = about 1.3.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to esketamine and impurity D in the chromatogram obtained with reference solution (a),

— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (c).

Limit:

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *ketamine impurity A CRS* in the mobile phase (using ultrasound, if necessary) and dilute to 10 ml with the mobile phase. To 1 ml of the solution add 0.5 ml of the test solution and dilute to 100 ml with the mobile phase. Prepare immediately before use.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm,

— *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.95 g of *sodium hexanesulphonate R* in 1000 ml of a mixture of 25 volumes of *acetonitrile R* and 75 volumes of *water R* and add 4 ml of *acetic acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 10 times the retention time of esketamine.

Relative retention With reference to esketamine: impurity A = about 1.6; impurity B = about 3.3; impurity C = about 4.6.

System suitability Reference solution (a):

— *retention time*: esketamine = 3.0 min to 4.5 min,

— *resolution*: minimum 1.5 between the peaks due to impurity A and esketamine.

Limits:

— *impurities A, B, C*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.1 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dilute 12.5 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *methanol R* and add 1.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

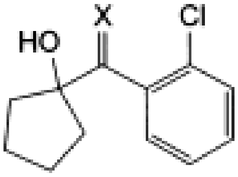
1 ml of *0.1 M sodium hydroxide* is equivalent to 27.42 mg of $C_{13}H_{17}Cl_2NO$.

STORAGE

Protected from light.

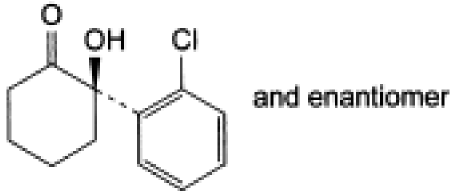
IMPURITIES

Specified impurities A, B, C, D.

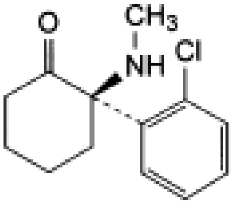


A. X = N-CH_3: 1-[(2-chlorophenyl)(methylimino)methyl]cyclopentanol,

C. X = O: (2-chlorophenyl)(1-hydroxycyclopentyl)methanone,



B. (2*RS*)-2-(2-chlorophenyl)-2-hydroxycyclohexanone,



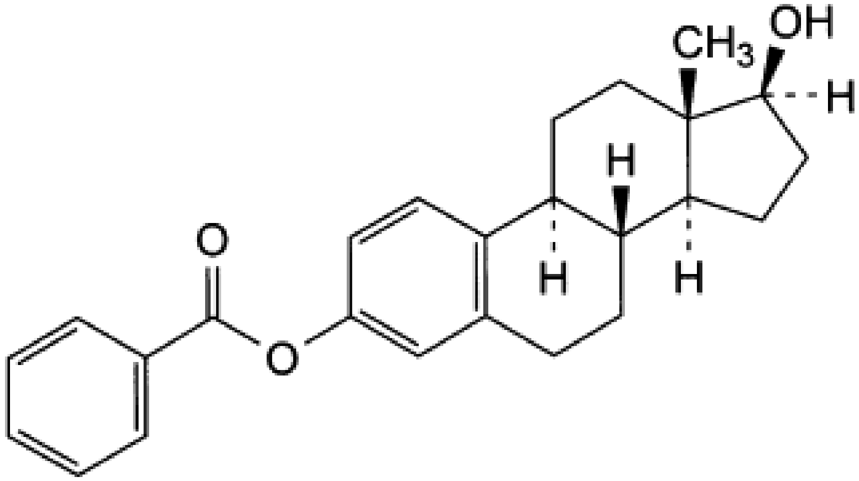
D. (2*R*)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone ((*R*)-ketamine).

Ph Eur

Estradiol Benzoate

General Notices

(Ph Eur monograph 0139)



$C_{25}H_{28}O_3$ 376.5 *50-50-0*

Action and use

Estrogen.

Preparation

Estradiol Injection

Ph Eur

DEFINITION

17β-Hydroxyestra-1,3,5(10)-trien-3-yl benzoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

Almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in methanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison estradiol benzoate CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation *(2.2.7)*

+ 55.0 to + 59.0 (dried substance).

Dissolve 0.250 g in *acetone R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*)

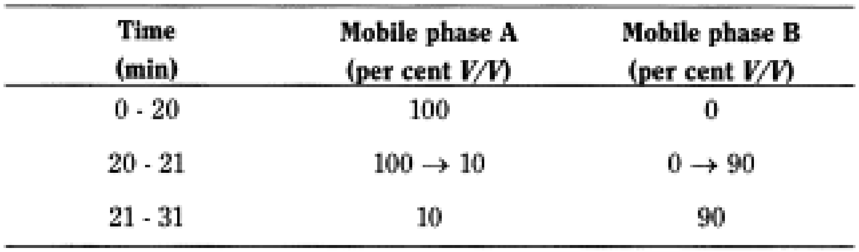
Test solution Dissolve 20 mg of the substance to be examined in *acetonitrile R1* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *estradiol benzoate for system suitability CRS* (containing impurities A, B, C, E and G) in *acetonitrile R1* and dilute to 2.5 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of the test solution to 100.0 ml with *acetonitrile R1.*

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *water R*, *acetonitrile R1* (40:60 *V/V*);
- *mobile phase B*: *acetonitrile R1;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 21	100 → 10	0 → 90
21 - 31	10	90

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *estradiol benzoate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, E and G.

Relative retention With reference to estradiol benzoate (retention time = about 19 min): impurity A = about 0.3; impurity E = about 1.1; impurity B = about 1.2; impurity G = about 1.3; impurity C = about 1.5.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to estradiol benzoate.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 3.3; impurity C = 0.7;

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities B, E, G*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity A*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

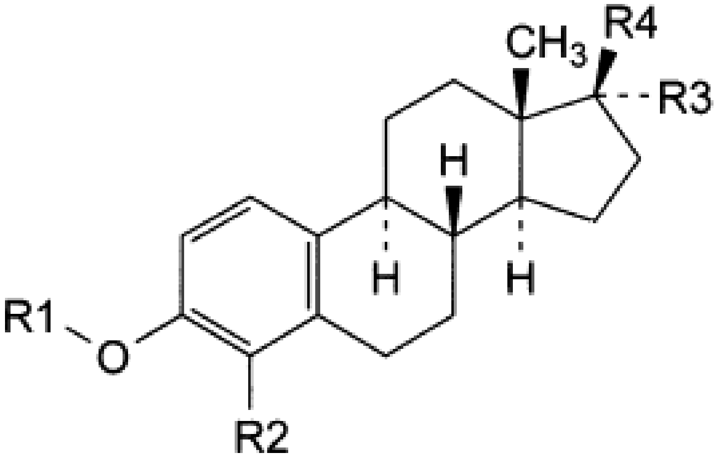
Dissolve 25.0 mg in *anhydrous ethanol R* and dilute to 250.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *anhydrous ethanol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 231 nm.

Calculate the content of $C_{25}H_{28}O_3$ taking the specific absorbance to be 500.

IMPURITIES

Specified impurities A, B, C, E, G.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D, F, H.*



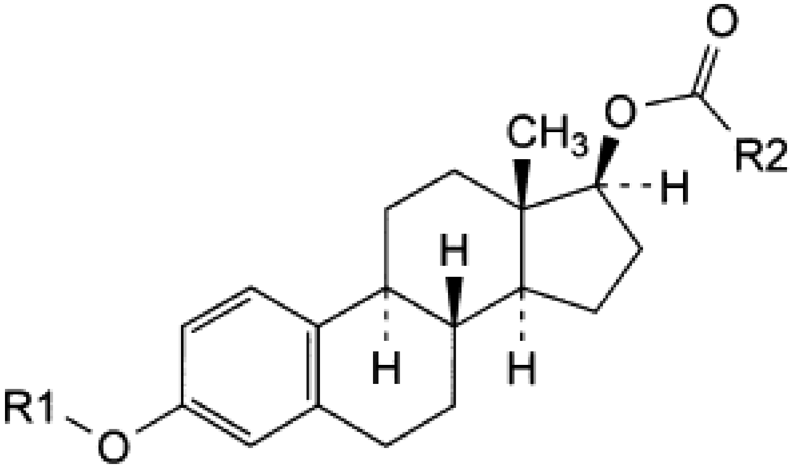
A. R1 = R2 = R3 = H, R4 = OH: estradiol,

B. R1 = CO-C_6H_5, R2 = CH_3, R3 = H, R4 = OH: 17β-hydroxy-4-methylestra-1,3,5(10)-trien-3-yl benzoate,

C. R1 = CO-C_6H_5, R2 = R3 = H, R4 = O-CO-C_6H_5: estra-1,3,5(10)-triene-3,17β-diyl dibenzoate,

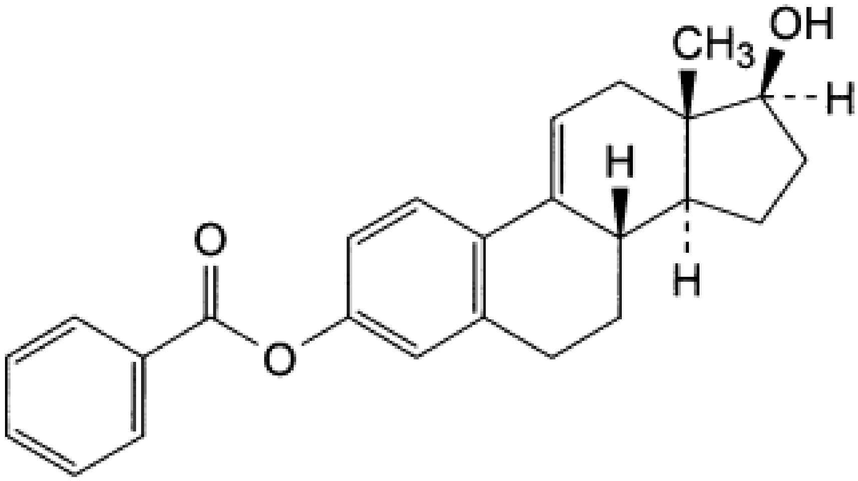
E. R1 = CO-C_6H_5, R2 = R4 = H, R3 = OH: 17α-hydroxyestra-1,3,5(10)-trien-3-yl benzoate,

G. R1 = CO-C_6H_5, R2 = H, R3 + R4 = O: 17-oxoestra-1,3,5(10)-trien-3-yl benzoate (estrone benzoate),



D. R1 = H, R2 = C_6H_5: 3-hydroxyestra-1,3,5(10)-trien-17β-yl benzoate,

H. R1 = CO-C_6H_5, R2 = CH_3: estra-1,3,5(10)-triene-3,17β-diyl 17-acetate 3-benzoate,



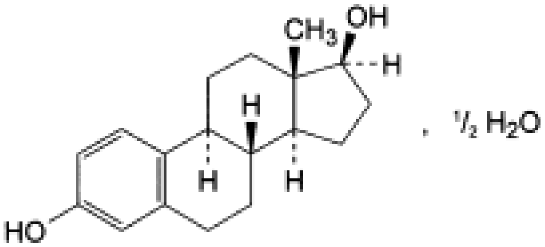
F. 17β-hydroxyestra-1,3,5(10),9(11)-tetraen-3-yl benzoate.

Ph Eur

Estradiol Hemihydrate

General Notices

(Ph Eur monograph 0821)



$C_{18}H_{24}O_2,½H_2O$ 281.4 *50-28-2*
(anhydrous)

Action and use

Estrogen.

Preparation

Estradiol Transdermal Patches

Estradiol and Norethisterone Tablets

Estradiol and Norethisterone Acetate Tablets

Ph Eur

DEFINITION

Estra-1,3,5(10)-triene-3,17β-diol hemihydrate.

Content

97.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, soluble in acetone, sparingly soluble in alcohol, slightly soluble in methylene chloride.

IDENTIFICATION

First identification B.

Second identification A, C, D, E.

A. Melting point *(2.2.14)*: 175 °C to 180 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *estradiol hemihydrate CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 50 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *estradiol hemihydrate CRS* in *methanol R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dissolve 25 mg of *ethinylestradiol CRS* in reference solution (a) and dilute to 25 ml with reference solution (a).

Plate *TLC silica gel plate R.*

Mobile phase *alcohol R, toluene R* (20:80 *V/V*).

Application 5 µl.

Development Over 3/4 of the plate.

Drying In air until the solvent has evaporated.

Detection Heat at 110 °C for 10 min. Spray the hot plate with *alcoholic solution of sulphuric acid R*. Heat again at 110 °C for 10 min. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

System suitability The chromatogram obtained with reference solution (b) shows 2 spots which may however not be completely separated.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 1 mg add 0.5 ml of freshly prepared *sulphomolybdic reagent R2*. A blue colour develops which in ultraviolet light at 365 nm has an intense green fluorescence. Add 1 ml of *sulphuric acid R* and 9 ml of *water R*. The colour becomes pink with a yellowish fluorescence.

E. It complies with the test for water (see Tests).

TESTS

Specific optical rotation *(2.2.7)*

+ 76.0 to + 83.0 (anhydrous substance).

Dissolve 0.250 g in *alcohol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in 10 ml of *acetonitrile R* and dilute to 25.0 ml with *methanol R2*.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 2 mg of *17α-estradiol R* in 5.0 ml of *acetonitrile R*. Mix 2.0 ml of this solution with 1.0 ml of the test solution and dilute to 5.0 ml with the mobile phase.

Reference solution (c) Mix equal volumes of a 1 mg/ml solution of the substance to be examined in *methanol R2* and of a 1 mg/ml solution of *2,3-dichloro-5,6-dicyanobenzoquinone R* in *methanol R2*. Allow to stand for 30 min before injection.

Reference solution (d) Dissolve 5 mg of *estradiol for peak identification CRS* (estradiol hemihydrate spiked with impurities A, B and C at about 0.5 per cent) in 2 ml of *acetonitrile R* and dilute to 5 ml with *methanol R2*.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase To 400 ml of *acetonitrile R* add 50 ml of *methanol R2* and 400 ml of *water R*; allow to stand for 10 min, dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Equilibration About 60 min.

Injection 20 µl.

Run time Twice the retention time of the principal peak.

Relative retention With reference to estradiol (retention time = about 13 min): impurity D = about 0.9; impurity B = about 1.1; impurity A = about 1.4; impurity C = about 1.9.

System suitability Reference solution (b):

- *resolution*: minimum 2.5 between the peaks due to estradiol and impurity B.

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity D by 0.4 (use the chromatogram obtained with reference solution (c) to identify this peak),
- *impurities A, B, C, D*: for each impurity, not more than 1.5 times the area of the principal peak obtained with reference solution (a) (0.3 per cent),
- *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak obtained with reference solution (a) (0.1 per cent),
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*

2.9 per cent to 3.5 per cent, determined on 0.500 g.

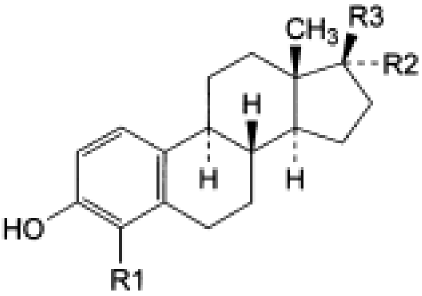
ASSAY

Dissolve 20.0 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *0.1 M sodium hydroxide*. Allow to cool to room temperature. Measure the absorbance *(2.2.25)* of the solution at the maximum at 238 nm.

Calculate the content of $C_{18}H_{24}O_2$ taking the specific absorbance to be 335.

IMPURITIES

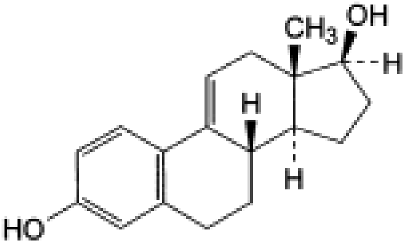
Specified impurities A, B, C, D.



A. R1 = H, R2 + R3 = O: 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),

B. R1 = R3 = H, R2 = OH: estra-1,3,5(10)-triene-3,17α-diol (17α-estradiol),

C. R1 = CH_3, R2 = H, R3 = OH: 4-methylestra-1,3,5(10)-triene-3,17β-diol,



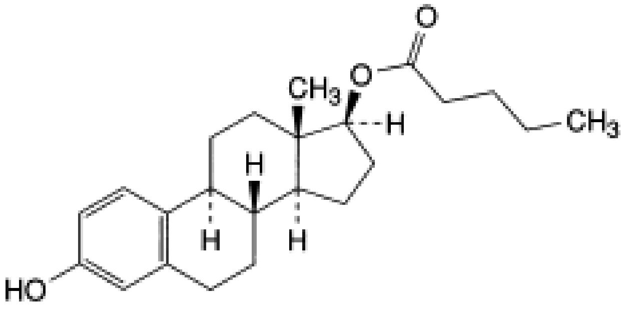
D. estra-1,3,5(10),9(11)-tetraene-3,17β-diol.

Ph Eur

Estradiol Valerate

General Notices

(Ph Eur monograph 1614)



$C_{23}H_{32}O_3$ 356.5 *979-32-8*

Action and use

Estrogen.

Ph Eur

DEFINITION

3-Hydroxyestra-1,3,5(10)-trien-17β-yl pentanoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, soluble in alcohol.

mp

About 145 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *estradiol valerate CRS.*

TESTS

Solution S

Dissolve 0.500 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

+ 41 to + 47 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture Mix 15 volumes of *water R* and 135 volumes of *acetonitrile R*.

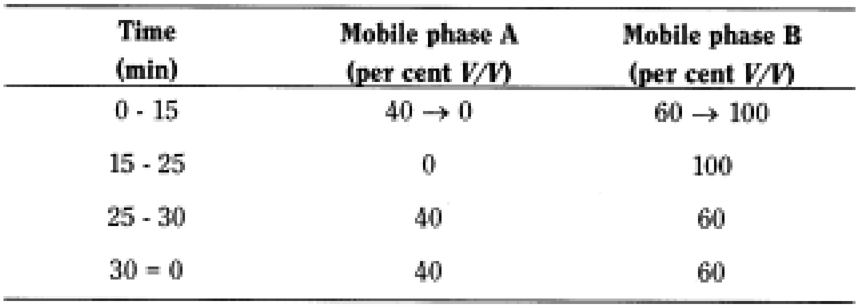
Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *estradiol valerate CRS* and 2 mg of *estradiol butyrate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dilute 0.5 ml of the test solution to 100.0 ml with the solvent mixture.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm,
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- — *temperature*: 40 °C.

Mobile phase:
- — *mobile phase A*: *water R*,
- — *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	40 → 0	60 → 100
15 - 25	0	100
25 - 30	40	60
30 = 0	40	60

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Relative retention With reference to estradiol valerate (retention time = about 12 min):

impurity F = about 0.9.

System suitability Reference solution (a):

— *resolution*: minimum of 5.0 between the peaks due to impurity F and to estradiol valerate.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

ASSAY

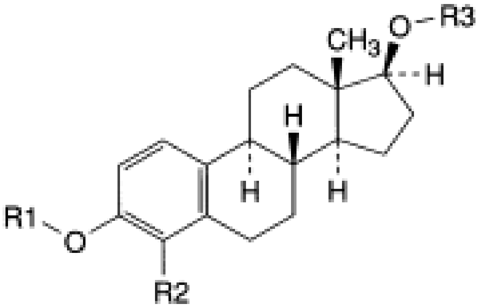
Dissolve 25.0 mg in *alcohol R* and dilute to 250.0 ml with the same solvent. Measure the absorbance *(2.2.25)* at the maximum at 280 nm.

Calculate the content of $C_{23}H_{32}O_3$ taking the specific absorbance to be 58.0.

STORAGE

Protected from light.

IMPURITIES



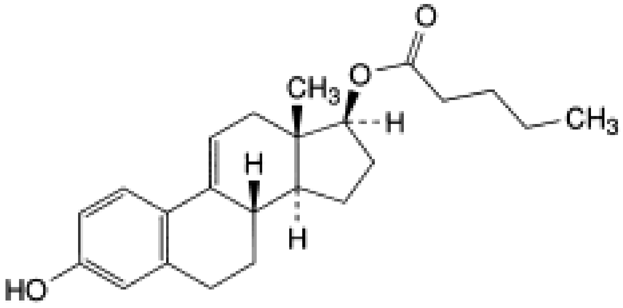
A. R1 = R2 = R3 = H: estradiol,

B. R1 = CO-$[CH_2]_3$-CH_3, R2 = R3 = H: 17β-hydroxyestra-1,3,5(10)-trien-3-yl pentanoate,

D. R1 = H,R2 = CH_3,R3 = CO-$[CH_2]_3$-CH_3: 3-hydroxy-4-methylestra-1,3,5(10)-trien-17β-yl pentanoate,

E. R1 = R3 = CO-$[CH_2]_3$-CH_3,R2 = H: estra-1,3,5(10)-trien-3,17β-diyl dipentanoate,

F. R1 = R2 = H,R3 = CO-$[CH_2]_2$-CH_3: 3-hydroxyestra-1,3,5(10)-trien-17β-yl butanoate (estradiol butyrate),

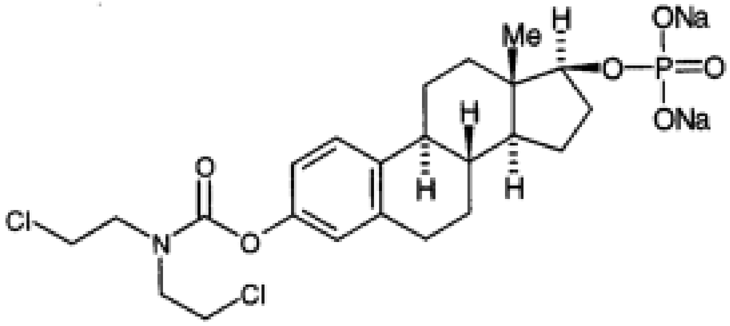

O
CH3
O
H
CH3
H
H
HO

C. 3-hydroxyestra-1,3,5(10),9(11)-tetraen-17β-yl pentanoate.

Ph Eur

Estramustine Sodium Phosphate

General Notices



$C_{23}H_{30}Cl_2NNa_2O_6P$ 564.4 *52205-73-9*

Action and use

Cytotoxic alkylating agent.

Preparation

Estramustine Phosphate Capsules

DEFINITION

Estramustine Sodium Phosphate is disodium 3-[bis(2-chloroethyl)carbamoyloxy]estra-1,3,5(10)-trien-17β-yl orthophosphate. It contains not less than 97.0% and not more than 103.0% of $C_{23}H_{30}Cl_2NNa_2O_6P$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white or almost white powder.

Freely soluble in *water* and in *methanol*; very slightly soluble in *absolute ethanol*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.05% w/v solution exhibits maxima at 267 nm and 275 nm. The *absorbance* at 267 nm is about 0.76 and at 275 nm is about 0.71.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of estramustine sodium phosphate *(RS 128)*. In preparing the potassium bromide disc precautions should be taken to exclude moisture and avoid excessive grinding; if necessary heat the prepared disc at 90° for 2 minutes.

C. A 1% w/v solution yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

pH of a 0.5% w/v solution, 8.5 to 10.0, Appendix V L.

Clarity and colour of solution

A 5.0% w/v solution is not more opalescent than *reference suspension II*, Appendix IV A, and is *colourless*, Appendix IV B, Method I.

Specific optical rotation

In a 2% w/v solution, +11 to +13, Appendix V F, calculated with reference to the anhydrous substance.

Ionisable chlorine

Dissolve 0.10 g in 10 ml of *water*, add carefully, with mixing, 0.1 ml of a mixture of 10 volumes of *silver nitrate solution* and 1 volume of *nitric acid* and examine immediately. Any opalescence produced is not more intense than that obtained by treating a solution containing 13.4 µg of *sodium chloride* in 10 ml in the same manner (0.1%).

Estradiol 17β-phosphate

Dissolve 50 mg in 5 ml of 0.2M *sodium hydroxide*, add sufficient *ethanol (96%)* to produce 10 ml, mix and immediately measure the *absorbance* at the maxima at 300 nm and 350 nm, Appendix II B. The difference between the two absorbances is not more than 0.34 (1.0%).

Inorganic phosphate

Dissolve 25 mg in 10 ml of *water*, add 4 ml of 1M *sulphuric acid* , 1 ml of a 10% w/v solution of *ammonium molybdate* and 2 ml of *methylaminophenol-sulphite reagent* and allow to stand for 15 minutes. Add sufficient *water* to produce 25 ml, allow to stand for 15 minutes and filter. The *absorbance* of the filtrate at 730 nm, Appendix II B, is not greater than the *absorbance* at 730 nm of a solution obtained by repeating the operation using 10 ml of a 0.00180% w/v solution of *potassium dihydrogen orthophosphate* and beginning at the words 'add 4 ml of 1M *sulphuric acid* …'.

Volatile matter

Carry out the method for *gas chromatography*, Appendix III B, using solutions in *water* containing (1) 0.0040% v/v of *pyridine*, 0.020% v/v of *absolute ethanol* and 0.020% v/v of *butan-1-ol* (internal standard), (2) 4.0% w/v of the substance being examined and (3) 4.0% w/v of the substance being examined and 0.020% v/v of the internal standard.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 20% w/w of polyethylene glycol (Carbowax 20M is suitable) and maintained at 120°.

In the chromatogram obtained with solution (1) the area of the peak due to pyridine is greater than the area of any corresponding peak in the chromatogram obtained with solution (3). In the chromatogram obtained with solution (1) the area of the peak due to ethanol is greater than the sum of the areas of any peaks with a retention time less than that of the peak due to the internal standard in the chromatogram obtained with solution (3).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of equal volumes of *butan-2-one*, *propan-2-ol* and *triethylamine hydrogen carbonate solution* as the mobile phase. Apply separately to the plate 10 µl of each of four freshly prepared solutions in a mixture of 49 volumes of *methanol* and 1 volume of *triethylamine* containing (1) 4.0% w/v of the substance being examined, (2) 0.020% w/v of the substance being examined, (3) 0.080% w/v of *17β,17′β-bis3-[bis(2-chloroethyl) carbamoyloxy]estra1,3,5(10)-trienyl pyrophosphate BPCRS* and (4) 0.040% of *estramustine BPCRS*. After removal of the plate, allow it to dry in air, spray with *methanolic sulphuric acid (20%)* and heat at 110° for 10 minutes. The principal spots in the chromatograms obtained with solutions (3) and (4) are more intense than any corresponding spots in the chromatogram obtained with solution (1) (2 and 1% respectively). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.2 g.

ASSAY

To 0.5 g add 40 ml of 1M *sodium hydroxide* and boil under a reflux condenser for 60 minutes. Cool and transfer the mixture to a 250 ml graduated flask with the aid of *water*. Add 100 ml of 0.1M *silver nitrate VS* and 10 ml of *nitric acid*, dilute to 250 ml with *water* and mix. Filter and titrate the excess of silver nitrate in 50 ml of the filtrate with 0.1M *ammonium thiocyanate VS* using 3 ml of *ammonium iron(III) sulphate solution R2* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 28.22 mg of $C_{23}H_{30}Cl_2NNa_2O_6P$.

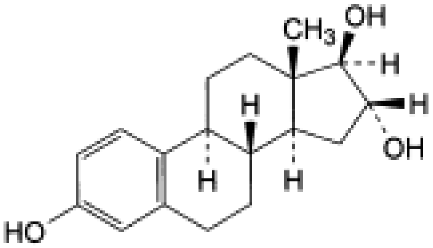
STORAGE

Estramustine Sodium Phosphate should be protected from light.

Estriol

General Notices

(Ph Eur monograph 1203)



$C_{18}H_{24}O_3$ 288.4 *50-27-1*

Action and use

Estrogen.

Preparation

Estriol Cream

Ph Eur

DEFINITION

Estra-1,3,5(10)-triene-3,16α,17β-triol.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in ethanol (96 per cent).

mp : about 282 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *estriol CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *estriol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *estradiol hemihydrate CRS* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate *TLC silica gel plate R.*

Mobile phase *ethanol (96 per cent) R, toluene R* (20:80 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *alcoholic solution of sulphuric acid R*. Heat at 100 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and ultraviolet light at 365 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the reference solution (a).

TESTS

Specific optical rotation *(2.2.7)*

+ 60 to + 65 (dried substance).

Dissolve 80 mg in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *2-propanol R1, heptane R* (20:80 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in 5 ml of *2-propanol R1* and dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *estriol CRS* and 2.0 mg of *estriol impurity A CRS* in 5 ml of *2-propanol R1*, then dilute to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.15 m, Ø = 4.0 mm;

— *stationary phase*: *diol silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: *heptane R*;

— *mobile phase B*: *2-propanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	95 → 88	5 → 12
10 - 20	88	12
20 - 30	88 → 95	12 → 5
30 - 35	95	5

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 280 nm.

Equilibration With the solvent mixture until a stable baseline is obtained.

Injection 20 µl; inject the solvent mixture as a blank.

Retention time Estriol = about 19 min; impurity A = about 21 min; if the retention times increase, wash the column first with *acetone R* and then with *heptane R*.

System suitability Reference solution (a):

— *resolution*: minimum 2.2 between the peaks due to estriol and impurity A; if the resolution decreases, wash the column first with *acetone R* and then with *heptane R*.

Limits:

— *impurity A*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurities B, C, D, E, F, G*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

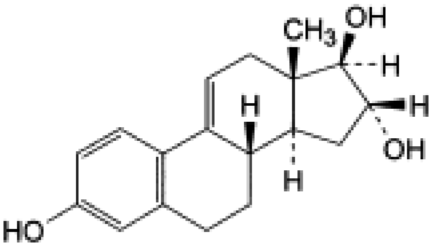
Dissolve 25.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Dilute 10.0 ml of this solution to 50.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 281 nm.

Calculate the content of $C_{18}H_{24}O_3$ taking the specific absorbance to be 72.5.

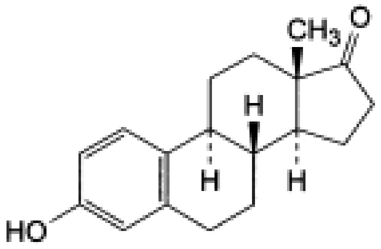
IMPURITIES

Specified impurities   *A, B, C, D, E, F, G.*

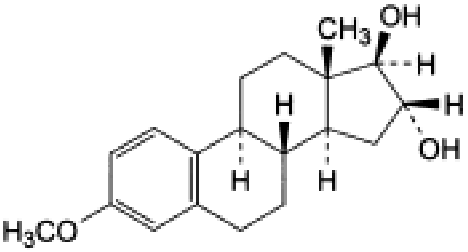
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): H, I*.



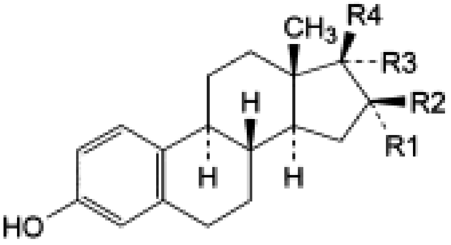
A. estra-1,3,5(10),9(11)-tetraene-3,16α,17β-triol (9,11-didehydroestriol),



B. 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),



C. 3-methoxyestra-1,3,5(10)-triene-16α,17β-diol (estriol 3-methyl ether),



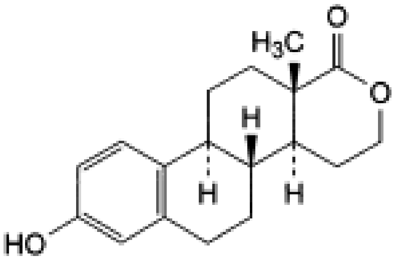
D. R1 = R2 = R3 = H, R4 = OH: estradiol,

E. R1 = R3 = OH, R2 = R4 = H: estra-1,3,5(10)-triene-3,16α,17α-triol (17-epi-estriol),

F. R1 = R3 = H, R2 = R4 = OH: estra-1,3,5(10)-triene-3,16β,17β-triol (16-epi-estriol),

G. R1 = R4 = H, R2 = R3 = OH: estra-1,3,5(10)-triene-3,16β,17α-triol (16,17-epi-estriol),

H. R1 = OH, R2 = H, R3 + R4 = O: 3,16α-dihydroxyestra-1,3,5(10)-trien-17-one,

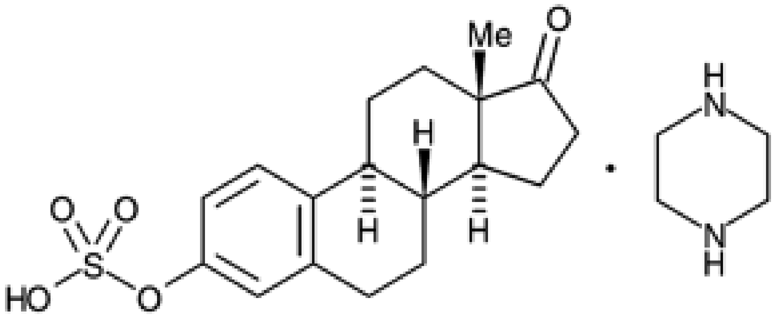


I. 3-hydroxy-17-oxa-*D*-homoestra-1,3,5(10)-trien-17a-one.

Ph Eur

Estropipate

General Notices



$C_{18}H_{22}O_5S,C_4H_{10}N_2$ 436.6 *7280-37-7*

Action and use

Estrogen.

Preparation

Estropipate Tablets

DEFINITION

Estropipate is piperazine 17-oxoestra-1,3,5-(10)-trien-3-yl hydrogen sulphate (1:1). It contains not less than 97.0% and not more than 103.0% of $C_{18}H_{22}O_5S,C_4H_{10}N_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Very slightly soluble in *water*, in *ethanol (96%)* and in *ether*.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of estropipate *(RS 129)*.

TESTS

Free estrone

Carry out the method for *liquid chromatography*, Appendix III D, using two solutions in *methanol* containing (1) 0.10% w/v of the substance being examined and (2) 0.0020% w/v of *estrone EPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography,* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 35 volumes of *acetonitrile* and 65 volumes of 0.025M *potassium dihydrogen orthophosphate* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 213 nm. The peak due to estrone has a retention time, relative to the peak due to estropipate, of about 5.

In the chromatogram obtained with solution (1), the area of any peak corresponding to estrone is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

Loss on drying

When dried at 105° for 1 hour, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.5%, Appendix IX A. Use 1 g.

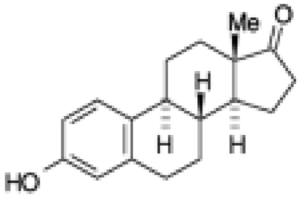
ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using two solutions in *methanol* containing (1) 0.01% w/v of the substance being examined and (2) 0.01% w/v of *estropipate BPCRS*.

The chromatographic procedure described under Free estrone may be used.

Calculate the content of $C_{18}H_{22}O_5S,C_4H_{10}N_2$ in the substance being examined using the declared content of $C_{18}H_{22}O_5S,C_4H_{10}N_2$ in *estropipate BPCRS.*

IMPURITIES

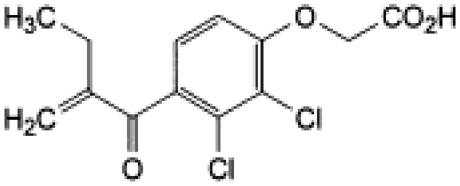


A. Estrone.

Etacrynic Acid

General Notices

(Ph Eur monograph 0457)



$C_{13}H_{12}Cl_2O_4$ 303.1 *58-54-8*

Action and use

Loop diuretic.

Preparation

Sodium Etacrynate Injection

Ph Eur

DEFINITION

[2,3-Dichloro-4-(2-ethylpropenoyl)phenoxy]acetic acid.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in alcohol. It dissolves in ammonia and in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification C.

Second identification A, B, D, E.

A. Melting point *(2.2.14)*: 121 °C to 124 °C.

B. Dissolve 50.0 mg in a mixture of 1 volume of a 103 g/l solution of *hydrochloric acid R* and 99 volumes of *methanol R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 10.0 ml of this solution to 100.0 ml with a mixture of 1 volume of a 103 g/l solution of *hydrochloric acid R* and 99 volumes of *methanol R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 270 nm and a shoulder at about 285 nm. The specific absorbance at the maximum at 270 nm is 110 to 120.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *etacrynic acid CRS.*

D. Dissolve about 30 mg in 2 ml of *aldehyde-free alcohol R*. Dissolve 70 mg of *hydroxylamine hydrochloride R* in 0.1 ml of *water R*, add 7 ml of *alcoholic potassium hydroxide solution R* and dilute to 10 ml with *aldehyde-free alcohol R*. Allow to stand and add 1 ml of the supernatant liquid to the solution of the substance to be examined. Heat the mixture on a water-bath for 3 min. After cooling, add 3 ml of *water R* and 0.15 ml of *hydrochloric acid R*. Examined in ultraviolet light at 254 nm, the mixture shows an intense blue fluorescence.

E. Dissolve about 25 mg in 2 ml of a 42 g/l solution of *sodium hydroxide R* and heat in a water-bath for 5 min. Cool and add 0.25 ml of a mixture of equal volumes of *water R* and *sulphuric acid R*. Add 0.5 ml of a 100 g/l solution of *chromotropic acid*, *sodium salt R* and, carefully, 2 ml of *sulphuric acid R*. An intense violet colour is produced.

TESTS

Related substances

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.2 g of the substance to be examined in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 0.3 ml of the test solution to 100 ml with *alcohol R*.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *glacial acetic acid R, ethyl acetate R, methylene chloride R* (20:50:60 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Limits:

— *any impurity*: any spots, apart from the principal spot, are not more intense than the spot in the chromatogram obtained with the reference solution (0.3 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test F. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 2.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure of 0.1-0.5 kPa.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 100 ml of *methanol R* and add 5 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

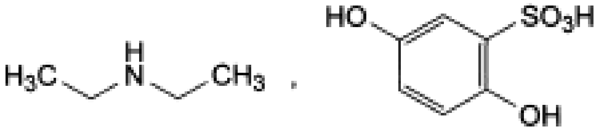
1 ml of *0.1 M sodium hydroxide* is equivalent to 30.31 mg of $C_{13}H_{12}Cl_2O_4$.

Ph Eur

Etamsylate

General Notices

(Ph Eur monograph 1204)



$C_{10}H_{17}NO_5S$ 263.3 *2624-44-4*

Action and use

Antifibrinolytic.

Ph Eur

DEFINITION

N-Ethylethanamine 2,5-dihydroxybenzenesulphonate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in methanol, soluble in anhydrous ethanol, practically insoluble in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 127 °C to 134 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison etamsylate CRS.

C. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 0.100 g in *water R* and dilute to 200.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. Examine immediately.

Spectral range 210-350 nm.

Absorption maxima At 221 nm and 301 nm.

Specific absorbance at the absorption maximum at 301 nm 145 to 151.

D. Into a test-tube, introduce 2 ml of freshly prepared solution S (see Tests) and 0.5 g of *sodium hydroxide R*. Warm the mixture and place a wet strip of *red litmus paper R* near the open end of the tube. The colour of the paper becomes blue.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S, when freshly prepared, is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 5.6 for solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Keep all solutions at 2-8 °C*.

Buffer solution Dissolve 1.2 g of *anhydrous sodium dihydrogen phosphate R* in 900 ml of *water for chromatography R*. Adjust to pH 6.5 with *disodium hydrogen phosphate solution R* and dilute to 1000 ml with *water for chromatography R*.

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 10 mg of *hydroquinone R* (impurity A) in *water R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 100 ml with *water R*.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: spherical end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R1*, buffer solution (10:90 *V/V*).

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Run time 2.5 times the retention time of etamsylate.

Relative retention With reference to etamsylate (retention time = about 6 min): impurity A = about 1.7.

System suitability Reference solution (b):

— *resolution*: minimum 8.0 between the peaks due to etamsylate and impurity A.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.5;

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Iron *(2.4.9)*

Maximum 10 ppm, determined on solution S.

Heavy metals *(2.4.8)*

Maximum 15 ppm.

1.0 g complies with test C. Prepare the reference solution using 1.5 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* in an oven at 60 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 10 ml of *water R* and 40 ml of *dilute sulphuric acid R*. Titrate with *0.1 M cerium sulphate*, determining the end-point potentiometrically *(2.2.20)*.

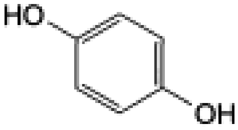
1 ml of *0.1 M cerium sulphate* is equivalent to 13.16 mg of $C_{10}H_{17}NO_5S$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities A.



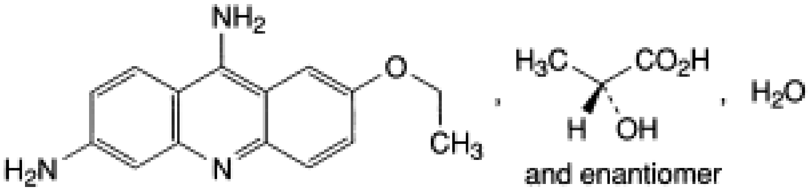
A. benzene-1,4-diol (hydroquinone).

Ph Eur

Ethacridine Lactate Monohydrate

General Notices

(Ph Eur monograph 1591)



$C_{15}H_{15}N_3O,C_3H_6O_3,H_2O$ 361.4   *1837-57-6*

Action and use

Antiseptic.

Ph Eur

DEFINITION

7-Ethoxyacridine-3,9-diamine (2*RS*)-2-hydroxypropanoate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow crystalline powder.

Solubility

Sparingly soluble in water, very slightly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification   *A.*

Second identification   *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   *ethacridine lactate monohydrate CRS.*

B. Mix 0.1 ml of solution S (see Tests) and 100 ml of *water R*. The solution is greenish-yellow and shows a strong green fluorescence in ultraviolet light at 365 nm. Add 5 ml of *1 M* *hydrochloric acid*. The fluorescence remains.

C. To 0.5 ml of solution S add 1.0 ml of *water R*, 0.1 ml of a 10 g/l solution of *cobalt chloride R* and 0.1 ml of a 50 g/l solution of *potassium ferrocyanide R*. The solution is green.

D. To 50 ml of solution S add 10 ml of *dilute sodium hydroxide solution R*. Filter. To 5 ml of the filtrate, add 1 ml of *dilute sulphuric acid R*. 5 ml of the solution obtained gives the reaction of lactates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 100.0 ml with the same solvent.

pH *(2.2.3)*

5.5 to 7.0 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.0 g of *sodium octanesulphonate R* in a mixture of 300 ml of *acetonitrile R* and 700 ml of *phosphate buffer solution pH 2.8 R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 268 nm.

Injection 10 µl.

Run time 3 times the retention time of ethacridine.

Retention time Ethacridine = about 15 min.

Limits:

- *any impurity*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent),
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 50 ppm.

1.0 g complies with limit test F. Prepare the standard using 5.0 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

4.5 per cent to 5.5 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

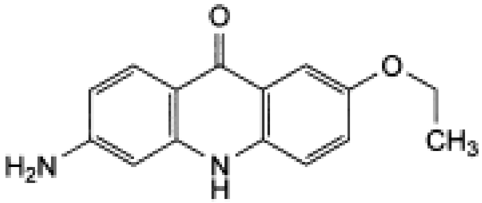
Dissolve 0.270 g in 5.0 ml of *anhydrous formic acid R*. Add 60.0 ml of *acetic anhydride R* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 34.34 mg of $C_{18}H_{21}N_3O_4$.

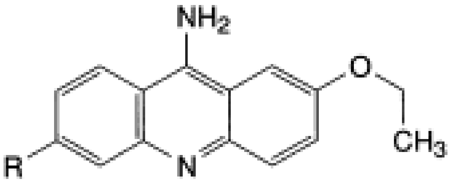
STORAGE

Protected from light.

IMPURITIES



A. 6-amino-2-ethoxyacridin-9(10*H*)-one,



B. R = Cl: 6-chloro-2-ethoxyacridin-9-amine,

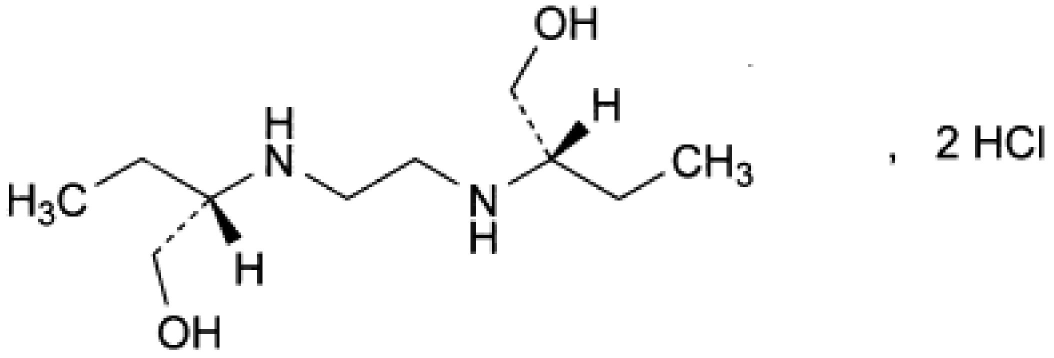
C. R = O-CH_2-CH_2-OH: 2-[(9-amino-7-ethoxyacridin-3-yl)oxy]ethanol.

Ph Eur

Ethambutol Hydrochloride

General Notices

(Ph Eur monograph 0553)



$C_{10}H_{26}Cl_2N_2O_2$ 277.2 *1070-11-7*

Action and use

Antituberculosis drug.

Preparation

Ethambutol Tablets

Ph Eur

DEFINITION

(2*S*,2′*S*)-2,2′-(ethylenediimino)dibutan-1-ol dihydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification   *A, D, E.*

Second identification   *B, C, D.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison   *ethambutol hydrochloride CRS.*

B. Examine the chromatograms obtained in the test for impurity A.

Results   The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (b).

C. Dissolve 0.1 g in 10 ml of *water R.* Add 0.2 ml of *copper sulphate solution R* and 0.5 ml of *dilute sodium hydroxide solution R*; a blue colour is produced.

D. It gives reaction (a) of chlorides (*2.3.1*).

E. Related substances (see Tests).

TESTS

pH (*2.2.3*)

3.7 to 4.0.

Dissolve 0.2 g in 10 ml of *carbon dioxide-free water R.*

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution (a)   Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b)   Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a)   Dissolve 50.0 mg of *2-aminobutanol R* (impurity A) in *methanol R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R.*

Reference solution (b)   Dissolve 50 mg of *ethambutol hydrochloride CRS* and 5 mg of *2-aminobutanol R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate   *TLC silica gel plate R.*

Mobile phase   *concentrated ammonia R*, *water R*, *methanol R* (10:15:75 *V/V/V*).

Application   2 µl.

Development   Over 2/3 of the plate.

Drying   In air; heat at 110 °C for 10 min.

Detection   Cool then spray with *ninhydrin solution R1*; heat at 110 °C for 5 min.

System suitability   Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity A*: any spot due to impurity A in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Suspend 4.0 mg of the substance to be examined in 4.0 ml of *acetonitrile R1* and add 100 µl of *triethylamine R*. Sonicate the mixture for 5 min. Add 15 µl of *(R)-(+)-α-methylbenzyl isocyanate R* and heat at 70 °C for 20 min.

Reference solution (a) Dilute 0.50 ml of the test solution to 100.0 ml with *acetonitrile R1.*

Reference solution (b) Treat 4.0 mg of *ethambutol for system suitability CRS* (containing impurity B) as described for the test solution.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm;

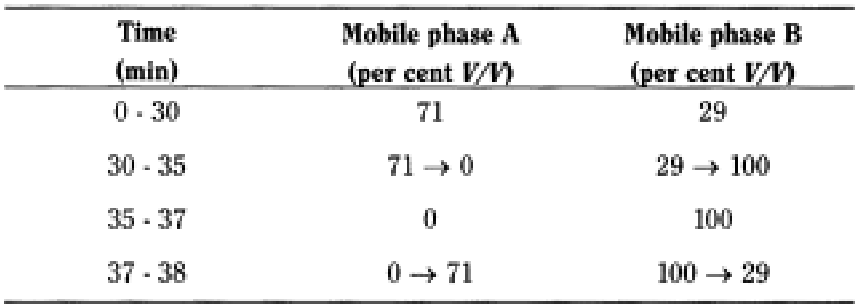
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: *methanol R*, *water R* (50:50 *V/V*);

— *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	71	29
30 - 35	71 → 0	29 → 100
35 - 37	0	100
37 - 38	0 → 71	100 → 29

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 10 µl.

Relative retention With reference to ethambutol (retention time = about 14 min): impurity B = about 1.3.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to ethambutol and impurity B.

Limits:

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *unspecified impurities with a relative retention of 0.75 to 1.5 with reference to ethambutol*: for each impurity, not more than 0.2 times the area of the peak due to ethambutol in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total* (impurity B and unspecified impurities with a relative retention of 0.75 to 1.5 with reference to ethambutol): not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the peak due to ethambutol in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity D (1,2-dichloroethane) ***(2.4.24)***

Maximum 5 ppm.

Heavy metals ***(2.4.8*****)**

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *water R* and add 1.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 27.72 mg of $C_{10}H_{26}Cl_2N_2O_2$.

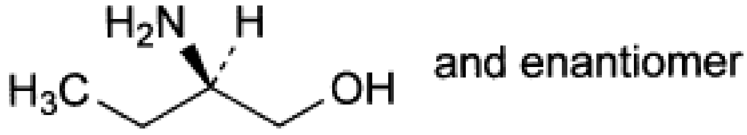
STORAGE

In an airtight container .

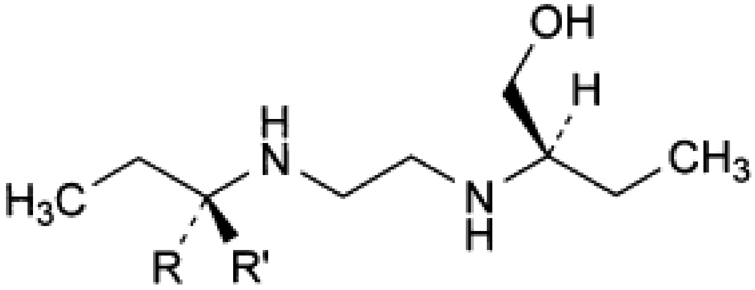
IMPURITIES

Specified impurities A, B, D.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C.*

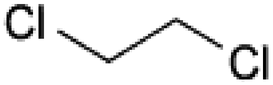


A. 2-aminobutan-1-ol,



B. R = CH_2-OH, R′ = H: (2*R*,2′*S*)-2,2′-(ethylenediimino)dibutan-1-ol (meso-ethambutol),

C. R = H, R′ = CH_2-OH: (2*R*,2′*R*)-2,2′-(ethylenediimino)dibutan-1-ol ((*R*,*R*)-ethambutol),



D. 1,2-dichloroethane (ethylene chloride).

Ph Eur

Ethanol (96 per cent)

General Notices

Alcohol (96 per cent)

(Ph Eur monograph 1317)

Ph Eur

DEFINITION

Content:

— ethanol (C_2H_6O; M_r 46.07): 95.1 per cent *V/V* (92.6 per cent *m/m*) to 96.9 per cent *V/V* (95.2 per cent *m/m*) at 20 °C, calculated from the relative density using the alcoholimetric tables (5.5),

— water.

CHARACTERS

Appearance

Colourless, clear, volatile, flammable liquid, hygroscopic.

Solubility

Miscible with water and with methylene chloride.

It burns with a blue, smokeless flame.

bp: about 78 °C.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. It complies with the test for relative density (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum ethanol (96 per cent).

C. Mix 0.1 ml with 1 ml of a 10 g/l solution of *potassium permanganate R* and 0.2 ml of *dilute sulphuric acid R* in a test-tube. Cover immediately with a filter paper moistened with a freshly prepared solution containing 0.1 g of *sodium nitroprusside R* and 0.5 g of *piperazine hydrate R* in 5 ml of *water R*. After a few minutes, an intense blue colour appears on the paper and becomes paler after 10-15 min.

D. To 0.5 ml add 5 ml of *water R*, 2 ml of *dilute sodium hydroxide solution R*, then slowly add 2 ml of *0.05 M iodine*. A yellow precipitate is formed within 30 min.

TESTS

Appearance

It is clear *(2.2.1)* and colourless *(2.2.2, Method II)* when compared with *water R*. Dilute 1.0 ml to 20 ml with *water R*. After standing for 5 min, the dilution remains clear *(2.2.1)* when compared with *water R*.

Acidity or alkalinity

To 20 ml add 20 ml of *carbon dioxide-free water R* and 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Add 1.0 ml of *0.01 M sodium hydroxide*. The solution is pink (30 ppm, expressed as acetic acid).

Relative density *(2.2.5)*

0.805 to 0.812.

Absorbance *(2.2.25)*

Maximum 0.40 at 240 nm, 0.30 between 250 nm and 260 nm and 0.10 between 270 nm and 340 nm. The absorption curve is smooth.

Examine between 235 nm and 340 nm, in a 5 cm cell using *water R* as the compensation liquid.

Volatile impurities

Gas chromatography *(2.2.28)*.

Test solution (a) The substance to be examined.

Test solution (b) Add 150 µl of *4-methylpentan-2-ol R* to 500.0 ml of the substance to be examined.

Reference solution (a) Dilute 100 µl of *anhydrous methanol R* to 50.0 ml with the substance to be examined. Dilute 5.0 ml of the solution to 50.0 ml with the substance to be examined.

Reference solution (b) Dilute 50 µl of *anhydrous methanol R* and 50 µl of *acetaldehyde R* to 50.0 ml with the substance to be examined. Dilute 100 µl of the solution to 10.0 ml with the substance to be examined.

Reference solution (c) Dilute 150 µl of *acetal R* to 50.0 ml with the substance to be examined. Dilute 100 µl of the solution to 10.0 ml with the substance to be examined.

Reference solution (d) Dilute 100 µl of *benzene R* to 100.0 ml with the substance to be examined. Dilute 100 µl of the solution to 50.0 ml with the substance to be examined.

Column:
— *material*: fused silica,
— *size: l* = 30 m, Ø = 0.32 mm,
— *stationary phase*: *poly[(cyanopropyl)(phenyl)][dimethyl]siloxane R* (film thickness 1.8 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 35 cm/s.

Split ratio 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 12	40
	12 - 32	40 → 240
	32 - 42	240
Injection port		200
Detector		280

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the first peak (acetaldehyde) and the second peak (methanol).

Limits:

— *methanol* in the chromatogram obtained with test solution (a): not more than half the area of the corresponding peak in the chromatogram obtained with reference solution (a) (200 ppm *V/V*),

— *acetaldehyde + acetal*: maximum 10 ppm *V/V*, expressed as acetaldehyde.

Calculate the sum of the contents of acetaldehyde and acetal in parts per million (V/V) using the following expression:

$$\frac{10 \times A_E}{A_T - A_E} + \frac{30 \times C_E}{C_T - C_E}$$

A_E = area of the acetaldehyde peak in the chromatogram obtained with test solution (a) ,

A_T = area of the acetaldehyde peak in the chromatogram obtained with reference solution (b),

C_E = area of the acetal peak in the chromatogram obtained with test solution (a),

C_T = area of the acetal peak in the chromatogram obtained with reference solution (c).

— *benzene*: maximum 2 ppm *V/V*.

Calculate the content of benzene in parts per million (V/V) using the following expression:

$$\frac{2B_E}{B_T - B_E}$$

B_E = area of the benzene peak in the chromatogram obtained with the test solution (a),

B_T = area of the benzene peak in the chromatogram obtained with reference solution (d).

If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).

— *total of other impurities* in the chromatogram obtained with test solution (b): not more than the area of the peak due to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (300 ppm),

— *disregard limit*: 0.03 times the area of the peak corresponding to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (9 ppm).

Residue on evaporation

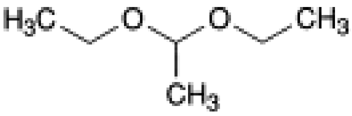
Maximum 25 ppm *m/V*.

Evaporate 100 ml to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs a maximum of 2.5 mg.

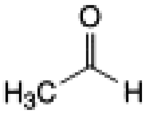
STORAGE

Protected from light.

IMPURITIES

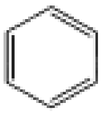


A. 1,1-diethoxyethane (acetal),

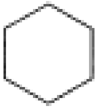


B. acetaldehyde,

C. acetone,

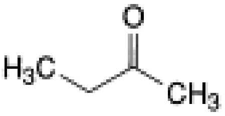


D. benzene,

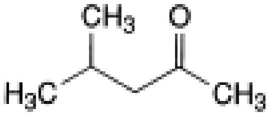


E. cyclohexane,

F. CH_3-OH: methanol,



G. butan-2-one (methyl ethyl ketone),

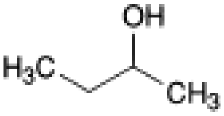


H. 4-methylpentan-2-one (methyl isobutyl ketone),

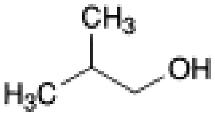
I. CH_3-$(CH_2)_2$-OH: propanol,

J. propan-2-ol (isopropyl alcohol),

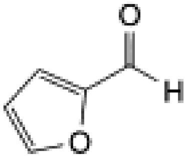
K. CH_3-$(CH_2)_3$-OH: butanol,



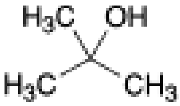
L. butan-2-ol,



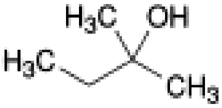
M. 2-methylpropanol (isobutanol),



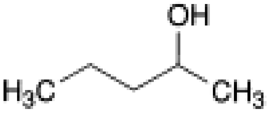
N. furane-2-carbaldehyde (furfural),



O. 2-methylpropan-2-ol (1,1-dimethylethyl alcohol),



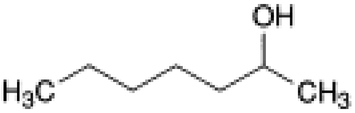
P. 2-methylbutan-2-ol,



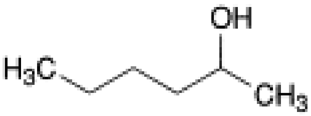
Q. pentan-2-ol,

R. CH_3-$(CH_2)_4$-OH: pentanol,

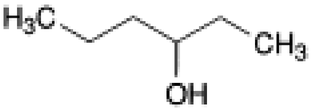
S. CH_3-$(CH_2)_5$-OH: hexanol,



T. heptan-2-ol,



U. hexan-2-ol,



V. hexan-3-ol.

Ph Eur

Ethanol

General Notices

Absolute Alcohol; Dehydrated Alcohol

(Anhydrous Ethanol, Ph Eur monograph 1318)

C_2H_6O 46.07 *64-17-5*

Ph Eur

DEFINITION

Content

Not less than 99.5 per cent *V/V* of C_2H_6O (99.2 per cent *m/m*), at 20 °C, calculated from the relative density using the alcoholimetric tables *(5.5)*.

CHARACTERS

Appearance

Colourless, clear, volatile, flammable liquid, hygroscopic.

Solubility

Miscible with water and with methylene chloride.

It burns with a blue, smokeless flame.

bp : about 78 °C.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. It complies with the test for relative density (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of anhydrous ethanol .

C. Mix 0.1 ml with 1 ml of a 10 g/l solution of *potassium permanganate R* and 0.2 ml of *dilute sulphuric acid R* in a test-tube. Cover immediately with a filter paper moistened with a freshly prepared solution containing 0.1 g of *sodium nitroprusside R* and 0.5 g of *piperazine hydrate R* in 5 ml of *water R*. After a few minutes, an intense blue colour appears on the paper and becomes paler after 10-15 min.

D. To 0.5 ml add 5 ml of *water R*, 2 ml of *dilute sodium hydroxide solution R*, then slowly add 2 ml of *0.05 M iodine*. A yellow precipitate is formed within 30 min.

TESTS

Appearance

It is clear *(2.2.1)* and colourless *(2.2.2, Method II)* when compared with *water R*. Dilute 1.0 ml to 20 ml with *water R*. After standing for 5 min, the dilution remains clear *(2.2.1)* when compared with *water R*.

Acidity or alkalinity

To 20 ml add 20 ml of *carbon dioxide-free water R* and 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Add 1.0 ml of *0.01 M sodium hydroxide*. The solution is pink (30 ppm, expressed as acetic acid).

Relative density *(2.2.5)*

0.790 to 0.793.

Absorbance *(2.2.25)*

Maximum 0.40 at 240 nm, 0.30 between 250 nm and 260 nm, and 0.10 between 270 nm and 340 nm. The absorption curve is smooth.

Examined between 235 nm and 340 nm in a 5 cm cell using *water R* as the compensation liquid.

Volatile impurities

Gas chromatography *(2.2.28)*.

Test solution (a) The substance to be examined.

Test solution (b) Add 150 µl of *4-methylpentan-2-ol R* to 500.0 ml of the substance to be examined.

Reference solution (a) Dilute 100 µl of *anhydrous methanol R* to 50.0 ml with the substance to be examined. Dilute 5.0 ml of the solution to 50.0 ml with the substance to be examined.

Reference solution (b) Dilute 50 µl of *anhydrous methanol R* and 50 µl of *acetaldehyde R* to 50.0 ml with the substance to be examined. Dilute 100 µl of the solution to 10.0 ml with the substance to be examined.

Reference solution (c) Dilute 150 µl of *acetal R* to 50.0 ml with the substance to be examined. Dilute 100 µl of the solution to 10.0 ml with the substance to be examined.

Reference solution (d) Dilute 100 µl of *benzene R* to 100.0 ml with the substance to be examined. Dilute 100 µl of the solution to 50.0 ml with the substance to be examined.

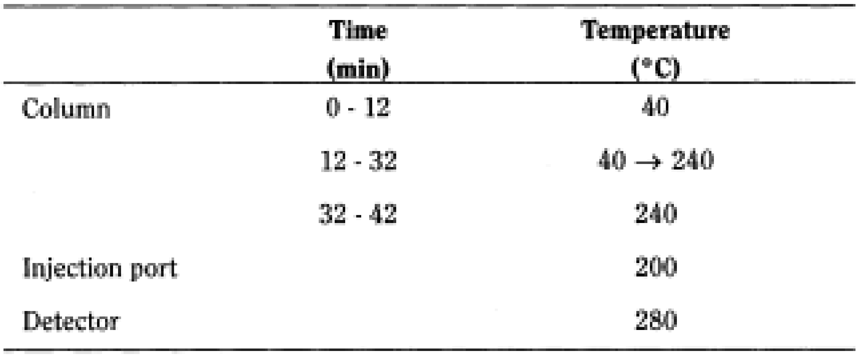
Column:
— *material*: fused silica,
— *size:* l = 30 m, Ø = 0.32 mm,
— *stationary phase*: *poly[(cyanopropyl)(phenyl)][dimethyl]siloxane R* (film thickness 1.8 µm).

Carrier gas helium for chromatography *R.*

Linear velocity 35 cm/s.

Split ratio 1:20.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 12	40
	12 - 32	40 → 240
	32 - 42	240
Injection port		200
Detector		280

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the first peak (acetaldehyde) and the second peak (methanol).

Limits:

— *methanol*: in the chromatogram obtained with test solution (a): not more than half the area of the corresponding peak in the chromatogram obtained with reference solution (a) (200 ppm *V/V*) .

— *acetaldehyde + acetal*: maximum of 10 ppm *V/v*, expressed as acetaldehyde.

Calculate the sum of the contents of acetaldehyde and acetal in parts per million (*V/V*) using the following expression:

$$\frac{10 \times A_E}{A_T - A_E} + \frac{30 \times C_E}{C_T - C_E}$$

A_E = area of the acetaldehyde peak in the chromatogram obtained with test solution (a),

A_T = area of the acetaldehyde peak in the chromatogram obtained with reference solution (b),

C_E = area of the acetal peak in the chromatogram obtained with test solution (a),

C_T = area of the acetal peak in the chromatogram obtained with reference solution (c).

— *benzene*: maximum 2 ppm V/V.

Calculate the content of benzene in parts per million (*V/V*) using the following expression:

$$\frac{2B_E}{B_T - B_E}$$

B_E = area of the benzene peak in the chromatogram obtained with the test solution (a),

B_T = area of the benzene peak in the chromatogram obtained with reference solution (d).

If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).

— *total of other impurities* in the chromatogram obtained with test solution (b): not more than the area of the peak due to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (300 ppm),

— *disregard limit*: 0.03 times the area of the peak corresponding to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (9 ppm).

Residue on evaporation

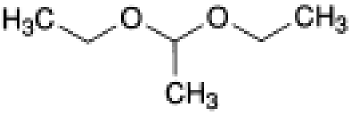
Maximum 25 ppm *m/V*.

Evaporate 100 ml to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs a maximum of 2.5 mg.

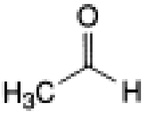
STORAGE

Protected from light.

IMPURITIES

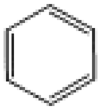


A. 1,1-diethoxyethane (acetal),

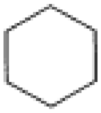


B. acetaldehyde,

C. acetone,

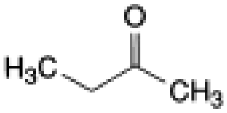


D. benzene,

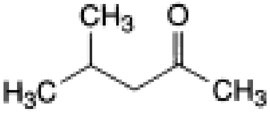


E. cyclohexane,

F. CH_3-OH: methanol,



G. butan-2-one (methyl ethyl ketone),

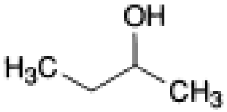


H. 4-methylpentan-2-one (methyl isobutyl ketone),

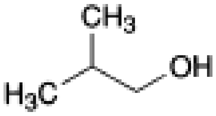
I. CH_3-$(CH_2)_2$-OH: propanol,

J. propan-2-ol (isopropyl alcohol),

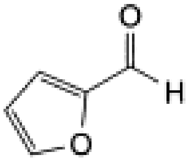
K. CH_3-$(CH_2)_3$-OH: butanol,



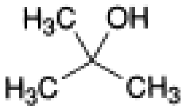
L. butan-2-ol,



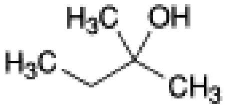
M. 2-methylpropanol (isobutanol),



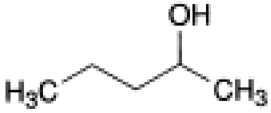
N. furane-2-carbaldehyde (furfural),



O. 2-methylpropan-2-ol (1,1-dimethylethyl alcohol),



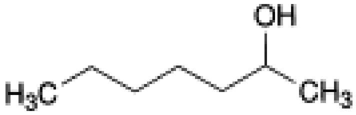
P. 2-methylbutan-2-ol,



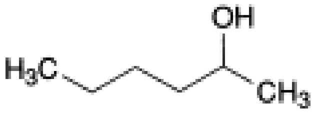
Q. pentan-2-ol,

R. CH_3-$(CH_2)_4$-OH: pentanol,

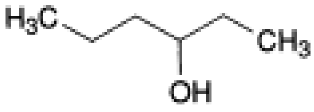
S. CH_3-$(CH_2)_5$-OH: hexanol,



T. heptan-2-ol,



U. hexan-2-ol,



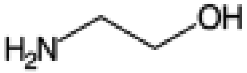
V. hexan-3-ol.

Ph Eur

Ethanolamine

General Notices

Monoethanolamine



C_2H_7NO 61.08 *141-43-5*

Action and use

Sclerosant.

Preparation

Ethanolamine Oleate Injection

DEFINITION

Ethanolamine is 2-aminoethanol. It contains not less than 98.0% and not more than 100.5% of C_2H_7NO.

CHARACTERISTICS

A clear, colourless or pale yellow liquid.

Miscible with *water* and with *ethanol (96%)*; slightly soluble in *ether*.

IDENTIFICATION

A. To 0.1 ml add 0.3 g of *picric acid* and 1 ml of *water* and evaporate to dryness on a water bath. The *melting point* of the residue, after recrystallisation from *ethanol (96%)* and drying at 105°, is about 160°, Appendix V A.

B. When freshly distilled the second half of the distillate freezes at about 10°.

C. It is alkaline to *litmus solution*.

TESTS

Refractive index

1.453 to 1.459, Appendix V E.

Weight per ml

1.014 to 1.023 g, Appendix V G.

Related substances

Prepare a 0.1% w/v solution of *3-aminopropan-1-ol* (internal standard) in *dichloromethane* (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions prepared in suitable sealed reaction vials. For solution (1) prepare a solution containing 0.05% w/v of *ethanolamine* and 0.1% w/v each of *diethanolamine* and *triethanolamine* in solution A. To 0.5 ml of this solution add 0.5 ml of *trifluoroacetic anhydride*, mix and allow to stand for 10 minutes. For solution (2) prepare a 10% w/v solution of the substance being examined in solution A. To 0.5 ml of this solution add 0.5 ml of *trifluoroacetic anhydride*, mix and allow to stand for 10 minutes.

The chromatographic procedure may be carried out using (a) a fused silica capillary column (25 m × 0.22 mm) bonded with a 0.25-µm layer of dimethylpolysiloxane, (b) *helium* as the carrier gas at a flow rate of 1.0 ml per minute with a flow rate of the make up gas of 20 ml per minute and (c) a split injection system with a split ratio of 40 to 1 maintained at 240° and a detector temperature of 250°. Maintain the temperature of the column at 80° for 2 minutes, then increase to 200° at a rate of 8° per minute and maintain this temperature for 10 minutes.

In the chromatogram obtained with solution (1) the peaks eluting after the solvent peak in order of emergence are due to (a) ethanolamine, (b) 3-aminopropan-1-ol, (c) diethanolamine and (d) triethanolamine. In the chromatogram obtained with solution (2) calculate the content of diethanolamine and triethanolamine by reference to the corresponding peaks in the chromatogram obtained with solution (1). Calculate the content of any other impurity by reference to the peak due to ethanolamine in the chromatogram obtained with solution (1). The content of diethanolamine and triethanolamine is not more than 1.0% w/w of each, the content of any other impurity is not more than 0.5% w/w and the sum of the contents of all the impurities is not more than 2.0% w/w.

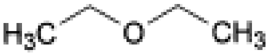
ASSAY

Dissolve 2.5 g in 50 ml of 1M *hydrochloric acid VS* and titrate the excess of acid with 1M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 1M *hydrochloric acid VS* is equivalent to 61.08 mg of C_2H_7NO.

Ether

General Notices

(Ph Eur monograph 0650)



$C_4H_{10}O$ 74.1 *60-29-7*

Ph Eur

DEFINITION

Diethyl ether.

It may contain a suitable non-volatile antioxidant at a suitable concentration.

CHARACTERS

Appearance

Clear, colourless liquid, volatile.

Solubility

Soluble in water, miscible with ethanol (96 per cent), with methylene chloride and with fatty oils.

It is highly flammable.

IDENTIFICATION

A. Relative density (see Tests).

B. Distillation range (see Tests).

TESTS

Acidity

To 20 ml of *ethanol (96 per cent) R* add 0.25 ml of *bromothymol blue solution R1* and, dropwise, *0.02 M sodium hydroxide* until a blue colour persists for 30 s. Add 25 ml of the substance to be examined, shake and add, dropwise, *0.02 M sodium hydroxide* until the blue colour reappears and persists for 30 s. Not more than 0.4 ml of *0.02 M sodium hydroxide* is required.

Relative density *(2.2.5)*

0.714 to 0.716.

Distillation range *(2.2.11)*

Do not distil if the substance to be examined does not comply with the test for peroxides. It distils completely between 34.0 °C and 35.0 °C. Carry out the test using a suitable heating device and taking care to avoid directly heating the flask above the level of the liquid.

Aldehydes

To 10.0 ml in a ground-glass-stoppered cylinder add 1 ml of *alkaline potassium tetraiodomercurate solution R* and shake for 10 s. Allow to stand for 5 min, protected from light. The lower layer may show a yellow or reddish-brown opalescence but not a grey or black opalescence.

Peroxides

Place 8 ml of *potassium iodide and starch solution R* in a 12 ml ground-glass-stoppered cylinder about 15 mm in diameter. Fill completely with the substance to be examined, mix and allow to stand protected from light for 5 min. No colour develops.

Non-volatile matter

Maximum 20 mg/l.

After ensuring that the substance to be examined complies with the test for peroxides, evaporate 50 ml to dryness on a water-bath and dry the residue in an oven at 100-105 °C. The residue weighs a maximum of 1 mg.

Substances with a foreign odour

Moisten a disc of filter paper 80 mm in diameter with 5 ml of the substance to be examined and allow to evaporate. No foreign odour is perceptible immediately after the evaporation.

Water *(2.5.12)*

Maximum 2 g/l, determined on 20 ml.

STORAGE

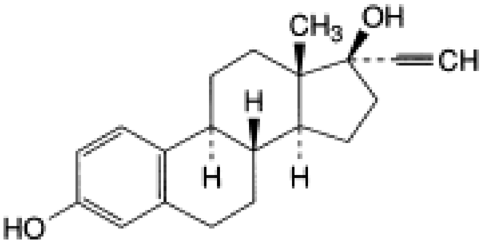
In an airtight container, protected from light, at a temperature of 8 °C to 15 °C.

Ph Eur

Ethinylestradiol

General Notices

(Ph Eur monograph 0140)



$C_{20}H_{24}O_2$ 296.4 *57-63-6*

Action and use

Estrogen.

Preparations

Ethinylestradiol Tablets

Levonorgestrel and Ethinylestradiol Tablets

Ph Eur

DEFINITION

19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish-white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in alcohol. It dissolves in dilute alkaline solutions.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ethinylestradiol CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 25 ml with the same mixture of solvents.

Reference solution Dissolve 25 mg of *ethinylestradiol CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 25 ml with the same mixture of solvents.

Plate *TLC silica gel G plate R.*

Mobile phase *alcohol R, toluene R* (10:90 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air until the solvent has evaporated.

Detection Heat at 110 °C for 10 min, spray the hot plate with *alcoholic solution of sulphuric acid R* and heat again at 110 °C for 10 min. Examine in daylight and in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour, fluorescence and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Specific optical rotation *(2.2.7)*

- 27 to - 30 (dried substance).

Dissolve 1.25 g in *pyridine R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 10 mg of *estradiol R* in the mobile phase, add 10.0 ml of the test solution and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 10.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water R* (45:55 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Run time 2.5 times the retention time of ethinylestradiol.

Relative retention With reference to ethinylestradiol (retention time = about 4.6 min): impurity D = about 0.76, impurity B = about 0.94.

System suitability Reference solution (a):

— *resolution*: minimum 3.5 between the peaks due to impurity D and ethinylestradiol.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *any other impurity*: not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent),

— *total* of other impurities: not more than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent)

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent)

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

ASSAY

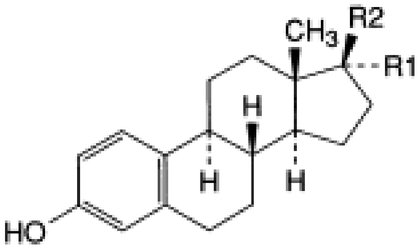
Dissolve 0.200 g in 40 ml of *tetrahydrofuran R* and add 5 ml of a 100 g/l solution of *silver nitrate R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 29.64 mg of $C_{20}H_{24}O_2$.

STORAGE

Protected from light.

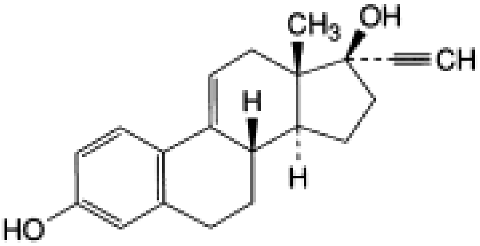
IMPURITIES



A. R1 = OH, R2 = C≡CH: 19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol (17β-ethinylestradiol),

C. R1 + R2 = O: 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),

D. R1 = H, R2 = OH: estradiol,



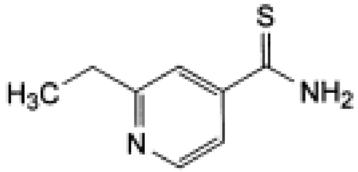
B. 19-nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol.

Ph Eur

Ethionamide

General Notices

(Ph Eur monograph 0141)



$C_8H_{10}N_2S$ 166.2 *536-33-4*

Action and use

Antituberculosis drug.

Ph.Eur

DEFINITION

Ethionamide contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 2-ethylpyridine-4-carbothioamide, calculated with reference to the dried substance.

CHARACTERS

A yellow, crystalline powder or small, yellow crystals, practically insoluble in water, soluble in methanol, sparingly soluble in alcohol.

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Melting point *(2.2.14)* 158 °C to 164 °C.

B. Dissolve 10.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *methanol R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 290 nm. The specific absorbance at the maximum is 380 to 440.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *ethionamide CRS*.

D. Dissolve about 10 mg in 5 ml of *methanol R*. Add 5 ml of *silver nitrate solution R2*. A dark-brown precipitate is formed.

TESTS

Appearance of solution

Dissolve 0.5 g in 10 ml of *methanol R*, heating to about 50 °C. Allow to cool to room temperature. The solution is not more opalescent than reference suspension II *(2.2.1)*.

Acidity

Dissolve 2.0 g in 20 ml of *methanol R*, heating to about 50 °C, and add 20 ml of *water R*. Cool slightly while shaking until crystallisation begins and then allow to cool to room temperature. Add 60 ml of *water R* and 0.2 ml of *cresol red solution R*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to red.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 0.5 ml of the test solution to 100 ml with *acetone R*.

Reference solution (b) Dilute 0.2 ml of the test solution to 100 ml with *acetone R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R* and 90 volumes of *chloroform R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most 1 such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

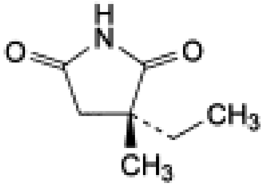
1 ml of *0.1 M perchloric acid* is equivalent to 16.62 mg of $C_8H_{10}N_2S$.

Ph Eur

Ethosuximide

General Notices

(Ph Eur monograph 0764)



$C_7H_{11}NO_2$ 141.2 *77-67-8*

Action and use

Antiepileptic.

Preparations

Ethosuximide Capsules

Ethosuximide Oral Solution

Ph Eur

DEFINITION

(*RS*)-3-Ethyl-3-methylpyrrolidine-2,5-dione.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, powder or waxy solid.

Solubility

Freely soluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification A, C.

Second identification *A, B, D, E.*

A. Melting point *(2.2.14)* 45 °C to 50 °C.

B. Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Examined between 230 nm and 300 nm *(2.2.25)*, the solution shows an absorption maximum at 248 nm. The specific absorbance at the absorption maximum is 8 to 9.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *ethosuximide CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

D. Dissolve 0.1 g in 3 ml of *methanol R*. Add 0.05 ml of a 100 g/l solution of *cobalt chloride R* and 0.05 ml of a 100 g/l solution of *calcium chloride R* and add 0.1 ml of *dilute sodium hydroxide solution R*. A purple colour develops and no precipitate is formed.

E. To about 10 mg add 10 mg of *resorcinol R* and 0.2 ml of *sulphuric acid R*. Heat at 140 °C for 5 min and cool. Add 5 ml of *water R* and 2 ml of *concentrated ammonia R1*. A brown colour is produced. Add about 100 ml of *water R*. A green fluorescence is produced.

TESTS

Solution S

Dissolve 2.5 g in water R and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Cyanide

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 0.125 g of *potassium cyanide R* in *water R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *water R*. Dilute 0.5 ml of this solution to 10.0 ml with *water R*.

Reference solution (b) Dissolve 0.50 g of the substance to be examined in *water R*, add 0.5 ml of reference solution (a) and dilute to 10.0 ml with *water R*.

Column:

— *size:* *l* = 0.075 m, Ø = 7.5 mm,

— *stationary phase*: *spherical weak anion exchange resin R* (10 µm).

Mobile phase Dissolve 2.1 g of *lithium hydroxide R* and 85 mg of *sodium edetate R* in *water for chromatography R* and dilute to 1000.0 ml with the same solvent.

Flow rate 2.0 ml/min.

Detection Electrochemical detector (direct amperometry) with a silver working electrode, a silver-silver chloride reference electrode, held at + 0.05 V oxidation potential, and a detector sensitivity of 20 nA full scale.

Injection 20 µl of the test solution and reference solution (b).

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 3, where H_r = height above the baseline of the peak due to cyanide and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ethosuximide.

Limit:

— *cyanide*: not more than 0.5 times the height of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 ppm).

Related substances

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 20 mg of *myristyl alcohol R* in *anhydrous ethanol R* and dilute to 10.0 ml with the same solvent.

Test solution Dissolve 1.00 g of the substance to be examined in *anhydrous ethanol R* add 1.0 ml of the internal standard solution and dilute to 20.0 ml with *anhydrous ethanol R*.

Reference solution (a) Dissolve 10.0 mg of *ethosuximide impurity A CRS* in *anhydrous ethanol R* and dilute to 5.0 ml with the same solvent. To 0.5 ml of the solution add 1.0 ml of the internal standard solution and dilute to 20.0 ml with *anhydrous ethanol* R.

Reference solution (b) Dissolve 0.500 g of the substance to be examined in *anhydrous ethanol R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of the solution to 50.0 ml with *anhydrous ethanol R*. To 2.0 ml of this solution add 1.0 ml of the internal standard solution and dilute to 20.0 ml with *anhydrous ethanol R*.

Column:

— *material*: fused silica,

— *size:* l = 30 m, Ø = 0.25 mm,

— *stationary phase*: *poly(cyanopropyl)(phenylmethyl) siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 ml/min.

Split ratio 1:67.

Temperature:

— *column*: 175 °C,

— *injection port and detector*: 240 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 1.5 times the retention time of ethosuximide.

Relative retention With reference to the internal standard (retention time = about 8 min): impurity A = about 0.7; ethosuximide = about 1.1.

System suitability Reference solution (b):

— *resolution*: minimum 5 between the peaks due to the internal standard and ethosuximide.

Limits:

- — *impurity A*: calculate the ratio (*R*) of the area of the peak due to impurity A to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (a); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak due to impurity A to the area of the peak due to the internal standard: this ratio is not greater than *R* (0.1 per cent);
- — *any other impurity*: calculate the ratio (*R*) of half the area of the peak due to ethosuximide to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak, apart from the principal peak and the peaks due to impurity A and to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (0.1 per cent);
- — *total*: calculate the ratio (*R*) of the area of the peak due to ethosuximide to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (0.2 per cent);
- — *disregard limit*: calculate the ratio (*R*) of 0.25 times the area of the peak due to impurity A to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (a); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: disregard any peak which has a ratio less than *R* (0.025 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.120 g in 20 ml of *dimethylformamide R* and carry out a potentiometric titration *(2.2.20)* using *0.1 M tetrabutylammonium hydroxide*. Protect the solution from atmospheric carbon dioxide throughout the titration. Carry out a blank titration.

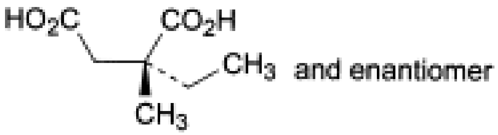
1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 14.12 mg of $C_7H_{11}NO_2$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A.


and enantiomer

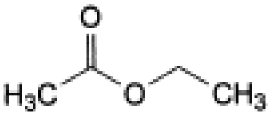
A. (2*RS*)-2-ethyl-2-methylbutanedioic acid.

Ph Eur

Ethyl Acetate

General Notices

(Ph Eur monograph 0899)



$C_4H_8O_2$ 88.1 *141-78-6*

Action and use

Excipient.

Ph Eur

DEFINITION

Ethyl ethanoate.

CHARACTERS

Appearance

Clear, colourless, volatile liquid.

Solubility

Soluble in water, miscible with acetone, with ethanol (96 per cent) and with methylene chloride.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Boiling point *(2.2.12)* 76 °C to 78 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of ethyl acetate.

C. It gives the reaction of acetyl *(2.3.1)*.

D. It gives the reaction of esters *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Mix 1 ml of the substance to be examined and 15 ml of *water R*.

Acidity

To 10 ml of *ethanol (96 per cent) R* add 0.1 ml of *phenolphthalein solution R and 0.01 M sodium hydroxide* until the colour changes to pink. Add 5.5 ml of the substance to be examined and 0.25 ml of *0.02 M sodium hydroxide*. The solution remains pink for not less than 15 s.

Relative density *(2.2.5)*

0.898 to 0.902.

Refractive index *(2.2.6)*

1.370 to 1.373.

Reaction with sulphuric acid

Carefully add 2 ml to 10 ml of *sulphuric acid R*. After 15 min, the interface between the 2 liquids is not coloured.

Related substances

Gas chromatography *(2.2.28)*.

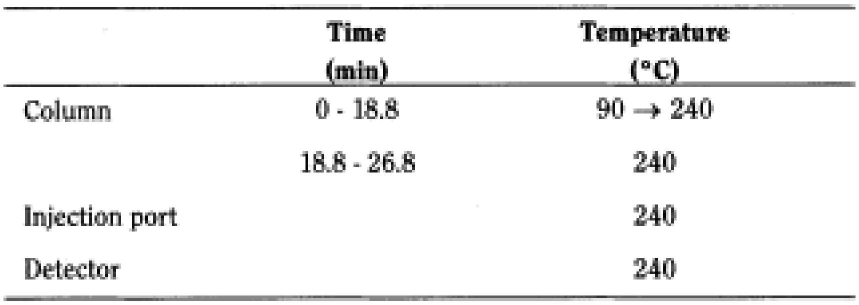
Test solution The substance to be examined.

Column:
- *material*: glass;
- *size: l* = 2 m, Ø = 2 mm;
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (136-173 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 18.8	90 → 240
	18.8 - 26.8	240
Injection port		240
Detector		240

Detection Flame ionisation.

Injection 1 µl.

Limit:

- — *total*: not more than 0.2 per cent of the area of the principal peak.

Residue on evaporation

Maximum 30 ppm.

Evaporate 100.0 g to dryness on a water-bath and dry in an oven at 100-105 °C. The residue weighs not more than 3 mg.

Water *(2.5.12)*

Maximum 0.1 per cent, determined on 10.0 ml.

STORAGE

Protected from light, at a temperature not exceeding 30 °C.

IMPURITIES

A. methyl acetate,

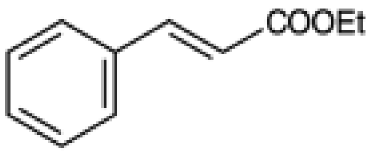
B. ethanol,

C. methanol.

Ph Eur

Ethyl Cinnamate

General Notices



$C_{11}H_{12}O_2$ 176.2 *103-36-6*

DEFINITION

Ethyl Cinnamate is predominantly ethyl (*E*)-3-phenylprop-2-enoate. It contains not less than 99.0% and not more than 100.5% of $C_{11}H_{12}O_2$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A clear, colourless or almost colourless liquid.

Practically insoluble in *water*; miscible with most organic solvents.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of ethyl cinnamate *(RS 136)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in *ethanol (96%)* exhibits a maximum only at 276 nm. The *absorbance* at the maximum is about 1.23.

C. To 1 g add 25 ml of 1M *sodium hydroxide*, boil under a reflux condenser for 1 hour, cool and acidify with *hydrochloric acid*. The *melting point* of the resulting precipitate, after filtration, washing with *water* and drying at 60° at a pressure not exceeding 0.7 kPa, is about 133°, Appendix V A.

TESTS

Acidity

Mix 30 g with 150 ml of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1*. Not more than 1.0 ml of 0.1M *sodium hydroxide VS* is required for neutralisation using *phenolphthalein solution R1* as indicator.

Refractive index

1.558 to 1.560, Appendix V E.

Weight per ml

1.048 to 1.051 g, Appendix V G.

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Solution (1) contains 1.0% w/v of the substance being examined in *chloroform*. Solution (2) is the substance being examined.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* coated with 3% w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225 is suitable) and maintained at 150°.

In the chromatogram obtained with solution (2) the sum of the areas of any *secondary peaks* is not greater than 1% by *normalisation*.

Sulphated ash

Not more than 0.1%, Appendix IX A.

Water

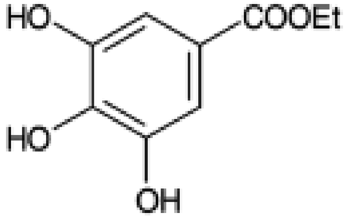
Not more than 0.1% w/w, Appendix IX C. Use 5 g.

ASSAY

In a borosilicate glass flask dissolve 2.5 g of the substance being examined in 5 ml of carbon dioxide-free ethanol prepared by boiling *ethanol (96%)* thoroughly and neutralising to *phenolphthalein solution R1*. Neutralise the free acid in the solution with 0.1M *ethanolic potassium hydroxide VS* using 0.2 ml of *phenolphthalein solution R1* as indicator. Add 50 ml of 0.5M *ethanolic potassium hydroxide* VS and boil under a reflux condenser on a water bath for 1 hour. Add 20 ml of *water* and titrate the excess of alkali with 0.5M *hydrochloric acid VS* using a further 0.2 ml of *phenolphthalein solution R1* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the alkali required to saponify the esters. Each ml of 0.5M *ethanolic potassium hydroxide VS* is equivalent to 88.11 mg of $C_{11}H_{12}O_2$.

Ethyl Gallate

General Notices



$C_9H_{10}O_5$ 198.2 *831-61-8*

Action and use

Antioxidant.

DEFINITION

Ethyl Gallate is ethyl 3,4,5-trihydroxybenzoate.

CHARACTERISTICS

A white to creamy white, crystalline powder.

Slightly soluble in *water*; freely soluble in *ethanol (96%)* and in *ether*; practically insoluble in arachis oil.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in *methanol* exhibits a maximum only at 275 nm. The *absorbance* at the maximum is about 1.08.

B. Carry out the method for *gas chromatography*, Appendix III B, using a solution prepared in the following manner. Boil 0.5 g with 50 ml of 5M *sodium hydroxide* under a reflux condenser for 10 minutes and distil 5 ml.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 10% w/w of free fatty acid phase (Supelco FFAP is suitable) and maintained at 80°.

The principal peak in the chromatogram has the same retention time as that of *absolute ethanol*, examined under the same conditions.

C. Dissolve 5 mg in a mixture of 25 ml of *acetone* and 25 ml of *water* and add 0.05 ml of *iron(III) chloride solution*. A purplish black colour is produced which rapidly becomes bluish black.

TESTS

Melting point

151° to 154°, Appendix V A.

Acidity

Dissolve 0.4 g in 100 ml of warm *carbon dioxide-free water*, cool and titrate with 0.1M *sodium hydroxide VS* using *bromocresol green solution* as indicator. Not more than 0.1 ml of 0.1M *sodium hydroxide VS* is required.

Chloride

Shake 0.50 g with 50 ml of *water* for 5 minutes and filter. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

STORAGE

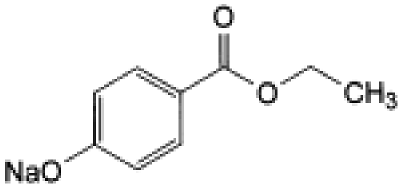
Ethyl Gallate should be protected from light. Contact with metals should be avoided.

Ethyl Hydroxybenzoate Sodium

General Notices

Ethylparaben Sodium

(Ethyl Parahydroxybenzoate Sodium, Ph Eur monograph 2134)



$C_9H_9NaO_3$ 188.2   *35285-68-8*

Ph Eur

DEFINITION

Sodium 4-(ethoxycarbonyl)phenolate.

Content

99.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, soluble in anhydrous ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification   *A, B, E.*

Second identification   *A, C, D, E.*

A. Dissolve 0.5 g in 50 ml of *water R*. Immediately add 5 ml of *hydrochloric acid R*1. Filter and wash the precipitate with *water R*. Dry *in vacuo* at 80 °C for 2 h. It melts *(2.2.14)* at 115 °C to 118 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation   The precipitate obtained in identification A.

Comparison *ethyl parahydroxybenzoate CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (c).

D. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution R*, boil for 30 s and cool. Add 5 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* and mix. An orange or red colour develops.

E. To 1 ml of solution S (see Tests) add 1 ml of *water R*. The solution gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S examined immediately after preparation is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

9.5 to 10.5.

Dilute 1 ml of solution S to 100 ml with *carbon dioxide-free water R*.

Related substances

Thin-layer chromatography (*2.2.27)*.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10 ml of *water R*. Immediately add 2 ml of *hydrochloric acid R* and shake with 50 ml of *methylene chloride R*. Evaporate the lower layer to dryness and take up the residue with 10 ml of *acetone R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *acetone R*.

Reference solution (a) Dissolve 34.3 mg of *4-hydroxybenzoic acid R* (impurity A) in *acetone R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100 ml with *acetone R*.

Reference solution (c) Dissolve 5 mg of *ethyl parahydroxybenzoate CRS* in *acetone R* and dilute to 5 ml with the same solvent.

Reference solution (d) Dissolve 5 mg of *methyl parahydroxybenzoate CRS* (impurity B) in 0.5 ml of test solution (a) and dilute to 5 ml with *acetone R*.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R.*

Mobile phase *glacial acetic acid R, water R, methanol R* (1:30:70 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (d):

— the chromatogram shows 2 clearly separated principal spots.

Limits Test solution (a):

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (4 per cent);

— *any other impurity*: any spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides *(2.4.4)*

Maximum 350 ppm.

To 10 ml of solution S add 30 ml of *water R* and 1 ml of *nitric acid R* and dilute to 50 ml with *water R*. Shake and filter. Dilute 10 ml of the filtrate to 15 ml with *water R*. The solution complies with the test. Prepare the standard using a mixture of 1 ml of *water R* and 14 ml of *chloride standard solution (5 ppm Cl) R*.

Sulphates *(2.4.13)*

Maximum 300 ppm.

To 25 ml of solution S add 5 ml of *distilled water R* and 10 ml of *hydrochloric acid R* and dilute to 50 ml with *distilled water R*. Shake and filter. Dilute 10 ml of the filtrate to 15 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20.0 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R*.

After the addition of *buffer solution pH 3.5 R*, the substance precipitates. Dilute each solution to 40 ml with *anhydrous ethanol R*: the substance dissolves completely. Continue the test as described for Method A. Filter the solutions through a membrane filter (pore size 0.45 µm) to evaluate the result.

Water *(2.5.12)*

Maximum 5.0 per cent, determined on 0.500 g.

ASSAY

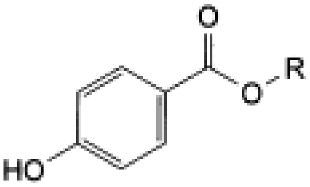
Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 18.82 mg of $C_9H_9NaO_3$.

STORAGE

In an airtight container.

IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

B. R = CH_3: methyl 4-hydroxybenzoate,

C. R = CH_2-CH_2-CH_3: propyl 4-hydroxybenzoate,

D. R = CH_2-CH_2-CH_2-CH_3: butyl 4-hydroxybenzoate.

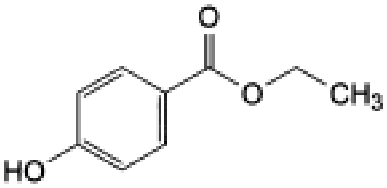
Ph Eur

Ethyl Hydroxybenzoate

General Notices

Ethyl paraben

(Ethyl Parahydroxybenzoate, Ph Eur monograph 0900)



$C_9H_{10}O_3$ 166.2 *120-47-8*

Ph Eur

DEFINITION

Ethyl 4-hydroxybenzoate.

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very slightly soluble in water, freely soluble in alcohol and in methanol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)* 115 °C to 118 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ethyl parahydroxybenzoate CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in

position and size to the principal spot in the chromatogram obtained with reference solution (b).

D. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution R*, boil for 30 s and cool (solution A). To a further 10 mg in a similar test-tube add 1 ml of *sodium carbonate solution R*; the substance partly dissolves (solution B). Add at the same time to solution A and solution B 5 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* and mix. Solution B is yellow to orange-brown. Solution A is orange to red, the colour being clearly more intense than any similar colour which may be obtained with solution B.

TESTS

Solution S

Dissolve 1.0 g in *alcohol R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity

To 2 ml of solution S add 3 ml of *alcohol R*, 5 ml of *carbon dioxide-free water R* and 0.1 ml of *bromocresol green solution R*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with acetone R.

Reference solution (a) Dilute 0.5 ml of test solution (a) to 100 ml with *acetone R*.

Reference solution (b) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of *methyl parahydroxybenzoate R* in 1 ml of test solution (a) and dilute to 10 ml with *acetone R*.

Plate Suitable octadecylsilyl silica gel with a fluorescent indicator having an optimal intensity at 254 nm as the coating substance.

Mobile phase *glacial acetic acid R, water R, methanol R* (1:30:70 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated principal spots.

Limits:

- *any impurity*: any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Sulphated ash *(2.4.14)*

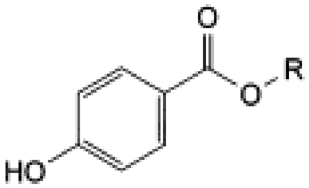
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 1.000 g add 20.0 ml of *1 M sodium hydroxide*. Heat at about 70 °C for 1 h. Cool rapidly in an ice bath. Prepare a blank in the same manner. Carry out the titration on the solutions at room temperature. Titrate the excess sodium hydroxide with *0.5 M sulphuric acid*, continuing the titration until the second point of inflexion and determining the end-point potentiometrically *(2.2.20)*.

1 ml of *1 M sodium hydroxide* is equivalent to 166.2 mg of $C_9H_{10}O_3$.

IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

B. R = CH_3: methyl 4-hydroxybenzoate,

C. R = CH_2-CH_2-CH_3: propyl 4-hydroxybenzoate,

D. R = CH_2-CH_2-CH_2-CH_3: butyl 4-hydroxybenzoate.

Ph Eur

Ethyl Oleate

General Notices

(Ph Eur monograph 1319)

Ph Eur

DEFINITION

Mixture consisting of the ethyl esters of fatty acids, mainly oleic (*cis*-9-octadecenoic) acid.

A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, pale yellow or colourless liquid.

Solubility

Practically insoluble in water, miscible with ethanol (96 per cent), with methylene chloride and with light petroleum (40-60 °C).

IDENTIFICATION

A. Relative density (see Tests).

B. Saponification value (see Tests).

C. Oleic acid (see Tests).

TESTS

Relative density *(2.2.5)*

0.866 to 0.874.

Acid value *(2.5.1)*

Maximum 0.5, determined on 10.0 g.

Iodine value (*2.5.4, Method A)*

75 to 90.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0.

Saponification value *(2.5.6)*

177 to 188, determined on 2.0 g.

Oleic acid ***(2.4.22, Method A)***

Minimum 60.0 per cent in the fatty acid fraction of the substance to be examined.

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.00 g.

Total ash ***(2.4.16)***

Maximum 0.1 per cent, determined on 2.0 g.

STORAGE

Protected from light.

Ph Eur

Ethylcellulose

General Notices

(Ph Eur monograph 0822)

Action and use

Excipient.

Ph Eur

DEFINITION

Partly *O*-ethylated cellulose.

Content

44.0 per cent to 51.0 per cent of ethoxy (-OC_2H_5) groups (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder or granular powder, odourless or almost odourless.

Solubility

Practically insoluble in water, soluble in methylene chloride and in a mixture of 20 g of ethanol (96 per cent) and 80 g of toluene, slightly soluble in ethyl acetate and in methanol, practically insoluble in glycerol (85 per cent) and in propylene glycol. The solutions may show a slight opalescence.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of ethylcellulose.

B. It complies with the limits of the assay.

TESTS

Acidity or alkalinity

To 0.5 g add 25 ml of *carbon dioxide-free water R* and shake for 15 min. Filter through a sintered-glass filter (40) *(2.1.2)*. To 10 ml of the solution add 0.1 ml of *phenolphthalein solution R* and 0.5 ml of *0.01 M sodium hydroxide*. The solution is pink. To 10 ml of the solution add *0.1 ml of methyl red solution R* and 0.5 ml of *0.01 M hydrochloric acid*. The solution is red.

Viscosity ***(2.2.9)***

80.0 per cent to 120.0 per cent of that stated on the label for a nominal viscosity greater than 6 mPa·s; 75.0 per cent to 140.0 per cent of that stated on the label for a nominal viscosity not greater than 6 mPa·s.

Shake a quantity of the substance to be examined equivalent to 5.00 g of the dried substance with 95 g of a mixture of 20 g of *ethanol (96 per cent) R* and 80 g of *toluene R* until the substance is dissolved. Determine the viscosity in mPa·s at 25 °C using a capillary viscometer.

Acetaldehyde

Maximum 100 ppm.

Introduce 3.0 g into a 250 ml conical flask with a ground-glass stopper, add 10 ml of *water R* and stir mechanically for 1 h. Allow to stand for 24 h, filter and dilute the filtrate to 100.0 ml with *water R*. Transfer 5.0 ml of the filtrate to a 25 ml volumetric flask, add 5 ml of a 0.5 g/l solution of *methylbenzothiazolone hydrazone hydrochloride R* and heat in a water-bath at 60 °C for 5 min. Add 2 ml of *ferric chloride-sulphamic acid reagent R* and heat again in a water-bath at 60 °C for 5 min. Cool and dilute to 25.0 ml with *water R*. The solution is not more intensely coloured than a standard prepared at the same time and in the same manner using instead of the 5.0 ml of filtrate, 5.0 ml of a reference solution prepared by diluting 3.0 ml of *acetaldehyde standard solution (100 ppm* C_2H_4O*) R1 to 100.0 ml with water R*.

Chlorides ***(2.4.4)***

Maximum 0.1 per cent.

Disperse 0.250 g in 50 ml of *water R*, heat to boiling and allow to cool, shaking occasionally. Filter and discard the first 10 ml of the filtrate. Dilute 10 ml of the filtrate to 15 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Gas chromatography *(2.2.28)*.

CAUTION: hydriodic acid and its reaction by-products are highly toxic. Perform all steps for preparation of the test and reference solutions in a properly functioning hood.

Internal standard solution Dilute 120 µl of *toluene R* to 10 ml with *o-xylene R*.

Test solution Transfer 50.0 mg of the substance to be examined, 50.0 mg of *adipic acid R* and 2.0 ml of the internal standard solution into a suitable 5 ml thick-walled reaction vial with a pressure-tight septum-type closure. Cautiously add 2.0 ml of *hydriodic acid R*, immediately close the vial tightly and weigh the contents and the vial accurately. Shake the vial for 30 s, heat to 125 °C for 10 min, allow to cool for 2 min, shake again for 30 s and heat to 125 °C for 10 min. Afterwards allow to cool for 2 min and repeat shaking and heating for a 3rd time. Allow the vial to cool for 45 min and reweigh. If the loss is greater than 10 mg, discard the mixture and prepare another. Use the upper layer.

Reference solution Transfer 100.0 mg of *adipic acid R*, 4.0 ml of the internal standard solution and 4.0 ml of *hydriodic acid R* into a suitable 10 ml thick-walled reaction vial with a pressure-tight septum-type closure. Close the vial tightly and weigh the vial and contents accurately. Afterwards inject 50 µl of *iodoethane R* through the septum with a syringe, weigh the vial again and calculate the mass of iodoethane added, by difference. Shake well and allow the layers to separate. Use the upper layer.

Column:
— *material*: stainless steel,

— *size:* l = 5.0 m, Ø = 2 mm,

— *stationary phase*: *diatomaceous earth for gas chromatography R* (150-180 µm) impregnated with 3 per cent m/m of *poly(dimethyl)siloxane R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 15 ml/min.

Temperature:
— *column*: 80 °C;

— *injection port and detector*: 200 °C.

Detection Fame ionisation.

Injection 1 µl.

Relative retention With reference to toluene: iodoethane = about 0.6; *o*-xylene = about 2.3.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the peaks due to iodoethane and toluene.

Calculate the percentage content of ethoxy groups using the following expression:

$$\frac{Q_1 \times m_2 \times 45.1 \times 100 \times 100}{2 \times Q_2 \times m_1 \times 156.0 \times (100 - d)}$$

Q_1 = ratio of iodoethane peak area to toluene peak area in the chromatogram obtained with the test solution,

Q_2 = ratio of iodoethane peak area to toluene peak area in the chromatogram obtained with the reference solution,

m_1 = mass of the substance to be examined used in the test solution, in milligrams,

m_2 = mass of iodoethane used in the reference solution, in milligrams,

d = percentage loss on drying.

LABELLING

The label states the nominal viscosity in millipascal seconds for a 5 per cent *m/m* solution.

Ph Eur

Ethylene Glycol Monopalmitostearate

General Notices

Ethylene Glycol Monostearate

(Ph Eur monograph 1421)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of ethylene glycol mono- and diesters of stearic (octadecanoic) and palmitic (hexadecanoic) acids, produced from the condensation of ethylene glycol and stearic acid 50 of vegetable or animal origin (see *Stearic acid (1474)*).

Content

Minimum of 50.0 per cent of monoesters.

CHARACTERS

Appearance

White or almost white, waxy solid.

Solubility

Practically insoluble in water, soluble in acetone and in hot alcohol.

IDENTIFICATION

A. It complies with the test for melting point (see Tests).

B. It complies with the test for composition of fatty acids (see Tests).

C. It complies with the assay (monoesters content).

TESTS

Melting point *(2.2.15)*

54 °C to 60 °C.

Acid value *(2.5.1)*

Maximum 3.0, determined on 10.0 g.

Iodine value *(2.5.4)*

Maximum 3.0.

Saponification value *(2.5.6)*

170 to 195, determined on 2.0 g.

Composition of fatty acids *(2.4.22, Method A)*

The fatty acid fraction has the following composition:

- — *stearic acid*: 40.0 per cent to 60.0 per cent,
- — *sum of contents of palmitic acid and stearic acid*: minimum 90.0 per cent.

Free ethylene glycol

Maximum 5.0 per cent, determined as prescribed under Assay.

Total ash *(2.4.16)*

Mximum 0.1 per cent, determined on 1.0 g.

ASSAY

Size-exclusion chromatography *(2.2.30)*.

Test solution Into a 15 ml flask, weigh about 0.2 g (m), to the nearest 0.1 mg. Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Heat gently, if necessary. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 ml flasks, weigh, to the nearest 0.1 mg, about 2.5 mg, 5.0 mg, 10.0 mg and 20.0 mg of *ethylene glycol R*. Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Weigh the flasks again and calculate the concentration of ethylene glycol in milligrams per gram for each reference solution.

Column:

- — *size:* l = 0.6 m, Ø = 7 mm,
- — *stationary phase*: *styrene-divinylbenzene copolymer R* (particle diameter 5 µm and pore size 10 nm).

Mobile phase *tetrahydrofuran R.*

Flow rate 1 ml/min.

Detection Dfferential refractometer.

Injection 40 µl.

Relative retention With reference to ethylene glycol: diesters = about 0.76, monoesters = about 0.83.

Limits:

- — *free ethylene glycol*: from the calibration curve obtained with the reference solutions, determine the concentration (*C*) in milligrams per gram in the test solution and calculate the percentage content in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\frac{A}{A+B} \times (100 - D)$$

A = area of the peak due to the monoesters,

B = area of the peak due to the diesters,

D = percentage content of free ethylene glycol + percentage content of free fatty acids which may be determined using the following expression:

$$\frac{I_A \times 270}{561.1}$$

I_A = acid value.

STORAGE

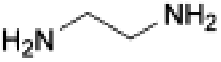
Protected from light.

Ph Eur

Ethylenediamine

General Notices

(Ph Eur monograph 0716)



$C_2H_8N_2$ 60.1 *107-15-3*

Ph Eur

DEFINITION

Ethane-1,2-diamine.

Content

98.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

Clear, colourless or slightly yellow liquid, hygroscopic.

Solubility

Miscible with water and with anhydrous ethanol.

On exposure to air, white fumes are evolved. On heating, it evaporates completely.

IDENTIFICATION

A. Relative density *(2.2.5)*: 0.895 to 0.905.

B. Boiling point *(2.2.12)*: 116 °C to 118 °C.

C. To 0.2 ml add 0.5 ml of *acetic anhydride R*. Boil. A crystalline mass forms after cooling, which dissolves in 5 ml of *2-propanol R* with heating. Cool the solution and add 5 ml of *ether R*. If necessary, initiate crystallisation by scratching the walls of the test-tube with a glass rod. Filter through a sintered-glass filter *(2.1.2)*, wash with several portions of *ether R* and dry at 100-105 °C. The residue melts *(2.2.14)* at 173 °C to 177 °C.

TESTS

Solution S

Mix 10 g with *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than the reference solution BY_6 *(2.2.2, Method II)*.

Carbonate

A mixture of 4 ml of solution S and 6 ml of *calcium hydroxide solution R* is not more opalescent than reference suspension II *(2.2.1)*.

Chlorides *(2.4.4)*

Maximum 100 ppm.

To 5 ml of solution S add 5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Ammonia and other bases

Dissolve 1.2 g in 20 ml of *ethanol (96 per cent) R* and add, dropwise with stirring, 4.5 ml of *hydrochloric acid R*. Evaporate to dryness on a water-bath, breaking up any resulting cake with a glass rod, and dry at 100-105 °C for 1 h. 1 g of the residue is equivalent to 0.4518 g of $C_2H_8N_2$. Calculate the percentage content of $C_2H_8N_2$: it does not vary by more than 0.5 from the percentage content determined in the assay.

Iron *(2.4.9)*

Maximum 10 ppm, determined on solution S.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Residue on evaporation

Maximum 0.3 per cent.

Evaporate 5.00 g to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs a maximum of 15 mg.

ASSAY

Place 25.0 ml of *1 M hydrochloric acid* and 0.2 ml of *methyl red mixed solution R* in a flask. Add 0.600 g of the substance to be examined. Titrate with *1 M sodium hydroxide* until the colour changes from violet-red to green.

1 ml of *1 M hydrochloric acid* is equivalent to 30.05 mg of $C_2H_8N_2$.

STORAGE

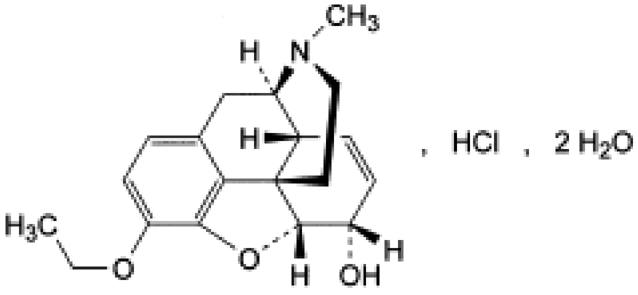
In an airtight container, protected from light.

Ph Eur

Ethylmorphine Hydrochloride

General Notices

(Ph Eur monograph 0491)



$C_{19}H_{23}NO_3,HCl,2H_2O$ 385.9 *125-30-4*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

7,8-Didehydro-4,5α-epoxy-3-ethoxy-17-methylmorphinan-6α-ol hydrochloride dihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water and in alcohol, insoluble in cyclohexane.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of ethylmorphine hydrochloride* .

B. In a test-tube, dissolve 0.5 g in 6 ml of *water R* and add 15 ml of *0.1 M sodium hydroxide*. Scratch the wall of the tube with a glass rod. A white, crystalline precipitate is formed. Collect the precipitate, wash and dissolve in 20 ml of *water R* heated to 80 °C. Filter and cool in iced water. The crystals, after drying *in vacuo* for 12 h, melt *(2.2.14)* at 85 °C to 89 °C.

C. To about 10 mg add 1 ml of *sulphuric acid R* and 0.05 ml of *ferric chloride solution R2*. Heat on a water-bath. A blue colour develops. Add 0.05 ml of *nitric acid R*. The colour becomes red.

D. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *methyl red solution R* and 0.2 ml of *0.02 M hydrochloric acid* , the solution is red. Add 0.4 ml of *0.02 M sodium hydroxide*, the solution becomes yellow.

Specific optical rotation *(2.2.7)*

- 102 to - 105 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 25.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 12.5 mg of *codeine R* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (c) Dilute 0.5 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d) To 1.0 ml of the test solution, add 1.0 ml of reference solution (b) and dilute to 50.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase Add 1.25 g of *sodium heptanesulphonate R* to a mixture of 12.5 ml of *glacial acetic acid R* and 5 ml of a 20 per cent *V/V* solution of *triethylamine R* in a mixture of equal volumes of *methanol R* and *water R*. Dilute to 1000 ml with *water R*. To 550 ml of this solution add 450 ml of *methanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

Run time 4 times the retention time of ethylmorphine.

Relative retention With reference to ethylmorphine (retention time = about 6.2 min): impurity B = about 0.7; impurity C = about 0.8; impurity D = about 1.3; impurity A = about 2.5.

System suitability Reference solution (d):

— *resolution*: minimum 5 between the peaks due to ethylmorphine and impurity C.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 0.4;

— *impurities A, B, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total of impurities other than C*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*

8.0 per cent to 10.0 per cent, determined on 0.250 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5 ml of *0.01 M hydrochloric acid* and 30 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

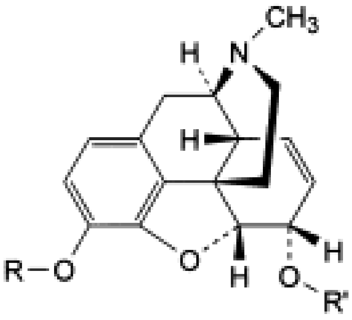
1 ml of *0.1 M sodium hydroxide* is equivalent to 34.99 mg of $C_{19}H_{24}ClNO_3$.

STORAGE

Protected from light.

IMPURITIES

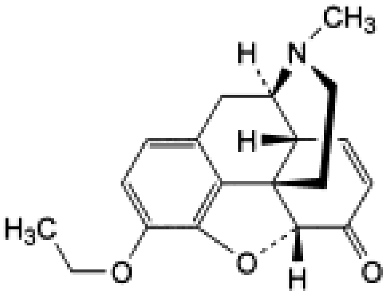
Specified impurities A, B, C, D.


CH3
H
N
H
R–O
O
H
H
O
R'

A. R = R′ = C_2H_5: 7,8-didehydro-4,5α-epoxy-3,6α-diethoxy-17-methylmorphinan,

B. R = R′ = H: morphine,

C. R = CH_3, R′ = H: codeine,


CH3
H
N
H
H3C
O
O
H
O

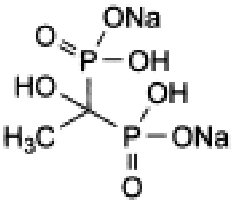
D. 7,8-didehydro-4,5α-epoxy-3-ethoxy-17-methylmorphinan-6-one (ethylmorphinone).

Ph Eur

Etidronate Disodium

General Notices

(Ph Eur monograph 1778)



$C_2H_6Na_2O_7P_2$ 250.0 *7414-83-7*

Action and use

Bisphosphonate; treatment of osteoporosis; Paget's disease.

Ph Eur

DEFINITION

Disodium dihydrogen (1-hydroxyethylidene)bisphosphonate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish, hygroscopic powder.

Solubility

Freely soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *etidronate disodium CRS*.

The transmittance at about 2000 cm^{-1} (5 µm) is not less than 40 per cent without compensation.

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

pH *(2.2.3)*

4.2 to 5.2.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Impurities A and B

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution To 2.0 ml of a 0.3 g/l solution of *phosphoric acid R* add 2.0 ml of a 0.25 g/l solution of *phosphorous acid R* and dilute to 50.0 ml with *water R*.

Column:
- *size:* *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *anion exchange resin R* (5 µm);
- *temperature*: 35 °C.

Mobile phase Mix 0.2 ml of *anhydrous formic acid R* and 1000 ml of *water R*; adjust to pH 3.5 with an 80 g/l solution of *sodium hydroxide R*.

Flow rate 1.0 ml/min.

Detection Differential refractometer.

Injection 100 µl.

System suitability Reference solution:
- *resolution*: minimum 2.5 between the peaks due to impurity A and impurity B.

Limits:
- *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.32)*

Maximum 5.0 per cent.

Dissolve 50.0 mg in a mixture of equal volumes of *anhydrous acetic acid R* and *formamide R* and dilute to 5.0 ml with the same mixture of solvents. Use 1.0 ml of the solution.

ASSAY

Dissolve 0.100 g in 2 ml of *formic acid R* and dilute to 50 ml with *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 12.50 mg of $C_2H_6Na_2O_7P_2$.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities *A, B*.

A. phosphoric acid,

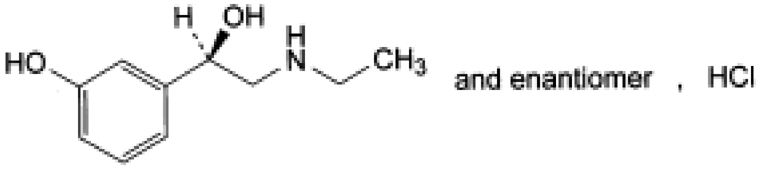
B. phosphorous acid.

Ph Eur

Etilefrine Hydrochloride

General Notices

(Ph Eur monograph 1205)



$C_{10}H_{15}NO_2$,HCl 217.7 *943-17-9*

Action and use

Adrenoceptor agonist.

Ph Eur

DEFINITION

(1*RS*)-2-(Ethylamino)-1-(3-hydroxyphenyl)ethanol hydrochloride.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. Melting point *(2.2.14)*: 118 °C to 122 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium chloride R*.

Comparison *etilefrine hydrochloride CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Prepare the solutions protected from bright light and develop the chromatograms protected from light.

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *etilefrine hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *phenylephrine hydrochloride CRS* in 2 ml of reference solution (a) and dilute to 10 ml with *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R*, *methanol R*, *methylene chloride R* (5:25:70 V/V/V).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with a 10 g/l solution of *potassium permanganate R*; examine in daylight after 15 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.2 ml of solution S (see Tests), add 1 ml of *water R*, 0.1 ml of *copper sulphate solution R* and 1 ml of *strong sodium hydroxide solution R*. A blue colour is produced. Add 2 ml of *ether R* and shake. The upper layer is colourless.

E. Dilute 1 ml of solution S to 10 ml with *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Dilute 4 ml of solution S to 10 ml with *carbon dioxide-free water R*. Add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with *water R*. Dilute 1.0 ml of this solution to 50.0 ml with *water R*.

Reference solution (b) Dissolve 10.0 mg of *etilefrine impurity A CRS* in *water R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 50.0 ml with *water R*.

Reference solution (c) To 10.0 ml of reference solution (a) add 5.0 ml of reference solution (b) and dilute to 20.0 ml with *water R*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 1.1 g/l solution of *sodium laurilsulfate R* adjusted to pH 2.3 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection
20 µl.

Run time 5 times the retention time of etilefrine.

Relative retention With reference to etilefrine (retention time = about 9 min): impurity E = about 0.5; impurity C = about 0.8; impurity B = about 0.9; impurity A = about 1.2; impurity F = about 1.7; impurity D = about 4.5.

System suitability Reference solution (c):

— *resolution*: minimum 2.5 between the peaks due to etilefrine and impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *impurities B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *sum of impurities other than A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent); disregard any peak due to the solvent.

Sulphates *(2.4.13)*

Maximum 200 ppm, determined on 15 ml of solution S.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with limit test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in a mixture of 20 ml of *anhydrous acetic acid R* and 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 21.77 mg of $C_{10}H_{16}ClNO_2$.

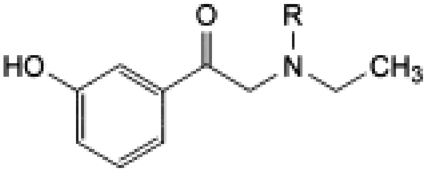
STORAGE

In an airtight container, protected from light.

IMPURITIES

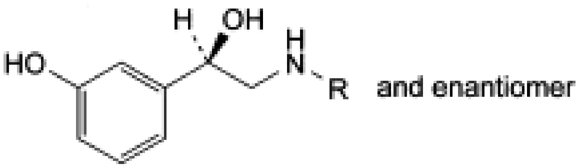
Specified impurities *A, B, C, D, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : F*.



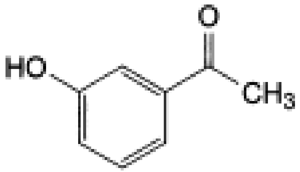
A. R = H: 2-(ethylamino)-1-(3-hydroxyphenyl)ethanone (etilefrone),

D. R = CH_2-C_6H_5: 2-(benzylethylamino)-1-(3-hydroxyphenyl)ethanone (benzyletilefrone),

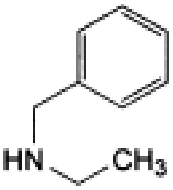


B. R = CH_3: (1*RS*)-1-(3-hydroxyphenyl)-2-(methylamino)ethanol (phenylephrine),

C. R = H: (1*RS*)-2-amino-1-(3-hydroxyphenyl)ethanol (norfenefrine),



E. 1-(3-hydroxyphenyl)ethanone (3-hydroxyacetophenone),



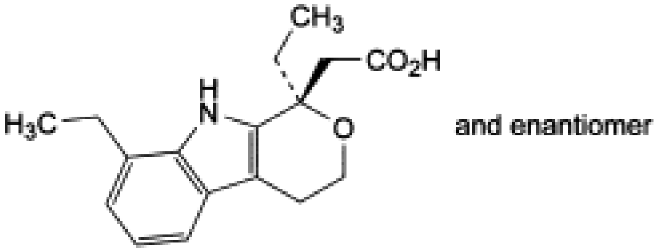
F*. N*-benzylethanamine (benzylethylamine).

Ph Eur

Etodolac

General Notices

(Ph Eur monograph 1422)



$C_{17}H_{21}NO_3$ 287.4 *41340-25-4*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Etodolac Capsules

Etodolac Tablets

Ph Eur

DEFINITION

2-[(1*RS*)-1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

First identification B.

Second identification A, C.

A. Melting point *(2.2.14)*: 144 °C to 150 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *etodolac CRS*.

C. Thin-layer chromatography *(2.2.27).*

Test solution Dissolve 10 mg of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *etodolac CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R* previously activated by heating at 105 °C for 1 h.

Place the plate in an unsaturated chamber containing a mixture of 20 volumes of a 25 g/l solution of *ascorbic acid R* and 80 volumes of *methanol R*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 min.

Mobile phase *glacial acetic acid R*, *anhydrous ethanol R*, *toluene R* (0.5:30:70 *V/V/V*).

Application 10 µl.

Development 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in *acetonitrile R1* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with *acetonitrile R1*. Dilute 1.0 ml of this solution to 20.0 ml with *acetonitrile R1*.

Reference solution (b) Dissolve 4 mg of *etodolac impurity H CRS* in the test solution and dilute to 10 ml with the same solution. Dilute 0.5 ml of this solution to 50 ml with *acetonitrile R1*.

Reference solution (c) Dissolve 4 mg of *etodolac for peak identification CRS* (containing impurities A, B, C, D, E, F, G, H, I and K) in 10 ml of *acetonitrile R1.*

Column:

- — *size*: *l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (3.5 µm);
- — *temperature*: 35 °C.

Mobile phase A 0.77g/l solution of *ammonium acetate R*;

Mobile phase B mobile phase A, *acetonitrile R1* (10:90 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	80 → 50	20 → 50
25 - 42	50	50
42 - 48	50 → 80	50 → 20

Flow rate 1 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 5 µl.

Identification of impurities Use the chromatogram supplied with *etodolac for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D, E, F, G, H, I and K.

Relative retention With reference to etodolac (retention time = about 16.7 min): impurity A = about 0.68; impurity B = about 0.83; impurity C = about 0.85; impurity H = about 1.09; impurity D = about 1.17; impurity G = about 1.19; impurity E = about 1.20; impurity F = about 1.22; impurity I = about 1.50; impurity K = about 2.37.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to etodolac and impurity H.

Limits:

— *impurity C*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurities A, B, D, E, F, G, H, I, K*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides

Maximum 300 ppm.

Dissolve 1.0 g of the substance to be examined in 60 ml of *methanol R*, add 10 ml of *water R* and 20 ml of *dilute nitric acid R*. Titrate with *0.01 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.01 M silver nitrate* is equivalent to 0.3545 mg of Cl.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

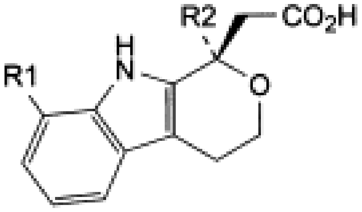
Dissolve 0.250 g in 60 ml of *methanol R*. Titrate with *0.1 M tetrabutylammonium hydroxide* determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 28.74 mg of $C_{17}H_{21}NO_3$.

IMPURITIES

Specified impurities   A, B, C, D, E, F, G, H, I, K.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): J, L*.



and enantiomer

A. R1 = H, R2 = CH_2-CH_3: 2-[(1*RS*)-1-ethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid (8-desethyl etodolac),

B. R1 = CH_3, R2 = CH_2-CH_3: 2-[(1*RS*)-1-ethyl-8-methyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid (8-methyl etodolac),

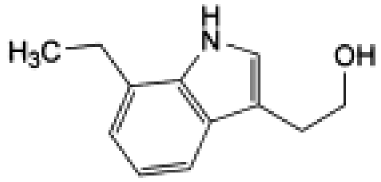
C. R1 = CH_2-CH_3, R2 = CH_3: 2-[(1*RS*)-8-ethyl-1-methyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid (1-methyl etodolac),

D. R1 = $CH(CH_3)_2$, R2 = CH_2-CH_3: 2-[(1*RS*)-1-ethyl-8-(1-methylethyl)-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid (8-isopropyl etodolac),

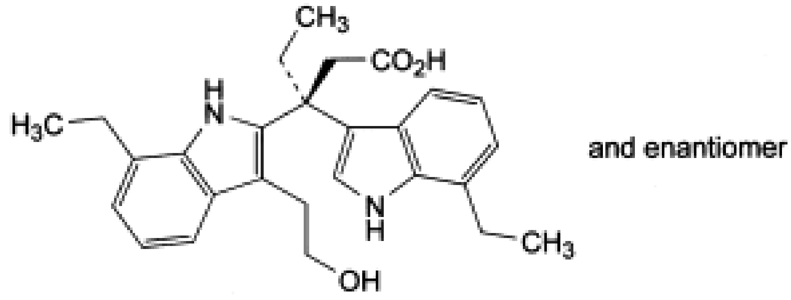
E. R1 = CH_2-CH_2-CH_3, R2 = CH_2-CH_3: 2-[(1*RS*)-1-ethyl-8-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid (8-propyl etodolac),

F. R1 = CH_2-CH_3, R2 = $CH(CH_3)_2$: 2-[(1*R*S)-8-ethyl-1-(1-methylethyl)-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid (1-isopropyl etodolac),

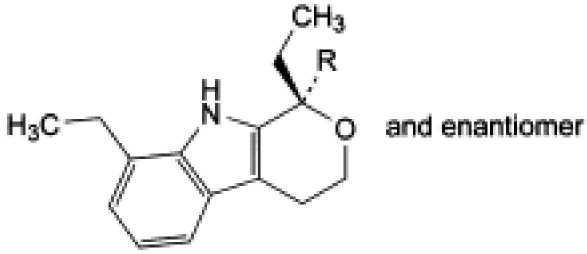
G. R1 = CH_2-CH_3, R2 = CH_2-CH_2-CH_3: 2-[(1*RS*)-8-ethyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid (1-propyl etodolac),



H. 2-(7-ethyl-1*H*-indol-3-yl)ethanol,

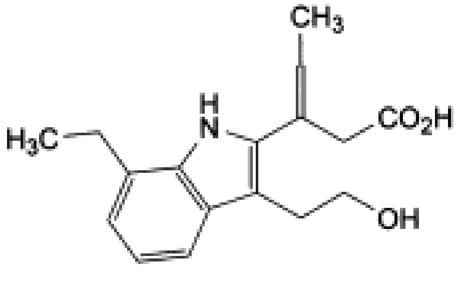


I. (3*RS*)-3-[7-ethyl-3-(2-hydroxyethyl)-1*H*-indol-2-yl]-3-(7-ethyl-1*H*-indol-3-yl)pentanoic acid (etodolac dimer),



J. R = CH_3: (1*RS*)-1,8-diethyl-1-methyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (decarboxy etodolac),

K. R = CH_2-CO-O-CH_3: methyl 2-[(1*RS*)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl] acetate (etodolac methyl ester),



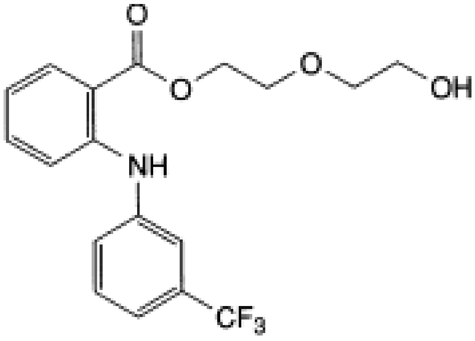
L. (*EZ*)-3-[7-ethyl-3-(2-hydroxyethyl)-1*H*-indol-2-yl]pent-3-enoic acid.

Ph Eur

Etofenamate

General Notices

(Ph Eur monograph 1513)



$C_{18}H_{18}F_3NO_4$ 369.4 *30544-47-9*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

2-(2-Hydroxyethoxy)ethyl 2-[[3-(trifluoromethyl)phenyl]amino]benzoate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellowish viscous liquid.

Solubility

Practically insoluble in water, miscible with alcohol and with ethyl acetate.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *etofenamate CRS.*

Preparation Films.

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution GY_1 *(2.2.2, Method II)*.

Impurity F

Gas chromatography *(2.2.28)*.

Internal standard *tetradecane* R.

Solution A Dissolve 6.0 mg of *tetradecane R* in *hexane R* and dilute to 10.0 ml with the same solvent.

Solution B To 6.0 mg of *diethylene glycol R* in a 10 ml volumetric flask add 3 ml of *N-methyltrimethylsilyl-trifluoroacetamide R* and heat for 30 min at 50 °C. After cooling dilute to 10.0 ml with *N-methyltrimethylsilyl-trifluoroacetamide R*.

Test solution To 0.200 g of the substance to be examined add 10 µl of solution A. Add 2 ml of *N-methyltrimethylsilyl-trifluoroacetamide R* and heat for 30 min at 50 °C.

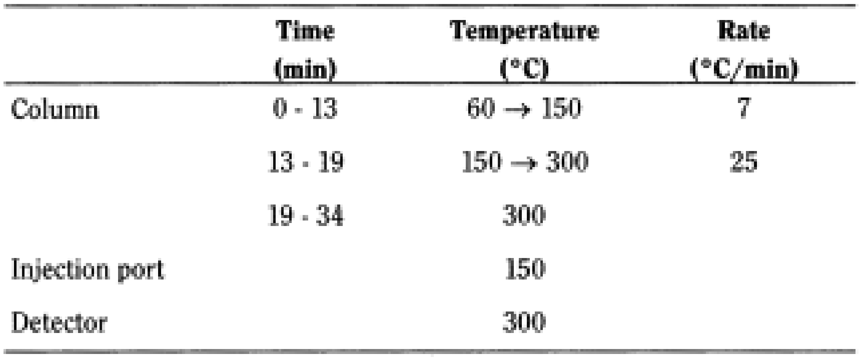
Reference solution To 2.0 ml of *N-methyltrimethylsilyl-trifluoroacetamide R* add 10 µl of solution A and 10 µl of solution B.

Column:
- *size:* *l* = 25 m, Ø = 0.20 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.33 µm).

Carrier gas *hydrogen for chromatography R*.

Flow rate 0.9 ml/min.

Temperature:



	Time (min)	Temperature (°C)	Rate (°C/min)
Column	0 - 13	60 → 150	7
	13 - 19	150 → 300	25
	19 - 34	300	
Injection port		150	
Detector		300	

Detection Flame ionisation.

Injection 0.2 µl.

Limit:
- *impurity F*: maximum 0.1 per cent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in 30 ml of *methanol R* and dilute to 50.0 ml with *water R*.

Reference solution (a) Dissolve 10.0 mg of *etofenamate impurity G CRS* in *methanol R* and dilute to 20.0 ml with the same solvent. Dilute 0.2 ml of the solution to 50.0 ml with a mixture of 40 volumes of *water R* and 60 volumes of *methanol R*.

Reference solution (b) Dilute 0.2 ml of the test solution to 100.0 ml with a mixture of 40 volumes of *water R* and 60 volumes of *methanol R*.

Reference solution (c) To 5.0 ml of reference solution (a), add 5.0 ml of reference solution (b).

Reference solution (d) Dissolve 10.0 mg of *etofenamate for peak identification CRS* (contains etofenamate spiked with about 1 per cent of impurities A, B, C, D and E) in 6.0 ml of *methanol R* and dilute to 10.0 ml with *water R*.

Column:

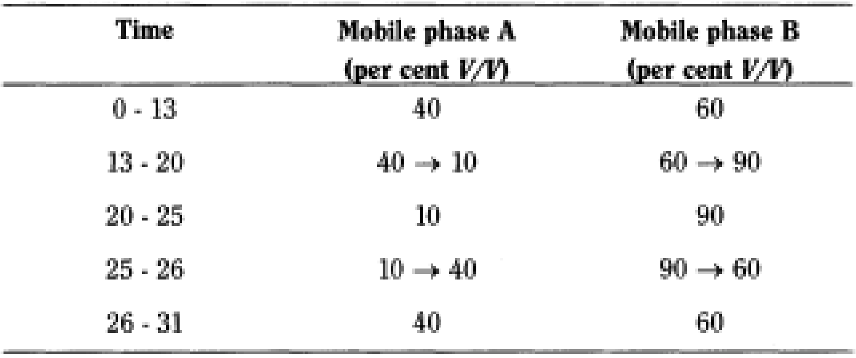
— *size:* *l* = 0.10 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 40 °C.

Mobile phase A Dissolve 1.3 g of *ammonium phosphate R* and 4.0 g of *tetrabutylammonium hydroxide R* in 900 ml of *water R*. Adjust the pH to 8.0 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R*,

Mobile phase B *methanol R*,



Time	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 13	40	60
13 - 20	40 → 10	60 → 90
20 - 25	10	90
25 - 26	10 → 40	90 → 60
26 - 31	40	60

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 286 nm.

Injection 20 µl.

Relative retention With reference to etofenamate (retention time = about 13 min): impurity A = about 0.2; impurity C = about 0.7; impurity G = about 0.85; impurity E = about 1.5; impurity B = about 1.6; impurity D = about 1.7.

System suitability Reference solution (c):

— *resolution*: minimum of 2.3 between the peaks due to impurity G and to etofenamate.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.62; impurity C = 0.45; impurity D = 0.77;

— *impurity A*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity D*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.2 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash *(2.4.14)*

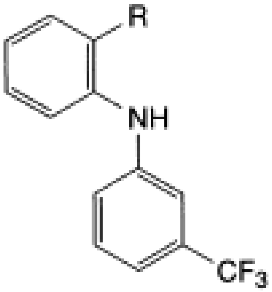
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 3.000 g add 20 ml of *2-propanol R* and 20.0 ml of *1 M sodium hydroxide* and heat under reflux for 2 h. Add 0.1 ml of *bromothymol blue solution R1*. Titrate after cooling with *1 M hydrochloric acid* until the colour disappears. Carry out a blank titration.

1 ml of *1 M sodium hydroxide* is equivalent to 0.3694 g of $C_{18}H_{18}F_3NO_4$.

IMPURITIES



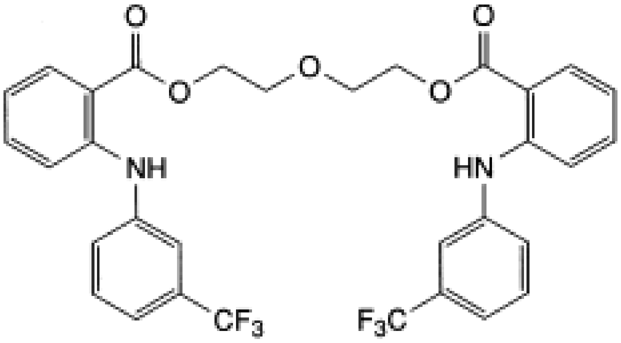
A. R = CO_2H: 2-[[3-(trifluoromethyl)phenyl]amino]benzoic acid (flufenamic acid),

B. R = $CO\text{-}O\text{-}C_4H_9$: butyl 2-[[3-(trifluoromethyl)phenyl]amino]benzoate (butyl flufenamate),

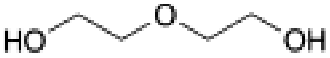
C. R = H: *N*-phenyl-3-(trifluoromethyl)aniline,

E. R = $CO\text{-}[O\text{-}CH_2\text{-}CH_2]_3\text{-}CH_2\text{-}CH_3$: 2-(2-butoxyethoxy)ethyl 2-[[3-(trifluoromethyl)phenyl]amino]benzoate,

G. R = $CO\text{-}O\text{-}CH_2\text{-}CH_2\text{-}OH$: 2-hydroxyethyl 2-[[3-(trifluoromethyl)phenyl]amino]benzoate,



D. 2,2′-oxybis(ethylene) bis[2-[[3-(trifluoromethyl)phenyl]amino]benzoate],



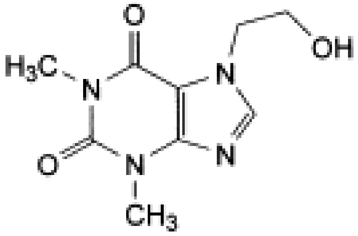
F. 2,2′-oxydiethanol.

Ph Eur

Etofylline

General Notices

(Ph Eur monograph 0492)



$C_9H_{12}N_4O_3$ 224.2 *519-37-9*

Action and use

Non-selective phosphodiestarase inhibitor (xanthine); treatment of reversible airways obstruction.

Ph Eur

DEFINITION

Etofylline contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7-(2-hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification B, C.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 161 °C to 166 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *etofylline CRS* . Examine the substances as discs prepared using 0.5 mg to 1 mg of the substance to be examined in 0.3 g of *potassium bromide R*.

C. Dissolve 1 g in 5 ml of *acetic anhydride R* and boil under a reflux condenser for 15 min. Allow to cool and add 100 ml of a mixture of 20 volumes of *ether R* and 80 volumes of *light petroleum R*. Cool in iced water for at least 20 min, shaking from time to time. Filter, wash the precipitate with a mixture of 20 volumes of *ether R* and 80 volumes of *light petroleum R*, recrystallise from *alcohol R* and dry *in vacuo*. The crystals melt *(2.2.14)* at 101 °C to 105 °C.

D. It gives the reaction of xanthines *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Acidity or alkalinity

To 10 ml of solution S add 0.25 ml of *bromothymol blue solution R1*. The solution is yellow or green. Not more than 0.4 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* HF_{254} *R* as the coating substance.

Test solution Dissolve 0.3 g of the substance to be examined in a mixture of 20 volumes of *water R* and 30 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents. Prepare immediately before use.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *methanol R*.

Reference solution (b) Dilute 0.2 ml of the test solution to 100 ml with *methanol R*.

Reference solution (c) Dissolve 10 mg of *theophylline R* in *methanol R*, add 0.3 ml of the test solution and dilute to 10 ml with *methanol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 10 volumes of *ethanol R* and 90 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

Dilute 2.5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (400 ppm).

Heavy metals *(2.4.8)*

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.200 g in 3.0 ml of *anhydrous formic acid R* and add 50.0 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 22.42 mg of $C_9H_{12}N_4O_3$.

STORAGE

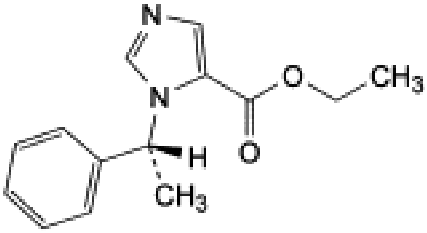
Store protected from light.

Ph Eur

Etomidate

General Notices

(Ph Eur monograph 1514)



$C_{14}H_{16}N_2O_2$ 244.3   *33125-97-2*

Action and use

Intravenous general anaesthetic.

Ph Eur

DEFINITION

Ethyl 1-[(1*R*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

mp : about 68 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   *etomidate CRS*.

B. Specific optical rotation (see Tests).

TESTS

Solution S

Dissolve 0.25 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

+ 67 to + 70 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *anhydrous ethanol R*, *water R* (50:50 V/V).

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution(a) Dissolve 5.0 mg of *etomidate CRS* and 5.0 mg of *etomidate impurity B CRS* in the solvent mixture, then dilute to 250.0 ml with the solvent mixture.

Reference solution(b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 25.0 ml with the solvent mixture.

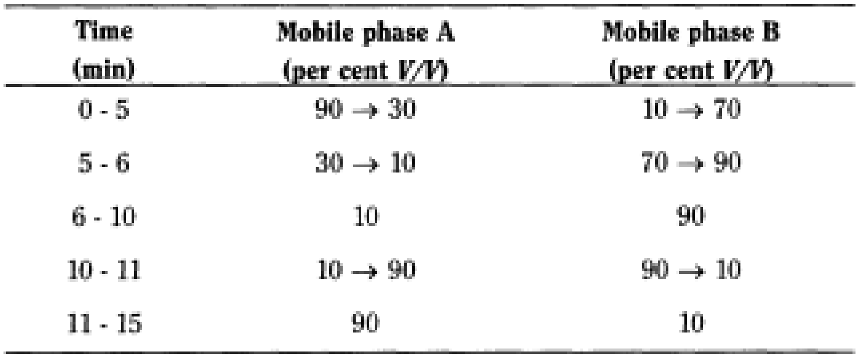
Column

— *size*: *l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase A 5 g/l solution of *ammonium carbonate R*,

Mobile phase B *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90 → 30	10 → 70
5 - 6	30 → 10	70 → 90
6 - 10	10	90
10 - 11	10 → 90	90 → 10
11 - 15	90	10

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 235 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl.

Retention time Impurity B = about 4.5 min; etomidate = about 5.0 min.

System suitability Reference solution(a):

— *resolution*: minimum 5.0 between the peaks due to impurity B and etomidate; if necessary, adjust the concentration of ammonium carbonate in the mobile phase or the time programme of the linear gradient.

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 40 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with 0.1 *M perchloric acid* using 0.2 ml of *naphtholbenzein solution R* as indicator.

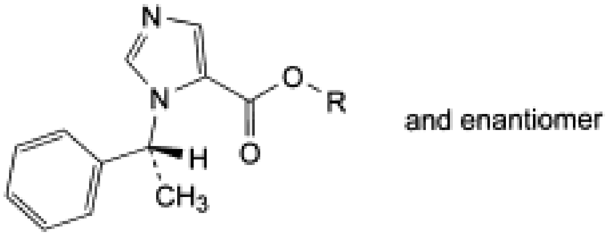
1 ml of *0.1 M perchloric acid* is equivalent to 24.43 mg of $C_{14}H_{16}N_2O_2$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C.*



A. R = H: 1-[(1*RS*)-1-phenylethyl]-1*H*-imidazole-5-carboxylic acid,

B. R = CH_3: methyl 1-[(1*RS*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate (metomidate),

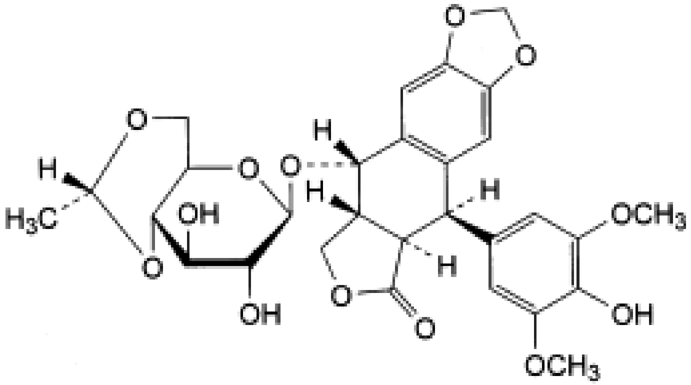
C. R = $CH(CH_3)_2$: 1-methylethyl 1-[(1*RS*)-1-phenylethyl]-1*H*-imidazole-5-carboxylate.

Ph Eur

Etoposide

General Notices

(Ph Eur monograph 0823)



$C_{29}H_{32}O_{13}$ 588.6 *33419-42-0*

Action and use

Inhibitor of DNA topoisomerase type II; cytotoxic.

Preparations

Etoposide Capsules

Etoposide Intravenous Infusion.

Ph Eur

DEFINITION

(5*R*,5a*R*,8a*R*,9*S*)-9-[[4,6-O-[(*R*)-Ethylidene]-β-D-glucopyranosyl]oxy]-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in methanol, slightly soluble in alcohol and in methylene chloride.

IDENTIFICATION

First identification A, B.

Second identification C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *etoposide CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 2 ml with the same mixture of solvents.

Reference solution Dissolve 10 mg of *etoposide CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 2 ml with the same mixture of solvents.

Plate Plate with *silica gel H R* as coating substance.

Mobile phase *water R*, *glacial acetic acid R*, *acetone R*, *methylene chloride R* (1.5:8:20:100 *V/V/V/V*).

Application 5 µl as 10 mm bands.

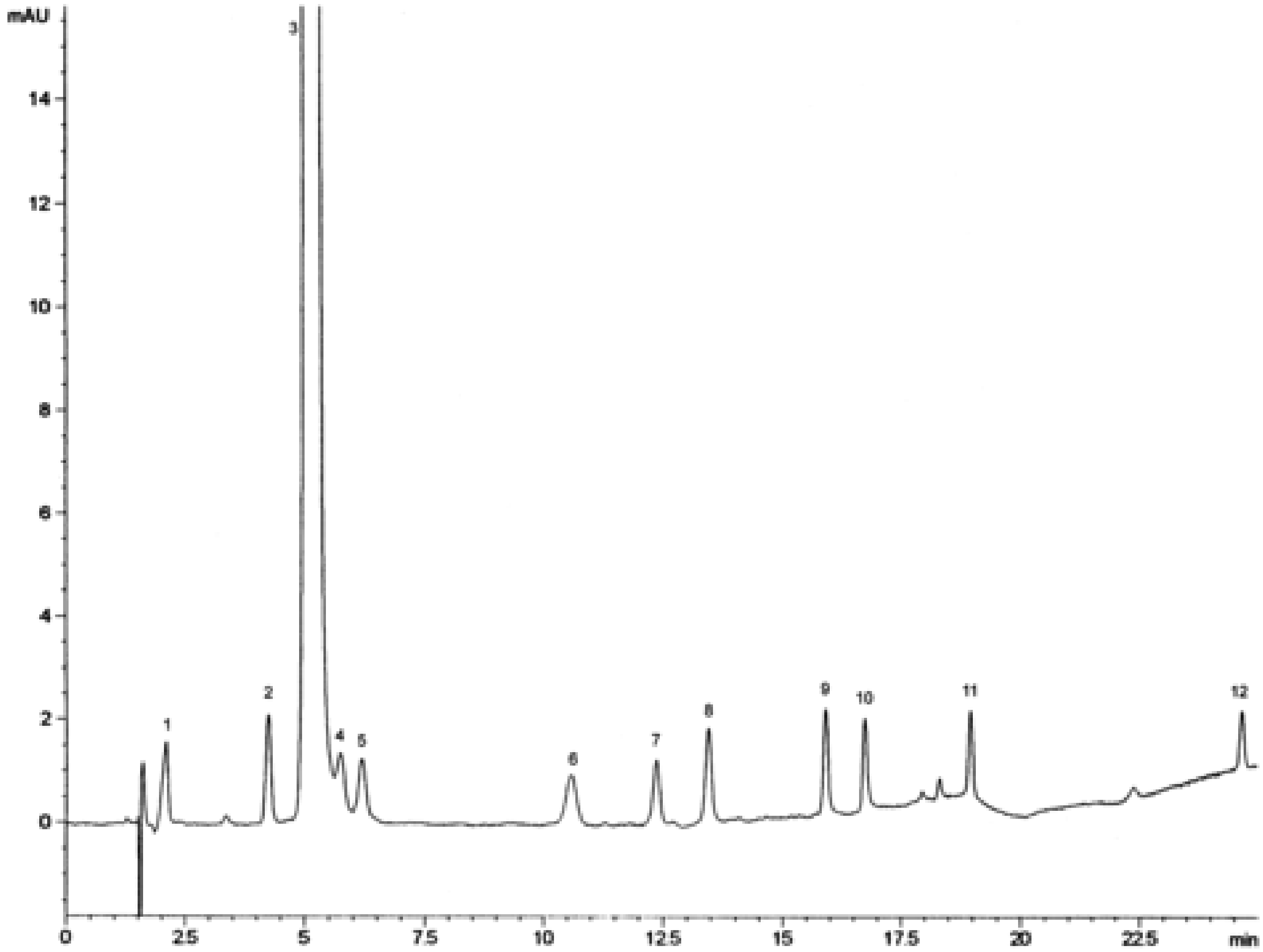
Development Immediately, over a path of 17 cm.

Drying In a current of warm air for 5 min.

Detection Spray with a mixture of 1 volume of *sulphuric acid R* and 9 volumes of *alcohol R* and heat at 140 °C for 15 min. Cover the plate immediately with a glass plate of the same size. Examine in daylight.

Results The principal band in the chromatogram obtained with the test solution is similar in position, colour and size to the principal band in the chromatogram obtained with the reference solution.

D. In a test-tube dissolve about 5 mg in 5 ml of *glacial acetic acid R* and add 0.05 ml of *ferric chloride solution R1*. Mix and cautiously add 2 ml of *sulphuric acid R*. Avoid mixing the 2 layers. Allow to stand for about 30 min; a pink to reddish-brown ring develops at the interface and the upper layer is yellow.



1. impurity D
2. impurity E
3. etoposide
4. impurity C
5. impurity B
6. impurity M
7. impurity I
8. impurity J
9. impurity H
10. impurity K
11. impurity A
12. impurity G

Figure 0823.-1. – *Chromatogram for the test for related substances of etoposide*

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 or BY_6 *(2.2.2, Method II)*.

Dissolve 0.6 g in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 20 ml with the same mixture of solvents.

Specific optical rotation *(2.2.7)*

- 106 to - 114 (anhydrous substance).

Dissolve 50 mg in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10.0 ml with the same mixture of solvents.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 40 mg of the substance to be examined in a mixture of equal volumes of mobile phase A and mobile phase B and dilute to 10.0 ml with the same mixture of mobile phases.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in a mixture of equal volumes of mobile phase A and mobile phase B and dilute to 50.0 ml with the same mixture of mobile phases.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 10.0 ml with a mixture of equal volumes of mobile phase A and mobile phase B. Dilute 1.0 ml of this solution to 20.0 ml with a mixture of equal volumes of mobile phase A and mobile phase B.

Reference solution (b) Dilute 4.0 ml of reference solution (a) to 10.0 ml with a mixture of equal volumes of mobile phase A and mobile phase B.

Reference solution (c) Dissolve 50.0 mg of *etoposide CRS* in a mixture of equal volumes of mobile phase A and mobile phase B and dilute to 50.0 ml with the same mixture of mobile phases.

Reference solution (d) To 10 ml of test solution (b), add 0.1 ml of a 4 per cent V/V solution of *glacial acetic acid R* and 0.1 ml of *phenolphthalein solution R*. Add *1 M sodium hydroxide* until the solution becomes faintly pink (about 0.15 ml). After 15 min, add 0.1 ml of a 4 per cent *V/V* solution of *glacial acetic acid R*.

Column:
— *size: l* = 0.125 m, Ø = 4.6 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
— *temperature:* 40 °C.

Mobile phase:
— *mobile phase A*: *triethylamine R*, *anhydrous formic acid R*, *water R* (1:1:998 *V/V/V*),
— *mobile phase B*: *triethylamine R*, *anhydrous formic acid R*, *acetonitrile R* (1:1:998 *V/V/V*),

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	75	25
7 - 23	75 → 27	25 → 73
23 - 25	27 → 75	73 → 25
25 - 40	75	25

Flow rate 1 ml/min.

Detection Spectrophotometer at 285 nm.

Injection 10 µl; inject test solution (a) and reference solutions (a), (b) and (d).

Retention times The retention times and the elution order of the peaks are similar to those shown in the chromatogram (Figure 0823.-1).

System suitability Reference solution (d): continue the chromatography until the peak due to phenolphtalein is eluted.

— the chromatogram shows 2 principal peaks corresponding to etoposide and to impurity B. Disregard any peak due to phenolphthalein.

— *resolution*: minimum 3.0 between the peaks due to etoposide and to impurity B.

If necessary, increase slightly the proportion of mobile phase A during the isocratic phase of the gradient. When the chromatograms are recorded under the prescribed conditions, the retention times of the peaks in the chromatogram obtained with reference solution (d) are similar to those shown in the chromatogram (Figure 0823.-2).

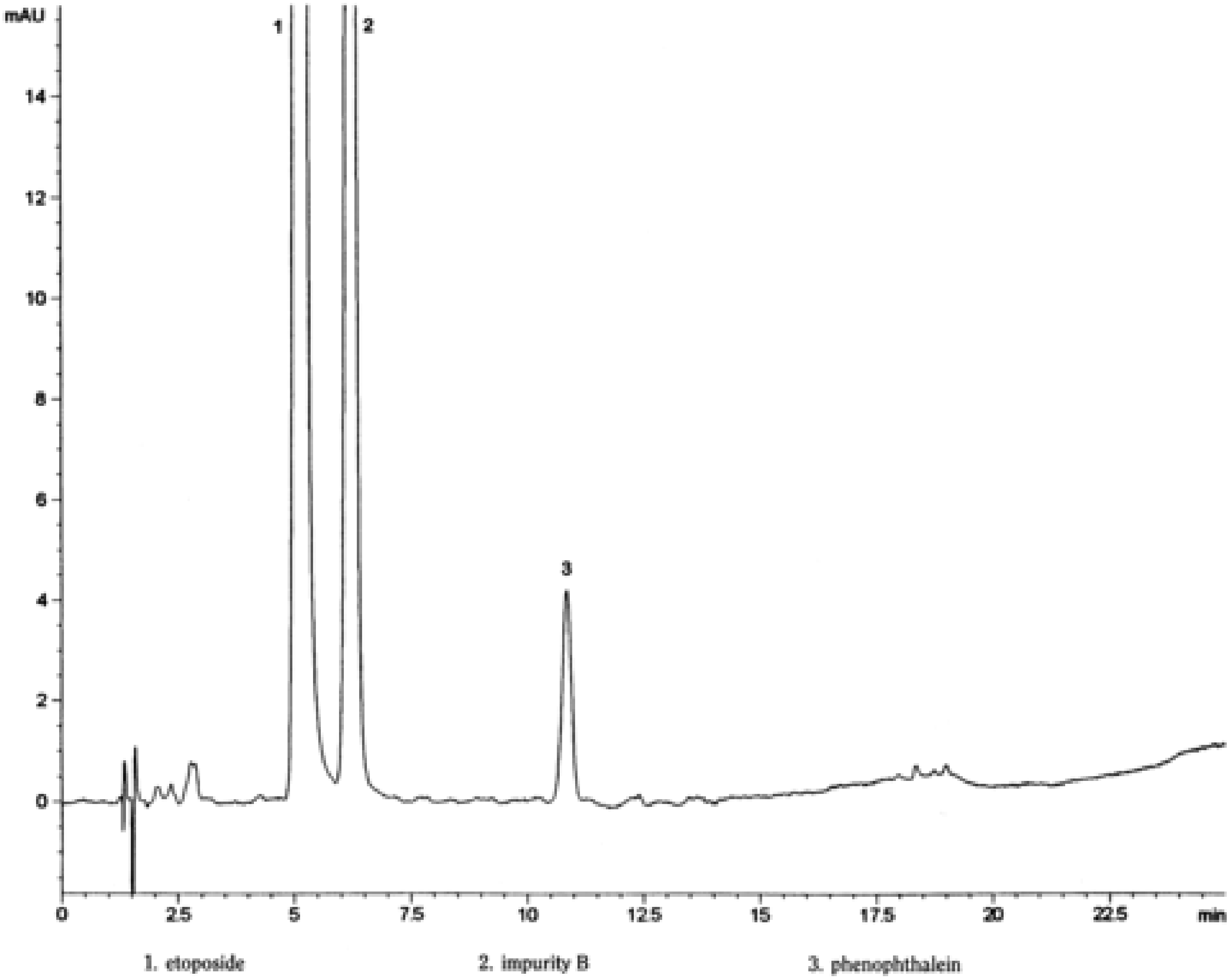


Figure 0823.-2. – *Chromatogram for the test for related substances of etoposide: reference solution (d)*

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than 2 such peaks have an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a). Disregard any peak due to the solvent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 6.0 per cent, determined on 0.250 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (c).

System suitability:

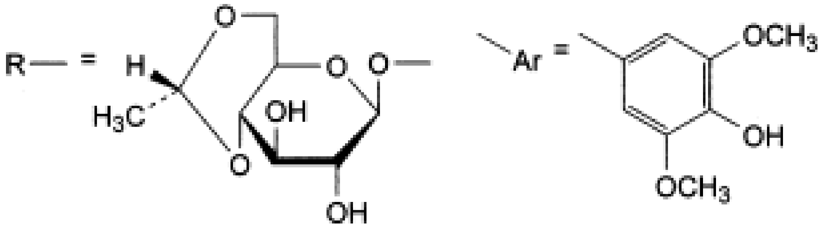
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (c).

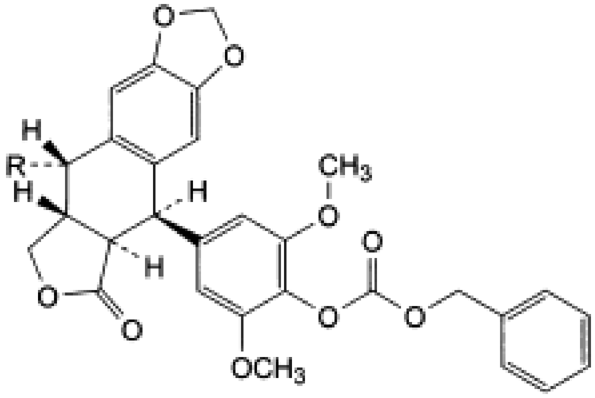
Calculate the percentage content of $C_{29}H_{32}O_{13}$ from the areas of the peaks and the declared content of *etoposide CRS*.

STORAGE

In an airtight container .

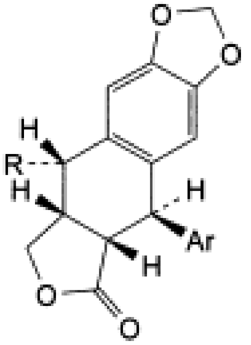
IMPURITIES



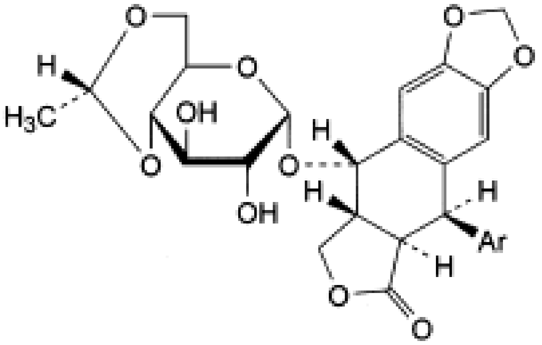


A. (5*R*,5a*R*,8a*R*,9*S*)-5-[4-[[(benzyloxy)carbonyl]oxy]-3,5-dimethoxyphenyl]-9-[[4,6-*O*-[(*R*)-ethylidene]-β-D-glucopyranosyl]oxy]-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-

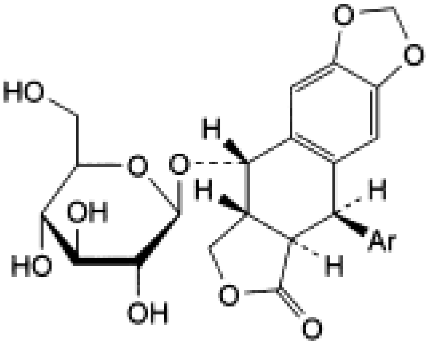
6(5a*H*)-one (4′-carbobenzoyloxyethylidene-lignan P),


O
O
H
R
H
H
Ar
H
O
O

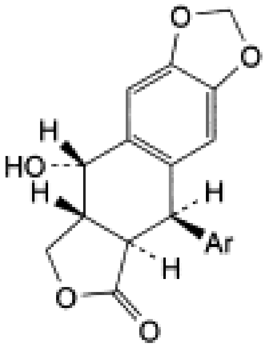
B. (5*R*,5a*S*,8a*R*,9*S*)-9-[[4,6-*O*-[(*R*)-ethylidene]-β-D-glucopyranosyl]oxy]-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one (picroethylidene-lignan P; *cis*-etoposide),


O
O
H
H_3C
OH
O
O
OH
O
H
H
H
Ar
H
O
O
O
O

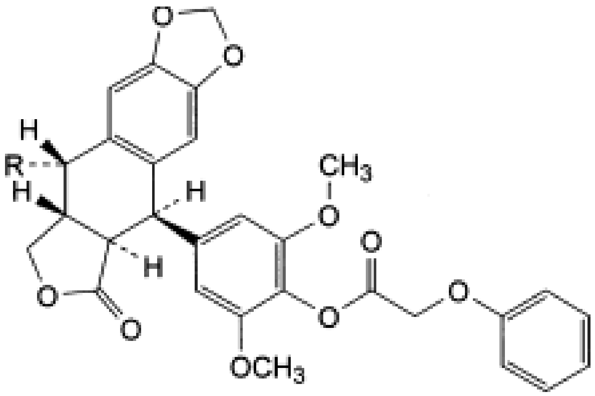
C. (5*R*,5a*R*,8a*R*,9*S*)-9-[[4,6-*O*-[(*R*)-ethylidene]-α-D-glucopyranosyl]oxy]-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one (α-etoposide),


HO
O
O
O
OH
HO
OH
H
H
H
Ar
H
O
O
O
O

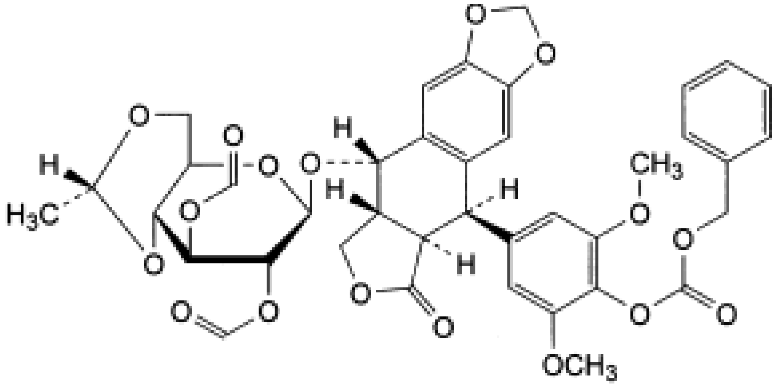
D. (5*R*,5a*R*,8a*R*,9*S*)-9-(β-D-glucopyranosyloxy)-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a, 9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one (lignan P),



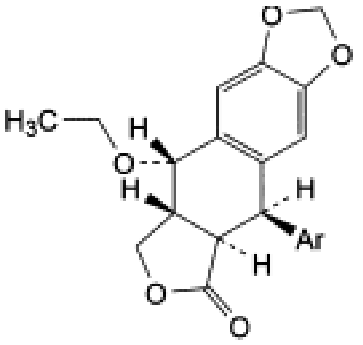
E. (5*R*,5a*R*,8a*R*,9*S*)-9-hydroxy-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one (4′-demethylepipodophyllotoxin),



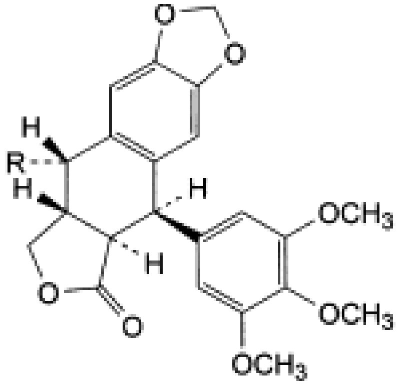
F. (5*R*,5a*R*,8a*R*,9*S*)-9-[[4,6-*O*-[(*R*)-ethylidene]-β-D-glucopyranosyl]oxy]-5-[4-[(phenoxyacetyl)oxy]-3,5-dimethoxyphenyl]-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one (4′-phenoxyacetyletoposide),



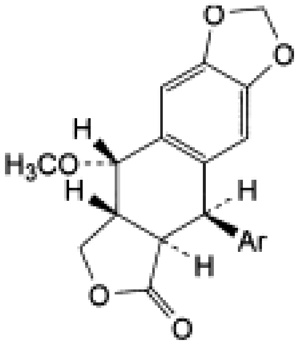
G. (5*R*,5a*R*,8a*R*,9*S*)-5-[4-[[(benzyloxy)carbonyl]oxy]-3,5-dimethoxyphenyl]-9-[[4,6-*O*-[(*R*)-ethylidene]-2,3-di-*O*-formyl-β-D-glucopyranosyl]oxy]-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one (4′-carbobenzoyloxydiformylethylidene-lignan P),



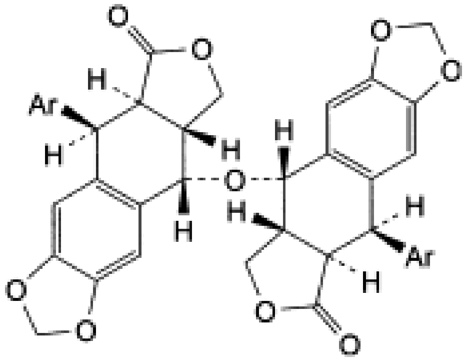
H. (5*R*,5a*R*,8a*R*,9*S*)-9-ethoxy-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*i*][1,3]benzodioxol-6(5a*H*)-one (4′-*O*-demethyl-1-*O*-ethylepipodophyllotoxin),



I. (5*R*,5a*R*,8a*R*,9*S*)-9-[[4,6-*O*-[(*R*)-ethylidene]-β-D-glucopyranosyl]oxy]-5-(3,4,5-trimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*i*][1,3]benzodioxol-6(5a*H*)-one (4-*O*-methylethylidene-lignan P),

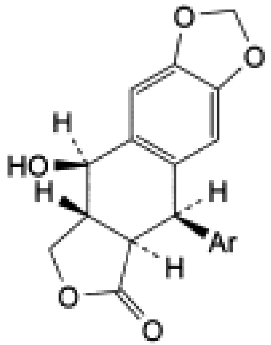


J. (5*R*,5a*R*,8a*R*,9*S*)-5-(4-hydroxy-3,5-dimethoxyphenyl)-9-methoxy-5,8,8a,9-tetrahydroisobenzofuro[5,6-*i*][1,3]benzodioxol-6(5a*H*)-one (4′-*O*-demethyl-1-*O*-methylepipodophyllotoxin),

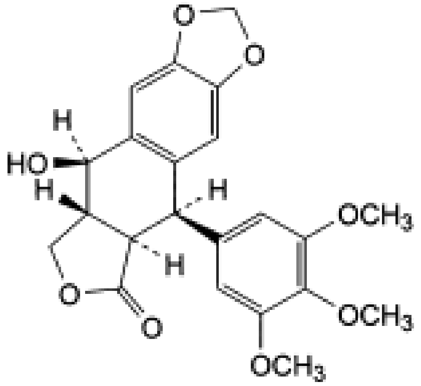


Ar
H
H
H
O
H
H
H
H
Ar
H

K. 9,9′-oxybis[(5*R*,5a*R*,8a*R*,9*S*)-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one] (di-4′-*O*-demethylepipodophyllotoxin),

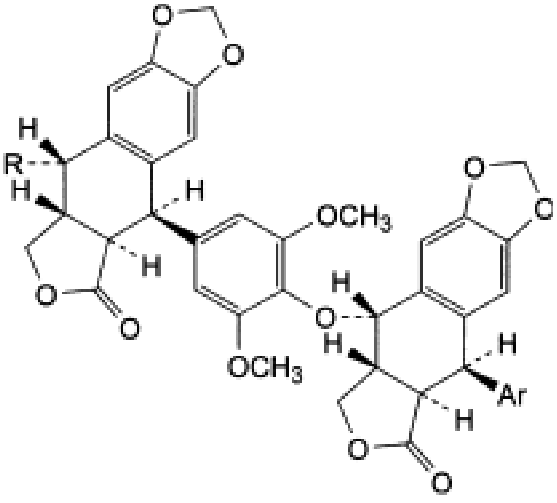


L. (5*R*,5a*R*,8a*R*,9*R*)-9-hydroxy-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one (4′-*O*-demethylpodophyllotoxin),



H
HO
H
H
OCH3
H
OCH3
OCH3

M. (5*R*,5a*R*,8a*R*,9*R*)-9-hydroxy-5-(3,4,5-trimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro [5,6-*f*][1,3]benzodioxol-6(5a*H*)-one (podophyllotoxin),

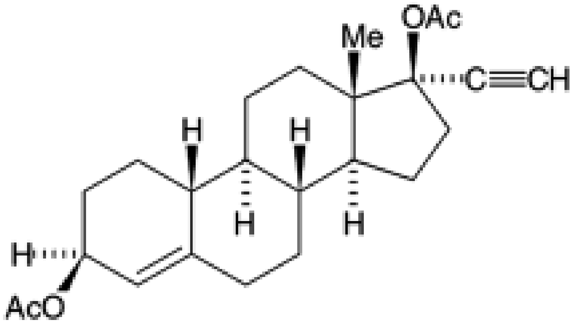

H
R
H
H
H
OCH3
OCH3
O
H
H
H
Ar
H
O
O
O
O
O
O
O
O
O
O

N. (5*R*,5a*R*,8a*R*,9*S*)-9-[[4,6-*O*-[(*R*)-ethylidene]-β-D-glucopyranosyl]oxy]-5-[4-[[(5*R*,5a*R*,8a*R*,9*S*)-5-(4-hydroxy-3,5-dimethoxyphenyl)-6-oxo-5,5a,6,8,8a,9-hexahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-9-yl]oxy]-3,5-dimethoxyphenyl]-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one.

Ph Eur

Etynodiol Diacetate

General Notices



$C_{24}H_{32}O_4$ 384.5 *297-76-7*

Action and use

Progestogen.

DEFINITION

Etynodiol Diacetate is 19-nor-17α-pregn-4-en-20-yne-3β,17β-diyl diacetate. It contains not less than 97.0% and not more than 102.0% of $C_{24}H_{32}O_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Very slightly soluble in *water*; freely soluble in *ether*; soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 350 nm of the solution obtained in the test for Light absorption exhibits a maximum only at 236 nm and shoulders at 229 and 243 nm.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of etynodiol diacetate *(RS 138)*.

C. Yields the reaction characteristic of *acetyl groups*, Appendix VI.

TESTS

Light absorption

Dissolve 50 mg in sufficient *methanol* to produce 50 ml (solution A). To 10 ml of solution A add 40 ml of *methanol* and a mixture of 3 ml of *hydrochloric acid* and 2 ml of *water*, mix and boil on a water bath for exactly 10 minutes. Cool, dilute to 100 ml with *methanol* and dilute 10 ml of the solution to 100 ml with *methanol*. The *absorbance* of the resulting solution at the maximum at 236 nm, Appendix II B, is 0.47 to 0.50, calculated with reference to the dried substance, using in the reference cell a solution prepared by diluting 1 ml of solution A to 100 ml with *methanol*.

Melting point

126° to 131°, Appendix V A.

Specific optical rotation

In a 1% w/v solution in *chloroform*, –70 to –76, Appendix V F, calculated with reference to the dried substance.

Conjugated compounds

Absorbance of a 0.050% w/v solution in *methanol* at 236 nm, not more than 0.47, calculated with reference to the dried substance, Appendix II B.

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.2 g in 40 ml of *tetrahydrofuran*, add 10 ml of a 10% w/v solution of *silver nitrate* and titrate with 0.1M *sodium hydroxide VS*, determining the end point potentiometrically. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 38.45 mg of $C_{24}H_{32}O_4$.

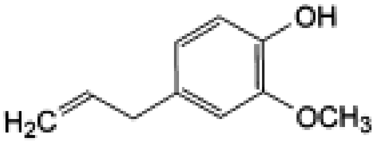
STORAGE

Etynodiol Diacetate should be protected from light.

Eugenol

General Notices

(Ph Eur monograph 1100)



$C_{10}H_{12}O_2$ 164.2 *97-53-0*

Ph Eur

DEFINITION

2-Methoxy-4-(prop-2-enyl)phenol.

CHARACTERS

Appearance

Colourless or pale yellow, clear liquid, darkening on exposure to air.

It has a strong odour of clove.

Solubility

Practically insoluble in water, freely soluble in ethanol (70 per cent *V/V*), practically insoluble in glycerol, miscible with ethanol (96 per cent), with glacial acetic acid, with methylene chloride and with fatty oils.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Refractive index (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *eugenol CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 µl of the substance to be examined in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.

Reference solution Dissolve 50 µl of *eugenol CRS* in *ethanol (96 per cent) R* and dilute to 25

ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (10:90 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of cold air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

Detection B Spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 0.05 ml in 2 ml of *ethanol (96 per cent)* R and add 0.1 ml of *ferric chloride solution R1*. A dark green colour is produced which changes to yellowish-green within 10 min.

TESTS

Relative density *(2.2.5)*

1.066 to 1.070.

Refractive index *(2.2.6)*

1.540 to 1.542.

Dimeric and oligomeric compounds

Dissolve 0.150 g in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. The absorbance *(2.2.25)* of the solution at 330 nm is not greater than 0.25.

Related substances

Gas chromatography (2.2.28) Use the normalisation procedure.

Test solution Dissolve 1.00 g of the substance to be examined in *anhydrous ethanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *anhydrous ethanol R*.

Reference solution (b) Dissolve 50 mg of *vanillin R* (impurity H) in 1 ml of the test solution and dilute to 5 ml with *anhydrous ethanol R*.

Column:

— *material*: fused silica;

— *size:* l = 30 m, Ø = 0.25 mm;

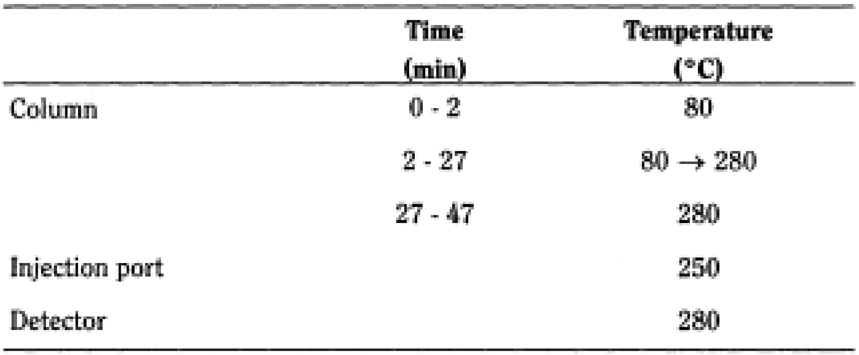
— *stationary phase*: *polymethylphenylsiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 ml/min.

Split ratio 1:40.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	80
	2 - 27	80 → 280
	27 - 47	280
Injection port		250
Detector		280

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

— *relative retention* with reference to eugenol: impurity H = minimum 1.1.

Limits:

— *any impurity*: for each impurity, maximum 0.5 per cent;

— *sum of impurities with a relative retention greater than 2.0 with reference to eugenol* : maximum 1.0 per cent;

— *total*: maximum 3.0 per cent;

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Hydrocarbons

Dissolve 1 ml in 5 ml of *dilute sodium hydroxide solution R* and add 30 ml of *water R* in a stoppered test-tube. Examined immediately, the solution is yellow and clear *(2.2.1)*.

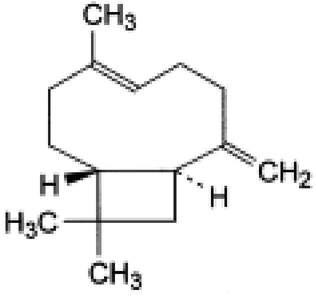
Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

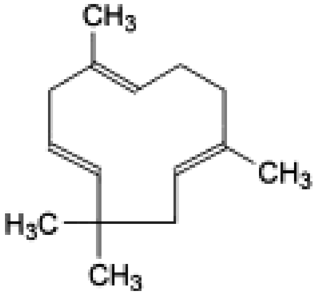
STORAGE

In a well-filled container, protected from light.

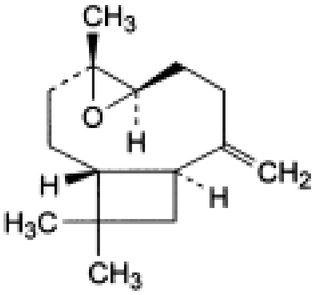
IMPURITIES


CH3
H
CH2
H
H3C
CH3

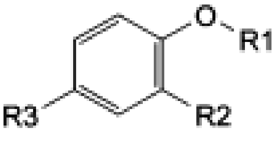
A. (1*R*,4*E*,9*S*)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene (β-caryophyllene),


CH3
CH3
H3C
CH3

B. (1*E*,4*E*,8*E*)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene (α-humulene, α-caryophyllene),


CH3
O
H
H
CH2
H
H3C
CH3

C. (1*R*,4*R*,6*R*,10*S*)-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.$0^{4,6}$]dodecane (β-caryophyllene oxide),



D. R1 = H, R2 = H, R3 = CH_2-CH=CH_2: 4-(prop-2-enyl)phenol,

E. R1 = CH_3, R2 = OCH_3, R3 = CH_2-CH=CH_2: 1,2-dimethoxy-4-(prop-2-enyl)benzene (eugenol methyl ether),

F. R1 = H, R2 = OCH_3, R3 = CH=CH-CH_3 (*cis*): 2-methoxy-4-[(Z)-prop-1-enyl]phenol (*cis*-isoeugenol),

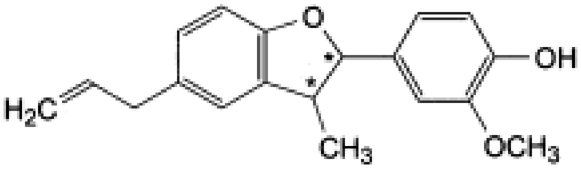
G. R1 = H, R2 = OCH_3, R3 = CH=CH-CH_3 (*trans*): 2-methoxy-4-[(E)-prop-1-enyl]phenol (*trans*-isoeugenol),

H. R1 = H, R2 = OCH_3, R3 = CHO: 4-hydroxy-3-methoxybenzaldehyde (vanillin),

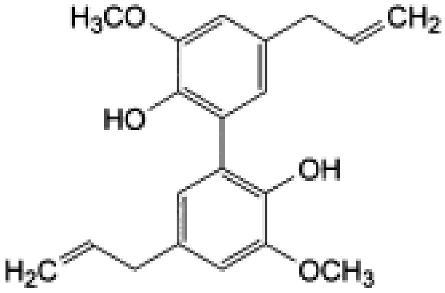
I. R1 = $CO\text{-}CH_3$, R2 = OCH_3, R3 = $CH_2\text{-}CH{=}CH_2$: 2-methoxy-4-(prop-2-enyl)phenyl acetate (acetyleugenol),

J. R1 = H, R2 = OCH_3, R3 = $CO\text{-}CH{=}CH_2$: 1-(4-hydroxy-3-methoxyphenyl)prop-2-enone,

K. R1 = H, R2 = OCH_3, R3 = CH=CH-CHO: (*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enal (*trans*-coniferyl aldehyde),

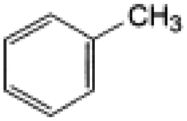


L. 2-methoxy-4-[3-methyl-5-(prop-2-enyl)-2,3-dihydrobenzofuran-2-yl]phenol (dehydrodi-isoeugenol),



M. 3,3′-dimethoxy-5,5′-bis(prop-2-enyl)biphenyl-2,2′-diol (dehydrodieugenol),

N. O. 2 further unknown dimeric compounds,



P. toluene.

Ph Eur

Refined Evening Primrose Oil

General Notices

(Ph Eur monograph 2104)

Ph Eur

DEFINITION

Fatty oil obtained from seeds of *Oenothera biennis* L. or *Oenothera lamarkiana* L. by extraction and/or expression. It is then refined. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, light yellow or yellow liquid.

Solubility

Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C).

Relative density

About 0.923.

Refractive index

About 1.478.

IDENTIFICATION

First identification *B*.

Second identification *A*.

A. Identification of fatty oils by thin-layer chromatography *(2.3.2)*.

The chromatogram obtained is similar to the type chromatogram for refined evening primrose oil.

B. Composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 0.5. If intended for use in the manufacture of parenteral dosage forms: maximum 0.3.

Peroxide value ***(2.5.5, Method A)***

Maximum 10.0. If intended for use in the manufacture of parenteral dosage forms: maximum 5.0.

Unsaponifiable matter ***(2.5.7)***

Maximum 2.5 per cent, determined on 5.0 g.

Alkaline impurities ***(2.4.19)***

It complies with the test for alkaline impurities in fatty oils.

Composition of fatty acids ***(2.4.22, Method A)***

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

— *saturated fatty acids of chain length less than* C_{16}: maximum 0.3 per cent,

— *palmitic acid*: 4.0 per cent to 10.0 per cent,

— *stearic acid*: 1.0 per cent to 4.0 per cent,

— *oleic acid*: 5.0 per cent to 12.0 per cent,

— *linoleic acid*: 65.0 per cent to 85.0 per cent,

— *gamma-linolenic acid* (equivalent chain length on macrogol 20 000: 19.0): 7.0 per cent to 14.0 per cent,

— *alpha-linolenic acid*: maximum 0.5 per cent.

Brassicasterol ***(2.4.23)***

Maximum 0.3 per cent in the sterol fraction of the oil.

Water ***(2.5.32)***

Maximum 0.1 per cent, if intended for use in the manufacture of parenteral dosage forms, determined on 5.00 g.

STORAGE

Under an inert gas, in a well-filled, airtight container, protected from light.

LABELLING

The label states, where applicable, that the oil is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Dried Ferrous Sulphate

General Notices

(Ph Eur monograph 2340)

$FeSO_4$ 151.9 *13463-43-9*

Ph Eur

DEFINITION

Hydrated ferrous sulphate from which part of the water of hydration has been removed by drying.

Content

86.0 per cent to 90.0 per cent.

CHARACTERS

Appearance

Greyish-white powder.

Solubility

Slowly but freely soluble in water, very soluble in boiling water, practically insoluble in ethanol (96 per cent).

It is oxidised in moist air, becoming brown.

IDENTIFICATION

A. It gives the reactions of sulphates *(2.3.1)*.

B. It gives reaction (a) of iron *(2.3.1)*.

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 2.00 g in a 5 per cent *V/V* solution of *lead-free nitric acid R* and dilute to 100.0 ml with the same acid.

pH *(2.2.3)*

3.0 to 4.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Chlorides *(2.4.4)*

Maximum 300 ppm.

Dissolve 2.5 g in *water R*, add 0.5 ml of *dilute sulphuric acid R* and dilute to 50 ml with *water R*. Dilute 3.3 ml of this solution to 10 ml with *water R* and add 5 ml of *dilute nitric acid R*. Prepare the standard using a mixture of 10 ml of *chloride standard solution (5 ppm Cl) R* and 5 ml of *dilute nitric acid R*. Use 0.15 ml of *silver nitrate solution R2* in this test.

Chromium

Maximum 1.00×10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *chromium standard solution (100 ppm Cr) R*, diluted as necessary with a 5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Chromium hollow-cathode lamp using a transmission band preferably of 1 nm.

Wavelength 357.9 nm.

Atomisation device Air-acetylene flame.

Copper

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *copper standard solution (0.1 per cent Cu) R*, diluted as necessary with a 5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Copper hollow-cathode lamp using a transmission band preferably of 1 nm.

Wavelength 324.7 nm.

Atomisation device Air-acetylene flame.

Ferric ions

Maximum 0.50 per cent.

In a ground-glass-stoppered flask, dissolve 5.00 g in a mixture of 10 ml of *hydrochloric acid R* and 100 ml of *carbon dioxide-free water R*. Add 3 g of *potassium iodide R*, close the flask and allow to stand in the dark for 5 min. Titrate the liberated iodine with *0.1 M sodium thiosulphate*, using 0.5 ml of *starch solution R*, added towards the end of titration, as indicator. Carry out a blank test in the same conditions. Not more than 4.5 ml of *0.1 M sodium thiosulphate* is used.

Manganese

Maximum 0.10 per cent.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dilute 1.0 ml of solution S to 20.0 ml with a 5 per cent *V/V* solution of *lead-free nitric acid R*.

Reference solutions Prepare the reference solutions using *manganese standard solution (1000 ppm Mn) R*, diluted as necessary with a 5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Manganese hollow-cathode lamp using a transmission band preferably of 1 nm.

Wavelength 279.5 nm.

Atomisation device Air-acetylene flame.

Nickel

Maximum 1.00 × 102 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *nickel standard solution (10 ppm Ni) R*, diluted as necessary with a 5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Nickel hollow-cathode lamp using a transmission band preferably of 1 nm.

Wavelength 232.0 nm.

Atomisation device Air-acetylene flame.

Zinc

Maximum 1.00 × 10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *zinc standard solution (100 ppm Zn) R*, diluted as necessary with a 5 per cent *V/V* solution of *lead-free nitric acid R*.

Source Zinc hollow-cathode lamp using a transmission band preferably of 1 nm.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame.

ASSAY

Dissolve 2.5 g of *sodium hydrogen carbonate R* in a mixture of 150 ml of *water R* and 10 ml of *sulphuric acid R*. When the effervescence ceases, add to the solution 0.140 g of the substance to be examined and dissolve with gentle shaking. Add 0.1 ml of *ferroin R* and titrate with *0.1 M ammonium and cerium nitrate* until the red colour disappears.

1 ml of *0.1 M ammonium and cerium nitrate* is equivalent to 15.19 mg of $FeSO_4$.

STORAGE

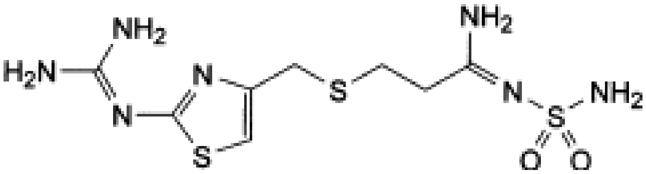
In an airtight container.

Ph Eur

Famotidine

General Notices

(Ph Eur monograph 1012)



$C_8H_{15}N_7O_2S_3$ 337.5 *76824-35-6*

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

Preparation

Famotidine Tablets

Ph Eur

DEFINITION

3-[[[2-[(Diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]-*N′*-sulphamoylpropanimidamide.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder or crystals.

Solubility

Very slightly soluble in water, freely soluble in glacial acetic acid, very slightly soluble in anhydrous ethanol, practically insoluble in ethyl acetate. It dissolves in dilute mineral acids.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *famotidine CRS.*

If the spectra obtained show differences, suspend 0.10 g of the substance to be examined and 0.10 g of the reference substance separately in 5 ml of *water R*. Heat to boiling and allow to cool, scratching the wall of the tube with a glass rod to initiate crystallisation. Filter, wash the crystals with 2 ml of iced *water R* and dry in an oven at 80 °C at a pressure not exceeding 670 Pa for 1 h. Record new spectra using the residues.

TESTS

Appearance of solution

Dissolve 0.20 g in a 50 g/l solution of *hydrochloric acid R*, heating to 40 °C if necessary, and dilute to 20 ml with the same acid. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 12.5 mg of the substance to be examined in mobile phase A and dilute to 25.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 100.0 ml with mobile phase A.

Reference solution (b) Dissolve 2.5 mg of *famotidine impurity D CRS* in *methanol R* and dilute to 10.0 ml with the same solvent. To 1.0 ml of the solution add 0.50 ml of the test solution and dilute to 100.0 ml with mobile phase A.

Reference solution (c) Dissolve 5.0 mg of *famotidine for system suitability CRS* (famotidine containing impurities A, B, C, D, E, F, G) in mobile phase A and dilute to 10.0 ml with mobile phase A.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

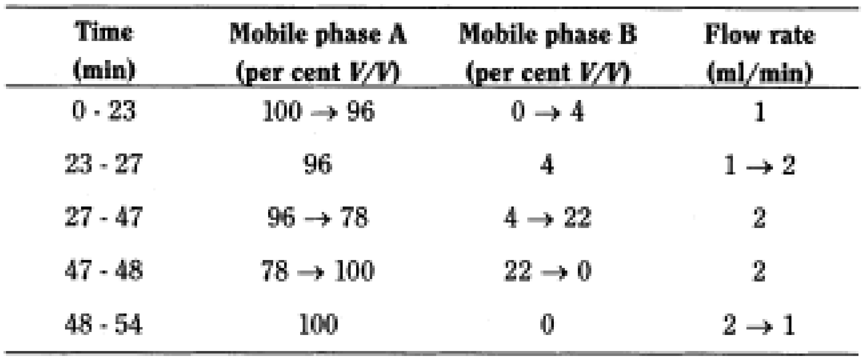
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 50 °C.

Mobile phase:

— *mobile phase A*: mix 6 volumes of *methanol R*, 94 volumes of *acetonitrile R* and 900 volumes of a 1.882 g/l solution of *sodium hexanesulphonate R* previously adjusted to pH 3.5 with *acetic acid R*,

— *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)	Flow rate (ml/min)
0 - 23	100 → 96	0 → 4	1
23 - 27	96	4	1 → 2
27 - 47	96 → 78	4 → 22	2
47 - 48	78 → 100	22 → 0	2
48 - 54	100	0	2 → 1

Detection Spectrophotometer at 265 nm.

Injection 20 µl.

Relative retention with reference to famotidine (retention time = about 21 min): impurity D = about 1.1; impurity C = about 1.2; impurity G = about 1.4; impurity F = about 1.5; impurity A = about 1.6; impurity B = about 2.0; impurity E = about 2.1.

System suitability:

— the chromatogram obtained with reference solution (c) is similar to the chromatogram supplied with *famotidine for system suitability CRS*;

— *retention time*: famotidine = 19-23 min in all the chromatograms; impurity E = maximum 48 min in the chromatogram obtained with reference solution (c);

— *resolution*: minimum 3.5 between the peaks due to famotidine and impurity D in the chromatogram obtained with reference solution (b).

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.9; impurity B = 2.5; impurity C = 1.9; impurity F = 1.7; impurity G = 1.4;

— *impurities A, G*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities B, C, D, E*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), and not more than 3 such peaks have an area greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) for the peaks eluting by 25 min, and not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) for the peaks eluting after 25 min (0.1 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 80 °C at a pressure not exceeding 670 Pa for 5 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.120 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

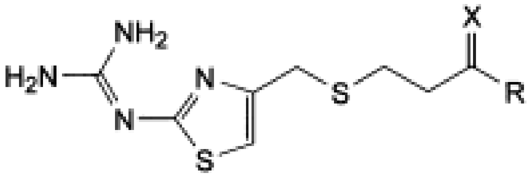
1 ml of *0.1 M perchloric acid* is equivalent to 16.87 mg of $C_8H_{15}N_7O_2S_3$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G.



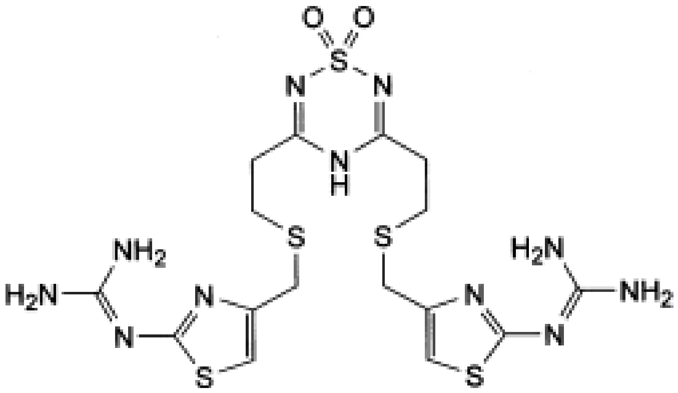
A. R = NH_2, X = NH: 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl] propanimidamide,

C. R = $NH-SO_2-NH_2$, X = O: 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl] -*N*-sulphamoylpropanamide,

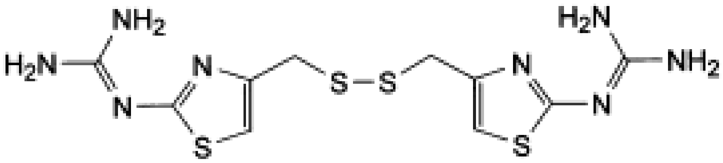
D. R = NH_2, X = O: 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl] propanamide,

F. R = OH, X = O: 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]propanoic acid,

G. R = NH-CN, X = NH: *N*-cyano-3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl] sulphanyl]propanimidamide,



B. 3,5-bis[2-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]ethyl]-4*H*-1,2,4,6-thiatriazine 1,1-dioxide,



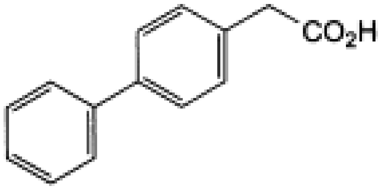
E. 2,2′-[disulphanediylbis(methylenethiazole-4,2-diyl)]diguanidine.

Ph Eur

Felbinac

General Notices

(Ph Eur monograph 2304)



$C_{14}H_{12}O_2$ 212.2 *5728-52-9*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

(Biphenyl-4-yl)acetic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in methanol, sparingly soluble in ethanol (96 per cent).

mp: about 164 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison felbinac CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Protect the solutions from light and inject within 20 min of preparation.*

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 5.0 mg of *felbinac impurity A CRS* and 5.0 mg of *biphenyl R* (impurity B) in *methanol R*, add 0.5 ml of the test solution and dilute to 50.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Column:
- *size:* l = 0.15 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 45 volumes of a 0.1 per cent *V/V* solution of *glacial acetic acid R* and 55 volumes of *methanol R*.

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 3.5 times the retention time of felbinac.

Relative retention With reference to felbinac (retention time = about 15 min): impurity A = about 1.3; impurity B = about 2.8.

System suitability Reference solution:

- *resolution*: minimum 3.0 between the peaks due to felbinac and impurity A.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent);
- *impurity B*: not more than the area of the peak due to felbinac in the chromatogram obtained with the reference solution (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the peak due to felbinac in the chromatogram obtained with the reference solution (0.10 per cent);
- *total*: not more than twice the area of the peak due to felbinac in the chromatogram obtained with the reference solution (0.2 per cent);
- *disregard limit*: 0.5 times the area of the peak due to felbinac in the chromatogram obtained with the reference solution (0.05 per cent).

Chlorides

Maximum 110 ppm.

Dissolve 1.0 g in 40 ml of *acetone R*, add 6 ml of a 10 per cent *V/V* solution of *nitric acid R*, dilute to 50.0 ml with *water R* and mix. Pour 15.0 ml of this solution as a single addition into 1 ml of *0.1 M silver nitrate* and allow to stand for 5 min protected from light. When viewed horizontally against a black background, any opalescence produced is not more intense than that obtained by treating in the same manner 15.0 ml of a mixture of 1.5 ml of *0.002 M hydrochloric acid*, 40 ml of *acetone R*, 6 ml of 10 per cent *V/V* solution of *nitric acid R*, diluted to 50.0 ml with *water R*.

Sulphates

Maximum 130 ppm.

Dissolve 1.5 g in 40 ml of *dimethylformamide R*, add 1 ml of a 10 per cent *V/V* solution of *hydrochloric acid R*, dilute to 50.0 ml with *dimethylformamide R* and mix. To 15.0 ml of this solution add 2.0 ml of a 120 g/l solution of *barium chloride R* and allow to stand for 5 min. Any opalescence produced is not more intense than that of a standard prepared in the same manner but using 2.0 ml of *0.001 M sulphuric acid* instead of the substance to be examined.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

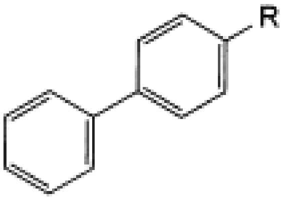
ASSAY

Dissolve 0.160 g in 50 ml of *methanol R*. Titrate with *0.1 M alcoholic potassium hydroxide* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M alcoholic potassium hydroxide* is equivalent to 21.23 mg of $C_{14}H_{12}O_2$.

IMPURITIES

Specified impurities *A, B.*



A. R = $CO\text{-}CH_3$: 4-acetyl biphenyl,

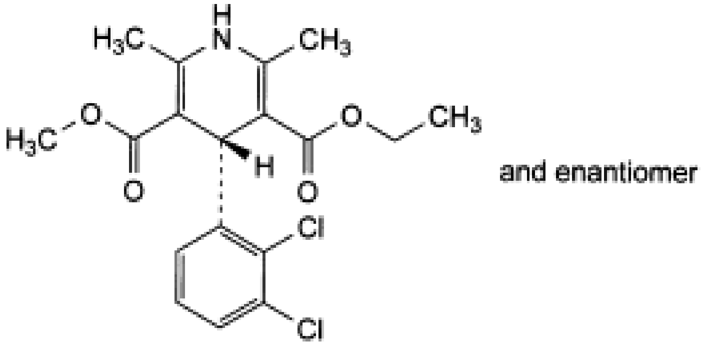
B. R = H: biphenyl.

Ph Eur

Felodipine

General Notices

(Ph Eur monograph 1013)



$C_{18}H_{19}Cl_2NO_4$ 384.3 *72509-76-3*

Action and use

Calcium channel blocker.

Preparation

Prolonged-release Felodipine Tablets

Ph Eur

DEFINITION

Ethyl methyl (4*RS*)-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or light yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol, in methanol and in methylene chloride.

IDENTIFICATION

First identification *B*.

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50 mg in *methanol R* and dilute to 100 ml with the same solvent. Dilute 3 ml of this solution to 100 ml with *methanol R*.

Spectral range 220-400 nm.

Absorption maxima At 238 nm and 361 nm.

Absorbance ratio $A_{361} / A_{238} = 0.34$ to 0.36.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *felodipine CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *felodipine CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *nifedipine CRS* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *ethyl acetate R*, *cyclohexane R* (40:60 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 0.150 g in a mixture of 25 ml of *2-methyl-2-propanol R* and 25 ml of *perchloric acid solution R*. Add 10 ml *of 0.1 M cerium sulphate*, allow to stand for 15 min, add 3.5 ml of *strong sodium hydroxide solution R* and neutralise with *dilute sodium hydroxide solution R*. Shake with 25 ml of *methylene chloride R*. Evaporate the lower layer to dryness on a water-bath under nitrogen (the residue is also used in the test for related substances). Dissolve about 20 mg of the residue in *methanol R* and dilute to 50 ml with the same solvent. Dilute 2 ml of this solution to 50 ml with *methanol R*.

Spectral range 220-400 nm.

Absorption maximum At 273 nm.

TESTS

Solution S

Dissolve 1.00 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

Absorbance *(2.2.25)*

Maximum 0.10, determined at 440 nm on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 50.0 mg of the residue obtained in identification test D (impurity A) and 25.0 mg of *felodipine CRS* in the mobile phase, then dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Column:
- *size:* l = 0.125-0.15 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *methanol R*, 40 volumes of *acetonitrile R* and 40 volumes of a phosphate buffer solution pH 3.0 containing 0.8 g/l of *phosphoric acid R* and 8 g/l of *sodium dihydrogen phosphate R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time Twice the retention time of felodipine.

Elution order Impurity B, impurity A, felodipine, impurity C.

Retention time Felodipine = about 12 min.

System suitability Reference solution (c):

- *resolution*: minimum 2.5 between the peaks due to impurity A and felodipine.

Limits:
- *sum of impurities B and C*: not more than the area of the principal peak in the

chromatogram obtained with reference solution (a) (1.0 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *sum of impurities other than B and C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g in a mixture of 25 ml of *2-methyl-2-propanol R* and 25 ml of *perchloric acid solution R*. Add 0.05 ml of *ferroin R*. Titrate with *0.1 M cerium sulphate* until the pink colour disappears. Titrate slowly towards the end of the titration.

1 ml of *0.1 M cerium sulphate* is equivalent to 19.21 mg of $C_{18}H_{19}C_{l2}NO_4$.

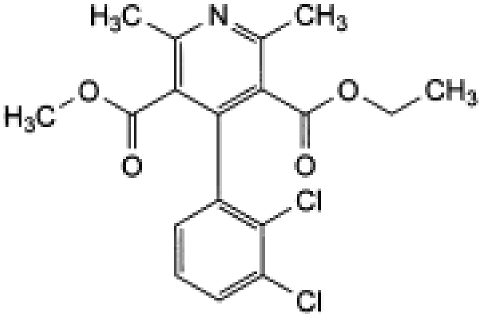
STORAGE

Protected from light.

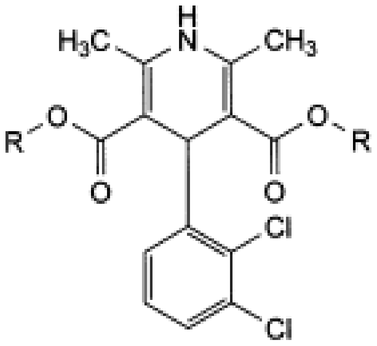
IMPURITIES

Specified impurities *B, C.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A*.



A. ethyl methyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate,


H3C
H
N
CH3
R
O
O
O
O
R
Cl
Cl

B. R = CH_3: dimethyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate,

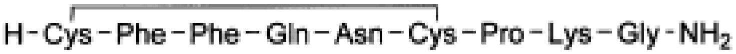
C. R = C_2H_5: diethyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

Ph Eur

Felypressin

General Notices

(Ph Eur monograph 1634)



$C_{46}H_{65}N_{13}O_{11}S_2$ 1039 *56-59-7*

Action and use

Vasopressin analogue; vasoconstrictor in local anaesthesia.

Ph Eur

DEFINITION

L-Cysteinyl-L-phenylalanyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-lysylglycinamide cyclic (1,6)-disulphide.

Synthetic nonapeptide having a vasoconstricting activity. It is available as an acetate.

Content

95.0 per cent to 102.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance

White or almost white, powder or flakes.

Solubility

Freely soluble in water, practically insoluble in acetone and ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis *(2.2.56)*. For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of amino acids, taking one-seventh of the sum of the number of moles of glutamic acid, aspartic acid, proline, lysine, glycine and phenylalanine as equal to one. The values fall within the following limits: aspartic acid: 0.9 to 1.1; glutamic acid: 0.9 to 1.1; proline: 0.9 to 1.1; glycine: 0.9 to 1.1; phenylalanine: 1.8 to 2.2; half-cystine: 1.8 to 2.2; lysine: 0.9 to 1.1.

TESTS

Specific optical rotation *(2.2.7)*

- 35 to - 29, determined at 25 °C (anhydrous and acetic acid-free substance).

Dissolve 20.0 mg in a 1 per cent *V/V* solution of *glacial acetic acid R* and dilute to 10.0 ml with the same solution.

Related substances

Liquid chromatography *(2.2.29)*; use the normalisation procedure. *The solutions are stable for 24 h at room temperature or for 1 week at 2-8 °C.*

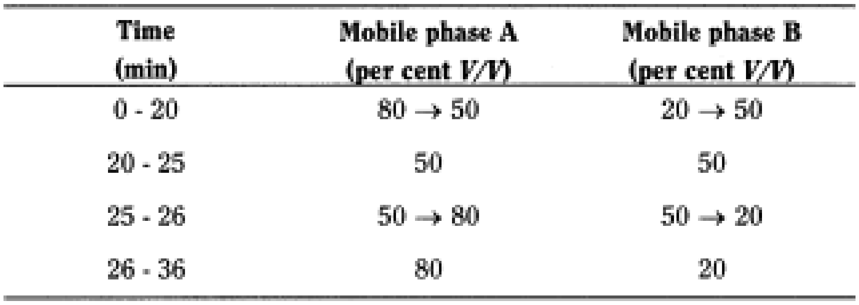
Test solution (a) Dissolve 5.0 mg of the substance to be examined in 5.0 ml of mobile phase A.

Test solution (b) Dilute 1.0 ml of test solution (a) to 5.0 ml with mobile phase A.

Reference solution Dissolve the contents of a vial of *felypressin CRS* in mobile phase A to obtain a concentration of 0.2 mg/ml.

Column:
- *size: l* = 0.15 m, Ø = 3.9 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 50 °C.

Mobile phase:
- *mobile phase A*: dissolve 3.62 g of *tetramethylammonium hydroxide R* in 900 ml *water R*; adjust to pH 2.5 with *phosphoric acid R* and dilute to 1000 ml with *water R*;
- *mobile phase B*: dissolve 1.81 g of *tetramethylammonium hydroxide R* in 450 ml of a 50 per cent *V/V* solution of *acetonitrile for chromatography R*; adjust to pH 2.5 with *phosphoric acid R* and dilute to 500 ml with a 50 per cent *V/V* solution of *acetonitrile for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	80 → 50	20 → 50
20 - 25	50	50
25 - 26	50 → 80	50 → 20
26 - 36	80	20

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl of test solution (a) and 50 µl of the reference solution.

Identification of impurities Use the chromatogram supplied with *felypressin CRS* to identify the peaks due to impurities A to F.

Relative retention with reference to felypressin: impurity A = about 0.9; impurity B = about 1.1; impurity F = about 1.2; impurity C = about 1.3; impurity D = about 1.4; impurity E = about 2.1.

System suitability Reference solution:

- — *retention time*: felypressin = about 7.5 min;
- — *resolution*: minimum 1.5 between the peaks due to impurity C and impurity D.

Limits:

- — *impurities A, B, C, D, E, F*: for each impurity, maximum 0.5 per cent,
- — *any other impurity*: for each impurity, maximum 0.1 per cent,
- — *total*: maximum 3.0 per cent,
- — *disregard limit*: 0.05 per cent.

Acetic acid *(2.5.34)*

9.0 per cent to 13.0 per cent.

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Water *(2.5.32)*

Maximum 7.0 per cent.

Bacterial endotoxins *(2.6.14)*

Less than 100 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection 10 µl of test solution (b) and of the reference solution.

Calculate the content of felypressin ($C_{46}H_{65}N_{13}O_{11}S_2$) from the areas of the peaks and the declared content of $C_{46}H_{65}N_{13}O_{11}S_2$ in *felypressin CRS*.

STORAGE

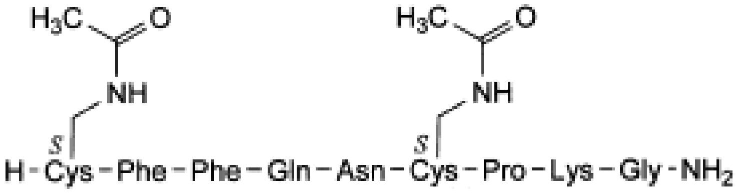
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

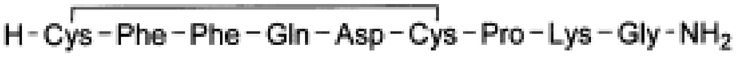
The label states the mass of peptide in the container.

IMPURITIES

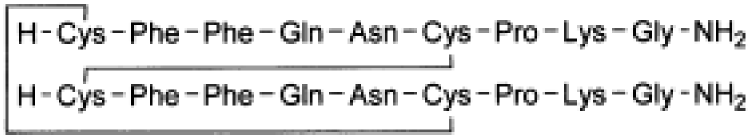
Specified impurities A, B, C, D, E, F.



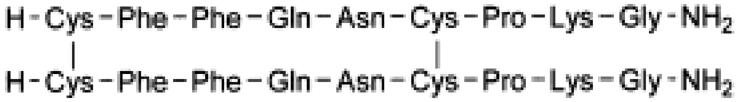
A. *S*[1],*S*[6]-bis[(acetylamino)methyl]-(reduced felypressin),



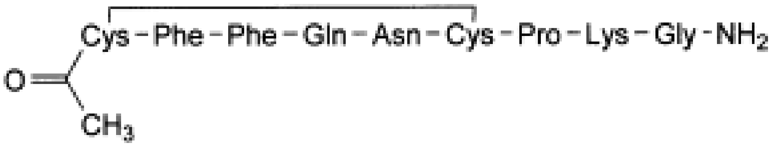
B. [5-aspartic acid]felypressin,



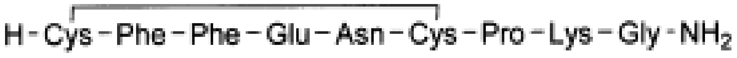
C. bis(reduced felypressin) (1,6′),(1′,6)-bis(disulfide),



D. bis(reduced felypressin) (1,1′),(6,6′)-bis(disulfide),



E. *N*[1]-acetylfelypressin,



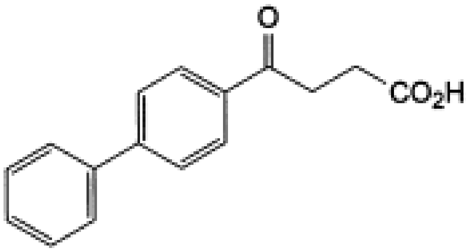
F. [4-glutamic acid]felypressin.

Ph Eur

Fenbufen

General Notices

(Ph Eur monograph 1209)



$C_{16}H_{14}O_3$ 254.3 *36330-85-5*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Fenbufen Capsules

Fenbufen Tablets

Ph Eur

DEFINITION

4-(Biphenyl-4-yl)-4-oxobutanoic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, fine, crystalline powder.

Solubility

Very slightly soluble in water, slightly soluble in acetone, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Melting point *(2.2.14)*: 186 °C to 189 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fenbufen CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *fenbufen CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *ketoprofen CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent. To 5 ml of this solution, add 5 ml of reference solution (a).

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *anhydrous acetic acid R*, *ethyl acetate R*, *hexane R* (5:25:75 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *dimethylformamide R*, mobile phase A (40:60 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dilute 0.5 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 25 mg of *fenbufen CRS* and 6 mg of *ketoprofen CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture. Dilute 1 ml of this solution to 100 ml with the solvent mixture.

Column:

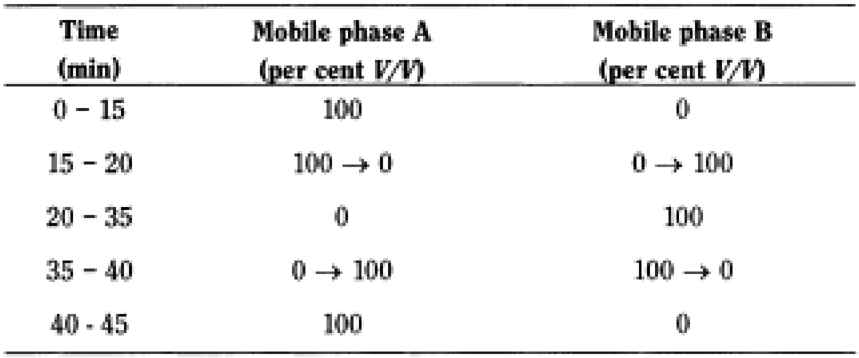
— *size:* l = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 32 volumes of *acetonitrile R* and 68 volumes of a mixture of 1 volume of *glacial acetic acid R* and 55 volumes of *water R*;

— *mobile phase B*: mix 45 volumes of *acetonitrile R* and 55 volumes of a mixture of 1 volume of *glacial acetic acid R* and 55 volumes of *water R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 15	100	0
15 – 20	100 → 0	0 → 100
20 – 35	0	100
35 – 40	0 → 100	100 → 0
40 - 45	100	0

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to ketoprofen and fenbufen.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

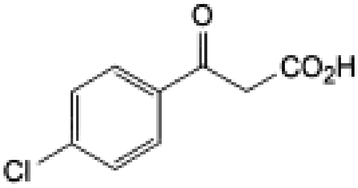
ASSAY

Dissolve 0.200 g in 75 ml of *acetone R* previously neutralised with *phenolphthalein solution*

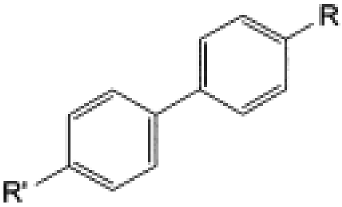
R1 and add 50 ml of *water R*. Add 0.2 ml of *phenolphthalein solution R1* and titrate with *0.1 M sodium hydroxide*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 25.43 mg of $C_{16}H_{14}O_3$.

IMPURITIES



A. 3-(4-chlorophenyl)-3-oxopropanoic acid,



B. R = CO-CH=CH-CO_2H, R′ = H: 4-(biphenyl-4-yl)-4-oxobut-2-enoic acid,

C. R = R′ = H: biphenyl,

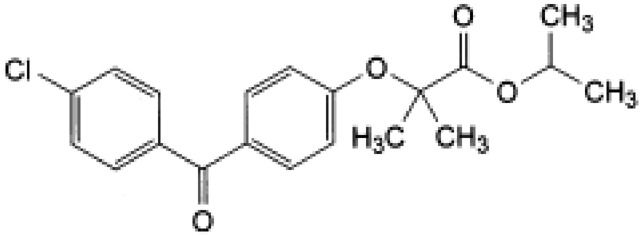
D. R = CO-CH_2-CH_2-CO_2H, R′ = OH: 4-(4′-hydroxybiphenyl-4-yl)-4-oxobutanoic acid.

Ph Eur

Fenofibrate

General Notices

(Ph Eur monograph 1322)



$C_{20}H_{21}ClO_4$ 360.8 *49562-28-9*

Action and use

Fibrate; lipid-regulating drug.

Ph Eur

DEFINITION

1-Methylethyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Melting point *(2.2.14)*: 79 °C to 82 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *fenofibrate CRS .*

TESTS

Solution S

To 5.0 g, add 25 ml of *distilled water R* and heat at 50 °C for 10 min. Cool and dilute to 50.0 ml with the same solvent. Filter. Use the filtrate as solution S.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY$_6$ *(2.2.2, Method II)*.

Dissolve 0.50 g in *acetone R* and dilute to 10.0 ml with the same solvent.

Acidity

Dissolve 1.0 g in 50 ml of *ethanol (96 per cent) R* previously neutralised using 0.2 ml of *phenolphthalein solution R1*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *fenofibrate CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *fenofibrate CRS*, 5.0 mg of *fenofibrate impurity A CRS*, 5.0 mg of *fenofibrate impurity B CRS* and 10.0 mg of *fenofibrate impurity G CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *water R* acidified to pH 2.5 with *phosphoric acid R* and 70 volumes of *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 286 nm.

Injection 20 µl of the test solution and reference solution (b).

Run time Twice the retention time of fenofibrate.

Relative retention with reference to fenofibrate:

impurity A = about 0.34; impurity B = about 0.36; impurity C = about 0.50; impurity D = about 0.65; impurity E = about 0.80, impurity F = about 0.85; impurity G = about 1.35.

System suitability Reference solution (b):

- *resolution*: minimum 1.5 between the peaks due to impurities A and B.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity G*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to fenofibrate in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 5 times the area of the peak due to fenofibrate in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the peak due to fenofibrate in the chromatogram obtained with reference solution (b) (0.01 per cent).

Halides expressed as chlorides *(2.4.4)*

Maximum 100 ppm.

To 5 ml of solution S add 10 ml of *distilled water R*.

Sulphates *(2.4.13)*

Maximum 100 ppm, determined on solution S.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection 5 µl of the test solution and reference solution (a).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

STORAGE

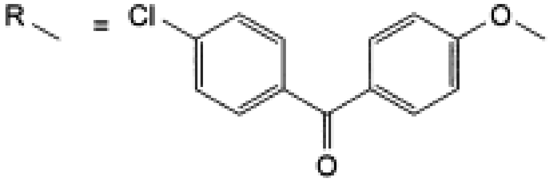
Protected from light.

IMPURITIES

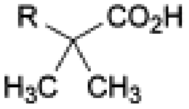
Specified impurities *A, B, G.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general

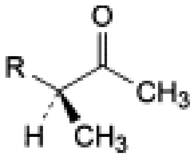
acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C, D, E, F.*



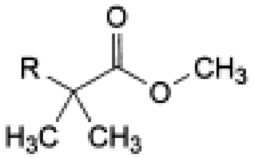
A. R-H: (4-chlorophenyl)(4-hydroxyphenyl)methanone,



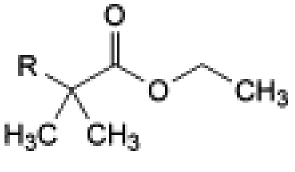
B. 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid (fenofibric acid),



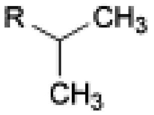
C. (3*RS*)-3-[4-(4-chlorobenzoyl)phenoxy]butan-2-one,



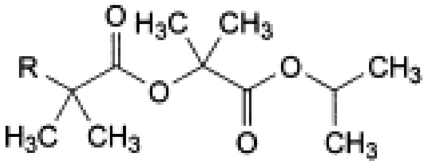
D. methyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate,



E. ethyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate,



F. (4-chlorophenyl)[4-(1-methylethoxy)phenyl]methanone,

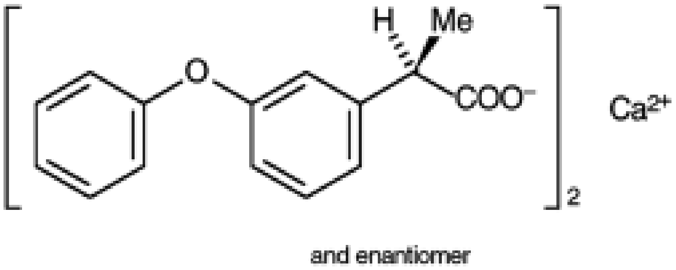


G. 1-methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate.

Ph Eur

Fenoprofen Calcium

General Notices



$(C_{15}H_{13}O_3)_2Ca,2H_2O$ 558.6   *34957-40-5*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparation

Fenoprofen Tablets

DEFINITION

Fenoprofen Calcium is calcium (*RS*)-2-(3-phenoxyphenyl)propionate dihydrate. It contains not less than 97.5% and not more than 101.0% of $(C_{15}H_{13}O_3)_2Ca$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Slightly soluble in *water*; soluble in *ethanol (96%)*.

IDENTIFICATION

A. Dissolve 0.1 g in 5 ml of *glacial acetic acid* and add sufficient *methanol* to produce 100 ml. Dilute 5 ml of this solution to 50 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 272 nm and 278 nm, and a shoulder at 266 nm. The *absorbance* at the maximum at 272 nm is about 0.70 and at the maximum at 278 nm is about 0.65.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of fenoprofen calcium *(RS 142)*.

C. The residue on ignition yields the reactions characteristic of *calcium salts*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using three solutions in the mobile phase containing (1) 0.0025% w/v of the substance being examined, (2) 0.50% w/v of the substance being examined and (3) 0.04% w/v of *fenoprofen calcium* and 0.0015% w/v of *4,4′-dimethoxybenzophenone*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (7 to 8 µm) (Zorbax ODS is suitable), (b) a mixture of 61 volumes of *water*, 30 volumes of *acetonitrile*, 7 volumes of *tetrahydrofuran* and 2 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 270 nm. For solution (2) allow the chromatography to proceed for 3 times the retention time of the peak due to fenoprofen.

The test is not valid unless the *resolution factor* between the peaks corresponding to fenoprofen and 4,4′-dimethoxybenzophenone in the chromatogram obtained with solution (3) is at least 3.0.

In the chromatogram obtained with solution (2) the area of any *secondary peak* is not greater than twice the area of the peak in the chromatogram obtained with solution (1) (1%), not more than one such peak has an area greater than the area of the peak in the chromatogram obtained with solution (1) (0.5%) and the sum of the areas of all such peaks is not greater than four times the area of the peak in the chromatogram obtained with solution (1) (2%).

Water

5.0 to 8.0% w/w, Appendix IX C. Use 0.2 g.

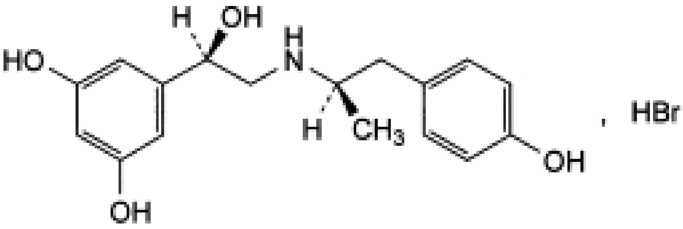
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.5 g and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 26.13 mg of $(C_{15}H_{13}O_3)_2Ca$.

Fenoterol Hydrobromide

General Notices

(Ph Eur monograph 0901)



$C_{17}H_{21}NO_4,HBr$ 384.3   *1944-12-3*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Preparation

Fenoterol Pressurised Inhalation

Ph Eur

DEFINITION

(1*RS*)-1-(3,5-Dihydroxyphenyl)-2-[[(1*RS*)-2-(4-hydroxyphenyl)-1-methylethyl]amino]ethanol hydrobromide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification   *B, E.*

Second identification   *A, C, D, E.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in *dilute hydrochloric acid R1* and dilute to 50.0 ml with the same acid. Dilute 5.0 ml of this solution to 50.0 ml with *dilute hydrochloric acid R1*.

Spectral range 230-350 nm.

Absorption maximum At 275 nm.

Shoulder At about 280 nm.

Specific absorbance at the absorption maximum 80 to 86.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fenoterol hydrobromide CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *fenoterol hydrobromide CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R*, *water R*, *aldehyde-free methanol R* (1.5:10:90 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 10 g/l solution of *potassium permanganate R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in a 20 g/l solution of *disodium tetraborate R* and dilute to 50 ml with the same solution. Add 1 ml of a 10 g/l solution of *aminopyrazolone R*, 10 ml of a 2 g/l solution of *potassium ferricyanide R* and 10 ml of *methylene chloride R*. Shake and allow to separate. A reddish-brown colour develops in the lower layer.

E. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.00 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.2 to 5.2 for solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 24.0 mg of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dissolve 24.0 mg of *fenoterol hydrobromide CRS* (containing impurity A) in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (b) Dissolve the contents of a vial of *fenoterol for peak identification CRS* (containing impurities B and C) in 1.0 ml of *water R*.

Reference solution (c) Dilute 10.0 ml of the test solution to 50.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 24 g of *disodium hydrogen phosphate R* in 1000 ml of *water R*. Mix 69 volumes of this solution with 1 volume of a 9 g/l solution of *potassium of hydrogen phosphate R*, adjust to pH 8.5 with *phosphoric acid R* and add 35 volumes of *methanol R2*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 3 times the retention time of fenoterol.

Relative retention with reference to fenoterol (retention time = about 7 min): impurity A = about 1.3; impurity B = about 2.0; impurity C = about 2.2.

System suitability:
- *resolution*:
  - minimum 3 between the peaks due to fenoterol and impurity A in the chromatogram obtained with reference solution (a);
  - minimum 1.5 between the peaks due to impurities B and C in the chromatogram obtained with reference solution (b).

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity B by 0.6;
- *impurity A*: maximum 4.0 per cent, calculated from the area of the corresponding peak in the chromatogram obtained with reference solution (a) and taking into account the declared content of impurity A in *fenoterol hydrobromide CRS*;
- *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *sum of impurities other than A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Iron *(2.4.9)*

Maximum 10 ppm.

Dissolve the residue obtained in the test for sulphated ash in 2.5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.600 g in 50 ml of *water R* and add 5 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *ferric ammonium sulphate solution R2*. Shake and titrate with *0.1 M ammonium thiocyanate* until an orange colour is obtained. Carry out a blank titration.

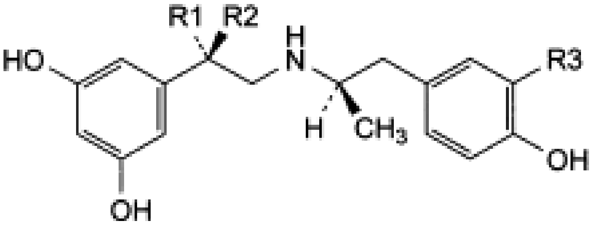
1 ml of *0.1 M silver nitrate* is equivalent to 38.43 mg of $C_{17}H_{22}BrNO_4$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C.



A. R1 = OH, R2 = R3 = H: (1*RS*)-1-(3,5-dihydroxyphenyl)-2-[[(1*SR*)-2-(4-hydroxyphenyl)-1-methylethyl]amino]ethanol,

B. R1 + R2 = O, R3 = H: 1-(3,5-dihydroxyphenyl)-2-[[(1*RS*)-2-(4-hydroxyphenyl)-1-methylethyl]amino]ethanone,

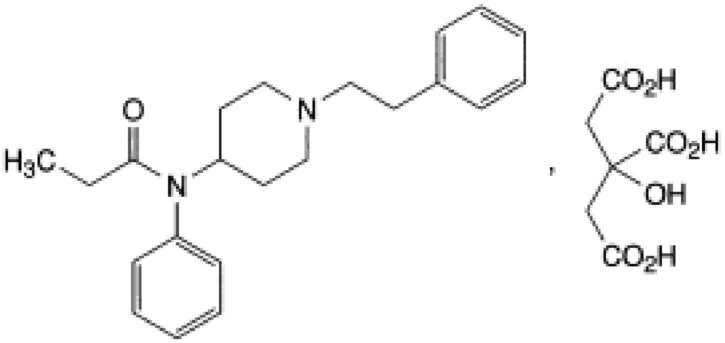
C. R1 = H, R2 = OH, R3 = CH_3: (1*RS*)-1-(3,5-dihydroxyphenyl)-2-[[(1*RS*)-2-(4-hydroxy-3-methylphenyl)-1-methylethyl]amino]ethanol.

Ph Eur

Fentanyl Citrate

General Notices

(Ph Eur monograph 1103)



$C_{22}H_{28}N_2O,C_6H_8O_7$ 528.6 *990-73-8*

Action and use

Opioid receptor agonist; analgesic.

Preparation

Fentanyl Injection

Ph Eur

DEFINITION

N-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]propanamide dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water, freely soluble in methanol, sparingly soluble in ethanol (96 per cent).

mp: about 152 °C, with decomposition.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of fentanyl citrate* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.2 g of the substance to be examined in *water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

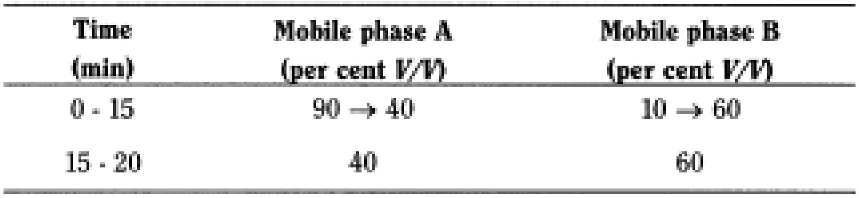
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) In order to prepare the *in situ* degradation compound (impurity D), dissolve 10 mg of the substance to be examined in 10.0 ml of *dilute hydrochloric acid R*. Heat on a water-bath under a reflux condenser for 4 h. Neutralise with 10.0 ml of *dilute sodium hydroxide solution R*. Evaporate to dryness on a water-bath. Cool and take up the residue in 10 ml of *methanol R*. Filter.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol R*.

Column:
- *size: l* = 0.1 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: 5 g/l solution of *ammonium carbonate R* in a mixture of 10 volumes of *tetrahydrofuran R* and 90 volumes of *water R*;
- *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 40	10 → 60
15 - 20	40	60

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Equilibration With *acetonitrile R* for at least 30 min, and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Fentanyl = about 10 min; impurity D = about 12 min.

System suitability Reference solution (a):

— *resolution*: minimum 8.0 between the peaks due to fentanyl and impurity D; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient elution.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak with a retention time relative to the principal peak of 0.05 or less.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid* using 0.2 ml of *naphtholbenzein solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 52.86 mg of $C_{28}H_{36}N_2O_8$.

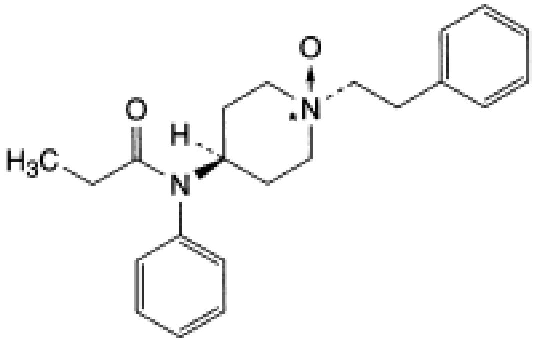
STORAGE

Protected from light.

IMPURITIES

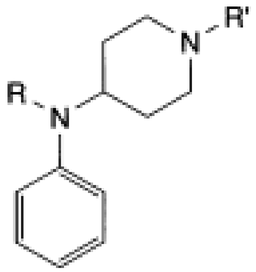
Specified impurities *A, B, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : E.*



and epimer at N*

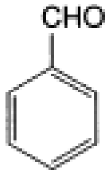
A. *N*-phenyl-*N*-[*cis*,*trans*-1-oxido-1-(2-phenylethyl)piperidin-4-yl]propanamide,



B. R = CO-C_2H_5, R′ = H: *N*-phenyl-*N*-(piperidin-4-yl)propanamide,

C. R = CO-CH_3, R′ = CH_2-CH_2-C_6H_5: *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]acetamide,

D. R = H, R′ = CH_2-CH_2-C_6H_5: *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine,



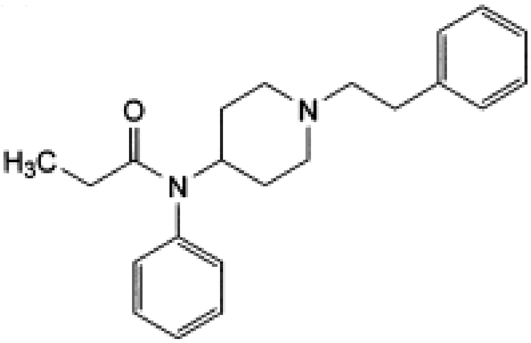
E. benzaldehyde.

Ph Eur

Fentanyl

General Notices

(Ph Eur monograph 1210)



$C_{22}H_{28}N_2O$ 336.5 *437-38-7*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

N-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]propanamide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent) and in methanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of fentanyl .

If the spectrum obtained in the solid state shows differences, dissolve the substance to be examined in the minimum volume of *anhydrous ethanol R*, evaporate to dryness at room temperature under an air-stream and record the spectrum again using the residue.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

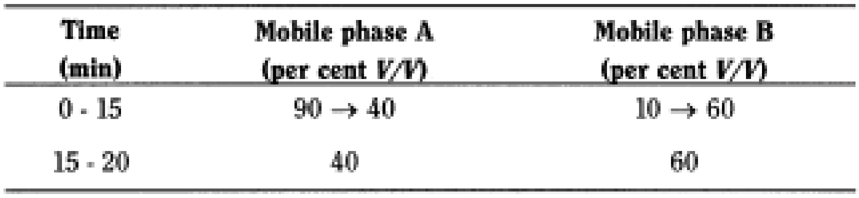
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) In order to prepare the *in situ* degradation compound (impurity D), dissolve 10 mg of the substance to be examined in 10.0 ml of *dilute hydrochloric acid R*. Heat on a water-bath under a reflux condenser for 4 h. Neutralise with 10.0 ml of *dilute sodium hydroxide solution R*. Evaporate to dryness on a water-bath. Cool and take up the residue in 10 ml of *methanol R*. Filter.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol R*.

Column:
- *size: l* = 0.1 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: 5 g/l solution of *ammonium carbonate R* in a mixture of 10 volumes of *tetrahydrofuran R* and 90 volumes of *water R*;
- *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 40	10 → 60
15 - 20	40	60

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Fentanyl = about 10 min; impurity D = about 12 min.

System suitability Reference solution (a):

- *resolution*: minimum 8.0 between the peaks due to fentanyl and impurity D; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient elution.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 50 °C.

ASSAY

Dissolve 0.200 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R* and titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 33.65 mg of $C_{22}H_{28}N_2O$.

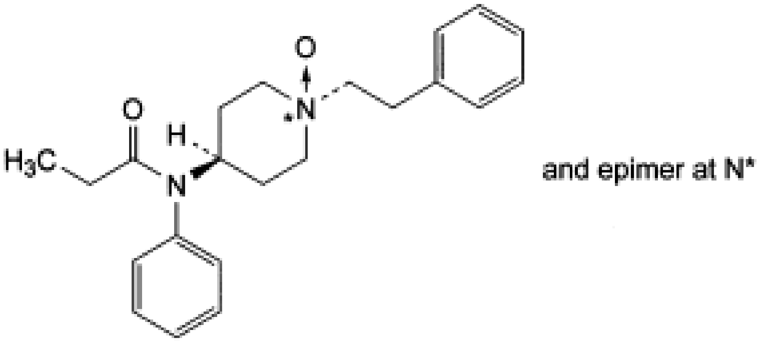
STORAGE

Protected from light.

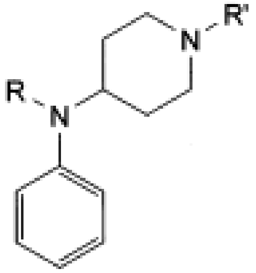
IMPURITIES

Specified impurities *A, B, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : E, F, G.*



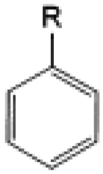
A. *N*-phenyl-*N*-[*cis*,*trans*-1-oxido-1-(2-phenylethyl)piperidin-4-yl]propanamide,



B. R = $CO-C_2H_5$, R′ = H: *N*-phenyl-*N*-(piperidin-4-yl)propanamide,

C. R = $CO-CH_3$, R′ = $CH_2-CH_2-C_6H_5$: *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]acetamide,

D. R = H, R′ = $CH_2-CH_2-C_6H_5$: *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine,



E. R = CHO: benzaldehyde,

F. R = NH_2: aniline (phenylamine),

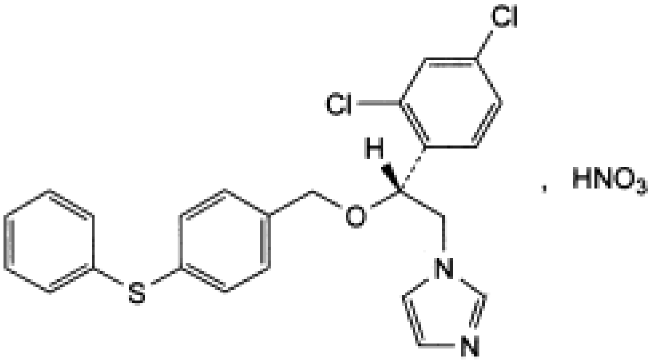
G. R = $NH-CO-C_2H_5$: *N*-phenylpropanamide.

Ph Eur

Fenticonazole Nitrate

General Notices

(Ph Eur monograph 1211)



$C_{24}H_{20}Cl_2N_2OS,HNO_3$ 518.4 *73151-29-8*

Action and use

Antifungal.

Ph Eur

DEFINITION

1-[(2*RS*)-2-(2,4-Dichlorophenyl)-2-[[4-(phenylsulphanyl)benzyl]oxy]ethyl]-1*H*-imidazole nitrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide and in methanol, sparingly soluble in anhydrous ethanol.

IDENTIFICATION

First identification C, D.

Second identification A, B, D.

A. Melting point *(2.2.14)*: 134 °C to 137 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 20.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *anhydrous ethanol R*.

Spectral range 230-350 nm.

Absorption maximum At 252 nm.

Shoulder At about 270 nm.

Absorption minimum At 236 nm.

Specific absorbance at the absorption maximum 260 to 280.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fenticonazole nitrate CRS* .

D. It gives the reaction of nitrates *(2.3.1)*.

TESTS

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 0.10 g in *methanol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b) Dilute 10.0 ml of reference solution (a) to 25.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (d) To 5 ml of the test solution add 5.0 mg of *fenticonazole impurity D CRS* , dissolve in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Mix 70 volumes of *acetonitrile R1* and 30 volumes of a phosphate buffer solution prepared by dissolving 3.4 g of *potassium dihydrogen phosphate R* in 900 ml of *water R*, adjusting to pH 3.0 with *phosphoric acid R* and diluting to 1000 ml with *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 229 nm.

Injection 10 µl.

Run time 5.5 times the retention time of fenticonazole.

System suitability:
- — *resolution*: minimum 2.0 between the peaks due to impurity D and fenticonazole in the chromatogram obtained with reference solution (d);
- — *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (c).

Limits:
- — *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- — *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- — *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peak due to the nitric ion (which corresponds to the dead volume of the column).

Toluene

Head-space gas chromatography *(2.2.28)*: use the standard additions method.

Test solution Disperse 0.2 g of the substance to be examined in a 10 ml vial with 5 ml of *water R*.

Reference solution Mix 4 mg of *toluene R* with *water R* and dilute to 1000 ml with the same solvent. Place 5 ml of this solution in a 10 ml vial.

Column:
- — *size: l* = 25 m, Ø = 0.32 mm;
- — *stationary phase*: *poly(cyanopropyl)(7)(phenyl)(7)(methyl)(86)siloxane R R* (film thickness 1.2 µm).

Carrier gas *helium for chromatography R.*

Split ratio 1:25.

Column head pressure 40 kPa.

Static head-space conditions which may be used:
- — *equilibration temperature:* 90 °C;
- — *equilibration time:* 1 h.

Temperature:
- — *column:* 80 °C;
- — *injection port:* 180 °C;
- — *detector:* 220 °C.

Detection Flame ionisation.

Injection 1 ml of the gaseous phase.

Limit:

— *toluene*: maximum 100 ppm.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.450 g in 50 ml of a mixture of equal volumes of *anhydrous acetic acid R* and *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

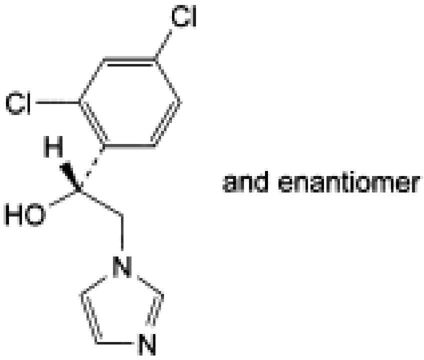
1 ml of *0.1 M perchloric acid* is equivalent to 51.84 mg of $C_{24}H_{21}Cl_2N_3O_4S$.

STORAGE

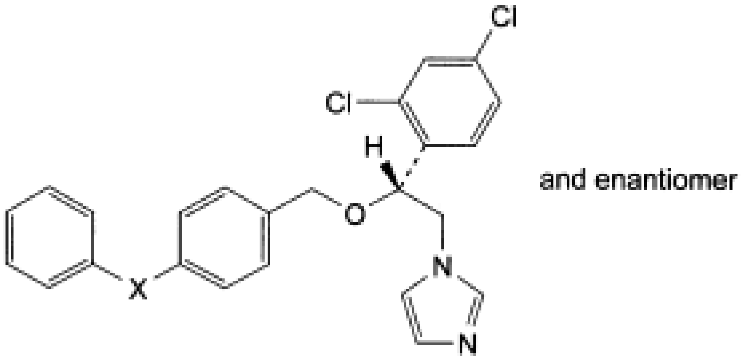
Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*



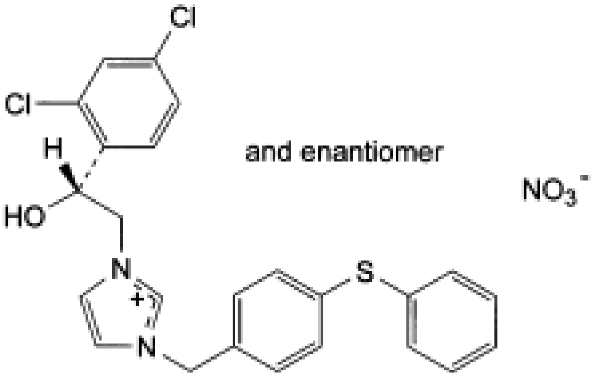
A. (*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,



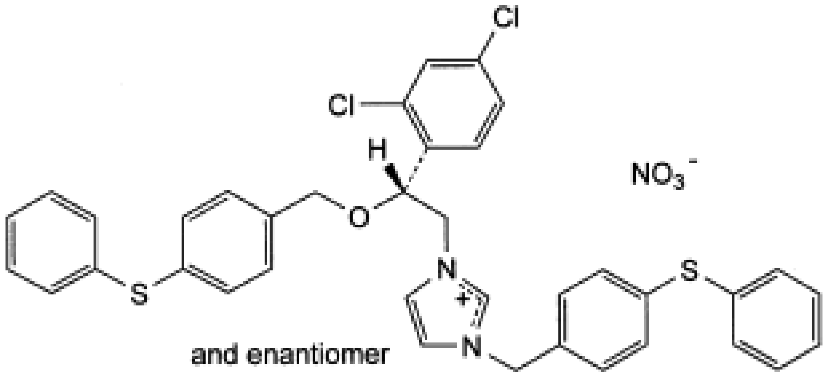
B. X = SO: 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[4-(phenylsulphinyl)benzyl]oxy]ethyl]-1*H*-imidazole,

C. X = SO_2: 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[4-(phenylsulphonyl)benzyl]oxy]ethyl]-1*H*-

imidazole,


and enantiomer

D. (*RS*)-1-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-3-[4-(phenylsulphanyl)benzyl]imidazolium nitrate,


and enantiomer

E. (*RS*)-1-[2-(2,4-dichlorophenyl)-2-[4-(phenylsulphanyl)benzyloxy]ethyl]-3-[4-(phenylsulphanyl)benzyl]imidazolium nitrate.

Ph Eur

Ferric Chloride Hexahydrate

General Notices

(Ph Eur monograph 1515)

$FeCl_3,6H_2O$ 270.3 *10025-77-1*

Ph Eur

DEFINITION

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

Crystalline mass or orange-yellow or brownish-yellow crystals, very hygroscopic.

Solubility

Very soluble in water and in ethanol (96 per cent), freely soluble in glycerol.

IDENTIFICATION

A. It gives reaction (a) of chlorides *(2.3.1)*.

B. It gives reaction (c) of iron *(2.3.1)*.

TESTS

Solution S

Dissolve 10 g in *distilled water R* and dilute to 100 ml with the same solvent.

Acidity

In a suitable polyethylene container, dissolve 3.0 g of *potassium fluoride R* in 15 ml of *water R*. Titrate with *0.1 M sodium hydroxide* using 0.1 ml of *phenolphthalein solution R* as indicator until a pink colour is obtained. Add 10 ml of solution S and allow to stand for 3 h. Filter and use 12.5 ml of the filtrate. Not more than 0.30 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Free chlorine

Heat 5 ml of solution S. The vapour does not turn *starch iodide paper R* blue.

Sulphates *(2.4.13)*

Maximum 100 ppm.

Heat 15 ml of solution S on a water-bath and add 5 ml of *strong sodium hydroxide solution R*. Allow to cool and filter. Neutralise the filtrate to *blue litmus paper R* using *hydrochloric acid R1* and evaporate to 15 ml.

Ferrous ions

Maximum 50 ppm.

To 10 ml of solution S, add 1 ml of *water R*, and 0.05 ml of *potassium ferricyanide solution R* followed by 4 ml of *phosphoric acid R*. After 10 min, any blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of *water R* and 1 ml of a freshly prepared 0.250 g/l solution of *ferrous sulphate R*.

Heavy metals *(2.4.8)*

Maximum 50 ppm.

Dissolve 1.0 g in 10 ml of *hydrochloric acid R1*. Add 2 ml of *strong hydrogen peroxide solution R*, then evaporate to 5 ml. Allow to cool and dilute to 20 ml with *hydrochloric acid R1* and transfer the solution to a separating funnel. Shake 3 times, for 3 min each time, with 20 ml of *methyl isobutyl ketone R1*. Separate the lower phase, reduce to half its volume by evaporation and dilute to 25 ml with *water R*. Neutralise 10 ml of the solution with *dilute ammonia R1* to *red litmus paper R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

In a conical flask with a ground-glass stopper, dissolve 0.200 g in 20 ml of *water R*. Add 10 ml of *dilute hydrochloric acid R* and 2 g of *potassium iodide R*. Allow the stoppered flask to stand for 1 h protected from light. Titrate with *0.1 M sodium thiosulphate*, adding 5 ml of *starch solution R* towards the end of the titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 27.03 mg of $FeCl_3,6H_2O$.

STORAGE

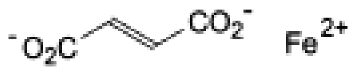
In an airtight container, protected from light.

Ph Eur

Ferrous Fumarate

General Notices

(Ph Eur monograph 0902)



$C_4H_2FeO_4$ 169.9 *141-01-5*

Action and use

Used in prevention and treatment of anaemias.

Preparations

Ferrous Fumarate Capsules

Ferrous Fumarate Oral Suspension

Ferrous Fumarate Tablets

Ferrous Fumarate and Folic Acid Tablets

Ferrous Fumarate contains in 200 mg about 65 mg of iron.

Ph Eur

DEFINITION

Iron(II) (*E*)-butenedioate.

Content

93.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Fine, reddish-orange or reddish-brown powder.

Solubility

Slightly soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g add 25 ml of a mixture of equal volumes of *hydrochloric acid R* and *water R* and heat on a water-bath for 15 min. Cool and filter. Use the filtrate for identification test C. Wash the residue with 50 ml of a mixture of 1 volume of *dilute hydrochloric acid R* and 9 volumes of *water R* and discard the washings. Dry the residue at 100-105 °C. Dissolve 20 mg of the residue in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *fumaric acid CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *anhydrous formic acid R*, *methylene chloride R*, *butanol R*, *heptane R* (12:16:32:44 *V/V/V/V*).

Application 5 µl.

Development In an unsaturated tank, over a path of 10 cm.

Drying At 105 °C for 15 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

B. Mix 0.5 g with 1 g of *resorcinol R*. To 0.5 g of the mixture in a crucible add 0.15 ml of *sulphuric acid R* and heat gently. A dark red semi-solid mass is formed. Add the mass, with care, to 100 ml of *water R*. An orange-yellow colour develops and the solution shows no fluorescence.

C. The filtrate obtained during preparation of the test solution in identification test A gives reaction (a) of iron *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in a mixture of 10 ml of *lead-free hydrochloric acid R* and 80 ml of *water R*, heating slightly if necessary. Allow to cool, filter if necessary and dilute to 100 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 0.2 per cent.

Heat 0.15 g with 8 ml of *dilute hydrochloric acid R* and 20 ml of *distilled water R*. Cool in iced water, filter and dilute to 30 ml with *distilled water R*.

Arsenic *(2.4.2, Method A)*

Maximum 5 ppm.

Mix 1.0 g with 15 ml of *water R* and 15 ml of *sulphuric acid R*. Warm to precipitate the fumaric acid completely. Cool and add 30 ml of *water R*. Filter. Wash the precipitate with *water R*. Dilute the combined filtrate and washings to 125 ml with *water R*. 25 ml of the solution complies with the test.

Ferric ion

Maximum 2.0 per cent.

In a flask with a ground-glass stopper, dissolve 3.0 g in a mixture of 10 ml of *hydrochloric acid R* and 100 ml of *water R* by heating rapidly to boiling. Boil for 15 s. Cool rapidly, add 3 g of *potassium iodide R,* stopper the flask and allow to stand protected from light for 15 min. Add 2 ml of *starch solution R* as indicator. Titrate the liberated iodine with *0.1 M sodium thiosulphate*. Carry out a blank test. The difference between the volumes used in the 2 titrations corresponds to the amount of iodine liberated by ferric ion.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 5.585 mg of ferric ion.

Cadmium

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R* and diluting with a 10 per cent *V/V* solution of *lead-free hydrochloric acid R*.

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Air-acetylene flame.

Chromium

Maximum 2.00×10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *chromium standard solution (0.1 per cent Cr) R* and diluting with a 10 per cent *V/V* solution of *lead-free hydrochloric acid R*.

Source Chromium hollow-cathode lamp.

Wavelength 357.9 nm.

Atomisation device Air-acetylene flame.

Lead

Maximum 20.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *lead standard solution (10 ppm Pb) R* and diluting with a 10 per cent *V/V* solution of *lead-free hydrochloric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm.

Atomisation device Air-acetylene flame.

Mercury

Maximum 1.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *mercury standard solution (10 ppm Hg) R* and diluting with a 25 per cent *V/V* solution of *lead-free hydrochloric acid R*.

Source Mercury hollow-cathode lamp.

Wavelength 253.7 nm.

Following the recommendations of the manufacturer, introduce 5 ml of solution S or 5 ml of the reference solutions into the reaction vessel of the cold-vapour mercury assay accessory, add 10 ml of *water R* and 1 ml of *stannous chloride solution R1*.

Nickel

Maximum 2.00×10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *nickel standard solution (10 ppm Ni) R* and diluting with a 10 per cent *V/V* solution of *lead-free hydrochloric acid R.*

Source Nickel hollow-cathode lamp.

Wavelength 232 nm.

Atomisation device Air-acetylene flame.

Zinc

Maximum 5.00×10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Solution S diluted to 10 volumes.

Reference solutions Prepare the reference solutions using *zinc standard solution (10 ppm Zn) R* and diluting with a 1 per cent *V/V* solution of *lead-free hydrochloric acid R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve with slight heating 0.150 g in 7.5 ml of *dilute sulphuric acid R*. Cool and add 25 ml of *water R*. Add 0.1 ml of *ferroin R*. Titrate immediately with *0.1 M cerium sulphate* until the colour changes from orange to light bluish-green.

1 ml of *0.1 M cerium sulphate* is equivalent to 16.99 mg of $C_4H_2FeO_4$.

STORAGE

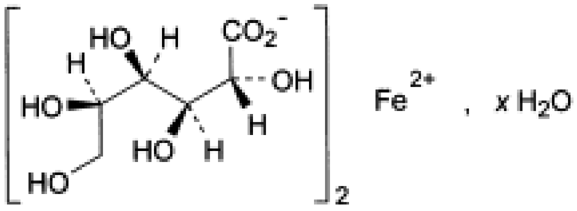
In an airtight container, protected from light.

Ph Eur

Ferrous Gluconate

General Notices

(Ph Eur monograph 0493)



$C_{12}H_{22}FeO_{14},xH_2O$ 446.1 *299-29-6*
(anhydrous) *(anhydrous)*

Action and use

Used in prevention and treatment of iron deficiency.

Preparation

Ferrous Gluconate Tablets

Ferrous Gluconate contains in 600 mg about 70 mg of iron.

Ph Eur

DEFINITION

Iron(II) di(D-gluconate).

Content

11.8 per cent to 12.5 per cent of iron(II) (dried substance). It contains a variable amount of water.

CHARACTERS

Appearance

Greenish-yellow or grey powder or granules.

Solubility

Freely but slowly soluble in water giving a greenish-brown solution, more readily soluble in hot water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in 2 ml of *water R*, heating if necessary in a water-bath at 60 °C.

Reference solution Dissolve 20 mg of *ferrous gluconate CRS* in 2 ml of *water R*, heating if necessary in a water-bath at 60 °C.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R*, *ethyl acetate R*, *water R*, *ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying At 100-105 °C for 20 min.

Detection Allow to cool and spray with a 50 g/l solution of *potassium dichromate R* in a 40 per cent *m/m* solution of *sulphuric acid R*.

Results After 5 min, the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. 1 ml of solution S (see Tests) gives reaction (a) of iron *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and heated to about 60 °C, allow to cool and dilute to 50 ml with *carbon dioxide-free water R* prepared from *distilled water R*.

Appearance of solution

The solution is clear *(2.2.1)*.

Dilute 2 ml of solution S to 10 ml with *water R*. Examine the solution against the light.

pH *(2.2.3)*

4.0 to 5.5 for solution S, measured 3-4 h after preparation.

Sucrose and reducing sugars

Dissolve 0.5 g in 10 ml of warm *water R* and add 1 ml of *dilute ammonia R1*. Pass *hydrogen sulphide R* through the solution and allow to stand for 30 min. Filter and wash the precipitate with 2 quantities, each of 5 ml, of *water R*. Acidify the combined filtrate and washings to *blue litmus paper R* with *dilute hydrochloric acid R* and add 2 ml in excess. Boil until the vapour no longer darkens *lead acetate paper R* and continue boiling, if necessary, until the volume is reduced to about 10 ml. Cool, add 15 ml of *sodium carbonate solution R*, allow to stand for 5 min and filter. Dilute the filtrate to 100 ml with *water R*. To 5 ml of this solution add 2 ml of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 1 min. No red precipitate is formed.

Chlorides ***(2.4.4)***

Maximum 0.06 per cent.

Dilute 0.8 ml of solution S to 15 ml with *water R*.

Oxalates

Dissolve 5.0 g in a mixture of 10 ml of *dilute sulphuric acid R* and 40 ml of *water R*. Shake the solution with 50 ml of *ether R* for 5 min. Separate the aqueous layer and shake it with 20 ml of *ether R* for 5 min. Combine the ether layers, evaporate to dryness and dissolve the residue in 15 ml of *water R*. Filter, boil the filtrate until the volume is reduced to 5 ml and add 1 ml of *dilute acetic acid R* and 1.5 ml of *calcium chloride solution R*. Allow to stand for 30 min. No precipitate is formed.

Sulphates ***(2.4.13)***

Maximum 500 ppm.

To 3.0 ml of solution S add 3 ml of *acetic acid R* and dilute to 15 ml with *distilled water R*. Examine the solutions against the light.

Arsenic ***(2.4.2, Method A)***

Maximum 2 ppm, determined on 0.5 g.

Barium

Dilute 10 ml of solution S to 50 ml with *distilled water R* and add 5 ml of *dilute sulphuric acid R*. Allow to stand for 5 min. Any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution S and 45 ml of *distilled water R*.

Ferric ion

Maximum 1.0 per cent.

In a ground-glass-stoppered flask, dissolve 5.00 g in a mixture of 10 ml of *hydrochloric acid R* and 100 ml of *carbon dioxide-free water R*. Add 3 g of *potassium iodide R*, close the flask and allow to stand protected from light for 5 min. Titrate with *0.1 M sodium thiosulphate*, using 0.5 ml of *starch solution R,* added towards the end of the titration, as indicator. Carry out a blank titration. Not more than 9.0 ml of *0.1 M sodium thiosulphate* is used.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

Thoroughly mix 2.5 g with 0.5 g of *magnesium oxide R1* in a silica crucible. Ignite to dull redness until a homogeneous mass is obtained. Heat at 800 ± 50 °C for about 1 h, allow to cool and take up the residue in 20 ml of hot *hydrochloric acid R*. Allow to cool. Transfer the liquid to a separating funnel and shake for 3 min with 3 quantities, each of 20 ml, of methyl isobutyl ketone saturated with hydrochloric acid (prepared by shaking 100 ml of freshly distilled *methyl isobutyl ketone R* with 1 ml of *hydrochloric acid R*). Allow to stand, separate the aqueous layer, reduce to half its volume by boiling, allow to cool and dilute to 25 ml with *water R*. Neutralise 10 ml of this solution to *red litmus paper R* using dilute *ammonia R1* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

5.0 per cent to 10.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 5 h.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count.

ASSAY

Dissolve 0.5 g of *sodium hydrogen carbonate R* in a mixture of 30 ml of *dilute sulphuric acid R* and 70 ml of *water R*. When the effervescence stops, dissolve 1.00 g of the substance to be examined with gentle shaking. Using 0.1 ml of *ferroin R* as indicator, titrate with *0.1 M ammonium and cerium nitrate* until the red colour disappears.

1 ml of *0.1 M ammonium and cerium nitrate* is equivalent to 5.585 mg of iron(II).

STORAGE

Protected from light.

Ph Eur

Ferrous Sulphate Heptahydrate

General Notices

(Ph Eur monograph 0083)

$FeSO_4,7H_2O$ 278.0 *7720-78-7*

Action and use

Used in prevention and treatment of anaemias.

Preparation

Paediatric Ferrous Sulphate Oral Solution

Ferrous Sulphate contains in 300 mg about 60 mg of iron.

Ph Eur

DEFINITION

Content

98.0 per cent to 105.0 per cent.

CHARACTERS

Appearance

Light green, crystalline powder or bluish-green crystals, efflorescent in air.

Solubility

Freely soluble in water, very soluble in boiling water, practically insoluble in alcohol.

Ferrous sulphate is oxidised in moist air, becoming brown.

IDENTIFICATION

A. It gives the reaction of sulphates *(2.3.1)*.

B. It gives reaction (a) of iron *(2.3.1)*.

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R*, add 0.5 ml of *dilute sulphuric acid R* and dilute to 50 ml with *water R.*

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)*.

pH ***(2.2.3)***

3.0 to 4.0.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Chlorides ***(2.4.4)***

Maximum 300 ppm.

Dilute 3.3 ml of solution S to 10 ml with *water R* and add 5 ml of *dilute nitric acid R*. The solution complies with the limit test for chlorides. Prepare the standard using a mixture of 10 ml of *chloride standard solution (5 ppm Cl) R* and 5 ml of *dilute nitric acid R*. Use 0.15 ml of *silver nitrate solution R2* in this test.

Ferric ions

Maximum 0.5 per cent.

In a ground-glass-stoppered flask, dissolve 5.00 g in a mixture of 10 ml of *hydrochloric acid R* and 100 ml of *carbon dioxide-free water R*. Add 3 g of *potassium iodide R*, close the flask and allow to stand in the dark for 5 min. Titrate the liberated iodine with *0.1 M sodium thiosulphate*, using 0.5 ml of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank test in the same conditions. Not more than 4.5 ml of *0.1 M sodium thiosulphate* is used, taking into account the blank titration.

Manganese

Maximum 0.1 per cent.

Dissolve 1.0 g in 40 ml of *water R*, add 10 ml of *nitric acid R* and boil until red fumes are evolved. Add 0.5 g of *ammonium persulphate R* and boil for 10 min. Discharge any pink colour by adding dropwise a 50 g/l solution of *sodium sulphite R* and boil until any odour of sulphur dioxide is eliminated. Add 10 ml of *water R*, 5 ml of *phosphoric acid R* and 0.5 g of *sodium periodate R*, boil for 1 min and allow to cool. The solution is not more intensely coloured than a standard prepared at the same time in the same manner using 1.0 ml of *0.02 M potassium permanganate* and adding the same volumes of the same reagents.

Zinc

Maximum 500 ppm.

To 5 ml of solution A obtained in the test for heavy metals add 1 ml of *potassium ferrocyanide solution R* and dilute to 13 ml with *water R*. After 5 min, any turbidity produced is not more intense than that of a standard prepared at the same time by mixing 10 ml of *zinc standard solution (10 ppm Zn) R*, 2 ml of *hydrochloric acid R1* and 1 ml of *potassium ferrocyanide solution R*.

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

Dissolve 1.0 g in 10 ml of *hydrochloric acid R1*, add 2 ml of *strong hydrogen peroxide solution R* and boil until the volume is reduced to 5 ml. Allow to cool, dilute to 20 ml with *hydrochloric acid R1*, transfer the solution to a separating funnel and shake for 3 min with 3 quantities, each of 20 ml, of methyl isobutyl ketone saturated with hydrochloric acid (prepared by shaking

100 ml of freshly distilled *methyl isobutyl ketone R* with 1 ml of *hydrochloric acid R1*). Allow to stand, separate the aqueous layer and reduce to half its volume by boiling, allow to cool and dilute to 25 ml with *water R* (solution A). Neutralise 10 ml of solution A to *litmus paper R* using *dilute ammonia R1* and dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 2.5 g of *sodium hydrogen carbonate R* in a mixture of 150 ml of *water R* and 10 ml of *sulphuric acid R*. When the effervescence ceases add to the solution 0.500 g of the substance to be examined and dissolve with gentle shaking. Add *0.1 ml of ferroin R* and titrate with *0.1 M ammonium and cerium nitrate* until the red colour disappears.

1 ml of *0.1 M ammonium and cerium nitrate* is equivalent to 27.80 mg of $FeSO_4,7H_2O$.

STORAGE

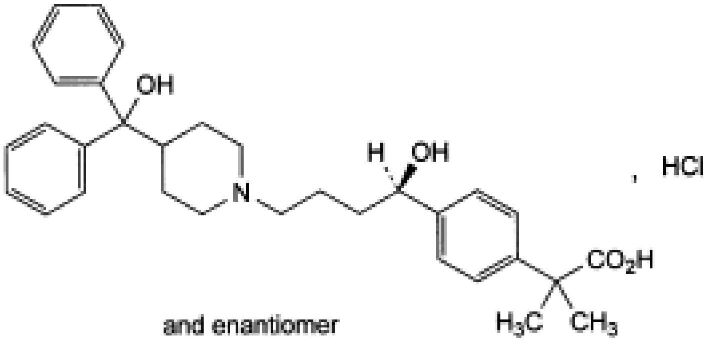
In an airtight container.

Ph Eur

Fexofenadine Hydrochloride

General Notices

(Ph Eur monograph 2280)



$C_{32}H_{39}NO_4,HCl$ 538.1 *153439-40-8*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

2-[4-[(1*RS*)-1-hydroxy-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butyl]phenyl]-2-methylpropanoic acid hydrochloride.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in methanol, very slightly soluble in acetone.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fexofenadine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Dissolve 30 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R*; sonicate if necessary and dilute to 2 ml with the same mixture of solvents. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Impurity B

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve the contents of a vial of *fexofenadine impurity B CRS* in the test solution and dilute to 2.0 ml with the test solution.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: silica gel BC for chiral chromatography R1* (5 µm).

Mobile phase Mix 20 volumes of *acetonitrile for chromatography R* and 80 volumes of a buffer solution prepared as follows: to 1.15 ml of *glacial acetic acid R* add *water for chromatography R*, adjust to pH 4.0 ± 0.1 with *dilute ammonia R1* and dilute to 1000 ml with *water for chromatography R*.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 1.2 times the retention time of fexofenadine.

Relative retention With reference to fexofenadine (retention time = about 20 min):impurity B = about 0.7.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to fexofenadine and impurity B.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 1.3;

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Buffer solution Dissolve 6.64 g of *sodium dihydrogen phosphate monohydrate R* and 0.84 g of *sodium perchlorate R* in *water for chromatography R*, adjust to pH 2.0 ± 0.1 with *phosphoric acid R* and dilute to 1000 ml with *water for chromatography R*.

Solvent mixture Mix equal volumes of *acetonitrile for chromatography R* and the buffer solution.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 25.0 ml of the solvent mixture.

Test solution (b) Dilute 3.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *fexofenadine hydrochloride CRS* in the solvent mixture and dilute to 25.0 ml with the solvent mixture. Dilute 3.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 1 mg each of *fexofenadine impurity A CRS* and *fexofenadine impurity C CRS* in 20 ml of reference solution (a) and dilute to 200.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: phenylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 350 volumes of *acetonitrile for chromatography R* and 650 volumes of the buffer solution; add 3 volumes of *triethylamine R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (a) and reference solutions (b) and (c).

Relative retention With reference to fexofenadine (retention time = about 9 min): impurity A = about 1.7; impurity D = about 2.3; impurity C = about 3.2.

Run time 6 times the retention time of fexofenadine for test solution (a) and reference solution (c), twice the retention time of fexofenadine for reference solution (b).

System suitability Reference solution (c):

— *resolution*: minimum 10 between the peaks due to fexofenadine and impurity A.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.4;

— *impurities A, C, D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 1.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using 5 ml of *lead standard solution (1 ppm Pb) R*.

Water ***(2.5.32)***

Maximum 0.5 per cent.

Dissolve 1.000 g in *anhydrous methanol R* and dilute to 5.0 ml with the same solvent. Use 1.0 ml of this solution.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

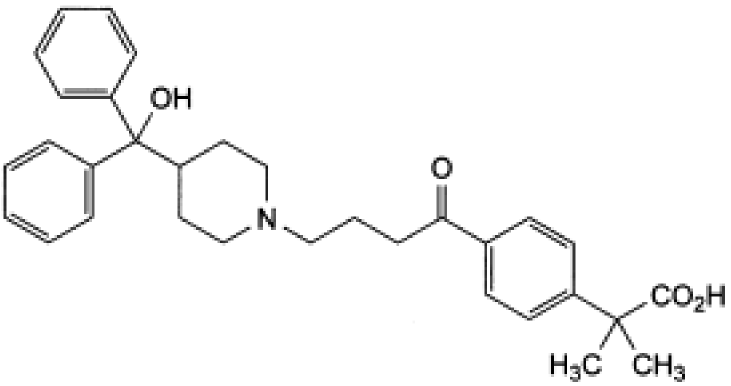
Run time Twice the retention time of fexofenadine.

Calculate the percentage content of fexofenadine hydrochloride from the declared content of *fexofenadine hydrochloride CRS*.

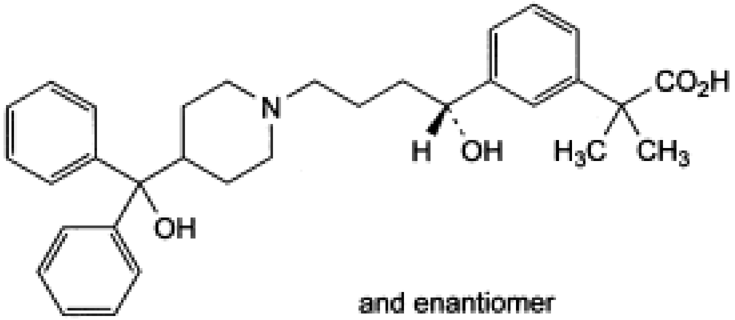
IMPURITIES

Specified impurities A, B, C, D.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *E, F, G*.

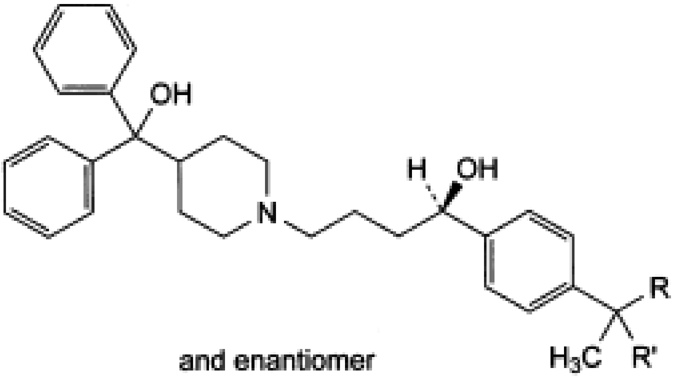


A. 2-[4-[4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butanoyl]phenyl]-2-methylpropanoic acid,



CO₂H
N
H OH
H₃C CH₃
OH
and enantiomer

B. 2-[3-[(1*RS*)-1-hydroxy-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butyl]phenyl]-2-methylpropanoic acid,

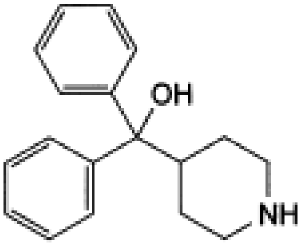


OH
N
H OH
R
and enantiomer
H₃C R'

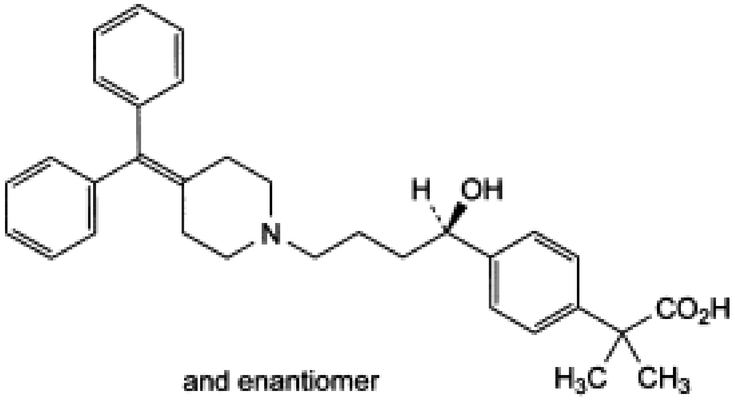
C. R = H, R′ = CH_3: (1*RS*)-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]-1-[4-(1-methylethyl) phenyl]butan-1-ol,

D. R = CO-OCH_3, R′ = CH_3: methyl 2-[4-[(1*RS*)-1-hydroxy-4-[4-(hydroxydiphenylmethyl) piperidin-1-yl]butyl]phenyl]-2-methylpropanoate,

F. R = CO_2H, R′ = H: 2-[4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butyl] phenyl]propanoic acid,



E. diphenyl(piperidin-4-yl)methanol,


H
OH
N
CO2H
H3C
CH3
and enantiomer

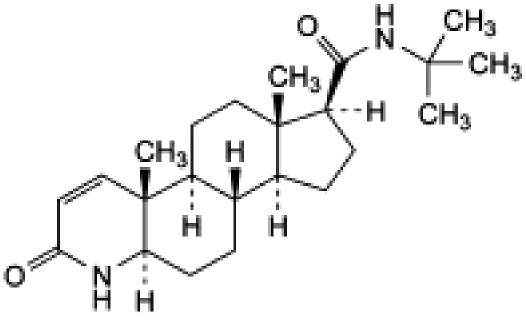
G. 2-[4-[(1*RS*)-4-[4-(diphenylmethylidene)piperidin-1-yl]-1-hydroxybutyl]phenyl]-2-methylpropanoic acid.

Ph Eur

Finasteride

General Notices

(Ph Eur monograph 1615)



$C_{23}H_{36}N_2O_2$ 372.6 *98319-26-7*

Action and use

5-Alpha reductase inhibitor; treatment of benign prostatic hyperplasia.

Preparation

Finasteride Tablets

Ph Eur

DEFINITION

N-(1,1-Dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *finasteride CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation *(2.2.7)*

+ 12.0 to + 14.0 (dried substance).

Dissolve 0.250 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 50.0 ml with the same mixture of solvents.

Test solution (b) Dissolve 0.100 g of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 25.0 mg of *finasteride CRS* in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (b) Dissolve 50.0 mg of *finasteride for system suitability CRS* in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 5.0 ml with the same mixture of solvents.

Reference solution (c) Dilute 2.0 ml of test solution (b) to 100.0 ml in a mixture of equal volumes of *acetonitrile R* and *water R*. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column:

— *size:* l = 0.25 m, Ø = 4.0 mm,

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a ratio of specific surface area (m^2g^{-1})/carbon-percentage less than 20,

— *temperature*: 60 °C.

Mobile phase *acetonitrile R*, *tetrahydrofuran R*, *water R* (10:10:80 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 15 µl; inject test solution (b) and reference solutions (b) and (c).

Run time Twice the retention time of finasteride.

Relative retention With reference to finasteride (retention time = about 28 min): impurity A = about 0.94; impurity B = about 1.22; impurity C = about 1.36.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity A, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to finasteride.

Limits:

- — *impurity A*: maximum 0.3 per cent, calculated from the area of the corresponding peak in the chromatogram obtained with reference solution (b) and taking into account the assigned value of impurity A in *finasteride for system suitability CRS*;
- — *impurity B*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- — *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- — *any other impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- — *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent);
- — *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

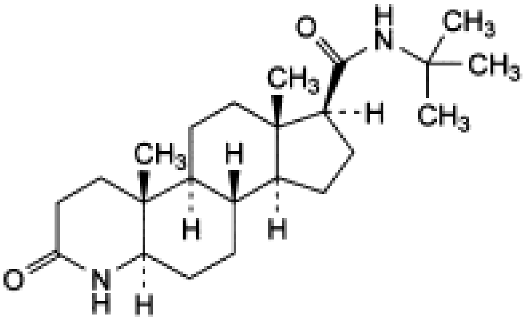
Injection Test solution (a) and reference solution (a).

Calculate the percentage content of $C_{23}H_{36}N_2O_2$.

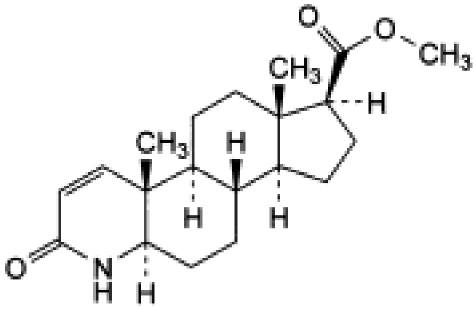
STORAGE

Protected from light.

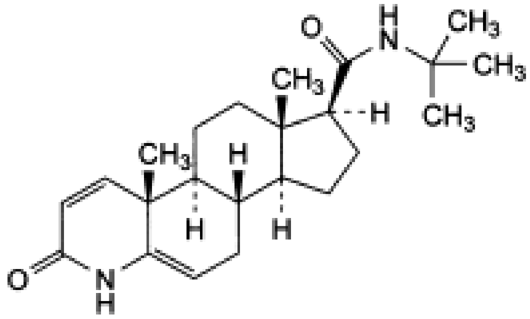
IMPURITIES



A. *N*-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androstane-17β-carboxamide (dihydrofinasteride),



B. methyl 3-oxo-4-aza-5α-androst-1-ene-17β-carboxylate (Δ-1-aza ester),



C. *N*-(1,1-dimethylethyl)-3-oxo-4-azaandrosta-1,5-diene-17β-carboxamide (Δ-1,5-aza amide)
.

Ph Eur

Fish Oil, Rich in Omega-3-Acids

General Notices

(Ph Eur monograph 1912)

Ph Eur

DEFINITION

Purified, winterised and deodorised fatty oil obtained from fish of the families *Engraulidae*, *Carangidae*, *Clupeidae*, *Osmeridae*, *Scombridae* and *Ammodytidae*. The omega-3 acids are defined as the following acids: *alpha*-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA).

Content:

— *EPA, expressed as triglycerides:* minimum 13.0 per cent,

— *DHA, expressed as triglycerides*: minimum 9.0 per cent,

— *total omega-3 acids, expressed as triglycerides*: minimum 28.0 per cent.

Authorised antioxidants in concentrations not exceeding the levels specified by the competent authorities may be added.

CHARACTERS

Appearance

Pale yellow liquid.

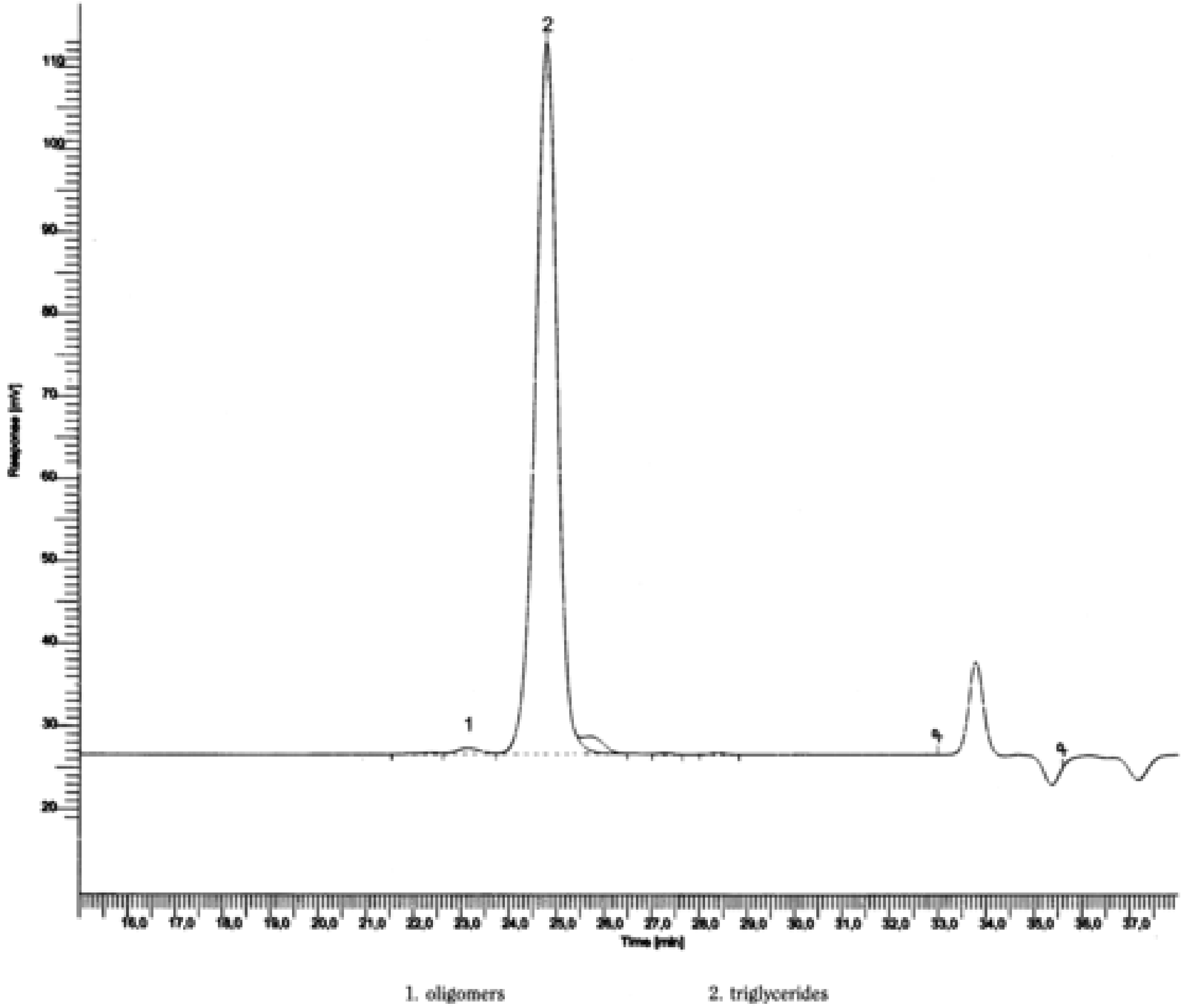


Figure 1912.-1. – *Chromatogram of the test for oligomers in fish oil rich in omega-3 acids*

Solubility

Practically insoluble in water, very soluble in acetone and in heptane, slightly soluble in anhydrous ethanol.

IDENTIFICATION

Examine the chromatograms obtained in the assay for EPA and DHA.

Results The peaks due to eicosapentaenoic acid methyl ester and to docosahexaenoic acid methyl ester in the chromatogram obtained with test solution (b) are similar in retention time to the corresponding peaks in the chromatogram obtained with reference solution (a).

TESTS

Appearance

The substance to be examined is not more intensely coloured than a reference solution prepared as follows: to 3.0 ml of red primary solution add 25.0 ml of yellow primary solution and dilute to 50.0 ml with a 10 g/l solution of *hydrochloric acid R* (*2.2.2, Method II)*.

Absorbance ***(2.2.25)***

Maximum 0.70 at 233 nm.

Dilute 0.300 g of the substance to be examined to 50.0 ml with *trimethylpentane R*. Dilute 2.0 ml of this solution to 50.0 ml with *trimethylpentane R*.

Acid value ***(2.5.1)***

Maximum 0.5, determined on 20.0 g in 50 ml of the prescribed mixture of solvents.

Anisidine value

Maximum 30.0.

The anisidine value is defined as 100 times the absorbance measured in a 1 cm cell filled with a solution containing 1 g of the substance to be examined in 100 ml of a mixture of solvents and reagents according to the method described below.

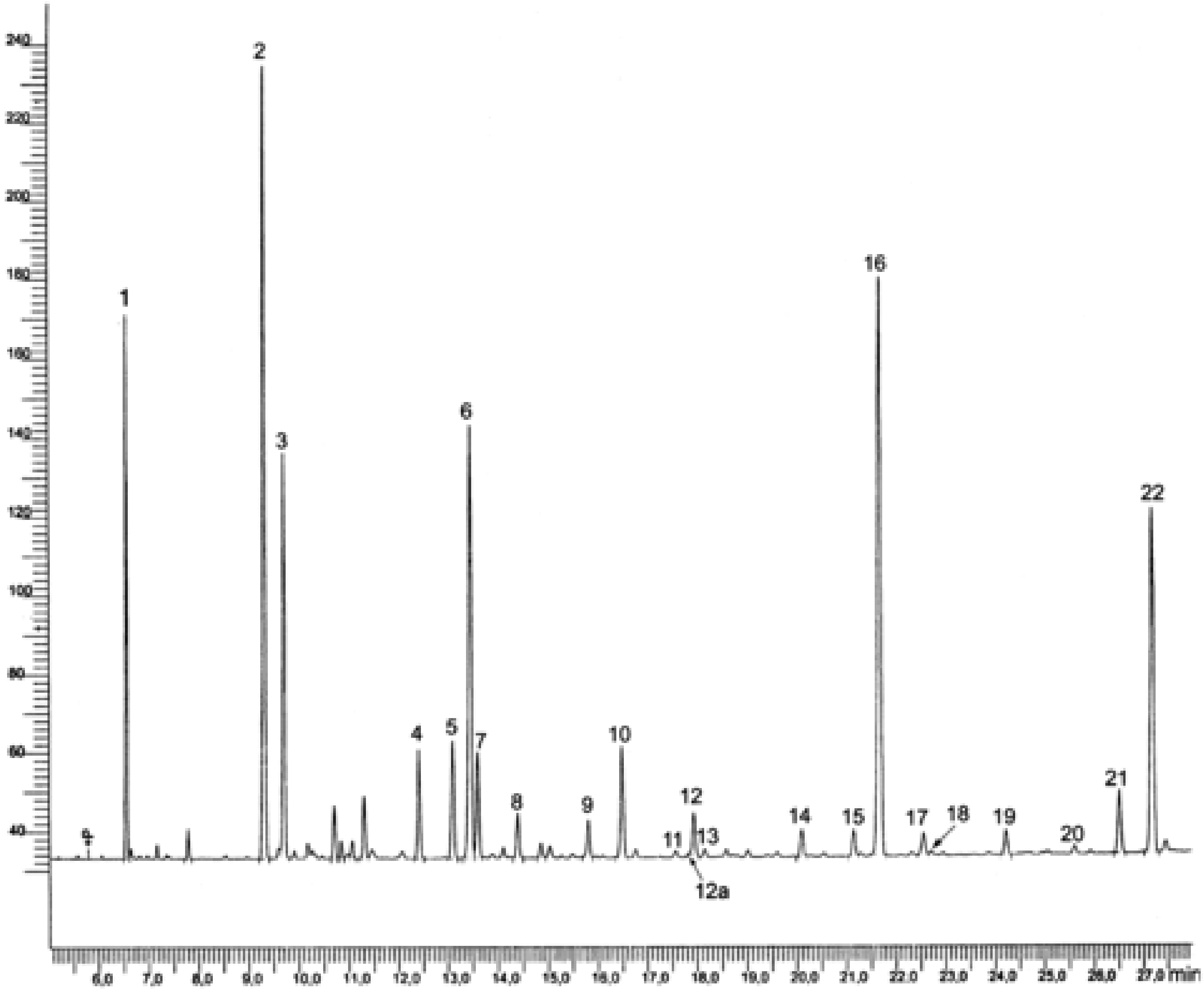
Carry out the operations as rapidly as possible, avoiding exposure to actinic light.

Test solution (a) Dilute 0.500 g of the substance to be examined to 25.0 ml with *trimethylpentane R*.

Test solution (b) To 5.0 ml of test solution (a) add 1.0 ml of a 2.5 g/l solution of *p-anisidine R* in *glacial acetic acid R*, shake and store protected from light.

Reference solution To 5.0 ml of *trimethylpentane R* add 1.0 ml of a 2.5 g/l solution of *p-anisidine R* in *glacial acetic acid R,* shake and store protected from light.

Measure the absorbance *(2.2.25)* of test solution (a) at 350 nm using *trimethylpentane R* as the compensation liquid. Measure the absorbance of test solution (b) at 350 nm exactly 10 min after its preparation, using the reference solution as the compensation liquid.



1. C14:0
2. C16:0
3. C16:1 n-7
4. C16:4 n-1
5. C18:0
6. C18:1 n-9
7. C18:1 n-7
8. C18:2 n-6
9. C18:3 n-3
10. C18:4 n-3
11. C20:0
12. C20:1 n-9

12a. C20:1 n-11

13. C20:1 n-7
14. C20:4 n-6
15. C20:4 n-3
16. C20:5 n-3
17. C22:1 n-11
18. C22:1 n-9
19. C21:5 n-3
20. C22:5 n-6
21. C22:5 n-3
22. C22:6 n-3

Figure 1912.-2. – *Chromatogram for the assay of total omega-3 acids in fish oil rich in omega-3 acids*

Calculate the anisidine value from the expression:

$$\frac{25 \times (1.2\,A_s - A_b)}{m}$$

A_s = absorbance of test solution (b),

A_b = absorbance of test solution (a),

m = mass of the substance to be examined in test solution (a), in grams.

Peroxide value ***(2.5.5, Method A)***

Maximum 10.0.

Unsaponifiable matter ***(2.5.7)***

Maximum 1.5 per cent, determined on 5.0 g.

Stearin

10 ml remains clear after cooling at 0 °C for 3 h.

Oligomers

Size-exclusion chromatography *(2.2.30)*.

Test solution Dilute 10.0 mg of the substance to be examined to 10.0 ml with *tetrahydrofuran R*.

Reference solution In a 100 ml volumetric flask dissolve 50 mg of *monodocosahexaenoin R*, 30 mg of *didocosahexaenoin R* and 20 mg of *tridocosahexaenoin R* in *tetrahydrofuran R* and dilute to 100.0 ml with the same solvent.

Column 1:

— *size:* l = 0.3 m, Ø = 7.8 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 10 nm.

Columns 2 and 3, placed closest to the injector:

— *size:* l = 0.3 m, Ø = 7.8 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 50 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 0.8 ml/min.

Detection Differential refractometer.

Injection 40 µl.

System suitability Reference solution:

— *elution order*: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin.

— *resolution*: minimum of 2.0 between the peaks due to monodocosahexaenoin and to didocosahexaenoin and minimum of 1.0 between the peaks due to didocosahexaenoin and to tridocosahexaenoin.

Identify the peaks from the chromatogram (Figure 1912.-1). Calculate the percentage content of oligomers using the following expression:

$$\frac{B}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram, *B* = area of the peak with a retention time less than the retention time of the triglyceride peak.

Limit:

— *oligomers*: maximum 1.5 per cent.

ASSAY

EPA and DHA *(2.4.29)*

See Figure 1912.-2.

Total omega-3 acids *(2.4.29)*

See Figure 1912.-2.

STORAGE

In an airtight, well-filled container, protected from light, under inert gas.

LABELLING

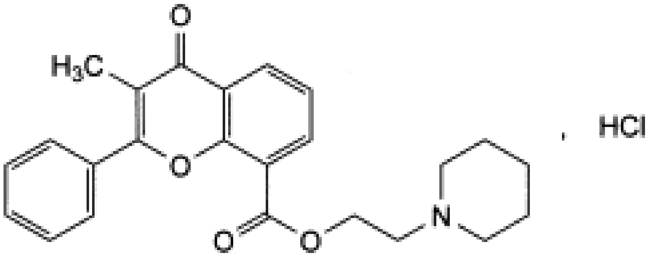
The label states the concentration of EPA, DHA and total omega-3 acids, expressed as triglycerides.

Ph Eur

Flavoxate Hydrochloride

General Notices

(Ph Eur monograph 1692)



$C_{24}H_{25}NO_4,HCl$ 427.9 *3717-88-2*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

2-(Piperidin-1-yl)ethyl 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylate hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison flavoxate hydrochloride CRS.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Use freshly prepared solutions*.

Solvent mixture Mix 20 volumes of a 0.4 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R* and 80 volumes of *acetonitrile R*.

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

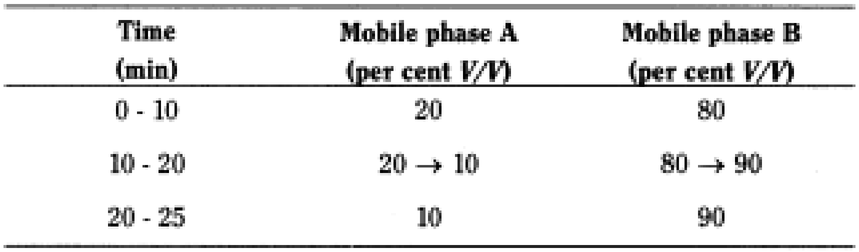
Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 6.0 mg of *flavoxate impurity A CRS* and 3.0 mg of *flavoxate impurity B CRS* in the solvent mixture, add 2.0 ml of the test solution and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: spherical end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: 0.435 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 7.5 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	20	80
10 - 20	20 → 10	80 → 90
20 - 25	10	90

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Relative retention With reference to flavoxate (retention time = about 10 min)*:* impurity A = about 0.2; impurity B = about 0.8.

System suitability Reference solution (c):
- *resolution*: minimum 4.0 between the peaks due to impurity B and flavoxate.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total of unspecified impurities*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.350 g in 10 ml of *anhydrous formic acid R* and add 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 42.79 mg of $C_{24}H_{26}ClNO_4$.

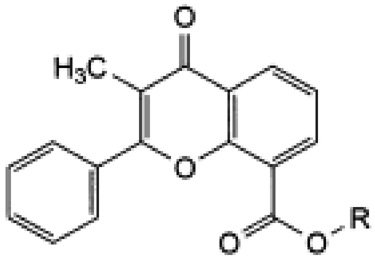
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C*.



A. R = H: 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylic acid,

B. R = C_2H_5: ethyl 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylate,

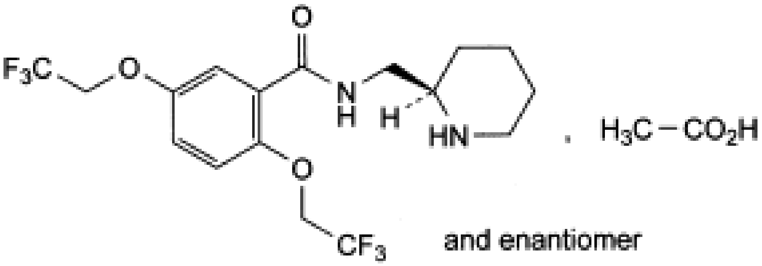
C. R = $CH(CH_3)_2$: 1-methylethyl 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylate.

Ph Eur

Flecainide Acetate

General Notices

(Ph Eur monograph 1324)



$C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ 474.4 *54143-56-5*

Action and use

Class I antiarrhythmic.

Preparations

Flecainide Injection

Flecainide Tablets

Ph Eur

DEFINITION

N-[(*RS*)-(Piperidin-2-ylmethyl)]-2,5-bis(2,2,2-trifluoroethoxy)benzamide acetate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, very hygroscopic, crystalline powder.

Solubility

Soluble in water and in anhydrous ethanol. It is freely soluble in dilute acetic acid and practically insoluble in dilute hydrochloric acid.

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Melting point *(2.2.14)*: 146 °C to 152 °C, with a melting range not greater than 3 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50 mg in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with *ethanol (96 per cent) R*.

Spectral range 230-350 nm.

Absorption maximum At 298 nm.

Specific absorbance at the absorption maximum 61 to 65.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison
flecainide acetate CRS .

D. It gives reaction (b) of acetates *(2.3.1)*.

TESTS

Appearance of solution.

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.25 g in *water R*, add 0.05 ml of *glacial acetic acid R* and dilute to 10 ml with *water R.*

pH *(2.2.3)*

6.7 to 7.1.

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Impurity B

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 2 ml with the same solvent.

Reference solution Dissolve 10 mg of *flecainide impurity B CRS* in *methanol R* and dilute to 100 ml with the same solvent (solution A). Dissolve 0.10 g of *flecainide acetate CRS* in solution A and dilute to 2 ml with the same solution.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Freshly prepared mixture of 5 volumes of concentrated *ammonia R* and 95 volumes of *acetone R*.

Application 5 µl.

Development Over a path of 10 cm.

Drying At 100-105 °C until the ammonia has evaporated.

Detection Examine in ultraviolet light at 254 nm to establish the position of the flecainide spot, then spray with a freshly prepared 2 g/l solution of *ninhydrin R* in *methanol R* and heat at

100-110 °C for 2-5 min; examine in daylight.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity B*: any spot due to impurity B is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.2 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.25 g of the substance to be examined in *methanol R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Reference solution (b) Dissolve 25 mg of *flecainide acetate CRS* and 25 mg of *flecainide impurity A CRS* in *methanol R* and dilute to 25.0 ml with the same solvent.

Column:

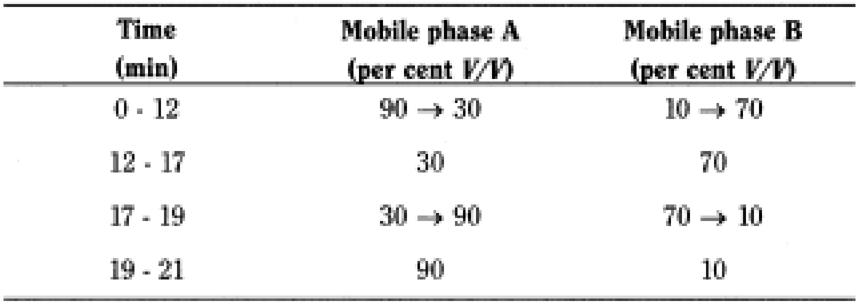
— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 2 ml of *concentrated ammonia R*, 4 ml of *triethylamine R* and 985 ml of *water R*; add 6 ml of *phosphoric acid R* and adjust to pH 2.8 with concentrated *ammonia R*;

— *mobile phase B*: *acetonitrile* R;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	90 → 30	10 → 70
12 - 17	30	70
17 - 19	30 → 90	70 → 10
19 - 21	90	10

If a suitable baseline cannot be obtained, use another grade of triethylamine.

Flow rate 2 ml/min.

Detection Spectrophotometer at 300 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 4 between the peaks due to flecainide and impurity A.

Limits:

— *impurities A, C, D, E*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 60 °C under a pressure not exceeding 0.6 kPa for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.400 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

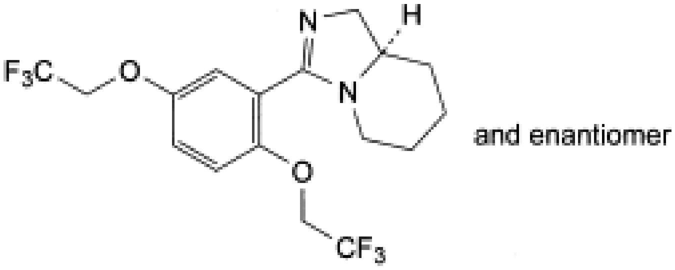
1 ml of *0.1 M perchloric acid* is equivalent to 47.44 mg of $C_{19}H_{24}F_6N_2O_5$.

STORAGE

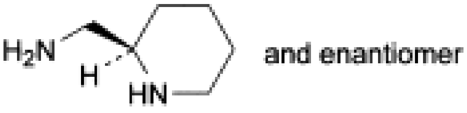
Protected from light.

IMPURITIES

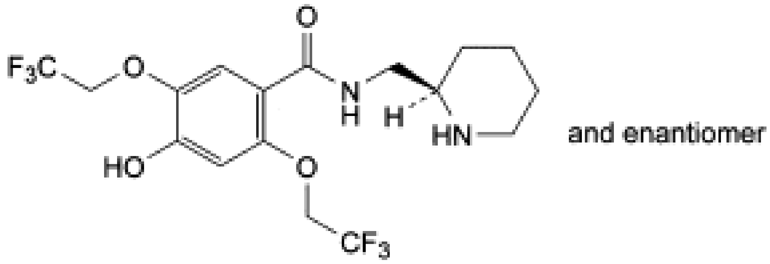
Specified impurities A, B, C, D, E.



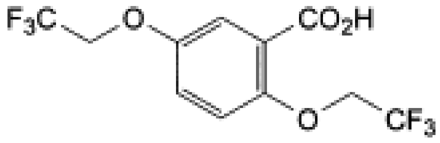
A. (8a*RS*)-3-[2,5-bis(2,2,2-trifluoroethoxy)phenyl]-1,5,6,7,8,8a-hexahydroimidazo[1,5-*a*] pyridine,



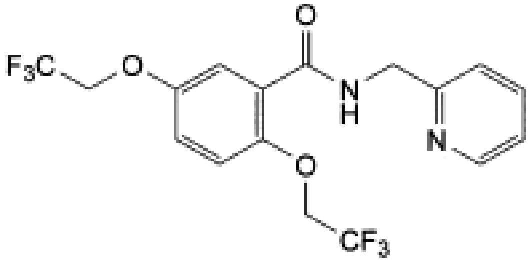
B. (*RS*)-(piperidin-2-yl)methanamine,



C. (*RS*)-4-hydroxy-*N*-(piperidin-2-ylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide,



D. 2,5-bis(2,2,2-trifluoroethoxy)benzoic acid,



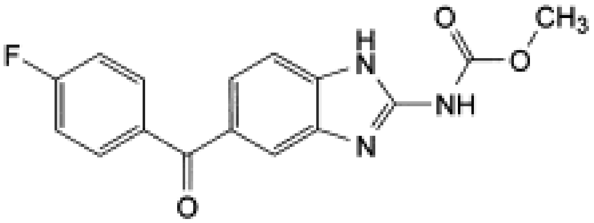
E. *N*-(pyridin-2-ylmethyl)-2,5-bis(2,2,2-trifluoroethoxy) benzamide.

Ph Eur

Flubendazole

General Notices

(Ph Eur monograph 1721)



$C_{16}H_{12}FN_3O_3$ 313.3   *31430-15-6*

Action and use

Benzimadazole antihelminthic.

Ph Eur

DEFINITION

Methyl [5-(4-fluorobenzoyl)-1*H*-benzimidazol-2-yl]carbamate

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, in alcohol and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*, without recrystallisation.

Comparison   flubendazole CRS .

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *dimethylformamide R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *flubendazole for system suitability CRS* in *dimethylformamide R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide R*. Dilute 5.0 ml of this solution to 20.0 ml with *dimethylformamide R.*

Column:

— *size: l* = 0.10 m, Ø = 4.6 mm,

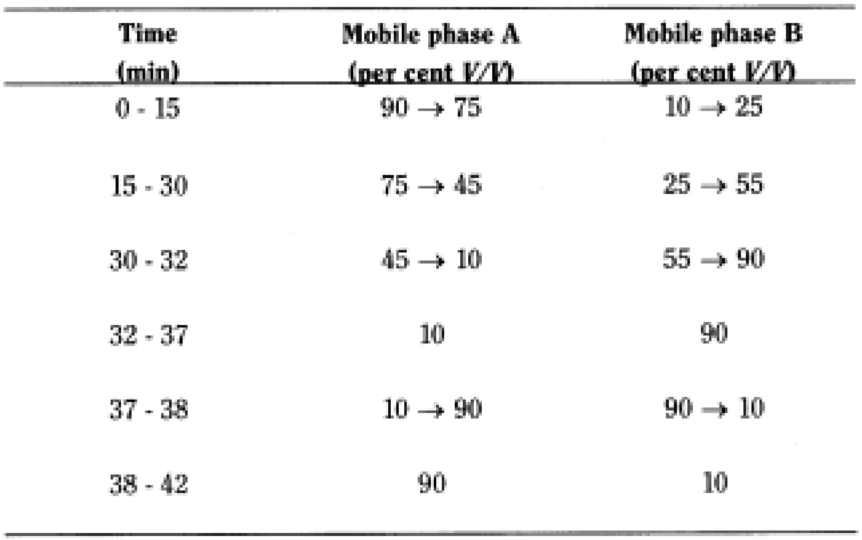
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm),

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: 7.5 g/l solution of ammonium *acetate R*,

— *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 75	10 → 25
15 - 30	75 → 45	25 → 55
30 - 32	45 → 10	55 → 90
32 - 37	10	90
37 - 38	10 → 90	90 → 10
38 - 42	90	10

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µl.

System suitability Reference solution (a):

— the chromatogram obtained is similar to the chromatogram supplied with *flubendazole for system suitability CRS* .

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.4; impurity C = 1.3; impurity D = 1.3; impurity G = 1.4,

— *impurities A, B, C, D, E, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent),

— *impurity F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *any other impurity with a relative retention between 1.2 and 1.3*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent),

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent),

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C, for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 3 ml of *anhydrous formic acid R* and add 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

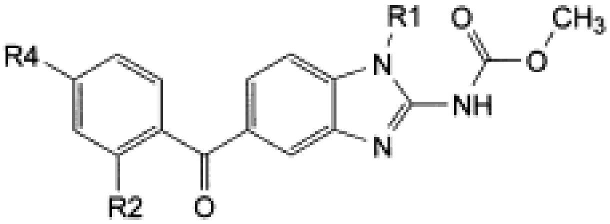
1 ml of *0.1 M perchloric acid* is equivalent to 31.33 mg of $C_{16}H_{12}FN_3O_3$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G.

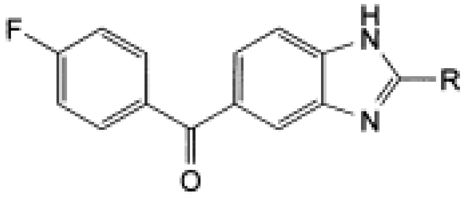


A. R1 = R2 = H, R4 = NH-CHO: methyl [5-[4-(formylamino)benzoyl]-1*H*-benzimidazol-2-yl] carbamate,

E. R1 = R4 = H, R2 = F: methyl [5-(2-fluorobenzoyl)-1*H*-benzimidazol-2-yl]carbamate,

F. R1 = CH_3, R2 = H, R4 = F: methyl [5-(4-fluorobenzoyl)-1-methyl-1*H*-benzimidazol-2-yl] carbamate,

G. R1 = R2 = H, R4 = O-CH(CH_3)$_2$: methyl [5-[4-(1-methylethoxy)benzoyl]-1*H*-benzimidazol-2-yl]carbamate,



B. R = NH_2: (2-amino-1*H*-benzimidazol-5-yl)(4-fluorophenyl)methanone,

C. R = OH: (4-fluorophenyl)(2-hydroxy-1*H*-benzimidazol-5-yl)methanone,

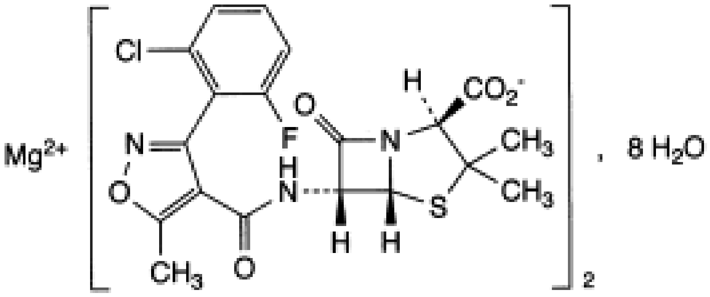
D. R = H: (1*H*-benzimidazol-5-yl)(4-fluorophenyl)methanone.

Ph Eur

Flucloxacillin Magnesium Octahydrate

General Notices

Flucloxacillin Magnesium
(Ph Eur monograph 2346)



$C_{38}H_{32}Cl_2F_2MgN_6O_{10}S_2,8H_2O$ 1074   *58486-36-5*

Action and use

Penicillin antibacterial.

Preparations

Flucloxacillin Oral Suspension

Co-fluampicil Oral Suspension

Ph Eur

DEFINITION

Magnesium bis[(2*S*,5*R*,6*R*)-6-[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate] octahydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in methanol.

IDENTIFICATION

First identification *A, C.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *flucloxacillin magnesium octahydrate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *water R*.

Reference solution (a) Dissolve 25 mg of *flucloxacillin sodium CRS* in 5 ml of *water R*.

Reference solution (b) Dissolve 25 mg of *cloxacillin sodium CRS*, 25 mg of *dicloxacillin sodium CRS* and 25 mg of *flucloxacillin sodium CRS* in 5 ml of *water R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose the plate to iodine vapour until the spots appear.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. It gives the reaction of magnesium *(2.3.1)*.

TESTS

pH *(2.2.3)*

4.5 to 6.5.

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 163 to + 175 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and

dilute to 50.0 ml with the mobile phase.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *flucloxacillin sodium CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of test solution (b) to 50.0 ml with the mobile phase.

Reference solution (c) In order to prepare impurity A *in situ*, add 1 ml of *sodium carbonate solution R* to 10 mg of the substance to be examined, dilute to 25 ml with *water R* and place in an oven at 70 °C for 20 min.

Reference solution (d) Dilute 1 ml of reference solution (c) to 10 ml with a 27 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*.

Reference solution (e) In order to prepare impurity B *in situ*, add 5 ml of *dilute hydrochloric acid R* to 10 ml of reference solution (c), dilute to 25 ml with *water R* and place in an oven at 70 °C for 1 h. Dilute 1 ml of this solution to 5 ml with a 27 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 7.0 with *phosphoric acid R*.

Reference solution (f) Dilute 2 ml of reference solution (a) to 10 ml with reference solution (e) .

Reference solution (g) Dissolve 1.5 mg of *flucloxacillin impurity C CRS* in 1 ml of the mobile phase and dilute to 50 ml with the mobile phase.

Reference solution (h) Dissolve 1 mg of *flucloxacillin impurity D CRS* in 100 ml of the mobile phase.

Reference solution (i) Dissolve 1 mg of *flucloxacillin impurity E CRS* in 100 ml of the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 25 volumes of *acetonitrile R1* and 75 volumes of a 2.7 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 5.0 with *dilute sodium hydroxide solution R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl of test solution (a) and reference solutions (b), (d), (e), (f), (g), (h) and (i).

Run time 7 times the retention time of flucloxacillin.

Identification of impurities Use the chromatograms obtained with reference solutions (d), (e), (g), (h) and (i) to identify the peaks due to impurities A, B, C, D and E respectively.

Relative retention WIth reference to flucloxacillin (retention time = about 8 min): impurity C = about 0.2; impurity A (isomer 1) = about 0.3; impurity A (isomer 2) = about 0.5; impurity D = about 0.6; impurity B (isomer 1) = about 0.8; impurity B (isomer 2) = about 0.9; impurity E = about 6.

System suitability Reference solution (f):

— *resolution*: minimum 2.0 between the 2nd peak due to impurity B (isomer 2) and the peak due to flucloxacillin.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 3.3;

— *impurity A* (sum of the 2 isomers): the sum of the areas of the 2 peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *impurity B* (sum of the 2 isomers): the sum of the areas of the 2 peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurities D, E*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *any other impurity*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.8 per cent m/m.

Water *(2.5.12)*

12.0 per cent to 15.0 per cent, determined on 0.100 g.

ASSAY

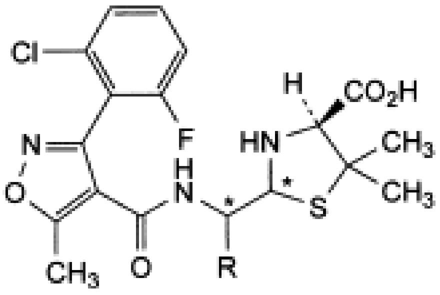
Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{38}H_{32}Cl_2F_2MgN_6O_{10}S_2$ from the declared content of *flucloxacillin sodium CRS*, multiplying by 0.9773.

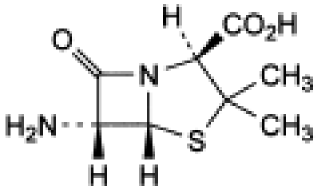
IMPURITIES

Specified impurities A, B, C, D, E.

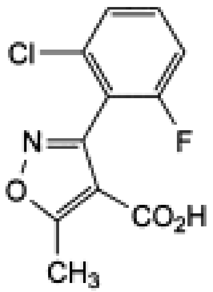


A. R = CO_2H: (4*S*)-2-[carboxy[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of flucloxacillin),

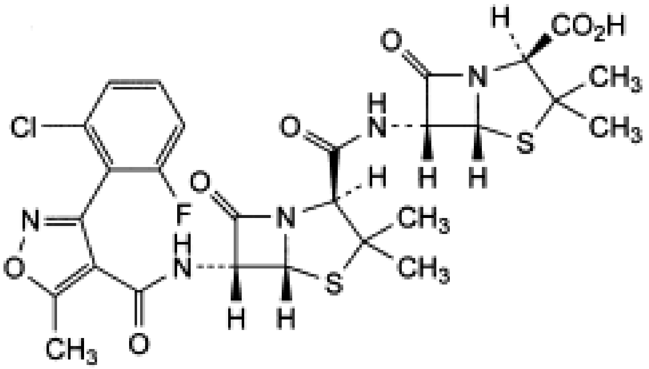
B. R = H: (2*RS*,4*S*)-2-[[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of flucloxacillin),



C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



D. 3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-4-carboxylic acid,



E. (2*S*,5*R*,6*R*)-6-[[[(2*S*,5*R*,6*R*)-6-[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-3,3-

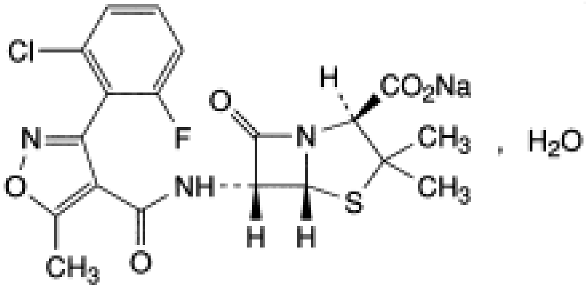
dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA flucloxacillin amide).

Ph Eur

Flucloxacillin Sodium

General Notices

(Ph Eur monograph 0668)



$C_{19}H_{16}ClFN_3NaO_5S,H_2O$ 493.9 *1847-24-1*

Action and use

Penicillin antibacterial.

Preparations

Flucloxacillin Capsules

Co-fluampicil Capsules

Flucloxacillin Injection

Flucloxacillin Oral Solution

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-6-[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water and in methanol, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *flucloxacillin sodium CRS* .

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *water R*.

Reference solution (a) Dissolve 25 mg of *flucloxacillin sodium CRS* in 5 ml of *water R*.

Reference solution (b) Dissolve 25 mg of *cloxacillin sodium CRS*, 25 mg of *dicloxacillin sodium CRS* and 25 mg of *flucloxacillin sodium CRS* in 5 ml of *water R*.

Plate *TLC silanised silica gel plate R*.

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R* adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the colour of the solution is slightly greenish-yellow. Place the test-tube in a water-bath for 1 min; the solution becomes yellow.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 430 nm is not greater than 0.04.

pH *(2.2.3)*

5.0 to 7.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 158 to + 168 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *flucloxacillin sodium CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 5 mg of *flucloxacillin sodium CRS* and 5 mg of *cloxacillin sodium CRS* in the mobile phase, then dilute to 50.0 ml with the mobile phase.

Column:
- — *size:* l = 0.25 m, Ø = 4 mm;
- — *stationary phase*: octadecylsilyl *silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of *acetonitrile R1* and 75 volumes of a 2.7 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 5.0 with *dilute sodium hydroxide solution R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl of test solution (a) and reference solutions (b) and (c).

Run time 6 times the retention time of flucloxacillin.

System suitability Reference solution (c):

- — *resolution*: minimum 2.5 between the peaks due to cloxacillin (1st peak) and flucloxacillin (2nd peak).

Limits:
- — *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- — *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent);
- — *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

***N,N*-Dimethylaniline** *(2.4.26, Method B)*

Maximum 20 ppm.

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.8 per cent m/m.

Water *(2.5.12)*

3.0 per cent to 4.5 per cent, determined on 0.300 g.

Pyrogens *(2.6.8)*

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test. Inject per kilogram of the rabbit's mass 1 ml of a solution in *water for injections R* containing 20 mg of the substance to be examined per millilitre.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

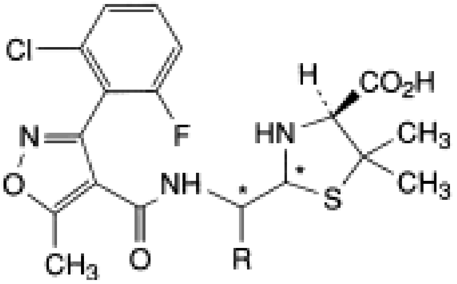
Calculate the percentage content of $C_{19}H_{16}ClFN_3NaO_5S$ from the declared content of *flucloxacillin sodium CRS*.

STORAGE

In an airtight container, at a temperature not exceeding 25 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

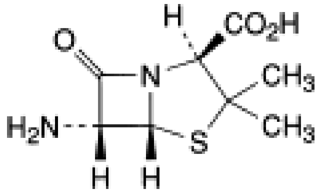
IMPURITIES

Specified impurities A, B, C, D, E.

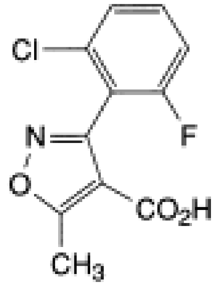


A. R = CO_2H: (4*S*)-2-[carboxy[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of flucloxacillin),

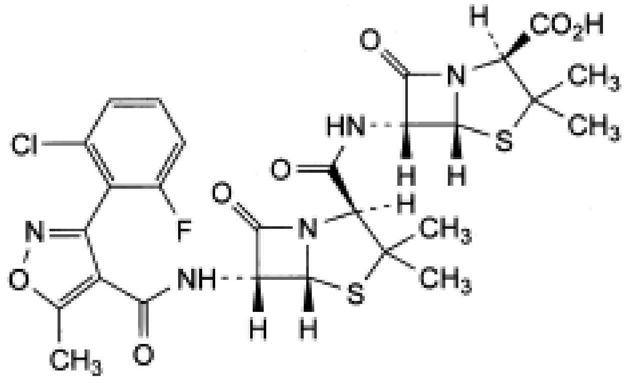
B. R = H: (2*RS*,4*S*)-2-[[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of flucloxacillin),



C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



D. 3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-4-carboxylic acid,



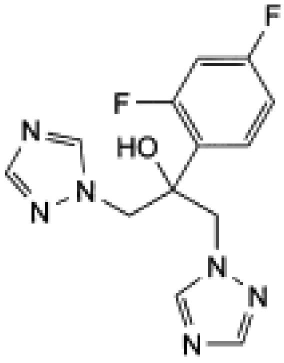
E. (2*S*,5*R*,6*R*)-6-[[[(2*S*,5*R*,6*R*)-6-[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl] carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Ph Eur

Fluconazole

General Notices

(Ph Eur monograph 2287)



$C_{13}H_{12}F_2N_6O$ 306.3   *86386-73-4*

Action and use

Antifungal.

Ph Eur

DEFINITION

2-(2,4-Difluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in methanol, soluble in acetone.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fluconazole CRS* .

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methylene chloride R*, evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase, sonicate if necessary, and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *fluconazole for peak identification CRS* (containing impurity A) in the mobile phase, sonicate if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (c) Dissolve 3.0 mg of *fluconazole impurity B CRS* in the mobile phase, sonicate if necessary and, dilute to 100.0 ml with the mobile phase.

Reference solution (d) Dissolve 2.0 mg of *fluconazole impurity C CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. To 1.0 ml of this solution add 1.0 ml of the test solution and dilute to 10.0 ml with the mobile phase.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm);

— *temperature*: 40 °C.

Mobile phase *acetonitrile R*, 0.63 g/l solution of *ammonium formate R* (14:86 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µl.

Run time 3.5 times the retention time of fluconazole.

Identification of impurities Use the chromatogram supplied with *fluconazole for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B and the chromatogram obtained with reference solution (d) to identify the peak due to impurity C.

Relative retention With reference to fluconazole (retention time = about 11 min): impurity B = about 0.4; impurity A = about 0.5; impurity C = about 0.8.

System suitability Reference solution (d):

— *resolution*: minimum 3.0 between the peaks due to impurity C and fluconazole.

Limits:

— *impurity A*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20.0 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.125 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 15.32 mg of $C_{13}H_{12}F_2N_6O$.

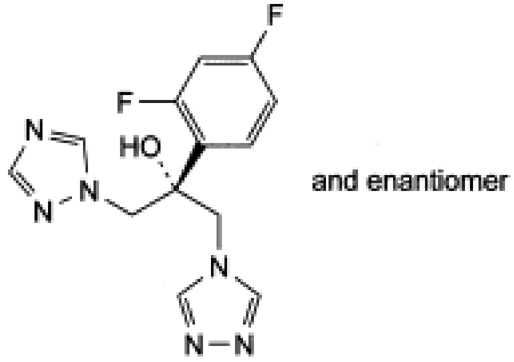
STORAGE

In an airtight container.

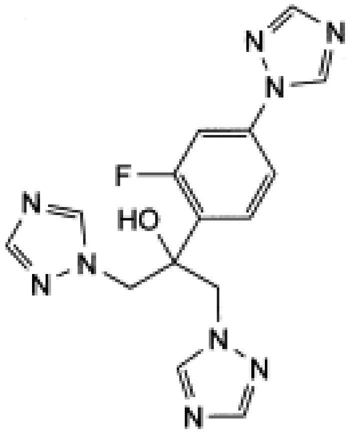
IMPURITIES

Specified impurities A, B, C.

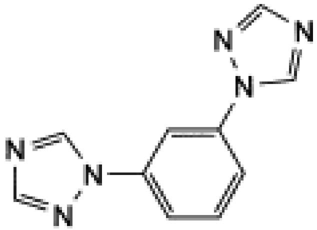
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : D, E, F, G, H, I.*



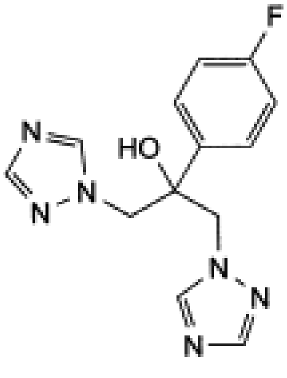
A. (2*RS*)-2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl)propan-2-ol,



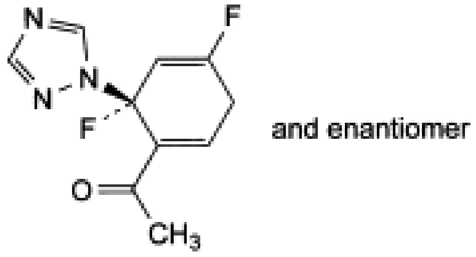
B. 2-[2-fluoro-4-(1*H*-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol,



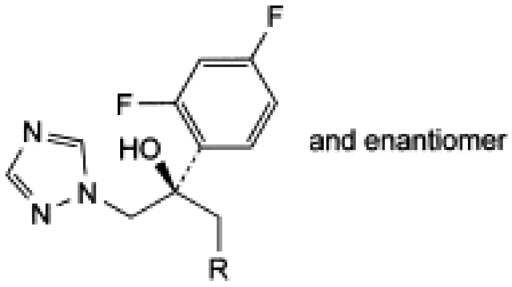
C. 1,1′-(1,3-phenylene)di-1*H*-1,2,4-triazole,



D. 2-(4-fluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol,

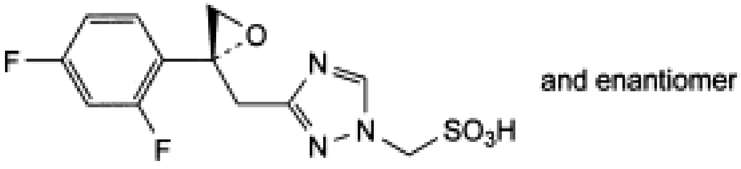

and enantiomer

E. 1-[(6*RS*)-4,6-difluoro-6-(1*H*-1,2,4-triazol-1-yl)cyclohexa-1,4-dienyl]ethanone,

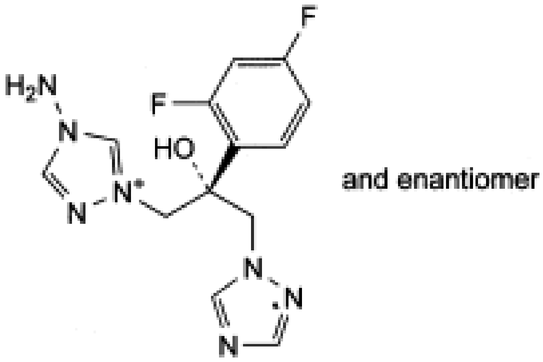

and enantiomer

F. R = OH: (2*RS*)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propane-1,2-diol,

H. R = Br: (2*RS*)-1-bromo-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol,


and enantiomer

G. [3-[[(2*RS*)-2-(2,4-difluorophenyl)oxiran-2-yl]methyl]1*H*-1,2,4-triazol-1-yl]methanesulphonic acid,

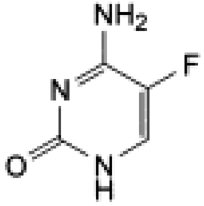

and enantiomer

I. 4-amino-1-[(2*RS*)-2-(2,4-difluorophenyl)-2-hydroxy-3(1*H*-1,2,4-triazol-1-yl)propyl]-4*H*-1,2,4-triazolium.

Flucytosine

General Notices

(Ph Eur monograph 0766)



$C_4H_4FN_3O$ 129.1 *2022-85-7*

Action and use

Antifungal.

Preparation

Flucytosine Tablets

Ph Eur

DEFINITION

Flucytosine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 4-amino-5-fluoropyrimidin-2(1*H*)-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, sparingly soluble in water, slightly soluble in ethanol.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *flucytosine CRS*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R*1 and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter and add to the filtrate a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The colour of the solution changes from red to yellow.

D. To 5 ml of solution S (see Tests) add 0.15 ml of *bromine water R* and shake. The colour of the solution is discharged.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 or Y_7 *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 50 mg of the substance to be examined in a mixture of 10 volumes of *water R* and 15 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 10 volumes of *water R* and 15 volumes of *methanol R.*

Reference solution (a) Dissolve 10 mg of *flucytosine CRS* in a mixture of 10 volumes of *water R* and 15 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dilute 1 ml of test solution (b) to 100 ml with a mixture of 10 volumes of *water R* and 15 volumes of *methanol R.*

Reference solution (c) Dissolve 5 mg of *fluorouracil CRS* in 5 ml of reference solution (a).

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm in an unsaturated tank using a mixture of 1 volume of *anhydrous formic acid R*, 15 volumes of *water R*, 25 volumes of *methanol R* and 60 volumes of *ethyl acetate R*. Allow the solvents to evaporate. At the bottom of a chromatography tank place an evaporating dish containing a mixture of 2 volumes of a 15 g/l solution of *potassium permanganate R*, 1 volume of *hydrochloric acid R1* and 1 volume of *water R*, close the tank and allow to stand for 15 min. Place the dried plate in the tank and close the tank. Leave the plate in contact with the chlorine vapour for 5 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of the coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution R.* Spray with *potassium iodide and starch solution R*. Examine the plate in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Fluoride

Not more than 200 ppm. Carry out a potentiometric determination of fluoride ion, using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode.

Prepare and store all solutions in plastic containers.

Buffer solution Dissolve 58 g of *sodium chloride R* in 500 ml of *water R*. Add 57 ml of *glacial acetic acid R* and 200 ml of a 100 g/l solution of *cyclohexylenedinitrilotetra-acetic acid R* in *1 M sodium hydroxide*. Adjust the pH to 5.0 to 5.5 with a 200 g/l solution of *sodium hydroxide R* and dilute to 1000.0 ml with *water R*.

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Dissolve 4.42 g of *sodium fluoride R*, previously dried at 120 °C for 2 h, in 300 ml of *water R* and dilute to 1000.0 ml with the same solvent (solution (a): 1.9 g/l of fluoride). Prepare three reference solutions by dilution of solution (a) 1 in 100, 1 in 1000 and 1 in 10 000.

To 20.0 ml of each reference solution, add 10.0 ml of the buffer solution and stir with a magnetic stirrer. Introduce the electrodes into the solution and allow to stand for 5 min with constant stirring. Determine the potential difference between the electrodes. Plot on semi-logarithmic graph paper the potential difference obtained for each solution as a function of concentration of fluoride. Using exactly the same conditions, determine the potential difference obtained with the test solution and calculate the content of fluoride.

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). *Use aplatinum crucible*. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.100 g in 40 ml of *anhydrous acetic acid R* and add 100 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 12.91 mg of $C_4H_4FN_3O$.

STORAGE

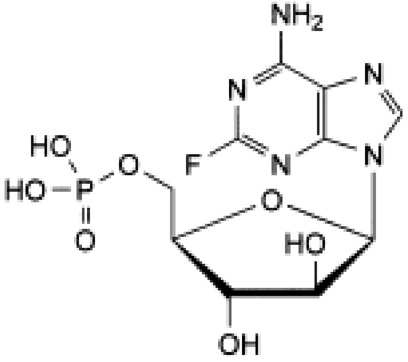
Store protected from light.

Ph Eur

Fludarabine Phosphate

General Notices

(Ph Eur monograph 1781)



$C_{10}H_{13}FN_5O_7P$ 365.2 *75607-67-9*

Purine analogue; cytotoxic.

Ph Eur

DEFINITION

2-Fluoro-9-(5-*C*-phosphono-β-D-arabinofuranosyl)-9*H*-purin-6-amine.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, hygroscopic.

Solubility

Slightly soluble in water, freely soluble in dimethylformamide, very slightly soluble in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fludarabine phosphate CRS* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Dissolve 50 mg in 5.0 ml of *dimethylformamide R* with the aid of ultrasound.

Specific optical rotation *(2.2.7)*

+ 10.0 to + 14.0 (anhydrous substance).

Dissolve 0.100 g in *water R* and dilute to 20.0 ml with the same solvent with the aid of ultrasound.

Related substances

Liquid chromatography *(2.2.29)*: use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution With the aid of ultrasound, dissolve 20 mg of the substance to be examined in 50 ml of *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) With the aid of ultrasound, dissolve 20 mg of *fludarabine phosphate CRS* in 50 ml of *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) With the aid of ultrasound, dissolve 20 mg of the substance to be examined in 20 ml of *0.1 M hydrochloric acid*. Heat in a water-bath at 80 °C for 15 min, cool to room temperature, mix and dilute to 100.0 ml with *water R*.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 20.0 ml with *water R*.

Blank solution *0.02 M hydrochloric acid*.

A. Early eluting impurities.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 60 volumes of *methanol R* and 940 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 260 nm and 292 nm.

Injection 10 µl; inject the solutions and record the chromatograms at 260 nm.

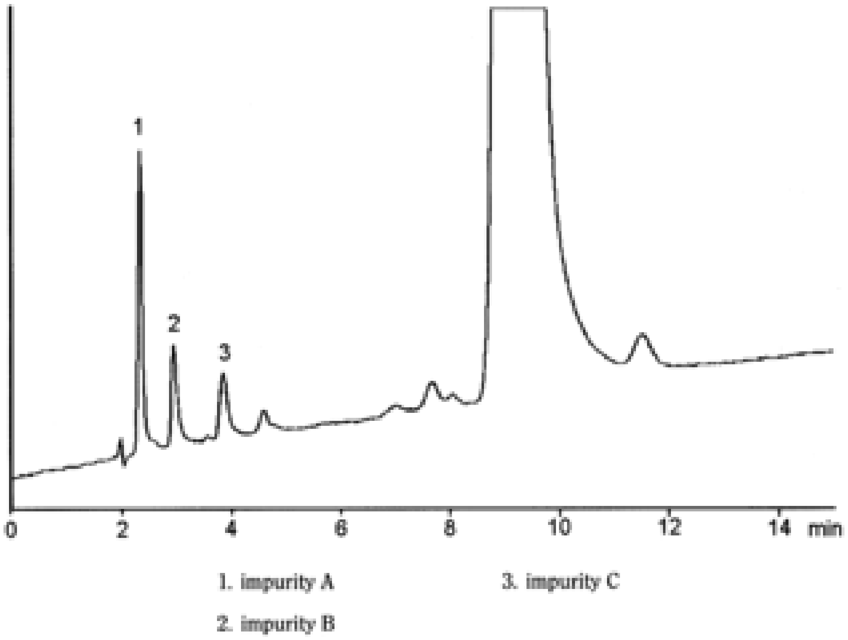


Figure 1781.-1. – *Chromatogram for test A for related substances of fludarabine phosphate: reference solution (a) at 260 nm*

Run time 4.5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Identification of impurities Identify the impurity peaks in the chromatogram obtained with reference solution (a) and in the chromatogram obtained with the test solution by comparison with Figure 1781.-1. Additionally, inject the test solution and reference solution (a) and record the chromatograms at 292 nm to identify impurities A and B, the response of which is much higher than that at 260 nm.

Relative retention With reference to fludarabine phosphate (retention time = about 9 min): impurity A = about 0.26; impurity B = about 0.34; impurity C = about 0.42.

System suitability Reference solution (b) at 292 nm:

— *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Limits At 260 nm:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 4.0; impurity B = 2.5; impurity C = 1.9;

— *impurity A*: maximum 0.8 per cent;

— *impurity B*: maximum 0.2 per cent;

— *impurity C*: maximum 0.4 per cent;

— *any other impurity preceding fludarabine phosphate* : maximum 0.1 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent), and any peak eluting after fludarabine phosphate.

B. Late eluting impurities.

Conditions as described under Test A with the following modifications.

Mobile phase Mix 200 volumes of *methanol R* and 800 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R*.

Detection Spectrophotometer at 260 nm.

Injection 10 µl.

Run time 8 times the retention time of the principal peak in the chromatogram obtained with the test solution.

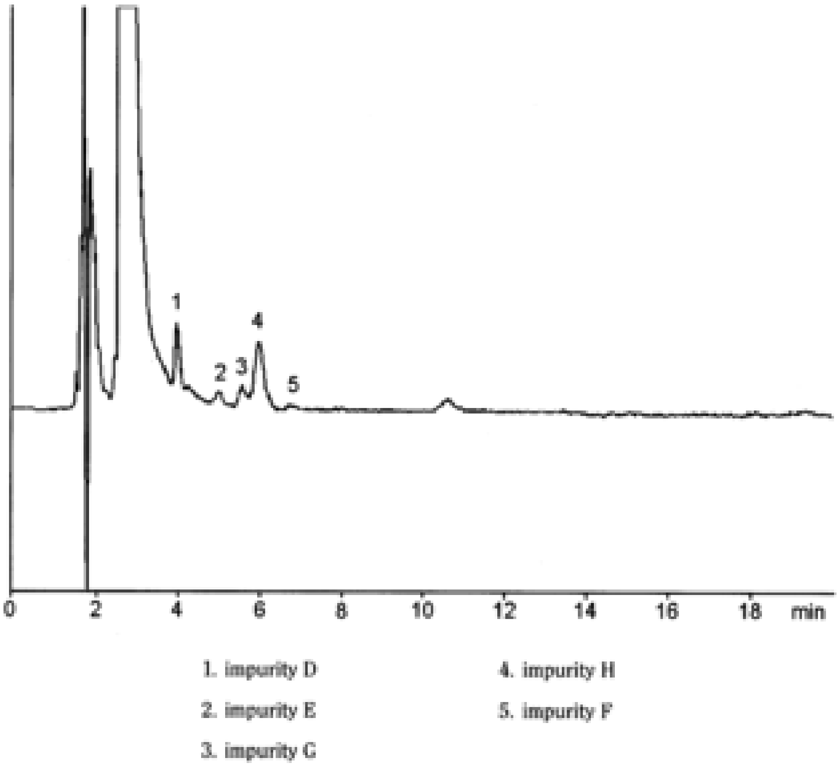


Figure 1781.-2. – *Chromatogram for test B for related substances of fludarabine phosphate: reference solution (a) at 260 nm.*

Identification of impurities Identify the impurity peaks in the chromatogram obtained with reference solution (a) and in the chromatogram obtained with the test solution by comparison with Figure 1781.-2.

Relative retention With reference to fludarabine phosphate (retention time = about 2.5 min): impurity D = about 1.5; impurity E = about 1.9; impurity G = about 2.2; impurity H = about 2.4; impurity F = about 2.5.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 2.0, where H_r = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity H.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.5; impurity E = 0.6; impurity F = 1.8;

— *impurity D*: maximum 0.1 per cent;

— *impurity E*: maximum 0.2 per cent;

— *impurity F*: maximum 0.2 per cent;

— *any other impurity eluting after fludarabine phosphate* : maximum 0.1 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent), and any peak eluting before fludarabine phosphate.

Total of impurities eluting before fludarabine phosphate in test A, apart from impurities A, B and C, and of impurities eluting after fludarabine phosphate in test B, apart from impurities D, E and F Maximum 0.5 per cent.

Total of all impurities eluting before fludarabine phosphate in test A and after fludarabine phosphate in test B Maximum 2.0 per cent.

Ethanol *(2.4.24, System A)*

Maximum 1.0 per cent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g by heating in 10 ml of *water R*. Allow to cool. Add *ammonia R* until the litmus paper reaction is slightly alkaline. Adjust to pH 3.0-4.0 with *dilute acetic acid R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 0.200 g (ground to a very fine powder). Stir the substance in 15 ml of *anhydrous methanol R* for about 15 s before titrating.

ASSAY

Liquid chromatography *(2.2.29)* as described in test A for related substances with the following modifications.

Test solution With the aid of ultrasound, dissolve 24.0 mg of the substance to be examined in 50 ml of *water R* and dilute to 100.0 ml with the same solvent. Dilute 25.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution With the aid of ultrasound, dissolve 24.0 mg of *fludarabine phosphate CRS* in 50 ml of *water R* and dilute to 100.0 ml with the same solvent. Dilute 25.0 ml of the solution to 100.0 ml with the mobile phase.

Detection Spectrophotometer at 260 nm.

Injection 10 µl.

Calculate the percentage content of $C_{10}H_{13}FN_5O_7P$ using the chromatograms obtained with the test solution and the reference solution, and the declared content of *fludarabine phosphate CRS.*

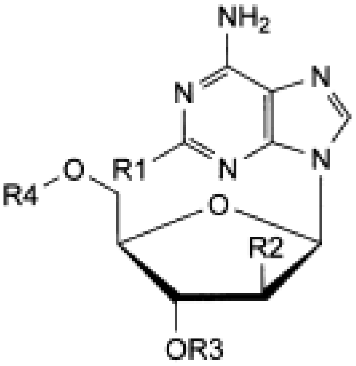
STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES

Specified impurities   *A, B, C, D, E, F, G.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : H, I, J.*



A. R1 = R2 = OH, R3 = H, R4 = PO_3H_2: 6-amino-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purin-2-ol,

C. R1 = F, R2 = OH, R3 = R4 = PO_3H_2: 9-(3,5-di-*O*-phosphono-β-D-arabinofuranosyl)-2-fluoro-9*H*-purin-6-amine,

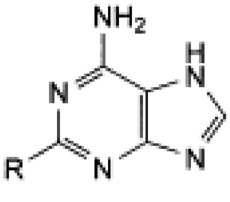
E. R1 = F, R2 = OH, R3 = R4 = H: 9-β-D-arabinofuranosyl-2-fluoro-9*H*-purin-6-amine,

F. R1 = O-C_2H_5, R2 = OH, R3 = H, R4 = PO_3H_2: 2-ethoxy-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purin-6-amine,

G. R1 = F, R2 = Cl, R3 = H, R4 = PO_3H_2: 9-(2-chloro-2-deoxy-5-*O*-phosphono-β-D-arabinofuranosyl)-2-fluoro-9*H*-purin-6-amine,

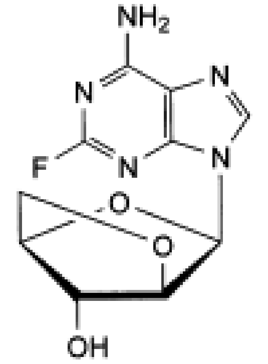
I. R1 = NH_2, R2 = OH, R3 = H, R4 = PO_3H_2: 9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purine-2,6-diamine,

J. R1 = OCH_3, R2 = OH, R3 = H, R4 = PO_3H_2: 2-methoxy-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-9*H*-purin-6-amine,



B. R = OH: 6-amino-7*H*-purin-2-ol,

D. R = F: 2-fluoro-7*H*-purin-6-amine,



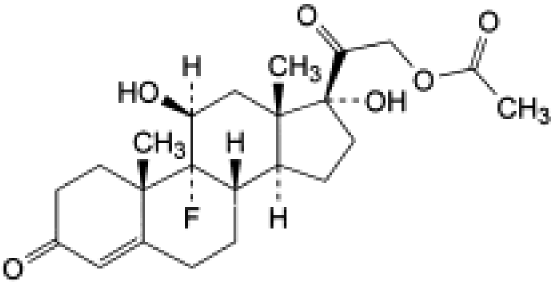
H. 9-(2,5-anhydro-β-D-arabinofuranosyl)-2-fluoro-9*H*-purin-6-amine.

Ph Eur

Fludrocortisone Acetate

General Notices

(Ph Eur monograph 0767)



$C_{23}H_{31}FO_6$ 422.5   *514-36-3*

Action and use

Mineralocorticoid.

Preparation

Fludrocortisone Tablets

Ph Eur

DEFINITION

9-Fluoro-11β,17-dihydroxy-3,20-dioxopregn-4-en-21-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in anhydrous ethanol.

IDENTIFICATION

First identification   *A, B.*

Second identification *C, D, E.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fludrocortisone acetate CRS* .

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *fludrocortisone acetate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *cortisone acetate CRS* in 5 ml of reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

C. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Test solution (b) Transfer 2 ml of solution A to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a stream of *nitrogen R* through the solution for 5 min. Stopper the tube. Heat on a water-bath at 45 °C protected from light for 2 h 30 min. Allow to cool.

Reference solution (a) Dissolve 25 mg of *fludrocortisone acetate CRS* in *methanol R* and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of this solution to 10 ml with *methylene chloride R.*

Reference solution (b) Transfer 2 ml of solution B to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a stream of *nitrogen R* through the solution for 5 min. Stopper the tube. Heat on a water bath at 45 °C protected from light for 2 h 30 min. Allow to cool.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of ether R and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have R_F values distinctly lower than those of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter and add to the filtrate a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R.* Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The colour of the solution to be examined changes from red to yellow.

E. About 10 mg gives the reaction of acetyl *(2.3.1)*.

TESTS

Specific optical rotation *(2.2.7)*

+ 148 to + 156 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *fludrocortisone acetate CRS* and 2.0 mg of *hydrocortisone acetate CRS* in the mobile phase, then dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:

- *size: l* = 0.2 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R*.

Mobile phase *tetrahydrofuran R*, *water R* (35:65 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time Twice the retention time of fludrocortisone acetate.

Retention time Hydrocortisone acetate = about 8.5 min; fludrocortisone acetate = about 10 min.

System suitability Reference solution (a):

- *resolution*: minimum 1.0 between the peaks due to hydrocortisone acetate and fludrocortisone acetate; if necessary, adjust slightly the concentration of tetrahydrofuran in the mobile phase (an increase in the concentration of tetrahydrofuran reduces the retention times).

Limits:

- *any impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *total*: not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
- *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 10.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 238 nm.

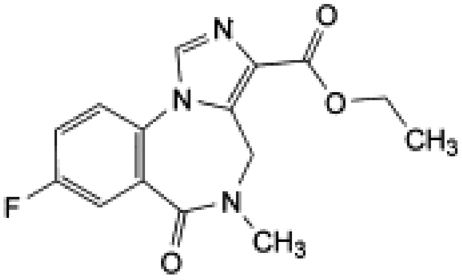
Calculate the content of $C_{23}H_{31}FO_6$ taking the specific absorbance to be 405.

Ph Eur

Flumazenil

General Notices

(Ph Eur monograph 1326)



$C_{15}H_{14}FN_3O_3$ 303.3 *78755-81-4*

Action and use

Benzodiazepine receptor antagonist.

Ph Eur

DEFINITION

Ethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in methylene chloride, sparingly soluble in methanol.

mp

198 °C to 202 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of flumazenil*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and is not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 0.10 g in *methanol R* and dilute to 10 ml with the same solvent.

Impurity C

Maximum 1 per cent.

Dissolve 0.10 g in 0.5 ml of *methylene chloride R* and dilute to 10 ml with *butanol R*. To 5.0 ml of this solution add 2.0 ml of *ninhydrin solution R* and heat in a water-bath at 95 °C for 15 min. Any blue-purple colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 5.0 ml of a 0.1 g/l solution of *dimethylformamide diethylacetal R* in *butanol R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in 5 ml of *methanol R* and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *flumazenil impurity B CRS* and 2.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 25.0 ml with the mobile phase.

Reference solution (b) Dilute 10.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase To 800 ml of *water R* adjusted to pH 2.0 with *phosphoric acid R*, add 130 ml of *methanol R* and 70 ml of *tetrahydrofuran R* and mix.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 3 times the retention time of flumazenil.

Relative retention With reference to flumazenil (retention time = about 14 min): impurity A = about 0.4; impurity D = about 0.5; impurity E = about 0.6; impurity B = about 0.7; impurity F = about 2.4.

System suitability Reference solution (a):

- *resolution*: minimum 3.0 between the peaks due to impurity B and flumazenil.

Limits:

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

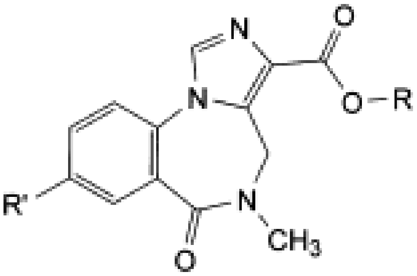
Dissolve 0.250 g in 50 ml of a mixture of 2 volumes of *acetic anhydride R* and 3 volumes of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 30.33 mg of $C_{15}H_{14}FN_3O_3$.

IMPURITIES

Specified impurities B, C.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : A, D, E, F.*

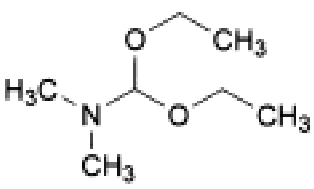


A. R = H, R′ = F: 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid,

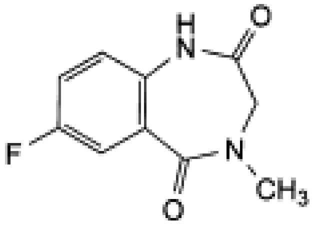
B. R = C_2-H_5, R′ = OH: ethyl 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4] benzodiazepine-3-carboxylate,

E. R = C_2H_5, R′ = H: ethyl 5-methyl-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4] benzodiazepine-3-carboxylate,

F. R = C_2H_5, R′ = Cl: ethyl 8-chloro-5-methyl-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4] benzodiazepine-3-carboxylate,


O
CH3
H3C
N
O
CH3
CH3

C. diethoxy-*N*,*N*-dimethylmethanamine,



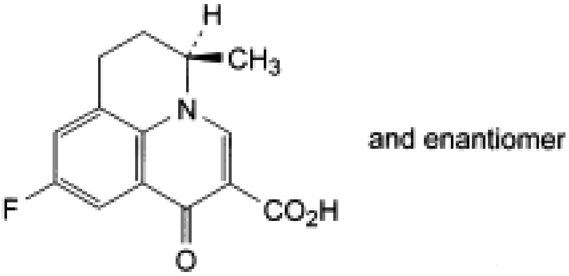
D. 7-fluoro-4-methyl-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione.

Ph Eur

Flumequine

General Notices

(Ph Eur monograph 1517)



$C_{14}H_{12}FNO_3$ 261.3 *42835-25-6*

Action and use

Antibacterial.

Ph Eur

DEFINITION

(*RS*)-9-Fluoro-5-methyl-1-oxo-6,7-dihydro-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, microcrystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in methylene chloride, very slightly soluble in methanol. It is freely soluble in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A, B.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *flumequine CRS.*

B. Optical rotation (see Tests).

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in 10 ml of *methylene chloride R*.

Reference solution Dissolve 5 mg of *flumequine CRS* in 10 ml of *methylene chloride R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ammonia R*, *water R*, *ethanol (96 per cent) R* (10:10:90 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 2 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter and add to the filtrate a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution changes from red to yellow and the blank remains red.

TESTS

Solution S

Dissolve 5.00 g in *0.5 M sodium hydroxide* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 35.0 mg of the substance to be examined in *dimethylformamide R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *flumequine impurity B CRS* in 2.0 ml of a 50 µg/ml solution of *flumequine CRS* in *dimethylformamide R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with *dimethylformamide*

R.

Column:

— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, 1.36 g/l solution of *potassium dihydrogen phosphate R* (49:51 *V/V*).

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 313 nm.

Injection 10 µl; inject *dimethylformamide R* as a blank.

Run time 3 times the retention time of flumequine.

Relative retention With reference to flumequine (retention time = about 13 min): impurity A = about 0.67; impurity B = about 0.85.

System suitability: Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to impurity B and flumequine.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

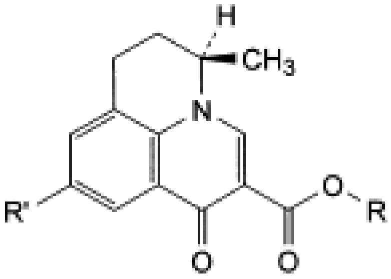
ASSAY

Dissolve 0.500 g in 50 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 26.13 mg of $C_{14}H_{12}FNO_3$.

IMPURITIES

Specified impurities *A, B*.



and enantiomer

A. R = R′ = H: (*RS*)-5-methyl-1-oxo-6,7-dihydro-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid (defluoroflumequine),

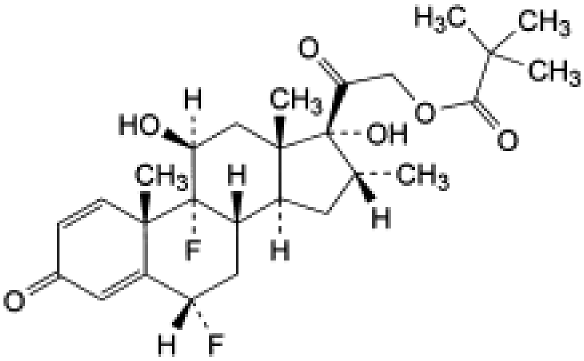
B. R = C_2H_5, R′ = F: ethyl (*RS*)-9-fluoro-5-methyl-1-oxo-6,7-dihydro-1*H*,5*H*-benzo[*i*,*j*] quinolizine-2-carboxylate (flumequine ethyl ester).

Ph Eur

Flumetasone Pivalate

General Notices

(Flumethasone Pivalate, Ph Eur monograph 1327)



$C_{27}H_{36}F_2O_6$ 494.6 *2002-29-1*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

6α,9-Difluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in acetone, slightly soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification A, B.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *flumetasone pivalate CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *flumetasone pivalate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *desoxycortone acetate CRS* in *acetone R* and dilute to 10 ml with the same solvent. Dilute 5 ml of this solution to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

C. Add about 2 mg to 2 ml of a mixture of 0.5 ml of *water R* and 1.5 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a pink colour develops. Add this solution to 10 ml of *water R* and mix. The colour fades and a clear solution remains.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R* add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Solution S

Dissolve 0.50 g in *acetone R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

+ 69 to + 77 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 10 mg of *dexamethasone pivalate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. To 5.0 ml of this solution, add 5.0 ml of the test solution, mix and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *tetrahydrofuran R*, *acetonitrile R*, *water R*, *methanol R* (5:30:30:35 *V/V/V/V*).

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 1.5 times the retention time of flumetasone pivalate.

Relative retention With reference to flumetasone pivalate: impurity C = about 1.1.

System suitability Reference solution (a):

- *resolution*: minimum 2.8 between the peaks due to flumetasone pivalate and impurity C; if necessary, adjust the concentration of tetrahydrofuran in the mobile phase.

Limits:

- *impurities A, B, C, D*: for each impurity, not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);
- *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 4 h.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 239 nm.

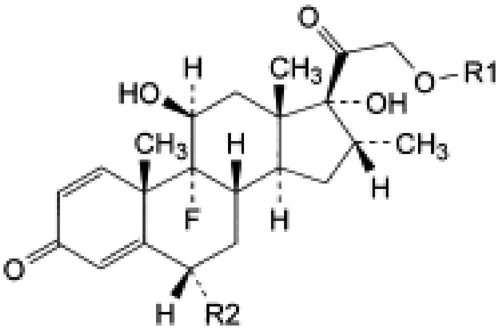
Calculate the content of $C_{27}H_{36}F_2O_6$ taking the specific absorbance to be 336.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D.



A. R1 = H, R2 = F: 6α,9-difluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione (flumetasone),

B. R1 = CO-CH_3, R2 = F: 6α,9-difluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate (flumetasone acetate),

C. R1 = CO-$C(CH_3)_3$, R2 = H: 9-fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate (dexamethasone pivalate),

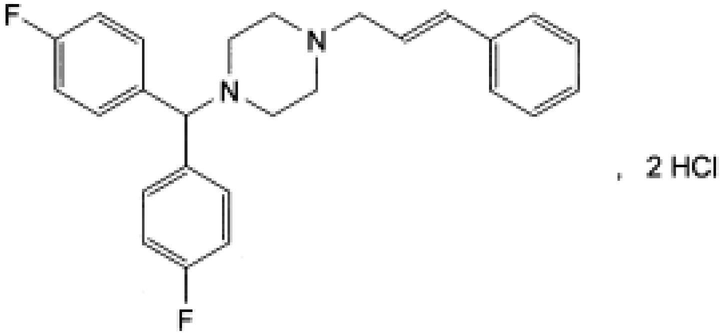
D. R1 = CO-$C(CH_3)_3$, R2 = Cl: 6α-chloro-9-fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate (chlordexamethasone pivalate).

Ph Eur

Flunarizine Dihydrochloride

General Notices

(Ph Eur monograph 1722)



$C_{26}H_{28}Cl_2F_2N_2$ 477.4 *30484-77-6*

Action and use

Calcium channel blocker.

Ph Eur

DEFINITION

1-[Bis(4-fluorophenyl)methyl]-4-[(2*E*)-3-phenylprop-2-enyl]piperazine dihydrochloride.

Content

99.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Slightly soluble in water, sparingly soluble in methanol, slightly soluble in alcohol and in methylene chloride.

mp

About 208 °C, with decomposition.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of flunarizine dihydrochloride* .

B. Dissolve 25 mg in 2 ml of *methanol R* and add 0.5 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *flunarizine dihydrochloride for system suitability CRS* in *methanol R* and dilute to 1.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol R*.

Column:

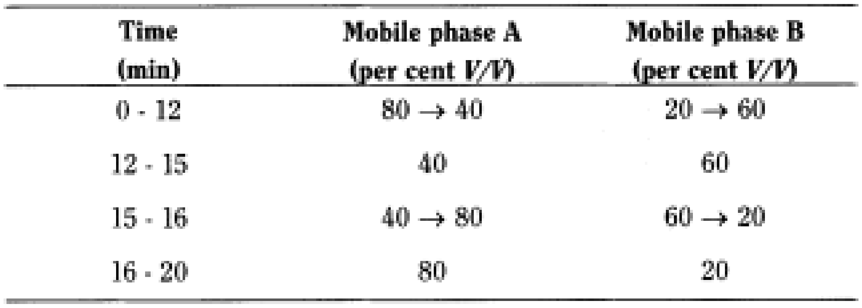
— *size:* *l* = 0.10 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: solution containing 23.8 g/l of *tetrabutylammonium hydrogen sulphate R* and 7 g/l of *ammonium acetate R*,

— *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 12	80 → 40	20 → 60
12 - 15	40	60
15 - 16	40 → 80	60 → 20
16 - 20	80	20

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to flunarizine,

— the chromatogram obtained is concordant with the chromatogram supplied with *flunarizine dihydrochloride for system suitability CRS* .

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.5,
- *impurities A, D*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent),
- *any other impurity*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.200 g in 70 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added at the second point of inflexion. Carry out a blank titration.

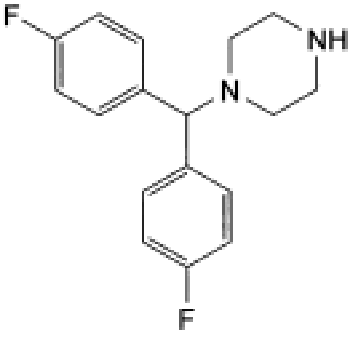
1 ml of *0.1 M sodium hydroxide* is equivalent to 23.87 mg of $C_{26}H_{28}Cl_2F_2N_2$.

STORAGE

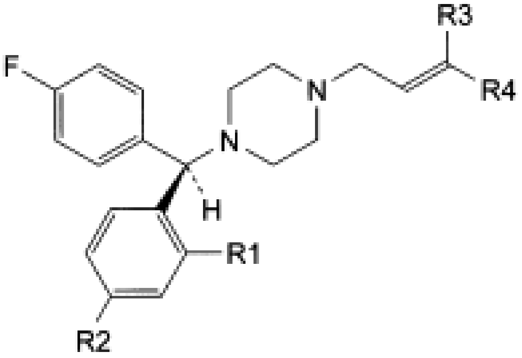
In an airtight container, protected from light.

IMPURITIES

Specified impurities A, B, C, D.



A. 1-[bis(4-fluorophenyl)methyl]piperazine,



and enantiomer

B. R1 = R2 = R3 = H, R4 = C_6H_5: 1-[(*RS*)-(4-fluorophenyl)phenylmethyl]-4-[(2*E*)-3-phenylprop-2-enyl]piperazine,

C. R1 = F, R2 = R3 = H, R4 = C_6H_5: 1-[(*RS*)-(2-fluorophenyl)(4-fluorophenyl)methyl]-4-[(2*E*)-3-phenylprop-2-enyl]piperazine,

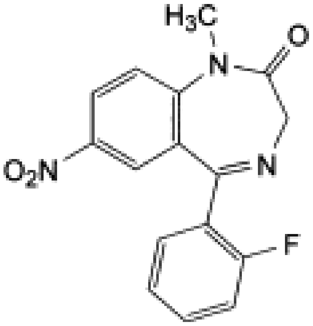
D. R1 = R4 = H, R2 = F, R3 = C_6H_5: 1-[bis(4-fluorophenyl)methyl]-4-[(2*Z*)-3-phenylprop-2-enyl]piperazine.

Ph Eur

Flunitrazepam

General Notices

(Ph Eur monograph 0717)



$C_{16}H_{12}FN_3O_3$ 313.3 *1622-62-4*

Action and use

Benzodiazepine.

Ph Eur

DEFINITION

5-(2-Fluorophenyl)-1-methyl-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone, slightly soluble in alcohol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of flunitrazepam.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 100.0 mg of the substance to be examined in 10 ml of *acetonitrile R* and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 4 mg of the substance to be examined and 4 mg of *nitrazepam R* in 5 ml of *acetonitrile R* and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Column:
- *size:* l = 0.15 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *acetonitrile R*, *water R* (50:305:645 *V/V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 6 times the retention time of flunitrazepam.

Relative retention With reference to flunitrazepam (retention time = about 11 min): impurity A = about 0.2; impurity B = about 0.6; impurity C = about 2.3; impurity D = about 4.0.

System suitability Reference solution (b):

- *resolution*: minimum 4.0 between the peaks due to nitrazepam and flunitrazepam.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity C by 2.44,
- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 20 ml of *anhydrous acetic acid R* and add 50 ml of *acetic anhydride R*.

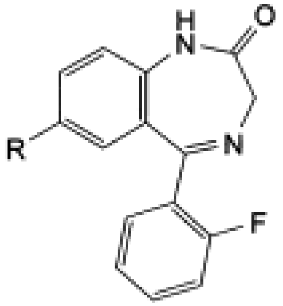
Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 31.33 mg of $C_{16}H_{12}FN_3O_3$.

STORAGE

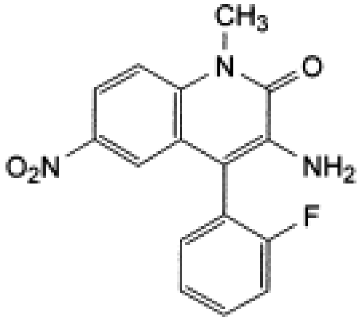
Protected from light.

IMPURITIES

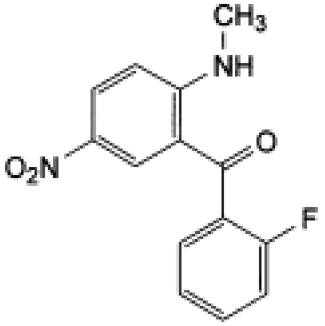


A. R = NH_2: 7-amino-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (7-aminodemethylflunitrazepam),

B. R = NO_2: 5-(2-fluorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (demethylflunitrazepam),



C. 3-amino-4-(2-fluorophenyl)-1-methyl-6-nitroquinolin-2(1*H*)-one,



D. (2-fluorophenyl)[2-(methylamino)-5-nitrophenyl]methanone.

Ph Eur

Fluocinolone Acetonide Dihydrate

General Notices

$C_{24}H_{30}F_2O_6,2H_2O$ 488.5 *67-73-2*
(*anhydrous*)

Action and use

Glucocorticoid.

Preparations

Fluocinolone Cream

Fluocinolone Ointment

DEFINITION

Fluocinolone Acetonide Dihydrate is 6α,9α-difluoro-11β,21-dihydroxy-16α-,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione dihydrate. It contains not less than 96.0% and not more than 104.0% of $C_{24}H_{30}F_2O_6$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Practically insoluble in *water*; freely soluble in *acetone*; soluble in *absolute ethanol*; sparingly soluble in *dichloromethane* and in *methanol*; practically insoluble in *hexane*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of fluocinolone acetonide dihydrate *(RS 147)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase H*. Apply 5 µl of each of the three solutions.

C. Complies with the test for *identification of steroids*, Appendix III A, using the conditions specified in test B but using solutions prepared in the following manner. For solution (1) dissolve 10 mg in 1.5 ml of *glacial acetic acid* in a separating funnel, add 0.5 ml of a 2% w/v solution of *chromium(VI) oxide* and allow to stand for 30 minutes. Add 5 ml of *water* and 2 ml of *dichloromethane* and shake vigorously for 2 minutes. Allow to separate and use the lower layer. Prepare solution (2) in the same manner but using 10 mg of *fluocinolone acetonide BPCRS*.

TESTS

Light absorption

Dissolve 15 mg in sufficient *absolute ethanol* to produce 100 ml. Dilute 10 ml of the solution to 100 ml with *absolute ethanol*. The A(1%, 1 cm) of the resulting solution at the maximum at

239 nm is 345 to 375, calculated with reference to the anhydrous substance, Appendix II B.

Specific optical rotation

In a 1% w/v solution in *1,4-dioxan*, +92 to +96, calculated with reference to the anhydrous substance, Appendix V F.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions of the substance being examined in *chloroform* containing (1) 1.0% w/v, (2) 0.020% w/v and (3) 0.010% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2.0%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1.0%).

Water

7.0 to 8.5% w/w, Appendix IX C. Use 0.5 g.

ASSAY

Carry out the *tetrazolium assay of steroids*, Appendix VIII J, and calculate the content of $C_{24}H_{30}F_2O_6$ from the *absorbance* obtained by repeating the operation using *fluocinolone acetonide BPCRS* in place of the substance being examined.

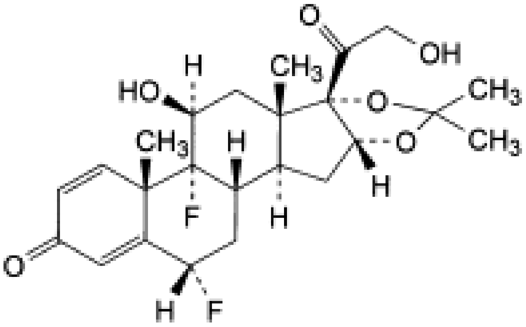
STORAGE

Fluocinolone Acetonide Dihydrate should be protected from light.

Fluocinolone Acetonide

General Notices

(Ph Eur monograph 0494)



$C_{24}H_{30}F_2O_6$ 452.5 *67-73-2*

Action and use

Glucocorticoid.

Preparations

Fluocinolone Cream

Fluocinolone Ointment

Ph Eur

DEFINITION

6α,9-Difluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone and in ethanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fluocinolone acetonide CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethanol R*, evaporate to dryness and record new spectra using the residues.

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the reference solution (b) is similar in retention time to the peak due to *fluocinolone acetonide CRS* in the chromatogram obtained with the reference solution (a).

TESTS

Specific optical rotation *(2.2.7)*

+ 100 to + 104 (dried substance).

Dissolve 0.100 g in *ethanol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light*.

Test solution Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *fluocinolone acetonide CRS* and 2.5 mg of *triamcinolone acetonide R* in 45 ml of *acetonitrile R* and dilute to 100.0 ml with *water R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 450 ml of *acetonitrile R* with 500 ml of *water R* and allow to equilibrate; adjust the volume to 1000.0 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 238 nm.

Injection 20 µl.

Run time 4 times the retention time of fluocinolone acetonide.

Retention times Triamcinolone acetonide = about 8.5 min; fluocinolone acetonide = about 10 min.

System suitability:

— *resolution*: minimum of 3.0 between the peaks due to triamcinolone acetonide and fluocinolone acetonide in the chromatogram obtained with reference solution (a).

Limits:

- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent) and not more than 1 such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent),
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Protect the solutions from light throughout the assay.

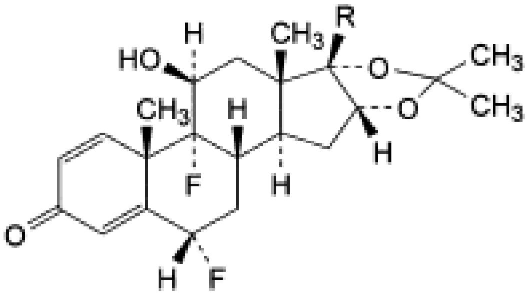
Dissolve 50.0 mg in *alcohol R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *alcohol R*. Measure the absorbance *(2.2.25)* at the maximum at 238 nm.

Calculate the content of $C_{24}H_{30}F_2O_6$ taking the specific absorbance to be 355.

STORAGE

Protected from light.

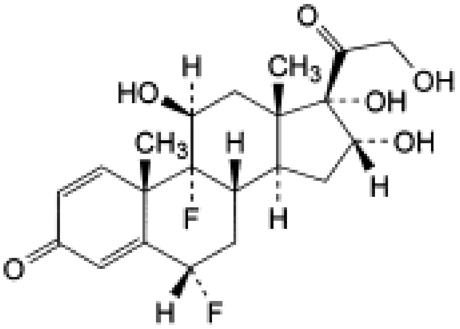
IMPURITIES



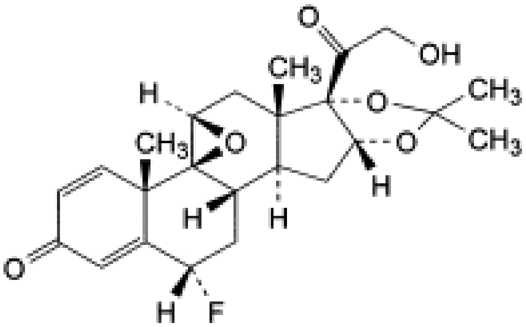
A. R = CO-CO_2H: 6α,9-difluoro-11β-hydroxy-16α,17-(1-methylethylidenedioxy)-3,20-dioxopregna-1,4-dien-21-oic acid,

B. R = CO_2H: 6α,9-difluoro-11β-hydroxy-16α,17-(1-methylethylidenedioxy)-3-oxoandrosta-1,4-diene-17β-carboxylic acid,

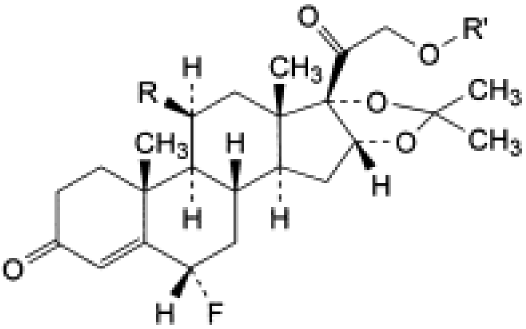
D. R = CO-CH=O: 6α,9-difluoro-11β-hydroxy-16α,17-(1-methylethylidenedioxy)-3,20-dioxopregna-1,4-dien-21-al,



C. 6α,9-difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione (fluocinolone),



E. 9,11β-epoxy-6α-fluoro-21-hydroxy-16α,17-(1-methylethylidenedioxy)-9β-pregna-1,4-diene-3,20-dione,



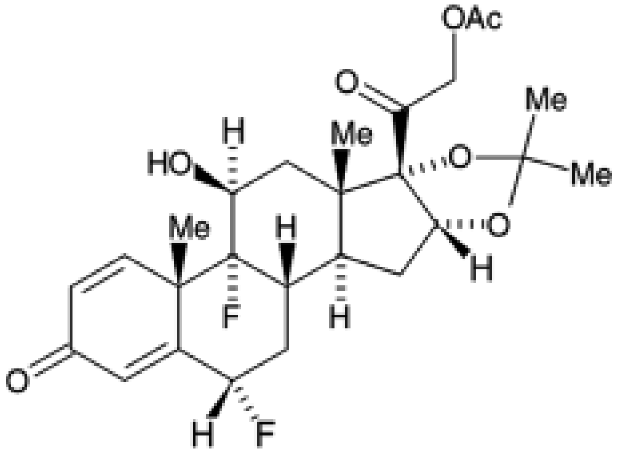
F. R = R′ = H: 6α-fluoro-21-hydroxy-16α,17-(1-methylethylidenedioxy)pregn-4-ene-3,20-dione,

G. R = OH, R′ = CO-CH_3: 6α-fluoro-11β-hydroxy-16α,17-(1-methylethylidenedioxy)-3,20-dioxopregn-4-en-21-yl acetate.

Ph Eur

Fluocinonide

General Notices



$C_{26}H_{32}F_2O_7$ 494.5 *356-12-7*

Action and use

Glucocorticoid.

Preparations

Fluocinonide Cream

Fluocinonide Ointment

DEFINITION

Fluocinonide is 6α,9α-difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-3,20-dioxopregna-1,4-dien-21-yl acetate. It contains not less than 97.0% and not more than 103.0% of $C_{26}H_{32}F_2O_7$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Practically insoluble in *water*; slightly soluble in *absolute ethanol*. It melts at about 220°, with decomposition.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of fluocinonide *(RS 148)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254}

precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 12 volumes of *water*, 80 volumes of *methanol*, 150 volumes of *ether* and 770 volumes of *dichloromethane* as the mobile phase; mix the water and the methanol before adding to the remaining components of the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dissolve 25 mg of the substance being examined in 5 ml of *methanol* (solution A); dilute 2 ml of solution A to 10 ml with *chloroform*. For solution (2) transfer 2 ml of solution A to a 15 ml glass tube with a ground-glass stopper, add 10 ml of *saturated methanolic potassium hydrogen carbonate solution* and immediately pass a current of *nitrogen* briskly through the solution for 2 minutes. Stopper the tube, heat in a water bath at 45° protected from light for 2.5 hours and allow to cool. For solution (3) dissolve 25 mg of *fluocinonide BPCRS* in 5 ml of *methanol* (solution B); dilute 2 ml of solution B to 10 ml with *chloroform*. Prepare solution (4) in the same manner as solution (2) but use 2 ml of solution B in place of 2 ml of solution A. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spots in each of the chromatograms obtained with solutions (1) and (2) are similar in position and size to those in the chromatograms obtained with solutions (3) and (4), respectively.

TESTS

Specific optical rotation

In a 1.0% w/v solution in *chloroform*, +81 to +89, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the precoated plate and the mobile phase specified in test B for Identification. Apply separately to the plate 5 µl of each of four solutions in *chloroform* containing (1) 0.50% w/v of the substance being examined, (2) 0.010% w/v of the substance being examined, (3) 0.0050% w/v of the substance being examined and (4) 0.010% w/v of *fluocinolone acetonide BPCRS*. After removal of the plate, allow it to dry in air, heat the plate at 105° for 10 minutes, cool and spray with *alkaline tetrazolium blue solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%). The test is not valid unless the principal spot in the chromatogram obtained with solution (2) has an R_f value relative to the spot in the chromatogram obtained with solution (4) of at least 1.5.

Loss on drying

When dried at 100° to 105° for 3 hours, loses not more than 1.0% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using two solutions in *methanol* containing (1) 0.012% w/v of the substance being examined and (2) 0.012% w/v of *fluocinonide BPCRS*.

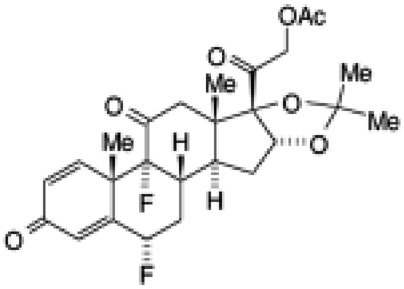
The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 1 volume of *glacial acetic acid*, 450 volumes of *acetonitrile* and 550 volumes of *water* and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{26}H_{32}F_2O_7$ using the declared content of $C_{26}H_{32}F_2O_7$ in *fluocinonide BPCRS*.

STORAGE

Fluocinonide should be protected from light.

IMPURITIES


OAc
O
Me
Me
O
Me
O
Me
H
O
O
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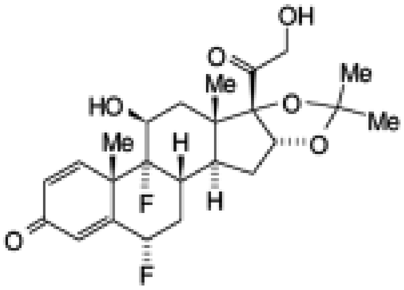
H
F

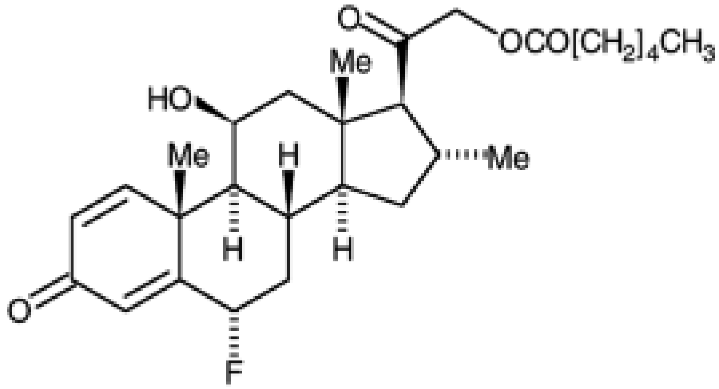
A. 6α,9α-difluoro-16α,17α-isopropylidene-3,11,20-trioxopregna-1,4-dien-21-yl acetate,


OH
O
Me
Me
HO
O
Me
Me
H
O
O
F
H
F

B. 6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenepregna-1,4-diene-3,20dione.

Fluocortolone Hexanoate

General Notices



$C_{28}H_{39}FO_5$ 474.6 *303-40-2*

Action and use

Glucocorticoid.

Preparation

Fluocortolone Cream

DEFINITION

Fluocortolone Hexanoate is 6α-fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl hexanoate. It contains not less than 97.0% and not more than 103.0% of $C_{28}H_{39}FO_5$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or creamy white, crystalline powder.

Practically insoluble in *water* and in *ether*; slightly soluble in *acetone* and in *1,4-dioxan*; very slightly soluble in *ethanol (96%)* and in *methanol*.

It exhibits polymorphism.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of fluocortolone hexanoate *(RS 149)*.

TESTS

Light absorption

Ratio of the *absorbance* of the solution prepared as directed in the Assay at the maximum at 242 nm to that at 263 nm, 2.15 to 2.35, Appendix II B.

Specific optical rotation

In a 1% w/v solution in *1,4-dioxan*, prepared with the aid of heat, +97 to +103, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.04% w/v of the substance being examined in a mixture of 1 volume of *water* and 9 volumes of *acetonitrile*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 1 volume of *water* and 9 volumes of *acetonitrile*. Solution (3) contains 0.002% w/v each of *fluocortolone pivalate BPCRS* and *fluocortolone hexanoate BPCRS* in a mixture of 1 volume of *water* and 9 volumes of *acetonitrile*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 25 volumes of *methanol*, 32 volumes of *water* and 50 volumes of *acetonitrile* and (c) a detection wavelength of 242 nm.

Inject solution (2). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50% of the full scale of the recorder.

Inject solution (3). The test is not valid unless, in the chromatogram obtained, the *resolution factor* between the two principal peaks is at least 6.0.

Inject solution (1). Continue the chromatography for twice the retention time of fluocortolone hexanoate. In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%). Disregard any peak due to the solvent and any peak with an area less than 0.025 times that of the principal peak in the chromatogram obtained with solution (2) (0.025%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

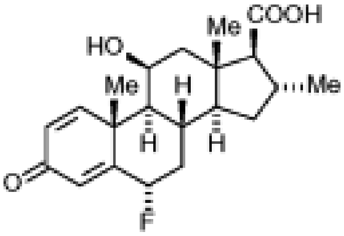
ASSAY

Dissolve 15 mg in sufficient *methanol* to produce 100 ml, dilute 20 ml to 100 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 242 nm, Appendix II B. Calculate the content of $C_{28}H_{39}FO_5$ taking 340 as the value of A(1%, 1 cm) at the maximum at 242 nm.

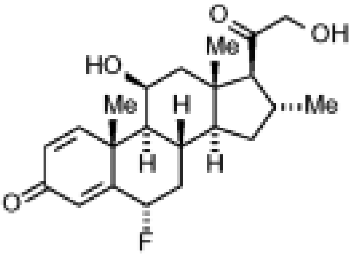
STORAGE

Fluocortolone Hexanoate should be protected from light.

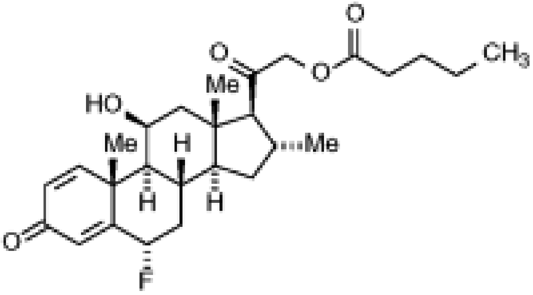
IMPURITIES



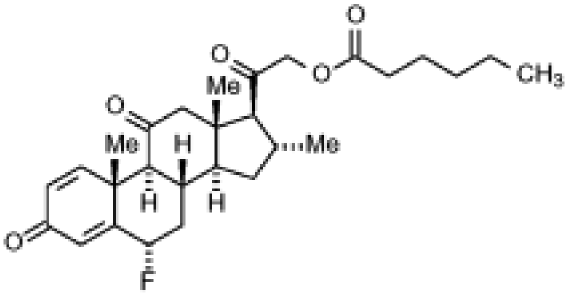
A. 6α-fluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid,



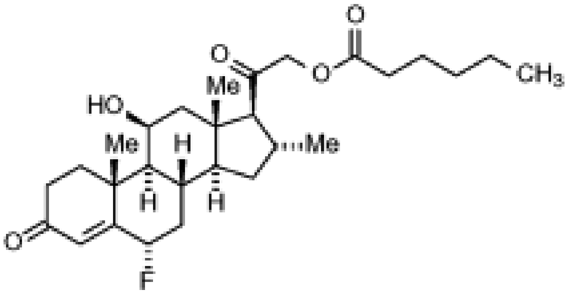
B. 6α-fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione,



C. 6α-fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl pentanoate,



D. 6α-fluoro-16α-methyl-3,11,20-trioxopregna-1,4-dien-21-yl hexanoate,


O
O
O
CH3
Me
HO
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H
Me
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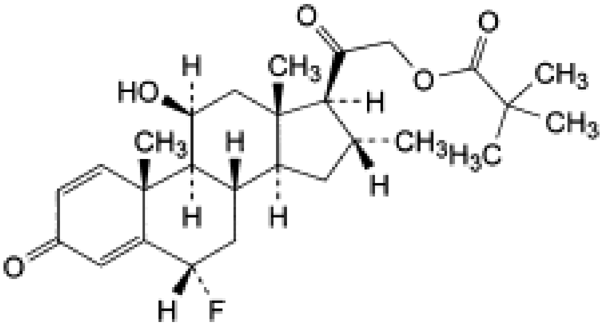
O
F

E. 6α-fluoro-11β-hydroxy-16α-methyl-3,11-dioxopregnen-21-yl hexanoate.

Fluocortolone Pivalate

General Notices

(Ph Eur monograph 1212)



$C_{27}H_{37}FO_5$ 460.6 *29205-06-9*

Action and use

Glucocorticoid.

Preparation

Fluocortolone Cream

Ph Eur

DEFINITION

6α-Fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride and in dioxan, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fluocortolone pivalate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *fluocortolone pivalate CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *norethisterone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

C. To about 1 mg add 2 ml of a mixture of 2 volumes of *glacial acetic acid R* and 3 volumes of *sulphuric acid R* and heat for 1 min on a water-bath. A red colour is produced. Add 5 ml of *water R*, the colour changes to violet-red.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter and add to the filtrate a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Specific optical rotation *(2.2.7)*

+ 100 to + 105 (dried substance).

Dissolve 0.25 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*.

Reference solution (b) Dissolve 2 mg of *fluocortolone pivalate CRS* and 2 mg of *prednisolone hexanoate CRS* in *acetonitrile R*, then dilute to 100 ml with the same solvent.

Column:
- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *acetonitrile R*, *water R* (25:30:32 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 243 nm.

Injection 20 µl.

Run time Twice the retention time of fluocortolone pivalate.

System suitability Reference solution (b):
- *resolution*: minimum 5.0 between the peaks due to fluocortolone pivalate and prednisolone hexanoate.

Limits:
- *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent);
- *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 30.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *anhydrous ethanol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 242 nm.

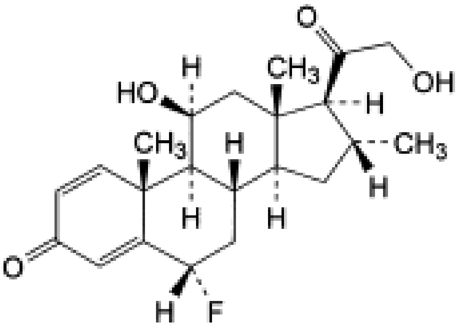
Calculate the content of $C_{27}H_{37}FO_5$ taking the specific absorbance to be 350.

STORAGE

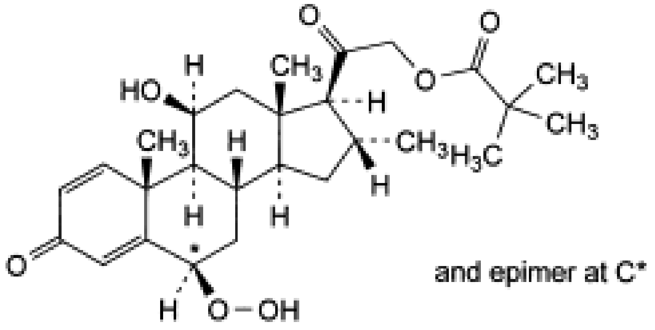
Protected from light.

IMPURITIES

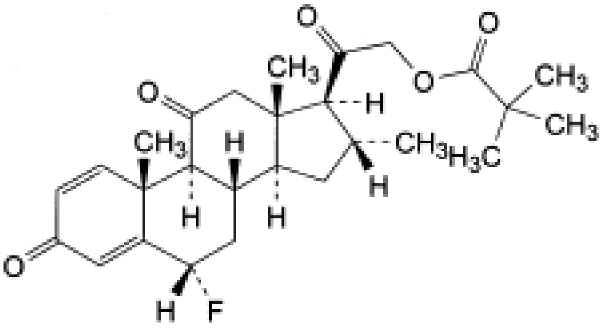
Specified impurities   A, B, C, D.



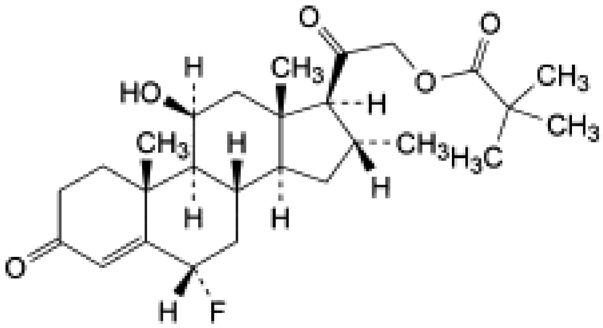
A. 6α-fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione (fluocortolone),



B. 6-hydroperoxy-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate,



C. 6α-fluoro-16α-methyl-3,11,20-trioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate,



O
O
H
CH3
HO
H
CH3
H
CH3
CH3
CH3H3C
H
O
H
H
H
H
F

D. 6α-fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-4-en-21-yl 2,2-dimethylpropanoate.

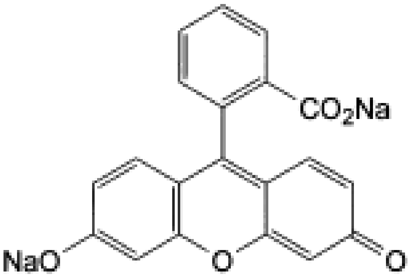
Ph Eur

Fluorescein Sodium

General Notices

Soluble Fluorescein

(Ph Eur monograph 1213)



$C_{20}H_{10}Na_2O_5$ 376.3 *518-47-8*

Action and use

Detection of corneal lesions, retinal angiography and pancreatic function testing.

Preparations

Fluorescein Eye Drops

Fluorescein Injection

Ph Eur

DEFINITION

Disodium 2-(6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate.

Content

95.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

Orange-red, fine powder, hygroscopic.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent), practically insoluble in hexane and in methylene chloride.

IDENTIFICATION

First identification   *B, D.*

Second identification   *A, C, D.*

A. Dilute 0.1 ml of solution S (see Tests) to 10 ml with *water R*. The solution shows yellowish-green fluorescence. The fluorescence disappears on addition of 0.1 ml of *dilute hydrochloric acid R* and reappears on addition of 0.2 ml of *dilute sodium hydroxide solution R*.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation   Discs.

Comparison   *Ph. Eur. reference spectrum of fluorescein sodium.*

C. The absorption by a piece of filter paper of 0.05 ml of the solution prepared for identification A (before the addition of *dilute hydrochloric acid R*) colours the paper yellow. On exposing the moist paper to bromine vapour for 1 min and then to ammonia vapour, the colour becomes deep pink.

D. Ignite 0.1 g in a porcelain crucible. Dissolve the residue in 5 ml of *water R* and filter. 2 ml of the filtrate gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and orange-yellow with yellowish-green fluorescence.

pH *(2.2.3)*

7.0 to 9.0 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a)   Dissolve 0.100 g of the substance to be examined in a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A and dilute to 100.0 ml with the same mixture of solvents.

Test solution (b)   Dilute 5.0 ml of test solution (a) to 250.0 ml with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A.

Reference solution (a)   Dissolve 55.0 mg of *diacetylfluorescein CRS* in a mixture of 1 ml of *2.5 M sodium hydroxide* and 5 ml of *ethanol (96 per cent) R*, heat on a water-bath for 20 min mixing frequently, cool and dilute to 50.0 ml with *water R*. Dilute 5.0 ml of the solution to 250.0 ml with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A.

Reference solution (b)   Dissolve 10.0 mg of *phthalic acid R* (impurity B) and 10.0 mg of *resorcinol R* (impurity A) in a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A and dilute to 100.0 ml with the same mixture of solvents. Dilute 5.0 ml of the solution to 100.0 ml with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A.

Reference solution (c) Dilute 5.0 ml of test solution (b) to 20.0 ml with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 35 °C.

Mobile phase:

- *mobile phase A*: dissolve 0.610 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000 ml with the same solvent; adjust to pH 2.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 20	85 → 20	15 → 80
20 - 29	20	80
29 - 30	20 → 85	80 → 15
30 - 35	85	15

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (a) and reference solutions (b) and (c).

Relative retention With reference to fluorescein (retention time = about 15 min): impurity A = about 0.44; impurity B = about 0.50; impurity C = about 0.87.

System suitability Reference solution (b):

- *resolution*: minimum 1.5 between the peaks due to impurity A and impurity B.

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity C by 1.6,
- *Impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),
- *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),
- *sum of impurities other than A, B, C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Chlorides *(2.4.4)*

Maximum 0.25 per cent.

To 10 ml of solution S add 90 ml of *water R* and 1 ml of *dilute nitric acid R*, wait for at least 10 min and filter. Dilute 10 ml of the filtrate to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 1.0 per cent.

To 5 ml of solution S add 90 ml of *distilled water R*, 2.5 ml of *dilute hydrochloric acid R* and dilute to 100 ml with *distilled water R*. Filter. 15 ml of the filtrate complies with the test.

Zinc

Dilute 5 ml of solution S to 10 ml with *water R*. Add 2 ml of *hydrochloric acid R1*, filter and add 0.1 ml of *potassium ferrocyanide solution R*. No turbidity or precipitate is formed immediately.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{20}H_{10}Na_2O_5$ using the chromatogram obtained with reference solution (a) and the declared content of *diacetylfluorescein CRS*.

1 mg of *diacetylfluorescein CRS* is equivalent to 0.9037 mg of $C_{20}H_{10}Na_2O_5$.

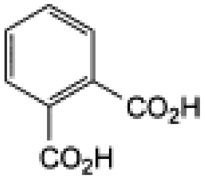
STORAGE

In an airtight container, protected from light.

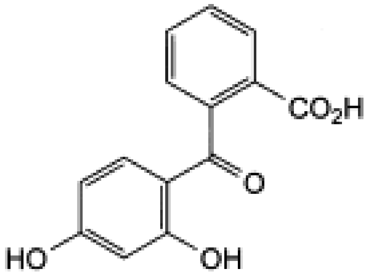
IMPURITIES

Specified impurities *A, B, C.*

A. resorcinol,



B. benzene-1,2-dicarboxylic acid (phthalic acid),



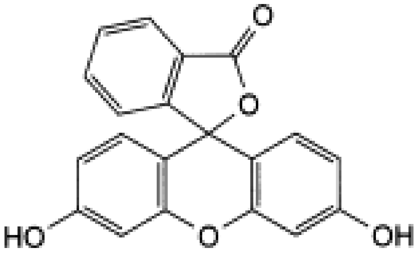
C. 2-(2,4-dihydroxybenzoyl)benzoic acid.

Ph Eur

Fluorescein

General Notices

(Ph Eur monograph 2348)



$C_{20}H_{12}O_5$ 332.3   *2321-07-5*

Action and use

Detection of corneal lesions, retinal angiography and pancreatic function testing.

Ph Eur

DEFINITION

3′,6′-Dihydroxy-3*H*-spiro[isobenzofuran-1,9′-xanthen]-3-one.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Orange-red, fine powder.

Solubility

Practically insoluble in water, soluble in hot ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification   *A, D.*

Second identification   *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fluorescein CRS.*

Dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness and record the spectra using the residues.

B. Dilute 0.1 ml of solution S (see Tests) to 10 ml with *water R*. The solution shows a yellowish-green fluorescence. The fluorescence disappears on addition of 0.1 ml of *dilute hydrochloric acid R* and reappears on addition of 0.2 ml of *dilute sodium hydroxide solution R*.

C. The absorption by a piece of filter paper of 0.05 ml of the solution prepared for identification B (before the addition of *dilute hydrochloric acid R*) colours the paper yellow. On exposing the moist paper to bromine vapour for 1 min and then to ammonia vapour, the colour becomes deep pink.

D. Suspend 0.5 g in 50 ml of *water R* and shake for 10 min. The substance does not completely dissolve.

TESTS

Solution S

Suspend 1.0 g in 35.0 ml of *water R* and add dropwise with shaking 4.5 ml of *1 M sodium hydroxide*. Adjust to pH 8.5-9.0 with *1 M sodium hydroxide* and dilute to 50.0 ml with *water R* to obtain a clear solution.

Appearance of solution

Solution S is clear *(2.2.1)* and orange-yellow with yellowish-green fluorescence.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile for chromatography R*, mobile phase A (30:70 *V/V*).

Test solution (a) Disperse 50.0 mg of the substance to be examined in 15.0 ml of *ethanol (96 per cent) R*. Sonicate and dilute to 50.0 ml with the solvent mixture.

Test solution (b) Dilute 5.0 ml of test solution (a) to 250.0 ml with the solvent mixture.

Reference solution (a) Disperse 50.0 mg of *fluorescein CRS* in 15.0 ml of *ethanol (96 per cent) R*. Sonicate and dilute to 50.0 ml with *water R*. Dilute 5.0 ml of this solution to 250.0 ml with the solvent mixture.

Reference solution (b) Dissolve 10.0 mg of *phthalic acid CRS* (impurity B) and 10.0 mg of *resorcinol CRS* (impurity A) in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (c) Dilute 5.0 ml of test solution (b) to 20.0 ml with the solvent mixture.

Reference solution (d) Dilute 10.0 ml of reference solution (c) to 100.0 ml with the solvent mixture.

Reference solution (e) Dissolve the contents of a vial of *fluorescein impurity C CRS* in 1 ml of the solvent mixture.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;

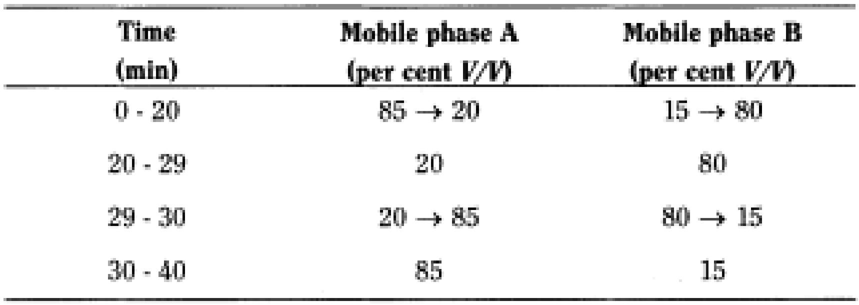
— *stationary phase: octylsilyl silica gel for chromatography R3* (5 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: dissolve 0.610 g of *potassium dihydrogen phosphate R* in *water for chromatography R*, adjust to pH 2.0 with *phosphoric acid R* and dilute to 1000.0 ml with *water for chromatography R*;

— *mobile phase B*: *acetonitrile for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	85 → 20	15 → 80
20 - 29	20	80
29 - 30	20 → 85	80 → 15
30 - 40	85	15

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (a) and reference solutions (b), (c), (d) and (e).

Identification of impurity C Use the chromatogram obtained with reference solution (e) to identify the peak due to impurity C.

Relative retention With reference to fluorescein (retention time = about 15 min): impurity A = about 0.42; impurity B = about 0.48; impurity C = about 0.86.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 1.9;

— *impurity C*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent);

— *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *sum of impurities other than A, B and C*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Chlorides ***(2.4.4)***

Maximum 0.25 per cent.

To 10.0 ml of solution S add 90.0 ml of *water R* and 3.0 ml of *dilute nitric acid R*, wait for at least 30 min and filter. Dilute 10.0 ml of the filtrate to 15.0 ml with *water R*.

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{20}H_{12}O_5$ from the declared content of *fluorescein CRS*.

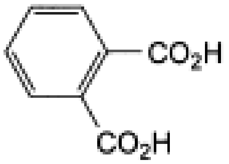
STORAGE

Protected from light.

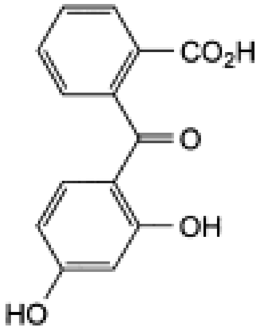
IMPURITIES

Specified impurities A, B, C.

A. resorcinol,



B. benzene-1,2-dicarboxylic acid (phthalic acid),

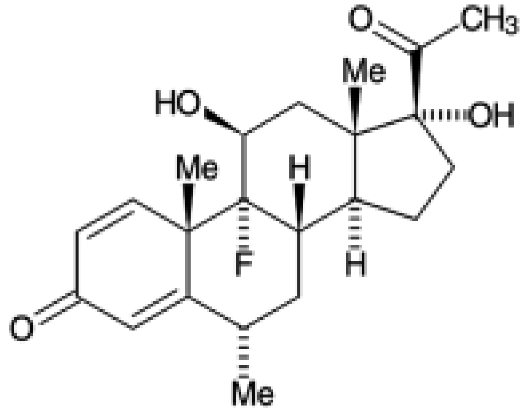


C. 2-(2,4-dihydroxybenzoyl)benzoic acid.

Ph Eur

Fluorometholone

General Notices



$C_{22}H_{29}FO_4$ 376.5 *426-13-1*

Action and use

Glucocorticoid.

Preparation

Fluorometholone Eye Drops

DEFINITION

Fluorometholone is 9α-fluoro-11β,17α-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione. It contains not less than 97.0% and not more than 103.0% of $C_{22}H_{29}FO_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to yellowish white, crystalline powder. It melts at about 280°, with decomposition.

Practically insoluble in *water*; slightly soluble in *absolute ethanol* and in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of fluorometholone *(RS 152)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Specific optical rotation

In a 1% w/v solution in *pyridine*, +52 to +60, Appendix V F, calculated with reference to the dried substance.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in *methanol* containing (1) 0.010% w/v of the substance being examined, (2) 0.00005% w/v of the substance being examined and (3) 0.00005% w/v each of *deltamedrane BPCRS* and *fluorometholone BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 40 volumes of *water* and 60 volumes of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

The test is not valid unless in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to deltamedrane and fluorometholone is at least 1.5.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

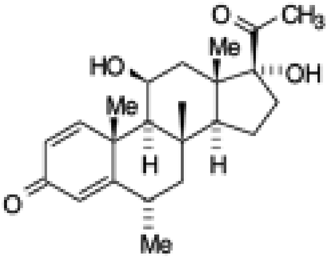
Carry out the method for *liquid chromatography*, Appendix III D, using two solutions in *methanol* containing (1) 0.005% w/v of the substance being examined, (2) 0.005% w/v of *fluorometholone BPCRS* and (3) 0.00005% w/v each of *deltamedrane BPCRS* and *fluorometholone BPCRS*.

The chromatographic procedure described under Related substances may be used.

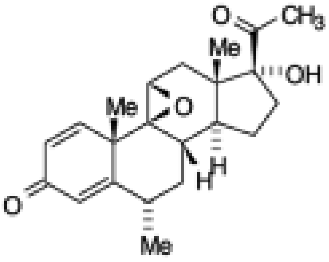
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to deltamedrane and fluorometholone is at least 1.5.

Calculate the content of $C_{22}H_{29}FO_4$ from the chromatograms obtained and using the declared content of $C_{22}H_{29}FO_4$ in *fluorometholone BPCRS.*

IMPURITIES



A. 11β,17α-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione *(deltamedrane)*

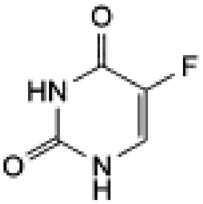


B. 9β,11β-epoxy-17α-hydroxy-6α-methylpregna-1,4-diene-3,20-dione *(epoxymedradiene)*

Fluorouracil

General Notices

(Ph Eur monograph 0611)



$C_4H_3FN_2O_2$ 130.1 *51-21-8*

Action and use

Pyrimidine analogue; cytotoxic.

Preparations

Fluorouracil Cream

Fluorouracil Injection

Ph Eur

DEFINITION

5-Fluoropyrimidine-2,4(1*H*,3*H*)-dione.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison fluorouracil CRS.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 or Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 5.0 for solution S.

Impurities F and G

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.10 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 5.0 mg of *fluorouracil impurity F CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 200.0 ml with the same mixture of solvents.

Reference solution (b) Dissolve 20.0 mg of *urea R* (impurity G) in *methanol R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *methanol R*.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *methanol R, water R*, *ethyl acetate R* (15:15:70 *V/V/V*).

Application 10 µl.

Development Over a path of 2/3 of the plate.

Drying In air.

Detection:

— *impurity F*: examine in ultraviolet light at 254 nm;

— *impurity G*: spray with a mixture of 200 ml of a 10 g/l solution of *dimethylaminobenzaldehyde R* in *anhydrous ethanol R* and 20 ml of *hydrochloric acid R*; dry in an oven at 80 °C for 3-4 min, then examine in daylight (impurity G produces a yellow spot and fluorouracil is not detected by the spray).

System suitability The chromatogram shows 2 clearly separated spots after both detections.

Limits:

— *impurity F*: any spot due to impurity F is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *impurity G*: any spot due to impurity G is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and

dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *fluorouracil impurity C CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *fluorouracil impurity A CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (d) Dissolve 5.0 mg of *fluorouracil impurity B CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (e) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (f) To 1 ml of reference solution (a) add 1 ml of the test solution and dilute to 10 ml with the mobile phase.

Reference solution (g) Dissolve the contents of a vial of *fluorouracil impurity mixture CRS* (containing impurities D and E) in 1.0 ml of the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 6.805 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 5.7 ± 0.1 with *5 M potassium hydroxide*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 266 nm.

Injection 20 µl.

Run time 3 times the retention time of fluorouracil.

Identification of impurities Use the chromatogram supplied with *fluorouracil impurity mixture CRS* and the chromatogram obtained with reference solution (g) to identify the peaks due to impurities D and E.

Relative retention With reference to fluorouracil (retention time = about 6 min): impurity A = about 0.5; impurity B = about 0.7; impurity C = about 0.9; impurity D = about 1.6; impurity E = about 1.9.

System suitability Reference solution (f):

— *resolution*: minimum 2 between the peaks due to impurity C and fluorouracil.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 1.5; impurity E = 1.3;

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram

obtained with reference solution (d) (0.1 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurities D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Use a platinum crucible. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.100 g in 80 ml of *dimethylformamide R*, warming gently. Cool and titrate with *0.1 M tetrabutylammonium hydroxide*, using 0.25 ml of a 10 g/l solution of *thymol blue R* in *dimethylformamide R* as indicator. Carry out a blank titration.

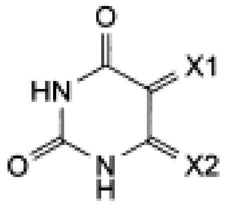
1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 13.01 mg of $C_4H_3FN_2O_2$.

STORAGE

Protected from light.

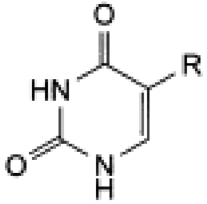
IMPURITIES

Specified impurities A, B, C, D, E, F, G.



A. X1 = H_2, X2 = O: pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (barbituric acid),

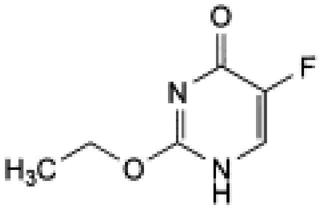
B. X1 = O, X2 = H_2: dihydropyrimidine-2,4,5(3*H*)-trione (isobarbituric acid or 5-hydroxyuracil),



C. R = H: pyrimidine-2,4(1*H*,3*H*)-dione (uracil),

D. R = OCH_3: 5-methoxypyrimidine-2,4(1*H*,3*H*)-dione (5-methoxyuracil),

E. R = Cl: 5-chloropyrimidine-2,4(1*H*,3*H*)-dione (5-chlorouracil),



F. 2-ethoxy-5-fluoropyrimidin-4(1*H*)-one (2-ethoxy-5-fluorouracil),

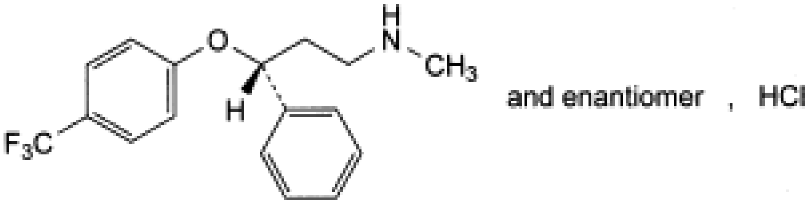
G. urea.

Ph Eur

Fluoxetine Hydrochloride

General Notices

(Ph Eur monograph 1104)



$C_{17}H_{18}F_3NO,HCl$ 345.8 *59333-67-4*

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

Preparations

Fluoxetine Capsules

Fluoxetine Oral Solution

Ph Eur

DEFINITION

(3*RS*)-*N*-Methyl-3-phenyl-3-[4-trifluoromethyl)phenoxy]propan-1-amine hydrochloride.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in methanol, sparingly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometer *(2.2.24)*.

Preparation Discs.

Comparison *fluoxetine hydrochloride CRS* .

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R*, then dilute to 100 ml with the same mixture of solvents.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 6.5.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Optical rotation *(2.2.7)*

- 0.05° to + 0.05°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 55.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Test solution (b) Dilute 2.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution Dissolve 22.0 mg of *fluoxetine hydrochloride CRS* in 10.0 ml of *0.5 M sulphuric acid* . Heat at about 85 °C for 3 h. Allow to cool. The resulting solution contains considerable quantities of impurity A and usually also contains 4-trifluoromethylphenol. To 0.4 ml of this solution add 28.0 mg of *fluoxetine hydrochloride CRS* , about 1 mg of *fluoxetine impurity B CRS* and about 1 mg of *fluoxetine impurity C CRS* , then dilute to 25.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 8 volumes of *methanol R*, 30 volumes of *tetrahydrofuran R* and 62 volumes of a solution of *triethylamine R* prepared as follows: to 10 ml of *triethylamine R*, add 980 ml of *water R*, mix and adjust to pH 6.0 with *phosphoric acid R* (about 4.5 ml) and dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 10 µl.

Run time 3 times the retention time of fluoxetine.

Relative retention

With reference to fluoxetine: impurity A = about 0.24; impurity B = about 0.27; impurity C = about 0.94.

System suitability Reference solution:

— *retention time*: fluoxetine = 10 min to 18 min; 4-trifluoromethylphenol: maximum 35 min; if no peak due to 4-trifluoromethylphenol is observed, inject a 0.02 per cent solution of *4-trifluoromethylphenol R* in the mobile phase;

— *peak-to-valley ratio*: maximum 1.1, where H_p = height above the baseline of the peak due to impurity C and H_v = height between the top of the peak due to impurity C and the lowest point of the valley between this peak and the peak due to fluoxetine; if necessary, reduce the volume of methanol and increase the volume of the solution of triethylamine in the mobile phase.

Limit Test solution (b):

— *impurity C*: not more than 0.0015 times the area of the principal peak (0.15 per cent).

Limits Test solution (a):

— *impurities A, B*: for each impurity, not more than 0.0125 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.25 per cent);

— *unspecified impurities*: for each impurity, not more than 0.005 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.10 per cent);

— *total:* not more than 0.025 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.5 per cent);

— *disregard limit*: 0.0025 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.05 per cent).

Acetonitrile

Gas chromatography *(2.2.28)*.

Test solution Dissolve 50 mg of the substance to be examined in *dimethylformamide R* and dilute to 5.0 ml with the same solvent.

Reference solution To 1.0 g of *acetonitrile R*, add *dimethylformamide R*, mix and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 1000.0 ml with *dimethylformamide R*.

Column:

— *material*: fused silica;

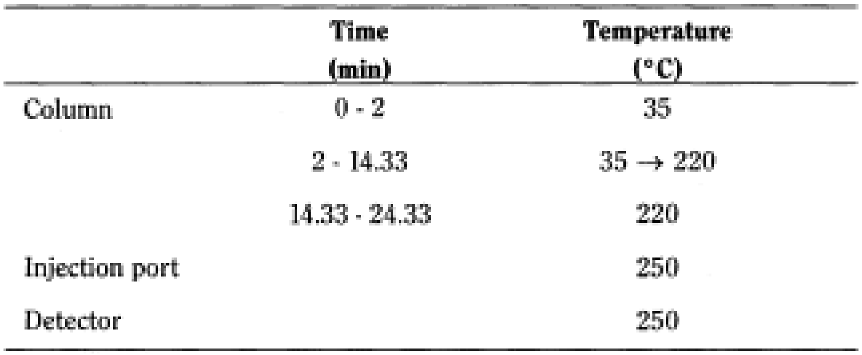
— *size:* *l* = 30 m, Ø = 0.53 mm;

— *stationary phase*: *macrogol 20 000 R* (film thickness 1 µm).

Carrier gas *helium for chromatography R*.

Flow rate 10 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	35
	2 - 14.33	35 → 220
	14.33 - 24.33	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl; inject *dimethylformamide R* as a blank.

In the chromatogram obtained with *dimethylformamide R*, verify that there is no peak with the same retention time as acetonitrile.

Limit:

— *acetonitrile*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Test solution Dissolve 55.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution Dissolve 55.0 mg of *fluoxetine hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Detection Spectrophotometer at 227 nm.

Retention time Fluoxetine = 10 min to 18 min; if necessary, adjust the volumes of methanol and of the solution of triethylamine in the mobile phase.

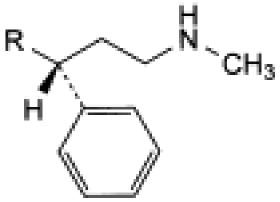
System suitability Reference solution:

— *symmetry factor*: maximum 2.0 calculated at 10 per cent of the height of the peak due to fluoxetine.

Calculate the content of $C_{17}H_{19}ClF_3NO$ from the declared content of *fluoxetine hydrochloride CRS.*

IMPURITIES

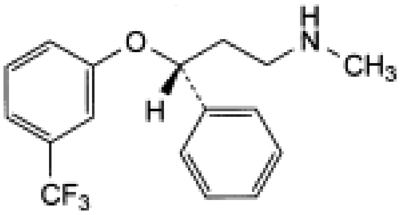
Specified impurities *A, B, C*.



and enantiomer

A. R = OH: (1*RS*)-3-(methylamino)-1-phenylpropan-1-ol,

B. R = H: *N*-methyl-3-phenylpropan-1-amine,



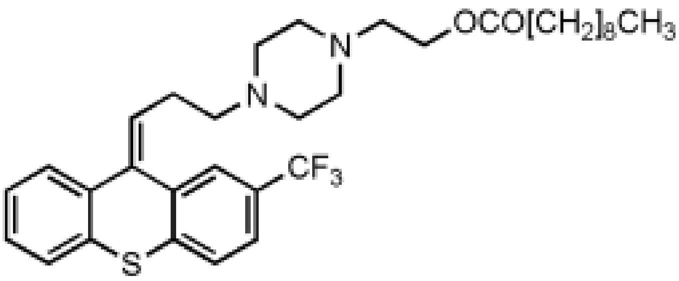
and enantiomer

C. (3*RS*)-*N*-methyl-3-phenyl-3-[3-(trifluoromethyl)phenoxy]propan-1-amine.

Ph Eur

Flupentixol Decanoate

General Notices



$C_{33}H_{43}F_3N_2O_2S$ 588.82 *30909-51-4*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Flupentixol Injection

DEFINITION

Flupentixol Decanoate is (*Z*)-2-{4-[3-(2-trifluoromethylthioxanthen-9-ylidene)propyl]piperazin-1-yl}ethyl decanoate. It contains not less than 98.0% and not more than 101.0% of $C_{33}H_{43}F_3N_2O_2S$, calculated with reference to the dried substance.

CHARACTERISTICS

A yellow, viscous oil.

Very slightly soluble in *water*; soluble in *ethanol (96%)*; freely soluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 350 nm of a 0.0015% w/v solution in *ethanol (96%)* exhibits two maxima at 230 nm and 264 nm. The *absorbances* at the maxima are about 0.85 and about 0.35, respectively.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of flupentixol decanoate *(RS 154)*.

TESTS

Heavy metals

1.0 g complies with *limit test C for heavy metals*, Appendix VII (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

Related substances

Carry out the method for *liquid chromatography,* Appendix III D**,** using the following solutions in *acetonitrile,* protected from light. Solution (1) contains 0.25% w/v of the substance being examined. Solution (2) contains 0.000625% w/v of cis*-flupentixol BPCRS.* Solution (3) contains 0.000625% w/v of *2-trifluoromethylthioxanthone BPCRS.* Solution (4) contains 0.0025% w/v of trans*-flupentixol decanoate dihydrochloride BPCRS.* Solution (5) contains 0.25% w/v of the substance being examined and 0.000625% w/v each of cis*-flupentixol BPCRS, 2-trifluoromethylthioxanthone BPCRS* and 0.0025% w/v of trans-*flupentixol decanoate dihydrochloride BPCRS.*

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable) maintained at a temperature of 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 0.1 volume of *orthophosphoric acid ,* 25 volumes of a 20 millimole solution of *dioctyl sodium sulphosuccinate* (prepared by dissolving 8.89 g of *dioctyl sodium sulphosuccinate* in 500 ml of *water,* stirring for 6 to 8 hours and diluting to 1000 ml with *water)* and *75* volumes of *ethanol (96%)* and (c) a detection wavelength of 270 nm.

Inject solution (1) and allow the chromatography to proceed for 1.5 times the retention time of the principal peak. Inject 20 µl of each solution. The substances are eluted in the following order: 2-trifluoromethylthioxanthone, *cis*-flupentixol (free alcohol), flupentixol decanoate and *trans*-flupentixol decanoate. The test is not valid unless the solution obtained with solution (5) shows clearly separated peaks.

In the chromatogram obtained with solution (1) the areas of any peaks corresponding to *cis*-flupentixol and 2-trifluoromethylthioxanthone respectively are not greater than the areas of the principal peaks in the chromatograms obtained with solutions (2) and (3) respectively (0.25% each) and the area of any peak corresponding to *trans*-flupentixol decanoate dihydrochloride is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1.0%).

Loss on drying

When dried at 60° at a pressure of 0.7 kPa for 3 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

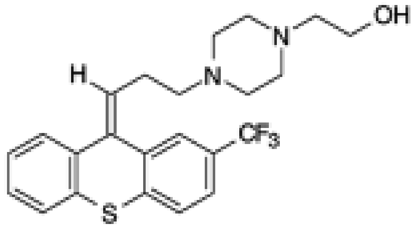
ASSAY

Dissolve 0.2 g in 50 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 29.44 mg of $C_{33}H_{43}F_3N_2O_2S$.

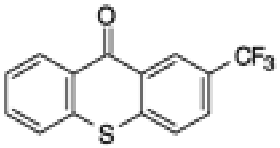
STORAGE

Flupentixol Decanoate should be protected from light and stored at a temperature below -15°.

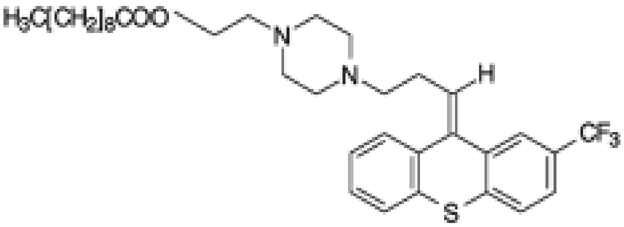
IMPURITIES



A. *cis*-flupentixol (free alcohol),



B. 2-trifluoromethylthioxanthone,

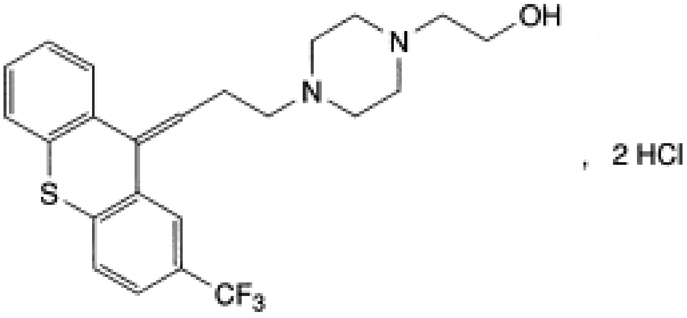


C. *trans*-flupentixol decanoate.

Flupentixol Hydrochloride

General Notices

(Flupentixol Dihydrochloride, Ph Eur monograph 1693)



$C_{23}H_{25}F_3N_2OS,2HCl$ *507.4* *2413-38-9*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

2-[4-[3-[(*EZ*)-2-(trifluoromethyl)-9*H*-thioxanthen-9-ylidene]propyl]piperazin-1-yl]ethanol dihydrochloride.

Content

- — flupentixol dihydrochloride: 98.0 per cent to 101.5 per cent (dried substance),
- — *Z*-isomer: 42.0 per cent to 52.0 per cent.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very soluble in water, soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *flupentixol dihydrochloride CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *flupentixol dihydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *water R*, *diethylamine R*, *methyl ethyl ketone R* (1:4:95 *V/V/V*).

Application 2 µl.

Development Twice over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution. Doubling of the spot may be observed in both chromatograms.

Detection B Spray with *alcoholic solution of sulphuric acid R*; heat at 110 °C for 5 min and allow to cool; examine in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution. Doubling of the spot may be observed in both chromatograms.

C. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter and add to the filtrate a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R.* Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow. The blank is red.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution GY_6 *(2.2.2, Method II)*.

Dissolve 2.0 g of the substance to be examined in *water R* and dilute to 20 ml with the same solvent.

pH *(2.2.3)*

2.0 to 3.0.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Related substances

Thin-layer chromatography *(2.2.27)*. *Carry out the test protected from light and prepare the solutions immediately before use.*

Test solution (a) Dissolve 0.40 g of the substance to be examined in *alcohol R* and dilute to 20 ml with the same solvent.

Test solution (b) Dilute 2.0 ml of test solution (a) to 20.0 ml with *alcohol R*.

Reference solution (a) Dilute 1.0 ml of test solution (b) to 50.0 ml with *alcohol R*.

Reference solution (b) Dilute 2.0 ml of reference solution (a) to 20.0 ml with *alcohol R*.

Reference solution (c) Dissolve 10 mg of *flupentixol impurity D CRS* in *alcohol R*, add 0.5 ml of test solution (a) and dilute to 20.0 ml with *alcohol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *diethylamine R*, *toluene R*, *ethyl acetate R* (10:20:70 *V/V/V*).

Application 5 µl.

Development In an unsaturated tank over a path of 10 cm.

Drying In air.

Detection Spray with *alcoholic solution of sulphuric acid R,* heat at 110 °C for 5 min and allow to cool; examine in ultraviolet light at 365 nm. Doubling of the spot due to flupentixol may be observed.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Limits:

— in the chromatogram obtained with test solution (a): any spots, apart from the principal spot, are not more intense than the spot, or spots in the chromatogram obtained with reference solution (a) (0.2 per cent),

— in the chromatogram obtained with test solution (b): any spots, apart from the principal spot, are not more intense than the spot or spots in the chromatogram obtained with reference solution (b) (0.2 per cent).

Impurity F

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light and prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution Dissolve 10.0 mg of *flupentixol dihydrochloride CRS* and 10.0 mg of *flupentixol impurity F CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Column:

— *size:* l = 0.125 m, Ø = 4.6 mm,

— *stationary phase*: *octylsilyl silica gel for chromatography R* (3 µm)

Mobile phase Mix 10 volumes of *acetonitrile R*, 55 volumes of *methanol R* and 35 volumes of a solution containing 8.72 g/l *of potassium dihydrogen phosphate R*, 0.37 g/l of *anhydrous disodium hydrogen phosphate R* and 0.77 g/l of *dodecyltrimethylammonium bromide R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µl.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the 2nd of the peaks due to impurity F and the 1st of the peaks due to flupentixol. Peak splitting may not always occur.

Limit:
— *impurity F*: not more than the area of the corresponding peak or peaks in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Flupentixol dihydrochloride . Dissolve 0.200 g in 30 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 50.74 mg of $C_{23}H_{27}Cl_2F_3N_2OS$.

Z-Isomer

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution Dissolve 20.0 mg of *flupentixol dihydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.25 m, Ø = 4.0 mm,

— *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase *water R*, *concentrated ammonia R*, *2-propanol R*, *heptane R* (2:4:150:850 *V/V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

System suitability Reference solution:

— *resolution*: minimum 3.0 between the peaks due to *Z*-isomer (1st peak) and to *E*-isomer (2nd peak).

Results:

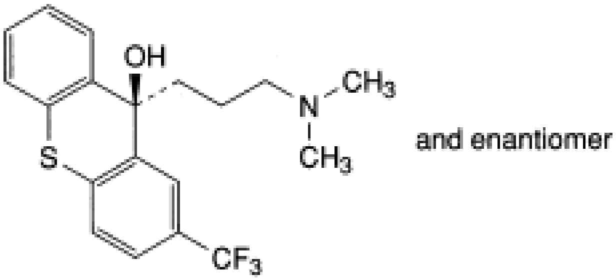
— calculate the percentage content of *Z*-isomer taking into account the assigned content of *Z*-isomer in *flupentixol dihydrochloride CRS* ,

— calculate the ratio of the area of the peak due to the *E*-isomer to the area of the peak due to the *Z*-isomer: this ratio is 0.9 to 1.4.

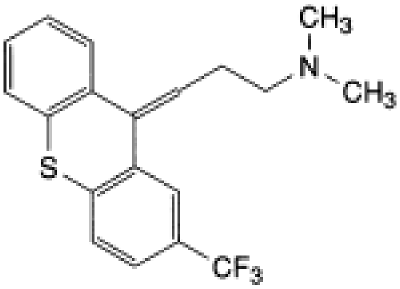
STORAGE

Protected from light.

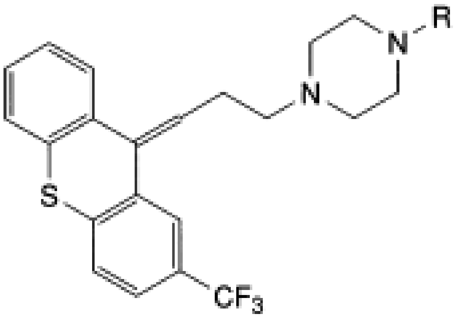
IMPURITIES



A. (9*RS*)-9-[3-(dimethylamino)propyl]-2-(trifluoromethyl)-9*H*-thioxanthen-9-ol,



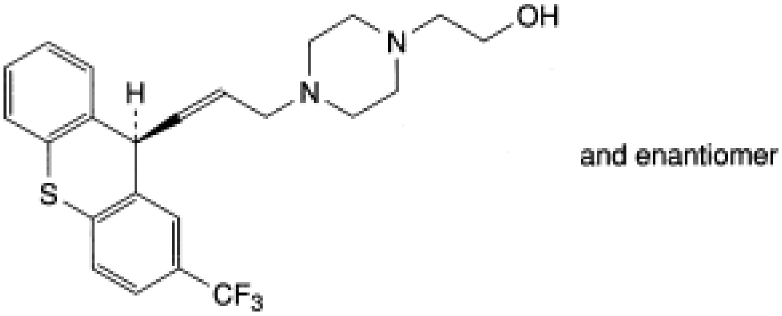
B. *N*,*N*-dimethyl-3-[(*EZ*)-2-(trifluoromethyl)-9*H*-thioxanthen-9-ylidene]propan-1-amine,



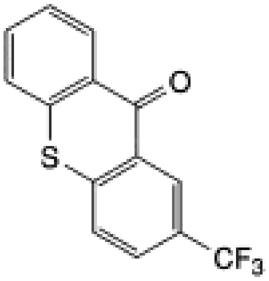
C. R = H: 1-[3-[(*EZ*)-2-(trifluoromethyl)-9*H*-thioxanthen-9-ylidene]propyl]piperazine,

D. R = CH_2-CH_2-O-CH_2-CH_2-OH: 2-[2-[4-[3-[(*EZ*)-2-(trifluoromethyl)-9*H*-thioxanthen-9-ylidene]propyl]piperazin-1-yl]ethoxy]ethanol,

E. R = CH_2-CH_2-O-CO-CH_3: 2-[4-[3-[(*EZ*)-2-(trifluoromethyl)-9*H*-thioxanthen-9-ylidene]propyl] piperazin-1-yl]ethyl acetate,



F. 2-[4-[(*EZ*)-3-[(9*RS*)-2-(trifluoromethyl)-9*H*-thioxanthen-9-yl]prop-2-enyl]piperazin-1-yl] ethanol,



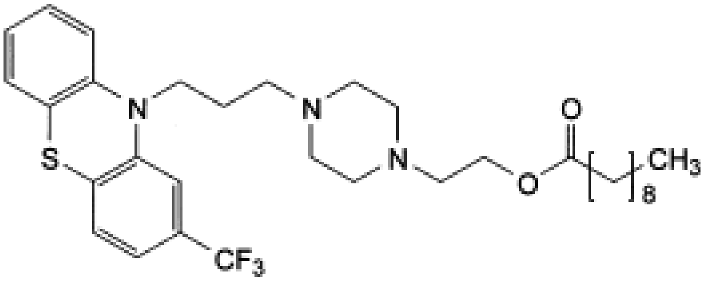
G. 2-(trifluoromethyl)-9*H*-thioxanthen-9-one.

Ph Eur

Fluphenazine Decanoate

General Notices

(Ph Eur monograph 1014)



$C_{32}H_{44}F_3N_3O_2S$ *591.8* *5002-47-1*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Fluphenazine Decanoate Injection

Ph Eur

DEFINITION

2-[4-[3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]piperazin-1-yl]ethyl decanoate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Pale yellow, viscous liquid or yellow solid.

Solubility

Practically insoluble in water, very soluble in ethanol and in methylene chloride, freely soluble in methanol.

IDENTIFICATION

First identification B, C.

Second identification A, C.

A. Dissolve 50.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 50.0 ml with *methanol R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 260 nm and a broad absorption maximum at about 310 nm. The specific absorbance at the maximum at 260 nm is 570 to 630.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Apply 50 µl of a 25 g/l solution in *methylene chloride R* to a disc of *potassium bromide R*. Dry the discs at 60 °C for 1 h before use.

Comparison *fluphenazine decanoate CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *fluphenazine decanoate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *fluphenazine enantate CRS* in reference solution (a) and dilute to 5 ml with the same solution.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R*.

Mobile phase *concentrated ammonia R1*, *water R*, *methanol R* (1:4:95 *V/V/V*).

Application 2 µl.

Development Over a path of 8 cm.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light and prepare the solutions immediately before use.*

Test solution Dissolve 10.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *fluphenazine octanoate CRS* and 5 mg of *fluphenazine enantate CRS* in *acetonitrile R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with a mixture of 5 volumes of mobile phase A and 95 volumes of mobile phase B. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of 5 volumes of mobile phase A and 95 volumes of mobile phase B.

Reference solution (c) Dissolve 11.7 mg of *fluphenazine dihydrochloride CRS* and 5.0 mg of *fluphenazine sulphoxide CRS* in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 1.0 ml to 50.0 ml with a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R.*

Column:

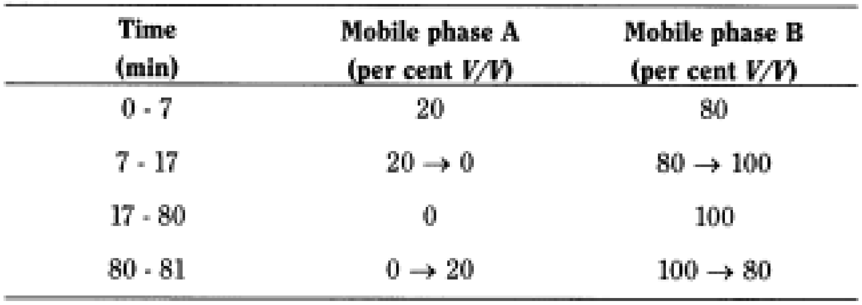
— *size: l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: 10 g/l solution of *ammonium carbonate R* adjusted to pH 7.5 with *dilute hydrochloric acid R*,

— *mobile phase B*: mobile phase A, *acetonitrile R*, *methanol R* (7.5:45:45 *V/V/V*),



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	20	80
7 - 17	20 → 0	80 → 100
17 - 80	0	100
80 - 81	0 → 20	100 → 80

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 260 nm.

Equilibration At least 30 min with the mobile phase at the initial composition.

Injection 10 µl.

Relative retention With reference to fluphenazine decanoate (retention time = about 34 min): impurity A = about 0.13; impurity B = about 0.33; impurity C = about 0.76; impurity D = about 0.82.

System suitability Reference solution (a):

— *resolution*: minimum 6 between the peaks due to impurity C and impurity D.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *total*: not more than 2.0 per cent,

— *disregard limit for any other impurity*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10* *ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

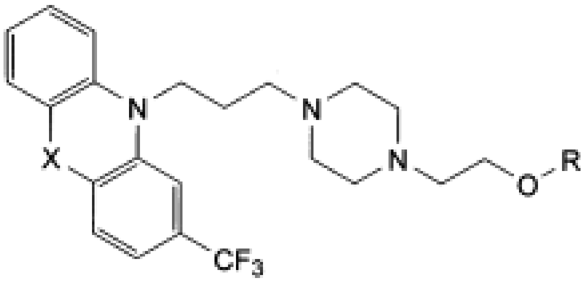
Dissolve 0.250 g in 30 ml of *glacial acetic acid R*. Using 0.05 ml of *crystal violet solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from violet to green.

1 ml of *0.1 M perchloric acid* is equivalent to 29.59 mg of $C_{32}H_{44}F_3N_3O_2S$.

STORAGE

Protected from light.

IMPURITIES



A. X = SO, R = H: fluphenazine *S*-oxide,

B. X = S, R = H: fluphenazine,

C. X = S, R = CO-$[CH_2]_5$-CH_3: fluphenazine enantate,

D. X = S, R = CO-$[CH_2]_6$-CH_3: fluphenazine octanoate,

E. X = S, R = CO-$[CH_2]_7$-CH_3: fluphenazine nonanoate,

F. X = S, R = CO-$[CH_2]_9$-CH_3: fluphenazine undecanoate,

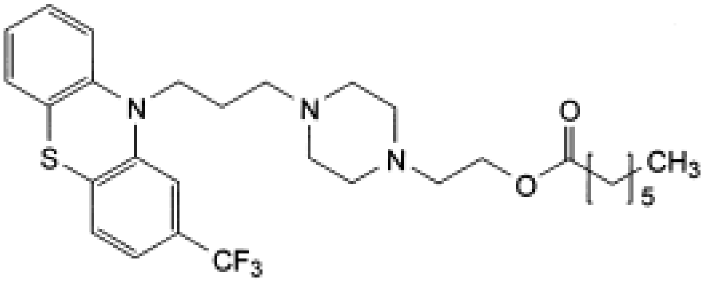
G. X = S, R = CO-$[CH_2]_{10}$-CH_3: fluphenazine dodecanoate.

Ph Eur

Fluphenazine Enantate

General Notices

(Ph Eur monograph 1015)



$C_{29}H_{38}F_3N_3O_2S$ *549.7* *2746-81-8*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

2-[4-[3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]piperazin-1-yl]ethyl heptanoate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Pale yellow, viscous liquid or yellow solid.

Solubility

Practically insoluble in water, very soluble in ethanol and in methylene chloride, freely soluble in methanol.

IDENTIFICATION

First identification B, C.

Second identification A, C.

A. Dissolve 50.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0

ml to 50.0 ml with *methanol R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 260 nm and a broad absorption maximum at about 310 nm. The specific absorbance at the maximum at 260 nm is 610 to 670.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Apply 50 µl of a 25 g/l solution in *methylene chloride R* to a disc of *potassium bromide R.* Dry the discs at 60 °C for 1 h before use.

Comparison *fluphenazine enantate CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *fluphenazine enantate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *fluphenazine decanoate CRS* in reference solution (a) and dilute to 5 ml with the same solution.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R*.

Mobile phase *concentrated ammonia R1*, *water R*, *methanol R* (1:4:95 *V/V/V*).

Application 2 µl.

Development Over a path of 8 cm.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light and prepare the solutions immediately before use.*

Test solution Dissolve 10.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *fluphenazine octanoate CRS* and 5 mg of *fluphenazine enantate CRS* in *acetonitrile R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with a mixture of 5 volumes of mobile phase A and 95 volumes of mobile phase B. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of 5 volumes of mobile phase A and 95 volumes of mobile phase B.

Reference solution (c) Dissolve 5.0 mg of *fluphenazine sulphoxide CRS* in *acetonitrile R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 50.0 ml with *acetonitrile R*.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: 10 g/l solution of *ammonium carbonate R* adjusted to pH 7.5 with *dilute hydrochloric acid R,*

— *mobile phase B*: mobile phase A, *acetonitrile R*, *methanol R* (7.5:45:45 *V/V/V*),

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	20	80
7 - 17	20 → 0	80 → 100
17 - 80	0	100
80 - 81	0 → 20	100 → 80

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 260 nm.

Equilibration At least 30 min with the mobile phase at the initial composition.

Injection 10 µl.

Relative retention With reference to fluphenazine enantate (retention time = about 25 min): impurity A = about 0.2; impurity D = about 1.1.

System suitability Reference solution (a):

— *resolution*: minimum 6 between the peaks due to fluphenazine enantate and impurity D.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *total*: not more than 1.6 per cent,

— *disregard limit for any other impurity*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

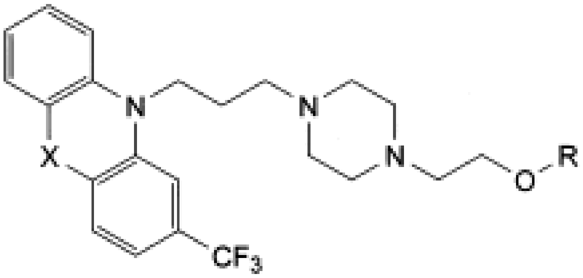
Dissolve 0.250 g in 30 ml of *glacial acetic acid R*. Using 0.05 ml of *crystal violet solution R* as indicator titrate with *0.1 M perchloric acid* until the colour changes from violet to green.

1 ml of *0.1 M perchloric acid* is equivalent to 27.49 mg of $C_{29}H_{38}F_3N_3O_2S$.

STORAGE

Protected from light.

IMPURITIES



A. X = SO, R = H: fluphenazine *S*-oxide,

B. X = S, R = H: fluphenazine,

C. X = S, R = CO-$[CH_2]_8$-CH_3: fluphenazine decanoate,

D. X = S, R = CO-$[CH_2]_6$-CH_3: fluphenazine octanoate,

E. X = S, R = CO-$[CH_2]_7$-CH_3: fluphenazine nonanoate,

F. X = S, R = CO-$[CH_2]_9$-CH_3: fluphenazine undecanoate,

G. X = S, R = CO-$[CH_2]_{10}$-CH_3: fluphenazine dodecanoate.

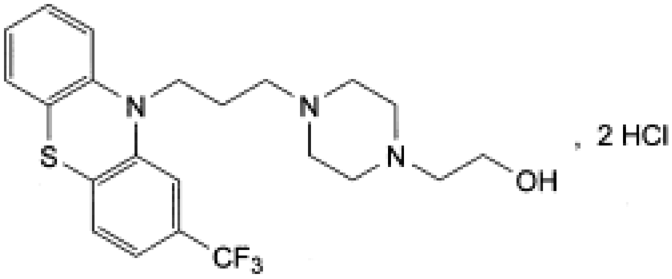
Ph Eur

Fluphenazine Hydrochloride

General Notices

Fluphenazine Dihydrochloride

(Fluphenazine Dihydrochloride, Ph Eur monograph 0904)



$C_{22}H_{26}F_3N_3OS,2HCl$ 510.5 *146-56-5*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Fluphenazine Tablets

Ph Eur

DEFINITION

2-[4-[3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]piperazin-1-yl]ethanol dihydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *methanol R*.

Spectral range 230-350 nm.

Absorption maxima At 260 nm and at about 310 nm (broad band).

Specific absorbance at the absorption maximum at 260 nm 630 to 700.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fluphenazine dihydrochloride CRS* .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *fluphenazine dihydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *perphenazine CRS* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R.*

Mobile phase *concentrated ammonia R1*, *water R*, *methanol R* (1:4:95 *V/V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

1.9 to 2.4.

Dissolve 0.5 g in 10 ml of *water R*.

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light and prepare the solutions immediately before use.*

Test solution Dissolve 25.0 mg of the substance to be examined in mobile phase B and dilute to 50.0 ml with mobile phase B.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase B.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 25.0 ml with mobile phase B.

Reference solution (c) Dissolve the contents of a vial of *fluphenazine impurity mixture CRS* (containing impurities A, B, C and D) in 1 ml of the test solution.

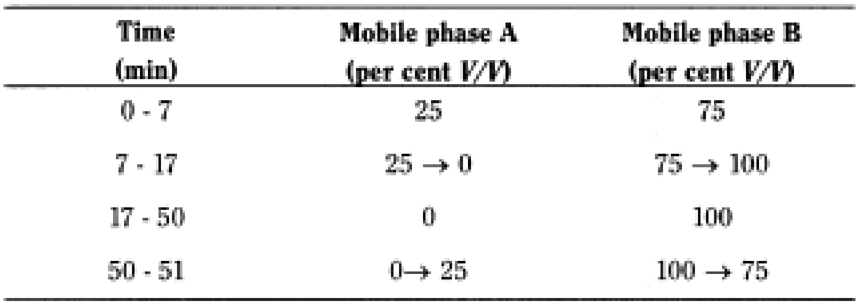
Reference solution (d) Dissolve 5.0 mg of *fluphenazine sulphoxide CRS* (impurity A) in mobile phase B and dilute to 50.0 ml with mobile phase B. Dilute 1.0 ml of this solution to 100.0 ml with mobile phase B.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 10 g/l solution of *ammonium carbonate R* adjusted to pH 7.5 with *dilute hydrochloric acid R*;

— *mobile phase B*: mobile phase A, *acetonitrile R*, *methanol R* (7.5:45:45 *V/V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	25	75
7 - 17	25 → 0	75 → 100
17 - 50	0	100
50 - 51	0→ 25	100 → 75

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 260 nm and at 274 nm.

Injection 10 µl of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *fluphenazine impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C and D.

Relative retention With reference to fluphenazine (retention time = about 14.5 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 1.8; impurity D = about 2.2.

System suitability Reference solution (c):

— *resolution*: minimum 2.5 between the peaks due to impurities A and B.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.3; impurity C = 0.6;

— *impurity A* at 274 nm: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.2 per cent);

— *impurity B* at 274 nm: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities C, D at 260 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities at 260 nm*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *sum of the impurities at 260 nm and impurities A and B at 274 nm*: maximum 1.0 per cent;

— *disregard limit at 260 nm*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 65 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

In order to avoid overheating during the titration, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.220 g in a mixture of 10 ml of *anhydrous formic acid R* and 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

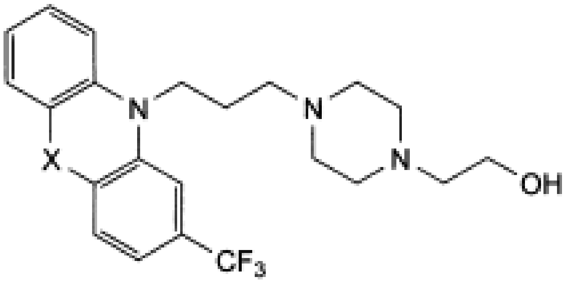
1 ml of *0.1 M perchloric acid* is equivalent to 25.52 mg of $C_{22}H_{28}C_{l2}F_{3}N_{3}OS$.

STORAGE

Protected from light.

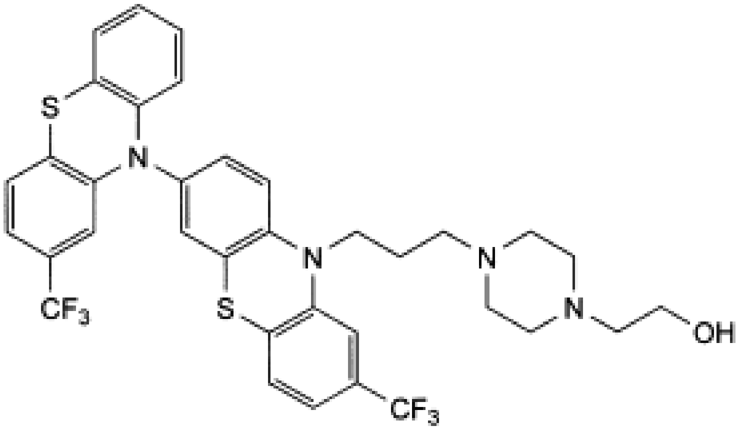
IMPURITIES

Specified impurities *A, B, C, D.*

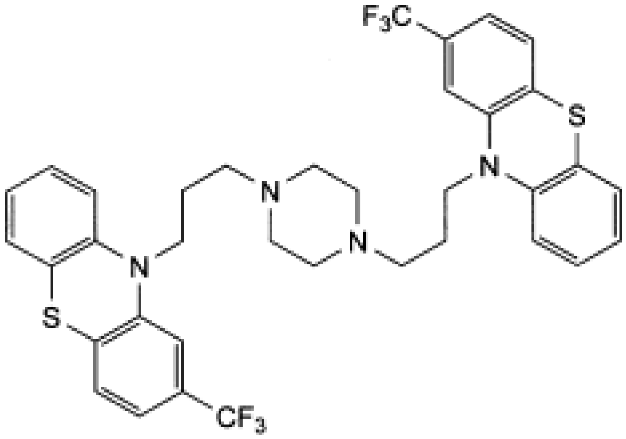


A. X = SO: 2-[4-[3-[5-oxo-2-(trifluoromethyl)-10*H*-5^4-phenothiazin-10-yl]propyl]piperazin-1-yl] ethanol (fluphenazine *S*-oxide),

B. X = SO_2: 2-[4-[3-[5,5-dioxo-2-(trifluoromethyl)-10*H*-5^6-phenothiazin-10-yl]propyl]piperazin-1-yl]ethanol (fluphenazine *S*,*S*-dioxide),



C. 2-[4-[3-[2′,8-bis(trifluoromethyl)-10*H*-3,10′-biphenothiazin-10-yl]propyl]piperazin-1-yl] ethanol,



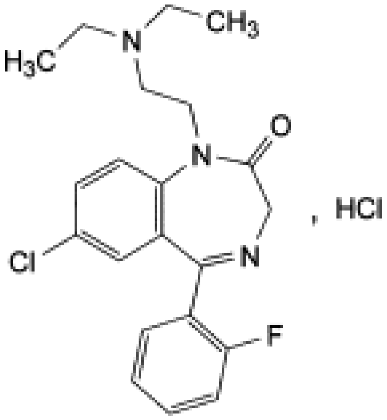
D. 10,10′-[piperazine-1,4-diylbis(propane-3,1-diyl)]bis[2-(trifluoromethyl)-10*H*-phenothiazine]
.

Ph Eur

Flurazepam Monohydrochloride

General Notices

(Ph Eur monograph 0905)



$C_{21}H_{23}ClFN_3O,HCl$ 424.3 *36105-20-1*

Action and use

Benzodiazepine.

Preparation

Flurazepam Capsules

Ph Eur

DEFINITION

7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one monohydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of flurazepam monohydrochloride*.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

5.0 to 6.0.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *oxazepam R* in 10 ml of *acetonitrile R* and dilute to 50.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 350 volumes of *acetonitrile R* and 650 volumes of a 10.5 g/l solution of *potassium dihydrogen phosphate R* and ajust to pH 6.1 with a 40 g/l solution of *sodium hydroxide R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 239 nm.

Injection 20 µl.

Run time 6 times the retention time of flurazepam.

Relative retention With reference to flurazepam (retention time = about 7 min): impurity C = about 1.5; impurity B = about 1.9; impurity A = about 2.4.

System suitability Reference solution (b):

— *resolution*: minimum of 4.5 between the peaks due to flurazepam and to oxazepam.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.61; impurity C = 0.65,

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Fluorides *(2.4.5)*

Maximum 500 ppm.

0.10 g complies with the limit test for fluorides.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

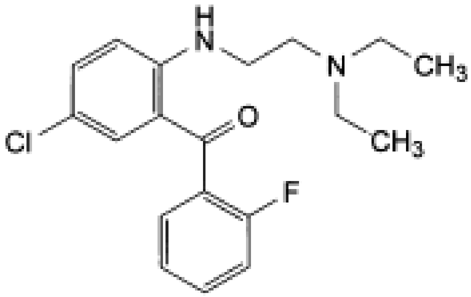
Dissolve 0.350 g in a mixture of 1.0 ml of *0.1 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 42.43 mg of $C_{21}H_{24}Cl_2FN_3O$.

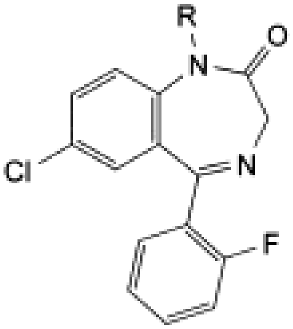
STORAGE

Protected from light.

IMPURITIES



A. [5-chloro-2-[[2-(diethylamino)ethyl]amino]phenyl](2-fluorophenyl)methanone,



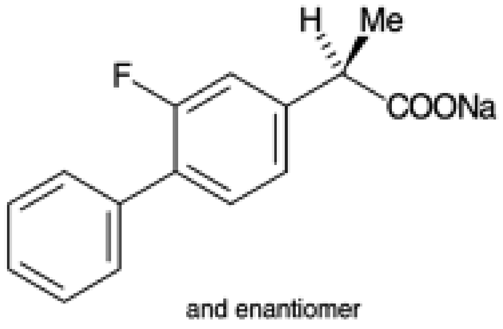
B. R = H: 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,

C. R = $CHOH\text{-}CH_3$: 7-chloro-5-(2-fluorophenyl)-1-[(1*RS*)-1-hydroxyethyl]-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Ph Eur

Flurbiprofen Sodium

General Notices



$C_{15}H_{12}FNaO_2,2H_2O$ 302.3 *56767-76-1*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparation

Flurbiprofen Eye Drops

DEFINITION

Flurbiprofen Sodium is sodium (*RS*)-2-(2-fluorobiphenyl-4-yl)propionate dihydrate. It contains not less than 98.5% and not more than 101.5% of $C_{15}H_{12}FNaO_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to creamy-white, crystalline powder.

Sparingly soluble in *water*; soluble in *ethanol (96%)*; practically insoluble in *dichloromethane*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of flurbiprofen sodium *(RS 157)*.

B. Heat 0.2 g over a flame until charred and then heat at 600° for 2 hours. The residue yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in a mixture of

25 volumes of *water* and 50 volumes of *methanol* containing (1) 0.10% w/v of the substance being examined, (2) 0.00020% w/v of the substance being examined, (3) 0.00050% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS* and (4) 0.00050% w/v of the substance being examined and 0.00050% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Resolve 5µ is suitable), (b) a mixture of 5 volumes of *glacial acetic acid*, 35 volumes of *acetonitrile* and 60 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm. Adjust the sensitivity so that the heights of the principal peaks in the chromatogram obtained with solution (4) are about 40% of full-scale deflection on the chart paper.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 1.5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(biphenyl-4-yl)propionic acid is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any *secondary peaks* is not greater than five times the area of the peak in the chromatogram obtained with solution (2) (1%).

Heavy metals

12 ml of a 20% w/v solution in *methanol* complies with *limit test A for heavy metals*, Appendix VII (10 ppm). Use 10 ml of the solution obtained by diluting 10 ml of *lead standard solution (20 ppm Pb)* to 100 ml with *methanol* to prepare the standard and 10 ml of *methanol* and 2 ml of the solution of the substance being examined to prepare the reagent blank.

Loss on drying

11.3% to 12.5% when determined by drying over *phosphorus pentoxide* at 60° at a pressure of 2 kPa for 18 hours. Use 1 g.

ASSAY

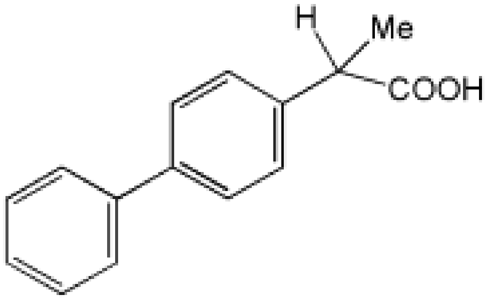
Carry out the method for *liquid chromatography*, Appendix III D, using solutions in a mixture of 25 volumes of *water* and 50 volumes of *methanol* containing (1) 0.015% w/v of the substance being examined, (2) 0.015% w/v of *flurbiprofen sodium BPCRS* and (3) 0.00075% w/v of the substance being examined and 0.00075% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS*.

The chromatographic procedure described under Related substances may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.5.

Calculate the content of $C_{15}H_{12}FNaO_2$ in the substance being examined using the declared content of $C_{15}H_{12}FNaO_2$ in *flurbiprofen sodium BPCRS*.

IMPURITIES

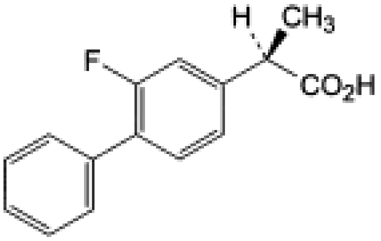


A. 2-(biphenyl-4-yl)propionic acid

Flurbiprofen

General Notices

(Ph Eur monograph 1519)



and enantiomer

$C_{15}H_{13}FO_2$ 244.3 *5104-49-4*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Flurbiprofen Suppositories

Flurbiprofen Tablets

Ph Eur

DEFINITION

(2*RS*)-2-(2-Fluorobiphenyl-4-yl)propanoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride. It dissolves in aqueous solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification C, D.

Second identification A, B, D.

A. Melting point *(2.2.14)*: 114 °C to 117 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 0.10 g in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same alkaline solution. Dilute 1.0 ml of this solution to 100.0 ml with *0.1 M sodium hydroxide*.

Spectral range 230-350 nm.

Absorption maximum At 247 nm.

Specific absorbance at the absorption maximum 780 to 820.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *flurbiprofen CRS*.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R* add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method I)*.

Dissolve 1.0 g in *methanol R* and dilute to 10 ml with the same solvent.

Optical rotation *(2.2.7)*

- 0.1° to + 0.1°.

Dissolve 0.50 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile R*, *water R* (45:55 *V/V*).

Test solution Dissolve 0.20 g of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 10.0 mg of *flurbiprofen impurity A CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 10.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (c) Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with reference solution (b).

Column:

- *size: l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R*, *acetonitrile R*, *water R* (5:35:60 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time Twice the retention time of flurbiprofen.

System suitability Reference solution (c):

- *resolution*: minimum 1.5 between the peaks due to impurity A and flurbiprofen.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurities B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *sum of impurities other than A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 10 volumes of *water R* and 90 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 10 volumes of *water R* and 90 volumes of *methanol R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

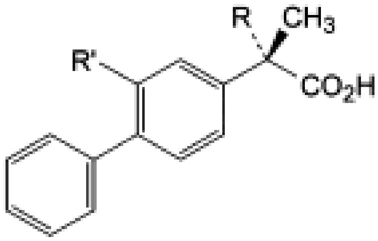
ASSAY

Dissolve 0.200 g in 50 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 24.43 mg of $C_{15}H_{13}FO_2$.

IMPURITIES

Specified impurities *A, B, C, D, E*.

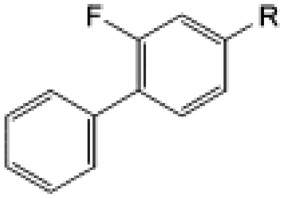


and enantiomer

A. R = R′ = H: (2*RS*)-2-(biphenyl-4-yl)propanoic acid,

B. R = CH(CH$_3$)-CO$_2$H: 2-(2-fluorobiphenyl-4-yl)-2,3-dimethylbutanedioic acid,

C. C. R = OH, R′ = F: (2*RS*)-2-(2-fluorobiphenyl-4-yl)-2-hydroxypropanoic acid,



D. R = CO-CH$_3$: 1-(2-fluorobiphenyl-4-yl)ethanone,

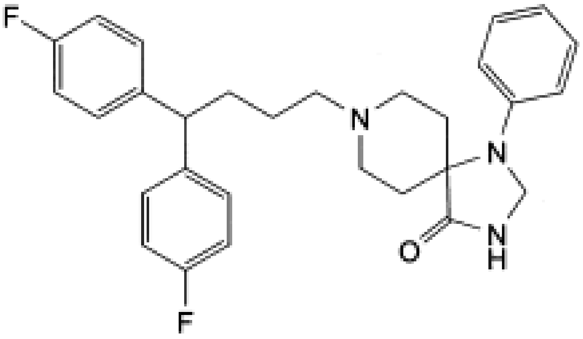
E. R = CO$_2$H: 2-fluorobiphenyl-4-carboxylic acid.

Ph Eur

Fluspirilene

General Notices

(Ph Eur monograph 1723)



$C_{29}H_{31}F_2N_3O$ *475.6* 1841-19-6

Action and use

Antipsychotic.

Ph Eur

DEFINITION

8-[4,4-bis(4-Fluorophenyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in methylene chloride, slightly soluble in alcohol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *fluspirilene CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, gently evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.25 g in 25 ml of *methylene chloride R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *dimethylformamide R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *fluspirilene impurity C CRS* in *dimethylformamide R*, add 0.5 ml of the test solution and dilute to 100.0 ml with *dimethylformamide R*.

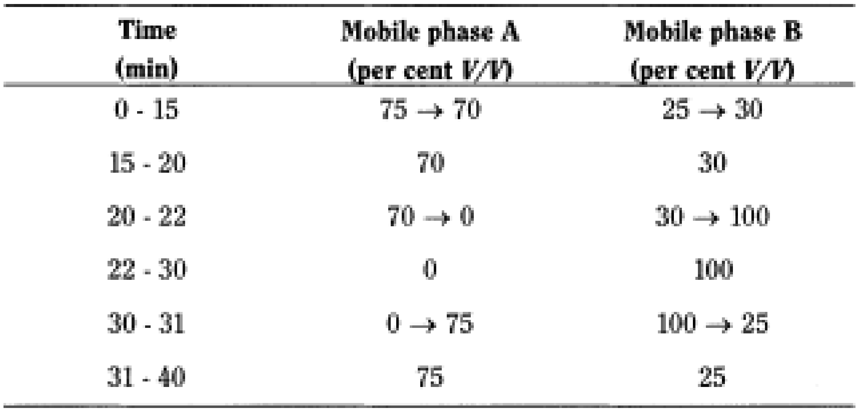
Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *dimethylformamide R*. Dilute 1.0 ml of this solution to 25.0 ml with *dimethylformamide R*.

Column:

- *size:* *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase A 13.6 g/l solution of tetrabutylammonium hydrogen sulphate R,

Mobile phase B *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	75 → 70	25 → 30
15 - 20	70	30
20 - 22	70 → 0	30 → 100
22 - 30	0	100
30 - 31	0 → 75	100 → 25
31 - 40	75	25

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µl.

Relative retention With reference to fluspirilene (retention time = about 15 min): impurity A = about 0.8; impurity B = about 0.93; impurity C = 0.97.

System suitability Reference solution (a):

— *resolution*: minimum 2.2 between the peaks due to impurity C and fluspirilene.

Limits:

— *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.350 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

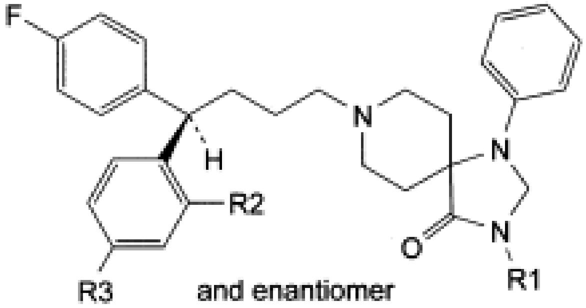
1 ml of *0.1 M perchloric acid* is equivalent to 47.56 mg of $C_{29}H_{31}F_2N_3O$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C.



A. R1 = R2 = R3 = H: 8-[(4*RS*)-4-(4-fluorophenyl)-4-phenylbutyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one,

B. R1 = R3 = H, R2 = F: 8-[(4*RS*)-4-(2-fluorophenyl)-4-(4-fluorophenyl)butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one,

C. R1 = CH_2OH, R2 = H, R3 = F: 8-[4,4-bis(4-fluorophenyl)butyl]-3-(hydroxymethyl)-1-

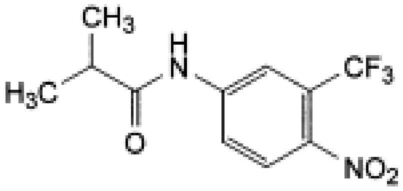
phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

Ph Eur

Flutamide

General Notices

(Ph Eur monograph 1423)



$C_{11}H_{11}F_3N_2O_3$ *276.2* 13311-84-7

Action and use

Antiandrogen.

Ph Eur

DEFINITION

2-Methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

Pale yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent).

mp: about 112 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison flutamide CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 2 mg of *flutamide CRS* and 2 mg of *flutamide impurity C CRS* in the mobile phase, then dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *water R* (50:50 *V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time 1.5 times the retention time of flutamide.

Retention time Impurity C = about 14 min; flutamide = about 19 min.

Relative retention With reference to flutamide: impurity C = about 0.72.

System suitability Reference solution (a):

— *resolution*: minimum 10.5 between the peaks due to impurity C and flutamide.

Limits:
— *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities A, B, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 25.0 mg in *methanol R* and dilute to 25.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *methanol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 295 nm.

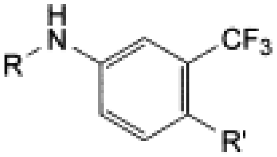
Calculate the content of $C_{11}H_{11}F_3N_2O_3$ taking the specific absorbance to be 295.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F.

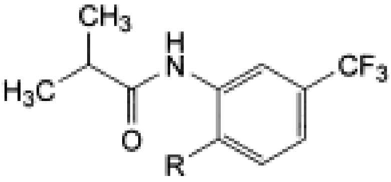


A. R = H, R′ = NO_2: 4-nitro-3-(trifluoromethyl)aniline,

B. R = CO-CH_3, R′ = NO_2: *N*-[4-nitro-3-(trifluoromethyl)phenyl]acetamide,

C. R = CO-CH_2-CH_3, R′ = NO_2: *N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide,

D. R = R′ = H: 3-(trifluoromethyl)aniline,



E. R = H: 2-methyl-*N*-[3-(trifluoromethyl)phenyl]propanamide,

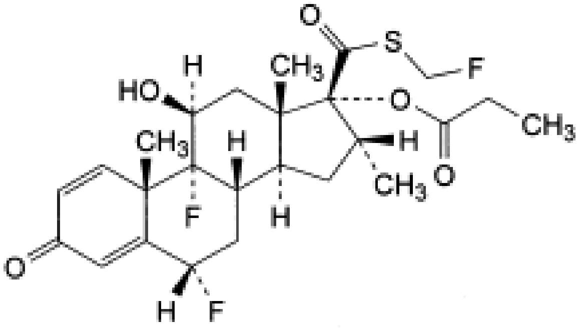
F. R = NO_2: 2-methyl-*N*-[2-nitro-5-(trifluoromethyl)phenyl]propanamide.

Ph Eur

Fluticasone Propionate

General Notices

(Ph Eur monograph 1750)



$C_{25}H_{31}F_3O_5S$ 500.6 *80474-14-2*

Action and use

Glucocorticoid.

Preparations

Fluticasone Cream

Futicasone Nasal Drops

Fluticasone Nasal Spray

Fluticasone Ointment

Ph Eur

DEFINITION

6α,9-Difluoro-17-[[(fluoromethyl)sulphanyl]carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl propanoate.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fluticasone propionate CRS*.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (b).

TESTS

Specific optical rotation *(2.2.7)*

+ 32 to + 36 (anhydrous substance).

Dissolve 0.25 g in *methylene chloride R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*: use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in a mixture of equal volumes of mobile phase A and mobile phase B and dilute to 100.0 ml with the same mixture of mobile phases.

Reference solution (a) Dissolve 4 mg of *fluticasone impurity D CRS* in a mixture of equal volumes of mobile phase A and mobile phase B and dilute to 100.0 ml with the same mixture of mobile phases.

Reference solution (b) Dissolve 20 mg of *fluticasone propionate CRS* in a mixture of equal volumes of mobile phase A and mobile phase B, add 1.0 ml of reference solution (a) and dilute to 100.0 ml with a mixture of equal volumes of mobile phase A and mobile phase B.

Column:

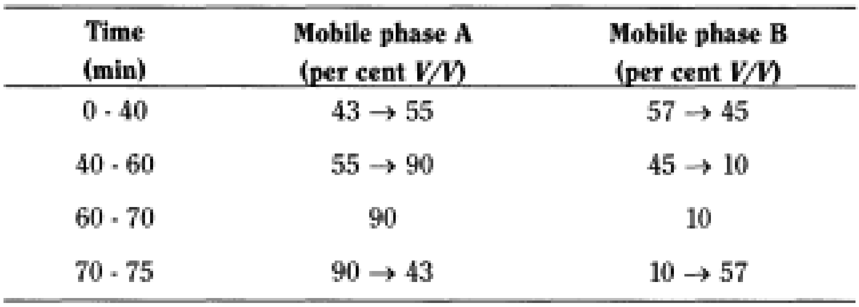
— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase A A solution containing 0.05 per cent *V/V* of *phosphoric acid R* and 3.0 per cent *V/V* of *methanol R* in *acetonitrile R*,

Mobile phase B A solution containing 0.05 per cent *V/V* of *phosphoric acid R* and 3.0 per cent *V/V* of *methanol R* in *water R*,



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 40	43 → 55	57 → 45
40 - 60	55 → 90	45 → 10
60 - 70	90	10
70 - 75	90 → 43	10 → 57

Flow rate 1 ml/min.

Detection Spectrophotometer at 239 nm.

Injection 50 µl; inject the test solution and reference solution (b).

Relative retention With reference to fluticasone propionate (retention time = about 30 min): impurity A = about 0.38; impurity B = about 0.46; impurity C = about 0.76; impurity D = about 0.95; impurity E = about 1.12; impurity F = about 1.18; impurity G = about 1.33; impurity H = about 1.93; impurity I = about 2.01.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity D and to fluticasone propionate.

Limits:

— *impurities D, G*: for each impurity, maximum 0.3 per cent;

— *impurities A, B, C, E, F, H, I*: for each impurity, maximum 0.2 per cent;

— *impurity with relative retention at about 1.23*: maximum 0.2 per cent;

— *any other impurity*: maximum 0.1 per cent;

— *total*: maximum 1.2 per cent;

— *disregard limit*: 0.05 per cent.

Acetone

Gas chromatography *(2.2.28)*.

Internal standard solution Dilute 0.5 ml of *tetrahydrofuran R* to 1000 ml with *dimethylformamide R*.

Test solution Dissolve 0.50 g of the substance to be examined in the internal standard solution and dilute to 10.0 ml with the same solution.

Reference solution Dilute 0.40 g of *acetone R* to 100.0 ml with the internal standard solution. Dilute 1.0 ml to 10.0 ml with the internal standard solution.

Column:

— *material*: fused silica;

— *size: l* = 25 m, Ø = 0.53 mm;

— *stationary phase*: cross-linked *macrogol 20 000 R* (film thickness 2 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 5.5 ml/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 3.5	60
	3.5 - 7.5	60 → 180
	7.5 - 10.5	180
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 0.1 µl.

Limit:

— *acetone*: maximum 1.0 per cent *m/m*.

Water *(2.5.12)*

Maximum 0.5 per cent determined on 0.250 g.

Use as solvent a mixture of equal volumes of *chloroform R* and *methanol R*.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *fluticasone propionate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 4.0 mg of *fluticasone impurity D CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. To 1.0 ml of this solution, add 1.0 ml of reference solution (a) and dilute to 10.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 15 volumes of *acetonitrile R*, 35 volumes of a 1.15 g/l solution of *ammonium dihydrogen phosphate R* adjusted to pH 3.5 and 50 volumes of *methanol R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 239 nm.

Injection 20 µl; inject the test solution and reference solutions (b) and (c).

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to impurity D and to fluticasone propionate.

If necessary, adjust the ratio of acetonitrile to methanol in the mobile phase.

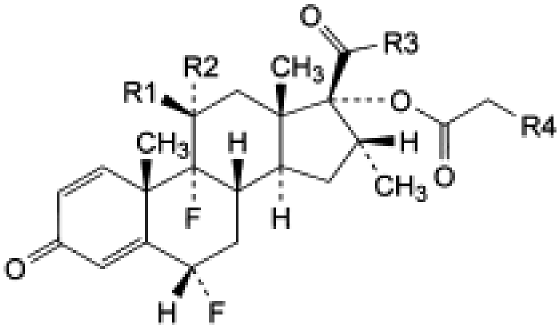
Calculate the percentage content of $C_{25}H_{31}F_3O_5S$ using the chromatograms obtained with the test solution and reference solution (b), and the declared content of *fluticasone propionate CRS.*

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I.



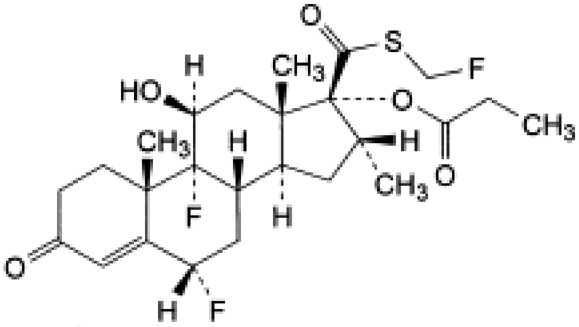
A. R1 = R3 = OH, R2 = H, R4 = CH_3: 6α,9-difluoro-11β-hydroxy-16α-methyl-3-oxo-17-(propanoyloxy)androsta-1,4-diene-17β-carboxylic acid,

B. R1 = OH, R2 = H, R3 = S-OH, R4 = CH_3: [[6α,9-difluoro-11β-hydroxy-16α-methyl-3-oxo-17-(propanoyloxy)androsta-1,4-dien-17β-yl]carbonyl]sulphenic acid,

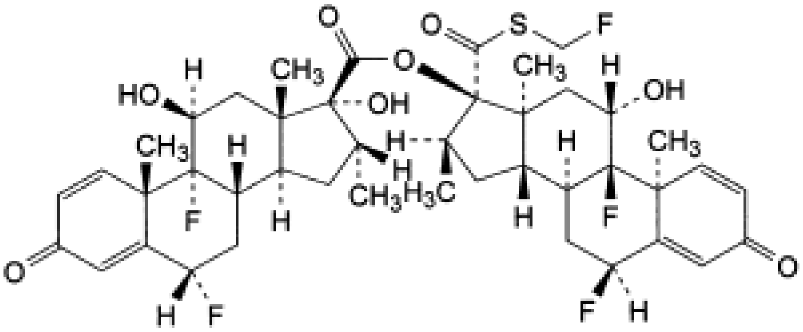
C. R1 = OH, R2 = R4 = H, R3 = S-CH_2-F: 6α,9-difluoro-17-[[(fluoromethyl)sulphanyl]carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl acetate,

D. R1 = OH, R2 = H, R3 = S-CH_3, R4 = CH_3: 6α,9-difluoro-17-[(methylsulphanyl)carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl propanoate,

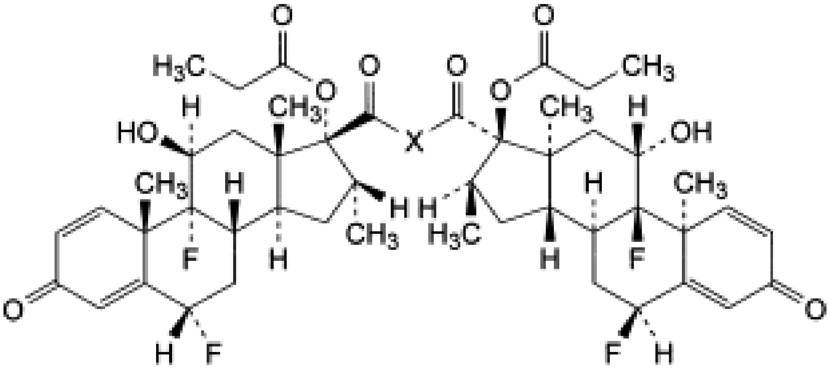
F. R1 + R2 = O, R3 = S-CH_2-F, R4 = CH_3: 6α,9-difluoro-17-[[(fluoromethyl)sulphanyl]carbonyl]-16α-methyl-3,11-dioxoandrosta-1,4-dien-17α-yl propanoate,



E. 6α,9-difluoro-17-[[(fluoromethyl)sulphanyl]carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrost-4-en-17α-yl propanoate,



G. 6α,9-difluoro-17-[[(fluoromethyl)sulphanyl]carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl 6α,9-difluoro-11β,17-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate,



H. X = S-S: 17,17′-(disulphanediyldicarbonyl)bis(6α,9-difluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl) dipropanoate,

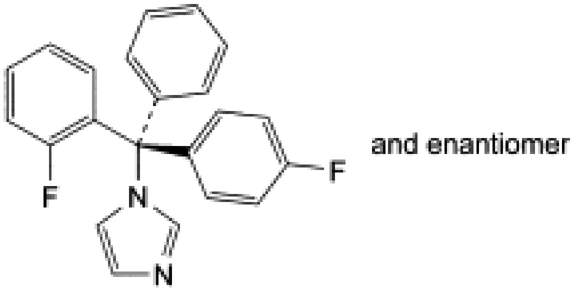
I. X = S-S-S: 17,17′-(trisulphanediyldicarbonyl)bis(6α,9-difluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl) dipropanoate.

Ph Eur

Flutrimazole

General Notices

(Ph Eur monograph 1424)



$C_{22}H_{16}F_2N_2$ *346.4* 119006-77-8

Action and use

Antifungal.

Ph Eur

DEFINITION

(*RS*)-1-[(2-Fluorophenyl)(4-fluorophenyl)phenylmethyl]-1*H*-imidazole.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in tetrahydrofuran, soluble in methanol.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 161 °C to 166 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *flutrimazole CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *flutrimazole CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *flutrimazole CRS* and 10 mg of *metronidazole benzoate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R*.

Pretreatment Heat the plate at 110 °C for 1 h.

Mobile phase *2-propanol R*, *ethyl acetate R* (10:90 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Solution S

Dissolve 1.00 g in *methanol R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.05° to + 0.05°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *imidazole CRS* (impurity A) in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 30.0 mg of *flutrimazole impurity B CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (c) Mix 2.0 ml of reference solution (a) and 2.0 ml of reference solution (b) and dilute to 50.0 ml with the mobile phase.

Reference solution (d) Dilute 10.0 ml of reference solution (c) to 50.0 ml with the mobile phase.

Reference solution (e) Mix 2.0 ml of the test solution and 10.0 ml of reference solution (c) and dilute to 50.0 ml with the mobile phase.

Reference solution (f) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.2 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *0.03 M phosphate buffer solution pH 7.0 R*, *acetonitrile R* (40:60 *V/V*).

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 2.5 times the retention time of flutrimazole.

System suitability Reference solution (e):

— *resolution*: minimum 2.0 between the peaks due to impurity A (1st peak) and impurity B (2nd peak); minimum 1.5 between the peaks due to impurity B and flutrimazole (3rd peak);

— *symmetry factors*: maximum 2.0 for the peaks due to impurities A and B.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (f) (0.10 per cent);

— *sum of impurities other than B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (f) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained

with reference solution (f) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test F. Use a platinum crucible. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 34.64 mg of $C_{22}H_{16}F_2N_2$.

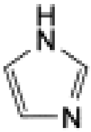
STORAGE

Protected from light.

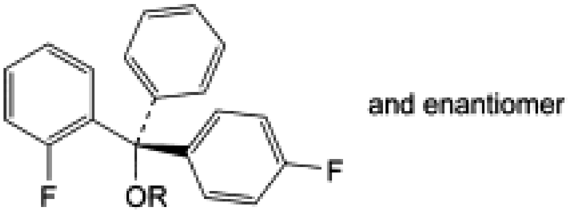
IMPURITIES

Specified impurities *A, B*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) C*.



A. imidazole,



B. R = H: (*RS*)-(2-fluorophenyl)(4-fluorophenyl)phenylmethanol,

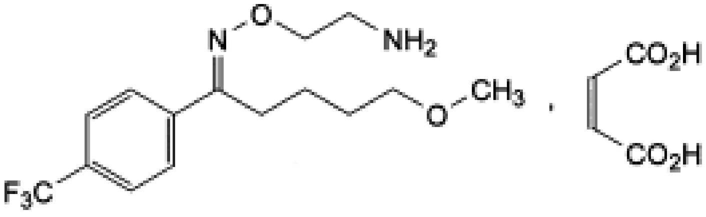
C. R = CH_3: (*RS*)-(2-fluorophenyl)(4-fluorophenyl)methoxyphenylmethane.

Ph Eur

Fluvoxamine Maleate

General Notices

(Ph Eur monograph 1977)



$C_{19}H_{25}F_3N_2O_6$ 434.4 *61718-82-9*

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

Preparation

Fluvoxamine Tablets

Ph Eur

DEFINITION

2-[[[(1*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene]amino]oxy]ethanamine (*Z*)-butenedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of aziridine. Where necessary, a validated test for the substance is carried out or the production method is validated to demonstrate acceptable clearance.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fluvoxamine maleate CRS.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the test solution immediately before use*.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 25 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *fluvoxamine for system suitability CRS* (containing impurities A, B, C and F) in the mobile phase and dilute to 5 ml with the mobile phase.

Reference solution (c) Dissolve 3.0 mg of *fluvoxamine impurity D CRS* in 5 ml of the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 370 volumes of *acetonitrile R1* and 630 volumes of a buffer solution containing 1.1 g/l of *potassium dihydrogen phosphate R* and 1.9 g/l of *sodium pentanesulphonate R* in *water R*, previously adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 234 nm.

Injection 20 µl.

Run time 6 times the retention time of fluvoxamine.

Identification of impurities Use the chromatogram supplied with *fluvoxamine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and F.

Relative retention With reference to fluvoxamine (retention time = about 15 min): maleic acid = about 0.15; impurities F and G = about 0.5; impurity C = about 0.6; impurity B = about 0.8; impurity A = about 2.5; impurity D = about 5.4.

System suitability Reference solution (b):

- *resolution*: minimum 1.5 between the peaks due to impurities F and C.

Limits:

- *impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurity C*: not more than 3 times the area of the principal peak in the chromatogram

obtained with reference solution (a) (0.3 per cent);

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent);

— *sum of impurities F and G*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to maleic acid.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test B. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

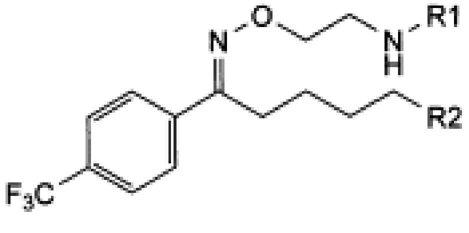
Dissolve 0.350 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 43.44 mg of $C_{19}H_{25}F_3N_2O_6$.

IMPURITIES

Specified impurities *A, B, C, D, F, G.*

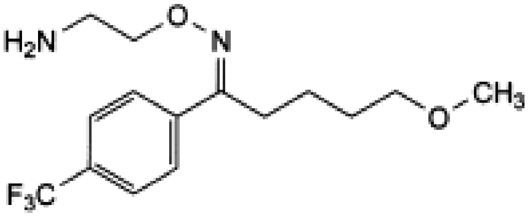
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, I, J*.



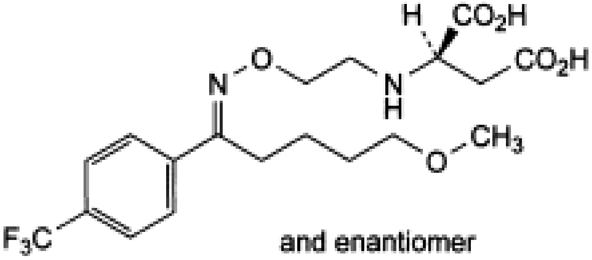
A. R1 = R2 = H: 2-[[[(1*E*)-1-[4-(trifluoromethyl)phenyl]pentylidene]amino]oxy]ethanamine,

F. R1 = CH_2-CH_2-NH_2, R2 = OCH_3: *N*-[2-[[[(1*E*)-5-methoxy-1[4-(trifluoromethyl)phenyl] pentylidene]amino]oxy]ethyl]ethane-1,2-diamine,

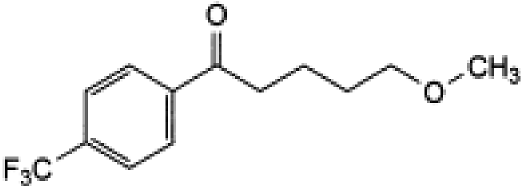
G. R1 = H, R2 = OH: (5*E*)-5-[(2-aminoethoxy)imino]-5-[4-(trifluoromethyl)phenyl]pentan-1-ol,



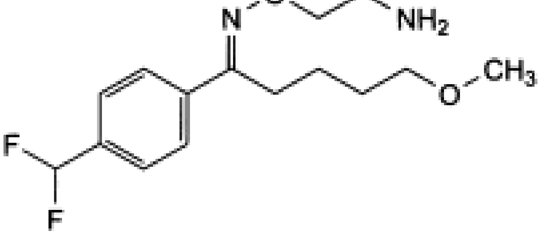
B. 2-[[[(1*Z*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene]amino]oxy]ethanamine,



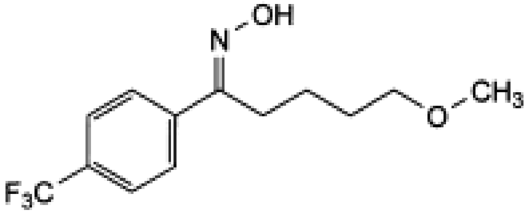
C. (2*RS*)-2-[[2-[[[(1*E*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene]amino]oxy]ethyl] amino]butanedioic acid,



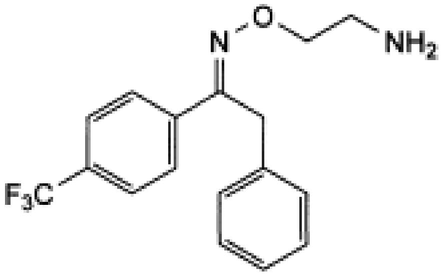
D. 5-methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one,



E. 2-[[[(1*E*)-1-[4-(difluoromethyl)phenyl]-5-methoxypentylidene]amino]oxy]ethanamine,



I. (*E*)-*N*-[5-methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene]hydroxylamine,



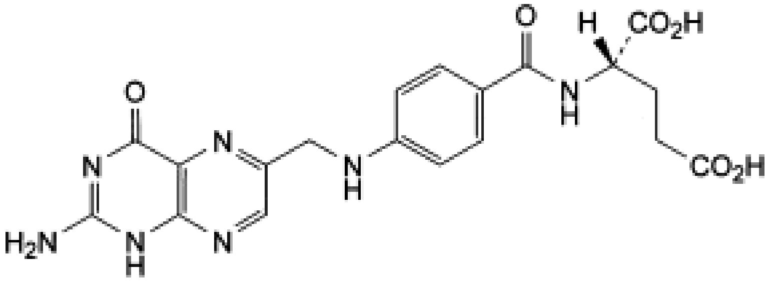
J. 2-[[[(1*E*)-2-phenyl-1-[4-(trifluoromethyl)phenyl]ethylidene]amino]oxy]ethanamine.

Ph Eur

Folic Acid

General Notices

(Ph Eur monograph 0067)



$C_{19}H_{19}N_7O_6$ *441.4* 59-30-3

Action and use

Vitamin B component.

Preparations

Folic Acid Tablets

Ferrous Fumarate and Folic Acid Tablets

Ph Eur

DEFINITION

(2*S*)-2-[[4-[[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellowish or orange, crystalline powder.

Solubility

Practically insoluble in water and in most organic solvents. It dissolves in dilute acids and in alkaline solutions.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. Specific optical rotation *(2.2.7)*: + 18 to + 22 (anhydrous substance).

Dissolve 0.25 g in *0.1 M sodium hydroxide* and dilute to 25.0 ml with the same solvent.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in a mixture of 2 volumes of *concentrated ammonia R* and 9 volumes of *methanol R* and dilute to 100 ml with the same mixture of solvents.

Reference solution Dissolve 50 mg of *folic acid CRS* in a mixture of 2 volumes of *concentrated ammonia R* and 9 volumes of *methanol R* and dilute to 100 ml with the same mixture of solvents.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R*, *propanol R*, *ethanol (96 per cent) R* (20:20:60 *V/V/V*).

Application 2 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in 5 ml of a 28.6 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 0.100 g of *folic acid CRS* in 5 ml of a 28.6 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) To 20 mg of *pteroic acid R*, add 5 ml of a 28.6 g/l solution of *sodium carbonate R*, dilute to 100.0 ml with the mobile phase and mix until completely dissolved. Mix 1.0 ml of this solution with 1.0 ml of reference solution (a) and dilute to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (d) Dissolve 10.0 mg of *N-(4-aminobenzoyl)-L-glutamic acid R* in 1 ml of a 28.6 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (e) To 12.0 mg of *pteroic acid R* , add 1 ml of a 28.6 g/l solution of *sodium carbonate R*, dilute to 100.0 ml with the mobile phase and mix until completely dissolved. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:
— *size:* l = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *spherical octylsilyl silica gel for chromatography R* (5 µm) with a carbon loading of 12.5 per cent, a specific surface of 350 m^2/g and a pore size of 10 nm.

Mobile phase Mix 12 volumes of *methanol R* and 88 volumes of a solution containing 11.16 g/l of *potassium dihydrogen phosphate R* and 5.50 g/l of *dipotassium hydrogen phosphate R*.

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 5 µl of the test solution and reference solutions (b), (c) (d) and (e).

Run time 3 times the retention time of folic acid.

Relative retention With reference to folic acid (retention time = about 8.5 min): impurity A = about 0.5; impurity B = about 0.6; impurity C = about 0.9; impurity E = about 1.27; impurity D = about 1.33; impurity F = about 2.2.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to folic acid and impurity D.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.6 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *Total of other impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water *(2.5.12)*

5.0 per cent to 8.5 per cent, determined on 0.150 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

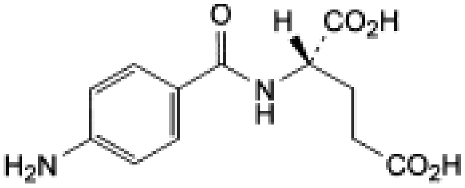
Injection Test solution and reference solution (a).

STORAGE

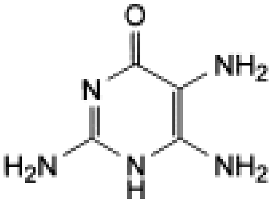
Protected from light.

IMPURITIES

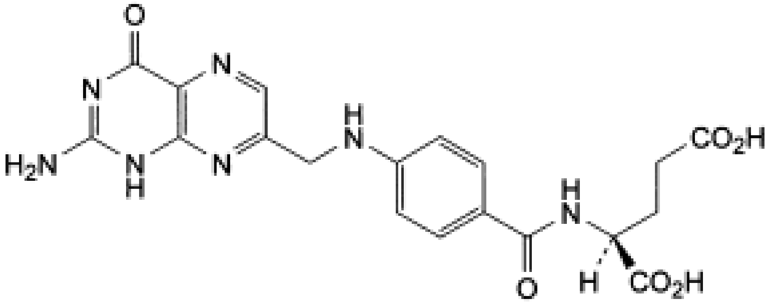
Specified impurities A, B, C, D, E, F.



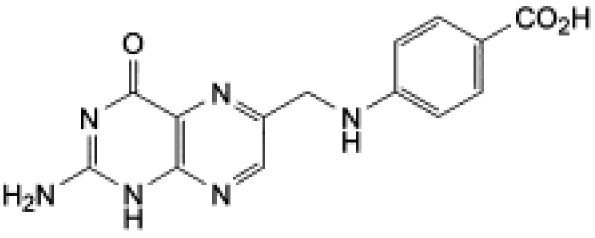
A. (2*S*)-2-[(4-aminobenzoyl)amino]pentanedioic acid (*N*-(4-aminobenzoyl)-L-glutamic acid),



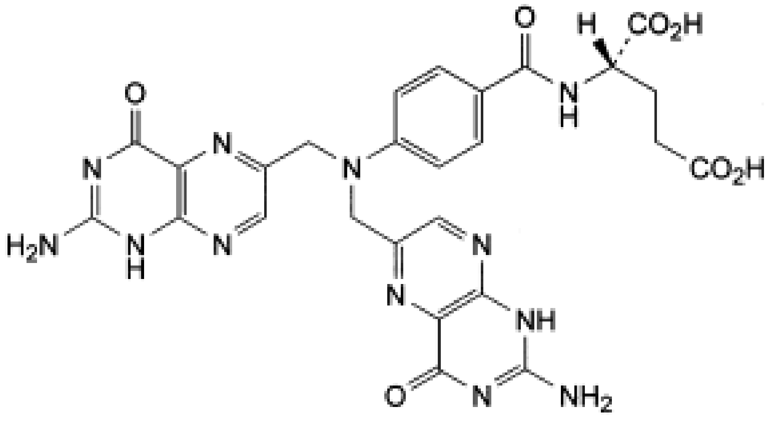
B. 2,5,6-triaminopyrimidin-4(1*H*)-one,



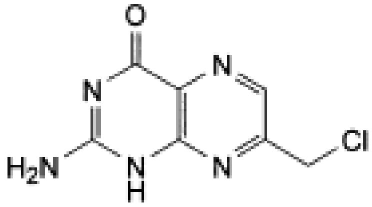
C. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-7-yl)methyl]amino]benzoyl]amino] pentanedioic acid (isofolic acid),



D. 4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzoic acid (pteroic acid),



E. (2*S*)-2-[[4-[bis[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzoyl]amino] pentanedioic acid (6-pterinylfolic acid),



F. 2-amino-7-(chloromethyl)pteridin-4(1*H*)-one.

Ph Eur

Formaldehyde Solution

General Notices

Formalin

(Formaldehyde Solution (35 per cent), Ph Eur monograph 0826)

NOTE: The name Formalin as a synonym for Formaldehyde Solution may be used freely in many countries, including the United Kingdom, but in other countries exclusive proprietary rights in this name are claimed.

CH_2O 30.03 *50-00-0*

Action and use

When suitably diluted, used in the treatment of warts.

Ph Eur

DEFINITION

Content

34.5 per cent *m/m* to 38.0 per cent *m/m* of formaldehyde (CH_2O; M_r 30.03).

It contains methanol as stabiliser.

CHARACTERS

Appearance

Clear, colourless liquid.

Solubility

Miscible with water and with ethanol (96 per cent).

It may be cloudy after storage.

IDENTIFICATION

A. Dilute 1 ml of solution S (see Tests) to 10 ml with *water R*. To 0.05 ml of the solution add 1 ml of a 15 g/l solution of *chromotropic acid sodium salt R*, 2 ml of *water R* and 8 ml of *sulphuric acid R*. A violet-blue or violet-red colour develops within 5 min.

B. To 0.1 ml of solution S add 10 ml of *water R*. Add 2 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R*, prepared immediately before use, 1 ml of *potassium ferricyanide solution R* and 5 ml of *hydrochloric acid R*. An intense red colour is formed.

C. Mix 0.5 ml with 2 ml of *water R* and 2 ml of *silver nitrate solution R2* in a test-tube. Add *dilute ammonia R2* until slightly alkaline. Heat on a water-bath. A grey precipitate or a silver mirror is formed.

D. It complies with the limits of the assay.

TESTS

Solution S

Dilute 10 ml, filtered if necessary, to 50 ml with *carbon dioxide-free water R*.

Appearance of solution

Solution S is colourless *(2.2.2, Method II)*.

Acidity

To 10 ml of solution S add 1 ml of *phenolphthalein solution R*. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to red.

Methanol

Gas chromatography *(2.2.28)*.

Internal standard solution Dilute 10 ml of *ethanol R1* to 100 ml with *water R*.

Test solution To 10.0 ml of the solution to be examined add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *water R*.

Reference solution To 1.0 ml of *methanol R* add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *water R*.

Column:

- *material*: glass;
- *size:* *l* = 1.5-2.0 m, Ø = 2-4 mm;
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (150-180 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 30-40 ml/min.

Temperature:

- *column*: 120 °C;
- *injection port and detector*: 150 °C.

Detection Flame ionisation.

Injection 1 µl of the test solution and the reference solution.

System suitability Reference solution:

- *resolution*: minimum 2.0 between the peaks due to methanol and ethanol.

Limit:

- *methanol*: 9.0 per cent *V/V* to 15.0 per cent *V/V*.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Into a 100 ml volumetric flask containing 2.5 ml of *water R* and 1 ml of *dilute sodium hydroxide solution R*, introduce 1.000 g of the solution to be examined, shake and dilute to 100.0 ml with *water R*. To 10.0 ml of the solution add 30.0 ml of *0.05 M iodine*. Mix and add 10 ml of *dilute sodium hydroxide solution R*. After 15 min, add 25 ml of dilute *sulphuric acid R* and 2 ml of *starch solution R*. Titrate with *0.1 M sodium thiosulphate*.

1 ml of *0.05 M iodine* is equivalent to 1.501 mg of CH_2O.

STORAGE

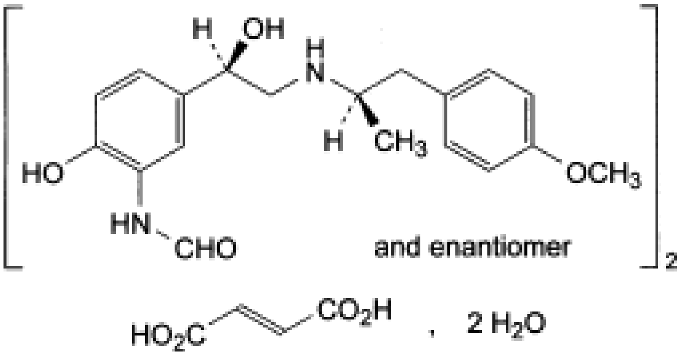
Protected from light, at a temperature of 15 °C to 25 °C.

Ph Eur

Formoterol Fumarate Dihydrate

General Notices

(Ph Eur monograph 1724)



$C_{42}H_{52}N_4O_{12},2H_2O$ 841 *43229-80-7*

(anhydrous)

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Ph Eur

DEFINITION

N-[2-Hydroxy-5-[(1*RS*)-1-hydroxy-2-[[(1*RS*)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide (*E*)-butenedioate dihydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white or slightly yellow powder.

Solubility

Slightly soluble in water, soluble in methanol, slightly soluble in 2-propanol, practically insoluble in acetonitrile.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison formoterol fumarate dihydrate CRS .

TESTS

pH *(2.2.3)*

5.5 to 6.5.

Dissolve 20 mg in *carbon dioxide-free water R* while heating to about 40 °C, allow to cool and dilute to 20 ml with the same solvent.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 0.25 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Solution A Dissolve 6.10 g of *sodium dihydrogen phosphate monohydrate R* and 1.03 g of *disodium hydrogen phosphate dihydrate R* in *water R* and dilute to 1000 ml with the same solvent. The pH is 6.0 ± 0.1.

Solvent mixture acetonitrile R, solution A (16:84 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture. *Inject within 4 h of preparation, or within 24 h if stored protected from light at 4 °C.*

Reference solution (a) Dissolve 5 mg of *formoterol fumarate for system suitability CRS* (containing impurities A, B, C, D, E, F and G) in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 25.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Column:

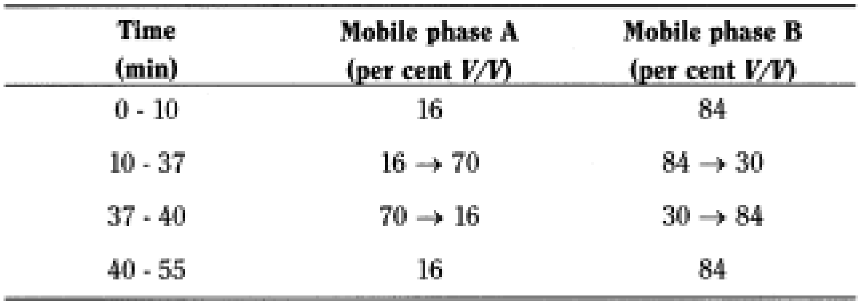
— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: spherical *octylsilyl silica gel for chromatography R3* (5 µm) with a pore size of 8 nm.

Mobile phase:

— *mobile phase A*: *acetonitrile R1;*

— *mobile phase B*: dissolve 3.73 g of *sodium dihydrogen phosphate monohydrate R* and 0.35 g of *phosphoric acid R* in *water R* and dilute to 1000 ml with the same solvent; the pH is 3.1 ± 0.1;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 10	16	84
10 - 37	16 → 70	84 → 30
37 - 40	70 → 16	30 → 84
40 - 55	16	84

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl; inject the solvent mixture until a repeatable profile is obtained.

Identification of impurities Use the chromatogram obtained with reference solution (a) and the chromatogram supplied with *formoterol for system suitability CRS* to identify the peaks.

Relative retention With reference to formoterol (retention time = about 12 min): impurity G = about 0.4; impurity A = about 0.5; impurity B = about 0.7; impurity C = about 1.2; impurity D = about 1.3; impurity E = about 1.8; impurity F = about 2.0; impurity H = about 2.2.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurity G and impurity A.

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to formoterol.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.75;

— *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities B, C, D, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity E*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity I

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 5.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent. Sonicate if necessary.

Reference solution (a) Dissolve 5.0 mg of *formoterol for impurity I identification CRS* in *water R* and dilute to 50.0 ml with the same solvent. Sonicate if necessary.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *water R*. Dilute 1.0 ml of this solution to 25.0 ml with *water R*.

Column:

- *size:* l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecyl vinyl polymer for chromatography R*.

Mobile phase Mix 12 volumes of *acetonitrile R1* with 88 volumes of a 5.3 g/l solution of *tripotassium phosphate trihydrate R* previously adjusted to pH 12.0 ± 0.1 with a 280 g/l solution of *potassium hydroxide R* or *phosphoric acid R*.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl.

Elution order Formoterol, impurity I.

System suitability Reference solution (a):

- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity I and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to formoterol.

Limit:

- *impurity I*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent).

Water *(2.5.12)*

4.0 per cent to 5.0 per cent, determined on 0.100 g.

ASSAY

Dissolve 0.350 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 40.24 mg of $C_{42}H_{52}N_4O_{12}$.

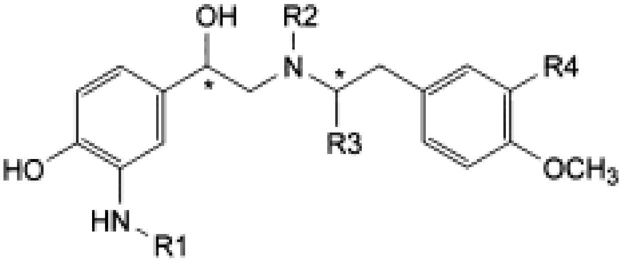
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F, I.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : G, H.*



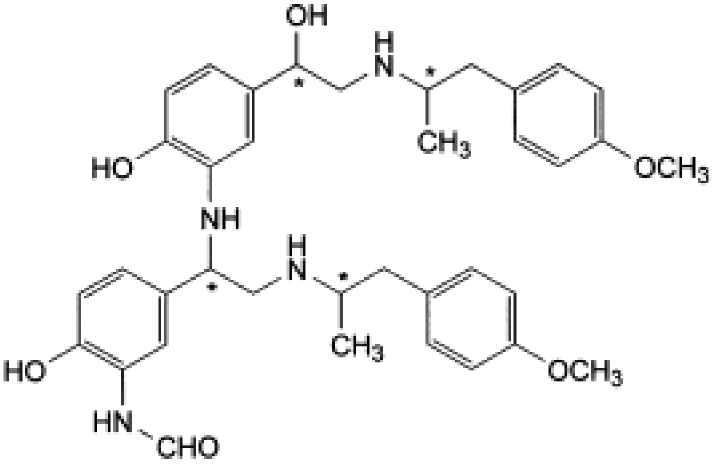
A. R1 = R2 = R4 = H, R3 = CH_3: 1-(3-amino-4-hydroxyphenyl)-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethanol,

B. R1 = CHO, R2 = R3 = R4 = H: *N*-[2-hydroxy-5-[(1*RS*)-1-hydroxy-2-[[2-(4-methoxyphenyl) ethyl]amino]ethyl]phenyl]formamide,

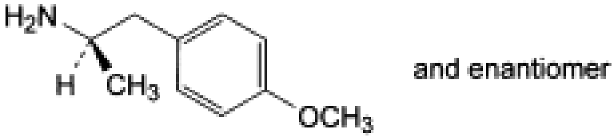
C. R1 = CO-CH_3, R2 = R4 = H, R3 = CH_3: *N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]acetamide,

D. R1 = CHO, R2 = R3 = CH_3, R4 = H: *N*-[2-hydroxy-5-[1-hydroxy-2-[methyl[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide,

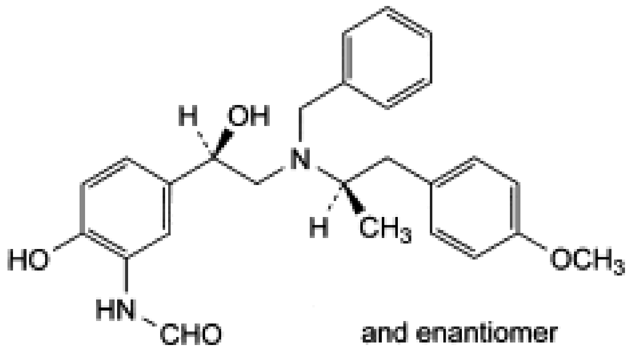
E. R1 = CHO, R2 = H, R3 = R4 = CH_3: *N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxy-3-methylphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide,



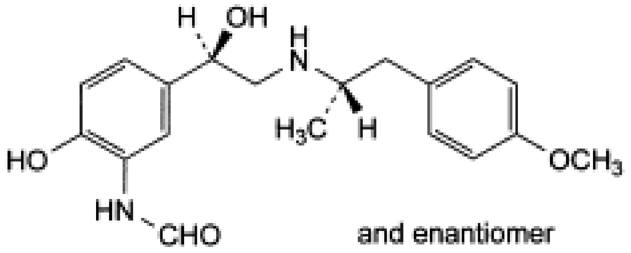
F. *N*-[2-hydroxy-5-[1-[[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino] ethyl]phenyl]amino]-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide,



G. (2*RS*)-1-(4-methoxyphenyl)propan-2-amine,


and enantiomer

H. *N*-[5-[(1*RS*)-2-[benzyl[(1*RS*)-2-(4-methoxyphenyl)-1-methylethyl]amino]-1-hydroxyethyl]-2-hydroxyphenyl]formamide (monobenzyl analogue),


and enantiomer

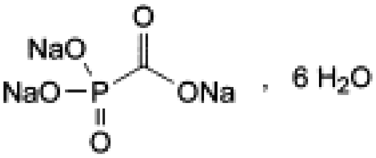
I. *N*-[2-hydroxy-5-[(1*RS*)-1-hydroxy-2-[[(1*SR*)-2-(4-methoxyphenyl)-1-methylethyl]amino] ethyl]phenyl]formamide (diastereoisomer).

Ph Eur

Foscarnet Sodium

General Notices

(Foscarnet Sodium Hexahydrate, Ph Eur monograph 1520)



$CNa_3O_5P,6H_2O$ 300.0 *34156-56-4*

Action and use

Antiviral (cytomegalovirus).

Preparation

Foscarnet Intravenous Infusion

Ph Eur

DEFINITION

Trisodium phosphonatoformate hexahydrate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison foscarnet sodium hexahydrate CRS .

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension I *(2.2.1)* and is colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

9.0 to 11.0 for solution S.

Impurity D

Gas chromatography *(2.2.28)*.

Test solution Dissolve 0.25 g of the substance to be examined in 9.0 ml of *0.1 M acetic acid* using a magnetic stirrer. Add 1.0 ml of *anhydrous ethanol R* and mix.

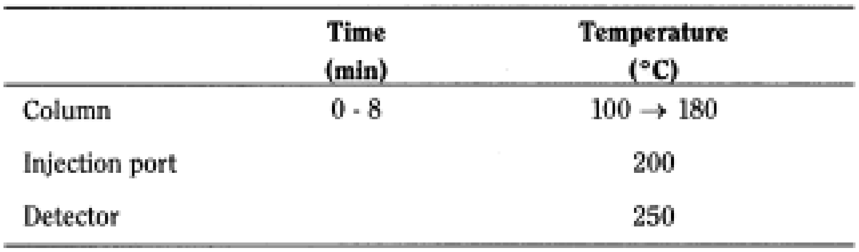
Reference solution Dissolve 25 mg of *triethyl phosphonoformate R* in *anhydrous ethanol R* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *anhydrous ethanol R*.

Column:
- *material*: fused silica;
- *size:* *l* = 25 m, Ø = 0.31 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)(divinyl)siloxane R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Split ratio 1:20.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 8	100 → 180
Injection port		200
Detector		250

Detection Flame ionisation.

Injection 3 µl

Limit:
- *impurity D*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *foscarnet impurity B CRS* in the mobile phase, add 2.0 ml of the test solution and dilute to 50.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve 3.22 g of *sodium sulphate decahydrate R* in *water R*, add 3 ml of *glacial acetic acid R*, 6 ml of a 44.61 g/l solution of *sodium pyrophosphate R* and dilute to 1000 ml with *water R* (solution A); dissolve 3.22 g of *sodium sulphate decahydrate R* in *water R*, add 6.8 g of *sodium acetate R* and 6 ml of a 44.61 g/l solution of *sodium pyrophosphate R* and dilute to 1000 ml with *water R* (solution B). Mix about 700 ml of solution A and about 300 ml of solution B to obtain a solution of pH 4.4. To 1000 ml of this solution, add 0.25 g of *tetrahexylammonium hydrogen sulphate R* and 100 ml of *methanol R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 2.5 times the retention time of foscarnet.

System suitability Reference solution (b):

— *resolution*: minimum 7 between the peaks due to foscarnet and impurity B.

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.04 per cent); disregard any peak with a relative retention time less than 0.6.

Phosphate and phosphite

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 60.0 mg of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dissolve 28 mg of *sodium dihydrogen phosphate monohydrate R* in *water R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dissolve 43 mg of *sodium phosphite pentahydrate R* in *water R* and dilute to 100 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 25 ml with *water R*.

Reference solution (d) Dilute 3 ml of reference solution (a) and 3 ml of reference solution (b) to 25 ml with *water R*.

Column:

— *size: l* = 0.05 m, Ø = 4.6 mm;

— *stationary phase*: *anion exchange resin R*.

Mobile phase Dissolve 0.102 g of *potassium hydrogen phthalate R* in *water R*, add 2.5 ml of *1 M nitric acid* and dilute to 1000 ml with *water R*.

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 290 nm (indirect detection).

Injection 20 µl of the test solution and reference solutions (c) and (d).

System suitability Reference solution (d):

— *resolution*: minimum 2.0 between the peaks due to phosphate (1st peak) and phosphite;

— *signal-to-noise ratio*: minimum 10 for the principal peak.

Limits:

— *phosphate*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *phosphite*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent).

Heavy metals

Maximum 10 ppm.

Dissolve 1.25 g in 12.5 ml of *1 M hydrochloric acid*. Warm on a water-bath for 3 min and cool to room temperature. Transfer to a beaker and adjust the pH to about 3.5 with *dilute ammonia R1* and dilute to 25 ml with *water R* (solution A). To 12 ml of solution A, add 2.0 ml of *buffer solution pH 3.5 R*. Rapidly pour the mixture into a test tube containing 1 drop of *sodium sulphide solution R*. The solution is not more intensely coloured than a reference solution prepared simultaneously and in the same manner pouring a mixture of 5.0 ml of *lead standard solution (1 ppm Pb) R*, 5.0 ml of *water R*, 2.0 ml of solution A and 2.0 ml of *buffer solution pH 3.5 R* into a test tube containing 1 drop of *sodium sulphide solution R*.

Loss on drying *(2.2.32)*

35.0 per cent to 37.0 per cent, determined on 1.000 g by drying in an oven at 150 °C.

Bacterial endotoxins *(2.6.14)*

Less than 83.3 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.200 g in 50 ml of *water R*. Titrate with *0.05 M sulphuric acid*, determining the end-point potentiometrically *(2.2.20)* at the 1st inflexion point.

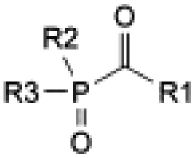
1 ml of *0.05 M sulphuric acid* is equivalent to 19.20 mg of CNa_3O_5P.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D.*



A. R1 = OC_2H_5, R2 = R3 = ONa: disodium (ethoxycarbonyl)phosphonate,

B. R1 = R2 = ONa, R3 = OC_2H_5: disodium (ethoxyoxydophosphanyl)formate,

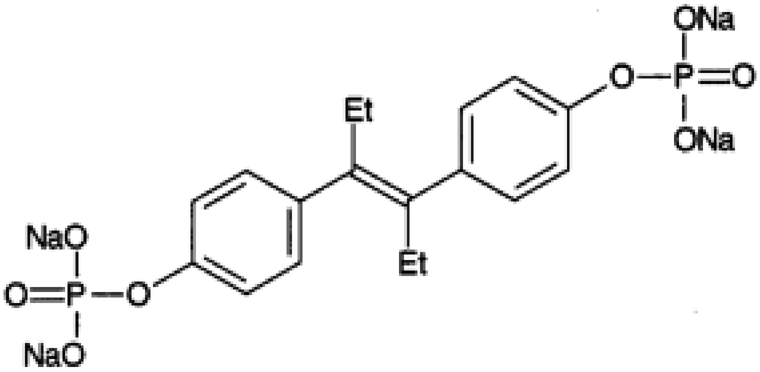
C. R1 = R2 = OC_2H_5, R3 = ONa: ethyl sodium (ethoxycarbonyl)phosphonate,

D. R1 = R2 = R3 = OC_2H_5: methyl (diethoxyphosphoryl)formate.

Ph Eur

Fosfestrol Sodium

General Notices



$C_{18}H_{18}Na_4O_8P_2,xH_2O$ 516.2(anhydrous) *23519-26-8*

Action and use

Estrogen.

Preparations

Fosfestrol Injection

Fosfestrol Tablets

DEFINITION

Fosfestrol Sodium is a hydrate of tetrasodium (*E*)-4,4′-(1,2-diethylvinylene)bis(phenyl orthophosphate). It contains not less than 98.0% and not more than 101.0% of $C_{18}H_{18}Na_4O_8P_2$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white or almost white powder.

Freely soluble in *water*; practically insoluble in *absolute ethanol* and in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of fosfestrol sodium *(RS 161)*.

B. Yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a 5% w/v solution, 7.0 to 9.0, Appendix V L.

To 20 ml of a 2.5% w/v solution, add 3.0 ml of 0.01M *sodium hydroxide VS*. The pH of the resulting solution is not less than 8.8.

Light absorption

Measure the *absorbance* of a 0.0050% w/v solution in 0.1M *sodium hydroxide* at the maximum at 242 nm, Appendix II B. The A(1%, 1 cm) at the maximum is 280 to 320, calculated with reference to the anhydrous substance.

Chloride

Dissolve 1.0 g in 10 ml of *water*, add 10 ml of 2M *nitric acid*, filter, wash the precipitate with 25 ml of *water* and dilute the combined filtrate and washings to 100 ml with *water*. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (350 ppm).

Inorganic phosphate

Dissolve 0.5 g in 20 ml of *water*, add 5 ml of 2M *hydrochloric acid* and extract with four 30 ml quantities of *ether*. Discard the ether extracts and heat the aqueous solution on a water bath until any remaining ether has evaporated. Allow to cool, add sufficient *water* to produce 100 ml (solution A) and then add 4 ml of *sulphomolybdic reagent R3*, shake, add 0.1 ml of a mixture of 1 volume of freshly prepared *tin(II) chloride solution* and 9 volumes of 2M *hydrochloric acid*, shake again and examine 20 ml. Any colour produced is not more intense than that of 20 ml of a solution obtained by repeating the operation using a mixture of 5 ml of a 0.01% w/v solution of *potassium dihydrogen orthophosphate* and 95 ml of *water* in place of solution A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 15 volumes each of *pentan-1-ol*, *triethylamine* and *water* and 55 volumes of *propan-1-ol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of three solutions of the substance being examined in *methanol (50%)* containing (1) 2.0% w/v (2) 0.030% w/v and (3) 0.010% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Free diethylstilbestrol

Dissolve a quantity of the substance being examined containing the equivalent of 2 g of anhydrous fosfestrol sodium in 50 ml of *water*, extract with two 20 ml quantities of *dichloromethane* and retain the aqueous phase. Wash the combined dichloromethane extracts with 30 ml of *water* and filter through *anhydrous sodium sulphate*. Extract the water wash with 10 ml of *dichloromethane*, filter the dichloromethane extract after filtration through *anhydrous sodium sulphate,* add to the original extract and add the aqueous extract to the original aqueous phase. Reserve the aqueous phase for the test for Diethylstilbestrol disodium monophosphate. Evaporate the combined filtrates to dryness on a water bath and dissolve the residue in 20 ml of *absolute ethanol*. To 10 ml of the resulting solution add 10 ml of a solution prepared by dissolving 1 g of *dipotassium hydrogen orthophosphate* in 55 ml of *water*. Transfer a portion of the mixture to a 1-cm closed silica cell, place the cell 10 cm from a 15-watt, short-wave ultraviolet lamp and irradiate for 30 minutes. The *absorbance* of the irradiated solution measured at the maximum at 418 nm, Appendix II B, is not greater than that obtained by repeating the operation using 10 ml of a 0.00150% w/v solution of *diethylstilbestrol EPCRS* in *absolute ethanol* , beginning at the words 'add 10 ml of a solution …' (0.015%).

Diethylstilbestrol disodium monophosphate

Dilute the combined aqueous phase reserved in the test for Free diethylstilbestrol to 100 ml with *water* (solution A). Prepare a solution containing 0.020% w/v of *diethylstilbestrol EPCRS* in *ethanol (96%) (*solution B). To 2 ml of each solution add 3 ml of *phosphomolybdotungstic reagent*, dilute to 50 ml with a 5% w/v solution of *anhydrous sodium carbonate*, mix thoroughly and allow to stand for 30 minutes; filter the solutions through a polytetrafluoroethylene filter (0.45 µm) and measure the *absorbance*, Appendix II B, at 660 nm using *water* in the reference cell. The *absorbance* of solution A, multiplied by 1.46, is not greater than that obtained for solution B (1%).

Water

13.0 to 16.5% w/w, Appendix IX C. Use 0.2 g.

ASSAY

Heat 0.2 g in a Kjeldahl flask with 2 ml of *sulphuric acid* and 2.5 ml of *nitric acid* until brown fumes cease to be evolved, allow to cool, add 1 ml of *nitric acid* and heat again. Continue adding *nitric acid* and heating until brown fumes are no longer evolved and the solution is colourless when cold. Heat until dense, white fumes are evolved, cool, transfer the solution to a flask with the aid of 150 ml of *water*, add 50 ml of *citric-molybdic acid solution* and heat slowly to boiling point. Swirling the flask continuously, add 25 ml of *quinoline solution*, at first drop wise and then in a steady stream, heat on a water bath for 5 minutes and cool. Filter, wash the precipitate with *water* until free from acid, transfer the precipitate to a flask with the aid of 100 ml of *water*, add 50 ml of 0.5M *sodium hydroxide VS* and shake until dissolved. Titrate the excess of alkali with 0.5M *hydrochloric acid VS* using *phenolphthalein-thymol blue solution* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 4.964 mg of $C_{18}H_{18}Na_4O_8P_2$.

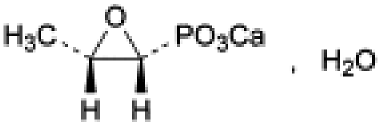
STORAGE

Fosfestrol Sodium should be protected from light.

Fosfomycin Calcium

General Notices

(Ph Eur monograph 1328)



$C_3H_5CaO_4P,H_2O$ 194.1 *26016-98-8*

Action and use

Phosphonic acid derivative; antibacterial.

Ph Eur

DEFINITION

Calcium (2*R*,3*S*)-(3-methyloxiran-2-yl)phosphonate monohydrate.

Substance produced by certain strains of *Streptomyces fradiae* or obtained by any other means.

Content

95.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, practically insoluble in acetone, in methanol and in methylene chloride.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison   Ph. Eur. reference spectrum of fosfomycin calcium.

B. Dissolve about 0.1 g in 3 ml of a 25 per cent *V/V* solution of *perchloric acid R*. Add 1 ml of *0.1 M sodium periodate* and heat on a water-bath for 30 min. Allow to cool and add 50 ml of *water R*. Neutralise with a saturated solution of *sodium hydrogen carbonate R* and add 1 ml of a freshly prepared 400 g/l solution of *potassium iodide R*. Prepare a blank at the same time and in the same manner. The test solution remains colourless and the blank is orange.

C. To about 8 mg add 2 ml of *water R*, 1 ml of *perchloric acid R* and 2 ml of *0.1 M sodium periodate*. Heat on a water-bath for 10 min and add, without cooling, 1 ml of *ammonium molybdate solution R5* and 1 ml of *aminohydroxynaphthalenesulphonic acid solution R*. Allow to stand for 30 min. A blue colour develops.

D. It gives reaction (a) of calcium *(2.3.1)*.

TESTS

pH *(2.2.3)*

8.1 to 9.6.

Dissolve 20 mg in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 11.0 to - 13.0 (anhydrous substance).

Dissolve 2.5 g in a 125 g/l solution of *sodium edetate R* previously adjusted to pH 8.5 with *strong sodium hydroxide solution R*, and dilute to 50.0 ml with the same solution. Measure at 405 nm using a mercury lamp.

Impurity A

Maximum 1.5 per cent.

In a glass-stoppered flask, dissolve 0.200 g in 100.0 ml of *water R*. Add 50 ml of *0.5 M phthalate buffer solution pH 6.4 R* and 5.0 ml of *0.005 M sodium periodate*, close and shake. Allow to stand protected from light for 90 min. Add 10 ml of a freshly prepared 400 g/l solution of *potassium iodide R*, close and shake for 2 min. Titrate with *0.0025 M sodium arsenite* until the yellow colour almost disappears. Add 2 ml of *starch solution R* and slowly continue the titration until the colour is completely discharged. Carry out a blank test under the same conditions. Calculate the percentage content of $C_3H_7CaO_5P$ using the following expression:

$$\frac{(n_1 - n_2) \times c \times 97}{m\,(100 - H)} \times 100$$

m = mass of the substance to be examined, in milligrams,

n_1 = volume *of 0.0025 M sodium arsenite* used in the blank titration,

n_2 = volume of *0.0025 M sodium arsenite* used in the titration of the test solution,

c = molarity of the sodium arsenite solution,

H = percentage content of water.

Chlorides *(2.4.4)*

Maximum 0.2 per cent.

Dissolve 0.500 g in *water R*, add 2 ml of *nitric acid R* and dilute to 50 ml with the same acid. To 2.5 ml of this solution add 12.5 ml of *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.5 g in 6 ml of *glacial acetic acid R* and dilute to 25.0 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Water *(2.5.12)*

8.5 per cent to 11.5 per cent, determined on 0.250 g. Use as the solvent a mixture of 1 volume of *pyridine R* and 3 volumes of *ethylene glycol R*.

ASSAY

In a glass-stoppered flask, dissolve 0.120 g in 20.0 ml of *0.1 M sodium periodate*. Add 5 ml of a 50 per cent *V/V* solution of *perchloric acid R* and shake. Heat in a water-bath at 37 °C for 105 min. Add 50 ml of *water R* and immediately adjust to pH 6.4 with a saturated solution of *sodium hydrogen carbonate R*. Add 10 ml of a freshly prepared 400 g/l solution of *potassium iodide R*, close and allow to stand for 2 min. Titrate with *0.1 M sodium arsenite* until the yellow colour almost disappears. Add 2 ml of *starch solution R* and slowly continue the titration until the colour is completely discharged. Carry out a blank test under the same conditions.

Calculate the percentage content of $C_3H_5CaO_4P$ using the following expression:

$$\frac{(n_1 - n_2) \times c \times 88 \times 100}{m\,(100 - H)} \times 100 - G$$

m = mass of the substance to be examined, in milligrams,

n_1 = volume of *0.1 M sodium arsenite* used in the blank titration,

n_2 = volume of *0.1 M sodium arsenite* used in the titration of the test solution,

c = molarity of the sodium arsenite solution,

G = percentage content of impurity A,

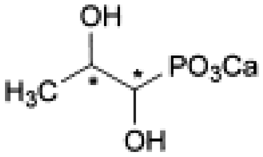
H = percentage content of water.

STORAGE

In an airtight container , protected from light.

IMPURITIES

Specified impurities *A*.



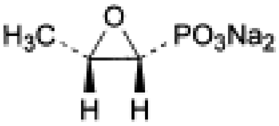
A. calcium (1,2-dihydroxypropyl)phosphonate.

Ph Eur

Fosfomycin Sodium

General Notices

(Ph Eur monograph 1329)



$C_3H_5Na_2O_4P$ 182.0 *26016-99-9*

Action and use

Phosphonic acid derivative; antibacterial.

Ph Eur

DEFINITION

Disodium (2*R*,3*S*)-(3-methyloxiran-2-yl)phosphonate.

Substance produced by certain strains of *Streptomyces fradiae* or obtained by any other means.

Content

95.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, very hygroscopic powder.

Solubility

Very soluble in water, sparingly soluble in methanol, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison Ph. Eur. reference spectrum of fosfomycin sodium.

B. Dissolve about 0.1 g in 3 ml of a 25 per cent *V/V* solution of *perchloric acid R*. Add 1 ml of *0.1 M sodium periodate* and heat on a water-bath for 30 min. Allow to cool and add 50 ml of *water R*. Neutralise with a saturated solution of *sodium hydrogen carbonate R* and add 1 ml of a freshly prepared 400 g/l solution of *potassium iodide R*. Prepare a blank at the same time and in the same manner. The test solution remains colourless and the blank is orange.

C. To about 8 mg add 2 ml of *water R*, 1 ml of *perchloric acid R* and 2 ml of *0.1 M sodium periodate*. Heat on a water-bath for 10 min and add, without cooling, 1 ml of *ammonium molybdate solution R5* and 1 ml of *aminohydroxynaphthalenesulphonic acid solution R*. Allow to stand for 30 min. A blue colour develops.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_9 *(2.2.2, Method II)*.

pH *(2.2.3)*

9.0 to 10.5.

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7)*

- 13.0 to - 15.0 (anhydrous substance).

Dissolve 2.5 g in *water R* and dilute to 50.0 ml with the same solvent. Measure at 405 nm using a mercury lamp.

Impurity A

Maximum 1.0 per cent.

In a glass-stoppered flask, dissolve 0.200 g in 100.0 ml of *water R*. Add 50 ml of *0.5 M phthalate buffer solution pH 6.4 R* and 5.0 ml of *0.005 M sodium periodate*, close and shake. Allow to stand protected from light for 90 min. Add 10 ml of a freshly prepared 400 g/l solution of *potassium iodide R*, close and shake for 2 min. Titrate with *0.0025 M sodium arsenite* until the yellow colour almost disappears. Add 2 ml of *starch solution R* and slowly continue the titration until the colour is completely discharged. Carry out a blank test under the same conditions.

Calculate the percentage content of $C_3H_7Na_2O_5P$ using the following expression:

$$\frac{(n_1 - n_2) \times c \times 100}{m\,(100 - H)} \times 100$$

m = mass of the substance to be examined, in milligrams,

n_1 = volume of *0.0025 M sodium arsenite* used in the blank titration,

n_2 = volume of *0.0025 M sodium arsenite* used in the titration of the test solution,

c = molarity of the sodium arsenite solution,

H = percentage content of water.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 0.50 g. Use as the solvent a mixture of 1 volume of *pyridine R* and 3 volumes of *ethylene glycol R*.

Bacterial endotoxins ***(2.6.14)***

Less than 0.083 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

In a glass-stoppered flask, dissolve 0.120 g in 20.0 ml of *0.1 M sodium periodate*. Add 5 ml of a 50 per cent *V/V* solution of *perchloric acid R* and shake. Heat on a water-bath at 37 °C for 105 min. Add 50 ml of *water R* and immediately adjust to pH 6.4 with a saturated solution of *sodium hydrogen carbonate R*. Add 10 ml of a freshly prepared 400 g/l solution of *potassium iodide R*, close and allow to stand for 2 min. Titrate with *0.1 M sodium arsenite* until the yellow colour almost disappears. Add 2 ml of *starch solution R* and slowly continue the titration until the colour is completely discharged. Carry out a blank test under the same conditions.

Calculate the percentage content of $C_3H_5Na_2O_4P$ using the following expression:

$$\frac{(n_1 - n_2) \times c \times 91 \times 100}{m\,(100 - H)} \times 100 - G$$

m = mass of the substance to be examined, in milligrams,

n_1 = volume of *0.1 M sodium arsenite* used in the blank titration,

n_2 = volume of *0.1 M sodium arsenite* used in the titration of the test solution,

c = molarity of the sodium arsenite solution,

G = percentage content of impurity A,

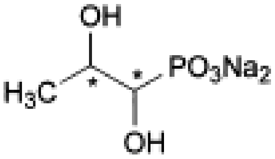
H = percentage content of water.

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities A.



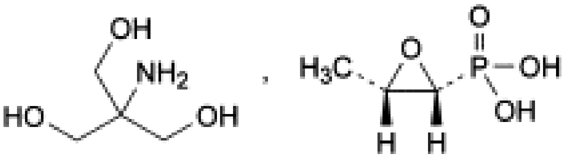
A. disodium (1,2-dihydroxypropyl)phosphonate.

Ph Eur

Fosfomycin Trometamol

General Notices

(Ph Eur monograph 1425)



$C_7H_{18}NO_7P$ 259.2 *78964-85-9*

Action and use

Phosphonic acid derivative; antibacterial.

Ph Eur

DEFINITION

2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrogen (2*R*,3*S*)-(3-methyloxiran-2-yl) phosphonate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Very soluble in water, slightly soluble in ethanol (96 per cent) and in methanol, practically insoluble in acetone.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *fosfomycin trometamol CRS* .

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *fosfomycin trometamol CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate *cellulose for chromatography R* as the coating substance.

Mobile phase *concentrated ammonia R*, *water R*, *2-propanol R* (10:20:70 *V/V/V*).

Application 10 µl.

Development Over 3/4 of the plate.

Drying In a current of warm air.

Detection Expose to iodine vapour until the spots appear.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 15 mg add 2 ml of *water R*, 1 ml of *perchloric acid R* and 2 ml of *0.1 M sodium periodate*. Heat on a water-bath for 10 min and add, without cooling, 1 ml of *ammonium molybdate solution R5* and 1 ml of *aminohydroxynaphthalenesulphonic acid solution R*. Allow to stand for 30 min. A blue colour develops.

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH *(2.2.3)*

3.5 to 5.5 for solution S.

Specific optical rotation *(2.2.7)*

- 13.5 to - 12.5 (anhydrous substance), determined on solution S at 365 nm using a mercury lamp.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 0.600 g of the substance to be examined in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (a) Dissolve 0.600 g of *fosfomycin trometamol CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 3.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Wet 0.3 g of the substance to be examined with 60 µl of *water R* and heat in an oven at 60 °C for 24 h. Dissolve the residue in the mobile phase and dilute to 20.0 ml with the mobile phase (solution A). Dissolve 0.6 g of the substance to be examined in solution A and dilute to 5.0 ml with the same solution (*in situ* degradation to obtain impurities

A, B, C and D).

Blank solution The mobile phase.

Column:
- *size:* *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 10.89 g/l solution of *potassium dihydrogen phosphate R in water for chromatography R*.

Flow rate 1.0 ml/min.

Detection Differential refractometer at 35 °C.

Injection 10 µl of the blank solution, the test solution and reference solutions (b) and (c).

Run time Twice the retention time of fosfomycin.

Relative retention With reference to fosfomycin (retention time = about 9 min): trometamol (2 peaks) = about 0.3; impurity B = about 0.48; impurity C = about 0.54; impurity A = about 0.88; impurity D = about 1.27.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to impurity A and fosfomycin,

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *impurities C, D*: for each impurity, not more than 0.33 times the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *unspecified impurities*: for each impurity, not more than 0.33 times the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than 1.67 times the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.17 times the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the 2 peaks due to trometamol and any peak due to the blank.

Phosphates

Maximum 500 ppm.

Dissolve 0.1 g in 3 ml of *dilute nitric acid R* and dilute to 10 ml with *water R*. To 5 ml of this solution add 5 ml of *water R* and 5 ml of *molybdovanadic reagent R*. Shake vigorously. After 5 min, any colour in the test solution is not more intense than that in a standard prepared at the same time in the same manner, using 5 ml of *phosphate standard solution (5 ppm PO_4) R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection 5 µl of the test solution and reference solution (a).

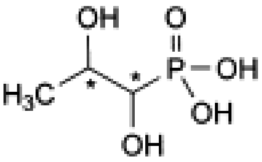
Calculate the percentage content of $C_7H_{18}NO_7P$ from the areas of the peaks due to fosfomycin and the declared content of *fosfomycin trometamol CRS* .

STORAGE

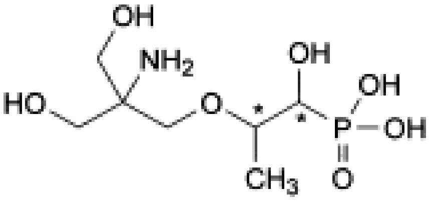
In an airtight container .

IMPURITIES

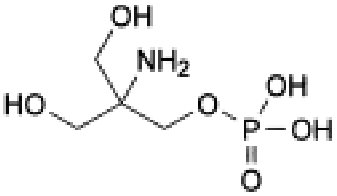
Specified impurities *A, B, C, D.*



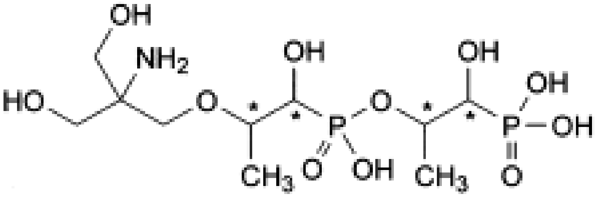
A. (1,2-dihydroxypropyl)phosphonic acid,



B. [2-[2-amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropyl]phosphonic acid,



C. 2-amino-3-hydroxy-2-(hydroxymethyl)propyl dihydrogen phosphate (trometamol phosphoric acid monoester),



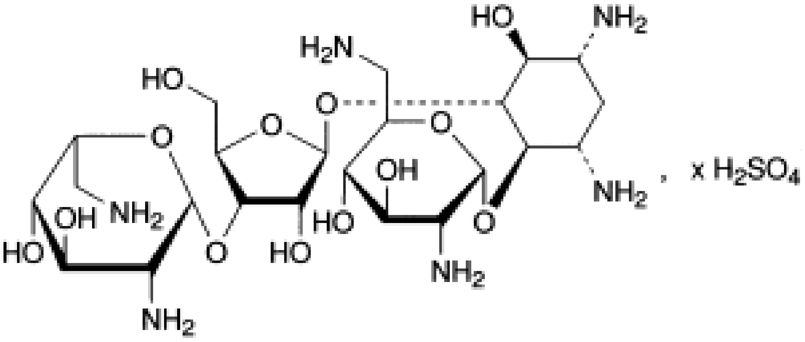
D. [2-[[[2-[2-amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropyl]hydroxyphosphoryl]oxy]-1-hydroxypropyl]phosphonic acid (trometamoyloxy fosfomycin dimer).

Ph Eur

Framycetin Sulphate

General Notices

(Ph Eur monograph 0180)



$C_{23}H_{46}N_6O_{13},xH_2SO_4$ 615 *4146-30-9*
(base)

Action and use

Antibacterial.

Ph Eur

DEFINITION

Sulphate of 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B).

Substance produced by the growth of selected strains of *Streptomyces fradiae* or *Streptomyces decaris* or obtained by any other means.

Content

Minimum of 630 IU/mg (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder, hygroscopic.

Solubility

Freely soluble in water, very slightly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances.

Results:

— the retention time of the principal peak in the chromatogram obtained with the test solution is approximately the same as that of the principal peak in the chromatogram obtained with reference solution (a),

— it complies with the limit given for impurity C.

B. It gives reaction (a) of sulphates *(2.3.1)*.

TESTS

pH *(2.2.3)*

6.0 to 7.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 52.5 to + 55.5 (dried substance).

Dissolve 1.00 g in *water R* and dilute to 10.0 ml with the same solvent

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve the contents of a vial of *framycetin sulphate CRS* in the mobile phase and dilute with the mobile phase to obtain a solution containing 0.5 mg/ml.

Reference solution (b) Dilute 3.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (d Dissolve the contents of a vial of *neamine CRS* (corresponding to 0.5 mg) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (e) Dissolve 10 mg of *neomycin sulphate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 25 °C.

Mobile phase Mix 20.0 ml of *trifluoroacetic acid R*, 6.0 ml of *carbonate-free sodium hydroxide solution R* and 500 ml of *water R*, allow to equilibrate, dilute to 1000 ml with *water R* and degas.

Flow rate 0.7 ml/min.

Post-column solution *carbonate-free sodium hydroxide solution R* diluted 1 in 25 previously degassed, which is added pulse-less to the column effluent using a 375 µl polymeric mixing coil.

Flow rate 0.5 ml/min.

Detection Pulsed amperometric detector with a gold working electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively 0.00 V detection, + 0.80 V oxidation and - 0.60 V reduction potentials, with pulse durations according to the instrument used.

Injection 10 µl.

Run time 1.5 times the retention time of neomycin B.

Relative retention With reference to neomycin B (retention time = about 10 min): impurity A = about 0.65; impurity C = about 0.9; impurity G = about 1.1.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to impurity C and to neomycin B in the chromatogram obtained with reference solution (e); if necessary, adjust the volume of the carbonate-free sodium hydroxide solution in the mobile phase,

— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (c).

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) and taking into account the declared content of *neamine CRS* (1.0 per cent),

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),

— *total of other impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

Sulphate

27.0 per cent to 31.0 per cent (dried substance).

Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 ml of *alcohol R* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Loss on drying *(2.2.32)*

Maximum 8.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 1.0 per cent, determined on 1.0 g.

Sterility ***(2.6.1)***

If intended for introduction into body cavities without a further appropriate sterilisation procedure, it complies with the test for sterility.

Bacterial endotoxins ***(2.6.14, Method D)***

Less than 1.3 IU/mg if intended for introduction into body cavities without a further appropriate procedure for the removal of bacterial endotoxins.

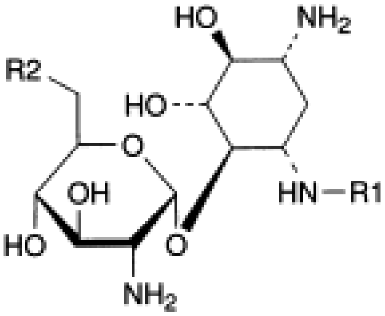
ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)*. Use *framycetin sulphate CRS* as the reference substance.

STORAGE

In an airtight container , protected from light. If the substance is intended for introduction into body cavities, store in a sterile, tamper-proof container .

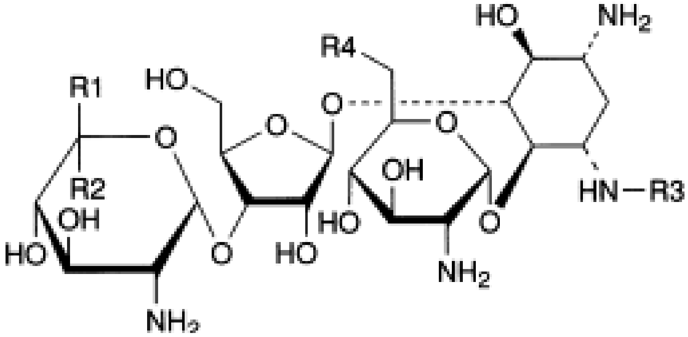
IMPURITIES



A. R1 = H, R_2 = NH_2: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (neamine or neomycin A-LP),

B. R1 = CO-CH_3, R2 = NH_2: 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (3-acetylneamine),

D. R1 = H, R2 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine (paromamine or neomycin D),



C. R1 = CH_2-NH_2, R2 = R3 = H, R4 = NH_2: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-O-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin C),

E. R1 = R3 = H, R2 = CH_2-NH_2, R4 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin I or neomycin E),

F. R1 = CH_2-NH_2, R2 = R3 = H, R4 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin II or neomycin F),

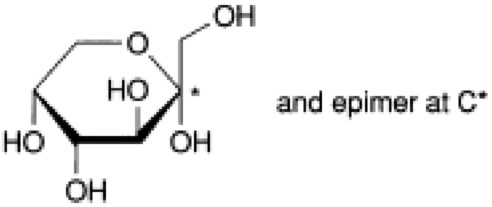
G. R1 = H, R2 = CH_2-NH_2, R3 = CO-CH_3, R4 = NH_2: 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B-LP).

Ph Eur

Fructose

General Notices

(Ph Eur monograph 0188)



$C_6H_{12}O_6$ 180.2 *57-48-7*

Preparation

Fructose Intravenous Infusion

Ph Eur

DEFINITION

(–)-D-Arabino-hex-2-ulopyranose.

The substance described in this monograph is not necessarily suitable for parenteral use.

CHARACTERS

Appearance

White or almost white, crystalline powder.

It has a very sweet taste.

Solubility

Very soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *water R*, *methanol R* (2:3 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *fructose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg each of *fructose CRS*, *glucose CRS*, *lactose CRS* and *sucrose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Plate *TLC silica gel G plate R*.

Mobile phase *water R*, *methanol R*, *anhydrous acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*). Measure the volumes accurately since a slight excess of water produces cloudiness.

Application 2 µl; thoroughly dry the starting points.

Development A Over a path of 15 cm.

Drying A In a current of warm air.

Development B Immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*. Heat at 130 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

B. Dissolve 0.1 g in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. A red precipitate is formed.

C. To 1 ml of solution S (see Tests) add 9 ml of *water R*. To 1 ml of the solution add 5 ml of *hydrochloric acid R* and heat to 70 °C. A brown colour develops.

D. Dissolve 5 g in *water R* and dilute to 10 ml with the same solvent. To 0.5 ml of the solution add 0.2 g of *resorcinol R* and 9 ml of *dilute hydrochloric acid R* and heat on a water-bath for 2 min. A red colour develops.

TESTS

Solution S

Dissolve 10.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Dissolve 5.0 g in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)*. Add 10 ml of *water R*. The solution is colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Dissolve 6.0 g in 25 ml of *carbon dioxide-free water R* and add 0.3 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation *(2.2.7)*

- 91.0 to - 93.5 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, add 0.2 ml of *dilute ammonia R1*, allow to stand for 30 min and dilute to 100.0 ml with *water R*.

Foreign sugars

Dissolve 5.0 g in *water R* and dilute to 10 ml with the same solvent. To 1 ml of the solution add 9 ml of *ethanol (96 per cent) R*. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of the initial solution and 9 ml of *water R*.

5-Hydroxymethylfurfural and related compounds

To 5 ml of solution S add 5 ml of *water R*. The absorbance *(2.2.25)* measured at 284 nm is not greater than 0.32.

Barium

To 10 ml of solution S add 1 ml of *dilute sulphuric acid R*. When examined immediately and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Lead *(2.4.10)*

Maximum 0.5 ppm.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash

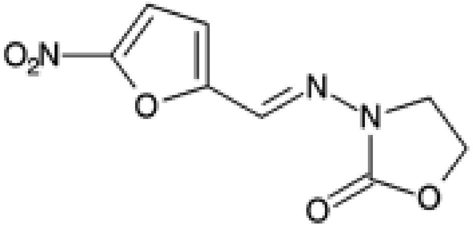
Maximum 0.1 per cent.

Dissolve 5.0 g in 10 ml of *water R*, add 2 ml of *sulphuric acid R*, evaporate to dryness on a water-bath and ignite to constant mass.

Ph Eur

Furazolidone

General Notices



$C_8H_7N_3O_5$ 225.2 *67-45-8*

Action and use

Antiprotozoal; antibacterial.

DEFINITION

Furazolidone is 3-(5-nitrofurfurylideneamino)oxazolidin-2-one. It contains not less than 97.0% and not more than 103.0% of $C_8H_7N_3O_5$, calculated with reference to the dried substance.

CHARACTERISTICS

A yellow, crystalline powder.

Very slightly soluble in *water* and in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of furazolidone *(RS 164)*.

B. Dissolve 1 mg in 1 ml of *dimethylformamide* and add 0.05 ml of 1M *ethanolic potassium hydroxide*. A deep blue colour is produced.

TESTS

Acidity or alkalinity

Shake 1 g for 15 minutes with 100 ml of *carbon dioxide-free water* and filter. The pH of the filtrate is 4.5 to 7.0, Appendix V L.

Nitrofurfural diacetate

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 95 volumes of *toluene* and 5 volumes of *1,4-dioxan* as the mobile phase. Apply separately to the plate 20 µl of solution (1) and 10 µl of solution (2). For solution (1) dissolve 50 mg of the substance being examined in 5 ml of *dimethylformamide* by heating on a water bath for a few minutes, allow to cool and dilute to 10 ml with *acetone*. Solution (2) contains 0.010% w/v solution of *nitrofurfural diacetate BPCRS* in a mixture of equal volumes of *dimethylformamide* and *acetone*. After removal of the plate, heat it at 105° for 5 minutes and spray with a solution prepared by dissolving 0.75 g of *phenylhydrazine hydrochloride* in 10 ml of *ethanol (96%)*, diluting to 50 ml with *water*, adding *activated charcoal*, filtering and then adding 25 ml of *hydrochloric acid* and sufficient *water* to produce 200 ml. Any spot corresponding to nitrofurfural diacetate in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Carry out the following procedure protected from light. To 80 mg add 150 ml of *dimethylformamide*, swirl to dissolve and add sufficient *water* to produce 500 ml. Dilute 5 ml to 100 ml with *water* and mix. Measure the *absorbance* of the resulting solution at the maximum at 367 nm, Appendix II B. Calculate the content of $C_8H_7N_3O_5$ taking 750 as the value of A(1%, 1 cm) at the maximum at 367 nm.

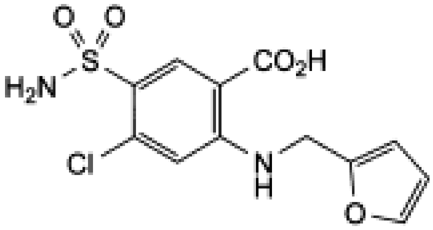
STORAGE

Furazolidone should be protected from light.

Furosemide

General Notices

(Ph Eur monograph 0391)



$C_{12}H_{11}ClN_2O_5S$ 330.7 *54-31-9*

Action and use

Loop diuretic.

Preparations

Co-amilofruse Tablets

Furosemide Injection

Furosemide Tablets

Ph Eur

DEFINITION

4-Chloro-2-[(furan-2-ylmethyl)amino]-5-sulphamoylbenzoic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

mp

About 210 °C, with decomposition.

IDENTIFICATION

First identification *B*.

Second identification *A, C.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50 mg in a 4 g/l solution of *sodium hydroxide R* and dilute to 100 ml with the same solution. Dilute 1 ml of this solution to 100 ml with a 4 g/l solution of *sodium hydroxide R*.

Spectral range 220-350 nm.

Absorption maxima At 228 nm, 270 nm and 333 nm.

Absorbance ratio A_{270} / A_{228} = 0.52 to 0.57.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *furosemide CRS .*

C. Dissolve about 25 mg in 10 ml of *ethanol (96 per cent) R*. To 5 ml of this solution add 10 ml of *water R*. To 0.2 ml of the solution add 10 ml of *dilute hydrochloric acid R* and heat under a reflux condenser for 15 min. Allow to cool and add 18 ml of *1 M sodium hydroxide* and 1 ml of a 5 g/l solution of *sodium nitrite R*. Allow to stand for 3 min, add 2 ml of a 25 g/l solution of *sulphamic acid R* and mix. Add 1 ml of a 5 g/l solution of *naphthylethylenediamine dihydrochloride R*. A violet-red colour develops.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *furosemide impurity A CRS* in the mobile phase and dilute to 2.0 ml with the mobile phase.

Reference solution (b) Dilute a mixture of 1.0 ml of the test solution and 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.2 g of *potassium dihydrogen phosphate R* and 0.25 g of *cetrimide R* in 70 ml of *water R*; adjust to pH 7.0 with *ammonia R* and add 30 ml of *propanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 238 nm.

Injection 20 µl of the test solution and reference solution (b).

Run time 3 times the retention time of furosemide.

System suitability Reference solution (b):

— *resolution*: minimum 4 between the peaks due to impurity A (1st peak) and furosemide (2nd peak).

Limits:

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) (0.025 per cent).

Chlorides ***(2.4.4)***

Maximum 200 ppm.

To 0.5 g add a mixture of 0.2 ml of *nitric acid R* and 30 ml of *water R* and shake for 5 min. Allow to stand for 15 min and filter.

Sulphates ***(2.4.13)***

Maximum 300 ppm.

To 1.0 g add a mixture of 0.2 ml of *acetic acid R* and 30 ml of *distilled water R* and shake for 5 min. Allow to stand for 15 min and filter.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 20 ml of *dimethylformamide R*. Titrate with *0.1 M sodium hydroxide* using 0.2 ml of *bromothymol blue solution R2*. Carry out a blank titration.

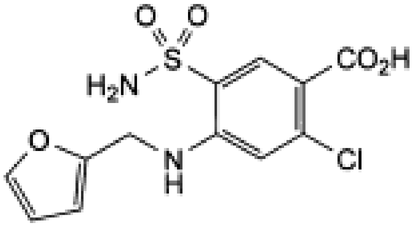
1 ml of *0.1 M sodium hydroxide* is equivalent to 33.07 mg of $C_{12}H_{11}ClN_2O_5S$.

STORAGE

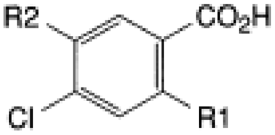
Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E.



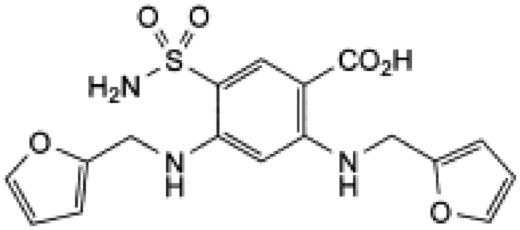
A. 2-chloro-4-[(furan-2-ylmethyl)amino]-5-sulphamoylbenzoic acid,



B. R1 = Cl, R2 = SO_2-NH_2: 2,4-dichloro-5-sulphamoylbenzoic acid,

C. R1 = NH_2, R2 = SO_2-NH_2: 2-amino-4-chloro-5-sulphamoylbenzoic acid,

E. R1 = Cl, R2 = H: 2,4-dichlorobenzoic acid,



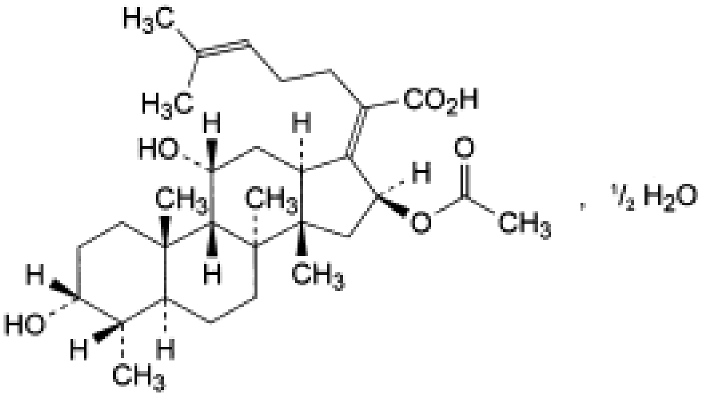
D. 2,4-bis[(furan-2-ylmethyl)amino]-5-sulphamoylbenzoic acid.

Ph Eur

Fusidic Acid

General Notices

(Ph Eur monograph 0798)



$C_{31}H_{48}O_6,½H_2O$ 525.7 *6990-06-3*

Action and use

Antibacterial.

Preparations

Fusidic Acid Cream

Fusidic Acid Eye Drops

Fusidic Acid Oral Suspension

Ph Eur

DEFINITION

ent-(17*Z*)-16α-(Acetyloxy)-3β,11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20), 24-dien-21-oic acid hemihydrate.

Antimicrobial substance produced by the growth of certain strains of *Fusidium coccineum* or by any other means.

Content

97.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of fusidic acid .*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 24 mg of *diethanolamine fusidate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *methanol R*, *cyclohexane R*, *glacial acetic acid R* , *chloroform R* (2.5:10:10:80 *V/V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *3-ketofusidic acid CRS* in 5 ml of the mobile phase. To 1.0 ml of this solution add 0.20 ml of the test solution and dilute to 20.0 ml with the mobile phase.

Reference solution (b) Dilute 20 µl of the test solution to 100.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.125-0.15 m, Ø = 4-5 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *10 g/l solution of phosphoric acid R* , *water R*, *acetonitrile R* (10:20:20:50 *V/V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl.

Run time 3.5 times the retention time of fusidic acid.

System suitability:
- — *resolution*: minimum 2.5 between the peaks due to 3-ketofusidic acid and fusidic acid in the chromatogram obtained with reference solution (a);
- — *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:
- — *total*: not more than twice the area of the peak due to fusidic acid in the chromatogram obtained with reference solution (a) (2.0 per cent);
- — *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Water *(2.5.12)*

1.4 per cent to 2.0 per cent, determined on 0.50 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 10 ml of *ethanol (96 per cent) R*. Add 0.5 ml of *phenolphthalein solution R*. Titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 51.67 mg of $C_{31}H_{48}O_6$.

STORAGE

Protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Hard Fat

General Notices

(Ph Eur monograph 0462)

Ph Eur

DEFINITION

Mixture of triglycerides, diglycerides and monoglycerides, which may be obtained either by esterification of fatty acids of natural origin with glycerol or by transesterification of natural fats.

Each type of hard fat is characterised by its melting point, its hydroxyl value and its saponification value.

It contains no added substances.

CHARACTERS

Appearance

White or almost white, waxy, brittle mass.

Solubility

Practically insoluble in water, slightly soluble in anhydrous ethanol.

When heated to 50 °C, it melts giving a colourless or slightly yellowish liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 1.0 g of the substance to be examined in *ethylene chloride R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel G plate R.

Mobile phase ether R, *ethylene chloride R* (10:90 *V/V*).

Application 2 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

Results The chromatogram shows a spot with an R_F value of about 0.6 due to triglycerides (R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.5) and to 1,2-diglycerides (R_{st} 0.3). A spot due to 1-monoglycerides may also be visible (R_{st} 0.05).

TESTS

Alkaline impurities

Dissolve 2.00 g in a mixture of 1.5 ml of *ethanol (96 per cent) R* and 3.0 ml of *ether R*. Add 0.05 ml of *bromophenol blue solution R*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Melting point *(2.2.15)*

30 °C to 45 °C, and within 2 °C of the nominal value.

Introduce the melted substance into the capillary tube and allow to stand at a temperature below 10 °C for 24 h.

Acid value *(2.5.1)*

Maximum 0.5.

Dissolve 5.0 g in 50 ml of the prescribed mixture of solvents.

Hydroxyl value *(2.5.3, Method A)*

Maximum 50, and within 5 units of the nominal value; maximum 5 if the nominal value is less than 5.

Iodine value *(2.5.4, Method A)*

Maximum 3.

Peroxide value *(2.5.5, Method A)*

Maximum 3.

Saponification value *(2.5.6)*

210 to 260, and within 5 per cent of the nominal value, determined on 2.0 g.

Unsaponifiable matter *(2.5.7)*

Maximum 0.6 per cent, determined on 5.0 g.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Total ash *(2.4.16)*

Maximum 0.05 per cent, determined on 2.00 g.

STORAGE

Protected from light and heat.

LABELLING

The label states:

— the nominal melting point,

— the nominal hydroxyl value,

— the nominal saponification value.

Ph Eur

Products of Fermentation

General Notices

(Ph Eur monograph 1468)

Ph Eur

This monograph applies to indirect gene products obtained by fermentation. It is not applicable to:

- *— monographs in the Pharmacopoeia concerning vaccines for human or veterinary use;*
- *— products derived from continuous cell lines of human or animal origin;*
- *— direct gene products that result from the transcription and translation from nucleic acid to protein, whether or not subject to post-translational modification;*
- *— products obtained by semi-synthesis from a product of fermentation and those obtained by biocatalytic transformation;*
- *— whole broth concentrates or raw fermentation products.*

This monograph provides general requirements for the development and manufacture of products of fermentation. These requirements are not necessarily comprehensive in a given case and requirements complementary or additional to those prescribed in this monograph may be imposed in an individual monograph or by the competent authority.

DEFINITION

For the purposes of this monograph, products of fermentation are active or inactive pharmaceutical substances produced by controlled fermentation as indirect gene products. They are primary or secondary metabolites of micro-organisms such as bacteria, yeasts, fungi and micro-algae, whether or not modified by traditional procedures or recombinant DNA (rDNA) technology. Such metabolites include vitamins, amino acids, antibiotics, alkaloids and polysaccharides.

They may be obtained by batch or continuous fermentation processes followed by procedures such as extraction, concentration, purification and isolation.

PRODUCTION

Production is based on a process that has been validated and shown to be suitable. The extent of validation depends on the critical nature of the respective process step.

CHARACTERISATION OF THE PRODUCER MICRO-ORGANISM

The history of the micro-organism used for production is documented. The micro-organism is adequately characterised. This may include determination of the phenotype of the micro-organism, macroscopic and microscopic methods and biochemical tests and, if appropriate, determination of the genotype of the micro-organism and molecular genetic tests.

PROCESSES USING A SEED-LOT SYSTEM

The *master cell bank* is a homogeneous suspension or lyophilisate of the original cells distributed into individual containers for storage. The viability and productivity of the cells under the selected storage conditions and their suitability for initiating a satisfactory production process after storage must be demonstrated.

Propagation of the master cell bank may take place through a seed-lot system that uses a working cell bank.

The *working cell bank* is a homogeneous suspension or lyophilisate of the cell material derived from the master cell bank, distributed in equal volumes into individual containers for storage (for example, in liquid nitrogen).

Production may take place by batch or continuous culture and may be terminated under defined conditions.

All containers in a cell bank are stored under identical conditions. Once removed from storage, the individual ampoules, vials or culture straws are not returned to the cell bank.

PROCESSES USING STAGED GROWTH IN CULTURES

The contents of a container of the working cell bank are used, if necessary after resuspension, to prepare an inoculum in a suitable medium. After a suitable period of growth, the cultures are used to initiate the fermentation process, if necessary following preculture in a prefermentor. The conditions to be used at each stage of the process are defined and must be met with each production run.

CHANGE CONTROL

If the production process is altered in a way that causes a significant change in the impurity profile of the product, the critical steps associated with this change in impurity profile are revalidated.

If a significant change has taken place in the micro-organism used for production that causes a significant change in the impurity profile of the product, the critical steps of the production process associated with this change, particularly the procedure for purification and isolation, are revalidated.

Revalidation includes demonstration that new impurities present in the product as a result of the change are adequately controlled by the test procedures. If necessary, additional or alternative tests must be introduced with appropriate limits. If the change in the process or in the micro-organism results in an increase in the level of an impurity already present, the acceptability of such an increase is addressed.

When a master cell bank is replaced, the critical steps of the production process must be revalidated to the extent necessary to demonstrate that no adverse change has occurred in the quality and safety of the product. Particular attention must be given to possible changes in the impurity profile of the product if a modified or new micro-organism is introduced into the process.

RAW MATERIALS

The raw materials employed in the fermentation and/or down-stream processing are of suitable quality for the intended purpose. They are tested to ensure that they comply with written specifications.

Levels of bioburden in media or in the inlet air for aeration are reduced to an adequately low level to ensure that if microbiological contamination occurs, it does not adversely affect the quality, purity and safety of the product. Addition of components such as nutrients, precursors, and substrates during fermentation takes place aseptically.

IN-PROCESS CONTROLS

In-process controls are in place to ensure the consistency of the conditions during fermentation and down-stream processing and of the quality of the isolated product. Particular attention must be paid to ensure that any microbial contamination that adversely affects the quality, purity and safety of the product is detected by the controls applied.

Production conditions may be monitored, as appropriate, by suitable procedures for example to control and check:

— temperature,

— pH,

— rate of aeration,

— rate of agitation,

— pressure,

and to monitor the concentration of the required product.

DOWN-STREAM PROCESSING

At the end of fermentation, the producer micro-organism is inactivated or removed. Further processing is designed to reduce residues originating from the culture medium to an acceptable level and to ensure that the desired product is recovered with consistent quality.

Various purification processes may be used, for example, charcoal treatment, ultrafiltration and solvent extraction. It must be demonstrated that the process or processes chosen reduce to a minimum or remove:

— residues from the producer micro-organism, culture media, substrates and precursors,

— unwanted transformation products of substrates and precursors.

If necessary, suitable tests are performed either as in-process controls or on the isolated product of fermentation.

IDENTIFICATION, TESTS AND ASSAY

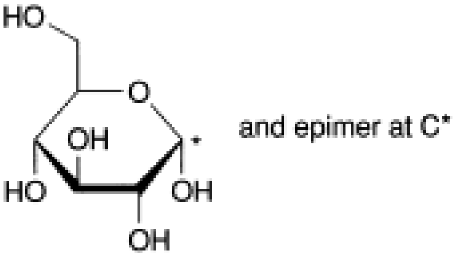
The requirements with which the product must comply throughout its period of validity, as well as specific test methods, are stated in the individual monographs.

Ph Eur

Anhydrous Glucose

General Notices

(Ph Eur monograph 0177)



$C_6H_{12}O_6$ 180.2 *50-99-7*

Preparations

Glucose Intravenous Infusion

Glucose Irrigation Solution

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution

Oral Rehydration Salts

Potassium Chloride and Glucose Intravenous Infusion

Potassium Chloride, Sodium Chloride and Glucose Intravenous Infusion

Sodium Chloride and Glucose Intravenous Infusion

Ph Eur

DEFINITION

(+)-D-Glucopyranose.

CHARACTERS

Appearance

White or almost white, crystalline powder.

It has a sweet taste.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *water R*, *methanol R* (2:3 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *glucose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg each of *fructose CRS*, *glucose CRS* , *lactose CRS* and *sucrose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Plate *TLC silica gel G plate R.*

Mobile phase *water R*, *methanol R*, *anhydrous acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application 2 µl; thoroughly dry the starting points.

Development A Over a path of 15 cm.

Drying A In a current of warm air.

Development B Immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*. Heat at 130 °C for 10 min.

System suitability Test solution (b):

— the chromatogram shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. A red precipitate is formed.

TESTS

Solution S

Dissolve 10.0 g in distilled *water R* and dilute to 100 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 10.0 g in 15 ml of *water R*.

Acidity or alkalinity

Dissolve 6.0 g in 25 ml of *carbon dioxide-free water R* and add 0.3 ml of *phenolphthalein* *solution R*. The solution is colourless. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation *(2.2.7)*

+ 52.5 to + 53.3 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, add 0.2 ml of *dilute ammonia R1*, allow to stand for 30 min and dilute to 100.0 ml with *water R*.

Foreign sugars, soluble starch, dextrins

Dissolve 1.0 g by boiling in 30 ml of *ethanol (90 per cent V/V) R*. Cool; the appearance of the solution shows no change.

Sulphites

Maximum 15 ppm, expressed as SO_2.

Test solution Dissolve 5.0 g in 40 ml of *water R*, add 2.0 ml of *0.1 M sodium hydroxide* and dilute to 50.0 ml with *water R*. To 10.0 ml of the solution, add 1 ml of a 310 g/l solution of *hydrochloric acid R*, 2.0 ml of *decolorised fuchsin solution R1* and 2.0 ml of a 0.5 per cent *V/V* solution of *formaldehyde R*. Allow to stand for 30 min.

Reference solution Dissolve 76 mg of *sodium metabisulphite R* in *water R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. To 3.0 ml of this solution add 4.0 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with *water R*. Immediately add to 10.0 ml of this solution 1 ml of a 310 g/l solution of *hydrochloric acid R*, 2.0 ml of *decolorised fuchsin solution R1* and 2.0 ml of a 0.5 per cent V/V solution of *formaldehyde R*. Allow to stand for 30 min.

Measure the absorbance *(2.2.25)* of the 2 solutions at the absorption maximum at 583 nm using for both measurements a solution prepared in the same manner using 10.0 ml of *water R* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution.

Chlorides *(2.4.4)*

Maximum 125 ppm.

Dilute 4 ml of solution S with 15 ml of *water R*.

Sulphates *(2.4.13)*

Maximum 200 ppm.

Dilute 7.5 ml of solution S with 15 ml of *distilled water R*.

Arsenic *(2.4.2, Method A)*

Maximum 1 ppm, determined on 1.0 g.

Barium

To 10 ml of solution S add 1 ml of *dilute sulphuric acid R*. When examined immediately and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Calcium *(2.4.3)*

Maximum 200 ppm.

Dilute 5 ml of solution S with 15 ml of *distilled water R*.

Lead ***(2.4.10)***

Maximum 0,5 ppm.

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 0.50 g.

Sulphated ash

Maximum 0.1 per cent.

Dissolve 5.0 g in 5 ml of *water R*, add 2 ml of *sulphuric acid R*, evaporate to dryness on a water-bath and ignite to constant mass. If necessary, repeat the heating with *sulphuric acid R*.

Pyrogens ***(2.6.8)***

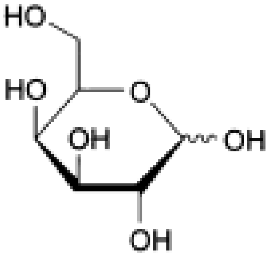
If intended for use in the manufacture of large-volume parental dosage forms without a further appropriate procedure for the removal of pyrogens, the competent authority may require that it comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 ml of a solution in *water for injections R* containing 50 mg of the substance to be examined per millilitre.

Ph Eur

Galactose

General Notices

(Ph Eur monograph 1215)



$C_6H_{12}O_6$ 180.2 *59-23-4*

Ph Eur

DEFINITION

D-Galactopyranose.

CHARACTERS

Appearance

White or almost white, crystalline or finely granulated powder.

Solubility

Freely soluble or soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *galactose CRS* .

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (a) Dissolve 10 mg of *galactose CRS* in a mixture of 2 volumes of *water*

R and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *galactose CRS* , 10 mg of *glucose CRS* and 10 mg of *lactose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Plate Suitable silica gel as the coating substance.

Mobile phase *water R*, *propanol R* (15:85 *V/V*).

Application 2 µl; thoroughly dry the starting points.

Development In an unsaturated tank over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*. Heat in an oven at 130 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. An orange or red precipitate is formed.

TESTS

Solution S

Dissolve, with heating in a water-bath at 50 °C, 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_8 *(2.2.2, Method II)*.

Acidity or alkalinity

To 30 ml of solution S add 0.3 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 1.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation *(2.2.7)*

+ 78.0 to + 81.5 (anhydrous substance).

Dissolve 10.00 g in 80 ml of *water R* and add 0.2 ml of *dilute ammonia R1*. Allow to stand for 30 min and dilute to 100.0 ml with *water R*.

Barium

Dilute 5 ml of solution S to 10 ml with *distilled water R*. Add 1 ml of *dilute sulphuric acid R*. When examined immediately and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 5 ml of solution S and 6 ml of *distilled water R*.

Lead ***(2.4.10)***

Maximum 0.5 ppm.

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.00 g.

Sulphated ash

Maximum 0.1 per cent.

To 5 ml of solution S add 2 ml of *sulphuric acid R*, evaporate to dryness on a water-bath and ignite to constant mass. The residue weighs a maximum of 1 mg.

Microbial contamination

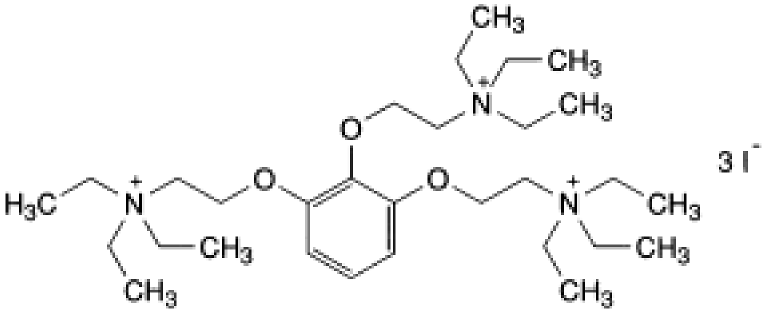
Total viable aerobic count *(2.6.12)* not more than 10^2 micro-organisms per gram.

Ph Eur

Gallamine Triethiodide

General Notices

(Ph Eur monograph 0181)



$C_{30}H_{60}I_3N_3O_3$ 892 *65-29-2*

Action and use

Non-depolarizing neuromuscular blocker.

Preparation

Gallamine Injection

Ph Eur

DEFINITION

2,2′,2″-[Benzene-1,2,3-triyltris(oxy)]tris(*N*,*N*,*N*-triethylethanaminium) triiodide.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50 mg in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid. Dilute 1.0 ml of this solution to 100.0 ml with *0.01 M hydrochloric acid*.

Spectral range 220-350 nm.

Absorption maximum At 225 nm.

Specific absorbance at the absorption maximum 500 to 550.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *gallamine triethiodide CRS.*

C. To 5 ml of solution S (see Tests) add 1 ml of *potassium tetraiodomercurate solution R*. A yellow precipitate is formed.

D. Dilute 0.5 ml of solution S to 2 ml with *water R*. Add 0.2 ml of *dilute nitric acid R*. The solution gives reaction (a) of iodides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.6 g in *water R* and dilute to 30 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and immediately after preparation is not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Acidity or alkalinity

To 50 ml of *water R* add 0.2 ml of *methyl red solution R*. Add either *0.01 M sulphuric acid* or *0.02 M sodium hydroxide* until an orange-yellow colour is obtained. Add 1.0 g of the substance to be examined and dissolve by shaking. Not more than 0.2 ml of *0.01 M sulphuric acid* or *0.02 M sodium hydroxide* is required to restore the orange-yellow colour.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 14 g of *sodium perchlorate R* in 850 ml of *phosphate buffer pH 3.0 R* and add 150 ml of *methanol R2*.

Flow rate 1 ml/min.

Detection   Spectrophotometer at 205 nm.

Injection   20 µl.

Run time   1.5 times the retention time of triethylgallamine as perchlorate.

Relative retention with reference to triethylgallamine as perchlorate (retention time = about 40 min): impurity A = about 0.45; impurity B = about 0.50; impurity C = about 0.65; impurity D = about 0.75; impurity E = about 0.85; impurity F = about 0.90.

Limits:

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with the reference solution (1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2 per cent);

— *disregard limit*: disregard the peak due to iodide with a retention time of zero.

Loss on drying *(2.2.32)*

Maximum 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.270 g in a mixture of 5.0 ml of *anhydrous formic acid R* and 50.0 ml of *acetic anhydride R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

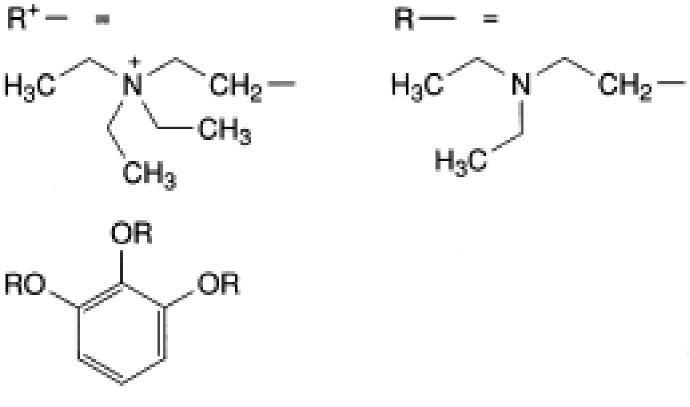
1 ml of *0.1 M perchloric acid* is equivalent to 29.72 mg of $C_{30}H_{60}I_3N_3O_3$.

STORAGE

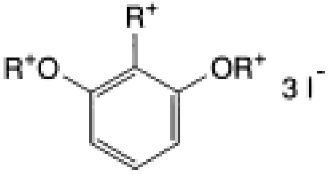
In an airtight container, protected from light.

IMPURITIES

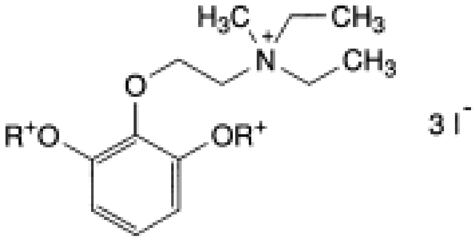
Specified impurities   *A, B, C, D, E, F*.



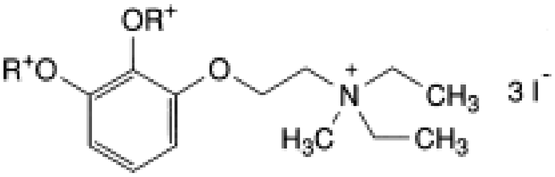
A. 2,2′,2′′-[benzene-1,2,3-triyltris(oxy)]tris(*N*,*N*-diethylethanamine),



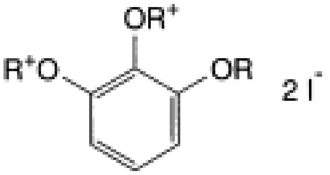
B. 2,2′-[2-[2-(triethylammonio)ethyl]-1,3-phenylenebis(oxy)]bis(*N*,*N*,*N*-triethylethanaminium) triiodide,



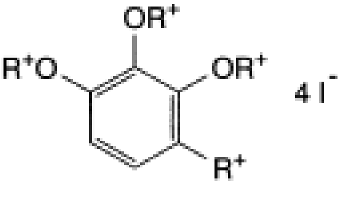
C. 2,2′-[2-[2-(diethylmethylammonio)ethoxy]-1,3-phenylenebis(oxy)]bis(*N*,*N*,*N*-triethylethanaminium) triiodide,



D. 2,2′-[3-[2-(diethylmethylammonio)ethoxy]-1,2-phenylenebis(oxy)]bis(*N*,*N*,*N*-triethylethanaminium) triiodide,



E. 2,2′-[3-[2-(diethylamino)ethoxy]-1,2-phenylenebis(oxy)]bis(*N*,*N*,*N*-triethylethanaminium) diiodide,



F. 2,2′,2′′-[4-[2-(triethylammonio)ethyl]benzene-1,2,3triyltris(oxy)]tris(*N*,*N*,*N*-triethylethanaminium) tetraiodide.

Ph Eur

Gelatin

General Notices

(Ph Eur monograph 0330)

Action and use

Excipient.

Ph Eur

DEFINITION

Purified protein obtained either by partial acid hydrolysis (type A), partial alkaline hydrolysis (type B) or enzymatic hydrolysis of collagen from animals (including fish and poultry); it may also be a mixture of different types.

The hydrolysis leads to gelling or non-gelling product grades. Both product grades are covered by this monograph.

Gelatin described in this monograph is not suitable for parenteral use or for other special purposes.

CHARACTERS

Appearance

Faintly yellow or light yellowish-brown, solid, usually occurring as translucent sheets, shreds, granules or powder.

Solubility

Practically insoluble in common organic solvents; gelling grades swell in cold water and give on heating a colloidal solution which on cooling forms a more or less firm gel.

The isoelectric point is a relevant quality parameter for use of gelatin in different applications: for type A gelatin it is typically between pH 6.0 and pH 9.5 and for type B gelatin is typically between pH 4.7 and pH 5.6. These ranges cover a variety of different gelatins and for specific applications a narrower tolerance is usually applied.

Different gelatins form aqueous solutions that vary in clarity and colour. For a particular application, a suitable specification for clarity and colour is usually applied.

IDENTIFICATION

A. To 2 ml of solution S (see Tests) add 0.05 ml of *copper sulphate solution R*. Mix and add 0.5 ml of *dilute sodium hydroxide solution R*. A violet colour is produced.

B. To 0.5 g in a test-tube add 10 ml of *water R*. Allow to stand for 10 min, heat at 60 °C for 15 min and keep the tube upright at 0 °C for 6 h. Invert the tube; the contents immediately flow out for non-gelling grades and do not flow out immediately for gelling grades.

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* at about 55 °C, dilute to 100 ml with the same solvent and keep the solution at this temperature to carry out the tests.

pH *(2.2.3)*

3.8 to 7.6 for solution S.

Conductivity *(2.2.38)*

Maximum 1 mS·cm^{-1}, determined on a 1.0 per cent solution at 30 ± 1.0 °C.

Sulphur dioxide *(2.5.29)*

Maximum 50 ppm.

Peroxides

Maximum 10 ppm, determined using *peroxide test strips R*.

Peroxidase transfers oxygen from peroxides to an organic redox indicator which is converted to a blue oxidation product. The intensity of the colour obtained is proportional to the quantity of peroxide and can be compared with a colour scale provided with the test strips, to determine the peroxide concentration.

Suitability test Dip a test strip for 1 s into *hydrogen peroxide standard solution (10 ppm H_2O_2) R*, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and compare the reaction zone after 15 s with the colour scale provided with the test strips used. The colour must match that of the 10 ppm concentration, otherwise the test is invalid.

Test Weigh 20.0 ± 0.1 g of the substance to be tested in a beaker and add 80.0 ± 0.2 ml of *water R*. Stir to moisten all gelatin and allow the sample to stand at room temperature for 1-3 h. Cover the beaker with a watch-glass. Place the beaker for 20 ± 5 min in a water bath at 65 ± 2 °C to dissolve the sample. Stir the contents of the beaker with a glass rod to achieve a homogeneous solution. Dip a test strip for 1 s into the test solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and compare the reaction zone after 15 s with the colour scale provided with the test strips used. Multiply the concentration read from the colour scale by a factor of 5 to calculate the concentration in parts per million of peroxide in the test substance.

Gel strength (Bloom value)

80 to 120 per cent of the labelled nominal value.

The gel strength is expressed as the mass in grams necessary to produce the force which, applied to a plunger 12.7 mm in diameter, makes a depression 4 mm deep in a gel having a concentration of 6.67 per cent *m/m* and matured at 10 °C.

Apparatus Texture analyser or gelometer with:

— a cylindrical piston 12.7 ± 0.1 mm in diameter with a plane pressure surface with a sharp bottom edge,

— a bottle 59 ± 1 mm in internal diameter and 85 mm high.

Adjust the apparatus according to the manufacturer's manual. Settings are: distance 4 mm, test speed 0.5 mm/s.

Method Perform the test in duplicate. Place 7.5 g of the substance to be tested in each bottle. Add 105 ml of *water R*, place a watch-glass over each bottle and allow to stand for 1-4 h. Heat in a water-bath at 65 ± 2 °C for 15 min. While heating, gently stir with a glass rod. Ensure that the solution is uniform and that any condensed water on the inner walls of the bottle is incorporated. Allow to cool at room temperature for 15 min and transfer the bottles to a thermostatically controlled bath at 10.0 ± 0.1 °C, and fitted with a device to ensure that the platform on which the bottles stand is perfectly horizontal. Close the bottles with a rubber stopper and allow to stand for 17 ± 1 h. Remove the sample bottles from the bath and quickly wipe the water from the exterior of the bottle. Centre consecutively the 2 bottles on the platform of the apparatus so that the plunger contacts the sample as nearly at its midpoint as possible and start the measurement. Report the result as the average of the 2 measurements.

Iron

Maximum 30.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution To 5.00 g of the substance to be examined, in a conical flask, add 10 ml of *hydrochloric acid R*. Close the flask and place in a water-bath at 75-80 °C for 2 h. Allow to cool and adjust the content of the flask to 100.0 g with *water R*.

Reference solutions Prepare the reference solutions using *iron standard solution (8 ppm Fe) R*, diluted as necessary with *water R*.

Wavelength 248.3 nm.

Chromium

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Test solution described in the test for iron.

Reference solutions Prepare the reference solutions using *chromium standard solution (100 ppm Cr) R*, diluted if necessary with *water R*.

Wavelength 357.9 nm.

Zinc

Maximum 30.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Test solution described in the test for iron.

Reference solutions Prepare the reference solutions using *zinc standard solution (10 ppm Zn) R*, diluted if necessary with *water R*.

Wavelength 213.9 nm.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 1.000 g, by drying in an oven at 105 °C.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

STORAGE

Protect from heat and moisture.

LABELLING

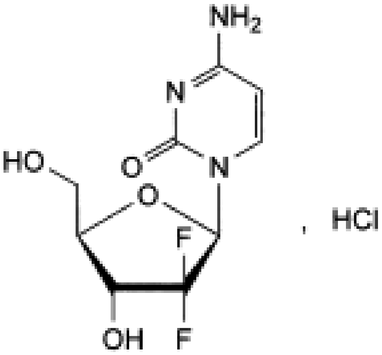
The label states the gel strength (Bloom value) or that it is a non-gelling grade.

Ph Eur

Gemcitabine Hydrochloride

General Notices

(Ph Eur monograph 2306)



$C_9H_{11}F_2N_3O_4,HCl$ 299.7 *122111-03-9*

Action and use

Pyrimidine analogue; cytotoxic.

Ph Eur

DEFINITION

4-Amino-1-(2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl)pyrimidin-2(1*H*)-one hydrochloride.

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water, slightly soluble in methanol, practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison gemcitabine hydrochloride CRS .

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

2.0 to 3.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 43.0 to + 50.0, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Test solution (b) Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 200.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of the substance to be examined and 10.0 mg of *gemcitabine impurity A CRS* in *water R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 200.0 ml with *water R*.

Reference solution (b) Dissolve 20.0 mg of *gemcitabine hydrochloride CRS* in *water R* and dilute to 200.0 ml with the same solvent.

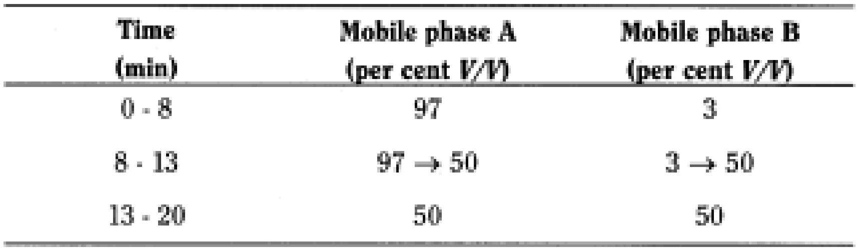
Reference solution (c) Place 10 mg of the substance to be examined in a small vial. Add 4 ml of a 168 g/l solution of *potassium hydroxide R* in *methanol R*, sonicate for 5 min then seal with a cap. The mixture may be cloudy. Heat at 55 °C for a minimum of 6 h to produce impurity B. Allow to cool, then transfer the entire contents of the vial to a 100 ml volumetric flask by successively washing with a 1 per cent *V/V* solution of *phosphoric acid R*. Dilute to 100 ml with a 1 per cent *V/V* solution of *phosphoric acid R* and mix.

Column:

- — *size: l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- — *mobile phase A*: 13.8 g/l solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 2.5 ± 0.1 with *phosphoric acid R*;
- — *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	97	3
8 - 13	97 → 50	3 → 50
13 - 20	50	50

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (c).

Relative retention with reference to gemcitabine (retention time = about 8 min): impurity A = about 0.4; impurity B = about 0.7.

System suitability Reference solution (c):

— *resolution*: minimum 8.0 between the peaks due to impurity B and gemcitabine.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to gemcitabine in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the peak due to gemcitabine in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the peak due to gemcitabine in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 1.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using 5 ml of *lead standard solution (1 ppm Pb) R,* 5 ml of *water R* and 2 ml of the aqueous solution to be examined. If necessary, filter the solutions and compare the spots on the membrane filter.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

Bacterial endotoxins *(2.6.14)*

Less than 0.05 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Mobile phase A.

Injection Test solution (b) and reference solutions (b) and (c).

Relative retention with reference to gemcitabine (retention time = about 10 min): impurity B = about 0.5.

System suitability Reference solution (c):

— *resolution*: minimum 8.0 between the peaks due to impurity B and gemcitabine.

Calculate the percentage content of $C_9H_{12}ClF_2N_3O_4$ from the declared content *of gemcitabine hydrochloride CRS* .

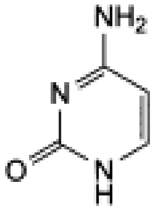
STORAGE

If the substance is sterile, store in a sterile, airtight, tamper-proof container.

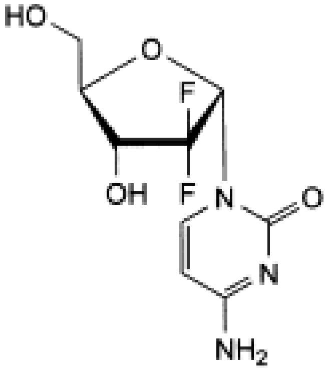
IMPURITIES

Specified impurities *A*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B.*



A. 4-aminopyrimidin-2(1*H*)-one (cytosine),



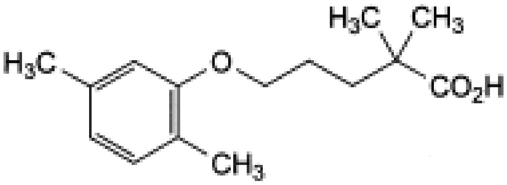
B. 4-amino-1-(2-deoxy-2,2-difluoro-α-D-*erythro*-pentofuranosyl)pyrimidin-2(1*H*)-one (gemcitabine α-anomer).

Ph Eur

Gemfibrozil

General Notices

(Ph Eur monograph 1694)



$C_{15}H_{22}O_3$ 250.3 *25812-30-0*

Action and use

Fibrate; lipid-regulating drug.

Preparations

Gemfibrozil Capsules

Gemfibrozil Tablets

Ph Eur

DEFINITION

5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, waxy, crystalline powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, freely soluble in anhydrous ethanol and in methanol.

IDENTIFICATION

A. Melting point *(2.2.14)*: 58 °C to 61 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *gemfibrozil CRS.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 40 mg of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A.

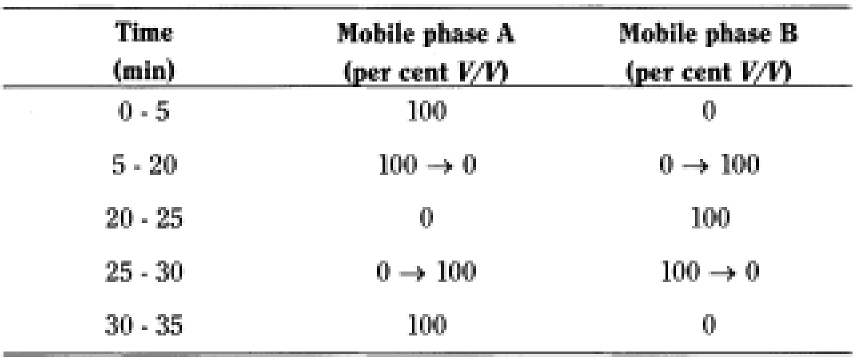
Reference solution (a) Dissolve the contents of a vial of *gemfibrozil for system suitability CRS* (containing impurities C, D and E) in 2 ml of *acetonitrile R.*

Reference solution (b) Dilute 1.0 ml of test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (c) Dissolve 5 mg of *2,5-dimethylphenol R* (impurity A) in mobile phase A and dilute to 10 ml with mobile phase A.

Column:
- *size:* *l* = 0.250 m, Ø = 4.0 mm,
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: dissolve 0.27 g of *potassium dihydrogen phosphate R* in 400 ml of *water R*, adjust to pH 4.0 with *phosphoric acid R* and add 600 ml of *acetonitrile R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 20	100 → 0	0 → 100
20 - 25	0	100
25 - 30	0 → 100	100 → 0
30 - 35	100	0

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 276 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *gemfibrozil for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C, D and E. Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention with reference to gemfibrozil (retention time = about 7 min): impurity A = about 0.4; impurity C = about 1.3; impurity D = about 1.5; impurity E = about 1.7; impurity I = about 2.0; impurity H = about 2.9.

System suitability Reference solution (a):

- — *resolution*: minimum 6.0 between the peaks due to gemfibrozil and impurity C, and minimum 2.0 between the peaks due to impurity D and impurity E.

Limits:

- — *correction factors*: for the calculations of content multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.5; impurity D = 3.3; impurity E = 0.2; impurity I = 2;
- — *impurities E, I*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- — *impurities A, D, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- — *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 0.25 per cent, determined on 2.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 2.0 g. Allow to stand for 1 h after the first moistening before heating.

ASSAY

Dissolve 0.200 g in 25 ml of *methanol R*. Add 25 ml of *water R* and 1 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)* using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 25.03 mg of $C_{15}H_{22}O_3$.

STORAGE

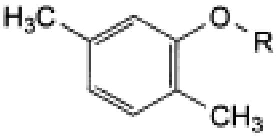
Protected from light.

IMPURITIES

Specified impurities *A, D, E, H, I*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph

Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *B, C, F, G.*

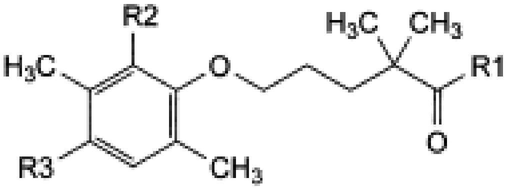


A. R = H: 2,5-dimethylphenol (*p*-xylenol),

C. R = $[CH_2]_3$-O-$[CH_2]$2-O-C_2H_5: 2-[3-(2-ethoxyethoxy)propoxy]-1,4-dimethylbenzene,

F. R = $[CH_2]_4$-C_6H_5: 1,4-dimethyl-2-(4-phenylbutoxy)benzene,

G. R = CH_2-CH=CH_2: 1,4-dimethyl-2-(prop-2-enyloxy)benzene,

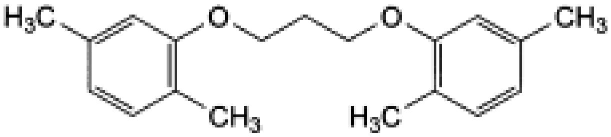


B. R1 = NH_2, R2 = R3 = H: 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanamide,

D. R1 = OH, R2 = CH=CH-CH_3, R3 = H: 5-[3,6-dimethyl-2-(prop-1-enyl)phenoxy]-2,2-dimethylpentanoic acid,

E. R1 = OH, R2 = H, R3 = CH=CH-CH_3: 5-[2,5-dimethyl-4-(prop-1-enyl)phenoxy]-2,2-dimethylpentanoic acid,

I. R1 = OCH_3, R2 = R3 = H: methyl 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate,



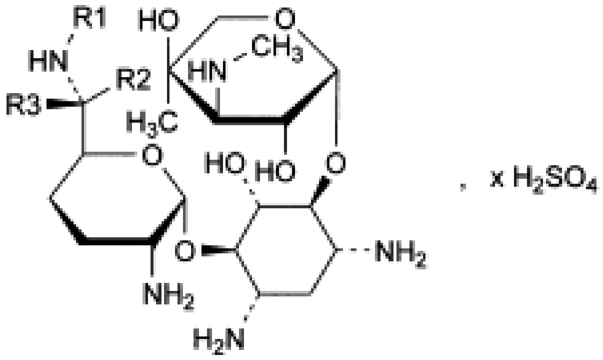
H. 1,3-bis(2,5-dimethylphenoxy)propane.

Ph Eur

Gentamicin Sulphate

General Notices

(Ph Eur monograph 0331)



Gentamicin	Mol. Formula	R1	R2	R3
C1	$C_{21}H_{43}N_5O_7$	CH_3	CH_3	H
C1a	$C_{19}H_{39}N_5O_7$	H	H	H
C2	$C_{20}H_{41}N_5O_7$	H	CH_3	H
C2a	$C_{20}H_{41}N_5O_7$	H	H	CH_3
C2b	$C_{20}H_{41}N_5O_7$	CH_3	H	H

1405-41-0

Action and use

Aminoglycoside antibacterial.

Preparations

Gentamicin Cream

Gentamicin Ear Drops

Gentamicin and Hydrocortisone Acetate Ear Drops

Gentamicin Eye Drops

Gentamicin Injection

Gentamicin Ointment

Ph Eur

DEFINITION

Mixture of the sulphates of antimicrobial substances produced by *Micromonospora purpurea*, the main components being gentamicins C1, C1a, C2, C2a and C2b.

Content

Minimum 590 IU/mg (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water, practically insoluble in alcohol.

IDENTIFICATION

First identification *C, D.*

Second identification *A, B, D.*

A. Dissolve about 10 mg in 1 ml of *water R* and add 5 ml of a 400 g/l solution of *sulphuric acid R*. Heat on a water-bath for 100 min, cool and dilute to 25 ml with *water R*. Examined between 240 nm and 330 nm *(2.2.25)*, the solution shows no absorption maximum.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve the contents of a vial of *gentamicin sulphate CRS* in *water R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase The lower layer of a mixture of equal volumes of *concentrated ammonia R*, *methanol R* and *methylene chloride R*.

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The 3 principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the 3 principal spots in the chromatogram obtained with the reference solution.

C. Examine the chromatograms obtained in the test for composition.

Results The chromatogram obtained with the test solution shows 5 principal peaks having the same retention times as the 5 principal peaks in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 0.8 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

pH *(2.2.3)*

3.5 to 5.5 for solution S.

Specific optical rotation *(2.2.7)*

+ 107 to + 121 (anhydrous substance).

Dissolve 2.5 g in *water R* and dilute to 25.0 ml with the same solvent.

Composition

Liquid chromatography *(2.2.29)*: use the normalisation procedure taking into account only the peaks due to gentamicins C1, C1a, C2, C2a and C2b; use the chromatogram supplied with *gentamicin sulphate CRS* to identify the corresponding peaks.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve the content of a vial of *gentamicin sulphate CRS* in the mobile phase and dilute with the mobile phase to obtain a solution containing 0.5 mg/ml.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm,
- *temperature*: 55 °C.

Mobile phase A mixture prepared with *carbon dioxide-free water R* containing 60 g/l of *anhydrous sodium sulphate R*, 1.75 g/l of *sodium octanesulphonate R*, 8 ml/l of *tetrahydrofuran R*, 50 ml/l of *0.2 M potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *dilute phosphoric acid R* and degassed.

Flow rate 1.0 ml/min.

Post-column solution A *carbonate-free sodium hydroxide solution R* diluted 1 to 25, previously degassed, which is added pulse-less to the column effluent using a 375 µl polymeric mixing coil.

Flow rate 0.3 ml/min.

Detection Pulsed amperometric detector or equivalent with a gold indicator electrode, a silver-silver chloride reference electrode, and a stainless steel auxiliary electrode which is the cell body, held at respectively + 0.05 V detection, + 0.75 V oxidation and - 0.15 V reduction potentials, with pulse durations according to the instrument used.

Injection 20 µl.

Run time 1.2 times the retention time of gentamicin C1.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 2.0 where H_p = height above the baseline of the peak due to gentamicin C2a, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to gentamicin C2.

Limits:

— *gentamicin C1* : 20.0 per cent to 40.0 per cent,

— *gentamicin C1a* : 10.0 per cent to 30.0 per cent,

— *sum of gentamicins C2, C2a, and C2b*: 40.0 per cent to 60.0 per cent,

— *disregard limit*: the area of the peak due to gentamicin C1a in the chromatogram obtained with reference solution (b).

Related substances

Liquid chromatography *(2.2.29)* as described in the test for composition.

Limits (for related substances eluting before gentamicin C1a):

— *any impurity*: maximum 3.0 per cent,

— *total*: maximum 10.0 per cent.

Methanol *(2.4.24, System B)*

Maximum 1.0 per cent.

Sulphate

32.0 per cent to 35.0 per cent (anhydrous substance).

Dissolve 0.250 g in 100 ml of *distilled water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate*, adding 50 ml of *alcohol R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Water *(2.5.12)*

Maximum 15.0 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 1.0 per cent, determined on 0.50 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.71 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)*.

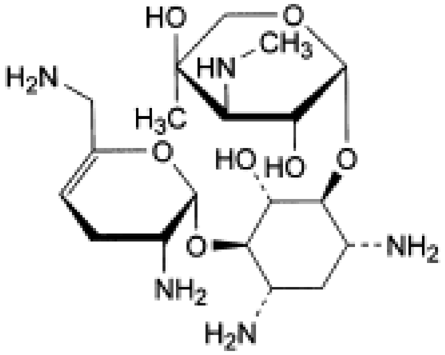
STORAGE

In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

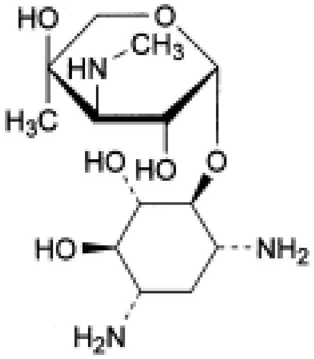
IMPURITIES

Specified impurities A, B, C.

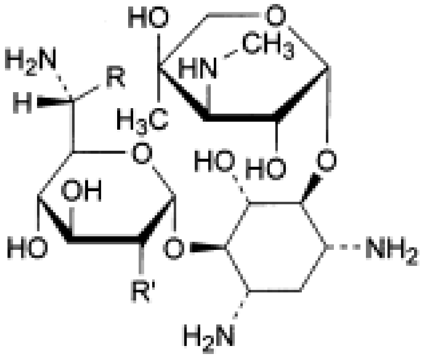
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use) : D, E.*



A. 2-deoxy-4-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-6-*O*-(2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl)-L-streptamine (sisomicin),

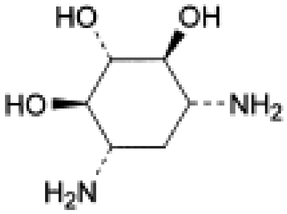


B. 2-deoxy-4-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-L-streptamine (garamine),



C. R = CH_3, R′ = OH: 4-*O*-(6-amino-6,7-dideoxy-D-*glycero*-α-D-*gluco*-heptopyranosyl)-2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-D-streptamine (gentamicin B $_1$),

D. R = H, R′ = NH_2: 2-deoxy-4-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-6-*O*-(2,6-diamino-2,6-dideoxy-α-D-*gluco*-hexopyranosyl)-L-streptamine,



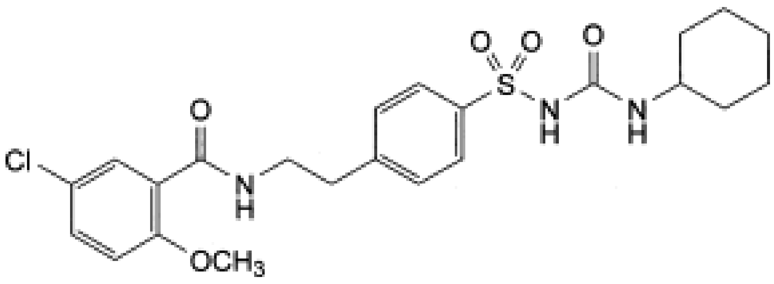
E. 2-deoxystreptamine.

Ph Eur

Glibenclamide

General Notices

(Ph Eur monograph 0718)



$C_{23}H_{28}ClN_3O_5S$ 494.0 *10238-21-8*

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

Preparation

Glibenclamide Tablets

Ph Eur

DEFINITION

1-[[4-[2-[(5-Chloro-2-methoxybenzoyl)amino]ethyl]phenyl]sulphonyl]-3-cyclohexylurea.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol and in methanol.

IDENTIFICATION

First identification A, C.

Second identification A, B, D, E.

A. Melting point *(2.2.14)*: 169 °C to 174 °C.

B. Dissolve 50.0 mg in *methanol R*, with the aid of ultrasound if necessary, and dilute to 50.0 ml with the same solvent. To 10.0 ml of the solution add 1.0 ml of a 103 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with *methanol R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 300 nm and a less intense maximum at 275 nm. The specific absorbances at the maxima are 61 to 65 and 27 to 32, respectively.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *glibenclamide CRS* .

If the spectra obtained show differences, moisten separately the substance to be examined and the reference substance with *methanol R*, triturate, dry at 100-105 °C and record the spectra again.

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 10 mg of *glibenclamide CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *alcohol R*, *glacial acetic acid R*, *cyclohexane R*, *methylene chloride R* (5:5:45:45 *V/V/V/V*).

Application 10 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

E. Dissolve 20 mg in 2 ml of *sulphuric acid R*. The solution is colourless and shows blue fluorescence in ultraviolet light at 365 nm. Dissolve 0.1 g of *chloral hydrate R* in the solution. Within about 5 min, the colour changes to deep yellow and, after about 20 min, develops a brownish tinge.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent. Prepare immediately before use.

Reference solution (a) Dissolve 5.0 mg of *glibenclamide impurity A CRS* and 5.0 mg of *glibenclamide impurity B CRS* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 20.0 ml with *methanol R*.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 50.0 ml with *methanol R*.

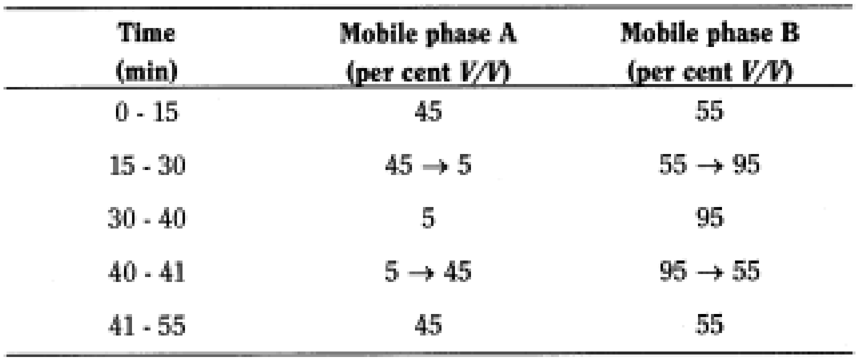
Reference solution (c) Dissolve 5 mg of *gliclazide CRS* in *methanol R*, add 2 ml of the test solution and dilute to 100 ml with *methanol R*. Dilute 1 ml of this solution to 10 ml with *methanol R.*

Column:

- *size: l* = 0.10 m, Ø = 4.6 mm,
- *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm),
- *temperature*: 35 °C.

Mobile phase:

- *mobile phase A*: mix 20 ml of a 101.8 g/l solution of freshly distilled *triethylamine R* adjusted to pH 3.0 using *phosphoric acid R*, and 50 ml of *acetonitrile R*; dilute to 1000 ml with *water R*,
- *mobile phase B*: mobile phase A, *water R*, *acetonitrile R* (20:65:915 *V/V/V*),



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	45	55
15 - 30	45 → 5	55 → 95
30 - 40	5	95
40 - 41	5 → 45	95 → 55
41 - 55	45	55

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

Relative retention With reference to glibenclamide (retention time = about 5 min): impurity A = about 0.5; impurity B = about 0.6.

System suitability Reference solution (c):

- *resolution*: minimum 5.0 between the peaks due to glibenclamide and gliclazide.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- *any other impurity*: not more than the area of the principal peak in the chromatogram

obtained with reference solution (b) (0.2 per cent), and not more than 2 such peaks have an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total of other impurities*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

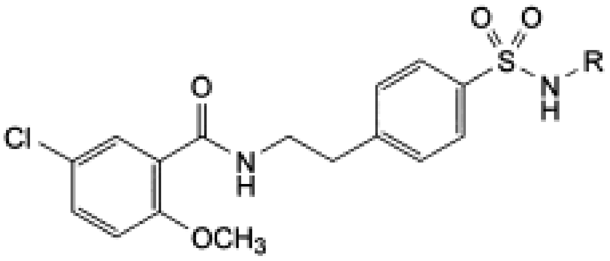
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g with heating in 100 ml of *alcohol R*. Titrate with *0.1 M sodium hydroxide*, using 1.0 ml of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 49.40 mg of $C_{23}H_{28}ClN_3O_5S$.

IMPURITIES



A. R = H: 5-chloro-2-methoxy-*N*-[2-(4-sulphamoylphenyl)ethyl]benzamide,

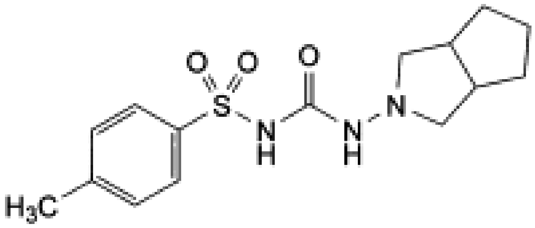
B. R = CO-OCH_3: methyl [[4-[2-[(5-chloro-2-methoxybenzoyl)amino]ethyl]phenyl]sulphonyl] carbamate.

Ph Eur

Gliclazide

General Notices

(Ph Eur monograph 1524)



$C_{15}H_{21}N_3O_3S$ 323.4 *21187-98-4*

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

Preparation

Gliclazide Tablets

Ph Eur

DEFINITION

1-(Hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(4-methylphenyl)sulphonyl]urea.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *gliclazide CRS* .

TESTS

Related substances Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Solvent mixture *acetonitrile R*, *water R* (45:55 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in 23 ml of *acetonitrile R* and dilute to 50.0 ml with *water R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 10.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 15 mg of *gliclazide impurity F CRS* in 23 ml of *acetonitrile R* and dilute to 50 ml with *water R*. Dilute 1 ml of this solution to 20 ml with the solvent mixture.

Reference solution (c) Dissolve 10.0 mg of *gliclazide impurity F CRS* in 45 ml of *acetonitrile R* and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

Column:
— *size:* *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *triethylamine R*, *trifluoroacetic acid R*, *acetonitrile R*, *water R* (0.1:0.1:45:55 *V/V/V/V*).

Flow rate 0.9 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl.

Run time Twice the retention time of gliclazide.

Relative retention With reference to gliclazide (retention time = about 16 min): impurity F = about 0.9.

System suitability Reference solution (b):

— *resolution*: minimum 1.8 between the peaks due to impurity F and gliclazide.

Limits:
— *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities other than F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Impurity B

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Test solution Dissolve 0.400 g of the substance to be examined in 2.5 ml of *dimethyl sulphoxide R* and dilute to 10.0 ml with *water R*. Stir for 10 min, store at 4 °C for 30 min and filter.

Reference solution Dissolve 20.0 mg of *gliclazide impurity B CRS* in *dimethyl sulphoxide R* and dilute to 100.0 ml with the same solvent. To 1.0 ml of the solution, add 12 ml of *dimethyl sulphoxide R* and dilute to 50.0 ml with *water R*. To 1.0 ml of this solution, add 12 ml of *dimethyl sulphoxide R* and dilute to 50.0 ml with *water R*.

Injection 50 µl.

Retention time Impurity B = about 8 min.

Limit:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (2 ppm).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

1.5 g complies with test F. Prepare the reference solution using 1.5 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.25 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

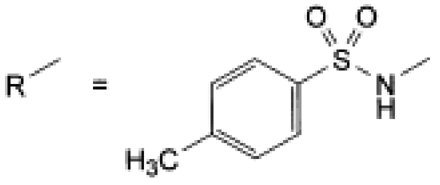
Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 32.34 mg of $C_{15}H_{21}N_3O_3S$.

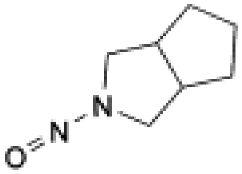
IMPURITIES

Specified impurities *B, F*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance*. See also 5.10. Control of impurities in substances for pharmaceutical use): A, C, D, E, G*.

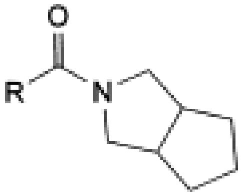


A. R-H: 4-methylbenzenesulphonamide,

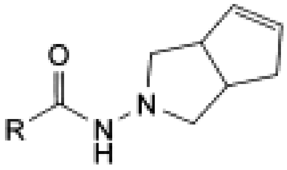


B. 2-nitroso-octahydrocyclopenta[*c*]pyrrole,

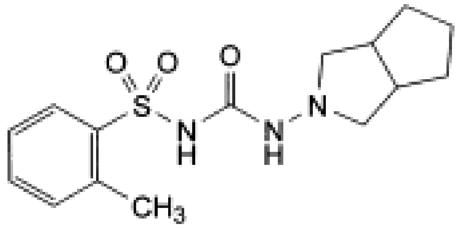
C. R-CO-O-C_2H_5: ethyl [(4-methylphenyl)sulphonyl]carbamate,



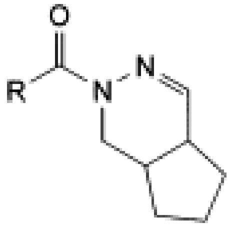
D. *N*-[(4-methylphenyl)sulphonyl]hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-carboxamide,



E. 1-[(4-methylphenyl)sulphonyl]-3-(3,3a,4,6a-tetrahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)urea,



F. 1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(2-methylphenyl)sulphonyl]urea,



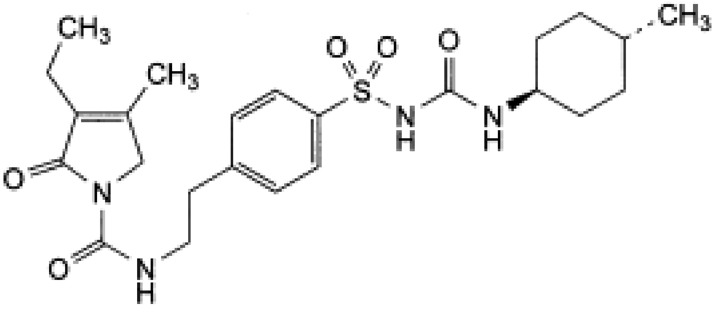
G. *N*-[(4-methylphenyl)sulphonyl]-1,4a,5,6,7,7a-hexahydro-2*H*-cyclopenta[*d*]pyridazine-2-carboxamide.

Ph Eur

Glimepiride

General Notices

(Ph Eur monograph 2223)



$C_{24}H_{34}N_4O_5S$ 490.6   *93479-97-1*

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

Ph Eur

DEFINITION

1-[[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl]phenyl]sulphonyl]-3-*trans*-(4-methylcyclohexyl)urea.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in dimethylformamide, slightly soluble in methylene chloride, very slightly soluble in methanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison glimepiride CRS .

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *dimethylformamide R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography *(2.2.29) Store the solutions at a temperature not exceeding 12 °C and for not more than 15 h.*

Solvent mixture water for chromatography R, *acetonitrile for chromatography R* (1:4 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *glimepiride for system suitability CRS* (containing impurities B, C and D) in 2.0 ml of the test solution.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 20.0 mg of *glimepiride CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Column:
- *size:* *l* = 0.25 m, Ø = 4 mm,
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase Dissolve 0.5 g of *sodium dihydrogen phosphate R* in 500 ml of *water for chromatography R* and adjust to pH 2.5 with *phosphoric acid R*. Add 500 ml of *acetonitrile for chromatography R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 228 nm.

Injection 20 µl of the test solution and reference solutions (a) and (b).

Run time 2.5 times the retention time of glimepiride.

Relative retention With reference to glimepiride (retention time = about 17 min): impurity B = about 0.2; impurity C = about 0.3; impurity D = about 1.1.

System suitability Reference solution (a):

- *resolution*: minimum 4.0 between the peaks due to impurity B and impurity C.

Limits:
- *impurity B*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent),
- *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *sum of impurities other than B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity A

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 10.0 mg of the substance to be examined in 5 ml of *methylene chloride R* and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 0.8 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 2.0 mg of *glimepiride CRS* (containing impurity A) in 1 ml of *methylene chloride R* and dilute to 4.0 ml with the mobile phase.

Column:
— *size:* l = 0.15 m, Ø = 4 mm,

— *stationary phase*: *diol silica gel for chromatography R* (5 µm).

Mobile phase *anhydrous acetic acid R*, *2-propanol R*, *heptane R* (1:100:899 *V/V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 228 nm.

Injection 10 µl.

Run time 1.5 times the retention time of glimepiride.

Identification of impurities Use the chromatogram supplied with *glimepiride CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to glimepiride (retention time = about 14 min): impurity A = about 0.9.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to glimepiride.

Limit:
— *impurity* A: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent).

Water *(2.5.32)*

Maximum 0.5 per cent.

Dissolve 0.250 g in *dimethylformamide R* and dilute to 5.0 ml with the same solvent. Carry out the test on 1.0 ml of solution. Carry out a blank test.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

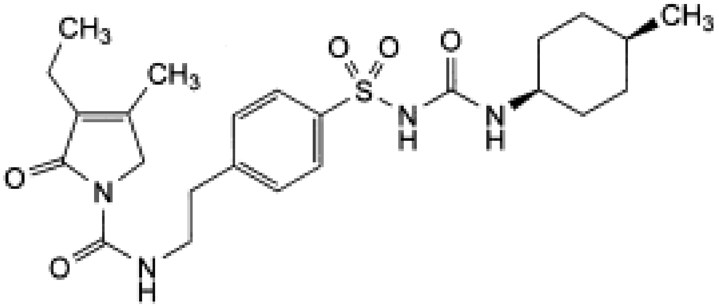
Injection Test solution and reference solution (c).

Calculate the percentage content of $C_{24}H_{34}N_4O_5S$ from the areas of the peaks and the declared content of *glimepiride CRS* .

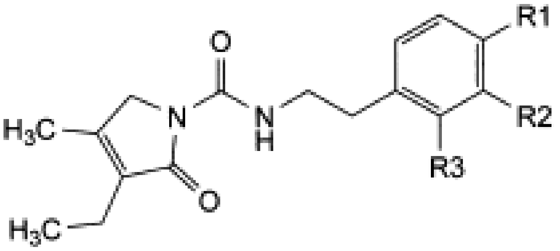
IMPURITIES

Specified impurities *A, B, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C, E, F, G, H, I, J.*



A. 1-[[4-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl] sulphonyl]-3-(*cis*-4-methylcyclohexyl)urea,



B. R1 = SO_2-NH_2, R2 = R3 = H: 3-ethyl-4-methyl-2-oxo-*N*-[2-(4-sulphamoylphenyl)ethyl]-2,3-dihydro-1*H*-pyrrole-1-carboxamide,

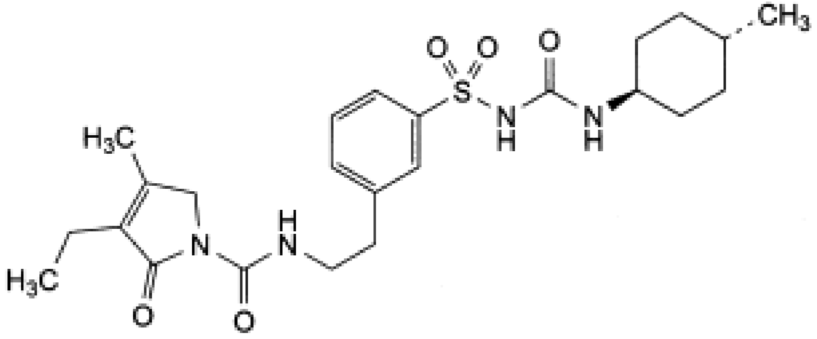
C. R1 = SO_2-NH-CO-OCH_3, R2 = R3 = H: methyl [[4-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]carbamate,

E. R1 = R3 = H, R2 = SO_2-NH_2: 3-ethyl-4-methyl-2-oxo-*N*-[2-(3-sulphamoylphenyl)ethyl]-2,3-dihydro-1*H*-pyrrole-1-carboxamide,

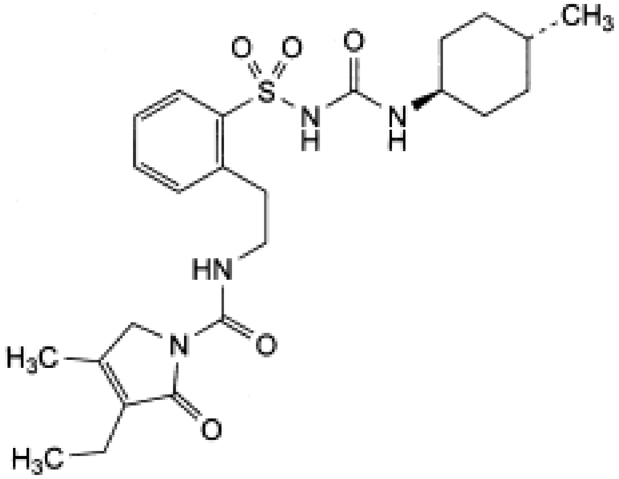
F. R1 = R2 = H, R3 = SO_2-NH-CO-OCH_3: methyl [[2-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]carbamate,

G. R1 = SO_2-N(CH_3)-CO-OCH_3, R2 = R3 = H: methyl [[4-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]methylcarbamate,

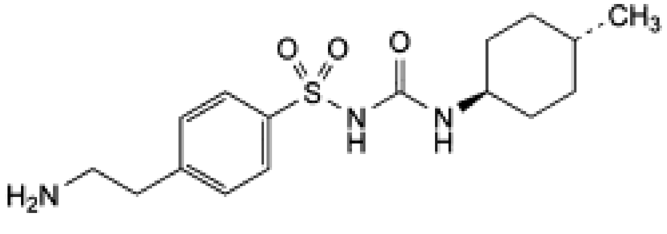
H. R1 = SO_2-NH-CO-NH-C_6H_4-CH_3, R2 = R3 = H: 1-[[4-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]-3-(4-methylphenyl)urea,



D. 1-[[3-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl] sulphonyl]-3-(*trans*-4-methylcyclohexyl)urea,



I. 1-[[2-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl] sulphonyl]-3-(*trans*-4-methylcyclohexyl)urea,



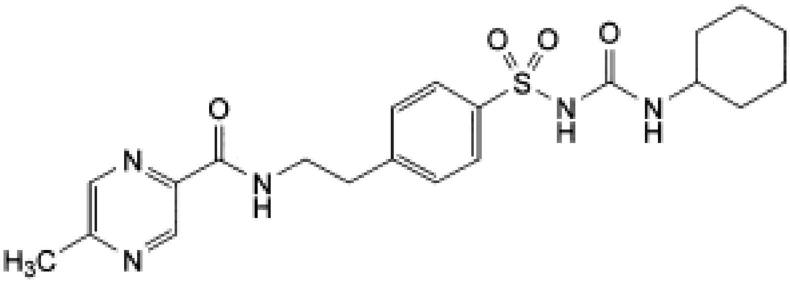
J. 1-[[4-(2-aminoethyl)phenyl]sulphonyl]-3-(*trans*-4-methylcyclohexyl)urea.

Ph Eur

Glipizide

General Notices

(Ph Eur monograph 0906)



$C_{21}H_{27}N_5O_4S$ 445.5 *29094-61-9*

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

Preparation

Glipizide Tablets

Ph Eur

DEFINITION

1-Cyclohexyl-3-[[4-[2-[[(5-methylpyrazin-2-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]urea.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very slightly soluble in methylene chloride and in acetone, practically insoluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve about 2 mg in *methanol R* and dilute to 100 ml with the same solvent.

Spectral range 220-350 nm.

Absorption maxima At 226 nm and 274 nm.

Absorbance ratio A_{226}/A_{274} = 2.0 to 2.4.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *glipizide CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 10 mg of *glipizide CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *anhydrous formic acid R*, *ethyl acetate R*, *methylene chloride R* (25:25:50 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances.

Liquid chromatography *(2.2.29)*.

Test solution Place 25 mg of the substance to be examined into a volumetric flask and add 75 ml of the mobile phase. Dissolve using sonication. Dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *glipizide impurity A CRS* and 5 mg of *glipizide impurity D CRS* in the mobile phase and dilute to 100 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 17 volumes of *acetonitrile R* and 83 volumes of a 3.5 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 8.0 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 274 nm.

Injection 50 µl.

Run time 1.5 times the retention time of glipizide.

Relative retention With reference to glipizide (retention time = about 24 min): impurity A = about 0.3; impurity D = about 0.4; impurity E = about 1.1.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurities A and D.

Limits:

— *impurity A*: not more 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),

— *impurities D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity B

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 25 mg of *decane R* in *methylene chloride R* and dilute to 100 ml with the same solvent. Dilute 5 ml of this solution to 100 ml with *methylene chloride R*.

Test solution (a) Dissolve 1.0 g of the substance to be examined in 50 ml of a 12 g/l solution of *sodium hydroxide R* and shake with 2 quantities, each of 5.0 ml of *methylene chloride R*. Use the combined lower layers.

Test solution (b) Dissolve 1.0 g of the substance to be examined in 50 ml of a 12 g/l solution of *sodium hydroxide R* and shake with 2 quantities, each of 5.0 ml of the internal standard solution. Use the combined lower layers.

Reference solution Dissolve 10.0 mg of *cyclohexylamine R* (impurity B) in a 17.5 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid. To 1.0 ml of this solution add 50 ml of a 12 g/l solution of *sodium hydroxide R* and shake with 2 quantities, each of 5.0 ml of the internal standard solution. Use the combined lower layers.

Column:

— *material*: fused silica,

— *size*: *l* = 25 m, Ø = 0.32 mm,

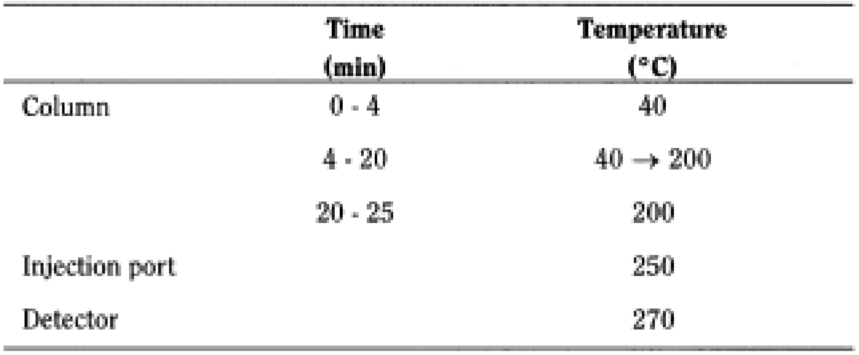
— *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.8 ml/min.

Split ratio 1:11.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 4	40
	4 - 20	40 → 200
	20 - 25	200
Injection port		250
Detector		270

Detection Flame ionisation.

Injection 1 µl.

Elution order Impurity B, decane.

System suitability:

- *resolution*: minimum 7 between the peaks due to impurity B and the internal standard in the chromatogram obtained with the reference solution,
- there is no peak with the same retention time as that of the internal standard in the chromatogram obtained with test solution (a).

Limit:

- *impurity B*: calculate the ratio (*R*) of the area of the peak due to impurity B to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with test solution (b), calculate the ratio of the area of any peak due to impurity B to the area of the peak due to the internal standard: this ratio is not greater than *R* (100 ppm).

Impurity C

Liquid chromatography *(2.2.29)*.

Solvent mixture Mixture of equal volumes of *acetonitrile R* and *water R* previously adjusted to pH 3.5 with *acetic acid R*.

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution Dissolve 5.0 mg of *glipizide impurity C CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. To 5.0 ml of the solution add 1.0 ml of the test solution and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R1* (3 µm).

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of *water R* previously adjusted to pH 3.5 with *acetic acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 50 µl.

Run time Twice the retention time of glipizide.

Relative retention With reference to glipizide (retention time = about 6 min): impurity C = about 1.4.

System suitability Reference solution:

— *resolution*: minimum 4.0 between the peaks due to glipizide and impurity C,

— *symmetry factor*: maximum 1.2 for the peak due to glipizide.

Limit:
— *impurity C*: not more than 0.4 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

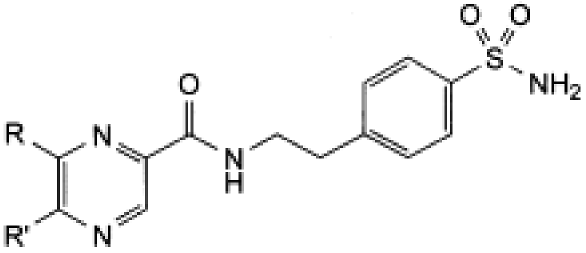
ASSAY

Dissolve 0.400 g in 50 ml of *dimethylformamide R*. Add 0.2 ml of *quinaldine red solution R*. Titrate with *0.1 M lithium methoxide* until the colour changes from red to colourless.

1 ml of *0.1 M lithium methoxide* is equivalent to 44.55 mg of $C_{21}H_{27}N_5O_4S$.

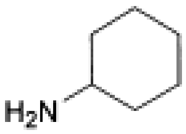
IMPURITIES

Specified impurities A, B, C, D, E.

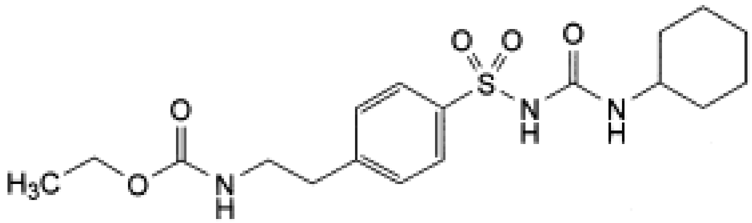


A. R = H, R′ = CH_3: 5-methyl-*N*-[2-(4-sulphamoylphenyl)ethyl]pyrazine-2-carboxamide,

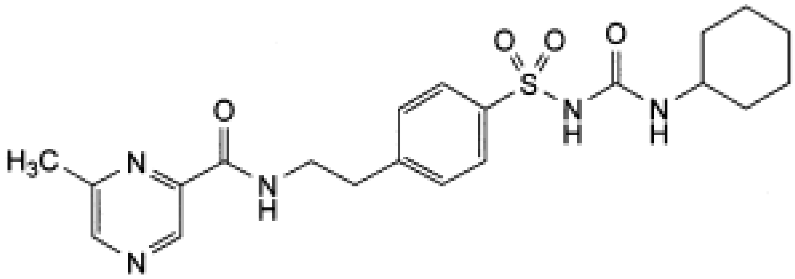
D. R = CH_3, R′ = H: 6-methyl-*N*-[2-(4-sulphamoylphenyl)ethyl]pyrazine-2-carboxamide,



B. cyclohexanamine,



C. ethyl [2-[4-[(cyclohexylcarbamoyl)sulphamoyl]phenyl]ethyl]carbamate,

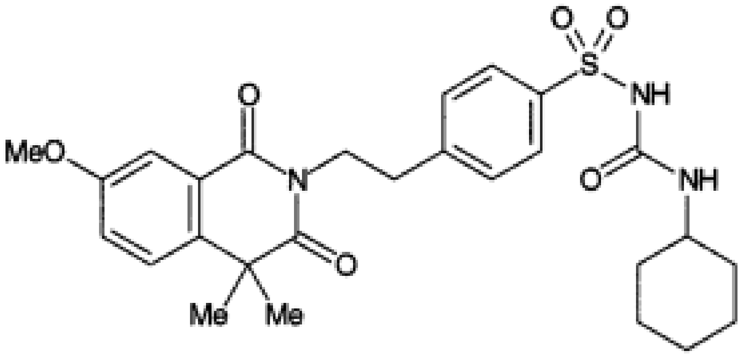


E. 1-cyclohexyl-3-[[4-[2-[[(6-methylpyrazin-2-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]urea.

Ph Eur

Gliquidone

General Notices



$C_{27}H_{33}N_3O_6S$ 527.6 *33342-05-1*

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

Preparation

Gliquidone Tablets

DEFINITION

Gliquidone is 1-cyclohexyl-3-*p*-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo2(1*H*)-isoquinolyl)ethyl]phenylsulphonylurea. It contains not less than 98.5% and not more than 101.5% of $C_{27}H_{33}N_3O_6S$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Practically insoluble in *water*; slightly soluble in *ethanol (96%)* and in *methanol*; soluble in *acetone*; freely soluble in *dimethylformamide*.

IDENTIFICATION

A. Dissolve 30 mg in 10 ml of *methanol*. Evaporate the methanol using a rotary evaporator and dry the residue at a temperature of 50° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of gliquidone *(RS 170)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Melting point

176° to 181°, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel 60F* as the coating substance and a mixture of 5 volumes of *glacial acetic acid*, 5 volumes of *ethanol (96%)*, 45 volumes of *chloroform* and 45 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of five solutions in a mixture of 50 volumes of *dichloromethane* and 50 volumes of *methanol* containing (1) 1.0% w/v of the substance being examined, (2) 1.0% w/v of *gliquidone BPCRS*, (3) 0.0030% w/v of *gliquidone BPCRS*, (4) 0.0030% w/v of *gliquidone sulphonamide BPCRS* and (5) a mixture of 1 volume of solution (3) and 1 volume of solution (4). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to gliquidone sulphonamide is not more intense than the spot in the chromatogram obtained with solution (4) (0.3%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%). The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated principal spots.

Heavy metals

1 g complies with *limit test C for heavy metals*, Appendix VII. Use 1 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

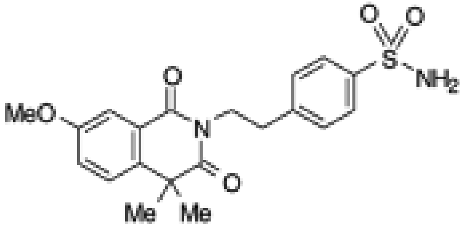
Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.3 g in 70 ml of *dimethylformamide* and immediately carry out Method II for *non-aqueous titration*, Appendix VIII A, in an atmosphere of nitrogen using 0.1M *tetrabutylammonium hydroxide VS* as titrant and a 0.3% w/v solution of *thymol blue* as indicator. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 52.76 mg of $C_{27}H_{33}N_3O_6S$.

IMPURITIES

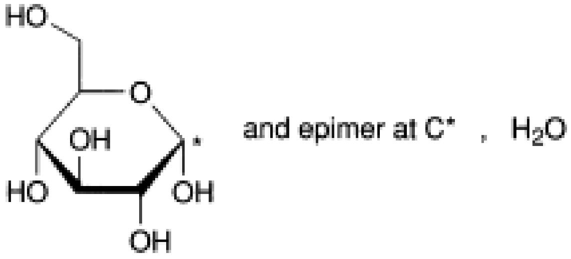


A. *p*-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo-2(1H)isoquinolyl)ethyl] benzenesulphonamide *(gliquidone sulphonamide)*

Glucose

General Notices

(Glucose Monohydrate, Ph Eur monograph 0178)



$C_6H_{12}O_6,H_2O$ 198.2 *5996-10-1*

Preparations

Glucose Intravenous Infusion

Glucose Irrigation Solution

Oral Rehydration Salts

Potassium Chloride and Glucose Intravenous Infusion

Potassium Chloride, Sodium Chloride and Glucose Intravenous Infusion

Sodium Chloride and Glucose Intravenous Infusion

Ph Eur

DEFINITION

(+)-D-Glucopyranose monohydrate.

CHARACTERS

Appearance

White or almost white, crystalline powder.

It has a sweet taste.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *water R*, *methanol R* (2:3 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *glucose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg each of *fructose CRS*, *glucose CRS* , *lactose CRS* and *sucrose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Plate *TLC silica gel G plate R*.

Mobile phase *water R*, *methanol R*, *anhydrous acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application 2 µl; thoroughly dry the starting points.

Development A Over a path of 15 cm.

Drying A In a current of warm air.

Development B Immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*; heat at 130 °C for 10 min.

System suitability Test solution (b):

— the chromatogram shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. A red precipitate is formed.

TESTS

Solution S

Dissolve 10.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 10.0 g in 15 ml of *water R*.

Acidity or alkalinity

Dissolve 6.0 g in 25 ml of *carbon dioxide-free water R* and add 0.3 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation *(2.2.7)*

+ 52.5 to + 53.3 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, add 0.2 ml of *dilute ammonia R1*, allow to stand for 30 min and dilute to 100.0 ml with *water R*.

Foreign sugars, soluble starch, dextrins

Dissolve 1.0 g by boiling in 30 ml of *ethanol (90 per cent V/V) R*. Cool; the appearance of the solution shows no change.

Sulphites

Maximum 15 ppm, expressed as SO_2.

Test solution Dissolve 5.0 g in 40 ml of *water R*, add 2.0 ml of *0.1 M sodium hydroxide* and dilute to 50.0 ml with *water R*. To 10.0 ml of the solution, add 1 ml of a 310 g/l solution of *hydrochloric acid R*, 2.0 ml of *decolorised fuchsin solution R1* and 2.0 ml of a 0.5 per cent *V/V* solution of *formaldehyde R*. Allow to stand for 30 min.

Reference solution Dissolve 76 mg of *sodium metabisulphite R* in *water R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. To 3.0 ml of this solution add 4.0 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with *water R*. Immediately add to 10.0 ml of this solution 1 ml of a 310 g/l solution of *hydrochloric acid R*, 2.0 ml of *decolorised fuchsin solution R1* and 2.0 ml of a 0.5 per cent *V/V* solution of *formaldehyde R*. Allow to stand for 30 min.

Measure the absorbance *(2.2.25)* of the 2 solutions at the absorption maximum at 583 nm using for both measurements a solution prepared in the same manner using 10.0 ml of *water R* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution.

Chlorides *(2.4.4)*

Maximum 125 ppm.

Dilute 4 ml of solution S with 15 ml of *water R*.

Sulphates *(2.4.13)*

Maximum 200 ppm.

Dilute 7.5 ml of solution S with 15 ml of *distilled water R*.

Arsenic *(2.4.2, Method A)*

Maximum 1 ppm, determined on 1.0 g.

Barium

To 10 ml of solution S add 1 ml of *dilute sulphuric acid R*. When examined immediately and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Calcium *(2.4.3)*

Maximum 200 ppm.

Dilute 5 ml of solution S with 15 ml of *distilled water R*.

Lead ***(2.4.10)***

Maximum 0.5 ppm.

Water ***(2.5.12)***

7.0 per cent to 9.5 per cent, determined on 0.50 g.

Sulphated ash

Maximum 0.1 per cent.

Dissolve 5.0 g in 5 ml of *water R*, add 2 ml of *sulphuric acid R*, evaporate to dryness on a water-bath and ignite to constant mass. If necessary, repeat the heating with *sulphuric acid R*.

Pyrogens ***(2.6.8)***

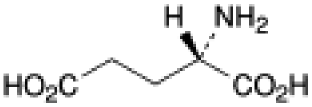
If intended for use in the manufacture of large-volume parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, the competent authority may require that it comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 ml of a solution in *water for injections R* containing 55 mg of the substance to be examined per millilitre.

Ph Eur

Glutamic Acid

General Notices

(Ph Eur monograph 0750)



$C_5H_9NO_4$ 147.1 *56-86-0*

Action and use

Amino acid.

Ph Eur

DEFINITION

Glutamic acid contains not less than 98.5 per cent and not more than the equivalent of 100.5 per cent of (2*S*)-2-aminopentanedioic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in boiling water, slightly soluble in cold water, practically insoluble in acetic acid, in acetone and in alcohol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *glutamic acid CRS*. Examine the substances prepared as discs. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum quantity of *water R*, evaporate to dryness at 60 °C and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 2.0 ml of solution S (see Tests) add 0.1 ml of *phenolphthalein solution R* and 3.0 ml to 3.5 ml of *1 M sodium hydroxide* to change the colour of the indicator to red. Add a mixture of

3 ml of *formaldehyde solution R*, 3 ml of *carbon dioxide-free water R* and 0.1 ml of *phenolphthalein solution R*, to which sufficient *1 M sodium hydroxide* has been added to produce a pink colour. The solution is decolourised. Add *1 M sodium hydroxide* until a red colour is produced. The total volume of *1 M sodium hydroxide* used is 4.0 ml to 4.7 ml.

TESTS

Solution S

Dissolve 5.00 g in *1 M hydrochloric acid* with gentle heating, and dilute to 50.0 ml with the same acid.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(Method II, 2.2.2)*.

Specific optical rotation *(2.2.7)*

+ 30.5 to + 32.5, determined on solution S and calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 5 ml of *dilute ammonia R2* and dilute to 10 ml with *water R*.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *glutamic acid CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *glutamic acid CRS* and 10 mg of *aspartic acid CRS* in *water R* and dilute to 25 ml with the same solvent.

Apply to the plate 5 µl of each solution. Dry the plate in a current of air for 15 min. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Chlorides *(2.4.4)*

Dissolve 0.25 g in 3 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution, to which 1 ml of *water R* is added instead of *dilute nitric acid R*, complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dilute 5 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals *(2.4.8)*

2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.130 g in 50 ml of *carbon dioxide-free water R* with gentle heating. Cool. Using 0.1 ml of *bromothymol blue solution R1* as indicator, titrate with *0.1 M sodium hydroxide* until the colour changes from yellow to blue.

1 ml of *0.1 M sodium hydroxide* is equivalent to 14.71 mg of $C_5H_9NO_4$.

STORAGE

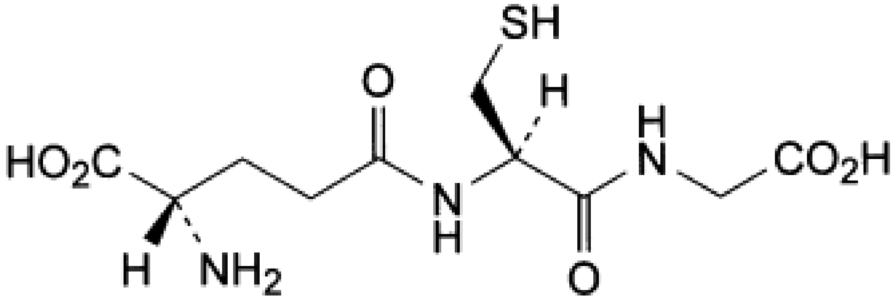
Protected from light.

Ph Eur

Glutathione

General Notices

(Ph Eur monograph 1670)



$C_{10}H_{17}N_3O_6S$ 307.3 *70-18-8*

Ph Eur

DEFINITION

L-γ-Glutamyl-L-cysteinylglycine.

Fermentation product.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison glutathione CRS.

TESTS

Solution S

Dissolve 5.0 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

- 15.5 to - 17.5 (dried substance).

Dissolve 1.0 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Capillary electrophoresis (*2.2.47*). *Prepare the solutions immediately before use.*

Internal standard solution (a) Dissolve 0.100 g of *phenylalanine R* in the electrolyte solution and dilute to 50.0 ml with the same solution.

Internal standard solution (b) Dilute 10.0 ml of internal standard solution (a) to 100.0 ml with the electrolyte solution.

Test solution (a) Dissolve 0.200 g of the substance to be examined in the electrolyte solution and dilute to 10.0 ml with the same solution.

Test solution (b) Dissolve 0.200 g of the substance to be examined in internal standard solution (b) and dilute to 10.0 ml with the same solution.

Reference solution (a) Dissolve 20.0 mg of the substance to be examined in internal standard solution (a) and dilute to 10.0 ml with the same solution.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the electrolyte solution.

Reference solution (c) Dissolve 0.200 g of the substance to be examined in 5 ml of the electrolyte solution. Add 1.0 ml of internal standard solution (a), 0.5 ml of a 2 mg/ml solution of *L-cysteine R* (impurity B) in the electrolyte solution, 0.5 ml of a 2 mg/ml solution of *oxidised L-glutathione R* (impurity C) in the electrolyte solution and 0.5 ml of a 2 mg/ml solution of *L-γ-glutamyl-L-cysteine R* (impurity D) in the electrolyte solution. Dilute to 10.0 ml with the electrolyte solution.

Capillary:

- *material*: uncoated fused silica;
- *size*: length to the detector cell = 0.5 m; total length = 0.6 m; Ø = 75 µm.

Temperature 25 °C.

Electrolyte solution Dissolve 1.50 g of *anhydrous sodium dihydrogen phosphate R* in 230 ml of *water R* and adjust to pH 1.80 with *phosphoric acid R.* Dilute to 250.0 ml with *water R*. Check the pH and, if necessary, adjust with *phosphoric acid R* or *dilute sodium hydroxide solution R.*

Detection Spectrophotometer at 200 nm.

Preconditioning of a new capillary: rinse the new capillary before the first injection with *0.1 M* *hydrochloric acid* at 138 kPa for 20 min and with *water R* at 138 kPa for 10 min; for complete equilibration, condition the capillary with the electrolyte solution at 350 kPa for 40 min, and subsequently at a voltage of 20 kV for 60 min.

Preconditioning of the capillary Rinse the capillary with the electrolyte solution at 138 kPa for 40 min.

Between-run rinsing Rinse the capillary with *water R* at 138 kPa for 1 min, with *0.1 M sodium hydroxide* at 138 kPa for 2 min, with *water R* at 138 kPa for 1 min, with *0.1 M hydrochloric acid* at 138 kPa for 3 min and with the electrolyte solution at 138 kPa for 10 min.

Injection Test solutions (a) and (b), reference solutions (b) and (c) and the electrolyte solution (blank): under pressure (3.45 kPa) for 5 s.

Migration Apply a voltage of 20 kV.

Run time 45 min.

Relative migration With reference to the internal standard (about 14 min): impurity A = about 0.77; impurity B = about 1.04; impurity E = about 1.2; impurity C = about 1.26; impurity D = about 1.3.

System suitability:

— *resolution*: minimum 1.5 between the peaks due to the internal standard and impurity B in the chromatogram obtained with reference solution (c); if necessary, increase the pH with *dilute sodium hydroxide solution R*;

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to glutathione in the chromatogram obtained with reference solution (c); if necessary, lower the pH with *phosphoric acid R;*

— check that in the electropherogram obtained with test solution (a) there is no peak with the same migration time as the internal standard (in such case correct the area of the phenylalanine peak).

Limits Test solution (b):

— *corrected areas*: divide all the peak areas by the corresponding migration times;

— *correction factors*: for the calculation of content, multiply the ratio of time-corrected peak areas of impurity and the internal standard by the corresponding correction factor: impurity B = 3.0; impurity D = 1.4;

— *impurity C:* not more than 1.5 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (1.5 per cent);

— *impurity* D: not more than the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (1.0 per cent);

— *impurities A, B, E*: for each impurity, not more than 0.5 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.2 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than 2.5 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (2.5 per cent);

— *disregard limit*: 0.05 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (0.05 per cent).

Chlorides ***(2.4.4)***

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R.*

Sulphates ***(2.4.13)***

Maximum 300 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Ammonium ***(2.4.1, Method B*****)**

Maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R.*

Iron ***(2.4.9)***

Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers, add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the test.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In a ground-glass-stoppered flask, dissolve 0.500 g of the substance to be examined and 2 g of *potassium iodide R* in 50 ml of *water R*. Cool the solution in iced water and add 10 ml of *hydrochloric acid R1* and 20.0 ml of *0.05 M iodine*. Stopper the flask and allow to stand in the dark for 15 min. Titrate with *0.1 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

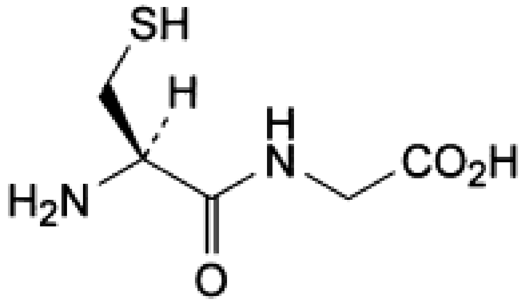
1 ml of *0.05 M iodine* is equivalent to 30.73 mg of $C_{10}H_{17}N_3O_6S$.

STORAGE

Protected from light.

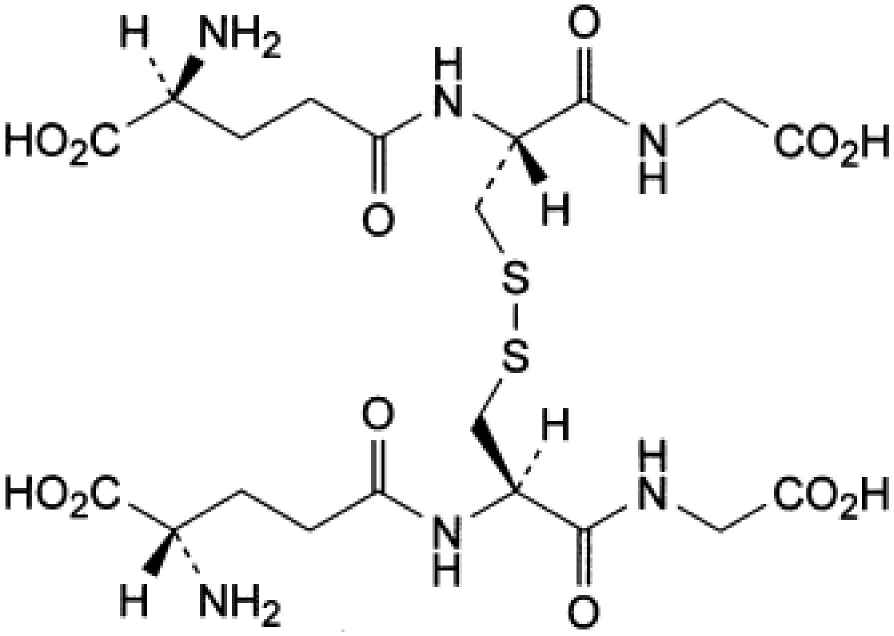
IMPURITIES

Specified impurities *A, B, C, D, E*.

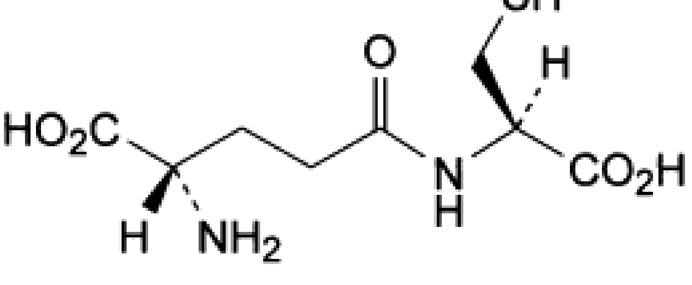


A. L-cysteinylglycine,

B. cysteine,



C. bis(L-γ-glutamyl-L-cysteinylglycine) disulfide (L-glutathione oxidised),



D. L-γ-glutamyl-L-cysteine,

E. unknown structure (product of degradation).

Ph Eur

Glycerol (85 per cent)

General Notices

(Ph Eur monograph 0497)

Ph Eur

DEFINITION

Aqueous solution of propane-1,2,3-triol.

Content

83.5 per cent *m/m* to 88.5 per cent *m/m* of propane-1,2,3-triol ($C_3H_8O_3$; M_r 92.1).

CHARACTERS

Aspect

Syrupy liquid, unctuous to the touch, colourless or almost colourless, clear, very hygroscopic.

Solubility

Miscible with water and with alcohol, slightly soluble in acetone, practically insoluble in fatty oils and in essential oils.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. It complies with the test for refractive index (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of glycerol (85 per cent).

C. Mix 1 ml with 0.5 ml of *nitric acid R*. Superimpose 0.5 ml of *potassium dichromate solution R*. A blue ring develops at the interface of the liquids. Within 10 min, the blue colour does not diffuse into the lower layer.

D. Heat 1 ml with 2 g of *potassium hydrogen sulphate R* in an evaporating dish. Vapours (acrolein) are evolved which blacken filter paper impregnated with *alkaline potassium tetraiodomercurate solution R.*

TESTS

Solution S

Dilute 117.6 g to 200.0 ml with *carbon dioxide-free water R*.

Appearance of solution

Solution S is clear *(2.2.1)*. Dilute 10 ml of solution S to 25 ml with *water R*. The solution is colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 50 ml of solution S add 0.5 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Refractive index *(2.2.6)*

1.449 to 1.455.

Aldehydes

Maximum 10 ppm.

Place 7.5 ml of solution S in a ground-glass-stoppered flask and add 7.5 ml of *water R* and 1.0 ml of *decolorised pararosaniline solution R*. Close the flask and allow to stand for 1 h at a temperature of 25 ± 1 °C. The absorbance *(2.2.25)* of the solution measured at 552 nm is not greater than that of a standard prepared at the same time and in the same manner using 7.5 ml of *formaldehyde standard solution (5 ppm CH_2O) R* and 7.5 ml of *water R*. The test is not valid unless the standard is pink.

Esters

Add 10.0 ml of *0.1 M sodium hydroxide* to the final solution obtained in the test for acidity or alkalinity. Boil under a reflux condenser for 5 min. Cool. Add 0.5 ml of *phenolphthalein solution R* and titrate with *0.1 M hydrochloric acid*. Not less than 8.0 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Impurity A and related substances

Gas chromatography *(2.2.28)*.

Test solution Dilute 10.0 ml of solution S to 100.0 ml with *water R*.

Reference solution (a) Dilute 11.8 g of *glycerol (85 per cent) R1* to 20.0 ml with *water R*. Dilute 10.0 ml of the solution to 100.0 ml with water R.

Reference solution (b) Dissolve 1.000 g of *diethylene glycol R* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with reference solution (a). Dilute 1.0 ml of this solution to 20.0 ml with reference solution (a).

Reference solution (d) Mix 1.0 ml of the test solution and 5.0 ml of reference solution (b) and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Reference solution (e) Dilute 5.0 ml of reference solution (b) to 100.0 ml with *water R*.

Column:

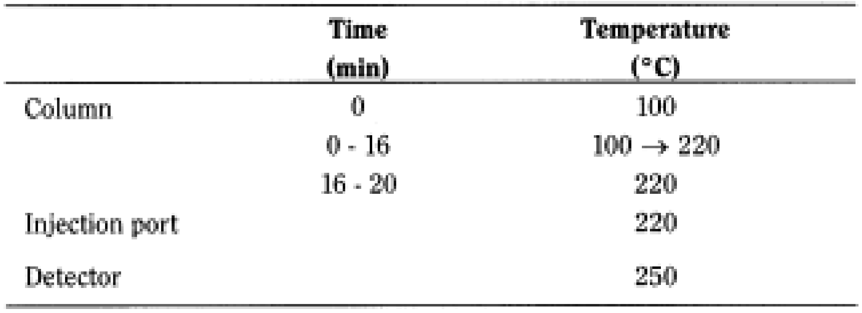
- *size:* l = 30 m, Ø = 0.53 mm,
- *stationary phase*: 6 per cent polycyanolpropylphenyl siloxane and 94 per cent of polydimethylsiloxane.

Carrier gas *helium for chromatography R*.

Split ratio 1:10.

Linear velocity 38 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0	100
	0 - 16	100 → 220
	16 - 20	220
Injection port		220
Detector		250

Detection Flame ionisation.

Injection 0.5 µl.

Elution order Impurity A, glycerol.

System suitability Reference solution (d):

— *resolution*: minimum 7.0 between the peaks due to impurity A and glycerol.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *any other impurity with a retention time less than the retention time of glycerol*: not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *total of all impurities with retention times greater than the retention time of glycerol*: not more than 5 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *disregard limit*: 0.05 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.05 per cent).

Halogenated compounds

Maximum 30 ppm.

To 10 ml of solution S add 1 ml of *dilute sodium hydroxide solution R*, 5 ml of *water R* and 50 mg of *halogen-free nickel-aluminium alloy R*. Heat on a water-bath for 10 min, allow to cool and filter. Rinse the flask and the filter with *water R* until 25 ml of filtrate is obtained. To 5 ml of the filtrate add 4 ml of *alcohol R*, 2.5 ml of *water R*, 0.5 ml of *nitric acid R* and 0.05 ml of *silver nitrate solution R2* and mix. Allow to stand for 2 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time by mixing 7.0 ml of *chloride standard solution (5 ppm Cl) R*, 4 ml of *alcohol R*, 0.5 ml of *water R*, 0.5 ml of *nitric acid R* and 0.05 ml of *silver nitrate solution R2*.

Sugars

To 10 ml of solution S add 1 ml of *dilute sulphuric acid R* and heat on a water-bath for 5 min. Add 3 ml of *carbonate-free dilute sodium hydroxide solution R* (prepared by the method described for carbonate-free *1 M sodium hydroxide (4.2.2)*), mix and add dropwise 1 ml of freshly prepared *copper sulphate solution R*. The solution is clear and blue. Continue heating on the water-bath for 5 min. The solution remains blue and no precipitate is formed.

Chlorides ***(2.4.4)***

Maximum 10 ppm.

1 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides. Prepare the standard using 1 ml of *chloride standard solution (5 ppm Cl) R* diluted to 15 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 5 ppm.

Dilute 8 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water ***(2.5.12)***

12.0 per cent to 16.0 per cent, determined on 0.200 g.

Sulphated ash ***(2.4.14)***

Maximum 0.01 per cent, determined on 5.0 g after heating to boiling and ignition.

ASSAY

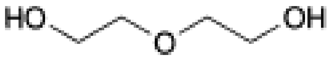
Thoroughly mix 0.075 g with 45 ml of *water R*. Add 25.0 ml of a mixture of 1 volume of *0.1 M sulphuric acid* and 20 volumes of *0.1 M sodium periodate*. Allow to stand protected from light for 15 min. Add 5.0 ml of a 500 g/l solution of *ethylene glycol R* and allow to stand protected from light for 20 min. Using 0.5 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 9.21 mg of $C_3H_8O_3$.

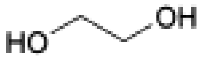
STORAGE

In an airtight container .

IMPURITIES



A. 2,2′-oxydiethanol (diethylene glycol),



B. ethane-1,2-diol (ethylene glycol),

C. propylene glycol.

Ph Eur

Glycerol Dibehenate

General Notices

(Ph Eur monograph 1427)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of diacylglycerols, mainly dibehenylglycerol, together with variable quantities of mono- and triacylglycerols, obtained by esterification of *glycerol (0496)* with behenic (docosanoic) acid.

Content:

— *monoacylglycerols*: 15.0 per cent to 23.0 per cent,

— *diacylglycerols*: 40.0 per cent to 60.0 per cent,

— *triacylglycerols*: 21.0 per cent to 35.0 per cent.

CHARACTERS

Appearance

Hard, waxy mass or powder or white or almost white, unctuous flakes.

Solubility

Practically insoluble in water, soluble in methylene chloride, partly soluble in hot ethanol (96 per cent).

IDENTIFICATION

A. Melting point *(2.2.14)*: 65 °C to 77 °C.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 1.0 g of the substance to be examined in *toluene R* with gentle heating and dilute to 20 ml with the same solvent.

Reference solution Dissolve 1.0 g of *glycerol dibehenate CRS* in *toluene R* with gentle heating and dilute to 20 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase hexane R, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R*; examine in ultraviolet light at 365 nm.

Results The spots in the chromatogram obtained with the test solution are similar in position to the spots in the chromatogram obtained with the reference solution.

C. Composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 4.0, determined on 1.0 g, using a mixture of equal volumes of *ethanol (96 per cent) R* and *toluene R* as solvent and with gentle heating.

Iodine value *(2.5.4, Method A)*

Maximum 3.0.

Saponification value *(2.5.6)*

145 to 165.

Carry out the titration with heating.

Free glycerol

Maximum 1.0 per cent, determined as described under Assay.

Composition of fatty acids *(2.4.22, Method C)*

Raise the temperature of the column to 240 °C and use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty acid fraction of the substance:

- *palmitic acid*: maximum 3.0 per cent;
- *stearic acid*: maximum 5.0 per cent;
- *arachidic acid*: maximum 10.0 per cent;
- *behenic acid*: minimum 83.0 per cent;
- *erucic acid*: maximum 3.0 per cent;
- *lignoceric acid*: maximum 3.0 per cent.

Nickel *(2.4.31)*

Maximum 1 ppm.

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 1.00 g. Use *pyridine R* as the solvent.

Total ash *(2.4.16)*

Maximum 0.1 per cent, determined on 1.00 g.

ASSAY

Size-exclusion chromatography *(2.2.30)*.

Stock solution Place 0.100 g of *glycerol R* in a flask and dilute to 25.0 ml with *tetrahydrofuran R.*

Test solution In a 15 ml flask, weigh 0.2 g (*m*) of the substance to be examined. Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Heat gently, at about 35 °C. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 ml flasks, introduce respectively 0.25 ml, 0.5 ml, 1.0 ml and 2.5 ml of the stock solution and add 5.0 ml of *tetrahydrofuran R*. Weigh each flask and calculate the concentration of glycerol in milligrams per gram of each reference solution.

Column:

- *size: l* = 0.6 m, Ø = 7 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 1 ml/min.

Detection Differential refractive index.

Injection 40 µl; when injecting the test solution, maintain the flask at about 35 °C to avoid precipitation.

Relative retention With reference to glycerol (retention time = about 15 min): triacylglycerols = about 0.73; diacylglycerols = about 0.76; monoacylglycerols = about 0.82.

Calculations:

- *free glycerol*: from the calibration curve obtained with the reference solutions, determine the concentration (*C*) in milligrams per gram in the test solution and calculate the percentage content in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

- *monoacylglycerols*: calculate the percentage content of monoacylglycerols using the following expression:

$$A - D$$

A = percentage content of monoacylglycerols determined by the normalisation procedure,

D = percentage content of free fatty acids.

Calculate the percentage content of free fatty acids using the following expression:

$$\frac{I_A \times 340}{561.1}$$

I_A = acid value.

— *diacylglycerols, triacylglycerols*: determine the percentage content of each by the normalisation procedure.

Ph Eur

Glycerol Monocaprylate

General Notices

(Ph Eur monograph 2213)

Ph Eur

DEFINITION

Mixture of monoacylglycerols, mainly mono-*O*-octanoylglycerol, containing variable quantities of di- and triacylglycerols, obtained by direct esterification of glycerol with caprylic (octanoic) acid, followed by a distillation step in the case of glycerol monocaprylate (type II).

Content:

— *glycerol monocaprylate (type I)*:
  — *monoacylglycerols*: 45.0 per cent to 75.0 per cent;
  — *diacylglycerols*: 20.0 per cent to 50.0 per cent;
  — *triacylglycerols*: maximum 10.0 per cent;

— *glycerol monocaprylate (type II)*:
  — *monoacylglycerols*: minimum 80.0 per cent;
  — *diacylglycerols*: maximum 20.0 per cent;
  — *triacylglycerols*: maximum 5.0 per cent.

CHARACTERS

Appearance

Colourless or slightly yellow, oily liquid or soft mass.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent) and freely soluble in methylene chloride.

IDENTIFICATION

A. Composition of fatty acids (see Tests).

B. It complies with the limits of the assay (monoacylglycerols).

TESTS

Acid value *(2.5.1)*

Maximum 3.0.

Composition of fatty acids *(2.4.22, Method C)*

Use the mixture of calibrating substances in Table 2.4.22.-2.

Composition of the fatty acid fraction of the substance:

- — *caproic acid*: maximum 1.0 per cent;
- — *caprylic acid*: minimum 90.0 per cent;
- — *capric acid*: maximum 10.0 per cent;
- — *lauric acid*: maximum 1.0 per cent;
- — *myristic acid*: maximum 0.5 per cent.

Free glycerol

Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 ml of *methylene chloride R*. Heat to about 50 °C then allow to cool. Add 100 ml of *water R*. Shake and add 25.0 ml of periodic *acetic acid solution R*. Shake again and allow to stand for 30 min. Add 40 ml of a 75 g/l solution of *potassium iodide R* and allow to stand for 1 min. Add 1 ml of *starch solution R*. Titrate with *0.1 M sodium thiosulfate* until the aqueous phase becomes colourless. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulfate* is equivalent to 2.3 mg of glycerol.

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 1.00 g.

Total ash *(2.4.16)*

Maximum 0.5 per cent.

ASSAY

Gas chromatography (2.2.28) Use the normalisation procedure.

Test solution To 0.25 g of the substance to be examined, add 5.0 ml of *tetrahydrofuran R* and shake to dissolve.

Reference solution (a) To 0.25 g of *glycerol monocaprylate CRS*, add 5.0 ml of *tetrahydrofuran R* and shake to dissolve.

Reference solution (b) To 50 mg of *glycerol 1-octanoate R* and 50 mg of *glycerol 1-decanoate R*, add 2.5 ml of *tetrahydrofuran R* and shake to dissolve.

Column:

- — *size: l* = 10 m, Ø = 0.32 mm;
- — *stationary phase: poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 2.3 ml/min.

Split ratio 1:50.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 3	60
	3 - 38	60 → 340
	38 - 50	340
Injection port		350
Detector		370

Detection Flame ionisation.

Injection 1 µl.

Identification of peaks Use the chromatogram supplied with *glycerol monocaprylate CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to mono-, di- and triacylglycerols.

System suitability Reference solution (b):

— *resolution*: minimum 5 between the peaks due to glycerol 1-octanoate and glycerol 1-decanoate.

For the calculation of the contents of mono-, di- and triacylglycerols, disregard the peaks with a retention time less than that of the monoacylglycerols, which are due to impurities of the solvent and to the free fatty acids.

Calculate the percentage content of free fatty acids (C) using the following expression:

$$\frac{I_A \times 144}{561.1}$$

I_A = acid value of glycerol monocaprylate.

Calculate the content of mono-, di- and triacylglycerols using the following equations:

$$\text{Monoacylglycerols content} = \frac{X \times (100 - A - B - C)}{100}$$

$$\text{Diacylglycerols content} = \frac{Y \times (100 - A - B - C)}{100}$$

$$\text{Triacylglycerols content} = \frac{Z \times (100 - A - B - C)}{100}$$

A = percentage content of free glycerol (see Tests);

B = percentage content of water;

X = monoacylglycerols content obtained by normalisation;

Y = diacylglycerols content obtained by normalisation;

Z = triacylglycerols content obtained by normalisation.

LABELLING

The label states the type of glycerol monocaprylate (type I or II).

Ph Eur

Glycerol Monocaprylocaprate

General Notices

(Ph Eur monograph 2392)

Ph Eur

DEFINITION

Mixture of monoacylglycerols, mainly mono-*O*-octanoylglycerol and mono-*O*-decanoylglycerol, containing variable quantities of di- and triacylglycerols, obtained by direct esterification of glycerol with caprylic (octanoic) and capric (decanoic) acids, followed by a distillation step in the case of glycerol monocaprylocaprate (type II).

Content:

— *glycerol monocaprylocaprate (type I)*:
  — *monoacylglycerols*: 45.0 per cent to 75.0 per cent;
  — *diacylglycerols*: 20.0 per cent to 50.0 per cent;
  — *triacylglycerols*: maximum 10.0 per cent;

— *glycerol monocaprylocaprate (type II)*:
  — *monoacylglycerols*: minimum 80.0 per cent;
  — *diacylglycerols*: maximum 20.0 per cent;
  — *triacylglycerols*: maximum 5.0 per cent.

CHARACTERS

Appearance

Colourless or slightly yellow, oily liquid or soft mass.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent) and freely soluble in methylene chloride.

IDENTIFICATION

A. Composition of fatty acids (see Tests).

B. It complies with the limits of the assay (monoacylglycerols).

TESTS

Acid value *(2.5.1)*

Maximum 3.0.

Composition of fatty acids *(2.4.22, Method C)*

Use the mixture of calibrating substances in Table 2.4.22.-2.

Composition of the fatty acid fraction of the substance:

- *caproic acid*: maximum 3.0 per cent;
- *caprylic acid*: 50.0 per cent to 90.0 per cent;
- *capric acid*: 10.0 per cent to 50.0 per cent;
- *lauric acid*: maximum 3.0 per cent;
- *myristic acid*: maximum 1.0 per cent.

Free glycerol

Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 ml of *methylene chloride R*. Heat to about 50 °C and allow to cool. Add 100 ml of *water R*, shake and add 25.0 ml of *periodic acetic acid solution R*. Shake again and allow to stand for 30 min. Add 40 ml of a 75 g/l solution of *potassium iodide R* and allow to stand for 1 min. Add 1 ml of *starch solution R*. Titrate with *0.1 M sodium thiosulphate* until the aqueous phase becomes colourless. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 2.3 mg of glycerol.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.00 g.

Total ash *(2.4.16)*

Maximum 0.5 per cent.

ASSAY

Gas chromatography (2.2.28). Use the normalisation procedure.

Test solution. To 0.25 g of the substance to be examined, add 5.0 ml of *tetrahydrofuran R* and shake to dissolve.

Reference solution (a). To 0.25 g of *glycerol monocaprylocaprate CRS*, add 5.0 ml of *tetrahydrofuran R* and shake to dissolve.

Reference solution (b). To 50 mg of *glycerol 1-octanoate R* and 50 mg of *glycerol 1-decanoate R*, add 2.5 ml of *tetrahydrofuran R* and shake to dissolve.

Column:

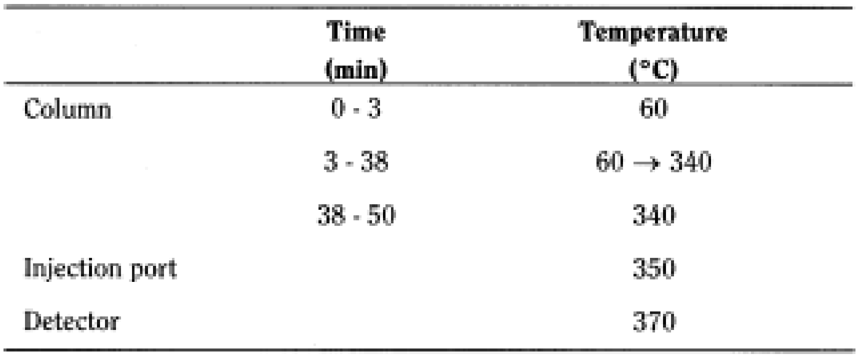
- *size*: *l* = 10 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.1 µm).

Carrier gas: *helium for chromatography R.*

Flow rate: 2.3 ml/min.

Split ratio: 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 3	60
	3 - 38	60 → 340
	38 - 50	340
Injection port		350
Detector		370

Detection Flame ionisation.

Injection 1 µl.

Identification of peaks Use the chromatogram supplied with *glycerol monocaprylocaprate CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to mono-, di- and triacylglycerols.

System suitability Reference solution (b):

— *resolution*: minimum 5 between the peaks due to glycerol 1-octanoate and glycerol 1-decanoate.

For the calculation of the contents of mono-, di- and triacylglycerols, disregard the peaks with a retention time less than that of the monoacylglycerols, which are due to the impurities of the solvent and to the free fatty acids.

Calculate the percentage content of free fatty acids *(C)* using the following equations:

$$\frac{I_A \times 144}{561.1}$$

I_A = acid value of the glycerol monocaprylocaprate.

Calculate the content of mono-, di- and triacylglycerols using the following equations:

$$\text{Monoacylglycerols content} = \frac{X \times (100 - A - B - C)}{100}$$

$$\text{Diacylglycerols content} = \frac{Y \times (100 - A - B - C)}{100}$$

$$\text{Triacylglycerols content} = \frac{Z \times (100 - A - B - C)}{100}$$

A = percentage content of free glycerol (see Tests);

B = percentage content of water;

X = monoacylglycerols content obtained by normalisation;

Y = diacylglycerols content obtained by normalisation;

Z = triacylglycerols content obtained by normalisation.

LABELLING

The labelling states the type of glycerol monocaprylocaprate (type I or II).

Ph Eur

Glycerol Monolinoleate

General Notices

(Ph Eur monograph 1429)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of monoacylglycerols, mainly mono-oleoyl- and monolinoleoylglycerol, together with variable quantities of di- and triacylglycerols, obtained by partial glycerolysis of vegetable oils mainly containing triacylglycerols of linoleic (*cis*,*cis*-9,12-octadecadienoic) acid. A suitable antioxidant may be added.

Content:

- *monoacylglycerols*: 32.0 per cent to 52.0 per cent;
- *diacylglycerols*: 40.0 per cent to 55.0 per cent;
- *triacylglycerols*: 5.0 per cent to 20.0 per cent.

CHARACTERS

Appearance

Amber, oily liquid which may be partially solidified at room temperature.

Solubility

Practically insoluble in water, freely soluble in methylene chloride.

IDENTIFICATION

A. Iodine value (see Tests).

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 ml with the same solvent.

Reference solution Dissolve 1.0 g of *glycerol monolinoleate CRS* in *methylene chloride* R and dilute to 20 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase hexane R, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The spots in the chromatogram obtained with the test solution are similar in position to those in the chromatogram obtained with the reference solution.

C. Composition of fatty acids (see Tests).

TESTS

Acid value ***(2.5.1)***

Maximum 6.0, determined on 1.0 g.

Iodine value ***(2.5.4, Method A)***

100 to 140.

Peroxide value ***(2.5.5, Method A)***

Maximum 12.0, determined on 2.0 g.

Saponification value ***(2.5.6)***

160 to 180, determined on 2.0 g.

Free glycerol

Maximum 6.0 per cent, determined as described in the assay.

Composition of fatty acids ***(2.4.22, Method C)*****.**

Composition of the fatty acid fraction of the substance:

- — *palmitic acid*: 4.0 per cent to 20.0 per cent;
- — *stearic acid*: maximum 6.0 per cent;
- — *oleic acid*: 10.0 per cent to 35.0 per cent;
- — *linoleic acid*: minimum 50.0 per cent;
- — *linolenic acid*: maximum 2.0 per cent;
- — *arachidic acid*: maximum 1.0 per cent;
- — *eicosenoic acid*: maximum 1.0 per cent.

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.00 g. Use as the solvent a mixture of equal volumes of *anhydrous methanol R* and *methylene chloride R*.

Total ash ***(2.4.16)***

Maximum 0.1 per cent.

ASSAY

Size-exclusion chromatography *(2.2.30)*.

Test solution Into a 15 ml flask, weigh about 0.2 g (*m*), to the nearest 0.1 mg. Add 5 ml of *tetrahydrofuran R* and shake to dissolve. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 ml flasks, respectively weigh, to the nearest 0.1 mg, about 2.5 mg, 5 mg, 10 mg and 20 mg of *glycerol R*. Add 5 ml of *tetrahydrofuran R* and shake until well mixed. Weigh the flasks again and calculate the concentration of glycerol in milligrams per gram for each reference solution.

Column:

— *size:* l = 0.6 m, Ø = 7 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 1 ml/min.

Detection Differential refractometer.

Injection 40 µl.

Relative retention With reference to glycerol (retention time = about 15.6 min): triacylglycerols = about 0.76; diacylglycerols = about 0.80; monoacylglycerols = about 0.86.

Calculations:

— *free glycerol* : from the calibration curve obtained with the reference solutions, determine the concentration (*C*) of glycerol in milligrams per gram in the test solution and calculate the percentage content of free glycerol in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *mono-, di- and triacylglycerols*: calculate the percentage content of mono-, di- and triacylglycerols using the normalisation procedure.

STORAGE

In an airtight container , protected from light.

Ph Eur

Glycerol Mono-oleate

General Notices

(Ph Eur monograph 1430)

Action and use

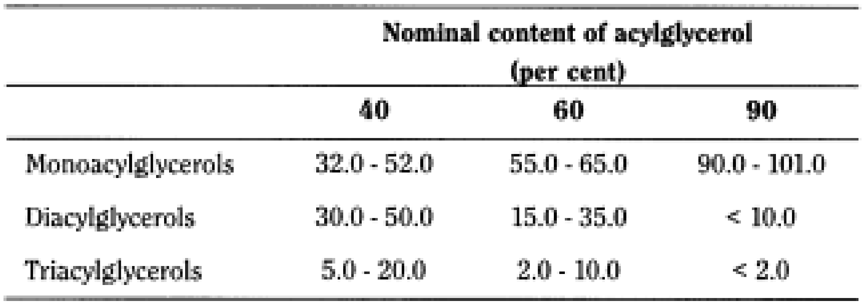
Excipient.

Ph Eur

DEFINITION

Mixture of monoacylglycerols, mainly mono-oleoylglycerol, together with variable quantities of di- and triacylglycerols. It is defined by the nominal content of monoacylglycerols and obtained by partial glycerolysis of vegetable oils mainly containing triacylglycerols of oleic (*cis*-9-octadecenoic) acid or by esterification of glycerol by oleic acid, this fatty acid being of vegetable or animal origin. A suitable antioxidant may be added.

Content:



	Nominal content of acylglycerol (per cent)		
	40	60	90
Monoacylglycerols	32.0 - 52.0	55.0 - 65.0	90.0 - 101.0
Diacylglycerols	30.0 - 50.0	15.0 - 35.0	< 10.0
Triacylglycerols	5.0 - 20.0	2.0 - 10.0	< 2.0

CHARACTERS

Appearance

Amber, oily liquid which may be partially solidified at room temperature.

Solubility

Practically insoluble in water, freely soluble in methylene chloride.

IDENTIFICATION

A. Iodine value (see Tests).

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 ml with the same solvent.

Reference solution Dissolve 1.0 g of *glycerol mono-oleate CRS* in *methylene chloride R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R*, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The spots in the chromatogram obtained with the test solution are similar in position to those in the chromatogram obtained with the reference solution.

C. It complies with the limits of the assay (monoacylglycerol content).

TESTS

Acid value *(2.5.1)*

Maximum 6.0, determined on 1.0 g.

Iodine value *(2.5.4, Method A)*

65.0 to 95.0.

Peroxide value *(2.5.5, Method A)*

Maximum 12.0, determined on 2.0 g.

Saponification value *(2.5.6)*

150 to 170, determined on 2.0 g.

Free glycerol

Maximum 6.0 per cent, determined as described in the assay.

Composition of fatty acids *(2.4.22, Method C)*.

Composition of the fatty acid fraction of the substance:

- — *palmitic acid*: maximum 12.0 per cent,
- — *stearic acid*: maximum 6.0 per cent,
- — *oleic acid*: minimum 60.0 per cent,
- — *linoleic acid*: maximum 35.0 per cent,
- — *linolenic acid*: maximum 2.0 per cent,
- — *arachidic acid*: maximum 2.0 per cent,
- — *eicosenoic acid*: maximum 2.0 per cent.

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 1.00 g. Use as the solvent a mixture of equal volumes of *anhydrous methanol R* and *methylene chloride R*.

Total ash *(2.4.16)*

Maximum 0.1 per cent.

ASSAY

Size-exclusion chromatography *(2.2.30)*.

Test solution Into a 15 ml flask, weigh about 0.2 g (*m*), to the nearest 0.1 mg. Add 5 ml of *tetrahydrofuran R* and shake to dissolve. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 ml flasks, respectively weigh, to the nearest 0.1 mg, about 2.5 mg, 5 mg, 10 mg and 20 mg of *glycerol R* . Add 5 ml of *tetrahydrofuran R* and shake until well mixed. Weigh the flasks again and calculate the concentration of glycerol in milligrams per gram for each reference solution.

Column:

- *size:* l = 0.6 m, Ø = 7 mm,
- *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R.*

Flow rate 1 ml/min.

Detection Differential refractometer.

Injection 40 µl.

Relative retention With reference to glycerol (retention time = about 15.6 min): triacylglycerols = about 0.76; diacylglycerols = about 0.79; monoacylglycerols = about 0.85.

Calculations:

- *free glycerol* : from the calibration curve obtained with the reference solutions determine the concentration (*C*) of glycerol in milligrams per gram in the test solution and calculate the percentage content of free glycerol in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

- *mono-, di- and triacylglycerols*: calculate the percentage content of mono-, di- and triacylglycerols using the normalisation procedure.

STORAGE

In an airtight container , protected from light.

LABELLING

The label states the nominal content of monoacylglycerol.

Ph Eur

Glycerol Monostearate 40-55

General Notices

(Ph Eur monograph 0495)

31566-31-1

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of monoacylglycerols, mainly monostearoylglycerol, together with variable quantities of di- and triacylglycerols. It is obtained by partial glycerolysis of vegetable oils mainly containing triacylglycerols of palmitic (hexadecanoic) or stearic (octadecanoic) acid or by esterification of glycerol with stearic acid. The fatty acids may be of vegetable or animal origin.

Content:

— *monoacylglycerols*: 40.0 per cent to 55.0 per cent;

— *diacylglycerols*: 30.0 per cent to 45.0 per cent;

— *triacylglycerols*: 5.0 per cent to 15.0 per cent.

CHARACTERS

Appearance

Hard, waxy mass or unctuous powder or flakes, white or almost white.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) at 60 °C.

IDENTIFICATION

First identification *C, D*.

Second identification *A, B*.

A. Melting point *(2.2.15)*: 54 °C to 66 °C.

Introduce the melted substance into the capillary tubes and allow to stand for 24 h in a well-closed container.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.5 g of the substance to be examined in *methylene chloride R*, with gentle heating, and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.5 g of *glycerol monostearate 40-55 CRS* in *methylene chloride R*, with gentle heating, and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *hexane R, ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Suitability system Reference solution:

— the chromatogram shows 4 clearly separated spots.

Results The spots in the chromatogram obtained with the test solution are similar in position to those in the chromatogram obtained with the reference solution.

C. Composition of fatty acids (see Tests) according to the type stated on the label.

D. It complies with the limits of the assay (monoacylglycerol content).

TESTS

Acid value *(2.5.1)*

Maximum 3.0, determined on 1.0 g.

Use a mixture of equal volumes of *ethanol (96 per cent) R* and *toluene R* as solvent and heat gently.

Iodine value *(2.5.4, Method A)*

Maximum 3.0.

Saponification value *(2.5.6)*

158 to 177, determined on 2.0 g. Carry out the titration with heating.

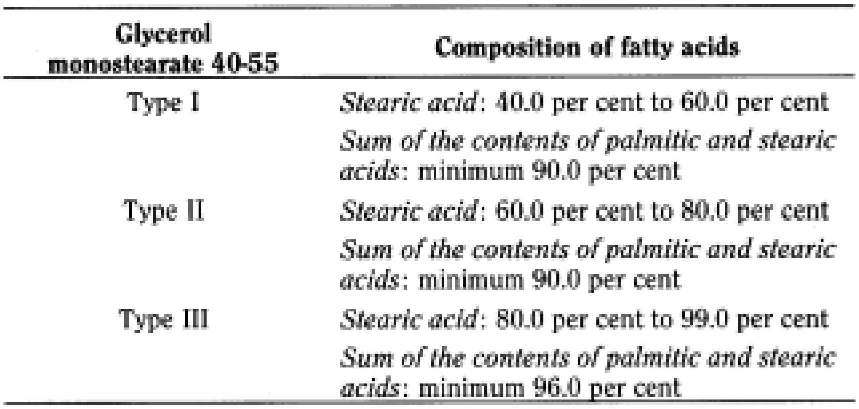
Free glycerol

Maximum 6.0 per cent, determined as described under Assay.

Composition of fatty acids *(2.4.22, Method C)*

Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty-acid fraction of the substance:



Glycerol monostearate 40-55	Composition of fatty acids
Type I	*Stearic acid*: 40.0 per cent to 60.0 per cent *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent
Type II	*Stearic acid*: 60.0 per cent to 80.0 per cent *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent
Type III	*Stearic acid*: 80.0 per cent to 99.0 per cent *Sum of the contents of palmitic and stearic acids*: minimum 96.0 per cent

Nickel ***(2.4.31)***

Maximum 1 ppm.

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.00 g. Use *pyridine R* as the solvent and heat gently.

Total ash ***(2.4.16)***

Maximum 0.1 per cent.

ASSAY

Size-exclusion chromatography *(2.2.30)*.

Test solution Into a 15 ml flask, weigh 0.200 g (*m*). Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 ml flasks, respectively weigh 2.5 mg, 5.0 mg, 10.0 mg and 20.0 mg of *glycerol R*, and add 5.0 ml of *tetrahydrofuran R* to each flask. Weigh the flasks again and calculate the concentration of glycerol in milligrams per gram for each reference solution.

Column:
- — *size*: l = 0.6 m, Ø = 7 mm;
- — *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 1 ml/min.

Detection Differential refractometer.

Injection 40 µl.

Relative retention With reference to glycerol (retention time = about 15 min): triacylglycerols = about 0.75; diacylglycerols = about 0.80; monoacylglycerols = about 0.85.

Calculations:
- — *free glycerol*: from the calibration curve obtained with the reference solutions, determine the concentration (*C*) in milligrams per gram in the test solution and calculate the percentage content in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *mono-, di- and triacylglycerols*: calculate the percentage contents by the normalisation procedure.

LABELLING

The label states the type of glycerol monostearate 40-55.

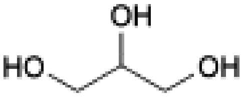
Ph Eur

Glycerol

General Notices

Glycerin

(Ph Eur monograph 0496)



$C_3H_8O_3$ 92.1 *56-81-5*

Action and use

Lubricant; laxative.

Preparations

Glycerol Eye Drops

Glycerol Suppositories

Ph Eur

DEFINITION

Propane-1,2,3-triol.

Content

98.0 per cent *m/m* to 101.0 per cent *m/m* (anhydrous substance).

CHARACTERS

Aspect

Syrupy liquid, unctuous to the touch, colourless or almost colourless, clear, very hygroscopic.

Solubility

Miscible with water and with alcohol, slightly soluble in acetone, practically insoluble in fatty oils and in essential oils.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. It complies with the test for refractive index (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation To 5 ml add 1 ml of *water R* and mix carefully.

Comparison Ph. Eur. reference spectrum of glycerol (85 per cent).

C. Mix 1 ml with 0.5 ml of *nitric acid R*. Superimpose 0.5 ml of *potassium dichromate solution R.* A blue ring develops at the interface of the liquids. Within 10 min, the blue colour does not diffuse into the lower layer.

D. Heat 1 ml with 2 g of *potassium hydrogen sulphate R* in an evaporating dish. Vapours (acrolein) are evolved which blacken filter paper impregnated with *alkaline potassium tetraiodomercurate solution R*.

TESTS

Solution S

Dilute 100.0 g to 200.0 ml with *carbon dioxide-free water R*.

Appearance of solution

Solution S is clear *(2.2.1)*. Dilute 10 ml of solution S to 25 ml with *water R*. The solution is colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 50 ml of solution S add 0.5 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Refractive index *(2.2.6)*

1.470 to 1.475.

Aldehydes

Maximum 10 ppm.

Place 7.5 ml of solution S in a ground-glass-stoppered flask and add 7.5 ml of *water R* and 1.0 ml of *decolorised pararosaniline solution R*. Close the flask and allow to stand for 1 h at a temperature of 25 ± 1 °C. The absorbance *(2.2.25)* of the solution measured at 552 nm is not greater than that of a standard prepared at the same time and in the same manner using 7.5 ml of *formaldehyde standard solution (5 ppm CH_2O) R* and 7.5 ml of *water R*. The test is not valid unless the standard is pink.

Esters

Add 10.0 ml of *0.1 M sodium hydroxide* to the final solution obtained in the test for acidity or alkalinity. Boil under a reflux condenser for 5 min. Cool. Add 0.5 ml of *phenolphthalein solution R* and titrate with *0.1 M hydrochloric acid*. Not less than 8.0 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Impurity A and related substances

Gas chromatography *(2.2.28)*.

Test solution Dilute 10.0 ml of solution S to 100.0 ml with *water R*.

Reference solution (a) Dilute 10.0 g of *glycerol R1* to 20.0 ml with *water R*. Dilute 10.0 ml of the solution to 100.0 ml with *water R*.

Reference solution (b) Dissolve 1.000 g of *diethylene glycol R* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with reference solution (a). Dilute 1.0 ml of this solution to 20.0 ml with reference solution (a).

Reference solution (d) Mix 1.0 ml of the test solution and 5.0 ml of reference solution (b) and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Reference solution (e) Dilute 5.0 ml of reference solution (b) to 100.0 ml with *water R*.

Column:

— *size: l* = 30 m, Ø = 0.53 mm,

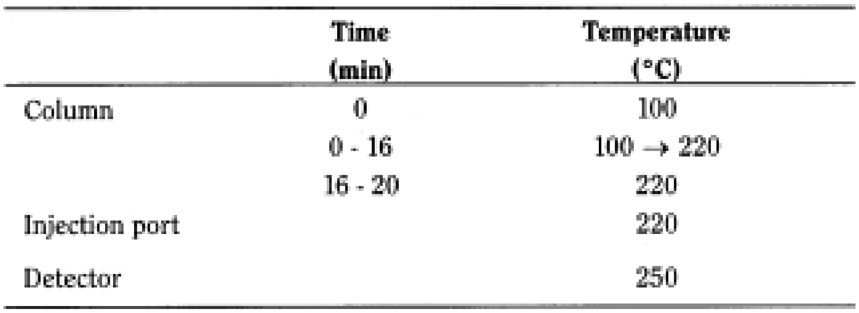
— *stationary phase*: 6 per cent polycyanopropylphenyl siloxane and 94 per cent of polydimethylsiloxane.

Carrier gas *helium for chromatography R*.

Split ratio 1:10.

Linear velocity 38 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0	100
	0 - 16	100 → 220
	16 - 20	220
Injection port		220
Detector		250

Detection Flame ionisation.

Injection 0.5 µl.

Elution order Impurity A, glycerol.

System suitability Reference solution (d):

— *resolution*: minimum 7.0 between the peaks due to impurity A and glycerol.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *any other impurity with a retention time less than the retention time of glycerol*: not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.1 per cent),

— *total of all impurities with retention times greater than the retention time of glycerol*: not more than 5 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *disregard limit*: 0.05 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.05 per cent).

Halogenated compounds

Maximum 35 ppm.

To 10 ml of solution S add 1 ml of *dilute sodium hydroxide solution R*, 5 ml of *water R* and 50 mg of *halogen-free nickel-aluminium alloy R*. Heat on a water-bath for 10 min, allow to cool and filter. Rinse the flask and the filter with *water R* until 25 ml of filtrate is obtained. To 5 ml of the filtrate add 4 ml of *alcohol R*, 2.5 ml of *water R*, 0.5 ml of *nitric acid R* and 0.05 ml of *silver nitrate solution R2* and mix. Allow to stand for 2 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time by mixing 7.0 ml of *chloride standard solution (5 ppm Cl) R*, 4 ml of *alcohol R*, 0.5 ml of *water R*, 0.5 ml of *nitric acid R* and 0.05 ml of *silver nitrate solution R2*.

Sugars

To 10 ml of solution S add 1 ml of *dilute sulphuric acid R* and heat on a water-bath for 5 min. Add 3 ml of *carbonate-free dilute sodium hydroxide solution R* (prepared by the method described for carbonate-free *1 M sodium hydroxide (4.2.2)*), mix and add dropwise 1 ml of freshly prepared *copper sulphate solution R*. The solution is clear and blue. Continue heating on the water-bath for 5 min. The solution remains blue and no precipitate is formed.

Chlorides *(2.4.4)*

Maximum 10 ppm.

1 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides. Prepare the standard using 1 ml of *chloride standard solution (5 ppm Cl) R* diluted to 15 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 5 ppm.

Dilute 8 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.01 per cent, determined on 5.0 g after heating to boiling and ignition.

ASSAY

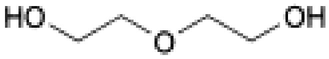
Thoroughly mix 0.075 g with 45 ml of *water R*. Add 25.0 ml of a mixture of 1 volume of *0.1 M sulphuric acid* and 20 volumes of *0.1 M sodium periodate*. Allow to stand protected from light for 15 min. Add 5.0 ml of a 500 g/l solution of *ethylene glycol R* and allow to stand protected from light for 20 min. Using 0.5 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 9.21 mg of $C_3H_8O_3$.

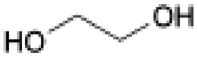
STORAGE

In an airtight container .

IMPURITIES



A. 2,2'-oxydiethanol (diethylene glycol),



B. ethane-1,2-diol (ethylene glycol),

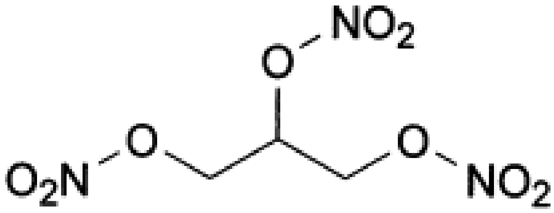
C. propylene glycol.

Ph Eur

Glyceryl Trinitrate Solution

General Notices

(Ph Eur monograph 1331)



$C_3H_5N_3O_9$ 227.1

Action and use

Vasodilator.

Preparations

Glyceryl Trinitrate Sublingual Spray

Glyceryl Trinitrate Tablets

Glyceryl Trinitrate Transdermal Patches

When concentrated glyceryl trinitrate solution is demanded, the intention of the purchaser, with respect to the strength expressed, should be ascertained.

Ph Eur

DEFINITION

Ethanolic solution of glyceryl trinitrate.

Content

1 per cent *m/m* to 10 per cent *m/m* of propane-1,2,3-triyl trinitrate and 96.5 per cent to 102.5 per cent of the declared content of glyceryl trinitrate stated on the label.

CHARACTERS

Appearance

Clear, colourless or slightly yellow solution.

Solubility

Miscible with acetone and with anhydrous ethanol.

Solubility of pure glyceryl trinitrate: practically insoluble in water, freely soluble in anhydrous ethanol, miscible with acetone.

IDENTIFICATION

First identification *A, C.*

Second identification *B, C.*

Upon diluting glyceryl trinitrate solution, care must be taken to always use anhydrous ethanol , otherwise droplets of pure glyceryl trinitrate may precipitate from the solution.

After examination, the residues and the solutions obtained in both the identification and the test sections must be heated on a water-bath for 5 min with dilute sodium hydroxide solution R.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Place 50 µl of a solution diluted, if necessary, with *anhydrous ethanol R*, to contain 10 g/l of glyceryl trinitrate, on a disc of *potassium bromide R* and evaporate the solvent *in vacuo.*

Comparison *Ph. Eur. reference spectrum of glyceryl trinitrate.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dilute a quantity of the substance to be examined corresponding to 50 mg of glyceryl trinitrate in *acetone R* and dilute to 100 ml with the same solvent.

Reference solution Dilute 0.05 ml of *glyceryl trinitrate solution CRS* to 1 ml with *acetone R*.

Plate *TLC silica gel G plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (20:80 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with freshly prepared *potassium iodide and starch solution R;* expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It complies with the limits of the assay.

TESTS

Upon diluting glyceryl trinitrate solution, care must be taken always to use anhydrous ethanol , otherwise droplets of pure glyceryl trinitrate may precipitate from the solution.

After examination, the residues and the solutions obtained in both the identification and the test sections must be heated on a water-bath for 5 min with dilute sodium hydroxide solution R.

Appearance of solution

If necessary dilute the solution to be examined to a concentration of 10 g/l with *anhydrous ethanol R*. The solution is not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Inorganic nitrates

Thin-layer chromatography *(2.2.27)*.

Test solution If necessary dilute the solution to be examined to a concentration of 10 g/l with *anhydrous ethanol R.*

Reference solution Dissolve 5 mg of *potassium nitrate R* in 1 ml *of water R* and dilute to 100 ml with *ethanol (96 per cent) R.*

Plate TLC silica gel plate R.

Mobile phase glacial acetic acid R, acetone R, *toluene R* (15:30:60 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of air until the acetic acid is completely removed.

Detection Spray intensively with freshly prepared *potassium iodide and starch solution R*; expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Limit:
- *nitrate ion*: any spot due to the nitrate ion in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent of the content of glyceryl trinitrate calculated as potassium nitrate).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve a quantity of the substance to be examined equivalent to 2 mg of glyceryl trinitrate in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 0.10 g of *glyceryl trinitrate solution CRS* and a quantity of *diluted pentaerythrityl tetranitrate CRS* equivalent to 1.0 mg of pentaerythrityl tetranitrate in the mobile phase and dilute to 100.0 ml with the mobile phase. Sonicate and filter if necessary.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase acetonitrile R, *water R* (50:50 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Run time 3 times the retention time of the principal peak.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to glyceryl trinitrate and to pentaerythrityl tetranitrate.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent, expressed as glyceryl trinitrate),

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent, expressed as glyceryl trinitrate),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

ASSAY

Test solution Prepare a solution containing 1.0 mg of glyceryl trinitrate in 250.0 ml of *methanol R.*

Reference solution Dissolve 70.0 mg of *sodium nitrite R* in *methanol R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 500.0 ml with *methanol R.*

Into three 50 ml volumetric flasks introduce 10.0 ml of the test solution, 10.0 ml of the reference solution and 10 ml of *methanol R* as a blank. To each flask add 5 ml of *dilute sodium hydroxide solution R*, close the flask, mix and allow to stand at room temperature for 30 min. Add 10 ml of *sulphanilic acid solution R* and 10 ml of *dilute hydrochloric acid R* and mix. After exactly 4 min, add 10 ml of *naphthylethylenediamine dihydrochloride solution R,* dilute to volume with *water R* and mix. After 10 min read the absorbance (*2.2.25*) of the test solution and the reference solution at 540 nm using the blank solution as the compensation liquid.

Calculate the amount of glyceryl trinitrate in milligrams in the test solution using the following expression:

$$\frac{A_T \times m_S \times C}{A_R \times m_T \times 60.8}$$

A_T = absorption of the test solution

m_T = mass of the substance to be examined, in milligrams

C = percentage content of sodium nitrite used as reference

A_R = absorption of the reference solution

m_S = mass of sodium nitrite, in milligrams.

STORAGE

Store the diluted solutions (10 g/l) protected from light, at a temperature of 2 °C to 15 °C.

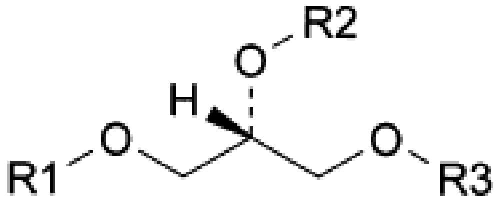
Store more concentrated solutions protected from light, at a temperature of 15 °C to 20 °C.

LABELLING

The label states the declared content of glyceryl trinitrate.

IMPURITIES

A. inorganic nitrates,



and enantiomer

B. $R1 = NO_2$, $R2 = R3 = H$: (2*RS*)-2,3-dihydroxypropyl nitrate,

C. $R1 = R3 = H$, $R2 = NO_2$: 2-hydroxy-1-(hydroxymethyl)ethyl nitrate,

D. $R1 = R2 = NO_2$, $R3 = H$: (2*RS*)-3-hydroxypropane-1,2-diyl dinitrate,

E. $R1 = R3 = NO_2$, $R2 = H$: 2-hydroxypropane-1,3-diyl dinitrate.

Ph Eur

Glycine

General Notices

(Ph Eur monograph 0614)

$C_2H_5NO_2$ 75.1 *56-40-6*

Action and use

Amino acid used for bladder irrigation during surgery.

Preparation

Glycine Irrigation Solution

Ph Eur

DEFINITION

2-Aminoacetic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison glycine CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (60 per cent V/V) R*, evaporate to dryness and record the spectra again.

B. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 50 mg in 5 ml of *water R*, add 1 ml of *strong sodium hypochlorite solution R* and boil for 2 min. Add 1 ml of *hydrochloric acid R* and boil for 4-5 min. Add 2 ml of *hydrochloric acid R* and 1 ml of a 20 g/l solution of *resorcinol R*, boil for 1 min and cool. Add 10 ml of *water R* and mix. To 5 ml of the solution add 6 ml of *dilute sodium hydroxide solution R*. The solution is violet with greenish-yellow fluorescence. After a few minutes, the colour becomes orange and then yellow and an intense fluorescence remains.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

5.9 to 6.4.

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Ninhydrin-positive substances

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *glycine CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 200 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *glycine CRS* and 10 mg of *alanine CRS* in *water R* and dilute to 25 ml with the same solvent.

Plate *cellulose for chromatography R* as the coating substance.

Mobile phase *glacial acetic acid R*, *water R*, *butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying At 80 °C for 30 min.

Detection Spray with *ninhydrin solution R* and dry at 100-105 °C for 15 min.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Limits In the chromatogram obtained with test solution (a):

— *any impurity*: any spots, apart from the principal spot, are not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides ***(2.4.4)***

Maximum 75 ppm.

Dissolve 0.67 g in *water R* and dilute to 15 ml with the same solvent.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 70.0 mg in 3 ml of *anhydrous formic acid R* and add 30 ml of *anhydrous acetic acid R*. Immediately after dissolution, titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

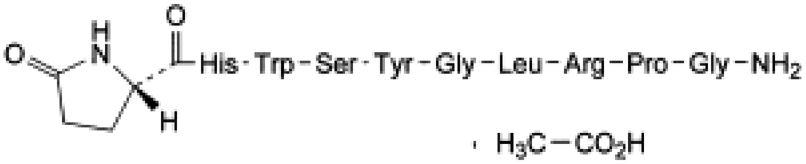
1 ml of *0.1 M perchloric acid* is equivalent to 7.51 mg of $C_2H_5NO_2$.

Ph Eur

Gonadorelin Acetate

General Notices

(Ph Eur monograph 0827)



$C_{55}H_{75}N_{17}O_{13},xC_2H_4O_2$ 1242 *52699-48-6*

Action and use

Gonadotropin-releasing hormone; treatment of prostate cancer.

Preparation

Gonadorelin Injection

Ph Eur

DEFINITION

Gonadorelin acetate is the acetate form of a hypothalamic peptide that stimulates the release of follicle-stimulating hormone and luteinising hormone from the pituitary gland. It contains not less than 95.0 per cent and not more than the equivalent of 102.0 per cent of the peptide $C_{55}H_{75}N_{17}O_{13}$, calculated with reference to the anhydrous, acetic acid-free substance. It is obtained by chemical synthesis.

CHARACTERS

A white or slightly yellowish powder, soluble in water and in a 1 per cent *V/V* solution of glacial acetic acid, sparingly soluble in methanol.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay. The retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel G plate R*.

Use the test solution and reference solution (a) prepared under Assay.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 6 volumes of *glacial acetic acid R*, 14 volumes of *water R*, 45 volumes of *methanol R* and 60 volumes of *methylene chloride R*. Allow the plate to dry in air for 5 min. At the bottom of a chromatography tank, place an evaporating dish containing a mixture of 10 ml of a 50 g/l solution of *potassium permanganate R* and 3 ml of *hydrochloric acid R*, close the tank and allow to stand. Place the dried plate in the tank and close the tank. Leave the plate in contact with the chlorine vapour for 2 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the points of application no longer gives a blue colour with 0.05 ml of *potassium iodide and starch solution R*. Spray with *potassium iodide and starch solution R*. The principal spot in the chromatogram obtained with the test solution corresponds in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

A 10 g/l solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Specific optical rotation

(2.2.7). Dissolve 10.0 mg in 1.0 ml of a 1 per cent *V/V* solution of *glacial acetic acid R*. The specific optical rotation is - 54 to - 66, calculated on the basis of the peptide content as determined in the assay.

Absorbance *(2.2.25)*

Dissolve 10.0 mg in *water R* and dilute to 100.0 ml with the same solvent. The absorbance, determined at the maximum at 278 nm, corrected to a 10 mg/100 ml solution on the basis of the peptide content determined in the assay, is 0.55 to 0.61.

Amino acids

Examine by means of an amino-acid analyser. Standardise the apparatus with a mixture containing equimolar amounts of ammonia, glycine and the L-form of the following amino acids:

lysine
histidine
arginine
aspartic acid
threonine
serine
glutamic acid
proline
alanine
valine
methionine
isoleucine
leucine
tyrosine
phenylalanine

together with half the equimolar amount of L-cystine. For the validation of the method, an appropriate internal standard, such as *DL-norleucine R*, is used.

Test solution Place 1.0 mg of the substance to be examined in a rigorously cleaned hard-glass tube 100 mm long and 6 mm in internal diameter. Add a suitable amount of a 50 per cent *V/V* solution of *hydrochloric acid R*. Immerse the tube in a freezing mixture at - 5 °C, reduce the pressure to below 133 Pa and seal. Heat at 110 °C to 115 °C for 16 h. Cool, open the tube, transfer the contents to a 10 ml flask with the aid of five quantities, each of 0.2 ml, of *water R* and evaporate to dryness over *potassium hydroxide R* under reduced pressure. Take up the residue in *water R* and evaporate to dryness over *potassium hydroxide R* under reduced pressure; repeat these operations once. Take up the residue in a buffer solution suitable for the amino-acid analyser used and dilute to a suitable volume with the same buffer solution. Apply a suitable volume to the amino-acid analyser.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking one-eighth of the sum of the number of moles of histidine, glutamic acid, leucine, proline, glycine, tyrosine and arginine as equal to one. The values fall within the following limits: serine 0.7 to 1.05; glutamic acid 0.95 to 1.05; proline 0.95 to 1.05; glycine 1.9 to 2.1; leucine 0.9 to 1.1; tyrosine 0.7 to 1.05; histidine 0.95 to 1.05 and arginine 0.95 to 1.05. Lysine and isoleucine are absent; not more than traces of other amino acids are present, with the exception of tryptophan.

Related substances

Examine by liquid chromatography *(2.2.29)* as described under Assay.

Inject 20 µl of reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full scale of the recorder.

Inject 20 µl of the test solution. Continue the chromatography for twice the retention time of gonadorelin. In the chromatogram obtained with the test solution: the area of any peak apart from the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent); the sum of the areas of the peaks, apart from the principal peak, is not greater than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent). Disregard any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Acetic acid *(2.5.34)*

4.0 per cent to 7.5 per cent.

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of solvents.

Water *(2.5.12)*

Not more than 7.0 per cent, determined on 0.200 g by the semi-micro determination of water.

Bacterial endotoxins *(2.6.14)*

Less than 70 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 5.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *gonadorelin CRS* in *water R* to obtain a concentration of 0.5 mg/ml.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *water R*.

Reference solution (c) Dissolve 2.5 mg of the substance to be examined in 1 ml of *0.1 M hydrochloric acid* and heat in a water-bath at 65 °C for 4 h. Add 1 ml of *0.1 M sodium* *hydroxide* and dilute to 5.0 ml with *water R*.

The chromatographic procedure may be carried out using:

- — a stainless steel column 0.12 m long and 4.0 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),
- — as mobile phase at a flow rate of 1.5 ml/min a mixture of 13 volumes of *acetonitrile R* and 87 volumes of a 1.18 per cent *V/V* solution of *phosphoric acid R* (adjusted to pH 2.3 with *triethylamine R*),
- — as detector a spectrophotometer set at 215 nm.

Inject 20 µl of reference solution (c). The test is not valid unless the resolution between the first and second peaks is at least 2.0.

Inject 20 µl of the test solution and 20 µl of reference solution (a).

Calculate the content of gonadorelin ($C_{55}H_{75}N_{17}O_{13}$) from the peak areas in the chromatograms obtained with the test solution and reference solution (a) and the declared content of $C_{55}H_{75}N_{17}O_{13}$ in *gonadorelin CRS* .

STORAGE

Store in an airtight container , protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

LABELLING

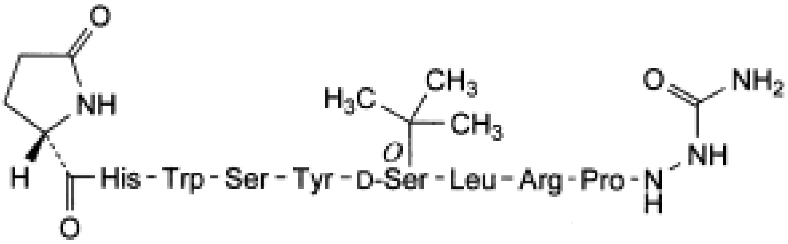
The label states the mass of peptide in the container.

Ph Eur

Goserelin

General Notices

(Ph Eur monograph 1636)



$C_{59}H_{84}N_{18}O_{14}$ 1269 *65807-02-5*

Action and use

Gonadotropin-releasing hormone, gonadorelin analogue; treatment of prostate cancer.

Ph Eur

DEFINITION

1-Carbamoyl-2-[5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-*C*-(1,1-dimethylethyl)-D-seryl-L-leucyl-L-arginyl-L-prolyl]diazane.

Synthetic nonapeptide analogue of the hypothalamic decapeptide, gonadorelin. It is obtained by chemical synthesis and is available as an acetate.

Content

94.5 per cent to 103.0 per cent of the peptide $C_{59}H_{84}N_{18}O_{14}$ (anhydrous and acetic-acid free substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water, freely soluble in glacial acetic acid. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

A. Nuclear magnetic resonance spectrometry *(2.2.33)*.

Preparation 40 mg/ml solution of the substance to be examined in *deuterium oxide R* adjusted to pH 4.0 with *deuterated acetic acid R*.

Results The ^{13}C, proton decoupled NMR spectrum obtained is qualitatively similar to the *Ph. Eur. reference spectrum of goserelin.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Amino acid analysis *(2.2.56)*. For protein hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking one sixth of the sum of the number of moles of glutamic acid, histidine, tyrosine, leucine, arginine, proline as equal to 1. The values fall within the following limits: glutamic acid, histidine, tyrosine, leucine, arginine and proline 0.9 to 1.1; serine 1.6 to 2.2. Not more than traces of other amino acids are present, with the exception of tryptophan.

TESTS

Specific optical rotation *(2.2.7)*

- 52 to - 56 (anhydrous and acetic-acid free substance).

Dissolve the substance to be examined in *water R* to obtain a concentration of 2 mg/ml.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve the substance to be examined in *water R* to obtain a concentration of 1.0 mg/ml.

Reference solution (a) Dissolve the contents of a vial of *goserelin CRS* in *water R* to obtain a concentration of 1.0 mg/ml.

Reference solution (b) Dilute 1.0 ml of the test solution to 100 ml with *water R*.

Reference solution (c) Dilute 1.0 ml of the test solution to 10.0 ml with *water R*.

Resolution solution (a) Dissolve the contents of a vial of *4-D-Ser-goserelin CRS* in *water R* to obtain a concentration of 0.1 mg/ml. Mix equal volumes of this solution and of reference solution (c).

Resolution solution (b) Dissolve the contents of a vial of *goserelin validation mixture CRS* with 1.0 ml of *water R*.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl amorphous organosilica polymer R* (3.5 µm) with a pore size of 12.5 nm,

— *temperature*: 50-55 °C.

Mobile phase *trifluoroacetic acid R*, *acetonitrile for chromatography R*, *water R* (0.5:200:800 *V/V/V*).

Flow rate 0.7-1.2 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl of the test solution, reference solution (b) and the resolution solutions.

Run time 90 min.

Relative retention With reference to goserelin: impurity A = about 0.67; impurity C = about 0.78; impurity B = about 0.79; impurity D = about 0.85; impurity E = about 0.89; impurity F = about 0.92; impurity G = about 0.94; impurity H = about 0.98; impurity I = about 1.43; impurity J = about 1.53; impurity K = about 1.67; impurity L = about 1.77.

System suitability:

— *retention time*: goserelin = 40 min to 50 min in the chromatogram obtained with resolution solution (b); adjust the flow rate of the mobile phase if necessary; if adjusting the flow rate does not result in a correct retention time of the principal peak, change the composition of acetonitrile in the mobile phase to obtain the requested retention time for goserelin;

— *resolution*: minimum 7.0 between the peaks due to impurity A and goserelin in the chromatogram obtained with resolution solution (a);

— *symmetry factor*: 0.8 to 2.5 for the peaks due to impurity A and goserelin in the chromatogram obtained with resolution solution (a);

— the chromatogram obtained with resolution solution (b) is similar to the chromatogram supplied with *goserelin validation mixture CRS*. 2 peaks eluting prior to the principal peak and corresponding to impurity E and impurity G, are clearly visible. 3 peaks eluting after the principal peak are clearly visible.

Limits:

— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent),

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Acetic acid ***(2.5.34)***

4.5 per cent to 15.0 per cent.

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Water ***(2.5.32)***

Maximum 10.0 per cent.

Bacterial endotoxins ***(2.6.14)***

Less than 16 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

Run time 60 min.

Calculate the content of goserelin ($C_{59}H_{84}N_{18}O_{14}$) using the chromatograms obtained with the test solution and reference solution (a) and the declared content of $C_{59}H_{84}N_{18}O_{14}$ in *goserelin CRS.*

STORAGE

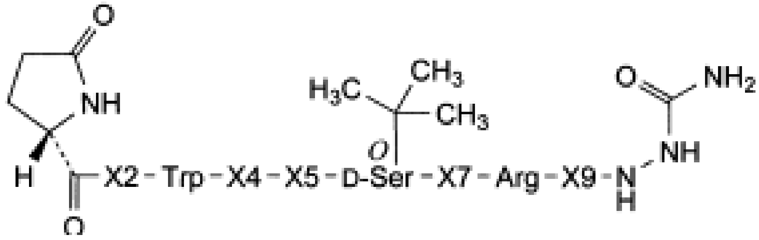
In an airtight container , protected from light, at a temperature of 2 °C to 8 °C.

LABELLING

The label states the mass of peptide in the container.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J, K, L.


O
NH
H
O
H3C
CH3
CH3
O
X2 - Trp - X4 - X5 - D-Ser - X7 - Arg - X9 - N
H
O
NH2
NH

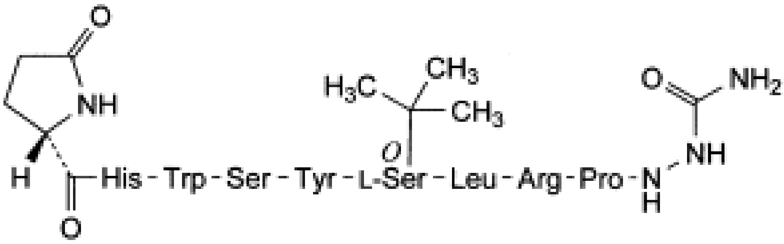
A. X2 = L-His, X4 = D-Ser, X5 = L-Tyr, X7 = L-Leu, X9 = L-Pro: [4-D-serine]goserelin,

C. X2 = L-His, X4 = L-Ser, X5 = L-Tyr, X7 = L-Leu, X9 = D-Pro: [9-D-proline]goserelin,

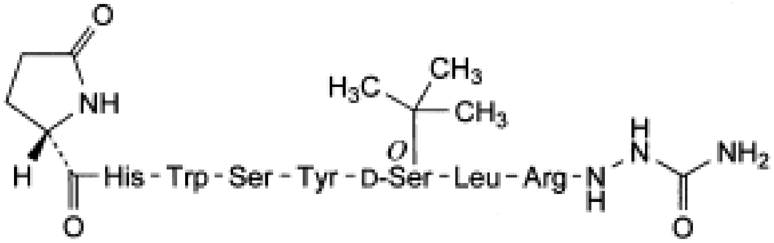
F. X2 = L-His, X4 = L-Ser, X5 = D-Tyr, X7 = L-Leu, X9 = L-Pro: [5-D-tyrosine]goserelin,

G. X2 = D-His, X4 = L-Ser, X5 = L-Tyr, X7 = L-Leu, X9 = L-Pro: [2-D-histidine]goserelin,

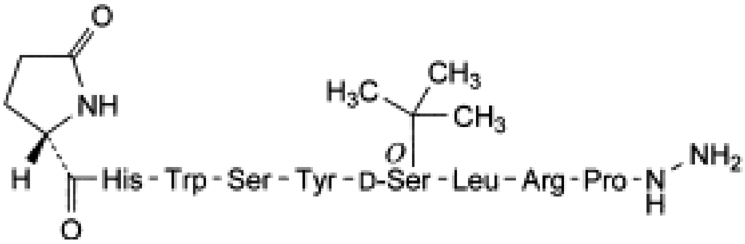
L. X2 = L-His, X4 = L-Ser, X5 = L-Tyr, X7 = D-Leu, X9 = L-Pro: [7-D-leucine]goserelin,


O
NH
H
O
H3C
CH3
CH3
O
His - Trp - Ser - Tyr - L-Ser - Leu - Arg - Pro - N
H
O
NH2
NH

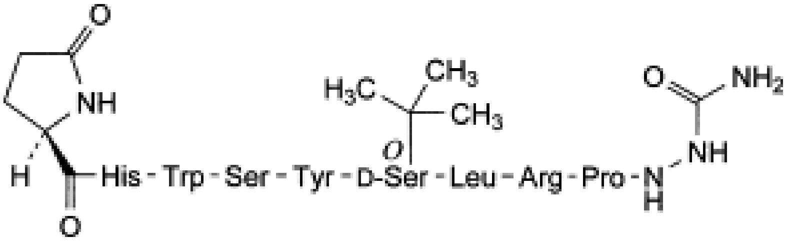
B. [6-[*O*-(1,1-dimethylethyl)-l-serine]]goserelin,


O
NH
H
O
His - Trp - Ser - Tyr - D-Ser - Leu - Arg
H3C
CH3
CH3
O
H
N
NH2
N
H
O

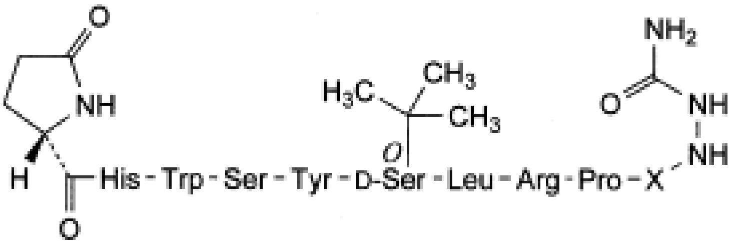
D. 1-carbamoylyl-2-[5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-*O*-(1,1-dimethylethyl)-D-seryl-L-leucyl-L-arginyl]diazane,


O
NH
H
O
His - Trp - Ser - Tyr - D-Ser - Leu - Arg - Pro
H3C
CH3
CH3
O
NH2
N
H

E. 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-*O*-(1,1-dimethylethyl)-D-seryl-L-leucyl-L-arginyl-L-prolinohydrazide,

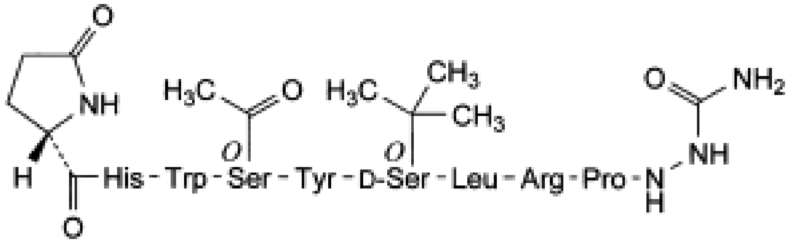

O
NH
H
O
His - Trp - Ser - Tyr - D-Ser - Leu - Arg - Pro
H3C
CH3
CH3
O
O
NH2
NH
N
H

H. [1-(5-oxo-d-proline)]goserelin,


O
NH
H
O
His - Trp - Ser - Tyr - D-Ser - Leu - Arg - Pro - X
H3C
CH3
CH3
O
NH2
O
NH
NH

I. X = Pro-Pro: endo-8a,8b-DI-L-proline-goserelin,

J. X = Pro: endo-8a-L-proline-goserelin,



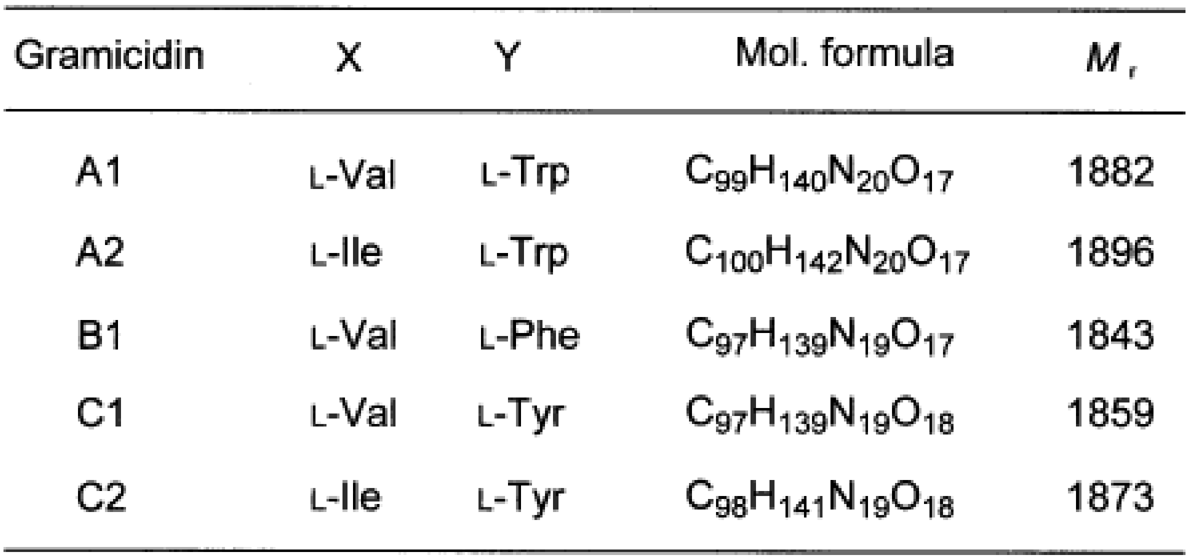
K. C^4-acetylgoserelin.

Ph Eur

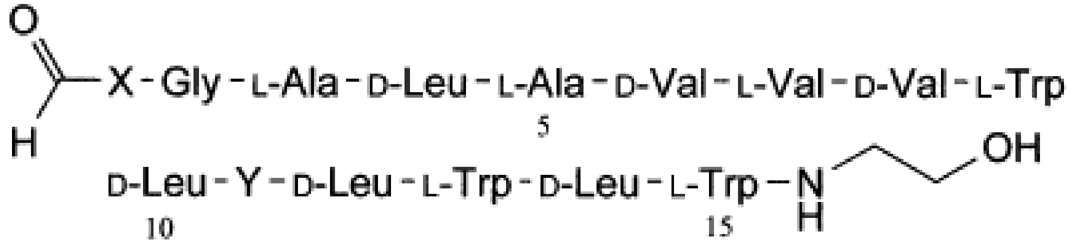
Gramicidin

General Notices

(Ph Eur monograph 0907)



Gramicidin	X	Y	Mol. formula	M_r
A1	L-Val	L-Trp	$C_{99}H_{140}N_{20}O_{17}$	1882
A2	L-Ile	L-Trp	$C_{100}H_{142}N_{20}O_{17}$	1896
B1	L-Val	L-Phe	$C_{97}H_{139}N_{19}O_{17}$	1843
C1	L-Val	L-Tyr	$C_{97}H_{139}N_{19}O_{18}$	1859
C2	L-Ile	L-Tyr	$C_{98}H_{141}N_{19}O_{18}$	1873


O
H
X - Gly - L-Ala - D-Leu - L-Ala - D-Val - L-Val - D-Val - L-Trp
5
D-Leu - Y - D-Leu - L-Trp - D-Leu - L-Trp - N
H
OH
10
15

1405-97-6

Action and use

Polypeptide antibacterial.

Ph Eur

DEFINITION

Gramicidin consists of a family of antimicrobial linear polypeptides, usually obtained by extraction from tyrothricin, the complex isolated from the fermentation broth of *Brevibacillus brevis* Dubos. The main component is gramicidin A1, together with gramicidins A2, B1, C1 and C2 in particular.

Content

Minimum 900 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, slightly hygroscopic.

Solubility

Practically insoluble in water, soluble in methanol, sparingly soluble in alcohol.

mp

About 230 °C.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B.*

A. Dissolve 0.100 g in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *alcohol R*. Examined between 240 nm and 320 nm *(2.2.25)*, the solution shows 2 absorption maxima, at 282 nm and 290 nm, a shoulder at about 275 nm and an absorption minimum at 247 nm. The specific absorbance at the maximum at 282 nm is 105 to 125.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in 6.0 ml of *alcohol R*.

Reference solution (a) Dissolve 5 mg of *gramicidin CRS* in 6.0 ml of *alcohol R*.

Reference solution (b) Dissolve 5 mg of *tyrothricin CRS* in 6.0 ml of *alcohol R*.

Plate *TLC silica gel plate R*.

Mobile phase *methanol R*, *butanol R*, *water R*, *glacial acetic acid R*, *butyl acetate R* (3:9:15:24:49 V/V/V/V/V).

Application 1 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Dip the plate into *dimethylaminobenzaldehyde solution R2*. Heat at 90 °C until the spots appear.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots or 2 clearly separated groups of spots.

Results The principal spot or group of principal spots in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot or group of principal spots in the chromatogram obtained with reference solution (a) and to the spot or group of spots with the highest R_F value in the chromatogram obtained with reference solution (b).

C. Examine the chromatograms obtained in the test for composition.

Results The 3 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the 3 principal peaks in the chromatogram obtained with reference solution (a).

TESTS

Composition Liquid chromatography *(2.2.29)*: use the normalisation procedure.

Test solution Dissolve 25 mg of the substance to be examined in 10 ml of *methanol R* and dilute to 25 ml with the mobile phase.

Reference solution (a) Dissolve 25 mg of *gramicidin CRS* in 10 ml of *methanol R* and dilute to 25 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 50 °C.

Mobile phase *water R*, *methanol R* (29:71 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 282 nm.

Injection 20 µl.

Run time 2.5 times the retention time of gramicidin A1.

Relative retention With reference to gramicidin A1 (retention time = about 22 min): gramicidin C1 = about 0.7; gramicidin C2 = about 0.8; gramicidin A2 = about 1.2; gramicidin B1 = about 1.9.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to gramicidin A1 and gramicidin A2,

— the chromatogram obtained is concordant with the chromatogram supplied with *gramicidin CRS* .

Composition:

— *sum of the contents of gramicidins A1, A2, B1, C1 and C2*: minimum 95.0 per cent,

— *ratio of the content of gramicidin A1 to the sum of the contents of gramicidins A1, A2, B1, C1 and C2* : minimum 60.0 per cent,

— *disregard limit*: the area of the peak due to gramicidin A1 in the chromatogram obtained with reference solution (b).

Related substances

Liquid chromatography *(2.2.29)* as described in the test for composition.

Limit:

— *any impurity*: maximum 2.0 per cent and not more than 1 peak is more than 1.0 per cent; disregard the peaks due to gramicidins A1, A2, B1, C1 and C2.

Loss on drying ***(2.2.32)***

Maximum 3.0 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at 60 °C at a pressure not exceeding 0.1 kPa for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 1.0 per cent, determined on 1.0 g.

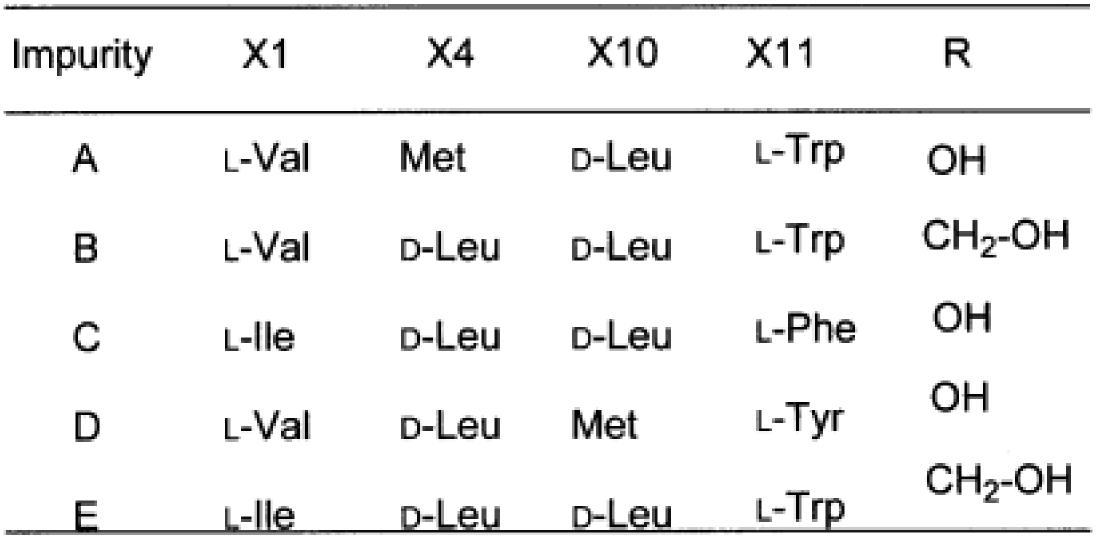
ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)*, using the turbidimetric method. Use *gramicidin CRS* as the reference substance.

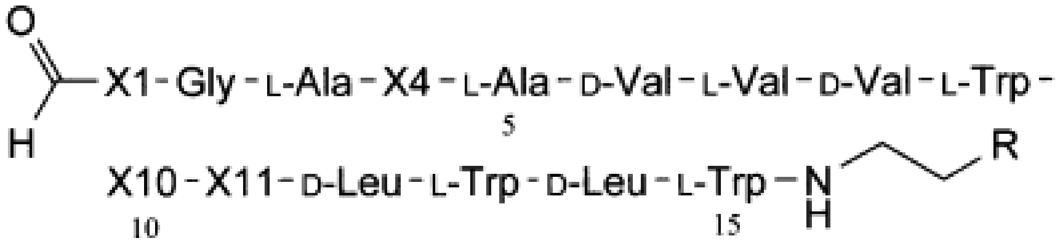
STORAGE

In an airtight container , protected from light.

IMPURITIES



Impurity	X1	X4	X10	X11	R
A	L-Val	Met	D-Leu	L-Trp	OH
B	L-Val	D-Leu	D-Leu	L-Trp	CH_2-OH
C	L-Ile	D-Leu	D-Leu	L-Phe	OH
D	L-Val	D-Leu	Met	L-Tyr	OH
E	L-Ile	D-Leu	D-Leu	L-Trp	CH_2-OH


O
H
X1-Gly-L-Ala-X4-L-Ala-D-Val-L-Val-D-Val-L-Trp-
5
X10-X11-D-Leu-L-Trp-D-Leu-L-Trp-N
H
R
10
15

A. [4-methionine]gramicidin A1,

B. gramicidin A1 3-hydroxypropyl,

C. gramicidin B2,

D. [10-methionine]gramicidin C1,

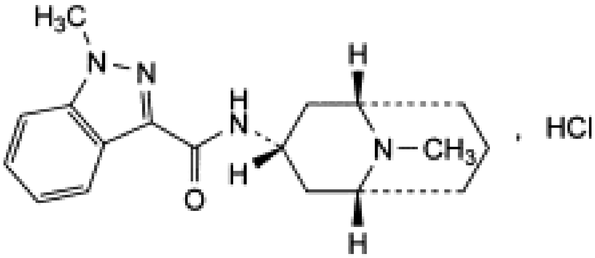
E. gramicidin A2 3-hydroxypropyl.

Ph Eur

Granisetron Hydrochloride

General Notices

(Ph Eur monograph 1695)



$C_{18}H_{24}N_4O,HCl$ 348.9 *107007-99-8*

Action and use

Serotonin $5HT_3$ receptor antagonist; treatment of nausea and vomiting.

Ph Eur

DEFINITION

1-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide hydrochloride.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, sparingly soluble in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison granisetron hydrochloride CRS .

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.0 to 6.5 for solution S.

Impurity E

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.25 g of the substance to be examined in a mixture of 20 volumes of *water R* and 80 volumes of *acetonitrile R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dissolve 5 mg of *granisetron impurity E CRS* in a mixture of 20 volumes of *water R* and 80 volumes of *acetonitrile R* and dilute to 20 ml with the same mixture of solvents.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R*, *2-propanol R*, *ethyl acetate R* (6.5:30:50 *V/V/V*).

Application 2 µl.

Development Over half of the plate.

Drying In air.

Detection Expose to iodine vapour for 30 min.

Limit:

— *impurity E*: any spot due to impurity E is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Transfer 2 ml of the test solution to a colourless glass vial, stopper and expose the solution either to sunlight for 4 h or under a UV lamp for 16 h (partial degradation of granisetron to impurity C). A degradation of at least about 0.3 per cent of granisetron to impurity C must be obtained as shown by appearance of a corresponding peak in the chromatogram. If not, expose the solution once again to sunlight or under a UV lamp.

Reference solution (c) Dissolve 50.0 mg of *granisetron hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (d) Dissolve the contents of a vial of *granisetron impurity A CRS* in 1 ml of the mobile phase.

Reference solution (e) Dissolve the contents of a vial of *granisetron impurity B CRS* in 1 ml of the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 40 °C.

Mobile phase Dilute 1.6 ml of *phosphoric acid R* to 800 ml with *water R*, add 200 ml of *acetonitrile R* and mix. Add 1.0 ml of *hexylamine R* and mix. Adjust to pH 7.5 ± 0.05 with freshly distilled *triethylamine R* (about 4 ml).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 305 nm.

Injection 10 µl of the test solution and reference solutions (a), (b), (d) and (e).

Run time Twice the retention time of granisetron.

Relative retention With reference to granisetron (retention time = about 7 min): impurity D = about 0.4; impurity B = about 0.5; impurity A = about 0.7; impurity C = about 0.8.

System suitability:

- *resolution*: minimum 3.5 between the peaks due to impurity C and granisetron in the chromatogram obtained with reference solution (b),
- *symmetry factor*: maximum 2.0 for the peak due to granisetron.

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity B by 1.7,
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- *impurity C*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
- *impurity D*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak due to the blank.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

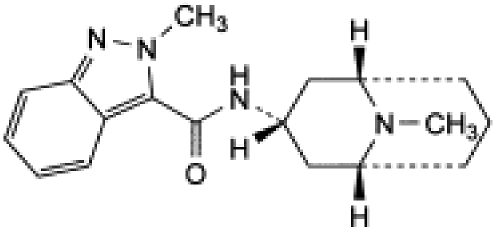
Injection Test solution and reference solution (c).

Calculate the percentage content of $C_{18}H_{25}ClN_4O$ using the declared content of *granisetron hydrochloride CRS* .

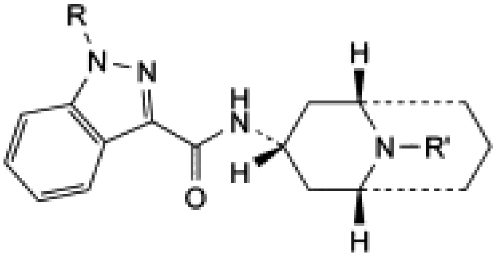
IMPURITIES

Specified impurities A, B, C, D, E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F, G, H, I.*

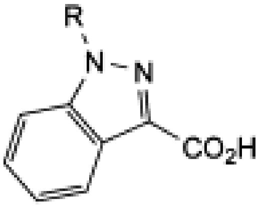


A. 2-methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide,



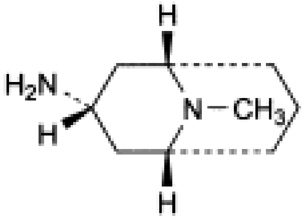
B. R = H, R′ = CH_3: *N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide,

C. R = CH_3, R′ = H: *N*-[(1*R*,3*r*,5*S*)-9-azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide,

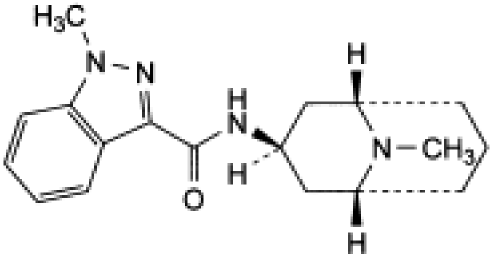


D. R = CH_3: 1-methyl-1*H*-indazole-3-carboxylic acid,

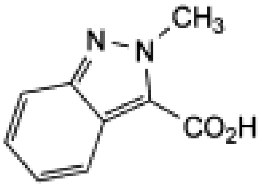
H. R = H: 1*H*-indazole-3-carboxylic acid,



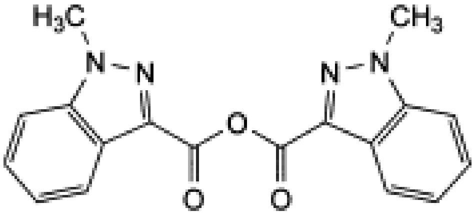
E. (1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine,



F. 1-methyl-*N*-[(1*R*,3*s*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide (exo-granisetron),



G. 2-methyl-2*H*-indazole-3-carboxylic acid,



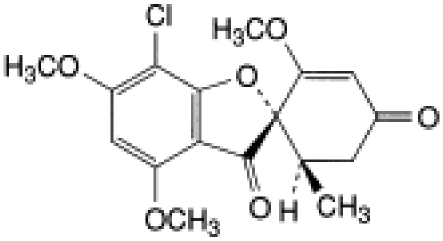
I. 1-methyl-1*H*-indazole-3-carboxylic anhydride.

Ph Eur

Griseofulvin

General Notices

(Ph Eur monograph 0182)



$CH_{17}ClO_6$ 352.8   *126-07-8*

Action and use

Antibacterial.

Preparation

Griseofulvin Tablets

Ph Eur

DEFINITION

(1′*S*,3-6′*R*)-7-Chloro-2′,4,6-trimethoxy-6′-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione.

Substance produced by the growth of certain strains of *Penicillium griseofulvum* or obtained by any other means.

Content

97.0 per cent to 102.0 per cent (dried substance).

PRODUCTION

The method of manufacture is validated to demonstrate that the product if tested would comply with the following test.

Abnormal toxicity

To each of 5 healthy mice, each weighing 17-22 g, administer orally a suspension of 0.1 g of the substance to be examined in 0.5-1 ml of *water R*. None of the mice dies within 48 h.

CHARACTERS

Appearance

White or yellowish-white, microfine powder , the particles of which generally have a maximum dimension of up to 5 µm, although larger particles that may exceed 30 µm may occasionally be present.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide and in tetrachloroethane, slightly soluble in anhydrous ethanol and in methanol.

mp: about 220 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison griseofulvin CRS .

B. Dissolve about 5 mg in 1 ml of *sulphuric acid R* and add about 5 mg of powdered *potassium dichromate R*. A dark red colour develops.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_4 *(2.2.2, Method II)*.

Dissolve 0.75 g in *dimethylformamide R* and dilute to 10 ml with the same solvent.

Acidity

Suspend 0.25 g in 20 ml of *ethanol (96 per cent) R* and add *0.1 ml of phenolphthalein solution R*. Not more than 1.0 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation *(2.2.7)*

+ 354 to + 364 (dried substance).

Dissolve 0.250 g in *dimethylformamide R* and dilute to 25.0 ml with the same solvent.

Related substances

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.2 g of *diphenylanthracene R* in *acetone R* and dilute to 100.0 ml with the same solvent.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 0.10 g of the substance to be examined in *acetone R*, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *acetone R*.

Reference solution Dissolve 5.0 mg of *griseofulvin CRS* in *acetone R*, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *acetone R*.

Column:

- *material*: glass;
- *size: l* = 1 m, Ø = 4 mm;
- *stationary phase*: *diatomaceous earth for gas chromatography R* impregnated with 1 per cent *m/m* of *poly[(cyanopropyl)(methyl)][(phenyl)(methyl)]siloxane R*.

Carrier gas *nitrogen for chromatography R.*

Flow rate 50-60 ml/min.

Temperature:
- *column*: 250 °C;
- *injection* port: 270 °C;
- *detector*: 300 °C.

Detection Flame ionisation.

Run time 3 times the retention time of griseofulvin.

Relative retention With reference to griseofulvin (retention time = about 11 min): dechloro-griseofulvin = about 0.6; dehydrogriseofulvin = about 1.4.

Calculate the ratio (*R*) of the area of the peak due to griseofulvin to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution.

Limits:
- *dechloro-griseofulvin* : calculate the ratio of the area of the peak due to dechloro-griseofulvin to the area of the peak due to the internal standard in the chromatogram obtained with test solution (b): this ratio is not greater than 0.6 *R* (3.0 per cent);
- *dehydrogriseofulvin*: calculate the ratio of the area of the peak due to dehydrogriseofulvin to the area of the peak due to the internal standard in the chromatogram obtained with test solution (b): this ratio is not greater than 0.15 *R* (0.75 per cent).

Substances soluble in light petroleum

Maximum 0.2 per cent.

Shake 1.0 g with 20 ml of *light petroleum R*. Boil under a reflux condenser for 10 min. Cool, filter and wash with 3 quantities, each of 15 ml, of *light petroleum R*. Combine the filtrate and washings, evaporate to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs not more than 2 mg.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

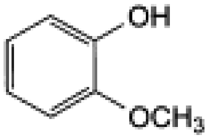
Dissolve 80.0 mg in *anhydrous ethanol R* and dilute to 200.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *anhydrous ethanol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 291 nm.

Calculate the content of $C_{17}H_{17}ClO_6$, taking the specific absorbance to be 686.

Guaiacol

General Notices

(Ph Eur monograph 1978)



$C_7H_8O_2$ 124.1 *90-05-1*

Ph Eur

DEFINITION

2-Methoxyphenol.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Crystalline mass or colourless or yellowish liquid, hygroscopic.

Solubility

Sparingly soluble in water, very soluble in methylene chloride, freely soluble in ethanol (96 per cent).

mp

About 28 °C.

IDENTIFICATION

First identification *A*.

Second identification *B.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *guaiacol CRS .*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.5 g of the substance to be examined in *methanol R* and dilute to 25

ml with the same solvent.

Reference solution Dissolve 0.5 g of *guaiacol CRS* in *methanol R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous acetic acid R*, *methanol R*, *toluene R* (6:14:80 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ferric chloride solution R1*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 1.00 g in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method I)*.

Acidity or alkalinity

To 5.0 ml of solution S, add 10 ml of *carbon dioxide-free water R* and 0.1 ml of *methyl red mixed solution R*. Not more than 0.05 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Impurity A

Liquid chromatography *(2.2.29)*.

Solvent mixture *phosphoric acid R*, *water R*, *methanol R* (0.1:49.9:50 *V/V/V*).

Test solution (a) Dissolve 1.0 g of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Test solution (b) Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Reference solution (b) Dissolve 0.200 g of *pyrocatechol R* (impurity A) and 0.200 g of *phenol R* (impurity B) in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 0.200 g of *guaiacol CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *mobile phase*: *phosphoric acid R*, *methanol R*, *water R* (1:150:849 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (b).

Run time 1.5 times the retention time of guaiacol.

Retention time Guaiacol = about 20 min.

System suitability Reference solution (b):

— *resolution*: minimum 5 between the peaks due to impurity A (1st peak) and impurity B (2nd peak).

Limit:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 1.00 g of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 0.200 g of *phenol R* (impurity B) and 0.400 g of *methyl benzoate R* (impurity E) in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 20.0 ml with *acetonitrile R*.

Reference solution (b) Dilute 0.5 ml of the test solution to 100.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 10.0 ml with *acetonitrile R*.

Reference solution (c) Dissolve 10 mg of *veratrole R* (impurity C) in *acetonitrile R* and dilute to 10 ml with the same solvent.

Column:

— *material*: fused silica;

— *size:* *l* = 25 m, Ø = 0.53 mm;

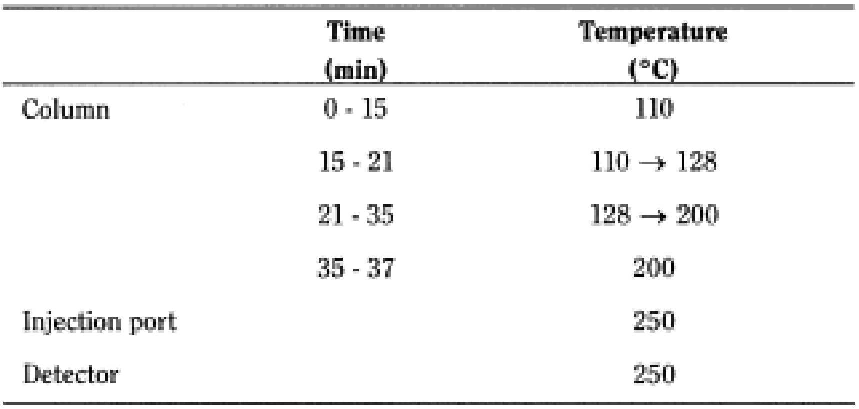
— *stationary phase*: *poly(cyanopropyl)(7)(phenyl)(7)(methyl)(86)siloxane R* (film thickness 2 µm).

Carrier gas *helium for chromatography R.*

Flow rate 6 ml/min.

Split ratio 1:5.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 15	110
	15 - 21	110 → 128
	21 - 35	128 → 200
	35 - 37	200
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to guaiacol (retention time = about 14 min): impurity E = 0.86; impurity B = about 0.88; impurity C = about 1.2.

System suitability Reference solution (a):

— *resolution*: minimum 1.0 between the peaks due to impurity E (1st peak) and impurity B (2nd peak).

Limits:

— *impurity B*: maximum 0.1 per cent;

— *impurity C*: maximum 0.4 per cent;

— *impurity E*: maximum 0.2 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b).

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 2.000 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for impurity A with the following modifications.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_7H_8O_2$ from the declared content of *guaiacol CRS* .

STORAGE

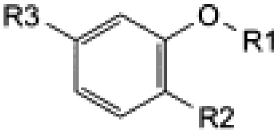
In an airtight container , protected from light.

IMPURITIES

Specified impurities *A, B, C, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general

acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D.*

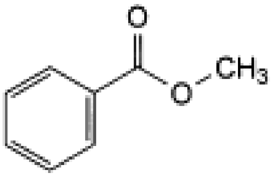


A. R1 = R3 = H, R2 = OH: benzene-1,2-diol (pyrocatechol),

B. R1 = R2 = R3 = H: phenol,

C. R1 = CH_3, R2 = OCH_3, R3 = H: 1,2-dimethoxybenzene (veratrole),

D. R1 = H, R2 = R3 = OCH_3: 2,5-dimethoxyphenol,



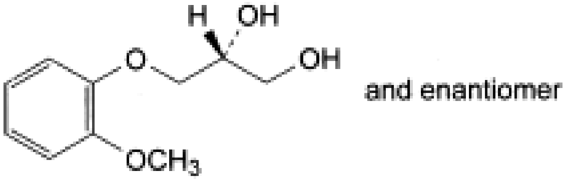
E. methyl benzoate.

Ph Eur

Guaifenesin

General Notices

(Ph Eur monograph 0615)



$C_{10}H_{14}O_4$ 198.2 *93-14-1*

Action and use

Expectorant.

Ph Eur

DEFINITION

(2*RS*)-3-(2-Methoxyphenoxy)propane-1,2-diol.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, soluble in alcohol.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Melting point *(2.2.14)*: 79 °C to 83 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison guaifenesin CRS .

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 30 mg of *guaifenesin CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *methylene chloride R*, *propanol R* (20:80 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a mixture of equal volumes of a 10 g/l solution of *potassium ferricyanide R*, a 200 g/l solution of *ferric chloride R* and *alcohol R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R*, heating gently if necessary, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *phenolphthalein solution R1*. Not more than 0.1 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator. To 10 ml of solution S add 0.15 ml of *methyl red solution R*. Not more than 0.1 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 20.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 10.0 ml with *acetonitrile R*.

Reference solution (b) Dissolve 10.0 mg of *guaiacol R* in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 0.5 ml of this solution to 50.0 ml with *acetonitrile R*.

Reference solution (c) Dissolve 50.0 mg of *guaiacol R* in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 10.0 ml with the test solution.

Column:

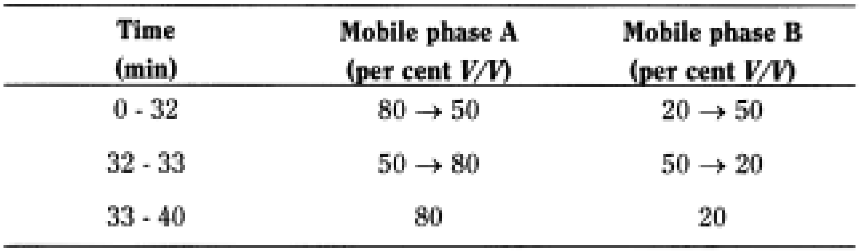
— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *glacial acetic acid R*, *water R* (10:990 *V/V*),

— *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 32	80 → 50	20 → 50
32 - 33	50 → 80	50 → 20
33 - 40	80	20

Flow rate 1 ml/min.

Detection Spectrophotometer at 276 nm.

Injection 10 µl.

Relative retention With reference to guaifenesin (retention time = about 8 min): impurity B = about 0.9; impurity A = about 1.4; impurity C = about 3.1; impurity D = about 3.7.

System suitability Reference solution (c):

— *resolution*: minimum 3.0 between the peaks due to guaifenesin and impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *total (excluding impurity B):* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *disregard level*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides and monochlorhydrins

Maximum of 250 ppm.

To 10 ml of solution S add 2 ml of *dilute sodium hydroxide solution R* and heat on a water-bath for 5 min. Cool and add 3 ml of *dilute nitric acid R*. The resulting solution complies with the limit test for chlorides *(2.4.4)*.

Heavy metals *(2.4.8)*

Maximum of 25 ppm.

Dissolve 2.0 g in a mixture of 1 volume of *water R* and 9 volumes of *alcohol R* and dilute to 25 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (2 ppm Pb) prepared by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 1 volume of *water R* and 9 volumes of *alcohol R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

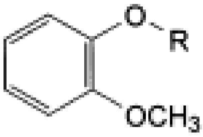
ASSAY

To 0.500 g (*m* g) add 10.0 ml of a freshly prepared mixture of 1 volume of *acetic anhydride R* and 7 volumes of *pyridine R*. Boil under a reflux condenser for 45 min. Cool and add 25 ml of *water R*. Using 0.25 ml of *phenolphthalein solution R* as indicator, titrate with *1 M sodium hydroxide* (n_1 ml). Carry out a blank titration (n_2 ml).

Calculate the percentage content of $C_{10}H_{14}O_4$ from the expression:

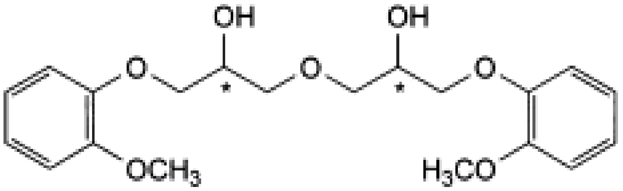
$$\frac{19.82\,(n_2 - n_1)}{2m}$$

IMPURITIES

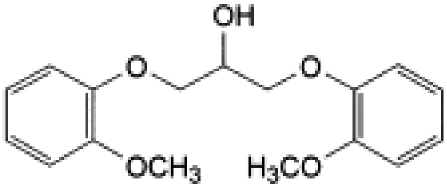


A. R = H: 2-methoxyphenol (guaiacol),

B. R = $CH(CH_2OH)_2$: 2-(2-methoxyphenoxy)propane-1,3-diol (B-isomer),



C. 1,1′-oxybis[3-(2-methoxyphenoxy)propan-2-ol] (bisether),

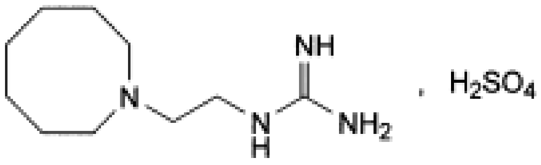


D. 1,3-bis(2-methoxyphenoxy)propan-2-ol.

Guanethidine Monosulphate

General Notices

(Ph Eur monograph 0027)



$C_{10}H_{22}N_4,H_2SO_4$ 296.4 *645-43-2*

Action and use

Adrenergic neuron blocker.

Preparation

Guanethidine Tablets

Ph Eur

DEFINITION

1-[2-(Hexahydroazocin-1(2*H*)-yl)ethyl]guanidine monosulphate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Colourless, crystalline powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

mp: about 250 °C, with decomposition.

IDENTIFICATION

A. Dissolve about 25 mg in 25 ml of *water R*, add 20 ml of *picric acid solution R* and filter. The precipitate, washed with *water R* and dried at 100-105 °C, melts *(2.2.14)* at about 154 °C.

B. Dissolve about 25 mg in 5 ml of *water R*. Add 1 ml of *strong sodium hydroxide solution R*, 1 ml of α-*naphthol solution R* and, dropwise with shaking, 0.5 ml of *strong sodium hypochlorite solution R*. A bright pink precipitate is formed and becomes violet-red on standing.

C. It gives the reactions of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 0.4 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is not more intensely coloured than reference solution GY_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.7 to 5.5 for solution S.

Oxidisable substances

In a conical, ground-glass-stoppered flask, dissolve 1.0 g in 25 ml of *water R* and add 25 ml of *dilute sodium hydroxide solution R*. Allow to stand for 10 min and add 1 g of *potassium bromide R* and 1 ml of *0.0083 M potassium bromate*. Acidify with 30 ml of *dilute hydrochloric acid R*. Mix and allow to stand in the dark for 5 min. Add 2 g of *potassium iodide R* and shake. Allow to stand for 2 min and titrate the liberated iodine with *0.05 M sodium thiosulphate*, using *starch solution R* as indicator. Not less than 0.3 ml of *0.05 M sodium thiosulphate* is required to decolorise the solution.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g, warming if necessary, in 30 ml of *anhydrous acetic acid R* and add 15 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 29.64 mg of $C_{10}H_{24}N_4O_4S$.

STORAGE

Protected from light.

Guar Galactomannan

General Notices

(Ph Eur monograph 0908)

Action and use

Excipient.

Ph Eur

DEFINITION

Guar galactomannan is obtained from the seeds of *Cyamopsis tetragonolobus* (L.) Taub. by grinding of the endosperms and subsequent partial hydrolysis. The main components are polysaccharides composed of D-galactose and D-mannose at molecular ratios of 1:1.4 to 1:2. The molecules consist of a linear main chain of β-(1→4)-glycosidically linked mannopyranoses and single α-(1→6)-glycosidically linked galactopyranoses.

CHARACTERS

Appearance

Yellowish-white powder.

Solubility

Soluble in cold water and in hot water, practically insoluble in organic solvents.

IDENTIFICATION

A. Mix 5 g of solution S (see Tests) with 0.5 ml of a 10 g/l solution of *disodium tetraborate R*. A gel forms within a short time.

B. Heat 20 g of solution S in a water-bath for 10 min. Allow to cool and adjust to the original mass with *water R*. The solution does not gel.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 10 mg of the substance to be examined in a thick-walled centrifuge tube add 2 ml of a 230 g/l solution of *trifluoroacetic acid R*, shake vigorously to dissolve the forming gel, stopper the tube and heat the mixture at 120 °C for 1 h. Centrifuge the hydrolysate, transfer the clear supernatant liquid carefully into a 50 ml flask, add 10 ml of *water R* and evaporate the solution to dryness under reduced pressure. Take up the residue in 10 ml of *water R* and evaporate again to dryness under reduced pressure. To the resulting clear film, which has no odour of acetic acid, add 0.1 ml of *water R* and 1 ml of *methanol R*. Centrifuge to separate the amorphous precipitate. Dilute the supernatant liquid, if necessary, to 1 ml with *methanol R*.

Reference solution Dissolve 10 mg of *galactose R* and 10 mg of *mannose R* in 2 ml of *water R* and dilute to 10 ml with *methanol R*.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, acetonitrile R* (15:85 *V/V*).

Application 5 µl, as bands of 20 mm by 3 mm.

Development Over a path of 15 cm.

Detection Spray with *aminohippuric acid reagent R* and heat at 120 °C for 5 min.

Results The chromatogram obtained with the reference solution shows in the lower part 2 clearly separated brownish zones (galactose and mannose in order of increasing R_F value). The chromatogram obtained with the test solution shows 2 zones due to galactose and mannose.

TESTS

Solution S

Moisten 1.0 g with 2 ml of *2-propanol R*. While stirring, dilute to 100 g with *water R* and stir until the substance is uniformly dispersed. Allow to stand for at least 1 h. If the apparent viscosity is below 200 mPa·s, use 3.0 g of substance instead of 1.0 g.

pH *(2.2.3)*

5.5 to 7.5 for solution S.

Apparent viscosity *(2.2.10)*

75 per cent to 140 per cent of the value stated on the label.

Moisten a quantity of the substance to be examined equivalent to 2.00 g of the dried substance with 2.5 ml of *2-propanol R* and, while stirring, dilute to 100.0 ml with *water R*. After 1 h, determine the viscosity at 20 °C using a rotating viscometer and a shear rate of 100 s^{-1}.

Insoluble matter

Maximum 7.0 per cent.

In a 250 ml flask disperse, while stirring, 1.50 g in a mixture of 1.6 ml of *sulphuric acid R* and 150 ml of *water R* and weigh. Immerse the flask in a water-bath and heat under a reflux condenser for 6 h. Adjust to the original mass with *water R*. Filter the hot solution through a tared, sintered-glass filter (160) *(2.1.2)*. Rinse the filter with hot *water R* and dry at 100-105 °C. The residue weighs a maximum of 105 mg.

Protein

Maximum 5.0 per cent.

Carry out the determination of nitrogen by sulphuric acid digestion *(2.5.9)*, using 0.400 g. Multiply the result by 6.25.

Tragacanth, sterculia gum, agar, alginates and carrageenan

To a small amount of the substance to be examined, add 0.2 ml of freshly prepared *ruthenium red solution R*. Examined under a microscope, none of the structures are red.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Total ash *(2.4.16)*

Maximum 1.8 per cent, determined on 1.00 g after wetting with 10 ml of *water R*.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count. It complies with the tests for *Escherichia coli* and for *Salmonella (2.6.13)*.

LABELLING

The label states the apparent viscosity in millipascal seconds for a 20 g/l solution.

Ph Eur

Guar

General Notices

(Ph Eur monograph 1218)

Ph Eur

DEFINITION

Guar is obtained by grinding the endosperms of seeds of *Cyamopsis tetragonolobus* (L.) Taub. It consists mainly of guar galactomannan.

CHARACTERS

Appearance

White or almost white powder.

Solubility

It yields a mucilage of variable viscosity when dissolved in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Examined under a microscope in *glycerol R*, the substance to be examined (125) *(2.9.12)* shows pyriform or ovoid cells, usually isolated, having very thick walls around a central somewhat elongated lumen with granular contents, and smaller polyhedral cells, isolated or in clusters, with thinner walls.

B. In a conical flask place 2 g, add rapidly 45 ml of *water R* and stir vigorously for 30 s. After 5-10 min a stiff gel forms which does not flow when the flask is inverted.

C. Mix a suspension of 0.1 g in 10 ml of *water R* with 1 ml of a 10 g/l solution of *disodium tetraborate R*; the mixture soon gels.

D. Thin-layer chromatography *(2.2.27)*.

Test solution To 10 mg in a thick-walled centrifuge test tube add 2 ml of a 100 g/l solution of *trifluoroacetic acid R*, shake vigorously to dissolve the forming gel, stopper the test tube and heat the mixture at 120 °C for 1 h. Centrifuge the hydrolysate, transfer the clear supernatant liquid carefully into a 50 ml flask, add 10 ml of *water R* and evaporate the solution to dryness under reduced pressure. To the resulting clear film add 0.1 ml of *water R* and 0.9 ml of *methanol R*. Centrifuge to separate the amorphous precipitate. Dilute the supernatant liquid, if necessary, to 1 ml with *methanol R*.

Reference solution Dissolve 10 mg of *galactose R* and 10 mg of *mannose R* in 2 ml of *water R*, then dilute to 20 ml with *methanol R*.

Plate TLC silica gel plate R.

Mobile phase *water R, acetonitrile R* (15:85 *V/V*).

Application 5 µl, as bands.

Development Over a path of 15 cm.

Detection Spray with *aminohippuric acid reagent R* and dry at 120 °C for 5 min.

Results The chromatogram obtained with the reference solution shows, in the lower part 2 clearly separated brownish zones due to galactose and mannose in order of increasing R_F value. The chromatogram obtained with the test solution shows 2 zones due to galactose and mannose.

TESTS

Tragacanth, sterculia gum, agar, alginates, carrageenan

To a small amount of the substance to be examined add 0.2 ml of freshly prepared *ruthenium red solution R*. Examined under a microscope the cell walls do not stain red.

Protein

Maximum 8.0 per cent.

Carry out the determination of nitrogen by the method of sulphuric acid digestion *(2.5.9)* using 0.170 g. Multiply the result by 6.25.

Apparent viscosity *(2.2.10)*

85 per cent to 115 per cent of the value stated on the label.

Moisten a quantity equivalent to 1.00 g of the dried substance with 2.5 ml of *2-propanol R*. While stirring, dilute to 100.0 ml with *water R*. After 1 h, determine the viscosity at 20 °C using a rotating viscometer and a shear rate of 100 s^{-1}.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Total ash *(2.4.16)*

Maximum 1.8 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 micro-organisms per gram, determined by plate-count. It complies with the tests *Escherichia coli* and for *Salmonella (2.6.13)*.

LABELLING

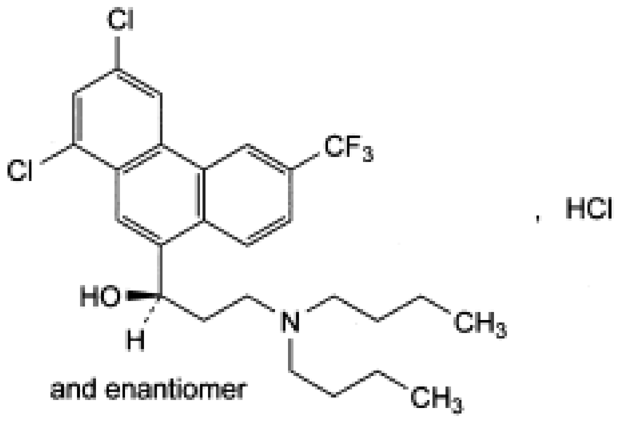
The label states the apparent viscosity in millipascal seconds for a 10 g/l solution.

Ph Eur

Halofantrine Hydrochloride

General Notices

(Ph Eur monograph 1979)



$C_{26}H_{30}Cl_2F_3NO,HCl$ 536.9   *36167-63-2*

Action and use

Antiprotozoal (malaria).

Ph Eur

DEFINITION

(1*RS*)-3-(Dibutylamino)-1-[1,3-dichloro-6-(trifluoromethyl)phenanthren-9-yl]propan-1-ol hydrochloride.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methanol, sparingly soluble in alcohol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *halofantrine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methyl ethyl ketone R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Optical rotation *(2.2.7)*

- 0.10 ° to + 0.10 °.

Dissolve 1.00 g in *alcohol R* and dilute to 100.0 ml with the same solvent.

Absorbance *(2.2.25)*

Maximum 0.085 at 450 nm.

Dissolve 0.200 g in *methanol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 40.0 mg of *halofantrine hydrochloride CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (d) Dissolve 10.0 mg of *halofantrine impurity C CRS* in the mobile phase and dilute to 25 ml with the mobile phase. To 5.0 ml of the solution, add 5.0 ml of reference solution (a) and dilute to 50.0 ml with the mobile phase.

Column:

— *size:* l = 0.30 m, Ø = 3.9 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm) of irregular type, with a specific surface of 330 m^2/g, a pore size of 12.5 nm and a carbon loading of 9.8 per cent.

Mobile phase Mix 250 ml of a 2.0 g/l solution of *sodium hydroxide R*, previously adjusted to pH 2.5 with *perchloric acid R* and 750 ml of *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µl; inject the test solution (a) and reference solutions (c) and (d).

Run time 5 times the retention time of halofantrine which is about 6 min.

System suitability:

- *resolution*: minimum 3.3 between the peaks due to halofantrine and impurity C in the chromatogram obtained with reference solution (d).

Limits:

- *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

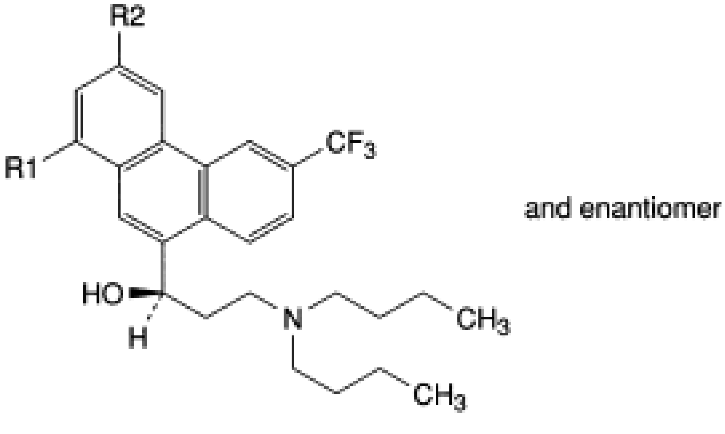
Injection Test solution (b) and reference solution (b).

Calculate the percentage content of halofantrine hydrochloride.

STORAGE

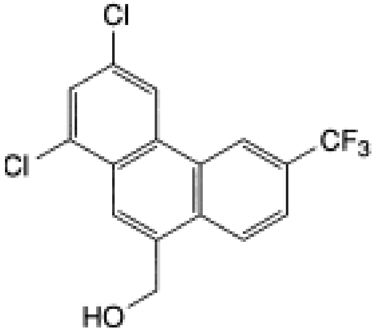
Protected from light.

IMPURITIES



A. R1 = H, R2 = Cl: (1*RS*)-1-[3-chloro-6-(trifluoromethyl)phenanthren-9-yl]-3-(dibutylamino) propan-1-ol (1-dechlorohalofantrine),

B. R1 = Cl, R2 = H: (1*RS*)-1-[1-chloro-6-(trifluoromethyl)phenanthren-9-yl]-3-(dibutylamino) propan-1-ol (3-dechlorohalofantrine),



C. [1,3-dichloro-6-(trifluoromethyl)phenanthren-9-yl]methanol.

Ph Eur

Human Glucagon

General Notices

(Glucagon, human, Ph Eur monograph 1635)

H-His–Ser–Gln–Gly–Thr–Phe–Thr–Ser–Asp–Tyr–
10
Ser–Lys–Tyr–Leu–Asp–Ser–Arg–Arg–Ala–Gln–
20
Asp–Phe–Val–Gln–Trp–Leu–Met–Asn–Thr-OH

$C_{153}H_{225}N_{43}O_{49}S$ 3483 *16941-32-5*

Action and use

Hormone; treatment of hypoglycaemia.

Ph Eur

DEFINITION

Polypeptide having the same structure (29 amino acids) as the hormone produced by the α-cells of the human pancreas, which increases the blood-glucose concentration by promoting rapid breakdown of liver glycogen.

Content

92.5 per cent to 105.0 per cent (anhydrous substance).

PRODUCTION

Human glucagon is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development it must be demonstrated that the manufacturing process produces a product having a biological activity of not less than 1 IU/mg using a suitable validated bioassay.

Host-cell-derived proteins

The limit is approved by the competent authority.

Host-cell- and vector-derived DNA

The limit is approved by the competent authority.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in most organic solvents. It is soluble in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

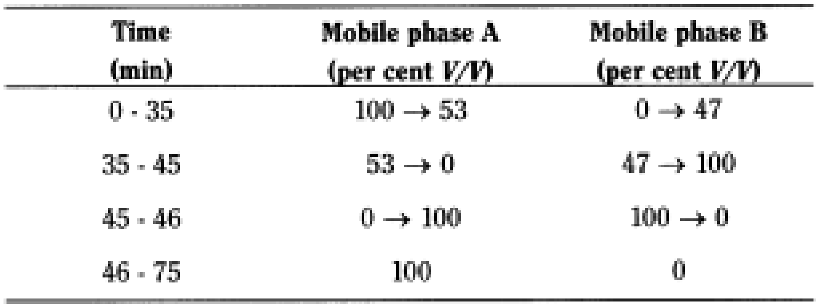
A. Peptide mapping. Liquid chromatography *(2.2.29)*.

Test solution Prepare a 5 mg/ml solution of the substance to be examined in *0.01 M hydrochloric acid*. Mix 200 µl of this solution with 800 µl of *0.1 M ammonium carbonate buffer solution pH 10.3 R* (diluted stock solution). Prepare a 2 mg/ml solution of α-*chymotrypsin for peptide mapping R* in *0.1 M ammonium carbonate buffer solution pH 10.3 R* and add 25 µl of this solution to the diluted stock solution. Place the solution in a closed vial at 37 °C for 2 h. Remove the vial and stop the reaction immediately by adding 120 µl of *glacial acetic acid R*.

Reference solution Prepare a 1 mg/ml solution of *human glucagon CRS* in *0.1 M ammonium carbonate buffer solution pH 10.3 R* (diluted stock solution) and continue as described for the test solution.

Column:
— *size: l* = 0.05 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: mix 500 µl of *trifluoroacetic acid R* and 1000 ml of *water R*;
— *mobile phase B*: mix 500 µl of *trifluoroacetic acid R* with 600 ml of *anhydrous ethanol R* and add 400 ml of *water R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100 → 53	0 → 47
35 - 45	53 → 0	47 → 100
45 - 46	0 → 100	100 → 0
46 - 75	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Equilibration With mobile phase A for at least 15 min.

Injection 20 µl.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Deamidated glucagon

Liquid chromatography *(2.2.29)*: use the normalisation procedure.

Test solution Dissolve the substance to be examined in *0.01 M hydrochloric acid* to obtain a concentration of 1.0 mg/ml.

Resolution solution Dissolve the substance to be examined in *0.1 M hydrochloric acid* to obtain a concentration of 1.0 mg/ml. Incubate in an oven at 60 °C for 2 h. Immediately after degradation, adjust to pH 2.5 with *1 M sodium hydroxide*.

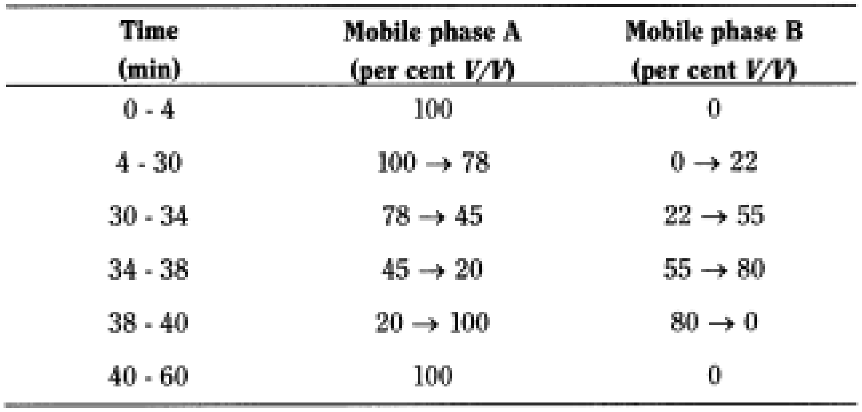
Column:
— *material*: glass;

— *size: l* = 0.05 m, Ø = 5 mm;

— *stationary phase*: *anion exchange resin R2*.

Mobile phase:
— *mobile phase A*: mix 1000 ml of *tris-hydrochloride buffer solution pH 8.3 R* and 1000 ml of *anhydrous ethanol R*;

— *mobile phase B*: dissolve 29.2 g of *sodium chloride R* in 1000 ml of *tris-hydrochloride buffer solution pH 8.3 R*; add 1000 ml of *anhydrous ethanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 30	100 → 78	0 → 22
30 - 34	78 → 45	22 → 55
34 - 38	45 → 20	55 → 80
38 - 40	20 → 100	80 → 0
40 - 60	100	0

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 230 nm.

Equilibration With mobile phase A for at least 15 min.

Injection 60 µl.

System suitability Resolution solution:

— *retention time*: glucagon = about 10 min; 4 deamidated forms: between 15 min and 40 min;

— *resolution*: baseline separation of the 4 deamidated forms and glucagon.

Limit:

— *sum of the 4 deamidated forms*: maximum 0.5 per cent, calculated from the peaks eluting between 15 min and 40 min.

Related proteins

Liquid chromatography *(2.2.29)*: use the normalisation procedure.

2.8 M urea solution Dissolve 16.8 g of *urea R* in 100 ml of *water R*.

Test solution Dissolve the substance to be examined in *0.01 M hydrochloric acid* to obtain a concentration of 0.5 mg/ml. *Maintain the solution at 2-8 °C.*

Reference solution Dissolve the contents of a vial of *human glucagon CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 0.5 mg/ml. *Maintain the solution at 2-8 °C.*

Resolution solution Dissolve 10 mg of the substance to be examined in 20 ml of the 2.8 M urea solution. Heat at 50 °C for 2 h. Cool and adjust to pH 2.2 with *1 M hydrochloric acid*. *Maintain the solution at 2-8 °C and use within 2 h, or maintain the solution below - 15 °C and then thaw and filter through a 0.22 µm filter before use.*

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

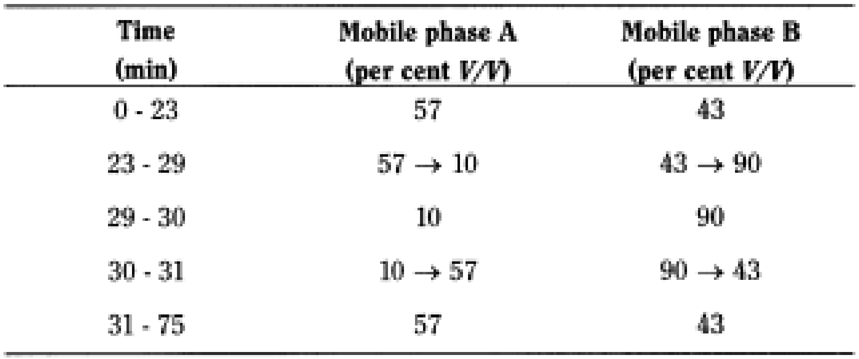
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm;

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A*: dissolve 13.6 g of *potassium dihydrogen phosphate R* in 400 ml of *water R*, adjust to pH 2.5 *(2.2.3)* with *phosphoric acid R*, and add 100 ml of *acetonitrile for chromatography R*;

— *mobile phase B*: acetonitrile *for chromatography R*, water R (40:60 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 23	57	43
23 - 29	57 → 10	43 → 90
29 - 30	10	90
30 - 31	10 → 57	90 → 43
31 - 75	57	43

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 50 µl of the test solution and the resolution solution.

Relative retention With reference to glucagon (retention time = about 20 min): carbamoylglucagon = about 1.1.

System suitability Resolution solution:

— *resolution*: minimum 2.0 between the peaks due to glucagon and carbamoylglucagon.

Limit:

- *total*: maximum 2.5 per cent.

Water *(2.5.12)*

Maximum 10 per cent, determined on 50 mg.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/mg.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related proteins with the following modification.

Injection Test solution and reference solution.

Calculate the content of human glucagon ($C_{153}H_{225}N_{43}O_{49}S$) from the declared content of $C_{153}H_{225}N_{43}O_{49}S$ in *human glucagon CRS* .

STORAGE

In an airtight container , protected from light, at a temperature lower than - 15 °C.

Ph Eur

Liquid Glucose

General Notices

(Ph Eur monograph 1330)

Action and use

Excipient.

Ph Eur

DEFINITION

Aqueous solution containing a mixture of glucose, oligosaccharides and polysaccharides obtained by hydrolysis of starch.

It contains a minimum of 70.0 per cent dry matter.

The degree of hydrolysis, expressed as dextrose equivalent (DE), is not less than 20 (nominal value).

CHARACTERS

Appearance

Clear, colourless or brown, viscous liquid.

Solubility

Miscible with water.

It may partly or totally solidify at room temperature and liquefies again when heated to 50 °C.

IDENTIFICATION

A. Dissolve 0.1 g in 2.5 ml of *water R* and heat with 2.5 ml of *cupri-tartaric solution R*. A red precipitate is formed.

B. Dip, for 1 s, a suitable stick with a reactive pad containing glucose-oxidase, peroxidase and a hydrogen-donating substance, such as tetramethylbenzidine, in a 5 g/l solution of the substance to be examined. Observe the colour of the reactive pad; within 60 s the colour changes from yellow to green or blue.

C. It is a clear, colourless or brown, viscous liquid, miscible with water. The substance may partly or totally solidify at room temperature and liquefies again when heated to 50 °C.

D. Dextrose equivalent (see Tests).

TESTS

Solution S

Dissolve 25.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

pH *(2.2.3)*

4.0 to 6.0.

Mix 1 ml of a 223.6 g/l solution of *potassium chloride R* and 30 ml of solution S.

Sulphur dioxide *(2.5.29)*

Maximum 20 ppm; maximum 400 ppm if intended for the production of lozenges or pastilles obtained by high boiling techniques, provided that the final product contains maximum 50 ppm of sulphur dioxide.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dilute 2 ml of solution S to 30 ml with *water R*. The solution complies with test E. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 30.0 per cent, determined on 1.000 g. Triturate the sample with 3.000 g of *kieselguhr G R*, previously dried at 80 °C under high vacuum for 2 h, and dry at 80 °C under high vacuum for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 1.0 g.

Dextrose equivalent (DE)

Within 10 per cent of the nominal value.

Weigh an amount of the substance to be examined equivalent to 2.85-3.15 g of reducing carbohydrates, calculated as dextrose equivalent, into a 500 ml volumetric flask. Dissolve in *water R* and dilute to 500.0 ml with the same solvent. Transfer the solution to a 50 ml burette.

Pipette 25.0 ml of *cupri-tartaric solution R* into a 250 ml flask and add 18.5 ml of the test solution from the burette, mix and add a few glass beads. Place the flask on a hot plate, previously adjusted so that the solution begins to boil after 2 min ± 15 s. Allow to boil for exactly 120 s, add 1 ml of a 1 g/l solution of *methylene blue R* and titrate with the test solution (V_1) until the blue colour disappears. Maintain the solution at boiling throughout the titration.

Standardise the cupri-tartaric solution using a 6.00 g/l solution of *glucose R* (V_0).

Calculate the dextrose equivalent using the following expression:

$$\frac{300 \times V_0 \times 100}{V_1 \times M \times D}$$

V_0 = total volume of glucose standard solution, in millilitres,

V_1 = total volume of test solution, in millilitres,

M = mass of the sample, in grams,

D = percentage content of dry matter in the substance.

LABELLING

The label states the dextrose equivalent (DE) (= nominal value).

Ph Eur

Self-emulsifying Glyceryl Monostearate

General Notices

Self-emulsifying Monostearin; Self-emulsifying Mono-and Diglycerides of Food Fatty Acids

Action and use

Excipient.

DEFINITION

Self-emulsifying Glyceryl Monostearate is a mixture consisting principally of mono-, di- and triglycerides of stearic and palmitic acids and of minor proportions of glycerides of other fatty acids; it may also contain free glycerol, free fatty acids and soap. It contains not less than 30.0% of monoglycerides, calculated as $C_{21}H_{42}O_4$, not more than 7.0% of free glycerol, calculated as $C_3H_8O_3$, and not more than 6.0% of soap, calculated as sodium oleate, $C_{18}H_{33}NaO_2$, all calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white to cream coloured, hard, waxy solid.

Dispersible in hot *water*; soluble in hot *absolute ethanol*, in hot *liquid paraffin* and, subject to turbidity at concentrations below 20%, in hot vegetable oils.

TESTS

Acid value

Not more than 6, Appendix X B.

Iodine value

Not more than 3 *(iodine monochloride method)*, Appendix X E.

Alkalinity

Shake 1 g with 20 ml of hot *carbon dioxide-free water* and allow to cool with continuous shaking. The pH of the aqueous layer is 8.0 to 10.0, Appendix V L.

Heavy metals

2.0 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Water

Not more than 2.0% w/w, Appendix IX C. Use 0.5 g and a mixture of 10 ml of *anhydrous methanol* and 10 ml of anhydrous *chloroform* as the solvent.

ASSAY

For free glycerol

Dissolve 0.4 g in 50 ml of *dichloromethane* in a ground-glass-stoppered separating funnel, cool if necessary, add 25 ml of *water* and shake vigorously for 1 minute; add 0.2 ml of *glacial acetic acid*, if necessary, to break the emulsion. Repeat the extraction a further three times using 25-, 20- and 20- ml quantities of *water* and reserve the dichloromethane solution for the Assay for monoglycerides. Filter the combined aqueous extracts through a filter paper moistened with *water*, wash the filter with two 5 ml quantities of *water* and dilute the combined filtrate and washings to 100 ml with *water*. To 50 ml of this solution add 25 ml of *periodic acetic acid solution*, shaking cautiously, allow to stand at 25° to 30° for 30 minutes and add 100 ml of *water* and 12 ml of *potassium iodide solution*. Titrate with 0.1M *sodium thiosulphate VS* using 1 ml of *starch solution* as indicator. Repeat the determination using 50 ml of *water* in place of the 50 ml of the solution being examined. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 2.3 mg of glycerol.

For monoglycerides

Filter the reserved dichloromethane solution obtained in the Assay for free glycerol through absorbent cotton and wash the separating funnel and the filter with three 5 ml quantities of *dichloromethane*. Dilute the combined filtrate and washings to 100 ml with *dichloromethane* and to 50 ml of the solution add 25 ml of *periodic acetic acid solution*, shaking cautiously. Allow to stand at 25° to 30° for 30 minutes and add 100 ml of *water* and 12 ml of *potassium iodide solution*. Titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using 1 ml of *starch solution* as indicator. Repeat the determination using 50 ml of *dichloromethane* in place of the 50 ml of the solution of the substance being examined. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 17.9 mg of 1-monoacylglycerols, calculated as $C_{21}H_{42}O_4$. The quantity of 0.1M *sodium thiosulphate VS* used in the assay is not less than 85% of the quantity of sodium thiosulphate used in the blank assay.

For soap

Add 10 g to a mixture of 60 ml of *acetone* and 0.15 ml of a 0.5% w/v solution of *bromophenol blue* in a mixture of 20 ml of *ethanol (20%)* and 80 ml of *water*, the solvent having been previously neutralised with 0.1M *hydrochloric acid VS* or 0.1M *sodium hydroxide VS*. Warm gently on a water bath until solution is complete and titrate with 0.1M *hydrochloric acid VS* until the blue colour is discharged. Allow to stand for 20 minutes, warm until any solidified matter has redissolved and, if the blue colour reappears, continue the titration. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 30.45 mg of $C_{18}H_{33}NaO_2$.

Spray-dried Liquid Glucose

General Notices

(Glucose, Liquid, Spray-dried, Ph Eur monograph 1525)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of glucose, oligosaccharides and polysaccharides, obtained by the partial hydrolysis of starch.

The degree of hydrolysis, expressed as dextrose equivalent (DE), is not less than 20 (nominal value).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic powder or granules.

Solubility

Freely soluble in water.

IDENTIFICATION

A. Dissolve 0.1 g in 2.5 ml of *water R* and heat with 2.5 ml of *cupri-tartaric solution R*. A red precipitate is formed.

B. Dip, for 1 s, a suitable stick with a reactive pad containing glucose-oxidase, peroxidase and a hydrogen-donating substance, such as tetramethylbenzidine, in a 5 g/l solution of the substance to be examined. Observe the colour of the reactive pad; within 60 s the colour changes from yellow to green or blue.

C. It is a powder or granules.

D. Dextrose equivalent (see Tests).

TESTS

Solution S

Dissolve 12.5 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

pH *(2.2.3)*

4.0 to 7.0.

Mix 1 ml of a 223.6 g/l solution of *potassium chloride R* and 30 ml of solution S.

Sulphur dioxide ***(2.5.29)***

Maximum 20 ppm.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dilute 4 ml of solution S to 30 ml with *water R*. The solution complies with test E. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 6.0 per cent, determined on 10.00 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.5 per cent, determined on 1.0 g.

Dextrose equivalent (DE)

Within 10 per cent of the nominal value.

Weigh an amount of the substance to be examined equivalent to 2.85-3.15 g of reducing carbohydrates, calculated as dextrose equivalent, into a 500 ml volumetric flask. Dissolve in *water R* and dilute to 500.0 ml with the same solvent. Transfer the solution to a 50 ml burette.

Pipette 25.0 ml of *cupri-tartaric solution R* into a 250 ml flask and add 18.5 ml of the test solution from the burette, mix and add a few glass beads. Place the flask on a hot plate, previously adjusted so that the solution begins to boil after 2 min ± 15 s. Allow to boil for exactly 120 s, add 1 ml of a 1 g/l solution of *methylene blue R* and titrate with the test solution (V_1) until the blue colour disappears. Maintain the solution at boiling throughout the titration.

Standardise the cupri-tartaric solution using a 6.00 g/l solution of *glucose R* (V_0).

Calculate the dextrose equivalent using the following expression:

$$\frac{300 \times V_0 \times 100}{V_1 \times M \times D}$$

V_0 = total volume of glucose standard solution, in millilitres,

V_1 = total volume of test solution, in millilitres,

M = mass of the sample, in grams,

D = percentage content of dry matter in the substance.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and not more than 10^2 fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella*

(2.6.13).

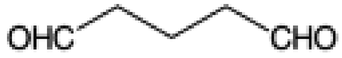
LABELLING

The label states the dextrose equivalent (DE) (= nominal value).

Ph Eur

Strong Glutaraldehyde Solution

General Notices



$C_5H_8O_2$ 100.1 *111-30-8*
(anhydrous)

Action and use

Used in treatment of warts.

Preparation

Glutaraldehyde Solution

DEFINITION

Strong Glutaraldehyde Solution is an aqueous solution of glutaraldehyde (pentanedial). It contains not less than 47.0% and not more than 53.0% w/w of glutaraldehyde, $C_5H_8O_2$.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Heat 1 ml with 10 ml of a solution containing 1 g of *hydroxylamine hydrochloride* and 2 g of *sodium acetate* in *water* on a water bath for 10 minutes, allow to cool and filter. The *melting point* of the residue, after washing with *water* and drying at 105°, is about 178°, Appendix V A.

B. Add 0.05 ml to 2 ml of *ammoniacal silver nitrate solution* and mix gently for a few minutes. Silver is deposited.

TESTS

Acidity

Dilute 10 ml with 10 ml of *carbon dioxide-free water* and titrate with 0.1M *sodium hydroxide VS* using *bromothymol blue solution R3* as indicator. Not more than 5.0 ml of 0.1M *sodium hydroxide VS* is required to change the colour of the solution.

Clarity and colour of solution

Dilute 1 volume with 4 volumes of *water*. The resulting solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* B_6, Appendix IV B, Method I.

Weight per ml

1.126 to 1.134 g, Appendix V G.

ASSAY

Dissolve 4 g in 100 ml of a 7% w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with 1M *sodium hydroxide VS* and allow to stand for 30 minutes. Add 20 ml of *petroleum spirit (boiling range, 40° to 60°)* and titrate with 1M *sodium hydroxide VS* until the colour of the aqueous phase matches that of a 7% w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with 1M *sodium hydroxide VS*. Each ml of 1M *sodium hydroxide VS* is equivalent to 50.05 mg of $C_5H_8O_2$.

STORAGE

Strong Glutaraldehyde Solution should be stored at a temperature not exceeding 15°.

Dilute Hydrochloric Acid

General Notices

(Ph Eur monograph 0003)

Ph Eur

DEFINITION

Content

9.5 per cent *m/m* to 10.5 per cent *m/m* of HCl (M_r 36.46).

PREPARATION

To 726 g of *water R* add 274 g of concentrated hydrochloric acid and mix.

IDENTIFICATION

A. It is strongly acid (*2.2.4*).

B. It gives the reactions of chlorides (*2.3.1*).

C. It complies with the limits of the assay.

TESTS

Appearance

It is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Free chlorine

Maximum 1 ppm.

To 60 ml add 50 ml of *carbon dioxide-free water R*, 1 ml of a 100 g/l solution of *potassium iodide R* and 0.5 ml of *iodide-free starch solution R*. Allow to stand in the dark for 2 min. Any blue colour disappears on the addition of 0.2 ml of *0.01 M sodium thiosulphate*.

Sulphates (*2.4.13*)

Maximum 5 ppm.

To 26 ml add 10 mg of *sodium hydrogen carbonate R* and evaporate to dryness on a water-bath. Dissolve the residue in 15 ml of *distilled water R.*

Heavy metals (*2.4.8*)

Maximum 2 ppm.

Dissolve the residue obtained in the test for residue on evaporation in 1 ml of *dilute hydrochloric acid R* and dilute to 25 ml with *water R*. Dilute 5 ml of this solution to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Residue on evaporation

Maximum 0.01 per cent.

Evaporate 100.0 g to dryness on a water-bath and dry at 100-105 °C. The residue weighs a maximum of 10 mg.

ASSAY

To 6.00 g add 30 ml of *water R*. Titrate with *1 M sodium hydroxide*, using *methyl red solution R* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 36.46 mg of HCl.

Ph Eur

Halibut-liver Oil

General Notices

Action and use

Source of vitamin A.

Preparation

Halibut-liver Oil Capsules

Halibut-liver Oil may contain up to about 3000 IU of vitamin D activity per g. When a statement is made of the vitamin D content, this is determined by an appropriate method. The method described under Cod-liver Oil (Type A) may be suitable.

DEFINITION

Halibut-liver Oil is the fixed oil extracted from the fresh, or suitably preserved, liver of the halibut species belonging to the genus *Hippoglossus* and refined. It contains in 1 g not less than 30,000 IU of vitamin A.

CHARACTERISTICS

A pale to golden yellow liquid; odour and taste, fishy, but not rancid.

Practically insoluble in *ethanol (96%)*; miscible with *ether* and with *petroleum spirit (boiling range, 40° to 60°)*.

TESTS

Acid value

Not more than 2.0, Appendix X B.

Iodine value

Not less than 122 *(iodine monochloride method)*, Appendix X E.

Iodine value of glycerides

112 to 150, when determined by the following method. Isolate the unsaponifiable matter as described in Appendix X H, but using 1 g of the oil and evaporating the acetone, drying the residue at 80° in a current of nitrogen and omitting the final titration. Weigh the residue and immediately determine the *iodine value*, Appendix X E, Method B.

Calculate the iodine value of the glycerides from the expression $(100x \uparrow Sy)/(100 \uparrow S)$, where x is the iodine value of the oil, y is the iodine value of the unsaponifiable matter and S is the percentage of unsaponifiable matter in the oil.

Saponification value

Not more than 180, Appendix X G.

Unsaponifiable matter

Not less than 7.0%, Appendix X H.

Weight per ml

0.915 to 0.925 g, Appendix V G.

ASSAY

Carry out the procedure as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper and iron) and acids.

Carry out Method A (ultraviolet absorption spectrophotometry) but if method A is found not to be valid, carry out method B (liquid chromatography).

Method A

To 1 g of the oil being examined in a round-bottomed flask, add 3 ml of a freshly prepared 50% w/w solution of *potassium hydroxide* and 30 ml of *ethanol*, boil under a reflux condenser in a current of *nitrogen* for 30 minutes and cool rapidly. Add 30 ml of *water* and extract with four 50 ml quantities of *ether* and discard the aqueous layer after complete separation of the final extract. Wash the combined ethereal layers with four 50 ml quantities of *water* and evaporate to dryness under a gentle current of *nitrogen* at a temperature not exceeding 30° or in a rotary evaporator at a temperature not exceeding 30° under reduced pressure. Dissolve the residue in sufficient *propan-2-ol R1* to give an expected concentration of vitamin A equivalent to 10 to 15 IU per ml. Measure the *absorbances* of the solution at 300 nm, 310 nm, 325 nm and 334 nm and at the wavelength of maximum absorption, Appendix II B, in a suitable spectrophotometer in 1-cm specially matched cells, using *propan-2-ol R1* in the reference cell.

Calculate the content of vitamin A, as all-*trans*-retinol, in IU per gram from the expression:

$$A_{325} \times \frac{1830}{100m} \times V$$

where A_{325} = absorbance at 325 nm,

m = weight of the oil being examined in grams,

V = total volume of solution containing 10 to 15 IU of vitamin A per ml,

1830 = conversion factor for the specific absorbance of all-*trans*-retinol in International Units.

The above expression can be used only if A_{325} has a value of not greater than $A_{325.corr}$/0.970 where $A_{325,corr}$ is the corrected absorbance at 325 nm and is given by the equation:

$$A_{325,corr} = 6.815A_{325}-2.555A_{310}-4.260A_{324}$$

A designates the absorbance at the wavelength indicated by the subscript.

If A_{325} has a value greater than $A_{325,corr}/0.970$, calculate the content of vitamin A from the expression:

$$A_{325,\mathrm{corr}} \times \frac{1830}{100m} \times V$$

The assay is not valid unless the wavelength of maximum absorption lies between 323 nm and 327 nm and the *absorbance* at 300 nm relative to that at 325 nm is at most 0.73.

Method B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare solution (1) in the following manner. To 2 g of the oil being examined in a round-bottomed flask add 5 ml of a freshly prepared 10% w/v solution of *ascorbic acid* and 10 ml of a freshly prepared 80% w/v solution of *potassium hydroxide* and 100 ml of *ethanol*, boil under a reflux condenser on a water-bath for 15 minutes. Add 100 ml of a 1% w/v solution of *sodium chloride* and cool. Transfer the solution to a 500 ml separating funnel rinsing the round-bottomed flask with about 75 ml of a 1% w/v solution of *sodium chloride* and then with 150 ml of a mixture of equal volumes of *light petroleum R3* and *ether*. Shake for 1 minute and when the layers have separated completely, discard the lower layer and wash the upper layer with 50 ml of a 3% w/v solution of *potassium hydroxide* in a 10% v/v solution of *ethanol* and then with three 50 ml quantities of a 1% w/v solution of *sodium chloride*. Filter the upper layer through 5 g of *anhydrous sodium sulphate* on a fast filter paper into a 250 ml flask suitable for a rotary evaporator. Wash the funnel with 10 ml of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30° under reduced pressure (water ejector) and fill with *nitrogen* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen* at a temperature not exceeding 30°. Dissolve the residue in *propan-2-ol*, transfer to a 25 ml graduated flask and dilute to 25 ml with *propan-2-ol*. Gentle heating with the aid of ultrasound may be required. (A large fraction of the white residue is cholesterol.) Solution (2) is a solution of *retinyl acetate EPCRS* in *propan-2-ol R1* containing about 1000 IU per ml of all-*trans*-retinol. The exact concentration of solution (2) is assessed by ultraviolet absorption spectrophotometry, Appendix II B. Dilute the solution with *propan-2-ol R1* to a presumed concentration of 10 to 15 IU per ml and measure the *absorbance* at 326 nm in matched 1-cm cells using *propan-2-ol R1* in the reference cell. Calculate the content of vitamin A in International Units per millilitre of solution (2) from the following expression, taking into account the assigned content of *retinyl acetate EPCRS*:

$$A_{326} \times \frac{1900 \times V_2}{100 \times V_1}$$

where A_{326} = absorbance at 326 nm,

V_2 = volume of the diluted solution,

V_1 = volume of reference solution (a) used,

1900 = conversion factor for the specific absorbance of *retinyl acetate EPCRS* in International Units.

Prepare solution (3) in the same manner as solution (1) but using 2 ml of solution (2) in place of the oil being examined. The exact concentration of solution (3) is assessed by ultraviolet absorption spectrophotometry, Appendix II B. Dilute solution (3) with *propan-2-ol R1* to a presumed concentration of 10 to 15 IU per ml of all-*trans*-retinol and measure the *absorbance* at 325 nm in matched 1-cm cells using *propan-2-ol R1* in the reference cell. Calculate the content of all-*trans*-retinol in International Units per millilitre of solution (3) from the expression:

$$A_{325} \times \frac{1830 \times V_4}{100 \times V_3}$$

where A_{325} = absorbance at 325 nm,

V_3 = volume of the diluted solution,

V_4 = volume of reference solution (b) used,

1830 = conversion factor for the specific absorbance of all-*trans*-retinol in International Units.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm), (b) as mobile phase at a flow rate of 1 ml per minute a mixture of 3 volumes of *water* and 97 volumes of *methanol* and (c) as detector a spectrophotometer set at 325 nm.

Inject in triplicate 10 µl of solutions (1) and (3). The retention time of all-*trans*-retinol is 4 to 6 minutes.

The assay is not valid unless (a) the chromatogram obtained with solution (1) shows a peak corresponding to that of all-*trans*-retinol in the chromatogram obtained with solution (3), (b) when using the method of standard additions to solution (1) there is greater than 95% recovery of the added *retinyl acetate EPCRS* and (c) the recovery of all-*trans*-retinol in solution (3) as assessed by direct absorption spectrophotometry is greater than 95%.

Calculate the content of vitamin A using the following expression:

$$A_1 \times \frac{C \times V}{A_2} \times \frac{1}{m}$$

where A_1 = area of the peak corresponding to all-*trans*-retinol in the chromatogram obtained with solution (1),

A_2 = area of the peak corresponding to all-*trans*-retinol in the chromatogram obtained with solution (3),

C = concentration of *retinyl acetate EPCRS* in solution (2) as assessed before the saponification in IU per ml (1000 IU per ml),

V = volume of solution (2) treated (2 ml),

m = weight of the oil being examined in solution (1) (2 g).

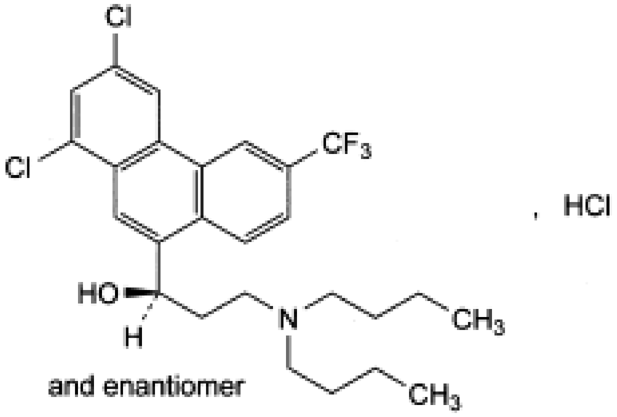
STORAGE

Halibut-liver Oil should be kept in a well-filled container and protected from light.

Halofantrine Hydrochloride

General Notices

(Ph Eur monograph 1979)



$C_{26}H_{30}Cl_2F_3NO,HCl$ 536.9 *36167-63-2*

Action and use

Antiprotozoal (malaria).

Ph Eur

DEFINITION

(1*RS*)-3-(Dibutylamino)-1-[1,3-dichloro-6-(trifluoromethyl)phenanthren-9-yl]propan-1-ol hydrochloride.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methanol, sparingly soluble in alcohol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *halofantrine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methyl ethyl ketone R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 1.00 g in *alcohol R* and dilute to 100.0 ml with the same solvent.

Absorbance *(2.2.25)*

Maximum 0.085 at 450 nm.

Dissolve 0.200 g in *methanol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 40.0 mg of *halofantrine hydrochloride CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (d) Dissolve 10.0 mg of *halofantrine impurity C CRS* in the mobile phase and dilute to 25 ml with the mobile phase. To 5.0 ml of the solution, add 5.0 ml of reference solution (a) and dilute to 50.0 ml with the mobile phase.

Column:

— *size:* l = 0.30 m, Ø = 3.9 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm) of irregular type, with a specific surface of 330 m^2/g, a pore size of 12.5 nm and a carbon loading of 9.8 per cent.

Mobile phase Mix 250 ml of a 2.0 g/l solution of *sodium hydroxide R*, previously adjusted to pH 2.5 with *perchloric acid R* and 750 ml of *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µl; inject the test solution (a) and reference solutions (c) and (d).

Run time 5 times the retention time of halofantrine which is about 6 min.

System suitability:

— *resolution*: minimum 3.3 between the peaks due to halofantrine and impurity C in the chromatogram obtained with reference solution (d).

Limits:

— *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

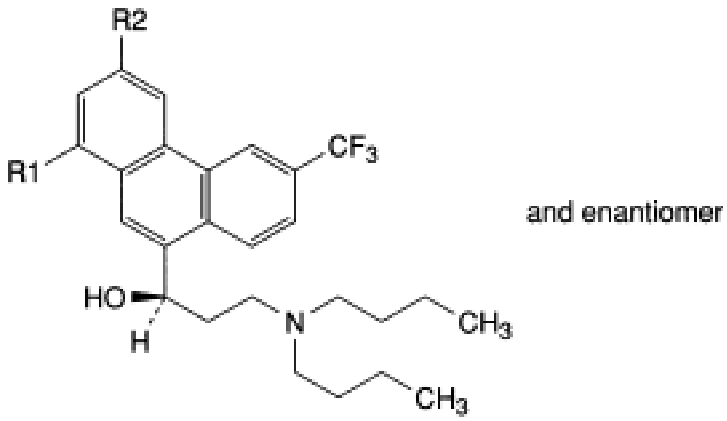
Injection Test solution (b) and reference solution (b).

Calculate the percentage content of halofantrine hydrochloride.

STORAGE

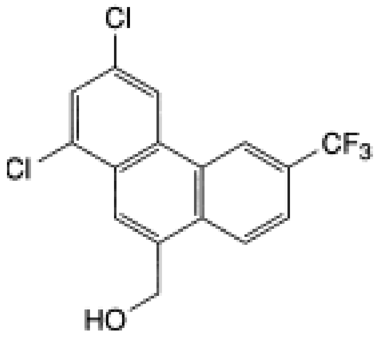
Protected from light.

IMPURITIES



A. R1 = H, R2 = Cl: (1*RS*)-1-[3-chloro-6-(trifluoromethyl)phenanthren-9-yl]-3-(dibutylamino) propan-1-ol (1-dechlorohalofantrine),

B. R1 = Cl, R2 = H: (1*RS*)-1-[1-chloro-6-(trifluoromethyl)phenanthren-9-yl]-3-(dibutylamino) propan-1-ol (3-dechlorohalofantrine),



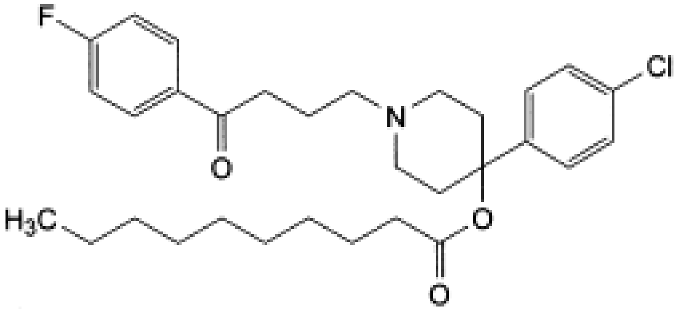
C. [1,3-dichloro-6-(trifluoromethyl)phenanthren-9-yl]methanol.

Ph Eur

Haloperidol Decanoate

General Notices

(Ph Eur monograph 1431)



$C_{31}H_{41}ClFNO_3$ 530.1 *74050-97-8*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

4-(4-Chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent), in methanol and in methylene chloride.

mp: about 42 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Mulls in *liquid paraffin R*.

Comparison *haloperidol decanoate CRS.*

B. To 0.1 g in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_5 *(2.2.2, Method II)*.

Dissolve 2.0 g in *methylene chloride R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect from light.*

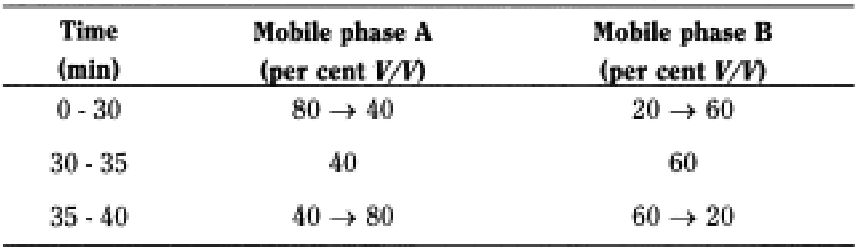
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *bromperidol decanoate CRS* and 2.5 mg of *haloperidol decanoate CRS* in *methanol R*, then dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Column:
- *size: l* = 0.1 m, Ø = 4.0 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: 27 g/l solution of *tetrabutylammonium hydrogen sulphate R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	80 → 40	20 → 60
30 - 35	40	60
35 - 40	40 → 80	60 → 20

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Haloperidol decanoate = about 24 min; bromperidol decanoate = about 24.5 min.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to haloperidol decanoate and bromperidol decanoate; if necessary, adjust the gradient or the time programme for the linear gradient elution.

Limits:

— *impurities A, B, C, D, E, F, G, H, I, J, K*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 30 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.425 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid* using 0.2 ml of *naphtholbenzein solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 53.01 mg of $C_{31}H_{41}ClFNO_3$.

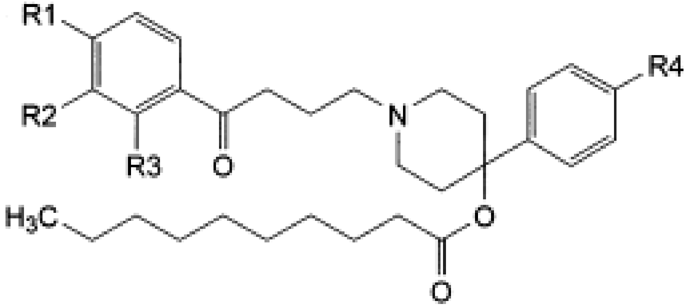
STORAGE

Protected from light, at a temperature below 25 °C.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J, K.

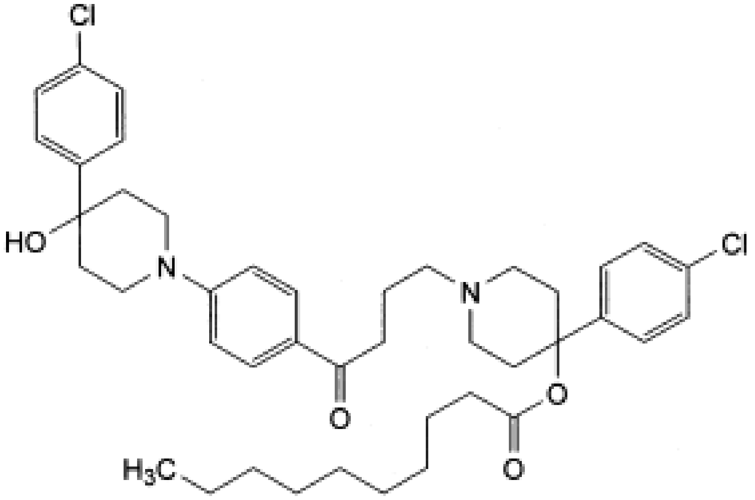
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use* (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): L.*



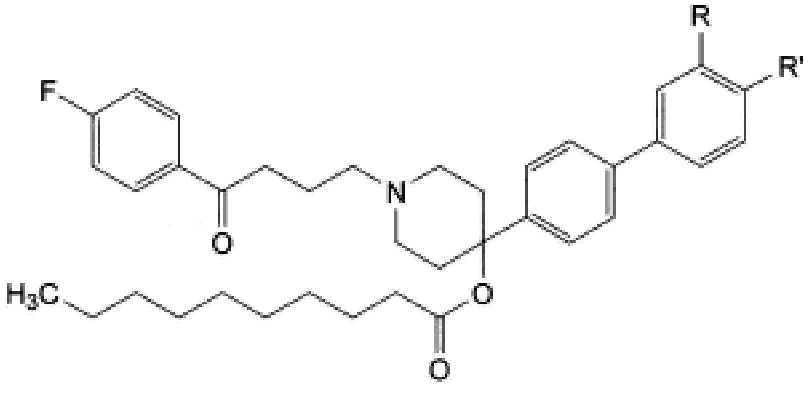
A. R1 = F, R2 = R3 = R4 = H: 1-[4-(4-fluorophenyl)-4-oxobutyl]-4-phenylpiperidin-4-yl decanoate,

B. R1 = R2 = H, R3 = F, R4 = Cl: 4-(4-chlorophenyl)-1-[4-(2-fluorophenyl)-4-oxobutyl] piperidin-4-yl decanoate,

C. R1 = F, R2 = C_2H_5, R3 = H, R4 = Cl: 4-(4-chlorophenyl)-1-[4-(3-ethyl-4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate,



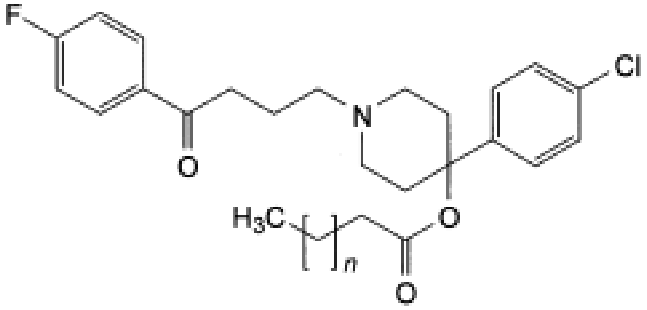
D. 4-(4-chlorophenyl)-1-[4-[4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]phenyl]-4-oxobutyl] piperidin-4-yl decanoate,



E. R = H, R′ = Cl: 4-(4′-chlorobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate,

F. R = Cl, R′ = H: 4-(3′-chlorobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate,

G. haloperidol,

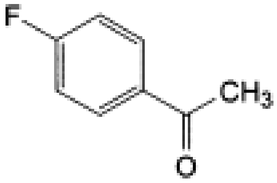


H. *n* = 5: 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl octanoate,

I. *n* = 6: 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl nonanoate,

J. *n* = 8: 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl undecanoate,

K. n = 9: 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl dodecanoate,



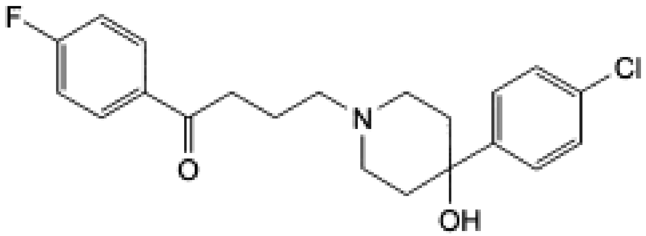
L. 1-(4-fluorophenyl)ethanone.

Ph Eur

Haloperidol

General Notices

(Ph Eur monograph 0616)



$C_{21}H_{23}ClFNO_2$ 375.9 *52-86-8*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparations

Haloperidol Capsules

Haloperidol Injection

Haloperidol Oral Solution

Strong Haloperidol Oral Solution

Haloperidol Tablets

Ph Eur

DEFINITION

4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent), in methanol and in methylene chloride.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Melting point *(2.2.14)*: 150 °C to 153 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *haloperidol CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *haloperidol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *haloperidol CRS* and 10 mg of *bromperidol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *tetrahydrofuran R*, *methanol R*, 58 g/l solution of *sodium chloride R* (10:45:45 *V/V/V*).

Application 1 µl.

Development In an unsaturated tank, over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 5 ml of *anhydrous ethanol R*. Add 0.5 ml of *dinitrobenzene solution R* and 0.5 ml of *2 M alcoholic potassium hydroxide R*. A violet colour is produced and becomes brownish-red after 20 min.

E. To 0.1 g in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Dissolve 0.2 g in 20 ml of a 1 per cent *V/V* solution of *lactic acid R*.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *haloperidol CRS* and 2.5 mg of *bromperidol CRS* in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Column:

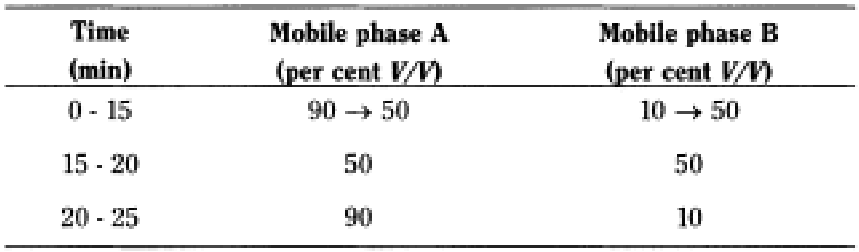
— *size*: *l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 17 g/l solution of *tetrabutylammonium hydrogen sulphate R1*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 50	10 → 50
15 - 20	50	50
20 - 25	90	10

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Haloperidol = about 5.5 min; bromperidol = about 6 min.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to haloperidol and bromperidol; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear-gradient elution.

Limits:

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g using a platinum crucible.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution R* as indicator.

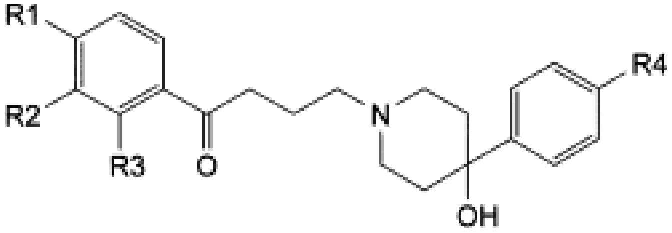
1 ml of *0.1 M perchloric acid* is equivalent to 37.59 mg of $C_{21}H_{23}ClFNO_2$.

STORAGE

Protected from light.

IMPURITIES

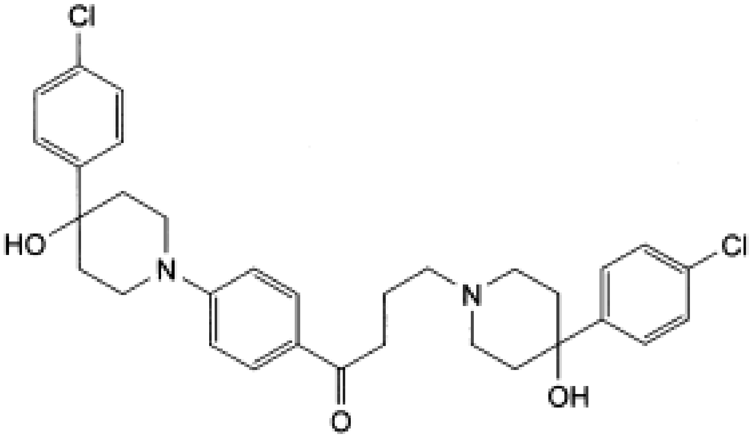
Specified impurities   A, B, C, D, E, F.



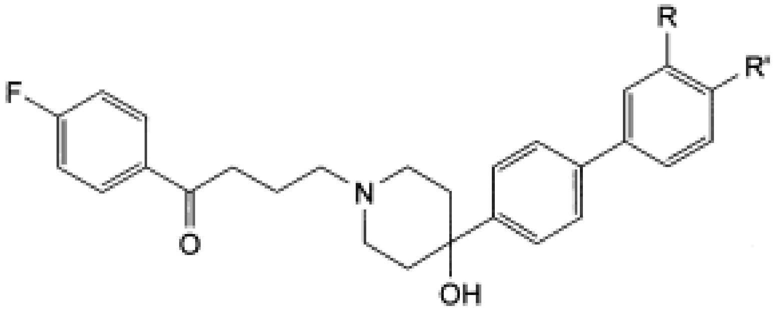
A. R1 = F, R2 = R3 = R4 = H: 1-(4-fluorophenyl)-4-(4-hydroxy-4-phenylpiperidin-1-yl)butan-1-one,

B. R1 = R2 = H, R3 = F, R4 = Cl: 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(2-fluorophenyl)butan-1-one,

C. R1 = F, R2 = C_2H_5, R3 = H, R4 = Cl: 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(3-ethyl-4-fluorophenyl)butan-1-one,



D. 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-[4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]phenyl]butan-1-one,



E. R = H, R′ = Cl: 4-[4-(4′-chlorobiphenyl-4-yl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl) butan-1-one,

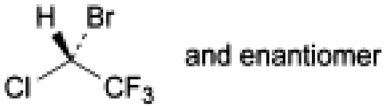
F. R = Cl, R′ = H: 4-[4-(3′-chlorobiphenyl-4-yl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl) butan-1-one.

Ph Eur

Halothane

General Notices

(Ph Eur monograph 0393)



$C_2HBrClF_3$ 197.4 *151-67-7*

Action and use

General anaesthetic.

Ph Eur

DEFINITION

(*RS*)-2-Bromo-2-chloro-1,1,1-trifluoroethane to which 0.01 per cent *m/m* of thymol has been added.

CHARACTERS

Appearance

Clear, colourless, mobile, heavy, non-flammable liquid.

Solubility

Slightly soluble in water, miscible with anhydrous ethanol and with trichloroethylene.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Distillation range (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Examine the substance in a 0.1 mm cell.

Comparison *Ph. Eur. reference spectrum of halothane.*

C. Add 0.1 ml to 2 ml of *2-methyl-2-propanol R* in a test-tube. Add 1 ml of *copper edetate solution R*, 0.5 ml of *concentrated ammonia R* and a mixture of 0.4 ml of *strong hydrogen peroxide solution R* and 1.6 ml of *water R* (solution A). Prepare a blank at the same time (solution B). Place both tubes in a water-bath at 50 °C for 15 min, cool and add 0.3 ml of *glacial acetic acid R*. To 1 ml of each of solutions A and B add 0.5 ml of a mixture of equal volumes of freshly prepared *alizarin S solution R* and *zirconyl nitrate solution R*. Solution A is yellow and solution B is red.

To 1 ml of each of solutions A and B add 1 ml of *buffer solution pH 5.2 R*, 1 ml of *phenol red solution R* diluted 1 to 10 with *water R* and 0.1 ml of *chloramine solution R*. Solution A is bluish-violet and solution B is yellow.

To 2 ml of each of solutions A and B add 0.5 ml of a mixture of 25 volumes of *sulphuric acid R* and 75 volumes of *water R*, 0.5 ml of *acetone R* and 0.2 ml of a 50 g/l solution of *potassium bromate R* and shake. Warm the tubes in a water-bath at 50 °C for 2 min, cool and add 0.5 ml of a mixture of equal volumes of *nitric acid R* and *water R* and 0.5 ml of *silver nitrate solution R2.* Solution A is opalescent and a white precipitate is formed after a few minutes; solution B remains clear.

TESTS

Acidity or alkalinity

To 20 ml add 20 ml of *carbon dioxide-free water R*, shake for 3 min and allow to stand. Separate the aqueous layer and add 0.2 ml of *bromocresol purple solution R*. Not more than 0.1 ml of *0.01 M sodium hydroxide* or 0.6 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Relative density *(2.2.5)*

1.872 to 1.877.

Distillation range *(2.2.11)*

It distils completely between 49.0 °C and 51.0 °C and 95 per cent distills within a range of 1.0 °C.

Volatile related substances

Gas chromatography *(2.2.28)*.

Internal standard *trichlorotrifluoroethane CRS.*

Test solution (a) The substance to be examined.

Test solution (b) Dilute 5.0 ml of *trichlorotrifluoroethane CRS* to 100.0 ml with the substance to be examined. Dilute 1.0 ml of the solution to 100.0 ml with the substance to be examined. Dilute 1.0 ml of this solution to 10.0 ml with the substance to be examined.

Column:

— *size:* l = 2.75 m, Ø = 5 mm;

— *stationary phase*: *silanised diatomaceous earth for gas chromatography R1* (180-250 µm), the first 1.8 m being impregnated with 30 per cent *m/m* of *macrogol 400 R* and the remainder with 30 per cent *m/m* of *dinonyl phthalate R*;

— *temperature*: 50 °C.

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 ml/min.

Detection Flame ionisation.

Injection 5 µl.

Limit Test solution (b):

- *total*: not more than the area of the peak due to the internal standard, corrected if necessary for any impurity with the same retention time as the internal standard (0.005 per cent).

Thymol

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.10 g of *menthol R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Test solution To 20.0 ml of the substance to be examined add 5.0 ml of the internal standard solution.

Reference solution Dissolve 20.0 mg of *thymol R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent. To 20.0 ml of this solution, add 5.0 ml of the internal standard solution.

Column:
- *material*: fused silica;
- *size:* l = 15 m, Ø = 0.53 mm;
- *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 1.5 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 15 ml/min.

Temperature:
- *column*: 150 °C;
- *injection port*: 170 °C;
- *detector*: 200 °C.

Detection Flame ionisation.

Injection 1.0 µl.

Limit:
- *thymol*: 0.75 times to 1.15 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.008 per cent *m/m* to 0.012 per cent *m/m*).

Bromides and chlorides

To 10 ml add 20 ml of *water R* and shake for 3 min. To 5 ml of the aqueous layer add 5 ml of *water R*, 0.05 ml of *nitric acid* R and 0.2 ml of *silver nitrate solution R1*. The solution is not more opalescent than a mixture of 5 ml of the aqueous layer and 5 ml of *water R*.

Bromine and chlorine

To 10 ml of the aqueous layer obtained in the test for bromides and chlorides add 1 ml of *potassium iodide and starch solution R*. No blue colour is produced.

Non-volatile matter

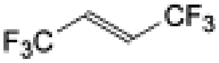
Maximum 20 mg/l.

Evaporate 50 ml to dryness on a water-bath and dry the residue in an oven at 100-105 °C for 2 h. The residue weighs a maximum of 1 mg.

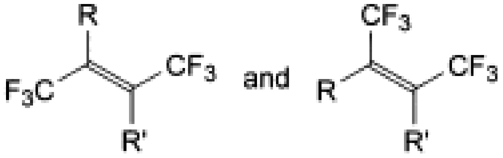
STORAGE

In an airtight container, protected from light, at a temperature not exceeding 25 °C. The choice of material for the container is made taking into account the particular reactivity of halothane with certain metals.

IMPURITIES

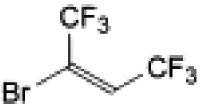


A. (*E*)-1,1,1,4,4,4-hexafluorobut-2-ene,

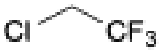


B. R = Cl, R′ = H: (*EZ*)-2-chloro-1,1,1,4,4,4-hexafluorobut-2-ene (*cis* and *trans*),

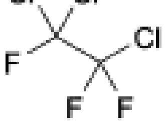
C. R = R′ = Cl: (*EZ*)-2,3-dichloro-1,1,1,4,4,4-hexafluorobut-2-ene (*cis* and *trans*),



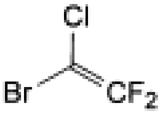
D. (*E*)-2-bromo-1,1,1,4,4,4-hexafluorobut-2-ene,



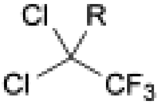
E. 2-chloro-1,1,1-trifluoroethane,



F. 1,1,2-trichloro-1,2,2-trifluoroethane,

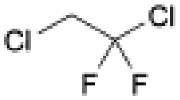


G. 1-bromo-1-chloro-2,2-difluoroethene,



H. R = H: 2,2-dichloro-1,1,1-trifluoroethane,

I. R = Br: 1-bromo-1,1-dichloro-2,2,2-trifluoroethane,



J. 1,2-dichloro-1,1-difluoroethane.

Ph Eur

Helium

General Notices

(Ph Eur monograph 2155)

He 4.00 *7440-59-7*

Ph Eur

DEFINITION

Content

Minimum 99.5 per cent *V/V* of He.

This monograph applies to helium obtained by separation from natural gas and intended for medicinal use.

CHARACTERS

Appearance

Colourless, inert gas.

IDENTIFICATION

Examine the chromatograms obtained in the assay. The retention time of the principal peak in the chromatogram obtained with the substance to be examined is approximately the same as that of the principal peak in the chromatogram obtained with the reference gas.

TESTS

Methane

Maximum 50.0 ppm *V/V*.

Infrared analyser.

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena (3 µm filter).

Reference gas (a) helium for chromatography R.

Reference gas (b) Mixture containing 50.0 ppm *V/V* of *methane R* in *helium for chromatography R*.

The infrared analyser generally comprises an infrared source emitting broadband infrared radiation, an optical device, a sample cell, a detector and in some analysers a reference cell. The optical device may be positioned either before or after the sample cell. It consists of one or more optical filters, through which the broadband radiation is passed. The optical device is selected for methane determination. The measurement light beam passes through the sample cell and may also pass through a reference cell if the analyser integrates such a feature. When methane is present in the sample cell, absorption of energy in the measurement light beam will occur according to the Beer-Lambert law, and this produces a change in the detector signal. This measurement signal is compared to a reference signal to generate an output related to the concentration of methane. The generated signal is linearised in order to determine the methane content.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the methane content in the gas to be examined.

Oxygen

Maximum 50.0 ppm *V/V*, determined using an oxygen analyser equipped with an electrochemical cell and a detector scale ranging from 0-100 ppm *V/V*.

The gas to be examined passes through a detection cell containing an aqueous solution of an electrolyte, generally potassium hydroxide. The presence of oxygen in the gas to be examined produces a variation in the electric signal recorded at the outlet of the cell that is proportional to the oxygen content.

Calibrate the analyser according to the instructions of the manufacturer. Pass the gas to be examined through the analyser using a suitable pressure regulator and airtight metal tubes and operating at the prescribed flow rates until constant readings are obtained.

Water *(2.5.28)*

Maximum 67 ppm *V/V*.

ASSAY

Gas chromatography *(2.2.28)*.

Gas to be examined The substance to be examined.

Reference gas *helium for chromatography R*.

Column:
— *size:* l = 2 m, Ø = 4.5 mm;

— *stationary phase*: *molecular sieve for chromatography R* (0.5 nm).

Carrier gas *argon for chromatography R*.

Flow rate 60 ml/min.

Temperature:
— *column:* 50 °C;

— *detector*: 150 °C.

Detection Thermal conductivity.

Injection 0.5 ml.

Inject the reference gas. Adjust the injected volumes and operating conditions so that the height of the peak due to helium in the chromatogram obtained is at least 35 per cent of the full scale of the recorder.

System suitability Reference gas:

— *symmetry factor*: minimum 0.6.

Calculate the content of He in the gas to be examined.

STORAGE

As compressed gas or liquid at cryogenic temperature, in appropriate containers, complying with the legal regulations.

IMPURITIES

Specified impurities A, B, C.

A. methane,

B. oxygen,

C. water.

Ph Eur

Heparin Calcium

General Notices

(Ph Eur monograph 0332)

Action and use

Anticoagulant.

Preparation

Heparin Injection

Ph Eur

DEFINITION

Heparin calcium is a preparation containing the calcium salt of a sulphated glucosaminoglycan present in mammalian tissues. On complete hydrolysis, it liberates D-glucosamine, D-glucuronic acid, L-iduronic acid, acetic acid and sulphuric acid. It has the characteristic property of delaying the clotting of freshly shed blood. The potency of heparin calcium intended for parenteral administration is not less than 150 IU/mg, calculated with reference to the dried substance. The potency of heparin calcium not intended for parenteral administration is not less than 120 IU/mg, calculated with reference to the dried substance.

PRODUCTION

It is prepared from the lungs of oxen or from the intestinal mucosae of oxen, pigs or sheep.

It is produced by methods of manufacturing designed to minimise or eliminate substances lowering blood pressure.

CHARACTERS

A white or almost white powder, hygroscopic, freely soluble in water.

IDENTIFICATION

A. It delays the clotting of recalcified citrated sheep plasma (see Assay).

B. Dissolve 0.40 g in *water R* and dilute to 10.0 ml with the same solvent. The specific optical rotation *(2.2.7)* is not less than + 35.

C. Examine by zone electrophoresis *(2.2.31)* using *agarose for electrophoresis R* as the supporting medium. To equilibrate the agarose and as electrolyte solution use a mixture of 50 ml of *glacial acetic acid R* and 800 ml of *water R* adjusted to pH 3 by addition of *lithium hydroxide R* and diluted to 1000.0 ml with *water R*.

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution Dilute *heparin sodium BRP* with an equal volume of *water R*.

Apply separately to the strip 2 µl to 3 µl of each solution. Pass a current of 1 mA to 2 mA per centimetre of strip width at a potential difference of 300 V for about 10 min. Stain the strips using a 1 g/l solution of *toluidine blue R* and remove the excess by washing. The ratio of the mobility of the principal band or bands in the electropherogram obtained with the test solution to the mobility of the band in the electropherogram obtained with the reference solution is 0.9 to 1.1.

D. It gives the reactions of calcium *(2.3.1)*.

TESTS

Appearance of solution

Dissolve a quantity equivalent to 50 000 IU in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than degree 5 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

pH *(2.2.3)*

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. The pH of the solution is 5.5 to 8.0.

Protein and nucleotidic impurities

Dissolve 40 mg in 10 ml of *water R*. The absorbance *(2.2.25)* measured at 260 nm is not greater than 0.20 and that measured at 280 nm is not greater than 0.15.

Nitrogen

Not more than 2.5 per cent, calculated with reference to the dried substance. Carry out the determination of nitrogen by sulphuric acid digestion *(2.5.9)*, using 0.100 g.

Calcium

9.5 per cent to 11.5 per cent of Ca, calculated with reference to the dried substance. Determine the calcium by complexometric titration *(2.5.11)*, using 0.200 g.

Heavy metals *(2.4.8)*

0.5 g complies with limit test C for heavy metals (30 ppm). Prepare the reference solution using 1.5 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 8.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash *(2.4.14)*

32 per cent to 40 per cent, determined on 0.20 g and calculated with reference to the dried substance.

Bacterial endotoxins *(2.6.14)*

Less than 0.01 IU per IU of heparin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins. The addition of divalent cations may be necessary in order to fulfil the validation criteria.

ASSAY

Carry out the assay of heparin *(2.7.5)*. The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits of the estimated potency ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the stated potency.

STORAGE

Store in an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

LABELLING

The label states:

— the number of International Units per milligram,

— the name and quantity of any added substance.

Ph Eur

Heparin Sodium

General Notices

(Ph Eur monograph 0333)

Action and use

Anticoagulant.

Preparation

Heparin Injection

Ph Eur

DEFINITION

Heparin sodium is a preparation containing the sodium salt of a sulphated glucosaminoglycan present in mammalian tissues. On complete hydrolysis, it liberates D-glucosamine, D-glucuronic acid, L-iduronic acid, acetic acid and sulphuric acid. It has the characteristic property of delaying the clotting of freshly shed blood. The potency of heparin sodium intended for parenteral administration is not less than 150 IU/mg, calculated with reference to the dried substance. The potency of heparin sodium not intended for parenteral administration is not less than 120 IU/mg, calculated with reference to the dried substance.

PRODUCTION

It is prepared from the lungs of oxen or from the intestinal mucosae of oxen, pigs or sheep.

It is produced by methods of manufacturing designed to minimise or eliminate substances lowering blood pressure.

CHARACTERS

A white or almost white powder, hygroscopic, freely soluble in water.

IDENTIFICATION

A. It delays the clotting of recalcified citrated sheep plasma (see Assay).

B. Dissolve 0.40 g in *water R* and dilute to 10.0 ml with the same solvent. The specific optical rotation *(2.2.7)* is not less than + 35.

C. Examine by zone electrophoresis *(2.2.31)* using *agarose for electrophoresis R* as the supporting medium. To equilibrate the agarose and as electrolyte solution use a mixture of 50 ml of *glacial acetic acid R* and 800 ml of *water R* adjusted to pH 3 by addition of *lithium hydroxide R* and diluted to 1000.0 ml with *water R*.

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution Dilute *heparin sodium BRP* with an equal volume of *water R*.

Apply separately to the strip 2 µl to 3 µl of each solution. Pass a current of 1 mA to 2 mA per centimetre of strip width at a potential difference of 300 V for about 10 min. Stain the strips using a 1 g/l solution of *toluidine blue R* and remove the excess by washing. The ratio of the mobility of the principal band or bands in the electropherogram obtained with the test solution to the mobility of the band in the electropherogram obtained with the reference solution is 0.9 to 1.1.

D. The residue obtained in the test for sulphated ash (see Tests) gives reaction (a) of sodium *(2.3.1)*.

TESTS

Appearance of solution

Dissolve a quantity equivalent to 50 000 IU in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

pH *(2.2.3)*

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. The pH of the solution is 5.5 to 8.0.

Protein and nucleotidic impurities

Dissolve 40 mg in 10 ml of *water R*. The absorbance *(2.2.25)* measured at 260 nm is not greater than 0.20 and that measured at 280 nm is not greater than 0.15.

Nitrogen

Not more than 2.5 per cent, calculated with reference to the dried substance. Carry out the determination of nitrogen by sulphuric acid digestion *(2.5.9)*, using 0.100 g.

Sodium

9.5 per cent to 12.5 per cent of Na, calculated with reference to the dried substance and determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 50 mg of the substance to be examined in *0.1 M hydrochloric acid* containing 1.27 mg of *caesium chloride R* per millilitre and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare reference solutions containing 25 ppm, 50 ppm and 75 ppm of Na, using *sodium standard solution (200 ppm Na) R* diluted with *0.1 M hydrochloric acid* containing 1.27 mg of *caesium chloride R* per millilitre.

Measure the absorbance at 330.3 nm using a sodium hollow-cathode lamp as the source of radiation and a flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Heavy metals *(2.4.8)*

0.5 g complies with limit test C for heavy metals (30 ppm). Prepare the reference solution using 1.5 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Not more than 8.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash ***(2.4.14)***

30 per cent to 43 per cent, determined on 0.20 g and calculated with reference to the dried substance.

Bacterial endotoxins ***(2.6.14)***

Less than 0.01 IU per IU of heparin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Carry out the assay of heparin *(2.7.5)*. The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits of the estimated potency ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the stated potency.

STORAGE

Store in an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

— the number of International Units per milligram,

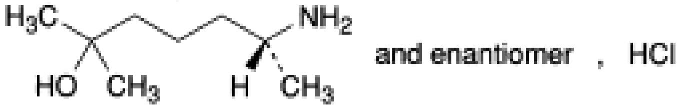
— the name and quantity of any added substance.

Ph Eur

Heptaminol Hydrochloride

General Notices

(Ph Eur monograph 1980)



$C_8H_{19}NO,HCl$ 181.7 *543-15-7*

Action and use

Non-selective phosphodiesterase inhibitor; treatment of reversible airways obstruction.

Ph Eur

DEFINITION

(6*RS*)-6-Amino-2-methylheptan-2-ol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. To 1 ml of solution S (see Tests) add 4 ml of *water R* and 2 ml of a 200 g/l solution of *ammonium and cerium nitrate R* in *4 M nitric acid*. An orange-brown colour develops.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *heptaminol hydrochloride CRS*.

C. Examine the chromatograms obtained in the test for related substances.

Detection Examine in daylight.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (b).

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* and 0.3 ml of *0.01 M hydrochloric acid*. The solution is red. Add 0.6 ml of *0.01 M sodium hydroxide*. The solution is yellow.

Related substances

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dilute 3.0 ml of test solution (a) to 10.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 50.0 ml with *methanol R*.

Reference solution (b) Dissolve 0.10 g of *heptaminol hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 10.0 mg of *heptaminol impurity A CRS* in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (d) Dilute 1.0 ml of reference solution (c) to 10.0 ml with *methanol R*.

Reference solution (e) To 2.5 ml of reference solution (c) add 0.5 ml of test solution (b) and dilute to 5 ml with *methanol R*.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia* R, *dioxan R*, *2-propanol R* (10:50:50 *V/V/V*).

Application 10 µl; apply test solutions (a) and (b) and reference solutions (a), (b), (d) and (e).

Development Over 2/3 of the plate.

Drying In air.

Detection Expose the plate to iodine vapour for at least 15 h.

System suitability The chromatogram obtained with reference solution (e) shows 2 clearly separated principal spots and the chromatogram obtained with reference solution (a) shows a single principal spot.

Limits In the chromatogram obtained with test solution (a):

— *impurity A*: any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.2 per cent),

— *any other impurity*: any spot, apart from the principal spot and any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.6 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

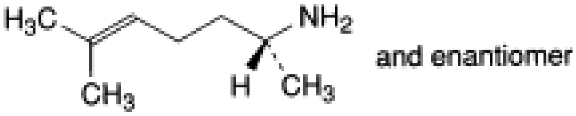
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.140 g in 50 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide.* Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 18.17 mg of $C_8H_{20}ClNO$.

IMPURITIES

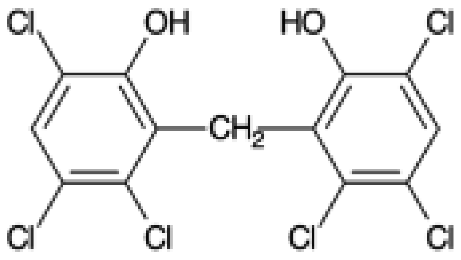


A. (2*RS*)-6-methylhept-5-en-2-amine.

Ph Eur

Hexachlorophene

General Notices



$C_{13}H_6Cl_6O_2$ 406.9 *70-30-4*

Action and use

Antiseptic.

Preparation

Hexachlorophene Dusting Powder

DEFINITION

Hexachlorophene is 2,2-methylenebis(3,4,6-trichlorophenol). It contains not less than 98.0% and not more than 100.5% of $C_{13}H_6Cl_6O_2$, calculated with reference to the dried substance.

PRODUCTION

A suitable test is carried out to demonstrate that the level of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin present does not exceed 2 ppb.

CHARACTERISTICS

A white or pale buff, crystalline powder.

Practically insoluble in *water*; very soluble in *acetone*; freely soluble in *ethanol (96%)*. It dissolves in dilute solutions of the alkali hydroxides.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hexachlorophene *(RS 174)*.

TESTS

Chloride

Dissolve 0.50 g in 2 ml of *ethanol (96%)*, dilute to 25 ml with *water* and filter. 5 ml of the clear filtrate diluted to 15 ml with *water* complies with the *limit test for chlorides*, Appendix VII (500 ppm).

Non-phenolic substances

Dissolve 5 g in 38 ml of *methanol*, add 125 ml of 0.25M *sodium hydroxide* and extract with three 15 ml quantities of n-*pentane*, retaining any foamy interphase with the aqueous layer. Dry the combined extracts over *anhydrous sodium sulphate* and evaporate to dryness at a pressure not exceeding 2 kPa. The residue weighs not more than 37.5 mg when dried to constant weight (0.75%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions of the substance being examined in *methanol* containing (1) 0.020% w/v and (2) 1.0% w/v.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography,* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 100 volumes of *methanol*, 20 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 300 nm.

Inject 20 µl of solutions (1) and (2). In the chromatogram obtained with solution (2) the sum of the areas of any *secondary peaks* with a retention time not more than 3 times that of the principal peak is not greater than twice the area of the principal peak in the chromatogram obtained with solution (1) (4.0%) and not more than one such peak has an area greater than half of the area of the principal peak in the chromatogram obtained with solution (1) (1.0%).

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 1.000 g in 25 ml of *ethanol (96%)* previously adjusted to pH 9.0 and titrate with 0.1M *sodium hydroxide VS* determining the end point potentiometrically. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 40.69 mg of $C_{13}H_6Cl_6O_2$.

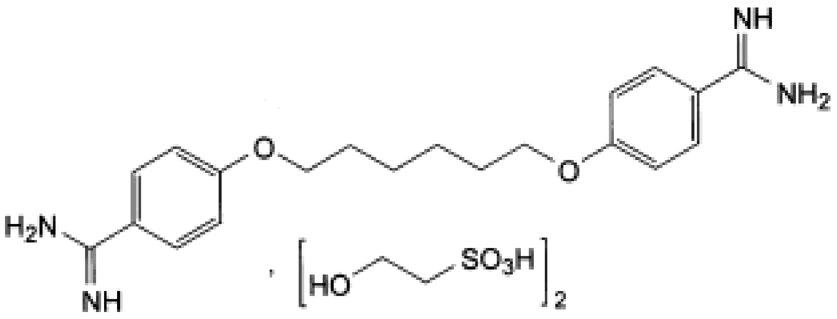
STORAGE

Hexachlorophene should be protected from light.

Hexamidine Isetionate

General Notices

(Hexamidine Diisetionate, Ph Eur monograph 1436)



$C_{24}H_{38}N_4O_{10}S_2$ 607 *659-40-5*

Action and use

Antiprotozoal.

Ph Eur

DEFINITION

4,4′-(Hexane-1,6-diylbisoxy)dibenzimidamide bis(2-hydroxyethanesulphonate).

Content

98.5 per cent to 101.5 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl isetionates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl isetionates are not detectable in the final product.

CHARACTERS

Appearance

White or slightly yellow powder, hygroscopic.

Solubility

Sparingly soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in

methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *hexamidine diisetionate CRS.*

B. Dissolve about 40 mg in 5 ml of *water R* and add dropwise with shaking 1 ml of a 100 g/l solution of *sodium chloride R*. Allow to stand for 5 min. An abundant, shimmering white precipitate is slowly formed.

TESTS

Appearance of solution

Dissolve 0.50 g in *carbon dioxide-free water R*, heating at about 70 °C and dilute to 10 ml with the same solvent. Allow to cool to room temperature for 10-15 min. The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Acidity or alkalinity

Dissolve 2.0 g in *water R* heating at about 50 °C and dilute to 20 ml with *water R* heating at about 50 °C. Allow to cool to about 35 °C, add 0.1 ml of *methyl red solution R*. Not more than 0.25 ml of *0.05 M hydrochloric acid* or *0.05 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with mobile phase A.

Reference solution (c) Dissolve 5 mg of the substance to be examined and 5 mg of *pentamidine diisetionate CRS* in mobile phase A and dilute to 100 ml with mobile phase A. Dilute 2 ml of the solution to 5 ml with mobile phase A.

Column:

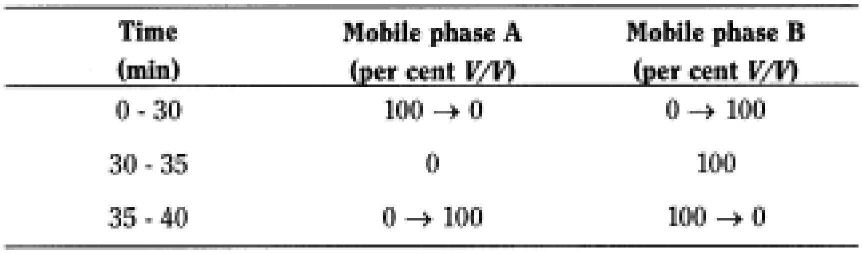
— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm).

Mobile phase:

— *mobile phase A*: mix 20 volumes of *acetonitrile R* and 80 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 using *phosphoric acid R,*

— *mobile phase B*: mix equal volumes of *acetonitrile R* and of a 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 using *phosphoric acid R,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	100 → 0	0 → 100
30 - 35	0	100
35 - 40	0 → 100	100 → 0

Flow rate 1 ml/min.

Detection Spectrophotometer at 263 nm.

Injection 20 µl.

Relative retention With reference to hexamidine (retention time = about 6 min): impurity B = about 1.7; impurity A = about 2.0; impurity C = about 3.7; impurity D = about 4.7.

System suitability Reference solution (c):

— *resolution*: minimum 5.0 between the peaks due to hexamidine and pentamidine.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *impurities C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *any other impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide* under a current of *nitrogen R*, determining the end-point potentiometrically *(2.2.20)*.

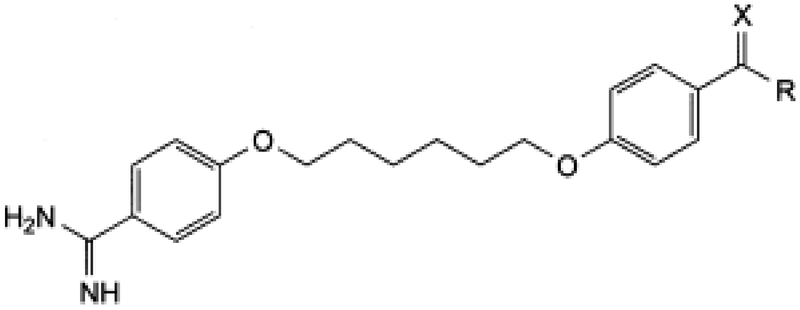
1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 30.35 mg of $C_{24}H_{38}N_4O_{10}S_2$.

STORAGE

In an airtight container.

IMPURITIES

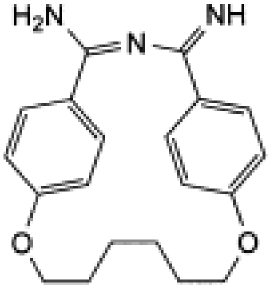
Specified impurities *A, B, C, D*.



A. X = O, R′ = NH_2: 4-[[6-(4-carbamimidoylphenoxy)hexyl]oxy]benzamide,

B. X = NH, R′ = OC_2H_5: ethyl 4-[[6-(4-carbamimidoylphenoxy)hexyl]oxy]benzimidoate,

D. X = O, R′ = OC_2H_5: ethyl 4-[[6-(4-carbamimidoylphenoxy)hexyl]oxy]benzoate,



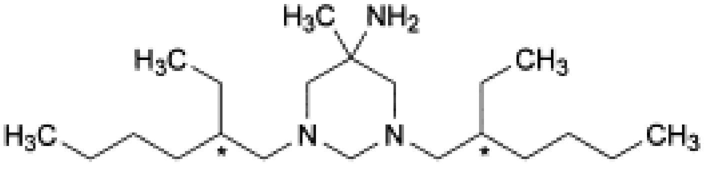
C. 4-imino-9,16-dioxa-3-azatricyclo[15.2.2.$2^{5,8}$]tricosa-1(19),2,5,7,17,20,22-heptaen-2-amine.

Ph Eur

Hexetidine

General Notices

(Ph Eur monograph 1221)



$C_{21}H_{45}N_3$ 339.6 *141-94-6*

Action and use

Antiseptic.

Ph Eur

DEFINITION

Hexetidine contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 1,3-bis(2-ethylhexyl)]-5-methylhexahydropyrimidin-5-amine.

CHARACTERS

An oily liquid, colourless or slightly yellow, very slightly soluble in water, very soluble in acetone, in alcohol and in methylene chloride. It dissolves in dilute mineral acids.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *hexetidine CRS*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 0.2 ml add 2 ml of *sulphuric acid R* and 2 mg of *chromotropic acid*, *sodium salt R*. Heat in a water-bath at 60 °C. A violet colour develops.

D. Dissolve 0.2 ml in 1 ml of *methylene chloride R*. Add 0.5 ml of *copper sulphate solution R*, 0.05 ml of *0.25 M alcoholic sulphuric acid R* and 5 ml of *water R*. Shake, then allow to stand. The lower layer becomes deep blue.

TESTS

Appearance

The substance to be examined is clear *(2.2.1)*and not more intensely coloured than reference solution Y_5 or reference solution GY_5 *(2.2.2, Method II)*.

Relative density *(2.2.5)*

0.864 to 0.870.

Refractive index *(2.2.6)*

1.461 to 1.467.

Optical rotation *(2.2.7)*

Dissolve 1.0 g in *ethanol R* and dilute to 10.0 ml with the same solvent. The angle of optical rotation is - 0.10 ° to + 0.10 °.

Absorbance *(2.2.25)*

Dissolve 0.50 g in *heptane R* and dilute to 50.0 ml with the same solvent. At wavelengths from 270 nm to 350 nm, the absorbance of the solution is not greater than 0.1.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel H R* as the coating substance. *Prepare the solutions immediately before use.*

Test solution (a) Dissolve 2.0 g of the substance to be examined in *heptane R* and dilute to 20 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *heptane R*.

Reference solution (a) Dissolve 20 mg of *hexetidine CRS* in *heptane R* and dilute to 2 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (a) to 100 ml with *heptane R*.

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with *heptane R*.

Reference solution (d) Dissolve 10 mg of *dehydrohexetidine CRS* in test solution (a) and dilute to 10 ml with the same solution.

Apply separately to the plate 1 µl of each solution. At the bottom of a chromatography tank, place an evaporating dish containing *concentrated ammonia R1*. Place the dried plate in the tank and close the tank. Leave the plate in contact with the ammonia vapour for 15 min. Withdraw the plate and place it in a current of air to remove the ammonia vapour. Develop over a path of 15 cm using a mixture of 20 volumes of *methanol R* and 80 volumes of *toluene R*. Allow the plate to dry in air. Expose the plate to iodine vapour for 30 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent) and at most two such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots.

Heavy metals *(2.4.8)*

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B for heavy metals (10 ppm). Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

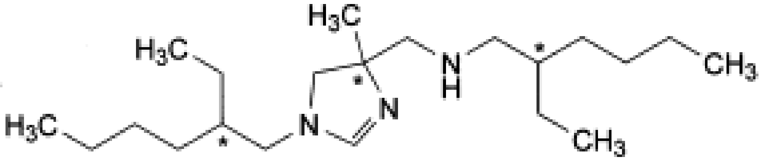
Dissolve 0.150 g in 80 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 16.98 mg of $C_{21}H_{45}N_3$.

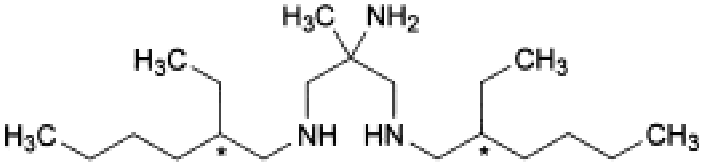
STORAGE

Store protected from light.

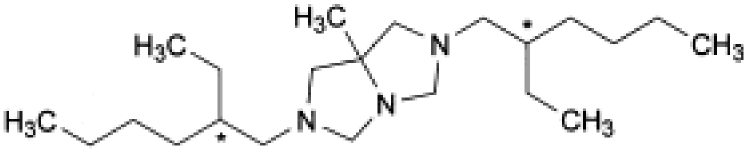
IMPURITIES



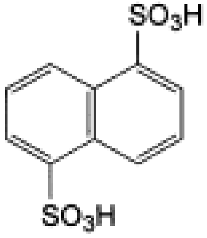
A. 2-ethyl-*N*-[[1-(2-ethylhexyl)-4-methyl-4,5-dihydro-1*H*-imidazol-4-yl]methyl]hexan-1-amine (dehydrohexetidine),



B. *N*^{1},*N*^{3}-bis(2-ethylhexyl)-2-methylpropane-1,2,3-triamine (triamine),



C. 2,6-bis(2-ethylhexyl)-7a-methylhexahydro-1*H*-imidazo[1,5-*c*]imidazole (hexedine),



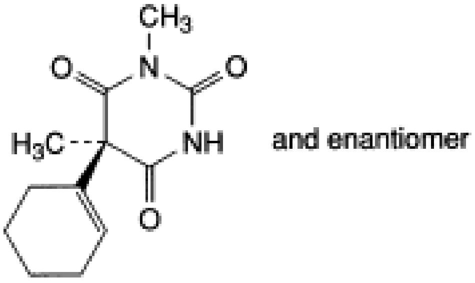
D. naphthalene-1,5-disulphonic acid.

Ph Eur

Hexobarbital

General Notices

(Ph Eur monograph 0183)



$C_{12}H_{16}N_2O_3$ 236.3 *56-29-1*

Action and use

Barbiturate.

Ph Eur

DEFINITION

Hexobarbital contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (5*RS*)-5-(cyclohex-1-enyl)-1,5-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, sparingly soluble in alcohol. It forms water-soluble compounds with alkali hydroxides and carbonates and with ammonia.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Determine the melting point *(2.2.14)* of the substance to be examined. Mix equal parts of the substance to be examined and *hexobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 146 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *hexobarbital CRS*.

C. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *chloroform R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 0.1 g of *hexobarbital CRS* in *chloroform R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To about 10 mg add 1.0 ml of a 10 g/l solution of *vanillin R* in *alcohol R* and 2 ml of a cooled mixture of 1 volume of *water R* and 2 volumes of *sulphuric acid R*. Shake and allow to stand for 5 min. A greenish-yellow colour develops. Heat on a water-bath for 10 min. The colour becomes dark red.

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 6 ml of *water R*. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6.*(2.2.2, Method II)*.

Acidity

Boil 1.0 g with 50 ml of *water R* for 2 min, allow to cool and filter. To 10 ml of the filtrate add 0.15 ml of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *chloroform R* and dilute to 100 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *chloroform R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 5 ml of *pyridine R*. Add 0.5 ml of *thymolphthalein solution R* and 10 ml of *silver nitrate solution in pyridine R*. Titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained. Carry out a blank titration.

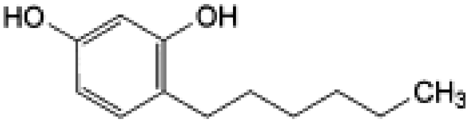
1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 23.63 mg of $C_{12}H_{16}N_2O_3$.

Ph Eur

Hexylresorcinol

General Notices

(Ph Eur monograph 1437)



$C_{12}H_{18}O_2$ 194.3 *136-77-6*

Action and use

Antihelminthic.

Ph Eur

DEFINITION

4-Hexylbenzene-1,3-diol.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Colourless, yellowish or reddish, crystalline powder or needles, turning brownish-pink on exposure to light or air.

Solubility

Very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 66 °C to 68 °C, melting may occur at about 60 °C, followed by solidification and a second melting between 66 °C and 68 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *hexylresorcinol CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately *in methanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dilute 0.1 ml of solution S (see Tests) to 10 ml with *ethanol (96 per cent) R*.

Reference solution (a) Dissolve 10 mg of *hexylresorcinol CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *hexylresorcinol CRS* and 10 mg of *resorcinol R* in *ethanol (96 per cent) R*, then dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *methyl ethyl ketone R*, *pentane R* (50:50 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air for 5 min.

Detection Spray with 3 ml of *anisaldehyde solution R* and heat at 100-105 °C for 5 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g in 1 ml of *ethanol (96 per cent) R*. Add one drop of *ferric chloride solution R1*. A green colour is produced. Add *dilute ammonia R1*. The solution becomes brown.

TESTS

Solution S

Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

Acidity

Dissolve 0.5 g in a mixture of 25 ml of *carbon dioxide-free water R* and 25 ml of *ether R* previously neutralised to *phenolphthalein solution R1* and titrate with *0.1 M sodium hydroxide*, shaking vigorously after each addition. Not more than 0.4 ml is required to change the colour of the solution.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.1 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b) Dissolve 20.0 mg of *phenol R* (impurity A) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 20.0 mg of *resorcinol R* (impurity B) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (d) To 8.0 ml of reference solution (a) add 2.0 ml of reference solution (b) , 2.0 ml of reference solution (c) and dilute to 20.0 ml with the mobile phase.

Column:
- — *size: l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of a 3.0 g/l solution of *glacial acetic acid R* adjusted to pH 5.9 with *dilute ammonia R1*, and 75 volumes of *methanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 281 nm.

Injection 20 µl.

Run time Twice the retention time of hexylresorcinol.

System suitability Reference solution (d):
- — *resolution*: minimum 5.0 between the peaks due to impurity A (2nd peak) and hexylresorcinol (3rd peak).

Limits:
- — *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.2 per cent);
- — *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- — *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- — *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 10 ml of *methanol R* in a ground-glass-stoppered flask, add 30.0 ml of *0.0167 M potassium bromate* and 2 g of *potassium bromide R*. Shake to dissolve the substance and add 15 ml of *dilute sulphuric acid R*. Stopper the flask, shake and allow to stand in the dark for 15 min, stirring continuously. Add 5 ml of *methylene chloride R* and a solution of 1 g of *potassium iodide R* in 10 ml of *water R*, allow to stand in the dark for 15 min, stirring continuously. Titrate with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution R*, shaking thoroughly. Carry out a blank titration under the same conditions.

1 ml of *0.0167 M potassium bromate* is equivalent to 4.857 mg of $C_{12}H_{18}O_2$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities A, B.

A. phenol,

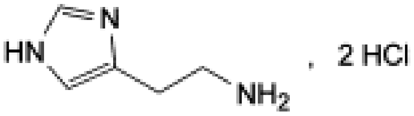
B. resorcinol.

Ph Eur

Histamine Dihydrochloride

General Notices

(Ph Eur monograph 0143)



$C_5H_9N_3$,2HCl 184.1 *56-92-8*

Ph Eur

DEFINITION

Histamine dihydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 2-(1*H*-imidazol-4-yl)ethanamine dihydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, hygroscopic, very soluble in water, soluble in alcohol.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *histamine dihydrochloride CRS*. Examine as discs prepared using 1 mg of substance.

B. Examine the chromatograms obtained in the test for histidine. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 7 ml of *water R* and add 3 ml of a 200 g/l solution of *sodium hydroxide R*. Dissolve 50 mg of *sulphanilic acid R* in a mixture of 0.1 ml of *hydrochloric acid R* and 10 ml of *water R* and add 0.1 ml of *sodium nitrite solution R*. Add the second solution to the first and mix. A red colour is produced.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 2.85 to 3.60.

Histidine

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel G plate R*.

Test solution (a) Dissolve 0.5 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 2 ml of test solution (a) to 10 ml with *water R*.

Reference solution (a) Dissolve 0.1 g of *histamine dihydrochloride CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 50 mg of *histidine monohydrochloride R* in *water R* and dilute to 100 ml with the same solvent.

Reference solution (c) Mix 1 ml of test solution (a) and 1 ml of reference solution (b).

Apply to the plate 1 µl of test solution (a), 1 µl of test solution (b), 1 µl of reference solution (a) , 1 µl of reference solution (b) and 2 µl of reference solution (c). Develop over a path of 15 cm using a mixture of 5 volumes of *concentrated ammonia R*, 20 volumes of *water R* and 75 volumes of *acetonitrile R*. Dry the plate in a current of air. Repeat the development in the same direction, dry the plate in a current of air and spray with *ninhydrin solution R1*. Heat the plate at 110 °C for 10 min. Any spot corresponding to histidine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Sulphates *(2.4.13)*

3 ml of solution S diluted to 15 ml with *distilled water R* complies with the limit test for sulphates (0.1 per cent).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 0.20 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 0.5 g.

ASSAY

Dissolve 0.080 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the first and third points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 9.203 mg of $C_5H_{11}Cl_2N_3$.

STORAGE

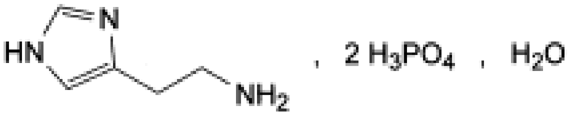
Store in an airtight container, protected from light.

Ph Eur

Histamine Phosphate

General Notices

(Ph Eur monograph 0144)



$C_5H_9N_3,2H_3PO_4,H_2O$ 325.2 *51-74-1*
(anhydrous)

Ph Eur

DEFINITION

Histamine phosphate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 2-(1*H*-imidazol-4-yl)ethanamine diphosphate, calculated with reference to the anhydrous substance.

CHARACTERS

Colourless, long prismatic crystals, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *histamine phosphate CRS*. Examine as discs prepared using 1 mg of substance.

B. Examine the chromatograms obtained in the test for histidine. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 7 ml of *water R* and add 3 ml of a 200 g/l solution of *sodium hydroxide R*. Dissolve 50 mg of *sulphanilic acid R* in a mixture of 0.1 ml of *hydrochloric acid R* and 10 ml of *water R* and add 0.1 ml of *sodium nitrite solution R*. Add the second solution to the first and mix. A red colour is produced.

D. It gives reaction (a) of phosphates *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 3.75 to 3.95.

Histidine

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.5 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 2 ml of test solution (a) to 10 ml with *water R*.

Reference solution (a) Dissolve 0.1 g of *histamine phosphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 50 mg of *histidine monohydrochloride R* in *water R* and dilute to 100 ml with the same solvent.

Reference solution (c) Mix 1 ml of test solution (a) and 1 ml of reference solution (b).

Apply separately to the plate 1 µl of test solution (a), 1 µl of test solution (b), 1 µl of reference solution (a), 1 µl of reference solution (b) and 2 µl of reference solution (c). Develop over a path of 15 cm using a mixture of 5 volumes of *concentrated ammonia R*, 20 volumes of *water R* and 75 volumes of *acetonitrile R*. Dry the plate in a current of air. Repeat the development in the same direction, dry the plate in a current of air and spray with *ninhydrin solution R1*. Heat at 110 °C for 10 min. Any spot corresponding to histidine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Sulphates *(2.4.13)*

3 ml of solution S diluted to 15 ml with *distilled water R* complies with the limit test for sulphates (0.1 per cent).

Water *(2.5.12)*

5.0 per cent to 6.2 per cent, determined on 0.30 g by the semi-micro determination of water.

ASSAY

Dissolve 0.140 g in 5 ml of *anhydrous formic acid R* and add 20 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 15.36 mg of $C_5H_{15}N_3O_8P_2$.

STORAGE

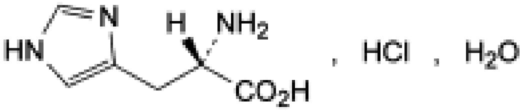
Store protected from light.

Ph Eur

Histidine Hydrochloride Monohydrate

General Notices

(Ph Eur monograph 0910)



$C_6H_9N_3O_2$,HCl,H_2O 209.6 *645-35-2*
(anhydrous)

Action and use

Amino acid.

Ph Eur

DEFINITION

Histidine hydrochloride monohydrate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of the hydrochloride of (*S*)-2-amino-3-(imidazol-4-yl) propanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification A, B, C, F.

Second identification A, B, D, E, F.

A. It complies with the test for specific optical rotation (see Tests).

B. It complies with the test for the pH (see Tests).

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *histidine hydrochloride monohydrate CRS*. Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

E. Dissolve 0.1 g in 7 ml of *water R* and add 3 ml of a 200 g/l solution of *sodium hydroxide*

R. Dissolve 50 mg of *sulphanilic acid R* in a mixture of 0.1 ml of *hydrochloric acid R* and 10 ml of *water R* and add 0.1 ml of *sodium nitrite solution R*. Add the second solution to the first and mix. An orange-red colour develops.

F. About 20 mg gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 3.0 to 5.0.

Specific optical rotation *(2.2.7)*

Dissolve 2.75 g in 12.0 ml of *hydrochloric acid R1* and dilute to 25.0 ml with *water R*. The specific optical rotation is + 9.2 to + 10.6, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R.*

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *histidine hydrochloride monohydrate CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *histidine hydrochloride monohydrate CRS* and 10 mg of *proline CRS* in *water R* and dilute to 25 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Dry the plate in a current of air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Sulphates *(2.4.13)*

Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals *(2.4.8)*

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

7.0 per cent to 10.0 per cent, determined on 1.000 g by drying in an oven at 145 °C to 150 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g in 50 ml of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 19.16 mg of $C_6H_{10}ClN_3O_2$.

STORAGE

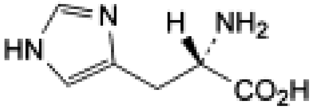
Store protected from light.

Ph Eur

Histidine

General Notices

(Ph Eur monograph 0911)



$C_6H_9N_3O_2$ 155.2 *71-00-1*

Action and use

Amino acid.

Ph Eur

DEFINITION

(*S*)-2-Amino-3-(imidazol-4-yl)propanoic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *histidine CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness at 60 °C and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g in 7 ml of *water R* and add 3 ml of a 200 g/l solution of *sodium hydroxide R*. Dissolve 50 mg of *sulphanilic acid R* in a mixture of 0.1 ml of *hydrochloric acid R* and 10 ml of *water R* and add 0.1 ml of *sodium nitrite solution R*. Add the second solution to the first and mix. An orange-red colour develops.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R*, heating in a water-bath and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

+ 11.4 to + 12.4 (dried substance).

Dissolve 2.75 g in 12.0 ml of *hydrochloric acid R1* and dilute to 25.0 ml with *water R*.

Ninhydrin-positive substances

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *histidine CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *histidine CRS* and 10 mg of *proline CRS* in *water R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R*, *water R*, *butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Limits:

— *any impurity*: any spots in the chromatogram obtained with test solution (a), apart from the principal spot, are not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides ***(2.4.4)***

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 300 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Ammonium ***(2.4.1, Method B)***

Maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron ***(2.4.9)***

Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 3 ml of *dilute hydrochloric acid R* and 15 ml of *water R*, with gentle warming if necessary, and dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.130 g in 50 ml of *water R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M hydrochloric acid* is equivalent to 15.52 mg of $C_6H_9N_3O_2$.

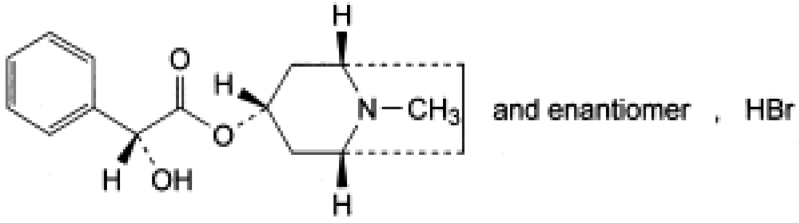
STORAGE

Protected from light.

Homatropine Hydrobromide

General Notices

(Ph Eur monograph 0500)



$C_{16}H_{21}NO_3,HBr$ 356.3 51-56-9

Action and use

Anticholinergic.

Preparation

Homatropine Eye Drops

Ph Eur

DEFINITION

(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-2-phenylacetate hydrobromide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, sparingly soluble in alcohol.

mp

About 215 °C, with decomposition.

IDENTIFICATION

First identification *A, C.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *homatropine hydrobromide CRS.*

B. Dissolve 50 mg in 1 ml of *water R* and add 2 ml of *dilute acetic acid R*. Heat and add 4 ml of *picric acid solution R*. Allow to cool, shaking occasionally. Collect the crystals, wash with 2 quantities, each of 3 ml, of iced *water R* and dry at 100-105 °C. The crystals melt *(2.2.14)* at 182 °C to 186 °C.

C. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.0 to 6.5 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 25.0 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *hyoscine hydrobromide CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. To 10.0 ml of this solution add 0.5 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Column:

— *size: l* = 0.1 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm),

— *temperature*: 40 °C.

Mobile phase Mix 33 volumes of *methanol R2* and 67 volumes of a solution prepared as follows: dissolve 6.8 g of *potassium dihydrogen phosphate R* and 7.0 g of *sodium heptanesulphonate monohydrate R* in 1000 ml of *water R* and adjust to pH 2.7 with a 330 g/l solution of *phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Run time 3 times the retention time of homatropine.

Relative retention With reference to homatropine (retention time = about 6.8 min): impurity C = about 0.2; impurity A = about 0.9; impurity B = about 1.1; impurity D = about 1.9.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to homatropine and impurity B,

— *symmetry* factor : maximum 2.5 for the peak due to homatropine.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *impurities B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent); disregard the peak due to the bromide ion which appears close to the peak due to the solvent,

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

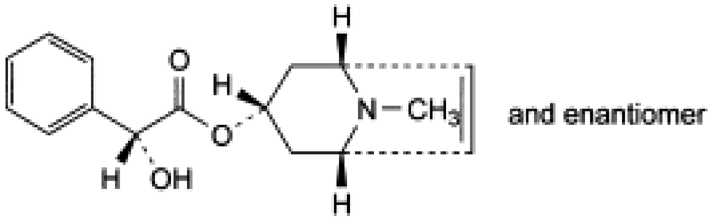
1 ml of *0.1 M sodium hydroxide* is equivalent to 35.63 mg of $C_{16}H_{22}BrNO_3$.

STORAGE

Protected from light.

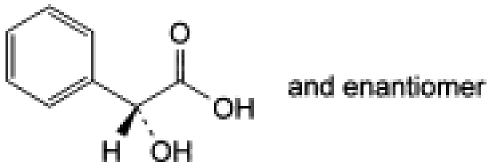
IMPURITIES

Specified impurities *A, B, C, D*.


and enantiomer

A. (1*R*,3*s*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-6-en-3-yl (2*RS*)-2-hydroxy-2-phenylacetate (dehydrohomatropine),

B. hyoscine,


and enantiomer

C. (2*RS*)-2-hydroxy-2-phenylacetic acid (mandelic acid),

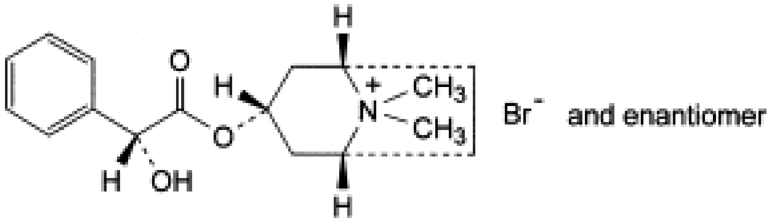
D. atropine.

Ph Eur

Homatropine Methylbromide

General Notices

(Ph Eur monograph 0720)



$C_{17}H_{24}BrNO_3$ 370.3 *80-49-9*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

(1*R*,3*r*,5*S*)-3-[[(2*RS*)-2-hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, soluble in alcohol.

mp

About 190 °C.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*,

Comparison *homatropine methylbromide CRS*.

B. Dissolve 50 mg in 1 ml of *water R* and add 2 ml of *dilute acetic acid R*. Heat and add 4 ml of *picric acid solution R*. Allow to cool, shaking occasionally. The crystals, washed with 2 quantities, each of 3 ml, of iced *water R* and dried at 100-105 °C melt *(2.2.14)* at 132 °C to 138 °C.

C. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 6.5 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile R*, mobile phase A (9:41 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 25.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *homatropine hydrobromide CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. To 10.0 ml of the solution add 0.5 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm,

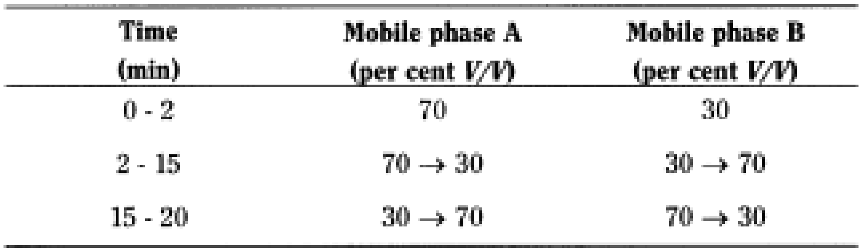
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm),

— *temperature*: 25 °C.

Mobile phase:

— *mobile phase A*: dissolve 3.4 g of *potassium dihydrogen phosphate R* and 5.0 g of *sodium heptanesulphonate monohydrate R* in 1000 ml of *water R*, and adjust to pH 3.0 with a 330 g/l solution of *phosphoric acid R*,

— *mobile phase B*: mix 400 ml of mobile phase A and 600 ml of *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	70	30
2 - 15	70 → 30	30 → 70
15 - 20	30 → 70	70 → 30

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Relative retention With reference to homatropine methylbromide (retention time = about 4.8 min): impurity C = about 0.7; impurity A = about 0.9; impurity B = about 1.2; impurity D = about 1.3; impurity E = about 1.4; impurity F = about 1.7.

System suitability Reference solution (c):

— *resolution*: minimum 2.5 between the peaks due to homatropine methylbromide and impurity B,

— *symmetry factor*: maximum 2.5 for the peak due to homatropine methylbromide.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *impurities C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent); disregard the peak due to the bromide ion which appears close to the peak due to the solvent,

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 10 ml of *water R*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20)*, using a silver indicator electrode and a silver-silver chloride reference electrode.

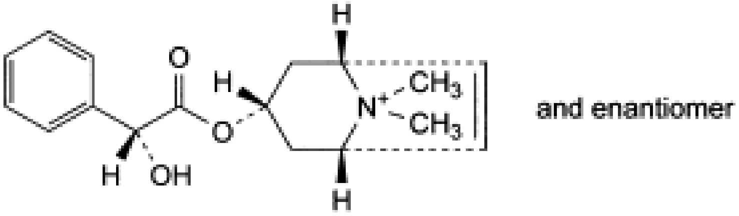
1 ml of *0.1 M silver nitrate* is equivalent to 37.03 mg of $C_{17}H_{24}BrNO_3$.

STORAGE

Protected from light.

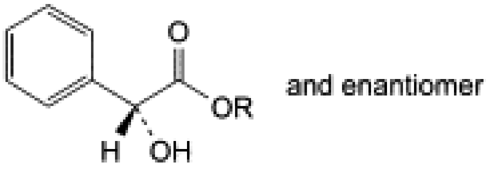
IMPURITIES

Specified impurities *A, B, C, D, E, F*.



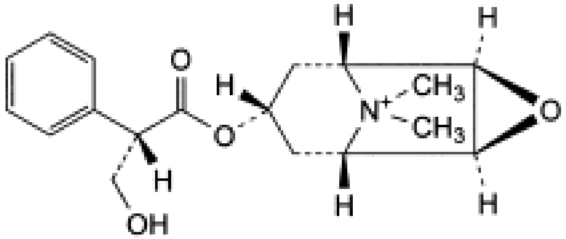
A. (1*R*,3*s*,5*S*)-3-[[(2*RS*)-2-hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1] oct-6-ene (methyldehydrohomatropine),

B. homatropine,



C. R = H: (2*RS*)-2-hydroxy-2-phenylacetic acid (mandelic acid),

F. R = CH_3: methyl (2*RS*)-2-hydroxy-2-phenylacetate (methyl mandelate),



D. (1*R*,2*R*,4*S*,5*S*,7*s*)-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane (methylhyoscine),

E. methylatropine.

Ph Eur

Honey

General Notices

(Ph Eur monograph 2051)

Ph Eur

DEFINITION

Honey is produced by bees (*Apis mellifera* L.) from the nectar of plants or from secretions of living parts of plants which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature.

PRODUCTION

If the bee has been exposed to treatment to prevent or cure diseases or to any substance intended for preventing, destroying or controlling any pest, unwanted species of plants or animals, appropriate steps are taken to ensure that the levels of residues are as low as possible.

CHARACTERS

Appearance

Viscous liquid which may be partly crystalline, almost white to dark brown.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.6 g of the substance to be examined in 50 ml of *ethanol (30 per cent V/V) R*.

Reference solution Dissolve 0.5 g of *fructose R,* 0.5 g of *glucose R* and 0.1 g of *sucrose R* in 100 ml of *ethanol (30 per cent V/V) R.*

Plate TLC silica gel plate R.

Mobile phase Water R, *acetonitrile R* (13:87 *V/V*).

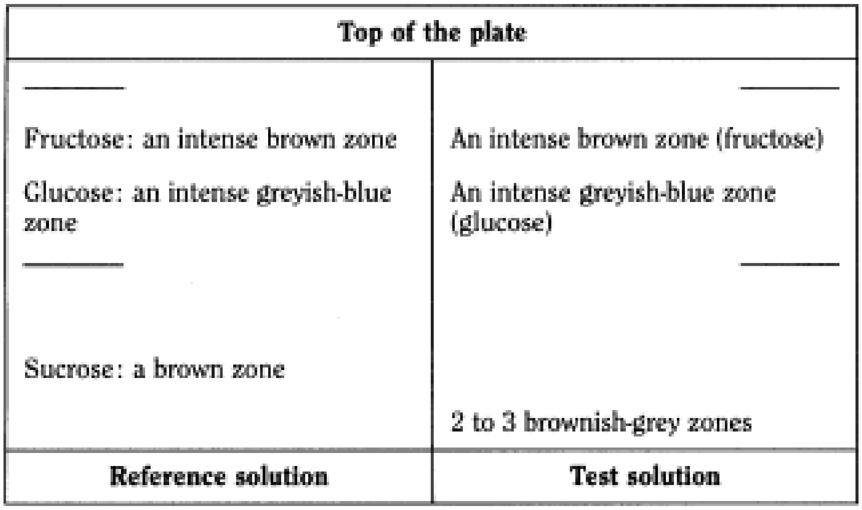
Application 5 µl as bands.

Development 3 times over a path of 15 cm.

Drying In warm air.

Detection Spray with a solution prepared as follows: dissolve 2 g of *diphenylamine R* and 2 ml of *aniline R* in 100 ml of *acetone R*. Add a 850 g/l solution of *phosphoric acid R* until the precipitate formed dissolves again (about 15-20 ml). Examine in daylight after heating at 100-105 °C for 5-10 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, the weak brown zone due to sucrose in the chromatogram obtained with the reference solution may be present in the chromatogram obtained with the test solution. One or more other weak zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Fructose: an intense brown zone	An intense brown zone (fructose)
Glucose: an intense greyish-blue zone	An intense greyish-blue zone (glucose)
———	———
Sucrose: a brown zone	
	2 to 3 brownish-grey zones
Reference solution	**Test solution**

TESTS

Refractive index *(2.2.6)*

Minimum 1.487 (equivalent to a maximum water content of 20 per cent).

Homogenise 100 g and transfer into a flask. Close tightly and place in a water-bath at 50 ± 0.2 °C until all sugar crystals have dissolved. Cool the solution to 20 °C and rehomogenise. Immediately after rehomogenisation, cover the surface of the refractometer prism evenly with the sample. Determine the refractive index after 2 min if using an Abbe refractometer and after 4 min if using a digital refractometer. Use the average value of 2 determinations.

Conductivity *(2.2.38)*

Maximum 800 µS·cm^{-1}.

Using the value obtained for the refractive index, determine the water content of the substance to be examined from Table 2051.-1. Using this information, dissolve an amount of the substance to be examined equivalent to 20.0 g of honey dry solids, in *water R* to produce 100.0 ml.

Optical rotation *(2.2.7)*

Maximum + 0.6°.

Using the value obtained for the refractive index, determine the water content of the substance to be examined from Table 2051.-1. Using this information, dissolve an amount of the substance to be examined, equivalent to 20.0 g of honey dry solids, in 50 ml of *water R*. Add 0.2 ml of *concentrated ammonia R* and dilute to 100.0 ml with *water R*. If necessary decolourise the solution with *activated charcoal R*.

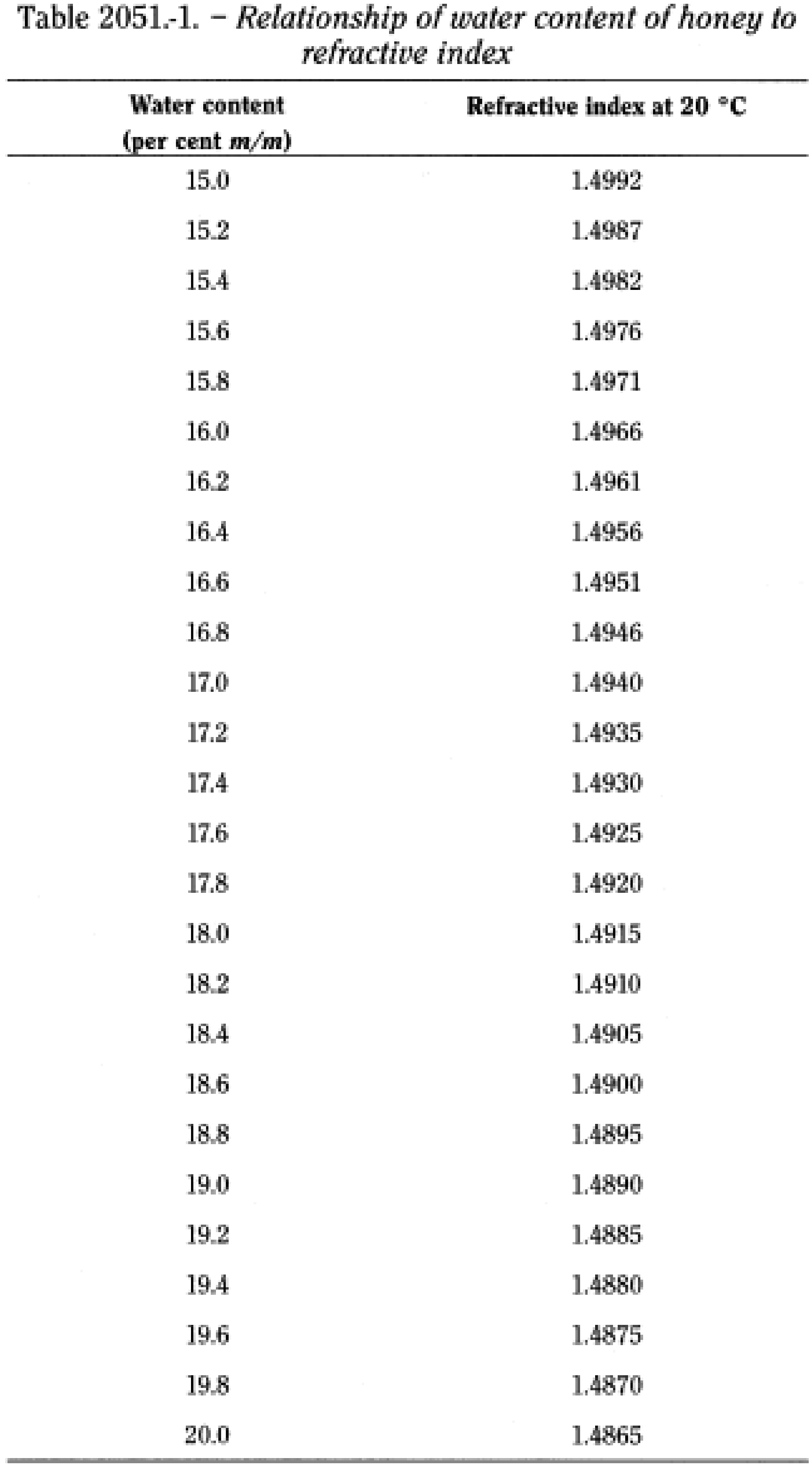


Table 2051.-1. – *Relationship of water content of honey to refractive index*

Water content (per cent *m/m*)	Refractive index at 20 °C
15.0	1.4992
15.2	1.4987
15.4	1.4982
15.6	1.4976
15.8	1.4971
16.0	1.4966
16.2	1.4961
16.4	1.4956
16.6	1.4951
16.8	1.4946
17.0	1.4940
17.2	1.4935
17.4	1.4930
17.6	1.4925
17.8	1.4920
18.0	1.4915
18.2	1.4910
18.4	1.4905
18.6	1.4900
18.8	1.4895
19.0	1.4890
19.2	1.4885
19.4	1.4880
19.6	1.4875
19.8	1.4870
20.0	1.4865

5-Hydroxymethylfurfural

Maximum 80 ppm, calculated on dry solids.

Using the value obtained for the refractive index, determine the water content of the substance to be examined from Table 2051.-1. Using this information, dissolve an amount of the substance to be examined, equivalent to 5.0 g of honey dry solids, in 25 ml of *water R* and transfer to a 50.0 ml volumetric flask with the same solvent. Add 0.5 ml of a 150 g/l solution of *potassium ferrocyanide R* and mix. Add 0.5 ml of a 300 g/l solution of *zinc acetate R*, mix and dilute to 50.0 ml with *water R* (a drop of *anhydrous ethanol R* may be added to avoid foaming). Filter. Transfer 5.0 ml of the filtered solution into each of 2 tubes. To one tube add 5.0 ml of *water R* (test solution). To the other tube add 5.0 ml of a 2.0 g/l solution of *sodium hydrogensulphite R* (reference solution). Determine the absorbance *(2.2.25)* of the test solution against the reference solution at 284 nm and 336 nm within 60 min. If the absorbance at 284 nm is greater than 0.8, dilute to the same extent the test solution with *water R* and the reference solution with a 2.0 g/l solution of *sodium hydrogensulphite R* so as to obtain an absorbance of less than 0.8.

Calculate the content of 5-hydroxymethylfurfural from the expression:

$$(A_1 - A_2) \times D \times 149.7$$

A_1 = absorbance at 284 nm,

A_2 = absorbance at 336 nm,

D = dilution factor, where applicable.

Chlorides ***(2.4.4)***

Maximum 350 ppm, determined on 15 ml of a 10 g/l solution.

Sulphates ***(2.4.13)***

Maximum 250 ppm, determined on 15 ml of a 40 g/l solution.

Ph Eur

Hyaluronidase

General Notices

(Ph Eur monograph 0912)

Action and use

Used to promote absorption of fluid into tissues.

Preparation

Hyaluronidase Injection

Ph Eur

DEFINITION

Hyaluronidase is an enzyme extracted from mammalian testes (for example bovine testes) and capable of hydrolysing mucopolysaccharides of the hyaluronic acid type. It contains not less than 300 IU of hyaluronidase activity per milligram, calculated with reference to the dried substance. It may contain a suitable stabiliser.

PRODUCTION

The animals from which hyaluronidase is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

A white or yellowish-white, amorphous powder, soluble in water, practically insoluble in acetone and in ethanol.

IDENTIFICATION

A solution containing the equivalent of 100 IU of hyaluronidase in 1 ml of a 9 g/l solution of *sodium chloride R* depolymerises an equal volume of a 10 g/l solution of *sodium hyaluronate BRP* in 1 min at 20 °C as shown by a pronounced decrease in viscosity. This action is destroyed by heating the hyaluronidase at 100 °C for 30 min.

TESTS

Appearance of solution

Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml. The solution is clear *(2.2.1)*.

pH *(2.2.3)*

Dissolve 30 mg in 10 ml of *carbon dioxide-free water R*. The pH of the solution is 4.5 to 7.5.

Loss on drying ***(2.2.32)***

Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C at a pressure not exceeding 670 Pa for 2 h.

Bacterial endotoxins ***(2.6.14)***

Less than 0.2 IU per IU of hyaluronidase.

ASSAY

The activity of hyaluronidase is determined by comparing the rate at which it hydrolyses *sodium hyaluronate BRP* with the rate obtained with the International Standard, or a reference preparation calibrated in International Units, using a slope-ratio assay.

Substrate solution To 0.10 g of *sodium hyaluronate BRP* in a 25 ml conical flask add slowly 20.0 ml of *water R* at 4 °C. The rate of addition must be slow enough to allow the substrate particles to swell (about 5 min). Maintain at 4 °C and stir for at least 12 h. Store at 4 °C and use within 4 days.

For the test solution and the reference solution, prepare the solution and carry out the dilution at 0 °C to 4 °C.

Test solution Dissolve a suitable amount of the substance to be examined in *hyaluronidase diluent R* so as to obtain a solution containing 0.6 ± 0.3 IU of hyaluronidase per millilitre.

Reference solution Dissolve a suitable amount of *hyaluronidase BRP* in *hyaluronidase diluent R* so as to obtain a solution containing 0.6 IU of hyaluronidase per millilitre.

In a reaction vessel, mix 1.50 ml of *phosphate buffer solution pH 6.4 R* and 1.0 ml of the substrate solution and equilibrate at 37 ± 0.1 °C. At time $t_1 = 0$ (first chronometer) add 0.50 ml of the test solution containing E_t mg of the enzyme to be examined, mix, measure the viscosity of the solution using a suitable viscometer maintained at 37 ± 0.1 °C and record the outflow time t_2 using a second chronometer (graduated in 0.1 second intervals), several times during about 20 min (read on the first chronometer). The following viscometer has been found suitable: Ubbelohde microviscometer (DIN 51 562, Part 2), capillary type MII, viscometer constant about 0.1 mm^2/s^2.

Repeat the procedure using 0.50 ml of the reference solution containing E_r mg of *hyaluronidase BRP*.

Calculate the viscosity ratio from the expression:

$$\eta_r = \frac{k \times t_2}{0.6915}$$

k = the viscometer constant in mm^2/s^2 (indicated on the viscometer);

t_2 = the outflow time (in seconds) of the solution;

0.6915 = the kinematic viscosity in mm^2/s of the buffer solution at 37 °C.

Since the enzymatic reaction continues during the outflow time measurements, the real reaction time equals $t_1 + t_2/2$, half of the outflow time ($t_2/2$) for which a certain measurement is valid being added to the time t_1 at which the measurement is started. Plot $(\ln \eta_r)^{-1}$ as a function of the reaction time ($t_1 + t_2/2$) in seconds. A linear relationship is obtained. Calculate the slope for the substance to be examined (b_t) and the reference preparation (b_r).

Calculate the specific activity in International Units per milligram from the expression:

$$\frac{b_t}{b_r} \times \frac{E_r}{E_t} \times A$$

A = the specific activity of *hyaluronidase BRP* in International Units per milligram.

Carry out the complete procedure at least three times and calculate the average activity of the substance to be examined.

STORAGE

Store in an airtight container at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, tamper-proof container.

LABELLING

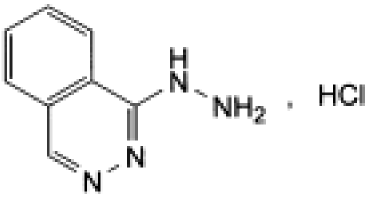
The label states the activity in International Units per milligram.

Ph Eur

Hydralazine Hydrochloride

General Notices

(Ph Eur monograph 0829)



$C_8H_8N_4$,HCl   196.6   *304-20-1*

Action and use

Vasodilator; treatment of hypertension.

Preparations

Hydralazine Injection

Hydralazine Tablets

Ph Eur

DEFINITION

1-Hydrazinophthalazine hydrochloride.

Content 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance White or almost white, crystalline powder.

Solubility Soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

mp: about 275 °C, with decomposition.

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50 mg in *water R* and dilute to 100 ml with the same solvent. Dilute 2 ml of this solution to 100 ml with *water R*.

Spectral range 220-350 nm.

Absorption maxima At 240 nm, 260 nm, 303 nm and 315 nm.

Absorbance ratio A_{240}/A_{303} = 2.0 to 2.2.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison hydralazine hydrochloride CRS.

C. Dissolve 0.5 g in a mixture of 8 ml of *dilute hydrochloric acid R* and 100 ml of *water R*. Add 2 ml of *sodium nitrite solution R*, allow to stand for 10 min and filter. The precipitate, washed with *water R* and dried at 100-105 °C, melts *(2.2.14)* at 209 °C to 212 °C.

D. Dissolve about 10 mg in 2 ml of *water R*. Add 2 ml of a 20 g/l solution of *nitrobenzaldehyde R* in *ethanol (96 per cent) R*. An orange precipitate is formed.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution GY_6 *(2.2.2, Method II)*.

Dilute 4 ml of solution S to 20 ml with *water R*.

pH *(2.2.3)*

3.5 to 4.2 for solution S.

Hydrazine

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.12 g of the substance to be examined in 4 ml of *water R* and add 4 ml of a 150 g/l solution of *salicylaldehyde R* in *methanol R* and 0.2 ml of *hydrochloric acid R*. Mix and keep at a temperature not exceeding 25 °C for 2-4 h, until the precipitate formed has sedimented. Add 4 ml of *toluene R*, shake vigorously and centrifuge. Transfer the clear supernatant liquid to a 100 ml separating funnel and shake vigorously, each time for 3 min, with 2 quantities, each of 20 ml, of a 200 g/l solution of *sodium metabisulphite R* and with 2 quantities, each of 50 ml, of *water R*. Separate the upper toluene layer which is the test solution.

Reference solution (a) Dissolve 12 mg of *hydrazine sulphate R* in *dilute hydrochloric acid R* and dilute to 100.0 ml with the same acid. Dilute 1.0 ml of this solution to 100.0 ml with *dilute hydrochloric acid R*.

Reference solution (b) Prepare the solution at the same time and in the same manner as for the test solution, using 1.0 ml of reference solution (a) and 3 ml of *water R*.

Plate TLC silica gel G plate R.

Mobile phase *ethanol (96 per cent) R*, *toluene R* (10:90 *V/V*).

Application 20 µl of the test solution and reference solution (b).

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Limit:

— *hydrazine*: any yellow fluorescent spot due to hydrazine is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (10 ppm).

Related substances

Liquid chromatography *(2.2.29)*. *The solutions must be injected within one working day.*

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 10.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *phthalazine R* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 4.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (d) Dilute a mixture of 4.0 ml of the test solution and 10.0 ml of reference solution (c) to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *nitrile silica gel for chromatography R1* (10 µm).

Mobile phase: mix 22 volumes of *acetonitrile R* and 78 volumes of a solution containing 1.44 g/l of *sodium laurilsulfate R* and 0.75 g/l of *tetrabutylammonium bromide R*, then adjust to pH 3.0 with *0.05 M sulphuric acid*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 3 times the retention time of hydralazine.

Retention time hydralazine = about 10 min to 12 min; if necessary, adjust the concentration of acetonitrile in the mobile phase.

System suitability:

— the chromatogram obtained with reference solution (d) shows 2 principal peaks;

— *resolution*: minimum 2.5 between the peaks due to hydralazine and phtalazine in the chromatogram obtained with reference solution (d);

— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (b).

Limit:

- *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo*.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 80.0 mg in 25 ml of *water R*. Add 35 ml of *hydrochloric acid R* and titrate with *0.05 M potassium iodate*, determining the end-point potentiometrically *(2.2.20)*, using a calomel reference electrode and a platinum indicator electrode.

1 ml of *0.05 M potassium iodate* is equivalent to 9.832 mg of $C_8H_9ClN_4$.

STORAGE

Protected from light.

Ph Eur

Hydrochloric Acid

General Notices

(Concentrated Hydrochloric Acid, Ph Eur monograph 0002)

HCl 36.46 *7647-01-0*

Preparation

Dilute Hydrochloric Acid

Ph Eur

DEFINITION

Content

35.0 per cent *m/m* to 39.0 per cent *m/m*.

CHARACTERS

Appearance

Clear, colourless, fuming liquid.

Solubility

Miscible with water.

Relative density

About 1.18.

IDENTIFICATION

A. Dilute with *water R*. The solution is strongly acid *(2.2.4)*.

B. It gives the reactions of chlorides *(2.3.1)*.

C. It complies with the limits of the assay.

TESTS

Appearance of solution

To 2 ml add 8 ml of *water R*. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Free chlorine

Maximum 4 ppm.

To 15 ml add 100 ml of *carbon dioxide-free water R*, 1 ml of a 100 g/l solution of *potassium* *iodide R* and 0.5 ml of *iodide-free starch solution R*. Allow to stand in the dark for 2 min. Any blue colour disappears on the addition of 0.2 ml of *0.01 M sodium thiosulphate*.

Sulphates ***(2.4.13)***

Maximum 20 ppm.

To 6.4 ml add 10 mg of *sodium hydrogen carbonate R* and evaporate to dryness on a water-bath. Dissolve the residue in 15 ml of *distilled water R*.

Heavy metals ***(2.4.8)***

Maximum 2 ppm.

Dissolve the residue obtained in the test for residue on evaporation in 1 ml of *dilute hydrochloric acid R* and dilute to 25 ml with *water R*. Dilute 5 ml of this solution to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Residue on evaporation

Maximum 0.01 per cent.

Evaporate 100.0 g to dryness on a water-bath and dry at 100-105 °C. The residue weighs a maximum of 10 mg.

ASSAY

Weigh accurately a ground-glass-stoppered flask containing 30 ml of *water R*. Introduce 1.5 ml of the acid to be examined and weigh again. Titrate with *1 M sodium hydroxide*, using *methyl red solution R* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 36.46 mg of HCl.

STORAGE

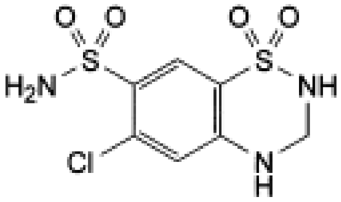
In a stoppered container made of glass or another inert material, at a temperature not exceeding 30 °C.

Ph Eur

Hydrochlorothiazide

General Notices

(Ph Eur monograph 0394)



$C_7H_8ClN_3O_4S_2$ 297.7 *58-93-5*

Action and use

Thiazide diuretic.

Preparations

Co-amilozide Oral Solution

Co-amilozide Tablets

Co-triamterzide Tablets

Hydrochlorothiazide Tablets

Ph Eur

DEFINITION

6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in 10 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with *water R*. Dilute 2.0 ml of this solution to 100.0 ml with *0.01 M sodium hydroxide*.

Spectral range 250-350 nm.

Absorption maxima At 273 nm and 323 nm.

Absorbance ratio A_{273}/A_{323} = 5.4 to 5.7.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *hydrochlorothiazide CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol R1*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *hydrochlorothiazide CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 25 mg of *chlorothiazide R* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethyl acetate R.*

Application 2 µl.

Development Over a path of 10 cm.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Gently heat about 1 mg with 2 ml of a freshly prepared 0.5 g/l solution of *chromotropic acid, sodium salt R* in a cooled mixture of 35 volumes of *water R* and 65 volumes of *sulphuric acid R*. A violet colour develops.

TESTS

Acidity or alkalinity

Shake 0.5 g of the powdered substance to be examined with 25 ml of *water R* for 2 min and filter. To 10 ml of the filtrate, add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution R*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture Dilute 50.0 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R* to 200.0 ml with *phosphate buffer solution pH 3.2 R1*.

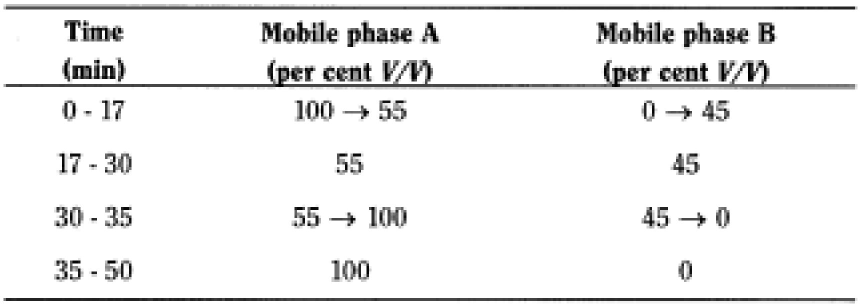
Test solution Dissolve 30.0 mg of the substance to be examined in 5 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R*, using sonication if necessary, and dilute to 20.0 ml with *phosphate buffer solution pH 3.2 R1*.

Reference solution (a) Dissolve 15 mg of *hydrochlorothiazide CRS* and 15 mg of *chlorothiazide CRS* (impurity A) in 25 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R*, using sonication if necessary, and dilute to 100 ml with *phosphate buffer solution pH 3.2 R1*. Dilute 5 ml of this solution to 100 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 20.0 ml with the solvent mixture.

Column:
- *size: l* = 0.1 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: to 940 ml of *phosphate buffer solution pH 3.2 R1* add 60.0 ml of *methanol R* and 10.0 ml of *tetrahydrofuran R* and mix;
- *mobile phase B*: to a mixture of 500 ml of *methanol R* and 500 ml of *phosphate buffer solution pH 3.2 R1* add 50.0 ml of *tetrahydrofuran R* and mix;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 17	100 → 55	0 → 45
17 - 30	55	45
30 - 35	55 → 100	45 → 0
35 - 50	100	0

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 224 nm.

Equilibration With mobile phase A for at least 20 min.

Injection 10 µl; inject the solvent mixture as a blank.

Retention time Impurity A = about 7 min; hydrochlorothiazide = about 8 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to impurity A and hydrochlorothiazide; if necessary, adjust slightly the composition of the mobile phase or the time programme of the linear gradient.

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides ***(2.4.4)***

Maximum 100 ppm.

Dissolve 1.0 g in 25 ml of *acetone R* and dilute to 30 ml with *water R*. Prepare the standard using 5 ml of *acetone R* containing 15 per cent *V/V* of *water R* and 10 ml of *chloride standard solution (5 ppm Cl) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

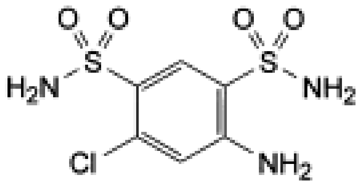
Dissolve 0.120 g in 50 ml of *dimethyl sulphoxide R*. Titrate with *0.1 M tetrabutylammonium hydroxide in 2-propanol*, determining the end-point potentiometrically *(2.2.20)* at the 2nd point of inflexion. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 14.88 mg of $C_7H_8ClN_3O_4S_2$.

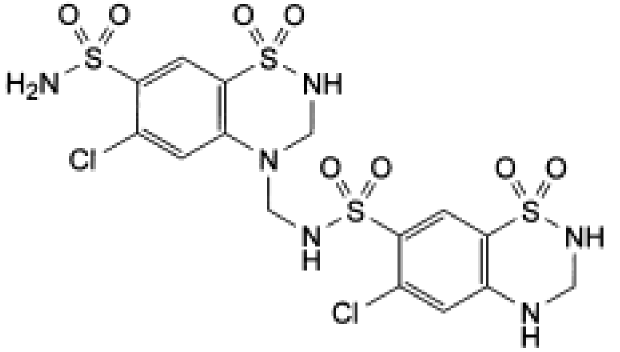
IMPURITIES

Specified impurities *A, B, C*.

A. chlorothiazide,



B. 4-amino-6-chlorobenzene-1,3-disulphonamide (salamide),



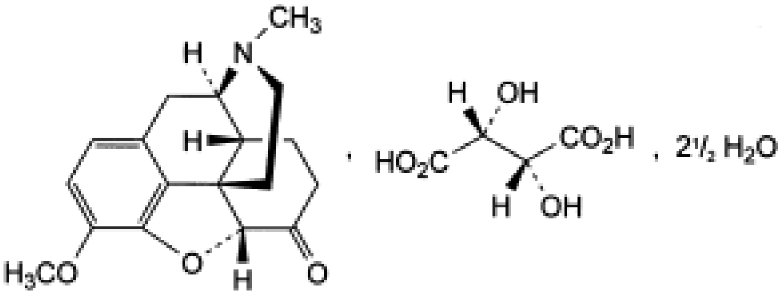
C. 6-chloro-*N*-[(6-chloro-7-sulphamoyl-2,3-dihydro-4*H*-1,2,4-benzothiadiazin-4-yl 1,1-dioxide)methyl]-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.

Ph Eur

Hydrocodone Hydrogen Tartrate Hydrate

General Notices

(Hydrocodone Hydrogen Tartrate 2.5-Hydrate, Ph Eur monograph 1784)



$C_{18}H_{21}NO_3,C_4H_6O_6,2½H_2O$ 494.5 *34195-34-1*

Action and use

Opioid receptor agonist; antitussive.

Ph Eur

DEFINITION

4,5α-Epoxy-3-methoxy-17-methylmorphinan-6-one hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate 2.5-hydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble or soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in cyclohexane.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison hydrocodone hydrogen tartrate 2.5-hydrate CRS.

If the spectra obtained in the solid state show differences, dry the substance to be examined and the reference substance at 105 °C and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Dissolve 0.5 g in *water R* and dilute to 10 ml with the same solvent.

pH *(2.2.3)*

3.2 to 3.8.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 87 to - 91 (anhydrous substance).

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a) Dissolve 5 mg of *oxycodone hydrochloride CRS* (impurity D) in mobile phase A, add 0.5 ml of the test solution and dilute to 5.0 ml with mobile phase A.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (c) Dissolve 20 mg of *benzophenone CRS* (impurity H) in 50.0 ml of *methanol R*. Dilute 1.0 ml of this solution to 20.0 ml with mobile phase A.

Reference solution (d) Dissolve the contents of a vial of *hydrocodone for peak identification CRS* in 1 ml of mobile phase A.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

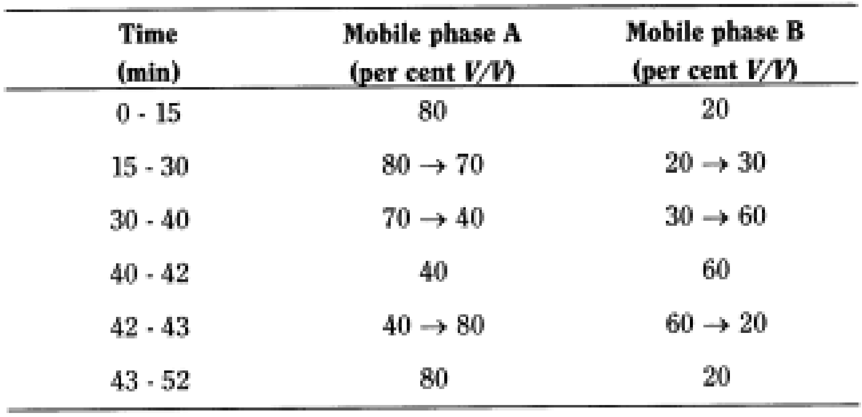
— *stationary phase*: *octadecylsilyl silica gel for chromatography end-capped R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: dissolve 1.08 g of *sodium octanesulphonate R* in *water R*, adjust to pH 2.0 with *phosphoric acid R* and dilute to 1000 ml with *water R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 15	80	20
15 - 30	80 → 70	20 → 30
30 - 40	70 → 40	30 → 60
40 - 42	40	60
42 - 43	40 → 80	60 → 20
43 - 52	80	20

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 283 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *hydrocodone for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, B, C, D, E, F and I.

Relative retention With reference to hydrocodone (retention time = about 14 min): impurity A = about 0.3; impurity K = about 0.43; impurity B = about 0.57; impurity C = about 0.61; impurity D = about 0.9; impurity E = about 1.1; impurity F = about 1.5; impurity I = about 2.0; impurity H = about 2.9.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to hydrocodone and impurity D.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity I by 0.2;

— *impurity I*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity H*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *impurities A, B, C, D, E, F, K*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

7.0 per cent to 12.0 per cent, determined on 0.100 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 44.95 mg of $C_{22}H_{27}NO_9$.

STORAGE

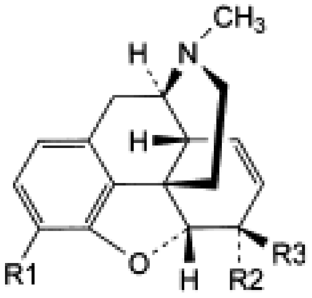
In an airtight container , protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F, H, I, K*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10*. *Control of impurities in substances for pharmaceutical use)*

G, J.

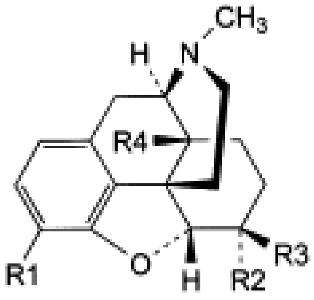


A. R1 = R2 = OH, R3 = H: morphine,

C. R1 = OCH_3, R2 = OH, R3 = H: codeine,

E. R1 = OCH_3, R2 + R3 = O: 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one (codeinone),

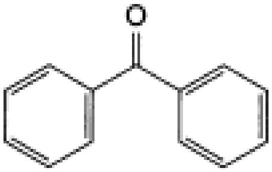
F. R1 = R2 = OCH_3, R3 = H: 7,8-didehydro-4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan (methylcodeine),



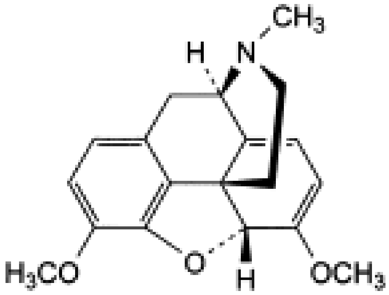
B. R1 = OCH_3, R2 = OH, R3 = R4 = H: 4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol (dihydrocodeine),

D. R1 = OCH_3, R2 + R3 = O, R4 = OH: 4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one (oxycodone),

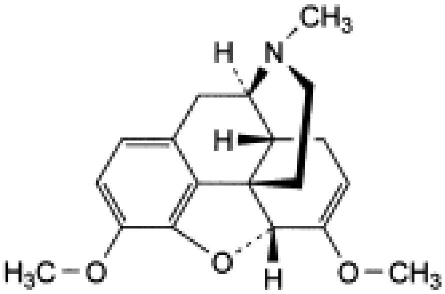
G. R1 = R2 = OCH_3, R3 = R4 = H: 4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan (tetrahydrothebaine),



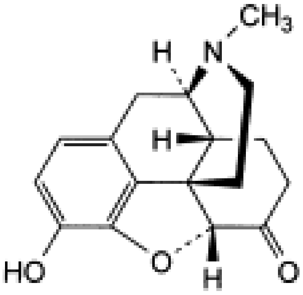
H. diphenylmethanone (benzophenone),


CH3
H
N
H3CO
O
H
OCH3

I. 6,7,8,14-tetradehydro-4,5α-epoxy-3,6-dimethoxy-17-methylmorphinan (thebaine),


CH3
H
N
H
H3C−O
O
H
O−CH3

J. (5α)-3,6-dimethoxy-17-methyl-6,7-didehydro-4,5-epoxymorphinan (dihydrothebaine),


CH3
H
N
H
HO
O
H
O

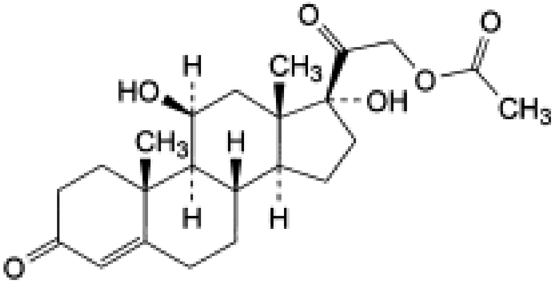
K. (5α)-3-hydroxy-17-methyl-4,5-epoxymorphinan-6-one (hydromorphone).

Ph Eur

Hydrocortisone Acetate

General Notices

(Ph Eur monograph 0334)



$C_{23}H_{32}O_6$ 404.5 *50-03-3*

Action and use

Corticosteroid.

Preparations

Gentamicin and Hydrocortisone Acetate Ear Drops

Hydrocortisone Acetate Cream

Hydrocortisone Acetate and Neomycin Ear Drops

Hydrocortisone Acetate and Neomycin Eye Drops

Hydrocortisone Acetate and Neomycin Eye Ointment

Hydrocortisone Acetate Injection

Hydrocortisone Acetate Ointment

Miconazole and Hydrocortisone Acetate Cream

Ph Eur

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregn-4-en-21-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in anhydrous ethanol and in methylene chloride.

mp: about 220 °C, with decomposition.

IDENTIFICATION

First identification *A, B.*

Second identification *C, D, E.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *hydrocortisone acetate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *hydrocortisone acetate CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *cortisone acetate R* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

C. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of this solution to 10 ml with *methylene chloride R.*

Test solution (b) Transfer 2 ml of solution A to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C protected from light for 2 h 30 min. Allow to cool.

Reference solution (a) Dissolve 25 mg of *hydrocortisone acetate CRS* in *methanol R* and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Reference solution (b) Transfer 2 ml of solution B to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C protected from light for 2 h 30 min. Allow to cool.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulphuric acid R* and heat at 120 °C for 10 min or until the spots appear and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_F value distinctly lower than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min an intense brownish-red colour develops with a green fluorescence which is particularly intense when viewed in ultraviolet light at 365 nm. Add this solution to 10 ml of *water R* and mix. The colour fades and the fluorescence in ultraviolet light does not disappear.

E. About 10 mg gives the reaction of acetyl *(2.3.1)*.

TESTS

Specific optical rotation *(2.2.7)*

+ 158 to + 167 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *hydrocortisone acetate CRS* and 2 mg of *cortisone acetate R* in the mobile phase, then dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 400 ml of *acetonitrile R* with 550 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time 2.5 times the retention time of hydrocortisone acetate.

Retention time Hydrocortisone acetate = about 10 min; cortisone acetate = about 12 min.

System suitability Reference solution (a):

- *resolution*: minimum 4.2 between the peaks due to hydrocortisone acetate and cortisone acetate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:
- *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), and not more than one such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 241.5 nm.

Calculate the content of $C_{23}H_{32}O_6$ taking the specific absorbance to be 395.

STORAGE

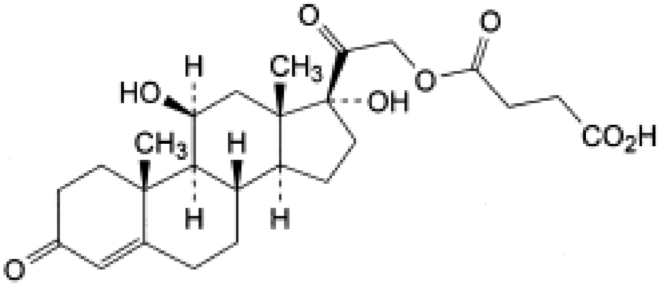
Protected from light.

Ph Eur

Hydrocortisone Hydrogen Succinate

General Notices

(Ph Eur monograph 0768)



$C_{25}H_{34}O_8$ 462.5 *2203-97-6*

Action and use

Corticosteroid.

Preparations

Hydrocortisone Sodium Succinate Injection

Hydrocortisone Oromucosal Tablets

Ph Eur

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregn-4-en-21-yl hydrogen butanedioate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in anhydrous ethanol. It dissolves in dilute solutions of alkali carbonates and alkali hydroxides.

IDENTIFICATION

First identification *A, B.*

Second identification *C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dry the substances before use at 100-105 °C for 3 h.

Comparison *hydrocortisone hydrogen succinate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *hydrocortisone hydrogen succinate CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *methylprednisolone hydrogen succinate CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *anhydrous formic acid R*, *anhydrous ethanol R*, *methylene chloride R* (0.1:1:15 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

C. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* with gentle heating and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Test solution (b) Transfer 2 ml of solution A to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of a 0.8 g/l solution of *sodium hydroxide R* in *methanol R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 30 min. Allow to cool.

Reference solution (a) Dissolve 25 mg of *hydrocortisone hydrogen succinate CRS* in *methanol R* with gentle heating and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Reference solution (b) Transfer 2 ml of solution B to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of a 0.8 g/l solution of *sodium hydroxide R* in *methanol R* and immediately pass a stream of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 30 min. Allow to cool.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Developement Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulphuric acid R.* Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) has an R_F value distinctly higher than that of the principal spot in each of the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, an intense brownish-red colour develops with a green fluorescence which is particularly intense when viewed in ultraviolet light at 365 nm. Add this solution to 10 ml of *water R* and mix. The colour fades and a clear solution remains. The fluorescence in ultraviolet light does not disappear.

TESTS

Appearance of solution

The solution is clear *(2.2.1)*.

Dissolve 0.10 g in 5 ml of *sodium hydrogen carbonate solution R*.

Specific optical rotation *(2.2.7)*

+ 147 to + 153 (dried substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 2 mg of *hydrocortisone hydrogen succinate CRS* and 2 mg of *dexamethasone CRS* in 50 ml of *acetonitrile R*, then dilute to 100.0 ml with *water R.*

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 330 ml of *acetonitrile R* with 600 ml of *water R* and 1.0 ml of *phosphoric acid R*, then allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time Twice the retention time of hydrocortisone hydrogen succinate.

Retention time Dexamethasone = about 12.5 min; hydrocortisone hydrogen succinate = about 15 min.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to dexamethasone and hydrocortisone hydrogen succinate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *impurities A, B*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 4.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent.

Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 241.5 nm.

Calculate the content of $C_{25}H_{34}O_8$ taking the specific absorbance to be 353.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities *A, B.*

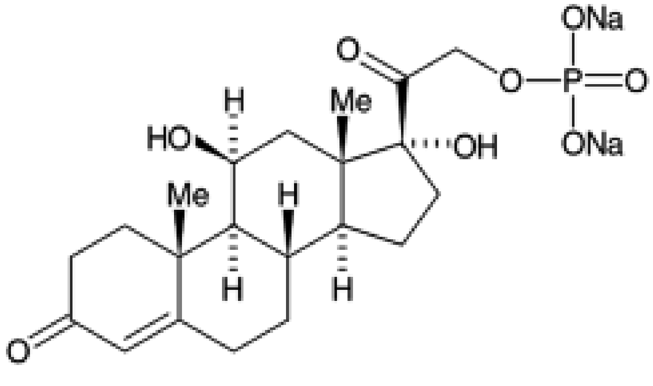
A. hydrocortisone,

B. hydrocortisone acetate.

Ph Eur

Hydrocortisone Sodium Phosphate

General Notices



$C_{21}H_{29}Na_2O_8P$ 486.4 *6000-74-4*

Action and use

Corticosteroid.

Preparations

Hydrocortisone Sodium Phosphate Injection

Hydrocortisone Sodium Phosphate Oral Solution

DEFINITION

Hydrocortisone Sodium Phosphate is disodium 11β,17α-dihydroxy-3,20-dioxopregn-4-en-21-yl orthophosphate. It contains not less than 96.0% and not more than 103.0% of $C_{21}H_{29}Na_2O_8P$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white or almost white powder; hygroscopic.

Freely soluble in *water*; practically insoluble in *absolute ethanol* .

IDENTIFICATION

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. The *infrared absorption spectrum*, Apppendix II A, is concordant with the *reference spectrum* of hydrocortisone sodium phosphate (*RS 386*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a freshly prepared mixture of 60 volumes of *butan-1-ol*, 20 volumes of *acetic anhydride* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. Solution (1) contains 0.25% w/v of the substance being examined in *methanol*. Solution (2) contains 0.25% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). Solution (4) is a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *betamethasone sodium phosphate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air until the solvent has evaporated, spray with *ethanolic sulphuric acid (20%)*, heat at 120° for 10 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot and the chromatogram obtained with solution (4) shows two principal spots with almost identical R_f values.

C. Dissolve 2 mg in 2 ml of *sulphuric acid*. A yellowish green fluorescence is produced immediately (distinction from betamethasone sodium phosphate, dexamethasone sodium phosphate and prednisolone sodium phosphate).

D. Heat gently 40 mg with 2 ml of *sulphuric acid* until white fumes are evolved, add *nitric acid* dropwise until oxidation is complete and cool. Add 2 ml of *water*, heat until white fumes are again evolved, cool, add 10 ml of *water* and neutralise to *litmus paper* with 5M *ammonia*. The resulting solution yields reaction A characteristic of *sodium salts* and reaction B characteristic of *phosphates*, Appendix VI.

TESTS

Alkalinity

pH of a 0.5% w/v solution, 7.5 to 9.0, Appendix V L.

Specific optical rotation

In a 1% w/v solution, +121 to +129, calculated with reference to the anhydrous substance, Appendix V F.

Inorganic phosphate

Dissolve 25 mg in 10 ml of *water*, add 4 ml of 1M *sulphuric acid*, 1 ml of a 10% w/v solution of *ammonium molybdate* and 2 ml of *methylaminophenol-sulphite reagent* and allow to stand for 15 minutes. Add sufficient *water* to produce 25 ml and allow to stand for a further 15 minutes. The *absorbance* of a 4-cm layer of the resulting solution at 730 nm, Appendix II B, is not more than that of a 4-cm layer of a solution prepared by treating 10 ml of a 0.0036% w/v solution of *potassium dihydrogen orthophosphate* in the same manner, beginning at the words 'add 4 ml …'.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 2 µl of each of three solutions in *methanol* containing (1) 1.0% w/v of the substance being examined, (2) 1.0% w/v of *hydrocortisone sodium phosphate BPCRS* and (3) 0.020% w/v of *hydrocortisone BPCRS*. After removal of the plate, allow it to dry in air for 5 minutes and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (2%).

Water

Not more than 10.0%, Appendix IX C. Use 0.4 g.

ASSAY

Dissolve 0.1 g in sufficient *water* to produce 200 ml. Dilute 5 ml to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B. Calculate the content of $C_{21}H_{29}Na_2O_8P$ taking 333 as the value of A(1%, 1 cm) at the maximum at 248 nm.

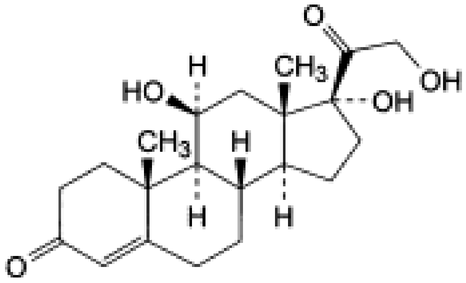
STORAGE

Hydrocortisone Sodium Phosphate should be protected from light.

Hydrocortisone

General Notices

(Ph Eur monograph 0335)



$C_{21}H_{30}O_5$ 362.5 *50-23-7*

Action and use

Corticosteroid.

Preparations

Hydrocortisone Cream

Hydrocortisone and Clioquinol Cream

Hydrocortisone and Neomycin Cream

Hydrocortisone Ointment

Hydrocortisone and Clioquinol Ointment

Miconazole and Hydrocortisone Cream

Miconazole and Hydrocortisone Ointment

Ph Eur

DEFINITION

Hydrocortisone contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of 11β,17,21-trihydroxypregn-4-ene-3,20-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in acetone and in alcohol, slightly soluble in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification A, B.

Second identification C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *hydrocortisone CRS*. If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dissolve 20 mg of *hydrocortisone CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *prednisolone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Apply to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R.* Develop over a path of 15 cm. Carry out a second development over a path of 15 cm using a mixture of 5 volumes of *butanol R* saturated with *water R*, 15 volumes of *toluene R* and 80 volumes of *ether R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine the chromatograms in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent. This solution is also used to prepare test solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Test solution (b) Transfer 0.4 ml of the solution obtained during preparation of test solution (a) to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension for 1 h in a mechanical shaker, protected from light. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 ml separating funnel, washing the filter with two quantities, each of 5 ml, of *water R*. Shake the clear filtrate with 10 ml of *methylene chloride R.* Wash the organic layer with 5 ml of *1 M sodium hydroxide* and two quantities, each of 5 ml, of *water R*. Dry over *anhydrous sodium sulphate R*.

Reference solution (a) Dissolve 25 mg of *hydrocortisone CRS* in *methanol R* and dilute to 5 ml with the same solvent. This solution is also used to prepare reference solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Reference solution (b) Transfer 0.4 ml of the solution obtained during preparation of reference solution (a) to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R.* Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension for 1 h in a mechanical shaker protected from light. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 ml separating funnel, washing the filter with two quantities, each of 5 ml, of *water R*. Shake the clear filtrate with 10 ml of *methylene chloride R*. Wash the organic layer with 5 ml of *1 M sodium hydroxide* and two quantities, each of 5 ml, of *water R*. Dry over *anhydrous sodium sulphate R.*

Apply to the plate 5 µl of test solution (a), 5 µl of reference solution (a), 25 µl of test solution (b) and 25 µl of reference solution (b), applying the latter two in small quantities to obtain small spots. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R.* Develop over a path of 15 cm. Carry out a second development over a path of 15 cm using a mixture of 5 volumes of *butanol R* saturated with *water R*, 15 volumes of *toluene R* and 80 volumes of *ether R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *alcoholic solution of sulphuric acid R* and heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine the plate in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_F value distinctly higher than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, an intense brownish-red colour develops with a green fluorescence which is particularly intense when examined in ultraviolet light at 365 nm. Add the solution to 10 ml of *water R* and mix. The colour fades and a clear solution remains. The fluorescence in ultraviolet light does not disappear.

TESTS

Specific optical rotation *(2.2.7)*

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 150 to + 156, calculated with reference to the dried substance.

Related substances

Examine by liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 25.0 mg of the substance to be examined in 2 ml of *tetrahydrofuran R* and dilute to 10.0 ml with *water R*.

Reference solution (a) Dissolve 2 mg of *hydrocortisone CRS* and 2 mg of *prednisolone CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1 ml/min a mixture prepared as follows: in a 1000 ml volumetric flask mix 220 ml of *tetrahydrofuran R* with 700 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again,

— as detector a spectrophotometer set at 254 nm, maintaining the temperature of the column at 45 °C.

Equilibrate the column with the mobile phase at a flow rate of 1 ml/min for about 30 min.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 20 µl of reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject 20 µl of reference solution (a). When the chromatograms are recorded in the prescribed conditions, the retention times are: prednisolone about 14 min and hydrocortisone about 15.5 min. The test is not valid unless the resolution between the peaks due to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of *tetrahydrofuran R* in the mobile phase.

Inject separately 20 µl of the solvent mixture of the test solution as a blank, 20 µl of the test solution and 20 µl of reference solution (b). Continue the chromatography of the test solution for four times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent): the sum of the areas of all the peaks, apart from the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Disregard any peak obtained with the blank run and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *alcohol R*. Measure the absorbance *(2.2.25)* at the maximum at 241.5 nm.

Calculate the content of $C_{21}H_{30}O_5$ taking the specific absorbance to be 440.

STORAGE

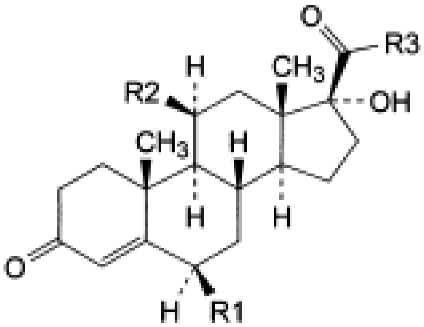
Store protected from light.

IMPURITIES

A. prednisolone,

B. cortisone,

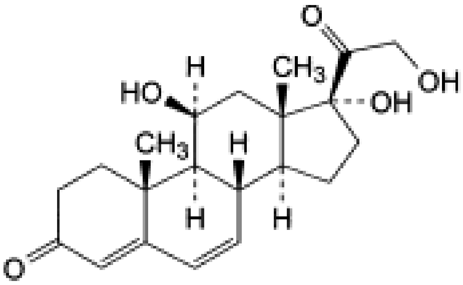
C. hydrocortisone acetate,



D. R1 = R2 = OH, R3 = CH_2OH: 6β,11β,17,21-tetrahydroxypregn-4-ene-3,20-dione (6β-hydroxyhydrocortisone),

F. R1 = R2 = H, R3 = CH_2OH: 17,21-dihydroxypregn-4-ene-3,20-dione (Reichstein's substance),

G. R1 = H, R2 = OH, R3 = CHO: 11β,17-dihydroxy-3,20-dioxopregn-4-en-21-al,

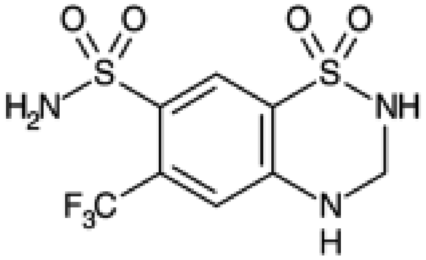


E. 11β,17,21-trihydroxypregna-4,6-diene-3,20-dione (Δ6-hydrocortisone).

Ph Eur

Hydroflumethiazide

General Notices



$C_8H_8F_3N_3O_4S_2$ 331.3 *135-09-1*

Action and use

Thiazide diuretic.

Preparation

Hydroflumethiazide Tablets

DEFINITION

Hydroflumethiazide is 3,4-dihydro-6-trifluoromethyl-2*H*-1,2,4-benzothiadiazine-7sulphonamide 1,1-dioxide. It contains not less than 98.0% and not more than 102.0% of $C_8H_8F_3N_3O_4S_2$, calculated with reference to the dried substance.

CHARACTERISTICS

White or almost white, glistening crystals or crystalline powder.

Practically insoluble in *water*; soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydroflumethiazide *(RS 181)*.

B. Dissolve 10 mg in 10 ml of 0.1M *sodium hydroxide*, add sufficient *water* to produce 100 ml and dilute 10 ml to 50 ml with 0.01M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 274 nm and 333 nm. The *absorbance* at the maxima is about 0.92 and about 0.19 respectively.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in *acetone* containing (1) 0.1% w/v of the substance being examined and (2) 0.1% w/v of *hydroflumethiazide BPCRS*. After removal of the plate, dry it in a current of air, examine under *ultraviolet light (254 nm)* and then reveal the spots by

Method I and examine again. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions of the substance being examined in *acetone* containing (1) 1.0% w/v and (2) 0.010% w/v. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried to constant weight at 105°C, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.3 g in 50 ml of *anhydrous pyridine* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 16.56 mg of $C_8H_8F_3N_3O_4S_2$.

Hydrogen Peroxide Solution (3 per cent)

General Notices

Dilute Hydrogen Peroxide Solution
(Ph Eur monograph 0395)

Action and use

Antiseptic; deodorant.

When hydrogen peroxide is prescribed or demanded, Hydrogen Peroxide Solution (6 per cent) shall be dispensed or supplied.

Ph Eur

DEFINITION

Content

2.5 per cent *m/m* to 3.5 per cent *m/m* of H_2O_2 (M_r 34.01).

1 volume of hydrogen peroxide solution (3 per cent) corresponds to about 10 times its volume of oxygen. A suitable stabiliser may be added.

CHARACTERS

Appearance

Colourless, clear liquid.

IDENTIFICATION

A. To 2 ml add 0.2 ml of *dilute sulphuric acid R* and 0.2 ml of *0.02 M potassium permanganate*. The solution becomes colourless or slightly pink within 2 min.

B. To 0.5 ml add 1 ml of *dilute sulphuric acid R*, 2 ml of *ether R* and 0.1 ml of *potassium chromate solution R* and shake. The ether layer is blue.

C. It complies with the requirement for the content of H_2O_2.

TESTS

Acidity

To 10 ml add 20 ml of *water R* and 0.25 ml of *methyl red solution R*. Not less than 0.05 ml and not more than 1.0 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Organic stabilisers

Maximum 250 ppm.

Shake 20 ml with 10 ml of *chloroform R* and then with 2 quantities, each of 5 ml, of *chloroform R*. Evaporate the combined chloroform layers under reduced pressure at a temperature not exceeding 25 °C and dry in a desiccator. The residue weighs a maximum of 5 mg.

Non-volatile residue

Maximum 2 g/l.

Allow 10 ml to stand in a platinum dish until all effervescence has ceased. Evaporate to dryness on a water-bath and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

ASSAY

Dilute 10.0 g to 100.0 ml with *water R*. To 10.0 ml of this solution add 20 ml of *dilute sulphuric acid R*. Titrate with *0.02 M potassium permanganate* until a pink colour is obtained.

1 ml of *0.02 M potassium permanganate* is equivalent to 1.701 mg of H_2O_2 or 0.56 ml of oxygen.

STORAGE

Protected from light, and if the solution does not contain a stabiliser, at a temperature below 15 °C.

LABELLING

If the solution contains a stabiliser, the label states that the contents are stabilised. The competent authority may require that the name of the stabiliser be stated on the label.

CAUTION

It decomposes in contact with oxidisable organic matter and with certain metals and if allowed to become alkaline.

Ph Eur

Hydrogen Peroxide Solution (30 per cent)

General Notices

(Ph Eur monograph 0396)

7722-84-1

Action and use

Antiseptic; deodorant.

When hydrogen peroxide is prescribed or demanded, Hydrogen Peroxide Solution (6 per cent) shall be dispensed or supplied.

Ph Eur

DEFINITION

Content

29.0 per cent *m/m* to 31.0 per cent *m/m* of H_2O_2 (M_r 34.01).

1 volume of hydrogen peroxyde solution (30 per cent) corresponds to about 110 times its volume of oxygen. A suitable stabiliser may be added.

CHARACTERS

Appearance

Colourless, clear liquid.

IDENTIFICATION

A. To 1 ml add 0.2 ml of *dilute sulphuric acid R* and 0.25 ml of *0.02 M potassium permanganate*. The solution becomes colourless with evolution of gas.

B. To 0.05 ml add 2 ml of *dilute sulphuric acid R*, 2 ml of *ether R* and 0.05 ml of *potassium chromate solution R* and shake. The ether layer is blue.

C. It complies with the requirement for the content of H_2O_2.

TESTS

Acidity

To 10 ml add 100 ml of *water R* and 0.25 ml of *methyl red solution R*. Not less than 0.05 ml and not more than 0.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Organic stabilisers

Maximum 500 ppm.

Shake 20 ml with 10 ml of *chloroform R* and then with 2 quantities, each of 5 ml, of *chloroform R*. Evaporate the combined chloroform layers under reduced pressure at a temperature not exceeding 25 °C and dry in a desiccator. The residue weighs a maximum of 10 mg.

Non-volatile residue

Maximum 2 g/l.

Allow 10 ml to stand in a platinum dish until all effervescence has ceased, cooling if necessary. Evaporate to dryness on a water-bath and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

ASSAY

Dilute 1.00 g to 100.0 ml with *water R*. To 10.0 ml of this solution add 20 ml of *dilute sulphuric acid R*. Titrate with *0.02 M potassium permanganate* until a pink colour is obtained.

1 ml of *0.02 M potassium permanganate* is equivalent to 1.701 mg of H_2O_2 or 0.56 ml of oxygen.

STORAGE

Protected from light, and if the solution does not contain a stabiliser, at a temperature below 15 °C.

LABELLING

If the solution contains a stabiliser, the label states that the contents are stabilised. The competent authority may require that the name of the stabiliser be stated on the label.

CAUTION

It decomposes vigorously in contact with oxidisable organic matter and with certain metals and if allowed to become alkaline.

Ph Eur

Hydrogen Peroxide Solution (6 per cent)

General Notices

Hydrogen Peroxide Solution

Action and use

Antiseptic; deodorant.

Preparation

Hydrogen Peroxide Mouthwash

When hydrogen peroxide is prescribed or demanded, Hydrogen Peroxide Solution (6 per cent) shall be dispensed or supplied.

DEFINITION

Hydrogen Peroxide Solution (6 per cent) is an aqueous solution of hydrogen peroxide containing not less than 5.0% w/v and not more than 7.0% w/v of H_2O_2 (34.01), corresponding to about 20 times its volume of available oxygen. It may contain a suitable stabilising agent.

CHARACTERISTICS

A clear, colourless liquid. It decomposes in contact with oxidisable organic matter and with certain metals and if allowed to become alkaline.

IDENTIFICATION

A. To 1 ml add 0.2 ml of 1M *sulphuric acid* and 0.25 ml of 0.02M *potassium permanganate*. The solution becomes colourless with evolution of gas.

B. Shake 0.05 ml with 2 ml of 1M *sulphuric acid*, 2 ml of *ether* and 0.05 ml of *potassium chromate solution*. The ether layer is blue.

C. Complies with the requirement for the content of H_2O_2.

TESTS

Acidity

Dilute 10 ml with 20 ml of *water* and add 0.25 ml of *methyl red solution*. Not less than 0.05 ml and not more than 1.0 ml of 0.1M *sodium hydroxide VS* is required to change the colour of the solution.

Organic stabilisers

Shake 20 ml with successive quantities of 10, 5 and 5 ml of *chloroform*. Evaporate the combined chloroform extracts at a temperature not exceeding 25° at a pressure of 2 kPa and dry in a desiccator. Any residue weighs not more than 5 mg (250 ppm).

Non-volatile matter

Place 10 ml in a platinum dish and allow to stand until effervescence has ceased, cooling if necessary. Evaporate the solution on a water bath. Any residue, when dried at 100°C to 105°C, weighs not more than 20 mg (0.2% w/v).

ASSAY

Dilute 10 ml to 100 ml with *water*. To 10 ml of the resulting solution add 20 ml of 1M *sulphuric acid* and titrate with 0.02M *potassium permanganate VS*. Each ml of 0.02M *potassium permanganate VS* is equivalent to 1.701 mg of H_2O_2 or 0.56 ml of oxygen.

STORAGE

Hydrogen Peroxide Solution (6 per cent) should be protected from light. If the solution does not contain a stabilising agent, it should be stored at a temperature not exceeding 15°C. It should not be stored for long periods.

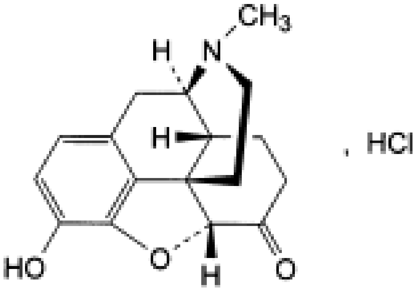
LABELLING

The label states, where applicable, that the solution contains a stabilising agent.

Hydromorphone Hydrochloride

General Notices

(Ph Eur monograph 2099)



$C_{17}H_{19}NO_3,HCl$ 321.8   *71-68-1*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   hydromorphone hydrochloride CRS.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.250 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Acidity or alkalinity

To 2 ml of solution S add 0.1 ml of *methyl red solution R*. The solution is not yellow. To 2 ml of solution S add 0.05 ml of *bromocresol green solution R*. The solution is not yellow.

Specific optical rotation *(2.2.7)*

- 136 to - 140 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *water R*, sonicating if necessary and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Reference solution (b) To 5 ml of the test solution add 5 mg of *naloxone hydrochloride dihydrate CRS* and dilute to 50 ml with *water R*.

Column:
— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 18.29 g of *diethylamine R* and 2.88 g of *sodium laurilsulfate R* in *water R* and dilute to 1000 ml with the same solvent. Adjust 800 ml of this solution to pH 3.0 with *phosphoric acid R.* Add 100 ml of *acetonitrile R* and 100 ml of *methanol R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 284 nm.

Injection 20 µl.

Run time 4 times the retention time of hydromorphone.

Relative retention with reference to hydromorphone (retention time = about 9 min): impurity D = about 0.72; impurity B = about 0.77; impurity C = about 0.82; impurity A = about 3.2.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to hydromorphone and naloxone.

Limits:
— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram

obtained with reference solution (a) (0.3 per cent),

— *impurities B, C, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 0.250 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

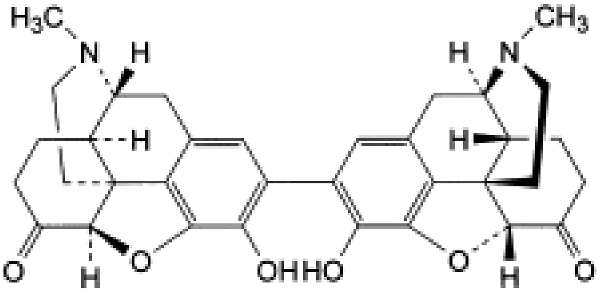
1 ml of *0.1 M sodium hydroxide* is equivalent to 32.18 mg of $C_{17}H_{20}ClNO_3$.

STORAGE

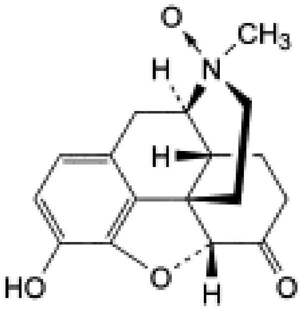
Protected from light.

IMPURITIES

Specified impurities A, B, C, D.

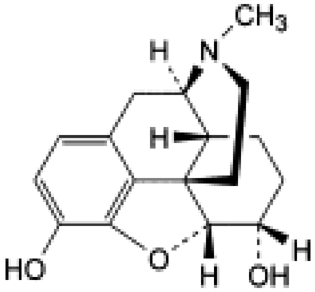


A. 4,5α:4′,5′α-diepoxy-3,3′-dihydroxy-17,17′-dimethyl-2,2′-bimorphinanyl-6,6′-dione (pseudohydromorphone),



B. 4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one 17-oxide (hydromorphone *N*-oxide),

C. morphine,



D. 4,5α-epoxy-17-methylmorphinan-3,6α-diol (dihydromorphine).

Ph Eur

Hydrotalcite

General Notices

$Mg_6Al_2(OH)_{16}CO_3,4H_2O$ 604.0 *12304-65-3*

Action and use

Antacid.

Preparation

Hydrotalcite Tablets

DEFINITION

Hydrotalcite is a hydrated form of an aluminium magnesium basic carbonate corresponding to the formula $Mg_6Al_2(OH)_{16}CO_3,4H_2O$. It contains not less than 15.3% and not more than 18.7% of Al_2O_3 and not less than 36.0% and not more than 44.0% of MgO. The ratio of the content of Al_2O_3 to the content of MgO is not less than 0.40 and not more than 0.45.

CHARACTERISTICS

A white or almost white, free-flowing, granular powder.

Practically insoluble in *water*. It dissolves in dilute mineral acids with slight effervescence.

IDENTIFICATION

A. Dissolve 1.0 g in 20 ml of 2M *hydrochloric acid*. Effervescence occurs. Add 30 ml of *water*, boil, add 2M *ammonia* until just alkaline to *methyl red solution*, continue boiling for 2 minutes and filter, reserving the filtrate for test B. Wash the precipitate with 50 ml of a hot 2% w/v solution of *ammonium chloride* and dissolve in 15 ml of 2M *hydrochloric acid*. The resulting solution yields the reaction characteristic of *aluminium salts*, Appendix VI.

B. Dilute 1 ml of the filtrate obtained in test A to 10 ml with *water*. The resulting solution yields the reactions characteristic of *magnesium salts*, Appendix VI.

TESTS

Alkalinity

pH of a 4% w/v suspension in *carbon dioxide-free water*, 8.0 to 10.0, Appendix V L.

Neutralising capacity

Mix 0.2 g with a small quantity of *water* to give a smooth paste and gradually add sufficient further quantities of *water* to produce 100 ml. Warm at 37°, add 100 ml of 0.1M *hydrochloric acid VS* previously heated to 37° and stir continuously for 1 hour using a paddle stirrer at a rate of about 200 revolutions per minute, maintaining the temperature at 37°, and titrate with 0.1M *sodium hydroxide VS* to pH 3.5. Subtract the volume of 0.1M *sodium hydroxide VS* from 100 ml to obtain the number of ml of 0.1M *hydrochloric acid VS* required for neutralisation. Not less than 260 ml of 0.1M *hydrochloric acid VS* is required to neutralise 1 g.

Arsenic

Dissolve 0.33 g in 5 ml of 2M *hydrochloric acid*. The resulting solution complies with the *limit test for arsenic*, Appendix VII (3 ppm).

Heavy metals

Dissolve 2.7 g in 20 ml of 5M *hydrochloric acid* and 10 ml of *water*, add 0.5 ml of *nitric acid* and boil for 30 seconds. Cool, add 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with three 10 ml quantities of a mixture of equal volumes of *isoamyl alcohol* and *ether*. Add to the aqueous layer 0.1 ml of *phenolphthalein solution* and 13.5M *ammonia* until a pink colour is produced. Cool, add *glacial acetic acid* until the solution is decolorised and add a further 5 ml of *glacial acetic acid*. Filter, if necessary, and dilute the solution to 40 ml with *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard (30 ppm) .

Sodium

Not more than 0.1% of Na when determined by Method II for *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm. To prepare the test solution dissolve 0.1 g in 4 ml of 5M *hydrochloric acid*, dilute to 200 ml with *water* and use *sodium standard solution (200 ppm Na)*, diluted if necessary with 0.1M *hydrochloric acid*, to prepare the standard solutions.

Chloride

Dissolve 0.18 g in 10 ml of 2M *nitric acid*, boil, allow to cool and dilute to 100 ml with *water*. To 10 ml add 5 ml of *water*. The resulting solution complies with the *limit test for chlorides*, Appendix VII (0.3%).

Sulphate

Dissolve 0.14 g in 15 ml of 1M *hydrochloric acid* and dilute to 100 ml with *water*. 15 ml of the resulting solution complies with the *limit test for sulphates*, Appendix VII (0.7%).

Loss on ignition

When ignited at 800°, loses 40.0 to 50.0% of its weight. Use 1 g.

ASSAY

For Al_2O_3

Dissolve 0.3 g in 2 ml of 7M *hydrochloric acid*, add 250 ml of *water* and 50 ml of 0.05M *disodium edetate VS* and neutralise with 1M *sodium hydroxide* using *methyl red solution* as indicator. Heat the solution on a water bath for 30 minutes and allow to cool. Add 3 g of *hexamine* and titrate the excess of disodium edetate with 0.05M *lead nitrate VS* using *xylenol orange solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

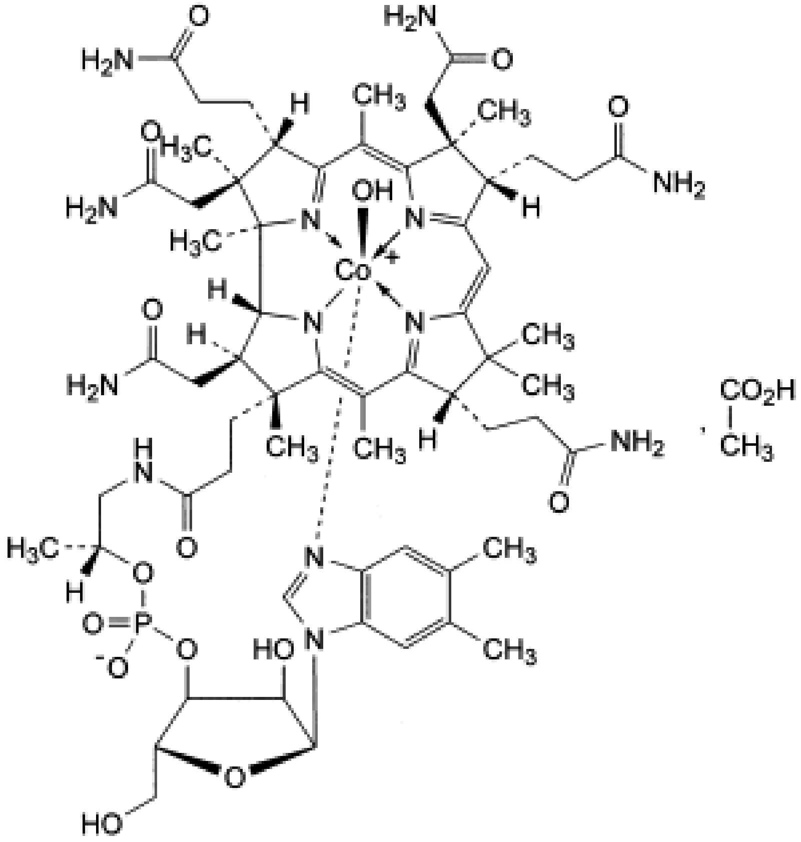
For MgO

Dissolve 0.125 g in the minimum volume of 7M *hydrochloric acid*, add 30 ml of *water*, 1 g of *ammonium chloride*, 10 ml of *triethanolamine*, 150 ml of *water* and 5 ml of *ammonia buffer pH 10.9* and titrate immediately with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.015 mg of MgO.

Hydroxocobalamin Acetate

General Notices

(Ph Eur monograph 0913)



$C_{62}H_{89}CoN_{13}O_{15}P,C_2H_4O_2$ 1406 *22465-48-1*

Action and use

Vitamin B_{12} analogue.

Preparation

Hydroxocobalamin Injection

Ph Eur

DEFINITION

Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-hydroxocobamide acetate.

Content

96.0 per cent to 102.0 per cent (dried substance).

This monograph applies to hydroxocobalamin acetate produced by fermentation.

CHARACTERS

Appearance

Dark red, crystalline powder or dark red crystals, very hygroscopic.

Solubility

Soluble in water.

Some decomposition may occur on drying.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 2.5 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R*, then dilute to 100 ml with the same solution.

Spectral range 260-610 nm.

Absorption maxima At 274 nm, 351 nm and 525 nm.

Absorbance ratio:

— A_{274}/A_{351} = 0.75 to 0.83;

— A_{525}/A_{351} = 0.31 to 0.35.

B. Thin-layer chromatography *(2.2.27)*. *Carry out the test protected from light.*

Test solution Dissolve 2 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *ethanol 96 per cent R* and *water R*.

Reference solution Dissolve 2 mg of *hydroxocobalamin CRS* in 1 ml of a mixture of equal volumes of *ethanol 96 per cent R* and *water R*.

Plate *TLC silica gel G plate R.*

Mobile phase *Dilute ammonia R1*, *methanol R* (25:75 *V/V*).

Application 10 µl.

Development In an unlined tank, over a path of 12 cm.

Drying In air.

Detection Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of acetates *(2.3.1)*.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Use freshly prepared solutions and protect them from bright light.*

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 25 mg of the substance to be examined in 10 ml of *water R*, warming if necessary. Allow to cool and add 1 ml of a 20 g/l solution of *chloramine R* and 0.5 ml of *0.05 M hydrochloric acid*. Dilute this solution to 25 ml with *water R*. Shake and allow to stand for 5 min. Inject immediately.

Column:
- *size:* l = 0.25 m, Ø = 4 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 19.5 volumes of *methanol R* and 80.5 volumes of a solution containing 15 g/l of *citric acid R* and 8.1 g/l of *disodium hydrogen phosphate R.*

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 351 nm.

Injection 20 µl.

Run time 4 times the retention time of hydroxocobalamine.

System suitability:
- the chromatogram obtained with reference solution (c) shows 3 principal peaks;
- *resolution*: minimum 3.0 between each pair of adjacent peaks in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (5 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Loss on drying *(2.2.32)*

8.0 per cent to 12.0 per cent, determined on 0.400 g by drying at 105 °C at a pressure not exceeding 0.7 kPa.

ASSAY

Protect the solutions from light throughout the assay. Dissolve 25.0 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R*, then dilute to 1000.0 ml with the same solution. Measure the absorbance *(2.2.25)* at the absorption maximum at 351 nm.

Calculate the content of $C_{64}H_{93}CoN_{13}O_{17}P$ taking the specific absorbance to be 187.

STORAGE

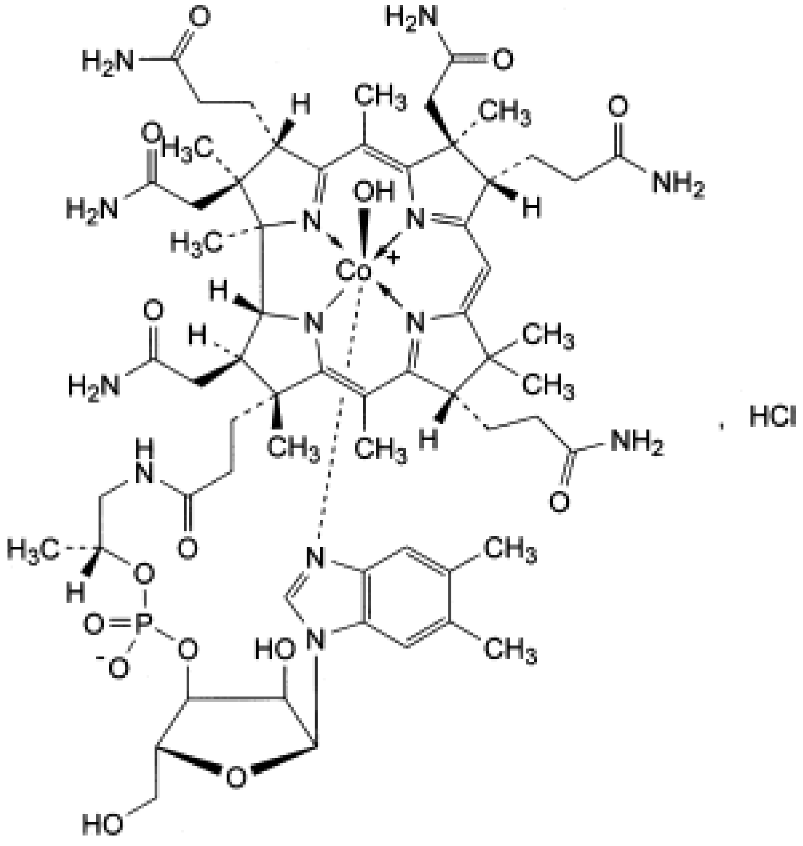
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Hydroxocobalamin Chloride

General Notices

(Ph Eur monograph 0914)



$C_{62}H_{89}CoN_{13}O_{15}P,HCl$ 1383 *58288-50-9*

Action and use

Vitamin B_{12} analogue.

Preparation

Hydroxocobalamin Injection

Ph Eur

DEFINITION

Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-hydroxocobamide chloride.

Fermentation product.

Content

96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Dark red crystalline powder or dark red crystals, very hygroscopic.

Solubility

Soluble in water.

Some decomposition may occur on drying.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 2.5 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R*, then dilute to 100 ml with the same solution.

Spectral range 260-610 nm.

Absorption maxima At 274 nm, 351 nm and 525 nm.

Absorbance ratio:
— A_{274}/A_{351} = 0.75 to 0.83;

— A_{525}/A_{351} = 0.31 to 0.35.

B. Thin-layer chromatography *(2.2.27)*. *Carry out the identification test protected from light.*

Test solution Dissolve 2 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *ethanol 96 per cent R* and *water R.*

Reference solution Dissolve 2 mg of *hydroxocobalamin CRS* in 1 ml of a mixture of equal volumes of *ethanol 96 per cent R* and *water R*.

Plate *TLC silica gel G plate R.*

Mobile phase *dilute ammonia R1*, *methanol R* (25:75 *V/V*).

Application 10 µl.

Development In an unlined tank, over a path of 12 cm.

Drying In air.

Detection Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Use freshly prepared solutions and protect them from bright light.*

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 25 mg of the substance to be examined in 10 ml of *water R*, warming if necessary. Allow to cool and add 1 ml of a 20 g/l solution of *chloramine R* and 0.5 ml of *0.05 M hydrochloric acid*. Dilute to 25 ml with *water R*. Shake and allow to stand for 5 min. Inject immediately.

Column:

— *size:* *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 19.5 volumes of *methanol R* and 80.5 volumes of a solution containing 15 g/l of *citric acid R* and 8.1 g/l of *disodium hydrogen phosphate R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 351 nm.

Injection 20 µl.

Run time 4 times the retention time of hydroxocobalamin.

System suitability:

— the chromatogram obtained with reference solution (c) shows 3 principal peaks;

— *resolution*: minimum 3.0 between each pair of adjacent peaks in the chromatogram obtained with reference solution (c);

— *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Loss on drying *(2.2.32)*

8.0 per cent to 12.0 per cent, determined on 0.400 g by drying at 105 °C at a pressure not exceeding 0.7 kPa.

ASSAY

Protect the solutions from light throughout the assay.

Dissolve 25.0 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R*, then dilute to 1000.0 ml with the same solution. Measure the absorbance *(2.2.25)* at the absorption maximum at 351 nm.

Calculate the content of $C_{62}H_{90}ClCoN_{13}O_{15}P$ taking the specific absorbance to be 190.

STORAGE

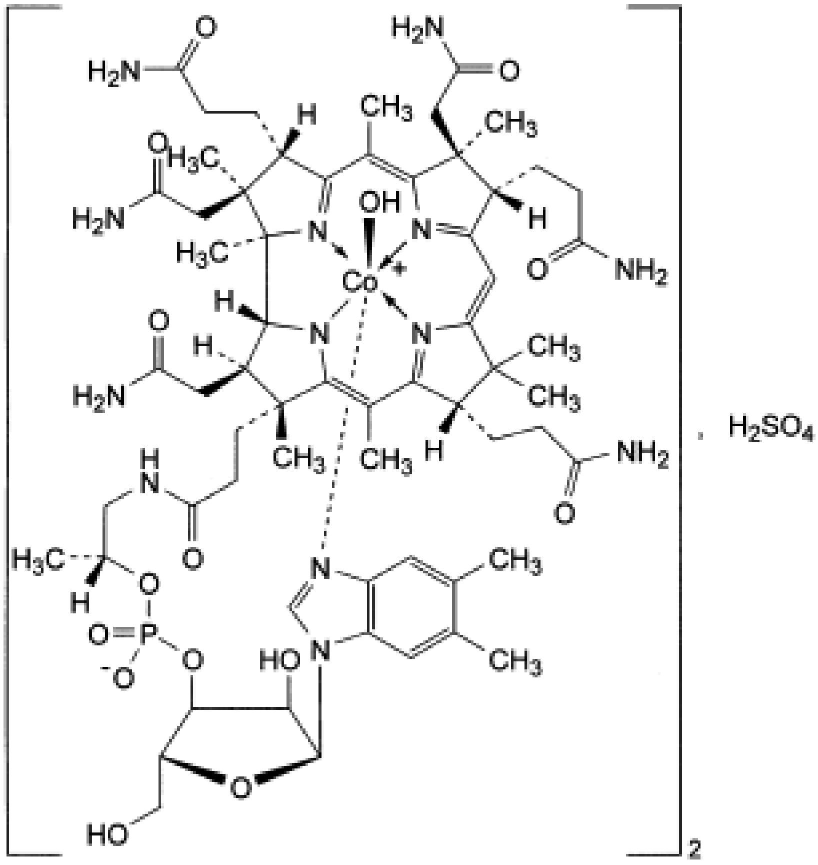
In an airtight container protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Hydroxocobalamin Sulphate

General Notices

(Ph Eur monograph 0915)



$(C_{62}H_{89}CoN_{13}O_{15}P)_2,H_2SO_4$ 2791

Action and use

Vitamin B_{12} analogue.

Preparation

Hydroxocobalamin Injection

Ph Eur

DEFINITION

Di-(Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-hydroxocobamide) sulphate.

Fermentation product.

Content

96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Dark red crystalline powder or dark red crystals, very hygroscopic.

Solubility

Soluble in water.

Some decomposition may occur on drying.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 2.5 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R*, then dilute to 100 ml with the same solution.

Spectral range 260-610 nm.

Absorption maxima At 274 nm, 351 nm and 525 nm.

Absorbance ratios:

— A_{274}/A_{351} = 0.75 to 0.83;

— A_{525}/A_{351} = 0.31 to 0.35.

B. Thin-layer chromatography *(2.2.27)*. *Carry out the test protected from light.*

Test solution Dissolve 2 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *ethanol 96 per cent R* and *water R*.

Reference solution Dissolve 2 mg of *hydroxocobalamin CRS* in 1 ml of a mixture of equal volumes of *ethanol 96 per cent R* and *water R*.

Plate *TLC silica gel G plate R*.

Mobile phase *dilute ammonia R1*, *methanol R* (25:75 *V/V*).

Application 10 µl.

Development In an unlined tank, over a path of 12 cm.

Drying In air.

Detection Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Use freshly prepared solutions and protect them from bright light.*

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 25 mg of the substance to be examined in 10 ml of *water R*, warming if necessary. Allow to cool and add 1 ml of a 20 g/l solution of *chloramine R* and 0.5 ml of *0.05 M hydrochloric acid*. Dilute to 25 ml with *water R*. Shake and allow to stand for 5 min. Inject immediately.

Column:

— *size:* *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 19.5 volumes of *methanol R* and 80.5 volumes of a solution containing 15 g/l of *citric acid R* and 8.1 g/l of *disodium hydrogen phosphate R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 351 nm.

Injection 20 µl.

Run time 4 times the retention time of hydroxocobalamin.

System suitability:

— the chromatogram obtained with reference solution (c) shows 3 principal peaks;

— *resolution*: minimum 3.0 between each pair of adjacent peaks in the chromatogram obtained with reference solution (c);

— *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Loss on drying *(2.2.32)*

8.0 per cent to 16.0 per cent, determined on 0.400 g by drying at 105 °C at a pressure not exceeding 0.7 kPa.

ASSAY

Protect the solutions from light throughout the assay.

Dissolve 25.0 mg in a solution containing 0.8 per cent *V/V* of *glacial acetic acid R* and 10.9 g/l of *sodium acetate R* and dilute to 1000.0 ml with the same solution. Measure the absorbance *(2.2.25)* at the absorption maximum at 351 nm.

Calculate the content of $C_{124}H_{180}Co_2N_{26}O_{34}P_2S$ taking the specific absorbance to be 188.

STORAGE

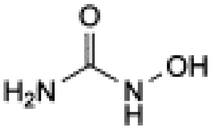
In an airtight container protected from light, at a temperature of 2 ℃ to 8 ℃.

Ph Eur

Hydroxycarbamide

General Notices

(Ph Eur monograph 1616)



$CH_4N_2O_2$ 76.05 *127-07-1*

Action and use

Cytotoxic alkylating drug.

Preparation

Hydroxycarbamide Capsules

Ph Eur

DEFINITION

N-Hydroxyurea.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, practically insoluble in alcohol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison hydroxycarbamide CRS.

If the spectra obtained in the solid state show differences dissolve the substance to be examined and the reference substance separately in *alcohol R*, evaporate to dryness and record new spectra using the residues.

B. Examine the chromatograms obtained in the test for urea.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (c).

TESTS

Urea

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *water R* and dilute to 1.0 ml with the same solvent.

Reference solution (a) Dissolve 12.5 mg of *urea R* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *urea R* in *water R* and dilute to 20 ml with the same solvent.

Reference solution (c) Dissolve 50 mg of *hydroxycarbamide CRS* in *water R* and dilute to 1 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *pyridine R*, *water R*, *ethyl acetate R* (2:2:10 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a 10 g/l solution of *dimethylaminobenzaldehyde R* in *1 M hydrochloric acid*.

System suitability The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Limit:

— *urea*: any spot corresponding to urea in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (a) (0.5 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the same mobile phase.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 0.100 g of *hydroxylamine hydrochloride R* and 5 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase. Prepare immediately before use.

Reference solution (b) Dilute 0.1 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 0.100 g of *hydroxycarbamide CRS* in the mobile phase and dilute to 10.0 ml with the same solvent. Dilute 5.0 ml to 50.0 ml with the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *water R* (5:95 *V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl; inject test solution (a) and reference solutions (a) and (b).

Run time 3 times the retention time of hydroxycarbamide which is about 5 min.

System suitability Reference solution (a):

- *resolution*: minimum of 1.0 between the peaks due to impurity A and to hydroxycarbamide.

Limits:
- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *total*: not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Chlorides *(2.4.4)*

Maximum 50 ppm.

Dissolve 1.0 g in *water R* and dilute to 15 ml with the same solvent. The solution complies with the limit test for chlorides.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 2.00 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

Injection Test solution (b) and reference solution (c).

STORAGE

In an airtight container, protected from light.

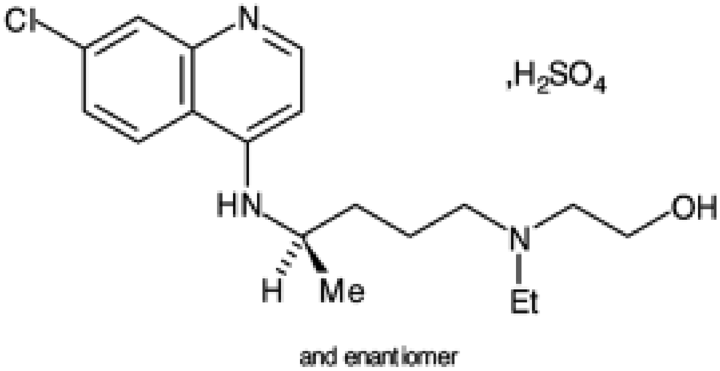
IMPURITIES

A. H_2N-OH: hydroxylamine.

Ph Eur

Hydroxychloroquine Sulphate

General Notices



$C_{18}H_{26}ClN_3O,H_2SO_4$ 434.0 *747-36-4*

Action and use

Antiprotozoal (malaria).

Preparation

Hydroxychloroquine Tablets

DEFINITION

Hydroxychloroquine Sulphate is (*RS*)-2-*N*-[4-(7-chloro-4-quinolylamino)pentyl]-*N*-ethylaminoethanol sulphate. It contains not less than 98.0% and not more than 100.5% of $C_{18}H_{26}ClN_3O,H_2SO_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Freely soluble in *water*; practically insoluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of 2M *sodium hydroxide* and extract with two 20 ml quantities of *dichloromethane*. Wash the dichloromethane extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of hydroxychloroquine *(RS 182)*.

B. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 3.5 to 5.5, Appendix V L.

Clarity and colour of solution

A 10.0% w/v solution is not more than slightly turbid and not more than slightly yellow.

Lead

Not more than 20 ppm when determined by the following method. Carefully heat 2.0 g for 10 minutes with 8 ml of *water* and 6 ml of *nitric acid* in a Kjeldahl flask. Cool, add 4 ml of *sulphuric acid* and heat until the mixture darkens. Continue heating, with the dropwise addition of *nitric acid*, until the liquid becomes colourless and white fumes of sulphur trioxide are produced. Add 3 ml of *water*, carefully evaporate until white fumes are again produced, cool and dilute to 18 ml with *water*. Add and dissolve 2 g of *citric acid*, make alkaline with 5M *ammonia* and add 1 ml of *potassium cyanide solution PbT*. Transfer to a separating funnel, add 10 ml of *dithizone solution*, shake vigorously and remove the lower layer. Repeat the extraction with two 5 ml quantities of *dithizone solution*. If, after the third extraction, the dichloromethane layer is bright red, continue the extraction with further 5 ml quantities of *dithizone solution* until the colour of the reagent no longer changes to bright red. Wash the combined dichloromethane solutions by shaking with 10 ml of *water* and then extract with two 10 ml quantities of 2M *hydrochloric acid*. Wash the combined acid solutions with 10 ml of *dichloromethane* and discard the dichloromethane. Transfer the solution to a *Nessler cylinder* and make alkaline with 5M *ammonia*. In a second *Nessler cylinder* mix 2 ml of 6M *acetic acid* with 20 ml of 2M *hydrochloric acid*, make alkaline with 5M *ammonia* and add 4 ml of *lead standard solution (10 ppm Pb)*.

Treat the contents of each cylinder as follows. Add 1 ml of *potassium cyanide solution PbT*; the solutions should not be more than faintly opalescent. If the colours of the solutions differ, equalise them by the addition of a few drops of a highly diluted solution of burnt sugar or other non-reactive substance. Dilute to 50 ml with *water*, add 0.1 ml of a solution prepared by dissolving 10 g of *sodium sulphide* in sufficient *water* to produce 100 ml and filtering and mix thoroughly. Compare the colours of the two solutions by a suitable method, such as by light reflected from a white tile through the Nessler cylinders. The colour of the solution in the first cylinder is not more intense than that of the solution in the second cylinder.

Chloride

Dissolve 0.50 g in 50 ml of *water*. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (350 ppm).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions of the substance being examined in *water*.

(1) 5.0% w/v.

(2) 0.050% w/v.

(3) 0.025% w/v.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 2 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

3 volumes of 13.5M *ammonia*, 25 volumes of *water* and 72 volumes of *methanol*,

LIMITS

In the chromatogram obtained with solution (1):

— any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%);

— not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Loss on drying

When dried to constant weight at 105°, loses not more than 2.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.2%, Appendix IX A.

ASSAY

Dissolve 0.5 g in 10 ml of *water*, add 20 ml of 1M *sodium hydroxide* and extract with four 25 ml quantities of *dichloromethane*. Combine the dichloromethane extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end-point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 21.70 mg of $C_{18}H_{26}ClN_3O,H_2SO_4$.

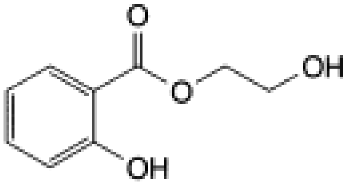
STORAGE

Hydroxychloroquine Sulphate should be protected from light.

Hydroxyethyl Salicylate

General Notices

(Ph Eur monograph 1225)



$C_9H_{10}O_4$ 182.2 *87-28-5*

Ph Eur

DEFINITION

2-Hydroxyethyl 2-hydroxybenzoate.

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

Oily, colourless or almost colourless liquid, or colourless crystals.

Solubility

Sparingly soluble in water, very soluble in acetone and in methylene chloride, freely soluble in alcohol.

mp

About 21 °C.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D, E.*

A. It complies with the test for refractive index (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Thin films.

Comparison *hydroxyethyl salicylate CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 1 ml of solution S (see Tests), add 1 ml of *water R* and 0.2 ml of *ferric chloride solution R2*. A violet-red colour appears which disappears immediately after the addition of 2 ml of *dilute acetic acid R*. A very faint violet colour may remain.

E. In a test tube 160 mm long, mix 1.0 g with 2.0 g of finely powdered *manganese sulphate R*. Insert 2 cm into the test-tube a strip of filter paper impregnated with a freshly prepared mixture of 1 volume of a 20 per cent *V/V* solution of *diethanolamine R* and 11 volumes of a 50 g/l solution of *sodium nitroprusside R* adjusted to pH 9.8 with *1 M hydrochloric acid*. Heat the test-tube over a naked flame for 1-2 min. The filter paper becomes blue.

TESTS

Solution S

Dissolve 2.5 g in 40 ml of *alcohol R* and dilute to 50 ml with *distilled water R*.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 2 ml of solution S add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.3 ml of *0.01 M hydrochloric acid*. The solution is red.

Relative density *(2.2.5)*

1.252 to 1.257.

Refractive index *(2.2.6)*

1.548 to 1.551.

Related substances

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 2 ml of test solution (a) to 50 ml with *methanol R*.

Reference solution (a) Dissolve 50.0 mg of *hydroxyethyl salicylate CRS* in *methanol R* and dilute to 25 ml with the same solvent.

Reference solution (b) Dilute 2.5 ml of test solution (b) to 10 ml with *methanol R*.

Reference solution (c) Dissolve 0.10 g of *ethylene glycol R* in *methanol R* and dilute to 50 ml with the same solvent. Dilute 1.25 ml of the solution to 10 ml with *methanol R*.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *ethyl acetate R*, *glacial acetic acid R*, *cyclohexane R* (20:20:60 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of cold air.

Detection A In ultraviolet light at 254 nm.

Limits A:

— *Any impurity*: any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent).

Detection B Spray the plate with *ammonium vanadate solution R* and heat at 100 °C for 10 min. Allow to cool for 10 min and examine in daylight.

Limits B In the chromatogram obtained with test solution (a):

— *impurity B*: any spot corresponding to impurity B is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent),

— *any other impurity*: any spot, apart from the principal spot and any spot corresponding to impurity B is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent).

System suitability The chromatogram obtained with reference solution (c) shows a clearly visible spot.

Chlorides ***(2.4.4)***

Maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides.

Sulphates ***(2.4.13)***

Maximum 250 ppm.

Dilute 12 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In a flask with a ground-glass stopper, dissolve 0.125 g in 30 ml of *glacial acetic acid R*. Add 10 ml of *dilute sulphuric acid R,* 1.5 g of *potassium bromide R* and 50.0 ml of *0.0167 M potassium bromate*. Immediately close the flask and allow to stand protected from light for 15 min. Add 1.5 g of *potassium iodide R* immediately after removing the stopper and titrate with *0.1 M sodium thiosulphate*, adding 1 ml of *starch solution R* towards the end of the titration. Carry out a blank titration.

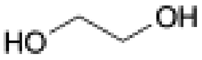
1 ml of *0.0167 M potassium bromate* is equivalent to 4.555 mg of $C_9H_{10}O_4$.

STORAGE

Protected from light.

IMPURITIES

A. salicylic acid,



B. ethane-1,2-diol (ethylene glycol).

Ph

Hydroxyethylcellulose

General Notices

(Ph Eur monograph 0336)

9004-62-0

Action and use

Excipient.

Ph Eur

DEFINITION

Partly *O*-(2-hydroxyethylated) cellulose.

CHARACTERS

Appearance

White, yellowish-white or greyish-white powder or granules.

Solubility

Soluble in hot and cold water giving a colloidal solution, practically insoluble in acetone, in ethanol (96 per cent) and in toluene.

IDENTIFICATION

A. Heat 10 ml of solution S (see Tests) to boiling. The solution remains clear.

B. To 10 ml of solution S add 0.3 ml of *dilute acetic acid R* and 2.5 ml of a 100 g/l solution of *tannic acid R*. A yellowish-white, flocculent precipitate is formed which dissolves in *dilute ammonia R1*.

C. In a test-tube about 160 mm in length, thoroughly mix 1 g with 2 g of finely powdered *manganese sulphate R*. Introduce to a depth of 2 cm into the upper part of the tube a strip of filter paper impregnated with a freshly prepared mixture of 1 volume of a 200 g/l solution of *diethanolamine R* and 11 volumes of a 50 g/l solution of *sodium nitroprusside R*, adjusted to about pH 9.8 with *1 M hydrochloric acid*. Insert the tube 8 cm into a silicone-oil bath and heat at 190-200 °C. The filter paper becomes blue within 10 min. Carry out a blank test.

D. Dissolve 0.2 g completely, without heating, in 15 ml of a 700 g/l solution of *sulphuric acid R*. Pour the solution with stirring into 100 ml of iced *water R* and dilute to 250 ml with iced *water R*. In a test-tube, mix thoroughly while cooling in iced water 1 ml of the solution with 8 ml of *sulphuric acid R*, added dropwise. Heat on a water-bath for exactly 3 min and immediately cool in iced water. While the mixture is cold, carefully add 0.6 ml of *ninhydrin solution R2* and mix well. Allow to stand at 25 °C. A pink colour is produced immediately and does not become violet within 100 min.

TESTS

Solution S

Disperse a quantity of the substance to be examined equivalent to 1.0 g of the dried substance in 50 ml of *carbon dioxide-free water R.* After 10 min, dilute to 100 ml with *carbon dioxide-free water R* and stir until dissolution is complete.

pH *(2.2.3)*

5.5 to 8.5 for solution S.

Apparent viscosity *(2.2.10)*

75 per cent to 140 per cent of the value stated on the label.

While stirring, introduce a quantity of the substance to be examined equivalent to 2.00 g of the dried substance into 50 g of *water R.* Dilute to 100.0 g with *water R* and stir until dissolution is complete. Determine the viscosity using a rotating viscometer at 25 °C and at a shear rate of 100 s^{-1} for substances with an expected viscosity up to 100 mPa·s, at a shear rate of 10 s^{-1} for substances with an expected viscosity between 100 mPa·s and 20 000 mPa·s and at a shear rate of 1 s^{-1} for substances with an expected viscosity above 20 000 mPa·s. If it is impossible to obtain a shear rate of exactly 1 s^{-1}, 10 s^{-1} or 100 s^{-1} respectively, use a rate slightly higher and a rate slightly lower and interpolate.

Chlorides *(2.4.4)*

Maximum 1.0 per cent.

Dilute 1 ml of solution S to 30 ml with *water R*. 15 ml of the solution complies with the limit test for chlorides.

Nitrates

Maximum 3.0 per cent (dried substance), if hydroxyethylcellulose has an apparent viscosity of 1000 mPa·s or less and maximum 0.2 per cent (dried substance), if hydroxyethylcellulose has an apparent viscosity of more than 1000 mPa·s.

Determine potentiometrically *(2.2.36, Method I)* using as indicator a nitrate selective electrode and a silver-silver chloride electrode with a 13.2 g/l solution of *ammonium sulphate R* as reference electrolyte.

Prepare the solutions immediately before use.

Buffer solution To a mixture of 50 ml of *1 M sulphuric acid* and 800 ml of *water R*, add 135 g of *potassium dihydrogen phosphate R* and dilute to 1000 ml with *water R*.

Buffered water Dilute 80 ml of buffer solution to 2000 ml with *water R*.

Nitrate standard solution (500 ppm NO_3) Dissolve 0.8154 g of *potassium nitrate R* in 500 ml of buffered water and dilute to 1000.0 ml with the same solvent.

Test solution Dissolve 0.50 g of the substance to be examined in buffered water and dilute to 100.0 ml with the same solvent.

Reference solutions If hydroxyethylcellulose has an apparent viscosity of 1000 mPa·s or less, dilute 10.0 ml, 20.0 ml and 40.0 ml of nitrate standard solution (500 ppm NO_3) to 100.0 ml with buffered water and mix.

If hydroxyethylcellulose has an apparent viscosity of more than 1000 mPa·s, dilute 1.0 ml, 2.0

ml and 4.0 ml of nitrate standard solution (500 ppm NO_3) to 100.0 ml with buffered water and mix.

Carry out the measurements for each solution. Calculate the concentration of nitrates using the calibration curve.

Glyoxal

Maximum 20 ppm.

Introduce 1.0 g into a test-tube with a ground-glass stopper and add 10.0 ml of *anhydrous ethanol R*. Stopper the tube and stir mechanically for 30 min. Centrifuge. To 2.0 ml of the supernatant liquid add 5.0 ml of a 4 g/l solution of *methylbenzothiazolone hydrazone hydrochloride R* in an 80 per cent *V/V* solution of *glacial acetic acid R* in *water R*. Shake to homogenise. After 2 h, the solution is not more intensely coloured than a standard prepared at the same time and in the same manner using 2.0 ml of *glyoxal standard solution (2 ppm* $C_2H_2O_2$*) R* instead of the 2.0 ml of supernatant liquid.

Ethylene oxide

Head-space gas chromatography *(2.4.25)*.

Test preparation Place 1.00 g of the substance to be examined in a 5 ml vial (other sizes may be used depending on the operating conditions) and add 1 ml of *water R*. It swells in water but does not dissolve.

Reference preparation (a) Place 1.00 g of the substance to be examined in an identical 5 ml vial. Add 0.1 ml of cooled *ethylene oxide solution R2* and 0.9 ml of *water R*. It swells in water but does not dissolve.

Reference preparation (b) To 0.1 ml of *ethylene oxide solution R2* in a 5 ml vial add 0.1 ml of a freshly prepared 10 mg/l solution of *acetaldehyde R.*

Close the vials immediately with a butyl rubber membrane stopper, coated with aluminium or polytetrafluoroethylene and secured with an aluminium crimped cap.

Limit:
— ethylene oxide: maximum 1 ppm.

2-Chloroethanol

Head-space gas chromatography *(2.2.28)*.

Test preparation To 50 mg of the substance to be examined in a 10 ml vial (other sizes may be used depending on the operating conditions), add 2 µl of *2-propanol R*. Seal the flask and mix.

Reference preparation (a) Dissolve 0.125 g of *2-chloroethanol R* and dilute to 50.0 ml with *2-propanol R*. Dilute 1.0 ml of the solution to 10.0 ml with *2-propanol R*.

Reference preparation (b) To 50 mg of the substance to be examined in an identical 10 ml vial, add 2 µl of reference solution (a). Seal the flask and mix.

Close the vials immediately with a butyl rubber membrane stopper, coated with aluminium or polytetrafluoroethylene and secured with an aluminium crimped cap.

Column:
— *size: l* = 50 m, Ø = 0.32 mm,
— *stationary phase*: *poly(dimethyl)siloxane R* (1.2 µm).

Carrier gas *helium for chromatography R*.

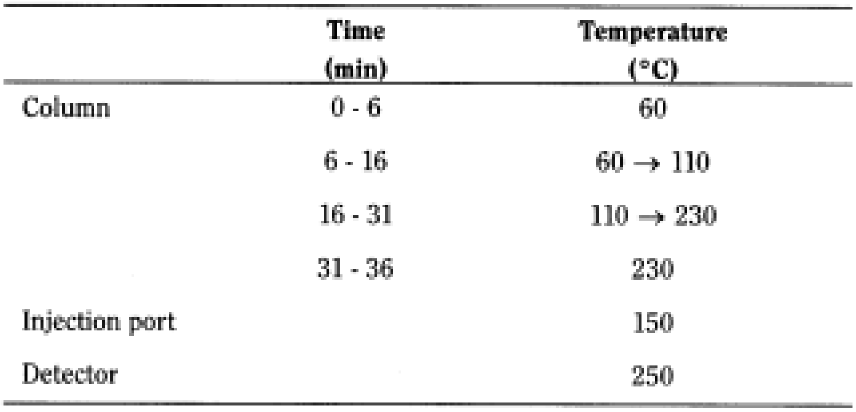
Flow rate 25-35 cm/s.

Split ratio 1:10.

Static head-space conditions which may be used:

- — *equilibration temperature*: 110 °C,
- — *equilibration time*: 20 min,
- — *temperature of injection system*: 115 °C.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	60
	6 - 16	60 → 110
	16 - 31	110 → 230
	31 - 36	230
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 2 ml.

Retention time 2-chloroethanol = about 7.8 min.

Limit:

- — *2-chloroethanol*: not more than 0.5 times the area of the peak due to 2-chloroethanol in the chromatogram obtained with reference solution (b) (10 ppm).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 4.0 per cent, determined on 1.0 g.

LABELLING

The label states the apparent viscosity, in millipascal seconds for a 2 per cent *m/m* solution.

Ph Eur

Hydroxyethylmethylcellulose

General Notices

(Methylhydroxyethylcellulose, Ph Eur monograph 0346)

Action and use

Excipient.

Ph Eur

DEFINITION

Partly *O*-methylated and *O*-(2-hydroxyethylated) cellulose.

CHARACTERS

Appearance

White, yellowish-white or greyish-white powder or granules, hygroscopic after drying.

Solubility

Practically insoluble in hot water, in acetone, in anhydrous ethanol and in toluene. It dissolves in cold water giving a colloidal solution.

IDENTIFICATION

A. Heat 10 ml of solution S (see Tests) in a water-bath while stirring. At a temperature above 50 °C, the solution becomes cloudy or a flocculent precipitate is formed. The solution becomes clear again on cooling.

B. To 10 ml of solution S add 0.3 ml of *dilute acetic acid R* and 2.5 ml of a 100 g/l solution of *tannic acid R*. A yellowish-white flocculent precipitate is formed which dissolves in *dilute ammonia R1.*

C. In a test-tube about 160 mm long, thoroughly mix 1 g with 2 g of finely powdered *manganese sulphate R*. Introduce to a depth of 2 cm into the upper part of the tube a strip of filter paper impregnated with a freshly prepared mixture of 1 volume of a 20 per cent *V/V* solution of *diethanolamine R* and 11 volumes of a 50 g/l solution of *sodium nitroprusside R*, adjusted to about pH 9.8 with *1 M hydrochloric acid*. Insert the tube 8 cm into a silicone-oil bath at 190-200 °C. The filter paper becomes blue within 10 min. Carry out a blank test.

D. Dissolve completely 0.2 g without heating in 15 ml of a 70 per cent *m/m* solution of *sulphuric acid R*. Pour the solution with stirring into 100 ml of iced *water R* and dilute to 250 ml with iced *water R*. In a test-tube, mix thoroughly while cooling in iced water 1 ml of this solution with 8 ml of *sulphuric acid R* added dropwise. Heat in a water-bath for exactly 3 min and immediately cool in iced water. While the mixture is cold, carefully add 0.6 ml of *ninhydrin solution R2* and mix well. Allow to stand at 25 °C. A pink colour is produced immediately and does not become violet within 100 min.

E. Place 1 ml of solution S on a glass plate. After evaporation of the water a thin film is formed.

TESTS

Solution S

While stirring, introduce a quantity of the substance to be examined equivalent to 1.0 g of the dried substance into 50 g of *carbon dioxide-free water R* heated to 90 °C. Allow to cool, adjust the mass of the solution to 100 g with *carbon dioxide-free water R* and stir until dissolution is complete.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH (2.2.3)

5.5 to 8.0 for solution S.

Apparent viscosity *(2.2.10)*

75 per cent to 140 per cent of the value stated on the label.

While stirring, introduce a quantity of the substance to be examined equivalent to 6.00 g of the dried substance into 150 g of *water R* heated to 90 °C. Stir with a propeller-type stirrer for 10 min, place the flask in a bath of iced water, continue the stirring and allow to remain in the bath of iced water for 40 min to ensure that dissolution is complete. Adjust the mass of the solution to 300 g and centrifuge the solution to expel any entrapped air. Adjust the temperature of the solution to 20 ± 0.1 °C. Determine the viscosity with a rotating viscometer at 20 °C and a shear rate of 10 s^{-1}.

Chlorides *(2.4.4)*

Maximum 0.5 per cent.

Dilute 1 ml of solution S to 15 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 1.0 per cent, determined on 1.000 g.

LABELLING

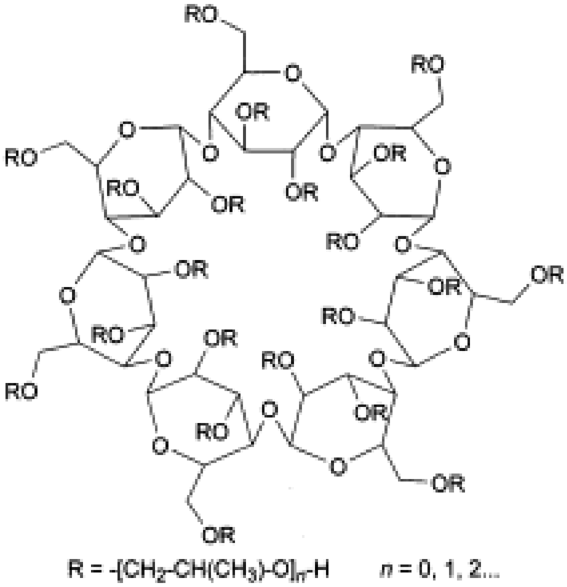
The label states the apparent viscosity in millipascal seconds for a 2 per cent *m/m* solution.

Ph Eur

Hydroxypropylbetadex

General Notices

(Ph Eur monograph 1804)



$C_{42}H_{70}O_{35}(C_3H_6O)_x$ with x = 7 *MS*

Action and use

Excipient.

Ph Eur

DEFINITION

Hydroxypropylbetadex (β-cyclodextrin, 2-hydroxypropyl ether) is a partially substituted poly (hydroxypropyl) ether of betadex. The number of hydroxypropyl groups per anhydroglucose unit, expressed as molar substitution (*MS*), is not less than 0.40 and not more than 1.50 and is within 10 per cent of the value stated on the label.

CHARACTERS

Appearance

White or almost white, amorphous or crystalline powder.

Solubility

Freely soluble in water and in propylene glycol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *hydroxypropylbetadex CRS.*

Results The spectrum obtained with the substance to be examined shows the same absorption bands as the spectrum obtained with *hydroxypropylbetadex CRS*. Due to the difference in the substitution of the substance, the intensity of some absorption bands can vary.

B. Appearance of solution (see Tests).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*, and remains so after cooling to room temperature.

Dissolve 1.0 g in 2.0 ml of *water R*, with heating.

Conductivity *(2.2.38)*

Maximum 200 µS·cm^{-1}.

Measure the conductivity of solution S, while gently stirring with a magnetic stirrer.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 2.50 g of the substance to be examined in *water R* with heating, cool, and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dissolve 0.15 g of *betadex CRS* and 0.25 g of *propylene glycol R* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water R*.

Precolumn:
— *stationary phase*: *phenylsilyl silica gel for chromatography R*.

Column:
— *size*: *l* = 0.30 m, Ø = 3.9 mm;
— *stationary phase*: *phenylsilyl silica gel for chromatography R*;
— *temperature*: 40 °C.

Mobile phase *water for chromatography R.*

Flow rate 1.5 ml/min.

Detection Differential refractometer, at 40 °C.

Injection 20 µl.

Run time 6 times the retention time of impurity A.

Relative retention With reference to impurity B (retention time = about 2.5 min): impurity A = about 4.2; hydroxypropylbetadex = about 6 for the beginning of the elution.

Hydroxypropylbetadex elutes as a very wide peak or several peaks.

System suitability Reference solution (a):

— *resolution*: minimum 4 between the peaks due to impurities A and B.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

— *any other impurity*: for each impurity, not more than 0.04 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *sum of impurities other than A and B*: not more than 0.4 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.02 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak eluting before impurity B or after impurity A.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 120 °C for 2 h.

Molar substitution

Nuclear magnetic resonance spectrometry *(2.2.33)*.

The molar substitution (*MS*) is calculated from the ratio between the signal from the 3 protons of the methyl group that is part of the hydroxypropyl group and the signal from the proton attached to the C1 carbon (glycosidic proton) of the anhydroglucose units.

Use a Fourier transform nuclear magnetic resonance spectrometer of minimum frequency 250 MHz, suited to record a proton spectrum and to carry out quantitative analysis, at a temperature of at least 25 °C.

Introduce not less than the equivalent of 10.0 mg of the substance to be examined (dried substance) into a 5 mm NMR tube, equipped with a spinner in order to record the spectrum in rotation. Add approximately 0.75 ml of *deuterium oxide R1*. Cap the tube, mix thoroughly and adapt the spinner.

Make the appropriate instrument settings (frequency, gain, digital resolution, sample rotation, shims, probe tuning, resolution/data point, receiver gain etc.) so as to obtain a suitable spectrum for quantitative analysis (good FID (Free Induction Decay), no distortion of the spectrum after Fourier transform and phase corrections). The relaxation delay must be adapted to the pulse angle in order to have sufficient relaxation of the protons concerned between 2 pulses (for example: 10 s for a 90° pulse).

Record the FID, with at least 8 scans, so as to obtain a spectral window comprised, at least, between 0 ppm and 6.2 ppm, referring to the signal of exchangeable protons (solvent) at 4.8 ppm (25 °C).

Make a zero filling of at least 3-fold in size relative to the acquisition data file and transform the FID to the spectrum without any correction of Gaussian broadening factor (GB = 0) and with a line broadening factor not greater than 0.2 (LB ≤ 0.2). Call the integration sub-routine after phase corrections and baseline correction between 0.5 ppm and 6.2 ppm.

Measure the peak areas of the doublet from the methyl groups at 1.2 ppm (A_1), and of the signals of the glycosidic protons between 5 ppm and 5.4 ppm (A_2).

The molar substitution is obtained using the following equation:

$$MS = \frac{A_1}{(3 \times A_2)}$$

A_1 = area of the signal due to the 3 protons of the methyl groups that are part of the hydroxypropyl groups;

A_2 = area of the signals due to the glycosidic protons.

The degree of substitution is the number of hydroxypropyl groups per molecule of β-cyclodextrin and is obtained by multiplying the *MS* by 7.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate count. If intended for use in the manufacture of parenteral preparations, the total viable aerobic count is not more than 10^2 bacteria and 10^2 fungi per gram. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/g, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

LABELLING

The label states:

— the molar substitution (*MS*);

— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES

A. betadex,

B. propylene glycol.

Hydroxypropylcellulose

General Notices

(Ph Eur monograph 0337)

9004-64-2

Action and use

Excipient.

Ph Eur

DEFINITION

Partly *C*-(2-hydroxypropylated) cellulose.

It may contain maximum 0.6 per cent of silica (SiO_2).

CHARACTERS

Appearance

White or yellowish-white powder or granules, hygroscopic after drying.

Solubility

Soluble in cold water, in glacial acetic acid, in anhydrous ethanol, in methanol and in propylene glycol and in a mixture of 10 parts of methanol and 90 parts of methylene chloride giving colloidal solutions, sparingly soluble or slightly soluble in acetone depending on the degree of substitution, practically insoluble in hot water, in ethylene glycol and in toluene.

IDENTIFICATION

A. Heat 10 ml of solution S (see Tests) in a water-bath while stirring. At a temperature above 40 °C the solution becomes cloudy or a flocculent precipitate is formed. The solution becomes clear again on cooling.

B. To 10 ml of solution S add 0.3 ml of *dilute acetic acid R* and 2.5 ml of a 100 g/l solution of *tannic acid R*. A yellowish-white flocculent precipitate is formed which dissolves in *dilute ammonia R1*.

C. In a test-tube about 160 mm long, thoroughly mix 1 g with 2 g of finely powdered *manganese sulphate R*. Introduce to a depth of 2 cm into the upper part of the tube a strip of filter paper impregnated with a freshly prepared mixture of 1 volume of a 20 per cent *V/V* solution of *diethanolamine R* and 11 volumes of a 50 g/l solution of *sodium nitroprusside R*, adjusted to about pH 9.8 with *1 M hydrochloric acid*. Insert the tube 8 cm into a silicone-oil bath at 190-200 °C. The filter paper becomes blue within 10 min. Carry out a blank test.

D. Dissolve completely 0.2 g without heating in 15 ml of a 70 per cent *m/m* solution of *sulphuric acid R*. Pour the solution with stirring into 100 ml of iced *water R* and dilute to 250 ml with iced *water R*. In a test-tube, mix thoroughly while cooling in iced water 1 ml of this solution with 8 ml of *sulphuric acid R* added dropwise. Heat in a water-bath for exactly 3 min and immediately cool in iced water. While the mixture is cold, carefully add 0.6 ml of *ninhydrin solution R2* and mix well. Allow to stand at 25 °C. A pink colour is produced immediately and becomes violet within 100 min.

E. Place 1 ml of solution S on a glass plate. After evaporation of the water a thin film is formed.

F. 0.2 g does not dissolve in 10 ml of *toluene R* but dissolves completely in 10 ml of *anhydrous ethanol R*.

TESTS

Solution S

While stirring, introduce a quantity of the substance to be examined equivalent to 1.0 g of the dried substance into 50 g of *carbon dioxide-free water R* heated to 90 °C. Allow to cool, adjust the mass of the solution to 100 g with *carbon dioxide-free water R* and stir until dissolution is complete.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

5.0 to 8.5 for solution S.

Apparent viscosity *(2.2.10)*

75 per cent to 140 per cent of the value stated on the label.

While stirring, introduce a quantity of the substance to be examined equivalent to 6.00 g of the dried substance into 150 g of *water R* heated to 90 °C. Stir with a propeller-type stirrer for 10 min, place the flask in a bath of iced water, continue the stirring and allow to remain in the bath of iced water for 40 min to ensure that dissolution is complete. Adjust the mass of the solution to 300 g and centrifuge the solution to expel any entrapped air. Adjust the temperature of the solution to 20 ± 0.1 °C. Determine the viscosity with a rotating viscometer at 20 °C and a shear rate of $10\ s^{-1}$.

For a product of low viscosity, use a quantity of the substance to be examined sufficient to prepare a solution of the concentration stated on the label.

Silica

Maximum 0.6 per cent.

To the residue obtained in the test for sulphated ash add sufficient *ethanol (96 per cent) R* to moisten the residue completely. Add 6 ml of *hydrofluoric acid R* in small portions. Evaporate to dryness at 95-105 °C, taking care to avoid loss from sputtering. Cool and rinse the wall of the platinum crucible with 6 ml of *hydrofluoric acid R*. Add 0.5 ml of *sulphuric acid R* and evaporate to dryness. Progressively increase the temperature, ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh. The difference between the mass of the residue obtained in the test for sulphated ash and the mass of the final residue is equal to the amount of silica in the substance to be examined.

Chlorides *(2.4.4)*

Maximum 0.5 per cent.

Dilute 1 ml of solution S to 15 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 7.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 1.6 per cent, determined on 1.0 g using a platinum crucible.

LABELLING

The label states:

— the apparent viscosity in millipascal seconds for a 2 per cent m/m solution,

— for a product of low viscosity, the concentration of the solution to be used and the apparent viscosity in millipascal seconds,

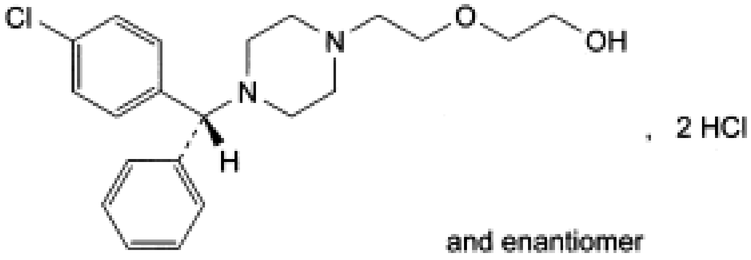
— where applicable, that the substance contains silica.

Ph Eur

Hydroxyzine Hydrochloride

General Notices

(Ph Eur monograph 0916)



$C_{21}H_{27}ClN_2O_2,2HCl$ 447.8 *2192-20-3*

Action and use

Histamine H_1 receptor antagonist.

Ph Eur

DEFINITION

(*RS*)-2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]ethanol dihydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water and in ethanol (96 per cent), very slightly soluble in acetone.

mp: about 200 °C, with decomposition.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *hydroxyzine hydrochloride CRS*.

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R*, *methylene chloride R* (50:50 *V/V*).

Test solution Dissolve 0.50 g of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 0.50 g of *hydroxyzine hydrochloride CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 0.50 g of *meclozine hydrochloride R* in the solvent mixture and dilute to 10 ml with the solvent mixture. Dilute 1 ml of this solution to 2 ml with reference solution (a).

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R*, *ethanol (96 per cent) R*, *toluene R* (1:24:75 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *potassium iodobismuthate solution R2*.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in *ethanol (96 per cent) R* and dilute to 15 ml with the same solvent. Add 15 ml of a saturated solution of *picric acid R* in *ethanol (96 per cent) R*. Allow to stand for 15 min. A precipitate is formed. Filter. Recrystallise from *ethanol (96 per cent) R*. Initiate crystallisation, if necessary, by scratching the wall of the tube with a glass rod. The crystals melt *(2.2.14)* at 189 °C to 192 °C.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *hydroxyzine hydrochloride CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 3.0 ml of the test solution to 200.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 25.0 ml with the mobile phase.

Column:

- *size:* l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve 0.5 g of *sodium methanesulphonate R* in a mixture of 14 ml of *triethylamine R,* 300 ml of *acetonitrile R* and 686 ml of *water R*, then adjust to pH 2.7 with *sulphuric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 2.5 times the retention time of hydroxyzine.

System suitability Reference solution (a):

- *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak immediately before the peak due to hydroxyzine and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to hydroxyzine.

Limits:

- *any impurity*: for each impurity, not more than 1/3 of the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

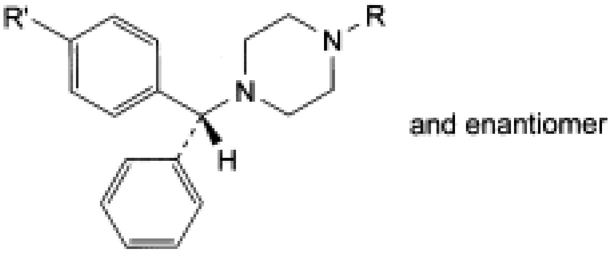
Dissolve 0.200 g in 10 ml of *anhydrous acetic acid R*. Add 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 22.39 mg of $C_{21}H_{29}Cl_3N_2O_2$.

STORAGE

In an airtight container , protected from light.

IMPURITIES



A. R = H, R′ = Cl: (*RS*)-1-[(4-chlorophenyl)phenylmethyl]piperazine,

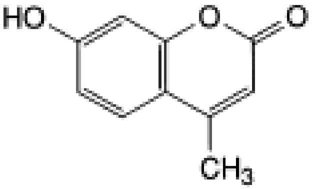
B. R = CH_2-CH_2-O-CH_2-CH_2-OH, R′ = H: 2-[2-[4-(diphenylmethyl)piperazin-1-yl]ethoxy] ethanol (decloxizine).

Ph Eur

Hymecromone

General Notices

(Ph Eur monograph 1786)



$C_{10}H_8O_3$ 176.2 *90-33-5*

Action and use

Choleretic; antispasmodic.

Ph Eur

DEFINITION

7-Hydroxy-4-methyl-2*H*-1-benzopyran-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Almost white crystalline powder.

Solubility

Very slightly soluble in water, sparingly soluble in methanol, slightly soluble in methylene chloride. It dissolves in dilute solutions of ammonia.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison hymecromone CRS.

TESTS

Absorbance *(2.2.25)*

Dissolve 50 mg in 10 ml of *ammonium chloride buffer solution pH 10.4 R* and dilute to 100.0 ml with *water R*. To 1.0 ml of the solution, add 10 ml of *ammonium chloride buffer solution pH 10.4 R* and dilute to 100.0 ml with *water R*. Examined between 200 nm and 400 nm, the solution shows 2 absorption maxima, at 229 nm and 360 nm, and an absorption minimum at 276 nm. The specific absorbance at the maximum at 360 nm is 1020 to 1120.

Related substances

Liquid chromatography *(2.2.29)*.

Buffer solution To 280 ml of a 1.56 g/l solution of *sodium dihydrogen phosphate R*, add 720 ml of a 3.58 g/l solution of *disodium hydrogen phosphate R*. Adjust to pH 7 with a 100 g/l solution of *phosphoric acid R*.

Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 20 mg of *hymecromone CRS*, 10 mg of *hymecromone impurity A CRS* and 10 mg of *hymecromone impurity B CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 200.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.25 m, Ø = 4 mm,
- *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase *methanol R*, *buffer solution* (465:535 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µl.

Run time 1.5 times the retention time of hymecromone.

Relative retention with reference to hymecromone (retention time = about 6 min): impurity A = about 0.5; impurity B = about 0.7.

System suitability Reference solution (a):

- *resolution*: minimum of 2 between the peaks due to impurity A and to impurity B and minimum of 3 between the peaks due to impurity B and to hymecromone.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.05 per cent),
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.05 per cent),
- *any other impurity*: not more than the area of the peak due to hymecromone in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *total*: not more than twice the area of the peak due to hymecromone in the chromatogram obtained with reference solution (b) (0.2 per cent),
- *disregard limit*: 0.1 times the area of the peak due to hymecromone in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 1.5 g in a mixture of 15 volumes of *water R* and 85 volumes of *dimethylformamide R* and dilute to 18 ml with the same mixture of solvents. The solution complies with limit test B. Prepare the standard using a lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *dimethylformamide R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 80 ml of *2-propanol R*. Titrate with *0.1 M tetrabutylammonium hydroxide in 2-propanol* determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

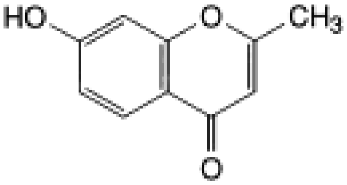
1 ml of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 17.62 mg of $C_{10}H_8O_3$.

STORAGE

Protected from light.

IMPURITIES

A. resorcinol,



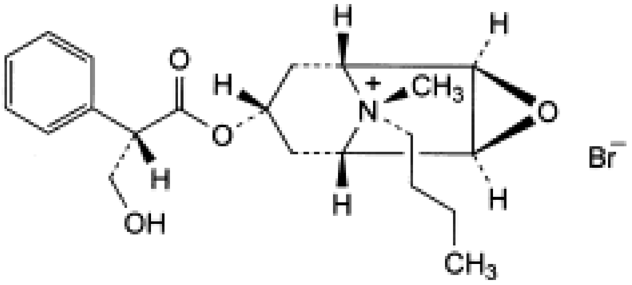
B. 7-hydroxy-2-methyl-4*H*-1-benzopyran-4-one.

Ph Eur

Hyoscine Butylbromide

General Notices

(Ph Eur monograph 0737)



$C_{21}H_{30}BrNO_4$ 440.4 *149-64-4*

Action and use

Anticholinergic.

Preparations

Hyoscine Butylbromide Injection

Hyoscine Butylbromide Tablets

Ph Eur

DEFINITION

(1*R*,2*R*,4*S*,5*S*,7*s*,9*r*)-9-Butyl-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9-methyl-3-oxa-9-azoniatricyclo[3.3.1.0^{2},^{4}]nonane bromide.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methylene chloride, sparingly soluble in anhydrous ethanol.

IDENTIFICATION

First identification A, C, F.

Second identification A, B, D, E, F.

A. It complies with the test for specific optical rotation (see Tests).

B. Melting point *(2.2.14)*: 139 °C to 141 °C.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *hyoscine butylbromide CRS.*

D. To about 1 mg add 0.2 ml of *nitric acid R* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.1 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

E. To 5 ml of solution S (see Tests) add 2 ml of *dilute sodium hydroxide solution R*. No precipitate is formed.

F. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH (2.2.3)

5.5 to 6.5 for solution S.

Specific optical rotation *(2.2.7)*

- 18 to - 20 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 10.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *hyoscine butylbromide impurity E CRS* in the mobile phase, add 1.0 ml of the test solution and dilute to 10.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Column:
— *size: l* = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (4 µm);
— *temperature*: 25 ± 1 °C.

Mobile phase Dissolve 5.8 g of *sodium dodecyl sulphate R* in a mixture of 410 ml of *acetonitrile R* and 605 ml of a 7.0 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.3 with *0.05 M phosphoric acid*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Run time 3.5 times the retention time of butylhyoscine.

Relative retention With reference to butylhyoscine (retention time = about 7.0 min): impurity B = about 0.1; impurity A = about 0.36; impurity C = about 0.40; impurity D = about 0.7; impurity E = about 0.8; impurity F = about 0.9; impurity G = about 3.0.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to butylhyoscine and impurity E;

— *symmetry factor*: maximum 2.5 for the peak due to butylhyoscine.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.3; impurity G = 0.6;

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurities B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent); disregard any peak due to the bromide ion which appears close to the solvent peak;

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 2.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 0.5 g.

ASSAY

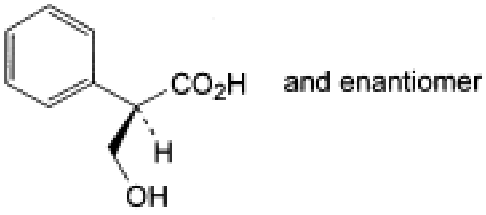
Dissolve 0.400 g in 50 ml of *water R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)* using a silver indicator electrode and a silver-silver chloride reference electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 44.04 mg of $C_{21}H_{30}BrNO_4$.

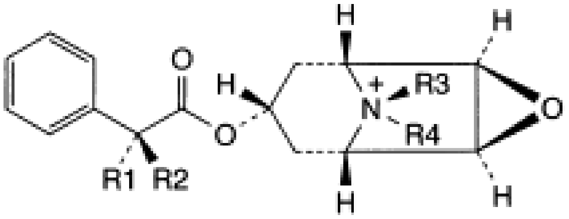
IMPURITIES

Specified impurities *A, B, C, D, E, F, G.*

A. hyoscine,



B. (2*RS*)-3-hydroxy-2-phenylpropanoic acid (dl-tropic acid),

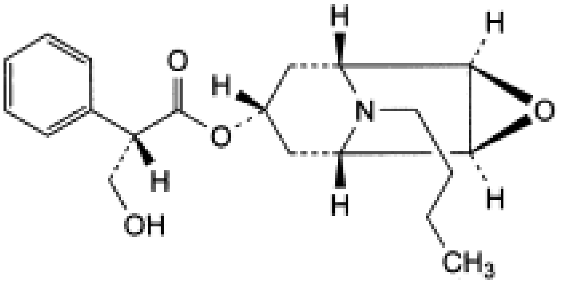


C. R1 = CH_2OH, R2 = H, R3 = R4 = CH_3: (1*R*,2*R*,4*S*,5*S*,7*s*)-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane (methylhyoscine),

D. R1 = CH_2OH, R2 = H, R3 = CH_3, R4 = CH_2-CH_2-CH_3: (1*R*,2*R*,4*S*,5*S*,7*s*,9*r*)-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9-methyl-9-propyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane (propylhyoscine),

F. R1 = CH_2OH, R2 = H, R3 = CH_2-CH_2-CH_2-CH_3, R4 = CH_3: (1*R*,2*R*,4*S*,5*S*,7*s*,9*s*)-9-butyl-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9-methyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane (pseudo-isomer),

G. R1 + R2 = CH_2 , R3 = CH_3, R4 = CH_2-CH_2-CH_2-CH_3: (1*R*,2*R*,4*S*,5*S*,7*s*,9*r*)-9-butyl-9-methyl-7-[(2-phenylprop-2-enoyl)oxy]-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane (apo-*N*-butylhyoscine);



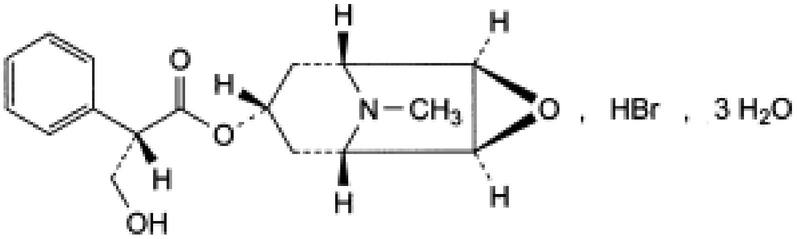
E. (1*R*,2*R*,4*S*,5*S*,7*s*)-9-butyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]nonan-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate (*N*-butylhyoscine).

Ph Eur

Hyoscine Hydrobromide

General Notices

(Ph Eur monograph 0106)



$C_{17}H_{21}NO_4,HBr,3H_2O$ 438.3 *6533-68-2*

Action and use

Anticholinergic.

Preparations

Hyoscine Eye Drops

Hyoscine Injection

Hyoscine Tablets

Ph Eur

DEFINITION

(1*R*,2*R*,4*S*,5*S*,7*s*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate hydrobromide trihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, efflorescent.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison hyoscine hydrobromide CRS.

If the spectra obtained in the solid state show differences, proceed as follows: dissolve 3 mg of the substance to be examined in 1 ml of *ethanol (96 per cent) R* and evaporate to dryness on a water-bath; dissolve the residue in 0.5 ml of *methylene chloride R* and add 0.2 g of *potassium bromide R* and 15 ml of *ether R*; allow to stand for 5 min shaking frequently; decant; dry the residue on a water-bath until the solvents have evaporated; using the residue prepare a disc and dry at 100-105 °C for 3 h. Repeat the procedure with *hyoscine hydrobromide CRS* and record the spectra.

C. Dissolve about 50 mg in 5 ml of *water R* and add 5 ml of *picric acid solution R* dropwise and with shaking. The precipitate, washed with *water R* and dried at 100-105 °C for 2 h, melts *(2.2.14)* at 188 °C to 193 °C.

D. To about 1 mg add 0.2 ml of *fuming nitric acid R* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.1 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

E. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

pH *(2.2.3)*

4.0 to 5.5 for solution S.

Specific optical rotation *(2.2.7)*

- 24 to - 27 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 70.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 25.0 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *hyoscine hydrobromide impurity B CRS* in the mobile phase, add 5.0 ml of the test solution and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 25 ± 1 °C.

Mobile phase Mix 330 ml of *acetonitrile R* with 670 ml of a 2.5 g/l solution of *sodium dodecyl sulphate R* previously adjusted to pH 2.5 with *3 M phosphoric acid*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 5 µl.

Run time 3 times the retention time of hyoscine.

Relative retention With reference to hyoscine (retention time = about 5.0 min): impurity D = about 0.2; impurity B = about 0.9; impurity A = about 1.3; impurity C = about 2.4.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to impurity B and hyoscine;

— *symmetry factor*: maximum 2.5 for the peak due to hyoscine.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.3; impurity C = 0.6;

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurities A, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 1.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent); disregard any peak due to the bromide ion which appears close to the solvent peak;

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

10.0 per cent to 13.0 per cent, determined on 0.20 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide* free from carbonate. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 38.43 mg of $C_{17}H_{22}BrNO_4$.

STORAGE

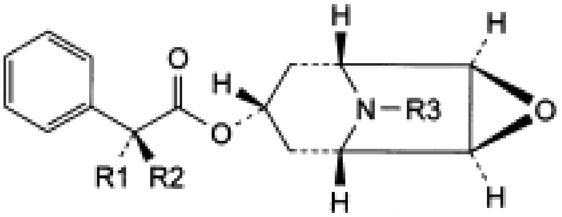
In a well-filled, airtight container of small capacity, protected from light.

IMPURITIES

Specified impurities

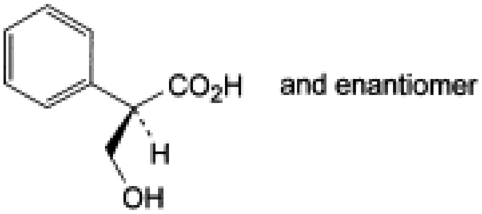
A, B, C, D.

A. hyoscyamine,



B. R1 = CH_2OH, R2 = R3 = H: (1*R*,2*R*,4*S*,5*S*,7*s*)-3-oxa-9-azatricyclo[3.3.1.02,4]non-7-yl (2S)-3-hydroxy-2-phenylpropanoate (norhyoscine),

C. R1 + R2 = CH_2, R3 = CH_3: (1*R*,2*R*,4*S*,5*S*,7*s*)-9-methyl-3-oxa-9-azatricyclo[3.3.1.02,4]non-7-yl 2-phenylprop-2-enoate (apohyoscine),



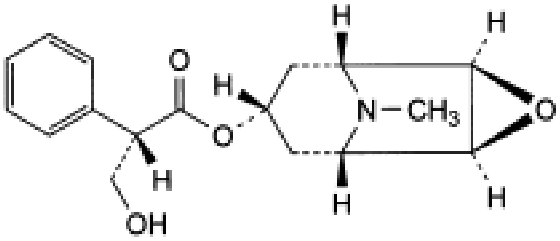
D. (2*RS*)-3-hydroxy-2-phenylpropanoic acid (dl-tropic acid).

Ph Eur

Hyoscine

General Notices

(Ph Eur monograph 2167)



$C_{17}H_{21}NO_4$ 303.4 *51-34-3*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

(1*R*,2*R*,4*S*,5*S*,7*s*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Soluble in water, freely soluble in ethanol (96 per cent).

mp

66 °C to 70 °C.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison hyoscine CRS.

TESTS

Specific optical rotation *(2.2.7)*

- 33 to - 39 (anhydrous substance).

Dissolve 1.00 g in *dilute hydrochloric acid R* and dilute to 25.0 ml with the same acid.

Related substances

Liquid chromatography (2.2.29).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *hyoscine impurity A CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 25.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (d) Mix 2.0 ml of reference solution (b) and 1.0 ml of the test solution and dilute to 10.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Mix 33 volumes of *acetonitrile R* and 67 volumes of a 2.5 g/l solution of *sodium dodecyl sulphate R* previously adjusted to pH 2.5 with a 346 g/l solution of *phosphoric acid R.*

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 5 µl.

Run time 3 times the retention time of hyoscine.

Relative retention With reference to hyoscine (retention time = about 5 min): impurity C = about 0.2; impurity A = about 0.9; impurity D = about 1.3; impurity B = about 2.5.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to impurity A and hyoscine.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.6; impurity C = 0.3;

— *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *impurities B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

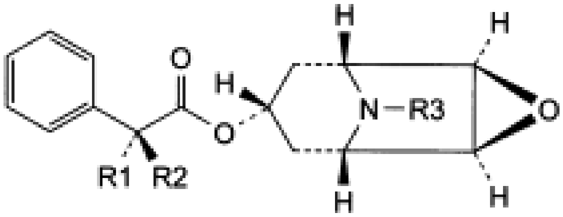
ASSAY

Dissolve 0.250 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 30.34 mg of $C_{17}H_{21}NO_4$.

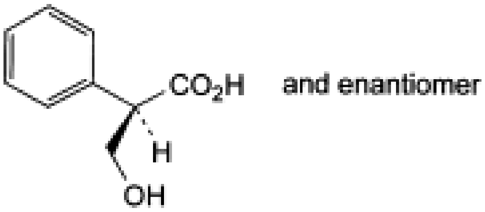
IMPURITIES

Specified impurities   A, B, C, D.



A. R1 = CH_2OH, R2 = R3 = H: (1*R*,2*R*,4*S*,5*S*,7*s*)-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate (norhyoscine),

B. R1 + R2 = CH_2, R3 = CH_3: (1*R*,2*R*,4*S*,5*S*,7*s*)-9-methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]non-7-yl 2-phenylprop-2-enoate (apohyoscine),



C. (2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid),

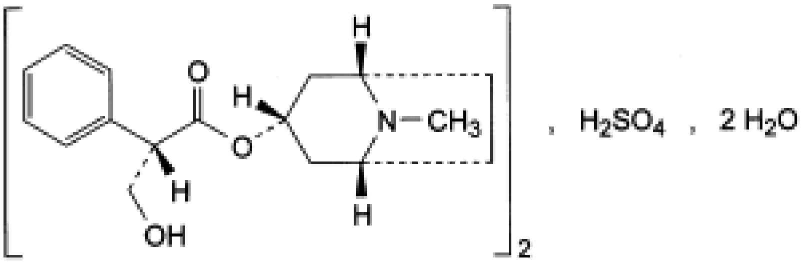
D. hyoscyamine.

Ph Eur

Hyoscyamine Sulphate

General Notices

(Ph Eur monograph 0501)



$(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ 713   *6835-16-1*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

Bis[(1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate] sulphate dihydrate.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless needles.

Solubility

Very soluble in water, sparingly soluble or soluble in ethanol (96 per cent).

IDENTIFICATION

First identification   A, B, E.

Second identification   C, D, E.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *hyoscyamine sulphate CRS.*

C. To 0.5 ml of solution S (see Tests) add 2 ml of *dilute acetic acid R* and heat. To the hot solution add 4 ml of *picric acid solution R*. Allow to cool, shaking occasionally. Collect the crystals, wash with 2 quantities, each of 3 ml, of iced *water R* and dry at 100-105 °C. The crystals melt *(2.2.14)* at 164 °C to 168 °C.

D. To about 1 mg add 0.2 ml of *fuming nitric acid R* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.2 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

E. It gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.50 g in *water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 6.2.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 24 to - 29 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 60.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A. Dilute 10.0 ml of the solution to 50.0 ml with mobile phase A.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 5.0 ml of this solution to 50.0 ml with mobile phase A.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 25.0 ml with mobile phase A.

Reference solution (c) Dissolve 5.0 mg of *hyoscyamine impurity E CRS* in the test solution and dilute to 20.0 ml with the test solution. Dilute 5.0 ml of this solution to 25.0 ml with mobile phase A.

Column:

— *size:* l = 0.10 m, Ø = 4.6 mm;

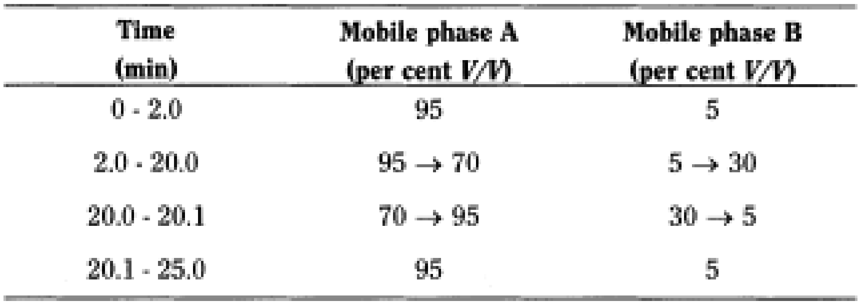
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 25 ± 1 °C.

Mobile phase:

— *mobile phase A*: dissolve 3.5 g of *sodium dodecyl sulphate R* in 606 ml of a 7.0 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.3 with *0.05 M phosphoric acid* and mix with 320 ml of *acetonitrile R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2.0	95	5
2.0 - 20.0	95 → 70	5 → 30
20.0 - 20.1	70 → 95	30 → 5
20.1 - 25.0	95	5

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Relative retention With reference to hyoscyamine (retention time = about 10.5 min): impurity A = about 0.2; impurity B = about 0.67; impurity C = about 0.72; impurity D = about 0.8; impurity E = about 0.9; impurity F = about 1.1; impurity G = about 1.8.

System suitability Reference solution (c):

— *resolution*: minimum 2.5 between the peaks due to hyoscyamine and impurity E;

— *symmetry factor*: maximum 2.5 for the peak due to hyoscyamine.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.3; impurity G = 0.6;

— *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities A, B, C, D, F, G*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*

2.0 per cent to 5.5 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

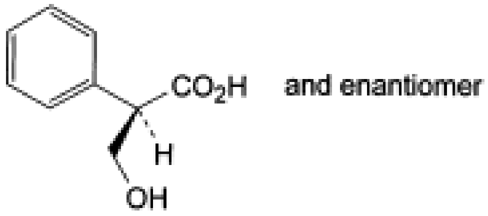
1 ml of *0.1 M perchloric acid* is equivalent to 67.7 mg of $C_{34}H_{48}N_2O_{10}S$.

STORAGE

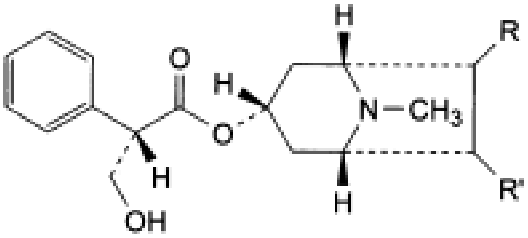
In an airtight container, protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G.



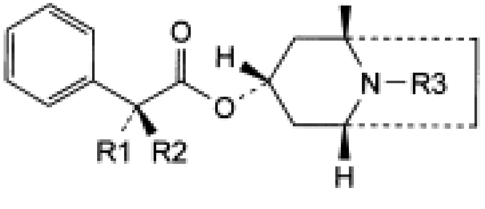
A. (2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid),



B. R = OH, R′ = H: (1*R*,3*S*,5*R*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (7-hydroxyhyoscyamine),

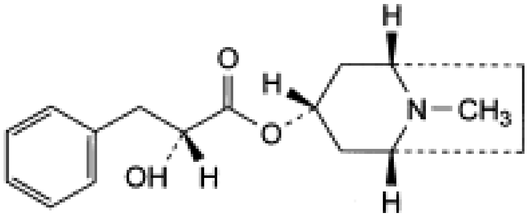
C. R = H, R′ = OH: (1*S*,3*R*,5*S*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (6-hydroxyhyoscyamine),

D. hyoscine,



E. R1 = CH_2OH, R2 = R3 = H: (1*R*,3*r*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (norhyoscyamine),

G. R1 + R2 = CH_2, R3 = CH_3: (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-phenylprop-2-enoate (apoatropine),



F. (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*R*)-2-hydroxy-3-phenylpropanoate (littorine).

Ph Eur

Hypromellose Phthalate

General Notices

(Ph Eur monograph 0347)

Action and use

Artificial tears.

Ph Eur

DEFINITION

Hydroxypropylmethylcellulose phtalate.

Monophthalic acid ester of hypromellose, containing methoxy (-OCH_3), 2-hydroxypropoxy (-$OCH_2CHOHCH_3$) and phthaloyl (*o*-carboxybenzoyl $C_8H_5O_3$) groups.

CHARACTERS

Appearance

White or almost white, free-flowing flakes or granular powder.

Solubility

Practically insoluble in water, soluble in a mixture of equal volumes of acetone and methanol and in a mixture of equal volumes of methanol and methylene chloride, very slightly soluble in acetone and in toluene, practically insoluble in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison   *hypromellose phthalate CRS.*

TESTS

Free phthalic acid

Liquid chromatography (*2.2.29*).

Test solution   Dissolve 0.20 g of the substance to be examined in about 50 ml of *acetonitrile R* with the aid of ultrasound. Add 10 ml of *water R*, cool to room temperature, dilute to 100.0 ml with *acetonitrile R* and mix.

Reference solution   Dissolve 12.5 mg of *phthalic acid R* in 125 ml of *acetonitrile R.* Add 25 ml of *water R*, dilute to 250.0 ml with *acetonitrile R* and mix.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase *acetonitrile R*, 1 g/l solution of *trifluoroacetic acid R* (1:9 *V/V*).

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µl.

System suitability Reference solution:

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 2 injections.

Limit:

— *phthalic acid*: not more than 0.4 times the area of the corresponding peak in the chromatogram obtained with the reference solution (1.0 per cent).

Chlorides

Maximum 0.07 per cent.

Dissolve 1.0 g in 40 ml of *0.2 M sodium hydroxide*, add 0.05 ml of *phenolphthalein solution R* and add *dilute nitric acid R* dropwise, with stirring, until the red colour disappears. Add an additional 20 ml of *dilute nitric acid R* with stirring. Heat on a water-bath with stirring until the gel-like precipitate formed becomes granular. Cool and centrifuge. Separate the liquid phase and wash the residue with 3 quantities, each of 20 ml, of *water R*, separating the washings by centrifugation. Combine the liquid phases, dilute to 200 ml with *water R,* mix and filter. To 50 ml of this solution, add 1 ml of *0.1 M silver nitrate*. The solution is not more opalescent than a standard prepared by mixing 0.5 ml of *0.01 M hydrochloric acid* with 10 ml of *0.2 M sodium hydroxide*, adding 7 ml of *dilute nitric acid R* and 1 ml of *0.1 M silver nitrate*, and diluting to 50 ml with *water R.*

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 5.0 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

STORAGE

In an airtight container .

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the *manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.*

The following characteristics may be relevant for hypromellose phthalate used as a gastro-resistant coating agent.

Apparent viscosity ***(2.2.9)***

80 per cent to 120 per cent of the nominal value.

Dissolve 10 g, previously dried at 105 °C for 1 h, in 90 g of a mixture of equal masses of *methanol R* and *methylene chloride R* by mixing and shaking.

Solubility

0.2 g does not dissolve in *0.1 M hydrochloric acid* but dissolves quickly and completely in 100 ml of *phosphate buffer solution pH 6.8 R* with stirring.

Phthaloyl groups

Typically 21.0 per cent to 35.0 per cent (anhydrous substance).

Dissolve 1.000 g in 50 ml of a mixture of 1 volume of *water R*, 2 volumes of *acetone R* and 2 volumes of *ethanol (96 per cent) R*. Add 0.1 ml of *phenolphthalein solution R* and titrate with *0.1 M sodium hydroxide* until a faint pink colour is obtained. Carry out a blank titration.

Calculate the percentage content of phthaloyl groups using the following expression:

$$\frac{149n}{(100-a)\,m} - 1.795S$$

a = percentage content of water

m = mass of the substance to be examined, in grams

n = volume of *0.1 M sodium hydroxide* used, in millilitres

S = percentage content of free phthalic acid (see Tests).

Ph Eur

Hypromellose

General Notices

(Ph Eur monograph 0348)

9004-65-3

Action and use

Artificial tears.

Preparation

Hypromellose Eye Drops

Ph Eur

DEFINITION

Hydroxypropylmethylcellulose.

Partly *O*-methylated and *O*-(2-hydroxypropylated) cellulose.

CHARACTERS

Appearance

White, yellowish-white or greyish-white powder or granules, hygroscopic after drying.

Solubility

Practically insoluble in hot water, in acetone, in anhydrous ethanol and in toluene. It dissolves in cold water giving a colloidal solution.

IDENTIFICATION

A. Evenly distribute 1.0 g on the surface of 100 ml of *water R* in a beaker, tapping the top of the beaker, gently if necessary to ensure a uniform layer on the surface. Allow to stand for 1-2 min: the powdered material aggregates on the surface.

B. Evenly distribute 1.0 g into 100 ml of boiling *water R*, and stir the mixture using a magnetic stirrer with a bar 25 mm long: a slurry is formed and the particles do not dissolve. Allow the slurry to cool to 10 °C and stir using a magnetic stirrer: a clear or slightly turbid solution occurs with its thickness dependent on the viscosity grade.

C. To 0.1 ml of the solution obtained in identification B add 9 ml of a 90 per cent *V/V* solution of *sulphuric acid R*, shake, heat on a water-bath for exactly 3 min, immediately cool in an ice-bath, carefully add 0.6 ml of a 20 g/l solution of *ninhydrin R,* shake and allow to stand at 25 °C: a red colour develops at first and changes to purple within 100 min.

D. Place 2-3 ml of the solution obtained in identification B onto a glass slide as a thin film and allow the water to evaporate: a coherent, clear film forms on the glass slide.

E. Add exactly 50 ml of the solution obtained in identification B to exactly 50 ml of *water R* in a beaker. Insert a thermometer into the solution. Stir the solution on a magnetic stirrer/hot plate and begin heating, increasing the temperature at a rate of 2-5 °C per minute. Determine the temperature at which a turbidity increase begins to occur and designate the temperature as the flocculation temperature: the flocculation temperature is higher than 50 °C.

TESTS

Solution S

While stirring, introduce a quantity of the substance to be examined equivalent to 1.0 g of the dried substance into 50 g of *carbon dioxide-free water R* heated to 90 °C. Allow to cool, adjust the mass of the solution to 100 g with *carbon dioxide-free water R* and stir until dissolution is complete.

Appearance of solution

Solution S is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH *(2.2.3)*

5.0 to 8.0 for the solution prepared as described under Apparent viscosity.

Carry out the test at 20 ± 2 °C and read the indicated pH value after the probe has been immersed for 5 ± 0.5 min.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulphated ash *(2.4.14)*

Maximum 1.5 per cent, determined on 1.0 g.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for hypromellose used as binder, viscosity-increasing agent or film former.

Apparent viscosity

Minimum 80 per cent and maximum 120 per cent of the nominal value for samples with a viscosity less than 600 mPa·s (Method 1); minimum 75 per cent and maximum 140 per cent of the nominal value for samples with a viscosity of 600 mPa·s or higher (Method 2).

Method 1, to be applied to samples with a viscosity of less than 600 mPa·s Weigh accurately a quantity of the substance to be examined equivalent to 4.000 g of the dried substance. Transfer into a wide-mouthed bottle, and adjust the mass to 200.0 g with hot *water R*. Capping the bottle, stir by mechanical means at 400 ± 50 r/min for 10-20 min until the particles are thoroughly dispersed and wetted. Scrape down the insides of the bottle with a spatula if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water-bath maintained at a temperature below 10 °C for another 20-40 min. Adjust the solution mass if necessary to 200.0 g using cold *water R*. Centrifuge the solution if necessary to expel any entrapped air bubbles. Using a spatula, remove any foam, if present. Determine the viscosity of this solution using the capillary viscometer method (*2.2.9*) to obtain the kinematic viscosity (ν). Separately, determine the density (ρ) (*2.2.5*) of the solution and calculate the dynamic viscosity (η), as $\eta = \rho\nu$.

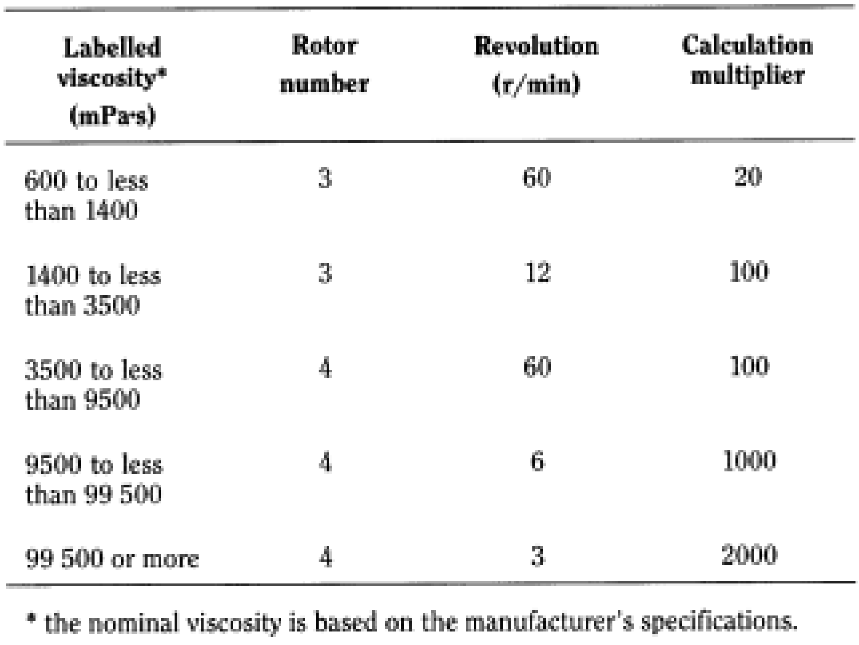
Method 2, to be applied to samples with a viscosity of 600 mPa·s or higher Weigh accurately a quantity of the substance to be examined equivalent to 10.00 g of the dried substance. Transfer into a wide-mouthed bottle, and adjust the mass to 500.0 g with hot *water R*. Capping the bottle, stir by mechanical means at 400 ± 50 r/min for 10-20 min until the particles are thoroughly dispersed and wetted. Scrape down the insides of the bottle with a spatula if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water-bath maintained at a temperature below 10 °C for another 20-40 min. Adjust the solution mass if necessary to 500.0 g using cold *water R*. Centrifuge the solution if necessary to expel any entrapped air bubbles. Using a spatula, remove any foam, if present. Determine the viscosity (*2.2.10*) of this solution at 20 ± 0.1 °C using a rotating viscometer.

Apparatus Single-cylinder type spindle viscometer.

Rotor number, revolution and calculation multiplier Apply the conditions specified in Table 0348.-1.

Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period of 2 min between subsequent measurements. Repeat the measurement twice and determine the mean of the 3 readings.

Table 0348.-1.



Labelled viscosity* (mPa·s)	Rotor number	Revolution (r/min)	Calculation multiplier
600 to less than 1400	3	60	20
1400 to less than 3500	3	12	100
3500 to less than 9500	4	60	100
9500 to less than 99 500	4	6	1000
99 500 or more	4	3	2000

* the nominal viscosity is based on the manufacturer's specifications.

Degree of substitution

Gas chromatography (*2.2.28*).

Apparatus:

— *reaction vial*: a 5 ml pressure-tight vial, 50 mm in height, 20 mm in external diameter and 13 mm in internal diameter at the mouth, equipped with a pressure-tight butyl rubber membrane stopper coated with polytetrafluoroethylene and secured with an aluminium crimped cap or another sealing system providing a sufficient air-tightness;

— *heater*: a heating module with a square aluminium block having holes 20 mm in diameter and 32 mm in depth, so that the reaction vials fit; mixing of the contents of the vial is effected using a magnetic stirrer equipped in the heating module or using a reciprocal shaker that performs approximately 100 cycles/min.

Internal standard solution 30 g/l solution of *octane R* in *xylene R.*

Test solution Weigh 65.0 mg of the substance to be examined, place in a reaction vial, add 0.06-0.10 g of *adipic acid R*, 2.0 ml of the internal standard solution and 2.0 ml of a 570 g/l solution of *hydriodic acid R*, immediately cap and seal the vial, and weigh accurately. Mix the contents of the vial continuously for 60 min while heating the block so that the temperature of the contents is maintained at 130 ± 2 °C. If a reciprocal shaker or magnetic stirrer cannot be used, shake the vial well by hand at 5-minute intervals during the initial 30 min of the heating time. Allow the vial to cool, and again weigh accurately. If the loss of mass is less than 0.50 per cent of the contents and there is no evidence of a leak, use the upper layer of the mixture as the test solution.

Reference solution Place 0.06-0.10 g of *adipic acid R*, 2.0 ml of the internal standard solution and 2.0 ml of *hydroiodic acid R* in another reaction vial, cap and seal the vial, and weigh accurately. Add 15-22 µl of *isopropyl iodide R* through the septum with a syringe, weigh accurately, add 45 µl of *methyl iodide R* in the same manner, and weigh accurately. Shake the reaction vial well, and use the upper layer as the reference solution.

Column:

— *size*: *l* = 1.8-3 m, Ø = 3-4 mm;

— *stationary phase*: *diatomaceous earth for gas chromatography R* impregnated with 10-20 per cent of *poly(dimethyl)(75)(diphenyl)(25)siloxane R* (film thickness 125-150 µm);

— *temperature*: 100 °C.

Carrier gas *helium for chromatography R* (thermal conductivity); *helium for chromatography R* or *nitrogen for chromatography R* (flame ionisation).

Flow rate Adjusted so that the retention time of the internal standard is about 10 min.

Detection Flame ionisation or thermal conductivity.

Injection 1-2 µl.

System suitability Reference solution:

— *resolution*: well resolved peaks of methyl iodide (1st peak), isopropyl iodide (2nd peak) and internal standard (3rd peak).

Calculation:

— *methoxy and hydroxypropoxy groups*: calculate the ratios (Q_1 and Q_2) of the areas of the peaks due to methyl iodide and isopropyl iodide to the area of the peak due to the internal standard in the chromatogram obtained with the test solution, and the ratios (Q_3 and Q_4) of the areas of the peaks due to methyl iodide and isopropyl iodide to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution.

Calculate the percentage content of methoxy groups using the following expression:

$$\frac{Q_1}{Q_3} \times \frac{m_1}{m} \times 21.864$$

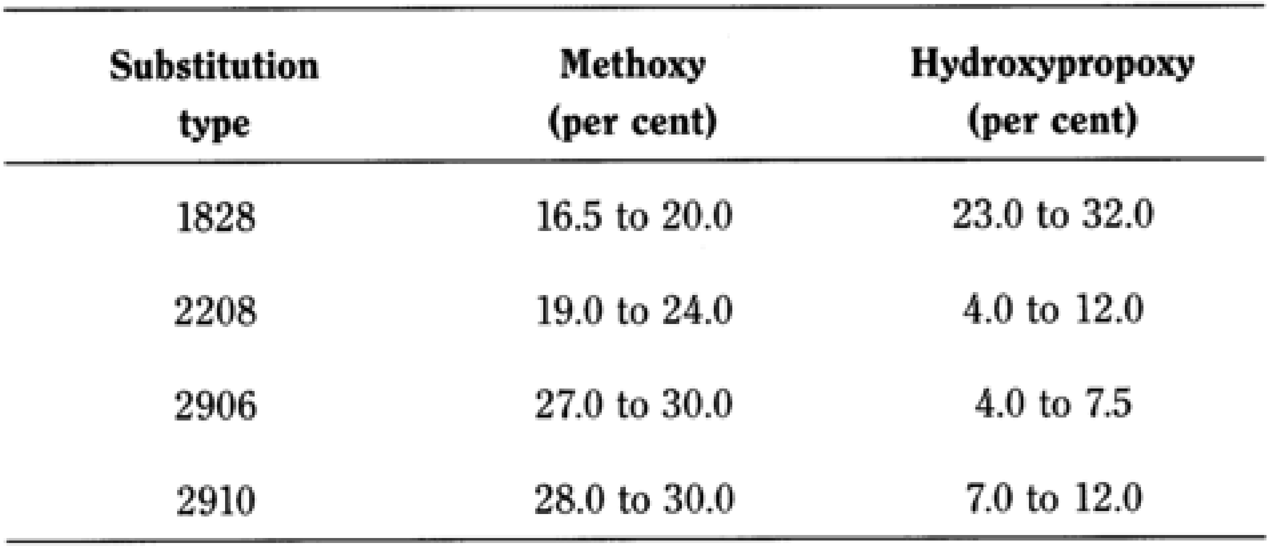
Calculate the percentage content of hydroxypropoxy groups using the following expression:

$$\frac{Q_2}{Q_4} \times \frac{m_2}{m} \times 44.17$$

m_1 = mass of methyl iodide in the reference solution, in milligrams

m_2 = mass of isopropyl iodide in the reference solution, in milligrams

m = mass of the sample (dried substance), in milligrams.



Substitution type	Methoxy (per cent)	Hydroxypropoxy (per cent)
1828	16.5 to 20.0	23.0 to 32.0
2208	19.0 to 24.0	4.0 to 12.0
2906	27.0 to 30.0	4.0 to 7.5
2910	28.0 to 30.0	7.0 to 12.0

The following characteristics may be relevant for hypromellose used as matrix former in prolonged-release tablets.

Apparent viscosity

See test above.

Degree of substitution

See test above.

Molecular mass distribution *(2.2.30)*.

Particle-size distribution *(2.9.31* or *2.9.38)*.

Powder flow *(2.9.36)*.

Ph Eur

Low-molecular-weight Heparins

General Notices

(Low-molecular-mass Heparins, Ph Eur monograph 0828)

Action and use

Anticoagulant.

Ph Eur

DEFINITION

Salts of sulphated glucosaminoglycans having a mass-average relative molecular mass less than 8000 and for which at least 60 per cent of the total mass has a relative molecular mass less than 8000. Low-molecular-mass heparins display different chemical structures at the reducing, or the non-reducing end of the polysaccharide chains.

The potency is not less than 70 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, is not less than 1.5.

PRODUCTION

Low-molecular-mass heparins are obtained by fractionation or depolymerisation of heparin of natural origin that complies with the monograph on *Heparin sodium (0333)* or *Heparin calcium (0332)*, whichever is appropriate, for parenteral use, unless otherwise justified and authorised. For each type of low-molecular-mass heparin the batch-to-batch consistency is ensured by demonstrating, for example, that the mass-average relative molecular mass and the mass percentage within defined relative molecular-mass ranges lower than 8000 are not less than 75 per cent and not more than 125 per cent of the mean value stated as type specification. The same limits apply also to the ratio of anti-factor Xa activity to anti-factor IIa activity.

Nucleotide and protein impurities of the source material

Dissolve 40 mg of the source material before fractionation in 10 ml of *water R*. The absorbance *(2.2.25)* measured at 260 nm and 280 nm is not greater than 0.20 and 0.15, respectively.

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Freely soluble in water.

IDENTIFICATION

A. Nuclear magnetic resonance spectrometry *(2.2.33)*.

Preparation Dissolve 0.200 g of the substance to be examined in a mixture of 0.2 ml of *deuterium oxide R* and 0.8 ml of *water R*.

Comparison Dissolve 0.200 g of the appropriate specific low-molecular-mass heparin reference standard in a mixture of 0.2 ml of *deuterium oxide R* and 0.8 ml of *water R*.

Operating conditions Use a pulsed (Fourier transform) spectrometer operating at 75 MHz for ^{13}C. Record the spectra at 40 °C, using cells 5 mm in diameter. Use *deuterated methanol R* as internal reference at $\delta = 50.0$ ppm.

Results The spectrum obtained is similar to the appropriate specific low-molecular-mass heparin reference standard.

B. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, is not less than 1.5.

C. Size-exclusion chromatography *(2.2.30)*.

Test solution Dissolve 20 mg of the substance to be examined in 2 ml of the mobile phase.

Reference solution Dissolve 20 mg of *heparin low-molecular-mass for calibration CRS* in 2 ml of the mobile phase.

Column:

— *size*: *l* = 0.30 m, Ø = 7.5 mm;

— *stationary phase*: appropriate porous silica beads (5 µm) with a fractionation range for proteins of approximately 15 000 to 100 000;

— *number of theoretical plates*: minimum of 20 000 per metre.

Mobile phase 28.4 g/l solution of *anhydrous sodium sulphate R* adjusted to pH 5.0 using *dilute sulphuric acid R*.

Flow rate 0.5 ml/min.

Detection Differential refractometer.

Injection 25 µl.

Calibration For detection, use a differential refractometer (RI) detector connected in series to a ultraviolet spectrophotometer (UV) set at 234 nm such that the UV monitor is connected to the column outlet, and the RI detector to the UV-monitor outlet.

It is necessary to measure the time lapse between the 2 detectors accurately, so that their chromatograms can be aligned correctly. The retention times used in the calibration must be those from the RI detector.

The normalisation factor used to calculate the relative molecular mass from the RI/UV ratio is obtained as follows: calculate the total area under the UV_{234} (ΣUV_{234}) and the RI (ΣRI) curves by numerical integration over the range of interest (i.e. excluding salt and solvent peaks at the end of the chromatogram). Calculate the ratio r using the following expression:

$$\frac{\sum \mathrm{RI}}{\sum \mathrm{UV}_{234}}$$

Calculate the factor f using the following expression:

$$\frac{M_{na}}{r}$$

M_{na} = assigned number-average relative molecular mass of the *Heparin low-molecular-mass for calibration CRS* found in the leaflet supplied with the CRS.

Provided the UV_{234} and the RI responses are aligned, the relative molecular mass M at any point is calculated using the following expression:

$$f\frac{\mathrm{RI}}{\mathrm{UV}_{234}}$$

The resulting table of retention times and relative molecular masses may be used to derive a calibration for the chromatographic system by fitting a suitable mathematical relationship to the data. A polynomial of the 3rd degree is recommended. *It must be stressed that the extrapolation of this fitted calibration curve to higher molecular masses is not valid*.

Inject 25 µl of the test solution and record the chromatogram for a period of time, ensuring complete elution of sample and solvent peaks.

The mass-average relative molecular mass is defined by the following expression:

$$\frac{\sum(\mathrm{RI}_i M_i)}{\sum \mathrm{RI}_i}$$

RI_i = mass of substance eluting in the fraction i;

M_i = relative molecular mass corresponding to fraction i.

Any low-molecular-mass heparin covered by a specific monograph complies with the requirements for identification C prescribed in the corresponding monograph.

Where no specific monograph exists for the low-molecular-mass heparin to be examined, the mass-average relative molecular mass is not greater than 8000 and at least 60 per cent of the total mass has a relative molecular mass lower than 8000. In addition, the molecular mass parameters (mass-average molecular mass and mass percentages of chains comprised between specified values) correspond to that of the manufacturer's reference preparation.

D. It gives reaction (a) of sodium or the reactions of calcium (as appropriate) *(2.3.1)*.

TESTS

pH *(2.2.3)*

5.5 to 8.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Nitrogen *(2.5.9)*

1.5 per cent to 2.5 per cent (dried substance).

Calcium *(2.5.11)*

9.5 per cent to 11.5 per cent (dried substance), if prepared from heparin complying with the monograph on *Heparin calcium (0332)*. Use 0.200 g.

Sodium

9.5 per cent to 12.5 per cent (dried substance), if prepared from heparin complying with the monograph on *Heparin sodium (0333)*.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 50 mg in *0.1 M hydrochloric acid* containing 1.27 mg of *caesium chloride R* per millilitre and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare reference solutions (25 ppm, 50 ppm and 75 ppm) using *sodium standard solution (200 ppm Na) R* diluted with *0.1 M hydrochloric acid* containing 1.27 mg of *caesium chloride R* per millilitre.

Source Sodium hollow-cathode lamp.

Wavelength 330.3 nm.

Atomisation device Flame of suitable composition (for example, 11 litres of air and 2 litres of acetylene per minute).

Molar ratio of sulphate ions to carboxylate ions *(2.2.38)*

Minimum 1.8.

The sample of heparin used in this titration must be free from ionisable impurities, particularly salts.

Weigh 0.100 g of the substance to be examined taking the necessary measures to avoid the problems linked to hygroscopicity.

Take up into about 20 ml of double-glass-distilled *water R*. Cool to 4 °C and apply 2.0 ml of this solution to a pre-cooled column (approximately 10 × 1 cm), packed with a suitable *cation exchange resin R*. Wash through with double-glass-distilled *water R* into the titration vessel up to a final volume of about 10-15 ml (*the titration vessel must be just large enough to hold the electrodes from the conductivity meter, a small stirrer bar and a fine flexible tube from the outlet of a 2 ml burette*). Stir magnetically. When the conductivity reading is constant, note it and titrate with *0.05 M sodium hydroxide* added in approximately 50 µl portions. Record the burette level and the conductivity meter reading a few seconds after each addition until the end-point is reached.

For each measured figure, calculate the number of milliequivalents of sodium hydroxide added from the volume and the known concentration of the sodium hydroxide solution. Plot on a graph the figures for conductivity (as *y*-axis) against the figures of milliequivalent of sodium hydroxide (as *x*-axis). The graph will have 3, approximately linear sections: an initial steep downward slope, a middle slight rise and a final steep rise. Estimate the best straight lines through these 3 parts of the graph. At the points where the 1st and 2nd lines intersect, and where the 2nd and 3rd lines intersect, draw perpendiculars to the *x*-axis to estimate the milliequivalents of sodium hydroxide taken up by the sample at those points. The point where the 1st and 2nd lines intersect will give the number of milliequivalents of sodium hydroxide taken up by the sulphate groups, and the point where the 2nd and 3rd lines intersect will give the number of milliequivalents taken up by the sulphate and carboxylate groups together. The difference between the 2 will therefore give the number of milliequivalents taken up by the carboxylate groups.

Heavy metals ***(2.4.8)***

Maximum 30 ppm.

0.5 g complies with test C. Prepare the reference solution using 1.5 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 10.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.67 kPa for 3 h.

Bacterial endotoxins ***(2.6.14)***

Less than 0.01 IU per International Unit of anti-Xa activity, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins. The addition of divalent cations may be necessary to fulfil the validation criteria.

ASSAY

The anticoagulant activity of low-molecular-mass heparins is determined *in vitro* by 2 assays which determine its ability to accelerate the inhibition of factor Xa (anti-Xa assay) and thrombin, factor IIa (anti-IIa assay), by antithrombin III.

The International Units for anti-Xa and anti-IIa activity are the activities contained in a stated amount of the International Standard for low-molecular-mass heparin.

Heparin low-molecular-mass for assay BRP, calibrated in International Units by comparison with the International Standard using the 2 assays given below, is used as reference preparation.

ANTI-FACTOR XA ACTIVITY

Reference and test solutions

Prepare 4 independent series of 4 dilutions each, of the substance to be examined and of the reference preparation of low-molecular-mass heparin in *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R*; the concentration range should be within 0.025 IU to 0.2 IU of anti-factor Xa activity per millilitre and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Procedure

Label 16 tubes in duplicate: T_1, T_2, T_3, T_4 for the dilutions of the substance to be examined and S_1, S_2, S_3, S_4 for the dilutions of the reference preparation. To each tube add 50 µl of *antithrombin III solution R1* and 50 µl of the appropriate dilution of the substance to be examined, or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order S_1, S_2, S_3, S_4, T_1, T_2, T_3, T_4, T_1, T_2, T_3, T_4, S_1, S_2, S_3, S_4, allow to equilibrate at 37 °C (water-bath or heating block) for 1 min and add to each tube 100 µl of *bovine factor Xa solution R*. Incubate for exactly 1 min and add 250 µl of *chromogenic substrate R1*. Stop the reaction after exactly 4 min by adding 375 µl of *acetic acid R*. Transfer the mixtures to semi-micro cuvettes and measure the absorbance *(2.2.25)* at 405 nm using a suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R* instead of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the substance to be examined and of the reference preparation of low-molecular-mass heparins and calculate the potency of the substance to be examined in International Units of anti-factor Xa activity per millilitre using the usual statistical methods for parallel-line assays.

ANTI-FACTOR IIA ACTIVITY

Reference and test solutions

Prepare 4 independent series of 4 dilutions each, of the substance to be examined and of the reference preparation of low molecular-mass heparin in *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R*; the concentration range should be within 0.015 IU to 0.075 IU of anti-factor IIa activity per millilitre and the dilutions chosen should give a linear response when results are plotted as absorbance against log concentration.

Procedure

Label 16 tubes in duplicate: T_1, T_2, T_3, T_4 for the dilutions of the substance to be examined and S_1, S_2, S_3, S_4 for the dilutions of the reference preparation. To each tube add 50 µl of *antithrombin III solution R2* and 50 µl of the appropriate dilution of the substance to be examined or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in the order S_1, S_2, S_3, S_4, T_1, T_2, T_3, T_4, T_1, T_2, T_3, T_4, S_1, S_2, S_3, S_4, allow to equilibrate at 37 °C (water-bath or heating block) for 1 min and add to each tube 100 µl of *human thrombin solution R*. Incubate for exactly 1 min and add 250 µl of *chromogenic substrate R2*. Stop the reaction after exactly 4 min by adding 375 µl of *acetic acid R*. Transfer the mixtures to semi-micro cuvettes and measure the absorbance *(2.2.25)* at 405 nm using a suitable reading device. Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R* instead of the reference and test solutions; the 2 blank values do not differ significantly. Calculate the regression of the absorbance on log concentrations of the solutions of the substance to be examined and of the reference preparation of low-molecular-mass heparins, and calculate the potency of the substance to be examined in International Units of anti-factor IIa activity per millilitre using the usual statistical methods for parallel-line assays.

LABELLING

The label states:

— the number of International Units of anti-factor Xa activity per milligram;

— the number of International Units of anti-factor IIa activity per milligram;

— the mass-average molecular mass and the percentage of molecules within defined molecular mass ranges;

— where applicable, that the contents are the sodium salt;

— where applicable, that the contents are the calcium salt.

STORAGE

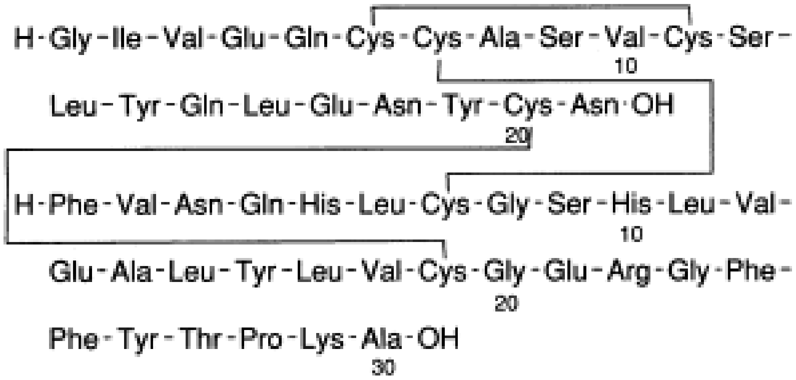
In an airtight tamper-proof container. If the product is sterile and free of bacterial endotoxins, store in a sterile and apyrogenic container.

Ph Eur

Bovine Insulin

General Notices

(Ph Eur monograph 1637)


H-Gly-Ile-Val-Glu-Gln-Cys-Cys-Ala-Ser-Val-Cys-Ser-
10
Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn-OH
20
H-Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-Leu-Val-
10
Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-
20
Phe-Tyr-Thr-Pro-Lys-Ala-OH
30

$C_{254}H_{377}N_{65}O_{75}S_6$ 5734 *11070-73-8*

Action and use

Hormone; treatment of diabetes mellitus.

Preparation

Insulin Preparations

Ph Eur

DEFINITION

Bovine insulin is the natural antidiabetic principle obtained from beef pancreas and purified.

Content:

— *sum of bovine insulin ($C_{254}H_{377}N_{65}O_{75}S_6$) and A21 desamido bovine insulin*: 93.0 per cent to 105.0 per cent (dried subtance).

By convention, for the purpose of labelling insulin preparations, 0.0342 mg of bovine insulin is equivalent to 1 IU of insulin.

PRODUCTION

The animals from which bovine insulin is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in ethanol. It dissolves in dilute mineral acids and with decomposition in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The retention time of the principal peak in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with reference solution (c).

B. Peptide mapping.

Test solution Prepare a 2.0 mg/ml solution of the substance to be examined in *0.01 M hydrochloric acid* and transfer 500 µl of this solution to a clean tube. Add 2.0 ml of *HEPES buffer solution pH 7.5 R* and 400 µl of a 1 mg/ml solution of *Staphylococcus aureus strain V8 protease R*. Cap the tube and incubate at 25 °C for 6 h. Stop the reaction by adding 2.9 ml of *sulphate buffer solution pH 2.0 R.*

Reference solution Prepare at the same time and in the same manner as for the test solution but using *bovine insulin CRS* instead of the substance to be examined.

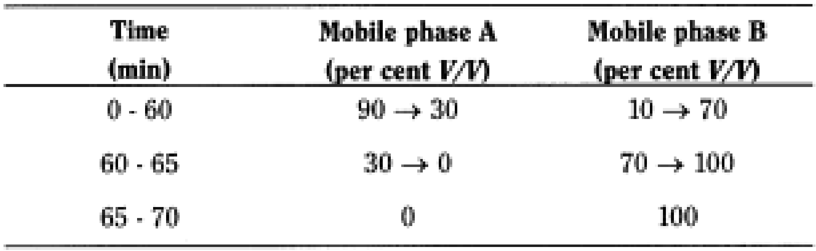
Examine the digests by liquid chromatography *(2.2.29)*.

Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature:* 40 °C.

Mobile phase:

- *mobile phase A*: mix 100 ml of *acetonitrile for chromatography R*, 700 ml of *water R* and 200 ml of *sulphate buffer solution pH 2.0 R*; filter and degas;
- *mobile phase B:* mix 400 ml of *acetonitrile for chromatography R*, 400 ml of *water R* and 200 ml of *sulphate buffer solution pH 2.0 R*; filter and degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	90 → 30	10 → 70
60 - 65	30 → 0	70 → 100
65 - 70	0	100

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.

Injection 50 µl.

System suitability The chromatograms obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of bovine insulin digest supplied with *bovine insulin CRS*. In the chromatogram obtained with the reference solution, identify the peaks due to digest fragments I, II and III. The symmetry factor of the peaks due to fragments II and III is not greater than 1.5, and the resolution between the 2 peaks is at least 1.9.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

NOTE: The retention time of fragment I is the same for porcine insulin and for human insulin. The retention times of fragments II and IV are the same for all insulins. The retention time of fragment III is the same for bovine insulin and for porcine insulin.

TESTS

Impurities with molecular masses greater than that of insulin

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure. *Maintain the solutions at 2-10 °C and use within 7 days. If an automatic injector is used, maintain the temperature at 2-10 °C.*

Test solution Dissolve 4 mg of the substance to be examined in 1.0 ml of *0.01 M hydrochloric acid*.

Resolution solution Use a solution of insulin (approximately 4 mg/ml), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R,* containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about 10 days.

Column:

- *size*: *l* = 0.3 m, Ø = at least 7.5 mm;
- *stationary phase*: *hydrophilic silica gel for chromatography R* (5-10 µm), of a grade suitable for the separation of insulin monomer from dimer and polymers.

Mobile phase Mix of 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile R* and 65 volumes of a 1.0 g/l solution of *arginine R*; filter and degas.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 276 nm.

Equilibration Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least 3 injections of the resolution solution. The column is equilibrated when repeatable results are obtained from 2 subsequent injections.

Injection 100 µl.

Run time About 35 min.

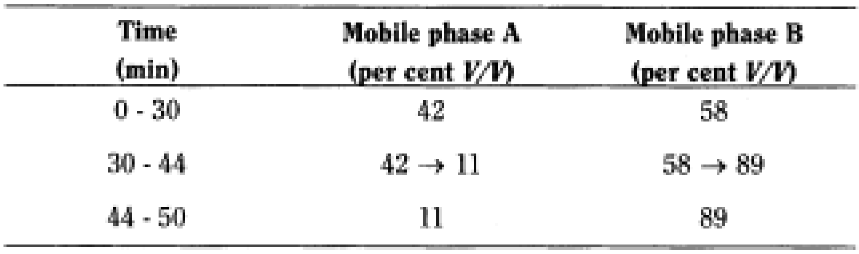
Retention times Polymeric insulin complexes = 13 min to 17 min; covalent insulin dimer = about 17.5 min; insulin monomer = about 20 min; salts = about 22 min.

System suitability Resolution solution:

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limits The sum of the areas of any peaks with a retention time less than that of the principal peak is not greater than 1.0 per cent of the total area of the peaks; disregard any peak with a retention time greater than that of the insulin peak.

Related proteins Liquid chromatography *(2.2.29)* as described under Assay, following the elution conditions as described in the table below.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	42	58
30 - 44	42 → 11	58 → 89
44 - 50	11	89

Maintain the solutions at 2-10 °C and use within 24 h. Perform a system suitability test (resolution, linearity) as described under Assay. If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µl of reference solution (c) and 20 µl of the test solution. If necessary, adjust the injection volume to between 10 µl and 20 µl in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for approximately 50 min. In the chromatogram obtained with reference solution (c), A21 desamido bovine insulin appears as a small peak after the principal peak and has a relative retention of about 1.3 with reference to the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to A21 desamido bovine insulin is not greater than 3.0 per cent of the total area of the peaks; the sum of the areas of all the peaks, apart from those due to bovine insulin and A21 desamido bovine insulin, is not greater than 3.0 per cent of the total area of the peaks.

Bovine proinsulin-like immunoreactivity (PLI)

Maximum 10 ppm (dried substance).

Use a suitably sensitive immunochemical method *(2.7.1)* such as radio-immunoassay, using the International Reference Reagent for bovine proinsulin to calibrate the method.

Zinc

Maximum 1.0 per cent (dried substance).

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid. Dilute if necessary to a suitable concentration (for example, 0.4 µg to 1.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/ml Zn) R* with *0.01 M hydrochloric acid*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Flame Air-acetylene flame of suitable composition (for example, 11 litres of air and 2 litres of acetylene per minute).

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 0.200 g by drying in an oven at 105 °C for 24 h.

Sulphated ash *(2.4.14)*

Maximum 2.5 per cent (dried substance), determined on 0.200 g.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Dissolve a suitable amount of the substance to be examined in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (a) Dissolve the contents of a vial of *human insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (b) Dissolve the contents of a vial of *porcine insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (c) Dissolve the contents of a vial of *bovine insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (d) Dilute 1.0 ml of reference solution (c) to 10.0 ml with *0.01 M hydrochloric acid*.

Resolution solution Mix 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b).

Maintain the solutions at 2-10 °C and use within 48 h. If an automatic injector is used, maintain the temperature at 2-10 °C.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 42 volumes of mobile phase A and 58 volumes of mobile phase B, adjusting the composition of the mixture if necessary.

Prepare and maintain the following solutions at a temperature of at least 20 °C:

— *mobile phase A*: dissolve 28.4 g of *anhydrous sodium sulphate R* in *water R* and dilute to 1000 ml with the same solvent; add 2.7 ml of *phosphoric acid R;* adjust to pH 2.3, if necessary, with *ethanolamine R*; filter and degas;

— *mobile phase B*: mix 550 ml of mobile phase A with 450 ml of *acetonitrile R*. Warm the solution to a temperature of at least 20 °C in order to avoid precipitation (mixing of mobile phase A with acetonitrile is endothermic); filter and degas.

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

System suitability:

— *resolution*: inject 20 µl of the resolution solution and 20 µl of reference solution (b). Record the chromatogram of the resolution solution until the peak corresponding to the principal peak in the chromatogram obtained with reference solution (b) is clearly visible. In the chromatogram obtained with the resolution solution, identify the peaks due to porcine insulin and human insulin. The test is not valid unless the resolution between the peaks corresponding to human insulin and porcine insulin is at least 1.2. If necessary, adjust the concentration of acetonitrile in the mobile phase until this resolution is achieved;

— *linearity*: inject 20 µl each of reference solutions (c) and (d). The test is not valid unless the area of the principal peak in the chromatogram obtained with reference solution (c) is 10 ± 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (d). If this test fails, adjust the injection volume to between 10 µl and 20 µl, in order that the responses are within the linearity range of the detector.

Injection 20 µl of the test solution.

Calculate the content of bovine insulin $C_{254}H_{377}N_{65}O_{75}S_6$ plus A21 desamido bovine insulin from the area of the principal peak and the area of the peak corresponding to A21 desamido bovine insulin in the chromatograms obtained with the test solution and reference solution (c) and the declared content of bovine insulin plus A21 desamido bovine insulin in *bovine insulin CRS.*

STORAGE

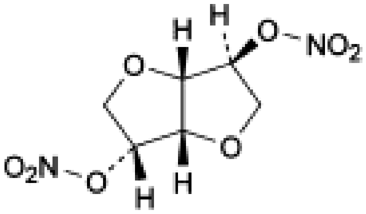
In an airtight container, protected from light, at - 20 °C until released by the manufacturer. When thawed, insulin may be stored at 5 ± 3 °C and used for manufacturing preparations within a short period of time. To avoid absorption of humidity from the air during weighing, the insulin must be at room temperature.

Ph Eur

Diluted Isosorbide Dinitrate

General Notices

(Ph Eur monograph 1117)



$C_6H_8N_2O_8$ 236.1   *87-33-2*

Action and use

Nitric acid analogue; treatment of angina pectoris.

Preparation

Isosorbide Dinitrate Tablets

Ph Eur

DEFINITION

Dry mixture of isosorbide dinitrate and *Lactose monohydrate (0187)* or *Mannitol (0559)*.

Content

95.0 per cent *m/m* to 105.0 per cent *m/m* of the content of 1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate stated on the label.

CAUTION: undiluted isosorbide dinitrate may explode if subjected to percussion or excessive heat. Appropriate precautions must be taken and only very small quantities handled.

CHARACTERS

Appearance

Undiluted isosorbide dinitrate is a fine, white or almost white, crystalline powder.

Solubility

Undiluted isosorbide dinitrate is very slightly soluble in water, very soluble in acetone, sparingly soluble in ethanol (96 per cent).

The solubility of the diluted product depends on the diluent and its concentration.

IDENTIFICATION

First identification *A, C, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs prepared with the residue obtained in identification test D.

Comparison *isosorbide dinitrate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Shake a quantity of the substance to be examined corresponding to 10 mg of isosorbide dinitrate with 10 ml of *ethanol (96 per cent) R* for 5 min and filter.

Reference solution Shake a quantity of *isosorbide dinitrate CRS* corresponding to 10 mg of isosorbide dinitrate with 10 ml of *ethanol (96 per cent) R* for 5 min and filter.

Plate *TLC silica gel G plate R*.

Mobile phase *methanol R, methylene chloride R* (5:95 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of air.

Detection Spray with freshly prepared *potassium iodide* and *starch solution R*; expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Shake a quantity of the substance to be examined corresponding to 0.10 g of lactose or mannitol with 10 ml of *water R.* Filter if necessary.

Reference solution (a) Dissolve 0.10 g of *lactose R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 0.10 g of *mannitol R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (c) Mix equal volumes of reference solutions (a) and (b).

Plate *TLC silica gel G plate R.*

Mobile phase *water R, methanol R, anhydrous acetic acid R, ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application 1 µl; thoroughly dry the starting points.

Development A Over a path of 15 cm.

Drying A In a current of warm air.

Development B Immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with *4-aminobenzoic acid solution R*, dry in a current of cold air until the acetone is removed, then heat at 100 °C for 15 min; allow to cool, spray with a 2 g/l solution of *sodium periodate R*, dry in a current of cold air, and heat at 100 °C for 15 min.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) for lactose or to the principal spot in the chromatogram obtained with reference solution (b) for mannitol.

D. Shake a quantity of the substance to be examined corresponding to 25 mg of isosorbide dinitrate with 10 ml of *acetone R* for 5 min. Filter, evaporate to dryness at a temperature below 40 °C and dry the residue over *diphosphorus pentoxide R* at a pressure of 0.7 kPa for 16 h. The melting point *(2.2.14)* of the residue is 69 °C to 72 °C.

TESTS

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Shake a quantity of the substance to be examined corresponding to 0.10 g of isosorbide dinitrate with 5 ml of *ethanol (96 per cent) R* and filter.

Reference solution Dissolve 10 mg of *potassium nitrate R* in 1 ml of *water R* and dilute to 100 ml with *ethanol (96 per cent) R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R*, *acetone R*, *toluene R* (15:30:60 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of air until the acetic acid is completely removed.

Detection Spray copiously with freshly prepared *potassium iodide and starch solution R*; expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

Impurities B and C

Liquid chromatography *(2.2.29)*.

Test solution (a) Sonicate a quantity of the substance to be examined corresponding to 25.0 mg of isosorbide dinitrate with 20 ml of the mobile phase for 15 min and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a) Sonicate a quantity of *isosorbide dinitrate CRS* corresponding to 25.0 mg of *isosorbide dinitrate CRS* with 20 ml of the mobile phase for 15 min and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 10.0 mg of *isosorbide 2-nitrate CRS* (impurity B) in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (d) Dissolve 10.0 mg of *isosorbide mononitrate CRS* (impurity C) in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (e) Dissolve 5 mg of *isosorbide 2-nitrate CRS* (impurity B) in the mobile phase and dilute to 10 ml with the mobile phase. To 1 ml of this solution add 0.5 ml of reference solution (a) and dilute to 10 ml with the mobile phase.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: aminopropylmethylsilyl silica gel for chromatography R* (10 µm).

Mobile phase *anhydrous ethanol R, trimethylpentane R* (15:85 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 210-215 nm.

Injection 10 µl of test solution (a) and reference solutions (c), (d) and (e).

Retention time Isosorbide dinitrate = about 5 min; impurity B = about 8 min; impurity C = about 11 min.

System suitability Reference solution (e):

— *resolution*: minimum 6.0 between the peaks due to isosorbide dinitrate and impurity B.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for impurities B and C with the following modifications.

Detection Spectrophotometer at 230 nm.

Injection 20 µl of test solution (b) and reference solution (b).

If the areas of the peaks from 2 successive injections of reference solution (b) do not agree to within 1.0 per cent, then inject a further 4 times and calculate, for the 6 injections, the relative standard deviation.

System suitability Reference solution (b):

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections.

Calculate the content of isosorbide dinitrate as a percentage of the declared content.

STORAGE

Protected from light.

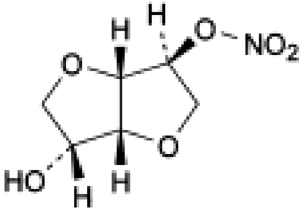
LABELLING

The label states the percentage content of isosorbide dinitrate.

IMPURITIES

Specified impurities *A, B, C.*

A. inorganic nitrates,



B. isosorbide 2-nitrate,

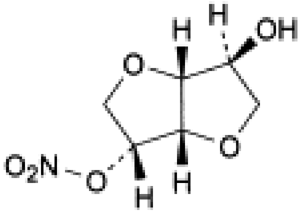
C. isosorbide mononitrate (isosorbide 5-nitrate).

Ph Eur

Diluted Isosorbide Mononitrate

General Notices

(Ph Eur monograph 1118)



$C_6H_9NO_6$ 191.1 *16051-77-7*

Action and use

Nitric acid analogue; treatment of angina pectoris.

Preparations

Isosorbide Mononitrate Tablets

Prolonged-release Isosorbide Mononitrate Tablets

Ph Eur

DEFINITION

Dry mixture of isosorbide mononitrate and *Lactose monohydrate (0187)* or *Mannitol (0559)*.

Content

95.0 per cent *m/m* to 105.0 per cent *m/m* of the content of 1,4:3,6-dianhydro-D-glucitol 5-nitrate stated on the label.

CHARACTERS

Appearance

Undiluted isosorbide mononitrate is a white or almost white, crystalline powder.

Solubility

Undiluted isosorbide mononitrate is freely soluble in water, in acetone, in ethanol (96 per cent) and in methylene chloride.

The solubility of the diluted product depends on the diluent and its concentration.

IDENTIFICATION

First identification A, C, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs prepared with the residue obtained in identification test D.

Comparison *isosorbide mononitrate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Shake a quantity of the substance to be examined corresponding to 10 mg of isosorbide mononitrate with 10 ml of *ethanol (96 per cent) R* for 5 min and filter.

Reference solution Dissolve 10 mg of *isosorbide mononitrate CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *methanol R, methylene chloride R* (5:95 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of air.

Detection Spray with freshly prepared *potassium iodide and starch solution R*. Expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Shake a quantity of the substance to be examined corresponding to 0.10 g of lactose or mannitol with 10 ml of *water R*; filter if necessary.

Reference solution (a) Dissolve 0.10 g of *lactose R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 0.10 g of *mannitol R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (c) Mix equal volumes of reference solutions (a) and (b).

Plate *TLC silica gel G plate R*.

Mobile phase *water R*, *methanol R*, *anhydrous acetic acid R*, *ethylene chloride* R (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application 1 µl; thoroughly dry the starting points.

Development A Over a path of 15 cm.

Drying A In a current of warm air.

Development B Immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with *4-aminobenzoic acid solution R* and dry in a current of cold air until the acetone is removed; heat at 100 °C for 15 min and allow to cool; spray with a 2 g/l solution of *sodium periodate R* and dry in a current of cold air; heat at 100 °C for 15 min.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) for lactose or to the principal spot in the chromatogram obtained with reference solution (b) for mannitol.

D. Shake a quantity of the substance to be examined corresponding to 25 mg of isosorbide mononitrate with 10 ml of *acetone R* for 5 min. Filter, evaporate to dryness at a temperature below 40 °C and dry the residue over *diphosphorus pentoxide R* at a pressure of 0.7 kPa for 16 h. The melting point *(2.2.14)* of the residue is 89 °C to 91 °C.

TESTS

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Shake a quantity of the substance to be examined corresponding to 0.10 g of isosorbide mononitrate with 5 ml of *ethanol (96 per cent) R* and filter.

Reference solution Dissolve 10 mg of *potassium nitrate R* in 1 ml of *water R* and dilute to 100 ml with *ethanol (96 per cent) R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R, acetone R, toluene R* (15:30:60 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of air until the acetic acid is completely removed.

Detection Spray copiously with freshly prepared *potassium iodide and starch solution R*; expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

Impurities B and C

Liquid chromatography *(2.2.29)*.

Test solution (a) Sonicate a quantity of the substance to be examined corresponding to 25.0 mg of isosorbide mononitrate with 20 ml of the mobile phase for 15 min and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *isosorbide mononitrate CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *isosorbide-2-nitrate CRS* (impurity C) in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c) Sonicate a quantity of *isosorbide dinitrate CRS* (impurity B) corresponding to 10.0 mg of isosorbide dinitrate in 15 ml of the mobile phase for 15 min and dilute to 20.0 ml with the mobile phase. Filter the solution through a suitable membrane filter. Dilute 0.1 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (d) Dissolve 5 mg of *isosorbide mononitrate CRS* and 5 mg of *isosorbide-2-nitrate CRS* (impurity C) in the mobile phase and dilute to 10 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: aminopropylmethylsilyl silica gel for chromatography R* (10 µm).

Mobile phase *anhydrous ethanol R, trimethylpentane R* (15:85 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 210-215 nm.

Injection 10 µl of test solution (a) and reference solutions (b), (c) and (d).

Retention time Impurity B = about 5 min; impurity C = about 8 min; isosorbide 5-nitrate = about 11 min.

System suitability Reference solution (d):

— *resolution*: minimum 4.0 between the peaks due to impurity C and isosorbide 5-nitrate.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *impurity C:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for impurities B and C with the following modifications.

Detection Spectrophotometer at 230 nm.

Injection 20 µl of test solution (b) and reference solution (a).

If the areas of the peaks from 2 successive injections of reference solution (a) do not agree to within 1.0 per cent, then inject a further 4 times and calculate, for the 6 injections, the relative standard deviation.

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections.

Calculate the content of isosorbide mononitrate as a percentage of the declared content.

STORAGE

Protected from light.

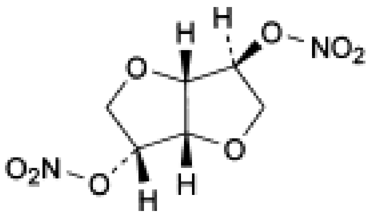
LABELLING

The label states the percentage content of isosorbide mononitrate.

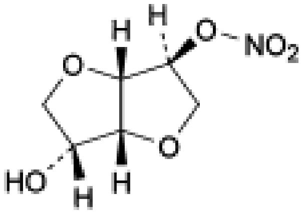
IMPURITIES

Specified impurities   A, B, C.

A. inorganic nitrates,



B. isosorbide dinitrate,



C. isosorbide 2-nitrate.

Ph Eur

Heavy Kaolin

General Notices

(Ph Eur monograph 0503)

Preparation

Kaolin Poultice

When kaolin is prescribed or demanded, Light Kaolin shall be dispensed or supplied, unless it is ascertained that Light Kaolin (Natural) is required.

Ph Eur

DEFINITION

Purified, natural, hydrated aluminium silicate of variable composition.

CHARACTERS

Appearance

Fine, white or greyish-white, unctuous powder.

Solubility

Practically insoluble in water and in organic solvents.

IDENTIFICATION

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate R* and 3 g of *sodium carbonate R* and heat until the mixture melts. Allow to cool. To the residue add 20 ml of boiling *water R*, mix and filter. Wash the residue with 50 ml of *water R*. To the residue add 1 ml of *hydrochloric acid R* and 5 ml of *water R*. Filter. To the filtrate add 1 ml of *strong sodium hydroxide solution R* and filter. To the filtrate add 3 ml of *ammonium chloride solution R*. A gelatinous white precipitate is formed.

B. Add 2.0 g in 20 portions to 100 ml of a 10 g/l solution of *sodium laurilsulfate R* in a 100 ml graduated cylinder about 30 mm in diameter. Allow 2 min between additions for each portion to settle. Allow to stand for 2 h. The apparent volume of the sediment is not greater than 5 ml.

C. 0.25 g gives the reaction of silicates *(2.3.1)*.

TESTS

Solution S

To 4 g add a mixture of 6 ml of *acetic acid R* and 34 ml of *distilled water R*, shake for 1 min and filter.

Acidity or alkalinity

To 1.0 g add 20 ml of *carbon dioxide-free water R*, shake for 2 min and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.25 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Organic impurities

Heat 0.3 g to redness in a calcination tube. The residue is only slightly more coloured than the original substance.

Adsorption power

To 1.0 g in a ground-glass-stoppered test-tube add 10.0 ml of a 3.7 g/l solution of *methylene blue R* and shake for 2 min. Allow to settle. Centrifuge and dilute the solution 1 to 100 with *water R*. The solution is not more intensely coloured than a 0.03 g/l solution of *methylene blue R.*

Swelling power

Triturate 2 g with 2 ml of *water R*. The mixture does not flow.

Substances soluble in mineral acids

Maximum 1 per cent.

To 5.0 g add 7.5 ml of *dilute hydrochloric acid R* and 27.5 ml of *water R* and boil for 5 min. Filter, wash the residue on the filter with *water R* and dilute the combined filtrate and washings to 50.0 ml with *water R.* To 10.0 ml of the solution add 1.5 ml of *dilute sulphuric acid R*, evaporate to dryness on a water-bath and ignite. The residue weighs a maximum of 10 mg.

Chlorides ***(2.4.4)***

Maximum 250 ppm.

Dilute 2 ml of solution S to 15 ml with *water R.*

Sulphates ***(2.4.13)***

Maximum 0.1 per cent.

Dilute 1.5 ml of solution S to 15 ml with *distilled water R.*

Calcium ***(2.4.3)***

Maximum 250 ppm.

Dilute 4 ml of solution S to 15 ml with *distilled water R*.

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

To 5 ml of the solution prepared for the test for substances soluble in mineral acids add 5 ml of *water R*, 10 ml of *hydrochloric acid R* and 25 ml of *methyl isobutyl ketone R*. Shake for 2 min. Separate the layers. Evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *acetic acid R* and dilute to 25 ml with *water R*. Filter. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

If intended for internal use, the above test is replaced by the following test for heavy metals *(2.4.8)*: maximum 25 ppm.

To 10 ml of the solution prepared for the test for substances soluble in mineral acids add 10 ml of *water R*, 20 ml of *hydrochloric acid R* and 25 ml of *methyl isobutyl ketone R*. Shake for 2 min. Separate the layers. Evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *acetic acid R* and dilute to 25 ml with *water R.* Filter. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate-count.

LABELLING

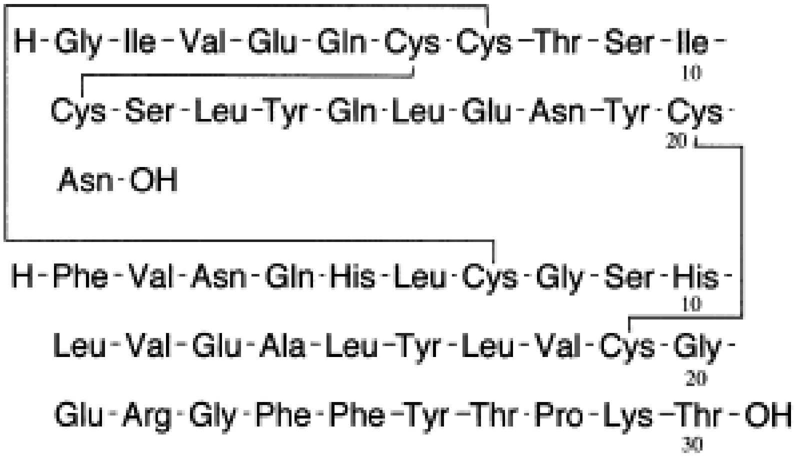
The label states, where applicable, that the substance is suitable for internal use.

Ph Eur

Human Insulin

General Notices

(Ph Eur monograph 0838)


H-Gly-Ile-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-
10
Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-
20
Asn-OH
H-Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-
10
Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-
20
Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr-OH
30

$C_{257}H_{383}N_{65}O_{77}S_6$ 5808 *11061-68-0*

Action and use

Hormone; treatment of diabetes mellitus.

Preparations

Insulin Preparations

Ph Eur

DEFINITION

Human insulin is a 2-chain peptide having the structure of the antidiabetic hormone produced by the human pancreas.

Content

95.0 per cent to 105.0 per cent of human insulin $C_{257}H_{383}N_{65}O_{77}S_6$ plus A21 desamido human insulin (dried substance).

By convention, for the purpose of labelling insulin preparations, 0.0347 mg of human insulin is equivalent to 1 IU of insulin.

PRODUCTION

Human insulin is produced either by enzymatic modification and suitable purification of insulin obtained from the pancreas of the pig or by a method based on recombinant DNA (rDNA) technology.

Human insulin is produced under conditions designed to minimise the degree of microbial contamination.

For human insulin produced by enzymatic modification of insulin obtained from the pancreas of the pig, the manufacturing process is validated to demonstrate removal of any residual proteolytic activity. The competent authority may require additional tests.

For human insulin produced by a method based on rDNA technology, prior to release the following tests are carried out on each batch of the final bulk product, unless exemption has been granted by the competent authority.

Host-cell-derived proteins

The limit is approved by the competent authority.

Single chain precursor

The limit is approved by the competent authority. Use a suitably sensitive method.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute mineral acids and with decomposition in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. Peptide mapping *(2.2.55)*.

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a 2.0 mg/ml solution of the substance to be examined in *0.01 M hydrochloric acid* and transfer 500 µl of this solution to a clean tube. Add 2.0 ml of *HEPES buffer solution pH 7.5 R* and 400 µl of a 1 mg/ml solution of *Staphylococcus aureus strain V8 protease R*. Cap the tube and incubate at 25 °C for 6 h. Stop the reaction by adding 2.9 ml of *sulphate buffer solution pH 2.0 R*.

Reference solution Prepare at the same time and in the same manner as for the test solution but using *human insulin CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATON

Liquid chromatography *(2.2.29)*.

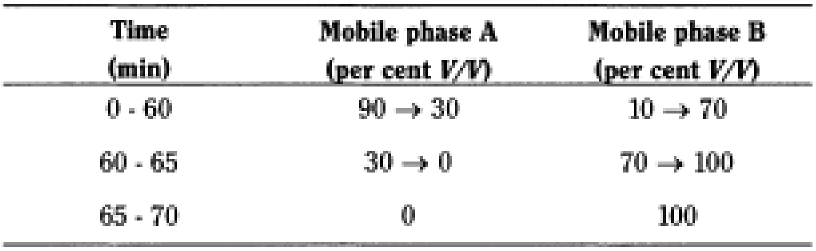
Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm,

- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm) with a pore size of 8 nm,
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: mix 100 ml of *acetonitrile for chromatography R*, 200 ml of *sulphate buffer solution pH 2.0 R* and 700 ml of *water R*; filter and degas;
- *mobile phase B*: mix 200 ml of *sulphate buffer solution pH 2.0 R*, 400 ml of *acetonitrile for chromatography R* and 400 ml of *water R*; filter and degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	90 → 30	10 → 70
60 - 65	30 → 0	70 → 100
65 - 70	0	100

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.

Injection 50 µl.

System suitability:

- the chromatograms obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of human insulin digest supplied with *human insulin CRS*,
- in the chromatogram obtained with the reference solution, identify the peaks due to digest fragments I, II and III:

Symmetry factor Maximum 1.5 for the peaks due to fragments II and III,

Resolution Minimum 3.4 between the peaks due to fragments II and III.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

NOTE: the retention time of fragment I is the same for porcine insulin and for human insulin. The retention times of fragments II and IV are the same for all insulins. The retention time of fragment III is the same for bovine insulin and for porcine insulin.

TESTS

Impurities with molecular masses greater than that of insulin

Size-exclusion chromatography *(2.2.30)*: use the normalisation procedure.

Test solution Prepare a solution containing 4 mg/ml of the substance to be examined in *0.01 M hydrochloric acid*.

Resolution solution Use a solution of insulin (about 4 mg/ml), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about 10 days.

Maintain the solutions at 2-8 °C and use within 7 days. If an automatic injector is used, maintain the temperature at 2-8 °C.

Column:

- *size*: *l* = 0.3 m, Ø = minimum 7.5 mm,
- *stationary phase*: *hydrophilic silica gel for chromatography R* (5-10 µm) with a pore size of 12-12.5 nm, of a grade suitable for the separation of insulin monomer from dimer and polymers.

Mobile phase Mix 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile R* and 65 volumes of a 1.0 g/l solution of *arginine R*; filter and degas.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 276 nm.

Equilibration Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least 3 injections of the resolution solution. The column is equilibrated when repeatable results are obtained from 2 subsequent injections.

Injection 100 µl.

Run time About 35 min.

Retention time Polymeric insulin complexes = 13-17 min; covalent insulin dimer = about 17.5 min; insulin monomer = about 20 min; salts = about 22 min.

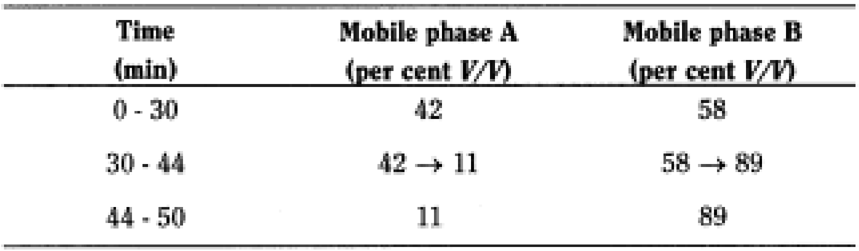
System suitability
Resolution solution:

- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limits The sum of the areas of any peaks with a retention time less than that of the principal peak is not greater than 1.0 per cent of the total area of the peaks. Disregard any peak with a retention time greater than that of the peak due to insulin.

Related proteins

Liquid chromatography *(2.2.29)* as described under Assay, following the elution conditions as described below:



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	42	58
30 - 44	42 → 11	58 → 89
44 - 50	11	89

Maintain the solutions at 2-8 °C and use within 24 h. Perform a system suitability test (resolution, linearity) as described in the assay. If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µl of reference solution (a), 20 µl of reference solution (b), 20 µl of reference solution (c) and 20 µl of the test solution. If necessary, adjust the injection volume to a volume between 10 µl and 20 µl in accordance with the results obtained in the test for linearity as described in the assay. Record the chromatograms for approximately 50 min. In the chromatogram obtained with reference solution (a), A21 desamido human insulin appears as a small peak after the principal peak and has a retention time of about 1.3 relative to the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to A21 desamido human insulin is not greater than 2.0 per cent of the total area of the peaks; the sum of the areas of all peaks, apart from those due to human insulin and that due to A21 desamido human insulin, is not greater than 2.0 per cent of the total area of the peaks. For semisynthetic human insulin only: in the chromatogram obtained with the test solution, the area of any peak corresponding to the principal peak in the chromatogram obtained with reference solution (b) is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent of porcine insulin in human insulin).

The following test applies only to human insulin produced by enzymatic modification of porcine insulin.

Proinsulin-like immunoreactivity (PLI)

Maximum 10 ppm, calculated with reference to the dried substance and determined by a suitably sensitive immunochemical method *(2.7.1)* such as radio-immunoassay. Use the International Reference Reagent for porcine proinsulin to calibrate the method.

Zinc

Maximum 1.0 per cent (dried substance).

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution

Dissolve 50.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid. Dilute if necessary to a suitable concentration (for example, 0.4-1.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions

Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/ml Zn) R* with *0.01 M hydrochloric acid*.

Source

Zinc hollow-cathode lamp.

Wavelength

213.9 nm.

Atomisation device: air-acetylene flame of suitable composition (for example, 11 litres of air and 2 litres of acetylene per minute).

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 0.200 g by drying in an oven at 105 °C for 24 h.

Sulphated ash *(2.4.14)*

Maximum 2.5 per cent, determined on 0.200 g (dried substance).

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 40.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *human insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (b) Dissolve the contents of a vial of *porcine insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 50.0 ml with *0.01 M hydrochloric acid*. To 1.0 ml of this solution add 1.0 ml of reference solution (a).

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 10.0 ml with *0.01 M hydrochloric acid*.

Resolution solution Mix 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b).

Maintain the solutions at 2-8 °C and use within 48 h. If an automatic injector is used, maintain at 2-8 °C.

Column:

- *size*: *l* = 0.25 , Ø = 4.6 mm,
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 40 °C.

Mobile phase Mix 42 volumes of mobile phase A and 58 volumes of mobile phase B, adjusting the composition of the mixture if necessary.

Prepare and maintain the following solutions at a temperature of at least 20 °C:

- *mobile phase A*: dissolve 28.4 g of *anhydrous sodium sulphate R* in *water R* and dilute to

1000 ml with the same solvent; add 2.7 ml of *phosphoric acid R*; adjust to pH 2.3, if necessary, with *ethanolamine R*; filter and degas;

— *mobile phase B*: mix 550 ml of mobile phase A with 450 ml of *acetonitrile R*. Warm the solution to a temperature of at least 20 °C in order to avoid precipitation (mixing of mobile phase A with acetonitrile is endothermic); filter and degas.

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

System suitability:

— *resolution*: inject 20 µl of the resolution solution and 20 µl of reference solution (b). Record the chromatogram of the resolution solution until the peak corresponding to the principal peak in the chromatogram obtained with reference solution (b) is clearly visible. In the chromatogram obtained with the resolution solution, identify the peaks due to porcine insulin and human insulin. The test is not valid unless the resolution between the peaks due to human insulin and porcine insulin is at least 1.2. If necessary, adjust the concentration of acetonitrile in the mobile phase until this resolution is achieved.

— *linearity*: inject 20 µl each of reference solutions (a) and (d). The test is not valid unless the area of the principal peak in the chromatogram obtained with reference solution (a) is 10 ± 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (d). If this test fails, adjust the injection volume to between 10 µl and 20 µl, in order that the responses are within the linearity range of the detector.

Injection 20 µl of the test solution and reference solution (a).

Calculate the content of human insulin $C_{257}H_{383}N_{65}O_{77}S_6$ plus A21 desamido human insulin using the areas of the corresponding peaks in the chromatograms obtained with the test solution and reference solution (a) and the declared content of human insulin plus A21 desamido human insulin in *human insulin CRS.*

STORAGE

In an airtight container, protected from light, at - 18 °C or below, until released by the manufacturer. When thawed, insulin is stored at 5 ± 3 °C and used for manufacturing preparations within a short period of time. To avoid absorption of humidity from the air during weighing, the insulin must be at room temperature.

LABELLING

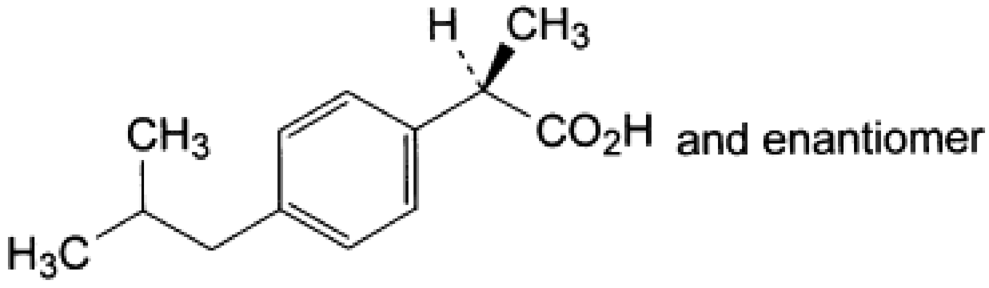
The label states whether the substance is produced by enzymatic modification of porcine insulin or by rDNA technology.

Ph Eur

Ibuprofen

General Notices

(Ph Eur monograph 0721)



$C_{13}H_{18}O_2$ 206.3 *15687-27-1*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory;.

Preparations

Ibuprofen Cream

Ibuprofen Gel

Ibuprofen Oral Suspension

Ibuprofen Tablets

Ph Eur

DEFINITION

(2*RS*)-2-[4-(2-Methylpropyl)phenyl]propanoic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in acetone, in methanol and in methylene chloride.

It dissolves in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D.*

A. Melting point *(2.2.14)*: 75 °C to 78 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in a 4 g/l solution of *sodium hydroxide R* and dilute to 100.0 ml with the same alkaline solution.

Spectral range 240-300 nm, using a spectrophotometer with a band width of 1.0 nm and a scan speed of not more than 50 nm/min.

Absorption maxima At 264 nm and 272 nm.

Shoulder At 258 nm.

Absorbance ratio:
— A_{264} / A_{258} = 1.20 to 1.30;
— A_{272} / A_{258} = 1.00 to 1.10.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ibuprofen CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *ibuprofen CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous acetic acid R*, *ethyl acetate R*, *hexane R* (5:24:71 *V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying At 120 °C for 30 min.

Detection Lightly spray with a 10 g/l solution of *potassium permanganate R* in *dilute sulphuric acid R* and heat at 120 °C for 20 min; examine in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 2.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.05° to + 0.05°.

Dissolve 0.50 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in 2 ml of *acetonitrile R1* and dilute to 10.0 ml with mobile phase A.

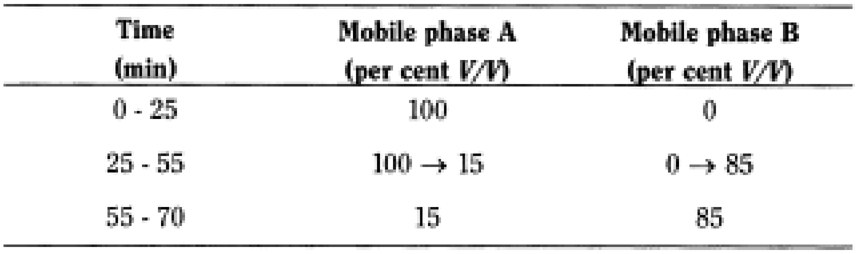
Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dilute 1.0 ml of *ibuprofen impurity B CRS* to 10.0 ml with *acetonitrile R1* (solution A). Dissolve 20 mg of *ibuprofen CRS* in 2 ml of *acetonitrile R1*, add 1.0 ml of solution A and dilute to 10.0 ml with mobile phase A.

Reference solution (c) Dissolve the contents of a vial of *ibuprofen for peak identification CRS* (mixture of impurities A, J and N) in 1 ml of *acetonitrile R1* and dilute to 5 ml with mobile phase A.

Column:
- — *size*: *l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- — *mobile phase A*: mix 0.5 volumes of *phosphoric acid R*, 340 volumes of *acetonitrile R1* and 600 volumes of *water R*; allow to equilibrate and dilute to 1000 volumes with *water R*;
- — *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 55	100 → 15	0 → 85
55 - 70	15	85

Flow rate 2 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *ibuprofen for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, J and N.

Relative retention With reference to ibuprofen (retention time = about 16 min): impurity J = about 0.2; impurity N = about 0.3; impurity A = about 0.9; impurity B = about 1.1.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity B, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ibuprofen. If necessary, adjust the concentration of acetonitrile in mobile phase A.

Limits:

— *impurities A, J, N*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Impurity F

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Methylating solution Dilute 1 ml of *N,N-dimethylformamide dimethyl acetal R* and 1 ml of *pyridine R* to 10 ml with *ethyl acetate R*.

Test solution Weigh about 50.0 mg of the substance to be examined into a sealable vial, dissolve in 1.0 ml of *ethyl acetate R*, add 1 ml of the methylating solution, seal and heat at 100 °C in a block heater for 20 min. Allow to cool. Remove the reagents under a stream of nitrogen at room temperature. Dissolve the residue in 5 ml of *ethyl acetate R.*

Reference solution (a) Dissolve 0.5 mg of *ibuprofen impurity F CRS* in *ethyl acetate R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Weigh about 50.0 mg of *ibuprofen CRS* into a sealable vial, dissolve in 1.0 ml of reference solution (a), add 1 ml of the methylating solution, seal and heat at 100 °C in a block heater for 20 min. Allow to cool. Remove the reagents under a stream of nitrogen at room temperature. Dissolve the residue in 5 ml of *ethyl acetate R.*

Column:

— *material*: fused silica;

— *size*: *l* = 25 m, Ø = 0.53 mm;

— *stationary phase*: *macrogol 20 000 R* (film thickness 2 µm).

Carrier gas *helium for chromatography R*.

Flow rate 5.0 ml/min.

Temperature:

— *column*: 150 °C;

— *injection port*: 200 °C;

— *detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µl of the test solution and reference solution (b).

Run time Twice the retention time of ibuprofen.

System suitability:
— *relative retention* with reference to ibuprofen (retention time = about 17 min): impurity F = about 1.5.

Limit:
— *impurity F*: maximum 0.1 per cent.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with *methanol R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo.*

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

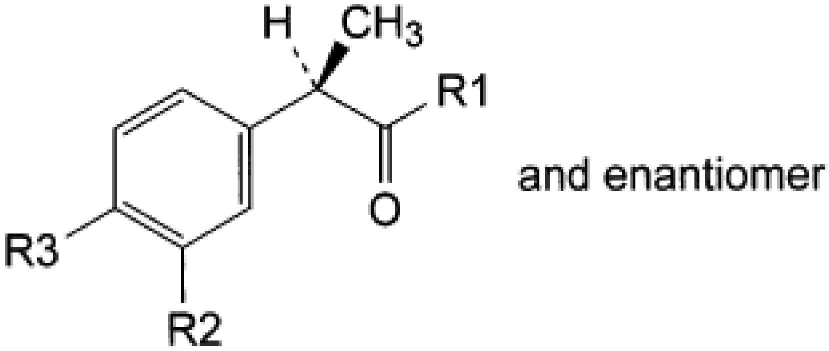
Dissolve 0.450 g in 50 ml of *methanol R*. Add 0.4 ml of *phenolphthalein solution R1*. Titrate with *0.1 M sodium hydroxide* until a red colour is obtained. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 20.63 mg of $C_{13}H_{18}O_2$.

IMPURITIES

Specified impurities *A, F, J, N.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E, G, H, I, K, L, M, O, P, Q, R.*

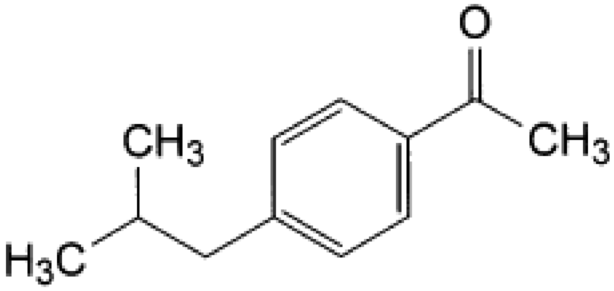


A. R1 = OH, R2 = CH_2-$CH(CH_3)_2$, R3 = H: (2*RS*)-2-[3-(2-methylpropyl)phenyl]propanoic acid,

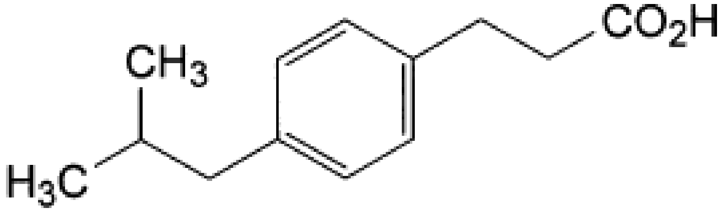
B. R1 = OH, R2 = H, R3 = $[CH_2]_3$-CH_3: (2*RS*)-2-(4-butylphenyl)propanoic acid,

C. R1 = NH2, R2 = H, R3 = CH_2-$CH(CH_3)_2$: (2*RS*)-2-[4-(2-methylpropyl)phenyl]propanamide,

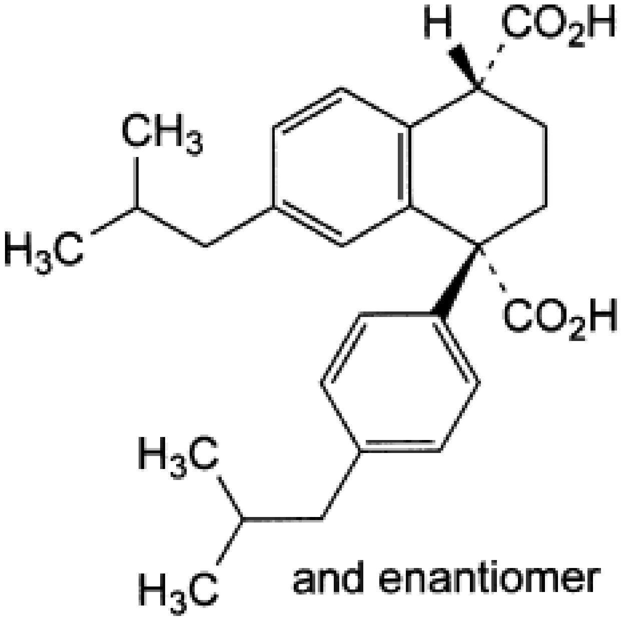
D. R1 = OH, R2 = H, R3 = CH_3: (2*RS*)-2-(4-methylphenyl)propanoic acid,


O
CH3
CH3
H3C

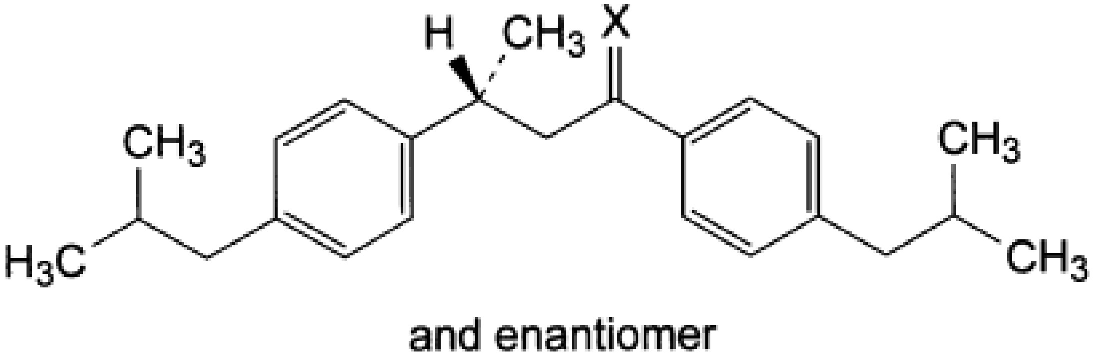
E. 1-[4-(2-methylpropyl)phenyl]ethanone,


CO2H
CH3
H3C

F. 3-[4-(2-methylpropyl)phenyl]propanoic acid,

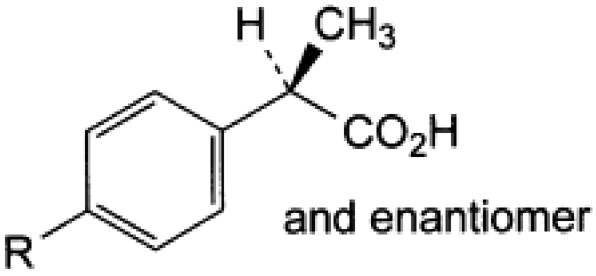

H
CO2H
CH3
H3C
CO2H
H3C
H3C
and enantiomer

G. (1*RS*,4*RS*)-7-(2-methylpropyl)-1-[4-(2-methylpropyl)phenyl]-1,2,3,4-tetrahydronaphthalene-1,4-dicarboxylic acid,



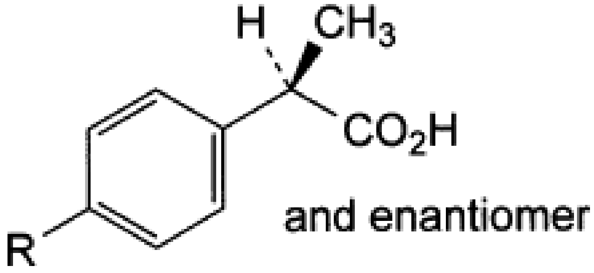
H. X = O: (3*RS*)-1,3-bis[4-(2-methylpropyl)phenyl]butan-1-one,

I. X = H_2: 1-(2-methylpropyl)-4-[(3*RS*)-3-[4-(2-methylpropyl)phenyl]butyl]benzene,



J. R = CO-CH$(CH_3)_2$: (2*RS*)-2-[4-(2-methylpropanoyl)phenyl]propanoic acid,

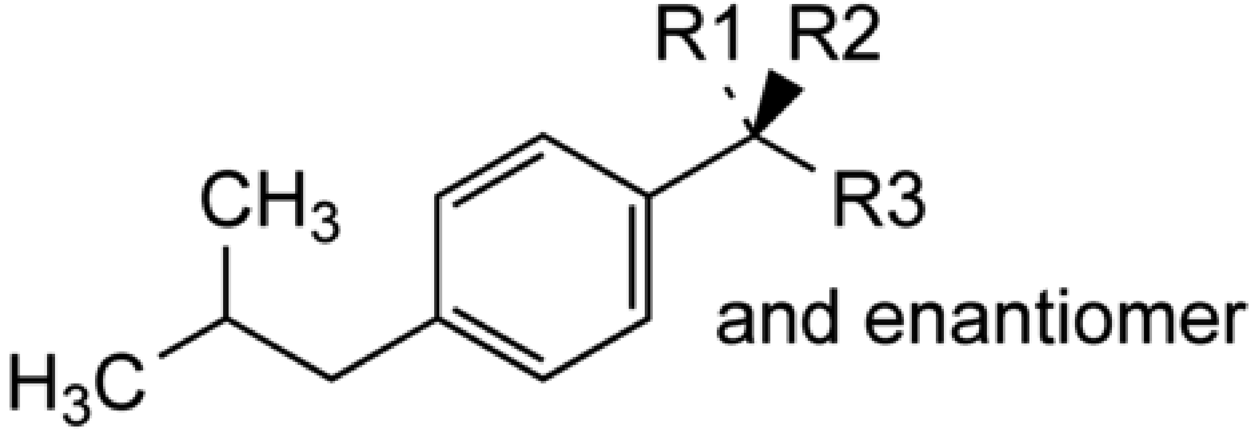
N. R = C_2H_5: (2*RS*)-2-(4-ethylphenyl)propanoic acid,



K. R = CHO: (2*RS*)-2-(4-formylphenyl)propanoic acid,

L. R = CHOH-CH$(CH_3)_2$: 2-[4-(1-hydroxy-2-methylpropyl)phenyl]propanoic acid,

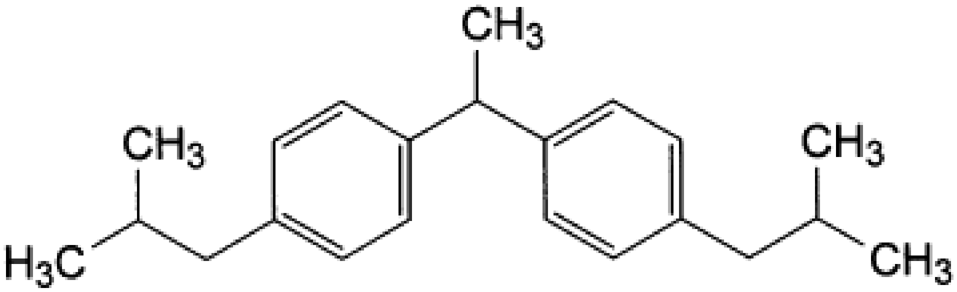
O. R = CH(CH_3)-C_2H_5: 2-[4-(1-methylpropyl)phenyl]propanoic acid,


R1
R2
CH3
R3
and enantiomer
H3C

M. R1 = OH, R2 = CH_3, R3 = CO_2H: (2*RS*)-2-hydroxy-2-[4-(2-methylpropyl)phenyl]propanoic acid,

P. R1 = H, R2 = CH_3, R3 = CH_2OH: (2*RS*)-2-[4-(2-methylpropyl)phenyl]propan-1-ol,

Q. R1 = R2 = H, R3 = CH_2OH: 2-[4-(2-methylpropyl)phenyl]ethanol,


CH3
CH3
CH3
H3C
CH3

R. 1,1′-(ethane-1,1-diyl)-4,4′-(2-methylpropyl)dibenzene.

Ph Eur

Ichthammol

General Notices

Ammonium Ichthosulphonate

(Ph Eur monograph 0917)

Action and use

Chronic lichenified eczema.

Preparation

Zinc and Ichthammol Cream

Ph Eur

DEFINITION

Ichthammol is obtained by distillation from certain bituminous schists, sulphonation of the distillate and neutralisation of the product with ammonia.

Content:

— *dry matter*: 50.0 per cent *m/m* to 56.0 per cent *m/m*,

— *total ammonia* (NH_3; M_r 17.03): 4.5 per cent *m/m to* 7.0 per cent *m/m* (dried substance),

— *organically combined sulphur*: minimum 10.5 per cent *m/m* (dried substance),

— *sulphur in the form of sulphate*: maximum 20.0 per cent *m/m.*

CHARACTERS

Appearance

Dense, blackish-brown liquid.

Solubility

Miscible with water and with glycerol, slightly soluble in ethanol (96 per cent), in fatty oils and in liquid paraffin. It forms homogeneous mixtures with wool fat and soft paraffin.

IDENTIFICATION

A. Dissolve 1.5 g in 15 ml *of water R* (solution A). To 2 ml of solution A add 2 ml of *hydrochloric acid R*. A resinous precipitate is formed. Decant the supernatant liquid. The precipitate is partly soluble in *ether R*.

B. 2 ml of solution A, obtained in identification test A, gives the reaction of ammonium salts and salts of volatile bases *(2.3.1).*

C. Evaporate and ignite the mixture of solution A and *dilute sodium hydroxide solution R* obtained in identification test B. Take up the residue with 5 ml of *dilute hydrochloric acid R.* Gas is evolved which turns *lead acetate paper R* brown or black. Filter the solution. The filtrate gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Acidity or alkalinity

To 10.0 ml of the clear filtrate obtained in the assay of total ammonia add 0.05 ml of *methyl red solution R*. Not more than 0.2 ml of *0.02 M hydrochloric acid* or *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Relative density *(2.2.5)*

1.040 to 1.085, determined on a mixture of equal volumes of the substance to be examined and *water R*.

Sulphated ash *(2.4.14)*

Maximum 0.3 per cent, determined on 1.00 g.

ASSAY

Dry matter

Weigh 1.000 g in a tared flask containing 2 g of *sand R*, previously dried to constant mass, and a small glass rod. Heat on a water-bath for 2 h with frequent stirring and dry in an oven at 100-105 °C until 2 consecutive weighings do not differ by more than 2.0 mg; the 2nd weighing is carried out after drying again for 1 h.

Total ammonia

Dissolve 2.50 g in 25 ml of warm *water R*. Rinse the solution into a 250 ml volumetric flask, add 200 ml of *sodium chloride solution R* and dilute to 250.0 ml with *water R.* Filter the solution, discarding the first 20 ml of filtrate. To 100.0 ml of the clear filtrate add 25 ml of *formaldehyde solution R*, neutralised to *phenolphthalein solution R1*. Titrate with *0.1 M sodium hydroxide* until a faint pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 1.703 mg of NH_3.

Organically combined sulphur

Mix 0.500 g with 4 g of *anhydrous sodium carbonate R* and 3 ml of *methylene chloride R* in a porcelain crucible of about 50 ml capacity, warm and stir until all the methylene chloride has evaporated. Add 10 g of coarsely powdered *copper nitrate R*, mix thoroughly and heat the mixture very gently using a small flame. When the initial reaction has subsided, increase the temperature slightly until most of the material has blackened. Cool, place the crucible in a large beaker, add 20 ml of *hydrochloric acid R* and, when the reaction has ceased, add 100 ml of *water R* and boil until all the copper oxide has dissolved. Filter the solution, add 400 ml of *water R*, heat to boiling and add 20 ml of *barium chloride solution R1.* Allow to stand for 2 h, filter, wash with *water R,* dry and ignite at about 600 ± 50 °C until 2 successive weighings do not differ by more than 0.2 per cent of the mass of the residue.

1 g of residue is equivalent to 0.1374 g of total sulphur.

Calculate the percentage content of total sulphur and subtract the percentage content of sulphur in the form of sulphate.

Sulphur in the form of sulphate

Dissolve 2.000 g in 100 ml of *water R*, add 2 g of *cupric chloride R* dissolved in 80 ml of *water R* and dilute to 200.0 ml with *water R.* Shake and filter. Heat 100.0 ml of the filtrate almost to boiling, add *1 ml of hydrochloric acid R* and 5 ml of *barium chloride solution R1* dropwise and heat on a water-bath. Filter, wash the precipitate with *water R*, dry and ignite at about 600 ± 50 °C until 2 successive weighings do not differ by more than 0.2 per cent of the mass of the residue.

1 g of residue is equivalent to 0.1374 g of sulphur present in the form of sulphate.

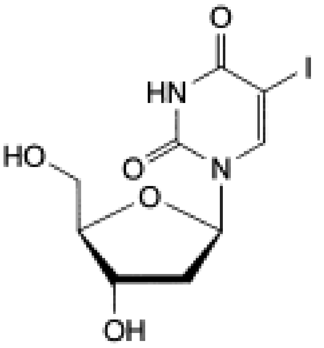
Calculate the percentage content of sulphur in the form of sulphate.

Ph Eur

Idoxuridine

General Notices

(Ph Eur monograph 0669)



$C_9H_{11}IN_2O_5$ 354.1 *54-42-2*

Action and use

Pyrimidine nucleoside analogue; antiviral (herpesviruses).

Preparation

Idoxuridine Eye Drops

Ph Eur

DEFINITION

Idoxuridine contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 5-iodo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water and in alcohol. It dissolves in dilute solutions of alkali hydroxides.

It melts at about 180 °C, with decomposition.

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *idoxuridine CRS*. Examine the substances as discs prepared using 1 mg of the substance to be examined and of the reference substance each in 0.3 g of *potassium bromide R*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (c).

C. Heat about 5 mg in a test-tube over a naked flame. Violet vapour is evolved.

D. Disperse about 2 mg in 1 ml of *water R* and add 2 ml of *diphenylamine solution R2*. Heat in a water-bath for 10 min. A persistent light-blue colour develops.

TESTS

Solution S

Dissolve 0.500 g in *1 M sodium hydroxide* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent. The pH of the solution is 5.5 to 6.5.

Specific optical rotation *(2.2.7)*

+ 28 to + 32, determined on solution S and calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using as coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 0.20 g of the substance to be examined in a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R.*

Reference solution (a) Dissolve 20 mg of *5-iodouracil R*, 20 mg of *2′-deoxyuridine R* and 20 mg of *5-bromo-2′-deoxyuridine R* in a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (b) Dissolve 0.20 g of the substance to be examined in 5 ml of reference solution (a).

Reference solution (c) Dissolve 20 mg of *idoxuridine CRS* in a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (d) Dilute 1 ml of test solution (b) to 20 ml with a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R*.

Apply separately to the plate 5 µl of each solution. Develop twice over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R*, 40 volumes of *chloroform R* and 50 volumes of *2-propanol R*, drying the plate in a current of cold air after each development. Examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a): any spots corresponding to 5-iodouracil, 2′-deoxyuridine and 5-bromo-2′-deoxyuridine are not more intense than the corresponding spots in the chromatogram obtained with reference solution (a) (0.5 per cent); any spot, apart from the principal spot and the spots corresponding to 5-iodouracil, 2′-deoxyuridine and 5-bromo-2′-deoxyuridine, is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows four clearly separated spots.

Iodide

Dissolve 0.25 g in 25 ml of *0.1 M sodium hydroxide*, add 5 ml of *dilute hydrochloric acid R* and dilute to 50 ml with *water R*. Allow to stand for 10 min and filter. To 25 ml of the filtrate add 5 ml of *dilute hydrogen peroxide solution R* and 10 ml of *chloroform R* and shake. Any pink colour in the organic layer is not more intense than that in a standard prepared at the same time in the same manner using 1 ml of a 0.33 g/l solution of *potassium iodide R* instead of the substance to be examined (0.1 per cent).

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.3000 g in 20 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 35.41 mg of $C_9H_{11}IN_2O_5$.

STORAGE

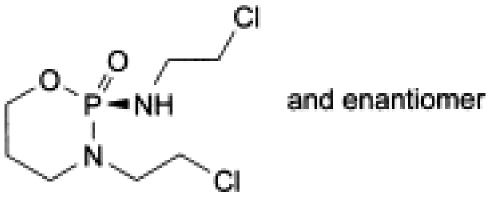
Store protected from light.

Ph Eur

Ifosfamide

General Notices

(Ph Eur monograph 1529)



$C_7H_{15}Cl_2N_2O_2P$ 261.1   *3778-73-2*

Action and use

Cytotoxic alkylating agent.

Preparation

Ifosfamide Injection

Ph Eur

DEFINITION

Ifosfamide contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (*RS*)-*N*,3-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, fine, crystalline powder, hygroscopic, soluble in water, freely soluble in methylene chloride.

IDENTIFICATION

Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the *Ph. Eur. reference spectrum of ifosfamide*. Examine the substance prepared as a disc.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II).*

Acidity or alkalinity

Dilute 5 ml of solution S to 50 ml with *carbon dioxide-free water R*. To 10 ml of this solution add 0.1 ml of *methyl red solution R.* Not more than 0.1 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red. To another 10 ml of the solution add 0.1 ml of *phenolphthalein solution R.* Not more than 0.3 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Optical rotation *(2.2.7).*

The angle of optical rotation, determined on solution S, is - 0.10 ° to + 0.10 °.

Related substances

A. Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution Dissolve 1.00 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dissolve 25 mg of *ifosfamide impurity A CRS* and 25 mg of *chloroethylamine hydrochloride R* (impurity C) in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (b) Dissolve 15 mg of *ifosfamide impurity B CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (c) Dissolve 5 mg of *ethanolamine R* (impurity D), 20 mg of *ifosfamide impurity A CRS* and 80 mg of *chloroethylamine hydrochloride R* (impurity C) in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100 ml with the same mixture of solvents.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *water R,* 15 volumes of *methanol R*, 25 volumes of *anhydrous acetic acid R* and 50 volumes of *methylene chloride R*. Dry the plate at 115 °C for 45 min. At the bottom of a chromatography tank, place an evaporating dish containing a 3.2 g/l solution of *potassium permanganate R* and add an equal volume of *dilute hydrochloric acid R*, close the tank and allow to stand for 10 min. Place the plate whilst still hot in the tank, avoiding contact of the stationary phase with the solution, and close the tank. Leave the plate in contact with the chlorine vapour for 20 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed (about 20 min) and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide* and *starch solution R*. Avoid prolonged exposure to cold air. Immerse the plate in a 1 g/l solution of *tetramethylbenzidine R* in *alcohol R* for 5 s. Allow the plate to dry and examine. In the chromatogram obtained with the test solution: any spot corresponding to impurity A or impurity C is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.25 per cent); any spot corresponding to impurity B is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.15 per cent); any other spot is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.15 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 3 clearly separated spots.

B. Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution Dissolve 0.200 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dissolve 5 mg of *ifosfamide impurity E CRS* and 5 mg of *ifosfamide impurity F CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *ifosfamide impurity E CRS* and 10 mg of *ifosfamide CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 100 ml with the same mixture of solvents.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *methylene chloride R* and 10 volumes of *acetone R*. Dry the plate at 115 °C for 45 min. Proceed as described in Related substances test A. Any spot corresponding to impurity E or impurity F in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Chlorides ***(2.4.4)***

Dilute 5 ml of solution S to 15 ml with *water R*. The freshly prepared solution complies with the limit test for chlorides (100 ppm).

Heavy metals ***(2.4.8)***

12 ml of solution S complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water ***(2.5.12)***

Not more than 0.5 per cent, determined on 1.00 g by the semi-micro determination of water.

ASSAY

Examine by liquid chromatography *(2.2.29). Use the solutions within 24 h*.

Solution A Dissolve 50.0 mg of *ethyl parahydroxybenzoate R* in 25 ml of *alcohol R*, dilute to 100.0 ml with *water R* and mix.

Test solution To 0.150 g of the substance to be examined add 10.0 ml of solution A and dilute to 250.0 ml with *water R*.

Reference solution To 15.0 mg of *ifosfamide CRS* add 1.0 ml of solution A and dilute to 25.0 ml with *water R.*

The chromatography may be carried out using:

- a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),
- as mobile phase at a flow rate of 1.5 ml/min a mixture of 30 volumes of *acetonitrile R* and 70 volumes of *water R*,
- as detector a spectrophotometer set at 195 nm.

Inject 1 µl of the reference solution six times. The assay is not valid unless the resolution between the peaks due to ifosfamide and to ethyl parahydroxybenzoate is not less than 6.0 and the relative standard deviation of the peak area for ifosfamide is at most 2.0 per cent.

Inject 1 µl of the test solution. Calculate the percentage content of $C_7H_{15}Cl_2N_2O_2P$ from the area of the corresponding peak in the chromatogram obtained and the declared content of *ifosfamide CRS*.

STORAGE

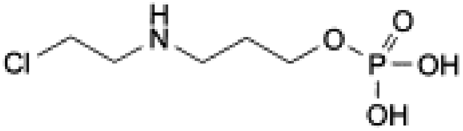
Store in an airtight container .

IMPURITIES

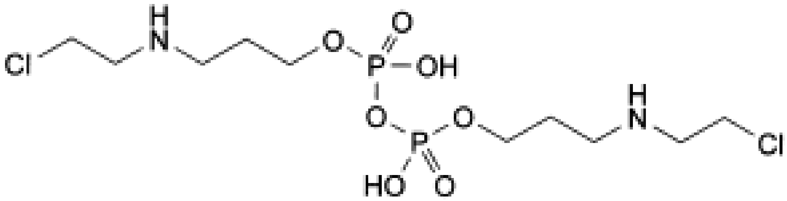
Specified impurities *A, B, C, E, F.*

Other detectable impurities *D.*

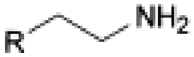
Related substances test A



A. 3-[(2-chloroethyl)amino]propyl dihydrogen phosphate,



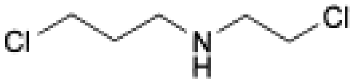
B. bis[3-[(2-chloroethyl)amino]propyl] dihydrogen diphosphate,



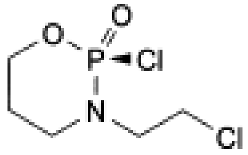
C. R = Cl: 2-chloroethanamine,

D. R = OH: 2-aminoethanol.

Related substances test B



E. 3-chloro-*N*-(2-chloroethyl)propan-1-amine,



and enantiomer

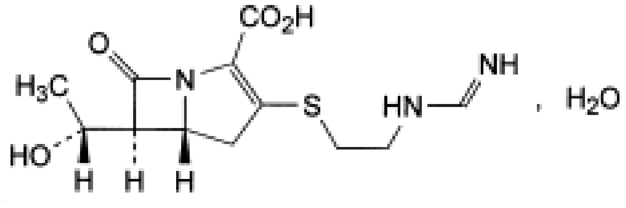
F. (*RS*)-2-chloro-3-(2-chloroethyl)-1,3,2-oxazaphosphinane 2-oxide.

Ph Eur

Imipenem

General Notices

(Ph Eur monograph 1226)



$C_{12}H_{17}N_3O_4S,H_2O$ 317.4 *64221-86-9*

Action and use

Carbapenem antibacterial.

Ph Eur

DEFINITION

(5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-3-[[2-[(iminomethyl)amino]-ethyl]sulphanyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white or pale yellow powder.

Solubility

Sparingly soluble in water, slightly soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24).*

Comparison

imipenem CRS.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than intensity 6 of the range of the reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Dissolve 0.500 g in *phosphate buffer solution pH 7.0 R3* and dilute to 50 ml with the same solution.

pH *(2.2.3)*

4.5 to 7.0.

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 100.0 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 84 to + 89 (anhydrous substance), measured at 25 °C.

Dissolve 0.125 g in *phosphate buffer solution pH 7.0 R3* and dilute to 25.0 ml with the same solution.

Related substances

Liquid chromatography *(2.2.29)*. *Keep the solutions in an ice-bath and use within 8 h of preparation.*

Solvent mixture Mix 0.7 volumes of *acetonitrile R* and 99.3 volumes of a 0.135 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 6.8 with *dilute phosphoric acid R*.

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dissolve 40.0 mg of *imipenem CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (c) Heat 20 ml of the test solution, previously adjusted to pH 10 with *sodium hydroxide solution R,* at 80 °C for 5 min (*in situ* preparation of impurity A).

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 0.7 volumes of *acetonitrile R* and 99.3 volumes of a 8.7 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 7.3 with *dilute phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of imipenem.

Relative retention with reference to imipenem (retention time = about 9 min): impurity A = about 0.8.

System suitability Reference solution (c):

— *resolution*: minimum 3.5 between the peaks due to impurity A and imipenem.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *any other impurity*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Water *(2.5.12)*

5.0 per cent to 8.0 per cent, determined on 0.200 g. Use an iodosulphurous reagent containing imidazole instead of pyridine and a clean container for each determination.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.17 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

System suitability Reference solution (a):

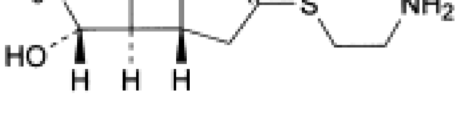
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

STORAGE

In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities A.



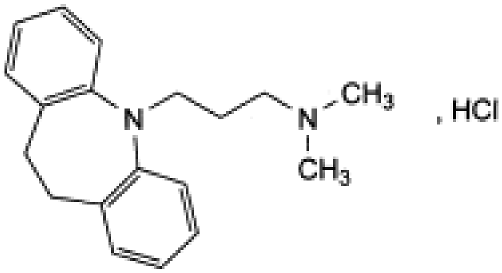
A. (5*R*,6*S*)-3-[(2-aminoethyl)sulphanyl]-6-[(*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (thienamycin).

Ph Eur

Imipramine Hydrochloride

General Notices

(Ph Eur monograph 0029)



$C_{19}H_{24}N_2$,HCl 316.9 *113-52-0*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Imipramine Tablets

Ph Eur

DEFINITION

3-(10,11-Dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Freely soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 170 °C to 174 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *imipramine hydrochloride CRS.*

C. Dissolve about 5 mg in 2 ml of *nitric acid R*. An intense blue colour develops.

D. About 20 mg gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

To 3.0 g add 20 ml of *carbon dioxide-free water R*, dissolve rapidly by shaking and triturating with a glass rod and dilute to 30 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*. Immediately after preparation, dilute solution S with an equal volume of *water R*. This solution is not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

3.6 to 5.0 for solution S, measured immediately after preparation.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution Dissolve 5 mg of *imipramine for system suitability CRS* (containing impurity B) in the mobile phase and dilute to 5.0 ml with the mobile phase.

Column:
- *size*: l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 40 volumes of *acetonitrile R1* with 60 volumes of a 5.2 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 7.0 with *phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Run time 2.5 times the retention time of imipramine.

Relative retention With reference to imipramine (retention time = about 7 min): impurity B = about 0.7.

System suitability Reference solution:

— *resolution*: minimum 5.0 between the peaks due to impurity B and imipramine.

Limits:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to imipramine in the chromatogram obtained with the reference solution (0.10 per cent);

— *total*: not more than 3 times the area of the peak due to imipramine in the chromatogram obtained with the reference solution (0.3 per cent);

— *disregard limit*: 0.5 times the area of the peak due to imipramine in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Test solution Dissolve 0.500 g of the substance to be examined in 20 ml of *water R*.

Reference solution Dilute 10 ml of *lead standard solution (1 ppm Pb) R* to 20 ml with *water R*.

Blank solution 20 ml of *water R*.

Monitor solution Dissolve 0.500 g of the substance to be examined in 10 ml of *lead standard solution (1 ppm Pb) R* and dilute to 20 ml of *water R*.

To each solution add 2 ml of *buffer solution pH 3.5 R*. Mix and add to 1.2 ml of *thioacetamide reagent R*. Mix immediately. Filter the solutions through a suitable membrane filter (pore size 0.45 µm). Compare the spots on the filters obtained with the different solutions. The test is invalid if the reference solution and the monitor solution do not show a slight brown colour compared to the blank solution. The substance to be examined complies with the test if the brown colour of the spot resulting from the test solution is not more intense than that of the spot resulting from the reference solution.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.69 mg of $C_{19}H_{25}ClN_2$.

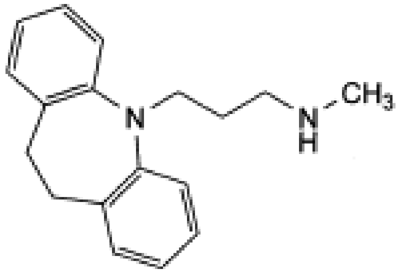
STORAGE

Protected from light.

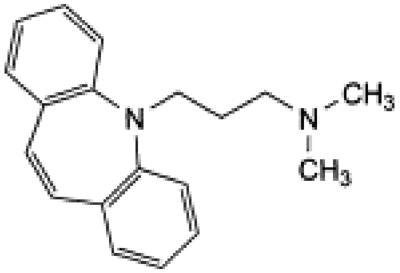
IMPURITIES

Specified impurities *B.*

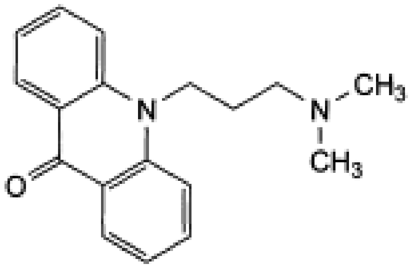
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, C.*



A. 3-(10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N*-methylpropan-1-amine (desipramine),



B. 3-(5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine (depramine),



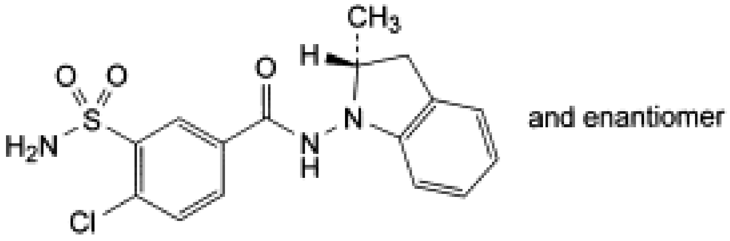
C. 10-[3-(dimethylamino)propyl]acridin-9(10*H*)-one.

Ph Eur

Indapamide

General Notices

(Ph Eur monograph 1108)



$C_{16}H_{16}ClN_3O_3S$ 365.8 *26807-65-8*

Action and use

Thiazide-like diuretic.

Preparation

Indapamide Tablets

Ph Eur

DEFINITION

4-Chloro-*N*-[(2*RS*)-2-methyl-2,3-dihydro-1*H*-indol-1-yl]-3-sulphamoylbenzamide.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*.

Spectral range 220-350 nm.

Absorption maximum At 242 nm.

Shoulders At 279 nm and 287 nm.

Specific absorbance at the absorption maximum 590 to 630.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *indapamide CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *indapamide CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg *of indometacin R* in 5 ml of reference solution (a) and dilute to 10 ml with *ethanol (96 per cent) R.*

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *glacial acetic acid R, acetone R, toluene R* (1:20:79 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability
Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Optical rotation *(2.2.7)*

- 0.02 ° to + 0.02 °.

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light and prepare the* *solutions immediately before use or maintain them at 4 °C.*

Test solution Dissolve 20.0 mg of the substance to be examined in 7 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R* and dilute to 20.0 ml with a 0.2 g/l solution of *sodium edetate R.*

Reference solution (a) Dissolve 3.0 mg of *indapamide impurity B CRS* in 3.5 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R* and dilute to 10.0 ml with a 0.2 g/l solution of *sodium edetate R.* To 1.0 ml of this solution, add 35 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R* and dilute to 100.0 ml with a 0.2 g/l solution of *sodium edetate R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with a mixture of 17.5 volumes of *acetonitrile R,* 17.5 volumes of *methanol R* and 65 volumes of a 0.2 g/l solution of *sodium edetate R*. Dilute 1.0 ml of this solution to 20.0 ml with a mixture of 17.5 volumes of *acetonitrile R*, 17.5 volumes of *methanol R* and 65 volumes of a 0.2 g/l solution of *sodium edetate R.*

Reference solution (c) Dissolve 20.0 mg of *indapamide CRS* in 7 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R* and dilute to 20.0 ml with a 0.2 g/l solution of *sodium edetate R*.

Reference solution (d) Dissolve 25.0 mg of *indapamide CRS* and 45.0 mg of *methylnitrosoindoline CRS* (impurity A) in 17.5 ml of a mixture of equal volumes of *acetonitrile R* and *methanol R* and dilute to 50.0 ml with a 0.2 g/l solution of *sodium edetate R.*

Column:

— *size*: *l* = 0.20 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *glacial acetic acid R, acetonitrile R, methanol R*, 0.2 g/l solution of *sodium edetate R* (0.1:17.5:17.5:65 *V/V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 2.5 times the retention time of indapamide.

Retention time Indapamide = about 11 min.

System suitability:

— *resolution*: minimum 4.0 between the peaks due to indapamide and impurity A in the chromatogram obtained with reference solution (d);

— *signal-to-noise ratio*: minimum 6 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity A

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in 1 ml of *acetonitrile R* and dilute to 10.0 ml with *water R*. Shake for 15 min. Allow to stand at 4 °C for 1 h and filter.

Reference solution Dissolve 25.0 mg of the substance to be examined in 1.0 ml of a 0.125 mg/l solution of *methylnitrosoindoline CRS* (impurity A) in *acetonitrile R* and dilute to 10.0 ml with *water R*. Shake for 15 min. Allow to stand at 4 °C for 1 h and filter.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 7 volumes of *acetonitrile R*, 20 volumes of *tetrahydrofuran R* and 73 volumes of a 1.5 g/l solution of *triethylamine R* adjusted to pH 2.8 with *phosphoric acid R.*

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 305 nm.

Injection 0.1 ml.

System suitability Reference solution:

— *signal-to-noise ratio*: minimum 3 for the peak due to impurity A appearing just before the peak due to indapamide;

— *peak-to-valley-ratio:* minimum 6.7, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to indapamide.

Limit:
— *impurity A:* not more than the difference between the areas of the peaks due to impurity A in the chromatograms obtained with the reference solution and the test solution (5 ppm).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 0.100 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection The test solution and reference solution (c).

System suitability Reference solution (c)

- — *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections; if necessary, adjust the integrator parameters.

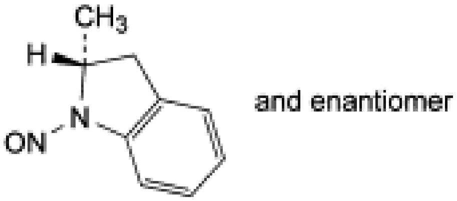
Calculate the percentage content of $C_{16}H_{16}ClN_3O_3S$ from the declared content of *indapamide CRS*.

STORAGE

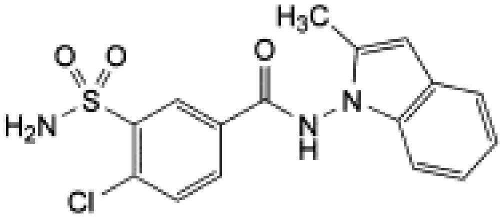
Protected from light.

IMPURITIES

Specified impurities A, B.



A. (2*RS*)-2-methyl-1-nitroso-2,3-dihydro-1*H*-indole,



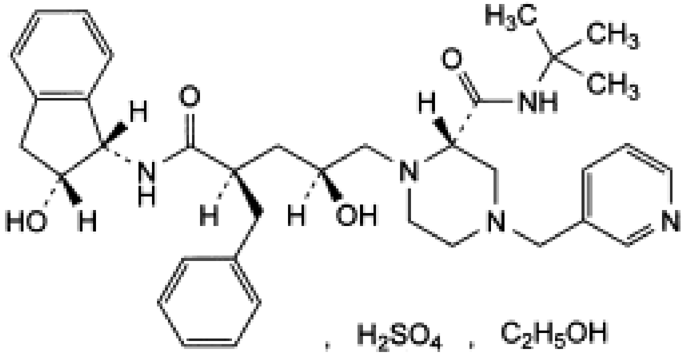
B. 4-chloro-*N*-(2-methyl-1*H*-indol-1-yl]-3-sulphamoylbenzamide.

Ph Eur

Indinavir Sulphate

General Notices

(Ph Eur monograph 2214)



$C_{36}H_{47}N_5O_4,H_2SO_4,C_2H_6O$ 758 *157810-81-6*

Action and use

Protease inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

(2*S*)-1-[(2*S*,4*R*)-4-Benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide sulphate ethanolate.

Content

98.0 per cent to 102.0 per cent (anhydrous and ethanol-free substance).

PRODUCTION

A test for enantiomeric purity is carried out unless it has been demonstrated that the manufacturing process is enantioselective for the substance.

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water, soluble in methanol, practically insoluble in heptane.

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: + 122 to + 129 (anhydrous and ethanol-free substance), determined at 365 nm and at 25 °C.

Dissolve 0.500 g in *water R* and dilute to 50.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of indinavir sulphate*.

C. It gives reaction (a) of sulphates *(2.3.1)*.

D. It complies with the test for ethanol (see Tests).

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Solution A Thoroughly mix equal volumes of mobile phase A and *acetonitrile R1*.

Test solution Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 100.0 ml with the same solution.

Reference solution (a) Dissolve 4 mg of *indinavir for system suitability CRS* (containing impurities B, C and E) in solution A and dilute to 10 ml with the same solution.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with solution A. Dilute 1.0 ml of this solution to 10.0 ml with solution A.

Reference solution (c) Dissolve 5.0 mg of *cis-aminoindanol R* (impurity A) in solution A and dilute to 10.0 ml with the same solution. Dilute 1.0 ml of the solution to 100.0 ml with solution A. Dilute 1.0 ml of this solution to 10.0 ml with solution A.

Reference solution (d) To 30 mg of the substance to be examined add 0.25 ml of *2 M hydrochloric acid R* and allow to stand at room temperature for 1 h. Dilute to 100 ml with a mixture of 2 volumes of *acetonitrile R1* and 3 volumes of mobile phase A and mix (*in situ* degradation to obtain impurity D).

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: solution containing 0.27 g/l of *potassium dihydrogen phosphate R* and 1.40 g/l of *dipotassium hydrogen phosphate R*; filter and degas;

— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 40	80 → 30	20 → 70
40 - 45	30	70
45 - 47	30 → 80	70 → 20
47 - 52	80	20

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *indinavir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, C and E; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity D.

Relative retention With reference to indinavir (retention time = about 25 min): impurity A = about 0.2; impurity B = about 0.8; impurity C = about 0.98; impurity D = about 1.1; impurity E = about 1.3.

System suitability Reference solution (a):

— *resolution*: minimum 1.8 between the peaks due to impurity C and indinavir.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 1.8;

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities B, C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Ethanol

Gas chromatography *(2.2.28)*.

Internal standard solution Dilute 1.0 ml of *propanol R* to 200.0 ml with *water R*.

Test solution Dissolve 0.400 g of the substance to be examined in 50.0 ml of *water R*, add

8.0 ml of the internal standard solution and dilute to 100.0 ml with *water R*.

Reference solution Dilute 1.0 ml of *anhydrous ethanol R* to 200.0 ml. Dilute 2.0 ml of this solution and 2.0 ml of the internal standard solution to 25.0 ml with *water R*.

Column:
- *material*: fused silica;
- *size:* l = 30 m, Ø = 0.53 mm;
- *stationary phase: macrogol 20 000 R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R.*

Flow rate 10 ml/min.

Split ratio 1:10.

Temperature:
- *column*: 35 °C;
- *injection port*: 140 °C;
- *detector*: 220 °C.

Detection Flame ionisation.

Injection 1.0 µl.

System suitability Reference solution:
- *retention time*: ethanol = 2 min to 4 min;
- *resolution*: minimum 5.0 between the peaks due to ethanol and propanol.

Calculate the percentage content of ethanol taking the density *(2.2.5)* to be 0.790 g/ml.

Limit:
- *ethanol* : 5.0 per cent to 8.0 per cent (*m/m*).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

Maximum 1.5 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)*.

Solution B Add 20 ml of *dibutylammonium phosphate for ion-pairing R* to 1000 ml of *water R*. Adjust to pH 6.5 with *1 M sodium hydroxide*.

Test solution Dissolve 60.0 mg of the substance to be examined in the mobile phase and

dilute to 100.0 ml with the mobile phase.

Reference solution Dissolve 50.0 mg of *indinavir CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: base-deactivated octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *acetonitrile R, solution B* (45:55 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 10 µl.

Run time Twice the retention time of indinavir.

Retention time Indinavir = about 10 min.

Calculate the percentage content of $C_{36}H_{49}N_5O_8S$ using the declared content of *indinavir CRS* and multiplying by a correction factor of 1.1598.

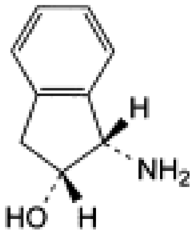
STORAGE

In an airtight container, protected from light.

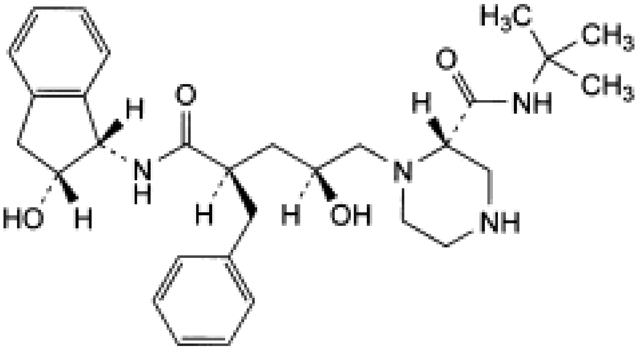
IMPURITIES

Specified impurities *A, B, C, D, E.*

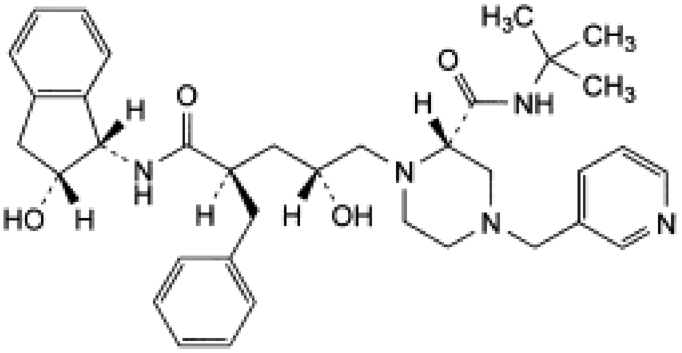
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F.*



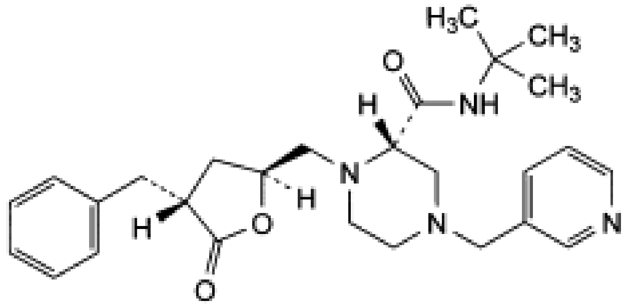
A. (1*S*,2*R*)-1-amino-2,3-dihydro-1*H*-inden-2-ol (*cis*-aminoindanol),



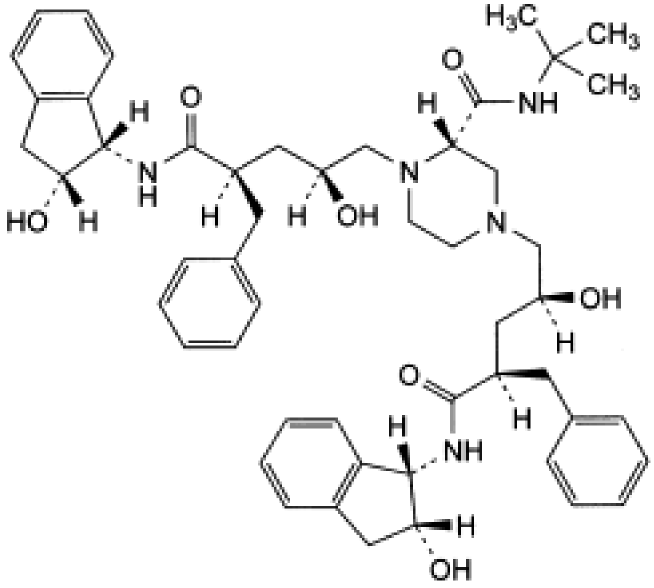
B. (2*S*)-1-[(2*S*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)piperazine-2-carboxamide,



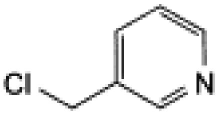
C. (2*S*)-1-[(2*R*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide,



D. (3*R*,5*S*)-3-benzyl-5-[[(2*S*)-2-[(1,1-dimethylethyl)carbamoyl]-4-(pyridin-3-ylmethyl)piperazin-1-yl]methyl]-4,5-dihydrofuran-2(3*H*)-one,



E. (2*S*)-1,4-bis[(2*S*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)piperazine-2-carboxamide,



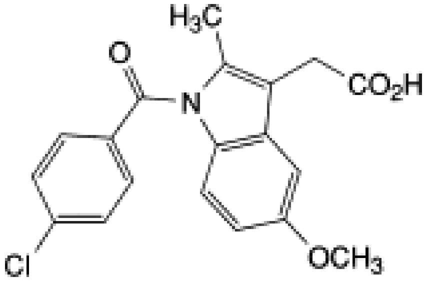
F. 3-(chloromethyl)pyridine (nicotinyl chloride).

Ph Eur

Indometacin

General Notices

(Ph Eur monograph 0092)



$C_{19}H_{16}ClNO_4$ 357.8   *53-86-1*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Indometacin Capsules

Indometacin Suppositories

Ph Eur

DEFINITION

Indometacin contains not less than 98.5 per cent and not more than the equivalent of 100.5 per cent of [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid, calculated with reference to the dried substance.

CHARACTERS

A white or yellow, crystalline powder, practically insoluble in water, sparingly soluble in alcohol.

IDENTIFICATION

First identification   *A, C.*

Second identification   *A, B, D, E.*

A. Melting point *(2.2.14)*: 158 °C to 162 °C.

B. Dissolve 25 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 9 volumes of *methanol R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 10.0 ml of the solution to 100.0 ml with a mixture of 1 volume of *1 M hydrochloric acid* and 9 volumes of *methanol R*. Examined between 300 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 318 nm. The specific absorbance at the maximum is 170 to 190.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *indometacin CRS*. Examine the substances in the solid state without recrystallisation.

D. Dissolve 0.1 g in 10 ml of *alcohol R*, heating slightly if necessary. To 0.1 ml of the solution add 2 ml of a freshly prepared mixture of 1 volume of a 250 g/l solution of *hydroxylamine hydrochloride R* and 3 volumes of *dilute sodium hydroxide solution R*. Add 2 ml of *dilute hydrochloric acid R* and 1 ml of *ferric chloride solution R2* and mix. A violet-pink colour develops.

E. To 0.5 ml of the solution in alcohol prepared in identification test D, add 0.5 ml of *dimethylaminobenzaldehyde solution R2*. A precipitate is formed that dissolves on shaking. Heat on a water-bath. A bluish-green colour is produced. Continue to heat for 5 min and cool in iced water for 2 min. A precipitate is formed and the colour changes to light greyish-green. Add 3 ml of *alcohol R*. The solution is clear and violet-pink in colour.

TESTS

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* HF_{254} *R* as the coating substance. Prepare the slurry using a 46.8 g/l solution of *sodium dihydrogen phosphate R*.

Test solution Dissolve 0.2 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent. Prepare immediately before use.

Reference solution Dilute 1 ml of the test solution to 200 ml with *methanol R.*

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of *light petroleum R* and 70 volumes of *ether R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals *(2.4.8)*

2.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 4 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

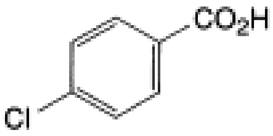
Dissolve 0.300 g in 75 ml of *acetone R,* through which *nitrogen R*, free from carbon dioxide, has been passed for 15 min. Maintain a constant stream of nitrogen through the solution. Add 0.1 ml of *phenolphthalein solution R*. Titrate with *0.1 M sodium hydroxide*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 35.78 mg of $C_{19}H_{16}ClNO_4$.

STORAGE

Store protected from light.

IMPURITIES

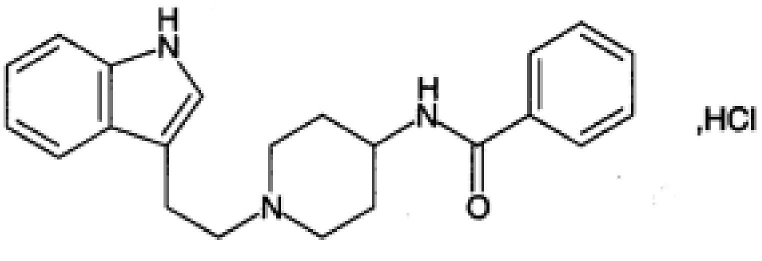


A. 4-chlorobenzoic acid.

Ph Eur

Indoramin Hydrochloride

General Notices



$C_{22}H_{25}N_3O,HCl$ 383.9 *33124-53-7*

Action and use

Alpha$_1$-adrenoceptor antagonist.

Preparation

Indoramin Tablets

DEFINITION

Indoramin Hydrochloride is *N*-1-[2-(indol-3-yl)ethyl]-4-piperidylbenzamide hydrochloride. It contains not less than 98.5% and not more than 100.5% of $C_{22}H_{25}N_3O,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder. It exhibits polymorphism.

Slightly soluble in *water*; sparingly soluble in *ethanol (96%)*; soluble in *methanol*; very slightly soluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.0045% w/v solution in *ethanol (96%)* exhibits three maxima, at 273, 280 and 290 nm. The *absorbances* at the maxima are about 0.76, 0.77 and 0.64, respectively.

B. Dissolve 50 mg in 30 ml of *water*, make the solution alkaline by the addition of 5M *ammonia* and shake with 50 ml of *dichloromethane*. Dry the dichloromethane layer with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of indoramin *(RS 188)*.

C. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 2% w/v suspension in *water*, 4.0 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *silica gel F_{254}* precoated plate (Merck silica gel 60 F_{254} plates are suitable)and a mixture of 1 volume of 18M *ammonia,* 20 volumes of *absolute ethanol* and 79 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of three solutions of the substance being examined in *ethanol (96%)* containing (1) 1.0% w/v, (2) 0.0050% w/v and (3) 0.0010% w/v. After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

Loss on drying

When dried at 100° to 105° for 4 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

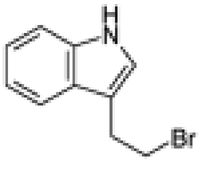
ASSAY

Dissolve 0.2 g in 30 ml of *anhydrous acetic acid*, add 6 ml of *acetic anhydride* and 6 ml of *mercury(II) acetate* solution. Titrate with 0.1M *perchloric acid VS* determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 38.39 mg of $C_{22}H_{25}N_3O,HCl$.

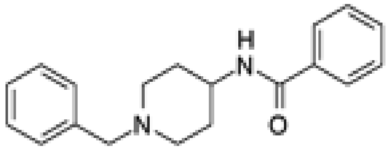
STORAGE

Indoramin Hydrochloride should be protected from light.

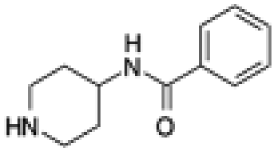
IMPURITIES



A. 3-(2-bromoethyl)indole



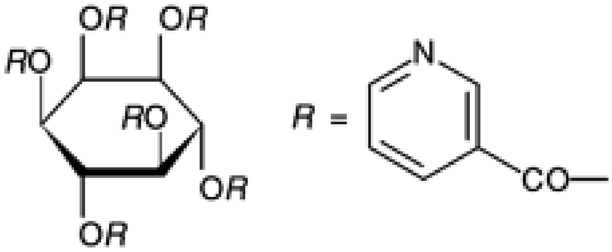
B. *N*-(1-benzyl-4-piperidyl)benzamide



C. *N*-(4-piperidyl)benzamide

Inositol Nicotinate

General Notices



$C_{42}H_{30}N_6O_{12}$ 810.7 *6556-11-2*

Action and use

Vasodilator.

Preparation

Inositol Nicotinate Tablets

DEFINITION

Inositol Nicotinate is *myo*-inositol hexanicotinate. It contains not less than 98.0% and not more than 101.0% of $C_{42}H_{30}N_6O_{12}$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Practically insoluble in *water*; practically insoluble in *acetone*, in *ethanol (96%)* and in *ether*. It dissolves in dilute mineral acids.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of inositol nicotinate *(RS 190)*.

TESTS

Clarity and colour of solution

A 5.0% w/v solution in 0.5M *sulphuric acid* is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* BY_6, Appendix IV B, Method II.

Heavy metals

2.0 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Chloride

Dissolve 0.14 g in a sufficient quantity of 2M *nitric acid* and dilute to 16 ml with *water*. The resulting solution complies with the *limit test for chlorides*, Appendix VII, beginning at the words 'pour the mixture as a single addition...' (350 ppm).

Free nicotinic acid

To 1 g add 75 ml of *water*, shake for 15 minutes and titrate with 0.02M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 0.8 ml of 0.02M *sodium hydroxide VS* is required to produce the first pink colour.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a plate 200 mm × 200 mm in size and *silica gel* GF_{254} as the coating substance. For the first development use a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol* as the mobile phase. Apply to the bottom right-hand corner of the plate 5 µl of solution (1) containing 5.0% w/v of the substance being examined in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* and develop over a path of 12 cm. After removal of the plate, allow it to dry in air and turn the plate through 90° in a clockwise direction. Apply separately to the bottom right-hand corner of the plate, and to the right of the solvent front, 5 µl of each of two solutions of the substance being examined in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* containing (2) 0.075% w/v and (3) 0.050% w/v. For the second development use a mixture of 50 volumes of *ethyl acetate* and 5 volumes each of *glacial acetic acid*, *ethanol (96%)* and *water* as the mobile phase. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

Acetone

Prepare a 0.020% v/v solution of *butan-2-one* (internal standard) in *dimethylformamide* (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Solution (1) contains 0.020% v/v of *acetone* in solution A. For solution (2) add 5 ml of *dimethylformamide* to 0.20 g of the substance being examined contained in a suitable vessel, stopper securely, suspend in a water bath until solution is complete and allow to cool. Prepare solution (3) in the same manner as solution (2) but using 5 ml of solution A in place of the dimethylformamide.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* coated with 10% w/w of polyethylene glycol 1000 and maintained at 60°.

In the chromatogram obtained with solution (3) the ratio of the area of any peak corresponding to acetone to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with solution (1).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

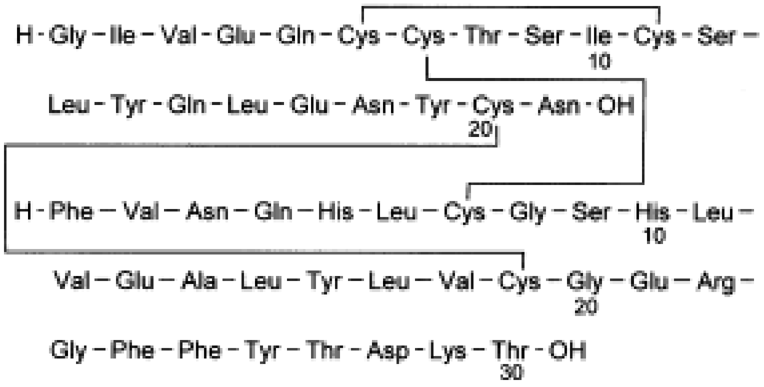
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.2 g and *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 13.51 mg of $C_{42}H_{30}N_6O_{12}$.

Insulin Aspart

General Notices

(Ph Eur monograph 2084)


H - Gly – Ile – Val - Glu - Gln - Cys - Cys - Thr - Ser - Ile - Cys - Ser –
10
Leu - Tyr - Gln - Leu - Glu – Asn - Tyr - Cys - Asn - OH
20
H - Phe – Val – Asn – Gln – His - Leu – Cys - Gly – Ser – His - Leu –
10
Val – Glu – Ala - Leu – Tyr – Leu – Val - Cys – Gly – Glu – Arg –
20
Gly – Phe – Phe - Tyr - Thr - Asp - Lys – Thr - OH
30

$C_{256}H_{381}N_{65}O_{79}S_6$ 5826 *116094-23-6*

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

DEFINITION

28^B-L-Aspartate insulin (human).

Insulin aspart is a 2-chain peptide containing 51 amino acids. The A-chain is composed of 21 amino acids and the B-chain is composed of 30 amino acids. It is identical in primary structure to human insulin, except that it has aspartic acid instead of proline at position 28 of the B-chain. As in human insulin, insulin aspart contains 2 interchain disulphide bonds and 1 intrachain disulphide bond.

Content

90.0 per cent to 104.0 per cent of insulin aspart $C_{256}H_{381}N_{65}O_{79}S_6$ plus A21Asp insulin aspart, B3Asp insulin aspart, B3isoAsp insulin aspart and B28isoAsp insulin aspart (dried substance).

By convention, for the purpose of labelling insulin aspart preparations, 0.0350 mg of insulin aspart is equivalent to 1 unit.

PRODUCTION

Insulin aspart is produced by a method based on recombinant DNA (rDNA) technology under conditions designed to minimise the degree of microbial contamination.

Prior to release the following tests are carried out on each batch of the final bulk product, unless exemption has been granted by the competent authority.

Host-cell-derived proteins

The limit is approved by the competent authority.

Single-chain precursor

The limit is approved by the competent authority. Use a suitably sensitive method.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in ethanol (96 per cent), in methanol and in aqueous solutions with a pH around 5.1. In aqueous solutions below pH 3.5 or above pH 6.5, the solubility is greater than or equal to 25 mg/ml.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. Peptide mapping *(2.2.55)*.

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a 2.0 mg/ml solution of the substance to be examined in *0.01 M hydrochloric acid* and transfer 25 µl of this solution to a clean tube. Add 100 µl of *HEPES buffer solution pH 7.5 R* and 20 µl of a 1 mg/ml solution of *Staphylococcus aureus strain V8 protease R.* Cap the tube and incubate at 25 °C for 6 h. Stop the reaction by adding 145 µl of *sulphate buffer solution pH 2.0 R*.

Reference solution Prepare at the same time and in the same manner as for the test solution, but using *insulin aspart CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION

Liquid chromatography *(2.2.29)*.

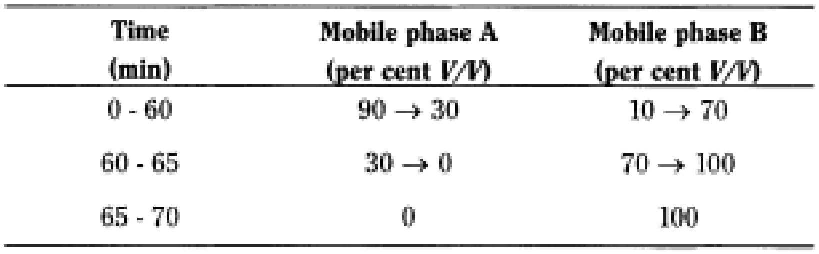
Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm,
- *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm) with a pore size of 8 nm,
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A:* mix 100 ml of *acetonitrile for chromatography R,* 200 ml of *sulphate buffer solution pH 2.0 R* and 700 ml of *water R;* filter and degas;

— *mobile phase B*: mix 200 ml of *sulphate buffer solution pH 2.0 R*, 400 ml of *acetonitrile for chromatography R* and 400 ml of *water R*; filter and degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	90 → 30	10 → 70
60 - 65	30 → 0	70 → 100
65 - 70	0	100

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.

Injection 50 µl.

System suitability:

— the chromatograms obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of insulin aspart digest supplied with *insulin aspart CRS*,

— in the chromatogram obtained with the reference solution, identify the peaks due to digest fragments I, II and III:

Symmetry factor Maximum 1.5, for the peaks due to fragments II and III,

Resolution Minimum 8.0, between the peaks due to fragments II and III.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

NOTE: the retention times of fragments I, II and IV are the same as for human insulin. The retention time of fragment III differs from human insulin due to substitution of proline by aspartic acid.

TESTS

Impurities with molecular masses greater than that of insulin aspart

Size-exclusion chromatography *(2.2.30)*: use the normalisation procedure.

Test solution Prepare a solution containing 4 mg/ml of the substance to be examined in *0.01 M hydrochloric acid*. Maintain the solution at 2-8 °C and use within 48 h.

Resolution solution Use a solution of insulin (about 4 mg/ml), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid* may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about 10 days. Maintain the solution at 2-8 °C and use within 7 days.

Column:

- *size*: *l* = 0.3 m, Ø = 7.8 mm,
- *stationary phase*: *hydrophilic silica gel for chromatography R* (5-10 µm) with a pore size of 12-12.5 nm, of a grade suitable for the separation of insulin monomer from dimer and polymers.

Mobile phase Mix 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile for chromatography R* and 65 volumes of a 1.0 g/l solution of *arginine R*; filter and degas.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 276 nm.

Equilibration At least 3 injections of the resolution solution; the column is equilibrated when repeatable results are obtained from 2 subsequent injections.

Injection 100 µl.

Run time About 35 min.

Retention time Insulin aspart polymers = 13-17 min; insulin aspart dimer = about 17.5 min; insulin aspart monomer = about 20 min; salts = about 22 min.

System suitability Resolution solution:

- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limits The sum of the areas of the peaks with a retention time less than that of the principal peak is not more than 0.5 per cent of the total area of the peaks. Disregard any peak with a retention time greater than that of the peak due to insulin aspart monomer.

Related proteins

Liquid chromatography *(2.2.29)* as described under Assay: use the normalisation procedure.

Limits:

- *B28isoAsp insulin aspart*: maximum 1.0 per cent,
- *total of the peaks due to A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart*: maximum 2.0 per cent,
- *total of other impurities*: maximum 1.5 per cent.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 0.200 g by drying in an oven at 105 °C for 24 h.

Sulphated ash *(2.4.14)*

Maximum 6.0 per cent, determined on 0.200 g (dried substance).

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Dissolve the substance to be examined in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml. Maintain the solution at 2-8 °C and use within 24 h.

Reference solution Dissolve the contents of a vial of *insulin aspart CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml. Maintain the solution at 2-8 °C and use within 48 h.

Resolution solution Use an appropriate solution with a content of B3Asp insulin aspart and A21Asp insulin aspart of not less than 1 per cent. This may be achieved by storing reference solution at room temperature for about 1-3 days. Maintain the solution at 2-8 °C and use within 72 h.

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm,

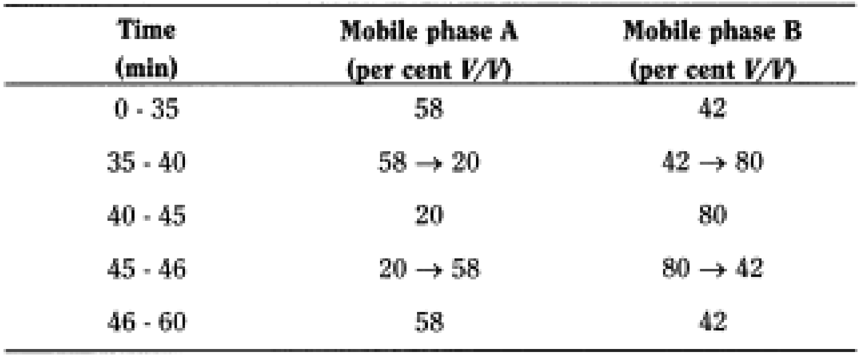
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: dissolve 142.0 g of *anhydrous sodium sulphate R* in *water R*; add 13.5 ml of *phosphoric acid R* and dilute to 5000 ml with *water R*; adjust to pH 3.6, if necessary, with *strong sodium hydroxide solution R*; filter and degas; mix 9 volumes of the solution with 1 volume of *acetonitrile for chromatography R*; filter and degas;

— *mobile phase B*: mix equal volumes of *water R* and *acetonitrile for chromatography R*; filter and degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	58	42
35 - 40	58 → 20	42 → 80
40 - 45	20	80
45 - 46	20 → 58	80 → 42
46 - 60	58	42

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 10 µl.

Relative retention with reference to insulin aspart (retention time = 20-24 min): B28isoAsp insulin aspart = about 0.9; B3Asp insulin aspart plus A21Asp insulin aspart (generally coeluted) = about 1.3; B3isoAsp insulin aspart = about 1.5.

System suitability Resolution solution:

— *resolution*: minimum 2.0 between the peak due to insulin aspart and the peak due to A21Asp insulin aspart and to B3Asp insulin aspart.

Calculate the content of insulin aspart $C_{256}H_{381}N_{65}O_{79}S_6$, plus B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart using the areas of the corresponding peaks in the chromatograms obtained with the test solution and reference solution and the declared content of insulin aspart plus B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart in *insulin aspart CRS*.

STORAGE

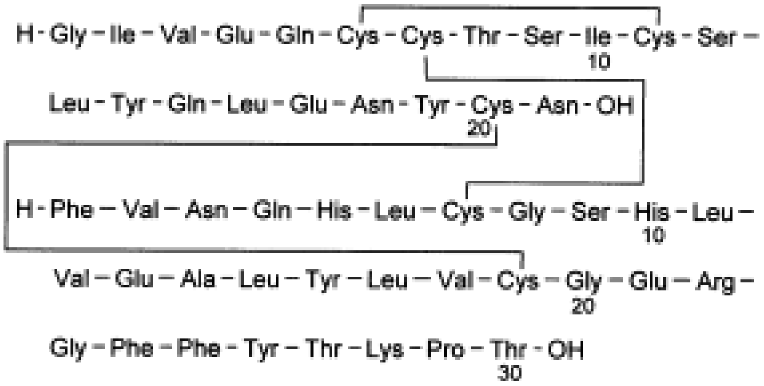
In an airtight container, protected from light, at or below - 18 °C until released by the manufacturer. When thawed, insulin aspart is stored at 5 ± 3 °C and used for manufacturing preparations within a short period of time. To avoid absorption of humidity from the air during weighing, insulin aspart must be at room temperature before opening the container.

Ph Eur

Insulin Lispro

General Notices

(Ph Eur monograph 2085)



$C_{257}H_{383}N_{65}O_{77}S_6$ 5808 *133107-64-9*

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

DEFINITION

28^B-L-Lysine-29^B-L-proline insulin (human).

Insulin lispro is a 2-chain peptide containing 51 amino acids. The A-chain is composed of 21 amino acids and the B-chain is composed of 30 amino acids. It is identical in primary structure to human insulin, only differing in amino acid sequence at positions 28 and 29 of the B-chain. Human insulin is Pro(B28), Lys(B29), whereas insulin lispro is Lys(B28), Pro(B29). As in human insulin, insulin lispro contains 2 interchain disulphide bonds and 1 intrachain disulphide bond.

Content

94.0 per cent to 104.0 per cent (dried substance).

By convention, for the purpose of labelling insulin lispro preparations, 0.0347 mg of insulin lispro is equivalent to 1 unit.

PRODUCTION

Insulin lispro is produced by a method based on recombinant DNA (rDNA) technology under conditions designed to minimise the degree of microbial contamination.

Prior to release the following tests are carried out on each batch of final bulk product, unless exemption has been granted by the competent authority.

Host-cell-derived proteins

The limit is approved by the competent authority.

Single-chain precursor

The limit is approved by the competent authority. Use a suitably sensitive method.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute mineral acids and with decomposition in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. Peptide mapping *(2.2.55)*.

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a 2.0 mg/ml solution of the substance to be examined in *0.01 M hydrochloric acid* and transfer 500 µl of this solution to a clean tube. Add 2.0 ml of *HEPES buffer solution pH 7.5 R* and 400 µl of a 1 mg/ml solution of *Staphylococcus aureus strain V8 protease R*. Cap the tube and incubate at 25 °C for 6 h. Stop the reaction by adding 2.9 ml of *sulphate buffer solution pH 2.0 R*.

Reference solution Prepare at the same time and in the same manner as for the test solution but using *insulin lispro CRS* instead of the substance to be examined.

CHROMATOGRAPIC SEPARATION

Liquid chromatography *(2.2.29)*.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm,

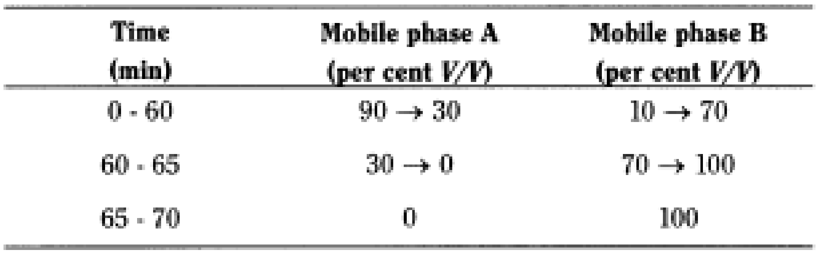
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm) with a pore size of 8 nm,

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: mix 100 ml of *acetonitrile for chromatography R*, 200 ml of *sulphate buffer solution pH 2.0 R* and 700 ml of *water R*; filter and degas;

— *mobile phase B*: mix 200 ml of *sulphate buffer solution pH 2.0 R*, 400 ml of *acetonitrile for chromatography R* and 400 ml of *water R*; filter and degas;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 60	90 → 30	10 → 70
60 - 65	30 → 0	70 → 100
65 - 70	0	100

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.

Injection 50 µl.

System suitability:

— the chromatograms obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of insulin lispro digest supplied with *insulin lispro CRS,*

— in the chromatogram obtained with the reference solution, identify the peaks due to digest fragments I, II and III

Symmetry factor Maximum 1.5 for the peaks due to fragments II and III,

Resolution Minimum 8.0 between the peaks due to fragments II and III.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

NOTE: the retention times of fragments I, II and IV are the same as for human insulin. The retention time of fragment III differs from human insulin due to differences in sequence at positions 28 and 29 of the B-chain.

TESTS

Impurities with molecular masses greater than that of insulin lispro

Size-exclusion chromatography *(2.2.30)*: use the normalisation procedure.

Test solution Prepare a solution containing 4 mg/ml of the substance to be examined in *0.01 M hydrochloric acid*. Maintain the solution at 2-8 °C and use within 48 h.

Resolution solution Use a solution of insulin (about 4 mg/ml), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about 10 days. Maintain the solution at 2-8 °C and use within 8 days.

Column:

— *size*: *l* = 0.30 m, Ø = 7.8 mm,

- *stationary phase*: *hydrophilic silica gel for chromatography R* (5-10 µm) with a pore size of 12-12.5 nm, of a grade suitable for the separation of insulin monomer from dimer and polymers.

Mobile phase Mix 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile for chromatography R* and 65 volumes of a 1.0 g/l *solution of arginine R*; filter and degas.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 276 nm.

Equilibration At least 3 injections of the resolution solution; the column is equilibrated when repeatable results are obtained for 2 subsequent injections.

Injection 100 µl.

Run time About 35 min.

Retention time Insulin lispro polymers = 13-17 min; insulin lispro dimer = about 17.5 min; insulin lispro monomer = about 20 min; salts = about 22 min.

System suitability Resolution solution:

- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer,
- *symmetry factor*: maximum 2.0 for the peak due to insulin lispro.

Limits The sum of the areas of the peaks with a retention time less than that of the principal peak is not more than 0.25 per cent of the total area of the peaks. Disregard any peak with a retention time greater than that of the peak due to insulin lispro monomer.

Related proteins

Liquid chromatography *(2.2.29)*: use the normalisation procedure.

Test solution Dissolve 3.5 mg of the substance to be examined in 1.0 ml of *0.01 M hydrochloric acid*. Maintain the solution at 2-8 °C and use within 56 h.

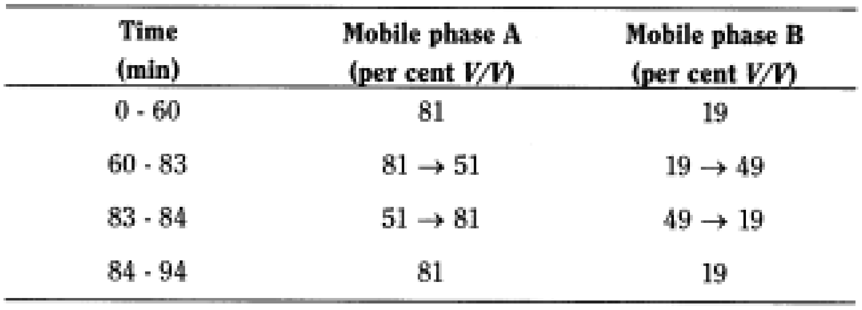
Resolution solution Dissolve 3.5 mg of the substance to be examined in 1.0 ml of *0.01 M hydrochloric acid*. Allow to stand at room temperature to obtain a solution containing between 0.8 per cent and 11 per cent of A21 desamido insulin lispro.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm,
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: mix 82 volumes of a 28.4 g/l solution of *anhydrous sodium sulphate R* adjusted to pH 2.3 with *phosphoric acid R* and 18 volumes of *acetonitrile for chromatography R*; filter and degas;
- *mobile phase B*: mix equal volumes of a 28.4 g/l solution of *anhydrous sodium sulphate R* adjusted to pH 2.3 with *phosphoric acid R* and *acetonitrile for chromatography R*; filter and degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	81	19
60 - 83	81 → 51	19 → 49
83 - 84	51 → 81	49 → 19
84 - 94	81	19

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl.

Retention time Adjust the mobile phase composition to obtain a retention time of about 41 min for insulin lispro; A21 desamido insulin lispro elutes near the start of the gradient elution.

System suitability Resolution solution:

— *resolution*: minimum 1.5 between the 1st peak (insulin lispro) and the 2nd peak (A21 desamido insulin lispro),

— *symmetry factor*: maximum 2.0 for the peak due to insulin lispro.

Limits:

— *A21 desamido insulin lispro*: maximum 1.0 per cent,

— *any other impurity*: maximum 0.50 per cent,

— *total (excluding A21)*: maximum 2.0 per cent.

Zinc

Maximum 1.0 per cent (dried substance).

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve at least 50 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25 ml with the same acid. Dilute if necessary to a suitable concentration (for example 0.4-0.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions Use solutions of concentrations which bracket the expected zinc concentration of the samples, for example, 0.2-0.8 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/ml Zn) R* with *0.01 M hydrochloric acid*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame of suitable composition (for example, 11 litres of air and 2 litres of acetylene per minute).

Loss on drying ***(2.2.32)***

Maximum 10.0 per cent, determined on 0.200 g by drying in an oven at 105 °C for 16 h.

Sulphated ash ***(2.4.14)***

Maximum 2.5 per cent, determined on 0.200 g (dried substance).

Bacterial endotoxins *(2.6.14, Method D)*

Less than 10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve the substance to be examined in *0.01 M hydrochloric acid* to obtain a concentration of 0.8 mg/ml. Maintain the solution at 2-8 °C and use within 48 h.

Reference solution Dissolve the contents of a vial *of insulin lispro CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 0.8 mg/ml. Maintain the solution at 2-8 °C and use within 48 h.

Resolution solution Dissolve about 10 mg of the substance to be examined in 10 ml of *0.01 M hydrochloric acid*. Allow to stand at room temperature to obtain a solution containing between 0.8 per cent and 11 per cent of A21 desamido insulin lispro. Maintain the solution at 2-8 °C and use within 14 days.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm) with a pore size of 8 nm,

— *temperature*: 40 °C.

Mobile phase Mix 745 volumes of a 28.4 g/l solution of *anhydrous sodium sulphate R* adjusted to pH 2.3 with *phosphoric acid R* and 255 volumes of *acetonitrile for chromatography R;* filter and degas.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl.

Retention time Insulin lispro = about 24 min.

System suitability:

— *resolution*: minimum 1.8 between the 1st peak (insulin lispro) and the 2nd peak (A21 desamido insulin lispro), in the chromatogram obtained with the resolution solution,

— *repeatability*: maximum relative standard deviation of 1.1 per cent after 3 injections of the reference solution.

Calculate the content of insulin lispro $C_{257}H_{383}N_{65}O_{77}S_6$ using the chromatograms obtained with the test solution and the reference solution and the declared content of $C_{257}H_{383}N_{65}O_{77}S_6$ in *insulin lispro CRS*.

STORAGE

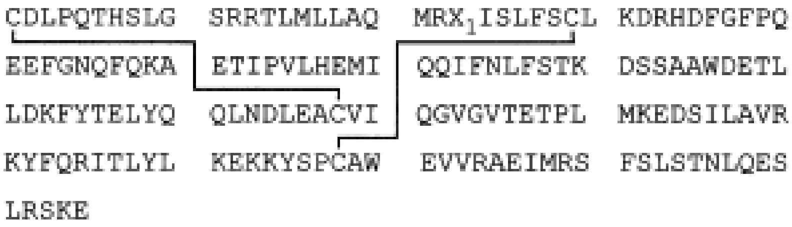
In an airtight container, protected from light, at or below - 18 °C. When thawed, insulin lispro is stored and weighed under conditions defined by the manufacturer to maintain the quality attributes of the drug substance and is used for manufacturing preparations within a short period of time. To avoid absorption of humidity from the air during weighing, insulin lispro must be at room temperature before opening the container.

Ph Eur

Interferon Alfa-2 Concentrated Solution

General Notices

(Ph Eur monograph 1110)



CDLPQTHSLG SRRTLMLLAQ MRX_1ISLFSCL KDRHDFGFPQ
EEFGNQFQKA ETIPVLHEMI QQIFNLFSTK DSSAAWDETL
LDKFYTELYQ QLNDLEACVI QGVGVTETPL MKEDSILAVR
KYFQRITLYL KEKKYSPCAW EVVRAEIMRS FSLSTNLQES
LRSKE

alfa-2a: $C_{860}H_{1353}N_{227}O_{255}S_9$ 19,241 *76543-85-9*

alfa-2b: $C_{860}H_{1353}N_{229}O_{255}S_9$ 19,269 *99210-65-8*

Action and use

Cytokine.

Ph Eur

DEFINITION

Interferon alfa-2 concentrated solution is a solution of a protein that is produced according to the information coded by the alfa-2 sub-species of interferon alfa gene and that exerts non-specific antiviral activity, at least in homologous cells, through cellular metabolic processes involving synthesis of both ribonucleic acid and protein. Interferon alfa-2 concentrated solution also exerts antiproliferative activity. Different types of alfa-2 interferon, varying in the amino acid residue at position 23, are designated by a letter in lower case.

Designation	Residue at position 23 (X_1)
alfa-2a	Lys
alfa-2b	Arg

This monograph applies to interferon alfa-2a and -2b concentrated solutions.

The potency of interferon alfa-2 concentrated solution is not less than 1.4 × 10^8 IU per milligram of protein. Interferon alfa-2 concentrated solution contains not less than 2 × 10^8 IU of interferon alfa-2 per millilitre.

PRODUCTION

Interferon alfa-2 concentrated solution is produced by a method based on recombinant DNA (rDNA) technology using bacteria as host cells. It is produced under conditions designed to minimise microbial contamination of the product.

Interferon alfa-2 concentrated solution complies with the following additional requirements.

Host-cell-derived proteins

The limit is approved by the competent authority.

Host-cell- or vector-derived DNA

The limit is approved by the competent authority.

CHARACTERS

A clear, colourless or slightly yellowish liquid.

IDENTIFICATION

A. It shows the expected biological activity (see Assay).

B. Examine by isoelectric focusing.

Test solution Dilute the preparation to be examined with *water R* to a protein concentration of 1 mg/ml.

Reference solution Prepare a 1 mg/ml solution of the appropriate *interferon alfa-2 CRS* in *water R*.

Isoelectric point calibration solution pI range 3.0 to 10.0. Prepare and use according to the manufacturer's instructions.

Use a suitable apparatus connected with a recirculating temperature controlled water-bath set at 10 °C and gels for isoelectric focusing with a pH gradient from 3.5 to 9.5. Operate the apparatus in accordance with the manufacturer's instructions. Use as the anode solution *phosphoric acid R* (98 g/l H_3PO_4) and as the cathode solution *1 M sodium hydroxide*. Samples are applied to the gel by filter papers. Place sample application filters on the gel close to the cathode.

Apply 15 µl of the test solution and 15 µl of the reference solution. Start the isoelectric focusing at 1500 V and 50 mA. Turn off the power after 30 min, remove the application filters and reconnect the power supply for 1 h. Keep the power constant during the focusing process. After focusing, immerse the gel in a suitable volume of a solution containing 115 g/l of *trichloroacetic acid R* and 34.5 g/l of *sulphosalicylic acid R* in *water R* and agitate the container gently for 60 min. Transfer the gel to a mixture of 32 volumes of *glacial acetic acid R*, 100 volumes of *anhydrous ethanol R* and 268 volumes of *water R*, and soak for 5 min. Immerse the gel for 10 min in a staining solution prewarmed to 60 °C in which 1.2 g/l of *acid blue 83 R* has been added to the previous mixture of glacial acetic acid, ethanol and water. Wash the gel in several containers with the previous mixture of glacial acetic acid, ethanol and water and keep the gel in this mixture until the background is clear (12 h to 24 h). After adequate destaining, soak the gel for 1 h in a 10 per cent *V/V* solution of *glycerol R* in the previous mixture of glacial acetic acid, ethanol and water.

The principal bands of the electropherogram obtained with the test solution correspond in position to the principal bands of the electropherogram obtained with the reference solution. Plot the migration distances of the isoelectric point markers versus their isoelectric points and determine the isoelectric points of the principal components of the test solution and the reference solution. They do not differ by more than 0.2 pI units. The test is not valid unless the isoelectric point markers are distributed along the entire length of the gel and the isoelectric points of the principal bands in the electropherogram obtained with the reference solution are between 5.8 and 6.3.

C. Examine the electropherograms obtained under reducing conditions in the test for impurities of molecular masses differing from that of interferon alfa-2. The principal band in the electropherogram obtained with test solution (a) corresponds in position to the principal band in the electropherogram obtained with reference solution (a).

D. Examine by peptide mapping.

Test solution Dilute the preparation to be examined in *water R* to a protein concentration of 1.5 mg/ml. Transfer 25 µl to a polypropylene or glass tube of 1.5 ml capacity. Add 1.6 µl of *1 M phosphate buffer solution pH 8.0 R*, 2.8 µl of a freshly prepared 1.0 mg/ml solution of *trypsin for peptide mapping R* in *water R* and 3.6 µl of *water R* and mix vigorously. Cap the tube and place it in a water-bath at 37 °C for 18 h, then add 100 µl of a 573 g/l solution of *guanidine hydrochloride R* and mix well. Add 7 µl of 154.2 g/l solution of *dithiothreitol R* and mix well. Place the capped tube in boiling water for 1 min. Cool to room temperature.

Reference solution Prepare at the same time and in the same manner as for the test solution but use a 1.5 mg/ml solution of the appropriate *interferon alfa-2 CRS* in *water R*.

Examine by liquid chromatography *(2.2.29)*.

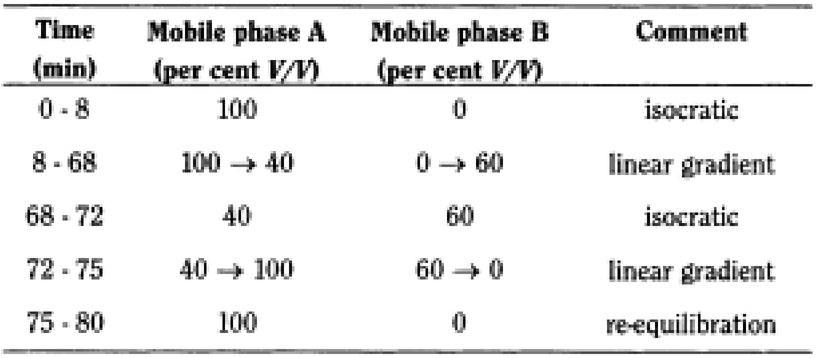
The chromatographic procedure may be carried out using:

— a stainless steel column 0.10 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm,

— as mobile phase at a flow rate of 1.0 ml/min:

Mobile phase A Dilute 1 ml of *trifluoroacetic acid R* to 1000 ml with *water R*,

Mobile phase B To 100 ml of *water R* add 1 ml of *trifluoroacetic acid R* and dilute to 1000 ml with *acetonitrile for chromatography R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 8	100	0	isocratic
8 - 68	100 → 40	0 → 60	linear gradient
68 - 72	40	60	isocratic
72 - 75	40 → 100	60 → 0	linear gradient
75 - 80	100	0	re-equilibration

— as detector a spectrophotometer set at 214 nm,

maintaining the temperature of the column at 30 °C.

Equilibrate the column with mobile phase A for at least 15 min.

Inject 100 µl of the test solution and 100 µl of the reference solution. The test is not valid unless the chromatogram obtained with each solution is qualitatively similar to the chromatogram of interferon alfa-2 digest supplied with the appropriate *interferon alfa-2 CRS*. The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

TESTS

Impurities of molecular masses differing from that of interferon alfa-2

Examine by SDS polyacrylamide gel electrophoresis *(2.2.31)*. The test is performed under both reducing and non-reducing conditions, using resolving gels of 14 per cent acrylamide and silver staining as the detection method.

Sample buffer (non-reducing conditions) Mix equal volumes of *water R* and *concentrated SDS PAGE sample buffer R*.

Sample buffer (reducing conditions) Mix equal volumes of *water R* and *concentrated SDS PAGE sample buffer for reducing conditions R* containing 2-mercaptoethanol as the reducing agent.

Test solution (a) Dilute the preparation to be examined in sample buffer to a protein concentration of 0.5 mg/ml.

Test solution (b) Dilute 0.20 ml of test solution (a) to 1 ml with sample buffer.

Reference solution (a) Prepare a 0.625 mg/ml solution of the appropriate *interferon alfa-2 CRS* in sample buffer.

Reference solution (b) Dilute 0.20 ml of reference solution (a) to 1 ml with sample buffer.

Reference solution (c) Dilute 0.20 ml of reference solution (b) to 1 ml with sample buffer.

Reference solution (d) Dilute 0.20 ml of reference solution (c) to 1 ml with sample buffer.

Reference solution (e) Dilute 0.20 ml of reference solution (d) to 1 ml with sample buffer.

Reference solution (f) Use a solution of molecular mass standards suitable for calibrating SDS-PAGE gels in the range 15 kDa to 67 kDa.

Place test and reference solutions, contained in covered test-tubes, on a water-bath for 2 min.

Apply 10 µl of reference solution (f) and 50 µl of each of the other solutions to the stacking gel wells. Perform the electrophoresis under the conditions recommended by the manufacturer of the equipment. Detect proteins in the gel by silver staining.

The test is not valid unless: the validation criteria are met *(2.2.31)*; a band is seen in the electropherogram obtained with reference solution (e); and a gradation of intensity of staining is seen in the electropherograms obtained, respectively, with test solution (a) and test solution (b) and with reference solutions (a) to (e).

The electropherogram obtained with test solution (a) under reducing conditions may show, in addition to the principal band, less intense bands with molecular masses lower than the principal band. No such band is more intense than the principal band in the electropherogram obtained with reference solution (d) (1.0 per cent) and not more than 3 such bands are more intense than the principal band in the electropherogram obtained with reference solution (e) (0.2 per cent).

The electropherogram obtained with test solution (a) under non-reducing conditions may show, in addition to the principal band, less intense bands with molecular masses higher than the principal band. No such band is more intense than the principal band in the electropherogram obtained with reference solution (d) (1.0 per cent) and not more than 3 such bands are more intense than the principal band in the electropherogram obtained with reference solution (e) (0.2 per cent).

Related proteins

Examine by liquid chromatography *(2.2.29)*.

Test solution Dilute the preparation to be examined with *water R* to a protein concentration of 1 mg/ml.

0.25 per cent m/m hydrogen peroxide solution. Dilute *dilute hydrogen peroxide solution R* in *water R* in order to obtain a 0.25 per cent m/m solution.

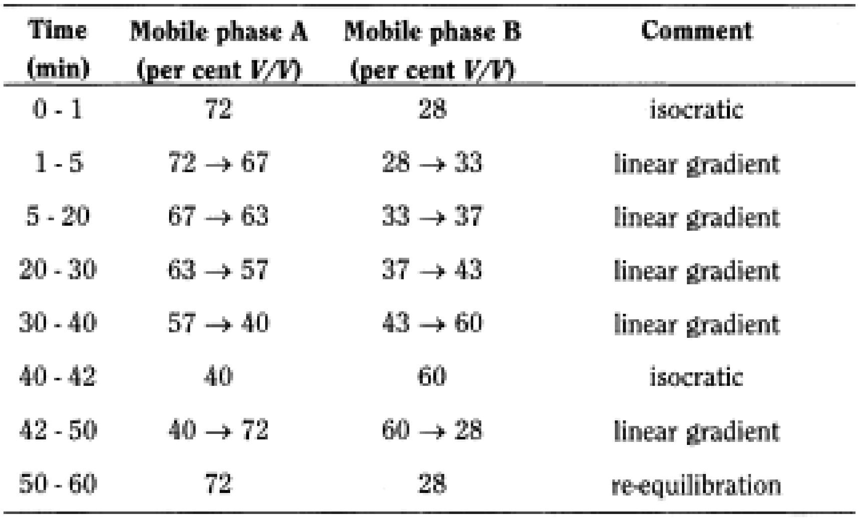
Reference solution To a volume of the test solution, add a suitable volume of 0.25 per cent *m/m* hydrogen peroxide solution to give a final hydrogen peroxide concentration of 0.005 per cent *m/m*, and allow to stand at room temperature for 1 h, or for the length of time that will generate about 5 per cent oxidised interferon. Add 12.5 mg of *L-methionine R* per millilitre of solution. Allow to stand at room temperature for 1 h. Store the solutions for not longer than 24 h at a temperature of 2-8 °C.

The chromatographic procedure may be carried out using:

- — a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm,
- — as mobile phase at a flow rate of 1.0 ml/min:

Mobile phase A To 700 ml of *water R* add 2 ml of *trifluoroacetic acid R* and 300 ml of *acetonitrile for chromatography R,*

Mobile phase B To 200 ml of *water R* add 2 ml of *trifluoroacetic acid R* and 800 ml of *acetonitrile for chromatography R,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 1	72	28	isocratic
1 - 5	72 → 67	28 → 33	linear gradient
5 - 20	67 → 63	33 → 37	linear gradient
20 - 30	63 → 57	37 → 43	linear gradient
30 - 40	57 → 40	43 → 60	linear gradient
40 - 42	40	60	isocratic
42 - 50	40 → 72	60 → 28	linear gradient
50 - 60	72	28	re-equilibration

- — as detector a spectrophotometer set at 210 nm.

Equilibrate the column with the mobile phases in the initial gradient ratio for at least 15 min. Inject 50 µl of each solution.

In the chromatograms obtained, interferon alfa-2 elutes at a retention time of about 20 min. In the chromatogram obtained with the reference solution a peak related to oxidised interferon appears at a retention time of about 0.9 relative to the principal peak. The test is not valid unless the resolution between the peaks corresponding to oxidised interferon and interferon is at least 1.0. Consider only the peaks whose retention time is 0.7 to 1.4 relative to that of the principal peak. In the chromatogram obtained with the test solution, the area of any peak, apart from the principal peak, is not greater than 3.0 per cent of the total area of all of the peaks. The sum of the areas of any peaks other than the principal peak is not greater than 5.0 per cent of the total area of all of the peaks.

Bacterial endotoxins *(2.6.14)*

Less than 100 IU in the volume that contains 1.0 mg of protein.

ASSAY

Protein

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of about 0.5 mg/ml of interferon alfa-2.

Reference solutions Prepare a stock solution of 0.5 mg/ml of *bovine albumin R*. Prepare 8 dilutions of the stock solution containing between 3 µg/ml and 30 µg/ml of *bovine albumin R*.

Prepare 30-fold and 50-fold dilutions of the test solution. Add 1.25 ml of a mixture prepared the same day by combining 2.0 ml of a 20 g/l solution of *copper sulphate R* in *water R*, 2.0 ml of a 40 g/l solution of *sodium tartrate R* in *water R* and 96.0 ml of a 40 g/l solution of *sodium carbonate R* in *0.2 M sodium hydroxide* to test-tubes containing 1.5 ml of *water R* (blank), 1.5 ml of the different dilutions of the test solution or 1.5 ml of the reference solutions. Mix after each addition. After approximately 10 min, add to each test-tube 0.25 ml of a mixture of equal volumes of *water R* and *phosphomolybdotungstic reagent R.* Mix after each addition. After approximately 30 min, measure the absorbance *(2.2.25)* of each solution at 750 nm using the blank as the compensation liquid. Draw a calibration curve from the absorbances of the 8 reference solutions and the corresponding protein contents and read from the curve the content of protein in the test solution.

Potency

The potency of interferon alfa-2 is estimated by comparing its effect to protect cells against a viral cytopathic effect with the same effect of the appropriate International Standard of human recombinant interferon alfa-2 or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Carry out the assay by a suitable method, based on the following design.

Use, in standard culture conditions, an established cell line sensitive to the cytopathic effect of a suitable virus (a human diploid fibroblast cell line, free of microbial contamination, responsive to interferon and sensitive to encephalomyocarditis virus, is suitable).

The following cell cultures and virus have been shown to be suitable: MDBK cells (ATCC No. CCL22), or Mouse L cells (NCTC clone 929; ATCC No. CCL 1) as the cell culture and vesicular stomatitis virus VSV, Indiana strain (ATCC No. VR-158) as the infective agent; or A-549 cells (ATCC No. CCL-185) responsive to interferon as the cell culture, and encephalomyocarditis virus (ATCC No. VR-129B) as the infective agent.

Incubate in at least 4 series, cells with 3 or more different concentrations of the preparation to be examined and the reference preparation in a microtitre plate and include in each series appropriate controls of untreated cells. Choose the concentrations of the preparations such that the lowest concentration produces some protection and the largest concentration produces less than maximal protection against the viral cytopathic effect. Add at a suitable time the cytopathic virus to all wells with the exception of a sufficient number of wells in all series, which are left with uninfected control cells. Determine the cytopathic effect of virus quantitatively with a suitable method. Calculate the potency of the preparation to be examined by the usual statistical methods for a parallel line assay.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits of the estimated potency ($P = 0.95$) are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE

Store in an airtight container, protected from light, at or below - 20 °C.

LABELLING

The label states:

— the type of interferon (alfa-2a or alfa-2b),

— the type of production.

Ph Eur

Interferon Gamma-1b Concentrated Solution

General Notices

(Ph Eur monograph 1440)

$C_{734}H_{1166}N_{204}O_{216}S_{5}$ 16,465 *98059-61-1*

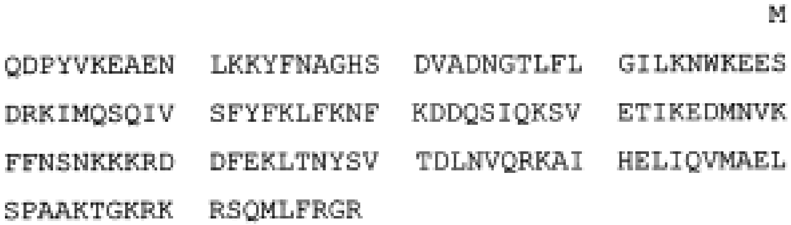
Action and use

Cytokine.

Ph Eur

DEFINITION

Interferon gamma-1b concentrated solution is a solution of the *N*-terminal methionyl form of interferon gamma, a protein which is produced and secreted by human antigen-stimulated T lymphocytes in response to viral infections and various other inducers. It has specific immunomodulatory properties, such as potent phagocyte-activating effects. The protein consists of non-covalent dimers of two identical monomers. The formula of the monomer is as follows:



```
                                              M
QDPYVKEAEN  LKKYFNAGHS  DVADNGTLFL  GILKNWKEES
DRKIMQSQIV  SFYFKLFKNF  KDDQSIQKSV  ETIKEDMNVK
FFNSNKKKRD  DFEKLTNYSV  TDLNVQRKAI  HELIQVMAEL
SPAAKTGKRK  RSQMLFRGR
```

The potency of interferon gamma-1b is not less than 20 × 10^6 IU per milligram of protein. Interferon gamma-1b concentrated solution contains not less than 30 × 10^6 IU of interferon gamma-1b per millilitre.

PRODUCTION

Interferon gamma-1b concentrated solution is produced by a method based on recombinant DNA technology, using bacteria as host-cells. It is produced under conditions designed to minimise microbial contamination.

Interferon gamma-1b concentrated solution complies with the following additional requirements.

Host-cell derived proteins

The limit is approved by the competent authority.

Host-cell- and vector-derived DNA

The limit is approved by the competent authority.

CHARACTERS

A clear, colourless or slightly yellowish liquid.

IDENTIFICATION

A. It shows the expected biological activity when tested as prescribed in the assay.

B. Examine the electropherograms obtained in the test for impurities of molecular masses differing from that of interferon gamma-1b. The principal bands in the electropherogram obtained with the test solution correspond in position to the principal bands in the electropherogram obtained with reference solution (a).

C. Examine by peptide mapping.

Solution A Prepare a solution containing 1.2 g/l of *tris(hydroxymethyl)aminomethane R*, 8.2 g/l of *anhydrous sodium acetate R*, 0.02 g/l of *calcium chloride R* and adjust to pH 8.3 (*2.2.3*) with *dilute acetic acid R*. Add *polysorbate 20 R* to a concentration of 0.1 per cent *V/V*.

Test solution Desalt a volume of the preparation to be examined containing 1 mg of protein by a suitable procedure. For example, filter in a microcentrifuge tube and reconstitute with 500 µl of solution A. Add 10 µl of a freshly prepared 1 mg/ml solution of *trypsin for peptide mapping R* in *water R* and mix gently by inversion. Incubate at 30 °C to 37 °C for 24 h, add 100 µl of *phosphoric acid R* per millilitre of digested sample and mix by inversion.

Reference solution Dilute *interferon gamma-1b CRS* in *water R* to obtain a concentration of 1 mg/ml. Prepare as for the test solution, ensuring that all procedures are carried out simultaneously and under identical conditions.

Examine by liquid chromatography *(2.2.29)*.

The chromatographic procedure may be carried out using:

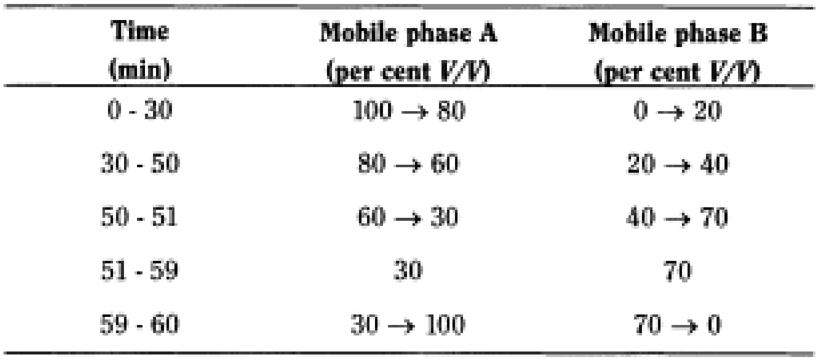
— a stainless steel column, 0.15 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (10 µm),

— as mobile phase at a flow rate of 1.0 ml/min:

Mobile phase A (0.05 M sodium phosphate buffer solution pH 3.3) Solution I: Dissolve 7.80 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000.0 ml with the same solvent. Solution II: dilute 0.33 ml of *phosphoric acid R* to 100.0 ml with *water R*. Mix 920 ml of solution I and 80 ml of solution II. Adjust the pH *(2.2.3)* if necessary,

Mobile phase B *Acetonitrile for chromatography R*,

With the following elution conditions (if necessary, the gradient may be modified to improve the separation of the digest):



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 30	100 → 80	0 → 20
30 - 50	80 → 60	20 → 40
50 - 51	60 → 30	40 → 70
51 - 59	30	70
59 - 60	30 → 100	70 → 0

— as detector a spectrophotometer set at 214 nm, maintaining the temperature of the column at 40 °C.

Equilibrate the column for at least 15 min at the initial elution composition. Carry out a blank run using the above-mentioned gradient.

Inject 100 µl of the test solution and 100 µl of the reference solution. The test is not valid unless the chromatogram obtained with each solution is qualitatively similar to the chromatogram of interferon gamma-1b digest supplied with *interferon gamma-1b CRS*. The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Examine by *N*-terminal sequence analysis.

Use an automated solid-phase sequencer, operated in accordance with the manufacturer's instructions.

Equilibrate by a suitable procedure the equivalent of 100 µg of interferon gamma-1b in a 10 g/l solution of *ammonium hydrogen carbonate R*, pH 9.0.

Identify the phenylthiohydantoin (PTH)-amino acids released at each sequencing cycle by reverse-phase liquid chromatography. The procedure may be carried out using the column and reagents recommended by the manufacturer of the sequencing equipment for the separation of PTH-amino acids.

The separation procedure is calibrated using:

— the mixture of PTH-amino acids provided by the manufacturer, with the gradient conditions adjusted as indicated to achieve optimum resolution of all amino acids,

— a sample from a blank sequencing cycle, obtained as recommended by the equipment manufacturer.

The first fifteen amino acids are:

Met-Gln-Asp-Pro-Tyr-Val-Lys-Glu-Ala-Glu-Asn-Leu-Lys-Lys-Tyr.

TESTS

Appearance

The preparation to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of the preparation to be examined is 4.5 to 5.5.

Covalent dimers and oligomers

Not greater than 2 per cent, determined by size-exclusion chromatography *(2.2.30)*.

Test solution Dilute the preparation to be examined with the mobile phase to a protein concentration of 0.1 mg/ml.

Reference solution (a) Dilute *interferon gamma-1b CRS* with the mobile phase to a protein concentration of 0.1 mg/ml.

Reference solution (b) Prepare a mixture of the following molecular mass standards: bovine albumin, ovalbumin, trypsinogen, lysozyme, at a concentration of 0.1 mg/ml to 0.2 mg/ml for each standard.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.3 m long and 7.8 mm in internal diameter packed with *hydrophilic silica gel for chromatography R*, of a grade suitable for fractionation of globular proteins in the molecular weight range of 10 000 to 500 000 (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture prepared as follows (0.2 M sodium phosphate buffer solution pH 6.8). Solution I: dissolve 31.2 g of *sodium dihydrogen phosphate R* and 1.0 g of *sodium dodecyl sulphate R* in *water R* and dilute to 1000.0 ml with the same solvent. Solution II: dissolve 28.4 g of *anhydrous disodium hydrogen phosphate R* and 1.0 g of *sodium dodecyl sulphate R* in *water R* and dilute to 1000.0 ml with the same solvent. Mix 450 ml of solution I and 550 ml of solution II. Adjust the pH *(2.2.3)* if necessary,

— as detector a spectrophotometer set at 210 nm to 214 nm.

Inject 200 µl of each solution. The test is not valid unless: the molecular mass standards in reference solution (b) are well separated; the retention time of the principal peak in the chromatogram obtained with reference solution (a) is between the retention time of trypsinogen and lysozyme in the chromatogram obtained with reference solution (b).

Compare the chromatograms obtained with the test solution and with reference solution (a). There are no additional shoulders or peaks in the chromatogram obtained with the test solution compared with the chromatogram obtained with reference solution (a).

Calculate the percentage content of covalent dimers and oligomers.

Monomer and aggregates

Examine by size-exclusion chromatography *(2.2.30)*. The content of monomer and aggregates is not greater than 2 per cent.

Solution A Prepare a solution of the following composition: 0.59 g/l of *succinic acid R* and 40 g/l of *mannitol R*, adjusted to pH 5.0 *(2.2.3)* with *sodium hydroxide solution R*.

Test solution Dilute the preparation to be examined with solution A to a protein concentration of 1 mg/ml.

Reference solution Dilute *interferon gamma-1b CRS* with solution A to a protein concentration of 1 mg/ml.

Resolution solution Prepare 500 µl of a mixture consisting of 0.04 mg/ml of *bovine albumin R* and 0.2 mg/ml of *interferon gamma-1b CRS* in solution A. Use this solution within 24 h of preparation.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.3 m long and 7.8 mm in internal diameter packed with *hydrophilic silica gel for chromatography R*, of a grade suitable for fractionation of globular proteins in the molecular weight range of 10 000 - 300 000 (5 µm),

— as mobile phase at a flow rate of 0.8 ml/min a 89.5 g/l solution of *potassium chloride R* (1.2 M),

— as detector a spectrophotometer set at 214 nm.

Inject 20 µl of the resolution solution. In the chromatogram obtained, the retention time of the principal peak, corresponding to the native interferon gamma-1b dimer, is about 10 min. bovine albumin elutes at a relative retention time of about 0.85, relative to the main peak. The test is not valid unless the resolution between the peaks corresponding to bovine albumin and interferon gamma-1b is at least 1.5.

Inject 20 µl of the test solution and 20 µl of the reference solution. The chromatograms obtained show principal peaks with identical retention times. Calculate the percentage content of monomer and aggregates from the peak area of the monomer peak and of peaks which elute prior to the native interferon gamma-1b peak in the chromatogram obtained with the test solution, by the normalisation procedure, disregarding any peak due to the solvent.

Deamidated and oxidised forms and heterodimers

Examine by liquid chromatography *(2.2.29)*. The content of deamidated and oxidised forms is not greater than 10 per cent. The content of heterodimers is not greater than 3 per cent.

Test solution Dilute the preparation to be examined with *water R* to a protein concentration of 1 mg/ml.

Reference solution Dilute *interferon gamma-1b CRS* with *water R* to a protein concentration of 1 mg/ml.

Resolution solution Use *interferon gamma-1b validation solution CRS*.

The chromatographic procedure may be carried out using:

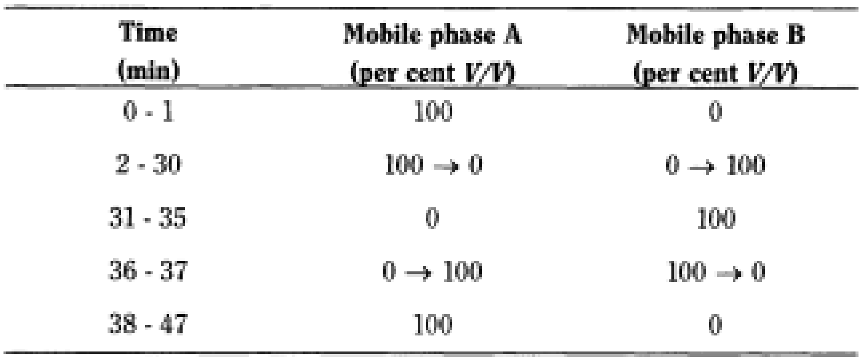
— a stainless steel column 0.075 m long and 7.5 mm in internal diameter packed with an appropriate hydrophilic polymethacrylate, strong cation exchange gel (10 µm, 100 nm),

— as mobile phase at a flow rate of 1.2 ml/min:

Mobile phase A (0.05 M ammonium acetate buffer pH 6.5) A 3.86 g/l solution of *ammonium acetate R*, adjusted to pH 6.5 with *dilute acetic acid R*,

Mobile phase B (1.2 M ammonium acetate buffer pH 6.5) A 92.5 g/l solution of *ammonium acetate R*, adjusted to pH 6.5 with *dilute acetic acid R,*

With the following elution conditions (if necessary, the slope of the gradient may be modified to improve the separation).



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 1	100	0
2 - 30	100 → 0	0 → 100
31 - 35	0	100
36 - 37	0 → 100	100 → 0
38 - 47	100	0

— as detector a spectrophotometer set at 280 nm,

maintaining the temperature of the column at 35 °C.

Inject 25 µl of the resolution solution. In the chromatogram obtained, the retention time of the principal peak is about 26 min. Deamidated and oxidised forms co-elute at a relative retention time of about 0.95, relative to the principal peak. The test is not valid unless the resolution, defined by the ratio of the height of the peak corresponding to the deamidated and oxidised forms to the height above the baseline of the valley separating the two peaks, is at least 1.2.

Inject 25 µl of the test solution and 25 µl of the reference solution. The chromatograms obtained show principal peaks with identical retention times. Calculate the percentage content of deamidated and oxidised interferon gamma-1b as a percentage of the area of the main peak. Heterodimers have relative retention times of 0.7 and 0.85 relative to the main peak. Calculate the percentage of heterodimers as a percentage of the sum of the areas of all peaks.

Impurities of molecular masses differing from that of interferon gamma-1b

Examine by polyacrylamide gel electrophoresis *(2.2.31)*. The test is performed under both reducing and non-reducing conditions, using resolving gels of 15 per cent acrylamide and silver staining as the detection method.

Sample buffer (non-reducing conditions) Dissolve 3.78 g of *tris(hydroxymethyl) aminomethane R*, 10.0 g of *sodium dodecyl sulphate R* and 0.100 g of *bromophenol blue R* in *water R.* Add 50.0 ml of *glycerol R* and dilute to 80 ml with *water R*. Adjust the pH *(2.2.3)* to 6.8 with *hydrochloric acid R* and dilute to 100 ml with *water R*.

Sample buffer (reducing conditions) Dissolve 3.78 g of *tris(hydroxymethyl)aminomethane R*, 10.0 g of *sodium dodecyl sulphate R* and 0.100 g of *bromophenol blue R* in *water R.* Add 50.0 ml of *glycerol R* and dilute to 80 ml with *water R.* Adjust the pH *(2.2.3)* to 6.8 with *hydrochloric acid R* and dilute to 100 ml with *water R*. Immediately before use, add *dithiothreitol R* to a final concentration of 250 mM.

Test solution Dilute the preparation to be examined in *water R* to a protein concentration of 1 mg/ml. Dilute 150 µl of the solution with 38 µl of sample buffer.

Reference solution (a) Prepare in the same manner as for the test solution, but using *interferon gamma-1b CRS* instead of the preparation to be examined.

Reference solution (b) (5 ng control) Mix 50 µl of a 0.01 mg/ml solution of *bovine albumin R* with 2000 µl of *water R* and 450 µl of sample buffer.

Reference solution (c) (2 ng control) Mix 20 µl of a 0.01 mg/ml solution of *bovine albumin R* with 2000 µl of *water R* and 450 µl of sample buffer.

Reference solution (d) Use a solution of molecular mass standards suitable for calibrating SDS-polyacrylamide gels in the range of 10 kDa to 70 kDa.

Leave each solution, contained in a test tube, at ambient temperature for 15 min, then store on ice.

Apply 25 µl of each solution to the stacking gel wells. Perform the electrophoresis under the conditions recommended by the manufacturer of the equipment. Detect proteins in the gel by silver staining.

The test is not valid unless: the validation criteria are met *(2.2.31)*; a band is seen in the electropherograms obtained with reference solutions (b) and (c).

The principal band in the electropherogram obtained with the test solution is similar in intensity to the principal band in the electropherogram obtained with reference solution (a). In the electropherogram obtained with the test solution, no significant bands are observed that are not present in the electropherogram obtained with reference solution (a) (0.01 per cent). A significant band is defined as any band whose intensity is greater than or equal to that of the band in the electropherogram obtained with reference solution (c).

Norleucine

Not more than 0.2 mole of norleucine per mole of interferon gamma-1b, determined by amino acid analysis.

Test solution Add 2.5 ml of the preparation to be examined onto a column suitable for the desalting of proteins previously equilibrated with 25 ml of a 10 per cent *V/V* solution of *acetic acid R*. Elute the sample with another 2.5 ml of a 10 per cent *V/V* solution of *acetic acid R*. Determine the protein content by measuring the absorbance of this solution as described under Protein, in the Assay section. Pipette a volume containing the equivalent of 100 µg of interferon gamma-1b into each of three reaction vials. Evaporate to dryness under reduced pressure.

Perform the hydrolysis of the three samples as follows. Add to each reaction vial 200 µl of a 50 per cent *V/V* solution of *hydrochloric acid R* containing 1 per cent *V/V* of *phenol R,* evacuate the samples, purge with nitrogen and hydrolyse in the gas phase. Heat the reaction vials at 110 °C for 22 h. After hydrolysis evaporate to dryness under reduced pressure.

Perform the derivatisation of the samples as follows. Prepare immediately before use a mixture consisting of two volumes of *ethanol R*, one volume of *water R* and one volume of *triethylamine R.* Add 50 µl of this solution to each reaction vial and shake lightly. Evaporate to dryness under reduced pressure. Add to each vial 50 µl of a mixture consisting of 7 volumes of *ethanol R*, one volume of *water R*, one volume of *triethylamine R* and one volume of *phenyl isothiocyanate R*. Shake lightly and allow to stand at room temperature for about 15 min. Evaporate to dryness under reduced pressure. Reconstitute the samples in 250 µl of mobile phase A.

Norleucine stock solution Prepare a 250 nmol/ml solution of *DL-norleucine R* in *0.01 M hydrochloric acid. This solution may be kept for two months at 4 ‘C.*

Leucine stock solution Prepare a 250 nmol/ml solution of *leucine R* in *0.01 M hydrochloric acid. This solution may be kept at 4 ‘C for two months.*

Reference solution Mix 10 µl of norleucine stock solution with 100 µl of leucine stock solution in each of the three reaction vials. Evaporate to dryness under reduced pressure. Perform the derivatisation of the samples as described for the preparation of the test solution.

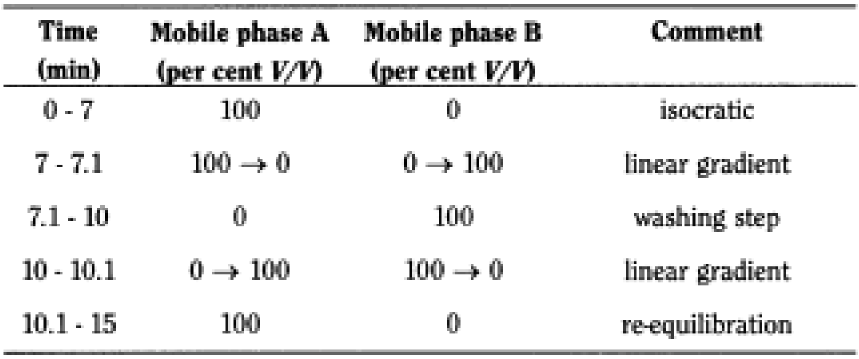
Examine by liquid chromatography *(2.2.29).*

The chromatographic procedure may be carried out using:

- — a stainless steel column 0.15 m long and 3.9 mm in diameter packed with *octadecylsilyl silica gel for chromatography R* (4 µm),
- — as mobile phase at a flow rate of 1.0 ml/min:

Mobile phase A Mix 70 volumes of a 19 g/l solution of *sodium acetate R* containing 0.05 per cent *V/V* of *triethylamine R* and adjusted to pH 6.4 with *dilute acetic acid R* and 30 volumes of mobile phase B,

Mobile phase B Mix 40 volumes of *water R* and 60 volumes of *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 7	100	0	isocratic
7 - 7.1	100 → 0	0 → 100	linear gradient
7.1 - 10	0	100	washing step
10 - 10.1	0 → 100	100 → 0	linear gradient
10.1 - 15	100	0	re-equilibration

- — as detector a spectrophotometer set at 254 nm,

maintaining the temperature of the column at 43 °C.

Inject 50 µl of each solution.

In the chromatograms obtained with the test solution, identify the peaks corresponding to leucine and norleucine. The retention time of norleucine is 6.2 min to 7 min.

Calculate the content of norleucine (in moles of norleucine per mole of interferon gamma-1b) from the peak areas of leucine and norleucine in the chromatograms obtained with the reference and test solutions, considering that there are 10 moles of leucine per mole of interferon gamma-1b.

Bacterial endotoxins *(2.6.14)*

Less than 5 IU in the volume that contains 20 × 10^6 IU of interferon gamma-1b.

ASSAY

Protein *(2.2.25)*

Dilute the substance to be examined in *water R* to obtain a concentration of 1 mg/ml. Record the absorbance spectrum between 220 nm and 340 nm. Measure the value at the absorbance maximum of 280 nm, after correction for any light scattering due to turbidity measured at 316 nm. Calculate the concentration of interferon gamma-1b using a specific absorbance value of 7.5.

Potency

The potency of interferon gamma-1b is estimated by evaluating the increase of the expression of human-leukocyte-antigen-DR (HLA-DR) due to the interferon gamma-1b present in test solutions during cultivation of the cells, and comparing this increase with the same effect of the appropriate International Standard of human recombinant interferon gamma or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Carry out the assay by a suitable method, based on the following design.

Use COLO 205 cells under standard culture conditions. Trypsinise a 3- to 5-day-old flask of COLO 205 cells and prepare a cell suspension at a concentration of 1.0×10^6 cells/ml.

Add 100 µl of the dilution medium to all wells of a 96-well microtitre plate. Add an additional 100 µl of this solution to the wells designed for the blanks. Add 100 µl of each solution to be tested onto the plate and carry out a series of twofold dilution steps in order to obtain a standard curve. Then add 100 µl of the cell suspension to all wells and incubate the plate under appropriate conditions for cell cultivation.

After cultivation remove the growth medium and wash and fix cells to the plate. Add an antibody able to detect HLA-DR expressed due to the presence of interferon gamma-1b and incubate under appropriate conditions. After washing the plate, incubate with an antibody conjugated to a marker enzyme which is able to detect the anti-HLA-DR antibody. After this incubation step, wash the plate and add an appropriate substrate solution. Stop the reaction. Measure the absorbance of the solution and calculate the potency of the preparation to be examined by the usual statistical methods.

The estimated specific activity is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 70 per cent and not more than 140 per cent of the estimated potency.

STORAGE

Store in an airtight container , protected from light and at a temperature of - 70 °C.

Ph Eur

Inulin

General Notices

Action and use

″Diagnostic agent used in the determination of renal function (glomerular filtration rate)″.

Preparation

Inulin Injection

DEFINITION

Inulin consists of polysaccharide granules obtained from the tubers of *Dahlia variabilis*, *Helianthus tuberosus* and other genera of the family Compositae.

CHARACTERISTICS

A white, amorphous, granular powder; hygroscopic.

Microscopical When mounted in *absolute ethanol*, appears as large, very irregular, angular masses, whole or fragmented, with occasional smaller, spherical to ovoid particles.

Slightly soluble in *water*; freely soluble in hot *water*; slightly soluble in organic solvents.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a suspension of *silica gel G* in a 0.3% w/v solution of *sodium acetate* to coat the plate, but spreading a layer 0.5 mm thick. Use a mixture of 70 volumes of *glacial acetic acid*, 60 volumes of *chloroform* and 10 volumes of *water* as the mobile phase. Apply separately to the plate 1 µl of each of three solutions in *water* containing (1) 2.5% w/v of the substance being examined, prepared using heat, (2) 2.5% w/v of the substance being examined and 10% w/v of *oxalic acid*, prepared by boiling for 10 minutes and cooling, and (3) 2.5% w/v of D-*fructose* and 0.1% w/v of D-*glucose monohydrate*. Develop the chromatograms in a continuous elution tank for about 4 hours. After removal of the plate, evaporate the solvent in a current of warm air and spray with a solution in *acetone* containing 1% v/v of *diphenylamine*, 1% v/v of *aniline* and 1% v/v of *orthophosphoric acid* and heat for 10 minutes at 130°. The two principal spots in the chromatogram obtained with solution (2) correspond to those in the chromatogram obtained with solution (3). The spot in the chromatogram obtained with solution (1) remains on the line of application.

B. Dissolve 10 mg in 2 ml of hot *water*, add 3 ml of a 0.15% w/v solution of *resorcinol* in *ethanol (96%)* followed by 3 ml of *hydrochloric acid*, mix and heat at 80°. A red colour is produced.

C. Boil 5 ml of a 10% w/v solution for 2 minutes with 0.5 ml of *hydrochloric acid*, cool and neutralise to *litmus paper* with 5M *sodium hydroxide*. Add 0.5 ml of *cupri-tartaric solution R1* and heat. A red precipitate is produced.

TESTS

Acidity

Dissolve 5 g in 50 ml of freshly boiled *water* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 0.20 ml is required to change the colour of the solution to pink.

Clarity and colour of solution

A 10.0% w/v solution in hot *water* is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Specific optical rotation

In a 2% w/v solution, prepared using heat, -36.5 to -40.5, calculated with reference to the dried substance, Appendix V F.

Arsenic

1.0 g dissolved in 25 ml of hot *water* complies with the *limit test for arsenic*, Appendix VII (1 ppm).

Calcium

Dissolve 1.0 g in 10 ml of hot *water*, place the solution in a water bath at 40°, add 0.5 ml of a 2.5% w/v solution of *ammonium oxalate* and allow to stand for 15 minutes. Any turbidity produced is not greater than that obtained by treating 10 ml of a 0.010% w/v solution of *calcium chloride* in the same manner (270 ppm).

Lead

Dissolve 2.5 g in 20 ml of hot *water* and transfer to a separating funnel with the aid of 5 ml of *water*. Add 2.5 ml of *hydrochloric acid* and shake for 5 minutes. Add 0.25 ml of *bromothymol blue solution R3* and sufficient 13.5M *ammonia* to produce a full blue colour and add 1.5 ml in excess. Add 1 ml of a 25% w/v solution of *citric acid* previously adjusted to pH 11 with 13.5M *ammonia* (ammonium citrate solution), 1 ml of *potassium cyanide solution PbT* and sufficient of a 0.002% w/v solution of *dithizone* in *chloroform* until, on shaking, the chloroform layer becomes purple or blue. Separate the chloroform layer, add to the aqueous layer 2 ml of the dithizone solution, shake and separate the chloroform layer. To the combined chloroform solutions add 10 ml of a 1% v/v solution of *nitric acid*, shake until the chloroform layer becomes green, allow to separate and discard the chloroform layer. To the aqueous layer add 0.2 ml of the ammonium citrate solution, 0.25 ml of 13.5M *ammonia*, 0.2 ml of *potassium cyanide solution PbT* and sufficient of the dithizone solution until, on shaking, the chloroform layer becomes purple or blue. Transfer the contents of the separating funnel to a stoppered tube. Repeat the operations without the substance being examined and using the same quantities of the reagents. To the tube containing the reference solution add *lead standard solution (10 ppm Pb)* dropwise until, on shaking, the colour of the chloroform layer matches that of the chloroform layer obtained from the substance being examined. Not more than 0.5 ml of *lead standard solution (10 ppm Pb)* is required (2 ppm).

Chloride

Dissolve 2.0 g in hot *water*, cool and dilute to 100 ml with *water*. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (170 ppm).

Oxalate

Dissolve 1.0 g in 10 ml of hot *water*, place the solution in a water bath at 40°, add 0.5 ml of a 7% w/v solution of *calcium chloride* and allow to stand for 15 minutes. Any turbidity produced is not greater than that obtained by treating 10 ml of a 0.003% w/v solution of *oxalic acid* in the same manner.

Sulphate

Dissolve 2.3 g in 20 ml of hot *water*, cool, add 2 ml of 2M *hydrochloric acid* and dilute to 45 ml with *water*. The solution complies with the *limit test for sulphates*, Appendix VII (200 ppm).

Reducing sugars

Heat 10 ml of *cupri-tartaric solution R1* and titrate with a 10% w/v solution of the substance being examined, adding a few ml at a time and boiling for 10 to 15 seconds between each addition. When the solution becomes greenish yellow, add 0.25 ml of a 1% w/v solution of *methylene blue* and continue the titration until the solution becomes orange. Repeat the operation using a 0.20% w/v solution of D-*fructose* in place of the solution of the substance being examined. The volume of the fructose solution required is not greater than that of the solution of the substance being examined.

Loss on drying

When dried to constant weight at 105°, loses not more than 10.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

Iodine

General Notices

(Ph Eur monograph 0031)

I_2 253.8 *7553-56-2*

Action and use

Antiseptic; antithyroid.

Preparations

Alcoholic Iodine Solution

Aqueous Iodine Oral Solution

Ph Eur

DEFINITION

Content

99.5 per cent to 100.5 per cent of I.

CHARACTERS

Appearance

Greyish-violet, brittle plates or fine crystals with a metallic sheen.

Solubility

Very slightly soluble in water, very soluble in concentrated solutions of iodides, soluble in ethanol (96 per cent), slightly soluble in glycerol.

It volatilises slowly at room temperature.

IDENTIFICATION

A. Heat a few fragments in a test-tube. Violet vapour is evolved and a bluish-black crystalline sublimate is formed.

B. To a saturated solution add *starch solution R*. A blue colour is produced. Heat until decolourised. On cooling, the colour reappears.

TESTS

Solution S

Triturate 3.0 g with 20 ml of *water R*, filter, wash the filter with *water R* and dilute the filtrate to 30 ml with the same solvent. To the solution add 1 g of *zinc powder R*. When the solution is decolourised, filter, wash the filter with *water R* and dilute to 40 ml with the same solvent.

Bromides and chlorides

Maximum 250 ppm.

To 10 ml of solution S add 3 ml of *ammonia R* and 6 ml of *silver nitrate solution R2*. Filter, wash the filter with *water R* and dilute the filtrate to 20 ml with the same solvent. To 10 ml of the solution add 1.5 ml of *nitric acid R*. After 1 min, any opalescence in the solution is not more intense than that in a standard prepared at the same time by mixing 10.75 ml of *water R*, 0.25 ml *of 0.01 M hydrochloric acid*, 0.2 ml of *dilute nitric acid R* and 0.3 ml of *silver nitrate solution R2.*

Non-volatile substances

Maximum 0.1 per cent.

Heat 1.00 g in a porcelain dish on a water-bath until the iodine has volatilised. Dry the residue at 100-105 °C. The residue weighs a maximum of 1 mg.

ASSAY

Introduce 0.200 g into a flask containing 1 g of *potassium iodide R* and 2 ml of *water R* and add 1 ml of *dilute acetic acid R.* When dissolution is complete, add 50 ml *of water R* and titrate with *0.1 M sodium thiosulphate*, using *starch solution R* as indicator.

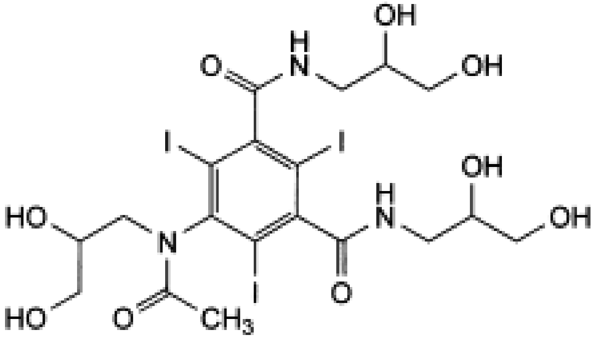
1 ml of *0.1 M sodium thiosulphate* is equivalent to 12.69 mg of I.

Ph Eur

Iohexol

General Notices

(Ph Eur monograph 1114)



$C_{19}H_{26}I_3N_3O_9$ 821 *66108-95-0*

Action and use

Iodinated contrast medium.

Ph Eur

DEFINITION

5-[Acetyl(2,3-dihydroxypropyl)amino]-*N*,*N′*-bis(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide.

The substance is a mixture of diastereoisomers and atropisomers.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or greyish-white, hygroscopic powder.

Solubility

Very soluble in water, freely soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison iohexol CRS.

B. Examine the chromatograms obtained in test A for related substances (see Tests).

Results The principal peaks in the chromatogram obtained with reference solution (b) are similar in retention time and size to the peaks due to iohexol in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 5.0 g in *water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Related substances

A. Liquid chromatography *(2.2.29)*.

NOTE: iohexol gives rise to 2 non-resolved peaks in the chromatogram due to endo-exo isomerism. In addition, a small peak (also due to iohexol) usually appears at the leading edge of the 1st principal peak. This small peak has a retention time about 1.2 min less than the 1st principal peak.

Test solution Dissolve 0.150 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 15.0 mg of *iohexol CRS* and 15.0 mg of *iohexol impurity A CRS* in a mixture of 1-2 drops of *dilute sodium hydroxide solution R* and 10 ml of *water R* and dilute to 100.0 ml with w*ater R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *water R*.

Reference solution (c) Dissolve 5.0 mg of *iohexol for peak identification CRS* (containing impurities B, C, D and E) in *water R* and dilute to 5.0 ml with the same solvent.

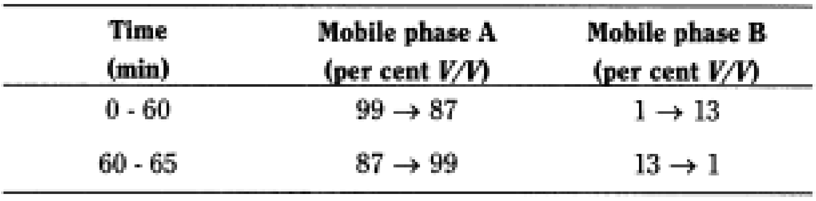
Blank solution water R.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A: water R*;

— *mobile phase B: acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	99 → 87	1 → 13
60 - 65	87 → 99	13 → 1

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration At the initial eluent composition for at least 10 min.

Injection 10 µl.

Retention times Impurity A and impurity H = about 17 min; iohexol (peaks corresponding to *endo-exo* isomerism) = about 20 min.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peak due to impurity A and the 2nd and greater peak due to iohexol.

Limits:

— *sum of impurities B, C, D and E* (relative retention with reference to the 2nd and greater peak due to iohexol between 1.1 and 1.4): not more than 0.6 times the total area of the principal peaks in the chromatogram obtained with reference solution (b) (0.6 per cent); use the chromatogram obtained with reference solution (c) to identify the corresponding peaks;

— *sum of impurities A and H*: not more than 0.5 times the total area of the principal peaks in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities M, N, O, P, Q:* for each impurity, not more than 0.1 times the total area of the principal peaks in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than 0.1 times the total area of the principal peaks in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 1.5 times the total area of the principal peaks in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.03 times the total area of the principal peaks in the chromatogram obtained with reference solution (b) (0.03 per cent); disregard any peak observed with the blank.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *iohexol impurity J CRS* and 50 mg of *iohexol CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with *water R*. Dilute 1.0 ml of this solution to 50.0 ml with *water R*.

Plate *TLC silica gel* F_{254} *plate R*.

Preconditioning Wash the plate with the mobile phase, dry at room temperature for 30 min, then at 90 °C for 1 h.

Mobile phase *Concentrated ammonia R, methanol R, 2-propanol R, acetone R* (16:16:28:40 *V/V/V/V*).

Application 10 µl.

Development Over half of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (a):

— the chromatogram shows 2 clearly separated spots.

Limits:

— *any impurity*: any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

3-Chloropropane-1,2-diol

Gas chromatography *(2.2.28)*.

Test solution Dissolve 1.0 g of the substance to be examined in 1.0 ml of *water R*. Shake with 4 quantities, each of 2 ml, of *methyl acetate R*. Dry the combined upper layers over *anhydrous sodium sulphate R*. Filter and concentrate to about 0.7 ml using a warm water bath at 60 °C and a stream of nitrogen and dilute to 1.0 ml with *methyl acetate R*.

Reference solution Dissolve 0.25 g of *3-chloropropane-1,2-diol R* in 100.0 ml of *methyl acetate R*. Dilute 1.0 ml of this solution with 100.0 ml of *methyl acetate R*.

Column:

— *material*: fused silica,

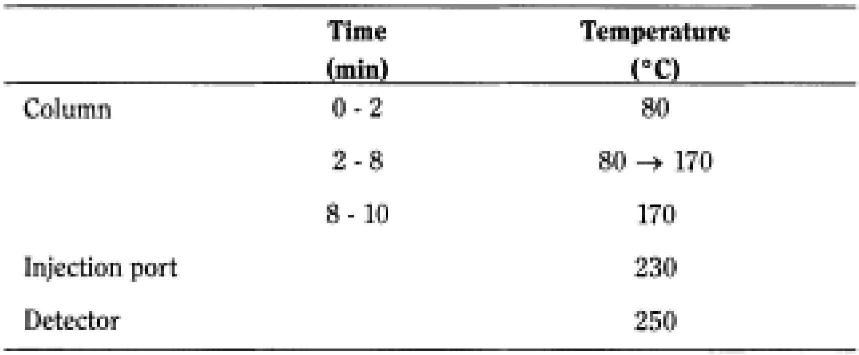
— *size*: *l* = 25 m, Ø = 0.33 mm,

— *stationary phase: polymethylphenylsiloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	80
	2 - 8	80 → 170
	8 - 10	170
Injection port		230
Detector		250

Detection Flame ionisation.

Injection 2 µl (splitless for 30 s).

System suitability Reference solution:

— *retention time*: 3-chloropropane-1,2-diol = about 8 min.

Limit:

— *3-chloropropane-1,2-diol*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (25 ppm).

Free aromatic amine

Maximum 500 ppm.

Test solution Transfer 0.200 g of the substance to be examined to a 25 ml volumetric flask and dissolve in 15.0 ml of *water R*.

Reference solution Dissolve 5.0 mg of *iohexol impurity J CRS* in *water R* and dilute to 5.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*. Mix 10.0 ml of this solution with 5.0 ml of *water R* in a 25 ml volumetric flask.

Blank solution Transfer 15.0 ml of *water R* to a 25 ml volumetric flask.

In conducting the following steps, keep the flasks in iced water and protected as much as possible from light until all of the reagents have been added.

Place the 3 flasks containing respectively the test solution, the reference solution and the blank solution in iced water, protected from light, for 5 min. Add 1.5 ml of *hydrochloric acid R1* and mix by swirling. Add 1.0 ml of a 20 g/l solution of *sodium nitrite R*, mix and allow to stand for 4 min. Add 1.0 ml of a 40 g/l solution of *sulphamic acid R,* swirl gently until gas liberation has ceased and allow to stand for 1 min. *(CAUTION: considerable pressure is produced)*. Add 1.0 ml of a freshly prepared 3 g/l solution of *naphthylethylenediamine dihydrochloride R* in a mixture of 30 volumes of *water R* and 70 volumes of *propylene glycol R* and mix. Remove the flasks from the iced water, dilute to 25.0 ml with *water R*, mix and allow to stand for 5 min. Simultaneously determine the absorbance *(2.2.25)* at 495 nm of the solutions obtained from the test solution and the reference solution in 5 cm cells, using the blank as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution.

Iodide:

Maximum 10 ppm.

Dissolve 6.000 g in *water R* and dilute to 20 ml with the same solvent. Add 2.0 ml of *0.001 M potassium iodide*. Titrate with *0.001 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20)*, using a silver indicator electrode and an appropriate reference electrode. Subtract the volume of titrant corresponding to the 2.0 ml of *0.001 M potassium iodide,* determined by titrating a blank to which is added 2.0 ml *of 0.001 M potassium iodide* and use the residual value to calculate the iodide content.

1 ml of *0.001 M silver nitrate* is equivalent to 126.9 µg of I-.

Ionic compounds ***(2.2.38)***

Maximum 0.01 per cent *m/m* calculated as sodium chloride.

Rinse all glassware with distilled water R 5 times before use.

Test solution Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution Dissolve 20.0 mg of *sodium chloride R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *water R*.

Measure the conductivity of the test solution and the reference solution using a suitable conductivity meter. The conductivity of the test solution is not greater than that of the reference solution.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 4.0 per cent, determined on 1.00 g.

ASSAY

To 0.500 g in a 125 ml round-bottomed flask add 25 ml of a 50 g/l solution of *sodium hydroxide R*, 0.5 g of *zinc powder R* and a few glass beads. Boil under a reflux condenser for 30 min. Allow to cool and rinse the condenser with 20 ml of *water R*, adding the rinsings to the flask. Filter through a sintered-glass filter *(2.1.2)* and wash the filter with several quantities of *water R.* Collect the filtrate and washings. Add 5 ml of *glacial acetic acid R* and titrate immediately with *0.1 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20).*

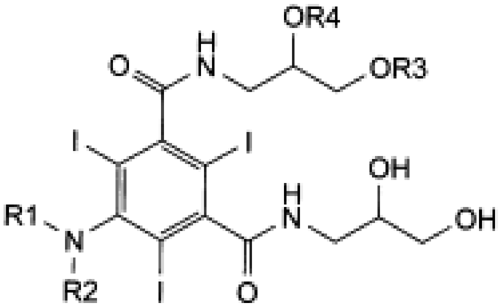
1 ml of *0.1 M silver nitrate* is equivalent to 27.37 mg of $C_{19}H_{26}I_3N_3O_9$.

STORAGE

In an airtight container, protected from light and moisture.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q.

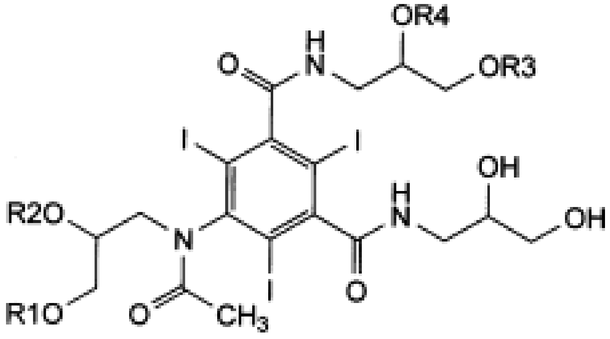


A. R1 = CO-CH_3, R2 = R3 = R4 = H: 5-(acetylamino)-*N,N′*-bis(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

J. R1 = R2 = R3 = R4 = H: 5-amino-*N,N′*-bis(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

P. R1 = R2 = CO-CH_3, R3 = CH_2-CHOH-CH_2OH, R4 = H: 5-(diacetylamino)-*N*-[3-(2,3-dihydroxypropoxy)-2-hydroxypropyl]-*N′*-(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

Q. R1 = R2 = CO-CH_3, R3 = H, R4 = CH_2-CHOH-CH_2OH: 5-(diacetylamino)-*N*-[2-(2,3-dihydroxypropoxy)-3-hydroxypropyl]-*N′*-(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,



B. R1 = CH_2-CHOH-CH_2OH, R2 = R3 = R4 = H: 5-[acetyl[3-(2,3-dihydroxypropoxy)-2-hydroxypropyl]amino]-*N,N′*-bis(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

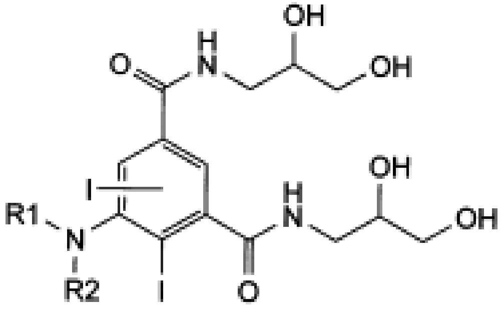
C. R2 = CH_2-CHOH-CH_2OH, R1 = R3 = R4 = H: 5-[acetyl[2-(2,3-dihydroxypropoxy)-3-hydroxypropyl]amino]-*N,N′*-bis(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

D. R3 = CH_2-CHOH-CH_2OH, R1 = R2 = R4 = H: 5-[acetyl(2,3-dihydroxypropyl)amino]-*N*-[3-(2,3-dihydroxypropoxy)-2-hydroxypropyl]-*N′*-(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

E. R4 = CH_2-CHOH-CH_2OH, R1 = R2 = R3 = H: 5-[acetyl(2,3-dihydroxypropyl)amino]-*N*-[2-(2,3-dihydroxypropoxy)-3-hydroxypropyl]-*N′*-(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

N. R4 = CO-CH_3, R1 = R2 = R3 = H: 5-[acetyl(2,3-dihydroxypropyl)amino]-*N*-[2-(acetyloxy)-3-hydroxypropyl]-*N′*-(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

O. R3 = CO-CH_3, R1 = R2 = R4 = H: 5-[acetyl(2,3-dihydroxypropyl)amino]-*N*-[3-(acetyloxy)-2-hydroxypropyl]-*N′*-(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide,

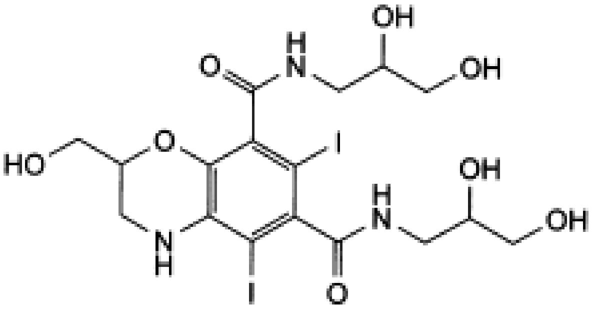


F. R1 = R2 = H: 5-amino-*N,N′*-bis(2,3-dihydroxypropyl)diiodobenzene-1,3-dicarboxamide,

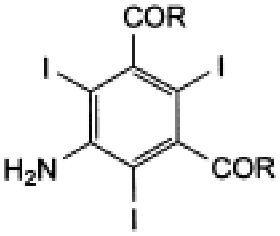
G. R1 = H, R2 = CO-CH_3: 5-(acetylamino)-*N,N′*-bis(2,3-dihydroxypropyl)diiodobenzene-1,3-dicarboxamide,

H. R1 = CH_2-CHOH-CH_2OH, R2 = CO-CH_3: 5-[acetyl(2,3-dihydroxypropyl)amino]-*N,N′*-bis(2,3-dihydroxypropyl)diiodobenzene-1,3-dicarboxamide,

M. R1 = CH_2-CHOH-CH_2OH, R2 = H: *N,N′*-bis(2,3-dihydroxypropyl)-5-[(2,3-dihydroxypropyl) amino]diiodobenzene-1,3-dicarboxamide,



I. *N,N′*-bis(2,3-dihydroxypropyl)-2-(hydroxymethyl)-5,7-diiodo-3,4-dihydro-2H-1,4-benzoxazine-6,8-dicarboxamide,



K. R = OH: 5-amino-2,4,6-triiodobenzene-1,3-dicarboxylic acid,

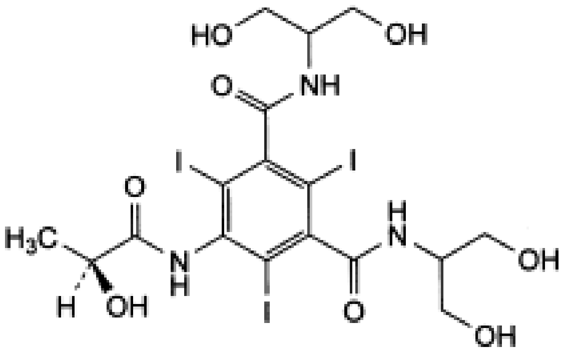
L. R = Cl: 5-amino-2,4,6-triiodobenzene-1,3-dicarbonyl dichloride.

Ph Eur

Iopamidol

General Notices

(Ph Eur monograph 1115)



$C_{17}H_{22}I_3N_3O_8$ 777 *60166-93-0*

Action and use

Iodinated contrast medium.

Ph Eur

DEFINITION

N,*N′*-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[[(2*S*)-2-hydroxypropanoyl]amino]-2,4,6-triiodobenzene-1,3-dicarboxamide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, very slightly soluble in methanol, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   *iopamidol CRS.*

B. It complies with the test for loss on drying (see Tests).

C. It complies with the test for specific optical rotation (see Tests).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1 g in *water R* and dilute to 50 ml with the same solvent.

Acidity or alkalinity

Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent. Not more than 0.75 ml of *0.01 M hydrochloric acid* or 1.4 ml of *0.01 M sodium hydroxide* is required to adjust to pH 7.0 *(2.2.3)*.

Specific optical rotation *(2.2.7)*

- 4.6 to - 5.2 (dried substance), determined at 436 nm.

Dissolve 10.0 g, with heating if necessary, in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution   Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a)   Dissolve 5.0 mg of *iopamidol impurity H CRS* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b)   Dilute 2.0 ml of the test solution to 20.0 ml with *water R*. Dilute 1.0 ml of this solution to 50.0 ml with *water R*.

Reference solution (c)   Add 0.1 ml of the test solution to 20 ml of reference solution (a) and dilute to 50 ml with *water R*.

Column:
2 columns coupled in series,

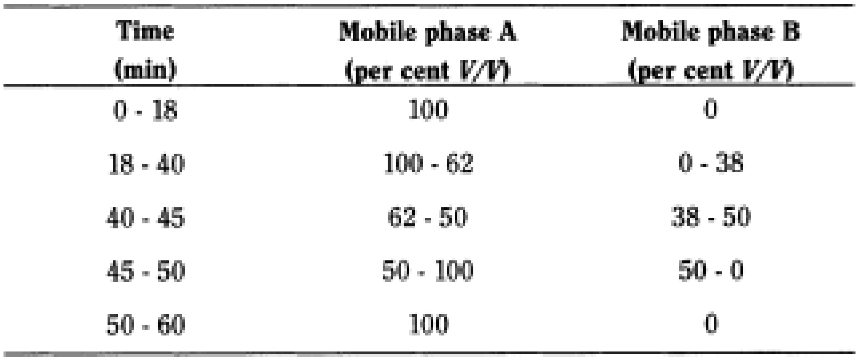
— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *phenylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 60 °C.

Mobile phase:
— *mobile phase A*: *water R*,

— *mobile phase B: acetonitrile R, water R* (50:50 *V/V*),



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 18	100	0
18 - 40	100 - 62	0 - 38
40 - 45	62 - 50	38 - 50
45 - 50	50 - 100	50 - 0
50 - 60	100	0

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Relative retention with reference to iopamidol (retention time = about 14.6 min): impurity D = about 0.1; impurity B = about 0.6; impurities I and H = about 0.9; impurity G = about 1.1; impurity K = about 1.2; impurity C = about 1.3; impurity J = about 1.5; impurity A = about 1.8; impurity E = about 2.2; impurity F = about 2.3.

System suitability Reference solution (c):

— *resolution*: minimum 2.0 between the peaks due to impurity H and iopamidol.

Limits:

— *sum of impurities H and I*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *impurities A, B, C, D, E, F, G, J, K*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *sum of impurities other than H and I*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Free aromatic amines

Maximum 200 ppm.

Keep the solutions and reagents in iced water, protected from bright light.

Test solution In a 25 ml volumetric flask, dissolve 0.500 g of the substance to be examined in 20.0 ml of *water R*.

Reference solution In a 25 ml volumetric flask, mix 4.0 ml of a 25.0 mg/l solution of *iopamidol impurity A CRS* with 16.0 ml of *water R*.

Blank solution Place 20.0 ml of *water R* in a 25 ml volumetric flask.

Place the flasks in iced water, protected from light, for 5 min. Add *1.0 ml of hydrochloric acid R* to each flask, mix and allow to stand for 5 min. Add 1.0 ml of a 20 g/l solution of *sodium nitrite R* prepared immediately before use, mix and allow to stand for 5 min. Add 1.0 ml of a

120 g/l solution of *ammonium sulphamate R*, swirl gently until gas liberation has ceased, and allow to stand for 5 min. *(CAUTION: considerable pressure is produced)*. Add 1.0 ml of a freshly prepared 1 g/l solution of *naphthylethylenediamine dihydrochloride R* and mix. Remove the flasks from the iced water and allow to stand for 10 min. Dilute to 25.0 ml with *water R* and mix. Measure immediately the absorbance *(2.2.25)* at 500 nm of the solutions obtained from the test solution and the reference solution using, as the compensation liquid, the solution obtained from the blank solution.

The absorbance of the test solution is not greater than that of the reference solution.

Free iodine

Maximum 10 ppm.

Dissolve 2.0 g in 25 ml of *water R* in a ground-glass stoppered centrifuge tube. Add 5 ml of *toluene R* and 5 ml of *diluted sulphuric acid R*. Shake and centrifuge. Any red colour of the upper layer is not more intense than that of the upper phase obtained in the same way from 22 ml of *water R*, 2 ml of *iodide standard solution (10 ppm I) R*, 5 ml of *dilute sulphuric acid R,* 1 ml of *concentrated hydrogen peroxide solution R* and 5 ml of *toluene R.*

Iodide

Maximum 10 ppm.

Dissolve 6.000 g in *water R* and dilute to 20 ml with the same solvent. Add 2.0 ml of *0.001 M potassium iodide*. Carry out a potentiometric titration *(2.2.20)* with *0.001 M silver nitrate* using a silver indicator electrode and an appropriate reference electrode. Subtract the volume of titrant corresponding to the 2.0 ml of *0.001 M potassium iodide*, determined by titrating a blank to which is added 2.0 ml of *0.001 M potassium iodide* and use the residual value to calculate the iodide content.

1 ml of *0.001 M silver nitrate* is equivalent to 126.9 µg of iodide.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 1.4 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

To 0.300 g in a 250 ml round-bottomed flask add 5 ml of *strong sodium hydroxide solution R*, 20 ml of *water R*, 1 g of *zinc powder R* and a few glass beads. Boil under a reflux condenser for 30 min. Allow to cool and rinse the condenser with 20 ml of *water R*, adding the rinsings to the flask. Filter through a sintered-glass filter *(2.1.2)* and wash the filter with several quantities of *water R.* Collect the filtrate and washings. Add 5 ml of *glacial acetic acid R* and titrate immediately with *0.1 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20)* using a suitable electrode system such as silver-silver chloride.

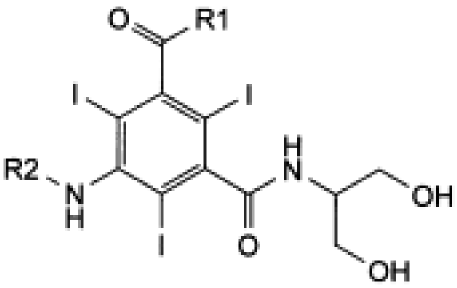
1 ml of *0.1 M silver nitrate* is equivalent to 25.90 mg of $C_{17}H_{22}I_3N_3O_8$.

STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J, K.



A. R1 = NH-CH(CH_2OH)$_2$, R2 = H: 5-amino-*N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodobenzene-1,3-dicarboxamide,

B. R1 = NH-CH(CH_2OH)$_2$, R2 = CO-CH_2OH: 5-[(hydroxyacetyl)amino]-*N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodobenzene-1,3-dicarboxamide,

C. R1 = NH-CH(CH_2OH)$_2$, R2 = CO-CH_3: 5-(acetylamino)-*N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodobenzene-1,3-dicarboxamide,

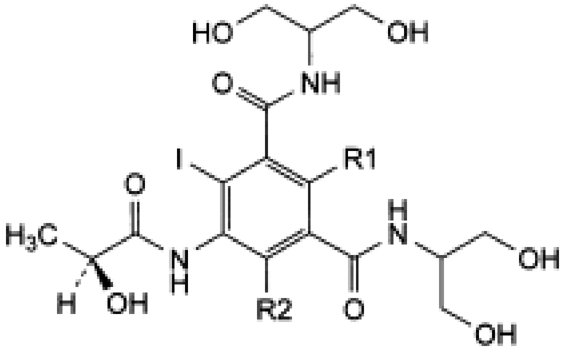
D. R1 = OH, R2 = CO-CHOH-CH_3: 3-[[2-hydroxy-1-(hydroxymethyl)ethyl]carbamoyl]-5-[[(2*S*)-2-hydroxypropanoyl]amino]-2,4,6-triiodobenzoic acid,

E. R1 = NH-CH(CH_2OH)$_2$, R2 = CO-CH(CH_3)-O-CO-CH_3: (1*S*)-2-[[3,5-bis[[2-hydroxy-1-(hydroxymethyl)ethyl]carbamoyl]-2,4,6-triiodophenyl]amino]-1-methyl-2-oxoethyl acetate,

F. R1 = N(CH_3)$_2$, R2 = CO-CHOH-CH_3: *N'*-[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[[(2*S*)-2-hydroxypropanoyl]amino]-2,4,6-triiodo-*N,N*-dimethylbenzene-1,3-dicarboxamide,

G. R1 = NH-CH_2-CHOH-CH_2OH, R2 = CO-CHOH-CH_3: *N*-(2,3-dihydroxypropyl)-*N'*-[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[[(2*S*)-2-hydroxypropanoyl]amino]-2,4,6-triiodobenzene-1, 3-dicarboxamide,

J. R1 = NH-CH_2-CH_2OH, R2 = CO-CHOH-CH_3: *N*-(2-hydroxyethyl)-*N'*-[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[[(2*S*)-2-hydroxypropanoyl]amino]-2,4,6-triiodobenzene-1,3-dicarboxamide,



H. R1 = I, R2 = Cl: 4-chloro-*N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[[(2*S*)-2-hydroxypropanoyl]amino]-2,6-diiodobenzene-1,3-dicarboxamide,

I. R1 = Cl, R2 = I: 2-chloro-*N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[[(2*S*)-2-hydroxypropanoyl]amino]-4,6-diiodobenzene-1,3-dicarboxamide,

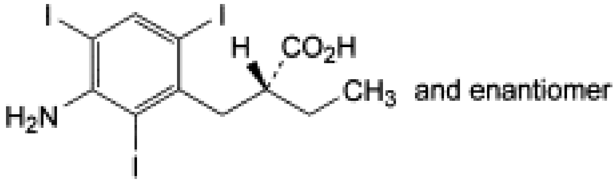
K. R1 = I, R2 = H: *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[[(2*S*)-2-hydroxypropanoyl] amino]-2,4-diiodobenzene-1,3-dicarboxamide.

Ph Eur

Iopanoic Acid

General Notices

(Ph Eur monograph 0700)



$C_{11}H_{12}I_3NO_2$ 571 *96-83-3*

Action and use

Iodinated contrast medium.

Preparation

Iopanoic Acid Tablets

Ph Eur

DEFINITION

Iopanoic acid contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*RS*)-2-(3-amino-2,4,6-tri-iodobenzyl)butanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or yellowish-white powder, practically insoluble in water, soluble in ethanol and in methanol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point *(2.2.14):* about 155 °C, with decomposition.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *iopanoic acid CRS*.

C. Examine the chromatograms obtained in the test for related substances (see Tests). Spray the plate with a 1 g/l solution of *4-dimethylaminocinnamaldehyde R* in a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *alcohol R*. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Heat 50 mg carefully in a small porcelain dish over a flame. Violet vapour is evolved.

TESTS

Appearance of solution

Dissolve 1.0 g in *1 M sodium hydroxide* and dilute to 20 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_3 (*2.2.2, Method II).*

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 1.0 g of the substance to be examined in a mixture of 3 volumes of *ammonia R* and 97 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 3 volumes of *ammonia R* and 97 volumes of *methanol R*.

Reference solution (a) Dissolve 50 mg of *iopanoic acid CRS* in a mixture of 3 volumes of *ammonia R* and 97 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (b) Dilute 1 ml of test solution (b) to 50 ml with a mixture of 3 volumes of *ammonia R* and 97 volumes of *methanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of *concentrated ammonia R*, 20 volumes of *methanol R*, 20 volumes of *toluene R* and 50 volumes of *dioxan R*. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Halides

To 0.46 g add 10 ml of *nitric acid R* and 15 ml of *water R*. Shake for 5 min and filter. 15 ml of the filtrate complies with the limit test for chlorides *(2.4.4)* (180 ppm, expressed as chloride).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

To 0.150 g in a 250 ml round-bottomed flask add 5 ml of *strong sodium hydroxide solution R*, 20 ml of *water R*, 1 g of *zinc powder R* and a few glass beads. Boil under a reflux condenser for 60 min. Allow to cool and rinse the condenser with 20 ml of *water R*, adding the rinsings to the flask. Filter through a sintered-glass filter *(2.1.2)* and wash the filter with several quantities of *water R*. Collect the filtrate and washings. Add 40 ml of *dilute sulphuric acid R* and titrate immediately with *0.1 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20)*, using a suitable electrode system such as silver-mercurous sulphate.

1 ml of *0.1 M silver nitrate* is equivalent to 19.03 mg of $C_{11}H_{12}I_3NO_2$.

STORAGE

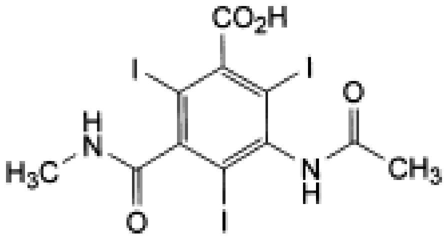
Store protected from light.

Ph Eur

Iotalamic Acid

General Notices

(Ph Eur monograph 0751)



$C_{11}H_9I_3N_2O_4$ 614 *2276-90-6*

Action and use

Iodinated contrast medium.

Ph Eur

DEFINITION

Iotalamic acid contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 3-(acetylamino)-2,4,6-tri-iodo-5-(methylcarbamoyl)benzoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, slightly soluble in water and in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *iotalamic acid CRS*.

B. Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel* GF_{254} *plate R.*

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* containing 3 per cent *V/V* of *ammonia R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 50 mg of *iotalamic acid CRS* in *methanol R* containing 3 per cent *V/V* of *ammonia R* and dilute to 5 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *anhydrous formic acid R*, 25 volumes of *methyl ethyl ketone R* and 60 volumes of *toluene R*. Allow the plate to dry until the solvents have evaporated and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Heat 50 mg gently in a small porcelain dish over a flame. Violet vapour is evolved.

TESTS

Appearance of solution

Dissolve 1.0 g in *1 M sodium hydroxide* and dilute to 20 ml with the same solvent. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel* GF_{254} *plate R.*

Test solution Dissolve 1.0 g of the substance to be examined in *methanol R* containing 3 per cent *V/V* of *ammonia R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 50 ml with *water R*. Dilute 1 ml of the solution to 10 ml with *water R*.

Reference solution (b) Dissolve 1 mg of *iotalamic acid impurity A CRS* in 5 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 1 volume of *glacial acetic acid R*, 1 volume of *anhydrous formic acid R*, 1 volume of *methanol R*, 5 volumes of *ether R* and 10 volumes of *methylene chloride R*. Allow the plate to dry until the solvents have evaporated. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

Halides

Dissolve 0.55 g in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 15 ml of *water R*. Add 6 ml of *dilute nitric acid R* and filter. 15 ml of the filtrate complies with the limit test for chlorides *(2.4.4)* (150 ppm, expressed as chloride).

Impurity A

Not more than 0.05 per cent *m/m* determined by absorption spectrophotometry *(2.2.25)*.

Test solution Transfer 0.500 g of the substance to be examined to a 50 ml volumetric flask, add 14 ml of *water R*, shake and add 1 ml of *dilute sodium hydroxide solution R*.

Reference solution Prepare the reference solution by mixing 10.0 ml of a 8.5 g/l solution of *sodium hydroxide R* containing 25 µg/ml of *iotalamic acid impurity A CRS* with 5 ml of *water R* in a 50 ml volumetric flask.

Blank solution Transfer 14 ml of *water R* and 1 ml of *dilute sodium hydroxide solution R* to a 50 ml volumetric flask.

In conducting the following steps, keep the flasks in iced water and protected as far as *possible from light until all of the reagents have been added.*

Place all three of the flasks containing the test solution, the reference solution and the blank solution in iced water, protected from light. Add 5 ml of a 5 g/l solution of *sodium nitrite R* and 12 ml of *dilute hydrochloric acid R*. Shake gently and allow to stand for exactly 2 min after adding the hydrochloric acid. Add 10 ml of a 20 g/l solution of *ammonium sulphamate R*. Allow to stand for 5 min shaking frequently *(CAUTION: considerable pressure is produced)*, and add 0.15 ml of a 100 g/l solution of α-*naphthol R* in *alcohol R*. Shake and allow to stand for 5 min. Add 3.5 ml of *buffer solution pH 10.9 R*, mix and dilute to 50.0 ml with *water R*. Concomitantly and within 20 min determine the absorbance at 485 nm of the solutions obtained from the test solution and the reference solution in 5 cm cells, using the blank as the compensation liquid.

Calculate the content of impurity A.

Iodide

Not more than 20 ppm, determined by potentiometric titration *(2.2.20)*. Dissolve 6.000 g in 20 ml of *1 M sodium hydroxide*, add 10 ml of *water R* and adjust to pH 4.5 to 5.5 with *acetic acid R*. Add 2.0 ml of *0.001 M potassium iodide*. Titrate with *0.001 M silver nitrate* using a silver indicator electrode and an appropriate reference electrode. Subtract the volume of titrant corresponding to the 2.0 ml of *0.001 M potassium iodide*, determined by titrating a blank to which is added 2.0 ml of *0.001 M potassium iodide* and use the residual value to calculate the iodide content.

1 ml of *0.001 M silver nitrate* is equivalent to 126.9 µg of iodide.

Heavy metals *(2.4.8)*

Dissolve 2.0 g in 4 ml of *dilute sodium hydroxide solution R* and dilute to 20 ml with *water R*. 12 ml of this solution complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 0.300 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

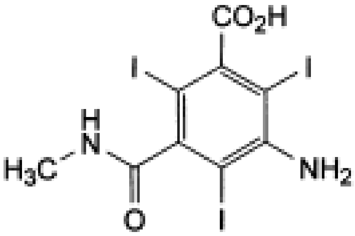
To 0.150 g in a 250 ml round-bottomed flask add 5 ml of *strong sodium hydroxide solution R*, 20 ml of *water R*, 1 g of *zinc powder R* and a few glass beads. Boil under a reflux condenser for 30 min. Allow to cool and rinse the condenser with 20 ml of *water R,* adding the rinsings to the flask. Filter through a sintered-glass filter *(2.1.2)* and wash the filter with several quantities of *water R*. Collect the filtrate and washings. Add 40 ml of *dilute sulphuric acid R* and titrate immediately with *0.1 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20)*, using a suitable electrode system such as silver-mercurous sulphate.

1 ml of *0.1 M silver nitrate* is equivalent to 20.47 mg of $C_{11}H_9I_3N_2O_4$.

STORAGE

Store protected from light.

IMPURITIES



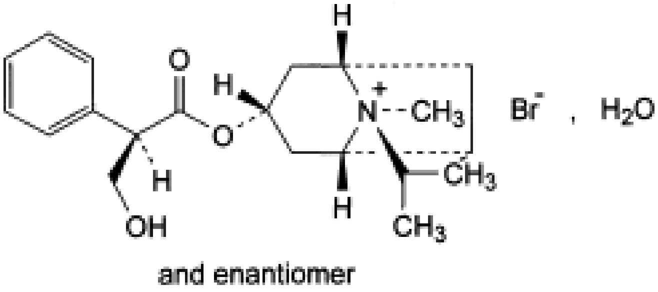
A. 3-amino-2,4,6-triiodo-5-(methylcarbamoyl)benzoic acid.

Ph Eur

Ipratropium Bromide

General Notices

(Ph Eur monograph 0919)



$C_{20}H_{30}BrNO_3,H_2O$ 430.4 *66985-17-9*

Action and use

Anticholinergic (antimuscarinic) bronchodilator.

Preparations

Ipratropium Nebuliser Solution

Ipratropium Powder for Inhalation

Ipratropium Pressurised Inhalation

Ph Eur

DEFINITION

(1*R*,3r,5*S*,8*r*)-3-[[(2*RS*)-3-Hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane bromide monohydrate.

Content

99.0 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in methanol, slightly soluble in ethanol (96 per cent).

mp: about 230 °C, with decomposition.

IDENTIFICATION

First identification *A, E.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ipratropium bromide CRS.*

B. Examine the chromatograms obtained in the test for impurity A.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 5 ml of solution S (see Tests), add 2 ml of *dilute sodium hydroxide solution R*. No precipitate is formed.

D. To about 1 mg add 0.2 ml of *nitric acid R* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.1 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

E. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution GY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

5.0 to 7.5 for solution S.

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 1.0 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *ipratropium bromide CRS* in *methanol R* and dilute to 1.0 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *methylatropine bromide CRS* in 1.0 ml of reference solution (a).

Reference solution (c) Dissolve 5 mg of *ipratropium impurity A CRS* in 100.0 ml of *methanol R*. Dilute 2.0 ml of the solution to 5.0 ml with *methanol R*.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *anhydrous formic acid R, water R, ethanol (96 per cent) R, methylene chloride*

R (1:3:18:18 *V/V/V/V*).

Application 1 µl.

Development Over a path of 6 cm.

Drying At 60 °C for 15 min.

Detection Spray with *potassium iodobismuthate solution R*, allow the plate to dry in air, spray with a 50 g/l solution of *sodium nitrite R* and protect immediately with a sheet of glass.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated principal spots.

Limit:

- *impurity A*: any spot due to impurity A is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *ipratropium bromide CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *ipratropium bromide CRS* and 5 mg of *ipratropium impurity B CRS* in 1 ml of *methanol R* and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase Dissolve 12.4 g of *sodium dihydrogen phosphate R* and 1.7 g of *tetrapropylammonium chloride R* in 870 ml of *water R*; adjust to pH 5.5 with a 180 g/l solution of *disodium hydrogen phosphate R* and add 130 ml of *methanol R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 5 µl.

Run time 6 times the retention time of ipratropium.

Relative retention With reference to ipratropium (retention time = about 4.9 min): impurity C = about 0.7; impurity B = about 1.2; impurity D = about 1.8; impurity E = about 2.3; impurity F = about 5.1.

System suitability Reference solution (b):

- *resolution*: minimum 3.0 between the peaks due to impurity B and ipratropium;
- *symmetry factor*: maximum 2.5 for the principal peak.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.3; impurity D = 0.2; impurity F = 0.5;
- *impurity D*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *impurities B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);
- *disregard limit*: one-third of the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent); disregard the peak due to the bromide ion.

Water *(2.5.12)*

3.9 per cent to 4.4 per cent, determined on 0.50 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

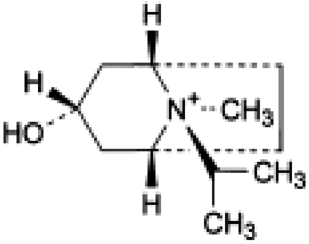
Dissolve 0.350 g in 50 ml of *water R* and add 3 ml of *dilute nitric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M silver nitrate* is equivalent to 41.24 mg of $C_{20}H_{30}BrNO_3$.

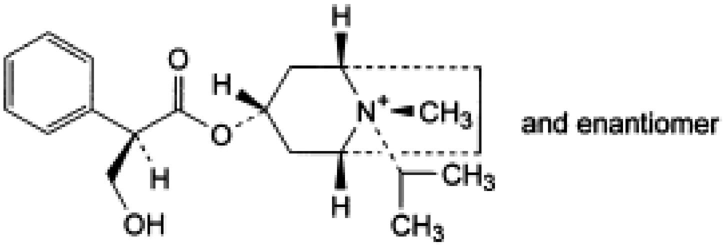
IMPURITIES

Specified impurities A, B, C, D.

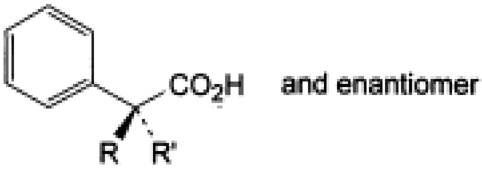
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, F.*



A. (1*R*,3*r*,5*S*,8*r*)-3-hydroxy-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane,

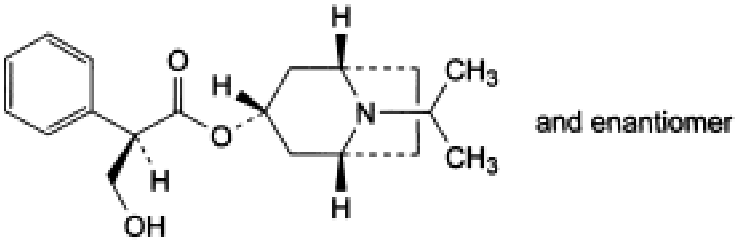

H
O
H
N⁺
CH3
and enantiomer
H
CH3
H
OH
CH3

B. (1*R*,3*r*,5*S*,8*s*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane,

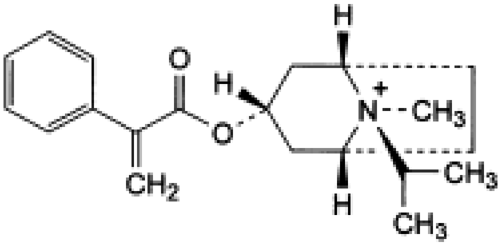

CO2H
and enantiomer
R
R'

C. R = CH_2-OH, R′ = H: (2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid),

D. R + R′ = CH_2: 2-phenylpropenoic acid (atropic acid),


H
O
H
CH3
N
and enantiomer
CH3
H
H
OH

E. (1*R*,3*r*,5*S*)-8-(1-methylethyl)-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate,


H
O
H
N⁺
CH3
CH3
CH2
H
CH3

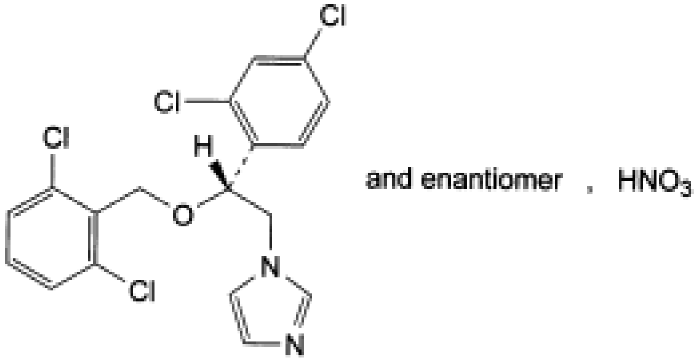
F. (1*R*,3*r*,5*S*,8*r*)-8-methyl-8-(1-methylethyl)-3-[(2-phenylpropenoyl)oxy]-8-azoniabicyclo [3.2.1]octane.

Ph Eur

Isoconazole Nitrate

General Notices

(Ph Eur monograph 1017)



$C_{18}H_{14}Cl_4N_2O,HNO_3$ 479.1 *40036-10-0*

Action and use

Antifungal.

Preparation

Isoconazole Pessaries

Ph Eur

DEFINITION

1-[(2*RS*)-2-[(2,6-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, soluble in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point *(2.2.14)* 178 °C to 182 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *isoconazole nitrate CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 30 mg of *isoconazole nitrate CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 30 mg of *isoconazole nitrate CRS* and 30 mg of *econazole nitrate CRS* in *methanol R*, then dilute to 5 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *ammonium acetate solution R*, *dioxan R*, *methanol R* (20:40:40 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives the reaction of nitrates *(2.3.1)*.

TESTS

Solution S

Dissolve 0.20 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of *isoconazole nitrate CRS* and 2.5 mg of *econazole nitrate CRS* in the mobile phase, then dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve 6.0 g of *ammonium acetate R* in a mixture of 300 ml of *acetonitrile R*, 320 ml of *methanol R* and 380 ml of *water R*.

Flow rate 2 ml/min.

Detection Spectrophotometer at 235 nm.

Equilibration With the mobile phase for about 30 min.

Injection 10 µl.

Run time 1.5 times the retention time of *isoconazole.*

Retention time econazole = about 10 min; isoconazole = about 14 min.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to econazole and isoconazole; if necessary, adjust the composition of the mobile phase.

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to the nitrate ion.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 75 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

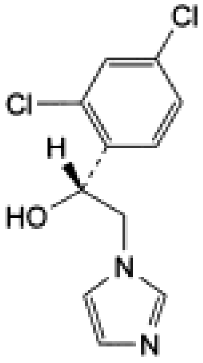
1 ml of *0.1 M perchloric acid* is equivalent to 47.91 mg of $C_{18}H_{15}Cl_4N_3O_4$.

STORAGE

Protected from light.

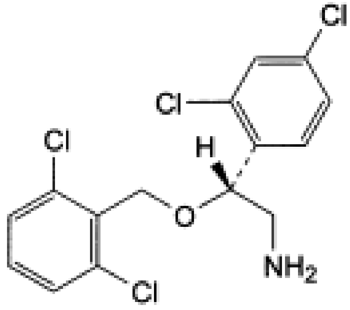
IMPURITIES

Specified impurities *A, B, C.*



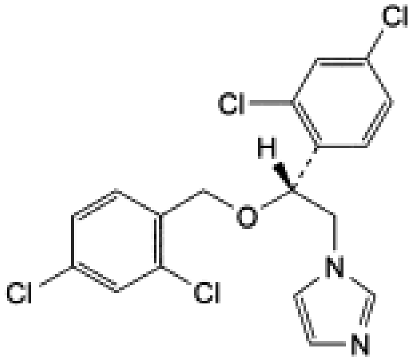
and enantiomer

A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,



and enantiomer

B. (2*RS*)-2-[(2,6-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethanamine,



and enantiomer

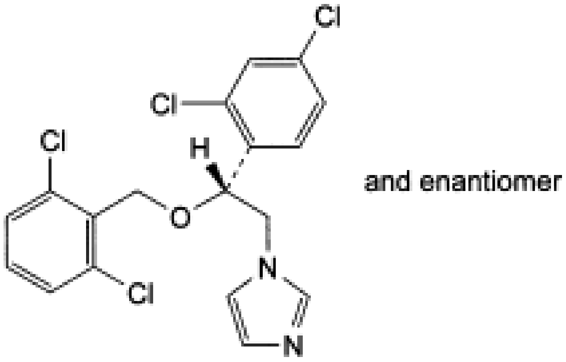
C. 1-[(2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Ph Eur

Isoconazole

General Notices

(Ph Eur monograph 1018)



$C_{18}H_{14}Cl_4N_2O$ 416.1   *27523-40-6*

Action and use

Antifungal.

Ph Eur

DEFINITION

1-[(2*RS*)-2-[(2,6-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, very soluble in methanol, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification   *A, B.*

Second identification   *A, C, D.*

A. Melting point *(2.2.14)*: 111 °C to 115 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *isoconazole CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 30 mg of *isoconazole CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 30 mg of *isoconazole CRS* and 30 mg of *econazole nitrate CRS* in *methanol R*, then dilute to 5 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *ammonium acetate solution R, dioxan R, methanol R* (20:40:40 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.20 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in 3.2 ml of *methanol R*. Add 3.0 ml of *acetonitrile R* and dilute to 10.0 ml with a solution of *ammonium acetate R* (6.0 g in 380 ml of *water R).*

Reference solution (a) Dissolve 2.5 mg of *isoconazole CRS* and 2.5 mg of *econazole nitrate CRS* in the mobile phase, then dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve 6.0 g of *ammonium acetate R* in a mixture of 300 ml of *acetonitrile R*, 320 ml of *methanol R* and 380 ml of *water R*.

Flow rate 2 ml/min.

Detection Spectrophotometer at 235 nm.

Equilibration With the mobile phase for about 30 min.

Injection 10 µl.

Run time 1.5 times the retention time of isoconazole.

Retention time econazole = about 10 min; isoconazole = about 14 min.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to econazole and isoconazole; if necessary, adjust the composition of the mobile phase.

Limits:

— *impurities B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Using 0.2 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from orange-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 41.61 mg of $C_{18}H_{14}Cl_4N_2O$.

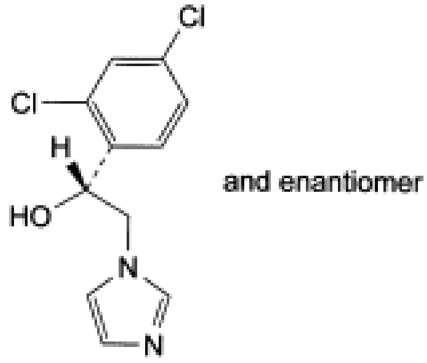
STORAGE

Protected from light.

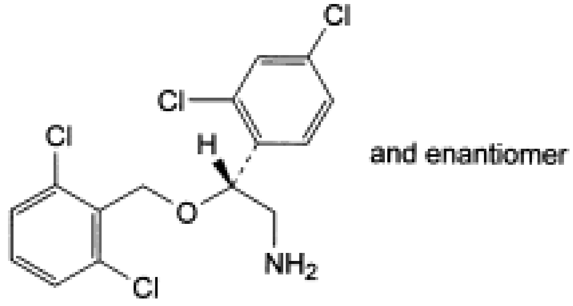
IMPURITIES

Specified impurities *B, C, D.*

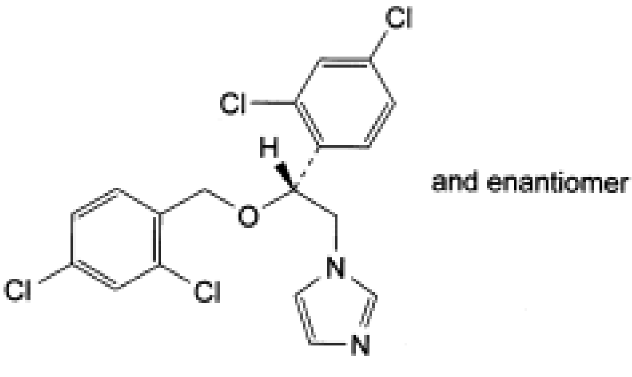
A. deleted,


and enantiomer

B. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,


and enantiomer

C. (2*RS*)-2-[(2,6-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethanamine,


and enantiomer

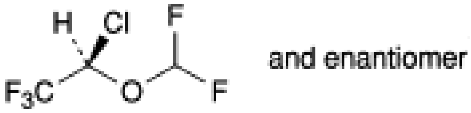
D. 1-[(2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Ph Eur

Isoflurane

General Notices

(Ph Eur monograph 1673)



$C_3H_2ClF_5O$ 184.5   *26675-46-7*

Action and use

General anaesthetic.

Ph Eur

DEFINITION

(2*RS*)-2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane.

CHARACTERS

Appearance

Clear, colourless, mobile, heavy liquid.

Solubility

Practically insoluble in water, miscible with ethanol and trichloroethylene.

bp: about 48 °C.

It is non-flammable.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Examine the substance in the gaseous state.

Comparison *Ph. Eur. reference spectrum of isoflurane.*

TESTS

Acidity or alkalinity

To 20 ml add 20 ml of *carbon dioxide-free water R*, shake for 3 min and allow to stand.

Collect the upper layer and add 0.2 ml of *bromocresol purple solution R*. Not more than 0.1 ml of *0.01 M sodium hydroxide* or 0.6 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Related substances

Gas chromatography *(2.2.28).*

Test solution The substance to be examined.

Reference solution To 80 ml of *ethanol R*, add 1.0 ml of the substance to be examined and 1.0 ml of *acetone R*, avoiding loss by evaporation. Dilute to 100.0 ml with *ethanol R*. Dilute 1.0 ml of the solution to 100.0 ml with *ethanol R*.

Column:
— *material*: fused silica,

— *size*: *l* = 30 m, Ø = 0.32 mm,

— *stationary phase: macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.0 ml/min.

Split ratio 1:25.

Temperature:
— *column*: 35 °C,

— *injection port*: 150 °C,

— *detector*: 250 °C.

Detection Flame ionisation.

Injection 1.0 µl of each solution and 1.0 µl of *ethanol R* as a blank.

Run time Until elution of the ethanol peak in the chromatogram obtained with the reference solution.

Relative retention with reference to isoflurane (retention time = about 3.8 min): acetone = about 0.75.

System suitability Reference solution:

— *resolution*: minimum of 5 between the peaks due to acetone and to isoflurane,

— *repeatability*: maximum relative standard deviation 15.0 per cent for the peak due to isoflurane after 3 injections.

Limits:
— *acetone*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.01 per cent),

— *any other impurity*: not more than the area of the peak due to isoflurane in the chromatogram obtained with the reference solution (0.01 per cent),

— *total*: not more than 3 times the area of the peak due to isoflurane in the chromatogram obtained with the reference solution (0.03 per cent),

— *disregard limit*: 0.1 times the area of the peak due to isoflurane in the chromatogram obtained with the reference solution (0.001 per cent).

Chlorides ***(2.4.4)***

Maximum 10 ppm.

To 10 ml add 10 ml *of 0.01 M sodium hydroxide* and shake for 3 min. To 5 ml of the upper layer add 10 ml of *water R*. The solution complies with the limit test for chlorides.

Fluorides

Maximum 10 ppm.

Determine by potentiometry *(2.2.36, Method I)* using a fluoride-selective indicator-electrode and a silver-silver chloride reference electrode.

Test solution To 10.0 ml in a separating funnel, add 10 ml of a mixture of 30.0 ml of *dilute ammonia R2* and 70.0 ml of *distilled water R*. Shake for 1 min and collect the upper layer. Repeat this extraction procedure twice collecting the upper layer each time. Adjust the combined upper layers to pH 5.2 using *dilute hydrochloric acid R*. Add 5.0 ml of *fluoride standard solution (1 ppm F) R* and dilute to 50.0 ml with *distilled water R.* To 20.0 ml of the solution add 20.0 ml of *total-ionic-strength-adjustment buffer R* and dilute to 50.0 ml with *distilled water R*.

Reference solutions To each of 5.0 ml, 4.0 ml, 3.0 ml, 2.0 ml and 1.0 ml of *fluoride standard solution (10 ppm F) R add* 20.0 ml of *total-ionic-strength-adjustment buffer R* and dilute to 50.0 ml with *distilled water R.*

Carry out the measurements on 20 ml of each solution. Calculate the concentration of fluorides using the calibration curve, taking into account the addition of fluoride to the test solution.

Non-volatile matter

Maximum 200 mg/l.

Evaporate 10.0 ml to dryness with the aid of a stream of cold air and dry the residue at 50 °C for 2 h. The residue weighs a maximum of 2.0 mg.

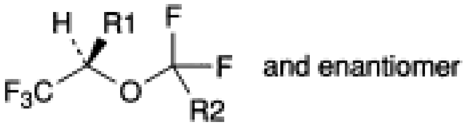
Water ***(2.5.12)***

Maximum 1.0 mg/ml, determined on 10.0 ml.

STORAGE

In an airtight container, protected from light.

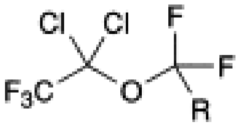
IMPURITIES



A. R1 = H, R2 = Cl: 2-(chlorodifluoromethoxy)-1,1,1-trifluoroethane,

B. R1 = R2 = H: 2-(difluoromethoxy)-1,1,1-trifluoroethane,

C. R1 = R2 = Cl: (2*RS*)-2-chloro-2-(chlorodifluoromethoxy)-1,1,1-trifluoroethane,



D. R = H: 1,1-dichloro-1-(difluoromethoxy)-2,2,2-trifluoroethane,

E. R = Cl: 1,1-dichloro-1-(chlorodifluoromethoxy)-2,2,2-trifluoroethane,

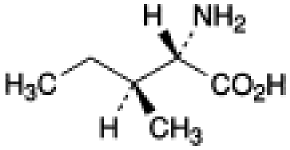
F. acetone.

Ph Eur

Isoleucine

General Notices

(Ph Eur monograph 0770)



$C_6H_{13}NO_2$ 131.2   *73-32-5*

Action and use

Amino acid.

Ph Eur

DEFINITION

Isoleucine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*S*,3*S*)-2-amino-3-methylpentanoic acid, calculated with reference to the dried substance.

CHARACTERS

White or almost white, crystalline powder or flakes, sparingly soluble in water, slightly soluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification   A, C.

Second identification   A, B, D.

A. It complies with the test for specific optical rotation (see Tests).

B. Dissolve 0.5 g in *water R* and dilute to 25 ml with the same solvent. The solution is dextrorotatory.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *isoleucine CRS*. Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 10 ml with the same acid. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 1.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 40.0 to + 43.0, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R.*

Test solution (a) Dissolve 0.10 g of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *isoleucine CRS* in *0.1 M hydrochloric acid* and dilute to 50 ml with the same acid.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R.*

Reference solution (c) Dissolve 10 mg of *isoleucine CRS* and 10 mg of *valine CRS* in *0.1 M hydrochloric acid* and dilute to 25 ml with the same acid.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Chlorides *(2.4.4)*

Dissolve 0.25 g in *water R* and dilute to 15 ml with the same solvent. The solution complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dissolve 0.5 g in 3 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals *(2.4.8)*

2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Using 0.1 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

STORAGE

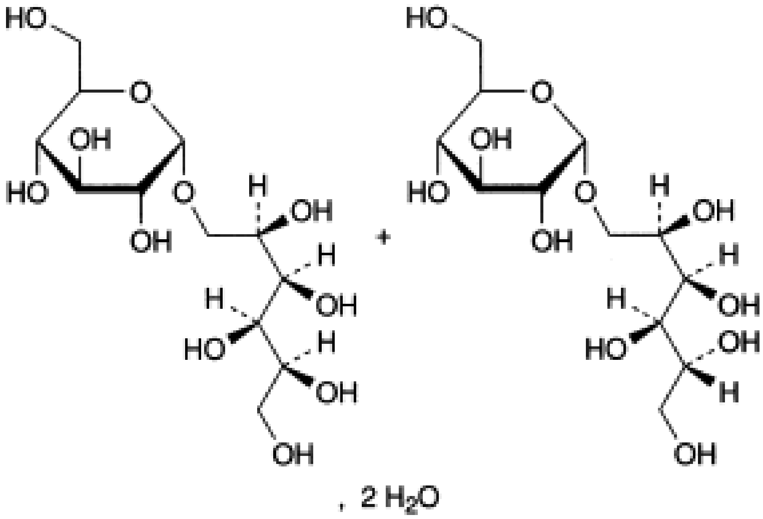
Store protected from light.

Ph Eur

Isomalt

General Notices

(Ph Eur monograph 1531)



$C_{12}H_{24}O_{11}$ 344.3

$C_{12}H_{24}O_{11},2H_2O$ 380.3

Action and use

Sweetening agent.

Ph Eur

DEFINITION

Mixture of 6-*C*-α-D-glucopyranosyl-D-glucitol (6-*C*-α-D-glucopyranosyl-D-sorbitol; 1,6-GPS) and 1-*O*-α-D-glucopyranosyl-D-mannitol (1,1-GPM).

Content

98.0 per cent to 102.0 per cent for the mixture of 1,6-GPS and 1,1-GPM and neither of the 2 components is less than 3.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder or granules.

Solubility

Freely soluble in water, practically insoluble in anhydrous ethanol.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the 2 principal peaks in the chromatogram obtained with reference solution (a).

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *isomalt CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *acetic acid R, propionic acid R, water R, ethyl acetate R, pyridine R* (5:5:10:50:50 *V/V/V/V/V*).

Application 1 µl; thoroughly dry the starting points in warm air.

Development Over a path of 10 cm.

Drying In a current of warm air.

Detection Dip for 3 s in a 1 g/l solution of *sodium periodate R* and dry in a current of hot air; dip for 3 s in a mixture of 1 volume of *acetic acid R,* 1 volume of *anisaldehyde* R, 5 volumes of *sulphuric acid R* and 90 volumes of *anhydrous ethanol R*; dry in a current of hot air until coloured spots become visible; the background colour may be brightened in warm steam; examine in daylight.

Results The chromatogram obtained with the reference solution shows 2 blue-grey spots with R_F values of about 0.13 (1,6-GPS) and 0.16 (1,1-GPM). The chromatogram obtained with the test solution shows principal spots similar in position and colour to the principal spots in the chromatogram obtained with the reference solution.

C. To 3 ml of a freshly prepared 100 g/l solution of *pyrocatechol R* add 6 ml of *sulphuric acid R* while cooling in iced water. To 3 ml of the cooled mixture add 0.3 ml of a 100 g/l solution of the substance to be examined. Heat gently over a naked flame for about 30 s. A pink colour develops.

TESTS

Conductivity *(2.2.38)*

Maximum 20 µS·cm^{-1}.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.3 per cent, expressed as glucose equivalent.

Dissolve 3.3 g in 10 ml of *water R* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that the boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R*. When the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R* as indicator, added towards the end of the titration. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Related products

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 1.00 g of the substance to be examined in 20 ml of *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 1.00 g of *isomalt CRS* in 20 ml of *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dissolve 10.0 mg of *sorbitol CRS* (impurity C) and 10.0 mg of *mannitol CRS* (impurity B) in 20 ml of *water R* and dilute to 100.0 ml with the same solvent.

Precolumn:
— *size*: *l* = 30 mm, Ø = 4.6 mm;

— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);

— *temperature*: 80 ± 1 °C.

Column:
— *size*: *l* = 0.3 m, Ø = 7.8 mm;

— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);

— *temperature*: 80 ± 1 °C.

Mobile phase degassed *water R*.

Flow rate 0.5 ml/min.

Detection Differential refractometer maintained at a constant temperature.

Injection 20 µl of the test solution and reference solution (b).

Run time Until impurity C is completely eluted (about 25 min).

Relative retention with reference to 1,1-GPM (retention time = about 12.3 min): impurity A = about 0.8; 1,6-GPS = about 1.2; impurity B = about 1.6; impurity C = about 2.0.

Limits:
— *impurities B, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 4 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (2 per cent);

— *disregard limit*: 0.2 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (0.1 per cent).

Lead *(2.4.10)*

Maximum 0.5 ppm.

Nickel *(2.4.15)*

Maximum 1 ppm.

Water *(2.5.12)*

Maximum 7.0 per cent, determined on 0.3 g. As solvent, use a mixture of 20 ml of *anhydrous methanol R* and 20 ml of *formamide R* at 50 ± 5 °C.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related products with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of isomalt (1,1-GPM and 1,6-GPS) from the declared content of 1,1-GPM and 1,6-GPS in *isomalt CRS*.

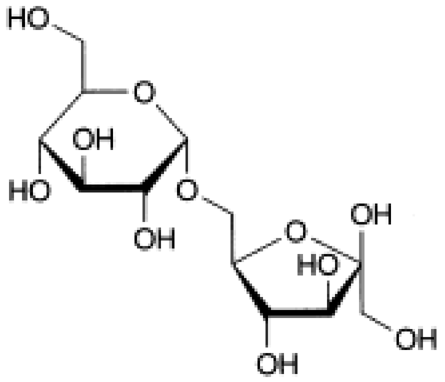
LABELLING

The label states the percentage content of 1,6-GPS and 1,1-GPM.

IMPURITIES

Specified impurities *B, C.*

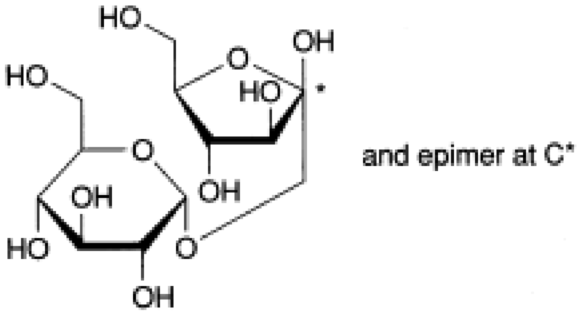
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, D*.



A. 6-*O*-α-D-glucopyranosyl-β-D-*arabino*-hex-2-ulofuranose (isomaltulose),

B. mannitol,

C. sorbitol,

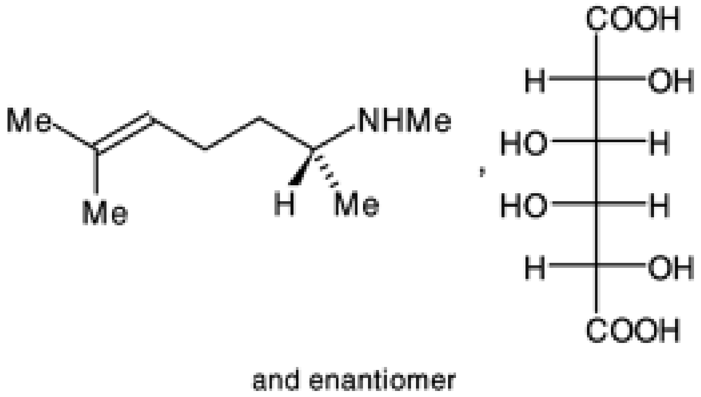

and epimer at C*

D. 1-*C*-α-D-glucopyranosyl-D-*arabino*-hex-2-ulofuranose (trehalulose).

Ph Eur

Isometheptene Mucate

General Notices



$(C_9H_{19}N)_2,C_6H_{10}O_8$ 492.7 *7492-31-1*

Action and use

Adrenoceptor agonist.

DEFINITION

Isometheptene Mucate is (*RS*)-1,5-dimethylhex-4-enyl(methyl)amine *galacto*-2,3,4,5-tetrahydroxyadipate. It contains not less than 98.5% and not more than 100.5% of $(C_9H_{19}N)_2$, $C_6H_{10}O_8$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Very soluble in *water*; slightly soluble in *absolute ethanol*; practically insoluble in *ether*.

IDENTIFICATION

A. Dissolve 0.2 g in 10 ml of *water*, make the solution alkaline with 2M *sodium hydroxide*, extract with 20 ml of *dichloromethane*, dry the dichloromethane layer over *anhydrous sodium sulphate*, filter and evaporate to dryness at a pressure of 2 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of isometheptene *(RS 195)*.

B. Prepare a 5.0% w/v solution of the substance being examined in *carbon-dioxide free water* (solution A) and acidify 10 ml with 6M *hydrochloric acid*, scratch the side of the tube with a glass rod to initiate crystallisation, filter, wash the precipitate with *water* and dry at 105° for 10 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mucic acid *(RS 377)*.

TESTS

Acidity

pH of solution A, 5.4 to 6.6, Appendix V L.

Clarity and colour of solution

Solution A is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Related substances

Dissolve 20 mg of *linalool* (internal standard) in sufficient *dichloromethane* to produce 100 ml (solution B). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 1 ml of 5M *sodium hydroxide* to 5 ml of a 0.050% w/v solution of the substance being examined, extract with 10 ml of solution B followed by 10 ml of *dichloromethane*, combine the dichloromethane layers and filter through *anhydrous sodium sulphate*. For solution (2) dissolve 0.5 g of the substance being examined in 5 ml of *water*, add 1 ml of 5M *sodium hydroxide*, extract with two 10 ml quantities of *dichloromethane*, combine the dichloromethane layers and filter through *anhydrous sodium sulphate*. Prepare solution (3) in the same manner as solution (2) but using 10 ml of solution B and 10 ml of *dichloromethane* in place of the two 10 ml quantities of *dichloromethane*. For solution (4) dissolve 10 mg of *1,5-dimethylhexyl(methyl)amine BPCRS* and 10 mg of *2-methyl-6-methylaminoheptan-2-ol BPCRS* in 50 ml of *dichloromethane*. For solution (5) dissolve 5 mg of the substance being examined and 5 mg of *linalool* in 25 ml of solution (4) and filter, if necessary.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) (Chromosorb W-HP is suitable) coated with 10% w/w of polyethylene glycol (Carbowax 20M is suitable) and 5% w/w of *potassium hydroxide* at a temperature increased from 80° to 140° at a rate of 4° per minute and then maintained at 140° for 10 minutes or the time required for the emergence of the peak due to 2-methyl-6-methylaminoheptan-2-ol and with the inlet port at 190°, the detector at 200° and a flow rate of 40 ml per minute for the carrier gas.

The test is not valid unless the chromatogram obtained with solution (5) resembles the reference chromatogram provided with *1,5-dimethylhexyl(methyl)amine BPCRS* and *2-methyl-6-methylaminoheptan-2-ol BPCRS*.

Calculate the ratio (*a*) of the area of the peak due to isometheptene to that of the peak due to the internal standard in the chromatogram obtained with solution (1). In the chromatogram obtained with solution (3) the ratio of the area of any peak corresponding to 1,5-dimethylhexyl-(methyl)amine to the area of the peak due to the internal standard is not greater than 2*a*, the ratio of the area of any other *secondary peak* to the area of the peak due to the internal standard is not greater than *a* and the ratio of the sum of the areas of any secondary peaks to the area of the peak due to the internal standard is not greater than 3*a*.

Loss on drying

When dried to constant weight over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

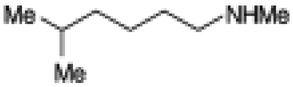
ASSAY

Dissolve 0.2 g in 25 ml of *water* and add 20 ml of 0.0167M *potassium bromate VS*, 10 ml of a 10% w/v solution of *potassium bromide* and 8 ml of *hydrochloric acid*. Allow to stand for 5 minutes in a stoppered flask, add 10 ml of *dilute potassium iodide solution*, allow to stand for 5 minutes and titrate the resulting solution with 0.1M *sodium thiosulphate VS* using *starch solution* as indicator. Repeat the procedure using 25 ml of *water* in place of the solution of the substance being examined. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.0167M *potassium bromate VS* is equivalent to 12.32 mg of $(C_9H_{19}N)_2,C_6H_{10}O_8$.

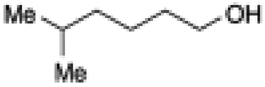
STORAGE

Isometheptene Mucate should be kept in an airtight container and protected from light.

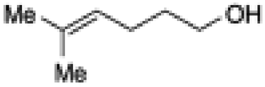
IMPURITIES



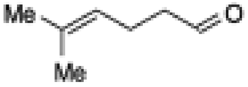
A. 1,5-dimethylhexyl(methyl)amine,



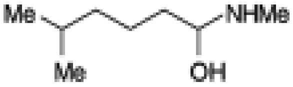
B. 2-methylheptan-6-ol,



C. 2-methylhept-2-en-6-ol,



D. 2-methylhept-2-en-6-one,

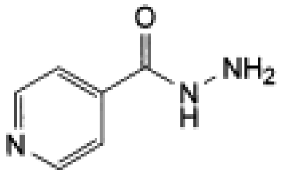


E. 2-methyl-6-methylaminoheptan-2-ol.

Isoniazid

General Notices

(Ph Eur monograph 0146)



$C_6H_7N_3O$ 137.1 *54-85-3*

Action and use

Antituberculosis drug.

Preparations

Isoniazid Injection

Isoniazid Tablets

Ph Eur

DEFINITION

Isoniazid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of pyridine-4-carbohydrazide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, sparingly soluble in alcohol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. Melting point *(2.2.14)*: 170 °C to 174 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *isoniazid CRS*.

C. Dissolve 0.1 g in 2 ml of *water R* and add 10 ml of a warm 10 g/l solution of *vanillin R*. Allow to stand and scratch the wall of the test tube with a glass rod. A yellow precipitate is formed, which, after recrystallisation from 5 ml of *alcohol (70 per cent V/V) R* and drying at

100 °C to 105 °C, melts *(2.2.14)* at 226 °C to 231 °C.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1*) and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 6.0 to 8.0.

Hydrazine and related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in a mixture of equal volumes of *acetone R* and *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution Dissolve 50.0 mg of *hydrazine sulphate R* in 50 ml of *water R* and dilute to 100.0 ml with *acetone R*. To 10.0 ml of this solution add 0.2 ml of the test solution and dilute to 100.0 ml with a mixture of equal volumes of *acetone R* and *water R*.

Apply separately to the plate 5 µl of each solution and develop over a path of 15 cm using a mixture of 10 volumes of *water R*, 20 volumes of *acetone R,* 20 volumes of *methanol R* and 50 volumes of *ethyl acetate R.* Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.2 per cent). Spray the plate with *dimethylaminobenzaldehyde solution R1*. Examine in daylight. An additional spot, corresponding to hydrazine, appears in the chromatogram obtained with the reference solution. Any corresponding spot in the chromatogram obtained with the test solution is not more intense than the spot corresponding to hydrazine in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals *(2.4.8)*

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32*)

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in *water R* and dilute to 100.0 ml with the same solvent. To 20.0 ml of the solution add 100 ml of *water R,* 20 ml of *hydrochloric acid R*, 0.2 g of *potassium bromide R* and 0.05 ml of *methyl red solution R*. Titrate dropwise with *0.0167 M potassium bromate*, shaking continuously, until the red colour disappears.

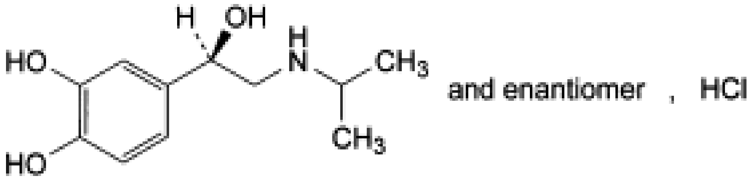
1 ml of *0.0167 M potassium bromate* is equivalent to 3.429 mg of $C_6H_7N_3O$.

Ph Eur

Isoprenaline Hydrochloride

General Notices

(Ph Eur monograph 1332)



$C_{11}H_{17}NO_3,HCl$ 247.7 *51-30-9*

Action and use

Adrenoceptor agonist.

Preparation

Isoprenaline Injection

Ph Eur

DEFINITION

(1*RS*)-1-(3,4-Dihydroxyphenyl)-2-[(1-methylethyl)amino]ethanol hydrochloride.

Content

98.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification B, C, E.

Second identification A, C, D, E.

A. Melting point *(2.2.14)*: 165 °C to 170 °C, with decomposition.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *isoprenaline hydrochloride CRS.*

C. Optical rotation (see Tests).

D. To 0.1 ml of solution S (see Tests) add 0.05 ml of *ferric chloride solution R1* and 0.9 ml of *water R*. A green colour is produced. Add dropwise *sodium hydrogen carbonate solution R*. The colour becomes blue then red.

E. To 0.5 ml of solution S add 1.5 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Prepare the solutions immediately before use.

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 or BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.3 to 5.5.

Mix 5 ml of solution S and 5 ml of *carbon dioxide-free water R*.

Optical rotation *(2.2.7)*

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Test solution (b) Dilute 0.5 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of *isoprenaline hydrochloride CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 2.5 mg of *orciprenaline sulphate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (c) To 1.0 ml of test solution (b) add 1.0 ml of reference solution (b) and dilute to 20.0 ml with the mobile phase.

Column:

— *size*: l = 0.125 m, Ø = 4.0 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R,* 11.5 g/l solution of *phosphoric acid R* (5:95 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (c).

Run time 7 times the retention time of isoprenaline.

Retention time Isoprenaline = about 3 min; if necessary, adjust the concentration of methanol in the mobile phase.

System suitability Reference solution (c):

— *resolution*: minimum 3 between the peaks due to isoprenaline and orciprenaline;

— *signal-to-noise ratio*: minimum 3 for the peak due to isoprenaline.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 15-25 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.150 g in 10 ml of *anhydrous formic acid R* and add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

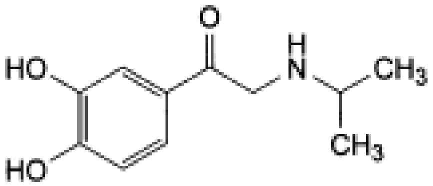
1 ml of *0.1 M perchloric acid* is equivalent to 24.77 mg of $C_{11}H_{18}ClNO_3$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities *A.*



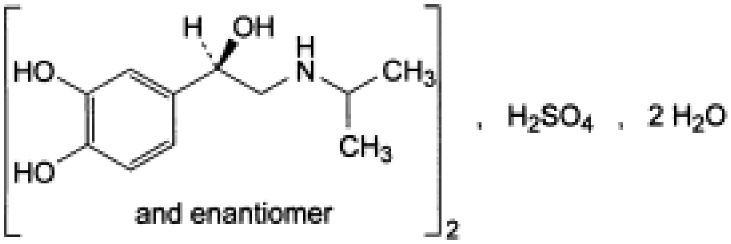
A. 1-(3,4-dihydroxyphenyl)-2-[(1-methylethyl)amino]ethanone.

Ph Eur

Isoprenaline Sulphate

General Notices

(Ph Eur monograph 0502)



$C_{11}H_{17}NO_3,H_2SO_4,2H_2O$ 556.6 *6700-39-6*

Action and use

Adrenoceptor agonist.

Ph Eur

DEFINITION

Bis[(1*RS*)-1-(3,4-dihydroxyphenyl)-2-[(1-methylethyl)amino]ethanol] sulphate dihydrate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

mp

About 128 °C, with decomposition.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Dissolve 0.5 g in 1.5 ml of *water R* and add 3.5 ml of *2-propanol R*. Scratch the wall of the tube with a glass rod to initiate crystallisation. Collect the crystals and dry *in vacuo* at 60 °C over *diphosphorus pentoxide R*.

Comparison Repeat the operations using 0.5 g of *isoprenaline sulphate CRS*.

B. To 0.1 ml of solution S (see Tests) add 0.9 ml of *water R* and 0.05 ml of *ferric chloride solution R1*. A green colour is produced. Add dropwise *sodium hydrogen carbonate solution R*. The colour becomes blue and then red.

C. Dilute 1 ml of solution S to 10 ml with *water R* and add 0.25 ml of *silver nitrate solution R1*. A shining, grey, fine precipitate is formed within 10 min and the solution becomes pink.

D. Solution S gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent. Use within 2 h of preparation.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.3 to 5.5.

Dilute 5 ml of solution S to 10 ml with *carbon dioxide-free water R*.

Isoprenalone

The absorbance *(2.2.25)* is not greater than 0.20 at 310 nm.

Dissolve 0.20 g in *0.005 M sulphuric acid* and dilute to 100.0 ml with the same acid.

Water *(2.5.12)*

5.0 per cent to 7.5 per cent, determined on 0.200 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 20 ml of *anhydrous acetic acid R*, warming gently if necessary and add 20 ml of *methyl isobutyl ketone R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 52.06 mg of $C_{22}H_{36}N_2O_{10}S$.

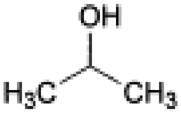
STORAGE

In an airtight container, protected from light.

Isopropyl Alcohol

General Notices

(Ph Eur monograph 0970)



C_3H_8O 60.1 *67-63-0*

Ph Eur

DEFINITION

Propan-2-ol.

CHARACTERS

Appearance

Clear, colourless liquid.

Solubility

Miscible with water and with alcohol.

IDENTIFICATION

A. Relative density *(2.2.5)*: 0.785 to 0.789.

B. Refractive index *(2.2.6)*: 1.376 to 1.379.

C. To 1 ml add 2 ml of *potassium dichromate solution R* and 1 ml of *dilute sulphuric acid R*. Boil. Vapour is produced which changes the colour of a piece of filter paper impregnated with *nitrobenzaldehyde solution R* to green. Moisten the filter paper with *dilute hydrochloric acid R*. The colour changes to blue.

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and colourless *(2.2.2, Method II).* Dilute 1 ml to 20 ml with *water R.* After 5 min, the solution is clear *(2.2.1)*.

Acidity or alkalinity

Gently boil 25 ml for 5 min. Add 25 ml of *carbon dioxide-free water R* and allow to cool protected from carbon dioxide in the air. Add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.6 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pale pink.

Absorbance *(2.2.25)*

Maximum 0.30 at 230 nm, 0.10 at 250 nm, 0.03 at 270 nm, 0.02 at 290 nm and 0.01 at 310 nm.

The absorbance is measured between 230 nm and 310 nm using *water R* as the compensation liquid. The absorption curve is smooth.

Benzene and related substances

Gas chromatography *(2.2.28*).

Test solution (a) The substance to be examined.

Test solution (b) Dilute 1.0 ml of *2-butanol R1* to 50.0 ml with test solution (a). Dilute 5.0 ml of the solution to 100.0 ml with test solution (a).

Reference solution (a) Dilute 0.5 ml of *2-butanol R1* and 0.5 ml of *propanol R* to 50.0 ml with test solution (a). Dilute 5.0 ml of the solution to 50.0 ml with test solution (a).

Reference solution (b) Dilute 100 µl of *benzene R* to 100.0 ml with test solution (a). Dilute 0.20 ml of the solution to 100.0 ml with test solution (a).

Column:

— *material*: fused silica,

— *size*: *l* = 30 m, Ø = 0.32 mm,

— *stationary phase: poly[(cyanopropyl)(phenyl)][dimethyl]siloxane R* (film thickness 1.8 µm).

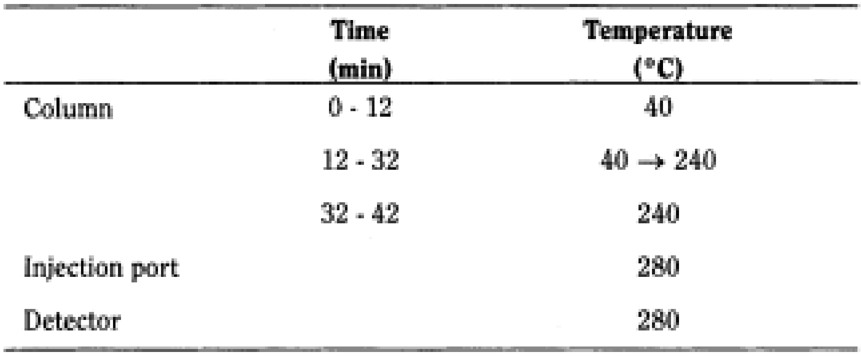
Carrier gas *helium for chromatography R.*

Auxiliary gas *nitrogen for chromatography R* or *helium for chromatography R*.

Linear velocity 35 cm/s.

Split ratio 1:5.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 12	40
	12 - 32	40 → 240
	32 - 42	240
Injection port		280
Detector		280

Detection Flame ionisation.

Injection 1 µl.

Retention time benzene = about 10 min.

System suitability Reference solution (a):

— *resolution*: minimum of 10 between the first peak (propanol) and the second peak (2-butanol).

Limits

— *benzene* (test solution (a)): not more than half of the area of the corresponding peak in the chromatogram obtained with reference solution (b) (2 ppm), after the sensitivity has been adjusted so that the height of the peak due to benzene in the chromatogram obtained with reference solution (b) represents at least 10 per cent of the full scale of the recorder.

— *total of impurities apart from 2-butanol* (test solution (b)): not more than 3 times the area of the peak due to 2-butanol in the chromatogram obtained with test solution (b) (0.3 per cent), after the sensitivity has been adjusted so that the height of the 2 peaks following the principal peak in the chromatogram obtained with reference solution (a) represents at least 50 per cent of the full scale of the recorder.

Peroxides

In a 12 ml test-tube with a ground-glass stopper and a diameter of about 15 mm, introduce 8 ml of *potassium iodide and starch solution R.* Fill completely with the substance to be examined. Shake vigorously and allow to stand protected from light for 30 min. No colour develops.

Non-volatile substances

Maximum 20 ppm.

Evaporate 100 g to dryness on a water-bath *after having verified that it complies with the test for peroxides* and dry in an oven at 100-105 °C. The residue weighs a maximum of 2 mg.

Water ***(2.5.12)***

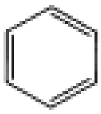
Maximum 0.5 per cent, determined on 5.0 g.

STORAGE

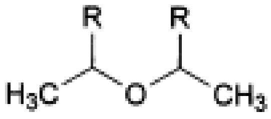
Protected from light.

IMPURITIES

A. acetone,



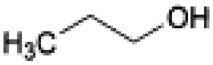
B. benzene,



C. R = CH_3: 2-(1-methylethoxy)propane (diisopropyl ether),

D. R = H: ethoxyethane (diethyl ether),

E. CH_3-OH: methanol,



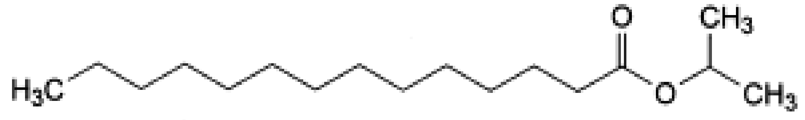
F. propan-1-ol (*n*-propanol).

Ph Eur

Isopropyl Myristate

General Notices

(Ph Eur monograph 0725)



$C_{17}H_{34}O_2$

$C_{17}H_{34}O_2$ 270.5 *110-27-0*

Action and use

Excipient.

Ph Eur

DEFINITION

1-Methylethyl tetradecanoate together with variable amounts of other fatty acid isopropyl esters.

Content

Minimum 90.0 per cent of $C_{17}H_{34}O_2$.

CHARACTERS

Appearance

Clear, colourless, oily liquid.

Solubility

Immiscible with water, miscible with ethanol (96 per cent), with methylene chloride, with fatty oils and with liquid paraffin.

Relative density

About 0.853.

IDENTIFICATION

First identification B.

Second identification A, C.

A. It complies with the test for saponification value (see Tests).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

C. Superpose 2 ml of a 1 g/l solution in *ethanol (96 per cent) R* on a freshly prepared solution of 20 mg of *dimethylaminobenzaldehyde R* in 2 ml of *sulphuric acid R*. After 2 min, a yellowish-red colour appears at the junction of the 2 liquids and gradually becomes red.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Dissolve 2.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Refractive index *(2.2.6)*

1.434 to 1.437.

Viscosity *(2.2.9)*

5 mPa·s to 6 mPa·s.

Acid value *(2.5.1)*

Maximum 1.0.

Iodine value *(2.5.4)*

Maximum 1.0.

Saponification value *(2.5.6)*

202 to 212.

Water *(2.5.12)*

Maximum 0.1 per cent, determined on 5.0 g.

Total ash *(2.4.16)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 50.0 mg of *tricosane R* in *heptane R* and dilute to 250.0 ml with the same solvent.

Test solution Dissolve 20.0 mg of the substance to be examined in the internal standard solution and dilute to 100.0 ml with the same solution.

Reference solution Dissolve 20.0 mg of *isopropyl tetradecanoate CRS* in the internal standard solution and dilute to 100.0 ml with the same solution.

Column:

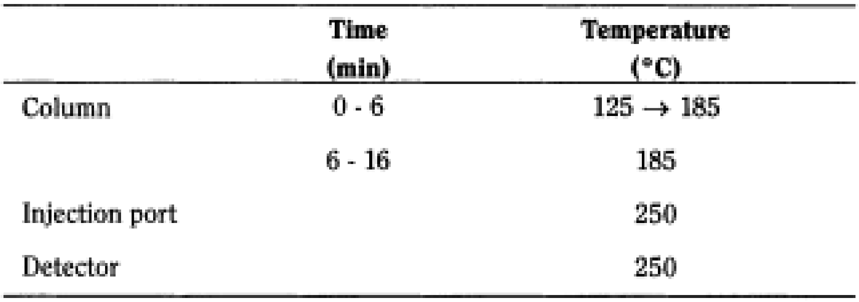
- *material*: fused silica,
- *size*: *l* = 50 m, Ø = 0.2 mm,
- *stationary phase: poly(cyanopropyl)siloxane R* (film thickness 0.2 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 ml/min.

Split ratio 1:40.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	125 → 185
	6 - 16	185
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 2 µl.

Calculate the percentage content of $C_{17}H_{34}O_2$ in the substance to be examined.

STORAGE

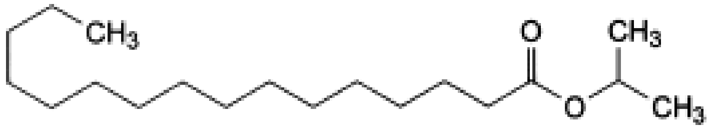
Protected from light.

Ph Eur

Isopropyl Palmitate

General Notices

(Ph Eur monograph 0839)



$C_{19}H_{38}O_2$ 298.5 *142-91-6*

Action and use

Excipient.

Ph Eur

DEFINITION

1-Methylethyl hexadecanoate together with varying amounts of other fatty acid isopropyl esters.

Content

Minimum 90.0 per cent of $C_{19}H_{38}O_2$.

CHARACTERS

Appearance

Clear, colourless, oily liquid.

Solubility

Immiscible with water, miscible with ethanol (96 per cent), with methylene chloride, with fatty oils and with liquid paraffin.

Relative density

About 0.854.

IDENTIFICATION

First identification B.

Second identification A, C.

A. It complies with the test for saponification value (see Tests).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

C. Superpose 2 ml of a 1 g/l solution in *ethanol (96 per cent) R* on a freshly prepared solution of 20 mg of *dimethylaminobenzaldehyde R* in 2 ml of *sulphuric acid R.* After 2 min, a yellowish-red colour appears at the junction of the 2 liquids which gradually becomes red.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II).*

Dissolve 2.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Refractive index *(2.2.6)*

1.436 to 1.440.

Viscosity *(2.2.9)*

5 mPa·s to 10 mPa·s.

Acid value *(2.5.1)*

Maximum 1.0.

Iodine value *(2.5.4)*

Maximum 1.0.

Saponification value *(2.5.6)*

183 to 193.

Water *(2.5.12)*

Maximum 0.1 per cent, determined on 5.0 g.

Total ash *(2.4.16)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 50.0 mg of *tricosane R* in *heptane R* and dilute to 250.0 ml with the same solvent.

Test solution Dissolve 20.0 mg of the substance to be examined in the internal standard solution and dilute to 100.0 ml with the same solution.

Reference solution Dissolve 20.0 mg of *isopropyl hexadecanoate CRS* in the internal standard solution and dilute to 100.0 ml with the same solution.

Column:

— *material:* fused silica,

— *size*: *l* = 50 m, Ø = 0.2 mm,

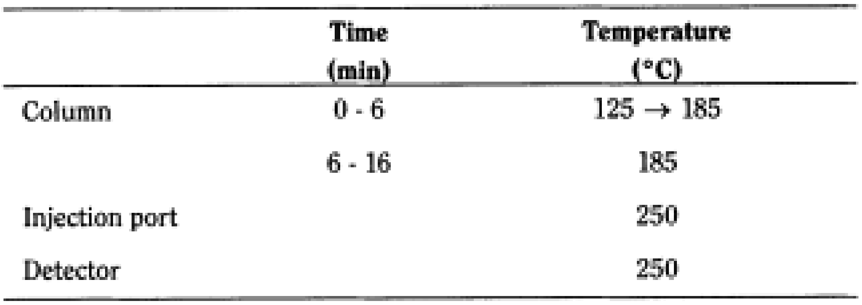
— *stationary phase: poly(cyanopropyl)siloxane R* (film thickness 0.2 µm).

Carrier gas helium for chromatography R.

Flow rate 1 ml/min.

Split ratio 1:40.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	125 → 185
	6 - 16	185
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 2 µl.

Calculate the percentage content of $C_{19}H_{38}O_2$ in the substance to be examined.

STORAGE

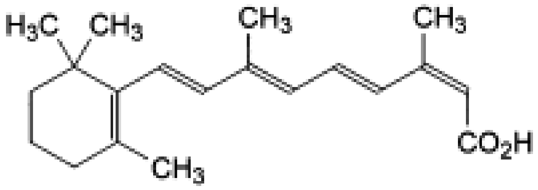
Protected from light.

Ph Eur

Isotretinoin

General Notices

(Ph Eur monograph 1019)



$C_{20}H_{28}O_2$ 300.4 *4759-48-2*

Action and use

Vitamin A analogue (retinoid); treatment of acne.

Preparations

Isotretinoin Capsules

Isotretinoin Gel

Ph Eur

DEFINITION

Isotretinoin contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid, calculated with reference to the dried substance.

CHARACTERS

A yellow or light-orange, crystalline powder, practically insoluble in water, soluble in methylene chloride, slightly soluble in alcohol. It is sensitive to air, heat and light, especially in solution.

Carry out all operations as rapidly as possible and avoid exposure to actinic light; use freshly prepared solutions.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Dissolve 75.0 mg in 5 ml of *methylene chloride R* and dilute immediately to 100.0 ml with acidified 2-propanol (prepared by diluting 1 ml of *0.01 M hydrochloric acid* to 1000 ml with

2-propanol R). Dilute 5.0 ml of this solution to 100.0 ml with the acidified 2-propanol (solution A). Dilute 5.0 ml of solution A to 50.0 ml with the acidified 2-propanol. Examined between 300 nm and 400 nm *(2.2.25)*, the solution shows an absorption maximum at 354 nm. The specific absorbance at the maximum is 1290 to 1420.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *isotretinoin CRS*. Examine the substances prepared as discs.

C. Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel* GF_{254} *plate R*.

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *isotretinoin CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *isotretinoin CRS* and 10 mg of *tretinoin CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 2 volumes of *glacial acetic acid R*, 4 volumes of *acetone R*, 40 volumes of *peroxide-free ether R* and 54 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

D. Dissolve about 5 mg in 2 ml of *antimony trichloride solution R*. An intense red colour develops and later becomes violet.

TESTS

Related substances

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *tretinoin CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 25.0 ml with *methanol R*.

Reference solution (c) Mix 1.0 ml of reference solution (a) with 0.5 ml of the test solution and dilute to 25.0 ml with *methanol R*.

Reference solution (d) Dilute 0.5 ml of the test solution to 100.0 ml with *methanol R.*

The chromatographic procedure may be carried out using:

— a stainless steel column 0.15 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (3 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture of 5 volumes of *glacial acetic acid R*, 225 volumes of *water R* and 770 volumes of *methanol R*.

— as detector a spectrophotometer set at 355 nm.

Inject separately 10 µl of each of reference solutions (b), (c) and (d) and of the test solution. Adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 70 per cent of the full scale of the recorder. The test is not valid unless the resolution between the peaks due to isotretinoin and tretinoin in the chromatogram obtained with reference solution (c) is at least 2.0. In the chromatogram obtained with the test solution: the area of any peak due to tretinoin is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent); the sum of the areas of any peaks, apart from the principal peak and any peak due to tretinoin, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

Heavy metals *(2.4.8)*

0.5 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying *in vacuo* for 16 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 70 ml of *acetone R*. Titrate with *0.1 M tetrabutylammonium hydroxide* determining the end-point potentiometrically (*2.2.20).*

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 30.04 mg of $C_{20}H_{28}O_2$.

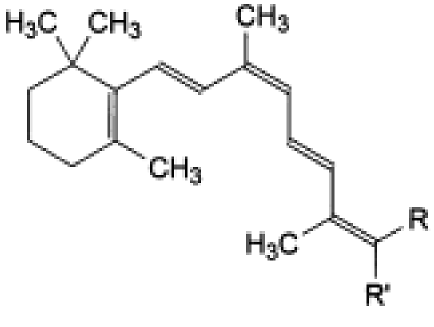
STORAGE

Store in an airtight container, protected from light, at a temperature not exceeding 25 °C.

It is recommended that the contents of an opened container be used as soon as possible and any unused part be protected by an atmosphere of an inert gas.

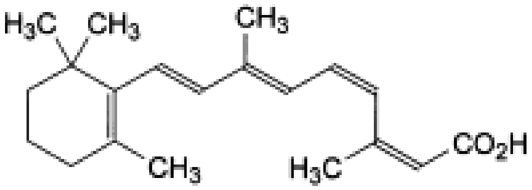
IMPURITIES

A. tretinoin,



B. R = CO_2H, R′ = H: (2*Z*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (9,13-di-*cis*-retinoic acid),

D. R = H, R′ = CO_2H: (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (9-*cis*-retinoic acid),


H3C
CH3
CH3
CH3
H3C
CO2H

C. (2*Z*,4*Z*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (11,13-di-*cis*-retinoic acid),

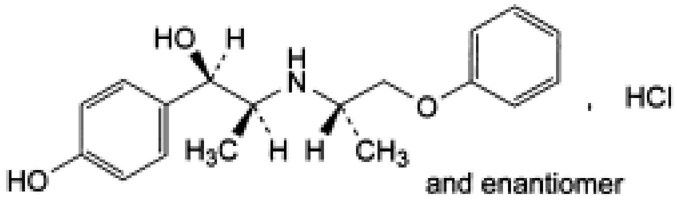
E. oxidation products of isotretinoin.

Ph Eur

Isoxsuprine Hydrochloride

General Notices

(Ph Eur monograph 1119)



$C_{18}H_{23}NO_3,HCl$ 337.8 *579-56-6*

Action and use

Beta$_2$-adrenoceptor agonist.

Ph Eur

DEFINITION

(1*RS*,2*SR*)-1-(4-Hydroxyphenyl)-2-[[(1*SR*)-1-methyl-2-phenoxyethyl]amino]propan-1-ol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

mp

About 205 °C, with decomposition.

IDENTIFICATION

First identification B, E.

Second identification *A, C, D, E*.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in *0.1 M hydrochloric acid* and dilute to 50.0 ml with the same acid. Dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*.

Spectral range 230-350 nm.

Absorption maxima At 269 nm and 275 nm.

Resolution (2.2.25) Minimum 1.7 for the absorbance ratio.

Specific absorbance at the absorption maxima:
— at 269 nm: 71 to 74;

— at 275 nm: 70 to 73.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *isoxsuprine hydrochloride CRS.*

If the spectra obtained show differences, dissolve 50 mg of the substance to be examined and of the reference substance separately in 2 ml of *methanol R*, add 15 ml of *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *isoxsuprine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (0.25:15:85 *V/V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In a current of warm air.

Detection Spray with a 10 g/l solution of *potassium permanganate R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 1 ml of solution S (see Tests) add 0.05 ml of *copper sulphate solution R* and 0.5 ml of *strong sodium hydroxide solution R*. The solution becomes blue. Add 1 ml of *ether R* and shake. Allow to separate. The upper layer remains colourless.

E. 2 ml of solution S gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.50 g, with gentle heating if necessary, in *carbon dioxide-free water R*, cool and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II*).

pH *(2.2.3)*

4.5 to 6.0 for solution S.

Optical rotation *(2.2.7)*

- 0.05 ° to + 0.05 °, determined on solution S.

Phenones

Maximum 1.0 per cent, calculated as impurity B.

Dissolve 10.0 mg in *water R* and dilute to 100.0 ml with the same solvent. The absorbance *(2.2.25)* of the solution measured at the absorption maximum at 310 nm is not greater than 0.10.

Related substances

Gas chromatography *(2.2.28)*. *Prepare the solutions immediately before use.*

Internal standard solution (a) Dissolve 0.1 g of *hexacosane R* in *trimethylpentane R* and dilute to 20 ml with the same solvent.

Internal standard solution (b) Dilute 1 ml of internal standard solution (a) to 50 ml with *trimethylpentane R*.

Test solution To 10.0 mg of the substance to be examined, add 0.5 ml of *N-trimethylsilylimidazole R*. Heat to 65 °C for 10 min. Allow to cool, then add 2.0 ml of the internal standard solution (b) and 2.0 ml of *water R.* Shake. Use the upper layer.

Reference solution (a) To 10.0 mg of the substance to be examined, add 0.5 ml of *N-trimethylsilylimidazole R*. Heat to 65 °C for 10 min. Allow to cool, then add 2.0 ml of the internal standard solution (a) and 2.0 ml of *water R*. Shake. Dilute 1.0 ml of the upper layer to 50.0 ml with *trimethylpentane R*.

Reference solution (b) To 10.0 mg of the substance to be examined, add 0.5 ml of *N-trimethylsilylimidazole R*. Heat to 65 °C for 10 min. Allow to cool, then add 2.0 ml of *trimethylpentane R* and 2.0 ml of *water R.* Shake. Use the upper layer.

Column:

— *material: glass*;

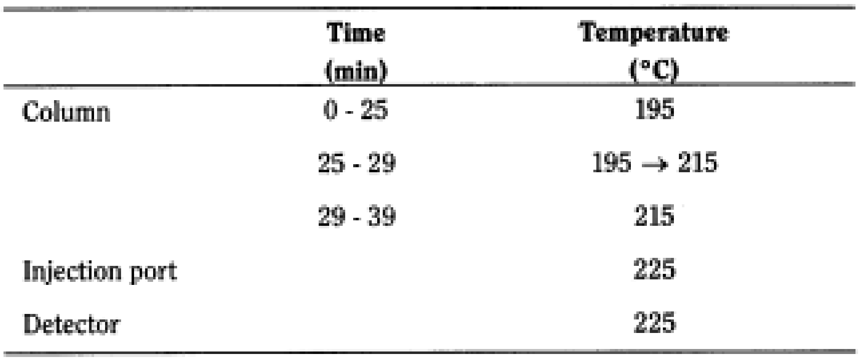
— *size*: *l* = 1.5 m, Ø = 4 mm;

— *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (125-135 µm) impregnated with 3 per cent *m/m* of *poly(dimethyl)siloxane R*.

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 25	195
	25 - 29	195 → 215
	29 - 39	215
Injection port		225
Detector		225

Detection Flame ionisation.

Injection 1 µl.

Elution order Isoxsuprine, hexacosane.

System suitability:
- *resolution*: minimum 5.0 between the peaks due to isoxsurpine and hexacosane in the chromatogram obtained with reference solution (a);
- in the chromatogram obtained with reference solution (b), there is no peak with the same retention time as the internal standard.

Limit:
- *total*: calculate the ratio (*R*) of the area of the peak due to the trimethylsilyl derivative of isoxsuprine to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (a); from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (2.0 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 80 ml of *ethanol (96 per cent) R* and add 1.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 33.78 mg of $C_{18}H_{24}ClNO_3$.

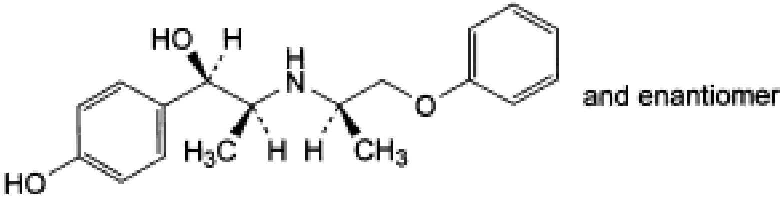
STORAGE

Protected from light.

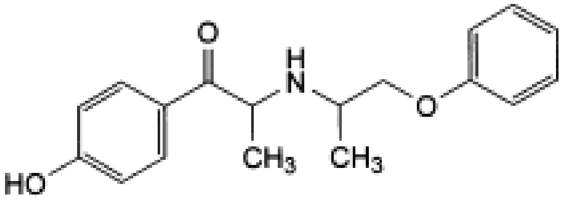
IMPURITIES

Specified impurities *B.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A*.



A. (1*RS*,2*SR*)-1-(4-hydroxyphenyl)-2-[[(1*RS*)-1-methyl-2-phenoxyethyl]amino]propan-1-ol,



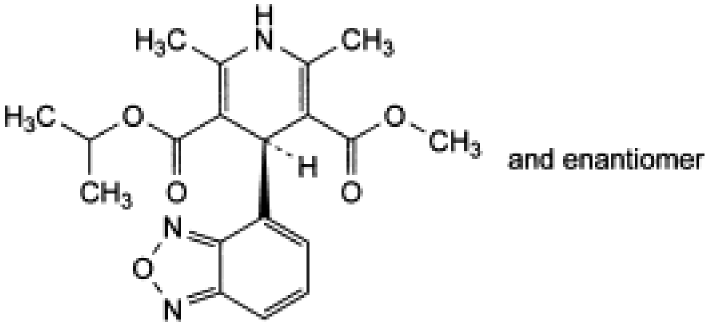
B. 1-(4-hydroxyphenyl)-2-[(1-methyl-2-phenoxyethyl)amino]propan-1-one.

Ph Eur

Isradipine

General Notices

(Ph Eur monograph 2110)



$C_{19}H_{21}N_3O_5$ 371.4 *75695-93-1*

Action and use

Calcium channel blocker.

Preparation

Isradipine Tablets

Ph Eur

DEFINITION

Methyl 1-methylethyl (4*RS*)-4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in methanol.

mp

About 168 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison isradipine CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in 1 ml of *methanol R*, using an ultrasonic bath if necessary, and dilute to 25.0 ml with the mobile phase.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in 2 ml of *methanol R* and dilute to 250.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 2 mg of the substance to be examined and 2 mg of *isradipine impurity D CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *isradipine CRS* in 2 ml of *methanol R* and dilute to 250.0 ml with the mobile phase.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, tetrahydrofuran R, water R* (125:270:625 *V/V/V*).

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (b).

Run time 5 times the retention time of isradipine.

Identification of impurities Use the chromatogram supplied with *isradipine CRS* to identify the peaks due to impurities A and B.

Relative retention with reference to isradipine (retention time = about 7 min): impurity A = about 0.8; impurity D = about 0.9; impurity B = about 1.8.

System suitability Reference solution (b):

- *resolution*: minimum 2.0 between the peaks due to isradipine and impurity D.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity D by 1.4;
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity B*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent);

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.2 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 326 nm.

Injection Test solution (b) and reference solution (c).

Run time Twice the retention time of isradipine.

Calculate the percentage content of isradipine from the areas of the peaks and the declared content of *isradipine CRS*.

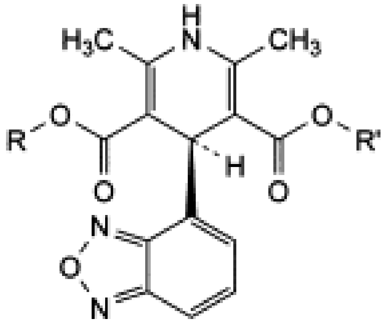
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, D.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C, E.*

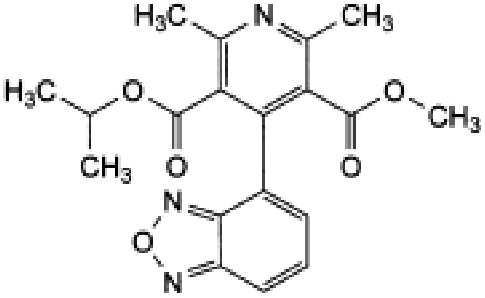


and enantiomer

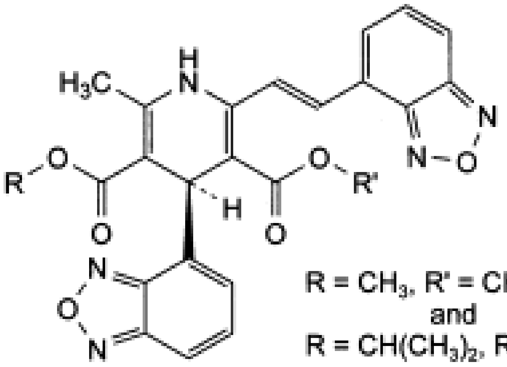
A. R = C_2H_5, R' = CH_3: ethyl methyl (4*RS*)-4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate,

B. R = R' = $CH(CH_3)_2$: bis(1-methylethyl) (4*RS*)-4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1, 4-dihydropyridine-3,5-dicarboxylate,

C. R = R' = CH_3: dimethyl (4*RS*)-4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate,



D. methyl 1-methylethyl 4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate,



its (*Z*) isomer and their enantiomers

R = CH_3, R' = $CH(CH_3)_2$
and
R = $CH(CH_3)_2$, R' = CH_3

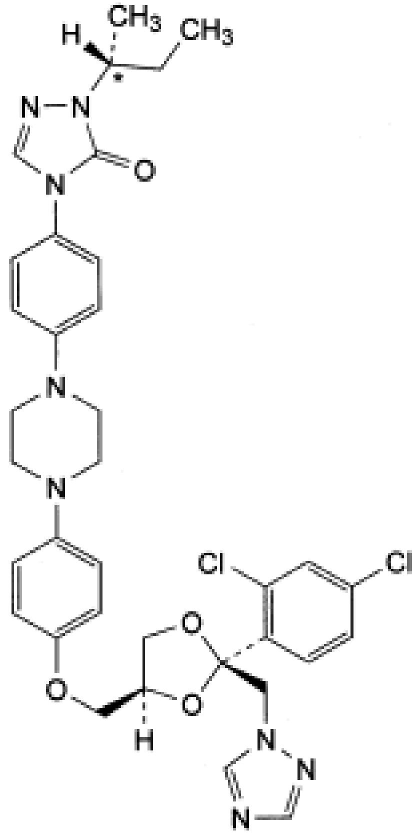
E. methyl 1-methylethyl (4*RS*)-4-(2,1,3-benzoxadiazol-4-yl)-2-[(*EZ*)-2-(2,1,3-benzoxadiazol-4-yl)ethenyl]-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.

Ph Eur

Itraconazole

General Notices

(Ph Eur monograph 1335)



its epimer at C*

and their enantiomers

$C_{35}H_{38}Cl_2N_8O_4$ 706 *84625-61-6*

Action and use

Antifungal.

Ph Eur

DEFINITION

Itraconazole contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of 4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in tetrahydrofuran, very slightly soluble in alcohol.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)*: 166 °C to 170 °C.

B. Examine by infrared spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *itraconazole CRS*. Examine the substance prepared as discs.

C. Examine by thin-layer chromatography *(2.2.27)*, using a suitable *octadecylsilyl silica gel* as the coating substance.

Test solution Dissolve 30 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (a) Dissolve 30 mg of *itraconazole CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (b) Dissolve 30 mg of *itraconazole CRS* and 30 mg of *ketoconazole CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Apply to the plate 5 µl of each solution. Develop in an unsaturated tank over a path of 10 cm using a mixture of 20 volumes of *ammonium acetate solution R,* 40 volumes of *dioxan R* and 40 volumes of *methanol R.* Dry the plate in a current of warm air for 15 min and expose it to iodine vapour until the spots appear. Examine in daylight. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

D. To 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II).*

Optical rotation *(2.2.7)*

The angle of optical rotation is - 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *tetrahydrofuran R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 5.0 mg of *itraconazole CRS* and 5.0 mg of *miconazole CRS* in a mixture of equal volumes of *methanol R* and *tetrahydrofuran R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *methanol R* and *tetrahydrofuran R*. Dilute to 5.0 ml of this solution to 10.0 ml with the same mixture of solvents.

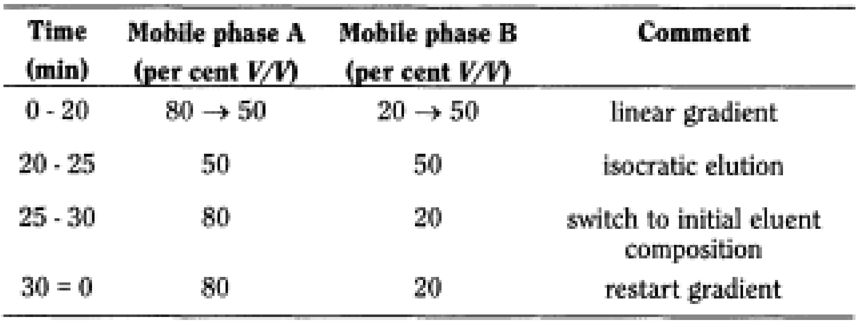
The chromatographic procedure may be carried out using:

— a stainless steel column 0.1 m long and 4.0 mm in internal diameter packed with *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm);

— as mobile phase at a flow rate of 1.5 ml/min a gradient programme using the following conditions:

Mobile phase A A 27.2 g/l solution of *tetrabutylammonium hydrogen sulphate R,*

Mobile phase B *Acetonitrile R,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 20	80 → 50	20 → 50	linear gradient
20 - 25	50	50	isocratic elution
25 - 30	80	20	switch to initial eluent composition
30 = 0	80	20	restart gradient

— as detector a spectrophotometer set at 225 nm.

Equilibrate the column for at least 30 min with *acetonitrile R* at a flow rate of 1.5 ml/min and then equilibrate at the initial eluent composition for at least 5 min.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 10 µl of reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject 10 µl of reference solution (a). When the chromatogram is recorded in the prescribed conditions, the retention times are: miconazole about 10.5 min and itraconazole about 11 min. The test is not valid unless the resolution between the peaks corresponding to miconazole and itraconazole is at least 2.0. If necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient elution.

Inject separately 10 µl of the mixture of equal volumes of *methanol R* and *tetrahydrofuran R* as a blank, 10 µl of the test solution and 10 µl of reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than that of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.25 per cent). Disregard any peak obtained with the blank run and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

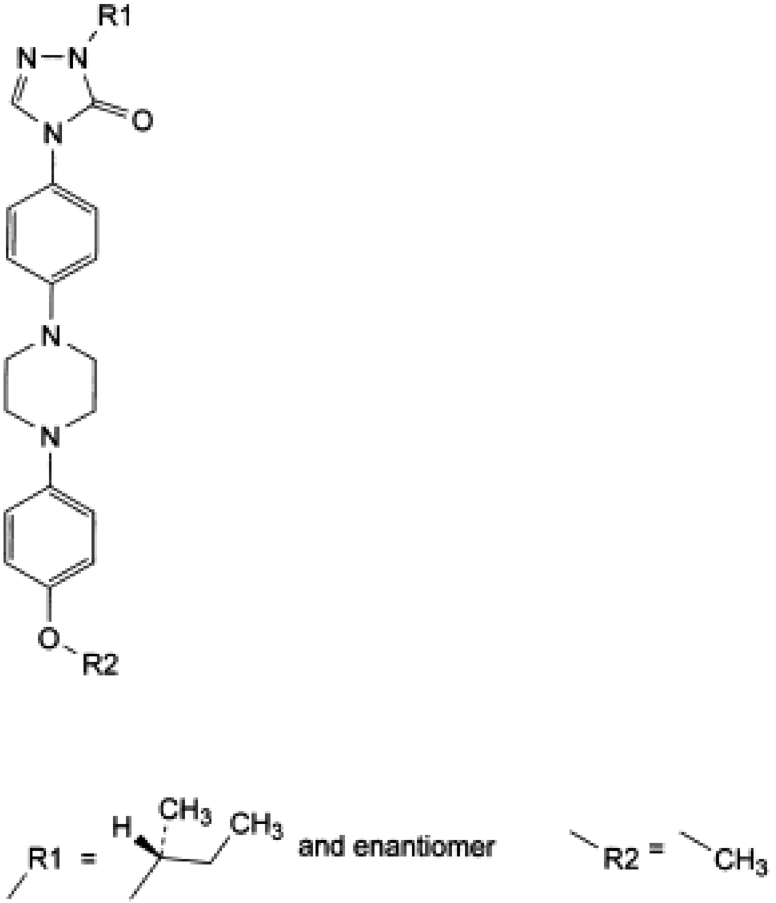
Dissolve 0.300 g in 70 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically at the second point of inflexion *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 35.3 mg of $C_{35}H_{38}Cl_2N_8O_4$.

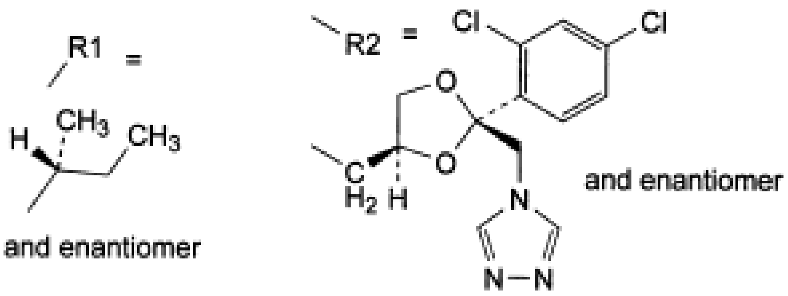
STORAGE

Store protected from light.

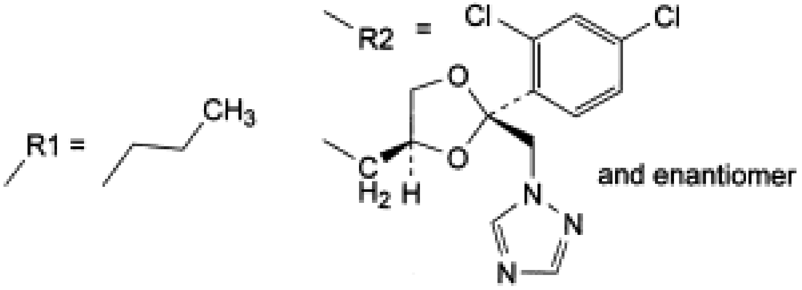
IMPURITIES



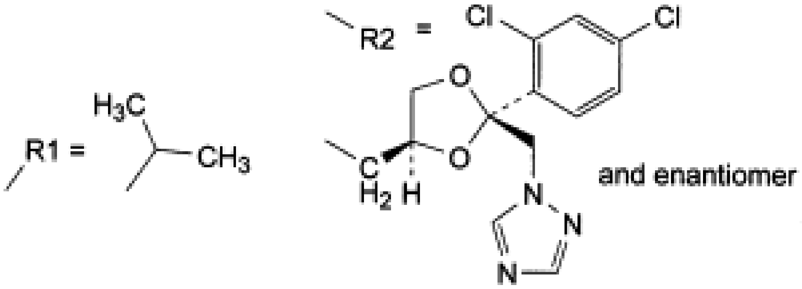
A. 4-[4-[4-(4-methoxyphenyl)piperazin-1-yl]phenyl]-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one,


R1 =
H
CH3
CH3
and enantiomer
R2 =
Cl
Cl
O
O
C
H2 H
N
N–N
and enantiomer

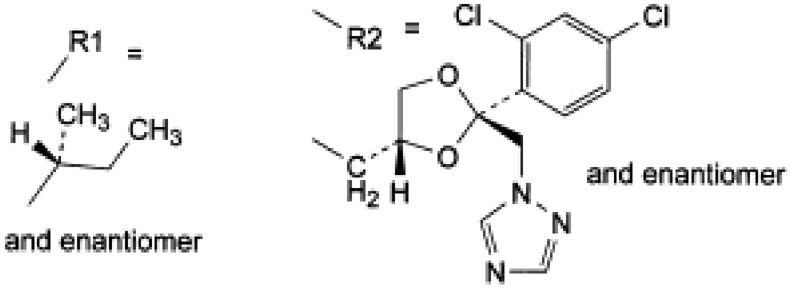
B. 4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(4*H*-1,2,4-triazol-4-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one,


R1 =
CH3
R2 =
Cl
Cl
O
O
C
H2 H
N
N
N
and enantiomer

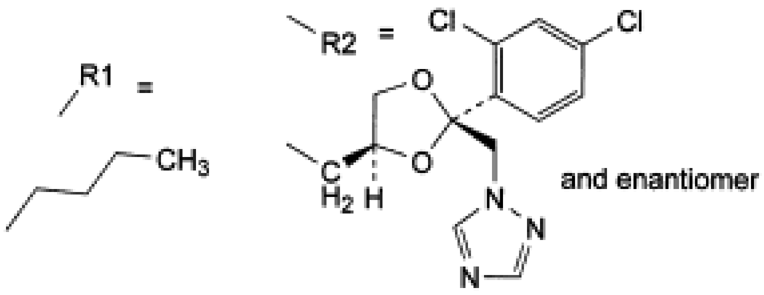
C. 4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenyl]piperazin-1-yl]phenyl]-2-propyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one,


R1 =
H3C
CH3
R2 =
Cl
Cl
O
O
C
H2 H
N
N
N
and enantiomer

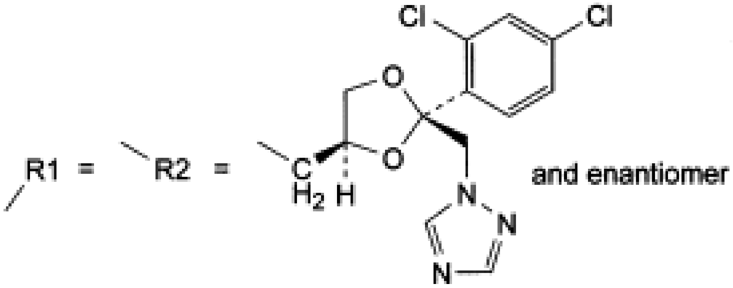
D. 4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenyl]piperazin-1-yl]phenyl]-2-(1-methylethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one,


R1 =
H
CH3
CH3
and enantiomer
R2 =
Cl
Cl
O
O
C
H2 H
N
N
N
and enantiomer

E. 4-[4-[4-[4-[[*trans*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol- 3-one,


R1 =
CH3
R2 =
Cl
Cl
O
O
C
H2 H
N
N
N
and enantiomer

F. 2-butyl-4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one,


R1 =
R2 =
Cl
Cl
O
O
C
H2 H
N
N
N
and enantiomer

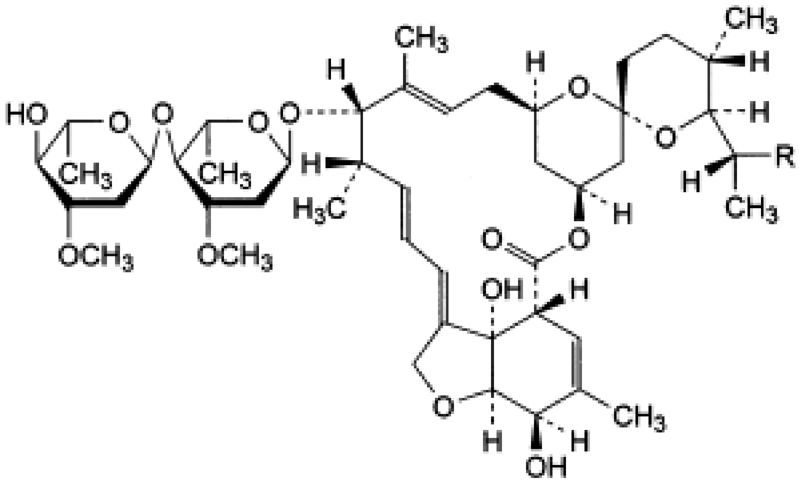
G. 4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenyl]piperazin-1-yl]phenyl]-2-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

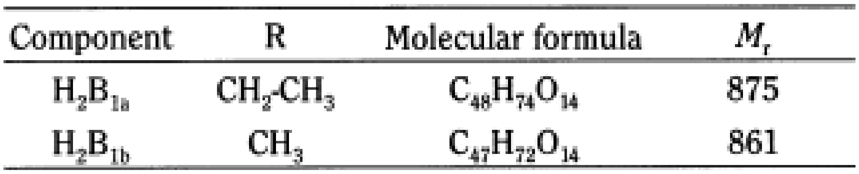
Ph Eur

Ivermectin

General Notices

(Ph Eur monograph 1336)





Component	R	Molecular formula	M_r
H_2B_{1a}	CH_2-CH_3	$C_{48}H_{74}O_{14}$	875
H_2B_{1b}	CH_3	$C_{47}H_{72}O_{14}$	861

70161-11-4 (ivermectin B_{1a})
70209-81-3 (ivermectin B_{1b})

Action and use

Antihelminthic.

Ph Eur

DEFINITION

Mixture of (2a*E*,4*E*,5′*S*,6*S*,6′*R*,7*S*,8*E*,11*R*,13*R*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-7-[[2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranosyl]oxy]-20,20b-dihydroxy-5′,6,8,19-tetramethyl-6′-[(1*S*)-1-methylpropyl]-3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-tetradecahydrospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecene-13,2′-[2*H*]pyran]-17-one (or 5-*O*-demethyl-22,23-dihydroavermectin A_{1a}) (component H_2B_{1a}) and (2a*E*,4*E*,5′*S*,6*S*,6′*R*,7*S*,8*E*,11*R*,13*R*,15*S*, 17a*R*,20*R*,20a*R*,20b*S*)-7-[[2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranosyl]oxy]-20,20b-dihydroxy-5′,6,8,19-tetramethyl-6′-(1-methylethyl)-3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-tetradecahydrospiro [11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecene-13,2′-[2*H*]pyran]-17-one (or 5-*O*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)-22,23-dihydroavermectin A_{1a}) (component H_2B_{1b}).

Semi-synthetic product derived from a fermentation product.

Content:

- ivermectin (H_2B_{1a} + H_2B_{1b}): 95.0 per cent to 102.0 per cent (anhydrous substance);
- ratio $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$ (areas by liquid chromatography): minimum 90.0 per cent.

CHARACTERS

Appearance

White or yellowish-white, crystalline powder, slightly hygroscopic.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ivermectin CRS.*

B. Examine the chromatograms obtained in the assay.

Results The retention times and sizes of the 2 principal peaks in the chromatogram obtained with the test solution are similar to those of the 2 principal peaks in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 1.0 g in 50 ml of *toluene R*.

Specific optical rotation *(2.2.7)*

- 17 to - 20 (anhydrous substance).

Dissolve 0.250 g in *methanol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 40.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 40.0 mg of *ivermectin CRS* in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol R*.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 100.0 ml with *methanol R*.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R, methanol R, acetonitrile R* (15:34:51 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

System suitability:
- — *resolution*: minimum of 3.0 between the first peak (component H_2B_{1b}) and the second peak (component H_2B_{1a}) in the chromatogram obtained with reference solution (a);
- — *signal-to-noise ratio*: minimum of 10 for the principal peak in the chromatogram obtained with reference solution (c);
- — *symmetry factor*: maximum of 2.5 for the principal peak in the chromatogram obtained with reference solution (a).

Limits:
- — *impurity with a relative retention of 1.3 to 1.5* with reference to the principal peak: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);
- — *any other impurity* (apart from the 2 principal peaks): not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- — *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent);
- — *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Ethanol and formamide

Gas chromatography *(2.2.28)*.

Internal standard solution Dilute 0.5 ml of *propanol R* to 100 ml with *water R*.

Test solution In a centrifuge tube, dissolve 0.120 g of the substance to be examined in 2.0 ml of *m-xylene R* (if necessary heat in a water-bath at 40-50 °C). Add 2.0 ml of *water R*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 ml of *water R*. Discard the upper layer and combine the aqueous layers. Add 1.0 ml of the internal standard solution. Centrifuge and discard any remaining *m*-xylene.

Reference solution (a) Dilute 3.0 g of *ethanol R* to 100.0 ml with *water R*.

Reference solution (b) Dilute 1.0 g of *formamide R* to 100.0 ml with *water R.*

Reference solution (c) Dilute 5.0 ml of reference solution (a) and 5.0 ml of reference solution (b) to 50.0 ml with *water R*. Introduce 2.0 ml of this solution into a centrifuge tube, add 2.0 ml of *m-xylene R*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 ml of *water R*. Discard the upper layer and combine the aqueous layers. Add 1.0 ml of the internal standard solution. Centrifuge and discard any remaining *m*-xylene.

Reference solution (d) Dilute 10.0 ml of reference solution (a) and 10.0 ml of reference solution (b) to 50.0 ml with *water R*. Treat as prescribed for reference solution (c) (from ″Introduce 2.0 ml of this solution...″).

Column:

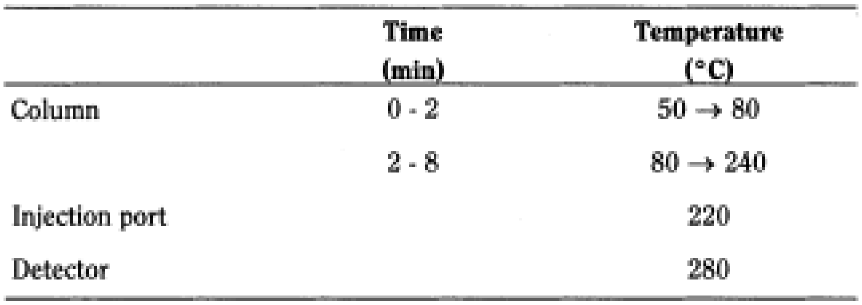
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.53 mm;
- *stationary phase: macrogol 20 000 R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 7.5 ml/min.

Split ratio 1:10.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	50 → 80
	2 - 8	80 → 240
Injection port		220
Detector		280

Detection Flame ionisation.

Injection 1 µl; inject the test solution and reference solutions (c) and (d).

Limits:

- *ethanol* : maximum 5.0 per cent;
- *formamide*: maximum 3.0 per cent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 0.50 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

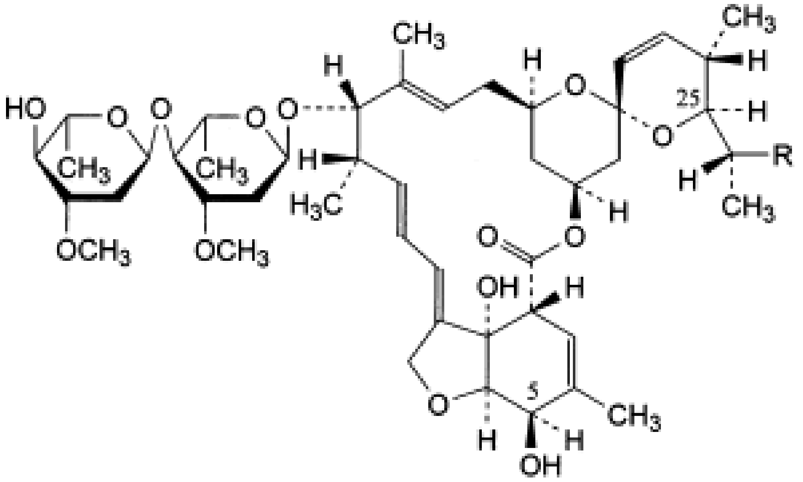
Injection 20 µl; inject the test solution and reference solution (a).

Calculate the percentage contents of ivermectin (H_2B_{1a} + H_2B_{1b}) and the ratio $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$ using the declared contents of *ivermectin CRS*.

STORAGE

In an airtight container .

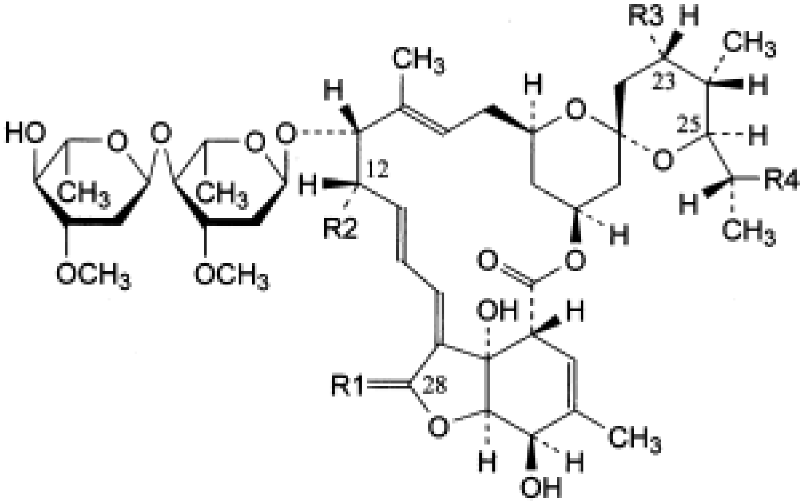
IMPURITIES



CH3
H
H
CH3
H
HO
O
O
O
O
25
H
CH3
CH3
H
O
R
H
H3C
H
CH3
OCH3
OCH3
O
O
OH
H
5
O
CH3
H
H
OH

A. R = C_2H_5: 5-*C*-demethylavermectin A_{1a} (avermectin B_{1a}),

B. R = CH_3: 5-*C*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)avermectin A_{1a} (avermectin B_{1b}),



R3 H
CH3
CH3
23
H
H
HO
O
O
O
O
25
H
CH3
CH3
12
H
O
R4
H
R2
H
CH3
OCH3
OCH3
O
O
OH
H
R1
28
O
CH3
H
H
OH

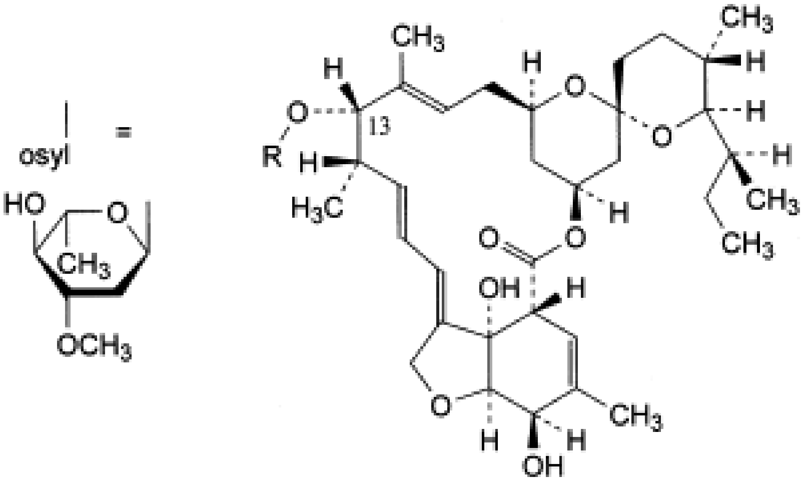
C. R1 = H_2, R2 = CH_3, R3 = OH, R4 = C_2H_5: (23*S*)-5-*C*-demethyl-23-hydroxy-22,23-dihydroavermectin A_{1a} (avermectin B_{2a}),

D. R1 = O, R2 = CH_3, R3 = H, R4 = C_2H_5: 5-*C*-demethyl-28-oxo-22,23-dihydroavermectin A_{1a}

(28-oxoH$_2$B$_{1a}$),

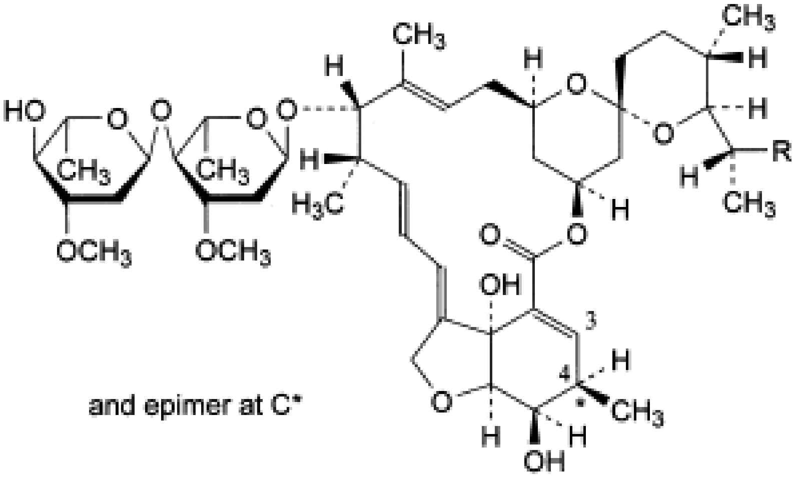
E. R1 = H$_2$, R2 = C$_2$H$_5$, R3 = H, R4 = C$_2$H$_5$: 5-*C*,12-didemethyl-12-ethyl-22,23-dihydroavermectin A$_{1a}$ (12-demethyl-12-ethyl-H$_2$B$_{1a}$),

F. R1 = H$_2$, R2 = C$_2$H$_5$, R3 = H, R4 = CH$_3$: 5-*C*,12-didemethyl-25-de(1-methylpropyl)-12-ethyl-25-(1-methylethyl)-22,23-dihydroavermectin A$_{1a}$ (12-demethyl-12-ethyl-H$_2$B$_{1b}$),



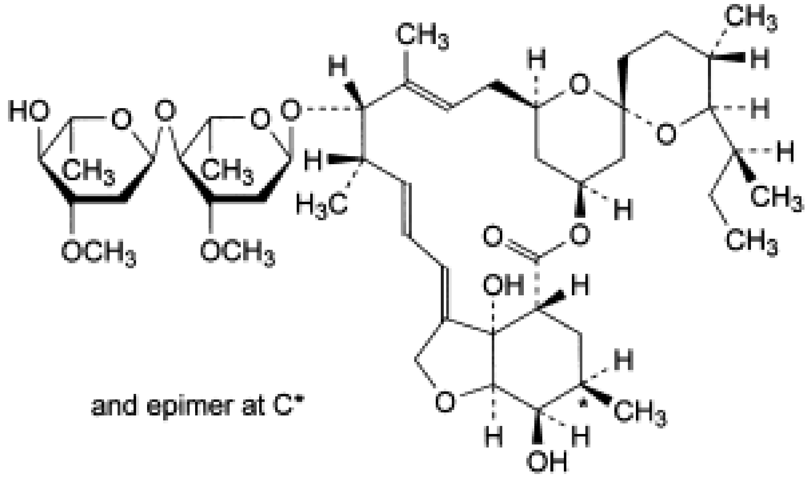
G. R = H: (6*R*,13*S*,25*R*)-5-*C*-demethyl-28-deoxy-6,28-epoxy-13-hydroxy-25-[(1*S*)-1-methylpropyl]milbemycin B (H$_2$B$_{1a}$ aglycone),

H. R = osyl: 4′-*C*-de(2,6-dideoxy-3-*C*-methyl-α-L-*arabino*-hexopyranosyl)-5-*C*-demethyl-22, 23-dihydroavermectin A$_{1a}$,



I. R = C$_2$H$_5$: 2,3-didehydro-5-*C*-demethyl-3,4,22,23-tetrahydroavermectin A$_{1a}$ ($\Delta^{2,3}$ H$_2$B$_{1a}$),

J. R = CH$_3$: 2,3-didehydro-5-*C*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)-3,4,22, 23-tetrahydroavermectin A$_{1a}$ ($\Delta^{2,3}$ H$_2$B$_{1b}$),



and epimer at C*

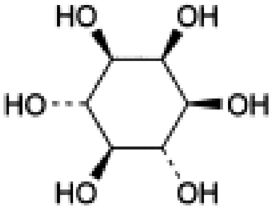
K. (4*R*) and (4*S*)-5-*C*-demethyl-3,4,22,23-tetrahydroavermectin A_{1a} (H_4B_{1a} isomers).

Ph Eur

myo -Inositol

General Notices

(Ph Eur monograph 1805)



$C_6H_{12}O_6$ 180.2 *87-89-8*

Action and use

Vasodilator.

Ph Eur

DEFINITION

Cyclohexane-1,2,3,5/4,6-hexol.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *myo-inositol CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference

solution (a).

TESTS

Solution S

Dissolve 10.0 g in *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Conductivity *(2.2.38)*

Maximum 30 µS·cm^{-1}.

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R,* with gentle warming if necessary, and dilute to 50.0 ml with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 0.500 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 0.500 g of *myo-inositol CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with *water R*. Dilute 5.0 ml of this solution to 100.0 ml with *water R*.

Reference solution (c) Dissolve 0.5 g of *myo-inositol R* and 0.5 g of *mannitol R* in *water R* and dilute to 10 ml with the same solvent.

Column:

- *size*: *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase: strong cation exchange resin (calcium form) R* (9 µm);
- *temperature:* 85 °C.

Mobile phase *water R.*

Flow rate 0.5 ml/min.

Detection Refractometer maintained at a constant temperature (at about 30-35 °C for example).

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of *myo*-inositol.

Relative retention with reference to *myo*-inositol (retention time = about 17.5 min): impurity A = about 1.3; impurity B = about 1.4.

System suitability Reference solution (c):

- *resolution*: minimum 4 between the peaks due to *myo*-inositol and impurity A.

Limits:

— *impurities A, B*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Barium

To 10 ml of solution S add 1 ml of *dilute sulphuric acid R*. When examined immediately, and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Lead *(2.4.10)*

Maximum 0.5 ppm.

Prepare the test solution by dissolving 20.0 g of the substance to be examined in 100 ml of *water R*, heating if necessary, and diluting to 200.0 ml with *dilute acetic acid R*.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.00 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_6H_{12}O_6$ from the declared content of *myo-inositol CRS.*

IMPURITIES

Specified impurities A, B.

A. mannitol,

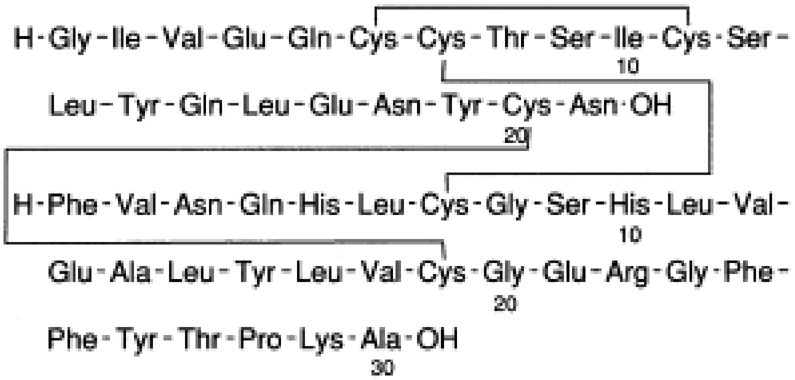
B. glycerol.

Ph Eur

Porcine Insulin

General Notices

(Ph Eur monograph 1638)


H-Gly-Ile-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-
10
Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn-OH
20
H-Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-Leu-Val-
10
Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-
20
Phe-Tyr-Thr-Pro-Lys-Ala-OH
30

$C_{256}H_{381}N_{65}O_{76}S_6$ 5778 *12584-58-6*

Action and use

Hormone; treatment of diabetes mellitus.

Preparations

Insulin Preparations

Ph Eur

DEFINITION

Porcine insulin is the natural antidiabetic principle obtained from pork pancreas and purified.

Content:

— *sum of porcine insulin ($C_{256}H_{381}N_{65}O_{76}S_6$) and A21 desamido porcine insulin*: 95.0 per cent to 105.0 per cent (dried substance).

By convention, for the purpose of labelling insulin preparations, 0.0345 mg of porcine insulin is equivalent to 1 IU of insulin.

PRODUCTION

The animals from which porcine insulin is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in ethanol. It dissolves in dilute mineral acids and with decomposition in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The retention time of the principal peak in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with reference solution (b).

B. Peptide mapping.

Test solution Prepare a 2.0 mg/ml solution of the substance to be examined in *0.01 M hydrochloric acid* and transfer 500 µl of this solution to a clean tube. Add 2.0 ml of *HEPES buffer solution pH 7.5 R* and 400 µl of a 1 mg/ml solution of *Staphylococcus aureus strain V8 protease R*. Cap the tube and incubate at 25 °C for 6 h. Stop the reaction by adding 2.9 ml of *sulphate buffer solution pH 2.0 R*.

Reference solution Prepare at the same time and in the same manner as for the test solution but using *porcine insulin CRS* instead of the substance to be examined.

Examine the digests by liquid chromatography (*2.2.29*).

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm;

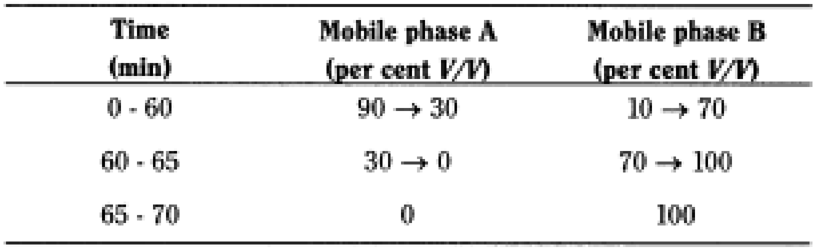
— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: mix 100 ml of *acetonitrile for chromatography R*, 700 ml of *water R* and 200 ml of *sulphate buffer solution pH 2.0 R*; filter and degas;

— *mobile phase B*: mix 400 ml of *acetonitrile for chromatography R*, 400 ml of *water R* and 200 ml of *sulphate buffer solution pH 2.0 R*; filter and degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	90 → 30	10 → 70
60 - 65	30 → 0	70 → 100
65 - 70	0	100

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.

Injection 50 µl.

System suitability The chromatograms obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of porcine insulin digest supplied with *porcine insulin CRS*. In the chromatogram obtained with the reference solution, identify the peaks due to digest fragments I, II and III. The symmetry factor of the peaks due to fragments II and III is not greater than 1.5, and the resolution between the 2 peaks is at least 1.9.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

NOTE: the retention time of fragment I is the same for porcine insulin and for human insulin. The retention times of fragments II and IV are the same for all insulins. The retention time of fragment III is the same for bovine insulin and for porcine insulin.

TESTS

Impurities with molecular masses greater than that of insulin

Size-exclusion chromatography *(2.2.30)*: use the normalisation procedure. *Maintain the solutions at 2-10 °C and use within 7 days. If an automatic injector is used, maintain the temperature at 2-10 °C.*

Test solution Dissolve 4 mg of the substance to be examined in 1.0 ml of *0.01 M hydrochloric acid*.

Resolution solution Use a solution of insulin (approximately 4 mg/ml), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about 10 days.

Column:

— *size*: *l* = 0.3 m, Ø = at least 7.5 mm;

— *stationary phase*: *hydrophilic silica gel for chromatography R* (5-10 µm), of a grade suitable for the separation of insulin monomer from dimer and polymers.

Mobile phase Mix 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile R* and 65 volumes of a 1.0 g/l solution of *arginine R*; filter and degas.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 276 nm.

Equilibration Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least 3 injections of the resolution solution. The column is equilibrated when repeatable results are obtained from 2 subsequent injections.

Injection 100 µl.

Run time About 35 min.

Retention times Polymeric insulin complexes = 13 min to 17 min; covalent insulin dimer = about 17.5 min; insulin monomer = about 20 min; salts = about 22 min.

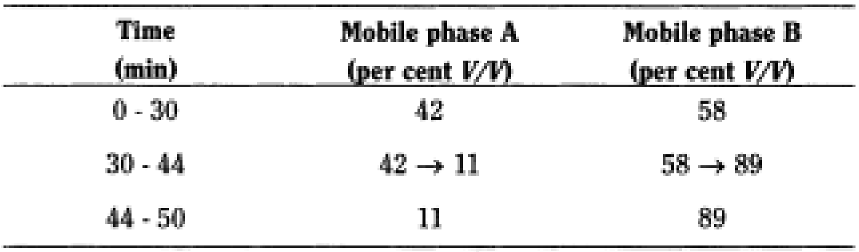
System suitability Resolution solution:

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limits The sum of the areas of any peaks with a retention time less than that of the principal peak is not greater than 1.0 per cent of the total area of the peaks; disregard any peak with a retention time greater than that of the insulin peak.

Related proteins

Liquid chromatography *(2.2.29)* as described under Assay, following the elution conditions as described in the table below.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	42	58
30 - 44	42 → 11	58 → 89
44 - 50	11	89

Maintain the solutions at 2-10 °C and use within 24 h. Perform a system suitability test (resolution, linearity) as described under Assay. If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µl of reference solution (b) and 20 µl of the test solution. If necessary, adjust the injection volume to between 10 µl and 20 µl in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for approximately 50 min. In the chromatogram obtained with reference solution (b), A21 desamido porcine insulin appears as a small peak after the principal peak and has a relative retention of about 1.3 with reference to the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to A21 desamido porcine insulin is not greater than 2.0 per cent of the total area of the peaks; the sum of the areas of all the peaks, apart from those due to porcine insulin and A21 desamido porcine insulin, is not greater than 2.0 per cent of the total area of the peaks.

Porcine proinsulin-like immunoreactivity (PLI)

Maximum 10 ppm (dried substance).

Use a suitably sensitive immunochemical method *(2.7.1)* such as radio-immunoassay, using the International Reference Reagent for porcine proinsulin to calibrate the method.

Zinc

Maximum 1.0 per cent (dried substance).

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid. Dilute if necessary to a suitable concentration (for example, 0.4 µg to 1.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/ml Zn) R* with *0.01 M hydrochloric acid*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Flame Air-acetylene flame of suitable composition (for example, 11 litres of air and 2 litres of acetylene per minute).

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 0.200 g by drying in an oven at 105 °C for 24 h.

Sulphated ash *(2.4.14)*

Maximum 2.5 per cent (dried substance), determined on 0.200 g.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 40.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *human insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (b) Dissolve the contents of a vial of *porcine insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with *0.01 M hydrochloric acid*.

Resolution solution Mix 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b).

Maintain the solutions at 2-10 °C and use within 48 h. If an automatic injector is used, maintain the temperature at 2-10 °C.

Column:

- *size*: *l* = 0.25 , Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 42 volumes of mobile phase A and 58 volumes of mobile phase B, adjusting the composition of the mixture if necessary.

Prepare and maintain the following solutions at a temperature of at least 20 °C:

— *mobile phase A*: dissolve 28.4 g of *anhydrous sodium sulphate R* in *water R* and dilute to 1000 ml with the same solvent; add 2.7 ml of *phosphoric acid R*; adjust to pH 2.3, if necessary, with *ethanolamine R;* filter and degas;

— *mobile phase B*: mix 550 ml of mobile phase A with 450 ml of *acetonitrile R*. Warm the solution to a temperature of at least 20 °C in order to avoid precipitation (mixing of mobile phase A with acetonitrile is endothermic); filter and degas.

Flow rate: 1 ml/min.

Detection Spectrophotometer at 214 nm.

System suitability:

— *resolution*: inject 20 µl of the resolution solution and 20 µl of reference solution (b). Record the chromatogram of the resolution solution until the peak corresponding to the principal peak in the chromatogram obtained with reference solution (b) is clearly visible. In the chromatogram obtained with the resolution solution, identify the peaks due to porcine insulin and human insulin. The test is not valid unless the resolution between the peaks corresponding to human insulin and porcine insulin is at least 1.2. If necessary, adjust the concentration of acetonitrile in the mobile phase until this resolution is achieved.

— *linearity*: inject 20 µl each of reference solutions (b) and (c). The test is not valid unless the area of the principal peak in the chromatogram obtained with reference solution (b) is 10 ± 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c). If this test fails, adjust the injection volume to between 10 µl and 20 µl, in order that the responses are within the linearity range of the detector.

Injection 20 µl of the test solution.

Calculate the content of porcine insulin $C_{256}H_{381}N_{65}O_{76}S_6$ plus A21 desamido porcine insulin from the area of the principal peak and the area of the peak corresponding to A21 desamido porcine insulin in the chromatograms obtained with the test solution and reference solution (b) and the declared content of porcine insulin plus A21 desamido porcine insulin in *porcine insulin CRS.*

STORAGE

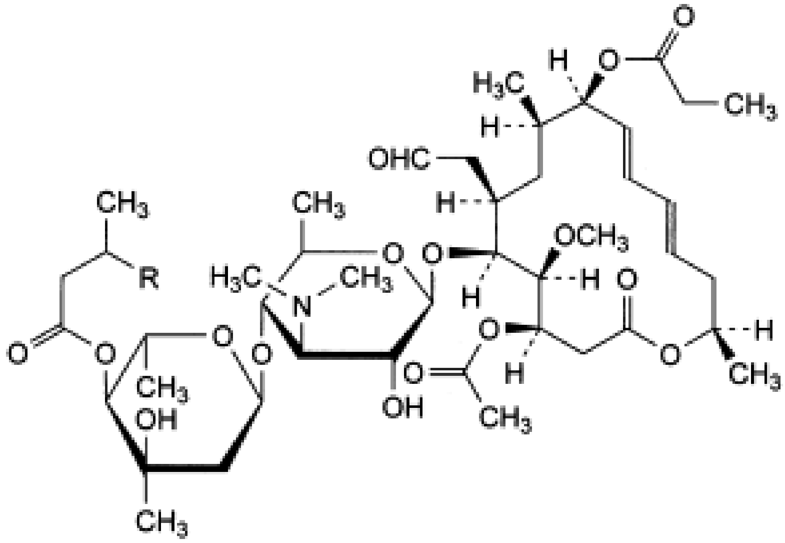
In an airtight container, protected from light, at - 20 °C until released by the manufacturer. When thawed, insulin may be stored at 5 ± 3 °C and used for manufacturing preparations within a short period of time. To avoid absorption of humidity from the air during weighing, the insulin must be at room temperature.

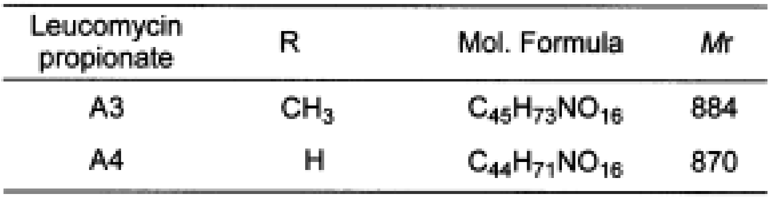
Ph Eur

Josamycin Propionate

General Notices

(Ph Eur monograph 1982)





Leucomycin propionate	R	Mol. Formula	*Mr*
A3	CH_3	$C_{45}H_{73}NO_{16}$	884
A4	H	$C_{44}H_{71}NO_{16}$	870

40922-77-8
(leucomycin A3 propionate)

Action and use

Antibacterial.

Ph Eur

DEFINITION

Propionyl ester of a macrolide antibiotic produced by certain strains of *Streptomyces narbonensis* var. *josamyceticus* var. *nova*, or obtained by any other means. The main component is (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)-10-(propanoyloxy) oxacyclohexadeca-11,13-dien-2-one propionate (leucomycin A3 propionate).

Semi-synthetic product derived from a fermentation product.

Content:

— minimum 843 Ph. Eur. U./mg (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline, slightly hygroscopic powder.

Solubility

Practically insoluble in water, freely soluble in methanol and in methylene chloride, soluble in acetone.

IDENTIFICATION

First identification *A, B.*

Second identification *B, C.*

Prepare solutions in methanol immediately before use.

A. Dissolve 0.10 g in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 50.0 ml with *methanol R*. Examined between 220 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 231 nm. The specific absorbance at the absorption maximum is 310 to 350.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol* R and dilute to 1 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *josamycin propionate CRS* in *methanol R* and dilute to 1 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *josamycin CRS* in *methanol R* and dilute to 1 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of *spiramycin CRS* in *methylene chloride R* and dilute to 1 ml with the same solvent.

Reference solution (d) Mix 0.5 ml of reference solution (a) with 0.5 ml of reference solution (b).

Plate *TLC silica gel G plate R.*

Mobile phase *methanol R, acetone R, ethyl acetate R, toluene R, hexane R* (8:10:20:25:30 *V/V/V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying At 100 °C for 10 min.

Detection Spray with *dilute sulphuric acid R* and heat at 100 °C for 10 min.

System suitability The chromatogram obtained with reference solution (d) shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) and its position is different from that of the principal spot in the chromatograms obtained with reference solutions (b) and (c).

C. Dissolve about 10 mg in 5 ml of *hydrochloric acid R1* and allow to stand for 10-20 min. A pink colour develops, turning brown.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_4 *(2.2.2, Method II)*.

Dissolve 1 g in *methanol R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 65 to - 75 (dried substance).

Dissolve 1.000 g in *methanol R* and dilute to 100.0 ml with the same solvent. Allow to stand for 30 min before measuring the angle of rotation.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *acetonitrile for chromatography R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *josamycin propionate CRS* in *acetonitrile for chromatography R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of the substance to be examined in 10 ml of *methanol R* and add 40 µl of *dilute phosphoric acid R*. Mix, allow to stand for 5 min and inject.

Reference solution (c) Dilute 2.0 ml of reference solution (a) to 100.0 ml with *acetonitrile for chromatography R.*

Column:

— *size*: *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R*, a 15.4 g/l solution of *ammonium acetate R* previously adjusted to pH 6.0 with *dilute phosphoric acid R* (60:40 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 232 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time 3 times the retention time of leucomycin A3 propionate.

Relative retention with reference to leucomycin A3 propionate (retention time = about 18 min): impurity E = about 0.2; impurity A = about 0.3; impurity B = about 0.5; leucomycin A4 propionate = about 0.7; impurity C = about 1.4; impurity D = about 2.0.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the 2 peaks eluting with a relative retention with reference to leucomycin A3 propionate of about 0.5 and 0.7 respectively.

Limits:

— *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c),

— *impurities A, B, C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c),

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c),

— *total:* not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (c),

— *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 60 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 40.0 mg in 20 ml of *methanol R* and dilute to 100.0 ml with *phosphate buffer solution pH 5.6 R.*

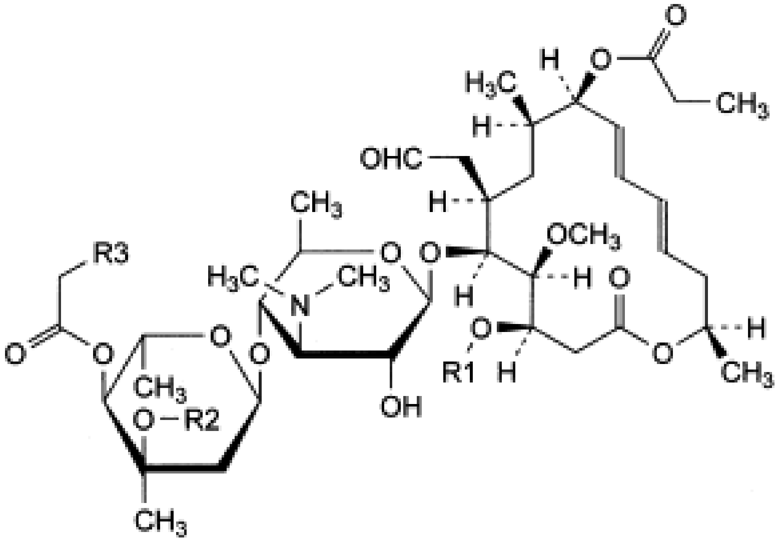
Carry out the microbiological assay of antibiotics *(2.7.2)*.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities A, B, C, D, E.


OHC
H
H₃C
H
O
O
CH₃
OCH₃
H
H
O
R1
H
O
O
H
CH₃
CH₃
R3
H₃C
N
CH₃
O
O
O
O
O
CH₃
O−R2
OH
CH₃

A. R1 = CO-CH_3, R2 = R3 = H: leucomycin A8 9-propionate,

B. R1 = R2 = H, R3 = C_2H_5: leucomycin A5 9-propionate,

C. R1 = CO-C_2H_5, R2 = H, R3 = $CH(CH_3)_2$: platenomycin A1 9-propionate,

D. R1 = CO-CH_3, R2 = CO-C_2H_5, R3 = $CH(CH_3)_2$: leucomycin A3 3″,9-dipropionate,

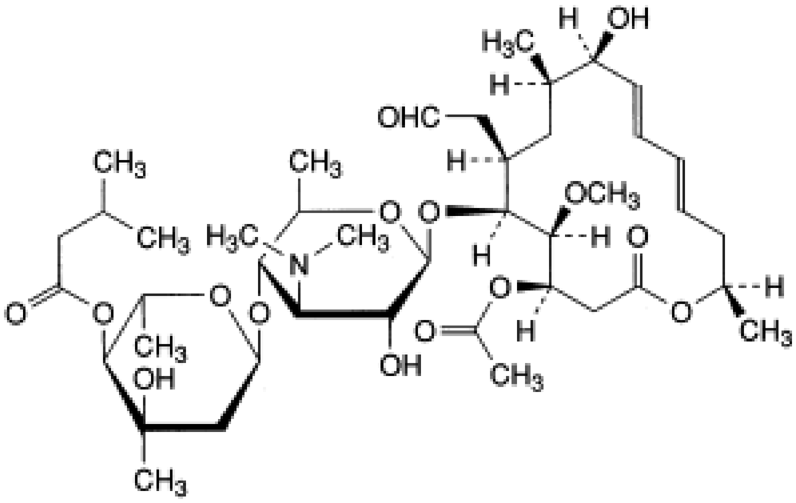
E. josamycin.

Ph Eur

Josamycin

General Notices

(Ph Eur monograph 1983)



$C_{42}H_{69}NO_{15}$ 828.0 *56689-45-3*

Action and use

Antibacterial.

Ph Eur

DEFINITION

Josamycin is a macrolide antibiotic produced by certain strains of *Streptomyces narbonensis* var. *josamyceticus* var. *nova*, or obtained by any other means. The main component is (4*R*, 5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one.

Content

Minimum 900 Ph. Eur. U./mg (dried substance).

CHARACTERS

Appearance

White or slightly yellowish powder, slightly hygroscopic.

Solubility

Very slightly soluble in water, freely soluble in methanol and in methylene chloride, soluble in acetone.

IDENTIFICATION

First identification *A, B.*

Second identification *B, C.*

A. Dissolve 0.10 g in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 50.0 ml with *methanol R*. Examined between 220 nm and 350 nm *(2.2.25),* the solution shows an absorption maximum at 232 nm. The specific absorbance at the maximum is 330 to 370.

B. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) and its position is different from that of the principal spot in the chromatograms obtained with reference solutions (d) and (e).

C. Dissolve about 10 mg in 5 ml of *hydrochloric acid R1* and allow to stand for 10-20 min. A pink colour develops, turning brown.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_4 *(2.2.2, Method II)*.

Dissolve 1.0 g in *methanol R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 65 to - 75 (dried substance).

Dissolve 1.000 g in *methanol R* and dilute to 100.0 ml with the same solvent. Allow to stand for 30 min before measuring the angle of rotation.

Related substances

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 0.10 g of *josamycin CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with *methanol R*.

Reference solution (c) Dilute 1 ml of reference solution (a) to 20 ml with *methanol R*.

Reference solution (d) Dissolve 0.10 g of *josamycin propionate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (e) Dissolve 0.10 g of *spiramycin CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (f) Dissolve 10 mg of *josamycin CRS* and 10 mg of *josamycin propionate CRS* in 1 ml of *methanol R*.

Plate *TLC silica gel G plate R*.

Mobile phase *methanol R, acetone R, ethyl acetate R, toluene R, hexane R* (8:10:20:25:30 *V/V/V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying At 100 °C for 10 min.

Detection Spray with *dilute sulphuric acid R* and heat at 100 °C for 10 min.

System suitability The chromatogram obtained with reference solution (f) shows 2 clearly separated principal spots.

Limits:

— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (10 per cent) and not more than 1 such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (5 per cent).

Heavy metals *(2.4.8)*

Maximum 30 ppm.

1.0 g complies with limit test C. Prepare the standard using 3 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 60 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 30.0 mg in 5 ml of *methanol R* and dilute to 100.0 ml with *water R*.

Carry out the microbiological assay of antibiotics *(2.7.2)*.

STORAGE

In an airtight container .

Ph Eur

Heavy Kaolin

General Notices

(Ph Eur monograph 0503)

Preparation

Kaolin Poultice

When kaolin is prescribed or demanded, Light Kaolin shall be dispensed or supplied, unless it is ascertained that Light Kaolin (Natural) is required.

Ph Eur

DEFINITION

Purified, natural, hydrated aluminium silicate of variable composition.

CHARACTERS

Appearance

Fine, white or greyish-white, unctuous powder.

Solubility

Practically insoluble in water and in organic solvents.

IDENTIFICATION

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate R* and 3 g of *sodium carbonate R* and heat until the mixture melts. Allow to cool. To the residue add 20 ml of boiling *water R*, mix and filter. Wash the residue with 50 ml of *water R*. To the residue add 1 ml of *hydrochloric acid R* and 5 ml of *water R*. Filter. To the filtrate add 1 ml of *strong sodium hydroxide solution R* and filter. To the filtrate add 3 ml of *ammonium chloride solution R*. A gelatinous white precipitate is formed.

B. Add 2.0 g in 20 portions to 100 ml of a 10 g/l solution of *sodium laurilsulfate R* in a 100 ml graduated cylinder about 30 mm in diameter. Allow 2 min between additions for each portion to settle. Allow to stand for 2 h. The apparent volume of the sediment is not greater than 5 ml.

C. 0.25 g gives the reaction of silicates *(2.3.1)*.

TESTS

Solution S

To 4 g add a mixture of 6 ml of *acetic acid R* and 34 ml of *distilled water R*, shake for 1 min and filter.

Acidity or alkalinity

To 1.0 g add 20 ml of *carbon dioxide-free water R*, shake for 2 min and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.25 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Organic impurities

Heat 0.3 g to redness in a calcination tube. The residue is only slightly more coloured than the original substance.

Adsorption power

To 1.0 g in a ground-glass-stoppered test-tube add 10.0 ml of a 3.7 g/l solution of *methylene blue R* and shake for 2 min. Allow to settle. Centrifuge and dilute the solution 1 to 100 with *water R*. The solution is not more intensely coloured than a 0.03 g/l solution of *methylene blue R.*

Swelling power

Triturate 2 g with 2 ml of *water R*. The mixture does not flow.

Substances soluble in mineral acids

Maximum 1 per cent.

To 5.0 g add 7.5 ml of *dilute hydrochloric acid R* and 27.5 ml of *water R* and boil for 5 min. Filter, wash the residue on the filter with *water R* and dilute the combined filtrate and washings to 50.0 ml with *water R.* To 10.0 ml of the solution add 1.5 ml of *dilute sulphuric acid R*, evaporate to dryness on a water-bath and ignite. The residue weighs a maximum of 10 mg.

Chlorides *(2.4.4)*

Maximum 250 ppm.

Dilute 2 ml of solution S to 15 ml with *water R.*

Sulphates *(2.4.13)*

Maximum 0.1 per cent.

Dilute 1.5 ml of solution S to 15 ml with *distilled water R.*

Calcium *(2.4.3)*

Maximum 250 ppm.

Dilute 4 ml of solution S to 15 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 50 ppm.

To 5 ml of the solution prepared for the test for substances soluble in mineral acids add 5 ml of *water R*, 10 ml of *hydrochloric acid R* and 25 ml of *methyl isobutyl ketone R*. Shake for 2 min. Separate the layers. Evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *acetic acid R* and dilute to 25 ml with *water R*. Filter. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

If intended for internal use, the above test is replaced by the following test for heavy metals *(2.4.8)*: maximum 25 ppm.

To 10 ml of the solution prepared for the test for substances soluble in mineral acids add 10 ml of *water R*, 20 ml of *hydrochloric acid R* and 25 ml of *methyl isobutyl ketone R*. Shake for 2 min. Separate the layers. Evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *acetic acid R* and dilute to 25 ml with *water R.* Filter. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate-count.

LABELLING

The label states, where applicable, that the substance is suitable for internal use.

Ph Eur

Kanamycin Acid Sulphate

General Notices

(Ph Eur monograph 0033)

Action and use

Aminoglycoside antibacterial.

Ph Eur

DEFINITION

Kanamycin acid sulphate is a form of kanamycin sulphate prepared by adding sulphuric acid to a solution of kanamycin monosulphate and drying by a suitable method. The potency is not less than 670 IU/mg, calculated with reference to the dried substance.

Fermentation product.

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

The method of manufacture is validated to demonstrate that the product if tested would comply with the following test.

Abnormal toxicity *(2.6.9)*

Inject into each mouse 0.5 ml of a solution containing 2 mg per millilitre of the substance to be examined.

CHARACTERS

A white or almost white powder, hygroscopic, soluble in about 1 part of water, practically insoluble in acetone and in alcohol.

IDENTIFICATION

A. Examine by thin-layer chromatography *(2.2.27),* using a plate coated with a 0.75 mm layer of the following mixture: mix 0.3 g of *carbomer R* with 240 ml of *water R* and allow to stand, with moderate shaking, for 1 h; adjust to pH 7 by the gradual addition, with continuous shaking, of *dilute sodium hydroxide solution R* and add 30 g of *silica gel H R*.

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *kanamycin monosulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *kanamycin monosulphate CRS*, 10 mg of *neomycin sulphate CRS* and 10 mg of *streptomycin sulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm using a 70 g/l solution of *potassium dihydrogen phosphate R*. Dry the plate in a current of warm air and spray with a mixture of equal volumes of a 2 g/l solution of *dihydroxynaphthalene R* in *alcohol R* and a 460 g/l solution of *sulphuric acid R*. Heat at 150 °C for 5 min to 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

B. Dissolve 0.5 g in 10 ml of *water R*. Add 10 ml of *picric acid solution R*. Initiate crystallisation if necessary by scratching the wall of the tube with a glass rod and allow to stand. Collect the crystals, wash with 20 ml of *water R* and filter. Dry at 100 °C. The crystals melt *(2.2.14)* at about 235 °C, with decomposition.

C. Dissolve about 50 mg in 2 ml of *water R*. Add 1 ml of a 10 g/l solution of *ninhydrin R* and heat for a few minutes on a water-bath. A violet colour develops.

D. It gives the reactions of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH *(2.2.3)*

The pH of solution S is 5.5 to 7.5.

Specific optical rotation *(2.2.7)*

+103 to +115, determined on solution S and calculated with reference to the dried substance.

Kanamycin B

Examine by thin-layer chromatography *(2.2.27)*, using a plate prepared as prescribed under identification test A.

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 0.11 g of the substance to be examined in *water R* and dilute to 20 ml with the same solvent.

Reference solution Dissolve 4 mg of *kanamycin B sulphate CRS* in *water R* and dilute to 20 ml with the same solvent.

Apply separately to the plate 4 µl of each solution. Develop over a path of 12 cm using a 70 g/l solution of *potassium dihydrogen phosphate R*. Dry the plate in a current of warm air and spray with *ninhydrin and stannous chloride reagent R*. Heat the plate at 110 °C for 15 min. Any spot corresponding to *kanamycin B* in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (4.0 per cent).

Loss on drying *(2.2.32)*

Not more than 5.0 per cent, determined on 1.00 g by drying at 60 °C at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash ***(2.4.14)***

Not more than 0.5 per cent, determined on 1.0 g.

Sulphate

23.0 per cent to 26.0 per cent of sulphate (SO_4), calculated with reference to the dried substance. Dissolve 0.175 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml *of 0.1 M barium chloride* and about 0.5 mg of *phthalein purple R.* Titrate with *0.1 M sodium edetate* adding 50 ml of *alcohol R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of sulphate (SO_4).

Pyrogens ***(2.6.8)***

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 ml of a solution in *water for injections R* containing 10 mg per millilitre of the substance to be examined.

ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)*. Use *kanamycin monosulphate CRS* as the reference substance.

STORAGE

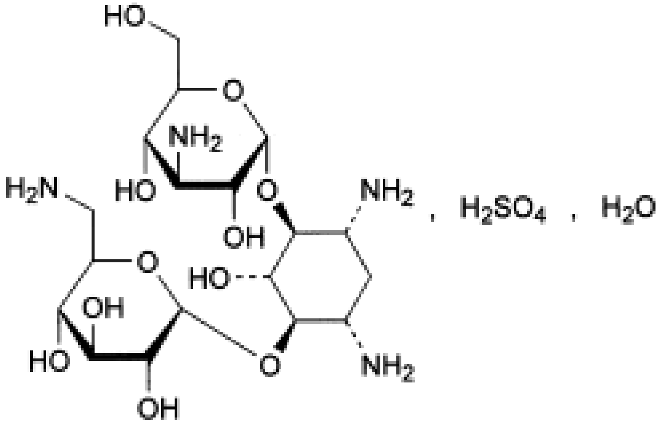
If the substance is sterile, store in a sterile, tamper-proof container.

Ph Eur

Kanamycin Sulphate

General Notices

(Kanamycin Monosulphate, Ph Eur monograph 0032)



$C_{18}H_{36}N_4O_{11},H_2SO_4,H_2O$ 601 *25389-94-0*
(anhydrous)

Action and use

Aminoglycoside antibacterial.

Ph Eur

DEFINITION

Kanamycin monosulphate is 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine sulphate, an antimicrobial substance produced by the growth of certain strains of *Streptomyces kanamyceticus*. The potency is not less than 750 IU/mg, calculated with reference to the dried substance.

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

The method of manufacture is validated to demonstrate that the product if tested would comply with the following test:

Abnormal toxicity *(2.6.9)*

Inject into each mouse 0.5 ml of a solution containing 2 mg per millilitre of the substance to be examined.

CHARACTERS

A white or almost white, crystalline powder, soluble in about 8 parts of water, practically insoluble in acetone and in alcohol.

IDENTIFICATION

A. Examine by thin-layer chromatography *(2.2.27)*, using a plate coated with a 0.75 mm layer of the following mixture: mix 0.3 g of *carbomer R* with 240 ml of *water R* and allow to stand, with moderate shaking, for 1 h; adjust to pH 7 by the gradual addition, with continuous shaking, of *dilute sodium hydroxide solution R* and add 30 g of *silica gel H R*.

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *kanamycin monosulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *kanamycin monosulphate CRS*, 10 mg of *neomycin sulphate CRS* and 10 mg of *streptomycin sulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm using a 70 g/l solution of *potassium dihydrogen phosphate R.* Dry the plate in a current of warm air and spray with a mixture of equal volumes of a 2 g/l solution of *dihydroxynaphthalene R* in *alcohol R* and a 460 g/l solution of *sulphuric acid R*. Heat at 150 °C for 5 min to 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.5 g in 10 ml of *water R*. Add 10 ml of *picric acid solution R*. Initiate crystallisation if necessary by scratching the wall of the tube with a glass rod and allow to stand. Collect the crystals, wash with 20 ml of *water R* and filter. Dry at 100 °C. The crystals melt *(2.2.14)* at about 235 °C, with decomposition.

C. Dissolve about 50 mg in 2 ml of *water R*. Add 1 ml of a 10 g/l solution of *ninhydrin R* and heat for a few minutes on a water-bath. A violet colour develops.

D. It gives the reactions of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH *(2.2.3)*

The pH of solution S is 6.5 to 8.5.

Specific optical rotation *(2.2.7)*

+112 to +123, determined on solution S and calculated with reference to the dried substance.

Kanamycin B

Examine by thin-layer chromatography *(2.2.27),* using a plate prepared as prescribed under identification test A. Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 0.1 g of the substance to be examined in *water R* and dilute to 20 ml with the same solvent.

Reference solution Dissolve 4 mg of *kanamycin B sulphate CRS* in *water R* and dilute to 20 ml with the same solvent.

Apply separately to the plate 4 µl of each solution. Develop over a path of 12 cm using a 70 g/l solution of *potassium dihydrogen phosphate R*. Dry the plate in a current of warm air and spray with *ninhydrin and stannous chloride reagent R*. Heat the plate at 110 °C for 15 min. Any spot corresponding to *kanamycin B* in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Loss on drying *(2.2.32)*

Not more than 1.5 per cent, determined on 1.00 g by drying at 60 °C at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash *(2.4.14)*

Not more than 0.5 per cent, determined on 1.0 g.

Sulphate

15.0 per cent to 17.0 per cent of sulphate (SO_4), calculated with reference to the dried substance. Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R.* Titrate with *0.1 M sodium edetate* adding 50 ml of *alcohol R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of sulphate (SO_4).

Pyrogens *(2.6.8)*

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 ml of a solution in *water for injections R* containing 10 mg per millilitre of the substance to be examined.

ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)*.

STORAGE

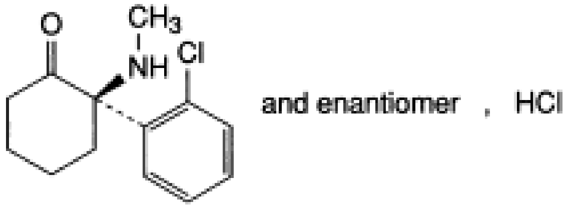
If the substance is sterile, store in a sterile, tamper-proof container.

Ph Eur

Ketamine Hydrochloride

General Notices

(Ph Eur monograph 1020)



$C_{13}H_{17}Cl2NO,HCl$ 274.2 *1867-66-9*

Ph Eur

DEFINITION

(*RS*)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride.

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, soluble in ethanol (96 per cent).

mp

About 260 °C, with decomposition.

IDENTIFICATION

A. Optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison ketamine hydrochloride CRS.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

3.5 to 4.1

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Optical rotation (*2.2.7*)

- 0.2° to + 0.2°.

Dilute 2.5 ml of solution S to 25.0 ml with *water R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *ketamine impurity A CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase (using ultrasound if necessary). To 1.0 ml of the solution, add 0.5 ml of the test solution and dilute to 100.0 ml with the mobile phase. Prepare immediately before use.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Precolumn:

— *size:* *l* = 4 mm, Ø = 4.0 mm;

— *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

— *size:* *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.95 g of *sodium hexanesulphonate R* in 1 litre of a mixture of 25 volumes of *acetonitrile R* and 75 volumes of *water R* and add 4 ml of *acetic acid R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 10 times the retention time of ketamine.

System suitability Reference solution (a):

— *retention time*: ketamine = 3 min to 4.5 min;

— *resolution*: minimum 1.5 between the peaks due to impurity A and ketamine.

Limits:

- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

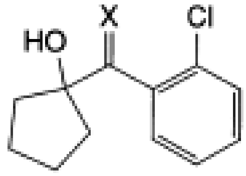
Dissolve 0.200 g in 50 ml of *methanol R* and add 1.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 27.42 mg of $C_{13}H_{17}C_{l2}NO$.

STORAGE

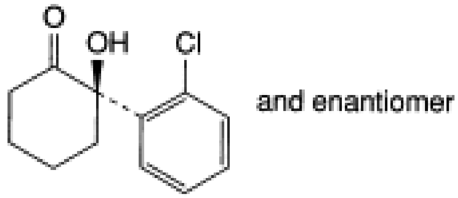
Protected from light.

IMPURITIES



A. X = N-CH_3: 1-[(2-chlorophenyl)(methylimino)methyl]cyclopentanol,

C. X = O: (2-chlorophenyl)(1-hydroxycyclopentyl)methanone,



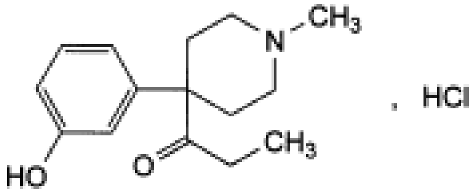
B. (2*RS*)-2-(2-chlorophenyl)-2-hydroxycyclohexanone.

Ph Eur

Ketobemidone Hydrochloride

General Notices

(Ph Eur monograph 1746)



$C_{15}H_{21}NO_2,HCl$ 283.8 *469-79-4 (base)*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

1-[4-(3-Hydroxyphenyl)-1-methylpiperidin-4-yl]propan-1-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, soluble in alcohol, very slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of ketobemidone hydrochloride.

B. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.250 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_8 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 5.5 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Solution A 1.54 g/l solution of *ammonium acetate R* adjusted to pH 8.0 with *dilute ammonia R1*.

Test solution Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 25.0 ml with the same solution.

Reference solution (a) Dissolve 1 mg of *ketobemidone impurity B CRS* and 1 mg of *ketobemidone impurity C CRS* in solution A and dilute to 25 ml with the same solution.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with solution A. Dilute 20.0 ml of this solution to 100.0 ml with solution A.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: phenylhexylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase *acetonitrile R*, solution A (20:80 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 278 nm.

Injection 20 µl.

Run time 4.5 times the retention time of ketobemidone.

Relative retention with reference to ketobemidone (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.6; impurity C = about 0.7; impurity D = about 3.5; impurity E = about 4.2.

System suitability Reference solution (a):

- *resolution*: minimum 4.0 between the peaks due to impurity B and impurity C.

Limits:

- *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
- *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *total*: not more than 3.5 times the area of the principal peak in the chromatogram

obtained with reference solution (b) (0.7 per cent),

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 0.50 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R.* Carry out a potentiometric titration *(2.2.20)* using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

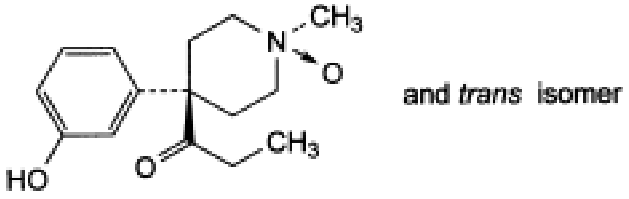
1 ml of *0.1 M sodium hydroxide* is equivalent to 28.38 mg of $C_{15}H_{22}ClNO_2$.

IMPURITIES

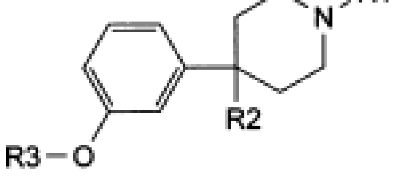
Specified impurities *A, B, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*:

E.



A. 1-[4-(3-hydroxyphenyl)-1-methyl-1-oxidopiperidin-4-yl]propan-1-one (*cis* and *trans* isomers),



B. R1 = CH_3, R2 = $CO\text{-}CH_3$, R3 = H: 1-[4-(3-hydroxyphenyl)-1-methylpiperidin-4-yl]ethanone,

C. R1 = R3 = H, R2 = $CO\text{-}CH_2\text{-}CH_3$: 1-[4-(3-hydroxyphenyl)piperidin-4-yl]propan-1-one,

D. R1 = R3 = CH_3, R2 = $CO\text{-}CH_2\text{-}CH_3$: 1-[4-(3-methoxyphenyl)-1-methylpiperidin-4-yl]propan-1-one,

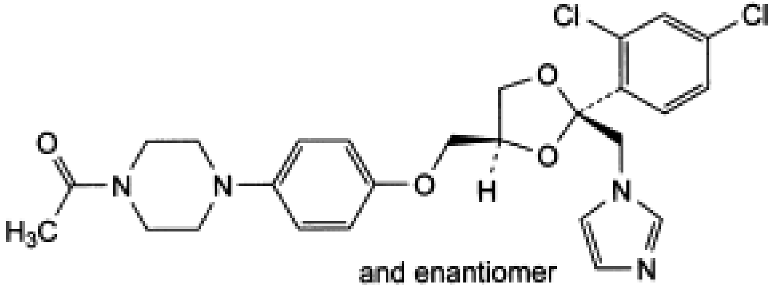
E. R1 = CH_3, R2 = CN, R3 = H: 4-(3-hydroxyphenyl)-1-methylpiperidin-4-carbonitrile.

Ph Eur

Ketoconazole

General Notices

(Ph Eur monograph 0921)



$C_{26}H_{28}Cl_2N_4O_4$ 531.4 *65277-42-1*

Action and use

Antifungal.

Ph Eur

DEFINITION

1-Acetyl-4-[4-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)1,3-dioxolan-4-yl] methoxy]phenyl]piperazine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in methanol, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point *(2.2.14)*: 148 °C to 152 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *ketoconazole CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 5 ml with the mobile phase.

Reference solution (a) Dissolve 30 mg of *ketoconazole CRS* in the mobile phase and dilute to 5 ml with the mobile phase.

Reference solution (b) Dissolve 30 mg of *ketoconazole CRS* and 30 mg of *econazole nitrate CRS* in the mobile phase, then dilute to 5 ml with the mobile phase.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *ammonium acetate solution R, dioxan R, methanol R* (20:40:40 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *methylene chloride R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_4 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

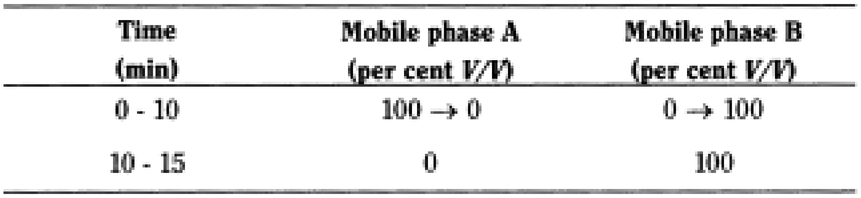
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *ketoconazole CRS* and 2.5 mg of *loperamide hydrochloride CRS* in *methanol R*, then dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: *acetonitrile R1*, 3.4 g/l solution of *tetrabutylammonium hydrogen sulphate R* (5:95 *V/V*);
- *mobile phase B: acetonitrile R1*, 3.4 g/l solution of *tetrabutylammonium hydrogen sulphate R* (50:50 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100 → 0	0 → 100
10 - 15	0	100

Flow rate 2 ml/min.

Detection Spectrophotometer at 220 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with mobile phase A for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Ketoconazole = about 6 min; loperamide = about 8 min.

System suitability Reference solution (a):

- *resolution*: minimum 15 between the peaks due to ketoconazole and loperamide; if necessary, adjust the final concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient elution.

Limits:
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

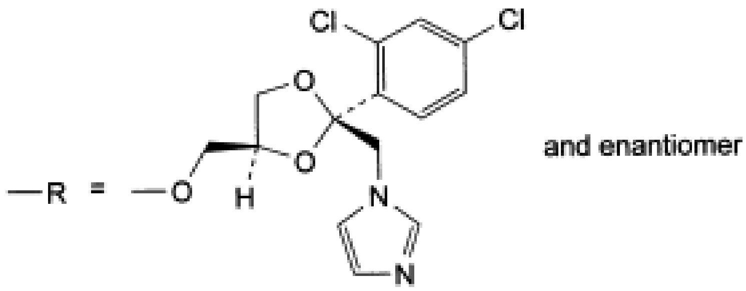
Dissolve 0.200 g in 70 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

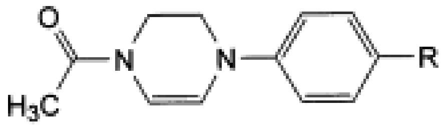
1 ml of *0.1 M perchloric acid* is equivalent to 26.57 mg of $C_{26}H_{28}Cl_2N_4O_4$.

STORAGE

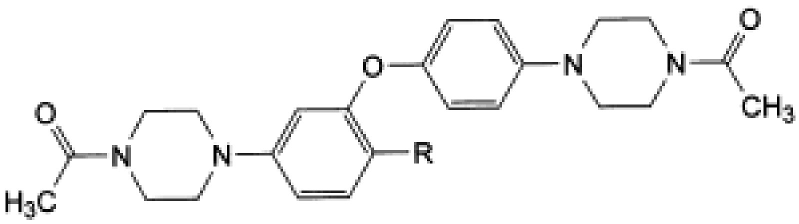
Protected from light.

IMPURITIES

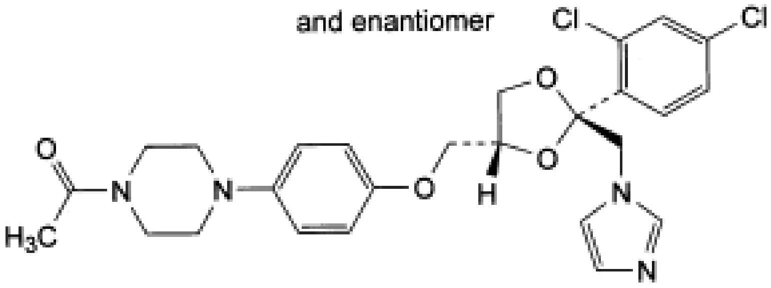




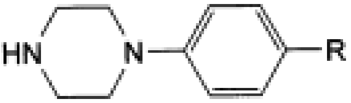
A. 1-acetyl-4-[4-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]1,2,3,4-tetrahydropyrazine,



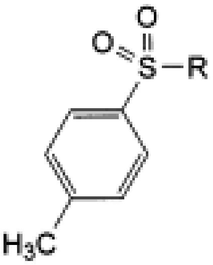
B. 1-acetyl-4-[4-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]-3-[4-(4-acetylpiperazin-1-yl)phenoxy]phenyl]piperazine,



C. 1-acetyl-4-[4-[[(2*RS*,4*RS*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine,



D. 1-[4-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenyl]piperazine,



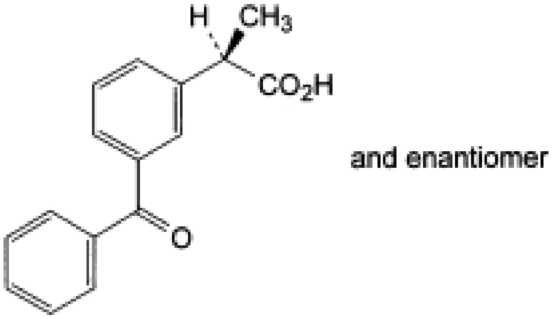
E. [(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl 4-methylbenzenesulphonate.

Ph Eur

Ketoprofen

General Notices

(Ph Eur monograph 0922)



$C_{16}H_{14}O_3$ 254.3 *22071-15-4*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Ketoprofen Capsules

Ketoprofen Gel

Ph Eur

DEFINITION

(2*RS*)-2-(3-Benzoylphenyl)propanoic acid.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Melting point *(2.2.14)*: 94 °C to 97 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with *ethanol (96 per cent) R*.

Spectral range 230-350 nm.

Absorption maximum At 255 nm.

Specific absorbance at the absorption maximum 615 to 680.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *ketoprofen CRS.*

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *ketoprofen CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *indometacin CRS* in *acetone R* and dilute to 10 ml with the same solvent. To 1 ml of this solution add 1 ml of reference solution (a).

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *glacial acetic acid R, methylene chloride R, acetone R* (1:49:50 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 1.0 g in *acetone R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *ketoprofen impurity A CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *ketoprofen impurity C CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. To 1.0 ml of this solution add 1.0 ml of reference solution (b).

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m2/g and a pore size of 10 nm.

Mobile phase Mix 2 volumes of freshly prepared *phosphate buffer solution pH 3.5 R*, 43 volumes of *acetonitrile R* and 55 volumes of *water R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 233 nm.

Injection 20 µl.

Run time 7 times the retention time of ketoprofen.

Relative retention With reference to ketoprofen (retention time = about 7 min): impurity C = about 0.34; impurity H = about 0.39; impurity G = about 0.46; impurity E = about 0.69; impurity B = about 0.73; impurity D = about 1.35; impurity I = about 1.43; impurity A = about 1.50; impurity J = about 1.86; impurity F = about 1.95; impurity K = about 2.27; impurity L = about 2.49.

System suitability Reference solution (d):

— *resolution*: minimum 7.0 between the peaks due to ketoprofen and impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatograms obtained with reference solution (b) (0.2 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatograms obtained with reference solution (c) (0.2 per cent);

— *impurities B, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatograms obtained with reference solution (a) (0.2 per cent);

— *sum of impurities other than A and C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g at 60 °C at a pressure not exceeding 670 Pa.

Sulphated ash (2.4.14)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

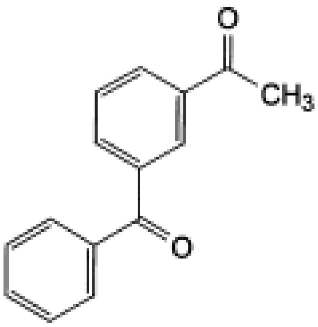
Dissolve 0.200 g in 25 ml of *ethanol (96 per cent)* R. Add 25 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20).*

1 ml of *0.1 M sodium hydroxide* is equivalent to 25.43 mg of $C_{16}H_{14}O_3$.

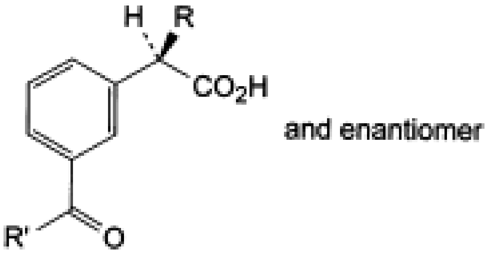
IMPURITIES

Specified impurities A, B, C, D, E, F.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10*. *Control of impurities in substances for pharmaceutical use): G, H, I, J, K, L.*

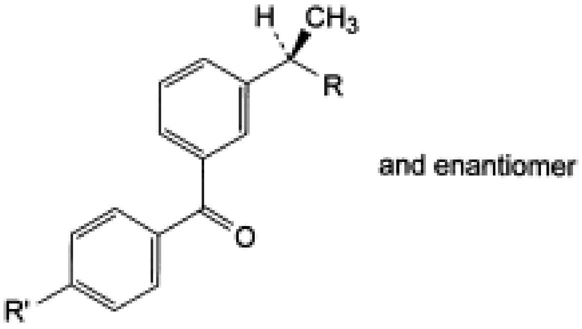


A. 1-(3-benzoylphenyl)ethanone,



B. R = H, R′ = C_6H_5: (3-benzoylphenyl)acetic acid,

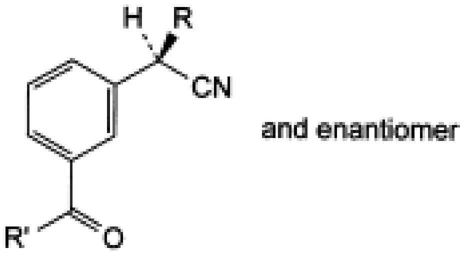
C. R = CH_3, R′ = OH: 3-[(1*RS*)-1-carboxyethyl]benzoic acid,


H
CH3
R
O
R'
and enantiomer

D. R = CO_2H, R′ = CH_3: (2*RS*)-2-[3-(4-methylbenzoyl)phenyl]propanoic acid,

E. R = CO-NH_2, R′ = H: (2*RS*)-2-(3-benzoylphenyl)propanamide,

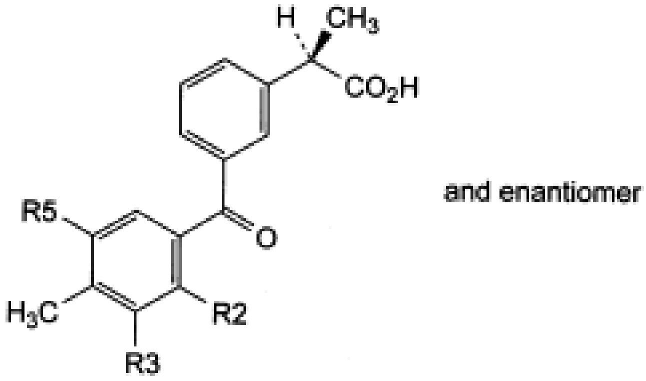
F. R = CN, R′ = H: (2*RS*)-2-(3-benzoylphenyl)propanenitrile,


H
R
CN
R'
O
and enantiomer

G. R = CH_3, R′ = OH: 3-[(1*RS*)-1-cyanoethyl]benzoic acid,

H. R = H, R′ = OH: 3-(cyanomethyl)benzoic acid,

I. R = H, R′ = C_6H_5: (3-benzoylphenyl)ethanenitrile,


H
CH3
CO2H
R5
O
H3C
R2
R3
and enantiomer

J. R2 = CH_3, R3 = R5 = H: (2*RS*)-2-[3-(2,4-dimethylbenzoyl)phenyl]propanoic acid,

K. R2 = R3 = CH_3, R5 = H + R2 = H, R3 = R5 = CH_3: mixture of (2*RS*)-2-[3-(2,3,4-trimethylbenzoyl)phenyl]propanoic acid and (2*RS*)-2-[3-(3,4,5-trimethylbenzoyl)phenyl] propanoic acid,

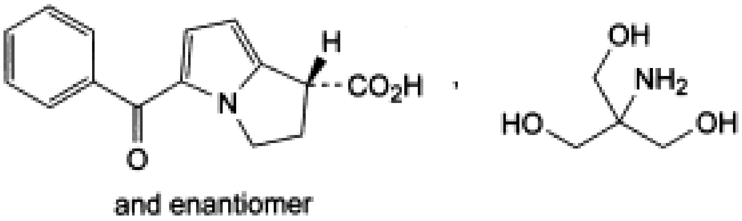
L. R2 = R5 = CH_3, R3 = H: (2*RS*)-2-[3-(2,4,5-trimethylbenzoyl)phenyl]propanoic acid.

Ph Eur

Ketorolac Trometamol

General Notices

(Ph Eur monograph 1755)



$C_{15}H_{13}NO_3,C_4H_{11}NO_3$ 376.4 *74103-07-4*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

2-Amino-2-(hydroxymethyl)propane-1,3-diol (1*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison ketorolac trometamol CRS.

TESTS

Solution S

Dissolve 0.75 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

pH *(2.2.3)*

5.7 to 6.7.

Dilute 5 ml of solution S to 15 ml with *carbon dioxide-free water R*.

Absorbance *(2.2.25)*

Maximum 0.10, determined at 430 nm for solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Protect the solutions from bright light.*

Solvent mixture *tetrahydrofuran R, water R* (30:70 *V/V*).

Test solution Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 50 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dissolve 2 mg of *ketorolac trometamol for peak identification CRS* (containing impurities A, B, C and D) in 5 ml of the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 30 volumes of *tetrahydrofuran R* with 70 volumes of a solution prepared as follows: dissolve 5.75 g of *ammonium dihydrogen phosphate R* in 900 ml of *water R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 ml with *water R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 313 nm.

Injection 10 µl.

Run time 3 times the retention time of ketorolac.

Identification of impurities Use the chromatogram supplied with *ketorolac trometamol for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and D.

Relative retention with reference to ketorolac (retention time = about 10 min): impurity C = about 0.5; impurity A = about 0.6; impurity D = about 0.7; impurity B = about 0.9.

System suitability Reference solution (b):

- *resolution:* minimum 1.5 between the peaks due to impurity B and ketorolac.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.67; impurity B = 0.52; impurity C = 2.2;
- *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 37.64 mg of $C_{19}H_{24}N_2O_6$.

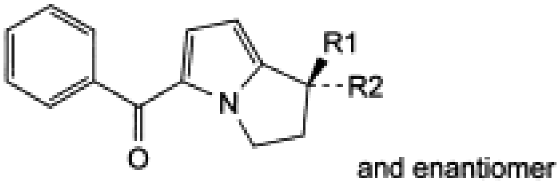
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F, G, H, I, J.*



A. R1 = H, R2 = OH: (1*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-ol,

B. R1 + R2 = O: 5-benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-one,

D. R1 = CO_2H, R2 = OCH_3: (1*RS*)-5-benzoyl-1-methoxy-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid,

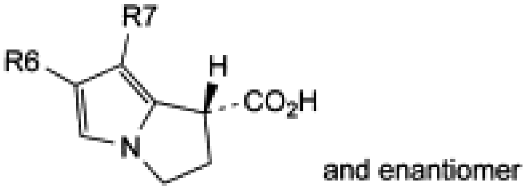
E. R1 = H, R2 = CO-NH-C(CH_2OH)$_3$: (1*RS*)-5-benzoyl-N-[2-hydroxy-1,1-bis(hydroxymethyl) ethyl]-2,3-dihydro-1H-pyrrolizine-1-carboxamide,

G. R1 = CO_2CH_3, R2 = OH: methyl (1*RS*)-5-benzoyl-1-hydroxy-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate,

H. R1 = H, R2 = CO_2CH_3: methyl (1*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate,

I. R1 = R2 = H: phenyl(2,3-dihydro-1*H*-pyrrolizin-5-yl)methanone,

J. R1 = H, R2 = $CO_2C_2H_5$: ethyl (1*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate,



C. R6 = CO-C_6H_5, R7 = H: (1*RS*)-6-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid,

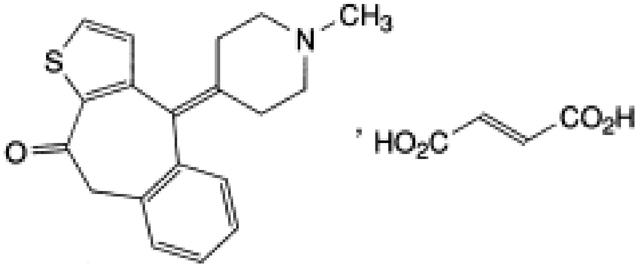
F. R6 = H, R7 = CO-C_6H_5: (1*RS*)-7-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid.

Ph Eur

Ketotifen Fumarate

General Notices

(Ketotifen Hydrogen Fumarate, Ph Eur monograph 1592)



$C_{19}H_{19}NOS,C_4H_4O_4$ 425.5 *34580-14-8*

Action and use

Histamine H_1 receptor antagonist.

Ph Eur

DEFINITION

4-(1-Methylpiperidin-4-ylidene)-4,9-dihydro-10*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-10-one hydrogen (*E*)-butenedioate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White to brownish-yellow, fine, crystalline powder.

Solubility

Sparingly soluble in water, slightly soluble in methanol, very slightly soluble in acetonitrile.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of ketotifen hydrogen fumarate.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 11 mg of *fumaric acid CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *cellulose for chromatography F_{254} R* as the coating substance.

Mobile phase *water R*, *anhydrous formic acid R*, *di-isopropyl ether R* (3:7:90 *V/V/V*).

Application 5 µl.

Development Over a path of 17 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm. Spray lightly with a 5 g/l solution of *potassium permanganate R* in a 1.4 per cent *V/V* solution of *sulphuric acid R*. Examine in daylight by transparency.

Results The spot due to fumaric acid in the chromatogram obtained with the test solution is similar in position, colour and intensity to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_4, BY_4 or B_4 (*2.2.2, Method II)*.

Dissolve 0.2 g in *methanol R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 30.0 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with a mixture of equal volumes of *methanol R* and *water R*. Dilute 1.0 ml to 10.0 ml with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution (b) Dissolve 3.0 mg of *ketotifen impurity G CRS* in 10 ml of *methanol R* and dilute to 20.0 ml with *water R*. *Protect the solution from light.*

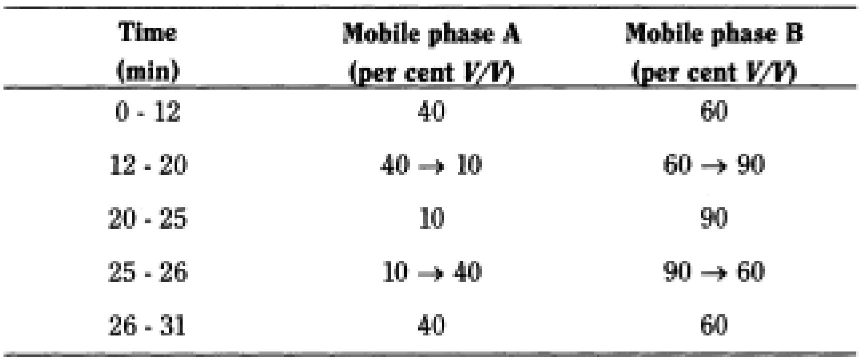
Reference solution (c) To 1.5 ml of reference solution (b) add 1.0 ml of the test solution and dilute to 10.0 ml with a mixture of equal volumes of *methanol R* and *water R*. *Protect the solution from light.*

Reference solution (d) Dilute 0.5 ml of reference solution (b) to 50.0 ml with a mixture of equal volumes of *methanol R* and *water R*. *Protect the solution from light.*

Column:
- *size*: *l* = 0.15 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 40 °C.

Mobile phase:

- — *mobile phase A*: mix 175 µl of *triethylamine R* and 500 ml of *water R;*
- — *mobile phase B*: mix 175 µl of *triethylamine R* and 500 ml of *methanol R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	40	60
12 - 20	40 → 10	60 → 90
20 - 25	10	90
25 - 26	10 → 40	90 → 60
26 - 31	40	60

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 297 nm.

Injection 20 µl; inject the test solution and reference solutions (a), (c) and (d).

Relative retentions with reference to ketotifen: impurity D = about 0.31; impurity C = about 0.61; impurity G = about 0.86; impurity E = about 1.18; impurity F = about 1.36; impurity B = about 1.72; impurity A = about 2.15.

System suitability:

- — *resolution*: minimum of 1.5 between the peaks due to ketotifen and to impurity G in the chromatogram obtained with reference solution (c);
- — *signal-to-noise ratio*: minimum of 70 for the principal peak in the chromatogram obtained with reference solution (d).

Limits:

- — *correction factor*: for the calculation of contents, multiply the area of the corresponding peak by the following correction factor: impurity G = 1.36;
- — *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- — *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

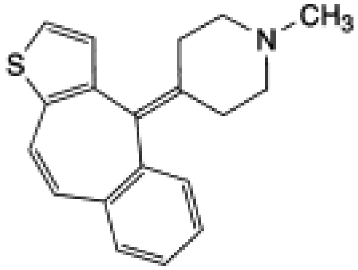
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

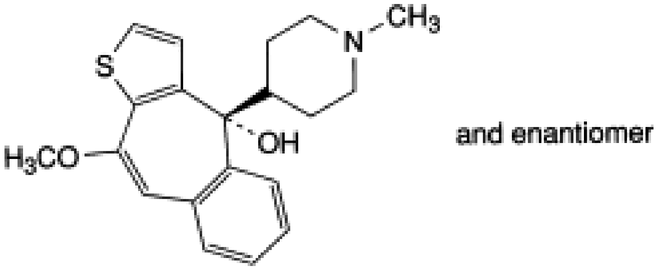
Dissolve 0.350 g in a mixture of 30 ml of *anhydrous acetic acid R* and 30 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 42.55 mg of $C_{23}H_{23}NO_5S$.

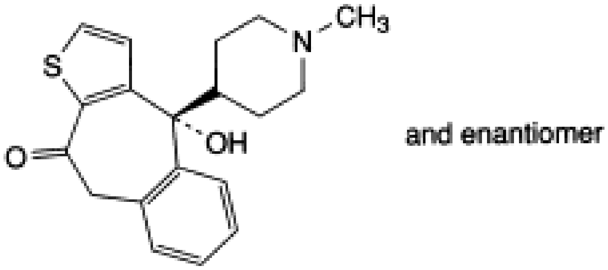
IMPURITIES



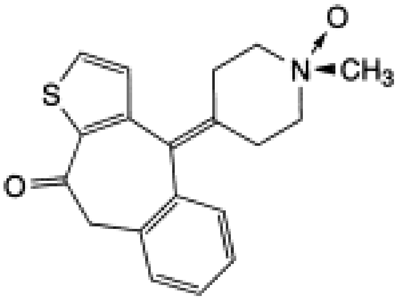
A. 4-(4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-4-ylidene)-1-methylpiperidine,


S
N
CH3
H3CO
OH
and enantiomer

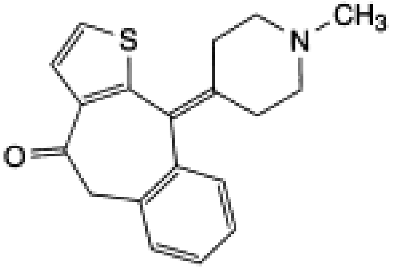
B. (4*RS*)-10-methoxy-4-(1-methylpiperidin-4-yl)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-4-ol,


S
N
CH3
O
OH
and enantiomer

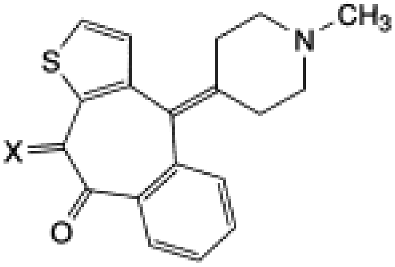
C. (4*RS*)-4-hydroxy-4-(1-methylpiperidin-4-yl)-4,9-dihydro-10*H*-benzo[4,5]cyclohepta[1,2-*b*] thiophen-10-one,



D. 4-[(a*R*a*S*)-1-methylpiperidin-4-ylidene]-4,9-dihydro-10*H*-benzo[4,5]cyclohepta[1,2-*b*] thiophen-10-one *N*-oxide (ketotifen N-oxide),



E. 10-(1-methylpiperidin-4-ylidene)-5,10-dihydro-4*H*-benzo[5,6]cyclohepta[1,2-*b*]thiophen-4-one,



F. X = H_2: 4-(1-methylpiperidin-4-ylidene)-4,10-dihydro-9*H*-benzo[4,5]cyclohepta[1,2-*b*] thiophen-9-one,

G. X = O: 4-(1-methylpiperidin-4-ylidene)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-9,10-dione.

Ph Eur

Light Kaolin (Natural)

General Notices

Action and use

Antidiarrhoeal.

Preparations

Kaolin Mixture

Kaolin and Morphine Mixture

When Kaolin or Light Kaolin is prescribed or demanded, Light Kaolin shall be dispensed or supplied unless it is ascertained that Light Kaolin (Natural) is required.

DEFINITION

Light Kaolin (Natural) is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. It does not contain a dispersing agent.

CHARACTERISTICS

A light, white powder free from gritty particles; odourless or almost odourless; unctuous.

Practically insoluble in *water* and in mineral acids.

IDENTIFICATION

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate* and 3 g of *sodium carbonate* and heat until the mixture melts. Allow to cool. To the residue add 20 ml of boiling *water*, mix and filter. Wash the residue with 50 ml of *water*, add to the residue 1 ml of *hydrochloric acid* and 5 ml of *water*, mix and filter. To the filtrate add 1 ml of *strong sodium hydroxide solution*, filter and add to the filtrate 3 ml of *ammonium chloride solution*. A gelatinous white precipitate is produced.

B. 0.25 g yields the reaction characteristic of *silicates*, Appendix VI.

C. Triturate 2 g with 2 ml of *water*. The resulting mixture does not flow.

TESTS

Coarse particles; Arsenic; Heavy metals; Chloride; Loss on drying; Loss on ignition; Soluble matter

Complies with the requirements stated under Light Kaolin.

Fine particles

Disperse 5 g in 250 ml of *water* containing 50 mg of *sodium pyrophosphate* by shaking vigorously for 2 minutes in a stoppered flask, pour immediately into a glass cylinder 5 cm in diameter and transfer 20 ml to a glass dish using a pipette. Evaporate to dryness and dry to constant weight at 105°. Allow the remainder of the suspension to stand for 4 hours at 20°

and withdraw a second 20 ml portion using a pipette with its tip exactly 5 cm below the surface and without disturbing the sediment. Transfer the second portion to a glass dish, evaporate to dryness and dry to constant weight at 105°. The weight of the residue from the second portion is not less than 70% of the weight of the residue from the first portion.

Light Kaolin

General Notices

Action and use

Antidiarrhoeal.

Preparations

Kaolin Mixture

Kaolin and Morphine Mixture

When Kaolin or Light Kaolin is prescribed or demanded, Light Kaolin shall be dispensed or supplied unless it is ascertained that Light Kaolin (Natural) is required.

DEFINITION

Light Kaolin is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. It contains a suitable dispersing agent.

CHARACTERISTICS

A light, white powder free from gritty particles; odourless or almost odourless; unctuous.

Practically insoluble in *water* and in mineral acids.

IDENTIFICATION

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate* and 3 g of *sodium carbonate* and heat until the mixture melts. Allow to cool. To the residue add 20 ml of boiling *water*, mix and filter. Wash the residue with 50 ml of *water*, add to the residue 1 ml of *hydrochloric acid* and 5 ml of *water*, mix and filter. To the filtrate add 1 ml of *strong sodium hydroxide solution*, filter and add to the filtrate 3 ml of *ammonium chloride solution*. A gelatinous white precipitate is produced.

B. 0.25 g yields the reaction characteristic of *silicates*, Appendix VI.

C. Triturate 2 g with 2 ml of *water*. The resulting mixture flows.

TESTS

Coarse particles

Transfer 5 g to a stoppered cylinder (about 16 cm × 35 mm), add 60 ml of a 1% w/v solution of *sodium pyrophosphate*, shake thoroughly and allow to stand for 5 minutes. Using a pipette, withdraw 50 ml from a point about 5 cm below the surface of the liquid. To the remaining liquid add 50 ml of *water*, shake, allow to stand for 5 minutes and withdraw 50 ml in the same manner as before. Repeat the operation until a total of 400 ml of suspension has been withdrawn under the prescribed conditions. Transfer the remainder to an evaporating dish and evaporate to dryness on a water bath. The residue, after drying at 105°, weighs not more than 25 mg.

Fine particles

Disperse 5 g in 250 ml of *water* by shaking vigorously for 2 minutes in a stoppered flask, pour immediately into a glass cylinder 5 cm in diameter and transfer 20 ml to a glass dish using a pipette. Evaporate to dryness and dry to constant weight at 105°. Allow the remainder of the suspension to stand for 4 hours at 20° and withdraw a second 20 ml portion using a pipette with its tip exactly 5 cm below the surface and without disturbing the sediment. Transfer the second portion to a glass dish, evaporate to dryness and dry to constant weight at 105°. The weight of the residue from the second portion is not less than 70% of the weight of the residue from the first portion.

Arsenic

0.50 g dispersed in 25 ml of *water* complies with the *limit test for arsenic*, Appendix VII (2 ppm).

Heavy metals

Heat 6.0 g for 15 minutes under a reflux condenser on a water bath with a mixture of 70 ml of *water* and 10 ml of *hydrochloric acid* and filter. To 40 ml of the filtrate add 0.5 ml of *nitric acid* and evaporate to low bulk. Add 20 ml of *water*, 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with two 10 ml quantities of a mixture of equal volumes of *isoamyl alcohol* and *ether*. To the aqueous layer add 2 g of *citric acid* and sufficient *water* to produce 60 ml. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (20 ppm).

Chloride

Boil 1.0 g with 80 ml of *water* and 20 ml of 2M *nitric acid* under a reflux condenser for 5 minutes, cool and filter. 15 ml of the filtrate complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Loss on drying

When dried to constant weight at 105°, loses not more than 1.5% of its weight. Use 1 g.

Loss on ignition

When ignited at 600°, loses not more than 15.0% of its weight. Use 1 g.

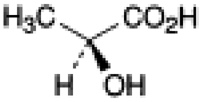
Soluble matter

Boil 2 g with 100 ml of 0.2M *hydrochloric acid* under a reflux condenser for 5 minutes, cool, filter and evaporate 50 ml of the filtrate to dryness. The residue, after ignition at about 600° for 30 minutes, weighs not more than 10 mg.

(*S*)-Lactic Acid

General Notices

(Ph Eur monograph 1771)



$C_3H_6O_3$ 90.1 *77-33-4*

Ph Eur

DEFINITION

Mixture of (*S*)-2-hydroxypropanoic acid, its condensation products, such as lactoyl-lactic acid and polylactic acids, and water. The equilibrium between lactic acid and polylactic acids depends on the concentration and temperature.

Content

88.0 per cent *m/m* to 92.0 per cent *m/m* of $C_3H_6O_3$, not less than 95.0 per cent of which is the (*S*)-enantiomer.

CHARACTERS

Appearance

Colourless or slightly yellow, syrupy liquid.

Solubility

Miscible with water and with ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in 10 ml of *water R*. The solution is strongly acidic *(2.2.4)*.

B. Relative density *(2.2.5)*: 1.20 to 1.21.

C. It gives the reaction of lactates *(2.3.1)*.

D. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 5.0 g in 42 ml of *1 M sodium hydroxide* and dilute to 50 ml with *distilled water R*.

Appearance

The substance to be examined is not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Ether-insoluble substances

Dissolve 1.0 g in 25 ml of *ether R*. The solution is not more opalescent than the solvent used for the test.

Sugars and other reducing substances

To 1 ml of solution S add 1 ml of *1 M hydrochloric acid*, heat to boiling, allow to cool and add 1.5 ml of *1 M sodium hydroxide* and 2 ml of *cupri-tartaric solution R*. Heat to boiling. No red or greenish precipitate is formed.

Methanol ***(2.4.24)***

Maximum 50 ppm, if intended for use in the manufacture of parenteral dosage forms.

Citric, oxalic and phosphoric acids

To 5 ml of solution S add *dilute ammonia R1* until slightly alkaline *(2.2.4)*. Add 1 ml of *calcium chloride solution R.* Heat on a water-bath for 5 min. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *water R* and 5 ml of solution S.

Sulphates ***(2.4.13)***

Maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*.

Calcium ***(2.4.3)***

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins ***(2.6.14)***

Less than 5 IU/g if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins. Before use, neutralise the test solution to pH 7.0-7.5 with *strong sodium hydroxide solution R* and shake vigorously.

ASSAY

Place 1.000 g in a ground-glass-stoppered flask and add 10 ml of *water R* and 20.0 ml of *1 M* *sodium hydroxide*. Close the flask and allow to stand for 30 min. Using 0.5 ml of *phenolphthalein solution R* as indicator, titrate with *1 M hydrochloric acid* until the pink colour is discharged.

1 ml of *1 M sodium hydroxide* is equivalent to 90.1 mg of $C_3H_6O_3$.

(*S*)-enantiomer

Transfer an amount of the substance to be examined equivalent to 2.00 g of lactic acid into a round-bottomed flask, add 25 ml of *1 M sodium hydroxide* and boil gently for 15 min. Cool down and adjust to pH 7.0 using *1 M hydrochloric acid*. Add 5.0 g of *ammonium molybdate R*, dissolve and dilute to 50.0 ml with *water R*. Filter and measure the angle of optical rotation *(2.2.7)*. Calculate the percentage content of (*S*)-enantiomer using the expression:

$$50 + \left(24.18 \times \alpha \times \frac{2.222}{m} \times \frac{90}{c}\right)$$

α = angle of optical rotation (absolute value),

m = mass of the substance to be examined, in grams,

c = percentage content of $C_3H_6O_3$ in the substance to be examined.

The complex of (*S*)-lactic acid formed under these test conditions is laevorotatory.

LABELLING

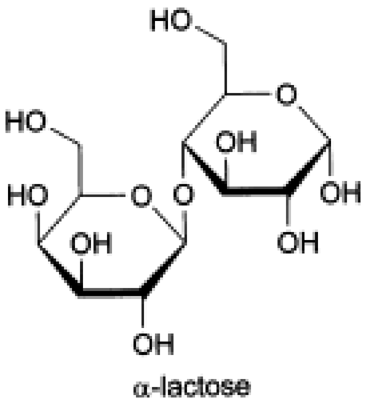
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

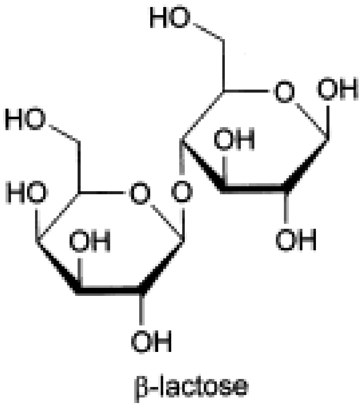
Ph Eur

Anhydrous Lactose

General Notices

(Ph Eur monograph 1061)


α-lactose


β-lactose

$C_{12}H_{22}O_{11}$ 342.3 *63-42-3*

Action and use

Excipient.

Ph Eur

DEFINITION

O-β-D-Galactopyranosyl-(1→4)-β-D-glucopyranose or mixture of *O*-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose and *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely but slowly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *anhydrous lactose CRS.*

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *water R*, *methanol R* (2:3 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *anhydrous lactose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *anhydrous lactose CRS*, 10 mg of *fructose CRS*, 10 mg of *glucose CR*S and 10 mg of *sucrose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Plate *TLC silica gel G plate R.*

Mobile phase *water R*, *methanol R*, *glacial acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately, as a slight excess of water produces cloudiness.

Application 2 µl; thoroughly dry the starting points.

Development A Over a path of 15 cm.

Drying A In a current of warm air.

Development B Immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*; heat at 130 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.25 g in 5 ml of *water R*. Add 5 ml of *ammonia R* and heat in a water-bath at 80 °C for 10 min. A red colour develops.

D. Water (see Tests).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 1.0 g in boiling *water R* and dilute to 10 ml with the same solvent.

Acidity or alkalinity

Dissolve 6.0 g by heating in 25 ml of *carbon dioxide-free water R*, cool and add 0.3 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation (*2.2.7*)

+ 54.4 to + 55.9 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, heating to 50 °C. Allow to cool and add 0.2 ml of *dilute ammonia R1*. Allow to stand for 30 min and dilute to 100.0 ml with *water R.*

Absorbance (*2.2.25*).

Test solution (a) Dissolve 1.0 g in boiling *water R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *water R*.

Spectral range 400 nm for test solution (a) and 210-300 nm for test solution (b).

Results:

— at 400 nm: maximum 0.04 for test solution (a);

— from 210 nm to 220 nm: maximum 0.25 for test solution (b);

— from 270 nm to 300 nm: maximum 0.07 for test solution (b).

Heavy metals (*2.4.8*)

Maximum 5 ppm.

2.0 g complies with test C. Prepare the reference solution using 1.0 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.50 g, using a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as the solvent.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination.

Total viable aerobic count (*2.6.12*) not more than 10^2 micro-organisms per gram, determined by plate-count. It complies with the test for *Escherichia coli* (*2.6.13*)

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for anhydrous lactose used as a filler/diluent in solid dosage forms (compressed and powder).

Particle size distribution (*2.9.31* or *2.9.38*).

Bulk and tapped density (*2.9.15*)

Determine the bulk density and the tapped density. Calculate the Hausner index using the following expression:

$$\frac{V_0}{V_f}$$

V_0 = volume of bulk substance;

V_f = volume of tapped substance.

α-Lactose and β-lactose

Gas chromatography (*2.2.28*).

Silylation reagent Mix 28 volumes of *N-trimethylsilylimidazole R* and 72 volumes of *pyridine R.*

Test solution Dissolve about 1 mg of the substance to be examined in 0.45 ml of *dimethyl sulphoxide R*. Add 1.8 ml of the silylation reagent. Mix gently and allow to stand for 20 min.

Reference solution Prepare a mixture of α-*lactose monohydrate R* and β-*lactose R* having an anomeric ratio of about 1:1 based on the labelled anomeric contents of the α-lactose monohydrate and β-lactose. Dissolve about 1 mg of this mixture in 0.45 ml of *dimethyl sulphoxide R*. Add 1.8 ml of the silylation reagent. Mix gently and allow to stand for 20 min.

Column:

— *material*: glass;

— *size:* l = 0.9 m, Ø = 4 mm;

— *stationary phase*: *silanised diatomaceous earth for gas chromatography R* impregnated with 3 per cent *m/m* of *poly[(cyanopropyl)(methyl)][(phenyl)(methyl)] siloxane R.*

Carrier gas *helium for chromatography R*.

Flow rate 40 ml/min.

Temperature:

— *column*: 215 °C;

— *injection port and detector*: 275 °C.

Detection Flame ionisation.

Injection 2 µl.

System suitability Reference solution:

— *relative retention* with reference to β-lactose: α-lactose = about 0.7;

— *resolution*: minimum 3.0 between the peaks due to α-lactose and β-lactose.

Calculate the percentage content of α-lactose from the following expression:

$$\frac{100S_a}{S_a + S_b}$$

Calculate the percentage content of β-lactose from the following expression:

$$\frac{100S_b}{S_a + S_b}$$

S_a = area of the peak due to α-lactose,

S_b = area of the peak due to β-lactose.

Loss on drying ***(2.2.32)***

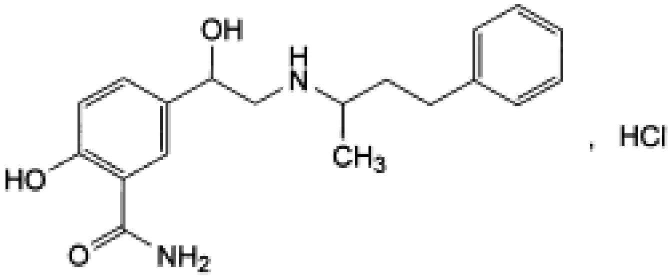
Determine on 1.000 g by drying in an oven at 80 °C for 2 h.

Ph Eur

Labetalol Hydrochloride

General Notices

(Ph Eur monograph 0923)



$C_{19}H_{24}N_2O_3,HCl$ 364.9 *32780-64-6*

Action and use

Alpha-and beta-adrenoceptor antagonist.

Preparations

Labetalol Injection

Labetalol Tablets

Ph Eur

DEFINITION

2-Hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]benzamide hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Sparingly soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, C, E.

Second identification A, B, D, E.

A. Optical rotation *(2.2.7)*: - 0.05 ° to + 0.05 °, determined on solution S (see Tests).

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 25.0 mg in *0.1 M hydrochloric acid* and dilute to 250.0 ml with the same acid.

Spectral range 230-350 nm.

Absorption maximum At 302 nm.

Specific absorbance at the absorption maximum 83 to 88.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *labetalol hydrochloride CRS.*

D. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in 1 ml of *ethanol (96 per cent) R.*

Reference solution (a) Dissolve 10 mg of *labetalol hydrochloride CRS* in 1 ml of *ethanol (96 per cent) R.*

Reference solution (b) Dissolve 10 mg of *labetalol hydrochloride CRS* and 10 mg of *propranolol hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R*.

Mobile phase *perchloric acid R*, *water R*, *methanol R* (0.5:50:80 *V/V/V*).

Application 2 µl.

Development Place the plate in a chromatographic tank immediately after the addition of the mobile phase, close the tank and develop over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent. *Solution S must be freshly prepared.*

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

pH *(2.2.3)*

4.0 to 5.0 for solution S.

Diastereoisomer ratio

Gas chromatography *(2.2.28)*.

Test solution Dissolve 2.0 mg of the substance to be examined in 1.0 ml of a 12.0 g/l solution of *butylboronic acid R* in *anhydrous pyridine R* and allow to stand for 20 min.

Column:
- *material*: glass;
- *size:* *l* = 1.5 m, Ø = 4 mm;
- *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (125-150 µm) impregnated with 3 per cent *m/m* of *polymethylphenylsiloxane R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 40 ml/min.

Temperature:
- *column, injection port and detector*: 300 °C.

Detection Flame ionisation.

Injection 2 µl.

System suitability:
- the height of the trough separating the 2 peaks due to the pairs of diastereoisomers is less than 5 per cent of the full scale of the recorder.

Limit:
- *each pair of diastereoisomers*: for the area of each peak, 45 per cent to 55 per cent of the total area of the 2 peaks.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution Dilute 0.5 ml of the test solution to 100.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 150 ml of *tetrahydrofuran R*, 300 ml of *methanol R*, 550 ml of *water R*, 0.82 g of *tetrabutylammonium hydrogen sulphate R*, 1 g of *sodium octyl sulphate R* and 10 ml of a 10 per cent *V/V* solution of *sulphuric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 229 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time 3 times the retention time of labelatol.

Retention time Labelatol = 10 min to 15 min; if necessary, adjust the water content of the mobile phase ensuring that the 2:1 ratio of methanol to tetrahydrofuran is maintained.

Limits:

— *impurities A, B*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

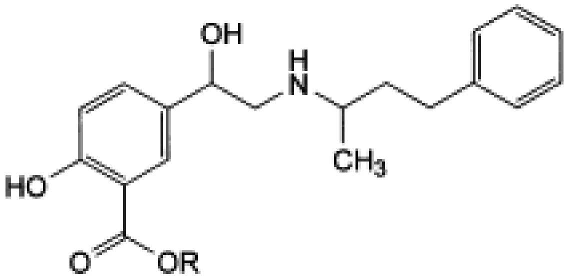
In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.200 g in a mixture of 10 ml of *anhydrous formic acid R* and 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 36.49 mg of $C_{19}H_{25}ClN_2O_3$.

IMPURITIES

Specified impurities *A, B*.



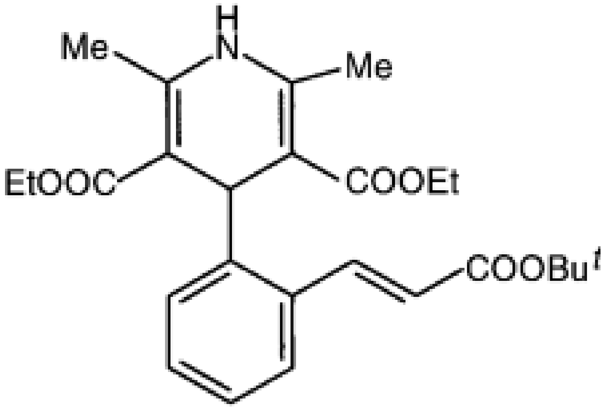
A. R = H: 2-hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)-amino]ethyl]benzoic acid,

B. R = CH_3: methyl 2-hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl] benzoate.

Ph Eur

Lacidipine

General Notices



$C_{26}H_{33}NO_6$ 455.6 *103890-78-4*

Action and use

Calcium channel blocker.

Preparation

Lacidipine Tablets

DEFINITION

Lacidipine is diethyl (*E*)-4-{2-[(*tert*-butoxycarbonyl)vinyl]phenyl}-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate. It contains not less than 97.5% and not more than 102.0% of $C_{26}H_{33}NO_6$, calculated with reference to the anhydrous, propan-2-ol–free substance.

CHARACTERISTICS

A white to pale yellow crystalline powder. It melts at about 178°.

Practically insoluble in *water*; freely soluble in *acetone*; sparingly soluble in *absolute ethanol*.

Carry out all of the following procedures protected from light and prepare solutions immediately before use.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lacidipine (*RS 407*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Propan-2-ol

Carry out the method for *gas chromatography*, Appendix III B. Prepare a 0.002% v/v solution of *1,4-dioxan* (internal standard) in *dimethylacetamide* (solution A).

(1) 0.002% v/v solution of *propan-2-ol* in solution A.

(2) 2% w/v of the substance being examined in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (60 m × 0.32 mm) bonded with a film (5 µm) of *polymethylsiloxane* (CP-Sil 5CB is suitable).

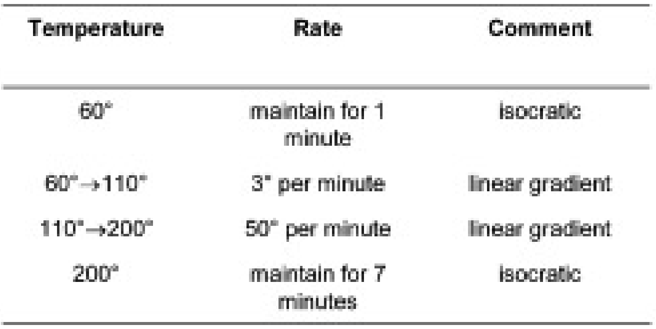
(b) Use *helium* as the carrier gas at 1.7 ml per minute.

(c) Use a temperature gradient as described below.

(d) Use an injection temperature of 170°.

(e) Use a detector temperature of 250°.

(f) Inject 1 µl of each solution.



Temperature	Rate	Comment
60°	maintain for 1 minute	isocratic
60°→110°	3° per minute	linear gradient
110°→200°	50° per minute	linear gradient
200°	maintain for 7 minutes	isocratic

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (1) shows two clearly separated peaks. The retention time for propan-2-ol is about 6.2 minutes and that for dioxan is about 15 minutes.

LIMIT

In the chromatogram obtained with solution (2):

— the percentage content of propan-2-ol by normalisation is not more than 0.5% w/w.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute 1 volume of a 0.1% w/v solution of the substance being examined in *absolute ethanol* to 5 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 500 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *lacidipine impurity standard BPCRS* in *absolute*

ethanol to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µl of each solution.

(g) If necessary adjust the composition of the mobile phase so that, in the chromatogram obtained with solution (3), the retention time of the peak due to lacidipine is about 10 minutes.

(h) For solution (1) allow the chromatography to proceed for 2 times the retention time of the principal peak.

MOBILE PHASE

3 volumes of *absolute ethanol* and 97 volumes of n-*hexane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lacidipine impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak due to lacidipine impurity B is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%, taking into account the correction factor of 2);

— the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

— the total nominal content of impurities is not greater than 0.5%.

Water

Not more than 0.2% w/w, Appendix IX C. Use 0.5 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute 5 volumes of a 0.1% w/v solution of the substance being examined in *absolute ethanol* to 100 volumes with the mobile phase.

(2) Dilute 5 volumes of a 0.1% w/v solution of *lacidipine BPCRS* in *absolute ethanol* to 100 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *lacidipine impurity standard BPCRS* in *absolute ethanol* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

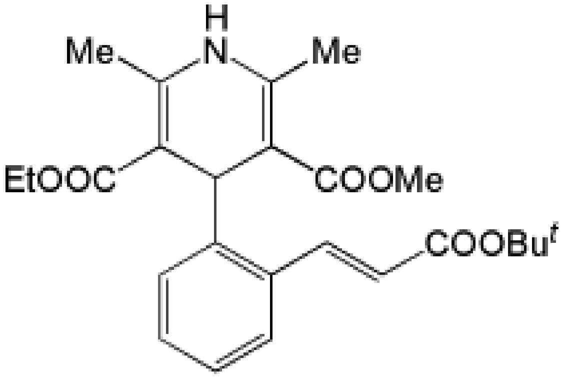
SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the corresponding chromatogram supplied with *lacidipine impurity standard BPCRS*.

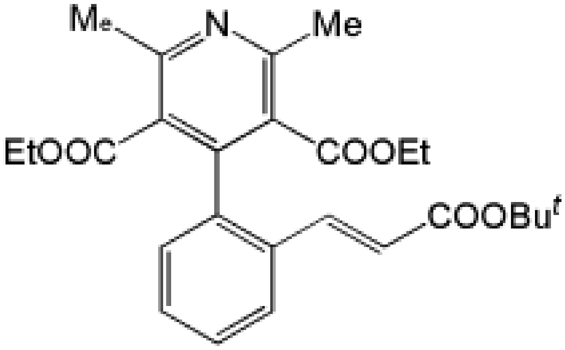
DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{33}NO_6$ from the chromatograms obtained and using the declared content of $C_{26}H_{33}NO_6$ in *lacidipine BPCRS*.

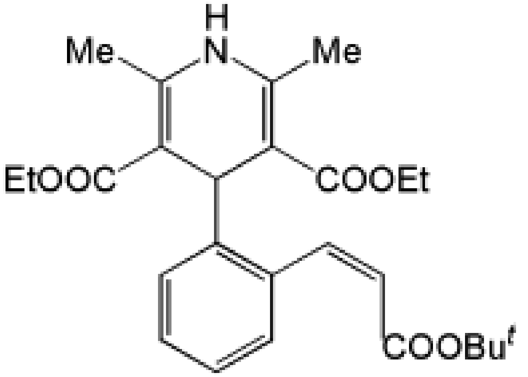
IMPURITIES



A. Ethyl methyl (*E*)-4-{2-[2-(*tert*-butoxycarbonyl)vinyl]phenyl}-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate,



B. Diethyl (*E*)-4-{2-[2-(*tert*-butoxycarbonyl)vinyl]phenyl}-2,6-dimethylpyridine-3,5-dicarboxylate,

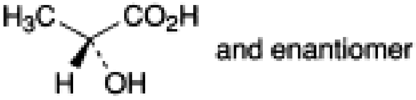

Me
H
N
Me
EtOOC
COOEt
COOBu^t

C. Diethyl (*Z*)-4-{2-[2-(*tert*-butoxycarbonyl)vinyl]phenyl}-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate.

Lactic Acid

General Notices

(Ph Eur monograph 0458)



$C_3H_6O_3$ 90.1 *50-21-5*

Preparations

Sodium Lactate Intravenous Infusion

Compound Sodium Lactate Intravenous Infusion

Lactic Acid Pessaries

Ph Eur

DEFINITION

Mixture of 2-hydroxypropanoic acid, its condensation products, such as lactoyl-lactic acid and polylactic acids, and water. The equilibrium between lactic acid and polylactic acids depends on the concentration and temperature. It is usually the racemate ((*RS*)-lactic acid).

Content

88.0 per cent *m/m* to 92.0 per cent *m/m* of $C_3H_6O_3$.

CHARACTERS

Appearance

Colourless or slightly yellow, syrupy liquid.

Solubility

Miscible with water and with ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in 10 ml of *water R*. The solution is strongly acidic *(2.2.4)*.

B. Relative density *(2.2.5)*: 1.20 to 1.21.

C. It gives the reaction of lactates *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in 42 ml of *1 M sodium hydroxide* and dilute to 50 ml with *distilled water R*.

Appearance

The substance to be examined is not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Ether-insoluble substances

Dissolve 1.0 g in 25 ml of *ether R*. The solution is not more opalescent than the solvent used for the test.

Sugars and other reducing substances

To 1 ml of solution S add 1 ml of *1 M hydrochloric acid*, heat to boiling, allow to cool and add 1.5 ml of *1 M sodium hydroxide* and 2 ml of *cupri-tartaric solution R*. Heat to boiling. No red or greenish precipitate is formed.

Methanol *(2.4.24)*

Maximum 50 ppm, if intended for use in the manufacture of parenteral dosage forms.

Citric, oxalic and phosphoric acids

To 5 ml of solution S add *dilute ammonia R1* until slightly alkaline *(2.2.4)*. Add 1 ml of *calcium chloride solution R*. Heat on a water-bath for 5 min. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *water R* and 5 ml of solution S.

Sulphates *(2.4.13)*

Maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R.*

Calcium *(2.4.3)*

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Sulphated ash *(2.4.14)*: maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 5 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins. Before use, neutralise the test solution to pH 7.0-7.5 with *strong sodium hydroxide solution R* and shake vigorously.

ASSAY

Place 1.000 g in a ground-glass-stoppered flask and add 10 ml of *water R* and 20.0 ml of *1 M sodium hydroxide*. Close the flask and allow to stand for 30 min. Using 0.5 ml of *phenolphthalein solution R* as indicator, titrate with *1 M hydrochloric acid* until the pink colour is discharged.

1 ml of *1 M sodium hydroxide* is equivalent to 90.1 mg of $C_3H_6O_3$.

LABELLING

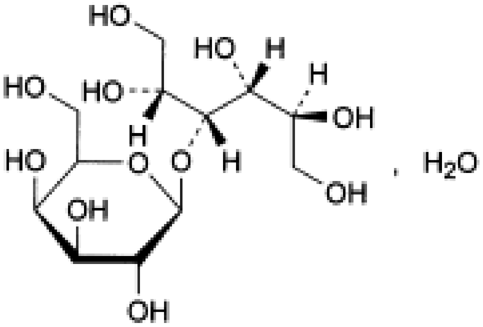
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Lactitol Monohydrate

General Notices

(Ph Eur monograph 1337)



$C_{12}H_{24}O_{11},H_2O$ 362.3 *81025-04-9*
(anhydrous)

Action and use

Osmotic laxative.

Ph Eur

DEFINITION

4-*O*-(β-D-galactopyranosyl)-D-glucitol.

Content

96.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, slightly soluble in alcohol, practically insoluble in methylene chloride

IDENTIFICATION

First identification B.

Second identification A, C.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *lactitol monohydrate CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *lactitol monohydrate CRS* in *methanol R* and dilute to 2 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *sorbitol CRS* in 2 ml of reference solution (a) and dilute to 20 ml with *methanol R*.

Plate *TLC silica gel G plate R*.

Mobile phase *water R*, *acetonitrile R* (25:75 *V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R*. Dry the plate in a current of cold air until the solvent is removed. Heat at 100 °C for 15 min. Allow to cool and spray with a 2 g/l solution of *sodium periodate R*. Dry the plate in a current of cold air. Heat at 100 °C for 15 min.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 5.000 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 10 ml of *carbon dioxide-free water R*. To 10 ml of this solution add 0.05 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink. To a further 10 ml of the solution add 0.05 ml of *methyl red solution R*. Not more than 0.3 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Specific optical rotation*(2.2.7)*

+ 13.5 to + 15.5 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dilute 2.0 ml of test solution (a) to 50.0 ml with *water R*.

Reference solution (a) Dissolve 5.0 mg of *lactitol monohydrate CRS* and 5 mg of *glycerol R* in *water R* and dilute to 25.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 100.0 ml with *water R*. Dilute 5.0 ml of this solution to 100.0 ml with *water R*.

Reference solution (c) Dilute 2.5 ml of reference solution (a) to 10.0 ml with *water R*.

Column:
- *size*: *l* = 0.30 m, Ø = 7.8 mm;
- *stationary phase*: *strong cation exchange resin (calcium form) R*;
- *temperature*: 60 °C.

Mobile phase *water R*.

Flow rate 0.6 ml/min.

Detection Refractive index detector maintained at a constant temperature.

Injection 100 µl; inject test solution (a) and reference solutions (b) and (c).

Run time 2.5 times the retention time of lactitol.

Relative retention With reference to lactitol (retention time = about 13 min): impurity A = about 0.7; impurity B = about 0.8; glycerol = about 1.3; impurity C = about 1.5; impurity D = about 1.8; impurity E = about 1.9.

System suitability Reference solution (c):
- *resolution*: minimum 5 between the peaks due to lactitol and glycerol.

Limits:
- *impurity B*: not more than the area of the peak due to lactitol in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *total of other impurities*: not more than the area of the peak due to lactitol in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the solvent.

Reducing sugars

Maximum 0.2 per cent.

Dissolve 5.0 g in 3 ml of *water R* with gentle heating. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R*. When the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R* added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Lead ***(2.4.10)***

Maximum 0.5 ppm.

Nickel ***(2.4.15)***

Maximum 1 ppm.

Water ***(2.5.12)***

4.5 per cent to 5.5 per cent, determined on 0.30 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram. It complies with the tests for *Escherichia coli*, *Salmonella* and *Pseudomonas aeruginosa (2.6.13)*.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

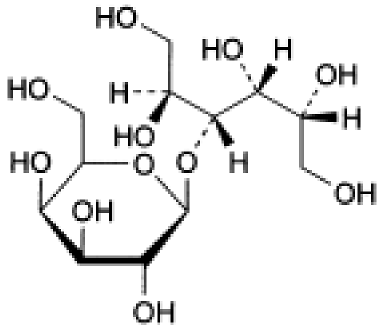
Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{12}H_{24}O_{11}$ using the chromatograms obtained with test solution (b) and reference solution (a) and the declared content of *lactitol monohydrate CRS*.

IMPURITIES

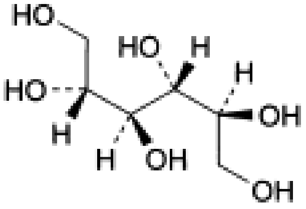
Specified impurities A, B, C, D, E.

A. lactose,



B. lactulitol,

C. mannitol,



D. dulcitol (galactitol),

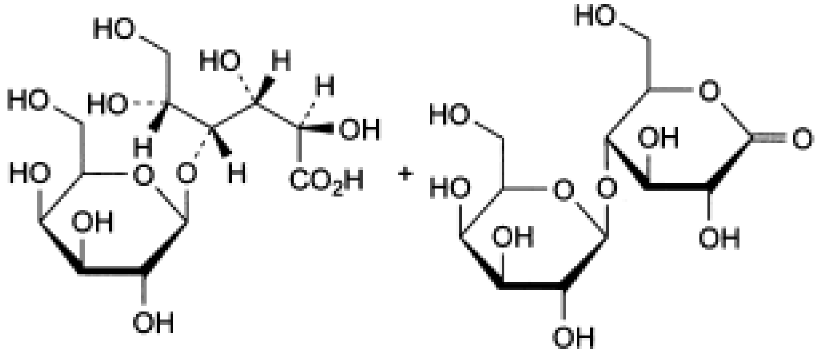
E. sorbitol.

Ph Eur

Lactobionic Acid

General Notices

(Ph Eur monograph 1647)



$C_{12}H_{22}O_{12}$ (acid form) 358.3 *96-82-2*

$C_{12}H_{20}O_{11}$ (δ-lactone) 340.3 *5965-65-1*

Ph Eur

DEFINITION

Mixture in variable proportions of 4-*O*-β-D-galactopyranosyl-D-gluconic acid and 4-*O*-β-D-galactopyranosyl-D-glucono-1,5-lactone.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, slightly soluble in glacial acetic acid, in anhydrous ethanol and in methanol.

mp

About 125 °C with decomposition.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *lactobionic acid CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *water R*, dry at 105 °C and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 1 ml with the same solvent.

Reference solution Dissolve 10 mg of *lactobionic acid CRS* in *water R* and dilute to 1 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R1*, *ethyl acetate R*, *water R*, *methanol R* (2:2:2:4 *V/V/V/V*).

Application 5 µl.

Development Over 3/4 of the plate.

Detection Spray 3 times with *ammonium molybdate solution R6* and heat in an oven at 110 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Dissolve 3.0 g in 25 ml of *water R*.

Specific optical rotation *(2.2.7)*

+ 23.0 to + 29.0 (anhydrous substance).

Dissolve 1.0 g in 80 ml of *water R* and dilute to 100.0 ml with the same solvent. Allow to stand for 24 h.

Reducing sugars

Maximum 0.2 per cent, calculated as glucose.

Dissolve 5.0 g in 25 ml of *water R* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R,* added towards the end of the titration, as indicator. Not less than 12.8 ml of 0.05 *M sodium thiosulphate* is required.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test E. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 5.0 per cent, determined on 0.50 g.

Use a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as solvent.

Total ash *(2.4.16)*

Maximum 0.2 per cent.

ASSAY

Dissolve 0.350 g in 50 ml of *carbon dioxide-free water R*, previously heated to 30 °C. Immediately titrate with *0.1 M sodium hydroxide* and determine the 2 equivalence points potentiometrically *(2.2.20)*.

The first equivalence point (V_1) corresponds to the acid form of lactobionic acid and the second equivalence point ($V_2 - V_1$) corresponds to the δ-lactone form.

1 ml of *0.1 M sodium hydroxide* is equivalent to 35.83 mg of $C_{12}H_{22}O_{12}$.

1 ml of *0.1 M sodium hydroxide* is equivalent to 34.03 mg of $C_{12}H_{20}O_{11}$.

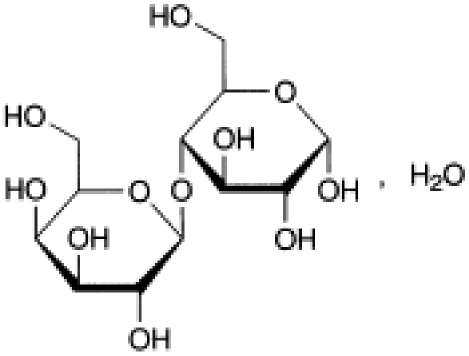
The sum of the 2 results is expressed as a percentage content of lactobionic acid.

Ph Eur

Lactose

General Notices

(Lactose Monohydrate, Ph Eur monograph 0187)



$C_{12}H_{22}O_{11},H_2O$ 360.3 *10039-26-6*

Action and use

Excipient.

Ph Eur

DEFINITION

C-β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely but slowly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison lactose CRS.

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *water R, methanol R* (2:3 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *lactose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *fructose CRS*, 10 mg of *glucose CRS*, 10 mg of *lactose CRS* and 10 mg of *sucrose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Plate *TLC silica gel G plate R*.

Mobile phase *water R*, *methanol R*, *glacial acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately, as a slight excess of water produces cloudiness.

Application 2 µl; thoroughly dry the starting points.

Development A Over a path of 15 cm.

Drying A In a current of warm air.

Development B Immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*; heat at 130 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.25 g in 5 ml of *water R*. Add 5 ml of *ammonia R* and heat in a water-bath at 80 °C for 10 min. A red colour develops.

D. Water (see Tests).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 1.0 g in boiling *water R* and dilute to 10 ml with the same solvent.

Acidity or alkalinity

Dissolve 6.0 g by heating in 25 ml of *carbon dioxide-free water R*, cool and add 0.3 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation (*2.2.7*)

+ 54.4 to + 55.9 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, heating to 50 °C. Allow to cool and add 0.2 ml of *dilute ammonia R1*. Allow to stand for 30 min and dilute to 100.0 ml with *water R.*

Absorbance (*2.2.25*).

Test solution (a) Dissolve 1.0 g in boiling *water R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *water R*.

Spectral range 400 nm for test solution (a) and 210-300 nm for test solution (b).

Results:
— *at 400 nm*: maximum 0.04 for test solution (a);

— *from 210 nm to 220 nm*: maximum 0.25 for test solution (b);

— *from 270 nm to 300 nm*: maximum 0.07 for test solution (b).

Heavy metals (*2.4.8*)

Maximum 5 ppm.

Dissolve 4.0 g in *water R* with warming, add 1 ml of *0.1 M hydrochloric acid* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*)

4.5 per cent to 5.5 per cent, determined on 0.50 g, using a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as the solvent.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 10^2 micro-organisms per gram, determined by plate-count. It complies with the test for *Escherichia coli* (*2.6.13*).

STORAGE

In an airtight container.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for lactose monohydrate used as a filler/diluent in solid dosage forms (compressed and powder).

Particle size distribution *(2.9.31* or *2.9.38)*.

Bulk and tapped density (*2.9.15*)

Determine the bulk density and the tapped density. Calculate the Hausner Index using the following expression:

$$\frac{V_0}{V_f}$$

V_0 = volume of bulk substance;

V_f = volume of tapped substance.

Ph Eur

Lactulose Solution

General Notices

(Liquid Lactulose, Ph Eur monograph 0924)

Action and use

Osmotic laxative.

Ph Eur

DEFINITION

Aqueous solution of 4-*C*-(β-D-galactopyranosyl)-D-*arabino*-hex-2-ulofuranose normally prepared by alkaline isomerisation of lactose. It may contain lesser amounts of other sugars including lactose, epilactose, galactose, tagatose and fructose.

Content

Minimum 620 g/l of lactulose ($C_{12}H_{22}O_{11}$; M_r 342.3) and 95.0 per cent to 105.0 per cent of the content of lactulose stated on the label.

It may contain a suitable antimicrobial preservative.

CHARACTERS

Appearance

Clear, viscous liquid, colourless or pale brownish-yellow.

Solubility

Miscible with water. It may be a supersaturated solution or may contain crystals which disappear on heating.

A 10 per cent *V/V* solution is laevorotatory.

IDENTIFICATION

First identification B, C, D.

Second identification A, C, D.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dilute 0.50 g of the substance to be examined to 50 ml with *water R*.

Reference solution Dissolve 60 mg of *lactulose CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel G plate R.

Mobile phase *glacial acetic acid R*, 50 g/l solution of *boric acid R*, *methanol R*, *ethyl acetate R* (10:15:20:55 *V/V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying At 100-105 °C for 5 min and allow to cool.

Detection Spray with a 1.0 g/l solution of *1,3-dihydroxynaphthalene R* in a mixture of 10 volumes of *sulphuric acid R* and 90 volumes of *methanol R*. Heat at 110 °C for 5 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (b).

C. To 0.1 g add 10 ml of *water R* and 3 ml of *cupri-tartaric solution R* and heat. A red precipitate is formed.

D. To 0.25 g add 5 ml of *water R* and 5 ml of *ammonia R*. Heat in a water-bath at 80 °C for 10 min. A red colour develops.

TESTS

Solution S

Mix 10 g with *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

3.0 to 7.0.

To 10 ml of solution S, add 0.1 ml of a saturated solution of *potassium chloride R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Mix 4.00 g of the substance to be examined with 20 ml of *water R*. Add 25.0 ml of *acetonitrile R* with gentle heating and dilute to 50.0 ml with *water R*.

Reference solution (a) To 5 ml of the test solution, add 47.5 ml of *acetonitrile R* with gentle heating and dilute to 100.0 ml with *water R*.

Reference solution (b) Dissolve 2.00 g of *lactulose CRS* in 20 ml of *water R*. Add 25.0 ml of *acetonitrile R* with gentle heating and dilute to 50.0 ml with *water R*.

Reference solution (c) Dissolve the contents of a vial of *lactulose for system suitability CRS* in 1 ml of a mixture of equal volumes of *acetonitrile R* and *water R*.

Precolumn:

— *size:* *l* = 0.05 m, Ø = 4.6 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 38 ± 1 °C.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 38 ± 1 °C.

Mobile phase Dissolve 0.253 g of *sodium dihydrogen phosphate R* in 220 ml of *water R* and add 780 ml of *acetonitrile R*.

Flow rate 1.0 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl of the test solution and reference solutions (a) and (c).

Run time 2.5 times the retention time of lactulose.

Relative retention With reference to lactulose (retention time = about 18 min): impurity E = about 0.38; impurity D = about 0.42; impurity B = about 0.57; impurity A = about 0.90; impurity C = about 1.17.

System suitability Reference solution (c):

— *resolution*: minimum 1.3 between the peaks due to lactulose and impurity A; if necessary, adjust the concentration of acetonitrile in the mobile phase to between 75.0 per cent *V/V* and 82.0 per cent *V/V*;

— the chromatogram obtained is similar to the chromatogram supplied with *lactulose for system suitability CRS.*

Limits:

— *impurities A, C*: for each impurity, not more than twice the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (10 per cent);

— *impurity B*: not more than 3 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (15 per cent);

— *impurity D*: not more than 0.2 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (1 per cent);

— *impurity E*: not more than 0.8 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (4 per cent).

Methanol

Head-space gas chromatography (*2.2.28*).

Internal standard solution Mix 0.5 ml of *propanol R* with 100.0 ml of *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*. Dilute 5.0 ml of the solution to 50.0 ml with *water R*.

Test solution To 0.13 g of the substance to be examined in a 20 ml vial add 1.0 ml of the internal standard solution and 5 µl of a 0.1 per cent *V/V* solution of *methanol R*.

Reference solution To 1.0 ml of the internal standard solution in a 20 ml vial add 5 µl of a 0.1 per cent *V/V* solution of *methanol R*.

Column:
- *size:* *l* = 2 m, Ø = 2 mm;
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (180 µm).

Carrier gas *helium for chromatography R*.

Flow rate 30 ml/min.

Static head-space conditions which may be used:
- *equilibration temperature*: 60 °C;
- *equilibration time*: 1 h;
- *pressurisation time*: 1 min.

Temperature:
- *column*: 140 °C;
- *injection port*: 200 °C;
- *detector*: 220 °C.

Detection Flame ionisation.

Injection 1 ml of the gaseous phase.

Calculate the content of methanol taking its density (*2.2.5*) at 20 °C to be 0.79 g/ml.

Limit:
- *methanol*: calculate the ratio (*R*) of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution; calculate the ratio of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the test solution: this ratio is not greater than 2 *R* (30 ppm).

Sulphites

Maximum 30 ppm.

Mix 5.0 g with 40 ml of *water R*, add 2.0 ml of *0.1 M sodium hydroxide* and dilute to 100 ml with *water R*. To 10.0 ml of this solution, add 1.0 ml of *hydrochloric acid R1*, 2.0 ml of *decolorised fuchsin solution R1* and 2.0 ml of a 0.5 per cent *V/V* solution of *formaldehyde R*. Allow to stand for 30 min and measure the absorbance (*2.2.25*) at 583 nm using as the compensation liquid a solution prepared at the same time and in the same manner with 10.0 ml of *water R* instead of the solution of the substance to be examined. The absorbance is not greater than that of a reference solution prepared at the same time and in the same manner using 10.0 ml of *sulphite standard solution (1.5 ppm* SO_2*) R* instead of the solution of the substance to be examined.

Boron

Maximum 5 ppm.

Avoid where possible the use of glassware.

Reference solution Dissolve 56.0 mg of *boric acid R in water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. *Keep in a well-closed polyethylene container.*

In 4 polyethylene 25 ml flasks, place separately:

- 1.00 g of the substance to be examined and 1 ml of *water R* (solution A);
- 1.00 g of the substance to be examined and 1 ml of the reference solution (solution B);
- 1 ml of the reference solution and 1 ml of *water R* (solution C);
- 2 ml of *water R* (solution D).

To each flask, add 4.0 ml of *acetate-edetate buffer solution pH 5.5 R*. Mix and add 4.0 ml of freshly prepared *azomethine H solution R*. Mix and allow to stand for 1 h. Measure the absorbance (*2.2.25*) of solutions A, B and C at 420 nm, using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is at least 0.25. The absorbance of solution B is not less than twice that of solution A.

Lead (*2.4.10*)

Maximum 0.5 ppm, calculated with reference to the declared content of lactulose.

Sulphated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.5 g and calculated with reference to the declared content of lactulose.

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 10^2 micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli* (*2.6.13*).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection The test solution and reference solution (b).

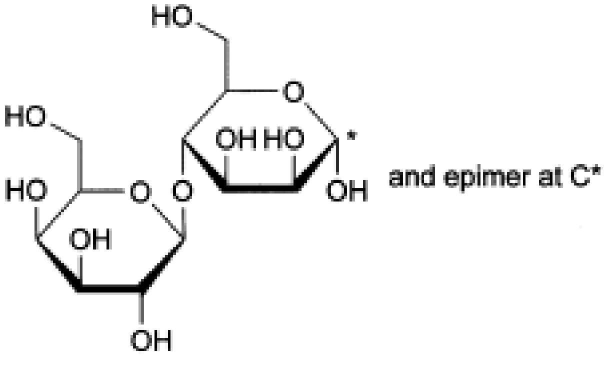
Calculate the percentage content of $C_{12}H_{22}O_{11}$ from the declared content of *lactulose CRS*.

LABELLING

The label states the declared content of lactulose.

IMPURITIES

Specified impurities A, B, C, D, E.

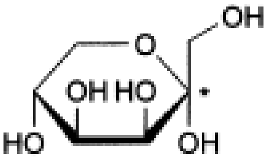


A. 4-*O*-(β-D-galactopyranosyl)-D-mannopyranose (epilactose),

B. galactose,

C. lactose,

D. fructose,



OH
O
OH HO
*
HO
OH

and epimer at C*

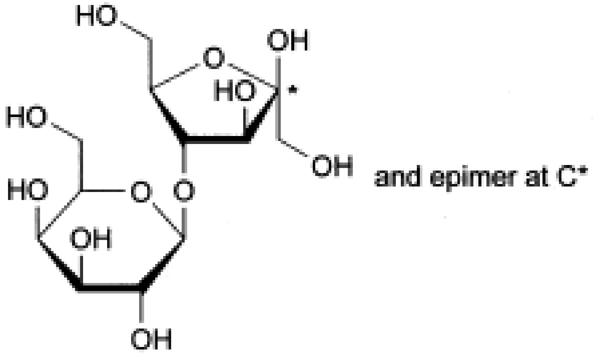
E. D-*lyxo*-hex-2-ulopyranose (tagatose).

Ph Eur

Lactulose

General Notices

(Ph Eur monograph 1230)



$C_{12}H_{22}O_{11}$ 342.3 *4618-18-2*

Action and use

Osmotic laxative.

Preparation

Lactulose Oral Powder

Ph Eur

DEFINITION

4-*O*-(β-D-Galactopyranosyl)-D-*arabino*-hex-2-ulofuranose.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in methanol, practically insoluble in toluene.

mp: about 168 °C.

IDENTIFICATION

First identification *B, C, D, E.*

Second identification *A, C, D, E.*

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 50.0 mg of *lactulose CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *glacial acetic acid R*, 50 g/l solution of *boric acid R*, *methanol R*, *ethyl acetate R* (10:15:20:55 *V/V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying At 100-105 °C for 5 min and allow to cool.

Detection Spray with a 1.0 g/l solution of *1,3-dihydroxynaphthalene R* in a mixture of 10 volumes of *sulphuric acid R* and 90 volumes of *methanol R*. Heat at 110 °C for 5 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

C. Dissolve 50 mg in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. A red precipitate is formed.

D. Dissolve 0.125 g in 5 ml of *water R*. Add 5 ml of *ammonia R*. Heat on a water-bath at 80 °C for 10 min. A red colour develops.

E. Specific optical rotation (see Tests).

TESTS

Solution S

Dissolve 3.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

3.0 to 7.0.

To 10 ml of solution S add 0.1 ml of a saturated solution of *potassium chloride R*.

Specific optical rotation (*2.2.7*)

- 46.0 to - 50.0 (anhydrous substance).

Dissolve 1.25 g in *water R*, add 0.2 ml of *concentrated ammonia R* and dilute to 25.0 ml with *water R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.00 g of the substance to be examined in 10 ml of *water R*. Add 12.5 ml of *acetonitrile R* with gentle heating and dilute to 25.0 ml with *water R*.

Reference solution (a) To 3 ml of the test solution add 47.5 ml of *acetonitrile R* with gentle heating and dilute to 100.0 ml with *water R*.

Reference solution (b) Dissolve 1.00 g of *lactulose CRS* in 10 ml of *water R*. Add 12.5 ml of *acetonitrile R* with gentle heating and dilute to 25.0 ml with *water R*.

Reference solution (c) Dissolve the contents of a vial of *lactulose for system suitability CRS* in 1 ml of a mixture of equal volumes of *acetonitrile R* and *water R*.

Precolumn:

— *size: l* = 0.05 m, Ø = 4.6 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 38 ± 1 °C.

Column:

— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 38 ± 1 °C.

Mobile phase Dissolve 0.253 g of *sodium dihydrogen phosphate R* in 220 ml of *water R* and add 780 ml of *acetonitrile R*.

Flow rate 1.0 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl of the test solution and reference solutions (a) and (c).

Run time 2.5 times the retention time of lactulose.

Relative retention With reference to lactulose (retention time = about 18.3 min): impurity E = about 0.38; impurity D = about 0.42; impurity B = about 0.57; impurity A = about 0.90; impurity C = about 1.17.

System suitability Reference solution (c):

— *resolution*: minimum 1.3 between the peaks due to lactulose and impurity A; if necessary, adjust the concentration of acetonitrile in the mobile phase to between 75.0 per cent *V/V* and 82.0 per cent *V/V*;

— the chromatogram obtained is similar to the chromatogram supplied with *lactulose for system suitability CRS*.

Limit:

- *sum of impurities A, B, C, D, E*: not more than the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (3 per cent).

Methanol

Head-space gas chromatography (*2.2.28*).

Internal standard solution Mix 0.5 ml of *propanol R* with 100.0 ml of *water R.* Dilute 1.0 ml of this solution to 100.0 ml with *water R*. Dilute 5.0 ml of the solution to 50.0 ml with *water R*.

Test solution To 79 mg of the substance to be examined in a 20 ml vial add 1.0 ml of the internal standard solution and 5 µl of a 0.1 per cent *V/V* solution of *methanol R*.

Reference solution To 1.0 ml of the internal standard solution in a 20 ml vial add 5 µl of a 0.1 per cent *V/V* solution of *methanol R*.

Column:
- *size: l* = 2 m, Ø = 2 mm;
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (180 µm).

Carrier gas *helium for chromatography R*.

Flow rate 30 ml/min.

Static head-space conditions which may be used:
- *equilibration temperature*: 60 °C;
- *equilibration time*: 1 h;
- *pressurisation time*: 1 min.

Temperature:
- *column*: 140 °C;
- *injection port*: 200 °C;
- *detector*: 220 °C.

Detection Flame ionisation.

Injection 1 ml of the gaseous phase.

Calculate the content of methanol taking its density (*2.2.5*) at 20 °C to be 0.79 g/ml.

Limit:
- *methanol*: calculate the ratio (*R*) of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution; calculate the ratio of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the test solution: this ratio is not greater than 2 *R* (50 ppm).

Boron

Maximum 9 ppm.

Avoid where possible the use of glassware.

Reference solution Dissolve 50.0 mg of *boric acid R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. *Keep in a well-closed polyethylene container.*

In 4 polyethylene 25 ml flasks, place separately:

— 0.50 g of the substance to be examined dissolved in 2.0 ml of *water R* (solution A);

— 0.50 g of the substance to be examined dissolved in 1.0 ml of the reference solution and 1.0 ml of *water R* (solution B);

— 1.0 ml of the reference solution and 1.0 ml of *water R* (solution C);

— 2.0 ml of *water R* (solution D).

To each flask, add 4.0 ml of *acetate-edetate buffer solution pH 5.5 R*. Mix and add 4.0 ml of freshly prepared *azomethine H solution R*. Mix and allow to stand for 1 h. Measure the absorbance (*2.2.25*) of solutions A, B and C at 420 nm, using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is at least 0.25. The absorbance of solution B is not less than twice that of solution A.

Lead (2.4.10)

Maximum 0.5 ppm.

Water (*2.5.12*)

Maximum 2.5 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 10^2 micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli* (*2.6.13*).

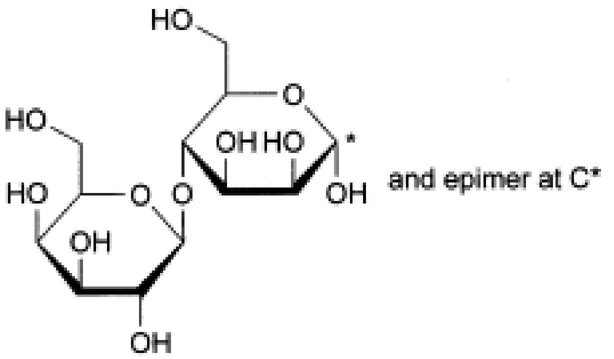
ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection The test solution and reference solution (b).

Calculate the percentage content of $C_{12}H_{22}O_{11}$ from the declared content of *lactulose CRS*.

IMPURITIES

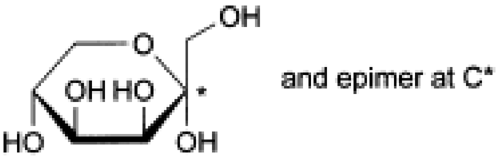


A. 4-*O*-(β-D-galactopyranosyl)-D-mannopyranose (epilactose),

B. galactose,

C. lactose,

D. fructose,


and epimer at C*

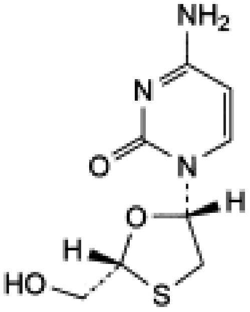
E. D-*lyxo*-hex-2-ulopyranose (tagatose).

Ph Eur

Lamivudine

General Notices

(Ph Eur monograph 2217)



$C_8H_{11}N_3O_3S$ 229.3 *134678-17-4*

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

4-Amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *B, C.*

Second identification *A, B.*

A. Specific optical rotation (*2.2.7*): - 97 to - 99 (dried substance).

Dissolve 0.250 g in *water R* and dilute to 50.0 ml with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lamivudine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Enantiomeric purity (see Tests).

TESTS

Absorbance (*2.2.25*)

Maximum 0.3 at 440 nm, using a path length of 4 cm.

Dissolve 1.00 g in *water R*, using sonication if necessary, and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *salicylic acid R* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *lamivudine CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (d) Dissolve 5 mg of *cytosine R* and 5 mg of *uracil R* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (e) Dissolve 5 mg of *lamivudine for system suitability 1 CRS* (containing impurities A and B) in 2 ml of mobile phase. Add 1.0 ml of reference solution (d) and dilute to 10.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase Mix 5 volumes of *methanol R* and 95 volumes of a 1.9 g/l solution of *ammonium acetate R*, previously adjusted to pH 3.8 with *glacial acetic acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 277 nm.

Injection 10 µl.

Run time 3 times the retention time of lamivudine.

Identification of impurities Use the chromatograms obtained with reference solutions (e) and (b) to identify the peaks due to impurities A, B, E, F and C.

Relative retention With reference to lamivudine (retention time = about 9 min): impurity E = about 0.28; impurity F = about 0.32; impurity A = about 0.36; impurity B = about 0.91; impurity J = about 1.45; impurity C = about 2.32.

System suitability Reference solution (e):

— *resolution*: minimum 1.5 between the peaks due to impurity F and impurity A; minimum 1.5 between the peaks due to impurity B and lamivudine.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.6; impurity F = 2.2; impurity J = 2.2;

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Enantiomeric purity

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution Dissolve the contents of a vial of *lamivudine for system suitability 2 CRS* (containing impurity D) in 1.0 ml of *water R*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel BC for chiral chromatography R*,

— *temperature*: maintain at constant temperature between 15 °C and 30 °C; the temperature may be adjusted to optimise the resolution between lamivudine and impurity D; a lower temperature favours improved resolution.

Mobile phase Mix 5 volumes of *methanol* R and 95 volumes of a 7.7 g/l solution of *ammonium acetate R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µl.

Run time Twice the retention time of lamivudine.

Relative retention With reference to lamivudine (retention time = about 8 min): impurity D = about 1.2; impurity B and enantiomer = about 1.3 and 1.5.

System suitability Reference solution:

— *peak-to-valley-ratio*: minimum 15, where H_r = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lamivudine.

Calculate the sum of the percentage contents of all impurity peaks with a relative retention from 1.2 to 1.5. Subtract the percentage content of impurity B as obtained in the test for related substances.

Limit:
— *impurity* D: maximum 0.3 per cent.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

Calculate the percentage content of $C_8H_{11}N_3O_3S$ using the chromatograms obtained with the test solution and reference solution (c) and the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine CRS.*

STORAGE

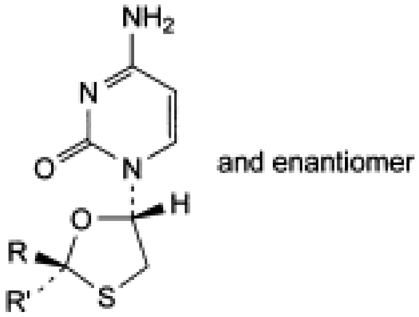
Protected from light.

IMPURITIES

Specified impurities A, B, C, D.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these

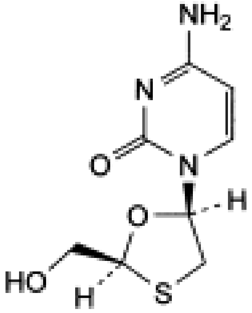
impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *E, F, G, H, I, J.*



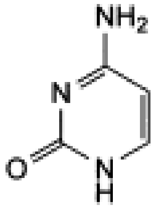
A. R = H, R′ = CO_2H: (2*RS*,5*SR*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid,

B. R = CH_2OH, R′ = H: 4-amino-1-[(2*RS*,5*RS*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidin-2(1*H*)-one ((±)-*trans*-lamivudine),

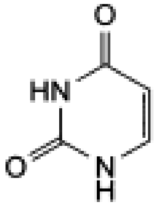
C. salicylic acid,



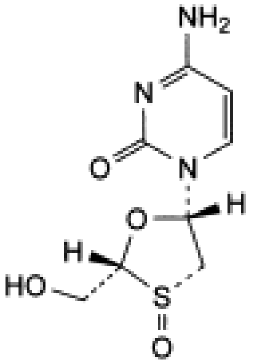
D. 4-amino-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one,



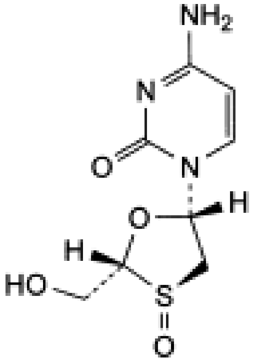
E. 4-aminopyrimidin-2(1*H*)-one (cytosine),



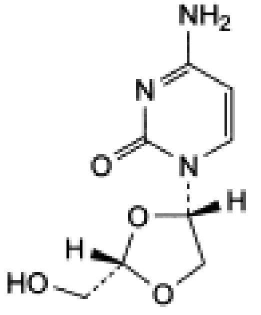
F. pyrimidine-2,4(1*H*,3*H*)-dione (uracil),



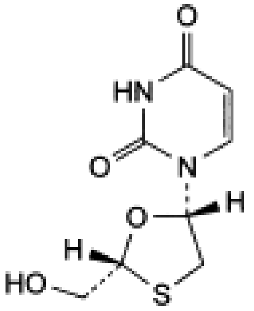
G. 4-amino-1-[(2*R*,3*S*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one *S*-oxide,



H. 4-amino-1-[(2*R*,3*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one *S*-oxide,



I. 4-amino-1-[(2*S*,4*S*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl]pyrimidin-2(1*H*)-one,



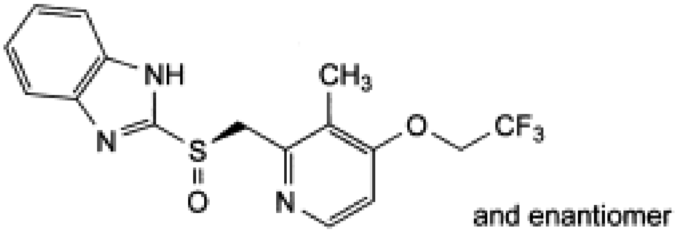
J. 1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidine-2,4(1*H*,3*H*)-dione.

Ph Eur

Lansoprazole

General Notices

(Ph Eur monograph 2219)



$C_{16}H_{14}F_3N_3O_2S$ 369.4 *103577-45-3*

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

Ph Eur

DEFINITION

2-[(*RS*)-[[3-Methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl]sulphinyl]-1*H*-benzimidazole.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or brownish powder.

Solubility

Practically insoluble in water, soluble in anhydrous ethanol, very slightly soluble in acetonitrile.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison lansoprazole CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_2 or BY_2 *(2.2.2, Method II)*.

Dissolve 1.0 g in *dimethylformamide R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect them from light*.

Solvent mixture Mix 1 volume of *triethylamine R* with 60 volumes of *water R*; adjust the pH to 10.5 using *phosphoric acid R*; mix the solution obtained with 40 volumes of *acetonitrile R1*.

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *lansoprazole for peak identification CRS* (containing impurities A and B) in 1.0 ml of the solvent mixture.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *2-mercaptobenzimidazole R* (impurity E) and 5 mg of *2-hydroxybenzimidazole R* (impurity D) in the solvent mixture and dilute to 100 ml with the solvent mixture. Dilute 1 ml of this solution to 10 ml with the solvent mixture.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: amidohexadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 1 volume of *triethylamine R* with 60 volumes of *water R*; adjust the pH to 6.2 using *phosphoric acid R*; mix the solution obtained with 40 volumes of *acetonitrile R1*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 285 nm.

Injection 10 µl.

Run time 3 times the retention time of lansoprazole.

Identification of impurities Use the chromatogram supplied with *lansoprazole for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B; use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and E.

Relative retention With reference to lansoprazole (retention time = about 7 min): impurity D = about 0.4; impurity A = about 0.5; impurity E = about 0.6; impurity B = about 1.2.

System suitability Reference solution (a):

- *resolution*: minimum 3.0 between the peaks due to lansoprazole and impurity B.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity E by 0.4;

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *impurities A, D, E*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water ***(2.5.32)***

Maximum 0.1 per cent, determined on 1.000 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 40 ml of *ethanol (96 per cent) R* and dilute to 50 ml with *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 36.94 mg of $C_{16}H_{14}F_3N_3O_2S$.

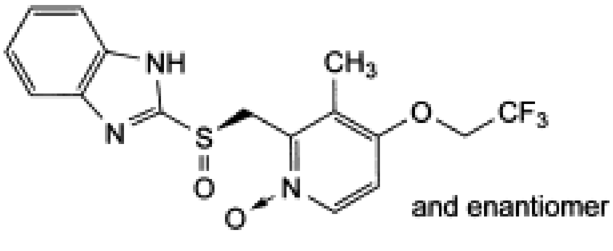
STORAGE

In an airtight container , protected from light.

IMPURITIES

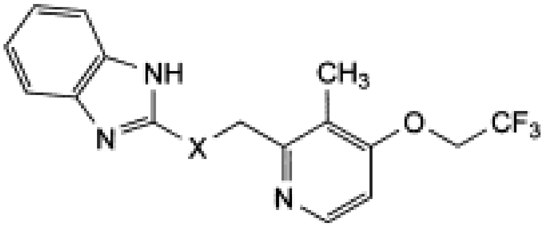
Specified impurities A, B, D, E.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C, F*.



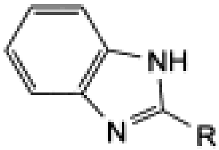
A. 2-[(*RS*)-[[3-methyl-1-oxido-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl]sulphinyl]-1*H*-

benzimidazole,


NH
CH3
O
CF3
N
X
N

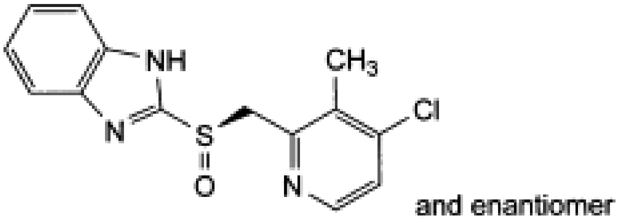
B. X = SO_2: 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl]sulphonyl]-1*H*-benzimidazole,

C. X = S: 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl]sulphanyl]-1*H*-benzimidazole,



D. R = OH: 1*H*-benzimidazol-2-ol,

E. R = SH: 1*H*-benzimidazole-2-thiol,


NH
CH3
Cl
N
S
O
N
and enantiomer

F. 2-[(*RS*)-[(4-chloro-3-methylpyridin-2-yl)methyl]sulphinyl]-1*H*-benzimidazole.

Ph Eur

Lauroyl Macrogolglycerides

General Notices

(Ph Eur monograph 1231)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols with a mean relative molecular mass between 300 and 1500.

They are obtained by partial alcoholysis of saturated oils mainly containing triglycerides of lauric (dodecanoic) acid, using macrogol, or by esterification of glycerol and macrogol with saturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of these hydrogenated oils.

CHARACTERS

Appearance

Pale yellow waxy solid.

Solubility

Dispersible in hot water, freely soluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *hexane R*, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9

(R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.7), to 1,2-diglycerides (R_{st} 0.6), to monoglycerides (R_{st} 0.1) and to esters of macrogol (R_{st} 0).

B. Hydroxyl value (see Tests).

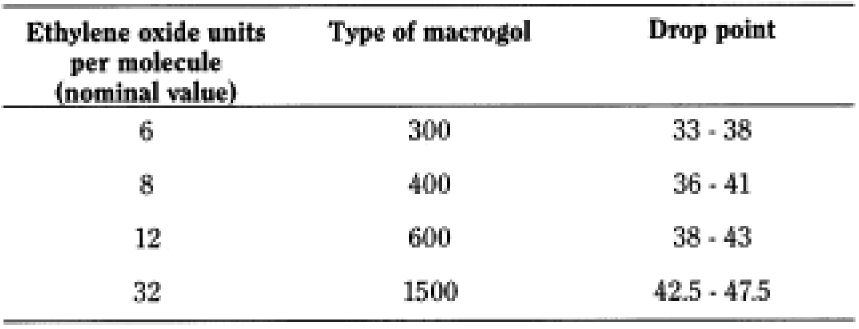
C. Saponification value (see Tests).

D. Fatty acid composition (see Tests).

TESTS

Drop point (*2.2.17*)

Introduce into the cup the substance to be examined, which has been melted by heating for 1 h in an oven at 100 ± 2 °C, and allow to stand for 5 h at about 5 °C.



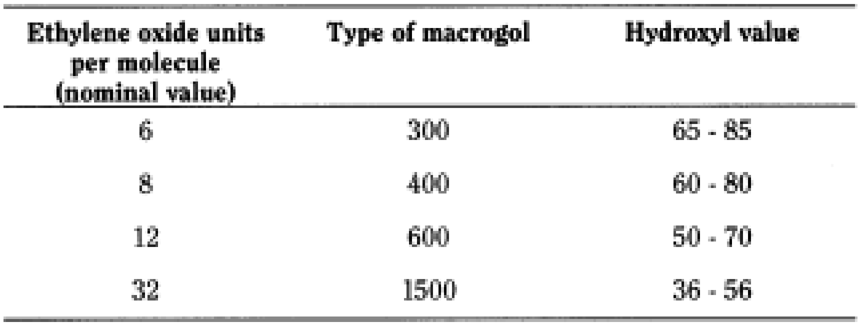
Ethylene oxide units per molecule (nominal value)	Type of macrogol	Drop point
6	300	33 - 38
8	400	36 - 41
12	600	38 - 43
32	1500	42.5 - 47.5

Acid value (*2.5.1*)

Maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)

Use 1.0 g.



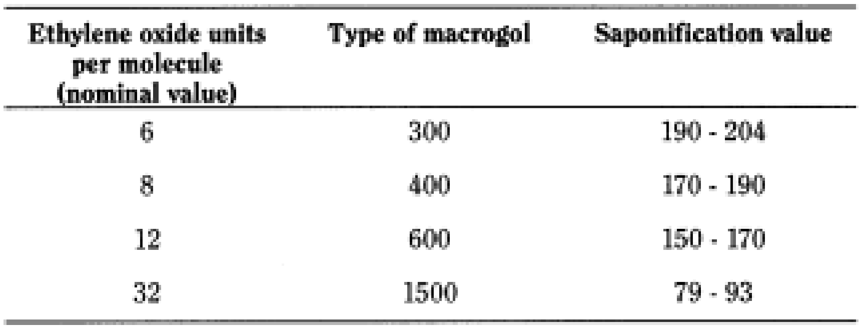
Ethylene oxide units per molecule (nominal value)	Type of macrogol	Hydroxyl value
6	300	65 - 85
8	400	60 - 80
12	600	50 - 70
32	1500	36 - 56

Peroxide value (*2.5.5, Method A*)

Maximum 6.0, determined on 2.0 g.

Saponification value (*2.5.6*)

Use 2.0 g.



Ethylene oxide units per molecule (nominal value)	Type of macrogol	Saponification value
6	300	190 - 204
8	400	170 - 190
12	600	150 - 170
32	1500	79 - 93

Alkaline impurities

Introduce 5.0 g into a test tube and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 ml of *water R* and 10 ml of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 ml of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol

Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 ml of *methylene chloride R*. Heat if necessary. After cooling, add 100 ml of *water R*. Shake and add 25.0 ml of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 ml of a 75 g/l solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 ml of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulphate*. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:

— *caprylic acid*: maximum 15.0 per cent;

— *capric acid*: maximum 12.0 per cent;

— *lauric acid*: 30.0 per cent to 50.0 per cent;

— *myristic acid*: 5.0 per cent to 25.0 per cent;

— *palmitic acid*: 4.0 per cent to 25.0 per cent;

— *stearic acid*: 5.0 per cent to 35.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.0 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash (*2.4.16*)

Maximum 0.1 per cent.

LABELLING

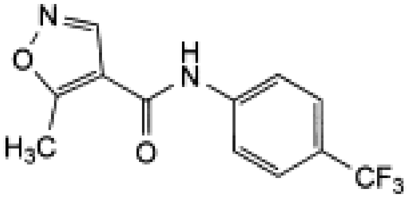
The label states the type of macrogol used (mean relative molecular mass) or the number of units of ethylene oxide per molecule (nominal value).

Ph Eur

Leflunomide

General Notices

(Ph Eur monograph 2330)



$C_{12}H_9F_3N_2O_2$ 270.2 *75706-12-6*

Action and use

Immunomodulator.

Ph Eur

DEFINITION

5-Methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methanol, sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Heat the substance to be examined and the reference substance at 130 °C for 10 min.

Comparison *leflunomide CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Store all solutions protected from light*.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 5 ml of *acetonitrile for chromatography R* and dilute to 50.0 ml with the mobile phase.

Test solution (b) Dissolve 0.125 g of the substance to be examined in 5 ml of *acetonitrile for chromatography R* and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 12.5 mg of *leflunomide impurity A CRS* in 5 ml of *acetonitrile for chromatography R* and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *leflunomide CRS* in 5 ml of *acetonitrile for chromatography R* and dilute to 50.0 ml with the mobile phase.

Reference solution (d) Dissolve the contents of 1 vial of *leflunomide for peak identification CRS* (containing impurities B and C) in 2.0 ml of the mobile phase and sonicate for 10 min.

Column:

- *size:* l = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 5 volumes of *triethylamine R* with 650 volumes of *water for chromatography R,* adjust to pH 3.4 ± 0.1 with *phosphoric acid R* and add 350 volumes of *acetonitrile for chromatography R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl of test solutions (a) and (b) and reference solutions (a), (b) and (d).

Run time Twice the retention time of leflunomide.

Identification of impurities Use the chromatogram supplied with *leflunomide for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B and C.

Relative retention with reference to leflunomide (retention time = about 25 min): impurity B = about 0.2; impurity A = about 0.4; impurity C = about 0.9.

System suitability Reference solution (d):

- *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to leflunomide.

Limits Test solution (a):

- *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *sum of impurities C and E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities other than B*: not more twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Limit Test solution (b):

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.3 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (a) and reference solution (c).

Calculate the percentage content of $C_{12}H_9F_3N_2O_2$ from the declared content of *leflunomide CRS.*

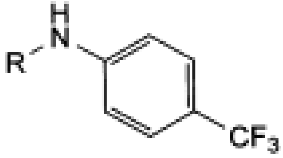
STORAGE

Protected from light.

IMPURITIES

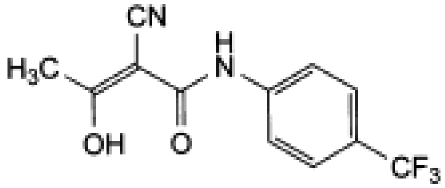
Specified impurities A, B.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10*. *Control of impurities in substances for pharmaceutical use)*: *C, D, E, F, G, H.*

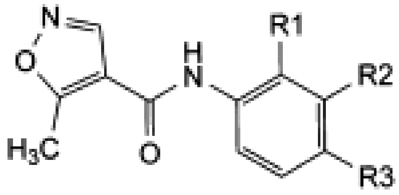


A. R = H: 4-(trifluoromethyl)aniline,

H. R = CO-CH_2-CN: 2-cyano-*N*-[4-(trifluoromethyl)phenyl]acetamide,



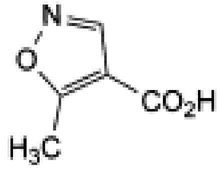
B. (2*Z*)-2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]but-2-enamide (teriflunomide),



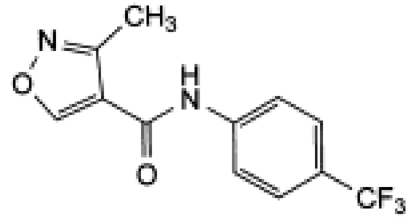
C. R1 = R3 = H, R2 = CF_3: 5-methyl-*N*-[3-(trifluoromethyl)phenyl]isoxazole-4-carboxamide,

F. R1 = CF_3, R2 = R3 = H: 5-methyl-*N*-[2-(trifluoromethyl)phenyl]isoxazole-4-carboxamide,

G. R1 = R2 = H, R3 = CH_3: 5-methyl-*N*-(4-methylphenyl)isoxazole-4-carboxamide,



D. 5-methylisoxazole-4-carboxylic acid,



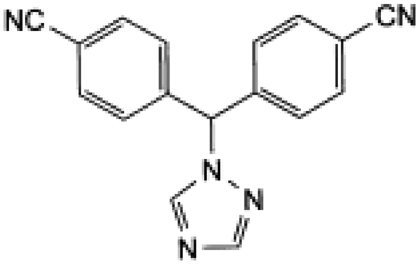
E. 3-methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide.

Ph Eur

Letrozole

General Notices

(Ph Eur monograph 2334)



$C_{17}H_{11}N_5$ 285.3 *112809-51-5*

Action and use

Aromatase inhibitor; treatment of breast carcinoma.

Ph Eur

DEFINITION

4,4′-(1*H*-1,2,4-Triazol-1-ylmethylene)dibenzonitrile.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison letrozole CRS.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 15 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*.

Test solution (b) To 2.0 ml of test solution (a) add 30 ml of *acetonitrile R1* and dilute to 100.0 ml with *water R*.

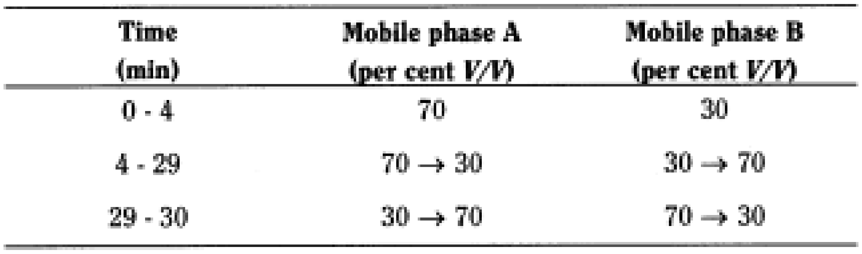
Reference solution (a) Dissolve 5.0 mg of *letrozole CRS* (containing impurities A and B) in 3 ml of *acetonitrile R1* and dilute to 10.0 ml with *water R*.

Reference solution (b) To 2.0 ml of test solution (a) add 30 ml of *acetonitrile R1* and dilute to 100.0 ml with *water R*. To 1.0 ml of this solution add 6 ml of *acetonitrile R1* and dilute to 20.0 ml with *water R*.

Reference solution (c) Dissolve 25.0 mg of *letrozole CRS* in 15 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*. To 2.0 ml of this solution add 30 ml of *acetonitrile R1* and dilute to 100.0 ml with *water R*.

Column:
- *size: l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *water R*;
- *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	70	30
4 - 29	70 → 30	30 → 70
29 - 30	30 → 70	70 → 30

Flow rate 1.0 ml/min.

Detection Sspectrophotometer at 230 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (b).

Relative retention With reference to letrozole (retention time = about 13 min): impurity A = about 0.6; impurity B = about 1.9.

System suitability Reference solution (a):

- *resolution*: minimum 5 between the peaks due to impurity A and letrozole;
- the chromatogram obtained is similar to the chromatogram supplied with *letrozole CRS*.

Limits:
- *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.3 per cent, determined on 1.000 g. Use a validated pyridine-free iodosulphurous reagent.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (c).

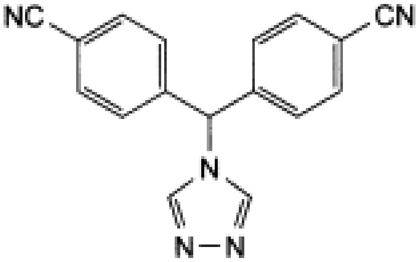
System suitability Reference solution (c):

- *symmetry factor*: maximum 1.7 for the peak due to letrozole.

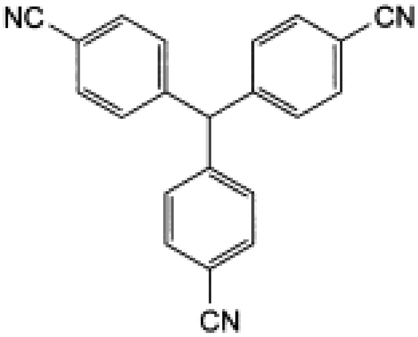
Calculate the percentage content of $C_{17}H_{11}N_5$ from the declared content of *letrozole CRS*.

IMPURITIES

Specified impurities A, B.



A. 4,4′-(4*H*-1,2,4-triazol-4-ylmethylene)dibenzonitrile,



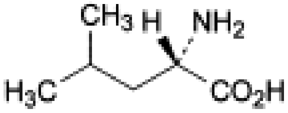
B. 4,4′,4″-methanetriyltribenzonitrile.

Ph Eur

Leucine

General Notices

(Ph Eur monograph 0771)



$C_6H_{13}NO_2$ 131.2 *61-90-5*

Action and use

Amino acid.

Ph Eur

DEFINITION

Leucine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2-amino-4-methylpentanoic acid, calculated with reference to the dried substance.

CHARACTERS

White or almost white crystalline powder or shiny flakes, sparingly soluble in water, practically insoluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Dissolve 0.50 g in *water R* and dilute to 25 ml with the same solvent. The solution is laevorotatory.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *leucine CRS*. Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 10 ml with the same acid. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

Dissolve 1.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 14.5 to + 16.5, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *leucine CRS* in *0.1 M hydrochloric acid* and dilute to 50 ml with the same acid.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *leucine CRS* and 10 mg of *valine CRS* in *0.1 M hydrochloric acid* and dilute to 25 ml with the same acid.

Apply separately to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (*2.4.4*)

Dissolve 0.25 g in *water R* and dilute to 15 ml with the same solvent. The solution complies with the limit test for chlorides (200 ppm).

Sulphates (*2.4.13*)

Dissolve 0.5 g in 3 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium (*2.4.1*)

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*)

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals (*2.4.8*)

2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 ml of *anhydrous formic acid R.* Add 30 ml of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid* using 0.1 ml of *naphtholbenzein solution R* as indicator, until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

STORAGE

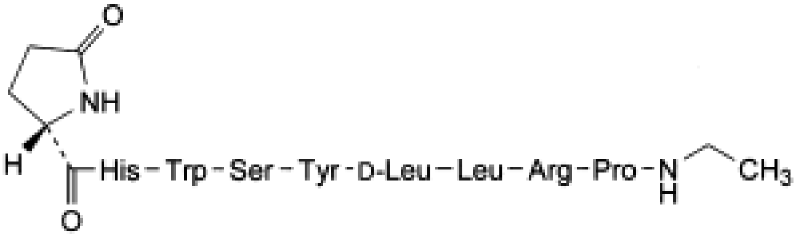
Store protected from light.

Ph Eur

Leuprorelin

General Notices

(Ph Eur monograph 1442)



$C_{59}H_{84}N_{16}O_{12}$ 1209 *53714-56-0*

Action and use

Gonadotropin releasing hormone (gonadorelin) analogue; treatment of prostate cancer.

Ph Eur

DEFINITION

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.

Synthetic nonapeptide analogue of the hypothalamic peptide, gonadorelin. It is obtained by chemical synthesis and is available as an acetate.

Content

97.0 per cent to 103.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance

Hygroscopic, white or almost white powder.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Comparison *Ph. Eur. reference spectrum of leuprorelin.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

C. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking one seventh of the sum of the number of moles of histidine, glutamic acid, leucine, proline, tyrosine and arginine as equal to 1. The values fall within the following limits: serine present; glutamic acid = 0.85 to 1.1; proline = 0.85 to 1.1; leucine = 1.8 to 2.2; tyrosine = 0.85 to 1.1; histidine = 0.85 to 1.1 and arginine = 0.85 to 1.1. Not more than traces of other amino acids are present, with the exception of tryptophan.

TESTS

Specific optical rotation (*2.2.7*)

- 38.0 to - 42.0 (anhydrous and acetic acid-free substance).

Dissolve the substance to be examined in a 1 per cent *V/V* solution of *glacial acetic acid R* to obtain a concentration of 10.0 mg/ml.

Related substances

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution (a) Dissolve the substance to be examined in the mobile phase to obtain a concentration of 1.0 mg/ml.

Test solution (b) Dilute 0.5 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve *leuprorelin CRS* in the mobile phase to obtain a concentration of 1.0 mg/ml.

Reference solution (b) Dilute 0.5 ml of reference solution (a) to 10.0 ml with the mobile phase.

Resolution solution Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water R*. To 5 ml of the solution add 100 µl of *1 M sodium hydroxide* and shake vigorously. Heat in an oven at 100 °C for 60 min, cool immediately and add 50 µl of *dilute phosphoric acid R*. Shake vigorously.

Column:

— *size:* *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve about 15.2 g of *triethylamine R* in 800 ml of *water R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 ml with *water R*. Add 850 ml of this solution to 150 ml of a mixture of 2 volumes of *propanol R* and 3 volumes of *acetonitrile R*.

Flow rate 1.0-1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (a) and the resolution solution.

Run time 90 min.

Relative retention With reference to leuprorelin (retention time = 41-49 min): impurity E = about 0.7; impurity F = about 0.7; impurity H = about 0.78; impurity A = about 0.8; impurity B =

about 0.9; impurity I = about 0.94; impurity J = about 1.09; impurity C = about 1.2; impurity G = about 1.3; impurity K = about 1.31; impurity D = about 1.5.

System suitability Resolution solution:

— *resolution*: minimum 1.5 between the peaks due to impurity B and leuprorelin.

Limits:

— *impurity D*: maximum 1.0 per cent;

— *impurities A, B, C*: for each impurity, maximum 0.5 per cent;

— *unspecified impurities*: for each impurity, maximum 0.5 per cent;

— *total*: maximum 2.5 per cent;

— *disregard limit*: 0.1 per cent.

Acetic acid (*2.5.34*)

4.7 per cent to 9.0 per cent.

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Water (*2.5.32*)

Maximum 5.0 per cent.

Sulphated ash (*2.4.14*)

Maximum 0.3 per cent.

Bacterial endotoxins (*2.6.14, Method D*)

Less than 16.7 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Run time 60 min.

Injection 20 µl of test solution (b) and reference solution (b).

Calculate the content of leuprorelin ($C_{59}H_{84}N_{16}O_{12}$) using the areas of the peaks and the declared content of $C_{59}H_{84}N_{16}O_{12}$ in *leuprorelin CRS*.

STORAGE

In an airtight container, protected from light, at a temperature not exceeding 30 °C.

If the substance is sterile, store in a sterile, airtight, tamper-proof container.

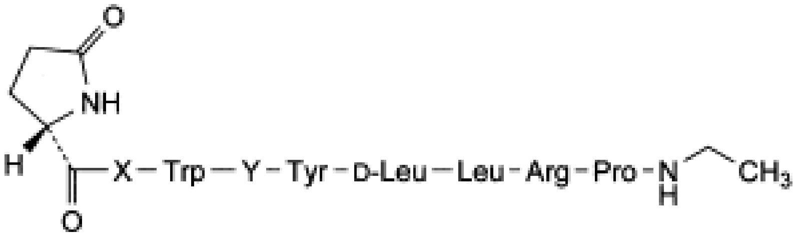
LABELLING

The label states the mass of peptide in the container.

IMPURITIES

Specified impurities   A, B, C, D.

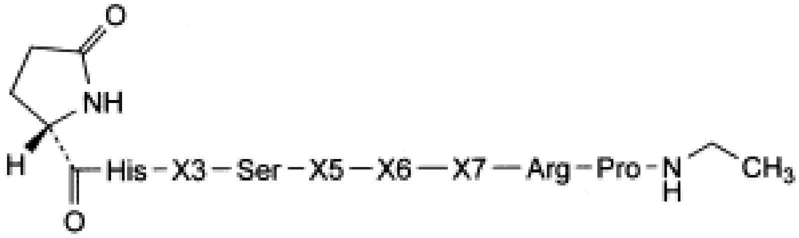
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10*. *Control of impurities in substances for pharmaceutical use)*: *E, F, G, H, I, J, K.*


O
NH
H
O
X – Trp – Y – Tyr – D-Leu – Leu – Arg – Pro – N
H
CH3

A. X = L-His, Y = D-Ser: [4-D-serine]leuprorelin,

B. X = D-His, Y = L-Ser: [2-D-histidine]leuprorelin,

F. X = D-His, Y = D-Ser: [2-D-histidine,4-D-serine]leuprorelin,

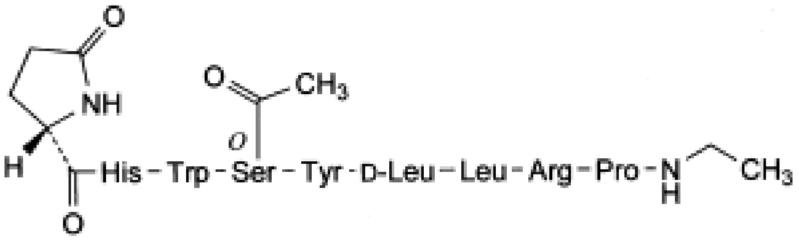

O
NH
H
O
His – X3 – Ser – X5 – X6 – X7 – Arg – Pro – N
H
CH3

C. X3 = L-Trp, X5 = L-Tyr, X6 = X7 = L-Leu: [6-L-leucine]leuprorelin,

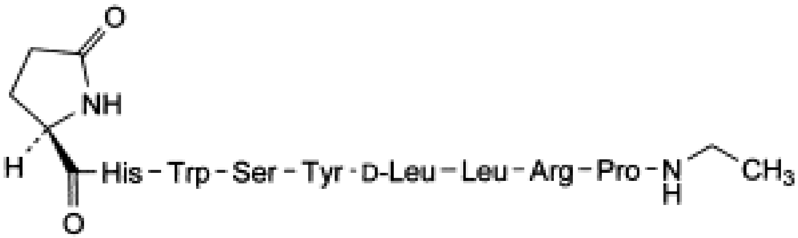
E. X3 = D-Trp, X5 = L-Tyr, X6 = D-Leu, X7 = L-Leu: [3-D-tryptophane]leuprorelin,

G. X3 = L-Trp, X5 = D-Tyr, X6 = D-Leu, X7 = L-Leu: [5-D-tyrosine]leuprorelin,

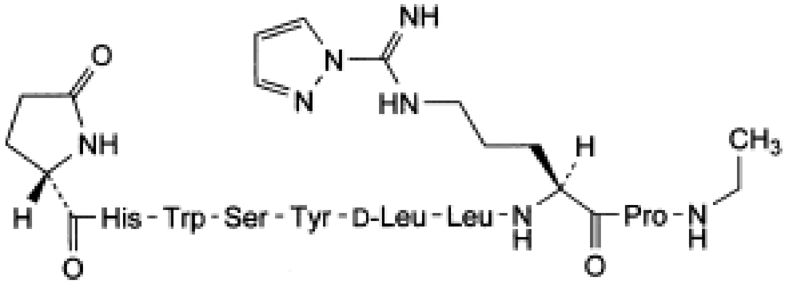
H. X3 = L-Trp, X5 = L-Tyr, X6 = X7 = D-Leu: [7-D-leucine]leuprorelin,


O
NH
O
CH3
O
H
O
His – Trp – Ser – Tyr – D-Leu – Leu – Arg – Pro – N
H
CH3

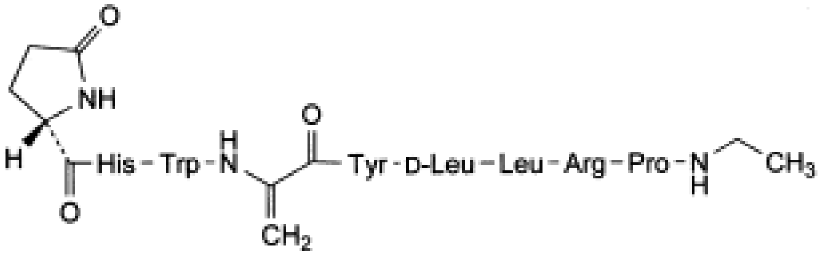
D. [4-(*O*-acetyl-L-serine)]leuprorelin,



I. [1-(5-oxo-D-proline)]leuprorelin,



J. [8-[5-*N*-[imino(1*H*-pyrazol-1-yl)methyl]-L-ornithine]]leuprorelin,



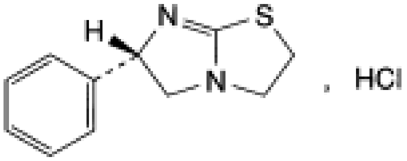
K. [4-dehydroalanine]leuprorelin.

Ph Eur

Levamisole Hydrochloride

General Notices

(Ph Eur monograph 0726)



$C_{11}H_{12}N_2S,HCl$ 240.8 *16595-80-5*

Action and use

Immunostimulant; antihelminthic.

Ph Eur

DEFINITION

(6*S*)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Freely soluble in water, soluble in alcohol, slightly soluble in methylene chloride.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *levamisole hydrochloride CRS.*

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)

3.0 to 4.5 for solution S.

Specific optical rotation (*2.2.7*)

- 121 to - 128(dried substance), determined on solution S.

Related substances

Liquid chromatography (2.2.29). *Prepare the solutions immediately before use, protect from light and keep below 25 °C.*

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R*, add 1.0 ml of *concentrated ammonia R* and dilute to 10.0 ml with *methanol R*.

Reference solution (a) Dissolve 50 mg of *levamisole hydrochloride for system suitability CRS* in *methanol R*, add 0.5 ml of *concentrated ammonia R* and dilute to 5.0 ml with *methanol R*.

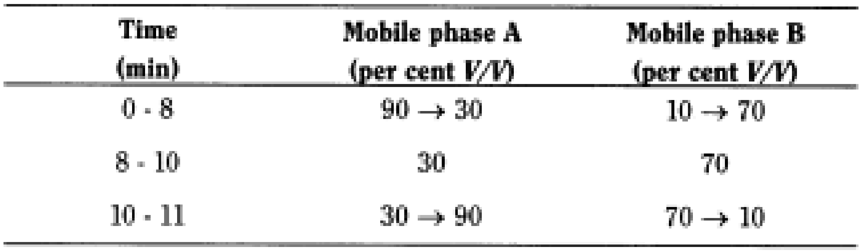
Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of the solution to 25.0 ml with *methanol R*.

Column:
— *size:* *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: dissolve 0.5 g of *ammonium dihydrogen phosphate R* in 90 ml of *water R*; adjust to pH 6.5 with a 40 g/l solution of *sodium hydroxide R* and dilute to 100 ml with *water R*;

— *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 8	90 → 30	10 → 70
8 - 10	30	70
10 - 11	30 → 90	70 → 10

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 215 nm.

Equilibration At least 4 min with the mobile phase at the initial composition.

Injection 10 µl.

Relative retention With reference to levamisole (retention time = about 3 min): impurity A = about 0.9; impurity B = about 1.4; impurity C = about 1.5; impurity D = about 1.6; impurity E = about 2.0.

System suitability Reference solution (a):

— the chromatogram obtained is similar to the chromatogram supplied with levamisole *hydrochloride for system suitability CRS*.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 2.0; impurity B = 1.7; impurity C = 2.9; impurity D = 1.3; impurity E = 2.7;

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *any other impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

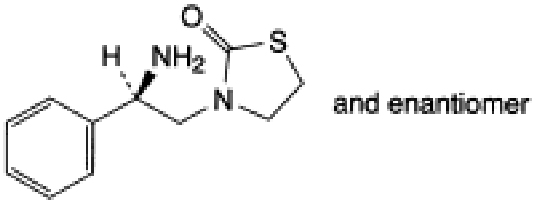
Dissolve 0.200 g in 30 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 24.08 mg of $C_{11}H_{13}ClN_2S$.

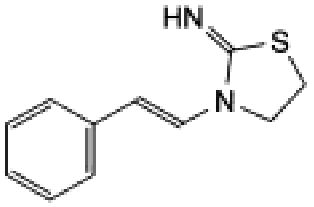
STORAGE

Protected from light.

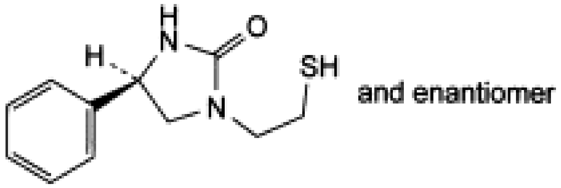
IMPURITIES


and enantiomer

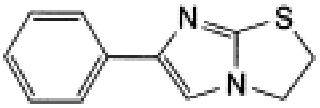
A. 3-[(2*RS*)-2-amino-2-phenylethyl]thiazolidin-2-one,



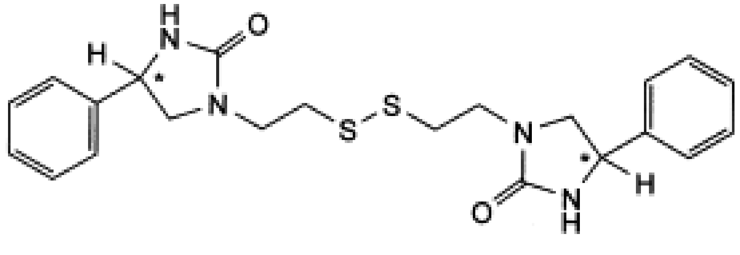
B. 3-[(*E*)-2-phenylethenyl]thiazolidin-2-imine,


and enantiomer

C. (4*RS*)-4-phenyl-1-(2-sulphanylethyl)imidazolidin-2-one,



D. 6-phenyl-2,3-dihydroimidazo[2,1-*b*]thiazole,

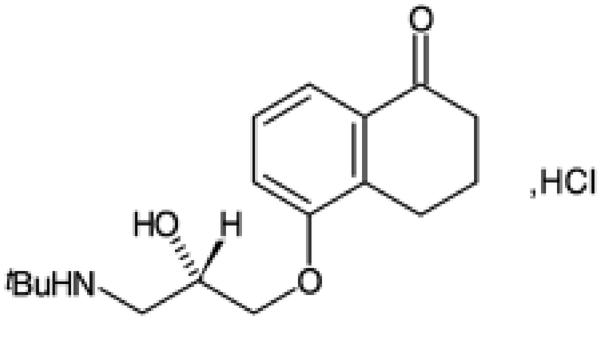


E. 1,1′-[(disulphane-1,2-diyl)bis(ethylene)]bis[(4*RS*)-4-phenylimidazolidin-2-one].

Ph Eur

Levobunolol Hydrochloride

General Notices



$C_{17}H_{25}NO_3,HCl$ 327.9 *27912-14-7*

Action and use

Beta-adrenoceptor antagonist.

Preparation

Levobunolol Eye Drops

DEFINITION

Levobunolol Hydrochloride is (*S*)-5-(3-*tert*-butylamino-2-hydroxypropoxy)-1,2,3,4-tetrahydronaphthalen-1-one hydrochloride. It contains not less than 98.5% and not more than 101.0% of $C_{17}H_{25}NO_3,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or pinkish white, crystalline powder.

Freely soluble in *water*; sparingly soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of levobunolol hydrochloride *(RS 200)*.

B. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 5% w/v solution, 4.5 to 6.5, Appendix V L.

Specific optical rotation

In a 3% w/v solution in *methanol*, –19.0 to –20.0, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in the mobile phase containing (1) 0.10% w/v of the substance being examined, (2) 0.00050% w/v of the substance being examined, (3) 0.0050% w/v of each of *levobunolol hydrochloride BPCRS* and *atenolol*.

The chromatographic procedure described under Assay may be used.

For solution (1) allow the chromatography to proceed for 3 times the retention time of the principal peak. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 8.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried over *phosphorus pentoxide* at 110° at a pressure not exceeding 2 kPa for 4 hours, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in the mobile phase containing (1) 0.01% w/v of the substance being examined, (2) 0.01% w/v of *levobunolol hydrochloride BPCRS* and (3) 0.0050% w/v of each of *levobunolol hydrochloride BPCRS* and *atenolol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 3.9 mm) packed with *end-capped octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP 8 is suitable), (b) a solution prepared by mixing 53 volumes of 0.005M *sodium heptanesulphonate* in *methanol* with 47 volumes of 0.005M *sodium heptanesulphonate* in *water* containing 1 ml of 0.5M *sulphuric acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 223 nm.

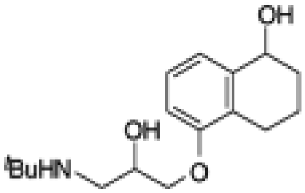
The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 8.

Calculate the content of $C_{17}H_{25}NO_3,HCl$ from the declared content of $C_{17}H_{25}NO_3,HCl$ in *levobunolol hydrochloride BPCRS*.

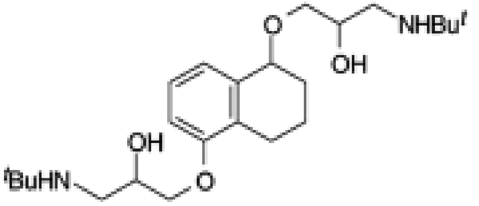
STORAGE

Levobunolol Hydrochloride should be protected from light.

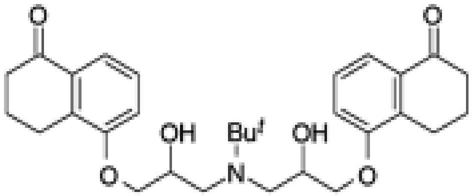
IMPURITIES



A. 5-(3-*tert*-butylamino-2-hydroxypropoxy)-1,2,3,4-tetrahydro-1-naphthol



B. 1,1′-(1,2,3,4-tetrahydro-1,5-naphthalenedioxy)bis(3-*tert*-butylamino)-2-propanol

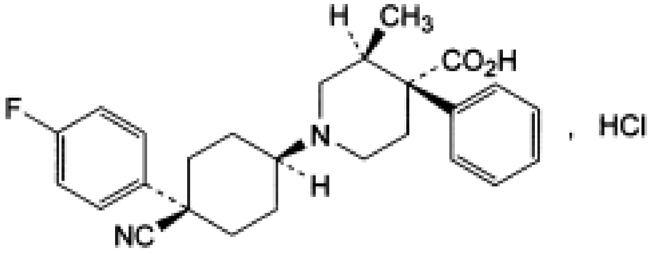


C. *meso*-5,5′-[(3,3′-*tert*-butylamino)bis(2-hydroxypropoxy)]bis-3,4-dihydronaphthalen-1(2 *H*)-one

Levocabastine Hydrochloride

General Notices

(Ph Eur monograph 1484)



$C_{26}H_{29}FN_2O_2,HCl$ 457.0 *79547-78-7*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

(3*S*,4*R*)-1-[*cis*-4-Cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid monohydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent) and in a 2 g/l solution of sodium hydroxide.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *levocabastine hydrochloride CRS.*

B. Dissolve 50 mg in a mixture of 0.4 ml of *ammonia R* and 2 ml of *water R*. Mix, allow to stand for 5 min and filter. Acidify the filtrate with *dilute nitric acid R*. It gives reaction (a) of chlorides (*2.3.1*).

C. Specific optical rotation (see Tests).

TESTS

Solution S

Dissolve 0.250 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

- 102 to - 106 (dried substance), determined on solution S.

Related substances

Capillary electrophoresis (*2.2.47*). *Prepare the solutions immediately before use*.

Test solution Dissolve 25.0 mg of the substance to be examined in a 2 g/l solution of *sodium hydroxide R* and dilute to 10.0 ml with the same solution.

Reference solution (a) Dissolve 2.5 mg of *levocabastine hydrochloride CRS* and 2.5 mg of *levocabastine impurity D CRS* in a 2 g/l solution of *sodium hydroxide R* and dilute to 200.0 ml with the same solution.

Reference solution (b) Dilute 5.0 ml of this test solution to 100.0 ml with a 2 g/l solution of *sodium hydroxide R.* Dilute 1.0 ml of this solution to 10.0 ml with a 2 g/l solution of *sodium hydroxide R.*

Blank solution A 2 g/l solution of *sodium hydroxide R*.

Capillary:

— *material*: uncoated fused silica;

— *size*: effective length = 0.5 m, Ø = 75 µm.

Temperature 50 °C.

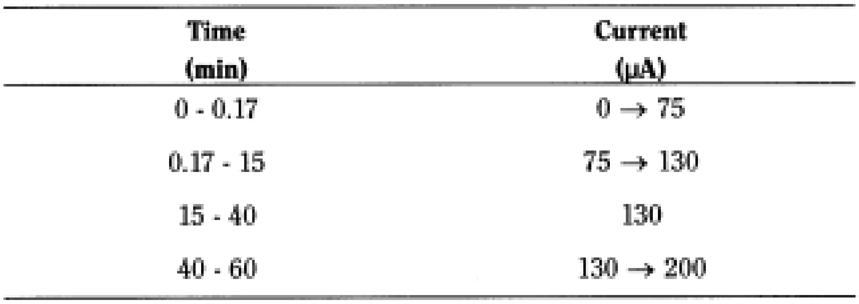
Electrolyte solution Dissolve 1.08 g of *sodium dodecyl sulphate R* and 0.650 g of *hydroxypropyl-*β*-cyclodextrin R* in 5 ml of *2-propanol R* and dilute to 50.0 ml with buffer solution pH 9.0 prepared as follows: dissolve 1.39 g of *boric acid R* in *water R* and adjust to pH 9.0 with *1 M sodium hydroxide* (about 9 ml). Dilute to 100.0 ml with *water R*.

Detection Spectrophotometer at 214 nm.

Preconditioning of the capillary Rinse the capillary for 2 min with a 2 g/l solution of *sodium hydroxide R* and for at least 5 min with the electrolyte solution.

Injection Under pressure (3,45 kPa) for 5 s.

Migration:



Time (min)	Current (µA)
0 - 0.17	0 → 75
0.17 - 15	75 → 130
15 - 40	130
40 - 60	130 → 200

Migration times Levocabastine = about 28 min; impurity D = about 30 min.

System suitability Reference solution (a):

— *resolution*: minimum 4 between the peaks due to levocabastine and impurity D; if necessary adjust the current gradient.

Limits:

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the electropherogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the electropherogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the electropherogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.175 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 1st and 3rd point of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 22.85 mg of $C_{26}H_{30}ClFN_2O_2$.

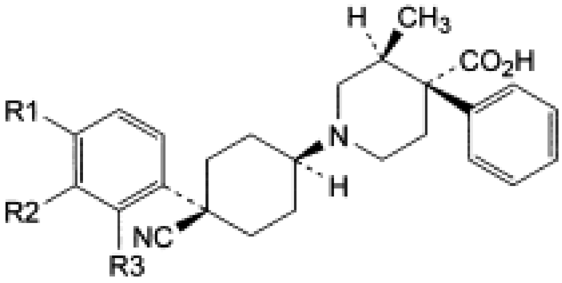
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E.

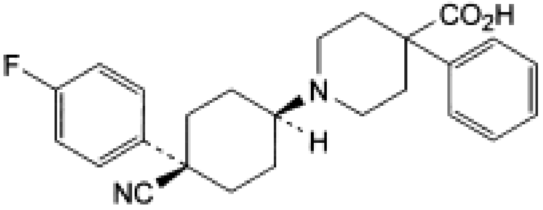
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *F, G, H, I.*


H
CH3
CO2H
R1
R2
R3
NC
N
H

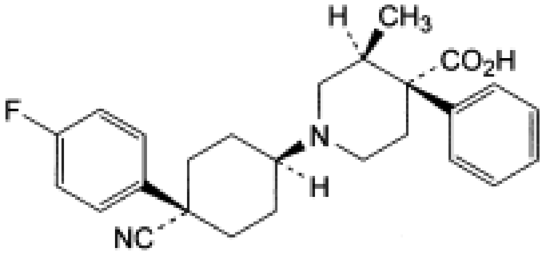
A. R1 = R2 = R3 = H: (3*S*,4*R*)-1-(*cis*-4-cyano-4-phenylcyclohexyl)-3-methyl-4-phenylpiperidine-4-carboxylic acid,

B. R1 = R2 = H, R3 = F: (3*S*,4*R*)-1-[*cis*-4-cyano-4-(2-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,

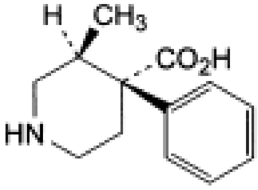
C. R1 = H, R2 = F, R3 = H: (3*S*,4*R*)-1-[*cis*-4-cyano-4-(3-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,



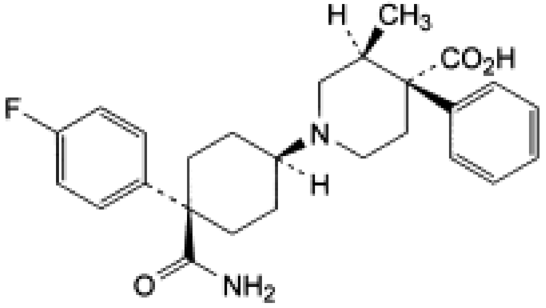
D. 1-[*cis*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-4-phenylpiperidine-4-carboxylic acid,


H
CH3
CO2H
F
N
H
NC

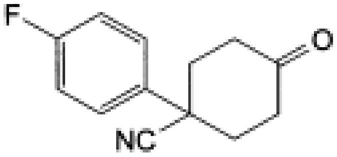
E. (3*S*,4*R*)-1-[*trans*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,


H
CH3
CO2H
HN

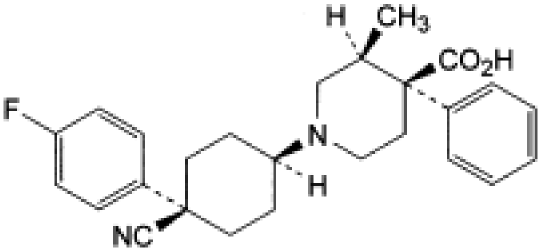
F. (3*S*,4*R*)-3-methyl-4-phenylpiperidine-4-carboxylic acid,


H
CH3
CO2H
F
N
H
O
NH2

G. (3*S*,4*R*)-1-[*cis*-4-carbamoyl-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,



H. 1-(4-fluorophenyl)-4-oxocyclohexanecarbonitrile,


H
CH3
CO2H
F
N
H
NC

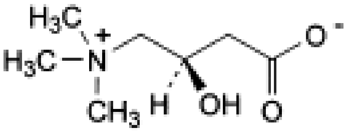
I. (3*S*,4*S*)-1-[*cis*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid.

Ph Eur

Levocarnitine

General Notices

(Ph Eur monograph 1339)



$C_7H_{15}NO_3$ 161.2 *541-15-1*

Action and use

Carnitine substitute.

Ph Eur

DEFINITION

(3*R*)-3-Hydroxy-4-(trimethylammonio)butanoate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Freely soluble in water, soluble in warm ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs, prepared using substance previously dried *in vacuo* at 50 °C for 5 h.

Comparison *levocarnitine CRS.*

C. To 1 ml of solution S (see Tests) add 9 ml of *water R*, 10 ml of *dilute sulphuric acid R* and 30 ml of *ammonium reineckate solution R*. A pink precipitate is formed. Allow to stand for 30 min. Filter and wash with *water R*, with *ethanol (96 per cent) R* and then with *acetone R* and dry at 80 °C. The precipitate melts (*2.2.14*) at 147 °C to 150 °C.

TESTS

Solution S

Dissolve 5.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

6.5 to 8.5.

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R.*

Specific optical rotation (*2.2.7*)

- 29.0 to - 32.0 (anhydrous substance), determined on solution S at 25 °C.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 12.5 mg of *levocarnitine impurity A CRS* in *water R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 10.0 mg of *levocarnitine impurity A CRS* in *water R* and dilute to 10.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (d) Dissolve 0.100 g of *levocarnitine CRS* in reference solution (c) and dilute to 10.0 ml with the same solution.

Column:

— *size:* *l* = 0.30 m, Ø = 3.9 mm;

— *stationary phase*: *aminopropylmethylsilyl silica gel for chromatography R* (10 µm);

— *temperature*: 30 °C.

Mobile phase Mix 35 volumes of a 6.81 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 4.7 with *dilute sodium hydroxide solution R*, and 65 volumes of *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 25 µl of the test solution and reference solutions (a), (b) and (d).

Retention time Levocarnitine = about 9.6 min; impurity A = about 10.6 min.

System suitability Reference solution (d):

— *resolution*: minimum 0.9 between the peaks due to levocarnitine and impurity A when the chromatogram is recorded over 15 min.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 300 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 2.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.125 g in a mixture of 3 volumes of *anhydrous formic acid R* and 50 volumes of *anhydrous acetic acid R*. Add 0.2 ml of *crystal violet solution R*. Titrate with *0.1 M perchloric acid* until the colour changes from violet to green.

1 ml of *0.1 M perchloric acid* is equivalent to 16.12 mg of $C_7H_{15}NO_3$.

STORAGE

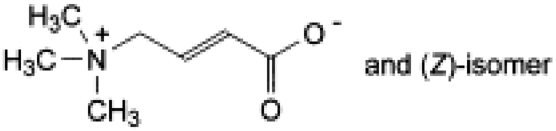
In an airtight container .

IMPURITIES

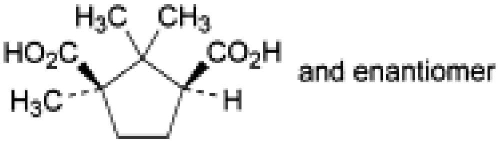
Specified impurities A.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general

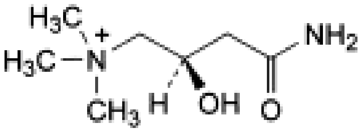
acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10*. *Control of impurities in substances for pharmaceutical use*): *B, C, D*.



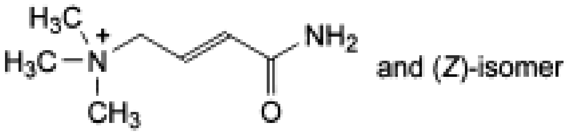
A. (*E*)- or (*Z*)-4-(trimethylammonio)but-2-enoate,



B. (1*RS*,3*SR*)-1,2,2-trimethylcyclopentane-1,3-dicarboxylic acid (camphoric acid),



C. (2*R*)-4-amino-2-hydroxy-*N*,*N*,*N*-trimethyl-4-oxobutan-1-aminium (carnitinamide),



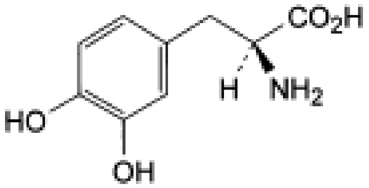
D. (*E*)- or (*Z*)-4-amino-*N*,*N*,*N*-trimethyl-4-oxobut-2-en-1-aminium.

Ph Eur

Levodopa

General Notices

(Ph Eur monograph 0038)



$C_9H_{11}NO_4$ 197.2 *59-92-7*

Action and use

Dopamine precursor; treatment of Parkinson's disease.

Preparations

Co-beneldopa Capsules

Dispersible Co-beneldopa Tablets

Co-careldopa Tablets

Levodopa Capsules

Levodopa Tablets

When L-dopa is prescribed or demanded, Levodopa shall be dispensed or supplied.

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)propanoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, practically insoluble in ethanol (96 per cent). It is freely soluble in *1 M* *hydrochloric acid* and sparingly soluble in *0.1 M hydrochloric acid*.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *levodopa CRS*.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 1.0 g in a 103 g/l solution of *hydrochloric acid R* and dilute to 25 ml with the same solution.

pH (*2.2.3*)

4.5 to 7.0.

Shake 0.10 g with 10 ml of *carbon dioxide-free water R* for 15 min.

Related substances

Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*

Solution A 10.3 g/l solution of *hydrochloric acid R*.

Test solution Dissolve 0.100 g of the substance to be examined in solution A and dilute to 25 ml with solution A.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with solution A. Dilute 5.0 ml of this solution to 100.0 ml with solution A.

Reference solution (b) Dissolve 8 mg of *tyrosine R* (impurity B) and 4 mg of *3-methoxy-l-tyrosine R* (L-isomer of impurity C) in 2 ml of the test solution and dilute to 50 ml with solution A. Dilute 5 ml of this solution to 100 ml with solution A.

Column:

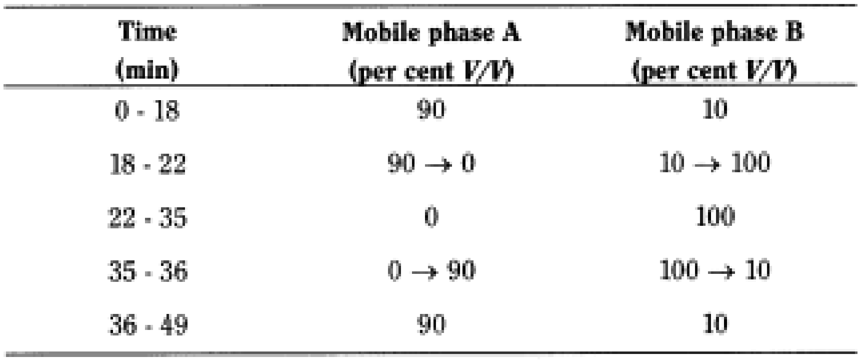
— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: spherical *di-isobutyloctadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 8 nm.

Mobile phase:

— *mobile phase A*: *0.1 M phosphate buffer solution pH 3.0*;

— *mobile phase B*: *methanol R*, *0.1 M phosphate buffer solution pH 3.0* (18:85 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 18	90	10
18 - 22	90 → 0	10 → 100
22 - 35	0	100
35 - 36	0 → 90	100 → 10
36 - 49	90	10

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Relative retention With reference to levodopa (retention time = about 6 min): impurity A = about 0.7; impurity B = about 2; impurity C = about 3.5.

System suitability Reference solution (b):

— *resolution*: minimum 10 between the peaks due to levodopa and impurity B.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 2.2;

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Enantiomeric purity

Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 25 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *D-dopa R* (impurity D) in 10 ml of the test solution. Dilute 1 ml of this solution to 100 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m2/g and a pore size of 10 nm.

Mobile phase Dissolve separately 200 mg of *copper acetate R* and 387 mg of *N,N-dimethyl-l-phenylalanine R* in *water R*; mix the 2 solutions and adjust immediately to pH 4.0 with *acetic acid R*; add 50 ml of *methanol R* and dilute to 1000 ml with *water R*; mix and filter.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Run time Twice the retention time of levodopa.

Relative retention With reference to levodopa (retention time = about 7 min): impurity D = about 0.4.

System suitability Reference solution (b):

— *resolution*: minimum 5 between the peaks due to impurity D and levodopa.

Limit:

— *impurity D:* not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.180 g, heating if necessary, in 5 ml of *anhydrous formic acid R*. Add 25 ml of *anhydrous acetic acid R*. Add 25 ml of *dioxan R* and 0.1 ml of *crystal violet solution R*. Titrate with *0.1 M perchloric acid*, until a green colour is obtained.

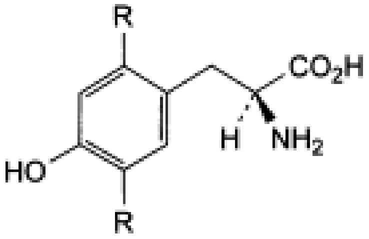
1 ml of *0.1 M perchloric acid* is equivalent to 19.72 mg of $C_9H_{11}NO_4$.

STORAGE

Protected from light.

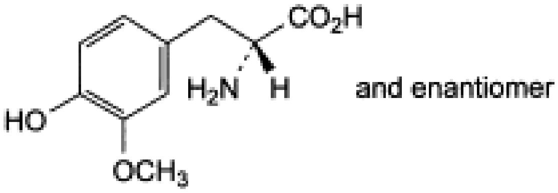
IMPURITIES

Specified impurities *A, B, C, D.*

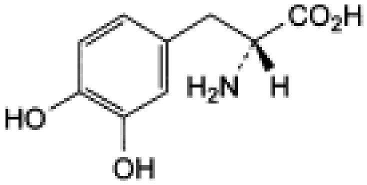

R
CO_2H
H
NH_2
HO
R

A. R = OH: (2*S*)-2-amino-3-(2,4,5-trihydroxyphenyl)propanoic acid,

B. R = H: (2*S*)-2-amino-3-(4-hydroxyphenyl)propanoic acid (tyrosine),


CO_2H
H_2N
H
and enantiomer
HO
OCH_3

C. (2*RS*)-2-amino-3-(4-hydroxy-3-methoxyphenyl)propanoic acid (3-methoxy-DL-tyrosine),


CO_2H
H_2N
H
HO
OH

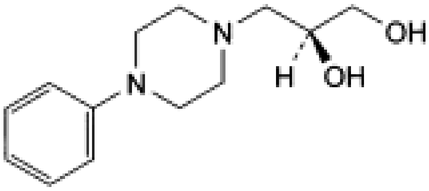
D. (2*R*)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid (D-dopa).

Ph Eur

Levodropropizine

General Notices

(Ph Eur monograph 1535)



$C_{13}H_{20}N_2O_2$ 236.3 *99291-25-5*

Action and use

Cough suppressant.

Ph Eur

DEFINITION

(2*S*)-3-(4-Phenylpiperazin-1-yl)propane-1,2-diol.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in dilute acetic acid and in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): - 30.0 to - 33.5 (dried substance).

Dissolve 1.50 g in a 21 g/l solution of *hydrochloric acid R* and dilute to 50.0 ml with the same acid.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison levodropropizine CRS.

TESTS

pH (*2.2.3*)

9.2 to 10.2.

Suspend 2.5 g in *carbon dioxide-free water R*, heat to dissolve, cool to room temperature and dilute to 100 ml with the same solvent.

Impurity B and related substances Liquid chromatography (*2.2.29*).

Test solution Dissolve 24.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 12.0 mg of *1-phenylpiperazine R* (impurity B) in methanol R and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Mix 2 ml of the test solution with 2 ml of reference solution (a) and dilute to 10 ml with the mobile phase.

Column:
- — *size: l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 12 volumes of *methanol R* and 88 volumes of a 6.81 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

System suitability Reference solution (b):

- — *resolution*: minimum 2.0 between the peaks due to levodropropizine and impurity B.

Limits:
- — *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- — *unspecified impurities*: for each impurity, not more than 0.2 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *disregard limit*: 0.02 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (0.01 per cent).

Impurity C

Gas chromatography (*2.2.28*). *Prepare the solutions immediately before use.*

Test solution Dissolve 0.50 g of the substance to be examined in *methylene chloride R* and dilute to 2.5 ml with the same solvent.

Reference solution (a) Dissolve 0.20 g of *glycidol R* (impurity C) in *methylene chloride R* and dilute to 100.0 ml with the same solvent. Dilute 0.5 ml of this solution to 100.0 ml with *methylene chloride R.*

Reference solution (b) Dissolve 0.50 g of the substance to be examined in *methylene* *chloride R*, add 0.5 ml of reference solution (a) and dilute to 2.5 ml with *methylene chloride R*.

Column:
- *material*: fused silica;
- *size:* l = 30 m, Ø = 0.53 mm;
- *stationary phase*: *poly[(cyanopropyl)(phenyl)][dimethyl]siloxane R* (film thickness 3 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.5 ml/min.

Split ratio 1:8.

Temperature:
- *column*: 140 °C;
- *injection port*: 170 °C;
- *detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µl of the test solution and reference solution (b).

Use a split-liner consisting of a column about 1 cm long packed with glass wool.

At the end of a series of tests, heat the column at 250 °C for 4-6 h.

Limit:
- *impurity C*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (10 ppm).

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Solvent mixture *anhydrous ethanol R*, *hexane R* (40:60 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in 10.0 ml of the solvent mixture. Dilute 1.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 10.0 mg of *levodropropizine CRS* in 10.0 ml of the solvent mixture. Dilute 1.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (b) Dissolve 10.0 mg of *levodropropizine impurity A CRS* in 10.0 ml of the solvent mixture. Dilute 1.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 50.0 ml with the solvent mixture.

Reference solution (d) Dilute 0.5 ml of reference solution (b) to 25 ml with reference solution (a).

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel OD for chiral separations R*.

Mobile phase *diethylamine R*, *anhydrous ethanol R*, *hexane R* (0.2:5:95 *V/V/V*).

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (a), (c) and (d).

Elution order Impurity A, levodropropizine.

System suitability:

— *retention times*: the retention times of the principal peaks in the chromatograms obtained with the test solution and reference solution (a) are similar;

— *resolution*: minimum 1.3 between the peaks due to impurity A and levodropropizine in the chromatogram obtained with reference solution (d).

Limit:

— *impurity A:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (2 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying *in vacuo* at 60 °C over *diphosphorus pentoxide R* at a pressure of 0.15-0.25 kPa for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 50 ml of *anhydrous acetic acid R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M perchloric acid*. Read the volume added at the 2nd point of inflexion.

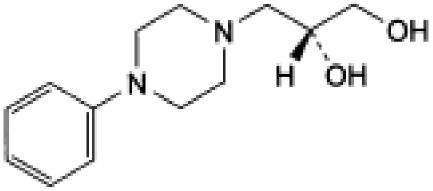
1 ml of *0.1 M perchloric acid* is equivalent to 11.82 mg of $C_{13}H_{20}N_2O_2$.

STORAGE

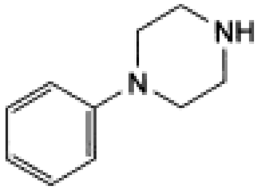
Protected from light.

IMPURITIES

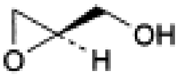
Specified impurities A, B, C.



A. (2*R*)-3-(4-phenylpiperazin-1-yl)propane-1,2-diol (dextrodropropizine),



B. 1-phenylpiperazine,



and enantiomer

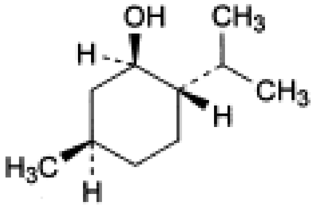
C. [(2*RS*)-oxiran-2-yl]methanol (glycidol).

Ph Eur

Levomenthol

General Notices

(Ph Eur monograph 0619)



$C_{10}H_{20}O$ 156.3 *2216-51-5*

Action and use

Decongestant.

Preparations

Levomenthol Cream

Menthol and Benzoin Inhalation

Ph Eur

DEFINITION

(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexanol.

CHARACTERS

Appearance

Prismatic or acicular, colourless, shiny crystals.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent) and in light petroleum, freely soluble in fatty oils and in liquid paraffin, very slightly soluble in glycerol.

mp: about 43 °C.

IDENTIFICATION

First identification A, C.

Second identification B, D.

A. Specific optical rotation (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 25 mg of *menthol CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air, until the solvents have evaporated.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in position and approximate dimensions to the principal peak in the chromatogram obtained with reference solution (c).

D. Dissolve 0.20 g in 0.5 ml of *anhydrous pyridine R*. Add 3 ml of a 150 g/l solution of *dinitrobenzoyl chloride R* in *anhydrous pyridine R*. Heat on a water-bath for 10 min. Add 7.0 ml of *water R* in small quantities with stirring and allow to stand in iced water for 30 min. A precipitate is formed. Allow to stand and decant the supernatant liquid. Wash the precipitate with 2 quantities, each of 5 ml, of iced *water R*, recrystallise from 10 ml of *acetone R*, wash with iced *acetone R* and dry at 75 °C at a pressure not exceeding 2.7 kPa for 30 min. The crystals melt (*2.2.14*) at 154 °C to 157 °C.

TESTS

Solution S

Dissolve 2.50 g in 10 ml of *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent. Add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation (*2.2.7*)

- 48 to - 51, determined on solution S.

Related substances

Gas chromatography (*2.2.28*).

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *methylene chloride R*.

Reference solution (a) Dissolve 40.0 mg of the substance to be examined and 40.0 mg of *isomenthol R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 0.10 ml of test solution (a) to 100.0 ml with *methylene chloride R*.

Reference solution (c) Dissolve 40.0 mg of *menthol CRS* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Column:
- *material*: glass;
- *size:* l = 2.0 m, Ø = 2 mm;
- *stationary phase*: *diatomaceous earth for gas chromatography R* impregnated with 15 per cent *m/m* of *macrogol 1500 R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 ml/min.

Temperature:
- *column*: 120 °C;
- *injection port*: 150 °C;
- *detector*: 200 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time Twice the retention time of menthol.

System suitability:
- *resolution*: minimum 1.4 between the peaks due to menthol and isomenthol in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits Test solution (a):
- *total*: not more than 1 per cent of the area of the principal peak;
- *disregard limit*: 0.05 per cent of the area of the principal peak.

Residue on evaporation

Maximum 0.05 per cent.

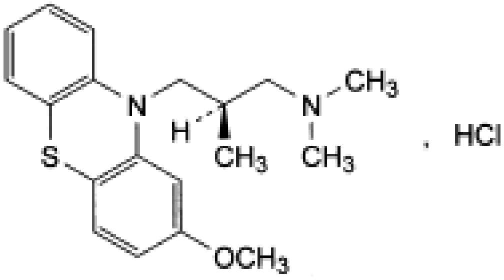
Evaporate 2.00 g on a water-bath and heat in an oven at 100-105 °C for 1 h. The residue weighs not more than 1.0 mg.

Ph Eur

Levomepromazine Hydrochloride

General Notices

(Ph Eur monograph 0505)



$C_{19}H_{24}N_2OS,HCl$ 364.9 *4185-80-2*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Levomepromazine Injection

Ph Eur

DEFINITION

Levomepromazine hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*R*)-3-(2-methoxy-10*H*-phenothiazin-10-yl)-*N*,*N*,2-trimethylpropan-1-amine hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or very slightly yellow, crystalline powder, slightly hygroscopic, freely soluble in water and in alcohol. It deteriorates when exposed to air and light. It exists in two forms, one melting at about 142 °C and the other at about 162 °C.

IDENTIFICATION

A. *Prepare the solution protected from bright light and carry out the measurements immediately.* Dissolve 50.0 mg in *water R* and dilute to 500.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water R*. Examined between 230 nm and 340 nm (*2.2.25*), the solution shows two absorption maxima, at 250 nm and 302 nm. The specific absorbance at the maximum at 250 nm is 640 to 700.

B. It complies with the identification test for phenothiazines by thin-layer chromatography (*2.3.3*): use *levomepromazine hydrochloride CRS* to prepare the reference solution.

C. Introduce 0.2 g into a 100 ml separating funnel. Add 5 ml of *water R* and 0.5 ml of *strong sodium hydroxide solution R*. Shake vigorously with two quantities, each of 10 ml, of *ether R.* Combine the ether layers, dry over *anhydrous sodium sulphate R* and evaporate to dryness. Keep the residue at 100 °C to 105 °C for 15 min and allow to crystallise in iced water. Initiate crystallisation if necessary by scratching the wall of the flask with a glass rod. Dry the crystals at 60 °C for 2 h. The crystals melt (*2.2.14*) at 122 °C to 128 °C.

D. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *bromocresol green solution R*. Not more than 0.5 ml of *0.01 M sodium hydroxide* or 1.0 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

+ 9.5 to + 11.5, determined on solution S and calculated with reference to the dried substance.

Related substances

Carry out the test protected from bright light. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents. Prepare immediately before use.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *acetone R*, 10 volumes of *diethylamine R* and 80 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 5 ml of *water R* and add 50 ml of *2-propanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* is equivalent to 36.49 mg of $C_{19}H_{25}ClN_2OS$.

STORAGE

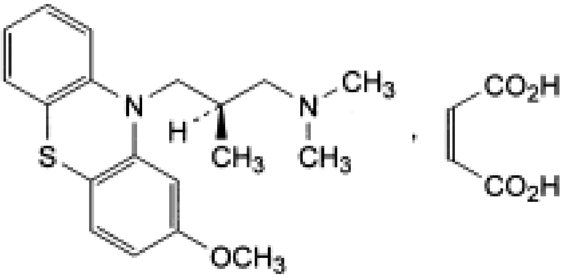
Store in an airtight container , protected from light.

Ph Eur

Levomepromazine Maleate

General Notices

(Ph Eur monograph 0925)



$C_{19}H_{24}N_2OS,C_4H_4O_4$ 444.6 *7104-38-3*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Levomepromazine Tablets

Ph Eur

DEFINITION

Levomepromazine maleate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*R*)-3-(2-methoxy-10*H*-phenothiazin-10-yl)-*N*,*N*,2-trimethylpropan-1-amine (*Z*)-butenedioate, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, slightly soluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol. It deteriorates when exposed to air and light.

It melts at about 186 °C, with decomposition.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *levomepromazine maleate CRS*. Examine the substances prepared as discs.

C. It complies with the identification test for phenothiazines by thin-layer chromatography (*2.3.3*): use *levomepromazine maleate CRS* to prepare the reference solution.

D. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.20 g of the substance to be examined in a mixture of 10 volumes of *water R* and 90 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 50 mg of *maleic acid CRS* in a mixture of 10 volumes of *water R* and 90 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Apply separately to the plate as bands 10 mm by 2 mm 5 µl of each solution. Develop over a path of 12 cm using a mixture of 3 volumes of *water R*, 7 volumes of *anhydrous formic acid R* and 90 volumes of *di-isopropyl ether R*. Dry the plate at 120 °C for 10 min and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution shows a band at the starting-point and another band similar in position and size to the principal band in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

Carry out the test protected from bright light. Introduce 0.50 g into a conical flask and add 25.0 ml of *carbon dioxide-free water R*. Shake and allow the solids to settle. The pH of the supernatant solution is 3.5 to 5.5.

Specific optical rotation (*2.2.7*)

Dissolve 1.25 g in *dimethylformamide R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is - 7.0 to - 8.5, calculated with reference to the dried substance.

Related substances

Carry out the test protected from bright light and prepare the solutions immediately before use. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.20 g of the substance to be examined in a mixture of 10 volumes of *water R* and 90 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with a mixture of 10 volumes of *water R* and 90 volumes of *acetone R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *acetone R*, 10 volumes of *diethylamine R* and 80 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

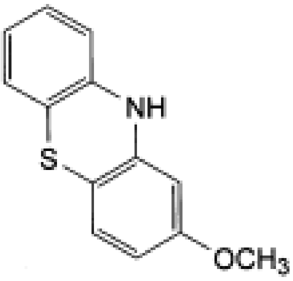
Dissolve 0.350 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 44.46 mg of $C_{23}H_{28}N_2O_5S$.

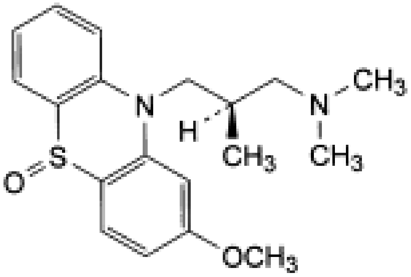
STORAGE

Store protected from light.

IMPURITIES



A. 2-methoxyphenothiazine,



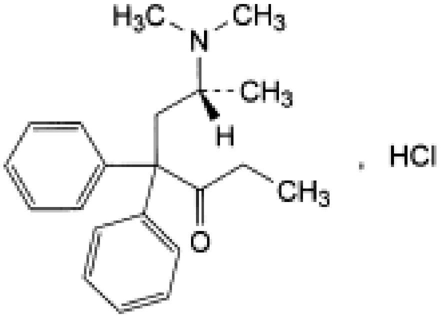
B. 10-[(2*R*)-3-(dimethylamino)-2-methylpropyl]-2-methoxy-10*H*-phenothiazine 5-oxide.

Ph Eur

Levomethadone Hydrochloride

General Notices

(Ph Eur monograph 1787)



$C_{21}H_{27}NO,HCl$ 345.9 *125-58-6*

Action and use

Opioid analgesic.

Ph Eur

DEFINITION

(6*R*)-6-(Dimethylamino)-4,4-diphenylheptan-3-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification A, C, D.

Second identification A, B, D.

A. Specific optical rotation (see Tests).

B. Melting point (*2.2.14*): 239 °C to 242 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of methadone hydrochloride.*

D. Dilute 1 ml of solution S (see Tests) to 5 ml with *water R* and add 1 ml of *dilute ammonia R1*. Mix, allow to stand for 5 min and filter. The filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dilute 10 ml of solution S to 25 ml with *carbon dioxide-free water R*. To 10 ml of the solution add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Specific optical rotation (*2.2.7*)

- 125 to - 135 (dried substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 12.0 mg of *imipramine hydrochloride CRS* in the mobile phase and dilute to 10 ml with the mobile phase. To 1 ml of the solution add 5 ml of the test solution and dilute to 10 ml with the mobile phase.

Column:
- *size:* *l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 25 °C.

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of an 11.5 g/l solution of *phosphoric acid R* adjusted to pH 3.6 with *tetraethylammonium hydroxide solution R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Equilibration About 30 min.

Injection 10 µl.

Run time 7 times the retention time of levomethadone.

Retention time Levomethadone = about 5 min.

System suitability Reference solution (b):

- *resolution*: minimum 2.5 between the peaks due to imipramine and levomethadone.

Limits:

- *any impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Dextromethadone

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *2-hydroxypropylbetadex for chromatography R* (5 µm);
- *temperature*: 10 °C.

Mobile phase Mix 1 volume of *triethylamine R* adjusted to pH 4.0 with *phosphoric acid R*, 15 volumes of *acetonitrile R* and 85 volumes of a 13.6 g/l solution of *potassium dihydrogen phosphate R*.

Flow rate 0.7 ml/min.

Detection Spectrophotometer at 210 nm.

Equilibration About 30 min.

Injection 10 µl.

Relative retention With reference to levomethadone: dextromethadone = about 1.4.

System suitability Test solution:

- *number of theoretical plates*: minimum 2000, calculated for the peak due to levomethadone;
- *tailing factor*: maximum 3 for the peak due to levomethadone.

Limit:

- *dextromethadone*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 40 ml of *water R* and 5 ml of *acetic acid R*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (*2.2.20*), using a silver electrode.

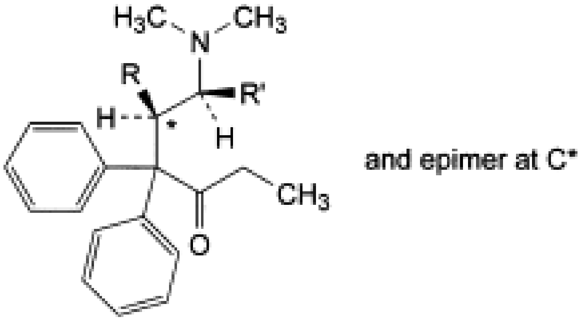
1 ml of *0.1 M silver nitrate* is equivalent to 34.59 mg of $C_{21}H_{28}ClNO$.

STORAGE

Protected from light.

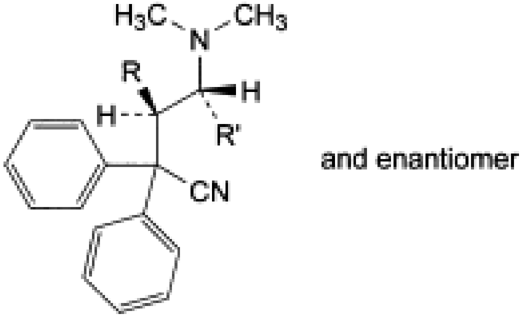
IMPURITIES

Specified impurities A, B, C, D, E, F.



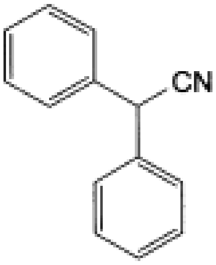
A. R = H, R′ = CH_3: (6*S*)-6-(dimethylamino)-4,4-diphenylheptan-3-one,

D. R = CH_3, R′ = H: (5*RS*)-6-(dimethylamino)-5-methyl-4,4-diphenylhexan-3-one,

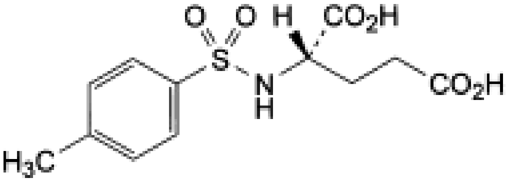


B. R = H, R′ = CH_3: (4*RS*)-4-(dimethylamino)-2,2-diphenylpentanenitrile,

C. R = CH_3, R′ = H: (3*RS*)-4-(dimethylamino)-3-methyl-2,2-diphenylbutanenitrile,



E. diphenylacetonitrile,



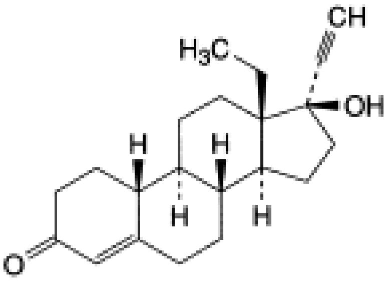
F. (2*S*)-2-[[(4-methylphenyl)sulphonyl]amino]pentanedioic acid (*N*-*p*-tosyl-L-glutamic acid).

Ph Eur

Levonorgestrel

General Notices

(Ph Eur monograph 0926)



$C_{21}H_{28}O_2$ 312.5 *797-63-7*

Action and use

Progestogen.

Preparations

Levonorgestrel Tablets

Levonorgestrel and Ethinylestradiol Tablets

Ph Eur

DEFINITION

Levonorgestrel contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 13β-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *levonorgestrel CRS*.

TESTS

Specific optical rotation (*2.2.7*)

Dissolve 0.200 g in *chloroform R* and dilute to 10.0 ml with the same solvent. The specific optical rotation is - 30 to - 35.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *chloroform R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 10 ml with *methylene chloride R*. Dilute 1 ml of this solution to 20 ml with *methylene chloride R*.

Reference solution (b) Dilute 4 ml of reference solution (a) to 10 ml with *methylene chloride R*.

Reference solution (c) Dissolve 5 mg of *levonorgestrel CRS* and 5 mg of *ethinylestradiol CRS* in *methylene chloride R* and dilute to 50 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *ethyl acetate R* and 80 volumes of *methylene chloride R.* Allow the plate to dry in air, spray with a 100 g/l solution of *phosphomolybdic acid R* in *alcohol R*, heat at 100 °C to 105 °C for 15 min and examine immediately. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most two such spots are more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

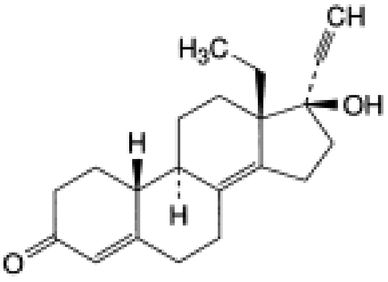
Dissolve 0.200 g in 45 ml of *tetrahydrofuran R*. Add 10 ml of a 100 g/l solution of *silver nitrate R*. After 1 min, titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.25 mg $C_{21}H_{28}O_2$.

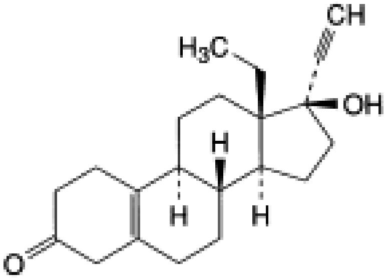
STORAGE

Store protected from light.

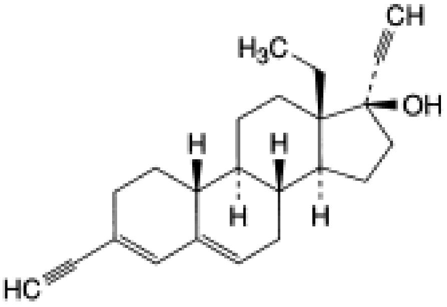
IMPURITIES


CH
H₃C
OH
H
H
O

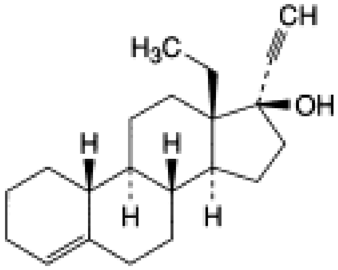
A. 3-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,8(14)-dien-20-yn-3-one,


CH
H₃C
OH
H
H
H
O

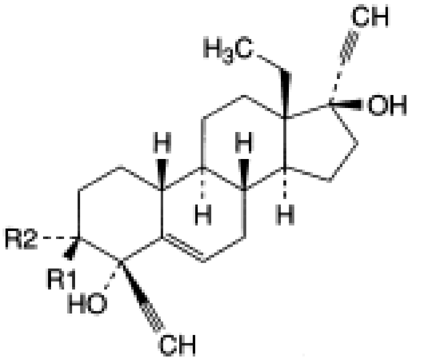
B. 3-ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(10)-en-20-yn-3-one,


CH
H₃C
OH
H
H
H
H
HC

C. 3-ethyl-3-ethynyl-18,19-dinor-17α-pregna-3,5-dien-20-yn-17-ol,


CH
H₃C
OH
H
H
H
H

D. 3-ethyl-18,19-dinor-17α-pregn-4-en-20-yn-17-ol,


CH
H$_3$C
OH
H
H
H
H
R2
R1
HO
CH

E. R1 = OH, R2 = C≡CH: 13-ethyl-3,4-diethynyl-18,19-dinor-17α-pregn-5-en-20-yn-3β,4α,17-triol,

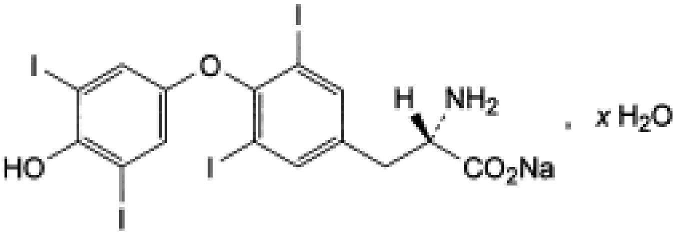
F. R1 = C≡CH, R2 = OH: 13-ethyl-3,4-diethynyl-18,19-dinor-17α-pregn-5-en-20-yn-3α,4α,17-triol.

Ph Eur

Levothyroxine Sodium

General Notices

(Ph Eur monograph 0401)



$C_{15}H_{10}I_4NNaO_4,xH_2O$ 799 *55-03-8*

Action and use

Thyroid hormone replacement

Preparation

Levothyroxine Tablets

Ph Eur

DEFINITION

Levothyroxine sodium contains not less than 97.0 per cent and not more than the equivalent of 102.0 per cent of sodium (2*S*)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propanoate, calculated with reference to the dried substance. It contains a variable amount of water.

CHARACTERS

An almost white or slightly brownish-yellow powder, or a fine, crystalline powder, very slightly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum

obtained with *levothyroxine sodium CRS*.

C. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 5 mg of the substance to be examined in a mixture of 5 volumes of *concentrated ammonia R* and 70 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (a) Dissolve 5 mg of *levothyroxine sodium CRS* in a mixture of 5 volumes of *concentrated ammonia R* and 70 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (b) Dissolve 5 mg of *liothyronine sodium CRS* in a mixture of 5 volumes of *concentrated ammonia R* and 70 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents. Mix 1 ml of this solution and 1 ml of the test solution.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *concentrated ammonia R*, 35 volumes of *2-propanol R* and 55 volumes of *ethyl acetate R*. Allow the plate to dry in air and spray with *ninhydrin solution R*. Heat at 100-105 °C until the spots appear. Examine in daylight. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

D. To about 50 mg in a porcelain dish add a few drops of *sulphuric acid R* and heat. Violet vapour is evolved.

E. To 200 mg add 2 ml of *dilute sulphuric acid R*. Heat on a water-bath and then carefully over a naked flame, increasing the temperature gradually up to about 600 ± 50 °C. Continue the ignition until most of the black particles have disappeared. Dissolve the residue in 2 ml of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 0.500 g in 23 ml of a gently boiling mixture of 1 volume of *1 M hydrochloric acid* and 4 volumes of *ethanol (96 per cent) R*. Cool and dilute to 25.0 ml with the same mixture of solvents.

Appearance of solution

Freshly prepared solution S is not more intensely coloured than reference solution BY_3 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

+ 16 to + 20, determined on solution S and calculated with reference to the dried substance.

Liothyronine and other related substances. Examine the chromatograms obtained in the assay. In the chromatogram obtained with test solution (a), the area of any peak due to liothyronine is not greater than that of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the peaks apart from the principal peak and any peak due to liothyronine is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Disregard any peak with an area less than that of the peak in the chromatogram obtained with reference solution (d).

Loss on drying (*2.2.32*)

6.0 per cent to 12.0 per cent, determined on 0.100 g by drying in an oven at 105 °C.

ASSAY

Examine by liquid chromatography (*2.2.29*). *Protect the solutions from light throughout the assay.*

Test solution (a) Dissolve 20.0 mg of the substance to be examined in *methanolic sodium hydroxide solution R* and dilute to 100.0 ml with the same solvent.

Test solution (b) Dilute 2.0 ml of test solution (a) to 200 ml with *methanolic sodium hydroxide solution R*.

Reference solution (a) Dissolve 20.0 mg of *levothyroxine sodium CRS* in *methanolic sodium hydroxide solution R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 200 ml with *methanolic sodium hydroxide solution R*.

Reference solution (b) Dissolve 5 mg of *liothyronine sodium CRS* in *methanolic sodium hydroxide solution R* and dilute to 50.0 ml with the same solvent. Dilute 10.0 ml of the solution to 50 ml with *methanolic sodium hydroxide solution R*. Dilute 10.0 ml of this solution to 100 ml with *methanolic sodium hydroxide solution R*.

Reference solution (c) Mix equal volumes of reference solution (a) and reference solution (b) .

Reference solution (d) Dilute 1 ml of reference solution (a) to 10 ml with *methanolic sodium hydroxide solution R*.

The chromatographic procedure may be carried out using:

— a column 0.25 m long and 4 mm in internal diameter packed with *nitrile silica gel for chromatography R* (5-10 µm);

— as mobile phase at a flow rate of 1 ml/min a mixture of 1 volume of *phosphoric acid R*, 300 volumes of *acetonitrile R* and 700 volumes of *water R*;

— as detector a spectrophotometer set at 225 nm;

— a loop injector.

Inject separately 50 µl of each solution. Continue the chromatography for 3.5 times the retention time of the principal peak. The assay is not valid unless the resolution between the peaks due to levothyroxine and liothyronine in the chromatogram obtained with reference solution (c) is at least 4 and the principal peak in the chromatogram obtained with reference solution (d) has a signal-to-noise ratio of at least 5.

Calculate the percentage content of $C_{15}H_{10}I_4NNaO_4$ from the declared content of *levothyroxine sodium CRS*.

STORAGE

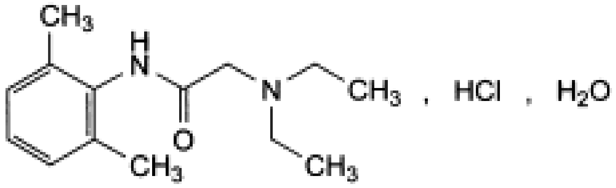
In an airtight container , protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Lidocaine Hydrochloride

General Notices

(Ph Eur monograph 0227)



$C_{14}H_{22}N_2O,HCl,H_2O$ 288.8 *6108-05-0*

Action and use

Local anaesthetic; Class I antiarrhythmic.

Preparations

Lidocaine Gel

Lidocaine and Chlorhexidine Gel

Lidocaine Injection

Lidocaine and Adrenaline Injection/Lidocaine and Epinephrine Injection

Sterile Lidocaine Solution

Ph Eur

DEFINITION

2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide hydrochloride monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Melting point (*2.2.14*): 74 °C to 79 °C, determined without previous drying.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lidocaine hydrochloride CRS*.

C. To about 5 mg add 0.5 ml of *fuming nitric acid R*. Evaporate to dryness on a water-bath, cool and dissolve the residue in 5 ml of *acetone R*. Add 0.2 ml of *alcoholic potassium hydroxide solution R*. A green colour is produced.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

4.0 to 5.5.

Dilute 1 ml of solution S to 10 ml with *carbon dioxide-free water R.*

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *2,6-dimethylaniline R* (impurity A) in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *2-chloro-N-(2,6-dimethylphenyl)acetamide R* (impurity H) in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase.

Reference solution (d) Mix 1.0 ml of reference solution (a), 1.0 ml of reference solution (b) and 1.0 ml of reference solution (c) and dilute to 100.0 ml with the mobile phase.

Column:

- *size:* l = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm);
- *temperature*: 30 °C.

Mobile phase Mix 30 volumes of *acetonitrile for chromatography R* and 70 volumes of a 4.85

g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 8.0 with *strong sodium hydroxide solution R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 3.5 times the retention time of lidocaine.

Relative retention with reference to lidocaine (retention time = about 17 min): impurity H = about 0.37; impurity A = about 0.40.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to impurities H and A.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.01 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.10 per cent);

— *total*: not more than 5 times the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 5 ppm.

Dissolve 1.0 g in *water R* and dilute to 25 ml with the same solvent. Carry out the prefiltration. 10 ml of the prefiltrate complies with test E. Prepare the reference solution using 2 ml of *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

5.5 per cent to 7.0 per cent, determined on 0.25 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.220 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 27.08 mg of $C_{14}H_{23}ClN_2O$.

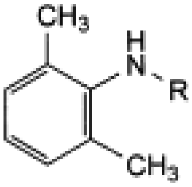
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10*. *Control of impurities in substances for pharmaceutical use)*: B, C, D, E, F, G, H, I, J, K.



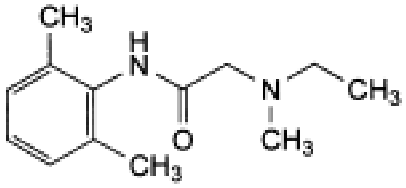
A. R = H: 2,6-dimethylaniline,

C. R = CO-CH_3: *N*-(2,6-dimethylphenyl)acetamide,

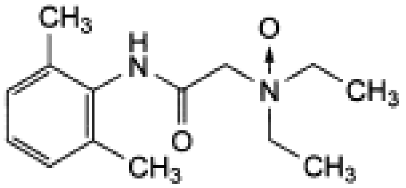
D. R = CO-CH_2-NH-C_2H_5: *N*-(2,6-dimethylphenyl)-2-(ethylamino)acetamide,

G. R = CO-CH_2-NH-CH$(CH_3)_2$: *N*-(2,6-dimethylphenyl)-2-[(1-methylethyl)amino]acetamide,

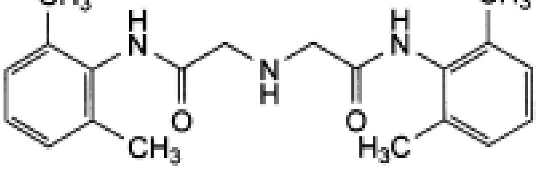
H. R = CO-CH_2-Cl: 2-chloro-*N*-(2,6-dimethylphenyl)acetamide,



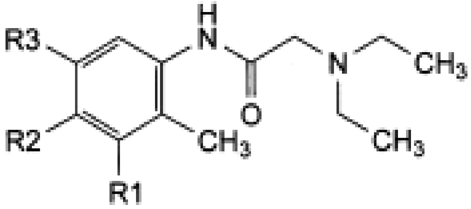
K. R = CO-CH_2-N$(CH_3)C_2H_5$: *N*-(2,6-dimethylphenyl)-2-(ethylmethylamino)acetamide,



B. 2-(diethylazinoyl)-*N*-(2,6-dimethylphenyl)acetamide (lidocaine N^2-oxide),



E. 2-2′-(azanediyl)bis[*N*-(2,6-dimethylphenyl)acetamide],


R3
R2
R1
H
N
O
N
CH3
CH3
CH3

F. R1 = CH_3, R2 = R3 = H: 2-(diethylamino)-*N*-(2,3-dimethylphenyl)acetamide,

I. R1 = R3 = H, R2 = CH_3: 2-(diethylamino)-*N*-(2,4-dimethylphenyl)acetamide,

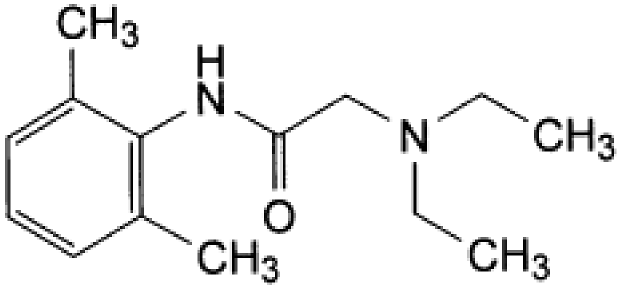
J. R1 = R2 = H, R3 = CH_3: 2-(diethylamino)-*N*-(2,5-dimethylphenyl)acetamide.

Ph Eur

Lidocaine

General Notices

(Ph Eur monograph 0727)



$C_{14}H_{22}N_2O$ 234.3 *137-58-6*

Action and use

Local anaesthetic; Class I antiarrhythmic.

Preparation

Lidocaine Ointment

Ph Eur

DEFINITION

2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification A.

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *lidocaine CRS.*

B. Melting point *(2.2.14)*: 66 °C to 70 °C, determined without previous drying.

C. To about 5 mg add 0.5 ml of *fuming nitric acid R*. Evaporate to dryness on a water-bath, cool, and dissolve the residue in 5 ml of *acetone R*. Add 0.2 ml of *alcoholic potassium hydroxide solution R*. A green colour develops.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *2,6-dimethylaniline R* (impurity A) in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *2-chloro-N-(2,6-dimethylphenyl)acetamide R* (impurity H) in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase.

Reference solution (d) Mix 1.0 ml of reference solution (a), 1.0 ml of reference solution (b) and 1.0 ml of reference solution (c), then dilute to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm);
- *temperature*: 30 °C.

Mobile phase Mix 30 volumes of *acetonitrile for chromatography R* and 70 volumes of a 4.85 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 8.0 with *strong sodium hydroxide solution R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 3.5 times the retention time of lidocaine.

Relative retention With reference to lidocaine (retention time = about 17 min): impurity H = about 0.37; impurity A = about 0.40.

System suitability Reference solution (d):

- *resolution*: minimum 1.5 between the peaks due to impurities H and A.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram

obtained with reference solution (d) (0.01 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.10 per cent);

— *total*: not more than 5 times the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.05 per cent).

Chlorides ***(2.4.4)***

Maximum 35 ppm.

Dissolve 1.4 g in a mixture of 3 ml of *dilute nitric acid R* and 12 ml of *water R.*

Sulphates ***(2.4.13)***

Maximum 0.1 per cent.

Dissolve 0.2 g in 5 ml of *ethanol (96 per cent) R* and dilute to 20 ml with *distilled water R.*

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.00 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

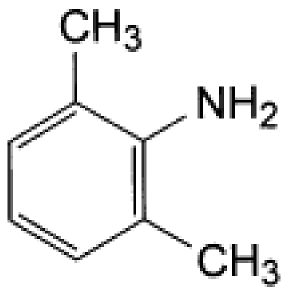
To 0.200 g add 50 ml of *anhydrous acetic acid R* and stir until dissolution is complete. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 23.43 mg of $C_{14}H_{22}N_2O$.

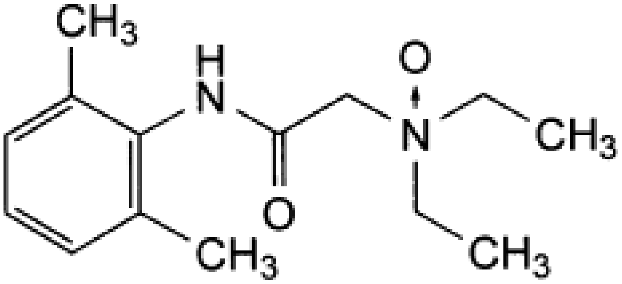
IMPURITIES

Specified impurities *A.*

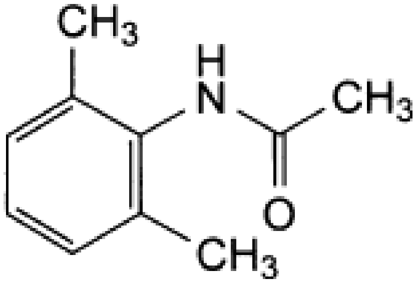
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E, F, G, H, I, J.*



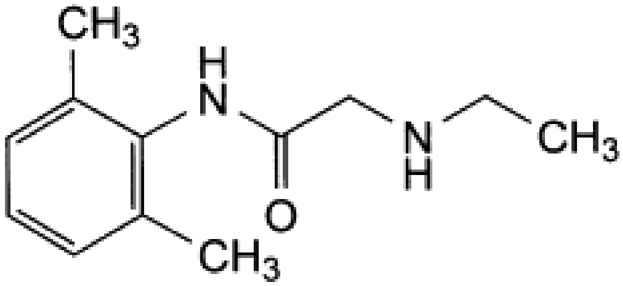
A. 2,6-dimethylaniline,


CH3
H
N
O
N
CH3
O
CH3
CH3

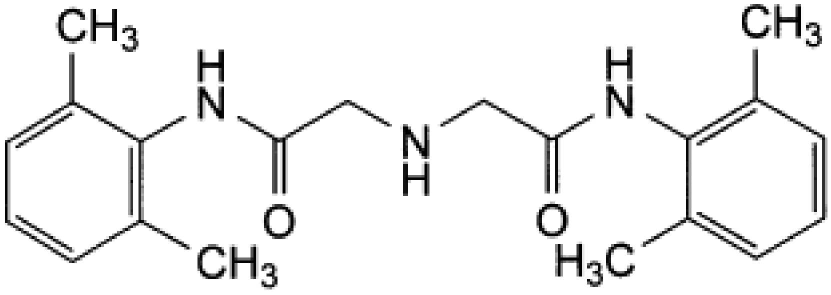
B. 2-(diethylazinoyl)-*N*-(2,6-dimethylphenyl)acetamide (lidocaine *N*-oxide),


CH3
H
N
CH3
O
CH3

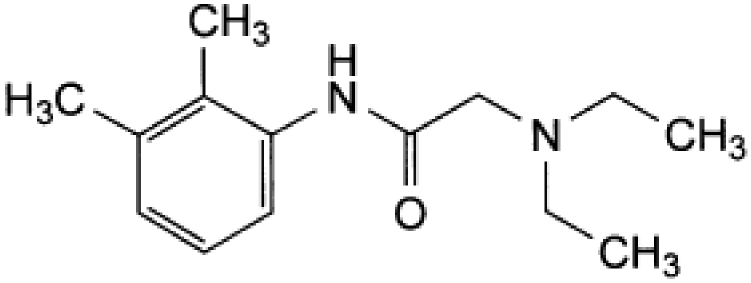
C. *N*-(2,6-dimethylphenyl)acetamide,


CH3
H
N
N
H
CH3
O
CH3

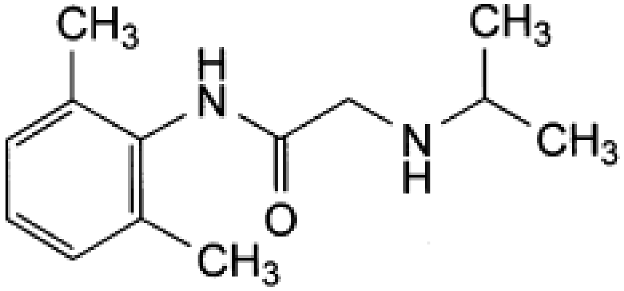
D. *N*-(2,6-dimethylphenyl)-2-(ethylamino)acetamide,


CH3
H
N
N
H
H
N
CH3
O
O
CH3
H3C

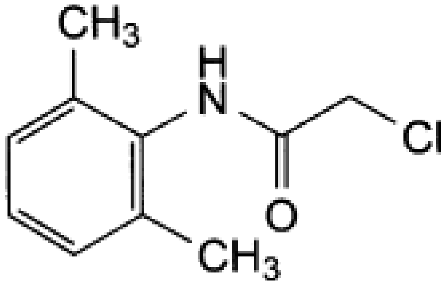
E. 2,2′-iminobis(*N*-(2,6-dimethylphenyl)acetamide),



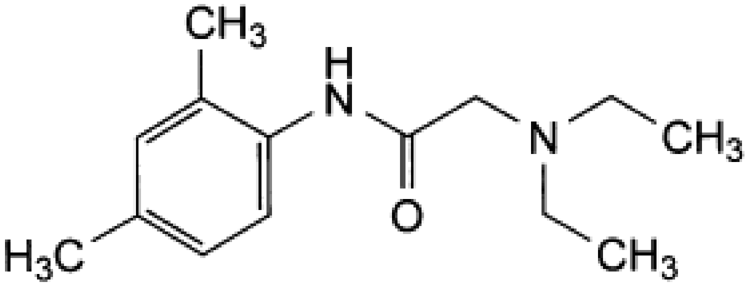
F. 2-(diethylamino)-*N*-(2,3-dimethylphenyl)acetamide,



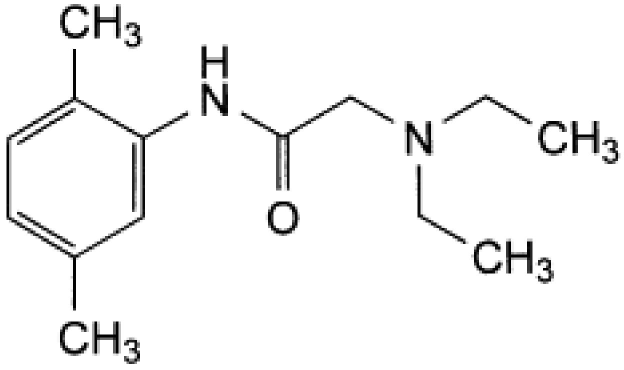
G. *N*-(2,6-dimethylphenyl)-2-((1-methylethyl)amino) acetamide,



H. 2-chloro-*N*-(2,6-dimethylphenyl)acetamide,



I. 2-(diethylamino)-*N*-(2,4-dimethylphenyl)acetamide,


CH3
H
N
N
CH3
O
CH3
CH3

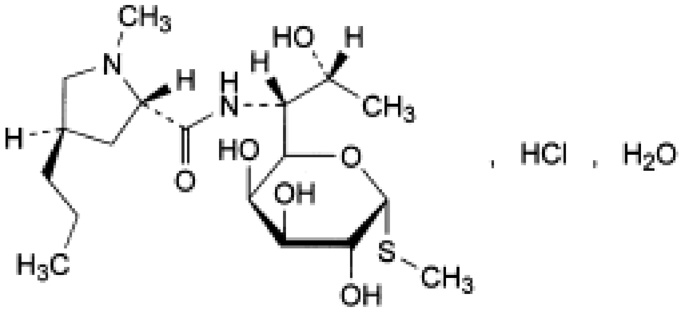
J. 2-(diethylamino)-*N*-(2,5-dimethylphenyl)acetamide.

Ph Eur

Lincomycin Hydrochloride

General Notices

(Ph Eur monograph 0583)



$C_{18}H_{34}N_2O_6S,HCl,H_2O$ 461.0 *7179-49-9*

Action and use

Antibacterial.

Preparations

Lincomycin Capsules

Lincomycin Injection

Ph Eur

DEFINITION

Lincomycin hydrochloride consists mainly of the methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside hydrochloride, an antimicrobial substance produced by *Streptomyces lincolnensis* var. *lincolnensis* or by any other means. It contains not less than 89.5 per cent and not more than 102.0 per cent of lincomycin hydrochloride ($C_{18}H_{35}ClN_2O_6S$), calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, very soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in acetone.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *lincomycin hydrochloride CRS*.

B. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *lincomycin hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *lincomycin hydrochloride CRS* and 10 mg of *clindamycin hydrochlo*ride *CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using the upper layer from a mixture of 20 volumes of *2-propanol R*, 40 volumes of a 150 g/l solution of *ammonium acetate R* previously adjusted to pH 9.6 with *ammonia R* and 45 volumes of *ethyl acetate R*. Allow the plate to dry in air and spray with a 1 g/l solution of *potassium permanganate R*. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve about 10 mg in 2 ml of *dilute hydrochloric acid R* and heat in a water-bath for 3 min. Add 3 ml of *sodium carbonate solution R* and 1 ml of a 20 g/l solution of *sodium nitroprusside R*. A violet-red colour develops.

D. Dissolve 0.1 g in *water R* and dilute to 10 ml with the same solvent. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3)*

The pH of solution S is 3.5 to 5.5.

Specific optical rotation (*2.2.7*)

Dissolve 1.000 g in *water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 135 to + 150, calculated with reference to the anhydrous substance.

Lincomycin B

Examine the chromatogram obtained in the assay with test solution (a). The area of the peak due to *lincomycin B*, which is eluted just before lincomycin, is not more than 5 per cent of the area of the peak due to lincomycin.

Heavy metals (*2.4.8*)

2.0 g complies with test C for heavy metals (5 ppm). Prepare the reference solution using 1.0 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*)

3.1 per cent to 4.6 per cent, determined on 0.500 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

Not more than 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.50 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Examine by gas chromatography (*2.2.28*), using *dotriacontane R* as the internal standard.

Internal standard solution Dissolve 0.200 g of *dotriacontane R* in *chloroform R* and dilute to 25.0 ml with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in a 20 g/l solution of *imidazole R* in *chloroform R* and dilute to 100.0 ml with the same solution. Shake until dissolution is complete. Place 4.0 ml of the solution in a ground-glass-stoppered 15 ml centrifuge tube. Add 1.0 ml of a mixture of 1 volume of *chlorotrimethylsilane R* and 99 volumes of *N,O-bis(trimethylsilyl)acetamide R* and swirl gently. Position the glass stopper loosely in the tube and heat at 65 °C for 30 min.

Test solution (b) Prepare as described for test solution (a) but add 10.0 ml of the internal standard solution before dissolution of the substance to be examined.

Reference solution Prepare as described for test solution (a) using 0.100 g of *lincomycin hydrochloride CRS* instead of the substance to be examined and adding 10.0 ml of the internal standard solution before dissolution of the reference substance.

The chromatographic procedure may be carried out using:

— a glass column 1.5 m long and 3 mm in internal diameter packed with *silanised diatomaceous earth for gas chromatography R* impregnated with 3 per cent *m/m* of *poly (methylphenylsiloxane) R*;

— *helium for chromatography R* as the carrier gas at a flow rate of about 45 ml/min;

— a flame-ionisation detector,

maintaining the temperature of the column at 260 °C and that of the injection port and of the detector between 260 °C and 290 °C. Inject the chosen volume of the test solutions and the reference solution.

STORAGE

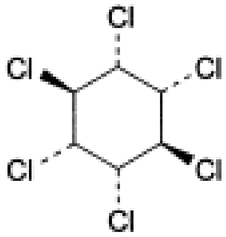
Store in an airtight container at a temperature not exceeding 30 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

Ph Eur

Lindane

General Notices

(Ph Eur monograph 0772)



$C_6H_6Cl_6$ 290.8 *58-89-9*

Action and use

Insecticide.

Ph Eur

DEFINITION

Lindane contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of *r*-1,*c*-2,*t*-3,*c*-4,*c*-5,*t*-6-hexachlorocyclohexane.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone, soluble in ethanol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 112 °C to 115 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *lindane CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in 4 ml of *alcohol R*. Add 1 ml of *0.5 M potassium hydroxide alcoholic*. Allow to stand for 10 min. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

Dissolve 0.50 g in *acetone R* and dilute to 10 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B7 (*2.2.2, Method II*).

Related substances Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 1.0 g of the substance to be examined in *chloroform R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *chloroform R*.

Reference solution (a) Dissolve 0.1 g of *lindane CRS* in *chloroform R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 10 ml with *chloroform R*.

Reference solution (c) Dissolve 10 mg of α*-hexachlorocyclohexane CRS* in test solution (a) and dilute to 5 ml with the same solvent.

Apply separately to the plate 1 µl of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *chloroform R* and 90 volumes of *cyclohexane R*. Dry the plate in a current of air, irradiate with ultraviolet light at 254 nm for 15 min and spray with a 6 g/l solution of *dicarboxidine hydrochloride R* in alcohol (90 per cent *V/V*) *R*. Examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (*2.4.4*)

To 0.75 g, finely powdered, add 15 ml of *water R*. Boil for 1 min. Allow to cool, shaking frequently, and filter. To 10 ml of the filtrate add 3 ml of *water R* and 2 ml of *alcohol R*. The solution complies with the limit test for chlorides (100 ppm).

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

To 0.200 g add 10 ml of *alcohol R* and warm on a water-bath until dissolved. Cool, add 20 ml of *0.5 M alcoholic potassium hydroxide* and allow to stand for 10 min, swirling frequently. Add 50 ml of *water R*, 20 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 5 ml of *ferric ammonium sulphate solution R2*. Titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 9.694 mg of $C_6H_6Cl_6$.

STORAGE

Store protected from light.

Ph Eur

Linoleoyl Macrogolglycerides

General Notices

(Ph Eur monograph 1232)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols.

They are obtained by partial alcoholysis of an unsaturated oil mainly containing triglycerides of linoleic (*cis*, *cis*-9,12-octadecadienoic) acid, using macrogol with a mean relative molecular mass between 300 and 400, or by esterification of glycerol and macrogol with unsaturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of this unsaturated oil.

CHARACTERS

Appearance

Amber, oily liquid which may give rise to a deposit after prolonged periods at 20 °C.

Solubility

Practically insoluble but dispersible in water, freely soluble in methylene chloride.

Viscosity

About 35 mPa·s at 40 °C.

Relative density

About 0.95 at 20 °C.

Refractive index

About 1.47 at 20 °C.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *hexane R*, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9 (R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.7), to 1,2-diglycerides (R_{st} 0.6), to monoglycerides (R_{st} 0.1) and to esters of macrogol (R_{st} 0).

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Fatty acid composition (see Tests).

TESTS

Acid value (*2.5.1*)

Maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)

45 to 65, determined on 1.0 g.

Iodine value (*2.5.4, Method A*)

90 to 110.

Peroxide value (*2.5.5, Method A*)

Maximum 12.0, determined on 2.0 g.

Saponification value (*2.5.6*)

150 to 170, determined on 2.0 g.

Alkaline impurities

Into a test tube introduce 5.0 g and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 ml of *water R* and 10 ml of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 ml of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol

Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 ml of *methylene chloride R*. Heat if necessary. After cooling, add 100 ml of *water R*. Shake and add 25.0 ml of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 ml of a 75 g/l solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 ml of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulphate*. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:

— *palmitic acid* : 4.0 per cent to 20.0 per cent;

— *stearic acid* : maximum 6.0 per cent;

— *oleic acid* : 20.0 per cent to 35.0 per cent;

— *linoleic acid* : 50.0 per cent to 65.0 per cent;

— *linolenic acid* : maximum 2.0 per cent;

— *arachidic acid*: maximum 1.0 per cent;

— *eicosenoic acid*: maximum 1.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.0 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash (*2.4.16*)

Maximum 0.1 per cent.

STORAGE

Protected from light.

LABELLING

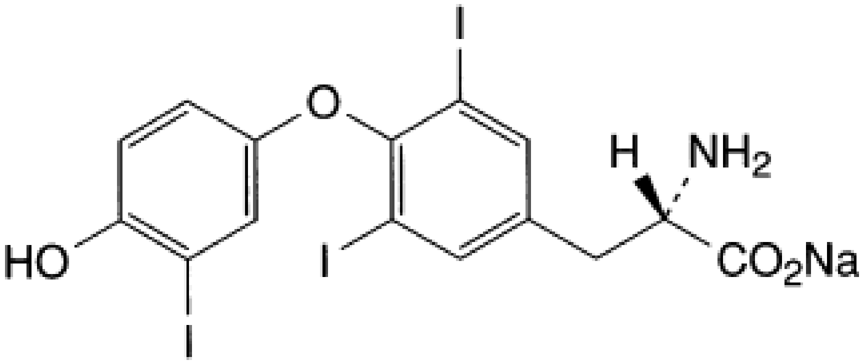
The label states the type of macrogol used (mean relative molecular mass) or the number of units of ethylene oxide per molecule (nominal value).

Ph Eur

Liothyronine Sodium

General Notices

(Ph Eur monograph 0728)



$C_{15}H_{11}I_3NNaO_4$ 673 *55-06-1*

Action and use

Thyroid hormone replacement.

Preparation

Liothyronine Tablets

Ph Eur

DEFINITION

Sodium (2*S*)-2-amino-3-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]propanoate.

Content

95.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly coloured powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A, C, E.

Second identification A, B, D, E.

A. Specific optical rotation (see Tests).

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 10.0 mg in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same solvent.

Spectral range 230-350 nm.

Absorption maximum At 319 nm.

Specific absorbance at the absorption maximum 63 to 69 (dried substance).

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison liothyronine sodium CRS.

D. To about 50 mg in a porcelain dish add a few drops of *sulphuric acid R* and heat. Violet vapour is evolved.

E. To 200 mg add 2 ml of *dilute sulphuric acid R*. Heat on a water-bath and then carefully over a naked flame, increasing the temperature gradually up to about 600 °C. Continue the ignition until most of the particles have disappeared. Dissolve the residue in 2 ml of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS

Specific optical rotation *(2.2.7)*

+ 18.0 to + 22.0 (dried substance).

Dissolve 0.200 g in a mixture of 1 volume of *1 M hydrochloric acid* and 4 volumes of *ethanol (96 per cent) R* and dilute to 20.0 ml with the same mixture of solvents.

Related substances

Liquid chromatography *(2.2.29)*. *Protect the solutions from light throughout the test.*

Solution A Mix 10 volumes of mobile phase A with 90 volumes of *methanol R.*

Solution B Mix 30 volumes of mobile phase B and 70 volumes of mobile phase A. Mix equal volumes of this solution with solution A.

Test solution Dissolve 20.0 mg of the substance to be examined in 20 ml of solution A. Dilute 4.0 ml of this solution to 20.0 ml with solution B.

Reference solution (a) Dissolve 2.5 mg of *levothyroxine sodium CRS* (impurity A) and 2.5 mg of *liothyronine sodium CRS* in solution A and dilute to 25 ml with the same solution. Dilute 1.0 ml of this solution to 50.0 ml with solution B.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with solution B.

Reference solution (c) Dissolve the contents of a vial of *liothyronine for peak identification CRS* (containing impurities A, B, C, D and E) in solution B and dilute to 1.0 ml with the same solution.

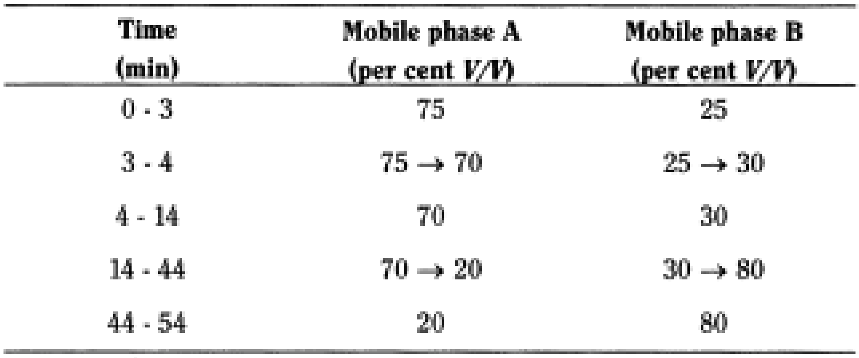
Reference solution (d) Dissolve 20.0 mg of *liothyronine sodium CRS* in 20 ml of solution A.

Dilute 4.0 ml of this solution to 20.0 ml with solution B.

Blank solution Solution B.

Column:
- *size*: *l* = 0.15 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: dissolve 9.7 g of *sulphamic acid R* in *water R* and dilute to 2000 ml with the same solvent; add 1.5 g of *sodium hydroxide R* and adjust to pH 2.0 with *2 M sodium hydroxide*;
- *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	75	25
3 - 4	75 → 70	25 → 30
4 - 14	70	30
14 - 44	70 → 20	30 → 80
44 - 54	20	80

Flow rate 1 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 25 µl of the test solution and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D and E.

- *relative retention* with reference to liothyronine (retention time = about 14 min): impurity B = about 0.2; impurity E = about 0.5; impurity A = about 1.4; impurity C = about 2; impurity D = about 2.4.

System suitability:
- *resolution*: minimum 5.0 between the peaks due to impurity A and liothyronine in the chromatogram obtained with reference solution (a).

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *impurity E*: not more than 5 times the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurities B, C*: for each impurity, not more than 3 times the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *impurity D*: not more than twice the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the peak due to liothyronine in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *disregard limit*: 0.5 times the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides

Maximum 2.0 per cent, expressed as NaCl (dried substance).

Dissolve 0.500 g in a 2 g/l solution of *sodium hydroxide R* and dilute to 100 ml with the same solvent. Add 15 ml of *dilute nitric acid R* and titrate with *0.05 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.05 M silver nitrate* is equivalent to 2.93 mg of NaCl.

Loss on drying *(2.2.32)*

Maximum 4.0 per cent, determined on 0.500 g by drying *in vacuo* at 60 °C for 4 h.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (d).

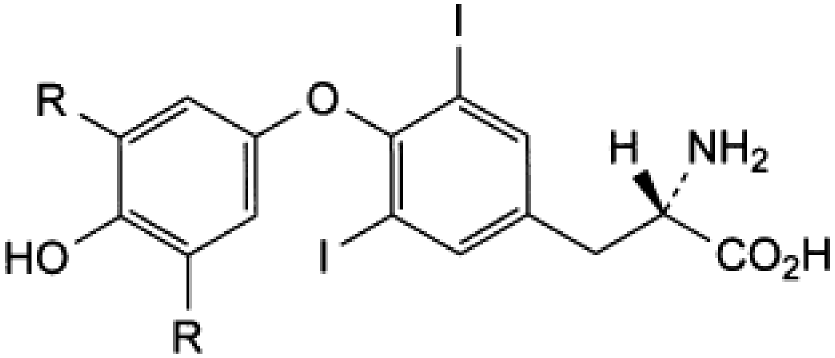
Calculate the percentage content of $C_{15}H_{11}I_3NNaO_4$ from the declared content of *liothyronine sodium CRS.*

STORAGE

In an airtight container , protected from light, at a temperature between 2 °C and 8 °C.

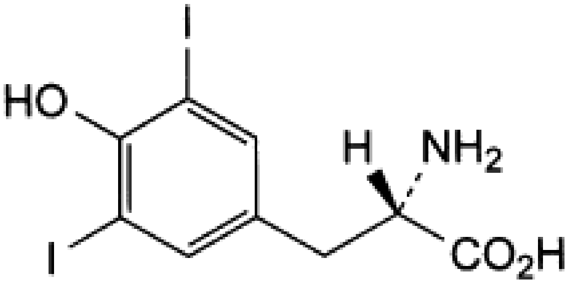
IMPURITIES

Specified impurities *A, B, C, D, E.*

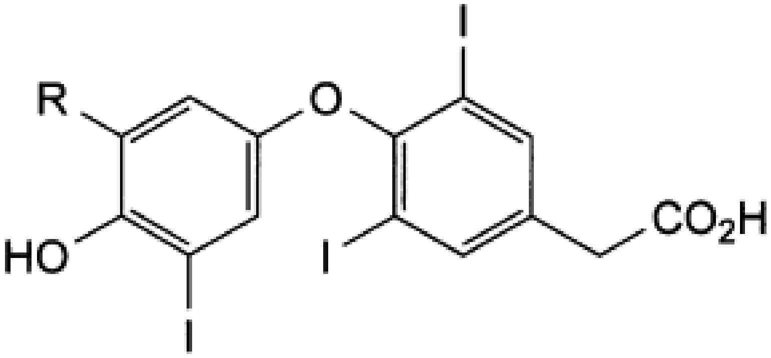


A. R = I: levothyroxine,

E. R = H: (2*S*)-2-amino-3-[4-(4-hydroxyphenoxy)-3,5-diiodophenyl]propanoic acid (diiodothyronine).



B. (2*S*)-2-amino-3-(4-hydroxy-3,5-diiodophenyl)propanoic acid (diiodotyrosine),



C. R = H: [4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]acetic acid (triiodothyroacetic acid) ,

D. R = I: [4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid (tetraiodothyroacetic acid),

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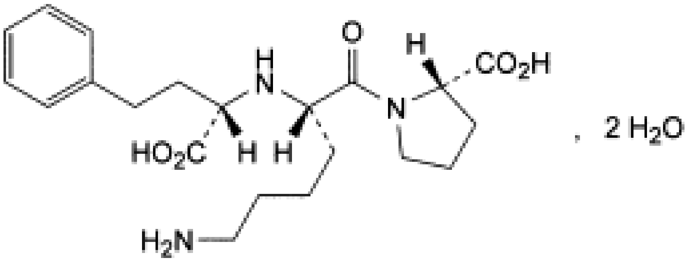
Ph Eur

Lisinopril Dihydrate

General Notices

(Ph Eur monograph 1120)



$C_{21}H_{31}N_3O_5,2H_2O$ 441.5 *83915-83-7*

Action and use

Angiotensin converting enzyme inhibitor.

Preparation

Lisinopril Tablets

Ph Eur

DEFINITION

(2*S*)-1-[(2*S*)-6-Amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, sparingly soluble in methanol, practically insoluble in acetone and in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *lisinopril dihydrate CRS.*

TESTS

Specific optical rotation (*2.2.7*)

- 43 to - 47 (anhydrous substance).

Dissolve 0.5 g in *zinc acetate solution R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a) Dissolve the contents of 1 vial of *lisinopril dihydrate for performance test CRS* with 1.0 ml of mobile phase A.

Reference solution (b) Dilute 0.5 ml of the test solution to 50.0 ml with mobile phase A.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

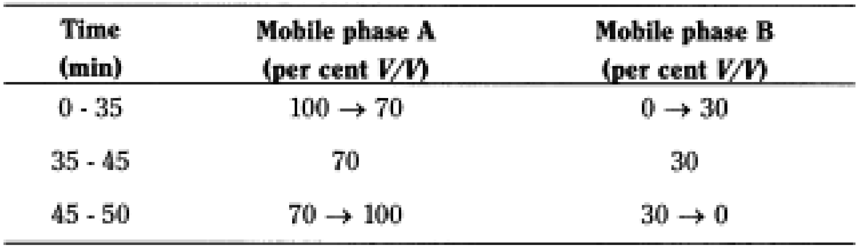
— *stationary phase*: *octylsilyl silica gel for chromatography R*;

— *temperature*: 50 °C.

Mobile phase:

— *mobile phase A*: mix 30 volumes of *acetonitrile R* and 970 volumes of a 3.12 g/l *sodium dihydrogen phosphate R* solution adjusted to pH 5.0 with a 50 g/l solution of *sodium hydroxide R*;

— *mobile phase B*: mix 200 volumes of *acetonitrile R* and 800 volumes of a 3.12 g/l *sodium dihydrogen phosphate R* solution adjusted to pH 5.0 with a 50 g/l solution of *sodium hydroxide R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100 → 70	0 → 30
35 - 45	70	30
45 - 50	70 → 100	30 → 0

Flow rate 1.8 ml/min.

Detection Spectrophotometer at 210 nm.

Equilibration With mobile phase A for at least 30 min.

Injection 20 µl.

System suitability Reference solution (a):

— the chromatogram obtained is similar to the chromatogram supplied with *lisinopril dihydrate for performance test CRS* in that the peaks due to impurity A and impurity E fall on either side of the peak due to lisinopril;

— *peak-to-valley ratio*: minimum 9, where H_r = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lisinopril, and minimum 9 where H_r = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lisinopril; if necessary, adjust the pH of the mobile phase to 4.5 with *phosphoric acid R*; a further adjustment to pH 4.0 may be necessary with some columns before satisfactory separation of impurity A, lisinopril and impurity E is obtained; if, after adjustment, the retention time of the peak due to impurities C and D becomes extended to the point where integration becomes difficult, increase the content of mobile phase B from 30 per cent to 40 per cent over the interval from 35-45 min from the start of the chromatogram; maintain this concentration for a further 10 min and return the concentration of mobile phase A to 100 per cent over the next 10 min prior to the next injection.

Limits:

— *impurities A, B, C, D, E, F*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *sum of impurities other than E*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak occurring in the first 3 min.

Water (*2.5.12*)

8.0 per cent to 9.5 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

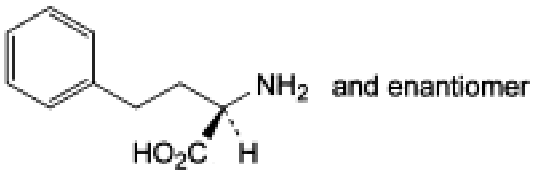
ASSAY

Dissolve 0.350 g in 50 ml of *distilled water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

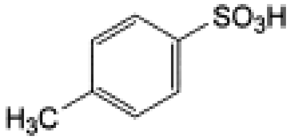
1 ml of *0.1 M sodium hydroxide* is equivalent to 40.55 mg of $C_{21}H_{31}N_3O_5$.

IMPURITIES

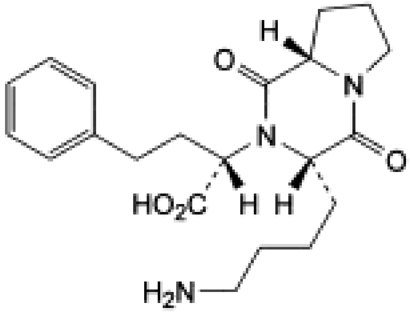
Specified impurities A, B, C, D, E, F.



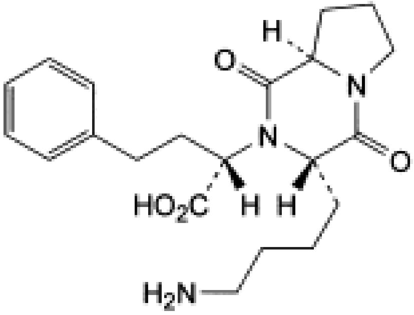
A. (2*RS*)-2-amino-4-phenylbutanoic acid,



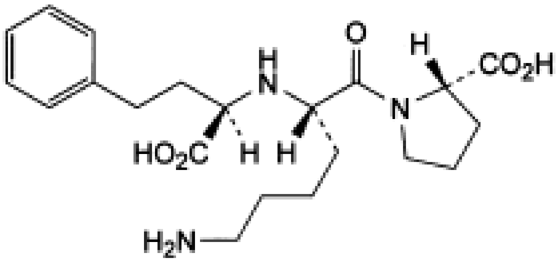
B. 4-methylbenzenesulphonic acid,



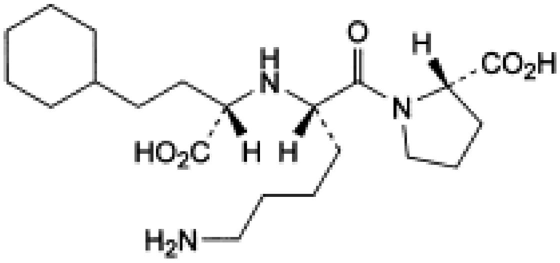
C. (2*S*)-2-[(3*S*,8aS)-3-(4-aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid (*S*,*S*,*S*-diketopiperazine),



D. (2*S*)-2-[(3*S*,8a*R*)-3-(4-aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid (*R*,*S*,*S*-diketopiperazine),



E. (2*S*)-1-[(2*S*)-6-amino-2-[[(1*R*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid (lisinopril *R*,*S*,*S*-isomer),



F. (2*S*)-1-[(2*S*)-6-amino-2-[[(1*S*)-1-carboxy-3-cyclohexylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid (cyclohexyl analogue).

Ph Eur

Lithium Carbonate

General Notices

(Ph Eur monograph 0228)

Li_2CO_3 73.9 *554-13-2*

Action and use

Prophylaxis of affective disorders.

Preparations

Lithium Carbonate Tablets

Prolonged-release Lithium Carbonate Tablets

Ph Eur

DEFINITION

Content: 98.5 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. When moistened with *hydrochloric acid R*, it gives a red colour to a non-luminous flame.

B. Dissolve 0.2 g in 1 ml of *hydrochloric acid R*. Evaporate to dryness on a water-bath. The residue dissolves in 3 ml of *ethanol (96 per cent) R*.

C. It gives the reaction of carbonates (*2.3.1*).

TESTS

Solution S

Suspend 10.0 g in 30 ml of *distilled water R* and dissolve by the addition of 22 ml of *nitric acid R*. Add *dilute sodium hydroxide solution R* until the solution is neutral and dilute to 100 ml with *distilled water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 200 ppm.

Disperse 1.25 g in 5 ml of *distilled water R* and dissolve by adding 5 ml of *hydrochloric acid R1*. Boil for 2 min. Cool and add *dilute sodium hydroxide solution R* until neutral. Dilute to 25 ml with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 0.5 g.

Calcium (*2.4.3*)

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Magnesium (*2.4.6*)

Maximum 150 ppm.

Dilute 1 ml of solution S to 10 ml with *water R*. Dilute 6.7 ml of this solution to 10 ml with *water R.*

Potassium

Maximum 3.00 × 102 ppm.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.0 g in 10 ml of *hydrochloric acid R1* and dilute to 50.0 ml with *water R*.

Reference solutions Prepare the reference solutions using a solution of *potassium chloride R* containing 500 µg of K per millilitre, diluted as necessary with *water R*.

Wavelength 766.5 nm.

Sodium

Maximum 3.00 × 10^2 ppm.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.0 g in 10 ml of *hydrochloric acid R1* and dilute to 50.0 ml with *water R*.

Reference solutions Prepare the reference solutions using a solution of *sodium chloride R* containing 500 µg of Na per millilitre, diluted as necessary with *water R*.

Wavelength 589 nm.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

ASSAY

Dissolve 0.500 g in 25.0 ml of *1 M hydrochloric acid*. Titrate with *1 M sodium hydroxide*, using *methyl orange solution R* as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 36.95 mg of Li_2CO_3.

Ph Eur

Lithium Citrate

General Notices

(Ph Eur monograph 0621)

$C_6H_5Li_3O_7,4H_2O$ 282.0 *6080-58-6*

Action and use

Prophylaxis of affective disorders.

Preparation

Lithium Citrate Oral Solution

Ph Eur

DEFINITION

Trilithium 2-hydroxypropane-1,2,3-tricarboxylate tetrahydrate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, fine crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. When moistened with *hydrochloric acid R*, it gives a red colour to a non-luminous flame.

B. Dilute 3 ml of solution S (see Tests) to 10 ml with *water R*. Add 3 ml of *potassium ferriperiodate solution R*. A white or yellowish-white precipitate is formed.

C. To 1 ml of solution S add 4 ml of *water R*. The solution gives the reaction of citrates (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Readily carbonisable substances

To 0.20 g of the powdered substance to be examined add 10 ml of *sulphuric acid R* and heat in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly. The solution is not more intensely coloured than reference solution Y_2 or GY_2 (*2.2.2, Method II*).

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Oxalates

Maximum 300 ppm, calculated as anhydrous oxalate ion.

Dissolve 0.50 g in 4 ml of *water R*, add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules and heat on a water-bath for 1 min. Allow to stand for 2 min, decant the liquid into a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 4 ml of a 0.05 g/l solution of *oxalic acid R*.

Sulphates (*2.4.13*)

Maximum 500 ppm.

To 3 ml of solution S add 2 ml of *hydrochloric acid R1* and dilute to 17 ml with *distilled water R*. Prepare the reference solution using 15 ml of a mixture of 2 ml of *hydrochloric acid R1* and 15 ml of *sulphate standard solution (10 ppm* SO_4*) R* and compare the opalescence after 15 min.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*)

24.0 per cent to 27.0 per cent, determined on 0.100 g. After adding the substance to be examined, stir for 15 min before titrating. Carry out a blank titration.

ASSAY

Dissolve 80.0 mg in 50 ml of *anhydrous acetic acid R*, heating to about 50 °C. Allow to cool. Titrate with *0.1 M perchloric acid*, using 0.25 ml of *naphtholbenzein solution R* as indicator, until the colour changes from yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 7.00 mg of $C_6H_5Li_3O_7$.

STORAGE

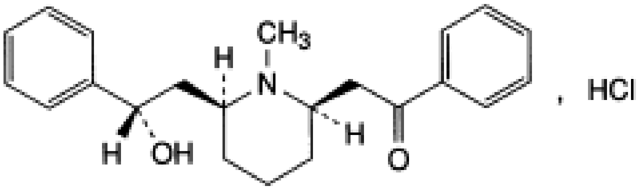
In an airtight container .

Ph Eur

Lobeline Hydrochloride

General Notices

(Ph Eur monograph 1988)



$C_{22}H_{27}NO_2,HCl$ 373.9 *134-63-4*

Action and use

Respiratory stimulant.

Ph Eur

DEFINITION

2-[(2*R*,6*S*)-6-[(2*S*)-2-Hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, microcrystalline powder.

Solubility

Sparingly soluble in water, freely soluble in alcohol, soluble in methylene chloride.

IDENTIFICATION

First identification A, B.

Second identification B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison lobeline hydrochloride CRS.

B. Solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

C. Examine the chromatograms obtained in the test for foreign alkaloids.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

TESTS

Solution S

Dissolve 0.250 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

4.6 to 6.4 for solution S.

Specific optical rotation (*2.2.7*)

- 55 to - 59 (dried substance), determined on solution S.

Foreign alkaloids

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R.*

Reference solution (a) Dilute 0.1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (b) Dissolve 10 mg of *lobeline hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *diethylamine R*, *cyclohexane R* (10:90 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying At 120 °C.

Detection Examine in ultraviolet light at 254 nm.

Limits In the chromatogram obtained with test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and

dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *phenytoin CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. To 1 ml of the solution add 0.1 ml of the test solution and dilute to 25 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.0 g of *sodium methanesulphonate R* and 2.50 g of *disodium hydrogen phosphate dihydrate R* in a mixture of 3 volumes of a 6.7 per cent *V/V* solution of *phosphoric acid R*, 29 volumes of *acetonitrile R* and 70 volumes of *water R* and dilute to 1000 ml with the same mixture of solvents.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Run time 2 times the retention time of lobeline which is about 17 min.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to phenytoin and to lobeline.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: maximum of 2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard level*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphates (*2.4.13*)

Maximum 0.1 per cent.

15 ml of solution S complies with the limit test for sulphates.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g in vacuo.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

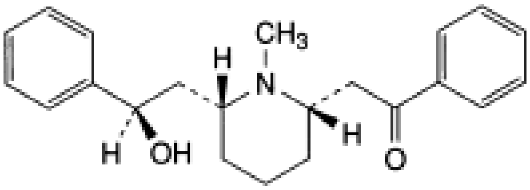
Dissolve 0.300 g in 50 ml of *alcohol R*. Add 5 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 37.39 mg of $C_{22}H_{28}ClNO_2$.

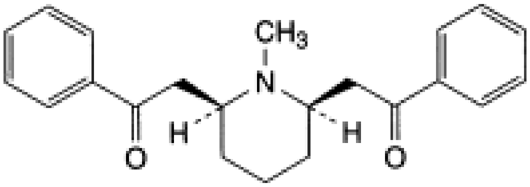
STORAGE

Protected from light.

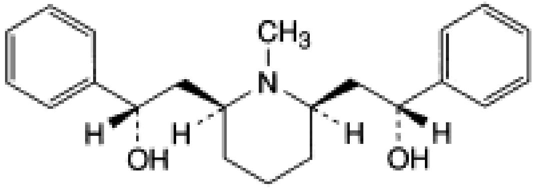
IMPURITIES



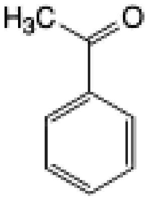
A. 2-[(2*S*,6*R*)-6-[(2*R*)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone ((+)-lobeline),



B. 2,2′-[(2*R*,6*S*)-1-methylpiperidine-2,6-diyl]bis(1-phenylethanone) (lobelanine),



C. *meso*-(1*R*,1′*S*)-2,2′-[(2*R*,6*S*)-1-methylpiperidine-2,6-diyl]bis(1-phenylethanol) (lobelanidine),

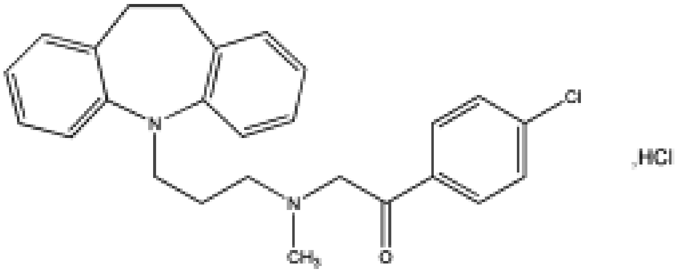


D. acetophenone.

Ph Eur

Lofepramine Hydrochloride

General Notices



$C_{26}H_{27}ClN_2O,HCl$ 455.4   *26786-32-3*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Lofepramine Tablets

DEFINITION

Lofepramine Hydrochloride is 5-(3-[*N*-(4-chlorophenacyl)-*N*-methylamino]propyl)10,11-dihydro-5*H*-dibenz[*b,f*]azepine hydrochloride. It contains not less than 98.5% and not more than 101.0% of $C_{26}H_{27}ClN_2O,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A fine, yellowish white to greenish yellow powder.

Very soluble in *ethanol (96%)* and in *methanol*; slightly soluble in *acetone*; very slightly soluble in *water*.

It exhibits polymorphism.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the reference spectrum of *lofepramine hydrochloride (form A) (RS 399A)*.

B. 2 ml of a 1% w/v solution in *ethanol (96%)* complies with the test for *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution (1) contains 0.1% w/v of the substance being examined in the mobile phase. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.0002% w/v each of *desipramine hydrochloride EPCRS* and *imipramine hydrochloride EPCRS* in the mobile phase.

Inject 20 µl of solutions (1) and (2). Inject 20 µl of solution (3) and allow the chromatography to continue for 4 times the retention time of the principal peak.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable) maintained at 50°, (b) as the mobile phase with a flow rate of 1.5 ml per minute a 0.09% w/v solution of *sodium dodecyl sulphate* in a mixture of 550 volumes of *acetonitrile*, 325 volumes of *water* and 125 volumes of a buffer solution of pH 1.0 containing 0.015% w/v of *glycine*, 0.018% w/v of *sodium chloride* and 0.44% w/v of *hydrochloric acid* and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 0.9.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried at a temperature of 100° at a pressure not exceeding 0.2 kPa, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of the substance being examined in the mobile phase. Solution (2) contains 0.02% w/v of *lofepramine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure described under the test for Related substances may be used.

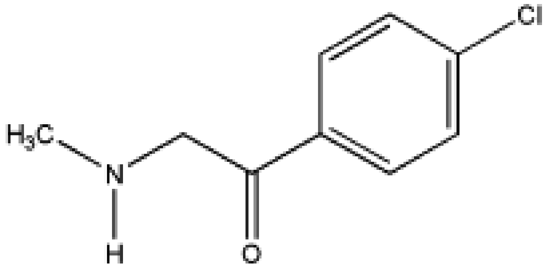
Inject 20 µl of each solution.

Calculate the content of $C_{26}H_{27}ClN_2O,HCl$ from the chromatograms obtained and using the declared content of $C_{26}H_{27}ClN_2O,HCl$ in *lofepramine hydrochloride BPCRS*.

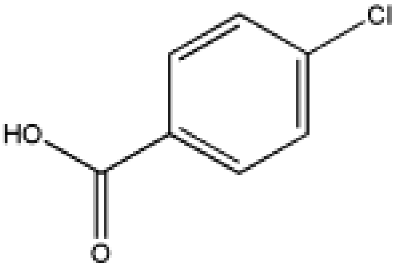
STORAGE

Lofepramine Hydrochloride should be kept in an airtight container and protected from light.

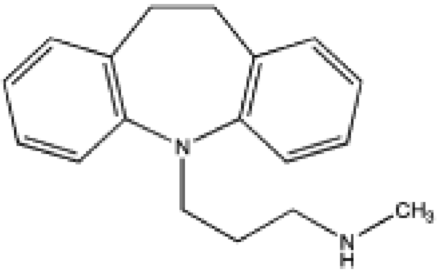
IMPURITIES



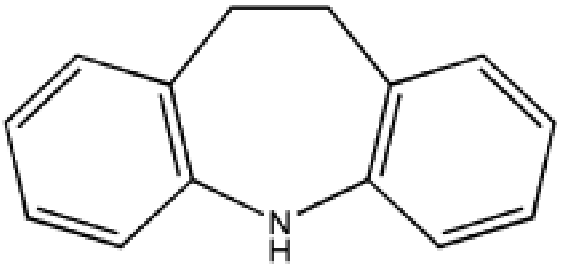
A. 1-methylamino-4′-chloroacetophenone,



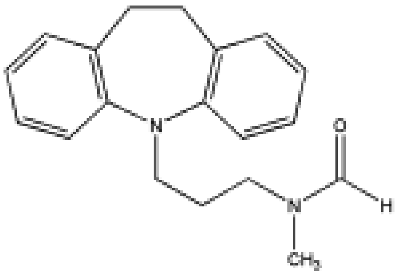
B. 4-chlorobenzoic acid,



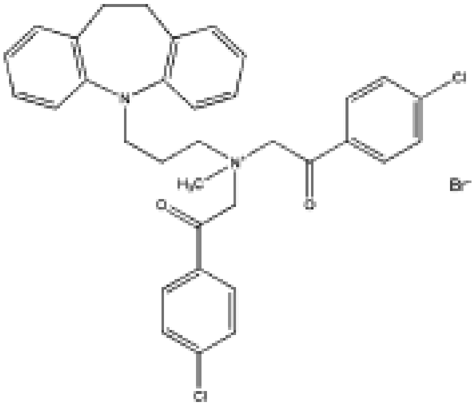
C. desipramine,



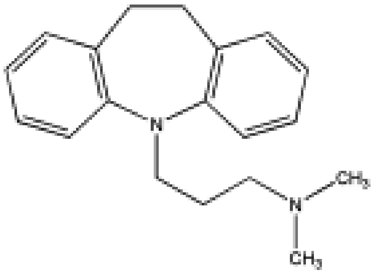
D. iminodibenzyl,



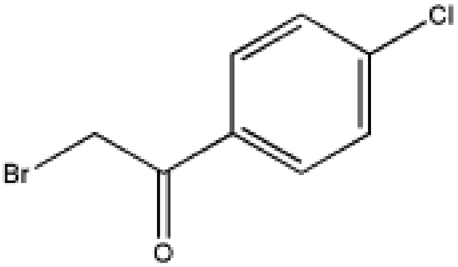
E. *N*-formyldesipramine,


Cl
N
H3C
N+
O
O
Br−
Cl

F. *N,N*-bis(4′-chlorophenacyl)desipramine bromide,



G. imipramine,

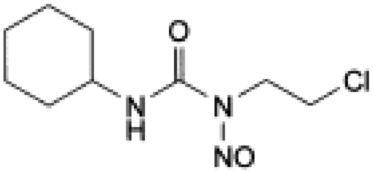


H. 2-bromo-4′-chloroacetophenone.

Lomustine

General Notices

(Ph Eur monograph 0928)



$C_9H_{16}ClN_3O_2$ 233.7 *13010-47-4*

Action and use

Cytotoxic alkylating agent.

Preparation

Lomustine Capsules

Ph Eur

DEFINITION

1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea.

Content

98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in methylene chloride, soluble in ethanol (96 per cent).

Carry out the tests protected from light and prepare all the solutions immediately before use.

IDENTIFICATION

First identification *C.*

Second identification *A, B, D, E.*

A. Melting point (*2.2.14*): 89 °C to 91 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*.

Spectral range 220-350 nm.

Absorption maximum At 230 nm.

Specific absorbance at the absorption maximum 250 to 270.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *lomustine CRS*.

D. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

E. Dissolve about 25 mg in 1 ml of *methanol R*, add 0.1 ml of *dilute sodium hydroxide solution R* and 2 ml of *water R.* Acidify by adding *dropwise dilute nitric acid R*. Filter. The filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances

A. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.25 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 25 ml with *methanol R*.

Reference solution (a) Dissolve 10 mg of *lomustine CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 10 ml with *methanol R*.

Reference solution (c) Dilute 1 ml of test solution (b) to 20 ml with *methanol R*.

Reference solution (d) Dissolve 10 mg of *lomustine CRS* and 10 mg of *dicyclohexylurea R* (impurity C) in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *glacial acetic acid R*, *toluene R* (20:80 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying At 110 °C for 1 h.

Chlorine treatment Place at the bottom of a chromatography tank an evaporating dish containing a mixture of 1 volume of *hydrochloric acid R1*, 1 volume of *water R* and 2 volumes of a 15 g/l solution of *potassium permanganate R*; close the tank and allow to stand for 15 min; place the dried plate in the tank and close the tank; leave the plate in contact with the chlorine vapour for 5 min; withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution R*.

Detection Spray with *potassium iodide and starch solution R*.

System suitability Reference solution (d):

— the chromatogram shows 2 clearly separated principal spots.

Limit Test solution (a):

— *impurities A, B, C*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.4 per cent) and at most one such spot is more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

B. Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.25 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*.

Column:
— *size:* l = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase *methanol R*, *water R* (50:50 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Limits:
— *total*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (1 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Chlorides (*2.4.4*)

Maximum 500 ppm.

Dissolve 0.24 g in 4 ml of *methanol R* and add 20 ml of *water R*. Allow to stand for 20 min and filter. To 10 ml of the filtrate, add 5 ml of *methanol R*. When preparing the standard, replace the 5 ml of *water R* with 5 ml of *methanol R*.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in a desiccator over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 24 h.

ASSAY

Dissolve 0.200 g in about 3 ml of *ethanol (96 per cent) R* and add 20 ml of a 200 g/l solution of *potassium hydroxide R* and boil under a reflux condenser for 2 h. Add 75 ml of *water R* and 4 ml of *nitric acid R*. Cool and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

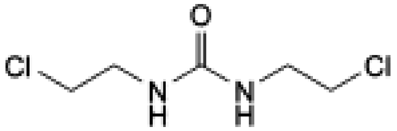
1 ml of *0.1 M silver nitrate* is equivalent to 23.37 mg of $C_9H_{16}ClN_3O_2$.

STORAGE

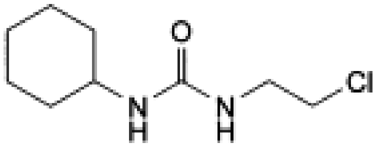
Protected from light.

IMPURITIES

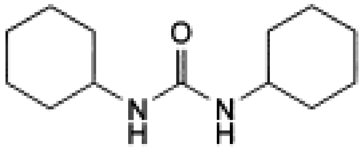
Specified impurities A, B, C.



A. 1,3-bis(2-chloroethyl)urea,



B. 1-(2-chloroethyl)-3-cyclohexylurea,



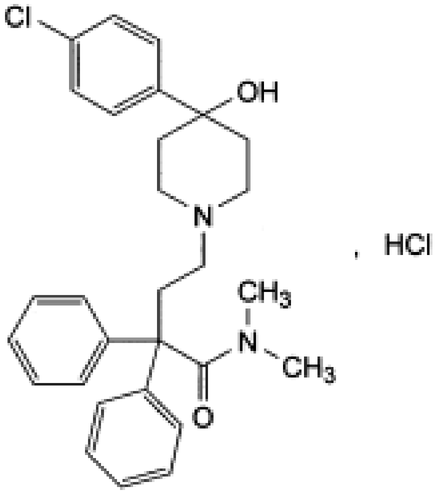
C. 1,3-dicyclohexylurea.

Ph Eur

Loperamide Hydrochloride

General Notices

(Ph Eur monograph 0929)



$C_{29}H_{33}ClN_2O_2,HCl$ 513.5 *34552-83-5*

Action and use

Inhibitor of mitochondriol function; cytotoxic.

Preparation

Loperamide Capsules

Ph Eur

DEFINITION

4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in alcohol and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *loperamide hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

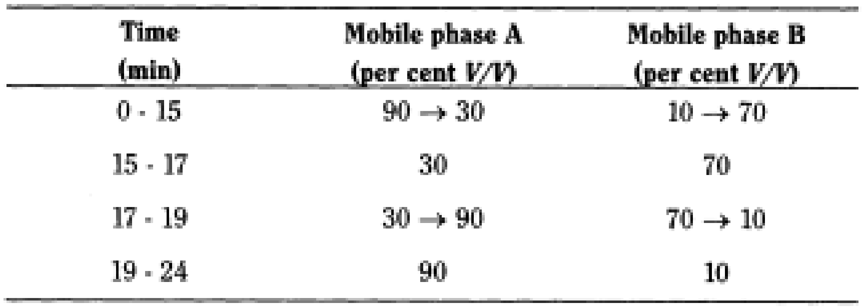
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *loperamide hydrochloride for system suitability CRS* in *methanol R* and dilute to 1.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 25.0 ml with *methanol R*.

Column:
- *size: l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: 17.0 g/l solution of *tetrabutylammonium hydrogen sulphate R1*;
- *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 30	10 → 70
15 - 17	30	70
17 - 19	30 → 90	70 → 10
19 - 24	90	10

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity H;

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A;

— the chromatogram obtained is concordant with the chromatogram supplied with *loperamide hydrochloride for system suitability CRS*.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.3; impurity D = 1.7;

— *impurities A, B, C, D, E, F, G, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

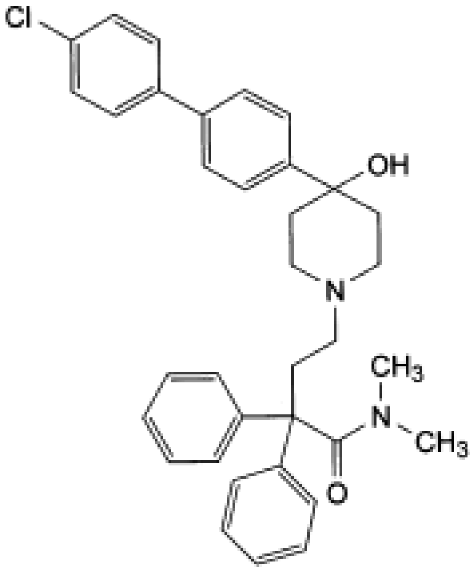
1 ml of *0.1 M sodium hydroxide* is equivalent to 51.35 mg of $C_{29}H_{34}Cl_2N_2O_2$.

STORAGE

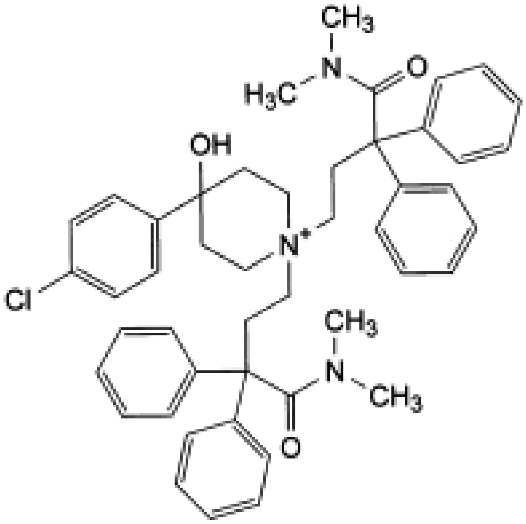
Protected from light.

IMPURITIES

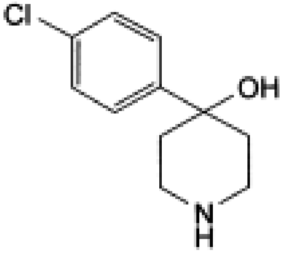
Specified impurities A, B, C, D, E, F, G, H.


Cl
OH
N
CH3
N
CH3
O

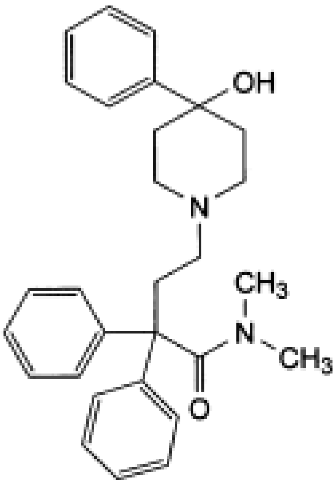
A. 4-[4-(4′-chlorobiphenyl-4-yl)-4-hydroxypiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide,


CH3
N
H3C
O
OH
N+
Cl
CH3
N
CH3
O

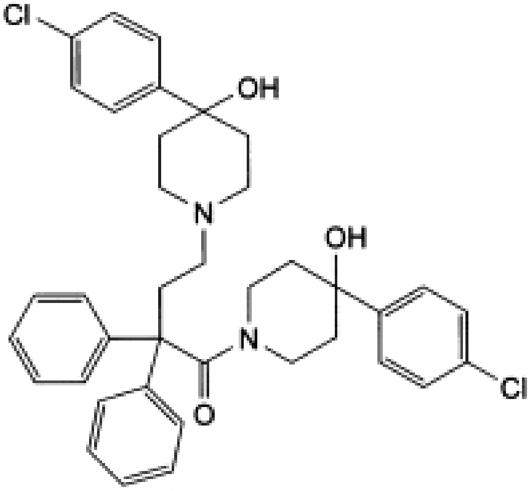
B. 4-(4-chlorophenyl)-1,1-bis[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidinium,



C. 4-(4-chlorophenyl)piperidin-4-ol,

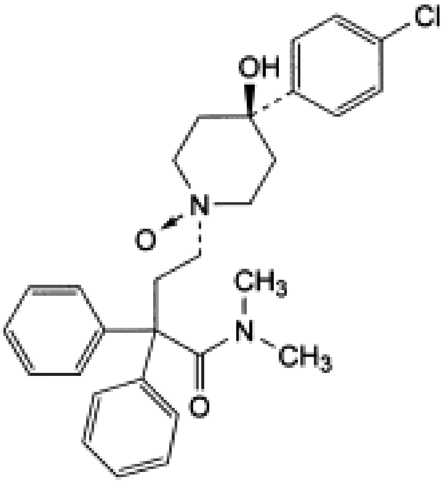


D. 4-(4-hydroxy-4-phenylpiperidin-1-yl)-*N*,*N*-dimethyl-2,2-diphenylbutanamide,

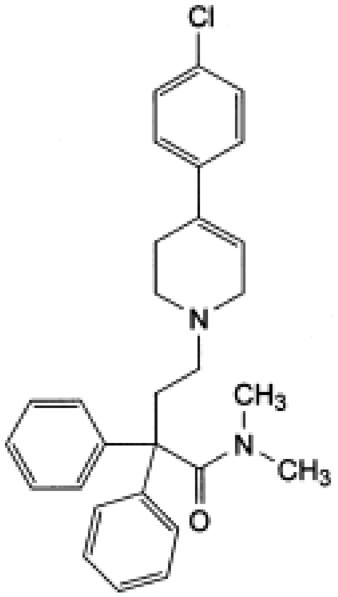


E. 4-(4-chlorophenyl)-1-[4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-2,2-diphenylbutanoyl] piperidin-4-ol,

F. loperamide oxide,



G. 4-[*cis*-4-(4-chlorophenyl)-4-hydroxy-1-oxidopiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide,



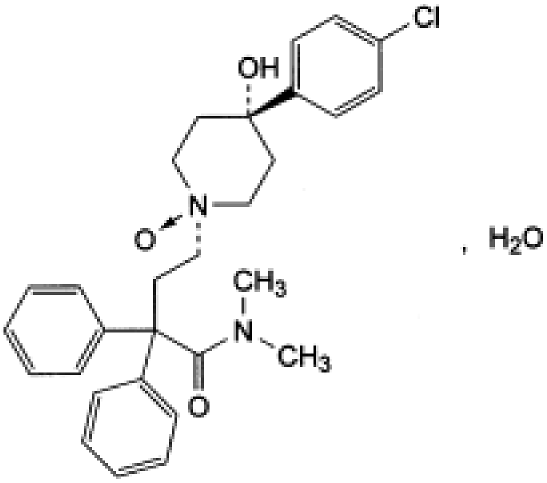
H. 4-[4-(4-chlorophenyl)-3,6-dihydropyridin-1(2*H*)-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide.

Ph Eur

Loperamide Oxide Monohydrate

General Notices

(Ph Eur monograph 1729)



$C_{29}H_{33}ClN_2O_3,H_2O$ 511.1   *106900-12-3*

Action and use

Opioid receptor agonist; antidiarrhoeal.

Ph Eur

DEFINITION

4-[*trans*-4-(4-Chlorophenyl)-4-hydroxy-1-oxidopiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, slightly hygroscopic.

Solubility

Practically insoluble in water, freely soluble in alcohol and in methylene chloride.

mp

About 152 °C, with decomposition.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison loperamide oxide monohydrate CRS.

TESTS

Related substances Liquid chromatography (*2.2.29*).

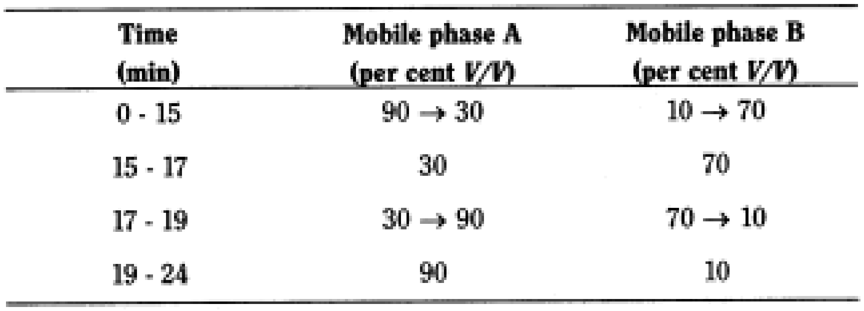
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *loperamide hydrochloride CRS* in *methanol R*, add 0.5 ml of the test solution and dilute to 100.0 ml with *methanol R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 25.0 ml with *methanol R*.

Column:
- — *size: l* = 0.10 m, Ø = 4.6 mm;
- — *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm);
- — *temperature*: 35 °C.

Mobile phase:
- — *mobile phase A*: 17.0 g/l solution of *tetrabutylammonium hydrogen sulphate R1*;
- — *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 30	10 → 70
15 - 17	30	70
17 - 19	30 → 90	70 → 10
19 - 24	90	10

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Relative retention With reference to loperamide oxide (retention time = about 7 min): impurity A = about 0.9; impurity B = about 1.11; impurity C = about 1.13.

System suitability Reference solution (a):

- — *resolution*: minimum 3.8 between the peaks due to loperamide oxide and impurity A.

Limits:
- — *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the

chromatogram obtained with reference solution (b) (0.2 per cent);

— *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

3.4 per cent to 4.2 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid* using 0.2 ml of *naphtholbenzein solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 49.30 mg of $C_{29}H_{33}ClN_2O_3$.

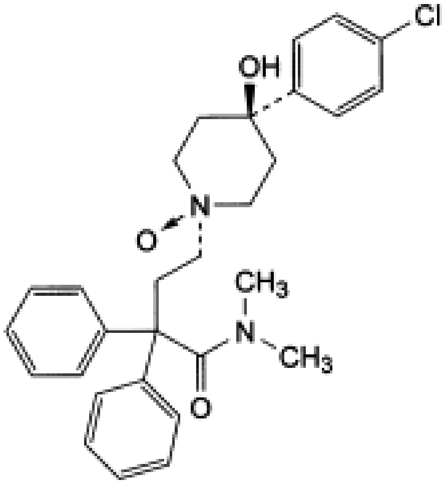
STORAGE

In an airtight container , protected from light.

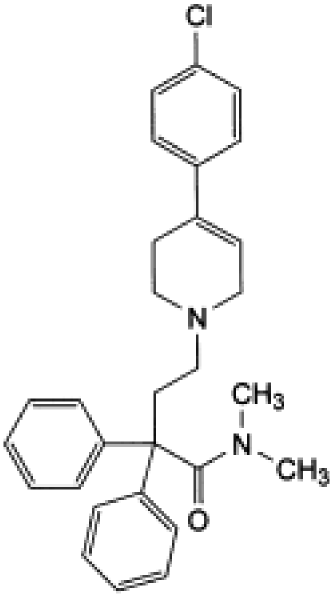
IMPURITIES

Specified impurities A, B, C.

A. loperamide,



B. 4-[*cis*-4-(4-chlorophenyl)-4-hydroxy-1-oxidopiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide,

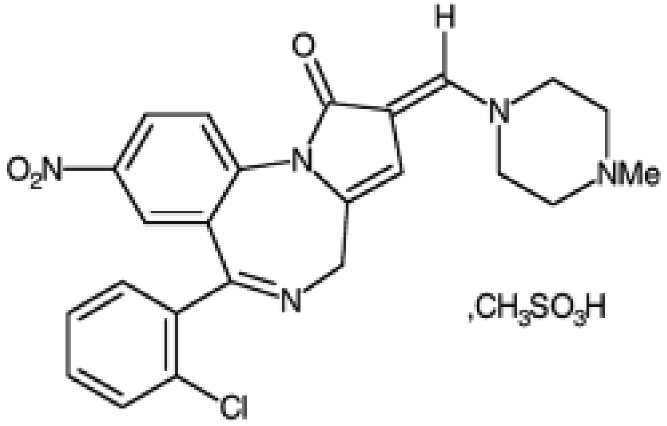

Cl
N
CH3
N
CH3
O

C. 4-[4-(4-chlorophenyl)-3,6-dihydropyridin-1(2*H*)-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide.

Ph Eur

Loprazolam Mesilate

General Notices



$C_{23}H_{21}ClN_6O_3,CH_4O_3S,H_2O$ 579.1 *70111-54-5*

Action and use

Benzodiazepine.

Preparation

Loprazolam Tablets

DEFINITION

Loprazolam Mesilate is (*Z*)-6-(2-chlorophenyl)-2,4-dihydro-2-(4-methylpiperazin-1-ylmethylene)-8-nitroimidazo[1,2-*a*][1,4]benzodiazepin-1-one methanesulphonate monohydrate. It contains not less than 98.5% and not more than 101.0% of $C_{23}H_{21}ClN_6O_3,CH_4O_3S$, calculated with reference to the dried substance.

CHARACTERISTICS

A yellow, crystalline powder.

Slightly soluble in *water* and in *ethanol (96%)*; very slightly soluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of loprazolam mesilate *(RS 205)*.

B. The *light absorption*, Appendix II B, in the range 210 to 370 nm of a 0.001% w/v solution in *ethanol (96%)* exhibits a maximum at 330 nm and a shoulder at 240 nm. The *absorbance* at the maximum is about 0.7.

TESTS

N-Methylpiperazine

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 80 volumes of *chloroform*, 20 volumes of *methanol* and 2 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in a mixture of equal volumes of *chloroform* and *methanol* containing (1) 2.0% w/v of the substance being examined and (2) 0.0050% w/v of N-*methylpiperazine*. After removal of the plate, allow it to dry in air, heat at 110° for 15 minutes and spray with a mixture of 50 volumes of a 2% w/v solution of *potassium iodide* and 1 volume of a 10% w/v solution of *chloroplatinic(IV) acid*. Any spot corresponding to *N*-methylpiperazine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 80 volumes of *chloroform* and 20 volumes of *methanol* as the mobile phase. Before use, stand the plate in *methanol*, allowing the solvent front to ascend 17 cm, allow to dry in air, heat the plate at 100° to 105° for 1 hour and use with the flow of mobile phase in the same direction as that used for the pretreatment. Apply separately to the plate 10 µl of each of three solutions in a mixture of 46 volumes of *chloroform*, 46 volumes of *methanol* and 8 volumes of *water* containing (1) 2.0% w/v of the substance being examined, (2) 0.0020% w/v of the substance being examined and (3) 0.010% w/v of *6-(2-chlorophenyl)-2,4-dihydro-2-[(dimethylamino)methylene]-8-nitroimidazo[1,2-*a*][1,4]benzodiazepin-1-one BPCRS* (dimethylamino analogue). After removal of the plate, allow it to dry in air, spray the plate with a 5% w/v solution of *titanium(III) chloride* in a solution of hydrochloric acid containing 10% w/v of HCl and then spray with a solution containing 0.4 g of *4-dimethyl-aminocinnamaldehyde* in a mixture of 20 ml of 6M *hydrochloric acid* and 100 ml of *ethanol (96%)*. Heat at 100° until spots appear (about 10 minutes). Any spot corresponding to the dimethylamino analogue in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%).

Loss on drying

When dried at 100° to 105° for 3 hours, loses 2.5 to 4.5% of its weight. Use 1 g.

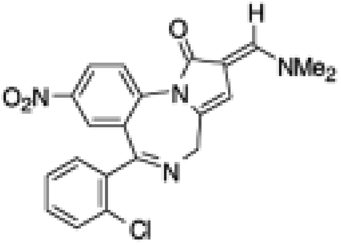
Sulphated ash

Not more than 0.1%, Appendix IX A.

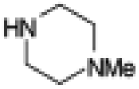
ASSAY

Dissolve 0.25 g in 60 ml of a 50% v/v solution of *propan-2-ol* and titrate with 0.05M *sodium hydroxide VS* determining the end point potentiometrically. Each ml of 0.05M *sodium hydroxide VS* is equivalent to 28.05 mg of $C_{23}H_{21}ClN_6O_3,CH_4O_3S$.

IMPURITIES



A. 6-(2-chlorophenyl)-2,4-dihydro-2-[(dimethylamino)methylene]-8-nitroimidazo[1,2-*a*][1,4] benzodiazepin-1-one,

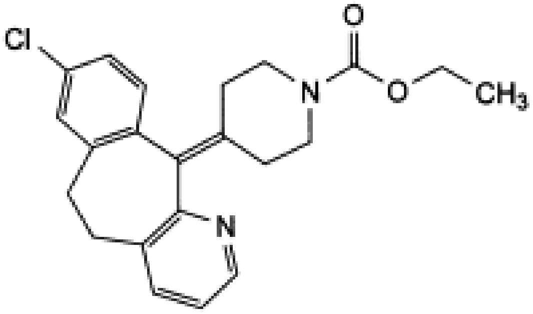


B. *N*-methylpiperazine.

Loratadine

General Notices

(Ph Eur monograph 2124)



$C_{22}H_{23}ClN_2O_2$ 382.9 *79794-75-5*

Action and use

Histamine H1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

Ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidine-1-carboxylate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison   *loratadine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Impurity H

Gas chromatography (*2.2.28*).

Internal standard solution   Dissolve 25 mg of *isoamyl benzoate R* in *methylene chloride R* and dilute to 100 ml with the same solvent. Dilute 5.0 ml of this solution to 50 ml with *methylene chloride R*.

Test solution   Dissolve 25.0 mg of the substance to be examined in *methylene chloride R*, add 1.0 ml of reference solution (a) and 1.0 ml of the internal standard solution and dilute to 5.0 ml with *methylene chloride R*.

Reference solution (a)   Dissolve 25.0 mg of *loratadine impurity H CRS* in *methylene chloride R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with *methylene chloride R*.

Reference solution (b)   To 1.0 ml of reference solution (a) add 1.0 ml of the internal standard solution and dilute to 5.0 ml with *methylene chloride R*.

Column:

— *material*: fused silica;

— *size: l* = 25 m, Ø = 0.32 mm;

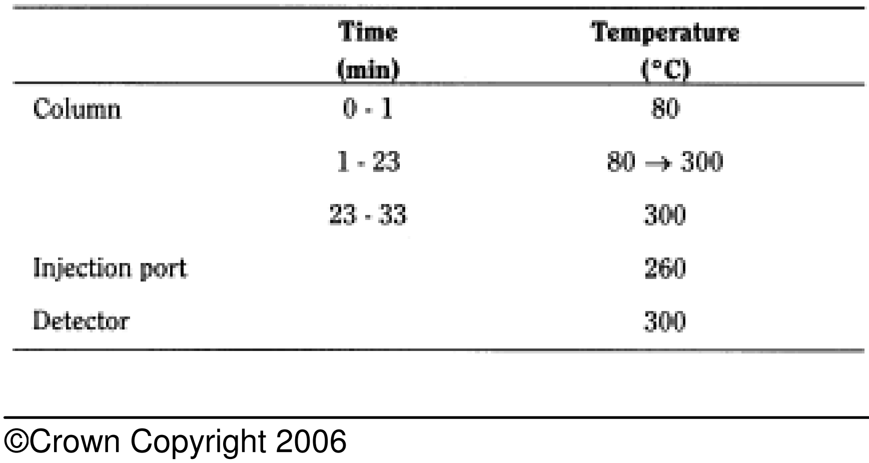
— *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 0.52 µm).

Carrier gas   *helium for chromatography R.*

Flow rate   1.0 ml/min.

Split ratio   1:30.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	80
	1 - 23	80 → 300
	23 - 33	300
Injection port		260
Detector		300

Detection Flame ionisation.

Injection 1 µl of the test solution and reference solution (b).

Relative retention With reference to loratadine (retention time = about 32 min): impurity H = about 0.33; isoamyl benzoate = about 0.37.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity H and isoamyl benzoate;

— *signal-to-noise ratio*: minimum 10 for the peak due to impurity H.

Limit:

— *impurity H*: calculate the ratio (*R*) of the area of the peak due to impurity H to the area of the peak due to isoamyl benzoate from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to impurity H to the area of the peak due to isoamyl benzoate: this ratio is not greater than twice *R* (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *loratadine impurity F CRS* in the mobile phase and dilute to 25 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *loratadine for system suitability CRS* (containing impurities A and E) in the mobile phase, add 0.5 ml of reference solution (a) and dilute to 5 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with very low silanol activity;

— *temperature*: 40 °C.

Mobile phase Mix 30 volumes of *methanol R*, 35 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.80 ± 0.05 with *phosphoric acid R* and 40 volumes of *acetonitrile R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time 5 times the retention time of loratadine.

Identification of impurities Use the chromatogram supplied with *loratadine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and E.

Relative retention With reference to loratadine (retention time = about 12 min): impurity D = about 0.2; impurity B = about 0.4; impurity F = about 0.9; impurity E = about 1.1; impurity A = about 2.4; impurity C = about 2.7.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to loratadine.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.7; impurity E = 3.4; impurity F = 1.6;

— *impurity F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulphates (*2.4.13*)

Maximum 150 ppm.

Ignite 1.33 g at 800 ± 25 °C and take up the residue with 20 ml of *distilled water R.* Filter, if necessary, through paper free from sulphates. Repeat the filtration with new paper filters until the filtrate is no longer turbid.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

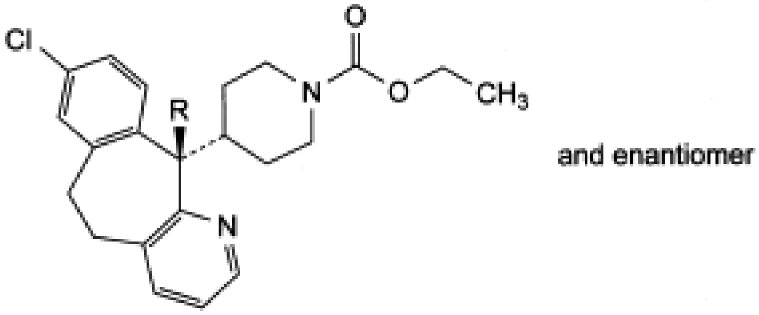
1 ml of *0.1 M perchloric acid* is equivalent to 38.29 mg of $C_{22}H_{23}ClN_2O_2$.

IMPURITIES

Specified impurities A, B, C, D, E, F, H.

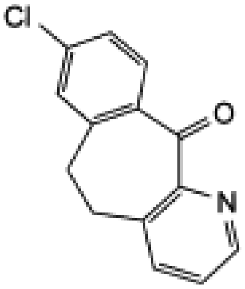
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general

acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10*. *Control of impurities in substances for pharmaceutical use)*: *G*.

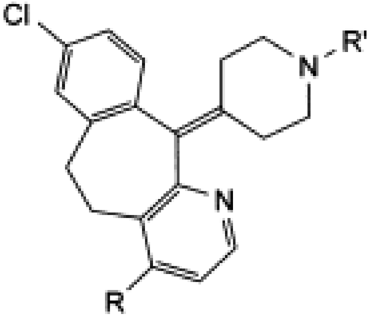


A. R = OH: ethyl 4-[(11*RS*)-8-chloro-11-hydroxy-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]piperidine-1-carboxylate,

F. R = F: ethyl 4-[(11*RS*)-8-chloro-11-fluoro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*] pyridin-11-yl]piperidine-1-carboxylate,



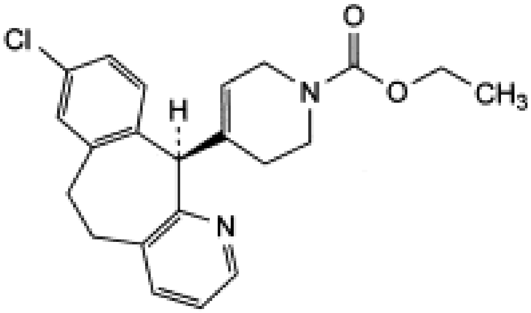
B. 8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-one,



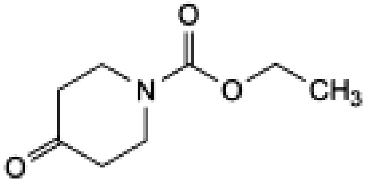
C. R = Cl, R′ = CO-OC_2H_5: ethyl 4-(4,8-dichloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidine-1-carboxylate,

D. R = R′ = H: 8-chloro-11-(piperidin-4-ylidene)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*] pyridine,

G. R = H, R′ = CH_3: 8-chloro-11-(1-methylpiperidin-4-ylidene)-6,11-dihydro-5*H*-benzo[5,6] cyclohepta[1,2-*b*]pyridine,



E. ethyl 4-[(11*RS*)-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]-3,6-dihydropyridine-1(2*H*)-carboxylate,



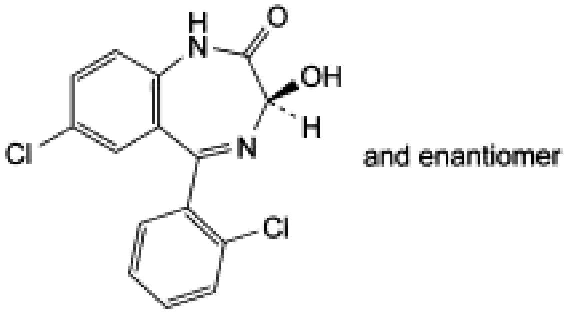
H. ethyl 4-oxopiperidine-1-carboxylate.

Ph Eur

Lorazepam

General Notices

(Ph Eur monograph 1121)



$C_{15}H_{10}Cl_2N_2O_2$ 321.2 *846-49-1*

Action and use

Benzodiazepine.

Preparations

Lorazepam Injection

Lorazepam Tablets

Ph Eur

DEFINITION

(3*RS*)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content

98.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in ethanol (96 per cent), sparingly soluble or slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Spectral range 600-2000 cm^{-1}.

Comparison *lorazepam CRS.*

TESTS

Related substances Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Test solution Dissolve 40.0 mg of the substance to be examined in 25 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (b) Dissolve the contents of a vial of *lorazepam for system suitability CRS* (containing impurities B and D) in 1.0 ml of a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (c) Dissolve 4.0 mg of *lorazepam impurity D CRS* in 25 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Column:

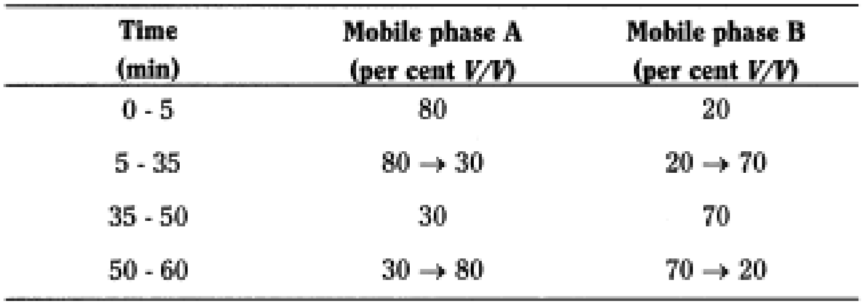
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) resistant to bases up to pH 11.5.

Mobile phase:

— *mobile phase A*: dissolve 3.48 g of *dipotassium hydrogen phosphate R* in a mixture of 50 ml of *acetonitrile R1* and 850 ml of *water R*; adjust the apparent pH to 10.5 with a 40 g/l solution of *sodium hydroxide R* and dilute to 1000 ml with *water R*;

— *mobile phase B*: *acetonitrile R1*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 35	80 → 30	20 → 70
35 - 50	30	70
50 - 60	30 → 80	70 → 20

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl.

Relative retention With reference to lorazepam (retention time = about 17 min): impurity D = about 0.9; impurity B = about 1.1.

System suitability Reference solution (b):

— *resolution*: minimum 4.5 between the peaks due to impurity D and lorazepam;

— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lorazepam.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g under high vacuum at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

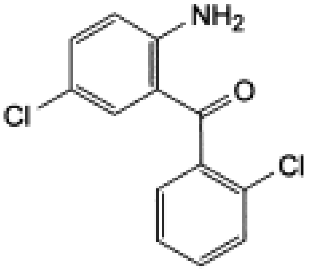
Dissolve 0.250 g in 30 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Protect the solution from atmospheric carbon dioxide throughout the titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 32.12 mg of $C_{15}H_{10}Cl_2N_2O_2$.

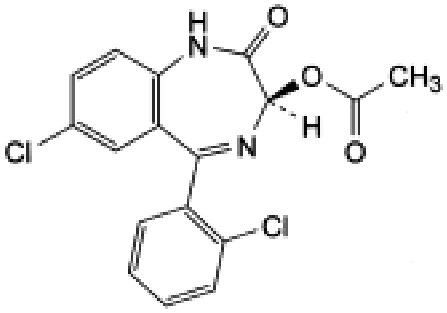
IMPURITIES

Specified impurities *B, D*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use)*: *A, C, E*.

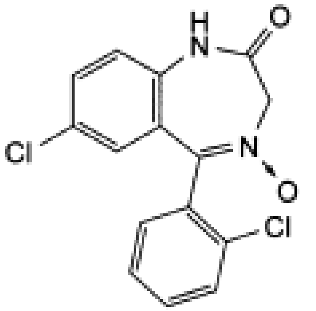


A. (2-amino-5-chlorophenyl)(2-chlorophenyl)methanone,

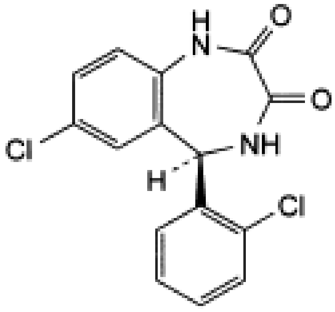


and enantiomer

B. (3*RS*)-7-chloro-5-(2-chlorophenyl)-2-oxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl acetate,

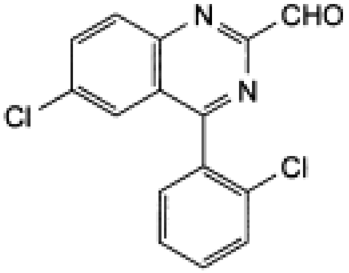


C. 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide,



and enantiomer

D. (5*RS*)-7-chloro-5-(2-chlorophenyl)-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione,

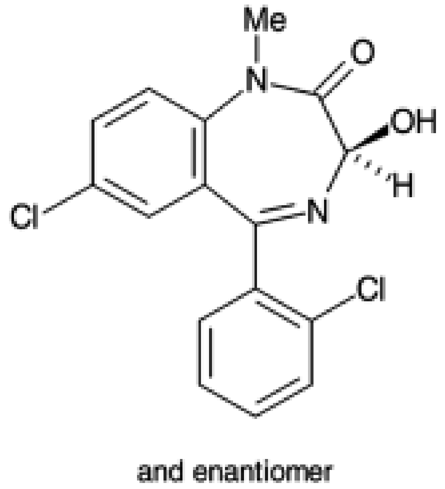


E. 6-chloro-4-(2-chlorophenyl)quinazoline-2-carbaldehyde.

Ph Eur

Lormetazepam

General Notices



$C_{16}H_{12}Cl_2N_2O_2$ 335.2 *848-75-9*

Action and use

Benzodiazepine.

Preparation

Lormetazepam Tablets

DEFINITION

Lormetazepam is (*RS*)-7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-1,4-benzodiazepin-2-one. It contains not less than 99.0% and not more than 101.0% of $C_{16}H_{12}Cl_2N_2O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Practically insoluble in *water*; soluble in *ethanol (96%)* and in *methanol*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lormetazepam *(RS 207)*.

B. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using five solutions in *methanol (70%)* containing (1) 0.25% w/v of the substance being examined, (2) 0.0005% w/v of the substance being examined, (3) 0.00025% w/v of the substance being examined, (4) 0.0005% w/v of *lormetazepam BPCRS* and (5) 0.00025% w/v each of *lormetazepam BPCRS* and *lorazepam BPCRS*.

The chromatographic procedure may be carried out using (a) a column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 48 volumes of *methanol* and 52 volumes of a phosphate buffer prepared by dissolving 4.91 g of *sodium dihydrogen orthophosphate* and 0.633 g of *disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 ml and (c) a detection wavelength of 230 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (5) is at least 4.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than that of the principal peak in the chromatogram obtained with solution (2) (0.2%) and not more than two such peaks have an area greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). The sum of the areas of all such peaks is not greater than 2.5 times the area of the principal peak obtained with solution (2) (0.5%).

Loss on drying

When dried to constant weight at 105° for 3 hours, loses not more 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

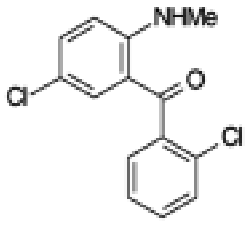
ASSAY

Dissolve 0.5 g in 50 ml of *nitroethane* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 33.52 mg of $C_{16}H_{12}Cl_2N_2O_2$.

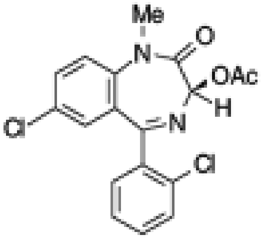
STORAGE

Lormetazepam should be protected from light.

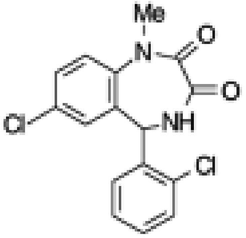
IMPURITIES



A. 2-methylamino-2′,5-dichlorobenzophenone


Me
O
N
OAc
H
N
Cl
Cl

B. *O*^{3}acetyl-lormetazepam

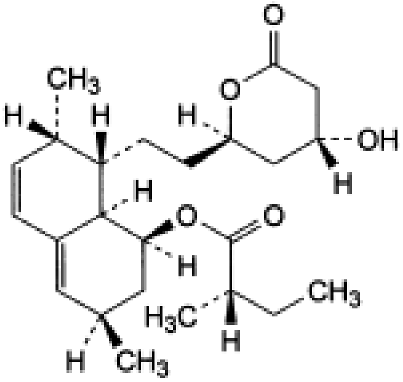

Me
O
N
O
NH
Cl
Cl

C. 7-chloro-1-methyl-5-(2-chlorophenyl)-4,5-dihydro-2 *H*-1,4-benzodiazepin-2,3-(1 *H*)-dione

Lovastatin

General Notices

(Ph Eur monograph 1538)



$C_{24}H_{36}O_5$ 404.5 *75330-75-5*

Action and use

HMG Co-A reductase inhibitor; lipid-regulating drug.

Ph Eur

DEFINITION

(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone, sparingly soluble in anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *lovastatin CRS.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 or BY_6 (*2.2.2, Method II*).

Dissolve 0.200 g in *acetonitrile R* and dilute to 20.0 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 325 to + 340 (dried substance).

Dissolve 0.125 g in *acetonitrile R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 20.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dilute 10.0 ml of test solution (a) to 20.0 ml with *acetonitrile R*.

Reference solution (a) Dissolve 10.0 mg of *lovastatin CRS* in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100.0 ml with *acetonitrile R*.

Reference solution (c) To 5.0 ml of reference solution (a) add 1 mg of *simvastatin CRS* and dilute to 50.0 ml with *acetonitrile R*.

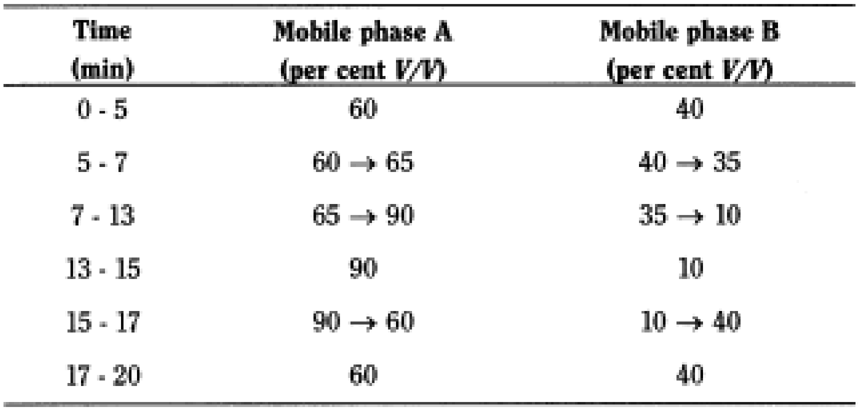
Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *acetonitrile R*;

— *mobile phase B*: 0.1 per cent *V/V* solution of *phosphoric acid R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	60	40
5 - 7	60 → 65	40 → 35
7 - 13	65 → 90	35 → 10
13 - 15	90	10
15 - 17	90 → 60	10 → 40
17 - 20	60	40

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 238 nm.

Injection 10 µl of test solution (a) and reference solutions (b) and (c).

Relative retention With reference to lovastatin (retention time = about 7 min): impurity B = about 0.6; impurity A = about 0.8; simvastatin = about 1.1; impurity C = about 1.2; impurity D = about 2.3.

System suitability Reference solution (c):

— *resolution*: minimum 5.0 between the peaks due to lovastatin and simvastatin.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator under high vacuum at 60 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

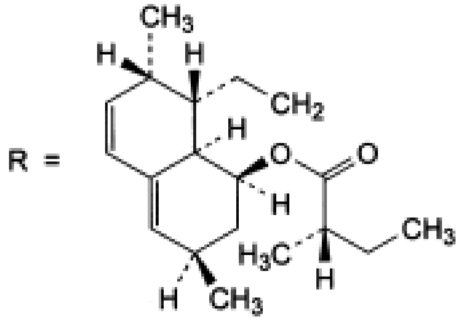
Calculate the content of $C_{24}H_{36}O_5$ from the declared content of *lovastatin CRS*.

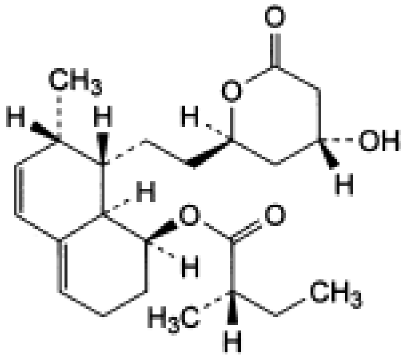
STORAGE

Under nitrogen at a temperature of 2 °C to 8 °C.

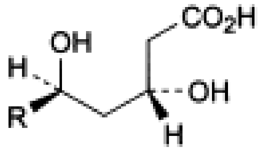
IMPURITIES

Specified impurities   A, B, C, D.

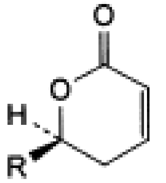

R =
CH3
H
H
CH2
H
O
O
H
H3C
CH3
H
H
CH3


O
CH3
H
H
H
O
OH
H
H
O
O
H
H3C
CH3
H

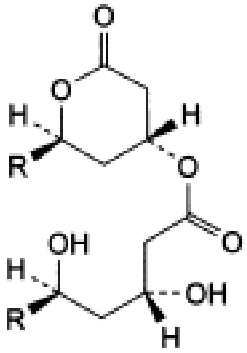
A. (1*S*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (mevastatin),


CO2H
OH
H
R
OH
H

B. (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid (hydroxyacid lovastatin),



C. (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-dimethyl-8-[2-[(2*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl]-1,2,3, 7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (dehydrolovastatin),



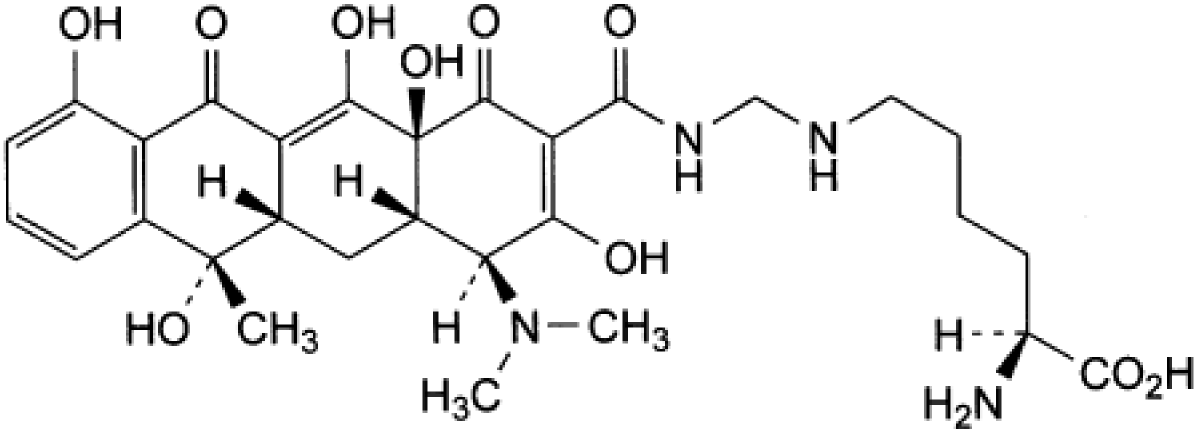
D. (2*R*,4*R*)-2-[2-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]-6-oxotetrahydro-2*H*-pyran-4-yl (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate (lovastatin dimer).

Ph Eur

Lymecycline

General Notices

(Ph Eur monograph 1654)



$C_{29}H_{38}N_4O_{10}$ 603 *992-21-2*

Action and use

Tetracycline antibacterial.

Preparation

Lymecycline Capsules

Ph Eur

DEFINITION

(2*S*)-2-Amino-6-[[[[(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracen-2-yl]carbonyl]amino]methyl]amino] hexanoic acid (reaction product of formaldehyde, lysine and tetracycline).

Semi-synthetic product derived from a fermentation product.

Content

81.0 per cent to 102.0 per cent (equivalent to 60.0 per cent to 75.0 per cent of tetracycline) (anhydrous substance).

CHARACTERS

Appearance

Yellow, hygroscopic powder.

Solubility

Very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *tetracycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *tetracycline hydrochloride CRS*, 5 mg of *demeclocycline hydrochloride R* and 5 mg of *oxytetracycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R* (2-10 µm).

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of o*xalic acid R* previously adjusted to pH 2.0 with *concentrated ammonia R.*

Application 2 µl.

Development Over half of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in 50 ml of *water R.*

Reference solution (a) Dissolve 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *arginine CRS* and 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R*, *2-propanol R* (30:70 *V/V*).

Application 5 µl.

Development Over 3/4 of the plate.

Drying At 100-105 °C until the ammonia disappears completely.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.2 g in 5 ml of *water R,* add 0.3 ml of *orthophosphoric acid R* and distil. To 1 ml of the distillate add 10 ml of *chromotropic acid-sulphuric acid solution R*. A violet colour is produced.

D. Specific optical rotation (see Tests).

TESTS

pH *(2.2.3)*

7.8 to 8.2.

Dissolve 0.1 g in 10 ml of *carbon dioxide-free water R.*

Specific optical rotation *(2.2.7)*

- 180 to - 210 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 50.0 ml with the same solvent.

Free tetracycline (impurity H)

Maximum 2.5 per cent (anhydrous and methanol-free substance).

To 0.5 g add 50 ml of *butyl acetate R* and allow to stand at 25 °C for 1 h. Filter and extract the filtrate with 2 quantities, each of 25 ml, of *0.1 M hydrochloric acid*. Combine the extracts and dilute to 50.0 ml with *0.1 M hydrochloric acid*. Dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*. The absorbance (*2.2.25*) measured at 355 nm is not greater than 0.64.

Light-absorbing impurities

The absorbance *(2.2.25)* is not greater than 0.50 at 430 nm (anhydrous and methanol-free substance).

Dissolve 25.0 mg in *0.01 M hydrochloric acid* and dilute to 10.0 ml with the same acid.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 0.125 g of the substance to be examined in 5.0 ml of *water R*. Add 1.0 ml of a 40 g/l solution of *sodium metabisulphite R* and allow to stand in the dark at 20-25 °C for 16-24 h, without stirring. Add 50 ml of *0.05 M hydrochloric acid*, shake to dissolve the precipitate and dilute to 100.0 ml with *water R.*

Reference solution (a) Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 12.5 mg of *4-epitetracycline hydrochloride CRS* (impurity A) in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solution (c) Dissolve 10.0 mg of *anhydrotetracycline hydrochloride CRS* (impurity C) in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid.

Reference solution (d) Dissolve 10.0 mg of *4-epianhydrotetracycline hydrochloride CRS* (impurity D) in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solution (e) Mix 1 ml of reference solution (a), 2 ml of reference solution (b) and 5 ml of reference solution (d) and dilute to 25 ml with *0.01 M hydrochloric acid.*

Reference solution (f) Mix 40.0 ml of reference solution (b), 20.0 ml of reference solution (c) and 5.0 ml of reference solution (d) and dilute to 200.0 ml with *0.01 M hydrochloric acid.*

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 10 nm;

— *temperature*: 60 °C.

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R*; add 100 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *dilute phosphoric acid R*, 200 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R,* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 ml with *water R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (e) and (f).

Run time 5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Relative retention With reference to tetracycline (retention time = about 8 min): impurity E = about 0.50; impurity A = about 0.6; impurity F = about 0.68; impurity B (eluting on the tail of the principal peak) = about 1.2; impurity D = about 1.45; impurity G = about 1.45; impurity C = about 2.95.

System suitability Reference solution (e):

— *resolution*: minimum 3.0 between the 1st peak (impurity A) and the 2nd peak (tetracycline) and minimum 5.0 between the 2nd peak and the 3rd peak (impurity D); adjust the concentration of 2-methyl-2-propanol in the mobile phase if necessary;

— *symmetry factor*: maximum 1.25 for the peak due to tetracycline.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (5.0 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (1.0 per cent);

— *impurities B, E, F*: for each impurity, not more than 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.5 per cent);

— *sum of impurities D and G*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.04 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.2 per cent);

— *total*: not more than 1.6 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (8.0 per cent);

— *disregard limit*: 0.02 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.1 per cent).

Methanol ***(2.4.24, System A)***

Maximum 1.5 per cent.

Water ***(2.5.12)***

Maximum 5.0 per cent, determined on 0.20 g.

Sulphated ash ***(2.4.14)***

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

System suitability:

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

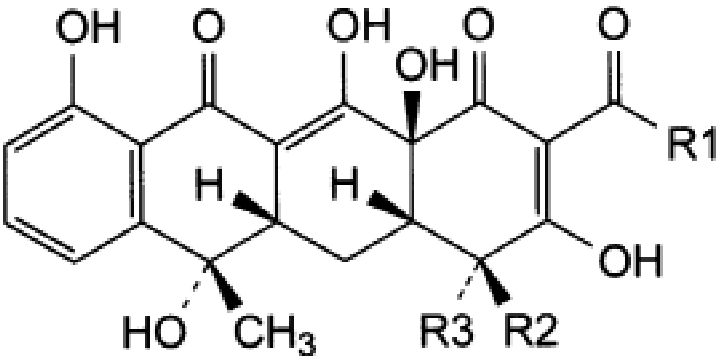
Calculate the percentage content of tetracycline and multiply it by 1.356 to obtain the percentage content of lymecycline.

STORAGE

In an airtight container, protected from light.

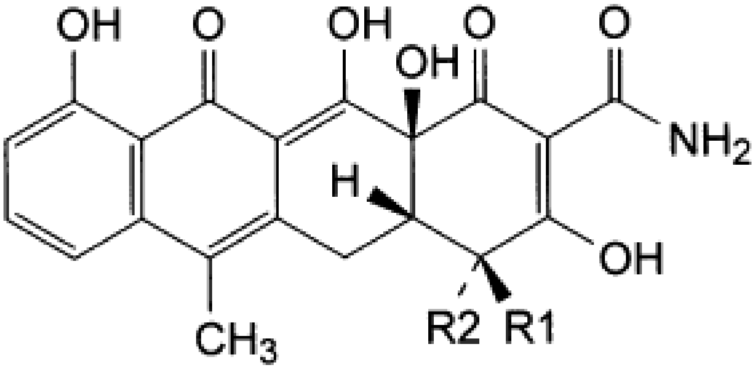
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H.*



A. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$: (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),

B. R1 = CH_3, R2 = $N(CH_3)_2$, R3 = H: (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyltetracycline),



C. R1 = $N(CH_3)_2$, R2 = H: (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

D. R1 = H, R2 = $N(CH_3)_2$: (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline),

E. unknown structure,

F. unknown structure,

G. chlortetracycline,

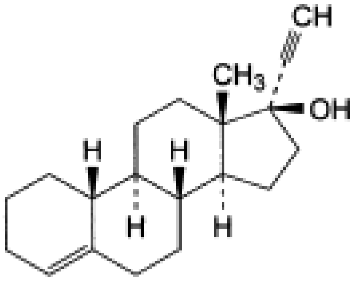
H. tetracycline.

Ph Eur

Lynestrenol

General Notices

(Ph Eur monograph 0558)



$C_{20}H_{28}O$ 284.4   *52-76-6*

Action and use

Progestogen.

Ph Eur

DEFINITION

Lynestrenol contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 19-nor-17α-pregn-4-en-20-yn-17-ol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, soluble in acetone and in alcohol.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Melting point (*2.2.14*): 161 °C to 165 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *lynestrenol CRS*.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with reference solution (b).

TESTS

Appearance of solution

Dissolve 0.2 g in *alcohol R* and dilute to 10 ml with the same solvent. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

Dissolve 0.900 g in *alcohol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is - 9.5 to - 11, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.125 g of the substance to be examined in *chloroform R* and dilute to 25 ml with the same solvent.

Test solution (b) Dilute 5 ml of test solution (a) to 10 ml with *chloroform R*.

Reference solution (a) Dilute 1 ml of test solution (a) to 100 ml with *chloroform R*. Dilute 5 ml of the solution to 10 ml with *chloroform R*.

Reference solution (b) Dissolve 25 mg of *lynestrenol CRS* in *chloroform R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *acetone R* and 80 volumes of *heptane R*. Allow the plate to dry in air, spray with *0.25 M alcoholic sulphuric acid R* and heat at 105 °C for 10 min. Examine in ultraviolet light at 365 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.150 g in 40 ml of *tetrahydrofuran R* and add 5.0 ml of a 100 g/l solution of silver *nitrate R*. Titrate with *0.1 M sodium hydroxide*. Determine the end-point potentiometrically (*2.2.20*), using a glass indicator electrode and as comparison electrode a silver-silver chloride double-junction electrode with a saturated solution of *potassium nitrate R* as junction liquid. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 28.44 mg of $C_{20}H_{28}O$.

STORAGE

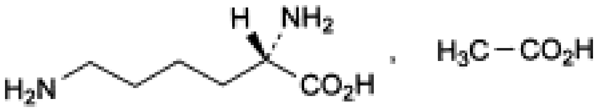
Store protected from light.

Ph Eur

Lysine Acetate

General Notices

(Ph Eur monograph 2114)



$C_6H_{14}N_2O_2,C_2H_4O_2$ 206.2 *57282-49-2*

Action and use

Amino acid.

Ph Eur

DEFINITION

(2*S*)-2,6-Diaminohexanoic acid acetate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lysine acetate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness at 60 °C and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 ml of solution S (see Tests) add 2 ml of *water R* and 1 ml of a 50 g/l solution of *phosphomolybdic acid R*. A yellowish-white precipitate is formed.

E. It gives reaction (a) of acetates (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

+ 8.5 to + 10.0 (dried substance), determined on solution S.

Ninhydrin-positive substances

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *lysine acetate CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *lysine acetate CRS* and 10 mg of *arginine CRS* in *water R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R*, *2-propanol R* (30:70 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying At 100-105 °C until the ammonia has evaporated.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Limits Test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 300 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Ammonium (*2.4.1, Method B*)

Maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron (*2.4.9*)

Maximum 30 ppm.

In a separating funnel, dissolve 0.33 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the test.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 60 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 80.0 mg in 3 ml of *anhydrous formic acid R*. Add 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 10.31 mg of $C_8H_{18}N_2O_4$.

STORAGE

Protected from light.

IMPURITIES

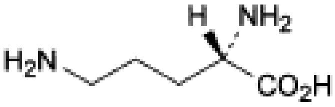
Specified impurities A, B, C, D, E, F.

A. aspartic acid,

B. glutamic acid,

C. alanine,

D. valine,



E. (2*S*)-2,5-diaminopentanoic acid (ornithine),

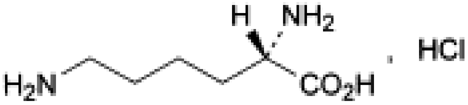
F. arginine.

Ph Eur

Lysine Hydrochloride

General Notices

(Ph Eur monograph 0930)



$C_6H_{14}N_2O_2$,HCl 182.7 *657-27-2*

Action and use

Amino acid.

Ph Eur

DEFINITION

Lysine hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2,6-diaminohexanoic acid hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *lysine hydrochloride CRS*. Examine the substances prepared as discs. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness at 60 °C, and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 ml of solution S (see Tests) add 2 ml of *water R* and 1 ml of a 50 g/l solution of *phosphomolybdic acid R*. A yellowish-white precipitate is formed.

E. To 0.1 ml of solution S add 2 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 or GY_7 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

Dissolve 2.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 21.0 to + 22.5, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *lysine hydrochloride CRS* and 10 mg of *arginine CRS* in *water R* and dilute to 25 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of *concentrated ammonia R* and 70 volumes of *2-propanol R*. Dry the plate at 100 °C to 105 °C until the ammonia disappears completely. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Sulphates (*2.4.13*)

Dilute 5 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium (*2.4.1*)

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH4) R*.

Iron (*2.4.9*)

In a separating funnel, dissolve 0.33 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (30 ppm).

Heavy metals (*2.4.8*)

12 ml of solution S complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 5 ml of *anhydrous formic acid R*. Add 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 18.27 mg of $C_6H_{15}ClN_2O_2$.

STORAGE

Store protected from light.

Ph Eur

Virgin Linseed Oil

General Notices

(Ph Eur monograph 1908)

Ph Eur

DEFINITION

Virgin oil obtained by cold expression from ripe seeds of *Linum usitatissimum* L. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, yellow or brownish-yellow liquid, on exposure to air turning dark and gradually thickening. When cooled, it becomes a soft mass at about - 20 °C.

Solubility

Very slightly soluble in alcohol, miscible with light petroleum.

Relative density

About 0.931.

Refractive index

About 1.480.

IDENTIFICATION

First identification *B, C.*

Second identification *A, B.*

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*). The chromatogram obtained is similar to the type chromatogram for linseed oil.

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)

Maximum 4.5.

Iodine value (*2.5.4*)

160 to 200.

Peroxide value (*2.5.5*)

Maximum 15.0.

Saponification value (*2.5.6*)

188 to 195. Carry out the saponification for 1 h.

Unsaponifiable matter (*2.5.7*)

Maximum 1.5 per cent, determined on 5.0 g.

Composition of fatty acids

Gas chromatography (*2.4.22, Method C*). Use the calibration mixture in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

— *fatty acids with a chain length less than* C_{16}: maximum 1.0 per cent;

— *palmitic acid*: 3.0 per cent to 8.0 per cent;

— *palmitoleic acid* (equivalent chain length on polyethyleneglycol adipate 16.3): maximum 1.0 per cent;

— *stearic acid*: 2.0 per cent to 8.0 per cent;

— *oleic acid* (equivalent chain length on polyethyleneglycol adipate 18.3): 11.0 per cent to 35.0 per cent;

— *linoleic acid* (equivalent chain length on polyethyleneglycol adipate 18.9): 11.0 per cent to 24.0 per cent;

— *linolenic acid* (equivalent chain length on polyethyleneglycol adipate 19.7): 35.0 per cent to 65.0 per cent;

— *arachidic acid*: maximum 1.0 per cent.

Cadmium (*2.4.27*)

Maximum 0.5 ppm.

Water (*2.5.32*)

Maximum 0.1 per cent, determined on 5.00 g.

STORAGE

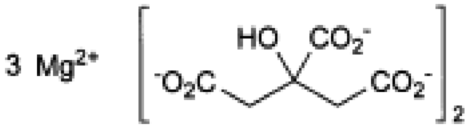
In an airtight container, protected from light.

Ph Eur

Anhydrous Magnesium Citrate

General Notices

(Ph Eur monograph 2339)



$Mg_3(C_6H_5O_7)_2$ 451.1 *3344-18-1*

Ph Eur

DEFINITION

Trimagnesium bis(2-hydroxypropane-1,2,3-tricarboxylate).

Content

15.0 per cent to 16.5 per cent of Mg (dried substance).

CHARACTERS

Appearance

White or almost white, fine, slightly hygroscopic powder.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent). It dissolves in dilute hydrochloric acid.

IDENTIFICATION

A. It gives the reaction of citrates *(2.3.1)*.

B. It gives the reaction of magnesium *(2.3.1)*.

C. pH (see Tests).

D. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R*, heating at 60 °C, cool and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solutions Y_7 or BY_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

6.0 to 8.5 for solution S.

Oxalates

Maximum 280 ppm.

Dissolve 0.50 g in 4 ml of *water R*. Add 3 ml of *hydrochloric acid R* and 1 g of *activated zinc R*. Allow to stand for 5 min. Transfer the liquid to a tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R*. Heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 4 ml of a 50 mg/l solution of *oxalic acid R*.

Sulphates *(2.4.13)*

Maximum 0.2 per cent.

Dilute 1.5 ml of solution S to 15 ml with *distilled water R*.

Calcium *(2.4.3)*

Maximum 0.2 per cent.

Dilute 1.0 ml of solution S to 15 ml with *distilled water R*.

Iron *(2.4.9)*

Maximum 100 ppm.

Dilute 2.0 ml of solution S to 10 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 5.0 g in 15 ml of *dilute hydrochloric acid R* with heating. Adjust to pH 3.5 with *ammonia R* and dilute to 50 ml with *distilled water R*. 12 ml of this solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 3.5 per cent, determined on 1.000 g by drying in an oven at 180 ± 10 °C for 5 h.

ASSAY

Dissolve 0.150 g in 50 ml of *water R*. Carry out the complexometric titration of magnesium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

STORAGE

In a non-metallic, airtight container .

Ph Eur

Basic Butylated Methacrylate Copolymer

General Notices

(Ph Eur monograph 1975)

Action and use

Excipient.

Ph Eur

DEFINITION

Copolymer of 2-(dimethylamino)ethyl methacrylate, butyl methacrylate and methyl methacrylate having a mean relative molecular mass of about 150 000. The ratio of 2-dimethylaminoethyl methacrylate groups to butyl methacrylate and methyl methacrylate groups is about 2:1:1.

Content of dimethylaminoethyl groups

20.8 per cent to 25.5 per cent (dried substance).

CHARACTERS

Appearance

Colourless or yellowish granules or white or almost white powder, slightly hygroscopic.

Solubility

Practically insoluble in water, freely soluble in methylene chloride. It dissolves slowly in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of basic butylated methacrylate copolymer.

B. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 12.5 g in a mixture of 35.0 g of *acetone R* and 52.5 g of *2-propanol R*.

Viscosity (*2.2.10*)

3 mPa·s to 6 mPa·s, determined on solution S.

Apparatus Rotating viscosimeter.

Dimensions:

— *spindle*: diameter = 25.15 mm, height = 90.74 mm, shaft diameter = 4 mm;

— *cylinder*: diameter = 27.62 mm, height = 0.135 m.

Stirring speed 30 r/min.

Volume of solution 16 ml of solution S.

Temperature 20 C.

Absorbance (*2.2.25*)

Maximum 0.30 at 420 nm, determined on solution S.

Appearance of film

Spread evenly 1.0 ml of solution S on a glass plate. Upon drying a clear film is formed.

Monomers

Maximum 0.3 per cent, for the sum of contents of butyl methacrylate, methyl methacrylate and 2-dimethylaminoethyl methacrylate calculated by procedures A and B.

A. Butyl methacrylate and methyl methacrylate. Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.00 g of the substance to be examined in *phosphate buffer solution pH 2.0 R* and dilute to 50.0 ml with the same buffer solution.

Reference solution Dissolve 10.0 mg of *butyl methacrylate R* and 10.0 mg of *methyl methacrylate R* in 10.0 ml of *acetonitrile R* and dilute to 50.0 ml with *water R*. Dilute 1.0 ml of the solution to 50.0 ml with *water R*.

Column:

— *size: l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase *phosphate buffer solution pH 2.0 R*, *methanol R* (45:55 *V/V*).

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 50 µl.

B. 2-Dimethylaminoethyl methacrylate. Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.00 g of the substance to be examined in *tetrahydrofuran R* and dilute to 50.0 ml with the same solvent.

Reference solution Dissolve 10.0 mg of *2-(dimethylamino)ethyl methacrylate R* in *tetrahydrofuran R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of the solution to 50.0 ml with *tetrahydrofuran R*.

Column:

— *size: l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 25 volumes of a 3.404 g/l solution of *potassium dihydrogen phosphate R* and 75 volumes of *tetrahydrofuran R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 50 µl.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

2.0 g complies with limit test C. Prepare the standard using 4.0 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 110 C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

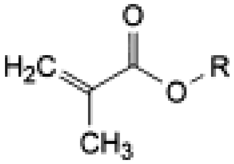
Dissolve 0.200 g in a mixture of 4 ml of *water R* and 96 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 7.21 mg of $C_4H_{10}N$.

STORAGE

In an airtight container .

IMPURITIES



A. R = $[CH_2]_3$-CH_3: butyl methacrylate,

B. R = CH_3: methyl methacrylate,

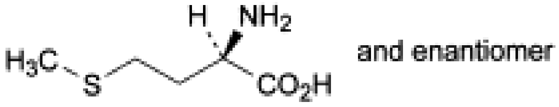
C. R = CH_2-CH_2-$N(CH_3)_2$: 2-(dimethylamino)ethyl methacrylate.

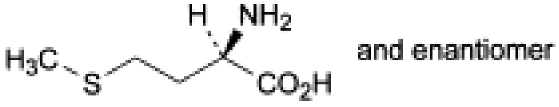
Ph Eur

DL- Methionine

General Notices

(Ph Eur monograph 0624)





$C_5H_{11}NO_2S$ 149.2 *59-51-8*

Action and use

Used in treatment of paracetamol overdose.

Ph Eur

DEFINITION

DL-Methionine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*)-2-amino-4-(methylsulphanyl)butanoic acid, calculated with reference to the dried substance.

CHARACTERS

Almost white, crystalline powder or small flakes, sparingly soluble in water, very slightly soluble in alcohol. It dissolves in dilute acids and in dilute solutions of the alkali hydroxides.

It melts at about 270 °C (instantaneous method).

IDENTIFICATION

First identification A, C.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *DL-methionine CRS*. Dry the substances at 105 °C.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 2.50 g in *1 M hydrochloric acid* and dilute to 50.0 ml with the same acid. The angle of optical rotation (*2.2.7*) is - 0.05° to + 0.05°.

D. Dissolve 0.1 g of the substance to be examined and 0.1 g of *glycine R* in 4.5 ml of *dilute sodium hydroxide solution R.* Add 1 ml of a 25 g/l solution of *sodium nitroprusside R*. Heat to 40 °C for 10 min. Allow to cool and add 2 ml of a mixture of 1 volume of *phosphoric acid* R and 9 volumes of *hydrochloric acid R*. A deep-red colour develops.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 5.4 to 6.1.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 20 mg of *dl-methionine CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with *water R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air and spray with *ninhydrin solution R*. Heat the plate at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Chlorides

Dissolve 0.25 g in 35 ml of *water R*. Add 5 ml of *dilute nitric acid R* and 10 ml of *silver nitrate solution R2*. Allow to stand protected from light for 5 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 10 ml of *chloride standard solution (5 ppm Cl) R* and 25 ml of *water R* (200 ppm). Examine the tubes laterally against a black background.

Sulphates (*2.4.13*)

Dissolve 1.0 g in 20 ml of *distilled water R*, heating to 60 °C. Cool to 10 °C and filter. 15 ml of the solution complies with the limit test for sulphates (200 ppm).

Heavy metals (*2.4.8*)

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.140 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Immediately after dissolution, titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.

STORAGE

Store protected from light.

Ph Eur

Dried Magnesium Sulphate

General Notices

Dried Epsom Salts

Preparation

Magnesium Sulphate Paste

DEFINITION

Dried Magnesium Sulphate contains not less than 62.0% and not more than 70.0% of $MgSO_4$. It may be prepared by drying magnesium sulphate at 100° until it has lost approximately 25% of its weight.

CHARACTERISTICS

A white powder.

Freely soluble in *water*; dissolves more rapidly in hot *water*.

IDENTIFICATION

Yields the reactions characteristic of *magnesium salts* and of *sulphates*, Appendix VI.

TESTS

Acidity or alkalinity

To 10 ml of a 7.5% w/v solution in *carbon dioxide-free water* add 0.05 ml of *phenol red solution*. Not more than 0.2 ml of either 0.01M *hydrochloric acid VS* or 0.01M *sodium hydroxide VS* is required to change the colour of the solution.

Arsenic

0.33 g dissolved in 25 ml of *water* complies with the *limit test for arsenic*, Appendix VII (3 ppm)
.

Heavy metals

Dissolve 1.3 g in 20 ml of *water* and add 1 g of *ammonium chloride*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (15 ppm).

Iron

0.33 g dissolved in 10 ml of *water* complies with the *limit test for iron*, Appendix VII (30 ppm).

Chloride

0.13 g dissolved in 15 ml of *water* complies with the *limit test for chlorides*, Appendix VII (400 ppm).

Insoluble matter

7.5 g dissolves in 20 ml of *water*, producing a solution which may be slightly turbid at first but which becomes clear in a few minutes.

ASSAY

Dissolve 0.3 g in 50 ml of *water* and carry out the *complexometric titration of magnesium*, Appendix VIII D. Each ml of 0.1M *disodium edetate VS* is equivalent to 12.04 mg of $MgSO_4$.

Heavy Magnesium Carbonate

General Notices

(Ph Eur monograph 0043)

546-93-0

Action and use

Antacid; osmotic laxative.

Ph Eur

DEFINITION

Hydrated basic magnesium carbonate.

Content

40.0 per cent to 45.0 per cent, calculated as MgO (M_r 40.30).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water. It dissolves in dilute acids with effervescence.

IDENTIFICATION

A. Bulk density *(2.9.34)*: minimum 0.25 g/ml.

B. It gives the reaction of carbonates *(2.3.1)*.

C. Dissolve about 15 mg in 2 ml of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in 100 ml of *dilute acetic acid R*. When the effervescence has ceased, boil for 2 min, allow to cool and dilute to 100 ml with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate.

Appearance of solution

Solution S is not more intensely coloured than reference solution B_4 *(2.2.2, Method II)*.

Soluble substances

Maximum 1.0 per cent.

Mix 2.00 g with 100 ml of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) *(2.1.2)*, allow to cool and dilute to 100 ml with *water R*. Evaporate 50 ml of the filtrate to dryness and dry at 100-105 °C. The residue weighs not more than 10 mg.

Substances insoluble in acetic acid

Maximum 0.05 per cent.

Any residue obtained during the preparation of solution S, washed, dried, and ignited at 600 ± 50 °C, weighs not more than 2.5 mg.

Chlorides *(2.4.4)*

Maximum 700 ppm.

Dilute 1.5 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 0.6 per cent.

Dilute 0.5 ml of solution S to 15 ml with *distilled water R*.

Arsenic *(2.4.2, Method A)*

Maximum 2 ppm, determined on 10 ml of solution S.

Calcium *(2.4.3)*

Maximum 0.75 per cent.

Dilute 2.6 ml of solution S to 150 ml with *distilled water R*. 15 ml of the solution complies with the test.

Iron *(2.4.9)*

Maximum 400 ppm.

Dissolve 0.1 g in 3 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. Dilute 2.5 ml of the solution to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

To 20 ml of solution S add 15 ml of *hydrochloric acid R1* and shake with 25 ml of *methyl isobutyl ketone R* for 2 min. Allow to stand, separate the aqueous lower layer and evaporate to dryness. Dissolve the residue in 1 ml of *acetic acid R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 0.150 g in a mixture of 2 ml of *dilute hydrochloric acid R* and 20 ml of *water R*. Carry out the complexometric titration of magnesium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.The following characteristics may be relevant for heavy magnesium carbonate used as a filler in tablets.

Particle-size distribution ***(2.9.31 or 2.9.38)*****.**

Bulk and tapped density ***(2.9.34)*****.**

Ph Eur

Heavy Magnesium Oxide

General Notices

(Ph Eur monograph 0041)

MgO 40.30 *1309-48-4*

Action and use

Antacid; osmotic laxative.

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent of MgO (ignited substance).

CHARACTERS

Appearance

Fine, white or almost white powder.

Solubility

Practically insoluble in water. It dissolves in dilute acids with at most slight effervescence.

IDENTIFICATION

A. 15 g has an apparent volume (*2.9.15*) before settling of not more than 60 ml.

B. Dissolve about 15 mg in 2 ml of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

C. Loss on ignition (see Tests).

TESTS

Solution S

Dissolve 5.0 g in a mixture of 30 ml of *distilled water R* and 70 ml of *acetic acid R*, boil for 2 min, cool and dilute to 100 ml with *dilute acetic acid R.* Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate.

Appearance of solution

Solution S is not more intensely coloured than reference solution B_3 (*2.2.2, Method II*).

Soluble substances

Maximum 2.0 per cent.

To 2.00 g add 100 ml of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 ml with *water R*. Evaporate 50 ml of the filtrate to dryness and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

Substances insoluble in acetic acid

Maximum 0.1 per cent.

Any residue obtained during the preparation of solution S, washed, dried, and ignited at 600 ± 50 °C, weighs a maximum of 5 mg.

Chlorides (*2.4.4*)

Maximum 0.1 per cent.

Dilute 1 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 1.0 per cent.

Dilute 0.3 ml of solution S to 15 ml with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 4 ppm, determined on 5 ml of solution S.

Calcium (*2.4.3*)

Maximum 1.5 per cent.

Dilute 1.3 ml of solution S to 150 ml with *distilled water R*. 15 ml of the solution complies with the test.

Iron (*2.4.9*)

Maximum 0.07 per cent.

Dissolve 0.15 g in 5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. Dilute 1 ml of the solution to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 30 ppm.

To 20 ml of solution S add 15 ml of *hydrochloric acid R1* and shake with 25 ml of *methyl isobutyl ketone R* for 2 min. Allow to stand, then separate and evaporate the aqueous layer to dryness. Dissolve the residue in 1 ml of *acetic acid R* and dilute to 30 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

Maximum 8.0 per cent, determined on 1.00 g at 900 ± 25 °C.

ASSAY

Dissolve 0.320 g in 20 ml of *dilute hydrochloric acid R* and dilute to 100.0 ml with *water R*. Using 20.0 ml of the solution, carry out the complexometric titration of magnesium (*2.5.11*).

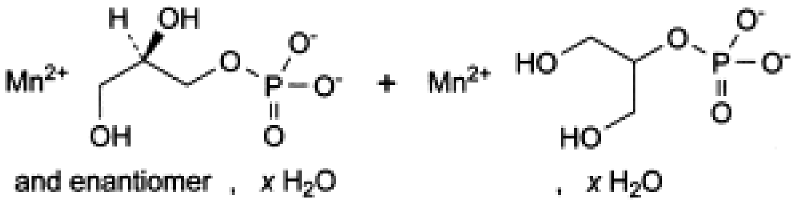
1 ml of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

Ph Eur

Hydrated Manganese Glycerophosphate

General Notices

(Ph Eur monograph 2163)



$C_3H_7MnO_6P,xH_2O$ 225.0

Ph Eur

DEFINITION

Mixture of variable proportions of hydrated manganese (II) (2*RS*)-2,3-dihydroxypropyl phosphate and hydrated manganese (II) 2-hydroxy-1-(hydroxymethyl)ethyl phosphate.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or pale pink, hygroscopic powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It is freely soluble in dilute mineral acids.

IDENTIFICATION

A. Mix 1 g with 1 g of *potassium hydrogen sulphate R* in a test tube fitted with a delivery tube. Heat strongly and direct the white vapour towards a piece of filter paper impregnated with a freshly prepared 10 g/l solution of *sodium nitroprusside R*. The filter paper develops a blue colour in contact with *piperidine R*.

B. Disperse 50 mg in 5 ml of *water R*. Add 0.5 ml of *ammonium sulphide solution R*. A pale pink precipitate is formed that dissolves on the addition of 1 ml of *acetic acid R*.

C. Ignite 0.1 g in a crucible. Take up the residue with 5 ml of *nitric acid R* and heat on a water-bath for 1 min. Filter. The filtrate gives reaction (b) of phosphates (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in 20 ml of *dilute hydrochloric acid R*. Filter if necessary. Add *dilute ammonia R1* until a precipitate is formed. Dissolve the precipitate by adding the minimum quantity needed of *dilute hydrochloric acid R* and dilute to 100 ml with *distilled water R*.

Glycerol and ethanol (96 per cent)-soluble substances

Maximum 1.0 per cent.

Shake 1.00 g with 25 ml of *ethanol (96 per cent) R* for 1 min. Filter. Evaporate the filtrate to dryness on a water-bath and dry the residue at 70 °C for 1 h. The residue weighs a maximum of 10 mg.

Chlorides (*2.4.4*)

Maximum 0.15 per cent.

Dissolve 0.22 g in a mixture of 1 ml of *nitric acid R* and 10 ml of *water R* and dilute to 100 ml with *water R*.

Phosphates (*2.4.11*)

Maximum 0.3 per cent.

Dilute 1.0 ml of solution S to 100.0 ml with *water R*. To 10 ml of this solution add 140 ml of *water R*.

Sulphates (*2.4.13*)

Maximum 0.2 per cent.

Dilute 5 ml of solution S to 50 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 50 ppm.

Dilute 4 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

ASSAY

To 0.200 g add 50 ml of *water R* and stir until dissolution. Add 10 mg of *ascorbic acid R*, 20 ml of *ammonium chloride buffer solution pH 10.0 R* and 0.3 ml of a 2 g/l solution of *mordant black 11 R* in *triethanolamine R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

1 ml of *0.1 M sodium edetate* is equivalent to 22.50 mg of $C_3H_7MnO_6P$.

STORAGE

In an airtight container .

Ph Eur

Industrial Methylated Spirit (Ketone-free)

General Notices

Industrial Denatured Alcohol (Ketone-free)

DEFINITION

Industrial Methylated Spirit (Ketone-free) is a mixture of nineteen volumes of ethanol of an appropriate strength with one volume of approved wood naphtha substantially free from ketones. Two strengths are available containing 99% by volume and 95% by volume of alcohol (also known as 74 OP and 66 OP respectively).

CHARACTERISTICS; IDENTIFICATION; TESTS

Acidity or alkalinity; Clarity of solution; Apparent density; Aldehydes; Non-volatile matter

Complies with the requirements stated under Industrial Methylated Spirit.

Ketones

Dilute 5 ml to 10 ml with *water*, add 1 ml of a 1.0% w/v solution of *2-nitrobenzaldehyde* in *ethanol (50%)* followed by 1 ml of a 15% w/v solution of *sodium hydroxide* in *water* and allow to stand for 15 minutes. Any colour produced is not more intense than that produced by treating in the same manner 10 ml of a 0.025% v/v solution of *acetone* in *ethanol (50%)* (500 ppm).

Industrial Methylated Spirit

General Notices

Industrial Methylated Spirits; IMS
Industrial Denatured Alcohol

DEFINITION

Industrial Methylated Spirit is a mixture of nineteen volumes of ethanol of an appropriate strength with one volume of approved wood naphtha. Two strengths are available containing 99% by volume and 95% by volume of alcohol (also known as 74 OP and 66 OP respectively) .

CHARACTERISTICS

A colourless, clear, mobile, volatile liquid, boiling at about 78°.

IDENTIFICATION

Mix 0.1 ml with 0.05 ml of an 11% w/w solution of *orthophosphoric acid* and 0.25 ml of *dilute potassium permanganate solution*. After 1 minute add a few mg of *sodium metabisulphite* and shake until the mixture is decolorised. Add 1.5 ml of a 50% v/v solution of *sulphuric acid* and a few mg of finely powdered *chromotropic acid sodium salt*, shake well and heat on a water bath for 5 minutes. A deep violet colour is produced.

TESTS

Acidity or alkalinity

25 ml requires not more than 0.2 ml of 0.1M *sodium hydroxide VS* to produce a pink colour with *phenolphthalein solution R1* and not more than 1.0 ml of 0.1M *hydrochloric acid VS* to produce a red colour with *methyl red solution*.

Clarity of solution

Dilute 5.0 ml to 100 ml with *water*. The solution is *clear*, Appendix IV A.

Apparent density

For '66 OP' grade, not greater than 811.6 kg m^{-3}, and for '74 OP' grade, not greater than 792.8 kg m^{-3}, Appendix V G.

Aldehydes

To 5.0 ml add 5 ml of *water* and 1 ml of *decolorised fuchsin solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that obtained by treating in the same manner 5 ml of a 0.005% w/v solution of redistilled *acetaldehyde* in *aldehyde-free ethanol (96%)* (50 ppm).

Non-volatile matter

When evaporated and dried at 105°, leaves not more than 0.01% w/v of residue.

Light Magnesium Carbonate

General Notices

(Ph Eur monograph 0042)

546-93-0

Action and use

Antacid; osmotic laxative.

Preparation

Aromatic Magnesium Carbonate Mixture

Ph Eur

DEFINITION

Hydrated basic magnesium carbonate.

Content

40.0 per cent to 45.0 per cent, calculated as MgO (M_r 40.30).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water. It dissolves in dilute acids with effervescence.

IDENTIFICATION

A. 15 g has an apparent volume (*2.9.15*) before settling of at least 100 ml.

B. It gives the reaction of carbonates (*2.3.1*).

C. Dissolve about 15 mg in 2 ml of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in 100 ml of *dilute acetic acid R*. When the effervescence has ceased, boil for 2 min, allow to cool and dilute to 100 ml with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate.

Appearance of solution

Solution S is not more intensely coloured than reference solution B_4 (*2.2.2, Method II*).

Soluble substances

Maximum 1.0 per cent.

Mix 2.00 g with 100 ml of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 ml with *water R*. Evaporate 50 ml of the filtrate to dryness and dry at 100-105 °C. The residue weighs a maximum of 10 mg.

Substances insoluble in acetic acid

Maximum 0.05 per cent.

Any residue obtained during the preparation of solution S, washed, dried and ignited at 600 ± 50 °C, weighs a maximum of 2.5 mg.

Chlorides (*2.4.4*)

Maximum 700 ppm.

Dilute 1.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 0.3 per cent.

Dilute 1 ml of solution S to 15 ml with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 10 ml of solution S.

Calcium (*2.4.3*)

Maximum 0.75 per cent.

Dilute 2.6 ml of solution S to 150 ml with *distilled water R*. 15 ml of the solution complies with the test.

Iron (*2.4.9*)

Maximum 400 ppm.

Dissolve 0.1 g in 3 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. Dilute 2.5 ml of the solution to 10 ml with *water R.*

Heavy metals (*2.4.8*)

Maximum 20 ppm.

To 20 ml of solution S add 15 ml of *hydrochloric acid R1* and shake with 25 ml of *methyl isobutyl ketone R* for 2 min. Allow to stand, separate the aqueous lower layer and evaporate to dryness. Dissolve the residue in 1 ml of *acetic acid R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 0.150 g in a mixture of 2 ml of *dilute hydrochloric acid R* and 20 ml of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

Ph Eur

Light Magnesium Oxide

General Notices

Light Magnesia

(Ph Eur monograph 0040)

MgO 40.30 *1309-48-4*

Action and use

Antacid; osmotic laxative.

Preparation

Magnesium Hydroxide Mixture

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent of MgO (ignited substance).

CHARACTERS

Appearance

Fine, white or almost white, amorphous powder.

Solubility

Practically insoluble in water. It dissolves in dilute acids with at most slight effervescence.

IDENTIFICATION

A. 15 g has an apparent volume (*2.9.15*) before settling of at least 100 ml.

B. Dissolve about 15 mg in 2 ml of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

C. Loss on ignition (see Tests).

TESTS

Solution S

Dissolve 5.0 g in a mixture of 30 ml of *distilled water R* and 70 ml of *acetic acid R*, boil for 2 min, cool and dilute to 100 ml with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of a suitable porosity to give a clear filtrate.

Appearance of solution

Solution S is not more intensely coloured than reference solution B_2 (*2.2.2, Method II*).

Soluble substances

Maximum 2.0 per cent.

To 2.00 g add 100 ml of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 ml with *water R*. Evaporate 50 ml of the filtrate to dryness and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

Substances insoluble in acetic acid

Maximum 0.1 per cent.

Any residue obtained during the preparation of solution S, washed, dried, and ignited at 600 ± 50 °C, weighs a maximum of 5 mg.

Chlorides (*2.4.4*)

Maximum 0.15 per cent.

Dilute 0.7 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 1.0 per cent.

Dilute 0.3 ml of solution S to 15 ml with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 4 ppm, determined on 5 ml of solution S.

Calcium (*2.4.3*)

Maximum 1.5 per cent.

Dilute 1.3 ml of solution S to 150 ml with *distilled water R*. 15 ml of the solution complies with the test.

Iron (*2.4.9*)

Maximum 0.1 per cent.

Dissolve 50 mg in 5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. Dilute 2 ml of t his solution to 10 ml with *water R.*

Heavy metals (*2.4.8*)

Maximum 30 ppm.

To 20 ml of solution S add 15 ml of *hydrochloric acid R1* and shake with 25 ml of *methyl isobutyl ketone R* for 2 min. Allow to stand, then separate and evaporate the aqueous layer to dryness. Dissolve the residue in 1.5 ml of *acetic acid R* and dilute to 30 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

Maximum 8.0 per cent, determined on 1.00 g at 900 ± 25 °C.

ASSAY

Dissolve 0.320 g in 20 ml of *dilute hydrochloric acid R* and dilute to 100.0 ml with *water R.* Using 20.0 ml of the solution, carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

Ph Eur

Liquid Maltitol

General Notices

(Ph Eur monograph 1236)

Ph Eur

DEFINITION

Aqueous solution of a hydrogenated, partly hydrolysed starch, composed of a mixture of mainly 4-*C*-α-D-glucopyranosyl-D-glucitol (D-maltitol) with D-glucitol (D-sorbitol) and hydrogenated oligo- and polysaccharides.

Content:

— *D-maltitol* ($C_{12}H_{24}O_{11}$): minimum 50.0 per cent *m/m* (anhydrous substance) and 95.0 per cent to 105.0 per cent of the content stated on the label;

— *D-sorbitol* ($C_6H_{14}O_6$): maximum 8.0 per cent *m/m* (anhydrous substance);

— *anhydrous substance*: 68.0 per cent *m/m* to 85.0 per cent *m/m*.

CHARACTERS

Appearance

Clear, colourless, syrupy liquid.

Solubility

Miscible with water and with glycerol.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. Thin-layer chromatography (*2.2.27*).

Test solution Dilute 0.35 g of the substance to be examined to 100 ml with *water R*.

Reference solution (a) Dissolve 20 mg of *maltitol CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *maltitol CRS* and 20 mg of *sorbitol CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *water R*, *ethyl acetate R*, *propanol R* (10:20:70 *V/V/V*)

Application 2 µl.

Development Over a path of 17 cm.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R*. Dry in a current of cold air until the acetone is removed. Heat at 100-105 °C for 15 min. Allow to cool and spray with a 2 g/l solution of *sodium periodate R*. Dry in a current of cold air. Heat at 100 °C for 15 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with reference solution (a).

C. To 3 ml of a freshly prepared 100 g/l solution of *pyrocatechol R*, add 6 ml of *sulphuric acid R* while cooling in iced water. To 3 ml of the cooled mixture, add 0.3 ml of solution S (see Tests). Heat gently over a naked-flame for about 30 s. A pink colour develops.

TESTS

Solution S

Dilute 7.0 g to 50 ml with *water R.*

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Conductivity (*2.2.38*)

Maximum 10 µS·cm^{-1}, measured on undiluted liquid maltitol while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent, calculated as glucose equivalent.

To 5.0 g add 6 ml of *water R*, 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine.* With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Lead (*2.4.10*)

Maximum 0.5 ppm.

Nickel (*2.4.15*)

Maximum 1 ppm.

Water (*2.5.12*)

15.0 per cent *m/m* to 32.0 per cent *m/m*, determined on 0.100 g. Use as solvent a mixture of equal volumes of *anhydrous methanol R* and *formamide R*. Carry out the titration at about 50 °C.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Mix 1.00 g of the solution to be examined with 20 ml of *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *maltitol CRS* in 2 ml of *water R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dissolve 8.0 mg of *sorbitol CRS* in 2 ml of *water R* and dilute to 5.0 ml with the same solvent.

Reference solution (c) Dissolve 50 mg of *maltitol R* and 50 mg of *sorbitol R* in 2 ml of *water R* and dilute to 5.0 ml with the same solvent.

Column:

- *size:* *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *strong cation exchange resin (calcium form) R* (9 µm);
- *temperature*: 85 ± 2 °C.

Mobile phase *degassed water R.*

Flow rate 0.5 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl.

Run time 3 times the retention time of maltitol.

Relative retention with reference to maltitol (retention time = about 16 min): sorbitol = about 1.8.

System suitability Reference solution (c):

- *resolution*: minimum 2 between the peaks due to sorbitol and maltitol.

Calculate the percentage contents of D-maltitol and D-sorbitol from the declared contents of *maltitol CRS* and *sorbitol CRS.*

LABELLING

The label states the content of D-maltitol.

Ph Eur

Macrogol 15 Hydroxystearate

General Notices

(Ph Eur monograph 2052)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of mainly monoesters and diesters of 12-hydroxystearic (12-hydroxyoctadecanoic) acid and macrogols obtained by ethoxylation of 12-hydroxystearic acid. The number of moles of ethylene oxide reacted per mole of 12-hydroxystearic acid is 15 (nominal value). It contains free macrogols.

CHARACTERS

Appearance

Yellowish, waxy mass.

Solubility

Very soluble in water, soluble in ethanol (96 per cent), insoluble in liquid paraffin.

It solidifies at about 25 °C.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g add 100 ml of a 100 g/l solution of *potassium hydroxide R* and boil under a reflux condenser for 30 min. Acidify the warm solution with 20 ml of *hydrochloric acid R* and cool to room temperature. Shake the mixture with 50 ml of *ether R* and allow to stand until a separation of the layers is visible. Separate the clear upper layer, add 5 g of *anhydrous sodium sulphate R*, wait for 30 min, filter and evaporate to dryness on a water-bath. Dissolve 50 mg of the residue in 25 ml of *ether R*.

Reference solution Dissolve 50 mg of *12-hydroxystearic acid R* in 25 ml of *methylene chloride R*.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *methylene chloride R*, *glacial acetic acid R*, *acetone R* (10:40:50 *V/V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Drying In a current of cold air.

Detection Spray with a 80 g/l solution of *phosphomolybdic acid R* in *2-propanol R* and heat at 120 °C for 1-2 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve 15.0 g in 50 ml of *water R*. The viscosity (*2.2.9*) has a maximum of 20 mPa·s.

C. Free macrogols (see Tests).

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solution B_6 or BY_6 (*2.2.2, Method II*).

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent.

Acid value (2.5.1) Maximum 1.0, determined on 2.0 g.

Hydroxyl value (2.5.3, Method A) 90 to 110.

Iodine value (2.5.4, Method A) Maximum 2.0.

Peroxide value (2.5.5, Method A) Maximum 5.0.

Saponification value (2.5.6) 53 to 63.

Free macrogols Size-exclusion chromatography (*2.2.30*).

Test solution Dissolve 1.20 g of the substance to be examined in the mobile phase and dilute to 250.0 ml with the mobile phase.

Reference solution (a) Dissolve about 0.4 g of *macrogol 1000 R* in the mobile phase and dilute to 250.0 ml with the mobile phase.

Reference solution (b) Dilute 50.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Precolumns (2):

— *size:* *l* = 0.125 m, Ø = 4 mm;

— *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm.

Column:

— *size:* *l* = 0.30 m, Ø = 7.8 mm;

— *stationary phase*: *hydroxylated polymethacrylate gel R* (6 µm) with a pore size of 12 nm.

Connect both precolumns to the column using a 3-way valve and switch the mobile phase flow according to the following programme:

— 0-114 s: precolumn 1 and column;

— 115 s to the end: precolumn 2 and column;

— 115 s to 7 min: flow back of precolumn 1.

Mobile phase *water R*, *methanol R* (2:8 *V/V*).

Flow rate 1.1 ml/min.

Detection Refractometer.

Injection 50 µl.

Calculate the percentage content of free macrogols using the following expression:

$$\frac{A_1 \times m_2 \times 200}{m_1 \times (A_2 + 2A_3)}$$

m_1 = mass of the substance to be examined in the test solution, in grams,

m_2 = mass of *macrogol 1000 R* in reference solution (a), in grams,

A_1 = area of the peak due to free macrogols in the substance to be examined in the chromatogram obtained with the test solution,

A_2 = area of the peak due to macrogol 1000 in the chromatogram obtained with reference solution (a),

A_3 = area of the peak due to macrogol 1000 in the chromatogram obtained with reference solution (b).

Limit:

— *free macrogols*: 27.0 per cent to 39.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 50 ppm of dioxan.

Nickel (*2.4.31*)

Maximum 1 ppm.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 2.00 g.

Total ash (*2.4.16*)

Maximum 0.3 per cent, determined on 1.0 g.

STORAGE

In an airtight container .

Ph Eur

Macrogol 20 Glycerol Monostearate

General Notices

(Ph Eur monograph 2044)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Macrogol 20 glycerol monostearate is obtained by ethoxylation with ethylene oxide of different types of glycerol stearates, mainly *Glycerol monostearate 40-55 (0495)*. The number of moles of ethylene oxide reacted per mole of glycerol stearate is 20 (nominal value).

CHARACTERS

Appearance

Pale yellow, oily liquid or gel.

Solubility

Soluble in water at 40 °C and above and in ethanol (96 per cent), practically insoluble in light liquid paraffin and in fatty oils.

Relative density

About 1.07.

IDENTIFICATION

A. Hydroxyl value (see Tests).

B. Saponification value (see Tests).

C. Composition of fatty acids (see Tests).

D. Place 1 g in a test tube and add 0.1 ml of *sulphuric acid R*. Heat the tube until white fumes appear. The fumes turn filter paper impregnated with *alkaline potassium tetraiodomercurate solution R* black.

TESTS

Acid value *(2.5.1)*

Maximum 2.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

65 to 85, determined on 0.350 g.

Iodine value (*2.5.4, Method A*)

Maximum 2.0.

Peroxide value (*2.5.5, Method A*)

Maximum 6.0.

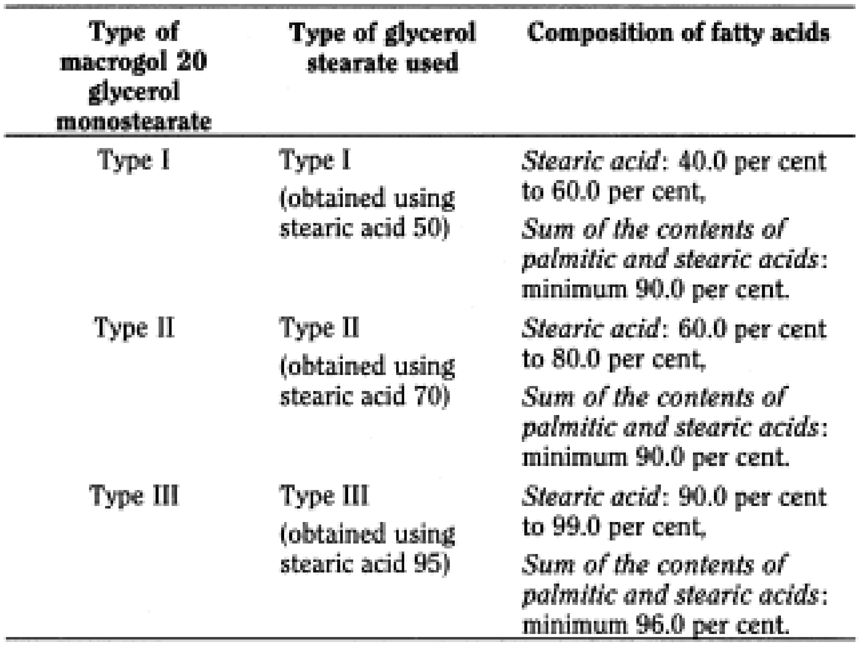
Saponification value (*2.5.6*)

40 to 60.

Composition of fatty acids

Gas chromatography (*2.4.22, Method C*).

Composition of the fatty-acid fraction of the substance:



Type of macrogol 20 glycerol monostearate	Type of glycerol stearate used	Composition of fatty acids
Type I	Type I (obtained using stearic acid 50)	*Stearic acid*: 40.0 per cent to 60.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.
Type II	Type II (obtained using stearic acid 70)	*Stearic acid*: 60.0 per cent to 80.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.
Type III	Type III (obtained using stearic acid 95)	*Stearic acid*: 90.0 per cent to 99.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 96.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals *(2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)

Maximum 0.2 per cent.

STORAGE

Protected from light.

LABELLING

The label states the type of macrogol 20 glycerol monostearate.

Ph Eur

Macrogol 40 Sorbitol Heptaoleate

General Notices

(Ph Eur monograph 2396)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of esters of fatty acids, mainly *Oleic acid (0799)*, and sorbitol ethoxylated with approximately 40 moles of ethylene oxide for each mole of sorbitol. 7 moles of oleic acid are used for each mole of sorbitol. It also contains macrogol fatty acid esters.

CHARACTERS

Appearance

Clear or slightly opalescent, yellowish, viscous, hygroscopic liquid.

Solubility

Dispersible in water, soluble in isopropyl myristate, in isopropyl palmitate, in mineral oils and in vegetable fatty oils.

Relative density

About 1.0.

Viscosity *(2.2.9)*

About 175 mPa·s at 25 °C.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison macrogol 40 sorbitol heptaoleate CRS.

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 12.0, determined on 3.0 g.

Hydroxyl value *(2.5.3, Method A)*

22 to 55.

Peroxide value

Maximum 10.0.

Introduce 10.0 g into a 100 ml beaker, dissolve with *glacial acetic acid R* and dilute to 20 ml with the same acid. Add 1 ml of *saturated potassium iodide solution R* and allow to stand for 1 min. Add 50 ml of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulphate*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulphate* required for the titration of the substance to be examined, in millilitres;

n_2 = volume of *0.01 M sodium thiosulphate* required for the blank titration, in millilitres;

M = molarity of the sodium thiosulphate solution;

m = mass of the substance to be examined, in grams.

Saponification value *(2.5.6)*

90 to 110, determined on 4.0 g.

Use 30.0 ml of *0.5 M alcoholic potassium hydroxide*, heat under reflux for 60 min and add 50 ml of *anhydrous ethanol R* before carrying out the titration.

Composition of fatty acids *(2.4.22, Method C)*

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the substance:

- — *myristic acid*: maximum 5.0 per cent;
- — *palmitic acid*: maximum 16.0 per cent;
- — *palmitoleic acid*: maximum 8.0 per cent;
- — *stearic acid*: maximum 6.0 per cent;
- — *oleic acid*: minimum 58.0 per cent;
- — *linoleic acid*: maximum 18.0 per cent;

— *linolenic acid* : maximum 4.0 per cent.

Ethylene oxide and dioxan *(2.4.25, Method A)*

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 0.50 g.

Sulphated ash

Maximum 0.25 per cent.

Heat a silica crucible to redness for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.0 g of the substance to be examined in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 ± 25 °C, until the substance is thoroughly charred. Carry out the test for sulphated ash *(2.4.14)* on the residue obtained, starting from "Moisten the substance to be examined...".

STORAGE

In an airtight container , protected from light.

Ph Eur

Macrogol 6 Glycerol Caprylocaprate

General Notices

(Ph Eur monograph 1443)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of mainly mono- and diesters of polyoxyethylene glycerol ethers mainly with caprylic (octanoic) and capric (decanoic) acids. The average number of moles of ethylene oxide reacted per mole of substance is 6.

Macrogol 6 glycerol caprylocaprate may be obtained by ethoxylation of glycerol and esterification with distilled coconut or palm kernel fatty acids, or by ethoxylation of mono- and diglycerides of caprylic and capric acids.

CHARACTERS

Appearance

Pale yellow liquid.

Solubility

Partly soluble in water, freely soluble in castor oil, in glycerol, in isopropanol and in propylene glycol.

Viscosity

About 145 mPa·s.

IDENTIFICATION

A. Dissolve 1.0 g in 99 g of a mixture of 10 volumes of *2-propanol R* and 90 volumes of *water R*. Heat the solution obtained to about 40 °C. A turbidity is produced. Allow to cool until the turbidity disappears. The cloud point is between 15 °C and 35 °C.

B. Saponification value (see Tests).

C. Composition of fatty acids (see Tests).

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_2 (*2.2.2, Method I*).

Alkalinity

Dissolve 2.0 g in a hot mixture of 10 ml of *ethanol (96 per cent) R* and 10 ml of *water R*. Add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 5.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

165 to 225.

Saponification value (*2.5.6*)

85 to 105, determined on 2.0 g.

Composition of fatty acids

Gas chromatography (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:

— *caproic acid*: maximum 2.0 per cent;

— *caprylic acid*: 50.0 per cent to 80.0 per cent;

— *capric acid*: 20.0 per cent to 50.0 per cent;

— *lauric acid*: maximum 3.0 per cent;

— *myristic acid*: maximum 1.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)

Maximum 0.3 per cent.

Ph Eur

Macrogol Cetostearyl Ether

General Notices

(Ph Eur monograph 1123)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of ethers of mixed macrogols with linear fatty alcohols, mainly cetostearyl alcohol. It may contain some free macrogols and it contains various amounts of free cetostearyl alcohol. The number of moles of ethylene oxide reacted per mole of cetostearyl alcohol is 2 to 33 (nominal value).

CHARACTERS

Appearance

White or yellowish-white, waxy, unctuous mass, pellets, microbeads or flakes.

Solubility:

— macrogol cetostearyl ether with low numbers of moles of ethylene oxide reacted per mole: practically insoluble in water, soluble in ethanol (96 per cent) and in methylene chloride;

— macrogol cetostearyl ether with higher numbers of moles of ethylene oxide reacted per mole: dispersible or soluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

It solidifies at 32 °C to 52 °C.

IDENTIFICATION

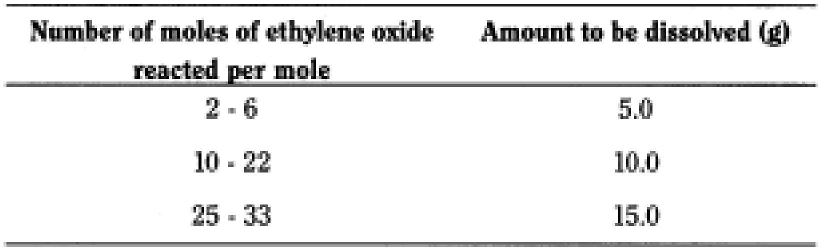
A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Saponification value (see Tests).

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve the prescribed amount of substance to be examined (see table below) in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 75 ml with the same mixture of solvents.



Number of moles of ethylene oxide reacted per mole	Amount to be dissolved (g)
2 - 6	5.0
10 - 22	10.0
25 - 33	15.0

Add 60 ml of *hexane R* and shake for 3 min. The formation of foam can be reduced by the addition of some drops of *ethanol (96 per cent) R*. Filter the upper layer through *anhydrous sodium sulphate R*, wash the filter with 3 quantities, each of 10 ml, of *hexane R* and evaporate the combined filtrates to dryness. Dissolve 0.05 g of the residue in 10 ml of *methanol R* (the solution may be opalescent).

Reference solution Dissolve 25 mg of *stearyl alcohol CRS* in *methanol R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R.*

Application 20 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with vanillin-sulphuric acid reagent prepared as follows: dissolve 0.5 g of *vanillin R* in 50 ml of *ethanol (96 per cent) R* and dilute to 100 ml with *sulphuric acid R*; allow to dry in air; heat at about 130 °C for 15 min and allow to cool in air.

Results The chromatogram obtained with the test solution shows several spots; one of these spots corresponds to the principal spot in the chromatogram obtained with the reference solution.

E. Dissolve or disperse 0.1 g in 5 ml of *ethanol (96 per cent) R*, add 2 ml of *water R*, 10 ml of *dilute hydrochloric acid R*, 10 ml of *barium chloride solution R1* and 10 ml of a 100 g/l solution of *phosphomolybdic acid R*. A precipitate is formed.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 5.0 g in *ethanol (96 per cent) R* and dilute to 50 ml with the same solvent.

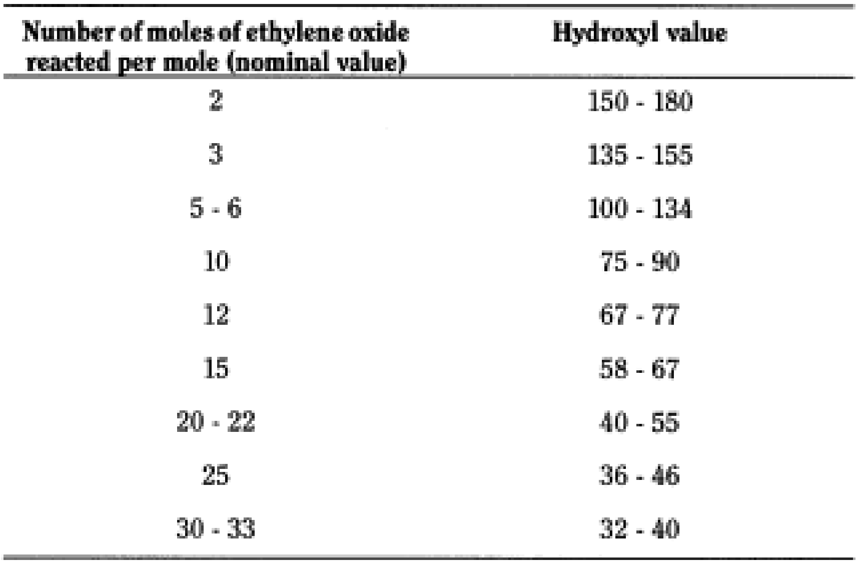
Alkalinity

Dissolve 2.0 g in a hot mixture of 10 ml of *ethanol (96 per cent) R* and 10 ml of *water R*. Add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*).



Number of moles of ethylene oxide reacted per mole (nominal value)	Hydroxyl value
2	150 - 180
3	135 - 155
5 - 6	100 - 134
10	75 - 90
12	67 - 77
15	58 - 67
20 - 22	40 - 55
25	36 - 46
30 - 33	32 - 40

Iodine value (*2.5.4, Method A*)

Maximum 2.0.

Saponification value (*2.5.6*)

Maximum 3.0, determined on 10.0 g.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 2.00 g.

Total ash (*2.4.16*)

Maximum 0.2 per cent, determined on 2.0 g.

STORAGE

In an airtight container .

LABELLING

The label states the number of moles of ethylene oxide reacted per mole of cetostearyl alcohol (nominal value).

Ph Eur

Macrogol Glycerol Cocoates

General Notices

(Ph Eur monograph 1122)

Action and use

Pharmaceutical aids.

Ph Eur

DEFINITION

Mixtures of mono-, di- and triesters of ethoxylated glycerol with fatty acids of vegetable origin having a composition corresponding to the fatty acid composition of the oil extracted from the hard, dried fraction of the endosperm of *Cocos nucifera* L. The average number of moles of ethylene oxide reacted per mole of substance (nominal value) is either 7 (macrogol 7 glycerol cocoate) or 23 (macrogol 23 glycerol cocoate).

CHARACTERS

Appearance

Clear, yellowish, oily liquid.

Solubility

Soluble in water and in ethanol (96 per cent) and practically insoluble in light petroleum (50-70 °C) for macrogol 7 glycerol cocoate and macrogol 23 glycerol cocoate.

Relative density

About 1.05 for macrogol 7 glycerol cocoate; about 1.09 for macrogol 23 glycerol cocoate.

IDENTIFICATION

A. Dissolve 1.0 g of macrogol 7 glycerol cocoate in 99 g of a mixture of 10 volumes of *2-propanol R* and 90 volumes of *water R*. Heat the solution to about 65 °C. A turbidity is produced. Allow to cool until the turbidity disappears. The cloud point is between 35 °C and 54 °C.

Heat a 10 g/l solution of macrogol 23 glycerol cocoate in a 100 g/l solution of *sodium chloride R* to about 90 °C. A turbidity is produced. Allow to cool until the turbidity disappears. The cloud point is between 65 °C and 85 °C.

B. Iodine value (see Tests).

C. Saponification value (see Tests).

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_2 (*2.2.2, Method I*).

Alkalinity

Dissolve 2.0 g in a hot mixture of 10 ml of *ethanol (96 per cent) R* and 10 ml of *water R*. Add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 5.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

See Table 1122.-1.

Saponification value (*2.5.6*)

See Table 1122.-1.

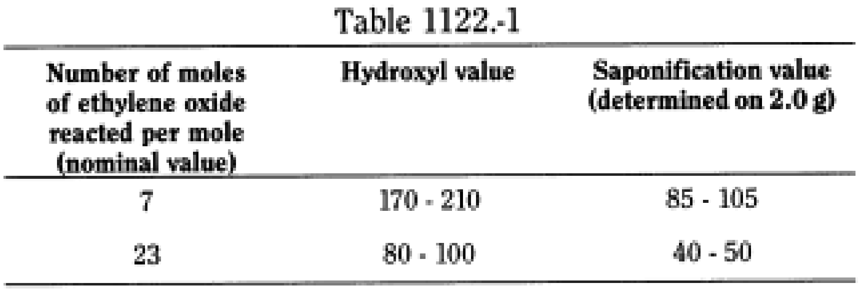


Table 1122.-1

Number of moles of ethylene oxide reacted per mole (nominal value)	Hydroxyl value	Saponification value (determined on 2.0 g)
7	170 - 210	85 - 105
23	80 - 100	40 - 50

Iodine value (*2.5.4, Method A*)

Maximum 5.0.

Composition of fatty acids

Gas chromatography (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:

— *caproic acid*: maximum 1.0 per cent;

— *caprylic acid*: 5.0 per cent to 10.0 per cent;

— *capric acid*: 4.0 per cent to 10.0 per cent;

— *lauric acid*: 40.0 per cent to 55.0 per cent;

— *myristic acid*: 14.0 per cent to 23.0 per cent;

— *palmitic acid*: 8.0 per cent to 12.0 per cent;

— *stearic acid*: 1.0 per cent to 5.0 per cent;

— *oleic acid*: 5.0 per cent to 10.0 per cent;

— *linoleic acid*: maximum 3.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.0 g.

Total ash (*2.4.16*)

Maximum 0.3 per cent.

LABELLING

The label states the number of moles of ethylene oxide reacted per mole of substance (nominal value).

Ph Eur

Macrogol Lauryl Ether

General Notices

(Ph Eur monograph 1124)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of ethers of mixed macrogols with fatty alcohols, mainly $C_{12}H_{26}O$. It contains a variable amount of free $C_{12}H_{26}O$ and it may contain free macrogols. The number of moles of ethylene oxide reacted per mole of $C_{12}H_{26}O$ is 3 to 23 (nominal value).

CHARACTERS

— Macrogol lauryl ether with 3 to 5 units of ethylene oxide per molecule.

Appearance

Colourless liquid.

Solubility

Practically insoluble in water, soluble or dispersible in alcohol, practically insoluble in light petroleum.

— Macrogol lauryl ether with 9 to 23 units of ethylene oxide per molecule.

Appearance

White or almost white, waxy mass.

Solubility

Soluble or dispersible in water, soluble in alcohol, practically insoluble in light petroleum.

IDENTIFICATION

A. It complies with the test for hydroxyl value (see Tests).

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for saponification value (see Tests).

D. Dissolve or disperse 0.1 g in 5 ml of *alcohol R*, add 10 ml of *dilute hydrochloric acid R*, 10 ml of *barium chloride solution R1* and 10 ml of a 100 g/l solution of *phosphomolybdic acid R*. A precipitate is formed.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 5.0 g in *alcohol R* and dilute to 50 ml with the same solvent.

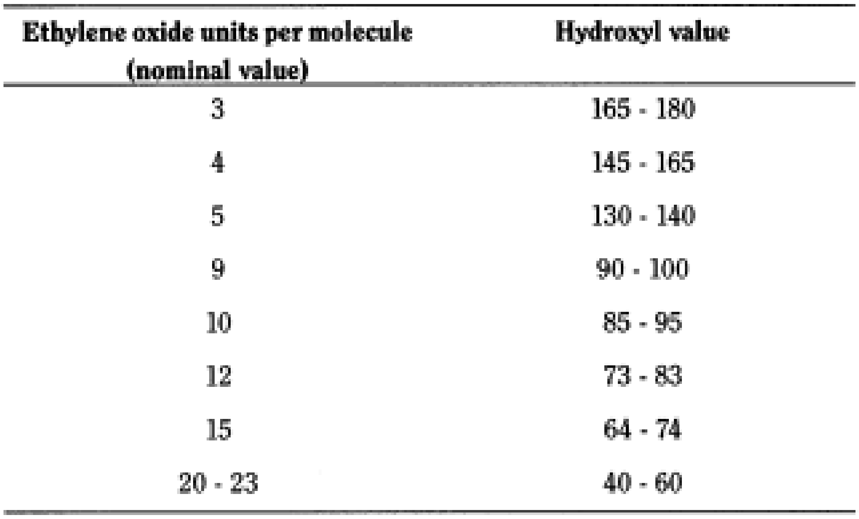
Alkalinity

Dissolve 2.0 g in a hot mixture of 10 ml of *water R* and 10 ml of *alcohol R*. Add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*).



Ethylene oxide units per molecule (nominal value)	Hydroxyl value
3	165 - 180
4	145 - 165
5	130 - 140
9	90 - 100
10	85 - 95
12	73 - 83
15	64 - 74
20 - 23	40 - 60

Iodine value (*2.5.4*)

Maximum 2.0.

Saponification value (*2.5.6*)

Maximum 3.0, determined on 10.0 g.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 2.00 g.

Total ash (*2.4.16*)

Maximum 0.2 per cent, determined on 2.0 g.

STORAGE

In an airtight container .

LABELLING

The label states the number of moles of ethylene oxide reacted per mole of $C_{12}H_{26}O$ (nominal value).

Ph Eur

Macrogol Oleate

General Notices

(Ph Eur monograph 1618)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

A mixture of monoesters and diesters of mainly oleic (*cis*-9-octadecenoic) acid and macrogols. It may be obtained by ethoxylation of *Oleic acid (0799)* or by esterification of macrogols with oleic acid of animal or vegetable origin. It may contain free macrogols. The average polymer length is equivalent to 5-6 or 10 moles of ethylene oxide per mole (nominal value). A suitable antioxidant may be added.

CHARACTERS

Appearance

Slightly yellowish, viscous liquid.

Solubility

Dispersible in water, soluble in ethanol (96 per cent) and in 2-propanol, dispersible in oils, miscible with fatty oils and with waxes.

Refractive index

About 1.466.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B.*

A. Saponification value (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution To 20 mg add 10 ml of *methylene chloride R* and mix.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase 25 per cent *V/V* solution of *concentrated ammonia R*, *2-propanol R* (20:80 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *potassium iodobismuthate solution R4*; examine the plate about 10 min later.

Results The chromatogram obtained shows 3 principal spots, corresponding, in order of increasing R_F value, to free macrogol, macrogol mono-oleate and macrogol dioleate.

C. Composition of fatty acids (see Tests).

TESTS

Alkalinity

Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 20 ml with the same solvent. To 2 ml of this solution add 0.05 ml of *phenol red solution R*. The solution is not red.

Acid value (*2.5.1*)

Maximum 2.0.

Hydroxyl value (*2.5.3, Method A*)

See Table 1618.-1.

Iodine value (*2.5.4, Method A*)

See Table 1618.-1.

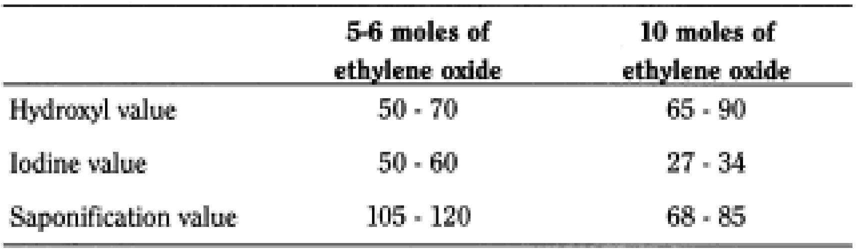
Peroxide value (*2.5.5, Method A*)

Maximum 12.0.

Saponification value (*2.5.6*)

See Table 1618.-1.

Table 1618.-1



	5-6 moles of ethylene oxide	**10 moles of ethylene oxide**
Hydroxyl value	50 - 70	65 - 90
Iodine value	50 - 60	27 - 34
Saponification value	105 - 120	68 - 85

Composition of fatty acids

Gas chromatography (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:

— *myristic acid*: maximum 5.0 per cent;

— *stearic acid*: maximum 6.0 per cent;

— *palmitic acid*: maximum 16.0 per cent;

— *palmitoleic acid*: maximum 8.0 per cent;

— *oleic acid*: 65.0 per cent to 88.0 per cent;

— *linoleic acid*: maximum 18.0 per cent;

— *linolenic acid*: maximum 4.0 per cent;

— *fatty acids with a chain length greater than* C_{18}: maximum 4.0 per cent.

Residual ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of residual ethylene oxide and 10 ppm of residual dioxan.

Water (*2.5.12*)

Maximum 2.0 per cent, determined on 1.00 g using *anhydrous methanol R* as the solvent.

Total ash (*2.4.16*)

Maximum 0.3 per cent, determined on 1.0 g.

STORAGE

In an airtight container .

LABELLING

The label states the number of moles of ethylene oxide per mole (nominal value).

Ph Eur

Macrogol Oleyl Ether

General Notices

(Ph Eur monograph 1125)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of ethers of mixed macrogols with linear fatty alcohols, mainly oleyl alcohol. It contains a variable amount of free oleyl alcohol and it may contain free macrogols. The number of moles of ethylene oxide reacted per mole of oleyl alcohol is 2 to 20 (nominal value) . A suitable antioxidant may be added.

CHARACTERS

— Macrogol oleyl ether with 2 to 5 units of ethylene oxide per molecule.

Appearance

Yellow liquid.

Solubility

Practically insoluble in water, soluble in alcohol, practically insoluble in light petroleum.

— Macrogol oleyl ether with 10 to 20 units of ethylene oxide per molecule.

Appearance

Yellowish-white waxy mass.

Solubility

Dispersible or soluble in water, soluble in alcohol, practically insoluble in light petroleum.

IDENTIFICATION

A. It complies with the test for hydroxyl value (see Tests).

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for saponification value (see Tests).

D. Dissolve or disperse 0.1 g in 5 ml of *alcohol R*, add 2 ml of *water R*, 10 ml of *dilute hydrochloric acid R*, 10 ml of *barium chloride solution R1* and 10 ml of a 100 g/l solution of *phosphomolybdic acid R*. A precipitate is formed.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 5.0 g in *alcohol R* and dilute to 50 ml with the same solvent.

Alkalinity

Dissolve 2.0 g in a hot mixture of 10 ml of *water R* and 10 ml of *alcohol R*. Add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

See Table 1125.-1.

Iodine value (*2.5.4*)

See Table 1125.-1.

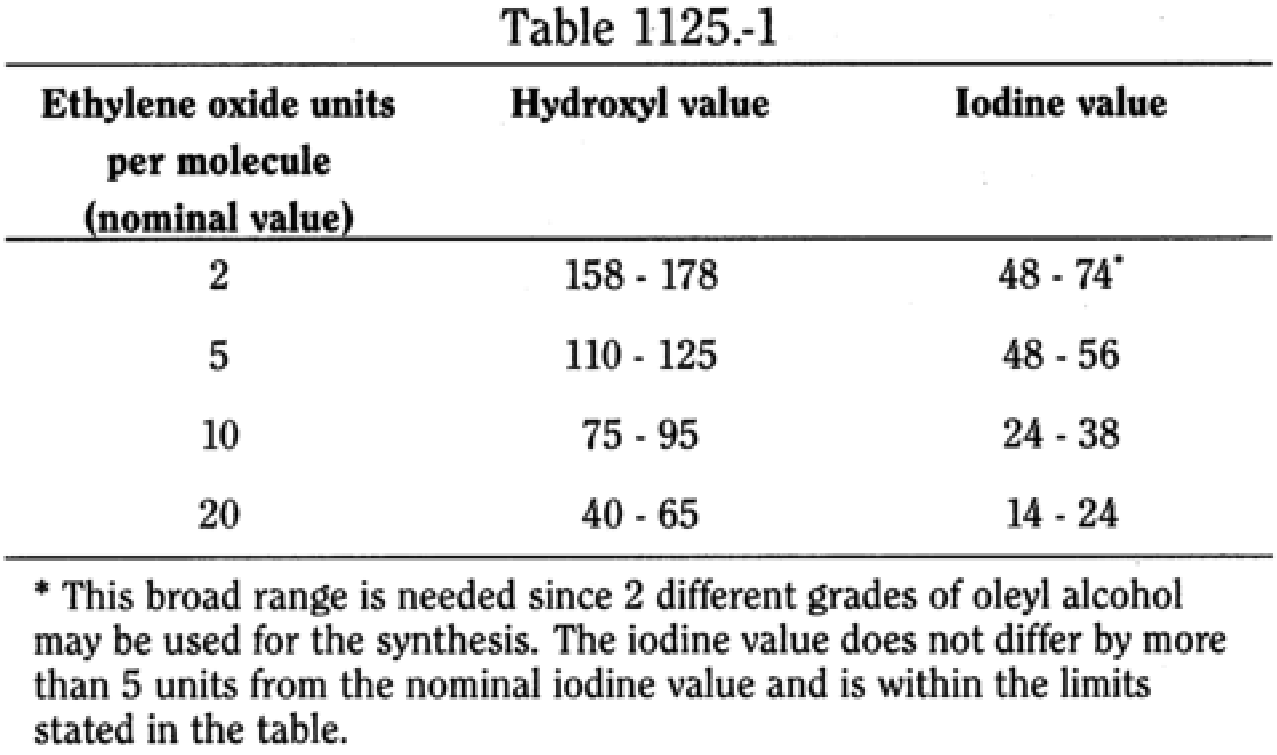


Table 1125.-1

Ethylene oxide units per molecule (nominal value)	Hydroxyl value	Iodine value
2	158 - 178	48 - 74*
5	110 - 125	48 - 56
10	75 - 95	24 - 38
20	40 - 65	14 - 24

* This broad range is needed since 2 different grades of oleyl alcohol may be used for the synthesis. The iodine value does not differ by more than 5 units from the nominal iodine value and is within the limits stated in the table.

Peroxide value (*2.5.5*)

Maximum 10.0.

Saponification value (*2.5.6*)

Maximum 3.0.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 2.00 g.

Total ash (*2.4.16*)

Maximum 0.2 per cent, determined on 2.0 g.

STORAGE

In an airtight container , protected from light.

LABELLING

The label states:

— the number of moles of ethylene oxide reacted per mole of oleyl alcohol (nominal value);

— the nominal iodine value for the type with 2 units of ethylene oxide per molecule.

Ph Eur

Macrogol Stearate

General Notices

(Ph Eur monograph 1234)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of monoesters and diesters of mainly stearic (octadecanoic) acid and/or palmitic (hexadecanoic) acid and macrogols. It may be obtained by ethoxylation or by esterification of macrogols with stearic acid 50 (type I) or stearic acid 95 (type II) (see *Stearic acid (1474)*). It may contain free macrogols. The average polymer length is equivalent to 6 to 100 ethylene oxide units per molecule (nominal value).

CHARACTERS

Appearance

White or slightly yellowish waxy mass.

Solubility

Soluble in alcohol and in 2-propanol. Macrogol stearate corresponding to a product with 6 to 9 units of ethylene oxide per molecule is practically insoluble, but freely dispersible in water and miscible with fatty oils and with waxes. Macrogol stearate corresponding to a product with 20 to 100 units of ethylene oxide per molecule is soluble in water and practically insoluble in fatty oils and in waxes.

IDENTIFICATION

A. It complies with the test for saponification value (see Tests).

B. It complies with the test for composition of fatty acids (see Tests).

TESTS

Alkalinity

Dissolve 2.0 g in *alcohol R* and dilute to 20 ml with the same solvent. To 2 ml of this solution add 0.05 ml of *phenol red solution R*. The solution is not red.

Melting point (*2.2.15*)

See Table 1234.-1.

Melt about 10 g at 80-90 °C. Introduce into the tube by capillary action, a sufficient amount of the substance, to form in the tube a column of the prescribed height. Allow to stand at 0 °C for 2 h.

Acid value (*2.5.1*)

Maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)

See Table 1234.-1.

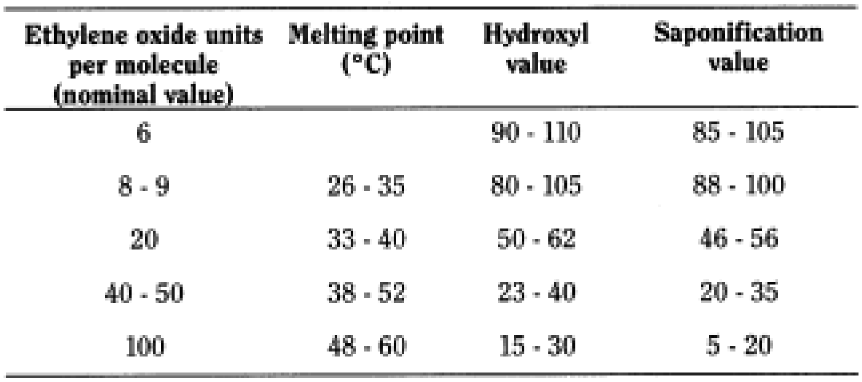
Iodine value (*2.5.4*)

Maximum 2.0.

Saponification value (*2.5.6*)

See Table 1234.-1.

Table 1234.-1



Ethylene oxide units per molecule (nominal value)	Melting point (°C)	Hydroxyl value	Saponification value
6		90 - 110	85 - 105
8 - 9	26 - 35	80 - 105	88 - 100
20	33 - 40	50 - 62	46 - 56
40 - 50	38 - 52	23 - 40	20 - 35
100	48 - 60	15 - 30	5 - 20

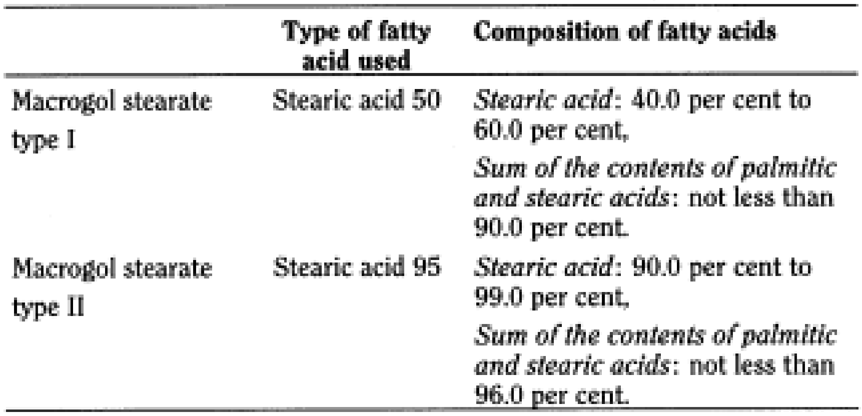
Reducing substances

Dissolve or disperse 2.0 g in *water R* and dilute to 20 ml with the same solvent. Mix 1.0 ml of the solution with 9 ml of *0.1 M sodium hydroxide* and 0.5 ml of *triphenyltetrazolium chloride solution R*. Heat in a water-bath at 70 °C. After 5 min, the solution is not more intensely coloured than a mixture of 0.15 ml of yellow primary solution, 0.9 ml of red primary solution and 8.95 ml of a 10 g/l solution of *hydrochloric acid R* (*2.2.2, Method II*).

Composition of fatty acids

Gas chromatography (*2.4.22, Method C*).

Composition of the fatty acid fraction of the substance:



	Type of fatty acid used	Composition of fatty acids
Macrogol stearate type I	Stearic acid 50	*Stearic acid*: 40.0 per cent to 60.0 per cent, *Sum of the contents of palmitic and stearic acids*: not less than 90.0 per cent.
Macrogol stearate type II	Stearic acid 95	*Stearic acid*: 90.0 per cent to 99.0 per cent, *Sum of the contents of palmitic and stearic acids*: not less than 96.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 0.50 g. Use as the solvent a mixture of equal volumes of *anhydrous methanol R* and *methylene chloride R*.

Total ash (*2.4.16*)

Maximum 0.3 per cent, determined on 1.0 g.

STORAGE

In an airtight container .

LABELLING

The label states:

— the number of ethylene oxide units per molecule (nominal value);

— the type of macrogol stearate.

Ph Eur

Macrogol Stearyl Ether

General Notices

(Ph Eur monograph 1340)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of ethers obtained by ethoxylation of stearyl alcohol. It may contain some free macrogols and various amounts of free stearyl alcohol. The number of moles of ethylene oxide reacted per mole of stearyl alcohol is 2 to 20 (nominal value).

CHARACTERS

Appearance

White or yellowish-white, waxy, unctuous mass, pellets, microbeads or flakes.

Solubility:

— macrogol stearyl ether with 2 moles of ethylene oxide reacted per mole: practically insoluble in water, soluble in ethanol (96 per cent) with heating and in methylene chloride;

— macrogol stearyl ether with 10 moles of ethylene oxide reacted per mole: soluble in water and in ethanol (96 per cent);

— macrogol stearyl ether with 20 moles of ethylene oxide reacted per mole: soluble in water, in ethanol (96 per cent) and in methylene chloride.

After melting, it solidifies at about 45 °C.

IDENTIFICATION

A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Saponification value (see Tests).

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10.0 g in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 75 ml with the same mixture of solvents. Add 60 ml of *heptane R* and shake for 3 min. The formation of foam can be reduced by the addition of a few drops of *ethanol (96 per cent) R*. Filter the upper layer through *anhydrous sodium sulphate R*, wash the filter with 3 quantities, each of 10 ml, of *heptane R* and evaporate the combined filtrates to dryness.

Dissolve 50 mg of the residue in 10 ml of *methanol R* (the solution may be opalescent).

Reference solution Dissolve 25 mg of *stearyl alcohol CRS* in *methanol R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R.*

Application 20 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with vanillin-sulphuric acid reagent prepared as follows: dissolve 0.5 g of *vanillin R* in 50 ml of *ethanol (96 per cent) R* and dilute to 100 ml with *sulphuric acid R*; allow to dry in air; heat at about 130 °C for 15 min and allow to cool in air.

Results The chromatogram obtained with the test solution shows several spots; one of these spots corresponds to the principal spot in the chromatogram obtained with the reference solution.

E. Dissolve or disperse 0.1 g in 5 ml of *ethanol 96 per cent R*, add 2 ml of *water R*, 10 ml of *dilute hydrochloric acid R*, 10 ml of *barium chloride solution R1* and 10 ml of a 100 g/l solution of *phosphomolybdic acid R*. A precipitate is formed.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 5.0 g in *ethanol (96 per cent) R* and dilute to 50 ml with the same solvent.

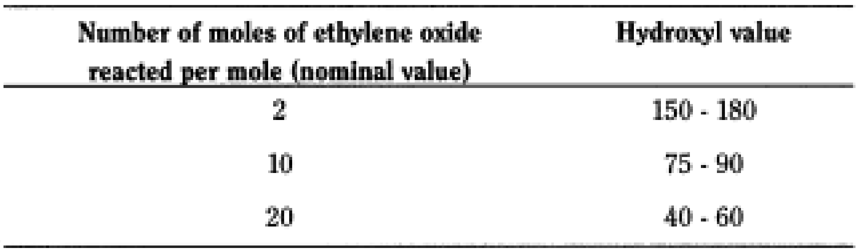
Alkalinity

Dissolve 2.0 g in a hot mixture of 10 ml of *ethanol (96 per cent) R* and 10 ml of *water R*. Add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*).



Number of moles of ethylene oxide reacted per mole (nominal value)	Hydroxyl value
2	150 - 180
10	75 - 90
20	40 - 60

Iodine value (*2.5.4, Method A*)

Maximum 2.0.

Saponification value (*2.5.6*)

Maximum 3.0, determined on 10.0 g.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 1.00 g.

STORAGE

In an airtight container .

LABELLING

The label states the number of moles of ethylene oxide reacted per mole of stearyl alcohol (nominal value).

Ph Eur

Macrogols

General Notices

(Ph Eur monograph 1444)

Action and use

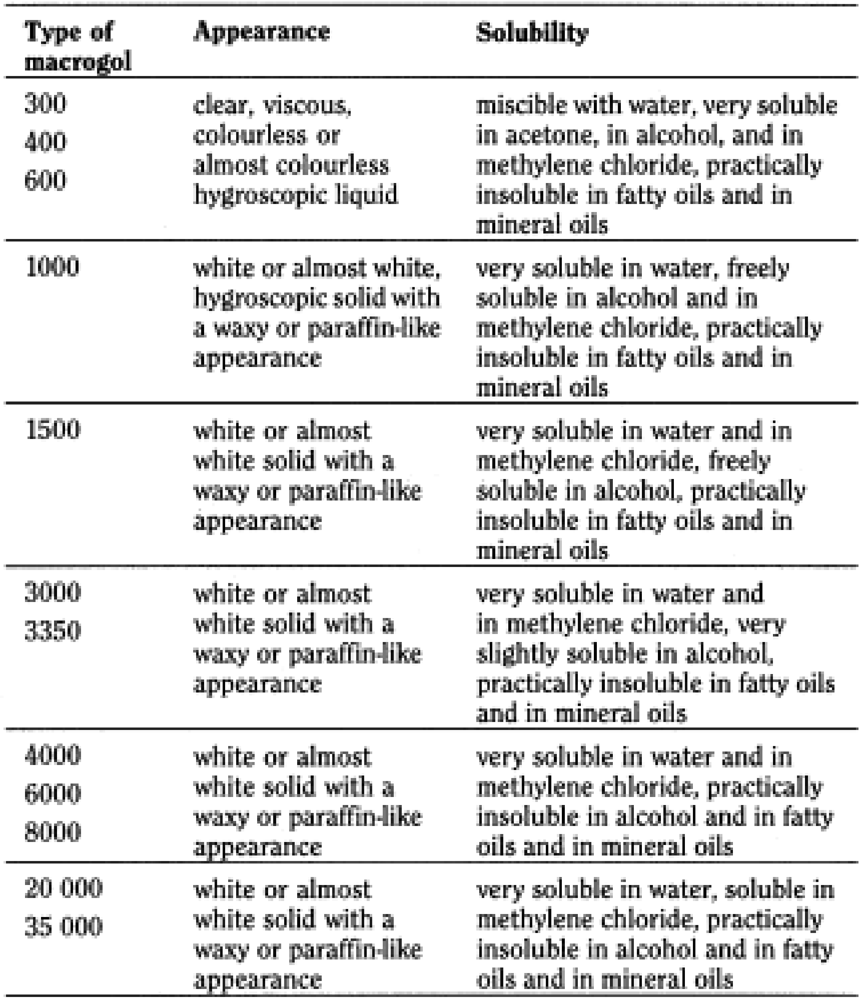
Non-ionic surfactant.

Ph Eur

DEFINITION

Mixtures of polymers with the general formula $H\text{-}(OCH_2\text{-}CH_2)_n\text{-}OH$ where *n* represents the average number of oxyethylene groups. The type of macrogol is defined by a number that indicates the average relative molecular mass. A suitable stabiliser may be added.

CHARACTERS



Type of macrogol	Appearance	Solubility
300 400 600	clear, viscous, colourless or almost colourless hygroscopic liquid	miscible with water, very soluble in acetone, in alcohol, and in methylene chloride, practically insoluble in fatty oils and in mineral oils
1000	white or almost white, hygroscopic solid with a waxy or paraffin-like appearance	very soluble in water, freely soluble in alcohol and in methylene chloride, practically insoluble in fatty oils and in mineral oils
1500	white or almost white solid with a waxy or paraffin-like appearance	very soluble in water and in methylene chloride, freely soluble in alcohol, practically insoluble in fatty oils and in mineral oils
3000 3350	white or almost white solid with a waxy or paraffin-like appearance	very soluble in water and in methylene chloride, very slightly soluble in alcohol, practically insoluble in fatty oils and in mineral oils
4000 6000 8000	white or almost white solid with a waxy or paraffin-like appearance	very soluble in water and in methylene chloride, practically insoluble in alcohol and in fatty oils and in mineral oils
20 000 35 000	white or almost white solid with a waxy or paraffin-like appearance	very soluble in water, soluble in methylene chloride, practically insoluble in alcohol and in fatty oils and in mineral oils

IDENTIFICATION

A. It complies with the test for viscosity (see Tests).

B. To 1 g in a test-tube add 0.5 ml of *sulphuric acid R*, close the test-tube with a stopper fitted with a bent delivery tube and heat until white fumes are evolved. Collect the fumes via the delivery tube into 1 ml of *mercuric chloride solution R*. An abundant white, crystalline precipitate is formed.

C. To 0.1 g add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R* and mix thoroughly with a glass rod. Add 5 ml of *methylene chloride R* and shake. The liquid phase becomes blue.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 12.5 g in *water R* and dilute to 50 ml with the same solvent.

Acidity or alkalinity

Dissolve 5.0 g in 50 ml of *carbon dioxide-free water R* and add 0.15 ml of *bromothymol blue* *solution R1*. The solution is yellow or green. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Viscosity (*2.2.9*)

The viscosity is calculated using a density given in Table 1444.-1.

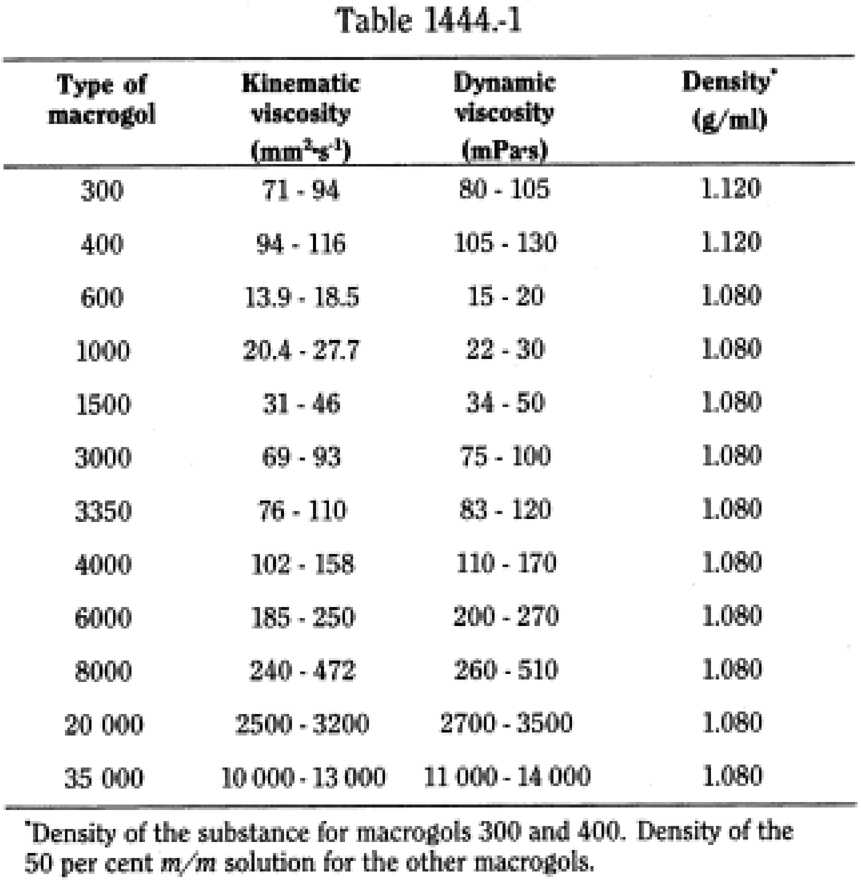


Table 1444.-1

Type of macrogol	Kinematic viscosity ($mm^2 s^{-1}$)	Dynamic viscosity (mPa·s)	Density* (g/ml)
300	71 - 94	80 - 105	1.120
400	94 - 116	105 - 130	1.120
600	13.9 - 18.5	15 - 20	1.080
1000	20.4 - 27.7	22 - 30	1.080
1500	31 - 46	34 - 50	1.080
3000	69 - 93	75 - 100	1.080
3350	76 - 110	83 - 120	1.080
4000	102 - 158	110 - 170	1.080
6000	185 - 250	200 - 270	1.080
8000	240 - 472	260 - 510	1.080
20 000	2500 - 3200	2700 - 3500	1.080
35 000	10 000 - 13 000	11 000 - 14 000	1.080

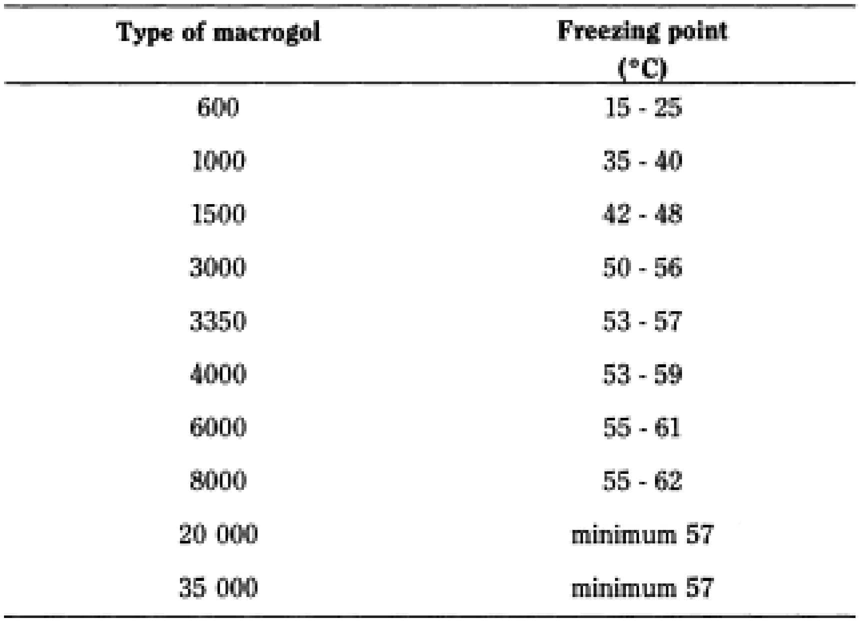
*Density of the substance for macrogols 300 and 400. Density of the 50 per cent *m/m* solution for the other macrogols.

For macrogols having a relative molecular mass greater than 400, determine the viscosity on a 50 per cent m/m solution of the substance to be examined.

Freezing point (*2.2.18*)

See Table 1444.-2.

Table 1444.-2



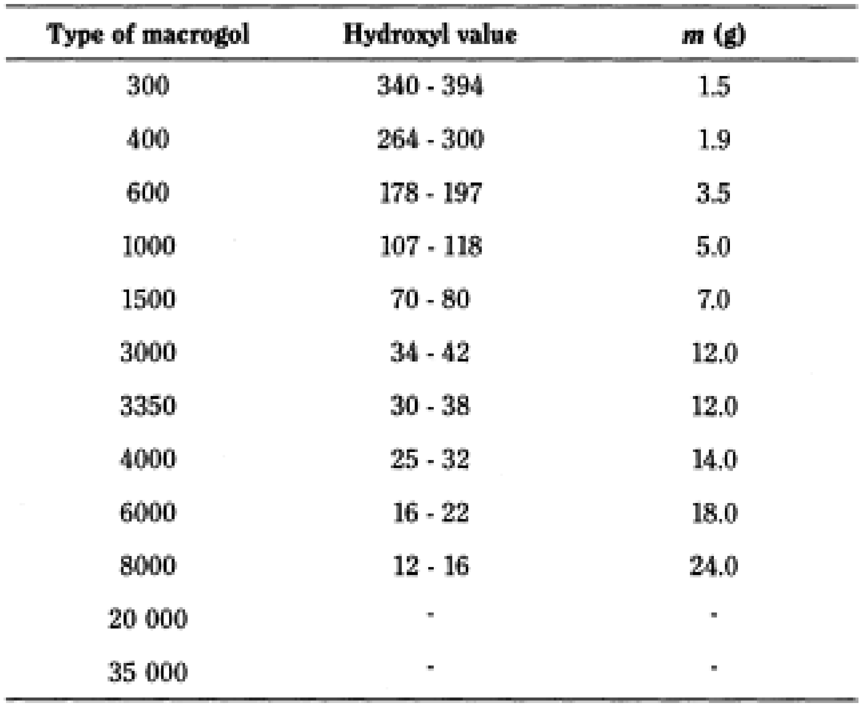
Type of macrogol	Freezing point (°C)
600	15 - 25
1000	35 - 40
1500	42 - 48
3000	50 - 56
3350	53 - 57
4000	53 - 59
6000	55 - 61
8000	55 - 62
20 000	minimum 57
35 000	minimum 57

Hydroxyl value

Introduce *m* g (see Table 1444.-3) into a dry conical flask fitted with a reflux condenser. Add 25.0 ml of *phthalic anhydride solution R*, swirl to dissolve and boil under a reflux condenser on a hot plate for 60 min. Allow to cool. Rinse the condenser first with 25 ml of *pyridine R* and then with 25 ml of *water R*, add 1.5 ml of *phenolphthalein solution R* and titrate with *1 M sodium hydroxide* until a faint pink colour is obtained (n_1 ml). Carry out a blank test (n_2 ml). Calculate the hydroxyl value using the expression:

$$\frac{56.1 \times (n_2 - n_1)}{m}$$

Table 1444.-3



Type of macrogol	Hydroxyl value	*m* (g)
300	340 - 394	1.5
400	264 - 300	1.9
600	178 - 197	3.5
1000	107 - 118	5.0
1500	70 - 80	7.0
3000	34 - 42	12.0
3350	30 - 38	12.0
4000	25 - 32	14.0
6000	16 - 22	18.0
8000	12 - 16	24.0
20 000	-	-
35 000	-	-

For macrogols having a relative molecular mass greater than 1000, if the water content is more than 0.5 per cent, dry a sample of suitable mass at 100-105 °C for 2 h and carry out the determination of the hydroxyl value on the dried sample.

Reducing substances

Dissolve 1 g in 1 ml of a 10 g/l solution of *resorcinol R* and warm gently if necessary. Add 2 ml of *hydrochloric acid R*. After 5 min the solution is not more intensely coloured than reference solution R_3 (*2.2.2, Method I*).

Formaldehyde

Maximum 30 ppm.

Test solution To 1.00 g add 0.25 ml of chromotropic acid, *sodium salt solution R*, cool in iced water and add 5.0 ml of *sulphuric acid R*. Allow to stand for 15 min and complete slowly to 10 ml with *water R*.

Reference solution Dilute 0.860 g of *formaldehyde solution R* to 100 ml with *water R*. Dilute 1.0 ml of this solution to 100 ml with *water R*. In a 10 ml flask, mix 1.00 ml of this solution with 0.25 ml of *chromotropic acid, sodium salt solution R*, cool in iced water and add 5.0 ml of *sulphuric acid R*. Allow to stand for 15 min and complete slowly to 10 ml with *water R*.

Blank solution In a 10 ml flask mix 1.00 ml of *water R* with 0.25 ml of chromotropic acid, *sodium salt solution R*, cool in iced water and add 5.0 ml of *sulphuric acid R*. Complete slowly to 10 ml with water *R*.

Determine the absorbance (*2.2.25*) of the test solution at 567 nm, against the blank solution. It is not higher than that of the reference solution.

If the use of macrogols with a higher content of formaldehyde may have adverse effects, the competent authority may impose a limit of not more than 15 ppm.

Ethylene glycol and diethylene glycol

Carry out this test only if the macrogol has a relative molecular mass below 1000.

Gas chromatography (*2.2.28*).

Test solution Dissolve 5.00 g of the substance to be examined in *acetone R* and dilute to 100.0 ml with the same solvent.

Reference solution Dissolve 0.10 g of *ethylene glycol R* and 0.50 g of *diethylene glycol R* in *acetone R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *acetone R*.

Column:
- *material*: glass;
- *size: l* = 1.8 m, Ø = 2 mm;
- *stationary phase*: *silanised diatomaceous earth for gas chromatography R*, impregnated with 5 per cent *m/m* of *macrogol 20 000 R*,

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 ml/min.

Temperature:
- *column*: if necessary, precondition the column by heating at 200 °C for about 15 h; adjust the initial temperature of the column to obtain a retention time of 14-16 min for diethylene glycol; raise the temperature of the column by about 30 °C at a rate of 2 °C/min but without exceeding 170 °C;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 2 µl.

Carry out 5 replicate injections to check the repeatability of the response.

Limit Maximum 0.4 per cent, calculated as the sum of the contents of ethylene glycol and diethylene glycol.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Water (*2.5.12*)

Maximum 2.0 per cent for macrogol with a relative molecular mass not greater than 1000 and maximum 1.0 per cent for macrogol with a relative molecular mass greater than 1000, determined on 2.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

STORAGE

In an airtight container.

LABELLING

The label states:

- — the type of macrogol;
- — the content of formaldehyde.

Ph Eur

Magaldrate

General Notices

(Ph Eur monograph 1539)

$Al_5Mg_{10}(OH)_{31}(SO_4)_2,xH_2O$ (anhydrous) 1097 *74978-16-8*

Action and use

Antacid.

Preparation

Magaldrate Oral Suspension

Ph Eur

DEFINITION

Magaldrate is composed of aluminium and magnesium hydroxides and sulphates. Its composition corresponds approximately to the formula $Al_5Mg_{10}(OH)_{31}(SO_4)_2,xH_2O$.

Content

90.0 per cent to 105.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It is soluble in dilute mineral acids.

IDENTIFICATION

A. Dissolve 0.6 g in 20 ml of *3 M hydrochloric acid R*, add about 30 ml of *water R* and heat to boiling. Adjust to pH 6.2 with *dilute ammonia R1*, continue boiling for a further 2 min, filter and retain the precipitate and the filtrate. To 2 ml of the filtrate add 2 ml of *ammonium chloride solution R* and neutralise with a solution prepared by dissolving 2 g of *ammonium carbonate R* and 2 ml of *dilute ammonia R1* in 20 ml of *water R*; no precipitate is produced. Add *disodium hydrogen phosphate solution R*; a white, crystalline precipitate is produced which does not dissolve in *dilute ammonia R1*.

B. The precipitate retained in identification test A gives the reaction of aluminium (*2.3.1*).

C. The filtrate retained in identification test A gives reaction (a) of sulphates (*2.3.1*).

TESTS

Soluble chlorides

Maximum 3.5 per cent.

Disperse 1 g in 50 ml of *water R*, boil for 5 min, cool, dilute again to 50.0 ml, mix and filter. To 25.0 ml of the filtrate add 0.2 ml of *potassium chromate solution R* and titrate with *0.1 M silver nitrate* until a persistent violet-red colour is obtained. Not more than 5.0 ml of *0.1 M silver nitrate* is required.

Soluble sulphates

Maximum 1.9 per cent.

To 2.5 ml of the filtrate obtained in the test for soluble chlorides, add 30 ml of *water R*, neutralise to *blue litmus paper R* with *hydrochloric acid R*, add 3 ml of *1 M hydrochloric acid*, 3 ml of a 120 g/l solution of *barium chloride R* and dilute to 50 ml with *water R*. Mix and allow to stand for 10 min. Any opalescence in the test solution is not more intense than that of a standard prepared at the same time in the same manner using 1 ml of *0.01 M sulphuric acid* instead of 2.5 ml of filtrate.

Sulphates

16.0 per cent to 21.0 per cent (dried substance).

Dissolve 0.875 g in a mixture of 5 ml of *glacial acetic acid R* and 10 ml of *water R* and dilute to 25 ml with *water R*. Prepare a chromatographic column of 1 cm in internal diameter containing 15 ml of *cation exchange resin R* (150-300 µm), previously washed with 30 ml of *water R*. Transfer 5.0 ml of the solution to be examined to the column and elute with 15 ml of *water R*. To the eluate add 5 ml of a 53.6 g/l solution of *magnesium acetate R*, 32 ml of *methanol R* and 0.2 ml of *alizarin S solution R*. Add from a burette about 4.0 ml of *0.05 M barium chloride*, add a further 0.2 ml of *alizarin S solution R* and slowly complete the titration until the yellow colour disappears and a violet-red tinge is visible.

1 ml of *0.05 M barium chloride* is equivalent to 4.803 mg of SO_4.

Aluminium hydroxide

32.1 per cent to 45.9 per cent (dried substance).

Dissolve 0.800 g in 10 ml of *dilute hydrochloric acid R1*, heating on a water-bath. Cool and dilute to 50.0 ml with *water R*. To 10.0 ml of this solution, add *dilute ammonia R1* until a precipitate begins to appear. Add the smallest quantity of *dilute hydrochloric acid R* needed to dissolve the precipitate and dilute to 20 ml with *water R*. Carry out the complexometric titration of aluminium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 7.80 mg of $Al(OH)_3$.

Magnesium hydroxide

49.2 per cent to 66.6 per cent (dried substance).

Dissolve 0.100 g in 2 ml of *dilute hydrochloric acid R* and carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 5.832 mg of $Mg(OH)_2$.

Sodium

Maximum 0.10 per cent.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Weigh 2.00 g into a 100 ml volumetric flask, place in an ice-bath, add 5 ml of *nitric acid R* and swirl to mix. Allow to warm to room temperature and dilute to 100 ml with *water R*. Filter, if necessary, to obtain a clear solution. Dilute 10.0 ml of the filtrate to 100.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *dilute nitric acid R*.

Source Sodium hollow-cathode lamp.

Wavelength 589 nm.

Atomisation device Air-acetylene flame.

Heavy metals (*2.4.8*)

Maximum 30 ppm.

Dissolve 2.0 g in 30 ml of *hydrochloric acid R1* and shake with 50 ml of *methyl isobutyl ketone R* for 2 min. Allow to stand, then separate and evaporate the aqueous layer to dryness. Dissolve the residue in 30 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying (*2.2.32*)

10.0 per cent to 20.0 per cent, determined on 1.000 g by drying in an oven at 200 °C for 4 h.

ASSAY

To 1.500 g add 50.0 ml of *1 M hydrochloric acid*. Titrate the excess hydrochloric acid with *1 M sodium hydroxide* to pH 3.0, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *1 M hydrochloric acid* is equivalent to 35.40 mg of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Ph Eur

Magnesium Acetate Tetrahydrate

General Notices

(Ph Eur monograph 2035)

$Mg(CH_3COO)_2,4H_2O$ 214.5 *16674-78-5*

Action and use

Used in dialysis solutions.

Ph Eur

DEFINITION

Content

98.0 per cent to 101.0 per cent of magnesium acetate (anhydrous substance).

CHARACTERS

Appearance

Colourless crystals or white or almost white, crystalline powder.

Solubility

Freely soluble in water and in alcohol.

IDENTIFICATION

A. Dissolve about 100 mg in 2 ml of *water R*. Add 1 ml of *dilute ammonia R1* and heat. A white precipitate is formed that dissolves slowly on addition of 5 ml of *ammonium chloride solution R*. Add 1 ml of *disodium hydrogen phosphate solution R*. A white crystalline precipitate is formed.

B. It gives reaction (b) of acetates (*2.3.1*).

TESTS

pH (*2.2.3*)

7.5 to 8.5.

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Chlorides (*2.4.4*)

Maximum 330 ppm.

Dissolve 1.0 g in *water R* and dilute to 100 ml with the same solvent. 15 ml of the solution complies with the limit test for chlorides.

Nitrates

Maximum 3 ppm.

Dissolve 1.0 g in *distilled water R* and dilute to 10 ml with the same solvent, add 5 mg of *sodium chloride R*, 0.05 ml of *indigo carmine solution R* and while stirring, 10 ml of *nitrogen-free sulphuric acid R*. A blue colour is produced which persists for at least 10 min.

Sulphates (*2.4.13*)

Maximum 600 ppm.

Dissolve 0.25 g in *distilled water R* and dilute to 15 ml with the same solvent.

Aluminium (*2.4.17*)

Maximum 1 ppm.

Prescribed solution Dissolve 4.0 g in *water R* and dilute to 100 ml with the same solvent. Add 10 ml of acetate buffer solution pH 6.0 R.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Calcium (*2.4.3*)

Maximum 100 ppm.

Dissolve 1.0 g in *distilled water R* and dilute to 15 ml with the same solvent.

Potassium

Maximum 0.10 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 0.5 g in *water R* and dilute to 100 ml with the same solvent.

Reference solutions Prepare the reference solutions using *potassium standard solution (600 ppm K) R*, diluted as necessary with *water R*.

Wavelength 766.5 nm.

Sodium

Maximum 0.50 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 1.0 g in *water R* and dilute to 100 ml with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589.0 nm.

Heavy metals (*2.4.8*)

Maximum 40 ppm.

Dissolve 1.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Readily oxidisable substances

Dissolve 2.0 g in 100 ml of boiling *water R*, add 6 ml of a 150 g/l solution of *sulphuric acid R* and 0.3 ml of *0.02 M potassium permanganate*. Mix and boil gently for 5 min. The pink colour is not completely discharged.

Water (*2.5.12*)

33.0 per cent to 35.0 per cent, determined on 0.100 g.

ASSAY

Dissolve 0.150 g in 300 ml of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

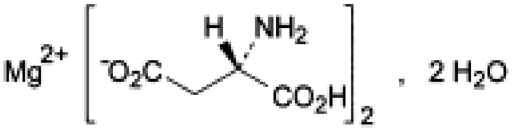
1 ml of *0.1 M sodium edetate* is equivalent to 14.24 mg of $C_4H_6MgO_4$.

Ph Eur

Magnesium Aspartate

General Notices

(Magnesium Aspartate Dihydrate, Ph Eur monograph 1445)



$C_8H_{12}MgN_2O_8,2H_2O$ 324.5 *72231-13-1*

Ph Eur

DEFINITION

Magnesium aspartate dihydrate contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of magnesium di[(*S*)-2-aminohydrogenobutane-1,4-dioate], calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Ignite about 15 mg until a white residue is obtained. Dissolve the residue in 1 ml of *dilute hydrochloric acid R*, neutralise to *red litmus paper R* by the addition of *dilute sodium hydroxide solution R* and filter if necessary. The solution gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 6.0 to 8.0.

Specific optical rotation (*2.2.7*)

Dissolve 0.50 g in a 515 g/l solution of *hydrochloric acid R* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 20.5 to + 23.0, calculated with reference to the anhydrous substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *magnesium aspartate dihydrate CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *magnesium aspartate dihydrate CRS* and 10 mg of *glutamic acid CRS* in 2 ml of *water R* and dilute to 25 ml with the same solvent.

Apply to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air and spray with *ninhydrin solution R*. Heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Chlorides (*2.4.4*)

Dilute 10 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates (*2.4.13*)

Dilute 12 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (500 ppm). Carry out the evaluation of the test after 30 min.

Ammonium (*2.4.1*)

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*)

In a separating funnel, dissolve 0.20 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (50 ppm).

Heavy metals (*2.4.8*)

Dissolve 2.0 g with gentle heating in 20 ml of *water R*. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*)

10.0 per cent to 14.0 per cent, determined on 0.100 g by the semi-micro determination of water. Dissolve the substance in 10 ml of *formamide R1* at 50 °C protected from moisture, add 10 ml of anhydrous *methanol R* and allow to cool. Carry out a blank determination.

ASSAY

Dissolve 0.260 g in 10 ml of *water R* and carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 28.85 mg of $C_8H_{12}MgN_2O_8$.

IMPURITIES

aspartic acid.

Ph Eur

Magnesium Chloride Hexahydrate

General Notices

(Ph Eur monograph 0402)

$M_gCl_2,6H_2O$ 203.3 *7791-18-6*

Action and use

Used in the treatment of electrolyte deficiencies and in dialysis solutions.

Preparation

Magnesium Chloride Injection

Ph Eur

DEFINITION

Content

98.0 per cent to 101.0 per cent of $MgCl_2,6H_2O$.

CHARACTERS

Appearance

Colourless crystals, hygroscopic.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Water (see Tests).

B. It gives reaction (a) of chlorides (*2.3.1*).

C. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 5 ml of solution S add 0.05 ml of *phenol red solution R*. Not more than 0.3 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides

Maximum 500 ppm.

Dilute 2.0 ml of solution S to 10.0 ml with *water R*. To 1.0 ml of this solution add 4.0 ml of *water R*, 2.0 ml of *phenol red solution R3* and 1.0 ml of *chloramine solution R2* and mix immediately. After exactly 2 min, add 0.30 ml of *0.1 M sodium thiosulphate*, mix and dilute to 10.0 ml with *water R*. The absorbance (*2.2.25*) of the solution measured at 590 nm, using *water R* as the compensation liquid, is not greater than that of a standard prepared at the same time and in the same manner using 5.0 ml of a 3 mg/l solution of *potassium bromide R*.

Sulphates (*2.4.13*)

Maximum 100 ppm, determined on solution S.

Aluminium (*2.4.17*)

Maximum 1 ppm, if intended for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions, or haemofiltration solutions.

Prescribed solution Dissolve 4 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 0.5 g.

Calcium (*2.4.3*)

Maximum 0.1 per cent.

Dilute 1 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S.

Potassium

Maximum 5.0×10^2 ppm, if intended for use in the manufacture of parenteral dosage forms.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using the following solution, diluted as necessary with *water R*: dissolve 1.144 g of *potassium chloride R*, previously dried at 100-105 °C for 3 h in *water R* and dilute to 1000.0 ml with the same solvent (600 µg of K per millilitre).

Wavelength 766.5 nm.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

51.0 per cent to 55.0 per cent, determined on 50.0 mg.

ASSAY

Dissolve 0.300 g in 50 ml of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 20.33 mg of $MgCl_2,6H_2O$.

STORAGE

In an airtight container .

LABELLING

The label states:

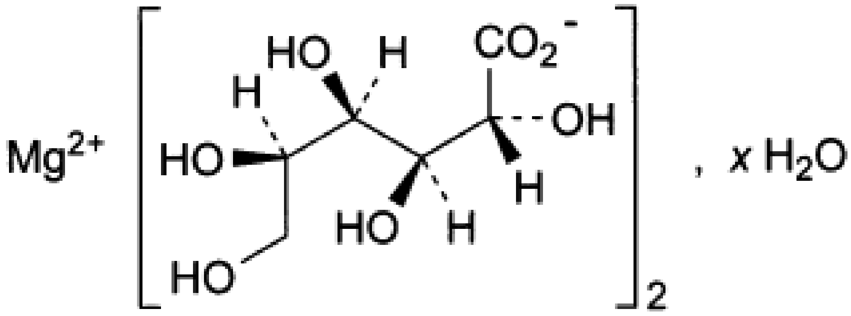
- — where applicable, that the substance is suitable for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions or haemofiltration solutions;
- — where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Magnesium Gluconate

General Notices

(Ph Eur monograph 2161)



$C_{12}H_{22}MgO_{14},xH_2O$ 414.6 *3632-91-5*
(anhydrous)

Ph Eur

DEFINITION

Anhydrous or hydrated magnesium D-gluconate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, amorphous, hygroscopic, crystalline or granular powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in 1 ml of *water R.*

Reference solution Dissolve 20 mg of *calcium gluconate CRS* in 1 ml of *water R*, heating if necessary in a water-bath at 60 °C.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *concentrated ammonia R*, *ethyl acetate R*, *water R*, *ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 1 µl.

Development Over 3/4 of the plate.

Drying At 100-105 °C for 20 min, then allow to cool to room temperature.

Detection Spray with a solution containing 25 g/l of *ammonium molybdate R* and 10 g/l of *cerium sulphate R* in *dilute sulphuric acid R*, then heat at 100-105 °C for about 10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. To 10 ml of solution S (see Tests) add 3 ml of *ammonium chloride solution R.* A slight opalescence may be observed. Add 10 ml of *disodium hydrogen phosphate solution R*. A white precipitate is formed that does not dissolve upon the addition of 2 ml of *dilute ammonia R1*.

TESTS

Solution S

Dissolve 1.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Sucrose and reducing sugars

Dissolve 0.5 g in a mixture of 2 ml of *hydrochloric acid R1* and 10 ml of *water R.* Boil for 5 min, allow to cool, add 10 ml of *sodium carbonate solution R* and allow to stand for 10 min. Dilute to 25 ml with *water R* and filter. To 5 ml of the filtrate add 2 ml of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 2 min. No red precipitate is formed.

Chlorides *(2.4.4)*

Maximum 500 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 500 ppm.

Dissolve 2.0 g in a mixture of 10 ml of *acetic acid R* and 90 ml of *distilled water R.*

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water *(2.5.32)*

Maximum 12.0 per cent, determined on 80 mg.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count.

ASSAY

Dissolve 0.350 g in 100 ml of *water R* and carry out the complexometric titration of magnesium *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 41.46 mg of $C_{12}H_{22}MgO_{14}$.

STORAGE

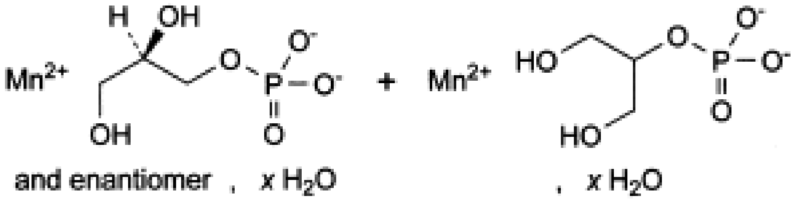
In an airtight container .

Ph Eur

Magnesium Glycerophosphate

General Notices

(Ph Eur monograph 1446)



$C_3H_7MgO_6P$ 194.4 *927-20-8*

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture, in variable proportions, of magnesium salts of (*RS*)-2,3-dihydroxypropyl phosphate and 2-hydroxy-1-(hydroxymethyl)ethyl phosphate, which may be hydrated.

Content

11.0 per cent to 12.5 per cent of Mg (dried substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Practically insoluble in ethanol (96 per cent). It dissolves in dilute solutions of acids.

IDENTIFICATION

A. Mix 1 g with 1 g of *potassium hydrogen sulphate R* in a test tube fitted with a glass tube. Heat strongly and direct the white vapour towards a piece of filter paper impregnated with a freshly prepared 10 g/l solution of *sodium nitroprusside R*. The filter paper develops a blue colour in contact with *piperidine R*.

B. Ignite 0.1 g in a crucible. Take up the residue with 5 ml of *nitric acid R* and heat on a water-bath for 1 min. Filter. The filtrate gives reaction (b) of phosphates (*2.3.1*).

C. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension III (*2.2.1*).

Acidity

Dissolve 1.0 g in 100 ml of *carbon dioxide-free water R*. Add 0.1 ml of *phenolphthalein solution R*. Not more than 1.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Glycerol and ethanol (96 per cent)-soluble substances

Maximum 1.5 per cent.

Shake 1.0 g with 25 ml of *ethanol (96 per cent) R* for 2 min. Filter and wash the residue with 5 ml of *ethanol (96 per cent) R*. Combine the filtrate and the washings, evaporate to dryness on a water-bath and dry the residue at 70 °C for 1 h. The residue weighs a maximum of 15 mg.

Chlorides (*2.4.4*)

Maximum 0.15 per cent.

Dissolve 1.0 g in *water R* and dilute to 100 ml with the same solvent. Dilute 3.5 ml of this solution to 15 ml with *water R.*

Phosphates (*2.4.11*)

Maximum 0.5 per cent.

Dilute 4 ml of solution S to 100 ml with *water R*. Dilute 1 ml of this solution to 100 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 0.1 per cent.

Dilute 3 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 150 ppm.

Dissolve 67 mg in *water R* and dilute to 10 ml with the same solvent.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

To 20 ml of solution S add 15 ml of *hydrochloric acid R* and shake with 25 ml of *methyl isobutyl ketone R* for 2 min. Allow to stand, then separate and evaporate the aqueous layer to dryness. Dissolve the residue in 2.5 ml of *acetic acid R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g by drying in an oven at 150 °C for 4 h.

ASSAY

Dissolve 0.200 g in 40 ml of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

STORAGE

In an airtight container .

Ph Eur

Magnesium Hydroxide

General Notices

(Ph Eur monograph 0039)

$Mg(OH)_2$ 58.32 *1309-42-8*

Action and use

Antacid; osmotic laxative.

Preparations

Co-magaldrox Oral Suspension

Co-magaldrox Tablets

Ph Eur

DEFINITION

Content

95.0 per cent to 100.5 per cent of $Mg(OH)_2$.

CHARACTERS

Appearance

White or almost white, fine, amorphous powder.

Solubility

Practically insoluble in water. It dissolves in dilute acids.

IDENTIFICATION

A. Dissolve about 15 mg in 2 ml of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

B. It complies with the test for loss on ignition (see Tests).

TESTS

Solution S

Dissolve 5.0 g in a mixture of 50 ml of *acetic acid R* and 50 ml of *distilled water R*. Not more than slight effervescence is produced. Boil for 2 min, cool and dilute to 100 ml with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate.

Appearance of solution

Solution S is not more intensely coloured than reference solution B_3 (*2.2.2, Method II*).

Soluble substances

Maximum 2.0 per cent.

Mix 2.00 g with 100 ml of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 ml with *water R*. Evaporate 50 ml of the filtrate to dryness and dry at 100-105 °C. The residue weighs not more than 20 mg.

Substances insoluble in acetic acid

Maximum 0.1 per cent.

Any residue obtained during the preparation of solution S, washed, dried, and ignited at 600 ± 50 °C, weighs not more than 5 mg.

Chlorides (*2.4.4*)

Maximum 0.1 per cent.

1 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides.

Sulphates (*2.4.13*)

Maximum 0.5 per cent.

0.6 ml of solution S diluted to 15 ml with *distilled water R* complies with the limit test for sulphates.

Arsenic (*2.4.2*)

Maximum 4 ppm.

5 ml of solution S complies with limit test A.

Calcium (*2.4.3*)

Maximum 1.5 per cent.

Dilute 1.3 ml of solution S to 150 ml with *distilled water R*. 15 ml of the solution complies with the limit test for calcium.

Iron (*2.4.9*)

Maximum 0.07 per cent.

Dissolve 0.15 g in 5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. 1 ml of this solution diluted to 10 ml with *water R* complies with the limit test for iron.

Heavy metals (*2.4.8*)

Maximum 30 ppm.

Dissolve 2.0 g in 20 ml of *hydrochloric acid R1* and shake with 25 ml of *methyl isobutyl ketone R* for 2 min. Allow to stand, separate the aqueous layer and evaporate to dryness. Dissolve the residue in 30 ml of *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Loss on ignition

29.0 per cent to 32.5 per cent.

Heat 0.5 g gradually to 900 ± 50 °C and ignite to constant mass.

ASSAY

Dissolve 0.100 g in a mixture of 20 ml of *water R* and 2 ml of *dilute hydrochloric acid R* and carry out the complexometric titration of magnesium (*2.5.11*).

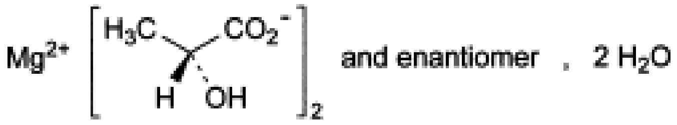
1 ml of *0.1 M sodium edetate* is equivalent to 5.832 mg of $Mg(OH)_2$.

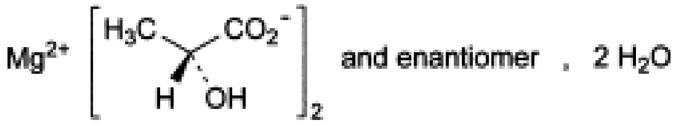
Ph Eur

Magnesium Lactate Dihydrate

General Notices

(Ph Eur monograph 2160)





$C_6H_{10}MgO_6,2H_2O$ 238.5 *18917-93-6*

Ph Eur

DEFINITION

Magnesium bis(2-hydroxypropanoate) or mixture of magnesium (2*R*)-, (2*S*)- and (2*RS*)-2-hydroxypropanoate dihydrate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline or granular powder.

Solubility

Slightly soluble in water, soluble in boiling water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives the reaction of lactates (*2.3.1*).

B. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g with heating in *carbon dioxide-free water R* prepared from *distilled water R*, allow to cool and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

6.5 to 8.5 for solution S.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 400 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 50 ppm.

Dilute 4 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

14.0 per cent to 17.0 per cent, determined on 0.500 g by drying in an oven at 125 °C.

ASSAY

Dissolve 0.180 g in *water R* and dilute to 300 ml with the same solvent. Carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 20.25 mg of $C_6H_{10}MgO_6$.

Ph Eur

Magnesium Peroxide

General Notices

(Ph Eur monograph 1540)

Ph Eur

DEFINITION

Mixture of magnesium peroxide and magnesium oxide.

Content

22.0 per cent to 28.0 per cent of MgO2 (M_r 56.30).

CHARACTERS

Appearance

White or slightly yellow, amorphous, light powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION

A. Dissolve about 15 mg in 2 ml of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

B. Dissolve 0.1 g in 2 ml of *dilute sulphuric acid R* and dilute to 10 ml with *water R*. Shake 1 ml of this solution with 5 ml of *ether R* and 0.5 ml of *potassium dichromate solution R1*. The ether layer is blue.

TESTS

Solution S1

Dissolve cautiously 5.0 g in 40 ml of *hydrochloric acid R1*. Cautiously evaporate the solution to 10 ml and dilute to 100 ml with a mixture of equal volumes of *acetic acid R* and distilled *water R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate. Keep the residue for the test for acid insoluble substances.

Solution S2

Dilute 5 ml of solution S1 to 25 ml with *distilled water R*.

Appearance of solution

Solution S1 is not more intensely coloured than reference solution B_4 (*2.2.2, Method II*).

Acidity or alkalinity

To 2.0 g add 100 ml of *carbon dioxide-free water R* and heat to boiling for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 ml with *carbon dioxide-free water R*. To 15 ml of the filtrate, add 0.1 ml of *phenolphthalein solution R*. The solution is red. Not more than 0.2 ml of *0.1 M hydrochloric acid* is necessary to change the colour of the indicator. Keep the filtrate for the test for soluble substances.

Acid insoluble substances

Maximum 0.1 per cent.

Any residue obtained during the preparation of solution S1, washed, dried and ignited at 600 ± 50 °C, weighs a maximum of 5 mg.

Soluble substances

Maximum 1.5 per cent.

Take 50 ml of the filtrate obtained in the test for acidity or alkalinity, evaporate to dryness and dry at 100-105 °C. The residue weighs a maximum of 15 mg.

Chlorides (*2.4.4*)

Maximum 0.1 per cent.

Dissolve 50 mg in 5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 0.5 per cent.

Dilute 3 ml of solution S2 to 15 ml with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 4 ppm, determined on 5 ml of solution S1.

Calcium (*2.4.3*)

Maximum 1.0 per cent.

Dilute 1 ml of solution S2 to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 500 ppm.

Dilute 2 ml of solution S2 to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 30 ppm.

To 20 ml of solution S1 add 15 ml of *hydrochloric acid R1* and shake with 25 ml of *methyl isobutyl ketone R* for 2 min. Allow to stand, then separate and evaporate the aqueous layer to dryness. Dissolve the residue in 1.5 ml of *acetic acid R* and dilute to 30 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 80.0 mg, shaking cautiously, in a mixture, previously cooled to 20 °C, of 10 ml of *sulphuric acid R* and 90 ml of *water R*. Titrate with *0.02 M potassium permanganate* until a pink colour is obtained.

1 ml of *0.02 M potassium permanganate* is equivalent to 2.815 mg of MgO_2.

STORAGE

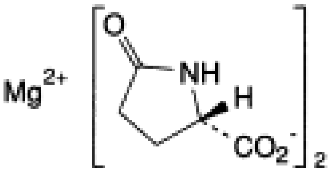
Protected from light.

Ph Eur

Magnesium Pidolate

General Notices

(Ph Eur monograph 1619)



$C_{10}H_{12}N_2O_6Mg$ 280.5 *62003-27-4*

Ph Eur

DEFINITION

Magnesium bis[(2*S*)-5-oxopyrrolidine-2-carboxylate].

Content

8.49 per cent to 8.84 per cent of Mg (A_r = 24.31) (anhydrous substance).

CHARACTERS

Appearance

Amorphous, white or almost white powder, hygroscopic.

Solubility

Very soluble in water, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 60 mg in 2 ml of *water R* and dilute to 10 ml with *methanol R*.

Reference solution Dissolve 55 mg of *pidolic acid CRS* in 2 ml of *water R* and dilute to 10 ml with *methanol R*.

Plate TLC silica gel plate R.

Mobile phase methanol R, *glacial acetic acid R*, *methylene chloride R* (15:20:65 *V/V/V*).

Application 1 µl.

Development Over 2/3 of the plate.

Drying At 100-105 °C for 15 min.

Detection Spray with *concentrated sodium hypochlorite solution R*. Allow to stand for 10 min and spray abundantly with *glacial acetic acid R*. Allow to stand again for 10 min and dry the plate at 100-105 °C for 2 min. Spray with *potassium iodide and starch solution R* until spots appear.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution. The chromatogram obtained with the test solution may show 2 faint secondary spots.

B. To 0.15 ml of solution S (see Tests) add 1.8 ml of *water R*. The solution gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 5.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method I*).

pH (*2.2.3*)

5.5 to 7.0 for solution S.

Specific optical rotation (*2.2.7*)

- 23.3 to - 26.5 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.500 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 50.0 mg *pidolate impurity B CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c) Dilute 10.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of *nitrate standard solution (100 ppm NO_3) R* to 100.0 ml with the mobile phase.

Reference solution (e) Dilute 6.0 ml of reference solution (a) to 10.0 ml with reference solution (b).

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.56 g of *sodium dihydrogen phosphate R* in 1000 ml of *water R* and adjust to pH 2.5 with a 10 per cent *V/V* solution of *phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl loop injector; inject the test solution and reference solutions (b), (c), (d) and (e).

Run time 4 times the retention time of pidolic acid.

Retention times Pidolic acid = about 4.5 min; impurity B = about 7.5 min.

System suitability Reference solution (e):

— *resolution*: minimum 10 between the peaks due to pidolic acid and to impurity B.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *total of other impurities*: not more than half of the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak corresponding to the nitrate ion (NO_3^-).

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.250 g of the substance to be examined in 4 ml of *water R* and dilute to 50.0 ml with *methanol R*.

Reference solution (a) Dissolve 60.0 mg of *glutamic acid R* in 50 ml of *water R* and dilute to 100.0 ml with *methanol R*. Dilute 1.0 ml of the solution to 20.0 ml with *methanol R*.

Reference solution (b) Dissolve 10 mg of *glutamic acid R* and 10 mg of *aspartic acid R* in *water R* and dilute to 25 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *water R.*

Plate TLC silica gel plate R.

Mobile phase glacial acetic acid R, *water R*, *butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Limit:

— *impurity A*: any spot corresponding to impurity A in the chromatogram obtained with the test solution is not more intense that the spot in the chromatogram obtained with reference solution (a) (0.6 per cent).

Chlorides (*2.4.4*)

Maximum 500 ppm.

Dilute 1.0 ml of solution S to 15.0 ml of *water R*. The solution complies with the limit test for chlorides.

Nitrates

Examine the chromatogram obtained with the test solution in the test for related substances.

Limit:

— *nitrates*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (200 ppm).

Sulphates (*2.4.13*)

Maximum 0.1 per cent.

Dilute 1.5 ml of solution S to 15.0 ml with *distilled water R*. The solution complies with the limit test for sulphates.

Arsenic (*2.4.2*)

Maximum 2 ppm.

5.0 ml of solution S complies with limit test A.

Iron (*2.4.9*)

Maximum 200 ppm.

Dilute 0.5 ml of solution S to 10 ml of *water R*. The solution complies with the limit test for iron.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Water (*2.5.12*)

Maximum 8.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.300 g in 50 ml of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

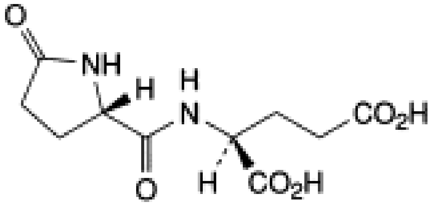
1 ml of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

STORAGE

In an airtight container .

IMPURITIES

A. glutamic acid,


O
NH
H
H
N
CO_2H
O
H
CO_2H

B. (2*S*)-2-[[[(2*S*)-5-oxopyrrolidin-2-yl]carbonyl]amino]pentanedioic acid.

Ph Eur

Magnesium Stearate

General Notices

(Ph Eur monograph 0229)

Action and use

Excipient.

Ph Eur

DEFINITION

Magnesium stearate is a mixture of magnesium salts of different fatty acids consisting mainly of stearic (octadecanoic) acid [$(C_{17}H_{35}COO)_2Mg$; M_r 591.3] and palmitic (hexadecanoic) acid [$(C_{15}H_{31}COO)_2Mg$; M_r 535.1] with minor proportions of other fatty acids. It contains not less than 4.0 per cent and not more than 5.0 per cent of Mg (A_r 24.30), calculated with reference to the dried substance. The fatty acid fraction contains not less than 40.0 per cent of stearic acid and the sum of stearic acid and palmitic acid is not less than 90.0 per cent.

CHARACTERS

A white or almost white, very fine, light powder, greasy to the touch, practically insoluble in water and in ethanol.

IDENTIFICATION

First identification C, D.

Second identification A, B, D.

A. The residue obtained in the preparation of solution S (see Tests) has a freezing point (*2.2.18*) not lower than 53 °C.

B. The acid value of the fatty acids (*2.5.1*) is 195 to 210, determined on 0.200 g of the residue obtained in the preparation of solution S dissolved in 25 ml of the prescribed mixture of solvents.

C. Examine the chromatograms obtained in the test for fatty acid composition. The retention times of the principal peaks in the chromatogram obtained with the test solution are approximately the same as those of the principal peaks in the chromatogram obtained with the reference solution.

D. 1 ml of solution S gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

To 5.0 g add 50 ml of *peroxide-free ether R*, 20 ml of *dilute nitric acid R* and 20 ml of *distilled water R* and heat under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 4 ml, of *distilled water R*. Combine the aqueous layers, wash with 15 ml of *peroxide-free ether R* and dilute to 50 ml with *distilled water R* (solution S). Evaporate the organic layer to dryness and dry the residue at 100-105°C. Keep the residue for identification tests A and B.

Acidity or alkalinity

To 1.0 g add 20 ml of *carbon dioxide-free water R* and boil for 1 min with continuous stirring. Cool and filter. To 10 ml of the filtrate add 0.05 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*)

0.5 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides (0.1 per cent).

Sulphates (*2.4.13*)

0.3 ml of solution S diluted to 15 ml with *distilled water R* complies with the limit test for sulphates (0.5 per cent).

Cadmium

Not more than 3.0 ppm of Cd, determined by atomic absorption spectrometry (*2.2.23, Methoa II*).

Test solution Place 50.0 mg of the substance to be examined in a polytetrafluoroethylene digestion bomb and add 0.5 ml of a mixture of 1 volume of *hydrochloric acid R* and 5 volumes of *cadmium- and lead-free nitric acid R*. Allow to digest at 170 °C for 5 h. Allow to cool. Dissolve the residue in *water R* and dilute to 5.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *cadmium standard solution (10 ppm Cd) R,* diluted as necessary with a 1 per cent *V/V* solution of *hydrochloric acid R*.

Measure the absorbance at 228.8 nm, using a cadmium hollow-cathode lamp as a source of radiation and a graphite furnace as atomic generator.

Lead Not more than 10.0 ppm of Pb, determined by atomic absorption spectrometry (*2.2.23*, *Method II*).

Test solution Use the solution described in the test for cadmium.

Reference solutions Prepare the reference solutions using *lead standard solution (10 ppm Pb) R*, diluted as necessary with *water R*.

Measure the absorbance at 283.3 nm, using a lead hollow-cathode lamp as a source of radiation and a graphite furnace as atomic generator, depending on the apparatus the line at 217.0 nm may be used.

Nickel

Not more than 5.0 ppm of Ni, determined by atomic absorption spectrometry (*2.2.23, Methoa II*).

Test solution Use the solution described in the test for cadmium.

Reference solutions Prepare the reference solutions using *nickel standard solution (10 ppm*

Ni) R, diluted as necessary with *water R*.

Measure the absorbance at 232.0 nm, using a nickel hollow-cathode lamp as a source of radiation and a graphite furnace as atomic generator.

Loss on drying (*2.2.32*)

Not more than 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 10^3 micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli* (*2.6.13*).

ASSAY

MAGNESIUM

To 0.500 g in a 250 ml conical flask add 50 ml of a mixture of equal volumes of *butanol R* and *ethanol R*, 5 ml of *concentrated ammonia R*, 3 ml of *ammonium chloride buffer solution pH 10.0 R*, 30.0 ml of *0.1 M sodium edetate* and 15 mg of *mordant black 11 triturate R*. Heat to 45-50 °C until the solution is clear and titrate with *0.1 M zinc sulphate* until the colour changes from blue to violet. Carry out a blank titration.

1 ml of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

FATTY ACID COMPOSITION

Examine by gas chromatography (*2.2.28*).

Test solution In a conical flask fitted with a reflux condenser, dissolve 0.10 g of the substance to be examined in 5 ml of *boron trifluoride-methanol solution R*. Boil under a reflux condenser for 10 min. Add 4 ml of *heptane R* through the condenser and boil again under a reflux condenser for 10 min. Allow to cool. Add 20 ml of a *saturated sodium chloride solution R*. Shake and allow the layers to separate. Remove about 2 ml of the organic layer and dry over 0.2 g of *anhydrous sodium sulphate R*. Dilute 1.0 ml of the solution to 10.0 ml with *heptane R*.

Reference solution Prepare the reference solution in the same manner as the test solution using 50.0 mg of *palmitic acid CRS* and 50.0 mg of *stearic acid CRS* instead of magnesium stearate.

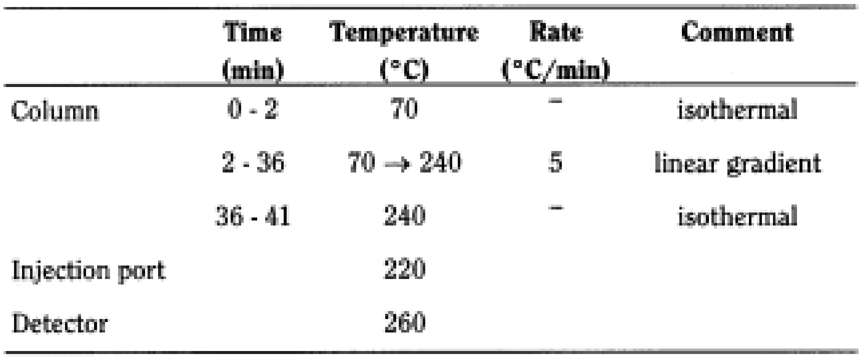
The chromatographic procedure may be carried out using:

— a fused-silica column 30 m long and 0.32 mm in internal diameter coated with macrogol 20 000 R (film thickness 0.5 µm);

— *helium for chromatography R* as the carrier gas at a flow rate of 2.4 ml/min;

— a flame-ionisation detector,

with the following temperature programme:



	Time (min)	Temperature (°C)	Rate (°C/min)	Comment
Column	0 - 2	70	–	isothermal
	2 - 36	70 → 240	5	linear gradient
	36 - 41	240	–	isothermal
Injection port		220		
Detector		260		

Inject 1 µl of the reference solution. When the chromatogram is recorded in the prescribed conditions, the relative retention of methyl palmitate to that of methyl stearate is about 0.88. The test is not valid unless, in the chromatogram obtained with the reference solution, the resolution between the peaks corresponding to methyl stearate and methyl palmitate is at least 5.0.

Inject 1 µl of the test solution. Calculate the percentage content of stearic acid and palmitic acid from the areas of the peaks in the chromatogram obtained with the test solution by the normalisation procedure, disregarding the peak due to the solvent.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for magnesium stearate used as a lubricant in solid dosage forms (compressed and powder).

Specific surface area (*2.9.26, Method I*)

Determine the specific surface area in the P/P_o range of 0.05 to 0.15.

Sample outgassing 2 h at 40 °C.

Ph Eur

Magnesium Sulphate Heptahydrate

General Notices

Epsom Salts

(Ph Eur monograph 0044)

$MgSO_4,7H_2O$ 246.5 *10034-99-8*

Action and use

Osmotic laxative; used in treatment of electrolyte deficiency.

Preparations

Magnesium Sulphate Injection

Magnesium Sulphate Mixture

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or brilliant, colourless crystals.

Solubility

Freely soluble in water, very soluble in boiling water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives the reactions of sulphates (*2.3.1*).

B. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *phenol red solution R*. Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*)

Maximum 300 ppm.

Dilute 1.7 ml of solution S to 15 ml with *water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 0.5 g.

Iron (*2.4.9*)

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

48.0 per cent to 52.0 per cent, determined on 0.500 g by drying in an oven at 110-120 °C for 1 h and then at 400 °C to constant mass.

ASSAY

Dissolve 0.450 g in 100 ml of *water R* and carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 12.04 mg of $MgSO_4$.

Ph Eur

Magnesium Trisilicate

General Notices

(Ph Eur monograph 0403)

Action and use

Antacid.

Preparations

Magnesium Trisilicate Mixture

Compound Magnesium Trisilicate Oral Powder

Compound Magnesium Trisilicate Tablets

Ph Eur

DEFINITION

It has a variable composition corresponding approximately to $Mg_2Si_3O_8,xH_2O$.

Content:

— *magnesium oxide* (MgO; M_r 40.30): minimum 29.0 per cent (ignited substance),

— *silicon dioxide* (SiO_2; M_r 60.1): minimum 65.0 per cent (ignited substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. 10.25 g gives the reaction of silicates (*2.3.1*).

B. 1 ml of solution S (see Tests) neutralised with *dilute sodium hydroxide solution R* gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

To 2.0 g add a mixture of 4 ml of *nitric acid R* and 4 ml of *distilled water R*. Heat to boiling with frequent shaking. Add 12 ml of *distilled water R* and allow to cool. Filter or centrifuge to obtain a clear solution and dilute to 20 ml with *distilled water R*.

Alkalinity

To 10.0 g in a 200 ml conical flask, add 100.0 g of *water R* and heat on a water-bath for 30 min. Allow to cool and make up to the initial mass with *water R*. Allow to stand and filter or centrifuge until a clear liquid is obtained. To 10 ml of this liquid add 0.1 ml of *phenolphthalein solution R*. Not more than 1.0 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Water-soluble salts

Maximum 1.5 per cent.

In a platinum dish, evaporate to dryness on a water-bath 20.0 ml of the liquid obtained in the test for alkalinity. The residue, ignited to constant mass at 900 ± 50 °C, weighs a maximum of 30 mg.

Chlorides (*2.4.4*)

Maximum 500 ppm.

Dilute 0.5 ml of solution S to 15 ml with *water R*. Prepare the standard using a mixture of 5 ml of *chloride standard solution (5 ppm Cl) R* and 10 ml of *water R*.

Sulphates (*2.4.13*)

Maximum 0.5 per cent.

Dilute 0.3 ml of solution S to 15 ml with *distilled water R*.

Arsenic (*2.4.2, method A*)

Maximum 4 ppm, determined on 2.5 ml of solution S.

Heavy metals (*2.4.8*)

Maximum 40 ppm.

Neutralise 10 ml of solution S with *dilute ammonia R1*, using *metanil yellow solution R* as an external indicator. Dilute to 20 ml with *water R* and filter if necessary. 12 ml of this solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Loss on ignition

17 per cent to 34 per cent, determined on 0.5 g by ignition to constant mass at 900 ± 50 °C in a platinum crucible.

Acid-absorbing capacity

Suspend 0.25 g in *0.1 M hydrochloric acid*, dilute to 100.0 ml with the same acid and allow to stand for 2 h in a water-bath at 37 ± 0.5 °C, with frequent shaking. Allow to cool. To 20.0 ml of the supernatant solution add 0.1 ml of *bromophenol blue solution R* and titrate with *0.1 M sodium hydroxide* until a blue colour is obtained. The acid-absorbing capacity is not less than 100.0 ml of *0.1 M hydrochloric acid* per gram.

ASSAY

Magnesium oxide

To 1.000 g in a 200 ml conical flask, add 35 ml of *hydrochloric acid R* and 60 ml of *water R* and heat in a water-bath for 15 min. Allow to cool, filter, wash the conical flask and the residue with *water R* and dilute the combined filtrate and washings to 250.0 ml with *water R*. Neutralise 50.0 ml of the solution with *strong sodium hydroxide solution R* (about 8 ml). Carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

Silicon dioxide

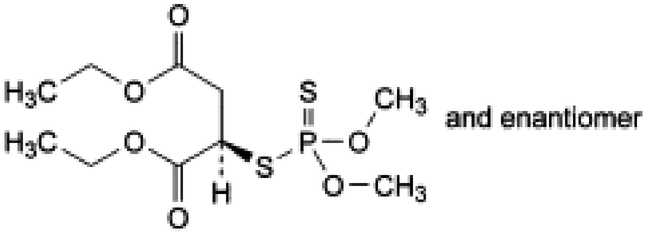
To 0.700 g add 10 ml of *dilute sulphuric acid R* and 10 ml of *water R*. Heat for 90 min on a water-bath with frequent shaking, replacing the evaporated water. Allow to cool and decant onto an ashless filter paper (diameter 7 cm). Wash the precipitate by decantation with 3 quantities, each of 5 ml, of hot *water R*, transfer it to the filter and wash it with hot *water R* until 1 ml of the filtrate remains clear after the addition of 0.05 ml of *dilute hydrochloric acid R* and 2 ml of *barium chloride solution R1*. Incinerate the filter and its contents in a platinum crucible, then ignite the residue (SiO_2) at 900 ± 50 °C to constant mass.

Ph Eur

Malathion

General Notices

(Ph Eur monograph 1343)



$C_{10}H_{19}O_6PS_2$ 330.4 *121-75-5*

Action and use

Organophosphorus insecticide.

Ph Eur

DEFINITION

Diethyl (2*RS*)-2-(dimethoxyphosphinodithioyl)butanedioate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless or slightly yellowish liquid.

Solubility

Slightly soluble in water, miscible with acetone, with cyclohexane, with ethanol (96 per cent) and with vegetable oils.

It solidifies at about 3 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison malathion CRS.

TESTS

Relative density (*2.2.5*)

1.220 to 1.240.

Optical rotation (*2.2.7*)

- 0.1 ° to + 0.1 °.

Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *water R*, *acetonitrile R* (1:3 *V/V*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 0.100 g of *malathion CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *malathion impurity A CRS* and 5.0 mg of *malathion impurity B CRS* in the solvent mixture, then dilute 50.0 ml with the solvent mixture.

Reference solution (d) Dilute 2.0 ml of reference solution (c) to 10.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm);

— *temperature*: 35 °C.

Mobile phase *acetonitrile R*, *water R* (45:55 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl of test solution (a) and reference solutions (b), (c) and (d).

Retention time Impurity B = about 3.5 min; impurity A = about 5 min; malathion = about 16 min.

System suitability Reference solution (c):

— *resolution*: minimum 2.0 between the peaks due to impurities B and A.

Limits:

— *impurity A*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *sum of impurities other than A and B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.1 per cent, determined on 2.000 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{10}H_{19}O_6PS_2$ from the declared content of *malathion CRS.*

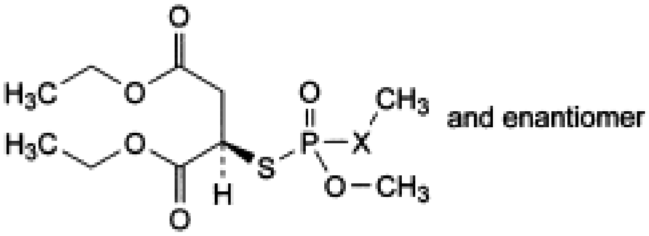
STORAGE

In an airtight container, protected from light.

IMPURITIES

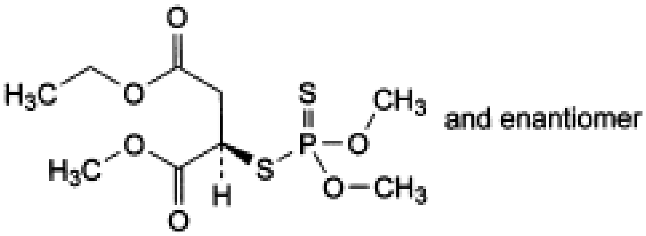
Specified impurities A, B.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C.*



A. X = S: diethyl (2*RS*)-2-[(methoxy)(methylsulphanyl)-*S*-phosphinothioyl]butanedioate (isomalathion),

B. X = O: diethyl (2*RS*)-2-(dimethoxy-*S*-phosphinothioyl)butanedioate (maloxon),


and enantiomer

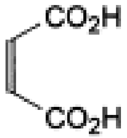
C. ethyl and methyl (2*RS*)-2-(dimethoxyphosphinodithioyl)butanedioate (methyl analogue).

Ph Eur

Maleic Acid

General Notices

(Ph Eur monograph 0365)



$C_4H_4O_4$ 116.1 *110-16-7*

Ph Eur

DEFINITION

Maleic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (*Z*)-butenedioic acid, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water and in alcohol.

IDENTIFICATION

A. Dilute 5 ml of solution S (see Tests) to 10 ml with *water R*. The pH of the dilution is less than 2.

B. Examine the chromatograms obtained in the test for fumaric acid. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 10 ml of *water R* (solution a). To 0.3 ml of solution (a) add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min; no colour develops. To 3 ml of solution (a) add 1 ml of *bromine water R*. Heat on a water-bath to remove the bromine (15 min), heat to boiling and cool. To 0.2 ml of this solution add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min. A violet-pink colour develops.

TESTS

Solution S

Dissolve 5.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2,* *Method II*).

Fumaric acid

Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution (a) Dissolve 0.5 g of the substance to be examined in *acetone R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *acetone R*.

Reference solution (a) Dissolve 20 mg of *maleic acid CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 15 mg of *fumaric acid CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (c) Mix 5 ml of reference solution (a) and 5 ml of reference solution (b).

Apply separately to the plate 5 µl of test solutions (a) and (b), 5 µl of reference solutions (a) and (b) and 10 µl of reference solution (c). Develop in a non-saturated tank over a path of 10 cm using a mixture of 12 volumes of *anhydrous formic acid R*, 16 volumes of *chloroform R*, 32 volumes of *butanol R* and 44 volumes of *heptane R*. Dry the plate at 100 °C for 15 min and examine in ultraviolet light at 254 nm. Any spot corresponding to fumaric acid in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Iron

To 10 ml of solution S add 2 ml of *dilute hydrochloric acid R* and 0.05 ml of *bromine water R*. After 5 min, remove the excess of bromine by passing a current of air and add 3 ml of *potassium thiocyanate solution R*. Shake. Prepare a standard at the same time and in the same manner, using a mixture of 5 ml of *iron standard solution (1 ppm Fe) R*, 1 ml of *dilute hydrochloric acid R*, 6 ml of *water R* and 0.05 ml of *bromine water R*. Allow both solutions to stand for 5 min. Any red colour in the test solution is not more intense than that in the standard (5 ppm).

Heavy metals (*2.4.8*)

1.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*)

Not more than 2.0 per cent, determined on 1.00 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 50 ml of *water R*. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution R* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 58.04 mg of $C_4H_4O_4$.

STORAGE

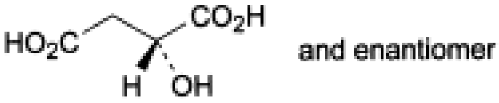
Store in a glass container, protected from light.

Ph Eur

Malic Acid

General Notices

(Ph Eur monograph 2080)



$C_4H_6O_5$ 134.1 *6915-15-7*

Action and use

Excipient.

Ph Eur

DEFINITION

(2*RS*)-2-Hydroxybutanedioic acid.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in alcohol, sparingly soluble in acetone.

IDENTIFICATION

A. Melting point (*2.2.14*): 128 °C to 132 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of malic acid.

TESTS

Solution S

Dissolve 5.00 g in *water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Optical rotation (*2.2.7*)

- 0.10° to + 0.10°, determined on solution S.

Water-insoluble substances

Maximum 0.1 per cent.

Dissolve 25.0 g in 100 ml of *water R*, filter the solution through a tared sintered-glass filter (16) (*2.1.2*), wash the filter with hot *water R* and dry at 100-105 °C to constant weight. The residue weighs a maximum of 25 mg.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 100.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *fumaric acid R* and 4.0 mg of *maleic acid R* in 25 ml of the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 2.5 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 20.0 mg of the substance to be examined in the mobile phase, add 1.0 ml of reference solution (a) and dilute to 20.0 ml with the mobile phase.

Column:
- — *size:* l = 0.30 m, Ø = 7.8 mm;
- — *stationary phase*: *ion-exclusion resin for chromatography R* (9 µm);
- — *temperature*: 37 °C.

Mobile phase *0.005 M sulphuric acid .*

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Run time Twice the retention time of the principal peak in the chromatogram obtained with the test solution.

Relative retention With reference to malic acid (retention time = about 10 min): impurity B = about 0.8; impurity A = about 1.5.

System suitability Reference solution (c):

- — *resolution*: minimum 2.5 between the peaks due to impurity B and malic acid.

Limits:
- — *impurity A*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- — *impurity B*: not more than 0.25 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.05 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total of other impurities*: not more than 2.5 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.02 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test F. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 2.0 per cent, determined on 1.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

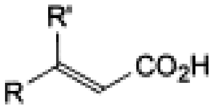
ASSAY

Dissolve 0.500 g in 50 ml of *carbon dioxide-free water R*. Titrate with *1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20*).

1 ml of *1 M sodium hydroxide* is equivalent to 67.05 mg of $C_4H_6O_5$.

IMPURITIES

Specified impurities   A, B.



A. R = CO_2H, R′ = H: (*E*)-butenedioic acid (fumaric acid),

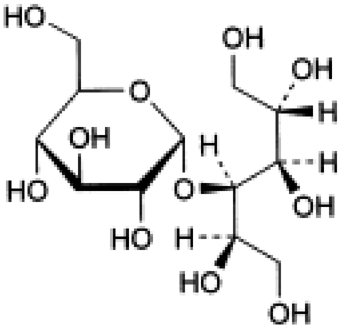
B. R = H, R′ = CO_2H: (*Z*)-butenedioic acid (maleic acid).

Ph Eur

Maltitol

General Notices

(Ph Eur monograph 1235)



$C_{12}H_{24}O_{11}$ 344.3 *585-88-6*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

4-*O*-α-D-Glucopyranosyl-D-glucitol (D-maltitol).

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Very soluble in water, practically insoluble in anhydrous ethanol.

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *maltitol CRS*.

B. Melting point (*2.2.14*): 148 °C to 151 °C.

C. Specific optical rotation (*2.2.7*): + 105.5 to + 108.5 (anhydrous substance).

Dissolve 5.00 g in *water R* and dilute to 100.0 ml with the same solvent.

D. Thin-layer chromatography (*2.2.27*) .

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *maltitol CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 25 mg of *maltitol CRS* and 25 mg of *sorbitol CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *water R*, *ethyl acetate R*, *propanol R* (10:20:70 *V/V/V*).

Application 2 µl.

Development Over a path of 17 cm.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R*. Dry in a current of cold air until the acetone is removed. Heat at 100-105 °C for 15 min. Allow to cool and spray with a 2 g/l solution of *sodium periodate R*. Dry in a current of cold air. Heat at 100 °C for 15 min.

System suitability Test solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 5.0 g in *water R* and dilute to 50 ml with the same solvent.

Conductivity (*2.2.38*)

Maximum 20 µS·cm^{-1}.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent. Measure the conductivity of the solution, while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent, expressed as glucose equivalent.

Dissolve 5.0 g in 6 ml of *water R* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration as indicator. Not less than 12.8 ml of *0.05 M sodium* thiosulphate is required.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 5.0 g of the substance to be examined in 20 ml of *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 0.50 g of *maltitol CRS* in 2.0 ml of *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *water R*.

Reference solution (c) Dilute 10.0 ml of reference solution (b) to 100.0 ml with *water R*.

Reference solution (d) Dissolve 0.5 g of *maltitol R* and 0.5 g of *sorbitol R* in 5 ml of *water R* and dilute to 10.0 ml with the same solvent.

Column:

— *size: l* = 0.3 m, Ø = 7.8 mm;

— *stationary phase*: *strong cation exchange resin (calcium form) R* (9 µm);

— *temperature*: 85 ± 1 °C.

Mobile phase *degassed water R.*

Flow rate 0.5 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl of the test solution and reference solutions (b), (c) and (d).

Run time 3 times the retention time of maltitol.

Relative retention With reference to maltitol (retention time = about 16 min): impurity B = about 0.8; impurity A = about 1.8.

System suitability Reference solution (d):

— *resolution*: minimum 2 between the peaks due to maltitol and impurity A.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Lead (*2.4.10*)

Maximum 0.5 ppm.

Nickel (*2.4.15*)

Maximum 1 ppm.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.00 g.

Microbial contamination

If intended for use in the manufacture of parenteral dosage forms, the total viable aerobic count (*2.6.12*) is not more than 10^2 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella* (*2.6.13*).

Bacterial endotoxins (*2.6.14*)

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins:

— less than 4 IU/g for parenteral dosage forms having a concentration of less than 100 g/l of maltitol,

— less than 2.5 IU/g for parenteral dosage forms having a concentration of 100 g/l or more of maltitol.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of D-maltitol from the declared content of *maltitol CRS*.

LABELLING

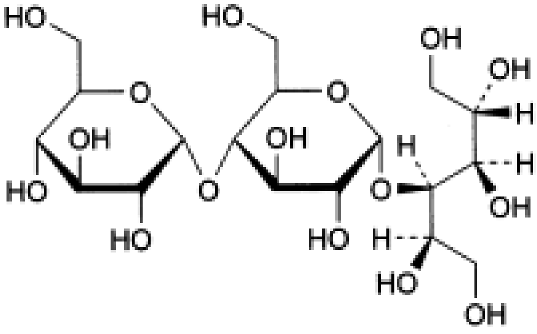
The label states:

— where applicable, the maximum concentration of bacterial endotoxins,;

— where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES

A. sorbitol,



B. *C*-α-D-glucopyranosyl-(1→4)-*C*-α-D-glucopyranosyl-(1→4)-D-glucitol (maltotriitol).

Ph Eur

Maltodextrin

General Notices

(Ph Eur monograph 1542)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of glucose, disaccharides and polysaccharides, obtained by the partial hydrolysis of starch.

The degree of hydrolysis, expressed as dextrose equivalent (DE), is not greater than 20 (nominal value).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic powder or granules.

Solubility

Freely soluble in water.

IDENTIFICATION

A. Dissolve 0.1 g in 2.5 ml of *water R* and heat with 2.5 ml of *cupri-tartaric solution R*. A red precipitate is formed.

B. Dip, for 1 s, a suitable stick with a reactive pad containing glucose-oxidase, peroxidase and a hydrogen-donating substance, such as tetramethylbenzidine, in a 100 g/l solution of the substance to be examined. Observe the colour of the reactive pad; within 60 s the colour changes from yellow to green or blue.

C. It is a powder or granules.

D. Dextrose equivalent (see Tests).

TESTS

Solution S

Dissolve 12.5 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

pH (*2.2.3*)

4.0 to 7.0.

Mix 1 ml of a 223.6 g/l solution of *potassium chloride R* and 30 ml of solution S.

Sulphur dioxide (*2.5.29*)

Maximum 20 ppm.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dilute 4 ml of solution S to 30 ml with *water R*. The solution complies with test E. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 6.0 per cent, determined on 10.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

Dextrose equivalent (DE)

Within 2 DE units of the nominal value.

Weigh an amount of the substance to be examined equivalent to 2.85-3.15 g of reducing carbohydrates, calculated as dextrose equivalent, into a 500 ml volumetric flask. Dissolve in water R and dilute to 500.0 ml with the same solvent. Transfer the solution to a 50 ml burette.

Pipette 25.0 ml of *cupri-tartaric solution R* into a 250 ml flask and add 18.5 ml of the test solution from the burette, mix and add a few glass beads. Place the flask on a hot plate, previously adjusted so that the solution begins to boil within 2 min ± 15 s. Allow to boil for exactly 120 s, add 1 ml of a 1 g/l solution of *methylene blue R* and titrate with the test solution (V_1) until the blue colour disappears. Maintain the solution at boiling throughout the titration.

Standardise the cupri-tartaric solution using a 6.00 g/l solution of *glucose R* (V_0).

Calculate the dextrose equivalent using the following expression:

$$\frac{300 \times V_0 \times 100}{V_1 \times M \times D}$$

V_0 = total volume of glucose standard solution, in millilitres,

V_1 = total volume of test solution, in millilitres,

M = sample mass, in grams,

D = percentage content of dry matter in the substance.

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella*

(*2.6.13*).

LABELLING

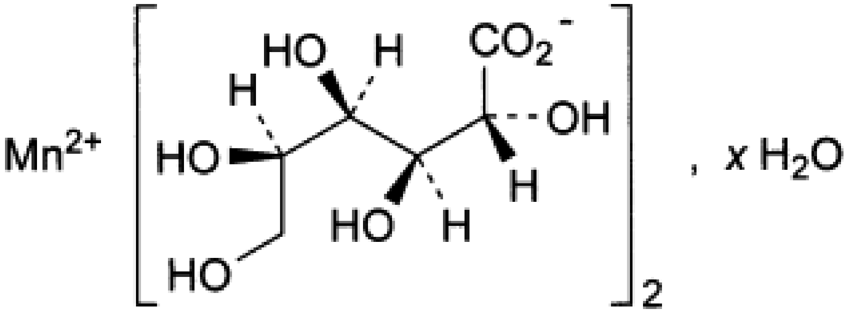
The label states the dextrose equivalent (DE) (= nominal value).

Ph Eur

Manganese Gluconate

General Notices

(Ph Eur monograph 2162)



$C_{12}H_{22}MnO_{14,}xH_20$ 445.2 *6485-39-8*
(anhydrous)

Ph Eur

DEFINITION

Anhydrous or hydrated manganese(II) D-gluconate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or pale pink, slightly hygroscopic, crystalline powder.

Solubility

Soluble in water, practically insoluble in anhydrous ethanol, insoluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in 1 ml of *water R*.

Reference solution Dissolve 20 mg of *calcium gluconate CRS* in 1 ml of *water R*, heating if necessary in a water-bath at 60 °C.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *concentrated ammonia R*, *ethyl acetate R*, *water R*, *ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 1 µl.

Development Over 3/4 of the plate.

Drying At 100-105 °C for 20 min, then allow to cool to room temperature.

Detection Spray with a solution containing 25 g/l of *ammonium molybdate R* and 10 g/l of *cerium sulphate R* in *dilute sulphuric acid R*, and heat at 100-105 °C for about 10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve 50 mg in 5 ml of *water R*. Add 0.5 ml of *ammonium sulphide solution R*. A pale pink precipitate is formed that dissolves upon the addition of 1 ml of *glacial acetic acid R.*

TESTS

Solution S

Dissolve 1.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Sucrose and reducing sugars

Dissolve 0.5 g in a mixture of 2 ml of *hydrochloric acid R1* and 10 ml of *water R*. Boil for 5 min, allow to cool, add 10 ml of *sodium carbonate solution R* and allow to stand for 10 min. Dilute to 25 ml with *water R* and filter. To 5 ml of the filtrate add 2 ml of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 2 min. No red precipitate is formed.

Chlorides *(2.4.4)*

Maximum 500 ppm.

Dilute 5 ml of solution S to 15 ml with *water R.*

Sulphates *(2.4.13)*

Maximum 500 ppm.

Dissolve 2.0 g in a mixture of 10 ml of *acetic acid R* and 90 ml of *distilled water R.*

Zinc

Maximum 50 ppm.

To 10 ml of solution S add 1 ml of *sulphuric acid R* and 0.1 ml of *potassium ferrocyanide solution R*. After 30 s, any opalescence in the solution is not more intense than that in a mixture of 1.0 ml of *zinc standard solution (10 ppm Zn) R*, 9 ml of *water R,* 1 ml of *sulphuric acid R* and 0.1 ml of *potassium ferrocyanide solution R.*

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R,* heating in a water-bath at 60 °C. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water ***(2.5.32)***

Maximum 9.0 per cent, determined on 80 mg.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count.

ASSAY

Dissolve 0.400 g in 50 ml of *water R*. Add 10 mg of *ascorbic acid R,* 20 ml of *ammonium chloride buffer solution pH 10.0 R* and 0.2 ml of a 2 g/l solution of *mordant black 11 R* in *triethanolamine R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

1 ml of *0.1 M sodium edetate* is equivalent to 44.52 mg of $C_{12}H_{22}MnO_{14}$.

STORAGE

In a non-metallic, airtight container .

Ph Eur

Manganese Sulphate Monohydrate

General Notices

(Ph Eur monograph 1543)

$MnSO_4,H_2O$ 169.0 *10034-96-5*

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent (ignited substance).

CHARACTERS

Appearance

Pale pink crystalline powder, slightly hygroscopic.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of sulphates (*2.3.1*).

B. Dissolve 50 mg in 5 ml of *water R*. Add 0.5 ml of *ammonium sulphide solution R*. A pale pink precipitate is formed which dissolves on the addition of 1 ml of *anhydrous acetic acid R*.

C. Loss on ignition (see Tests).

TESTS

Solution S

Dissolve 10.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*).

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S.

Zinc

Maximum 50 ppm.

To 10 ml of solution S add 1 ml of *sulphuric acid R* and 0.1 ml of *potassium ferrocyanide solution R*. After 30 s, any opalescence in the solution is not more intense than that in a mixture of 5 ml of *zinc standard solution (10 ppm Zn) R*, 5 ml of *water R*, 1 ml of *sulphuric acid R* and 0.1 ml of *potassium ferrocyanide solution R*.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Loss on ignition

10.0 per cent to 12.0 per cent, determined on 1.00 g at 500 ± 50 °C.

ASSAY

Dissolve 0.150 g in 50 ml of *water R*. Add 10 mg of *ascorbic acid R*, 20 ml of *ammonium chloride buffer solution pH 10.0 R* and 0.2 ml of a 2 g/l solution of *mordant black 11 R* in *triethanolamine R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

1 ml of *0.1 M sodium edetate* is equivalent to 15.10 mg of $MnSO_4$.

Ph Eur

Manganese Sulphate

General Notices

$MnSO_4,4H_2O$ 223.1 *7785-87-7*

DEFINITION

Manganese Sulphate is manganese(II) sulphate tetrahydrate. It contains not less than 98.0% and not more than 100.5% of $MnSO_4$, calculated with reference to the substance ignited at 450° to 500°.

CHARACTERISTICS

Pale pink crystals or crystalline powder.

Freely soluble in *water*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION

A. Dissolve 0.5 g in 10 ml of *water* and add 1 ml of *sodium sulphide solution*. A pink precipitate is produced which is soluble in 6M *acetic acid*.

B. To 0.1 g add 2 g of *lead(IV) oxide* and 5 ml of *nitric acid*, boil gently for a few minutes, add 100 ml of *water* and filter. A purple solution is produced.

C. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Arsenic

0.25 g complies with the *limit test for arsenic*, Appendix VII (4 ppm).

Heavy metals

Dissolve 0.50 g in 50 ml of *water*, add 1 ml of 1M *acetic acid* and pass *hydrogen sulphide* through the solution for 20 seconds. The colour produced within 2 minutes is not more intense than that obtained by treating 10 ml of *lead standard solution (2 ppm Pb)* diluted to 50 ml in the same manner (40 ppm).

Iron

Dissolve 1.0 g in 10 ml of *water* and add 2 ml of 1M *hydrochloric acid* and, dropwise, 0.05M *potassium permanganate* until a permanent pink colour is produced. Add 5 ml of a 10% w/v solution of *ammonium thiocyanate* and 20 ml of a mixture of equal volumes of *isoamyl alcohol* and *amyl acetate*, shake well and allow to separate. Any colour in the upper layer is not more intense than that obtained by treating 2 ml of *iron standard solution (20 ppm Fe)* diluted to 10 ml in the same manner (40 ppm).

Zinc

Dissolve 2.0 g in 10 ml of *water*, add 3 ml of 1M *hydrochloric acid* and 0.3 ml of a freshly prepared 3% w/v solution of *potassium hexacyanoferrate(II)*, mix and allow to stand for 15 minutes. Any turbidity produced is not more intense than that obtained by treating 10 ml of *zinc standard solution (100 ppm Zn)* in the same manner (500 ppm).

Chloride

15 ml of a 1.0% w/v solution complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Loss on ignition

When ignited to constant weight at 450° to 500°, loses 31.0 to 34.0% of its weight. Use 1 g.

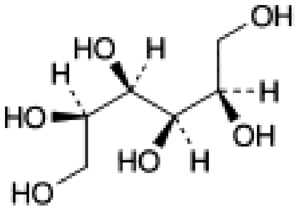
ASSAY

Dissolve 0.15 g in 40 ml of *water*, add 8 ml of freshly boiled and cooled *nitric acid*, cool, add 1.5 g of *sodium bismuthate* and shake for 2 minutes. Add 25 ml of a mixture of 3 volumes of *nitric acid* and 97 volumes of *water*, filter, wash the residue with 40 ml of the mixture, collecting the filtrate and washings in 50 ml of 0.1M *ammonium iron(II) sulphate VS*, and titrate immediately with 0.02M *potassium permanganate VS*. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of ammonium iron(II) sulphate required. Each ml of 0.1M *ammonium iron(II) sulphate VS* is equivalent to 3.020 mg of $MnSO_4$.

Mannitol

General Notices

(Ph Eur monograph 0559)



$C_6H_{14}O_6$ 182.2 *69-65-8*

Action and use

Diuretic.

Preparation

Mannitol Intravenous Infusion

Ph Eur

DEFINITION

D-Mannitol.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or free-flowing granules.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Specific optical rotation (*2.2.7*): + 23 to + 25 (anhydrous substance).

Dissolve 2.00 g of the substance to be examined and 2.6 g of *disodium tetraborate R* in about 20 ml of *water R* at 30 °C; shake continuously for 15-30 min without further heating. Dilute the resulting clear solution to 25.0 ml with *water R*.

B. Melting point (*2.2.14*): 165 °C to 170 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison mannitol CRS.

If the spectra obtained in the solid state show differences, dissolve separately in 2 glass vials 25 mg of the substance to be examined and 25 mg of the reference substance in 0.25 ml of *distilled water R* without heating. The solutions obtained are clear. Evaporate to dryness by heating in a microwave oven with a power range of 1000-1300 W for 15-30 min or by heating in an oven *in vacuo* at 100 °C. Non-sticky, white or slightly yellowish powders are obtained. Record new spectra using the residues.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *mannitol CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 25 mg of *mannitol R* and 25 mg of *sorbitol R* in *water R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel G plate R.

Mobile phase water R, *ethyl acetate R*, *propanol R* (10:20:70 *V/V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R*. Dry in a current of cold air until the acetone is removed. Heat at 100 °C for 15 min. Allow to cool and spray with a 2 g/l solution of *sodium periodate R*. Dry in a current of cold air. Heat at 100 °C for 15 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 5.0 g in *water R* and dilute to 50 ml with the same solvent.

Conductivity (*2.2.38*)

Maximum 20 µS·cm^{-1}.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* by heating at 40-50 °C and dilute to 100.0 ml with the same solvent. After cooling, measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent (calculated as glucose equivalent).

Dissolve 5.0 g in 25 ml of *water R* with the aid of gentle heating. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 5.0 g of the substance to be examined in 25 ml of *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 0.50 g of *mannitol CRS* in 2.5 ml of *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with *water R*.

Reference solution (c) Dilute 0.5 ml of reference solution (b) to 20.0 ml with *water R*.

Reference solution (d) Dissolve 0.5 g of *mannitol R* and 0.5 g of *sorbitol R* (impurity A) in 5 ml of *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (e) Dissolve 0.1 g of *maltitol R* (impurity B) and 0.1 g of *isomalt R* (impurity C) in 5 ml of *water R* and dilute to 100 ml with the same solvent.

Column:

- *size:* *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);
- *temperature*: 85 ± 1 °C.

Mobile phase *degassed water R.*

Flow rate 0.5 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl of the test solution and reference solutions (b), (c), (d) and (e).

Run time Twice the retention time of mannitol.

Relative retention With reference to mannitol (retention time = about 22 min): impurity C (eluted in 2 peaks) = about 0.7; impurity B = about 0.8; impurity A = about 1.2.

System suitability Reference solution (d):

— *resolution*: minimum 2 between the peaks due to mannitol and impurity A.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *impurity C*: for the sum of the areas of the 2 peaks, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *unspecified impurities*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Lead (*2.4.10*)

Maximum 0.5 ppm.

Dissolve the substance to be examined in 150.0 ml of the prescribed mixture of solvents.

Nickel (*2.4.15*)

Maximum 1 ppm.

Dissolve the substance to be examined in 150.0 ml of the prescribed mixture of solvents.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g. Use as solvent 40 ml of a mixture of equal volumes of *anhydrous methanol R* and *formamide R* at about 50 °C.

Microbial contamination

If intended for use in the manufacture of parenteral dosage forms: the total viable aerobic count (*2.6.12*) is not more than 10^2 bacteria and 10^2 fungi per gram, determined by plate count; it complies with the tests for *Escherichia coli* and *Salmonella* (*2.6.13*).

Bacterial endotoxins (*2.6.14*)

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins: less than 4 IU/g for parenteral dosage forms having a concentration of 100 g/l or less of mannitol, and less than 2.5 IU/g for parenteral dosage forms having a concentration of more than 100 g/l of mannitol.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of D-mannitol from the areas of the peaks and the declared content of *mannitol CRS*.

LABELLING

The label states:

— where applicable, the maximum concentration of bacterial endotoxins;

— where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES

Specified impurities   A, B, C.

A. sorbitol,

B. maltitol,

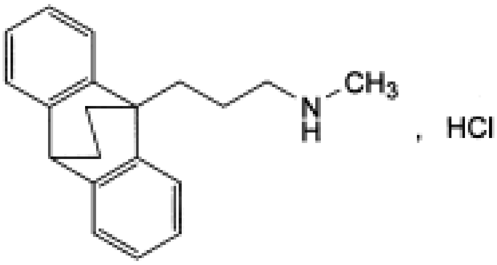
C. isomalt.

Ph Eur

Maprotiline Hydrochloride

General Notices

(Ph Eur monograph 1237)



$C_{20}H_{23}N,HCl$ 313.9 *10347-81-6*

Action and use

Antidepressant.

Ph Eur

DEFINITION

3-(9,10-Ethanoanthracen-9(10*H*)-yl)-*N*-methylpropan-1-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent), sparingly soluble in methylene chloride, very slightly soluble in acetone.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 10 mg in *1 M hydrochloric acid* and dilute to 100 ml with the same acid.

Spectral range 250-300 nm.

Absorption maxima At 265 nm and 272 nm.

Absorption minimum At 268 nm.

Absorbance ratio A_{272}/A_{265} = 1.1 to 1.3.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *maprotiline hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *maprotiline hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *maprotiline impurity D CRS* in reference solution (a) and dilute to 2 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethyl acetate R*, *dilute ammonia R1*, *2-butanol R* (4:5:14 *V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dilute 0.5 ml of solution S (see Tests) to 2 ml with *methanol R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 1.0 mg of *maprotiline impurity D CRS* in the test solution and dilute to 10.0 ml with the test solution.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase Dissolve about 0.580 g of *ammonium acetate R* in 200 ml of *water R* and add 2 ml of a 70 g/l solution of *concentrated ammonia R*; add 150 ml of *2-propanol R* and 650 ml of *methanol R*; the resulting apparent pH value is between 8.2 and 8.4.

Flow rate 1 ml/min.

Detection Spectrophotometer at 272 nm.

Injection 20 µl.

Run time 1.5 times the retention time of maprotiline.

Relative retention With reference to maprotiline (retention time = about 10.3 min): impurity A = about 0.3; impurity B = about 0.47; impurity C = about 0.74; impurity D = about 0.81; impurity E = about 1.26.

System suitability Reference solution (b):

- *resolution*: 1.8 to 3.2 between the peaks due to impurity D and maprotiline; if necessary, adjust the pH of the mobile phase, in steps of 0.1 pH unit, by adding a 50 per cent *V/V* solution of *acetic acid R* if the resolution is less than 1.8, or by adding a 70 g/l solution of *concentrated ammonia R* if the resolution is greater than 3.2.

Limits:

- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 80 °C at a pressure not exceeding 2.5 kPa for 6 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

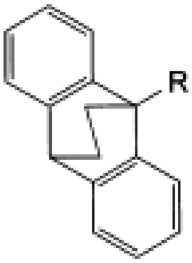
ASSAY

Dissolve 0.250 g in a mixture of 5 ml of *0.1 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.39 mg of $C_{20}H_{24}ClN$.

IMPURITIES

Specified impurities *A, B, C, D, E.*

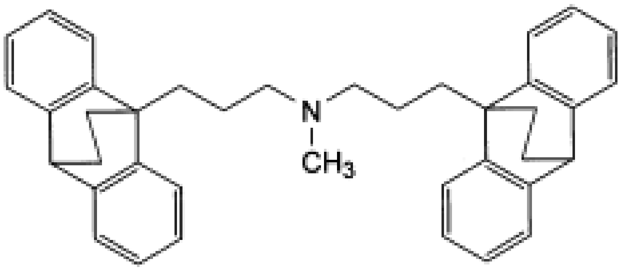


A. R = CH=CH-CH=O: 3-(9,10-ethanoanthracen-9(10*H*)-yl)prop-2-enal,

C. R = CH_2-CH_2-CH_2-NH_2: 3-(9,10-ethanoanthracen-9(10*H*)-yl)propan-1-amine,

D. R = CH=CH-CH_2-NH-CH_3: 3-(9,10-ethanoanthracen-9(10*H*)-yl)-*N*-methylprop-2-en-1-amine (dehydromaprotiline),

E. R = CH_2-CH_2-CH_2-$N(CH_3)_2$: 3-(9,10-ethanoanthracen-9(10*H*)-yl)-*N*,*N*-dimethylpropan-1-amine,



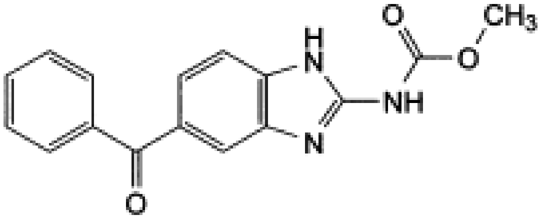
B. 3-(9,10-ethanoanthracen-9(10*H*)-yl)-*N*-[3-(9,10-ethanoanthracen-9(10*H*)-yl)propyl]-*N*-methylpropan-1-amine.

Ph Eur

Mebendazole

General Notices

(Ph Eur monograph 0845)



$C_{16}H_{13}N_3O_3$ 295.3 *31431-39-7*

Action and use

Benzimadazole anthelminthic.

Ph Eur

DEFINITION

Methyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, in alcohol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of mebendazole.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

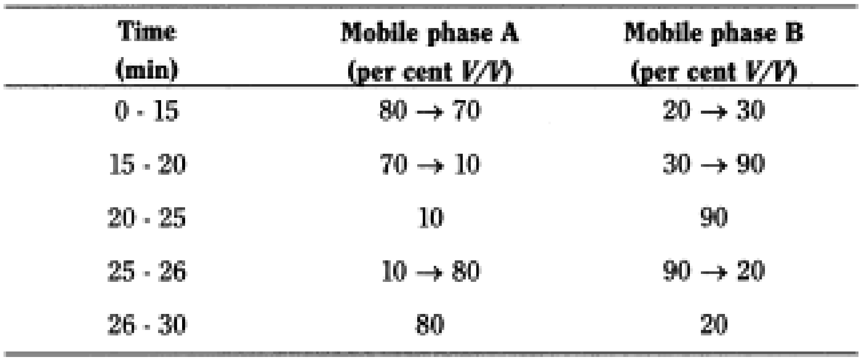
Test solution Dissolve 25.0 mg of the substance to be examined in *dimethylformamide* R and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *mebendazole for system suitability CRS* in *dimethylformamide R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide R*. Dilute 5.0 ml of this solution to 20.0 ml with *dimethylformamide R.*

Column:
- *size: l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: 7.5 g/l solution of *ammonium acetate R*;
- *mobile phase B*: *acetonitrile R*.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	80 → 70	20 → 30
15 - 20	70 → 10	30 → 90
20 - 25	10	90
25 - 26	10 → 80	90 → 20
26 - 30	80	20

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µl.

System suitability Reference solution (a):

- the chromatogram obtained is similar to the chromatogram supplied with *mebendazole for system suitability CRS.*

Limits:
- *correction facto*r: for the calculation of content, multiply the peak area of impurity G by 1.4;
- *impurity G*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

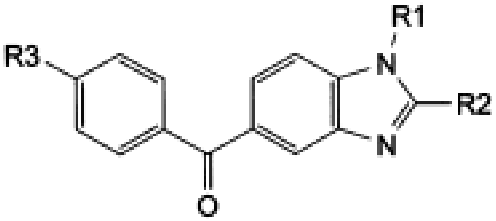
Dissolve 0.250 g in 3 ml of *anhydrous formic acid R* and add 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 29.53 mg of $C_{16}H_{13}N_3O_3$.

STORAGE

Protected from light.

IMPURITIES



A. R1 = R3 = H, R2 = NH_2: (2-amino-1*H*-benzimidazol-5-yl)phenylmethanone,

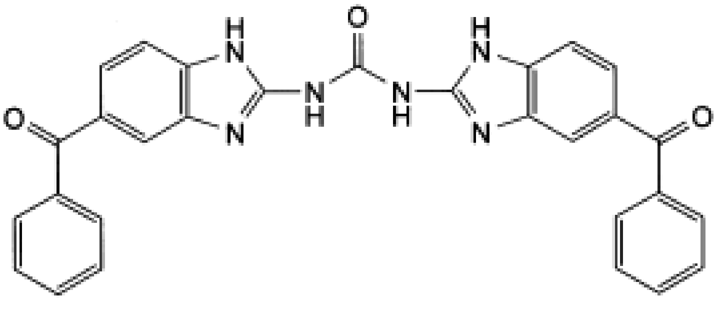
B. R1 = R3 = H, R2 = OH: (2-hydroxy-1*H*-benzimidazol-5-yl)phenylmethanone,

C. R1 = CH_3, R2 = NH_2, R3 = H: (2-amino-1-methyl-1*H*-benzimidazol-5-yl)phenylmethanone,

D. R1 = CH_3, R2 = NH-CO-OCH_3, R3 = H: methyl (5-benzoyl-1-methyl-1*H*-benzimidazol-2-yl) carbamate,

E. R1 = R3 = H, R2 = NH-CO-OC_2H_5: ethyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate,

F. R1 = H, R2 = NH-CO-OCH_3, R3 = CH_3: methyl [5-(4-methylbenzoyl)-1*H*-benzimidazol-2-yl] carbamate,

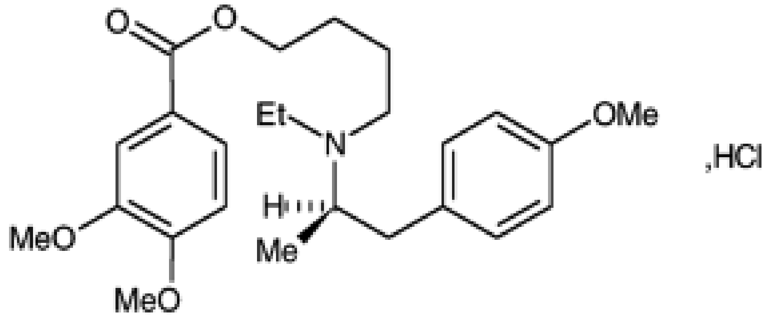


G. *N*,*N*′-bis(5-benzoyl-1*H*-benzimidazol-2-yl)urea.

Ph Eur

Mebeverine Hydrochloride

General Notices



and enantiomer

$C_{25}H_{35}NO_5,HCl$ 466.0 *2753-45-9*

Action and use

Smooth muscle relaxant; antispasmodic.

Preparation

Mebeverine Tablets

DEFINITION

Mebeverine Hydrochloride is (*RS*)-4-[ethyl(4-methoxy-α-methylphenethyl)amino]butyl veratrate hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{25}H_{35}NO_5,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Very soluble in *water*; freely soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mebeverine hydrochloride *(RS 209)*.

B. Dissolve 25 mg in 2 ml of *water*, acidify with 2M *nitric acid* and centrifuge. The supernatant liquid yields reaction A characteristic of *chlorides*, Appendix VI, beginning at the words 'add 0.4 ml of...'.

TESTS

Acidity

pH of a 2% w/v solution, 4.5 to 6.5, Appendix V L.

Ether-soluble extractive

Dissolve 40 mg in 25 ml of 2M *hydrochloric acid* and shake with 50 ml of *ether* for 1 minute. Wash the ether layer with three 25 ml quantities of *water*, evaporate the ether to dryness using a rotary evaporator and dissolve the residue in sufficient *methanol* to produce 20 ml. The *absorbance* of the resulting solution at 260 nm is not more than 0.23, Appendix II B.

Non-tertiary amine

Dissolve 0.5 g in 5 ml of *pyridine*, add 5 ml of *copper chloride-pyridine reagent* and heat at 50° for 30 minutes. Cool, add sufficient *acetone* to produce 50 ml and measure the *absorbance* of the resulting solution at 405 nm, Appendix II B, using in the reference cell a solution obtained by treating 5 ml of *pyridine* in the same manner. The absorbance is not more than that obtained by repeating the test using 5 ml of a 0.0060% w/v solution of *di*-n-*butylamine* in *pyridine* and beginning at the words 'add 5 ml of *copper chloride-pyridine reagent*...'.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 50 volumes of *absolute ethanol*, 50 volumes of *chloroform* and 1 volume of 18M *ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of three solutions in *acetone* containing (1) 2.0% w/v of the substance being examined, (2) 0.010% w/v of the substance being examined and (3) 0.0020% w/v of *veratric acid*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Expose the plate to iodine vapour for 1 hour. When viewed under ultraviolet light any spot corresponding to veratric acid in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). By each method of visualisation any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried at 105° for 1 hour, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.4 g, adding 7 ml of *mercury(II) acetate solution* and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 46.60 mg of $C_{25}H_{35}NO_5,HCl$.

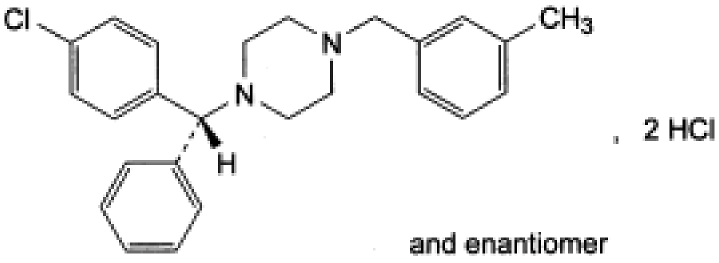
STORAGE

Mebeverine Hydrochloride should be kept in an airtight container, protected from light and stored at a temperature not exceeding 30°.

Meclozine Hydrochloride

General Notices

(Ph Eur monograph 0622)



$C_{25}H_{29}Cl_3N_2$ 463.9 *1104-22-9*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

Meclozine hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 1-[(*RS*-(4-chlorophenyl)phenylmethyl]-4-(3-methylbenzyl) piperazine dihydrochloride, calculated with reference to the anhydrous substance.

CHARACTERS

A yellow or yellowish-white, crystalline powder, slightly soluble in water, soluble in alcohol and in methylene chloride.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Dissolve 15.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 232 nm and weak absorbance without a defined maximum between 260 nm and 300 nm. The specific absorbance at the maximum is 345 to 380, calculated with reference to the anhydrous substance.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *meclozine hydrochloride CRS*. Examine the substances prepared as discs using *potassium chloride R*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 15 mg in 2 ml of *alcohol R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

Dissolve 0.50 g in *alcohol R* and dilute to 25 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity or alkalinity

Calculate the acidity or alkalinity from the titration volumes noted in the Assay using the equation:

$$A = V_2 - 2V_1$$

V_1 = volume of *0.1 M sodium hydroxide* added at the first point of inflexion,

V_2 = volume of *0.1 M sodium hydroxide* added at the second point of inflexion.

A is - 0.3 ml to 0.3 ml for 0.3500 g of the substance to be examined.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.50 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Reference solution (a) Dissolve 50 mg of *meclozine hydrochloride CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride* R and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dilute 0.5 ml of test solution (b) to 10 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 0.5 volumes of *concentrated ammonia R*, 5 volumes of *methanol R*, 30 volumes of toluene R and 60 volumes of *methylene chloride R*. Allow the plate to dry in air and spray with *dilute potassium iodobismuthate solution R.* Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Disregard any yellowish-white spot at the starting point.

Water (*2.5.12*)

Not more than 5.0 per cent, determined on 0.200 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.3500 g in 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 46.39 mg of $C_{25}H_{29}Cl_3N_2$.

STORAGE

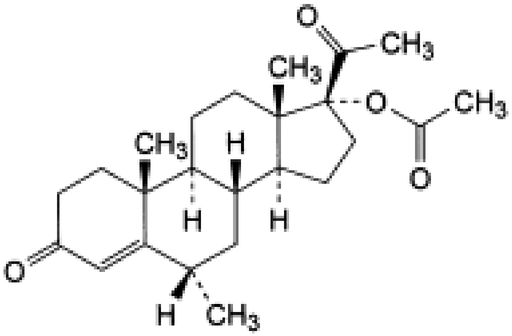
Store in an airtight container .

Ph Eur

Medroxyprogesterone Acetate

General Notices

(Ph Eur monograph 0673)



$C_{24}H_{34}O_4$ 386.5 *71-58-9*

Action and use

Progestogen.

Preparations

Medroxyprogesterone Injection

Medroxyprogesterone Tablets

Ph Eur

DEFINITION

6-Methyl-3,20-dioxopregn-4-en-17-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *medroxyprogesterone acetate CRS.*

TESTS

Specific optical rotation (*2.2.7*)

+ 47 to + 53 (dried substance).

Dissolve 0.250 g in *acetone R* and dilute to 25.0 ml with the same solvent.

Impurity F

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.200 g of the substance to be examined in *methylene chloride R* and dilute to 10.0 ml with the same solvent.

Reference solution To 20.0 mg of *medroxyprogesterone acetate for performance test CRS* (containing 0.5 per cent of impurity F) add 1.0 ml of *methylene chloride R* and dissolve.

Plate *TLC silica gel plate R*.

Mobile phase *tetrahydrofuran R*, *1,1-dimethylethyl methyl ether R*, *heptane R* (10:45:45 *V/V/V*).

Application 10 µl.

Development 1 Over a path of 10 cm.

Drying 1 In air.

Development 2 Over a path of 10 cm in the same direction with the same mobile phase.

Drying 2 At 120 °C for 10 min.

Detection Spray with a 200 g/l solution of *toluenesulphonic acid R* in *ethanol (96 per cent) R*. Heat at 120 °C for 10 min. Allow to cool. Examine the chromatograms in ultraviolet light at 365 nm.

System suitability Reference solution:

— the chromatogram obtained shows 2 clearly separated spots.

Limit:

— *impurity F*: in the chromatogram obtained with the test solution the blue fluorescent spot with an R_F value higher than the principal spot due to medroxyprogesterone acetate is not more intense than the corresponding blue fluorescent spot due to impurity F in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 4 mg of *medroxyprogesterone acetate for system suitability CRS* (containing impurities A, B, C, D, E, G and I) in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 2 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column:

— *size:* *l* = 0.25 m, Ø = 3.0 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 60 °C.

Mobile phase *tetrahydrofuran R*, *acetonitrile R*, *water R* (12:23:65 *V/V/V*).

Flow rate 0.9 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time Twice the retention time of medroxyprogesterone acetate.

Identification of impurities Use the chromatogram supplied with *medroxyprogesterone acetate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, G and I.

Relative retention With reference to medroxyprogesterone acetate (retention time = about 20 min): impurity A = about 0.3; impurity I = about 0.5; impurity H = about 0.65; impurity B = about 0.7; impurity C = about 0.8; impurity G = about 0.85; impurity D = about 0.9; impurity E = about 0.95.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to medroxyprogesterone acetate.

Limits:

— *correction factor*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.5; impurity G = 2.6;

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurity B*: not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *impurities C, E, G, I*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 241 nm.

Calculate the content of $C_{24}H_{34}O_4$ taking the specific absorbance to be 420.

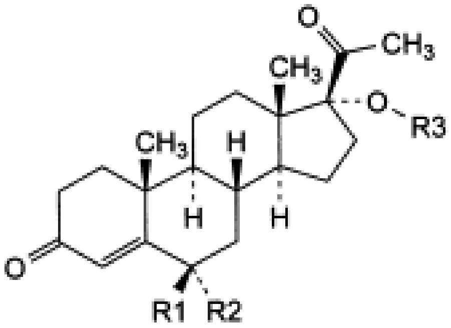
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, I.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): H.*



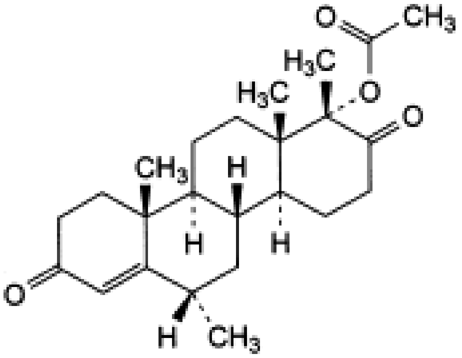
A. R1 = OH, R2 = CH_3, R3 = CO-CH_3: 6-hydroxy-6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-hydroxymedroxyprogesterone acetate),

B. R1 = R3 = H, R2 = CH_3: 17-hydroxy-6-methylpregn-4-ene-3,20-dione (medroxyprogesterone),

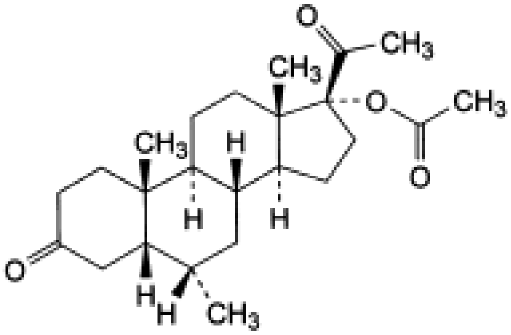
D. R1 = CH_3, R2 = H, R3 = CO-CH_3: 6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-epimedroxyprogesterone acetate),

E. R1 + R2 = CH_2, R3 = CO-CH_3: 6-methylidene-3,20-dioxopregn-4-en-17-yl acetate (6-methylenehydroxyprogesterone acetate),

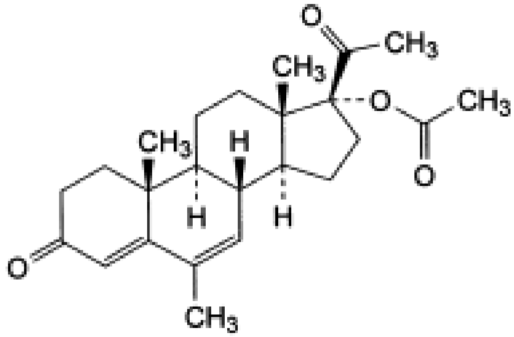
H. R1 = R2 = H, R3 = CO-CH_3: 3,20-dioxopregn-4-en-17-yl acetate (hydroxyprogesterone acetate),



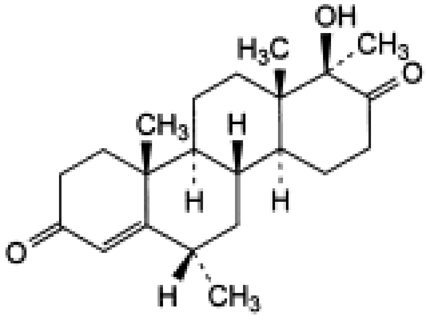
C. 6,17a-dimethyl-3,17-dioxo-*D*-homoandrost-4-en-17a-yl acetate,



F. 6-methyl-3,20-dioxo-5-pregnan-17-yl acetate (4,5-dihydromedroxyprogesterone acetate),



G. 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate),



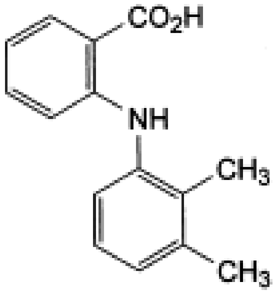
I. (17aβ)-17a-hydroxy-6,17a-dimethyl-*D*-homoandrost-4-ene-3,17-dione.

Ph Eur

Mefenamic Acid

General Notices

(Ph Eur monograph 1240)



$C_{15}H_{15}NO_2$ 241.3 *61-68-7*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Mefenamic Acid Capsules

Mefenamic Acid Tablets

Ph Eur

DEFINITION

Mefenamic acid contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of 2-[(2,3-dimethylphenyl)amino]benzoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, microcrystalline powder, practically insoluble in water, slightly soluble in alcohol and in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Dissolve 20 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 100 ml with the same mixture of solvents. Dilute 5 ml of the solution to 50 ml with a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R*. Examined between 250 nm and 380 nm (*2.2.25*), the solution shows two absorption maxima, at 279 nm and 350 nm. The ratio of the absorbance measured at the maximum at 279 nm to that measured at 350 nm is 1.1 to 1.3.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *mefenamic acid CRS*. Examine the substances prepared as discs. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *alcohol R*, evaporate to dryness and record new spectra using the residues.

C. Dissolve about 25 mg in 15 ml of *methylene chloride R* and examine in ultraviolet light at 365 nm; the solution exhibits a strong greenish-yellow fluorescence. Carefully add 0.5 ml of a saturated solution of *trichloroacetic acid R* dropwise and examine in ultraviolet light at 365 nm. The solution does not exhibit fluorescence.

D. Dissolve about 5 mg in 2 ml of *sulphuric acid R* and add 0.05 ml of *potassium dichromate solution R1*. An intense blue colour is produced, turning rapidly to brownish-green.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel* GF_{254} *plate R*.

Test solution Dissolve 0.125 g of the substance to be examined in a mixture of 1 volume of *methanol* R and 3 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (a) Dilute 1 ml of the test solution to 50 ml with a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R*. Dilute 1 ml of the solution to 10 ml with a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R*.

Reference solution (b) Dissolve 5 mg of *flufenamic acid R* and 5 mg of *mefenamic acid CRS* in a mixture of 1 volume of *methanol* R and 3 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Apply to the plate 20 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *glacial acetic acid R*, 25 volumes of *dioxan R* and 90 volumes of *toluene R*. Dry the plate in a current of warm air. Expose the plate to iodine vapour for 5 min and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

2,3-Dimethylaniline

Solution (a) Dissolve 0.250 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents. This solution is used to prepare the test solution.

Solution (b) Dissolve 50 mg of *2,3-dimethylaniline R* in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 100 ml with the same mixture of solvents. Dilute 1 ml of the solution to 100 ml with a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R*. This solution is used to prepare the standard.

Using three flat-bottomed tubes, place in the first 2 ml of solution (a), in the second 1 ml of solution (b) and 1 ml of a mixture of 1 volume of *methanol R* and 3 volumes of *methylene* *chloride R* and in the third 2 ml of a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* (used to prepare a blank). To each tube add 1 ml of a freshly prepared 10 g/l solution of *dimethylaminobenzaldehyde R* in *methanol R* and 2 ml of *glacial acetic acid R* and allow to stand at room temperature for 10 min. The intensity of the yellow colour of the test solution is between that of the blank and that of the standard (100 ppm).

Copper

Not more than 10.0 ppm of Cu, determined by atomic absorption spectrometry (*2.2.23*, *Method I*).

Test solution Place 1.00 g of the substance to be examined in a silica crucible, moisten with *sulphuric acid R,* heat cautiously on a flame for 30 min and then progressively to 650 ± 50 °C. Continue ignition until all black particles have disappeared. Allow to cool, dissolve the residue in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solutions Prepare the reference solutions using *copper standard solution (0.1 per cent Cu) R*, diluted as necessary with *0.1 M nitric acid*.

Measure the absorbance at 324.8 nm using a copper hollow-cathode lamp as a source of radiation and an air-acetylene flame.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying at 105 °C.

Sulphated ash (*2.4.14*)

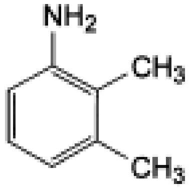
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

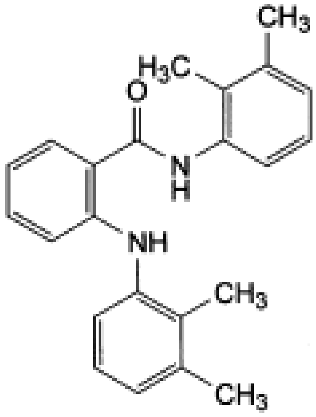
Dissolve with the aid of ultrasound 0.200 g in 100 ml of warm *ethanol R*, previously neutralised to *phenol red solution R*. Add 0.1 ml of *phenol red solution R* and titrate with *0.1 M sodium hydroxide.*

1 ml of *0.1 M sodium hydroxide* is equivalent to 24.13 mg of $C_{15}H_{15}NO_2$.

IMPURITIES



A. 2,3-dimethylaniline,



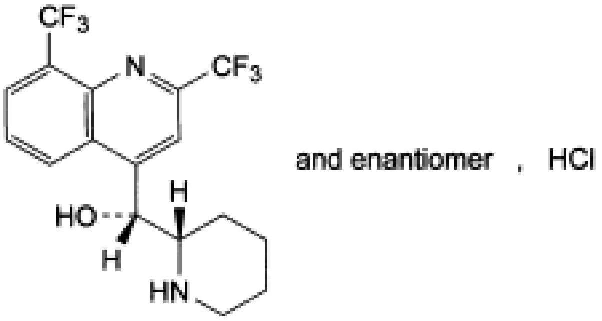
B. *N*-(2,3-dimethyphenyl)-2-[(2,3-dimethylphenyl)amino]benzamide.

Ph Eur

Mefloquine Hydrochloride

General Notices

(Ph Eur monograph 1241)



$C_{17}H_{17}ClF_6N_2O$ 414.8 *51773-92-3*

Action and use

Antiprotozoal (malaria).

Ph Eur

DEFINITION

(*RS*)-[2,8-Bis(trifluoromethyl)quinolin-4-yl][(2*SR*)-piperidin-2-yl]methanol hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

mp: about 260 °C, with decomposition.

IDENTIFICATION

First identification A, E.

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mefloquine hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 8 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 8 mg of *mefloquine hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 2.5 ml of the test solution to 100 ml with *methanol R*.

Reference solution (c) To 1 ml of reference solution (b) add 1 ml of a 0.016 g/l solution of *quinidine sulphate R* in *methanol R*.

Plate *TLC silica gel* F_{254} *plate R*.

Pretreatment Develop the plate with a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R*, and dry at 100-105 °C for 15 min before use.

Mobile phase *anhydrous acetic acid R*, *methanol R*, *methylene chloride R* (10:10:80 *V/V/V*).

Application 20 µl.

Development Over a path of 10 cm.

Drying In a current of warm air for 15 min.

Detection Examine in ultraviolet light at 254 nm; lightly spray with a mixture prepared immediately before use of 1 volume of *sulphuric acid R* and 40 volumes of *iodoplatinate reagent R*; spray with *strong hydrogen peroxide solution R*.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Mix about 10 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until a practically white residue is obtained. Allow to cool, then add 2 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to make the solution colourless. Filter. To the filtrate add a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with a blank prepared in the same manner. The test solution is yellow and the blank is red.

D. To about 20 mg add 0.2 ml of *sulphuric acid R*. Blue fluorescence appears in ultraviolet light at 365 nm.

E. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *methanol R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method I*).

Optical rotation (*2.2.7*)

- 0.2° to + 0.2°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 8 mg of *mefloquine hydrochloride CRS* and 8 mg of *quinidine sulphate R* in the mobile phase, then dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Precolumn:
- — *size: l* = 0.025 m, Ø = 4 mm;
- — *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Column:
- — *size: l* = 0.25 m, Ø = 4 mm;
- — *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography* R (5 µm).

Mobile phase Dissolve 1 g of *tetraheptylammonium bromide R* in a mixture of 200 volumes of *methanol R*, 400 volumes of a 1.5 g/l solution of *sodium hydrogen sulphate R* and 400 volumes of *acetonitrile R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 280 nm.

Equilibration With the mobile phase at a flow rate of 2 ml/min for about 30 min.

Injection 20 µl.

Run time 10 times the retention time of mefloquine.

Retention time Quinidine = about 2 min; mefloquine = about 4 min; impurity B = about 15 min; impurity A = about 36 min.

System suitability Reference solution (b):

- — *resolution*: minimum 8.5 between the peaks due to quinidine and mefloquine.

Limits:
- — *impurity with a relative retention with reference to mefloquine of about 0.7* : not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a)

(0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *sum of impurities other than the impurity with a relative retention with reference to mefloquine of about 0.7* : not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 1.000 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

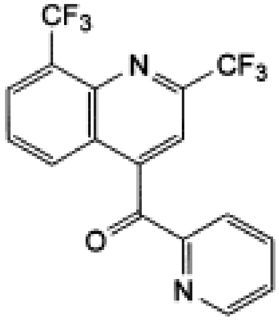
Dissolve 0.350 g in 15 ml of *anhydrous formic acid R* and add 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 41.48 mg of $C_{17}H_{17}ClF_6N_2O$.

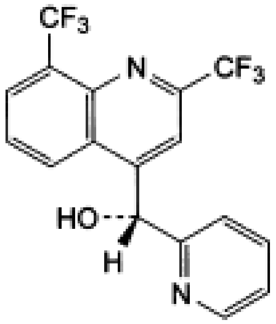
STORAGE

Protected from light.

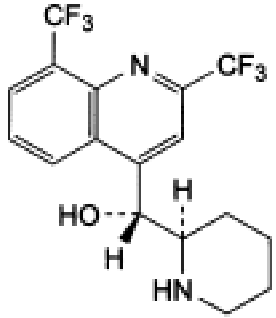
IMPURITIES



A. [2,8-bis(trifluoromethyl)quinolin-4-yl](pyridin-2-yl)methanone,


and enantiomer

B. (*RS*)-[2,8-bis(trifluoromethyl)quinolin-4-yl](pyridin-2-yl)methanol,


and enantiomer

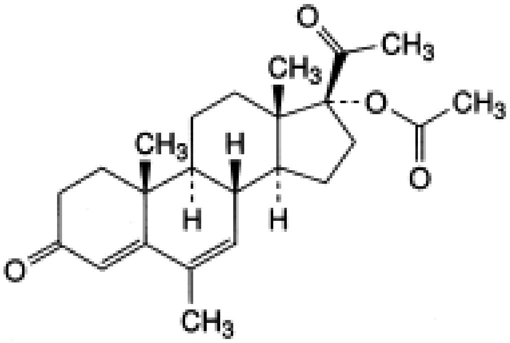
C. (*RS*)-[2,8-bis(trifluoromethyl)quinolin-4-yl][(2*RS*)-piperidin-2-yl]methanol.

Ph Eur

Megestrol Acetate

General Notices

(Ph Eur monograph 1593)



$C_{24}H_{32}O_4$ 384.5 *595-33-5*

Action and use

Progestogen.

Preparation

Megestrol Tablets

Ph Eur

DEFINITION

6-Methyl-3,20-dioxopregna-4,6-dien-17-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

A white or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone, sparingly soluble in alcohol.

mp

About 217 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of megestrol acetate.*

TESTS

Specific optical rotation (*2.2.7*)

+ 14.0 to + 17.0 (dried substance).

Dissolve 2.50 g in *methylene chloride R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in a mixture of 145 volumes of *tetrahydrofuran R* and 255 volumes of *acetonitrile R*, dilute to 20 ml with the same mixture of solvents and then dilute to 50.0 ml with *water R*.

Reference solution (a) Dissolve 25.0 mg of *medroxyprogesterone acetate CRS* in a mixture of 145 volumes of *tetrahydrofuran R* and 255 volumes of *acetonitrile R*, dilute to 20 ml with the same mixture of solvents and then dilute to 50.0 ml with *water R*.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 200.0 ml with the mobile phase.

Reference solution (c) To 3 ml of the test solution, add 1 ml of reference solution (a) and dilute to 50 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *tetrahydrofuran R*, *acetonitrile R*, *water R* (145:255:600 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Sensitivity Reference solution (b).

Run time 1.5 times the retention time of megestrol acetate.

System suitability Reference solution (c):

— *resolution*: minimum 4.0 between the peaks corresponding to impurity A and megestrol acetate.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total of other impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

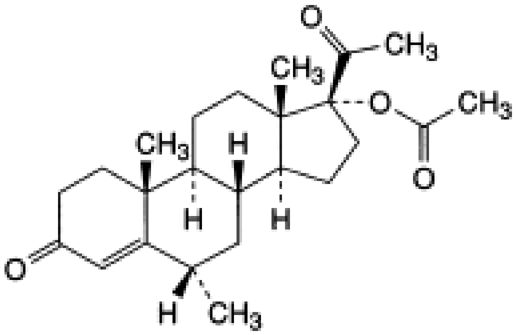
Dissolve 15.0 mg in *ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *ethanol R*. Measure the absorbance (*2.2.25*) at the maximum at 287 nm.

Calculate the content of $C_{24}H_{32}O_4$ taking the specific absorbance to be 640.

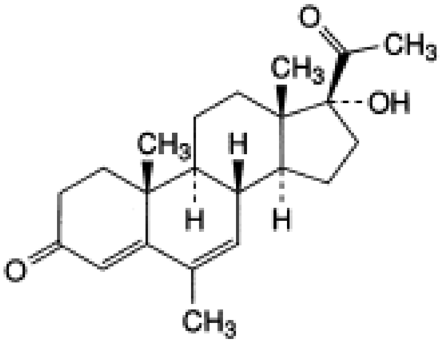
STORAGE

Protected from light.

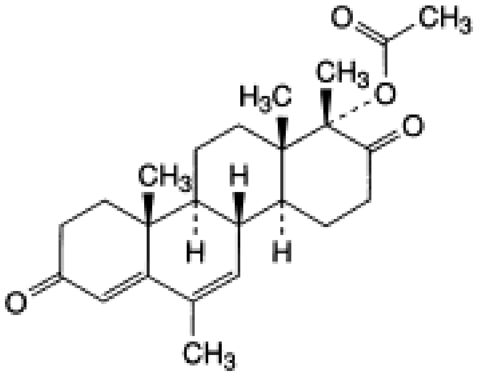
IMPURITIES



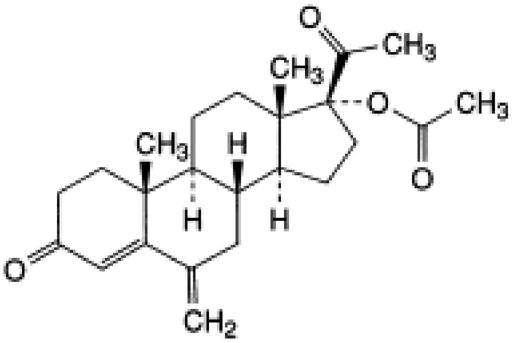
A. 6α-methyl-3,20-dioxopregn-4-en-17-yl acetate (medroxyprogesterone acetate),



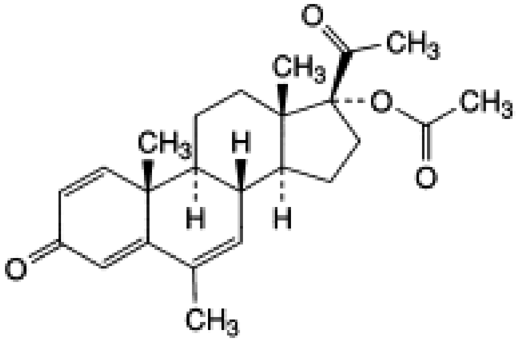
B. 6-methyl-17-hydroxypregna-4,6-diene-3,20-dione (megestrol),



C. 6,17a-dimethyl-3,17-dioxo-*D*-homoandrosta-4,6-dien-17aα-yl acetate (*D-homo* megestrol acetate),



D. 6-methylene-3,20-dioxopregn-4-en-17-yl acetate (6-methylene hydroxyprogesterone acetate),



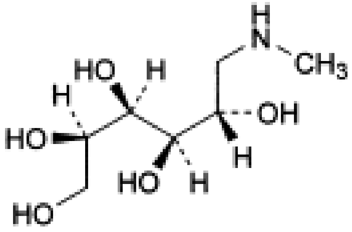
E. 6-methyl-3,20-dioxopregna-1,4,6-trien-17-yl acetate.

Ph Eur

Meglumine

General Notices

(Ph Eur monograph 2055)



$C_7H_{17}NO_5$ 195.2 *6284-40-8*

Action and use

Organic base used in the preparation of organic acids.

Ph Eur

DEFINITION

1-Deoxy-1-(methylamino)-D-glucitol.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

mp

About 128 °C.

IDENTIFICATION

First identification A.

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of meglumine.*

B. To 5 ml of *water R*, add 0.5 ml of *paraldehyde R* and 1 ml of *dilute sulphuric acid R*. Shake vigorously and heat on a water-bath until opalescence appears. Allow to stand for 15 min. To 1 ml of this solution add 0.2 ml of a freshly prepared 100 g/l solution of *sodium nitroprusside R*, 50 mg of the substance to be examined and 2 ml of a 50 g/l solution of *disodium tetraborate R*. A blue colour develops.

C. Dissolve 0.1 g in 2 ml of *nitric acid R*. Add 5 ml of a 6.4 g/l solution of *sodium periodate R*, then add 5 ml of *nitric acid R* and 25 ml of a 17 g/l solution of *silver nitrate R*. Allow to stand in the dark for 10 min. A white precipitate is formed.

D. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 20.0 g in *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and its absorbance *(2.2.25)* at 420 nm is not greater than 0.03.

Dissolve the residue obtained in the test for loss on drying in *water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 16.0 to - 17.0 (dried substance).

Dilute 12.5 ml of solution S to 25.0 ml with *water R*.

Reducing substances

Maximum 0.2 per cent, expressed as glucose.

Dilute 1.25 ml of solution S to 2.5 ml with *water R*, add 2 ml of *cupri-tartaric solution R*. Heat on a water-bath for 10 min. Cool under running water for 1 min and then sonicate the tube for 20 s. Immediately filter through a filter 25 mm in diameter and 0.5 µm in pore size. Rinse with 10 ml of *water R*. Prepare a standard in the same manner using 2.5 ml of a solution obtained by dissolving 20 mg of *glucose R* in *water R* and diluting to 100 ml with the same solvent. Any precipitate on the membrane filter obtained with the test solution is not more intensely coloured than the precipitate obtained with the standard.

Chlorides *(2.4.4)*

Maximum 100 ppm.

To 2.5 ml of solution S add 12.5 ml of *water R*.

Sulphates *(2.4.13)*

Maximum 150 ppm.

To 5 ml of solution S add 10 ml of *distilled water R*.

Iron

Maximum 10 ppm.

To 10 ml of solution S add about 0.8 ml of *hydrochloric acid R* and 0.05 ml of *bromine water R*. Allow to stand for 5 min, evaporate the excess of bromine in a current of air and add 3 ml of *potassium thiocyanate solution R*. Prepare a reference solution at the same time and in the same manner using 10 ml of *iron standard solution (2 ppm Fe) R*, to which 2 ml of *hydrochloric acid R* has been added. After 5 min, any red colour in the test solution is not more intense than that in the reference solution.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Adjust 10 ml of solution S to pH 3-4 with *dilute acetic acid R*. Dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 1.5 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

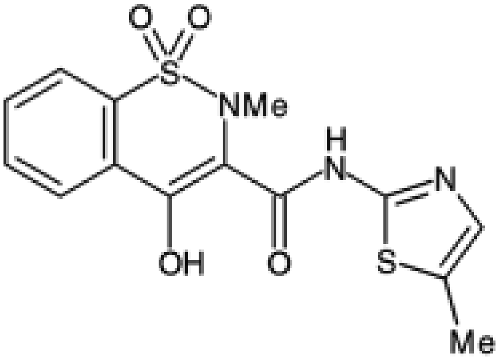
Dissolve 0.180 g in 30 ml of *water R*. Titrate with *0.05 M sulphuric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.05 M sulphuric acid* is equivalent to 19.52 mg of $C_7H_{17}NO_5$.

Ph Eur

Meloxicam

General Notices



$C_{14}H_{13}N_3O_4S_2$ 351.4 *71125-38-7*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparation

Meloxicam Tablets

DEFINITION

Meloxicam is 4-hydroxy-2-methyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2 *H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide. It contains not less than 99.0% and not more than 100.5% of $C_{14}H_{13}N_3O_4S_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A pale yellow powder.

Practically insoluble in *water*; slightly soluble in *acetone*; soluble in *dimethylformamide*; very slightly soluble in *ethanol (96%)* and in *methanol*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of meloxicam *(RS 374)*.

B. The *light absorption*, Appendix II B, in the range 240 nm to 450 nm of a 0.0015% w/v solution in *methanol* exhibits a maximum at 354 nm. The *absorbance* at 354 nm is about 0.8.

TESTS

Clarity of solution

A 5.0% w/v solution in *dimethylformamide* is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Prepare a 0.4% w/v solution of the substance being examined in a mixture of 50 volumes of *methanol (40%)* and 3 volumes of 0.4M *sodium hydroxide* and dilute a portion of this solution with an equal volume of *methanol (40%)*.

(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol (40%)* and further dilute 1 volume of this solution to 10 volumes with *methanol (40%)*.

(3) Prepare in the same manner as solution (1) using *meloxicam impurity standard BPCRS* in place of the substance being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS2 is suitable).

(b) Use linear gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use a column temperature of 45°.

(e) Use detection wavelengths of 260 and 350 nm.

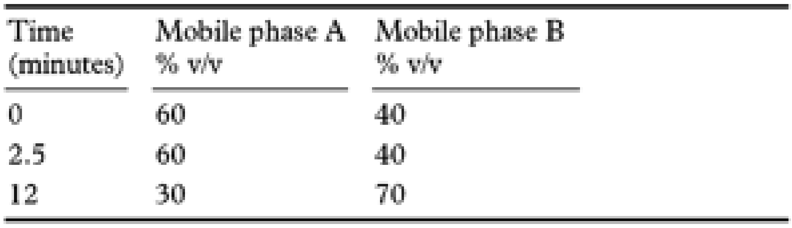
(f) Inject 10 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for 20 minutes or until impurity C has eluted.

MOBILE PHASE

Mobile phase A A 0.1% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 6.0 with *dilute sodium hydroxide solution.*

Mobile phase B *methanol*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v
0	60	40
2.5	60	40
12	30	70

SYSTEM SUITABILITY

The test is not valid unless, at each wavelength, the chromatogram obtained with solution (3) closely resembles the corresponding chromatogram supplied with *meloxicam impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1) at 350 nm:

— the areas of any peaks corresponding to impurity A and impurity C are not greater than half the area of the peak in the chromatogram obtained with solution (2) at 350 nm (0.1% of impurity A assuming a correction factor of 2.0, and 0.05% for impurity C).

In the chromatogram obtained with solution (1) at 260 nm:

— the area of any peak corresponding to impurity B is not greater than the area of the peak in the chromatogram obtained with solution (2) at 350 nm (0.1%).

In both the chromatograms obtained with solution (1) at 350 nm and at 260 nm:

— the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) at 350 nm (0.1%).

Calculate the percentage content of impurities A and C at 350 nm, the percentage content of impurity B at 260 nm and the percentage content of any other secondary peaks at the wavelength of higher response. The nominal total content of any such impurities is not greater than 0.3%.

Heavy metals

2.0 g complies with *limit test C for heavy metals*, Appendix VII (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

Loss on drying

When dried to a constant weight at 105°, loses not more than 0.5% of its weight. Use 3 g.

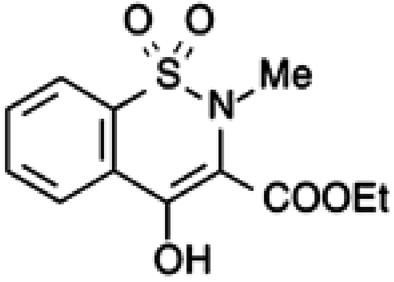
Sulphated ash

Not more than 0.1%, Appendix IX A. Use 1 g.

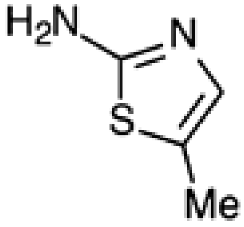
ASSAY

Dissolve 0.25 g in a mixture of 50 ml of *anhydrous acetic acid* and 5 ml of *anhydrous formic acid* and carry out the method for *non-aqueous titration*, Appendix VIII A, determining the end-point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 35.14 mg of $C_{14}H_{13}N_3O_4S_2$.

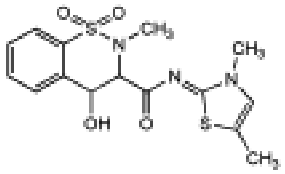
IMPURITIES



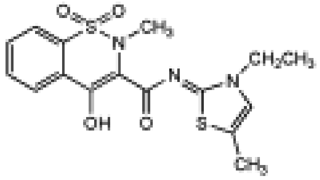
A. ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,



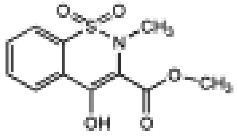
B. 5-methyl 1,3-thiazol-2-amine,



C. *N*-[(2*Z*)-3,5-dimethyl-1,3-thiazol-2(3*H*)-ylidene]-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide,



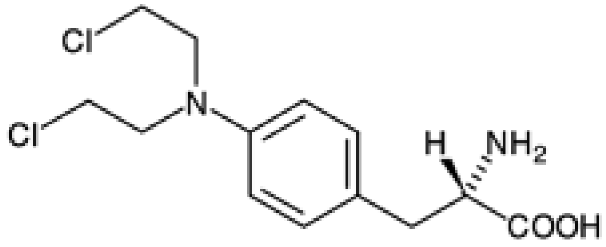
D. *N*-[(2*Z*)-3,ethyl-5-methyl-1,3-thiazol-2(3*H*)-ylidene]-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide,



E. methyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide.

Melphalan

General Notices



$C_{13}H_{18}Cl_2N_2O_2$ 305.2 *148-82-3*

Action and use

Cytotoxic alkylating agent.

Preparations

Melphalan Injection

Melphalan Tablets

DEFINITION

Melphalan is 4-bis(2-chloroethyl)amino-L-phenylalanine. It contains not less than 93.0% and not more than 100.5% of $C_{13}H_{18}Cl_2N_2O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Practically insoluble in *water*; slightly soluble in *methanol*; practically insoluble in *ether*. It dissolves in dilute mineral acids.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of melphalan (*RS 212*).

B. A 0.5% w/v solution in *methanol* is laevorotatory.

TESTS

Ionisable chlorine

Dissolve 0.4 g in a mixture of 75 ml of *water* and 2 ml of *nitric acid*, allow to stand for 2 minutes and titrate with 0.1M *silver nitrate VS* determining the end point potentiometrically. Not more than 0.8 ml is required.

Loss on drying

When dried at 100° at a pressure not exceeding 0.7 kPa for 2 hours, loses not more than 7.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.3%, Appendix IX A.

ASSAY

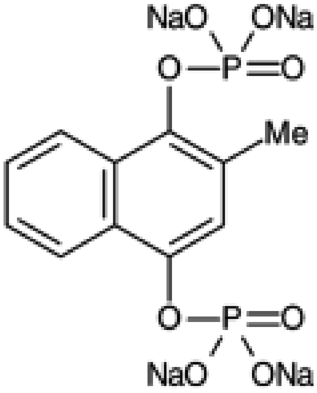
To 0.4 g add 20 ml of a 20% w/v solution of *potassium hydroxide*, heat on a water bath for 2 hours, replacing water lost by evaporation, and cool. Add 75 ml of *water* and 4 ml of *nitric acid*, cool and titrate with 0.1M *silver nitrate VS* determining the end point potentiometrically. Subtract the volume of 0.1M *silver nitrate VS* used in the test for Ionisable chlorine. Each ml of 0.1M *silver nitrate VS* represented by the difference is equivalent to 15.26 mg of $C_{13}H_{18}Cl_2N_2O_2$.

STORAGE

Melphalan should be protected from light.

Menadiol Sodium Phosphate

General Notices



$C_{11}H_8Na_4O_8P_2,6H_2O$ 530.2 *6700-42-1*

Action and use

Vitamin K analogue.

Preparations

Menadiol Phosphate Injection

Menadiol Phosphate Tablets

DEFINITION

Menadiol Sodium Phosphate is tetrasodium 2-methylnaphthalene-1,4-diyl di(orthophosphate) hexahydrate. It contains not less than 98.0% and not more than 100.5% of $C_{11}H_8Na_4O_8P_2$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white to pink, crystalline powder; hygroscopic.

Very soluble in *water*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of menadiol sodium phosphate *(RS 213)*.

B. To 10 ml of a 2% w/v solution add 10 ml of 1M *sulphuric acid*, 10 ml of 0.1M *cerium(IV) sulphate* and 1 ml of *hydrogen peroxide solution (20 vol)* and extract with two 10 ml quantities of *chloroform*. Evaporate the combined chloroform extracts to dryness on a water bath and dry the residue at 40° at a pressure not exceeding 0.7 kPa. The *infrared* *absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of menadione.

C. Dissolve 40 mg in 2 ml of *water*, heat gently with 2 ml of *sulphuric acid* until white fumes are evolved, add *nitric acid* dropwise until digestion is complete and cool. Add 2 ml of *water*, heat until white fumes are evolved again, cool, add a further 10 ml of *water* and neutralise to *litmus paper* with 5M *ammonia*. The solution yields reaction A characteristic of *sodium salts* and reaction B characteristic of *phosphates*, Appendix VI.

TESTS

Inorganic phosphate

Dissolve 25 mg in 10 ml of *water*, add 4 ml of 1M *sulphuric acid*, 1 ml of a 10% w/v solution of *ammonium molybdate* and 2 ml of *methylaminophenyl-sulphite reagent* and allow to stand for 15 minutes. The *absorbance* of the solution at 730 nm, Appendix II B, is not more than the *absorbance* of a solution prepared in the same manner but using 10 ml of a 0.0025% w/v solution of *potassium dihydrogen orthophosphate* in place of the solution of the substance being examined (0.7%).

Total phosphate

Dissolve 85 mg in 50 ml of *glacial acetic acid*, add 5 ml of *mercury(II) acetate solution* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Not less than 7.7 ml and not more than 8.3 ml of 0.1M *perchloric acid VS* is required.

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 50 volumes of *propan-1-ol*, 50 volumes of a 2% w/v solution of *ammonium chloride*, 5 volumes of *butan-1-ol* and 1.5 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions in *methanol (50%)* containing (1) 4.0 w/v of the substance being examined, (2) 0.020% w/v of the substance being examined and (3) 0.0080% w/v of *2-methyl-1,4-naphthaquinone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to 2-methyl-1,4-naphthaquinone (menadione) in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2).

Water

19.0 to 21.5% w/w, Appendix IX A. Use 0.25 g.

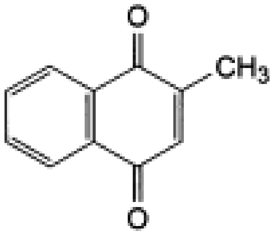
ASSAY

Dissolve 0.1 g in 25 ml of *water*, add 25 ml of *glacial acetic acid* and 25 ml of 3M *hydrochloric acid* and titrate with 0.02M *cerium(IV) sulphate VS* using platinum and calomel electrodes and determining the end point potentio-metrically. Each ml of 0.02M *cerium(IV) sulphate VS* is equivalent to 4.221 mg of $C_{11}H_8Na_4O_8P_2$.

Menadione

General Notices

(Ph Eur monograph 0507)



$C_{11}H_8O_2$ 172.2 *58-27-5*

Action and use

Vitamin K analogue.

Ph Eur

DEFINITION

Menadione contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 2-methylnaphthalene-1,4-dione, calculated with reference to the dried substance.

CHARACTERS

A pale-yellow, crystalline powder, practically insoluble in water, freely soluble in toluene, sparingly soluble in alcohol and in methanol. It is unstable in light.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 105 °C to 108 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *menadione CRS*.

C. Dissolve about 1 mg in 5 ml of *alcohol R*, add 2 ml of *ammonia R* and 0.2 ml of *ethyl cyanoacetate R*. An intense bluish-violet colour develops. Add 2 ml of *hydrochloric acid R*. The colour disappears.

D. Dissolve about 10 mg in 1 ml of *alcohol R*, add 1 ml of *hydrochloric acid R* and heat in a water-bath. A red colour develops.

TESTS

Related substances

Carry out the test protected from bright light. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *acetone R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *nitromethane R*, 2 volumes of *acetone R*, 5 volumes of ethylene *chloride R* and 90 volumes of *cyclohexane R*. Dry the plate in a current of hot air. Repeat the development and drying a further two times. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at a pressure of 2 kPa to 3 kPa for 4 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 15 ml of *glacial acetic acid R* in a flask with a stopper fitted with a valve. Add 15 ml of *dilute hydrochloric acid R* and 1 g of *zinc powder R*. Close the flask. Allow the mixture to stand for 60 min, protected from light, with occasional shaking. Filter the solution over a cotton wad, wash with three quantities, each of 10 ml, of *carbon dioxide-free water R*. Add 0.1 ml of *ferroin R* and immediately titrate the combined filtrate and washings with *0.1 M ammonium and cerium nitrate*.

1 ml of *0.1 M ammonium and cerium nitrate* is equivalent to 8.61 mg of $C_{11}H_8O_2$.

STORAGE

Store protected from light.

Ph Eur

Menotrophin

General Notices

9002-68-0

Action and use

1:1 ratio of follicle stimulating hormone and luteinizing hormone.

Preparation

Menotrophin Injection

DEFINITION

Menotrophin is a dry preparation containing glycoprotein gonadotrophins possessing follicle-stimulating and luteinising activities. It contains not less than 40 IU of follicle-stimulating hormone activity per mg. The ratio of IU of luteinising hormone (LH) activity to IU of follicle-stimulating hormone (FSH) activity is approximately 1. The preparation is exclusively or predominantly of pituitary origin and obtained from the urine of post-menopausal women but, where necessary, chorionic gonadotrophin obtained from the urine of pregnant women may be added to achieve the above ratio.

PRODUCTION

Menotrophin may be prepared by a suitable fractionation procedure followed by ion-exchange chromatography. It is prepared in conditions designed to minimise microbial contamination. The manufacturing process must have been shown to eliminate any adventitious viral contamination such as hepatitis virus or HIV by appropriate validated methods.

CHARACTERISTICS

An almost white or slightly yellow powder.

Soluble in *water*.

IDENTIFICATION

Causes enlargement of the ovaries of immature female rats and increases the weight of the seminal vesicles and prostate gland of immature male rats when administered as directed in the Assay.

TESTS

Hepatitis antigens

Examined by a suitably sensitive immunochemical method, Appendix XIV B, hepatitis antigens are not detected.

HIV antigens

Examined by a suitably sensitive immunochemical method, Appendix XIV B, HIV antigens are not detected.

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D. Use per kg of the rabbit's weight 1 ml of a solution in *sodium chloride injection* containing 5 IU of follicle stimulating hormone activity per ml.

Water

Not more than 5% w/w when determined by *gas chromatography*, Appendix III B, using throughout dry glassware which may be siliconised and the following solutions. For solution (1) dilute 15 µl of *anhydrous methanol* (internal standard) with sufficient *propan-2-ol R1* to produce 100 ml. For solution (2) dissolve 4 mg of the substance being examined in 0.5 ml of *propan-2-ol R1*. For solution (3) dissolve 4 mg of the substance being examined in 0.5 ml of solution (1). For solution (4) add 10 µl of *water* to 50 ml of solution (1).

The chromatographic procedure may be carried out using a stainless steel column (1 m × 2 mm) packed with porous polymer beads (60 to 80 mesh) (Chromosorb 102 is suitable), maintained at 120° with a thermal conductivity detector at 150° and using *helium* as the carrier gas.

From the chromatograms obtained and taking into account any water detectable in solution (1), calculate the percentage content of water taking 0.9972 g as its weight per ml at 20°.

ASSAY

Carry out the *biological assay of menotrophin* described below. For each component the estimated potency is not less than 80% and not more than 125% of the stated potency. The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE

Menotrophin should be kept in an airtight, tamper-evident container and protected from light. Under these conditions it may be expected to retain its potency for not less than 3 years.

LABELLING

The label states (1) the number of IU (Units) of follicle-stimulating hormone activity and the number of IU (Units) of luteinising hormone activity in the container; (2) the number of IU (Units) of follicle-stimulating hormone activity per mg and the number of IU (Units) of luteinising hormone activity per mg; (3) where applicable, the number of IU (Units) of chorionic gonadotrophin activity per mg; (4) the date after which the material is not intended to be used; (5) the conditions under which it should be stored; (6) where applicable, that it is sterile.

Menotrophin intended for use in the manufacture of a parenteral dosage form without a further appropriate sterilisation procedure complies with the following additional requirement

Sterility

Complies with the *test for sterility*, Appendix XVI A.

BIOLOGICAL ASSAY OF MENOTROPHIN

The potency of menotrophin with respect to its follicle-stimulating hormone activity is estimated by comparing its effect in enlarging the ovaries of immature female rats with that of the Standard Preparation of human urinary FSH and human urinary LH under the conditions of a suitable method of assay. The potency of menotrophin with respect to its luteinising hormone activity is estimated by comparing its effect in increasing the weight of the seminal vesicles or the prostate gland of immature male rats with that of the Standard Preparation of human urinary FSH and human urinary LH under the conditions of a suitable method of assay.

Standard Preparation

The Standard Preparation is the International Standard preparation of human urinary follicle-stimulating hormone and luteinising hormone, consisting of a freeze-dried extract of urine from post-menopausal women together with lactose, or another suitable preparation the potency of which has been determined in relation to the International Standard.

SUGGESTED METHOD

Follicle-stimulating hormone activity

Select female rats of the same strain, 19 to 28 days old and differing in age by not more than 3 days, and having weights such that the difference between the heaviest rat and the lightest rat is not more than 10 g. Assign the rats at random to six equal groups of at least five animals. If sets of six litter mates are available, allot one litter mate from each set to each group and mark according to litter.

Choose three doses of the Standard Preparation and three doses of the preparation being examined such that the smallest dose produces a positive response in some of the rats and the largest dose does not produce a maximum response in all of the rats. Use doses in geometric progression. As an initial approximation, total doses of 1.5, 3.0 and 6.0 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation being examined and of the Standard Preparation corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* containing not less than 70 IU of *chorionic gonadotrophin* per ml so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4% w/v of *phenol* or 0.002% w/v of *thiomersal*. Store the solutions at a temperature of 2° to 8°. Inject subcutaneously into each rat the daily dose allocated to its group. Repeat the procedure after 24 and 48 hours. About 24 hours after the last injection, kill the rats and remove the ovaries. Remove any extraneous fluid and tissue and immediately weigh the ovaries from each animal. Record the combined weight of both ovaries from each rat. Calculate the result of the assay by standard statistical methods using the weight of the ovaries as the response. (The precision of the assay may be improved by a suitable correction of the organ weight with reference to the weight of the animal from which it was taken; an analysis of covariance may be used).

Luteinising hormone activity

Select male rats of the same strain, approximately 19 to 28 days old and differing in age by not more than 3 days, and having weights such that the difference between the heaviest rat and the lightest rat is not more than 10 g. Assign the rats at random to six equal groups of at least five animals. If sets of six litter mates are available, allot one litter mate from each set to each group and mark according to litter.

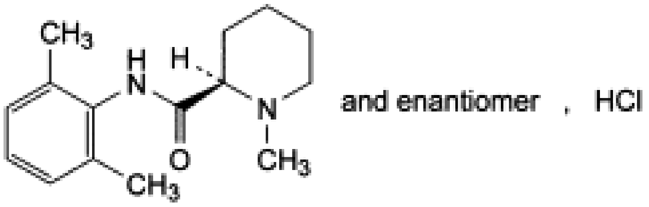
Choose three doses of the Standard Preparation and three doses of the preparation being examined such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximum response in all of the rats. Use doses in geometric progression. As an initial approximation, total doses of 7, 14 and 28 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation being examined and of the Standard Preparation corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4% w/v of *phenol* or 0.002% w/v of *thiomersal*. Store the solutions at a temperature of 2° to 8°. Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, kill the rats and remove the seminal vesicles or the prostate gland. Remove any extraneous fluid and tissue and weigh immediately the seminal vesicles or the prostate gland. Calculate the result of the assay by standard statistical methods, using the weight of the vesicles or the prostate gland as the response. (The precision of the assay may be improved by a suitable correction of the organ weight with reference to the weight of the animal from which it was taken; an analysis of covariance may be used.)

Mepivacaine Hydrochloride

General Notices

(Ph Eur monograph 1242)



$C_{15}H_{23}ClN_2O$ 282.8   *1722-62-9*

Action and use

Local anaesthetic.

Ph Eur

DEFINITION

(*RS*)-*N*-(2,6-Dimethylphenyl)-1-methylpiperidine-2-carboxamide hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in ethanol (96 per cent), very slightly soluble in methylene chloride.

mp: about 260 °C, with decomposition.

IDENTIFICATION

First identification   A, B, D.

Second identification   B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *mepivacaine hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *mepivacaine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *mepivacaine hydrochloride CRS* and 20 mg of *lidocaine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *concentrated ammonia R*, *methanol R*, *ether R* (1:5:100 *V/V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 5 ml of solution S (see Tests) add 1 ml of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 10 ml, of *ether R*. Dry the combined upper layers over *anhydrous sodium sulphate R*. Filter and evaporate the ether on a water-bath. Dry the residue at 100-105 °C for 2 h. The melting point (*2.2.14*) is 151 °C to 155 °C.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.5 g in *carbon dioxide-free water R* and dilute to 30 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

pH (*2.2.3*)

4.0 to 5.0.

Dilute 2 ml of solution S to 5 ml with *carbon dioxide-free water R*.

Optical rotation (*2.2.7*)

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of the substance to be examined and 30.0 mg of *mepivacaine impurity B CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size:* l = 0.125 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 2.25 g/l solution of *phosphoric acid R* adjusted to pH 7.6 with *strong sodium hydroxide solution R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 3 times the retention time of mepivacaine.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to impurity B and mepivacaine.

Limits:

— *impurities B, C, D, E*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), and not more than one of the peaks has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Impurity A

Maximum 100 ppm.

Dissolve 0.50 g in *methanol R* and dilute to 10 ml with the same solvent. To 2 ml of this solution add 1 ml of a freshly prepared 10 g/l solution of *dimethylaminobenzaldehyde R* in *methanol R* and 2 ml of *glacial acetic acid R* and allow to stand for 10 min. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 2 ml of a 5 mg/l solution of *2,6-dimethylaniline R* in *methanol R*.

Heavy metals (*2.4.8*)

Maximum 5 ppm.

Dissolve 1.0 g in *water R* and dilute to 25 ml with the same solvent. Carry out the prefiltration. 10 ml of the filtrate complies with test E. Prepare the reference solution using 2 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

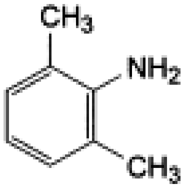
ASSAY

Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

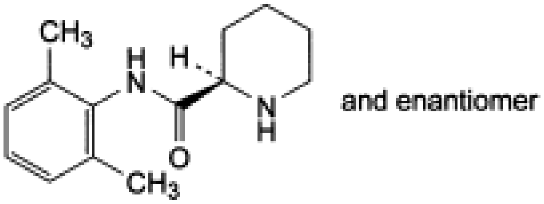
1 ml of *0.1 M sodium hydroxide* is equivalent to 28.28 mg of $C_{15}H_{23}ClN_2O$.

IMPURITIES

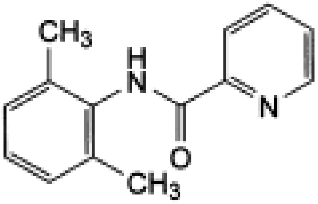
Specified impurities *A, B, C, D, E.*



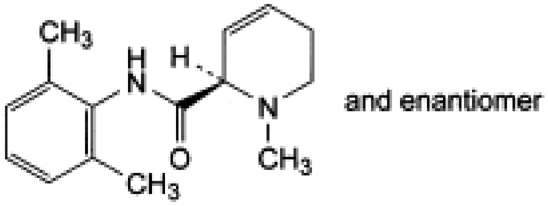
A. 2,6-dimethylaniline,



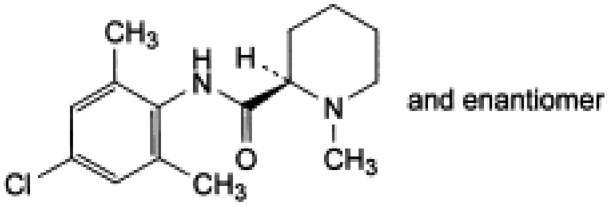
B. (*RS*)-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide,



C. *N*-(2,6-dimethylphenyl)pyridine-2-carboxamide,


CH3
H
N
H
O
N
CH3
CH3
and enantiomer

D. (*RS*)-*N*-(2,6-dimethylphenyl)-1-methyl-1,2,5,6-tetrahydropyridine-2-carboxamide,


CH3
H
N
H
O
N
CH3
Cl
CH3
and enantiomer

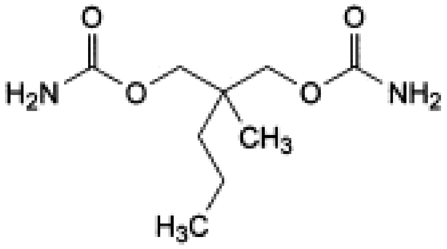
E. (*RS*)-*N*-(4-chloro-2,6-dimethylphenyl)-1-methylpiperidine-2-carboxamide.

Ph Eur

Meprobamate

General Notices

(Ph Eur monograph 0407)



$C_9H_{18}N_2O_4$ 218.3 *57-53-4*

Action and use

Hypnotic.

Ph Eur

DEFINITION

Meprobamate contains not less than 97.0 per cent and not more than the equivalent of 101.0 per cent of 2-methyl-2-propylpropane-1,3-diyl dicarbamate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, amorphous or crystalline powder, slightly soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 104 °C to 108 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *meprobamate CRS*.

C. To 0.5 g add 1 ml of *acetic anhydride R* and 0.05 ml of *sulphuric acid R*, mix and allow to stand for 30 min, shaking frequently. Pour the solution dropwise into 50 ml of *water R*, mix and allow to stand. Initiate crystallisation by scratching the wall of the tube with a glass rod. Collect the precipitate by filtration, wash and dry at 60 °C. The precipitate melts (*2.2.14*) at 124 °C to 128 °C.

D. Dissolve 0.2 g in 15 ml of *0.5 M alcoholic potassium hydroxide* and boil under a reflux condenser for 15 min. Add 0.5 ml of *glacial acetic acid R* and 1 ml of a 50 g/l solution of *cobalt nitrate R* in *ethanol R*. A deep-blue colour develops.

TESTS

Appearance of solution

Dissolve 1.0 g in 20 ml of *ethanol R*. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.20 g of the substance to be examined in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 0.1 ml of the test solution to 10 ml with *alcohol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *pyridine R*, 30 volumes of *acetone R* and 70 volumes of *hexane R*. Dry the plate at 120 °C for 30 min, allow to cool and spray with a solution of 0.25 g of *vanillin R* in a cooled mixture of 10 ml of *alcohol R* and 40 ml of *sulphuric acid R* and heat at 100 °C to 105 °C for 30 min. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (*2.4.8*)

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B for heavy metals (10 ppm). Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with the mixture of *water R* and *acetone R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulphated ash (*2.4.14*).

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

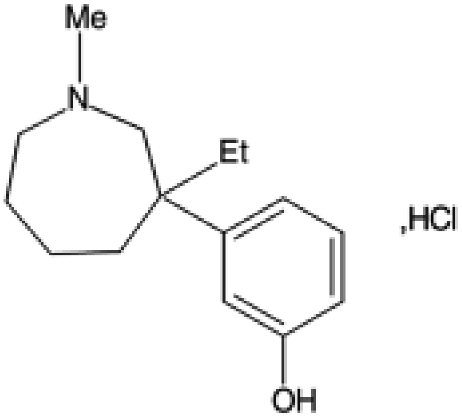
Dissolve 0.1000 g in 15 ml of a 25 per cent *V/V* solution of *sulphuric acid R* and boil under a reflux condenser for 3 h. Cool, dissolve by cautiously adding 30 ml of *water R*, cool again and place in a steam-distillation apparatus. Add 40 ml of *strong sodium hydroxide solution R* and distil immediately by passing steam through the mixture. Collect the distillate into 40 ml of a 40 g/l solution of *boric acid R* until the total volume in the receiver reaches about 200 ml. Add 0.25 ml of *methyl red mixed solution R*. Titrate with *0.1 M hydrochloric acid* until the colour changes from green to violet. Carry out a blank titration.

1 ml of *0.1 M hydrochloric acid* is equivalent to 10.91 mg of $C_9H_{18}N_2O_4$.

Ph Eur

Meptazinol Hydrochloride

General Notices



$C_{15}H_{23}NO,HCl$ 269.8 *59263-76-2*

Action and use

Opioid receptor partial agonist; analgesic.

Preparations

Meptazinol Injection

Meptazinol Tablets

DEFINITION

Meptazinol Hydrochloride is 3-(3-ethyl-1-methylperhydroazepin-3-yl)phenol hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{15}H_{23}NO,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Very soluble in *water* and in *methanol*; freely soluble in *ethanol (96%)*; very slightly soluble in *acetone*. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.01% w/v solution in *ethanol (96%)* exhibits a maximum only at 275 nm. The *absorbance* at 275 nm is about 0.80.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference* *spectrum* of meptazinol hydrochloride *(RS 215)*.

TESTS

Acidity or alkalinity

To 10 ml of a 2% w/v solution in *carbon dioxide-free water* add 0.2 ml of 0.01M *sodium hydroxide VS* and 0.1 ml of *methyl red solution*; the solution is yellow. Add 0.4 ml of 0.01M *hydrochloric acid VS*; the solution is red.

Colour of solution

A 10.0% w/v solution is not more intensely coloured than *reference solution* Y_6, Appendix IV B, Method II.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 18M *ammonia*, 30 volumes of *chloroform* and 70 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of three solutions of the substance being examined in *methanol* containing (1) 1.0% w/v, (2) 0.01% w/v and (3) 0.005% w/v, respectively. After removal of the plate, allow it to dry in air, examine under *ultraviolet light (254 nm)*, expose to iodine vapour for 2 hours and examine again. For each examination any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Loss on drying

When dried at 105° for 3 hours, loses not more than 0.5% of its weight. Use 1 g.

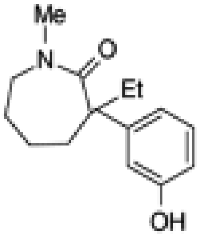
Sulphated ash

Not more than 0.1%, Appendix IX A.

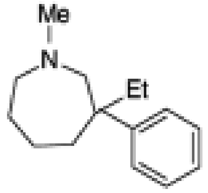
ASSAY

Dissolve 0.15 g in 50 ml of *anhydrous acetic acid* and add 5 ml of *mercury(II) acetate solution*. Carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 26.98 mg of $C_{15}H_{23}NO,HCl$.

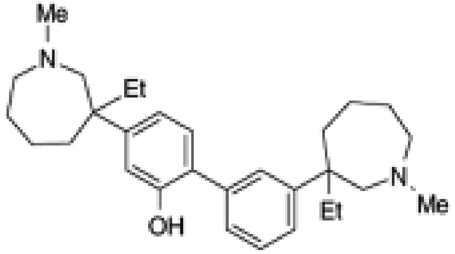
IMPURITIES



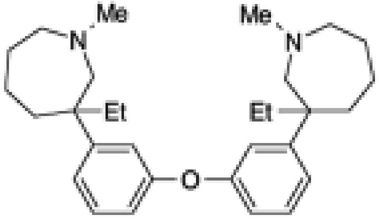
A. 3-ethyl-3-(3-hydroxyphenyl)-1-methylperhydroazepin-2-one



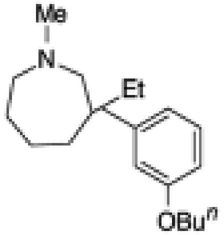
B. 3-ethyl-1-methyl-3-phenylperhydroazepine



C. 4-(3-ethyl-1-methylperhydroazepin-3-yl)-2-[3-(3-ethyl-1-methylperhydroazepin3-yl)phenyl]
phenol



D. 3,3′-oxybis(3-ethyl-1-methylperhydroazepin-3-ylbenzene)

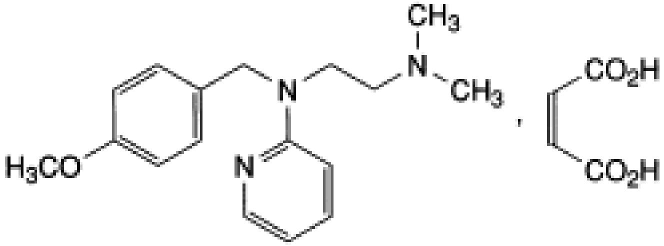


E. 3-ethyl-3-(3-butoxyphenyl)-1-methylperhydroazepine

Mepyramine Maleate

General Notices

(Ph Eur monograph 0278)



$C_{21}H_{27}N_3O_5$ 401.5 *59-33-6*

Action and use

Anti-emetic.

Preparation

Mepyramine Tablets

Ph Eur

DEFINITION

N-(4-Methoxybenzyl)-*N′*,*N′*-dimethyl-*N*-(pyridin-2-yl)ethane-1,2-diamine (*Z*)-butenedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B.*

Second identification *A, C, D, E.*

A. Melting point (*2.2.14*): 99 °C to 103 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation 50 g/l solutions in *methylene chloride R* using a 0.1 mm cell.

Comparison *mepyramine maleate CRS.*

C. Dissolve 0.100 g in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 1.0 ml of this solution to 100.0 ml with *0.01 M hydrochloric acid*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows 2 absorption maxima, at 239 nm and 316 nm. The specific absorbances at the absorption maxima are 431 to 477 and 196 to 220, respectively.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 40 mg of *mepyramine maleate CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *diethylamine R*, *ethyl acetate R* (2:100 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

E. Triturate 0.1 g with 3 ml of *water R* and 1 ml of *strong sodium hydroxide solution R*. Shake with 3 quantities, each of 5 ml, of *ether R*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min; no colour develops. To the rest of the aqueous layer add 1 ml of *bromine water R*. Heat on a water-bath for 15 min and then heat to boiling and cool. To 0.2 ml of this solution add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min; a violet-pink colour develops.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dilute 5 ml of solution S to 25 ml with *carbon dioxide-free water R*.

pH *(2.2.3)*

4.9 to 5.2.

Dilute 1.0 ml of solution S to 10 ml with *carbon dioxide-free water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *anisaldehyde R* (impurity B), 5.0 mg of *mepyramine impurity A CRS* and 5.0 mg of *mepyramine impurity C CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *phenylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase Mix 0.1 volume of *triethylamine R,* 40 volumes of a 0.771 g/l solution of *ammonium acetate R* and 60 volumes of *methanol R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 3 times the retention time of mepyramine.

Relative retention With reference to mepyramine (retention time = about 13 min): maleic acid = about 0.2; impurity C = about 0.3; impurity B = about 0.4; impurity A = about 0.5.

System suitability Reference solution (a):

- *resolution*: minimum 3.0 between the peaks due to impurities C and B.

Limits:
- *impurities A, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to maleic acid.

Chlorides ***(2.4.4)***

Maximum 100 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.25 per cent, determined on 1.000 g by drying in an oven at 80 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 0.150 g in 40 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

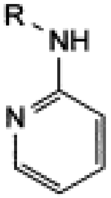
1 ml of *0.1 M perchloric acid* is equivalent to 20.07 mg of $C_{21}H_{27}N_3O_5$.

STORAGE

Protected from light.

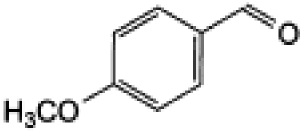
IMPURITIES

Specified impurities A, B, C.



A. R = CH_2-C_6H_4-p-OCH_3: *N*-(4-methoxybenzyl)pyridin-2-amine,

C. R = H: pyridin-2-amine,



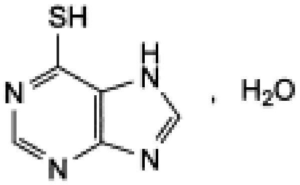
B. 4-methoxybenzaldehyde (anisaldehyde).

Ph Eur

Mercaptopurine

General Notices

(Ph Eur monograph 0096)



$C_5H_4N_4S,H_2O$ 170.2 *6112-76-1*

Action and use

Thiopurine cytotoxic.

Preparations

Mercaptopurine Oral Suspension

Mercaptopurine Tablets

Ph Eur

DEFINITION

Mercaptopurine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7*H*-purine-6-thiol, calculated with reference to the anhydrous substance.

CHARACTERS

A yellow, crystalline powder, practically insoluble in water, slightly soluble in alcohol. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

A. Dissolve 20 mg in 5 ml of *dimethyl sulphoxide R* and dilute to 100 ml with *0.1 M hydrochloric acid*. Dilute 5 ml of the solution to 200 ml with *0.1 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows only one absorption maximum, at 325 nm.

B. Dissolve about 20 mg in 20 ml of *alcohol R* heated to 60 °C and add 1 ml of a saturated solution of *mercuric acetate R* in *alcohol R*. A white precipitate is formed.

C. Dissolve about 20 mg in 20 ml of *alcohol R* heated to 60 °C and add 1 ml of a 10 g/l solution of *lead acetate R* in *alcohol R*. A yellow precipitate is formed.

TESTS

Hypoxanthine

Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF $_{254}$ R* as the coating substance.

Test solution Dissolve 50 mg of the substance to be examined in 1 ml of *dimethyl sulphoxide R* and dilute to 10 ml with *methanol R*.

Reference solution Dissolve 10 mg of *hypoxanthine R* in 10 ml of *dimethyl sulphoxide* R and dilute to 100 ml with *methanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 3 volumes of *concentrated ammonia R*, 7 volumes of *water R* and 90 volumes of *acetone R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot corresponding to hypoxanthine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (2.0 per cent).

Water (*2.5.12*)

10.0 per cent to 12.0 per cent, determined on 0.250 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 50 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium* hydroxide, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 15.22 mg of $C_5H_4N_4S$.

STORAGE

Store protected from light.

Ph Eur

Mercuric Chloride

General Notices

(Ph Eur monograph 0120)

$HgCl_2$ 271.5 *7487-94-7*

Ph Eur

DEFINITION

Content

99.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless or white or almost white crystals or heavy crystalline masses.

Solubility

Soluble in water and in glycerol, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives the reactions of chlorides (*2.3.1*).

B. Solution S (see Tests) gives the reactions of mercury (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R*. The solution is red. Add 0.5 g of *sodium chloride R*. The solution becomes yellow. Not more than 0.5 ml of *0.01 M hydrochloric acid* is required to change the colour to red.

Mercurous chloride

Dissolve 1.0 g in 30 ml of *ether R*. The solution shows no opalescence.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 2.00 g by drying *in vacuo* for 24 h.

ASSAY

Dissolve 0.500 g in 100 ml of *water R*. Add 20.0 ml of *0.1 M sodium edetate* and 5 ml of *buffer solution pH 10.9 R*. Allow to stand for 15 min. Add 0.1 g of *mordant black 11 triturate R* and titrate with *0.1 M zinc sulphate* until the colour changes to purple. Add 3 g of *potassium iodide R*, allow to stand for 2 min, add a further 0.1 g of *mordant black 11 triturate R* and titrate with *0.1 M zinc sulphate*.

1 ml of *0.1 M zinc sulphate* used in the second titration is equivalent to 27.15 mg of $HgCl_2$.

STORAGE

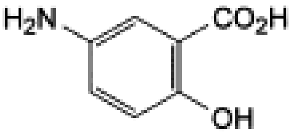
Protected from light.

Ph Eur

Mesalazine

General Notices

(Ph Eur monograph 1699)



$C_7H_7NO_3$ 153.1 *89-57-6*

Action and use

Aminosalicylate; treatment of ulcerative colitis.

Ph Eur

DEFINITION

5-Amino-2-hydroxybenzoic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Almost white or light grey or light pink powder or crystals.

Solubility

Very slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides and in dilute hydrochloric acid.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Dissolve 50.0 mg in 10 ml of a 10.3 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml to 200.0 ml with a 10.3 g/l solution of *hydrochloric acid R*. Examined between 210 nm and 250 nm *(2.2.25)*, the solution shows an absorption maximum at about 230 nm. The specific absorbance at the maximum is 430 to 450.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *mesalazine CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in 10 ml of a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 20.0 ml with *methanol R*.

Reference solution Dissolve 50 mg of *mesalazine CRS* in 10 ml of a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 20.0 ml with *methanol R*.

Plate A suitable silica gel as the coating substance.

Mobile phase *glacial acetic acid R*, *methanol R*, *methyl isobutyl ketone R* (10:40:50 *V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

Maintain the solutions at 40 °C during preparation and measurements. Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 20 ml with the same acid. The solution is clear *(2.2.1)*. Immediately measure the absorbance *(2.2.25)* of the solution at 440 nm and 650 nm. The absorbance is not greater than 0.15 at 440 nm and 0.10 at 650 nm.

Reducing substances

Dissolve 0.10 g in *dilute hydrochloric acid R* and dilute to 25 ml with the same acid. Add 0.2 ml of *starch solution R* and 0.25 ml of *0.01 M iodine*. Allow to stand for 2 min. The solution is blue or violet-brown.

Related substances

Liquid chromatography (*2.2.29*). *Use freshly prepared solutions and mobile phases*.

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b) Dissolve 5.0 mg of *3-aminobenzoic acid R* in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 1.0 ml to 25.0 ml with the test solution.

Reference solution (c) Dissolve 5.0 mg of *3-aminobenzoic acid R* in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 1.0 ml to 50.0 ml with mobile phase A.

Reference solution (d) Dissolve 10.0 mg of *3-aminophenol R* in mobile phase A and dilute to

100.0 ml with mobile phase A. Dilute 1.0 ml to 50.0 ml with mobile phase A.

Reference solution (e) Dissolve 5.0 mg of *2,5-dihydroxybenzoic acid R* in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 1.0 ml to 50.0 ml with mobile phase A.

Reference solution (f) Dissolve 15.0 mg of *salicylic acid R* in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 1.0 ml to 50.0 ml with mobile phase A.

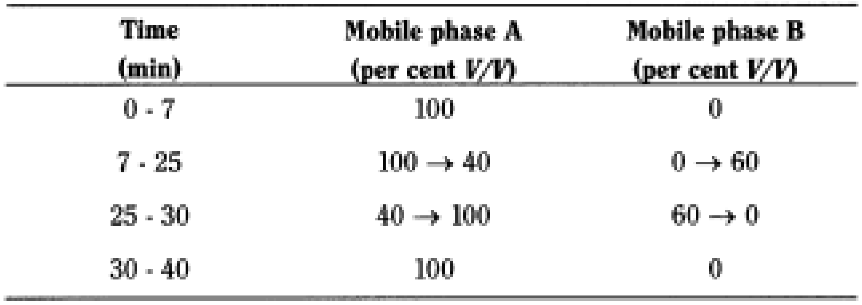
Blank solution Mobile phase A.

Column:

- — *size: l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: spherical *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- — *mobile phase A*: dissolve 2.2 g of *perchloric acid R* and 1.0 g of *phosphoric acid R* in *water R* and dilute to 1000.0 ml with the same solvent;
- — *mobile phase B*: dissolve 1.7 g of *perchloric acid R* and 1.0 g of *phosphoric acid R* in *acetonitrile R* and dilute to 1000.0 ml with the same solvent.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	100	0
7 - 25	100 → 40	0 → 60
25 - 30	40 → 100	60 → 0
30 - 40	100	0

Flow rate 1.25 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Relative retention With reference to mesalazine (retention time = about 5 min): impurity B = about 0.8; impurity D = about 1.2; impurity G = about 3.1; impurity H = about 3.9.

System suitability Reference solution (b):

- — *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

Limits:

- — *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent);
- — *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- — *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.1 per cent);
- — *impurity H*: not more than the area of the principal peak in the chromatogram obtained

with reference solution (f) (0.3 per cent);

— *any other impurity*: not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent). Disregard any peaks obtained with the blank solution.

Impurity A and impurity C

Liquid chromatography *(2.2.29)*. *Use freshly prepared mobile phases.*

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a) Dissolve 5.0 mg of *2-aminophenol R* in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 10.0 ml to 100.0 ml with mobile phase A.

Reference solution (b) Dissolve 5.0 mg of *4-aminophenol R* in mobile phase A and dilute to 250.0 ml with mobile phase A. To 1.0 ml of this solution, add 1.0 ml of reference solution (a) and dilute to 100.0 ml with mobile phase A.

Reference solution (c) Dilute 1.0 ml of the test solution to 200.0 ml with mobile phase A. To 5.0 ml of this solution add 5.0 ml of reference solution (a).

Column:

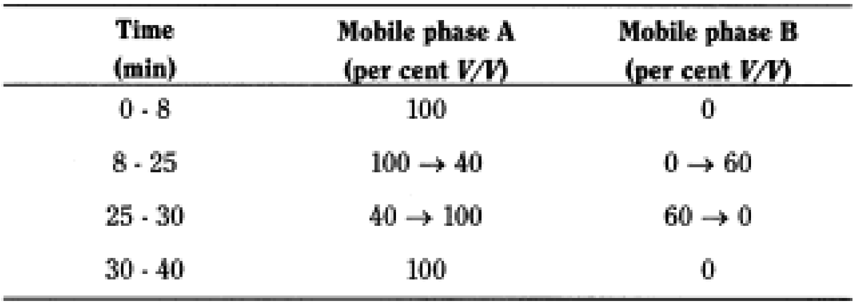
— *size: l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: dissolve 2.2 g of *perchloric acid R* and 1.0 g of *phosphoric acid R* in *water R* and dilute to 1000.0 ml with the same solvent;

— *mobile phase B*: dissolve 1.7 g of *perchloric acid R* and 1.0 g of *phosphoric acid R* in *acetonitrile R* and dilute to 1000.0 ml with the same solvent.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	100	0
8 - 25	100 → 40	0 → 60
25 - 30	40 → 100	60 → 0
30 - 40	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl; inject the test solution and reference solutions (b) and (c).

Relative retention With reference to mesalazine (retention time = about 9 min): impurity A =

about 0.5; impurity C = about 0.9.

System suitability Reference solution (c):

— *resolution*: minimum 3 between the peaks due to impurity C and mesalazine.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (200 ppm);

— *impurity C*: not more than 4 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (200 ppm).

Impurity K

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution Dissolve 27.8 mg of *aniline hydrochloride R* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 0.20 ml of this solution to 20.0 ml with the mobile phase. Dilute 0.20 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 15 volumes of *methanol R* and 85 volumes of a solution containing 1.41 g/l of *potassium dihydrogen phosphate R* and 0.47 g/l of *disodium hydrogen phosphate dihydrate R* previously adjusted to pH 8.0 with a 42 g/l solution of *sodium hydroxide R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 50 µl.

Retention time impurity K = about 15 min.

System suitability Reference solution:

— *signal-to-noise ratio*: minimum 10 for the principal peak.

Limit:

— *impurity K*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm).

Chlorides

Maximum 0.1 per cent.

Dissolve 1.50 g in 50 ml of *anhydrous formic acid R*. Add 100 ml of *water R* and 5 ml of *2 M nitric acid*. Titrate with *0.005 M silver nitrate* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.005 M silver nitrate* is equivalent to 0.1773 mg of Cl.

Sulphates ***(2.4.13)***

Maximum 200 ppm.

Shake 1.0 g with 20 ml of *distilled water R* for 1 min and filter. 15 ml of the filtrate complies with the limit test for sulphates.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with limit test F. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

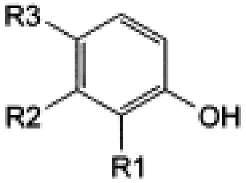
Dissolve 50.0 mg in 100 ml of boiling *water R*. Cool rapidly to room temperature and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 15.31 mg of $C_7H_7NO_3$.

STORAGE

In an airtight container , protected from light.

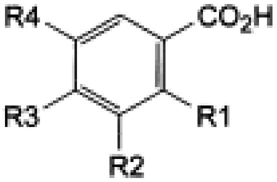
IMPURITIES



A. R1 = R2 = H, R3 = NH_2: 4-aminophenol,

B. R1 = R3 = H, R2 = NH_2: 3-aminophenol,

C. R1 = NH_2, R2 = R3 = H: 2-aminophenol,



D. R1 = R3 = R4 = H, R2 = NH_2: 3-aminobenzoic acid,

E. R1 = OH, R2 = R4 = H, R3 = NH_2: 4-amino-2-hydroxybenzoic acid (4-aminosalicylic acid),

F. R1 = OH, R2 = NH_2, R3 = R4 = H: 3-amino-2-hydroxybenzoic acid (3-aminosalicylic acid),

G. R1 = R4 = OH, R2 = R3 = H: 2,5-dihydroxybenzoic acid,

H. R1 = OH, R2 = R3 = R4 = H: 2-hydroxybenzoic acid (salicylic acid),

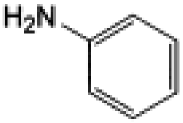
I. R1 = OH, R2 = R3 = H, R4 = N=N-C_6H_5: 2-hydroxy-5-(phenyldiazenyl)benzoic acid (phenylazosalicylic acid),

J. R1 = OH, R2 = R4 = NH_2, R3 = H: 3,5-diamino-2-hydroxybenzoic acid (diaminosalicylic acid),

L. R1 = Cl, R2 = R3 = R4 = H: 2-chlorobenzoic acid,

M. R1 = Cl, R2 = R3 = H, R4 = NO_2: 2-chloro-5-nitrobenzoic acid,

N. R1 = OH, R2 = R3 = H, R4 = NO_2: 2-hydroxy-5-nitrobenzoic acid (5-nitrosalicylic acid),



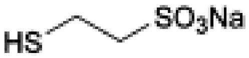
K. aniline.

Ph Eur

Mesna

General Notices

(Ph Eur monograph 1674)



$C_2H_5NaO_3S_2$ 164.2 *19767-45-4*

Action and use

Preventing adverse effects of cyclophosphamide and ifosfamide.

Ph Eur

DEFINITION

Sodium 2-sulphanylethanesulphonate.

Content

96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in cyclohexane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of mesna.

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 6.0.

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 4.0 mg of *mesna impurity C CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 6.0 mg of *mesna impurity D CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (c) Dilute 3.0 ml of the test solution to 10.0 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of reference solution (c) to 100.0 ml with the mobile phase.

Reference solution (e) Dilute 6.0 ml of reference solution (c) to 20.0 ml with the mobile phase. To 10 ml of the solution add 10 ml of reference solution (a).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Dissolve 2.94 g of *potassium dihydrogen phosphate R*, 2.94 g of *dipotassium hydrogen phosphate R* and 2.6 g of *tetrabutylammonium hydrogen sulphate R* in about 600 ml of *water R*. Adjust to pH 2.3 with *phosphoric acid R*, add 335 ml of *methanol R* and dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl.

Run time 4 times the retention time of mesna.

Relative retention With reference to mesna (retention time = about 4.8 min): impurities A and B = about 0.6; impurity E = about 0.8; impurity C = about 1.4; impurity D = about 2.3.

System suitability Reference solution (e):

— *resolution*: minimum 3.0 between the peaks due to mesna and impurity C.

Limits:
— *correction factors*: for the calculation of contents, multiply the peak areas of impurities A, B and E by 0.01;

— *impurity C:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *impurities A, B, E:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *any other impurity*: for each impurity, not more than one third of the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *sum of other impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *disregard limit*: 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.045 per cent).

Chlorides (*2.4.4*)

Maximum 250 ppm.

Dilute 1 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 300 ppm.

Dilute 5 ml of solution S to 30 ml with *distilled water R*. 15 ml of the solution complies with the test.

Disodium edentate

Maximum 500 ppm.

Dissolve 4.000 g in 90 ml of *water R* and adjust to pH 4.5 using *0.1 M hydrochloric acid*. Add 10 ml of *acetate buffer solution pH 4.5 R* and 50 ml of *2-propanol R*. Add 2 ml of a 0.25 g/l solution of *dithizone R* in *2-propanol R*. Titrate with *0.01 M zinc sulphate* until the colour changes from bluish-grey to pink.

1 ml of *0.01 M zinc sulphate* is equivalent to 3.72 mg of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g under high vacuum at 60 °C for 2 h.

ASSAY

Dissolve 0.120 g in 10 ml of *water R*. Add 10 ml of *1 M sulphuric acid* and 10.0 ml of *0.1 M iodine*. Titrate with *0.1 M sodium thiosulphate* adding 1 ml of *starch solution R* near the endpoint. Carry out a blank titration.

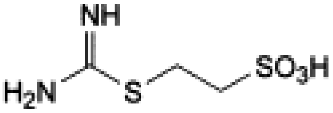
1 ml of *0.1 M sodium thiosulphate* is equivalent to 16.42 mg of $C_2H_5NaO_3S_2$.

STORAGE

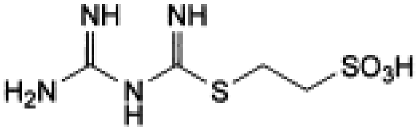
In an airtight container .

IMPURITIES

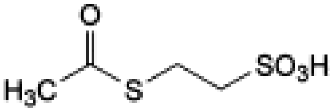
Specified impurities A, B, C, D, E.



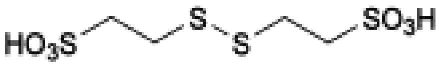
A. 2-(carbamimidoylsulphanyl)ethanesulphonic acid,



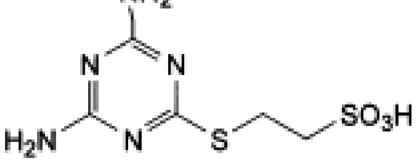
B. 2-[[(guanidino)(imino)methyl]sulphanyl]ethanesulphonic acid,



C. 2-(acetylsulphanyl)ethanesulphonic acid,



D. 2,2′-(disulphanediyl)bis(ethanesulphonic acid),



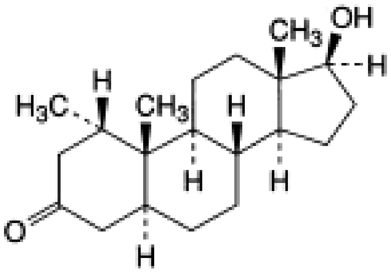
E. 2-(4,6-diamino-1,3,5-triazin-2-yl)sulphanylethanesulphonic acid.

Ph Eur

Mesterolone

General Notices

(Ph Eur monograph 1730)



$C_{20}H_{32}O_2$ 304.5 *1424-00-6*

Action and use

Androgen.

Ph Eur

DEFINITION

17β-Hydroxy-1α-methyl-5α-androstan-3-one.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in acetone, in ethyl acetate and in methanol.

IDENTIFICATION

A. Melting point *(2.2.14)*: 206 °C to 211 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *mesterolone CRS.*

TESTS

Specific optical rotation *(2.2.7)*

+ 20 to + 24 (dried substance).

Dissolve 0.200 g in *methylene chloride R* and dilute to 10.0 ml with the same solvent.

Impurity B

Maximum 0.5 per cent.

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 100.0 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Reference solution (b) Dissolve 5.0 mg of *mesterolone impurity A CRS* in reference solution (a) and dilute to 100.0 ml with the same solution.

Plate *TLC silica gel plate R*.

Mobile phase *methanol R*, *acetone R*, *toluene R* (2:15:85 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 366 nm; spray with a 200 g/l solution of *toluenesulphonic acid R* in *alcohol R* and heat the plate for 10 min at 120 °C.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots (blue spot due to mesterolone and yellow spot due to impurity A).

Limit:

— *impurity B*: any blue spot, apart from the main spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in a mixture of 20 volumes of *water R* and 80 volumes of *acetonitrile R* and dilute to 25.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 50.0 mg of *mesterolone CRS* in a mixture of 20 volumes of *water R* and 80 volumes of *acetonitrile R* and dilute to 25.0 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10.0 mg of *mesterolone impurity A CRS* in a mixture of 20 volumes of *water R* and 80 volumes of *acetonitrile R* and dilute to 5.0 ml with the same mixture of solvents.

Reference solution (c) Dilute 0.5 ml of reference solution (a) and 0.5 ml of reference solution (b) to 100.0 ml with a mixture of 20 volumes of *water R* and 80 volumes of *acetonitrile R*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase *acetonitrile R*, *water R*, *methanol R* (20:40:60 *V/V/V*).

Flow rate 0.9 ml/min.

Detection Spectrophotometer at 200 nm.

Injection 50 µl; inject the test solution and reference solution (c).

Run time 3 times the retention time of mesterolone.

Relative retention With reference to mesterolone (retention time = about 22 min): impurity A = about 0.7.

System suitability Reference solution (c):

— *resolution*: minimum 6.0 between the peaks due to impurity A and to mesterolone.

Limits:

— *impurity A*: not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *any other impurity*: not more than half the area of the peak due to mesterolone in the chromatogram obtained with reference solution (c) (0.25 per cent);

— *total*: not more than 1.5 times the area of the peak due to mesterolone in the chromatogram obtained with reference solution (c) (0.75 per cent);

— *disregard limit*: 0.1 times the area of the peak due to mesterolone in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

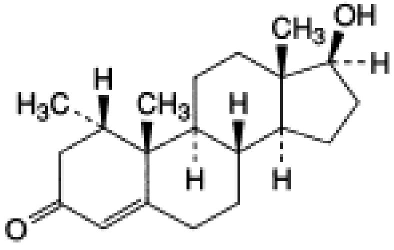
ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances.

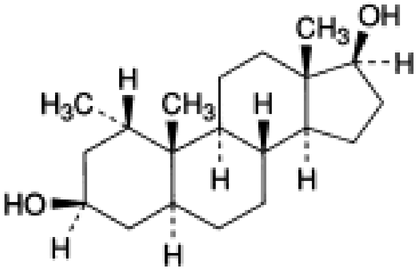
Injection 10 µl; inject the test solution and reference solution (a).

Calculate the percentage content of $C_{20}H_{32}O_2$.

IMPURITIES



A. 17β-hydroxy-1α-methylandrost-4-en-3-one,



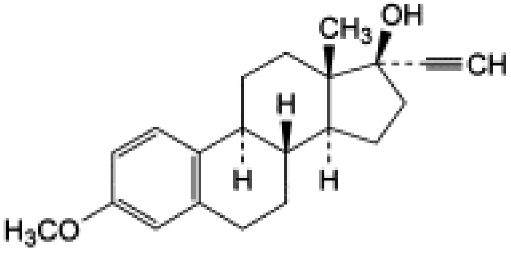
B. 1α-methyl-5α-androstane-3β,17β-diol.

Ph Eur

Mestranol

General Notices

(Ph Eur monograph 0509)



$C_{21}H_{26}O_2$ 310.4 *72-33-3*

Action and use

Estrogen.

Ph Eur

DEFINITION

Mestranol contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 3-methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in alcohol.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)*: 150 °C to 154 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *mestranol CRS*.

C. Examine the chromatograms obtained in the test for related substances in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour, fluorescence and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in 1 ml of *sulphuric acid R*. A red colour develops with a greenish-yellow fluorescence in ultraviolet light at 365 nm. Add the solution to 10 ml of *water R* and mix. The solution becomes pink and a pink to violet precipitate is formed on standing.

TESTS

Specific optical rotation *(2.2.7)*

Dissolve 0.100 g in *anhydrous pyridine R* and dilute to 10.0 ml with the same solvent. The specific optical rotation is - 20 to - 24, calculated with reference to the dried substance.

Absorbance *(2.2.25)*

Dissolve 25.0 mg in *alcohol R* and dilute to 25.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *alcohol R*. Examined between 260 nm and 310 nm, the solution shows two absorption maxima, at 279 nm and 288 nm, and a minimum at 286 nm. The specific absorbances at the maxima are 62 to 68 and 59 to 64, respectively.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *chloroform R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *chloroform R*.

Reference solution (a) Dissolve 10 mg of *mestranol CRS* in c*hloroform R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 10 ml with *chloroform R.*

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with *chloroform R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *alcohol R* and 90 volumes of *toluene R*. Allow the plate to dry in air until the solvent has evaporated. Heat at 110 °C for 10 min. Spray the hot plate with alcoholic solution of *sulphuric acid R*. Heat again at 110 °C for 10 min. Examine in daylight and in ultraviolet light at 365 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.200 g in 40 ml of *tetrahydrofuran R* and add 5 ml of a 100 g/l solution of *silver nitrate R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.04 mg of $C_{21}H_{26}O_2$.

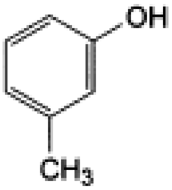
STORAGE

Store protected from light.

Metacresol

General Notices

(Ph Eur monograph 2077)



C_7H_8O 108.1 *108-39-4*

Action and use

Antiseptic; antimicrobial preservative.

Ph Eur

DEFINITION

3-Methylphenol.

CHARACTERS

Appearance

Colourless or yellowish liquid.

Solubility

Sparingly soluble in water, miscible with ethanol (96 per cent) and with methylene chloride.

Relative density

About 1.03.

mp

About 11 °C.

bp

About 202 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of metacresol.*

TESTS

Solution S

Dissolve 1.5 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Freshly prepared solution S is not more opalescent than reference suspension III *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Acidity

To 25 ml of solution S add 0.15 ml of *methyl red solution R*. The solution is red. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 1.00 g of the substance to be examined in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 0.10 g of *cresol R*, 0.10 g of *p-cresol R* and 0.10 g of the substance to be examined in *methanol R* and dilute to 20.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 20.0 ml with *methanol R*.

Column:

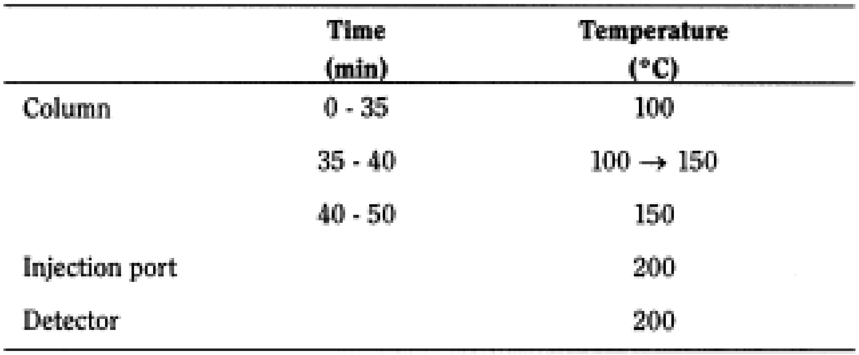
- *material*: fused silica;
- *size: l* = 25 m, Ø = 0.25 mm;
- *stationary phase*: *poly[(cyanopropyl)(methyl)][(phenyl)(methyl)]siloxane R* (0.2 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.8 ml/min.

Split ratio 1:30.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 35	100
	35 - 40	100 → 150
	40 - 50	150
Injection port		200
Detector		200

Detection Flame ionisation.

Injection 1.0 µl.

Relative retention With reference to metacresol (retention time = about 28 min): impurity B = about 0.75; impurity C = about 0.98.

System suitability Reference solution (a):

— *resolution*: minimum 1.4 between the peaks due to impurity C and metacresol.

Limits:

— *impurities B, C*: for each impurity, not more than 0.5 per cent;

— *any other impurity*: for each impurity, not more than 0.1 per cent;

— *total*: not more than 1.0 per cent;

— *disregard limit*: the area of the peak due to metacresol in the chromatogram obtained with reference solution (b) (0.05 per cent).

Residue on evaporation

Maximum 0.1 per cent.

Evaporate 2.0 g to dryness on a water-bath under a fume hood and dry at 100-105 °C for 1 h. The residue weighs a maximum of 2 mg.

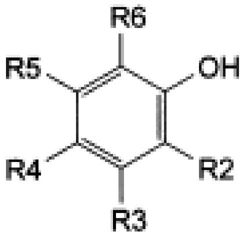
STORAGE

In an airtight container , protected from light.

IMPURITIES

Specified impurities *B, C.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, D, E, F, G, H, I, J, K, L, M.*



A. R2 = R3 = R4 = R5 = R6 = H: phenol,

B. R2 = CH_3, R3 = R4 = R5 = R6 = H: 2-methylphenol (*o*-cresol, cresol),

C. R2 = R3 = R5 = R6 = H, R4 = CH_3: 4-methylphenol (*p*-cresol),

D. R2 = R6 = CH_3, R3 = R4 = R5 = H: 2,6-dimethylphenol (2,6-xylenol),

E. R2 = C_2H_5, R3 = R4 = R5 = R6 = H: 2-ethylphenol (*o*-ethylphenol),

F. R2 = R4 = CH_3, R3 = R5 = R6 = H: 2,4-dimethylphenol (2,4-xylenol),

G. R2 = R5 = CH_3, R3 = R4 = R6 = H: 2,5-dimethylphenol (2,5-xylenol),

H. R2 = $CH(CH_3)_2$, R3 = R4 = R5 = R6 = H: 2-(1-methylethyl)phenol,

I. R2 = R3 = CH_3, R4 = R5 = R6 = H: 2,3-dimethylphenol (2,3-xylenol),

J. R2 = R4 = R6 = H, R3 = R5 = CH_3: 3,5-dimethylphenol (3,5-xylenol),

K. R2 = R3 = R5 = R6 = H, R4 = C_2H_5: 4-ethylphenol (*p*-ethylphenol),

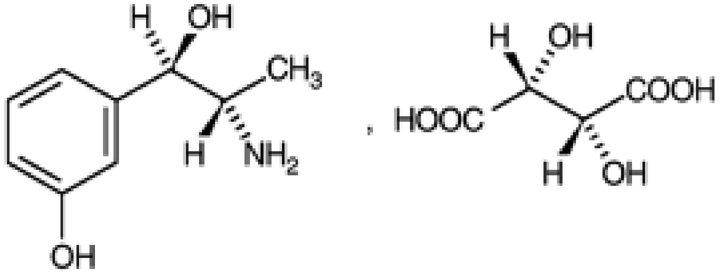
L. R2 = R5 = R6 = H, R3 = R4 = CH_3: 3,4-dimethylphenol (3,4-xylenol),

M. R2 = R3 = R5 = CH_3, R4 = R6 = H: 2,3,5-trimethylphenol.

Ph Eur

Metaraminol Tartrate

General Notices



$C_9H_{13}NO_2,C_4H_6O_6$ 317.3 *17171-57-2*

Action and use

Adrenoceptor agonist.

Preparation

Metaraminol Injection

DEFINITION

Metaraminol Tartrate is (1*R*,2*S*)-2-amino-1-(3-hydroxyphenyl)propan-1-ol hydrogen (2*R*,3*R*)-tartrate. It contains not less than 99.0% and not more than 101.0% of $C_9H_{13}NO_2,C_4H_6O_6$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Freely soluble in *water*; sparingly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. In the test for Related substances the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

B. To 0.5 ml of a 0.05% w/v solution add 0.5 ml of *phosphomolybdotungstic reagent* and 5 ml of *dilute sodium carbonate solution* and allow to stand for 5 minutes. An intense blue colour is produced.

C. To 4 ml of a 0.05% w/v solution add 5 ml of *borate buffer pH 9.6* and 1 ml of a freshly prepared 0.5% w/v solution of *sodium 1,2-naphthaquinone-4-sulphonate* and allow to stand for 1 minute. Add 0.2 ml of a 2% v/v solution of *benzalkonium chloride solution* and 5 ml of *toluene* and shake. A mauve colour is immediately produced in the toluene layer (distinction from phenylephrine).

TESTS

Acidity

pH of a 5% w/v solution, 3.2 to 3.5, Appendix V L.

Phenones

Absorbance of a 0.2% w/v solution at 310 nm, not more than 0.2, calculated with reference to the dried substance, Appendix II B.

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 80 volumes of *chloroform*, 80 volumes of *methanol* and 10 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions in *methanol*. Solutions (1), (2) and (3) are solutions of the substance being examined containing (1) 1.0% w/v, (2) 0.050% w/v and (3) 0.0050% w/v. Solution (4) contains 0.050% w/v of *metaraminol tartrate BPCRS*. After removal of the plate, allow it to dry in air and spray with a solution prepared in the following manner. Mix 25 ml of a 0.45% w/v solution of *sulphanilic acid* in 1M *hydrochloric acid* with 1.5 ml of a 5% w/v solution of *sodium nitrite*, allow to stand for 5 minutes and mix cautiously with 25 ml of 2M *sodium carbonate*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

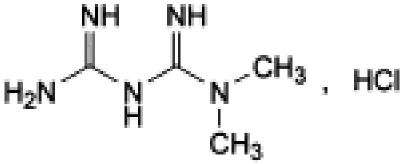
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.6 g and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 31.73 mg of $C_9H_{13}NO_2,C_4H_6O_6$.

Metformin Hydrochloride

General Notices

(Ph Eur monograph 0931)



$C_4H_{11}N_5,HCl$ 165.6   *1115-70-4*

Action and use

Biguanide; treatment of diabetes mellitus.

Preparation

Metformin Tablets

Ph Eur

DEFINITION

1,1-Dimethylbiguanide hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystals.

Solubility

Freely soluble in water, slightly soluble in alcohol, practically insoluble in acetone and in methylene chloride.

IDENTIFICATION

First identification   *B, E.*

Second identification   *A, C, D, E.*

A. Melting point (*2.2.14*): 222 °C to 226 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium chloride R*.

Comparison *metformin hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 20 mg of *metformin hydrochloride CRS* in *water R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase Upper layer of a mixture of 10 volumes of *glacial acetic acid R*, 40 volumes of *butanol R* and 50 volumes of *water R.*

Application 5 µl.

Development Over a path of 15 cm.

Drying At 100-105 °C for 15 min.

Detection Spray with a mixture of equal volumes of a 100 g/l solution of *sodium nitroprusside R*, a 100 g/l solution of *potassium ferricyanide R* and a 100 g/l solution of *sodium hydroxide R*, prepared 20 min before use.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 5 mg in *water R* and dilute to 100 ml with the same solvent. To 2 ml of the solution add 0.25 ml of *strong sodium hydroxide solution R* and 0.10 ml of *α-naphthol solution R*. Mix and allow to stand in iced water for 15 min. Add 0.5 ml of *sodium hypobromite solution R* and mix. A pink colour develops.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.50 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *cyanoguanidine R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 200.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 10.0 mg of *melamine R* in about 90 ml of *water R.* Add 5.0 ml of the test solution and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: irregular, porous silica gel to which benzenesulphonic acid groups have been chemically bonded (10 µm);

Or

— *size: l* = 0.11 m, Ø = 4.7 mm;

— *stationary phase*: regular, porous silica gel to which benzenesulphonic acid groups have been chemically bonded (5 µm).

Mobile phase 17 g/l solution of *ammonium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 218 nm.

Injection 20 µl.

Run time Twice the retention time of metformin hydrochloride.

System suitability Reference solution (c):

— *resolution*: minimum of 10 between the peaks due to melamine and to metformin hydrochloride.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.02 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

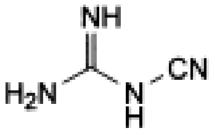
Dissolve 0.100 g in 4 ml of *anhydrous formic acid R*. Add 80 ml of *acetonitrile R*. Carry out the titration immediately. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 16.56 mg of $C_4H_{12}ClN_5$.

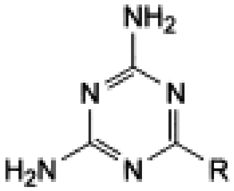
IMPURITIES

Specified impurities *A.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E, F.*



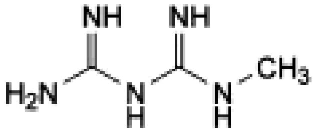
A. cyanoguanidine,



B. R = NH-C(=NH)-NH_2: (4,6-diamino-1,3,5-triazin-2-yl)guanidine,

C. R = $N(CH_3)_2$: *N*,*N*-dimethyl-1,3,5-triazine-2,4,6-triamine,

D. R = NH_2: 1,3,5-triazine-2,4,6-triamine (melamine),



E. 1-methylbiguanide,

F. CH_3-NH-CH_3: *N*-methylmethanamine.

Ph Eur

Methacrylic Acid - Ethyl Acrylate Copolymer (1:1) Dispersion 30 per cent

General Notices

(Ph Eur monograph 1129)

Action and use

Excipient.

Ph Eur

DEFINITION

Methacrylic acid - ethyl acrylate copolymer (1:1) dispersion 30 per cent is a dispersion in water of a copolymer of methacrylic acid and ethyl acrylate having a mean relative molecular mass of about 250 000. The ratio of carboxylic groups to ester groups is about 1:1. It may contain suitable surface-active agents such as sodium dodecyl sulphate and polysorbate 80. It contains not less than 46.0 per cent *m/m* and not more than 50.6 per cent *m/m* of methacrylic acid units, calculated with reference to the residue on evaporation.

CHARACTERS

An opaque, white, slightly viscous liquid, miscible with water. On addition of solvents such as acetone, ethanol or 2-propanol, a precipitate is formed which dissolves on addition of excess solvent. It is miscible with a 40 g/l solution of sodium hydroxide.

It is sensitive to spoilage by microbial contaminants.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the *Ph. Eur. reference spectrum of methacrylic acid - ethyl acrylate copolymer (1:1) dispersion 30 per cent.*

B. It complies with the limits of the assay.

TESTS

Apparent viscosity

Determine the viscosity *(2.2.10)* using a rotating viscometer at 20 °C. At a shear rate of 50 s^{-1}, the apparent viscosity is not more than 15 mPa·s.

Appearance of a film

Place 1 ml on a glass plate and allow to dry. A clear, brittle film is formed.

Particulate matter

Filter 100.0 g through a tared stainless steel sieve (90). Rinse with *water R* until a clear filtrate is obtained and dry at 100 °C to 105 °C. The mass of the residue is not more than 1.00 g.

Ethyl acrylate and methacrylic acid

Total content not more than 0.1 per cent, determined by liquid chromatography *(2.2.29)* and calculated with reference to the dried substance.

Blank solution To 50.0 ml of *methanol R* add 25.0 ml of mobile phase.

Test solution Dissolve 40 mg of the substance to be examined in 50.0 ml of *methanol R* and add 25.0 ml of mobile phase.

Reference solution Dissolve 10 mg each of *ethyl acrylate R* and *methacrylic acid R* in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 0.1 ml of this solution to 50.0 ml with *methanol R* and add 25.0 ml of mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.10 m long and 4 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 2.5 ml/min a mixture of 30 volumes of *methanol R* and 70 volumes of *phosphate buffer solution pH 2.0 R*,

as detector a spectrophotometer set at 202 nm.

Inject 50 µl of each solution. The test is not valid unless the resolution between the peaks corresponding to ethyl acrylate and methacrylic acid in the chromatogram obtained with the reference solution is at least 2.0. The test is not valid if the chromatogram obtained with the blank solution shows peaks with the same retention times as ethyl acrylate or methacrylic acid.

Calculate the percentage content of monomers from the area of the peaks in the chromatograms obtained with the test solution and the reference solution and from the content of monomers in the reference solution.

Residue on evaporation

Dry 1.000 g at 110 °C for 5 h. The residue weighs not less than 0.285 g and not more than 0.315 g.

Sulphated ash *(2.4.14)*

Not more than 0.2 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count.

ASSAY

Dissolve 1.500 g in a mixture of 40 ml of *water R* and 60 ml of *2-propanol R*. Titrate slowly while stirring with *0.5 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

1 ml of *0.5 M sodium hydroxide* is equivalent to 43.05 mg of $C_4H_6O_2$ (methacrylic acid units).

STORAGE

Store protected from freezing. Handle the substance so as to minimise microbial contamination.

LABELLING

The label states, where applicable, the name and concentration of any surface-active agents.

Ph Eur

Methacrylic Acid - Ethyl Acrylate Copolymer (1:1)

General Notices

(Ph Eur monograph 1128)

Action and use

Pharmaceutical aid.

Ph Eur

DEFINITION

Copolymer of methacrylic acid and ethyl acrylate having a mean relative molecular mass of about 250 000. The ratio of carboxylic groups to ester groups is about 1:1. The substance is in the acid form (type A) or partially neutralised using sodium hydroxide (type B). It may contain suitable surface-active agents such as sodium dodecyl sulphate and polysorbate 80.

Content:

— type A: 46.0 per cent to 50.6 per cent of methacrylic acid units (dried substance);

— type B: 43.0 per cent to 48.0 per cent of methacrylic acid units (dried substance).

CHARACTERS

Appearance

White or almost white, free-flowing powder.

Solubility

Practically insoluble in water (type A) or dispersible in water (type B), freely soluble in anhydrous ethanol, practically insoluble in ethyl acetate. It is freely soluble in a 40 g/l solution of sodium hydroxide.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 0.1 g of the substance to be examined in 1 ml of *ethanol (90 per cent V/V) R*, and place 2 drops of the solution on a sodium chloride plate; dry to allow the formation of a film and cover with another sodium chloride plate.

Comparison Methacrylic acid - ethyl acrylate copolymer (1:1) (type A or type B) CRS.

B. It complies with the limits of the assay.

C. Sulphated ash (see Tests).

TESTS

Ethyl acrylate and methacrylic acid

Liquid chromatography *(2.2.29)*.

Blank solution To 50.0 ml of *methanol R* add 25.0 ml of the mobile phase.

Test solution Dissolve 40 mg of the substance to be examined in 50.0 ml of *methanol R* and add 25.0 ml of the mobile phase.

Reference solution Dissolve 10 mg of *ethyl acrylate R* and 10 mg of *methacrylic acid R* in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 0.1 ml of this solution to 50.0 ml with *methanol R* and add 25.0 ml of the mobile phase.

Column:

— *size*: *l* = 0.10 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *phosphate buffer solution pH 2.0 R* (30:70 *V/V*).

Flow rate 2.5 ml/min.

Detection Spectrophotometer at 202 nm.

Injection 50 µl.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to ethyl acrylate and methacrylic acid in the chromatogram obtained with the reference solution;

— the chromatogram obtained with the blank solution does not show peaks with the same retention time as the peaks due to ethyl acrylate or methacrylic acid.

Limit:

— *sum of the contents of ethyl acrylate and methacrylic acid*: maximum 0.1 per cent.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 6 h.

Sulphated ash *(2.4.14)*

Maximum 0.4 per cent (type A) or 0.5 per cent to 3.0 per cent (type B), determined on 1.0 g.

ASSAY

Dissolve 1.000 g in a mixture of 40 ml of *water R* and 60 ml of *2-propanol R*. Titrate slowly while stirring with *0.5 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

1 ml of *0.5 M sodium hydroxide* is equivalent to 43.05 mg of $C_4H_6O_2$ (methacrylic acid units).

LABELLING

The label states the type (type A or type B).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the *characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.*

The following characteristics may be relevant for methacrylic acid - ethyl acrylate copolymer (1:1) used as gastro-resistant coating agent.

Apparent viscosity

— *Type A*: 100 mPa·s to 200 mPa·s.

Dissolve a quantity of the substance to be examined corresponding to 37.5 g of the dried substance in a mixture of 7.9 g of *water R* and 254.6 g of *2-propanol R*. Determine the viscosity *(2.2.10)* at 20 °C using a rotating viscometer at a shear rate of 10 s^{-1}.

— *Type B*: not more than 100 mPa·s.

Disperse a quantity of the substance to be examined corresponding to 80.0 g of the dried substance in *water R* and make up to 320 g with the same solvent. Stir for 3 h and determine the viscosity *(2.2.10)* at 23 °C using a rotating viscometer and a spindle rotating at 100 r/min (rotor 2).

Solubility of a film

Place 1 ml of the solution (type A) or dispersion (type B) prepared for the test on apparent viscosity on a glass plate and allow to dry. A clear brittle film is formed. Take a piece of the film and place it in a flask containing phosphate buffer solution pH 6.8. It dissolves.

Ph Eur

Methacrylic Acid - Methyl Methacrylate Copolymer (1:1)

General Notices

(Ph Eur monograph 1127)

Action and use

Excipient.

Ph Eur

DEFINITION

Copolymer of methacrylic acid and methyl methacrylate having a mean relative molecular mass of about 135 000. The ratio of carboxylic groups to ester groups is about 1:1.

Content

46.0 per cent to 50.6 per cent of methacrylic acid units (dried substance).

CHARACTERS

Appearance

White or almost white, free-flowing powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol and in 2-propanol, practically insoluble in ethyl acetate. It is freely soluble in a 40 g/l solution of sodium hydroxide.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of methacrylic acid - methyl methacrylate copolymer (1:1).

B. It complies with the limits of the assay.

TESTS

Apparent viscosity (*2.2.10*)

50 mPa·s to 200 mPa·s.

Dissolve a quantity of the substance to be examined corresponding to 37.5 g of the dried substance in a mixture of 7.9 g of *water R* and 254.6 g of *2-propanol R*. Determine the viscosity using a rotating viscometer at 20 °C and at a shear rate of 10 s^{-1}.

Appearance of a film

Place 1 ml of the solution prepared in the test for apparent viscosity on a glass plate and allow to dry. A clear brittle film is formed.

Methyl methacrylate and methacrylic acid

Liquid chromatography (*2.2.29*).

Blank solution To 50.0 ml of *methanol R* add 25.0 ml of the mobile phase.

Test solution Dissolve 40 mg of the substance to be examined in 50.0 ml of *methanol R* and add 25.0 ml of the mobile phase.

Reference solution Dissolve 10 mg of *methyl methacrylate R* and 10 mg of *methacrylic acid R* in *methanol R*, then dilute to 50.0 ml with the same solvent. Dilute 0.1 ml of this solution to 50.0 ml with *methanol R* and add 25.0 ml of the mobile phase.

Column:
- — *size:* *l* = 0.10 m, Ø = 4 mm;
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *phosphate buffer solution pH 2.0 R* (30:70 *V/V*).

Flow rate 2.5 ml/min.

Detection Spectrophotometer at 202 nm.

Injection 50 µl.

System suitability:
- — *resolution*: minimum 2.0 between the peaks due to methyl methacrylate and methacrylic acid in the chromatogram obtained with the reference solution;
- — the chromatogram obtained with the blank solution does not show peaks with the same retention times as methyl methacrylate or methacrylic acid.

Limit:
- — *sum of the contents of methyl methacrylate and methacrylic acid*: maximum 0.1 per cent.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 C for 6 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 1.000 g in a mixture of 40 ml of *water R* and 60 ml of *2-propanol R*. Titrate slowly while stirring with *0.5 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

1 ml of *0.5 M sodium hydroxide* is equivalent to 43.05 mg of $C_4H_6O_2$ (methacrylic acid units).

Ph Eur

Methacrylic Acid - Methyl Methacrylate Copolymer (1:2)

General Notices

(Ph Eur monograph 1130)

Action and use

Excipient.

Ph Eur

DEFINITION

Copolymer of methacrylic acid and methyl methacrylate having a mean relative molecular mass of about 135 000. The ratio of carboxylic groups to ester groups is about 1:2.

Content

27.6 per cent to 30.7 per cent of methacrylic acid units (dried substance).

CHARACTERS

Appearance

White or almost white, free-flowing powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol and in 2-propanol, practically insoluble in ethyl acetate. It is freely soluble in a 40 g/l solution of sodium hydroxide.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of methacrylic acid - methyl methacrylate copolymer (1:2).

B. It complies with the limits of the assay.

TESTS

Apparent viscosity (*2.2.10*)

50 mPa·s to 200 mPa·s.

Dissolve a quantity of the substance to be examined corresponding to 37.5 g of the dried substance in a mixture of 7.9 g of *water R* and 254.6 g of *2-propanol R*. Determine the viscosity using a rotating viscometer at 20 C and at a shear rate of 10 s^{-1}.

Appearance of a film

Place 1 ml of the solution prepared for the viscosity test on a glass plate and allow to dry. A clear brittle film is formed.

Methyl methacrylate and methacrylic acid

Liquid chromatography (*2.2.29*).

Blank solution To 50.0 ml of *methanol R* add 25.0 ml of the mobile phase.

Test solution Dissolve 40 mg of the substance to be examined in 50.0 ml of *methanol R* and add 25.0 ml of the mobile phase.

Reference solution Dissolve 10 mg of *methyl methacrylate R* and 10 mg of *methacrylic acid R* in *methanol R*, then dilute to 50.0 ml with the same solvent. Dilute 0.1 ml of this solution to 50.0 ml with *methanol R* and add 25.0 ml of the mobile phase.

Column:
- *size: l* = 0.10 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *phosphate buffer solution pH 2.0 R* (30:70 *V/V*).

Flow rate 2.5 ml/min.

Detection Spectrophotometer at 202 nm.

Injection 50 µl.

System suitability:
- *resolution*: minimum 2.0 between the peaks due to methyl methacrylate and methacrylic acid in the chromatogram obtained with the reference solution;
- the chromatogram obtained with the blank solution does not show peaks with the same retention times as methyl methacrylate or methacrylic acid.

Limit:
- *sum of the contents of methyl methacrylate and methacrylic acid*: maximum 0.1 per cent.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 C for 6 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 1.000 g in a mixture of 40 ml of *water R* and 60 ml of *2-propanol R*. Titrate slowly while stirring with *0.5 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

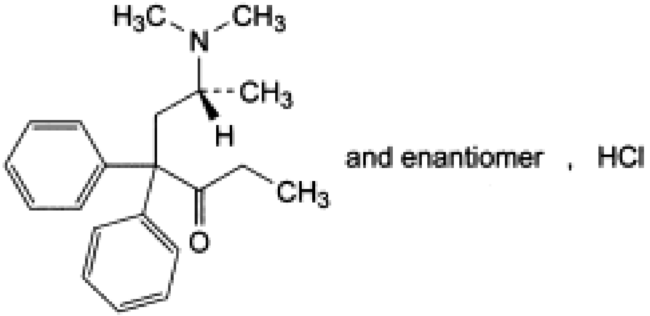
1 ml of *0.5 M sodium hydroxide* is equivalent to 43.05 mg of $C_4H_6O_2$ (methacrylic acid units).

Ph Eur

Methadone Hydrochloride

General Notices

(Ph Eur monograph 0408)



$C_{21}H_{28}ClNO$ 345.9 *1095-90-5*

Action and use

Opioid receptor agonist; analgesic.

Preparations

Methadone Injection

Methadone Linctus

Methadone Oral Solution (1 mg per ml)

Methadone Tablets

Ph Eur

DEFINITION

(6*RS*)-6-(Dimethylamino)-4,4-diphenylheptan-3-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, C, D.*

Second identification *A, B, D.*

A. Optical rotation (see Tests).

B. Melting point *(2.2.14)*: 233 C to 236 C.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of methadone hydrochloride.*

D. Dilute 1 ml of solution S (see Tests) to 5 ml with *water R* and add 1 ml of *dilute ammonia R1*. Mix, allow to stand for 5 min and filter. The filtrate gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Dilute 10 ml of solution S to 25 ml with *carbon dioxide-free water R*. To 10 ml of the solution add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Optical rotation *(2.2.7)*

- 0.05° to + 0.05°, determined on solution S in a 2 dm tube.

Related substances

Gas chromatography *(2.2.28)*.

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 100.0 ml with *methanol R*.

Reference solution (b) Dissolve 5 mg of *imipramine hydrochloride CRS* and 5 mg of *cyclobenzaprine hydrochloride CRS* in 100.0 ml of *methanol R.*

Column:

— *material*: fused silica;

— *size:* l = 50 m, Ø = 0.32 mm;

— *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 1.05 µm).

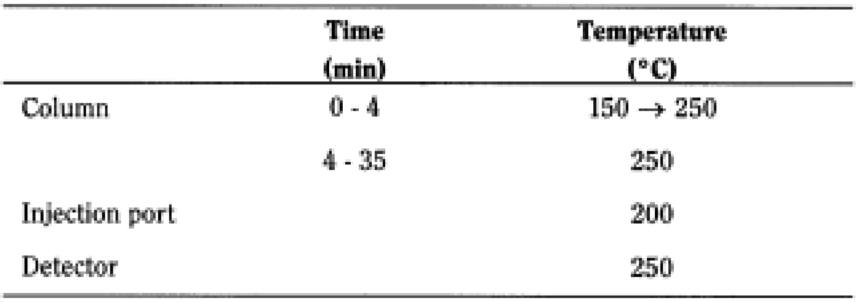
Carrier gas *helium for chromatography R.*

Flow rate 1.2 ml/min.

Injection liner Packed with deactivated glass wool to wipe the needle.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 4	150 → 250
	4 - 35	250
Injection port		200
Detector		250

Detection Flame ionisation.

Injection 2 µl.

Run time 1.5 times the retention time of methadone.

Relative retention With reference to methadone (retention time = about 25 min): impurity E = about 0.44; impurity C = about 0.81; impurity B = about 0.89; impurity D = about 0.98; impurity A = about 1.14; imipramine = about 1.19; cyclobenzaprine = about 1.24.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to imipramine and cyclobenzaprine.

Limits:

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5 ml of *0.01 M hydrochloric acid* and 50 ml of *anhydrous ethanol R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion. Carry out a blank titration.

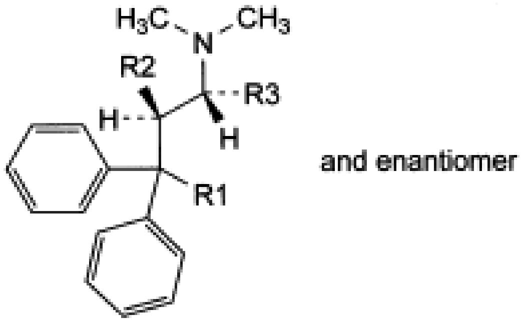
1 ml of *0.1 M sodium hydroxide* is equivalent to 34.59 mg of $C_{21}H_{28}ClNO$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities   A, B, C, D, E.

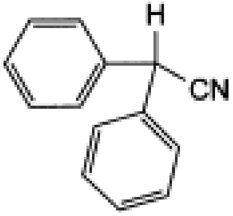


A. R1 = CNH-C_2H_5, R2 = CH_3, R3 = H: (2*RS*)-4-imino-*N*,*N*,2-trimethyl-3,3-diphenylhexan-1-amine (isomethadone ketimine),

B. R1 = CN, R2 = H, R3 = CH_3: (4*RS*)-4-(dimethylamino)-2,2-diphenylpentanenitrile (didiavalo),

C. R1 = CN, R2 = CH_3, R3 = H: (3*RS*)-4-(dimethylamino)-3-methyl-2,2-diphenylbutanenitrile (isodidiavalo),

D. R1 = CO-C_2H_5, R2 = CH_3, R3 = H: (5*RS*)-6-(dimethylamino)-5-methyl-4,4-diphenylhexan-3-one (isomethadone),



E. diphenylacetonitrile.

Ph Eur

Methanol

General Notices

Methyl Alcohol

(Ph Eur monograph 1989)

H_3C-OH

CH_4O 32.04 *67-56-1*

Ph Eur

DEFINITION

Methyl alcohol.

CHARACTERS

Appearance

Clear, colourless, volatile, hygroscopic liquid.

Solubility

Miscible with water and with methylene chloride.

bp: about 64 C.

It is flammable.

IDENTIFICATION

A. Refractive index (*2.2.6*): 1.328 to 1.330.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of methanol.

TESTS

Appearance

It is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 25 ml add 25 ml of *water R* and 0.25 ml of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.9 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Relative density (*2.2.5*)

0.791 to 0.793.

Absorbance (*2.2.25*)

Maximum 0.15 at 230 nm, maximum 0.05 at 250 nm, maximum 0.02 at 270 nm and maximum 0.01 at 290 nm.

Examine between 230 nm and 290 nm using *water R* as the compensation liquid. The absorption curve is smooth.

Impurity A

Gas chromatography (*2.2.28*).

Test solution (a) The substance to be examined.

Test solution (b) Dilute 1.0 ml of *4-methylpentan-2-ol R* to 50.0 ml with test solution (a). Dilute 5.0 ml of this solution to 100.0 ml with test solution (a).

Reference solution (a) To 50 µl of *anhydrous ethanol R* add 50 µl of *acetone R* and dilute to 50.0 ml with test solution (a). Dilute 100 µl of this solution to 10.0 ml with test solution (a).

Reference solution (b) Dilute 100 µl of *benzene R* to 100.0 ml with test solution (a). Dilute 0.20 ml to 100.0 ml with test solution (a).

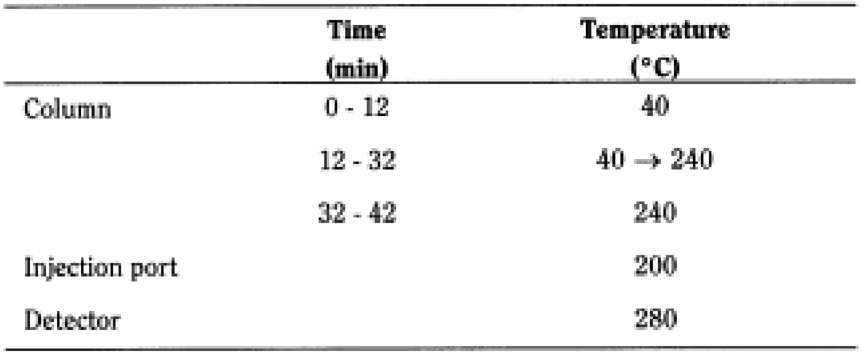
Column:
- *material*: fused silica;
- *size: l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *poly[(cyanopropyl)(phenyl)][dimethyl]siloxane R* (film thickness 1.8 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 35 cm/s.

Split ratio 1:20.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 12	40
	12 - 32	40 → 240
	32 - 42	240
Injection port		200
Detector		280

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to impurity B (1st peak) and impurity C (2nd peak).

Limit:

— *impurity A*: maximum 2 ppm *V/V*.

Calculate the content of impurity A in parts per million (*V/V*) using the following expression:

$$\frac{2 \times A_1}{A_2 - A_1}$$

A_1 = area of the peak due to impurity A in the chromatogram obtained with test solution (a),

A_2 = area of the peak due to impurity A in the chromatogram obtained with reference solution (b).

If necessary, the identity of impurity A can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).

Related substances

Gas chromatography (*2.2.28*) as described in the test for impurity A.

Limits:

— *any impurity*: for each impurity, not more than the area of the peak due to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (0.1 per cent);

— *total*: not more than 3 times the area of the peak due to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (0.3 per cent);

— *disregard limit*: 0.05 times the area of the peak due to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (50 ppm).

Reducing substances

To 20 ml add 0.1 ml of *0.02 M potassium permanganate*. The pink colour is not completely discharged within 5 min.

Residue on evaporation

Maximum 10 ppm.

Evaporate 100 g to dryness on a water bath and dry in an oven at 100-105 °C. The residue weighs a maximum of 1 mg.

Water (*2.5.12*)

Maximum 0.10 per cent, determined on 10.0 g.

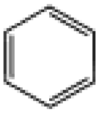
STORAGE

In an airtight container.

IMPURITIES

Specified impurities *A.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C.*



A. benzene,

B. ethanol,

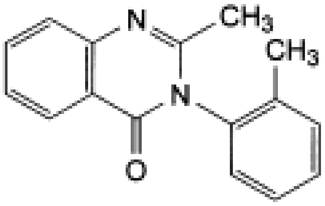
C. acetone.

Ph Eur

Methaqualone

General Notices

(Ph Eur monograph 0510)



$C_{16}H_{14}N_2O$ 250.3 *72-44-6*

Action and use

Hypnotic.

Ph Eur

DEFINITION

2-Methyl-3-(2-methylphenyl)quinazolin-4(3*H*)-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, soluble in ethanol (96 per cent). It dissolves in dilute sulphuric acid.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D.*

A. Melting point (*2.2.14*): 114 °C to 117 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *0.1 M hydrochloric acid*, warming if necessary, and dilute to 100.0 ml with the same acid. Dilute 1.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*.

Spectral range 220-300 nm.

Absorption maxima At 235 nm and 270 nm.

Specific absorbances at the absorption maxima:
— at 235 nm: 1270 to 1390,

— at 270 nm: 315 to 345.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of methaqualone.

D. Dissolve about 10 mg in 2 ml of *ethanol (96 per cent) R*. Add 1 ml of *dimethylaminobenzaldehyde solution R1* and heat on a water-bath for 5 min. A reddish-orange colour develops.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 1.0 g in *methanol R* and dilute to 25 ml with the same solvent.

Acidity

Shake 0.6 g with 30 ml of *carbon dioxide-free water R* for 5 min and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

2-Aminobenzoic acid

Maximum 20 ppm.

Test solution Dissolve 1.00 g in a mixture of 1 volume of *water R* and 3 volumes of *ethanol (96 per cent) R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution Dissolve 40 mg of *2-aminobenzoic acid R* in a mixture of 1 volume of *water R* and 3 volumes of *ethanol (96 per cent) R* and dilute to 100.0 ml with the same mixture of solvents; dilute 10.0 ml of this solution to 100.0 ml with a mixture of 1 volume of *water R* and 3 volumes of *ethanol (96 per cent) R*; dilute 0.5 ml of this solution to 50.0 ml with a mixture of 1 volume of *water R* and 3 volumes of *ethanol (96 per cent) R*.

To the test solution and to the reference solution add 5 ml of *dilute hydrochloric acid R* and cool in iced water for 5 min. Add 0.1 ml of *sodium nitrite solution R* and cool again in iced water for 5 min. Add 1.5 ml of a freshly prepared 10 g/l solution of *sulphamic acid R* and shake until the colour of the solution is discharged. Add 2.5 ml of a freshly prepared 2 g/l solution of *naphthylethylenediamine dihydrochloride R* and allow to stand for 2 h 30 min. The test solution is not more intensely coloured (*2.2.2, Method II*) than the reference solution.

o-Toluidine

Maximum 10 ppm.

Test solution Dissolve 0.50 g in *acetone R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 50 mg of freshly distilled *o-toluidine R* in 5 ml of *acetone R* and dilute to 100.0 ml with *dilute hydrochloric acid R*; dilute 10.0 ml of the solution to 100.0 ml with *dilute hydrochloric acid R*; dilute 0.1 ml of this solution to 10.0 ml with *acetone R*.

To the test solution and to the reference solution add 5 ml of *water R* and cool in iced water for 5 min. Add 0.1 ml of *sodium nitrite solution R* and cool again in iced water for 5 min. Add 10 ml of a freshly prepared 2 g/l solution of β*-naphthol R* in *dilute sodium hydroxide solution R*. The test solution is not more intensely coloured (*2.2.2, Method II*) than the reference solution.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 25.03 mg of $C_{16}H_{14}N_2O$.

STORAGE

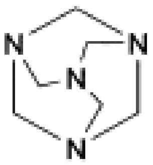
Protected from light.

Ph Eur

Methenamine

General Notices

(Ph Eur monograph 1545)



$C_6H_{12}N_4$ 140.2 *100-97-0*

Action and use

Anti-infective.

Ph Eur

DEFINITION

1,3,5,7-Tetraazotricyclo[3.3.1.1^{3,7}]decane.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison methenamine CRS.

B. To 1 ml of solution S (see Tests) add 1 ml of *sulphuric acid R* and immediately heat to boiling. Allow to cool. To 1 ml of the solution add 4 ml of *water R* and 5 ml of *acetylacetone* *reagent R1*. Heat on a water-bath for 5 min. An intense yellow colour develops.

C. To 1 ml of solution S add 1 ml of *dilute sulphuric acid R* and immediately heat to boiling. The solution gives the reaction of ammonium salts and salts of volatile bases (*2.3.1*).

D. Dissolve 10 mg in 5 ml of *water R* and acidify with *dilute hydrochloric acid R*. Add 1 ml of *potassium iodobismuthate solution R*. An orange precipitate is formed immediately.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 5 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Free formaldehyde

Maximum 50 ppm.

Dissolve 0.8 g in *water R* and dilute to 8 ml with the same solvent. Add 2 ml of *ammoniacal silver nitrate solution R*. After 5 min, any grey colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner with a mixture of 8 ml of freshly prepared *formaldehyde standard solution (5 ppm* CH_2O*) R* and 2 ml of *ammoniacal silver nitrate solution R*.

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 100 ppm, determined on solution S.

Ammonium (*2.4.1*)

Maximum 50 ppm.

Dilute 2 ml of freshly prepared solution S to 13 ml with *water R*. Add 2 ml of *dilute sodium hydroxide solution R.*

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 2.0 per cent, determined on 1.000 g by drying in a desiccator.

ASSAY

Dissolve 0.100 g in 30 ml of *methanol R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 14.02 mg of $C_6H_{12}N_4$.

STORAGE

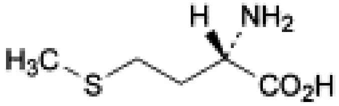
Protected from light.

Ph Eur

Methionine

General Notices

(Ph Eur monograph 1027)



$C_5H_{11}NO_2S$ 149.2 *63-68-3*

Action and use

Amino acid.

Ph Eur

DEFINITION

Methionine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*S*)-2-amino-4-(methylsulphanyl)butanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, soluble in water, very slightly soluble in alcohol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *methionine CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g of the substance to be examined and 0.1 g of *glycine R* in 4.5 ml of *dilute sodium hydroxide solution R*. Add 1 ml of a 25 g/l solution of *sodium nitroprusside R*. Heat to 40 C for 10 min. Allow to cool and add 2 ml of a mixture of 1 volume of *phosphoric acid R* and 9 volumes of *hydrochloric acid R*. A dark red colour develops.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 5.5 to 6.5.

Specific optical rotation (*2.2.7*)

Dissolve 1.00 g in *hydrochloric acid R1* and dilute to 50.0 ml with the same acid. The specific optical rotation is + 22.5 to + 24.0, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dilute hydrochloric acid R* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *methionine CRS* in a 10 g/l solution of *hydrochloric acid R* and dilute to 50 ml with the same acid solution.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *methionine CRS* and 10 mg of *serine CRS* in a 10 g/l solution of *hydrochloric acid R* and dilute to 25 ml with the same acid solution.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 C to 105 C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides

To 10 ml of solution S add 25 ml of *water R*, 5 ml of *dilute nitric acid R* and 10 ml of *silver nitrate solution R2*. Allow to stand protected from light for 5 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of *chloride standard solution (5 ppm Cl) R* (200 ppm). Examine the tubes laterally against a black background.

Sulphates (*2.4.13*)

Dissolve 0.5 g in 3 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium (*2.4.1*)

0.10 g complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.2 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*)

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined upper layers add 10 ml of *water R* and shake for 3 min. The lower layer complies with the limit test for iron (10 ppm).

Heavy metals (*2.4.8*)

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.125 g in 5 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.

STORAGE

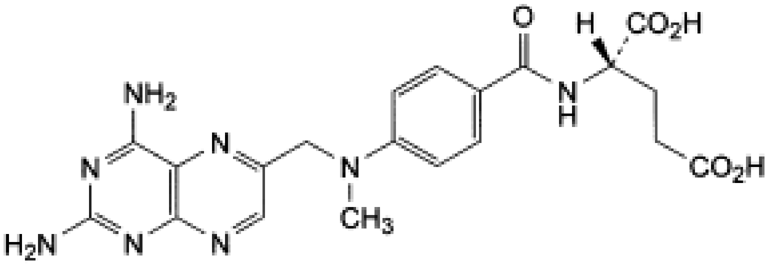
Store protected from light.

Ph Eur

Methotrexate

General Notices

(Ph Eur monograph 0560)



$C_{20}H_{22}N_8O_5$ 454.4 *59-05-2*

Action and use

Dihydrofolate reductase inhibitor; cytostatic.

Preparations

Methotrexate Injection

Methotrexate Tablets

Ph Eur

DEFINITION

(2*S*)-2-[[4-[[(2,4-Diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioic acid.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow or orange, crystalline, hygroscopic powder.

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methotrexate CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in a mixture of 0.5 ml of *dilute ammonia R1* and 5 ml of mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (a) Dissolve 40.0 mg of *methotrexate CRS* in a mixture of 0.5 ml of *dilute ammonia R1* and 5 ml of mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 5.0 ml of this solution to 50.0 ml with mobile phase A.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 25.0 ml with mobile phase A.

Reference solution (d) Dissolve 5 mg of the substance to be examined, 5 mg of *4-aminofolic acid R* (impurity B), 5 mg of *methotrexate impurity C CRS*, 5 mg of *methotrexate impurity D CRS* and 5 mg of *methotrexate impurity E CRS* in a mixture of 0.5 ml of *dilute ammonia R1* and 5 ml of mobile phase A and dilute to 100 ml with mobile phase A.

Reference solution (e) Dissolve 8 mg of *methotrexate for peak identification CRS* (containing impurities H and I) in a mixture of 0.1 ml of *dilute ammonia R1* and 1 ml of mobile phase A and dilute to 20 ml with mobile phase A.

Column:

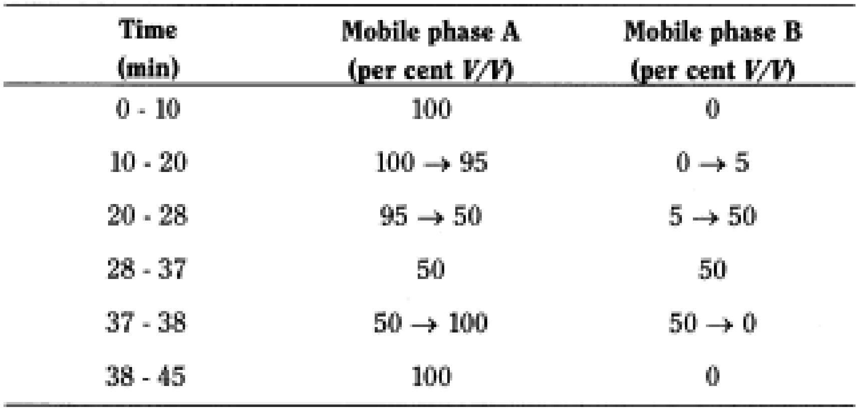
— *size:* l = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 5 volumes of *acetonitrile for chromatography R* and 95 volumes of a 3.4 g/l solution of *anhydrous sodium dihydrogen phosphate R* previously adjusted to pH 6.0 with a 42 g/l solution of *sodium hydroxide R*;

— *mobile phase B*: mix 50 volumes of *acetonitrile for chromatography R* and 50 volumes of a 3.4 g/l solution of *anhydrous sodium dihydrogen phosphate R* previously adjusted to pH 6.0 with a 42 g/l solution of *sodium hydroxide R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 20	100 → 95	0 → 5
20 - 28	95 → 50	5 → 50
28 - 37	50	50
37 - 38	50 → 100	50 → 0
38 - 45	100	0

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl of the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *methotrexate for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities H and I.

Relative retention Wth reference to methotrexate (retention time = about 18 min): impurity B = about 0.3; impurity C = about 0.4; impurity E = about 1.4; impurity I = about 1.5; impurity H = about 1.6.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to impurities B and C and minimum 1.5 between the peaks due to impurity D and methotrexate, in the chromatogram obtained with reference solution (d); minimum 1.5 between the peaks due to impurities I and H in the chromatogram obtained with reference solution (e); if the resolution between impurity D and methotrexate does not comply, increase the flow rate to meet the requirement.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.8; impurity I = 1.4;

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities B, E*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities H, I*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent);

— *sum of impurities other than B, C and E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent).

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 4.0 mg of *(RS)-methotrexate R* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 4.0 mm;

— *stationary phase*: *bovine albumin R* bound to *silica gel for chromatography R* (7 µm) with a pore size of 30 nm.

Mobile phase Add 500 ml of a 7.1 g/l solution of *anhydrous disodium hydrogen phosphate R* to 600 ml of a 6.9 g/l solution of *sodium dihydrogen phosphate monohydrate R*, mix, and adjust to pH 6.9 with *dilute sodium hydroxide solution R*; to 920 ml of this mixture add 80 ml of *propanol R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 302 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to methotrexate and impurity F.

Limit:

— *impurity F*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

Heavy metals (*2.4.8*)

Maximum 50 ppm.

1.0 g complies with test C. Prepare the reference solution using 5 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 13.0 per cent, determined on 0.10 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{20}H_{22}N_8O_5$ from the declared content of *methotrexate CRS.*

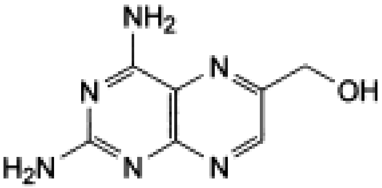
STORAGE

In an airtight container, protected from light.

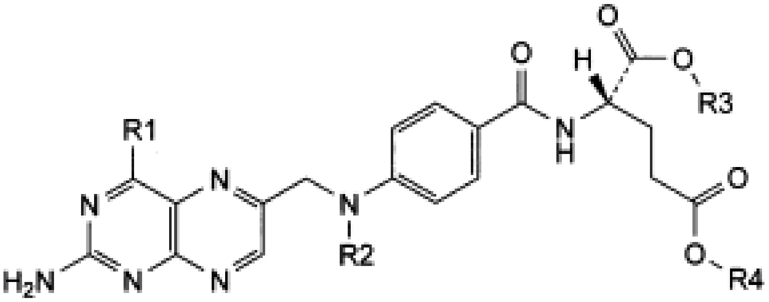
IMPURITIES

Specified impurities B, C, E, F, H, I.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, D, G, J, K, L.*



A. (2,4-diaminopteridin-6-yl)methanol,

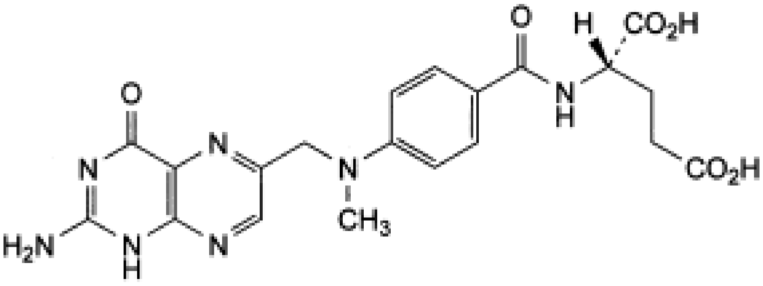


B. R1 = NH_2, R2 = R3 = R4 = H: (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid (4-aminofolic acid, aminopteridine),

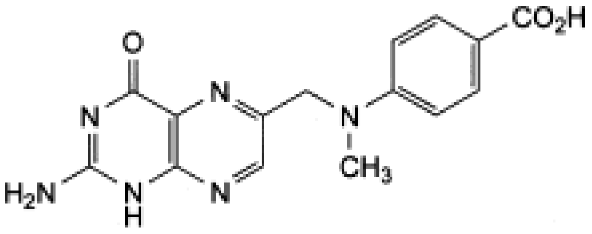
H. R1 = NH_2, R2 = R4 = CH_3, R3 = H: (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]-5-methoxy-5-oxopentanoic acid (methotrexate 5-methyl ester),

I. R1 = NH_2, R2 = R3 = CH_3, R4 = H: (4*S*)-4-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]-5-methoxy-5-oxopentanoic acid (methotrexate 1-methyl ester),

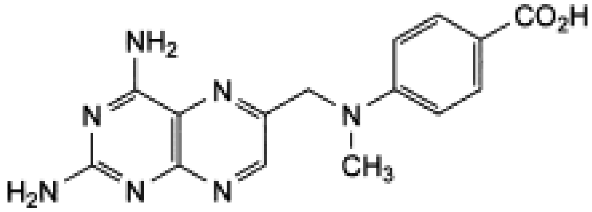
J. R1 = NH_2, R2 = R3 = R4 = CH_3: dimethyl (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioate (methotrexate dimethyl ester),



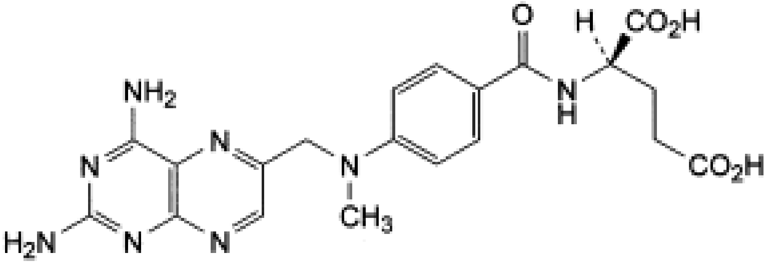
C. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioic acid (*N*-methylfolic acid, methopteridine),



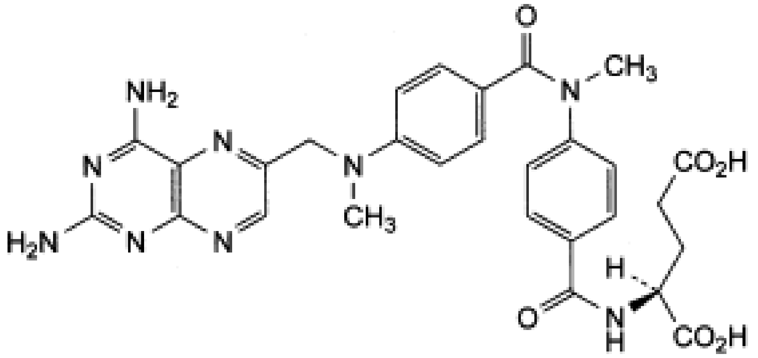
D. 4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino]benzoic acid (N^{10}-methylpteroic acid),



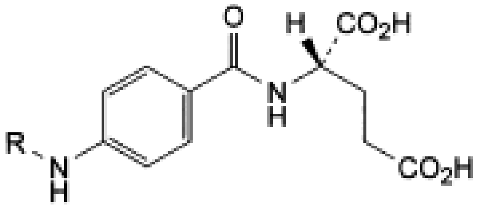
E. 4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoic acid (4-amino-N^{10}-methylpteroic acid, APA),



F. (2*R*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioic acid ((*R*)-methotrexate),



G. (2*S*)-2-[[4-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]methylamino] benzoyl]amino]pentanedioic acid,



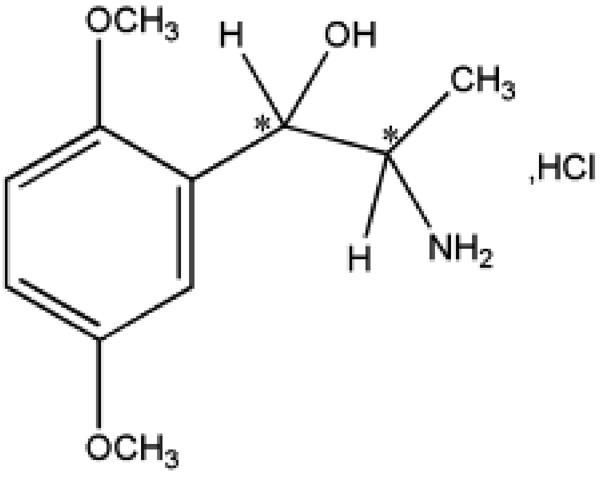
K. R = H: (2*S*)-2-[(4-aminobenzoyl)amino]pentanedioic acid,

L. R = CH_3: (2*S*)-2-[[4-(methylamino)benzoyl]amino]pentanedioic acid.

Ph Eur

Methoxamine Hydrochloride

General Notices



mixture of four stereoisomers

$C_{11}H_{17}NO_3,HCl$ 247.7   *61-16-5*

Action and use

Adrenoceptor agonist.

Preparation

Methoxamine Injection

DEFINITION

Methoxamine Hydrochloride is all-*rac*-2-amino-1-(2,5-dimethoxyphenyl)propan-1-ol hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{11}H_{17}NO_3,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

Colourless crystals or white, plate-like crystals or a white, crystalline powder.

Freely soluble in *water*; soluble in *ethanol (96%)*; very slightly soluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methoxamine hydrochloride *(RS 220)*.

B. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 2% w/v solution, 4.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 86 volumes of *chloroform*, 12 volumes of *methanol* and 2 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions in *methanol* containing (1) 2.0% w/v of the substance being examined, (2) 0.010% w/v of *2,5-dimethoxybenzaldehyde* and (3) 0.020% w/v of the substance being examined. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any spot corresponding to 2,5-dimethoxybenzaldehyde in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Spray the plate with a 0.3% w/v solution of *ninhydrin* in *butan-1-ol* containing 3% v/v of *glacial acetic acid* and heat at 105° for 5 minutes. Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

Loss on drying

When dried at 105° for 2 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

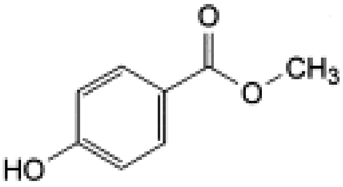
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.5 g and *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 24.77 mg of $C_{11}H_{17}NO_3,HCl$.

Methyl Hydroxybenzoate

General Notices

Methylparaben

(Methyl Parahydroxybenzoate, Ph Eur monograph 0409)



$C_8H_8O_3$ 152.1 *99-763-3*

Action and use

Antimicrobial preservative.

Ph Eur

DEFINITION

Methyl 4-hydroxybenzoate.

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very slightly soluble in water, freely soluble in alcohol and in methanol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point (*2.2.14*): 125 °C to 128 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methyl parahydroxybenzoate CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

D. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution R*, boil for 30 s and cool (solution A). To a further 10 mg in a similar test-tube add 1 ml of *sodium carbonate solution R*; the substance partly dissolves (solution B). Add at the same time to solution A and solution B 5 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* and mix. Solution B is yellow to orange-brown. Solution A is orange to red, the colour being clearly more intense than any similar colour which may be obtained with solution B.

TESTS

Solution S

Dissolve 1.0 g in *alcohol R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity

To 2 ml of solution S add 3 ml of *alcohol R*, 5 ml of *carbon dioxide-free water R* and 0.1 ml of *bromocresol green solution R*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *acetone R*.

Reference solution (a) Dilute 0.5 ml of test solution (a) to 100 ml with *acetone R*.

Reference solution (b) Dissolve 10 mg of *methyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in 1 ml of test solution (a) and dilute to 10 ml with *acetone R*.

Plate Suitable octadecylsilyl silica gel with a fluorescent indicator having an optimal intensity at 254 nm as the coating substance.

Mobile phase *glacial acetic acid R*, *water R*, *methanol R* (1:30:70 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated principal spots.

Limits:

— *any impurity*: any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Sulphated ash (*2.4.14*)

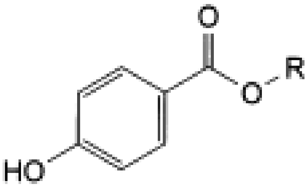
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 1.000 g add 20.0 ml of *1 M sodium hydroxide*. Heat at about 70 °C for 1 h. Cool rapidly in an ice bath. Prepare a blank in the same manner. Carry out the titration on the solutions at room temperature. Titrate the excess sodium hydroxide with *0.5 M sulphuric acid ,* continuing the titration until the second point of inflexion and determining the end-point potentiometrically (*2.2.20*).

1 ml of *1 M sodium hydroxide* is equivalent to 152.1 mg of $C_8H_8O_3$.

IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

B. R = CH_2-CH_3: ethyl 4-hydroxybenzoate,

C. R = CH_2-CH_2-CH_3: propyl 4-hydroxybenzoate,

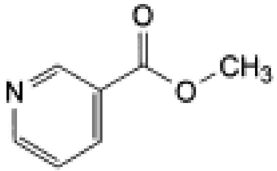
D. R = CH_2-CH_2-CH_2-CH_3: butyl 4-hydroxybenzoate.

Ph Eur

Methyl Nicotinate

General Notices

(Ph Eur monograph 2129)



$C_7H_7NO_2$ 137.1 *93-60-7*

Action and use

Vasodilator.

Ph Eur

DEFINITION

Methyl pyridine-3-carboxylate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 40 °C to 42 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison methyl nicotinate CRS.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 2 ml with the same solvent.

Reference solution Dissolve 10 mg of *methyl nicotinate CRS* in *methanol R* and dilute to 2 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methanol R*, *toluene R* (10:90 *V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 0.5 g add 0.1 g of *citric acid R* and 0.2 ml of *acetic anhydride R*. Heat cautiously for 1 min. A yellow colour is produced which turns first to orange, then to red and then to violet.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 25 mg of *nicotinic acid R* in the mobile phase and dilute to 25.0 ml with the mobile phase. To 0.5 ml of this solution add 0.5 ml of the test solution and dilute to 100 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetic acid R*, *water R*, *acetonitrile R* (1:29:70 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 261 nm.

Injection 20 µl.

Run time 3 times the retention time of methyl nicotinate.

Retention time Methyl nicotinate = about 3.3 min.

System suitability Reference solution (a):

— *resolution*: minimum 2 between the peaks due to impurity A and methyl nicotinate.

Limits:

- — *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- — *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- — *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dissolve 0.25 g in *water R* and dilute to 15 ml with the same solvent.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 2.000 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.120 g in 50 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 13.71 mg of $C_7H_7NO_2$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A.

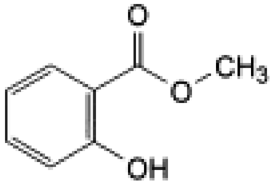
A. nicotinic acid.

Ph Eur

Methyl Salicylate

General Notices

(Ph Eur monograph 0230)



$C_8H_8O_3$ 152.1 *119-36-860-7*

Action and use

Counter-irritant.

Preparations

Methyl Salicylate Liniment

Methyl Salicylate Ointment

Ph Eur

DEFINITION

Methyl 2-hydroxybenzoate.

Content

99.0 per cent *m/m* to 100.5 per cent *m/m.*

CHARACTERS

Appearance

Colourless or slightly yellow liquid.

Solubility

Very slightly soluble in water, miscible with ethanol (96 per cent) and with fatty and essential oils.

IDENTIFICATION

A. Heat 0.25 ml with 2 ml of *dilute sodium hydroxide solution R* on a water-bath for 5 min. Add 3 ml of *dilute sulphuric acid R.* A crystalline precipitate is formed. Filter. The precipitate washed with *water R* and dried at 100-105 °C, melts (*2.2.14*) at 156 °C to 161 °C.

B. To 10 ml of a saturated solution add 0.05 ml of *ferric chloride solution R1*. A violet colour develops.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

To 2 ml add 10 ml of *ethanol (96 per cent) R*.

Acidity

Dissolve 5.0 g in a mixture of 0.2 ml of *bromocresol green solution R* and 50 ml of *ethanol (96 per cent) R* previously neutralised to a blue colour by addition of *0.1 M sodium hydroxide*. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required to restore the blue colour.

Relative density (*2.2.5*)

1.180 to 1.186.

Refractive index (*2.2.6*)

1.535 to 1.538.

ASSAY

Dissolve 0.500 g in 25 ml of *ethanol (96 per cent) R*. Add 0.05 ml of *phenol red solution R* and neutralise with *0.1 M sodium hydroxide*. To the neutralised solution add 50.0 ml of *0.1 M sodium hydroxide* and heat under a reflux condenser on a water-bath for 30 min. Cool and titrate with *0.1 M hydrochloric acid*. Calculate the volume of *0.1 M sodium hydroxide* used in the saponification. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 15.21 mg of $C_8H_8O_3$.

STORAGE

Protected from light.

Ph Eur

Methylcellulose

General Notices

(Ph Eur monograph 0345)

9004-67-5

Action and use

Bulk-forming laxative; excipient.

Preparations

Methylcellulose Granules

Methylcellulose Tablets

Ph Eur

DEFINITION

Partly *O*-methylated cellulose.

CHARACTERS

Appearance

White, yellowish-white or greyish-white powder or granules, hygroscopic after drying.

Solubility

Practically insoluble in hot water, in acetone, in anhydrous ethanol and in toluene. It dissolves in cold water giving a colloidal solution.

IDENTIFICATION

A. Evenly distribute 1.0 g onto the surface of 100 ml of *water R* in a beaker, tapping the top of the beaker gently if necessary to ensure a uniform layer on the surface. Allow to stand for 1-2 min: the powdered material aggregates on the surface.

B. Evenly distribute 1.0 g into 100 ml of boiling *water R*, and stir the mixture using a magnetic stirrer with a bar 25 mm long: a slurry is formed and the particles do not dissolve. Allow the slurry to cool to 5 °C and stir using a magnetic stirrer: a clear or slightly turbid solution occurs with its thickness dependent on the viscosity grade.

C. To 0.1 ml of the solution obtained in identification B add 9 ml of a 90 per cent *V/V* solution of *sulphuric acid R*, shake, heat on a water-bath for exactly 3 min, immediately cool in an ice-bath, carefully add 0.6 ml of a 20 g/l solution of *ninhydrin R*, shake and allow to stand at 25 °C: a red colour develops and does not change to purple within 100 min.

D. Place 2-3 ml of the solution obtained in identification B on a glass slide as a thin film and allow the water to evaporate: a coherent, clear film forms on the glass slide.

E. Add exactly 50 ml of the solution obtained in identification B to exactly 50 ml of *water R* in a beaker. Insert a thermometer into the solution. Stir the solution on a magnetic stirrer/hot plate and begin heating, increasing the temperature at a rate of 2-5 °C per minute. Determine the temperature at which a turbidity increase begins to occur and designate the temperature as the flocculation temperature: the flocculation temperature is higher than 50 °C.

TESTS

Solution S

While stirring, introduce a quantity of the substance to be examined equivalent to 1.0 g of the dried substance into 50 g of *carbon dioxide-free water R* heated to 90 °C. Allow to cool, adjust the mass of the solution to 100 g with *carbon dioxide-free water R* and stir until dissolution is complete. Allow to stand at 2-8 °C for 1 h before carrying out the test for appearance of solution.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

5.0 to 8.0 for the solution prepared as described under Apparent viscosity.

Read the pH after the probe has been immersed for 5 ± 0.5 min.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulphated ash *(2.4.14)*

Maximum 1.5 per cent, determined on 1.0 g.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for methylcellulose used as binder, viscosity-enhancing agent or film former.

Apparent viscosity

Minimum 80 per cent and maximum 120 per cent of the nominal value for samples with a viscosity of less than 600 mPa·s (Method 1); minimum 75 per cent and maximum 140 per cent of the nominal value for samples with a viscosity of 600 mPa·s or higher (Method 2).

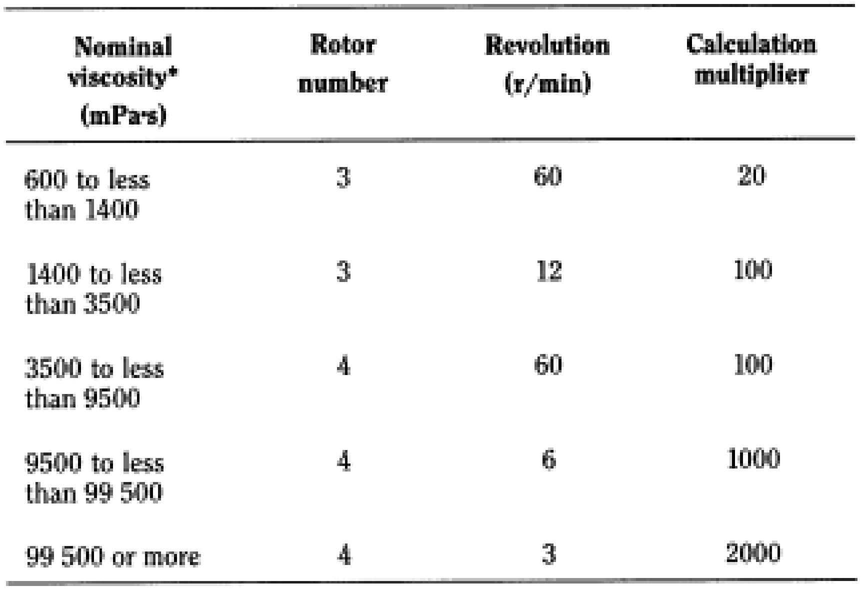
Method 1, to be applied to samples with a viscosity of less than 600 mPa·s Weigh accurately a quantity of the substance to be examined equivalent to 4.000 g of the dried substance. Transfer into a wide-mouthed bottle, and adjust the mass to 200.0 g with *water R*. Capping the bottle, stir by mechanical means at 400 ± 50 r/min for 10-20 min until the particles are thoroughly dispersed and wetted. Scrape down the insides of the bottle with a spatula if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water-bath maintained at a temperature below 5 °C for another 20-40 min. Adjust the solution mass if necessary to 200.0 g using cold *water R*. Centrifuge the solution if necessary to expel any entrapped air bubbles. Using a spatula remove any foam, if present. Determine the viscosity of this solution using the capillary viscometer method *(2.2.9)* to obtain the kinematic viscosity (ν). Separately, determine the density (ρ) *(2.2.5)* of the solution and calculate the dynamic viscosity (η), as $\eta = \rho\nu$.

Method 2, to be applied to samples with a viscosity of 600 mPa·s or higher Weigh accurately a quantity of the substance to be examined equivalent to 10.00 g of the dried substance. Transfer into a wide-mouthed bottle, and adjust the mass to 500.0 g with *water R*. Capping the bottle, stir by mechanical means at 400 ± 50 r/min for 10-20 min until the particles are thoroughly dispersed and wetted. Scrape down the insides of the bottle with a spatula if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water-bath maintained at a temperature below 5 °C for another 20-40 min. Adjust the solution mass if necessary to 500.0 g using cold *water R*. Centrifuge the solution if necessary to expel any entrapped air bubbles. Using a spatula, remove any foam, if present. Determine the viscosity *(2.2.10)* of this solution at 20 ± 0.1 °C using a rotating viscometer.

Apparatus Single-cylinder type spindle viscometer.

Rotor number, revolution and calculation multiplier Apply the conditions specified in Table 0345.-1.

Table 0345.-1.



Nominal viscosity* (mPa·s)	Rotor number	Revolution (r/min)	Calculation multiplier
600 to less than 1400	3	60	20
1400 to less than 3500	3	12	100
3500 to less than 9500	4	60	100
9500 to less than 99 500	4	6	1000
99 500 or more	4	3	2000

*the nominal viscosity is based on the manufacturer's specifications.

Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period of 2 min between subsequent measurements. Repeat the measurement twice and determine the mean of the 3 readings.

Degree of substitution

26.0 per cent to 33.0 per cent of methoxy groups (dried substance).

Gas chromatography *(2.2.28)*.

Apparatus:

— *reaction vial*: a 5 ml pressure-tight vial, 50 mm in height, 20 mm in external diameter and 13 mm in internal diameter at the mouth, equipped with a pressure-tight butyl rubber membrane stopper coated with polytetrafluoroethylene and secured with an aluminium crimped cap or another sealing system providing a sufficient air-tightness;

— *heater*: a heating module with a square aluminium block having holes 20 mm in diameter and 32 mm in depth, so that the reaction vials fit; mixing of the contents of the vial is effected using a magnetic stirrer equipped in the heating module or using a reciprocal shaker that performs approximately 100 cycles/min.

Internal standard solution 30 g/l solution of *octane R* in *xylene R.*

Test solution Weigh 65.0 mg of the substance to be examined, place in a reaction vial, add 0.06-0.10 g of *adipic acid R*, 2.0 ml of the internal standard solution and 2.0 ml of a 570 g/l solution of *hydriodic acid R*, immediately cap and seal the vial, and weigh accurately. Using a magnetic stirrer, mix the contents of the vial continuously for 60 min while heating the block so that the temperature of the contents is maintained at 130 ± 2 °C. If a reciprocal shaker or magnetic stirrer cannot be used, shake the vial well by hand at 5-minute intervals during the initial 30 min of the heating time. Allow the vial to cool, and again weigh accurately. If the loss of mass is less than 0.50 per cent of the contents and there is no evidence of a leak, use the upper layer of the mixture as the test solution.

Reference solution Place 0.06-0.10 g of *adipic acid R*, 2.0 ml of the internal standard solution and 2.0 ml of *hydroiodic acid R* in another reaction vial, cap and seal the vial, and weigh accurately. Add 45 µl of *methyl iodide R* through the septum with a syringe, and weigh accurately. Shake the reaction vial well, and use the upper layer as the reference solution.

Column:

— *size*: l = 1.8-3 m, Ø = 3-4 mm;

— *stationary phase*: *diatomaceous earth for gas chromatography R* impregnated with 10-20 per cent of *poly(dimethyl)(75)(diphenyl)(25)siloxane R* (film thickness 125-150 µm);

— *temperature*: 100 °C.

Carrier gas *helium for chromatography R* (thermal conductivity); *helium for chromatography R* or *nitrogen for chromatography R* (flame ionisation).

Flow rate Adjusted so that the retention time of the internal standard is about 10 min.

Detection Flame ionisation or thermal conductivity.

Injection 1-2 µl.

System suitability Reference solution:

— *resolution*: well-resolved peaks of methyl iodide (1st peak) and internal standard (2nd peak).

Calculation:

— *methoxy groups*: calculate the ratio (Q) of the area of the peak due to methyl iodide to the area of the peak due to the internal standard in the chromatogram obtained with the test solution, and the ratio (Q_1) of the area of the peak due to methyl iodide to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution.

Calculate the percentage content of methoxy groups using the following expression:

$$\frac{Q \times m_1}{Q_1 \times m} \times 21.864$$

m_1 = mass of methyl iodide in the reference solution, in milligrams.

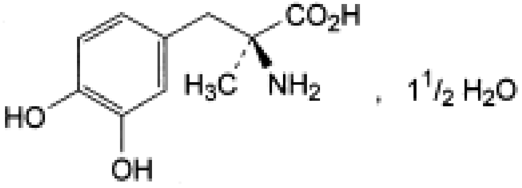
m = mass of the sample (dried substance), in milligrams.

Ph Eur

Methyldopa

General Notices

(Ph Eur monograph 0045)



$C_{10}H_{13}NO_4,1½H_2O$ 238.2 *41372-08-1*

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

Preparation

Methyldopa Tablets

Ph Eur

DEFINITION

Methyldopa contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*S*)-2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid, calculated with reference to the anhydrous substance.

CHARACTERS

A white or yellowish white, crystalline powder or colourless or almost colourless crystals, slightly soluble in water, very slightly soluble in alcohol. It is freely soluble in dilute mineral acids.

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *methyldopa CRS*.

B. Dissolve about 2 mg in 2 ml of *water R* and add 0.2 ml of *ferric chloride solution R2*. A green colour develops which changes to bluish-violet on the addition of 0.1 g of *hexamethylenetetramine R*.

C. Dissolve about 5 mg in a mixture of 5 ml of *1 M hydrochloric acid* and 5 ml of *water R*. Add 0.1 ml of *sodium nitrite solution R* containing 100 g/l of *ammonium molybdate R*. A yellow colour is produced which becomes brownish-red on the addition of *strong sodium hydroxide solution R*.

D. To about 5 mg add 1 ml of *water R*, 1 ml of *pyridine R* and about 5 mg of *nitrobenzoyl chloride R* and heat to boiling. Add, while shaking, 0.2 ml of *sodium carbonate solution R*. An orange or amber colour develops.

TESTS

Appearance of solution

Dissolve 1.0 g in *1 M hydrochloric acid* and dilute to 25 ml with the same solvent. The solution is not more intensely coloured than reference solution BY_6 or B_6 (*2.2.2, Method II*).

Acidity

Dissolve 1.0 g with heating in 100 ml of *carbon dioxide-free water R*. Add 0.1 ml of *methyl red solution R*. Not more than 0.5 ml of *0.1 M sodium hydroxide* is required to produce the pure yellow colour of the indicator.

Optical rotation (*2.2.7*)

Dissolve a quantity equivalent to 2.20 g of the anhydrous substance in *aluminium chloride solution R* and dilute to 50.0 ml with the same solution. The angle of optical rotation is - 1.10° to - 1.23 °.

Absorbance (*2.2.25*)

Dissolve 40.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined between 230 nm and 350 nm, the solution shows a single absorption maximum, at 280 nm. The specific absorbance at this maximum is 122 to 137, calculated with reference to the anhydrous substance.

Methoxymethyldopa and related substances

Examine by thin-layer chromatography (*2.2.27*), using *cellulose for chromatography R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in a mixture of 0.4 ml of *hydrochloric acid R1* and 9.6 ml of *methanol R*.

Reference solution (a) Dissolve 5 mg of *3-methoxymethyldopa CRS* in 100 ml of *methanol R*.

Reference solution (b) To 1 ml of the test solution add 1 ml of reference solution (a).

Apply separately to the plate 10 µl of the test solution, 10 µl of reference solution (a) and 20 µl of reference solution (b). Develop over a path of 10 cm using a mixture of 15 volumes of *glacial acetic acid R*, 25 volumes of *water R* and 65 volumes of *butanol R*. Dry the plate immediately in a current of warm air and spray with a mixture of 5 volumes of a 50 g/l solution of *sodium nitrite R* and 45 volumes of a 3 g/l solution of *nitroaniline R* in a mixture of 20 volumes of *water R* and 80 volumes of *hydrochloric acid R*. Dry the plate immediately in a current of warm air and spray with a 200 g/l solution of *sodium carbonate R*. Examine the chromatograms immediately. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Heavy metals (*2.4.8*)

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*)

10.0 per cent to 13.0 per cent, determined on 0.20 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 15 ml of *anhydrous formic acid R* 30 ml of *anhydrous acetic acid R* and 30 ml of *dioxan R*. Titrate with *0.1 M perchloric acid* until a green colour is obtained using 0.1 ml of *crystal violet solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 21.12 mg of $C_{10}H_{13}NO_4$.

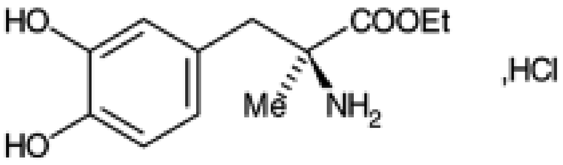
STORAGE

Store protected from light.

Ph Eur

Methyldopate Hydrochloride

General Notices



$C_{12}H_{17}NO_4,HCl$ 275.7 *2508-79-4*

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

Preparation

Methyldopate Injection

DEFINITION

Methyldopate Hydrochloride is ethyl 3-(3,4-dihydroxyphenyl)-2-methyl-L-alaninate hydrochloride. It contains not less than 98.5% and not more than 101.0% of $C_{12}H_{17}NO_4,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Freely soluble in *water*, in *ethanol (96%)* and in *methanol*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methyldopate hydrochloride *(RS 224)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.008% w/v solution in 0.1M *hydrochloric acid* exhibits a maximum only at 280 nm. The *absorbance* at the maximum at 280 nm is about 0.80.

C. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 3.0 to 5.0, Appendix V L.

Specific optical rotation

In a 4% w/v solution in 0.1M *hydrochloric acid* and determined at 405 nm, –13.5 to –14.9, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of equal volumes of *acetone*, *butan-1-ol*, *glacial acetic acid*, *toluene* and *water* as the mobile phase, but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 2 µl of each of three solutions in *methanol* containing (1) 10.0% w/v of the substance being examined, (2) 0.25% w/v of *methyldopa BPCRS* and (3) 0.040% w/v of *methyldopa BPCRS*. After removal of the plate, allow it to dry in air, heat at 105° for 10 minutes and examine under *ultraviolet light* (254 nm). Expose the plate to iodine vapour for 10 minutes and examine again. By each method of visualisation any spot corresponding to methyldopa in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.4%).

Loss on drying

When dried to constant weight at 105° at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.5 g and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 27.57 mg of $C_{12}H_{17}NO_4$,HCl.

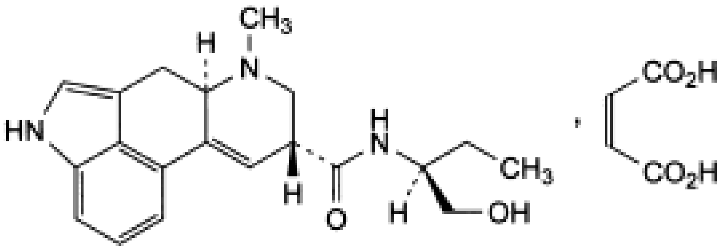
STORAGE

Methyldopate Hydrochloride should be protected from light.

Methylergometrine Maleate

General Notices

(Ph Eur monograph 1788)



$C_{20}H_{25}N_3O_2,C_4H_4O_4$ 455.5 *57432-61-8*

Action and use

Oxytocic.

Ph Eur

DEFINITION

(6a*R*,9*R*)-*N*-[(1*S*)-1-(Hydroxymethyl)propyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-fg]quinoline-9-carboxamide (2*Z*)-but-2-enedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Soluble in water, slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison methylergometrine maleate CRS.

TESTS

Solution S

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH *(2.2.3)*

4.4 to 5.2.

Dilute 2.0 ml of solution S to 50.0 ml with *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7)*

+ 44.0 to + 50.0 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*: use the normalisation procedure. *Carry out the test protected from light*.

Test solution Dissolve 25.0 mg of the substance to be examined in 15 ml of mobile phase B and dilute to 50.0 ml with *water R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 20.0 ml with *water R*.

Reference solution (b) Dissolve the contents of a vial of *methylergometrine for system suitability CRS* (containing impurities A, B, C, D, E, F, G, H and I) in 1.0 ml of a mixture of 30 volumes of mobile phase B and 70 volumes of *water R*.

Column:
- *size: l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:
- *mobile phase A*: 2 g/l solution of *ammonium carbamate R*;
- *mobile phase B*: *acetonitrile R, water R* (50:50 *V/V);*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	85 → 65	15 → 35
5 - 10	65	35
10 - 15	65 → 20	35 → 80
15 - 20	20	80

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 310 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *methylergometrine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D, E, F, G, H and I.

Relative retention With reference to methylergometrine (retention time = 11 min): impurity A = about 0.2; impurity B = about 0.5; impurity C = about 0.6; impurity D = about 0.7; impurity I = about 1.10; impurity E = about 1.14; impurity F = about 1.2; impurity G = about 1.3; impurity H = about 1.4.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to methylergometrine and impurity I;

— minimum 1.5 between the peaks due to impurities I and E.

Limits:

— *impurities A, B, C, D, E, G*: for each impurity, maximum 0.5 per cent;

— *sum of impurities F, H and I*: maximum 1.0 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 2.0 per cent;

— *disregard limit*: the area of the peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

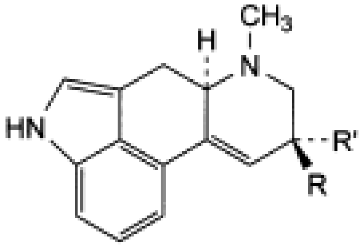
1 ml of *0.1 M perchloric acid* is equivalent to 45.55 mg of $C_{24}H_{29}N_3O_6$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H, I.*

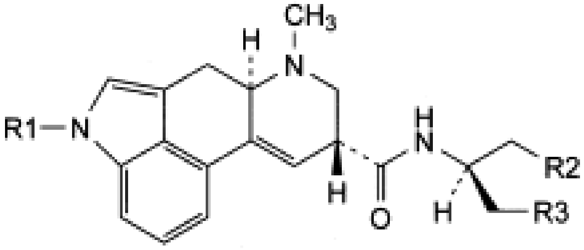


A. R = H, R′ = CO_2H: (6a*R*,9*R*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,

B. R = CO_2H, R′ =H: (6a*R*,9*S*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,

C. R = H, R′ = $CONH_2$: (6a*R*,9*R*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide,

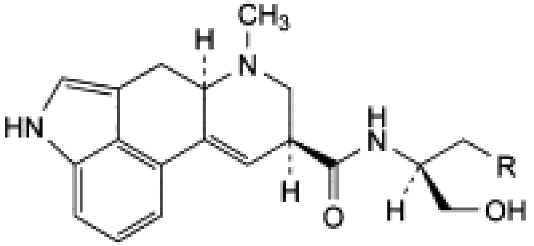
E. R = $CONH_2$, R′ = H: (6a*R*,9*S*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide,



D. R1 = R2 = H, R3 = OH: (6a*R*,9*R*)-*N*-[(1*S*)-2-hydroxy-1-methylethyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (ergometrine),

G. R1 = R2 = CH_3, R3 = OH: (6a*R*,9*R*)-*N*-[(1*S*)-1-(hydroxymethyl)propyl]-4,7-dimethyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (methysergide),

I. R1 = H, R2 = OH, R3 = CH_3: (6a*R*,9*R*)-*N*-[(1*R*)-1-(hydroxymethyl)propyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (1'-*epi*-methyergometrine),



F. R = H: (6a*R*,9*S*)-*N*-[(1*S*)-2-hydroxy-1-methylethyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (ergometrinine),

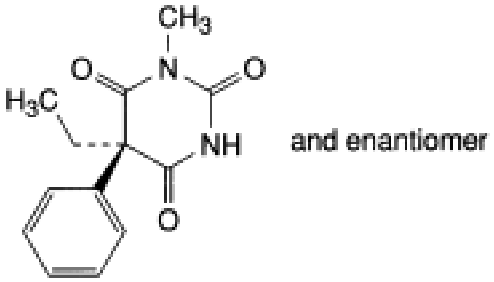
H. R = CH_3: (6a*R*,9*S*)-*N*-[(1*S*)-1-(hydroxymethyl)propyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (methylergometrinine).

Ph Eur

Methylphenobarbital

General Notices

(Ph Eur monograph 0189)



$C_{13}H_{14}N_2O_3$ 246.3 *115-38-8*

Action and use

Barbiturate.

Preparation

Methylphenobarbital Tablets

Ph Eur

DEFINITION

Methylphenobarbital contains not less than 99.0 per cent and not more than the equivalent of 102.0 per cent of (5*RS*)-5-ethyl-1-methyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, practically insoluble in water, very slightly soluble in ethanol. It forms water-soluble compounds with alkali hydroxides and carbonates and with ammonia.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Determine the melting point (*2.2.14*) of the substance to be examined. Mix equal parts of the substance to be examined and *methylphenobarbital CRS* and determine the melting point of the mixture. The difference between the two melting points (which are about 178 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *methylphenobarbital CRS*.

C. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *chloroform R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 0.1 g of *methylphenobarbital CRS* in *chloroform R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To about 10 mg add 0.2 ml of *sulphuric acid R* and 0.1 ml of *nitric acid R*. Heat on a water-bath for 10 min. Cool in iced water and add 5 ml of *water R* and 5 ml of strong *sodium hydroxide solution R*. Add 5 ml of *acetone R*, shake and allow to stand. A dark-red colour develops in the upper layer.

TESTS

Appearance of solution

Dissolve 1.0 g, with gentle heating, in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 6 ml of *water R*. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity

Boil 1.0 g with 50 ml of *water R* for 2 min, allow to cool and filter. To 10 ml of the filtrate add 0.15 ml of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *chloroform R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 0.1 g of *phenobarbital CRS* in *chloroform R* and dilute to 100 ml with the same solvent. Dilute 10 ml of the solution to 100 ml with *chloroform R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 5 ml of *pyridine R*. Add 0.5 ml of *thymolphthalein solution R* and 10 ml of *silver nitrate solution* in *pyridine R*. Titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained. Carry out a blank titration.

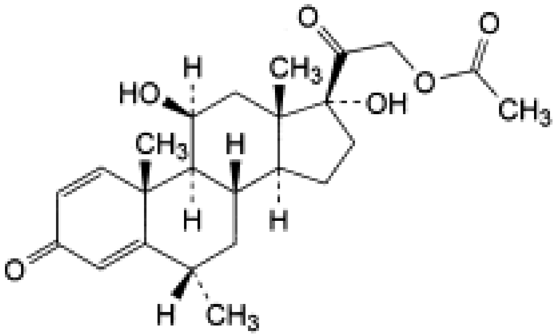
1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 24.63 mg of $C_{13}H_{14}N_2O_3$.

Ph Eur

Methylprednisolone Acetate

General Notices

(Ph Eur monograph 0933)



$C_{24}H_{32}O_6$ 416.5 *53-36-1*

Action and use

Glucocorticoid.

Preparation

Methylprednisolone Acetate Injection

Ph Eur

DEFINITION

11β,17-Dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

First identification A, B.

Second identification C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylprednisolone acetate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *methylprednisolone acetate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *prednisolone acetate CRS* and 10 mg of *methylprednisolone acetate CRS* in the solvent mixture, then dilute to 10 ml with the solvent mixture.

Plate *TLC silica gel GF_{254} plate R*.

Mobile phase *butanol R*, *toluene R*, *ether R* (5:10:85 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which, when examined in ultraviolet light at 365 nm, may not be completely separated.

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Test solution (b) Transfer 2 ml of solution A to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium* *hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C protected from light for 1 h. Allow to cool.

Reference solution (a) Dissolve 25 mg of *methylprednisolone acetate CRS* in *methanol R* and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of this solution to 10 ml with *methylene chloride R.*

Reference solution (b) Transfer 2 ml of solution B to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C protected from light for 1 h. Allow to cool.

Plate *TLC silica gel GF_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) has an R_F value distinctly lower than that of the principal spot in each of the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add this solution to 10 ml of *water R* and mix. The colour fades and there is a greenish-yellow fluorescence in ultraviolet light at 365 nm.

E. About 10 mg gives the reaction of acetyl (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)

+ 97 to + 105 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in 5 ml of *tetrahydrofuran R* and dilute to 10.0 ml with *water R*.

Reference solution (a) Dissolve 4 mg of *methylprednisolone acetate CRS* and 4 mg of *dexamethasone acetate CRS* in the mobile phase, then dilute to 20.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 260 ml *tetrahydrofuran R* and 700 ml of *water R*, then allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 45 min.

Injection 20 µl.

Run time 1.5 times the retention time of methylprednisolone acetate.

Retention time Methylprednisolone acetate = about 43 min; dexamethasone acetate = about 57 min.

System suitability Reference solution (a):

— *resolution*: minimum 6.5 between the peaks due to methylprednisolone acetate and dexamethasone acetate; if necessary, adjust the concentration of *water R* in the mobile phase.

Limits:

— *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

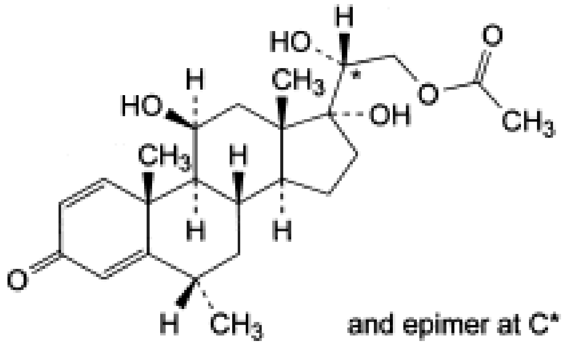
Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 243 nm.

Calculate the content of $C_{24}H_{32}O_6$ taking the specific absorbance to be 355.

STORAGE

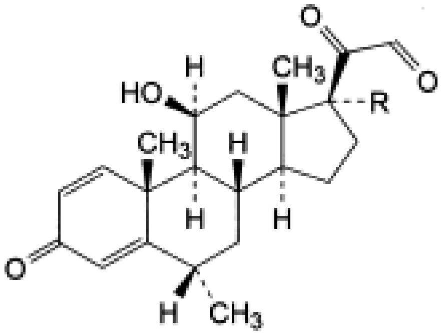
Protected from light.

IMPURITIES



A. (20*RS*)-11β,17,20-trihydroxy-6α-methyl-3-oxopregna-1,4-dien-21-yl acetate,

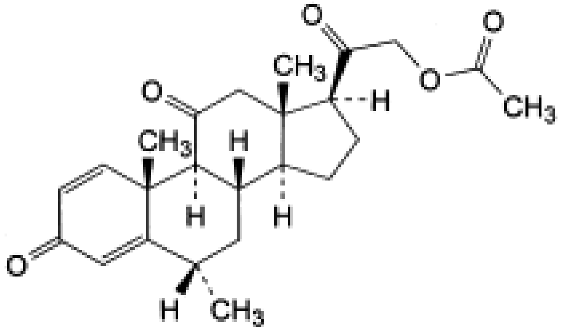
B. methylprednisolone,



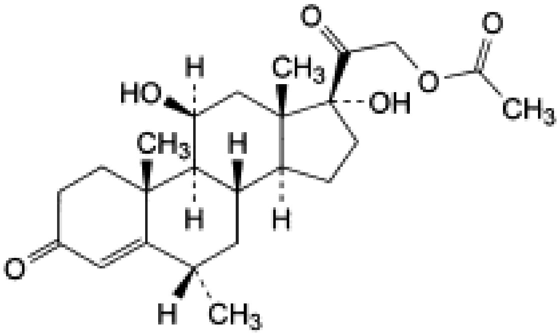
C. R = OH: 11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20,21-trione,

D. R = H: 11β-hydroxy-6α-methylpregna-1,4-diene-3,20,21-trione,

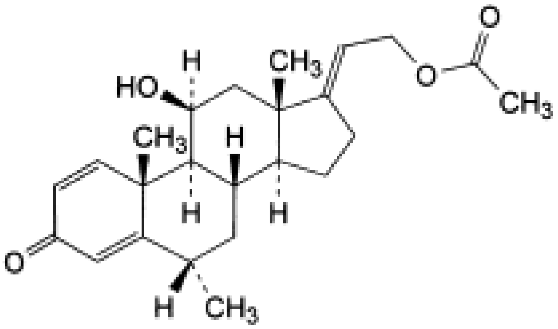
E. prednisolone acetate,



F. 6α-methyl-3,11,20-trioxopregna-1,4-dien-21-yl acetate,



G. 11β,17-dihydroxy-6α-methyl-3,20-dioxopregn-4-en-21-yl acetate,



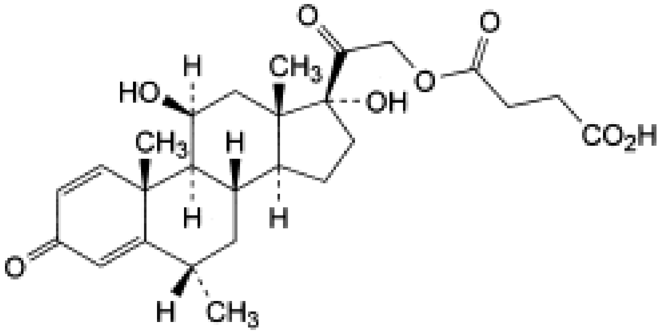
H. 11β-hydroxy-6α-methyl-3-oxopregna-1,4,17(20)-trien-21-yl acetate.

Ph Eur

Methylprednisolone Hydrogen Succinate

General Notices

(Ph Eur monograph 1131)



$C_{26}H_{34}O_8$ 474.6 *2921-57-5*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

4-[(11β,17-Dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-21-yl)oxy]-4-oxobutanoic acid.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Practically insoluble in water, slightly soluble in acetone and in anhydrous ethanol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A, B.*

Second identification *C, D.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylprednisolone hydrogen succinate CRS.*

B. Thin layer chromatography (*2.2.27*).

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *methylprednisolone hydrogen succinate CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *hydrocortisone hydrogen succinate CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *anhydrous formic acid R*, *anhydrous ethanol R*, *methylene chloride R* (0.1:1:15 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*; heat at 120 °C for 10 min or until the spots appear and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

C. Thin layer chromatography (*2.2.27*).

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* with gentle heating and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Test solution (b) Transfer 2 ml of solution A to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of a 0.8 g/l solution of *sodium hydroxide R* in *methanol R* and immediately pass a stream of *nitrogen R* through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 30 min. Allow to cool.

Reference solution (a) Dissolve 25 mg of *methylprednisolone hydrogen succinate CRS* in *methanol R* with gentle heating and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Reference solution (b) Transfer 2 ml of solution B to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of a 0.8 g/l solution of *sodium hydroxide R* in *methanol R* and immediately pass a stream of *nitrogen R* through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 30 min. Allow to cool.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) has an R_F value distinctly higher than that of the principal spot in each of the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min a reddish-brown colour develops. Add this solution to 10 ml of water R and mix. The colour fades and a precipitate is formed.

TESTS

Appearance of solution

The solution is clear (*2.2.1*).

Dissolve 0.100 g in 5 ml of *sodium hydrogen carbonate solution R*.

Specific optical rotation (*2.2.7*)

+ 87 to + 95 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 25 mg of *methylprednisolone hydrogen succinate for performance test CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, 3 per cent *V/V* solution of *glacial acetic acid R* (33:67 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time Twice the retention time of methylprednisolone hydrogen succinate.

Retention time Methylprednisolone hydrogen succinate = about 22 min; impurity D (eluting immediately after the main peak and appearing as a shoulder) = about 24 min.

System suitability Reference solution (a):

- *peak-to-valley ratio*: minimum 4, where H_r = height above the base line of the peak due to impurity D and H_v = height above the base line of the lowest point of the curve separating this peak from the peak due to methylprednisolone hydrogen succinate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

- *impurities A, B, C, D*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 50.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 243 nm.

Calculate the content of $C_{26}H_{34}O_8$ taking the specific absorbance to be 316.

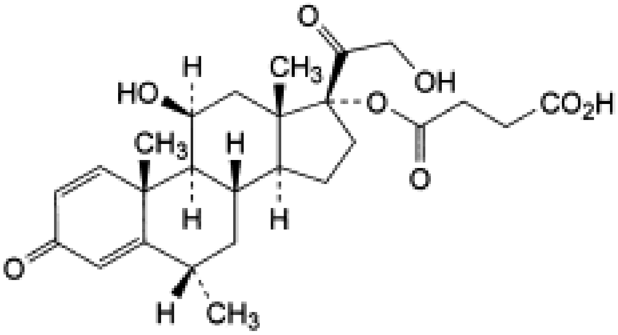
STORAGE

In an airtight container, protected from light.

IMPURITIES

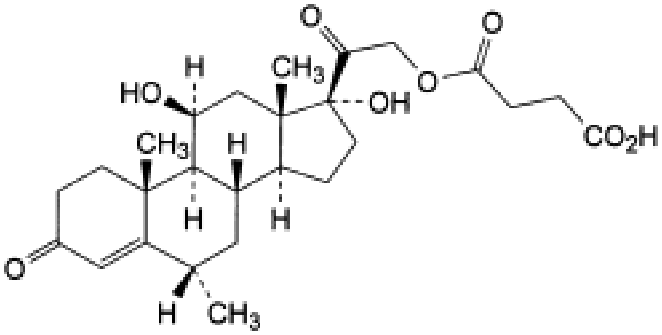
Specified impurities A, B, C, D.

A. methylprednisolone,



B. 4-[(11β,21-dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-17-yl)oxy]-4-oxobutanoic acid (methylprednisolone 17-(hydrogen succinate)),

C. methylprednisolone acetate,



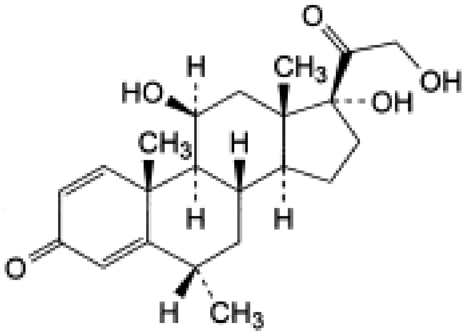
D. 4-[(11β,17-dihydroxy-6α-methyl-3,20-dioxopregn-4-en-21-yl)oxy]-4-oxobutanoic acid (methylhydrocortisone 21-(hydrogen succinate)).

Ph Eur

Methylprednisolone

General Notices

(Ph Eur monograph 0561)



$C_{22}H_{30}O_5$ 374.5 *83-43-2*

Action and use

Glucocorticoid.

Preparation

Methylprednisolone Tablets

Ph Eur

DEFINITION

11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in ethanol (96 per cent), slightly soluble in acetone and in methylene chloride.

It shows polymorphism (5.9).

IDENTIFICATION

First identification A, B.

Second identification C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylprednisolone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *methylprednisolone CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *hydrocortisone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R*.

Mobile phase A Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Mobile phase B *butanol R* saturated with *water R*, *toluene R*, *ether R* (5:15:80 *V/V/V*).

Application 5 µl.

1st development Over a path of 15 cm with mobile phase A.

2nd development Over a path of 15 cm with mobile phase B.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of solution A to 10 ml with *methylene chloride R*.

Test solution (b) Transfer 0.4 ml of solution A to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension in a mechanical shaker protected from light for 1 h. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 ml separating funnel, washing the filter with 2 quantities, each of 5 ml, of *water R*. Shake the clear filtrate with 10 ml of *methylene chloride R*. Wash the organic layer with 5 ml of *1 M sodium hydroxide* and 2 quantities, each of 5 ml, of *water R*. Dry over *anhydrous sodium sulphate R*.

Reference solution (a) Dissolve 25 mg of *methylprednisolone CRS* in *methanol R* and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of solution B to 10 ml with *methylene chloride R*.

Reference solution (b) Transfer 0.4 ml of solution B to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension in a mechanical shaker protected from light for 1 h. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 ml separating funnel, washing the filter with 2 quantities, each of 5 ml, of *water R*. Shake the clear filtrate with 10 ml of *methylene chloride R*. Wash the organic layer with 5 ml of *1 M sodium hydroxide* and 2 quantities, each of 5 ml, of *water R*. Dry over *anhydrous sodium sulphate R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *butanol R* saturated with *water R*, *toluene R*, *ether R* (5:10:85 *V/V/V*).

Application 5 µl of test solution (a) and reference solution (a), 10 µl of test solution (b) and reference solution (b), applying the latter 2 in small quantities in order to obtain small spots.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position, and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with alcoholic solution of *sulphuric acid R* and heat at 120 °C for 15 min. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) have an R_F value distinctly higher than that of the principal spot in each of the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, brownish-red fluorescence is seen. Add this solution to 10 ml of *water R* and mix. The colour fades and there is a yellowish-green fluorescence in ultraviolet light at 365 nm.

TESTS

Specific optical rotation (*2.2.7*)

+ 79.0 to + 86.0 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

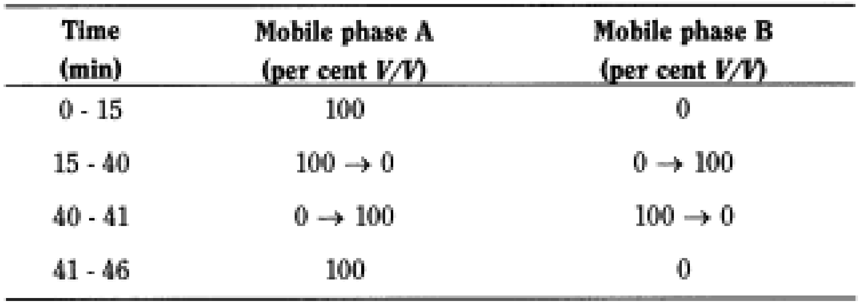
Test solution Dissolve 25.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *methanol R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 2 mg of *methylprednisolone CRS* and 2 mg of *betamethasone CRS* in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: in a 1000 ml volumetric flask mix 250 ml of *acetonitrile R* with 700 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 40	100 → 0	0 → 100
40 - 41	0 → 100	100 → 0
41 - 46	100	0

Flow rate 2.5 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With mobile phase B for at least 30 min, then with mobile phase A for 5 min. For subsequent chromatograms, use the conditions described from 40 min to 46 min.

Injection 20 µl; inject a mixture of equal volumes of *acetonitrile R* and *methanol R* as a blank.

Retention time Methylprednisolone = about 11.5 min; betamethasone = about 12.5 min.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to methylprednisolone and betamethasone; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 243 nm.

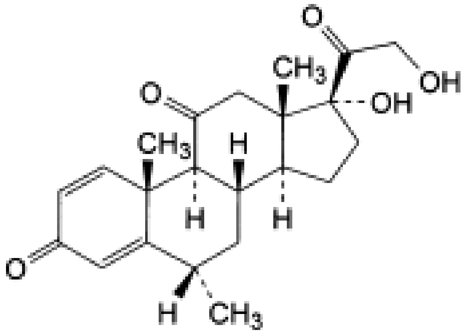
Calculate the content of $C_{22}H_{30}O_5$ taking the specific absorbance to be 395.

STORAGE

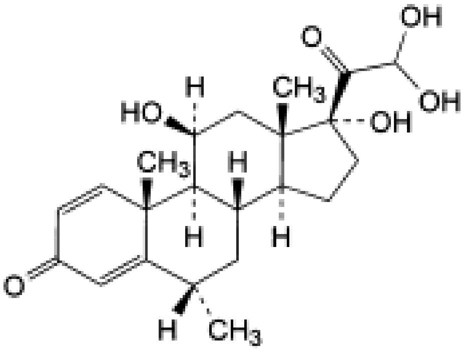
Protected from light.

IMPURITIES

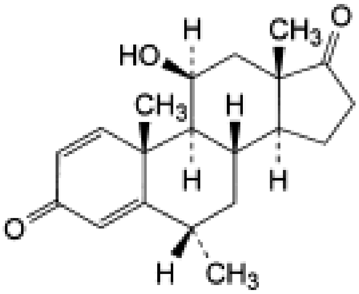
Specified impurities *A, B, C, D.*



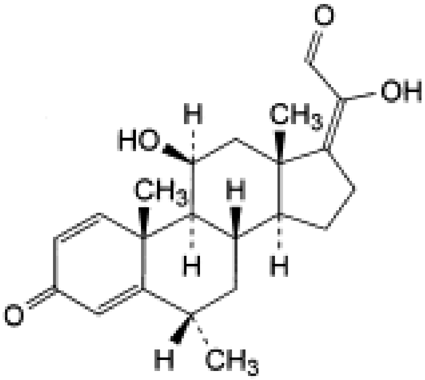
A. 17,21-dihydroxy-6α-methylpregna-1,4-diene-3,11,20-trione,



B. 11β,17,21,21-tetrahydroxy-6α-methylpregna-1,4-diene-3,20-dione,



C. 11β-hydroxy-6α-methylandrosta-1,4-diene-3,17-dione,



and (*Z*)-isomer

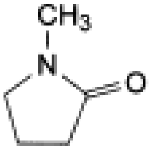
D. (*E*)- and (*Z*)-11β,20-dihydroxy-6α-methylpregna-1,4,17(20)-triene-3,21-dione.

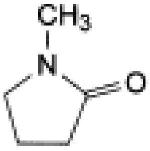
Ph Eur

Methylpyrrolidone

General Notices

(N-Methylpyrrolidone, Ph Eur monograph 1675)





C_5H_9NO *99.1* *872-50-4*

Ph Eur

DEFINITION

1-Methylpyrrolidin-2-one.

CHARACTERS

Appearance

Clear, colourless liquid.

Solubility

Miscible with water and with alcohol.

bp: about 204 °C.

Relative density

About 1.034.

Refractive index

About 1.469.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Films.

Comparison *Ph. Eur. reference spectrum of N-methylpyrrolidone.*

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Alkalinity

Dissolve 50 ml of the substance to be examined in 50 ml of *water R* previously adjusted with *0.02 M potassium hydroxide* or *0.02 M hydrochloric acid* until a yellow colour is obtained using 0.5 ml of *bromothymol blue solution R1* as indicator. Titrate with *0.02 M hydrochloric acid* to the initial coloration. Not more than 8.0 ml of *0.02 M hydrochloric acid* is required.

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution The substance to be examined.

Reference solution To 1 ml of the substance to be examined, add 1 ml of *2-pyrrolidone R* and dilute to 20 ml with *methylene chloride R.*

Column:

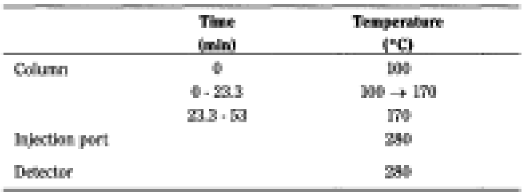
- *material*: fused silica;
- *size: l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)siloxane R* (5 µm).

Carrier gas *nitrogen for chromatography R*.

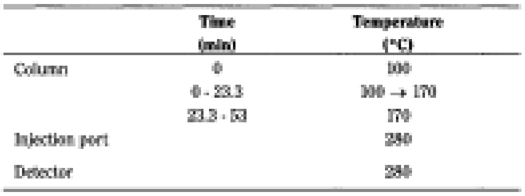
Linear velocity 20 cm/s.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0	100
	0 - 23.3	100 → 170
	23.3 - 53	170
Injection port		280
Detector		280



	Time (min)	Temperature (°C)
Column	0	100
	0 - 23.3	100 → 170
	23.3 - 53	170
Injection port		280
Detector		280

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution:

- *resolution*: minimum 2.0 between the peaks due to *N*-methylpyrrolidone and impurity G.

Limits:

- *any impurity*: maximum 0.1 per cent,
- *total*: maximum 0.3 per cent,
- *disregard limit*: 0.02 per cent.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 4.0 g in *water R* and dilute to 20.0 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Water (*2.5.32*)

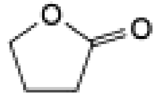
Maximum 0.1 per cent, determined on 1.000 g.

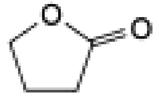
STORAGE

Protected from light.

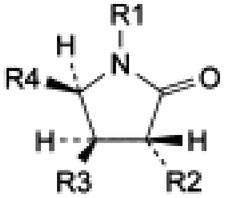
IMPURITIES

A. H_3C-NH_2: methanamine (methylamine),

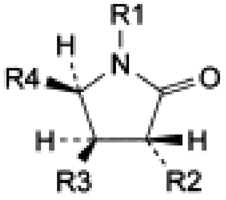




B. dihydrofuran-2(3*H*)-one (γ-butyrolactone),



and enantiomer



and enantiomer

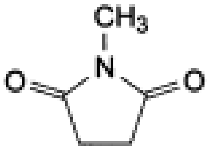
C. R1 = R2 = CH_3, R3 = R4 = H: (3*RS*)-1,3-dimethylpyrrolidin-2-one,

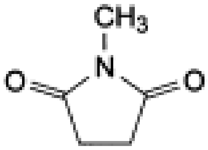
D. R1 = R3 = CH_3, R2 = R4 = H: (4*RS*)-1,4-dimethylpyrrolidin-2-one,

E. R1 = R4 = CH_3, R2 = R3 = H: (5*RS*)-1,5-dimethylpyrrolidin-2-one,

G. R1 = R2 = R3 = R4 = H: pyrrolidin-2-one (2-pyrrolidone),

F. HO-$[CH_2]_4$-OH: butane-1,4-diol,





H. 1-methylpyrrolidine-2,5-dione (*N*-methylsuccinimide),

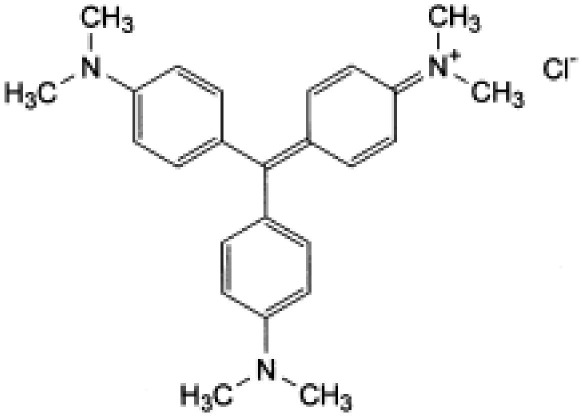
I. propylene glycol.

Ph Eur

Methylrosanilinium Chloride

General Notices

(Ph Eur monograph 1990)



$C_{25}H_{30}ClN_3$ 408.0 *548-62-9*

Action and use

Antiseptic dye (gentian violet).

Ph Eur

DEFINITION

N-[4-[Bis[4-(dimethylamino)phenyl]methylene]cyclohexa-2,5-dienylidene]-*N*-methylmethanaminium chloride (hexamethyl-*p*-rosanilinium chloride). It contains not more than 10 per cent of pentamethyl-*p*-rosanilinium chloride and is also known as crystal violet and gentian violet.

Content

95.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Dark green, shiny powder, hygroscopic.

Solubility

Sparingly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylrosanilinium chloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *methylrosanilinium chloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *glacial acetic acid R*, *water R*, *butanol R* (17:17:66 *V/V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution. In the chromatogram obtained with the test solution, a secondary spot may be observed.

C. Dissolve 50 mg in *water R* and dilute to 5 ml with the same solvent; add 3 ml of *dilute sulphuric acid R*, 1 g of *zinc powder R* and heat gently. The mixture decolourises. Filter. To 3 ml of the filtrate add 0.5 ml of *silver nitrate solution R1*. A white turbidity is produced which slowly forms a dark, coagulating precipitate.

TESTS

***N*,*N*-Dimethylaniline (*2.4.26, Method A*)**

Maximum 100 ppm.

Test solution Dissolve in a ground-glass-stoppered tube 0.50 g of the substance to be examined in 30.0 ml of *water R*. Add 1.0 ml of the internal standard solution. Adjust the solution to 26-28 °C. Add 1.0 ml of *strong sodium hydroxide solution R* and mix for 2 min. Add 2.0 ml of *trimethylpentane R*. Shake for 2 min and centrifuge. Use the upper layer.

Reference solution Dissolve 50.0 mg of *N,N-dimethylaniline R* in 4.0 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*. To 0.50 g of the substance to be examined add 5.0 ml of this solution and dilute to 30.0 ml with *water R*. Add 1.0 ml of the internal standard solution and 1.0 ml of *strong sodium hydroxide solution R*. Add 2.0 ml of *trimethylpentane R*. Shake for 2 min and centrifuge. Use the upper layer.

Limit:

— calculate the ratio (*R*) of the area of the peak due to *N*,*N*-dimethylaniline to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to *N*,*N*-dimethylaniline to the area of the peak due to the internal standard: this ratio is not greater than 0.5 *R*.

Pentamethyl-*p*-rosanilinium

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 30.0 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 3.0 mg of *methylrosanilinium for system suitability CRS* in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *ethanol (96 per cent) R*. Dilute 1.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *spherical octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R*, *water R*, *methanol R* (10:190:800 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 589 nm.

Injection 20 µl.

Run time 2.5 times the retention time of the principal peak.

System suitability Reference solution (a):
- *resolution*: peak due to pentamethyl-*p*-rosanilinium is baseline separated from the peak due to methylrosanilinium.

Locate the peak due to pentamethyl-*p*-rosanilinium using the chromatogram provided with *methylrosanilinium for system suitability CRS*.

Limits:
- *pentamethyl-p-rosanilinium*: maximum 10 per cent.

Related substances

Liquid chromatography (2.2.29) as described in the test for pentamethyl-*p*-rosanilinium.

Limits:
- *impurity A*: maximum 1.0 per cent;
- *any other impurity*: for each impurity, maximum 0.1 per cent;
- *sum of impurities other than A*: maximum 1.0 per cent;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to pentamethyl-*p*-rosanilinium.

Substances insoluble in ethanol (90 per cent *V/V*)

Maximum 0.5 per cent.

In a conical flask introduce 1.0 g and add 50 ml of *ethanol (90 per cent V/V) R*. Boil under a reflux condenser for 1 h. Filter the warm liquid through a weighed sintered glass filter (16) (*2.1.2*) previously dried at 100-105 °C. Wash with hot *ethanol (90 per cent V/V) R* until a colourless filtrate is obtained. Dry at 100-105 °C until constant weight.

Water (*2.5.12*)

Maximum 10.0 per cent, determined on 0.100 g.

Sulphated ash (*2.4.14*)

Maximum 1.5 per cent, determined on 1.0 g.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 250.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the maximum at 589 nm.

Calculate the content of $C_{25}H_{30}ClN_3$ taking the specific absorbance to be 2605.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities A.

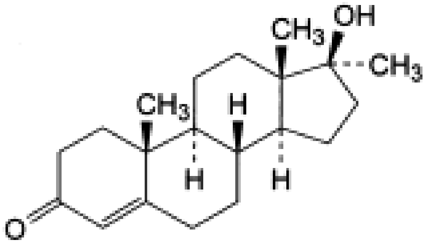
A. impurity of unknown structure with a relative retention of about 0.7.

Ph Eur

Methyltestosterone

General Notices

(Ph Eur monograph 0410)



$C_{20}H_{30}O_2$ 302.5 *58-18-4*

Action and use

Anabolic steroid.

Ph Eur

DEFINITION

17β-Hydroxy-17-methylandrost-4-en-3-one.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish-white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B.

Second identification A, C.

A. Melting point *(2.2.14)*: 162 °C to 168 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *methyltestosterone CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 20 mg of *methyltestosterone CRS* in 1 ml of a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R*.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *anhydrous acetic acid R, light petroleum R, butyl acetate R* (1:30:70 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm and spray with a saturated solution of *potassium dichromate R* in a mixture of 30 volumes of *water R* and 70 volumes of *sulphuric acid R*. Examine immediately in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Specific optical rotation *(2.2.7)*

+ 79 to + 85 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dilute 0.5 ml of the test solution to 100.0 ml with *methanol R*.

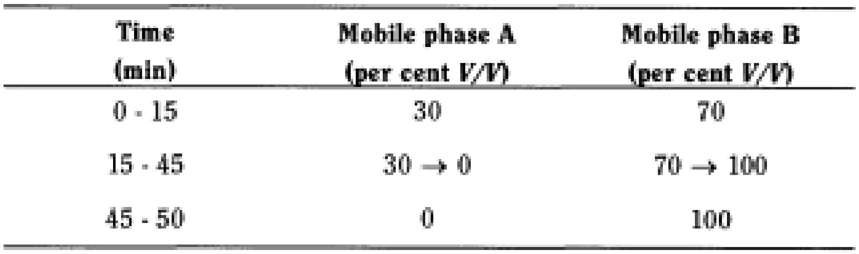
Reference solution (b) Dissolve 5 mg of *methyltestosterone for system suitability CRS* (containing impurity A) in *methanol R* and dilute to 10 ml with the same solvent.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *water R*;

— *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 15	30	70
15 - 45	30 → 0	70 → 100
45 - 50	0	100

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *methyltestosterone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to methyltestosterone (retention time = about 8 min): impurity A = about 1.5.

System suitability Reference solution (b):

— *resolution*: minimum 5 between the peaks due to methyltestosterone and impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *ethanol (96 per cent) R*. Dilute 10.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 241 nm.

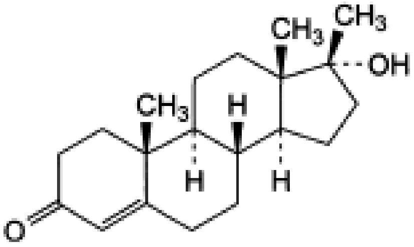
Calculate the content of $C_{20}H_{30}O_2$, taking the specific absorbance to be 540.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A.



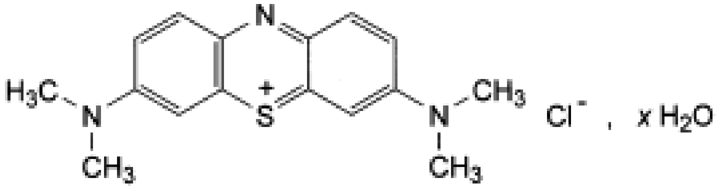
A. 17α-hydroxy-17-methylandrost-4-en-3-one.

Ph Eur

Methylthioninium Chloride

General Notices

(Ph Eur monograph 1132)



$C_{16}H_{18}ClN_3S,xH_2O$ 319.9 *61-73-4*
(anhydrous)

Action and use

Reducing agent; antidote to methaemoglobinaemia.

Preparation

Methylthioninium Injection

Ph Eur

DEFINITION

3,7-Bis(dimethylamino)phenothiazin-5-ylium chloride (methylene blue).

Content

95.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Dark blue, crystalline powder with a copper-coloured sheen, or green crystals with a bronze-coloured sheen.

Solubility

Soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 10 mg in *dilute hydrochloric acid R* and dilute to 100 ml with the same acid. Dilute 5 ml of the solution to 100 ml with *dilute hydrochloric acid R*.

Spectral range 240-800 nm.

Absorption maxima At 255-260 nm, 285-290 nm, 675-685 nm and 740-750 nm.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *methanol R*.

Reference solution Dissolve 10 mg of *methylthioninium chloride CRS* in *methanol R* and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R*, *propanol R* (20:80 *V/V*).

Application 2 µl.

Development Over a path of 8 cm.

Drying In air, protected from light.

Detection Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution. A secondary spot may appear above the principal spot in both chromatograms.

C. Dissolve about 1 mg in 10 ml of *water R*. Add 1 ml of *glacial acetic acid R* and 0.1 g of *zinc powder R*. Heat to boiling. The solution becomes colourless. Filter and shake the filtrate. It becomes blue on contact with air.

D. Ignite 50 mg with 0.5 g of *anhydrous sodium carbonate R*. Cool and dissolve the residue in 10 ml of *dilute nitric acid R*. Filter. The filtrate, without further addition of *dilute nitric acid R*, gives reaction (a) of chlorides (*2.3.1*).

TESTS

Methanol-insoluble substances

Maximum 10.0 mg (1.0 per cent).

To 1.0 g add 20 ml of *methanol R* and boil under a reflux condenser for 5 min. Filter through a tared sintered-glass filter (40) (*2.1.2*) and wash the filter with *methanol R* until a colourless filtrate is obtained. Dry the filter at 100 °C and weigh.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 15.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 7.5 mg of *methylthioninium impurity A CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. To 1.0 ml of this solution add 1.0 ml of the test solution and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 27 volumes of *acetonitrile R* and 73 volumes of a mixture of 3.4 ml of *phosphoric acid R* and 1000 ml of *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 246 nm.

Injection 20 µl.

Run time Twice the retention time of methylthioninium.

Relative retention with reference to methylthioninium (retention time = about 11 min): impurity A = about 0.7.

System suitability Reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to impurity A and methylthioninium. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

- *impurity A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent);
- *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

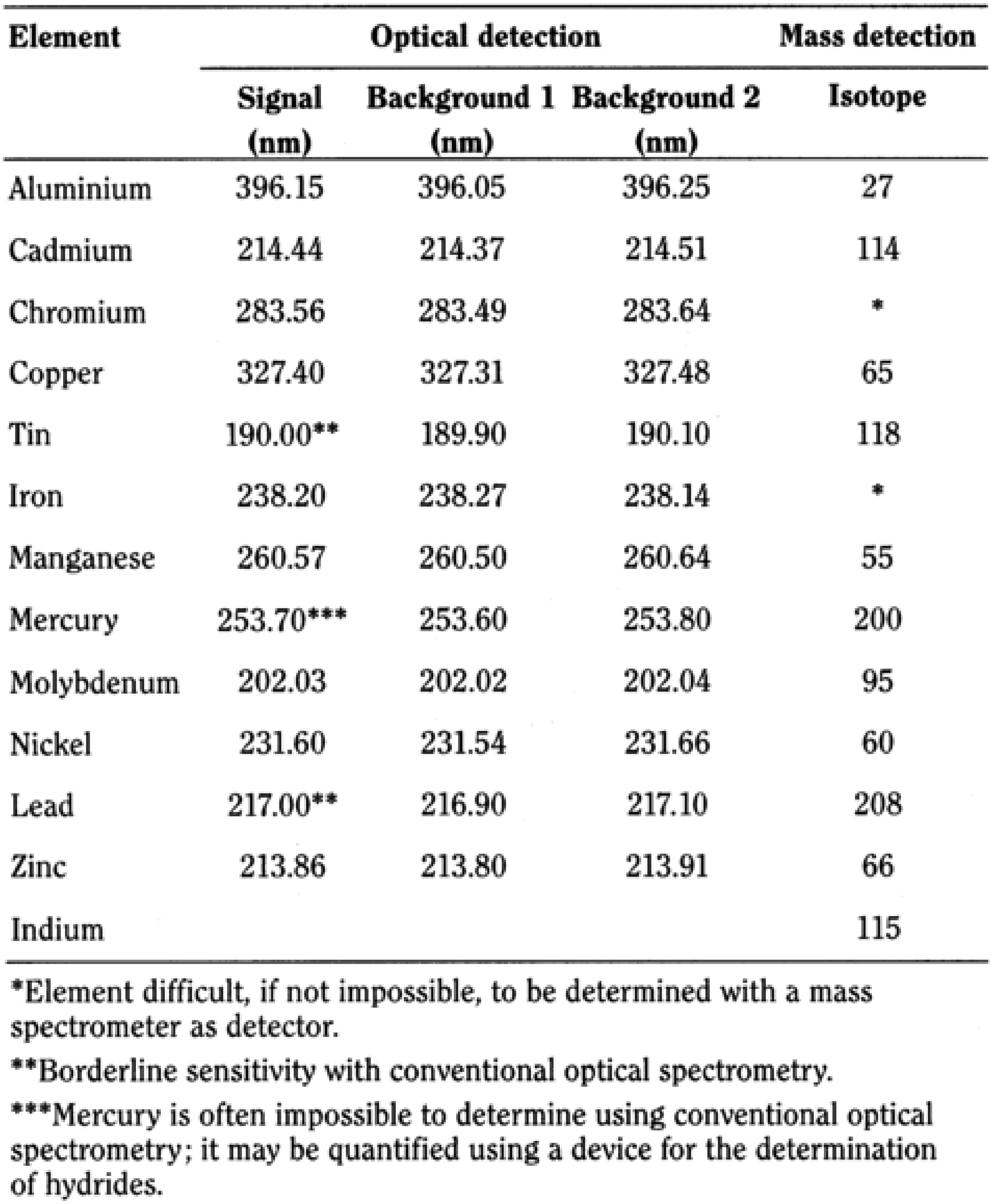
Metals

Atomic emission spectrometry (*2.2.22*) in argon plasma, using as detector a conventional optical system or a mass spectrometer; in the case of a mass spectrometer, use indium as internal standard.

Test solution In a 10 ml volumetric flask, dissolve with stirring 100 mg of the substance to be examined in 9 ml of *water R*, add 100.0 µl of a 10 µg/ml solution of indium prepared from indium *elementary standard solution for atomic spectrometry (1.000 g/l) R* in *nitric acid R* which has been diluted fifty-fold with *water R*. Dilute to 10.0 ml with *water R*.

Reference solutions Into a 100 ml volumetric flask, introduce 10.0 ml of a standard solution containing 1.00 µg/ml of each of the metals to be determined and prepared by dilution, with *water R*, of each *elementary standard solution for atomic spectrometry (1.000 g/l) R* for the corresponding elements. Add 1.00 ml of a 10 µg/ml solution of indium prepared from indium *elementary standard solution for atomic spectrometry (1.000 g/l) R* in *nitric acid R* which has been diluted fifty-fold with *water R*. Dilute to 100.0 ml with *water R*.

Blank solution Dilute one hundred-fold with *water R* the 10 µg/ml solution of indium used for the test and reference solutions.

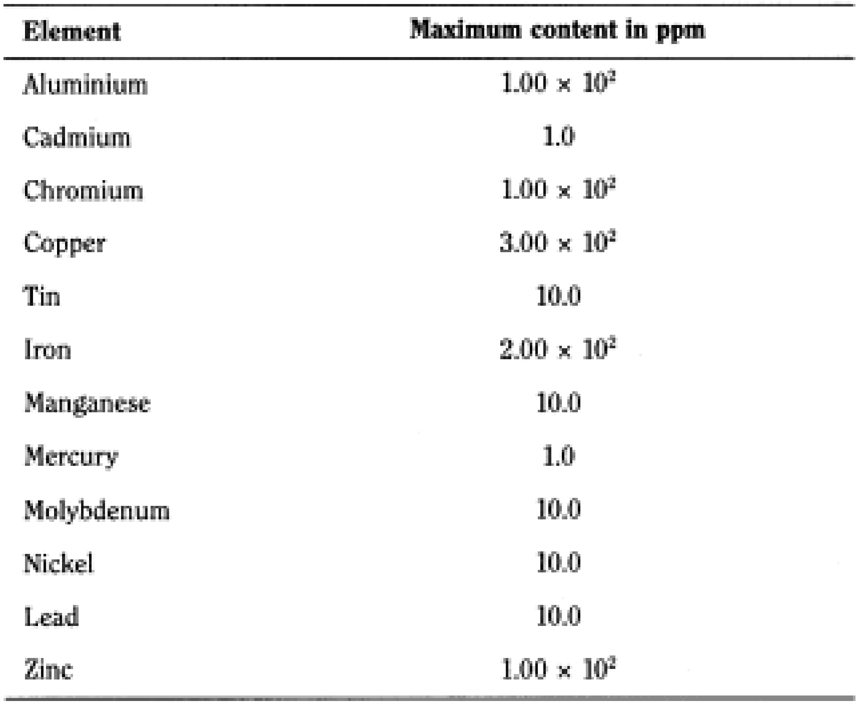


Element	Optical detection			Mass detection
	Signal (nm)	Background 1 (nm)	Background 2 (nm)	Isotope
Aluminium	396.15	396.05	396.25	27
Cadmium	214.44	214.37	214.51	114
Chromium	283.56	283.49	283.64	*
Copper	327.40	327.31	327.48	65
Tin	190.00**	189.90	190.10	118
Iron	238.20	238.27	238.14	*
Manganese	260.57	260.50	260.64	55
Mercury	253.70***	253.60	253.80	200
Molybdenum	202.03	202.02	202.04	95
Nickel	231.60	231.54	231.66	60
Lead	217.00**	216.90	217.10	208
Zinc	213.86	213.80	213.91	66
Indium				115

*Element difficult, if not impossible, to be determined with a mass spectrometer as detector.

**Borderline sensitivity with conventional optical spectrometry.

***Mercury is often impossible to determine using conventional optical spectrometry; it may be quantified using a device for the determination of hydrides.



Element	Maximum content in ppm
Aluminium	1.00×10^2
Cadmium	1.0
Chromium	1.00×10^2
Copper	3.00×10^2
Tin	10.0
Iron	2.00×10^2
Manganese	10.0
Mercury	1.0
Molybdenum	10.0
Nickel	10.0
Lead	10.0
Zinc	1.00×10^2

Loss on drying (*2.2.32*)

8.0 per cent to 22.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.25 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 30 ml of *water R* with heating. Cool, add 50.0 ml of *potassium dichromate solution R1* and dilute to 100.0 ml with *water R*. Allow to stand for 10 min. Filter and discard the first 20 ml of filtrate. Introduce 50.0 ml of the filtrate into a flask with a ground-glass neck, add 50 ml of *dilute sulphuric acid R* and 8.0 ml of *potassium iodide solution R*. Allow to stand protected from light for 5 min, then add 80 ml of *water R*. Titrate with *0.1 M sodium thiosulphate* using 2 ml of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

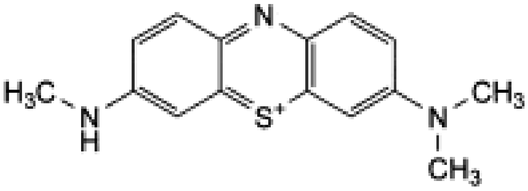
1 ml of *0.1 M sodium thiosulphate* is equivalent to 10.66 mg of $C_{16}H_{18}ClN_3S$.

STORAGE

In an airtight container , protected from light.

IMPURITIES

Specified impurities A.

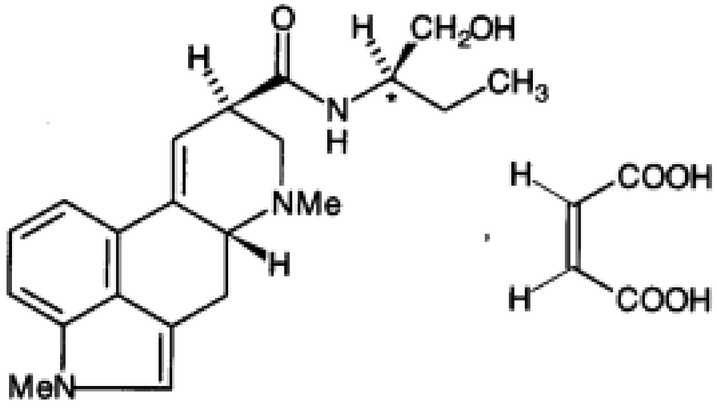

N
H3C
N
H
S+
N
CH3
CH3

A. 3-(dimethylamino)-7-(methylamino)phenothiazin-5-ylium.

Ph Eur

Methysergide Maleate

General Notices



and epimer at C*

$C_{21}H_{27}N_3O_2,C_4H_4O_4$ 469.5 *129-49-7*

Action and use

Non-selective 5HT receptor antagonist.

Preparation

Methysergide Tablets

DEFINITION

Methysergide Maleate is (1*RS*)-*N*-[1-(hydroxymethyl)propyl]-1-methyl-D-lysergamide hydrogen maleate. It contains not less than 98.0% and not more than 101.0% of $C_{21}H_{27}N_3O_2$, $C_4H_4O_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder which may have a yellow or pink tinge.

Slightly soluble in *water* and in *methanol*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methysergide maleate *(RS 227)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (8).

C. Dissolve 1 mg in 1 ml of *ethanol (96%)* and add 1 ml of *dimethylaminobenzaldehyde solution R6*. A brownish red to violet colour is produced.

TESTS

Acidity

pH of a 0.2% w/v solution, 3.7 to 4.7, Appendix V L.

Specific optical rotation

In a 0.25% w/v solution, +35.0 to +45.0, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using a suspension of *silica gel G* in 0.1M *sodium hydroxide* to prepare the plate and a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. Solution (1) contains 2.0% w/v of the substance being examined in a mixture of 1 volume of 13.5M *ammonia* and 100 volumes of *methanol*. Solutions (2) to (8) are solutions of *methysergide maleate BPCRS* in a mixture of 1 volume of 13.5M *ammonia* and 100 volumes of *methanol* containing (2) 0.0050% w/v, (3) 0.010% w/v, (4) 0.015% w/v, (5) 0.020% w/v, (6) 0.030% w/v, (7) 0.040% w/v and (8) 2.0% w/v respectively. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Assess the intensities of any *secondary spots* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with solutions (2) to (7), making allowance for area in assessing the intensities of spots of different R_f values and disregarding any spots less intense than the spot in the chromatogram obtained with solution (2). The total of the intensities so assessed does not exceed 2%.

Loss on drying

When dried to constant weight at 120° at a pressure not exceeding 0.7 kPa, loses not more than 7.0% of its weight. Use 1 g.

ASSAY

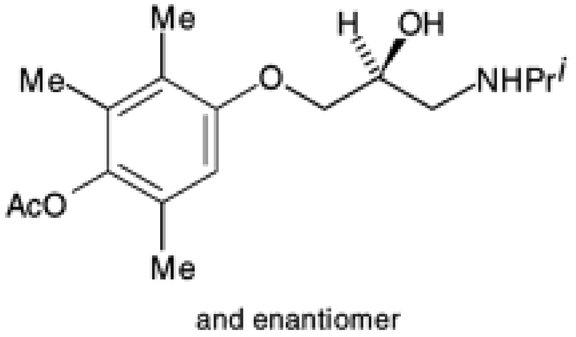
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.4 g and *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 46.95 mg of $C_{21}H_{27}N_3O_2,C_4H_4O_4$.

STORAGE

Methysergide Maleate should be protected from light and stored at a temperature of 2° to 8°.

Metipranolol

General Notices



$C_{17}H_{27}NO_4$ 309.4 *22664-55-7*

Action and use

Beta-adrenoceptor antagonist.

Preparation

Metipranolol Eye Drops

DEFINITION

Metipranolol is (*RS*)-4-(2-hydroxy-3-isopropylaminopropoxy)-2,3,6-trimethylphenyl acetate. It contains not less than 99.0% and not more than 101.0% of $C_{17}H_{27}NO_4$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white, crystalline powder; melting point, about 108°.

Practically insoluble in *water*; soluble in *ethanol (96%),* in *acetone* and in *methanol*. It dissolves in dilute mineral acids.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of metipranolol *(RS 375)*.

TESTS

Alkalinity

Shake 0.5 g with 20 ml of *water* for 5 minutes and filter. The pH of the filtrate is 9.0 to 10.0, Appendix V L.

Colour of solution

A 5.0% w/v solution in 1M *hydrochloric acid* is not more intensely coloured than *reference solution Y_6*, Appendix IV B.

Heavy metals

2 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Iron

Dissolve the residue obtained in the test for Sulphated ash in 1 ml of *hydrochloric acid*, evaporate to dryness, dissolve the residue in a mixture of 0.5 ml of 6M *acetic acid* and 7 ml of *water* and add sufficient *water* to produce 20 ml. 10 ml of this solution complies with the *limit test for iron*, Appendix VII (10 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol*. Solution (1) contains 0.10% w/v of the substance being examined. Solution (2) contains 0.0005% w/v of the substance being examined. Solution (3) contains 0.001% w/v of *desacetylmetipranolol BPCRS*. Solution (4) contains 0.02% w/v of the substance being examined and 0.02% w/v of *desacetylmetipranolol BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1 volume of *perchloric acid*, 45 volumes of *methanol* and 54 volumes of *water*, the pH of the mixture being adjusted to 3.0 with 13.5M *ammonia* and (c) a detection wavelength of 275 nm. For solution (1) allow the chromatography to proceed for at least 3 times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to desacetylmetipranolol is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%) and the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Sulphated ash

Not more than 0.1%, Appendix IX A. Use 2 g.

Water

Not more than 0.5% w/w, Appendix IX C. Use 1 g.

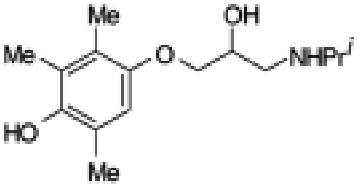
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.3 g and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 30.94 mg of $C_{17}H_{27}NO_4$.

STORAGE

Metipranolol should be protected from light.

IMPURITIES

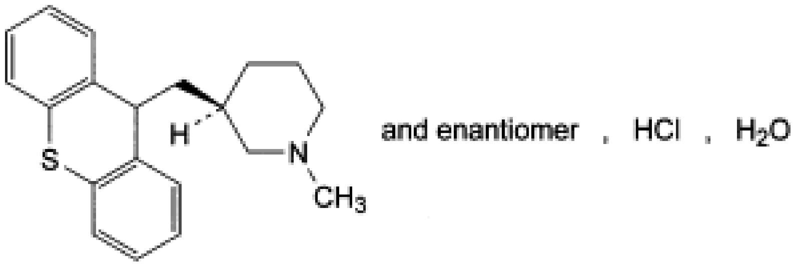

Me
Me
HO
Me
O
OH
NHPr^i

A. desacetylmetipranolol

Metixene Hydrochloride

General Notices

(Ph Eur monograph 1347)



$C_{20}H_{23}NS,HCl,H_2O$ 363.9   *7081-40-5*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

Metixene hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (*RS*)-1-methyl-3-[(9*H*-thioxanthen-9-yl)methyl]piperidine hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline or fine crystalline powder, soluble in water, soluble in alcohol and in methylene chloride.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *metixene hydrochloride CRS*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

Dissolve 0.40 g in *methanol R* and dilute to 20.0 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method I*).

pH (*2.2.3*)

Dissolve 0.18 g in *carbon dioxide-free water R* heating if necessary at about 50 °C, cool and dilute to 10.0 ml with the same solvent. The pH of the solution, measured immediately, is 4.4 to 5.8.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*. Carry out the test rapidly and protected from light.

Test solution Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *metixene hydrochloride CRS* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *thioxanthene CRS* in 50 ml of *methylene chloride R*. Dilute 1.0 ml of the solution to 20.0 ml with *methylene chloride R*.

Reference solution (c) Dissolve 5 mg of *thioxanthone CRS* in 50 ml of *methylene chloride R*. Dilute 1.0 ml of the solution to 20.0 ml with *methylene chloride R*.

Reference solution (d) Dilute 4 ml of reference solution (a) to 10.0 ml with *methylene chloride R.*

Apply to the plate as narrow bands 5 µl of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of *glacial acetic acid R*, 10 volumes of *methanol R* and 80 volumes of *methylene chloride R*. Dry the plate in a stream of cold air. Spray with a mixture of 1 volume of *sulphuric acid R* and 9 volumes of *alcohol R* and heat at 100 °C for 10 min. Allow the plate to cool and examine in ultraviolet light at 365 nm. Thioxanthene shows orange fluorescence and thioxanthone shows greenish-blue fluorescence. Any band corresponding to thioxanthene in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (b) (0.2 per cent); any band corresponding to thioxanthone in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (c) (0.05 per cent); any band, apart from the principal band and the bands corresponding to thioxanthene and thioxanthone, is not more intense than the band in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most one such band is more intense than the band in the chromatogram obtained with reference solution (d) (0.2 per cent). The test is not valid unless the bands in the chromatograms obtained with reference solutions (b) and (c) are clearly visible and differentiated.

Loss on drying (*2.2.32*)

Not less than 4.0 per cent and not more than 6.0 per cent, determined on 0.500 g by drying in an oven at 138-142 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

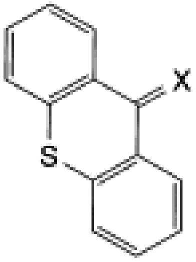
Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 34.59 mg of $C_{20}H_{24}ClNS$.

STORAGE

Store protected from light.

IMPURITIES



A. X = H_2: 9*H*-thioxanthene,

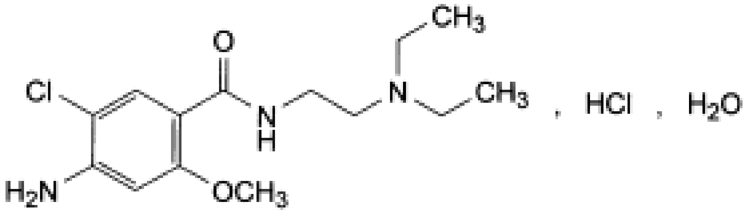
B. X = O: 9*H*-thioxanthen-9-one (thioxanthone).

Ph Eur

Metoclopramide Hydrochloride

General Notices

(Ph Eur monograph 0674)



$C_{14}H_{22}ClN_3O_2,HCl,H_2O$ 354.3 *54143-57-6*

Action and use

Dopamine receptor antagonist; antiemetic.

Preparations

Metoclopramide Injection

Metoclopramide Oral Solution

Metoclopramide Tablets

Ph Eur

DEFINITION

Metoclopramide hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-5-chloro- *N*-[2-(diethylamino)ethyl]-2-methoxybenzamide hydrochloride, calculated with reference to the anhydrous substance.

CHARACTERS

White or almost white, crystalline powder or crystals, very soluble in water, freely soluble in alcohol, sparingly soluble in methylene chloride.

It melts at about 183 °C with decomposition.

IDENTIFICATION

First identification A, B, D.

Second identification A, C, D, E.

A. The pH (*2.2.3*) of solution S (see Tests) is 4.5 to 6.0.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum

obtained with *metoclopramide hydrochloride CRS*. Examine the substances as discs prepared using *potassium chloride R*.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light before spraying with *dimethylaminobenzaldehyde solution R1*. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dilute 1 ml of solution S to 2 ml with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

E. Dissolve about 2 mg in 2 ml of *water R*. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* HF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.40 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a Dissolve 20 mg of *metoclopramide hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (a) to 100 ml with *methanol R*. Dilute 1 ml of this solution to 10 ml with *methanol R*.

Reference solution (c) Dissolve 10 mg of *N,N-diethylethylenediamine R* in *methanol R* and dilute to 50 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 12 cm using a mixture of 2 volumes of *concentrated ammonia R*, 10 volumes of *dioxan R*, 14 volumes of *methanol R* and 90 volumes of *methylene chloride R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Spray with *dimethylaminobenzaldehyde solution R1*. Allow the plate to dry in air. Any spot in the chromatogram obtained with test solution (a) that has not been visualised in ultraviolet light at 254 nm is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Heavy metals (*2.4.8*)

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Water (*2.5.12*)

4.5 per cent to 5.5 per cent, determined on 0.500 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.2500 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume of *0.1 M sodium hydroxide* added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 33.63 mg of $C_{14}H_{23}Cl_2N_3O_2$.

STORAGE

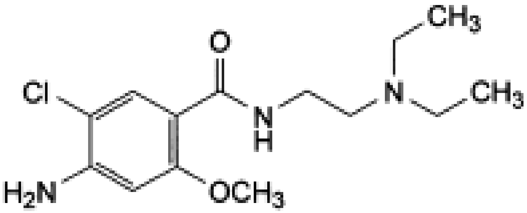
Store protected from light.

Ph Eur

Metoclopramide

General Notices

(Ph Eur monograph 1348)



$C_{14}H_{22}ClN_3O_2$ 299.8 *364-62-5*

Action and use

Dopamine receptor antagonist; antiemetic

Metoclopramide Hydrochloride formulations should reflect the Hydrochloride salt.

Ph Eur

DEFINITION

4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, fine powder.

Solubility

Practically insoluble in water, sparingly soluble or slightly soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

First identification A, B.

Second identification A, C.

A. Melting point *(2.2.14)*: 145 °C to 149 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison metoclopramide CRS.

C. Examine the chromatograms obtained in test A for related substances.

Detection Examine in ultraviolet light at 254 nm before spraying with *dimethylaminobenzaldehyde solution R1*.

Results The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The freshly prepared solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 2.5 g in 25 ml of *1 M hydrochloric acid*.

Related substances

A. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dissolve 0.160 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *metoclopramide CRS* and 10 mg of *sulpiride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *N,N-diethylethane-1,2-diamine R* (impurity E) in *methanol R* and dilute to 50 ml with the same solvent. Dilute 2 ml of this solution to 25 ml with *methanol R*.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *concentrated ammonia R, dioxan R, methanol R, methylene chloride R* (2:10:14:90 *V/V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm (identification C), then spray with *dimethylaminobenzaldehyde solution R1* and allow to dry in air.

System suitability Reference solution (a):

— the chromatogram shows 2 clearly separated spots.

Limit Test solution (b):

— *impurity E*: any spot due to impurity E (not visualised in ultraviolet light at 254 nm) is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

B. Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 0.2 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *metoclopramide impurity A CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Mix 1 ml of this solution with 0.1 ml of the test solution and dilute to 10 ml with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 6.8 g of *potassium dihydrogen phosphate R* in 700 ml of *water R*; add 0.2 ml of *N,N-dimethyloctylamine R* and adjust to pH 4.0 with *dilute phosphoric acid R*; dilute to 1000 ml with *water R*, add 250 ml of *acetonitrile R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl.

Run time 8 times the retention time of metoclopramide.

Relative retention With reference to metoclopramide (retention time = about 3.6 min): impurity A = about 0.82; impurity F = about 0.89; impurity H = about 0.91; impurity G = about 1.7; impurity C = about 2.7; impurity D = about 2.8; impurity B = about 6.4.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and metoclopramide .

Limits:
— *impurities A, B, C, D, F, G, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

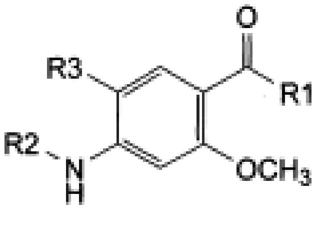
ASSAY

Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R* and add 5 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 29.98 mg of $C_{14}H_{22}ClN_3O_2$.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H.

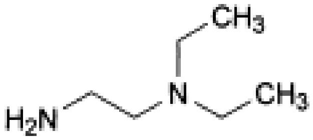


A. R1 = $NH-CH_2-CH_2-N(C_2H_5)_2$, R2 = $CO-CH_3$, R3 = Cl: 4-(acetylamino)-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide,

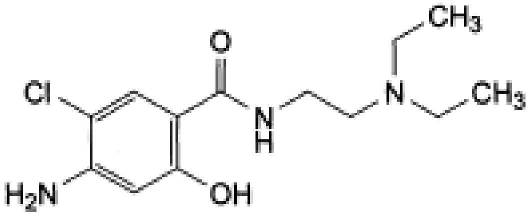
B. R1 = OCH_3, R2 = $CO-CH_3$, R3 = Cl: methyl 4-(acetylamino)-5-chloro-2-methoxybenzoate,

C. R1 = OH, R2 = H, R3 = Cl: 4-amino-5-chloro-2-methoxybenzoic acid,

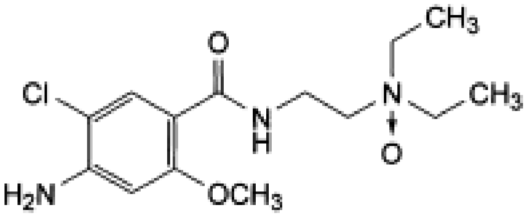
D. R1 = OCH_3, R2 = $CO-CH_3$, R3 = H: methyl 4-(acetylamino)-2-methoxybenzoate,



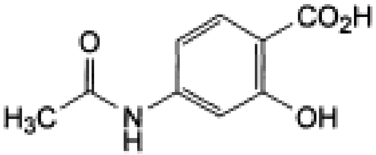
E. *N,N*-diethylethane-1,2-diamine,



F. 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-hydroxybenzamide,



G. *N′*-(4-amino-5-chloro-2-methoxybenzoyl)-*N,N*-diethylethane-1,2-diamine *N*-oxide,



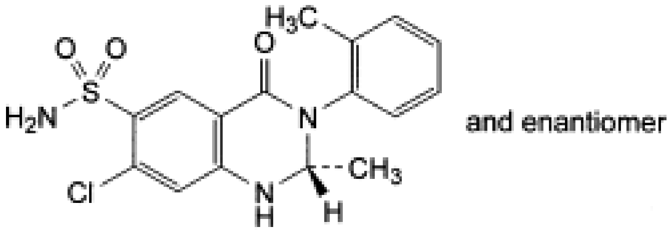
H. 4-(acetylamino)-2-hydroxybenzoic acid.

Ph Eur

Metolazone

General Notices

(Ph Eur monograph 1757)



$C_{16}H_{16}ClN_3O_3S$ 365.8 *17560-51-9*

Action and use

Thiazide-like diuretic.

Ph Eur

DEFINITION

(2*RS*)-7-Chloro-2-methyl-3-(2-methylphenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulphonamide.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder.

Solubility

Very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethyl acetate, very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison metolazone CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 30.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dilute 2.0 ml of test solution (a) to 100.0 ml with *methanol R*.

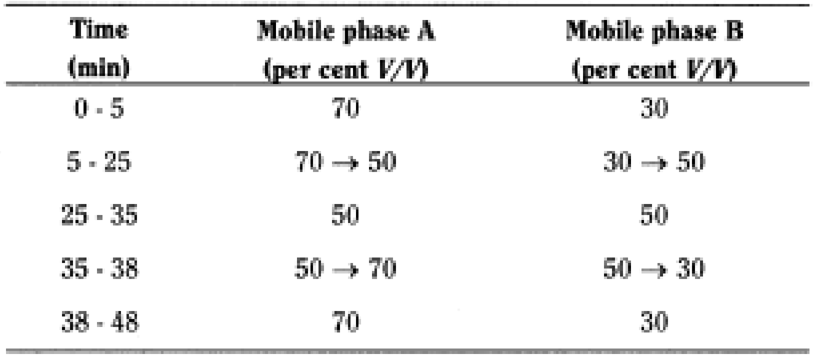
Reference solution (a) Dissolve 3.0 mg of *metolazone for system suitability CRS* (containing impurities A, B, C, D and E) in 1 ml of *methanol R*.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 10.0 ml with *methanol R*.

Reference solution (c) Dissolve 30.0 mg of *metolazone CRS* in *methanol R* and dilute to 10.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *methanol R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: 5.44 g/l solution of *potassium dihydrogen phosphate R*;
- *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	70	30
5 - 25	70 → 50	30 → 50
25 - 35	50	50
35 - 38	50 → 70	50 → 30
38 - 48	70	30

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *metolazone for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D and E.

System suitability Reference solution (a):

— *resolution*: minimum 1.6 between the peaks due to impurities E and C and minimum 1.5 between the peaks due to impurities A and B.

Limits:

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

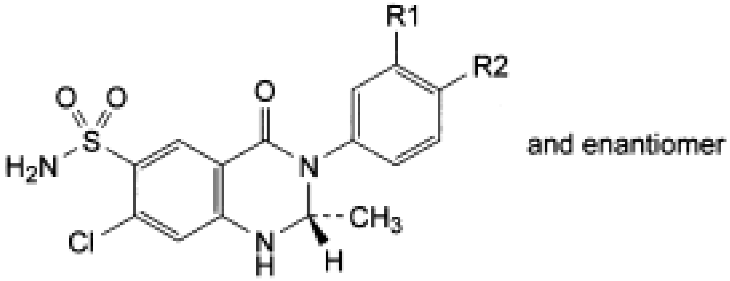
Calculate the percentage content of $C_{16}H_{16}ClN_3O_3S$ from the declared content of *metolazone CRS.*

STORAGE

Protected from light.

IMPURITIES

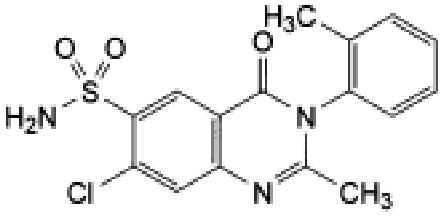
Specified impurities *A, B, C, D, E.*



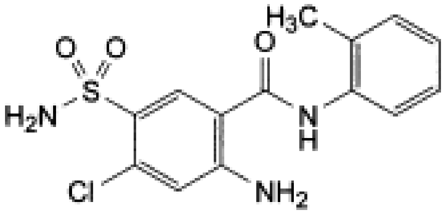
A. R1 = CH_3, R2 = H: (2*RS*)-7-chloro-2-methyl-3-(3-methylphenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulphonamide,

B. R1 = H, R2 = CH_3: (2*RS*)-7-chloro-2-methyl-3-(4-methylphenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulphonamide,

C. R1 = R2 = H: (2*RS*)-7-chloro-2-methyl-4-oxo-3-phenyl-1,2,3,4-tetrahydroquinazoline-6-sulphonamide,



D. 7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo-3,4-dihydroquinazoline-6-sulphonamide,



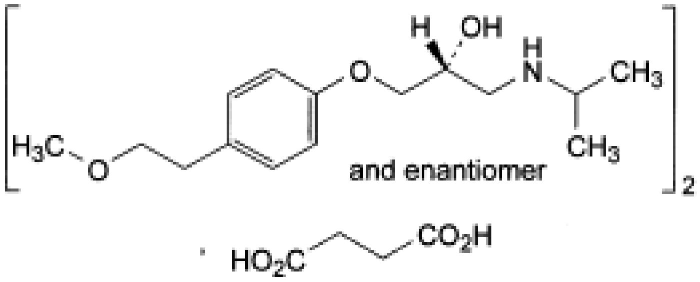
E. 2-amino-4-chloro-*N*-(2-methylphenyl)-5-sulphamoylbenzamide.

Ph Eur

Metoprolol Succinate

General Notices

(Ph Eur monograph 1448)



$C_{34}H_{56}N_2O_{10}$ 653 *98418-47-4*

Action and use

Beta-adrenoceptor antagonist.

Ph Eur

DEFINITION

Bis[(2*RS*)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol] butanedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, soluble in methanol, slightly soluble in alcohol, very slightly soluble in ethyl acetate.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of metoprolol succinate.

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and it is colourless (*2.2.2, Method II*).

pH (*2.2.3*)

7.0 to 7.6 for solution S.

Related substances

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 1 ml of the test solution to 50 ml with *methanol R*. Dilute 5 ml of this solution to 50 ml with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase Place 2 beakers each containing 30 volumes of *concentrated ammonia R* at the bottom of a chromatographic tank containing a mixture of 20 volumes of *methanol R* and 80 volumes of *ethyl acetate R*.

Application 10 µl.

Development Over a path of 12 cm in a tank saturated for at least 1 h.

Drying In air for at least 3 h.

Detection Expose the plate to iodine vapour for at least 15 h.

Limits:

— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.2 per cent);

— *disregard* any spot on the starting line.

B. Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of the substance to be examined and 3.0 mg of *metoprolol impurity A CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c) If this solution is required (see below), it is to be prepared in a fume cupboard. This solution is used only to identify the peak due to impurity C. Dissolve 10 mg of the substance to be examined in 10 ml of *0.1 M hydrochloric acid*. Transfer this solution to an evaporating dish 10 cm in diameter. Place the dish so that the surface of the solution is 5 cm from a lamp emitting ultraviolet light (*2.1.3*) at 254 nm for 6 h. Dilute 0.5 ml of this solution to

25 ml with the mobile phase.

Column:

- *size: l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Dissolve 3.9 g of *ammonium acetate R* in 810 ml of *water R*, add 2.0 ml of *triethylamine R*, 10.0 ml of *glacial acetic acid R*, 3.0 ml of *phosphoric acid R* and 146 ml of *acetonitrile R* and mix.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl; inject the test solution and reference solutions (a) and (b).

Run time 3 times the retention time of metoprolol.

Relative retention With reference to metoprolol (retention time = about 7 min): impurity C = about 0.3; impurity A = about 0.7.

System suitability Reference solution (a):

- *resolution*: minimum of 6.0 between the peaks due to impurity A and to metoprolol.

Limits:

- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to succinic acid.

If a peak occurs with a retention time of about 2.3 min (impurity C) which has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b), prepare and inject reference solution (c). For the chromatogram obtained with the test solution, multiply the peak area of impurity C by a correction factor of 0.1.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 40 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 32.64 mg of $C_{34}H_{56}N_2O_{10}$.

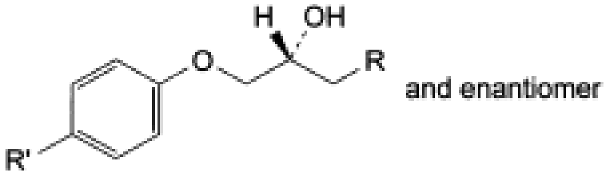
STORAGE

Protected from light.

IMPURITIES

By liquid chromatography A, B, C, D, E, F, G, H, J.

By thin-layer chromatography M, N, O.



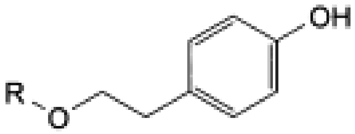
A. R = NH-CH_2-CH_3, R′ = CH_2-CH_2-OCH_3: (2*RS*)-1-(ethylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol,

C. R = NH-CH(CH_3)$_2$, R′ = CHO: 4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]benzaldehyde,

D. R = OH, R′ = CH_2-CH_2-OCH_3: (2*RS*)-3-[4-(2-methoxyethyl)phenoxy]propane-1,2-diol,

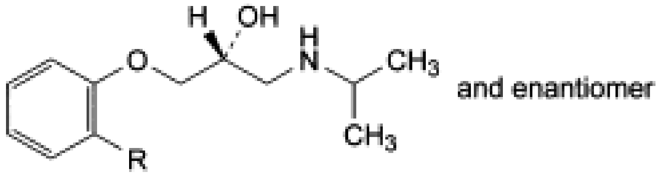
H. R = NH-CH(CH_3)$_2$, R′ = CH_2-CH_2-OH: (2*RS*)-1-[4-(2-hydroxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

J. R = O-CH_2-CHOH-CH_2-NH-CH(CH_3)$_2$, R′ = CH_2-CH_2-OCH_3: 1-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol,



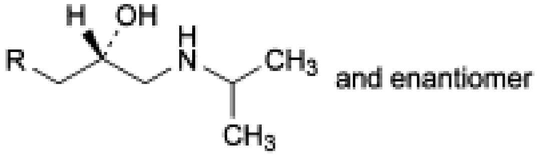
B. R = CH_3: 4-(2-methoxyethyl)phenol,

G. R = H: 2-(4-hydroxyphenyl)ethanol,



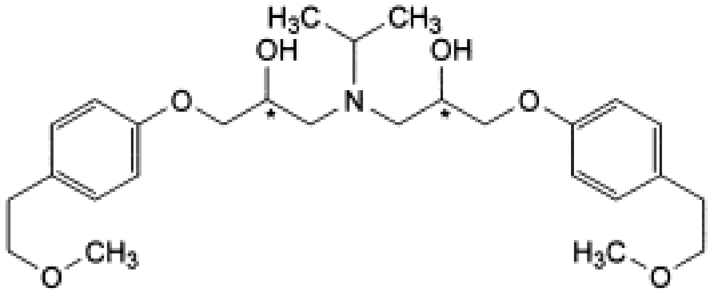
E. R = CH_2-CH_2-OCH_3: (2*RS*)-1-[2-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

F. R = H: (2*RS*)-1-[(1-methylethyl)amino]-3-phenoxypropan-2-ol,



M. R = NH-CH$(CH_3)_2$: 1,3-bis[(1-methylethyl)amino]propan-2-ol,

N. R = OH: (2*RS*)-3-[(1-methylethyl)amino]propane-1,2-diol,



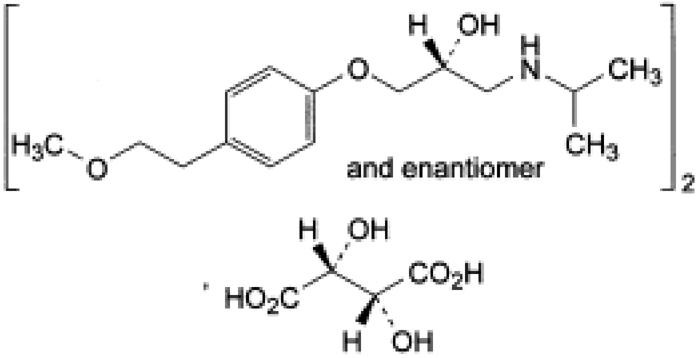
O. 1,1′-[(1-methylethyl)imino]bis[3-[4-(2-methoxyethyl)phenoxy]propan-2-ol].

Ph Eur

Metoprolol Tartrate

General Notices

(Ph Eur monograph 1028)



$(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ 685 *56392-17-7*

Action and use

Beta-adrenoceptor antagonist.

Preparations

Metoprolol Injection

Metoprolol Tartrate Tablets

Ph Eur

DEFINITION

Bis[(2*RS*)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol] (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, freely soluble in alcohol.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *metoprolol tartrate CRS.*

If the spectra obtained in the solid state show differences, record further spectra using discs prepared by placing 25 µl of a 100 g/l solution in *methylene chloride R* on a disc of *potassium bromide R* and evaporating the solvent. Examine immediately.

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method II*).

pH (*2.2.3*)

6.0 to 7.0 for solution S.

Specific optical rotation (*2.2.7*)

+ 7.0 to + 10.0 (dried substance), determined on solution S.

Related substances

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 20 ml with *methanol R*. Dilute 5 ml of this solution to 50 ml with *methanol R*.

Reference solution (b) Dilute 4 ml of reference solution (a) to 10 ml with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase Place 2 beakers each containing 30 volumes of *concentrated ammonia R* at the bottom of a chromatographic tank containing a mixture of 20 volumes of *methanol R* and 80 volumes of *ethyl acetate R*.

Application 5 µl.

Development Over a path of 12 cm in a tank saturated for at least 1 h.

Drying In air for at least 3 h.

Detection Expose the plate to iodine vapour for at least 15 h.

Limits:

— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most 1 such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard* any spot on the starting-line.

B. Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *metoprolol tartrate CRS* and 3.0 mg of *metoprolol impurity A CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 3.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c) If this solution is required (see below), it is to be prepared in a fume cupboard. This solution is used only to identify the peak due to impurity C. Dissolve 10 mg of *metoprolol tartrate CRS* in 10 ml of *0.1 M hydrochloric acid*. Transfer this solution to an evaporating dish 10 cm in diameter. Place the dish so that the surface of the solution is 5 cm from a lamp emitting ultraviolet light (*2.1.3*) at 254 nm for 6 h. Dilute 0.5 ml of this solution to 25 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Dissolve 3.9 g of *ammonium acetate R* in 810 ml of *water R*, add 2.0 ml of *triethylamine R*, 10.0 ml of *glacial acetic acid R*, 3.0 ml of *phosphoric acid R* and 146 ml of *acetonitrile R* and mix.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl; inject the test solution and reference solutions (a) and (b).

Run time 3 times the retention time of metoprolol.

Relative retention With reference to metoprolol (retention time = about 7 min): impurity C = about 0.3; impurity A = about 0.7.

System suitability Reference solution (a):

— *resolution*: minimum of 6.0 between the peaks due to impurity A and to metoprolol.

Limits:

— *any impurity (A to J)*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *total*: not more than 1.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.17 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to tartaric acid.

If a peak occurs with a retention time of about 2.3 min (impurity C) which has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b), prepare and inject reference solution (c). For the chromatogram obtained with the test solution, multiply the peak area of impurity C by a correction factor of 0.1.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* over *anhydrous calcium chloride R* for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 34.24 mg of $C_{34}H_{56}N_2O_{12}$.

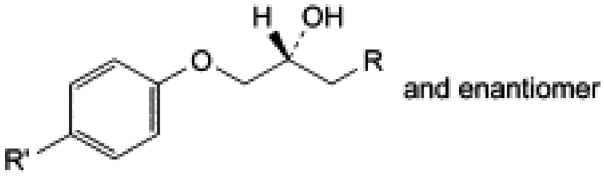
STORAGE

Protected from light.

IMPURITIES

By liquid chromatography A, B, C, D, E, F, G, H, J.

By thin-layer chromatography M, N, O.



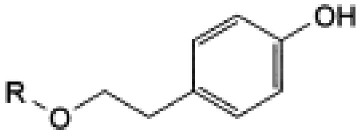
A. R = NH-CH_2-CH_3, R′ = CH_2-CH_2-OCH_3: (2*RS*)-1-(ethylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol,

C. R = NH-$CH(CH_3)_2$, R′ = CHO: 4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]benzaldehyde,

D. R = OH, R′ = CH_2-CH_2-OCH_3: (2*RS*)-3-[4-(2-methoxyethyl)phenoxy]propane-1,2-diol,

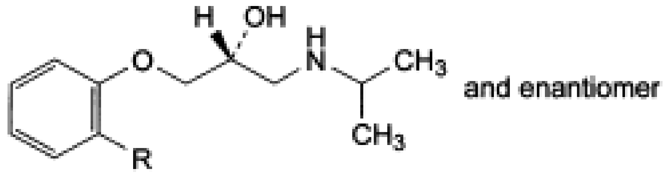
H. R = NH-$CH(CH_3)_2$, R′ = CH_2-CH_2-OH: (2*RS*)-1-[4-(2-hydroxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

J. R = O-CH_2-CHOH-CH_2-NH-$CH(CH_3)_2$, R′ = CH_2-CH_2-OCH_3: 1-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol,



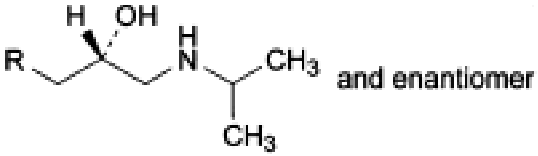
B. R = CH_3: 4-(2-methoxyethyl)phenol,

G. R = H: 2-(4-hydroxyphenyl)ethanol,



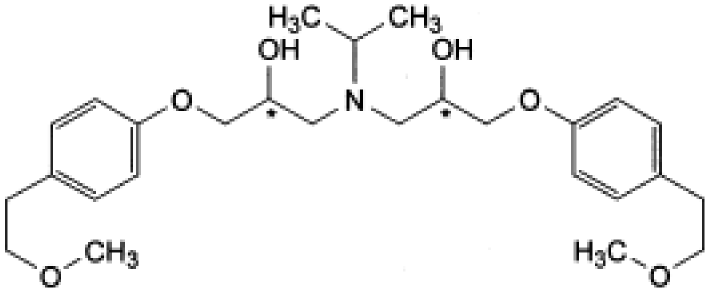
E. R = CH_2-CH_2-OCH_3: (2*RS*)-1-[2-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino] propan-2-ol,

F. R = H: (2*RS*)-1-[(1-methylethyl)amino]-3-phenoxypropan-2-ol,



M. R = NH-CH(CH_3)$_2$: 1,3-bis[(1-methylethyl)amino]propan-2-ol,

N. R = OH: (2*RS*)-3-[(1-methylethyl)amino]propane-1,2-diol,



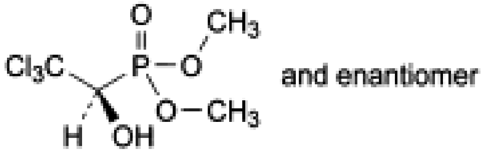
O. 1,1′-[(1-methylethyl)imino]bis[3-[4-(2-methoxyethyl)phenoxy]propan-2-ol].

Ph Eur

Metrifonate

General Notices

(Ph Eur monograph 1133)



$C_4H_8Cl_3O_4P$ 257.4 *52-68-6*

Action and use

Anthelminthic.

Ph Eur

DEFINITION

Dimethyl (*RS*)-(2,2,2-trichloro-1-hydroxyethyl)phosphonate.

Content

98.0 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very soluble in methylene chloride, freely soluble in acetone and in ethanol (96 per cent).

mp

76 °C to 81 °C.

IDENTIFICATION

First identification A, B.

Second identification B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *metrifonate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *metrifonate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R*, *dioxan R*, *toluene R* (5:25:70 *V/V/V*).

Application 10 µl.

Development In an unsaturated tank over a path of 15 cm.

Drying In air.

Detection Spray with a 50 g/l solution of *4-(4-nitrobenzyl)pyridine R* in *acetone R* and heat at 120 °C for 15 min; spray the still-warm plate with a 100 g/l solution of *tetraethylene pentamine R* in *acetone R* and examine immediately.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 20 mg in 1 ml of *dilute sodium hydroxide solution R*. Add 1 ml of *pyridine R*. Shake and heat on a water-bath for 2 min. A red colour develops in the upper layer.

D. To 0.1 g add 0.5 ml of *nitric acid R*, 0.5 ml of a 500 g/l solution of *ammonium nitrate R1* and 0.1 ml of *strong hydrogen peroxide solution R*. Heat on a water-bath for 10 min. Heat to boiling and add 1 ml of *ammonium molybdate solution R*. A yellow colour is produced or a yellow precipitate is formed.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Dissolve 5.0 g in 20 ml of *methanol R*.

Acidity

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent. Add 0.1 ml of *methyl red solution R.* Not more than 1.0 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Optical rotation (*2.2.7*)

- 0.10° to + 0.10°.

Dissolve 0.1 g in ethanol (96 per cent) and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (10:90 *V/V*).

Test solution Dissolve 0.20 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Use a freshly prepared solution. Dissolve 10.0 mg of *desmethylmetrifonate CRS* (impurity A) in the solvent mixture and dilute to 20.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 5.0 ml with the solvent mixture.

Reference solution (b) Dissolve 0.10 g of *dichlorvos R* (impurity B) in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (c) Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (d) Use a freshly prepared solution. Mix 1.0 ml of reference solution (a), 1.0 ml of reference solution (b) and 0.025 ml of the test solution.

Reference solution (e) Dilute 4.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

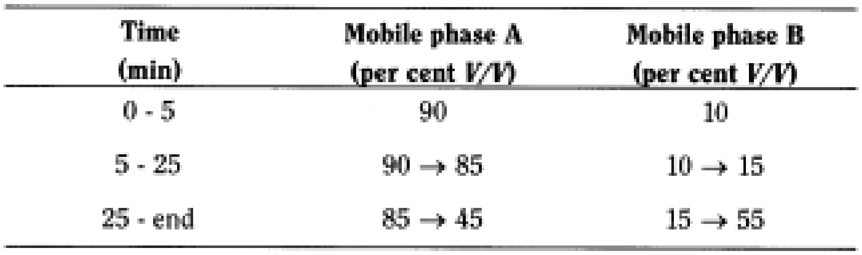
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: 1.36 g/l solution of *potassium dihydrogen phosphate R*, previously adjusted to pH 2.9 with *phosphoric acid R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 25	90 → 85	10 → 15
25 - end	85 → 45	15 → 55

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µl.

Run time 3 times the retention time of metrifonate.

Elution order Impurity A, metrifonate, impurity B.

System suitability Reference solution (d):

— *resolution*: minimum 3.0 between the peaks due to impurity A and metrifonate and minimum 4.5 between the peaks due to metrifonate and impurity B.

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *sum of impurities other than A and B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.04 per cent).

Chlorides

Maximum 500 ppm.

Dissolve 5.00 g in 30 ml of *ethanol (96 per cent) R* and add a mixture of 15 ml of *nitric acid R* and 100 ml of *water R*. Titrate with *0.01 M silver nitrate* determining the end-point potentiometrically (*2.2.20*), using a silver electrode.

1 ml of *0.01 M silver nitrate* is equivalent to 0.3546 mg of Cl.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*)

Maximum 0.3 per cent, determined on 3.000 g.

ASSAY

Dissolve 0.300 g in 30 ml of *ethanol (96 per cent) R*. Add 10 ml of *ethanolamine R* and allow to stand for 1 h at 20-22 °C. Add a chilled mixture of 15 ml of *nitric acid R* and 100 ml of *water R* maintaining the temperature of the mixture at 20-22 °C. Maintain at that temperature and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*), using a silver electrode.

Calculate the percentage content of $C_4H_8Cl_3O_4P$, taking into account the content of chloride and using the following expression:

$$\left[\frac{V_{\mathrm{P}}}{M_{\mathrm{P}}} - \frac{V_{\mathrm{Cl}} \times 0.1}{M_{\mathrm{Cl}}}\right] \times 25.74 \times 0.1$$

V_{P} = volume of silver nitrate used in the assay, in millilitres,

M_{P} = mass of substance used in the assay, in grams,

V_{Cl} = volume of silver nitrate used in the test for chlorides, in millilitres,

M_{Cl} = mass of substance used in the test for chlorides, in grams.

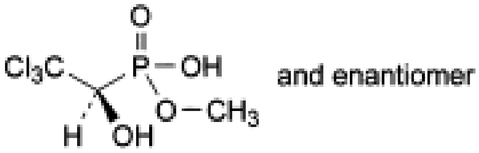
STORAGE

Protected from light.

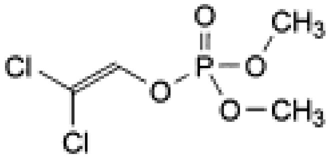
IMPURITIES

Specified impurities

A, B.



A. methyl (*RS*)-(2,2,2-trichloro-1-hydroxyethyl)phosphonate acid (desmethylmetrifonate),



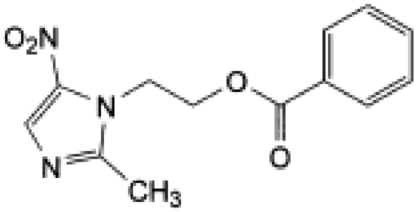
B. 2,2-dichloroethenyl dimethyl phosphate (dichlorvos).

Ph Eur

Metronidazole Benzoate

General Notices

(Ph Eur monograph 0934)



$C_{13}H_{13}N_3O_4$ 275.3 *13182-89-3*

Action and use

Imidazole antibacterial.

Preparation

Metronidazole Oral Suspension

Ph Eur

DEFINITION

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl benzoate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder or flakes.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, slightly soluble in alcohol.

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Melting point (*2.2.14*): 99 °C to 102 °C.

B. Dissolve 0.100 g in a 103 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid. Dilute 1.0 ml of the solution to 100.0 ml with a 103 g/l solution of *hydrochloric acid R*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows 2 absorption maxima, at 232 nm and 275 nm. The specific absorbance at the absorption maximum at 232 nm is 525 to 575.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of metronidazole benzoate*.

D. To about 10 mg add about 10 mg of *zinc powder R*, 1 ml of *water R* and 0.3 ml of *hydrochloric acid R*. Heat on a water-bath for 5 min and cool. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution GY_3 (*2.2.2, Method II*).

Dissolve 1.0 g in *dimethylformamide R* and dilute to 10 ml with the same solvent.

Acidity

Dissolve 2.0 g in a mixture of 20 ml of *dimethylformamide R* and 20 ml of *water R*, previously neutralised with *0.02 M hydrochloric acid* or *0.02 M sodium hydroxide* using 0.2 ml of *methyl red solution R*. Not more than 0.25 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mix 45 volumes of mobile phase B and 55 volumes of mobile phase A.

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 5.0 mg of *metronidazole CRS*, 5.0 mg of *2-methyl-5-nitroimidazole R* and 5.0 mg of *benzoic acid R* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Column:

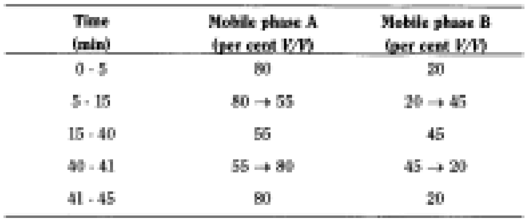
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *spherical di-isobutyloctadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 180 m2/g, a pore size of 8 nm and a carbon loading of 10 per cent.

Mobile phase:

— *mobile phase A*: 1.5 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.2 with *phosphoric acid R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 5	80	20
5 - 15	80 → 55	20 → 45
15 - 40	55	45
40 - 41	55 → 80	45 → 20
41 - 45	80	20

Flow rate 1 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µl.

Relative retention With reference to metronidazole benzoate (retention time = about 20 min): impurity B = about 0.17; impurity A = about 0.20; impurity C = about 0.7.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and impurity B.

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 80 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 27.53 mg of $C_{13}H_{13}N_3O_4$.

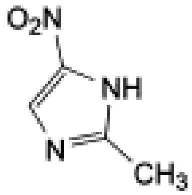
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C.*

A. metronidazole,



B. 2-methyl-5-nitroimidazole,

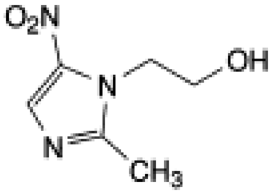
C. benzoic acid.

Ph Eur

Metronidazole

General Notices

(Ph Eur monograph 0675)



$C_6H_9N_3O_3$ 171.2 *443-48-1*

Action and use

Imidazole antibacterial.

Preparations

Metronidazole Gel

Metronidazole Intravenous Infusion

Metronidazole Suppositories

Metronidazole Tablets

Ph Eur

DEFINITION

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethanol.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder.

Solubility

Slightly soluble in water, in acetone, in alcohol and in methylene chloride.

IDENTIFICATION

First identification   *C.*

Second identification   *A, B, D.*

A. Melting point (*2.2.14*): 159 °C to 163 °C.

B. Dissolve 40.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 277 nm and a minimum at 240 nm. The specific absorbance at the maximum is 365 to 395.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation   Discs.

Comparison   *metronidazole CRS.*

D. To about 10 mg add about 10 mg of *zinc powder R*, 1 ml of *water R* and 0.25 ml of *dilute hydrochloric acid R*. Heat on a water-bath for 5 min. Cool. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method II*).

Dissolve 1.0 g in *1 M hydrochloric acid* and dilute to 20 ml with the same acid.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions protected from light.*

Test solution   Dissolve 0.05 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a)   Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase and dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b)   Dissolve 5.0 mg of *metronidazole impurity A CRS* in the mobile phase, add 10.0 ml of the test solution and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml to 100.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase   Mix 30 volumes of *methanol R* and 70 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R*,

Flow rate   1 ml/min.

Detection   Spectrophotometer at 315 nm.

Injection   10 µl.

Run time   3 times the retention time of metronidazole.

Relative retention   With reference to metronidazole (retention time = about 7 min): impurity A = about 0.7.

System suitability Reference solution (b):

- *resolution*: minimum of 2.0 between the peaks due to metronidazole and to impurity A.

Limits:

- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

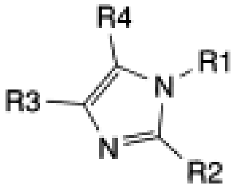
Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 17.12 mg of $C_6H_9N_3O_3$.

STORAGE

Protected from light.

IMPURITIES



A. R1 = R4 = H, R2 = CH_3, R3 = NO_2: 2-methyl-4-nitroimidazole,

B. R1 = R2 = R4 = H, R3 = NO_2: 4-nitroimidazole,

C. R1 = CH_2-CH_2-OH, R2 = R4 = H, R3 = NO_2: 2-(4-nitro-1*H*-imidazol-1-yl)ethanol,

D. R1 = CH_2-CH_2-OH, R2 = R3 = H, R4 = NO_2: 2-(5-nitro-1*H*-imidazol-1-yl)ethanol,

E. R1 = CH_2-CH_2-OH, R2 = CH_3, R3 = NO_2, R4 = H: 2-(2-methyl-4-nitro-1*H*-imidazol-1-yl) ethanol,

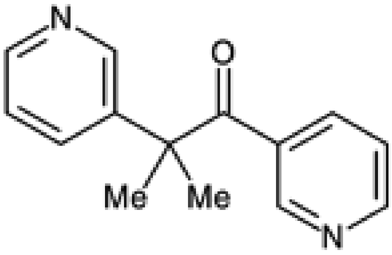
F. R1 = CH_2-CH_2-O-CH_2-CH_2-OH, R2 = CH_3, R3 = H, R4 = NO_2: 2-[2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy]ethanol,

G. R1 = CH_2-CO_2H, R2 = CH_3, R3 = H, R4 = NO_2: 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetic acid.

Ph Eur

Metyrapone

General Notices



$C_{14}H_{14}N_2O$ 226.3 *54-36-4*

Action and use

11-Beta-hydroxylase inhibitor; inhibition of the formation of corticosteroids.

Preparation

Metyrapone Capsules

DEFINITION

Metyrapone is 2-methyl-1,2-di(3-pyridyl)-propan-1-one. It contains not less than 97.0% and not more than 103.0% of $C_{14}H_{14}N_2O$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to light amber, crystalline powder.

Sparingly soluble in *water*; freely soluble in *ethanol (96%)*. It dissolves in dilute mineral acids.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with *reference spectrum 1* of metyrapone *(RS 230)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.1M *hydrochloric acid* exhibits a maximum only at 260 nm. The *absorbance* at 260 nm is about 1.0.

C. To 5 ml of a 1% w/v solution in 1M *sulphuric acid* add 0.2 ml of *potassium tetraiodomercurate solution*. A cream precipitate is produced.

TESTS

Melting point

50° to 53°, Appendix V A.

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 90 volumes of *propan-2-ol*, 5 volumes of 13.5M *ammonia* and 5 volumes of *water* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions of the substance being examined in *dichloromethane* containing (1) 5.0% w/v and (2) 0.010% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 6 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

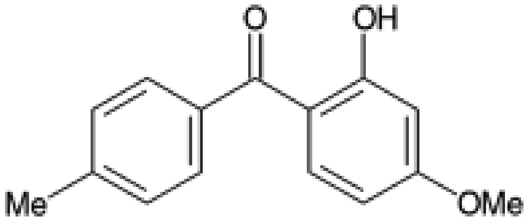
Carry out the following procedure protected from light. Dissolve 0.1 g in sufficient 0.1M *hydrochloric acid* to produce 100 ml. Dilute 5 ml to 50 ml with 0.1M *hydrochloric acid* and dilute 5 ml of this solution to 50 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{14}H_{14}N_2O$ taking 500 as the value of A(1%, 1 cm) at the maximum at 260 nm.

STORAGE

Metyrapone should be protected from light.

Mexenone

General Notices



$C_{15}H_{14}O_3$ 242.3 *1641-17-4*

Action and use

Sunscreen.

Preparation

Mexenone Cream

DEFINITION

Mexenone is 2-hydroxy-4-methoxy-4′-methylbenzophenone. It contains not less than 97.0% and not more than 103.0% of $C_{15}H_{14}O_3$, calculated with reference to the dried substance.

CHARACTERISTICS

A pale yellow, crystalline powder.

Practically insoluble in *water*; freely soluble in *acetone*; sparingly soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mexenone *(RS 232)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.0015% w/v solution in *methanol* exhibits three maxima, at 243, 287 and 325 nm. The *absorbances* at the maxima are about 0.53, about 0.90 and about 0.66, respectively.

C. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to the spot in the chromatogram obtained with solution (4).

TESTS

Melting point

99° to 102°, Appendix V A.

Iron

Ignite 1.0 g with 1 g of *anhydrous sodium carbonate*, cool, dissolve the residue in 5 ml of *hydrochloric acid* and dilute to 40 ml with *water*. 10 ml of the solution complies with the *limit test for iron*, Appendix VII (40 ppm).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 100 volumes of *toluene* and 10 volumes of *butan-2-one* as the mobile phase. Apply separately to the plate 5 µl of each of four solutions in *butan-2-one* containing (1) 10% w/v of the substance being examined, (2) 0.20% w/v of the substance being examined, (3) 0.10% w/v of the substance being examined and (4) 0.20% w/v of *mexenone BPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

Loss on drying

When dried to constant weight at 60° at a pressure not exceeding 3.5 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

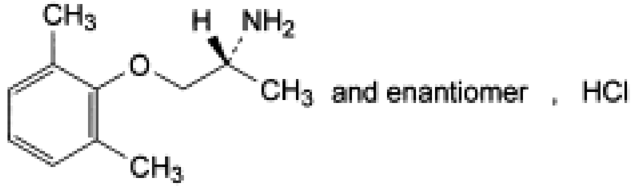
ASSAY

Dissolve 80 mg in sufficient *methanol* to produce 100 ml. Dilute 10 ml of the solution to 100 ml with *methanol* and further dilute 10 ml of this solution to 100 ml with *methanol*. Measure the *absorbance* of the resulting solution at the maximum at 287 nm, Appendix II B, and calculate the content of $C_{15}H_{14}O_3$ taking 640 as the value of A(1%, 1 cm) at the maximum at 287 nm.

Mexiletine Hydrochloride

General Notices

(Ph Eur monograph 1029)



$C_{11}H_{17}NO,HCl$ 215.7 *5370-01-4*

Action and use

Class I antiarrhythmic.

Preparations

Mexiletine Capsules

Mexiletine Injection

Ph Eur

DEFINITION

(2*RS*)-1-(2,6-Dimethylphenoxy)propan-2-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mexiletine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Dilute 1.5 ml of solution S (see Tests) to 15 ml with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dilute 5 ml of solution S to 10 ml with *water R*.

pH (*2.2.3*)

4.0 to 5.5 for solution S.

Impurity D

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.500 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve the content of a vial of *mexiletine impurity D CRS* in 4.0 ml of *methanol R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *methanol R*.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 5.0 ml with *methanol R*.

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 5.0 ml with reference solution (b).

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R*, *alcohol R*, *acetone R*, *toluene R* (3:7:45:45 *V/V/V/V*).

Application 5 µl of the test solution and reference solutions (c) and (d).

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *ninhydrin solution R3* and heat at 100-105 °C for 15 min or until the spots appear.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Limit:

- *impurity D*: any spot corresponding to impurity D in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve the content of a vial of *mexiletine impurity C CRS* in the mobile phase and transfer the solution quantitatively to a volumetric flask containing 16.0 mg of *2,6-dimethylphenol R*. Dilute to 20.0 ml with the mobile phase. Mix 1.0 ml of this solution with 2.0 ml of reference solution (a) and dilute the mixture to 100.0 ml with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 65 volumes of *methanol R2* and 35 volumes of a solution prepared as follows: dissolve 11.5 g of *anhydrous sodium acetate R* in 500 ml of *water R*, add 3.2 ml of *glacial acetic acid R*, mix and allow to cool; adjust to pH 4.8 with *glacial acetic acid R* and dilute to 1000 ml with *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 262 nm.

Injection 20 µl.

Run time 5.5 times the retention time of mexiletine.

Relative retention With reference to mexiletine (retention time = about 4 min): impurity C = about 0.7; impurity A = about 1.8.

System suitability Reference solution (b):

- *resolution*: minimum 5.0 between the peaks due to mexiletine and impurity C.

Limits:
- *impurity A*: not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *impurity C*: not more than 20 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *any other impurity*: for each impurity, not more than 0.5 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than 2.5 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.25 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10* *ppm Pb) R.*

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

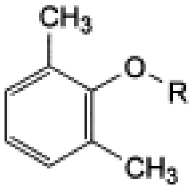
Dissolve 0.150 g in 50 ml of a mixture of equal volumes of *anhydrous acetic acid R* and *acetic anhydride R.* Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*) and completing the titration within 2 min.

1 ml of *0.1 M perchloric acid* is equivalent to 21.57 mg of $C_{11}H_{18}ClNO$.

IMPURITIES

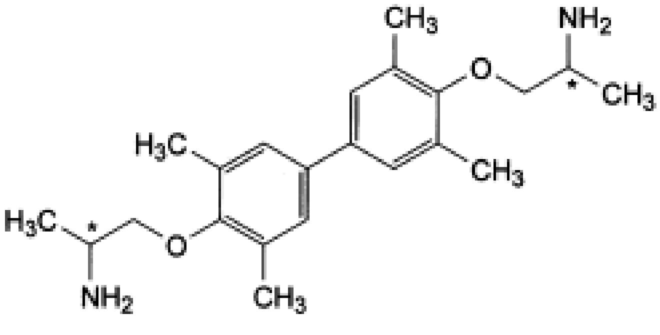
Specified impurities *A, C, D.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B.*

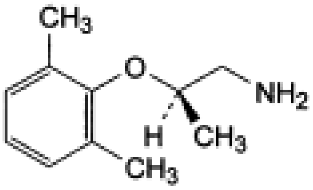


A. R = H: 2,6-dimethylphenol,

B. R = CH_2-CO-CH_3: 1-(2,6-dimethylphenoxy)propan-2-one,



C. 1,1′-[(3,3′,5,5′-tetramethylbiphenyl-4,4′-diyl)bisoxy]dipropan-2-amine,


and enantiomer

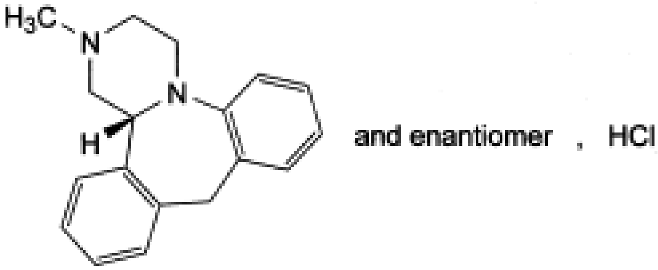
D. (2*RS*)-2-(2,6-dimethylphenoxy)propan-1-amine.

Ph Eur

Mianserin Hydrochloride

General Notices

(Ph Eur monograph 0846)



$C_{18}H_{20}N_2$,HCl 300.8 *21535-47-7*

Action and use

Monoamine reuptake inhibitor; tetracyclic antidepressant.

Preparation

Mianserin Tablets

Ph Eur

DEFINITION

Mianserin hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*RS*)-2-methyl-1,2,3,4,10,14b- hexahydrodibenzo[*c,f*]pyrazino [1,2-*a*]azepine hydrochloride, calculated with reference to the dried substance.

CHARACTERS

White or almost white, crystalline powder or crystals, sparingly soluble in water, soluble in methylene chloride, slightly soluble in alcohol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 50.0 mg in *water R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *water R*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 279 nm. The specific absorption at the maximum is 64 to 72.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum

obtained with *mianserin hydrochloride CRS*. Examine the substances as discs prepared using *potassium chloride R*. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *mianserin hydrochloride CRS* in *methylene chloride R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *mianserin hydrochloride CRS* and 10 mg of *cyproheptadine hydrochloride CRS* in *methylene chloride R* and dilute to 5 ml with the same solvent.

Apply to the plate 2 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *diethylamine R,* 20 volumes of *ether R* and 75 volumes of *cyclohexane R*. Examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. The pH of the solution is 4.0 to 5.5.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.20 g of the substance to be examined in a mixture of 1 volume of *ammonia R* and 4 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dilute 1 ml of the test solution to 200 ml with a mixture of 1 volume of *ammonia R* and 4 volumes of *methanol R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 25 ml with a mixture of 1 volume of *ammonia R* and 4 volumes of *methanol R.*

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R* and 90 volumes of *methylene chloride R*. Dry the plate in a current of cold air. Expose the plate to iodine vapour for 20 min. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at 65 °C at a pressure not exceeding 700 Pa for 3 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

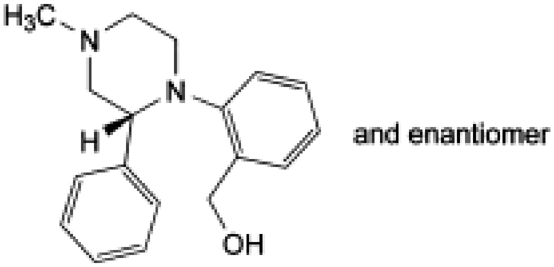
Dissolve 0.200 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

I ml of *0.1 M sodium hydroxide* is equivalent to 30.08 mg of $C_{18}H_{21}ClN_2$.

STORAGE

Store protected from light.

IMPURITIES



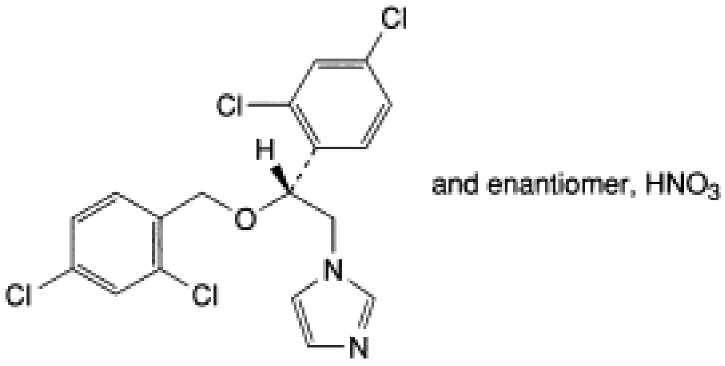
A. [2-[(2*RS*)-4-methyl-2-phenylpiperazin-1-yl]phenyl]methanol.

Ph Eur

Miconazole Nitrate

General Notices

(Ph Eur monograph 0513)



$C_{18}H_{14}Cl_4N_2O,HNO_3$ 479.1 *22832-87-7*

Action and use

Antifungal azole.

Preparation

Miconazole Cream

Miconazole and Hydrocortisone Cream

Miconazole and Hydrocortisone Acetate Cream

Miconazole and Hydrocortisone Ointment

Ph Eur

DEFINITION

1-[(2*RS*)-2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point (*2.2.14*): 178 °C to 184 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Comparison *miconazole nitrate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 5 ml with the mobile phase.

Reference solution (a) Dissolve 30 mg of *miconazole nitrate CRS* in the mobile phase and dilute to 5 ml with the mobile phase.

Reference solution (b) Dissolve 30 mg of *miconazole nitrate CRS* and 30 mg of *econazole nitrate CRS* in the mobile phase, then dilute to 5 ml with the mobile phase.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *ammonium acetate solution R*, *dioxan R, methanol R* (20:40:40 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives the reaction of nitrates (*2.3.1*).

TESTS

Solution S

Dissolve 0.1 g in *methanol R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of *miconazole nitrate CRS* and 2.5 mg of *econazole nitrate CRS* in the mobile phase, then dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve 6.0 g of *ammonium acetate R* in a mixture of 300 ml of *acetonitrile R*, 320 ml of *methanol R* and 380 ml of *water R*.

Flow rate 2 ml/min.

Detection Spectrophotometer at 235 nm.

Equilibration With the mobile phase for about 30 min.

Injection 10 µl.

Run time 1.2 times the retention time of miconazole.

Retention time Econazole = about 10 min; miconazole = about 20 min.

System suitability Reference solution (a):

- *resolution*: minimum 10 between the peaks due to econazole and miconazole; if necessary, adjust the composition of the mobile phase.

Limits:
- *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the nitrate ion.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 75 ml of *anhydrous acetic acid R*, with slight heating if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 47.91 mg of $C_{18}H_{15}Cl_4N_3O_4$.

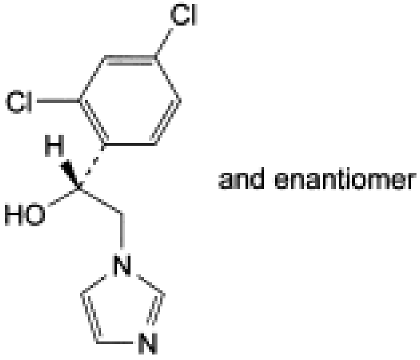
STORAGE

Protected from light.

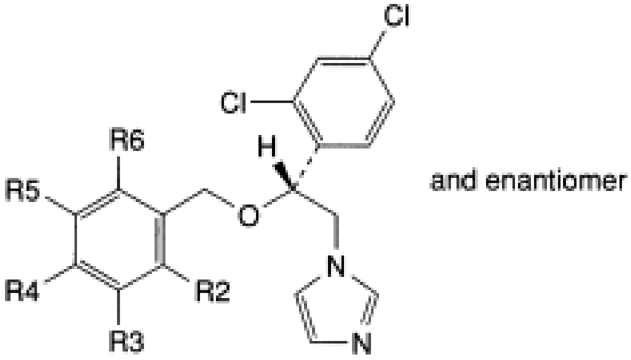
IMPURITIES

Specified impurities   A, B, C, D, E, F, G.

Other detectable impurities   (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): H, I.*



A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,



B. R2 = R3 = R5 = R6 = H, R4 = Cl: 1-[(2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethyl]-1*H*-imidazole,

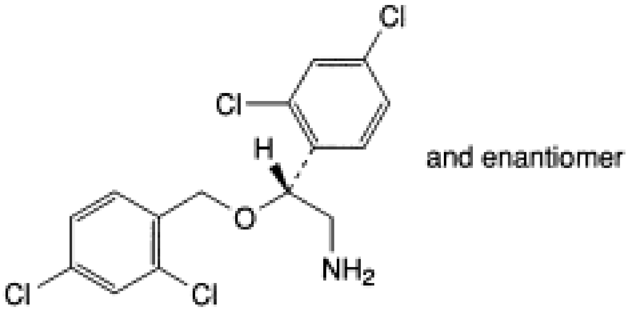
D. R2 = R6 = Cl, R3 = R4 = R5 = H: 1-[(2*RS*)-2-[(2,6-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

F. R2 = R5 = R6 = H, R3 = R4 = Cl: 1-[(2*RS*)-2-[(3,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

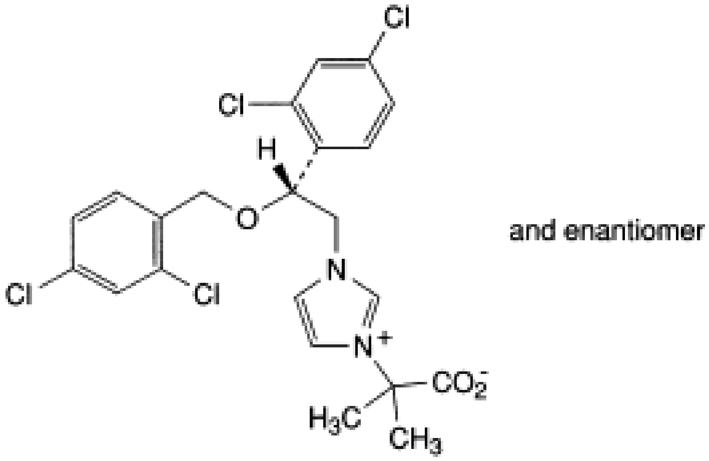
G. R2 = R5 = Cl, R3 = R4 = R6 = H: 1-[(2*RS*)-2-[(2,5-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

H. R2 = R3 = R4 = R5 = R6 = H: 1-[(2*RS*)-2-benzyloxy-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

I. R2 = Cl, R3 = R4 = R5 = R6 = H: 1-[(2*RS*)-2-[(2-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethyl]-1*H*-imidazole,


Cl
Cl
H
O
NH2
Cl
Cl
and enantiomer

C. (2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethanamine,


Cl
Cl
H
O
N
N+
CO2-
H3C
CH3
Cl
Cl
and enantiomer

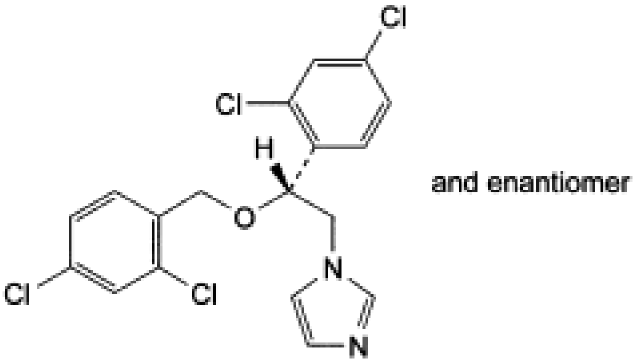
E. 2-[1-[(2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazol-3-io]-2-methylpropanoate.

Ph Eur

Miconazole

General Notices

(Ph Eur monograph 0935)



$C_{18}H_{14}Cl_4N_2O$ 416.1 *22916-47-8*

Action and use

Antifungal azole.

Preparation

Miconazole Oromucosal Gel

Ph Eur

DEFINITION

1-[(2*RS*)-2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point (*2.2.14*): 83 °C to 87 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Comparison *miconazole CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 5 ml with the mobile phase.

Reference solution (a) Dissolve 30 mg of *miconazole CRS* in the mobile phase and dilute to 5 ml with the mobile phase.

Reference solution (b) Dissolve 30 mg of *miconazole CRS* and 30 mg of *econazole nitrate CRS* in the mobile phase and dilute to 5 ml with the mobile phase.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *ammonium acetate solution R*, *dioxan R*, *methanol R* (20:40:40 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.1 g in *methanol R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of *miconazole CRS* and 2.5 mg of *econazole nitrate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

- *size:* l = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve 6.0 g of *ammonium acetate R* in a mixture of 300 ml of *acetonitrile R*, 320 ml of *methanol R* and 380 ml of *water R.*

Flow rate 2 ml/min.

Detection Spectrophotometer at 235 nm.

Equilibration With the mobile phase for about 30 min.

Injection 10 µl.

Run time 1.2 times the retention time of miconazole.

Retention time Econazole = about 10 min; miconazole = about 20 min.

System suitability Reference solution (a):

- *resolution*: minimum 10 between the peaks due to econazole and miconazole; if necessary, adjust the composition of the mobile phase.

Limits:

- *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit:* 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Using 0.2 ml of *naphtholbenzein solution R* as indicator, titrate with

0.1 M perchloric acid until the colour changes from orange-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 41.61 mg of $C_{18}H_{14}Cl_4N_2O$.

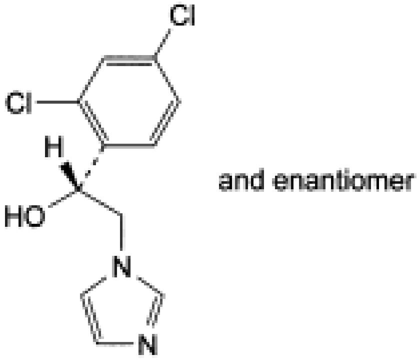
STORAGE

Protected from light.

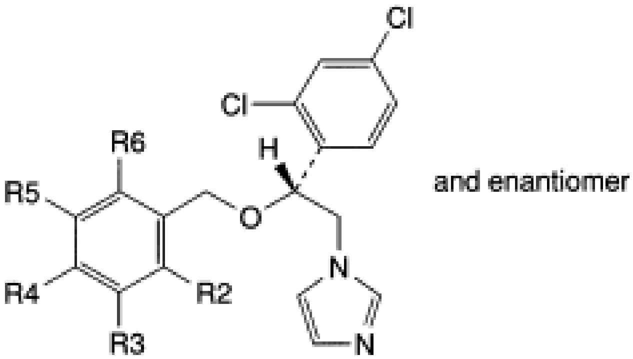
IMPURITIES

Specified impurities A, B, C, D, E, F, G.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): H, I.*



A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,



B. R2 = R3 = R5 = R6 = H, R4 = Cl: 1-[(2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethyl]-1*H*-imidazole,

D. R2 = R6 = Cl, R3 = R4 = R5 = H: 1-[(2*RS*)-2-[(2,6-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

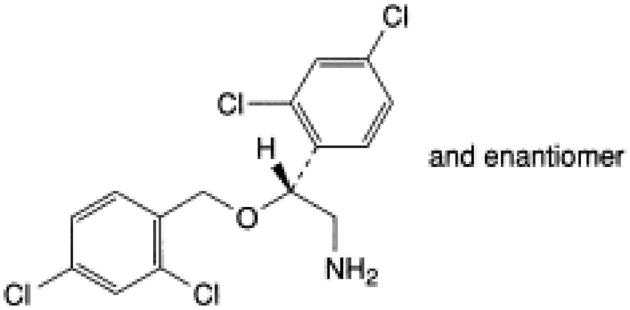
F. R2 = R5 = R6 = H, R3 = R4 = Cl: 1-[(2*RS*)-2-[(3,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

G. R2 = R5 = Cl, R3 = R4 = R6 = H: 1-[(2*RS*)-2-[(2,5-dichlorobenzyl)oxy]-2-(2,4-

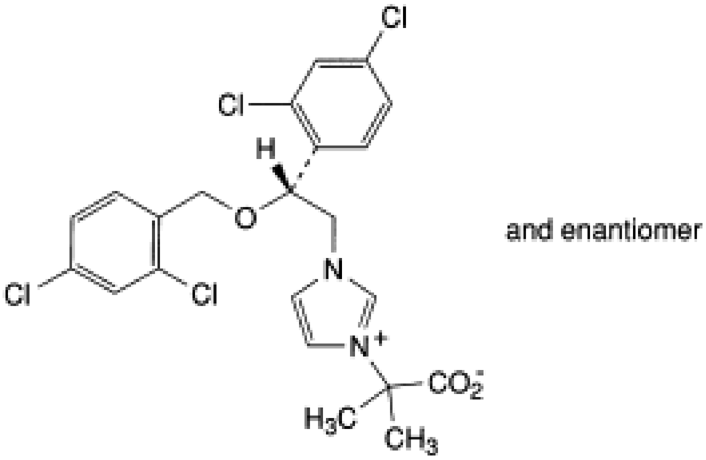
dichlorophenyl)ethyl]-1*H*-imidazole,

H. R2 = R3 = R4 = R5 = R6 = H: 1-[(2*RS*)-2-benzyloxy-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

I. R2 = Cl, R3 = R4 = R5 = R6 = H: 1-[(2*RS*)-2-[(2-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethyl]-1*H*-imidazole,


Cl
Cl
H
O
NH2
Cl
Cl
and enantiomer

C. (2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethanamine,


Cl
Cl
H
O
N
N+
CO2-
H3C
CH3
Cl
Cl
and enantiomer

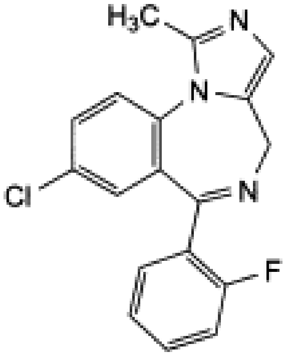
E. 2-[1-[(2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazol-3-io]-2-methylpropanoate.

Ph Eur

Midazolam

General Notices

(Ph Eur monograph 0936)



$C_{18}H_{13}ClFN_3$ 325.8 *59467-70-8*

Action and use

Benzodiazepine.

Preparation

Midazolam Injection

Ph Eur

DEFINITION

8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent), soluble in methanol.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D, E.*

A. Melting point (*2.2.14*): 161 °C to 164 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *midazolam CRS.*

C. Examine the chromatograms obtained in the test for impurity C.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

D. Mix 90 mg with 0.30 g of *anhydrous sodium carbonate R* and ignite in a crucible until an almost white residue is obtained (normally in less than 5 min). Allow to cool and dissolve the residue in 5 ml of *dilute nitric acid R*. Filter (the filtrate is also used in identification test E). Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank solution is red.

E. To 1 ml of the filtrate obtained in identification test D add 1 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.1 g in *0.1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Reference solution (b) Dissolve the contents of a vial of *midazolam for system suitability CRS* (containing impurities A, B, E, G and H) in 1.0 ml of *methanol R*.

Column:
— *size:* *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Prepare a solution containing 7.7 g/l of *ammonium acetate R* and 10 ml/l of *tetrabutylammonium hydroxide solution (400 g/l) R* and adjust to pH 5.3 with *glacial acetic acid R*. Mix 44 volumes of this solution with 56 volumes of *methanol R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 2.5 times the retention time of midazolam.

Relative retention With reference to midazolam (retention time = about 17 min): impurity I = about 0.25; impurity J (2 peaks) = about 0.3; impurity D = about 0.4; impurity E = about 0.5; impurity F = about 0.7; impurity A = about 0.9; impurity G = about 1.2; impurity H = about 1.9; impurity B = about 2.2.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 3.0 where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to midazolam.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 2; impurity E = 2; impurity H = 1.7;

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *impurities A, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity C

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.20 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *ethanol (96 per cent) R*.

Reference solution (a) Dissolve the contents of a vial of *midazolam impurity C CRS* in 2.0 ml of *methanol R*.

Reference solution (b) Dissolve 8 mg of *midazolam CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 40 mg of the substance to be examined in 1 ml of reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R*, *water R*, *methanol R*, *ethyl acetate R* (2:15:20:80 *V/V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity C*: any spot due to impurity C in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

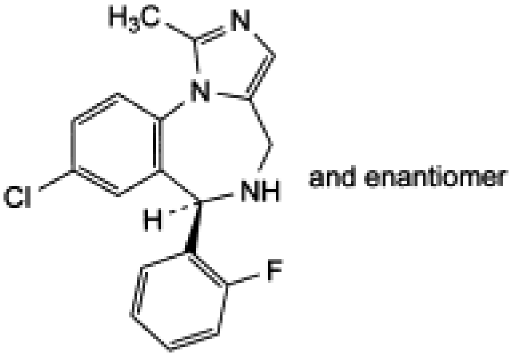
Dissolve 0.120 g in 30 ml of *anhydrous acetic acid R* and add 20 ml of *acetic anhydride R*. Titrate *with 0.1 M perchloric acid* to the 2nd point of inflexion, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 16.29 mg of $C_{18}H_{13}ClFN_3$.

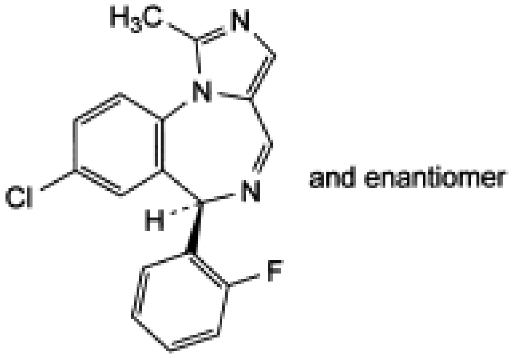
IMPURITIES

Specified impurities A, B, C, G.

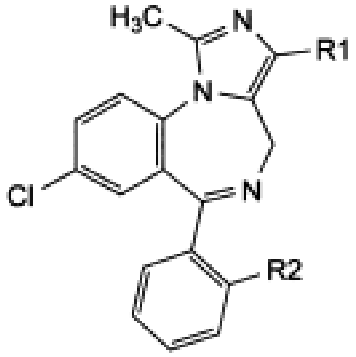
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D, E, F, H, I, J.*



A. (6*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-5,6-dihydro-4*H*-imidazo[1,5-a][1,4]benzodiazepine,

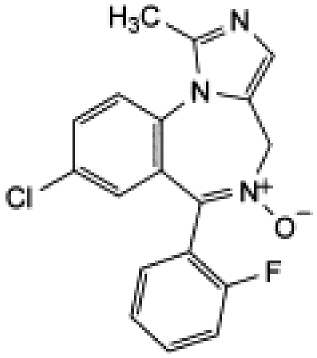


B. (6*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-6*H*-imidazo[1,5-*a*][1,4]benzodiazepine,

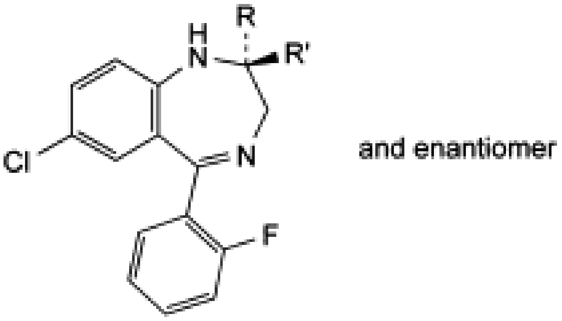


C. R1 = CO_2H, R2 = F: 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4] benzodiazepine-3-carboxylic acid,

G. R1 = R2 = H: 8-chloro-1-methyl-6-phenyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (desfluoromidazolam),



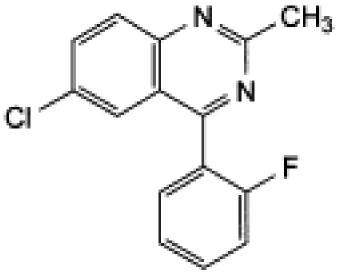
D. 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine 5-oxide,



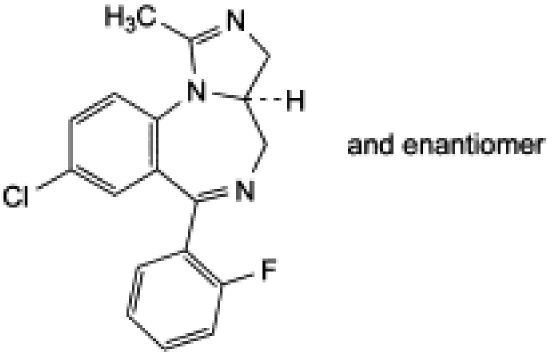
H
N
R
R'
Cl
N
F
and enantiomer

E. R = CH_2-NH_2, R′ = H: [(2*RS*)-7-chloro-5-(2-fluorophenyl)2,3-dihydro-1*H*-1,4-benzodiazepine-2-yl]methanamine,

F. R + R′ = O: 7-chloro-5-(2-fluoropheny)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (1-des[(diethylamino)ethyl]flurazepam),

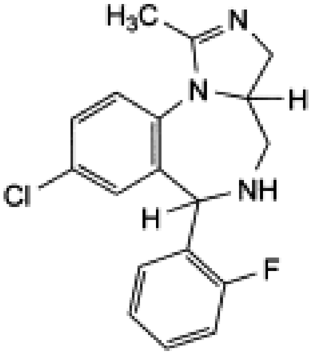


H. 6-chloro-4-(2-fluorophenyl)-2-methylquinazoline,



H_3C
N
N
H
Cl
N
F
and enantiomer

I. (3a*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-3a,4-dihydro-3*H*-imidazo[1,5-*a*][1,4] benzodiazepine,


H3C
N
N
H
Cl
NH
H
F

J. 8-chloro-6-(2-fluorophenyl)-1-methyl-3a,4,5,6-tetrahydro-3*H*-imidazo[1,5-*a*][1,4] benzodiazepine.

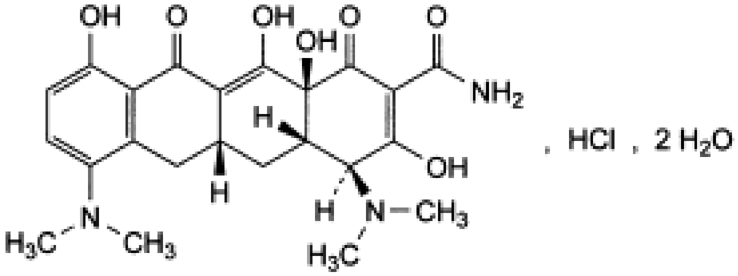
Ph Eur

Minocycline Hydrochloride Dihydrate

General Notices

Minocycline Hydrochloride

(Ph Eur monograph 1030)



$C_{23}H_{27}N_3O_7,HCl,2H_2O$ 530.0 *13614-98-7*

Action and use

Tetracycline antibacterial.

Preparations

Prolonged-release Minocycline Capsules

Minocycline Tablets

Ph Eur

DEFINITION

(4*S*,4a*S*,5a*R*,12a*S*)-4,7-Bis(dimethylamino)-3,10,12,12atetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride dihydrate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.5 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow, hygroscopic, crystalline powder.

Solubility

Sparingly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *minocycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *minocycline hydrochloride CRS* and 5 mg of *oxytetracycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µl

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A bright yellow colour develops. Add 2.5 ml of *water R* to the solution. The solution becomes pale yellow.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.200 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 450 nm using a 1 cm cell is not greater than 0.23.

Dilute 1.0 ml of solution S to 10.0 ml with *water R*.

pH (*2.2.3*)

3.5 to 4.5 for solution S.

Light-absorbing impurities

Carry out the measurement within 1 h of preparing solution S.

The absorbance (*2.2.25*) of solution S measured at 560 nm is not greater than 0.06.

Related substances

Liquid chromatography (*2.2.29*).

Carry out the test protected from bright light. Store the solutions at a temperature of 2-8 ‘C and use them within 3 h of preparation.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b) Dilute 10.0 ml of test solution (a) to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 12.5 mg of *minocycline hydrochloride CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 2.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.2 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (d) Dissolve 10 mg of *minocycline hydrochloride CRS* in 1 ml of *water R*. Boil the solution on a water-bath for 20 min. Dilute to 25 ml with the mobile phase.

Column:

- *size: l* = 0.20 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of a 4 g/l solution of *sodium edetate R*, 27 volumes of *dimethylformamide R* and 50 volumes of a 28 g/l solution of *ammonium oxalate R*, and adjust to pH 7.0 with *tetrabutylammonium hydroxide solution (104 g/l) R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl of test solution (a) and reference solutions (a), (b), (c) and (d).

Run time 1.5 times the retention time of minocycline.

System suitability:

- *resolution*: minimum 2.0 between the peaks due to impurity A and minocycline in the chromatogram obtained with reference solution (d);
- *number of theoretical plates*: minimum 15 000, calculated for the peak due to minocycline in the chromatogram obtained with reference solution (a).

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.2 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.2 per cent);
- *total of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Heavy metals (*2.4.8*)

Maximum 50 ppm.

0.5 g complies with test C. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

5.0 per cent to 8.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)

Less than 1.25 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

System suitability:

— *repeatability*: maximum relative standard deviation of the peak area for minocycline of 1.5 per cent after 6 injections of reference solution (a).

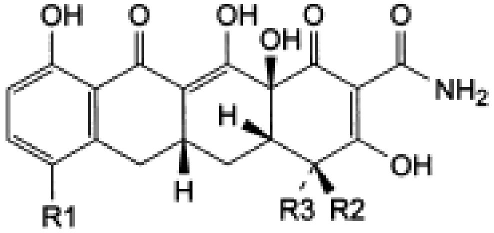
Calculate the percentage content of $C_{23}H_{28}ClN_3O_7$ from the declared content of *minocycline hydrochloride CRS.*

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities *A, B, C, D.*



A. R1 = R3 = $N(CH_3)_2$, R2 = H: (4*R*,4a*S*,5a*R*,12a*S*)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epiminocycline),

B. R1 = R3 = H, R2 = $N(CH_3)_2$: (4*S*,4a*S*,5a*R*,12a*S*)-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (sancycline),

C. R1 = NH-CH_3, R2 = $N(CH_3)_2$, R3 = H: (4*S*,4a*S*,5a*R*,12a*S*)-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-7-(methylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-

carboxamide (7-monodemethylminocycline),

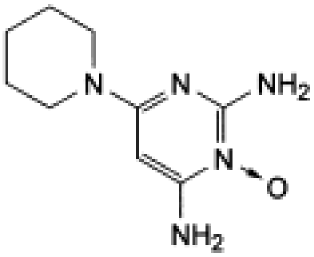
D. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = H: (4*S*,4a*S*,5a*R*,12a*S*)-7-amino-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (7-aminosancycline).

Ph Eur

Minoxidil

General Notices

(Ph Eur monograph 0937)



$C_9H_{15}N_5O$ 209.3 *38304-91-5*

Action and use

Vasodilator; treatment of hypertension; male pattern baldness.

Preparation

Minoxidil Scalp Application

Ph Eur

DEFINITION

6-(Piperidin-1-yl)pyrimidine-2,4-diamine 3-oxide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, soluble in methanol and in propylene glycol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a) Dissolve 20.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid (solution A). Dilute 2.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*.

Test solution (b) Dilute 2.0 ml of solution A to 100.0 ml with *0.1 M sodium hydroxide*.

Spectral range 200-350 nm.

Absorption maxima At 230 nm and 281 nm for test solution (a); at 230 nm, 262 nm and 288 nm for test solution (b).

Specific absorbances at the absorption maxima:
— at 230 nm: 1015 to 1120 for test solution (a); 1525 to 1685 for test solution (b);

— at 262 nm: 485 to 535 for test solution (b);

— at 281 nm: 1060 to 1170 for test solution (a);

— at 288 nm: 555 to 605 for test solution (b).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *minoxidil CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *minoxidil CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *concentrated ammonia R*, *methanol R* (1.5:100 *V/V*).

Application 2 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 1 ml of *methanol R*. Add 0.1 ml of *copper sulphate solution R*. A green colour develops. The solution becomes greenish-yellow on the addition of 0.1 ml of *dilute hydrochloric acid R*.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.5 g in 12.5 ml of *methanol R* and dilute to 25 ml with *water R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of 1 vial of *deoxyminoxidil CRS* (impurity E) with 1 ml of the mobile phase, add 1 ml of the test solution and dilute to 5 ml with the mobile phase.

Column:
- *size:* *l* = 0.10 m, Ø = 3 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.0 g of *docusate sodium R* in a mixture of 10 ml of *glacial acetic acid R* and 300 ml of *water R*, adjust to pH 3.0 with *perchloric acid R* and add 700 ml of *methanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl.

Run time Twice the retention time of the principal peak.

System suitability Reference solution (b):

- *resolution*: minimum 2.0 between the peaks due to minoxidil and impurity E.

Limits:
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

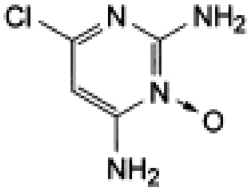
Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 20.93 mg of $C_9H_{15}N_5O$.

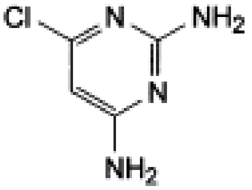
STORAGE

Protected from light.

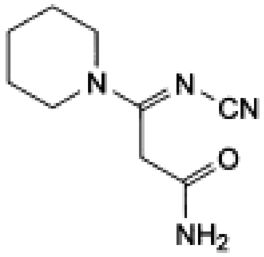
IMPURITIES



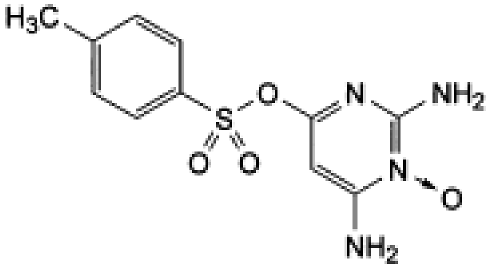
A. 6-chloropyrimidine-2,4-diamine 3-oxide,



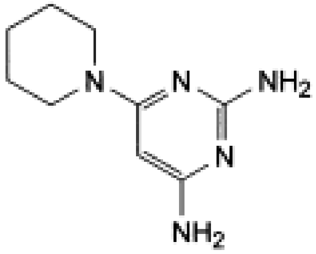
B. 6-chloropyrimidine-2,4-diamine,



C. 3-(cyanoimino)-3-(piperidin-1-yl)propanamide,



D. 6-[[(4-methylphenyl)sulphonyl]oxy]pyrimidine-2,4-diamine 3-oxide,



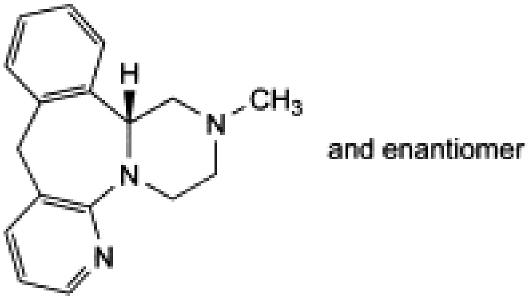
E. 6-(piperidin-1-yl)pyrimidine-2,4-diamine (desoxyminoxidil).

Ph Eur

Mirtazapine

General Notices

(Ph Eur monograph 2338)



$C_{17}H_{19}N_3$ 265.4 *61337-67-5*

Action and use

Inhibitor of 5HT and noradrenaline reuptake; antidepressant.

Ph Eur

DEFINITION

(14b*RS*)-2-Methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, slightly hygroscopic to hygroscopic.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mirtazapine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Optical rotation (*2.2.7*)

- 0.10° to + 0.10° (anhydrous substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, *water R* (50:50 *V/V*).

Buffer solution Dissolve 18.0 g of *tetramethylammonium hydroxide R* in 950 ml of *water R*. While stirring, adjust to pH 7.4 with *phosphoric acid R*, then dilute to 1000 ml with *water R* and mix.

Test solution Dissolve 30 mg of the substance to be examined in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (a) Dissolve 3 mg of *mirtazapine for system suitability CRS* (containing impurities A, B, C, D, E and F) in 2 ml of the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *tetrahydrofuran for chromatography R*, *methanol R*, *acetonitrile R*, buffer solution (7.5:12.5:15:65 *V/V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl.

Run time Twice the retention time of mirtazapine.

Identification of impurities Use the chromatogram supplied with *mirtazapine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E and F.

Relative retention With reference to mirtazapine (retention time = about 16 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.35; impurity D = about 0.4; impurity E = about 1.3; impurity F = about 1.35.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurities E and F.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.3; impurity B = 1.3; impurity F = 0.2;

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

Maximum 3.5 per cent, determined on 1.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 35 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

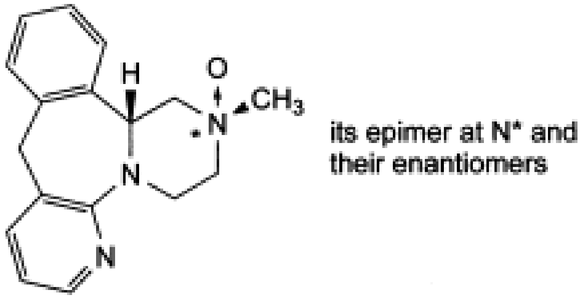
1 ml of *0.1 M perchloric acid* is equivalent to 13.27 mg of $C_{17}H_{19}N_3$.

STORAGE

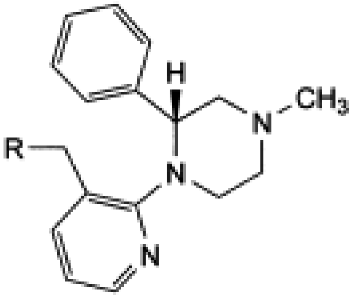
In an airtight container .

IMPURITIES

Specified impurities *A, B, C, D, E, F*.

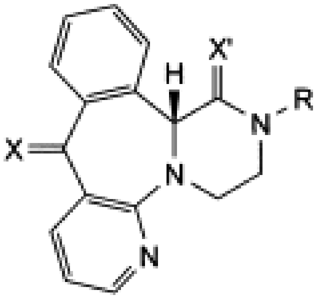


A. (14b*RS*)-2-methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide,



B. R = OH: [2-[(2*RS*)-4-methyl-2-phenylpiperazin-1-yl]pyridin-3-yl]methanol,

E. R = H: (2*RS*)-4-methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine,



C. R = CH3, X = H_2, X′ = O: (14b*RS*)-2-methyl-3,4,10,14b-tetrahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepin-1(2*H*)-one,

D. R = H, X = X′ = H_2: (14b*RS*)-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine,

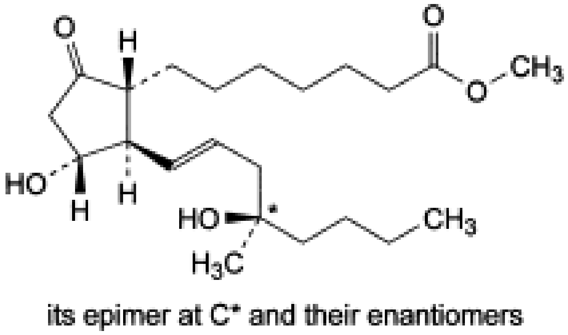
F. R = CH_3, X = O, X′ = H2: (14b*RS*)-2-methyl-1,3,4,14b-tetrahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepin-10(2*H*)-one.

Ph Eur

Misoprostol

General Notices

(Ph Eur monograph 1731)



$C_{22}H_{38}O_5$ 382.5 *59122-46-2*

Action and use

Prostaglandin (PGE_1) analogue.

Ph Eur

DEFINITION

Mixture of methyl 7-[(1*RS*,2*RS*,3*RS*)-3-hydroxy-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1*RS*,2*RS*,3*RS*)-3-hydroxy-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate.

The 4 stereoisomers are present in approximately equal proportions.

Content

96.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless or yellowish, oily liquid, hygroscopic.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent), sparingly soluble in acetonitrile.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison   *misoprostol CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution   Dissolve 10.00 mg of the substance to be examined in *acetonitrile R1* and dilute to 5.0 ml with the same solvent.

Reference solution (a)   Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R1*.

Reference solution (b)   Dilute 1.0 ml of reference solution (a) to 10.0 ml with *acetonitrile R1*.

Reference solution (c)   Dissolve 0.25 mg of *misoprostol impurity A CRS* in reference solution (a) and dilute to 10.0 ml with the same solution.

Reference solution (d)   Dissolve 10.00 mg of *misoprostol CRS* in *acetonitrile R1* and dilute to 5.0 ml with the same solvent.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: spherical partially *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 220 m2/g and a carbon loading of 7 per cent;

— *temperature*: 40 °C.

Mobile phase   Mix 45 volumes of *acetonitrile R1* and 55 volumes of *water R* and add 0.05 volumes of a 24.5 g/l solution of *phosphoric acid R*.

Flow rate   0.75 ml/min.

Detection   Spectrophotometer at 200 nm.

Injection   10 µl.

Run time   3 times the retention time of misoprostol.

Relative retention   With reference to misoprostol (retention time = about 20 min): impurity A = about 0.9; impurity E = about 0.9; impurity B (1st peak) = about 0.9; impurity B (2nd peak) = about 0.95.

System suitability   Reference solution (c):

— *resolution*: minimum 1.9 between the peaks due to impurity A and misoprostol.

Limits:

— *sum of impurities A, B and E*: not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.3 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Diastereoisomers

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 1.0 ml with the mobile phase.

Reference solution Dilute 0.1 ml of the test solution to 10.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 5 volumes of *2-propanol R* and 95 volumes of *heptane R* and add 0.01 volumes of *glacial acetic R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µl.

Run time 1.5 times the retention time of the 1st peak of misoprostol.

Retention time Misoprostol 1st peak = about 19 min; misoprostol 2nd peak = about 21 min.

System suitability Reference solution:

- *resolution*: minimum 2.3 between the 1st peak and the 2nd peak of misoprostol.

Limit:
- *1st peak of misoprostol*: 50 per cent to 55 per cent of the sum of the areas of the 2 peaks due to misoprostol.

Water (*2.5.32*)

Maximum 1.0 per cent.

Use 1.0 ml of a 10 mg/ml solution of the substance to be examined in *methanol R*.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (d).

Calculate the percentage content of $C_{22}H_{38}O_5$ using the declared content of *misoprostol CRS*.

STORAGE

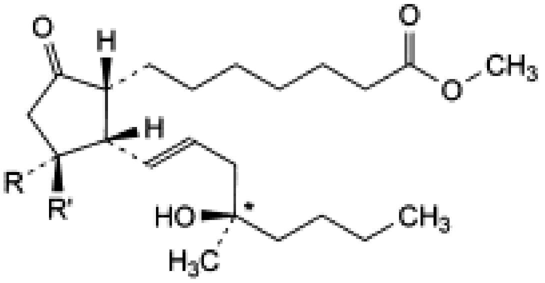
In an airtight container , at - 20 °C.

IMPURITIES

Specified impurities *A, B, E .*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general

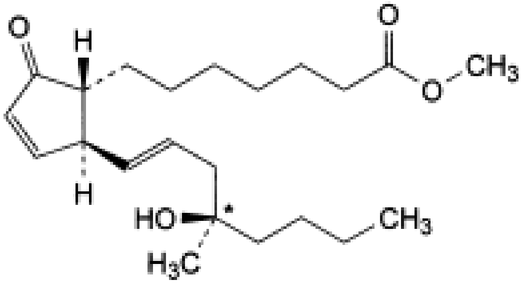
acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C, D.*



its epimer at C* and their enantiomers

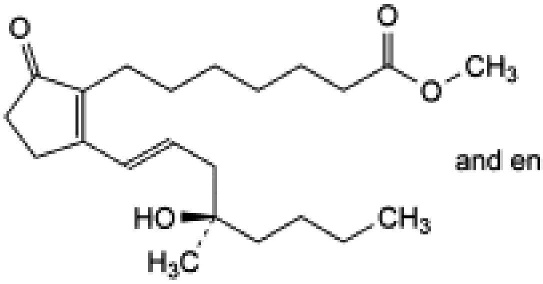
A. R = H, R′ = OH: mixture of methyl 7-[(1*RS*,2*SR*,3*SR*)-3-hydroxy-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1*RS*,2S*R*,3S*R*)-3-hydroxy-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate (8-epimisoprostol),

B. R = OH, R′ = H: mixture of methyl 7-[(1*RS*,2*SR*,3*RS*)-3-hydroxy-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1*RS*,2*SR*,3*RS*)-3-hydroxy-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate (12-epimisoprostol),



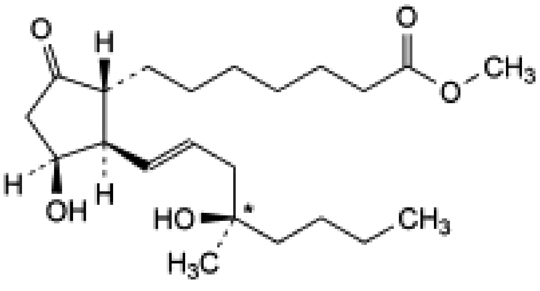
its epimer at C* and their enantiomers

C. mixture of methyl 7-[(1*RS*,2*SR*)-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl]heptanoate and methyl 7-[(1*RS*,2*SR*)-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl]heptanoate (misoprostol A),



and enantiomer

D. methyl 7-[2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-1-enyl]heptanoate (misoprostol B),



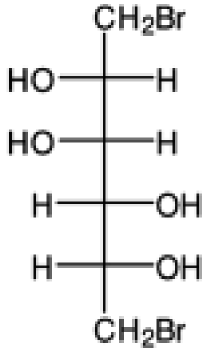
its epimer at C* and their enantiomers

E. mixture of methyl 7-[(1*RS*,2*RS*,3*SR*)-3-hydroxy-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1*RS*,2*RS*,3*SR*)-3-hydroxy-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate (11-epi misoprostol).

Ph Eur

Mitobronitol

General Notices



$C_6H_{12}Br_2O_4$ 308.0 *488-41-5*

Action and use

Cytotoxic.

Preparation

Mitobronitol Tablets

DEFINITION

Mitobronitol is 1,6-dibromo-1,6-dideoxy-D-mannitol. It contains not less than 98.5% and not more than 101.0% of $C_6H_{12}Br_2O_4$ calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline solid.

Slightly soluble in *water*, in *acetone* and in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mitobronitol *(RS 236)*.

B. Dissolve 0.1 g in 10 ml of 1M *sodium hydroxide*, boil, cool, acidify with 2M *nitric acid* and add 1 ml of *silver nitrate solution*. A pale yellow, curdy precipitate is produced.

C. Dissolve 20 mg in 2 ml of a mixture of 1 volume of *periodic acid solution* and 24 volumes of *water*. Add 1 ml of 0.25M *barium chloride* and shake well. A white, flocculent precipitate is produced.

TESTS

Acidity

Shake 2 g with 50 ml of *carbon dioxide-free water* for 15 minutes and filter. 40 ml of the filtrate requires not more than 0.3 ml of 0.02M *sodium hydroxide VS* for neutralisation using *phenolphthalein solution R1* as indicator.

Clarity and colour of solution

A 4.0% w/v solution in *dimethylformamide* is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Heavy metals

1.0 g complies with *limit test C for heavy metals*, Appendix VII (20 ppm). Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

Ionic halide

Shake 0.20 g with 30 ml of *water* for 5 minutes and filter. 15 ml of the filtrate complies with the *limit test for chlorides*, Appendix VII (500 ppm, calculated as Cl).

Loss on drying

When dried at 105° for 2 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.2%, Appendix IX A.

ASSAY

Dissolve 0.2 g in 20 ml of 1M *sodium hydroxide* by heating gently, cool, add 25 ml of 0.1M *silver nitrate VS* and acidify with 5 ml of 5M *nitric acid*. Add 2 ml of *ammonium iron(III) sulphate solution R2* and titrate the excess of silver nitrate with 0.1M *ammonium thiocyanate VS*. Each ml of 0.1M *silver nitrate VS* is equivalent to 15.40 mg of $C_6H_{12}Br_2O_4$.

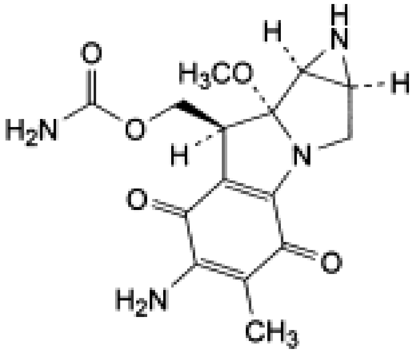
STORAGE

Mitobronitol should be protected from light.

Mitomycin

General Notices

(Ph Eur monograph 1655)



$C_{15}H_{18}N_4O_5$ 334.3 *50-07-7*

Action and use

Antibacterial; cytotoxic.

Ph Eur

DEFINITION

[(1a*S*,8*S*,8a*R*,8b*S*)-6-Amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-yl]methyl carbamate (mitomycin C).

Substance produced by a strain of *Streptomyces caespitotus*.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Blue-violet crystals or crystalline powder.

Solubility

Slightly soluble in water, freely soluble in dimethylacetamide, sparingly soluble in methanol, slightly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mitomycin CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

pH (*2.2.3*)

5.5 to 7.5.

Dissolve 10 mg in 10 ml of *carbon dioxide-free water R*.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 10.0 ml with *methanol R*.

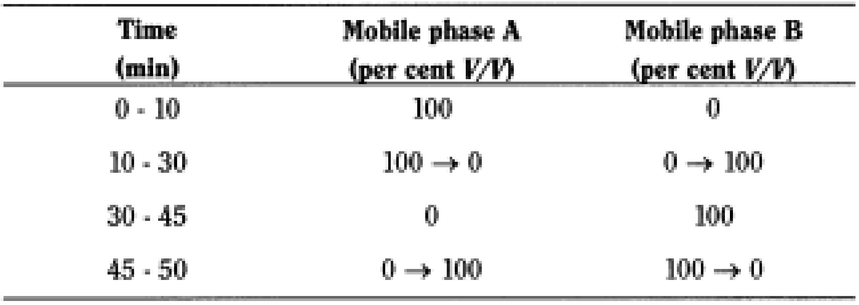
Reference solution (b) Dissolve 10 mg of *cinnamamide R* in *methanol R* and dilute to 10 ml with the same solvent. Mix 2 ml of this solution and 1 ml of the test solution and dilute to 10 ml with *methanol R*.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: *methanol R*, 0.77 g/l solution of *ammonium acetate R* (20:80 *V/V*);
- *mobile phase B*: *methanol R*, 0.77 g/l solution of *ammonium acetate R* (50:50 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 30	100 → 0	0 → 100
30 - 45	0	100
45 - 50	0 → 100	100 → 0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Relative retention With reference to mitomycin (retention time = about 21 min): impurity D = about 0.6; impurity C = about 1.2; impurity A = about 1.3; impurity B = about 1.6.

System suitability Reference solution (b):

— *resolution*: minimum 15.0 between the peaks due to mitomycin and impurity A.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.35;

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 2.5 per cent, determined on 0.30 g.

Bacterial endotoxins (*2.6.14, Method B*)

Less than 10 IU/ mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *dimethylacetamide R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *mitomycin CRS* in *dimethylacetamide R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *cinnamamide R* in *methanol R* and dilute to 20 ml with the same solvent. Mix 2 ml of this solution with 2 ml of reference solution (a).

Column:

— *size:* *l* = 0.30 m, Ø = 3.9 mm,

— *stationary phase*: *end-capped phenylsilyl silica gel for chromatography R* (10 µm) with a specific surface area of 330 m2/g, a carbon loading of 8 per cent and a pore size of 12.5 nm.

Mobile phase Mix 23 volumes of *methanol R*, 77 volumes of a solution containing 2.05 g/l of *ammonium acetate R* and 2.8 ml/l of *dilute acetic acid R*.

Flow rate 2.0 ml/min.

Detection Variable wavelength spectrophotometer capable of operating at 365 nm and 254 nm.

Injection 20 µl.

Run time Twice the retention time of mitomycin.

Relative retention With reference to mitomycin (retention time = about 8 min): impurity A = about 1.2.

System suitability:

— *resolution*: minimum 1.8 between the peaks due to mitomycin and impurity A in the chromatogram obtained with reference solution (b) at 254 nm;

— *symmetry factor*: maximum 1.3 for the principal peak in the chromatogram obtained with reference solution (a) at 365 nm.

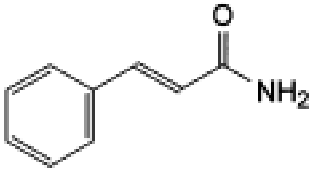
Calculate the percentage content of $C_{15}H_{18}N_4O_5$, from the chromatograms obtained at 365 nm and the declared content of *mitomycin CRS*.

STORAGE

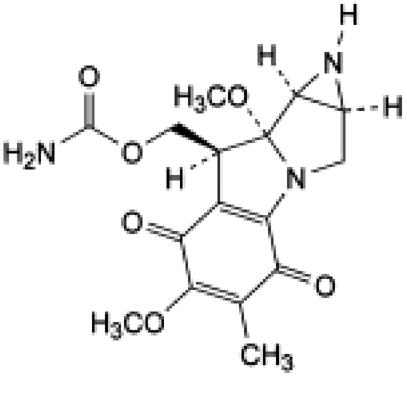
Protected from light.

IMPURITIES

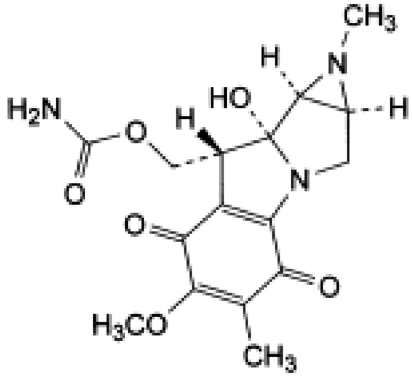
Specified impurities *A, B, C, D.*



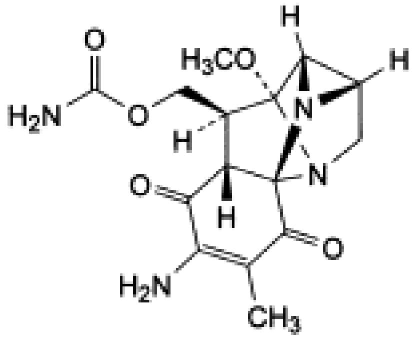
A. (*E*)-3-phenylprop-2-enamide (cinnamamide),



B. [(1a*S*,8*S*,8a*R*,8b*S*)-6,8a-dimethoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-yl]methyl carbamate (mitomycin A),



C. [(1a*S*,8*R*,8a*R*,8b*S*)-8a-hydroxy-6-methoxy-1,5-dimethyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-yl]methyl carbamate (mitomycin B),



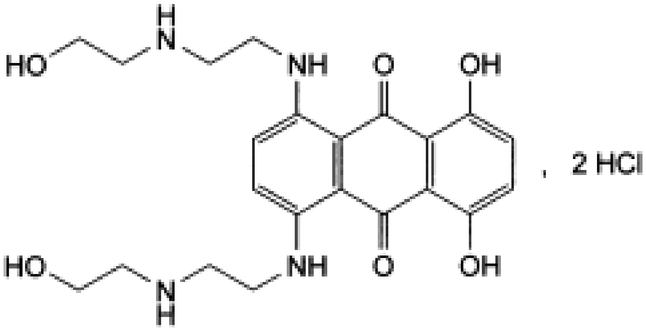
D. [(1*S*,2*S*,4*S*,5*R*,6*S*,6a*R*,10a*S*,11*S*)-8-amino-5-methoxy-9-methyl-7,10-dioxo-2,3,6,6a,7,10-hexahydro-1,2,5-metheno-1*H*,5*H*-imidazo[2,1-i]indol-6-yl]methyl carbamate (albomitomycin C).

Ph Eur

Mitoxantrone Hydrochloride

General Notices

(Ph Eur monograph 1243)



$C_{22}H_{28}N_4O_6,2HCl$ 517.4 *70476-82-3*

Action and use

Cytotoxic.

Preparation

Mitoxantrone Intravenous Infusion

Ph Eur

DEFINITION

1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]anthracene-9,10-dione dihydrochloride.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Dark blue, electrostatic, hygroscopic powder.

Solubility

Sparingly soluble in water, slightly soluble in methanol, practically insoluble in acetone.

CAUTION: Mitoxantrone hydrochloride and impurity A are electrostatic; the use of an *antistatic gun or other suitable method to discharge the solids before weighing or transfer is recommended.*

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 2-3 mg in 1 ml of *methanol R* by warming in a water-bath at 40-50 °C. Evaporate to dryness under a stream of dry nitrogen, warming gently if necessary. Examine the residue.

Comparison *mitoxantrone hydrochloride CRS.*

B. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in about 40 ml of the mobile phase, sonicating if necessary, and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *mitoxantrone hydrochloride CRS* in about 40 ml of the mobile phase, sonicating if necessary, and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Reference solution (c) Dissolve 2.0 mg of *mitoxantrone impurity A CRS* in 1.0 ml of reference solution (a).

Reference solution (d) Dilute 1 ml of reference solution (b) to 10 ml with the mobile phase.

Column:

— *size:* l = 0.30 m, Ø = 3.0 mm;

— *stationary phase*: *phenylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 750 volumes of *water R*, 250 volumes of *acetonitrile R* and 25 volumes of a solution prepared as follows: dissolve 22.0 g of *sodium heptanesulphonate R* in about 150 ml of *water R* and filter through a 0.45 µm filter; wash the filter with *water R* and combine the filtrate and washings; add 32.0 ml of *glacial acetic acid R* and dilute to 250 ml with *water R*.

Flow rate 3 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 50 µl of the test solution and reference solutions (b), (c) and (d).

Run time 3 times the retention time of mitoxantrone.

System suitability Reference solution (c):

— *resolution*: minimum 3.0 between the peaks due to mitoxantrone and impurity A.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Ethanol

Gas chromatography (*2.2.28*).

Internal standard solution Dilute 2.0 ml of *propanol R* to 100 ml with *water R*. Dilute 5.0 ml of this solution to 100 ml with *water R*.

Test solution Mix 0.100 g of the substance to be examined with 2.0 ml of the internal standard solution and dilute to 5.0 ml with *water R*. Place the flask in an ultrasonic bath for 2 min, then shake the flask for 2 min. If necessary, repeat the sonication and shaking until dissolution is complete.

Reference solution Dilute 2.0 ml of *anhydrous ethanol R* to 100.0 ml with *water R*. Dilute 5.0 ml of the solution to 100.0 ml with *water R*. Dilute 10.0 ml of this solution and 10.0 ml of the internal standard solution to 25.0 ml with *water R*.

Column:
— *size:* *l* = 2 m, Ø = 3 mm;

— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R*.

Carrier gas *helium for chromatography R.*

Flow rate 19 ml/min.

Temperature:
— *column*: 120 °C;

— *injection port*: 175 °C;

— *detector*: 210 °C.

Detection Flame ionisation.

Injection 1 µl.

Retention time Ethanol = about 1 min; propanol = about 2 min.

System suitability Reference solution:

— *resolution*: minimum 6 between the peaks due to ethanol and propanol.

Calculate the content of ethanol, taking its density (*2.2.5*) to be 0.790 g/ml at 20 °C.

Limit:
— *ethanol* : maximum 1.6 per cent *m/m*.

Water (*2.5.12*)

Maximum 6.0 per cent, determined on 0.300 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

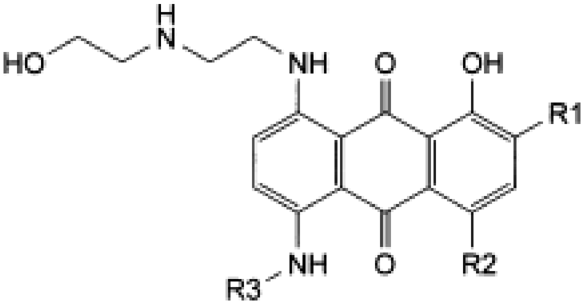
Calculate the percentage content of $C_{22}H_{30}Cl_2N_4O_6$ from the declared content of *mitoxantrone* *hydrochloride CRS.*

STORAGE

In an airtight container .

IMPURITIES

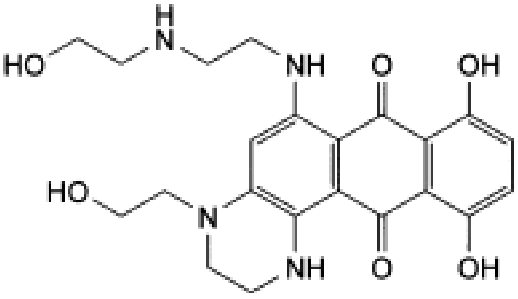
Specified impurities *A, B, C, D.*



A. R1 = R3 = H, R2 = OH: 1-amino-5,8-dihydroxy-4-[[2-[(2-hydroxyethyl)amino]ethyl]amino] anthracene-9,10-dione,

B. R1 = R2 = H, R3 = CH_2-CH_2-NH-CH_2-CH_2OH: 5-hydroxy-1,4-bis[[2-[(2-hydroxyethyl)amino] ethyl]amino]anthracene-9,10-dione,

C. R1 = Cl, R2 = OH, R3 = CH_2-CH_2NH-CH_2-CH_2OH: 2-chloro-1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]anthracene-9,10-dione,



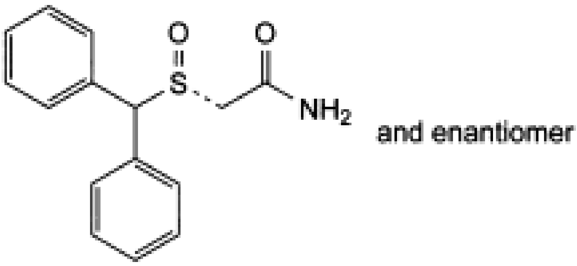
D. 8,11-dihydroxy-4-(2-hydroxyethyl)-6-[[2-[(2-hydroxyethyl)-amino]ethyl]amino]-1,2,3,4-tetrahydronaphtho[2,3-*i*]quinoxaline-7,12-dione.

Ph Eur

Modafinil

General Notices

(Ph Eur monograph 2307)



$C_{15}H_{15}NO_2S$ 273.4 *68693-11-8*

Action and use

Narcolepsy and sleep disorders.

Ph Eur

DEFINITION

2-[(*RS*)-(Diphenylmethyl)sulphinyl]acetamide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble or practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison modafinil CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1*, *water R* (35:65 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in 35 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *modafinil CRS* in 35 ml of *acetonitrile R1* and dilute to 50.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 20.0 ml with the solvent mixture.

Reference solution (c) Add 2.0 ml of the solvent mixture to a vial of *modafinil for system suitability CRS* (containing impurities A, B and C) and sonicate for 10 min.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.3 with *phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time 4 times the retention time of modafinil.

Identification of impurities Use the chromatogram supplied with *modafinil for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C.

Relative retention With reference to modafinil (retention time = about 4 min): impurity A = about 1.3; impurity B = about 1.8; impurity C = about 3.0.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to modafinil.

Limits:

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities B, C*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dissolve 1.0 g in 40 ml of *methanol R*, warming slightly. Add 7.5 ml of *water R*. Allow to cool, then dilute to 50.0 ml with *methanol R*. 12 ml of the solution complies with test B. Prepare the reference solution using 2 ml of *lead standard solution (2 ppm Pb) R*, 8 ml of *methanol R* and 2 ml of the test solution.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

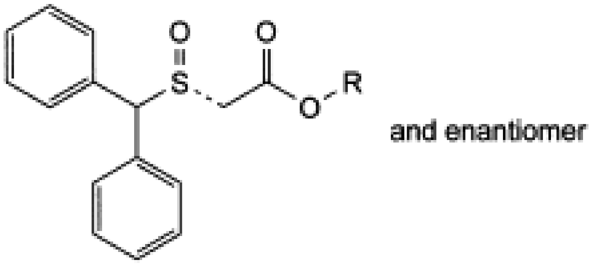
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of modafinil from the declared content of $C_{15}H_{15}NO_2S$ in *modafinil CRS.*

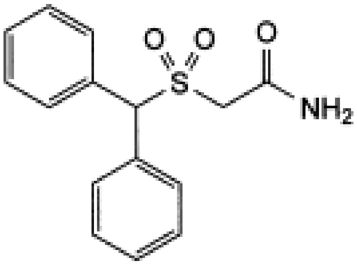
IMPURITIES

Specified impurities *A, B, C.*



A. R = H: [(*RS*)-(diphenylmethyl)sulphinyl]acetic acid,

C. R = CH_3: methyl [(*RS*)-(diphenylmethyl)sulphinyl]acetate,



B. 2-[(diphenylmethyl)sulphonyl]acetamide.

Ph Eur

Molgramostim Concentrated Solution

General Notices

(Ph Eur monograph 1641)

APARSPSPST QPWEHVNAIQ EARRLLNLSR
DTAAEMNETV EVISEMFDLQ EPTCLQTRLE
LYKQGLRGSL TKLKGPLTMM ASHYKQHCPP
TPETSCATQI ITFESFKENL KDFLLVIPFD
CWEPVQE

$C_{639}H_{1007}N_{171}O_{196}S_8$ 14,477 *99283-10-0*

Action and use

Recombinant granulocyte macrophage colony-stimulating factor.

Ph Eur

DEFINITION

Solution of a protein having the structure of the granulocyte macrophage colony stimulating factor which is produced and secreted by various human blood cell types. The protein stimulates the differentiation and proliferation of leucocyte stem cells into mature granulocytes and macrophages.

Content

Minimum 2.0 mg of protein per millilitre.

Potency

Minimum 0.7 × 10^7 IU per milligram of protein.

PRODUCTION

Molgramostim concentrated solution is produced by a method based on recombinant DNA (rDNA) technology, using bacteria as host cells. It is produced under conditions designed to minimise microbial contamination of the product.

Prior to release, the following tests are carried out on each batch of the final bulk product, unless exemption has been granted by the competent authority.

Host-cell derived proteins

The limit is approved by the competent authority.

Host-cell or vector derived DNA

The limit is approved by the competent authority.

CHARACTERS

Appearance

Clear, colourless liquid.

IDENTIFICATION

A. It shows the expected biological activity (see Assay).

B. Isoelectric focusing (*2.2.54*).

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of 0.25 mg/ml.

Reference solution (a) Dilute *molgramostim CRS* with *water R* to obtain a concentration of 0.25 mg/ml.

Reference solution (b) Use an isoelectric point (pI) calibration solution, in the pI range of 2.5-6.5, prepared according to the manufacturer's instructions.

Focusing:

— *pH gradient*: 4.0-6.5;

— *catholyte*: 8.91 g/l (0.1 M) solution of β*-alanine R*;

— *anolyte*: 14.7 g/l (0.1 M) solution of *glutamic acid R* in a 50 per cent *V/V* solution of *dilute phosphoric acid R* (0.5 M);

— *application*: 20 µl.

Detection Immerse the gel in a suitable volume of a solution containing 115 g/l of *trichloroacetic acid R* and 34.5 g/l of *sulphosalicylic acid R* and shake the container gently for 30 min. Transfer the gel to a mixture of 32 volumes of *glacial acetic acid R,* 100 volumes of *ethanol R* and 268 volumes of *water R* (mixture A) and rinse for 5 min. Immerse the gel for 10 min in a staining solution prewarmed to 60 °C and prepared by adding *brilliant blue R* at a concentration of 1.2 g/l to mixture A. Wash the gel in several containers with mixture A and keep the gel in this mixture until the background is clear (12-24 h). After adequate destaining, soak the gel for 1 h in a 10 per cent *V/V* solution of *glycerol R* in mixture A.

System suitability:

— in the electropherogram obtained with reference solution (b), the relevant isoelectric point markers are distributed along the entire length of the gel,

— in the electropherogram obtained with reference solution (a), the pI of the principal band is 4.9 to 5.4.

Results The principal band in the electropherogram obtained with the test solution corresponds in position to the principal band in the electropherogram obtained with reference solution (a). Plot the migration distances of the relevant pI markers versus their pI and determine the isoelectric points of the principal component of each of the test solution and reference solution (a). They do not differ by more than 0.2 pI units.

C. Examine the electropherograms obtained under reducing conditions in the test for impurities with molecular masses differing from that of molgramostim. The principal band in the electropherogram obtained with test solution (a) is similar in position to the principal band in the electropherogram obtained with reference solution (a).

D. Peptide mapping *(2.2.55)*.

Test solution Introduce 50 µl of *tris-hydrochloride buffer solution pH 8.0 R* and 50 µl of the preparation to be examined at a concentration of 2 mg/ml into a polypropylene tube of 0.5 ml capacity. Add 4 µl of a 1 mg/ml solution of *trypsin for peptide mapping R* in a 0.01 per cent *V/V* solution of *trifluoroacetic acid R*, cap tightly and mix well. Incubate at about 37 °C for 18 h. Add 125 µl of a 764 g/l (8 M) solution of *guanidine hydrochloride R* and mix well. Add 10 µl of a 154.2 g/l (1 M) solution of *dithiothreitol R* and mix well. Place the capped tube in boiling water for 1 min. Cool to room temperature.

Reference solution Prepare at the same time and in the same manner as for the test solution but use *molgramostim CRS* instead of the preparation to be examined.

Examine the 2 tryptic digests by liquid chromatography (*2.2.29*).

Column:

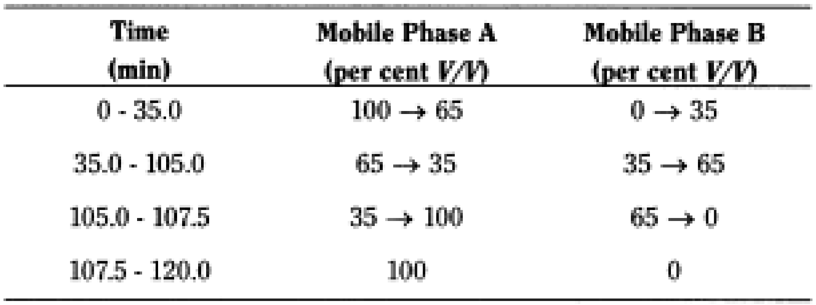
— *size:* *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm.

Mobile phase:

— *mobile phase* A: dilute 1 ml of *trifluoroacetic acid R* in 1000 ml of *water R*;

— *mobile phase B*: dilute 1 ml of *trifluoroacetic acid R* in 100 ml of *water R*; add 900 ml of *acetonitrile for chromatography R* and mix;



Time (min)	Mobile Phase A (per cent *V/V*)	Mobile Phase B (per cent *V/V*)
0 - 35.0	100 → 65	0 → 35
35.0 - 105.0	65 → 35	35 → 65
105.0 - 107.5	35 → 100	65 → 0
107.5 - 120.0	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 12 min.

Injection 200 µl.

System suitability The chromatograms obtained with the reference solution and the test solution are qualitatively similar to the *Ph. Eur. reference chromatogram of molgramostim* *digest.*

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

E. *N*-Terminal sequence analysis.

Perform the Edman degradation using an automated solid-phase sequencer, operated in accordance with the manufacturer's instructions.

Load about 1 nmol of the test preparation to a sequencing cartridge using the protocol provided by the manufacturer. Run 16 sequencing cycles, noting, if appropriate, the presence of proline at positions 2, 6, 8 and 12.

Identify the phenylthiohydantoin (PTH)-amino acids released at each sequencing cycle by reverse-phase liquid chromatography. The procedure may be carried out using the column and reagents recommended by the manufacturer of the sequencing equipment for the separation of PTH-amino acids.

The separation procedure is calibrated using:

— the mixture of PTH-amino acids provided by the manufacturer of the sequencer, with the gradient conditions adjusted as indicated to achieve optimum resolution of all amino acids;

— a sample obtained from a blank sequencing cycle obtained as recommended by the equipment manufacturer.

Results The first 16 amino acids are: Ala-Pro-Ala-Arg-Ser-Pro-Ser-Pro-Ser-Thr-Gln-Pro-Trp-Glu-His-Val.

TESTS

Impurities with molecular masses differing from that of molgramostim

Polyacrylamide gel electrophoresis (*2.2.31*) under both reducing and non-reducing conditions.

Gel dimensions 0.75 mm thick.

Resolving gel 14 per cent acrylamide.

Sample buffer A Mix equal volumes of *water R* and *concentrated SDS-PAGE sample buffer R*.

Sample buffer B (reducing conditions) Mix equal volumes of *water R* and *concentrated SDS-PAGE sample buffer for reducing conditions R.*

Test solution (a) Dilute the preparation to be examined in *water R* to obtain a concentration of 1.0 mg/ml. To 1 volume of this solution add 1 volume of *concentrated SDS-PAGE sample buffer R*.

Test solution (b) (2 per cent control) Dilute 0.020 ml of test solution (a) to 1.0 ml with sample buffer A.

Test solution (c) (1 per cent control) To 0.20 ml of test solution (b) add 0.20 ml of sample buffer A.

Test solution (d) (0.5 per cent control) To 0.20 ml of test solution (c) add 0.20 ml of sample buffer A.

Test solution (e) (0.25 per cent control) To 0.20 ml of test solution (d) add 0.20 ml of sample buffer A.

Test solution (f) (0.1 per cent control) To 0.20 ml of test solution (e) add 0.30 ml of sample buffer A.

Test solution (g) (0.05 per cent control) To 0.20 ml of test solution (f) add 0.20 ml of sample buffer A.

Test solution (h) (0.025 per cent control) To 0.20 ml of test solution (g) add 0.20 ml of sample buffer A.

Test solution (i) Prepare as for test solution (a), but using *concentrated SDS-PAGE sample buffer for reducing conditions R.*

Test solutions (j)-(p) Prepare as for test solutions (b)-(h), but using sample buffer B.

Reference solution (a) Dilute *molgramostim CRS* in *water R* to obtain a concentration of 0.02 mg/ml. Mix 1 volume of this solution with 1 volume of *concentrated SDS-PAGE sample buffer R*.

Reference solution (b) Prepare as for reference solution (a), but using *concentrated SDS-PAGE sample buffer for reducing conditions R.*

Reference solution (c) Use a solution of molecular mass markers suitable for calibrating SDS-PAGE gels in the range of 14 400-94 000. Dissolve in sample buffer or sample buffer (reducing conditions), as appropriate.

Sample treatment Boil for 3 min.

Application 50 µl; apply reduced and non-reduced solutions to separate gels.

Detection Silver staining as described below.

Immerse the gel overnight in a mixture of 10 volumes of *acetic acid R*, 40 volumes of *water R* and 50 volumes of *methanol R*. Transfer the gel to a 100 g/l solution of *glutaraldehyde R* and shake for about 30 min. Replace the glutaraldehyde solution with *water R*, and keep the gel in *water R* for 20 min. Repeat this washing-step twice. Transfer the gel to a mixture containing 0.75 g/l of *sodium hydroxide R*, 14 g/l of *concentrated ammonia R* and 8 g/l of *silver nitrate R*. This solution is prepared immediately before use. Place the gel on a shaker in the dark for 5 min. Wash the gel for 30 s in each of 3 containers with *water R* and shake the gel in a mixture consisting of 0.05 g/l of *citric acid R*, 0.05 per cent *V/V* of *formaldehyde R* and 0.005 per cent *V/V* of *methanol R* in *water R*. Protein bands become visible during this step. Keep the gel in the solution until sufficiently stained and then rinse the gel repeatedly with *water R* in a shaking water bath. Soak gels in a solution consisting of 10 per cent *V/V* of *acetic acid R* and 1 per cent *V/V* of *glycerol R*.

System suitability:

— the validation criteria are met (*2.2.31*);

— a band is seen in the electropherogram obtained with test solution (h);

— a gradation of intensity of staining is seen in the electropherograms obtained with test solutions (a)-(h) and (i)-(p);

— the molecular mass of the principal band in the electrophoregram obtained with reference solution (a) or (b) is within the range of 15 100 to 17 100.

Limits Compare the staining intensity of each non-molgramostim band observed in the electropherogram obtained with test solution (a) to the staining intensity of the principal band in the electropherograms obtained with test solutions (b)-(h). Proceed similarly with the electropherograms obtained with test solutions (i)-(p). The impurity level is estimated as the dilution, in percentage, of the solution giving the electropherogram with the closest intensity of staining.

Reducing conditions:

— *impurity with an apparent molecular mass of 20 000*: maximum 1 per cent;

— *impurity with an apparent molecular mass of 25 000*: maximum 0.1 per cent;

— *impurity with an apparent molecular mass of 30 000*: maximum 0.3 per cent;

— *total*: maximum 2 per cent.

Non-reducing conditions:

— *total of all impurities of molecular masses higher than 30 000*: maximum 1 per cent.

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution (a) Dilute the preparation to be examined with *0.05 M phosphate buffer solution pH 7.0 R* to obtain a concentration of 0.5 mg/ml.

Test solution (b) Mix 1 volume of test solution (a) with 4 volumes of a 0.125 mg/ml solution of *human albumin R* or *bovine albumin R* in *0.05 M phosphate buffer solution pH 7.0 R*.

Reference solution (a) Dilute *molgramostim CRS* with *0.05 M phosphate buffer solution pH 7.0 R* to obtain a concentration of 0.5 mg/ml.

Reference solution (b) Mix 1 volume of reference solution (a) with 4 volumes of a 0.125 mg/ml solution of *human albumin R* or *bovine albumin R* in *0.05 M phosphate buffer solution pH 7.0 R*.

Column:

— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *butylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm.

Mobile phase:

— *mobile phase A*: to about 800 ml of *water R* add 1.0 ml of *trifluoroacetic acid R* and dilute to 1000 ml with *water R*;

— *mobile phase B*: to 100 ml of *water R* add 1.0 ml of *trifluoroacetic acid R* and 900 ml of *acetonitrile for chromatography R*;

Time (min)	**Mobile Phase A (per cent *V/V*)**	**Mobile Phase B (per cent *V/V*)**
0 - 30	64 → 44	36 → 56
30 - 35	44 → 0	56 → 100
35 - 45	0	100
45 - 50	0 → 64	100 → 36
50 - 60	64	36

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µl of test solution (a), reference solutions (a) and (b).

System suitability Reference solution (b):

— *retention time*: molgramostin = about 22 min;

— *repeatability*: maximum relative standard deviation of 5.0 per cent after 4 injections;

— *resolution*: minimum 2 between the peaks due to albumin and molgramostim.

Limits:

— *any impurity*: for each impurity, maximum 1.5 per cent;

— *total of impurities eluting between 5 min and 30 min*: maximum 4 per cent.

Bacterial endotoxins (*2.6.14*)

Less than 5 IU in the volume that contains 1.0 mg of protein.

ASSAY

Protein

Liquid chromatography (*2.2.29*) as described in the test for related proteins.

Injection 150 µl of test solution (b) and reference solution (b).

Calculate the content of molgramostim using the declared content of molgramostim in *molgramostim CRS.*

Potency

Determination of the biological activity of molgramostim concentrated solution based on the stimulation of proliferation of TF-1 cells by molgramostim.

The following method uses the conversion of tetrazolium bromide (MTT) as a staining method. Validated alternative stains such as Almar blue have also been found suitable.

TF-1 cells are incubated with varying dilutions of test and reference preparations of molgramostim. They are then incubated with a solution of MTT. This cytochemical stain is converted by cellular dehydrogenases to a purple formazan product. The formazan is then measured spectrophotometrically. The potency of the preparation to be examined is determined by comparison of the dilutions of the test preparation with the dilutions of the appropriate International Standard of molgramostim or with a reference preparation calibrated in International Units, which yield the same response (50 per cent maximal stimulation).

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Add 50 µl of dilution medium to all wells of a 96-well microtitre plate. Add an additional 50 µl of this solution to the wells designed for the blanks. Add 50 µl of each solution to be tested in triplicate (test preparation and reference preparation at a concentration of about 65 IU/ml, plus a series of 10 twofold dilutions to obtain a standard curve). Then add to each well 50 µl of a TF-1 cell suspension containing 3×10^5 cells per millilitre, maintaining the cells in a uniform suspension during addition.

Incubate the plate at 36.0-38.0 °C for a minimum of 24 h in a humidified incubator using 6 ± 1 per cent CO_2. Add 25 µl of a 5.0 g/l sterile solution of *tetrazolium bromide R* to each well. Reincubate for 5 h. Remove the plates from the incubator and add to each well 100 µl of a 240 g/l solution of *sodium dodecyl sulphate R* previously adjusted to pH 2.7 with hydrochloric acid. Reincubate overnight.

Determine the relative quantity of purple formazan product formed in each well by measuring the absorbance (*2.2.25*) using a 96-well microtitre plate reader. Read each plate at 570 nm and at 690 nm. Subtract the reading at 690 nm from the reading at 570 nm. Analyse the data by fitting a sigmoidal dose-response curve to the data obtained and by using a suitable statistical method, for example the 4-parameter model (see *5.3*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) of the estimated potency are not less than 74 per cent and not more than 136 per cent of the stated potency.

STORAGE

In an airtight container, protected from light, at a temperature below - 65 °C.

LABELLING

The label states:

— the content, in milligrams of protein per millilitre;

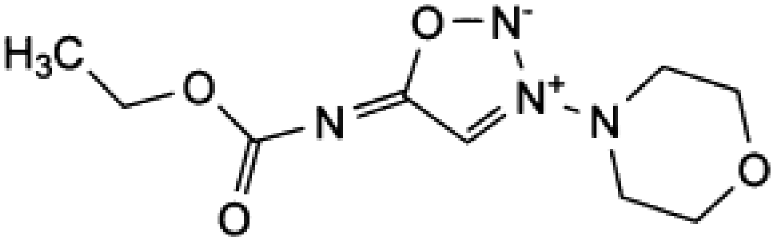
— the potency, in International Units per milligram of protein.

Ph Eur

Molsidomine

General Notices

(Ph Eur monograph 1701)



$C_9H_{14}N_4O_4$ 242.2   *25717-80-0*

Action and use

Nitric oxide donor; treatment of angina pectoris.

Ph Eur

DEFINITION

N-(Ethoxycarbonyl)-3-(morpholin-4-yl)sydnonimine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, soluble in anhydrous ethanol and in methylene chloride.

mp

About 142 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   molsidomine CRS.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

Dissolve 1.0 g in *anhydrous ethanol R* and dilute to 20.0 ml with the same solvent.

pH *(2.2.3)*

5.5 to 7.5.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Impurity B

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 240 nm.

Injection 20 µl of test solution (a) and reference solution (b).

Relative retention with reference to molsidomine (retention time = about 9 min): impurity B = about 0.43.

System suitability Reference solution (b):

— *signal-to-noise ratio*: minimum 20 for the principal peak.

Limit:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (3 ppm).

Impurity E

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *morpholine for chromatography R* in 500.0 ml of *water for chromatography R*. Dilute 20.0 ml of the solution to 500.0 ml with *water for chromatography R*. Dilute 5.0 ml of this solution to 100.0 ml with *water for chromatography R.*

Reference solution (b) Mix 10.0 ml of the test solution with 10.0 ml of reference solution (a).

Column:

— *size:* l = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *resin for reversed-phase ion chromatography R*;

— *temperature*: 25 °C.

Mobile phase Mix 3.0 ml of *methanesulphonic acid R* and 75 ml of *acetonitrile R* in *water for chromatography R* and dilute to 1000 ml with *water for chromatography R*.

Suppressor regenerant *water for chromatography R.*

Flow rate 1.0 ml/min.

Expected background conductivity Less than 0.5 µS.

Detection Conductivity detector at 10 µS.

Injection 50 µl.

Run time 20 min.

Relative retention with reference to molsidomine (retention time = about 3 min): impurity E = about 2.4.

System suitability Reference solution (b):

- *signal-to-noise ratio*: minimum 6 for the peak due to impurity E.

Limit:

- *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Solvent mixture *methanol R*, mobile phase A (10:90 *V/V*).

Test solution (a) Dissolve 0.200 g of the substance to be examined in 2.5 ml of *methanol R* and dilute to 5.0 ml with mobile phase A.

Test solution (b) Dilute 1.0 ml of test solution (a) to 20.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of test solution (b) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 2.4 mg of *molsidomine impurity B CRS* in 80 ml of *methanol R* and dilute to 100.0 ml with *methanol R*. Dilute 2.0 ml of the solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 20.0 ml with the solvent mixture.

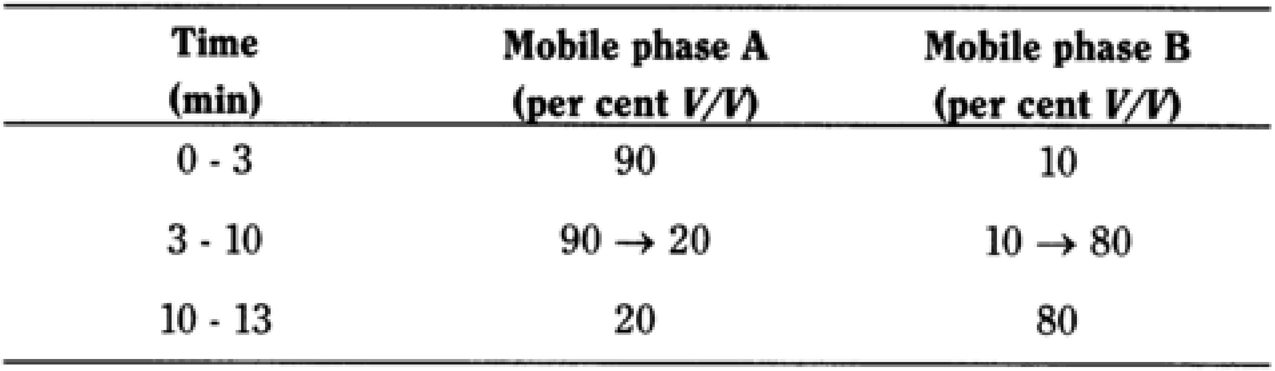
Reference solution (c) Dissolve 10 mg of *linsidomine hydrochloride R* (impurity A) and 5 mg of *molsidomine impurity D CRS* in 10 ml of *methanol R* and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Column:

- *size:* l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: dissolve 4.0 g of *potassium dihydrogen phosphate R* in *water for chromatography R* and dilute to 1000 ml with the same solvent;
- *mobile phase B*: *methanol R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	90	10
3 - 10	90 → 20	10 → 80
10 - 13	20	80

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl of test solution (b) and reference solutions (a) and (c).

Relative retention With reference to molsidomine (retention time = about 9 min): impurity A = about 0.2; impurity D = about 0.3.

System suitability Reference solution (c):

— *resolution*: minimum 3.5 between the peaks due to impurities A and D.

Limits:

— *unspecified impurities*: for each impurity, not more than the area of the peak due to molsidomine in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the peak due to molsidomine in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the peak due to molsidomine in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals

Maximum 20 ppm.

Prescribed solution Dissolve 0.5 g in 20 ml of *ethanol (96 per cent) R.*

Test solution 12 ml of the prescribed solution.

Reference solution Mix 6 ml of lead standard solution (1 ppm Pb) (obtained by diluting *lead standard solution (100 ppm Pb) R* with *ethanol (96 per cent) R*) with 2 ml of the prescribed solution and 4 ml of *water R.*

Blank solution Mix 10 ml of ethanol (96 per cent) R and 2 ml of the prescribed solution.

To each solution, add 2 ml of *buffer solution pH 3.5 R*. Mix and add to 1.2 ml of *thioacetamide reagent R*. Mix immediately. Filter the solutions through a membrane filter (pore size 0.45 µm) *(2.4.8)*. Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston. Compare the spots on the filters obtained with the different solutions. The test is invalid if the reference solution does not show a slight brown colour compared to the blank solution. The substance to be examined complies with the test if the brown colour of the spot resulting from the test solution is not more intense than that of the spot resulting from the reference solution.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 5 ml of *acetic anhydride R* and 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 24.22 mg of $C_9H_{14}N_4O_4$.

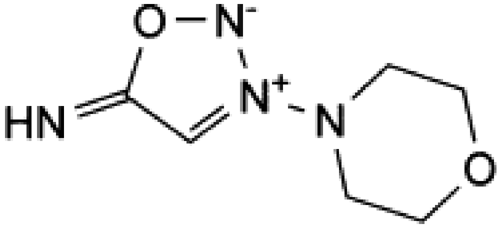
STORAGE

Protected from light.

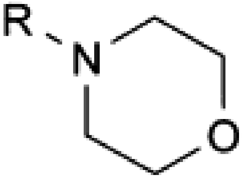
IMPURITIES

Specified impurities *B, E.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, C, D.*



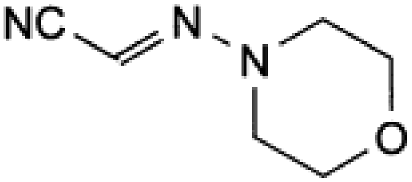
A. 3-(morpholin-4-yl)sydnonimine (linsidomine),



B. R = NO: 4-nitrosomorpholine,

D. R = CHO: morpholine-4-carbaldehyde,

E. R = H: morpholine,



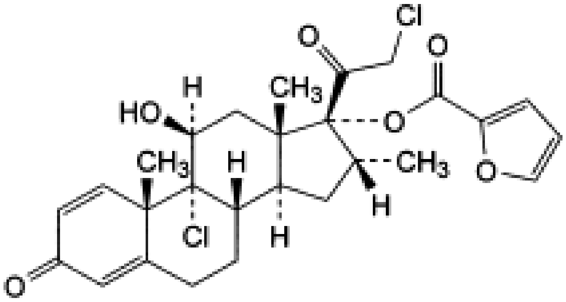
C. (2*E*)-(morpholin-4-ylimino)acetonitrile.

Ph Eur

Mometasone Furoate

General Notices

(Ph Eur monograph 1449)



$C_{27}H_{30}Cl_2O_6$ 521.4 *83919-23-7*

Action and use

Glucocorticoid.

Preparations

Mometasone Cream

Mometasone Aqueous Nasal Spray

Mometasone Ointment

Mometasone Scalp Application

Ph Eur

DEFINITION

9,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in acetone and in methylene chloride, slightly soluble in ethanol (96 per cent).

mp: about 220 °C, with decomposition.

IDENTIFICATION

First identification A, B.

Second identification B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *mometasone furoate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *mometasone furoate CRS* in *methylene chloride R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *anhydrous beclometasone dipropionate CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with alcoholic solution of *sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which, when examined in ultraviolet light at 365 nm, may not be completely separated.

C. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 15 min a light yellow colour develops. When examined in ultraviolet light at 365 nm, no fluorescence is seen. Add this solution to 10 ml of *water R* and mix. The colour fades and there is no fluorescence.

D. Mix 80 mg with 0.30 g of *anhydrous sodium carbonate R* and ignite in a crucible until an almost white residue is obtained. Allow to cool and dissolve the residue in 5 ml of *dilute nitric acid R*; filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)

+ 50 to + 55 (dried substance).

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Solvent mixture Mix 50 ml of *acetonitrile R* and 50 ml of *water R*, then add 0.1 ml of *acetic acid R*.

Test solution Dissolve 20.0 mg of the substance to be examined in 4.0 ml of *acetonitrile R* and dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *mometasone furoate CRS* and 6 mg of *anhydrous beclometasone dipropionate CRS* in the solvent mixture, then dilute to 10.0 ml with the solvent mixture. Dilute 0.25 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:
- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *water R* (50:50 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time Twice the retention time of mometasone furoate.

Retention time Mometasone furoate = about 17 min; beclometasone dipropionate = about 22 min.

System suitability Reference solution (a):

- *resolution*: minimum 6 between the peaks due to mometasone furoate and beclometasone dipropionate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:
- *impurities A, B, C, D, E, F, G, H, I*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *total*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

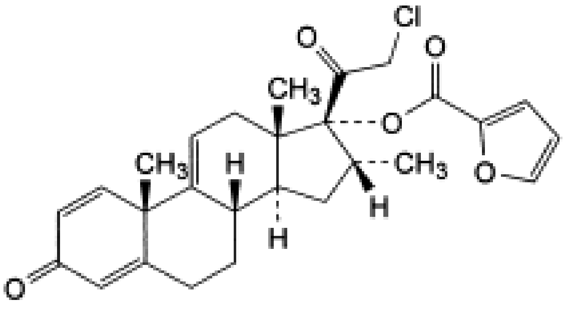
ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 249 nm.

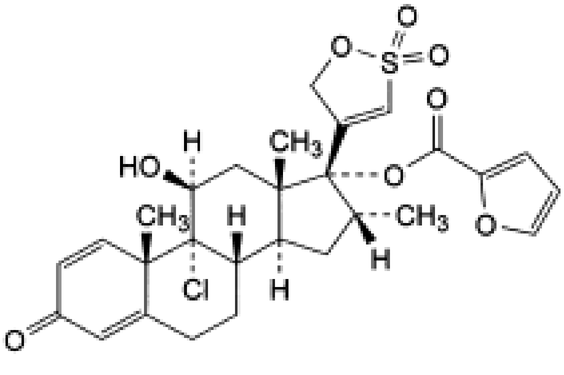
Calculate the content of $C_{27}H_{30}Cl_2O_6$ taking the specific absorbance to be 481.

IMPURITIES

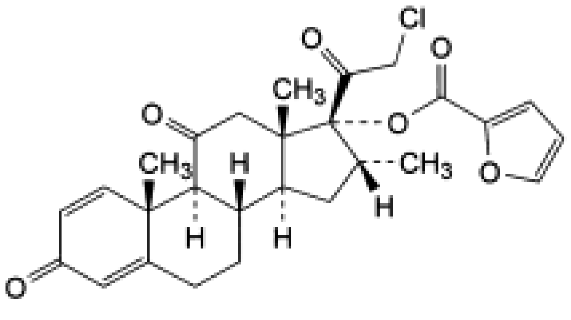
Specified impurities A, B, C, D, E, F, G, H, I.



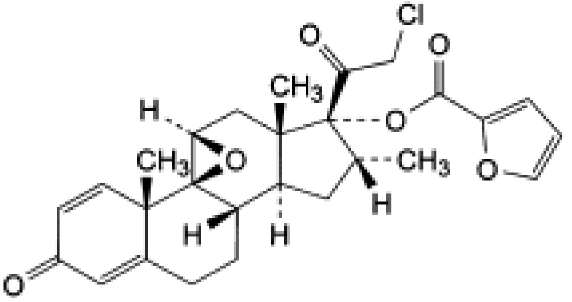
A. 21-chloro-16α-methyl-3,20-dioxopregna-1,4,9(11)-trien-17-yl furan-2-carboxylate,



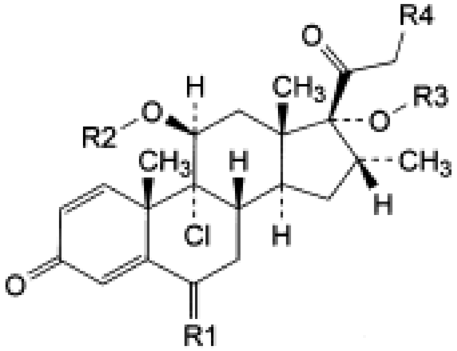
B. 4-[9-chloro-17-[(furan-2-ylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17β-yl]-5*H*-1,2-oxathiole 2,2-dioxide,

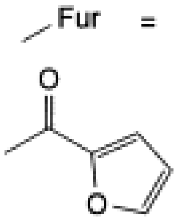


C. 21-chloro-16α-methyl-3,11,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,



D. 21-chloro-9,11β-epoxy-16α-methyl-3,20-dioxo-9β-pregna-1,4-dien-17-yl furan-2-carboxylate,



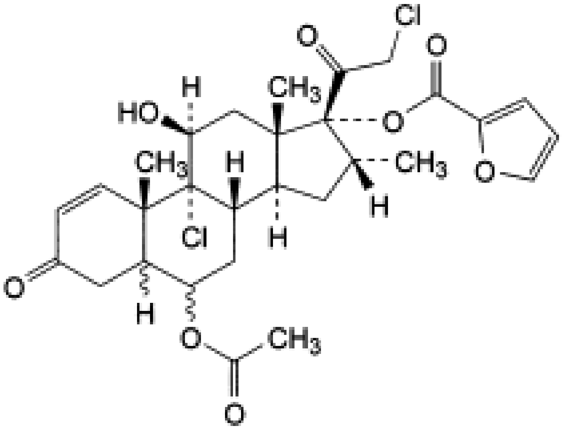


E. R1 = H_2, R2 = R3 = Fur, R4 = Cl: 9,21-dichloro-16α-methyl-3,20-dioxopregna-1,4-diene-11β,17-diyl bis(furan-2-carboxylate),

F. R1 = O, R2 = H, R3 = Fur, R4 = Cl: 9,21-dichloro-11β-hydroxy-16α-methyl-3,6,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,

G. R1 = H_2, R2 = R3 = H, R4 = Cl: 9,21-dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione (mometasone),

H. R1 = H_2, R2 = H, R3 = Fur, R4 = OH: 9-chloro-11β,21-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



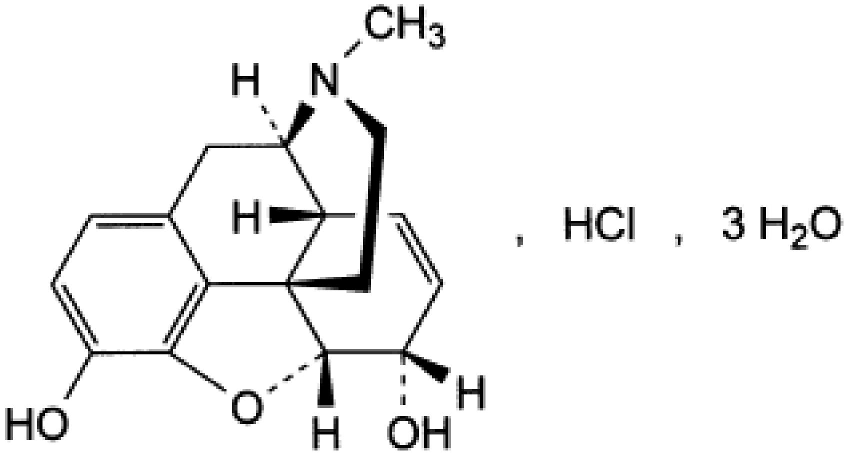
I. 9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxo-5ξ-pregn-1-ene-6ξ,17-diyl 6-acetate 17-(furan-2-carboxylate).

Ph Eur

Morphine Hydrochloride

General Notices

(Ph Eur monograph 0097)



$C_{17}H_{19}NO_3,HCl,3H_2O$ 375.8   *52-26-6*
(anhydrous)

Action and use

Opioid receptor agonist; analgesic.

Preparations

Chloroform and Morphine Tincture

Morphine Suppositories

Ph Eur

DEFINITION

7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol hydrochloride trihydrate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless, silky needles or cubical masses, efflorescent in a dry atmosphere.

Solubility

Soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in toluene.

IDENTIFICATION

First identification *A, E.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *morphine hydrochloride CRS.*

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Solution A Dissolve 25.0 mg in *water R* and dilute to 25.0 ml with the same solvent.

Test solution (a) Dilute 10.0 ml of solution A to 100.0 ml with *water R.*

Test solution (b) Dilute 10.0 ml of solution A to 100.0 ml with *0.1 M sodium hydroxide*.

Spectral range 250-350 nm for test solutions (a) and (b).

Absorption maximum At 285 nm for test solution (a); at 298 nm for test solution (b).

Specific absorbance at the absorption maximum 37 to 43 for test solution (a); 64 to 72 for test solution (b).

C. To about 1 mg of powdered substance in a porcelain dish add 0.5 ml of *sulphuric acid-formaldehyde reagent R*. A purple colour develops and becomes violet.

D. It gives the reaction of alkaloids (*2.3.1*).

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.2 ml of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation *(2.2.7)*

- 110 to - 115 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.125 g of the substance to be examined in a 1 per cent *V/V* solution of *acetic acid R* and dilute to 50 ml with the same solution.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with a 1 per cent *V/V* solution of *acetic acid R.* Dilute 2.0 ml of this solution to 10.0 ml with a 1 per cent *V/V* solution of *acetic acid R*.

Reference solution (b) Dissolve 5 mg of *morphine for system suitability CRS* (containing impurities B, C, E and F) in a 1 per cent *V/V* solution of *acetic acid R* and dilute to 2 ml with the same solution.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

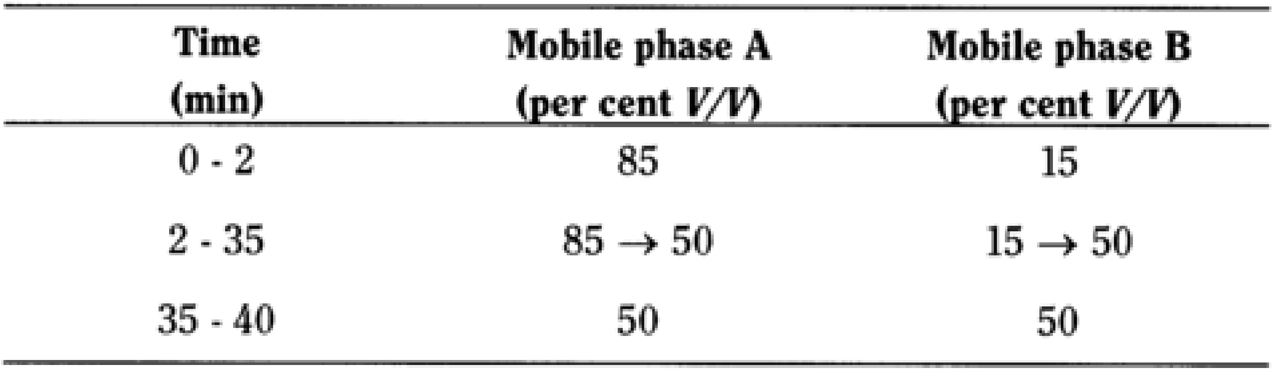
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: 1.01 g/l solution of *sodium heptanesulphonate R* adjusted to pH 2.6 with a 50 per cent *V/V* solution of *phosphoric acid R*;

— *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 35	85 → 50	15 → 50
35 - 40	50	50

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *morphine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, E and F.

Relative retention With reference to morphine (retention time = about 12.5 min): impurity F = about 0.95; impurity E = about 1.1; impurity C = about 1.6; impurity B = about 1.9.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2, where H_r = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to morphine.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.25; impurity C = 0.4;

impurity E = 0.5;

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *impurities C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Water *(2.5.12)*

12.5 per cent to 15.5 per cent, determined on 0.10 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5 ml of *0.01 M hydrochloric acid* and 30 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 32.18 mg of $C_{17}H_{20}ClNO_3$.

STORAGE

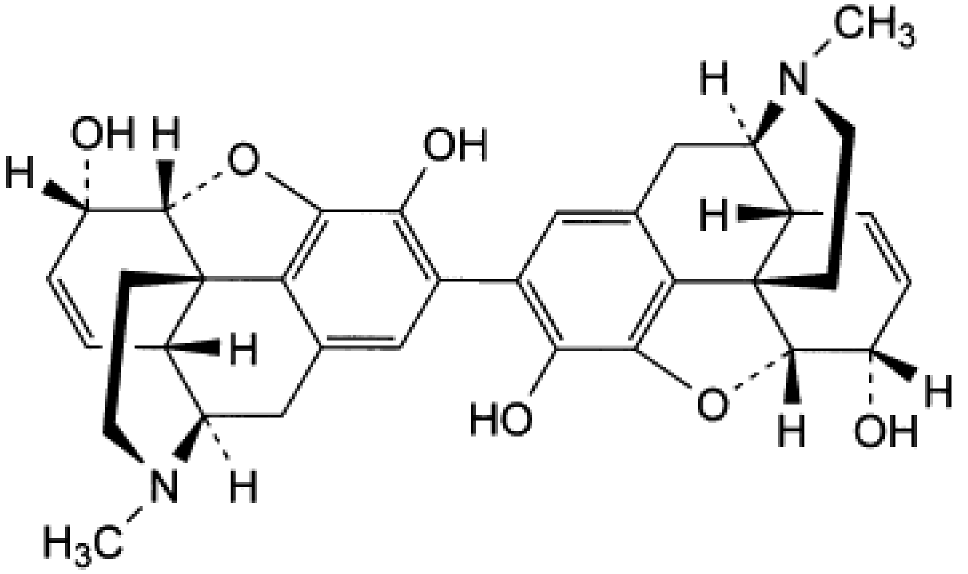
Protected from light.

IMPURITIES

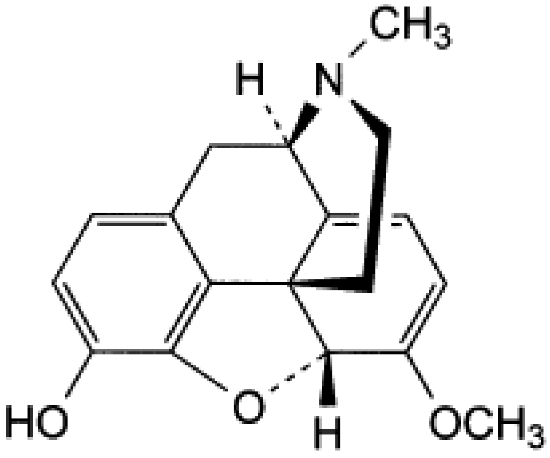
Specified impurities *B, C, E.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, D, F.*

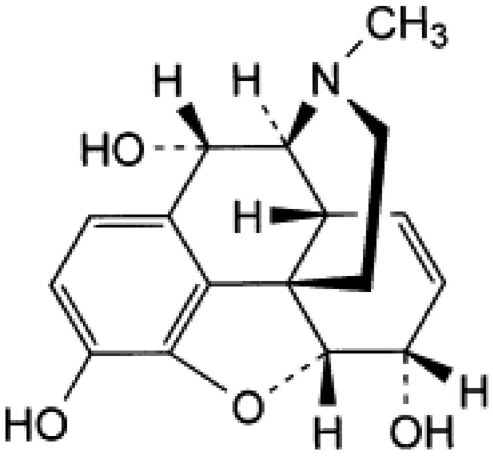
A. codeine,



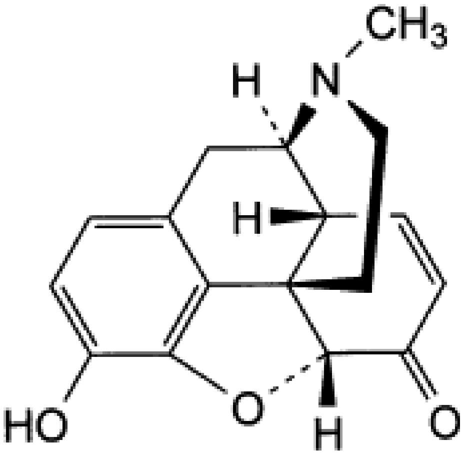
B. 7,7′,8,8′-tetradehydro-4,5α:4′,5′α-diepoxy-17,17′-dimethyl-2,2′-bimorphinanyl-3,3′,6α,6′α-tetrol (2,2′-bimorphine),



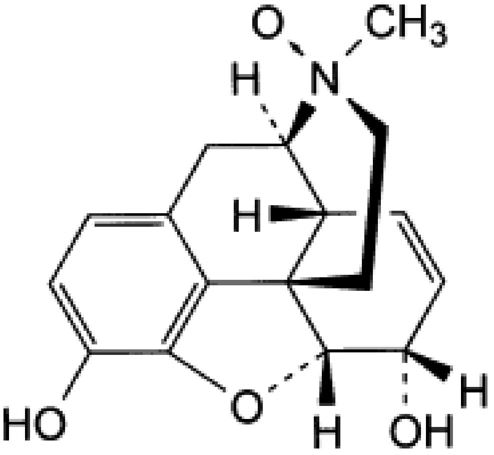
C. 6,7,8,14-tetradehydro-4,5α-epoxy-6-methoxy-17-methylmorphinan-3-ol (oripavine),



D. 7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α,10α-triol (10*S*-hydroxymorphine),



E. 7,8-didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one (morphinone),



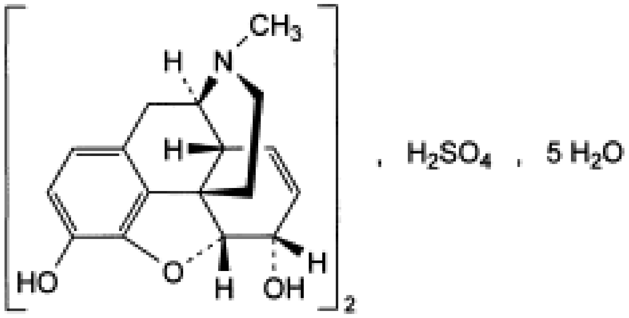
F. (17*S*)-7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol 17-oxide (morphine *N*-oxide).

Ph Eur

Morphine Sulphate

General Notices

(Ph Eur monograph 1244)



$C_{34}H_{40}N_2O_{10}S,5H_2O$ 759 *6211-15-0*

Action and use

Opioid receptor agonist; analgesic.

Preparations

Morphine Sulphate Injection

Morphine and Atropine Injection

Morphine Suppositories

Morphine Tablets

Prolonged-release Morphine Tablets

Ph Eur

DEFINITION

Di(7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol) sulphate pentahydrate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in toluene.

IDENTIFICATION

First identification *A, E.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 20 mg in 1 ml of *water R*, add 0.05 ml of *1 M sodium hydroxide* and shake. A precipitate is formed. Filter, wash with 2 quantities, each of 0.5 ml, of *water R* and dry the precipitate at 145 °C for 1 h. Prepare discs using the dried precipitate.

Comparison Repeat the operations using 20 mg of *morphine sulphate CRS*.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Solution A Dissolve 25.0 mg in *water R* and dilute to 25.0 ml with the same solvent.

Test solution (a) Dilute 10.0 ml of solution A to 100.0 ml with *water R*.

Test solution (b) Dilute 10.0 ml of solution A to 100.0 ml with *0.1 M sodium hydroxide*.

Spectral range 250-350 nm for test solutions (a) and (b).

Absorption maximum At 285 nm for test solution (a); at 298 nm for test solution (b).

Specific absorbance at the absorption maximum 37 to 43 for test solution (a); 64 to 72 for test solution (b).

C. To about 1 mg of powdered substance in a porcelain dish add 0.5 ml of *sulphuric acid-formaldehyde reagent R*. A purple colour develops and becomes violet.

D. It gives the reaction of alkaloids *(2.3.1)*.

E. It gives the reactions of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 or BY_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.2 ml of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation *(2.2.7)*

- 107 to - 110 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.125 g of the substance to be examined in a 1 per cent *V/V* solution of *acetic acid R* and dilute to 50 ml with the same solution.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with a 1 per cent *V/V* solution of *acetic acid R*. Dilute 2.0 ml of this solution to 10.0 ml with a 1 per cent *V/V* solution of *acetic acid R*.

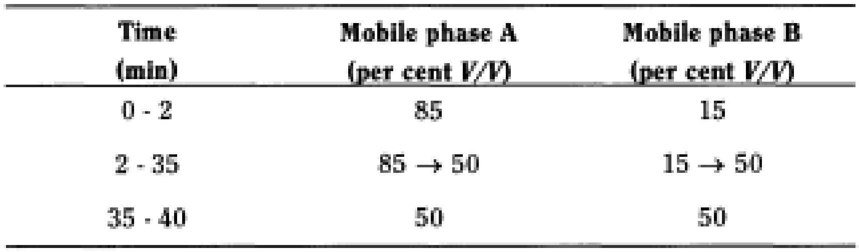
Reference solution (b) Dissolve 5 mg of *morphine for system suitability CRS* (containing impurities B, C, E and F) in a 1 per cent *V/V* solution of *acetic acid R* and dilute to 2 ml with the same solution.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase:

- *mobile phase A*: 1.01 g/l solution of *sodium heptanesulphonate R* adjusted to pH 2.6 with a 50 per cent *V/V* solution of *phosphoric acid R*;
- *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 35	85 → 50	15 → 50
35 - 40	50	50

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *morphine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, E and F.

Relative retention With reference to morphine (retention time = about 12.5 min): impurity F = about 0.95; impurity E = about 1.1; impurity C = about 1.6; impurity B = about 1.9.

System suitability Reference solution (b):

- *peak-to-valley ratio*: minimum 2, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to morphine.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.25; impurity C = 0.4; impurity E = 0.5;

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *impurities C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Iron *(2.4.9)*

Maximum 5 ppm.

Dissolve the residue from the test for sulphated ash in *water R* and dilute to 10.0 ml with the same solvent.

Water *(2.5.12)*

10.4 per cent to 13.4 per cent, determined on 0.10 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.500 g in 120 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 66.88 mg of $C_{34}H_{40}N_2O_{10}S$.

STORAGE

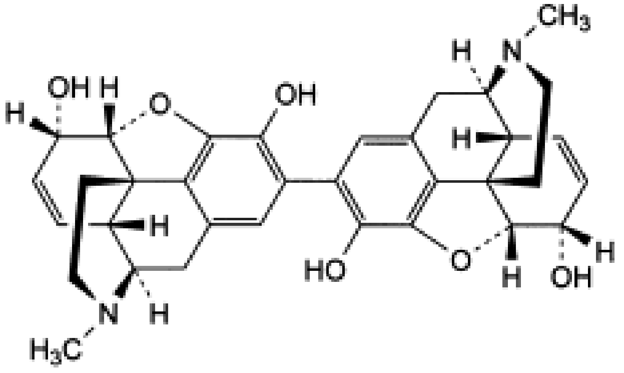
Protected from light.

IMPURITIES

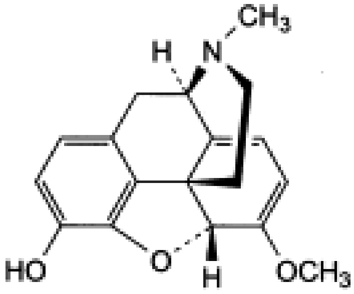
Specified impurities *B, C, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, D, F.*

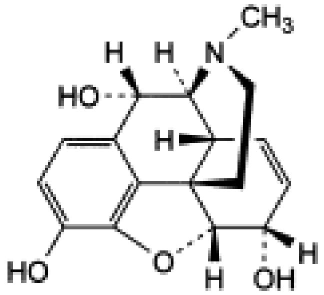
A. codeine,



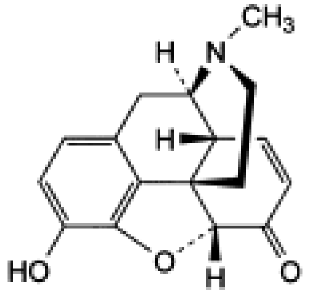
B. 7,7′,8,8′-tetradehydro-4,5α:4′,5′α-diepoxy-17,17′-dimethyl-2,2′-bimorphinanyl-3,3′,6α,6′α-tetrol (2,2′-bimorphine),



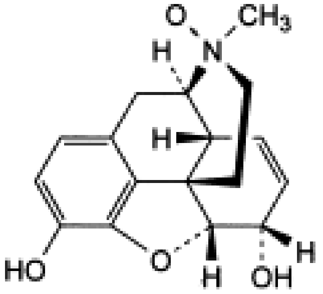
C. 6,7,8,14-tetradehydro-4,5α-epoxy-6-methoxy-17-methylmorphinan-3-ol (oripavine),



D. 7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α,10α-triol (10*S*-hydroxymorphine),



E. 7,8-didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one (morphinone),


O
CH3
H
N
H
HO
O
H
OH
H

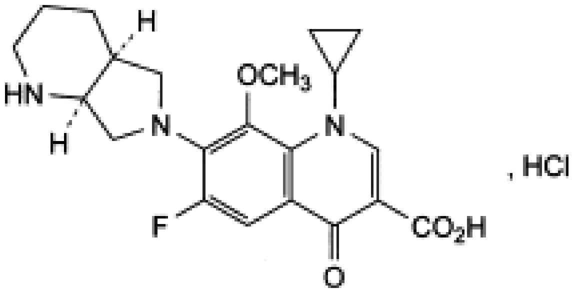
F. (17*S*)-7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol 17-oxide (morphine *N*-oxide).

Ph Eur

Moxifloxacin Hydrochloride

General Notices

(Ph Eur monograph 2254)



$C_{21}H_{25}ClFN_3O_4$ 437.9 *186826-86-8*

Action and use

Fluoroquinolone antibacterial.

Ph Eur

DEFINITION

1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

The production method is validated to demonstrate the satisfactory enantiomeric purity of the final product.

CHARACTERS

Appearance

Light yellow or yellow powder or crystals, slightly hygroscopic.

Solubility

Sparingly soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *moxifloxacin hydrochloride CRS.*

C. Dissolve 50 mg in 5 ml of *water R*, add 1 ml of *dilute nitric acid R*, mix, allow to stand for 5 min and filter. The filtrate gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution GY_2 *(2.2.2, Method II)*. If intended for use in the manufacture of parenteral dosage forms, the solution is clear *(2.2.1)* and not more intensely coloured than reference solution GY_2 *(2.2.2, Method II)*.

Dissolve 1.0 g in 20 ml of *dilute sodium hydroxide solution R*.

pH *(2.2.3)*

3.9 to 4.6.

Dissolve 0.10 g in 50 ml of *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7)*

- 125 to - 138 (anhydrous substance).

Dissolve 0.200 g in 20.0 ml of a mixture of equal volumes of *acetonitrile R* and *water R*.

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light.*

Solution A Dissolve 0.50 g of *tetrabutylammonium hydrogen sulphate R* and 1.0 g of *potassium dihydrogen phosphate R* in about 500 ml of *water R*. Add 2 ml of *phosphoric acid R* and 0.050 g of *anhydrous sodium sulphite R*, then dilute to 1000.0 ml with *water R*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 50.0 ml with the same solution.

Test solution (b) Dilute 2.0 ml of test solution (a) to 20.0 ml with solution A.

Reference solution (a) Dissolve 50.0 mg of *moxifloxacin hydrochloride CRS* in solution A and dilute to 50.0 ml with the same solution. Dilute 2.0 ml of this solution to 20.0 ml with solution A.

Reference solution (b) Dissolve 5 mg of *moxifloxacin for peak identification CRS* (containing impurities A, B, C, D and E) in solution A and dilute to 5.0 ml with the same solution.

Reference solution (c) Dilute 1.0 ml of test solution (a) to 100.0 ml with solution A. Dilute 1.0 ml of this solution to 10.0 ml with solution A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped phenylsilyl silica gel for chromatography R* (5 µm);

- *temperature*: 45 °C.

Mobile phase Mix 28 volumes of *methanol R* and 72 volumes of a solution containing 0.5 g/l of *tetrabutylammonium hydrogen sulphate R*, 1.0 g/l of *potassium dihydrogen phosphate R* and 3.4 g/l of *phosphoric acid R*.

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 293 nm.

Injection 10 µl of test solution (a) and reference solutions (b) and (c).

Run time 2.5 times the retention time of moxifloxacin.

Identification of impurities Use the chromatogram supplied with *moxifloxacin for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to moxifloxacin (retention time = about 14 min): impurity A = about 1.1; impurity B = about 1.3; impurity C = about 1.4; impurity D = about 1.6; impurity E = about 1.7.

System suitability Reference solution (b):

- *resolution*: minimum 1.5 between the peaks due to moxifloxacin and impurity A;
- the chromatogram obtained is similar to the chromatogram supplied with *moxifloxacin for peak identification CRS*.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.4; impurity E = 3.5;
- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water *(2.5.12)*

Maximum 4.5 per cent, determined on 0.200 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{21}H_{25}ClFN_3O_4$ from the declared content of *moxifloxacin hydrochloride CRS*.

STORAGE

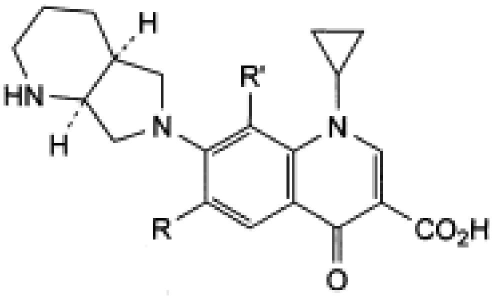
In an airtight container , protected from light.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES

Specified impurities   A, B, C, D, E.



A. R = R′ = F: 1-cyclopropyl-6,8-difluoro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

B. R = R′ = OCH_3: 1-cyclopropyl-6,8-dimethoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*] pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

C. R = F, R′ = OC_2H_5: 1-cyclopropyl-8-ethoxy-6-fluoro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3, 4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

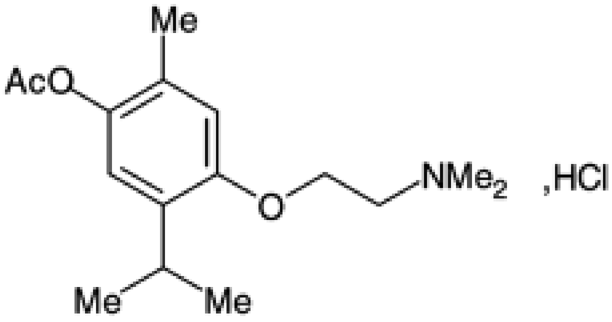
D. R = OCH_3, R′ = F: 1-cyclopropyl-8-fluoro-6-methoxy-7-[(4*aS*,7a*S*)-octahydro-6*H*-pyrrolo [3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

E. R = F, R′ = OH: 1-cyclopropyl-6-fluoro-8-hydroxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3, 4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

Ph Eur

Moxisylyte Hydrochloride

General Notices



$C_{16}H_{25}NO_3,HCl$ 315.8 *964-52-3*

Action and use

Alpha-adrenoceptor antagonist.

Preparation

Moxisylyte Tablets

DEFINITION

Moxisylyte Hydrochloride is 4-(2-dimethylaminoethoxy)-5-isopropyl-2-methylphenyl acetate hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{16}H_{25}NO_3,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Freely soluble in *water* and in *chloroform*; soluble in *ethanol (96%)*; practically insoluble in *ether* and in petroleum spirit.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of moxisylyte hydrochloride *(RS 238)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 5% w/v solution, 4.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in the mobile phase containing (1) 0.0010% w/v of *2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride BPCRS* and 0.00050% w/v of *2-thymoxyethyldimethylamine hydrochloride BPCRS* and (2) 0.2% w/v of the substance being examined. For solution (3) dilute 1 volume of solution (2) to 100 volumes. For solution (4) dilute 1 volume of solution (3) to 10 volumes.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) 0.005M *sodium hexanesulphonate* in a mixture of 2 volumes of *glacial acetic acid*, 185 volumes of *water* and 315 volumes of *methanol* as the mobile phase with a flow rate of 1.0 ml per minute and (c) a detection wavelength of 276 nm.

The peaks in the chromatogram obtained with solution (1) are due to (a) 2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride and (b) 2-thymoxyethyldimethylamine hydrochloride in order of their elution. In the chromatogram obtained with solution (2) the areas of any peaks corresponding to (a) and (b) are not greater than the areas of the corresponding peaks in the chromatogram obtained with solution (1) (0.5% of 2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride and 0.25% of 2-thymoxyethyldimethylamine hydrochloride respectively), the area of any peak with a retention time relative to moxisylyte of 2.3 is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1% of 2-(6-chlorothymoxy)ethyldimethyl-amine hydrochloride) and the area of any peak with a retention time relative to moxisylyte of 0.9 is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.1% of 2-(6-acetylthymoxy) ethyldimethylamine).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.5 g and *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 31.58 mg of $C_{16}H_{25}NO_3$,HCl.

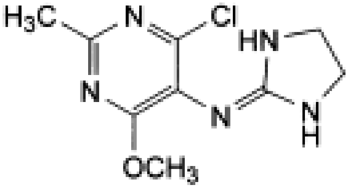
STORAGE

Moxisylyte Hydrochloride should be protected from light.

Moxonidine

General Notices

(Ph Eur monograph 1758)



$C_9H_{12}ClN_5O$ *241.7* *75438-57-2*

Action and use

Imidazoline I_1 receptor antagonist; treatment of hypertension.

Ph Eur

DEFINITION

4-Chloro-*N*-(imidazolidin-2-ylidene)-6-methoxy-2-methylpyrimidin-5-amine.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, sparingly soluble in methanol, slightly soluble in methylene chloride, very slightly soluble in acetonitrile.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *moxonidine CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 10.0 mg of *moxonidine CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with a mixture of equal volumes of *methanol R* and *water R*. Dilute 2.0 ml of this solution to 20.0 ml with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution (c) Dissolve 5.0 mg of *moxonidine impurity A CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (d) Dilute 6.0 ml of reference solution (c) to 100.0 ml with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution (e) Dilute 2.5 ml of reference solution (a) to 50.0 ml with reference solution (c).

Column:

— *size:* *l* = 0.25 m, Ø = 4 mm;

— *stationary phase: base-deactivated octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 136 volumes of *acetonitrile R* with 1000 volumes of a 3.48 g/l solution of *sodium pentanesulphonate R* previously adjusted to pH 3.5 with *dilute sulphuric acid R*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl; inject a blank, the test solution and reference solutions (b), (d) and (e).

Run time Twice the retention time of moxonidine.

Relative retentions With reference to moxonidine (retention time = about 11.6 min): impurity A = about 0.9; impurity B = about 1.7.

System suitability Reference solution (e):

— *resolution*: minimum of 2 between the peaks due to impurity A and moxonidine.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.05 per cent). Disregard any peak observed with the blank run.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

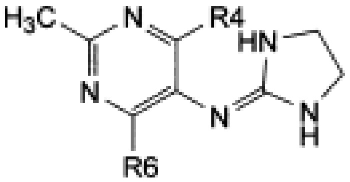
ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_9H_{12}ClN_5O$ from the areas of the peaks and the declared content of *moxonidine CRS*.

IMPURITIES



A. R4 = R6 = Cl: 4,6-dichloro-*N*-(imidazolidin-2-ylidene)-2-methylpyrimidin-5-amine (6-chloromoxonidine),

B. R4 = R6 = OCH_3: *N*-(imidazolidin-2-ylidene)-4,6-dimethoxy-2-methylpyrimidin-5-amine (4-methoxymoxonidine),

C. R4 = OH, R6 = OCH_3: 5-[(imidazolidin-2-ylidene)amino]-6-methoxy-2-methylpyrimidin-4-ol (4-hydroxymoxonidine),

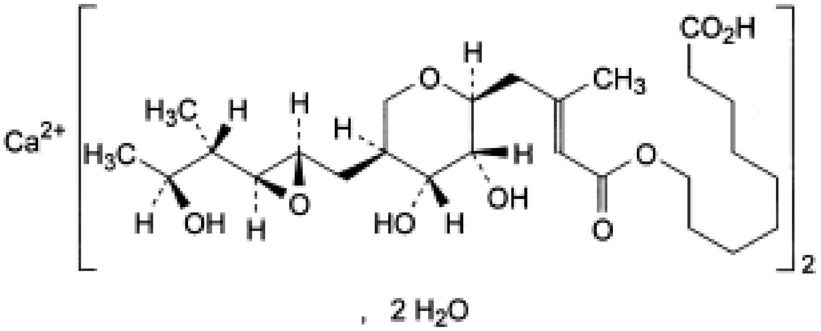
D. R4 = OH, R6 = Cl: 6-chloro-5-[(imidazolidin-2-ylidene)amino]-2-methylpyrimidin-4-ol (6-desmethylmoxonidine).

Ph Eur

Mupirocin Calcium

General Notices

(Ph Eur monograph 1451)



$C_{52}H_{86}O_{18}Ca,2H_2O$ 1075 *115074-43-6*

Action and use

Antibacterial.

Preparations

Mupirocin Cream

Mupirocin Nasal Ointment

Ph Eur

DEFINITION

Calcium bis[9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoate] dihydrate.

Substance produced by the growth of certain strains of *Pseudomonas fluorescens* or obtained by any other means.

Content

93.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, sparingly soluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of mupirocin calcium.*

B. It gives reaction (a) of calcium (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)

- 16 to - 20 (anhydrous substance).

Dissolve 0.50 g in *methanol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mix 50 volumes of *methanol R* and 50 volumes of a 13.6 g/l solution of *sodium acetate R* adjusted to pH 4.0 with *acetic acid R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture.

Reference solution (b) Adjust 10 ml of reference solution (a) to pH 2.0 with *hydrochloric acid R* and allow to stand for 20 h.

Reference solution (c) Dissolve 25 mg of *mupirocin lithium CRS* in the solvent mixture and dilute to 200.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *water R*, 30 volumes of *tetrahydrofuran R* and 50 volumes of a 10.5 g/l solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time 3.5 times the retention time of mupirocin.

Relative retention With reference to mupirocin: impurity C = about 0.75.

System suitability Reference solution (b):

— *resolution*: minimum 7.0 between the 2nd of the 2 peaks due to hydrolysis products and the peak due to mupirocin.

Limits:

- *impurity C*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent);
- *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- *total*: not more than 2.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (4.5 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Chlorides (*2.4.4*)

Maximum 0.5 per cent.

Dissolve 10.0 mg in a mixture of 1 ml of *dilute nitric acid R* and 15 ml of *methanol R*.

Water (*2.5.12*)

3.0 per cent to 4.5 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in 5 ml of *methanol R* and dilute to 200.0 ml with a 7.5 g/l solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Reference solution (a) Dissolve 25.0 mg of *mupirocin lithium CRS* in 5 ml of *methanol R* and dilute to 200.0 ml with a 7.5 g/l solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Reference solution (b) Adjust 10 ml of the test solution to pH 2.0 with *hydrochloric acid R* and allow to stand for 20 h.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 19 volumes of *water R*, 32 volumes of *tetrahydrofuran R* and 49 volumes of a 10.5 g/l solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

System suitability:

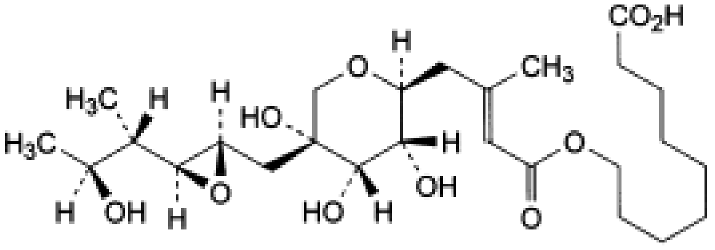
- *resolution*: minimum 7.0 between the 2nd of the 2 peaks due to hydrolysis products and the peak due to mupirocin in the chromatogram obtained with reference solution (b);
- *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

Calculate the percentage content of mupirocin calcium by multiplying the percentage content of mupirocin lithium by 1.038.

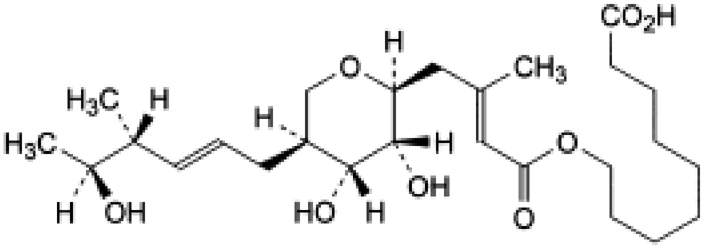
IMPURITIES

Specified impurities *C.*

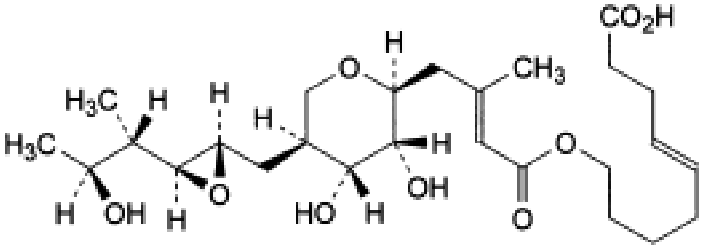
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, D, E, F, G, H, I.*



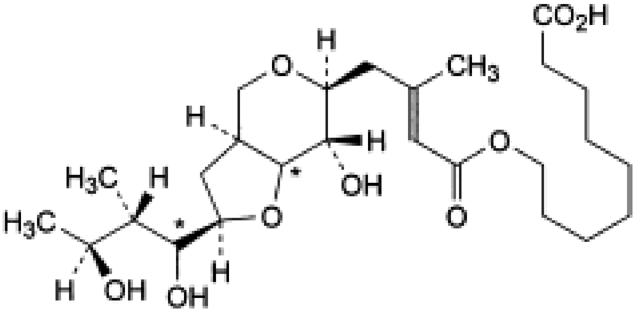
A. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*R*)-3,4,5-trihydroxy-5-[[(2*S*,3*S*)3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid (pseudomonic acid B),



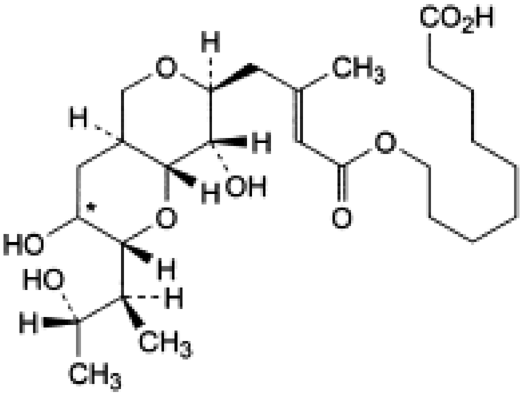
B. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(2*E*,4*R*,5*S*)-5-hydroxy-4-methylhex-2-enyl] tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid (pseudomonic acid C),



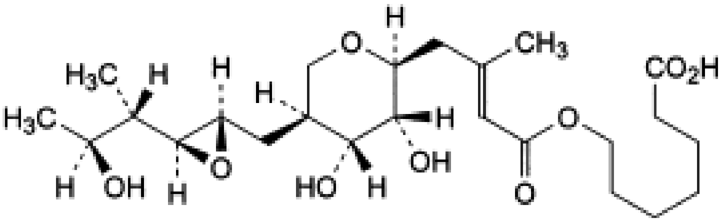
C. (4*E*)-9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]non-4-enoic acid (pseudomonic acid D),



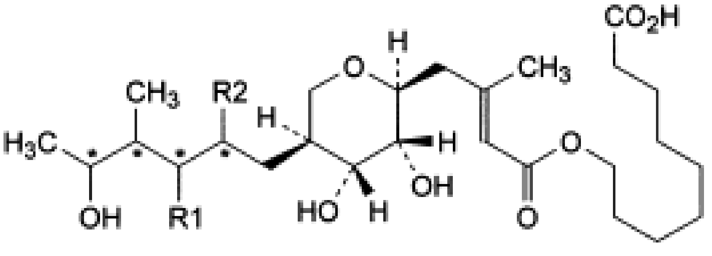
D. 9-[[(2*E*)-4-[(2*R*,3a*S*,6*S*,7*S*)-2-[(2*S*,3*S*)-1,3-dihydroxy-2-methylbutyl]-7-hydroxyhexahydro-4*H*-furo[3,2-*c*]pyran-6-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,



E. 9-[[(2*E*)-4-[(2*R*,3*RS*,4a*S*,7*S*,8*S*,8a*R*)-3,8-dihydroxy-2-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl] hexahydro-2*H*,5*H*-pyrano[4,3-*b*]pyran-7-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,

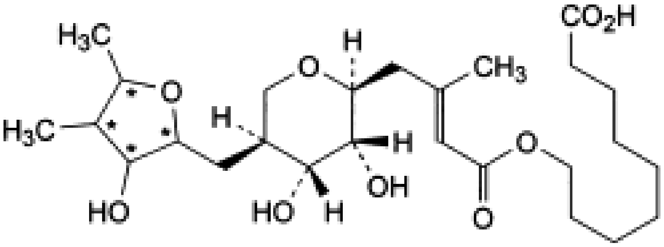


F. 7-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl] oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]heptanoic acid,



G. R1 = OH, R2 = Cl: 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-5-(2-chloro-3,5-dihydroxy-4-methylhexyl)-3, 4-dihydroxytetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,

H. R1 = Cl, R2 = OH: 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-5-(3-chloro-2,5-dihydroxy-4-methylhexyl)-3,4-dihydroxytetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,



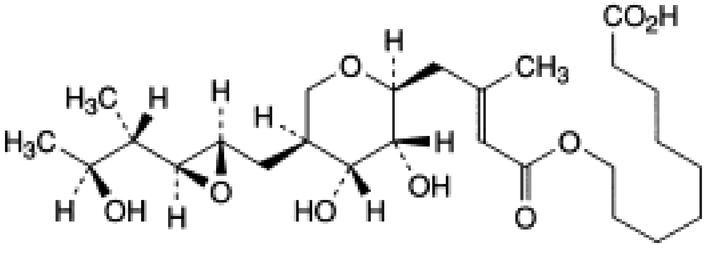
I. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(3-hydroxy-4,5-dimethyltetrahydrofuran-2-yl) methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid.

Ph Eur

Mupirocin

General Notices

(Ph Eur monograph 1450)



$C_{26}H_{44}O_9$ 500.6 *12650-69-0*

Action and use

Antibacterial.

Preparation

Mupirocin Ointment

Ph Eur

DEFINITION

9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl] oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid.

Substance produced by the growth of certain strains of *Pseudomonas fluorescens* or obtained by any other means.

Content

93.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in acetone, in anhydrous ethanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of mupirocin.*

TESTS

pH (*2.2.3*)

3.5 to 4.0 for a freshly prepared saturated solution (about 10 g/l) in *carbon dioxide-free water R.*

Specific optical rotation *(2.2.7)*

- 17 to - 21 (anhydrous substance).

Dissolve 0.50 g in *methanol R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mix 50 volumes of *methanol R* and 50 volumes of a 13.6 g/l solution of *sodium acetate R* adjusted to pH 4.0 with *acetic acid R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture.

Reference solution (b) Adjust 10 ml of reference solution (a) to pH 2.0 with *hydrochloric acid R* and allow to stand for 20 h.

Reference solution (c) Dissolve 25 mg of *mupirocin lithium CRS* in the solvent mixture and dilute to 200.0 ml with the solvent mixture.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *water R*, 30 volumes of *tetrahydrofuran R* and 50 volumes of a 10.5 g/l solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time 3.5 times the retention time of mupirocin.

Relative retention With reference to mupirocin: impurity C = about 0.75.

System suitability Reference solution (b):

— *resolution*: minimum 7.0 between the 2nd of the 2 peaks due to hydrolysis products and the peak due to mupirocin.

Limits:

- *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (4 per cent);
- *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (6 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in 5 ml of *methanol R* and dilute to 200.0 ml with a 7.5 g/l solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Reference solution (a) Dissolve 25.0 mg of *mupirocin lithium CRS* in 5 ml of *methanol R* and dilute to 200.0 ml with a 7.5 g/l solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Reference solution (b) Adjust 10 ml of the test solution to pH 2.0 with *hydrochloric acid R* and allow to stand for 20 h.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 19 volumes of *water R*, 32 volumes of *tetrahydrofuran R* and 49 volumes of a 10.5 g/l solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

System suitability:

- *resolution*: minimum 7.0 between the 2nd of the 2 peaks due to hydrolysis products and the peak due to mupirocin in the chromatogram obtained with reference solution (b);
- *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

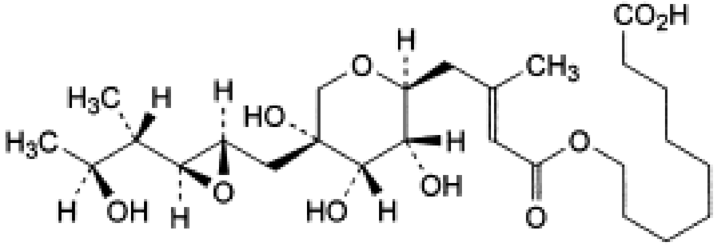
STORAGE

Protected from light.

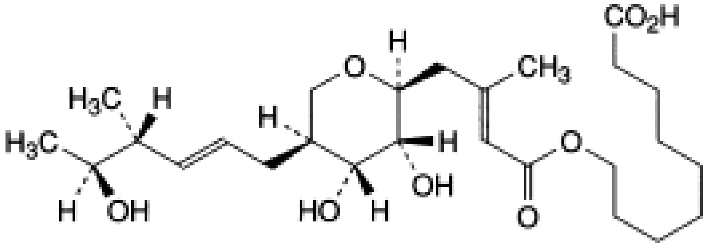
IMPURITIES

Specified impurities *C.*

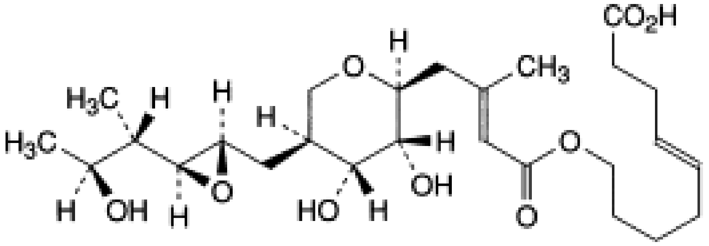
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, D, E, F.*



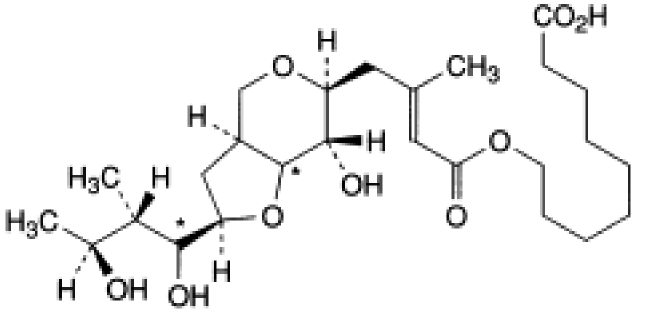
A. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*R*)-3,4,5-trihydroxy-5-[[(2*S*,3*S*)3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid (pseudomonic acid B),



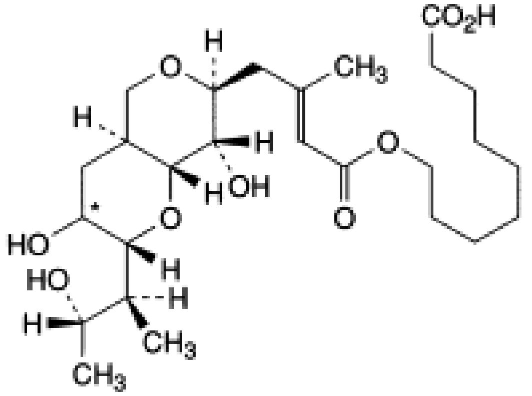
B. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(2*E*,4*R*,5*S*)-5-hydroxy-4-methylhex-2-enyl] tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid (pseudomonic acid C),



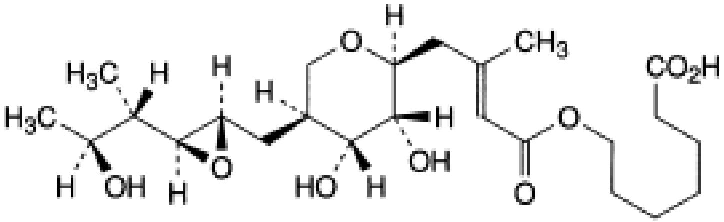
C. (4*E*)-9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]non-4-enoic acid (pseudomonic acid D),



D. 9-[[(2*E*)-4-[(2*R*,3a*S*,6*S*,7*S*)-2-[(2*S*,3*S*)-1,3-dihydroxy-2-methylbutyl]-7-hydroxyhexahydro-4H-furo[3,2-*c*]pyran-6-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,



E. 9-[[(2*E*)-4-[(2*R*,3*RS*,4a*S*,7*S*,8*S*,8a*R*)-3,8-dihydroxy-2-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl] hexahydro-2*H*,5*H*-pyrano[4,3-*b*]pyran-7-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,



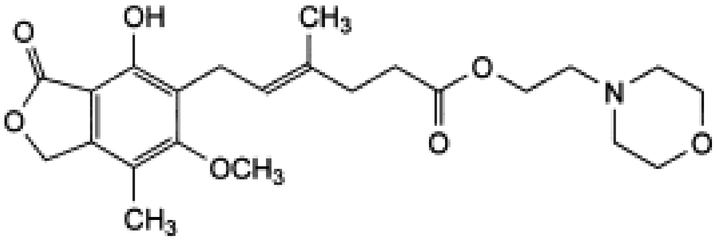
F. 7-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl] oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]heptanoic acid.

Ph Eur

Mycophenolate Mofetil

General Notices

(Ph Eur monograph 1700)



$C_{23}H_{31}NO_7$ 433.5 *115007-34-6*

Action and use

Inhibitor of nucleic acid synthesis; immunomodulator.

Ph Eur

DEFINITION

2-(Morpholin-4-yl)ethyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, sparingly soluble in anhydrous ethanol.

mp

About 96 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison   *mycophenolate mofetil CRS.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.10 g in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from light. Prepare the solutions immediately before use, or store them at 4-8 ‘C. Keep the temperature of the autosampler at 10 ‘C, allow the temperature of the solutions to equilibrate in the vials for 15 min before injection.*

Test solution   Dissolve 20 mg of the substance to be examined in *acetonitrile R* and dilute to 10 ml with the same solvent.

Reference solution (a)   Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 10.0 ml with *acetonitrile R*.

Reference solution (b)   Dissolve 5 mg of *mycophenolate mofetil for peak identification CRS* (mycophenolate mofetil with impurities A, B, D, E, F, G and H) in *acetonitrile R* and dilute to 2.5 ml with the same solvent.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 45 °C.

Mobile phase   Mix 350 ml of *acetonitrile R* with a mixture of 650 ml of *water R* and 2.0 ml of *triethylamine R* previously adjusted to pH 5.3 with *dilute phosphoric acid R*.

Flow rate   1.5 ml/min.

Detection   Spectrophotometer at 250 nm.

Injection   10 µl.

Run time   3 times the retention time of mycophenolate mofetil.

Relative retention   With reference to mycophenolate mofetil (retention time = about 22 min): impurity F = about 0.3; impurity A = about 0.4; impurity H = about 0.5; impurity G = about 0.6; impurity B = about 0.8; impurity D = about 1.2; impurity E = about 1.6.

System suitability   Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and impurity H;

— the chromatogram obtained is similar to the chromatogram supplied with *mycophenolate mofetil for peak identification CRS*.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 2.1;

— *impurity F*: not more than 5 times the area of the principal peak in the chromatogram

obtained with reference solution (a) (0.5 per cent);

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities A, D, E, G, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 43.35 mg of $C_{23}H_{31}NO_7$.

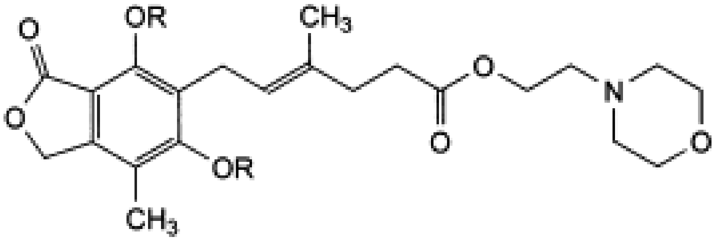
STORAGE

Protected from light.

IMPURITIES

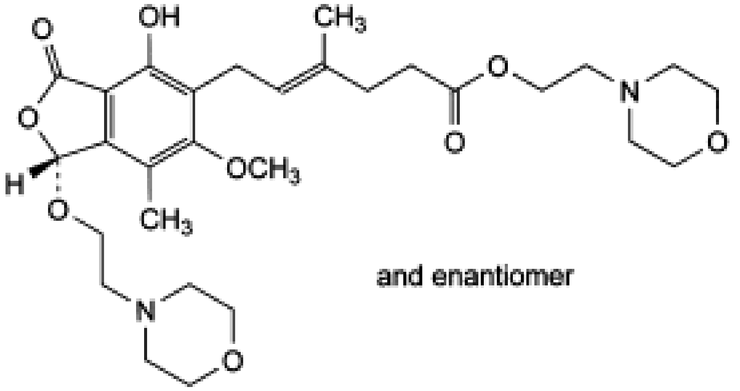
Specified impurities *A, B, D, E, F, G, H.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C.*

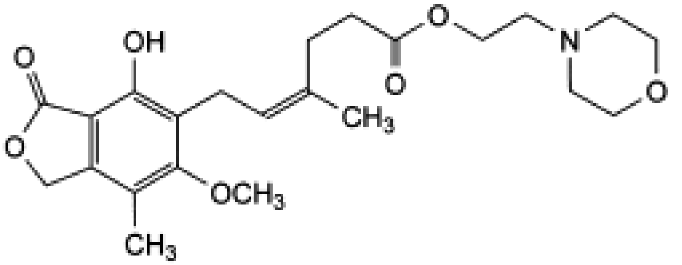


A. R = H: 2-(morpholin-4-yl)ethyl (4*E*)-6-(4,6-dihydroxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate,

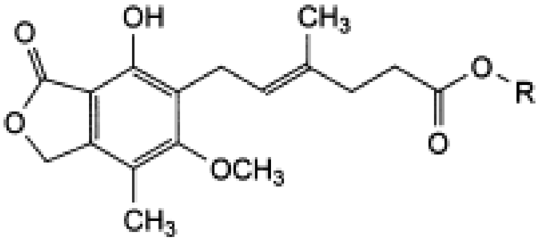
D. R = CH_3: 2-(morpholin-4-yl)ethyl (4*E*)-6-(4,6-dimethoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate,



B. 2-(morpholin-4-yl)ethyl (4*E*)-6-[(1*RS*)-4-hydroxy-6-methoxy-7-methyl-1-[2-(morpholin-4-yl) ethoxy]-3-oxo-1,3-dihydroisobenzofuran-5-yl]-4-methylhex-4-enoate,



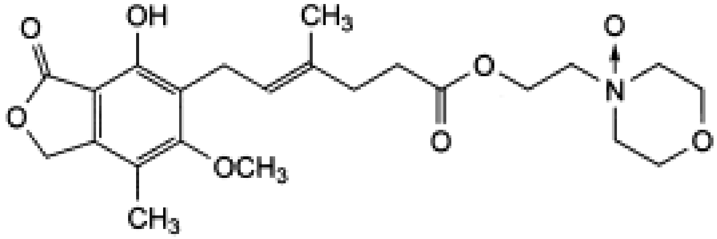
C. 2-(morpholin-4-yl)ethyl (4*Z*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate,



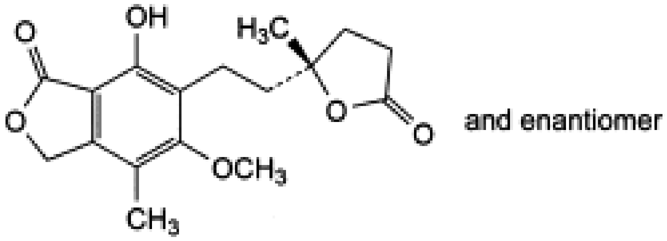
E. R = CH_3: methyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-

5-yl)-4-methylhex-4-enoate,

F. R = H: (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoic acid (mycophenolic acid),


O
OH
CH₃
O
O
N
O
O
O
OCH₃
CH₃

G. 2-(morpholin-4-yl)ethyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate *N*-oxide,


O
OH
H₃C
O
O
O
and enantiomer
OCH₃
CH₃

H. 7-hydroxy-5-methoxy-4-methyl-6-[2-[(2*RS*)-2-methyl-5-oxotetrahydrofuran-2-yl]ethyl] isobenzofuran-1(3*H*)-one.

Ph Eur

Partially Hydrated Magnesium Chloride

General Notices

(Magnesium Chloride 4.5-Hydrate, Ph Eur monograph 1341)

$MgCl_2$,~$4.5H_2O$ 95.21 (anhydrous)

Ph Eur

DEFINITION

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, granular powder, hygroscopic.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Water (see Tests).

B. It gives reaction (a) of chlorides (*2.3.1*).

C. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 5 ml of solution S add 0.05 ml of *phenol red solution R*. Not more than 0.3 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides

Maximum 500 ppm.

Dilute 2.0 ml of solution S to 10.0 ml with *water R*. To 1.0 ml of the solution add 4.0 ml of *water R*, 2.0 ml of *phenol red solution R3* and 1.0 ml of *chloramine solution R2* and mix immediately. After exactly 2 min, add 0.30 ml of *0.1 M sodium thiosulphate*, mix and dilute to 10.0 ml with *water R*. The absorbance (*2.2.25*) of the solution measured at 590 nm, using *water R* as the compensation liquid, is not greater than that of a standard prepared at the same time and in the same manner using 5.0 ml of a 3 mg/l solution of *potassium bromide R*.

Sulphates (*2.4.13*)

Maximum 100 ppm, determined on solution S.

Aluminium (*2.4.17*)

Maximum 1 ppm, if intended for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions, or haemofiltration solutions.

Prescribed solution Dissolve 4 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 0.5 g.

Calcium (*2.4.3*)

Maximum 0.1 per cent.

Dilute 1 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S.

Potassium

Maximum 5.00 × 10^2 ppm, if intended for use in the manufacture of parenteral dosage forms.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using the following solution, diluted as necessary with *water R*: dissolve 1.144 g of *potassium chloride R* , previously dried at 100-105 °C for 3 h, in *water R* and dilute to 1000.0 ml with the same solvent (600 µg of K per millilitre).

Wavelength 766.5 nm.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

44.0 per cent to 48.0 per cent, determined on 50.0 mg.

ASSAY

Dissolve 0.250 g in 50 ml of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 9.521 mg of $MgCl_2$.

STORAGE

In an airtight container .

LABELLING

The label states:

— where applicable, that the substance is suitable for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions or haemofiltration solutions;

— where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Refined Maize Oil

General Notices

(Ph Eur monograph 1342)

Ph Eur

DEFINITION

Fatty oil obtained from the seeds of *Zea mays* L. by expression or by extraction, then refined.

CHARACTERS

Appearance

Clear, light yellow or yellow oil.

Solubility

Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C) and with methylene chloride.

Relative density

About 0.920.

Refractive index

About 1.474.

IDENTIFICATION

A. Identification of fatty oils by thin-layer chromatography *(2.3.2)*.

Results The chromatogram obtained with the test solution is similar to that obtained with the reference solution.

B. Composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 0.5, or maximum 0.3 if intended for use in the manufacture of parenteral preparations, determined on 10.0 g.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0, or maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter ***(2.5.7)***

Maximum 2.8 per cent, determined on 5.0 g.

Alkaline impurities ***(2.4.19)***

It complies with the test.

Composition of fatty acids ***(2.4.22, Method A)***

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

— *fatty acids of chain length less than* C_{16}: maximum 0.6 per cent;

— *palmitic acid*: 8.6 per cent to 16.5 per cent;

— *stearic acid*: maximum 3.3 per cent;

— *oleic acid*: 20.0 per cent to 42.2 per cent;

— *linoleic acid*: 39.4 per cent to 65.6 per cent;

— *linolenic acid*: 0.5 per cent to 1.5 per cent;

— *arachidic acid*: maximum 0.8 per cent;

— *eicosenoic acid*: maximum 0.5 per cent;

— *behenic acid*: maximum 0.5 per cent;

— *other fatty acids*: maximum 0.5 per cent.

Sterols ***(2.4.23)***

Maximum 0.3 per cent of brassicasterol in the sterol fraction of the oil.

Water ***(2.5.32)***

Maximum 0.1 per cent if intended for use in the manufacture of parenteral preparations, determined on 5.00 g. Use a mixture of equal volumes of *anhydrous methanol R* and *decanol R* as the solvent.

STORAGE

Protected from light, at a temperature not exceeding 25 °C.

LABELLING

The label states:

— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;

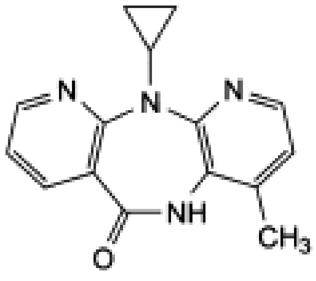
— whether the oil is obtained by mechanical expression or by extraction.

Ph Eur

Anhydrous Nevirapine

General Notices

(Ph Eur monograph 2255)



$C_{15}H_{14}N_4O$ 266.3 *129618-40-2*

Action and use

Non-nucleoside reverse transcriptase inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

11-Cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido [3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, sparingly soluble or slightly soluble in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison anhydrous nevirapine CRS.

B. Loss on drying (see Tests).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 24.0 mg of the substance to be examined in a mixture of 4 ml of *acetonitrile R* and 80 ml of the mobile phase and sonicate until dissolution is complete. Dilute to 100.0 ml with the mobile phase.

Test solution (b) Dilute 3.0 ml of test solution (a) to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Add 2.0 ml of the mobile phase to a vial of *nevirapine for peak identification CRS* (containing impurities A, B and C), mix and sonicate for 1 min.

Reference solution (c) Dissolve 24.0 mg of *anhydrous nevirapine CRS* in a mixture of 4 ml of *acetonitrile R* and 80 ml of the mobile phase and sonicate until complete dissolution. Dilute to 100.0 ml with the mobile phase. Dilute 3.0 ml of this solution to 25.0 ml with the mobile phase.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *hexadecylamidylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 35 °C.

Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a 2.88 g/l solution of *ammonium dihydrogen phosphate R*, previously adjusted to pH 5.0 using *dilute sodium hydroxide solution R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µl of test solution (a) and reference solutions (a) and (b).

Run time 10 times the retention time of nevirapine.

Identification of impurities Use the chromatogram supplied with *nevirapine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to nevirapine (retention time = about 8 min): impurity B = 0.7; impurity A = 1.5; impurity C = 2.8.

System suitability Reference solution (b):

— *resolution*: minimum 5 between the peaks due to impurity B and nevirapine.

Limits:
— *impurities A, B, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.6 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

0.50 g complies with test G. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

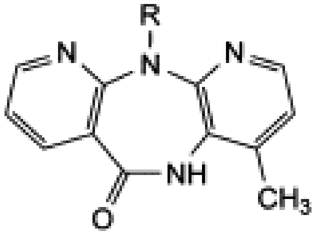
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 25 µl of test solution (b) and reference solution (c).

Calculate the percentage content of $C_{15}H_{14}N_4O$ from the declared content of *anhydrous nevirapine CRS.*

IMPURITIES

Specified impurities *A, B, C.*



A. R = C_2H_5: 11-ethyl-4-methyl-5,11-dihydro-6*H*-dipyrido [3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,

B. R = H: 4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,

C. R = CH_2-CH_2-CH_3: 4-methyl-11-propyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4] diazepin-6-one.

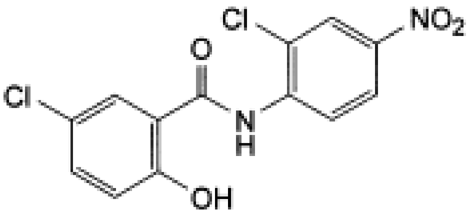
Ph Eur

Anhydrous Niclosamide

General Notices

Niclosamide

(Ph Eur monograph 0679)



$C_{13}H_8Cl_2N_2O_4$ 327.1   *50-65-7*

Action and use

Anthelminthic.

Preparation

Niclosamide Tablets

Ph Eur

DEFINITION

5-Chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellowish-white or yellowish, fine crystals.

Solubility

Practically insoluble in water, sparingly soluble in acetone, slightly soluble in anhydrous ethanol.

IDENTIFICATION

First identification B, E.

Second identification *A, C, D, E.*

A. Melting point (*2.2.14*): 227 °C to 232 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs prepared using about 0.5 mg of substance and 0.3 g of *potassium bromide R*.

Comparison *anhydrous niclosamide CRS.*

C. To 50 mg add 5 ml of *1 M hydrochloric acid* and 0.1 g of *zinc powder R*, heat in a water-bath for 10 min, cool and filter. To the filtrate add 1 ml of a 5 g/l solution of *sodium nitrite R* and allow to stand for 3 min; add 2 ml of a 20 g/l solution of *ammonium sulphamate R*, shake, allow to stand for 3 min and add 2 ml of a 5 g/l solution of *naphthylethylenediamine dihydrochloride R*. A violet colour is produced.

D. Heat the substance on a copper wire in a non-luminous flame. The flame becomes green.

E. Loss on drying (see Tests).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R*, heating gently, cool and dilute to 50.0 ml with the same solvent.

Reference solution Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 20.0 ml with *acetonitrile R*.

Column:
- *size*: *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mixture of equal volumes of *acetonitrile R* and a solution containing 2 g/l of *potassium dihydrogen phosphate R*, 1 g/l of *disodium hydrogen phosphate R* and 2 g/l of *tetrabutylammonium hydrogen sulphate R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time Twice the retention time of niclosamide.

Limits:
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.005 per cent).

5-Chlorosalicylic acid

Maximum 60 ppm.

Test solution To 1.0 g add 15 ml of *water R*, boil for 2 min, cool, filter through a membrane filter (nominal pore size: 0.45 µm), wash the filter and dilute the combined filtrate and washings to 20.0 ml with *water R*.

Reference solution Dissolve 30 mg of *5-chlorosalicylic acid R* in 20 ml of *methanol R* and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*.

To 10.0 ml of the test solution and to 10.0 ml of the reference solution add separately 0.1 ml of *ferric chloride solution R2*. Any violet colour in the test solution is not more intense than that in the reference solution.

2-Chloro-4-nitroaniline

Maximum 100 ppm.

Test solution To 0.250 g add 5 ml of *methanol R*, heat to boiling, cool, add 45 ml of *1 M hydrochloric acid*, heat again to boiling, cool, filter and dilute the filtrate to 50.0 ml with *1 M hydrochloric acid*.

Reference solution Dissolve 50 mg of *2-chloro-4-nitroaniline R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Dilute 2.0 ml of this solution to 20.0 ml with *1 M hydrochloric acid*.

To 10.0 ml of the test solution and to 10.0 ml of the reference solution add separately 0.5 ml of a 5 g/l solution of *sodium nitrite R* and allow to stand for 3 min. Add 1 ml of a 20 g/l solution of *ammonium sulphamate R*, shake, allow to stand for 3 min and add 1 ml of a 5 g/l solution of *naphthylethylenediamine dihydrochloride R*. Any pinkish-violet colour in the test solution is not more intense than that in the reference solution.

Chlorides (*2.4.4*)

Maximum 500 ppm.

To 2 g add a mixture of 1.2 ml of *acetic acid R* and 40 ml of *water R*, boil for 2 min, cool and filter. Dilute 2 ml of the filtrate to 15 ml with *water R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.3000 g in 80 ml of a mixture of equal volumes of *acetone R* and *methanol R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

STORAGE

In an airtight container, protected from light.

Ph Eur

Low-Oxygen Nitrogen

General Notices

(Nitrogen, Low-Oxygen, Ph Eur monograph 1685)

N_2 28.01

DEFINITION

This monograph applies to nitrogen which is used for inerting finished medicinal products which are particularly sensitive to degradation by oxygen. It does not necessarily apply to nitrogen used in earlier production steps.

Content

Minimum 99.5 per cent *V/V* of N_2, calculated by deduction of the sum of impurities found when performing the test for impurities.

CHARACTERS

Colourless and odourless gas.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 62 volumes of water and about 10 volumes of alcohol.

PRODUCTION

Oxygen

Maximum 5 ppm *V/V*, determined using an oxygen analyser with a detector scale ranging from 0 ppm *V/V* to 100 ppm *V/V* and equipped with an electrochemical cell.

The gas to be examined passes through a detection cell containing an aqueous solution of an electrolyte, generally potassium hydroxide. The presence of oxygen in the gas to be examined produces variation in the electric signal recorded at the outlet of the cell that is proportional to the oxygen content.

Calibrate the analyser according to the manufacturer's instructions. Pass the gas to be examined through the analyser using a suitable pressure regulator and airtight metal tubes and operating at the prescribed flow rates until constant readings are obtained.

Impurities

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas (a) Use ambient air.

Reference gas (b) Use *nitrogen R1* .

Column:

- *material*: stainless steel;
- *size: l* = 2 m, Ø = 2 mm;
- *stationary phase*: appropriate molecular sieve for chromatography (0.5 nm).

Carrier gas *helium for chromatography R*.

Flow rate 40 ml/min.

Temperature:

- *column*: 50 °C;
- *detector*: 130 °C.

Detection Thermal conductivity.

System suitability Reference gas (a): adjust the injected volumes and operating conditions so that the height of the peak due to nitrogen in the chromatogram obtained is at least 35 per cent of the full scale of the recorder:

- the chromatogram obtained shows a clear separation of oxygen and nitrogen.

Limit:

- *total*: not more than 0.5 per cent of the sum of the areas of all the peaks (0.5 per cent *V/V*).

IDENTIFICATION

First identification *A*.

Second identification *B, C*.

A. Examine the chromatograms obtained in the test for impurities (see Production).

Results The principal peak in the chromatogram obtained with the gas to be examined is similar in retention time to the principal peak in the chromatogram obtained with reference gas (b).

B. In a 250 ml conical flask replace the air by the gas to be examined. Place a burning or glowing splinter of wood in the flask. The splinter is extinguished.

C. In a suitable test tube, place 0.1 g of *magnesium R* in turnings. Close the tube with a two-hole stopper fitted with a glass tube reaching about 1 cm above the turnings. Pass the gas to be examined through the glass tube for 1 min without heating, then for 15 min while heating the test tube to a red glow. After cooling, add 5 ml of *dilute sodium hydroxide solution R*. The evolving vapours turn the colour of moistened *red litmus paper R* blue.

STORAGE

Where the gas has to be stored, store as a compressed gas or a liquid in appropriate containers complying with the legal regulations.

IMPURITIES

A. oxygen,

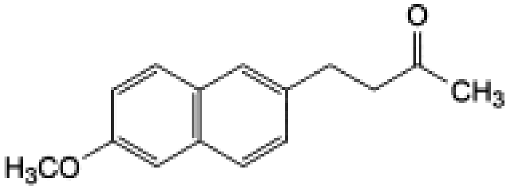
B. argon.

Ph Eur

Nabumetone

General Notices

(Ph Eur monograph 1350)



$C_{15}H_{16}O_2$ 228.3 *42924-53-8*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Nabumetone Oral Suspension

Nabumetone Tablets

Ph Eur

DEFINITION

4-(6-Methoxynaphthalen-2-yl)butan-2-one.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, slightly soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison nabumetone CRS.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 25.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 5.0 ml with *acetonitrile R*.

Reference solution (a) Dissolve 20.0 mg of *nabumetone CRS* in *acetonitrile R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with *acetonitrile R*.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100.0 ml with *acetonitrile R*.

Reference solution (c) Dissolve 1.5 mg of *nabumetone impurity F CRS* in *acetonitrile R* and dilute to 100.0 ml with the same solvent.

Reference solution (d) Dissolve 4 mg of *nabumetone impurity D CRS* in *acetonitrile R* and dilute to 100 ml with the same solvent. To 5 ml of this solution, add 5 ml of test solution (b).

Column:

— *size: l* = 0.15 m, Ø = 4.6 mm;

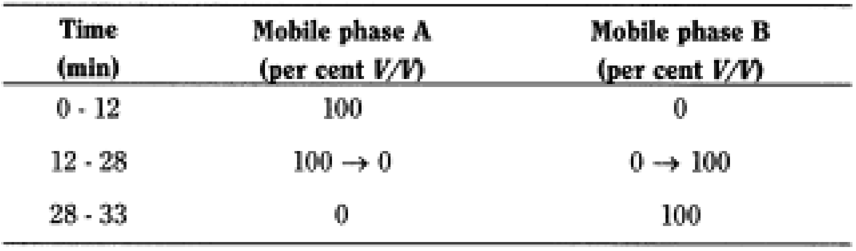
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (4 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: mix 12 volumes of *tetrahydrofuran R*, 28 volumes of *acetonitrile* for *chromatography R* and 60 volumes of a 0.1 per cent *V/V* solution of *glacial acetic acid R* in *carbon dioxide-free water R* prepared from *distilled water R*;

— *mobile phase B*: mix 24 volumes of *tetrahydrofuran R*, 56 volumes of acetonitrile for *chromatography R* and 20 volumes of a 0.1 per cent *V/V* solution of *glacial acetic acid R* in *carbon dioxide-free water R* prepared from *distilled water R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	100	0
12 - 28	100 → 0	0 → 100
28 - 33	0	100

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of test solution (a) and reference solutions (b), (c) and (d).

Retention time Nabumetone = about 11 min.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to nabumetone and impurity D.

Limits:

- *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *sum of impurities other than F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 0.2 per cent, determined on 1.000 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

System suitability Reference solution (a):

- *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{15}H_{16}O_2$ from the declared content of *nabumetone CRS.*

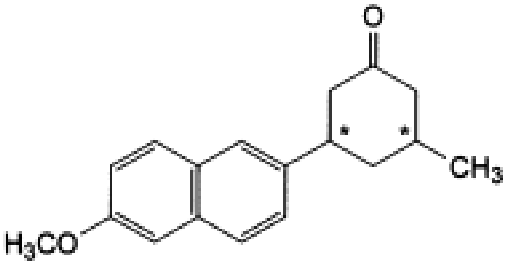
STORAGE

Protected from light.

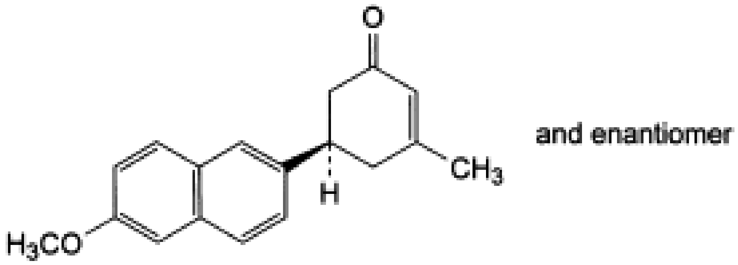
IMPURITIES

Specified impurities F.

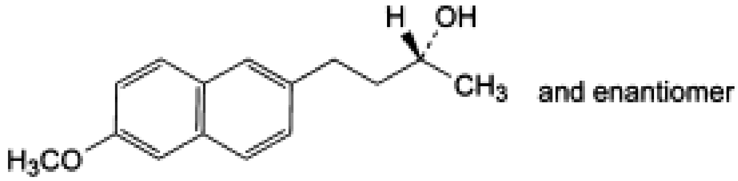
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, D, E.*



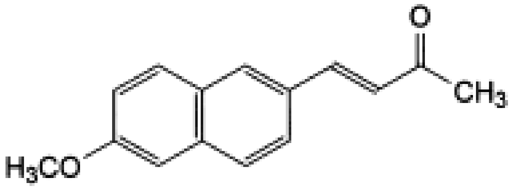
A. 3-(6-methoxynaphthalen-2-yl)-5-methylcyclohexanone,



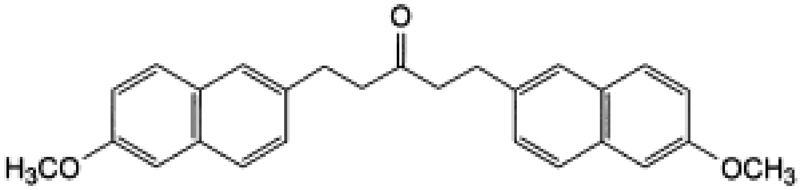
B. (5*RS*)-5-(6-methoxynaphthalen-2-yl)-3-methylcyclohex-2-enone,



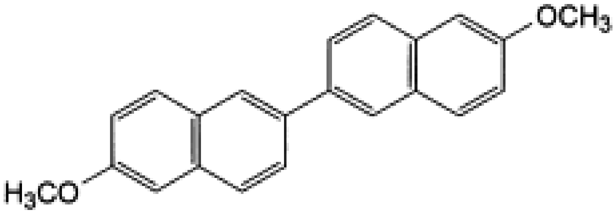
C. (2*RS*)-4-(6-methoxynaphthalen-2-yl)butan-2-ol,



D. (*E*)-4-(6-methoxynaphthalen-2-yl)but-3-en-2-one,



E. 1,5-bis(6-methoxynaphthalen-2-yl)pentan-3-one,



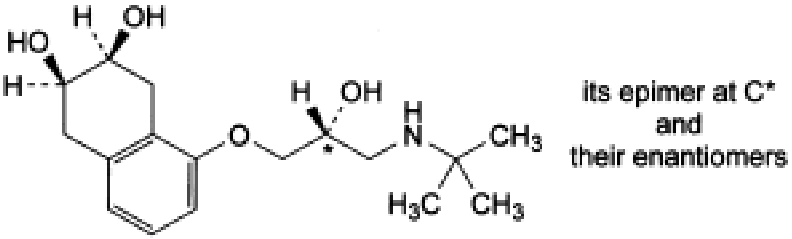
F. 6,6′-dimethoxy-2,2′-binaphthalenyl.

Ph Eur

Nadolol

General Notices

(Ph Eur monograph 1789)



$C_{17}H_{27}NO_4$ 309.4 *42200-33-9*

Action and use

Beta-adrenoceptor antagonist.

Ph Eur

DEFINITION

cis-5-[3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol.

It consists of 2 pairs of enantiomers which are present as 2 racemic compounds: racemate A and racemate B.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison nadolol CRS.

TESTS

Racemate content

Infrared absorption spectrophotometry (*2.2.24*).

Prepare a mull in *liquid paraffin R* of the substance to be examined (dried substance), adjusting the thickness of the mull to give an absorbance reading of 0.6 ± 0.1 at 1587 cm^{-1}. Record the spectrum from 1667 to 1111 cm^{-1}, using *liquid paraffin R* as reference. Measure the absorbance A_a, corresponding to racemate A, at the maximum at 1266 cm^{-1} and the absorbance A_b, corresponding to racemate B, at the maximum at 1250 cm^{-1}. The ratio A_a/A_b is 0.72 to 1.08 (corresponding to racemate A content of between 40 per cent and 60 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in 4.0 ml of *methanol R* and dilute to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*. Dilute 5.0 ml of the solution to 50.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Reference solution (b) Dissolve 20 mg of *pindolol CRS* and 20 mg of the substance to be examined in 20 ml of *methanol R* and dilute to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*. Dilute 1.0 ml of the solution to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

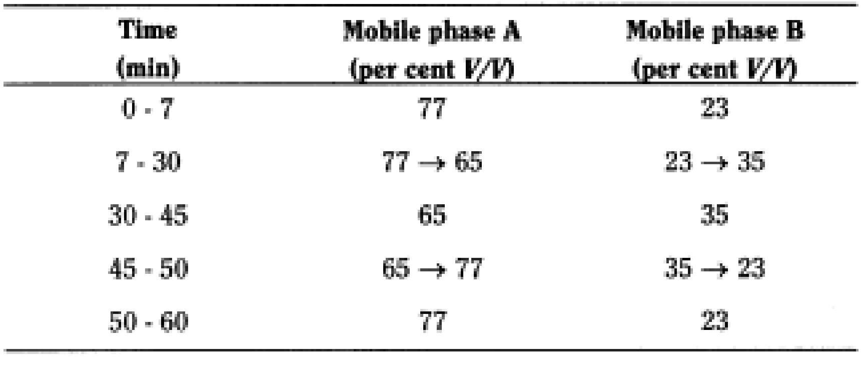
Reference solution (c) Dilute 5.0 ml of reference solution (a) to 20.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Column:

- *size:* l = 0.30 m, Ø = 3.9 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm), with a specific surface area of 700 m2/g, a pore size of 0.9 nm and a carbon loading of 12 per cent;
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: 5.6 g/l solution of *sodium octanesulphonate R* adjusted to pH 3.5 with a 300 g/l solution of *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	77	23
7 - 30	77 → 65	23 → 35
30 - 45	65	35
45 - 50	65 → 77	35 → 23
50 - 60	77	23

Flow rate 1 ml/min.

Detection Spectrophotometer at 206 nm.

Injection 20 µl.

Relative retention With reference to nadolol (retention time = about 18 min): impurity A = 0.25; impurity B = 0.4; impurity C (doublet) = 0.6 and 0.7; impurity D = 1.4; impurity E = 1.6; impurity F = 2.2; impurity G = 2.6.

System suitability Reference solution (b):

— *resolution*: minimum of 8.0 between the peaks due to nadolol and to pindolol.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.7; impurity B = 1.7; impurity C = 1.7 (multiply the sum of the areas of the 2 peaks);

— *impurity A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 30 ppm.

1.0 g complies with limit test D. Prepare the standard using 3 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 2.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash (*2.4.14*)

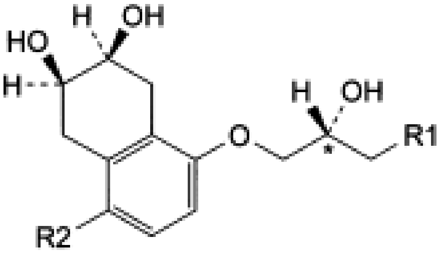
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 100 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

I ml of *0.1 M perchloric acid* is equivalent to 30.94 mg of $C_{17}H_{27}NO_4$.

IMPURITIES

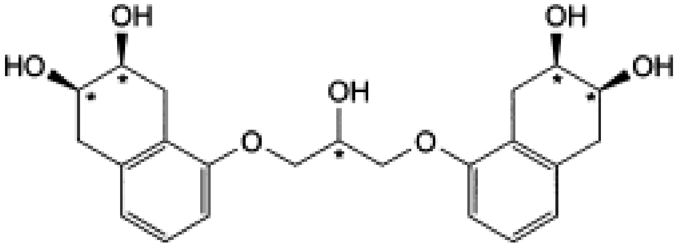


its epimer at C*
and
their enantiomers

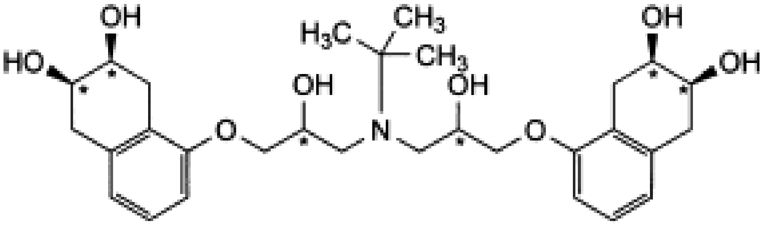
A. R1 = OH, R2 = H: *cis*-5-[(2*RS*)-2,3-dihydroxypropoxy]-1,2,3,4-tetrahydronaphtalene-2,3-diol (tetraol),

B. R1 = OCH_3, R2 = H: *cis*-5-[(2*RS*)-2-hydroxy-3-methoxypropoxy]-1,2,3,4-tetrahydronaphtalene-2,3-diol,

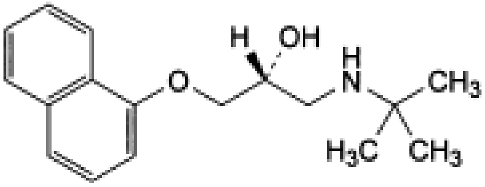
E. R1 = NH-$C(CH_3)_3$, R2 = I: *cis*-5-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-8-iodo-1,2,3,4-tetrahydronaphtalene-2,3-diol,



C. 5,5′-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(*cis*-1,2,3,4-tetrahydronaphthalene-2,3-diol),

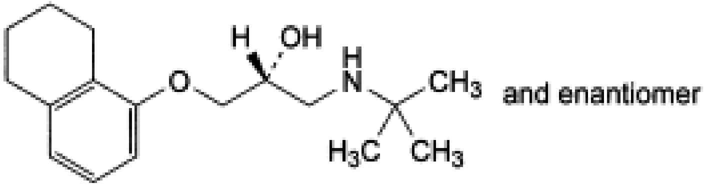


D. 5,5′-[[(1,1-dimethylethyl)imino]bis[(2-hydroxypropane-1,3-diyl)oxy]]bis(*cis*-1,2,3,4-tetrahydronaphthalene-2,3-diol),



and enantiomer

F. (2*RS*)-1-[(1,1-dimethylethyl)amino]-3-(naphthalen-1-yloxy)propan-2-ol,


and enantiomer

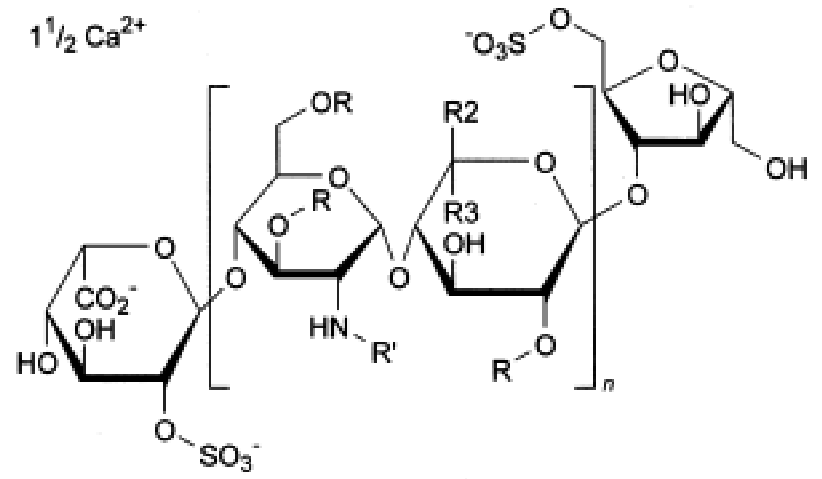
G. (2*RS*)-1-[(1,1-dimethylethyl)amino]-3-[(5,6,7,8-tetrahydronaphtalen-1-yl)oxy]propan-2-ol.

Ph Eur

Nadroparin Calcium

General Notices

(Ph Eur monograph 1134)



R = H or $SO_3(\frac{1}{2}Ca)$, R' = H or $SO_3(\frac{1}{2}Ca)$ or $CO\text{-}CH_3$

R2 = H and R3 = $CO_2(\frac{1}{2}Ca)$ or R2 = $CO_2(\frac{1}{2}Ca)$ and R3 = H

Action and use

Low molecular weight heparin.

Ph Eur

DEFINITION

Nadroparin calcium is the calcium salt of low-molecular-mass heparin obtained by nitrous acid depolymerisation of heparin from pork intestinal mucosa, followed by fractionation to eliminate selectively most of the chains with a molecular mass lower than 2000. The majority of the components have a 2-*O*-sulpho-α-L-idopyranosuronic acid structure at the non-reducing end and a 6-*O*-sulpho-2,5-anhydro-D-mannitol structure at the reducing end of their chain.

Nadroparin calcium complies with the monograph Low-molecular-mass heparins (0828) with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 3600 and 5000 with a characteristic value of about 4300.

The degree of sulphatation is about 2 per disaccharide unit.

The potency is not less than 95 IU and not more than 130 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 2.5 and 4.0.

IDENTIFICATION

Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *nadroparin calcium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. The following requirements apply.

The mass-average relative molecular mass ranges between 3600 and 5000. The mass percentage of chains lower than 2000 is not more than 15 per cent. The mass percentage of chains between 2000 and 8000 ranges between 75 per cent and 95 per cent. The mass percentage of chains between 2000 and 4000 ranges between 35 per cent and 55 per cent.

TESTS

Appearance of solution

Dissolve 0.5 g of the substance to be examined in 10 ml of *water R*. The solution is not more intensely opalescent than the reference suspension II (*2.2.1*) and not more intensely coloured than the reference solution Y_5 (*2.2.2, Method II*).

Ethanol

Not more than 1.0 per cent *m/m*, determined by head-space gas chromatography (*2.2.28*), using *2-propanol R* as the internal standard.

Internal standard solution Dilute 1.0 ml of *2-propanol R* to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 50.0 ml with *water R*.

Reference solution Dilute 1.0 ml of *ethanol R* to 100.0 ml with *water R*. Dilute 0.5 ml of this solution to 20.0 ml with *water R*.

Vial filling Place the following into 4 separate vials which can be crimp-sealed and which are compatible with the injection system:

— 1.0 ml of *water R* (blank vial);

— 0.50 ml of the reference solution and 0.50 ml of the internal standard solution (reference vial);

— 10.0 mg of the substance to be examined. Add 1.0 ml of *water R* (test vial A);

— 10.0 mg of the substance to be examined. Add 0.50 ml of *water R* and 0.50 ml of the internal standard solution (test vial B).

The chromatographic procedure may be carried out using:

— a nickel column 1.5 m long and 2 mm in internal diameter packed with *ethylvinylbenzene divinylbenzene copolymer R* (150 µm to 180 µm);

— *helium for chromatography R* or *nitrogen for chromatography R* as the carrier gas at a flow rate of 30 ml/min;

— a flame-ionisation detector.

Maintain the temperature of the column at 150 °C and those of the injection port and the detector at 250 °C.

Equilibrate each vial in the head-space system at 90 °C for 15 min. The pre-injection pressurisation time is 1 min.

The chromatogram obtained with the reference vial shows 2 peaks which correspond in order of increasing retention time to ethanol and 2-propanol (with retention times of approximately 2.5 min and 4 min). Calculate the content of ethanol (*m/m*) taking its density at 20 °C to be 0.792 g/ml.

N-NO groups

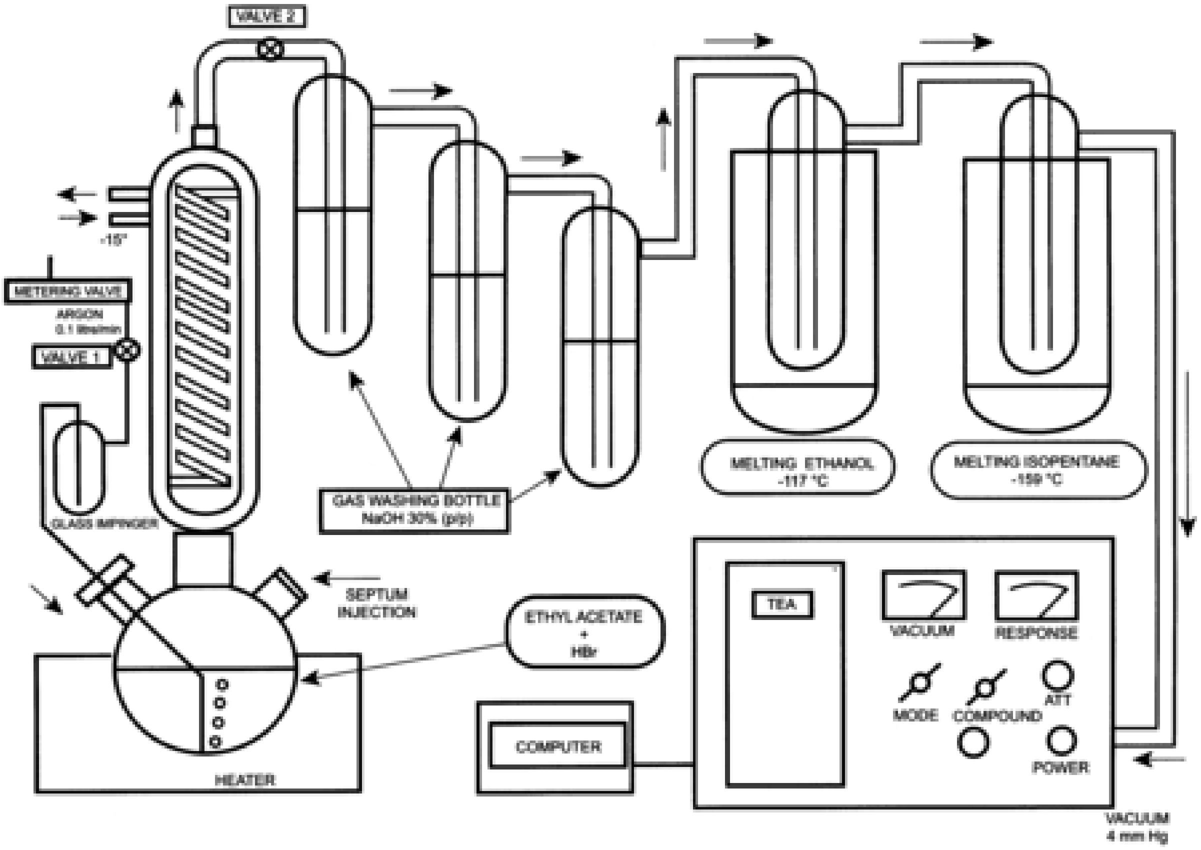
Not more than 0.25 ppm, determined by cleavage of the N-NO bond with hydrobromic acid in ethyl acetate under a reflux condenser and detection of the released NO by chemiluminescence.

Description of the apparatus (Figure 1134-1) Use a 500 ml borosilicate glass round-bottomed flask, above which is attached a condenser which is equipped with:

— on one side, a torion joint through which a stream of *argon R* can be introduced via a cannula;

— on the other side, a screw joint with a piston equipped with a septum through which the reference solution and test solution will be injected.

The round-bottomed flask is connected in series to 3 bubble traps which are themselves connected to 2 cold traps, which are in turn connected to a chemiluminescence detector. Suitable tubing ensures the junctions are leak-free.


VALVE 2
-15°
METERING VALVE
ARGON
0.1 litres/min
VALVE 1
GLASS IMPINGER
GAS WASHING BOTTLE
NaOH 30% (p/p)
SEPTUM
INJECTION
ETHYL ACETATE
+
HBr
HEATER
MELTING ETHANOL
-117 °C
MELTING ISOPENTANE
-159 °C
TEA
VACUUM
RESPONSE
MODE
COMPOUND
ATT
POWER
COMPUTER
VACUUM
4 mm Hg

Bubble traps. Height: 24 cm, internal diameter: 2.5 cm, internal tubing 23 cm in length by 0.5 cm internal diameter. Centrally positioned Rotulex mounting. Equipped with torion joints on the inlet and outlet.

Chemiluminescence detector.

Cold trap. Height: 16.5 cm, internal diameter: 4 cm, internal tubing 14 cm in length and internal diameter 1.3 cm. Equipped with torion joints on the inlet and outlet.

Condenser. Height: 21 cm, internal diameter: 3 cm. Lower rodavis joint and upper torion joint.

Flask. Round-bottomed borosilicate glass flask equipped with a central rodavis joint, a torion joint on the left neck and a 15 cm screw joint on the right neck.

Isothermic flask. Internal depth: 22 cm, internal diameter: 8 cm.

Septum. Silicone material, diameter: 14 mm, thickness: 3.5 mm.

Torion joint.

Tubing. Polytetrafluoroethylene FEP material, internal diameter: 3.2 mm, thickness: 0.8 mm.

Figure 1134.-1. – *Apparatus used for the assay of N-NO groups*

Preparation of the chemiluminescence detector Switch on the chemiluminescence detector 48 h before use and start the vacuum pump. The vacuum must be less than 0.5 mm Hg. 1 h before use, open the oxygen valve at a pressure of 0.2 MPa and a flow rate of 9.4 ml/min.

Preparation of the bubble trap In each bubble trap, place 30 ml of a 300 g/l solution of *sodium hydroxide R* in *water R*.

Preparation of the cold traps

— Trap at - 120 °C: Slowly add liquid nitrogen to an isothermic flask containing 250 ml of *ethanol R* whilst stirring with a wooden spatula until a paste is obtained. Place the cold trap in the isothermic flask prepared as described.

— Trap at - 160 °C: Slowly add liquid nitrogen to an isothermic flask containing 250 ml of *2-methylbutane R* whilst stirring with a wooden spatula until a paste is obtained. Place the cold trap in the isothermic flask prepared as described.

Drying of the 500 ml borosilicate-glass round-bottomed flask and condenser Boil 50 ml of *ethyl acetate R* under reflux for 1 h under *argon R* without connecting the system to the chemiluminescence detector.

Test solution Dry the substance to be examined for 12 h over *diphosphorus pentoxide R* at 60 °C under vacuum. Dissolve 0.10 g of the treated substance to be examined in 1.0 ml of *treated formamide R*. Shake the solution obtained for 30 min.

Reference solution Dilute 0.1 ml of *nitrosodipropylamine solution R* in 6.0 ml of *ethanol R*. Dilute 0.1 ml of the solution obtained in 1.0 ml of *treated formamide R*. (This solution is equivalent to 0.05 ppm of N-NO groups).

Place 50 ml of *treated ethyl acetate R* in the dry 500 ml borosilicate glass round-bottomed flask equipped with a septum. Connect the round-bottomed flask to the condenser which has been previously cooled to - 15 °C for 2 h.

Connect the *argon R* cannula and adjust the flow rate to 0.1 litre/min. Check that the system is leak-free. Only the connector to the chemiluminescence detector remains open in order to avoid excess pressure.

Heat the treated *ethyl acetate R* to boiling.

Evacuate the system by slowly turning the valve of the chemiluminescence detector. At the same time tighten the inlet on the chemiluminescence detector.

When the system is equilibrated, the vacuum reaches 4 mm Hg.

The signal of the zero adjuster on the chemiluminescence detector is set to 10 per cent of the full scale of the recorder.

Through the septum of the 500 ml borosilicate glass round-bottomed flask, sequentially inject 0.5 ml of *water R*, 2.0 ml of *dilute hydrobromic acid R* and then another 2.0 ml of *dilute hydrobromic acid R*, making sure that the recorder pen has returned to the baseline between each injection.

Inject 50.0 µl of the reference solution, then 50.0 µl of the test solution after the recorder pen has returned to the baseline.

Calculate the content of N-NO groups of the substance to be examined.

Free sulphates

Not more than 0.5 per cent, determined by liquid chromatography (*2.2.29*) using an instrument equipped with a conductivity detector.

Test solution Dissolve 30.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 1.4787 g of *anhydrous sodium sulphate R* in *water R* and dilute to 1000.0 ml with the same solvent. Dilute 1.0 ml of this solution to 200.0 ml with *distilled water R* (5 ppm of sulphate ions).

The chromatographic procedure may be carried out using:

— an anion separation column 50 mm long and 4.6 mm in internal diameter;

— a chemical neutralisation system: neutralisation micromembrane in line with the mobile phase for anion detection;

— elute with a 1.91 g solution of *disodium tetraborate R* in 1000 ml of *water R* as the mobile phase for 15 min; change to 100 per cent of *0.1 M sodium hydroxide* for a period of 0.5 min;

elute with this solution for 10 min; return to the initial conditions for a period of 0.5 min; the flow rate is 1.0 ml/min,

— a detector with a sensitivity of 30 µS.

Continuously pump the chemical neutralisation system in counter-flow with a 2.45 g/l solution of *sulphuric acid R*, at a flow rate of 4 ml/min.

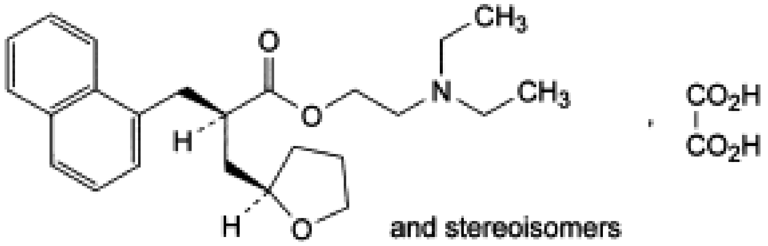
Inject 50 µl of each solution. The chromatogram obtained with the reference solution shows a principal peak which corresponds to the sulphate ion (retention time of about 7.5 min). Change the composition of the mobile phase, if necessary, to obtain the prescribed retention time. Calculate the sulphate content of the substance to be examined.

Ph Eur

Naftidrofuryl Oxalate

General Notices

(Naftidrofuryl Hydrogen Oxalate, Ph Eur monograph 1594)



$C_{24}H_{33}NO_3,C_2H_2O_4$ 473.6 *3200-06-4*

Action and use

Vasodilator.

Preparation

Naftidrofuryl Capsules

Ph Eur

DEFINITION

Mixture of 4 stereoisomers of 2-(diethylamino)ethyl 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoate hydrogen oxalate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, freely soluble or soluble in alcohol, slightly or sparingly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 1.0 g in *water R* and dilute to 50 ml with the same solvent. Add 2 ml of *concentrated ammonia R* and shake with 3 quantities, each of 10 ml, of *methylene chloride R*. To the combined lower layers, add *anhydrous sodium sulphate R*, shake, filter and evaporate the filtrate at a temperature not exceeding 30 °C, using a rotary evaporator. Use the residue obtained.

Comparison Ph. Eur. reference spectrum of naftidrofuryl.

B. Dissolve 0.5 g in *water R* and dilute to 10 ml with the same solvent. Add 2.0 ml of *calcium chloride solution R*. A white precipitate is formed. The precipitate dissolves after the addition of 3.0 ml of *hydrochloric acid R*.

TESTS

Absorbance (*2.2.25*)

Maximum 0.1 at 430 nm.

Dissolve 1.5 g in *water R* and dilute to 10 ml with the same solvent. If necessary use an ultrasonic bath.

Related substances

A. Liquid chromatography (*2.2.29*).

Test solution Dissolve 80.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase. Treat in an ultrasonic bath for 10 s. A precipitate is formed. Filter through a 0.45 µm membrane filter, discarding the first 5 millilitres. Use a freshly prepared solution.

Reference solution (a) Dissolve 5.0 mg of *naftidrofuryl impurity A CRS* in *acetonitrile R* and dilute to 25.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *naftidrofuryl impurity B CRS* and 5 mg of the substance to be examined in *acetonitrile R* and dilute to 50 ml with the same solvent. Dilute 1 ml of this solution to 50 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m2/g, a pore size of 10 nm and a carbon loading of 14 per cent.

Mobile phase Mix 60 ml of *methanol R* with 150 ml of *tetrabutylammonium buffer solution pH 7.0 R* and dilute to 1000 ml with *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 283 nm.

Injection 20 µl.

Run time 2.3 times the retention time of naftidrofuryl.

Relative retention With reference to naftidrofuryl (retention time = about 7 min): impurity A = about 0.5; impurity B = about 0.8; impurity C = about 1.8.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to naftidrofuryl and impurity B.

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

B. Gas chromatography (*2.2.28*).

Test solution (a) Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 50 ml with the same solvent. Add 2 ml of *concentrated ammonia R* and shake with 3 quantities, each of 10 ml, of *methylene chloride R*. To the combined lower layers, add *anhydrous sodium sulphate R*, shake, filter and evaporate the filtrate at a temperature not exceeding 30 °C, using a rotary evaporator. Take up the residue with *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *methylene chloride R*.

Reference solution Dissolve 5 mg of *naftidrofuryl impurity F CRS* in *methylene chloride R* and dilute to 50 ml with the same solvent.

Column:

— *material*: fused silica;

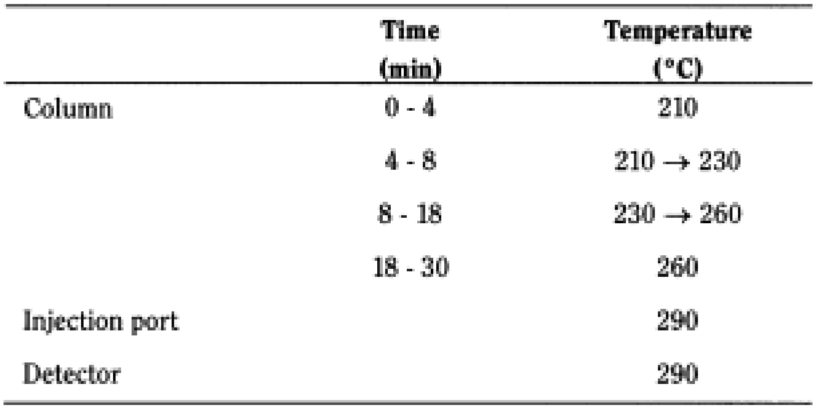
— *size: l* = 25 m, Ø = 0.32 mm;

— *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.45 µm).

Carrier gas *helium for chromatography R*.

Splitter flow rate 25 ml/min.

Flow rate 2.9 ml/min.



	Time (min)	Temperature (°C)
Column	0 - 4	210
	4 - 8	210 → 230
	8 - 18	230 → 260
	18 - 30	260
Injection port		290
Detector		290

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to the second eluting peak of naftidrofuryl: impurity D = about 0.14; impurity B = about 0.55 (for the second eluting peak); impurity E = about 0.86; impurity F = about 1.04 (for the second eluting peak).

System suitability Test solution (b):

— *resolution*: minimum 1.0 between the 2 peaks due to the diastereoisomers of naftidrofuryl.

Limits Test solution (a):

— *impurity F*: for the sum of the areas of the 2 peaks, maximum 0.20 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.20 per cent);

— *impurity E*: maximum 0.20 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.20 per cent);

— *impurity D*: maximum 0.10 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.10 per cent);

— *any other impurity*: for each impurity, maximum 0.10 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.10 per cent);

— *total*: maximum 0.50 per cent of the sum of the areas of the 2 peaks due to naftidroduryl (0.50 per cent);

— *disregard limit*: 0.02 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.02 per cent); disregard any peaks due to impurity B.

Diastereoisomer ratio

Gas chromatography (*2.2.28*) as described in test B for related substances.

Limits Test solution (b):

— *first eluting naftidrofuryl diastereoisomer* : minimum 30 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

In a silica crucible, mix thoroughly 1.0 g of the substance to be examined with 0.5 g of *magnesium oxide R1*. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained. If after 30 min of ignition the mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary repeat the operation. Heat at 800 ± 50 °C for about 1 h. Take up the residue with 2 quantities, each of 5 ml, of a mixture of equal volumes of *hydrochloric acid R1* and *water R*. Add 0.1 ml of *phenolphthalein solution R* and then *concentrated ammonia R* until a pink colour is obtained. Cool, add *glacial acetic acid R* until the solution is decolorised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with *water R*. The solution complies with limit test E. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

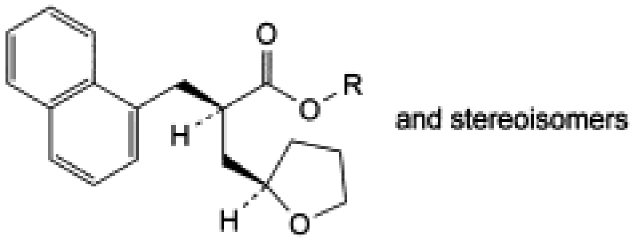
ASSAY

Dissolve 0.350 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 47.36 mg of $C_{26}H_{35}NO_7$.

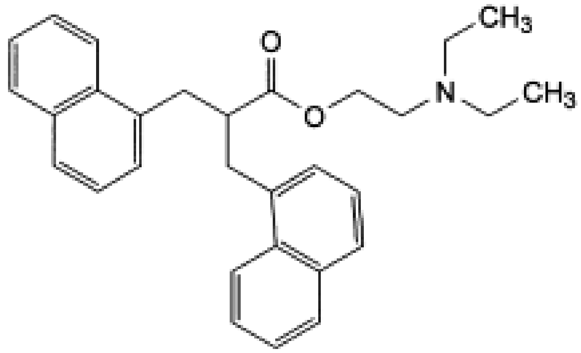
IMPURITIES

Specified impurities A, B, C, D, E, F.

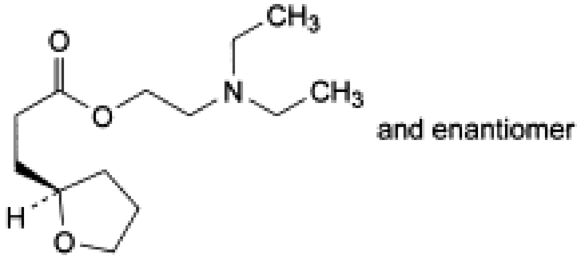

O
R
O
H
H
O
and stereoisomers

A. R = H: 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoic acid,

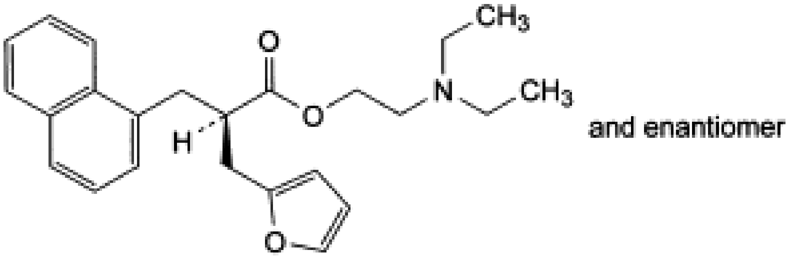
B. R = C_2H_5: ethyl 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoate,



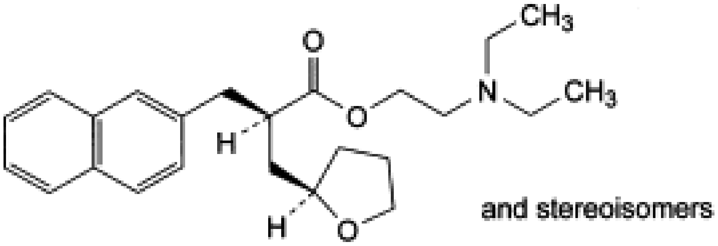
C. 2-(diethylamino)ethyl 3-(naphthalen-1-yl)-2-[(naphthalen-1-yl)methyl]propanoate,


O
CH3
O
N
CH3
and enantiomer
H
O

D. 2-(diethylamino)ethyl 3-[(2*RS*)-tetrahydrofuran-2-yl]propanoate,


and enantiomer

E. 2-(diethylamino)ethyl (2*RS*)-2-[(furan-2-yl)methyl]-3-(naphthalen-1-yl)propanoate,


and stereoisomers

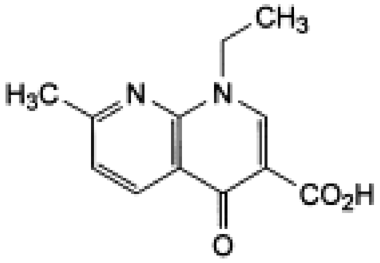
F. 2-(diethylamino)ethyl 2-[(naphthalen-2-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoate.

Ph Eur

Nalidixic Acid

General Notices

(Ph Eur monograph 0701)



$C_{12}H_{12}N_2O_3$ 232.2 *389-08-2*

Action and use

Quinolone antibacterial.

Preparations

Nalidixic Acid Oral Suspension

Nalidixic Acid Tablets

Ph Eur

DEFINITION

Nalidixic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid, calculated with reference to the dried substance.

CHARACTERS

An almost white or pale yellow, crystalline powder, practically insoluble in water, soluble in methylene chloride, slightly soluble in acetone and in alcohol. It dissolves in dilute solutions of alkali hydroxides.

It melts at about 230 °C.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Dissolve 12.5 mg in *0.1 M sodium hydroxide* and dilute to 50.0 ml with the same solvent.

Dilute 2.0 ml of this solution to 100.0 ml with *0.1 M sodium hydroxide*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows two absorption maxima, at 258 nm and 334 nm. The ratio of the absorbance measured at 258 nm to that measured at 334 nm is 2.2 to 2.4.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *nalidixic acid CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with the test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g in 2 ml of *hydrochloric acid R*. Add 0.5 ml of a 100 g/l solution of *β-naphthol R* in *alcohol R*. An orange-red colour develops.

TESTS

Absorbance

Dissolve 1.50 g in *methylene chloride R* and dilute to 50.0 ml with the same solvent. The absorbance (*2.2.25*) measured at 420 nm is not greater than 0.10.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel* F_{254} *plate R.*

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 20 ml with *methylene chloride R*.

Reference solution (a) Dissolve 20 mg of *nalidixic acid CRS* in *methylene chloride R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dilute 2 ml of test solution (b) to 10 ml with *methylene chloride R*.

Reference solution (c) Dilute 1 ml of reference solution (b) to 10 ml with *methylene chloride R.*

Reference solution (d) Dilute 1 ml of reference solution (b) to 25 ml with *methylene chloride R.*

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *dilute ammonia R1*, 20 volumes of *methylene chloride R* and 70 volumes of *alcohol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (d).

Heavy metals (*2.4.8*)

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 10 ml of *methylene chloride R* and add 30 ml of *2-propanol R* and 10 ml of *carbon dioxide-free water R*. Keep the titration vessel covered and pass *nitrogen R* through the solution throughout the titration. Keep the temperature of the solution between 15 °C and 20 °C. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*) using a silver-silver chloride comparison electrode with a sleeve diaphragm or a capillary tip, filled with a saturated solution of *lithium chloride R* in *ethanol R*, and a glass electrode as indicator electrode.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 23.22 mg of $C_{12}H_{12}N_2O_3$.

STORAGE

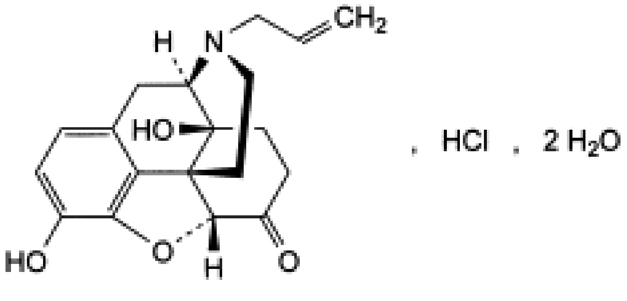
Store in an airtight container, protected from light.

Ph Eur

Naloxone Hydrochloride

General Notices

(Naloxone Hydrochloride Dihydrate, Ph Eur monograph 0729)



$C_{19}H_{21}NO_4,HCl,2H_2O$ 399.9 *51481-60-8*

Action and use

Opioid receptor antagonist.

Preparations

Naloxone Injection

Neonatal Naloxone Injection

Ph Eur

DEFINITION

4,5α-Epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one hydrochloride dihydrate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent), practically insoluble in toluene.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *naloxone hydrochloride dihydrate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 8 mg of the substance to be examined in 0.5 ml of *water R* and dilute to 1 ml with *methanol R*.

Reference solution Dissolve 8 mg of *naloxone hydrochloride dihydrate CRS* in 0.5 ml of *water R* and dilute to 1 ml with *methanol R*.

Plate *TLC silica gel G plate R*.

Mobile phase Mix 5 volumes of *methanol R* and 95 volumes of the upper layer from a mixture of 60 ml of *dilute ammonia R2* and 100 ml of *butanol R*.

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a freshly prepared 5 g/l solution of *potassium ferricyanide R* in *ferric chloride solution R1*; examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10.0 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.2 ml of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

- 170 to - 181 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.125 g of the substance to be examined *in 0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 5 mg of *naloxone for peak identification CRS* (containing impurities A, B, C, D, E and F) in 1 ml of *0.1 M hydrochloric acid*.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *0.1 M hydrochloric acid*. Dilute 1.0 ml of this solution to 25.0 ml with *0.1 M hydrochloric acid*.

Solution A Dissolve 1.10 g of *sodium octanesulphonate R* in 1000 ml of *water R*, adjust to pH 2.0 with a 50 per cent *V/V* solution of *phosphoric acid R* and filter.

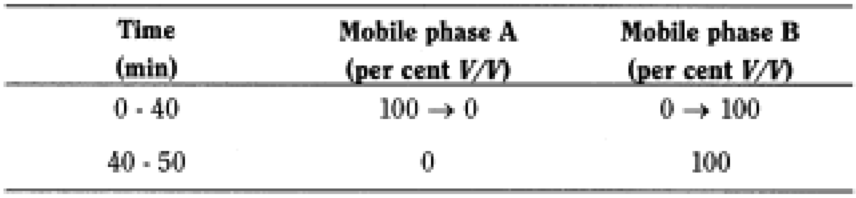
Column:
— *size:* l = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R*, *tetrahydrofuran R*, solution A (20:40:940 *V/V/V*);

— *mobile phase B*: *tetrahydrofuran R*, *acetonitrile R*, solution A (40:170:790 *V/V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	100 → 0	0 → 100
40 - 50	0	100

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Relative retention With reference to naloxone (retention time = about 11 min): impurity C = about 0.6; impurity A = about 0.8; impurity F = about 0.9; impurity D = about 1.1; impurity E = about 3.0; impurity B = about 3.2.

Identification of impurities Use the chomatogram supplied with *naloxone for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E and F.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 2.0, where $H_p =$ height above the baseline of the peak due to impurity D and $H_v =$ height above the baseline of the lowest point of the curve separating this peak from the peak due to naloxone.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity E by 0.5;

— *impurities A, B, C, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

7.5 per cent to 11.0 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 0.50 g.

ASSAY

Dissolve 0.300 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric* acid. Carry out a potentiometric titration (*2.2.20*), using *0.1 M ethanolic sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 36.38 mg of $C_{19}H_{22}ClNO_4$.

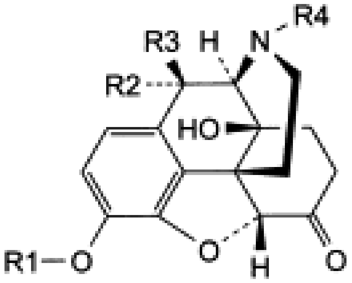
STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): G.*



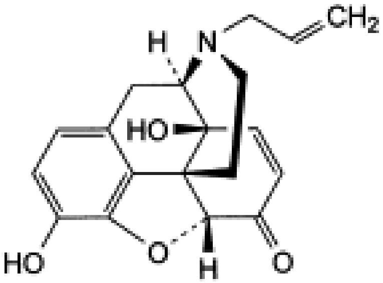
A. R1 = R2 = R3 = R4 = H: 4,5α-epoxy-3,14-dihydroxymorphinan-6-one (noroxymorphone),

B. R1 = R4 = CH_2-CH=CH_2, R2 = R3 = H: 4,5α-epoxy-14-hydroxy-17-(prop-2-enyl)-3-(prop-2-enyloxy)morphinan-6-one (3-*O*-allylnaloxone),

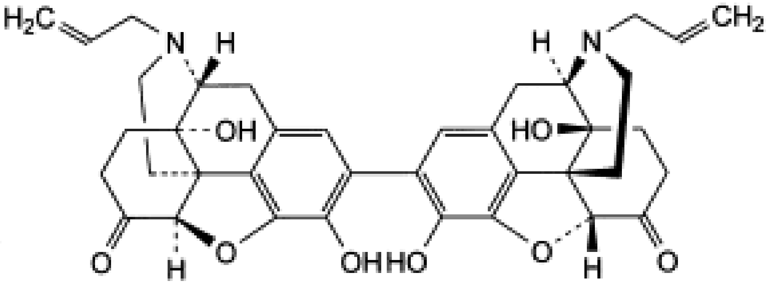
C. R1 = R3 = H, R2 = OH, R4 = CH_2-CH=CH_2: 4,5α-epoxy-3,10α,14-trihydroxy-17-(prop-2-enyl)morphinan-6-one (10α-hydroxynaloxone),

F. R1 = R2 = H, R3 = OH, R4 = CH_2-CH=CH_2: 4,5α-epoxy-3,10β,14-trihydroxy-17-(prop-2-enyl)morphinan-6-one (10β-hydroxynaloxone),

G. R1 = CH_3, R2 = R3 = H, R4 = CH_2-CH=CH_2: 4,5α-epoxy-14-hydroxy-3-methoxy-17-(prop-2-enyl)morphinan-6-one (3-*C*-methylnaloxone),



D. 7,8-didehydro-4,5α-epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one (7,8-didehydronaloxone),



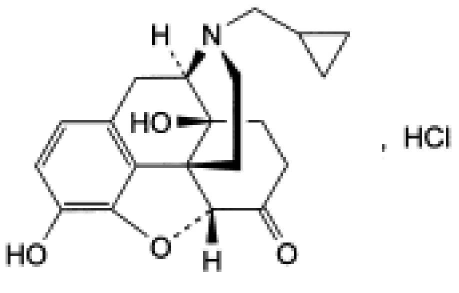
E. 4,5α:4′,5′α-diepoxy-3,3′,14,14′-tetrahydroxy-17,17′-bis(prop-2-enyl)-2,2′-bimorphinanyl-6,6′-dione (2,2′-binaloxone).

Ph Eur

Naltrexone Hydrochloride

General Notices

(Ph Eur monograph 1790)



$C_{20}H_{24}ClNO_4$ 377.9 *16676-29-2*

Action and use

Opioid receptor antagonist.

Ph Eur

DEFINITION

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride. It may be anhydrous, a monohydrate or a dihydrate, a mixture or a solvate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, very hygroscopic.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Dissolve 20 mg in *water R* and dilute to 5 ml with the same solvent. Make alkaline with *dilute ammonia R1*. Shake with 10 ml of *methylene chloride R*, separate the organic layer and evaporate the solvent. Dry the residue obtained *in vacuo*.

Comparison *naltrexone hydrochloride CRS*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.40 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or B_6 (*2.2.2, Method II*).

Acidity and alkalinity

To 10 ml of solution S, add 0.05 ml of *methyl red solution R*. Not more than 0.2 ml of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

- 187 to - 195 (anhydrous substance).

Dissolve 0.40 g in *water R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *naltrexone impurity C CRS* in *0.1 M hydrochloric acid* and dilute to 2.5 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution and 1.0 ml of reference solution (a) to 100.0 ml with *0.1 M hydrochloric acid*. Dilute 1.0 ml of this solution to 10.0 ml with *0.1 M hydrochloric acid*.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

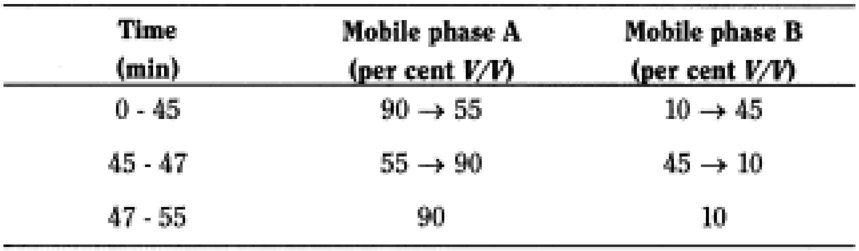
— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: 1.1 g/l solution of *sodium octanesulphonate R* adjusted to pH 2.3 with *phosphoric acid R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 45	90 → 55	10 → 45
45 - 47	55 → 90	45 → 10
47 - 55	90	10

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 230 nm.

Equilibration 8 min.

Injection 10 µl.

Relative retention With reference to naltrexone (retention time = about 16 min): impurity A = about 0.4; impurity B = about 0.7; impurity F = about 0.8; impurity G = about 0.9; impurity C = about 1.05; impurity H = about 1.1; impurity I = about 1.2; impurity J = about 1.3; impurity D = about 1.4; impurity E = 1.7.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to naltrexone and impurity C.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 0.4;

— *impurities C, D, E, F, G*: for each impurity, not more than twice the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities A, B, H, I, J:* for each impurity, not more than the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 10 times the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol (*2.4.24, System A*)

Maximum 3.0 per cent.

Test solution Dissolve 0.25 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution Dilute 0.750 g of *anhydrous ethanol R* to 1000.0 ml with *water R*.

Water (*2.5.12*)

Maximum 10.0 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 60 ml of *ethanol (96 per cent) R*, add 1.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. The curve shows 3 points of inflexion. Read the volume added between the first 2 points of inflexion.

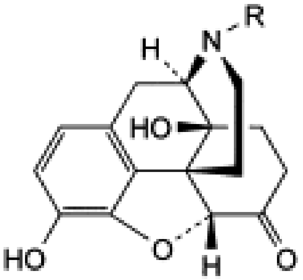
1 ml of *0.1 M sodium hydroxide* is equivalent to 37.79 mg of $C_{20}H_{24}ClNO_4$.

STORAGE

In an airtight container . Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J.

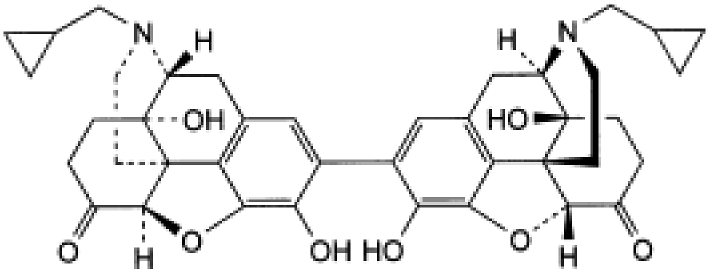


A. R = CHO: 17-formyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one,

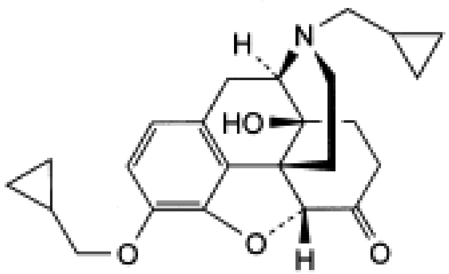
B. R = H: 4,5α-epoxy-3,14-dihydroxymorphinan-6-one (noroxymorphone),

C. R = CH_2-CH_2-CH=CH_2: 17-but-3-enyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one,

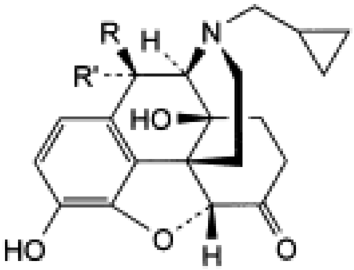
H. R = CH_2-CH_2-CH_2-CH_3: 17-butyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one,



D. 17,17′-bis(cyclopropylmethyl)-4,5α:4′,5′α-diepoxy-3,3′,14,14′-tetrahydroxy-2,2′-bimorphinanyl-6,6′-dione (pseudonaltrexone),



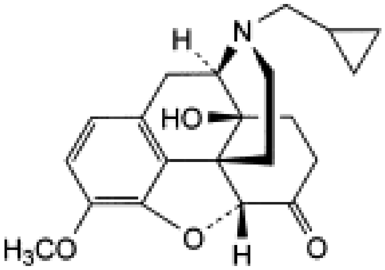
E. 3-(cyclopropylmethoxy)-17-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxymorphinan-6-one,



F. R = H, R′ = OH: 17-(cyclopropylmethyl)-4,5α-epoxy-3,10α,14-trihydroxymorphinan-6-one,

G. R = OH, R′ = H: 17-(cyclopropylmethyl)-4,5α-epoxy-3,10β,14-trihydroxymorphinan-6-one,

I. R + R′ = O: 17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6,10-dione,



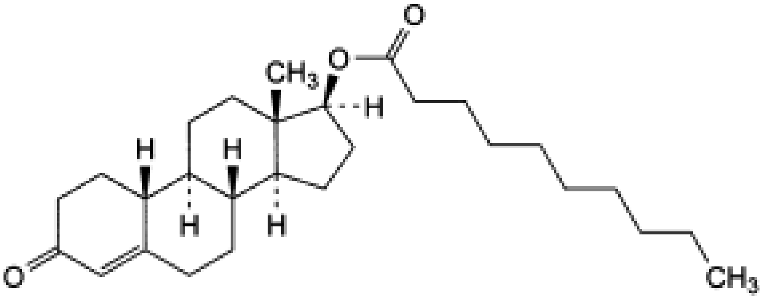
J. 7-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxy-3-methoxymorphinan-6-one.

Ph Eur

Nandrolone Decanoate

General Notices

(Ph Eur monograph 1992)



$C_{28}H_{44}O_3$ 428.7 *360-70-3*

Action and use

Anabolic steroid; androgen.

Ph Eur

DEFINITION

3-Oxoestr-4-en-17β-yl decanoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Melting point (*2.2.14*): 34 °C to 38 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison nandrolone decanoate CRS.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.20 g in 10 ml of *methanol R*.

Specific optical rotation (*2.2.7*)

+ 35.0 to + 40.0 (dried substance).

Dissolve 0.200 g in *anhydrous ethanol R* and dilute to 20.0 ml with the same solvent.

Impurities A, B, C

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with *methylene chloride R*.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with *methylene chloride R*.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 20.0 ml with *methylene chloride R*.

Reference solution (d) Dissolve 5 mg of *nandrolone decanoate for system suitability CRS* (containing impurities A, B, C) in 0.5 ml of *methylene chloride R*.

Plate *TLC silica gel plate R*.

Mobile phase *acetone R*, *heptane R* (30:70 *V/V*).

Application 10 µl of the test solution and reference solutions (b), (c) and (d).

Development Over 2/3 of the plate.

Drying In air.

Detection Treat with *alcoholic solution of sulphuric acid R* and heat at 130 °C until the spots appear. Examine in ultraviolet light at 366 nm.

Retardation factors Nandrolone decanoate = about 0.37; impurity A = about 0.45; impurity B = about 0.55; impurity C = about 0.58.

System suitability Reference solution (d):

— the chromatogram shows 4 clearly separated spots.

Limits:

— *impurity A*: any spot due to impurity A is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurities B, C*: any spot due to impurity B or C is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Related substances

Liquid chromatography (*2.2.29*).

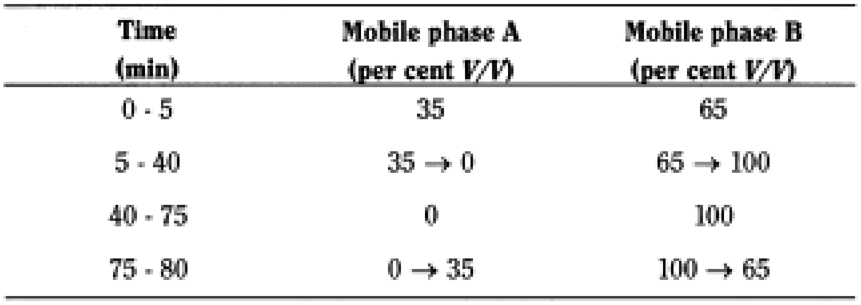
Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Reference solution (b) Dissolve 5 mg of *nandrolone decanoate for peak identification CRS* (containing impurities D, F, G, H, I, K, L) in *methanol R* and dilute to 2.0 ml with the same solvent.

Column:
- *size: l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *water R;*
- *mobile phase B*: *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	35	65
5 - 40	35 → 0	65 → 100
40 - 75	0	100
75 - 80	0 → 35	100 → 65

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Relative retention With reference to nandrolone decanoate (retention time = about 30 min): impurity D = about 0.05; impurity F = about 0.6; impurity K = about 0.7; impurity L = about 0.9; impurity G = about 0.97; impurity H = about 1.1; impurity I = about 1.2.

System suitability Reference solution (b):

- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nandrolone decanoate.

Limits:
- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.5; impurity F = 0.6; impurity H = 1.1; impurity I = 1.3; impurity K = 0.8;
- *impurities D, F, G, H, I, K, L*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in

the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R*, at a pressure not exceeding 0.7 kPa for 4 h at room temperature.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with *anhydrous ethanol R*. Measure the absorbance (2.2.25) at the absorption maximum at 240 nm. Calculate the content of $C_{28}H_{44}O_3$ taking the specific absorbance to be 407.

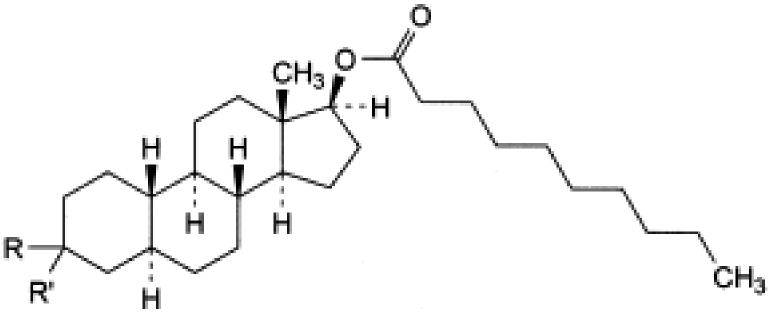
STORAGE

Under nitrogen, protected from light and at a temperature of 2 °C to 8 °C.

IMPURITIES

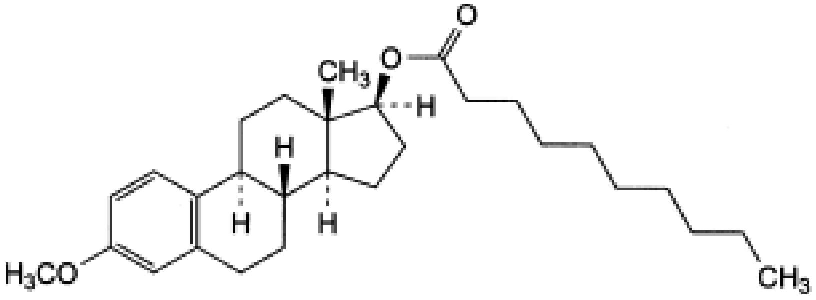
Specified impurities *A, B, C, D, F, G, H, I, K, L.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, J.*

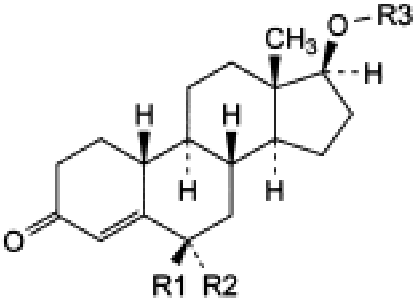


A. R + R′ = O: 3-oxo-5α-estran-17β-yl decanoate,

C. R = R′ = OCH_3: 3,3-dimethoxy-5α-estran-17β-yl decanoate,



B. 3-methoxyestra-1,3,5(10)-trien-17β-yl decanoate,



D. R1 = R2 = R3 = H: 17β-hydroxyestr-4-en-3-one,

E. R1 = H, R2 = OH, R3 = CO-$[CH_2]_8$-CH_3: 6α-hydroxy-3-oxoestr-4-en-17β-yl decanoate,

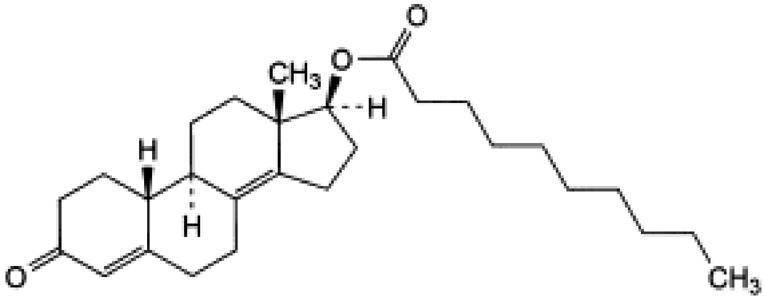
F. R1 + R2 = O, R3 = CO-$[CH_2]_8$-CH_3: 3,6-dioxoestr-4-en-17β-yl decanoate,

H. R1 = R2 = H, R3 = CO-$[CH_2]_9$-CH_3: 3-oxoestr-4-en-17β-yl undecanoate,

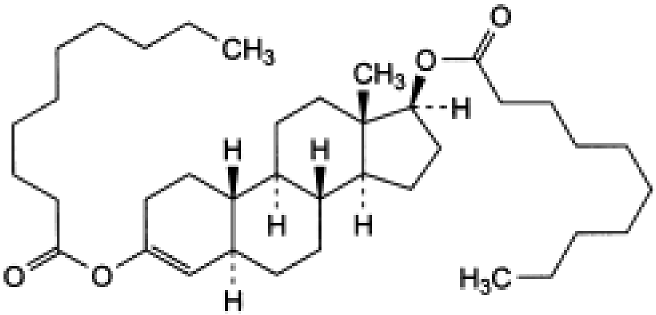
I. R1 = R2 = H, R3 = CO-$[CH_2]_{10}$-CH_3: 3-oxoestr-4-en-17β-yl dodecanoate,

K. R1 = R2 = H, R3 = CO-$[CH_2]_6$-CH_3: 3-oxoestr-4-en-17β-yl octanoate,

L. R1 = R2 = H, R3 = CO-$[CH_2]_7$-CH_3: 3-oxoestr-4-en-17β-yl nonanoate,



G. 3-oxoestra-4,8(14)-dien-17β-yl decanoate,

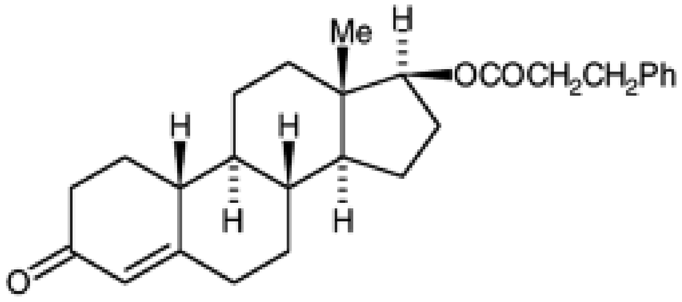


J. 5α-estr-3-ene-3,17β-diyl didecanoate.

Ph Eur

Nandrolone Phenylpropionate

General Notices



$C_{27}H_{34}O_3$ 406.6 *62-90-8*

Action and use

Anabolic steroid; androgen.

Preparation

Nandrolone Phenylpropionate Injection

DEFINITION

Nandrolone Phenylpropionate is 3-oxo-estr-4-en-17β-yl 3-phenylpropionate. It contains not less than 97.0% and not more than 103.0% of $C_{27}H_{34}O_3$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to creamy white, crystalline powder.

Practically insoluble in *water*; soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of nandrolone phenylpropionate *(RS 243)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate, the surface of which has been modified by chemically bonded octadecylsilyl groups (Whatman KC 18F plates are suitable) and a mixture of 60 volumes of *propan-2-ol*, 40 volumes of *acetonitrile* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions in *chloroform* containing (1) 0.5% w/v of the substance being examined, (2) 0.5% w/v of *nandrolone phenylpropionate BPCRS* and (3) equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air until the solvent has evaporated and heat at 100° for 10 minutes. Allow to cool and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

C. *Melting point*, about 97°, Appendix V A.

TESTS

Specific optical rotation

In a 1% w/v solution in *1,4-dioxan*, +48 to +51, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF$_{254}$* as the coating substance and a mixture of 70 volumes of *heptane* and 30 volumes of *acetone* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions in *chloroform* containing (1) 1.0% w/v of the substance being examined, (2) 0.0050% w/v of the substance being examined and (3) 0.010% w/v of *nandrolone BPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to nandrolone is not more intense than the spot in the chromatogram obtained with solution (3) (1.0%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Loss on drying

When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 4 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 10 mg in sufficient *absolute ethanol* to produce 100 ml, dilute 5 ml to 50 ml with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 240 nm, Appendix II B. Calculate the content of $C_{27}H_{34}O_3$ taking 430 as the value of A (1%, 1 cm) at the maximum at 240 nm.

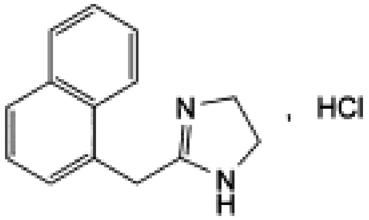
STORAGE

Nandrolone Phenylpropionate should be protected from light.

Naphazoline Hydrochloride

General Notices

(Ph Eur monograph 0730)



$C_{14}H_{15}ClN_2$ 246.7 *550-99-2*

Action and use

Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION

2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, soluble in alcohol.

mp

About 259 °C, with decomposition.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Dissolve 50.0 mg in *0.01 M hydrochloric acid* and dilute to 250.0 ml with the same acid. Dilute 25.0 ml of the solution to 100.0 ml with *0.01 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows 4 absorption maxima, at 270 nm, 280 nm, 287 nm and 291 nm. The ratios of the absorbances measured at the maxima at 270 nm, 287 nm and 291 nm to that measured at the maximum at 280 nm are 0.82 to 0.86, 0.67 to 0.70 and 0.65 to 0.69, respectively.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of naphazoline hydrochloride.*

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 20 ml of solution S add 0.2 ml of *0.01 M sodium hydroxide* and 0.1 ml of *methyl red solution R*. The solution is yellow. Not more than 0.6 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *1-naphthylacetic acid R* in the mobile phase, add 5 ml of the test solution and dilute to 100 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *naphazoline impurity A CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *base-deactivated end-capped octylsilyl silica gel for chromatography R* (4 µm) with a pore size of 6 nm.

Mobile phase Dissolve 1.1 g of *sodium octanesulphonate R* in a mixture of 5 ml of *glacial acetic acid R*, 300 ml of *acetonitrile R* and 700 ml of *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Run time 3 times the retention time of naphazoline.

Retention time Naphazoline = about 14 min.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to naphazoline and to impurity B.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity:* not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of alcohol R. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 24.67 mg of $C_{14}H_{15}ClN_2$.

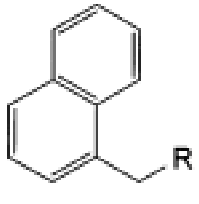
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D.*

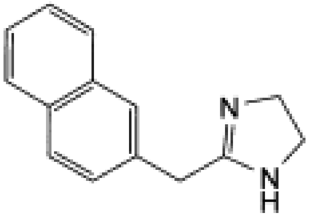
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D.*



A. R = CO-NH-$[CH_2]_2$-NH_2: *N*-(2-aminoethyl)-2-(naphthalen-1-yl)acetamide (naphthylacetylethylenediamine),

B. R = CO_2H: (naphthalen-1-yl)acetic acid (1-naphthylacetic acid),

C. R = CN: (naphthalen-1-yl)acetonitrile (1-naphthylacetonitrile),



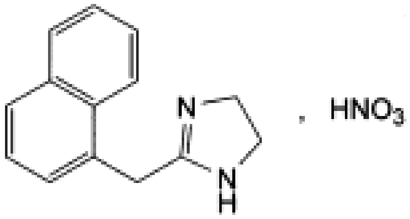
D. 2-(naphthalen-2-ylmethyl)-4,5-dihydro-1*H*-imidazole (β-naphazoline).

Ph Eur

Naphazoline Nitrate

General Notices

(Ph Eur monograph 0147)



$C_{14}H_{15}N_3O_3$ 273.3 *5144-52-5*

Action and use

Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION

2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole nitrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *C.*

Second identification A, B, D.

A. Melting point (*2.2.14*): 167 °C to 170 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *0.01 M hydrochloric acid* and dilute to 250.0 ml with the same acid. Dilute 25.0 ml of the solution to 100.0 ml with *0.01 M hydrochloric acid*.

Spectral range 230-350 nm.

Absorption maximum At 270 nm, 280 nm, 287 nm and 291 nm.

Absorbance ratio:

- A_{270}/A_{280} = 0.82 to 0.86;
- A_{291}/A_{280} = 0.65 to 0.69.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *naphazoline nitrate CRS.*

D. Dissolve 45 mg of the substance to be examined in 2 ml of *water R*. Add 1 ml of *sulphuric acid R*. Shake carefully and allow to cool. Add 1 ml of *ferrous sulphate solution R2* dropwise along the walls of the container. At the junction of the 2 liquids, a brown colour develops.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R*, warming gently, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

5.0 to 6.5 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *1-naphthylacetic acid R* in the mobile phase, add 5 ml of the test solution and dilute to 100 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *naphazoline impurity A CRS* in the mobile phase and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped base-deactivated octylsilyl silica gel for chromatography R* (4 µm) with a pore size of 6 nm.

Mobile phase Dissolve 1.1 g of *sodium octanesulphonate R* in a mixture of 5 ml of *glacial acetic acid R*, 300 ml of *acetonitrile R* and 700 ml of *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Run time 3 times the retention time of naphazoline.

Relative retention With reference to naphazoline (retention time = about 14 min): impurity A = about 0.76; impurity D = about 1.24; impurity B = about 1.27; impurity C = about 2.8.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to naphazoline and impurity B.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peak due to the nitrate ion.

Chlorides (*2.4.4*)

Maximum 330 ppm, determined on solution S.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 27.33 mg of $C_{14}H_{15}N_3O_3$.

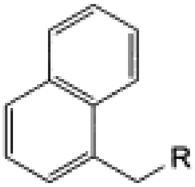
STORAGE

Protected from light.

IMPURITIES

Specified impurities A.

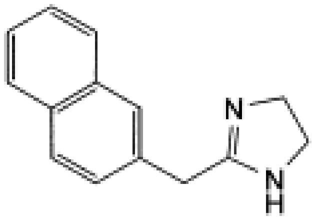
Other detectable impurities B, C, D.



A. R = $CO\text{-}NH\text{-}[CH_2]_2\text{-}NH_2$: *N*-(2-aminoethyl)-2-(naphthalen-1-yl)acetamide (naphthylacetylethylenediamine),

B. R = CO_2H: (naphthalen-1-yl)acetic acid (1-naphthylacetic acid),

C. R = CN: (naphthalen-1-yl)acetonitrile (1-naphthylacetonitrile),



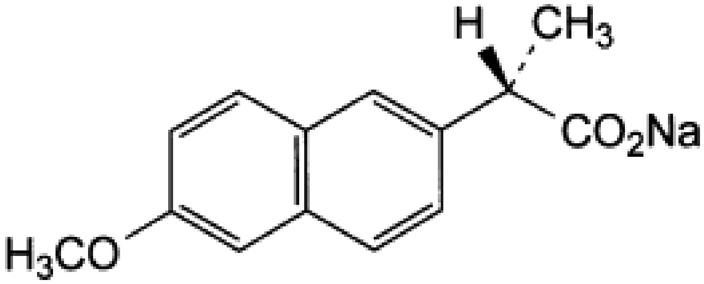
D. 2-(naphthalen-2-ylmethyl)-4,5-dihydro-1*H*-imidazole (β-naphazoline).

Ph Eur

Naproxen Sodium

General Notices

(Ph Eur monograph 1702)



$C_{14}H_{13}O_3Na$ 252.2 *26159-34-2*

Ph Eur

DEFINITION

Sodium (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, freely soluble or soluble in methanol, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, C, D.

Second identification A, B, D.

A. Specific optical rotation (*2.2.7*): - 14.7 to - 17.0 (dried substance).

Dissolve 0.50 g in a 4.2 g/l solution of *sodium hydroxide R* and dilute to 25.0 ml with the same solution.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 40.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *methanol R.*

Spectral range 230-350 nm.

Absorption maxima At 262 nm, 271 nm, 316 nm and 331 nm.

Specific absorbance at the absorption maxima:
- — at 262 nm: 207 to 227;
- — at 271 nm: 200 to 220;
- — at 316 nm: 56 to 68;
- — at 331 nm: 72 to 84.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 50 mg in 5 ml of *water R.* Add 1 ml of *dilute sulphuric acid R* and 5 ml of *ethyl acetate R*. Shake vigorously. Allow the 2 layers to separate. Evaporate the upper layer to dryness and subsequently dry at 60 °C for 15 min. Record the spectrum using the residue.

Comparison *naproxen CRS.*

D. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 1.25 g in *water R* and dilute to 25 ml with the same solvent.

pH *(2.2.3)*

7.0 to 9.8.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Enantiomeric purity

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in 15 ml of *water R* and add 1 ml of *hydrochloric acid R*. Shake with 2 quantities, each of 10 ml, of *ethyl acetate R*, combine the upper layers and evaporate to dryness under reduced pressure. Dissolve the residue in 50.0 ml of *tetrahydrofuran R.* Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 2.5 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *racemic naproxen CRS* in 10 ml of *tetrahydrofuran R* and dilute to 100 ml with the mobile phase.

Column:
- — *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel π-acceptor/π-donor for chiral separations R* (5 µm) (S,S);

— *temperature*: 25 °C.

Mobile phase *glacial acetic acid R*, *acetonitrile R*, *2-propanol R*, *hexane R* (5:50:100:845 *V/V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 263 nm.

Injection 20 µl.

Run time 1.5 times the retention time of naproxen (retention time = about 5 min).

System suitability Reference solution (b):

— *resolution*: minimum 3 between the peaks due to impurity G and naproxen.

Limit:

— *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Test solution Dissolve 12 mg of the substance to be examined in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 6 mg of *bromomethoxynaphthalene R* (impurity N), 6.0 mg of *naproxen impurity L CRS* and 6 mg of *(1RS)-1-(6-methoxynaphthalen-2-yl)ethanol R* (impurity K) in *acetonitrile R* and dilute to 10 ml with the same solvent. To 1 ml of the solution add 1 ml of the test solution and dilute to 50 ml with the mobile phase. Dilute 1 ml of this solution to 20 ml with the mobile phase.

Column:

— *size*: l = 0.10 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 50 °C.

Mobile phase Mix 42 volumes of *acetonitrile R* and 58 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R.*

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 1.5 times the retention time of impurity N.

Relative retention With reference to naproxen (retention time = about 2.5 min): impurity K = about 0.9; impurity L = about 1.4; impurity N = about 5.3.

System suitability Reference solution (b):

— *resolution*: minimum 2.2 between the peaks due to impurity K and naproxen.

Limits:

- *impurity L:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in 20.0 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

After the addition of *buffer solution pH 3.5 R*, the substance precipitates. Dilute each solution to 40 ml with *anhydrous ethanol R*: the substance dissolves completely. Proceed as described in the test, filtering the solutions to evaluate the result.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.200 g in 50 ml of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 25.22 mg of $C_{14}H_{13}O_3Na$.

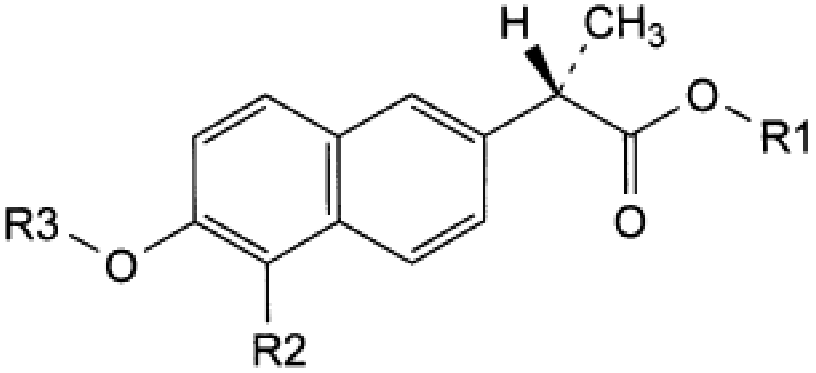
STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities G, L.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, D, E, F, H, I, J, K, M, N.*



A. R1 = R2 = R3 = H: (2*S*)-2-(6-hydroxynaphthalen-2-yl)propanoic acid,

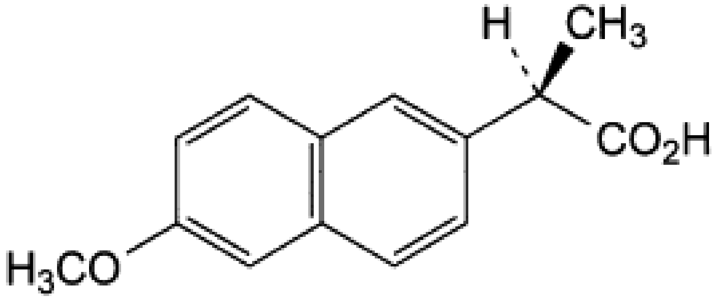
B. R1 = H, R2 = Cl, R3 = CH_3: (2*S*)-2-(5-chloro-6-methoxynaphthalen-2-yl)propanoic acid,

C. R1 = H, R2 = Br, R3 = CH_3: (2*S*)-2-(5-bromo-6-methoxynaphthalen-2-yl)propanoic acid,

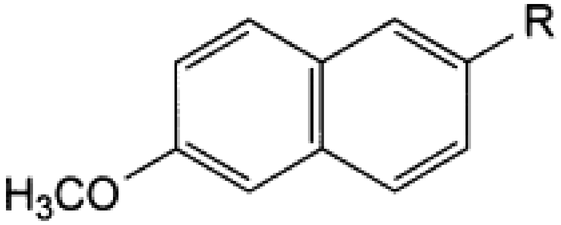
D. R1 = H, R2 = I, R3 = CH_3: (2*S*)-2-(5-iodo-6-methoxynaphthalen-2-yl)propanoic acid,

E. R1 = R3 = CH_3, R2 = H: methyl (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate,

F. R1 = C_2H_5, R2 = H, R3 = CH_3: ethyl (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate,



G. (2*R*)-2-(6-methoxynaphthalen-2-yl)propanoic acid,



H. R = OH: 6-methoxynaphthalen-2-ol,

I. R = CH_2-CO_2H: (6-methoxynaphthalen-2-yl)acetic acid,

J. R = C_2H_5: 2-ethyl-6-methoxynaphthalene,

K. R = CHOH-CH_3: (1*RS*)-1-(6-methoxynaphthalen-2-yl)ethanol,

L. R = CO-CH_3: 1-(6-methoxynaphthalen-2-yl)ethanone,

M. R = H: 2-methoxynaphthalene (nerolin),

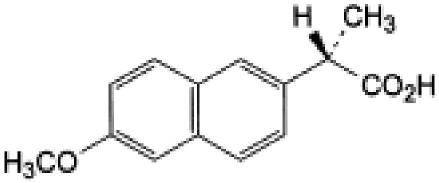
N. R = Br: 2-bromo-6-methoxynaphthalene.

Ph Eur

Naproxen

General Notices

(Ph Eur monograph 0731)



$C_{14}H_{14}O_3$ 230.3 *22204-53-1*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Naproxen Oral Suspension

Naproxen Suppositories

Naproxen Tablets

Gastro-resistant Naproxen Tablets

Ph Eur

DEFINITION

(2*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification A, D.

Second identification A, B, C.

A. Specific optical rotation *(2.2.7)*: + 59 to + 62 (dried substance).

Dissolve 0.50 g in *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

B. Melting point *(2.2.14)*: 154 °C to 158 °C.

C. Dissolve 40.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *methanol R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows 4 absorption maxima, at 262 nm, 271 nm, 316 nm and 331 nm. The specific absorbances at the absorption maxima are 216 to 238, 219 to 241, 61 to 69 and 79 to 87, respectively.

D. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *naproxen CRS.*

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 1.25 g in *methanol R* and dilute to 25 ml with the same solvent.

Enantiomeric purity

Liquid chromatography *(2.2.29)*. *Protect the solutions from light*.

Test solution Dissolve 25.0 mg of the substance to be examined in *tetrahydrofuran R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 2.5 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *racemic naproxen CRS* in 10 ml of *tetrahydrofuran R* and dilute to 100 ml with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel π-acceptor/π-donor for chiral separations R* (5 µm) (S,S);

— *temperature*: 25 °C.

Mobile phase *glacial acetic acid R, acetonitrile R, 2-propanol R, hexane R* (5:50:100:845 *V/V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 263 nm.

Injection 20 µl.

Run time 1.5 times the retention time of naproxen (retention time = about 5 min).

System suitability Reference solution (b):

— *resolution*: minimum 3 between the peaks due to impurity G and naproxen.

Limit:

- *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

Related substances

Liquid chromatography *(2.2.29)*. *Protect the solutions from light*.

Test solution Dissolve 12 mg of the substance to be examined in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 6 mg of *bromomethoxynaphthalene R* (impurity N), 6 mg of *1-(6-methoxynaphthalen-2-yl)ethanone R* (impurity L) and 6 mg of *(1RS)-1-(6-methoxynaphthalen-2-yl)ethanol R* (impurity K) in *acetonitrile R* and dilute to 10 ml with the same solvent. To 1 ml of this solution add 1 ml of the test solution and dilute to 50 ml with the mobile phase. Dilute 1 ml of this solution to 20 ml with the mobile phase.

Column:

- *size*: l = 0.10 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 50 °C.

Mobile phase Mix 42 volumes of *acetonitrile R* and 58 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 1.5 times the retention time of impurity N.

Relative retention With reference to naproxen (retention time = about 2.5 min): impurity K = about 0.9; impurity L = about 1.4; impurity N = about 5.3.

System suitability Reference solution (b):

- *resolution*: minimum 2.2 between the peaks due to impurity K and naproxen.

Limits:

- *impurity L*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 25 ml of *water R* and 75 ml of *methanol R*. Titrate with *0.1 M sodium hydroxide*, using 1 ml of *phenolphthalein solution R* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 23.03 mg of $C_{14}H_{14}O_3$.

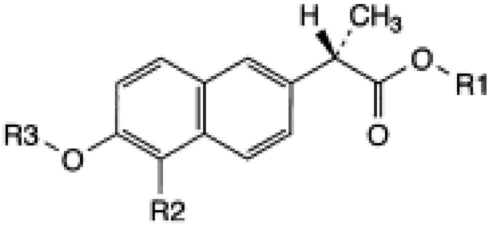
STORAGE

Protected from light.

IMPURITIES

Specified impurities *G, L.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F, H, I, J, K, M, N.*



A. R1 = R2 = R3 = H: (2*S*)-2-(6-hydroxynaphthalen-2-yl)propanoic acid,

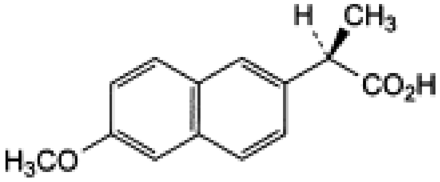
B. R1 = H, R2 = Cl, R3 = CH_3: (2*S*)-2-(5-chloro-6-methoxynaphthalen-2-yl)propanoic acid,

C. R1 = H, R2 = Br, R3 = CH_3: (2*S*)-2-(5-bromo-6-methoxynaphthalen-2-yl)propanoic acid,

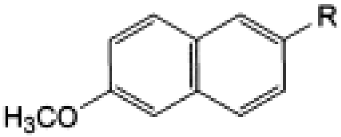
D. R1 = H, R2 = I, R3 = CH_3: (2*S*)-2-(5-iodo-6-methoxynaphthalen-2-yl)propanoic acid,

E. R1 = R3 = CH_3, R2 = H: methyl (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate,

F. R1 = C_2H_5, R2 = H, R3 = CH_3: ethyl (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate,



G. (2*R*)-2-(6-methoxynaphthalen-2-yl)propanoic acid ((*R*)-enantiomer),



H. R = OH: 6-methoxynaphthalen-2-ol,

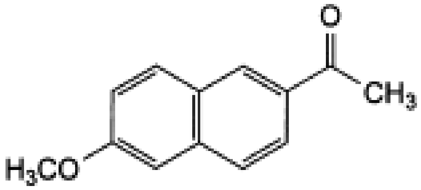
I. R = CH_2-CO_2H: (6-methoxynaphthalen-2-yl)acetic acid,

J. R = C_2H_5: 2-ethyl-6-methoxynaphthalene,

K. R = CHOH-CH_3: (1*RS*)-1-(6-methoxynaphthalen-2-yl)ethanol,

M. R = H: 2-methoxynaphthalene (nerolin),

N. R = Br: 2-bromo-6-methoxynaphthalene,



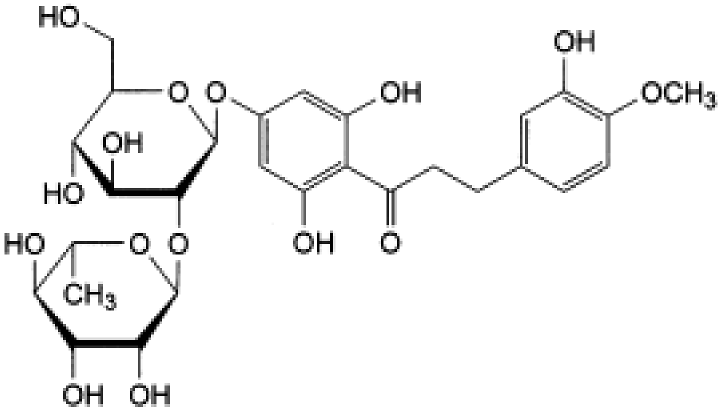
L. 1-(6-methoxynaphthalen-2-yl)ethanone.

Ph Eur

Neohesperidin-Dihydrochalcone

General Notices

(Ph Eur monograph 1547)



$C_{28}H_{36}O_{15}$ 613 *20702-77-6*

Ph Eur

DEFINITION

1-[4-[[2-*C*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one.

Content

96.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Practically insoluble in water, freely soluble in dimethyl sulphoxide, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *neohesperidin-dihydrochalcone CRS*.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_4 (*2.2.2, Method II*).

Dissolve 0.25 g in *methanol R* and dilute to 25 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dimethyl sulphoxide R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dilute 10.0 ml of test solution (a) to 20.0 ml with *dimethyl sulphoxide R*.

Reference solution (a) Dissolve 50.0 mg of *neohesperidin-dihydrochalcone CRS* in *dimethyl sulphoxide R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dissolve 4.0 mg of *neohesperidin-dihydrochalcone impurity B CRS* in *dimethyl sulphoxide R* and dilute to 100.0 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of test solution (a) to 100.0 ml with *dimethyl sulphoxide R*.

Reference solution (d) In order to prepare *in situ* impurity F and impurity G, suspend 0.10 g of the substance to be examined in 10.0 ml of a 100 g/l solution of *sulphuric acid R*. Heat the sample for 5 min on a water-bath. Dilute immediately 1.0 ml of the resulting solution to 50.0 ml with *dimethyl sulphoxide R*.

Column:

— *size:* *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (4 µm) with a carbon loading of 7 per cent;

— *temperature*: 30 °C.

Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a solution prepared by adding 5.0 ml of *glacial acetic acid R* to 1000.0 ml of *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 282 nm.

Injection 10 µl; inject test solution (a) and reference solutions (a), (b), (c) and (d).

Run time 5 times the retention time of neohesperidin-dihydrochalcone which is about 10 min.

Relative retention With reference to neohesperidin-dihydrochalcone: impurity B = about 0.4; impurity D = about 0.7; impurity F = about 1.2; impurity G = about 3.7.

System suitability:

- *resolution*: minimum of 2.5 between the first peak (neohesperidin-dihydrochalcone) and the second peak (impurity F) in the chromatogram obtained with reference solution (d);
- chromatogram obtained with reference solution (a) is similar to the chromatogram provided with *neohesperidin-dihydrochalcone CRS* .

Limits:

- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);
- *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2 per cent);
- *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *total of all impurities apart from impurity B*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (2.5 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 12.0 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances.

Injection 10 µl; inject test solution (b) and reference solutions (a) and (d).

System suitability:

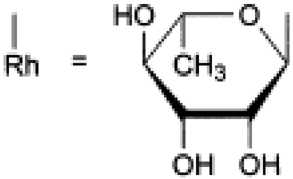
- *resolution*: minimum of 2.5 between the first peak (neohesperidin-dihydrochalcone) and the second peak (impurity F) in the chromatogram obtained with reference solution (d);
- *repeatability*: reference solution (a).

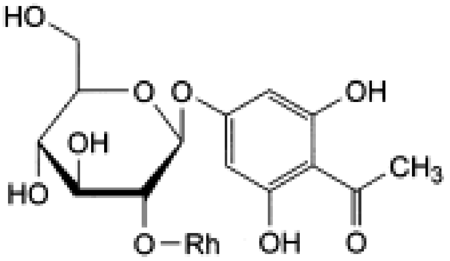
Calculate the percentage content of $C_{28}H_{36}O_{15}$ using the chromatogram obtained with reference solution (a) and the stated content of $C_{28}H_{36}O_{15}$ in *neohesperidin-dihydrochalcone CRS*, correcting for the water content of the substance to be examined.

STORAGE

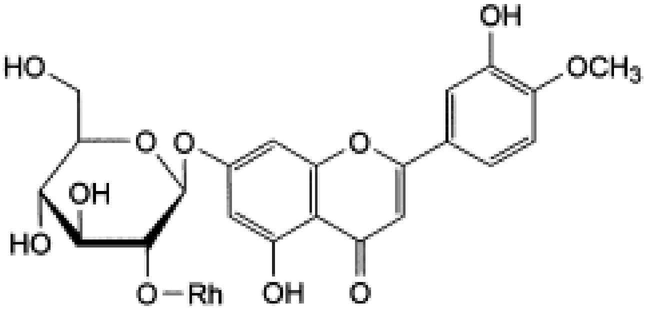
Protected from light.

IMPURITIES

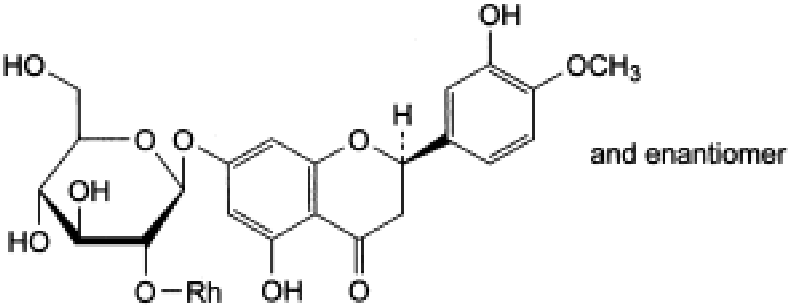

Rh =
HO
O
CH3
OH OH


HO
O
O
OH
OH
CH3
HO
O—Rh
OH
O

A. 1-[4-[[2-*C*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl] ethanone (phloroacetophenone neohesperidoside),

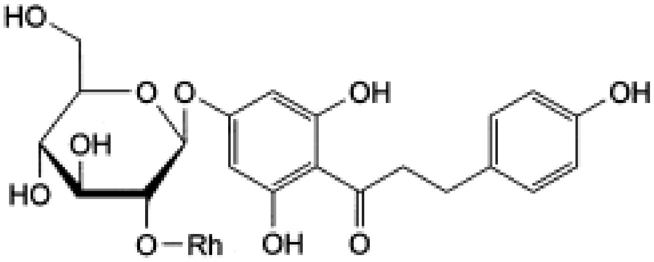

OH
OCH3
HO
O
O
O
OH
HO
O—Rh
OH
O

B. 7-[[2-*C*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (neodiosmin),

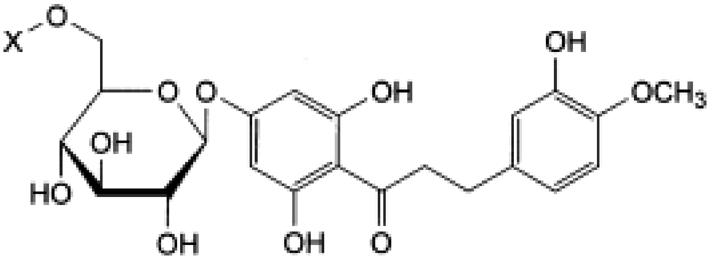

OH
OCH3
HO
H
O
O
O
OH
HO
O—Rh
OH
O

and enantiomer

C. (2*RS*)-7-[[2-*C*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (neohesperidin),

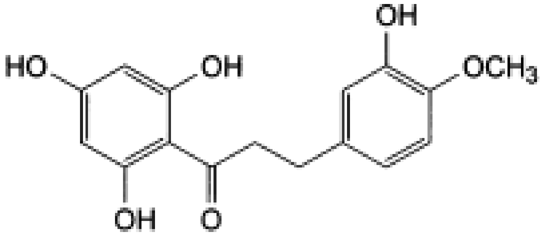


D. 1-[4-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(4-hydroxyphenyl)propan-1-one (naringin-dihydrochalcone),



E. X = Rh: 1-[4-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (hesperidin-dihydrochalcone)
,

F. X = H: 1-[4-(β-D-glucopyranosyloxy)-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl) propan-1-one (hesperetin-dihydrochalcone 7′-glucoside),



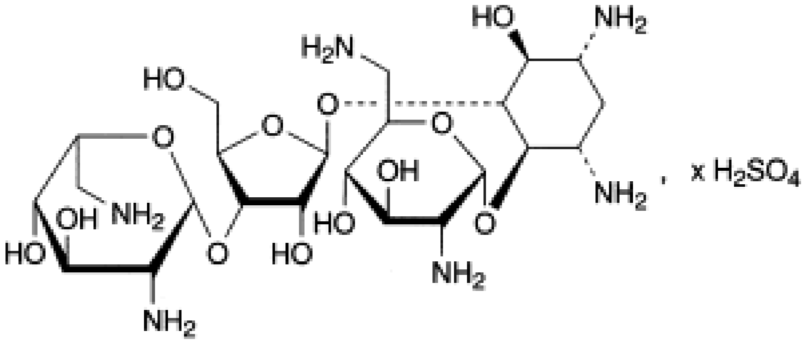
G. 3-(3-hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one (hesperetin-dihydrochalcone).

Ph Eur

Neomycin Sulphate

General Notices

(Ph Eur monograph 0197)



$C_{23}H_{46}N_6O_{13},xH_2SO_4$ 615 *(base)* *1405-10-3*

Action and use

Aminoglycoside antibacterial.

Preparations

Dexamethasone and Neomycin Ear Spray

Hydrocortisone and Neomycin Cream

Hydrocortisone Acetate and Neomycin Ear Drops

Hydrocortisone Acetate and Neomycin Eye Drops

Neomycin Eye Drops

Hydrocortisone Acetate and Neomycin Eye Ointment

Neomycin Eye Ointment

Neomycin Oral Solution

Neomycin Tablets

Ph Eur

DEFINITION

Mixture of sulphates of substances produced by the growth of certain selected strains of *Streptomyces fradiae*, the main component being the sulphate of 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B).

Content

Minimum of 680 IU/mg (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder, hygroscopic.

Solubility

Very soluble in water, very slightly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances.

Results:

— the retention time of the principal peak in the chromatogram obtained with the test solution is approximately the same as that of the principal peak in the chromatogram obtained with reference solution (e);

— it complies with the limits given for impurity C.

B. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

pH (*2.2.3*)

5.0 to 7.5.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 53.5 to + 59.0 (dried substance).

Dissolve 1.00 g in *water R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *framycetin sulphate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (d) Dissolve the contents of a vial of *neamine CRS* (corresponding to 0.5 mg) in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (e) Dissolve 10 mg of *neomycin sulphate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 25 °C.

Mobile phase Mix 20.0 ml of *trifluoroacetic acid R*, 6.0 ml of *carbonate-free sodium hydroxide solution R* and 500 ml of *water R*, allow to equilibrate, dilute to 1000 ml with *water R* and degas.

Flow rate 0.7 ml/min.

Post-column solution *Carbonate-free sodium hydroxide solution R* diluted 1 in 25 previously degassed, which is added pulse-less to the column effluent using a 375 µl polymeric mixing coil.

Flow rate 0.5 ml/min.

Detection Pulsed amperometric detector with a gold indicator electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively 0.00 V detection, + 0.80 V oxidation and - 0.60 V reduction potentials, with pulse durations according to the instrument used.

Injection 10 µl; inject the test solution and the reference solutions (b), (c), (d) and (e).

Run time 1.5 times the retention time of neomycin B.

Relative retention With reference to neomycin B (retention time = about 10 min): impurity A = about 0.65; impurity C = about 0.9; impurity G = about 1.1.

System suitability:

— *resolution*: minimum of 2.0 between the peaks due to impurity C and to neomycin B in the chromatogram obtained with reference solution (e); if necessary, adjust the volume of the carbonate-free sodium hydroxide solution in the mobile phase;

— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (c).

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent);

— *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (15.0 per cent) and not less than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent);

— *total of other impurities*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (15.0 per cent);

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

Sulphate

27.0 per cent to 31.0 per cent (dried substance).

Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 ml of *alcohol R* when the colour of the solution begins to change, continuing the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Loss on drying (*2.2.32*)

Maximum 8.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash (*2.4.14*)

Maximum 1.0 per cent, determined on 1.0 g.

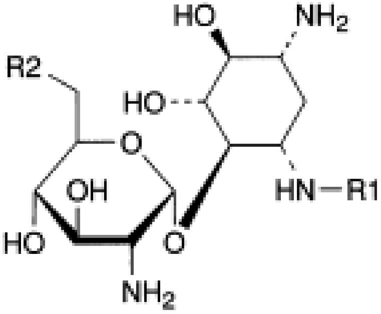
ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *neomycin sulphate for microbiological assay CRS* as the reference substance.

STORAGE

In an airtight container, protected from light.

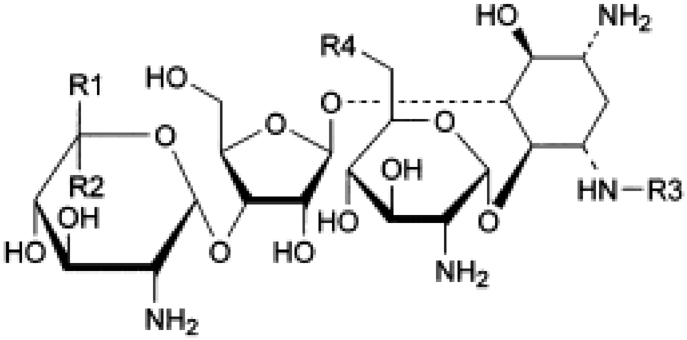
IMPURITIES



A. R1 = H, R2 = NH_2: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (neamine or neomycin A-LP),

B. R1 = CO-CH_3, R2 = NH_2: 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (3-acetylneamine),

D. R1 = H, R2 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine (paromamine or neomycin D),



C. R1 = CH_2-NH_2, R2 = R3 = H, R4 = NH_2: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin C),

E. R1 = R3 = H, R2 = CH_2-NH_2, R4 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin I or neomycin E),

F. R1 = CH_2-NH_2, R2 = R3 = H, R4 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin II or neomycin F),

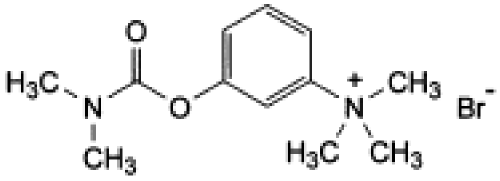
G. R1 = H, R2 = CH_2-NH_2, R3 = CO-CH_3, R4 = NH_2: 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B-LP).

Ph Eur

Neostigmine Bromide

General Notices

(Ph Eur monograph 0046)



$C_{12}H_{19}BrN_2O_2$ 303.2 *114-80-7*

Action and use

Cholinesterase inhibitor.

Preparation

Neostigmine Tablets

Ph Eur

DEFINITION

3-[(Dimethylcarbamoyl)oxy]-*N*,*N*,*N*-trimethylanilinium bromide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 20 mg in *0.5 M sulphuric acid* and dilute to 100 ml with the same acid.

Spectral range 230-350 nm.

Absorption maxima At 260 nm and 266 nm.

Specific absorbances at the absorption maxima:
— at 260 nm: about 16;

— at 266 nm: about 14.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison neostigmine bromide CRS.

C. Heat about 50 mg with a mixture of 0.4 g of *potassium hydroxide R* and 2 ml of *ethanol (96 per cent) R* on a water-bath for 3 min, replacing the evaporated ethanol (96 per cent). Cool and add 2 ml of *water R* and 2 ml of *diazobenzenesulphonic acid solution R1*. An orange-red colour develops.

D. It gives the reactions of bromides (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Impurity A

Maximum 0.33 per cent.

Dissolve 50 mg in a mixture of 1 ml of *sodium carbonate solution R* and 9 ml of *water R*. The absorbance (*2.2.25*) measured immediately at 294 nm is not greater than 0.25.

Sulphates (*2.4.13*)

Maximum 200 ppm, determined on solution S.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.225 g in 2 ml of *anhydrous formic acid R*. Add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

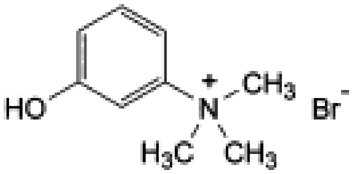
1 ml of *0.1 M perchloric acid* is equivalent to 30.32 mg of $C_{12}H_{19}BrN_2O_2$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A*.



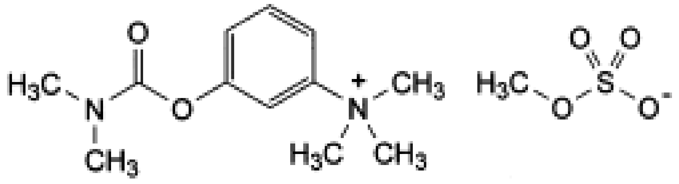
A. 3-hydroxy-*N*,*N*,*N*-trimethylanilinium bromide.

Ph Eur

Neostigmine Metilsulfate

General Notices

(Ph Eur monograph 0626)



$C_{13}H_{22}N_2O_6S$ 334.4 *51-60-5*

Action and use

Cholinesterase inhibitor.

Preparation

Neostigmine Injection

Ph Eur

DEFINITION

3-[(Dimethylcarbamoyl)oxy]-*N*,*N*,*N*-trimethylanilinium methyl sulphate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, C.

Second identification A, B, D, E.

A. Melting point (*2.2.14*): 144 °C to 149 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50 mg in *0.5 M sulphuric acid* and dilute to 100.0 ml with the same acid.

Spectral range 230-350 nm.

Absorption maxima At 261 nm and 267 nm.

Resolution (2.2.25) Minimum 1.9 for the absorbance ratio.

Absorbance ratio A_{267} / A_{261} = 0.84 to 0.87.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *neostigmine metilsulfate CRS.*

D. To 50 mg add 0.4 g of *potassium hydroxide R* and 2 ml of *ethanol (96 per cent) R* and heat on a water-bath for 3 min, replacing the evaporated ethanol (96 per cent). Cool and add 2 ml of *water R* and 2 ml of *diazobenzenesulphonic acid solution R1*. An orange-red colour develops.

E. Dissolve 0.1 g in 5 ml of *distilled water R* and add 1 ml of *barium chloride solution R1*. No precipitate is formed. Add 2 ml of *hydrochloric acid R* and heat in a water-bath for 10 min. A fine, white precipitate is formed.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 4.0 ml of solution S add 6.0 ml of *water R* and 0.1 ml of *phenolphthalein solution R1*. The solution is colourless. Add 0.3 ml of *0.01 M sodium hydroxide*; the solution becomes red. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution becomes colourless. Add 0.1 ml of *methyl red solution R*; the solution becomes red or yellowish-red.

(3-Hydroxyphenyl)trimethylammonium methyl sulphate

Dissolve 50 mg in a mixture of 1 ml of *sodium carbonate solution R* and 9 ml of *water R*. The absorbance (*2.2.25*) measured immediately at 294 nm is not greater than 0.20.

Sulphates (2.4.13)

Maximum 200 ppm, determined on solution S.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 150 ml of *water R* and add 100 ml of *dilute sodium hydroxide solution R*. Distil collecting the distillate in 40 ml of a 40 g/l solution of *boric acid R* until the total volume in the collecting vessel is about 250 ml. Titrate the solution in the collecting vessel with *0.1 M hydrochloric acid*, using 0.25 ml of *methyl red mixed solution R* as indicator. Carry out a blank test.

1 ml of *0.1 M hydrochloric acid* is equivalent to 33.44 mg of $C_{13}H_{22}N_2O_6S$.

STORAGE

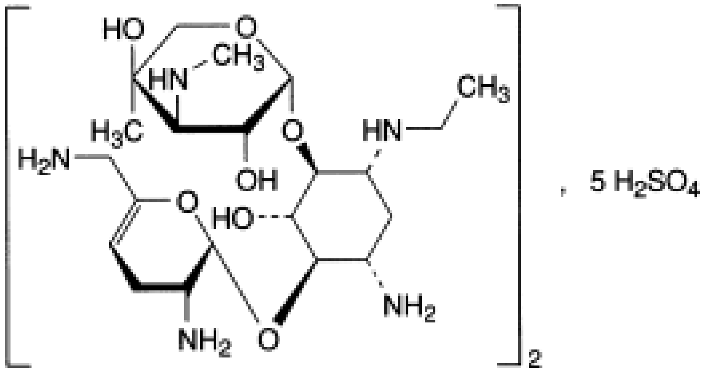
In a airtight container, protected from light.

Ph Eur

Netilmicin Sulphate

General Notices

(Ph Eur monograph 1351)



$C_{42}H_{82}N_{10}O_{14},5H_2SO_4$ 1442 *56391-57-2*

Action and use

Aminoglycoside antibacterial.

Ph Eur

DEFINITION

2-Deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-4-*O*-(2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl)-1-*N*-ethyl-D-streptamine sulphate.

Substance obtained by synthesis from sisomicin.

Semi-synthetic product derived from a fermentation product.

Content

Minimum 650 IU/mg (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder, very hygroscopic.

Solubility

Very soluble in water, practically insoluble in acetone and in alcohol.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances.

Results The retention time and size of the principal peak in the chromatogram obtained with test solution (a) are approximately the same as those of the principal peak in the chromatogram obtained with reference solution (a).

B. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

Solution S

Dissolve 0.80 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and its absorbance at 400 nm (*2.2.25*) has a maximum of 0.08.

pH (*2.2.3*)

3.5 to 5.5 for solution S.

Specific optical rotation (*2.2.7*)

+ 88.0 to + 96.0 (dried substance).

Dissolve 0.50 g in *water R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Test solution (b) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *netilmicin sulphate CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (b) Dissolve 25.0 mg of *sisomicin sulphate CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (c) Dissolve 20.5 mg of *1-N-ethylgaramine sulphate CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of reference solution (a), 1.0 ml of reference solution (b) and 1.0 ml of reference solution (c) to 100.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm;
- *temperature*: 50 °C.

Mobile phase Prepare a solution in *carbon dioxide-free water R* containing 35 g/l of *anhydrous sodium sulphate R*, 0.5 g/l of *sodium octanesulphonate R*, 10 ml/l of *tetrahydrofuran R*, 50 ml/l of 0.2 M *potassium dihydrogenphosphate R* previously adjusted to pH 3.0 with a 22.5 g/l solution of *phosphoric acid R* and degassed.

Flow rate 1.0 ml/min.

Post-column solution 20 g/l carbonate-free solution of *sodium hydroxide R* previously degassed, which is added pulse-less to the column effluent using a 375 µl polymeric mixing coil.

Flow rate 0.3 ml/min.

Detection Pulsed amperometric detector with a gold indicator electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively + 0.05 V detection, + 0.75 V oxidation and - 0.15 V reduction potentials, with pulse durations according to the instrument used.

Injection 20 µl; inject test solutions (a) and (b) and reference solution (d).

Run time 3 times the retention time of netilmicin.

Retention time Netilmicin = about 12 min.

System suitability:

— *resolution*: minimum of 2.0 between the peaks due to impurity B (first peak) and to impurity A (second peak); minimum of 3.0 between the peaks due to impurity A (second peak) and to netilmicin (third peak) in the chromatogram obtained with reference solution (d) . If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase;

— *signal-to-noise ratio*: minimum of 10 for the principal peak in the chromatogram obtained with test solution (b).

Limits:

— *impurity A*: not more than the area of the second peak in the chromatogram obtained with reference solution (d) and taking into account the declared content of *sisomicin sulphate CRS (1 per cent);*

— *impurity B*: not more than the area of the first peak in the chromatogram obtained with reference solution (d) and taking into account the declared content of *1-N-ethylgaramine sulphate CRS* (1 per cent);

— *any other impurity*: not more than the area of the third peak in the chromatogram obtained with reference solution (d) (1 per cent);

— *total of other impurities*: not more than twice the area of the third peak in the chromatogram obtained with reference solution (d) (2 per cent);

— *disregard limit*: any peak with an area less than that of the principal peak in the chromatogram obtained with test solution (b) (0.1 per cent).

Sulphate

31.5 per cent to 35.0 per cent (dried substance).

Dissolve 0.12 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 30.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 ml of *alcohol R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Loss on drying (*2.2.32*)

Maximum 15.0 per cent, determined on 0.500 g by drying at 110 °C under high vacuum for 3 h.

Sulphated ash (*2.4.14*)

Maximum 1.0 per cent, determined on 0.5 g.

Bacterial endotoxins (*2.6.14*)

Less than 1.25 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

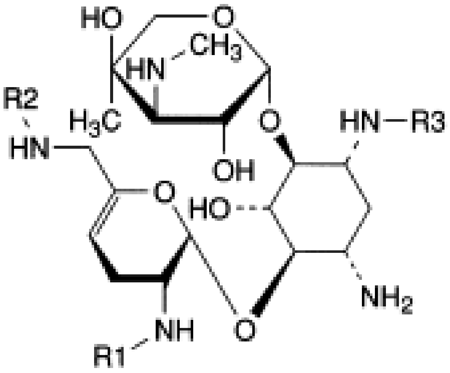
ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*), using the diffusion method.

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

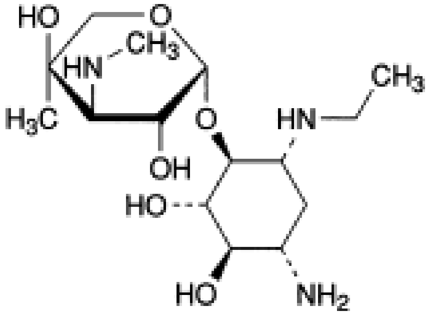
IMPURITIES



A. R1 = R2 = R3 = H: 2-deoxy-4-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-6-*O*-(2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl)-L-streptamine (sisomicin),

C. R1 = R3 = C_2H_5, R2 = H: 4-*O*-[6-amino-2,3,4,6-tetradeoxy-2-(ethylamino)-α-D-*glycero*-hex-4-enopyranosyl]-2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-1-*N*-ethyl-D-streptamine (2′-*N*-ethylnetilmicin),

D. R1 = H, R2 = R3 = C_2H_5: 4-*O*-[2-amino-2,3,4,6-tetradeoxy-6-(ethylamino)-α-D-*glycero*-hex-4-enopyranosyl]-2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-1-*N*-ethyl-D-streptamine (6′-*N*-ethylnetilmicin),



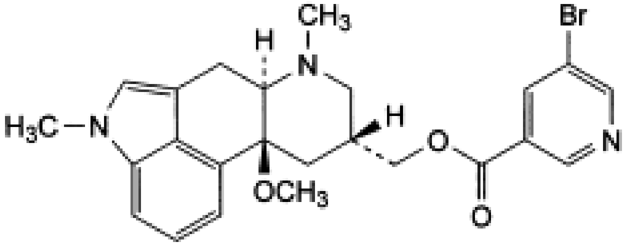
B. 2-deoxy-6-*C*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-1-*N*-ethyl-D-streptamine (1-*N*-ethylgaramine).

Ph Eur

Nicergoline

General Notices

(Ph Eur monograph 1998)



$C_{24}H_{26}BrN_3O_3$ 484.4 *27848-84-6*

Action and use

Ergot derivative.

Ph Eur

DEFINITION

[(6a*R*,9*R*,10a*S*)-10a-Methoxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]methyl 5-bromopyridine-3-carboxylate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Fine to granular, white or yellowish powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in alcohol.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Specific optical rotation (*2.2.7*): + 4.8 to + 5.8 (anhydrous substance).

Dissolve 0.50 g in *alcohol R* and dilute to 10.0 ml with the same solvent.

B. Dissolve 50.0 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml to 50.0 ml with *alcohol R*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 288 nm and an absorption minimum at 251 nm. The specific absorbance at the maximum at 288 nm is 175 to 185 (anhydrous substance).

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *Ph. Eur. reference spectrum of nicergoline.*

If the spectra obtained show differences, dissolve the substance to be examined in *alcohol R*, evaporate to dryness and record a further spectrum using the residue.

D. Dissolve 2 mg in 2 ml of *sulphuric acid R*. A blue colour develops.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Dissolve 0.5 g in *alcohol R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg in *acetonitrile R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*. Dilute 10.0 ml of this solution to 50.0 ml with *acetonitrile R*.

Reference solution (b) Dissolve 25.0 mg of the substance to be examined and 10.0 mg of *nicergoline impurity A CRS* in *acetonitrile R* and dilute to 25.0 ml with the same solvent. Dilute 1.0 ml to 100.0 ml with *acetonitrile R*.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *acetonitrile R*, 35 volumes of *methanol R* and 35 volumes of a freshly prepared solution of 6.8 g/l *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with *triethylamine R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 288 nm.

Injection 20 µl.

Run time Twice the retention time of nicergoline.

Relative retention With reference to nicergoline (retention time = about 25 min): impurity B = 0.5.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to nicergoline and impurity A.

Limits:

— *impurity B*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent);

— *any other impurity*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than 2 such peaks have an area greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 7.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.32*)

Maximum 0.5 per cent, determined on 0.100 g.

Sulphated ash (*2.4.14*)

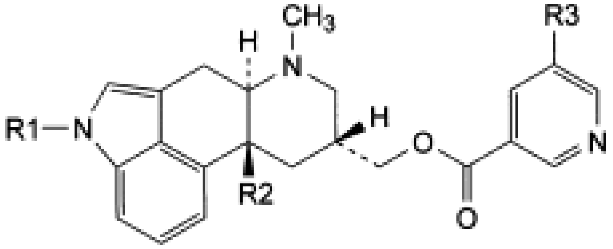
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *acetone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Titrate to the 1st point of inflexion.

1 ml of *0.1 M perchloric acid* is equivalent to 48.44 mg of $C_{24}H_{26}BrN_3O_3$.

IMPURITIES

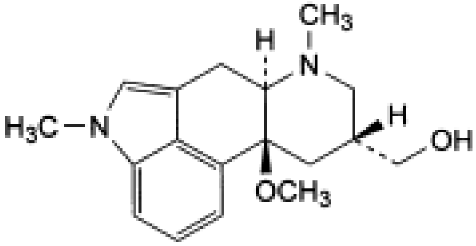


A. R1 = CH_3, R2 = OCH_3, R3 = Cl: [(6a*R*,9*R*,10a*S*)-10a-methoxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]methyl 5-chloropyridine-3-carboxylate,

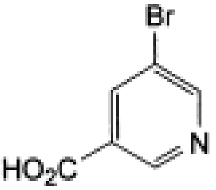
B. R1 = H, R2 = OCH_3, R3 = Br: [(6a*R*,9*R*,10a*S*)-10a-methoxy-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]methyl 5-bromopyridine-3-carboxylate,

E. R1 = CH_3, R2 = OH, R3 = Br: [(6a*R*,9*R*,10a*S*)-10a-hydroxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]methyl 5-bromopyridine-3-carboxylate,

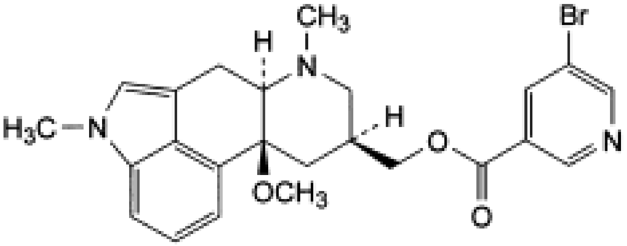
G. R1 = CH_3, R2 = H, R3 = Br: [(6a*R*,9*R*,10a*R*)-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]methyl 5-bromopyridine-3-carboxylate



C. [(6a*R*,9*R*,10a*S*)-10a-methoxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]methanol,



D. 5-bromopyridine-3-carboxylic acid,



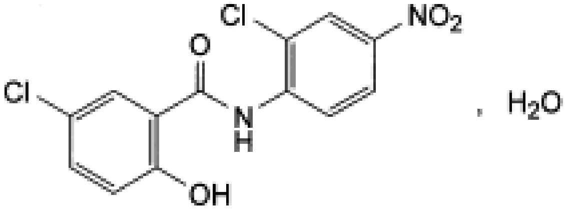
F. [(6a*R*,9*S*,10a*S*)-10a-methoxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]methyl 5-bromopyridine-3-carboxylate.

Ph Eur

Niclosamide Monohydrate

General Notices

(Ph Eur monograph 0680)



$C_{13}H_8Cl_2N_2O_4,H_2O$ 345.1 *50-65-7*
(anhydrous)

Action and use

Anthelminthic.

Preparation

Niclosamide Tablets

Ph Eur

DEFINITION

5-Chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide monohydrate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellowish, fine crystals.

Solubility

Practically insoluble in water, sparingly soluble in acetone, slightly soluble in anhydrous ethanol.

IDENTIFICATION

First identification B, E.

Second identification *A, C, D, E.*

A. Melting point (*2.2.14*): 227 °C to 232 °C, determined after drying at 100-105 °C for 4 h.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dry the substance to be examined at 100-105 °C for 4 h and examine as discs prepared using about 0.5 mg of substance and 0.3 g of *potassium bromide R.*

Comparison *anhydrous niclosamide CRS.*

C. To 50 mg add 5 ml of *1 M hydrochloric acid* and 0.1 g of *zinc powder R*, heat in a water-bath for 10 min, cool and filter. To the filtrate add 1 ml of a 5 g/l solution of *sodium nitrite R* and allow to stand for 3 min; add 2 ml of a 20 g/l solution of *ammonium sulphamate R*, shake, allow to stand for 3 min and add 2 ml of a 5 g/l solution of *naphthylethylenediamine dihydrochloride R*. A violet colour is produced.

D. Heat the substance on a copper wire in a non-luminous flame. The flame becomes green.

E. Loss on drying (see Tests).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R*, heating gently, cool and dilute to 50.0 ml with the same solvent.

Reference solution Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 20.0 ml with *acetonitrile R*.

Column:

— *size:* *l* = 0.125 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mixture of equal volumes of *acetonitrile R* and a solution containing 2 g/l of *potassium dihydrogen phosphate R*, 1 g/l of *disodium hydrogen phosphate R* and 2 g/l of *tetrabutylammonium hydrogen sulphate R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time Twice the retention time of niclosamide.

Limits:

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.005 per cent).

5-Chlorosalicylic acid

Maximum 60 ppm.

Test solution To 1.0 g add 15 ml of *water R*, boil for 2 min, cool, filter through a membrane filter (0.45 µm), wash the filter and dilute the combined filtrate and washings to 20.0 ml with *water R*.

Reference solution Dissolve 30 mg of *5-chlorosalicylic acid R* in 20 ml of *methanol R* and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*.

To 10.0 ml of the test solution and to 10.0 ml of the reference solution add separately 0.1 ml of *ferric chloride solution R2*. Any violet colour produced in the test solution is not more intense than that in the reference solution.

2-Chloro-4-nitroaniline

Maximum 100 ppm.

Test solution To 0.250 g add 5 ml of *methanol R*, heat to boiling, cool, add 45 ml of *1 M hydrochloric acid*, heat again to boiling, cool, filter and dilute the filtrate to 50.0 ml with *1 M hydrochloric acid*.

Reference solution Dissolve 50 mg of *2-chloro-4-nitroaniline R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Dilute 2.0 ml of this solution to 20.0 ml with *1 M hydrochloric acid*.

To 10.0 ml of the test solution and to 10.0 ml of the reference solution add separately 0.5 ml of a 5 g/l solution of *sodium nitrite R* and allow to stand for 3 min. Add 1 ml of a 20 g/l solution of *ammonium sulphamate R*, shake, allow to stand for 3 min and add 1 ml of a 5 g/l solution of *naphthylethylenediamine dihydrochloride R*. Any pinkish-violet colour produced in the test solution is not more intense than that in the reference solution.

Chlorides (*2.4.4*)

Maximum 500 ppm.

To 2 g add a mixture of 1.2 ml of *acetic acid R* and 40 ml of *water R,* boil for 2 min, cool and filter. Dilute 2 ml of the filtrate to 15 ml with *water R*.

Loss on drying (*2.2.32*)

4.5 per cent to 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.3000 g in 80 ml of a mixture of equal volumes of *acetone R* and *methanol R.* Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

STORAGE

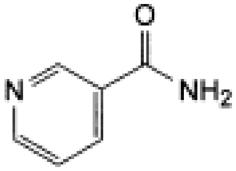
Protected from light.

Ph Eur

Nicotinamide

General Notices

(Ph Eur monograph 0047)



$C_6H_6N_2O$ 122.1 *98-92-0*

Action and use

Component of vitamin B.

Preparations

Nicotinamide Tablets

Vitamins B and C Injection

Ph Eur

DEFINITION

Nicotinamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of pyridine-3-carboxamide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water and in ethanol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 128 °C to 131 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *nicotinamide CRS*.

C. Boil 0.1 g with 1 ml of *dilute sodium hydroxide solution R*. Ammonia is evolved which is recognisable by its odour.

D. Dilute 2 ml of solution S (see Tests) to 100 ml with *water R*. To 2 ml of the solution, add 2 ml of *cyanogen bromide solution R* and 3 ml of a 25 g/l solution of *aniline R* and shake. A yellow colour develops.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

pH (2.2.3)

The pH of solution S is 6.0 to 7.5.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel* GF_{254} *plate R*.

Test solution Dissolve 0.4 g of the substance to be examined in a mixture of equal volumes of *alcohol R* and *water R* and dilute to 5.0 ml with the same mixture of solvents.

Reference solution Dilute 0.5 ml of the test solution to 200 ml with a mixture of equal volumes of *alcohol R* and *water R*.

Apply to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 4 volumes of *water R*, 45 volumes of *ethanol R* and 48 volumes of *chloroform R*. Allow the plate to dry and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.25 per cent).

Heavy metals (*2.4.8*)

Dilute 12 ml of solution S to 18 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (30 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.00 g by drying *in vacuo* for 18 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 20 ml of *anhydrous acetic acid R*, heating slightly if necessary, and add 5 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution R* as indicator until the colour changes to greenish-blue.

1 ml of *0.1 M perchloric acid* is equivalent to 12.21 mg of $C_6H_6N_2O$.

Ph Eur

Nicotine Resinate

General Notices

(Ph Eur monograph 1792)

Ph Eur

DEFINITION

Complex of nicotine (3-[(2*S*)-1-methylpyrrolidin-2-yl]pyridine) with a weak cationic exchange resin.

Content

95.0 per cent to 115.0 per cent of the declared content of nicotine stated on the label (anhydrous susbtance).

It may contain glycerol.

CHARACTERS

Appearance

White or slightly yellowish powder.

Solubility

Practically insoluble in water.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Shake a quantity of the substance to be examined equivalent to 100 mg of nicotine with a mixture of 10 ml of *dilute ammonia R2*, 10 ml of *water R*, 5 ml of *strong sodium hydroxide solution R* and 20 ml of *hexane R* for 5 min. Transfer the upper layer to a beaker and evaporate to produce an oily residue. Record the spectrum of the oily residue as a thin film between *sodium chloride R* plates.

Comparison Ph. Eur. reference spectrum of nicotine.

B. It complies with the test for nicotine release (see Tests).

TESTS

Nicotine release

Minimum 70 per cent of the content determined under Assay in 10 min.

Transfer an accurately weighed quantity of the substance to be examined equivalent to about 4 mg of nicotine, to a glass-stoppered test-tube, add 10.0 ml of a 9 g/l solution of *sodium* *chloride R* previously heated to 37 °C and shake vigorously for 10 min. Immediately filter the liquid through a dry filter paper discarding the first millilitre of filtrate. Transfer 1.0 ml of the filtrate to a 20 ml volumetric flask, dilute to volume with *0.1 M hydrochloric acid* and mix. Determine the absorbance (*2.2.25*) at the minima at about 236 nm and 282 nm and at the maximum at 259 nm using 1.0 ml of a 9 g/l solution of *sodium chloride R* diluted to 20 ml with *0.1 M hydrochloric acid* as compensation liquid.

Calculate the percentage of nicotine release from the expression:

$$\frac{20 \times 10^{6} \times \left(A_{259} - 0.5A_{236} - 0.5A_{282}\right)}{323 \times C \times m}$$

323 = specific absorbance of nicotine at 259 nm,

C = percentage of nicotine in the substance to be examined on the basis of the amount determined in the assay,

m = mass of the substance to be examined, in milligrams,

A_{259}, A_{236}, A_{282} = absorbances of the solution at the wavelength indicated by the subscript.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Accurately weigh a quantity of the substance to be examined equivalent to 20 mg of nicotine into a glass-stoppered test-tube, add 5.0 ml of *dilute ammonia R2*, 5.0 ml of *water R* and shake vigorously for 10 min. Centrifuge at about 2000 r/min for 10 min and dilute 3.0 ml of the clear solution to 10.0 ml with a 39.5 g/l solution of *phosphoric acid R*.

Reference solution (a) Weigh 60.0 mg of *nicotine ditartrate CRS* into a glass-stoppered test-tube, add 5.0 ml of *dilute ammonia R2*, 5.0 ml of *water R* and shake until dissolution is complete. Dilute 3.0 ml of the clear solution to 10.0 ml with a 39.5 g/l solution of *phosphoric acid R*.

Reference solution (b) Dissolve 5 mg of *myosmine R* in *acetonitrile R* and dilute to 5.0 ml with the same solvent. To 2.0 ml add 1.0 ml of the clear solution obtained during preparation of reference solution (a) and dilute to 10.0 ml with a 39.5 g/l solution of *phosphoric acid R*.

Reference solution (c) Dilute 1.0 ml of the test solution to 10.0 ml with a 39.5 g/l solution of *phosphoric acid R*. Dilute 1.0 ml to 20.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.10 m, Ø = 8 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase Dissolve 2.31 g of *sodium dodecyl sulphate R* in a mixture of 250 ml of *acetonitrile R* and 750 ml of a 13.6 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 4.5 with *dilute sodium hydroxide R* or *dilute phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 50 µl.

Run time Twice the retention time of nicotine.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity D and nicotine.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*)

Maximum 5.0 per cent.

Suspend 1.0 g in 20.0 ml of *methanol R*, shake for 30 min and allow to stand for 30 min. Use 10 ml of the methanol layer for the titration. Carry out a blank titration.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances.

Calculate the percentage content of nicotine using the chromatograms obtained with the test solution and reference solution (a) taking into account the declared content of *nicotine ditartrate CRS*.

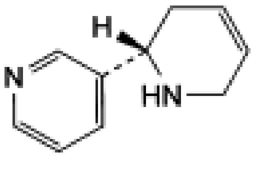
STORAGE

In an airtight container, protected from light.

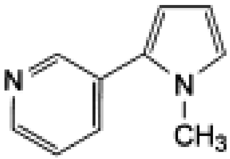
LABELLING

The label states the content of nicotine.

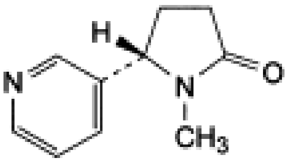
IMPURITIES



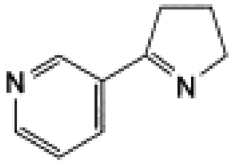
A. (2*S*)-1,2,3,6-tetrahydro-2,3′-bipyridyl (anatabine),



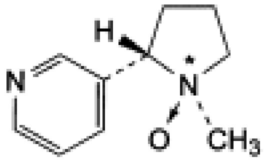
B. 3-(1-methyl-1*H*-pyrrol-2-yl)pyridine (β-nicotyrine),



C. (5*S*)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one (cotinine),



D. 3-(4,5-dihydro-3*H*-pyrrol-2-yl)pyridine (myosmine),



and epimer at N*

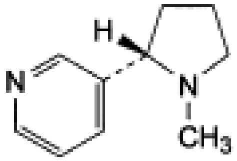
E. (1*RS*,2*S*)-1-methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide (nicotine *N*-oxide).

Ph Eur

Nicotine

General Notices

(Ph Eur monograph 1452)



$C_{10}H_{14}N_2$ 162.2 *54-11-5*

Action and use

Aid to smoking cessation.

Ph Eur

DEFINITION

Nicotine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3-[(2*S*)-1-methylpyrrolidin-2-yl]pyridine, calculated with reference to the anhydrous substance.

CHARACTERS

A colourless or brownish viscous liquid, volatile, hygroscopic, soluble in water, miscible with ethanol.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the *Ph. Eur. reference spectrum of nicotine.*

TESTS

Appearance of solution

Dissolve 1.0 g in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5, BY_5 or R_5 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 1.00 g in *ethanol R* and dilute to 50.0 ml with the same solvent. The specific optical rotation is -140 to -152.

Related substances

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 4 mg of *nicotine ditartrate CRS* and 2 mg of *myosmine R* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 0.4 ml of the test solution to 100.0 ml with the mobile phase.

The chromatography may be carried out using:

— a stainless steel column 0.10 m long and 8 mm in internal diameter, packed with *octadecylsilyl silica gel for chromatography R* (4 µm);

— as mobile phase at a flow rate of 1.5 ml/min a solution prepared as follows: dissolve 2.31 g of *sodium dodecyl sulphate R* in a mixture of 250 ml of *acetonitrile R* and 750 ml of a 13.6 g/l solution of *potassium dihydrogen phosphate R*, adjusted to pH 4.5 with *sodium hydroxide R* or *phosphoric acid R*;

— as detector a spectrophotometer set at 254 nm.

Inject 25 µl of reference solution (a). When the chromatograms are recorded in the prescribed conditions the retention times are: nicotine about 13 min and impurity D about 11 min. The test is not valid unless the resolution between the impurity D peak eluting closest to the nicotine peak and the peak due to nicotine is at least 1.5; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 25 µl of reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject 25 µl of the test solution and 25 µl of reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Water *(2.5.12)*

Not more than 0.5 per cent, determined on 1.00 g by the semi-micro determination of water.

ASSAY

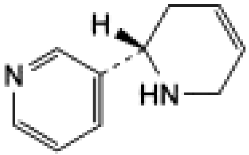
Dissolve 60.0 mg in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 8.11 mg of $C_{10}H_{14}N_2$.

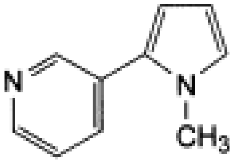
STORAGE

Store, under nitrogen, in an airtight container , protected from light.

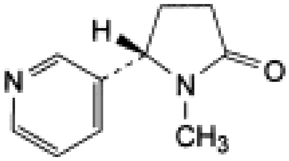
IMPURITIES



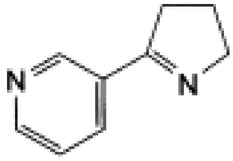
A. (2*S*)-1,2,3,6-tetrahydro-2,3′-bipyridyl (anatabine),



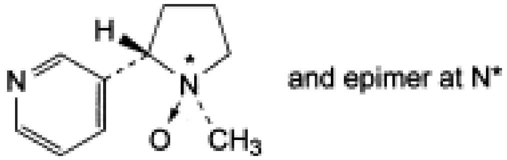
B. 3-(1-methyl-1*H*-pyrrol-2-yl)pyridine (β-nicotyrine),



C. (5*S*)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one (cotinine),



D. 3-(4,5-dihydro-3*H*-pyrrol-2-yl)pyridine (myosmine),



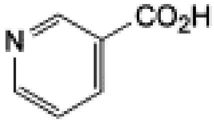
E. (1*RS*,2*S*)-1-methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide (nicotine *N*-oxide).

Ph Eur

Nicotinic Acid

General Notices

(Ph Eur monograph 0459)



$C_6H_5NO_2$ 123.1 *59-67-6*

Action and use

Component of vitamin B.

Preparation

Nicotinic Acid Tablets

Ph Eur

DEFINITION

Nicotinic acid contains not less than 99.5 per cent and not more than the equivalent of 100.5 per cent of pyridine-3-carboxylic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, soluble in boiling water and in boiling alcohol, sparingly soluble in water. It dissolves in dilute solutions of the alkali hydroxides and carbonates.

IDENTIFICATION

First identification A, B.

Second identification A, C.

A. Melting point (*2.2.14*): 234 °C to 240 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *nicotinic acid CRS*.

C. Dissolve about 10 mg in 10 ml of *water R*. To 2 ml of the solution add 2 ml of *cyanogen bromide solution R* and 3 ml of a 25 g/l solution of *aniline R* and shake. A yellow colour develops.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.5 g of the substance to be examined in *water R,* warming slightly if necessary, and dilute to 25 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *water R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *water R*, 10 volumes of *anhydrous formic acid R* and 85 volumes of *propanol R*. Dry the plate at 100 °C to 105 °C for 10 min and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Chlorides (*2.4.4*)

Dissolve 0.25 g in *water R*, heating on a water-bath, and dilute to 15 ml with the same solvent. The solution complies with the limit test for chlorides (200 ppm).

Heavy metals (*2.4.8*)

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, using 0.25 ml of *phenolphthalein solution R* as indicator, until a pink colour is obtained. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 12.31 mg of $C_6H_5NO_2$.

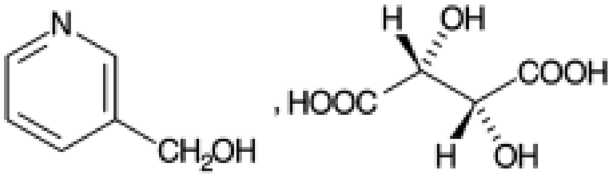
STORAGE

Store protected from light.

Ph Eur

Nicotinyl Alcohol Tartrate

General Notices



$C_6H_7NO,C_4H_6O_6$ 259.2   *6164-87-0*

Action and use

Vasodilator.

Preparation

Nicotinyl Alcohol Tablets

DEFINITION

Nicotinyl Alcohol Tartrate is 3-pyridylmethanol hydrogen (2*R*,3*R*)-tartrate. It contains not less than 98.5% and not more than 101.5% of $C_6H_7NO,C_4H_6O_6$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white crystalline powder.

Freely soluble in *water*; slightly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.004% w/v solution in 0.1 M *hydrochloric acid* exhibits a maximum only at 261 nm. The *absorbance* at 261 nm is about 0.84.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

C. Yields reaction B characteristic of *tartrates*, Appendix VI.

TESTS

Acidity

pH of a 5% w/v solution, 2.8 to 3.7, Appendix V L.

Clarity and colour of solution

A 5.0% w/v solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* Y_5, Appendix IV B, Method II.

Melting point

146° to 150°, Appendix V A.

Heavy metals

Dissolve the residue obtained in the test for Sulphated ash in 1 ml of 2M *hydrochloric acid* and dilute to 20 ml with *water*. 12 ml of the solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm)* to prepare the standard (20 ppm).

Nicotinaldehyde

Mix 10 ml of a 10% w/v solution of the substance being examined with 10 ml of a 1.0% w/v solution of *phenylhydrazine hydrochloride* in 3.6M *orthophosphoric acid*, dilute to 50 ml with *water* and allow to stand for 30 minutes. Measure the *absorbance* of the solution at the maximum at 370 nm, Appendix II B, using in the reference cell a 0.20% w/v solution of *phenylhydrazine hydrochloride* in 0.72M *orthophosphoric acid*. The absorbance is not more than that of a solution prepared by treating 10 ml of a 0.0010% w/v solution of *pyridine-3-carboxaldehyde* in the same manner.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 50 volumes of *dichloromethane*, 30 volumes of *1,4-dioxan*, 16 volumes of *methanol* and 4 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of four solutions in 0.1M *ammonia* containing (1) 25% w/v of the substance being examined, (2) 0.050% w/v of the substance being examined, (3) 0.050% w/v of *3-(aminomethyl)-pyridine* and (4) 0.050% w/v of *nicotinyl alcohol tartrate BPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (254 nm). Spray with a 2% w/v solution of *chloro-2,4,6-trinitrobenzene* in *absolute ethanol*, dry in a current of air and spray with a 5% w/v solution of *sodium carbonate*. Any spot corresponding to 3-(aminomethyl)pyridine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot due to tartaric acid on the line of application.

Loss on drying

When dried at 105° for 3 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A. Use 2.0 g.

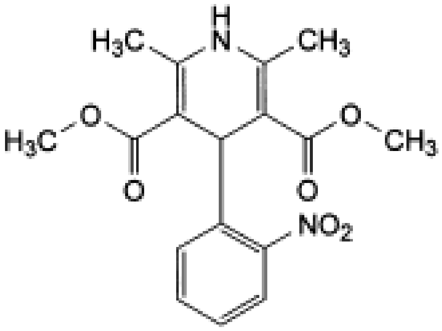
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 g and determining the end-point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 25.92 mg of $C_6H_7NO,C_4H_6O_6$.

Nifedipine

General Notices

(Ph Eur monograph 0627)



$C_{17}H_{18}N_2O_6$ 346.3 *21829-25-4*

Action and use

Calcium channel blocker.

Preparation

Nifedipine Capsules

Ph Eur

DEFINITION

Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol.

When exposed to daylight and to artificial light of certain wavelengths, it readily converts to a nitrosophenylpyridine derivative. Exposure to ultraviolet light leads to the formation of a nitrophenylpyridine derivative.

Prepare solutions immediately before use in the dark or under long-wavelength light (> 420 nm) and protect them from light.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Melting point (*2.2.14*): 171 °C to 175 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nifedipine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *nifedipine CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *ethyl acetate R*, *cyclohexane R* (40:60 *V/V*).

Application 5 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, appearance at 254 nm and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 25 mg in a test tube, add 10 ml of a mixture of 1.5 volumes of *hydrochloric acid R*, 3.5 volumes of *water R* and 5 volumes of *alcohol R* and dissolve with gentle heating. Add 0.5 g of *zinc R* in granules and allow to stand for 5 min with occasional swirling. Filter into a second test tube, add 5 ml of a 10 g/l solution of *sodium nitrite R* to the filtrate and allow to stand for 2 min. Add 2 ml of a 50 g/l solution of *ammonium sulphamate R*, shake vigorously with care and add 2 ml of a 5 g/l solution of *naphthylethylenediamine dihydrochloride R*. An intense red colour develops which persists for not less than 5 min.

TESTS

Impurity D and other basic impurities

Transfer 4 g to a 250 ml conical flask and dissolve in 160 ml of *glacial acetic acid R* using an ultrasonic bath. Titrate with *0.1 M perchloric acid* using 0.25 ml of *naphtholbenzein solution R* as indicator until the colour changes from brownish-yellow to green. Not more than 0.48 ml of *0.1 M perchloric acid* is required (0.14 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in 20 ml of *methanol R* and

dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 10 mg of *nifedipine impurity A CRS* in *methanol R* and dilute to 25.0 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *nifedipine impurity B CRS* in *methanol R* and dilute to 25.0 ml with the same solvent.

Reference solution (c) Mix 1.0 ml of reference solution (a), 1.0 ml of reference solution (b) and 0.1 ml of the test solution and dilute to 20.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *methanol R*, *water R* (9:36:55 *V/V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl; inject the test solution and reference solution (c).

Run time Twice the retention time of nifedipine.

Elution order Impurity A, impurity B, nifedipine.

Retention time Nifedipine = about 15.5 min.

System suitability Reference solution (c):

- *resolution*: minimum 1.5 between the peaks due to impurity A and impurity B and minimum 1.5 between the peaks due to impurity B and nifedipine.

Limits:

- *impurity A:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *any other impurity*: not more than the area of the peak due to nifedipine in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *total*: not more than 0.3 per cent;
- *disregard limit*: 0.1 times the area of the peak due to nifedipine in the chromatogram obtained with reference solution (c) (0.01 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.1300 g in a mixture of 25 ml of *2-methyl-2-propanol R* and 25 ml of *perchloric acid solution R*. Titrate with *0.1 M cerium sulphate* using 0.1 ml of *ferroin R* as indicator, until the

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pink colour disappears. Titrate slowly towards the end of the titration. Carry out a blank titration.

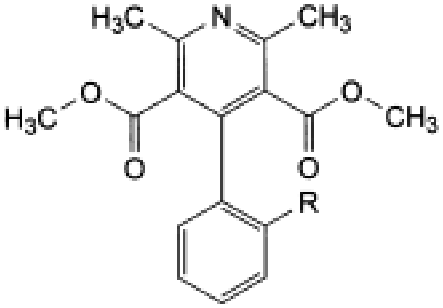
1 ml of *0.1 M cerium sulphate* is equivalent to 17.32 mg of $C_{17}H_{18}N_2O_6$.

STORAGE

Protected from light.

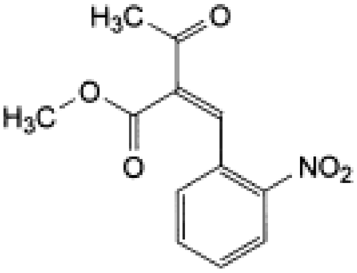
IMPURITIES

Specified impurities A, B, C, D.

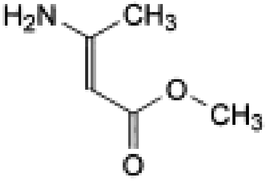


A. R = NO_2: dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (nitrophenylpyridine analogue),

B. R = NO: dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate (nitrosophenylpyridine analogue),



C. methyl 2-(2-nitrobenzylidene)-3-oxobutanoate,



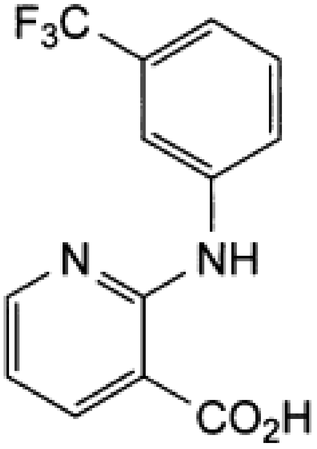
D. methyl 3-aminobut-2-enoate.

Ph Eur

Niflumic Acid

General Notices

(Ph Eur monograph 2115)



$C_{13}H_9F_3N_2O_2$ 282.2 *4394-00-7*

Ph Eur

DEFINITION

2-[[3-(Trifluoromethyl)phenyl]amino]pyridine-3-carboxylic acid.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Pale yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent) and in methanol.

mp

About 204 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *niflumic acid CRS.*

TESTS

Impurity C

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.50 g of the substance to be examined in 5 ml of *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 25 mg of *3-trifluoromethylaniline R* (impurity C) in 20 ml of *methanol R* and dilute to 100 ml with the same solvent. Dilute 1.0 ml of this solution to 100 ml with *methanol R.*

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetic acid R*, *ethyl acetate R*, *toluene R* (5:25:90 *V/V/V*).

Application 10 µl.

Development Over 3/4 of the plate.

Drying In air, until the solvents have evaporated.

Detection Spray with *4-dimethylaminocinnamaldehyde solution R* and heat at 60 °C for 10 min.

Limit:

— *impurity C*: any spot due to impurity C is not more intense than the principal spot in the chromatogram obtained with the reference solution (50 ppm).

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in 10 ml of *acetonitrile R* and dilute to 20.0 ml with *water R*.

Reference solution Dissolve 5.0 mg of *niflumic acid impurity A CRS*, 5.0 mg of *niflumic acid impurity B CRS* and 6.0 mg of *niflumic acid impurity E CRS* in 20 ml of *acetonitrile R,* add 5.0 ml of the test solution and dilute to 50.0 ml with *water R.* Dilute 1.0 ml of this solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R.*

Column:

— *size:* l = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 25 °C.

Mobile phase *phosphoric acid R*, *acetonitrile R*, *water R* (2.5:500:500 *V/V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 267 nm.

Injection 10 µl.

Run time 4 times the retention time of niflumic acid.

Relative retention With reference to niflumic acid (retention time = about 5.5 min): impurity A = about 0.25; impurity B = about 0.57; impurity E = about 0.64.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to impurities B and E.

Limits:

— *impurity B*: not more than 4 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.4 per cent);

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to niflumic acid in the chromatogram obtained with the reference solution (0.10 per cent);

— *sum of impurities other than B*: not more than twice the area of the peak due to niflumic acid in the chromatogram obtained with the reference solution (0.2 per cent);

— *disregard limit*: 0.5 times the area of the peak due to niflumic acid in the chromatogram obtained with the reference solution (0.05 per cent).

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dissolve 0.5 g in a mixture of 1 ml of *nitric acid R* and 10 ml of *methanol R*, and dilute to 20 ml with *water R*. To 10 ml of this solution add 5 ml of *water R.*

Phosphates *(2.4.11)*

Maximum 100 ppm.

Dilute 1.0 ml of the solution prepared in the test for heavy metals to 100 ml with *water R.*

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.3 per cent, determined on 2.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

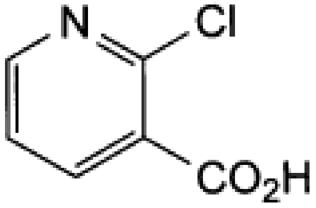
Dissolve 0.200 g in a mixture of 10 ml of *water R* and 40 ml of *ethanol (96 per cent) R.* Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 28.22 mg of $C_{13}H_9F_3N_2O_2$.

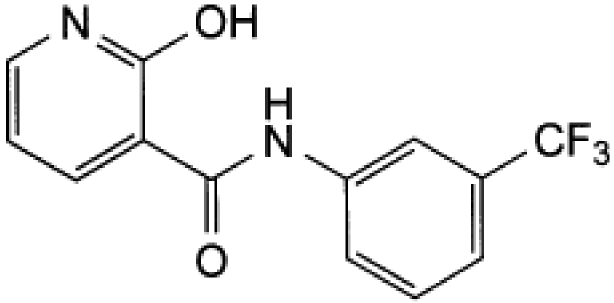
IMPURITIES

Specified impurities A, B, C.

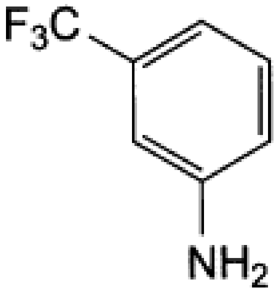
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, F.*



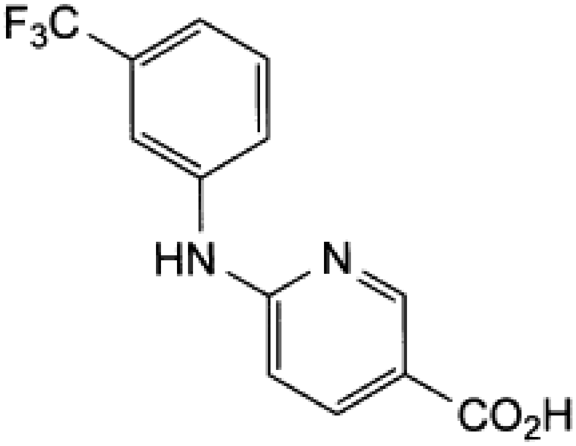
A. 2-chloropyridine-3-carboxylic acid,



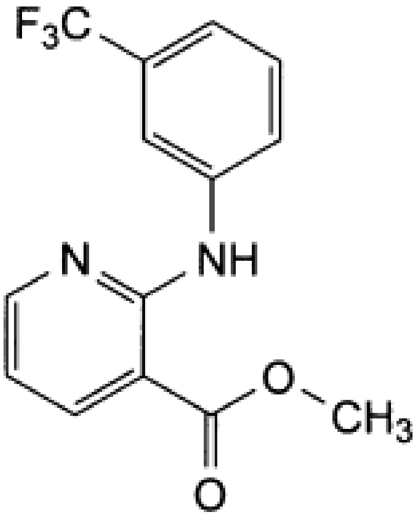
B. 2-hydroxy-*N*-[3-(trifluoromethyl)phenyl]pyridine-3-carboxamide,



C. 3-(trifluoromethyl)aniline,


F3C
HN
N
CO2H

E. 6-[[3-(trifluoromethyl)phenyl]amino]pyridine-3-carboxylic acid,


F3C
N
NH
O
CH3
O

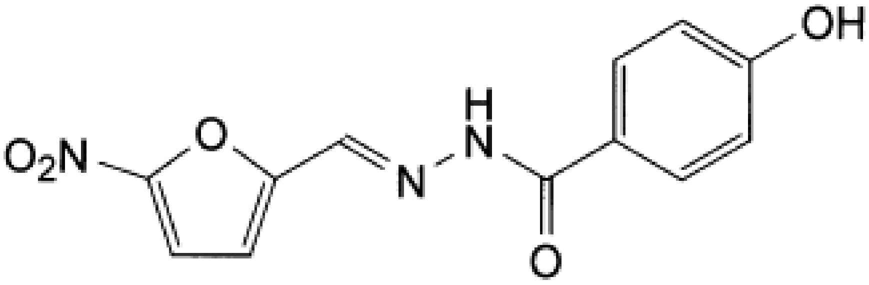
F. methyl 2-[[3-(trifluoromethyl)phenyl]amino]pyridine-3-carboxylate.

Ph Eur

Nifuroxazide

General Notices

(Ph Eur monograph 1999)



$C_{12}H_9N_3O_5$ 275.2 965-52-6

Action and use

Antibacterial.

Ph Eur

DEFINITION

(*E*)-4-Hydroxy-*N*′-[(5-nitrofuran-2-yl)methylidene]benzohydrazide.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Bright yellow, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison nifuroxazide CRS.

TESTS

Specific absorbance *(2.2.25)*

940 to 1000 at the absorption maximum at 367 nm.

Protected from light, dissolve 10.0 mg in 10 ml of *ethylene glycol monomethyl ether R* and dilute to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 100.0 ml with *methanol R.*

Impurity A

Maximum 0.05 per cent.

Test solution (a) Dissolve 1.0 g of the substance to be examined in *dimethyl sulphoxide R* and dilute to 10.0 ml with the same solvent.

Test solution (b) To 5.5 ml of test solution (a) add 50.0 ml of *water R* while stirring. Allow to stand for 15 min and filter.

Reference solution To 0.5 ml of test solution (a) add 5.0 ml of a 50 mg/l solution of *4-hydroxybenzohydrazide R* (impurity A) in *dimethyl sulphoxide R.* Add 50.0 ml of *water R* while stirring. Allow to stand for 15 min and filter.

Add 0.5 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *sodium carbonate solution R* separately to 10.0 ml of test solution (b) and to 10.0 ml of the reference solution. Allow to stand for 1 h. Examine the 2 solutions at 750 nm. The absorbance *(2.2.25)* of the solution obtained with test solution (b) is not greater than that obtained with the reference solution.

Related substances

Liquid chromatography *(2.2.29)*. *Use amber volumetric flasks, unless otherwise specified.*

Solvent mixture *acetonitrile R*, *water R* (40:60 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture, using sonication for not more than 5 min, and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) In order to prepare impurity E in situ, dissolve 5 mg of the substance to be examined in the solvent mixture in a colourless volumetric flask, using sonication for 5 min, and dilute to 50 ml with the solvent mixture. Allow to stand in ambient light for 1 h.

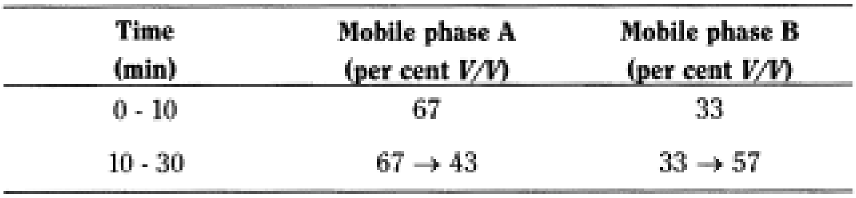
Reference solution (c) Dissolve 5.0 mg of *methyl parahydroxybenzoate CRS* (impurity B) in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 10 °C.

Mobile phase:

- *mobile phase A*: *tetrahydrofuran R*, *water R* (5:95 *V/V*);
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 10	67	33
10 - 30	67 → 43	33 → 57

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 50 µl.

Relative retention With reference to nifuroxazide (retention time = about 8 min): impurity A (keto-enol tautomers) = about 0.36 and 0.39; impurity E = about 0.9; impurity B = about 1.2; impurity C = about 2.6; impurity D = about 3.4.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity E and nifuroxazide.

Limits:

— *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities B, C, D*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent), and not more than 1 such peak has an area greater than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities other than E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peaks due to impurity A.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g, with heating if necessary, in 30 ml of *dimethylformamide R* and add 20 ml of *water R.* Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

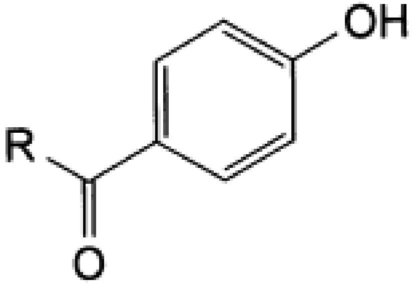
1 ml of *0.1 M sodium hydroxide* is equivalent to 27.52 mg of $C_{12}H_9N_3O_5$.

STORAGE

Protected from light.

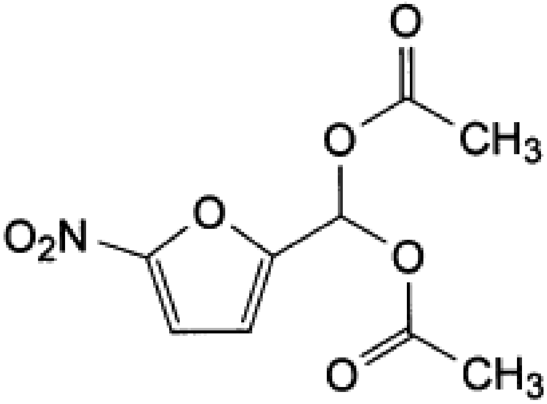
IMPURITIES

Specified impurities A, B, C, D, E.

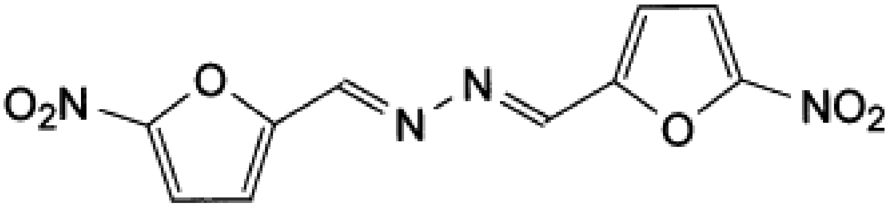


A. R = $NH\text{-}NH_2$: 4-hydroxybenzohydrazide (*p*-hydroxybenzohydrazide),

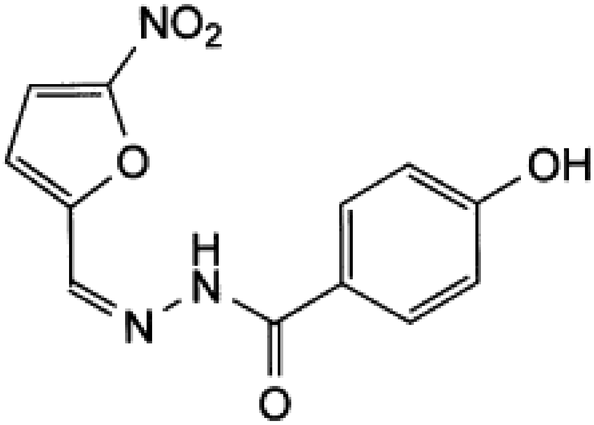
B. R = OCH_3: methyl 4-hydroxybenzoate (methyl parahydroxybenzoate),



C. (5-nitrofuran-2-yl)methylidene diacetate,



D. (*E*,*E*)-*N*,*N′*-bis[(5-nitrofuran-2-yl)methylidene]hydrazine (5-nitrofurfural azine),



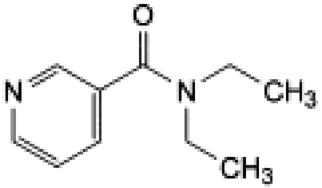
E. (*Z*)-4-hydroxy-*N′*-[(5-nitrofuran-2-yl)methylidene]benzohydrazide.

Ph Eur

Nikethamide

General Notices

(Ph Eur monograph 0233)



$C_{10}H_{14}N_2O$ 178.2 *59-26-7*

Action and use

Central nervous system stimulant.

Preparation

Nikethamide Injection

Ph Eur

DEFINITION

Nikethamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of *N*,*N*-diethylpyridine-3-carboxamide, calculated with reference to the anhydrous substance.

CHARACTERS

An oily liquid or a crystalline mass, colourless or slightly yellowish, miscible with water and with alcohol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Dissolve 0.15 g in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 1.0 ml of this solution to 100.0 ml with *0.01 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*) in a 2 cm cell, the solution shows a single absorption maximum, at 263 nm. The specific absorbance at the maximum is about 285.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *nikethamide CRS*.

C. Heat 0.1 g with 1 ml of *dilute sodium hydroxide solution R*. Diethylamine is evolved progressively and is recognisable by its characteristic odour and by its turning *red litmus paper R* blue.

D. Dilute 1 ml of solution S (see Tests) to 250 ml with *water R*. To 2 ml of this solution add 2 ml of *cyanogen bromide solution R*. Add 3 ml of a 25 g/l solution of *aniline R* and shake. A yellow colour develops.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Appearance

The substance to be examined, in liquid form or liquefied by slight heating, is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 6.0 to 7.8.

Refractive index (*2.2.6*)

1.524 to 1.526.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.4 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 40 mg of *ethylnicotinamide CRS* in *methanol R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 25 volumes of *propanol R* and 75 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spot corresponding to ethylnicotinamide is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent) and any spot, apart from the principal spot and the spot corresponding to ethylnicotinamide, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*)

Dilute 10 ml of solution S to 25 ml with *water R*. 12 ml of this solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

Not more than 0.3 per cent, determined on 2.00 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in a mixture of 5 ml of *acetic anhydride R* and 20 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

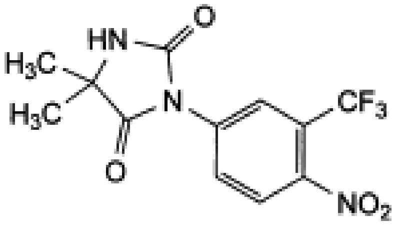
1 ml of *0.1 M perchloric acid* is equivalent to 17.82 mg of $C_{10}H_{14}N_2O$.

Ph Eur

Nilutamide

General Notices

(Ph Eur monograph 2256)



$C_{12}H_{10}F_3N_3O_4$ 317.2 *63612-50-0*

Action and use

Cytotoxic.

Ph Eur

DEFINITION

5,5-Dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl]imidazolidine-2,4-dione.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, freely soluble in acetone, soluble in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *nilutamide CRS.*

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Solvent mixture *acetonitrile for chromatography R, water R* (35:65 *V/V*).

Test solution Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 100 ml with the solvent mixture.

Reference solution (a) Dilute 20.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

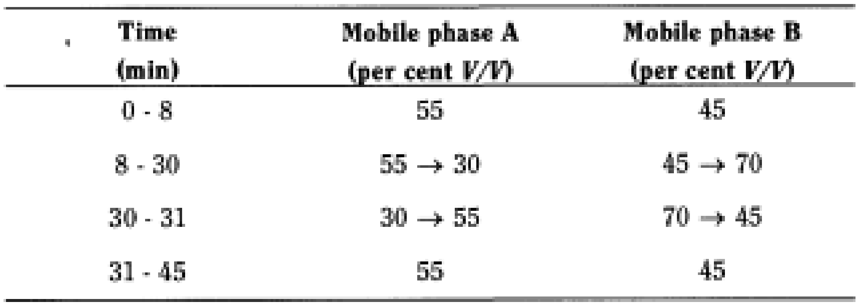
Reference solution (b) Dissolve 2 mg of the substance to be examined and 2 mg of *nilutamide impurity B CRS* in the solvent mixture and dilute to 50 ml with the solvent mixture.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase:* spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 2.0 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 7.5 with *1 M sodium hydroxide*;

— *mobile phase B: acetonitrile for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	55	45
8 - 30	55 → 30	45 → 70
30 - 31	30 → 55	70 → 45
31 - 45	55	45

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Relative retention With reference to nilutamide (retention time = about 5.3 min): impurity B = about 0.9.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to impurity B and nilutamide.

Limits:
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

It complies with test B with the following modifications.

Prescribed solution Dissolve 0.5 g in a mixture of 10 volumes of *water R* and 90 volumes of *acetone R* and dilute to 20 ml with the same mixture of solvents.

Test solution 12 ml of the prescribed solution.

Reference solution Dilute 0.5 ml of *lead standard solution (10 ppm Pb) R* to 10 ml with a mixture of 10 volumes of *water R* and 90 volumes of *acetone R* and add 2 ml of the prescribed solution.

Filter the solutions through a membrane filter (0.45 µm). Compare the spots on the filters obtained with the different solutions. The substance to be examined complies with the test if the brown colour of the spot obtained with the test solution is not more intense than that of the spot obtained with the reference solution.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography *(2.2.29)*. *The solutions are stable for 24 h at room temperature and in daylight.*

Solvent mixture *acetonitrile for chromatography R, water R* (35:65 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution Dissolve 50.0 mg of *nilutamide CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase:* spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a 2.0 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 7.5 with *1 M sodium hydroxide*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 267 nm.

Injection 20 µl.

Retention time About 9 min.

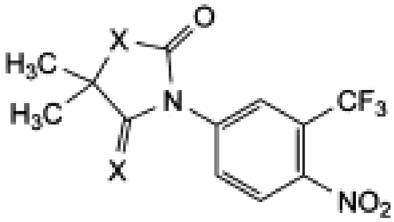
Calculate the percentage content of $C_{12}H_{10}F_3N_3O_4$ from the declared content of *nilutamide CRS*.

STORAGE

Protected from light.

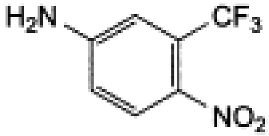
IMPURITIES

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D*.

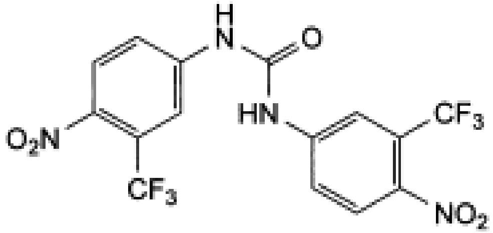


A. X = NH: 5-imino-4,4-dimethyl-1-[4-nitro-3-(trifluoromethyl)phenyl]imidazolidin-2-one,

C. X = O: 5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl]oxazolidine-2,4-dione,



B. 4-nitro-3-(trifluoromethyl)aniline (nifeline),



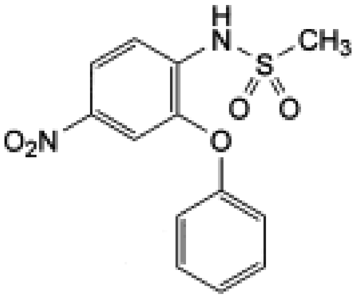
D. 1,3-bis[4-nitro-3-(trifluoromethyl)phenyl]urea.

Ph Eur

Nimesulide

General Notices

(Ph Eur monograph 1548)



$C_{13}H_{12}N_2O_5S$ 308.3 *51803-78-2*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

N-(4-Nitro-2-phenoxyphenyl)methanesulphonamide.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Yellowish crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, slightly soluble in anhydrous ethanol.

mp

About 149 °C.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *nimesulide CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Absorbance (*2.2.25*)

Maximum 0.50 at 450 nm.

Dissolve 1.0 g in *acetone R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in 8 ml of *acetonitrile R* and dilute to 20.0 ml with *water R*.

Reference solution (a) Dissolve 5 mg of *2-phenoxyaniline R* (impurity C) in 10 ml of *acetonitrile R* and dilute to 25.0 ml with *water R*. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase. Mix 1.0 ml of this solution with the contents of a vial of *nimesulide impurity D CRS* previously dissolved in 1.0 ml of *acetonitrile R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

— *dimensions*: l = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R*.

Mobile phase A mixture of 35 volumes of *acetonitrile R* and 65 volumes of a 1.15 g/l solution of *ammonium dihydrogen phosphate R* adjusted to pH 7.0 with *ammonia R*.

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 7 times the retention time of nimesulide.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the 2 principal peaks.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

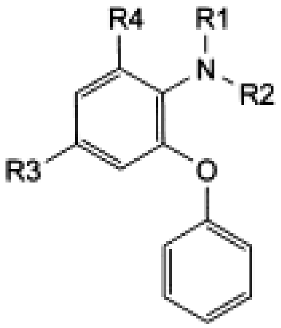
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.240 g in 30 ml of previously neutralised *acetone R* and add 20 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* is equivalent to 30.83 mg of $C_{13}H_{12}N_2O_5S$.

IMPURITIES



A. R1 = SO_2-CH_3, R2 = H, R3 = R4 = NO_2: *N*-(2,4-dinitro-6-phenoxyphenyl) methanesulphonamide,

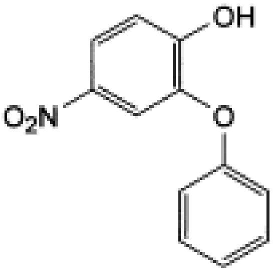
B. R1 = SO_2-CH_3, R2 = R3 = R4 = H: *N*-(2-phenoxyphenyl)methanesulphonamide,

C. R1 = R2 = R3 = R4 = H: 2-phenoxyaniline,

D. R1 = R2 = R4 = H, R3 = NO_2: 4-nitro-2-phenoxyaniline,

E. R1 = R2 = SO_2-CH_3, R3 = R4 = H: *N*,*N*-bis(methylsulphonyl)-2-phenoxyaniline,

F. R1 = R2 = SO_2-CH_3, R3 = NO_2, R4 = H: *N*,*N*-bis(methylsulphonyl)-4-nitro-2-phenoxyaniline,



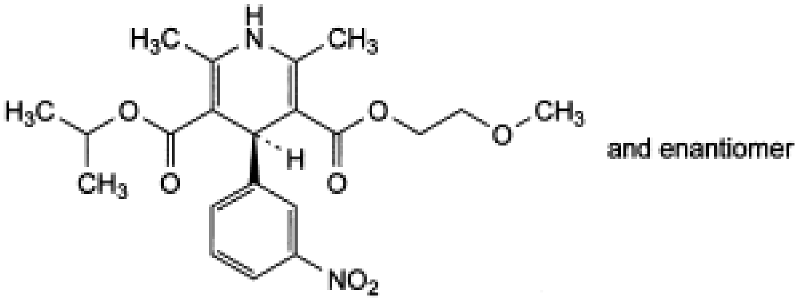
G. 4-nitro-2-phenoxyphenol.

Ph Eur

Nimodipine

General Notices

(Ph Eur monograph 1245)



$C_{21}H_{26}N_2O_7$ 418.4   *66085-59-4*

Action and use

Calcium channel blocker.

Preparations

Nimodipine Intravenous Infusion

Nimodipine Tablets

Ph Eur

DEFINITION

2-Methoxyethyl 1-methylethyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Light yellow or yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethyl acetate, sparingly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

Exposure to ultraviolet light leads to the formation of a nitrophenylpyridine derivative.

Prepare solutions immediately before use either protected from light or under long-wavelength light (> 420 nm).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nimodipine CRS.*

If the spectra obtained in the solid state show differences, record new spectra using 20 g/l solutions in *methylene chloride R* and a 0.2 mm cell.

TESTS

Solution S

Dissolve 1.0 g in *acetone R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*).

Optical rotation (*2.2.7*)

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in 2.5 ml of *tetrahydrofuran R* and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) *Nimodipine impurity A CRS.*

Reference solution (c) Dilute the test solution as described in the leaflet accompanying *nimodipine impurity A CRS*.

Reference solution (d) Mix reference solution (b) and reference solution (c) as described in the leaflet accompanying *nimodipine impurity A CRS*.

Column:

- *size:* l = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase *methanol R*, *tetrahydrofuran R*, *water R* (20:20:60 *V/V/V*).

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl of the test solution and reference solutions (a) and (d).

Run time 4 times the retention time of nimodipine.

Retention time Impurity A = about 7 min; nimodipine = about 8 min.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to impurity A and nimodipine.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *impurities B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the peak due to nimodipine in the chromatogram obtained with reference solution (d) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve with gentle heating 0.180 g in a mixture of 25 ml of *2-methyl-2-propanol R* and 25 ml of *perchloric acid solution R*. Add 0.1 ml of *ferroin R*. Titrate with *0.1 M cerium sulphate*. Titrate slowly towards the end of the titration. Carry out a blank titration.

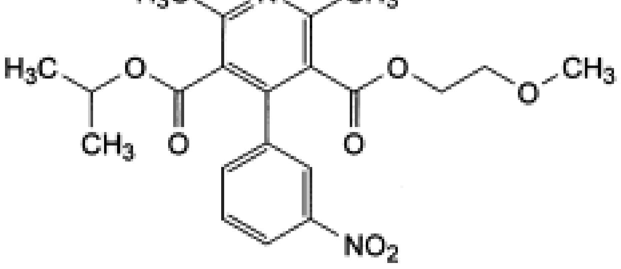
1 ml of *0.1 M cerium sulphate* is equivalent to 20.92 mg of $C_{21}H_{26}N_2O_7$.

STORAGE

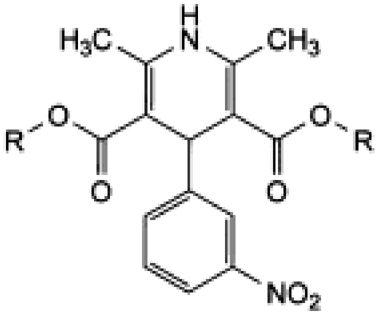
Protected from light.

IMPURITIES

Specified impurities *A, B, C*.



A. 2-methoxyethyl 1-methylethyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate,



B. R = $CH(CH_3)_2$: bis(1-methylethyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate,

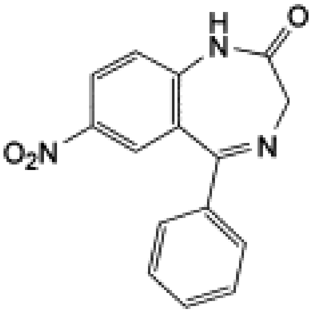
C. R = CH_2-CH_2-OCH_3: bis(2-methoxyethyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Ph Eur

Nitrazepam

General Notices

(Ph Eur monograph 0415)



$C_{15}H_{11}N_3O_3$ 281.3 *146-22-5*

Action and use

Benzodiazepine.

Preparations

Nitrazepam Oral Suspension

Nitrazepam Tablets

Ph Eur

DEFINITION

Nitrazepam contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, calculated with reference to the dried substance.

CHARACTERS

A yellow, crystalline powder, practically insoluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D, E.*

A. Melting point (*2.2.14*): 226 °C to 230 °C.

B. *Protect the solutions from light and measure the absorbances immediately*. Dissolve 25.0 mg in a 5 g/l solution of *sulphuric acid R* in *methanol R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with a 5 g/l solution of *sulphuric acid R* in *methanol R*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 280 nm. The specific absorbance at the maximum is 890 to 950.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *nitrazepam CRS*.

D. Dissolve about 20 mg in a mixture of 5 ml of *hydrochloric acid R* and 10 ml of *water R*. Boil for 5 min, cool and add 2 ml of a 1 g/l solution of *sodium nitrite R*. Allow to stand for 1 min and add 1 ml of a 5 g/l solution of *sulphamic acid R* and mix. Allow to stand for 1 min and add 1 ml of a 1 g/l solution of *naphthylethylenediamine dihydrochloride* R. A red colour is produced.

E. Dissolve about 10 mg in 1 ml of *methanol R*, warming if necessary, and add 0.05 ml of *dilute sodium hydroxide solution R*. An intense yellow colour is produced.

TESTS

Related substances

Carry out the test protected from light. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent. Prepare the solution immediately before use.

Reference solution (a) Dissolve 10 mg of *aminonitrobenzophenone R* in *acetone R* and dilute to 100 ml with the same solvent. Dilute 10 ml of the solution to 50 ml with *acetone R*.

Reference solution (b) Dissolve 10 mg of *nitrazepam impurity A CRS* in *acetone R* and dilute to 100 ml with the same solvent. Dilute 10 ml of the solution to 50 ml with *acetone R*.

Reference solution (c) Dilute 1 ml of the test solution to 20 ml with *acetone R*. Dilute 1 ml of this solution to 50 ml with *acetone R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm using a mixture of 15 volumes of *ethyl acetate R* and 85 volumes of *nitromethane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution: any spot corresponding to aminonitrobenzophenone is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent); any spot corresponding to nitrazepam impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent); any spot apart from the principal spot and the spots corresponding to aminonitrobenzophenone and nitrazepam impurity A is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

Heavy metals (*2.4.8*)

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

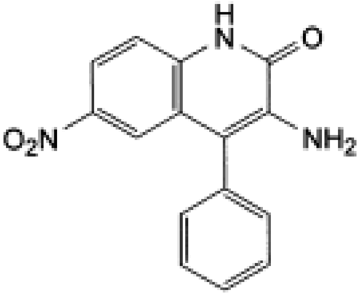
Dissolve 0.250 g in 25 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 28.13 mg of $C_{15}H_{11}N_3O_3$.

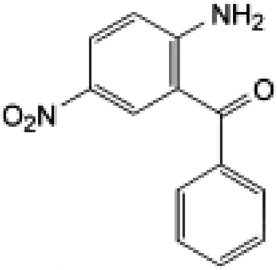
STORAGE

Store protected from light.

IMPURITIES



A. 3-amino-6-nitro-4-phenylquinolin-2(1*H*)-one,



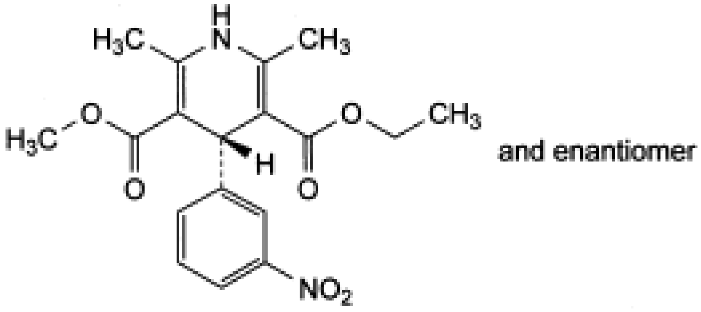
B. (2-amino-5-nitrophenyl)phenylmethanone.

Ph Eur

Nitrendipine

General Notices

(Ph Eur monograph 1246)



$C_{18}H_{20}N_2O_6$ 360.4 *39562-70-4*

Action and use

Calcium channel blocker.

Ph Eur

DEFINITION

Ethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethyl acetate, sparingly soluble in anhydrous ethanol and in methanol.

It shows polymorphism (*5.9*).

Exposure to ultraviolet light leads to the formation of a nitrophenylpyridine derivative

Prepare solutions immediately before use either protected from light or under long-wavelength light (> 420 nm).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nitrendipine CRS.*

If the spectra obtained in the solid state show differences, record new spectra using 20 g/l solutions in *methylene chloride R* and a 0.2 mm cell.

TESTS

Optical rotation (*2.2.7*)

- 0.10 ° to + 0.10 °.

Dissolve 0.2 g in *acetone R* and dilute to 10.0 ml with the same solvent.

Related substance

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in 2.5 ml of *tetrahydrofuran R* and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 25.0 ml with the mobile phase.

Reference solution (b) Dissolve 20.0 mg of *nitrendipine impurity A CRS* in 2.5 ml of *tetrahydrofuran R* and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c) Dilute 0.5 ml of the test solution to 20.0 ml with the mobile phase.

Reference solution (d) Mix 1.0 ml of reference solution (b) and 1.0 ml of reference solution (c), then dilute to 25.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.125 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *acetonitrile R*, *tetrahydrofuran R*, *water R* (14:22:64 *V/V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl of the test solution and reference solutions (a) and (d).

Run time 5 times the retention time of nitrendipine.

Retention time Impurity A = about 6 min; nitrendipine = about 8 min.

System suitability Reference solution (d):

— *resolution*: minimum 2.0 between the peaks due to impurity A and nitrendipine.

Limits:

— *impurities B, C*: for each impurity, not more than the area of the peak due to nitrendipine in the chromatogram obtained with reference solution (a) (0.8 per cent);

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.2 per cent);

— *disregard limit*: 0.5 times the area of the peak due to nitrendipine in the chromatogram obtained with reference solution (d) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g with gentle heating if necessary in a mixture of 25 ml of *2-methyl-2-propanol R* and 25 ml of *perchloric acid solution R.* Titrate with *0.1 M cerium sulphate*, using 0.1 ml of *ferroin R* as indicator. Titrate slowly towards the end of the titration. Carry out a blank titration.

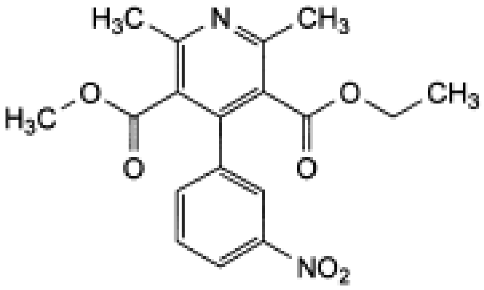
1 ml of *0.1 M cerium sulphate* is equivalent to 18.02 mg of $C_{18}H_{20}N_2O_6$.

STORAGE

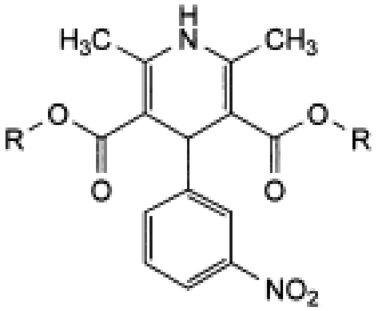
Protected from light.

IMPURITIES

Specified impurities   A, B, C.



A. ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate,



B. R = CH_3: dimethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate,

C. R = CH_2-CH_3: diethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Ph Eur

Nitric Acid

General Notices

(Ph Eur monograph 1549)

HNO_3 63.0 *7697-37-2*

Ph Eur

DEFINITION

Content

63.0 per cent *m/m* to 70.0 per cent *m/m* of HNO_3.

A clear, colourless or almost colourless liquid, miscible with water.

1.384 to 1.416.

A 10 g/l solution is strongly acid and gives the reaction of nitrates (*2.3.1*).

Appearance

Nitric acid is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*Method II, 2.2.2*).

Chlorides (*2.4.4*)

To 5 g add 10 ml of *water R* and 0.3 ml of *silver nitrate solution R2* and allow to stand for 2 min protected from light. Any opalescence is not more intense than that of a standard prepared in the same manner using 13 ml of *water R*, 0.5 ml of *nitric acid R* , 0.5 ml of *chloride standard solution (5 ppm Cl) R* and 0.3 ml of *silver nitrate solution R2* (0.5 ppm).

Sulphates (*2.4.13*)

Evaporate 10 g to dryness with 0.2 g of *sodium carbonate R*. Dissolve the residue in 15 ml of *distilled water R*. The solution complies with the limit test for sulphates (2 ppm). Prepare the standard using a mixture of 2 ml of *sulphate standard solution (10 ppm* SO_4*) R* and 13 ml of *distilled water R*.

Arsenic (*2.4.2*)

Gently heat 50 g with 0.5 ml of *sulphuric acid R* until white fumes begin to evolve. To the residue add 1 ml of a 100 g/l solution of *hydroxylamine hydrochloride R* and dilute to 2 ml with *water R*. The solution complies with limit test A for arsenic (0.02 ppm). Prepare the standard using 1.0 ml of *arsenic standard solution (1 ppm As) R*.

Heavy metals (*2.4.8*)

Dilute 10 ml of the solution prepared for the limit test for iron to 20 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (2 ppm). Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Iron (*2.4.9*)

Dissolve the residue from the determination of sulphated ash in 1 ml of *dilute hydrochloric acid R* and dilute to 50 ml with *water R*. 5 ml of the solution diluted to 10 ml with *water R* complies with the limit test for iron (1 ppm).

Sulphated ash

Carefully evaporate 100 g to dryness. Moisten the residue with a few drops of *sulphuric acid R* and heat to dull red. The residue does not exceed 0.001 per cent.

ASSAY

To 1.50 g add about 50 ml of *water R* and titrate with *1 M sodium hydroxide*, using 0.1 ml of *methyl red solution R* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 63.0 mg of HNO_3.

STORAGE

Protected from light.

Ph Eur

Nitric Oxide

General Notices

(Ph Eur monograph 1550)

NO 30.01 10102-43-9

Ph Eur

DEFINITION

Content

Minimum 99.0 per cent *V/V* of NO.

This monograph applies to nitric oxide for medicinal use.

CHARACTERS

Appearance

Colourless gas which turns brown when exposed to air.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 21 volumes of water.

PRODUCTION

Carbon dioxide

Gas chromatography *(2.2.28)*.

Gas to be examined The substance to be examined.

Reference gas Mixture containing 3000 ppm *V/V* of *carbon dioxide R1* in *nitrogen R*.

Column:

- *material*: stainless steel;
- *size*: *l* = 3.5 m, Ø = 2 mm;
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R*;
- *temperature*: 50 °C.

Carrier gas *helium for chromatography R.*

Flow rate 15 ml/min.

Detection Thermal conductivity.

Injection Loop injector.

System suitability:

— the chromatograms obtained show a clear separation of carbon dioxide from nitric oxide.

Limit:

— *carbon dioxide*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (3000 ppm *V/V*).

Nitrogen

Gas chromatography *(2.2.28)*.

Gas to be examined The substance to be examined.

Reference gas Mixture containing 3000 ppm *V/V* of *nitrogen R* in *helium for chromatography R*.

Column:

— *material*: stainless steel;

— *size*: *l* = 3.5 m, Ø = 2 mm;

— *stationary phase*: *molecular sieve for chromatography R* (0.5 nm);

— *temperature*: 50 °C.

Carrier gas *helium for chromatography R.*

Flow rate 15 ml/min.

Detection Thermal conductivity.

Injection Loop injector.

System suitability:

— the chromatograms obtained show a clear separation of nitrogen from nitric oxide.

Limit:

— *nitrogen*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (3000 ppm *V/V*).

Nitrogen dioxide

Maximum 400 ppm *V/V*.

Ultraviolet absorption spectrophotometry analyser.

Gas to be examined The substance to be examined.

Reference gas (a) *nitrogen R1.*

Reference gas (b) Mixture containing 400 ppm *V/V* of *nitrogen dioxide R* in *nitrogen R*.

Apparatus:

— an ultraviolet-visible light source (analytical wavelength about 400 nm);

— a sample gas cell through which the feed gas flows;

— a closed reference gas cell containing *nitrogen R1* in parallel with the sample gas cell;

— a rotating chopper which feeds light alternately through the reference gas cell and the sample gas cell;

— a semiconductor detector which generates a frequency modulated output whose amplitude is a measure of the difference of absorption of the sample gas and the reference gas.

Analysis:

— set the zero of the instrument using reference gas (a) through the sample gas cell at a flow rate of 1 litre/min;

— adjust the span while feeding reference gas (b) through the sample gas cell at a flow rate of 1 litre/min;

— feed the gas to be examined through the sample gas cell at a flow rate of 1 litre/min, read the value from the instrument output and calculate, if necessary, the concentration of nitrogen dioxide.

Nitrous oxide

Gas chromatography *(2.2.28)*.

Gas to be examined The substance to be examined.

Reference gas Mixture containing 3000 ppm *V/V* of *nitrous oxide R* in *nitrogen R*.

Column:

— *material*: stainless steel;

— *size*: *l* = 3.5 m, Ø = 2 mm;

— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R*;

— *temperature*: 50 °C.

Carrier gas *helium for chromatography R.*

Flow rate 15 ml/min.

Detection Thermal conductivity.

Injection Loop injector.

System suitability:

— the chromatograms obtained show a clear separation of nitrous oxide from nitric oxide.

Limit:

— *nitrous oxide*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (3000 ppm *V/V*).

Water *(2.5.28)*

Maximum 100 ppm *V/V*.

Assay

Determine the content of nitric oxide by difference using the mass balance equation after determining the sum of the impurities described under Production.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of nitric oxide.*

STORAGE

Compressed at a pressure not exceeding 2.5 MPa (25 bars) measured at 15 °C, in suitable containers complying with the legal regulations.

IMPURITIES

Specified impurities A, B, C, D, E.

A. carbon dioxide,

B. nitrogen,

C. nitrogen dioxide,

D. nitrous oxide,

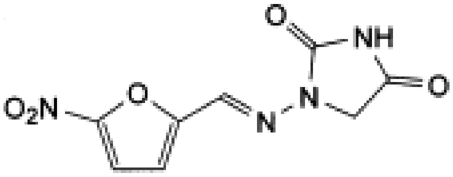
E. water.

Ph Eur

Nitrofurantoin

General Notices

(Ph Eur monograph 0101)



$C_8H_6N_4O_5$ 238.2 *67-20-9*

Action and use

Antibacterial.

Preparations

Nitrofurantoin Oral Suspension

Nitrofurantoin Tablets

Ph Eur

DEFINITION

Nitrofurantoin contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 1-[[(5-nitrofuran-2-yl)methylene]amino]imidazolidine-2,4-dione, calculated with reference to the dried substance.

CHARACTERS

A yellow, crystalline powder or yellow crystals, odourless or almost odourless, very slightly soluble in water and in alcohol, soluble in dimethylformamide.

IDENTIFICATION

A. *Carry out the test protected from bright light.* Use the solution prepared for the assay. Examined between 220 nm and 400 nm (*2.2.25*), the solution shows two absorption maxima, at 266 nm and 367 nm. The ratio of the absorbance at the maximum at 367 nm to that at the maximum at 266 nm is 1.36 to 1.42.

B. Dissolve about 10 mg in 10 ml of *dimethylformamide R*. To 1 ml of the solution add 0.1 ml of *0.5 M alcoholic potassium hydroxide*. A brown colour develops.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel HF_{254} R* as the coating substance.

Test solution Dissolve 0.25 g of the substance to be examined in a minimum of *dimethylformamide R* and dilute to 10 ml with *acetone R*.

Reference solution Dilute 1 ml of the test solution to 100 ml with *acetone R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R* and 90 volumes of *nitromethane R*. Allow the plate to dry in air and heat at 100 °C to 105 °C for 5 min. Examine in ultraviolet light at 254 nm. Spray with *phenylhydrazine hydrochloride solution R*. Heat the plate at 100 °C to 105 °C for a further 10 min. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the assay protected from bright light. Dissolve 0.120 g in 50 ml of *dimethylformamide R* and dilute to 1000.0 ml with *water R*. Dilute 5.0 ml of the solution to 100.0 ml with a solution containing 18 g/l of *sodium acetate R* and 0.14 per cent *V/V* of *glacial acetic acid R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 367 nm, using the sodium acetate solution described above as compensation liquid.

Calculate the content of $C_8H_6N_4O_5$, taking the specific absorbance to be 765.

STORAGE

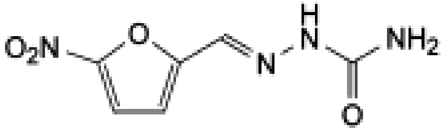
Store protected from light, at a temperature below 25 °C.

Ph Eur

Nitrofurazone

General Notices

(Nitrofural, Ph Eur monograph 1135)



$C_6H_6N_4O_4$ 198.1 *59-87-0*

Action and use

Antibacterial; topical antiprotozoal.

Ph Eur

DEFINITION

2-[(5-Nitrofuran-2-yl)methylene]diazanecarboxamide.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow or brownish-yellow, crystalline powder.

Solubility

Very slightly soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*). *Carry out the test protected from bright light.*

Test solution Use the solution prepared for the assay.

Spectral range 220-400 nm.

Absorption maxima At 260 nm and 375 nm.

Absorbance ratio A_{375}/A_{260} = 1.15 to 1.30.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *nitrofural CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *nitrofural CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *methanol R*, *nitromethane R* (10:90 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *phenylhydrazine hydrochloride solution R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 1 mg in 1 ml of *dimethylformamide R* and add 0.1 ml of *alcoholic potassium hydroxide solution R*. A violet-red colour is produced.

TESTS

pH (*2.2.3*)

5.0 to 7.0.

To 1.0 g add 100 ml of *carbon dioxide-free water R*. Shake and filter.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *(5-nitro-2-furyl)methylene diacetate R* (impurity B) in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *nitrofural CRS* and 10 mg of *nitrofurantoin R* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 5 ml of this solution to 100 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *water R* (40:60 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 310 nm.

Injection 20 µl.

Run time 10 times the retention time of nitrofural.

Retention time Nitrofural = about 3 min.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to nitrofurantoin and nitrofural.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the assay protected from bright light. Dissolve 60.0 mg in 20 ml of *dimethylformamide R* and dilute to 500.0 ml with *water R*. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. Prepare a reference solution in the same manner using 60.0 mg of *nitrofural CRS*. Measure the absorbances (*2.2.25*) of the 2 solutions at the absorption maximum at 375 nm.

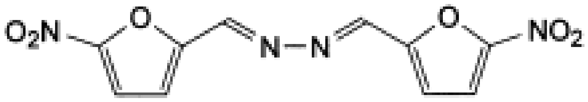
Calculate the content of $C_6H_6N_4O_4$ from the absorbances measured and the concentrations of the solutions.

STORAGE

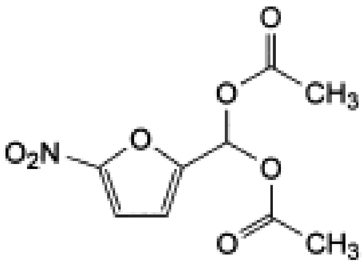
Protected from light.

IMPURITIES

Specified impurities A, B.



A. bis[(5-nitrofuran-2-yl)methylene]diazane,


O
O
CH3
O2N
O
O
O
CH3

B. (5-nitrofuran-2-yl)methylene diacetate.

Ph Eur

Nitrogen

General Notices

(Ph Eur monograph 1247)

N_2 28.01 7727-37-9

Nitrogen should be kept in approved metal cylinders, the shoulders of which are painted black and the remainder grey. The cylinder should carry a label stating 'Nitrogen'.

Ph Eur

DEFINITION

Content

Minimum 99.5 per cent *V/V* of N_2.

This monograph applies to nitrogen for medicinal use.

CHARACTERS

Appearance

Colourless, odourless gas.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 62 volumes of water and about 10 volumes of ethanol (96 per cent).

PRODUCTION

Carbon dioxide

Maximum 300 ppm *V/V*, determined using an infrared analyser *(2.5.24)*.

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Nitrogen R1.*

Reference gas (b) Mixture containing 300 ppm *V/V* of *carbon dioxide R1* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon dioxide in the gas to be examined.

Carbon monoxide

Maximum 5 ppm *V/V*, determined using an infrared analyser *(2.5.25)*.

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Nitrogen R1.*

Reference gas (b) Mixture containing 5 ppm *V/V* of *carbon monoxide R* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon monoxide in the gas to be examined.

Oxygen

Maximum 50 ppm *V/V*, determined using an oxygen analyser with a detector scale ranging from 0-100 ppm *V/V* and equipped with an electrochemical cell.

The gas to be examined passes through a detection cell containing an aqueous solution of an electrolyte, generally potassium hydroxide. The presence of oxygen in the gas to be examined produces variation in the electric signal recorded at the outlet of the cell that is proportional to the oxygen content.

Calibrate the analyser according to the instructions of the manufacturer. Pass the gas to be examined through the analyser using a suitable pressure regulator and airtight metal tubes and operating at the prescribed flow-rates until constant readings are obtained.

Water ***(2.5.28)***

Maximum 67 ppm *V/V*.

Assay

Gas chromatography *(2.2.28)*.

Gas to be examined The substance to be examined.

Reference gas (a) Ambient air.

Reference gas (b) *Nitrogen R1.*

Column:
- — *material*: stainless steel;
- — *size*: *l* = 2 m, Ø = 2 mm;
- — *stationary phase*: *molecular sieve for chromatography R* (0.5 nm).

Carrier gas *helium for chromatography R.*

Flow rate 40 ml/min.

Temperature:
- — *column*: 50 °C;
- — *detection*: 130 °C.

Detection Thermal conductivity.

Injection Loop injector.

Inject reference gas (a). Adjust the injected volumes and operating conditions so that the height of the peak due to nitrogen in the chromatogram obtained with the reference gas is at least 35 per cent of the full scale of the recorder.

System suitability:
- — the chromatograms obtained show a clear separation of oxygen and nitrogen.

Calculate the content of N_2 in the gas to be examined.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Examine the chromatograms obtained in the assay (see Production).

Results The principal peak in the chromatogram obtained with the substance to be examined is similar in retention time to the principal peak in the chromatogram obtained with reference gas (b).

B. In a 250 ml conical flask replace the air by the substance to be examined. Place a burning or glowing splinter of wood in the flask. The splinter is extinguished.

C. In a suitable test tube, place 0.1 g of *magnesium R* in turnings. Close the tube with a two-hole stopper fitted with a glass tube reaching about 1 cm above the turnings. Pass the substance to be examined through the glass tube for 1 min without heating, then for 15 min while heating the test tube to a red glow. After cooling, add 5 ml of *dilute sodium hydroxide solution R*. The evolving vapours change the colour of moistened *red litmus paper R* blue.

TESTS

Carbon dioxide *(2.1.6)*

Maximum 300 ppm *V/V*, determined using a carbon dioxide detector tube.

Carbon monoxide *(2.1.6)*

Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube.

Water vapour *(2.1.6)*

Maximum 67 ppm *V/V*, determined using a water vapour detector tube.

STORAGE

As a compressed gas or a liquid in appropriate containers complying with the legal regulations.

IMPURITIES

Specified impurities A, B, C, D.

A. carbon dioxide,

B. carbon monoxide,

C. oxygen,

D. water.

Ph Eur

Nitrous Oxide

General Notices

(Ph Eur monograph 0416)

N_2O 44.01 *10024-97-2*

Action and use

General anaesthetic; analgesic.

Nitrous oxide should be kept in approved metal cylinders which are painted blue and carry a label stating 'Nitrous Oxide'. In addition, 'Nitrous Oxide' or the symbol 'N_2O' should be stencilled in paint on the shoulder of the cylinder.

Ph Eur

DEFINITION

Content

Minimum 98.0 per cent *V/V* of N_2O in the gaseous phase, when sampled at 15 °C.

This monograph applies to nitrous oxide for medicinal use.

CHARACTERS

Appearance

Colourless gas.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 1.5 volumes of water.

PRODUCTION

Nitrous oxide is produced from ammonium nitrate by thermic decomposition.

Examine the gaseous phase.

If the test is performed on a cylinder, keep the cylinder at room temperature for at least 6 h before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost.

Carbon dioxide

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas A mixture containing 300 ppm *V/V* of *carbon dioxide R1* in *nitrous oxide R*.

Column:

- — *material*: stainless steel;
- — *size: l* = 3.5 m, Ø = 2 mm;
- — *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R*.

Carrier gas *helium for chromatography R*.

Flow rate 15 ml/min.

Temperature:

- — *column*: 40 °C;
- — *detector*: 90 °C.

Detection Thermal conductivity.

Injection Loop injector.

Adjust the injected volumes and operating conditions so that the height of the peak due to carbon dioxide in the chromatogram obtained with the reference gas is at least 35 per cent of the full scale of the recorder. The test is not valid unless the chromatograms obtained show a clear separation of carbon dioxide from nitrous oxide.

Limit:

- — *carbon dioxide*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (300 ppm *V/V*).

Carbon monoxide Gas chromatography (*2.2.28*). When the test is carried out on a cylinder, use the first portion of gas to be withdrawn.

Gas to be examined The substance to be examined.

Reference gas A mixture containing 5 ppm *V/V* of *carbon monoxide R* in *nitrous oxide R*.

Column:

- — *material*: stainless steel;
- — *size: l* = 2 m, Ø = 4 mm;
- — *stationary phase*: suitable molecular sieve for chromatography (0.5 nm).

Carrier gas *helium for chromatography R*.

Flow rate 60 ml/min.

Temperature:

- — *column*: 50 °C;
- — *injection port and detector*: 130 °C.

Detection Flame ionisation with methaniser.

Injection Loop injector.

Adjust the injected volumes and the operating conditions so that the height of the peak due to carbon monoxide in the chromatogram obtained with the reference gas is at least 35 per cent of the full scale of the recorder.

Limit:

- — *carbon monoxide*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (5 ppm *V/V*).

Nitrogen monoxide and nitrogen dioxide

Maximum 2 ppm *V/V* in total in the gaseous and liquid phases, determined using a chemiluminescence analyser (*2.5.26*).

Gas to be examined The substance to be examined.

Reference gas (a) Nitrous oxide R.

Reference gas (b) A mixture containing 2 ppm *V/V* of *nitrogen monoxide R* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of nitrogen monoxide and nitrogen dioxide, separately examining the samples collected from the gaseous phase and the liquid phase of the gas to be examined.

Multiply the result obtained by the quenching correction factor in order to correct the quenching effect on the analyser response caused by the nitrous oxide matrix effect.

The quenching correction factor is determined by applying a known reference mixture of nitrogen monoxide in nitrous oxide and comparing the actual content with the content indicated by the analyser which has been calibrated with an NO/N_2 reference mixture.

$$\text{Quenching correction factor} = \frac{\text{actual nitrogen monoxide content}}{\text{indicated nitrogen monoxide content}}$$

Water

Maximum 67 ppm *V/V*, determined using an electrolytic hygrometer (*2.5.28*).

ASSAY

Infrared analyser (*2.5.35*).

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) Nitrous oxide R.

Reference gas (b) A mixture containing 5.0 per cent *V/V* of *nitrogen R1* and 95.0 per cent *V/V* of *nitrous oxide R*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of nitrous oxide in the gas to be examined.

IDENTIFICATION

First identification A.

Second identification B, C.

A. It complies with the limits of the assay.

B. Place a glowing splinter of wood in the substance to be examined. The splinter bursts into flame.

C. Introduce the substance to be examined into *alkaline pyrogallol solution R*. A brown colour does not develop.

TESTS

Examine the gaseous phase.

If the test is performed on a cylinder, keep the cylinder of the substance to be examined at room temperature for at least 6 h before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost.

Carbon dioxide

Maximum 300 ppm *V/V*, determined using a carbon dioxide detector tube (*2.1.6*).

Carbon monoxide

Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube (*2.1.6*). *When the test is carried out on a cylinder, use the first portion of the gas to be withdrawn.*

Nitrogen monoxide and nitrogen dioxide

Maximum 2 ppm *V/V*, determined using a nitrogen monoxide and nitrogen dioxide detector tube (*2.1.6*).

Water vapour

Maximum 67 ppm *V/V*, determined using a water vapour detector tube (*2.1.6*).

STORAGE

Store liquefied under pressure in suitable containers complying with the legal regulations. The taps and valves are not greased or oiled.

IMPURITIES

Specified impurities *A, B, C, D, E*.

A. carbon dioxide,

B. carbon monoxide,

C. nitrogen monoxide,

D. nitrogen dioxide,

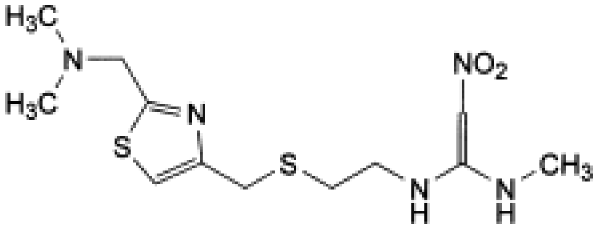
E. water.

Ph Eur

Nizatidine

General Notices

(Ph Eur monograph 1453)



$C_{12}H_{21}N_5O_2S_2$ 331.5 *76963-41-2*

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer.

Preparation

Nizatidine Intravenous Infusion

Ph Eur

DEFINITION

(*EZ*)-*N*-[2-[[[2-[(Dimethylamino)methyl]thiazol-4-yl]methyl]sulphanyl]ethyl]-*N′*-methyl-2-nitroethene-1,1-diamine.

Content

97.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Almost white or slightly brownish, crystalline powder.

Solubility

Sparingly soluble in water, soluble in methanol.

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Melting point (*2.2.14*): 131 °C to 134 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.10 g in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *methanol R*.

Spectral range 220-350 nm.

Absorption maxima At 242 nm and 325 nm.

Absorbance ratio A_{325}/A_{242} = 2.2 to 2.5.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *nizatidine CRS*.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *nizatidine CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 50 mg of *nizatidine CRS* and 50 mg of *ranitidine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *water R*, *concentrated ammonia R1*, *2-propanol R*, *ethyl acetate R* (2:4:15:25 *V/V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose to iodine vapour until the spots are clearly visible. Examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 0.2 g in a 10 g/l solution of *hydrochloric acid R* and dilute to 20 ml with the same acid solution.

pH (*2.2.3*)

8.5 to 10.0.

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in a mixture of 24 volumes of mobile phase B and 76 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Test solution (b) Dissolve 15.0 mg of the substance to be examined in a mixture of 24 volumes of mobile phase B and 76 volumes of mobile phase A and dilute to 50.0 ml with the same mixture of mobile phases.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with a mixture of 24 volumes of mobile phase B and 76 volumes of mobile phase A.

Reference solution (b) Dissolve 15.0 mg of *nizatidine CRS* in a mixture of 24 volumes of mobile phase B and 76 volumes of mobile phase A and dilute to 50.0 ml with the same mixture of mobile phases.

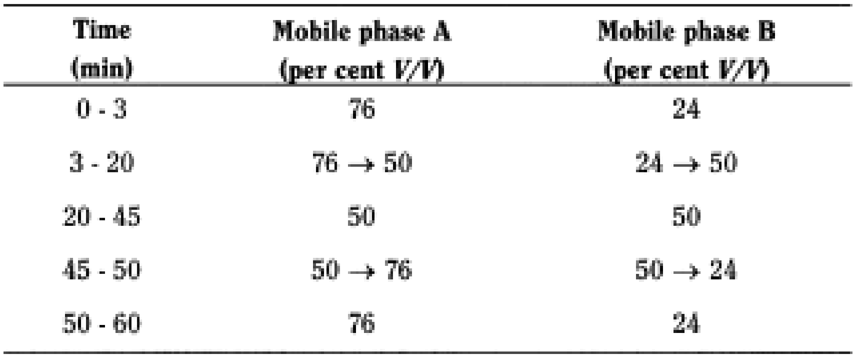
Reference solution (c) Dissolve 5 mg of *nizatidine CRS* and 0.5 mg of *nizatidine impurity F CRS* in a mixture of 24 volumes of mobile phase B and 76 volumes of mobile phase A and dilute to 100.0 ml with the same mixture of mobile phases.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: dissolve 5.9 g of *ammonium acetate R* in 760 ml of *water R*, add 1 ml of *diethylamine R*, and adjust to pH 7.5 with *acetic acid R*;
- *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	76	24
3 - 20	76 → 50	24 → 50
20 - 45	50	50
45 - 50	50 → 76	50 → 24
50 - 60	76	24

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (c).

System suitability:

— *retention time*: nizatidine = 10 min to 20 min in the chromatogram obtained with reference solution (a);

— *symmetry factor*: maximum 2.0 for the peak due to nizatidine in the chromatogram obtained with reference solution (a);

— *resolution*: minimum 2.0 between the peaks due to nizatidine (1st peak) and impurity F (2nd peak) in the chromatogram obtained with reference solution (c).

Limits:

— *impurities A, B, C, D, E, F, G, H, I, J, K*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.03 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mobile phase B, mobile phase A (35:65 *V/V*).

Injection Test solution (b) and reference solution (b).

System suitability Reference solution (b):

— *retention time*: nizatidine = 8 min to 10 min;

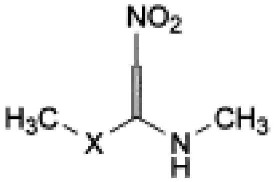
— *symmetry factor*: maximum 2.0 for the peak due to nizatidine;

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections.

Calculate the percentage content of nizatidine from the declared content of *nizatidine CRS*.

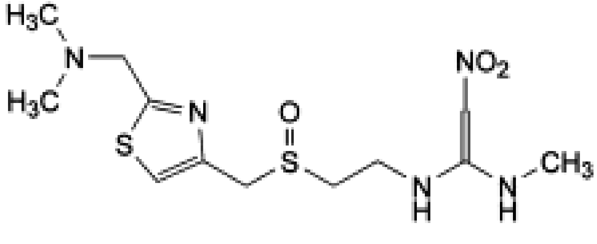
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H, I, J, K.*

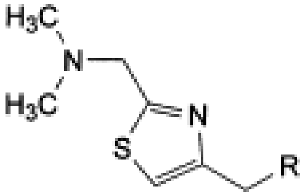


A. X = NH: *N*,*N′*-dimethyl-2-nitroethene-1,1-diamine,

B. X = S:(*EZ*)-*N*-methyl-1-(methylsulphanyl)-2-nitroethen-1-amine,



C. (*EZ*)-*N*-[2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl]sulphinyl]ethyl]-*N′*-methyl-2-nitroethene-1,1-diamine,

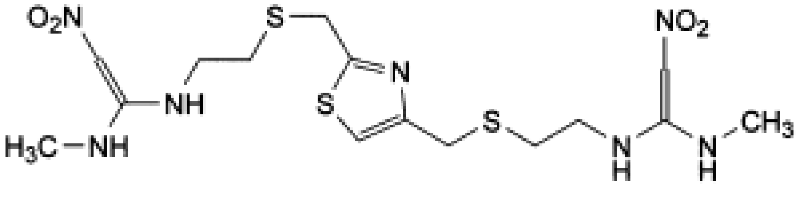


D. R = S-CH_2-CH_2-NH_2: 2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl]sulphanyl] ethanamine,

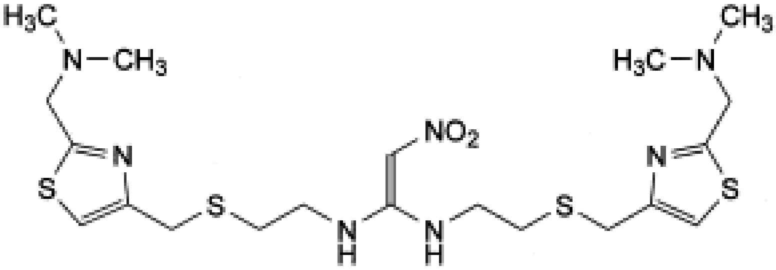
E. R = S-CH_2-CH_2-NH-CO-CH_2-NO_2:*N*-[2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl] sulphanyl]ethyl]-2nitroacetamide,

I. R = S-CH_2-CH_2-NH-CO-NH-CH_3:*N*-[2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl] sulphanyl]ethyl]-*N′*-methylurea,

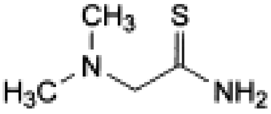
J. R = OH: [2-[(dimethylamino)methyl]thiazol-4-yl]methanol,



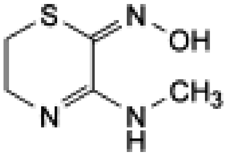
F. (*EZ*)-*N*[1],*N*[1′]-[thiazole-2,4-diylbis(methylenesulphanediylethylene)]bis(*N′*-methyl-2-nitroethene-1,1-diamine),



G. *N*,*N′*-bis[2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl]sulphanyl]ethyl]-2-nitroethene-1,1-diamine,



H. 2-(dimethylamino)thioacetamide,



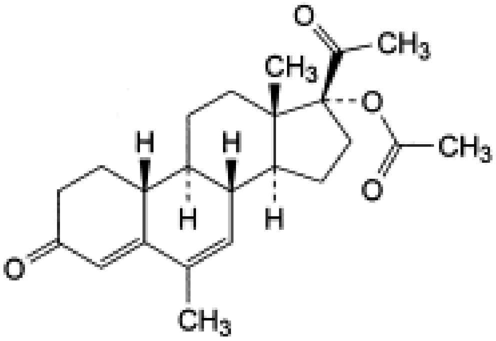
K. 3-(methylamino)-5,6-dihydro-2*H*-1,4-thiazin-2-one oxime.

Ph Eur

Nomegestrol Acetate

General Notices

(Ph Eur monograph 1551)



$C_{23}H_{30}O_4$ 370.5 *58652-20-3*

Action and use

Progestogen.

Ph Eur

DEFINITION

6-Methyl-3,20-dioxo-19-norpregna-4,6-dien-17-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison nomegestrol acetate CRS.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *methylene chloride R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*)

- 60.0 to - 64.0 (dried substance).

Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b) Dissolve 25.0 mg of *nomegestrol acetate impurity A CRS* in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (c) Dissolve 25.0 mg of *nomegestrol acetate CRS* in 20 ml of *methanol R,* add 0.25 ml of reference solution (b) and dilute to 50.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *methanol R*, *water R* (24:38:38 *V/V/V*).

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 245 nm and at 290 nm.

Injection 10 µl.

Run time 1.5 times the retention time of nomegestrol acetate.

Retention time At 245 nm: nomegestrol acetate = about 17 min; impurity A = about 18.5 min.

System suitability Reference solution (c) at 245 nm:

— *peak-to-valley ratio*: minimum 5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nomegestrol acetate.

Limits:

— *impurity A at 245 nm*: not more than 0.4 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *unspecified impurities at 245 nm*: for each impurity, not more than 0.2 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *unspecified impurities at 290 nm*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities other than A at 290 nm and 245 nm*: maximum 0.3 per cent;

— *disregard limit at 245 nm*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (c) (0.05 per cent);

— *disregard limit at 290 nm*: 0.04 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 50.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *anhydrous ethanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 287 nm.

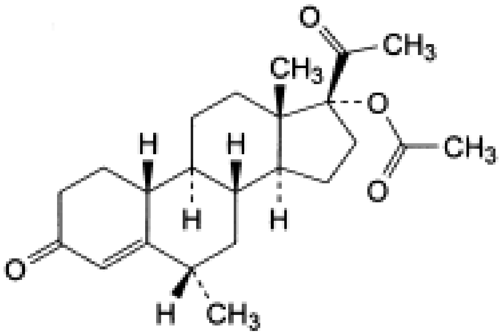
Calculate the content of $C_{23}H_{30}O_4$ taking the specific absorbance to be 685.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A*.



A. 6α-methyl-3,20-dioxo-19-norpregn-4-en-17-yl acetate.

Ph Eur

Nonoxinol 9

General Notices

(Ph Eur monograph 1454)

26027-38-3

Action and use

Spermatocide.

Ph Eur

DEFINITION

α-(4-Nonylphenyl)-ω-hydroxynona(oxyethylene).

Mixture consisting mainly of monononylphenyl ethers of macrogols corresponding to the formula: $C_9H_{19}C_6H_4\text{-}[OCH_2\text{-}CH_2]_n\text{-}OH$ where the average value of *n* is 9. It may contain free macrogols.

CHARACTERS

Appearance

Clear, colourless or light yellow, viscous liquid.

Solubility

Miscible with water, with ethanol (96 per cent) and with vegetable oils.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nonoxinol 9 CRS.*

Preparation Film between *sodium chloride R* plates.

B. Cloud point (see Tests).

TESTS

Acidity or alkalinity

Boil 1.0 g with 20 ml of *carbon dioxide-free water R* for 1 min, with constant stirring. Cool and filter. To 10 ml of the filtrate, add 0.05 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Hydroxyl value (*2.5.3, Method A*)

84 to 94.

Cloud point

52 °C to 58 °C.

Dissolve 1.0 g in 99 g of *water R*. Transfer about 30 ml of this solution into a test-tube, heat on a water-bath and stir continuously until the solution becomes cloudy. Remove the test-tube from the water-bath (ensuring that the temperature does not increase to more than 2 °C) and continue to stir. The cloud point is the temperature at which the solution becomes sufficiently clear that the entire thermometer bulb is plainly seen.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in *distilled water R* and dilute to 20.0 ml with the same solvent. 12 ml of this solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 2.00 g.

Total ash (*2.4.16*)

Maximum 0.4 per cent, determined on 1.0 g.

STORAGE

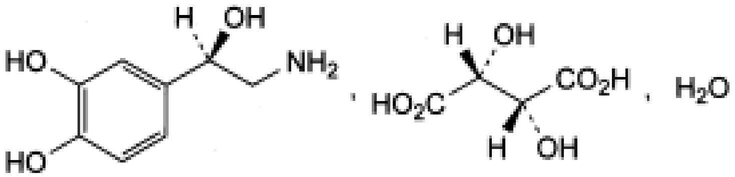
In an airtight container .

Ph Eur

Noradrenaline Acid Tartrate / Norepinephrine Acid Tartrate

General Notices

(Noradrenaline Tartrate, Ph Eur monograph 0285)



$C_{12}H_{17}NO_9,H_2O$ 337.3 *108341-18-0*

Action and use

Alpha-adrenoceptor agonist.

Preparation

Noradrenaline Injection/Norepinephrine Injection

Ph Eur

DEFINITION

(1*R*)-2-Amino-1-(3,4-dihydroxyphenyl)ethanol hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate monohydrate.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 2 g in 20 ml of a 5 g/l solution of *sodium metabisulphite R* and make alkaline by addition of *ammonia R*. Keep in iced water for 1 h and filter. Reserve the filtrate for identification test C. Wash the precipitate with 3 quantities, each of 2 ml, of *water R*, then with 5 ml of *ethanol (96 per cent) R* and finally with 5 ml of *methylene chloride R* and dry *in* *vacuo* for 3 h. The specific optical rotation (*2.2.7*) of the precipitate (noradrenaline base) is - 44 to - 48, determined using a 20.0 g/l solution in *0.5 M hydrochloric acid*.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of noradrenaline base prepared as described in identification test A.

Comparison Use noradrenaline base prepared as described in identification test A from a suitable amount of *noradrenaline tartrate CRS*.

C. 0.2 ml of the filtrate obtained in identification test A gives reaction (b) of tartrates (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 0.2 g in *water R* and dilute to 10 ml with the same solvent. Examine the solution immediately.

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from air. Remove oxygen from the mobile phases with nitrogen R immediately before use. Fill up the flasks.*

Test solution Dissolve 0.20 g of the substance to be examined in mobile phase A and dilute to 50 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 10 mg of the substance to be examined in 5 ml of *0.1 M hydrochloric acid*. To 1 ml of this solution add 0.1 ml of *strong hydrogen peroxide solution R* and expose to UV light at 254 nm for 90 min. Dilute to 10 ml with mobile phase A. The degradation of noradrenaline produces 2 peaks, one with a relative retention of about 1.2 (unidentified compound) and the other with a relative retention of about 1.5 (impurity B). Use this solution to identify the peak due to impurity B.

Reference solution (c) Dissolve 7.5 mg of *noradrenaline impurity D CRS* and 5 mg of *noradrenaline impurity E CRS* in mobile phase A and dilute to 100 ml with mobile phase A.

Reference solution (d) Dissolve 5 mg of *noradrenaline impurity F CRS* in mobile phase A and dilute to 10 ml with mobile phase A. To 1 ml of this solution, add 1 ml of reference solution (c) and dilute to 20 ml with mobile phase A.

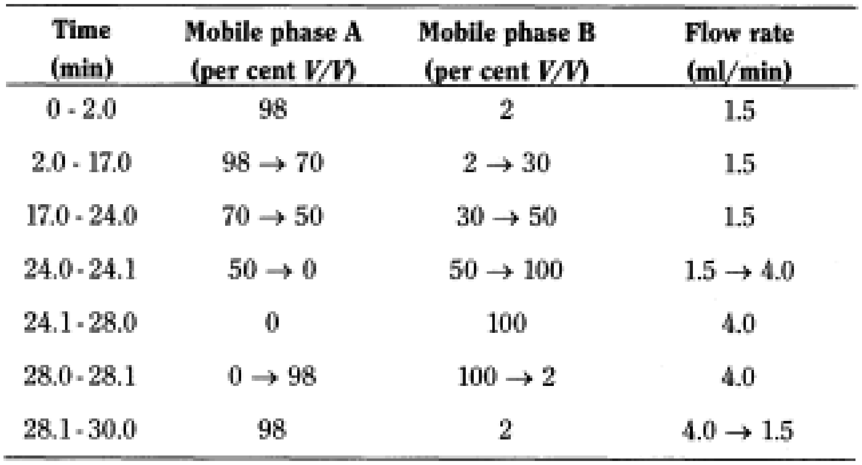
Column:

- *size:* l = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *monolithic octadecylsilyl silica gel for chromatography R*;
- *temperature*: 25 °C.

Mobile phase:

- *mobile phase A*: dissolve 0.50 g of *sodium heptanesulphonate R* in *water for chromatography R* and dilute to 1000 ml with the same solvent; adjust to pH 2.2 with *phosphoric acid R*;
- *mobile phase B*: dissolve 0.25 g of sodium *heptanesulphonate R* in *water for*

chromatography R and dilute to 500 ml with the same solvent; add 500 ml of *acetonitrile for chromatography R* and adjust the apparent pH to 2.4 with *phosphoric acid R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (ml/min)
0 - 2.0	98	2	1.5
2.0 - 17.0	98 → 70	2 → 30	1.5
17.0 - 24.0	70 → 50	30 → 50	1.5
24.0 - 24.1	50 → 0	50 → 100	1.5 → 4.0
24.1 - 28.0	0	100	4.0
28.0 - 28.1	0 → 98	100 → 2	4.0
28.1 - 30.0	98	2	4.0 → 1.5

Detection Spectrophotometer at 280 nm, except for impurity F: spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (a), (b) and (d).

Relative retention With reference to noradrenaline (retention time = about 3 min): impurity B = about 1.5; impurity D = about 2.8; impurity E = about 3.0; impurity F = about 6.9.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to impurities D and E.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.3; impurity E = 0.3; impurity F = 1.5;

— *impurity F* at 254 nm: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *impurities B, D, E at 280 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities at 280 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of the impurities at 280 nm and impurity F at 254 nm*: maximum 0.3 per cent;

— *disregard limit at 280 nm*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

4.5 per cent to 5.8 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*, heating gently if necessary. Titrate with *0.1 M perchloric acid* using 0.1 ml of *crystal violet solution R* as indicator, until a bluish-green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 31.93 mg of $C_{12}H_{17}NO_9$.

STORAGE

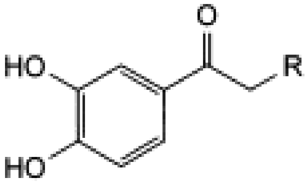
In an airtight container or preferably in a sealed tube under vacuum or under an inert gas, protected from light.

IMPURITIES

Specified impurities B, D, E, F.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, C, G.*

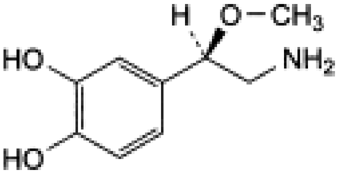
A. adrenaline,



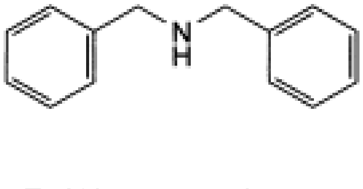
B. R = NH_2: 2-amino-1-(3,4-dihydroxyphenyl)ethanone (noradrenalone),

E. R = Cl: 2-chloro-1-(3,4-dihydroxyphenyl)ethanone,

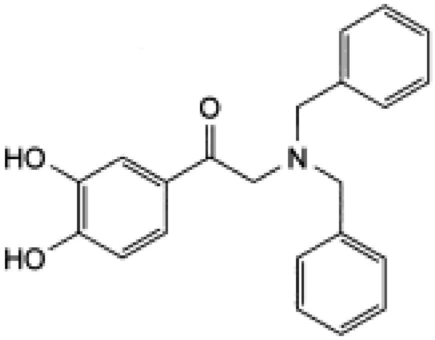
C. dopamine,



D. 4[(1*R*)-2-amino-1-methoxyethyl]benzene-1,2-diol (noradrenaline methyl ether),



F. *N*-benzyl-1-phenylmethanamine,



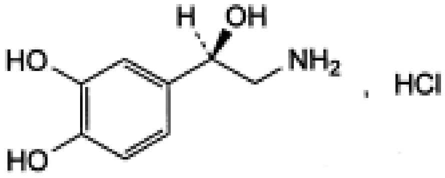
G. 2-(dibenzylamino)-1-(3,4-dihydroxyphenyl)ethanone.

Ph Eur

Noradrenaline Hydrochloride / Norepinephrine Hydrochloride

General Notices

(Noradrenaline Hydrochloride, Ph Eur monograph 0732)



$C_8H_{11}NO_3,HCl$ 205.6 *329-56-6*

Action and use

Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION

(1*R*)-2-Amino-1-(3,4-dihydroxyphenyl)ethanol hydrochloride.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or brownish-white, crystalline powder.

Solubility

Very soluble in water, slightly soluble in ethanol (96 per cent).

It becomes coloured on exposure to air and light.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of noradrenaline base prepared as follows. Dissolve 2 g in 20 ml of a 5 g/l solution of *sodium metabisulphite R* and make alkaline by addition of *ammonia R*. Keep in iced water for 1 h and filter. Wash the precipitate with 3 quantities, each of 2 ml, of *water R*, then with 5 ml of *ethanol 96 per cent R* and finally with 5 ml of *methylene chloride R* and dry *in vacuo* for 3 h.

Comparison Use noradrenaline base prepared as above from a suitable amount of *noradrenaline tartrate CRS*.

C. 0.2 ml of solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than a mixture of 0.2 ml of blue primary solution, 0.4 ml of yellow primary solution, 0.4 ml of red primary solution and 9 ml of a 13.7 per cent *V/V* solution of *dilute hydrochloric acid R* (*2.2.2, Method II*).

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. Examine the solution immediately.

pH (*2.2.3*)

3.5 to 4.5 for solution S.

Specific optical rotation (*2.2.7*)

- 37 to - 41 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from air. Remove oxygen from the mobile phases with nitrogen R immediately before use. Fill up the flasks.*

Test solution Dissolve 0.125 g of the substance to be examined in mobile phase A and dilute to 50 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 10 mg of the substance to be examined in 5 ml of *0.1 M hydrochloric acid*. To 1 ml of this solution add 0.1 ml of *strong hydrogen peroxide solution R* and expose to UV light at 254 nm for 90 min. Dilute to 10 ml with mobile phase A. The degradation of noradrenaline produces 2 peaks, one with a relative retention of about 1.2 (unidentified compound) and the other with a relative retention of about 1.5 (impurity B). Use this solution to identify the peak due to impurity B.

Reference solution (c) Dissolve 7.5 mg of *noradrenaline impurity D CRS* and 5 mg of *noradrenaline impurity E CRS* in mobile phase A and dilute to 100 ml with mobile phase A.

Reference solution (d) Dissolve 5 mg of *noradrenaline impurity F CRS* in mobile phase A and dilute to 10 ml with mobile phase A. To 1 ml of this solution, add 1 ml of reference solution (c) and dilute to 20 ml with mobile phase A.

Column:

— *size:* l = 0.10 m, Ø = 4.6 mm;

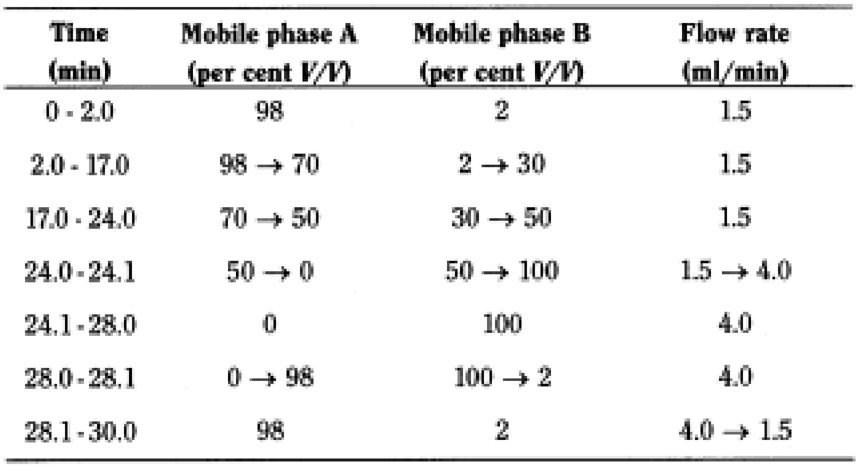
— *stationary phase*: *monolithic octadecylsilyl silica gel for chromatography R*;

— *temperature*: 25 °C.

Mobile phase:

— *mobile phase A*: dissolve 0.50 g of *sodium heptasulphonate R* in water for *chromatography R* and dilute to 1000 ml with the same solvent; adjust to pH 2.2 with *phosphoric acid R*;

— *mobile phase B*: dissolve 0.25 g of *sodium heptasulphonate R* in water for *chromatography R* and dilute to 500 ml with the same solvent; add 500 ml of *acetonitrile for chromatography R* and adjust the apparent pH to 2.4 with *phosphoric acid R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (ml/min)
0 - 2.0	98	2	1.5
2.0 - 17.0	98 → 70	2 → 30	1.5
17.0 - 24.0	70 → 50	30 → 50	1.5
24.0 - 24.1	50 → 0	50 → 100	1.5 → 4.0
24.1 - 28.0	0	100	4.0
28.0 - 28.1	0 → 98	100 → 2	4.0
28.1 - 30.0	98	2	4.0 → 1.5

Detection Spectrophotometer at 280 nm, except for impurity F: spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (a), (b) and (d).

Relative retention With reference to noradrenaline (retention time = about 3 min): impurity B = about 1.5; impurity D = about 2.8; impurity E = about 3.0; impurity F = about 6.9.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to impurities D and E.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.3; impurity E = 0.3; impurity F = 1.5;

— *impurity D at 280 nm*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity F at 254 nm*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *impurities B, E at 280 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities at 280 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities other than D at 280 nm*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *sum of the impurities at 280 nm and impurity F at 254 nm*: maximum 0.7 per cent;

— *disregard limit at 280 nm*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.000 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.180 g in 50 ml of *acetic anhydride R* and add 10 ml of *anhydrous formic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 20.56 mg of $C_8H_{12}ClNO_3$.

STORAGE

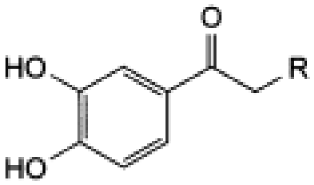
In an airtight container , or preferably in a sealed tube under vacuum or under an inert gas, protected from light.

IMPURITIES

Specified impurities *B, D, E, F*.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, C, G.*

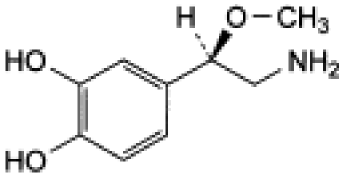
A. adrenaline,



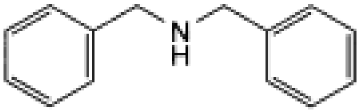
B. R = NH_2: 2-amino-1-(3,4-dihydroxyphenyl)ethanone (noradrenalone),

E. R = Cl: 2-chloro-1-(3,4-dihydroxyphenyl)ethanone,

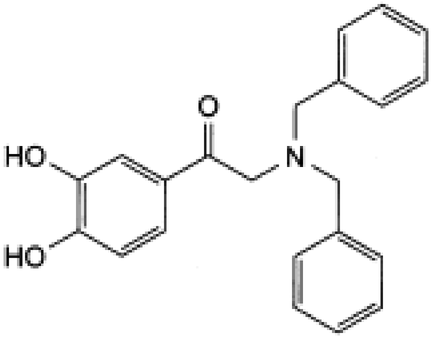
C. dopamine,



D. 4-[(1*R*)-2-amino-1-methoxyethyl]benzene-1,2-diol (noradrenaline methyl ether),



F. *N*-benzyl-1-phenylmethanamine,



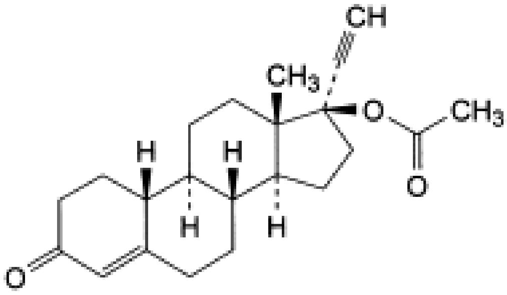
G. 2-(dibenzylamino)-1-(3,4-dihydroxyphenyl)ethanone.

Ph Eur

Norethisterone Acetate

General Notices

(Ph Eur monograph 0850)



$C_{22}H_{28}O_3$ 340.5 *51-98-9*

Action and use

Progestogen.

Preparation

Estradiol and Norethisterone Acetate Tablets

Ph Eur

DEFINITION

3-Oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in alcohol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *norethisterone acetate CRS.*

If the spectra show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS

Specific optical rotation (*2.2.7*)

- 30 to - 35 (dried substance).

Dissolve 0.500 g in *ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2 mg of *desoxycortone acetate CRS* and 2 mg of *norethisterone acetate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *water R* (60:40 *V/V*).

Flow rate 1.0 ml/min.

Detection Variable wavelength spectrophotometer capable of operating at 254 nm and at 210 nm.

Injection 20 µl.

Run time 3 times the retention time of norethisterone acetate.

Relative retention With reference to norethisterone acetate (retention time = about 10 min): impurity A = about 0.48; impurity D = about 0.65; impurity E = about 0.83; impurity C = about 1.35; impurity B = about 1.40.

System suitability Reference solution (a) at 254 nm:

— *resolution*: minimum of 3.5 between the peaks due to norethisterone acetate and to desoxycortone acetate.

Limits Spectrophotometer at 254 nm:

— *any impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Limits Spectrophotometer at 210 nm:

— *any impurity* with a relative retention between 1.0 and 1.6, with reference to norethisterone acetate (retention time = about 10 min): not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *total of these impurities*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.200 g in 40 ml of *tetrahydrofuran R*. Add 10 ml of a 100 g/l solution of *silver nitrate R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

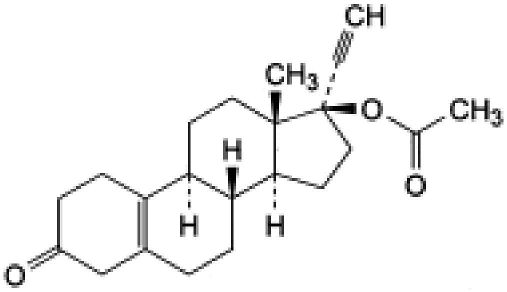
1 ml of *0.1 M sodium hydroxide* is equivalent to 34.05 mg of $C_{22}H_{28}O_3$.

IMPURITIES

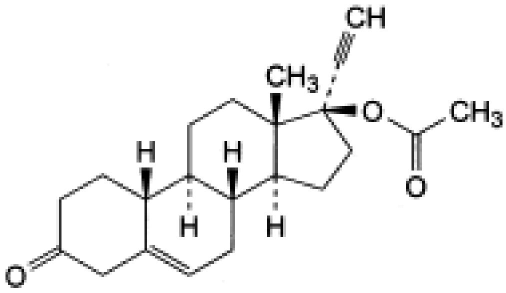
Specified impurities *A, B, C, D, E.*

Other detectable impurities *F, G.*

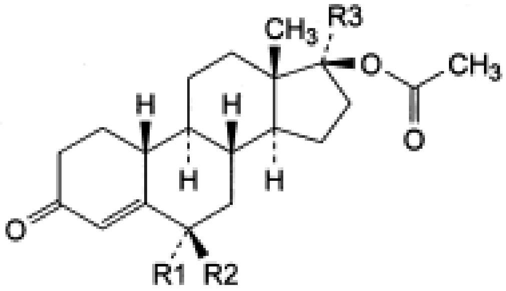
A. norethisterone,



B. 3-oxo-19-nor-17α-pregn-5(10)-en-20-yn-17-yl acetate,



C. 3-oxo-19-nor-17α-pregn-5-en-20-yn-17-yl acetate,



D. R1 = H, R2 = CO-CH_3, R3 = C≡CH: 6β-acetyl-3-oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate,

E. R1 = R2 = H, R3 = CO-CH_3: 3,20-dioxo-19-nor-17α-pregn-4-en-17-yl acetate,

F. R1 = H, R2 = OH, R3 = C≡CH: 6β-hydroxy-3-oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate,

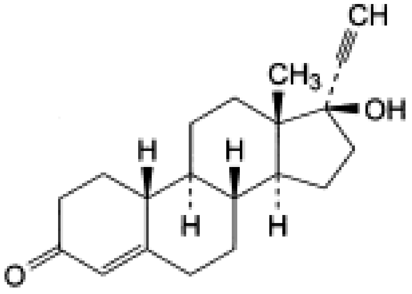
G. R1 + R2 = O, R3 = C≡CH: 3,6-dioxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate.

Ph Eur

Norethisterone

General Notices

(Ph Eur monograph 0234)



$C_{20}H_{26}O_2$ 298.4 *68-22-4*

Action and use

Progestogen.

Preparation

Norethisterone Tablets

Estradiol and Norethisterone Tablets

Ph Eur

DEFINITION

17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Practically insoluble in water, soluble in methylene chloride, sparingly soluble in acetone and in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *norethisterone CRS.*

TESTS

Specific optical rotation (*2.2.7*)

- 32.0 to - 37.0 (dried substance).

Dissolve 0.250 g in *acetone R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R1* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 5 mg of *norethisterone for system suitability CRS* (containing impurities A, B, C, D, E, F, G and H) in a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R1* and dilute to 2.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R1*. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R1*.

Column:

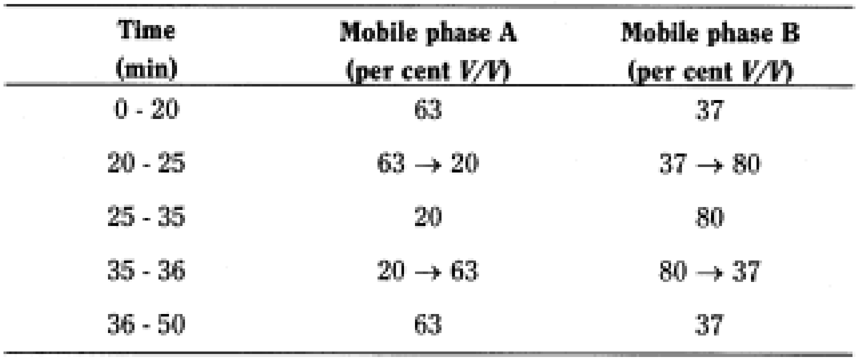
— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *water R*;

— *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	63	37
20 - 25	63 → 20	37 → 80
25 - 35	20	80
35 - 36	20 → 63	80 → 37
36 - 50	63	37

Flow rate 1.0 ml/min.

Detection Variable wavelength spectrophotometer capable of operating at 254 nm and at 210 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram obtained with reference solution (a) and the chromatogram supplied with *norethisterone for system suitability CRS* to identify the peaks due to the impurities A, B, C, D, E, F, G and H.

Relative retention at 254 nm With reference to norethisterone (retention time = about 10 min): impurity H = about 0.3; impurity A = about 0.8; impurity B = about 0.9; impurity G = about 1.5; impurity C (at 210 nm) = about 1.6; impurity D (at 210 nm) = about 1.7; impurity E = about 2.3; impurity F = about 2.4.

System suitability Reference solution (a) at 254 nm:

— *resolution*: baseline separation between the peaks due to impurity B and norethisterone;

— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

Limits Spectrophotometer at 254 nm:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 2.5; impurity E = 0.7; impurity F = 1.4; impurity H = 1.7;

— *impurities E, G, H*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities A, B, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Limits Spectrophotometer at 210 nm:

— *impurities C, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

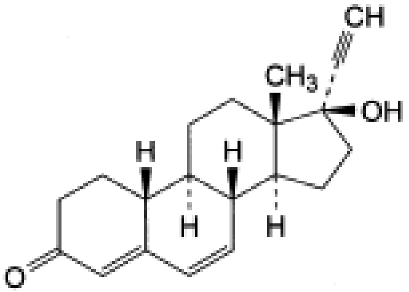
ASSAY

Dissolve 0.200 g in 40 ml of *tetrahydrofuran R*. Add 10 ml of a 100 g/l solution of *silver nitrate R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Rinse the electrode with *acetone R* after each titration.

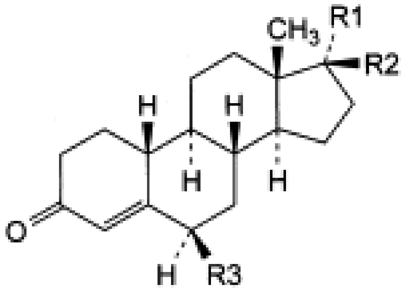
1 ml of *0.1 M sodium hydroxide* is equivalent to 29.84 mg of $C_{20}H_{26}O_2$.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H.



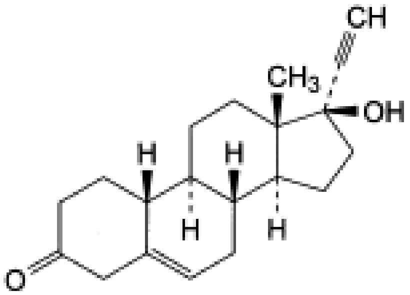
A. 17-hydroxy-19-nor-17α-pregna-4,6-dien-20-yn-3-one,



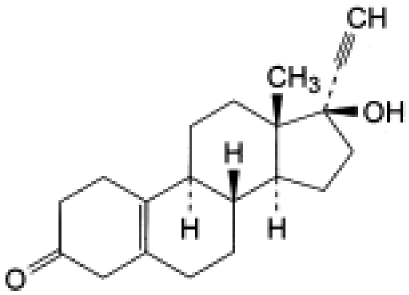
B. R1 + R2 = O, R3 = H: estr-4-ene-3,17-dione (norandrostenedione),

G. R1 = OH, R2 = C≡CH, R3 = H: 17-hydroxy-19-norpregn-4-en-20-yn-3-one (17-*epi*-norethisterone),

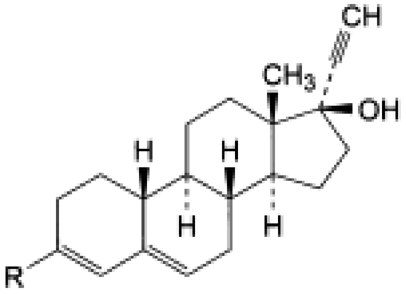
H. R1 = C≡CH, R2 = R3 = OH: 6β,17-dihydroxy-19-nor-17α-pregn-4-en-20-yn-3-one (6β-hydroxynorethisterone),



C. 17-hydroxy-19-nor-17α-pregn-5-en-20-yn-3-one,



D. 17-hydroxy-19-nor-17α-pregn-5(10)-en-20-yn-3-one,



E. R = C≡CH: 3-ethynyl-19-nor-17α-pregna-3,5-dien-20-yn-17-ol,

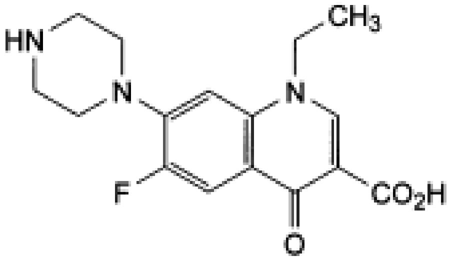
F. R = O-C_2H_5: 3-ethoxy-19-nor-17α-pregna-3,5-dien-20-yn-17-ol.

Ph Eur

Norfloxacin

General Notices

(Ph Eur monograph 1248)



$C_{16}H_{18}FN_3O_3$ 319.3 70458-96-7

Action and use

Fluoroquinolone antibacterial.

Preparations

Norfloxacin Eye Drops

Norfloxacin Tablets

Ph Eur

DEFINITION

1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or pale yellow, hygroscopic, photosensitive, crystalline powder.

Solubility

Very slightly soluble in water, slightly soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *norfloxacin CRS.*

TESTS

Appearance of solution

Dissolve 0.5 g in a previously filtered 4 g/l solution of *sodium hydroxide R* in *methanol R* and dilute to 50 ml with the same solution. The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

Related substances

Liquid chromatography *(2.2.29)*.

Solution A Mix 5 volumes of *acetonitrile R* and 95 volumes of *water R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Test solution Dissolve 20 mg of the substance to be examined in 25 ml of solution A. Sonicate for 5 min and dilute to 50.0 ml with solution A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with solution A. Dilute 1.0 ml of this solution to 10.0 ml with solution A.

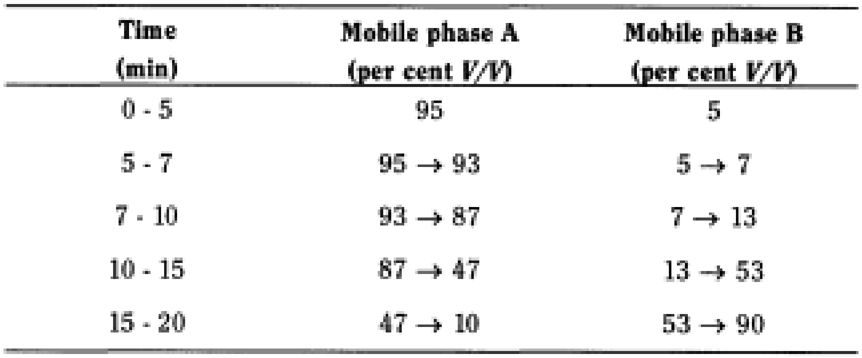
Reference solution (b) Dissolve 4 mg of *norfloxacin for system suitability CRS* (containing impurities A, E and H) in 5 ml of solution A. Sonicate for 5 min and dilute to 10 ml with solution A.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped hexadecylamidylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 60 °C.

Mobile phase:

- *mobile phase A*: *water R* adjusted to pH 2.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 7	95 → 93	5 → 7
7 - 10	93 → 87	7 → 13
10 - 15	87 → 47	13 → 53
15 - 20	47 → 10	53 → 90

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *norfloxacin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, E and H.

Relative retention With reference to norfloxacin (retention time = about 11 min): impurity E = about 0.97;

impurity A = about 1.5; impurity H = about 1.6.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to impurities A and H;

— *peak-to-valley ratio*: minimum 5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to norfloxacin.

Limits:

— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 15 ppm.

2.0 g complies with test D. Prepare the reference solution using 3 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying under high vacuum at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.240 g in 80 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 31.93 mg of $C_{16}H_{18}FN_3O_3$.

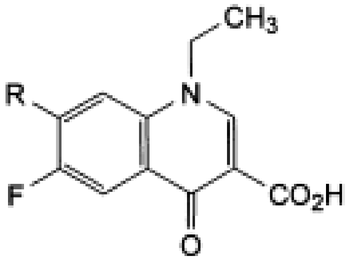
STORAGE

In an airtight container, protected from light.

IMPURITIES

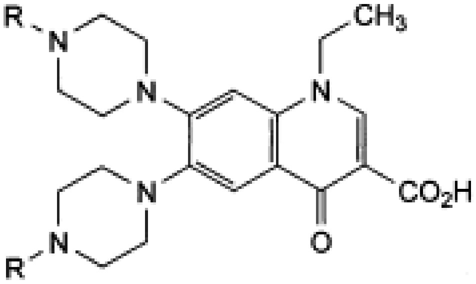
Specified impurities E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, D, F, G, H, I, J.*



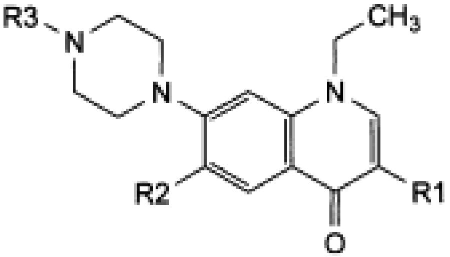
A. R = Cl: 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

B. R = NH-CH_2-CH_2-NH_2: 7-[(2-aminoethyl)amino]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



C. R = H: 1-ethyl-4-oxo-6,7-bis(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid,

J. R = CO-O-C_2H_5: 6,7-bis[4-(ethoxycarbonyl)piperazin-1-yl]-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



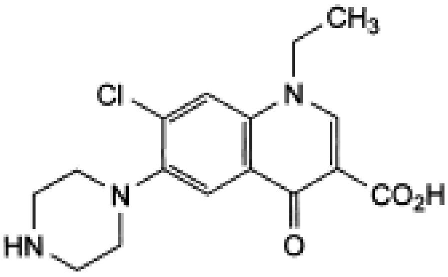
D. R1 = R3 = H, R2 = F: 1-ethyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1*H*)-one,

F. R1 = CO_2H, R2 = Cl, R3 = H: 6-chloro-1-ethyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid,

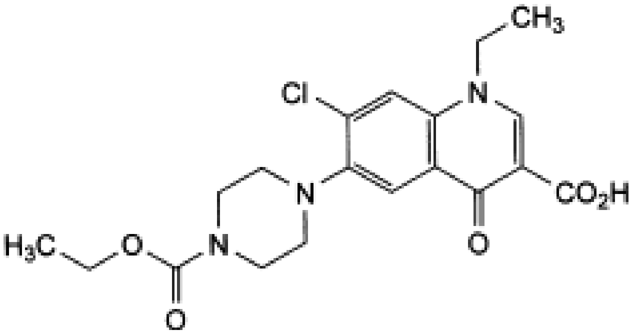
G. R1 = CO_2H, R2 = F, R3 = CHO: 1-ethyl-6-fluoro-7-(4-formylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

H. R1 = CO_2H, R2 = F, R3 = CO-O-C_2H_5: 7-[4-(ethoxycarbonyl)piperazin-1-yl]-1-ethyl-6-

fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,


CH3
Cl
N
N
HN
CO2H
O

E. 7-chloro-1-ethyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid,


CH3
Cl
N
N
H3C
O
N
O
CO2H
O

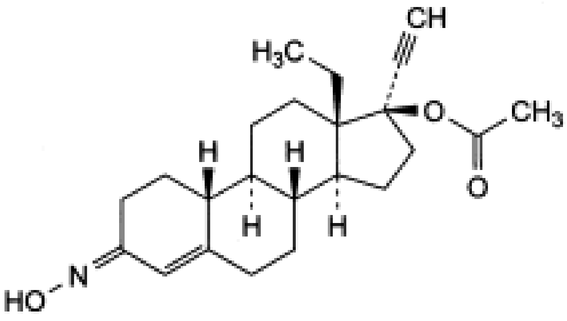
I. 7-chloro-6-[4-(ethoxycarbonyl)piperazin-1-yl]-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

Ph Eur

Norgestimate

General Notices

(Ph Eur monograph 1732)



$C_{23}H_{31}NO_3$ 369.5 *35189-28-7*

Action and use

Progestogen.

Ph Eur

DEFINITION

(3*EZ*)-13β-Ethyl-3-(hydroxyimino)-18,19-dinor-17α-pregn-4-en-20-yn-17-yl acetate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison norgestimate CRS.

TESTS

Specific optical rotation *(2.2.7)*

+ 42.0 to + 50.0 (dried substance).

Dissolve 0.200 g in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *water R, methanol R* (1:4 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Reference solution (b) Dissolve 2 mg of *norgestimate for system suitability CRS* (containing impurity A) in 4 ml of the solvent mixture.

Column:

— *size: l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase:* spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *acetonitrile R, tetrahydrofuran for chromatography R, water R* (18:22:60 *V/V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 244 nm.

Injection 25 µl.

Run time Twice the retention time of the (*E*)-isomer of norgestimate.

Identification of impurities Use the chromatogram supplied with *norgestimate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to the (*E*)-isomer of norgestimate (retention time = about 14 min): impurity A = about 0.7; (*Z*)-isomer of norgestimate = about 0.9.

System suitability: Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of the (*Z*)-isomer of norgestimate by 1.33;

— *impurity A*: not more than twice the sum of the areas of the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the sum of the areas of the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the sum of the areas of the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the sum of the areas of the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate in the chromatogram obtained with reference solution (a) (0.05 per cent).

Ratio of (*E*)- to (*Z*)-isomers

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection   Test solution.

Calculate the (*E*)- to (*Z*)-isomer ratio by dividing the area of the peak due to the (*E*)-isomer by 1.33 times the area of the peak due to the (*Z*)-isomer. The ratio is 1.27 to 1.78.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.300 g in 40 ml of *tetrahydrofuran R*. Add 10 ml of a 100 g/l solution of *silver nitrate R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Rinse the electrode with *acetone R* after each titration.

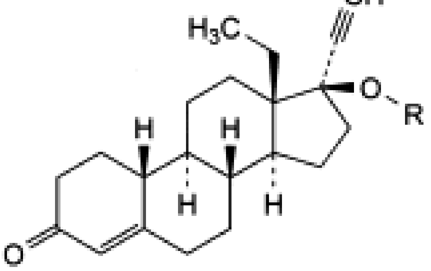
If necessary, after several titrations re-equilibrate the electrode in *water R* for 15 min to obtain sharper titration curves.

1 ml of *0.1 M sodium hydroxide* is equivalent to 36.95 mg of $C_{23}H_{31}NO_3$.

IMPURITIES

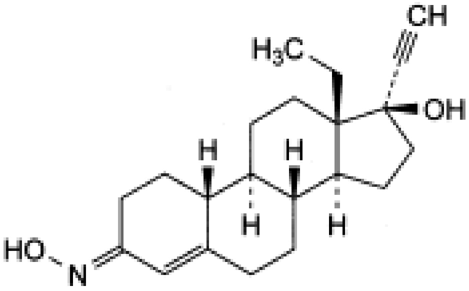
Specified impurities   *A.*

Other detectable impurities   (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D*.

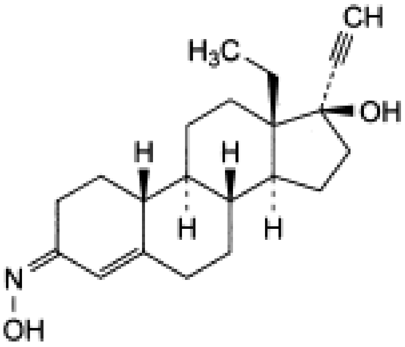


A. R = CO-CH_3: 13β-ethyl-3-oxo-18,19-dinor-17α-pregn-4-en-20-yn-17-yl acetate (levonorgestrel acetate),

B. R = H: levonorgestrel,



C. (3*E*)-13β-ethyl-3-(hydroxyimino)-18,19-dinor-17α-pregn-4-en-20-yn-17-ol ((*E*)-norelgestromin),



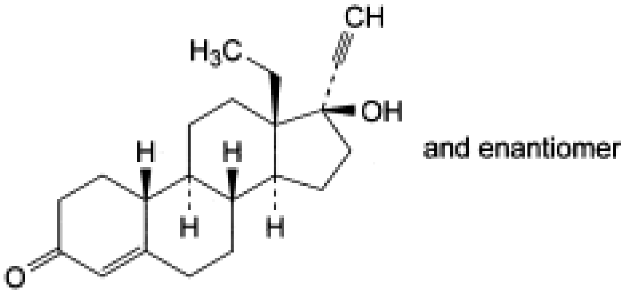
D. (3*Z*)-13β-ethyl-3-(hydroxyimino)-18,19-dinor-17α-pregn-4-en-20-yn-17-ol ((*Z*)-norelgestromin).

Ph Eur

Norgestrel

General Notices

(Ph Eur monograph 0940)



$C_{21}H_{28}O_2$ 312.5 *6533-00-2*

Action and use

Progestogen.

Preparation

Norgestrel Tablets

Ph Eur

DEFINITION

Norgestrel contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of *rac*-13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol.

IDENTIFICATION

A. Dissolve 0.5 g in *methylene chloride R* and dilute to 10.0 ml with the same solvent. The angle of optical rotation (*2.2.7*) is + 0.05 ° to - 0.05 °.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *norgestrel CRS*.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 10 ml with *methylene chloride R*. Dilute 1 ml of this solution to 20 ml with *methylene chloride R*.

Reference solution (b) Dilute 4 ml of reference solution (a) to 10 ml with *methylene chloride R*.

Reference solution (c) Dissolve 5 mg of *norgestrel CRS* and 5 mg of *ethinylestradiol CRS* in *methylene chloride R* and dilute to 50 ml with the same solvent.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *ethyl acetate R* and 80 volumes of *methylene chloride R*. Allow the plate to dry in air, spray with a 100 g/l solution of *phosphomolybdic acid R* in *alcohol R*, heat at 100-105 °C for 15 min and examine immediately. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most two such spots are more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash(*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 45 ml of *tetrahydrofuran R.* Add 10 ml of a 100 g/l solution of *silver nitrate R*. After 1 min, titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.25 mg of $C_{21}H_{28}O_2$.

STORAGE

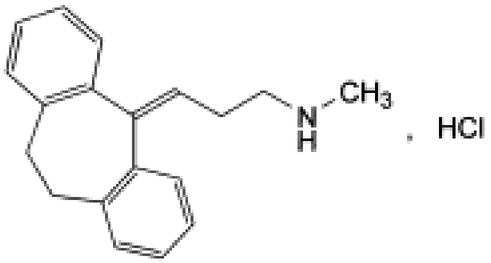
Store protected from light.

Ph Eur

Nortriptyline Hydrochloride

General Notices

(Ph Eur monograph 0941)



$C_{19}H_{21}N,HCl$ 299.8 *894-71-3*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparations

Nortriptyline Capsules

Nortriptyline Tablets

Ph Eur

DEFINITION

Nortriptyline hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*-methylpropan-1-amine hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, sparingly soluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification C, E.

Second identification A, B, D, E.

A. Melting point (*2.2.14*): 216 °C to 220 °C.

B. Dissolve 20.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *methanol R*. Examined between 230 nm and 350 nm

(*2.2.25*), the solution shows an absorption maximum at 239 nm. The specific absorbance at the absorption maximum is 465 to 495.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with *nortriptyline hydrochloride CRS*.

D. Dissolve 50 mg in 3 ml of warm *water R*, cool and add 0.05 ml of a 25 g/l solution of *quinhydrone R* in *methanol R*. A red colour develops slowly.

E. 50 mg gives reaction (b) of chlorides (*2.3.1*).

TESTS

Appearance of solution

Dissolve 0.5 g in *water R* with gentle heating and dilute to 25 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Acidity or alkalinity

Dissolve 0.2 g with gentle heating in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. Add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Related substances

Prepare the solutions in subdued light and develop the chromatograms protected from light. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 2 ml with *ethanol (96 per cent) R.*

Reference solution (a) Dissolve 10 mg of *dibenzosuberone CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 100 ml with *ethanol (96 per cent) R*.

Reference solution (b) Dissolve 10 mg of *norcyclobenzaprine CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 100 ml with *ethanol (96 per cent) R.*

Reference solution (c) To 0.1 ml of test solution (b) add 10 ml of reference solution (b).

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm in an unsaturated tank using a mixture of 3 volumes of *diethylamine R*, 15 volumes of *ethyl acetate R* and 85 volumes of *cyclohexane R*. Allow the plate to dry in air and spray with a freshly prepared mixture of 4 volumes of *formaldehyde solution R* and 96 volumes of *sulphuric acid R*. Examine immediately in ultraviolet light at 365 nm and then at 254 nm. In the chromatogram obtained with test solution (a): any spot due to dibenzosuberone, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.05 per cent); and any spot in the chromatogram obtained with test solution (b), apart from the principal spot and any spot due to dibenzosuberone, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Heavy metals (*2.4.8*)

1.0 g complies with test C for heavy metals (20 ppm). Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb)* R.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

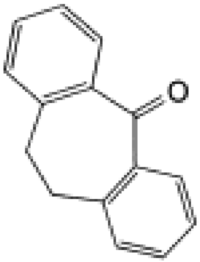
Dissolve 0.250 g in 30 ml of *ethanol (96 per cent) R*. Add 1.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 29.98 mg of $C_{19}H_{22}ClN$.

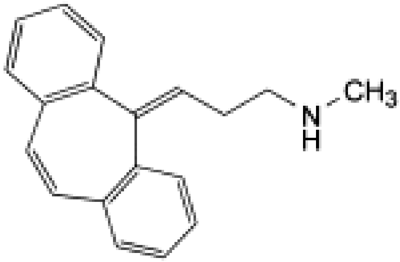
STORAGE

Protected from light.

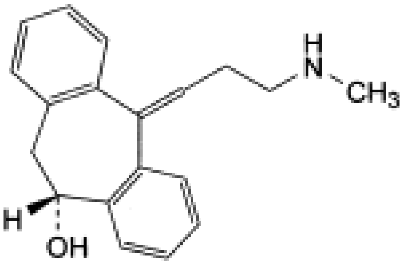
IMPURITIES



A. 10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-one(dibenzosuberone),



B. 3-(5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*-methylpropan-1-amine (norcyclobenzaprine),



and enantiomer

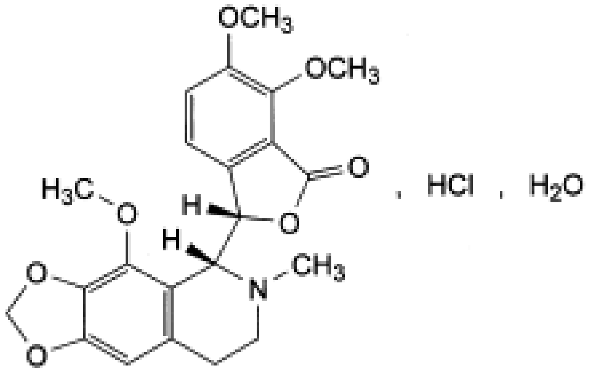
C. 10,11-dihydro-5-[3-(methylamino)propylidene]-5*H*-dibenzo[*a*,*d*][7]annulen-10-ol.

Ph Eur

Noscapine Hydrochloride

General Notices

(Ph Eur monograph 0515)



$C_{22}H_{23}NO_7,HCl,H_2O$ 467.9 *912-60-7*

Action and use

Opioid receptor agonist; cough suppressant.

Ph Eur

DEFINITION

(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one hydrochloride monohydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Freely soluble in water and in alcohol. Aqueous solutions are faintly acid; the base may be precipitated when the solutions are allowed to stand.

mp

About 200 °C, with decomposition.

IDENTIFICATION

First identification *C, E.*

Second identification *A, B, D, E.*

A. It complies with the test for specific optical rotation (see Tests).

B. Melting point (*2.2.14*) of the precipitate obtained in identification test E: 174 °C to 177 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Examine the precipitate obtained in identification test E.

Comparison *noscapine CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 22 mg of *noscapine CRS* in *acetone R* and dilute to 100 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R*, *alcohol R*, *acetone R*, *toluene R* (1:3:20:20 *V/V/V/V)* .

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *dilute potassium iodobismuthate solution R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

E. Dissolve about 40 mg in a mixture of 2 ml of *water R* and 3 ml of *alcohol R* and add 1 ml of *dilute ammonia R2*. Heat until dissolution is complete. Allow to cool, scratching the wall of the tube with a glass rod. Filter. The filtrate gives reaction (a) of chlorides (*2.3.1*). Wash the precipitate with *water R*, dry at 100-105 °C and reserve for identification tests B and C.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*).

Dissolve 0.5 g in *water R*, add 0.3 ml of *0.1 M hydrochloric acid* and dilute to 25 ml with *water R.*

pH (*2.2.3*)

Minimum 3.0.

Dissolve 0.2 g in 10 ml of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

+ 38.5 to + 44.0 (dried substance).

Dissolve 0.500 g in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined with gentle heating in 14 ml of *methanol R*, cool the solution and dilute to 20.0 ml with *phosphate buffer solution pH 6.0 R1*.

Reference solution (a) Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *papaverine hydrochloride R* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 1.5 mg of *papaverine hydrochloride R* in 10 ml of the test solution and dilute to 50 ml with the mobile phase.

Column:
- *size: l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *nitrile silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *phosphate buffer solution pH 6.0 R1* (350:650 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time 2.5 times the retention time of noscapine.

Relative retention With reference to noscapine (retention time = about 10 min): impurity A = about 1.3.

System suitability Reference solution (c):
- *resolution*: minimum 2 between the peaks due to noscapine and to impurity A.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *any other impurity*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total of other impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

2.5 per cent to 6.5 per cent, determined on 0.200 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 44.99 mg of $C_{22}H_{24}ClNO_7$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

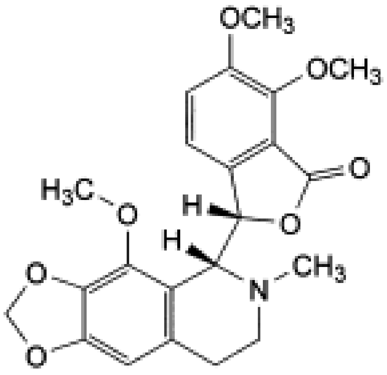
A. papaverine.

Ph Eur

Noscapine

General Notices

(Ph Eur monograph 0516)



$C_{22}H_{23}NO_7$ 413.4 *128-62-1*

Action and use

Opioid receptor agonist; cough suppressant.

Ph Eur

DEFINITION

(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, soluble in acetone, slightly soluble in alcohol. It dissolves in strong acids; on dilution of the solution with water, the base may be precipitated.

IDENTIFICATION

First identification *C, E.*

Second identification *A, B, D, E.*

A. It complies with the test for specific optical rotation (see Tests).

B. Melting point (*2.2.14*): 174 °C to 177 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *noscapine CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *acetone R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 25 mg of *noscapine CRS* in *acetone R* and dilute to 100 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R*, *alcohol R*, *acetone R*, *toluene R* (1:3:20:20 *V/V/V/V*) .

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with dilute potassium iodobismuthate solution R.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

E. To 20 mg add 10 ml of *water R* and shake. It does not dissolve.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.2 g in *acetone R* and dilute to 10 ml with the same solvent. Examine immediately after preparation.

Specific optical rotation (*2.2.7*)

+ 42 to + 48 (dried substance).

Dissolve 0.500 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined with gentle heating in 14 ml of *methanol R*, cool the solution and dilute to 20.0 ml with *phosphate buffer solution pH 6.0 R1*.

Reference solution (a) Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase.

Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *papaverine hydrochloride R* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 1.5 mg of *papaverine hydrochloride R* in 10 ml of the test solution and dilute to 50 ml with the mobile phase.

Column:

— *size:* *l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase*: *nitrile silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *phosphate buffer solution pH 6.0 R1* (350:650 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time 2.5 times the retention time of noscapine.

Relative retention With reference to noscapine (retention time = about 10 min): impurity A = about 1.3.

System suitability Reference solution (c):

— *resolution*: minimum 2 between the peaks due to noscapine and to impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity:* not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total of other impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 40 ml of *anhydrous acetic acid R*, warming gently. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 41.34 mg of $C_{22}H_{23}NO_7$.

STORAGE

Protected from light.

IMPURITIES

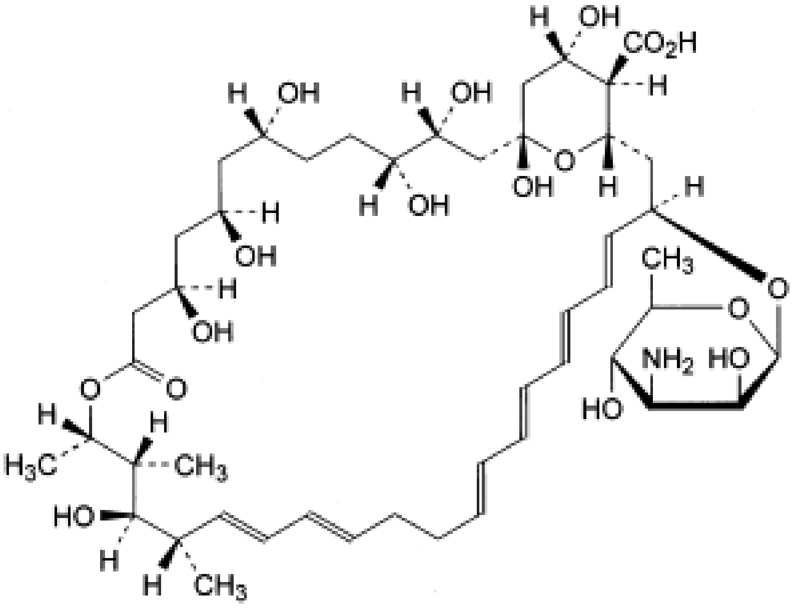
A. papaverine.

Ph Eur

Nystatin

General Notices

(Ph Eur monograph 0517)



$C_{47}H_{75}NO_{17}$ 926 *1400-61-9*

Action and use

Antifungal.

Preparations

Nystatin Ointment

Nystatin Oral Suspension

Nystatin Pastilles

Nystatin Pessaries

Nystatin Tablets

Ph Eur

DEFINITION

Antifungal substance obtained by fermentation using certain strains of Streptomyces noursei as the production micro-organism. It contains mainly tetraenes, the principal component being

(1*S*,3*R*,4*R*,7*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid (nystatin A1).

Content

Minimum 4400 IU/mg (dried substance) and minimum 5000 IU/mg (dried substance) if intended for oral administration.

PRODUCTION

If nystatin is not intended for cutaneous administration, the method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Abnormal toxicity (*2.6.9*)

Inject intraperitoneally into each mouse a quantity equivalent to not less than 600 IU suspended in 0.5 ml of a 5 g/l solution of *acacia R*.

CHARACTERS

Appearance

Yellow or slightly brownish powder, hygroscopic.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide and in dimethyl sulphoxide, slightly soluble in methanol, practically insoluble in alcohol.

IDENTIFICATION

First identification B, E.

Second identification A, C, D.

A. Examine the solution prepared in the test for absorbance between 220 nm and 350 nm (*2.2.25*). The solution shows 4 absorption maxima at 230 nm, 291 nm, 305 nm and 319 nm, and a shoulder at 280 nm. The ratios of the absorbances at the absorption maxima at 291 nm and 319 nm to the absorbance at the absorption maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. The ratio of the absorbance measured at the absorption maximum at 230 nm to that measured at the shoulder at 280 nm is 0.83 to 1.25.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison nystatin CRS.

C. To about 2 mg add 0.1 ml of *hydrochloric acid R*. A brown colour develops.

D. To about 2 mg add 0.1 ml of *sulphuric acid R*. A brown colour develops that becomes violet on standing.

E. Examine the chromatograms obtained in the test for composition.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Absorbance (*2.2.25*)

Dissolve 0.10 g in a mixture of 5.0 ml of *glacial acetic acid R* and 50 ml of *methanol R* and dilute to 100.0 ml with *methanol R*. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Determined at the maximum at 305 nm within 30 min of preparation of the solution, the absorbance is not less than 0.60.

Composition

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Carry out the test protected from light.*

Test solution Dissolve 20 mg of the substance to be examined in *dimethyl sulphoxide R* and dilute to 50 ml with the same solvent.

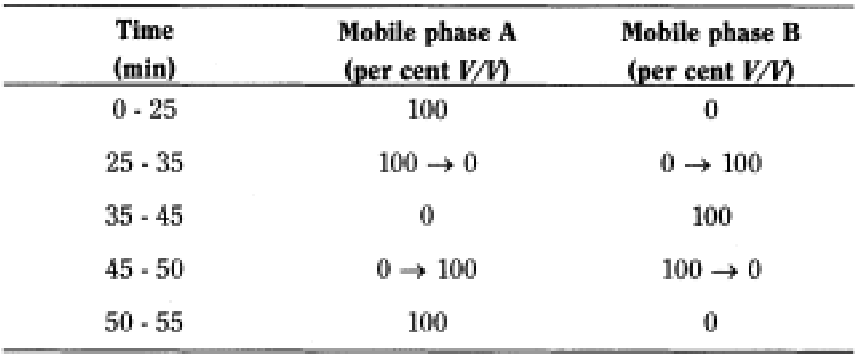
Reference solution (a) Dissolve 20 mg of *nystatin CRS* in *dimethyl sulphoxide R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of the substance to be examined in 25 ml of *methanol R* and dilute to 50 ml with *water R*. To 10.0 ml of the solution add 2.0 ml of *dilute hydrochloric acid R*. Allow to stand at room temperature for 1 h.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 100.0 ml with *dimethyl sulphoxide R*. Dilute 1.0 ml of this solution to 10.0 ml with *dimethyl sulphoxide R*.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile R*, 3.85 g/l solution of *ammonium acetate R* (29:71 *V/V*);
- *mobile phase B*: 3.85 g/l solution of *ammonium acetate R*, *acetonitrile R* (40:60 *V/V*),



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 35	100 → 0	0 → 100
35 - 45	0	100
45 - 50	0 → 100	100 → 0
50 - 55	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 305 nm.

Injection 20 µl

Retention time Nystatin A1 = about 14 min.

System suitability Reference solution (b):

— *resolution*: minimum 3.5 between the 2 principal peaks (retention time = about 13 min and 19 min).

Composition:

— *nystatin A1* : minimum 85.0 per cent;

— *any other compound*: maximum 4.0 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c); disregard any peak with a retention time of less than 2 min.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa for 3 h.

Sulphated ash (*2.4.14*)

Maximum 3.5 per cent, determined on 1.0 g.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). *Protect the solutions from light throughout the assay.*

Dissolve the substance to be examined and *nystatin CRS* separately in *dimethylformamide R* and dilute with a mixture of 5 volumes of *dimethylformamide R* and 95 volumes of buffer solution pH 6.0.

STORAGE

In an airtight container, protected from light.

LABELLING

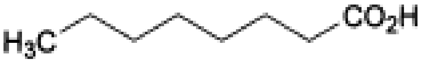
The label states where applicable, that the substance is only for cutaneous use.

Ph Eur

Octanoic Acid

General Notices

(Caprylic Acid, Ph Eur monograph 1401)



$C_8H_{16}O_2$ 144.2 *124-07-2*

Action and use

Excipient.

Ph Eur

DEFINITION

Octanoic acid.

Content

99.0 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless or slightly yellowish, oily liquid.

Solubility

Very slightly soluble in water, very soluble in acetone and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Relative density (see Tests).

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Relative density (*2.2.5*)

0.909 to 0.912.

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.10 g of the substance to be examined in *ethyl acetate R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 0.10 g of *caprylic acid CRS* in *ethyl acetate R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *ethyl acetate R*. Dilute 5.0 ml of this solution to 50.0 ml with *ethyl acetate R*.

Column:

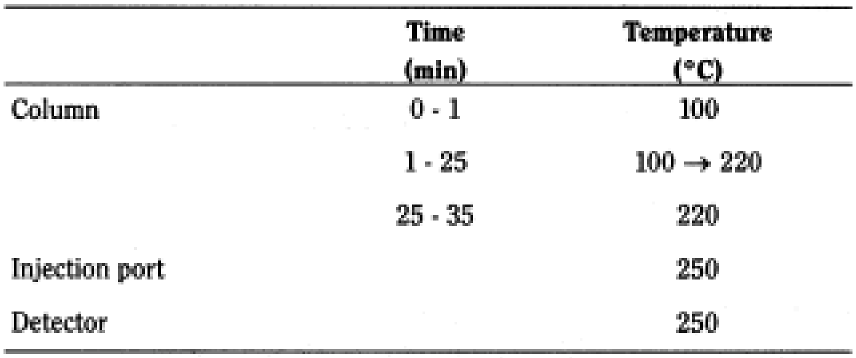
- *material*: fused silica;
- *size: l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 2-nitroterephthalate R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	100
	1 - 25	100 → 220
	25 - 35	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

- *signal-to-noise ratio*: minimum 5 for the principal peak.

Limits:

- *any impurity*: for each impurity, maximum 0.3 per cent;
- *total*: maximum 0.5 per cent;
- *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test B. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R* and 9 ml of *ethanol (96 per cent) R*.

Water (*2.5.12*)

Maximum 0.7 per cent, determined on 1.000 g.

Sulphated ash (*2.4.14*)

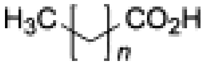
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.125 g in 25 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* is equivalent to 14.42 mg of $C_8H_{16}O_2$.

IMPURITIES



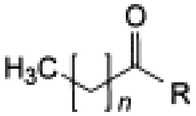
A. *n* = 4: hexanoic acid,

B. *n* = 5: heptanoic acid,

C. *n* = 7: nonanoic acid,

D. *n* = 8: decanoic acid,

E. valproic acid,

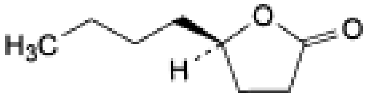


F. R = OCH_3, *n* = 6: methyl octanoate,

G. R = OC_2H_5, *n* = 6: ethyl octanoate,

H. R = OCH_3, *n* = 8: methyl decanoate,

I. R = CH_3, *n* = 8: undecan-2-one,



and enantiomer

J. 5-butyltetrahydrofuran-2-one (γ-hydroxyoctanoic acid lactone).

Ph Eur

Octoxinol 10

General Notices

(Ph Eur monograph 1553)

$C_{14}H_{21}$-$[C_2H_4O]_n$-OH

Action and use

Excipient.

Ph Eur

DEFINITION

α-[4-(1,1,3,3-Tetramethylbutyl)phenyl]-ω-hydroxydeca(oxyethylene).

Mixture consisting mainly of mono-octylphenyl ethers of macrogols corresponding to the formula $C_8H_{17}C_6H_4$-$[OCH_2$-$CH_2]_n$-OH where the average value of *n* is 10. It may contain free macrogols.

CHARACTERS

Appearance

Clear, colourless or light yellow, viscous liquid.

Solubility

Miscible with water, with ethanol (96 per cent) and with vegetable oils.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *octoxinol 10 CRS.*

Preparation Film between *sodium chloride R* plates.

B. It complies with the test for cloud point (see Tests).

TESTS

Acidity or alkalinity

Boil 1.0 g with 20 ml of *carbon dioxide-free water R* for 1 min, with constant stirring. Cool and filter. To 10 ml of the filtrate, add 0.05 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Hydroxyl value (*2.5.3, Method A*)

85 to 101.

Cloud point

63 °C to 70 °C.

Dissolve 1.0 g in 99 g of *water R*. Transfer about 30 ml of this solution to a test-tube, heat on a water-bath and stir continuously until the solution becomes cloudy. Remove the test-tube from the water-bath (ensuring that the temperature does not increase more than 2 °C), and continue to stir. The cloud point is the temperature at which the solution becomes sufficiently clear that the entire thermometer bulb is plainly seen.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in *distilled water R* and dilute to 20.0 ml with the same solvent. 12 ml of this solution complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 2.00 g.

Total ash (*2.4.16*)

Maximum 0.4 per cent, determined on 1.0 g.

STORAGE

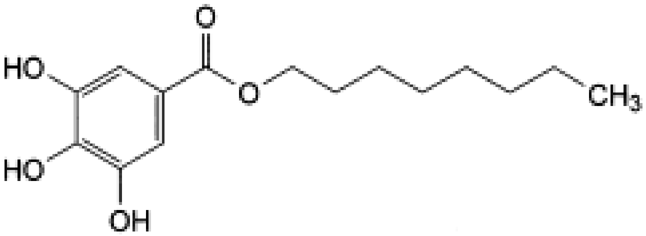
In an airtight container .

Ph Eur

Octyl Gallate

General Notices

(Ph Eur monograph 2057)



$C_{15}H_{22}O_5$ 282.3 *1034-01-1*

Action and use

Used in treatment of alcohol dependence.

Ph Eur

DEFINITION

Octyl 3,4,5-trihydroxybenzoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Melting point *(2.2.14)*.

Determine the melting point of the substance to be examined. Mix equal parts of the substance to be examined and *octyl gallate CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 101 °C) is not greater than 2 °C.

B. Examine the chromatograms obtained in the test for impurity A.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 20 ml with *acetone R*.

Reference solution (a) Dissolve 10 mg of *octyl gallate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *gallic acid R* in *acetone R* and dilute to 20 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10 ml with *acetone R*.

Reference solution (d) Dilute 1.0 ml of reference solution (b) to 5 ml with test solution (a).

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R*, *ethyl formate R*, *toluene R* (10:40:50 *V/V/V*).

Application 5 µl of test solutions (a) and (b) and reference solutions (a), (c) and (d).

Development Over 2/3 of the plate.

Drying In air for 10 min.

Detection Spray with a mixture of 1 volume of *ferric chloride solution R1* and 9 volumes of *ethanol (96 per cent) R.*

System suitability Reference solution (d):

— the chromatogram shows 2 clearly separated principal spots.

Limit Test solution (a):

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Chlorides *(2.4.4)*

Maximum 100 ppm.

To 1.65 g add 50 ml of *water R*. Shake for 5 min. Filter. 15 ml of the filtrate complies with the test.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 70 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in *methanol R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 200.0 ml with *methanol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 275 nm.

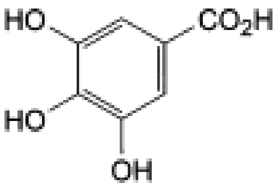
Calculate the content of $C_{15}H_{22}O_5$ taking the specific absorbance to be 387.

STORAGE

In a non-metallic container, protected from light.

IMPURITIES

Specified impurities *A.*



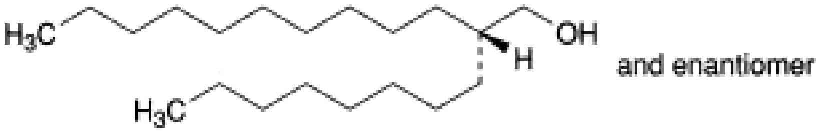
A. 3,4,5-trihydroxybenzoic acid (gallic acid).

Ph Eur

Octyldodecanol

General Notices

(Ph Eur monograph 1136)



Action and use

Excipient.

Ph Eur

DEFINITION

Condensation product of saturated liquid fatty alcohols.

Content

Minimum 90 per cent of (2*RS*)-2-octyldodecan-1-ol ($C_{20}H_{42}O$; M_r 298.6), the remainder consisting mainly of related alcohols.

CHARACTERS

Appearance

Clear, colourless or yellowish, oily liquid.

Solubility

Practically insoluble in water, miscible with ethanol (96 per cent).

Relative density

About 0.840.

Refractive index

About 1.455.

IDENTIFICATION

A. Hydroxyl value (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.20 g of the substance to be examined in *toluene R* and dilute to 20 ml with the same solvent.

Reference solution Dissolve 0.20 g of *octyldodecanol CRS* in *toluene R* and dilute to 20 ml with the same solvent.

Plate Suitable silica gel plate.

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

Application 2 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with about 7 ml of a mixture of 1 volume of a 25 g/l solution of *vanillin R* in *ethanol (96 per cent) R* and 4 volumes of *sulphuric acid R* and heat at 130 °C for 5-10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Acidity or alkalinity

Mix 5.0 g thoroughly for 1 min with a mixture of 0.1 ml of *bromothymol blue solution R1*, 2 ml of *heptane R* and 10 ml of *water R*. If the aqueous layer is blue, not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow. If the aqueous layer is yellow, add 0.45 ml of *0.01 M sodium hydroxide* and shake vigorously. After standing to ensure complete separation, the aqueous layer is blue.

Optical rotation (*2.2.7*)

- 0.10 ° to + 0.10 °.

Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.

Hydroxyl value (*2.5.3, Method A*)

175 to 190.

Iodine value (*2.5.4, Method A*)

Maximum 8.0.

Peroxide value (*2.5.5, Method A*)

Maximum 5.0.

Saponification value (*2.5.6*)

Maximum 5.0.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution*

(10 ppm Pb) R.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 2.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 0.4 g of *tetradecane R* in *hexane R* and dilute to 100.0 ml with the same solvent.

Test solution Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 10.0 ml with the same solution.

Reference solution Dissolve 0.100 g of *octyldodecanol CRS* in the internal standard solution and dilute to 10.0 ml with the same solution.

Column:

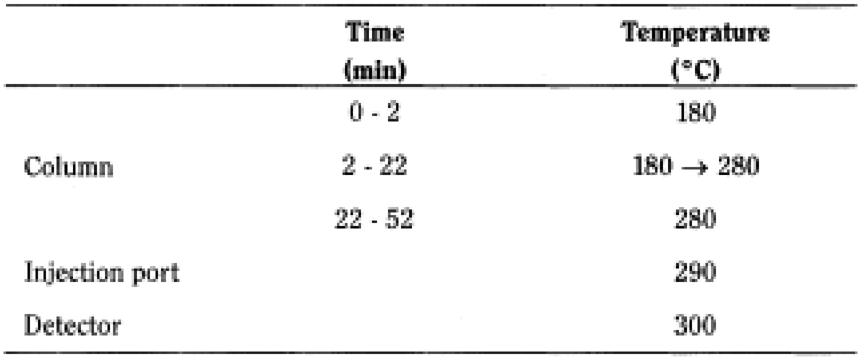
- *material*: stainless steel;
- *size*: *l* = 60 m, Ø = 0.25 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)(divinyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 0.68 ml/min.

Split ratio 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	180
	2 - 22	180 → 280
	22 - 52	280
Injection port		290
Detector		300

Detection Flame ionisation.

Injection 1 µl.

Calculate the content of $C_{20}H_{42}O$ in the substance to be examined.

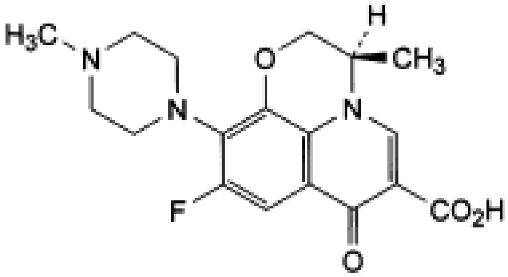
STORAGE

Protected from light.

Ofloxacin

General Notices

(Ph Eur monograph 1455)



and enantiomer

$C_{18}H_{20}FN_3O_4$ 361.4 *82419-36-1*

Action and use

Fluoroquinolone antibacterial.

Ph Eur

DEFINITION

(*RS*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Pale yellow or bright yellow, crystalline powder.

Solubility

Slightly soluble in water, soluble in glacial acetic acid, slightly soluble or soluble in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison ofloxacin CRS.

TESTS

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 0.300 g in a mixture of 10 volumes of *methanol R* and 40 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Absorbance *(2.2.25)*

Maximum 0.25 at 440 nm.

Dissolve 0.5 g in *0.1 M hydrochloric acid* and dilute to 100 ml with the same solvent.

Impurity A

Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R, methylene chloride R* (10:40 *V/V*).

Test solution Dissolve 0.250 g of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution Dissolve 10.0 mg of *ofloxacin impurity A CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Plate *TLC silica gel* GF_{254} *plate R* (2-10 µm).

Mobile phase *glacial acetic acid R, water R, ethyl acetate R* (10:10:20 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.2 per cent).

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Solvent mixture *acetonitrile R, water R* (10:60 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *ofloxacin impurity E CRS* in the solvent mixture and dilute to 100 ml with the solvent mixture. Mix 10 ml of the solution with 5 ml of the test solution and dilute to 50 ml with the solvent mixture. Dilute 1 ml of this solution to 50 ml with the solvent mixture.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 45 °C.

Mobile phase Dissolve 4.0 g of *ammonium acetate R* and 7.0 g of *sodium perchlorate R* in 1300 ml of *water R*; adjust to pH 2.2 with *phosphoric acid R* and add 240 ml of *acetonitrile R*.

Flow rate Adjust so that a retention time of about 20 min is obtained for ofloxacin.

Detection Spectrophotometer at 294 nm.

Injection 10 µl.

Run time 2.5 times the retention time of ofloxacin.

Relative retention With reference to ofloxacin (retention time = about 20 min): impurity B = about 0.3; impurity C = about 0.5; impurity D = about 0.7; impurity E = about 0.9; impurity F = about 1.6.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity E and ofloxacin.

Limits:

— *impurities B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.2 per cent, determined on 1.000 g by drying at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 100 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

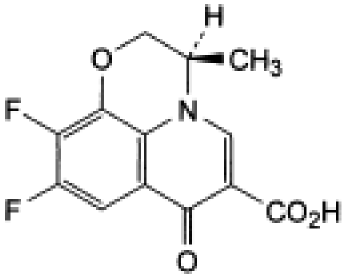
1 ml of *0.1 M perchloric acid* is equivalent to 36.14 mg of $C_{18}H_{20}FN_3O_4$.

STORAGE

In an airtight container, protected from light.

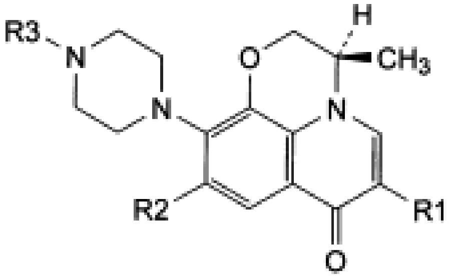
IMPURITIES

Specified impurities A, B, C, D, E, F.



and enantiomer

A. (*RS*)-9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid (FPA),

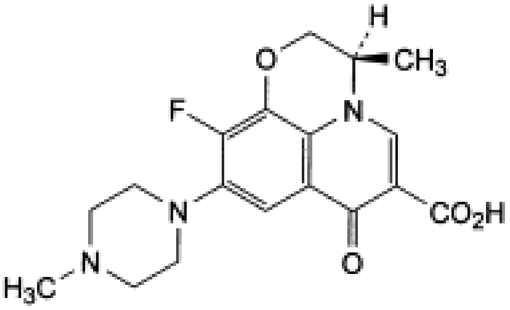


and enantiomer

B. R1 = H, R2 = F, R3 = CH_3: (*RS*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazin-7-one,

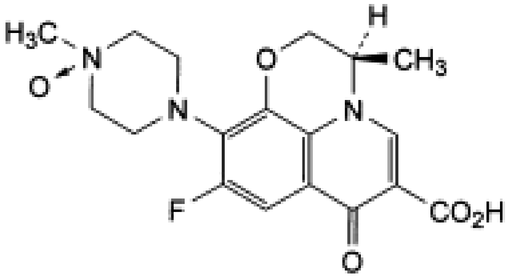
C. R1 = CO_2H, R2 = H, R3 = CH_3: (*RS*)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,

E. R1 = CO_2H, R2 = F, R3 = H: (*RS*)-9-fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



and enantiomer

D. (*RS*)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



and enantiomer

F. 4-[(*RS*)-6-carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-10-yl]-1-methylpiperazine 1-oxide.

Ph Eur

Oleic Acid

General Notices

(Ph Eur monograph 0799)

$C_{18}H_{34}O_2$ *282.5* *112-80-1*

Action and use

Excipient.

Ph Eur

DEFINITION

(*Z*)-Octadec-9-enoic acid ($C_{18}H_{34}O_2$; M_r 282.5), together with varying amounts of saturated and other unsaturated fatty acids. A suitable antioxidant may be added.

Content

65.0 per cent to 88.0 per cent of $C_{18}H_{34}O_2$.

CHARACTERS

Appearance

Clear, yellowish or brownish, oily liquid.

Solubility

Practically insoluble in water, miscible with alcohol and with methylene chloride.

Relative density

About 0.892.

IDENTIFICATION

A. It complies with the test for acid value (see Tests).

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

Margaric acid Maximum 0.2 per cent for oleic acid of vegetable origin and maximum 4.0 per cent for oleic acid of animal origin.

TESTS

Appearance

The substance to be examined is not more intensely coloured than reference solution Y_1 or

BY_1 *(2.2.2, Method I)*.

Acid value ***(2.5.1)***

195 to 204, determined on 0.5 g.

Iodine value ***(2.5.4)***

89 to 105.

Peroxide value ***(2.5.5)***

Maximum 10.0.

Composition of fatty acids

Gas chromatography *(2.4.22, Method C)*.

Test solution Prepare as described in the method but omitting the initial hydrolysis.

Composition of the fatty acid fraction of the substance:

— *myristic acid*: maximum 5.0 per cent;

— *palmitic acid*: maximum 16.0 per cent;

— *palmitoleic acid*: maximum 8.0 per cent;

— *stearic acid*: maximum 6.0 per cent;

— *oleic acid*: 65.0 per cent to 88.0 per cent;

— *linoleic acid*: maximum 18.0 per cent;

— *linolenic acid*: maximum 4.0 per cent;

— *fatty acids of chain length greater than* C_{18}: maximum 4.0 per cent.

Total ash ***(2.4.16)***

Maximum 0.1 per cent, determined on 2.00 g.

STORAGE

In an airtight, well-filled container, protected from light.

LABELLING

The label states the origin of oleic acid (animal or vegetable).

Ph Eur

Oleoyl Macrogolglycerides

General Notices

(Ph Eur monograph 1249)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols.

They are obtained by partial alcoholysis of an unsaturated oil mainly containing triglycerides of oleic (*cis*-9-octadecenoic) acid, using macrogol with a mean relative molecular mass between 300 and 400, or by esterification of glycerol and macrogol with unsaturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of this unsaturated oil.

CHARACTERS

Appearance

Amber oily liquid, which may give rise to a deposit after prolonged periods at 20 °C.

Solubility

Practically insoluble but dispersible in water, freely soluble in methylene chloride.

Viscosity

About 35 mPa·s at 40 °C.

Relative density

About 0.95 at 20 °C.

Refractive index

About 1.47 at 20 °C.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *hexane R*, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9 (R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.7), to 1,2-diglycerides (R_{st} 0.6), to monoglycerides (R_{st} 0.1) and to esters of macrogol (R_{st} 0).

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Fatty acid composition (see Tests).

TESTS

Acid value (*2.5.1*)

Maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)

45 to 65, determined on 1.0 g.

Iodine value (*2.5.4, Method A*)

75 to 95.

Peroxide value (*2.5.5, Method A*)

Maximum 12.0, determined on 2.0 g.

Saponification value (*2.5.6*)

150 to 170, determined on 2.0 g.

Alkaline impurities

Introduce 5.0 g into a test tube and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 ml of *water R* and 10 ml of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 ml of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol

Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 ml of *methylene chloride R*. Heat if necessary. After cooling, add 100 ml of *water R*. Shake and add 25.0 ml of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 ml of a 75 g/l solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 ml of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulphate*. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:

— *palmitic acid*: 4.0 per cent to 9.0 per cent;

— *stearic acid*: maximum 6.0 per cent;

— *oleic acid*: 58.0 per cent to 80.0 per cent;

— *linoleic acid*: 15.0 per cent to 35.0 per cent;

— *linolenic acid*: maximum 2.0 per cent;

— *arachidic acid*: maximum 2.0 per cent;

— *eicosenoic acid*: maximum 2.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.0 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash (*2.4.16*)

Maximum 0.1 per cent.

STORAGE

Protected from light.

LABELLING

The label states the type of macrogol used (mean relative molecular mass) or the number of units of ethylene oxide per molecule (nominal value).

Ph Eur

Oleyl Alcohol

General Notices

(Ph Eur monograph 2073)

Action and use

Nonionic surfactant.

Ph Eur

DEFINITION

Mixture of unsaturated and saturated long-chain fatty alcohols consisting mainly of octadec-9-enol (oleyl alcohol and elaidyl alcohol; $C_{18}H_{36}O$; M_r 268.5). It may be of vegetable or animal origin.

CHARACTERS

Appearance

Colourless or light yellow liquid.

IDENTIFICATION

A. It complies with the test for hydroxyl value (see Tests).

B. It complies with the test for composition of fatty alcohols (see Tests).

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Refractive index (*2.2.6*)

1.458 to 1.460, determined at 25 °C.

Cloud point

Maximum 10 °C.

Introduce about 60 g into a cylindrical flat-bottomed container, 30-33.5 mm internal diameter and 115-125 mm high. Heat to 30 °C, cool, and immerse the container in iced water with the surfaces of the water and the sample at the same level. Insert a thermometer and, using it as a stirring rod begin stirring rapidly and steadily when the temperature falls below 20 °C. Keep the thermometer immersed throughout the test, remove and examine the container at regular intervals. The cloud point is the temperature at which the immersed portion of the thermometer, positioned vertically in the centre of the container, is no longer visible when viewed horizontally through the container and sample.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

205 to 215.

Saponification value (*2.5.6*)

Maximum 2.0.

Composition of fatty alcohols

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Mix 25 mg of the substance to be examined with 1.0 ml of *methylene chloride R*.

Reference solution (a) Dissolve 25 mg of each of *arachidyl alcohol R*, *linolenyl alcohol R*, *linoleyl alcohol R*, oleyl *alcohol R*, *palmityl alcohol R* and *stearyl alcohol R* in *methylene chloride R* and dilute to 5 ml with the same solvent. Dilute 1 ml of this solution to 5 ml with *methylene chloride R*.

Reference solution (b) Dissolve 10 mg of *linoleyl alcohol R* and 1 g of *oleyl alcohol R* in *methylene chloride R* and dilute to 40 ml with the same solvent.

Column:

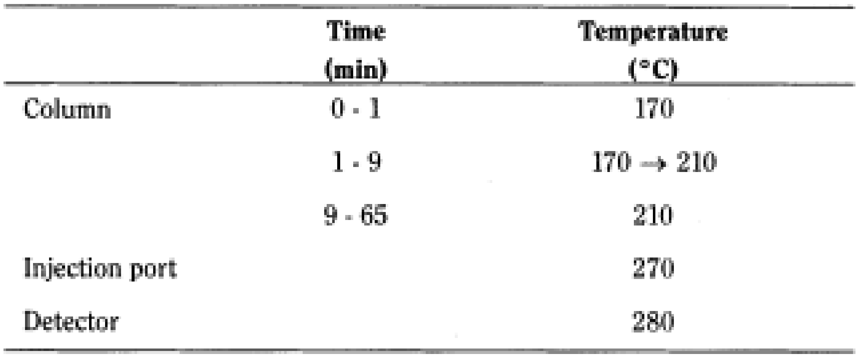
- *size: l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 ml/min.

Split ratio 1:11.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	170
	1 - 9	170 → 210
	9 - 65	210
Injection port		270
Detector		280

Detection Flame ionisation.

Injection 1 µl.

Identify the peaks using the chromatogram obtained with reference solution (a).

Relative retention With reference to oleyl alcohol (retention time = about 30 min): palmityl alcohol = about 0.6; linolenyl alcohol = about 0.8; linoleyl alcohol = about 0.9; stearyl alcohol = about 1.1; arachidyl alcohol = about 1.9 (elaidyl alcohol co-elutes with oleyl alcohol).

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 1.2 between the peaks due to linoleyl alcohol and oleyl alcohol.

Limits:

— *palmityl alcohol*: maximum 8.0 per cent,

— *stearyl alcohol*: maximum 5.0 per cent,

— *oleyl alcohol* (sum of oleyl and elaidyl alcohols): minimum 80.0 per cent,

— *linoleyl alcohol*: maximum 3.0 per cent,

— *linolenyl alcohol*: maximum 0.5 per cent,

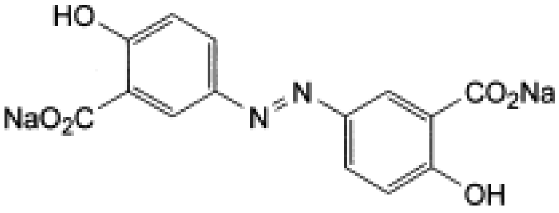
— *arachidyl alcohol*: maximum 0.3 per cent.

Ph Eur

Olsalazine Sodium

General Notices

(Ph Eur monograph 1457)



$C_{14}H_8N_2Na_2O_6$ 346.2 *6054-98-4*

Action and use

Aminosalicylate; treatment of ulcerative colitis.

Ph Eur

DEFINITION

Disodium 3,3′-diazenediylbis(6-hydroxybenzoate).

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, fine, crystalline powder.

Solubility

Sparingly soluble in water, soluble in dimethyl sulphoxide, very slightly soluble in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 40.0 mg in 5 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with a 7.8 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 7.2 with *strong sodium hydroxide solution R* (buffer solution). Dilute 2.0 ml of the solution to 100.0 ml with the buffer solution.

Spectral range 240 nm to 400 nm.

Absorption maxima At 255 nm and 362 nm.

Absorbance ratio A_{255}/A_{362} = 0.53 to 0.56.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison olsalazine sodium CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *dilute ammonia R2* and 4 volumes of *ethanol (96 per cent) R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dissolve 10 mg of *olsalazine sodium CRS* in a mixture of 1 volume of *dilute ammonia R2* and 4 volumes of *ethanol (96 per cent) R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 5 mg of *sulfasalazine CRS* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate TLC silica gel F_{254} *plate R.*

Mobile phase anhydrous formic acid R, *acetone R*, *methylene chloride R* (5:50:60 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.5 g add 2 ml of *sulphuric acid R*. Progressively heat to ignition and continue heating until an almost white or at most greyish residue is obtained. Carry out the ignition at a temperature up to 800 ± 50 °C. Dissolve the residue in 10 ml of boiling *water R* and filter. 2 ml of the filtrate gives reaction (a) of sodium (*2.3.1*).

TESTS

Acetate

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.125 g of the substance to be examined in 25.0 ml of *water R* and add 1.0 ml of *dilute hydrochloric acid R*. Centrifuge and then filter the solution through a 0.45 µm filter and also through an appropriate filter for removal of chlorides.

Reference solution (a) Dissolve 0.140 g of *sodium acetate R*, 0.150 g of *sodium formate R* and 0.180 g of *potassium sulphate R* in 100.0 ml of *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*.

Reference solution (b) Use suitable amounts of *sodium acetate R* to prepare not fewer than 5 reference solutions containing 10-50 µg/ml of acetate.

Column:
- *size:* l = 0.25 m, Ø = 9 mm;
- *stationary phase*: ion-exclusion resin for *chromatography R* with a capacity of about 27 meq/column.

Suppressor column:
Mobile phase 0.0001 M hydrochloric acid.

Flow rate 0.9 ml/min.

Detection Conductivity detector at 10 µs·cm^{-1}.

Injection 0.1 ml.

System suitability Reference solution (a):

- the chromatogram shows 3 separated peaks.

Determine the concentration of acetate in the test solution using the calibration curve generated by the average of the readings obtained with the reference solutions. Measure the peak area for acetate. Calculate the percentage content of acetate using the following expression:

$$\frac{2.6\,c}{m}$$

c = concentration of acetate in the test solution, in micrograms per millilitre, determined by linear interpolation of the standard curve for reference solution (b),

m = mass of sample, in milligrams.

Limit:
- *acetate*: maximum 1.0 per cent.

Methanesulphonic acid

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.25 g of the substance to be examined in 20 ml of *water R*, add 1.0 ml of *dilute hydrochloric acid R* and dilute to 25.0 ml with *water R*. Centrifuge and then filter the solution through a 0.45 µm filter and also through an appropriate filter for removal of chlorides.

Reference solution (a) Dissolve 0.25 g of *methanesulphonic acid R* in 50 ml of *water R*. Add

0.58 g of *sodium acetate R* and 0.08 g of *sodium chloride R* and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*.

Reference solution (b) Dissolve 0.10 g of *methanesulphonic acid R* in *water R* and dilute to 100.0 ml with *water R*. Dilute 3.0 ml of this solution to 100.0 ml with *water R*.

Precolumn:
- *size:* l = 0.035 m, Ø = 4 mm;
- *stationary phase*: *resin for reversed-phase ion chromatography R* (10 µm).

Column:
- *size:* l = 0.25 m, Ø = 4 mm;
- *stationary phase*: *resin for reversed-phase ion chromatography R* (10 µm).

Mobile phase Mix 10 volumes of *acetonitrile for chromatography R* and 990 volumes of a solution containing 1.6 g/l of *tetrabutylammonium hydroxide R* and 0.053 g/l of *anhydrous sodium carbonate R*.

Flow rate 1.0 ml/min.

Detection Conductivity detector at 50 µs·cm^{-1}.

Injection 100 µl.

System suitability Reference solution (a):
- the chromatogram shows 3 separated peaks.

Limit:
- *methanesulphonic acid*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in mobile phase A and dilute to 25.0 ml with mobile phase A.

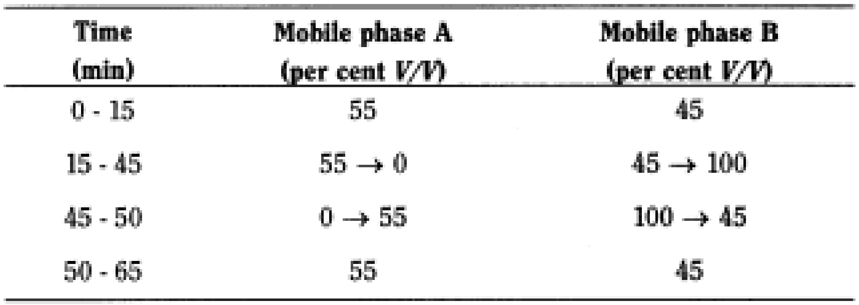
Reference solution (a) Dilute 0.5 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b) Dissolve 20.0 mg of *olsalazine sodium for performance test CRS* in mobile phase A and dilute to 25.0 ml with mobile phase A.

Column:
- *size:* l = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: dissolve 2.38 g of *tetrabutylammonium hydrogen sulphate R* and 3.6 g of *disodium hydrogen phosphate dihydrate R* in 900 ml of *water R*, adjust to pH 7.6 with *dilute sodium hydroxide solution R* and dilute to 1000.0 ml with *water R*; mix 700 ml of this buffer solution with 300 ml of *methanol R*;
- *mobile phase B*: dissolve 4.75 g of *tetrabutylammonium hydrogen sulphate R* and 3.6 g of *disodium hydrogen phosphate dihydrate R* in 900 ml of *water R*, adjust to pH 7.6 with *dilute sodium hydroxide solution R* and dilute to 1000.0 ml with *water R*; mix 350 ml of this

buffer solution with 650 ml of *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	55	45
15 - 45	55 → 0	45 → 100
45 - 50	0 → 55	100 → 45
50 - 65	55	45

Flow rate 1 ml/min.

Detection Spectrophotometer at 360 nm.

Injection 20 µl.

System suitability Reference solution (b):

— the chromatogram is similar to the chromatogram obtained with *olsalazine sodium for performance test CRS*.

Limits:

— *impurities A, B, C, D, E, F, G, H, I*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), and not more than one of the peaks has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 150 °C.

ASSAY

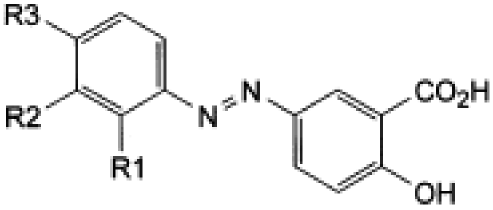
Dissolve 0.100 g in 15 ml of *ethylene glycol R*. Add 40 ml of *dioxan R* and 0.2 ml of a 224 g/l solution of *potassium chloride R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

Correct the volume consumed for the content of acetate, taking the molecular mass of acetate to be 59.0.

1 ml of *0.1 M hydrochloric acid* is equivalent to 17.31 mg of $C_{14}H_8N_2Na_2O_6$.

IMPURITIES

Specified impurities   *A, B, C, D, E, F, G, H, I.*



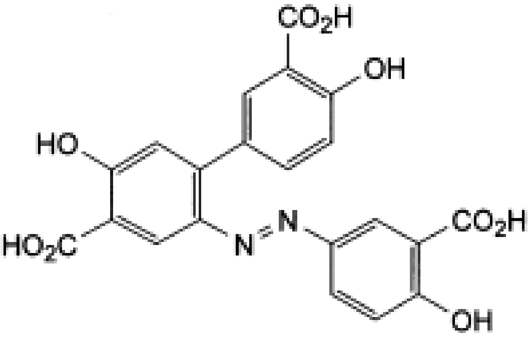
A. R1 = H, R2 = CO_2H, R3 = OCH_3: 6-hydroxy-6′-methoxy-3,3′-diazenediyldibenzoic acid,

B. R1 = OH, R2 = CO_2H, R3 = H: 2,6′-dihydroxy-3,3′-diazenediyldibenzoic acid,

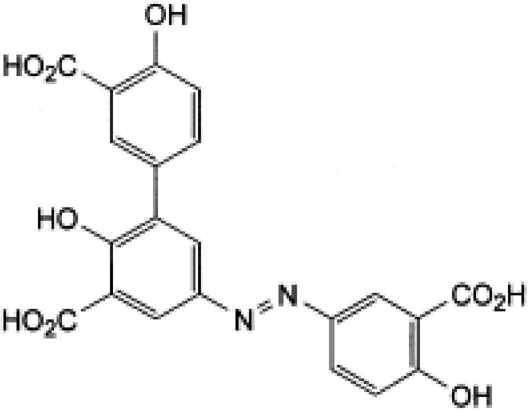
C. R1 = R2 = H, R3 = OH: 2-hydroxy-5-[(4-hydroxyphenyl)diazenyl]benzoic acid,

D. R1 = H, R2 = CO_2H, R3 = Cl: 6-chloro-6′-hydroxy-3,3′-diazenediyldibenzoic acid,

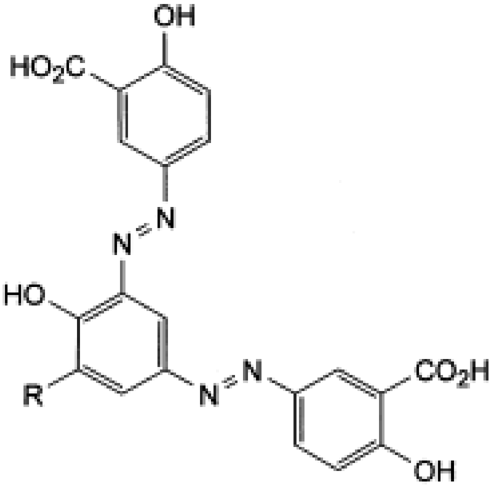
E. R1 = H, R2 = $CO\text{-}CH_2\text{-}SO_3H$, R3 = OH: 2-hydroxy-5-[[4-hydroxy-3-(sulphoacetyl)phenyl) diazenyl]benzoic acid,



F. 2′-[(3-carboxy-4-hydroxyphenyl)diazenyl]-4,5′-dihydroxybiphenyl-3,4′-dicarboxylic acid,



G. 5-[(3-carboxy-4-hydroxyphenyl)diazenyl]-2,4′-dihydroxybiphenyl-3,3′-dicarboxylic acid,



H. R = CO_2H: 3,3′-[5-carboxy-4-hydroxy-1,3-phenylenebis(diazenediyl)]bis(6-hydroxybenzoic) acid,

I. R = H: 3,3′-[4-hydroxy-1,3-phenylenebis(diazenediyl)]bis(6-hydroxybenzoic) acid.

Ph Eur

Omega-3-Acid Ethyl Esters 60

General Notices

(Ph Eur monograph 2063)

Action and use

Lipid-regulating drug.

Ph Eur

DEFINITION

Ethyl esters of *alpha*-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA). Omega-3-acid ethyl esters 60 are obtained by transesterification of the body oil of fat fish species coming from families like *Engraulidae, Carangidae, Clupeidae, Osmeridae, Salmonidae* and *Scombridae* and subsequent physico-chemical purification processes, including molecular distillation. The minimum content of total omega-3-acid ethyl esters and the minimum content of the omega-3 acids EPA and DHA ethyl esters are indicated in Table 2063.-1.

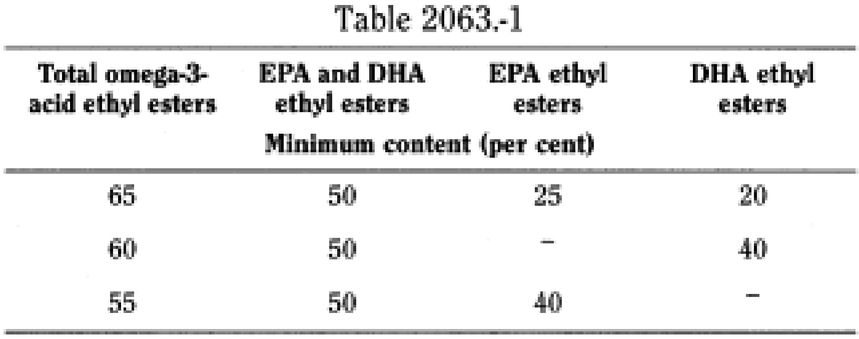


Table 2063.-1

Total omega-3-acid ethyl esters	EPA and DHA ethyl esters	EPA ethyl esters	DHA ethyl esters
	Minimum content (per cent)		
65	50	25	20
60	50	–	40
55	50	40	–

Tocopherol may be added as an antioxidant.

CHARACTERS

Appearance

Light yellow liquid.

It has a slight fish-like odour.

Solubility

Practically insoluble in water, very soluble in acetone, in ethanol (96 per cent), in heptane and in methanol.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay for EPA and DHA ethyl esters.

Results The peaks due to eicosapentaenoic acid ethyl ester and to docosahexaenoic acid ethyl ester in the chromatogram obtained with the test solution are similar in retention time to the corresponding peaks in the chromatogram obtained with the reference solution.

B. It complies with the limits of the assay for Total omega-3-acid ethyl esters.

TESTS

Absorbance (*2.2.25*)

Maximum 0.60 at 233 nm.

Dilute 0.300 g to 50.0 ml with *trimethylpentane R*. Dilute 2.0 ml of this solution to 50.0 ml with *trimethylpentane R*.

Acid value (*2.5.1*)

Maximum 2.0, determined on 10 g in 50 ml of the prescribed mixture of solvents.

Peroxide value (*2.5.5, Method A*)

Maximum 10.0.

Anisidine value

Maximum 20.0.

The anisidine value is defined as 100 times the absorbance measured in a 1 cm cell of a solution containing 1 g of the substance to be examined in 100 ml of a mixture of solvents and reagents according to the method described below.

Carry out the operations as rapidly as possible, avoiding exposure to actinic light.

Test solution (a) Dilute 0.500 g of the substance to be examined to 25.0 ml with *trimethylpentane R*.

Test solution (b) To 5.0 ml of test solution (a) add 1.0 ml of a 2.5 g/l solution of *p-anisidine R* in *glacial acetic acid R*, shake and protect from light.

Reference solution To 5.0 ml of trimethylpentane R add 1.0 ml of a 2.5 g/l solution of *p-anisidine R* in *glacial acetic acid R*, shake and protect from light.

Measure the absorbance (*2.2.25*) of test solution (a) at 350 nm using *trimethylpentane R1* as the compensation liquid. Measure the absorbance of test solution (b) at 350 nm exactly 10 min after its preparation, using the reference solution as the compensation liquid.

Calculate the anisidine value using the following expression:

$$\frac{25 \times (1.2\, A_s - A_b)}{m}$$

A_s = absorbance of test solution (b) at 350 nm,

A_t = absorbance of test solution (a) at 350 nm,

m = mass of the substance to be examined in test solution (a), in grams.

Oligomers and partial glycerides

Size exclusion chromatography (*2.2.30*).

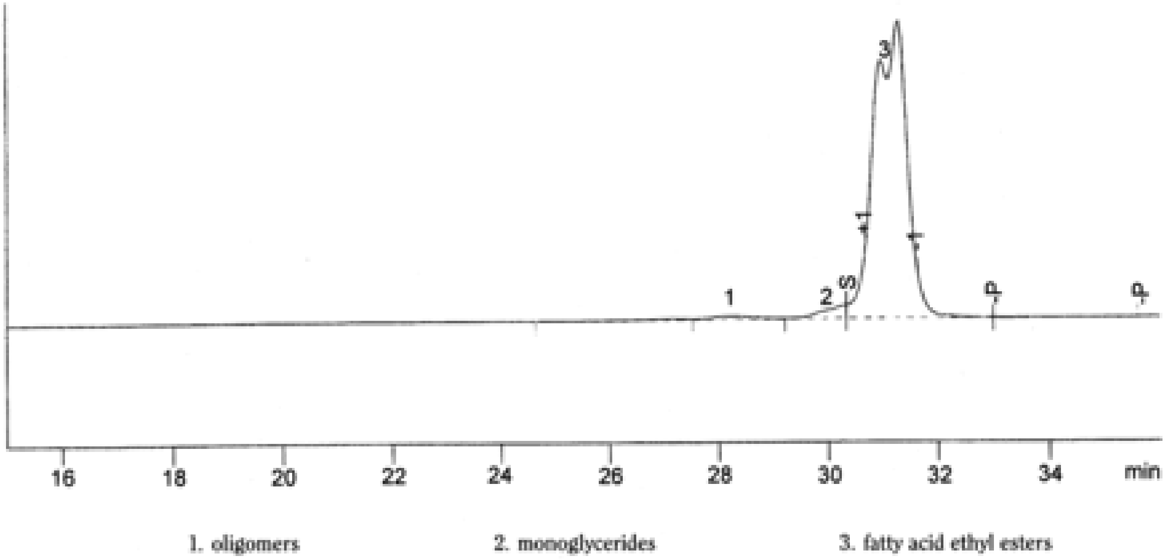


Figure 2063.-1. – *Chromatogram for the test for oligomers and partial glycerides in omega-3-acid ethyl esters 60*

Test solution Dilute 10.0 mg of the substance to be examined to 10.0 ml with *tetrahydrofuran R*.

Reference solution In a 100 ml volumetric flask dissolve 50 mg of *monodocosahexaenoin R*, 30 mg of *didocosahexaenoin R* and 20 mg of *tridocosahexaenoin R* in *tetrahydrofuran R* and dilute to 100.0 ml with the same solvent.

Column 1:
- *dimensions*: *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm), with a pore size of 10 nm.

Columns 2 and 3 placed closest to the injector:
- *dimensions: l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm), with a pore size of 50 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 0.8 ml/min.

Detection Differential refractometer.

Injection 40 µl.

System suitability:
- *elution order* in the chromatogram obtained with the reference solution: tridocosahexaenoin, didocosahexaenoin and monodocosahexaenoin;

- *resolution* in the chromatogram obtained with the reference solution: minimum 2.0 between the peaks due to monodocosahexaenoin and didocosahexaenoin; minimum 1.0 between the peaks due to didocosahexaenoin and tridocosahexaenoin;
- if the method of standard addition to the test solution is used, the recovery for the added *eicosapentaenoic acid ethyl ester CRS* or *docosahexaenoic acid ethyl ester CRS* is not less than 95 per cent.

Calculate the percentage content of oligomers plus partial glycerides using the following expression:

$$\frac{B}{A} \times 100$$

A = sum of areas of all the peaks in the chromatogram,

B = sum of the areas of the peaks with a retention time smaller than those of the ethyl ester peaks.

The ethyl ester peaks, which may be present in the form of an unresolved double peak, are identified as the major peaks in the chromatogram (Figure 2063.-1).

Limit:

- *oligomers + partial glycerides*: maximum 7.0 per cent.

ASSAY

EPA and DHA ethyl esters (*2.4.29*)

See Figure 2063.-2.

Total omega-3-acid ethyl esters (*2.4.29*)

See Figure 2063.-2.

STORAGE

Under an inert gas, in an airtight container , protected from light.

LABELLING

The label states:

- the content of total omega-3-acid ethyl esters;
- the content of EPA ethyl ester and DHA ethyl ester;
- the concentration of any added tocopherol.

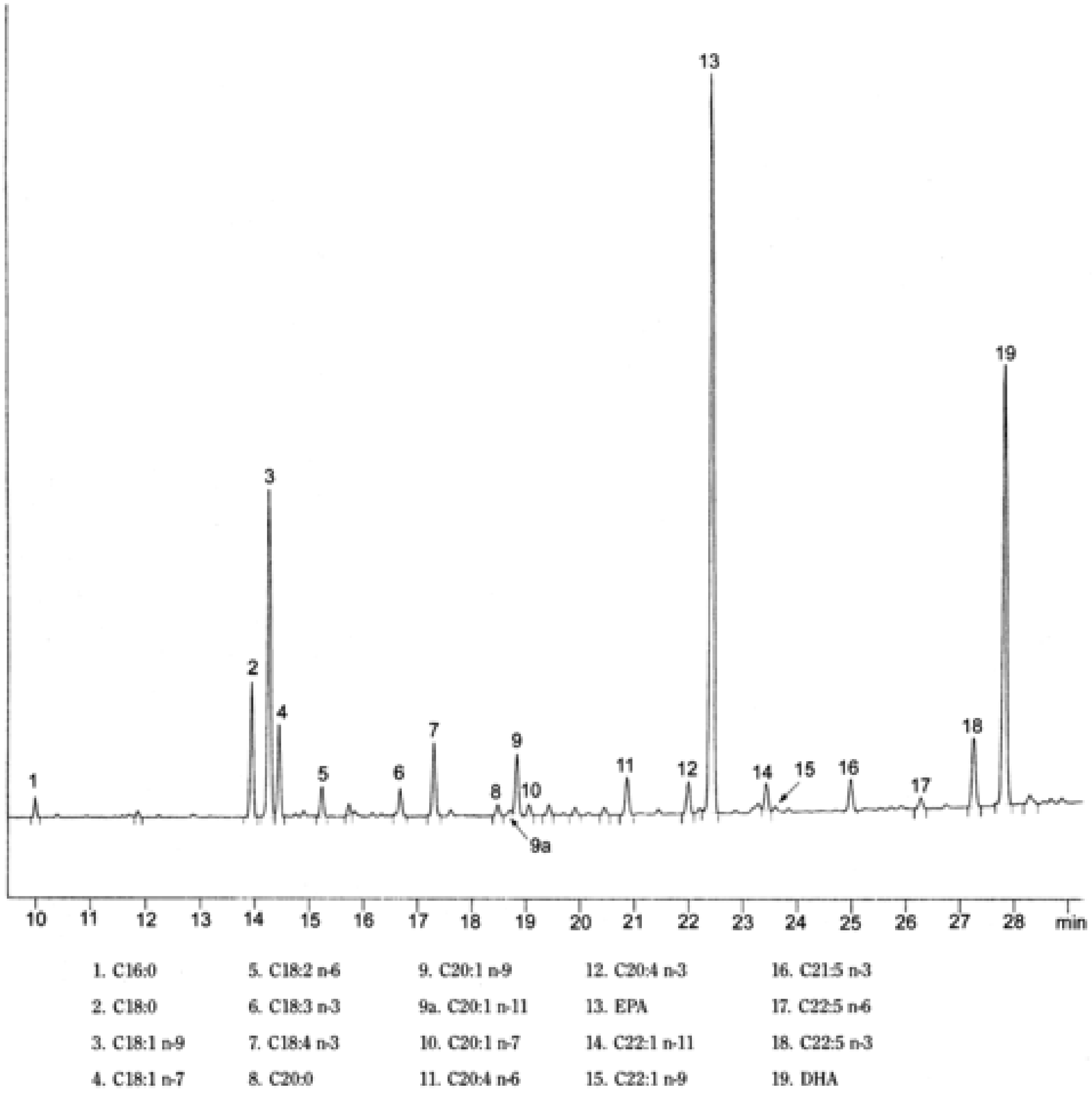


Figure 2063.-2. – *Chromatogram for the assay of total omega-3 acid ethyl esters in omega-3 acid ethyl esters 60*

Ph Eur

Omega-3-Acid Ethyl Esters 90

General Notices

(Ph Eur monograph 1250)

Action and use

Lipid-regulating drug.

Ph Eur

DEFINITION

Ethyl esters of alpha-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA). Omega-3-acid ethyl esters are obtained by transesterification of the body oil of fat fish species coming from families such as *Engraulidae, Carangidae, Clupeidae, Osmeridae*, *Salmonidae* and *Scombridae* and subsequent physico-chemical purification processes, including urea fractionation followed by molecular distillation.

Content:

— *EPA and DHA ethyl esters*: minimum 80 per cent, with minimum 40 per cent of EPA ethyl esters and minimum 34 per cent of DHA ethyl esters;

— *total omega-3-acid ethyl esters* : minimum 90 per cent.

Tocopherol may be added as an antioxidant.

CHARACTERS

Appearance

Light yellow liquid.

Solubility

Practically insoluble in water, very soluble in acetone, in ethanol (96 per cent), in heptane and in methanol.

IDENTIFICATION

Examine the chromatograms obtained in the assay for EPA and DHA ethyl esters.

Results The peaks due to eicosapentaenoic acid ethyl ester and to docosahexaenoic acid ethyl ester in the chromatogram obtained with the test solution are similar in retention time and size to the corresponding peaks in the chromatogram obtained with the reference solution.

TESTS

Absorbance (*2.2.25*)

Maximum 0.55 at 233 nm.

Dilute 0.300 g to 50.0 ml with *trimethylpentane R*. Dilute 2.0 ml of this solution to 50.0 ml with *trimethylpentane R*.

Acid value (*2.5.1*)

Maximum 2.0, determined on 10 g in 50 ml of the prescribed mixture of solvents.

Anisidine value (*2.5.36*)

Maximum 20.0.

Peroxide value (*2.5.5, Method A*)

Maximum 10.0.

Oligomers

Size-exclusion chromatography (*2.2.30*).

Test solution Dilute 10.0 mg of the substance to be examined to 10.0 ml with *tetrahydrofuran R*.

Reference solution In a 100 ml volumetric flask, dissolve 50 mg of *monodocosahexaenoin R*, 30 mg of *didocosahexaenoin R* and 20 mg of *tridocosahexaenoin R* in *tetrahydrofuran R* and dilute to 100.0 ml with the same solvent.

Column 1:
- *size:* *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 10 nm.

Columns 2 and 3 placed closest to the injector:
- *size:* *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 50 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 0.8 ml/min.

Detection Differential refractometer.

Injection 40 µl.

System suitability:
- *elution order* in the chromatogram obtained with the reference solution: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin;
- *resolution*: minimum 2.0 between the peaks due to monodocosahexaenoin and didocosahexaenoin and minimum 1.0 between the peaks due to didocosahexaenoin and tridocosahexaenoin in the chromatogram obtained with the reference solution.

Calculate the percentage content of oligomers using the following expression:

$$\frac{B}{A} \times 100$$

A = sum of areas of all the peaks in the chromatogram,

B = sum of the areas of the peaks with a retention time smaller than the retention time of the peaks due to ethyl esters.

The ethyl ester peaks, which may be present in the form of an unresolved double peak, are identified as the major peaks in the chromatogram (Figure 1250.-1).

When the result obtained exceeds the limit due to the presence of monoglycerides, the following procedure is carried out.

Test solution Weigh 10.0 mg of the substance to be examined into a quartz tube. Add 1.5 ml of a 20 g/l solution of *sodium hydroxide R* in *methanol R,* cover with *nitrogen R*, cap tightly with a polytetrafluoroethylene-lined cap, mix and heat on a water-bath for 7 min. Allow to cool. Add 2 ml of *boron trichloride-methanol solution R*, cover with *nitrogen R*, cap tightly, mix and heat on a water-bath for 30 min. Cool to 40-50 °C, add 1 ml of *trimethylpentane R*, cap and shake vigorously for at least 30 s. Immediately add 5 ml of a *saturated sodium chloride solution R*, cover with *nitrogen R*, cap and shake thoroughly for at least 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer once more with 1 ml of *trimethylpentane R.* Carefully evaporate the solvent under a current of *nitrogen R* then add 10.0 ml of *tetrahydrofuran R* to the residue. Add a small amount of *anhydrous sodium sulphate R* and filter.

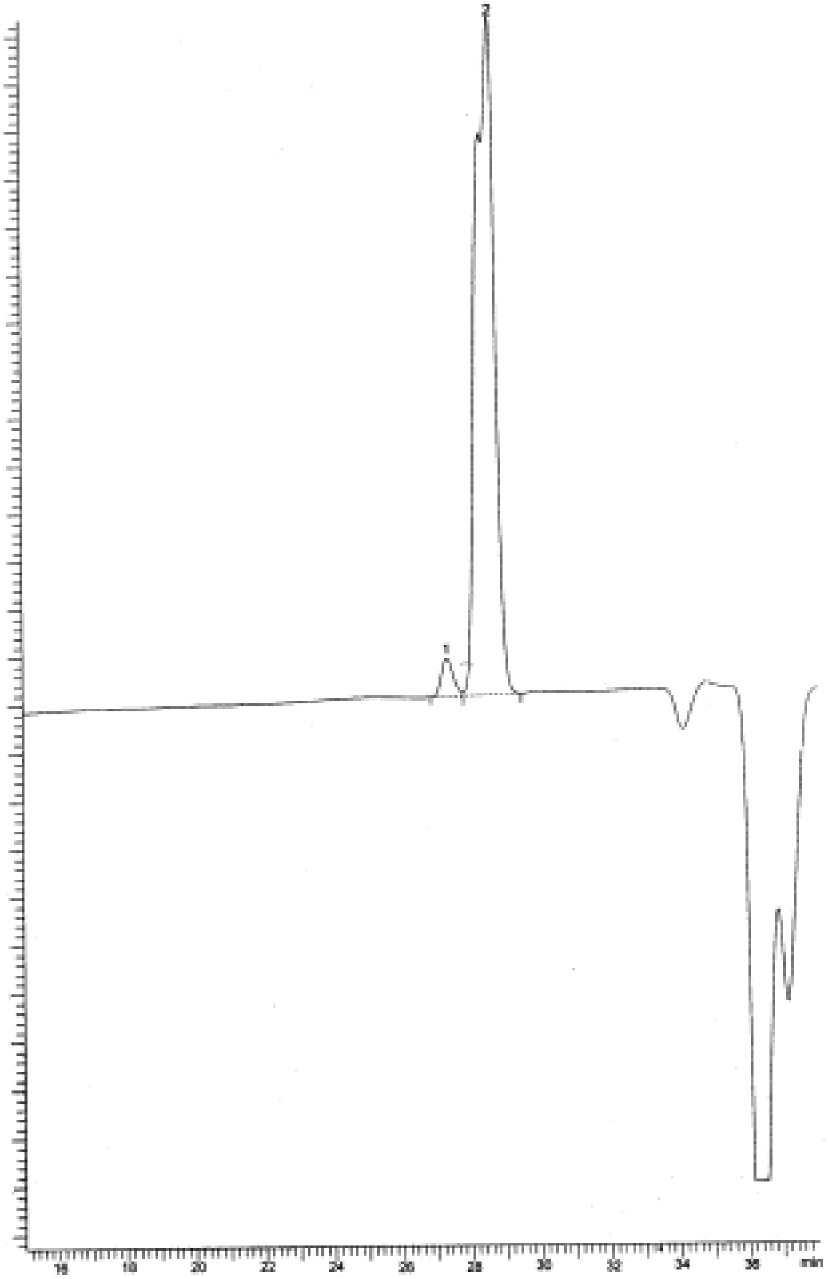
Calculate the percentage content of oligomers using the following expression:

$$\frac{B'}{A} \times 100$$

B′ = sum of the areas of the peaks with a retention time smaller than the retention time of the peaks due to methyl esters.

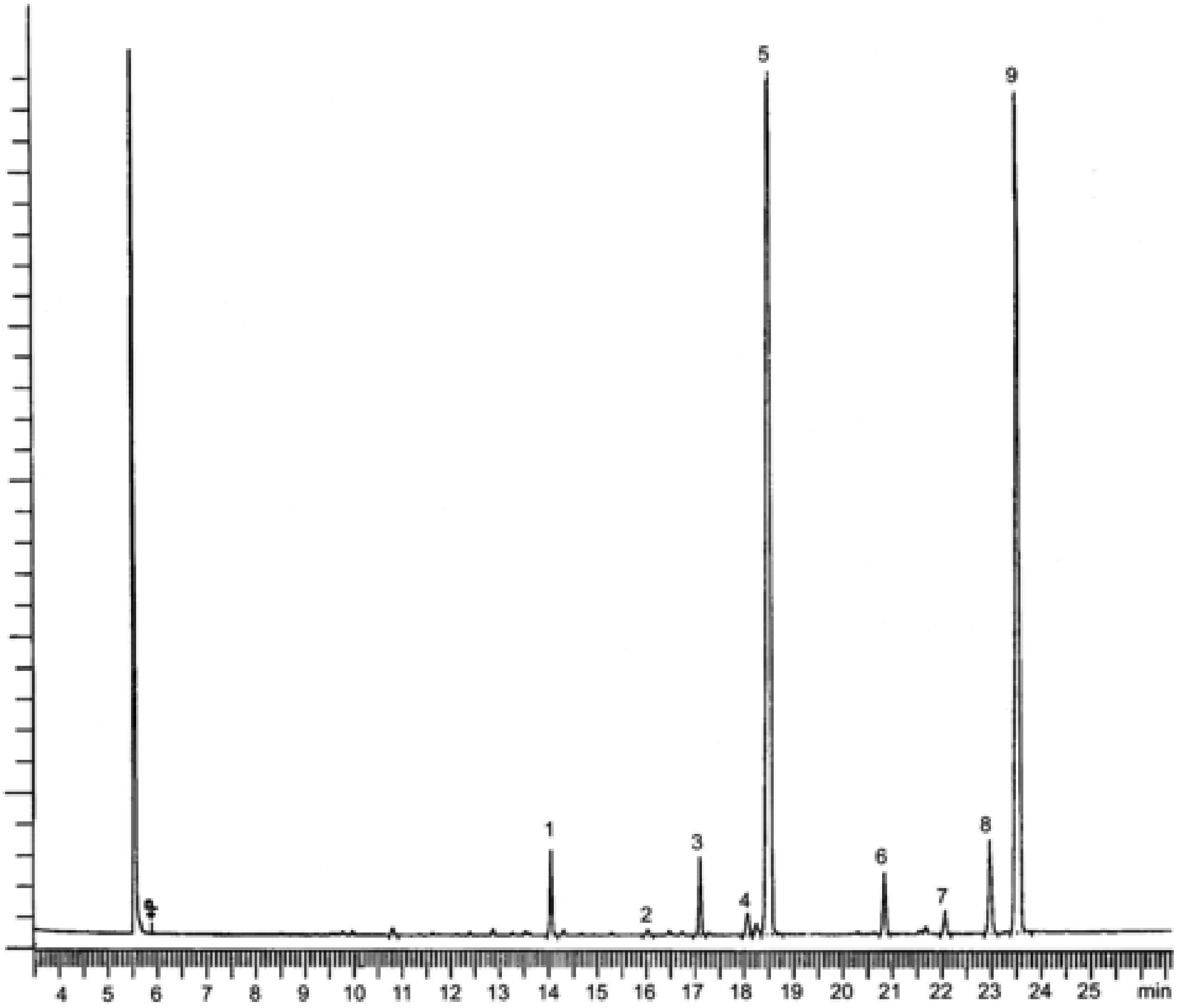
Limit:

— *oligomers*: maximum 1.0 per cent.



1. oligomers 2. ethyl esters

Figure 1250.-1. – *Chromatogram of the test for oligomers in omega-3-acid ethyl esters 90: spiked sample*



1. C18:4 n-3
2. C20:2 n-6
3. C20:4 n-6
4. C20:4 n-3
5. EPA
6. C21:5 n-3
7. C22:5 n-6
8. C22:5 n-3
9. DHA

Figure 1250.-2. - *Chromatogram for the assays*

ASSAY

EPA and DHA ethyl esters (*2.4.29*)

See Figure 1250.-2.

Total omega-3-acid ethyl esters (*2.4.29*)

See Figure 1250.-2.

STORAGE

Under an inert gas, in an airtight container , protected from light.

LABELLING

The label states the concentration of any added tocopherol.

Ph Eur

Omega-3-Marine Triglycerides

General Notices

(Omega-3-Acid Triglycerides, Ph Eur monograph 1352)

Action and use

Lipid-regulating drug.

Ph Eur

DEFINITION

Mixture of mono-, di- and triesters of omega-3 acids with glycerol containing mainly triesters and obtained either by esterification of concentrated and purified omega-3 acids with glycerol or by transesterification of the omega-3-acid ethyl esters with glycerol. The origin of the omega-3 acids is the body oil from fatty fish species coming from families like *Engraulidae*, *Carangidae, Clupeidae, Osmeridae, Salmonidae and Scombridae*. The omega-3 acids are identified as the following acids: alpha-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA).

Content:

- — sum of the contents of the omega-3 acids EPA and DHA, expressed as triglycerides: minimum 45.0 per cent;
- — total omega-3 acids, expressed as triglycerides: minimum 60.0 per cent.

Tocopherol may be added as an antioxidant.

CHARACTERS

Appearance

Pale yellow liquid.

Solubility

Practically insoluble in water, very soluble in acetone and in heptane, slightly soluble in anhydrous ethanol.

IDENTIFICATION

Examine the chromatograms obtained in the assay for EPA and DHA.

Results The peaks due to eicosapentaenoic acid methyl ester and to docosahexaenoic acid methyl ester in the chromatogram obtained with test solution (b) are similar in retention time and size to the corresponding peaks in the chromatogram obtained with reference solution (a)

.

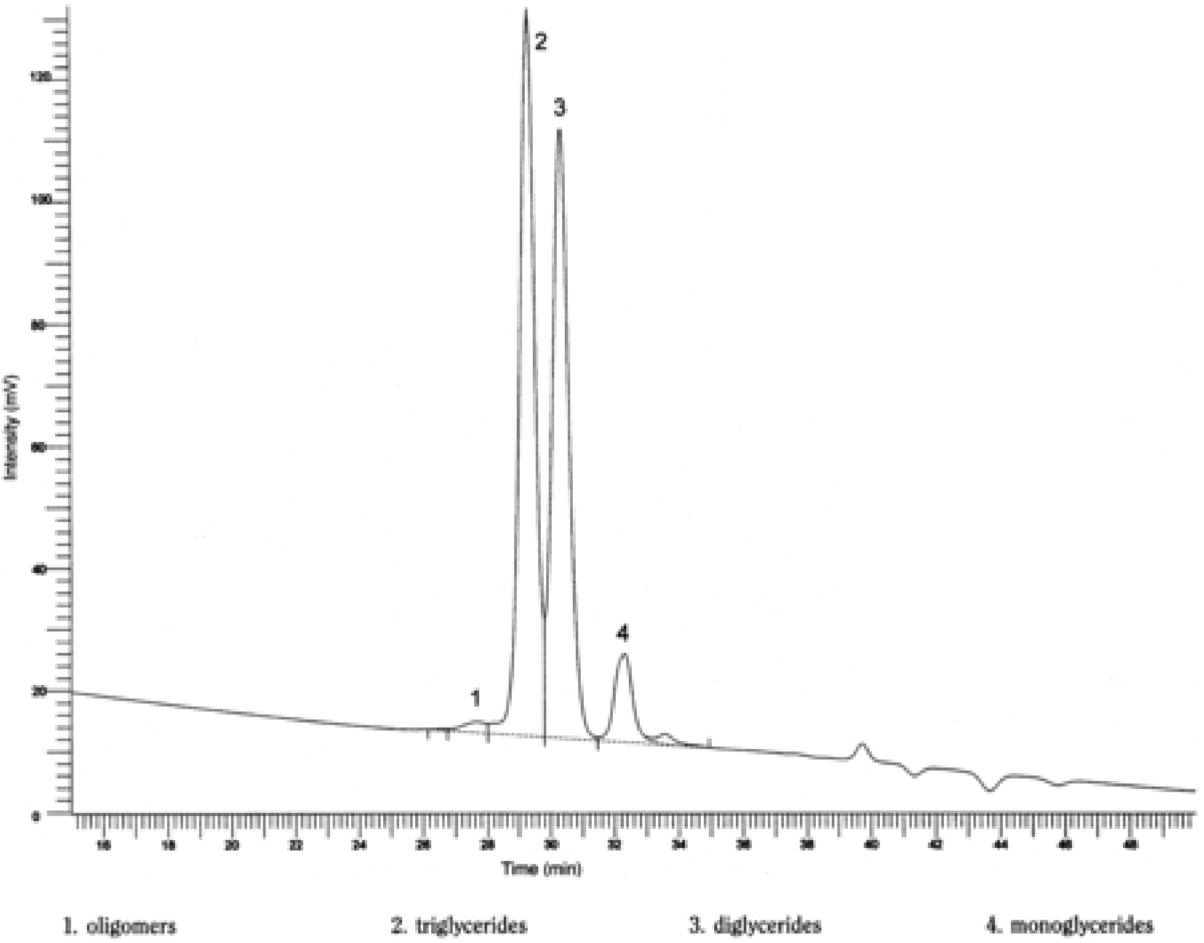


Figure 1352.-1. – *Chromatogram of the test for oligomers and partial glycerides in omega-3-acid triglycerides*

TESTS

Absorbance (*2.2.25*)

Maximum 0.73 at 233 nm.

Dilute 0.300 g of the substance to be examined to 50.0 ml with *trimethylpentane R*. Dilute 2.0 ml of this solution to 50.0 ml with *trimethylpentane R*.

Acid value (*2.5.1*)

Maximum 3.0, determined on 10.0 g in 50 ml of the prescribed mixture of solvents.

Anisidine value

Maximum 30.0.

The anisidine value is defined as 100 times the absorbance measured in a 1 cm cell filled with a solution containing 1 g of the substance to be examined in 100 ml of a mixture of solvents and reagents according to the method described below.

Carry out the operations as rapidly as possible, avoiding exposure to actinic light.

Test solution (a) Dilute 0.500 g of the substance to be examined to 25.0 ml with *trimethylpentane R*.

Test solution (b) To 5.0 ml of test solution (a) add 1.0 ml of a 2.5 g/l solution of *p-anisidine R* in *glacial acetic acid R,* shake and store protected from light.

Reference solution To 5.0 ml of *trimethylpentane R* add 1.0 ml of a 2.5 g/l solution of *p-anisidine R* in glacial *acetic acid R*, shake and store protected from light.

Measure the absorbance (*2.2.25*) of test solution (a) at 350 nm using *trimethylpentane R* as the compensation liquid. Measure the absorbance of test solution (b) at 350 nm exactly 10 min after its preparation, using the reference solution as the compensation liquid.

Calculate the anisidine value using the following expression:

$$\frac{25 \times (1.2A_s - A_b)}{m}$$

A_s = absorbance of test solution (b)

A_b = absorbance of test solution (a)

m = mass of the substance to be examined in test solution (a), in grams.

Peroxide value (*2.5.5, Method A*)

Maximum 10.0.

Oligomers and partial glycerides

Size-exclusion chromatography (*2.2.30*).

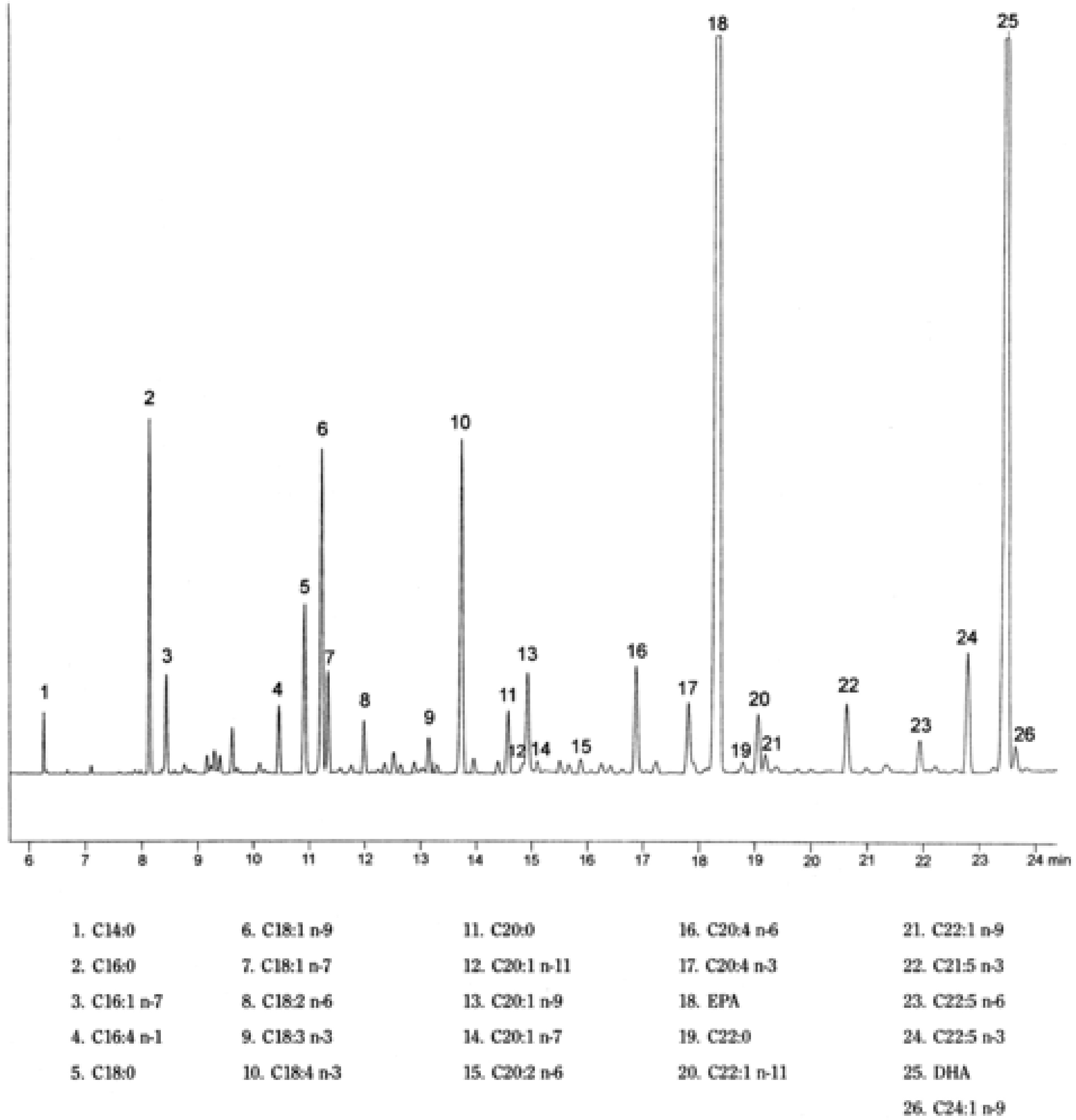


Figure 1352.-2. – *Chromatogram for the assay of total omega-3 acids in omega-3-acid triglycerides*

Test solution Dilute 10.0 mg of the substance to be examined to 10.0 ml with *tetrahydrofuran R*.

Reference solution In a 100 ml volumetric flask dissolve 50 mg of *monodocosahexaenoin R*, 30 mg of *didocosahexaenoin R* and 20 mg of *tridocosahexaenoin R* in *tetrahydrofuran R* and dilute to 100.0 ml with the same solvent.

Column 1:

— *dimensions: l* = 0.3 m, Ø = 7.8 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 10 nm.

Columns 2 and 3 placed closest to the injector:

— *dimensions:* l = 0.3 m, Ø = 7.8 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 50 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 0.8 ml/min.

Detection Differential refractometer.

Injection 40 µl.

System suitability Reference solution:

— *elution order*: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin;

— *resolution*: minimum of 2.0 between the peaks due to monodocosahexaenoin and to didocosahexaenoin and minimum of 1.0 between the peaks due to didocosahexaenoin and to tridocosahexaenoin.

Identify the peaks from the chromatogram (Figure 1352.-1). Calculate the percentage content of oligomers using the following expression:

$$\frac{B}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram,

B = area of the peak with a retention time smaller than the retention time of the triglyceride peak.

Calculate the percentage content of partial glycerides using the following expression:

$$\frac{C}{A} \times 100$$

C = (sum of the) area(s) of the peak(s) due to the mono- and diglycerides.

Limits:

— *oligomers*: maximum 3.0 per cent;

— *partial glycerides*: maximum 50.0 per cent.

ASSAY

EPA and DHA (*2.4.29*)

See Figure 1352.-2.

Total omega-3 acids (*2.4.29*)

See Figure 1352.-2.

STORAGE

In an airtight, well-filled container, protected from light, under an inert gas.

LABELLING

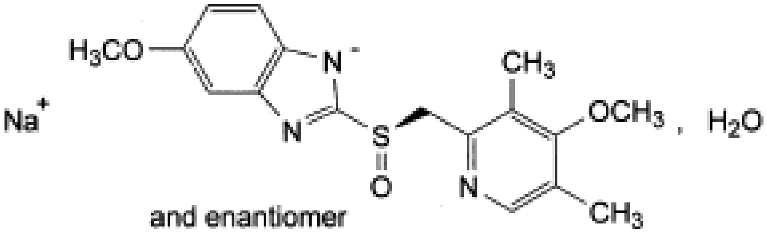
The label states the concentration of any added tocopherol.

Ph Eur

Omeprazole Sodium

General Notices

(Ph Eur monograph 1032)



$C_{17}H_{18}N_3NaO_3S,H_2O$ 385.4 *95510-70-6*

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

Ph Eur

DEFINITION

Sodium 5-methoxy-2-[(*RS*)-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulphinyl]-1*H*-benzimidazole monohydrate.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water and in ethanol (96 per cent), soluble in propylene glycol, very slightly soluble in methylene chloride.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 2.0 mg in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same solvent.

Spectral range 230-350 nm.

Absorption maxima At 276 nm and 305 nm.

Absorption ratio A_{305} / A_{276} = 1.6 to 1.8.

B. Examine the chromatograms obtained in the test for impurity C.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Place the plate in a tank saturated with vapour of *acetic acid R*. The spots rapidly turn brown.

C. Ignite *1 g* and cool. Add 1 ml of *water R* to the residue and neutralise with *hydrochloric acid R*. Filter and dilute the filtrate to 4 ml with *water R*. 0.1 ml of the solution gives reaction (b) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

pH (*2.2.3*)

10.3 to 11.3 for solution S.

Impurity C

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in 2.0 ml of *methanol R*.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 9 mg of *omeprazole CRS* in 2.0 ml of *methanol R*.

Reference solution (b) Dilute 1.0 ml of test solution (b) to 100 ml with *methanol R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Mix 20 volumes of *2-propanol R*, 40 volumes of *methylene chloride R* previously shaken with *concentrated ammonia R* (shake 100 ml of *methylene chloride R* with 30 ml of concentrated *ammonia R* in a separating funnel, allow the layers to separate and use the lower layer) and 40 volumes of *methylene chloride R*.

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Limit Test solution (a):

— *impurity C*: any spot with a higher R_F value than that of the spot due to omeprazole is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 3.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 1.0 mg of *omeprazole CRS* and 1.0 mg of *omeprazole impurity D CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size: l* = 0.15 m, Ø = 4 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 27 volumes of *acetonitrile R* and 73 volumes of a 1.4 g/l solution of *disodium hydrogen phosphate R*, previously adjusted to pH 7.6 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 40 µl.

Run time 3 times the retention time of omeprazole.

Relative retention With reference to omeprazole (retention time = about 9 min): impurity D = about 0.8.

System suitability Reference solution (a):

— *resolution*: minimum 3 between the peaks due to impurity D and omeprazole; if necessary adjust the pH of the mobile phase or the concentration of *acetonitrile R*; an increase in the pH will improve the resolution.

Limit:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml *of lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

4.5 per cent to 10.0 per cent, determined on 0.300 g.

ASSAY

Dissolve 0.300 g in 50 ml of *water R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M hydrochloric acid* corresponds to 36.74 mg of $C_{17}H_{18}N_3NaO_3S$.

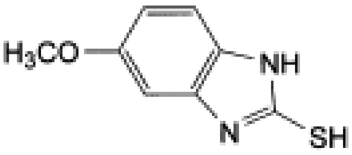
STORAGE

In an airtight container, protected from light.

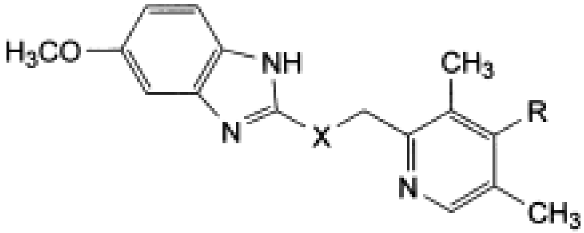
IMPURITIES

Specified impurities C.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, D, E.*



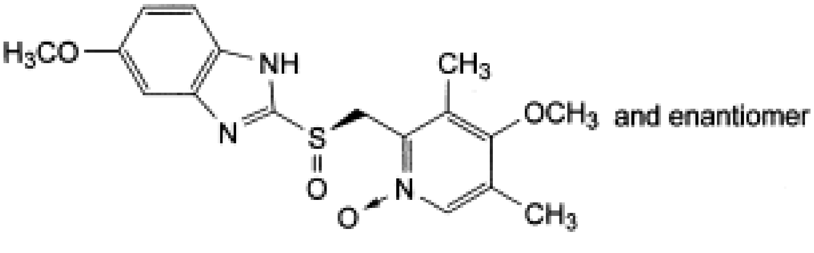
A. 5-methoxy-1*H*-benzimidazole-2-thiol,



B. R = H, X = SO: 2-[(*RS*)-[(3,5-dimethylpyridin-2-yl)methyl]sulphinyl]-5-methoxy-1*H*-benzimidazole,

C. R = OCH_3, X = S: 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]thio]-1*H*-benzimidazole (ufiprazole),

D. R = OCH_3, X = SO_2: 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole (omeprazole-sulphone),



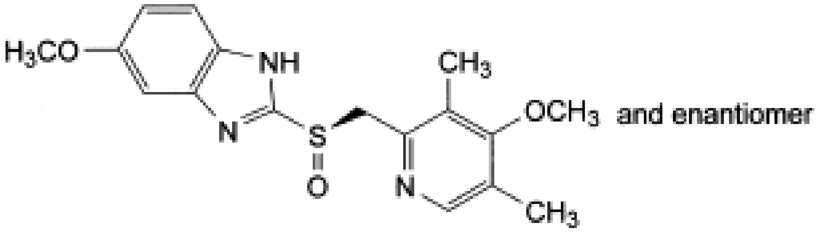
E. 4-methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl)sulphinyl]methyl]-3,5-dimethylpyridine 1-oxide.

Ph Eur

Omeprazole

General Notices

(Ph Eur monograph 0942)



$C_{17}H_{19}N_3O_3S$ 345.4 *73590-58-6*

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

Preparations

Gastro-resistant Omeprazole Capsules

Gastro-resistant Omeprazole Tablets

Ph Eur

DEFINITION

5-Methoxy-2-[(*RS*)-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulphinyl]-1*H*-benzimidazole.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, soluble in methylene chloride, sparingly soluble in ethanol (96 per cent) and in methanol. It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 2.0 mg in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same solvent.

Spectral range 230-350 nm.

Absorption maxima At 276 nm and 305 nm.

Absorbance ratio A_{305} / A_{276} = 1.6 to 1.8.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *omeprazole CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for impurity C.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). Place the plate in a tank saturated with vapour from *acetic acid R*. The spots rapidly turn brown.

TESTS

Solution S

Dissolve 0.50 g in *methylene chloride R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*).

Impurities F and G

Maximum 0.035 per cent for the sum of the contents.

The absorbance (*2.2.25*) of solution S measured at 440 nm is not greater than 0.10.

Impurity C

Thin-layer chromatography (*2.2.27*).

Solvent mixture *methanol R*, *methylene chloride R* (50:50 *V/V*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in 2.0 ml of the solvent mixture.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 10 mg of *omeprazole CRS* in 2.0 ml of *methanol R*.

Reference solution (b) Dilute 1 ml of test solution (a) to 10 ml with the solvent mixture. Dilute 1 ml of this solution to 100 ml with the solvent mixture.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Mix 20 volumes of *2-propanol R*, 40 volumes of *methylene chloride R* previously shaken with *concentrated ammonia R* (shake 100 ml of *methylene chloride R* with 30 ml of *concentrated ammonia R* in a separating funnel; allow the layers to separate and use the lower layer) and 40 volumes of *methylene chloride R*.

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Limits Test solution (a):

— *impurity C*: any spot with a higher R_F value than that of the spot due to omeprazole is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 3.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 1 mg of *omeprazole CRS* and 1 mg of *omeprazole impurity D CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size:* l = 0.15 m, Ø = 4 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 27 volumes of *acetonitrile R* and 73 volumes of a 1.4 g/l solution of *disodium hydrogen phosphate R* previously adjusted to pH 7.6 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 40 µl.

Run time 3 times the retention time of omeprazole.

Relative retention With reference to omeprazole (retention time = about 9 min): impurity A = about 0.4; impurity E = about 0.6; impurity D = about 0.8; impurity B = about 0.9.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to impurity D and omeprazole; if necessary, adjust the pH of the mobile phase or the concentration of *acetonitrile R*; an increase in the pH will improve the resolution.

Limits:

— *impurities A, B, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent).

Chloroform and methylene chloride

Head-space gas chromatography (*2.2.28*): use the standard additions method.

Test solution Place 0.50 g of the substance to be examined in a 10 ml vial. Add 4.0 ml of *dimethylacetamide R* and stopper the vial.

Column:
- *material*: fused silica;
- *size:* *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: cross-linked *poly[(cyanopropyl)(phenyl)][dimethyl]siloxane R* (film thickness 1.8 µm).

Carrier gas *nitrogen for chromatography R.*

Static head-space conditions that may be used:
- *equilibration temperature*: 80 °C;
- *equilibration time*: 1 h.

Detection Flame ionisation.

Limits:
- *methylene chloride*: maximum 100 ppm;
- *chloroform*: maximum 50 ppm.

Loss on drying (*2.2.32*)

Maximum 0.2 per cent, determined on 1.000 g by drying under high vacuum at 60 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 1.100 g in a mixture of 10 ml of *water R* and 40 ml of *ethanol (96 per cent) R*. Titrate with *0.5 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.1727 g of $C_{17}H_{19}N_3O_3S$.

STORAGE

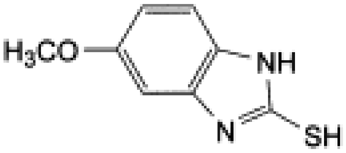
In an airtight container , protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES

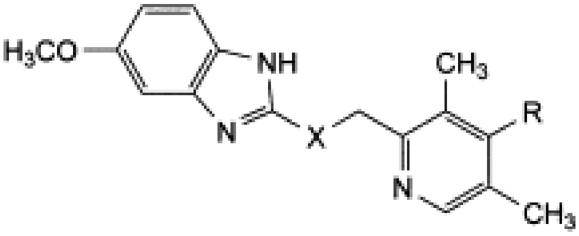
Specified impurities *A, B, C, D, E, F, G*.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances*

for pharmaceutical use): H, I.



A. 5-methoxy-1*H*-benzimidazole-2-thiol,

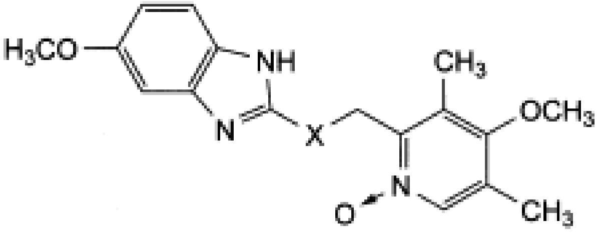


B. R = H, X = SO: 2-[(*RS*)-[(3,5-dimethylpyridin-2-yl)methyl]sulphinyl]-5-methoxy-1*H*-benzimidazole,

C. R = OCH_3, X = S: 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulphanyl]-1*H*-benzimidazole (ufiprazole),

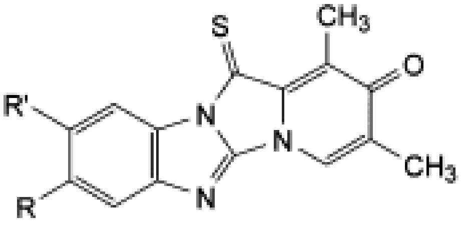
D. R = OCH_3, X = SO_2: 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulphonyl]-1*H*-benzimidazole (omeprazole sulphone),

H. R = Cl, X = SO: 2-[(*RS*)-[(4-chloro-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole,



E. X = SO: 4-methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl)sulphinyl]methyl]-3,5-dimethylpyridine 1-oxide,

I. X = SO_2: 4-methoxy-2-[[(5-methoxy-1*H*-benzimidazol-2-yl)sulphonyl]methyl]-3,5-dimethylpyridine 1-oxide,



F. R = OCH_3, R′ = H: 8-methoxy-1,3-dimethyl-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-a] benzimidazol-2(12*H*)-one,

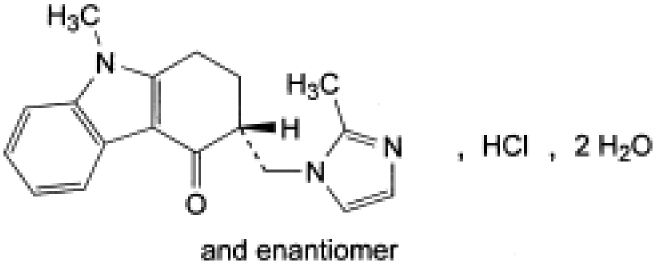
G. R = H, R′ = OCH_3: 9-methoxy-1,3-dimethyl-12-thioxopyrido[1′,2′:3,4]imidazo[1,2-*a*] benzimidazol-2(12*H*)-one.

Ph Eur

Ondansetron Hydrochloride Dihydrate

General Notices

(Ph Eur monograph 2016)



$C_{18}H_{20}ClN_3O,2H_2O$ 365.9 *103639-04-9*

Action and use

Serotonin $5HT_3$ antagonist; treatment of nausea and vomiting.

Preparations

Ondansetron Injection

Ondansetron Tablets

Ph Eur

DEFINITION

(3*RS*)-9-Methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride dihydrate.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Sparingly soluble in water and in alcohol, soluble in methanol, slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ondansetron hydrochloride dihydrate CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Impurity B

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.125 g of the substance to be examined in a mixture of 0.5 volumes of *concentrated ammonia R,* 100 volumes of *alcohol R* and 100 volumes of *methanol R*, and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 12.5 mg of *ondansetron for TLC system suitability CRS* in a mixture of 0.5 volumes of *concentrated ammonia R*, 100 volumes of *alcohol R* and 100 volumes of *methanol R*, and dilute to 1.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1 ml of the test solution to 100 ml with a mixture of 0.5 volumes of *concentrated ammonia R*, 100 volumes of *alcohol R* and 100 volumes of *methanol R*. Dilute 4.0 ml to 10.0 ml with a mixture of 0.5 volumes of *concentrated ammonia R*, 100 volumes of *alcohol R* and 100 volumes of *methanol R*.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *concentrated ammonia R*, *methanol R*, *ethyl acetate R*, *methylene chloride R* (2:40:50:90 *V/V/V/V*).

Application 20 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Order of elution Ondansetron, impurity B, impurity A.

System suitability The chromatogram obtained with reference solution (a) shows 3 clearly separated spots.

Limit:

— *impurity B*: any spot corresponding to impurity B in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.4 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b) Dissolve 90.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 10.0 ml to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *imidazole R* and 10.0 mg of *2-methylimidazole R* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *ondansetron for LC system suitability CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (d) Dissolve 5.0 mg of *ondansetron impurity D CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml to 100.0 ml with the mobile phase.

Reference solution (e) Dissolve 90.0 mg of *ondansetron hydrochloride dihydrate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: spherical *nitrile silica gel for chromatography R* (5 µm) with a specific surface area of 220 m2/g and a pore size of 8 nm.

Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a 2.8 g/l solution of *sodium dihydrogen phosphate monohydrate R* previously adjusted to pH 5.4 with a 40 g/l solution of *sodium hydroxide R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 216 nm.

Injection 20 µl; inject test solution (a) and reference solutions (a), (b), (c) and (d).

Run time 1.5 times the retention time of ondansetron.

Relative retentions With reference to ondansetron (retention time = about 18 min): impurity E = about 0.1; impurity F = about 0.2; impurity C = about 0.4; impurity D = about 0.5; impurity H = about 0.7; impurity A = about 0.8; impurity G = about 0.9.

System suitability:

— *resolution*: minimum of 1.3 between the peak due to impurity E (first peak) and the peak due to impurity F (second peak) in the chromatogram obtained with reference solution (b) and minimum of 2.5 between the peak due to impurity C (first peak) and the peak due to impurity D (second peak) in the chromatogram obtained with reference solution (c).

Limits:

— *correction factor*: for the calculation of contents, multiply the peak area of impurity C by 0.6;

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *impurity E*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.4 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.04 per cent).

Water (*2.5.12*)

9.0 per cent to 10.5 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

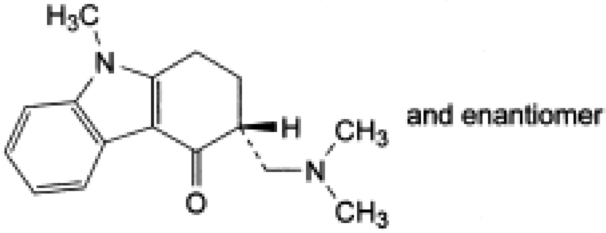
Injection Test solution (b) and reference solution (e).

Calculate the percentage content of $C_{18}H_{20}ClN_3O$.

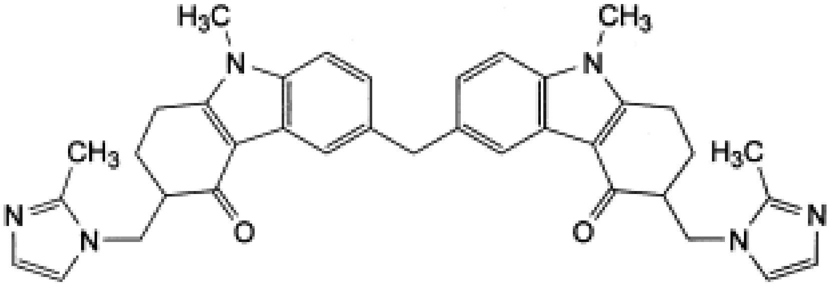
STORAGE

Protected from light.

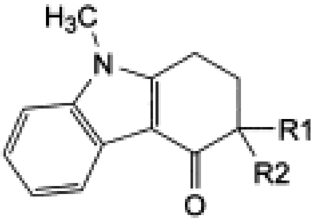
IMPURITIES



A. (3*RS*)-3-[(dimethylamino)methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one,

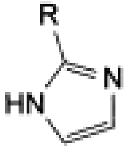


B. 6,6′-methylenebis[(3*RS*)-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one],



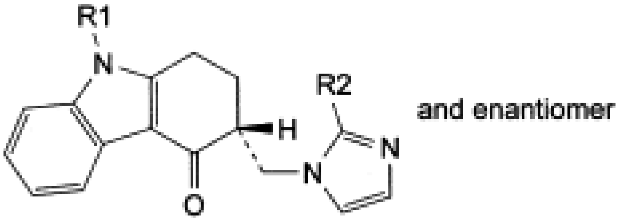
C. R1 = R2 = H: 9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one,

D. R1 + R2 = CH_2: 9-methyl-3-methylene-1,2,3,9-tetrahydro-4*H*-carbazol-4-one,



E. R = H: 1*H*-imidazole,

F. R = CH_3: 2-methyl-1*H*-imidazole,



R1
N
R2
H
N
N
O
and enantiomer

G. R1 = CH_3, R2 = H: (3*RS*)-3-[(1*H*-imidazol-1-yl)methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (*C*-demethylondansetron),

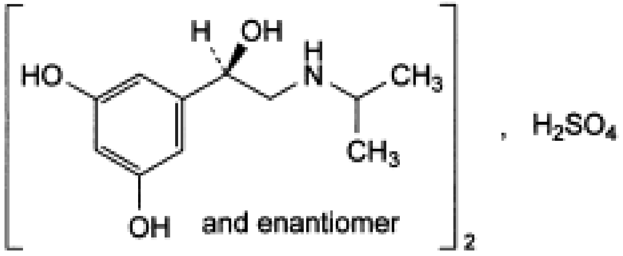
H. R1 = H, R2 = CH_3: (3*RS*)-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (*N*-demethylondansetron).

Ph Eur

Orciprenaline Sulphate

General Notices

(Ph Eur monograph 1033)



$(C_{11}H_{17}NO_3)_2,H_2SO_4$ 520.6 *5874-97-5*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Preparations

Orciprenaline Oral Solution

Orciprenaline Tablets

Ph Eur

DEFINITION

Bis[5-[(1*RS*)-1-hydroxy-2-[(1-methylethyl)amino]ethyl]benzene-1,3-diol] sulphate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in a 0.04 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50.0 ml with the same solution. Dilute 5.0 ml of this solution to 50.0 ml with a 0.04 per cent *V/V* solution of *hydrochloric acid R*.

Spectral range 240-350 nm.

Absorption maximum At 278 nm.

Specific absorbance at the absorption maximum 68.5 to 76.0 (anhydrous substance).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *orciprenaline sulphate CRS*.

If the spectra obtained show differences, dissolve separately, with heating, 50 mg of the substance to be examined and 50 mg of the reference substance, in the minimum volume of *water R*. Add 10 ml of *acetone R* and centrifuge. Dry the precipitates at 40 °C under reduced pressure for 3 h and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *orciprenaline sulphate CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *orciprenaline sulphate CRS* and 10 mg of *salbutamol CRS* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *ammonia R, water R, aldehyde-free methanol R* (1.5:10:90 *V/V/V*).

Application 2 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a 10 g/l solution of *potassium permanganate R*.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 20 mg in 2 ml of *ethanol (96 per cent) R*. Add 2 ml of a 1 g/l solution of *dichloroquinonechlorimide R* in *ethanol (96 per cent) R* and 1 ml of *sodium carbonate solution R*. A violet colour is produced, turning to brown.

E. It gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.0 to 5.5 for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 2 mg of *orciprenaline for system suitability CRS* (containing impurities A and B) in 2.0 ml of the mobile phase.

Column:

— *size: l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase: spherical end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 45 °C.

Mobile phase Dissolve 9.1 g of *potassium dihydrogen phosphate R* and 4.6 g of *sodium octanesulphonate R* in *water R*, adjust to pH 4.0 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R*. Add 140 ml of *acetonitrile R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µl.

Run time Twice the retention time of orciprenaline.

Identification of impurities Use the chromatogram supplied with *orciprenaline for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to orciprenaline (retention time = about 7 min): impurity A = about 0.9; impurity B = about 1.3.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and orciprenaline.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 0.3;

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the

chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Phenone

Maximum 0.1 per cent.

Dissolve 0.50 g in a 0.04 per cent *V/V* solution of *hydrochloric acid R* and dilute to 25.0 ml with the same solution. The absorbance *(2.2.25)* of the solution measured at 328 nm is not greater than 0.16.

Iron *(2.4.9)*

Maximum 20 ppm.

The residue obtained in the test for sulphated ash complies with the test. Prepare the reference solution using *iron standard solution (2 ppm Fe) R*.

Heavy metals

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 5 ml of *anhydrous formic acid R* and add 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* using 0.1 ml of *crystal violet solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 52.06 mg of $C_{22}H_{36}N_2O_{10}S$.

STORAGE

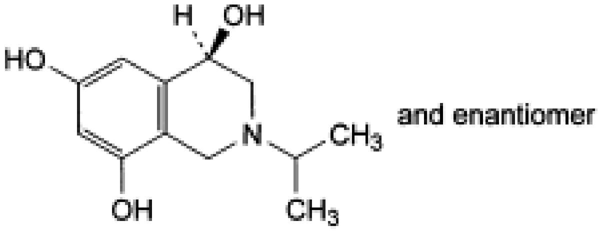
In an airtight container , protected from light.

IMPURITIES

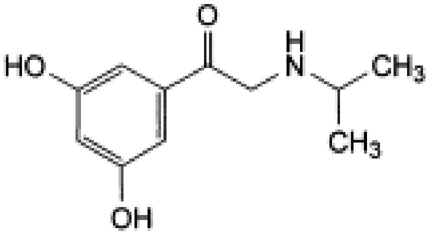
Specified impurities A, B.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these

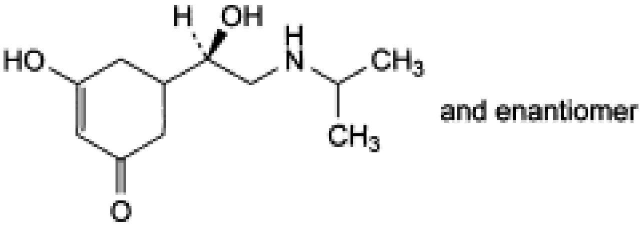
impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C*.


H
OH
HO
N
CH3
OH
CH3
and enantiomer

A. (4*RS*)-2-(1-methylethyl)-1,2,3,4-tetrahydroisoquinoline-4,6,8-triol,


O
HO
H
N
CH3
CH3
OH

B. 1-(3,5-dihydroxyphenyl)-2-[(1-methylethyl)amino]ethanone,


H
OH
HO
H
N
CH3
CH3
O
and enantiomer

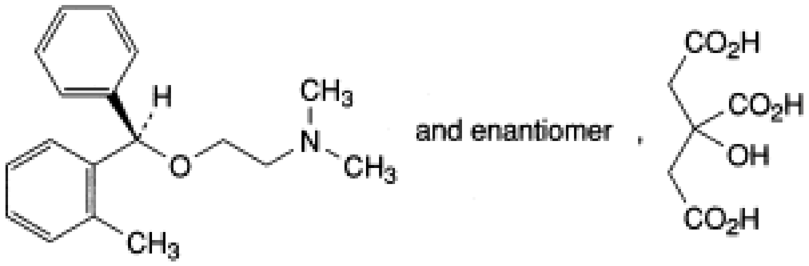
C. 3-hydroxy-5-[(1*RS*)-1-hydroxy-2-[(1-methylethyl)amino]ethyl]cyclohex-2-enone.

Ph Eur

Orphenadrine Citrate

General Notices

(Ph Eur monograph 1759)



$C_{18}H_{23}NO,C_6H_8O_7$ 461.5 *4682-36-4*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

(*RS*)-*N*,*N*-Dimethyl-2-[(2-methylphenyl)phenylmethoxy]ethanamine dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, slightly soluble in alcohol.

mp

About 137 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *orphenadrine citrate CRS.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 436 nm has a maximum of 0.050.

Dissolve 1.0 g in a 3.6 per cent *V/V* solution of *hydrochloric acid R* in *alcohol R* and dilute to 10.0 ml with the same acid solution.

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.500 g of the substance to be examined in *water R* and dilute to 50 ml with the same solvent. Add 2 ml of *concentrated ammonia R* and shake with 3 quantities, each of 10 ml, of *toluene R*. To the combined upper layers add anhydrous sodium sulphate R, shake, filter and evaporate the filtrate, at a temperature not exceeding 50 °C, using a rotary evaporator. Take up the residue with *toluene R* and dilute to 20.0 ml with the same solvent.

Reference solution Dissolve 30 mg of *orphenadrine citrate CRS* and 30 mg of *orphenadrine impurity E CRS* in 20 ml of *water R*. Add 1 ml of *concentrated ammonia R* and shake with 3 quantities, each of 5 ml, of *toluene R*. To the combined upper layers add *anhydrous sodium sulphate R*, shake, filter and evaporate the filtrate, at a temperature not exceeding 50 °C, using a rotary evaporator. Take up the residue with *toluene R* and dilute to 20.0 ml with the same solvent.

Column:
- *size:* *l* = 60 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 ml/min.

Split ratio 1:25.

Temperature:
- *column*: 240 °C;
- *injection port and detector*: 290 °C.

Detection Flame ionisation.

Injection 2 µl.

Run time 1.3 times the retention time of orphenadrine.

System suitability Reference solution:

- *resolution*: minimum of 1.5 between the peaks due to orphenadrine and to impurity E.

Limits:
- *any impurity*: maximum 0.3 per cent;
- *total*: maximum 1.0 per cent;
- *disregard limit*: 0.02 per cent.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

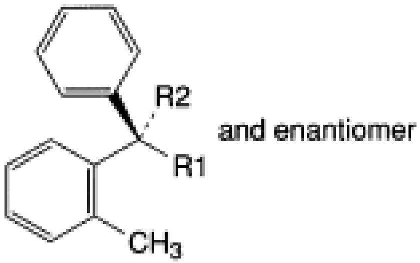
Dissolve 0.350 g in 50 ml of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 46.15 mg of $C_{24}H_{31}NO_8$.

STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container , protected from light.

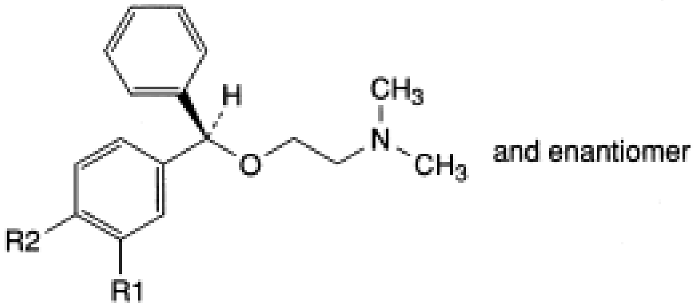
IMPURITIES



A. R1 = OH, R2 = H: (*RS*)-(2-methylphenyl)phenylmethanol (2-methylbenzhydrol),

B. R1 + R2 = O: (2-methylphenyl)phenylmethanone (2-methylbenzophenone),

C. R1 = O-CH_2-CH_2-NH_2, R2 = H: (*RS*)-2-[(2-methylphenyl)phenylmethoxy]ethanamine,



D. R1 = R2 = H: diphenhydramine,

E. R1 = CH_3, R2 = H: (*RS*)-*N*,*N*-dimethyl-2-[(3-methylphenyl)phenylmethoxy]ethanamine (*meta*-methylbenzyl isomer),

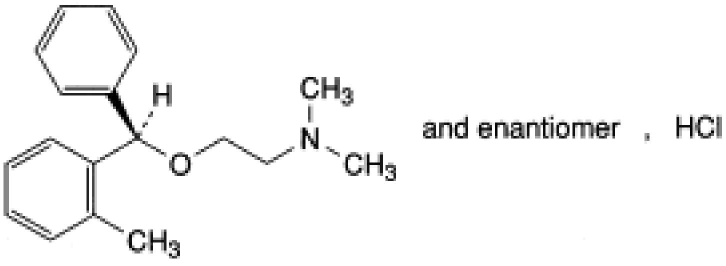
F. R1 = H, R2 = CH_3: (*RS*)-*N*,*N*-dimethyl-2-[(4-methylphenyl)phenylmethoxy]ethanamine (*para*-methylbenzyl isomer).

Ph Eur

Orphenadrine Hydrochloride

General Notices

(Ph Eur monograph 1760)



$C_{18}H_{23}NO,HCl$ 305.9 *341-69-5*

Action and use

Anticholinergic.

Preparation

Orphenadrine Hydrochloride Tablets

Ph Eur

DEFINITION

(*RS*)-*N*,*N*-Dimethyl-2-[(2-methylphenyl)phenylmethoxy]ethanamine hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in alcohol.

mp

About 160 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *orphenadrine hydrochloride CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 436 nm has a maximum of 0.050.

Dissolve 0.70 g in *alcohol R* and dilute to 10.0 ml with the same solvent.

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.300 g of the substance to be examined in *water R* and dilute to 50 ml with the same solvent. Add 2 ml of *concentrated ammonia R* and shake with 3 quantities, each of 10 ml, of *toluene R*. To the combined upper layers add *anhydrous sodium sulphate R*, shake, filter and evaporate the filtrate, at a temperature not exceeding 50 °C, using a rotary evaporator. Take up the residue with *toluene R* and dilute to 20.0 ml with the same solvent.

Reference solution Dissolve 20 mg of *orphenadrine hydrochloride CRS* and 20 mg of *orphenadrine impurity E CRS* in 20 ml of *water R*. Add 1 ml of *concentrated ammonia R* and shake with 3 quantities, each of 5 ml, of *toluene R*. To the combined upper layers add *anhydrous sodium sulphate R*, shake, filter and evaporate the filtrate, at a temperature not exceeding 50 °C, using a rotary evaporator. Take up the residue with *toluene R* and dilute to 20.0 ml with the same solvent.

Column:

— *size:* l = 60 m, Ø = 0.32 mm;

— *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 ml/min.

Split ratio 1:25.

Temperature:

— *column*: 240 °C;

— *injection port and detector*: 290 °C.

Detection Flame ionisation.

Injection 2 µl.

Run time 1.3 times the retention time of orphenadrine.

System suitability Reference solution:

— *resolution*: minimum of 1.5 between the peaks due to orphenadrine and to impurity E.

Limits:

— *any impurity*: maximum 0.3 per cent;

— *total*: maximum 1.0 per cent;

— *disregard limit*: 0.02 per cent.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

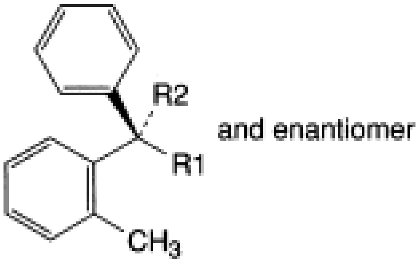
Dissolve 0.250 g in 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 30.59 mg of $C_{18}H_{24}ClNO$.

STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container , protected from light.

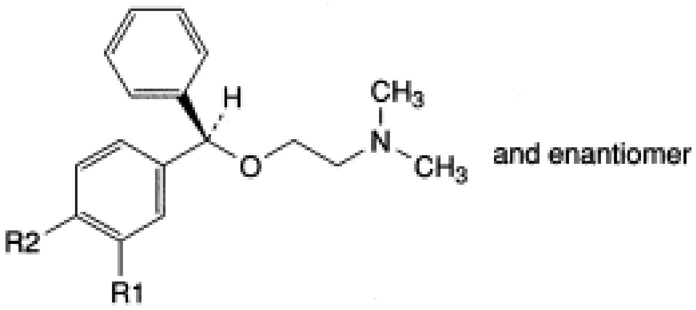
IMPURITIES



A. R1 = OH, R2 = H: (*RS*)-(2-methylphenyl)phenylmethanol (2-methylbenzhydrol),

B. R1+ R2 = O: (2-methylphenyl)phenylmethanone (2-methylbenzophenone),

C. R1 = O-CH_2-CH_2-NH_2, R2 = H: (*RS*)-2-[(2-methylphenyl)phenylmethoxy]ethanamine,


H
CH3
N
CH3
O
R2
R1
and enantiomer

D. R1 = R2 = H: diphenhydramine,

E. R1 = CH_3, R2 = H: (*RS*)-*N*,*N*-dimethyl-2-[(3-methylphenyl)phenylmethoxy]ethanamine (*meta*-methylbenzyl isomer),

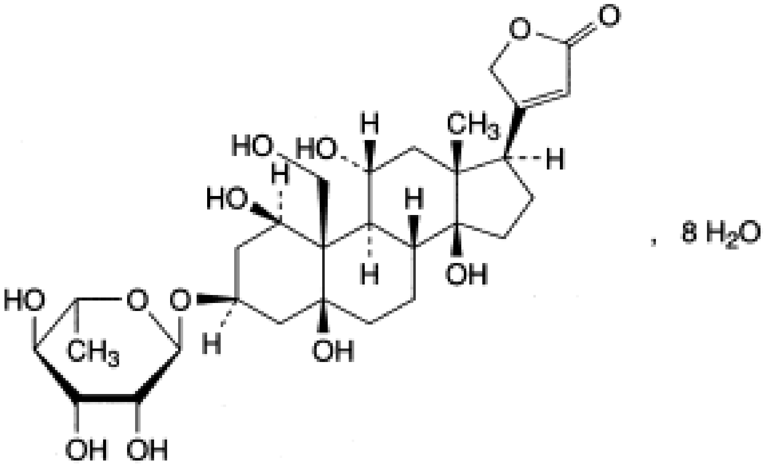
F. R1 = H, R2 = CH_3: (*RS*)-*N*,*N*-dimethyl-2-[(4-methylphenyl)phenylmethoxy]ethanamine (*para*-methylbenzyl isomer).

Ph Eur

Ouabain

General Notices

(Ph Eur monograph 0048)



$C_{29}H_{44}O_{12},8H_2O$ 729 *11018-89-6*

Ph Eur

DEFINITION

Ouabain contains not less than 96.0 per cent and not more than the equivalent of 104.0 per cent of 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-1β,5,11α,14,19-pentahydroxy-5β,14β-card-20(22)-enolide, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, sparingly soluble in water and in ethanol, practically insoluble in ethyl acetate.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the spot in the chromatogram obtained with reference solution (a).

B. Dissolve 2 mg to 3 mg in 2 ml of *sulphuric acid R*; a pink colour develops which quickly changes to red. The solution shows green fluorescence in ultraviolet light.

C. Dissolve about 1 mg in 1 ml of *dinitrobenzene solution R* and add 0.2 ml of *dilute sodium hydroxide solution R*. An intense blue colour develops.

D. Dissolve 0.1 g in 5 ml of a 150 g/l solution of *sulphuric acid R* and boil for a few minutes.

The solution becomes yellow and turbid. Filter and add to the filtrate 5 ml of a 120 g/l solution of *sodium hydroxide R* and 3 ml of *cupri-tartaric solution R*. Heat. A red precipitate is formed.

TESTS

Solution S

Dissolve 0.20 g in 15 ml of *water R*, heating on a water-bath. Allow to cool and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

- 30 to - 33, determined on solution S and calculated with reference to the anhydrous substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution Dissolve a quantity of the substance to be examined corresponding to 20 mg of the anhydrous substance in 1.0 ml of a mixture of 32 volumes of *water R*, 100 volumes of *chloroform R* and 100 volumes of *methanol R*.

Reference solution (a) Dissolve a quantity of *ouabain CRS* corresponding to 20 mg of the anhydrous substance in 1.0 ml of a mixture of 32 volumes of *water R*, 100 volumes of *chloroform R* and 100 volumes of *methanol R*.

Reference solution (b) Dissolve a quantity of *ouabain CRS* corresponding to 10 mg of the anhydrous substance in a mixture of 32 volumes of *water R*, 100 volumes of *chloroform R*, 100 volumes of *methanol R* and dilute to 25 ml with the same mixture of solvents.

Reference solution (c) Dilute 2.5 ml of reference solution (b) to 10 ml with a mixture of 32 volumes of *water R*, 100 volumes of *chloroform R* and 100 volumes of *methanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 13 cm using a homogeneous mixture of 4 volumes of *water R*, 15 volumes of *methanol R*, 15 volumes of *dimethyl sulphoxide R* and 70 volumes of *chloroform R*. Dry the plate immediately at 140 °C for 30 min in a ventilated drying oven. Allow to cool, spray with *alcoholic sulphuric acid solution R* and heat at 140 °C for 15 min. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (2.0 per cent). The test is not valid unless the principal spot in the chromatogram obtained with reference solution (a) and the principal spot in the chromatogram obtained with the test solution migrate over a distance sufficient to give unequivocal separation of the secondary spots and the spot in the chromatogram obtained with reference solution (c) is clearly visible.

Alkaloids and strophanthin-K

To 5.0 ml of solution S add 0.5 ml of a 100 g/l solution of *tannic acid R*. No precipitate is formed.

Water *(2.5.12)*

18.0 per cent to 22.0 per cent, determined on 0.100 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 40.0 mg in *alcohol R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *alcohol R*. Prepare a reference solution in the same manner using 40.0 mg of *ouabain CRS*. To 5.0 ml of each solution add 3.0 ml of *alkaline sodium picrate solution R*, allow to stand protected from bright light for 30 min and measure the absorbance (*2.2.25*) of each solution at the maximum at 495 nm using as the compensation liquid a mixture of 5.0 ml of *alcohol R* and 3.0 ml of *alkaline sodium picrate solution R* prepared at the same time.

Calculate the content of $C_{29}H_{44}O_{12}$ from the absorbances measured and the concentrations of the solutions.

STORAGE

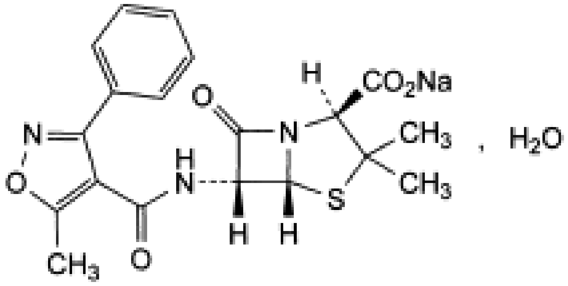
Store protected from light.

Ph Eur

Oxacillin Sodium Monohydrate

General Notices

(Ph Eur monograph 2260)



$C_{19}H_{18}N_3NaO_5S,H_2O$ 441.4   *7240-38-2*

Action and use

Penicillin antibacterial.

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-3,3-dimethyl-6-[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   oxacillin sodium monohydrate CRS.

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and its absorbance *(2.2.25)* at 430 nm is not greater than 0.10.

Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent.

pH *(2.2.3)*

4.5 to 7.5.

Dissolve 0.30 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 196 to + 212 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *oxacillin sodium monohydrate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of test solution (b) to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 5 mg of *cloxacillin sodium CRS* (impurity E) and 5 mg of *oxacillin sodium monohydrate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (d) In order to prepare impurities B and D *in situ*, dissolve 25 mg of the substance to be examined in 1 ml of *0.05 M sodium hydroxide*, allow to stand for 3 min, then dilute to 100 ml with the mobile phase. Inject immediately.

Reference solution (e) Dissolve 5 mg of *oxacillin for peak identification CRS* (containing impurities E, F, G, I and J) in 5 ml of the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of *acetonitrile R* and 75 volumes of a 2.7 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 5.0 with *dilute sodium hydroxide solution R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl of test solution (a) and reference solutions (b), (c), (d) and (e).

Run time 7 times the retention time of oxacillin.

Identification of impurities:

- — in the chromatogram obtained with reference solution (d), the 2 principal peaks eluting before the main peak are due to impurities B and D respectively;
- — use the chromatogram supplied with *oxacillin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities E, F, G, I and J.

Relative retention With reference to oxacillin (retention time = about 5 min): impurity A = about 0.3; impurity B (isomer 1) = about 0.4; impurity B (isomer 2) = about 0.5; impurity C = about 0.65; impurity D (2 isomers) = about 0.9; impurity E = about 1.5; impurity F = about 1.9; impurity G = about 2.1; impurity H = about 3.5; impurity I = about 3.8; impurity J = about 5.8.

System suitability:

- — *resolution*: minimum 2.5 between the peaks due to oxacillin and impurity E in the chromatogram obtained with reference solution (c);
- — the chromatogram obtained with reference solution (e) is similar to the chromatogram supplied with *oxacillin for peak identification CRS*.

Limits:

- — *impurity B*: for the sum of the areas of the 2 isomer peaks, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
- — *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- — *impurities D (sum of the 2 isomers), F, G, I, J*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- — *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- — *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);
- — *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethyl acetate and butyl acetate

Head-space gas chromatography *(2.2.28)*.

Test solution Dissolve 0.200 g of the substance to be examined in 6.0 ml of *water R*.

Reference solution Dissolve 83 mg of *ethyl acetate R* and 83 mg of *butyl acetate R* in *water R* and dilute to 250.0 ml with the same solvent. Use 6.0 ml of this solution.

Close the vials immediately with a rubber membrane stopper coated with polytetrafluoroethylene and secured with an aluminium crimped cap. Mix to obtain a homogeneous solution.

Column:

- — *material*: fused silica;

— *size: l* = 50 m, Ø = 0.32 mm;

— *stationary phase: poly(dimethyl)siloxane R* (film thickness 5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 2 ml/min.

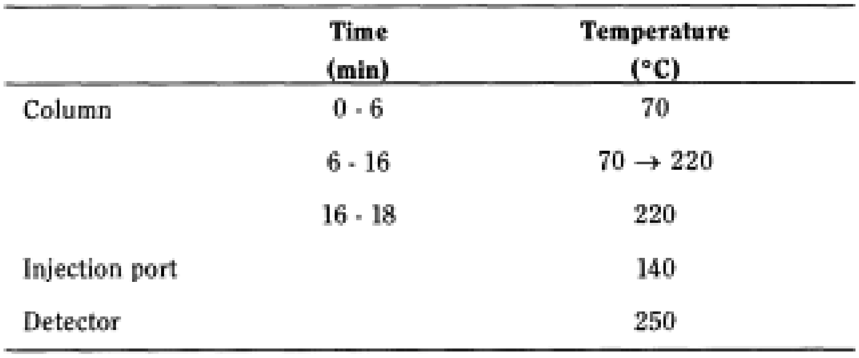
Static head-space conditions that may be used:
— *equilibration temperature*: 80 °C;

— *equilibration time*: 60 min;

— *transfer-line temperature*: 140 °C;

— *pressurisation time*: 30 s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	70
	6 - 16	70 → 220
	16 - 18	220
Injection port		140
Detector		250

Detection Flame ionisation.

Retention time Ethyl acetate = about 10 min; butyl acetate = about 15.5 min.

Limits:
— *ethyl acetate*: maximum 1.0 per cent,

— *butyl acetate*: maximum 1.0 per cent.

N,N*-Dimethylaniline *(2.4.26, Method B)

Maximum 20 ppm.

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.8 per cent.

Water *(2.5.12)*

3.5 per cent to 5.0 per cent, determined on 0.300 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.20 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

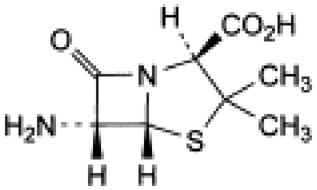
Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{19}H_{18}N_3NaO_5S$ from the declared content of *oxacillin sodium monohydrate CRS*.

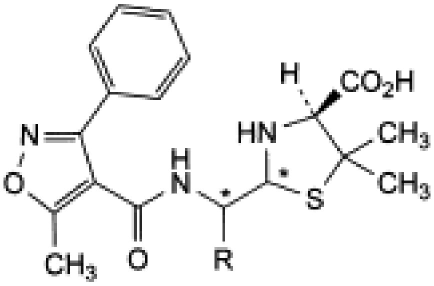
IMPURITIES

Specified impurities B, D, E, F, G, I, J.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, C, H.*

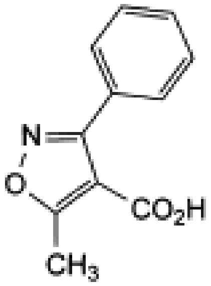


A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



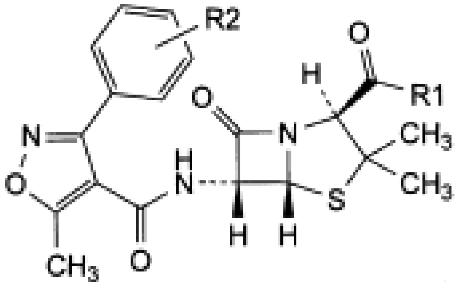
B. R = CO_2H: (4*S*)-2-[carboxy[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of oxacillin),

D. R = H: (2*RS*,4*S*)-5,5-dimethyl-2-[[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]methyl] thiazolidine-4-carboxylic acid (penilloic acids of oxacillin),



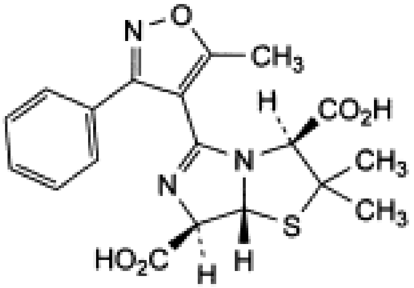
C. 5-methyl-3-phenylisoxazole-4-carboxylic acid,

E. cloxacillin,

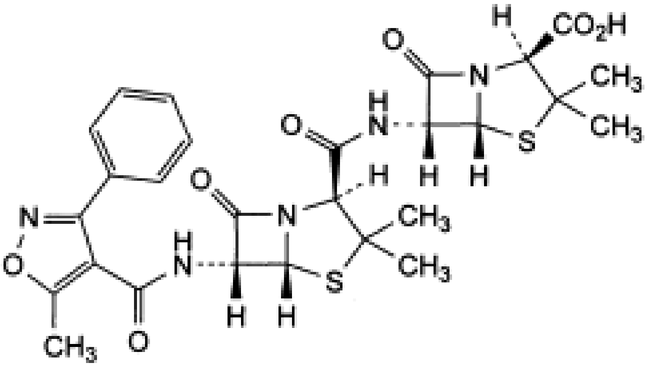

R2
O
H
R1
O
N
CH_3
N
O
H
N
CH_3
S
H
H
CH_3
O

F. R1 = SH, R2 = H: (2*R*,5*R*,6*R*)-3,3-dimethyl-6-[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbothioic acid (thiooxacillin),

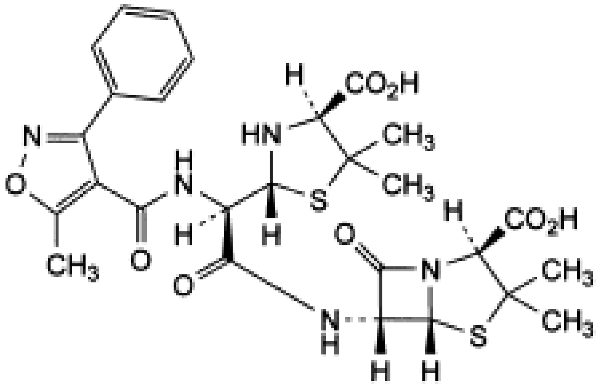
G. R1 = OH, R2 = Cl: (2*S*,5*R*,6*R*)-6-[[[3-(chlorophenyl)-5-methylisoxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (cloxacillin isomer),


N
O
CH_3
H
CO_2H
N
CH_3
N
CH_3
S
HO_2C
H
H

H. (3S,7R,7aR)-2,2-dimethyl-5-(5-methyl-3-phenylisoxazol-4-yl)-2,3,7,7a-tetrahydroimidazo[5,1-b]thiazole-3,7-dicarboxylic acid (penillic acid of oxacillin),


H
CO_2H
O
N
CH_3
H
O
N
S
CH_3
H
H
H
O
N
CH_3
N
H
O
N
S
CH_3
H
H
CH_3
O

I. (2*S*,5*R*,6*R*)-6-[[(2*S*,5*R*,6*R*)-3,3-dimethyl-6-[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA oxacillin amide),



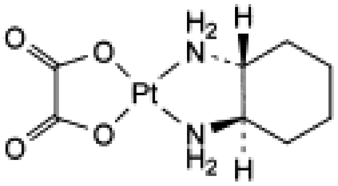
J. (2*S*,5*R*,6*R*)-6-[[(2*R*)-[(2*R*,4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl][[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid (ozolamide of 6-APA dimer).

Ph Eur

Oxaliplatin

General Notices

(Ph Eur monograph 2017)



$C_8H_{14}N_2O_4Pt$ 397.3 *61825-94-3*

Action and use

Platinum-containing cytotoxic.

Ph Eur

DEFINITION

(*SP*-4-2)-[(1*R*,2*R*)-Cyclohexane-1,2-diamine-κ*N*,κ*N′*] [ethanedioato(2-)-κO^1,κO^2]platinum.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, very slightly soluble in methanol, practically insoluble in ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison oxaliplatin CRS.

B. It complies with the test for specific optical rotation (see Tests).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.10 g in *water R* and dilute to 50 ml with the same solvent.

Acidity

Dissolve 0.10 g in *carbon dioxide-free water R*, dilute to 50 ml with the same solvent and add 0.5 ml of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.60 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation *(2.2.7)*

+ 74.5 to + 78.0 (dried substance).

Dissolve 0.250 g in *water R* and dilute to 50.0 ml with the same solvent.

Related substances

A. Impurity A. Liquid chromatography *(2.2.29)*. *Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the test solution within 20 min of preparation.*

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 14.0 mg of *oxalic acid R* (impurity A) in *water R* and dilute to 250.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 200.0 ml with *water R*.

Reference solution (c) Dissolve 12.5 mg of *sodium nitrate R* in *water R* and dilute to 250.0 ml with the same solvent. Dilute a mixture of 2.0 ml of this solution and 25.0 ml of reference solution (a) to 100.0 ml with *water R*.

Column:

— *size:* l = 25 cm, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Temperature 40 °C.

Mobile phase Mix 20 volumes of *acetonitrile R* with 80 volumes of a solution prepared as follows: to 10 ml of a 320 g/l solution of *tetrabutylammonium hydroxide R* add 1.36 g of *potassium dihydrogen phosphate R* and dilute to 1000 ml with *water R*; adjust this solution to pH 6.0 with *phosphoric acid R*.

Flow rate 2 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl; inject the test solution and reference solutions (b) and (c).

Run time Twice the retention time of impurity A.

Retention times Nitrate = about 2.7 min; impurity A = about 4.7 min.

System suitability:

— *resolution*: minimum 9 between the peaks due to nitrate and impurity A in the chromatogram obtained with reference solution (c);

— *signal-to-noise ratio*: minimum of 10 for the peak due to impurity A in the chromatogram obtained with reference solution (b).

Limits:

- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

B. Impurity B. Liquid chromatography (*2.2.29*). *Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the test solution within 20 min of preparation. Use suitable polypropylene containers for the preparation and injection of all solutions. Glass pipettes may be used for diluting solutions.*

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 12.5 mg of *oxaliplatin impurity B CRS* in 63 ml of *methanol R* and dilute to 250.0 ml with *water R*. Dilute 3.0 ml to 200.0 ml with *water R.*

Reference solution (b) In order to prepare in situ the degradation compound (impurity E) dissolve 12.5 mg of *oxaliplatin impurity B CRS* in 63 ml of *methanol R* and dilute to 250 ml with *water R*. Adjust to pH 6.0 with a 0.2 g/l solution of *sodium hydroxide R*. Heat for 4 h at 70 °C and allow to cool.

Column:

- *size:* l = 25 cm, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Temperature 40 °C.

Mobile phase Mix 20 volumes of *acetonitrile R* with 80 volumes of a solution prepared as follows: dissolve 1.36 g of *potassium dihydrogen phosphate R* and 1 g of *sodium heptanesulphonate R* in 1000 ml of *water R*; adjust this solution to pH 3.0 ± 0.05 with *phosphoric acid R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 2.5 times the retention time of impurity B.

Retention times Impurity B = about 4.3 min; impurity E = about 6.4 min.

System suitability:

- *resolution*: minimum 7 between the peaks due to impurity B and impurity E in the chromatogram obtained with reference solution (b);
- *signal-to-noise ratio*: minimum of 10 for the peak due to impurity B in the chromatogram obtained with reference solution (a).

Limits:

- *impurity B*: not more than 3.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

C. Impurity C and other related substances. Liquid chromatography (*2.2.29*). *Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the test solution within 20 min of preparation.*

Test solution (a) Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 500.0 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *oxaliplatin impurity C CRS* and 10 mg of *oxaliplatin CRS* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water R.*

Reference solution (c) Dissolve 5 mg of *dichlorodiaminocyclohexaneplatinum CRS* in *methanol R* and dilute to 50.0 ml with the same solvent. To 10.0 ml of this solution add 10.0 ml of reference solution (a) and dilute to 100.0 ml with *water R*.

Reference solution (d) Dissolve 50.0 mg of *oxaliplatin CRS* in *water R* and dilute to 500.0 ml with the same solvent.

Reference solution (e) Dissolve 5.0 mg of *dichlorodiaminocyclohexaneplatinum CRS* in reference solution (d) and dilute to 50.0 ml with the same solvent.

Reference solution (f) To 0.100 g of the substance to be examined add 1.0 ml of reference solution (a) and dilute to 50.0 ml with *water R*.

Column:

— *size:* l = 25 cm, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Temperature 40 °C.

Mobile phase Mixture of solutions A and B (99:1 *V/V*).

— *solution A*: dilute 0.6 ml of *dilute phosphoric acid R* in 1000 ml of *water R* and adjust to pH 3.0 with either *sodium hydroxide solution R* or *phosphoric acid R*;

— *solution B*: *acetonitrile R*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl; inject test solution (a) and reference solutions (b), (c) and (f).

Run time 3 times the retention time of oxaliplatin.

Retention times Impurity C = about 4.4 min; dichlorodiaminocyclohexaneplatinum = about 6.9 min; oxaliplatin = about 8.0 min.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to dichlorodiaminocyclohexaneplatinum and oxaliplatin in the chromatogram obtained with reference solution (c);

— *signal-to-noise ratio*: minimum 50 for the peak due to impurity C and minimum 10 for the peak due to oxaliplatin in the chromatogram obtained with reference solution (b).

Limits:

— *impurity C:* not more than half the area of the peak due to impurity C in the chromatogram obtained with reference solution (f) (0.1 per cent);

— *any other impurity*: not more than twice the area of the peak due to oxaliplatin in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total of other impurities*: not more than twice the area of the peak due to oxaliplatin in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *disregard limit*: the area of the peak due to oxaliplatin in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak with a retention time less than 2 min.

D. *Total of impurities*: the sum of impurities A, B, C and other related impurities is not greater than 0.30 per cent.

Impurity D

Liquid chromatography (*2.2.29*).

Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *oxaliplatin impurity D CRS* in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 15.0 ml of reference solution (a) to 50.0 ml with *methanol R*.

Reference solution (c) Dissolve 150.0 mg of *oxaliplatin CRS* in *methanol R* and dilute to 200.0 ml with the same solvent.

Reference solution (d) Dilute 5.0 ml of reference solution (c) to 100.0 ml with *methanol R*.

Reference solution (e) To 40 ml of reference solution (c) add 1.0 ml of reference solution (b) and dilute to 50.0 ml with *methanol R*.

Reference solution (f) Mix 4.0 ml of reference solution (a) and 5.0 ml of reference solution (d) and dilute to 50.0 ml with *methanol R*.

Column:

— *size:* *l* = 25 cm, Ø = 4.6 mm;

— *stationary phase*: *silica gel OC for chiral separations R*.

Temperature 40 °C.

Mobile phase *ethanol R*, *methanol R* (3:7 *V/V*).

Flow rate 0.3 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl; inject the test solution and reference solutions (e) and (f).

Run time Twice the retention time of oxaliplatin.

Retention times Oxaliplatin = about 14 min; impurity D = about 16 min.

System suitability:

— *resolution*: minimum 1.5 between the peaks due to oxaliplatin and impurity D in the chromatogram obtained with reference solution (f);

— *signal-to-noise ratio*: minimum 10 for the peak due to impurity D in the chromatogram obtained with reference solution (e).

Limits:

— *impurity D*: not more than twice the peak height of the corresponding peak in the chromatogram obtained with reference solution (e) (0.1 per cent).

Silver

Maximum 5.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Dissolve 0.1000 g of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent. Dilute 20 µl of this solution to 40 µl with *0.5 M nitric acid*.

Reference solution (a) Dilute a solution of *silver nitrate R* containing 1000 ppm of silver in *0.5 M nitric acid* with *0.5 M nitric acid* to obtain a solution which contains 10 ppb of silver.

Reference solution (b) Mix 20 µl of the test solution and 8 µl of reference solution (a) and dilute to 40 µl with *0.5 M nitric acid*.

Reference solution (c) Mix 20 µl of the test solution and 16 µl of reference solution (a) and dilute to 40 µl with *0.5 M nitric acid*.

Source Silver hollow-cathode lamp.

Wavelength 328.1 nm.

Atomisation device Furnace.

Measure the absorbance of the test solution and reference solutions (b) and (c).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Bacterial endotoxins (*2.6.14*)

Less than 1.0 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for impurity C and other related substances with the following modifications.

Injection 20 µl; inject test solution (b) and reference solutions (d) and (e).

System suitability:

— *resolution*: minimum 2.0 between the peaks due to dichlorodiaminocyclohexaneplatinum and oxaliplatin in the chromatogram obtained with reference solution (e);

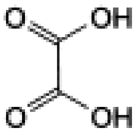
— *repeatability*: reference solution (d).

Calculate the percentage content of oxaliplatin using the chromatogram obtained with reference solution (d).

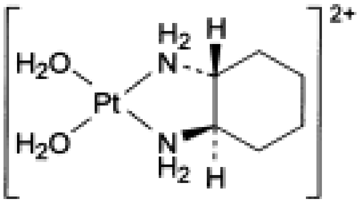
IMPURITIES

Specified impurities *A, B, C, D.*

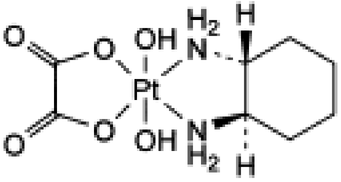
Other detectable impurities *E.*



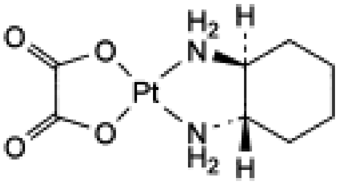
A. ethanedioic acid (oxalic acid),



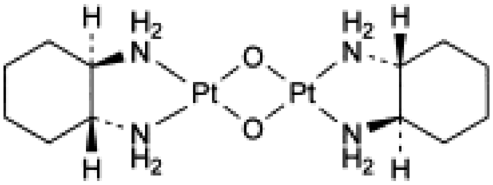
B. (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-κ*N*,κ*N*′]platinum (diaquodiaminocyclohexaneplatinum),



C. (*OC*-6-33)-[(1*R*,2*R*)-cyclohexane-1,2-diamine-κ*N*,κ*N*′][ethanedioato(2-)-κO^1,κO^2] dihydroxyplatinum,



D. (*SP*-4-2)-[(1*S*,2*S*)-cyclohexane-1,2-diamine-κ*N*,κ*N*′][ethanedioato(2-)-κO^1,κO^2]platinum (*S*,*S*-enantiomer of oxaliplatin),



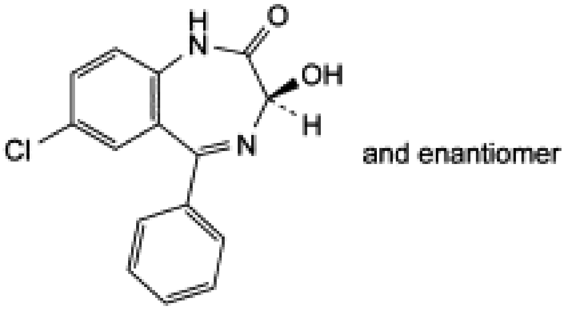
E. (*SP*-4-2)-di-μ-oxobis[(1*R*,2*R*)-cyclohexane-1,2-diamine-κ*N*,κ*N*′]diplatinum (diaquodiaminocyclohexaneplatinum dimer).

Ph Eur

Oxazepam

General Notices

(Ph Eur monograph 0778)



$C_{15}H_{11}ClN_2O_2$ 286.7 *604-75-1*

Action and use

Benzodiazepine.

Preparation

Oxazepam Tablets

Ph Eur

DEFINITION

(3*RS*)-7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison oxazepam CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 40.0 mg of the substance to be examined in 25 ml of a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*. Dilute 2.0 ml of this solution to 10.0 ml with a mixture of equal volumes of *acetonitrile R* and *water R*.

Reference solution (b) Dissolve the contents of a vial of *oxazepam for peak identification CRS* (containing impurities A, B, C, D and E) in 1.0 ml of the test solution.

Column:

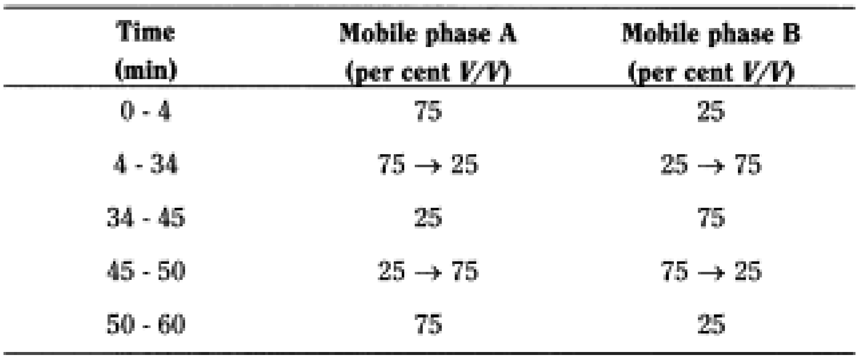
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) resistant to bases up to pH 11.

Mobile phase:

— *mobile phase A*: dissolve 3.48 g of *dipotassium hydrogen phosphate R* in 900 ml of *water R*, adjust to pH 10.5 with a 40 g/l solution of *sodium hydroxide R* and dilute to 1000 ml with *water R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	75	25
4 - 34	75 → 25	25 → 75
34 - 45	25	75
45 - 50	25 → 75	75 → 25
50 - 60	75	25

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram obtained with reference solution (b) and the chromatogram supplied with *oxazepam for peak identification CRS* to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to oxazepam (retention time = about 15 min): impurity E = about 0.7; impurity A = about 0.8; impurity B = about 1.2; impurity C = about 1.4; impurity D = about 2.0.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurities E and A.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 4.0; impurity B = 1.1;

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 10 ml of *anhydrous acetic acid R* and 90 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

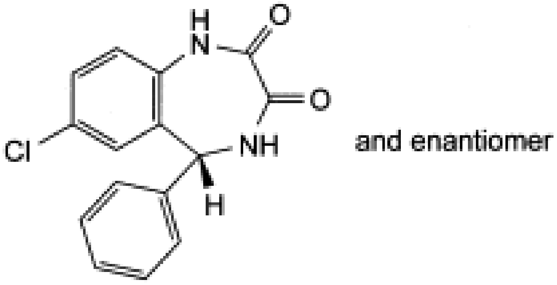
1 ml of *0.1 M perchloric acid* is equivalent to 28.67 mg of $C_{15}H_{11}ClN_2O_2$.

STORAGE

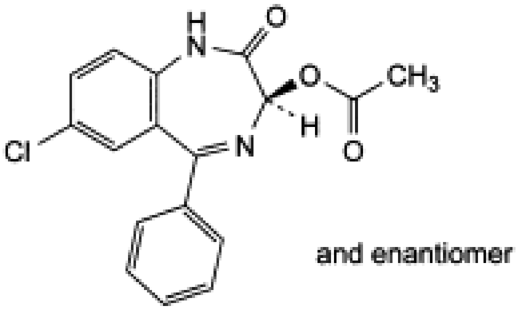
Protected from light.

IMPURITIES

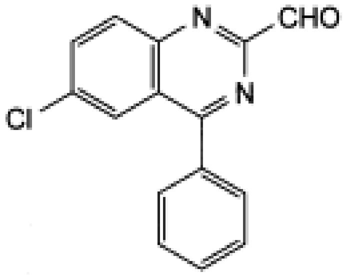
Specified impurities *A, B, C, D, E*.



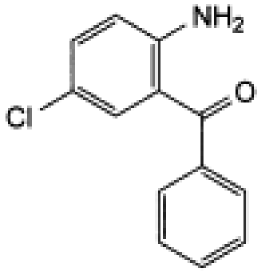
A. (5*RS*)-7-chloro-5-phenyl-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione,



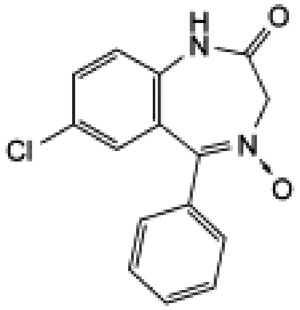
B. (3*RS*)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl acetate,



C. 6-chloro-4-phenylquinazoline-2-carbaldehyde,



D. (2-amino-5-chlorophenyl)phenylmethanone,



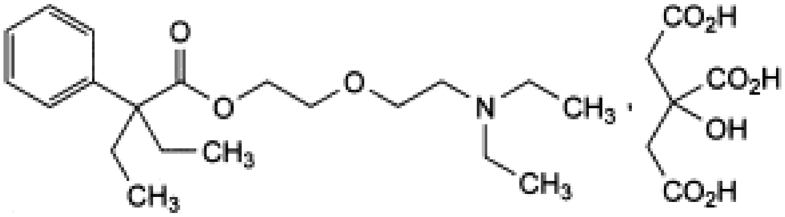
E. 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide.

Ph Eur

Oxeladin Hydrogen Citrate

General Notices

(Ph Eur monograph 1761)



$C_{20}H_{33}NO_3,C_6H_8O_7$ 527.6 *52432-72-1*

Action and use

Cough suppressant.

Ph Eur

DEFINITION

2-[2-(Diethylamino)ethoxy]ethyl 2-ethyl-2-phenylbutanoate dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly to very slightly soluble in ethyl acetate.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison oxeladin hydrogen citrate CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 2.0 g in *water R* and dilute to 10.0 ml with the same solvent.

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution Dissolve 0.500 g of the substance to be examined in *water R* and dilute to 50 ml with the same solvent. Add 1 ml of a 10.3 g/l solution of *hydrochloric acid R* and shake with 3 quantities, each of 10 ml, of *methylene chloride R*. Combine the lower layers. Add 5 ml of *concentrated ammonia R* to the aqueous layer and shake with 3 quantities, each of 10 ml, of *methylene chloride R*. Combine the lower layers obtained to the lower layers obtained previously, add *anhydrous sodium sulphate R*, shake, filter and evaporate the filtrate, at a temperature not exceeding 30 °C, using a rotary evaporator. Take up the residue with *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *oxeladin impurity D CRS* in 10 ml of *water R*, add 0.5 ml of *concentrated ammonia R* and shake with 3 quantities, each of 2 ml, of *methylene chloride R*. To the combined lower layers, add 0.2 ml of the test solution and dilute to 10.0 ml with *methylene chloride R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methylene chloride R*. Dilute 1.0 ml of this solution to 20.0 ml with *methylene chloride R*.

Reference solution (c) Dissolve 5 mg of *oxeladin impurity C CRS* in 10 ml of *water R*, add 0.5 ml of *concentrated ammonia R* and shake with 3 quantities, each of 2 ml, of *methylene chloride R*. Combine the lower layers and dilute to 10 ml with *methylene chloride R*.

Column:

— *material*: fused silica;

— *size*: l = 25 m, Ø = 0.32 mm;

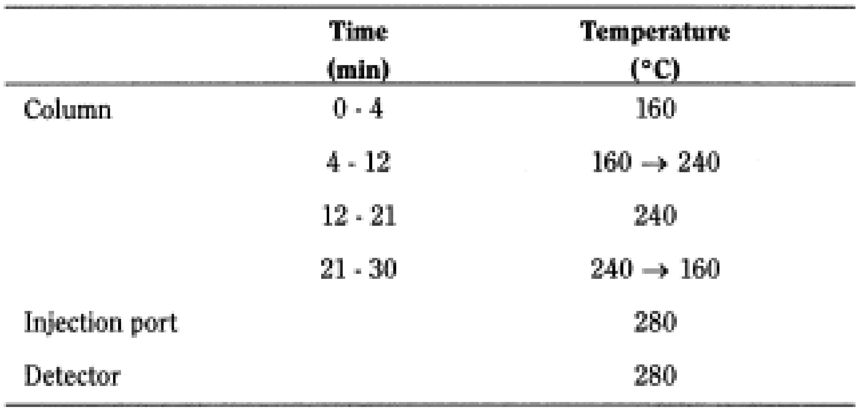
— *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.4 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.0 ml/min. Adjust the flow rate if necessary to obtain a retention time of about 13 min for oxeladin.

Split ratio 1:15.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 4	160
	4 - 12	160 → 240
	12 - 21	240
	21 - 30	240 → 160
Injection port		280
Detector		280

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to oxeladin (retention time = about 13 min): impurity A = about 0.2; impurity B = about 0.4; impurity C = about 0.8; impurity D = about 0.9.

System suitability Reference solution (a):

— *resolution*: minimum 10 between the peaks due to impurity D and oxeladin.

Limits:

— *impurity C*: maximum 0.2 per cent;

— *impurity D*: maximum 0.3 per cent;

— *any other impurity*: for each impurity, maximum 0.1 per cent;

— *total*: maximum 1.0 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

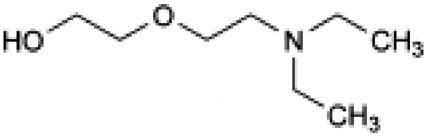
Dissolve 0.400 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 52.76 mg of $C_{26}H_{41}NO_{10}$.

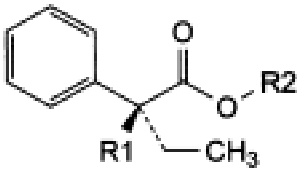
IMPURITIES

Specified impurities C, D.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph. *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B.*



A. 2-[2-(diethylamino)ethoxy]ethanol,


and enantiomer

B. R1 = C_2H_5, R2 = H: 2-ethyl-2-phenylbutanoic acid,

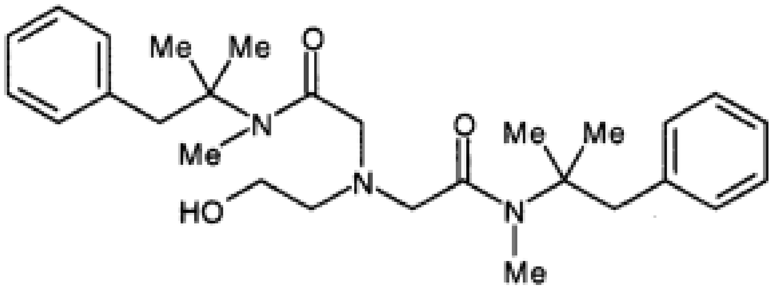
C. R1 = C_2H_5, R2 = $[CH_2]_2$-$N(C_2H_5)_2$: 2-(diethylamino)ethyl 2-ethyl-2-phenylbutanoate,

D. R1 = H, R2 = $[CH_2]_2$-O-$[CH_2]_2$-$N(C_2H_5)_2$: 2-[2-(diethylamino)ethoxy]ethyl (2*RS*)-2-phenylbutanoate.

Ph Eur

Oxetacaine

General Notices



$C_{28}H_{41}N_3O_3$ 467.6 *126-27-6*

Action and use

Local anaesthetic.

DEFINITION

Oxetacaine is 2,2′-(2-hydroxyethylimino)bis[*N*-(α,α-dimethylphenethyl)-*N*-methylacetamide]. It contains not less than 99.0% and not more than 100.5% of $C_{28}H_{41}N_3O_3$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Practically insoluble in *water*; freely soluble in *methanol*; soluble in *ethyl acetate*.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of oxetacaine *(RS 254)*.

TESTS

Melting point

100°C to 104°C, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 precoated plate (Merck plates are suitable) and a mixture of 79 volumes of *toluene*, 20 volumes of *absolute ethanol* and 1 volume of 18M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions of the substance being examined in *ethyl acetate* containing (1) 10.0% w/v, (2) 0.050% w/v and (3) 0.010% w/v. After removal of the plate, dry it in a current of warm air and spray liberally with a solution containing 6% w/v of *ammonium thiocyanate* and 2% w/v of *cobalt(II) chloride*. Carefully remove excess solution by applying filter paper to the plate and allow the plate to dry in air for 10 minutes or until spots appear. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

Loss on drying

When dried at 60°C at a pressure not exceeding 0.7 kPa for 4 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

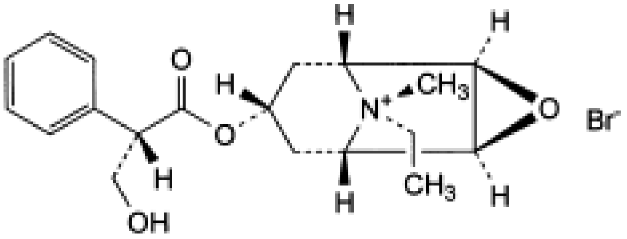
ASSAY

Dissolve 1 g in 50 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end-point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 46.76 mg of $C_{28}H_{41}N_3O_3$.

Oxitropium Bromide

General Notices

(Ph Eur monograph 2170)



$C_{19}H_{26}NO_4Br$ 412.3   *30286-75-0*

Action and use

Anticholinergic; treatment of reversible airways obstruction.

Ph Eur

DEFINITION

(1*R*,2*R*,4*S*,5*S*,7*s*,9*r*)-9-Ethyl-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9-methyl-3-oxa-9-azoniatricyclo[3.3.1.0^{2,4}]nonane bromide (ethylhyoscine).

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in methanol, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison   oxitropium bromide CRS.

If the spectra obtained in the solid state show differences at about 1700 cm^{-1} and about 3300 cm^{-1}, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (a) of bromides (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)

- 24 to - 26 (dried substance).

Dissolve 1.0 g in *water R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 75.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 7.5 mg of *oxitropium bromide impurity B CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) Mix 5.0 ml of the test solution and 5.0 ml of reference solution (a).

Reference solution (d) Dilute 15.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (e) Dilute 5.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m2/g and a pore size of 6 nm.

Mobile phase Mix 1 volume of *acetonitrile for chromatography R* with 10 volumes of a 7.8 g/l solution of *sodium dihydrogen phosphate R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µl of the test solution and reference solutions (b), (c), (d) and (e).

Relative retention With reference to oxitropium (retention time = about 6 min): impurity A = about 0.8; impurity B = about 0.9; impurity C = about 1.3.

System suitability Reference solution (c):

— *resolution*: minimum 1.6 between the peaks due to impurity B and oxitropium.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.1 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.5 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.10 per cent);

— *sum of unspecified impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Impurity D

Liquid chromatography (*2.2.29*).

Test solution Dissolve 75.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 6.0 mg of *oxitropium bromide impurity D CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 200.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) To 5.0 ml of the test solution add 5.0 ml of reference solution (a).

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 185 volumes of *acetonitrile for chromatography R* with 1000 volumes of a 7.8 g/l solution of *sodium dihydrogen phosphate R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µl of the test solution and reference solutions (b) and (c).

System suitability Reference solution (c):

— *resolution*: minimum 3.0 between the peaks due to impurity D and oxitropium.

Limit:

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

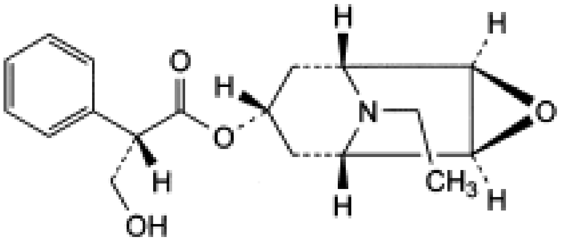
ASSAY

Dissolve 0.350 g in 100 ml of *water R* and add 5.0 ml of *dilute nitric acid R*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (*2.2.20*) using a silver indicator electrode and a silver-silver chloride reference electrode.

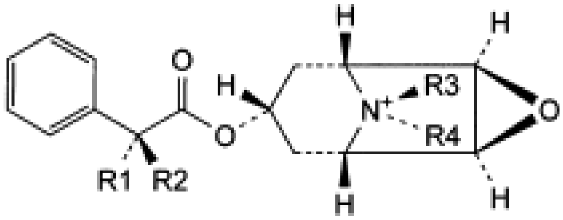
1 ml of *0.1 M silver nitrate* is equivalent to 41.23 mg of $C_{19}H_{26}BrNO_4$.

IMPURITIES

Specified impurities A, B, C, D.



A. (1*R*,2*R*,4*S*,5*S*,7*s*)-9-ethyl-3-oxa-9-azatricyclo[3.3.1.02,4]nonan-7yl (2*S*)-3-hydroxy-2-phenylpropanoate (*N*-ethylnorhyoscine),



B. R1 = CH_2OH, R2 = H, R3 = R4 = CH_3: (1*R*,2*R*,4*S*,5*S*,7*s*)-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.02,4]nonane (methylhyoscine),

C. R1 = CH_2OH, R2 = H, R3 = C_2H_5, R4 = CH_3: (1*R*,2*R*,4*S*,5*S*,7*s*,9*s*)-9-ethyl-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9-methyl-3-oxa-9-azoniatricyclo[3.3.1.02,4]nonane (pseudo-isomer),

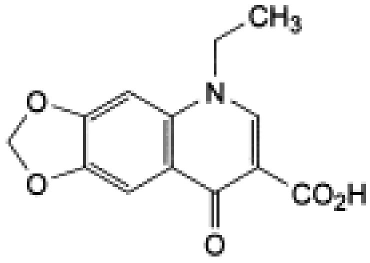
D. R1 + R2 = CH_2, R3 = CH_3, R4 = C_2H_5: (1*R*,2*R*,4*S*,5*S*,7*s*,9*r*)-9-ethyl-9-methyl-7-[(2-phenylprop-2-enoyl)oxy]-3-oxa-9-azoniatricyclo[3.3.1.02,4]nonane (apo-*N*-ethylhyoscine).

Ph Eur

Oxolinic Acid

General Notices

(Ph Eur monograph 1353)



$C_{13}H_{11}NO_5$ 261.2 *14698-29-4*

Action and use

Antibacterial.

Ph Eur

DEFINITION

Oxolinic acid contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 5-ethyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-*g*]quinoline-7-carboxylic acid, calculated with reference to the dried substance.

CHARACTERS

An almost white or pale yellow, crystalline powder, practically insoluble in water, very slightly soluble in methylene chloride, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Dissolve 25.0 mg in 5 ml of *0.1 M sodium hydroxide*, heating on a water-bath. Allow to cool and dilute to 100.0 ml with *methanol R*. Dilute 2.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows three absorption maxima, at 260 nm, 322 nm and 336 nm respectively. The ratio of the absorbance measured at the maximum at 260 nm to that measured at the maximum at 336 nm is 4.9 to 5.2.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *oxolinic acid CRS*. Examine the substances prepared as discs.

C. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution Dissolve 10 mg of the substance to be examined in 3 ml of *dilute sodium hydroxide solution R* and dilute to 20 ml with *alcohol R*.

Reference solution (a) Dissolve 10 mg of *oxolinic acid CRS* in 3 ml of *dilute sodium hydroxide solution R* and dilute to 20 ml with *alcohol R*.

Reference solution (b) Dissolve 5 mg of *ciprofloxacin hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 2 ml with reference solution (a).

Apply separately to the plate 10 µl of each solution. At the bottom of a chromatographic tank, place an evaporating disk containing 50 ml of *concentrated ammonia R*. Close the tank and expose the plate to the ammonia vapour for 15 min. Withdraw the plate and transfer to a chromatographic tank and develop over a path of 15 cm using a mixture of 10 volumes of *acetonitrile R*, 20 volumes of *concentrated ammonia R*, 40 volumes of *methanol R* and 40 volumes of *methylene chloride R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with reference solution (a). The identification is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

TESTS

Solution S

Dissolve 0.6 g in 20 ml of a 40 g/l solution of *sodium hydroxide R*.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable cellulose with a particle size of narrow distribution.

Test solution Dissolve 50 mg in 3 ml of *dilute sodium hydroxide solution R* and dilute to 10 ml with *alcohol R*.

Reference solution (a) Dilute 1 ml of the test solution to 50.0 ml with *alcohol R*. Dilute 1.0 ml of the solution to 5.0 ml with *alcohol R*.

Reference solution (b) Dissolve 2 mg of *oxolinic acid impurity B CRS* in *alcohol R* and dilute to 10 ml with the same solvent. Dilute 0.5 ml of the solution to 10 ml with *alcohol R*.

Reference solution (c) Dissolve 5 mg of the substance to be examined and 5 mg of *oxolinic acid impurity A CRS* in 2 ml of *dilute sodium hydroxide solution R* and dilute to 40 ml with *alcohol R*.

Apply separately to the plate 5 µl of each solution, in sufficiently small portions to obtain small spots. Develop over a path of 6 cm (corresponding to two thirds of the plate height) with a mixture of 15 volumes of *ammonia R*, 30 volumes of *water R* and 55 volumes of *propanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution: any spot corresponding to oxolinic acid impurity B is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent); any spot apart from the principal spot and any spot corresponding to oxolinic acid impurity B is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.4 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Heavy metals (*2.4.8*)

2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Not more than 0.5 per cent determined on 1.000 g by heating in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent determined on 1.0 g.

ASSAY

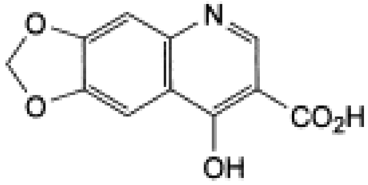
Dissolve 0.200 g in 150 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium* hydroxide, determining the end-point potentiometrically (*2.2.20*). Use a glass indicator electrode and a calomel reference electrode containing, as the electrolyte, a saturated solution of *potassium chloride R* in *methanol R*. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 26.12 mg of $C_{13}H_{11}NO_5$.

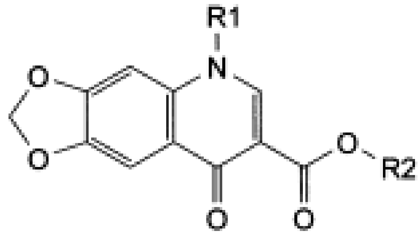
STORAGE

Store protected from light.

IMPURITIES



A. 8-hydroxy-1,3-dioxolo[4,5-*g*]quinoline-7-carboxylic acid,



B. R1 = R2 = C_2H_5: ethyl 5-ethyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-*g*]quinoline-7-carboxylate,

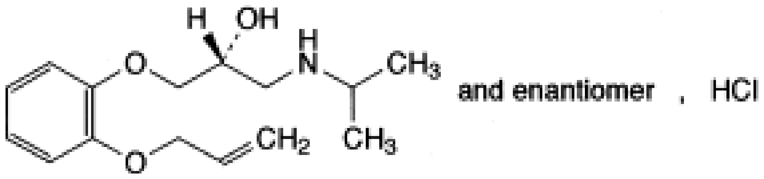
C. R1 = CH_3, R2 = H: 5-methyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-*g*]quinoline-7-carboxylic acid.

Ph Eur

Oxprenolol Hydrochloride

General Notices

(Ph Eur monograph 0628)



$C_{15}H_{23}NO_3$,HCl 301.8 *6452-73-9*

Action and use

Beta-adrenoceptor antagonist.

Preparation

Oxprenolol Tablets

Ph Eur

DEFINITION

(2*RS*)-1-[(1-methylethyl)amino]-3-[2-(prop-2-enyloxy)phenoxy]propan-2-ol hydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Melting point (*2.2.14*): 107 °C to 110 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *oxprenolol hydrochloride CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethyl acetate R*, evaporate to dryness and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 6.0 for freshly prepared solution S.

Related substances

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in 2 ml of a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (a) Dissolve 10 mg of *oxprenolol hydrochloride CRS* in 2 ml of a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (b) Dilute 0.4 ml of test solution (a) to 100 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (d) Dissolve 5 mg of *alprenolol hydrochloride CRS* in 1 ml of reference solution (a).

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R*, *methanol R*, *methylene chloride R* (2:12:88 *V/V/V*).

Application 2 µl; allow the spots to dry in air for 15 min.

Development Over a path of 13 cm.

Drying In a current of warm air for 10 min.

Detection Allow to cool and spray with *anisaldehyde solution R*. Heat at 100-105 °C for 5-10 min. Examine in daylight.

System suitability The test is not valid unless the chromatogram obtained with reference solution (d) shows 2 clearly separated spots.

Limits In the chromatogram obtained with test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.4 per cent); not more than 1 such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Lead

Maximum 5.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using 0.5 ml and 1.0 ml respectively of *lead standard solution (10 ppm Pb) R* diluted to 25.0 ml with *water R*.

Source Lead hollow-cathode lamp.

Wavelength 217.0 nm.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 6 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 30.18 mg of $C_{15}H_{24}ClNO_3$.

STORAGE

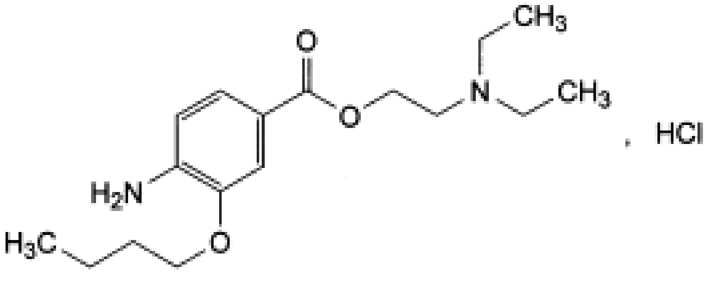
Protected from light.

Ph Eur

Oxybuprocaine Hydrochloride

General Notices

(Ph Eur monograph 1251)



$C_{17}H_{28}N_2O_3,HCl$ 344.9 *5987-82-6*

Action and use

Local anaesthetic.

Preparation

Oxybuprocaine Eye Drops

Ph Eur

DEFINITION

2-(Diethylamino)ethyl 4-amino-3-butoxybenzoate hydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Melting point (*2.2.14*): 158 °C to 162 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *oxybuprocaine hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 40 mg of *oxybuprocaine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *procaine hydrochloride R* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *anhydrous formic acid R*, *methanol R*, *water R*, *ethyl acetate R* (10:15:15:60 *V/V/V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In a current of warm air for 10 min.

Detection Spray with *dimethylaminobenzaldehyde solution R7* and examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dilute 0.2 ml of solution S (see Tests) to 2 ml with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 6.0 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Buffer solution pH 2.5 Add 6 ml of *perchloric acid solution R* and 12 ml of *dilute phosphoric acid R* to 950 ml of *water R*. Adjust to pH 2.5 with a 40 g/l solution of *sodium hydroxide R* and dilute to 1000.0 ml with *water R*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Mix 1.0 ml of the test solution with 1 ml of a 40 g/l solution of *sodium hydroxide R* and allow to stand for 20 min. Add 1 ml of *dilute phosphoric acid R* and dilute to 100.0 ml with the mobile phase. Dilute 25 ml of this solution to 100.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent;

— *temperature*: 35 °C.

Mobile phase *acetonitrile R*, buffer solution pH 2.5 (25:75 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 309 nm.

Injection 20 µl.

Run time 4 times the retention time of oxybuprocaine.

Retention time Oxybuprocaine = about 9 min.

System suitability Reference solution (b):

— *resolution*: minimum 12 between the peaks due to oxybuprocaine and impurity B (hydrolysis product).

Limits:

— *any impurity*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.0125 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

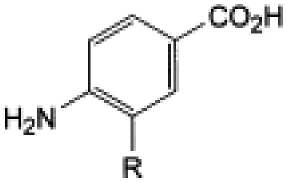
Dissolve 0.300 g in a mixture of 20 ml of *anhydrous acetic acid R* and 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 34.49 mg of $C_{17}H_{29}ClN_2O_3$.

STORAGE

Protected from light.

IMPURITIES



A. R = H: 4-aminobenzoic acid,

B. R = $O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$: 4-amino-3-butoxybenzoic acid,

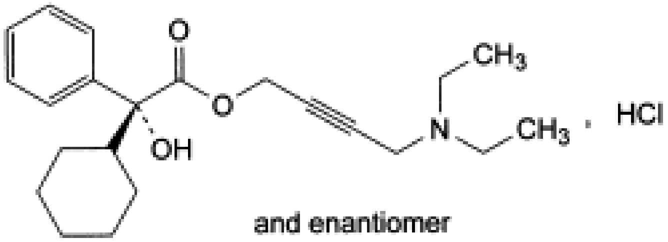
C. R = OH: 4-amino-3-hydroxybenzoic acid.

Ph Eur

Oxybutynin Hydrochloride

General Notices

(Ph Eur monograph 1354)



$C_{22}H_{31}NO_3,HCl$ 394.0 *1508-65-2*

Action and use

Anticholinergic.

Preparation

Oxybutynin Tablets

Ph Eur

DEFINITION

4-(Diethylamino)but-2-ynyl (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride.

Content

99.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in ethanol (96 per cent), soluble in acetone, practically insoluble in cyclohexane.

IDENTIFICATION

First identification *B, D.*

Second identification A, C, D.

A. Melting point (*2.2.14*): 124 °C to 129 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *oxybutynin hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *oxybutynin hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 2 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *methanol R.*

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour for 30 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.00 g in *water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *oxybutynin hydrochloride CRS* and 5.0 mg of *oxybutynin impurity A CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R2* (5 µm).

Mobile phase Mix 49 volumes of a solution containing 3.4 g/l of *potassium dihydrogen phosphate R* and 4.36 g/l of *dipotassium hydrogen phosphate R* and 51 volumes of *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Run time Twice the retention time of oxybutynin.

Retention time Oxybutynin = about 15 min; impurity A = about 24 min.

System suitability Reference solution (a):

- *resolution*: minimum 11.0 between the peaks due to oxybutynin and impurity A.

Limits:
- *impurity A*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
- *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.025 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 39.4 mg of $C_{22}H_{32}ClNO_3$.

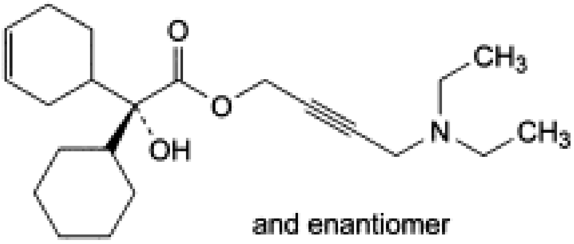
STORAGE

Protected from light.

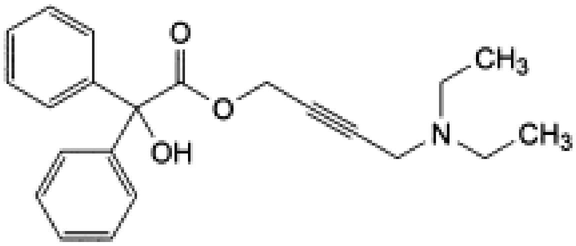
IMPURITIES

Specified impurities *A.*

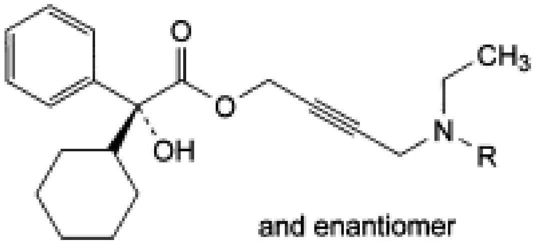
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E.*



A. 4-(diethylamino)but-2-ynyl (*RS*)-2-(cyclohex-3-enyl)-2-cyclohexyl-2-hydroxyacetate,

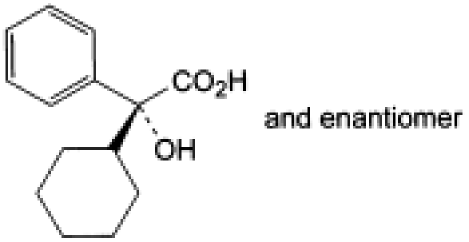


B. 4-(diethylamino)but-2-ynyl 2-hydroxy-2,2-diphenylacetate (diphenyl analogue of oxybutynin),



C. R = CH_3: 4-(ethylmethylamino)but-2-ynyl (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetate (methylethyl analogue of oxybutynin),

E. R = CH_2-CH_2-CH_3: 4-(ethylpropylamino)but-2-ynyl (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetate (ethylpropyl analogue of oxybutynin),


CO2H
OH
and enantiomer

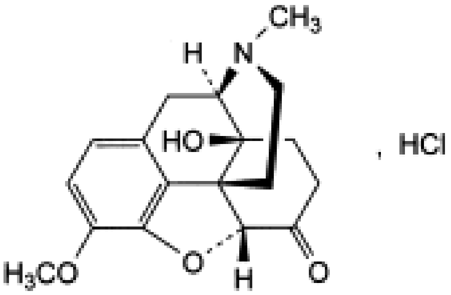
D. (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetic acid (phenylcyclohexylglycolic acid).

Ph Eur

Oxycodone Hydrochloride

General Notices

(Ph Eur monograph 1793)



$C_{18}H_{22}ClNO_4$ 351.9 *124-90-3*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in anhydrous ethanol, practically insoluble in toluene.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Dissolve 50 mg in *water R* and dilute to 5 ml with the same solvent. Render the solution alkaline with *dilute ammonia R1*. Allow the mixture to stand until a precipitate is formed. Filter, wash the precipitate with 10 ml of cold *water R*, and dry for 1 h at 105 °C. Examine the precipitate.

Comparison Repeat the operations using 50 mg of *oxycodone hydrochloride CRS*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.2 ml of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

- 140 to - 148 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions protected from light*.

Test solution Dissolve 0.100 g of the substance to be examined in a 1 per cent *V/V* solution of *dilute acetic acid R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *oxycodone impurity D CRS* in a 1 per cent *V/V* solution of *dilute acetic acid R* and dilute to 10.0 ml with the same solution.

Reference solution (b) To 1.0 ml of the test solution, add 1.0 ml of reference solution (a) and dilute to 100.0 ml with a 1 per cent *V/V* solution of *dilute acetic acid R*. Dilute 1.0 ml of the solution to 10.0 ml with a 1 per cent *V/V* solution of *dilute acetic acid R*.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

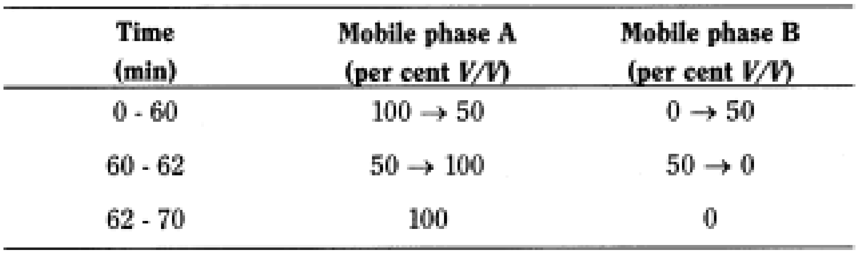
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: mix 830 ml of a 1.1 g/l solution of *sodium heptanesulphonate monohydrate R* previously adjusted to pH 2.0 with a mixture of equal volumes of *phosphoric acid R* and *water R*, with 70 ml of *acetonitrile R* and 100 ml of *methanol R*;

— *mobile phase B*: mix 600 ml of a 1.1 g/l solution of *sodium heptanesulphonate monohydrate R* previously adjusted to pH 2.0 with a mixture of equal volumes of *phosphoric acid R* and *water R*, with 150 ml of *acetonitrile R* and 250 ml of *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	100 → 50	0 → 50
60 - 62	50 → 100	50 → 0
62 - 70	100	0

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Relative retention With reference to oxycodone (retention time = about 24 min): impurity A = about 0.4; impurity B = about 0.7; impurity C = about 1.14; impurity D = about 1.18; impurity E = about 1.18; impurity F = about 2.4.

System suitability Reference solution (b):

— *resolution*: minimum 3 between the peaks due to oxycodone and impurity D.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity F by 0.5;

— *sum of impurities D and E*: not more than 10 times the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurities A, B, C, F*: for each impurity, not more than the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 15 times the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.5 times the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol (*2.4.24, System A*)

Maximum 1.0 per cent.

Water (*2.5.12*)

Maximum 7.0 per cent, determined on 0.250 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 60 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Measure the volume used between the 2 inflexion points.

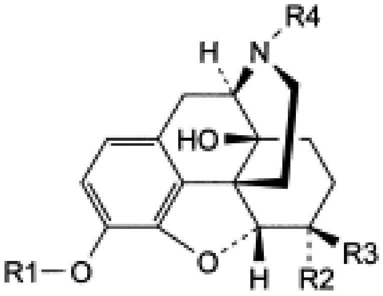
1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 35.19 mg of $C_{18}H_{22}ClNO_4$.

STORAGE

In an airtight container , protected from light.

IMPURITIES

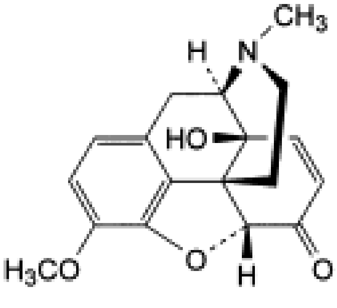
Specified impurities   A, B, C, D, E, F.



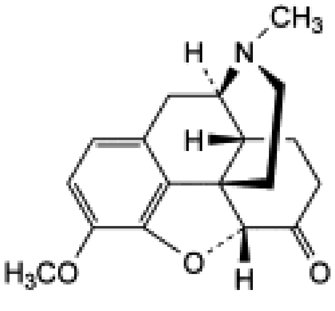
A. R1 = H, R2 = R3 = O, R4 = CH_3: 4,5α-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one (oxymorphone),

B. R1 = R4 = CH_3, R2 = OH, R3 = H: 4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,14-diol (7,8-dihydro-14-hydroxycodeine),

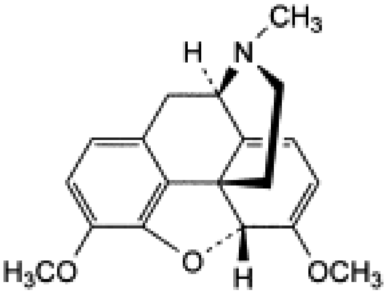
C. R1 = CH_3, R2 + R3 = O, R4 = H: 4,5α-epoxy-14-hydroxy-3-methoxymorphinan-6-one (noroxycodone),



D. 7,8-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one (14-hydroxycodeinone),



E. 4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one (hydrocodone),


CH3
H
N
H3CO
O
H
OCH3

F. 6,7,8,14-tetradehydro-4,5α-epoxy-3-6-dimethoxy-17-methylmorphinan (thebain).

Ph Eur

Oxygen

General Notices

(Ph Eur monograph 0417)

O_2 32.00 *7782-44-7*

Oxygen should be kept in approved metal cylinders, the shoulders of which are painted white and the remainder black. The cylinder should carry a label stating 'Oxygen'. In addition, 'Oxygen' or the symbol 'O_2' should be stencilled in paint on the shoulder of the cylinder.

When Oxygen is intended for use in a room in which magnetic resonance imaging (MRI) is being performed, the cylinder and fittings should be made from suitable non-ferromagnetic materials and labelled accordingly.

Ph Eur

DEFINITION

Content

Minimum 99.5 per cent *V/V* of O_2.

This monograph applies to oxygen for medicinal use.

CHARACTERS

Appearance

Colourless, odourless gas.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 32 volumes of water.

PRODUCTION

Carbon dioxide

Maximum 300 ppm *V/V*, determined using an infrared analyser *(2.5.24)*.

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Oxygen R.*

Reference gas (b) Mixture containing 300 ppm *V/V* of *carbon dioxide R1* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon dioxide in the gas to be examined.

Carbon monoxide

Maximum 5 ppm *V/V*, determined using an infrared analyser *(2.5.25)*.

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Oxygen R.*

Reference gas (b) Mixture containing 5 ppm *V/V* of *carbon monoxide R* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon monoxide in the gas to be examined.

Water *(2.5.28)*

Maximum 67 ppm *V/V*.

Assay

Determine the concentration of oxygen using a paramagnetic analyser *(2.5.27)*.

IDENTIFICATION

First identification *C.*

Second identification *A, B.*

A. Place a glowing splinter of wood in the substance to be examined. The splinter bursts into flame.

B. Shake with *alkaline pyrogallol solution R*. The substance to be examined is absorbed and the solution becomes dark brown.

C. It complies with the limits of the assay.

TESTS

Carbon dioxide *(2.1.6)*

Maximum 300 ppm *V/V*, determined using a carbon dioxide detector tube.

Carbon monoxide *(2.1.6)*

Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube.

Water vapour *(2.1.6)*

Maximum 67 ppm *V/V*, determined using a water vapour detector tube.

STORAGE

As a compressed gas or liquid in appropriate containers, complying with the legal regulations. Taps and valves are not to be greased or oiled.

IMPURITIES

Specified impurities *A, B, C.*

A. carbon dioxide,

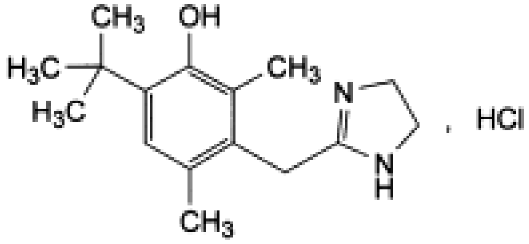
B. carbon monoxide,

C. water.

Oxymetazoline Hydrochloride

General Notices

(Ph Eur monograph 0943)



$C_{16}H_{24}N_2O,HCl$ 296.8 *2315-02-8*

Action and use

Alpha-adrenoceptor agonist; decongestant.

Ph Eur

Oxymetazoline hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethylphenol hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water and in alcohol.

IDENTIFICATION

First identification *A, D*.

Second identification *B, C, D*.

A. Examine by infrared absorption spectrophotometry *(2.2.24)* comparing with the spectrum obtained with *oxymetazoline hydrochloride CRS*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To a solution of about 2 mg in 1 ml of *water R* add 0.2 ml of a 50 g/l solution of *sodium nitroprusside R* and 0.2 ml of *dilute sodium hydroxide solution R*. Allow to stand for 10 min. Add 2 ml of *sodium hydrogen carbonate solution R*. A violet colour develops.

D. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Acidity or alkalinity

Dissolve 0.25 g in *water R* and dilute to 25 ml with the same solvent. Add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M hydrochloric acid*. The solution is red. Not more than 0.4 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.40 g of the substance to be examined in a mixture of equal volumes of *ethyl acetate R* and *methanol R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of equal volumes of *ethyl acetate R* and *methanol R*.

Reference solution (a) Dissolve 40 mg of *oxymetazoline hydrochloride CRS* in a mixture of equal volumes of *ethyl acetate R* and *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dilute 1 ml of test solution (b) to 20 ml with a mixture of equal volumes of *ethyl acetate R* and *methanol R*.

Reference solution (c) Dilute 1 ml of test solution (b) to 40 ml with a mixture of equal volumes of *ethyl acetate R* and *methanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 6 volumes of *diethylamine R*, 15 volumes of *cyclohexane R* and 79 volumes of *ethanol R*. Dry the plate in a current of warm air for 5 min. Allow to cool and spray with a freshly prepared 5.0 g/l solution of *potassium ferricyanide R* in *ferric chloride solution R2*. Examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

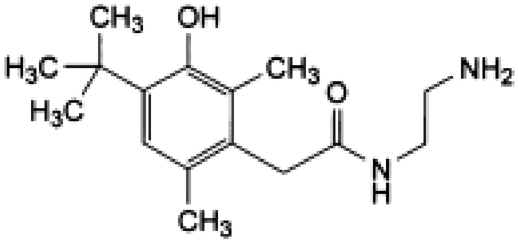
ASSAY

Dissolve 0.200 g in a mixture of 20 ml of *anhydrous acetic acid R* and 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically

(2.2.20).

1 ml of *0.1 M perchloric acid* is equivalent to 29.68 mg of $C_{16}H_{25}ClN_2O$.

IMPURITIES

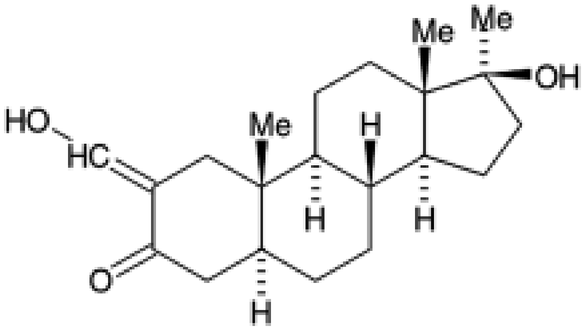

CH3
OH
H3C
H3C
CH3
O
NH2
N
H
CH3

A. *N*-(2-aminoethyl)-2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]acetamide.

Ph Eur

Oxymetholone

General Notices



$C_{21}H_{32}O_3$ 332.5 *434-07-1*

Action and use

Anabolic steroid; androgen.

Preparation

Oxymetholone Tablets

DEFINITION

Oxymetholone is 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one. It contains not less than 97.0% and not more than 103.0% of $C_{21}H_{32}O_3$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to creamy white crystalline powder. It exhibits polymorphism.

Practically insoluble in *water*; soluble in *ethanol (96%)*; slightly soluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.01M *ethanolic sodium hydroxide* exhibits a maximum only at 315 nm. The *absorbance* at 315 nm is about 1.1.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.003% w/v solution in 0.01M *ethanolic hydrochloric acid* exhibits a maximum only at 277 nm. The *absorbance* at 277 nm is about 1.0.

C. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of oxymetholone *(RS 256)*. If the spectra are not concordant, dissolve the substance in the minimum of *chloroform*, evaporate to dryness, dry the residue over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa and prepare a new spectrum.

D. Complies with the test for identification of steroids, Appendix III A, using impregnating solvent II and mobile phase D.

TESTS

Melting point

175° to 180°, Appendix V A.

Specific optical rotation

In a 2% w/v solution in *1,4-dioxan*, +34 to +38, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 98 volumes of *toluene* and 2 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions of the substance being examined in a mixture of equal volumes of *chloroform* and *ethanol (96%)* containing (1) 1.0% w/v and (2) 0.0050% w/v. After removal of the plate, allow it to dry in air and spray with *vanillin-ethanolic sulphuric acid reagent*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

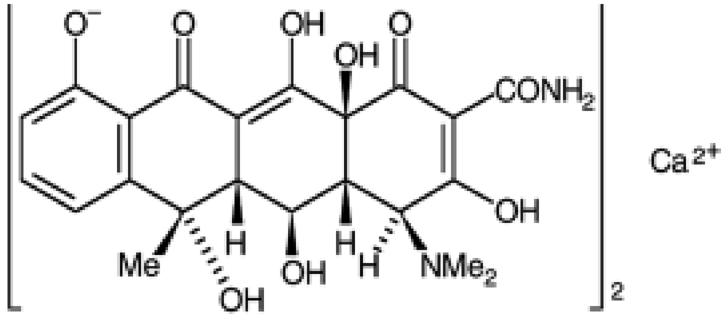
Dissolve 0.1 g in sufficient 0.01M *ethanolic sodium hydroxide* to produce 200 ml, dilute 5 ml to 250 ml with 0.01M *ethanolic sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 315 nm, Appendix II B. Calculate the content of $C_{21}H_{32}O_3$ taking 547 as the value of A(1%, 1 cm) at the maximum at 315 nm.

STORAGE

Oxymetholone should be kept free from contact with ferrous metals and protected from light.

Oxytetracycline Calcium

General Notices



$(C_{22}H_{23}N_2O_9)_2Ca$ 958.9 *15251-48-6*

Action and use

Tetracycline antibacterial.

DEFINITION

Oxytetracycline Calcium is the calcium salt of (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-dimethylamino-1, 4,4a,5, 5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11dioxonaphthacene-2-carboxamide, a substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means. It contains not less than 90.0% and not more than 100.5% of $(C_{22}H_{23}N_2O_9)_2Ca$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A pale yellow to greenish fawn, crystalline powder.

Practically insoluble in *water*; soluble in dilute acids. It dissolves slowly in 5M *ammonia*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 ml for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate at 110° for 1 hour. Apply separately to the plate 1 µl of each of three solutions in 0.01M *methanolic hydrochloric acid* containing (1) 0.05% w/v of the substance being examined, (2) 0.05% w/v of *oxytetracycline EPCRS* and (3) 0.05% w/v of each of *oxytetracycline EPCRS* and *demeclocycline hydrochloride EPCRS.* After removal of the plate, dry it in a current of air and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. To 2 mg add 5 ml of *sulphuric acid*; a deep red colour is produced. Add the solution to 2.5 ml of *water*; the colour changes to yellow.

C. Yields reaction B characteristic of *calcium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a 2.5% w/v suspension, 6.0 to 7.5, Appendix V L.

Light absorption

Absorbance of a 0.002% w/v solution in *0.1M chloride buffer pH 2.0* at the maximum at 353 nm, 0.56 to 0.61, calculated with reference to the anhydrous substance, Appendix II B.

Specific optical rotation

In a 1% w/v solution in 0.1M *hydrochloric acid*, -194 to -210, calculated with reference to the anhydrous substance, Appendix V F. Allow the solution to stand protected from light for 30 minutes before measurement.

Light-absorbing impurities

A. Dissolve 0.2 g in 6 ml of 1M *hydrochloric acid* and add sufficient *methanol* to produce 100 ml. The *absorbance* at 430 nm, when measured within 1 hour of preparing the solution, is not more than 0.30, calculated with reference to the anhydrous substance, Appendix II B.

B. Dissolve 1 g in 6 ml of 1M *hydrochloric acid* and add sufficient *methanol* to produce 100 ml. The *absorbance* at 490 nm, when measured within 1 hour of preparing the solution, is not more than 0.20, calculated with reference to the anhydrous substance, Appendix II B.

Calcium

3.90 to 4.20%, calculated with reference to the anhydrous substance, when determined by the following method. Transfer about 1 g, accurately weighed, to a Kjeldahl flask, cautiously add 10 ml of *nitric acid* and mix. Allow to stand for 5 minutes, add a glass bead and heat on a water bath for 5 minutes. Remove from the water bath, cautiously add 5 ml of 9M *perchloric acid* and heat, adding further 5 ml quantities of the perchloric acid at intervals until the liquid is almost colourless. Add 0.1 ml of *nitric acid* and allow any further reaction to subside. Do not allow the volume of the liquid in the flask to be reduced below 3 ml at any stage in the oxidation. Wash the walls of the flask with 40 ml of *water*, collecting the washings in the flask, and boil for 3 to 4 minutes to expel chlorine. Cool, transfer the contents of the flask to a conical flask with the aid of *water* and dilute to about 200 ml with *water*. Adjust to pH 9 with 5M *sodium hydroxide* and then add 100 ml of *water* followed by 12 ml of 10M *sodium hydroxide* and mix. Add about 15 mg of *calconcarboxylic acid triturate* and titrate with 0.05M *disodium edetate VS* until the colour changes from violet to full blue. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

Water

Not more than 15.0% w/w, Appendix IX C. Use 0.25 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For solution (1) dilute 1 volume of a 0.1% w/v solution of the substance being examined in 0.1M *hydrochloric acid* to 20 volumes with the mobile phase. For solution (2) dilute 1 volume of a 0.1% w/v solution of *oxytetracycline EPCRS* in 0.1M *hydrochloric acid* to 20 volumes with the mobile phase. Solution (3) contains 0.1% w/v of *4-epioxytetracycline EPCRS* in 0.1M *hydrochloric acid*. Solution (4) contains 0.1% w/v of *tetracycline hydrochloride BPCRS* in 0.1M *hydrochloric acid*. For solution (5) dilute a mixture containing 1.5 ml of a 0.1% w/v solution of *oxytetracycline EPCRS* in 0.1M *hydrochloric acid*, 1 ml of solution (3) and 3 ml of solution (4) to 25 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene copolymer* (8 to 10 µm) (Polymer Laboratories, PLRP-S 100A, is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared as described below and (c) a detection wavelength of 254 nm. To 50.0 g of *2-methyl-propan-2-ol* add 200 ml of *water*, 60 ml of *0.33*M *phosphate buffer pH 7.5*, 50 ml of a 1.0% w/v solution of *tetrabutylammonium hydrogen sulphate* previously adjusted to pH 7.5 with 2M *sodium hydroxide* and 10 ml of a 0.04% w/v solution of *disodium edetate* previously adjusted to pH 7.5 with 2M *sodium hydroxide* and dilute to 1 litre with *water*.

Inject solution (5). The assay is not valid unless (a) the *resolution factor* between the first peak (4-epioxytetracycline) and the second peak (oxytetracycline) is at least 4.0, (b) the *resolution factor* between the second peak and the third peak (tetracycline) is at least 5.0 (if necessary reduce the content of 2-methylpropan-2-ol in the mobile phase to increase the resolution) and (c) the *symmetry factor* of the peak due to oxytetracycline is not more than 1.25.

Calculate the content of $(C_{22}H_{23}N_2O_9)_2Ca$ from the declared content of $C_{22}H_{24}N_2O_9$ in *oxytetracycline EPCRS*. Each mg of $C_{22}H_{24}N_2O_9$ is equivalent to 1.041 mg of $(C_{22}H_{23}N_2O_9)_2Ca$.

STORAGE

Oxytetracycline Calcium should be protected from light and stored at a temperature of 2° to 8°.

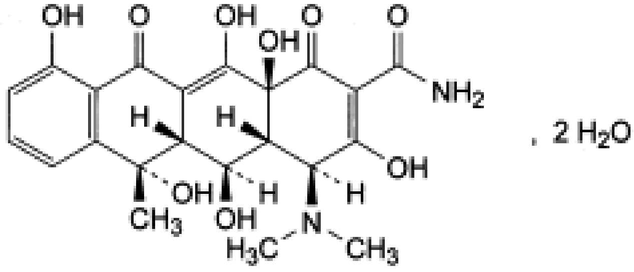
LABELLING

The label states (1) the date after which the material is not intended to be used; (2) the conditions under which it should be stored.

Oxytetracycline Dihydrate

General Notices

(Ph Eur monograph 0199)



$C_{22}H_{24}N_2O_9, 2H_2O$ 496.4 *6153-64-6*

Action and use

Tetracycline antibacterial.

Preparation

Oxytetracycline Tablets

Ph Eur

DEFINITION

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide dihydrate.

Substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Very slightly soluble in water. It dissolves in dilute acid and alkaline solutions.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *oxytetracycline CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *oxytetracycline CRS*, 5 mg of *tetracycline hydrochloride R* and 5 mg of *minocycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R*.

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A deep red colour develops. Add the solution to 2.5 ml of *water R*. The colour becomes yellow.

C. Dissolve about 10 mg in a mixture of 1 ml of *dilute nitric acid R* and 5 ml *of water R*. Shake and add 1 ml of *silver nitrate solution R2*. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of *dilute nitric acid R*, 5 ml of a 0.021 g/l solution of *potassium chloride R* and 1 ml of *silver nitrate solution R2*.

TESTS

pH (*2.2.3*)

4.5 to 7.5.

Suspend 0.1 g in 10 ml of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

- 203 to - 216 (anhydrous substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Specific absorbance (*2.2.25*)

290 to 310 determined at 353 nm (anhydrous substance).

Dissolve 20.0 mg in *buffer solution pH 2.0 R* and dilute to 100.0 ml with the same buffer solution. Dilute 10.0 ml of this solution to 100.0 ml with *buffer solution pH 2.0 R*.

Light-absorbing impurities

Carry out the measurements within 1 h of preparing the solutions.

Dissolve 20.0 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 10.0 ml with the same mixture of solvents. The absorbance (*2.2.25*), determined at 430 nm has a maximum of 0.25 (anhydrous substance).

Dissolve 0.100 g in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 10.0 ml with the same mixture of solvents. The absorbance (*2.2.25*) determined at 490 nm has a maximum of 0.20 (anhydrous substance).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 20.0 mg of *oxytetracycline CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 20.0 mg of *4-epioxytetracycline CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c) Dissolve 20.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (d) Mix 1.5 ml of reference solution (a), 1.0 ml of reference solution (b) and 3.0 ml of reference solution (c) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (e) Mix 1.0 ml of reference solution (b) and 4.0 ml of reference solution (c) and dilute to 200.0 ml with *0.01 M hydrochloric acid*.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm);

— *temperature*: 60 °C.

Mobile phase Weigh 60.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R*; add 60 ml of *0.33 M phosphate buffer solution pH 7.5 R*, 50 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R* and 10 ml of a 0.4 g/l solution of *sodium edetate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R*; dilute to 1000 ml with *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (d) and (e).

System suitability Reference solution (d):

— *resolution*: minimum 4.0 between the peaks due to impurity A (1st peak) and oxytetracycline (2nd peak) and minimum 5.0 between the peaks and due to oxytetracycline and impurity B (3rd peak); adjust the 2-methyl-2-propanol content in the mobile phase if necessary;

— *symmetry factor*: maximum 1.25 for the peak due to oxytetracycline.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (0.5 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per cent);

— *impurity C* (eluting on the tail of the principal peak): not more than 4 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (2.0 per cent);

— *disregard limit*: 0.02 times the area of the peak due to oxytetracycline in the chromatogram obtained with reference solution (d) (0.1 per cent).

Heavy metals (*2.4.8*)

Maximum 50 ppm.

0.5 g complies with test F. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

6.0 per cent to 9.0 per cent, determined on 0.250 g.

Sulphated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.2*9) as described in the test for related substances with the following modification.

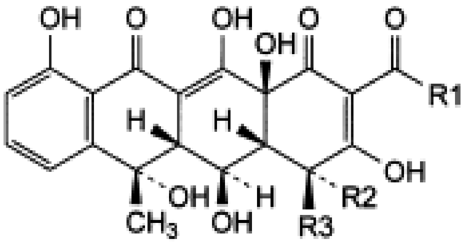
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{24}N_2O_9$.

STORAGE

In an airtight container , protected from light.

IMPURITIES



A. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = H: (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epioxytetracycline),

C. R1 = CH_3, R2 = H, R3 = $N(CH_3)_2$: (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-

3,5,6,10,12,12a-hexahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyloxytetracycline),

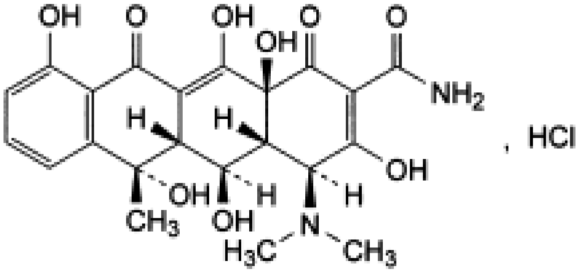
B. tetracycline.

Ph Eur

Oxytetracycline Hydrochloride

General Notices

(Ph Eur monograph 0198)



$C_{22}H_{24}N_2O_9$,HCl 496.9 *2058-46-0*

Action and use

Tetracycline antibacterial.

Preparation

Oxytetracycline Capsules

Ph Eur

DEFINITION

(4*S*,4a*R*,5*S,*5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent). Solutions in water become turbid on standing, owing to the precipitation of oxytetracycline.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *oxytetracycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *oxytetracycline hydrochloride CRS*, 5 mg of *tetracycline hydrochloride R* and 5 mg of *minocycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel* F_{254} *plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A deep red colour develops. Add the solution to 2.5 ml of *water R*. The colour becomes yellow.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)

2.3 to 2.9.

Dissolve 0.1 g in 10 ml of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

- 188 to - 200 (anhydrous substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Specific absorbance (*2.2.25*)

270 to 290 determined at 353 nm (anhydrous substance).

Dissolve 20.0 mg in *buffer solution pH 2.0 R* and dilute to 100.0 ml with the same buffer solution. Dilute 10.0 ml of the solution to 100.0 ml with *buffer solution pH 2.0 R*.

Light-absorbing impurities

Carry out the measurements within 1 h of preparing the solutions.

Dissolve 20.0 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 10.0 ml with the same mixture of solvents. The absorbance (*2.2.25*) determined at 430 nm has a maximum of 0.50 (anhydrous substance).

Dissolve 0.100 g in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 10.0 ml with the same mixture of solvents. The absorbance (*2.2.25*) determined at 490 nm has a maximum of 0.20 (anhydrous substance).

Related substances

Liquid chromatography (*2.2.29*)

Prepare the solutions immediately before use.

Test solution Dissolve 20.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 20.0 mg of *oxytetracycline CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 20.0 mg of *4-epioxytetracycline CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c) Dissolve 20.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (d) Dissolve 8.0 mg of *α-apo-oxytetracycline CRS* in 5 ml of *0.01 M sodium hydroxide* and dilute to 100.0 ml with *0.01 M hydrochloric acid*.

Reference solution (e) Dissolve 8.0 mg of *β-apo-oxytetracycline CRS* in 5 ml of *0.01 M sodium hydroxide* and dilute to 100.0 ml with *0.01 M hydrochloric acid*.

Reference solution (f) Mix 1.5 ml of reference solution (a), 1.0 ml of reference solution (b), 3.0 ml of reference solution (c), 3.0 ml of reference solution (d) and 3.0 ml of reference solution (e) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (g) Mix 1.0 ml of reference solution (b), 4.0 ml of reference solution (c) and 40.0 ml of reference solution (e) and dilute to 200.0 ml with *0.01 M hydrochloric acid*.

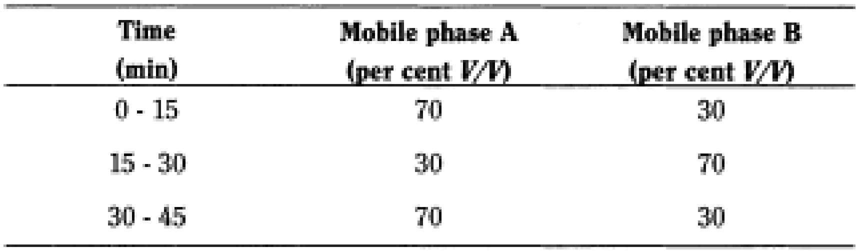
Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm);

— *temperature*: 60 °C.

Mobile phase Weigh 30.0 g (for mobile phase A) and 100.0 g (for mobile phase B) of *2-methyl-2-propanol R* and transfer separately to 1000 ml volumetric flasks with the aid of 200 ml of *water R*; to each flask add 60 ml of 0.33 M phosphate buffer solution pH 7.5 R, 50 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R* and 10 ml of a 0.4 g/l solution of *sodium edetate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R*; dilute each solution to 1000 ml with *water R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	70	30
15 - 30	30	70
30 - 45	70	30

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (f) and (g).

System suitability Reference solution (f):

— *resolution*: minimum 4.0 between the peaks due to impurity A (1st peak) and oxytetracycline (2nd peak), minimum 5.0 between the peaks due to oxytetracycline and impurity B (3rd peak) and minimum 3.5 between the peaks due to impurity D (4th peak) and impurity E (5th peak); if necessary, adapt the ratio mobile phase A: mobile phase B and/or adjust the time programme used to produce the 1-step gradient elution;

— *symmetry factor*: maximum 1.25 for the peak due to oxytetracycline.

Limits:

— *impurity A:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (g) (0.5 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (g) (2.0 per cent);

— *impurity C* (eluting on the tail of the main peak): not more than 4 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (g) (2.0 per cent);

— *total of impurities D, E and F* (eluting between the latter two): not more than the area of the peak due to impurity E in the chromatogram obtained with reference solution (g) (2.0 per cent);

— *disregard limit*: 0.02 times the area of the peak due to oxytetracycline in the chromatogram obtained with reference solution (f) (0.1 per cent).

Heavy metals (*2.4.8*)

Maximum 50 ppm.

0.5 g complies with test F. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 2.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.4 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

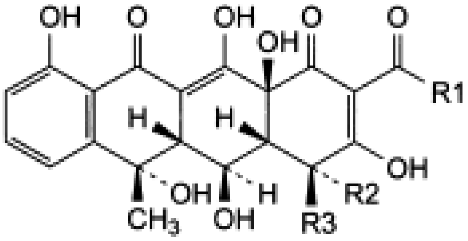
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{25}ClN_2O_9$ taking 1 mg of oxytetracycline as equivalent to 1.079 mg of oxytetracycline hydrochloride.

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

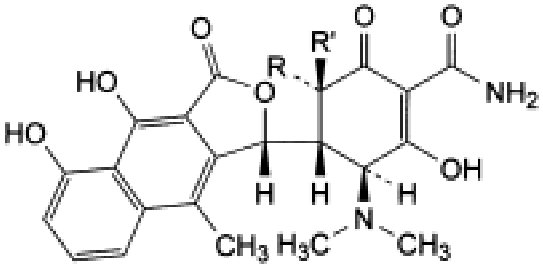
IMPURITIES



A. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = H: (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epioxytetracycline),

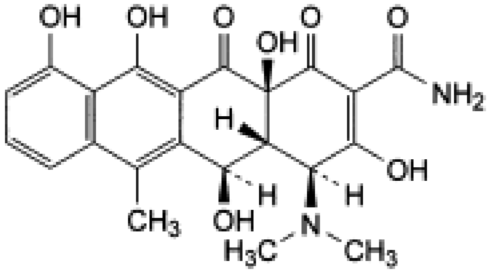
C. R1 = CH_3, R2 = H, R3 = $N(CH_3)_2$: (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyloxytetracycline),

B. tetracycline,



D. R = OH, R′ = H: (3*S*,4*S*,5*S*)-4-[(1*R*)-4,5-dihydroxy-9-methyl-3-oxo-1,3-dihydronaphtho[2,3-*c*]furan-1-yl]-3-(dimethylamino)-2,5-dihydroxy-6-oxocyclohex-1-enecarboxamide (α-apo-oxytetracycline),

E. R = H, R′ = OH: (3*S*,4*S*,5*R*)-4-[(1*R*)-4,5-dihydroxy-9-methyl-3-oxo-1,3-dihydronaphtho[2,3-*c*]furan-1-yl]-3-(dimethylamino)-2,5-dihydroxy-6-oxocyclohex-1-enecarboxamide (β-apo-oxytetracycline),



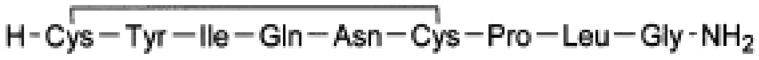
F. (4*S*,4a*R*,5*R*,12a*S*)-4-(dimethylamino)-3,5,10,11,12a-pentahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydro-oxytetracycline).

Ph Eur

Oxytocin Concentrated Solution

General Notices

Oxytocin Bulk Solution
(Ph Eur monograph 0779)



$\text{H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH}_2$

$C_{43}H_{66}N_{12}O_{12}S_2$ 1007

Action and use

Oxytocic.

Preparations

Ergometrine and Oxytocin Injection

Oxytocin Injection

Ph Eur

DEFINITION

L-Cysteinyl-L-tyrosyl-L-isoleucyl-L-glutamyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide cyclic (1→6)-disulfide.

Solution of oxytocin, a synthetic cyclic nonapeptide having the structure of the hormone produced by the posterior lobe of the pituitary gland that stimulates contraction of the uterus and milk ejection in receptive mammals. It is available as a solution with a stated concentration of not less than 0.25 mg of oxytocin per millilitre, in a solvent that may contain an appropriate antimicrobial preservative.

Content

95.0 per cent to 105.0 per cent of the amount of the peptide stated per millilitre.

By convention, for the purpose of labelling oxytocin preparations, 1 mg of oxytocin peptide ($C_{43}H_{66}N_{12}O_{12}S_2$) is equivalent to 600 IU of biological activity.

CHARACTERS

Appearance

Clear, colourless liquid.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine and leucine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half cystine: 1.4 to 2.1. Not more than traces of other amino acids are present.

TESTS

pH (*2.2.3*)

3.0 to 5.0.

Related substances

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution The preparation to be examined.

Resolution solution Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture CRS* in 1 ml of a 15.6 g/l solution of *sodium dihydrogen phosphate R*.

Column:

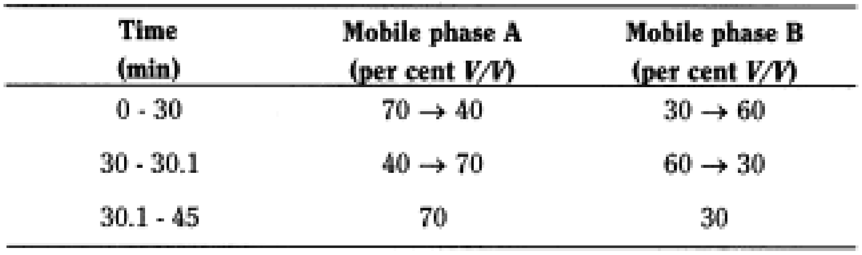
— *size*: *l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: 15.6 g/l solution of *sodium dihydrogen phosphate R*;

— *mobile phase B*: *acetonitrile for chromatography R*, *water R* (50:50 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	70 → 40	30 → 60
30 - 30.1	40 → 70	60 → 30
30.1 - 45	70	30

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µl.

Retention time Oxytocin = about 7.5 min; desmopressin = about 10 min.

System suitability Resolution solution:

— *resolution*: minimum 5.0 between the peaks due to desmopressin and oxytocin.

Limits:

- *any impurity*: maximum 1.5 per cent;
- *total*: maximum 5 per cent;
- *disregard limit*: 0.1 per cent.

Bacterial endotoxins (*2.6.14*)

Less than 300 IU in the volume that contains 1 mg of oxytocin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Reference solution Dissolve the contents of a vial of *oxytocin CRS* in a 15.6 g/l solution of *sodium dihydrogen phosphate R* to obtain a concentration of 0.25 mg/ml.

Injection 25 µl.

Calculate the content of oxytocin ($C_{43}H_{66}N_{12}O_{12}S_2$) from the declared content of $C_{43}H_{66}N_{12}O_{12}S_2$ in *oxytocin CRS*.

STORAGE

At a temperature of 2 °C to 8 °C, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

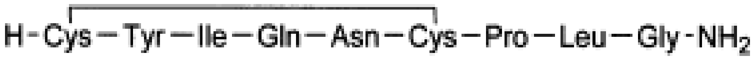
The label states the oxytocin peptide content in milligrams of $C_{43}H_{66}N_{12}O_{12}S_2$ per millilitre.

Ph Eur

Oxytocin

General Notices

(Ph Eur monograph 0780)



$\text{H-}\overbrace{\text{Cys-Tyr-Ile-Gln-Asn-Cys}}\text{-Pro-Leu-Gly-NH}_2$

$C_{43}H_{66}N_{12}O_{12}S_2$ 1007 *50-56-6*

Action and use

Oxytocic.

Preparations

Ergometrine and Oxytocin Injection

Oxytocin Injection

Ph Eur

DEFINITION

L-Cysteinyl-L-tyrosyl-L-isoleucyl-L-glutamyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide cyclic (1→6)-disulfide.

Synthetic cyclic nonapeptide having the structure of the hormone produced by the posterior lobe of the pituitary gland that stimulates contraction of the uterus and milk ejection in receptive mammals. It is available in the freeze-dried form as an acetate.

Content

93.0 per cent to 102.0 per cent (anhydrous and acetic acid-free substance).

By convention, for the purpose of labelling oxytocin preparations, 1 mg of oxytocin peptide ($C_{43}H_{66}N_{12}O_{12}S_2$) is equivalent to 600 IU of biological activity.

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Very soluble in water. It dissolves in dilute solutions of acetic acid and of ethanol (96 per cent)
.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine and leucine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half-cystine: 1.4 to 2.1. Not more than traces of other amino acids are present.

TESTS

pH (*2.2.3*)

3.0 to 6.0.

Dissolve 0.200 g in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Related substances

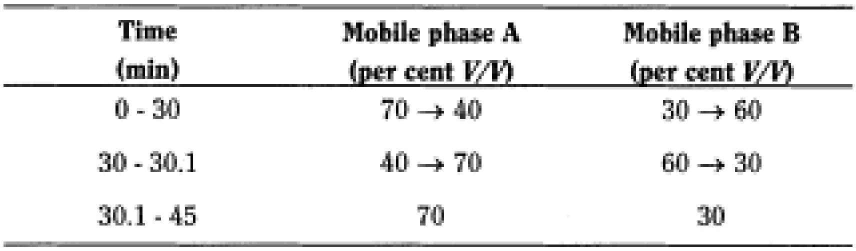
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Prepare a 0.25 mg/ml solution of the substance to be examined in a 15.6 g/l solution of *sodium dihydrogen phosphate R*.

Resolution solution Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture CRS* in 1 ml of a 15.6 g/l solution of *sodium dihydrogen phosphate R*.

Column:
- *size: l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: 15.6 g/l solution of *sodium dihydrogen phosphate R*;
- *mobile phase B*: *acetonitrile for chromatography R*, *water R* (50:50 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	70 → 40	30 → 60
30 - 30.1	40 → 70	60 → 30
30.1 - 45	70	30

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µl.

Retention time Oxytocin = about 7.5 min; desmopressin = about 10 min.

System suitability Resolution solution:

— *resolution*: minimum 5.0 between the peaks due to desmopressin and oxytocin.

Limits:

— *any impurity*: maximum 1.5 per cent;

— *total*: maximum 5 per cent;

— *disregard limit*: 0.1 per cent.

Acetic acid (*2.5.34*)

6.0 per cent to 10.0 per cent.

Test solution Dissolve 15.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Water (*2.5.12*)

Maximum 5.0 per cent, determined on at least 50 mg.

Bacterial endotoxins (*2.6.14*)

Less than 300 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Reference solution Dissolve the contents of a vial of *oxytocin CRS* in a 15.6 g/l solution of *sodium dihydrogen phosphate R* to obtain a concentration of 0.25 mg/ml.

Injection 25 µl.

Calculate the content of oxytocin ($C_{43}H_{66}N_{12}O_{12}S_2$) from the declared content of $C_{43}H_{66}N_{12}O_{12}S_2$ in oxytocin CRS.

STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states the oxytocin peptide content ($C_{43}H_{66}N_{12}O_{12}S_2$).

Ph Eur

Refined Olive Oil

General Notices

(Ph Eur monograph 1456)

Ph Eur

DEFINITION

Fatty oil obtained by refining of crude olive oil, obtained by cold expression or other suitable mechanical means from the ripe drupes of *Olea europaea* L. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, colourless or greenish-yellow transparent liquid.

Solubility

Practically insoluble in ethanol (96 per cent), miscible with light petroleum (50-70 °C).

When cooled, it begins to become cloudy at 10 °C and becomes a butter-like mass at about 0 °C.

Relative density

About 0.913.

IDENTIFICATION

A. Acid value (see Tests).

B. Identification of fatty oils by thin-layer chromatography *(2.3.2)*.

Results The chromatogram obtained shows spots corresponding to those in the typical chromatogram for olive oil (see Figure 2.3.2.-1). For certain types of olive oil, the difference in the size of spots E and F is less pronounced than in the typical chromatogram.

TESTS

Acid value *(2.5.1)*

Maximum 0.3, determined on 10.0 g.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0. If intended for use in the manufacture of parenteral preparations: maximum 5.0.

Unsaponifiable matter

Maximum 1.5 per cent.

Place 5.0 g (*m g*) in a 150 ml flask fitted with a reflux condenser. Add 50 ml of *2 M alcoholic potassium hydroxide R* and heat on a water-bath for 1 h, shaking frequently. Add 50 ml of *water R* through the top of the condenser, shake, allow to cool and transfer the contents of the flask to a separating funnel. Rinse the flask with several portions totalling 50 ml of *light petroleum R1* and add the rinsings to the separating funnel. Shake vigorously for 1 min. Allow to separate and transfer the aqueous layer to a 2nd separating funnel. If an emulsion forms, add small quantities of *ethanol (96 per cent) R* or a concentrated solution of *potassium hydroxide R*. Shake the aqueous layer with 2 quantities, each of 50 ml, of *light petroleum R1*. Combine the light petroleum layers in a 3rd separating funnel and wash with 3 quantities, each of 50 ml, of *ethanol (50 per cent V/V) R*. Transfer the light petroleum layer to a tared 250 ml flask. Rinse the separating funnel with small quantities of *light petroleum R1* and add to the flask. Evaporate the light petroleum on a water-bath and dry the residue at 100-105 °C for 15 min, keeping the flask horizontal. Allow to cool in a desiccator and weigh (*a g*). Repeat the drying for successive periods of 15 min until the loss of mass between 2 successive weighings does not exceed 0.1 per cent. Dissolve the residue in 20 ml of *ethanol (96 per cent) R*, previously neutralised to 0.1 ml of *bromophenol blue solution R*. If necessary, titrate with *0.1 M hydrochloric acid* (b ml).

Calculate the percentage content of unsaponifiable matter using the following expression:

$$\frac{100\,(a - 0.032b)}{m}$$

If 0.032*b* is greater than 5 per cent of a, the test is not valid and must be repeated.

Alkaline impurities *(2.4.19)*

It complies with the test.

Specific absorbance *(2.2.25)*

Maximum 1.20, determined at 270 nm.

Dissolve 1.00 g in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Composition of fatty acids *(2.4.22, Method A)*

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

- — *saturated fatty acids of chain length less than C_{16}*: maximum 0.1 per cent;
- — *palmitic acid*: 7.5 per cent to 20.0 per cent;
- — *palmitoleic acid* : maximum 3.5 per cent;
- — *stearic acid*: 0.5 per cent to 5.0 per cent;
- — *oleic acid* : 56.0 per cent to 85.0 per cent;
- — *linoleic acid* : 3.5 per cent to 20.0 per cent;
- — *linolenic acid* : maximum 1.2 per cent;
- — *arachidic acid*: maximum 0.7 per cent;

- *eicosenoic acid* : maximum 0.4 per cent;
- *behenic acid*: maximum 0.2 per cent;
- *lignoceric acid*: maximum 0.2 per cent.

Sterols *(2.4.23)*.

Composition of the sterol fraction of the oil:

- *cholesterol* : maximum 0.5 per cent;
- *campesterol*: maximum 4.0 per cent;
- *Δ7-stigmasterol* : maximum 0.5 per cent;
- *sum of contents of Δ5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol*: minimum 93.0 per cent.

The content of stigmasterol is not greater than that of campesterol.

Sesame oil

In a ground-glass-stoppered cylinder shake 10 ml for about 1 min with a mixture of 0.5 ml of a 0.35 per cent *V/V* solution of *furfural R* in *acetic anhydride R* and 4.5 ml of *acetic anhydride R*. Filter through a filter paper impregnated with *acetic anhydride R*. To the filtrate add 0.2 ml of *sulphuric acid R*. No bluish-green colour develops.

Water *(2.5.32)*

Maximum 0.1 per cent, determined on 5.0 g, if intended for use in the manufacture of parenteral preparations. Use a mixture of equal volumes of *anhydrous methanol R* and *decanol R* as solvent.

STORAGE

In a well-filled container, protected from light, at a temperature not exceeding 25 °C. If intended for use in the manufacture of parenteral preparations, store under an inert gas.

LABELLING

The label states:

- where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
- the name of the inert gas.

Ph Eur

Virgin Olive Oil

General Notices

Olive Oil

(Ph Eur monograph 0518)

Preparation

Olive Oil Ear Drops

Ph Eur

DEFINITION

Fatty oil obtained by cold expression or other suitable mechanical means from the ripe drupes of *Olea europaea* L.

CHARACTERS

Appearance

Clear, transparent, yellow or greenish-yellow liquid.

Solubility

Practically insoluble in ethanol (96 per cent), miscible with light petroleum (50-70 °C).

It has a characteristic odour.

When cooled, it begins to become cloudy at 10 °C and becomes a butter-like mass at about 0 °C.

Relative density

About 0.913.

IDENTIFICATION

Identification of fatty oils by thin-layer chromatography *(2.3.2)*.

Results The chromatogram obtained shows spots corresponding to those in the typical chromatogram for olive oil (see Figure 2.3.2.-1). For certain types of olive oil, the difference in the size of spots E and F is less pronounced than in the typical chromatogram.

TESTS

Acid value *(2.5.1)*

Maximum 2.0, determined on 5.0 g.

Peroxide value ***(2.5.5, Method A)***

Maximum 20.0.

Unsaponifiable matter

Maximum 1.5 per cent.

Place 5.0 g (*m* g) in a 150 ml flask fitted with a reflux condenser. Add 50 ml of *2 M alcoholic potassium hydroxide R* and heat on a water-bath for 1 h, shaking frequently. Add 50 ml of *water R* through the top of the condenser, shake, allow to cool and transfer the contents of the flask to a separating funnel. Rinse the flask with several portions totalling 50 ml of *light petroleum R1* and add the rinsings to the separating funnel. Shake vigorously for 1 min. Allow to separate and transfer the aqueous layer to a 2nd separating funnel. If an emulsion forms, add small quantities of *ethanol (96 per cent) R* or a concentrated solution of *potassium hydroxide R*. Shake the aqueous layer with 2 quantities, each of 50 ml, of *light petroleum R1*. Combine the light petroleum layers in a 3rd separating funnel and wash with 3 quantities, each of 50 ml, of *ethanol (50 per cent V/V) R*. Transfer the light petroleum layer to a tared 250 ml flask. Rinse the separating funnel with small quantities of *light petroleum R1* and add to the flask. Evaporate the light petroleum on a water-bath and dry the residue at 100-105 °C for 15 min, keeping the flask horizontal. Allow to cool in a desiccator and weigh (*a g*). Repeat the drying for successive periods of 15 min until the loss of mass between 2 successive weighings does not exceed 0.1 per cent. Dissolve the residue in 20 ml of *ethanol (96 per cent) R*, previously neutralised to 0.1 ml of *bromophenol blue solution R*. If necessary, titrate with *0.1 M hydrochloric acid* (*b* ml).

Calculate the percentage content of unsaponifiable matter using the following expression:

$$\frac{100(a - 0.032b)}{m}$$

If 0.032*b* is greater than 5 per cent of *a*, the test is not valid and must be repeated.

Absorbance ***(2.2.25)***

Maximum 0.20 at 270 nm. The ratio of the absorbance at 232 nm to that at 270 nm is greater than 8.

Dissolve 1.00 g in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Composition of fatty acids ***(2.4.22, Method A)***

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

- *saturated fatty acids of chain length less than C_{16}*: maximum 0.1 per cent;
- *palmitic acid*: 7.5 per cent to 20.0 per cent;
- *palmitoleic acid* : maximum 3.5 per cent;
- *stearic acid*: 0.5 per cent to 5.0 per cent;
- *oleic acid* : 56.0 per cent to 85.0 per cent;
- *linoleic acid* : 3.5 per cent to 20.0 per cent;
- *linolenic acid* : maximum 1.2 per cent;

— *arachidic acid*: maximum 0.7 per cent;

— *eicosenoic acid* : maximum 0.4 per cent;

— *behenic acid*: maximum 0.2 per cent;

— *lignoceric acid*: maximum 0.2 per cent.

Sterols *(2.4.23)*.

Composition of the sterol fraction of the oil:

— *cholesterol* : maximum 0.5 per cent;

— *campesterol*: maximum 4.0 per cent;

— *Δ7-stigmasterol* : maximum 0.5 per cent;

— *sum of contents of Δ5,23-stigmastadienol, clerosterol, β-sitosterol , sitostanol , Δ5-avenasterol and Δ5,24-stigmastadienol*: minimum 93.0 per cent.

The content of stigmasterol is not greater than that of campesterol.

Sesame oil

In a ground-glass-stoppered cylinder shake 10 ml for about 1 min with a mixture of 0.5 ml of a 0.35 per cent *V/V* solution of *furfural R* in *acetic anhydride R* and 4.5 ml of *acetic anhydride R*. Filter through a filter paper impregnated with *acetic anhydride R*. To the filtrate add 0.2 ml of *sulphuric acid R*. No bluish-green colour develops.

STORAGE

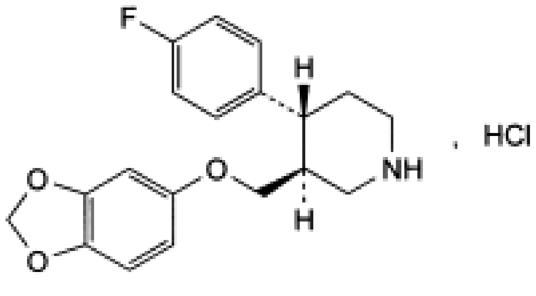
In a well-filled container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Anhydrous Paroxetine Hydrochloride

General Notices

(Ph Eur monograph 2283)



$C_{19}H_{20}FNO_3,HCl$ 365.8 *78246-49-8*

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

Preparation

Paroxetine Tablets

Ph Eur

DEFINITION

(3*S*,4*R*)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine hydrochloride anhydrous.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

Impurity G

Maximum 1 ppm, determined by a suitable, validated method.

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in methanol, sparingly soluble in anhydrous ethanol and in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *anhydrous paroxetine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, mix 1 part of the substance to be examined and 1 part of the reference substance separately with 30 parts of *anhydrous acetone R* and heat to boiling to dissolve. Recrystallise and record new spectra using the residues.

B. Water (see Tests).

C. It gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Impurity D

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 5 ml of *methanol R* and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *paroxetine impurity D CRS* in 2 ml of *methanol R* and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the test solution.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Column:

- *size:* l = 0.10 m, Ø = 4.0 mm;
- *stationary phase*: *silica gel AGP for chiral chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase Dissolve 8.7 g of *dipotassium hydrogen phosphate R* in 1000 ml of water for chromatography *R* and adjust to pH 6.5 with *phosphoric acid R*; mix 930 ml of this solution and 70 ml of *acetonitrile R*.

Flow rate 0.9 ml/min.

Detection Spectrophotometer at 295 nm.

Injection 20 µl of the test solution and reference solutions (b) and (c).

Run time 2.5 times the retention time of paroxetine which is about 12 min.

System suitability:

- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to paroxetine in the chromatogram obtained with reference solution (b);

— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (c);

— *symmetry factor*: the requirements stated in chapter 2.2.46 are not applicable.

Limit:

— *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent).

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *tetrahydrofuran R*, *water R* (10:90 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 5.0 ml of the test solution to 50.0 ml with the solvent mixture.

Reference solution (b) Dissolve 5.0 mg of *anhydrous paroxetine hydrochloride impurity H CRS* in 25 ml of *tetrahydrofuran R* and dilute to 50.0 ml with *water R*.

Reference solution (c) Dissolve 5 mg of *anhydrous paroxetine hydrochloride impurity C CRS* in 25 ml of *tetrahydrofuran R* and dilute to 50.0 ml with *water R*.

Reference solution (d) To 5.0 ml of reference solution (a) add 1.0 ml of reference solution (b) and dilute to 100.0 ml with the solvent mixture.

Reference solution (e) To 5.0 ml of reference solution (a) add 5.0 ml of reference solution (b) and 5.0 ml of reference solution (c). Dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (f) Dissolve 2.5 mg of *paroxetine impurity E CRS* in the solvent mixture, add 2.5 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Reference solution (g) Dissolve 5 mg of *paroxetine impurity A CRS* in the solvent mixture and dilute to 50 ml with the solvent mixture. Use this solution to identify the peak due to impurity A.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

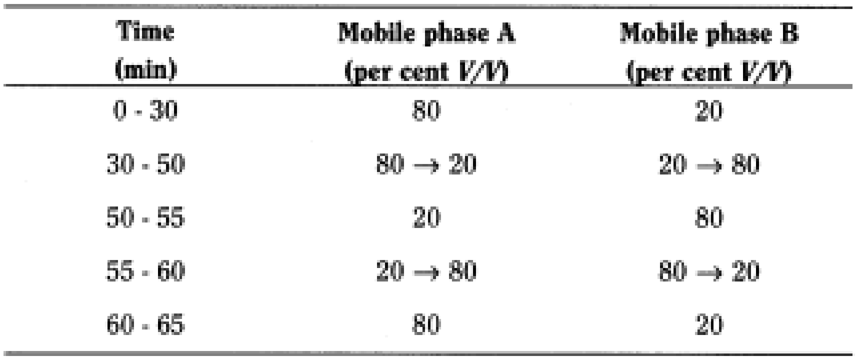
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: *trifluoroacetic acid R*, *tetrahydrofuran R*, *water R* (5:100:900 *V/V/V*);

— *mobile phase B*: *trifluoroacetic acid R*, *tetrahydrofuran R*, *acetonitrile R* (5:100:900 *V/V/V*) ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	80	20
30 - 50	80 → 20	20 → 80
50 - 55	20	80
55 - 60	20 → 80	80 → 20
60 - 65	80	20

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 295 nm.

Injection 20 µl of the test solution and reference solutions (d), (e), (f) and (g).

Relative retention With reference to paroxetine (retention time = about 28 min): impurity A = about 0.8; impurity E = about 0.9; impurity C = about 1.5.

Relative retention With reference to impurity C: impurity F = about 0.97; impurity J = about 1.02.

System suitability:

— *resolution*: minimum 3.5 between the peaks due to impurity E and paroxetine in the chromatogram obtained with reference solution (f);

— *signal-to-noise ratio*: minimum 3 for the peak due to paroxetine in the chromatogram obtained with reference solution (e).

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.6; impurity F = 1.7; impurity J = 1.3;

— *impurity A*: not more than 0.6 times the area of the peak due to paroxetine in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *impurities C, F, J*: for each impurity, not more than 0.2 times the area of the peak due to paroxetine in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the peak due to paroxetine in the chromatogram obtained with reference solution (d) (0.10 per cent);

— *total*: not more than the area of the peak due to paroxetine in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *disregard limit*: the area of the peak due to paroxetine in the chromatogram obtained with reference solution (e) (0.05 per cent).

Impurities H and I

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 263 nm.

Injection Test solution and reference solutions (d) and (e).

Relative retention With reference to paroxetine (retention time = about 28 min): impurity I = about 0.2; impurity H = about 0.4.

System suitability Reference solution (e):

— *signal-to-noise ratio*: minimum 3 for the peak due to impurity H.

Limits:

— *impurities H, I:* for each impurity, not more than the area of the peak due to impurity H in the chromatogram obtained with reference solution (d) (0.1 per cent).

Acetone *(2.4.24, System B)*

Maximum 3.5 per cent.

2-Propanol *(2.4.24, System B)*

Maximum 4.3 per cent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Use a platinum crucible. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

Maximum 1.5 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 51.2 mg of *paroxetine hydrochloride hemihydrate CRS* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dissolve 5.0 mg of *paroxetine hydrochloride hemihydrate CRS* and 5 mg of *paroxetine impurity A CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *trimethylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.85 g of *ammonium acetate R* in *water R*, adjust to pH 5.5 with *anhydrous acetic acid R* and dilute to 600 ml with *water R*; add 400 ml of *acetonitrile R*; slowly add, with stirring, 10 ml of *triethylamine R* and adjust to pH 5.5 with *anhydrous acetic acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 295 nm.

Injection 10 µl.

Run time Twice the retention time of paroxetine.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to paroxetine and impurity A.

Calculate the percentage content of $C_{19}H_{21}ClFNO_3$ using the chromatogram obtained with reference solution (a) and the declared content of *paroxetine hydrochloride hemihydrate CRS*.

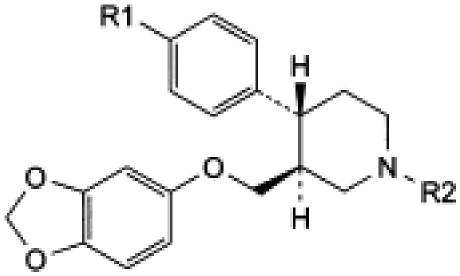
STORAGE

In an airtight container , at a temperature not exceeding 25 °C.

IMPURITIES

Specified impurities A, C, D, F, G, H, I, J.

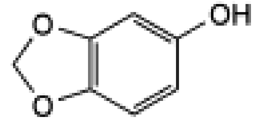
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, E.*



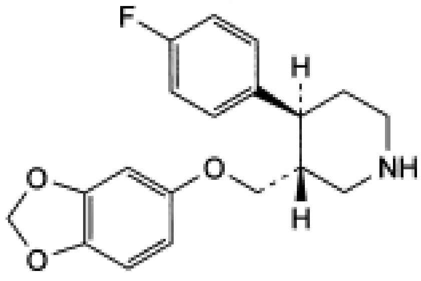
A. R1 = R2 = H: (3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-phenylpiperidine (desfluoroparoxetine),

C. R1 = F, R2 = CH_2-C_6H_5: (3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-1-benzyl-4-(4-fluorophenyl)piperidine (*N*-benzylparoxetine),

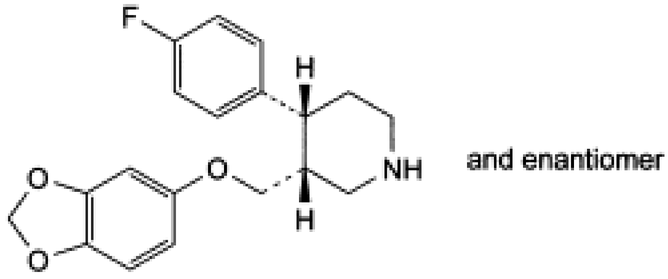
F. R1 = H, R2 = CH_2-C_6H_5: (3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-1-benzyl-4-phenylpiperidine (*N*-benzyldesfluoroparoxetine),



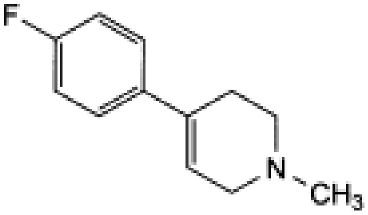
B. 1,3-benzodioxol-5-ol (sesamol),



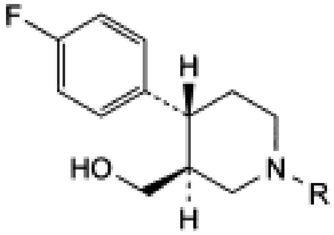
D. (3*R*,4*S*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine ((+)-*trans*-paroxetine),


F
H
O
NH
and enantiomer
O
O
H

E. (3*RS*,4*RS*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine (*cis*-paroxetine),

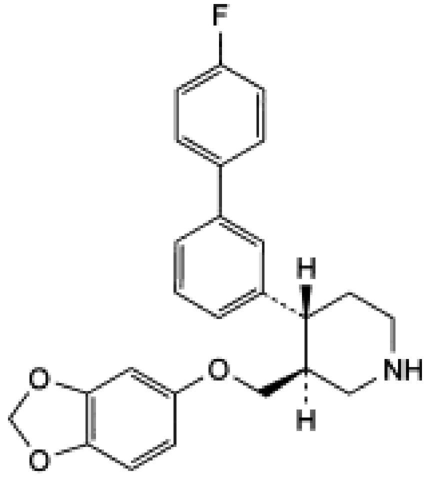


G. 4-(4-fluorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine,



H. R = CH_2-C_6H_5: [(3*S*,4*R*)-1-benzyl-4-(4-fluorophenyl)piperidin-3-yl]methanol,

I. R = H: [(3*S*,4*R*)-4-(4-fluorophenyl)piperidin-3-yl]methanol,



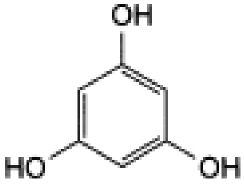
J. (3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4′-fluorobiphenyl-3-yl)piperidine.

Ph Eur

Anhydrous Phloroglucinol

General Notices

(Ph Eur monograph 2301)



$C_6H_6O_3$ 126.1 *108-73-6*

Action and use

Antispasmodic.

Ph Eur

DEFINITION

Benzene-1,3,5-triol.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Sparingly soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison anhydrous phloroglucinol CRS.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.20 g of *anhydrous phloroglucinol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate F_{254} R.*

Mobile phase *anhydrous formic acid R*, *hexane R*, *ethyl acetate R* (2:37.5:62.5 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

4.0 to 6.0.

Dilute 10 ml of solution S to 100 ml with *carbon dioxide-free water R.*

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 3 mg of *resorcinol R* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *nitrile silica gel for chromatography R* (5 µm).

Mobile phase Mix 3 volumes of *acetonitrile R* and 97 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 10 µl.

Run time 5 times the retention time of phloroglucinol.

Retention time Phloroglucinol = about 5 min.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to phloroglucinol and resorcinol.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 500 ppm.

Dilute 3 ml of solution S to 15 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 50 ml of *water R*. Titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *1 M sodium hydroxide* is equivalent to 63.05 mg of $C_6H_6O_3$.

STORAGE

Protected from light.

Ph Eur

Dilute Phosphoric Acid

General Notices

(Ph Eur monograph 0005)

Ph Eur

DEFINITION

Content

9.5 per cent *m/m* to 10.5 per cent *m/m* of H_3PO_4 (M_r 98.0).

PREPARATION

To 885 g of *water R* add 115 g of concentrated phosphoric acid and mix.

IDENTIFICATION

A. It is strongly acid (*2.2.4*).

B. Solution S (see Tests), neutralised with *dilute sodium hydroxide solution R*, gives the reactions of phosphates (*2.3.1*).

TESTS

Solution S

Dilute 86 g to 150 ml with *water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Substances precipitated with ammonia

To 10 ml of solution S add 8 ml of *dilute ammonia R1*. Any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution S and 8 ml of *water R*.

Hypophosphorous acid and phosphorous acid

To 5 ml of solution S add 2 ml of *silver nitrate solution R2* and heat on a water-bath for 5 min. The solution shows no change in appearance.

Chlorides (*2.4.4*)

Maximum 6 ppm, determined on solution S.

Sulphates (*2.4.13*)

Maximum 10 ppm, determined on the preparation to be examined.

Arsenic (*2.4.2, Method A*)

Maximum 0.2 ppm, determined on 7.5 ml of solution S.

Iron (*2.4.9*)

Maximum 6 ppm.

Dilute 3 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 1 ppm.

To 20 g add 4 ml of *dilute ammonia R1* and dilute to 25 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using a mixture of 2 ml of *water R* and 8 ml of *lead standard solution (1 ppm Pb) R*.

ASSAY

To 8.60 g add a solution of 10 g of *sodium chloride R* in 30 ml of *water R*. Titrate with *1 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

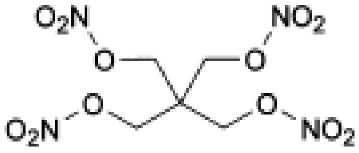
1 ml of *1 M sodium hydroxide* is equivalent to 49.00 mg of H_3PO_4.

Ph Eur

Diluted Pentaerythrityl Tetranitrate

General Notices

(Ph Eur monograph 1355)



$C_5H_8N_4O_{12}$ 316.1 *78-11-5*

Action and use

Vasodilator.

Ph Eur

DEFINITION

Dry mixture of 2,2-bis(hydroxymethyl)propane-1,3-diol tetranitrate (pentaerythrityl tetranitrate) and *Lactose monohydrate (0187)* or *Mannitol (0559).*

Content

95.0 per cent m/m to 105.0 per cent *m/m* of the declared content of pentaerythrityl tetranitrate.

CAUTION

Undiluted pentaerythrityl tetranitrate may explode if subjected to percussion or excessive heat. Appropriate precautions must be taken and only very small quantities handled.

CHARACTERS

Appearance of pentaerythrityl tetranitrate

White or slightly yellowish powder.

Solubility of pentaerythrityl tetranitrate

Practically insoluble in water, soluble in acetone, slightly soluble in ethanol (96 per cent).

The solubility of diluted pentaerythrityl tetranitrate depends on the diluent and its concentration.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Separately shake a quantity of the substance to be examined and a quantity of the reference substance, each corresponding to 25 mg of pentaerythrityl tetranitrate, with 10 ml of *acetone R* for 5 min; filter, evaporate to dryness at a temperature below 40 °C, and dry the residue over *diphosphorus pentoxide R* at a pressure of 0.7 kPa for 16 h. Examine the residues prepared as discs.

Comparison *Diluted pentaerythrityl tetranitrate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Shake a quantity of the substance to be examined corresponding to 10 mg of pentaerythrityl tetranitrate with 10 ml of *ethanol (96 per cent) R* for 5 min and filter.

Reference solution Shake a quantity of *diluted pentaerythrityl tetranitrate CRS* corresponding to 10 mg of pentaerythrityl tetranitrate with 10 ml of *ethanol (96 per cent) R* for 5 min and filter.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (20:80 *V/V).*

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with freshly prepared *potassium iodide and starch solution R*, expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Thin-layer chromatography (*2.2.27*).

Test solution Shake a quantity of the substance to be examined corresponding to 0.10 g of lactose or mannitol with 10 ml of *water R*. Filter if necessary.

Reference solution (a) Dissolve 0.10 g of *lactose R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 0.10 g of *mannitol R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (c) Mix equal volumes of reference solutions (a) and (b).

Plate *TLC silica gel G plate R.*

Mobile phase *water R*, *methanol R*, *anhydrous acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*). Measure the volumes accurately since a slight excess of water produces cloudiness.

Application 1 µl; thoroughly dry the starting points.

Development A Over 2/3 of the plate.

Drying A In a current of warm air.

Development B Immediately, over 2/3 of the plate, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with *4-aminobenzoic acid solution R*, dry in a current of cold air until the acetone is removed, then heat at 100 °C for 15 min; allow to cool, spray with a 2 g/l solution of *sodium periodate R*, dry in a current of cold air and heat at 100 °C for 15 min.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) for lactose or to the principal spot in the chromatogram obtained with reference solution (b) for mannitol.

TESTS

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution Shake a quantity of the substance to be examined corresponding to 0.10 g of pentaerythrityl tetranitrate with 5 ml of *ethanol (96 per cent) R* and filter.

Reference solution Dissolve 10 mg of *potassium nitrate R* in 1 ml of *water R* and dilute to 100 ml with *ethanol (96 per cent) R.*

Plate TLC silica gel plate R.

Mobile phase glacial acetic acid R, *acetone R*, *toluene R* (15:30:60 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of air until the acetic acid is completely removed.

Detection Spray copiously with freshly prepared *potassium iodide and starch solution R*, expose the plate to ultraviolet light at 254 nm for 15 min and examine in daylight.

Limit:

— *nitrate*: any spot due to nitrate is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Sonicate for 15 min a quantity of the substance to be examined corresponding to 25.0 mg of pentaerythrityl tetranitrate in 20 ml of the mobile phase and dilute to 25.0 ml with the mobile phase. Filter.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a) Sonicate for 15 min a quantity of *diluted pentaerythrityl tetranitrate CRS* corresponding to 25.0 mg of pentaerythrityl tetranitrate in 20 ml of the mobile phase and dilute to 25.0 ml with the mobile phase. Filter.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 0.3 ml of reference solution (b) to 10.0 ml with the mobile phase.

Reference solution (d) Dilute 200 µl of *glyceryl trinitrate solution CRS* to 25.0 ml with the mobile phase.

Reference solution (e) To 1 ml of reference solution (b) add 1 ml of reference solution (d) and dilute to 10 ml with the mobile phase.

Reference solution (f) Dilute 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase. Dilute 0.5 ml of this solution to 50.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R*, *acetonitrile R* (35:65 *V/V*).

Flow rate 1.4 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (a) and reference solutions (c), (e) and (f).

Run time 5 times the retention time of pentaerythrityl tetranitrate.

Relative retention With reference to pentaerythrityl tetranitrate (retention time = about 2.4 min): impurity B = about 0.7; impurity C = about 3.0.

System suitability Reference solution (e):

— *resolution*: minimum 3.0 between the peaks due to glyceryl trinitrate and pentaerythrityl tetranitrate.

Limits:

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (f) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (f) (0.05 per cent).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (b).

Calculate the percentage content of $C_5H_8N_4O_{12}$ from the declared content of *diluted pentaerythrityl tetranitrate CRS* .

STORAGE

Protected from light and heat.

LABELLING

The label states:

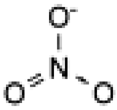
— the percentage content of pentaerythrityl tetranitrate;

— the diluent used.

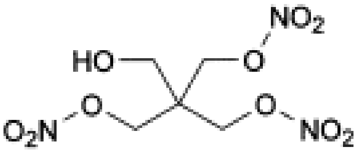
IMPURITIES

Specified impurities *A, C.*

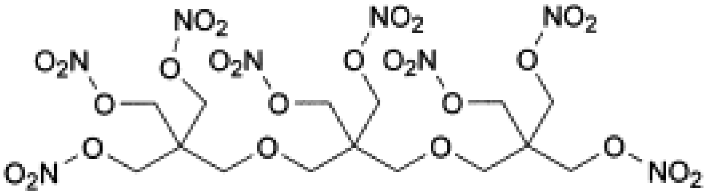
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, D.*



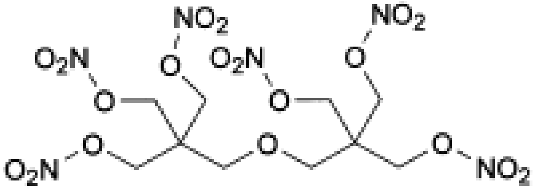
A. NO_3^-: inorganic nitrates,



B. 2,2-bis(hydroxymethyl)propane-1,3-diol trinitrate (pentaerythritol trinitrate),



C. 2,2-bis[[3-hydroxy-2,2-bis(hydroxymethyl)propoxy]methyl]propane-1,3-diol octanitrate (tripentaerythrityl octanitrate),



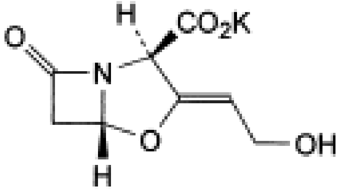
D. 2,2′-(oxybismethylene)bis[2-(hydroxymethyl)propane-1,3-diol] hexanitrate (dipentaerythrityl hexanitrate).

Ph Eur

Diluted Potassium Clavulanate

General Notices

(Ph Eur monograph 1653)



$C_8H_8KNO_5$ 237.3

Action and use

Beta-lactamase inhibitor.

Preparation

Co-amoxiclav Tablets

Ph Eur

DEFINITION

Dry mixture of *Potassium clavulanate (1140)* and *Cellulose, microcrystalline (0316)* or *Silica, colloidal anhydrous (0434)* or *Silica, colloidal hydrated (0738)*.

Content

91.2 per cent to 107.1 per cent of the content of potassium clavulanate stated on the label.

CHARACTERS

Appearance of diluted potassium clavulanate

White or almost white powder, hygroscopic.

Solubility of potassium clavulanate

Freely soluble in water, slightly soluble in alcohol, very slightly soluble in acetone.

The solubility of the diluted product depends on the diluent and its concentration.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. It gives reaction (b) of potassium (*2.3.1*).

C. Depending on the diluent used, carry out the corresponding identification test (a) or (b).

(a) A quantity of the substance to be examined, corresponding to 20 mg of cellulose, when placed on a watch-glass and dispersed in 4 ml of *iodinated zinc chloride solution R*, becomes violet-blue.

(b) It gives the reaction of silicates (*2.3.1*).

TESTS

pH (*2.2.3*)

4.8 to 8.0.

Suspend a quantity of the substance to be examined corresponding to 0.200 g of potassium clavulanate in 20 ml of *carbon dioxide-free water R*.

Absorbance (*2.2.25*)

Maximum 0.40 measured immediately at 278 nm.

Disperse a quantity of the substance to be examined corresponding to 50.0 mg of potassium clavulanate in 10 ml of *0.1 M phosphate buffer solution pH 7.0 R*, dilute to 50.0 ml with the same buffer solution and filter.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

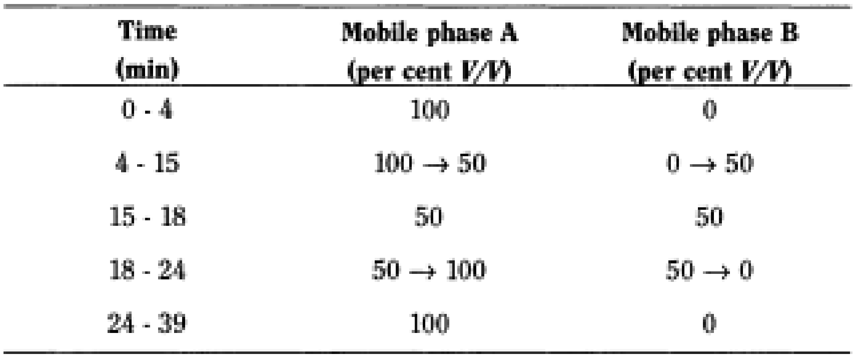
Test solution Disperse a quantity of the substance to be examined corresponding to 0.250 g of potassium clavulanate in 5 ml of mobile phase A, dilute to 25.0 ml with mobile phase A and filter.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b) Dissolve 10 mg of *amoxicillin trihydrate CRS* in 1 ml of the test solution and dilute to 100 ml with mobile phase A.

Column:
- *size:* l = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: 7.8 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 4.0 with *dilute phosphoric acid R*;
- *mobile phase B*: mixture of equal volumes of mobile phase A and *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 15	100 → 50	0 → 50
15 - 18	50	50
18 - 24	50 → 100	50 → 0
24 - 39	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum of 13 between the peak due to clavulanate (1st peak) and the peak due to amoxicillin (2nd peak).

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained disregard limit: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 2.5 per cent, determined on 1.000 g.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Test solution Disperse a quantity of the substance to be examined corresponding to 50.0 mg of potassium clavulanate in a 4.1 g/l solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R*, dilute to 50.0 ml with the same solution and filter.

Reference solution (a) Dissolve 50.0 mg of *lithium clavulanate CRS* in a 4.1 g/l solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R* and dilute to 50.0 ml with the same solution.

Reference solution (b) Dissolve 10 mg of *amoxicillin trihydrate CRS* in 10 ml of reference solution (a).

Column:

— *size: l* = 0.3 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 5 volumes of *methanol R1* and 95 volumes of a 15 g/l solution of *sodium dihydrogen phosphate R* previously adjusted to pH 4.0 with *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

System suitability Reference solution (b):

— *resolution*: minimum 3.5 between the peak due to clavulanate (1^{st} peak) and the peak due to amoxicillin (2^{nd} peak).

1 mg of $C_8H_9NO_5$ is equivalent to 1.191 mg of $C_8H_8KNO_5$.

STORAGE

In an airtight container .

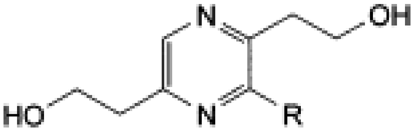
LABELLING

The label states the *m/m* percentage content of potassium clavulanate and the diluent used to prepare the mixture.

IMPURITIES

Specified impurities A, B, C, D, G.

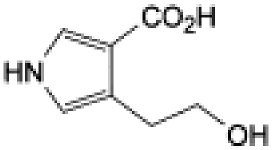
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F.*



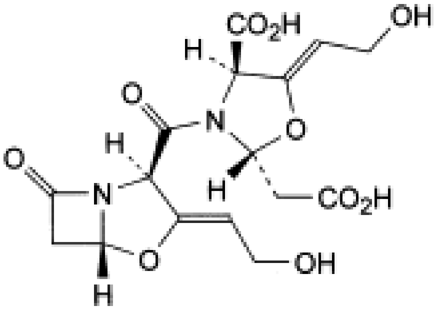
A. R = H: 2,2′-(pyrazine-2,5-diyl)diethanol,

B. R = CH_2-CH_2-CO_2H: 3-[3,6-bis(2-hydroxyethyl)pyrazin-2-yl]propanoic acid,

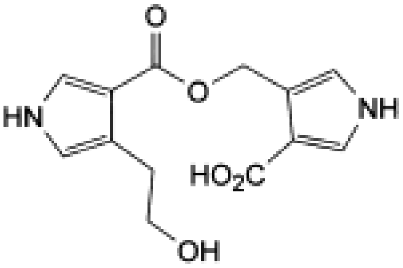
C. R = CH_2-CH_3: 2,2′-(3-ethylpyrazine-2,5-diyl)diethanol,



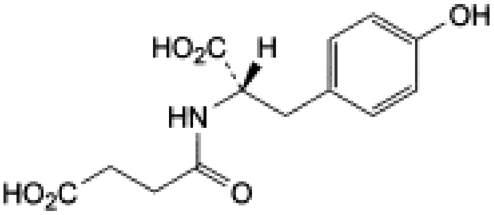
D. 4-(2-hydroxyethyl)-1*H*-pyrrole-3-carboxylic acid,



E. (2*R*,4*R*,5*Z*)-2-(carboxymethyl)-5-(2-hydroxyethylidene)-3-[[(2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxazolidine-4-carboxylic acid,



F. 4-[[[[4-(2-hydroxyethyl)-1*H*-pyrrol-3-yl]carbonyl]oxy]methyl]-1*H*-pyrrole-3-carboxylic acid,



G. 4-[[(1*S*)-1-carboxy-2-(4-hydroxyphenyl)ethyl]amino]-4-oxobutanoic acid (*N*-succinyltyrosine).

Ph Eur

Fractionated Palm Kernel Oil

General Notices

The standards of this monograph encompass several different suppository bases. The selection of a basis for a particular suppository formulation should be appropriate to the product concerned and it may be necessary to apply more restrictive standards for a particular application.

Action and use

Suppository basis.

DEFINITION

Fractionated Palm Kernel Oil is obtained by expression of the natural oil from the kernels of *Elaeis guineensis* Jacq. followed by selective solvent fractionation and hydrogenation.

CHARACTERISTICS

A white, solid, brittle fat; odourless or almost odourless.

Practically insoluble in *water*; miscible with *ether* and with *petroleum spirit (boiling range, 40° to 60°)*; practically insoluble in *ethanol (96%)*.

TESTS

Acid value

Not more than 0.2, Appendix X B.

Iodine value

Not more than 6.0 *(iodine bromide method)*, Appendix X E.

Melting point

31° to 36°, Appendix V A, Method IV. Prepare the substance being examined in the following manner. Melt about 30 g in an oven at a temperature of 55° to 60° and filter through a suitable dry filter paper, maintaining the temperature between 53° and 60°. Cool with occasional stirring until the temperature falls to between 32° and 34°, stir continuously with a mechanical stirrer until the first signs of cloudiness appear and continue to stir by hand until the substance has the consistence of a paste. Immediately transfer to a vessel previously kept at a temperature of 15° to 22° and allow to stand at this temperature for 24 hours before carrying out the test.

Refractive index

At 50°, 1.445 to 1.447, Appendix V E.

Saponification value

246 to 250, Appendix X G.

Peroxides

Dissolve 5 g in 15 ml of *chloroform*, add 20 ml of *glacial acetic acid* and 0.5 ml of a saturated solution of *potassium iodide*, mix well, allow to stand in the dark for exactly 1 minute, add 30 ml of *water* and titrate with 0.01M *sodium thiosulphate VS* using *starch mucilage* as indicator. Not more than 0.5 ml of 0.01M *sodium thiosulphate VS* is required.

Hard Paraffin

General Notices

(Ph Eur monograph 1034)

Ph Eur

DEFINITION

A purified mixture of solid saturated hydrocarbons generally obtained from petroleum. It may contain a suitable antioxidant.

CHARACTERS

Appearance

Colourless or white or almost white mass; the melted substance is free from fluorescence in daylight.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, C.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *hard paraffin CRS.*

Preparation Place about 2 mg on a sodium chloride plate, heat in an oven at 100 °C for 10 min, spread the melted substance with another sodium chloride plate and remove one of the plates.

B. Acidity or alkalinity (see Tests).

C. Melting point *(2.2.16)*: 50 °C to 61 °C.

TESTS

Acidity or alkalinity

To 15 g add 30 ml of boiling *water R* and shake vigorously for 1 min. Allow to cool and to separate. To 10 ml of the aqueous layer add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to red. To a further 10 ml of the aqueous layer add 0.1 ml of *methyl red solution R*. The solution is yellow. Not more than 0.5 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Polycyclic aromatic hydrocarbons

Use reagents for ultraviolet absorption spectrophotometry. Dissolve 0.50 g in 25 ml of *heptane R* and place in a 125 ml separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 5.0 ml of *dimethyl sulphoxide R*. Shake vigorously for 1 min and allow to stand until 2 clear layers are formed. Transfer the lower layer to a 2nd separating funnel, add 2 ml of *heptane R* and shake the mixture vigorously. Allow to stand until 2 clear layers are formed. Separate the lower layer and measure its absorbance (*2.2.25*) between 265 nm and 420 nm using as the compensation liquid the clear lower layer obtained by vigorously shaking 5.0 ml of *dimethyl sulphoxide R* with 25 ml of *heptane R* for 1 min. Prepare a 7.0 mg/l reference solution of *naphthalene R* in *dimethyl sulphoxide R* and measure the absorbance of this solution at the absorption maximum at 278 nm using *dimethyl sulphoxide R* as the compensation liquid. At wavelengths from 265 nm to 420 nm, the absorbance of the test solution is not greater than one-third that of the reference solution at 278 nm.

Sulphates *(2.4.13)*

Maximum 150 ppm.

Introduce 2.0 g of the melted substance to be examined into a 50 ml ground-glass-stoppered separating funnel. Add 30 ml of boiling *distilled water R*, shake vigorously for 1 min and filter.

STORAGE

Protected from light.

Ph Eur

Iodinated Povidone

General Notices

(Ph Eur monograph 1142)

Action and use

Antiseptic.

Preparations

Povidone-Iodine Mouthwash

Povidone-Iodine Solution

Ph Eur

DEFINITION

Complex of iodine and povidone.

Content

9.0 per cent to 12.0 per cent of available iodine (dried substance).

PRODUCTION

It is produced using povidone that complies with the monograph on *Povidone (0685)*, except that the povidone used may contain not more than 2.0 per cent of formic acid and not more than 8.0 per cent of water.

CHARACTERS

Appearance

Yellowish-brown or reddish-brown, amorphous powder.

Solubility

Soluble in water and in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *iodinated povidone CRS.*

B. Dissolve 10 mg in 10 ml of *water R* and add 1 ml of *starch solution R*. An intense blue colour is produced.

C. Dissolve 0.1 g in 5 ml of *water R* and add a 10 g/l solution of *sodium sulphite R* dropwise, until the solution becomes colourless. Add 2 ml of *potassium dichromate solution R* and 1 ml of *hydrochloric acid R*. A light brown precipitate is formed.

TESTS

pH (*2.2.3*)

1.5 to 5.0.

Dissolve 1.0 g in 10 ml of *carbon dioxide-free water R*.

Iodide

Maximum 6.0 per cent (dried substance).

Dissolve 0.500 g in 100 ml of *water R*. Add *sodium metabisulphite R* until the colour of the iodine has disappeared. Add 25.0 ml of *0.1 M silver nitrate*, 10 ml of *nitric acid R* and 5 ml of *ferric ammonium sulphate solution R2*. Titrate with *0.1 M ammonium thiocyanate*. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 12.69 mg of total iodine. From the percentage of total iodine, calculated with reference to the dried substance, subtract the percentage of available iodine as determined in the assay to obtain the percentage of iodide.

Loss on drying (*2.2.32*)

Maximum 8.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Transfer 1.000 g into a ground-glass-stoppered flask containing 150 ml of *water R* and stir for 1 h. Add 0.1 ml of *dilute acetic acid R* and titrate with *0.1 M sodium thiosulphate* using *starch solution R* as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 12.69 mg of available iodine.

STORAGE

Protected from light.

Ph Eur

Light Liquid Paraffin

General Notices

(Ph Eur monograph 0240)

Preparation

Light Liquid Paraffin Eye Drops

Ph Eur

DEFINITION

Purified mixture of liquid saturated hydrocarbons obtained from petroleum.

CHARACTERS

Appearance

Colourless, transparent, oily liquid, free from fluorescence in daylight.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent), miscible with hydrocarbons.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison Ph. Eur. reference spectrum of liquid paraffin.

B. In a test tube cautiously boil 1 ml with 1 ml of *0.1 M sodium hydroxide*, with continuous shaking, for about 30 s. On cooling to room temperature, 2 phases separate. To the aqueous phase add 0.1 ml of *phenolphthalein solution R*. The solution becomes red.

C. Viscosity (see Tests).

TESTS

Acidity or alkalinity

To 10 ml add 20 ml of boiling *water R* and shake vigorously for 1 min. Separate the aqueous layer and filter. To 10 ml of the filtrate, add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Relative density *(2.2.5)*

0.810 to 0.875.

Viscosity *(2.2.9)*

25 mPa·s to 80 mPa·s.

Polycyclic aromatic hydrocarbons

Use reagents for ultraviolet spectrophotometry. Introduce 25.0 ml into a 125 ml separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 25 ml of *hexane R* which has been previously shaken twice with one-fifth its volume of *dimethyl sulphoxide R*. Mix and add 5.0 ml of *dimethyl sulphoxide R*. Shake vigorously for 1 min and allow to stand until 2 clear layers are formed. Transfer the lower layer to a 2nd separating funnel, add 2 ml of *hexane R* and shake the mixture vigorously. Allow to stand until 2 clear layers are formed. Separate the lower layer and measure its absorbance *(2.2.25)* between 260 nm and 420 nm, using as the compensation liquid the clear lower layer obtained by vigorously shaking 5.0 ml of *dimethyl sulphoxide R* with 25 ml of *hexane R* for 1 min. Prepare a 7.0 mg/l reference solution of *naphthalene R* in *trimethylpentane R* and measure the absorbance of the solution at the absorption maximum at 275 nm, using *trimethylpentane R* as the compensation liquid. At no wavelength between 260 nm and 420 nm does the absorbance of the test solution exceed one-third that of the reference solution at 275 nm.

Readily carbonisable substances

Use a ground-glass-stoppered tube about 125 mm long and 18 mm in internal diameter, graduated at 5 ml and 10 ml; wash with hot *water R* (temperature at least 60 °C), *acetone R*, *heptane R* and finally with *acetone R*, dry at 100-110 °C. Cool in a desiccator. Introduce 5 ml of the substance to be examined and add 5 ml of *nitrogen-free sulphuric acid R1*. Insert the stopper and shake as vigorously as possible, in the longitudinal direction of the tube, for 5 s. Loosen the stopper, immediately place the tube in a water-bath, avoiding contact of the tube with the bottom or side of the bath, and heat for 10 min. After 2 min, 4 min, 6 min and 8 min, remove the tube from the bath and shake as vigorously as possible, in the longitudinal direction of the tube for 5 s. At the end of 10 min of heating, remove the tube from the water-bath and allow to stand for 10 min. Centrifuge at 2000 *g* for 5 min. The lower layer is not more intensely coloured *(2.2.2, Method I)* than a mixture of 0.5 ml of blue primary solution, 1.5 ml of red primary solution, 3.0 ml of yellow primary solution and 2 ml of a 10 g/l solution of *hydrochloric acid R*.

Solid paraffins

Dry a suitable quantity of the substance to be examined by heating at 100 °C for 2 h and cool in a desiccator over *sulphuric acid R*. Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 h, the liquid is sufficiently clear for a black line, 0.5 mm wide, to be easily seen against a white background held vertically behind the tube.

STORAGE

Protected from light.

Ph Eur

Liquefied Phenol

General Notices

Action and use

Antiseptic; antimicrobial preservative; antipruritic.

When Phenol is to be mixed with collodion, fixed oils or paraffins, melted Phenol should be used and not Liquefied Phenol.

DEFINITION

Phenol	800 g
Purified Water	Sufficient to produce 1000 g

Extemporaneous preparation

The following directions apply.

Warm the Phenol on a water bath until it is melted, add the Purified Water and mix thoroughly.

Content of phenol, C_6H_6O

77.0 to 81.5% w/w.

CHARACTERISTICS

A colourless or faintly coloured liquid; caustic.

Soluble in *water*; miscible with *ethanol (96%)*, with *ether* and with *glycerol*.

IDENTIFICATION

A. Dissolve 0.6 g in 2 ml of 13.5M *ammonia* and dilute to 100 ml with *water*. To 2 ml of the resulting solution add 0.05 ml of *sodium hypochlorite solution*. A blue colour is produced which becomes progressively more intense.

B. Dilute 1 ml of a 15% w/v solution to 10 ml and add 0.1 ml of *iron(III) chloride solution R1*. A violet colour is produced which is discharged on the addition of *propan-2-ol*.

C. To 1 ml of a 15% w/v solution add 10 ml of *water* and 1 ml of *bromine water*. A white or yellowish white precipitate is produced.

TESTS

Acidity

To 2 ml of a 15% w/v solution add 0.05 ml of *methyl orange solution*. The solution is yellow.

Clarity and colour of solution

A solution of 1.0 ml in 14 ml of *water*, at 20°, is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* R_7 or B_7, Appendix IV B, Method II.

Weight per ml

1.055 to 1.060 g, Appendix V G.

Non-volatile matter

When heated on a water bath and dried at 105°, leaves not more than 0.05% w/v of residue.

ASSAY

Dissolve 2.5 g in sufficient *water* to produce 1000 ml, transfer 25 ml to a 500 ml glass-stoppered flask and add 50 ml of 0.05M *bromine VS* and 5 ml of *hydrochloric acid*, stopper, swirl occasionally during 30 minutes and allow to stand for 15 minutes. Add 5 ml of a 20% w/v solution of *potassium iodide* taking care to avoid loss of bromine, shake thoroughly and titrate with 0.1M *sodium thiosulphate VS* until only a faint yellow colour remains. Add 0.1 ml of *starch mucilage* and 10 ml of *chloroform* and complete the titration with vigorous shaking. Repeat the operation without the material being examined. The difference between the titrations represents the amount of bromine required. Each ml of 0.05M *bromine VS* is equivalent to 1.569 mg of C_6H_6O.

STORAGE

Liquefied Phenol should be protected from light. Liquefied Phenol may congeal or deposit crystals if stored at a temperature below 4°. It should be completely melted before use.

Liquid Paraffin

General Notices

(Ph Eur monograph 0239)

Action and use

Faecal softener.

Preparations

Liquid Paraffin Oral Emulsion

Liquid Paraffin and Magnesium Hydroxide Oral Emulsion

Ph Eur

DEFINITION

Purified mixture of liquid saturated hydrocarbons obtained from petroleum.

CHARACTERS

Appearance

Colourless, transparent, oily liquid, free from fluorescence in daylight.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent), miscible with hydrocarbons.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of liquid paraffin.

B In a test tube cautiously boil 1 ml with 1 ml of *0.1 M sodium hydroxide*, with continuous shaking, for about 30 s. On cooling to room temperature, 2 phases separate. To the aqueous phase add 0.1 ml of *phenolphthalein solution R*. The solution becomes red.

C. Viscosity (see Tests).

TESTS

Acidity or alkalinity

To 10 ml add 20 ml of boiling *water R* and shake vigorously for 1 min. Separate the aqueous layer and filter. To 10 ml of the filtrate, add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Relative density *(2.2.5)*

0.827 to 0.890.

Viscosity *(2.2.9)*

110 mPa·s to 230 mPa·s.

Polycyclic aromatic hydrocarbons

Use reagents for ultraviolet spectrophotometry Introduce 25.0 ml into a 125 ml separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 25 ml of *hexane R* which has been previously shaken twice with one-fifth its volume of *dimethyl sulphoxide R*. Mix and add 5.0 ml of *dimethyl sulphoxide R*. Shake vigorously for 1 min and allow to stand until 2 clear layers are formed. Transfer the lower layer to a 2^{d} separating funnel, add 2 ml of *hexane R* and shake the mixture vigorously. Allow to stand until 2 clear layers are formed. Separate the lower layer and measure its absorbance *(2.2.25)* between 260 nm and 420 nm, using as the compensation liquid the clear lower layer obtained by vigorously shaking 5.0 ml of *dimethyl sulphoxide R* with 25 ml of *hexane R* for 1 min. Prepare a 7.0 mg/l reference solution of *naphthalene R* in *trimethylpentane R* and measure the absorbance of the solution at the absorption maximum at 275 nm, using *trimethylpentane R* as the compensation liquid. At no wavelength between 260 nm and 420 nm does the absorbance of the test solution exceed one-third that of the reference solution at 275 nm.

Readily carbonisable substances

Use a ground-glass-stoppered tube about 125 mm long and 18 mm in internal diameter, graduated at 5 ml and 10 ml; wash with hot *water R* (temperature at least 60 °C), *acetone R*, *heptane R* and finally with *acetone R*, dry at 100-110 °C. Cool in a desiccator. Introduce 5 ml of the substance to be examined and add 5 ml of *nitrogen-free sulphuric acid R1*. Insert the stopper and shake as vigorously as possible, in the longitudinal direction of the tube, for 5 s. Loosen the stopper, immediately place the tube in a water-bath, avoiding contact of the tube with the bottom or side of the bath, and heat for 10 min. After 2 min, 4 min, 6 min and 8 min, remove the tube from the bath and shake as vigorously as possible, in the longitudinal direction of the tube for 5 s. At the end of 10 min of heating, remove the tube from the water-bath and allow to stand for 10 min. Centrifuge at 2000 *g* for 5 min. The lower layer is not more intensely coloured *(2.2.2*, *Method I*) than a mixture of 0.5 ml of blue primary solution, 1.5 ml of red primary solution, 3.0 ml of yellow primary solution and 2 ml of a 10 g/l solution of *hydrochloric acid R*.

Solid paraffins

Dry a suitable quantity of the substance to be examined by heating at 100 °C for 2 h and cool in a desiccator over *sulphuric acid R*. Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 h, the liquid is sufficiently clear for a black line, 0.5 mm wide, to be easily seen against a white background held vertically behind the tube.

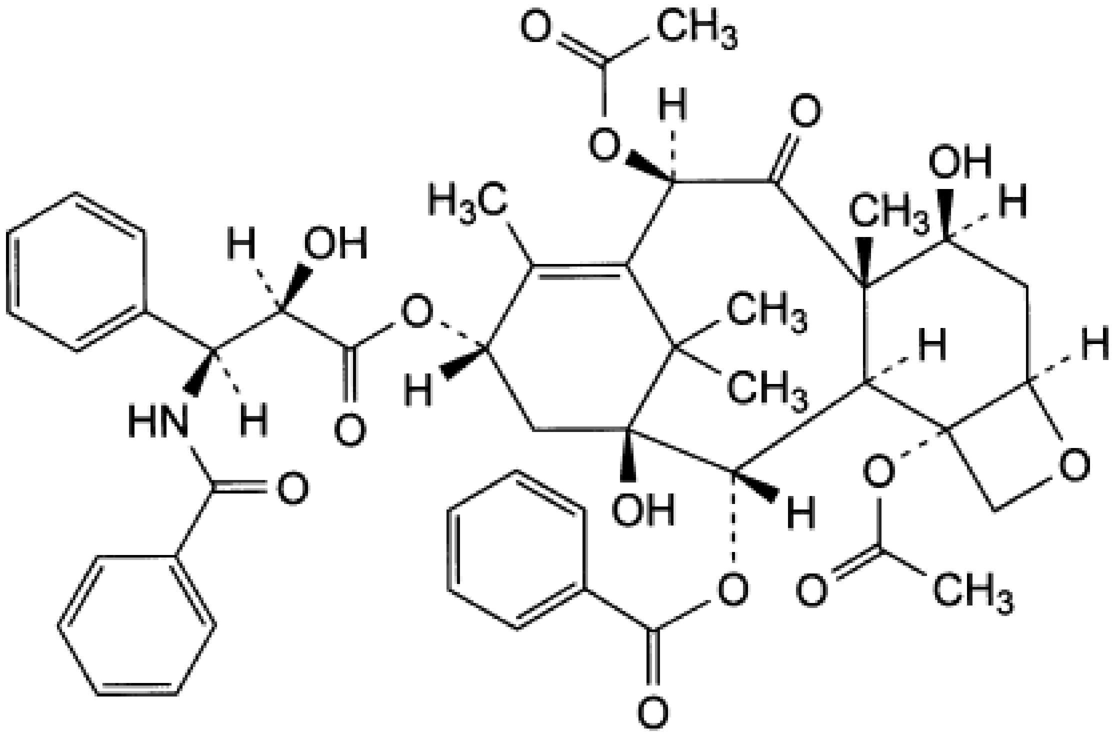
STORAGE

Protected from light.

Paclitaxel

General Notices

(Ph Eur monograph 1794)



$C_{47}H_{51}NO_{14}$ 854 *33069-62-4*

Action and use

Taxane cytotoxic.

Ph Eur

DEFINITION

5β,20-Epoxy-1,7β-dihydroxy-9-oxotax-11-ene-2α,4,10β,13α-tetrayl 4,10-diacetate 2-benzoate 13-[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoate].

It is isolated from natural sources or produced by fermentation or by a semi-synthetic process.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in methanol and freely soluble in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison paclitaxel CRS.

If the spectra obtained in the solid state show differences, dissolve 10 mg of the substance to be examined and the reference substance separately in 0.4 ml of *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of the solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.1 g in 10 ml of *methanol R*.

Specific optical rotation *(2.2.7)*

- 49.0 to - 55.0 (anhydrous substance).

Dissolve 0.250 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

A. Paclitaxel isolated from natural sources or produced by fermentation.

Test solution (a) Dissolve 20.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 20.0 ml with *acetonitrile R.*

Reference solution (a) Dilute 1.0 ml of test solution (a) to 10.0 ml with *acetonitrile R.* Dilute 1.0 ml of this solution to 100.0 ml with *acetonitrile R*.

Reference solution (b) Dissolve 5.0 mg of *paclitaxel CRS* in *acetonitrile R* and dilute to 5.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with *acetonitrile R.*

Reference solution (c) Dissolve 2.0 mg of *paclitaxel impurity C CRS* in *acetonitrile R* and dilute to 20.0 ml with the same solvent.

Reference solution (d) Dilute 1.0 ml of reference solution (c) to 50.0 ml with *acetonitrile R.*

Reference solution (e) To 1 ml of reference solution (b) add 1 ml of reference solution (c).

Reference solution (f) Dissolve 5 mg of *paclitaxel natural for peak identification CRS* (containing impurities A, B, C, D, E, F, H, O, P, Q and R) in *acetonitrile R* and dilute to 5 ml with the same solvent.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

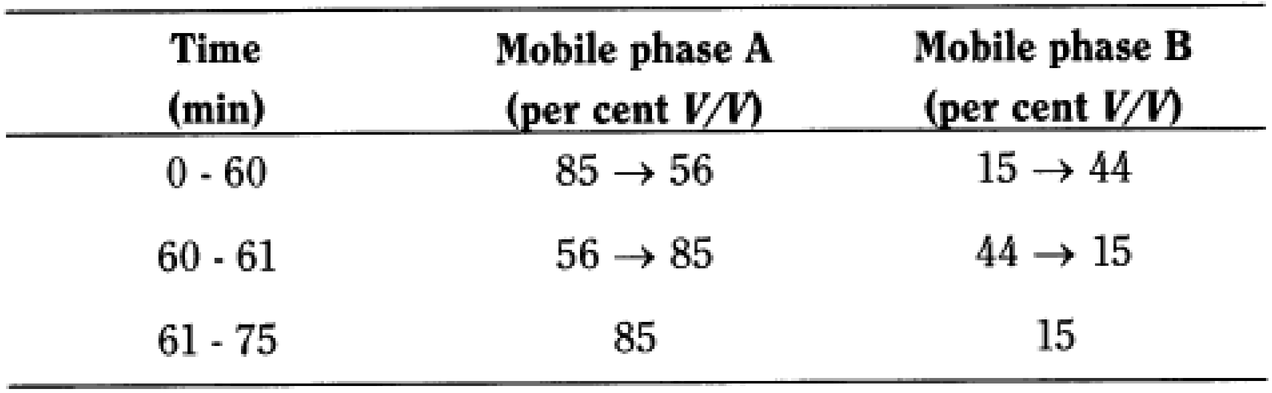
— *stationary phase*: *diisopropylcyanopropylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 180 m2/g and a pore size of 80 Å;

— *temperature*: 20 ± 1 °C.

Mobile phase:

— *mobile phase A*: *methanol R*, *water R* (200:800 *V/V*);

— *mobile phase B*: *methanol R*, *acetonitrile for chromatography R* (200:800 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	85 → 56	15 → 44
60 - 61	56 → 85	44 → 15
61 - 75	85	15

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 227 nm.

Injection 10 µl of test solution (a) and reference solutions (a), (d), (e) and (f).

Identification of impurities Use the chromatogram supplied with *paclitaxel natural for peak identification CRS* and the chromatogram obtained with reference solution (f) to identify the peaks due to impurities A, B, C, D, E, F, H, O, P, Q and R.

Relative retention With reference to paclitaxel (retention time = about 50 min): impurities A and B = about 0.90; impurity R = about 0.93; impurity H = about 0.96; impurities Q and P = about 1.02; impurity C = about 1.05; impurity D = about 1.07; impurities O and E = about 1.15; impurity F = about 1.20.

System suitability Reference solution (e):

— *resolution*: minimum 3.5 between the peaks due to paclitaxel and impurity C.

Limits:

— *sum of impurities A and B*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *impurity C*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *sum of impurities E and O*: not more than 5 times the area of the principal peak in the

chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *sum of impurities P and Q*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity R*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

B. Paclitaxel produced by a semi-synthetic process.

Test solution Dissolve 10.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 100.0 ml with *acetonitrile R.*

Reference solution (b) Dissolve 5.0 mg of *paclitaxel CRS* in *acetonitrile R* and dilute to 5.0 ml with the same solvent.

Reference solution (c) Dissolve 5 mg of *paclitaxel semi-synthetic for peak identification CRS* (containing impurities A, G, I and L) in *acetonitrile R* and dilute to 5 ml with the same solvent.

Reference solution (d) Dissolve 5.0 mg of *paclitaxel semi-synthetic for system suitability CRS* (containing impurities E, H and N) in *acetonitrile R* and dilute to 5.0 ml with the same solvent.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm) with a specific surface area of 300 m2/g and a pore size of 120 Å;

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile for chromatography R, water R* (400:600 *V/V*);

— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 20	100	0
20 - 60	100 → 10	0 → 90
60 - 62	10 → 100	90 → 0
62 - 70	100	0

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 227 nm.

Injection 15 µl of the test solution and reference solutions (a), (c) and (d).

Identification of impurities Use the chromatogram supplied with *paclitaxel semi-synthetic for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, G, I and L; use the chromatogram supplied with *paclitaxel semi-synthetic for system suitability CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities E, H and N.

Relative retention With reference to paclitaxel (retention time = about 23 min): impurity N = about 0.2; impurity G = about 0.5; impurity A = about 0.8; impurities M, J and H = about 0.9; impurity E = about 1.3; impurity I = about 1.4; impurity L = about 1.5; impurity K = about 2.2.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to impurity H and paclitaxel.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity N by 1.29;

— *impurity A*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

— *impurities E, I*: for each impurity, not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *impurities G, K, N*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *sum of impurities H, J and M*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *impurity L*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 12 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in *methanol R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test B. Prepare the reference solution using 10 ml of lead standard solution (1 ppm Pb), obtained by diluting *lead standard solution (100 ppm Pb) R* with *methanol R* and 2 ml of the test solution. To 12 ml of each solution, add 2 ml of *buffer solution pH 3.5 R*. Mix. Add 1.2 ml of *thioacetamide reagent R*. The substance will precipitate. Dilute to 40 ml with *methanol R*; the substance re-dissolves completely. Filter the solution through a membrane filter (pore size: 0.45 µm). Compare the spots on the filters obtained with the different solutions. The substance to be examined complies with the test if any brownish-black colour in the spot of the test solution is not more intense than that in the reference solution.

Water *(2.5.32)*

Maximum 3.0 per cent, determined on 0.050 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^2 bacteria and not more than 10^1 fungi per gram determined by plate count. It complies with the tests for *Escherichia coli, Salmonella*, *Pseudomonas aeruginosa* and *Staphylococccus aureus (2.6.13)*.

Bacterial endotoxins *(2.6.14)*

Less than 0.4 IU/mg.

ASSAY

A. Paclitaxel isolated from natural sources or produced by fermentation.

Liquid chromatography *(2.2.29)* as described in test A for related substances with the following modification.

Injection Test solution (b) and reference solution (b).

Calculate the percentage content of $C_{47}H_{51}NO_{14}$ from the declared content of *paclitaxel CRS*.

B. Paclitaxel produced by a semi-synthetic process.

Liquid chromatography *(2.2.29)* as described in test B for related substances with the following modification.

Injection 10 µl of the test solution and reference solution (b).

Calculate the percentage content of $C_{47}H_{51}NO_{14}$ from the declared content of *paclitaxel CRS*.

STORAGE

In an airtight container , protected from light.

LABELLING

The label states the origin of the substance:

— isolated from natural sources;

— produced by fermentation;

— produced by a semi-synthetic process.

IMPURITIES

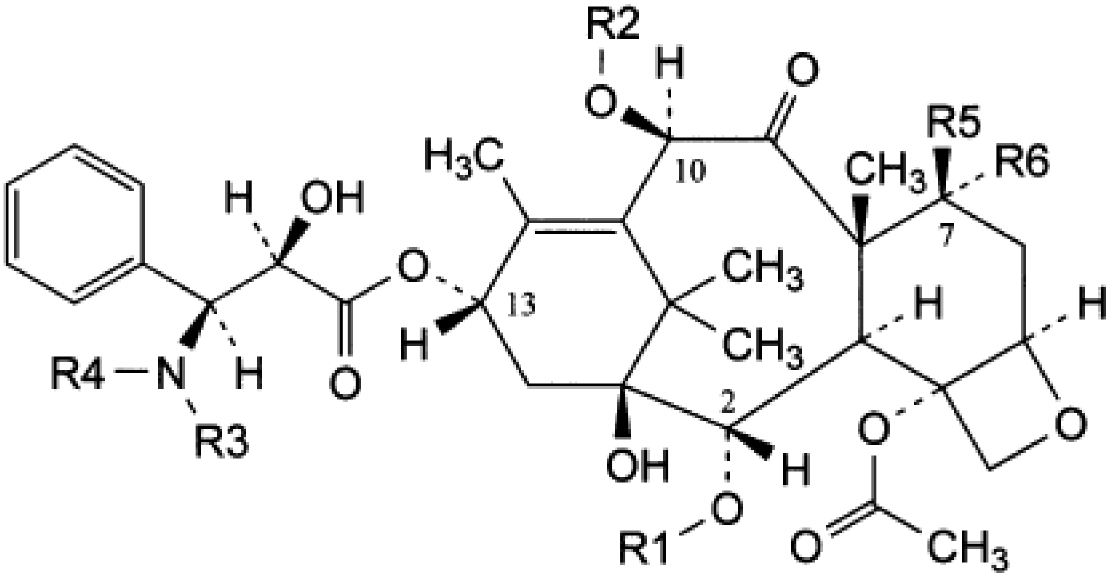
Test A

Specified impurities *A, B, C, D, E, F, O, P, Q, R.*

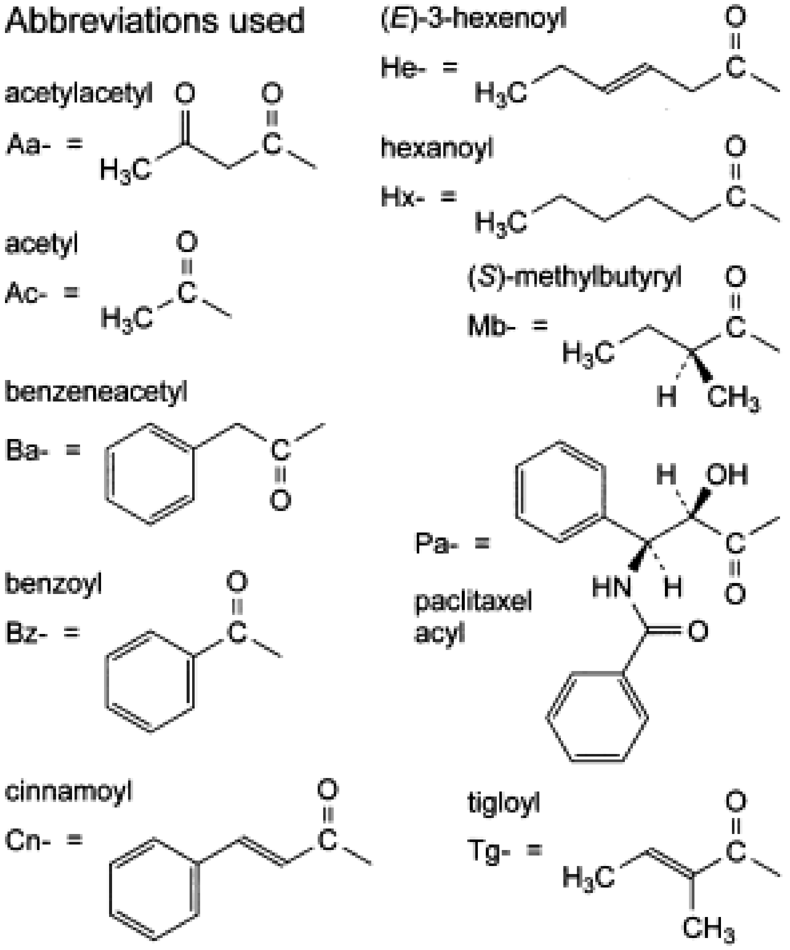
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): H.*

Test B

Specified impurities *A, E, G, H, I, J, K ,L, M, N.*



Abbreviations used


acetylacetyl
Aa- =
acetyl
Ac- =
benzeneacetyl
Ba- =
benzoyl
Bz- =
cinnamoyl
Cn- =
(E)-3-hexenoyl
He- =
hexanoyl
Hx- =
(S)-methylbutyryl
Mb- =
Pa- =
paclitaxel acyl
tigloyl
Tg- =

A. R1 = Tg, R2 = Ac, R3 = Bz, R4 = R6 = H, R5 = OH: 2-*O*-debenzoyl-2-*O*-tigloylpaclitaxel,

B. R1 = Bz, R2 = Ac, R3 = Tg, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-tigloylpaclitaxel (cephalomannine),

C. R1 = Bz, R2 = Ac, R3 = Hx, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-hexanoylpaclitaxel (paclitaxel C),

D. R1 = Bz, R2 = Ac, R3 = Tg, R4 = R5 = H, R6 = OH: *N*-debenzoyl-*N*-tigloyl-7-*epi*-paclitaxel (7-*epi*-cephalomannine),

E. R1 = R3 = Bz, R2 = Ac, R4 = R5 = H, R6 = OH: 7-*epi*-paclitaxel,

F. R1 = Bz, R2 = Ac, R3 = Hx, R4 = CH3, R5 = OH, R6 = H: *N*-debenzoyl-*N*-hexanoyl-*N*-methylpaclitaxel (*N*-methylpaclitaxel C),

G. R1 = R3 = Bz, R2 = R4 = R6 = H, R5 = OH: 10-*O*-deacetylpaclitaxel,

H. R1 = R3 = Bz, R2 = R4 = R5 = H, R6 = OH: 10-*O*-deacetyl-7-*epi*-paclitaxel,

I. R1 = R3 = Bz, R2 = Pa, R4 = R6 = H, R5 = OH: 10-*O*-[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]-10-*O*-deacetylpaclitaxel,

J. R1 = R3 = Bz, R2 = Aa, R4 = R6 = H, R5 = OH: 10-*O*-deacetyl-10-*O*-(3-oxobutanoyl) paclitaxel,

K. R1 = R3 = Bz, R2 = Ac, R4 = R6 = H, R5 = *O*-Si(C_2H_5)3: 7-*O*-(triethylsilanyl)paclitaxel,

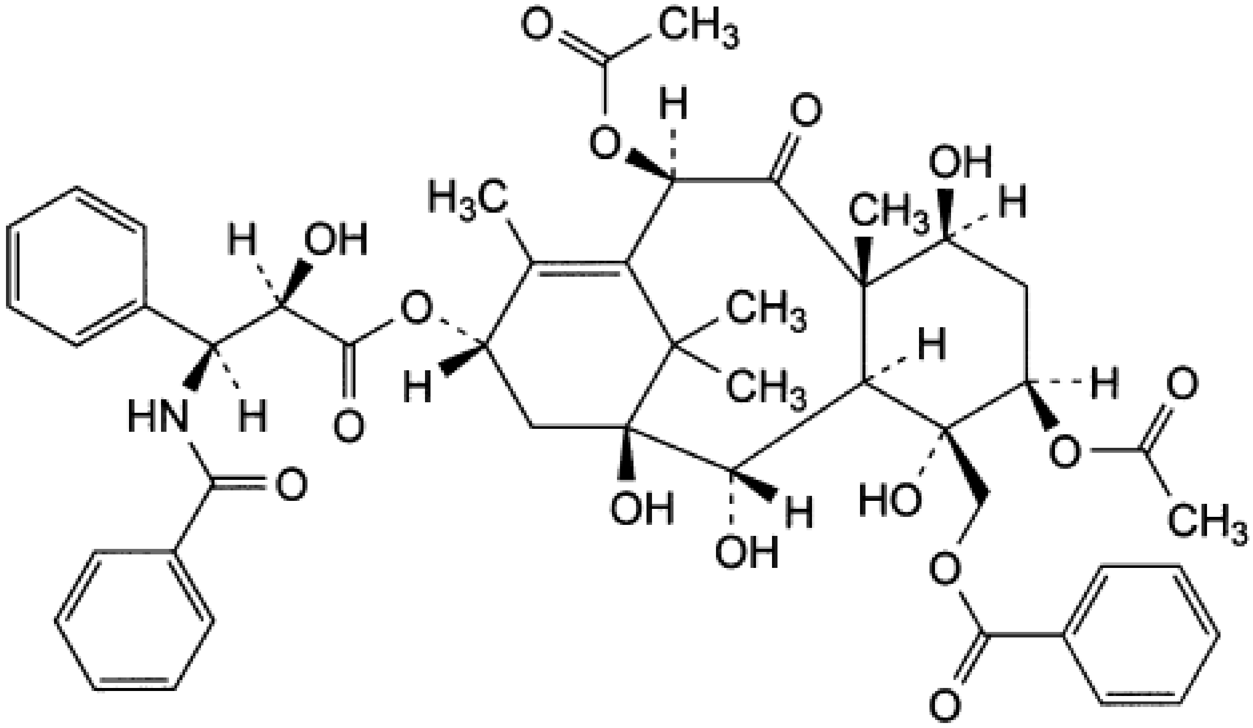
L. R1 = R3 = Bz, R2 = Ac, R4 = R6 = H, R5 = *O*-CO-CH_3: 7-*O*-acetylpaclitaxel,

O. R1 = Bz, R2 = Ac, R3 = Cn, R4 = R6 = H, R5 = OH: *N*-cinnamoyl-*N*-debenzoylpaclitaxel,

P. R1 = Bz, R2 = Ac, R3 = Ba, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-(phenylacetyl) paclitaxel,

Q. R1 = Bz, R2 = Ac, R3 = He, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-[(3*E*)-hex-3-enoyl] paclitaxel,

R. R1 = Bz, R2 = Ac, R3 = Mb, R4 = R6 = H, R5 = OH: *N*-debenzoyl-*N*-[(2*S*)-2-methylbutanoyl]paclitaxel,



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CH3
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CH3
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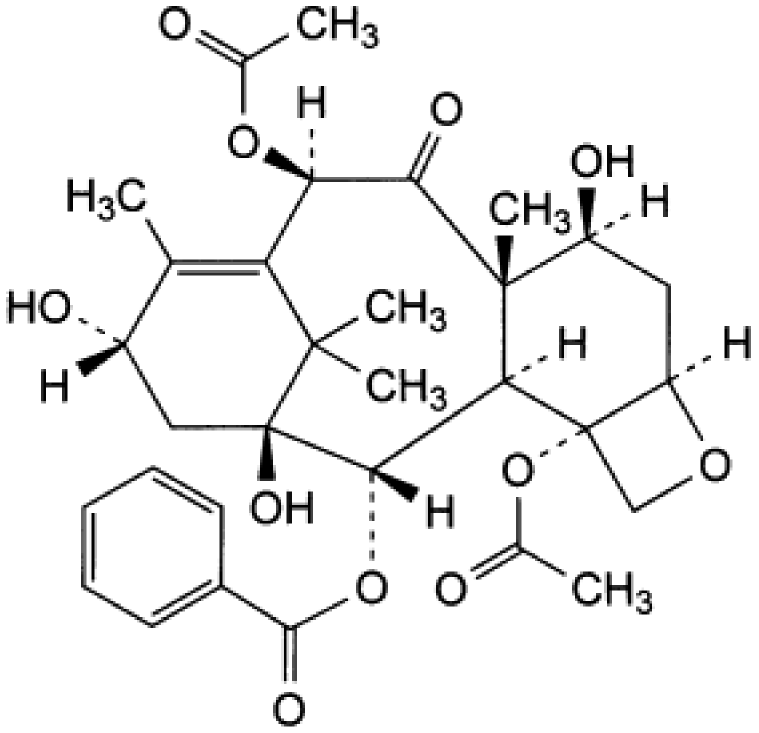
M. 1,2α,4,7β-dihydroxy-9-oxotax-11-ene-5β,10β,13α,20- tetrayl 5,10-diacetate 20-benzoate 13-[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoate],


O
CH3
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CH3
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N. 13-*O*-de[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]paclitaxel (baccatin III).

Ph Eur

Palmitic Acid

General Notices

(Ph Eur monograph 1904)

Action and use

Excipient.

Ph Eur

DEFINITION

Hexadecanoic acid ($C_{16}H_{32}O_2$; M_r 256.4), obtained from fats or oils of vegetable or animal origin.

Content

Minimum 92.0 per cent.

CHARACTERS

Appearance

White or almost white, waxy solid.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. Freezing point (see Tests).

B. Acid value *(2.5.1)*: 216 to 220, determined on 0.1 g.

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Appearance

Heat the substance to be examined to about 75 °C. The resulting liquid is not more intensely coloured than reference solution Y_7 or BY_7 *(2.2.2, Method I)*.

Acidity

Melt 5.0 g, stir for 2 min in 10 ml of *hot carbon dioxide-free water R*, cool slowly and filter. To the filtrate add 0.05 ml of *methyl orange solution R*. No red colour develops.

Freezing point ***(2.2.18)***

60 °C to 66 °C

Iodine value ***(2.5.4)***

Maximum 1.

Stearic acid

Maximum 6.0 per cent, determined as prescribed in the assay.

Nickel ***(2.4.31)***

Maximum 1 ppm

ASSAY

Gas chromatography *(2.4.22, Method C)*. Prepare the solutions as described in the method but omitting the initial hydrolysis.

Reference solution Prepare the reference solution in the same manner as the test solution using a mixture of 50 mg of *palmitic acid R* and 50 mg of *stearic acid R* instead of the substance to be examined.

Relative retention With reference to methyl stearate: methyl palmitate = about 0.9.

System suitability:

— *resolution*: minimum 5.0 between the peaks due to methyl stearate and methyl palmitate.

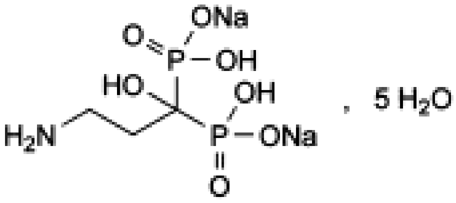
Ph Eur

Pamidronate Disodium Pentahydrate

General Notices

Disodium Pamidronate

(Ph Eur monograph 1779)



$C_3H_9NNa_2O_7P_2,5H_2O$ 369.1 *57248-88-1*

Action and use

Bisphosphonate; treatment of osteolytic lesions; Paget's disease; hypercalcaemia of malignancy.

Preparation

Pamidronate Disodium Intravenous Infusion

Ph Eur

DEFINITION

Disodium dihydrogen (3-amino-1-hydroxypropylidene)bisphosphonate pentahydrate.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, practically insoluble in methylene chloride. It is sparingly soluble in dilute mineral acids and dissolves in dilute alkaline solutions.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *pamidronate disodium pentahydrate CRS.*

B. Dissolve 0.5 g in 10 ml of *water R.* The solution gives reaction (a) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

pH *(2.2.3)*

7.8 to 8.8.

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 30 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 15 mg of β*-alanine R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R*, *di-isopropyl ether R*, *methanol R (4:8:9 V/V/V)*.

Application

10 µl.

Development

Over 2/3 of the plate.

Drying In a current of warm air.

Detection Spray with a *ninhydrin solution R*. Heat at 100-105 °C for 15 min.

Limit:
— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Impurities B and C

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution To 2.0 ml of a 0.3 g/l solution of *phosphoric acid R* add 2.0 ml of a 0.25 g/l solution of *phosphorous acid R* and dilute to 50.0 ml with *water R*.

Column:
— *size:* l = 0.10 m, Ø = 4.6 mm;

— *stationary phase: anion exchange resin R* (5 µm);

— *temperature:* 35 °C.

Mobile phase To 0.5 ml of *anhydrous formic acid R* add 2500 ml of *water R*; adjust to pH 3.5 with an 80 g/l solution of sodium *hydroxide R*.

Flow rate 1.0 ml/min.

Detection Refractometer.

Injection 100 µl.

Relative retention With reference to pamidronate (retention time = about 13 min): impurity B = about 1.3; impurity C = about 1.6.

System suitability Reference solution:

— *resolution*: minimum 2.5 between the peaks due to impurities B and C.

Limits:

— *impurities B, C*: for each impurity, not more than the area of the corresponding peaks in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

2.0 g complies with test C. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Water ***(2.5.12)***

23.0 per cent to 27.0 per cent, determined on 0.100 g.

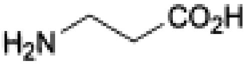
ASSAY

Dissolve 0.250 g in 70 ml of *water R.* Titrate with *0.1M hydrochloric acid* determining the end-point potentiometrically *(2.2.20).*

1 ml of *0.1M hydrochloric acid* is equivalent to 27.91 mg of $C_3H_9NNa_2O_7P_2$.

IMPURITIES

Specified impurities A, B, C.



A. 3-aminopropanoic acid (β-alanine),

B. phosphoric acid,

C. phosphorous acid.

Ph Eur

Pancreatic Extract

General Notices

(Pancreas Powder, Ph Eur monograph 0350)

Ph Eur

DEFINITION

Pancreas powder is prepared from the fresh or frozen pancreases of mammals. It contains various enzymes having proteolytic, lipolytic and amylolytic activities.

1 mg of pancreas powder contains not less than 1.0 Ph. Eur. U. of total proteolytic activity, 15 Ph. Eur. U. of lipolytic activity and 12 Ph. Eur. U. of amylolytic activity.

CHARACTERS

A slightly brown, amorphous powder, partly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Triturate 0.5 g with 10 ml of *water R* and adjust to pH 8 with *0.1 M sodium hydroxide*, using 0.1 ml of *cresol red solution R* as indicator. Divide the suspension into 2 equal parts (suspension (a) and suspension (b)). Boil suspension (a). To each suspension add 10 mg of *fibrin congo red R*, heat to 38-40 °C and maintain at this temperature for 1 h. Suspension (a) is colourless or slightly pink and suspension (b) is distinctly more red.

B. Triturate 0.25 g with 10 ml of *water R* and adjust to pH 8 with *0.1 M sodium hydroxide*, using 0.1 ml of *cresol red solution R* as indicator. Divide the suspension into 2 equal parts (suspension (a) and suspension (b)). Boil suspension (a). Dissolve 0.1 g of soluble *starch R* in 100 ml of boiling *water R*, boil for 2 min, cool and dilute to 150 ml with *water R*. To 75 ml of the starch solution add suspension (a) and to the remaining 75 ml add suspension (b). Heat each mixture to 38-40 °C and maintain at this temperature for 5 min.

To 1 ml of each mixture add 10 ml of *iodine solution R2*. The mixture obtained with suspension (a) has an intense blue-violet colour; the mixture obtained with suspension (b) has the colour of the iodine solution.

TESTS

Fat content

In an extraction apparatus, treat 1.0 g with *light petroleum R1* for 3 h. Evaporate the solvent and dry the residue at 100-105 °C for 2 h. The residue weighs not more than 50 mg (5.0 per cent).

Loss on drying *(2.2.32)*

Not more than 5.0 per cent, determined on 0.50 g by drying at 60 °C at a pressure not exceeding 670 Pa for 4 h.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 micro-organisms per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

Total proteolytic activity

The total proteolytic activity of pancreas powder is determined by comparing the quantity of peptides non-precipitable by a 50 g/l solution of *trichloroacetic acid R* released per minute from a substrate of casein solution with the quantity of such peptides released by *pancreas powder (protease) BRP* from the same substrate in the same conditions.

Casein solution Suspend a quantity of *casein BRP* equivalent to 1.25 g of dried substance in 5 ml of *water R*, add 10 ml of *0.1 M sodium hydroxide* and stir for 1 min. (Determine the water content of *casein BRP* prior to the test by heating at 60 °C *in vacuo* for 4 h.) Add 60 ml of *water R* and stir with a magnetic stirrer until the solution is practically clear. Adjust to pH 8.0 with *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid*. Dilute to 100.0 ml with *water R*. Use the solution on the day of preparation.

Enterokinase solution Dissolve 50 mg of *enterokinase BRP* in *0.02 M calcium chloride solution R* and dilute to 50.0 ml with the same solvent. Use the solution on the day of preparation.

For the test suspension and the reference suspension, prepare the suspension and carry out the dilution at 0-4 °C.

Test suspension Triturate 0.100 g of the substance to be examined for 5 min adding gradually 25 ml of *0.02 M calcium chloride solution R*. Transfer completely to a volumetric flask and dilute to 100.0 ml with *0.02 M calcium chloride solution R*. To 10.0 ml of this suspension add 10.0 ml of the enterokinase solution and heat on a water-bath at 35 ± 0.5 °C for 15 min. Cool and dilute with *borate buffer solution pH 7.5 R* at 5 ± 3 °C to a final concentration of about 0.065 Ph. Eur. U. of total proteolytic activity per millilitre calculated on the basis of the stated activity.

Reference suspension Prepare a suspension of *pancreas powder (protease) BRP* as described for the test suspension but without the addition of enterokinase so as to obtain a known final concentration of about 0.065 Ph. Eur. U. per millilitre calculated on the basis of the stated activity.

Designate tubes in duplicate T, T_b, S_1, S_{1b}, S_2, S_{2b}, S_3, S_{3b}; designate a tube B.

Add *borate buffer solution pH 7.5 R* to the tubes as follows:

B: 3.0 ml,

S_1 and S_{1b}: 2.0 ml,

S_2, S_{2b}, T and T_b: 1.0 ml.

Add the reference suspension to the tubes as follows:

S_1 and S_{1b}: 1.0 ml,

S_2 and S_{2b}: 2.0 ml,

S_3 and S_{3b}: 3.0 ml.

Add 2.0 ml of the test suspension to tubes T and T_b.

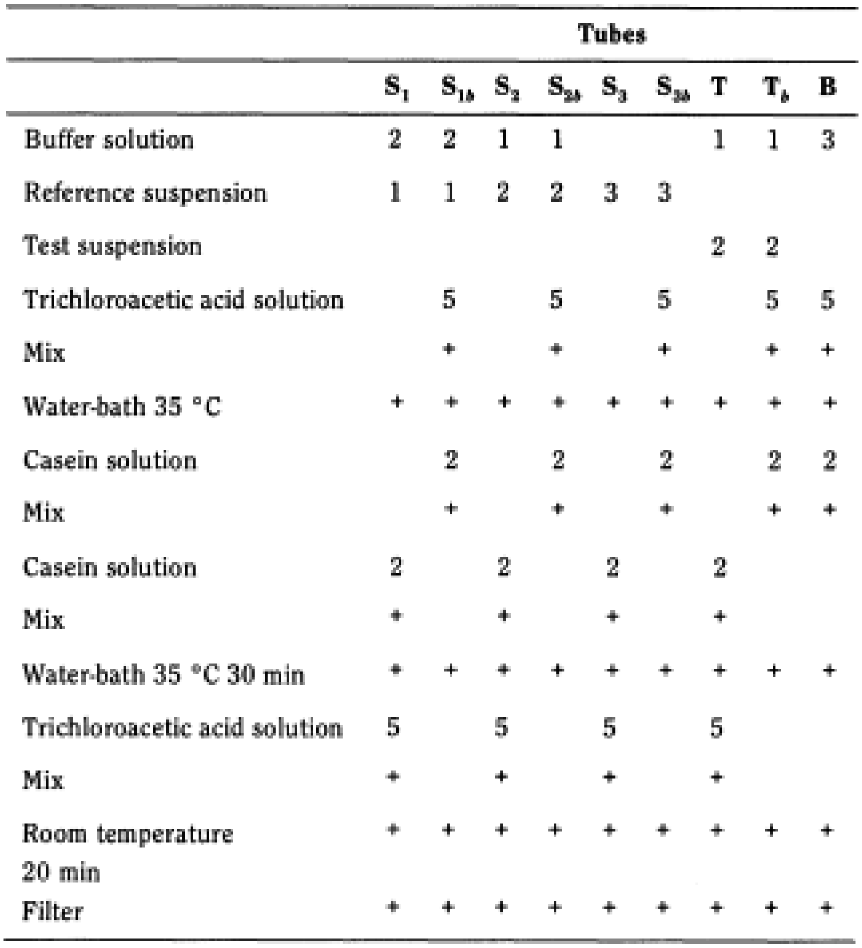
Add 5.0 ml of a 50 g/l solution of *trichloroacetic acid R* to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix by shaking.

Place the tubes and the casein solution in a water-bath at 35 ± 0.5 °C. Place a glass rod in each tube. When temperature equilibrium is reached, add 2.0 ml of the casein solution to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix. At time zero, add 2.0 ml of casein solution successively and at intervals of 30 s to tubes S_1, S_2, S_3 and T. Mix immediately after each addition. Exactly 30 min after addition of the casein solution, taking into account the regular interval adopted, add 5.0 ml of a 50 g/l solution of *trichloroacetic acid R* to tubes S_1, S_2, S_3 and T. Mix. Withdraw the tubes from the water-bath and allow to stand at room temperature for 20 min.

Filter the contents of each tube twice through the same suitable filter paper previously washed with a 50 g/l solution of *trichloroacetic acid R*, then with *water R* and dried.

A suitable filter paper complies with the following test: filter 5 ml of a 50 g/l solution of *trichloroacetic acid R* on a 7 cm disc of white filter paper; the absorbance *(2.2.25)* of the filtrate, measured at 275 nm using unfiltered trichloroacetic acid solution as the compensation liquid, is less than 0.04.

Table 0350.-1



	Tubes								
	S_1	S_{1b}	S_2	S_{2b}	S_3	S_{3b}	T	T_b	B
Buffer solution	2	2	1	1			1	1	3
Reference suspension	1	1	2	2	3	3			
Test suspension							2	2	
Trichloroacetic acid solution		5		5		5		5	5
Mix		+		+		+		+	+
Water-bath 35 °C	+	+	+	+	+	+	+	+	+
Casein solution		2		2		2		2	2
Mix		+		+		+		+	+
Casein solution	2		2		2		2		
Mix	+		+		+		+		
Water-bath 35 °C 30 min	+	+	+	+	+	+	+	+	+
Trichloroacetic acid solution	5		5		5		5		
Mix	+		+		+		+		
Room temperature 20 min	+	+	+	+	+	+	+	+	+
Filter	+	+	+	+	+	+	+	+	+

Measure the absorbance *(2.2.25)* of the filtrates at 275 nm using the filtrate obtained from tube B as the compensation liquid.

Correct the average absorbance values for the filtrates obtained from tubes S_1, S_2 and S_3 by subtracting the average values obtained for the filtrates from tubes S_{1b}, S_{2b} and S_{3b} respectively. Draw a calibration curve of the corrected values against volume of reference suspension used.

Determine the activity of the substance to be examined using the corrected absorbance for the test suspension ($T - T_b$) and the calibration curve and taking into account the dilution factors.

The test is not valid unless the corrected absorbance values are between 0.15 and 0.60.

Lipolytic activity

The lipolytic activity is determined by comparing the rate at which a suspension of pancreas powder hydrolyses a substrate of olive oil emulsion with the rate at which a suspension of *pancreas powder (amylase and lipase) BRP* hydrolyses the same substrate under the same conditions. The test is carried out under nitrogen.

Olive oil stock emulsion In an 800 ml beaker 9 cm in diameter, place 40 ml of *olive oil R*, 330 ml of *acacia solution R* and 30 ml of *water R*. Place an electric mixer at the bottom of the beaker. Place the beaker in a vessel containing *ethanol (96 per cent) R* and a sufficient quantity of ice as a cooling mixture. Emulsify using the mixer at an average speed of 1000-2000 r/min. Cool to 5-10 °C. Increase the mixing speed to 8000 r/min. Mix for 30 min keeping the temperature below 25 °C by the continuous addition of crushed ice into the cooling mixture. (A mixture of calcium chloride and crushed ice is also suitable). Store the stock emulsion in a refrigerator and use within 14 days. The emulsion must not separate into 2 distinct layers. Check the diameter of the globules of the emulsion under a microscope. At least 90 per cent have a diameter below 3 µm and none has a diameter greater than 10 µm. Shake the emulsion thoroughly before preparing the emulsion substrate.

Olive oil emulsion For 10 determinations, mix the following solutions in the order indicated: 100 ml of the stock emulsion, 80 ml of *tris(hydroxymethyl)aminomethane solution R1*, 20 ml of a freshly prepared 80 g/l of *sodium taurocholate BRP* and 95 ml of *water R*. Use on the day of preparation.

Apparatus Use a reaction vessel of about 50 ml capacity provided with:

— a device that will maintain a temperature of 37 ± 0.5 °C;

— a magnetic stirrer;

— a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration apparatus may be used. In the latter case, the burette is graduated in 0.005 ml and the pH-meter is provided with a wide reading scale and glass-calomel or glass-silver-silver chloride electrodes. After each test the reaction vessel is evacuated by suction and washed several times with *water R*, the washings being removed each time by suction.

Test suspension In a small mortar cooled to 0-4 °C, triturate carefully a quantity of the substance to be examined equivalent to about 2500 Ph. Eur. U. of lipolytic activity with 1 ml of cooled *maleate buffer solution pH 7.0 R* (lipase solvent) until a very fine suspension is obtained. Dilute the suspension with cold *maleate buffer solution pH 7.0 R*, transfer quantitatively to a volumetric flask and dilute to 100.0 ml with the cold buffer solution. Keep the flask containing the test suspension in iced water during the titration.

Reference suspension To avoid absorption of water formed by condensation, allow the reference preparation to reach room temperature before opening the container. Prepare a *suspension of pancreas powder (amylase and lipase) BRP* as described for the test suspension using a quantity equivalent to about 2500 Ph. Eur. U.

Carry out the titrations immediately after preparation of the test suspension and the reference suspension. Place 29.5 ml of olive oil emulsion in the reaction vessel equilibrated at 37 ± 0.5 °C. Fit the vessel with the electrodes, a stirrer and the burette (the tip being immersed in the olive oil emulsion).

Put the lid in place and switch on the apparatus. Carefully add *0.1 M sodium hydroxide* with stirring to adjust to pH 9.2. Using a rapid-flow graduated pipette transfer about 0.5 ml of the previously homogenised reference suspension, start the chronometer and add continuously *0.1 M sodium hydroxide* to maintain the pH at 9.0. After exactly 1 min, note the volume of *0.1 M sodium hydroxide* used. Carry out the measurement a further 4 times. Discard the first reading and determine the average of the 4 others (S_1). Make 2 further determinations (S_2 and S_3). Calculate the average of the values S_1, S_2 and S_3. The average volume of *0.1 M sodium hydroxide* used should be about 0.12 ml per minute with limits of 0.08 ml to 0.16 ml.

Carry out 3 determinations in the same manner for the test suspension (T_1, T_2 and T_3). If the quantity of *0.1 M sodium hydroxide* used is outside the limits of 0.08 ml to 0.16 ml per minute, the assay is repeated with a quantity of test suspension that is more suitable but situated between 0.4 ml and 0.6 ml. Otherwise the quantity of the substance to be examined is adjusted to comply with the conditions of the test. Calculate the average of the values T_1, T_2 and T_3.

Calculate the activity in European Pharmacopoeia Units per milligram using the following expression:

$$\frac{n \times m_1}{n_1 \times m} \times A$$

n = average volume of *0.1 M sodium hydroxide* used per minute during the titration of the test suspension, in millilitres;

n_1 = average volume of *0.1 M sodium hydroxide* used per minute during the titration of the reference suspension, in millilitres;

m = mass of the substance to be examined, in milligrams;

m_1 = mass of the reference preparation, in milligrams;

A = activity of *pancreas powder (amylase and lipase) BRP*, in European Pharmacopoeia Units per milligram.

Amylolytic activity

The amylolytic activity is determined by comparing the rate at which a suspension of pancreas powder hydrolyses a substrate of starch solution with the rate at which a suspension of *pancreas powder (amylase and lipase) BRP* hydrolyses the same substrate under the same conditions.

Starch solution To a quantity of *starch BRP* equivalent to 2.0 g of the dried substance add 10 ml of *water R* and mix. (Determine the water content of *starch BRP* prior to the test by heating at 120 °C for 4 h). Add this suspension, whilst stirring continuously, to 160 ml of boiling *water*

R. Wash the container several times with successive quantities, each of 10 ml, of *water R* and add the washings to the hot starch solution. Heat to boiling, stirring continuously. Cool to room temperature and dilute to 200 ml with *water R*. Use the solution on the day of preparation.

For the test suspension and the reference suspension, prepare the suspension and carry out the dilution at 0-4 °C.

Test suspension Triturate a quantity of the substance to be examined equivalent to about 1500 Ph. Eur. U. of amylolytic activity with 60 ml of *phosphate buffer solution pH 6.8 R1* for 15 min. Transfer quantitatively to a volumetric flask and dilute to 100.0 ml with *phosphate buffer solution pH 6.8 R1*.

Reference suspension Prepare a suspension of *pancreas powder (amylase and lipase) BRP* as described for the test suspension, using a quantity equivalent to about 1500 Ph. Eur. U.

In a test tube 200 mm long and 22 mm in diameter, fitted with a ground-glass stopper, place 25.0 ml of starch solution, 10.0 ml of *phosphate buffer solution pH 6.8 R1* and 1.0 ml of an 11.7 g/l solution of *sodium chloride R*. Close the tube, shake and place in a water-bath at 25.0 ± 0.1 °C. When the temperature equilibrium has been reached, add 1.0 ml of the test suspension and start the chronometer. Mix and place the tube in the water-bath. After exactly 10 min, add 2 ml of *1 M hydrochloric acid*. Transfer the mixture quantitatively to a 300 ml conical flask fitted with a ground-glass stopper. Whilst shaking continuously, add 10.0 ml of *0.05 M iodine* immediately followed by 45 ml of *0.1 M sodium hydroxide*. Allow to stand in the dark at a temperature between 15 °C and 25 °C for 15 min. Add 4 ml of a mixture of 1 volume of *sulphuric acid R* and 4 volumes of *water R*. Titrate the excess of iodine with *0.1 M sodium thiosulphate* using a microburette. Carry out a blank titration adding the 2 ml of *1 M hydrochloric acid* before introducing the test suspension. Carry out the titration of the reference suspension in the same manner.

Calculate the amylolytic activity in European Pharmacopoeia Units per milligram using the following expression:

$$\frac{(n' - n)\,m_1}{(n'_1 - n_1)\,m} \times A$$

n = volume of *0.1 M sodium thiosulphate* used in the titration of the test suspension, in millilitres;

n_1 = volume of *0.1 M sodium thiosulphate* used in the titration of the reference suspension, in millilitres;

n' = volume of *0.1 M sodium thiosulphate* used in the blank titration of the test suspension, in millilitres;

n'_1 = volume of *0.1 M sodium thiosulphate* used in the blank titration of the reference suspension, in millilitres;

m = mass of the substance to be examined, in milligrams;

m_1 = mass of the reference preparation, in milligrams;

A = activity of *pancreas powder (amylase and lipase) BRP*, in European Pharmacopoeia Units per milligram.

STORAGE

Store in an airtight container.

Ph Eur

Pancreatin

General Notices

Action and use

Enzyme; treatment of pancreatic exocrine deficiency.

Preparations

Pancreatin Granules

Gastro-resistant Pancreatin Tablets

DEFINITION

Pancreatin is a preparation of mammalian pancreas containing enzymes having protease, lipase and amylase activity. It may contain Sodium Chloride. Pancreatin contains in 1 mg not less than 1.4 Units of free protease activity, not less than 20 Units of lipase activity and not less than 24 Units of amylase activity.

PRODUCTION

Pancreatin is prepared in conditions designed to minimise the degree of microbial contamination.

CHARACTERISTICS

A white or buff amorphous powder.

Soluble or partly soluble in *water* forming a slightly turbid solution; practically insoluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. Triturate 0.5 g with 10 ml of *water* and adjust to pH 8.0 by the addition of 1M *sodium hydroxide* using *cresol red solution* as indicator. Divide the resulting solution into two equal portions. Boil one portion [solution (1)] and leave the other untreated [solution (2)]. To each add a few shreds of *congo red fibrin*, warm to 38° to 40° and maintain at this temperature for 1 hour. Solution (2) is stained red and solution (1) is colourless or not more than slightly pink.

B. Triturate 0.25 g with 10 ml of *water* and adjust to pH 8.0 by the addition of 1M *sodium hydroxide* using *cresol red solution* as indicator. Divide the resulting solution into two equal portions. Boil one portion [solution (1)] and leave the other untreated [solution (2)]. Dissolve 0.1 g of *soluble starch* in 100 ml of boiling *water*, boil for 2 minutes, cool and dilute to 150 ml with *water*. Add solution (1) to half the starch mucilage and solution (2) to the remainder and maintain the mixtures at 38° to 40° for 5 minutes. To 1 ml of each mixture add 10 ml of *iodinated potassium iodide solution*. The liquid containing solution (2) retains the colour of the solution of iodine and the liquid containing solution (1) acquires an intense blue colour.

TESTS

Fat

Extract 1 g with *petroleum spirit (boiling range, 40° to 60°)* for 3 hours in an apparatus for the *continuous extraction of drugs*, Appendix XI F, evaporate the extract and dry the residue at 105° for 2 hours. The residue weighs not more than 30 mg.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 4 hours, loses not more than 5.0% of its weight. Use 0.5 g.

Microbial contamination

1 g is free from *Escherichia coli*; 10 g is free from *Salmonella*, Appendix XVI B1.

ASSAY

Carry out the *assay of pancreatin* described below.

STORAGE

Pancreatin should be stored at a temperature not exceeding 15°.

LABELLING

The label states (1) the minimum number of Units of activity of free protease, lipase and amylase per mg; (2) the name of any added substance; (3) the date after which the material is not intended to be used; (4) the conditions under which it should be stored.

ASSAY OF PANCREATIN

The free protease, lipase and amylase activities of pancreatin are determined by the following methods.

STANDARD PREPARATION AND UNITS

The Standard Preparation is the appropriate FIP Standard which has been adopted as an official preparation by the European Pharmacopoeia Commission and is available as *pancreas powder (protease) EPBRP* or *pancreas powder (amylase and lipase) EPBRP* as appropriate.

The Unit of protease activity is contained in that amount of the Standard Preparation that, under the conditions of the assay, hydrolyses casein at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid that gives the same absorbance at 275 nm as one micromole of tyrosine. The Unit of lipase activity is contained in that amount of the Standard Preparation that, under the conditions of the assay, liberates one micro-equivalent of acid per minute at pH 9.0 and 37°. The Unit of amylase activity is contained in that amount of the standard preparation that, under the conditions of the assay, decomposes starch at an initial rate such that one micro-equivalent of glycosidic linkage is hydrolysed per minute.

FOR FREE PROTEASE ACTIVITY

Method

Solution of the standard preparation Triturate for 5 minutes a quantity of the Standard Preparation containing approximately 100 Units of protease activity with 25 ml of *calcium* *chloride solution* cooled to 5°. Dilute to 100 ml with the cooled *calcium chloride solution* and then dilute a sufficient quantity of the resulting suspension to 100 ml with *borate buffer pH 7.5*, cooled to 5°, so that 1 ml of the final solution contains 0.065 Units of protease activity.

Solution of the substance being examined Triturate for 5 minutes a quantity of the substance being examined containing approximately 100 Units of protease activity with 25 ml of *calcium chloride solution* cooled to 5°. Dilute to 100 ml with the cooled *calcium chloride solution* and then dilute a sufficient quantity of the resulting suspension to 100 ml with *borate buffer pH 7.5*, cooled to 5°, so that the estimated free protease activity corresponds approximately to the activity of the solution of the Standard Preparation.

Label 16 test tubes with the following identification in duplicate; S_1, S_2, S_3, S_1B, S_2B, S_3B, U and UB. To tubes S_1 and S_1B add 2.0 ml and to tubes S_2, S_2B, U and UB, 1.0 ml of *borate buffer pH 7.5*. Then to tubes S_1 and S_1B add 1.0 ml, to tubes S_2 and S_2B, 2.0 ml and to tubes S_3 and S_3B, 3.0 ml of the solution of the Standard Preparation. Add 2.0 ml of the solution of the substance being examined to tubes U and UB. To each of the control tubes (S_1B, S_2B, S_3B and UB) add 5.0 ml of a 5% w/v solution of *trichloroacetic acid* and mix. Place a stirring rod in each tube and warm to, and maintain at, 35° in a water bath. Add 5.0 ml of *concentrated casein substrate* to each of the control tubes and mix.

At accurately timed intervals add 5.0 ml of *concentrated casein substrate*, previously warmed to 35°, to tubes S_1, S_2, S_3 and U and mix immediately. After exactly 30 minutes, in the same order, stop the reaction in tubes S_1, S_2, S_3 and U by adding 5.0 ml of a 5% w/v solution of *trichloroacetic acid* and mix thoroughly. Remove all the test tubes from the water bath and allow to stand at room temperature for 20 minutes. Filter the contents of the tubes through suitable filter paper[1] , collect the filtrates and refilter through the same paper. The filtrates must be free from haze.

Measure the *absorbances* of the filtrates at the maximum at 275 nm, Appendix II B, using in the reference cell a mixture of 6.0 ml of *borate buffer pH 7.5* and 5.0 ml of the 5% w/v solution of *trichloroacetic acid* that has been filtered in the same way. Correct the mean absorbances of the filtrates from tubes S_1, S_2 and S_3 by subtracting the mean absorbances of the filtrates from the corresponding control tubes S_1B, S_2B and S_3B.

Prepare a reference curve by plotting the mean corrected absorbances against the potency of the dilution of the solution of the Standard Preparation used. Calculate the corrected mean absorbance of the substance being examined by subtracting the mean absorbance of the filtrates from tubes UB from that of the filtrates from tubes U. Using the corrected mean absorbance, determine the potency of the solution of the substance being examined from the reference curve and calculate the free protease activity per mg of the substance being examined by taking into account the dilution factors.

The test is not valid unless the corrected absorbances are between 0.15 and 0.60.

FOR LIPASE ACTIVITY

Apparatus

Use a reaction vessel of about 50 ml capacity fitted with a device that will maintain a temperature of 36.5° to 37.5°, a magnetic stirrer and a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents. An automatic or manual titration apparatus may be used. In the latter case, the burette is graduated in 5-µl divisions and the pH meter is provided with a wide reading scale and glass and calomel electrodes. After each test the reaction vessel is evacuated by suction and washed several times with water, the washings being removed each time by suction.

Method

Carry out the assay under nitrogen. In a small mortar cooled to 0° to 4° triturate carefully an amount of the substance being examined containing approximately 2500 Units of lipase activity with 1 ml of cooled *lipase solvent* until a very fine suspension is obtained (about 10 minutes). Dilute with cooled *lipase solvent*, transfer quantitatively to a graduated flask and dilute to 100.0 ml with the cooled solvent; use immediately.

Transfer 29.5 ml of *olive oil substrate emulsion* to the assembled reaction vessel equilibrated at 36.5° to 37.5° and adjust the pH to 9.2 with 0.1M *sodium hydroxide*. Add about 0.5 ml of the suspension of the substance being examined and record the time at which the pH reaches 9.0. Add continuously from a micrometer syringe sufficient 0.1M *sodium hydroxide VS* to maintain the pH at 9.0. Record the volume of 0.1M *sodium hydroxide VS* consumed at 1-minute intervals for 5 minutes. Discounting the first reading, calculate the mean rate of alkali consumption U. If necessary, dilute with sufficient *lipase solvent* to produce an average alkali consumption of 0.08 to 0.16 ml of 0.1M *sodium hydroxide VS* per minute. Repeat the procedure using the Standard Preparation in place of the substance being examined and calculate the mean rate of alkali consumption, S. Calculate the potency (P_L) of the substance being examined in Units per mg from the expression:

$$P_L = \frac{U}{S} \times \frac{w_s}{w} \times R$$

Where U = the mean volume in ml of 0.1M *sodium hydroxide VS* used per minute in the titration of the substance being examined,

S = the mean volume in ml of 0.1M *sodium hydroxide VS* used per minute in the titration of the Standard Preparation,

w = weight in mg of the substance being examined,

w_s = weight in mg of the Standard Preparation,

R = potency of the Standard Preparation in Units per mg.

Calculate the potency of the substance being examined using the average of three separate titrations for both the substance being examined and the Standard Preparation.

FOR AMYLASE ACTIVITY

Method

Triturate an amount of the substance being examined containing approximately 1500 Units of amylase activity with 60 ml of *0.2M mixed phosphate buffer pH 6.8* for 15 minutes and add sufficient *0.2*M *mixed phosphate buffer mixed phosphate buffer p*H 6.8 to produce 100 ml. To a stoppered tube (200 mm × 22 mm) add 25.0 ml of *starch substrate*, 10.0 ml of *0.2*M *mixed phosphate buffer pH 6.8* and 1.0 ml of 0.2M *sodium chloride*. Stopper the tube, mix the contents and place in a water bath at 24.9° to 25.1°. When the temperature of the mixture has reached 25° add 1.0 ml of the solution of the substance being examined and record the time of addition. Mix thoroughly and replace in the water bath. After exactly 10 minutes add 2 ml of 1M *hydrochloric acid* to stop the reaction. Transfer the contents of the tube to a stoppered 300 ml flask. While shaking continuously add 10.0 ml of 0.05M *iodine VS* followed immediately by 45 ml of 0.1M *sodium hydroxide*. Allow to stand in the dark at a temperature of 15° to 25° for 15 minutes. Add 4 ml of a mixture of 1 volume of *sulphuric acid* and 4 volumes of *water* and titrate with 0.1M *sodium thiosulphate VS*. Repeat the procedure but add the 2 ml of 1M *hydrochloric acid* before the addition of the solution of the substance being examined. Prepare a solution of the Standard Preparation in the same manner as described for the solution of the substance being examined and repeat the procedure beginning at the words 'To a stoppered tube …' but using 1.0 ml of this solution in place of the solution of the substance being examined.

Calculate the potency (P_A) of the substance being examined in Units per mg from the expression:

$$P_A = \frac{(B - A)w_s}{(B_s - A_s)w} \times Q$$

Where A = volume in ml of 0.1M *sodium thiosulphate VS* used in the titration of the substance being examined,

A_s = volume in ml of 0.1M *sodium thiosulphate VS* used in the titration of the Standard Preparation

B = volume in ml of 0.1M *sodium thiosulphate VS* used in the titration of the substance being examined inactivated by the addition of 1M hydrochloric acid,

B_s = volume in ml of 0.1M *sodium* thiosulphate VS used in the titration of the Standard Preparation inactivated by the addition of 1M hydrochloric acid,

Q = potency of the Standard Preparation in Units per mg

w = total weight in mg of the substance being examined in the solution prepared for assay,

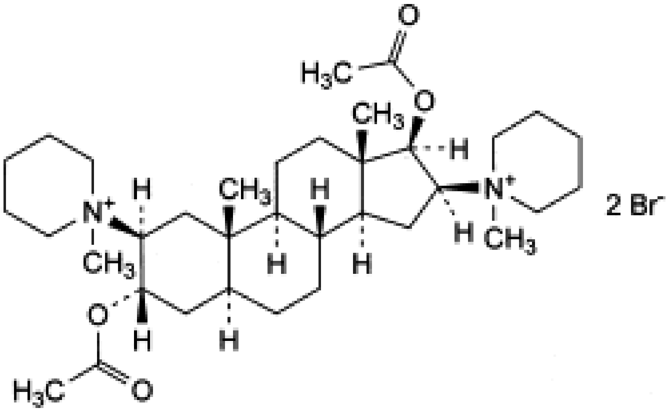
w_s = total weight in mg of the Standard Preparation in the solution prepared for assay.

[1] A suitable filter paper complies with the following test. Filter 5 ml of a 5% w/v solution of

Pancuronium Bromide

General Notices

(Ph Eur monograph 0681)



$C_{35}H_{60}Br_2N_2O_4$ 733 *15500-66-0*

Action and use

Non-depolarizing neuromuscular blocker.

Preparation

Pancuronium Injection

Ph Eur

DEFINITION

1,1′-[3α,17β-Bis(acetyloxy)-5α-androstane-2β,16β-diyl]bis(1-methylpiperidinium) dibromide.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White, yellowish-white or slightly pink, crystalline powder, hygroscopic.

Solubility

Very soluble or freely soluble in water, very soluble in methylene chloride, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison pancuronium bromide CRS.

B. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 50 mg in *water R* and dilute to 25 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 38.0 to + 42.0 (anhydrous substance).

Dissolve 0.75 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 50.0 mg of the substance to be examined in *methylene chloride R* and dilute to 5.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with *methylene chloride R*. Dilute 1.0 ml of this solution to 20.0 ml with *methylene chloride* R.

Reference solution (b) Dissolve 10.0 mg of *pancuronium bromide for system suitability CRS* (containing 1.0 per cent of impurity D) in 1.0 ml of *methylene chloride R*.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase 400 g/l solution of *sodium iodide R, acetonitrile R, 2-propanol R* (5:10:85 *V/V/V*).

Application 5 µl.

Development In an unlined and unsaturated tank over a path of 8 cm.

Drying In a current of air at room temperature.

Detection Spray with a 20 g/l solution of *sodium nitrite R* and allow to dry for 5 min. Then spray with *potassium iodobismuthate solution R5*. Cover the plate with a transparent glass cover.

System suitability:

— the chromatogram obtained with reference solution (b) shows 2 clearly separated spots due to pancuronium bromide (R_F = about 0.5) and impurity D (R_F = about 0.6);

— the chromatogram obtained with reference solution (a) shows a clearly visible spot.

Note Impurity A if present will co-migrate with impurity D.

Limits:

— *impurities A, D*: any spot due to impurities A and/or D is not more intense than the spot due to impurity D in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *unspecified impurities*: any other spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.10 per cent).

Water ***(2.5.12)***

Maximum 8.0 per cent, determined on 0.300 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *acetic anhydride R*, heating if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 36.63 mg of $C_{35}H_{60}Br_2N_2O_4$.

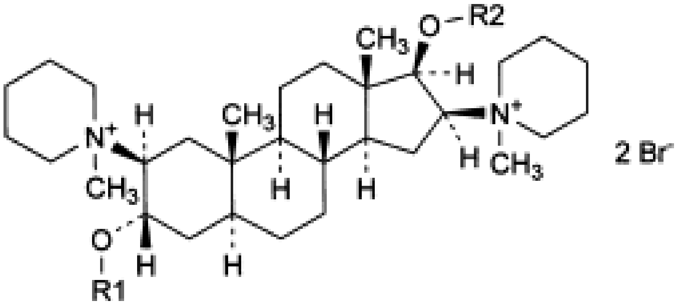
STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities *A, D.*

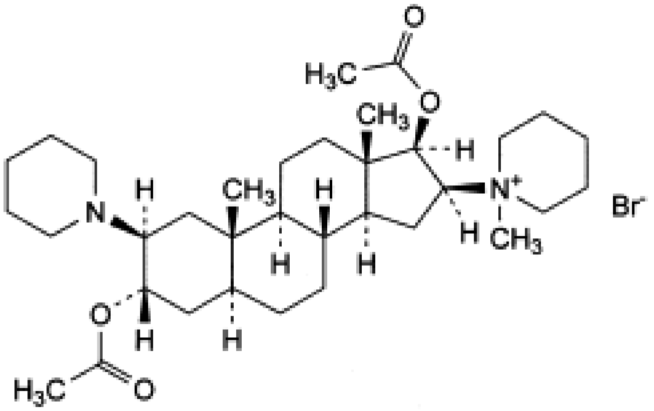
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, E.*



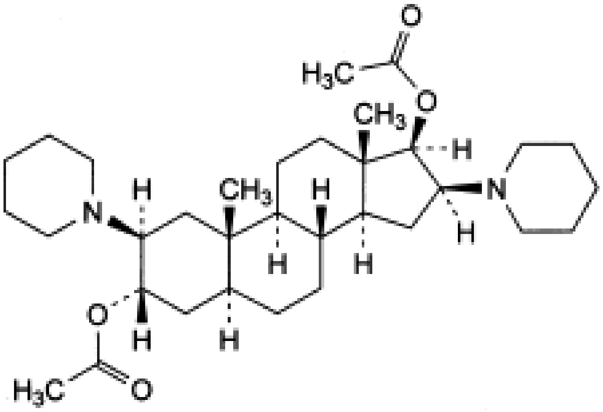
A. R1 = CO-CH$_3$, R2 = H: 1,1′-[3α-(acetyloxy)-17β-hydroxy-5α-androstane-2β,16β-diyl]bis(1-methylpiperidinium) dibromide (dacuronium bromide),

B. R1 = H, R2 = CO-CH$_3$: 1,1′-[17β-(acetyloxy)-3α-hydroxy-5α-androstane-2β,16β-diyl]bis(1-methylpiperidinium) dibromide,

C. R1 = R2 = H: 1,1′-(3α,17β-dihydroxy-5α-androstane-2β,16β-diyl)bis(1-methylpiperidinium) dibromide,



D. 1-[3α,17β-bis(acetyloxy)-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidinium bromide (vecuronium bromide),



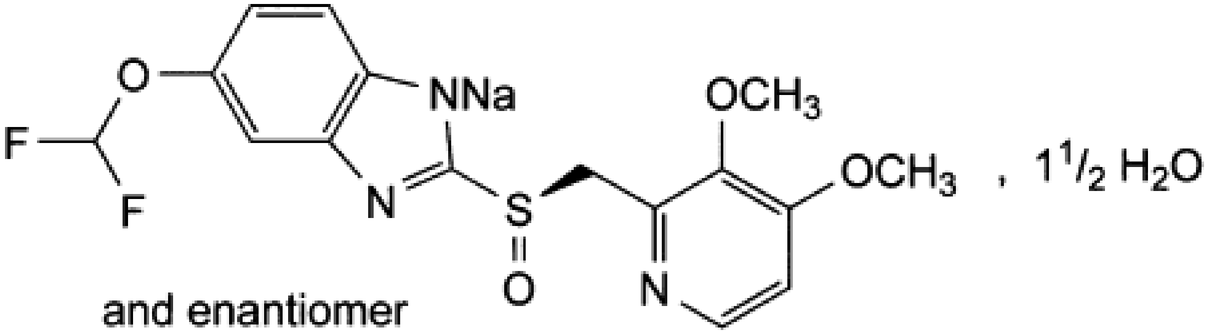
E. 2β,16β-bis(piperidin-1-yl)-5α-androstane-3α,17β-diyl diacetate.

Ph Eur

Pantoprazole Sodium Sesquihydrate

General Notices

(Ph Eur monograph 2296)



$C_{16}H_{14}F_2N_3NaO_4S$,1½ H_2O 432.4 *164579-32-2*

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

Ph Eur

DEFINITION

Sodium 5-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulphinyl] benzimidazol-1-ide sesquihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

PRODUCTION

It is produced by methods of manufacture designed to guarantee the proper hydrate form and it complies, if tested, with a suitable test that demonstrates its sesquihydrate nature (for example near-infrared spectrophotometry (*2.2.40*) or X-ray powder diffraction (*2.9.33*)).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water and in ethanol (96 per cent), practically insoluble in hexane.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *pantoprazole sodium sesquihydrate CRS.*

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

Dissolve 0.20 g in *water R* and dilute to 20.0 ml with the same solvent.

Optical rotation *(2.2.7)*

- 0.4° to + 0.4°.

Dissolve 0.2 g in 10 ml of *water R*. Adjust to pH 11.5-12.0 with an 8 g/l solution of *sodium hydroxide R*. Dilute to 20.0 ml with *water R.*

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *acetonitrile for chromatography R*, 40 mg/l solution of *sodium hydroxide R* (50:50 *V/V*).

Test solution Dissolve 23 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *pantoprazole for system suitability CRS* (containing impurities A, B, C, D and E) in 1.0 ml of the solvent mixture.

Column:

— *size*: *l* = 0.125 m, Ø = 4 mm;

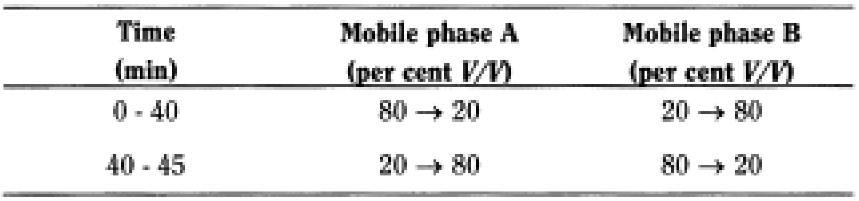
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: 1.74 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 7.00 ± 0.05 with a 330 g/l solution of *phosphoric acid R;*

— *mobile phase B*: *acetonitrile for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	80 → 20	20 → 80
40 - 45	20 → 80	80 → 20

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 290 nm and, for impurity C, at 305 nm.

Injection 20 µl.

Identification of impurities Use the chromatogram supplied with *pantoprazole for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D + F and E.

Relative retention With reference to pantoprazole (retention time = about 11 min): impurity C = about 0.6; impurity A = about 0.9; impurities D and F = about 1.2; impurity E = about 1.3; impurity B = about 1.5.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurities E and D + F;

— the chromatogram obtained is similar to the chromatogram supplied with *pantoprazole for system suitability CRS.*

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.3;

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *sum of impurities D and F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities B, C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

5.9 per cent to 6.9 per cent, determined on 0.150 g.

ASSAY

Dissolve 0.200 g in 80 ml of *anhydrous acetic acid R*, add 5 ml of *acetic anhydride R* and mix for at least 10 min. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

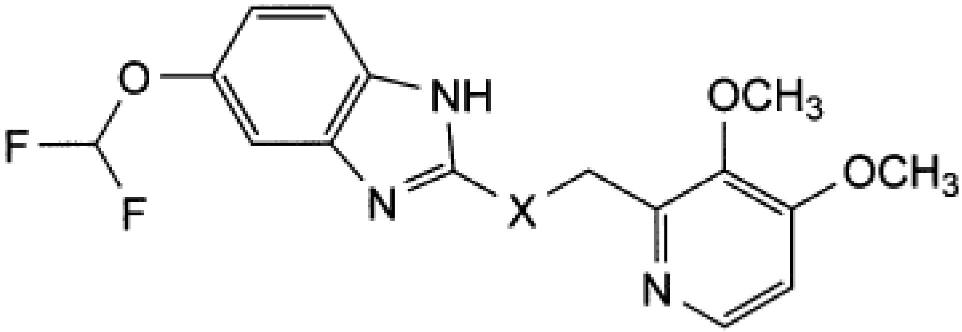
1 ml of *0.1 M perchloric acid* is equivalent to 20.27 mg of $C_{16}H_{14}F_2N_3NaO_4S$.

STORAGE

Protected from light.

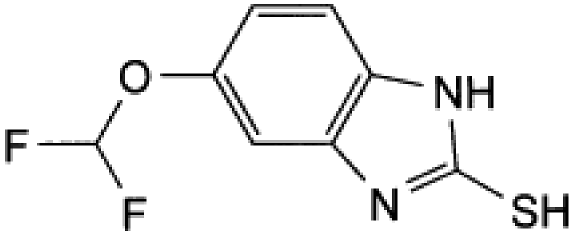
IMPURITIES

Specified impurities A, B, C, D, E, F.

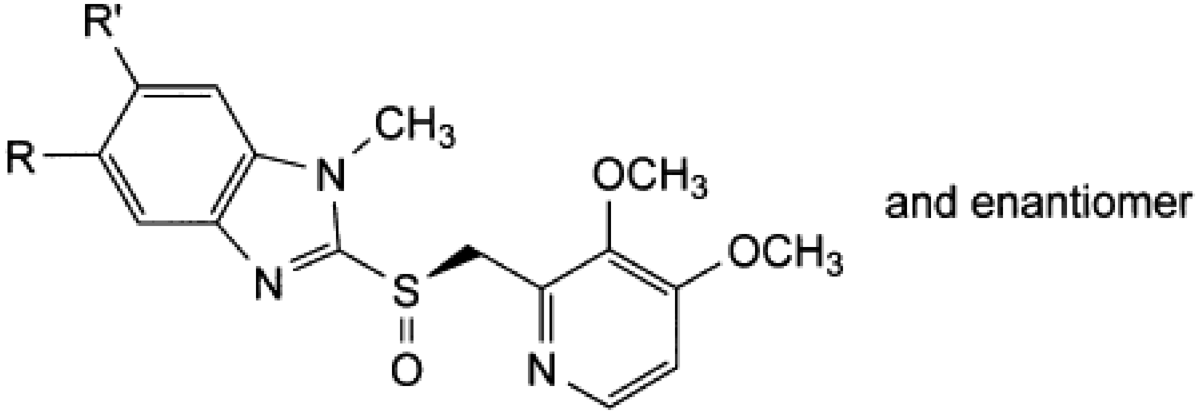

NH
OCH3
OCH3
O
F
F
N
X
N

A. X = SO_2: 5-(difluoromethoxy)-2-[[(3,4-dimethoxypyridin-2-yl)methyl]sulphonyl]-1*H*-benzimidazole,

B. X = S: 5-(difluoromethoxy)-2-[[(3,4-dimethoxypyridin-2-yl)methyl]sulphanyl]-1*H*-benzimidazole,

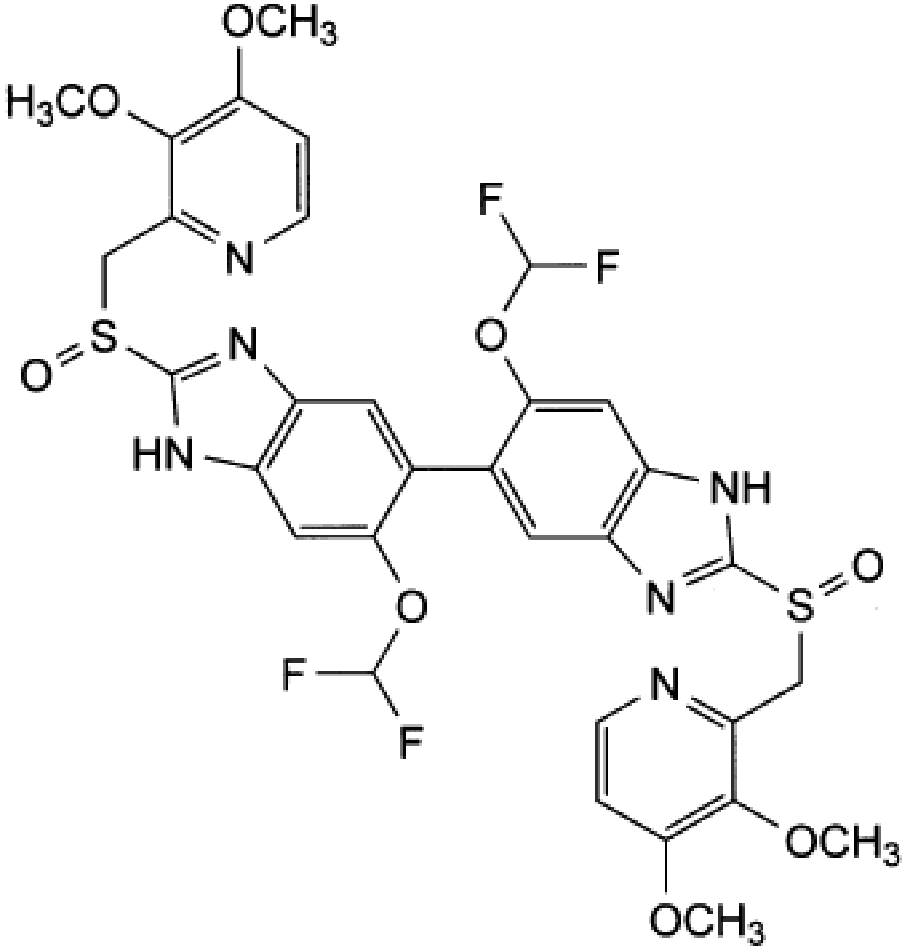


C. 5-(difluoromethoxy)-1*H*-benzimidazole-2-thiol,


R'
CH3
R
N
OCH3
and enantiomer
OCH3
N
S
O
N

D. R = $OCHF_2$, R′ = H: 5-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl] sulphinyl]-1-methyl-1*H*-benzimidazole,

F. R = H, R′ = $OCHF_2$: 6-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl] sulphinyl]-1-methyl-1*H*-benzimidazole,


OCH3
H3CO
N
F
F
O
O
S
N
HN
NH
N
S
O
O
F
F
N
OCH3
OCH3

E. mixture of the stereoisomers of 6,6′-bis(difluoromethoxy)-2,2′-bis[[(3,4-dimethoxypyridin-2-yl)methyl]sulphinyl]-1*H*,1′*H*-5,5′-bibenzimidazolyl.

Ph Eur

Papaveretum

General Notices

Action and use

Opioid receptor agonist; analgesic.

Preparation

Papaveretum Injection

DEFINITION

Papaveretum is a mixture of 253 parts of Morphine Hydrochloride ($C_{17}H_{19}NO_3,HCl,3H_2O$, 375.9), 23 parts of Papaverine Hydrochloride ($C_{20}H_{21}NO_4,HCl$, 375.9) and 20 parts of Codeine Hydrochloride ($C_{18}H_{21}NO_3,HCl,2H_2O$, 371.9). It contains not less than 80.0% and not more than 88.4% of $C_{17}H_{19}NO_3,HCl$, not less than 8.3% and not more than 9.2% of $C_{20}H_{21}NO_4,HCl$ and not less than 6.6% and not more than 7.4% of $C_{18}H_{21}NO_3,HCl$, calculated with reference to the dried material.

CHARACTERISTICS

A white or almost white, crystalline powder.

Soluble in *water*; sparingly soluble in *ethanol (96%)*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia,* 6 volumes of *ethanol (96%),* 40 volumes of *acetone* and 40 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of four solutions in *water* containing (1) 1.5% w/v of the substance being examined, (2) 1.28% w/v of *morphine sulphate BPCRS*, (3) 0.115% w/v of *papaverine hydrochloride BPCRS* and (4) 0.10% w/v of *codeine hydrochloride BPCRS*. After removal of the plate, dry it at 100° to 105° for 15 minutes, allow it to cool and spray with *potassium iodobismuthate solution* and then with a 0.4% v/v solution of *sulphuric acid*. The chromatogram obtained with solution (1) shows three principal spots corresponding to the principal spots in the chromatograms obtained with solutions (2), (3) and (4).

B. Yields the reactions characteristics of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1.5% w/v solution, 3.7 to 4.7, Appendix V L.

Clarity and colour of solution

A 1.5% w/v solution in *water* is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* BY_5, Appendix IV B, Method II.

Loss on drying

When dried to constant weight at 130°, loses not less than 10.0% and not more than 14.0% of its weight. Use 0.5 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using three solutions in *methanol (60%)* containing (1) 0.128% w/v of *morphine sulphate BPCRS*, 0.0115% w/v of *papaverine hydrochloride BPCRS* and 0.010% w/v of *codeine hydrochloride BPCRS*, (2) 0.128% w/v of *morphine sulphate BPCRS* and (3) 0.15% w/v of the substance being examined.

The chromatographic procedure may be carried out using a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid* and (c) a detection wavelength of 285 nm. If necessary, adjust the proportion of methanol in the mobile phase in the range 55% v/v to 65% v/v so that the retention time of morphine in solution (2) is 4 to 5 minutes. Adjust the pH of the mobile phase with either *glacial acetic acid* or 2M *sodium hydroxide* in order to obtain optimum separation of the three principal components in solution (1). The retention times of codeine and papaverine relative to that of morphine are about 1.3 and 1.7 respectively.

For anhydrous morphine hydrochloride

Calculate the content of $C_{17}H_{19}NO_3,HCl$ using the declared content of $C_{17}H_{19}NO_3$ in *morphine sulphate BPCRS*. Each mg of $C_{17}H_{19}NO_3$ is equivalent to 1.13 mg of $C_{17}H_{19}NO_3,HCl$.

For papaverine hydrochloride

Calculate the content of $C_{20}H_{21}NO_4,HCl$ using the declared content of $C_{20}H_{21}NO_4,HCl$ in *papaverine hydrochloride BPCRS*.

For anhydrous codeine hydrochloride

Calculate the content of $C_{18}H_{21}NO_3,HCl$ using the declared content of $C_{18}H_{21}NO_3,HCl$ in *codeine hydrochloride BPCRS*.

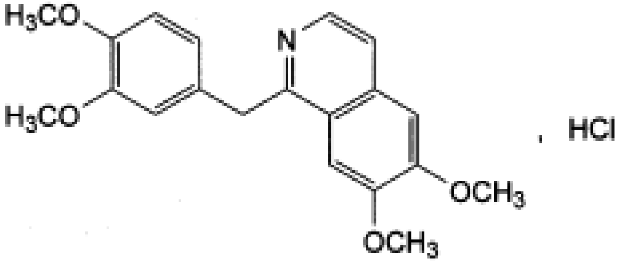
STORAGE

Papaveretum should be protected from light.

Papaverine Hydrochloride

General Notices

(Ph Eur monograph 0102)



$C_{20}H_{21}NO_4$,HCl 375.9 *61-25-6*

Action and use

Phosphodiesterase inhibitor; smooth muscle relaxant.

Preparation

Papaverine Injection

Ph Eur

DEFINITION

1-(3,4-Dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder or white or almost white crystals.

Solubility

Sparingly soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification

A, D.

Second identification   *B, C, D.*

A Infrared absorption spectrophotometry (*2.2.24*).

Comparison   *Papaverine hydrochloride CRS.*

B Thin-layer chromatography *(2.2.27)*.

Test solution   Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution   Dissolve 5 mg of *papaverine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate   *TLC silica gel* GF_{254} *plate R.*

Mobile phase   *Diethylamine R, ethyl acetate R*, *toluene R* (10:20:70 *V/V/V*).

Application   10 µl.

Development   Over 2/3 of the plate.

Drying   At 100-105 °C for 2 h.

Detection   Examine in ultraviolet light at 254 nm.

Results   The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 10 ml of solution S (see Tests) add 5 ml of *ammonia R* dropwise and allow to stand for 10 min. The precipitate, washed and dried, melts (*2.2.14*) at 146 °C to 149 °C.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.4 g in *carbon dioxide-free water R*, heating gently if necessary, and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II*).

pH *(2.2.3)*

3.0 to 4.0 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture   *acetonitrile R*, *mobile phase A* (20:80 *V/V*).

Test solution   Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a)

Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve 12 mg of *noscapine CRS* in 1.0 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Column:

— *size: l* = 0.25 m, Ø = 4.0 mm;

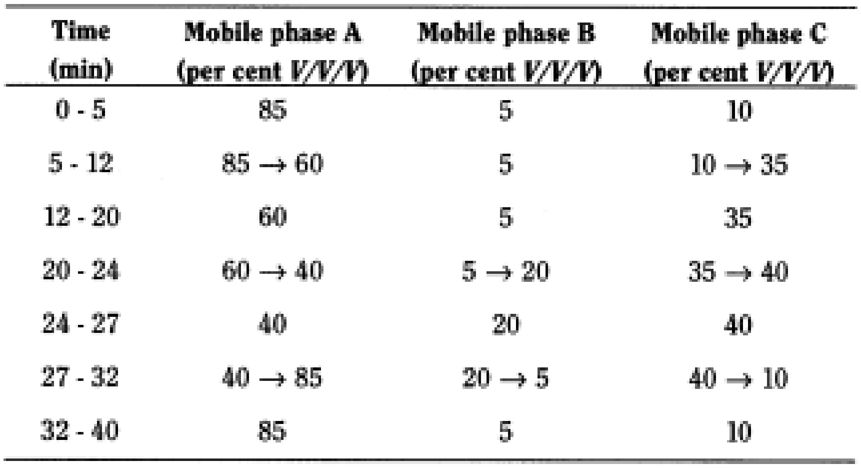
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: 3.4 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *dilute phosphoric acid R*;

— *mobile phase B*: *acetonitrile R*;

— *mobile phase C*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V/V*)	Mobile phase B (per cent *V/V/V*)	Mobile phase C (per cent *V/V/V*)
0 - 5	85	5	10
5 - 12	85 → 60	5	10 → 35
12 - 20	60	5	35
20 - 24	60 → 40	5 → 20	35 → 40
24 - 27	40	20	40
27 - 32	40 → 85	20 → 5	40 → 10
32 - 40	85	5	10

Flow rate 1 ml/min.

Detection Spectrophotometer at 238 nm.

Injection 10 µl.

Relative retention With reference to papaverine (retention time = about 23.4 min): impurity E = about 0.7; impurity C = about 0.75; impurity B = about 0.8; impurity A = about 0.9; impurity F = about 1.1; impurity D = about 1.2.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity A and papaverine.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 7; impurity D = 0.5; impurity A = 6.2;

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on the residue from the test for loss on drying.

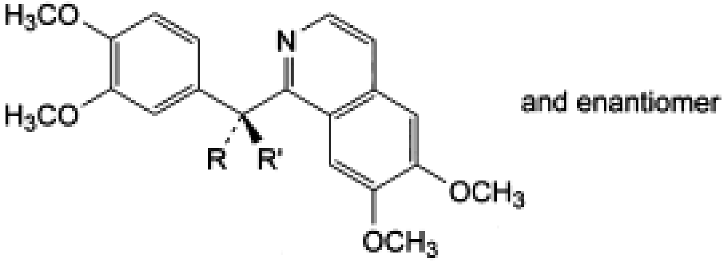
ASSAY

Dissolve 0.300 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 37.59 mg of $C_{20}H_{22}ClNO_4$.

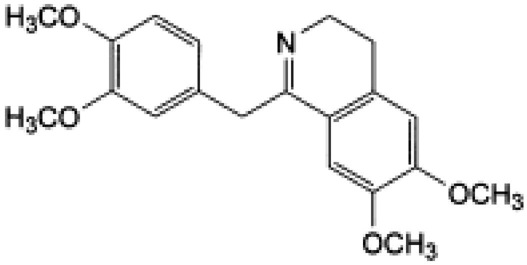
IMPURITIES

A. noscapine,

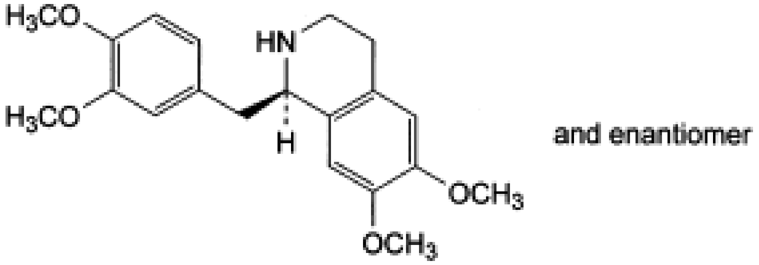


B. R = OH, R′ = H: (*RS*)-(3,4-dimethoxyphenyl)(6,7-dimethoxyisoquinolin-1-yl)methanol (papaverinol),

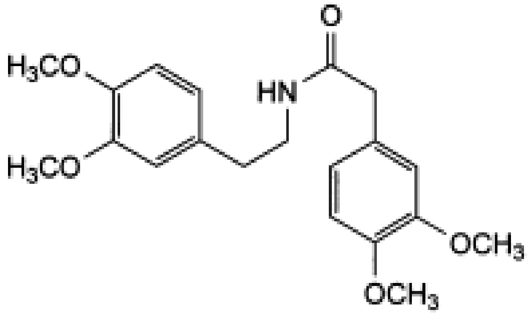
D. R + R′ = O: (3,4-dimethoxyphenyl)(6,7-dimethoxyisoquinolin-1-yl)methanone (papaveraldine),



C. 1-(3,4–dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline (dihydropapaverine),



E. (1*RS*)-1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (tetrahydropapaverine),



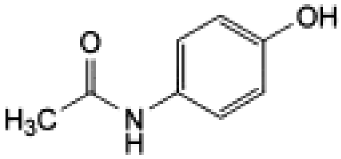
F. 2-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)ethyl]acetamide.

Ph Eur

Paracetamol

General Notices

(Ph Eur monograph 0049)



$C_8H_9NO_2$ 151.2 *103-90-2*

Action and use

Analgesic; antipyretic.

Preparations

Co-codamol Tablets

Co-codamol Capsules

Effervescent Co-codamol Tablets

Co-dydramol Tablets

Co-proxamol Tablets

Paracetamol Capsules

Paediatric Paracetamol Oral Solution

Paracetamol Oral Suspension

Paracetamol Suppositories

Paracetamol Tablets

Dispersible Paracetamol Tablets

Soluble Paracetamol Tablets

Paracetamol, Codeine Phosphate and Caffeine Capsules

Paracetamol, Codeine Phosphate and Caffeine Tablets

Ph Eur

DEFINITION

N-(4-Hydroxyphenyl)acetamide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in alcohol, very slightly soluble in methylene chloride.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D, E.*

A. Melting point *(2.2.14)*: 168 °C to 172 °C.

B. Dissolve 0.1 g in *methanol R* and dilute to 100.0 ml with the same solvent. To 1.0 ml of the solution add 0.5 ml of a 10.3 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with *methanol R*. Protect the solution from bright light and immediately measure the absorbance *(2.2.25)* at the absorption maximum at 249 nm. The specific absorbance at the maximum is 860 to 980.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *paracetamol CRS*.

D. To 0.1 g add 1 ml of *hydrochloric acid R*, heat to boiling for 3 min, add 1 ml of *water R* and cool in an ice bath. No precipitate is formed. Add 0.05 ml of a 4.9 g/l solution of *potassium dichromate R*. A violet colour develops which does not change to red.

E. It gives the reaction of acetyl *(2.3.1)*. Heat over a naked flame.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 0.200 g of the substance to be examined in 2.5 ml of *methanol R* containing 4.6 g/l of a 400 g/l solution of *tetrabutylammonium hydroxide R* and dilute to 10.0 ml with a mixture of equal volumes of a 17.9 g/l solution of *disodium hydrogen phosphate R* and of a 7.8 g/l solution of *sodium dihydrogen phosphate R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *4-aminophenol R*, 5 mg of *paracetamol CRS* and 5.0 mg of *chloroacetanilide R* in *methanol R* and dilute to 20.0 ml with the same solvent.

Dilute 1.0 ml to 250.0 ml with the mobile phase.

Reference solution (d) Dissolve 20.0 mg of *4-nitrophenol R* in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml to 20.0 ml with the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);
- *temperature:* 35 °C.

Mobile phase Mix 375 volumes of a 17.9 g/l solution of *disodium hydrogen phosphate R*, 375 volumes of a 7.8 g/l solution of *sodium dihydrogen phosphate R* and 250 volumes of *methanol R* containing 4.6 g/l of a 400 g/l solution of *tetrabutylammonium hydroxide R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 245 nm.

Injection 20 µl.

Run time 12 times the retention time of paracetamol.

Relative retention With reference to paracetamol (retention time = about 4 min): impurity K = about 0.8; impurity F = about 3; impurity J = about 7.

System suitability Reference solution (c):

- *resolution*: minimum 4.0 between the peaks due to impurity K and to paracetamol;
- *signal-to-noise ratio*: minimum 50 for the peak due to impurity J.

Limits:
- *impurity J*: not more than 0.2 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (10 ppm);
- *impurity K*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (50 ppm);
- *impurity F*: not more than half the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.05 per cent);
- *any other impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *total of other impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *disregard limit* for the calculation of the total of other impurities: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

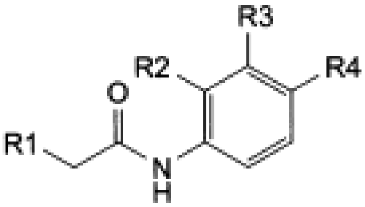
Dissolve 0.300 g in a mixture of 10 ml of *water R* and 30 ml of *dilute sulphuric acid R*. Boil under a reflux condenser for 1 h, cool and dilute to 100.0 ml with *water R*. To 20.0 ml of the solution add 40 ml of *water R*, 40 g of ice, 15 ml of *dilute hydrochloric acid R* and 0.1 ml of *ferroin R*. Titrate with *0.1 M cerium sulphate* until a greenish-yellow colour is obtained. Carry out a blank titration.

1 ml of *0.1 M cerium sulphate* is equivalent to 7.56 mg of $C_8H_9NO_2$.

STORAGE

Protected from light.

IMPURITIES



A. R1 = R3 = R4 = H, R2 = OH: *N*-(2-hydroxyphenyl)acetamide,

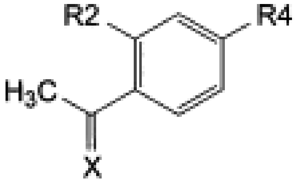
B. R1 = CH_3, R2 = R3 = H, R4 = OH: *N*-(4-hydroxyphenyl)propanamide,

C. R1 = R2 = H, R3 = Cl, R4 = OH: *N*-(3-chloro-4-hydroxyphenyl)acetamide,

D. R1 = R2 = R3 = R4 = H: *N*-phenylacetamide,

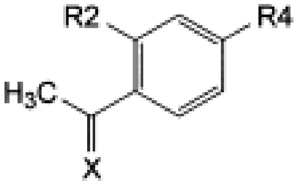
H. R1 = R2 = R3 = H, R4 = O-CO-CH_3: 4-(acetylamino)phenyl acetate,

J. R1 = R2 = R3 = H, R4 = Cl: *N*-(4-chlorophenyl)acetamide (chloroacetanilide),

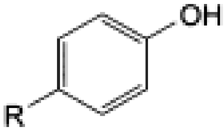


E. X = O, R2 = H, R4 = OH: 1-(4-hydroxyphenyl)ethanone,

G. X = N-OH, R2 = H, R4 = OH: 1-(4-hydroxyphenyl)ethanone oxime,



I. X = O, R2 = OH, R4 = H: 1-(2-hydroxyphenyl)ethanone,



F. R = NO_2: 4-nitrophenol,

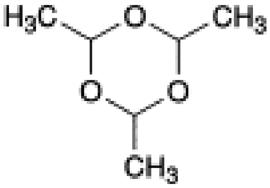
K. R = NH_2: 4-aminophenol.

Ph Eur

Paraldehyde

General Notices

(Ph Eur monograph 0351)



$C_6H_{12}O_3$ 132.2 *123-63-7*

Action and use

Anticonvulsant.

Preparation

Paraldehyde Injection

Ph Eur

DEFINITION

2,4,6-Trimethyl-1,3,5-trioxane (cyclic trimer of acetaldehyde).

It may contain a suitable quantity of an antioxidant.

CHARACTERS

Appearance

Colourless or slightly yellow, transparent liquid. It solidifies on cooling to form a crystalline mass.

Solubility

Soluble in water, but less soluble in boiling water, miscible with ethanol (96 per cent) and with essential oils.

IDENTIFICATION

A. Solution S (see Tests) is clear *(2.2.1)* but becomes turbid on warming.

B. To 5 ml add 0.1 ml of *dilute sulphuric acid R* and heat. Acetaldehyde, recognisable by its odour, is evolved.

C. To 5 ml of solution S in a test-tube add 5 ml of *ammoniacal silver nitrate solution R* and heat in a water-bath. Silver is deposited as a mirror on the wall of the tube.

TESTS

Solution S

Dissolve 20.0 ml in *carbon dioxide-free water R* and dilute to 200.0 ml with the same solvent.

Acidity

To 50.0 ml of solution S add 0.05 ml of *phenolphthalein solution R*. Not more than 1.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Refractive index ***(2.2.6***

1.403 to 1.406.

Relative density ***(2.2.5)***

0.991 to 0.996.

Distillation range *(2.2.11)*: a maximum of 10 per cent distils below 123 °C and a minimum of 95 per cent distils below 126 °C.

Freezing point ***(2.2.18)***

10 °C to 13 °C.

Acetaldehyde

To 5.0 ml add a mixture of 0.2 ml of *methyl orange solution R*, 5 ml of *ethanol (60 per cent V/V) R* and 5 ml of *alcoholic hydroxylamine solution R* and shake. Not more than 0.8 ml of *0.5 M sodium hydroxide* is required to change the colour of the indicator to pure yellow.

Peroxides

Place 50.0 ml of solution S in a ground-glass-stoppered flask, add 5 ml of *dilute sulphuric acid R* and 10 ml of *potassium iodide solution R*, close the flask and allow to stand protected from light for 15 min. Titrate with *0.1 M sodium thiosulphate* using 1 ml of *starch solution R* as indicator. Allow to stand for 5 min and, if necessary complete the titration. Not more than 2.0 ml of *0.1 M sodium thiosulphate* is required.

Non-volatile residue

Maximum 0.6 g/l.

Heat 5.0 ml in a tared evaporating dish on a water-bath and dry at 105 °C for 1 h. The residue weighs a maximum of 3 mg.

STORAGE

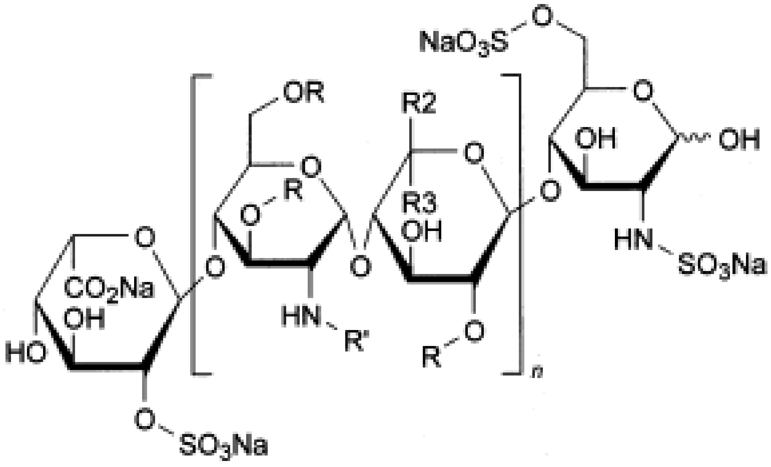
In a small, well-filled, airtight container, protected from light. If the substance has solidified the whole contents of the container must be liquefied before use.

Ph Eur

Parnaparin Sodium

General Notices

(Ph Eur monograph 1252)



n = 1 to 21 , R = H or SO_3Na , R' = SO_3Na or $CO\text{-}CH_3$
R2 = H and R3 = CO_2Na or R2 = CO_2Na and R3 = H

Action and use

Low molecular weight heparin.

Ph Eur

DEFINITION

Sodium salt of a low-molecular-mass heparin that is obtained by radical-catalysed depolymerisation, with hydrogen peroxide and with a cupric salt, of heparin from bovine or porcine intestinal mucosa. The majority of the components have a 2-*O*-sulpho-α-L-idopyranosuronic acid structure at the non-reducing end and a 2-*N*,6-*O*-disulpho-D-glucosamine structure at the reducing end of their chain.

Parnaparin sodium complies with the monograph *Low-molecular-mass heparins (0828)*, with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 4000 and 6000 with a characteristic value of about 5000.

The degree of sulphatation is 2.0 to 2.6 per disaccharide unit.

The potency is not less than 75 IU and not more than 110 IU of anti-factor Xa activity per milligram calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 3.0.

IDENTIFICATION

Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *parnaparin sodium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. In order to verify the suitability of the system in the lower molecular mass ranges (for example Mr 2000), a suitable reference preparation is used. The following requirements apply.

The mass-average relative molecular mass ranges between 4000 and 6000. The mass percentage of chains lower than 3000 is not more than 30 per cent. The mass percentage of chains between 3000 and 8000 ranges between 50 per cent and 60 per cent.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Dissolve 1.5 g in 10 ml of *water R*.

Copper

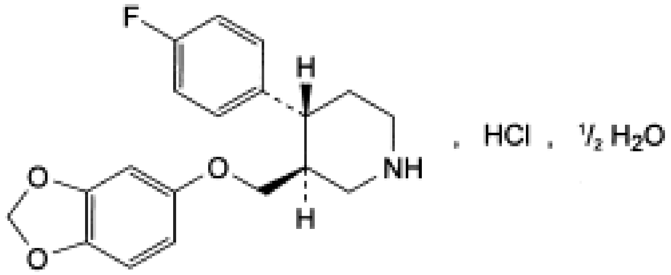
Maximum 10.0 ppm, determined by atomic absorption spectrometry *(2.2.23*, *Method I*) and calculated with reference to the dried substance.

Ph Eur

Paroxetine Hydrochloride Hemihydrate

General Notices

(Ph Eur monograph 2018)



$C_{19}H_{20}FNO_3,HCl,½H_2O$ 374.8 *110429-35-1*

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

Ph Eur

DEFINITION

(*3S,4R*)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine hydrochloride hemihydrate.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

Impurity G

Maximum 1 ppm, determined by a suitable, validated method.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in methanol, sparingly soluble in ethanol (96 per cent) and in methylene chloride.

It shows pseudopolymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *paroxetine hydrochloride hemihydrate CRS.*

If the spectra obtained show differences, dissolve 1 part of the substance to be examined and 1 part of the reference substance separately in 10 parts of a mixture of 1 volume of *water R* and 9 volumes of *2-propanol R* and heat to 70 °C to dissolve. Recrystallise and record new spectra using the residues.

B. Examine the chromatograms obtained in the test for impurity D.

Injection Test solution and reference solution (c).

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

C. Water (see Tests).

D. It gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Impurity D

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.1000 g of the substance to be examined in 20 ml of *methanol R* and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *paroxetine impurity D CRS* and 5 mg of *paroxetine hydrochloride hemihydrate CRS* in 2 ml of *methanol R* and dilute to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve 10 mg of *paroxetine hydrochloride hemihydrate CRS* in 2 ml of *methanol R* and dilute to 10.0 ml with the mobile phase.

Column:
— *size*: *l* = 0.10 m, Ø = 4.0 mm;

— *stationary phase: silica gel AGP for chiral chromatography R* (5 µm).

Mobile phase Mix 2 volumes of *methanol R* and 8 volumes of a 5.8 g/l solution of *sodium chloride R*.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 295 nm.

Injection 10 µl of the test solution and reference solutions (a) and (b).

Run time 2.5 times the retention time of paroxetine.

Retention time Paroxetine = about 30 min.

System suitability Reference solution (b):

— *resolution*: minimum 2.2 between the peaks due to impurity D and paroxetine.

Limit:

— *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Related subtances

Liquid chromatography (*2.2.29*).

Solvent mixture *tetrahydrofuran R*, *water R* (1:9 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the same solvent mixture.

Reference solution (a) Dilute 5.0 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 200.0 ml with the solvent mixture.

Reference solution (b) Dissolve 2 mg of *paroxetine for system suitability CRS* (containing impurity C) in the solvent mixture and dilute to 10 ml with the solvent mixture. Dilute 1 ml of this solution to 10 ml with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *paroxetine impurity A CRS* in the solvent mixture and dilute to 20 ml with the same solvent mixture.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

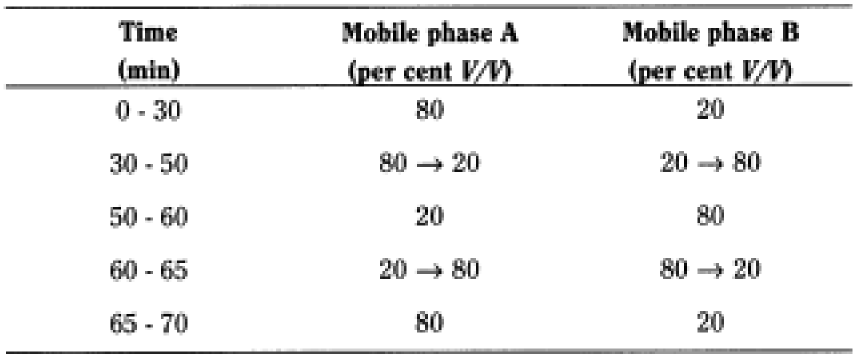
— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: *trifluoroacetic acid R*, t*etrahydrofuran R*, *water R* (5:100:900 *V/V/V*);

— *mobile phase B*: *trifluoroacetic acid R*, *tetrahydrofuran R*, *acetonitrile R* (5:100:900 *V/V/V*) ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	80	20
30 - 50	80 → 20	20 → 80
50 - 60	20	80
60 - 65	20 → 80	80 → 20
65 - 70	80	20

Flow rate 1 ml/min.

Detection Spectrophotometer at 295 nm.

Injection 20 µl.

Relative retention With reference to paroxetine: impurity A = about 0.8.

System suitability Reference solution (b):

— *resolution*: minimum 3.5 between the peaks due to impurity C and paroxetine.

Limits:

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Use a platinum crucible. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

2.2 per cent to 2.7 per cent, determined on 0.300 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *paroxetine hydrochloride hemihydrate CRS in water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dissolve 5.0 mg of *paroxetine hydrochloride hemihydrate CRS* and 5 mg of *paroxetine impurity A CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: trimethylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.85 g of *ammonium acetate R* in *water R*, adjust to pH 5.5 with *anhydrous acetic acid R* and dilute to 600 ml with the same solvent; add 400 ml of *acetonitrile R*; slowly add, with stirring, 10 ml of *triethylamine R* and readjust to pH 5.5 with *anhydrous acetic acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 295 nm.

Injection 10 µl.

Run time Twice the retention time of paroxetine.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to paroxetine and impurity A.

Calculate the percentage content of paroxetine hydrochloride using the chromatogram obtained with reference solution (a).

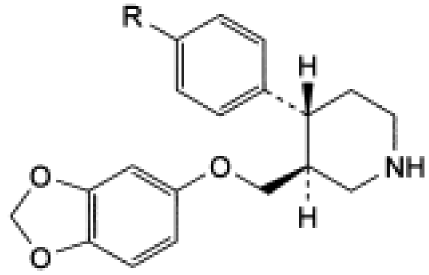
STORAGE

Protected from light.

IMPURITIES

Specified impurities A, D, G.

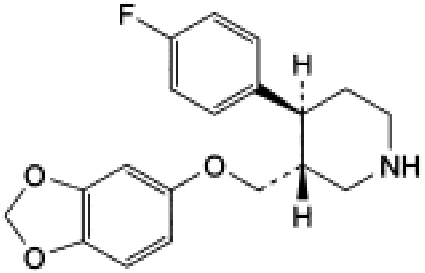
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, E, F.*



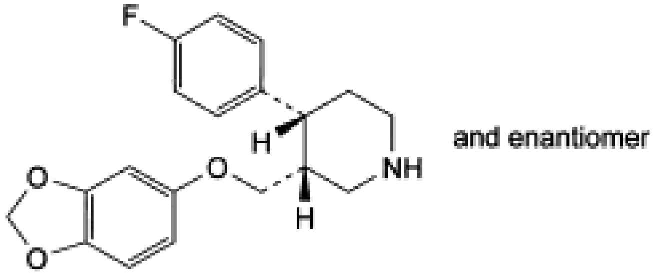
A. R = H: (3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-phenylpiperidine (desfluoroparoxetine),

B. R = OCH_3: (3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-methoxyphenyl)piperidine,

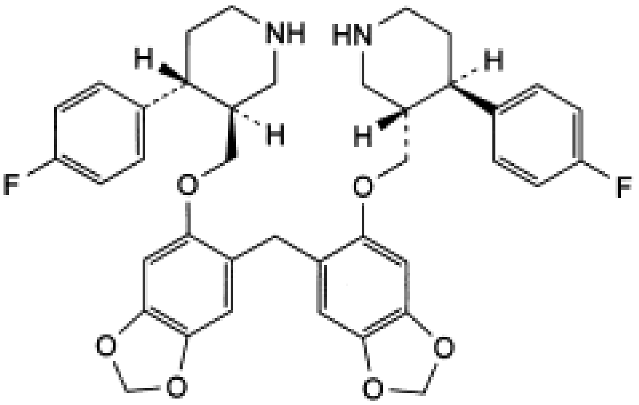
C. R = C_2H_5: (3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-ethoxyphenyl)piperidine,



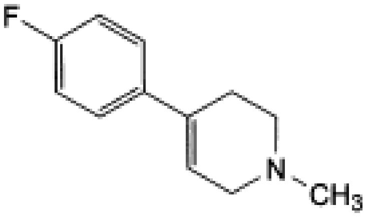
D. (3*R*,4*S*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine ((+)-*trans*-paroxetine),



E. (3*RS*,4*RS*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine (*cis*-paroxetine),



F. 3,3′-[methylenebis(1,3-benzodioxole-6,5-diyloxymethylene)]bis[3*S*,4*R*)4-(4-fluorophenyl) piperidine],



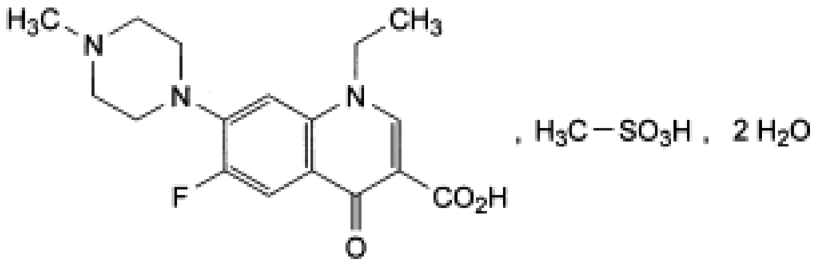
G. 4-(4-fluorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine.

Ph Eur

Pefloxacin Mesilate

General Notices

(Pefloxacin Mesilate Dihydrate, Ph Eur monograph 1460)



$C_{18}H_{24}FN_3O_6S,2H_2O$ 465.5 *70458-95-6*

Action and use

Antibacterial.

Ph Eur

DEFINITION

1-Ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid methanesulphonate dihydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

Fine, white or almost white powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 0.1 g in 10 ml of *water R*. Add 5 ml of *1 M sodium hydroxide*. Adjust to pH 7.4 ± 0.1 with *phosphoric acid R* and shake with 2 quantities, each of 30 ml, of *methylene chloride R*. Combine the organic layers and dry over *anhydrous sodium sulphate R*. Evaporate to dryness. Examine the residue as a disc of *potassium bromide R*.

Comparison Repeat the operations using 0.1 g of *pefloxacin mesilate dihydrate CRS*.

B. Thin-layer chromatography (*2.2.27)*.

Test solution Dissolve 40 mg in *water R* and dilute to 1 ml with the same solvent.

Reference solution Dissolve 60 mg of *methanesulphonic acid R* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *water R*, *ammonia R*, *butanol R*, *acetone R* (5:10:20:65 *V/V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.4 g/l solution of *bromocresol purple R* in *ethanol (50 per cent V/V) R*, adjusted to pH 10 using *1 M sodium hydroxide*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 1.0 g in carbon dioxide-free *water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution.

Examined within 1 h after its preparation, solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 3 of the range of reference solutions of the most appropriate colour *(2.2.2*, *Method II*).

pH

(*2.2.3*): 3.5 to 4.5.

Dilute 1 ml of solution S to 10 ml with carbon dioxide-free *water R*.

Related substances.

Liquid chromatography (*2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *pefloxacin impurity B CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase. In 2.0 ml of this solution, dissolve the contents of a vial of *pefloxacin impurity C CRS*.

Reference solution (b) Dissolve 10.0 mg of *norfloxacin impurity A CRS* (impurity F) in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:
— *size: l* = 0.15 m, Ø = 6 mm;
— *stationary phase*: *octadecylsilyl vinyl polymer for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *acetonitrile R*, 70 volumes of a solution containing 2.70 g/l of *cetyltrimethylammonium bromide R* and 6.18 g/l of *boric acid R* (exactly adjusted to pH 8.30 with *1 M sodium hydroxide*), and 0.2 volumes of *thiodiethylene glycol R*.

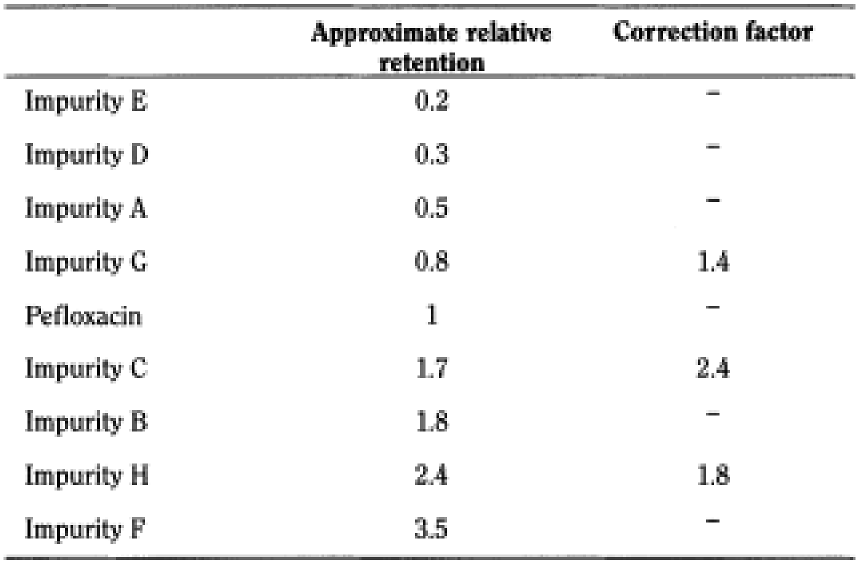
Flow rate 1 ml/min.

Detection Spectrophotometer at 258 nm and at 273 nm.

Injection 20 µl.

Run time 4 times the retention time of pefloxacin (about 60 min).

Relative retentions and correction factors



	Approximate relative retention	Correction factor
Impurity E	0.2	–
Impurity D	0.3	–
Impurity A	0.5	–
Impurity G	0.8	1.4
Pefloxacin	1	–
Impurity C	1.7	2.4
Impurity B	1.8	–
Impurity H	2.4	1.8
Impurity F	3.5	–

System suitability Reference solution (a) at 273 nm:

— *resolution*: minimum 1.5 between the peaks due to impurities C and B.

From the chromatogram obtained at 258 nm with the test solution, calculate the percentage content of impurities C, F, G and H using the area of the principal peak in the chromatogram obtained at 258 nm with reference solution (b) (external standardisation) taking into account the correction factors indicated in the table.

From the chromatogram obtained at 273 nm with the test solution, calculate the percentage content of impurities A, B, D and E and of any other impurity from the areas of the peaks in the chromatogram obtained with the test solution by the normalisation procedure.

Limits:

- *impurities A, B, D, E and any other impurity at 273 nm and impurities C, F, G, H at 258 nm*: for each impurity, maximum 0.5 per cent and not more than 3 impurities have a content between 0.2 per cent and 0.5 per cent;
- *total*: maximum 1.0 per cent;
- *disregard limit at 273 nm*: 0.0005 times the area of the principal peak in the chromatogram obtained with the test solution (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

1.0 g complies with test E. Prepare the reference solution using 10.0 ml of *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*)

7.0 per cent to 8.5 per cent, determined on 50.0 mg using a mixture of 10 volumes of *methanol R* and 50 volumes of *methylene chloride R*.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 15.0 ml of *anhydrous acetic acid R* and add 75.0 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

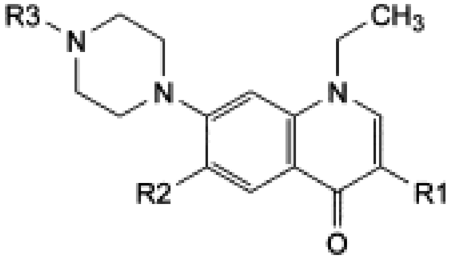
1 ml of *0.1 M perchloric acid* is equivalent to 21.48 mg of $C_{18}H_{24}FN_3O_6S$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

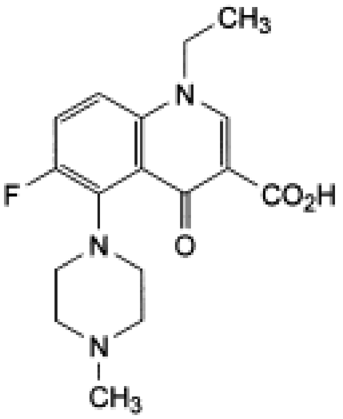
Specified impurities A, B, C, D, E, F, G, H.



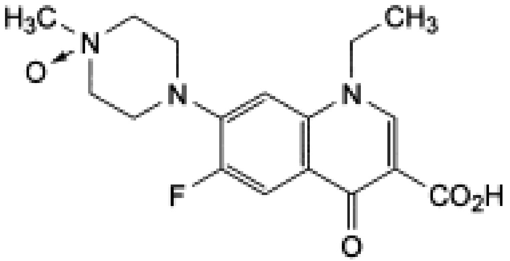
A. R1 = CO_2H, R2 = F, R3 = H: 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (demethylated pefloxacin or norfloxacin),

B. R1 = CO_2H, R2 = Cl, R3 = CH_3: 6-chloro-1-ethyl-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (chlorinated homologue of pefloxacin),

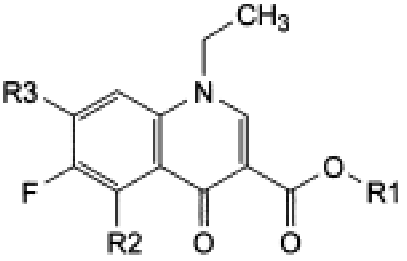
E. R1 = H, R2 = F, R3 = CH_3: 1-ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)quinoline-4(1*H*)-one (decarboxylated pefloxacin),



C. 1-ethyl-6-fluoro-5-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (isopefloxacin),



D. 4-(3-carboxy-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolin-7-yl)-1-methylpiperazine 1-oxide (*N*-oxide of pefloxacin),



F. R1 = R2 = H, R3 = Cl: 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (*N*-ethyl acid) (norfloxacin impurity A),

G. R1 = CH_5, R2 = H, R3 = Cl: ethyl 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (*N*-ethyl ester),

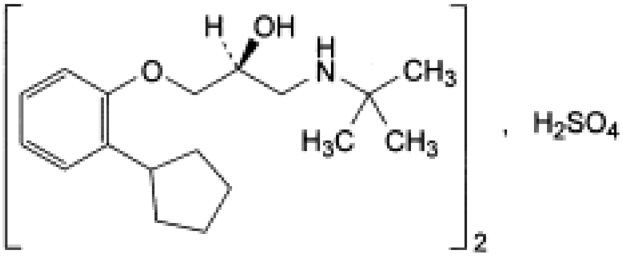
H. R1 = R3 = H, R2 = Cl: 5-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (iso-*N*-ethyl acid).

Ph Eur

Penbutolol Sulphate

General Notices

(Ph Eur monograph 1461)



$C_{36}H_{58}N_2O_4,H_2SO_4$ 681 *38363-32-5*

Action and use

Beta-adrenoceptor antagonist.

Ph Eur

DEFINITION

Di[(2*S*)-1-(2-cyclopentylphenoxy)-3-[(1,1-dimethylethyl)amino]propan-2-ol] sulphate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, soluble in methanol, practically insoluble in cyclohexane.

IDENTIFICATION

First identification *A, C, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Penbutolol sulphate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40 mg of the substance to be examined in 1 ml of *methanol R*.

Reference solution Dissolve 40 mg of *penbutolol sulphate CRS* in 1 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *Glacial acetic acid R*, *water R*, *butanol R*, *ethyl acetate R* (10:20:35:35 *V/V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 50 mg in a mixture of 5 ml of *water R* and 1 ml of *0.1 M hydrochloric acid*. The solution gives reaction (a) of sulphates (*2.3.1*).

D. Specific optical rotation (see Tests).

TESTS

Solution S

Dissolve 1.00 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Acidity or alkalinity

To 4 ml of solution S add 4 ml of *carbon dioxide-free water R*. Add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution is red.

Specific optical rotation (*2.2.7)*

- 23 to - 25 (dried substance), determined on solution S.

Related substances.

Liquid chromatography (*2.2.29*).

Solvent mixture: Mobile phase B, mobile phase A (40:60 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 4.0 mg of the substance to be examined and 1.0 mg of *penbutolol impurity A CRS* in 5.0 ml of the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with the solvent mixture.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with the solvent mixture.

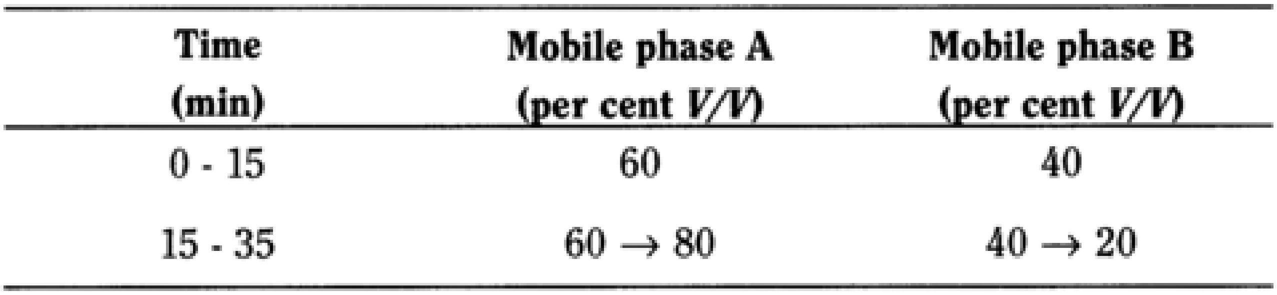
Reference solution (d) Dissolve 5.0 mg of *penbutolol impurity A CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *Mobile phase A*: *acetonitrile for chromatography R*, *methanol R* (39:61 *V/V*);
- *Mobile phase B*: dissolve 11 g of *sodium heptanesulphonate R* in 1000 ml of *water R*, add 5.0 ml of *triethylamine R* and adjust to pH 2.7 with *phosphoric acid R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	60	40
15 - 35	60 → 80	40 → 20

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µl.

System suitability Reference solution (a):

- *resolution*: minimum 3.0 between the 2 principal peaks.

Limits:

- *Impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);
- *Any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *Sum of impurities other than A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- *Disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

1.0 g complies with test F. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 40 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

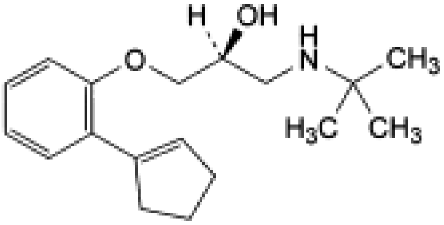
1 ml of *0.1 M perchloric acid* is equivalent to 68.10 mg of $C_{36}H_{60}N_2O_8S$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A.



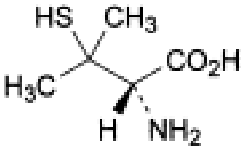
A. (2*S*)-1-[2-(cyclopent-1-enyl)phenoxy]-3-[(1,1-dimethylethyl)amino]propan-2-ol.

Ph Eur

Penicillamine

General Notices

(Ph Eur monograph 0566)



$C_5H_{11}NO_2S$ 149.2 *52-67-5*

Action and use

Disease-modifying antirheumatic drug; chelating agent; treatment of Wilson′s disease; heavy metal poisoning; cystinuria.

Preparation

Penicillamine Tablets

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-methyl-3-sulphanylbutanoic acid.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B, D.*

Second identification *A, C, D.*

A. Dissolve 0.5 g in a mixture of 0.5 ml of *hydrochloric acid R* and 4 ml of warm *acetone R*, cool in iced water and initiate crystallisation by scratching the wall of the tube with a glass rod. A white precipitate is formed. Filter with the aid of vacuum, wash with *acetone R* and dry with suction. A 10 g/l solution of the precipitate is dextrorotatory.

B. Examine the chromatograms obtained in the test for impurity A.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and approximate size to the principal peak in the chromatogram obtained with reference solution (a).

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 4 ml of *water R*.

Reference solution Dissolve 10 mg of *penicillamine CRS* in 4 ml of *water R*.

Plate *TLC silica gel G plate R*.

Mobile phase *Glacial acetic acid R, water R*, *butanol R* (18:18:72 *V/V/V*).

Application 2 µl.

Development Over a path of 10 cm.

Drying at 100-105 °C for 5-10 min.

Detection Expose to iodine vapour for 5-10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 40 mg in 4 ml of *water R* and add 2 ml of *phosphotungstic acid solution R*. Allow to stand for 5 min. A blue colour develops.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2*, *Method II*).

pH (*2.2.3*)

4.5 to 5.5.

Dilute 1 ml of solution S to 10 ml with *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7*)

- 61.0 to - 65.0 (dried substance).

Dissolve 0.500 g in *1 M sodium hydroxide* and dilute to 10.0 ml with the same solvent.

Ultraviolet-absorbing substances

Maximum 0.5 per cent of *penilloic acid*.

Dissolve 0.100 g in *water R* and dilute to 50.0 ml with the same solvent. The absorbance (*2.2.25*) of the solution at 268 nm is not greater than 0.07.

Impurity A

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 40 mg of *penicillamine CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 20 mg of *penicillamine disulphide CRS* (impurity A) in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 5 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Solution containing 0.1 g/l of *sodium edetate R* and 2 g/l of *methanesulphonic acid R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 220 nm.

Limit:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1 per cent).

Impurity B

Maximum 0.1 ppm.

Carry out all the operations in a penicillin-free atmosphere and with equipment reserved for this test. Sterilise the equipment at 180 °C for 3 h and the buffer solutions at 121 °C for 20 min before use.

Test solution (a). Dissolve 1.000 g in 8 ml of *buffer solution pH 2.5 R* and add 8 ml of *ether R*. Shake vigorously for 1 min. Repeat the extraction and combine the ether layers. Add 8 ml of *buffer solution pH 2.5 R.* Shake for 1 min, allow to settle and quantitatively separate the upper layer, taking care to eliminate the aqueous phase completely (penicillin is unstable at pH 2.5; carry out operations at this pH within 6-7 min). Add 8 ml of *phosphate buffer solution pH 6.0 R2* to the ether phase, shake for 5 min, allow to settle, then separate the aqueous layer and check that the pH is 6.0.

Test solution (b) To 2 ml of test solution (a) add 20 µl of *penicillinase solution R* and incubate at 37 °C for 1 h.

Reference solution (a) Dissolve 5 mg of *benzylpenicillin sodium R* in 500 ml of *phosphate buffer solution pH 6.0 R2*. Dilute 0.25 ml of the solution to 200.0 ml with *buffer solution pH 2.5 R*. Carry out the extraction using 8 ml of this solution as described for test solution (a).

Reference solution (b) To 2 ml of reference solution (a) add 20 µl of *penicillinase solution R* and incubate at 37 °C for 1 h.

Blank solution Prepare the solution as described for test solution (a) but omitting the substance to be examined.

Liquefy a suitable nutrient medium such as that described below and inoculate it at a suitable temperature with a culture of *Kocuria rhizophila* (ATCC 9341), to give 5×10^4 micro-organisms per millilitre or a different quantity if necessary to obtain the required sensitivity and formation of clearly defined inhibition zones of suitable diameter. Immediately pour the inoculated medium into 5 Petri dishes 10 cm in diameter to give uniform layers 2-5 mm deep. The medium may alternatively consist of 2 layers, only the upper layer being inoculated. Store the dishes so that no appreciable growth or death of the micro-organisms occurs before use and so that the surface of the agar is dry at the time of use. In each dish, place 5 stainless steel hollow cylinders 6 mm in diameter on the surface of the agar evenly spaced on a circle with a radius of about 25 mm and concentric with the dish. For each dish, place in separate cylinders 0.15 ml of test solutions (a) and (b), reference solutions (a) and (b) and the blank solution. Maintain at 30 °C for at least 24 h. Measure the diameters of the inhibition zones with a precision of at least 0.1 mm. The test is valid if reference solution (a) gives a clear inhibition zone and if reference solution (b) and the blank solution give no inhibition zone. If test solution (a) gives an inhibition zone, this is caused by penicillin if test solution (b) gives no inhibition zone. If this is so, the average diameter of the inhibition zones given by test solution (a) for the 5 Petri dishes is less than the average diameter of the inhibition zones given by reference solution (a) measured in the same conditions.

Nutrient medium (pH 6.0)

Peptone	5 g
Yeast extract	1.5 g
Meat extract	1.5 g
Sodium chloride	3.5 g
Agar	15 g
Distilled water R	1000 ml

Mercury

Maximum 10.0 ppm.

Atomic absorption spectrometry (*2.2.23*, *Method I*).

Test solution To 1.00 g add 10 ml of *water R* and 0.15 ml of *perchloric acid R* and swirl until dissolution is complete. Add 1.0 ml of a 10 g/l solution of *ammonium pyrrolidinedithiocarbamate R* which has been washed immediately before use 3 times, each time with an equal volume of *methyl isobutyl ketone R*. Mix and add 2.0 ml of *methyl isobutyl ketone R* and shake for 1 min. Dilute to 25.0 ml with *water R* and allow the 2 layers to separate; use the methyl isobutyl ketone layer.

Reference solutions Dissolve a quantity of *mercuric oxide R* equivalent to 0.108 g of HgO in the smallest necessary volume of *dilute hydrochloric acid R* and dilute to 1000.0 ml with *water R* (100 ppm Hg). Prepare the reference solutions in the same manner as the test solution but using instead of the substance to be examined suitable volumes of the solution containing 100 ppm of Hg.

Source Mercury hollow-cathode lamp.

Wavelength 254 nm.

Atomisation device Air-acetylene flame.

Set the zero of the instrument using a methyl isobutyl ketone layer obtained as described for the test solution but omitting the substance to be examined.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at 60 °C at a pressure not exceeding 0.67 kPa.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

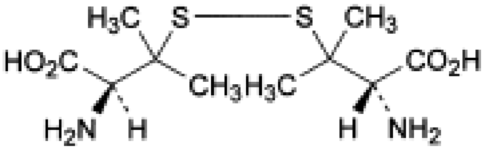
ASSAY

Dissolve 0.1000 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

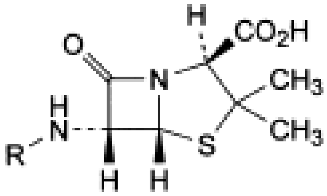
1 ml of *0.1 M perchloric acid* is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.

IMPURITIES

Specified impurities A, B.



A. 3,3′-(disulphanediyl)bis[(2*S*)-2-amino-3-methylbutanoic] acid (penicillamine disulphide),



B. penicillin.

Ph Eur

Pentagastrin

General Notices

Boc-βAla-Trp-Met-Asp-Phe

Boc=*tert*-butyloxycarbonyl

$C_{37}H_{49}N_7O_9S$ 767.9 *5534-95-2*

Action and use

Gastrin analogue.

Preparation

Pentagastrin Injection

DEFINITION

Pentagastrin is a pentapeptide that stimulates gastric secretion of acid. It contains not less than 97.0% and not more than 103.0% of $C_{37}H_{49}N_7O_9S$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Practically insoluble in *water*; soluble in 5M *ammonia* and in *dimethylformamide*; slightly soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.010% w/v solution in 0.01M *ammonia* exhibits maxima at 280 nm and 288 nm and an inflection at 275 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance on each of three plates and the following solvent systems as the mobile phases: (A) a mixture of 75 volumes of *butan-2-ol* and 25 volumes of a 3% v/v solution of 13.5M *ammonia*, (B) the upper layer produced by shaking together 50 volumes of *water*, 40 volumes of *butan-1-ol* and 10 volumes of *glacial acetic acid* and allowing to separate and (C) a mixture of 50 volumes of *glacial acetic acid*, 25 volumes of *ether* and 25 volumes of *water*. Apply separately to each plate 2 µl of each of two solutions in 0.1M *ammonia* containing (1) 0.5% w/v of the substance being examined and (2) *pentagastrin BPCRS* containing 0.5% w/v of pentagastrin. After removal of the plates, allow them to dry in air, heat at 100° for 2 minutes, spray with a 1.0% w/v solution of *4-dimethylaminobenzaldehyde* in a mixture of 3 volumes of *methanol* and 1 volume of *hydrochloric acid* and heat at 100° until purple spots are produced (about 2 minutes). Examine by transmitted light. On each plate, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

Light absorption

Ratio of the *absorbance* of a 0.010% w/v solution in 0.01M *ammonia* at the maximum at 280 nm to that at the maximum at 288 nm, 1.12 to 1.22, Appendix II B.

Specific optical rotation

In a 1% w/v solution in *dimethylformamide*, -25.0 to -29.0, calculated with reference to the dried substance, Appendix V F.

Amino acids

β-Alanine 11.0 to 12.2%, aspartic acid 16.4 to 18.2%, methionine 18.4 to 20.4%, phenylalanine, 20.4 to 22.6%, when determined by the following method. Examine using an amino acid analyser according to the manufacturer's instructions. Standardise the apparatus with 1 mg of an equimolar mixture of β-alanine, aspartic acid, methionine and phenylalanine. Place 1 mg of the substance being examined in a rigorously cleaned hard-glass tube (10 cm × 6 mm), add not less than 0.5 ml of 6M *hydrochloric acid*, immerse the tube in a freezing mixture at -5°, evacuate to a pressure not exceeding 0.133 kPa and seal. Heat for 16 hours at 110° to 115°, cool, open the tube, transfer the contents to a 10 ml flask with the aid of five 0.2 ml quantities of *water* and evaporate to dryness over *potassium hydroxide* at a pressure of 2 kPa. Take up the residue in *water* and repeat the evaporation; repeat these operations. Dissolve the residue in a suitable buffer solution (pH 2.2) and dilute to a suitable volume with the same buffer solution. Apply an aliquot of the solution to the amino acid analyser. Choose conditions such that the peak given by the amino acid present in the largest amount produces almost maximum deflection on the chart paper.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *ether*, 20 volumes of *glacial acetic acid* and 10 volumes of *water* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions of the substance being examined in a mixture of 24 volumes of *methanol* and 1 volume of 13.5M *ammonia* containing (1) 0.50% w/v and (2) 0.010% w/v. After removal of the plate, allow it to dry in air, heat at 100° for 2 minutes, spray with a 1.0% w/v solution of *4-dimethylaminobenzaldehyde* in a mixture of 3 volumes of *methanol* and 1 volume of *hydrochloric acid* and heat at 100° until purple spots are produced (about 2 minutes). Examine by transmitted light. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

Dissolve 5 mg in sufficient 0.01M *ammonia* to produce 100 ml and measure the *absorbance* at the maximum at 280 nm, Appendix II B. Calculate the content of $C_{37}H_{49}N_7O_9S$ taking 70.0 as the value of A (1%, 1 cm) at the maximum at 280 nm.

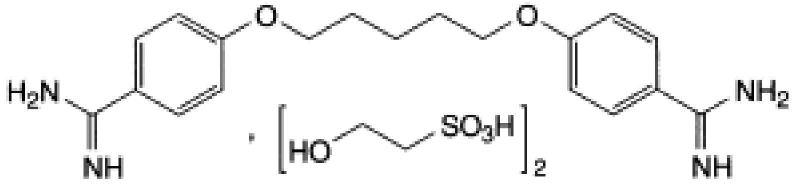
STORAGE

Pentagastrin should be protected from light.

Pentamidine Isetionate

General Notices

(Pentamidine Diisetionate, Ph Eur monograph 1137)



$C_{19}H_{24}N_4O_2,2C_2H_6O_4S$ 592.7 *140-64-7*

Action and use

Antiprotozoal.

Preparation

Pentamidine Injection

Ph Eur

DEFINITION

4,4′-[Pentane-1,5-diylbis(oxy)]dibenzamidine di(2-hydroxyethanesulphonate).

Content

98.5 per cent to 101.5 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl 2-hydroxyethanesulphonates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl 2-hydroxyethanesulphonates are not detectable in the final product.

CHARACTERS

Appearance

White or almost white powder or colourless crystals, hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in

methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison pentamidine diisetionate CRS.

B. Dissolve about 40 mg in 5 ml of *water R* and add dropwise with shaking 1 ml of a 10 g/l solution of *sodium chloride R*. Allow to stand for 5 min. The mixture remains clear.

C. Treat 0.15 g by the oxygen-flask method *(2.5.10)*. Use 10 ml of *dilute hydrogen peroxide solution R* to absorb the combustion products. The solution gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent.

pH *(2.2.3)*

4.5 to 6.5.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) To 0.1 g in a conical flask, add 40 ml of *water R* and glass beads. Adjust to pH 10.5 with dilute *sodium hydroxide solution R* and boil under reflux for 20 min. Cool and dilute to 50 ml with *water R.* Dilute 1 ml of the solution to 50 ml with the mobile phase.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase
Mix 65 volumes of *methanol R* and 35 volumes of a 30 g/l solution of *ammonium acetate R* previously adjusted to pH 7.5 using *triethylamine R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 10 µl.

Run time 3.5 times the retention time of pentamidine.

System suitability Reference solution (b):

- — the chromatogram obtained shows 2 principal peaks;
- — *resolution*: minimum 2.0 between the 2 principal peaks.

Limits:

- — *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- — *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 4.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

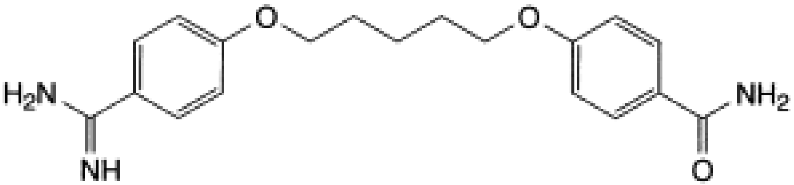
Dissolve 0.250 g in 50 ml of *dimethylformamide R*. Add 0.25 ml of *thymol blue solution R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, under a current of *nitrogen R*, until the colour of the indicator changes to blue. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 29.63 mg of $C_{23}H_{36}N_4O_{10}S_2$.

STORAGE

In an airtight container .

IMPURITIES



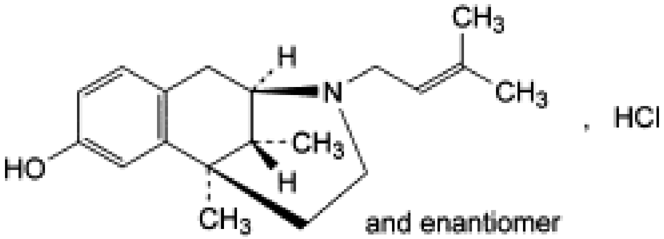
A. 4-[[5-(4-amidinophenoxy)pentyl]oxy]benzenecarboxamide.

Ph Eur

Pentazocine Hydrochloride

General Notices

(Ph Eur monograph 1463)



$C_{19}H_{27}NO,HCl$ 321.9 *64024-15-3*

Action and use

Opioid receptor agonist; analgesic.

Preparations

Pentazocine Capsules

Pentazocine Tablets

Ph Eur

DEFINITION

Pentazocine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*,6*RS*,11*RS*)-6,11-dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, sparingly soluble in water, soluble in ethanol (96 per cent) and sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the *Ph. Eur. reference spectrum of pentazocine hydrochloride.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)

Dissolve 0.1 g in 10 ml of *carbon dioxide-free water R*. The pH of the solution is 4.0 to 6.0.

Absorbance (*2.2.25*)

Dissolve 0.100 g in a mixture of 20 ml of *water R* and 1 ml of *1 M hydrochloric acid*, and dilute to 100.0 ml with *water R*. To 10.0 ml add 1 ml of *1 M hydrochloric acid* and dilute to 100.0 ml with *water R*. The absorbance at the absorption maximum at 278 nm is 0.59 to 0.63, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution Dissolve 0.20 g in 3 ml of *methanol R* and dilute to 10 ml with *methylene chloride R*.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *methylene chloride R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *methylene chloride R.*

Reference solution (c) Dilute 5 ml of reference solution (a) to 20 ml with *methylene chloride R*.

Apply to the plate 10 µl of each solution. Develop over a path corresponding to two-thirds of the plate height using a mixture of 3 volumes of *isopropylamine R*, 3 volumes of *methanol R* and 94 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Heat the plate at 100-105 °C for 15 min, allow to cool, expose to iodine vapour and re-examine under ultraviolet light at 254 nm. By each method of visualisation: any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot obtained with reference solution (a) (1 per cent); not more than 1 such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent); and not more than 4 such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 4 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *ethanol (96 per cent) R*. Add 5 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflection.

1 ml of *0.1 M sodium hydroxide* is equivalent to 32.19 mg of $C_{19}H_{28}ClNO$.

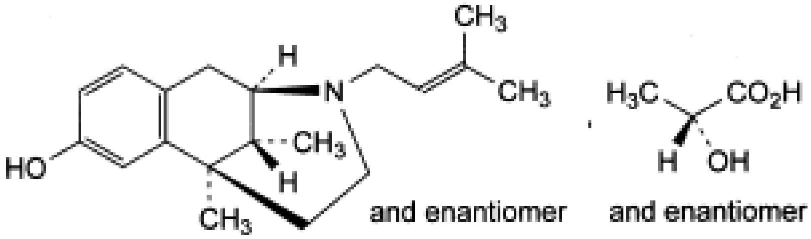
STORAGE

Store protected from light.

Pentazocine Lactate

General Notices

(Ph Eur monograph 2000)



$C_9H_{28}NO,C_3H_5O_3$ 375.5 *17146-95-1*

Action and use

Opioid receptor agonist; analgesic.

Preparations

Pentazocine Injection

Pentazocine Suppositories

Ph Eur

DEFINITION

(2*RS*,6*RS*,11*RS*)-6,11-Dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol (2*RS*)-2-hydroxypropanoate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Sparingly soluble in water, freely soluble in methanol, slightly soluble in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of pentazocine lactate.*

TESTS

pH *(2.2.3)*

5.5 to 6.5.

Dissolve 0.1 g in 10 ml of *carbon dioxide-free water R*.

Absorbance *(2.2.25)*

0.50 to 0.54, determined at the absorption maximum at 278 nm.

Dissolve 0.10 g in 10.0 ml of *1 M hydrochloric acid* and dilute to 100.0 ml with *water R*. Dilute 10.0 ml of the solution to 100.0 ml with *water R*.

Related substances

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 100 mg of the substance to be examined in *acetic anhydride R* and dilute to 5 ml with the same solvent. Heat at 80 °C for 10 min. Dilute 1 ml of the solution to 10 ml with *methanol R*. Mix 1 ml of this solution with 1 ml of the test solution.

Reference solution (b) Dilute 1 ml of the test solution to 100 ml with *methylene chloride R*. Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *isopropylamine R*, *methanol R*, *methylene chloride R* (3:3:94 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm. Heat at 100-105 °C for 15 min. Allow to cool. Expose to iodine vapour and re-examine in ultraviolet light at 254 nm.

System suitability Reference solution (a):

— the chromatogram shows 2 clearly separated spots.

Limits By each method of detection:

— *any impurity*: any spots, apart from the principal spot, are not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 30 ml of *anhydrous acetic acid R* and add 30 ml of *dioxan R*. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 37.55 mg of $C_{22}H_{33}NO_4$.

STORAGE

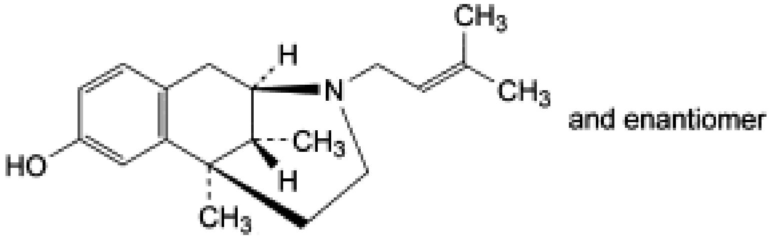
Protected from light.

Ph Eur

Pentazocine

General Notices

(Ph Eur monograph 1462)



$C_{19}H_{27}NO$ 285.4 *359-83-1*

Action and use

Opioid receptor agonist; analgesic.

Preparation

Pentazocine Injection

Ph Eur

DEFINITION

Pentazocine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*,6*RS*,11*RS*)-6,11-dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, practically insoluble in water, freely soluble in methylene chloride and soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the *Ph. Eur. reference spectrum for pentazocine (form A)*.

TESTS

Absorbance *(2.2.25)*

Dissolve 0.100 g in a mixture of 20 ml of *water R* and 1 ml of *1 M hydrochloric acid*, and dilute to 100.0 ml with *water R*. To 10.0 ml add 1 ml of *1 M hydrochloric acid* and dilute to 100.0 ml with *water R*. The absorbance at the absorption maximum at 278 nm is 0.67 to 0.71, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel F_{254} plate R*.

Test solution Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *methylene chloride R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *methylene chloride R*.

Reference solution (c) Dilute 5 ml of reference solution (a) to 20 ml with *methylene chloride R*.

Apply to the plate 10 µl of each solution. Develop over a path corresponding to two thirds of the plate height using a mixture of 3 volumes of *isopropylamine R*, 3 volumes of *methanol R* and 94 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Heat the plate at 100-105 °C for 15 min, allow to cool, expose to iodine vapour and re-examine under ultraviolet light at 254 nm. By each method of visualisation: any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot obtained with reference solution (a) (1 per cent); not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than 4 such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent) .

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 4 h.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of anhydrous acetic acid R. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 28.54 mg of $C_{19}H_27NO$.

STORAGE

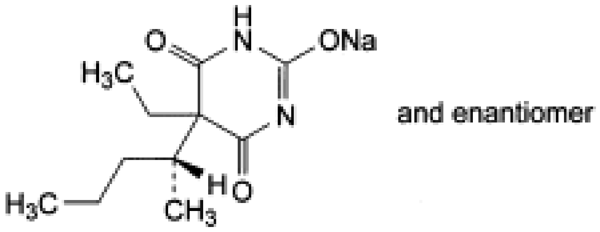
Store protected from light.

Ph Eur

Pentobarbital Sodium

General Notices

(Ph Eur monograph 0419)



$C_{11}H_{17}N_2NaO_3$ 248.3 *57-33-0*

Action and use

Barbiturate.

Preparation

Pentobarbital Tablets

Ph Eur

DEFINITION

Pentobarbital sodium contains not less than 99.0 per cent and not more than the equivalent of 101.5 per cent of the sodium derivative of 5-ethyl-5-[(1*RS*)-1-methylbutyl]pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, hygroscopic, very soluble in water.

IDENTIFICATION

A. Dissolve 1 g in 10 ml of *water R* and add 5 ml of *dilute acetic acid R*. A white, crystalline precipitate is formed. Filter, wash the precipitate with *water R* and dry at 100 °C to 105 °C. Determine the melting point (*2.2.14*) of the precipitate. Mix equal parts of the precipitate and *pentobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 131 °C) is not greater than 2 °C.

B. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel* GF_{254} *plate R*.

Test solution Dissolve 25 mg of the precipitate obtained in identification test A in *alcohol R* and dilute to 25 ml with the same solvent.

Reference solution Dissolve 25 mg of *pentobarbital CRS* in *alcohol R* and dilute to 25 ml with the same solvent.

Apply to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer from a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 10 mg add about 10 mg of *vanillin R* and 2 ml of *sulphuric acid R*. Mix and heat on a water-bath for 2 min. A reddish-brown colour develops. Cool and add cautiously 5 ml of *ethanol R*. The colour becomes violet and then blue.

D. Ignite 1 g. The residue gives reaction (a) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*)

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. The pH measured immediately after preparation of the solution is 9.6 to 11.0.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel GF_{254} plate R*.

Test solution Dissolve 0.2 g of the substance to be examined in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *alcohol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using the lower layer from a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent). Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately in daylight. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Free pentobarbital

Not more than 3.5 per cent. Dissolve 2.00 g in 75 ml of *dimethylformamide R*, heating gently if necessary. Titrate with *0.1 M sodium methoxide* until the colour changes from olive-green to blue, using 0.25 ml of a 10 g/l solution of *thymol blue R* in *dimethylformamide R* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium methoxide* is equivalent to 22.63 mg of pentobarbital.

Isomer

Dissolve 0.3 g in 5 ml of a 50 g/l solution of *anhydrous sodium carbonate R*. Add a solution of 0.3 g of *nitrobenzyl chloride R* in 10 ml of *alcohol R* and heat under a reflux condenser for 30 min. Cool to 25 °C, if necessary scratching the wall of the container with a glass rod to induce crystallisation. Filter and wash the precipitate with five quantities, each of 5 ml, of *water R*. In a small flask, heat the precipitate with 25 ml of *alcohol R* under a reflux condenser until dissolved (about 10 min). Cool to 25 °C, if necessary scratching the wall of the flask with a glass rod to induce crystallisation, and filter. The precipitate, washed with two quantities, each of 5 ml, of *water R* and dried at 100 °C to 105 °C for 30 min, melts (*2.2.14*) at 136 °C to 148 °C.

Heavy metals (*2.4.8*)

Dissolve 1.0 g in *water R* and dilute to 10.0 ml with the same solvent. To 9 ml of the solution, add 3 ml of *dilute acetic acid R* and 3 ml of *buffer solution pH 3.5 R* and filter. Dilute the filtrate to 18 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (20 ppm). In preparing the test solution, replace the buffer solution with *water R*. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 3.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.200 g in 15 ml of a 127.5 g/l solution of *silver nitrate R* in *pyridine R*. Titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained, using 0.5 ml of *thymolphthalein solution R* as indicator. Carry out a blank titration.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 24.83 mg of $C_{11}H_{17}N_2NaO_3$.

STORAGE

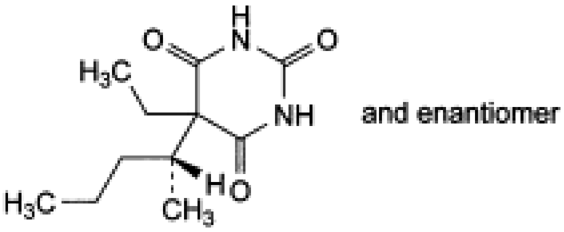
Store in an airtight container .

Ph Eur

Pentobarbital

General Notices

(Ph Eur monograph 0200)



$C_{11}H_{18}N_2O_3$ 226.3 *76-74-4*

Action and use

Barbiturate.

Ph Eur

DEFINITION

Pentobarbital contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 5-ethyl-5-[(1*RS*)-1-methylbutyl]pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, very slightly soluble in water, freely soluble in ethanol. It forms water-soluble compounds with alkali hydroxides and carbonates and with ammonia.

IDENTIFICATION

A. Determine the melting point (*2.2.14*) of the substance to be examined. Mix equal parts of the substance to be examined and *pentobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 133 °C) is not greater than 2 °C.

B. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 0.1 g of *pentobarbital CRS* in *alcohol R* and dilute to 100 ml with the same solvent.

Apply to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 10 mg add about 10 mg of *vanillin R* and 2 ml of *sulphuric acid R*. Mix and heat on a water-bath for 2 min. A reddish-brown colour develops. Cool and add cautiously 5 ml of *ethanol R*. The colour becomes violet and then blue.

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 6 ml of *water R*. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y6 (*2.2.2, Method II*).

Acidity

Boil 1.0 g with 50 ml of *water R* for 2 min, allow to cool and filter. To 10 ml of the filtrate add 0.15 ml of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *alcohol R*.

Apply to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Isomer

Dissolve 0.3 g in 5 ml of a 50 g/l solution of *anhydrous sodium carbonate R*, heating slightly if necessary. Add a solution of 0.3 g of *nitrobenzyl chloride R* in 10 ml of *alcohol R* and heat under a reflux condenser for 30 min. Cool to 25 °C, filter and wash the precipitate with five quantities, each of 5 ml, of *water R*. In a small flask, heat the precipitate with 25 ml of *alcohol R* under a reflux condenser until dissolved (about 10 min). Cool to 25 °C, if necessary scratching the wall of the flask with a glass rod to induce crystallisation, and filter. The precipitate, washed with two quantities, each of 5 ml, of *water R* and dried at 100 °C to 105 °C for 30 min, melts (*2.2.14*) at 136 °C to 148 °C.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 5 ml of *pyridine R*. Add 0.5 ml of *thymolphthalein solution R* and 10 ml of silver nitrate solution in *pyridine R*. Titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained. Carry out a blank titration.

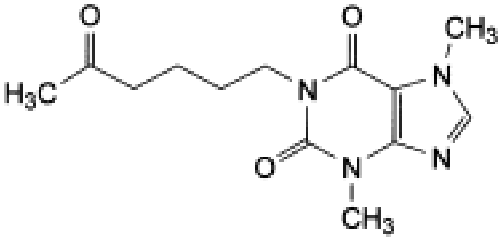
1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 11.31 mg of $C_{11}H_{18}N_2O_3$.

Ph Eur

Pentoxifylline

General Notices

(Ph Eur monograph 0851)



$C_{13}H_{18}N_4O_3$ 278.3 *6493-05-6*

Action and use

Vasodilator.

Ph Eur

DEFINITION

3,7-Dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 103 °C to 107 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pentoxifylline CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *pentoxifylline CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *methanol R*, *ethyl acetate R* (15:85 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of xanthines (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

A 40 per cent (*V/V*) solution of solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Acidity

To 8 ml of solution S add 12 ml of *carbon dioxide-free water R* and 0.05 ml of *bromothymol blue solution R1*. The solution is green or yellow. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture A mixture of equal volumes of a 5.44 g/l solution of *potassium dihydrogen phosphate R* and *methanol R.*

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 10.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *caffeine R* (impurity F) and 2 mg of *theophylline R* (impurity C) in the solvent mixture, add 1 ml of the test solution and dilute to 10 ml with the solvent mixture.

Reference solution (d) Dissolve 5.0 mg of *caffeine R* (impurity F), 5.0 mg of *theobromine R* (impurity A) and 5.0 mg of *theophylline R* (impurity C) in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml to 25.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

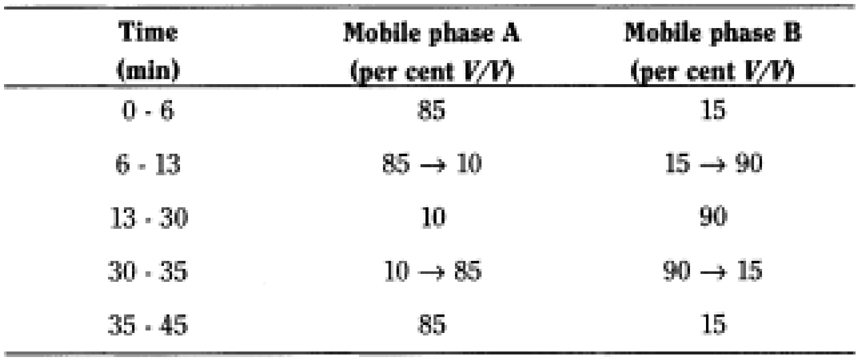
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: mix 30 volumes of *methanol R* and 70 volumes of a 5.44 g/l solution of *potassium dihydrogen phosphate R*;

— *mobile phase B*: mix 30 volumes of a 5.44 g/l solution of *potassium dihydrogen phosphate R* and 70 volumes of *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	85	15
6 - 13	85 → 10	15 → 90
13 - 30	10	90
30 - 35	10 → 85	90 → 15
35 - 45	85	15

Flow rate 1 ml/min.

Detection Spectrophotometer at 272 nm.

Injection 10 µl.

Relative retention With reference to pentoxifylline (retention time = about 12 min): impurity A = about 0.3; impurity C = about 0.4; impurity F = about 0.5; impurity J = about 1.6.

System suitability Reference solution (c):

— *retention time*: impurity F = 4 min to 7 min; pentoxifylline = 9 min to 13 min; if necessary adapt the mixing ratio of the mobile phases;

— *resolution*: minimum 4 between the peaks due to impurity C and impurity F.

Limits:

— *impurities A, C, F*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *impurity J*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the

chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Chlorides (*2.4.4*)

Maximum 100 ppm.

Place 20 ml of solution S in a separating funnel and shake with 2 quantities, each of 20 ml, of *2-methylpropan-1-ol R*. Dilute 10 ml of the aqueous layer to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 200 ppm, determined on 15 ml of solution S.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at 60 °C at a pressure not exceeding 700 Pa.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 5 ml of *anhydrous acetic acid R*. Add 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 27.83 mg of $C_{13}H_{18}N_4O_3$.

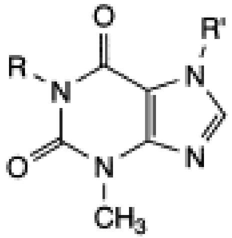
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, C, F, J.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, D, E, G, H, I, K.*



A. R = H, R′ = CH3: theobromine,

B. R = R′ = H: 3-methyl-3,7-dihydro-1*H*-purine-2,6-dione,

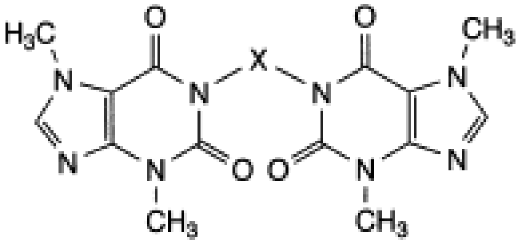
C. R = CH_3, R′ = H: theophylline,

D. R = CH_2-CH_2-CH_2-OH, R′ = CH_3: 1-(3-hydroxypropyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,

F. R = R′ = CH_3: caffeine,

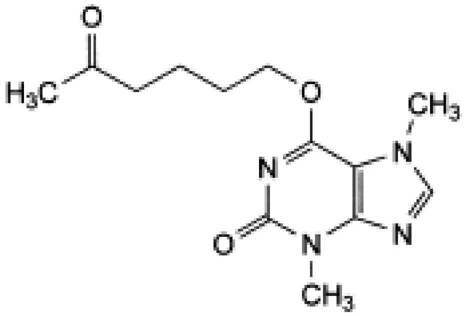
H. R = R′ = CH_2-$[CH_2]_3$-CO-CH_3: 3-methyl-1,7-bis(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione,

I. R = CH_2-C_6H5, R′ = CH_3: 1-benzyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,

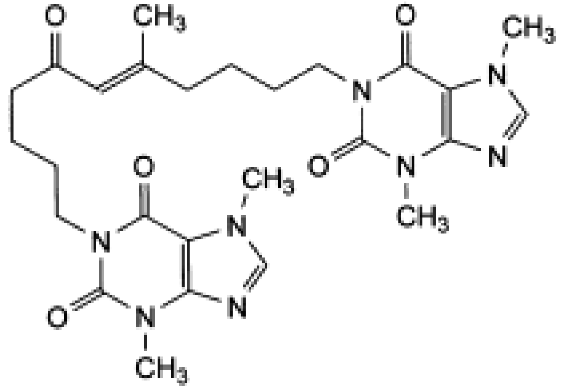


E. X = CH_2: 1,1′-methylenebis(3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione),

K. X = CH_2-CH_2-CH_2: 1,1′-(propane-1,3-diyl)bis(3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione),



G. 3,7-dimethyl-6-(5-oxohexyloxy)-3,7-dihydro-2*H*-purin-2-one,


O
CH3
O
CH3
N
N
N
N
O
CH3
O
CH3
N
N
N
N
O
CH3
CH3

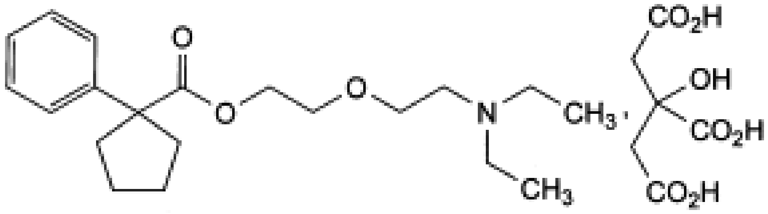
J. 1-[(5*E*)-11-(3,7-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-1-yl)-5-methyl-7-oxoundec-5-enyl]-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

Ph Eur

Pentoxyverine Citrate

General Notices

(Pentoxyverine Hydrogen Citrate, Ph Eur monograph 1621)



$C_{20}H_{31}NO_3,C_6H_8O_7$ 525.6 *23142-01-0*

Action and use

Cough suppressant.

Ph Eur

DEFINITION

2-[2-(Diethylamino)ethoxy]ethyl 1-phenylcyclopentanecarboxylate dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very soluble in glacial acetic acid, freely soluble in methanol, soluble in alcohol and in methylene chloride.

mp

About 93 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of pentoxyverine hydrogen citrate.*

B. Dissolve 0.25 g in 5 ml of *water R*. The solution gives the reaction of citrates (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)

3.3 to 3.7 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution Introduce 5.0 mg of *pentoxyverine impurity A CRS* and 5.0 mg of *pentoxyverine impurity B CRS* in a conical flask, add 5.0 ml of the test solution and dilute to 100.0 ml with the mobile phase. Dilute 3.0 ml of the solution to 50.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm and a carbon loading of 12 per cent;
- *temperature*: 50 °C.

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 1.5 g/l solution of *sodium heptanesulphonate R* adjusted to pH 3.0 with *dilute sulphuric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl.

Run time 3 times the retention time of pentoxyverine.

Relative retention With reference to pentoxyverine (retention time = about 6 min): impurity B = about 0.8; impurity A = about 1.5.

System suitability Reference solution:

- *resolution*: minimum of 5.0 between the peaks due to pentoxyverine and to impurity A;
- *signal-to-noise ratio*: minimum of 100 for the peak due to pentoxyverine;
- *symmetry factor*: maximum of 2.0 for the peak due to pentoxyverine.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram

obtained with the reference solution (0.3 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.3 per cent);

— *any other impurity*: not more than one-third of the area of the peak due to pentoxyverine in the chromatogram obtained with the reference solution (0.1 per cent);

— *total of any other impurity*: not more than the area of the peak due to pentoxyverine in the chromatogram obtained with the reference solution (0.3 per cent);

— *disregard limit*: 0.1 times the area of the peak due to pentoxyverine in the chromatogram obtained with the reference solution (0.03 per cent); disregard any peak with a retention time less than or equal to 2.5 min.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

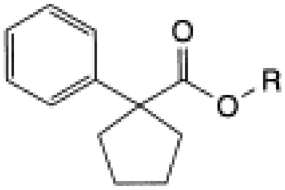
Dissolve 0.400 g in 70 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 52.56 mg of $C_{26}H_{39}NO_{10}$.

STORAGE

Protected from light.

IMPURITIES



A. R = H: 1-phenylcyclopentanecarboxylic acid,

B. R = CH_2-CH_2-N(CH_2-CH_3)$_2$: 2-(diethylamino)ethyl 1-phenylcyclopentanecarboxylate (caramiphen).

Ph Eur

Pepsin

General Notices

(Pepsin Powder, Ph Eur monograph 0682)

Action and use

Proteolytic enzyme.

Ph Eur

DEFINITION

Pepsin powder is prepared from the gastric mucosa of pigs, cattle or sheep. It contains gastric proteinases, active in acid medium (pH 1 to 5). It has an activity not less than 0.5 Ph. Eur. U./mg, calculated with reference to the dried substance.

PRODUCTION

The animals from which pepsin powder is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

A white or slightly yellow, crystalline or amorphous powder, hygroscopic, soluble in water, practically insoluble in ethanol (96 per cent). The solution in water may be slightly opalescent with a weak acidic reaction.

IDENTIFICATION

In a mortar, pound 30 mg of *fibrin blue R*. Suspend in 20 ml of *dilute hydrochloric acid R2*. Filter the suspension on a filter paper and wash with *dilute hydrochloric acid R2* until a colourless filtrate is obtained. Perforate the filter paper and wash the fibrin blue R through it into a conical flask using 20 ml of *dilute hydrochloric acid R2*. Shake before use. Dissolve a quantity of the substance to be examined, equivalent to not less than 20 Ph. Eur. U., in 2 ml of *dilute hydrochloric acid R2* and adjust to pH 1.6 ± 0.1. Add 1 ml of this solution to a test-tube containing 4 ml of the fibrin blue suspension, mix and place in a water-bath at 25 °C with gentle shaking. Prepare a blank solution at the same time and in the same manner using 1 ml of *water R*. After 15 min of incubation the blank solution is colourless and the test solution is blue.

TESTS

Loss on drying (*2.2.32*)

Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 4 h.

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 10^4 micro-organisms per gram, determined by plate-count. It complies with the tests for *Escherichia coli* and *Salmonella* (*2.6.13*).

ASSAY

The activity of pepsin powder is determined by comparing the quantity of peptides, non-precipitable by *trichloroacetic acid solution R* and assayed using the *phosphomolybdotungstic reagent R*, which are released per minute from a substrate of *haemoglobin solution R*, with the quantity of such peptides released by *pepsin powder BRP* from the same substrate in the same conditions.

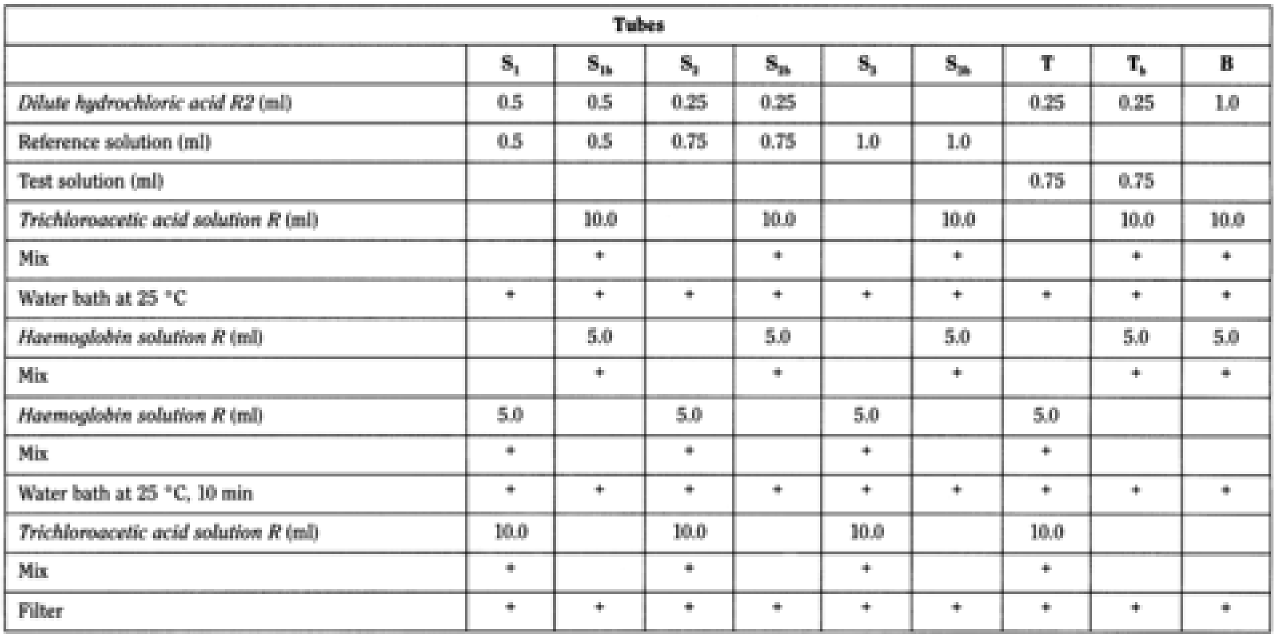
For the test solution and the reference solution, prepare the solution and carry out the dilution at 0 °C to 4 °C.

Avoid shaking and foaming during preparation of the test and reference solutions.

Test solution Immediately before use, prepare a solution of the substance to be examined expected to contain 0.5 Ph. Eur. U./ml in *dilute hydrochloric acid R2*; before dilution to volume, adjust to pH 1.6 ± 0.1, if necessary, using *1 M hydrochloric acid*.

Reference solution Less than 15 min before use, prepare a solution of *pepsin powder BRP* containing 0.5 Ph. Eur. U./ml in *dilute hydrochloric acid R2*; before dilution to volume, adjust to pH 1.6 ± 0.1, if necessary, using *1 M hydrochloric acid*.

Table 0682.-1



	Tubes								
	S_1	S_{1b}	S_2	S_{2b}	S_3	S_{3b}	T	T_b	B
Dilute hydrochloric acid R2 (ml)	0.5	0.5	0.25	0.25			0.25	0.25	1.0
Reference solution (ml)	0.5	0.5	0.75	0.75	1.0	1.0			
Test solution (ml)							0.75	0.75	
Trichloroacetic acid solution R (ml)		10.0		10.0		10.0		10.0	10.0
Mix		+		+		+		+	+
Water bath at 25 °C	+	+	+	+	+	+	+	+	+
Haemoglobin solution R (ml)		5.0		5.0		5.0		5.0	5.0
Mix		+		+		+		+	+
Haemoglobin solution R (ml)	5.0		5.0		5.0		5.0		
Mix	+		+		+		+		
Water bath at 25 °C, 10 min	+	+	+	+	+	+	+	+	+
Trichloroacetic acid solution R (ml)	10.0		10.0		10.0		10.0		
Mix	+		+		+		+		
Filter	+	+	+	+	+	+	+	+	+

Designate tubes in duplicate T, T_b, S_1, S_{1b}, S_2, S_{2b}, S_3, S_{3b}; designate a tube B.

Add *dilute hydrochloric acid R2* to the tubes as follows:

B: 1.0 ml

S_1 and S_{1b}: 0.5 ml

S_2, S_{2b} and T and T_b: 0.25 ml

Add the reference solution to the tubes as follows:

S_1 and S_{1b}: 0.5 ml

S_2 and S_{2b}: 0.75 ml

S_3 and S_{3b}: 1.0 ml

Add 0.75 ml of the test solution to tubes T and T_b.

Add 10.0 ml of *trichloroacetic acid solution R* to tubes S_{1b}, S_{2b}, S_{3b}, T_b and B. Mix by shaking.

Place the tubes and *haemoglobin solution R* in a water bath at 25 ± 0.1 °C. When temperature equilibrium is reached, add 5.0 ml of *haemoglobin solution R* to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix.

At time zero add 5.0 ml of *haemoglobin solution R* successively and at intervals of 30 s to tubes S_1, S_2, S_3 and T.

Mix immediately after each addition.

Exactly 10 min after adding the *haemoglobin solution R*, stop the reaction by adding, at intervals of 30 s, 10.0 ml of *trichloroacetic acid solution R* to tubes S_1, S_2, S_3 and T (the use of a fast-flowing or blow-out pipette is recommended) and mix.

Filter the contents of each tube (samples and blanks) twice through the same suitable filter paper previously washed with a 50 g/l solution of *trichloroacetic acid R,* then with *water R* and dried. Discard the first 5 ml of filtrate. Place 3.0 ml of each filtrate separately in a tube containing 20 ml of *water R*. Mix.

A suitable filter paper complies with the following test: filter 5 ml of a 50 g/l solution of *trichloroacetic acid R* through a 7 cm disc of white filter paper: the absorbance (*2.2.25*) of the filtrate, measured at 275 nm using unfiltered *trichloroacetic acid R* solution as the compensation liquid, is less than 0.04.

Add to each tube 1.0 ml of *sodium hydroxide solution R* and 1.0 ml of *phosphomolybdotungstic reagent R*, beginning with the blanks and then the samples of each set, in a known order.

A schematic presentation of the above operations is shown in Table 0682.-1.

After 15 min measure the absorbance (*2.2.25*) of solutions S_1, S_2, S_3, S_{1b}, S_{2b}, S_{3b} and T at 540 nm using the filtrate obtained from tube B as the compensation liquid. Correct the average absorbance values for the filtrates obtained from tubes S_1, S_2 and S_3 by subtracting the average values obtained for the filtrates from tubes S_{1b}, $S2_b$, S_{3b} respectively.

Draw a calibration curve of the corrected values against volume of reference solution used. Determine the activity of the substance to be examined using the corrected absorbance for the test solution ($T - T_b$) together with the calibration curve and taking into account the dilution factors.

STORAGE

Store in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

LABELLING

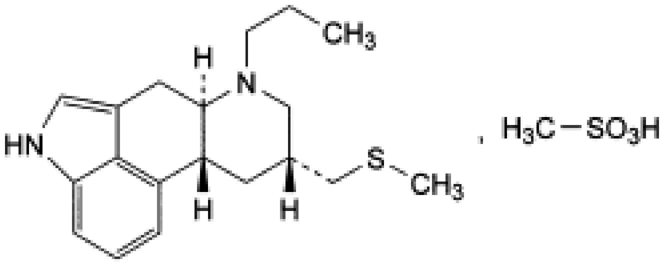
The label states the activity in European Pharmacopoeia Units per milligram.

Ph Eur

Pergolide Mesilate

General Notices

(Ph Eur monograph 1555)



$C_{20}H_{30}N_2O_3S_2$ 410.6 *66104-23-2*

Action and use

Dopamine receptor agonist; treatment of Parkinson's disease.

Ph Eur

DEFINITION

(6a*R*,9*R*,10a*R*)-9-[(Methylsulphanyl)methyl]-7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline monomethanesulphonate.

Content

97.5 per cent to 102.0 per cent (dried substance).

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent) and in methylene chloride, very slightly soluble in acetone.

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): - 17 to - 23 (dried substance).

Dissolve 0.25 g in *dimethylformamide R* and dilute to 25.0 ml with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *pergolide mesilate CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Reference solution (b) Dissolve 10 mg of *4,4′-dimethoxybenzophenone R* in *methanol R* and dilute to 10 ml with the same solvent. To 1 ml of the solution add 2 ml of the test solution and dilute to 100 ml with *methanol R*. Dilute 1 ml of this solution to 10 ml with *methanol R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

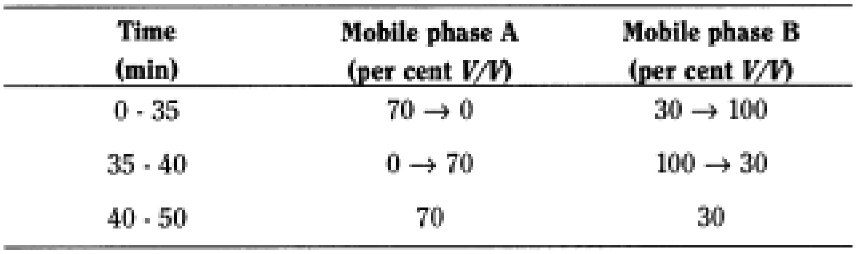
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: mix 5.0 ml of *morpholine for chromatography R* with 995 ml of *water R* and adjust to pH 7.0 with *phosphoric acid R*; use within 24 h;

— *mobile phase B*: *acetonitrile R*, *methanol R*, *tetrahydrofuran R* (1:1:1 *V/V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	70 → 0	30 → 100
35 - 40	0 → 70	100 → 30
40 - 50	70	30

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to 4,4′-dimethoxybenzophenone (1st peak) and pergolide (2nd peak).

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C for 1 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solution A

Dissolve 5.0 mg of *DL-methionine R* in 500 ml of *0.01 M hydrochloric acid*. Add 500 ml of *methanol R* and mix.

Test solution Dissolve 65.0 mg of the substance to be examined in solution A and dilute to 100.0 ml with solution A. Dilute 10.0 ml of this solution to 100.0 ml with solution A.

Reference solution Dissolve 65.0 mg of *pergolide mesilate CRS* in solution A and dilute to 100.0 ml with solution A. Dilute 10.0 ml of this solution to 100.0 ml with solution A.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 1 volume of *acetonitrile R*, 1 volume of *methanol R* and 2 volumes of a mixture prepared as follows: dissolve 2.0 g of *sodium octanesulphonate R* in *water R*, add 1.0 ml of *anhydrous acetic acid R* and dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Retention time Pergolide = about 9 min.

System suitability Reference solution:

— *symmetry factor*: maximum 1.5 for the peak due to pergolide.

Calculate the percentage content of $C_{20}H_{30}N_2O_3S_2$ from the declared content of *pergolide mesilate CRS* .

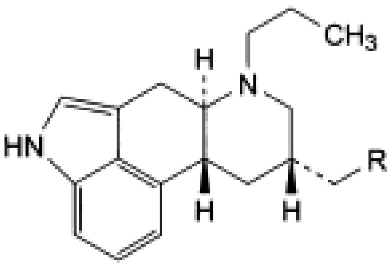
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B.*



A. R = $SO-CH_3$: (6a*R*,9*R*,10a*R*)-9-[(methylsulphinyl)methyl]-7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline (pergolide sulphoxide),

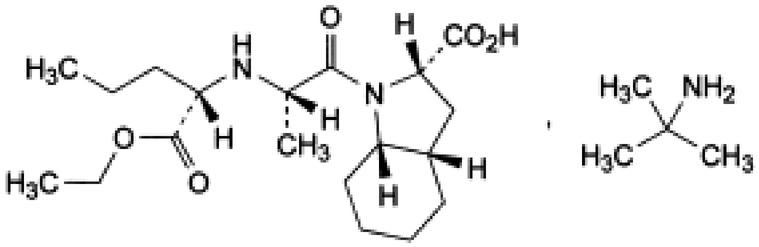
B. R = SO_2-CH_3: (6a*R*,9*R*,10a*R*)-9-[(methylsulphonyl)methyl]-7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline (pergolide sulphone).

Ph Eur

Perindopril Erbumine

General Notices

(Perindopril tert-Butylamine, Ph Eur monograph 2019)



$C_{19}H_{32}N_2O_5$, $C_4H_{11}N$ 441.6 *107133-36-8*

Action and use

Angiotensin converting enzyme inhibitor.

Ph Eur

DEFINITION

2-Methylpropan-2-amine (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, slightly hygroscopic.

Solubility

Freely soluble in water and in ethanol (96 per cent), sparingly soluble in methylene chloride.

It shows polymorphism.

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: - 66 to - 69 (anhydrous substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *perindopril tert-butylamine CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for impurity A.

Results In the chromatogram obtained with the test solution a spot is observed with the same R_f as the spot with the higher R_f in the chromatogram with reference solution (c) (*tert*-butylamine).

TESTS

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *perindopril impurity A CRS* in *methanol R* and dilute to 25.0 ml with the same solvent.

Reference solution (b) Dilute 5 ml of reference solution (a) to 20 ml with *methanol R*.

Reference solution (c) To 5 ml of reference solution (a) add 5 ml of a 20 g/l solution of *1,1-dimethylethylamine R* in *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, toluene R, methanol R* (1:40:60 *V/V/V*).

Application 10 µl of the test solution and reference solutions (b) and (c).

Development In a saturated tank, over 2/3 of the plate.

Drying In a current of warm air.

Detection Expose to iodine vapour for at least 20 h.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Limit:
— *impurity A:* any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Stereochemical purity

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with *ethanol (96 per cent) R*.

Reference solution (b) Dissolve 10 mg of *perindopril for stereochemical purity CRS* in *ethanol (96 per cent) R* and dilute to 5.0 ml with the same solvent.

Reference solution (c) Dilute 10.0 ml of reference solution (a) to 50.0 ml with *ethanol (96 per cent) R*.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase: spherical octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 450 m^2/g and a pore size of 10 nm,
- *temperature:* 50 °C for the column and at least 30 cm of the tubing preceding the column.

Mobile phase Mix, in the following order, 21.7 volumes of *acetonitrile R*, 0.3 volumes of *pentanol R* and 78 volumes of a 1.50 g/l solution of *sodium heptanesulphonate R*, previously adjusted to pH 2.0 with a mixture of equal volumes of *perchloric acid R* and *water R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 215 nm.

Equilibration Minimum 4 h.

Injection 10 µl.

Run time 1.5 times the retention time of perindopril.

Retention time Perindopril = about 100 min.

System suitability:

- *signal-to-noise ratio:* minimum 3 for the principal peak in the chromatogram obtained with reference solution (c),
- *peak-to-valley ratio:* minimum 3, where H_p = height above the baseline of the peak due to impurity I and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to perindopril in the chromatogram obtained with reference solution (b),
- the chromatogram obtained with reference solution (b) is similar to the chromatogram provided with *perindopril for stereochemical purity CRS*.

Limits:

- *any impurity:* not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard any peak with a retention time less than 0.6 times the retention time of perindopril and any peak with a retention time greater than 1.4 times the retention time of perindopril.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 60 mg of the substance to be examined in mobile phase A and dilute to 20.0 ml with mobile phase A.

Reference solution (a) Dissolve 15 mg of *perindopril for system suitability CRS* in mobile phase A and dilute to 5.0 ml with mobile phase A.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with mobile phase A.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

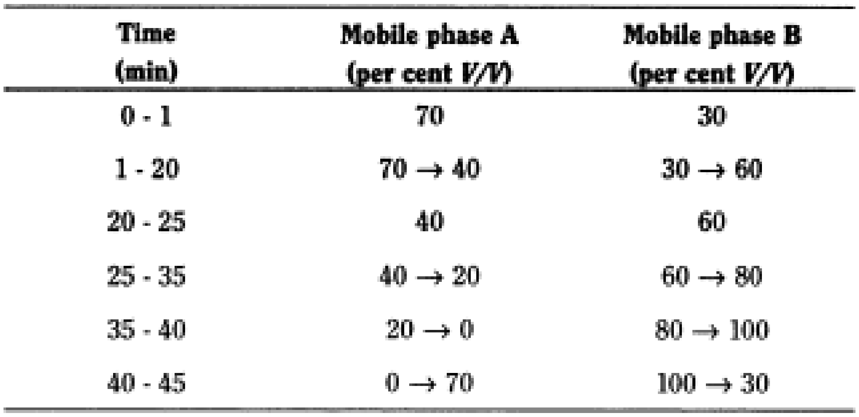
— *stationary phase: spherical octylsilyl silica gel for chromatography R* (5 µm),

— *temperature:* 70 °C.

Mobile phase:

— *mobile phase A:* dissolve 0.92 g of *sodium heptanesulphonate R* in 1000 ml of *water R*, add 1 ml of *triethylamine R* and adjust to pH 2.0 with a mixture of equal volumes of *perchloric acid R* and *water R*,

— *mobile phase B: acetonitrile R1,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	70	30
1 - 20	70 → 40	30 → 60
20 - 25	40	60
25 - 35	40 → 20	60 → 80
35 - 40	20 → 0	80 → 100
40 - 45	0 → 70	100 → 30

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Relative retention With reference to perindopril (retention time = about 8 min): impurity B = about 0.4; impurity C = about 0.8; impurity D = about 0.9; impurity E = about 1.4; impurity F = about 1.7; impurity G = about 2.2 and 2.3; impurity H = about 3.6 and 3.7.

System suitability Reference solution (a):

— *peak-to-valley ratio:* minimum 10, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to perindopril. If necessary, adjust the concentration of triethylamine R in mobile phase A.

Limits:

— *impurity B:* not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

— *impurity E:* not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent),

— *impurities F, H:* for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— *any other impurity:* not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),

— *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 0.50 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 22.08 mg of $C_{23}H_{43}N_3O_5$.

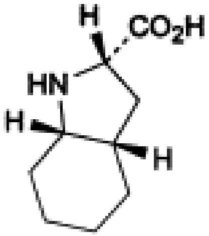
STORAGE

In an airtight container.

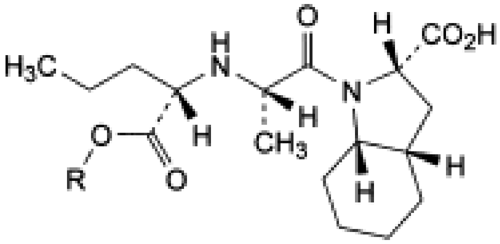
IMPURITIES

Specified impurities A, B, E, F, H, I.

Other detectable impurities C, D, G.

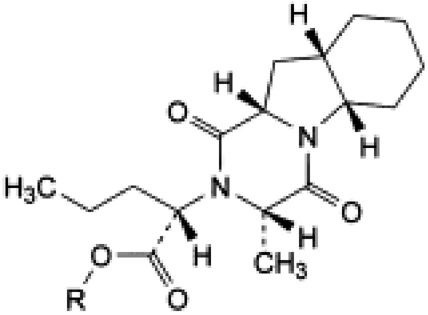


A. (2*S*,3a*S*,7a*S*)-octahydro-1*H*-indole-2-carboxylic acid,



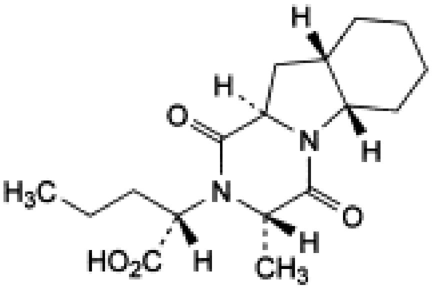
B. R = H: (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxybutyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,

E. R = $CH(CH_3)_2$: (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-[(1-methylethoxy)carbonyl]butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,

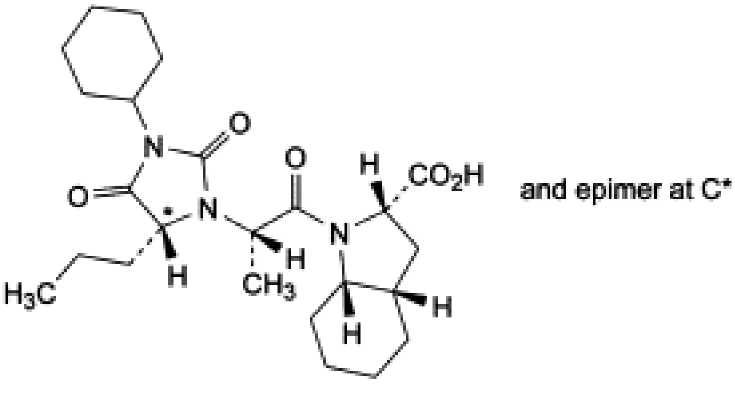


C. R = H: (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid,

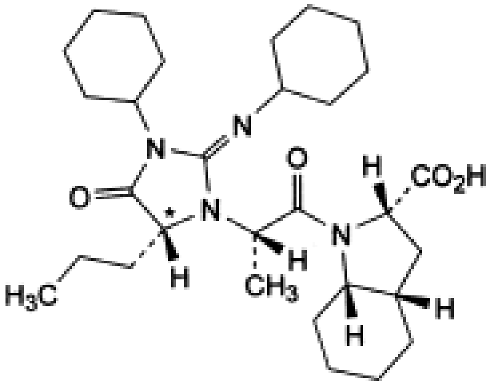
F. R = C_2H_5: ethyl (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoate,



D. (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*R*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid,

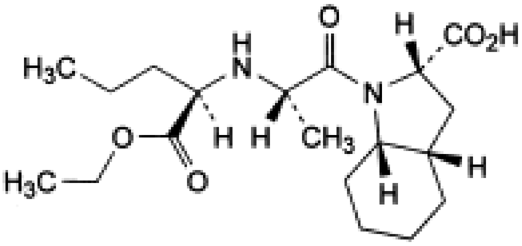


G. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[(5*RS*)-3-cyclohexyl-2,4-dioxo-5-propylimidazolidin-1-yl]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



and epimer at C*

H. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[(5*RS*)-3-cyclohexyl-2-(cyclohexylimino)-4-oxo-5-propylimidazolidin-1-yl]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



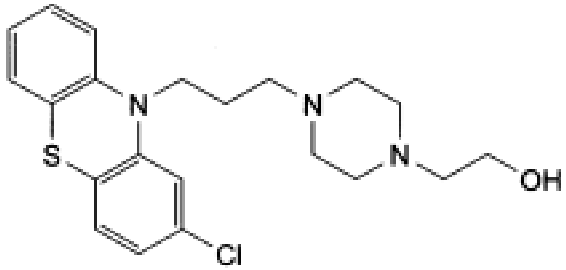
I. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*R*)-1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.

Ph Eur

Perphenazine

General Notices

(Ph Eur monograph 0629)



$C_{21}H_{26}ClN_3OS$ 404.0 *58-39-9*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Perphenazine Tablets

Ph Eur

DEFINITION

Perphenazine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 2-[4-[3-(2-chlorophenothiazin-10-yl)propyl]piperazin-1-yl]ethanol, calculated with reference to the dried substance.

CHARACTERS

A white or yellowish-white, crystalline powder, practically insoluble in water, freely soluble in methylene chloride, soluble in alcohol. It dissolves in dilute solutions of hydrochloric acid.

IDENTIFICATION

First identification *A, C*

Second identification *A, B, D.*

A. Melting point (*2.2.14*): 96 °C to 100 °C.

B. Dissolve 10 mg in *methanol R* and dilute to 100 ml with the same solvent. Dilute 10 ml of the solution to 100 ml with *methanol R*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows two absorption maxima, at 257 nm and 313 nm. The ratio of the absorbance measured at the maximum at 313 nm to that measured at the maximum at 257 nm is 0.120 to 0.128.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *perphenazine CRS* . Examine the substances prepared as discs.

D. Examine by thin-layer chromatography (*2.2.27*), using *kieselguhr G R* as the coating substance. Impregnate the plate by placing it in a closed tank containing the necessary quantity of the impregnation mixture containing 2.5 per cent *V/V* of *phenoxyethanol R* and 7.5 per cent *V/V* of *formamide R* in *acetone R* so that the plate dips about 5 mm beneath the surface of the liquid. When the impregnation mixture has risen at least 17 cm from the lower edge of the plate, remove the plate and use immediately for chromatography. Carry out the chromatography in the same direction as the impregnation.

Test solution Dissolve 20 mg of the substance to be examined in *chloroform R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *perphenazine CRS* in *chloroform R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 2 µl of each solution. Develop in the dark over a path of 15 cm using a mixture of 2 volumes of *diethylamine R* and 100 volumes of *light petroleum R*, saturated with phenoxyethanol (add *phenoxyethanol R* - 6 volumes to 8 volumes - to the above mixture of solvents until there is a persistent cloudiness after shaking, decant, and use the supernatant liquid, even if it is cloudy). Expose the plate to ultraviolet light at 365 nm and examine after a few minutes. The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with the reference solution. Dry the plate at 120 °C for 20 min, allow to cool and spray with a 10 per cent *V/V* solution of *sulphuric acid R* in *alcohol R*. The principal spot in the chromatogram obtained with the test solution is of the same colour as the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

Dissolve 0.20 g in 10 ml of *methanol R*. The solution is clear (*2.2.1*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance. Prepare the solutions immediately before use.

Test solution Dissolve 0.1 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 14 volumes of *water R* and 85 volumes of *butanol R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 65 °C for 4 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.1500 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 20.20 mg of $C_{21}H_{26}ClN_3OS$.

STORAGE

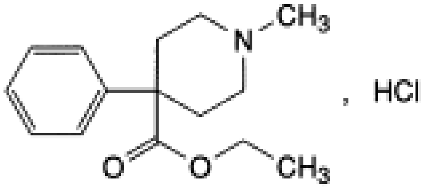
Store protected from light.

Ph Eur

Pethidine Hydrochloride

General Notices

(Ph Eur monograph 0420)



$C_{15}H_{22}ClNO_2$ 283.8 *50-13-5*

Action and use

Opioid receptor agonist; analgesic.

Preparations

Pethidine Injection

Pethidine Tablets

Ph Eur

DEFINITION

Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

If intended for use in the manufacture of parenteral dosage forms, the manufacturing process is validated to show that the content of impurity B is not more than 0.1 ppm.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Melting point (*2.2.14*): 187 °C to 190 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of pethidine hydrochloride.*

C. Dissolve 0.1 g in 10 ml of *ethanol R* and add 10 ml of *picric acid solution R*. A crystalline precipitate is formed which, when washed with *water R* and dried at 100-105 °C, melts (*2.2.14*) at 186 °C to 193 °C. Mix equal quantities of the precipitate and the substance to be examined and determine the melting point of the mixture. The melting point is at least 20 °C lower than that of the precipitate.

D. To 5 ml of solution S (see Tests) add 5 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.3 ml of *0.01 M hydrochloric acid*. The solution is red.

Impurity B

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 0.100 g of the substance to be examined in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 25.0 ml with the same mixture of solvents.

Test solution (b) Dissolve 0.125 g of the substance to be examined in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 0.5 ml of test solution (a) to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Reference solution (b) Dissolve 10.0 mg of *pethidine impurity A CRS* in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (c) Dissolve 12.5 mg of *1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine R* in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 10.0 ml with the same mixture of solvents. Dilute 1.0 ml of the solution to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

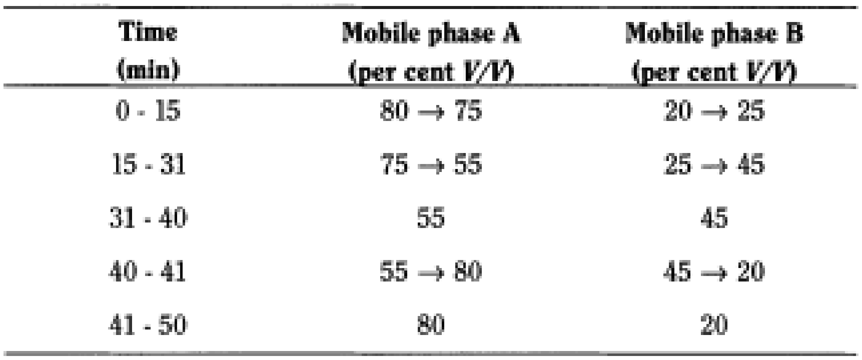
Reference solution (d) Dilute 5.0 ml of reference solution (b) and 1.0 ml of reference solution (c) to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Column:

- *size:* l = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 340 m2/g, a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase:

- *mobile phase A*: mix equal volumes of a 42.0 g/l solution of *sodium perchlorate R* and of a 11.6 g/l solution of *phosphoric acid R*, adjust to pH 2.0 with *triethylamine R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 15	80 → 75	20 → 25
15 - 31	75 → 55	25 → 45
31 - 40	55	45
40 - 41	55 → 80	45 → 20
41 - 50	80	20

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µl; inject test solution (b) and reference solution (d).

Relative retention With reference to pethidine (retention time = about 24 min): impurity B = about 0.66; impurity A = about 0.68.

System suitability Reference solution (d):

- *signal-to-noise ratio*: minimum 10 for the first peak;
- *peak-to-valley ratio*: minimum 4, where H_p = height above the baseline of the peak due to impurity B, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A.

Limit:

- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (10 ppm) if intended for non-parenteral use.

Related substances

Liquid chromatography (*2.2.29*) as described in the test for impurity B with the following modifications.

Injection 20 µl; inject test solution (a) and reference solution (a).

Limits:

- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.220 g in 50 ml of *alcohol R*. Add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 28.38 mg of $C_{15}H_{22}ClNO_2$.

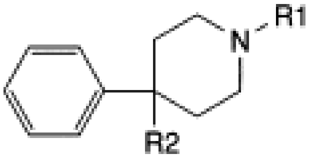
STORAGE

In an airtight container, protected from light.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES



A. R1 = CH_3, R2 = H: 1-methyl-4-phenylpiperidine (MPP),

C. R1 = CH_3, R2 = CO_2H: 1-methyl-4-phenylpiperidine-4-carboxylic acid,

D. R1 = CH_3, R2 = CO_2-CH_3: methyl 1-methyl-4-phenylpiperidine-4-carboxylate,

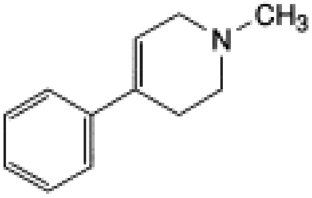
E. R1 = H, R2 = CO_2-CH_2-CH_3: ethyl 4-phenylpiperidine-4-carboxylate,

F. R1 = CH_2-C_6H_5, R2 = CO_2H: 1-benzyl-4-phenylpiperidine-4-carboxylic acid,

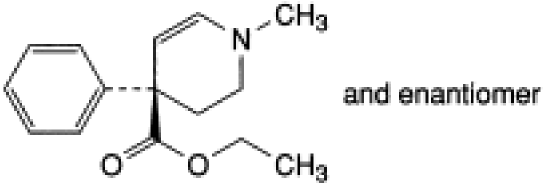
G. R1 = CH_3, R2 = CO_2-$CH(CH_3)_2$: 1-methylethyl 1-methyl-4-phenylpiperidine-4-carboxylate,

H. R1 = CH_2-C_6H_5, R2 = CO_2-CH_2-CH_3: ethyl 1-benzyl-4-phenylpiperidine-4-carboxylate,

J. R1 = CH_2-CH_3, R2 = CO_2-CH_2-CH_3: ethyl 1-ethyl-4-phenylpiperidine-4-carboxylate,



B. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),


and enantiomer

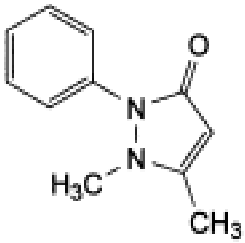
I. ethyl (4*RS*)-1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine-4-carboxylate.

Ph Eur

Phenazone

General Notices

(Ph Eur monograph 0421)



$C_{11}H_{12}N_2O$ 188.2 *60-80-0*

Action and use

Analgesic; used to test hepatic drug-metabolizing activity.

Ph Eur

DEFINITION

1,5-Dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 109 °C to 113 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Comparison *phenazone CRS.*

C. To 1 ml of solution S (see Tests) add 4 ml of *water R* and 0.25 ml of *dilute sulphuric acid R*. Add 1 ml of *sodium nitrite solution R*. A green colour develops.

D. To 1 ml of solution S add 4 ml of *water R* and 0.5 ml of *ferric chloride solution R2*. A red colour develops which is discharged on the addition of *dilute sulphuric acid R*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Add 0.2 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.25 ml of *methyl red solution R* and 0.4 ml of *0.01 M hydrochloric acid*. The solution is red or yellowish-red.

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 100 ppm.

Dissolve 1.5 g in *distilled water R* and dilute to 15 ml with the same solvent.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 6 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 20 ml of *water R*. Add 2 g of *sodium acetate R* and 25.0 ml of *0.05 M* iodine. Allow to stand protected from light for 30 min. Add 25 ml of *methylene chloride R* and shake until the precipitate dissolves. Titrate with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 9.41 mg of $C_{11}H_{12}N_2O$.

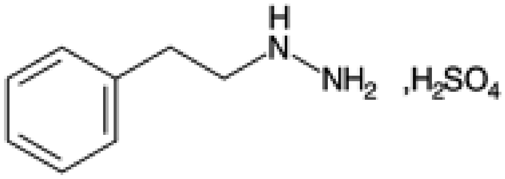
STORAGE

Protected from light.

Ph Eur

Phenelzine Sulphate

General Notices



$C_8H_{12}N_2,H_2SO_4$ 234.3 *156-51-4*

Action and use

Monoamine oxidase inhibitor; antidepressant.

Preparation

Phenelzine Tablets

DEFINITION

Phenelzine Sulphate is phenethylhydrazine hydrogen sulphate. It contains not less than 98.0% and not more than 100.5% of $C_8H_{12}N_2,H_2SO_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white powder or pearly platelets.

Freely soluble in *water*; practically insoluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.1% w/v solution in 0.05M *sulphuric acid* exhibits three well-defined maxima, at 252, 258 and 263 nm. The *absorbances* at the maxima are about 0.62, about 0.77 and about 0.58, respectively.

B. Dissolve 0.1 g in 5 ml of *water*, make alkaline with 5M *sodium hydroxide* and add 1 ml of *cupri-tartaric solution R1*. A red precipitate is produced.

C. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Melting point

164° to 168°, Appendix V A.

Loss on drying

When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

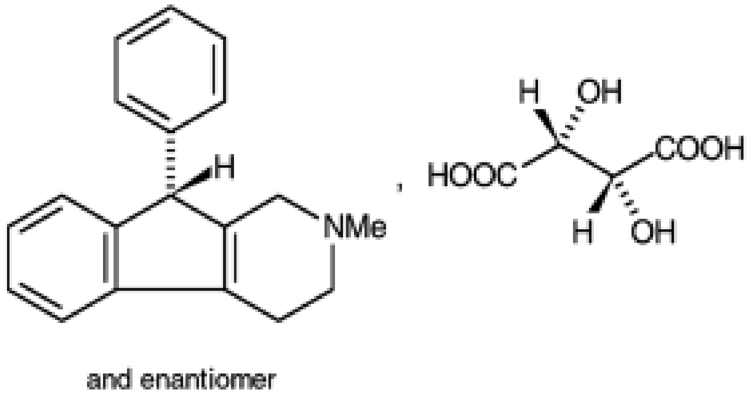
Dissolve 0.25 g in 50 ml of *water*, add 1.5 g of *sodium hydrogen carbonate* and 50 ml of 0.05M *iodine VS*, close the flask and allow to stand for 90 minutes. Add 20 ml of 2M *hydrochloric acid* and titrate with 0.1M *sodium thiosulphate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of iodine required. Each ml of 0.05M *iodine VS* is equivalent to 5.857 mg of $C_8H_{12}N_2,H_2SO_4$.

STORAGE

Phenelzine Sulphate should be protected from light.

Phenindamine Tartrate

General Notices



$C_{19}H_{19}N,C_4H_6O_6$ 411.5 *569-59-5*

Action and use

Histamine H_1 receptor antagonist.

DEFINITION

Phenindamine Tartrate is (*RS*)-2,3,4,9-tetrahydro-2-methyl-9-phenyl-1 *H*-indeno[2,1-*c*]pyridine hydrogen (2*R*,3*R*)-tartrate. It contains not less than 98.5% and not more than 101.0% of $C_{19}H_{19}N,C_4H_6O_6$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, voluminous powder.

Sparingly soluble in *water*; slightly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 300 nm of a 0.004% w/v solution exhibits a maximum only at 259 nm. The *absorbance* at the maximum is about 0.88.

B. Dissolve 25 mg in 5 ml of *sulphuric acid*. An orange-brown colour is produced which is discharged when the solution is carefully added to 20 ml of *water*.

C. Dissolve 0.5 g in 15 ml of hot *water*, add a slight excess of 5M *sodium hydroxide*, filter and neutralise the filtrate to *litmus paper* with 2M *hydrochloric acid*. The solution yields reaction B characteristic of *tartrates*, Appendix VI.

D. *Melting point*, 160° to 162°, Appendix V A. When heated to 163° it resolidifies and it melts again at about 168°, with decomposition.

TESTS

Acidity

pH of a 1% w/v solution, 3.4 to 3.9, Appendix V L.

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

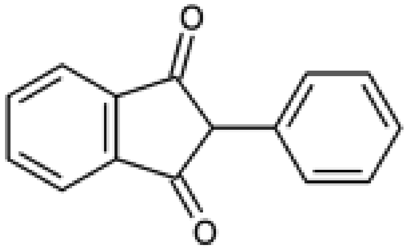
Dissolve 0.7 g in 40 ml of warm *water*, cool, add 10 ml of *dilute sodium carbonate solution* and extract with successive quantities of 25, 10 and 10 ml of *dichloromethane*, washing each extract with the same 15 ml of *water* and filtering into a dry flask. Titrate the combined extracts, which should be clear and free from droplets of water, with 0.05M *perchloric acid VS* determining the end-point potentiometrically. Each ml of 0.05M *perchloric acid VS* is equivalent to 20.57 mg of $C_{19}H_{19}N,C_4H_6O_6$.

STORAGE

Phenindamine Tartrate should be protected from light.

Phenindione

General Notices



$C_{15}H_{10}O_2$ 222.2 *83-12-5*

Action and use

Oral anticoagulant (indanedione).

Preparation

Phenindione Tablets

DEFINITION

Phenindione is 2-phenylindane-1,3-dione. It contains not less than 98.0% and not more than 100.5% of $C_{15}H_{10}O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

Soft, white or creamy white crystals.

Very slightly soluble in *water*; slightly soluble in *ethanol (96%)* and in *ether*. Solutions are yellow to red.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of phenindione *(RS 268)*.

B. Dissolve 0.1 g in 30 ml of *ethanol (96%)* with the aid of heat, cool and add sufficient *ethanol (96%)* to produce 50 ml. Dilute 10 ml to 250 ml with 0.1M *sodium hydroxide* and further dilute 5 ml to 100 ml with 0.1M *sodium hydroxide*. The *absorbance* of the resulting solution at the maximum at 278 nm is about 0.54 and at the maximum at 330 nm is about 0.16, Appendix II B.

C. To 1 g add 50 ml of *ethanol (96%)* and 0.5 ml of *aniline*, heat gently under a reflux condenser for 3 hours, cool in ice and filter. The *melting point* of the residue, after washing with 2 ml of *ethanol (96%)* and recrystallising from *chloroform*, is about 225°, Appendix V A.

TESTS

Melting point

148° to 151°, Appendix V A.

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and as the mobile phase a solution containing 0.02% w/v of *butylated hydroxytoluene* in a mixture of 80 volumes of *toluene*, 20 volumes of *ethyl acetate* and 4 volumes of *glacial acetic acid*. Allow the solvent front to ascend 4 cm, remove the plate and dry it in a current of cold air for 1 minute. Without delay apply separately to the plate 10 µl of each of three solutions of the substance being examined in *dichloromethane* containing (1) 1.0% w/v, (2) 0.020% w/v and (3) 0.0050% w/v and develop immediately. After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light* (254 nm). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Loss on drying

When dried at 105° for 2 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

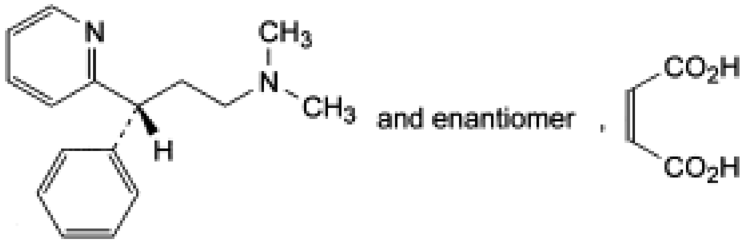
ASSAY

To 0.3 g add 50 ml of *ethanol (96%)* and warm until solution is effected. Cool to room temperature, add 10 ml of a 10% v/v solution of *bromine* in *ethanol (96%)* and allow to stand for 10 minutes, shaking occasionally. Add 1 g of *2-naphthol* and shake until the colour of the bromine is discharged. Remove any vapour of bromine in the flask with a current of air, add 50 ml of *water* and 10 ml of *dilute potassium iodide solution* and titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 11.11 mg of $C_{15}H_{10}O_2$.

Pheniramine Maleate

General Notices

(Ph Eur monograph 1357)



$C_{16}H_{20}N_2,C_4H_4O_4$ 356.5 *132-20-7*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

(3*RS*)-*N*,*N*-Dimethyl-3-phenyl-3-(pyridin-2-yl)propan-1-amine (*Z*)-butenedioate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), in methanol and in methylene chloride.

IDENTIFICATION

First identification C, D.

Second identification A, B, D.

A. Melting point (*2.2.14*): 106 °C to 109 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 40.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of this solution to 50.0 ml with *0.1 M hydrochloric acid*.

Spectral range 220-320 nm.

Absorption maximum At 265 nm.

Shoulder At 261 nm.

Specific absorbance at the absorption maximum 200 to 220.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *pheniramine maleate CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 65 mg of *maleic acid R* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 0.10 g of *pheniramine maleate CRS* in *methanol R* and dilute to 5.0 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *water R*, *anhydrous formic acid R*, *methanol R*, *di-isopropyl ether R* (3:7:20:70 *V/V/V/V*).

Application 5 µl.

Development Over a path of 12 cm.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution shows 2 clearly separated spots; the upper spot is similar in position and size to the spot in the chromatogram obtained with reference solution (a); the lower spot is similar in position and size to the spot in the chromatogram obtained with reference solution (b).

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*)and not more intensely coloured than reference solution BY_6 (*2.2.2*, *Method II*).

pH (*2.2.3*)

4.5 to 5.5.

Dissolve 0.20 g in 20.0 ml of *carbon dioxide-free water R*.

Optical rotation (*2.2.7*)

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, mobile phase A (1:9 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 10.0 mg of *2-benzylpyridine R* (impurity A) in 10.0 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

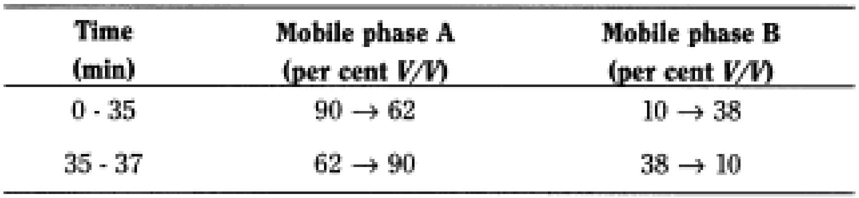
Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

- *size*: *l* = 0.30 m, Ø = 3.9 mm;
- *stationary phase*: *dimethyloctadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase:

- *mobile phase A*: 5.056 g/l solution of *sodium heptanesulphonate R* adjusted to pH 2.5 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	90 → 62	10 → 38
35 - 37	62 → 90	38 → 10

Flow rate 1 ml/min.

Detection Spectrophotometer at 264 nm.

Injection 20 µl.

System suitability Reference solution (a):

- the chromatogram obtained shows 3 principal peaks: maleic acid, impurity A and pheniramine, in order of elution;
- *resolution*: minimum 8 between the peaks due to impurity A and pheniramine.

Limits:

- *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); disregard the peak due to maleic acid.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* in an oven at 60 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.260 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

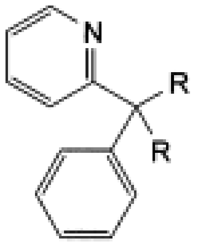
1 ml of *0.1 M perchloric acid* is equivalent to 17.82 mg of $C_{20}H_{24}N_2O_4$.

STORAGE

Protected from light.

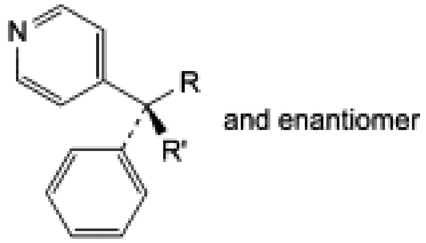
IMPURITIES

Specified impurities *A, B, C, D.*



A. R = H: 2-benzylpyridine,

D. R = CH_2-CH_2-N(CH_3)$_2$: *N*,*N*,*N′*,*N′*-tetramethyl-3-phenyl-3-(pyridin-2-yl)pentane-1,5-diamine,



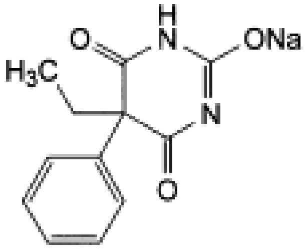
B. R = R′ = H: 4-benzylpyridine,

C. R = CH_2-CH_2-N(CH_3)$_2$, R′ = H: (3*RS*)-*N*,*N*-dimethyl-3-phenyl-3-(pyridin-4-yl)propan-1-amine.

Phenobarbital Sodium

General Notices

(Ph Eur monograph 0630)



$C_{12}H_{11}N_2NaO_3$ 254.2 *57-30-7*

Action and use

Barbiturate.

Preparations

Phenobarbital Injection

Paediatric Phenobarbital Oral Solution

Phenobarbital Sodium Tablets

Ph Eur

DEFINITION

Phenobarbital sodium contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of the sodium derivative of 5-ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, hygroscopic, freely soluble in carbon dioxide-free water (a small fraction may be insoluble), soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. Acidify 10 ml of solution S (see Tests) with *dilute hydrochloric acid R* and shake with 20 ml of *ether R*. Separate the ether layer, wash with 10 ml of *water R*, dry over *anhydrous* *sodium sulphate R* and filter. Evaporate the filtrate to dryness and dry the residue at 100 °C to 105 °C. Determine the melting point (*2.2.14*) of the test residue. Mix equal parts of the residue and of *phenobarbital CRS* and determine the melting point of the mixture. The difference between the two melting points (which are about 176 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing the residue obtained during identification test A with the spectrum obtained with *phenobarbital CRS.* If the spectra obtained in the solid state show differences, dissolve the test residue and the reference substance separately in *ethanol R*, evaporate to dryness and record the spectra again.

C. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.10 g of the substance to be examined in *alcohol (50 per cent V/V) R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 90 mg of *phenobarbital CRS* in *alcohol (50 per cent V/V) R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer from a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates (*2.3.1*).

E. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *alcohol (50 per cent V/V) R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)

Dissolve 5.0 g as completely as possible in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent. The pH of the solution is not greater than 10.2.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *alcohol (50 per cent V/V) R* and dilute to 100 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *alcohol (50 per cent V/V) R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer from a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent). Disregard any spot at the starting-point.

Loss on drying (*2.2.32*)

Not more than 7.0 per cent, determined on 0.500 g by drying in an oven at 150 °C for 4 h.

ASSAY

Dissolve 0.150 g in 2 ml of *water R* and add 8 ml of *0.05 M sulphuric acid*. Heat to boiling and cool. Add 30 ml of *methanol R* and shake until dissolution is complete. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. After the first point of inflexion, interrupt the addition of sodium hydroxide, add 10 ml of *pyridine R*, mix and continue the titration. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 25.42 mg of $C_{12}H_{11}N_2NaO_3$.

STORAGE

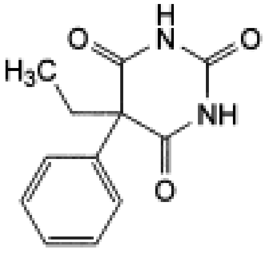
Store in an airtight container.

Ph Eur

Phenobarbital

General Notices

(Ph Eur monograph 0201)



$C_{12}H_{12}N_2O_3$ 232.2 *50-06-6*

Action and use

Barbiturate.

Preparations

Phenobarbital Elixir

Phenobarbital Tablets

Ph Eur

DEFINITION

Phenobarbital contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 5-ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, very slightly soluble in water, freely soluble in alcohol. It forms water-soluble compounds with alkali hydroxides and carbonates and with ammonia.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Determine the melting point (*2.2.14*) of the substance to be examined. Mix equal parts of the substance to be examined and *phenobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 176 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *phenobarbital CRS*.

C. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 0.1 g of *phenobarbital CRS* in *alcohol R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates (*2.3.1*).

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 6 ml of *water R*. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity

Boil 1.0 g with 50 ml of *water R* for 2 min, allow to cool and filter. To 10 ml of the filtrate add 0.15 ml of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *alcohol R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 5 ml of *pyridine R*. Add 0.5 ml of *thymolphthalein solution R* and 10 ml of silver nitrate solution in *pyridine R*. Titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained. Carry out a blank titration.

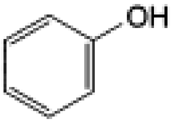
1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 11.61 mg of $C_{12}H_{12}N_2O_3$.

Ph Eur

Phenol

General Notices

(Ph Eur monograph 0631)



C_6H_6O 94.1 *108-95-2*

Action and use

Antiseptic; antimicrobial preservative; antipruritic.

Preparations

Aqueous Phenol Injection

Oily Phenol Injection

Phenol and Glycerol Injection

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

Colourless or faintly pink or faintly yellowish, crystals or crystalline masses, deliquescent.

Solubility

Soluble in water, very soluble in ethanol (96 per cent), in glycerol and in methylene chloride.

IDENTIFICATION

A. Dissolve 0.5 g in 2 ml of *concentrated ammonia R*. The substance dissolves completely. Dilute to about 100 ml with *water R*. To 2 ml of this solution add 0.05 ml of *strong sodium hypochlorite solution R*. A blue colour develops and becomes progressively more intense.

B. To 1 ml of solution S (see Tests) add 10 ml of *water R* and 0.1 ml of *ferric chloride* *solution R1*. A violet colour is produced which disappears on addition of 5 ml of 2-*propanol R*.

C. To 1 ml of solution S add 10 ml of *water R* and 1 ml of *bromine water R*. A pale-yellow precipitate is formed.

TESTS

Solution S

Dissolve 1.0 g in *water R* and dilute to 15 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Acidity

To 2 ml of solution S add 0.05 ml of *methyl orange solution R*. The solution is yellow.

Freezing point (*2.2.18*)

Minimum 39.5 °C.

Residue on evaporation

Maximum 0.05 per cent.

Evaporate 5.000 g to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs a maximum of 2.5 mg.

ASSAY

Dissolve 2.000 g in *water R* and dilute to 1000.0 ml with the same solvent. Transfer 25.0 ml of the solution to a ground-glass-stoppered flask and add 50.0 ml of *0.0167 M bromide-bromate* and 5 ml of *hydrochloric acid R*, close the flask, allow to stand with occasional swirling for 30 min. Then allow to stand for 15 min. Add 5 ml of a 200 g/l solution of *potassium iodide R*, shake and titrate with *0.1 M sodium thiosulphate* until a faint yellow colour remains. Add 0.5 ml of *starch solution R* and 10 ml of *chloroform R* and continue the titration with vigorous shaking. Carry out a blank titration.

1 ml of *0.0167 M bromide-bromate* is equivalent to 1.569 mg of C_6H_6O.

STORAGE

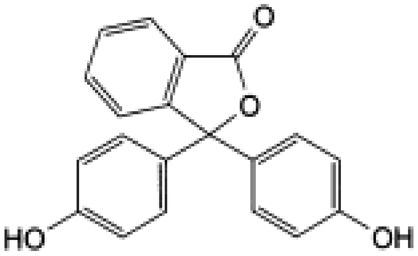
In an airtight container , protected from light.

Ph Eur

Phenolphthalein

General Notices

(Ph Eur monograph 1584)



$C_{20}H_{14}O_4$ 318.3 *77-09-8*

Action and use

Stimulant laxative.

Ph Eur

DEFINITION

Phenolphthalein contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 3,3-bis(4-hydroxyphenyl)isobenzofuran-1(3*H*)-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, practically insoluble in water, soluble in alcohol.

It melts at about 260 °C.

IDENTIFICATION

A. Dissolve 25.0 mg in *alcohol R* and dilute to 100.0 ml with the same solvent (solution A). To 2.0 ml of solution A add 5.0 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *alcohol R* (solution A_1). To 10.0 ml of solution A add 5.0 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *alcohol R* (solution A2). To 2.0 ml of solution A add 5.0 ml of *1 M sodium hydroxide* and dilute to 50.0 ml with *alcohol R* (solution B). Examined between 220 nm and 250 nm (*2.2.25*), solution A_1 shows an absorption maximum at 229 nm. The specific absorbance at the maximum at 229 nm is 922 to 1018. Examined between 250 nm and 300 nm, solution A_2 shows an absorption maximum at 276 nm. The specific absorbance at the maximum at 276 nm is 142 to 158. Examined between 230 nm and 270 nm, solution B shows an absorption maximum at 249 nm. The specific absorbance at the maximum at 249 nm is 744 to 822.

B. Dissolve about 10 mg in *alcohol R*. Add 1 ml of *dilute sodium hydroxide solution R*. The solution is red. Add 5 ml of *dilute sulphuric acid R*. The colour disappears.

TESTS

Solution S

To 2.0 g add 40 ml of *distilled water R* and heat to boiling. Cool and filter.

Appearance of solution

Dissolve 0.20 g in 5 ml of *alcohol R*. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.15 ml of *bromothymol blue solution R1*. Add 0.05 ml of *0.01 M hydrochloric acid*, the solution is yellow. Add 0.10 ml of *0.01 M sodium hydroxide*, the solution is blue.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution Dissolve 0.5 g of the substance to be examined in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 10 ml with *alcohol R*. Dilute 5 ml of this solution to 100 ml with alcohol R.

Reference solution (b) Dissolve 25 mg of *fluorene R* in *alcohol R*, add 0.5 ml of the test solution and dilute to 10 ml with *alcohol R*.

Apply to the plate 5 µl of the test solution and 5 µl of each of the reference solutions. Develop over a path corresponding to two-thirds of the plate height using a mixture of 50 volumes of *acetone R* and 50 volumes of *methylene chloride R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm and re-examine after exposure to ammonia vapour. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Chlorides (*2.4.4*)

Dilute 10 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (100 ppm).

Sulphates (*2.4.13*)

15 ml of solution S complies with the limit test for sulphates (200 ppm).

Heavy metals (*2.4.8*)

Heat 3 g with 50 ml of *dilute hydrochloric acid R* on a water-bath for 5 min and filter. Evaporate the filtrate almost to dryness and dissolve the residue in 30 ml of *water R*. 12 ml of this solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 5 ml of *dimethylformamide R*. Add 5 ml of *sodium carbonate solution R*, 10 ml of *sodium hydrogen carbonate solution R*, 35 ml of *water R* and 50.0 ml of *0.05 M* iodine. Add 10 ml of *methylene chloride R* and 20 ml of *dilute sulphuric acid R*. Titrate the excess of iodine with *0.1 M sodium thiosulphate*, using 0.3 ml of *starch solution R* added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 3.979 mg of $C_{20}H_{14}O_4$.

STORAGE

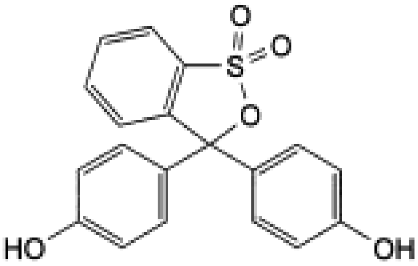
Store protected from light.

Ph Eur

Phenolsulphonphthalein

General Notices

(Ph Eur monograph 0242)



$C_{19}H_{14}O_5S$ 354.4 *143-74-8*

Action and use

Measurement of renal function; marker in drug absorption studies.

Ph Eur

DEFINITION

Phenolsulfonphthalein (phenol red) contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 3,3-bis(4-hydroxyphenyl)-3*H*-2,1-benzoxathiole 1,1-dioxide, calculated with reference to the dried substance.

CHARACTERS

A bright-red to dark-red, crystalline powder, very slightly soluble in water, slightly soluble in alcohol.

IDENTIFICATION

A. Dissolve 10 mg in a 10 g/l solution of *sodium carbonate R* and dilute to 200.0 ml with the sodium carbonate solution. Dilute 5.0 ml of the solution to 100.0 ml with a 10 g/l solution of *sodium carbonate R*. Examined between 400 nm and 630 nm (*2.2.25*), the solution shows an absorption maximum at 558 nm. The specific absorbance at the maximum is 1900 to 2100.

B. Dissolve about 10 mg in 1 ml of *dilute sodium hydroxide solution R* and add 9 ml of *water R*. The solution is deep red. To 5 ml of the solution add a slight excess of *dilute sulphuric acid R*. The colour becomes orange.

C. To 5 ml of the solution prepared for identification test B add 1 ml of *0.0167 M bromide-bromate* and 1 ml of *dilute hydrochloric acid R*, shake and allow to stand for 15 min. Make alkaline with *dilute sodium hydroxide solution R*. An intense violet-blue colour is produced.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *0.1 M sodium hydroxide* and dilute to 5 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *0.1 M sodium hydroxide*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 25 volumes of *glacial acetic acid R*, 25 volumes of *water R* and 100 volumes of *tert-pentyl alcohol R*. Allow the plate to dry in air until the solvent has evaporated and expose the plate to the vapour from *concentrated ammonia R*. Examine in ultraviolet light at 254 nm. Not more than one spot, apart from the principal spot, appears in the chromatogram obtained with the test solution and this spot is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Insoluble matter

To 1.0 g of the finely powdered substance to be examined add 12 ml of *sodium hydrogen carbonate solution R*. Allow to stand for 1 h, shaking frequently. Dilute to 100 ml with *water R* and allow to stand for 15 h. Centrifuge at 2000 g to 3000 g, for 30 min, decant the supernatant liquid and wash the residue with 25 ml of a 10 g/l solution of *sodium hydrogen carbonate R* and then 25 ml of *water R*. Dry at 100 °C to 105 °C. The residue weighs not more than 5 mg (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 1.00 g of the powdered substance to be examined by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.2 per cent, determined on 0.5 g.

ASSAY

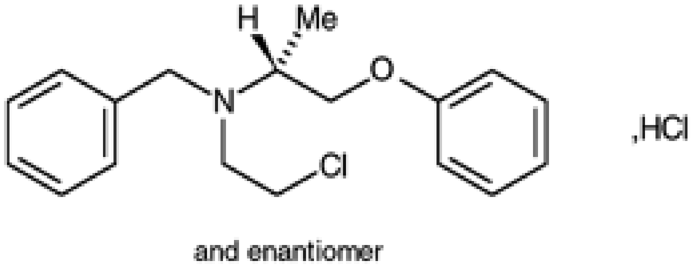
Dissolve 0.900 g in 15 ml of *1 M sodium hydroxide* and dilute to 250.0 ml with *water R*. To 10.0 ml of the solution in a glass-stoppered flask add 25 ml of *glacial acetic acid R*, 20.0 ml of *0.0167 M potassium bromate*, 5 ml of a 100 g/l solution of *potassium bromide R* and 5 ml of *hydrochloric acid* R. Allow to stand protected from light for 15 min, add 10 ml of a 100 g/l solution of *potassium iodide R* and titrate immediately with *0.1 M sodium thiosulphate*, using 0.1 ml of *starch solution R* as indicator.

1 ml of *0.0167 M potassium bromate* is equivalent to 4.43 mg of $C_{19}H_{14}O_5S$.

Ph Eur

Phenoxybenzamine Hydrochloride

General Notices



$C_{18}H_{22}ClNO,HCl$ 340.3 *63-92-3*

Action and use

Alpha-adrenoceptor antagonist.

Preparation

Phenoxybenzamine Capsules

DEFINITION

Phenoxybenzamine Hydrochloride is (*RS*)-benzyl(2-chloroethyl)1-methyl-2-phenoxyethylamine hydrochloride. It contains not less than 98.5% and not more than 101.0% of $C_{18}H_{22}ClNO,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Sparingly soluble in *water*; freely soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of phenoxybenzamine hydrochloride *(RS 271)*.

B. Dissolve 0.5 g in 50 ml of *ethanol-free chloroform* and extract with three 20-ml quantities of 0.01M *hydrochloric acid*. Filter the chloroform layer through absorbent cotton and dilute 5 ml of the filtrate to 250 ml with *ethanol-free chloroform*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits two maxima, at 272 nm and 279 nm. The *absorbances* at the maxima are about 1.1 and about 0.90, respectively.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Melting point

137.5° to 140°, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 80 volumes of *acetone* and 20 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of two freshly prepared solutions of the substance being examined in *methanol* containing (1) 2.0% w/v and (2) 0.010% w/v. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Loss on drying

When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

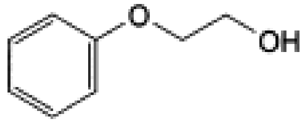
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.6 g and *oracet blue B solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 34.03 mg of $C_{18}H_{22}ClNO,HCl$.

Phenoxyethanol

General Notices

(Ph Eur monograph 0781)



$C_8H_{10}O_2$ 138.2 *122-99-6*

Action and use

Antimicrobial preservative; also used topically in treatment of bacterial infections.

Ph Eur

DEFINITION

2-Phenoxyethanol.

Content

99.0 per cent m/m to 100.5 per cent *m/m*.

CHARACTERS

Appearance

Colourless, slightly viscous liquid.

Solubility

Slightly soluble in water, miscible with acetone, with ethanol (96 per cent) and with glycerol, slightly soluble in arachis oil and in olive oil.

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Refractive index (*2.2.6*): 1.537 to 1.539.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 80.0 mg in *water R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water R*.

Spectral range 240-350 nm.

Absorption maxima At 269 nm and 275 nm.

Specific absorbances at the absorption maxima:
- at 269 nm: 95 to 105;
- at 275 nm: 75 to 85.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenoxyethanol CRS.*

D. Shake 2 ml with a mixture of 4 g of *potassium permanganate R*, 5.4 g of *sodium carbonate R* and 75 ml of *water R* for 30 min. Add 25 g of *sodium chloride R* and stir continuously for 60 min, filter and acidify with *hydrochloric acid R* to about pH 1.7. The melting point of the precipitate, after recrystallisation from *water R*, is 96 °C to 99 °C (*2.2.14*).

TESTS

Relative density (*2.2.5*)

1.105 to 1.110.

Related substances

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 1.25 g of *methyl laurate R* in *methylene chloride R* and dilute to 25 ml with the same solvent.

Test solution (a) Dissolve 5.0 g of the substance to be examined in *methylene chloride R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 5.0 g of the substance to be examined in *methylene chloride R*, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *methylene chloride R*.

Reference solution To 1.0 ml of test solution (a) add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *methylene chloride R*.

Column:
- *material*: glass;
- *size:* l = 1.5 m, Ø = 4 mm,
- *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (150-180 µm) impregnated with 3 per cent *m/m* of *polymethylphenylsiloxane R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 ml/min.

Temperature:
- *column*: 130 °C;
- *injection port and detector*: 200 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time 5 times the retention time of phenoxyethanol.

Elution order Phenoxyethanol, methyl laurate.

Retention time Phenoxyethanol = about 5 min.

System suitability:

— *resolution*: minimum 12 between the peaks due to phenoxyethanol and methyl laurate in the chromatogram obtained with the reference solution;

— in the chromatogram obtained with test solution (a) there is no peak with the same retention time as the internal standard.

Limit:

— *total*: calculate the ratio (*R*) of the area of the peak due to phenoxyethanol to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than *R* (1.0 per cent).

Phenol

Maximum 0.1 per cent.

Dissolve 1.00 g in 50 ml of *methylene chloride R*, add 1 ml of *dilute sodium hydroxide solution R* and 10 ml of *water R*. Shake. Wash the upper layer with 2 quantities, each of 20 ml, of *methylene chloride R* and dilute to 100.0 ml with *water R*. The absorbance (*2.2.25*) of the solution measured at the absorption maximum at 287 nm is not greater than 0.27.

ASSAY

To 2.000 g in an acetylation flask fitted with an air condenser, add 10.0 ml of freshly prepared *acetic anhydride solution R1* and heat with frequent shaking in a water-bath for 45 min. Cool and carefully add 10 ml of *water R*. Heat for a further 2 min. Cool, add 10 ml of *butanol R,* shake vigorously and titrate the excess of acetic acid with *1 M sodium hydroxide* using 0.2 ml of *phenolphthalein solution R* as indicator. Repeat the procedure without the substance to be examined. The difference between the volumes used in the titrations represents the amount of acetic anhydride required for the acetylation of the substance to be examined.

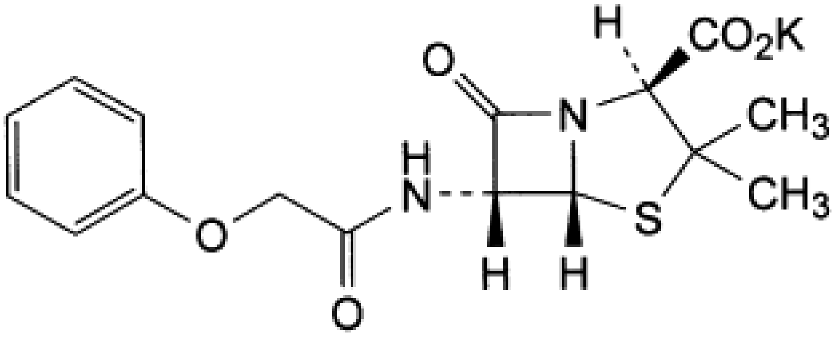
1 ml of *1 M sodium hydroxide* is equivalent to 0.1382 g of $C_8H_{10}O_2$.

Ph Eur

Phenoxymethylpenicillin Potassium

General Notices

(Ph Eur monograph 0149)



$C_{16}H_{17}KN_2O_5S$ 388.5 *132-98-9*

Action and use

Penicillin antibacterial.

Preparations

Phenoxymethylpenicillin Oral Solution

Phenoxymethylpenicillin Tablets

Ph Eur

DEFINITION

Potassium salt of (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Substance produced by the growth of certain strains of *Penicillium notatum* or related organisms on a culture medium containing an appropriate precursor, or obtained by any other means.

Content

95.0 per cent to 102.0 per cent for the sum of the percentage contents of phenoxymethylpenicillin potassium and 4-hydroxyphenoxymethylpenicillin potassium (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *phenoxymethylpenicillin potassium CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *water R*.

Reference solution (a) Dissolve 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 ml of *water R*.

Reference solution (b) Dissolve 25 mg of *benzylpenicillin potassium CRS* and 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 ml of *water R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R* adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is reddish-brown. Place the test-tube in a water-bath for 1 min; a dark reddish-brown colour develops.

D. It gives reaction (a) of potassium *(2.3.1)*.

TESTS

pH *(2.2.3)*

5.5 to 7.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 215 to + 230 (anhydrous substance).

Dissolve 0.250 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Dissolution mixture To 250 ml of *0.2 M potassium dihydrogen phosphate R* add 500 ml of *water R* and adjust to pH 6.5 with an 8.4 g/l solution of *sodium hydroxide R*. Dilute to 1000 ml with *water R*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the dissolution mixture and dilute to 50.0 ml with the dissolution mixture.

Test solution (b) *Prepare immediately before use*. Dissolve 80.0 mg of the substance to be examined in the dissolution mixture and dilute to 20.0 ml with the dissolution mixture.

Reference solution (a) Dissolve 50.0 mg of *phenoxymethylpenicillin potassium CRS* in the dissolution mixture and dilute to 50.0 ml with the dissolution mixture.

Reference solution (b) Dissolve 4.0 mg of *4-hydroxyphenoxymethylpenicillin potassium CRS* in the dissolution mixture and dilute to 10.0 ml with the dissolution mixture. Dilute 5.0 ml of this solution to 100.0 ml with the dissolution mixture.

Reference solution (c) Dissolve 10 mg of *phenoxymethylpenicillin potassium CRS* and 10 mg of *benzylpenicillin sodium CRS* (impurity A) in the dissolution mixture and dilute to 50 ml with the dissolution mixture.

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 20 ml with the dissolution mixture. Dilute 1.0 ml of this solution to 50 ml with the dissolution mixture.

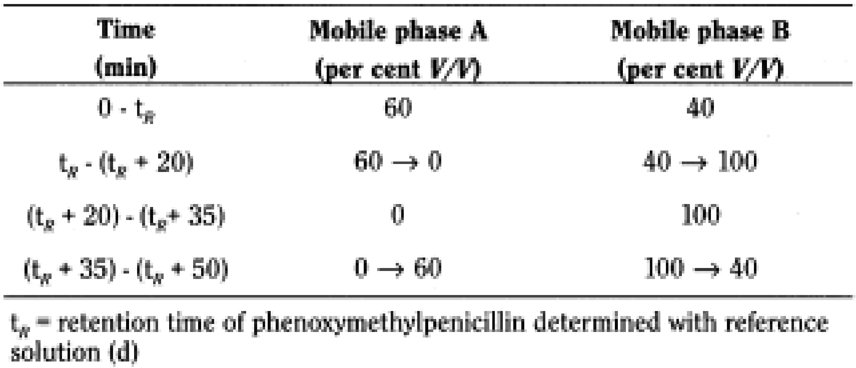
Reference solution (e) Dilute 1.0 ml of reference solution (a) to 25.0 ml with the dissolution mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: *phosphate buffer solution pH 3.5 R, methanol R, water R* (10:30:60 V/V/V);
- *mobile phase B*: *phosphate buffer solution pH 3.5 R, water R, methanol R* (10:35:55 V/V/V);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	60	40
t_R - (t_R + 20)	60 → 0	40 → 100
(t_R + 20) - (t_R+ 35)	0	100
(t_R + 35) - (t_R + 50)	0 → 60	100 → 40

t_R = retention time of phenoxymethylpenicillin determined with reference solution (d)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of reference solutions (c), (d) and (e) with isocratic elution at the initial mobile phase composition and 20 µl of test solution (b) according to the elution gradient described under Mobile phase; inject the dissolution mixture as a blank according to the elution gradient described under Mobile phase.

System suitability:

— *resolution*: minimum 6.0 between the peaks due to impurity A and phenoxymethylpenicillin in the chromatogram obtained with reference solution (c); if necessary, adjust the ratio A:B of the mobile phase;

— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (d);

— *mass distribution ratio*: 5.0 to 7.0 for the peak due to phenoxymethylpenicillin (2nd peak) in the chromatogram obtained with reference solution (c).

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (1 per cent);

— *disregard limit*: disregard the peak due to 4-hydroxyphenoxymethylpenicillin.

4-Hydroxyphenoxymethylpenicillin potassium

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solution (b).

Limit:

— 4-hydroxyphenoxymethylpenicillin potassium: maximum 4.0 per cent (anhydrous substance).

Calculate the percentage content by multiplying, if necessary, by the correction factor supplied with the CRS.

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.000 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solutions (a) and (b).

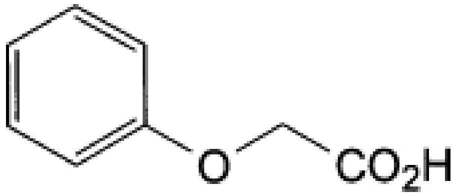
System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

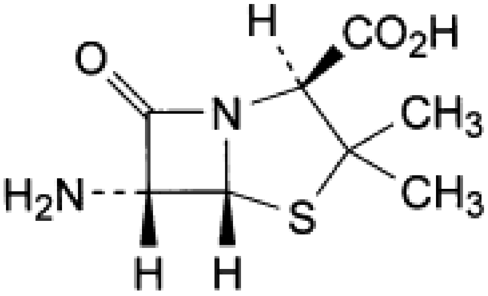
Calculate the percentage content of phenoxymethylpenicillin potassium and of 4-hydroxyphenoxymethylpenicillin potassium.

IMPURITIES

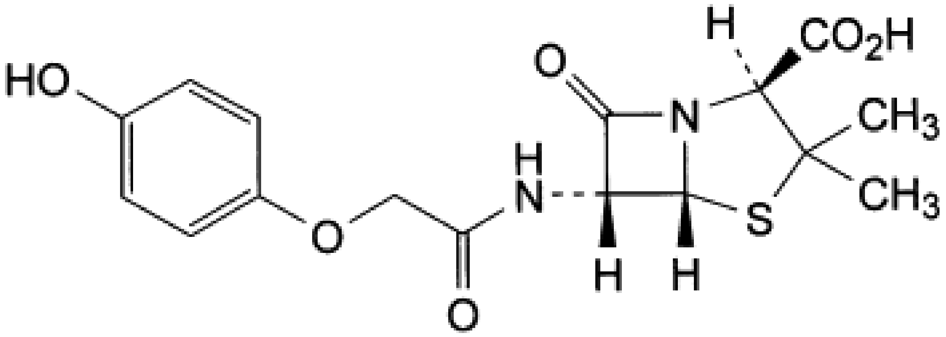
A. benzylpenicillin,



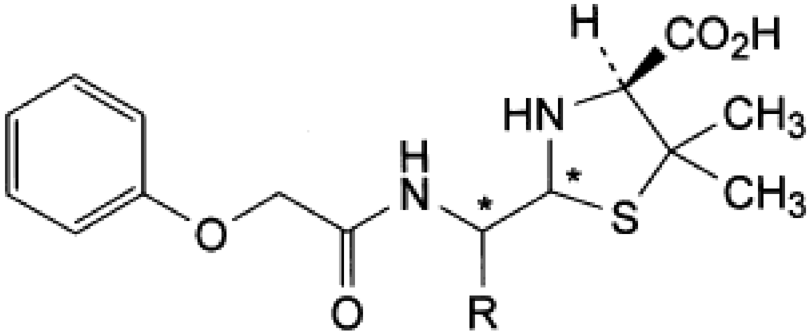
B. phenoxyacetic acid,



C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



D. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[[2-(4-hydroxyphenoxy)acetyl]amino]-4-thia- 1-azabicyclo[3.2.0]heptane-2-carboxylic acid (4-hydroxyphenoxymethylpenicillin),



E. R = CO_2H: (4*S*)-2-[carboxy[(phenoxyacetyl)amino]methyl]-5,5dimethylthiazolidine-4-carboxylic acid (penicilloic acids of phenoxymethylpenicillin),

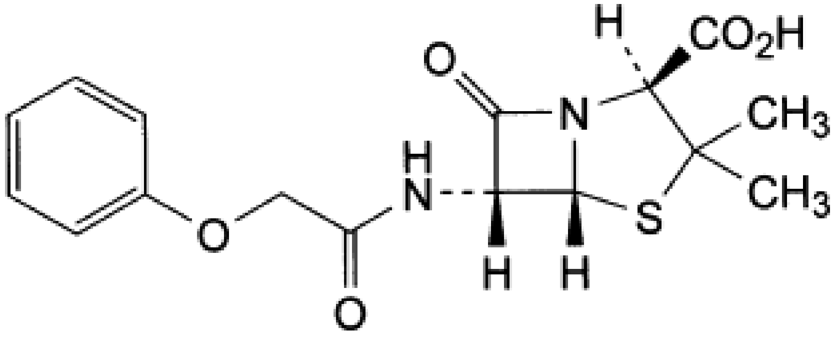
F. R = H: (2*RS*,4*S*)-5,5-dimethyl-2-[[(phenoxyacetyl)amino]methyl]thiazolidine-4-carboxylic acid (penilloic acids of phenoxymethylpenicillin).

Ph Eur

Phenoxymethylpenicillin

General Notices

(Ph Eur monograph 0148)



$C_{16}H_{18}N_2O_5S$ 350.4 *87-08-1*

Action and use

Penicillin antibacterial.

Ph Eur

DEFINITION

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Substance produced by the growth of certain strains of *Penicillium notatum* or related organisms on a culture medium containing an appropriate precursor, or obtained by any other means.

Content

95.0 per cent to 102.0 per cent for the sum of the percentage contents of phenoxymethylpenicillin and 4-hydroxyphenoxymethylpenicillin (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic, crystalline powder.

Solubility

Very slightly soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. pH (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *phenoxymethylpenicillin CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *acetone R*.

Reference solution (a) Dissolve 25 mg of *phenoxymethylpenicillin CRS* in 5 ml of *acetone R*.

Reference solution (b) Dissolve 25 mg of *benzylpenicillin potassium CRS* and 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 ml of *water R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R* adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is reddish-brown. Place the test-tube on a water-bath for 1 min; a dark reddish-brown colour develops.

TESTS

pH *(2.2.3)*

2.4 to 4.0.

Suspend 50 mg in 10 ml of *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7)*

+ 186 to + 200 (anhydrous substance).

Dissolve 0.250 g in *butanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Dissolution mixture To 250 ml of *0.2 M potassium dihydrogen phosphate R* add 500 ml of *water R*, adjust to pH 6.5 with an 8.4 g/l solution of *sodium hydroxide R* and dilute to 1000 ml with *water R*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the dissolution mixture and dilute to 50.0 ml with the dissolution mixture.

Test solution (b) *Prepare immediately before use*. Dissolve 80.0 mg of the substance to be examined in the dissolution mixture and dilute to 20.0 ml with the dissolution mixture.

Reference solution (a) Dissolve 55.0 mg of *phenoxymethylpenicillin potassium CRS* in the dissolution mixture and dilute to 50.0 ml with the dissolution mixture.

Reference solution (b) Dissolve 4.0 mg of *4-hydroxyphenoxymethylpenicillin potassium CRS* in the dissolution mixture and dilute to 10.0 ml with the dissolution mixture. Dilute 5.0 ml of this solution to 100.0 ml with the dissolution mixture.

Reference solution (c) Dissolve 10 mg of *phenoxymethylpenicillin potassium CRS* and 10 mg of *benzylpenicillin sodium CRS* (impurity A) in the dissolution mixture and dilute to 50 ml with the dissolution mixture.

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 20 ml with the dissolution mixture. Dilute 1.0 ml of this solution to 50 ml with the dissolution mixture.

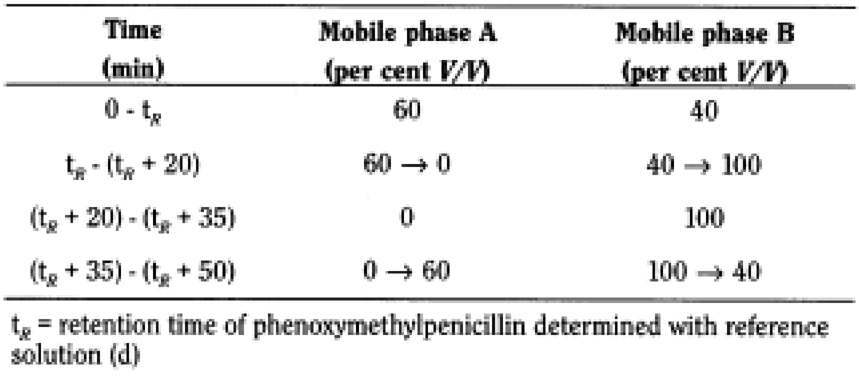
Reference solution (e) Dilute 1.0 ml of reference solution (a) to 25.0 ml with the dissolution mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: *phosphate buffer solution pH 3.5 R*, *methanol R*, *water R* (10:30:60 *V/V/V*);
- *mobile phase B*: *phosphate buffer solution pH 3.5 R*, *water R*, *methanol R* (10:35:55 *V/V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	60	40
t_R - (t_R + 20)	60 → 0	40 → 100
(t_R + 20) - (t_R + 35)	0	100
(t_R + 35) - (t_R + 50)	0 → 60	100 → 40

t_R = retention time of phenoxymethylpenicillin determined with reference solution (d)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of reference solutions (c), (d) and (e) with isocratic elution at the initial mobile phase composition and 20 µl of test solution (b) according to the elution gradient described under Mobile phase; inject the dissolution mixture as a blank according to the elution gradient described under Mobile phase.

System suitability:

— *resolution*: minimum 6.0 between the peaks due to impurity A and phenoxymethylpenicillin in the chromatogram obtained with reference solution (c); if necessary, adjust the ratio A:B of the mobile phase;

— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (d);

— *mass distribution ratio*: 5.0 to 7.0 for the peak due to phenoxymethylpenicillin (2nd peak) in the chromatogram obtained with reference solution (c).

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (1 per cent);

— *disregard limit*: disregard the peak due to 4-hydroxyphenoxymethylpenicillin.

4-Hydroxyphenoxymethylpenicillin

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solution (b).

Limit:

— *4-hydroxyphenoxymethylpenicillin*: maximum 4.0 per cent (anhydrous substance).

Calculate the percentage content by multiplying, if necessary, by the correction factor supplied with the CRS.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.000 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Injection Test solution (a) and reference solutions (a) and (b).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of phenoxymethylpenicillin by multiplying the percentage content of phenoxymethylpenicillin potassium by 0.902. Calculate the percentage content of

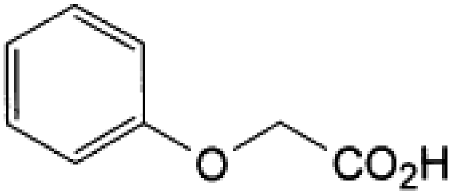
4-hydroxyphenoxymethylpenicillin by multiplying, if necessary, by the correction factor supplied with the CRS.

STORAGE

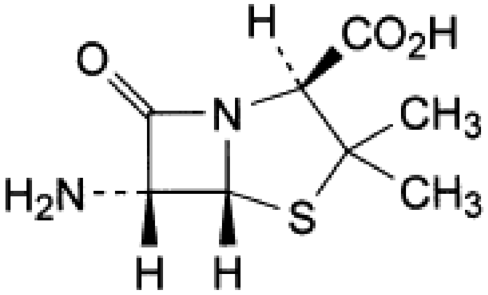
In an airtight container .

IMPURITIES

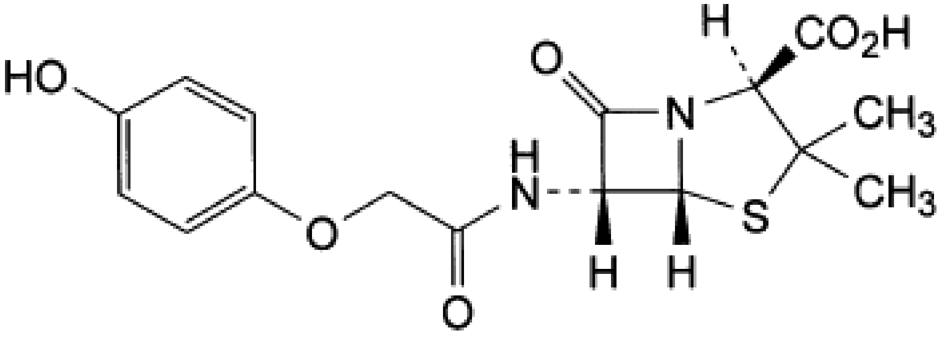
A. benzylpenicillin,



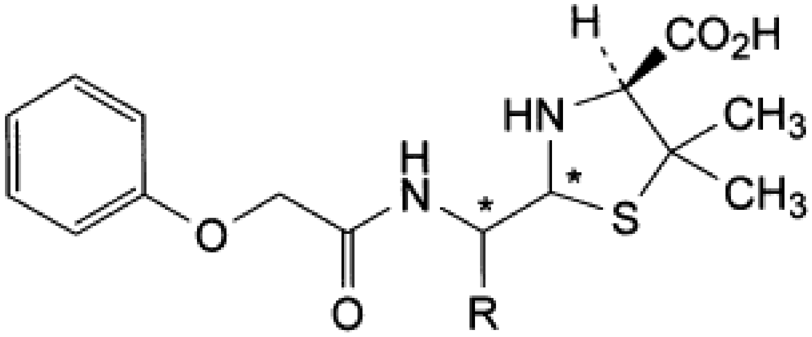
B. phenoxyacetic acid,


H
CO2H
O
N
CH3
H2N
S
CH3
H
H

C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),


H
CO2H
HO
O
N
CH3
H
N
S
CH3
O
H
H
O

D. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[[2-(4-hydroxyphenoxy)acetyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (4-hydroxyphenoxymethylpenicillin),



E. R = CO_2H: (4*S*)-2-[carboxy[(phenoxyacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of phenoxymethylpenicillin),

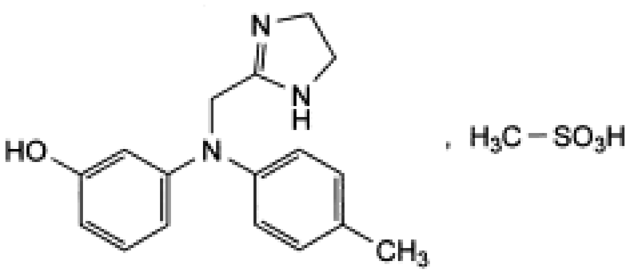
F. R = H: (2*RS*,4*S*)-5,5-dimethyl-2-[[(phenoxyacetyl)amino]methyl]thiazolidine-4-carboxylic acid (penilloic acids of phenoxymethylpenicillin).

Ph Eur

Phentolamine Mesilate

General Notices

(Ph Eur monograph 1138)



$C_{18}H_{23}N_3O_4S$ 377.5 *65-28-1*

Action and use

Alpha-adrenoceptor antagonist.

Preparation

Phentolamine Injection

Ph Eur

DEFINITION

Phentolamine mesilate contains not less than 98.0 per cent and not more than the equivalent of 100.5 per cent of 3-[[(4,5-dihydro-1*H*-imidazol-2-yl)methyl](4-methylphenyl)amino]phenol methanesulphonate, calculated with reference to the dried substance.

PRODUCTION

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

CHARACTERS

A white or almost white, crystalline powder, slightly hygroscopic, freely soluble in water and in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification C, E

Second identification *A, B, D, E.*

A. Melting point (*2.2.14*): 178 °C to 182 °C.

B. Dissolve 60.0 mg in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *water R*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 278 nm. The specific absorbance at the maximum is 220 to 245.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the *Ph. Eur. reference spectrum of phentolamine mesilate* .

D. Dissolve 0.5 g in a mixture of 5 ml of *alcohol R* and 5 ml of a 10 g/l solution of *hydrochloric acid R* and add 0.5 ml of a 5 g/l solution of *ammonium vanadate R*. A light green precipitate is produced.

E. Mix 50 mg with 0.2 g of *sodium hydroxide R*, heat to fusion and continue heating for a few seconds. Allow to cool and add 0.5 ml of warm *water R*. Acidify with *dilute hydrochloric acid R* and heat. Sulphur dioxide is evolved, which turns moistened *starch iodate paper R* blue.

TESTS

Acidity

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. Add 0.1 ml of *methyl red solution R*. If the solution is red, not more than 0.05 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.10 g of the substance to be examined in *alcohol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dilute 0.5 ml of the test solution to 100 ml with *alcohol R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *alcohol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *acetone R* and 85 volumes of *methyl ethyl ketone R*. Allow the plate to dry in air and spray with *dilute potassium iodobismuthate solution R*. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 100 ml of *2-propanol R1*. Titrate under a current of nitrogen with *0.1 M tetrabutylammonium hydroxide in 2-propanol* . Determine the end-point potentiometrically

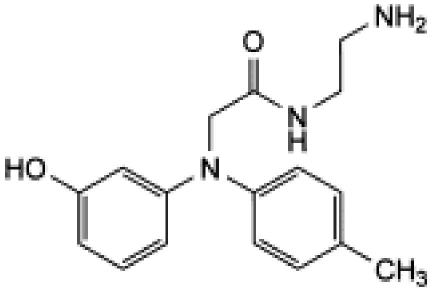
(*2.2.20*), using a glass electrode as indicator electrode and a calomel electrode containing a saturated solution of *tetramethylammonium chloride R* in *2-propanol R1* as the comparison electrode. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 37.75 mg of $C_{18}H_{23}N_3O_4S$.

STORAGE

In an airtight container, protected from light.

IMPURITIES



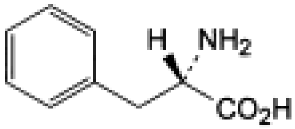
A. *N*-(2-aminoethyl)-2-[(3-hydroxyphenyl)(4-methylphenyl)amino]acetamide.

Ph Eur

Phenylalanine

General Notices

(Ph Eur monograph 0782)



$C_9H_{11}NO_2$ 165.2 *63-91-2*

Action and use

Amino acid.

Ph Eur

DEFINITION

Phenylalanine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2-amino-3-phenylpropanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, or shiny, white flakes, sparingly soluble in water, very slightly soluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *phenylalanine CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 10 mg add 0.5 g of *potassium nitrate R* and 2 ml of *sulphuric acid R*. Heat on a water-bath for 20 min. Allow to cool. Add 5 ml of a 50 g/l solution of *hydroxylamine hydrochloride R* and allow to stand in iced water for 10 min. Add 9 ml of *strong sodium* *hydroxide solution R*. A violet-red to violet-brown colour develops.

TESTS

Appearance of solution

Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 10 ml with the same acid. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

Dissolve 0.50 g in *water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is - 33.0 to - 35.5, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with a mixture of equal volumes of *glacial acetic acid R* and *water R*.

Reference solution (a) Dissolve 10 mg of *phenylalanine CRS* in a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 50 ml with the same mixture of solvents.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with a mixture of equal volumes of *glacial acetic acid R* and *water R*.

Reference solution (c) Dissolve 10 mg of *phenylalanine CRS* and 10 mg of *tyrosine CRS* in a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 25 ml with the same mixture of solvents.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (*2.4.4*)

Dissolve 0.25 g in 3 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution complies with the limit test for chlorides, without any further addition of nitric acid (200 ppm).

Sulphates (*2.4.13*)

Dissolve 0.5 g in a mixture of 5 volumes of *dilute hydrochloric acid R* and 25 volumes of *distilled water R* and dilute to 15 ml with the same mixture of solvents. The solution complies with the limit test for sulphates (300 ppm).

Ammonium (*2.4.1*)

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron (*2.4.9*)

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals (*2.4.8*)

2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* using 0.1 ml of *naphtholbenzein solution R* as indicator, until the colour changes from yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 16.52 mg of $C_9H_{11}NO_2$.

STORAGE

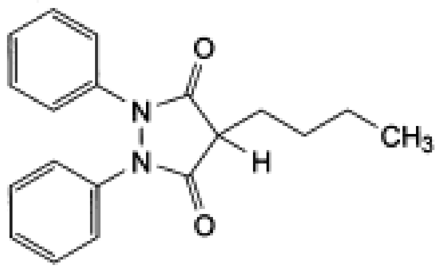
Store protected from light.

Ph Eur

Phenylbutazone

General Notices

(Ph Eur monograph 0422)



$C_{19}H_{20}N_2O_2$ 308.4 *50-33-9*

Action and use

Cyclo-oxygenase inhibitor; pyrazolone analgesic.

Ph Eur

DEFINITION

4-Butyl-1,2-diphenylpyrazolidine-3,5-dione.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in alcohol. It dissolves in alkaline solutions.

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Melting point (*2.2.14*): 104 °C to 107 °C.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in a mixture of equal volumes of *ethanol R* and *methylene chloride R* and dilute to 25 ml with the same mixture of solvents.

Reference solution Dissolve 25 mg of *phenylbutazone CRS* in a mixture of equal volumes of *ethanol R* and *methylene chloride R* and dilute to 25 ml with the same mixture of solvents.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *acetone R*, *methylene chloride R* (20:80 *V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenylbutazone CRS.*

D. To 0.1 g add 1 ml of *glacial acetic acid R* and 2 ml of *hydrochloric acid R* and heat the mixture under a reflux condenser for 30 min. Cool, add 10 ml of *water R* and filter. To the filtrate add 3 ml of a 7 g/l solution of *sodium nitrite R*. A yellow colour is produced. To 1 ml of the solution add a solution of 10 mg of β*-naphthol R* in 5 ml of *sodium carbonate solution R*. A brownish-red to violet-red precipitate is formed.

TESTS

Solution S

Dissolve 1.0 g with shaking in 20 ml of *dilute sodium hydroxide solution R* and maintain the solution at 25 °C for 3 h.

Appearance of solution

Solution S is clear (*2.2.1*).

Acidity or alkalinity

Heat to boiling 1.0 g in 50 ml of *water R*, cool with shaking in a closed flask and filter. To 25 ml of the filtrate add 0.5 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator. Add 0.6 ml of *0.01 M hydrochloric acid* and 0.1 ml of *methyl red solution R*; the solution is red or orange.

Absorbance (*2.2.25*)

Maximum 0.20 for solution S at 420 nm in a 4 cm cell.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 100.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*. Dilute 1.0 ml to 10.0 ml with *acetonitrile R*.

Reference solution (b) Dissolve 5 mg of *phenylbutazone impurity B CRS* and 5 mg of *1,2-diphenylhydrazine R* in *acetonitrile R*, add 0.5 ml of the test solution and dilute to 50 ml with *acetonitrile R*. Dilute 2.5 ml to 10 ml with *acetonitrile R*.

Reference solution (c) Dissolve 1.0 mg of *benzidine R* in *acetonitrile R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 100.0 ml with *acetonitrile R*. Dilute 5.0 ml to 10.0 ml with *acetonitrile R.*

Column:

— *size:* *l* = 0.125 m, Ø = 4.0 mm;

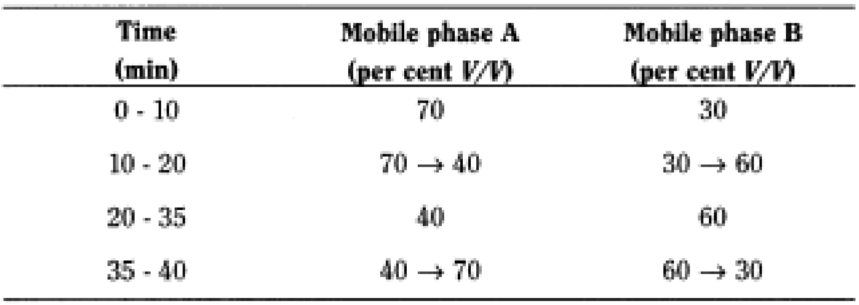
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: dissolve 1.36 g of *sodium acetate R* in *water R*, adjust to pH 5.2 with a 52.5 g/l solution of *citric acid R* and dilute to 1000 ml with *water R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	70	30
10 - 20	70 → 40	30 → 60
20 - 35	40	60
35 - 40	40 → 70	60 → 30

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl; inject the test solution and reference solutions (a) and (b).

Relative retentions With reference to phenylbutazone (retention time = about 13 min): impurity E = about 0.2; impurity A = about 0.5; impurity B = about 1.2; impurity C = about 1.3; impurity D = about 1.7.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to phenylbutazone and to impurity B.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.55;

— *impurities A, B*: for each impurity, not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *impurity C*: not more than twice the area of the principal peak in the chromatogram

obtained with reference solution (a) (0.20 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent); disregard any peak due to impurity E.

Impurity E

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 280 nm.

Injection Test solution and reference solution (c).

System suitability Reference solution (c):

— *signal-to-noise ratio*: minimum 10 for the principal peak.

Limit:

— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (5 ppm).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 0.2 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

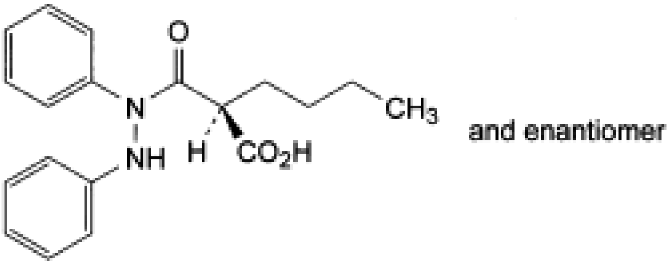
Dissolve 0.250 g in 25 ml of *acetone R* and add 0.5 ml of *bromothymol blue solution R1.* Titrate with *0.1 M sodium hydroxide* until a blue colour is obtained which persists for 15 s. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 30.84 mg of $C_{19}H_{20}N_2O_2$.

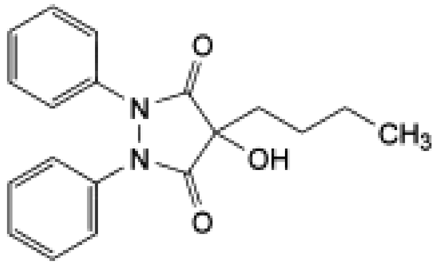
STORAGE

Protected from light.

IMPURITIES



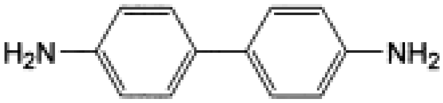
A. (2*RS*)-2-[(1,2-diphenyldiazanyl)carbonyl]hexanoic acid,



B. 4-butyl-4-hydroxy-1,2-diphenylpyrazolidine-3,5-dione,

C. C_6H_5-NH-NH-C_6H_5: 1,2-diphenyldiazane, (1,2-diphenylhydrazine),

D. C_6H_5-N=N-C_6H_5: 1,2-diphenyldiazene,



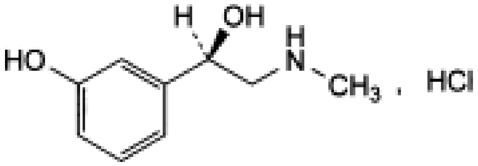
E. biphenyl-4,4′-diamine (benzidine).

Ph Eur

Phenylephrine Hydrochloride

General Notices

(Ph Eur monograph 0632)



$C_9H_{13}NO_2,HCl$ 203.7   *61-76-7*

Action and use

Alpha-adrenoceptor agonist.

Preparations

Phenylephrine Eye Drops

Phenylephrine Injection

Ph Eur

DEFINITION

(1*R*)-1-(3-Hydroxyphenyl)-2-(methylamino)ethanol hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in ethanol (96 per cent).

mp

About 143 °C.

IDENTIFICATION

First identification A, C, E.

Second identification A, B, D, E.

A. Specific optical rotation (see Tests).

B. Melting point (2.2.14): 171 °C to 176 °C.

Dissolve 0.3 g in 3 ml of *water R*, add 1 ml of *dilute ammonia R1* and initiate crystallisation by scratching the wall of the tube with a glass rod. Wash the crystals with iced *water R* and dry at 105 °C for 2 h.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison phenylephrine hydrochloride CRS.

D. Dissolve about 10 mg in 1 ml of *water R* and add 0.05 ml of a 125 g/l solution of *copper sulphate R* and 1 ml of a 200 g/l solution of *sodium hydroxide R*. A violet colour is produced. Add 1 ml of *ether R* and shake; the upper layer remains colourless.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Specific optical rotation (*2.2.7*)

- 43 to - 47 (dried substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (20:80 *V/V*).

Buffer solution pH 2.8 Dissolve 3.25 g of *sodium octanesulphonate R* in 1000 ml of *water R* by stirring for 30 min and adjust to pH 2.8 with *dilute phosphoric acid R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 100.0 ml with the solvent mixture.

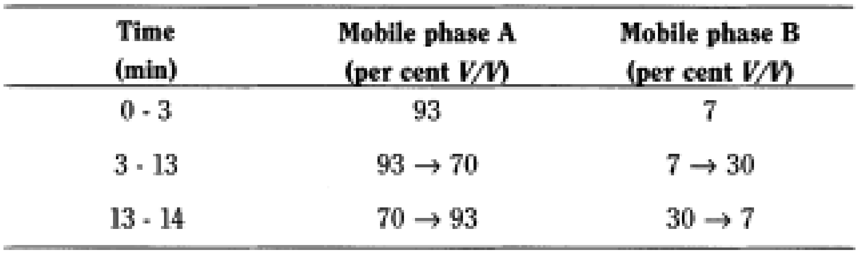
Reference solution (b) Dissolve the contents of a vial of *phenylephrine hydrochloride for peak identification CRS* (containing impurities C and E) in 2.0 ml of the solvent mixture.

Column:

- *size: l* = 0.055 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 45 °C.

Mobile phase:

- *mobile phase A*: *acetonitrile R1*, buffer solution pH 2.8 (10:90 *V/V*);
- *mobile phase B*: buffer solution pH 2.8, *acetonitrile R1* (10:90 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	93	7
3 - 13	93 → 70	7 → 30
13 - 14	70 → 93	30 → 7

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 10 µl.

Relative retention With reference to phenylephrine (retention time = about 2.8 min): impurity C = about 1.3; impurity E = about 3.6.

System suitability:

- *symmetry factor*: maximum 1.9 for the principal peak in the chromatogram obtained with the test solution;
- *peak-to-valley ratio*: minimum 5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to phenylephrine in the chromatogram obtained with reference solution (b).

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.5; impurity E = 0.5;
- *impurities C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphates (*2.4.13*)

Maximum 500 ppm, determined on solution S.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

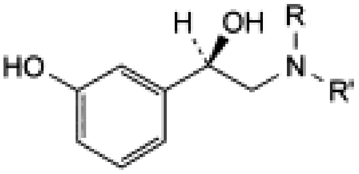
Dissolve 0.150 g in a mixture of 0.5 ml of *0.1 M hydrochloric acid* and 80 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*) using *0.1 M ethanolic sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 20.37 mg of $C_9H_{14}ClNO_2$.

IMPURITIES

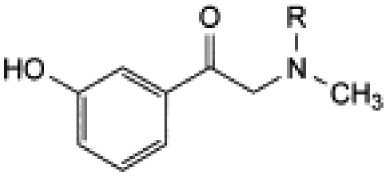
Specified impurities *C, E.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, D.*



A. R = R′ = H: (1*R*)-2-amino-1-(3-hydroxyphenyl)ethanol (norphenylephrine),

D. R = CH_2-C_6H_5, R′ = CH_3: (1*R*)-2-(benzylmethylamino)-1-(3-hydroxyphenyl)ethanol (benzylphenylephrine),



C. R = H: 1-(3-hydroxyphenyl)-2-(methylamino)ethanone (phenylephrone),

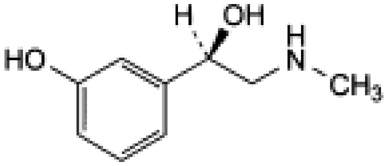
E. R = CH_2-C_6H_5: 2-(benzylmethylamino)-1-(3-hydroxyphenyl)ethanone (benzylphenylephrone).

Ph Eur

Phenylephrine

General Notices

(Ph Eur monograph 1035)



$C_9H_{13}NO_2$ 167.2 *59-42-7*

Action and use

Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION

(1*R*)-1-(3-Hydroxyphenyl)-2-(methylamino)ethanol.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent) . It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

mp

About 174 °C.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *phenylephrine CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Solvent mixture A mixture of equal volumes of *methylene chloride R* and methanolic hydrochloric acid (*hydrochloric acid R* diluted to 10 volumes with *methanol R*).

Test solution Dissolve 0.1 g of the substance to be examined in the solvent mixture and dilute to 5 ml with the solvent mixture.

Reference solution Dissolve 20 mg of *phenylephrine CRS* in the solvent mixture and dilute to 1 ml with the solvent mixture.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R*, *methanol R*, *methylene chloride R* (0.5:25:70 *V/V/V*) .

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of cold air.

Detection Examine in ultraviolet light at 254 nm; spray with a 1 g/l solution of *fast red B salt R* in a 50 g/l solution of *sodium carbonate R* and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 1 ml of *1 M hydrochloric acid*, add 0.05 ml of *copper sulphate solution R* and 1 ml of a 200 g/l solution of *sodium hydroxide R*. A violet colour develops. Add 1 ml of *ether R* and shake. The upper layer remains colourless.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Dissolve 1 g in *1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Specific optical rotation *(2.2.7)*

- 53 to - 57 (dried substance).

Dissolve 1.250 g in *1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *dilute hydrochloric acid R*, mobile phase B, mobile phase A (5:200:800 *V/V/V*).

Buffer solution pH 2.8 Dissolve 3.25 g of *sodium octanesulphonate R* in 1000 ml of *water R* by stirring for 30 min and adjust to pH 2.8 with *dilute phosphoric acid R*.

Test solution Dissolve 41.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *phenylephrine hydrochloride for peak identification CRS* (containing impurities C and E) in 2.0 ml of the solvent mixture.

Column:
— *size*: *l* = 0.055 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R1*, buffer solution pH 2.8 (10:90 *V/V*);
— *mobile phase B*: buffer solution pH 2.8, *acetonitrile R1* (10:90 *V/V*);

Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 3	93	7
3 - 13	93 → 70	7 → 30
13 - 14	70 → 93	30 → 7

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 10 µl.

Relative retention With reference to phenylephrine (retention time = about 2.8 min): impurity C = about 1.3; impurity E = about 3.6.

System suitability:
— *symmetry factor*: maximum 1.9 for the principal peak in the chromatogram obtained with the test solution;
— *peak-to-valley ratio*: minimum 5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to phenylephrine in the chromatogram obtained with reference solution (b).

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.5; impurity E = 0.5;
— *impurities C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 16.72 mg of $C_9H_{13}NO_2$.

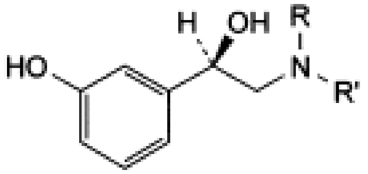
STORAGE

In an airtight container, protected from light.

IMPURITIES

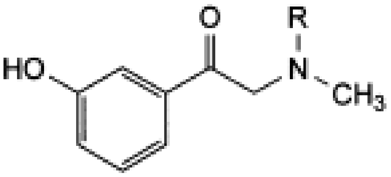
Specified impurities *C, E.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, D.*



A. R = R′ = H: (1*R*)-2-amino-1-(3-hydroxyphenyl)ethanol (norphenylephrine),

D. R = CH_2-C_6H_5, R′ = CH_3: (1*R*)-2-(benzylmethylamino)-1-(3-hydroxyphenyl)ethanol (benzylphenylephrine),



C. R = H: 1-(3-hydroxyphenyl)-2-(methylamino)ethanone (phenylephrone),

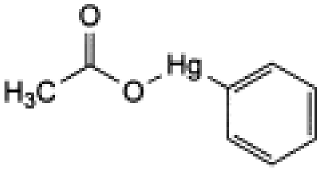
E. R = CH_2-C_6H_5: 2-(benzylmethylamino)-1-(3-hydroxyphenyl)ethanone (benzylphenylephrone).

Ph Eur

Phenylmercuric Acetate

General Notices

(Ph Eur monograph 2042)



$C_8H_8HgO_2$ 336.7 *62-38-4*

Action and use

Antiseptic, antimicrobial preservative.

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder or small, colourless crystals.

Solubility

Slightly soluble in water, soluble in acetone and in alcohol.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of phenylmercuric acetate.

B. To 5 ml of solution S (see Tests) add 5 ml of *water R* and 0.1 ml of *sodium sulphide solution R*. A white precipitate is formed that darkens slowly on heating.

C. To 10 ml of solution S add 2 ml of *potassium iodide solution R* and shake vigorously.

Filter. The filtrate gives reaction (b) of acetates (*2.3.1*).

TESTS

Solution S

Dissolve 0.250 g in 40 ml of *water R* by heating to boiling. Allow to cool and dilute to 50 ml with *water R*. *Prepare the solution immediately before use*.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

Ionised mercury

Maximum 0.2 per cent.

To 2 ml of solution S add 8 ml of *water R*, 2 ml of *potassium iodide solution R* and 3 ml of *dilute hydrochloric acid R*. Filter. The filtrate is not more coloured than the potassium iodide solution used. Wash the precipitate with 3 ml of *water R*. Combine the filtrate and the washings, add 2 ml of *dilute sodium hydroxide solution R* and dilute to 20 ml with *water R*. 12 ml of this solution complies with limit test A for heavy metals (*2.4.8*). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Polymercuric benzene compounds

Maximum 1.5 per cent.

Shake 0.2 g with 10 ml of *acetone R*. Filter. Wash the residue twice with 5 ml of *acetone R*. Dry the residue at 105 °C for 1 h. The residue weighs a maximum of 3 mg.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 45 °C for 15 h.

ASSAY

Dissolve with heating 0.300 g in 100 ml of *water R*. Cool and add 3 ml of *nitric acid R*. Titrate with *0.1 M ammonium thiocyanate* using 2 ml of *ferric ammonium sulphate solution R2* as indicator, until a persistent reddish-yellow colour is obtained.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 33.67 mg of phenylmercuric acetate.

STORAGE

Protected from light.

Ph Eur

Phenylmercuric Borate

General Notices

(Ph Eur monograph 0103)

8017-88-7

Action and use

Antiseptic; antimicrobial preservative.

Ph Eur

DEFINITION

Compound consisting of equimolecular proportions of phenylmercuric orthoborate and phenylmercuric hydroxide ($C_{12}H_{13}BHg_2O_4$; M_r 633) or of the dehydrated form (metaborate, $C_{12}H_{11}BHg_2O_3$; M_r 615) or of a mixture of the 2 compounds.

Content:

— *mercury* (Hg; A_r 200.6): 64.5 per cent to 66.0 per cent (dried substance);

— *borates expressed as* H_3BO_3: 9.8 per cent to 10.3 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder or colourless, shiny crystals.

Solubility

Slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *Ph. Eur. reference spectrum of phenylmercuric borate.*

B. To 2 ml of solution S (see Tests) add 8 ml of *water R* and 0.1 ml of *sodium sulphide solution R*. A white precipitate is formed that darkens slowly on heating.

C. Dissolve about 20 mg in 2 ml of *methanol R*. The solution is clear and colourless. Ignite; the solution burns with a green-edged flame.

TESTS

Solution S

Dissolve 0.25 g by sprinkling it on the surface of 25 ml of boiling *water R*, cool and dilute to 25 ml with *water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Ionised mercury

Maximum 0.01 per cent.

To 10 ml of solution S add 2 ml of *potassium iodide solution R* and 3 ml of *dilute hydrochloric acid R*. Filter. The filtrate is colourless. Wash the precipitate with 3 ml of *water R*. Combine the filtrate and the washings, add 2 ml of *dilute sodium hydroxide solution R* and dilute to 20 ml with *water R*. 12 ml of this solution complies with test A for heavy metals (*2.4.8*). Prepare the reference solution using a mixture of 2.5 ml of *lead standard solution (2 ppm Pb) R* and 7.5 ml of *water R*.

Loss on drying (*2.2.32*)

Maximum 3.5 per cent, determined on 0.50 g by drying in an oven at 45 °C for 15 h ± 30 min.

ASSAY

Mercury

Dissolve 0.300 g in 100 ml of *water R* and add 3 ml of *nitric acid R*. Titrate with *0.1 M ammonium thiocyanate*, using 2 ml of *ferric ammonium sulphate solution R2* as indicator, until a persistent reddish-yellow colour is obtained.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 20.06 mg of Hg.

Borates

Dissolve 0.600 g with heating in 25 ml of *water R*. Dissolve 10 g of *sorbitol R* in the hot solution and cool. Titrate with *0.1 M sodium hydroxide*, using 0.5 ml of *phenolphthalein solution R* as indicator, until a persistent pink colour is obtained. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 6.18 mg of H_3BO_3.

STORAGE

Protected from light.

Ph Eur

Phenylmercuric Nitrate

General Notices

(Ph Eur monograph 0783)

55-68-5

Action and use

Antiseptic; antimicrobial preservative.

Ph Eur

DEFINITION

Mixture of phenylmercuric nitrate ($C_6H_5HgNO_3$; *M*$_r$ 339.7) and phenylmercuric hydroxide (C_6H_5HgOH; *M*$_r$ 294.7).

Content

62.5 per cent to 64.0 per cent of Hg (*A*$_r$ 200.6) (dried substance).

CHARACTERS

Appearance

White or pale yellow powder.

Solubility

Very slightly soluble in water and in ethanol (96 per cent), slightly soluble in hot water. It dissolves in glycerol and in fatty oils.

IDENTIFICATION

A. To 5 ml of solution S (see Tests) add 8 ml of *water R* and 0.1 ml of *sodium sulphide solution R*. A white precipitate is formed that darkens slowly on heating.

B. To 1 ml of a saturated solution of the substance to be examined add 1 ml of *dilute hydrochloric acid R*. A white, flocculent precipitate is formed.

C. To 5 ml of solution S add 1 ml of *dilute hydrochloric acid R*, 2 ml of *methylene chloride R* and 0.2 ml of *dithizone solution R*. Shake. The lower layer is orange-yellow.

D. About 10 mg gives the reaction of nitrates (*2.3.1*).

TESTS

Solution S

To 0.1 g add 45 ml of *water R* and heat to boiling with shaking. Cool, filter and dilute to 50 ml with *water R*.

Appearance of solution

Solution S is colourless (*2.2.2, Method II*).

Inorganic mercuric compounds

Maximum 0.1 per cent.

To 10 ml of solution S add 2 ml of *potassium iodide solution R* and 3 ml of *dilute hydrochloric acid R*. Filter. The filtrate is colourless. Wash the precipitate with 2 ml of *water R*. Combine the filtrate and washings, add 2 ml of *dilute sodium hydroxide solution R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A for heavy metals (*2.4.8*). Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* for 24 h.

ASSAY

Dissolve 0.150 g in a mixture of 10 ml of *dilute nitric acid R* and 90 ml of *water R*, heating to boiling. Cool to 15-20 °C. Titrate with *0.1 M ammonium thiocyanate* using 2 ml of ferric *ammonium sulphate solution R2* as indicator, until a persistent reddish-yellow colour is obtained. Carry out a blank titration.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 20.06 mg of Hg.

STORAGE

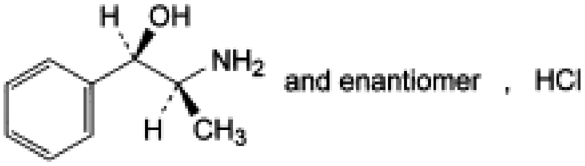
Protected from light.

Ph Eur

Phenylpropanolamine Hydrochloride

General Notices

(Ph Eur monograph 0683)



$C_9H_{13}NO,HCl$ 187.7 *154-41-6*

Action and use

Adrenoceptor agonist.

Ph Eur

DEFINITION

Phenylpropanolamine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.5 per cent of (1*RS*,2*SR*)-2-amino-1-phenylpropan-1-ol hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water and in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification B, E.

Second identification A, C, D, E.

A. Melting point (*2.2.14*): 194 °C to 197 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *phenylpropanolamine hydrochloride CRS*. Examine the substances prepared as discs without recrystallisation.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 50 mg in 5 ml of *water R*, add 0.2 ml of *copper sulphate solution R* and 0.3 ml of *dilute sodium hydroxide solution R*. A violet colour develops. Add 2 ml of *ether R* and shake. A violet precipitate is formed between the two layers.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.25 g in *water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is red.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel H R* as the coating substance.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *alcohol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *alcohol R*.

Reference solution (a) Dissolve 20 mg of *phenylpropanolamine hydrochloride CRS* in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with *alcohol R*.

Reference solution (c) Dissolve 20 mg of *norpseudoephedrine hydrochloride CRS* in *alcohol R*, add 1 ml of test solution (a) and dilute to 10 ml with *alcohol R*.

Reference solution (d) Dissolve 60 mg of *ammonium chloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Before applying the solutions, spray the plate with a 20 g/l solution of *disodium tetraborate R*, using 8 ml for a plate 100 mm by 200 mm and dry in a stream of cold air for 30 min. Apply separately to the plate as bands about 10 mm by 3 mm 10 µl of each solution. Develop over a path of 10 cm using a mixture of 6 volumes of *concentrated ammonia R*, 24 volumes of *alcohol R* and 70 volumes of *butanol R*. Dry the plate in a current of warm air until the solvents have evaporated, allow to cool, spray with a 2 g/l solution of *ninhydrin R* in *alcohol R* and heat at 110 °C for 15 min. Any spot in the chromatogram obtained with test solution (a) apart from the principal spot and the spot corresponding to ammonium chloride is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Phenylpropanonamine

Dissolve 1.0 g in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid. The absorbance (*2.2.25*) of the solution measured at 283 nm is not greater than 0.10.

Heavy metals (*2.4.8*)

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard

using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.1500 g in a mixture of 5 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

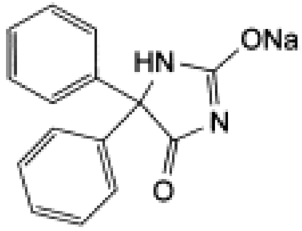
1 ml of *0.1 M sodium hydroxide* is equivalent to 18.77 mg of $C_9H_{14}ClNO$.

Ph Eur

Phenytoin Sodium

General Notices

(Ph Eur monograph 0521)



$C_{15}H_{11}N_2NaO_2$ 274.3 *630-93-3*

Action and use

Antiepileptic.

Preparations

Phenytoin Capsules

Phenytoin Injection

Phenytoin Tablets

Ph Eur

DEFINITION

Phenytoin sodium contains not less than 98.5 per cent and not more than the equivalent of 100.5 per cent of sodium 4-oxo-5,5-diphenyl-4,5-dihydro-1*H*-imidazol-2-olate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, slightly hygroscopic, soluble in water and in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Dissolve 0.1 g in 20 ml of *water R*. Acidify with *dilute hydrochloric acid R* and shake with three quantities, each of 30 ml, of *chloroform R*. Wash the combined chloroform layers with *water R*, evaporate to dryness and dry the residue at 100 °C to 105 °C (test residue).

Repeat the operations using 0.1 g of *phenytoin sodium CRS* (reference residue). Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *phenytoin sodium CRS*. Examine as discs prepared using *potassium bromide R*.

B. To about 10 mg add 1 ml of *water R* and 0.05 ml of *ammonia R*. Heat until boiling begins. Add 0.05 ml of a 50 g/l solution of *copper sulphate R* in *dilute ammonia R2* and shake. A pink, crystalline precipitate is formed.

C. Ignite 1 g and cool. Add 2 ml of *water R* to the residue and neutralise the solution with *hydrochloric acid R*. Filter and dilute the filtrate to 4 ml with *water R*. 0.1 ml of the solution gives reaction (b) of sodium (*2.3.1*).

TESTS

Appearance of solution

Suspend 1.0 g in 5 ml of *water R* and dilute to 20 ml with *0.1 M sodium hydroxide.* The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel containing a fluorescent indicator having an optimal intensity at 254 nm.

Test solution Dissolve 0.4 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *methanol R*.

Reference solution (b) Dissolve 20 mg of *benzophenone R* in *methanol R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution and dry the plate in a stream of cold air for 2 min. Develop over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R*, 45 volumes of *chloroform R* and 45 volumes of *2-propanol R*. Dry the plate in an oven at 80 °C for 5 min and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spot corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and any spot, apart from the principal spot and the spot corresponding to benzophenone, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent).

Free phenytoin

Dissolve 0.30 g in 10 ml of a mixture of equal volumes of *pyridine R* and *water R*. Add 0.5 ml of *phenolphthalein solution R* and 3 ml of silver nitrate solution in *pyridine R*. Not more than 1.0 ml of 0.1 M sodium hydroxide is required to change the colour of the indicator to pink.

Heavy metals (*2.4.8*)

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*)

Not more than 3.0 per cent, determined on 1.000 g by the semi-micro determination of water.

ASSAY

Suspend 0.180 g in 2 ml of *water R*. Add 8.0 ml of *0.05 M sulphuric acid* and heat gently for 1 min. Add 30 ml of *methanol R* and cool. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. After reaching the first point of inflexion, interrupt the addition of *0.1 M sodium hydroxide*, add 5 ml of silver nitrate solution in *pyridine R*, mix and continue the titration. Read the volume of *0.1 M sodium hydroxide* added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 27.43 mg of $C_{15}H_{11}N_2NaO_2$.

STORAGE

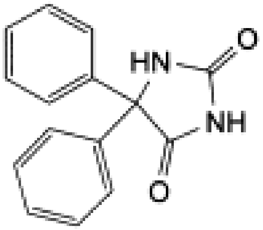
Store in an airtight container .

Ph Eur

Phenytoin

General Notices

(Ph Eur monograph 1253)



$C_{15}H_{12}N_2O_2$ 252.3   *57-41-0*

Action and use

Antiepileptic.

Preparation

Phenytoin Oral Suspension

Ph Eur

DEFINITION

Phenytoin contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 5,5-diphenylimidazolidine-2,4-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in alcohol, very slightly soluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification   A.

Second identification   B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *phenytoin CRS*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To about 10 mg add 1 ml of *water R* and 0.05 ml of *ammonia R*. Heat until boiling begins. Add 0.05 ml of a 50 g/l solution of *copper sulphate R* in *dilute ammonia R2* and shake. A pink, crystalline precipitate is formed.

D. It complies with the test for sulphated ash (see Tests).

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 5 ml of *1 M sodium hydroxide* and 20 ml of *water R*. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity or alkalinity

To 1.0 g add 45 ml of *water R* and boil for 2 min. Allow to cool and filter. Wash the filter with *carbon dioxide-free water R* and dilute the combined filtrate and washings to 50 ml with the same solvent. To 10 ml of the solution add 0.15 ml of *methyl red solution R*. Not more than 0.5 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red. To 10 ml of the solution add 0.15 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm. Before use, wash the plate with a mixture of 30 volumes of *dioxan R* and 75 volumes of *hexane R*. Allow the plate to dry in air.

Test solution (a) Dissolve 0.40 g of the substance to be examined in a mixture of equal volumes of *acetone R* and *methanol R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 20 ml with a mixture of equal volumes of *acetone R* and *methanol R.*

Reference solution (a) Dissolve 20 mg of *phenytoin CRS* in a mixture of equal volumes of *acetone R* and *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 8 mg of *benzophenone R* in a mixture of equal volumes of *acetone R* and *methanol R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (c) Dissolve 8 mg of *benzil R* in a mixture of equal volumes of *acetone R* and *methanol R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (d) Dilute 1 ml of test solution (a) to 100 ml with a mixture of equal volumes of *acetone R* and *methanol R*.

Reference solution (e) Mix 1 ml of reference solution (b) and 1 ml of reference solution (c).

Apply separately to the plate 10 µl of each solution and dry the plate in a stream of cold air for 2 min. Develop over a path of 15 cm using a mixture of 30 volumes of *dioxan R* and 75 volumes of *hexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a): any spot corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent); any spot corresponding to benzil is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent) and any spot, apart from the principal spot and any spot corresponding to benzo-phenone and benzil, is not more intense than the spot in the chromatogram obtained with reference solution (d) (1 per cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows two clearly separated principal spots.

Heavy metals (*2.4.8*)

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying(*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

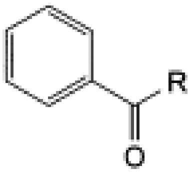
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *dimethylformamide R*. Titrate with *0.1 M sodium methoxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium methoxide* is equivalent to 25.23 mg of $C_{15}H_{12}N_2O_2$.

IMPURITIES



A. R = C_6H_5: diphenylmethanone (benzophenone),

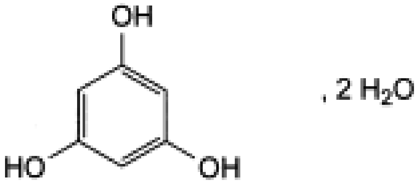
B. R = CO-C_6H_5: diphenylethanedione (benzil).

Ph Eur

Phloroglucinol Dihydrate

General Notices

(Ph Eur monograph 2302)



$C_6H_6O_3,2H_2O$ 162.1 *6099-90-7*

Action and use

Antispasmodic.

Ph Eur

DEFINITION

Benzene-1,3,5-triol dihydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Sparingly soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Previously dry the substance to be examined in an oven at 105 °C.

Comparison *anhydrous phloroglucinol CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10

ml with the same solvent.

Reference solution Dissolve 0.20 g of *anhydrous phloroglucinol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous formic acid R*, *hexane R*, *ethyl acetate R* (2:37.5:62.5 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2*, *Method II*).

pH (*2.2.3*)

4.0 to 6.0.

Dilute 10 ml of solution S to 100 ml with *carbon dioxide-free water R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 3 mg of *resorcinol R* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *nitrile silica gel for chromatography R* (5 µm).

Mobile phase Mix 3 volumes of *acetonitrile R* and 97 volumes of a 1.36 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 265 nm.

Injection 10 µl.

Run time 5 times the retention time of phloroglucinol.

Retention time Phloroglucinol = about 5 min.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to phloroglucinol and resorcinol.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 500 ppm.

Dilute 3 ml of solution S to 15 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

20.0 per cent to 23.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.600 g in 50 ml of *water R.* Titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *1 M sodium hydroxide* is equivalent to 63.05 mg of $C_6H_6O_3$.

STORAGE

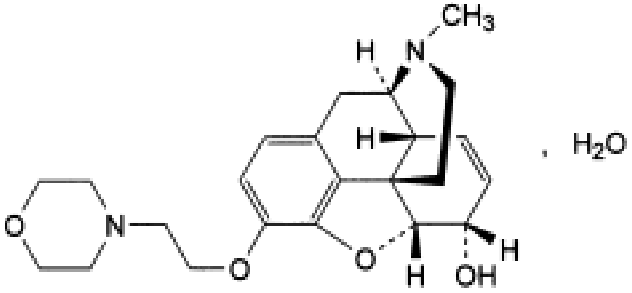
Protected from light.

Ph Eur

Pholcodine

General Notices

(Ph Eur monograph 0522)



$C_{23}H_{30}N_2O_4,H_2O$ 416.5 *509-67-1*

Action and use

Opioid receptor agonist; cough suppressant.

Preparations

Pholcodine Linctus

Strong Pholcodine Linctus

Ph Eur

DEFINITION

7,8-Didehydro-4,5α-epoxy-17-methyl-3-[2-(morpholin-4-yl)ethoxy]morphinan-6α-ol monohydrate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Sparingly soluble in water, freely soluble in acetone and in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pholcodine CRS.*

TESTS

Specific optical rotation (*2.2.7*)

- 94 to - 98 (dried substance).

Dissolve 1.000 g in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

0.02 M phosphate buffer solution

To 80.0 ml of *0.2 M sodium hydroxide* add 100.0 ml of *0.2 M potassium dihydrogen phosphate R* and dilute to 1000.0 ml with *water R*.

Solvent mixture Dilute 80 ml of *acetonitrile R* to 1000 ml with the *0.02 M phosphate buffer solution*.

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 50 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *codeine R* (impurity B) in the solvent mixture and dilute to 10 ml with the solvent mixture. To 0.5 ml of this solution add 0.5 ml of the test solution and dilute to 50 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *pholcodine for peak identification CRS* (containing impurities A, B and D) in the solvent mixture and dilute to 5 ml with the solvent mixture.

Column:

— *size:* *l* = 0.075 m, Ø = 4.6 mm;

— *stationary phase*: *spherical end-capped phenylhexylsilyl silica gel for chromatography R* (3 µm) with a specific surface area of 400 m2/g and a pore size of 10 nm;

— *temperature*: 35 °C.

Mobile phase To 50 ml of *tetrahydrofuran for chromatography R* add 75 ml of *acetonitrile R* and dilute to 1000 ml with the *0.02 M phosphate buffer solution*; adjust to pH 7.9 ± 0.05 with *0.2 M sodium hydroxide*; the pH must not exceed 8.0.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 238 nm.

Injection 20 µl.

Run time 5 times the retention time of pholcodine.

Identification of impurities Use the chromatogram supplied with *pholcodine for peak* *identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and D.

Relative retention With reference to pholcodine (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.8; impurity D = about 2.3.

System suitability Reference solution (a):

— *resolution*: minimum 3 between the peaks due to impurity B and pholcodine.

Limits:

— *impurities A, B, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

3.9 per cent to 4.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.180 g in 50 ml of *anhydrous acetic acid R*, warming gently. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*) at the 2nd point of inflexion.

1 ml of *0.1 M perchloric acid* is equivalent to 19.93 mg of $C_{23}H_{30}N_2O_4$.

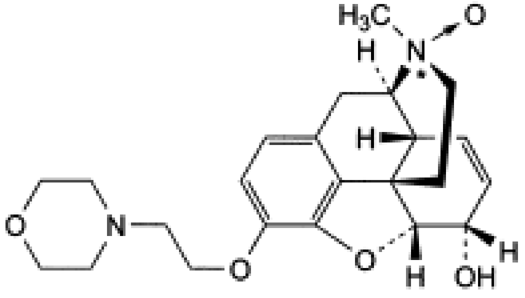
IMPURITIES

Specified impurities A, B, D.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C, E, F.*

A. morphine,

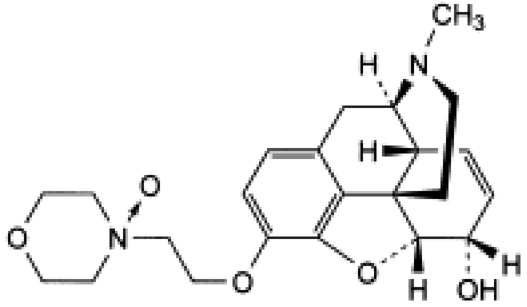
B. codeine,



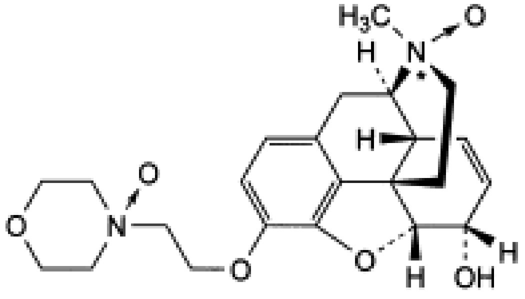
and epimer at N*

C. (17*RS*)-7,8-didehydro-4,5α-epoxy-17-methyl-3-[2-(morpholin-4-yl)ethoxy]morphinan-6α-ol 17-oxide (pholcodine *N*-oxide),

D. unknown structure,



E. 7,8-didehydro-4,5α-epoxy-17-methyl-3-[2-(4-oxidomorpholin-4-io)ethoxy]morphinan-6α-ol (pholcodine *N′*-oxide),



and epimer at N*

F. (17*RS*)-7,8-didehydro-4,5α-epoxy-17-methyl-3-[2-(4-oxidomorpholin-4-io)ethoxy] morphinan-6α-ol 17-oxide (pholcodine *N*,*N′*-dioxide).

Ph Eur

Phosphoric Acid

General Notices

(Concentrated Phosphoric Acid, Ph Eur monograph 0004)

H_3PO_4 98.0 *7664-38-2*

Ph Eur

DEFINITION

Content

84.0 per cent *m/m* to 90.0 per cent *m/m*.

CHARACTERS

Appearance

Clear, colourless, syrupy liquid, corrosive.

Solubility

Miscible with water and with ethanol (96 per cent).

When stored at a low temperature it may solidify into a mass of colourless crystals which do not melt at a temperature below 28 °C.

Relative density

About 1.7.

IDENTIFICATION

A. Dilute with *water R*. The solution is strongly acid (*2.2.4*).

B. Solution S (see Tests) neutralised with *dilute sodium hydroxide solution R* gives the reactions of phosphates (*2.3.1*).

TESTS

Solution S

Dilute 10.0 g to 150 ml with *water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Substances precipitated with ammonia

To 10 ml of solution S add 8 ml of *dilute ammonia R1*. Any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution S and 8 ml of *water R*.

Hypophosphorous acid and phosphorous acid

To 5 ml of solution S add 2 ml of *silver nitrate solution R2* and heat on a water-bath for 5 min. The solution shows no change in appearance.

Chlorides (*2.4.4*)

Maximum 50 ppm, determined on solution S.

Sulphates (*2.4.13*)

Maximum 100 ppm.

Dilute 1.5 g to 15 ml with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 7.5 ml of solution S.

Iron (*2.4.9*)

Maximum 50 ppm.

Dilute 3 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

To 2.5 g add 4 ml of *dilute ammonia R1* and dilute to 25 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb*) *R.*

ASSAY

To 1.000 g add a solution of 10 g of *sodium chloride R* in 30 ml of *water R*. Titrate with *1 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 49.00 mg of H_3PO_4.

STORAGE

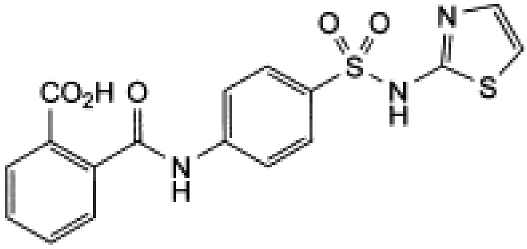
In a glass container.

Ph Eur

Phthalylsulfathiazole

General Notices

(Ph Eur monograph 0352)



$C_{17}H_{13}N_3O_5S_2$ 403.4 *85-73-4*

Action and use

Antibacterial.

Ph Eur

DEFINITION

2-[[4-(Thiazol-2-ylsulphamoyl)phenyl]carbamoyl]benzoic acid.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide, slightly soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B, E.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison phthalylsulfathiazole CRS.

B. To 1 g add 8.5 ml of *dilute sodium hydroxide solution R* and boil under a reflux condenser for 30 min. Cool and add 17.5 ml of *dilute hydrochloric acid R*. Shake vigorously and filter. Neutralise the filtrate with *dilute sodium hydroxide solution R*. Filter, wash the precipitate with *water R*, recrystallise from *water R* and dry the crystals at 100-105 °C. The crystals melt (*2.2.14*) at 200 °C to 203 °C.

C. To 0.1 g in a test-tube add 3 ml of *dilute sulphuric acid R* and 0.5 g of *zinc powder R*. Fumes are evolved which produce a black stain on *lead acetate paper R*.

D. To 0.1 g add 0.5 g of *resorcinol R* and 0.3 ml of *sulphuric acid R* and heat on a water-bath until a homogeneous mixture is obtained. Allow to cool. Add 5 ml of dilute *sodium hydroxide solution R*. Dilute 0.1 ml of this brownish-red mixture to 25 ml with *water R*. An intense green fluorescence appears which disappears on acidification.

E. Dissolve about 10 mg of the crystals obtained in identification test B in 200 ml of *0.1 M hydrochloric acid*. 2 ml of the solution gives the reaction of primary aromatic amines (*2.3.1*) with formation of an orange precipitate.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *1 M sodium hydroxide* and dilute to 20 ml with the same solvent.

Acidity

To 2.0 g add 20 ml of *water R*, shake continuously for 30 min and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Sulfathiazole and other primary aromatic amines: maximum 2.0 per cent.

Dissolve 5 mg in a mixture of 3.5 ml of *water R*, 6 ml of *dilute hydrochloric acid R* and 25 ml of *ethanol (96 per cent) R*, previously cooled to 15 °C. Place immediately in iced water and add 1 ml of a 2.5 g/l solution of *sodium nitrite R*. Allow to stand for 3 min, add 2.5 ml of a 40 g/l solution of *sulphamic acid R* and allow to stand for 5 min. Add 1 ml of a 4 g/l solution of *naphthylethylenediamine dihydrochloride R* and dilute to 50 ml with *water R*. Measured at 550 nm, the absorbance (*2.2.25*) is not greater than that of a standard prepared at the same time and in the same manner using a mixture of 1 ml of a 100 ml aqueous solution containing 10 mg of *sulfathiazole R* and 0.5 ml of *hydrochloric acid R;* 2.5 ml of *water R*; 6 ml of *dilute hydrochloric acid R*; and 25 ml of ethanol (96 per cent) R.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 2 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 40 ml of *dimethylformamide R*. Titrate with *0.1 M sodium hydroxide* until the colour becomes blue. using 0.2 ml of *thymolphthalein solution R* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 20.17 mg of $C_{17}H_{13}N_3O_5S_2$.

STORAGE

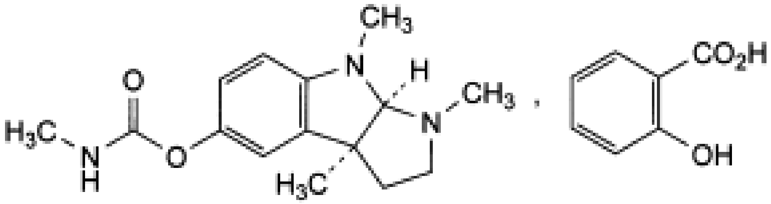
Protected from light.

Ph Eur

Physostigmine Salicylate

General Notices

(Ph Eur monograph 0286)



$C_{15}H_{21}N_3O_2,C_7H_6O_3$ 413.5 *57-64-7*

Action and use

Cholinesterase inhibitor.

Ph Eur

DEFINITION

Physostigmine salicylate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (3a*S*,8a*R*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-*b*]indol-5-yl methylcarbamate salicylate, calculated with reference to the dried substance.

CHARACTERS

Colourless or almost colourless crystals, sparingly soluble in water, soluble in alcohol. The crystals gradually become red when exposed to air and light; the colour develops more quickly when the crystals are also exposed to moisture. Aqueous solutions are unstable.

It melts at about 182 °C, with decomposition.

IDENTIFICATION

First identification A, B.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *physostigmine salicylate CRS*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Heat about 10 mg in a porcelain dish with a few drops of dilute ammonia R1. An orange colour develops. Evaporate the solution to dryness. The residue dissolves in *alcohol R* giving a blue solution. Add 0.1 ml of *glacial acetic acid R*. The colour becomes violet. Dilute with *water R.* An intense red fluorescence appears.

D. Solution S (see Tests) gives reaction (a) of salicylates (*2.3.1*).

TESTS

Solution S

Dissolve 0.900 g, without heating, in 95 ml of *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent. Prepare immediately before use.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 5.1 to 5.9.

Specific optical rotation (*2.2.7*)

- 90 to - 94, determined on solution S and calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g of the substance to be examined in *alcohol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 2.5 ml of test solution (a) to 50 ml with *alcohol R*.

Reference solution (a) Dissolve 10 mg of *physostigmine salicylate CRS* in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 2 ml of reference solution (a) to 20 ml with *alcohol R*.

Apply to the plate 20 µl of each solution. Develop over a path of 15 cm using a mixture of 2 volumes of *concentrated ammonia R*, 23 volumes of *2-propanol R* and 100 volumes of *cyclohexane R*. Dry the plate in a current of cold air and carry out a second development in the same direction. Allow the plate to dry in air and spray with freshly prepared potassium *iodobismuthate solution R* and then with *dilute hydrogen peroxide solution R.* Examine the plate within 2 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Eseridine

To 5 ml of solution S add a few crystals of *potassium iodate R*, 0.05 ml of *dilute hydrochloric acid R* and 2 ml of *chloroform R.* Shake. No violet colour develops in the chloroform layer within 1 min.

Sulphates (*2.4.13*)

15 ml of solution S complies with the limit test for sulphates (0.1 per cent).

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 0.350 g in 50 ml of a mixture of equal volumes of *anhydrous acetic acid R* and *chloroform R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 41.35 mg of $C_{22}H_{27}N_3O_5$.

STORAGE

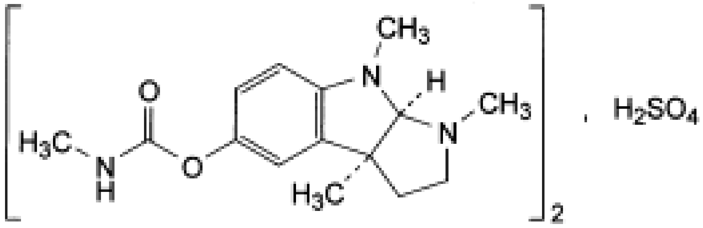
Store in an airtight container , protected from light.

Ph Eur

Physostigmine Sulphate

General Notices

(Ph Eur monograph 0684)



$(C_{15}H_{21}N_3O_2)_2,H_2SO_4$ 648.8 *64-47-1*

Action and use

Cholinesterase inhibitor.

Ph Eur

DEFINITION

Physostigmine sulphate contains not less than 97.0 per cent and not more than the equivalent of 101.0 per cent of di[(3a*S*,8a*R*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-*b*]indol-5-yl methylcarbamate] sulphate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, hygroscopic, very soluble in water, freely soluble in alcohol. It gradually becomes red when exposed to air and light; the colour develops more quickly when the substance is also exposed to moisture. Aqueous solutions are unstable.

It melts at about 145 °C, with decomposition.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *physostigmine sulphate CRS*. Examine the substances prepared as discs using *potassium bromide R*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Heat about 10 mg in a porcelain dish with 0.5 ml of *dilute ammonia R1*. An orange colour develops. Evaporate the solution to dryness. The residue dissolves in *alcohol R* giving a blue solution. Add 0.1 ml of *glacial acetic acid R*. The colour becomes violet. Dilute with water. An intense red fluorescence appears.

D. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

Solution S

Dissolve 0.500 g without heating in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent. Prepare immediately before use.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 3.5 to 5.5.

Specific optical rotation (*2.2.7*)

- 116 to - 120, determined on solution S and calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.15 g of the substance to be examined in *alcohol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *alcohol R*.

Reference solution (a) Dissolve 30 mg of *physostigmine sulphate CRS* in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 100 ml with *alcohol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 2 volumes of *concentrated ammonia R*, 23 volumes of *2-propanol R* and 100 volumes of *cyclohexane R*. Dry the plate in a current of cold air and carry out a second development in the same direction. Allow the plate to dry in air and spray with freshly prepared *potassium iodo-bismuthate solution R* and then with *dilute hydrogen peroxide solution R*. Examine the plate within 2 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Eseridine

To 5 ml of solution S add a few crystals of *potassium iodate R*, 0.05 ml of *dilute hydrochloric acid R* and 2 ml of *chloroform R* and shake. After 1 min, the chloroform layer is not more intensely coloured than a reference solution prepared at the same time in the same manner using 5 ml of *water R* instead of solution S.

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 0.5000 g in a mixture of 20 ml of *anhydrous acetic acid R* and 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)* at the first inflexion point.

1 ml of *0.1 M perchloric acid* is equivalent to 64.88 mg of $C_{30}H_{44}N_6O_8S$.

STORAGE

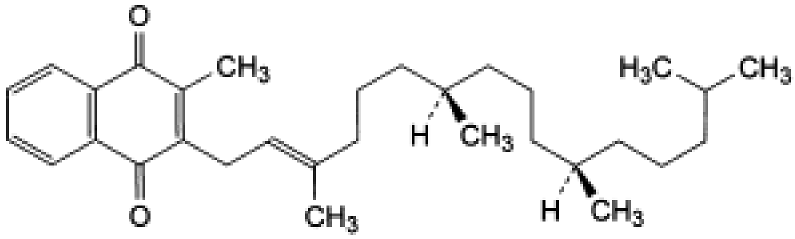
Store in a well-filled, airtight glass container, protected from light.

Ph Eur

Phytomenadione

General Notices

(Ph Eur monograph 1036)



$C_{31}H_{46}O_2$ 450.7 *84-80-0*

Action and use

Vitamin K analogue.

Preparations

Phytomenadione Injection

Phytomenadione Tablets

When vitamin K_1 is prescribed or demanded, Phytomenadione shall be dispensed or supplied.

Ph Eur

DEFINITION

Phytomenadione is a mixture of 2-methyl-3-[(2*E*)-(7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione (*trans*-phytomenadione), 2-methyl-3-[(2*Z*)-(7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione (*cis*-phytomenadione) and 2,3-epoxy-2-methyl-3-[(2*E*)-(7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enyl]-2,3-dihydronaphthalene-1,4-dione (*trans*-epoxyphytomenadione). It contains not more than 4.0 per cent of *trans*-epoxyphytomenadione and not less than 75.0 per cent of *trans*-phytomenadione. The total of the three components is not less than 97.0 per cent and not more than the equivalent of 103.0 per cent.

CHARACTERS

A clear, intense yellow, viscous, oily liquid, practically insoluble in water, sparingly soluble in ethanol (96 per cent), miscible with fatty oils.

It decomposes on exposure to actinic light.

The refractive index is about 1.526.

IDENTIFICATION

Carry out all operations as rapidly as possible avoiding exposure to actinic light.

A. Dissolve 10.0 mg in *trimethylpentane R* and dilute to 100.0 ml with the same solvent. Examined between 275 nm and 340 nm (*2.2.25*), the solution shows an absorption maximum at 327 nm and an absorption minimum at 285 nm. The specific absorbance at the maximum is 67 to 73. Dilute 10.0 ml of the solution to 50.0 ml with *trimethylpentane R*. Examined between 230 nm and 280 nm, the solution shows four absorbance maxima, at 243 nm, 249 nm, 261 nm and 270 nm respectively.

B. Examine the chromatograms obtained in the test for menadione and other related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 50 mg in 10 ml of *methanol R* and add 1 ml of a 200 g/l solution of *potassium hydroxide R* in *methanol R*. A green colour is produced which becomes violet-red on heating in a water-bath at 40 °C and then reddish-brown on standing.

TESTS

Appearance of solution

Dissolve 2.5 g in *trimethylpentane R* and dilute to 25 ml with the same solvent. The solution is clear (*2.2.1*).

Acid value (*2.5.1*)

Not more than 2.0, determined on 2.00 g.

Menadione and other related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution (a) Dissolve 0.40 g of the substance to be examined in *cyclohexane R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *cyclohexane R*.

Reference solution (a) Dissolve 40 mg of *phytomenadione CRS* in *cyclohexane R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 20 ml with *cyclohexane R*.

Reference solution (c) Dissolve 4.0 mg of *menadione R* in *cyclohexane R* and dilute to 50 ml with the same solvent.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *cyclohexane R* and 80 volumes of *toluene R*. Allow the plate to dry in air for 5 min. Examine in ultraviolet light at 254 nm and spray with a 100 g/l solution of *phosphomolybdic acid R* in *ethanol R*. Heat at 120 °C for 5 min. Examine in daylight. In the chromatogram obtained with test solution (a): any spot corresponding to menadione is not more intense that the spot in the chromatogram obtained with reference solution (c) (0.2 per cent); any spot, apart from the principal spot and any spot corresponding to menadione, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Disregard any spot below the principal spot, which may not be completely separated from the principal spot.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph Substances for pharmaceutical use (2034) do not apply.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Examine by liquid chromatography (*2.2.29*).

Test solution Dissolve 15.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 15.0 mg of *phytomenadione CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 15.0 mg of *phytomenadione CRS* and 4.0 mg of *trans-epoxyphytomenadione CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

- — a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with spherical *silica gel for chromatography R* (5 µm) with a porosity of 8 nm;
- — as mobile phase at a flow rate of 0.4 ml/min a mixture of 0.67 volumes of *octanol R,* 3.3 volumes of *di-isopropyl ether R* and 1000 volumes of *heptane R;*
- — as detector a spectrophotometer set at 254 nm;
- — a 20 µl loop injector.

Inject reference solution (b). When using a recorder, adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder. The test is not valid unless the order of elution of the peaks is: *trans*-epoxyphytomenadione, *cis*-phytomenadione and *trans*-phytomenadione. Carry out six replicate injections of reference solution (a). The assay is not valid unless the relative standard deviation of the peak area of the trans-isomer is less than 1.0 per cent and the resolution between the peaks corresponding to *trans*-phytomenadione and *cis*-phytomenadione is at least 2.5. Inject the test solution and reference solution (a) and calculate the percentage contents of *trans*-phytomenadione, *cis*-phytomenadione and *trans*-epoxyphytomenadione using the following expressions:

$$\textit{trans}\text{-phytomenadione} = \frac{m' \times A'_{trans} \times S_{trans}}{m \times S'_{trans}}$$

$$\textit{cis}\text{-phytomenadione} = \frac{m' \times A'_{cis} \times S_{cis}}{m \times S'_{cis}}$$

$$\textit{trans}\text{-epoxyphytomenadione} = \frac{m' \times A'_{epoxy} \times S_{epoxy}}{m \times S'_{epoxy}}$$

m' = mass of the reference substance in reference solution (a), in milligrams,

m = mass of the substance to be examined in the test solution, in milligrams,

A'_{trans} = percentage content of *trans*-phytomenadione in *phytomenadione CRS*

A'_{cis} = percentage content of *cis*-phytomenadione in *phytomenadione CRS*

A'_{epoxy} = percentage content of *trans*-epoxyphytomenadione in *phytomenadione CRS*,

S_{trans} = area of the peak corresponding to the *trans*-isomer in the chromatogram obtained with the test solution,

S_{cis} = area of the peak corresponding to the *cis*-isomer in the chromatogram obtained with the test solution,

S_{epoxy} = area of the peak corresponding to *trans*-epoxyphytomenadione in the chromatogram obtained with the test solution,

S'_{trans} = area of the peak corresponding to the *trans*-isomer in the chromatogram obtained with reference solution (a),

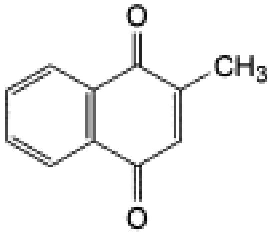
S'_{cis} = area of the peak corresponding to the *cis*-isomer in the chromatogram obtained with reference solution (a),

S'_{epoxy} = area of the peak corresponding to *trans*-epoxyphytomenadione in the chromatogram obtained with reference solution (a).

STORAGE

Store protected from light.

IMPURITIES



A. 2-methylnaphthalene-1,4-dione (menadione).

Ph Eur

Phytosterol

General Notices

(Ph Eur monograph 1911)

Ph Eur

DEFINITION

Natural mixture of sterols obtained from plants of the genera *Hypoxis*, *Pinus* and *Picea*.

Content

Minimum 70.0 per cent of β-sitosterol (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in tetrahydrofuran, sparingly soluble in ethyl acetate.

IDENTIFICATION

A. Mix 1 ml of *acetic anhydride R* with 0.5 ml of solution S (see Tests). After the addition of 0.1 ml of *sulphuric acid R* a red colour is produced, which changes rapidly to violet, then to blue and finally to green.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the peak in the chromatogram obtained with reference solution (b).

TESTS

Solution S

Dissolve 1.0 g in *tetrahydrofuran R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity or alkalinity

Shake 0.20 g with a mixture of 4.0 ml of *ethyl acetate R* and 10.0 ml of *carbon dioxide-free water R* for 3 min. Allow the layers to separate. To the aqueous layer add 0.1 ml of *bromothymol blue solution R1*. If the solution is yellow, not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour to blue. If the solution is blue, not more than 0.5 ml of *0.01 M hydrochloric acid* is required to change the colour to yellow.

Specific optical rotation (*2.2.7*)

- 15.0 to - 28.0 (dried substance).

Dissolve 0.500 g in *ethyl acetate R* and dilute to 10.0 ml with the same solvent.

Acid value (*2.5.1*)

Maximum 1.0, determined on 2.0 g.

Peroxide value (*2.5.5*)

Maximum 10.0.

Saponification value (*2.5.6*)

Maximum 1.0.

Carry out the test using 2.50 g of the substance to be examined, *0.1 M alcoholic potassium hydroxide*, *0.1 M hydrochloric acid*, and a factor of 5.61 (instead of 28.05).

Other sterols

Examine the chromatogram obtained with the test solution in the assay (Figure 1911.-1).

Composition of the other sterols:

— *cholesterol*: maximum 0.5 per cent;

— *brassicasterol*: maximum 0.5 per cent;

— *campesterol*: maximum 15.0 per cent;

— *campestanol*: maximum 5.0 per cent;

— *stigmasterol*: maximum 5.0 per cent;

— *sitostanol*: maximum 15.0 per cent;

— *Δ7-stigmastenol*: maximum 5.0 per cent.

Loss on drying (*2.2.32*)

Maximum 4.0 per cent, determined on 0.250 g by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Gas chromatography (*2.2.28*): use the normalisation procedure.

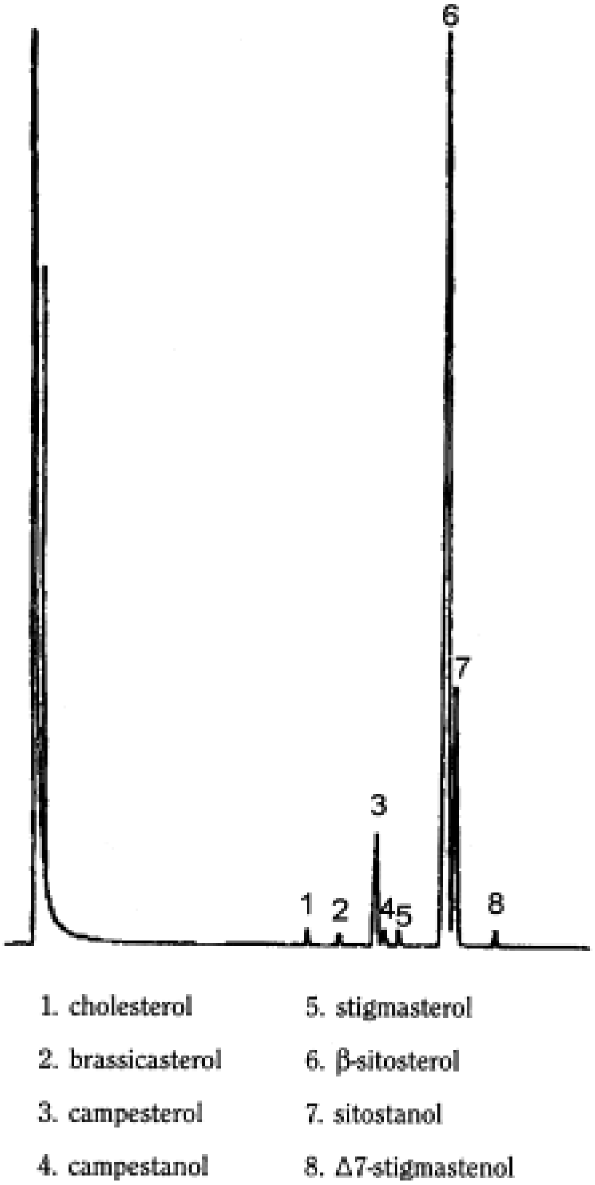


Figure 1911.-1. – *Chromatogram for the assay of phytosterol (trimethylsilyl derivatives)*

Test solution Dissolve 0.100 g in *tetrahydrofuran R* and dilute to 10.0 ml with the same solvent. Introduce 100 µl of this solution into a 3 ml flask and evaporate to dryness under *nitrogen R*. Add 100 µl of a freshly prepared mixture of 50 µl of *1-methylimidazole R* and 1.0 ml of *heptafluoro-N-methyl-N-(trimethylsilyl)butanamide R*, close the flask tightly and heat to 100 °C for 15 min. Allow to cool.

Reference solution (a) Dissolve 25 mg of β*-sitosterol R* and 25 mg of *sitostanol R* in *tetrahydrofuran R* and dilute to 10.0 ml with the same solvent. Introduce 100 µl of this solution into a 3 ml flask and evaporate to dryness under *nitrogen R*. Add 100 µl of a freshly prepared mixture of 50 µl of 1-methylimidazole and 1.0 ml of *heptafluoro-N-methyl-N-(trimethylsilyl) butanamide R*. Close the flask tightly and heat to 100 °C for 15 min. Allow to cool.

Reference solution (b) Dissolve 0.100 g of β*-sitosterol R* in *tetrahydrofuran R* and dilute to 10.0 ml with the same solvent. Introduce 100 µl of this solution into a 3 ml flask and evaporate to dryness under *nitrogen R*. Add 100 µl of a freshly prepared mixture of 50 µl of *1-methylimidazole R* and 1.0 ml of *heptafluoro-N-methyl-N-(trimethylsilyl)butanamide R*. Close the flask tightly and heat to 100 °C for 15 min. Allow to cool.

Column:
— *material*: quartz;

— *size: l* = 25 m, Ø = 0.3 mm;

— *stationary phase*: *poly(dimethyl)(diphenyl)(divinyl)siloxane R* (1 µm).

Carrier gas hydrogen for chromatography R.

Flow rate 2 ml/min.

Split ratio 1:20.

Temperature:
— *column*: 280 °C;

— *injection port and detector*: 300 °C.

Detection Flame ionisation.

Injection 1 µl.

Relative retentions With reference to β-sitosterol (retention time = about 16 min): cholesterol = about 0.7; brassicasterol = about 0.77; campesterol = about 0.84; campestanol = about 0.86; stigmasterol = about 0.9; sitostanol = about 1.02; Δ7-stigmastenol = about 1.1.

System suitability Reference solution (a):

— *resolution*: minimum 1.0 between the peaks due to β-sitosterol and sitostanol.

STORAGE

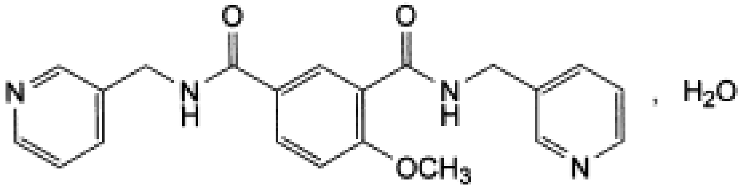
In an airtight container, protected from light.

Ph Eur

Picotamide Monohydrate

General Notices

(Ph Eur monograph 1358)



$C_{21}H_{20}N_4O_3,H_2O$ 394.4 *80530-63-8*

Action and use

Thromboxane Synthetase inhibitor; thromboxane receptor antagonist; antiplatelet agent.

Ph Eur

DEFINITION

Picotamide monohydrate contains not less than 98.0 per cent and not more than 101.0 per cent of 4-methoxy- *N*,*N′*-bis(pyridin-3-ylmethyl)benzene-1,3-dicarboxamide, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water, soluble in ethanol and in methylene chloride. It dissolves in dilute mineral acids.

It shows polymorphism (*5.9*).

IDENTIFICATION

Examine by infrared spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *picotamide monohydrate CRS* . If the spectra obtained in the solid state shows differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

Dissolve 2.5 g in *methanol R* and dilute to 50 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution Dissolve 0.5 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 10 ml with *methanol R*. Dilute 1 ml of the solution to 20 ml with *methanol R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *methanol R*.

Reference solution (c) Dissolve 0.5 g of the substance to be examined and 5 mg of *picotamide impurity A CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture 0.8 volumes of *glacial acetic acid R*, 1 volume of *water R*, 2.5 volumes of *methanol R* and 8 volumes of *butanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Chlorides (*2.4.4*)

Dissolve 0.25 g in a mixture of 2.5 ml of *dilute nitric acid R* and 12.5 ml of *water R*. The solution complies with the limit test for chlorides (200 ppm).

Heavy metals (*2.4.8*)

Dissolve 1.0 g by gently heating in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B for heavy metals (20 ppm). Prepare the standard, using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *methanol R*.

Water (*2.5.12*)

4.5 per cent to 5.0 per cent, determined on 0.300 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

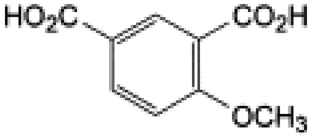
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

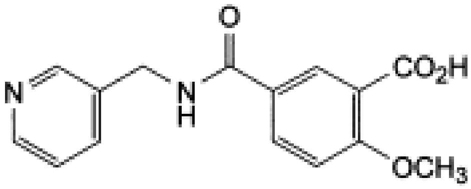
Dissolve 0.150 g in a mixture of 20 ml of *anhydrous acetic acid R* and 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 18.82 mg of $C_{21}H_{20}N_4O_3$.

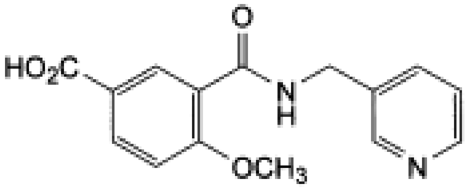
IMPURITIES



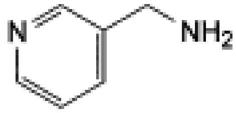
A. 4-methoxybenzene-1,3-dicarboxylic acid,



B. 2-methoxy-5-[[(pyridin-3-ylmethyl)amino]carbonyl]benzoic acid,



C. 4-methoxy-3-[[(pyridin-3-ylmethyl)amino]carbonyl]benzoic acid,



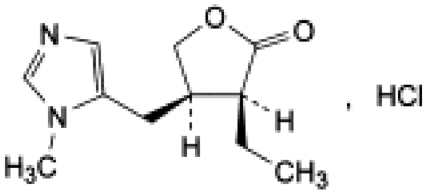
D. (pyridin-3-yl)methanamine.

Ph Eur

Pilocarpine Hydrochloride

General Notices

(Ph Eur monograph 0633)



$C_{11}H_{16}N_2O_2$,HCl   244.7   *54-71-7*

Action and use

Cholinoceptor agonist; treatment of glaucoma.

Preparation

Pilocarpine Hydrochloride Eye Drops

Ph Eur

DEFINITION

(3*S*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Very soluble in water and in ethanol (96 per cent).

mp: about 203 °C.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pilocarpine hydrochloride CRS.*

If the substances are examined as discs, prepare them using *potassium chloride R*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg in *methanol R* and dilute to 2 ml with the same solvent.

Reference solution Dissolve 10 mg of *pilocarpine hydrochloride CRS* in *methanol R* and dilute to 2 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R*, *methanol R*, *methylene chloride R* (1:14:85 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying At 100-105 °C for 10 min, then allow to cool.

Detection Spray with *dilute potassium iodobismuthate solution R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dilute 0.2 ml of solution S (see Tests) to 2 ml with *water R*. Add 0.05 ml of a 50 g/l solution of *potassium dichromate R*, 1 ml of *dilute hydrogen peroxide solution R* and 2 ml of *methylene chloride R* and shake. A violet colour develops in the organic layer.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)

3.5 to 4.5 for solution S.

Specific optical rotation (*2.2.7*)

+ 89 to + 93 (dried substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with *water R*. Dilute 2.0 ml of this solution to 20.0 ml with *water R*.

Reference solution (b) Dissolve 5.0 mg of pilocarpine nitrate for *system suitability CRS* (containing impurity A) in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (c) To 5 ml of the test solution, add 0.1 ml of *ammonia R* and heat the solution on a water-bath for 30 min, cool and dilute to 25 ml with *water R.* Dilute 3 ml of this solution to 25 ml with *water R*. Mainly pilocarpic acid (impurity B) is formed.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Mix 55 volumes of *methanol R*, 60 volumes of *acetonitrile R* and 885 volumes of a 0.679 g/l solution of *tetrabutylammonium dihydrogen phosphate R* previously adjusted to pH 7.7 with *dilute ammonia R2*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time Twice the retention time of pilocarpine.

Elution order Impurity B, impurity C, impurity A, pilocarpine.

Retention time Pilocarpine = about 40 min.

System suitability Reference solution (b):

— *resolution*: minimum 1.6 between the peaks due to impurity A and pilocarpine.

Limits:

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *sum of impurities A and B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *sum of impurities other than A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S. Prepare the standard using 5 ml of *iron standard solution (1 ppm Fe) R* and 5 ml of *water R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *ethanol (96 per cent) R* and add 5 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 24.47 mg of $C_{11}H_{17}ClN_2O_2$.

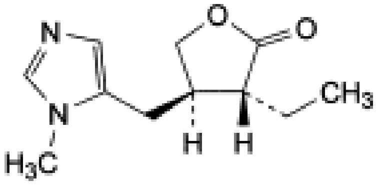
STORAGE

In an airtight container, protected from light.

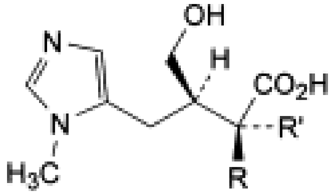
IMPURITIES

Specified impurities *A, B.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C.*



A. (3*R*,4*R*)-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one (isopilocarpine),



B. R = C_2H_5, R′ = H: (2*S*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl) butanoic acid (pilocarpic acid),

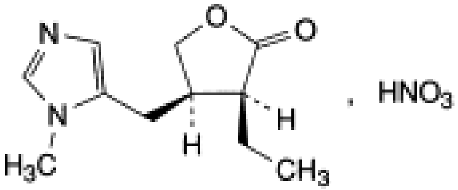
C. R = H, R′ = C_2H_5: (2*R*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl) butanoic acid (isopilocarpic acid).

Ph Eur

Pilocarpine Nitrate

General Notices

(Ph Eur monograph 0104)



$C_{11}H_{16}N_2O_2,HNO_3$ 271.3 *148-72-1*

Action and use

Cholinoceptor agonist; treatment of glaucoma.

Preparation

Pilocarpine Nitrate Eye Drops

Ph Eur

DEFINITION

(3*S*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one nitrate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, sensitive to light.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent).

mp

About 174 °C, with decomposition.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison pilocarpine nitrate CRS.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *pilocarpine nitrate CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel G plate R.

Mobile phase concentrated ammonia R, *methanol R*, *methylene chloride R* (1:14:85 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying At 100-105 °C for 10 min and allow to cool.

Detection Spray with *potassium iodobismuthate solution R*.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dilute 0.2 ml of solution S (see Tests) to 2 ml with *water R*. Add 0.05 ml of a 50 g/l solution of *potassium dichromate R*, 1 ml of *dilute hydrogen peroxide solution R* and 2 ml of *methylene chloride R* and shake. A violet colour develops in the organic layer.

E. It gives the reaction of nitrates (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent. Prepare immediately before use.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)

3.5 to 4.5 for solution S.

Specific optical rotation (*2.2.7*)

+ 80 to + 83 (dried substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 ml of the test solution to 100.0 ml with *water R*. Dilute 2.0 ml of this solution to 20.0 ml with *water R*.

Reference solution (b) Dissolve 5.0 mg of *pilocarpine nitrate for system suitability CRS* (containing impurity A) in water R and dilute to 50.0 ml with the same solvent.

Reference solution (c) To 5 ml of the test solution, add 0.1 ml of *ammonia R* and heat the solution on a water-bath for 30 min, cool and dilute to 25 ml with *water R*. Dilute 3 ml of this solution to 25 ml with *water R*. Mainly pilocarpic acid (impurity B) is formed.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Mix 55 volumes of *methanol R*, 60 volumes of *acetonitrile R* and 885 volumes of a 0.679 g/l solution of *tetrabutylammonium dihydrogen phosphate R* previously adjusted to pH 7.7 with *diluted ammonia R2*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time Twice the retention time of pilocarpine.

Elution order Impurity B, impurity C, impurity A, pilocarpine.

Retention time Pilocarpine = about 40 min.

System suitability Reference solution (b):

— *resolution*: minimum 1.6 between the peaks due to impurity A and pilocarpine.

Limits:

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *sum of impurities A and B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *sum of impurities other than A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent); disregard any peak due to the nitrate ion with a relative retention time with reference to pilocarpine of about 0.3.

Chlorides (*2.4.4*)

Maximum 70 ppm, determined on solution S.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S. Prepare the standard using 5 ml of *iron standard solution (1 ppm Fe) R* and 5 ml of *water R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 27.13 mg of $C_{11}H_{17}N_3O_5$.

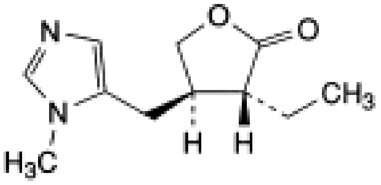
STORAGE

Protected from light.

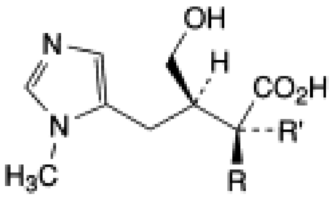
IMPURITIES

Specified impurities A, B.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C.*



A. (3*R*,4*R*)-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one (isopilocarpine),



B. R = C_2H_5, R′ = H: (2*S*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl) butanoic acid (pilocarpic acid),

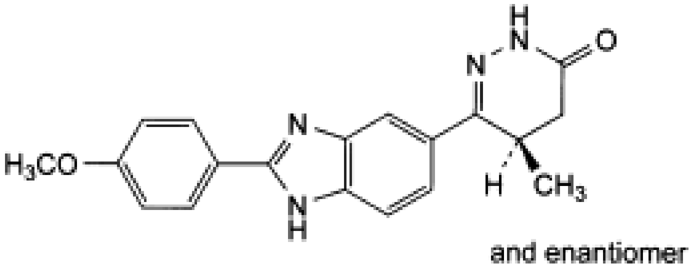
C. R = H, R′ = C_2H_5: (2*R*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl) butanoic acid (isopilocarpic acid).

Ph Eur

Pimobendan

General Notices

(Ph Eur monograph 2179)



$C_{19}H_{18}N_4O_2$ 334.4 *118426-36-7*

Action and use

Inhibitor of phosphodiesterase type III; calcium sensitizer.

Ph Eur

DEFINITION

(5*RS*)-6-[2-(4-Methoxyphenyl)-1*H*-benzimidazol-5-yl]-5-methyl-4,5-dihydropyridazin-3(2*H*)-one.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellowish powder, hygroscopic.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide, slightly soluble in acetone and in methanol.

mp

About 242 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pimobendan CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*.

Reference solution (b) Dissolve the contents of a vial of *pimobendan for system suitability CRS* (impurities A and B) in 1.0 ml of *methanol R*.

Column:

— *size: l* = 0.125 m, Ø = 4.6 mm;

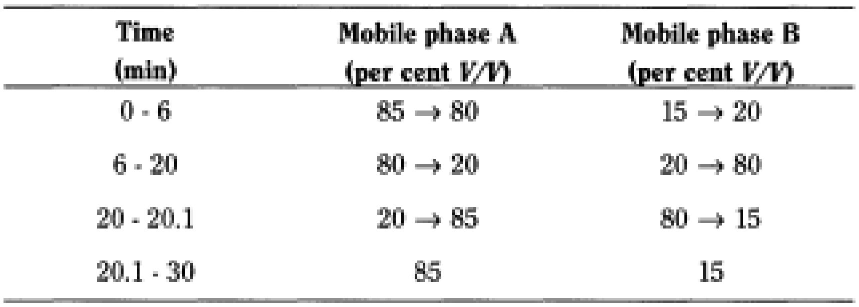
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A*: dissolve 3.0 g of *potassium dihydrogen phosphate R* in 950 ml of *water for chromatography R*, adjust to pH 2.5 with *dilute phosphoric acid R* and dilute to 1000 ml with *water for chromatography R*;

— *mobile phase B*: *acetonitrile for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	85 → 80	15 → 20
6 - 20	80 → 20	20 → 80
20 - 20.1	20 → 85	80 → 15
20.1 - 30	85	15

Flow rate 1 ml/min.

Detection Spectrophotometer at 290 nm.

Injection 10 µl.

Relative retention With reference to pimobendan (retention time = about 8.3 min): impurity A = about 1.3; impurity B = about 1.4.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and impurity B.

Limits:

— *impurities A, B*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 5 ml of *anhydrous formic acid R*. Add 10 ml of *acetic anhydride R* and 70 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

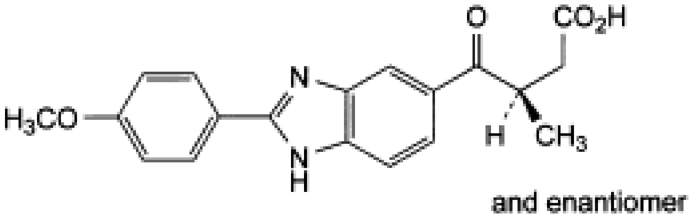
1 ml of *0.1 M perchloric acid* is equivalent to 33.44 mg of $C_{19}H_{18}N_4O_2$.

STORAGE

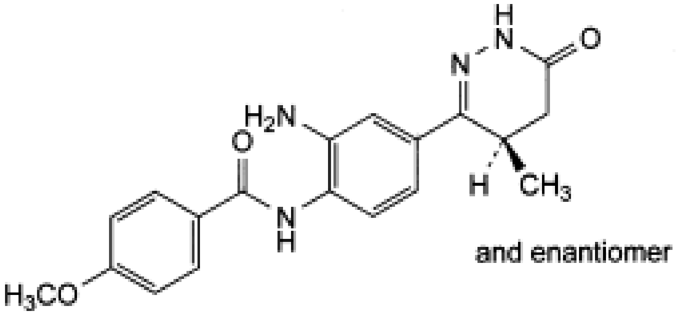
In an airtight container .

IMPURITIES

Specified impurities *A, B.*



A. (3*RS*)-4-[2-(4-methoxyphenyl)-1*H*-benzimidazol-5-yl]-3-methyl-4-oxobutanoic acid,


and enantiomer

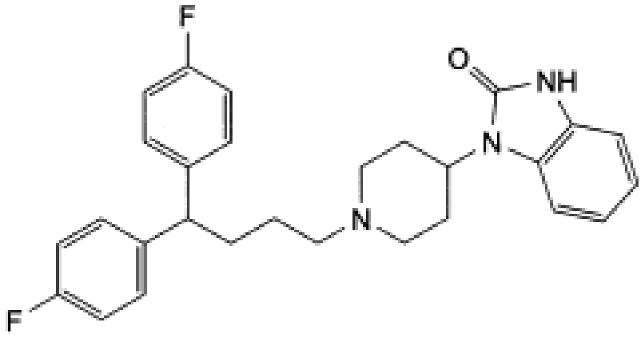
B. *N*-[2-amino-4-[(4*RS*)-4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl]phenyl]-4-methoxybenzamide.

Ph Eur

Pimozide

General Notices

(Ph Eur monograph 1254)



$C_{28}H_{29}F_2N_3O$ 461.6 *2062-78-4*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Pimozide Tablets

Ph Eur

DEFINITION

1-[1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in methylene chloride, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Melting point (*2.2.14*): 216 °C to 220 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *pimozide CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a) Dissolve 30 mg of *pimozide CRS* in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b) Dissolve 30 mg of *pimozide CRS* and 30 mg of *benperidol CRS* in the mobile phase and dilute to 10 ml with the mobile phase.

Plate *TLC silica gel plate R.*

Mobile phase *acetone R*, *methanol R* (1:9 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, then add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Dissolve 0.2 g in *methanol R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

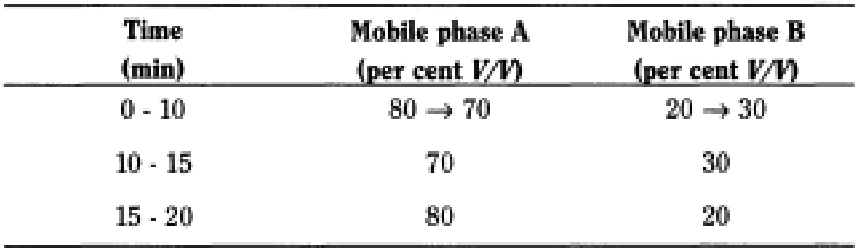
Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *pimozide CRS* and 2.0 mg of *mebendazole CRS* in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

Column:
- *size: l* = 0.1 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: solution containing 2.5 g/l of *ammonium acetate R* and 8.5 g/l of *tetrabutylammonium hydrogen sulphate R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	80 → 70	20 → 30
10 - 15	70	30
15 - 20	80	20

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 280 nm.

Equilibration With the mobile phase at the initial composition for at least 10 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Mebendazole = about 7 min; pimozide = about 8 min.

System suitability Reference solution (a):

- *resolution*: minimum 5.0 between the peaks due to mebendazole and pimozide; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear-gradient elution.

Limits:
- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution R* as indicator.

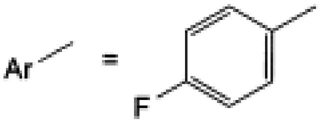
1 ml *0.1 M perchloric acid* is equivalent to 46.16 mg of $C_{28}H_{29}F_2N_3O$.

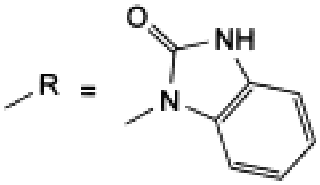
STORAGE

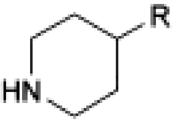
Protected from light.

IMPURITIES

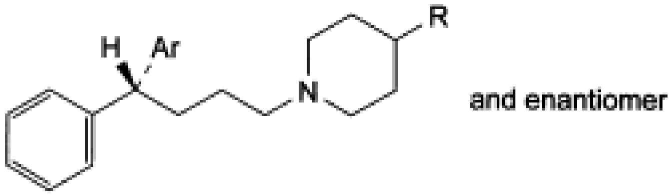
Specified impurities   A, B, C, D, E.



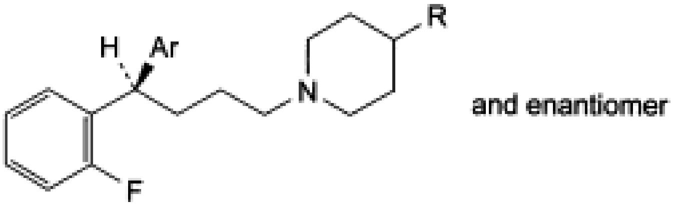




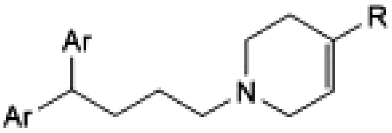
A. 1-(piperidin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one,



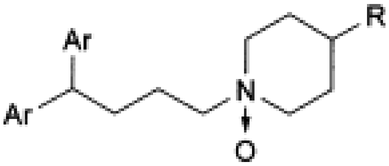
B. 1-[1-[(4*RS*)-4-(4-fluorophenyl)-4-phenylbutyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



C. 1-[1-[(4*RS*)-4-(2-fluorophenyl)-4-(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



D. 1-[1-[4,4-bis(4-fluorophenyl)butyl]-1,2,3,6-tetrahydropyridin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



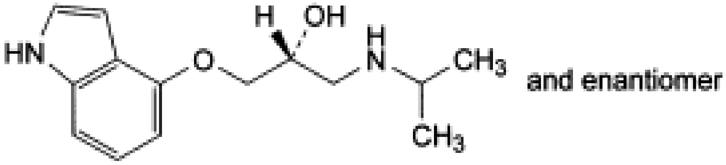
E. 1-[1-[4,4-bis(4-fluorophenyl)butyl]piperidin-4-yl 1-oxide]-1,3-dihydro-2*H*-benzimidazol-2-one.

Ph Eur

Pindolol

General Notices

(Ph Eur monograph 0634)



$C_{14}H_{20}N_2O_2$ 248.3 *13523-86-9*

Action and use

Beta-adrenoceptor antagonist.

Preparation

Pindolol Tablets

Ph Eur

DEFINITION

Pindolol contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*)-1-(1*H*-indol-4-yloxy)-3-[(1-methylethyl)amino]propan-2-ol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, slightly soluble in methanol. It dissolves in dilute mineral acids.

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Melting point (*2.2.14*): 169 °C to 174 °C.

B. Dissolve 20.0 mg in a 0.085 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* and dilute to 100.0 ml with the same solution. Dilute 10.0 ml of the solution to 100.0 ml with a 0.085 per cent *V/V* solution of *hydrochloric acid R* in *methanol R*. Examined between 230 nm and 320 nm (*2.2.25*), the solution shows two absorption maxima, at 264 nm and at 287 nm, and a shoulder at 275 nm. The specific absorbance at the maxima are 330 to 350 and 170 to 190, respectively.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *pindolol CRS*.

D. Examine in daylight the chromatograms on plate A obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

Dissolve 0.5 g in *dilute acetic acid R* and dilute to 10 ml with the same acid. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 or B_5 (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance. Carry out all operations as rapidly as possible, protected from light.

Test solution (a) Dissolve 0.10 g of the substance to be examined in a mixture of 1 volume of *anhydrous acetic acid R* and 99 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents. Prepare immediately before use and apply this solution to the plate last.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *anhydrous acetic acid R* and 99 volumes of *methanol R*.

Reference solution (a) Dissolve 20 mg of *pindolol CRS* in a mixture of 1 volume of *anhydrous acetic acid R* and 99 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.5 ml of reference solution (a) to 50 ml with a mixture of 1 volume of *anhydrous acetic acid R* and 99 volumes of *methanol R*.

A. Apply separately 5 µl of each solution. Develop the plate without delay over a path of 10 cm using a freshly prepared mixture of 4 volumes of *concentrated ammonia R*, 50 volumes of *ethyl acetate R* and 50 volumes of *methanol R*. Dry the plate briefly in a current of cold air. Spray the plate without delay with *dimethylaminobenzaldehyde solution R7* and heat to 50 °C for 20 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent).

B. Apply separately 10 µl of each solution. Develop the plate without delay over a path of 10 cm using a freshly prepared mixture of 4 volumes of *concentrated ammonia R*, 50 volumes of *ethyl acetate R* and 50 volumes of *methanol R*. Dry the plate briefly in a current of cold air. Examine the plate without delay in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot and the spots detected on plate A, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent).

Heavy metals (*2.4.8*)

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

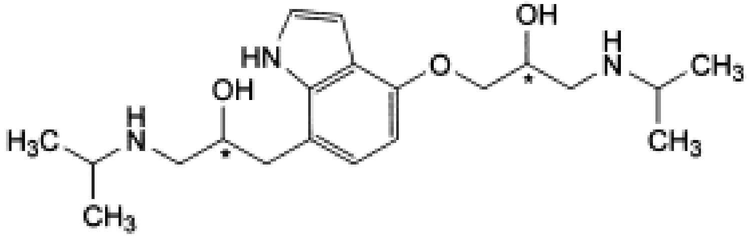
Dissolve 0.200 g in 80 ml of *methanol R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M hydrochloric acid* is equivalent to 24.83 mg of $C_{14}H_{20}N_2O_2$.

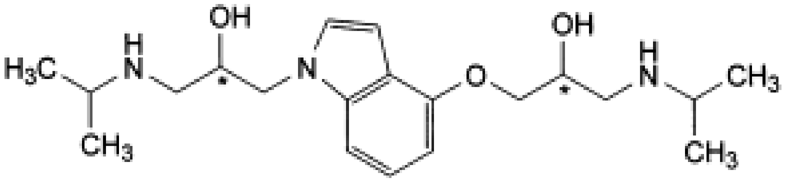
STORAGE

Store protected from light.

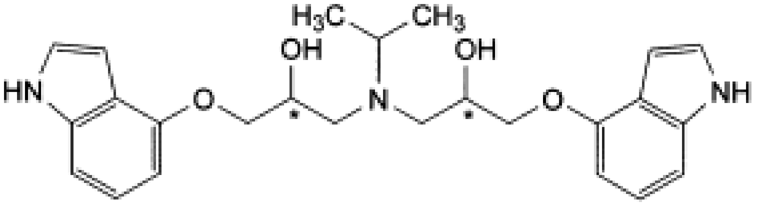
IMPURITIES



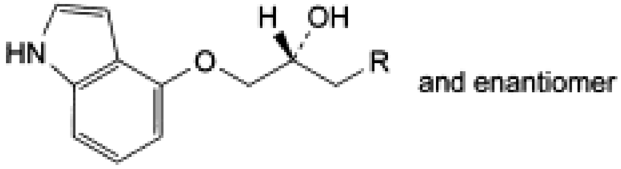
A. 1-[[7-[2-hydroxy-3-[(1-methylethyl)amino]propyl]-1*H*-indol-4-yl]oxy]-3-[(1-methylethyl)amino]propan-2-ol,



B. 1-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-1*H*-indol-1-yl]-3-[(1-methylethyl)amino]propan-2-ol,



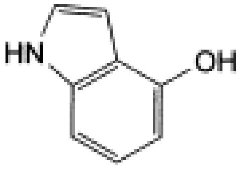
C. 1,1′-[(1-methylethyl)imino]bis[3-(1*H*-indol-4-yloxy)propan-2-ol],



and enantiomer

D. R = OH: (2*RS*)-3-(1*H*-indol-4-yloxy)propane-1,2-diol,

F. R = Cl: (2*RS*)-1-chloro-3-(1*H*-indol-4-yloxy)propan-2-ol,



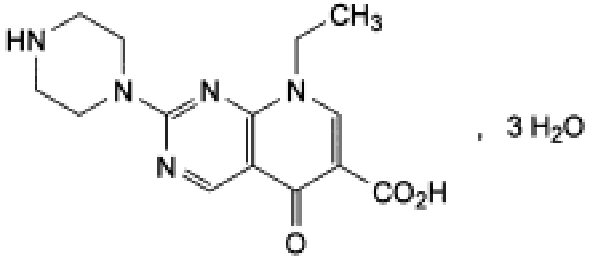
E. 1*H*-indol-4-ol.

Ph Eur

Pipemidic Acid Trihydrate

General Notices

(Ph Eur monograph 1743)



$C_{14}H_{17}N_5O_3,3H_2O$ 357.4 *51940-44-4*
(anhydrous)

Action and use

Antibacterial.

Ph Eur

DEFINITION

8-Ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido[2,3-d]pyrimidine-6-carboxylic acid trihydrate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Pale yellow or yellow, crystalline powder.

Solubility

Very slightly soluble in water. It dissolves in dilute solutions of acids and of alkali hydroxides.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of pipemidic acid trihydrate.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in 10 ml of the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *ethyl parahydroxybenzoate R* in 2.0 ml of the test solution and dilute to 20.0 ml with the mobile phase.

Column:
- *size:* *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 18 nm and a carbon loading of 13 per cent.

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a solution containing 5.7 g/l of *citric acid R* and 1.7 g/l of *sodium decanesulphonate R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µl.

Run time 2.5 times the retention time of pipemidic acid.

System suitability Reference solution (b):

- *resolution*: minimum 4.0 between the peaks due to pipemidic acid and to ethyl parahydroxybenzoate.

Limits:
- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2.0 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

14.0 per cent to 16.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

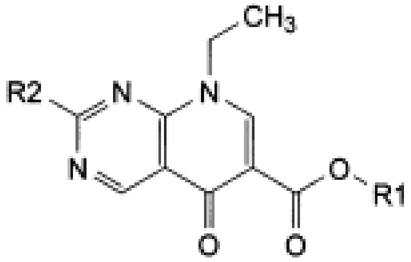
Dissolve 0.240 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 30.33 mg of $C_{14}H_{17}N_5O_3$.

STORAGE

Protected from light.

IMPURITIES

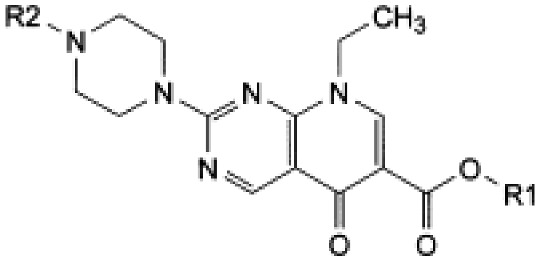


A. R1 = H, R2 = OH: 8-ethyl-2-hydroxy-5-oxo-5,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxylic acid,

B. R1 = H, R2 = OCH_3: 8-ethyl-2-methoxy-5-oxo-5,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxylic acid,

C. R1 = H, R2 = OC_2H_5: 2-ethoxy-8-ethyl-5-oxo-5,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxylic acid,

D. R1 = C_2H_5, R2 = Cl: ethyl 2-chloro-8-ethyl-5-oxo-5,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxylate,



E. R1 = C_2H_5, R2 = H: ethyl 8-ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido[2,3-*d*] pyrimidine-6-carboxylate,

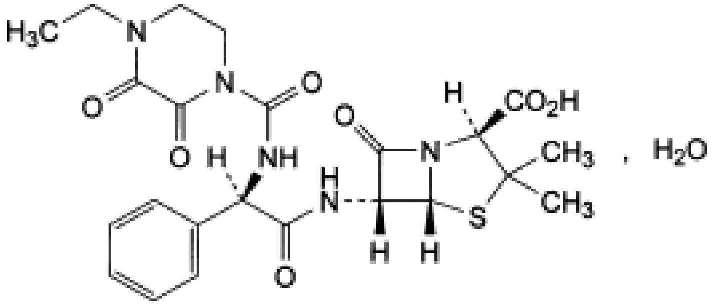
F. R1 = H, R2 = CO-CH_3: 2-(4-acetylpiperazin-1-yl)-8-ethyl-5-oxo-5,8-dihydropyrido[2,3-*d*] pyrimidine-6-carboxylic acid (acetylpipemidic acid).

Ph Eur

Piperacillin

General Notices

(Ph Eur monograph 1169)



$C_{23}H_{27}N_5O_7S,H_2O$ 535.6 *66258-76-2*

Action and use

Penicillin antibacterial.

Preparation

Piperacillin Intravenous infusion

Ph Eur

DEFINITION

(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(4-Ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in methanol, slightly soluble in ethyl acetate.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *piperacillin CRS.*

TESTS

Solution S

Dissolve 2.50 g in *sodium carbonate solution R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and its absorbance (*2.2.25*) at 430 nm is not greater than 0.10.

Specific optical rotation (*2.2.7*)

+ 165 to + 175 (anhydrous substance).

Dissolve 0.250 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, 31.2 g/l solution of *sodium dihydrogen phosphate R* (25:75 *V/V*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Test solution (b) *Prepare the solution immediately before use*. Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 25.0 mg of *piperacillin CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 25.0 ml with the solvent mixture.

Reference solution (c) Dissolve 10.0 mg of *piperacillin CRS* and 10.0 mg of *anhydrous ampicillin CRS* (impurity A) in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 50.0 ml with the solvent mixture.

Column:

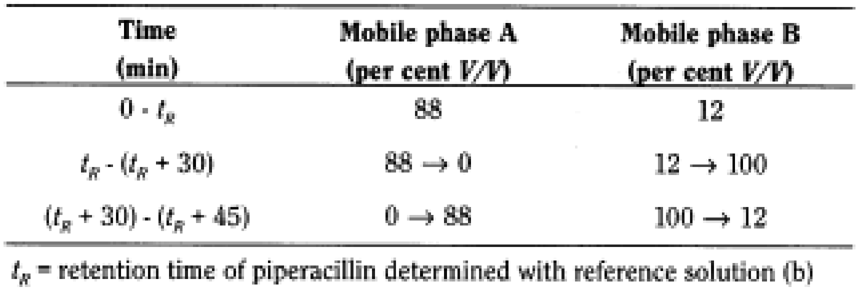
— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 576 ml of *water R*, 200 ml of a 31.2 g/l solution of *sodium dihydrogen phosphate R* and 24 ml of an 80 g/l solution of *tetrabutylammonium hydroxide R*; if necessary, adjust to pH 5.5 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*; add 200 ml of *acetonitrile R*;

— *mobile phase B*: mix 126 ml of *water R*, 200 ml of a 31.2 g/l solution of *sodium dihydrogen phosphate R* and 24 ml of an 80 g/l solution of *tetrabutylammonium hydroxide R*; if necessary, adjust to pH 5.5 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*; add 650 ml of *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	88	12
t_R - (t_R + 30)	88 → 0	12 → 100
(t_R + 30) - (t_R + 45)	0 → 88	100 → 12

t_R = retention time of piperacillin determined with reference solution (b)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of reference solutions (b), (c) and (d) with isocratic elution at the initial mobile phase composition and 20 µl of test solution (b) according to the elution gradient described under Mobile phase.

System suitability:

— *resolution*: minimum 10 between the peaks due to impurity A and piperacillin in the chromatogram obtained with reference solution (c); if necessary, adjust the ratio A:B of the mobile phase;

— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (d);

— *mass distribution ratio*: 2.0 to 3.0 for the peak due to piperacillin in the chromatogram obtained with reference solution (c).

Limit:

— *any impurity*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent).

_N,N_-Dimethylaniline (_2.4.26, Method A_)

Maximum 20 ppm.

Heavy metals (_2.4.8_)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (_2.5.12_)

2.0 per cent to 4.0 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Initial composition of the mixture of mobile phases A and B, adjusted where applicable.

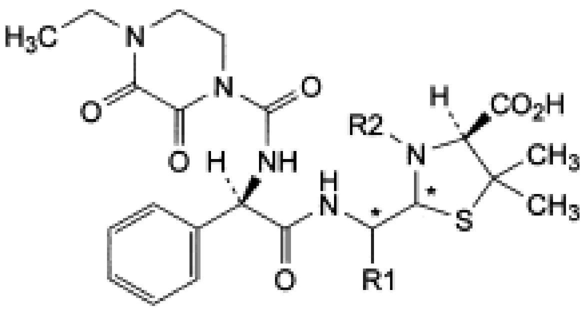
Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

IMPURITIES

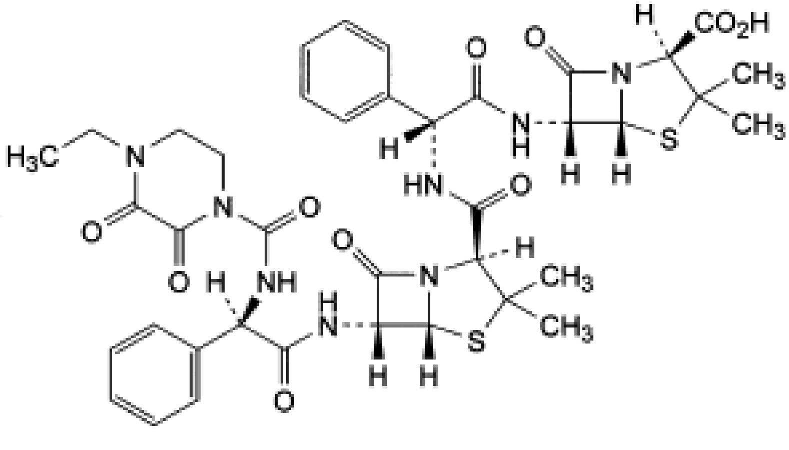
A. ampicillin,



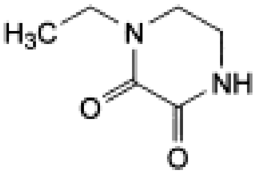
B. R1 = CO_2H, R2 = H: (4*S*)-2-[carboxy[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of piperacillin),

C. R1 = R2 = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of piperacillin),

F. R1 = CO_2H, R2 = CO-CH_3: (4*S*)-3-acetyl-2-[carboxy[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (acetylated penicilloic acids of piperacillin),



D. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl] carbonyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (piperacillinylampicillin),



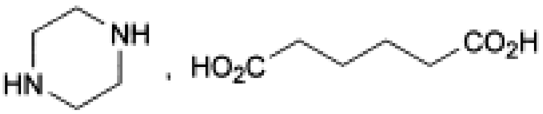
E. 1-ethylpiperazine-2,3-dione.

Ph Eur

Piperazine Adipate

General Notices

(Ph Eur monograph 0423)



$C_4H_{10}N_2,C_6H_{10}O_4$ 232.3   *142-88-1*

Action and use

Anthelminthic.

Ph Eur

DEFINITION

Piperazine adipate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of piperazine hexanedioate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white crystalline powder, soluble in water, practically insoluble in alcohol. It melts at about 250 °C, with decomposition.

IDENTIFICATION

First identification   A.

Second identification   B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *piperazine adipate CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances after spraying with the ninhydrin solutions. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 10 ml of solution S (see Tests) add 5 ml of *hydrochloric acid R* and shake with three quantities, each of 10 ml, of *ether R*. Evaporate the combined ether layers to dryness. The residue, washed with 5 ml of *water R* and dried at 100 °C to 105 °C, melts (*2.2.14*) at 150 °C to 154 °C.

TESTS

Solution S

Dissolve 2.5 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 1.0 g of the substance to be examined in 6 ml of *concentrated ammonia R* and dilute to 10 ml with *ethanol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R*.

Reference solution (a) Dissolve 0.1 g of *piperazine adipate CRS* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 25 mg of *ethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (c) Dissolve 25 mg of *triethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (d) Dissolve 12.5 mg of *triethylenediamine R* in 5.0 ml of test solution (a) and dilute to 50 ml with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a freshly prepared mixture of 20 volumes of *concentrated ammonia R* and 80 volumes of *acetone R*. Dry the plate at 105 °C and spray successively with a 3 g/l solution of *ninhydrin R* in a mixture of 3 volumes of *anhydrous acetic acid R* and 100 volumes of *butanol R* and a 1.5 g/l solution of *ninhydrin R* in *ethanol R*. Dry the plate at 105 °C for 10 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent) . Spray the plate with *0.05 M iodine* and allow to stand for about 10 min. Any spot corresponding to triethylenediamine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots. Disregard any spots remaining on the starting line.

Heavy metals (*2.4.8*)

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*)

Not more than 0.5 per cent, determined on 1.00 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 10 ml of *anhydrous acetic acid R* with gentle heating and dilute to 70 ml with the same acid. Titrate with *0.1 M perchloric acid* using 0.25 ml of *naphtholbenzein solution R* as indicator until the colour changes from brownish-yellow to green.

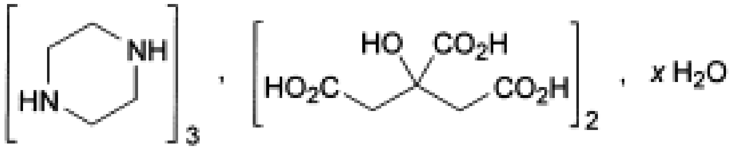
1 ml of *0.1 M perchloric acid* is equivalent to 11.61 mg of $C_{10}H_{20}N_2O_4$.

Ph Eur

Piperazine Citrate

General Notices

(Ph Eur monograph 0424)



$(C_4H_{10}N_2)_3,2C_6H_8O_7xH_20$ 643 *41372-10-5*

(anhydrous substance)

Action and use

Anthelminthic.

Preparation

Piperazine Citrate Elixir

Ph Eur

DEFINITION

Piperazine citrate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of tripiperazine bis(2-hydroxy-propane-1,2,3-tricarboxylate), calculated with reference to the anhydrous substance. It contains a variable quantity of water.

CHARACTERS

A white or almost white granular powder, freely soluble in water, practically insoluble in alcohol.

After drying at 100 °C to 105 °C, it melts at about 190 °C.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *piperazine citrate CRS*. Dry the substance to be examined and the reference substance at 120 °C for 5 h, powder the substances avoiding uptake of water, prepare discs and record the spectra without delay.

B. Examine the chromatograms obtained in the test for related substances after spraying with the ninhydrin solutions. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.5 g in *water R* and dilute to 5 ml with the same solvent. The solution gives the reaction of citrates *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_8 *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 1.0 g of the substance to be examined in 6 ml of *concentrated ammonia R* and dilute to 10 ml with *ethanol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R*.

Reference solution (a) Dissolve 0.1 g of *piperazine citrate CRS* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 25 mg of *ethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (c) Dissolve 25 mg of *triethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (d) Dissolve 12.5 mg of *triethylenediamine R* in 5.0 ml of test solution (a) and dilute to 50 ml with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a freshly prepared mixture of 20 volumes of *concentrated ammonia R* and 80 volumes of *acetone R*. Dry the plate at 105 °C and spray successively with a 3 g/l solution of *ninhydrin R* in a mixture of 3 volumes of *anhydrous acetic acid R* and 100 volumes of *butanol R* and a 1.5 g/l solution of *ninhydrin R* in *ethanol R*. Dry the plate at 105 °C for 10 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent) . Spray the plate with *0.05 M iodine* and allow to stand for about 10 min. Any spot corresponding to triethylenediamine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots. Disregard any spots remaining on the starting line.

Heavy metals *(2.4.8)*

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

10.0 per cent to 14.0 per cent, determined on 0.300 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 10 ml of *anhydrous acetic acid R* with gentle heating and dilute to 70 ml with the same acid. Titrate with *0.1 M perchloric acid* using 0.25 ml of *naphtholbenzein solution R* as indicator until the colour changes from brownish-yellow to green.

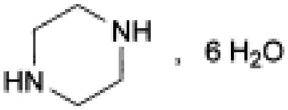
1 ml of *0.1 M perchloric acid* is equivalent to 10.71 mg of $C_{24}H_{46}N_6O_{14}$.

Ph Eur

Piperazine Hydrate

General Notices

(Ph Eur monograph 0425)



$C_4H_{10}N_2,6H_2O$ 194.2 *142-63-2*

Action and use

Anthelminthic.

Ph Eur

DEFINITION

Piperazine hydrate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of piperazine hexahydrate.

CHARACTERS

Colourless, deliquescent crystals, freely soluble in water and in alcohol.

It melts at about 43 °C.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *piperazine hydrate CRS*. Dry the substance to be examined and the reference substance over *diphosphorus pentoxide R in vacuo* for 48 h, powder the substances avoiding uptake of water, prepare discs and record the spectra without delay.

B. Examine the chromatograms obtained in the test for related substances after spraying with the ninhydrin solutions. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.5 g in 5 ml of *dilute sodium hydroxide solution R*. Add 0.2 ml of *benzoyl chloride R* and mix. Continue to add *benzoyl chloride R* in portions of 0.2 ml until no further precipitate is formed. Filter and wash the precipitate with a total of 10 ml of *water R* added in small portions. Dissolve the precipitate in 2 ml of hot *alcohol R* and pour the solution into

5 ml of *water R*. Allow to stand for 4 h, filter, wash the crystals with *water R* and dry at 100 °C to 105 °C. The crystals melt *(2.2.14)* at 191 °C to 196 °C.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_8 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 10.5 to 12.0.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 1.0 g of the substance to be examined in 6 ml of *concentrated ammonia R* and dilute to 10 ml with *ethanol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R*.

Reference solution (a) Dissolve 0.1 g of *piperazine hydrate CRS* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 25 mg of *ethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (c) Dissolve 25 mg of *triethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (d) Dissolve 12.5 mg of *triethylenediamine R* in 5.0 ml of test solution (a) and dilute to 50 ml with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R.*

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a freshly prepared mixture of 20 volumes of *concentrated ammonia R* and 80 volumes of *acetone R*. Dry the plate at 105 °C and spray successively with a 3 g/l solution of *ninhydrin R* in a mixture of 3 volumes of *anhydrous acetic acid R* and 100 volumes of *butanol R* and a 1.5 g/l solution of *ninhydrin R* in *ethanol R*. Dry the plate at 105 °C for 10 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent) . Spray the plate with *0.05 M iodine* and allow to stand for about 10 min. Any spot corresponding to triethylenediamine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots.

Heavy metals ***(2.4.8)***

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 80.0 mg in 10 ml of *anhydrous acetic acid R* with gentle heating and dilute to 70 ml with the same acid. Titrate with *0.1 M perchloric acid* using 0.25 ml of *naphtholbenzein solution R* as indicator until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 9.705 mg of $C_4H_{10}N_2,6H_2O$.

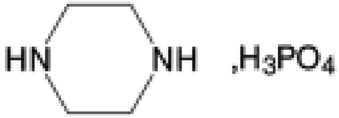
STORAGE

Store in an airtight container, protected from light.

Ph Eur

Piperazine Phosphate

General Notices



$C_4H_{10}N_2,H_3PO_4,H_2O$ 202.1 *18534-18-4*

Action and use

Anthelminthic.

Preparation

Piperazine Phosphate Tablets

DEFINITION

Piperazine Phosphate contains not less than 98.5% and not more than 100.5% of $C_4H_{10}N_2$, H_3PO_4, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white, crystalline powder; odourless or almost odourless.

Sparingly soluble in *water*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION

A. Dissolve 0.1 g in 5 ml of *water*, add 0.5 g of *sodium hydrogen carbonate*, 0.5 ml of a 5% w/v solution of *potassium hexacyanoferrate(III)* and 0.1 ml of *mercury*. Shake vigorously for 1 minute and allow to stand for 20 minutes. A reddish colour is produced slowly.

B. Dissolve 0.2 g in 5 ml of 2M *hydrochloric acid*, add with stirring 1 ml of a 50% w/v solution of *sodium nitrite* and cool in ice for 15 minutes, stirring if necessary to induce crystallisation. The *melting point* of the crystals, after washing with 10 ml of iced *water* and drying at 105°, is about 159°, Appendix V A.

C. A solution yields the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 6.0 to 6.5, Appendix V L.

Heavy metals

Dissolve 2.0 g in 20 ml of 2M *acetic acid*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard (20 ppm).

Water

8.0 to 9.5% w/w, Appendix IX C. Use 0.25 g.

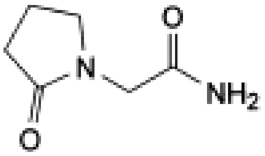
ASSAY

Dissolve 0.2 g in a mixture of 3.5 ml of 0.5M *sulphuric acid* and 10 ml of *water*. Add 100 ml of *picric acid solution R1*, heat on a water bath for 15 minutes and allow to stand for 1 hour. Filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable) and wash the residue with successive 10 ml quantities of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulphate. Wash the residue with five 10 ml quantities of *absolute ethanol* and dry to constant weight at 100° to 105°. Each g of residue is equivalent to 0.3382 g of $C_4H_{10}N_2,H_3PO_4$.

Piracetam

General Notices

(Ph Eur monograph 1733)



$C_6H_{10}N_2O_2$ 142.2 *7491-74-9*

Action and use

Nootropic; cortical myoclonus.

Ph Eur

DEFINITION

2-(2-Oxopyrrolidin-1-yl)acetamide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, powder.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison piracetam CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 2.0 g in *water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents.

Test solution (b) Dilute 10.0 ml of test solution (a) to 50.0 ml with a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water R*.

Reference solution (a) Dissolve 5 mg of the substance to be examined and 10 µl of *2-pyrrolidone R* in a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 100.0 ml with a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water R*. Dilute 5.0 ml of this solution to 50.0 ml with a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water R*.

Reference solution (c) Dissolve 50.0 mg of *piracetam CRS* in a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 10.0 ml of this solution to 50.0 ml with a mixture of 10 volumes of *acetonitrile R1* and 90 volumes of *water R*.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 10 volumes of *acetonitrile R1* and 90 volumes of a 1.0 g/l solution of *dipotassium hydrogen phosphate R*; adjust to pH 6.0 with *dilute phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl of test solution (a) and reference solutions (a) and (b).

Run time 8 times the retention time of piracetam.

Relative retention With reference to piracetam (retention time = about 4 min): impurity D = about 0.8; impurity A = about 1.15; impurity B = about 2.8; impurity C = about 6.3.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to piracetam and impurity A;

— *symmetry factor*: maximum 2.0 for the peak due to piracetam.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

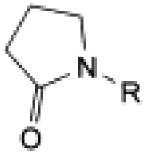
Calculate the percentage content of $C_6H_{10}N_2O_2$ from the areas of the peaks and the declared content of *piracetam CRS*.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D.



A. R = H: pyrrolidin-2-one (2-pyrrolidone),

B. R = CH_2-CO-O-CH_3: methyl (2-oxopyrrolidin-1-yl)acetate,

C. R = CH_2-CO-O-C_2H_5: ethyl (2-oxopyrrolidin-1-yl)acetate,

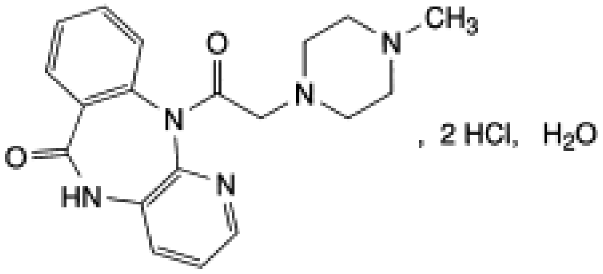
D. R = CH_2-CO_2H: (2-oxopyrrolidin-1-yl)acetic acid.

Ph Eur

Pirenzepine Hydrochloride

General Notices

(Pirenzepine Dihydrochloride Monohydrate, Ph Eur monograph 2001)



$C_{19}H_{21}N_5O_2,2HCl,H_2O$ 442.3 *29868-61-7*

Action and use

Muscarinic M_3 receptor antagonist.

Ph Eur

DEFINITION

11-[(4-Methylpiperazin-1-yl)acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one dihydrochloride monohydrate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 30.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *methanol R*. Examined between 240 nm and 360 nm (*2.2.25*), the solution shows an absorption maximum at 283 nm. The specific absorbance at the maximum is 190 to 205 (anhydrous substance).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison pirenzepine dihydrochloride monohydrate CRS.

C. Examine the chromatograms obtained in the test for impurity D.

Results The principal band obtained in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal band in the chromatogram obtained with reference solution (d).

D. To 0.2 ml of solution S (see Tests) add 1.8 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

1.0 to 2.0 for solution S.

Impurity D

Thin-layer chromatography (*2.2.27*).

Test solution (a) To 0.10 g add 0.1 ml of *concentrated ammonia R* and dilute to 10 ml with *methanol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) To 0.1 g of *pirenzepine dihydrochloride monohydrate CRS* add 0.1 ml of *concentrated ammonia R* and dilute to 10 ml with *methanol R*.

Reference solution (b) Dissolve 25 mg of *methylpiperazine R* in *methanol R* and dilute to 25 ml with the same solvent. Dilute 2.0 ml of the solution to 100 ml with *methanol R*.

Reference solution (c) Dilute 5 ml of test solution (a) to 100 ml with *methanol R*. Dilute 4 ml of this solution to 100 ml with *methanol R*. Mix 1 ml with 1 ml of reference solution (b).

Reference solution (d) Dilute 1 ml of reference solution (a) to 10 ml with *methanol R*.

Plate TLC silica gel plate R.

Mobile phase *concentrated ammonia R*, *methanol R*, *ethyl acetate R*, *toluene R* (7:25:28:40 *V/V/V/V*).

Application 20 µl as bands of 20 mm by 2 mm.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose the plate to iodine vapour until the band in the chromatogram obtained with reference solution (b) is clearly visible (at most 60 min).

System suitability The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated bands.

Limit:

- *impurity D*: any band corresponding to impurity D in the chromatogram obtained with test solution (a) is not more intense than the band in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.30 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent. To 1.0 ml of the solution add 5 ml of *methanol R* and dilute to 10.0 ml with mobile phase A.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

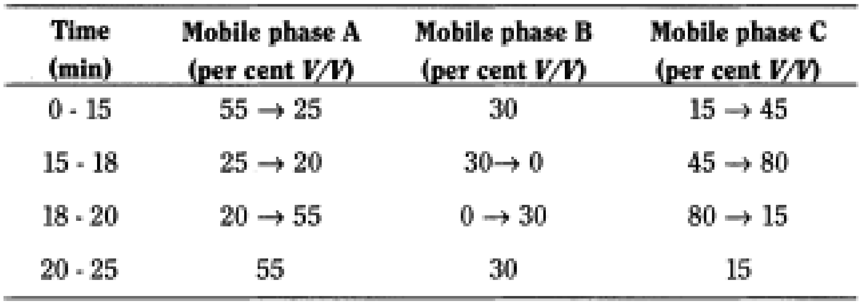
Reference solution (b) Dissolve 0.1 g of *1-phenylpiperazine R* in *methanol R* and dilute to 10 ml with the same solvent. Mix 1 ml of the solution with 1 ml of the test solution, add 5 ml of *methanol R* and dilute to 10 ml with mobile phase A.

Column:

- *size: l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: octadecylsilyl silica gel for chromatography R (5 µm).

Mobile phase:

- *mobile phase A*: dissolve 2.0 g of *sodium dodecyl sulphate R* in *water R*, adjust to pH 3.2 with *acetic acid R* and dilute to 1000 ml with *water R*;
- *mobile phase B*: *methanol R*;
- *mobile phase C*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 15	55 → 25	30	15 → 45
15 - 18	25 → 20	30→ 0	45 → 80
18 - 20	20 → 55	0 → 30	80 → 15
20 - 25	55	30	15

Flow rate 1 ml/min.

Detection Spectrophotometer at 283 nm.

Injection 10 µl.

System suitability Reference solution (b):

— *resolution*: minimum of 5.0 between the peaks corresponding to pirenzepine and to 1-phenylpiperazine.

Limits:

— *any impurity:* not more than the peak area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.04 per cent).

Water (*2.5.12*)

3.5 per cent to 5.0 per cent, determined on 0.250 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

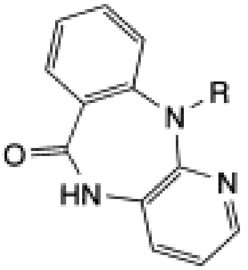
Dissolve 0.300 g in 50 ml of *water R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume at the first point of inflection.

1 ml of *0.1 M sodium hydroxide* is equivalent to 42.43 mg of $C_{19}H_{23}Cl_2N_5O_2$.

STORAGE

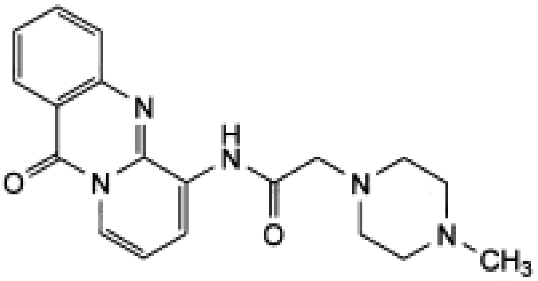
Protected from light.

IMPURITIES

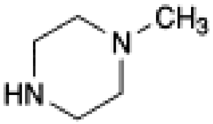


A. R = CO-CH_2-Cl: 11-(chloroacetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one,

B. R = H: 5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one,



C. 6-[[(4-methylpiperazin-1-yl)acetyl]amino]-11*H*-pyrido[2,1-*b*]quinazolin-11-one,



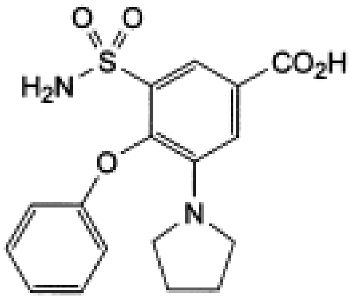
D. 1-methylpiperazine.

Ph Eur

Piretanide

General Notices

(Ph Eur monograph 1556)



$C_{17}H_{18}N_2O_5S$ 362.4 *55837-27-9*

Action and use

Thiazide diuretic.

Ph Eur

DEFINITION

4-Phenoxy-3-(pyrrolidin-1-yl)-5-sulphamoylbenzoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellowish-white or yellowish powder.

Solubility

Very slightly soluble in water, sparingly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *piretanide CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_4 (*2.2.2, Method II*).

Dissolve 0.1 g in *methanol R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *anhydrous ethanol R*, *acetonitrile R*, *water R* (10:45:45 *V/V/V*).

Test solution Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *piretanide CRS* and 3 mg of *piretanide impurity A CRS* in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (b) Dilute 0.3 ml of the test solution to 100.0 ml with the solvent mixture.

Column:
- *size:* *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase A mixture of 35 volumes of *acetonitrile R1* and 65 volumes of a solution prepared as follows: add 1 ml of *trifluoroacetic acid R* to 500 ml of *water for chromatography R,* add 1 ml of *triethylamine R* and dilute to 1000 ml with *water for chromatography R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 232 nm.

Injection 10 µl.

Run time 5 times the retention time of piretanide.

Relative retention With reference to piretanide (retention time = about 10 min): impurity A = about 0.9.

System suitability Reference solution (a):

- *resolution*: minimum 2 between the peaks due to impurity A and piretanide.

Limits:
- *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *total*: not more than 3.33 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

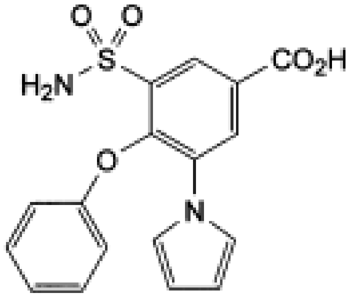
1 ml of *0.1 M perchloric acid* is equivalent to 36.24 mg of $C_{17}H_{18}N_2O_5S$.

STORAGE

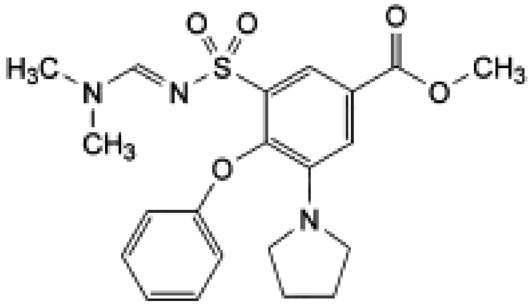
Protected from light.

IMPURITIES

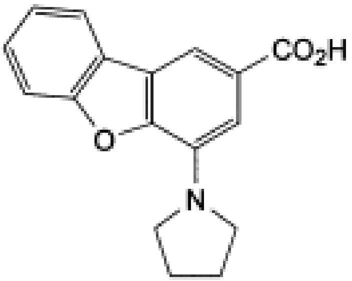
Specified impurities A, B, C.



A. 4-phenoxy-3-(1*H*-pyrrol-1-yl)-5-sulphamoylbenzoic acid,



B. methyl-3-[[(dimethylamino)methylene]sulphamoyl]-4-phenoxy-5-(pyrrolidin-1-yl)benzoate,



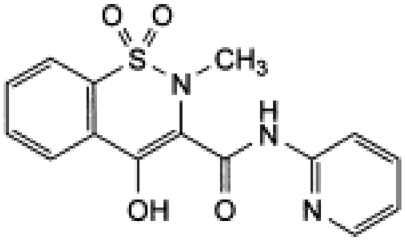
C. 4-(pyrrolidin-1-yl)dibenzo[*b*,*d*]furan-2-carboxylic acid.

Ph Eur

Piroxicam

General Notices

(Ph Eur monograph 0944)



$C_{15}H_{13}N_3O_4S$ 331.4 *36322-90-4*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Piroxicam Capsules

Piroxicam Gel

Ph Eur

DEFINITION

4-Hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Practically insoluble in water, soluble in methylene chloride, slightly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Comparison *piroxicam CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methylene chloride R*, evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 75 mg of the substance to be examined in *acetonitrile R*, warming slightly if necessary, and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *piroxicam for system suitability CRS* in *acetonitrile R* and dilute to 25.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 50.0 ml with *acetonitrile R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a 6.81 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Run time 5 times the retention time of piroxicam.

System suitability Reference solution (a):

— *relative retention* with reference to piroxicam: impurity B = about 0.85;

— *symmetry factor*: maximum 1.5 for the peak due to impurity B;

— the chromatogram obtained is similar to the chromatogram supplied with *piroxicam for system suitability CRS* .

Limits:
— *impurities A, B, C, D, E, F, G, H, I, J, K, L*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.02 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 60 ml of a mixture of equal volumes of *acetic anhydride R* and *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

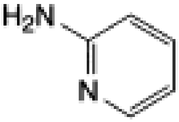
1 ml of *0.1 M perchloric acid* is equivalent to 33.14 mg of $C_{15}H_{13}N_3O_4S$.

STORAGE

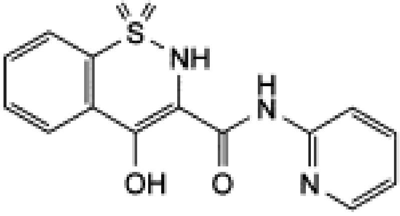
In an airtight container, protected from light.

IMPURITIES

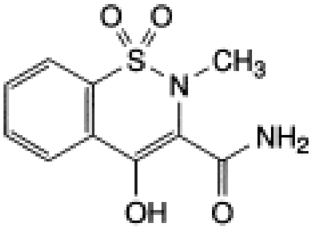
Specified impurities *A, B, C, D, E, F, G, H, I, J, K, L.*



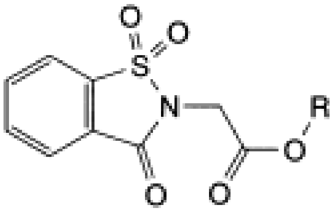
A. pyridin-2-amine,



B. 4-hydroxy-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide,



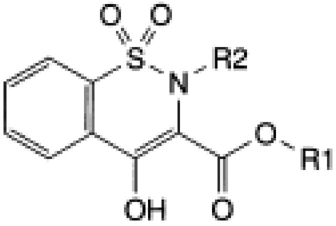
C. 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide,



D. R = CH_3: methyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)acetate,

E. R = C_2H_5: ethyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)acetate,

F. R = $CH(CH_3)_2$: 1-methylethyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)acetate,



G. R1 = CH_3, R2 = H: methyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,

H. R1 = C_2H_5, R2 = H: ethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,

I. R1 = $CH(CH_3)_2$, R2 = H: 1-methylethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,

J. R1 = R2 = CH_3: methyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,

K. R1 = C_2H_5, R2 = CH_3: ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,

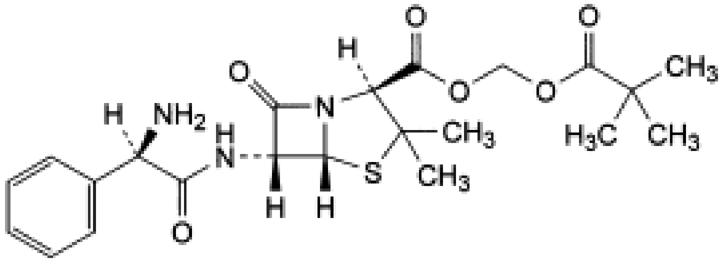
L. R1 = $CH(CH_3)_2$, R2 = CH_3: 1-methylethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide.

Ph Eur

Pivampicillin

General Notices

(Ph Eur monograph 0852)



$C_{22}H_{29}N_3O_6S$ 463.6 *33817-20-8*

Action and use

Antibacterial.

Ph Eur

DEFINITION

Methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 2,2-dimethylpropanoate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methanol, soluble in anhydrous ethanol. It dissolves in dilute acids.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pivampicillin CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 2 ml of *methanol R*.

Reference solution (a) Dissolve 10 mg of *pivampicillin CRS* in 2 ml of *methanol R*.

Reference solution (b) Dissolve 10 mg of *bacampicillin hydrochloride CRS*, 10 mg of *pivampicillin CRS* and 10 mg of *talampicillin hydrochloride CRS* in 2 ml of *methanol R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 10 volumes of a 272 g/l solution of *sodium acetate R* adjusted to pH 5.0 with *glacial acetic acid R*, 40 volumes of *water R* and 50 volumes of *ethanol (96 per cent) R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with *ninhydrin solution R1* and heat at 60 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is almost colourless. Place the test-tube in a water-bath for 1 min; a dark yellow colour develops.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method I*).

Dissolve 50 mg in 12 ml of *0.1 M hydrochloric acid*.

Specific optical rotation (*2.2.7*)

+ 208 to + 222 (anhydrous substance).

Dissolve 0.100 g in 5.0 ml of *ethanol (96 per cent) R* and dilute to 10.0 ml with *0.1 M hydrochloric acid*.

Triethanolamine

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.100 g of the substance to be examined in 1.0 ml of a mixture of 1 volume of *water R* and 9 volumes of *acetonitrile R*.

Reference solution Dissolve 5.0 mg of *triethanolamine R* in a mixture of 1 volume of *water R* and 9 volumes of *acetonitrile R* and dilute to 100 ml with the same mixture of solvents.

Plate *TLC silica gel plate R*.

Mobile phase *methanol R*, *butanol R*, *phosphate buffer solution pH 5.8 R*, *glacial acetic acid R*, *butyl acetate R* (5:15:24:40:80 *V/V/V/V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying At 110 °C for 10 min and allow to cool.

Chlorine treatment Place at the bottom of a chromatographic tank an evaporating dish containing a mixture of 1 volume of *hydrochloric acid R1*, 1 volume of *water R* and 2 volumes of a 15 g/l solution of *potassium permanganate R*; close the tank and allow to stand for 15 min; place the dried plate in the tank and close the tank; leave the plate in contact with the chlorine vapour in the tank for 15-20 min; withdraw the plate and allow it to stand in air for 2-3 min.

Detection Spray with *tetramethyldiaminodiphenylmethane reagent R*.

Limit:
— *triethanolamine*: any spot due to triethanolamine is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.05 per cent).

Related substances

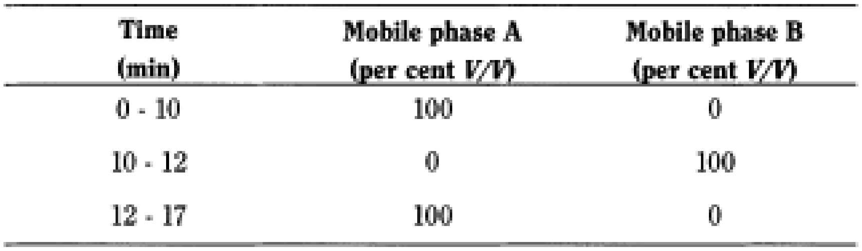
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in 10.0 ml of *acetonitrile R* and dilute to 20 ml with a 1 g/l solution of *phosphoric acid R*.

Reference solution Mix 2.0 ml of the test solution with 9.0 ml of *acetonitrile R* and 9.0 ml of a 1 g/l solution of *phosphoric acid R*.

Column:
— *size:* *l* = 0.125 m, Ø = 4 mm;
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R*.

Mobile phase:
— *mobile phase A*: mix 50 volumes of a 1.32 g/l solution of *ammonium phosphate R*, adjusted to pH 2.5 with a 100 g/l solution of *phosphoric acid R*, and 50 volumes of *acetonitrile R*;
— *mobile phase B*: mix 15 volumes of a 1.32 g/l solution of *ammonium phosphate R*, adjusted to pH 2.5 with a 100 g/l solution of *phosphoric acid R*, and 85 volumes of *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 12	0	100
12 - 17	100	0

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µl.

Retention time Pivampicillin dimer = about 5 min.

System suitability Reference solution:

— *ratio of the mass distribution ratio*: minimum 12 for the peak due to pivampicillin dimer to that of the peak due to pivampicillin (principal peak).

Limits:

— *total*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (3 per cent);

— *disregard limit*: 0.01 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

***N*,*N*-Dimethylaniline (*2.4.26, Method B*)**

Maximum 20 ppm.

Test solution To 1.00 g of the substance to be examined in a ground-glass-stoppered tube add 10 ml of *0.5 M sulphuric acid*. Heat the tube for 10 min in a water-bath, cool and add 15 ml of *1 M sodium hydroxide* and 1.0 ml of the internal standard solution. Stopper the tube and shake vigorously for 1 min. Centrifuge if necessary and use the upper layer.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.30 g.

Sulphated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*). Use the solutions within 2 h of preparation.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *pivampicillin CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 25.0 mg of *propyl parahydroxybenzoate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase. Mix 5.0 ml of this solution with 5.0 ml of reference solution (a).

Column:

— *size*: l = 0.125 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a 2.22 g/l solution of *phosphoric acid R* adjusted to pH 2.5 with *triethylamine R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

System suitability:

— *resolution*: minimum 5.0 between the peaks due to pivampicillin (1st peak) and propyl parahydroxybenzoate (2nd peak) in the chromatogram obtained with reference solution (b);

— *symmetry factor*: maximum 2.0 for the peak due to pivampicillin in the chromatogram obtained with reference solution (b);

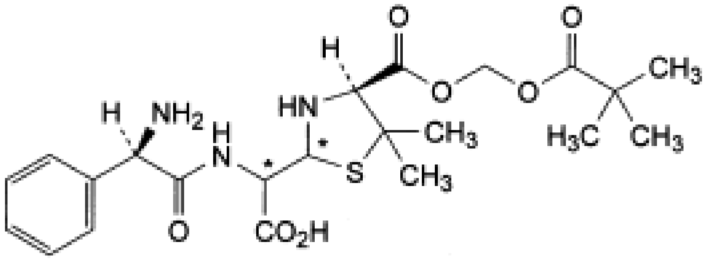
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

Calculate the percentage content of $C_{22}H_{29}N_3O_6S$ from the declared content of *pivampicillin CRS.*

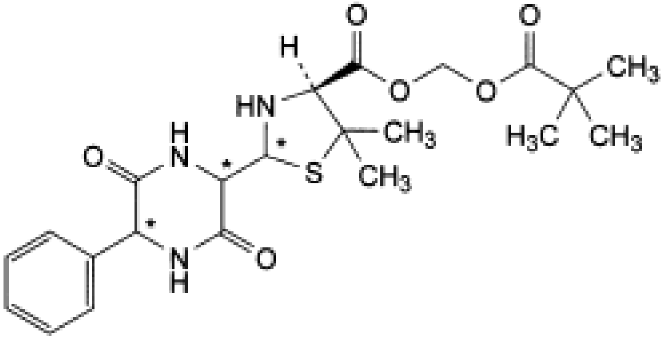
STORAGE

In an airtight container .

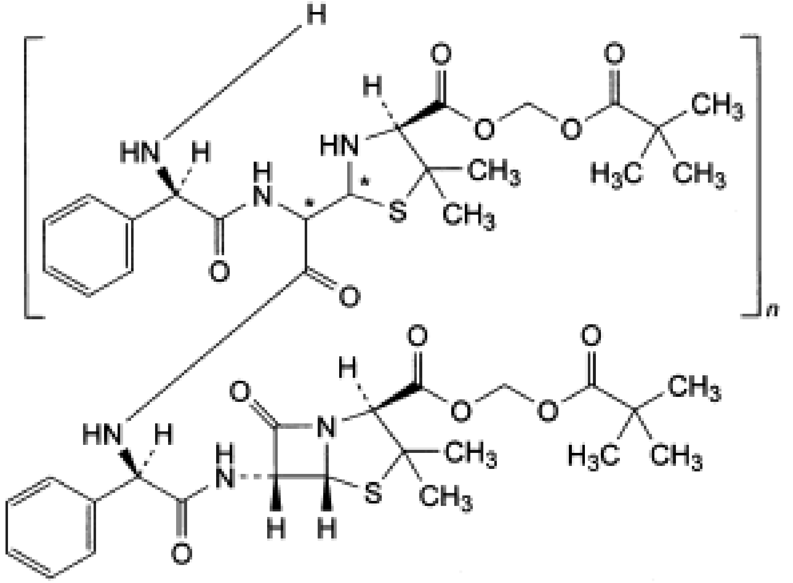
IMPURITIES



A. 2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-[(4*S*)-4-[[[(2,2-dimethylpropanoyl)oxy]methoxy] carbonyl]-5,5-dimethylthiazolidin-2-yl]acetic acid (penicilloic acids of pivampicillin),



B. methylene (4*S*)-5,5-dimethyl-2-(3,6-dioxo-5-phenylpiperazin-2-yl)thiazolidine-4-carboxylate 2,2-dimethylpropanoate (diketopiperazines of pivampicillin),



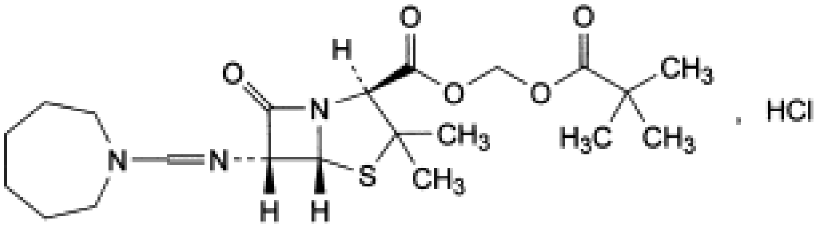
C. co-oligomers of pivampicillin and of penicilloic acids of pivampicillin.

Ph Eur

Pivmecillinam Hydrochloride

General Notices

(Ph Eur monograph 1359)



$C_{21}H_{33}N_{3}O_{5}S,HCl$ 476.0 *32887-03-9*

Action and use

Antibacterial.

Ph Eur

DEFINITION

Methylene 2,2-dimethylpropanoate (2*S*,5*R*,6*R*)-6-[[(hexahydro-1*H*-azepin-1-yl)methylene] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate hydrochloride.

Semi-synthetic product derived from a fermentation product.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, in anhydrous ethanol and in methanol, slightly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *pivmecillinam hydrochloride CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method I*).

Dissolve 0.5 g in *water R* and dilute to 10 ml with the same solvent.

pH (*2.2.3*)

2.8 to 3.8.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Solvent mixture To 45 volumes of *acetonitrile R* add 55 volumes of a 13.5 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *dilute phosphoric acid R*.

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 200.0 ml with the solvent mixture.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *pivmecillinam hydrochloride CRS* in the solvent mixture and dilute to 200.0 ml with the solvent mixture.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *pivmecillinam hydrochloride CRS* and 5 mg of *pivmecillinam impurity C CRS* in the solvent mixture, and dilute to 50 ml with the solvent mixture.

Column:

— *size: l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.55 g of *tetraethylammonium hydrogen sulphate R* and 1.0 g of *tetramethylammonium hydrogen sulphate R* in the solvent mixture and dilute to 1000 ml with the solvent mixture.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution (b) and reference solutions (b) and (c).

Run time 3 times the retention time of pivmecillinam.

System suitability Reference solution (c):

— *resolution*: minimum 3.5 between the peaks due to pivmecillinam (1^{st} peak) and impurity

C (2nd peak).

Limits:

— *any impurity*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

***N,N*-Dimethylaniline (*2.4.26, Method A*)**

Maximum 20 ppm.

Test solution Prepare as described in the general method but heat at about 27 °C after the addition of *strong sodium hydroxide solution R*, to dissolve the precipitate formed, then add the *trimethylpentane R.*

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dissolve 1.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):

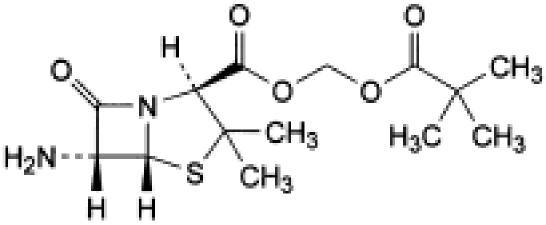
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{21}H_{34}ClN_3O_5S$ from the declared content of *pivmecillinam hydrochloride CRS.*

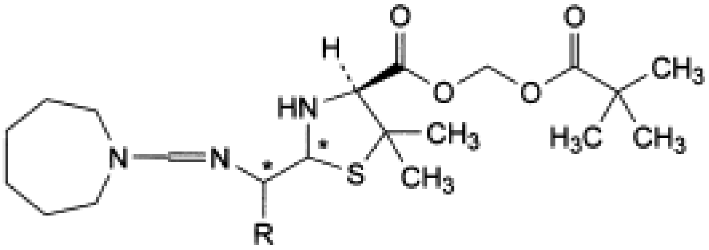
STORAGE

Protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES

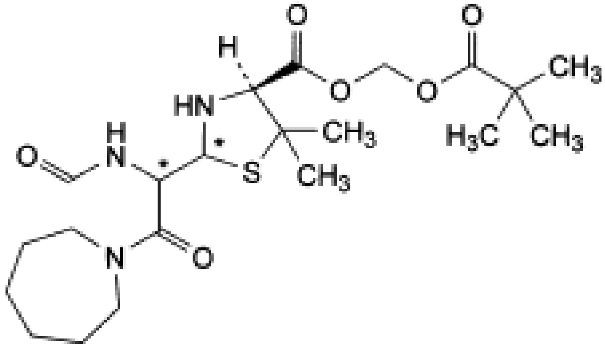


A. methylene (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 2,2-dimethylpropanoate (pivaloyloxymethyl 6-aminopenicillanate),



B. R = CO_2H: 2-[[(hexahydro-1*H*-azepin-1-yl)methylene]amino]-2-[(4*S*)-4-[[[(2,2-dimethylpropanoyl)oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetic acid (penicilloic acids of pivmecillinam),

C. R = H: methylene 2,2-dimethylpropanoate (2*RS*,4*S*)-2-[[[(hexahydro-1*H*-azepin-1-yl) methylene]amino]methyl]-5,5-dimethylthiazolidin-4-carboxylate,

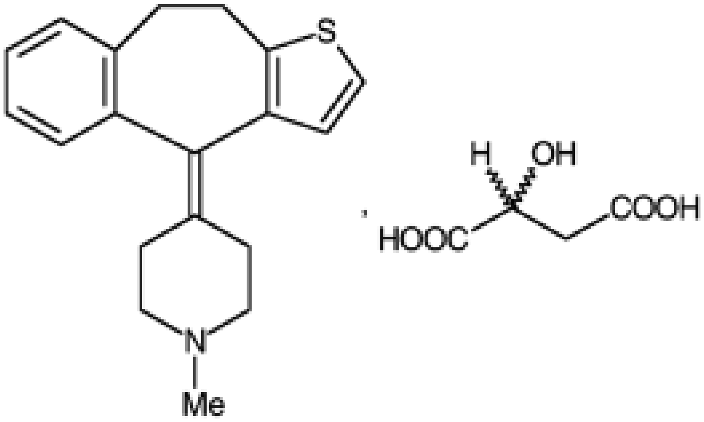


D. methylene 2,2-dimethylpropanoate (4*S*)-2-[1-(formylamino)-2-(hexahydro-1*H*-azepin-1-yl) -2-oxoethyl]-5,5-dimethylthiazolidin-4-carboxylate.

Ph Eur

Pizotifen Malate

General Notices



$C_{19}H_{21}NS,C_4H_6O_5$ 429.5 *5189-11-7*

Action and use

Serotonin (5HT) receptor partial agonist; prophylaxis of migraine.

Preparation

Pizotifen Tablets

DEFINITION

Pizotifen Malate is 9,10-dihydro-4-(1-methylpiperidin-4-ylidene)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophene hydrogen malate. It contains not less than 98.5% and not more than 101.5% of $C_{19}H_{21}NS,C_4H_6O_5$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or slightly yellowish white, crystalline powder.

Very slightly soluble in *water*; slightly soluble in *ethanol (96%)*; sparingly soluble in *methanol*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of pizotifen malate *(RS 277)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (5).

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 70 volumes of *isopropyl ether*, 25 volumes of *anhydrous formic acid* and 5 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dissolve 30 mg of the substance being examined in 1 ml of *ethanol (80%)*, heating if necessary. Solution (2) contains 1% w/v of *malic acid* in *ethanol (80%)*. After removal of the plate, dry it at 100° for 30 minutes, cool, spray with 0.02M *potassium permanganate* and dry in a current of warm air for about 1 minute. The chromatogram obtained with solution (1) exhibits a spot corresponding in position, colour and size to the spot in the chromatogram obtained with solution (2).

TESTS

Clarity and colour of solution

A 1.0% w/v solution in a mixture of equal volumes of *ethanol (96%)* and *water* is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* BY_6, Appendix IV B, Method II.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *toluene*, 60 volumes of *butan-1-ol*, 40 volumes of *hexane* and 3 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of five solutions in a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol* containing (1) 2.0% w/v, (2) 0.20% w/v, (3) 0.010% w/v and (4) 0.0050% w/v of the substance being examined and (5) 0.20% w/v of *pizotifen malate BPCRS*. After removal of the plate, dry it in a current of cold air for 5 minutes, spray with a mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of 2M *acetic acid* and then with *hydrogen peroxide solution (10 vol)*, cover immediately with a glass plate and examine in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4). Disregard any yellow spot or band remaining on the line of application.

Loss on drying

When dried to constant weight at 100° to 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.2%, Appendix IX A, Method II. Use the residue obtained in the test for Loss on drying.

ASSAY

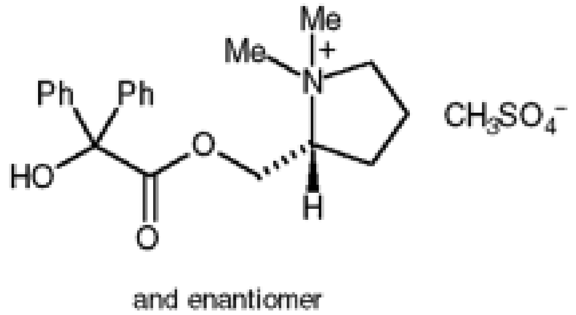
Dissolve 0.35 g in 60 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 42.95 mg of $C_{19}H_{21}NS,C_4H_6O_5$.

STORAGE

Pizotifen Malate should be protected from light.

Poldine Metilsulfate

General Notices



$C_{22}H_{29}NO_7S$ 451.5 *545-80-2*

Action and use

Anticholinergic.

Preparation

Poldine Tablets

DEFINITION

Poldine Metilsulfate is (*R*S)-2-benziloyloxy-methyl-1,1-dimethylpyrrolidinium methyl sulphate. It contains not less than 98.5% and not more than 100.5% of $C_{22}H_{29}NO_7S$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Freely soluble in *water*; soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of poldine metilsulfate *(RS 278)*.

B. The *light absorption*, Appendix II B, in the range 250 to 350 nm of a 0.1% w/v solution exhibits maxima at 252 nm and 258 nm and inflections at 262, 264 and 268 nm. The *absorbance* at the maximum at 252 nm is about 0.85 and at the maximum at 258 nm is about 0.99.

C. Dissolve 2 mg in 10 ml of *water*, add 20 ml of *ammonium cobaltothiocyanate solution* and 5 ml of *dichloromethane* and shake well. The dichloromethane layer becomes blue.

TESTS

Acidity or alkalinity

pH of a 1% w/v solution, 5.0 to 7.0, Appendix V L.

Melting point

137° to 142°, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 60 volumes of *dichloromethane*, 30 volumes of *methanol*, 5 volumes of *formic acid* and 5 volumes of *water* as the mobile phase. Apply separately to the plate 20 µl of each of two solutions of the substance being examined in a mixture of 5 volumes of *methanol* and 1 volume of *formic acid* containing (1) 1.0% w/v and (2) 0.010% w/v. After removal of the plate, allow it to dry in air, spray with *sulphuric acid (50%)* and heat at 110° for 10 minutes. Any pink *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried to constant weight at 80°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.5 g in 10 ml of *water* in a stoppered flask, add 0.05 ml of *cresol red solution* and, if necessary, sufficient 0.05M *sodium hydroxide* to produce a pale pink colour. Add 20 ml of 0.1M *sodium hydroxide VS*, stopper the flask and allow to stand for 10 minutes at 25°. Titrate with 0.1M *hydrochloric acid VS* using *cresol red solution* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of alkali required. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 45.15 mg of $C_{22}H_{29}NO_7S$.

Poloxamers

General Notices

(Ph Eur monograph 1464)

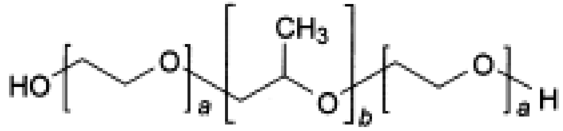
Action and use

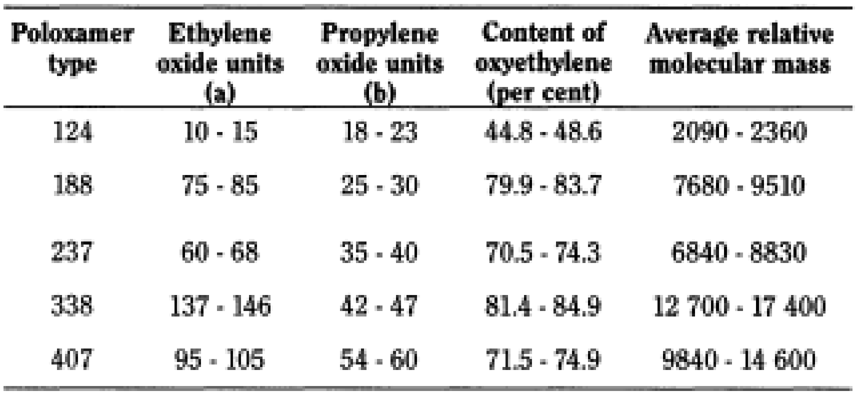
Non-ionic surfactant.

Ph Eur

DEFINITION

Synthetic block copolymer of ethylene oxide and propylene oxide, represented by the following general formula:





Poloxamer type	Ethylene oxide units (a)	Propylene oxide units (b)	Content of oxyethylene (per cent)	Average relative molecular mass
124	10 - 15	18 - 23	44.8 - 48.6	2090 - 2360
188	75 - 85	25 - 30	79.9 - 83.7	7680 - 9510
237	60 - 68	35 - 40	70.5 - 74.3	6840 - 8830
338	137 - 146	42 - 47	81.4 - 84.9	12 700 - 17 400
407	95 - 105	54 - 60	71.5 - 74.9	9840 - 14 600

A suitable antioxidant may be added.

CHARACTERS

Appearance:

— *poloxamer 124*: colourless or almost colourless liquid;

— *poloxamers 188, 237, 338, 407* : white or almost white, waxy powder, microbeads or flakes.

Solubility:

- *poloxamers 124, 237, 338, 407* : very soluble in water and in ethanol (96 per cent), practically insoluble in light petroleum (50-70 °C);
- *poloxamer 188*: soluble in water and in ethanol (96 per cent).

mp

About 50 °C for poloxamers 188, 237, 338 and 407.

IDENTIFICATION

First identification *A, B.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Chemical reference substance of the Ph. Eur. corresponding to the type of poloxamer to be examined.

B. Average relative molecular mass (see Tests).

C. Oxypropylene:oxyethylene ratio (see Tests).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

pH (*2.2.3*)

5.0 to 7.5 for solution S.

Ethylene oxide, propylene oxide and dioxin

Head-space gas chromatography (*2.2.28*).

Ethylene oxide stock solution Introduce 0.5 g of *ethylene oxide solution R5* into a vial and dilute to 50.0 ml with *dimethyl sulphoxide R1*. Mix carefully.

Ethylene oxide solution Dilute 1.0 ml of the ethylene oxide stock solution to 250 ml with *dimethyl sulphoxide R1*.

Propylene oxide stock solution Introduce about 7 ml of *methylene chloride R* into a volumetric flask, add 0.500 g (m) of *propylene oxide R* and dilute to 10.0 ml with *methylene chloride R*. Dilute 0.5 ml of this solution to 50.0 ml with *dimethyl sulphoxide R1*. Mix carefully. Calculate the exact concentration of propylene oxide in mg/ml using the following expression:

$$\frac{m \times 1000 \times 0.5}{10 \times 50}$$

Propylene oxide solution. Dilute 1.0 ml of the propylene oxide stock solution to 50.0 ml with *dimethyl sulphoxide R1*.

Calculate the exact concentration of propylene oxide in µg/ml using the following expression:

$$\frac{C \times 1000 \times 1}{50}$$

C = concentration of the propylene oxide stock solution in mg/ml.

Dioxan solution Introduce 0.100 g (m) of *dioxan R* into a flask and dilute to 50.0 ml with *dimethyl sulphoxide R1*. Dilute 2.50 ml of this solution to 100.0 ml with *dimethyl sulphoxide R1*.

Calculate the exact concentration of dioxan in µg/ml using the following expression:

$$\frac{m \times 2.50 \times 1000 \times 1000}{50 \times 100}$$

Mixture solution Dilute a mixture of 6.0 ml of the ethylene oxide solution, 6.0 ml of the propylene oxide solution and 2.5 ml of the dioxan solution to 25.0 ml with *dimethyl sulphoxide R1*.

Test solution To 1.000 g of the substance to be examined in a head-space vial, add 4.0 ml of *dimethyl sulphoxide R1* and close the vial immediately.

Reference solution To 1.000 g of the substance to be examined in a head-space vial, add 2.0 ml of *dimethyl sulphoxide R1* and 2.0 ml of the mixture solution. Close the vial immediately.

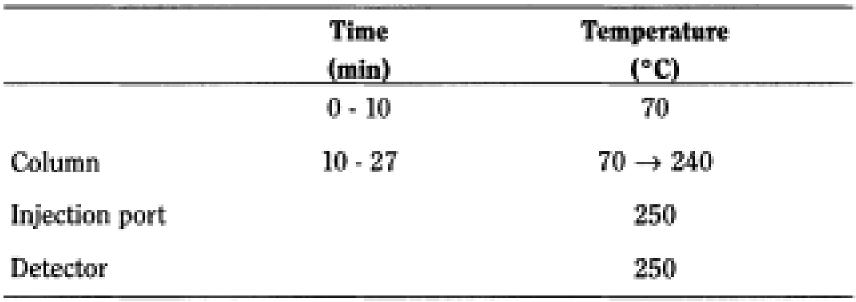
Column:
- — *material*: fused silica;
- — *size:* l = 50 m, Ø = 0.32 mm;
- — *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.4 ml/min.

Static head-space conditions:
- — *equilibrium temperature*: 110 °C;
- — *equilibration time*: 30 min;
- — *transfer-line temperature*: 140 °C;
- — *pressurisation time*: 1 min;
- — *injection time*: 0.05 min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	70
	10 - 27	70 → 240
Injection port		250
Detector		250

Detection Flame ionisation.

Injection Inject a suitable volume of the gaseous phase, for example 1 ml.

Relative retention With reference to ethylene oxide (retention time = about 6 min): propylene oxide = about 1.3; methylene chloride = about 1.6; dioxan = about 3.0; dimethyl sulphoxide = about 3.7.

Limits:

- *ethylene oxide*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (1 ppm);
- *propylene oxide*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (5 ppm);
- *dioxan*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm).

Average relative molecular mass

Weigh 15 g (m) of the substance to be examined into a 250 ml ground-glass-stoppered flask, add 25.0 ml of *phthalic anhydride solution R* and a few glass beads and swirl to dissolve. Boil gently under a reflux condenser for 1 h, allow to cool and add 2 quantities, each of 10 ml, of *pyridine R*, through the condenser. Add 10 ml of *water R*, mix and allow to stand for 10 min. Add 40.0 ml of *0.5 M sodium hydroxide* and 0.5 ml of a 10 g/l solution of *phenolphthalein R* in *pyridine R*. Titrate with *0.5 M sodium hydroxide* to a light pink endpoint that persists for 15 s and record the volume of sodium hydroxide used (S). Prepare a blank. Record the volume of sodium hydroxide used (B).

Calculate the average relative molecular mass using the following expression:

$$\frac{4000m}{B - S}$$

Oxypropylene:oxyethylene ratio

Nuclear magnetic resonance spectrometry (*2.2.33*).

Use a 100 g/l solution of the substance to be examined in *deuterated chloroform R*. Record the average area of the doublet appearing at about 1.08 ppm due to the methyl groups of the oxypropylene units (A_1) and the average area of the composite band from 3.2 ppm to 3.8 ppm due to CH_2O groups of both the oxyethylene and oxypropylene units and the CHO groups of the oxypropylene units (A_2) with reference to the internal standard.

Calculate the percentage of oxyethylene, by weight, in the sample being examined using the following expression:

$$\frac{3300\alpha}{33\alpha + 58}$$

where $\alpha = \frac{A_2}{A_1} - 1$

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.000 g.

Total ash (*2.4.16*)

Maximum 0.4 per cent, determined on 1.0 g.

STORAGE

In an airtight container.

LABELLING

The label states the type of poloxamer.

Ph Eur

Poly(vinyl acetate) Dispersion 30 per cent

General Notices

(Ph Eur monograph 2152)

Ph Eur

DEFINITION

Dispersion in water of poly(vinyl acetate) having a mean relative molecular mass of about 450 000. It may contain *Povidone (0685)* and a suitable surface-active agent, such as *Sodium laurilsulphate (0098)*, as stabilisers.

Content

25.0 per cent to 30.0 per cent of poly(vinyl acetate).

CHARACTERS

Appearance

Opaque, white or almost white, slightly viscous liquid.

Solubility

Miscible with water and with ethanol (96 per cent).

It is sensitive to spoilage by microbial contaminants.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dry 1 ml *in vacuo*, dissolve the residue in *acetone R*, and spread 1 drop of the solution between 2 *sodium chloride R* plates; remove 1 plate and allow the solvent to evaporate.

Comparison Repeat the operation using *poly(vinyl acetate) dispersion 30 per cent CRS*.

B. Place 3 ml on a glass plate and allow to dry. A clear film is formed.

C. 50 mg gives the reaction of acetyl *(2.3.1)*.

TESTS

Agglomerates

Filter 100.0 g through a tared stainless steel sieve (90). Rinse with *water R* until a clear filtrate is obtained and dry to constant mass at 100-105 °C. The mass of the residue is not greater than 0.500 g.

Vinyl acetate

Liquid chromatography *(2.2.29)*.

Test solution Introduce 0.250 g into a 10 ml volumetric flask and add about 1 ml of *methanol R2*. Sonicate. Add about 8 ml of *water for chromatography R*. Sonicate and dilute to 10.0 ml with *water for chromatography R*. Centrifuge for about 10 min and filter.

Reference solution (a) Dissolve 5.0 mg of *vinyl acetate CRS* in *methanol R2* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of the solution to 20.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) To 5 mg of *vinyl acetate R* and 5 mg of *1-vinylpyrrolidin-2-one R*, add 10 ml of *methanol R2* and sonicate. Dilute to 50 ml with mobile phase A. Dilute 1 ml of this solution to 20 ml with mobile phase A.

A precolumn containing *octadecylsilyl silica gel for chromatography R* (5 µm) may be used if a matrix effect is observed.

Column:

- *size: l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: *acetonitrile for chromatography R, methanol R2, water for chromatography R* (5:5:90 *V/V/V*);
- *mobile phase B: methanol R2, acetonitrile for chromatography R, water for chromatography R* (5:45:50 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 26	100 → 80	0 → 20
26 - 27	80 → 0	20 → 100
27 - 30	0 → 100	100 → 0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µl of the test solution and reference solutions (a) and (b).

System suitability Reference solution (b):

- *resolution*: minimum 5.0 between the peaks due to vinyl acetate and 1-vinylpyrrolidin-2-one.

Limit:

- *vinyl acetate*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (100 ppm).

Povidone

Maximum 4.0 per cent.

Carry out the determination of nitrogen by sulphuric acid digestion *(2.5.9)* on 0.25 g. Calculate the percentage content of povidone using the following expression:

$$\frac{N}{0.126}$$

N = percentage content of nitrogen.

Acetic acid

Liquid chromatography *(2.2.29)*.

Test solution Mix 0.200 g with *water for chromatography R*. Sonicate for about 10 min and dilute to 10.0 ml with *water for chromatography R*.

Reference solution Dissolve 30.0 mg of *acetic acid R* and 30 mg of *citric acid R* in the mobile phase. Shake gently to dissolve and dilute to 100.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *0.005 M sulphuric acid*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl. After each injection, rinse the column with a mixture of equal volumes of *acetonitrile for chromatography R* and *0.005 M sulphuric acid*.

Retention time Acetic acid = about 6 min; citric acid = about 8 min.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the peaks due to citric acid and acetic acid.

Limit:
— *acetic acid*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (1.5 per cent).

Residue on evaporation

0.285 g to 0.315 g, determined on 1.000 g at 110 °C for 5 h.

Sulphated ash

Maximum 0.5 per cent, determined on 1.0 g.

Heat a silica crucible to redness for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.00 g of the preparation to be examined in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 °C ± 25 °C, until the substance is thoroughly charred. Carry out the test for sulphated ash *(2.4.14)* on the residue obtained, starting with "Moisten the substance to be examined...".

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count.

ASSAY

Determine the saponification value *(2.5.6)* on 1.5 g and calculate the percentage content of poly(vinyl acetate) using the following expression:

$$I_s \times 0.1534$$

I_s = saponification value.

STORAGE

At a temperature of 5 °C to 30 °C. Handle the substance so as to minimise microbial contamination.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient. This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for poly(vinyl acetate) dispersion 30 per cent used in the manufacture of modified-release dosage forms and to mask taste.

Solubility of a film

Place the film obtained in identification test B in 50 ml of *phosphate buffer solution pH 6.8 R* whilst stirring continuously. The film does not dissolve within 30 min.

Apparent viscosity *(2.2.10)*

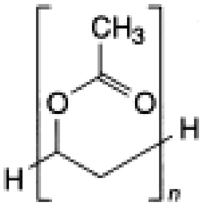
Maximum 100 mPa·s, determined using a rotating viscometer at 20 °C and a shear rate of 100 s^{-1}.

Ph Eur

Poly(vinyl acetate)

General Notices

(Ph Eur monograph 1962)



Ph Eur

DEFINITION

Poly(vinyl acetate) is a thermoplastic polymer obtained by polymerisation of vinyl acetate using a suitable starter, without solvent or with water or 2-propanol. The vast majority of the acetate moieties are attached to non-neighbouring carbon atoms of the chain.

The index *n* is about 100 - 17 000. The relative molecular mass lies between 10 000 and 1500 000. The viscosity is 4 to 250 mPa·s. The ester value, which characterises the degree of hydrolysis, is 615 to 675.

CHARACTERS

Appearance

White or almost white powder or colourless granules or beads.

Solubility

Practically insoluble in water, freely soluble in ethyl acetate, soluble in alcohol. It is hygroscopic and swells in water.

It softens at temperatures above 40-50 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs prepared as follows: dissolve about 200 mg in 5 ml of *acetone R*, place 2 drops on a *potassium bromide R* disc and dry to evaporate the solvent.

The spectrum obtained shows absorption maxima corresponding to poly(vinyl acetate) at 1750 cm^{-1}, 1400 cm^{-1} and 1020 cm^{-1}.

B. It complies with the test for viscosity (see Tests).

C. Saponify 0.500 g in a mixture of 25.0 ml of *0.5 M alcoholic potassium hydroxide R* and 25.0 ml of *water R* (*2.5.6*). The solution obtained gives the reaction of acetates (*2.3.1*).

TESTS

Solution S

Suspend 50.0 g in 100 ml of *ethyl acetate R* in a borosilicate glass flask with a ground-glass neck. Heat under a reflux condenser with constant stirring for 30 min. Allow to cool. Filter through a tared sintered glass filter (16) (*2.1.2*) and wash the residue with 50.0 ml of *ethyl acetate R*, pour the filtrate into a 250 ml graduated flask. Dilute to 250 ml with *ethyl acetate R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method I*).

Viscosity (*2.2.49*)

85 per cent to 115 per cent of the value stated on the label.

Determine the viscosity immediately after preparation of solution S at 20 ± 0.1 °C by using a rolling ball viscosimeter.

Acid value (*2.5.1*)

Maximum 2.0, determined on 5.0 g dissolved in 50 ml of *alcohol R* by shaking for 3 h.

Ester value (*2.5.2*)

615 to 675.

Saponify 0.500 g in a mixture of 25.0 ml of *0.5 M alcoholic potassium hydroxide* and 25.0 ml of *water R* (*2.5.6*).

Residual peroxides

Maximum 100 ppm, calculated as hydrogen peroxide.

Place 0.85 g in a borosilicate glass flask with a ground-glass neck. Add 10 ml of *ethyl acetate R* and heat under a reflux condenser with constant agitation. Allow to cool. Replace the air in the container with *oxygen-free nitrogen R* and add a solution of 1 ml of *glacial acetic acid R* and 0.5 g of *sodium iodide R* in 40 ml of *water R*. Shake thoroughly and allow to stand protected from light for 20 min. Titrate with *0.005 M sodium thiosulphate R* until the yellow colour is discharged. Carry out a blank titration. The difference between the titration volumes is not greater than 1.0 ml.

Vinyl acetate

Head-space gas chromatography (*2.2.28*).

Reference solution Place 15 ml of *dimethylformamide R* in a 20 ml vial, add 45 µl of *vinyl acetate R* and 50.0 µl of *butanal R* and dilute to volume with *dimethylformamide R*. Dilute 1 ml of the solution to 10 ml with *dimethylformamide R*.

Test solution (a) Place 0.2000 g of the substance to be examined in a 20 ml vial and add 1.00 ml of *dimethylformamide R*. Close the vial and secure the stopper. Shake, avoiding contact between the stopper and the liquid.

Test solution (b) Place 0.2000 g of the substance to be examined in a 20 ml vial and add 1.00 ml of the reference solution. Close the vial and secure the stopper. Shake, avoiding contact between the stopper and the liquid.

Column:
- — *material*: fused silica;
- — *size*: *l* = 25 m, Ø = 0.32 mm;
- — *stationary phase*: *poly(dimethyl)(diphenyl)(divinyl)siloxane R* (film thickness: 0.32 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 20 ml/min.

Static head-space conditions which may be used:
- — *equilibration temperature*: 60 °C;
- — *equilibration time*: 20 min;
- — *transfer-line temperature*: 120 °C;
- — *carrier gas*: *nitrogen for chromatography R*.

Temperature:
- — *column*: 155 °C;
- — *injection port*: 120 °C;
- — *detector*: 180 °C.

Detection Flame ionisation.

Injection 1.6 ml of the gaseous phase of test solutions (a) and (b).

System suitability Test solution (b):
- — *resolution*: minimum of 2.0 between the peaks due to vinyl acetate and butanal;
- — *signal-to-noise ratio*: minimum of 5 for the vinyl acetate peak.

Calculate the percentage of vinyl acetate from the expression:

$$\frac{V \times S_1 \times 0.931}{(m_1 S_2 - m_2 S_1) \times 2000}$$

S_1 = peak area or height for vinyl acetate in the chromatogram obtained with test solution (a),

S_2 = peak area or height for vinyl acetate in the chromatogram obtained with test solution (b),

m_1 = mass of the substance to be examined used to prepare test solution (a), in grams,

m_2 = mass of the substance to be examined used to prepare test solution (b), in grams, 0.931 = density of vinyl acetate, in grams per millilitre,

V = volume of vinyl acetate used to prepare the reference solution, in microlitres.

Limit:

— *vinyl acetate*: maximum 0.3 per cent.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

1.0 g complies with limit test D. Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

The following test concerning the pharmacotechnological properties may be carried out depending on the intended formulation. It is not a mandatory requirement.

Infrared absorption spectrum (*2.2.24*)

The determination of the spectrum and comparison with a suitable sample is useful for ensuring suitable functionality-related properties.

LABELLING

The label states:

— the nominal relative molecular mass;

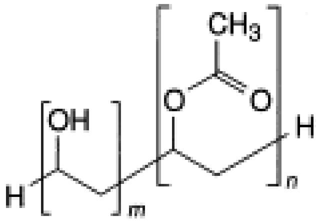
— the viscosity.

Ph Eur

Poly(vinyl alcohol)

General Notices

(Ph Eur monograph 1961)



Ph Eur

DEFINITION

Poly(vinyl alcohol) is obtained by polymerisation of vinyl acetate, followed by partial or almost complete hydrolysis of poly(vinyl acetate) in the presence of catalytic amounts of alkali or mineral acids.

Poly(vinyl alcohol) polymers comply with the following indices:

$$0 \leq \frac{n}{m} \leq 0.35$$

The mean relative molecular mass lies between 20 000 and 150 000. The viscosity is 3 to 70 mPa·s. The ester value which characterises the degree of hydrolysis is not greater than 280.

CHARACTERS

Appearance

Yellowish-white powder or translucent granules.

Solubility

Soluble in water, slightly soluble in ethanol, practically insoluble in acetone.

Various grades of poly(vinyl alcohol) are available. They differ in their degree of polymerisation and their degree of hydrolysis which determine the physical properties of the different grades. They are characterised by the viscosity and the ester value of the substance.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs; in some cases, the sample has to be milled, after cooling if necessary, before preparing the discs.

The spectrum obtained shows absorption maxima corresponding to poly(vinyl alcohol) at 2940 cm^{-1} and 2920 cm^{-1}.

B. It complies with the test for viscosity (see Tests).

TESTS

Solution S

Heat on a water-bath 250 ml of *water R* in a borosilicate round-bottomed flask attached to a reflux condenser with stirrer, add 10.0 g of the substance to be examined and continue heating for 30 min with continuous stirring. Remove the flask from the water-bath and continue stirring until room temperature is reached.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 6.5 for solution S.

Viscosity (*2.2.49*)

85 per cent to 115 per cent of the value stated on the label.

Determine the viscosity using a falling ball viscometer immediately after preparation of solution S at 20 ± 0.1 °C.

Acid value

Maximum 3.0.

Add 1 ml of *phenolphthalein solution R* to 50 ml of solution S and titrate with *0.05 M potassium hydroxide* until the pink colour persists for 15 s. Calculate the acid value from the expression:

$$\frac{2.805V}{2}$$

V = number of millilitres of 0.05 M potassium hydroxide used.

Ester value (*2.5.2*)

90 per cent to 110 per cent of the value stated on the label.

Saponify 1.00 g in a mixture of 25.0 ml of *0.5 M alcoholic potassium hydroxide* and 25.0 ml of *water R* (2.5.6).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

1.0 g complies with limit test D. Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 1.0 per cent, determined on 1.0 g.

The following test concerning the pharmacotechnological properties may be carried out depending on the intended formulation. It is not a mandatory requirement.

Infrared absorption spectrum (*2.2.24*)

The determination of the spectrum and comparison with a suitable sample is useful for ensuring suitable functionality-related properties. The intensities of the absorption maxima at 1720 cm^{-1} and 1260 cm^{-1} are inversely proportional to the degree of hydrolysis.

LABELLING

The label states:

- the viscosity for a 40 g/l solution;
- the ester value.

Ph Eur

Polyacrylate Dispersion (30 per cent)

General Notices

(Ph Eur monograph 0733)

Action and use

Excipient.

Ph Eur

DEFINITION

Dispersion in water of a copolymer of ethyl acrylate and methyl methacrylate having a mean relative molecular mass of about 800 000.

Content

28.5 per cent to 31.5 per cent (residue on evaporation).

It may contain a suitable emulsifier.

CHARACTERS

Appearance

Opaque, white or almost white, slightly viscous liquid.

Solubility

Miscible with water, soluble in acetone, in anhydrous ethanol and in 2-propanol.

IDENTIFICATION

First identification *A.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of polyacrylate.

B. To 1 g add 5 ml of *water R* and mix; the mixture remains opaque. Take 3 portions of 1 g and mix separately with 5 g of *anhydrous ethanol R*, 5 g of *acetone R* and 5 g of *2-propanol R*. Transparent solutions are obtained.

C. To 1 g add 10 ml of *0.1 M sodium hydroxide*. The mixture remains opaque.

D. Appearance of a film (see Tests).

E. Dry 4 g in a Petri dish at 60 °C in an oven for 4 h and transfer the resulting clear film to a small test-tube (100 mm × 12 mm). Heat over a flame and collect the fumes that evolve in a 2nd test-tube held over the mouth of the 1st tube. The condensate gives the reaction of esters (*2.3.1*).

TESTS

Relative density (*2.2.5*)

1.037 to 1.047.

Apparent viscosity (*2.2.10*)

Maximum 50 mPa·s, determined using a rotating viscometer at 20 °C and a shear rate of 10 s^{-1}.

Appearance of a film

Pour 1 ml on a glass plate and allow to dry. A clear elastic film is formed.

Particulate matter

Filter 100.0 g through a tared stainless steel sieve (90). Rinse with *water R* until a clear filtrate is obtained and dry at 80 °C to constant mass. The residue weighs not more than 0.500 g.

Residual monomers

Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.00 g of the substance to be examined in *tetrahydrofuran R* and dilute to 50.0 ml with the same solvent. To 5.0 ml of a 35 g/l solution of *sodium perchlorate R* add 10.0 ml of the solution dropwise whilst stirring continuously. Centrifuge and filter the clear supernatant liquid. Dilute 5.0 ml of this solution to 10.0 ml with *water R*.

Reference solution Dissolve 10 mg of *ethyl acrylate R* and 10 mg of *methyl methacrylate R* in *tetrahydrofuran R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *tetrahydrofuran R*. To 10.0 ml of the solution add 5.0 ml of a 35 g/l solution of *sodium perchlorate R* and mix. Dilute 5.0 ml of the mixture to 10.0 ml with *water R*.

Column:
— *size: l* = 0.12 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase *acetonitrile R1*, *water for chromatography R* (15:85 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 205 nm.

Injection About 50 µl.

Limit:
— *residual monomers*: maximum 100 ppm.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Sulphated ash (*2.4.14*)

Maximum 0.4 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 103 micro-organisms per gram, determined by plate count.

ASSAY

Dry 1.000 g at 110 °C for 3 h and weigh the residue.

STORAGE

At a temperature of 5 °C to 25 °C. Protect from freezing. Handle the substance so as to minimise microbial contamination.

LABELLING

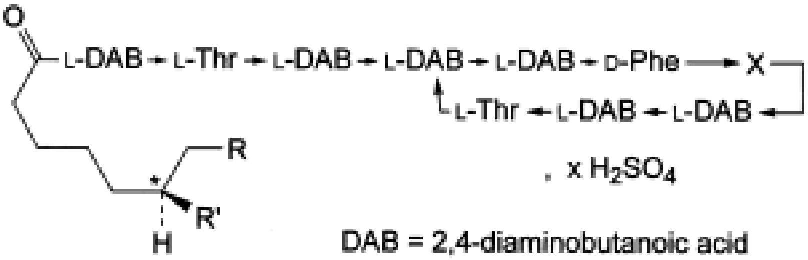
The label states, where applicable, the name and concentration of any added emulsifier.

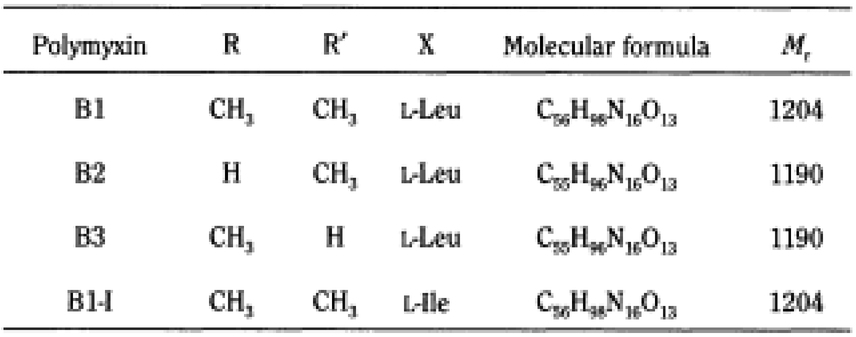
Ph Eur

Polymyxin B Sulphate

General Notices

(Ph Eur monograph 0203)


O
L-DAB
L-Thr
L-DAB
L-DAB
L-DAB
D-Phe
X
L-Thr
L-DAB
L-DAB
R
R'
H
, x H_2SO_4
DAB = 2,4-diaminobutanoic acid



Polymyxin	R	R′	X	Molecular formula	M_r
B1	CH_3	CH_3	L-Leu	$C_{56}H_{98}N_{16}O_{13}$	1204
B2	H	CH_3	L-Leu	$C_{55}H_{96}N_{16}O_{13}$	1190
B3	CH_3	H	L-Leu	$C_{55}H_{96}N_{16}O_{13}$	1190
B1-I	CH_3	CH_3	L-Ile	$C_{56}H_{98}N_{16}O_{13}$	1204

1405-20-5

Action and use

Antibacterial.

Preparation

Polymyxin and Bacitracin Eye Ointment

Ph Eur

DEFINITION

Mixture of the sulphates of polypeptides produced by the growth of certain strains of *Paenibacillus polymyxa*, or obtained by any other means, the main component being polymyxin B1.

Content:

— *sum of polymyxins B1, B2, B3 and B1-I*: minimum 80.0 per cent (dried substance);

— *polymyxin B3* : maximum 6.0 per cent (dried substance);

— *polymyxin B1-I* : maximum 15.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *hydrochloric acid R* and *water R*. Heat at 135 °C in a sealed tube for 5 h. Evaporate to dryness on a water-bath and continue the heating until the hydrochloric acid has evaporated. Dissolve the residue in 0.5 ml of *water R*.

Reference solution (a) Dissolve 20 mg of *leucine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *threonine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 20 mg of *phenylalanine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (d) Dissolve 20 mg of *serine R* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Carry out the following procedures protected from light.

Mobile phase *water R*, *phenol R* (25:75 *V/V*).

Application 5 µl as bands of 10 mm, then place the plate in the chromatographic tank so that it is not in contact with the mobile phase, and allow it to become impregnated with the vapour of the mobile phase for at least 12 h.

Development Over a path of 12 cm using the same mobile phase.

Drying At 100-105 °C.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The chromatogram obtained with the test solution shows zones corresponding to those in the chromatograms obtained with reference solutions (a), (b) and (c), but shows no zone corresponding to that in the chromatogram obtained with reference solution (d); the chromatogram obtained with the test solution also shows a zone with a very low R_F value (2, 4-diaminobutyric acid).

B. Examine the chromatograms obtained in the assay.

Results The peaks due to polymyxins B1, B2, B3 and B1-I in the chromatogram obtained with the test solution are similar in retention time to the corresponding peaks in the chromatogram obtained with reference solution (a).

C. Dissolve about 2 mg in 5 ml of *water R* and add 5 ml of a 100 g/l solution of *sodium hydroxide R*. Shake and add dropwise 0.25 ml of a 10 g/l solution of *copper sulphate R*, shaking after each addition. A reddish-violet colour develops.

D. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

pH *(2.2.3)*

5.0 to 7.0.

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 78 to - 90 (dried substance).

Dissolve 0.50 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*: use the normalisation procedure.

Test solution Dissolve 50.0 mg of the substance to be examined in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 50.0 mg of *polymyxin B sulphate CRS* in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a solution prepared as follows: dissolve 4.46 g of *anhydrous sodium sulphate R* in 900 ml of *water R*, adjust to pH 2.3 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection: 20 µl.

Run time 1.4 times the retention time of polymyxin B1.

Relative retention With reference to polymyxin B1 (retention time = about 35 min): polymyxin B2 = about 0.5; polymyxin B3 = about 0.6; polymyxin B1-I = about 0.8.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to polymyxin B2 and polymyxin B3.

Limits:

— *any impurity*: for each impurity, maximum 3.0 per cent;

— *total*: maximum 17.0 per cent;

— *disregard limit*: 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphate

15.5 per cent to 17.5 per cent (dried substance).

Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 with *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate*, adding 50 ml of *ethanol (96 per cent) R* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Loss on drying *(2.2.32)*

Maximum 6.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.75 per cent, determined on 1.0 g.

Pyrogens *(2.6.8)*

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject, per kilogram of the rabbit's mass, 1 ml of a solution in *water for injections R* containing 1.5 mg of the substance to be examined per millilitre.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of polymyxin B3, of polymyxin B1-I, and of the sum of polymyxins B1, B2, B3 and B1-I, using the following expression:

$$C_{\mathrm{B}i} = \frac{A_{\mathrm{B}i} \times m_2 \times D_{\mathrm{B}i}}{m_1 \times B_{\mathrm{B}i}}$$

C_{Bi} = percentage content of polymyxin B*i*,

A_{Bi} = area of the peak due to polymyxin B*i* in the chromatogram obtained with the test solution,

m_1 = mass of the substance to be examined (dried substance) in the test solution, in milligrams,

B_{Bi} = area of the peak due to polymyxin B *i* in the chromatogram obtained with reference solution (a),

m_2 = mass of *polymyxin B sulphate CRS* in reference solution (a), in milligrams,

D_{Bi} = declared percentage content for polymyxin B *i* in *polymyxin B sulphate CRS.*

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

Ph Eur

Polysorbate 20

General Notices

Polyoxyethylene 20 Sorbitan Monolaurate

(Ph Eur monograph 0426)

Action and use

Non-ionic surfactants.

Ph Eur

DEFINITION

Mixture of partial esters of fatty acids, mainly lauric (dodecanoic) acid, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

CHARACTERS

Appearance

Oily, yellow to brownish-yellow, clear or slightly opalescent liquid.

Solubility

Soluble in water, in ethanol, in ethyl acetate and in methanol, practically insoluble in fatty oils and in liquid paraffin.

Relative density

About 1.10.

Viscosity

About 400 mPa·s at 25 °C.

IDENTIFICATION

First identication A, D.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of polysorbate 20.

B. It complies with the test for hydroxyl value (see Tests).

C. It complies with the test for saponification value (see Tests).

D. It complies with the test for composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 ml of *methylene chloride R*. Add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R*. Stir with a glass rod. The solution becomes blue.

TESTS

Acid value (*2.5.1*)

Maximum 2.0.

Dissolve 5.0 g in 50 ml of the prescribed mixture of solvent.

Hydroxyl value (*2.5.3, Method A*)

96 to 108.

Peroxide value

Maximum 10.0.

Introduce 10.0 g into a 100 ml beaker, dissolve with *glacial acetic acid R* and dilute to 20 ml with the same solvent. Add 1 ml of *saturated potassium iodide solution R* and allow to stand for 1 min. Add 50 ml of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulphate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulphate* required for the substance to be examined, in millilitres,

n_2 = volume of *0.01 M sodium thiosulphate* required for the blank, in millilitres,

M = molarity of the sodium thiosulphate solution, in moles per litre,

m = mass of substance to be examined, in grams.

Saponification value (*2.5.6*)

40 to 50, determined on 4.0 g.

Use 15.0 ml of *0.5 M alcoholic potassium hydroxide* and dilute with 50 ml of *alcohol R* before carrying out the titration. Heat under reflux for 60 min.

Composition of fatty acids (*2.4.22, Method C*)

Prepare reference solution (a) as indicated in Table 2.4.22.-2.

Column:

— *material*: fused silica;

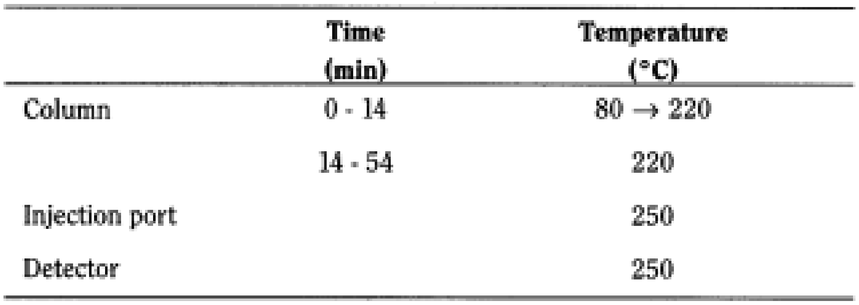
— *dimensions:* l = 30 m, Ø = 0.32 mm;

— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas helium for chromatography R.

Linear velocity 50 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
	14 - 54	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Composition of the fatty acid fraction of the substance:

- — *caproic acid*: maximum 1.0 per cent;
- — *caprylic acid*: maximum 10.0 per cent;
- — *capric acid:* maximum 10.0 per cent;
- — *lauric acid*: 40.0 per cent to 60.0 per cent;
- — *myristic acid*: 14.0 per cent to 25.0 per cent;
- — *palmitic acid*: 7.0 per cent to 15.0 per cent;
- — *stearic acid*: maximum 7.0 per cent;
- — *oleic acid*: maximum 11.0 per cent;
- — *linoleic acid*: maximum 3.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)

Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)

Maximum 0.25 per cent, determined on 2.0 g.

STORAGE

In an airtight container, protected from light.

Ph Eur

Polysorbate 40

General Notices

Polyoxyethylene 20 Sorbitan Monopalmitate
(Ph Eur monograph 1914)

Action and use

Non-ionic surfactants.

Ph Eur

DEFINITION

Mixture of partial esters of fatty acids, mainly *Palmitic acid (1904)*, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

CHARACTERS

Appearance

Oily, viscous, yellowish or brownish-yellow liquid.

Solubility

Miscible with water, with ethanol, with ethyl acetate and with methanol, practically insoluble in fatty oils and in liquid paraffin.

Relative density

About 1.10.

Viscosity

About 400 mPa·s at 30 °C.

IDENTIFICATION

First identication A, D.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of polysorbate 40.

B. It complies with the test for hydroxyl value (see Tests).

C. It complies with the test for saponification value (see Tests).

D. It complies with the test for composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 ml of *methylene chloride R*. Add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R*. Stir with a glass rod. The solution becomes blue.

TESTS

Acid value (*2.5.1*)

Maximum 2.0.

Dissolve 5.0 g in 50 ml of the prescribed mixture of solvents.

Hydroxyl value (*2.5.3, Method A*)

89 to 105.

Peroxide value

Maximum 10.0.

Introduce 10.0 g into a 100 ml beaker, dissolve with *glacial acetic acid R* and dilute to 20 ml with the same solvent. Add 1 ml of *saturated potassium iodide solution R* and allow to stand for 1 min. Add 50 ml of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulphate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulphate* required for the substance to be examined, in millilitres,

n_2 = volume of *0.01 M sodium thiosulphate* required for the blank, in millilitres,

M = molarity of the sodium thiosulphate solution, in moles per litre,

m = mass of substance to be examined, in grams.

Saponification value (*2.5.6*)

41 to 52, determined on 4.0 g.

Use 15.0 ml of *0.5 M alcoholic potassium hydroxide* and dilute with 50 ml of *alcohol R* before carrying out the titration. Heat under reflux for 60 min.

Composition of fatty acids (*2.4.22, Method C*)

Prepare reference solution (a) as indicated in Table 2.4.22.-1.

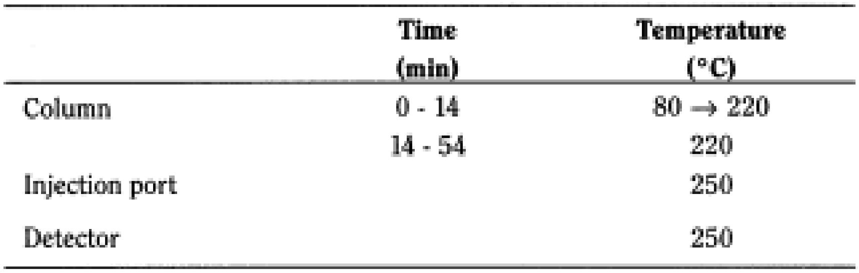
Column:

- *material*: fused silica;
- *size: l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Linear velocity 50 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
	14 - 54	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Composition of the fatty acid fraction of the substance:
— *palmitic acid*: minimum 92.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)

Maximum 0.25 per cent, determined on 2.0 g.

STORAGE

In an airtight container, protected from light.

Ph Eur

Polysorbate 60

General Notices

Polyoxyethylene 20 Sorbitan Monostearate

(Ph Eur monograph 0427)

Action and use

Non-ionic surfactants.

Ph Eur

DEFINITION

Mixture of partial esters of fatty acids, mainly *Stearic acid 50 (1474),* with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

CHARACTERS

Appearance

Yellowish-brown gelatinous mass which becomes a clear liquid at temperatures above 25 °C.

Solubility

Soluble in water, in ethanol, in ethyl acetate and in methanol, practically insoluble in fatty oils and in liquid paraffin.

Relative density

About 1.10.

Viscosity: about 400 mPa·s at 30 °C.

IDENTIFICATION

First identication A, D.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of polysorbate 60.

B. It complies with the test for hydroxyl value (see Tests).

C. It complies with the test for saponification value (see Tests).

D. It complies with the test for composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 ml of *methylene chloride R*. Add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R.* Stir with a glass rod. The solution becomes blue.

TESTS

Acid value (*2.5.1*)

Maximum 2.0.

Dissolve 5.0 g in 50 ml of the prescribed mixture of solvents.

Hydroxyl value (*2.5.3, Method A*)

81 to 96.

Peroxide value

Maximum 10.0.

Introduce 10.0 g into a 100 ml beaker, dissolve with *glacial acetic acid R* and dilute to 20 ml with the same solvent. Add 1 ml of *saturated potassium iodide solution R* and allow to stand for 1 min. Add 50 ml of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulphate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulphate* required for the substance to be examined, in millilitres,

n_2 = volume of *0.01 M sodium thiosulphate* required for the blank, in millilitres,

M = molarity of the sodium thiosulphate solution, in moles per litre,

m = mass of substance to be examined, in grams.

Saponification value (*2.5.6*)

45 to 55, determined on 4.0 g.

Use 15.0 ml of *0.5 M alcoholic potassium hydroxide* and dilute with 50 ml of *alcohol R* before carrying out the titration. Heat under reflux for 60 min.

Composition of fatty acids (*2.4.22, Method C*)

Prepare reference solution (a) as indicated in Table 2.4.22.-1.

Column:

— *material*: fused silica;

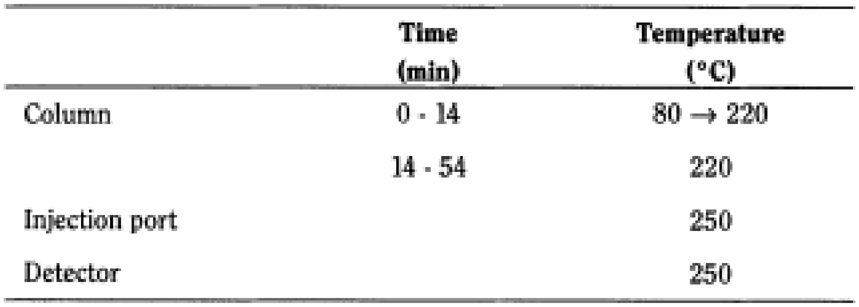
— *size:* *l* = 30 m, Ø = 0.32 mm;

— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 50 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
	14 - 54	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Composition of the fatty acid fraction of the substance:

— *stearic acid*: 40.0 per cent to 60.0 per cent;

— *sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)

Maximum 0.25 per cent, determined on 2.0 g.

STORAGE

In an airtight container , protected from light.

Ph Eur

Polysorbate 80

General Notices

Polyoxyethylene 20 Sorbitan Mono-oleate
(Ph Eur monograph 0428)

Action and use

Non-ionic surfactants.

Ph Eur

DEFINITION

Mixture of partial esters of fatty acids, mainly *Oleic acid (0799)*, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

CHARACTERS

Appearance

Oily, yellowish or brownish-yellow, clear or slightly opalescent liquid.

Solubility

Dispersible in water, in anhydrous ethanol, in ethyl acetate and in methanol, practically insoluble in fatty oils and in liquid paraffin.

Relative density

About 1.10.

Viscosity

About 400 mPa·s at 25 °C.

IDENTIFICATION

First identification A, D.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of polysorbate 80.

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 ml of *methylene chloride R*. Add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R*. Stir with a glass rod. The solution becomes blue.

TESTS

Acid value (*2.5.1*)

Maximum 2.0.

Dissolve 5.0 g in 50 ml of the prescribed mixture of solvents.

Hydroxyl value (*2.5.3, Method A*)

65 to 80.

Peroxide value

Maximum 10.0.

Introduce 10.0 g into a 100 ml beaker, dissolve with *glacial acetic acid R* and dilute to 20 ml with the same solvent. Add 1 ml of *saturated potassium iodide solution R* and allow to stand for 1 min. Add 50 ml of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulphate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of 0.01 M sodium thiosulphate required for the substance to be examined, in millilitres,

n_2 = volume of 0.01 M sodium thiosulphate required for the blank, in millilitres,

M = molarity of the sodium thiosulphate solution, in moles per litre,

m = mass of substance to be examined, in grams.

Saponification value (*2.5.6*)

45 to 55, determined on 4.0 g.

Use 30.0 ml of *0.5 M alcoholic potassium hydroxide,* heat under reflux for 60 min and add 50 ml of *anhydrous ethanol R* before carrying out the titration.

Composition of fatty acids

Gas chromatography (*2.4.22, Method C*). Use the mixture of calibrating substances in Table 2.4.22.-3.

Column:

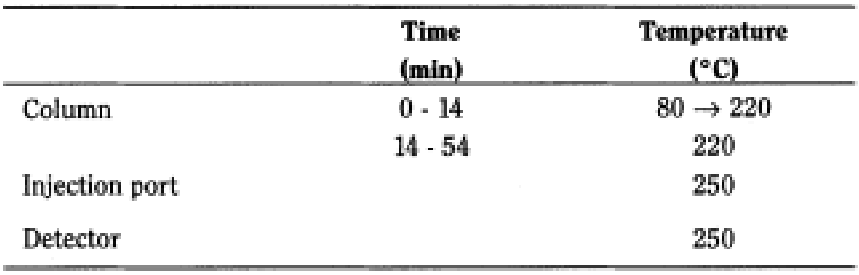
- — *material*: fused silica;
- — *size:* l = 30 m, Ø = 0.32 mm;

— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Linear velocity 50 cm/s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
	14 - 54	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Composition of the fatty acid fraction of the substance:

- — *myristic acid*: maximum 5.0 per cent;
- — *palmitic acid*: maximum 16.0 per cent;
- — *palmitoleic acid*: maximum 8.0 per cent;
- — *stearic acid*: maximum 6.0 per cent;
- — *oleic acid*: minimum 58.0 per cent;
- — *linoleic acid*: maximum 18.0 per cent;
- — *linolenic acid*: maximum 4.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)

Maximum 0.25 per cent, determined on 2.0 g.

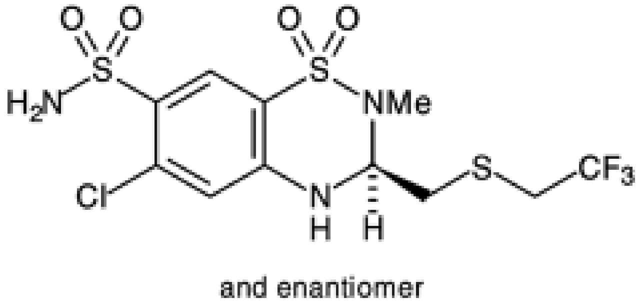
STORAGE

In an airtight container, protected from light.

Ph Eur

Polythiazide

General Notices



$C_{11}H_{13}ClF_3N_3O_4S_3$ 439.9 *346-18-9*

Action and use

Thiazide diuretic.

Preparation

Polythiazide Tablets

DEFINITION

Polythiazide is (*RS*)-3,4-dihydro-2-methyl-3-(2,2,2-trifluoroethylthiomethyl)-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide. It contains not less than 97.0% and not more than 102.0% of $C_{11}H_{13}ClF_3N_3O_4S_3$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Practically insoluble in *water*; sparingly soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of polythiazide *(RS 280)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in *methanol* exhibits a maximum at 268 nm and a less well-defined maximum at 317 nm. The *absorbance* at 268 nm is about 1.0.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 50 volumes of *toluene*, 30 volumes of *ether* and 20 volumes of *acetone* as the mobile phase. Apply separately to the plate 20 µl of each of two solutions in *methanol* containing (1) 0.02% w/v of the substance being examined and

(2) 0.02% w/v of *polythiazide BPCRS*. After removal of the plate, allow it to dry in air until the solvent has evaporated, examine under *ultraviolet light (254 nm)* and then treat the plate by *Method I* and examine again. The principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of solution (1) and quantities of 1, 2, 3, 4 and 5 µl of solution (2). Solutions (1) and (2) are solutions of the substance being examined in *acetone* containing (1) 1.0% w/v and (2) 0.020% w/v. After removal of the plate, allow it to dry in air and reveal the spots using *Method I*. Assess the intensity of any *secondary spot* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with the applications of solution (2). The sum of the intensities so assessed does not exceed 3% and no such spot is more intense than the spot in the chromatogram obtained with 5 µl of solution (2) (2%).

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.1 g in sufficient *methanol* to produce 250 ml, dilute 5 ml to 200 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 268 nm, Appendix II B. Calculate the content of $C_{11}H_{13}ClF_3N_3O_4S_3$ taking 500 as the value of A(1%, 1 cm) at the maximum at 268 nm.

Potassium Acetate

General Notices

(Ph Eur monograph 1139)

$C_2H_3O_2K$ 98.14 *127-08-2*

Action and use

Used in solutions for dialysis.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, deliquescent.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives reaction (a) of acetates (*2.3.1*).

B. It gives reaction (a) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

7.5 to 9.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Reducing substances

Dilute 10 ml of solution S to 100 ml with *water R*. Add 5 ml of *dilute sulphuric acid R* and 0.5 ml of a 0.32 g/l solution of *potassium permanganate R*. Mix and boil gently for 5 min. The solution remains pink.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R.*

Aluminium (*2.4.17*)

Maximum 1 ppm, if intended for use in the manufacture of peritoneal dialysis solutions, haemofiltration solutions or haemodialysis solutions.

Prescribed solution Dissolve 2.0 g in 50 ml of *water R* and add 5 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 1 ml of *aluminium standard solution (2 ppm Al) R*, 5 ml of *acetate buffer solution pH 6.0 R* and 49 ml of *water R*.

Blank solution Mix 5 ml of *acetate buffer solution pH 6.0 R* and 50 ml of *water R*.

Iron (*2.4.9*)

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Sodium

Maximum 0.50 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589 nm.

Heavy metals (*2.4.8*)

Maximum 4 ppm.

Dissolve 5.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 80.0 mg in 20 ml of *anhydrous acetic acid R*. Add 0.2 ml of *naphtholbenzein solution R*. Titrate with *0.1 M perchloric acid*. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 9.81 mg of $C_2H_3KO_2$.

STORAGE

In an airtight container .

Ph Eur

Potassium Bicarbonate

General Notices

(Potassium Hydrogen Carbonate, Ph Eur monograph 1141)

$KHCO_3$ 100.1 *298-14-6*

Action and use

Excipient.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

When heated in the dry state or in solution, it is gradually converted to potassium carbonate.

IDENTIFICATION

A. To 5 ml of solution S (see Tests) add 0.1 ml of *phenolphthalein solution R*. A pale pink colour is produced. Heat; gas is evolved and the colour becomes red.

B. It gives the reaction of carbonates and bicarbonates (*2.3.1*).

C. 1 ml of solution S gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in 90 ml of *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Carbonates

The pH (*2.2.3*) of freshly prepared solution S is not greater than 8.6.

Chlorides (*2.4.4*)

Maximum 150 ppm.

Dilute 7 ml of solution S to 15 ml with *dilute nitric acid R*.

Sulphates (*2.4.13*)

Maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *acetic acid R*. Prepare the standard using a mixture of 7.5 ml of *sulphate standard solution (10 ppm* SO_4*) R* and 7.5 ml of *distilled water R*.

Ammonium (*2.4.1*)

Maximum 20 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Calcium (*2.4.3*)

Maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *acetic acid R*. Prepare the standard using a mixture of 5 ml of *calcium standard solution (10 ppm Ca) R* and 10 ml of *distilled water R*.

Iron (*2.4.9*)

Maximum 20 ppm, determined on solution S.

Sodium

Maximum 0.50 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589 nm.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 2 ml of *hydrochloric acid R* and 18 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 0.800 g in 50 ml of *carbon dioxide-free water R*. Add 0.1 ml of *methyl orange solution R*. Titrate with *1 M hydrochloric acid* until the yellow colour begins to change to yellowish-pink. Heat cautiously and boil for at least 2 min. The solution becomes yellow. Cool and titrate until a yellowish-red colour is obtained.

1 ml of *1 M hydrochloric acid* is equivalent to 0.1001 g of $KHCO_3$.

Potassium Bromide

General Notices

(Ph Eur monograph 0184)

KBr 119.0 *7758-02-3*

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water and in glycerol, slightly soluble in alcohol.

IDENTIFICATION

A. It gives reaction (a) of bromides (*2.3.1*).

B. Solution S (see Tests) gives the reactions of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromates

To 10 ml of solution S add 1 ml of *starch solution R*, 0.1 ml of a 100 g/l solution of *potassium* *iodide R* and 0.25 ml of *0.5 M sulphuric acid* and allow to stand protected from light for 5 min. No blue or violet colour develops.

Chlorides

Maximum 0.6 per cent.

In a conical flask, dissolve 1.000 g in 20 ml of *dilute nitric acid R*. Add 5 ml of *strong hydrogen peroxide solution R* and heat on a water-bath until the solution is completely decolorised. Wash down the sides of the flask with a little *water R* and heat on a water-bath for 15 min. Allow to cool, dilute to 50 ml with *water R* and add 5.0 ml of *0.1 M silver nitrate* and 1 ml of *dibutyl phthalate R*. Shake and titrate with *0.1 M ammonium thiocyanate*, using 5 ml of *ferric ammonium sulphate solution R2* as indicator. Not more than 1.7 ml of *0.1 M silver nitrate* is used. Note the volume of *0.1 M silver nitrate* used (see Assay). Carry out a blank test.

Iodides

To 5 ml of solution S add 0.15 ml of *ferric chloride solution R1* and 2 ml of *methylene chloride R*. Shake and allow to separate. The lower layer is colourless (*2.2.2, Method I*).

Sulphates (*2.4.13*)

Maximum 100 ppm.

15 ml of solution S complies with the limit test for sulphates.

Iron (*2.4.9*)

Maximum 20 ppm.

5 ml of solution S diluted to 10 ml with *water R* complies with the limit test for iron.

Magnesium and alkaline-earth metals (*2.4.7*)

Maximum 200 ppm, calculated as Ca.

10.0 g complies with the limit test for magnesium and alkaline-earth metals. The volume of 0.01 M sodium edetate used does not exceed 5.0 ml.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 2.000 g in *water R* and dilute to 100.0 ml with the same solvent. To 10.0 ml of the solution add 50 ml of *water R*, 5 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R*. Shake. Titrate with *0.1 M ammonium thiocyanate*, using 2 ml of *ferric ammonium sulphate solution R2* as indicator and shaking vigorously towards the end-point.

1 ml of *0.1 M silver nitrate* is equivalent to 11.90 mg of KBr.

Calculate the percentage content of KBr from the expression:

$a - 3.357\ b$

a = percentage content of KBr and KCl obtained in the assay and calculated as KBr,

b = percentage content of Cl in the test for chlorides.

Ph Eur

Potassium Carbonate

General Notices

(Ph Eur monograph 1557)

K_2CO_3 138.2 *584-08-7*

Action and use

Excipient.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white granular powder, hygroscopic.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in 10 ml of *water R*. The solution is strongly alkaline (*2.2.4*).

B. 2 ml of the solution prepared for identification test A gives the reaction of carbonates and bicarbonates (*2.3.1*).

C. 1 ml of the solution prepared for identification test A gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in 25 ml of *distilled water R*. Slowly add 14 ml of *hydrochloric acid R*. When the effervescence has ceased, boil for a few minutes. Allow to cool and dilute to 50 ml with *distilled water R*.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dissolve 0.50 g in 10 ml of *water R*. Carefully add dropwise 1 ml of *nitric acid R*. Boil. Cool, add 5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 100 ppm.

Dilute 7.50 ml of solution S to 15 ml with *distilled water R*.

Calcium (*2.4.3*)

Maximum 100 ppm.

To 5 ml of solution S add 1 ml of *concentrated ammonia R*. Boil. Cool. Dilute to 15 ml with *distilled water R.*

Iron (*2.4.9*)

Maximum 10 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 0.300 g by drying in an oven at 120-125 °C for 5 h.

ASSAY

Dissolve 0.500 g in 50 ml of *carbon dioxide-free water R*. Carry out a potentiometric titration (*2.2.20*), using *1 M hydrochloric acid*. Read the volume added at the 2nd point of inflexion.

1 ml of *1 M hydrochloric acid* is equivalent to 69.1 mg of K_2CO_3.

STORAGE

In an airtight container.

Ph Eur

Potassium Chloride

General Notices

(Ph Eur monograph 0185)

KCl 74.6 *7447-40-7*

Action and use

Used in prevention and treatment of potassium deficiency and electrolyte imbalance.

Preparations

Bumetanide and Prolonged-release Potassium Tablets

Oral Rehydration Salts

Sterile Potassium Chloride Concentrate

Potassium Chloride and Glucose Intravenous Infusion

Potassium Chloride and Sodium Chloride Intravenous Infusion

Potassium Chloride, Sodium Chloride and Glucose Intravenous Infusion

Potassium Chloride Oral Solution

Prolonged-release Potassium Chloride Tablets

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent of KCl (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, practically insoluble in anhydrous ethanol.

IDENTIFICATION

A. It gives the reactions of chlorides *(2.3.1)*.

B. Solution S (see Tests) gives the reactions of potassium *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 50 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides

Maximum 0.1 per cent.

Dilute 1.0 ml of solution S to 50 ml with *water R*. To 5.0 ml of the solution add 2.0 ml of *phenol red solution R2* and 1.0 ml of *chloramine solution R1* and mix immediately. After exactly 2 min add 0.15 ml of *0.1 M sodium thiosulphate*, mix and dilute to 10.0 ml with *water R*. The absorbance *(2.2.25)* of the solution measured at 590 nm, using *water R* as the compensation liquid, is not greater than that of a standard prepared at the same time and in the same manner using 5 ml of a 3.0 mg/l solution of *potassium bromide R*.

Iodides

Moisten 5 g by the dropwise addition of a freshly prepared mixture of 0.15 ml of *sodium nitrite solution R*, 2 ml of *0.5 M sulphuric acid*, 25 ml of *iodide-free starch solution R* and 25 ml of *water R*. After 5 min, examine in daylight. The substance shows no blue colour.

Sulphates *(2.4.13)*

Maximum 300 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Aluminium *(2.4.17)*

Maximum 1.0 ppm, if intended for use in the manufacture of haemodialysis solutions.

Prescribed solution Dissolve 4 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Barium

To 5 ml of solution S add 5 ml of *distilled water R* and 1 ml of *dilute sulphuric acid R*. After 15 min, any opalescence in the solution is not more intense than that in a mixture of 5 ml of solution S and 6 ml of *distilled water R*.

Iron *(2.4.9)*

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Magnesium and alkaline-earth metals *(2.4.7)*

Maximum 200 ppm, calculated as Ca, determined on 10.0 g using 0.15 g of *mordant black 11 triturate R*. The volume of *0.01 M sodium edetate* used does not exceed 5.0 ml.

Sodium

Maximum 0.10 per cent, if intended for use in the manufacture of parenteral preparations or haemodialysis solutions.

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions by diluting as required a solution containing 200 µg of Na per millilitre, prepared as follows: dissolve in *water R* 0.5084 g of *sodium chloride R*, previously dried at 100-105 °C for 3 h, and dilute to 1000.0 ml with the same solvent.

Wavelength 589 nm.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 1.300 g in *water R* and dilute to 100.0 ml with the same solvent. To 10.0 ml of the solution add 50 ml of *water R*, 5 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R*. Shake. Titrate with *0.1 M ammonium thiocyanate*, using 2 ml of *ferric ammonium sulphate solution R2* as indicator and shaking vigorously towards the end-point.

1 ml of *0.1 M silver nitrate* is equivalent to 7.46 mg of KCl.

LABELLING

The label states:

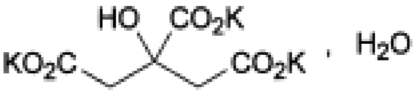
- where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
- where applicable, that the substance is suitable for use in the manufacture of haemodialysis solutions.

Ph Eur

Potassium Citrate

General Notices

(Ph Eur monograph 0400)



$C_6H_5K_3O_7,H_2O$ 324.4   *6100-05-6*

Action and use

Alkalisation of urine.

Preparation

Potassium Citrate Mixture

Ph Eur

DEFINITION

Tripotassium 2-hydroxypropane-1,2,3-tricarboxylate monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, granular powder or transparent crystals, hygroscopic.

Solubility

Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. To 1 ml of solution S (see Tests) add 4 ml of *water R*. The solution gives the reaction of citrates (*2.3.1*).

B. 0.5 ml of solution S gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Readily carbonisable substances

To 0.20 g of the powdered substance to be examined add 10 ml of *sulphuric acid R* and heat in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly. The solution is not more intensely coloured than reference solution Y_2 or GY_2 (*2.2.2, Method II*).

Chlorides (*2.4.4*)

Maximum 50 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Oxalates

Maximum 300 ppm.

Dissolve 0.50 g in 4 ml of *water R*, add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules and heat on a water-bath for 1 min. Allow to stand for 2 min, decant the liquid into a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 4 ml of a 0.05 g/l solution of *oxalic acid R*.

Sulphates (*2.4.13*)

Maximum 150 ppm.

To 10 ml of solution S add 2 ml of *hydrochloric acid R1* and dilute to 15 ml with *distilled water R.*

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Sodium

Maximum 0.30 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution To 10 ml of solution S add 1 ml of *dilute hydrochloric acid R* and dilute to 100 ml with *distilled water R.*

Reference solutions Prepare the reference solutions using a solution of *sodium chloride R* containing 1 mg of Na per millilitre diluted as necessary with *distilled water R*.

Wavelength 589 nm.

Water (*2.5.12*)

4.0 per cent to 7.0 per cent, determined on 0.500 g. After adding the substance to be examined, stir for 15 min before titrating.

ASSAY

Dissolve 0.150 g in 20 ml of *anhydrous acetic acid R*, heating to about 50 °C. Allow to cool. Titrate with 0.1 M perchloric acid using 0.25 ml of *naphtholbenzein solution R* as indicator until a green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 10.21 mg of $C_6H_5K_3O_7$.

STORAGE

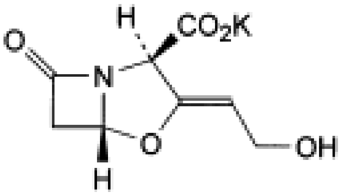
In an airtight container .

Ph Eur

Potassium Clavulanate

General Notices

(Ph Eur monograph 1140)



$C_8H_8KNO_5$ *237.3* 61177-45-5

Action and use

Beta-lactamase inhibitor, potentiation of the action of amoxicillin and tiarcillin.

Preparations

Co-amoxiclav Injection

Co-amoxiclav Tablets

Ph Eur

DEFINITION

Potassium (2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate, the potassium salt of a substance produced by the growth of certain strains of Streptomyces clavuligerus or obtained by any other means.

Content

96.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, slightly soluble in alcohol, very slightly soluble in acetone.

PRODUCTION

The method of production, extraction and purification are such that clavam-2-carboxylate is eliminated or present at a level not exceeding 0.01 per cent.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison   Ph. Eur. reference spectrum of potassium clavulanate.

B. It gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 0.400 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH (*2.2.3*)

5.5 to 8.0.

Dilute 5 ml of solution S to 10 ml with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

+ 53 to + 63 (anhydrous substance), determined on solution S.

Absorbance (*2.2.25*)

Maximum 0.40 at 278 nm.

Dissolve 50.0 mg in *0.1 M phosphate buffer solution pH 7.0 R* and dilute to 50.0 ml with the same solution. Measure immediately the absorbance of this solution.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution   Dissolve 0.250 g of the substance to be examined in mobile phase A and dilute to 25.0 ml with mobile phase A.

Reference solution (a)   Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b)   Dissolve 10 mg of *lithium clavulanate CRS* and 10 mg of *amoxicillin trihydrate CRS* in mobile phase A and dilute to 100 ml with mobile phase A.

Column:
— *size: l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: a 7.8 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 4.0 with *phosphoric acid R* and filtered through a 0.5 µm filter;

— *mobile phase B*: a mixture of equal volumes of mobile phase A and *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 15	100 → 50	0 → 50
15 - 18	50	50
18 - 24	50 → 100	50 → 0
24 - 39	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 13 between the first peak (clavulanate) and the second peak (amoxicillin).

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Aliphatic amines

Gas chromatography (*2.2.28*).

The method shown below can be used to determine the following aliphatic amines: 1,1-dimethylethylamine; diethylamine; *N*,*N*,*N′*,*N′*-tetramethylethylenediamine; 1,1,3,3-tetramethylbutylamine; *N*,*N′*-diisopropylethylenediamine; 2,2′-oxydi(*N*,*N*)dimethylethylamine.

Internal standard solution Dissolve 50 µl of *3-methylpentan-2-one R* in *water R* and dilute to 100.0 ml with the same solvent.

Test solution Weigh 1.00 g of the substance to be examined into a centrifuge tube. Add 5.0 ml of the internal standard solution, 5.0 ml of *dilute sodium hydroxide solution R*, 10.0 ml of *water R*, 5.0 ml of *2-methylpropanol R* and 5 g of *sodium chloride R*. Shake vigorously for 1 min. Centrifuge to separate the layers.

Reference solution Dissolve 80.0 mg of each of the following amines *1,1-dimethylethylamine R*; *diethylamine R*; *tetramethylethylenediamine R*; *1,1,3,3-tetramethylbutylamine R*; *N,N′-diisopropylethylenediamine R* and *2,2′-oxybis(N,N-dimethylethylamine) R* in dilute *hydrochloric acid R* and dilute to 200.0 ml with the same acid. Introduce 5.0 ml of this solution into a centrifuge tube. Add 5.0 ml of the internal standard solution, 10.0 ml of *dilute sodium hydroxide solution R*, 5.0 ml of *2-methylpropanol R* and 5 g of *sodium chloride R*. Shake vigorously for 1 min. Centrifuge to separate the layers.

Column:

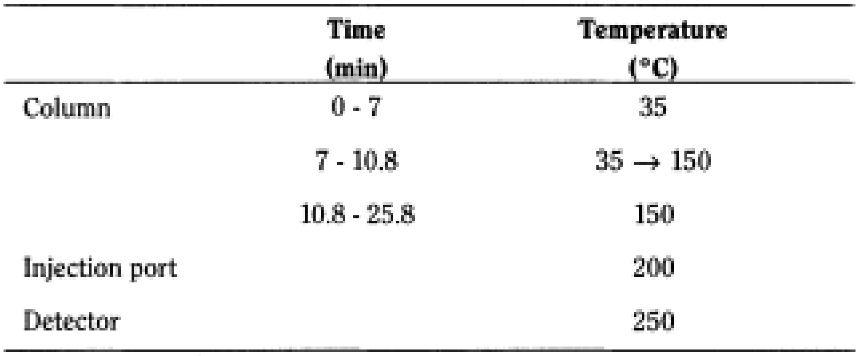
- *material*: fused silica;
- *size: l* = 50 m, Ø = 0.53 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 8 ml/min.

Split ratio 1:10.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 7	35
	7 - 10.8	35 → 150
	10.8 - 25.8	150
Injection port		200
Detector		250

Detection Flame ionisation.

Injection 1 µl of the upper layers obtained from test solution and reference solution.

Relative retention With reference to 3-methylpentan-2-one (retention time = about 11.4 min): impurity H = about 0.55; impurity I = about 0.76; impurity J = about 1.07; impurity K = about 1.13; impurity L = about 1.33; impurity M = about 1.57.

Limit:

- *aliphatic amines*: maximum 0.2 per cent.

2-Ethylhexanoic acid (*2.4.28*)

Maximum 0.8 per cent.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.03 IU/mg if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Test solution Dissolve 50.0 mg of the substance to be examined in a 4.1 g/l solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R*, and dilute to 50.0 ml with the same solution.

Reference solution (a) Dissolve 50.0 mg of *lithium clavulanate CRS* in a 4.1 g/l solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R* and dilute to 50.0 ml with the same solution.

Reference solution (b) Dissolve 50.0 mg of *lithium clavulanate CRS* and 50.0 mg of *amoxicillin trihydrate CRS* in a 4.1 g/l solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R* and dilute to 50.0 ml with the same solution.

Column:

- *size:* *l* = 0.3 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 5 volumes of *methanol R1* and 95 volumes of a 15 g/l solution of *sodium dihydrogen phosphate R* previously adjusted to pH 4.0 with *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

System suitability Reference solution (b):

- *resolution*: minimum 3.5 between the first peak (clavulanate) and the second peak (amoxicillin).

1 mg of clavulanate ($C_8H_9NO_5$) is equivalent to 1.191 mg of $C_8H_8KNO_5$.

STORAGE

In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

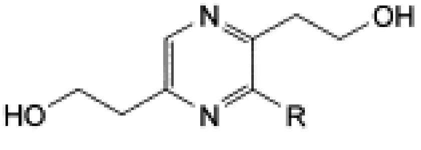
IMPURITIES

Specified impurities A, B, C, D, G, H, I, J, K, L, M.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F.*

By liquid chromatography A, B, C, D, E, F, G.

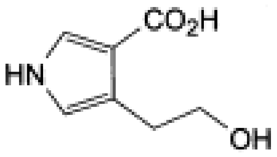
By gas chromatography H, I, J, K, L, M.



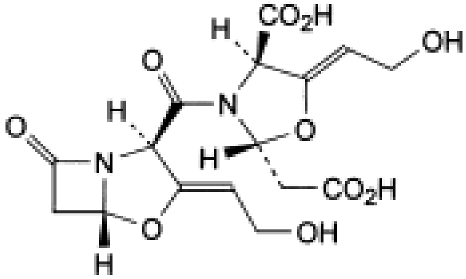
A. R = H: 2,2′-(pyrazine-2,5-diyl)diethanol,

B. R = CH_2-CH_2-CO_2H: 3-[3,6-bis(2-hydroxyethyl)pyrazin-2-yl]propanoic acid,

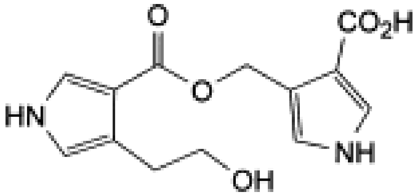
C. R = CH_2-CH_3: 2,2′-(3-ethylpyrazine-2,5-diyl)diethanol,



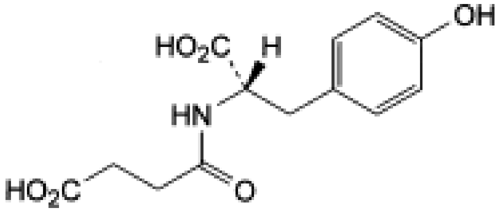
D. 4-(2-hydroxyethyl)pyrrole-3-carboxylic acid,



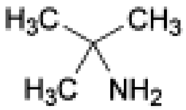
E. (2*R*,4*R*,5*Z*)-2-(carboxymethyl)-5-(2-hydroxyethylidene)-3-[[(2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabibyclo[3.2.0]hept-2-yl]carbonyl]oxazolidine-4-carboxylic acid,



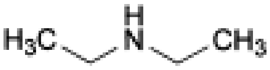
F. 4-[[[[4-(2-hydroxyethyl)-1*H*-pyrrol-3-yl]carbonyl]oxy]methyl]-1*H*-pyrrole-3-carboxylic acid,



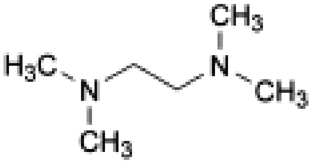
G. 4-[[(1*S*)-1-carboxy-2-(4-hydroxyphenyl)ethyl]amino]-4-oxobutanoic acid (*N*-succinyltyrosine),



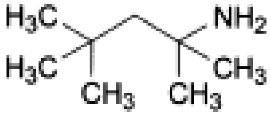
H. 2-amino-2-methylpropane (1,1-dimethylethylamine),



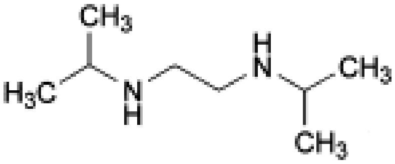
I. diethylamine,



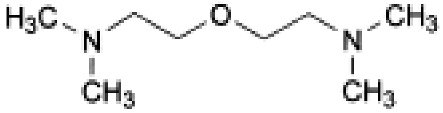
J. 1,2-bis(dimethylamino)ethane (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine),



K. 2-amino-2,4,4-trimethylpentane (1,1,3,3-tetramethylbutylamine),



L. *N*,*N*′-bis(1-methylethyl)-1,2-ethanediamine (*N*,*N*′-diisopropylethylenediamine),



M. bis(2-dimethylamino)ethyl ether [2,2′-oxybis(*N*,*N*-dimethylethylamine)].

Ph Eur

Potassium Dihydrogen Phosphate

General Notices

(Ph Eur monograph 0920)

KH_2PO_4 136.1 *7778-77-0*

Preparations

Sterile Potassium Dihydrogen Phosphate Concentrate

Potassium Dihydrogen Phosphate Oral Solution

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly acid (*2.2.4*).

B. Solution S gives reaction (b) of phosphates (*2.3.1*).

C. 0.5 ml of solution S gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

4.2 to 4.5.

To 5 ml of solution S add 5 ml of *carbon dioxide-free water R*.

Reducing substances

To 5 ml of solution S add 5 ml of *dilute sulphuric acid R* and 0.25 ml of *0.02 M potassium permanganate*. Heat on a water-bath for 5 min. The colour of the permanganate is not completely discharged.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 300 ppm.

To 5 ml of solution S add 0.5 ml of *hydrochloric acid R* and dilute to 15 ml with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 0.5 g.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S.

Sodium

Maximum 0.10 per cent, if intended for use in the manufacture of parenteral dosage forms.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using the following solution, diluted as necessary with *water R*: dissolve 0.5084 g of *sodium chloride R*, previously dried at 100-105 °C for 3 h, in *water R* and dilute to 1000.0 ml with the same solvent (200 µg of Na per millilitre).

Wavelength 589 nm.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 125-130 °C.

ASSAY

Dissolve 1.000 g in 50 ml of *carbon dioxide-free water R*. Titrate with carbonate-free *1 M* *sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *1 M sodium hydroxide* is equivalent to 0.1361 g of KH_2PO_4.

LABELLING

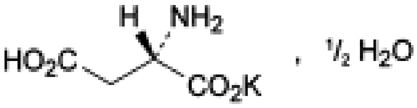
The label states, where applicable, that the substance is suitable for use in the manufacture of parental dosage forms.

Ph Eur

Potassium Hydrogen Aspartate Hemihydrate

General Notices

(Ph Eur monograph 2076)



$C_4H_6KNO_4,½H_2O$ 180.2

Action and use

Excipient.

Ph Eur

DEFINITION

Potassium hydrogen (2*S*)-2-aminobutanedioate hemihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, powder or crystalline powder, or colourless crystals.

Solubility

Very soluble in water, practically insoluble in alcohol and in methylene chloride.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. It gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

6.0 to 7.5 for solution S.

Specific optical rotation (*2.2.7*)

+ 18.0 to + 20.5 (anhydrous substance).

Dissolve 0.50 g in a mixture of equal volumes of *hydrochloric acid R* and *water R* and dilute to 25.0 ml with the same mixture of solvents.

Ninhydrin-positive substances

Thin-layer chromatography (*2.2.27*).

Test solution (a) Solution S.

Test solution (b) Dilute 1.0 ml of solution S to 10.0 ml with *water R*.

Reference solution (a) Dissolve 25 mg of *potassium hydrogen aspartate hemihydrate CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of test solution (b) to 20.0 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *glutamic acid CRS* and 10 mg of the substance to be examined in *water R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R*, *water R*, *butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated principal spots.

Limits Test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

To 10 ml of solution S add 5 ml of *water R*.

Sulphates (*2.4.13*)

Maximum 500 ppm.

To 12 ml of solution S add 3 ml of *distilled water R*.

Ammonium (*2.4.1, Method B*)

Maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron (*2.4.9*)

Maximum 30 ppm.

In a separating funnel, dissolve 0.33 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*)

4.0 per cent to 6.0 per cent, determined on 0.200 g.

Dissolve the substance to be examined in 10 ml of *formamide R1* and add 10 ml of *anhydrous methanol R*.

ASSAY

Dissolve 70.0 mg in 5 ml of anhydrous formic acid R, add 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 8.56 mg of $C_4H_6KNO_4$.

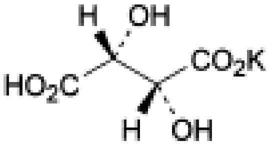
Ph Eur

Potassium Hydrogen Tartrate

General Notices

Cream of Tartar

(Ph Eur monograph 1984)



$C_4H_5KO_6$ 188.2 *868-14-4*

Action and use

Excipient.

Ph Eur

DEFINITION

Potassium hydrogen (2*R*,3*R*)-2,3-dihydroxybutane-1,4-dioate.

Content

99.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Suspend 0.5 g in 50 ml of *water R* and boil until dissolution is complete. Allow to cool (solution A). To 5 ml of solution A, add 0.1 ml of *methyl red solution R*. The solution is red.

C. Solution A gives reaction (a) of tartrates (*2.3.1*).

D. Solution A gives reaction (b) of potassium (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)

+ 8.0 to + 9.2 (dried substance).

Dissolve 2.50 g in 20 ml of *1 M hydrochloric acid* with heating. Allow to cool. Dilute to 25.0 ml with *water R*.

Oxalic acid

Maximum 500 ppm.

Dissolve 0.43 g in 4 ml of *water R*. Add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules and boil for 1 min. Allow to stand for 2 min. Collect the liquid in a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of a 50 g/l solution of *potassium ferricyanide R* . Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 1 ml of *water R* and 3 ml of a 0.1 g/l solution of *oxalic acid R*.

Chlorides (*2.4.4*)

Maximum 500 ppm.

Dissolve 1.0 g with heating in a mixture of 3 ml of *dilute nitric acid R* and 50 ml of *water R*. Dilute to 100 ml with *water R*. Dilute 10 ml of the solution to 15 ml with *water R*. The solution complies with the limit test for chlorides.

Sulphates (*2.4.13*)

Maximum 500 ppm.

Suspend 0.30 g in 3.0 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. Heat until dissolution is complete. The solution complies with the limit test for sulphates.

Barium

Suspend 0.50 g in a mixture of 1.5 ml of *dilute hydrochloric acid R* and 8.5 ml of *water R*. Heat until dissolution is complete. Allow to cool. Add 1 ml of *dilute sulphuric acid R*. The solution remains clear (*2.2.1*) on standing for 15 min.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.170 g in 100 ml of *water R* at 100 °C. Titrate the hot solution with *0.1 M sodium hydroxide*, using 0.3 ml of *phenolphthalein solution R* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 18.82 mg of $C_4H_5KO_6$.

Ph Eur

Potassium Hydroxide

General Notices

Caustic Potash

(Ph Eur monograph 0840)

KOH 56.11 *1310-58-3*

Preparation

Potassium Hydroxide Solution

Ph Eur

DEFINITION

Content

85.0 per cent to 100.5 per cent of total alkali, calculated as KOH.

CHARACTERS

Appearance

White or almost white, crystalline, hard masses, supplied as sticks, pellets or irregularly shaped pieces, deliquescent, hygroscopic, absorbing carbon dioxide.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. pH (*2.2.3*): minimum 10.5.

Dissolve 0.1 g in 10 ml of *water R* (solution A used for identification test B). Dilute 1 ml of this solution to 100 ml with *water R.*

B. 1 ml of solution A prepared in identification test A gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S1

Dissolve 2.5 g in 10 ml of *water R*. Carefully add 2 ml of *nitric acid R* while cooling, and dilute to 25 ml with *dilute nitric acid R*.

Solution S2

Dissolve 10 g in 15 ml of *distilled water R*. Carefully add 12 ml of *hydrochloric acid R* while cooling, and dilute to 50 ml with *dilute hydrochloric acid R*.

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Carbonates

Maximum 2.0 per cent, calculated as K_2CO_3 as determined in the assay.

Chlorides (*2.4.4*)

Maximum 50 ppm.

Dilute 10 ml of solution S1 to 15 ml with *water R*.

Phosphates (*2.4.11*)

Maximum 20 ppm.

Dilute 5 ml of solution S1 to 100 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 50 ppm, determined on solution S2.

Aluminium (*2.4.17*)

Maximum 0.2 ppm, if intended for use in the manufacture of haemodialysis solutions.

Prescribed solution Dissolve 20 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Iron (*2.4.9*)

Maximum 10 ppm.

Dilute 5 ml of solution S2 to 10 ml with *water R*.

Sodium

Maximum 1.0 per cent.

Atomic absorption spectrometry (*2.2.23, Method II*)

Test solution Dissolve 1.00 g in 50 ml of *water R*, add 5 ml of *sulphuric acid R* and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with water R.

Source Sodium hollow-cathode lamp.

Wavelength 589 nm.

Atomisation device Air-acetylene flame.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dilute 10 ml of solution S2 to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 2.000 g in 25 ml of *carbon-dioxide free water R*. Add 25 ml of freshly prepared *barium chloride solution R1* and 0.3 ml of *phenolphthalein solution R*. Add slowly while shaking 25.0 ml of *1 M hydrochloric acid* and continue the titration with *1 M hydrochloric acid* until the colour changes from pink to colourless. Add 0.3 ml of *bromophenol blue solution R* and continue the titration with *1 M hydrochloric acid* until the colour changes from violet-blue to yellow.

1 ml of *1 M hydrochloric acid* used in the 2nd part of the titration is equivalent to 69.11 mg of K_2CO_3.

1 ml of *1 M hydrochloric acid* used in the combined titrations is equivalent to 56.11 mg of total alkali, calculated as KOH.

STORAGE

In an airtight, non-metallic container.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of haemodialysis solutions.

Ph Eur

Potassium Hydroxyquinoline Sulphate

General Notices

Action and use

Used in treatment of acne.

Preparation

Potassium Hydroxyquinoline Sulphate and Benzoyl Peroxide Cream.

DEFINITION

Potassium Hydroxyquinoline Sulphate is an equimolecular mixture of quinolin-8-ol sulphate monohydrate, $(C_9H_7NO)_2,H_2SO_4,H_2O$, and potassium sulphate, K_2SO_4. It contains not less than 50.6% and not more than 52.6% of quinolin-8-ol, C_9H_7NO, and not less than 29.5% and not more than 32.5% of K_2SO_4, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A pale yellow, microcrystalline powder. It partly liquefies between 172° and 184°.

Freely soluble in *water*; insoluble in *ether*. On extraction with hot *absolute ethanol* a residue of potassium sulphate and a solution of quinolin-8-ol sulphate are obtained.

IDENTIFICATION

A. To 5 ml of a 5% w/v solution add drop wise, with shaking, 5M *sodium hydroxide* until a heavy precipitate is produced. Filter, wash with *water* and dry at a pressure not exceeding 0.7 kPa for 3 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of quinolin-8-ol *(RS 310)*.

B. To 5 ml of a 5% w/v solution add 0.5 ml of *iron(III) chloride solution R1*. A dark green colour is produced.

C. Yields reaction A characteristic of *potassium salts*, Appendix VI.

D. Yields reaction A characteristic of *sulphates*, Appendix VI.

TESTS

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

ASSAY

For quinolin-8-ol

Dissolve 0.35 g in 50 ml of *water* and 20 ml of *hydrochloric acid*, add 50 ml of 0.05M *bromine VS*, stopper the flask and shake for 15 minutes. Allow to stand for 15 minutes, add 80 ml of *water* and 10 ml of *dilute potassium iodide solution* and titrate with 0.1M *sodium thiosulphate*

VS using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of bromine required. Each ml of 0.05M *bromine VS* is equivalent to 3.629 mg of C_9H_7NO.

For potassium sulphate

Prepare a solution of suitable concentration with *water*. Carry out the method for *atomic emission spectrophotometry*, Appendix II D, measuring at 766.5 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions. Each mg of potassium is equivalent to 2.2284 mg of K_2SO_4.

Potassium Iodate

General Notices

KIO_3 214.0 *7758-05-6*

Action and use

Iodine supplement for emergency use.

Preparation

Potassium Iodate Tablets

DEFINITION

Potassium Iodate contains not less than 99.0% and not more than 101.0% of KIO_3, calculated with reference to the dried substance.

CHARACTERISTICS

A white crystalline powder.

Slowly soluble in water; insoluble in ethanol (96%).

Dissolve 10 g of the substance being examined in sufficient water to produce 200 ml (solution S1).

Add 25 ml of *hydrochloric acid* to 6 g of the substance being examined, evaporate to dryness and repeat. Heat until iodine is removed. Dissolve the residue in 2.5 ml of a 25% v/v solution of *hydrochloric acid* and dilute to 50 ml with *water* (solution S2).

IDENTIFICATION

A. 1 ml of solution S1 yields reaction B characteristic of *potassium salts*, Appendix VI.

B. Dissolve 0.1 g in 5 ml of *water*. Add 1 ml of *silver nitrate solution* followed by 1 ml of *sulphur dioxide solution*. A yellow precipitate is produced immediately.

TESTS

Acidity or alkalinity

pH of solution S1, 5.0 to 8.0, Appendix V L.

Clarity and colour of solution

Solution S1 is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Chloride, chlorate, bromide, bromate

Dilute 5 ml of solution S1 to 15 ml with *water*, add 20 ml of *sulphur dioxide solution* and heat on a water bath for 30 minutes. Heat to boiling, cool, add 10 ml of 18M *ammonia* and 20 ml of *silver nitrate solution R2* and dilute to 70 ml with *water*. Filter, transfer 35 ml of the filtrate to a

Nessler cylinder and acidify with 6 ml of *nitric acid*. After 5 minutes, any opalescence, when viewed vertically, is not greater than that produced by treating 5 ml of a 0.00165% w/v solution of *sodium chloride* at the same time and in the same manner (0.02%).

Iodide

Add 1 ml of 1.8M *sulphuric acid* to 25 ml of solution S1 and shake with 1 ml of *chloroform*. Any violet colour produced is not more intense than that of a solution prepared at the same time and in the same manner but using 5 ml of solution S1 and 2 ml of *iodide standard solution (10 ppm I)* (20 ppm).

Sulphate

Add 1 ml of a 25% w/v solution of *barium chloride* to 1.5 ml of *sulphate standard solution (10 ppm SO_4)*, shake and allow to stand for 1 minute. Add 12.5 ml of solution S2 diluted to 15 ml with *distilled water* and 0.5 ml of 5M *acetic acid* and allow to stand for 5 minutes. Any opalescence produced is not more intense than that of a standard prepared in the same manner but using 7.5 ml of *sulphate standard solution (10 ppm SO_4)* diluted to 15 ml with *distilled water* in place of the solution being examined (50 ppm).

Heavy metals

Adjust the pH of 20 ml of solution S2 to about 5 with 5M *ammonia*. The solution complies with *limit test A for heavy metals*, Appendix VII. Use 10 ml of *lead standard solution (2 ppm Pb)* to prepare the standard (20 ppm).

Loss on drying

When dried at 130° for 1 hour, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

To 1.5 g add sufficient *water* to produce 250 ml. To 25 ml of the resulting solution in an iodine flask add 3 g of *potassium iodide*, 100 ml of *water* and 10 ml of *hydrochloric acid*. Close the flask and stand in the dark for 5 minutes. Titrate the solution with 0.1M *sodium thiosulphate VS* to a light straw colour and then complete the titration to a colourless end point using *starch mucilage* as indicator. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 3.567 mg of KIO_3.

Potassium Iodide

General Notices

(Ph Eur monograph 0186)

KI 166.0 *7681-11-0*

Action and use

Antithyroid.

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or colourless crystals.

Solubility

Very soluble in water, freely soluble in glycerol, soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives the reactions of iodides (*2.3.1*).

B. Solution S gives the reactions of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Alkalinity

To 12.5 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Iodates

To 10 ml of solution S add 0.25 ml of *iodide-free starch solution R* and 0.2 ml of *dilute sulphuric acid R* and allow to stand protected from light for 2 min. No blue colour develops.

Sulphates (*2.4.13*)

Maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Thiosulphates

To 10 ml of solution S add 0.1 ml of *starch solution R* and 0.1 ml of *0.005 M iodine*. A blue colour is produced.

Iron (*2.4.9*)

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.00 g of previously powdered substance by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 1.500 g in *water R* and dilute to 100.0 ml with the same solvent. To 20.0 ml of the solution add 40 ml of *hydrochloric acid R* and titrate with *0.05 M potassium iodate* until the colour changes from red to yellow. Add 5 ml of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer is decolourised.

1 ml of *0.05 M potassium iodate* is equivalent to 16.60 mg of KI.

STORAGE

Protected from light.

Ph Eur

Potassium Metabisulphite

General Notices

(Ph Eur monograph 2075)

$K_2S_2O_5$ 222.3 *16731-55-8*

Action and use

Preservative.

Ph Eur

DEFINITION

Potassium metabisulphite (potassium disulphite).

Content

95.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white powder, or colourless crystals.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. pH (see Tests).

B. To 5 ml of solution S (see Tests), add 0.5 ml of *0.05 M iodine*. The mixture is colourless and gives reaction (a) of sulphates (*2.3.1*).

C. Solution S gives reaction (a) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method I*).

pH (*2.2.3*)

3.0 to 4.5 for solution S.

Thiosulphates

To 2.00 g add 25 ml of a 42.5 g/l solution of *sodium hydroxide R* and 75 ml of *water R*. Shake until dissolved and add 10 ml of *formaldehyde R* and 10 ml of *acetic acid R*. After 5 min, titrate with *0.05 M iodine* using 1 ml of *starch solution R*. Carry out a blank titration. The difference between the volumes consumed in the 2 titrations is not more than 0.15 ml.

Iron

Maximum 10.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 20 ml of solution S to 50 ml with *water R*.

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, diluted as necessary with *water R.*

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Selenium

Maximum 10 ppm.

To 3.0 g add 10 ml of *formaldehyde R*. Carefully add 2 ml of *hydrochloric acid R* in small portions. Heat on a water-bath for 20 min. Any pink colour in the solution is not more intense than that of a reference solution prepared at the same time in the same manner using 1.0 g of the substance to be examined to which 0.2 ml of *selenium standard solution (100 ppm Se) R* has been added.

Zinc

Maximum 25.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 20 ml of solution S to 50 ml with *water R*.

Reference solutions Prepare the reference solutions using *zinc standard solution (100 ppm Zn) R*, diluted as necessary with *water R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Introduce 40 ml of solution S into a silica crucible, add 10 ml of *hydrochloric acid R* and evaporate to dryness. Dissolve the residue in 19 ml of *water R* and add 1 ml of a 40 g/l solution of *sodium fluoride R*. The solution complies with test E. Prepare the reference solution using 20 ml of *lead standard solution (1 ppm Pb) R.*

ASSAY

In a 500 ml conical flask containing 50.0 ml of *0.05 M iodine* introduce 0.150 g and add 5 ml of *hydrochloric acid R.* Titrate the excess of iodine with *0.1 M sodium thiosulphate* using 0.1 ml of *starch solution R*.

1 ml of *0.05 M iodine* is equivalent to 5.558 mg of $K_2S_2O_5$.

STORAGE

In an airtight container , protected from light.

Ph Eur

Potassium Nitrate

General Notices

(Ph Eur monograph 1465)

KNO_3 101.1 *7757-79-1*

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, very soluble in boiling water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives the reaction of nitrates (*2.3.1*).

B. Solution S (see Tests) gives the reactions of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Reducible substances

To 10 ml of solution S, add 0.5 ml of *dilute sulphuric acid R* and 2 ml of *zinc iodide and starch solution R*. The solution does not become blue within 2 min.

Chlorides (*2.4.4*)

Maximum 20 ppm, if intended for ophthalmic use.

Dissolve 2.5 g in *water R* and dilute to 15 ml with the same solvent.

Sulphates (*2.4.13*)

Maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Ammonium (*2.4.1*)

Maximum 100 ppm, determined in 1 ml of solution S; maximum 50 ppm if intended for ophthalmic use.

Calcium (*2.4.3*)

Maximum 100 ppm; maximum 50 ppm if intended for ophthalmic use.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 20 ppm; maximum 10 ppm if intended for ophthalmic use.

Dilute 5 ml of solution S to 10 ml with *water R*.

Sodium

Maximum 0.10 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589 nm.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Prepare a chromatography column 0.3 m long and 10 mm in internal diameter and filled with 10 g of *strongly acidic cation-exchange resin R* covered with *carbon dioxide-free water R*. Maintain a 1 cm layer of liquid above the resin at all times. Allow 100 ml of *dilute hydrochloric acid R* to run through the column at a flow rate of about 5 ml/min. Wash the column (with the tap completely open) with *carbon dioxide-free water R* until neutral to *blue litmus paper R*. Dissolve 0.200 g of the substance to be examined in 2 ml of *carbon dioxide-free water R* in a beaker and transfer it to the column reservoir, allow the solution to run through the column at a flow rate of about 3 ml/min and collect the eluate. Wash the beaker with 10 ml of *carbon dioxide-free water R* and transfer this solution at the same flow rate to the column before it runs dry. Finally wash the column with 200 ml of *carbon dioxide-free water R* (with the tap completely open) until neutral to *blue litmus paper R*. Titrate the combined eluate and washings with *0.1 M sodium hydroxide*, using 1 ml of *phenolphthalein solution R* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 10.11 mg of KNO_3.

LABELLING

The label states, where applicable, that the substance is suitable for ophthalmic use.

Ph Eur

Potassium Perchlorate

General Notices

(Ph Eur monograph 1987)

$KClO_4$ 138.5 *7778-74-7*

Action and use

Diagnostic aid; treatment of hyperthyroidism.

Ph Eur

DEFINITION

Content

99.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Sparingly soluble in water, practically insoluble in alcohol.

IDENTIFICATION

A. Dissolve 0.1 g in 5 ml of *water R*. Add 5 ml of *indigo carmine solution R* and heat to boiling. The colour of the solution does not disappear.

B. It complies with the test for chlorates and chlorides (see Tests).

C. Heat 10 mg over a flame for 2 min. Dissolve the residue in 2 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

D. Dissolve 50 mg with heating in 5 ml of *water R*. Allow to cool to room temperature. The solution gives reaction (a) of potassium (*2.3.1*).

TESTS

Solution S

Suspend 5.0 g in 90 ml of *distilled water R* and heat to boiling. Allow to cool. Filter. Dilute the filtrate to 100 ml with *carbon dioxide-free water R*.

Appearance of solution

The solution is clear (*2.2.1*) and colourless (2*.2.2, Method II*).

Dissolve 0.20 g in *water R* and dilute to 20 ml with the same solvent.

Acidity or alkalinity

To 5 ml of solution S add 5 ml of *water R* and 0.1 ml of *phenolphthalein solution R*. Not more than 0.25 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator. To 5 ml of solution S, add 5 ml of *water R* and 0.1 ml of *bromocresol green solution R*. Not more than 0.25 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Chlorates and chlorides (*2.4.4*)

Maximum 100 ppm (calculated as chlorides).

To 5 ml of solution S, add 5 ml of *water R* and heat to boiling. Add 1 ml of *nitric acid R* and 0.1 g of *sodium nitrite R*. Allow to cool to room temperature. Dilute to 15 ml with *water R*. The solution complies with the limit test for chlorides. Prepare the standard using 5 ml of *chloride standard solution (5 ppm Cl)* R and 10 ml of *water R*, and adding only 1 ml of *dilute nitric acid R*.

Sulphates (*2.4.13*)

Maximum 100 ppm.

15 ml of solution S complies with the limit test for sulphates. Prepare the standard using a mixture of 7.5 ml of *sulphate standard solution (10 ppm* SO_4*) R* and 7.5 ml of *water R*.

Calcium (*2.4.3*)

Maximum 100 ppm.

15 ml of solution S complies with the limit test for calcium. Prepare the standard using a mixture of 7.5 ml of *calcium standard solution (10 ppm Ca) R*, 1 ml of *dilute acetic acid R* and 7.5 ml of *distilled water R*.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

ASSAY

Prepare a chromatography column 0.3 m long and 10 mm in internal diameter and filled with 10 g of *strongly acidic ion-exchange resin R* covered with *carbon dioxide-free water R*. Maintain a 1 cm layer of liquid above the resin throughout the determination. Allow 100 ml of *dilute hydrochloric acid R* to run through the column at a flow rate of about 5 ml/min. Wash the column (with the tap completely open) with *carbon dioxide-free water R* until the eluate is neutral to *blue litmus paper R*. Dissolve 0.100 g of the substance to be examined in 10 ml of *carbon dioxide-free water R* in a beaker and transfer it to the column reservoir, allow the solution to run through the column at a flow rate of about 3 ml/min and collect the eluate. Wash the beaker 3 times with 10 ml of *carbon dioxide-free water R* and transfer this solution at the same flow rate to the column before it runs dry. Finally, wash the column with 200 ml of *carbon dioxide-free water R* (with the tap completely open) until the eluate is neutral to *blue litmus paper R*. Titrate the combined eluate and washings with *0.1 M sodium hydroxide*, using 1 ml of *phenolphthalein solution R* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 13.86 mg of $KClO_4$.

Potassium Permanganate

General Notices

(Ph Eur monograph 0121)

$KMnO_4$ 158.0 *7722-64-7*

Action and use

Antiseptic.

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

Dark purple or brownish-black, granular powder or dark purple or almost black crystals, usually having a metallic lustre.

Solubility

Soluble in cold water, freely soluble in boiling water.

It decomposes on contact with certain organic substances.

IDENTIFICATION

A. Dissolve about 50 mg in 5 ml of *water R* and add 1 ml of *ethanol (96 per cent) R* and 0.3 ml of *dilute sodium hydroxide solution R*. A green colour develops. Heat to boiling. A dark brown precipitate is formed.

B. Filter the mixture obtained in identification test A. The filtrate gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 0.75 g in 25 ml of *distilled water R*, add 3 ml of *ethanol (96 per cent) R* and boil for 2-3 min. Cool, dilute to 30 ml with *distilled water R* and filter.

Appearance of solution

Solution S is colourless (*2.2.2, Method II*).

Substances insoluble in water

Maximum 1.0 per cent.

Dissolve 0.5 g in 50 ml of *water R* and heat to boiling. Filter through a tared sintered-glass filter (16) (*2.1.2*). Wash with *water R* until the filtrate is colourless and collect the residue on the filter. The residue, dried in an oven at 100-105 °C, weighs a maximum of 5 mg.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*)

Maximum 500 ppm.

Dilute 12 ml of solution S to 15 ml with *distilled water R*.

ASSAY

Dissolve 0.300 g in *water R* and dilute to 100.0 ml with the same solvent. To 20.0 ml of the solution add 20 ml of *water R*, 1 g of *potassium iodide R* and 10 ml of *dilute hydrochloric acid R*. Titrate the liberated iodine with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution R* as indicator.

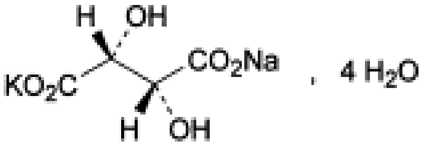
1 ml of *0.1 M sodium thiosulphate* is equivalent to 3.160 mg of $KMnO_4$.

Ph Eur

Potassium Sodium Tartrate Tetrahydrate

General Notices

(Ph Eur monograph 1986)



$C_4H_4KNaO_6,4H_2O$ 282.2   *6381-59-5*

Action and use

Excipient.

Ph Eur

DEFINITION

Potassium sodium (+)-(2*R*,3*R*)-2,3-dihydroxybutanedioate tetrahydrate.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless, transparent crystals.

Solubility

Very soluble in water, practically insoluble in alcohol.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. It gives reaction (b) of tartrates (*2.3.1*).

C. It gives reaction (b) of potassium (*2.3.1*).

D. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 5.000 g in *carbon dioxide-free water R*, prepared from *distilled water R*, and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 5 ml of solution S, add 0.1 ml of *phenolphthalein solution R*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

+ 28.0 to + 30.0 (anhydrous substance), determined on solution S.

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides.

Sulphates (*2.4.13*)

Maximum 50 ppm.

Dissolve 1.0 g in *distilled water R* and dilute to 15 ml with the same solvent. The solution complies with the limit test for sulphates. Prepare the reference solution with a mixture of 5 ml of *sulphate standard solution (10 ppm SO_4) R* and 10 ml of *distilled water R*.

Ammonium (*2.4.1*)

Maximum 40 ppm.

5 ml of solution S complies with the limit test for ammonium.

Barium and oxalates

To 5 ml of solution S, add 3 ml of *calcium sulphate solution R*. Allow to stand for 5 min. Any opalescence in the solution is not more intense than that in a mixture of 3 ml of *calcium sulphate solution R* and 5 ml of *distilled water R*.

Calcium (*2.4.3*)

Maximum 200 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for calcium.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*

Water (*2.5.12*)

24.0 per cent to 26.5 per cent, determined on 50.0 mg. Use 50 ml of *anhydrous methanol R*. Titrate slowly.

ASSAY

To 0.100 g of finely powdered substance add 40 ml of *anhydrous acetic acid R* and 20 ml of *acetic anhydride R*. Titrate slowly with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

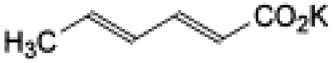
1 ml of *0.1 M perchloric acid* is equivalent to 10.51 mg of $C_4H_4KNaO_6$.

Ph Eur

Potassium Sorbate

General Notices

(Ph Eur monograph 0618)



$C_6H_7KO_2$ 150.2 *590-00-1*

Action and use

Antimicrobial preservative.

Ph Eur

DEFINITION

Potassium (*E*,*E*)-hexa-2,4-dienoate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or granules.

Solubility

Very soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *water R* and dilute to 250.0 ml with the same solvent. Dilute 2.0 ml of this solution to 200.0 ml with *0.1 M hydrochloric acid*.

Spectral range 230-350 nm.

Absorption maximum At 264 nm.

Specific absorbance at the absorption maximum 1650 to 1900.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison potassium sorbate CRS.

C. Dissolve 1.0 g in 50 ml of *water R*, add 10 ml of *dilute hydrochloric acid R* and shake. Filter the crystalline precipitate, wash with *water R* and dry *in vacuo* over *sulphuric acid R* for 4 h. The residue obtained melts (*2.2.14*) at 132 °C to 136 °C.

D. Dissolve 0.2 g in 2 ml of *water R* and add 2 ml of *dilute acetic acid R*. Filter. The solution gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Acidity or alkalinity

To 20 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 0.25 ml of *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Aldehydes

Maximum 0.15 per cent, expressed as C_2H_4O.

Dissolve 1.0 g in a mixture of 30 ml of *water R* and 50 ml of *2-propanol R*, adjust to pH 4 with *1 M hydrochloric acid* and dilute to 100 ml with *water R*. To 10 ml of the solution add 1 ml of *decolorised fuchsin solution R* and allow to stand for 30 min. Any colour in the solution is not more intense than that in a standard prepared at the same time by adding 1 ml of decolorised *fuchsin solution R* to a mixture of 1.5 ml of *acetaldehyde standard solution (100 ppm C_2H_4O) R*, 4 ml of *2-propanol R* and 4.5 ml of *water R*.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.120 g in 20 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* using 0.1 ml of *crystal violet solution R* as indicator until the colour changes from violet to bluish-green.

1 ml of *0.1 M perchloric acid* is equivalent to 15.02 mg of $C_6H_7KO_2$.

STORAGE

Protected from light.

Ph Eur

Potassium Sulphate

General Notices

(Ph Eur monograph 1622)

K_2SO_4 174.3 *7778-80-5*

Ph Eur

DEFINITION

Content

98.5 per cent to 101.0 per cent of K_2SO_4 (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Soluble in water, practically insoluble in ethanol.

IDENTIFICATION

A. It gives the reactions of sulphates (*2.3.1*).

B. It gives the reactions of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in 90 ml of *carbon dioxide-free water R* prepared from *distilled water R*, heating gently. Allow to cool and dilute to 100 ml with *carbon dioxide-free water R* prepared from *distilled water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*)

Maximum 40 ppm.

Dilute 12.5 ml of solution S to 15 ml with *water R*.

Calcium (*2.4.3*)

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 10 ppm, determined on 10 ml of solution S.

Magnesium

Maximum 20 ppm.

To 5 ml of solution S add 5 ml of *water R*, 1 ml of *glycerol (85 per cent) R*, 0.15 ml of titan *yellow solution R*, 0.25 ml of *ammonium oxalate solution R* and 5 ml of *dilute sodium hydroxide solution R* and shake. Any pink colour in the test solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 1 ml of *magnesium standard solution (10 ppm Mg) R* and 9 ml of *water R*.

Sodium

Maximum 0.10 per cent.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Dissolve in *water R* 0.50 g of *sodium chloride R*, previously dried at 100-105 °C for 3 h, and dilute to 1000.0 ml with the same solvent (200 µg of Na per millilitre). Dilute as required.

Wavelength 589 nm.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 4 h.

ASSAY

Dissolve 0.150 g in 40 ml of *water R.* Add 0.2 ml of *0.1 M hydrochloric acid* and 80 ml of *methanol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M lead nitrate* and as indicator electrode a lead-selective electrode and as reference electrode a silver-silver chloride electrode.

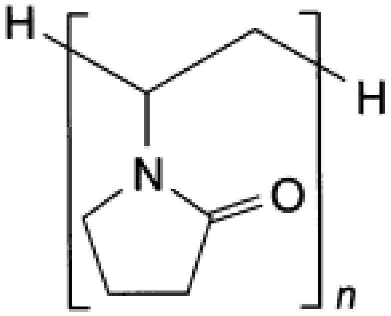
1 ml of *0.1 M lead nitrate* is equivalent to 17.43 mg of K_2SO_4.

Ph Eur

Povidone

General Notices

(Ph Eur monograph 0685)



$C_{6n}H_{9n+2}N_nO_n$ *9003-39-8*

Action and use

Disinfectant.

Ph Eur

DEFINITION

α-Hydro-ω-hydropoly[1-(2-oxopyrrolidin-1-yl)ethylene]. It consists of linear polymers of 1-ethenylpyrrolidin-2-one.

Content

11.5 per cent to 12.8 per cent of nitrogen (N; A_r 14.01) (anhydrous substance).

The different types of povidone are characterised by their viscosity in solution expressed as a K-value.

CHARACTERS

Appearance

White or yellowish-white, hygroscopic powder or flakes.

Solubility

Freely soluble in water, in ethanol (96 per cent) and in methanol, very slightly soluble in acetone.

IDENTIFICATION

First identification *A, E.*

Second identification *B, C, D, E.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dry the substances beforehand at 105 °C for 6 h. Record the spectra using 4 mg of substance.

Comparison *povidone CRS.*

B. To 0.4 ml of solution S1 (see Tests) add 10 ml of *water R*, 5 ml of *dilute hydrochloric acid R* and 2 ml of *potassium dichromate solution R*. An orange-yellow precipitate is formed.

C. To 1 ml of solution S1 add 0.2 ml of *dimethylaminobenzaldehyde solution R1* and 0.1 ml of *sulphuric acid R*. A pink colour is produced.

D. To 0.1 ml of solution S1 add 5 ml of *water R* and 0.2 ml of *0.05 M iodine*. A red colour is produced.

E. To 0.5 g add 10 ml of *water R* and shake. The substance dissolves.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. Add the substance to be examined to the water in small portions, stirring using a magnetic stirrer.

Solution S1

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent. Add the substance to be examined to the water in small portions, stirring using a magnetic stirrer.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_6, BY_6 or R_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

3.0 to 5.0 for solution S, for povidone having a stated *K*-value of not more than 30; 4.0 to 7.0 for solution S, for povidone having a stated *K*-value of more than 30.

Viscosity, expressed as *K*-value

For povidone having a stated value of 18 or less, use a 50 g/l solution. For povidone having a stated value of more than 18 and not more than 95, use a 10 g/l solution. For povidone having a stated value of more than 95, use a 1.0 g/l solution. Allow to stand for 1 h and determine the viscosity (*2.2.9*) of the solution at 25 °C, using viscometer No.1 with a minimum flow time of 100 s. Calculate the *K*-value using the following expression:

$$\frac{1.5\log\eta - 1}{0.15 + 0.003c} + \frac{\sqrt{300c\log\eta + (\mathrm{c} + 1.5c\log\eta)^2}}{0.15c + 0.003c^2}$$

c = concentration of the substance to be examined, calculated with reference to the anhydrous substance, in grams per 100 ml

η = kinematic viscosity of the solution relative to that of *water R.*

The *K*-value of povidone having a stated *K*-value of 15 or less is 85.0 per cent to 115.0 per cent of the stated value.

The *K*-value of povidone having a stated *K*-value or a stated *K*-value range with an average of more than 15 is 90.0 per cent to 108.0 per cent of the stated value or of the average of the stated range.

Aldehydes

Maximum 5.0×10^2 ppm, expressed as acetaldehyde.

Test solution Dissolve 1.0 g of the substance to be examined in *phosphate buffer solution pH 9.0 R* and dilute to 100.0 ml with the same solvent. Stopper the flask tightly and heat at 60 °C for 1 h. Allow to cool to room temperature.

Reference solution Dissolve 0.140 g of *acetaldehyde ammonia trimer trihydrate R* in *water R* and dilute to 200.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *phosphate buffer solution pH 9.0 R.*

Into 3 identical spectrophotometric cells with a path length of 1 cm, introduce separately 0.5 ml of the test solution, 0.5 ml of the reference solution and 0.5 ml of *water R* (blank). To each cell, add 2.5 ml of *phosphate buffer solution pH 9.0 R* and 0.2 ml of *nicotinamide-adenine dinucleotide solution R*. Mix and stopper tightly. Allow to stand at 22 ± 2 °C for 2-3 min and measure the absorbance (*2.2.25*) of each solution at 340 nm, using *water R* as the compensation liquid. To each cell, add 0.05 ml of *aldehyde dehydrogenase solution R,* mix and stopper tightly. Allow to stand at 22 ± 2 °C for 5 min. Measure the absorbance of each solution at 340 nm using *water R* as the compensation liquid.

Calculate the content of aldehydes using the following expression:

$$\frac{(A_{t2} - A_{t1}) - (A_{b2} - A_{b1})}{(A_{s2} - A_{s1}) - (A_{b2} - A_{b1})} \times \frac{100\,000 \times C}{m}$$

A_{t1} = absorbance of the test solution before the addition of aldehyde dehydrogenase

A_{t2} = absorbance of the test solution after the addition of aldehyde dehydrogenase

A_{S1} = absorbance of the reference solution before the addition of aldehyde dehydrogenase

A_{S2} = absorbance of the reference solution after the addition of aldehyde dehydrogenase

A_{b1} = absorbance of the blank before the addition of aldehyde dehydrogenase

A_{t2} = absorbance of the blank after the addition of aldehyde dehydrogenase

m = mass of povidone calculated with reference to the anhydrous substance, in grams

C = concentration of acetaldehyde in the reference solution, calculated from the weight of the acetaldehyde ammonia trimer trihydrate with the factor 0.72, in milligrams per millilitre.

Peroxides

Maximum 400 ppm, expressed as H_2O_2.

Dissolve a quantity of the substance to be examined equivalent to 4.0 g of the anhydrous substance in 100 ml of *water R*. To 25 ml of this solution, add 2 ml of *titanium trichloride-sulphuric acid reagent R*. Allow to stand for 30 min. The absorbance (*2.2.25*) of the solution, measured at 405 nm using a mixture of 25 ml of a 40 g/l solution of the substance to be examined and 2 ml of a 13 per cent *V/V* solution of *sulphuric acid R* as the compensation liquid, is not greater than 0.35.

Formic acid

Liquid chromatography *(2.2.29)*.

Test solution Dissolve a quantity of the substance to be examined equivalent to 2.0 g of the anhydrous substance in *water R* and dilute to 100.0 ml with the same solvent (test stock solution). Transfer a suspension of *strongly acidic ion exchange resin R* for column chromatography in *water R* to a glass tube 0.8 cm in internal diameter and about 20 mm long and keep the strongly acidic ion exchange resin layer constantly immersed in *water R*. Pour 5 ml of *water R* and adjust the flow rate so that the water drops at a rate of about 20 drops per min. When the level of the water comes down to near the top of the strongly acidic ion exchange resin layer, put the test stock solution into the column.

After dropping 2 ml of the solution, collect 1.5 ml of the solution and use this solution as the test solution.

Reference solution Dissolve 100 mg of *formic acid R* and dilute to 100.0 ml with *water R.* Dilute 1.0 ml of this solution to 100.0 ml with *water R.*

Column:
— *material*: stainless steel,

— *size*: *l* = 0.25-0.30 m, Ø = 4-8 mm,

— *stationary phase*: *strongly acidic ion exchange resin for chromatography R* (5-10 µm),

— *temperature*: 30 °C.

Mobile phase Dilute 5 ml of *perchloric acid R* to 1000 ml with *water R.*

Flow rate Adjusted so that the retention time of formic acid is about 11 min.

Detection Spectrophotometer at 210 nm.

Injection 50 µl each of the test and reference solutions.

System suitability:
— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of the

reference solution.

Limits:

— *formic acid*: not more than 10 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent).

Hydrazine

Thin-layer chromatography *(2.2.27)*. *Use freshly prepared solutions.*

Test solution Dissolve a quantity of the substance to be examined equivalent to 2.5 g of the anhydrous substance in 25 ml of *water R*. Add 0.5 ml of a 50 g/l solution of *salicylaldehyde R* in *methanol R*, mix and heat in a water-bath at 60 °C for 15 min. Allow to cool, add 2.0 ml of *toluene R,* shake for 2 min and centrifuge. Use the upper layer of the mixture.

Reference solution Dissolve 90 mg of *salicylaldehyde azine R* in *toluene R* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 100 ml with *toluene R.*

Plate *TLC silanised silica gel plate F_{254} R.*

Mobile phase *water R*, *methanol R* (1:2 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Retardation factor Salicylaldehyde azine = about 0.3.

Limit:

— *hydrazine*: any spot corresponding to salicylaldehyde azine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1 ppm).

Impurity A

Liquid chromatography *(2.2.29)*.

Test solution Dissolve a quantity of the substance to be examined equivalent to 0.250 g of the anhydrous substance in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 50 mg of *1-vinylpyrrolidin-2-one R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *1-vinylpyrrolidin-2-one R* and 0.5 g of *vinyl acetate R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Precolumn:

— *size*: *l* = 0.025 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *acetonitrile R*, *water R* (10:90 *V/V*).

Flow rate Adjusted so that the retention time of the peak corresponding to impurity A is about 10 min.

Detection Spectrophotometer at 235 nm.

Injection 50 µl. After injection of the test solution, wait for about 2 min and wash the precolumn by passing the mobile phase backwards, at the same flow rate applied in the test, for 30 min.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to impurity A and to vinyl acetate in the chromatogram obtained with reference solution (b);

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (a).

Limit:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (10 ppm).

Impurity B

Liquid chromatography *(2.2.29)*.

Test solution Dissolve a quantity of the substance to be examined equivalent to 100 mg of the anhydrous substance in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution Dissolve 100 mg of *2-pyrrolidone R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 3.0 ml of this solution to 50.0 ml with *water R.*

Precolumn:

— *size*: *l* = 0.025 m, Ø = 3 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

— *size*: *l* = 0.25 m, Ø = 3 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *water R*, adjusted to pH 2.4 with *phosphoric acid R*.

Flow rate Adjusted so that the retention time of impurity B is about 11 min.

Detection Spectrophotometer at 205 nm.

Injection 50 µl. After each injection of the test solution, wash away the polymeric material of povidone from the guard column by passing the mobile phase through the column backwards for about 30 min at the same flow rate as applied in the test.

System suitability:

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of the reference solution.

Limit:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (3.0 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2.0 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 5.0 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Place 100.0 mg of the substance to be examined (*m* mg) in a combustion flask, add 5 g of a mixture of 1 g of *copper sulphate R*, 1 g of *titanium dioxide R* and 33 g of *dipotassium sulphate R*, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of *water R*. Add 7 ml of *sulphuric acid R,* allowing it to run down the insides of the flask. Heat the flask gradually until the solution has a clear, yellowish-green colour, and the inside wall of the flask is free from a carbonised material, and then heat for a further 45 min. After cooling, add cautiously 20 ml of *water R*, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 ml of a 40 g/l solution of *boric acid R*, 3 drops of *bromocresol green-methyl red solution* R and sufficient water to immerse the lower end of the condenser tube. Add 30 ml of a solution of *strong sodium hydroxide solution R* through the funnel, rinse the funnel cautiously with 10 ml of *water R*, immediately close the clamp on the rubber tube, then start distillation with steam to obtain 80-100 ml of distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of *water R,* and titrate the distillate with *0.025 M sulphuric acid* until the colour of the solution changes from green through pale greyish blue to pale greyish reddish-purple. Carry out a blank determination.

1 ml of *0.025 M sulphuric acid* is equivalent to 0.7004 mg of N.

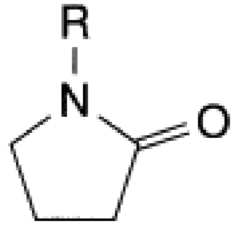
STORAGE

In an airtight container.

LABELLING

The label indicates the nominal *K*-value.

IMPURITIES



A. R = $CH=CH_2$: 1-ethenylpyrrolidin-2-one (1-vinylpyrrolidin-2-one),

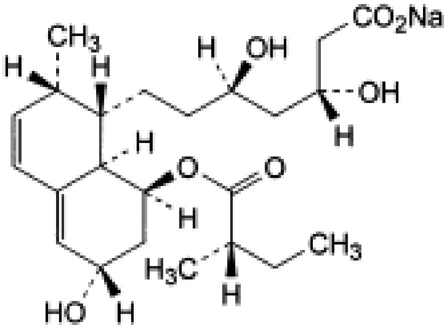
B. R = H: pyrrolidin-2-one (2-pyrrolidone).

Ph Eur

Pravastatin Sodium

General Notices

(Ph Eur monograph 2059)



$C_{23}H_{35}NaO_7$ 446.5 *81131-70-6*

Action and use

HMG Co-A reductase inhibitor; lipid-regulating drug.

Preparation

Pravastatin Tablets

Ph Eur

DEFINITION

Sodium (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoate.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White to yellowish-white powder or crystalline powder, hygroscopic.

Solubility

Freely soluble in water and in methanol, soluble in anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of pravastatin sodium.

C. 1 ml of solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dilute 2.0 ml of solution S to 10.0 ml with *water R*.

pH (*2.2.3*)

7.2 to 9.0 for solution S.

Specific optical rotation (*2.2.7*)

+ 153 to + 159 (anhydrous substance).

Dilute 2.0 ml of solution S to 20.0 ml with *water R*.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *methanol R*, *water R* (9:11 *V/V*).

Test solution (a) Dissolve 0.1000 g of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Test solution (b) Dilute 10.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *pravastatin impurity A CRS* in 1.0 ml of test solution (b).

Reference solution (b) Dilute 2.0 ml of test solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 12.4 mg of *pravastatin 1,1,3,3-tetramethylbutylamine* CRS in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Column:

— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 25 °C.

Mobile phase *glacial acetic acid R*, *triethylamine R*, *methanol R*, *water R* (1:1:450:550 *V/V/V/V*).

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 238 nm.

Injection 10 µl of test solution (a) and reference solutions (a) and (b).

Run time 2.5 times the retention time of pravastatin.

Relative retention With reference to pravastatin (retention time = about 21 min): impurity B = about 0.2; impurity E = about 0.3; impurity A = about 0.6; impurity D = about 1.9; impurity C = about 2.1.

System suitability Reference solution (a):

— *resolution*: minimum 7.0 between the peaks due to impurity A and pravastatin.

Limits:

— *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol (*2.4.24, System A*)

Maximum 3.0 per cent m/m.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *methanol R*.

Water (*2.5.12*)

Maximum 4.0 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{23}H_{35}NaO_7$ using the chromatogram obtained with reference solution (c) and the declared content of pravastatin in *pravastatin 1,1,3,3-tetramethylbutylamine CRS.*

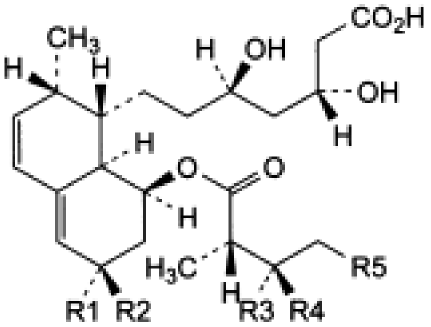
1 mg of pravastatin is equivalent to 1.052 mg of pravastatin sodium.

STORAGE

In an airtight container .

IMPURITIES

Specified impurities   A, B, C, D, E.

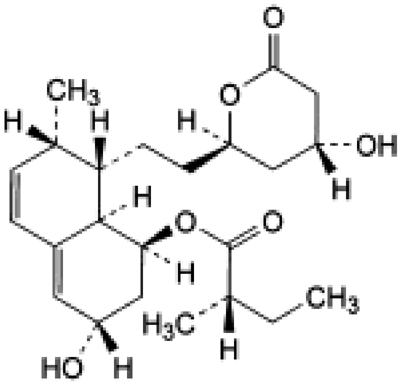


A. R1 = R3 = R4 = R5 = H, R2 = OH: (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl] heptanoic acid (6′-epipravastatin),

B. R1 = R4 = OH, R2 = R3 = R5 = H: (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-8-[[(2*S*,3*R*)-3-hydroxy-2-methylbutanoyl]oxy]-2-methyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoic acid (3″-(*R*)-hydroxypravastatin),

C. R1 = OH, R2 = R3 = R4 = H, R5 = CH_3: (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylpentanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl] heptanoic acid,

E. R1 = R3 = OH, R2 = R4 = R5 = H: (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-8-[[(2*S*,3*S*)-3-hydroxy-2-methylbutanoyl]oxy]-2-methyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoic acid (3″-(*S*)-hydroxypravastatin),



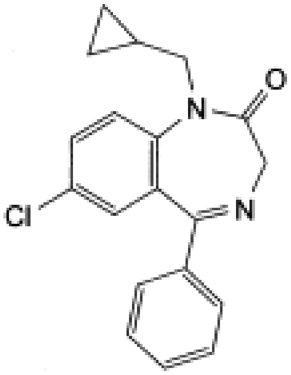
D. (1*S*,3*S*,7*S*,8*S*,8a*R*)-3-hydroxy-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl] ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (pravastatin lactone).

Ph Eur

Prazepam

General Notices

(Ph Eur monograph 1466)



$C_{19}H_{17}ClN_2O$ 324.8 *2955-38-6*

Action and use

Anxiolytic.

Ph Eur

DEFINITION

Prazepam contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7-chloro-1-(cyclopropylmethyl)-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol.

It melts at about 145 °C.

IDENTIFICATION

First identification B.

Second identification A, C.

A. Dissolve 30.0 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 20.0 ml of the solution to 100.0 ml (solution A) and 2.0 ml of the solution to 100.0 ml (solution B) with the same solvent. Examined between 300 nm and 350 nm (*2.2.25*), solution A shows an absorption maximum at 312 nm. Examined between 210 nm and 300 nm, solution B shows an absorption maximum at 228 nm and a point of inflexion at about 252 nm. The specific absorbance at the maximum at 228 nm is 900 to 940. The specific absorbance at the maximum at 312 nm is 59 to 63.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *prazepam CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, fluorescence at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (b).

TESTS

Appearance of solution

Dissolve 0.25 g in *alcohol R* and dilute to 10 ml with the same solvent. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 0.50 g of the substance to be examined in *acetone R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 100 ml with *acetone R*.

Reference solution (a) Dilute 1 ml of test solution (b) to 10 ml with *acetone R*.

Reference solution (b) Dissolve 10 mg of *prazepam CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 15 mg of *nordazepam CRS* in *acetone R* and dilute to 50 ml with the same solvent.

Reference solution (d) To 1 ml of reference solution (a) add 1 ml of reference solution (c) and mix.

Apply to the plate 5 µl of each solution. Develop over a path of 10 cm using a freshly prepared mixture of 50 volumes of *ethyl acetate R* and 50 volumes of *heptane R*. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm. In the chromatogram obtained with test solution (a): any spot corresponding to nordazepam is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.3 per cent); not more than four additional spots are present, none of which are more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots.

Heavy metals (*2.4.8*)

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

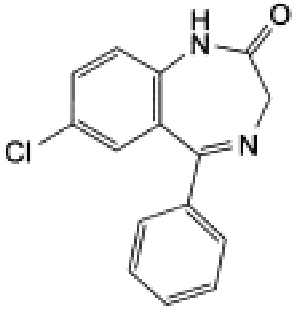
Dissolve 0.250 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 32.48 mg of $C_{19}H_{17}ClN_2O$.

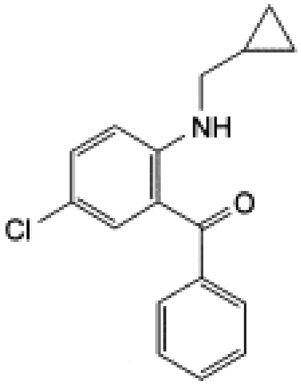
STORAGE

Store protected from light.

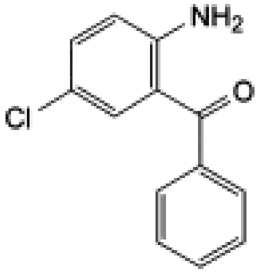
IMPURITIES



A. 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (nordazepam),



B. [5-chloro-2-[(cyclopropylmethyl)amino]phenyl]phenylmethanone,



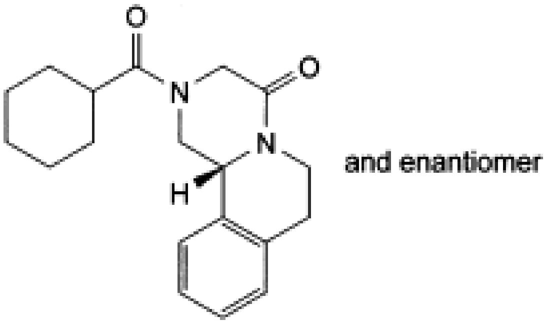
C. (2-amino-5-chlorophenyl)phenylmethanone.

Ph Eur

Praziquantel

General Notices

(Ph Eur monograph 0855)



$C_{19}H_{24}N_2O_2$ 312.4 *55268-74-1*

Action and use

Anthelminthic.

Ph Eur

DEFINITION

(11b*RS*)-2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in alcohol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *praziquantel CRS.*

If the spectra obtained show differences, dissolve 50 mg of the substance to be examined and 50 mg of the reference substance separately in 2 ml of *methanol R*. Evaporate and dry the residue at 60 °C at a pressure not exceeding 0.7 kPa. Record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Test solution (b) Dilute 1.0 ml of test solution (a) to 20.0 ml with the mobile phase.

Reference solution (a) Dissolve 40.0 mg of *praziquantel CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *praziquantel impurity A CRS* in reference solution (a) and dilute to 25.0 ml with reference solution (a). Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of test solution (a) to 20.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *water R* (45:55 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl; inject test solution (a) and reference solutions (b) and (c).

Run time 5 times the retention time of praziquantel (retention time = about 9 min).

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks corresponding to impurity A and praziquantel.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent), not more than 1 such peak having an area greater than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 50 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 2 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

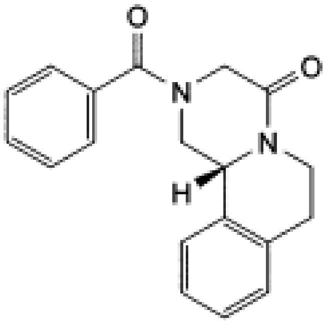
Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{19}H_{24}N_2O_2$.

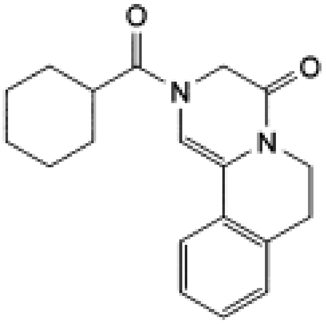
STORAGE

Protected from light.

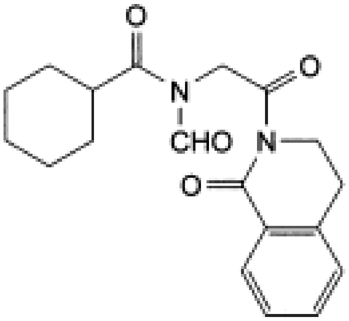
IMPURITIES



A. (11b*RS*)-2-benzoyl-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one,



B. 2-(cyclohexylcarbonyl)-2,3,6,7-tetrahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one,



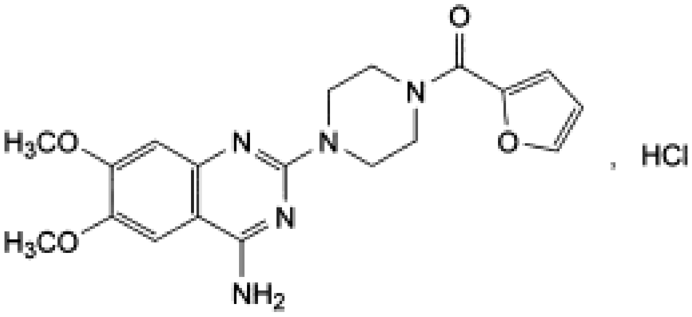
C. *N*-formyl-*N*-[2-oxo-2-(1-oxo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl] cyclohexanecarboxamide.

Ph Eur

Prazosin Hydrochloride

General Notices

(Ph Eur monograph 0856)



$C_{19}H_{21}N_5O_4$,HCl 419.9 *19237-84-4*

Action and use

Alpha$_1$-adrenoceptor antagonist.

Preparation

Prazosin Tablets

Ph Eur

DEFINITION

1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-4-(furan-2-ylcarbonyl)piperazine hydrochloride.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, slightly soluble in alcohol and in methanol, practically insoluble in acetone.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 50.0 mg in a 0.1 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* and dilute to 100.0 ml with the same acid solution. Dilute separately 1.0 ml and 5.0 ml of this solution to 100.0 ml with a 0.1 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* (solution A and solution B, respectively). Examined between 220 nm and 280 nm (*2.2.25*), solution A shows an absorption maximum at 247 nm. The specific absorbance at the maximum is 1320 to 1400. Examined between 280 nm and 400 nm, solution B shows 2 absorption maxima, at 330 nm and 343 nm. The specific absorbances at the maxima are 260 to 280 and 240 to 265, respectively.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation *discs of potassium chloride R*.

Comparison *prazosin hydrochloride CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *diethylamine R*, 10 volumes of *methanol R* and 10 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 10 mg of *prazosin hydrochloride CRS* in a mixture of 1 volume of *diethylamine R*, 10 volumes of *methanol R* and 10 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *diethylamine R*, *ethyl acetate R* (5:95 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 2 mg in 2 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 8 mg of *metoclopramide hydrochloride CRS* in 1 ml of the test solution and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Column:
- — *size:* l = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 50 volumes of *methanol R* and 50 volumes of a solution containing 3.484 g/l of *sodium pentanesulphonate R* and 3.64 g/l of *tetramethylammonium hydroxide* R adjusted to pH 5.0 with *glacial acetic acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 6 times the retention time of prazosin.

Retention times Prazosin = about 9 min; metoclopramide = about 5 min.

System suitability Reference solution (b):

- — *resolution*: minimum 8 between the peaks due to metoclopramide and to prazosin.

Limits:
- — *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Iron

Maximum 1.00 × 10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution To 1.0 g add dropwise about 1.5 ml of *nitric acid R*. After fuming has subsided, evaporate on a water-bath and ignite by gradually raising the temperature from 150 °C to 1000 ± 50 °C, maintaining the final temperature for 1 h. Cool, dissolve the residue in 20 ml of *dilute hydrochloric acid R*, evaporate to about 5 ml and dilute to 25.0 ml with *dilute hydrochloric acid R*.

Reference solutions Prepare the reference solutions *using iron standard solution (8 ppm Fe) R*, diluted as necessary with *water R*.

Source Iron hollow-cathode lamp.

Wavelength 248 nm.

Flame Air-acetylene.

Nickel

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use the test solution prepared in the test for iron.

Reference solutions Prepare the reference solutions using *nickel standard solution (10 ppm Ni) R*, diluted as necessary with *water R*.

Source Nickel hollow-cathode lamp.

Wavelength 232 nm.

Flame Air-acetylene.

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.000 g. Use a mixture of equal volumes of *methanol R* and *methylene chloride R* as the solvent.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

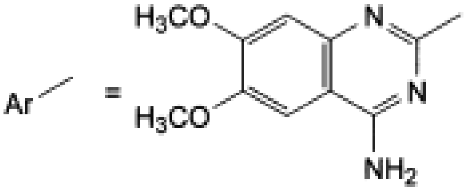
Dissolve 0.350 g in a mixture of 20 ml of *anhydrous formic acid R* and 30 ml of *acetic anhydride R*. Titrate quickly with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 41.99 mg of $C_{19}H_{22}ClN_5O_4$.

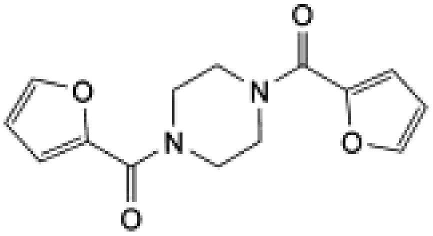
STORAGE

Protected from light.

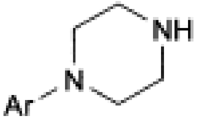
IMPURITIES



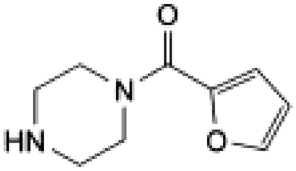
A. Ar-Cl: 2-chloro-6,7-dimethoxyquinazolin-4-amine,



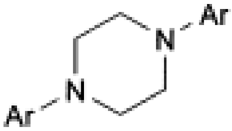
B. 1,4-bis(furan-2-ylcarbonyl)piperazine,



C. 6,7-dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine,



D. 1-(furan-2-ylcarbonyl)piperazine,



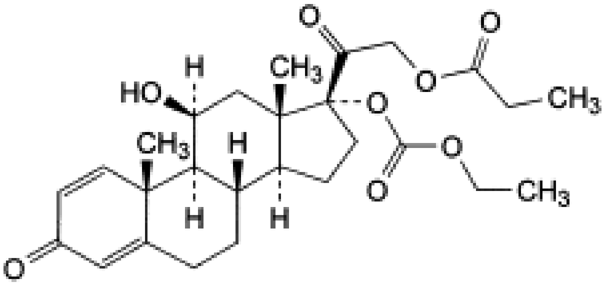
E. 2,2′-(piperazin-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine).

Ph Eur

Prednicarbate

General Notices

(Ph Eur monograph 1467)



$C_{27}H_{36}O_8$ 488.6 *73771-04-7*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

11β-Hydroxy-3,20-dioxopregna-1,4-diene-17,21-diyl 17-ethylcarbonate 21-propanoate.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent), sparingly soluble in propylene glycol.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison prednicarbate CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *prednicarbate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *prednisolone acetate CRS* in 5 ml of reference solution (a).

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with alcoholic solution of *sulphuric acid R*; heat at 120 °C for 10 min or until the spots appear and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

TESTS

Specific optical rotation (*2.2.7*)

+ 60 to + 66 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 3 mg of *prednicarbate impurity F CRS* in the mobile phase, add 5.0 ml of the test solution and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (c) Dissolve 30.0 mg of *prednicarbate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Column:
— *size: l* = 0.125 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *water R* (5:6 *V/V*).

Flow rate 0.7 ml/min.

Detection Spectrophotometer at 243 nm.

Injection 20 µl of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of prednicarbate.

Retention time Prednicarbate = about 17 min; impurity F = about 19 min.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to prednicarbate and impurity F; if necessary, adjust the composition of the mobile phase.

Limits:
— *impurity F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.0125 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

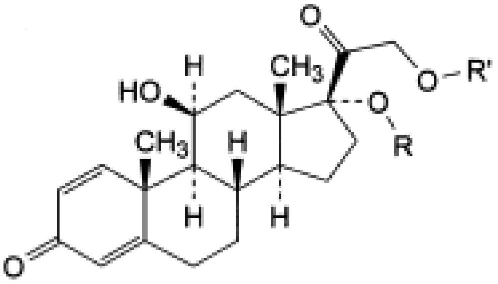
Calculate the percentage content of $C_{27}H_{36}O_8$ from the declared content of *prednicarbate CRS.*

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F.*


O
H
CH3
HO
O
R'
O
R
CH3
H
H
H
O

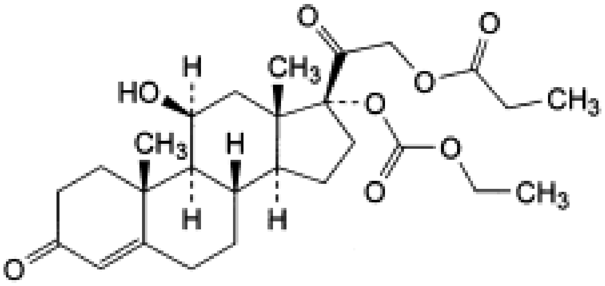
A. R = R′ = H: prednisolone,

B. R = CO-O-C_2H_5, R′ = H: prednisolone 17-ethylcarbonate,

C. R = H, R′ = CO-C_2H_5: prednisolone 21-propanoate,

D. R = H, R′ = CO-O-C_2H_5: prednisolone 21-ethylcarbonate,

E. R = CO-O-C_2H_5, R′ = CO-CH_3: prednisolone 21-acetate 17-ethylcarbonate,


O
O
H
CH3
HO
O
CH3
CH3
H
O
O
H
H
O
CH3
O

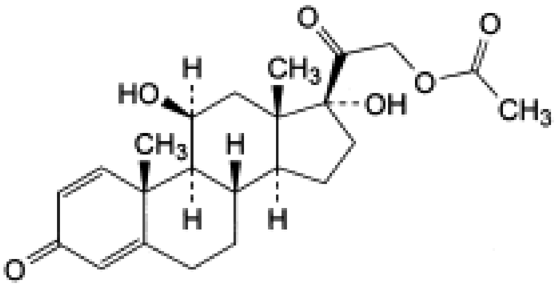
F. 11β-hydroxy-3,20-dioxopregn-4-ene-17,21-diyl 17-ethylcarbonate 21-propanoate(1,2-dihydroprednicarbate).

Ph Eur

Prednisolone Acetate

General Notices

(Ph Eur monograph 0734)



$C_{23}H_{30}O_6$ 402.5 *52-21-1*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *A, B.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *prednisolone acetate CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dissolve 20 mg of *prednisolone acetate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *prednisolone pivalate CRS* in reference solution (a) and dilute to 10 ml with the same solution.

Plate *TLC silica gel plate F_{254} R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 105 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

System suitability Reference solution (b):

— the chromatogram obtained shows 2 clearly separated spots.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add the solution to 10 ml of *water R* and mix. The colour fades and there is greenish-yellow fluorescence in ultraviolet light at 365 nm.

D. About 10 mg gives the reaction of acetyl (*2.3.1*).

TESTS

Specific optical rotation *(2.2.7)*

+ 128 to + 137 (dried substance).

Dissolve 70.0 mg in *methanol R2* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Buffer solution pH 4 Mix 1 volume of *dilute hydrochloric acid R*, 5 volumes of a 68.1 g/l solution of *sodium acetate R*, 15 volumes of a 37.3 g/l solution of *potassium chloride R* and 79 volumes of *water R*.

Solvent mixture Mix equal volumes of *acetonitrile R* and buffer solution pH 4.

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *prednisolone acetate CRS* and 2 mg of *hydrocortisone acetate CRS* (impurity A) in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *prednisolone acetate for peak identification CRS* (containing impurities A, B and C) in the solvent mixture and dilute to 50 ml with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase *acetonitrile R*, *water R* (350:650 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 2.5 times the retention time of prednisolone acetate.

Identification of impurities Use the chromatogram supplied with *prednisolone acetate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C.

Relative retention With reference to prednisolone acetate (retention time = about 17 min): impurity B = about 0.4; impurity A = about 1.1; impurity C = about 2.0.

System suitability Reference solution (a):

- *resolution*: minimum 2.0 between the peaks due to prednisolone acetate and impurity A.

Limits:

- *impurities A, B*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurity C*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 243 nm.

Calculate the content of $C_{23}H_{30}O_6$ taking the specific absorbance to be 370.

STORAGE

Protected from light.

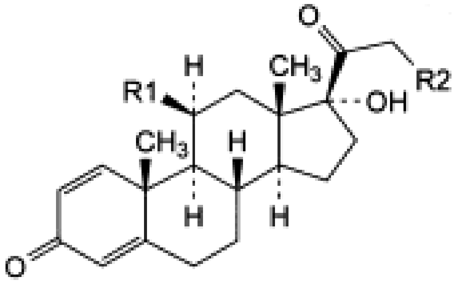
IMPURITIES

Specified impurities A, B, C.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D, E.*

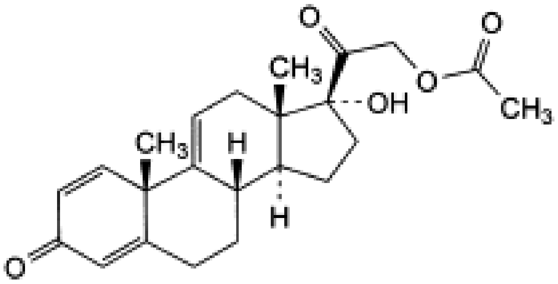
A. hydrocortisone acetate,

B. prednisolone,



C. R1 = R2 = O-CO-CH_3: 17-hydroxy-3,20-dioxopregna-1,4-diene-11β,21-diyl diacetate (prednisolone 11,21-diacetate),

D. R1 = OH, R2 = H: 11β,17-dihydroxypregna-1,4-diene3,20-dione,


O
O
CH3
OH
CH3
CH3
H
H
O

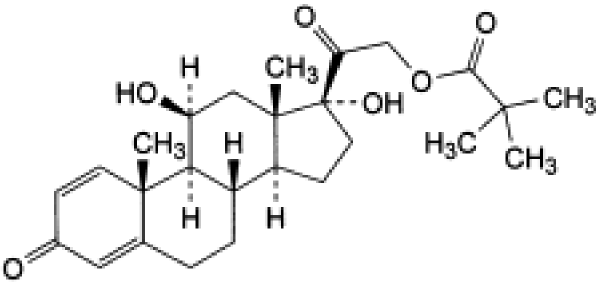
E. 17-hydroxy-3,20-dioxopregna-1,4,9(11)-trien-21-yl acetate.

Ph Eur

Prednisolone Pivalate

General Notices

(Ph Eur monograph 0736)



$C_{26}H_{36}O_6$ 444.6 *1107-99-9*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent), soluble in methylene chloride.

mp: about 229 °C, with decomposition.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D.*

A. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* at the absorption maximum at 415 nm is 0.20 to 0.30.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *prednisolone pivalate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R,* evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *prednisolone pivalate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *prednisolone acetate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture. Dilute 5 ml of this solution to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*, heat at 120 °C for 10 min or until the spots appear, and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

D. To 2 ml of *sulphuric acid R*, add about 2 mg and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add this solution to 10 ml of *water R* and mix. The colour fades and there is greenish-yellow fluorescence in ultraviolet light at 365 nm.

TESTS

Specific optical rotation *(2.2.7)*

+ 104 to + 112 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 62.5 mg of the substance to be examined in 2 ml of a mixture of 1 volume of *water R* and 4 volumes of *tetrahydrofuran R* and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 25 mg of *prednisolone acetate CRS*, 25 mg of *cortisone acetate CRS* and 25 mg of *prednisolone pivalate CRS* in 2 ml of a mixture of 1 volume of *water R* and 4 volumes of *tetrahydrofuran R* and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 25.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Carefully mix 19 ml of *butyl acetate R1* with 37 ml of *tetrahydrofuran R* and 213 ml of *ethylene glycol monomethyl ether R*, then add with 231 ml of *water R*; mix, allow to equilibrate for 1 h and filter through a 0.45 µm filter.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time 1.5 times the retention time of prednisolone pivalate.

Retention time Prednisolone acetate = about 3.5 min; cortisone acetate = about 4.5 min; prednisolone pivalate = about 13 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to prednisolone acetate and cortisone acetate; if necessary, adjust the concentration of water in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent), and not more than one such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 250.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 243 nm.

Calculate the content of $C_{26}H_{36}O_6$ taking the specific absorbance to be 337.

STORAGE

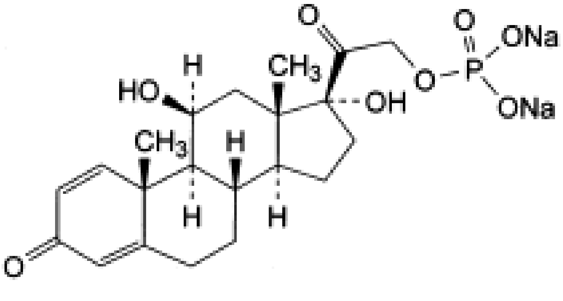
Protected from light.

Ph Eur

Prednisolone Sodium Phosphate

General Notices

(Ph Eur monograph 0735)



$C_{21}H_{27}Na_2O_8P$ 484.4   *125-02-0*

Action and use

Glucocorticoid.

Preparations

Prednisolone Enema

Prednisolone Sodium Phosphate Ear Drops

Prednisolone Sodium Phosphate Eye Drops

Ph Eur

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl disodium phosphate.

Content

96.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D, E.*

A. Dissolve 10.0 mg in 5 ml of *water R* and dilute to 100.0 ml with *anhydrous ethanol R*. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance *(2.2.25)* at the absorption maximum at 415 nm is 0.10 to 0.20.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *prednisolone sodium phosphate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of ethanol (96 per cent) R, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *prednisolone sodium phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *dexamethasone sodium phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent. Dilute 5 ml of this solution to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R*, *water R*, *butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*, heat at 120 °C for 10 min or until the spots appear, and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 spots which may, however, not be completely separated.

D. To 2 ml of *sulphuric acid R* add about 2 mg and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add this solution to 10 ml of *water R* and mix. The colour fades and there is a greenish-yellow fluorescence in ultraviolet light at 365 nm.

E. To about 40 mg add 2 ml of *sulphuric acid R* and heat gently until white fumes are evolved. Add *nitric acid R* dropwise, continue the heating until the solution is almost colourless, and cool. Add 2 ml of *water R*, heat until white fumes are again evolved, cool, add 10 ml of *water R* and neutralise to *red litmus paper R* with *dilute ammonia R1*. The solution gives reaction (a) of sodium (*2.3.1*) and reaction (b) of phosphates (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

pH *(2.2.3)*

7.5 to 9.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 94 to + 100 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 62.5 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 25 mg *of prednisolone sodium phosphate CRS* and 25 mg of *prednisolone CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 25.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Into a 250 ml conical flask weigh 1.360 g of *potassium dihydrogen phosphate R* and 0.600 g of *hexylamine R*, mix, allow to stand for 10 min, then dissolve in 185 ml of *water R*; add 65 ml of *acetonitrile R*, mix, and filter through a 0.45 µm filter.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl.

Run time 3 times the retention time of prednisolone sodium phosphate.

Retention time Prednisolone sodium phosphate = about 6.5 min; prednisolone = about 8.5 min.

System suitability Reference solution (a):

— *resolution*: minimum 4.5 between the peaks due to prednisolone sodium phosphate and prednisolone; if necessary, increase the concentration of *acetonitrile R* or *water R* in the mobile phase.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent), and not more than 1 such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Inorganic phosphate

Maximum 1 per cent.

Dissolve 50 mg in *water R* and dilute to 100 ml with the same solvent. To 10 ml of this solution add 5 ml of *molybdovanadic reagent R*, mix, and allow to stand for 5 min. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of *phosphate standard solution (5 ppm* PO_4*) R*.

Water *(2.5.12)*

Maximum 8.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.100 g in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 250.0 ml with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 247 nm.

Calculate the content of $C_{21}H_{27}Na_2O_8P$ taking the specific absorbance to be 312.

STORAGE

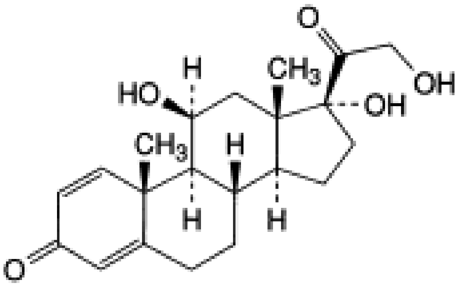
Protected from light.

Ph Eur

Prednisolone

General Notices

(Ph Eur monograph 0353)



$C_{21}H_{28}O_5$ 360.4 *50-24-8*

Action and use

Glucocorticoid.

Preparations

Prednisolone Tablets

Gastro-resistant Prednisolone Tablets

Ph Eur

DEFINITION

11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline, hygroscopic powder.

Solubility

Very slightly soluble in water, soluble in ethanol (96 per cent) and in methanol, sparingly soluble in acetone, slightly soluble in methylene chloride.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *prednisolone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a) Dissolve 20 mg of *prednisolone CRS* in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *hydrocortisone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *methanol R*, *methylene chloride R* (10:90 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*; heat at 120 °C for 10 min or until the spots appear and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

TESTS

Specific optical rotation *(2.2.7)*

+ 96 to + 102 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in 2 ml of *tetrahydrofuran R* and dilute to 10.0 ml with *water R*.

Reference solution (a) Dissolve 2 mg of *prednisolone CRS* and 2 mg of *hydrocortisone CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase In a 1000 ml volumetric flask, mix 220 ml of *tetrahydrofuran R* with 700 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µl; inject the solvent mixture of the test solution as a blank.

Run time 4.5 times the retention time of prednisolone.

Retention time Prednisolone = about 14 min; impurity A = about 15.5 min.

System suitability Reference solution (a):

- *resolution*: minimum 2.2 between the peaks due to prednisolone and impurity A; if necessary, adjust the concentration of *tetrahydrofuran R* in the mobile phase.

Limits:

- *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent) and not more than one such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 243.5 nm.

Calculate the content of $C_{21}H_{28}O_5$ taking the specific absorbance to be 415.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities A.

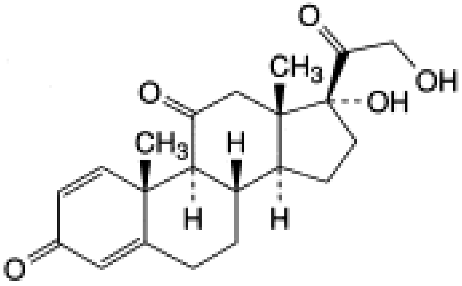
A. hydrocortisone.

Ph Eur

Prednisone

General Notices

(Ph Eur monograph 0354)



$C_{21}H_{26}O_5$ 358.4 *53-03-2*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

17,21-Dihydroxypregna-1,4-diene-3,11,20-trione.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *A, B.*

Second identification *C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *prednisone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R*, *methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 20 mg of *prednisone CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *betamethasone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

C. Thin-layer chromatography *(2.2.27)*.

Test solution (a) Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of this solution to 10 ml with *methylene chloride R.*

Test solution (b) Transfer 0.4 ml of solution A to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension in a mechanical shaker, protected from light, for 1 h. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 ml separating funnel, washing the filter with 2 quantities, each of 5 ml, of *water R*. Shake the clear filtrate with 10 ml of *methylene chloride R*. Wash the organic layer with 5 ml of *1 M sodium hydroxide* and 2 quantities, each of 5 ml, of *water R*. Dry over *anhydrous sodium sulphate R*.

Reference solution (a) Dissolve 25 mg of *prednisone CRS* in *methanol R* and dilute to 5 ml with the same solvent (solution B). Dilute 2 ml of this solution to 10 ml with *methylene chloride R*.

Reference solution (b) Transfer 0.4 ml of solution B to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension in a mechanical shaker, protected from light, for 1 h. Add 2 ml of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 ml separating funnel, washing the filter with 2 quantities, each of 5 ml, of *water R*. Shake the clear filtrate with 10 ml of *methylene chloride R*. Wash the organic layer with 5 ml of *1 M sodium hydroxide* and 2 quantities, each of 5 ml, of *water R*. Dry over *anhydrous sodium sulphate R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µl of test solution (a) and reference solution (a) and 50 µl of test solution (b) and reference solution (b), applying the latter 2 in small quantities in order to obtain small spots.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_F value distinctly higher than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a yellow colour develops with a blue fluorescence in ultraviolet light at 365 nm. Add this solution to 10 ml of *water R* and mix. The colour fades but the blue fluorescence in ultraviolet light does not disappear.

TESTS

Specific optical rotation *(2.2.7)*

+ 167 to + 175 (dried substance).

Dissolve 0.125 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *prednisone CRS* and 2 mg of *prednisolone CRS* in *methanol R* and dilute to 100.0 ml with the same solvent.

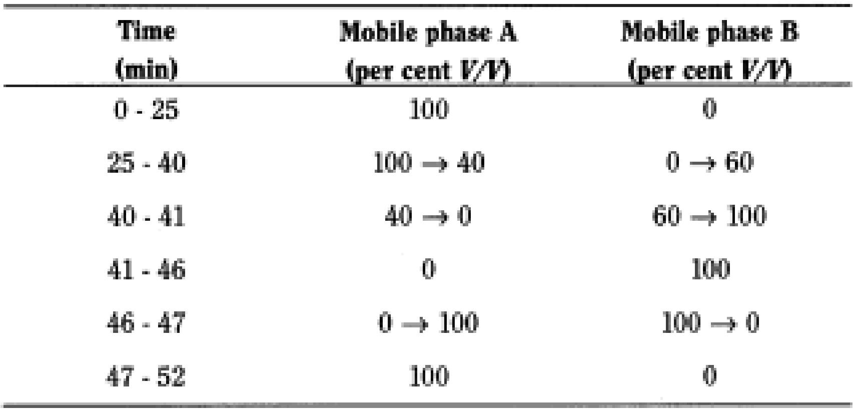
Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:

- *mobile phase A*: in a 1000 ml volumetric flask mix 100 ml of *acetonitrile R* with 200 ml of *methanol R* and 650 ml of *water R*; allow to equilibrate; adjust to 1000 ml with *water R* and mix again;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 40	100 → 40	0 → 60
40 - 41	40 → 0	60 → 100
41 - 46	0	100
46 - 47	0 → 100	100 → 0
47 - 52	100	0

Flow rate 2.5 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With mobile phase B for at least 30 min, and then with mobile phase A for 5 min. For subsequent chromatograms, use the conditions described from 40.0 min to 52.0 min.

Injection 20 µl; inject *methanol R* as a blank.

Retention time Prednisone = about 19 min; prednisolone = about 23 min.

System suitability Reference solution (a):

- *resolution*: minimum 2.7 between the peaks due to prednisone and prednisolone; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Limits:

— *any impurity*: for each impurity, not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R.* Measure the absorbance (*2.2.25*) at the absorption maximum at 238 nm.

Calculate the content of $C_{21}H_{26}O_5$ taking the specific absorbance to be 425.

STORAGE

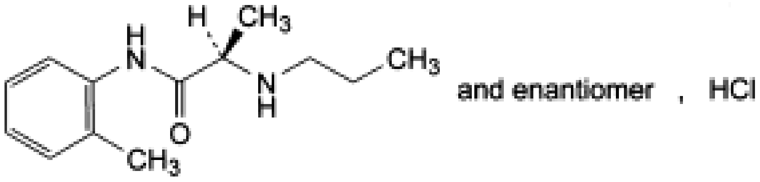
Protected from light.

Ph Eur

Prilocaine Hydrochloride

General Notices

(Ph Eur monograph 1363)



$C_{13}H_{20}N_2O,HCl$ 256.8 *1786-81-8*

Action and use

Local anaesthetic.

Preparation

Prilocaine Injection

Ph Eur

DEFINITION

(*RS*)-*N*-(2-Methylphenyl)-2-(propylamino)propanamide hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water and in ethanol (96 per cent), very slightly soluble in acetone.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Melting point (*2.2.14*): 168 °C to 171 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *prilocaine hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20.0 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *prilocaine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 20.0 mg of *prilocaine hydrochloride CRS* and 20.0 mg of *lidocaine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R*.

Mobile phase *concentrated ammonia R*, *methanol R*, *ether R* (1:5:100 *V/V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dilute 4 ml of solution S to 10 ml with *carbon dioxide-free water R*. Add 0.1 ml of *bromocresol green solution R* and 0.40 ml of *0.01 M sodium hydroxide*; the solution is blue. Add 0.80 ml of *0.01 M hydrochloric acid*; the solution is yellow.

Optical rotation (*2.2.7*)

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 3.0 mg of the substance to be examined and 3.0 mg of *prilocaine impurity E CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
- — *size*: *l* = 0.125 m, Ø = 4.6 mm;
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a solution prepared as follows: dissolve 0.180 g of *sodium dihydrogen phosphate monohydrate R* and 2.89 g of *disodium hydrogen phosphate dihydrate R* in 1000 ml of *water R* (pH 8.0).

Flow rate 1 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time Twice the retention time of prilocaine.

Retention time Prilocaine = about 7 min.

System suitability Reference solution (a):

- — *resolution*: minimum 3.0 between the peaks due to impurity E and prilocaine.

Limits:
- — *impurities A, C, D, E, F*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and not more than one such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- — *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- — *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Impurity B

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications. *Use freshly prepared solutions*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution Dissolve 10.0 mg of *o-toluidine hydrochloride R* (impurity B) in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Limit:
- — *impurity B*: not more than the area of the corresponding peak in the chromatogram

obtained with the reference solution (100 ppm).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

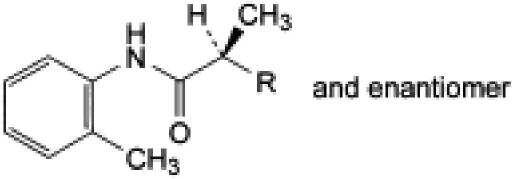
ASSAY

Dissolve 0.400 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 25.68 mg of $C_{13}H_{21}ClN_2O$.

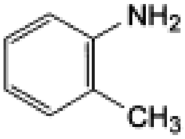
IMPURITIES

Specified impurities A, B, C, D, E, F.

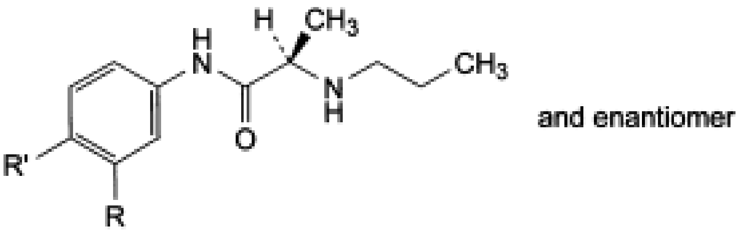


A. R = Cl: (*RS*)-2-chloro-*N*-(2-methylphenyl)propanamide,

C. R = NH-C_2H_5: (*RS*)-2-ethylamino-*N*-(2-methylphenyl)propanamide,



B. 2-methylbenzenamine (*o*-toluidine),



D. R = CH_3, R′ = H: (*RS*)-*N*-(3-methylphenyl)-2-(propylamino)propanamide,

E. R = H, R′ = CH_3: (*RS*)-*N*-(4-methylphenyl)-2-(propylamino)propanamide,

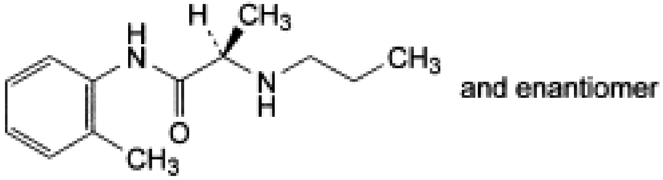
F. R = R′ = H: (*RS*)-*N*-phenyl-2-(propylamino)propanamide.

Ph Eur

Prilocaine

General Notices

(Ph Eur monograph 1362)



$C_{13}H_{20}N_2O$ 220.3 *721-50-6*

Action and use

Local anaesthetic.

Ph Eur

DEFINITION

(*RS*)-*N*-(2-Methylphenyl)-2-(propylamino)propanamide.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, very soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Melting point (*2.2.14*): 36 °C to 39 °C, determined without previous drying.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Apply 50 µl of a 30 g/l solution of the substance to be examined or the reference substance in *ether R* to *potassium bromide R* discs and evaporate the solvent.

Comparison *Prilocaine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20.0 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *prilocaine CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 20.0 mg of *prilocaine CRS* and 20.0 mg of *lidocaine CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel GF_{254} plate R*.

Mobile phase *concentrated ammonia R*, *methanol R*, *ether R* (1:5:100 *V/V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 2.50 g in 15 ml of *dilute hydrochloric acid R* and dilute to 50.0 ml with *water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Optical rotation (*2.2.7*)

- 0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of the substance to be examined and 3.0 mg of *prilocaine impurity E CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a solution prepared as follows: dissolve 0.180 g of *sodium dihydrogen phosphate monohydrate R* and 2.89 g of *disodium hydrogen phosphate dihydrate R* in 1000 ml of *water R* (pH 8.0).

Flow rate 1 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

Run time Twice the retention time of prilocaïne.

Retention time Prilocaïne = about 7 min.

System suitability Reference solution (a):

- *resolution*: minimum 3.0 between the peaks due to impurity E and prilocaine.

Limits:

- *impurities A, C, D, E, F*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and not more than one such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Impurity B

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications. *Use freshly prepared solutions*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution Dissolve 10.0 mg of *o-toluidine hydrochloride R* (impurity B) in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Limit:

- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (100 ppm).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

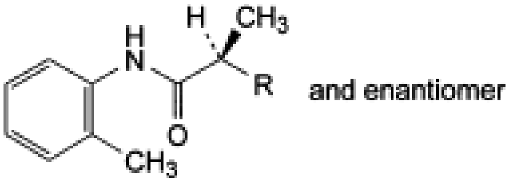
ASSAY

Dissolve 0.400 g in 20 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 22.03 mg of $C_{13}H_{20}N_2O$.

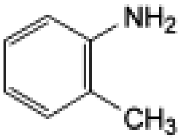
IMPURITIES

Specified impurities *A, B, C, D, E, F*.

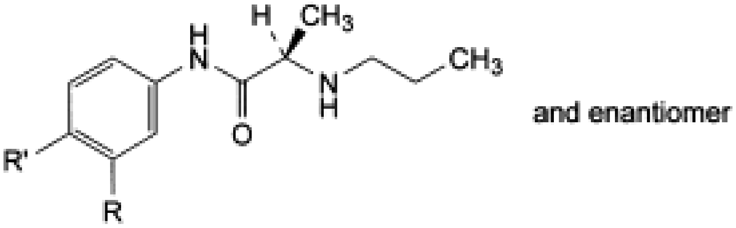

H
N
H
CH3
R
O
CH3
and enantiomer

A. R = Cl: (*RS*)-2-chloro-*N*-(2-methylphenyl)propanamide,

C. R = NH-C_2H_5: (*RS*)-2-(ethylamino)-*N*-(2-methylphenyl)propanamide,



B. 2-methylbenzenamine (*o*-toluidine),


H
N
H
CH3
CH3
N
H
O
R'
R
and enantiomer

D. R = CH_3, R′ = H: (*RS*)-*N*-(3-methylphenyl)-2-(propylamino)propanamide,

E. R = H, R′ = CH_3: (*RS*)-*N*-(4-methylphenyl)-2-(propylamino)propanamide,

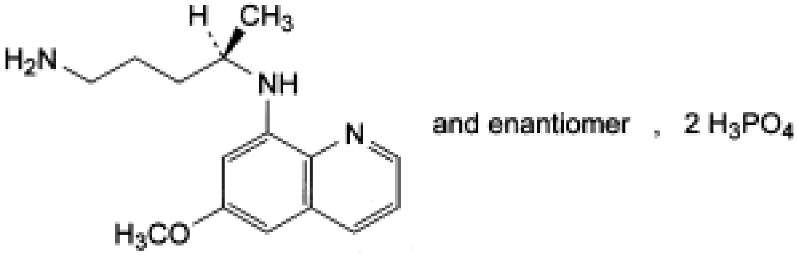
F. R = R′ = H: (*RS*)-*N*-phenyl-2-(propylamino)propanamide.

Ph Eur

Primaquine Phosphate

General Notices

(Primaquine Diphosphate, Ph Eur monograph 0635)



$C_{15}H_{21}N_3O,2H_3PO_4$ 455.3 *63-45-6*

Action and use

Antiprotozoal (malaria).

Ph Eur

DEFINITION

(4*RS*)-*N*^{4}-(6-Methoxyquinolin-8-yl)pentane-1,4-diamine bisphosphate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Orange crystalline powder.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

mp: about 200 °C, with decomposition.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a) Dissolve 15 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid.

Test solution (b) Dilute 5.0 ml of test solution (a) to 50.0 ml with *0.01 M hydrochloric acid*.

Spectral range 310-450 nm for test solution (a); 215-310 nm for test solution (b).

Absorption maxima At 332 nm and 415 nm for test solution (a); at 225 nm, 265 nm and 282 nm for test solution (b).

Specific absorbance at the absorption maxima:
— at 332 nm: 45 to 52 for test solution (a);
— at 415 nm: 27 to 35 for test solution (a);
— at 225 nm: 495 to 515 for test solution (b);
— at 265 nm: 335 to 350 for test solution (b);
— at 282 nm: 330 to 345 for test solution (b).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Dissolve separately 0.1 g of the substance to be examined and 0.1 g of the reference substance in 5 ml of *water R*, add 2 ml of *dilute ammonia R2* and 5 ml of *methylene chloride R*, then shake. Dry the methylene chloride layer over 0.5 g of *anhydrous sodium sulphate R*. Prepare a blank disc using about 0.3 g of *potassium bromide R*. Apply dropwise to the disc 0.1 ml of the methylene chloride layer, allowing the methylene chloride to evaporate between applications. Dry the disc at 50 °C for 2 min.

Comparison *primaquine diphosphate CRS.*

C. Thin-layer chromatography (*2.2.27*). Carry out all operations as rapidly as possible, protected from light. *Prepare the solutions immediately before use*.

Test solution Dissolve 0.20 g of the substance to be examined in 5 ml of *water R* and dilute to 10 ml with *methanol R*. Dilute 1 ml of this solution to 10 ml with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution Dissolve 20 mg of *primaquine diphosphate CRS* in 5 ml of *water R* and dilute to 10 ml with *methanol R*.

Plate *TLC silica gel* GF_{254} *plate R.*

Pretreatment Wash the plate with the mobile phase and allow to dry in air.

Mobile phase *concentrated ammonia R*, *methanol R*, *methylene chloride R* (1:40:60 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 50 mg in 5 ml of *water R*. Add 2 ml of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 5 ml, of *methylene chloride R*. The aqueous layer, acidified by addition of *nitric acid R*, gives reaction (b) of phosphates (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in *water R* and dilute to 5.0 ml with the same solvent. To 1.0 ml of this solution add 0.2 ml of *concentrated ammonia R* and shake with 10.0 ml of the mobile phase. Use the clear lower layer.

Reference solution (a) Dissolve 50 mg of *primaquine diphosphate CRS* in *water R* and dilute to 5.0 ml with the same solvent. To 1.0 ml of this solution add 0.2 ml of *concentrated ammonia R* and shake with 10.0 ml of the mobile phase. Use the clear lower layer.

Reference solution (b) Dilute 3.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Column:
- — *size:* l = 0.2 m, Ø = 4.6 mm;
- — *stationary phase*: *silica gel for chromatography R* (10 µm).

Mobile phase *concentrated ammonia R*, *methanol R*, *hexane R*, *methylene chloride R* (0.1:10:45:45 *V/V/V/V*).

Flow rate 3.0 ml/min.

Detection Spectrophotometer at 261 nm.

Injection 20 µl.

Run time At least twice the retention time of primaquine.

System suitability:
- — the chromatogram obtained with reference solution (a) shows just before the principal peak a peak whose area is about 6 per cent of that of the principal peak;
- — *resolution*: minimum 2.0 between the peak just before the principal peak and the principal peak in the chromatogram obtained with reference solution (a);
- — *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (c).

Limits:
- — *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);
- — *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.2000 g in 40 ml of *anhydrous acetic acid R*, heating gently. Allow to cool and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 22.77 mg of $C_{15}H_{27}N_3O_9P_2$.

STORAGE

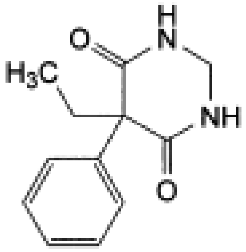
Protected from light.

Ph Eur

Primidone

General Notices

(Ph Eur monograph 0584)



$C_{12}H_{14}N_2O_2$ 218.3 *125-33-7*

Action and use

Antiepileptic.

Preparations

Primidone Oral Suspension

Primidone Tablets

Ph Eur

DEFINITION

5-Ethyl-5-phenyldihydropyrimidine-4,6(1*H*,5*H*)-dione.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in alkaline solutions.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Use the solution prescribed for the assay. Examined between 240 nm and 300 nm (*2.2.25*), the solution shows 3 absorption maxima, at 252 nm, 257 nm and 264 nm, and 2 absorption minima, at 254 nm and 261 nm. The ratio of the absorbance measured at the absorption maximum at 257 nm to that measured at the absorption minimum at 261 nm is 2.00 to 2.20. The identification is valid if, in the test for resolution (*2.2.25*), the ratio of the absorbances is not less than 2.0.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Comparison *primidone CRS.*

C. Dissolve 0.1 g in 5 ml of a 5 g/l solution of *chromotropic acid, sodium salt R* in a mixture of 4 volumes of *water R* and 9 volumes of *sulphuric acid R*. A pinkish-blue colour develops on heating.

D. Mix 0.2 g and 0.2 g of *anhydrous sodium carbonate R*. Heat until the mixture melts. Ammonia is evolved which is detectable by its alkaline reaction (*2.2.4*).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R1* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R1*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R1.*

Reference solution (b) Dissolve 5 mg of *primidone for peak identification CRS* (containing impurities A, B, C, D, E and F) in *methanol R1* and dilute to 5 ml with the same solvent.

Column:
— *size:* *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *monolithic octadecylsilyl silica gel for chromatography R*.

Mobile phase:
— *mobile phase A*: 1.36 g/l solution of *potassium dihydrogen phosphate R*;

— *mobile phase B*: *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	75	25
1 - 6	75 → 40	25 → 60
6 - 8	40	60
8 - 8.5	40 → 75	60 → 25
8.5 - 10	75	25

Flow rate 3.2 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *primidone for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks.

Relative retention With reference to primidone (retention time = about 2.2 min): impurity A = about 0.5; impurity B = about 1.4; impurity C = about 1.6; impurity D = about 1.75; impurity E = about 2.0; impurity F = about 2.8.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to impurity B and impurity C.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.5; impurity C = 1.5; impurity D = 1.4; impurity E = 1.3;

— *impurity F*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

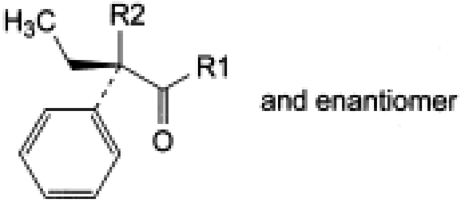
ASSAY

Dissolve 60.0 mg with heating in 70 ml of *ethanol (96 per cent) R*, cool and dilute to 100.0 ml with the same solvent. Prepare a reference solution in the same manner using 60.0 mg of *primidone CRS*. Measure the absorbance (*2.2.25*) of the 2 solutions at the absorption maximum at 257 nm.

Calculate the content of $C_{12}H_{14}N_2O_2$ from the absorbances measured and the concentrations of the solutions.

IMPURITIES

Specified impurities *A, B, C, D, E, F.*


H3C
R2
R1
O
and enantiomer

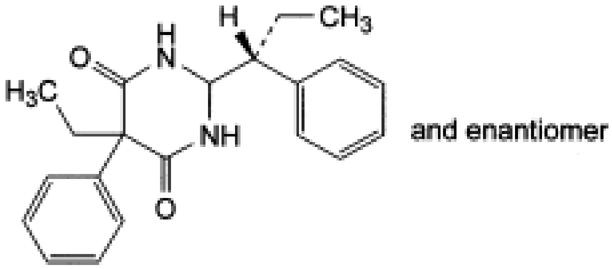
A. R1 = NH_2, R2 = CO-NH_2: 2-ethyl-2-phenylpropanediamide (ethylphenylmalonamide),

C. R1 = NH_2, R2 = H: (2*RS*)-2-phenylbutanamide,

D. R1 = NH_2, R2 = CN: (2*RS*)-2-cyano-2-phenylbutanamide,

E. R1 = OH, R2 = H: (2*RS*)-2-phenylbutanoic acid,

B. phenobarbital,


H
CH3
O
H
N
H3C
NH
O
and enantiomer

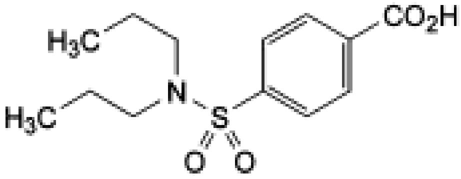
F. 5-ethyl-5-phenyl-2-[(1*RS*)-1-phenylpropyl]dihydropyrimidine-4,6(1*H*,5*H*)-dione.

Ph Eur

Probenecid

General Notices

(Ph Eur monograph 0243)



$C_{13}H_{19}NO_4S$ 285.4 *57-66-9*

Action and use

Uricosuric drug.

Preparation

Probenecid Tablets

Ph Eur

DEFINITION

Probenecid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-(dipropylsulphamoyl)benzoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or small crystals, practically insoluble in water, soluble in acetone, sparingly soluble in ethanol.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D.*

A. Melting point (*2.2.14*): 197 °C to 202 °C.

B. Dissolve 20 mg in a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *alcohol R* and dilute to 100.0 ml with the same mixture of solvents. Dilute 5.0 ml of the solution to 100.0 ml with a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *alcohol R*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows two absorption maxima, at 223 nm and 248 nm. The specific absorbance at the maximum at 248 nm is 310 to 350.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *probenecid CRS*.

D. Dissolve 0.2 g in the smallest necessary quantity of *dilute ammonia R2* (about 0.6 ml). Add 3 ml of *silver nitrate solution R2*. A white precipitate is formed which dissolves in an excess of ammonia.

TESTS

Appearance of solution

Dissolve 1.0 g in *1 M sodium hydroxide* and dilute to 10 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity

To 2.0 g add 100 ml of *water R* and heat on a water-bath for 30 min. Make up to the original volume with *water R*, allow to cool to room temperature and filter. To 50 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. Not more than 0.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *acetone R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *glacial acetic acid R*, 15 volumes of *chloroform R*, 20 volumes of *di-isopropyl ether R* and 55 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals (*2.4.8*)

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

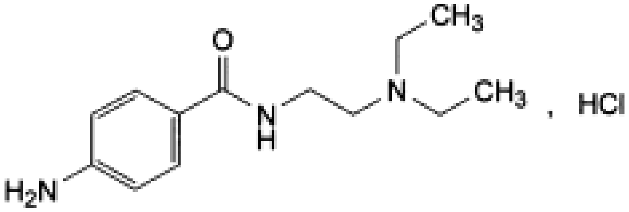
Dissolve 0.250 g in 50 ml of *alcohol R*, shaking and heating slightly if necessary. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* is equivalent to 28.54 mg of $C_{13}H_{19}NO_4S$.

Procainamide Hydrochloride

General Notices

(Ph Eur monograph 0567)



$C_{13}H_{21}N_3O,HCl$ 271.8 *614-39-1*

Action and use

Class I antiarrhythmic.

Preparations

Procainamide Injection

Procainamide Tablets

Ph Eur

DEFINITION

Procainamide hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino- *N*-[2-(diethylamino)ethyl]benzamide hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or very slightly yellow, crystalline powder, hygroscopic, very soluble in water, freely soluble in alcohol, slightly soluble in acetone.

IDENTIFICATION

First identification C, D.

Second identification A, B, D, E.

A. Melting point (*2.2.14*): 166 °C to 170 °C.

B. Dissolve 10.0 mg in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *0.1 M sodium hydroxide*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 273 nm. The specific absorbance at the maximum is 580 to 610.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *procainamide hydrochloride CRS*.

D. Dilute 1 ml of solution S to 5 ml with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

E. Dilute 1 ml of solution S (see Tests) to 2 ml with *water R*. 1 ml of this solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 5.6 to 6.3.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.10 g of the substance to be examined in *alcohol R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 1 ml of the test solution to 200 ml with *alcohol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 12 cm using a mixture of 15 volumes of *glacial acetic acid R*, 30 volumes of *water R* and 60 volumes of *butanol R*. Place the plate in a stream of cold air until the plate appears dry. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals (*2.4.8*)

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.2500 g in 50 ml of *dilute hydrochloric acid R*. Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*).

1 ml of *0.1 M sodium nitrite* is equivalent to 27.18 mg of $C_{13}H_{22}ClN_3O$.

STORAGE

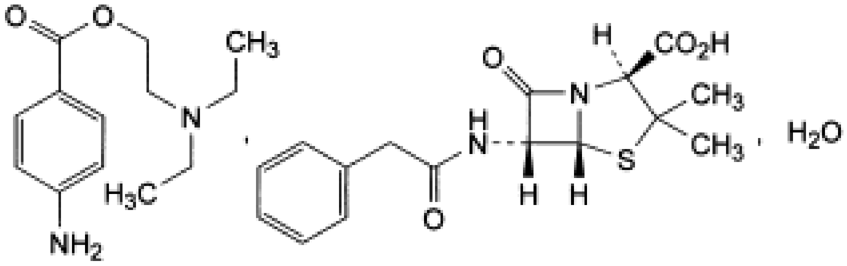
Store in an airtight container, protected from light.

Ph Eur

Procaine Benzylpenicillin

General Notices

(Ph Eur monograph 0115)



$C_{13}H_{20}N_2O_2,C_{16}H_{18}N_2O_4S,H_2O$ 588.7 *6130-64-9*

Action and use

Penicillin antibacterial.

Ph Eur

DEFINITION

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with 2-(diethylamino)ethyl 4-aminobenzoate monohydrate.

Substance produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Content:

- *procaine benzylpenicillin*: 96.0 per cent to 102.0 per cent (anhydrous substance);
- *procaine* ($C_{13}H_{20}N_2O_2$; M_r 236.3): 39.0 per cent to 42.0 per cent (anhydrous substance).

Dispersing or suspending agents (for example, lecithin and polysorbate 80) may be added.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *procaine benzylpenicillin CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in 5 ml of *acetone R*.

Reference solution Dissolve 25 mg of *procaine benzylpenicillin CRS* in 5 ml of *acetone R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R* previously adjusted to pH 7.0 with *ammonia R*.

Application 1 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated spots.

Results The 2 principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the 2 principal spots in the chromatogram obtained with the reference solution.

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube on a water-bath for 1 min; a reddish-brown colour develops.

D. Dissolve 0.1 g in 2 ml of *dilute hydrochloric acid R* and use the solution which may be turbid. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

pH *(2.2.3)*

5.0 to 7.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 15 ml with the same solvent. Shake until dissolution is complete.

Specific optical rotation *(2.2.7)*

+ 165 to + 180 (anhydrous substance).

Dissolve 0.250 g in a mixture of 2 volumes of *water R* and 3 volumes of *acetone R*, then dilute to 25.0 ml with the same mixture of solvents.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution (a) Dissolve 70.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b) Dissolve 70.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 70.0 mg of *procaine benzylpenicillin CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 4 mg of *4-aminobenzoic acid R* (impurity A) in reference solution (a) and dilute to 25 ml with reference solution (a).

Reference solution (c) Dissolve 16.8 mg of *4-aminobenzoic acid R* (impurity A) in *water R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *water R*. To 1.0 ml of this solution, add 1.0 ml of test solution (a) and dilute to 100.0 ml with the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 250 ml of *acetonitrile R1*, 250 ml of *water R* and 500 ml of a solution containing 14 g/l of *potassium dihydrogen phosphate R* and 6.5 g/l of *tetrabutylammonium hydroxide solution (400 g/l) R* adjusted to pH 7.0 with *1 M potassium hydroxide*; if necessary, adjust the mixture to pH 7.2 with *dilute phosphoric acid R*.

Flow rate 1.75 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl of test solution (a) and reference solutions (b) and (c).

Run time 1.5 time the retention time of benzylpenicillin.

Elution order Impurity A, procaine, benzylpenicillin.

System suitability Reference solution (b):

- *resolution*: minimum 2.0 between the peaks due to impurity A and procaine; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.024 per cent);
- *any other impurity*: for each impurity, not more than the area of the peak due to benzylpenicillin in the chromatogram obtained with reference solution (c) (1 per cent).

Water (*2.5.12*)

2.8 per cent to 4.2 per cent, determined on 0.500 g.

Bacterial endotoxins *(2.6.14, Method E)*

Less than 0.10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

System suitability Reference solution (a):

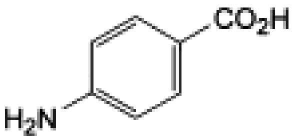
— *repeatability*: maximum relative standard deviation of 1.0 per cent for the 2 principal peaks after 6 injections.

Calculate the percentage contents of procaine and procaine benzylpenicillin.

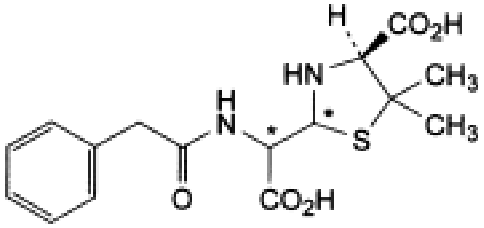
STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

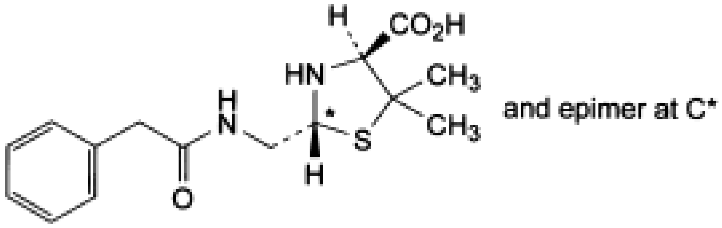
IMPURITIES



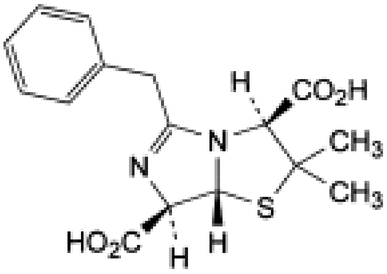
A. 4-aminobenzoic acid,



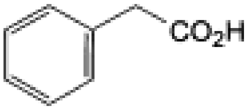
B. (4*S*)-2-[carboxy[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of benzylpenicillin),



C. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin),



D. (3*S*,7*R*,7a*R*)-5-benzyl-2,2-dimethyl-2,3,7,7a-tetrahydroimidazo[5,1-*b*]thiazole-3,7-dicarboxylic acid (penillic acid of benzylpenicillin),



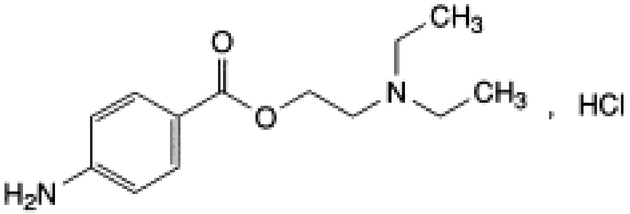
E. phenylacetic acid.

Ph Eur

Procaine Hydrochloride

General Notices

(Ph Eur monograph 0050)



$C_{13}H_{20}N_2O_2$,HCl,   272.8   *51-05-8*

Action and use

Local anaesthetic.

Ph Eur

DEFINITION

Procaine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 2-(diethylamino)ethyl 4-aminobenzoate hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, very soluble in water, soluble in alcohol.

IDENTIFICATION

First identification *A, B, E.*

Second identification *A, C, D, E, F.*

A. Melting point (*2.2.14*): 154 °C to 158 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *procaine hydrochloride CRS*.

C. To about 5 mg add 0.5 ml of *fuming nitric acid R*. Evaporate to dryness on a water-bath, allow to cool and dissolve the residue in 5 ml of *acetone R*. Add 1 ml of *0.1 M alcoholic potassium hydroxide*. Only a brownish-red colour develops.

D. To 0.2 ml of solution S (see Tests) add 2 ml of *water R* and 0.5 ml of *dilute sulphuric acid R* and shake. Add 1 ml of a 1 g/l solution of *potassium permanganate R*. The colour is immediately discharged.

E. It gives reaction (a) of chlorides (*2.3.1*).

F. Dilute 1 ml of solution S to 100 ml with *water R*. 2 ml of this solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

Dilute 4 ml of solution S to 10 ml with *carbon dioxide-free water R*. The pH of the solution is 5.0 to 6.5.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *4-aminobenzoic acid R* in *water R* and dilute to 100 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *water R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 4 volumes of *glacial acetic acid R*, 16 volumes of *hexane R* and 80 volumes of *dibutyl ether R*. Dry the plate at 100 °C to 105 °C for 10 min and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.05 per cent). The principal spot in the chromatogram obtained with the test solution remains on the starting point.

Heavy metals (*2.4.8*)

Dissolve 1.0 g in *water R* and dilute to 25.0 ml with the same solvent. Carry out the prefiltration. 10 ml of the prefiltrate complies with limit test E for heavy metals (5 ppm). Prepare the standard using 2 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *dilute hydrochloric acid R*. Carry out the determination of primary aromatic amino nitrogen (*2.5.8*).

1 ml of *0.1 M sodium nitrite* is equivalent to 27.28 mg of $C_{13}H_{21}ClN_2O_2$.

STORAGE

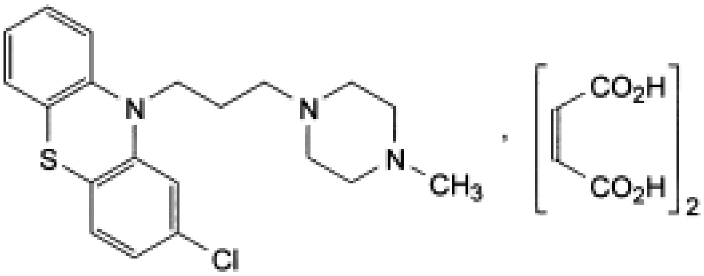
Store protected from light.

Ph Eur

Prochlorperazine Maleate

General Notices

(Ph Eur monograph 0244)



$C_{20}H_{24}ClN_3S,2C_4H_4O_4$ 606 *84-02-6*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparations

Prochlorperazine Tablets

Prochlorperazine Buccal Tablets

Ph Eur

DEFINITION

Prochlorperazine maleate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine bis[hydrogen (*Z*)-butenedioate], calculated with reference to the dried substance.

CHARACTERS

A white or pale-yellow, crystalline powder, very slightly soluble in water and in alcohol.

IDENTIFICATION

First identification *B, C, D.*

Second identification *A, C, D.*

A. Carry out the test protected from light. Dissolve 50 mg in *0.1 M hydrochloric acid* and dilute to 500.0 ml with the same acid. Examined immediately between 280 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 305 nm. Dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined immediately between 230 nm and 280 nm, the solution shows an absorption maximum at 255 nm. The specific absorbance at the maximum is 525 to 575.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *prochlorperazine maleate CRS*.

C. It complies with the identification of phenothiazines by thin-layer chromatography *(2.3.3)*, with the following modifications:

Test solution Dissolve 20 mg of the substance to be examined in a mixture of equal volumes of *chloroform R* and *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution Dissolve 20 mg of *prochlorperazine maleate CRS* in a mixture of equal volumes of *chloroform R* and *methanol R* and dilute to 20 ml with the same mixture of solvents.

Apply separately to the plate 4 µl of each solution.

D. Triturate 0.2 g with a mixture of 1 ml of *strong sodium hydroxide solution R* and 3 ml of *water R*. Shake with three quantities, each of 5 ml, of *ether R*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R.* Heat in a water-bath for 15 min. No colour develops. To the remainder of the aqueous layer add 2 ml of *bromine solution R*. Heat in a water-bath for 15 min and then heat to boiling. Cool. To 0.1 ml of the solution add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat in a water-bath for 15 min. A blue colour develops.

TESTS

pH *(2.2.3)*

The pH of a freshly prepared saturated solution in *carbon dioxide-free water R* is 3.0 to 4.0.

Related substances

Carry out the test protected from light. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents. Prepare immediately before use.

Reference solution Dilute 1 ml of the test solution to 200 ml with a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *acetone R*, 10 volumes of *diethylamine R* and 80 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent). Disregard any spots remaining at the starting-points.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g of the powdered substance to be examined in 50 ml of *anhydrous acetic acid R*, warming on a water-bath. Allow to cool to room temperature. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 30.31 mg of $C_{28}H_{32}ClN_3O_8S$.

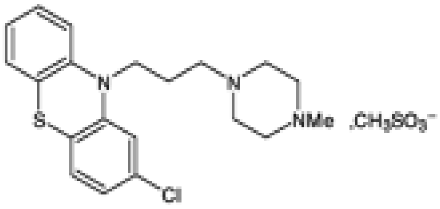
STORAGE

Store protected from light.

Ph Eur

Prochlorperazine Mesilate

General Notices



$C_{20}H_{24}ClN_3S,2CH_4SO_3$ 566.2   *5132-55-8*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparations

Prochlorperazine Injection

Prochlorperazine Oral Solution

DEFINITION

Prochlorperazine Mesilate is 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine di (methanesulphonate). It contains not less than 98.0% and not more than 101.0% of $C_{20}H_{24}ClN_3S,2CH_4SO_3$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Very soluble in *water*; sparingly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of prochlorperazine mesilate *(RS 290)*.

B. Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

TESTS

Acidity

pH of a 2% w/v solution, 2.0 to 3.0, Appendix V L.

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and dissolving the substance being examined in *methanol* containing 0.5% v/v of 13.5M *ammonia*.

Loss on drying

When dried to constant weight at 100° at a pressure not exceeding 0.7 kPa, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

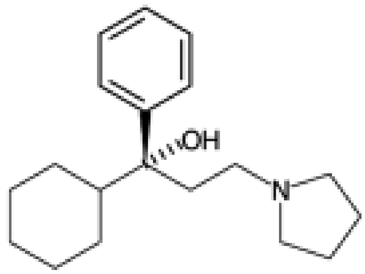
Dissolve 0.8 g in 10 ml of *water*, add 5 ml of 1M *sodium hydroxide* and extract by shaking with successive quantities of 50, 25, 25 and 25 ml of *ether*. Wash the combined ether extracts with 5 ml of *water*, shake the washings with 5 ml of *ether*, add the ether to the combined ether extracts and evaporate to dryness. Add 2 ml of *absolute ethanol* to the residue, evaporate to dryness and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 28.31 mg of $C_{20}H_{24}ClN_3S,2CH_4SO_3$.

STORAGE

Prochlorperazine Mesilate should be protected from light.

Procyclidine Hydrochloride

General Notices



$C_{19}H_{29}NO,HCl$ 323.9 *1508-76-5*

Action and use

Anticholinergic.

Preparations

Procyclidine Injection

Procyclidine Tablets

DEFINITION

Procyclidine Hydrochloride is (*RS*)-1-cyclohexyl-1-phenyl-3-pyrrolidin-1-ylpropan-1ol hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{19}H_{29}NO,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder; odourless or almost odourless.

Sparingly soluble in *water*; soluble in *ethanol (96%)*; practically insoluble in *acetone* and in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of procyclidine hydrochloride *(RS 292)*.

B. Dissolve 0.25 g in 10 ml of *water*, make alkaline with 5M *ammonia* and extract with three 10 ml quantities of *ether*. Dry the combined extracts over *anhydrous sodium sulphate*, filter, remove the ether and scratch the residue with a glass rod to induce solidification. The *melting point* of the residue is about 85°, Appendix V A.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 4.5 to 6.5, Appendix V L.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 100 volumes of *ether* and 1 volume of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions in *chloroform* containing (1) 2.0% w/v of the substance being examined, (2) 0.0040% w/v of *1-phenyl-3-pyrrolidinopropan-1-one hydrochloride BPCRS* and (3) 0.010% w/v of the substance being examined. After removal of the plate, dry it at 105° for 15 minutes and examine under *ultraviolet light (254 nm)*. Any spot corresponding to 1-phenyl-3-pyrrolidinopropan-1-one in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Spray the plate with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) .

B. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 5 ml of 1.25M *sodium hydroxide* to 20 ml of a 0.015% w/v solution of the substance being examined and mix. Extract with two 20 ml quantities of *ether*, add to the combined extracts 5 ml of a 0.06% w/v solution of *triphenylethylene* (internal standard) in *ether*, shake with *anhydrous sodium sulphate* and filter; evaporate the filtrate and dissolve the residue in 1 ml of *ether*. Prepare solution (2) in the same manner as solution (1) but using 20 ml of a 0.50% w/v solution of the substance being examined and omitting the addition of the internal standard solution. Prepare solution (3) in the same manner as solution (1) but using 20 ml of a 0.50% w/v solution of the substance being examined.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (HP Chromosorb W is suitable) (80 to 100 mesh), coated with 10% w/w of modified polyethylene glycol 20M (SP-1000 is suitable) and 2% w/w of *potassium hydroxide* and maintained at 240°, and using on-column injection.

The ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard in the chromatogram obtained with solution (3) is not more than the ratio of the area of the principal peak to the area of the internal standard peak in the chromatogram obtained with solution (1).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

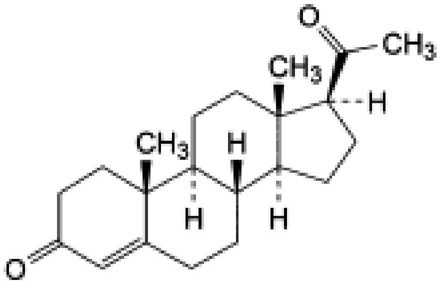
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.7 g and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 32.39 mg of $C_{19}H_{29}NO,HCl$.

Progesterone

General Notices

(Ph Eur monograph 0429)



$C_{21}H_{30}O_2$ 314.5 *57-83-0*

Action and use

Progestogen.

Preparation

Progesterone Injection

Ph Eur

DEFINITION

Pregn-4-ene-3,20-dione.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in ethanol, sparingly soluble in acetone and in fatty oils.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *progesterone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethanol R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*)

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 10 mg of *progesterone CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ethyl acetate R*, *methylene chloride R* (33:66 *V/V*).

Application 5 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Detection B Spray with alcoholic solution of *sulphuric acid R*, heat at 120 °C for 15 min and allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Specific optical rotation (*2.2.7*)

+ 186 to + 194 (dried substance).

Dissolve 0.250 g in *ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

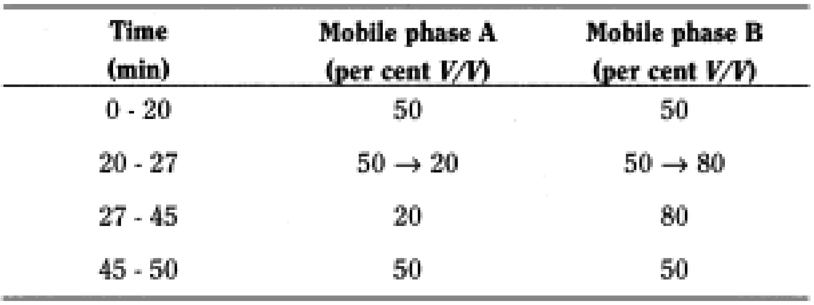
Test solution Dissolve 20.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 2.0 mg of *progesterone CRS* and 2.0 mg of *progesterone impurity C CRS* in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *water R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	50	50
20 - 27	50 → 20	50 → 80
27 - 45	20	80
45 - 50	50	50

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 241 nm.

Injection 10 µl.

System suitability Reference solution (a):
- *resolution*: minimum 1.5 between the peaks due to impurity C and to progesterone.

Limits:
- *any impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

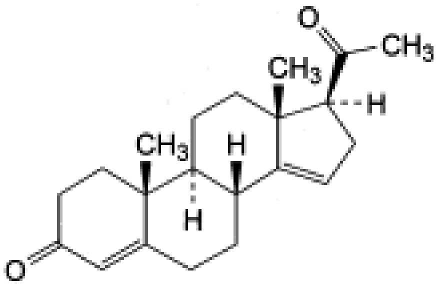
Dissolve 25.0 mg in *alcohol R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *alcohol R*. Measure the absorbance (*2.2.25*) at the maximum at 241 nm.

Calculate the content of $C_{21}H_{30}O_2$ taking the specific absorbance to be 535.

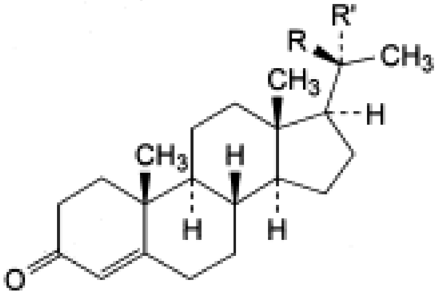
STORAGE

Protected from light.

IMPURITIES



A. pregna-4,14-diene-3,20-dione,

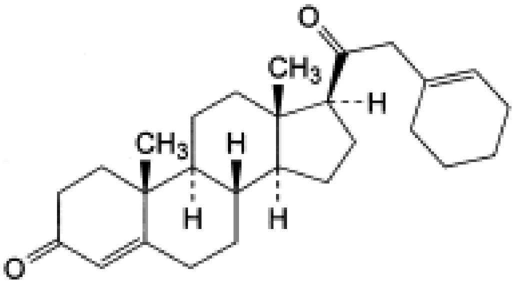


B. R = OH, R′ = H: (20*S*)-20-hydroxypregn-4-en-3-one,

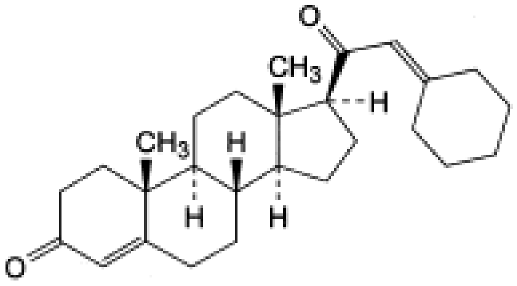
C. R = H, R′ = OH: (20*R*)-20-hydroxypregn-4-en-3-one,

D. R = O-CO-CH_3, R′ = H: (20*S*)-3-oxopregn-4-en-20-yl acetate,

E. R = H, R′ = O-CO-CH_3: (20*R*)-3-oxopregn-4-en-20-yl acetate,



F. 21-(cyclohex-1-enyl)pregn-4-ene-3,20-dione,



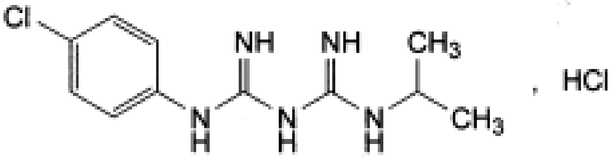
G. 21-(cyclohexylidene)pregn-4-ene-3,20-dione.

Ph Eur

Proguanil Hydrochloride

General Notices

(Ph Eur monograph 2002)



$C_{11}H_{17}Cl_2N_5$ 290.2 *637-32-1*

Action and use

Antiprotozoal (malaria).

Ph Eur

DEFINITION

1-(4-Chlorophenyl)-5-(1-methylethyl)biguanide hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of proguanil hydrochloride.

B. Dissolve 0.4 g in 50 ml of *water R* (solution A). To 15 ml of solution A add 2 ml of *dilute sodium hydroxide solution R*. Extract with 20 ml of *ethyl acetate R*. Wash the organic layer with *water R*, evaporate to dryness and dry at 105 °C. The melting point (*2.2.14*) of the residue is 130 °C to 133 °C.

C. To 10 ml of solution A, add 1 drop of *copper sulphate solution R* and 2 ml of *dilute ammonia R1*. Add 5 ml of *toluene R* and stir. Allow to stand until separation of the layers is obtained. The upper layer is violet-red.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Acidity or alkalinity

To 35 ml of *water R* maintained at 60-65 °C, add 0.2 ml of *methyl red mixed solution R*. Neutralise to a grey colour with either *0.01 M sodium hydroxide* or *0.01 M hydrochloric acid*. Add 0.4 g of the substance to be examined and stir until completely dissolved. The solution is grey or green. Not more than 0.2 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to reddish-violet.

Chloroaniline

Maximum 250 ppm.

Dissolve 0.10 g in 1 ml of *2 M hydrochloric acid R* and dilute to 20 ml with *water R*. Cool to 5 °C. Add 1 ml of a 3.45 g/l solution of *sodium nitrite R* and allow to stand at 5 °C for 5 min. Add 2 ml of a 50 g/l solution of *ammonium sulphamate R* and allow to stand for 10 min. Add 2 ml of *naphthylethylenediamine dihydrochloride solution R*, dilute to 50 ml with *water R* and allow to stand for 30 min. Any red colour produced is not more intense than that of a standard prepared at the same time and in the same manner, using 20 ml of a 1.25 mg/l solution of *chloroaniline R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *proguanil impurity C CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 0.1 ml to 10 ml with the mobile phase.

Reference solution (c) Dissolve 5 mg of *proguanil impurity D CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 0.1 ml to 10 ml with the mobile phase.

Reference solution (d) Dilute 1 ml of the test solution to 200 ml with the mobile phase. To 1 ml add 1 ml of reference solution (c) and mix.

Column:

— *size*: *l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.78 g of *sodium hexanesulphonate R* in a mixture of 10 volumes of *glacial acetic acid R*, 800 volumes of *water R* and 1200 volumes of *methanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm and 254 nm.

Injection 20 µl.

Run time 5 times the retention time of proguanil.

Retention time Proguanil = about 6 min.

System suitability Reference solution (d) at 230 nm:

— *resolution*: minimum 5 between the peaks due to impurity D and proguanil.

Limits:

— *impurity C*: not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) at 230 nm (0.7 per cent);

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) at 230 nm (0.2 per cent);

— *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) at 230 nm and at 254 nm (0.1 per cent);

— *total*: the sum of the calculated percentage contents of known and unknown impurities is not greater than 1 per cent, considering each peak at the wavelength at which the peak shows the higher value;

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

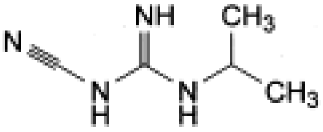
Suspend 0.100 g in 20 ml of *anhydrous acetic acid R*, shake and heat at 50 °C for 5 min. Cool to room temperature and add 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 14.51 mg of $C_{11}H_{17}Cl_2N_5$.

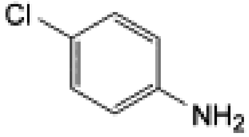
STORAGE

Protected from light.

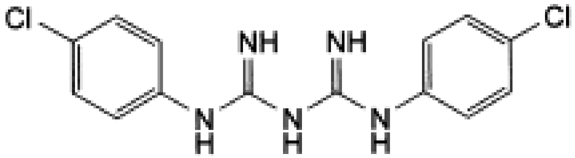
IMPURITIES



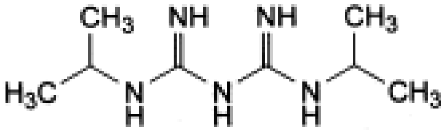
A. 1-cyano-3-(1-methylethyl)guanidine,



B. 4-chloroaniline,



C. 1,5-bis(4-chlorophenyl)biguanide,



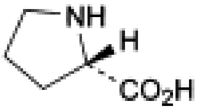
D. 1,5-bis(1-methylethyl)biguanide.

Ph Eur

Proline

General Notices

(Ph Eur monograph 0785)



$C_5H_9NO_2$ 115.1 *147-85-3*

Action and use

Amino acid.

Ph Eur

DEFINITION

Proline contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*S*)-pyrrolidine-2-carboxylic acid, calculated with reference to the dried substance.

CHARACTERS

White or almost white, crystalline powder or colourless crystals, very soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification A, B.

Second identification A, C.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *proline CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

Dissolve 1.00 g in *water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is - 84.0 to - 86.0, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *proline CRS* in *0.1 M hydrochloric acid* and dilute to 50 ml with the same acid.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *proline CRS* and 10 mg of *threonine CRS* in *0.1 M hydrochloric acid* and dilute to 25 ml with the same acid.

Apply to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (*2.4.4*)

Dilute 5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates (*2.4.13*)

Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium (*2.4.1*)

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*)

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals (*2.4.8*)

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using the *lead solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* using 0.1 ml of *naphtholbenzein solution R* as indicator, until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 11.51 mg of $C_5H_9NO_2$.

STORAGE

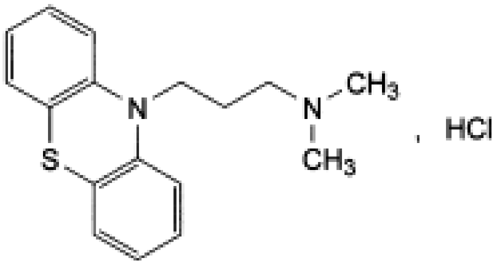
Store protected from light.

Ph Eur

Promazine Hydrochloride

General Notices

(Ph Eur monograph 1365)



$C_{17}H_{20}N_2S,HCl$ 320.9 *53-60-1*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparations

Promazine Injection

Promazine Oral Suspension

Promazine Tablets

Ph Eur

DEFINITION

Promazine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3-(10*H*-phenothiazin-10-yl)-*N*,*N*-dimethylpropan-1-amine hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, slightly hygroscopic, very soluble in water, in alcohol and in methylene chloride.

It melts at about 179 °C.

IDENTIFICATION

First identification A, B, D.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *promazine hydrochloride CRS*.

B. It complies with the identification test for phenothiazines by thin-layer chromatography (*2.3.3*). Use *promazine hydrochloride CRS* to prepare the reference solution.

C. Dissolve about 5 mg in 2 ml of *sulphuric acid R* and allow to stand for 5 min. An orange colour is produced.

D. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. The pH of the freshly prepared solution is 4.2 to 5.2.

Related substances

Carry out the test protected from bright light. Prepare the solutions immediately before use.

Examine by thin layer chromatography (*2.2.27*), using a *TLC silica gel* F_{254} *plate R*.

Test solution Dissolve 0.10 g of the substance to be examined in a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dilute 1 ml of the test solution to 200 ml with a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R*.

Reference solution (b) Dissolve 10 mg of *chlorprothixene hydrochloride CRS* in a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R*, add 1 ml of the test solution and dilute to 10 ml with the same mixture of solvents.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *acetone R*, 10 volumes of *diethylamine R* and 80 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (a) (0.5 per cent). Disregard any spot at the starting point. The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

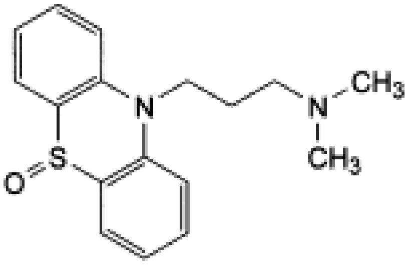
Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 32.09 mg of $C_{17}H_{21}ClN_2S$.

STORAGE

Store protected from light.

IMPURITIES



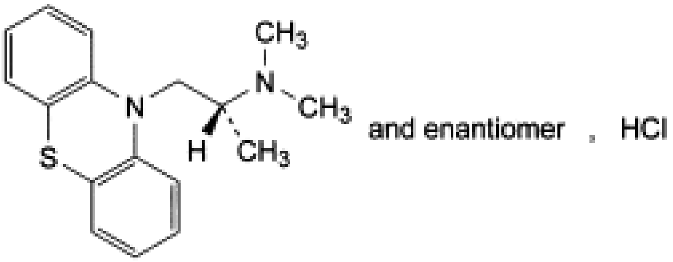
A. 3-(10*H*-phenothiazin-10-yl)-*N*,*N*-dimethylpropan-1-amine *S*-oxide (promazine sulphoxide).

Ph Eur

Promethazine Hydrochloride

General Notices

(Ph Eur monograph 0524)



$C_{17}H_{20}N_2S,HCl$ 320.9 *58-33-3*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparations

Promethazine Injection

Promethazine Oral Solution

Promethazine Hydrochloride Tablets

Ph Eur

DEFINITION

(2*RS*)-*N*,*N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or faintly yellowish, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

mp: about 222 °C, with decomposition.

IDENTIFICATION

First identification *A, B, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *promethazine hydrochloride CRS*.

B. It complies with the identification test for phenothiazines by thin-layer chromatography (*2.3.3*): use *promethazine hydrochloride CRS* to prepare the reference solution.

C. Dissolve 0.1 g in 3 ml of *water R*. Add dropwise 1 ml of *nitric acid R*. A precipitate is formed which rapidly dissolves to give a red solution, becoming orange and then yellow. Heat to boiling. The solution becomes orange and an orange-red precipitate is formed.

D. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)

4.0 to 5.0, measured immediately after preparation.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light and use freshly prepared solutions.*

Solvent mixture *triethylamine R*, *methanol R* (1:1000 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 2.5 mg of *promethazine for peak identification CRS* (containing impurities A, B and C) in the solvent mixture and dilute to 5 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *promethazine impurity D CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1 ml of this solution to 100 ml with the solvent mixture.

Column:

- *size:* l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography with polar incorporated groups R* (5 µm).

Mobile phase Mix 20 volumes of *methanol R*, 30 volumes of *acetonitrile R* and 50 volumes of a 3.4 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with *potassium hydroxide R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 2.5 times the retention time of promethazine.

Identification of impurities: use the chromatogram supplied with *promethazine for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to promethazine (retention time = about 18 min): impurity D = about 0.2; impurity C = about 0.5; impurity B = about 1.4; impurity A = about 1.8.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to impurities B and A in the chromatogram obtained with reference solution (a);

— the chromatogram obtained with reference solution (a) is similar to the chromatogram supplied with *promethazine for peak identification CRS*.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.5;

— *impurity B*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);

— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total:* not more than 12 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 1.0 g in 5 ml of *water R*, then add 5 ml of *acetone R* and 5 ml of *buffer solution pH 3.5 R*. Carry out the prefiltration. The prefiltrate complies with test E. Prepare the reference solution using 5 ml of *lead standard solution (2 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

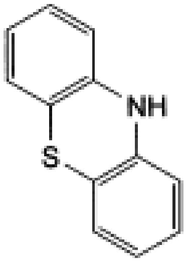
1 ml of *0.1 M sodium hydroxide* is equivalent to 32.09 mg of $C_{17}H_{21}ClN_2S$.

STORAGE

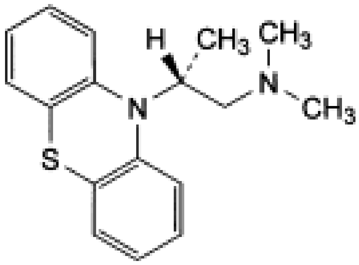
Protected from light.

IMPURITIES

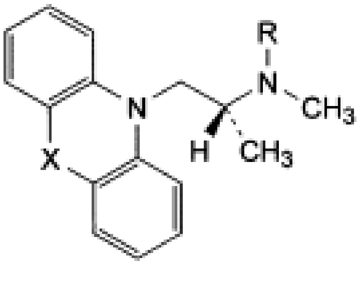
Specified impurities A, B, C, D.



A. phenothiazine,



B. (2*RS*)-*N*,*N*-dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine (isopromethazine),



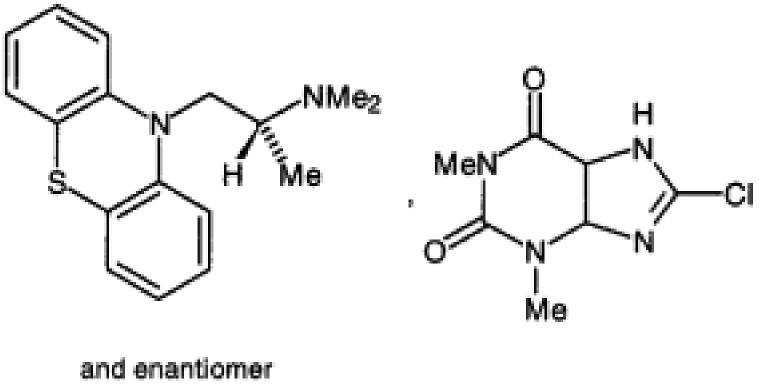
C. R = H, X = S: (2*RS*)-*N*-methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine,

D. R = CH_3, X = SO: (2*RS*)-*N*,*N*-dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine *S*-oxide.

Ph Eur

Promethazine Teoclate

General Notices



$C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$ 499.0 *17693-51-5*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Promethazine Teoclate Tablets

DEFINITION

Promethazine Teoclate is the (*RS*)-dimethyl(2-phenothiazin-10-ylpropyl)amine salt of 8-chlorotheophylline. It contains not less than 98.0% and not more than 101.0% of $C_{17}H_{20}N_2S$, $C_7H_7ClN_4O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Very slightly soluble in *water*; sparingly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. Shake 0.15 g with 2.5 ml of *water*, add 1 ml of 5M *ammonia* and extract with 30 ml of *ether*. Wash the ether extract with 10 ml of *water*, dry with *anhydrous sodium sulphate* and evaporate the ether to dryness. Dissolve the residue in 1 ml of *chloroform IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promethazine *(RS 297)*.

B. Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. Shake 0.4 g with 10 ml of *water*, add 4 ml of 5M *ammonia*, shake with two 30-ml quantities of *ether* and add 4 ml of *hydrochloric acid* to the aqueous solution. Filter the white precipitate, wash with *water* and dry at 105°. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness. A reddish residue remains which becomes purple on exposure to the vapour of ammonia.

TESTS

Chloride

Shake 0.3 g with 30 ml of *water* for 2 minutes and filter. 15 ml of the filtrate complies with the *limit test for chlorides*, Appendix VII, but using 2 ml of *nitric acid* in place of the 1 ml of *dilute nitric acid* (350 ppm).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *diethylamine*, 10 volumes of *acetone* and 85 volumes of *cyclohexane* as the mobile phase. Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank and allow the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. Solution (1) contains 2% w/v of the substance being examined. Solution (2) contains 0.02% w/v of *isopromethazine hydrochloride BPCRS*. For solution (3) dilute 1 volume of solution (1) to 200 volumes. For solution (4) dilute 1 volume of solution (1) to 500 volumes. Allow the plate to dry in air and examine under *ultraviolet light (254 nm).* In the chromatogram obtained with solution (1) any spot corresponding to isopromethazine is not more intense than the spot in the chromatogram obtained with solution (2) (1%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%). Disregard any spot remaining on the line of application.

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 1 g in 200 ml of *acetone* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 49.90 mg of $C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$.

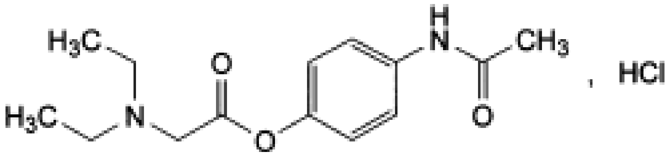
STORAGE

Promethazine Teoclate should be protected from light.

Propacetamol Hydrochloride

General Notices

(Ph Eur monograph 1366)



$C_{14}H_{20}N_2O_3$,HCl 300.8 *66532-86-3*

Action and use

Analgesic; antipyretic.

Ph Eur

DEFINITION

4-(Acetylamino)phenyl (diethylamino)acetate hydrochloride.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in anhydrous ethanol, practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of propacetamol hydrochloride.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Prepare the solution immediately before use. Dissolve 1.75 g in *water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*).

Absorbance (*2.2.25*)

Maximum 0.05, determined at 390 nm on solution S.

Impurity B

Thin-layer chromatography (*2.2.27*).

Test solution Suspend 4.00 g of the substance to be examined in 8 ml of *acetonitrile R*. Shake for 30 min and filter. Dilute to 10 ml with *acetonitrile R*.

Reference solution (a) Dissolve 25 mg of *4-aminophenol R* (impurity B) in *acetonitrile R* and dilute to 50 ml with the same solvent. Dilute 10 ml of this solution to 50 ml with *acetonitrile R*.

Reference solution (b) Dilute 5 ml of reference solution (a) to 50 ml with *acetonitrile R*.

Reference solution (c) Dilute 0.2 ml of reference solution (a) to 5 ml with the test solution.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *anhydrous formic acid R*, *water R*, *methanol R*, *methylene chloride R* (3:4:30:64 *V/V/V/V*).

Application 50 µl of the test solution and of reference solutions (b) and (c).

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm. Spray with a 10 g/l solution of *dimethylaminobenzaldehyde R* in *ethanol (96 per cent) R*.

Identification of spots Reference solution (c) shows 2 spots, one visible in ultraviolet light due to propacetamol and the other one yellow, visible after spraying due to impurity B. An additional spot may appear in ultraviolet light and corresponds to impurity A.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity B*: any yellow spot due to impurity B not visible in ultraviolet light is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (25 ppm).

Related substances

Liquid chromatography (*2.2.29*).

Solution A Dissolve 2.16 g of *sodium octanesulphonate R* in 900 ml of *water R* and dilute to 1000 ml with the same solvent. Adjust to pH 3.0 with *acetic acid R*.

Test solution Suspend 1.00 g of the substance to be examined in 10.0 ml of *acetonitrile R*.

Shake for 10 min. Allow to stand. Take 3.0 ml of the supernatant solution and dilute to 10.0 ml with solution A. Inject immediately.

Reference solution (a) Dissolve 50 mg of *paracetamol R* (impurity A) in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 50.0 ml with *acetonitrile R*. Dilute 3.0 ml of this solution to 10.0 ml with solution A.

Reference solution (b) Dissolve 10 mg of *paracetamol R* (impurity A) and 0.100 g of 4-*aminophenol R* (impurity B) in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 50.0 ml with *acetonitrile R*. Dilute 3.0 ml of this solution to 10.0 ml with solution A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, solution A (30:70 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 246 nm.

Injection 20 µl.

Run time Twice the retention time of propacetamol.

Identification of impurities The chromatogram obtained with reference solution (b) shows a peak due to impurity A (1st peak) and a peak due to impurity B (2nd peak).

Relative retention With reference to impurity A: impurity B = about 1.6.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (200 ppm);

— *unspecified impurities*: for each impurity, not more than 3.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent taking into account the response factor of paracetamol of 1.6);

— *total*: not more than 6.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent taking into account the relative response factor of paracetamol of 1.6);

— *disregard limit*: 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3 ppm taking into account the relative response factor of paracetamol of 1.6).

Methanol

Gas chromatography (*2.2.28*).

Internal standard solution Dilute 2.0 ml of *propanol R* to 20.0 ml with *water R*. Dilute 1.0 ml of the solution to 25.0 ml with *water R*. Dilute 1.0 ml of this solution to 25.0 ml with *water R*.

Test solution Dissolve 2.00 g of the substance to be examined in *water R*, add 2.0 ml of the internal standard solution and dilute to 10.0 ml with *water R*.

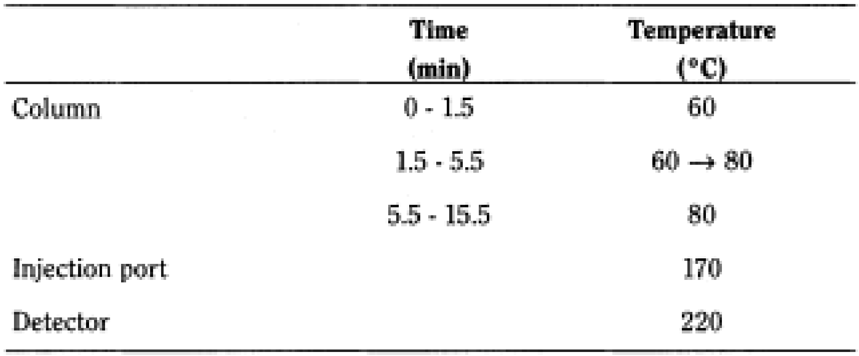
Reference solution Dilute 0.8 ml of *methanol R* to 50.0 ml with *water R*. Dilute 1.0 ml of the solution to 25.0 ml with *water R*. To 2.0 ml of this solution, add 2.0 ml of the internal standard solution and dilute to 10.0 ml with *water R*.

Column:

- *material*: glass;
- *size*: *l* = 2 m, Ø = 2 mm;
- *stationary phase*: carbon molecular sieve impregnated with 0.2 per cent of macrogol 1500.

Carrier gas   *nitrogen for chromatography R*.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1.5	60
	1.5 - 5.5	60 → 80
	5.5 - 15.5	80
Injection port		170
Detector		220

Detection   Flame ionisation.

Injection   2 µl.

Limit:

- *methanol*: calculate the ratio (*R*) of the area of the peak due to methanol to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak due to methanol to the area of the peak due to the internal standard: this ratio is not greater than *R* (500 ppm).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution 1 ppm (Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 25 ml of *anhydrous acetic acid R* and 25 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 30.08 mg of $C_{14}H_{21}ClN_2O_3$.

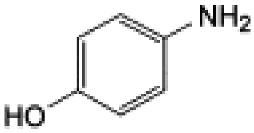
STORAGE

Protected from humidity.

IMPURITIES

Specified impurities *A, B*.

A. paracetamol,



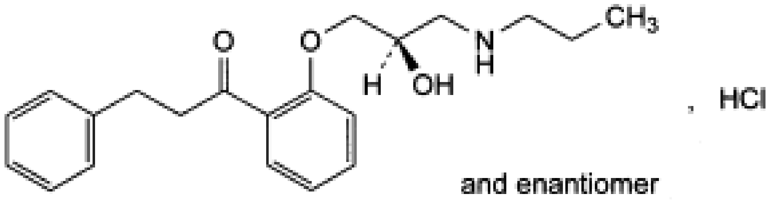
B. 4-aminophenol.

Ph Eur

Propafenone Hydrochloride

General Notices

(Ph Eur monograph 2103)



$C_{21}H_{27}NO_3,HCl$ 377.9 *34183-22-7*

Action and use

Class I antiarrhythmic.

Ph Eur

DEFINITION

1-[2-[(2*RS*)-2-Hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylpropan-1-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Colourless crystals or white or almost white powder.

Solubility

Slightly soluble in cold water, soluble in methanol and in hot water, practically insoluble in ethanol (96 per cent).

mp

About 173 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison propafenone hydrochloride CRS.

B. To 5.0 ml of solution S (see Tests) add 2 drops of *dilute nitric acid R*. A precipitate is formed. After 10 min, filter. 2.0 ml of the clear filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

To 0.500 g in a 100 ml volumetric flask add 50 ml of *water R*, and heat to boiling for 5 min. Allow to cool to room temperature and dilute to 100.0 ml with *carbon dioxide free water R*.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

pH (*2.2.3*)

5.0 to 6.2 for solution S.

Optical rotation (*2.2.7*)

- 0.05° to + 0.05°.

Dissolve 1.00 g in *methanol R* and dilute to 100.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (35:65 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of the substance to be examined and 5.0 mg of *propafenone impurity B CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

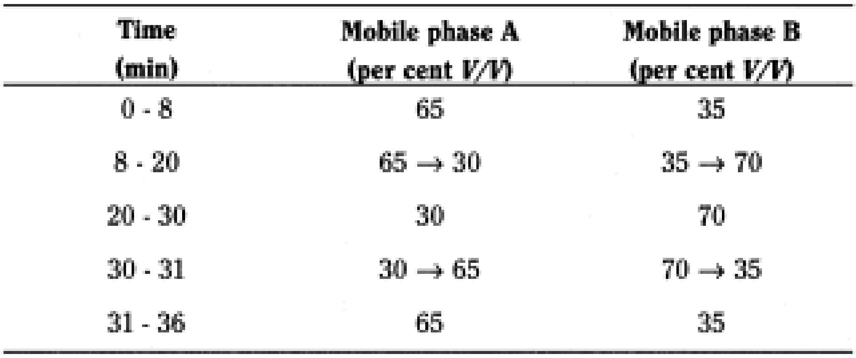
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 320-350 m2/g and a pore size of 12-13 nm;

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: 3.42 g/l solution of *dipotassium hydrogen phosphate trihydrate R* adjusted to pH 2.5 with *phosphoric acid R*;

— *mobile phase B*: *acetonitrile for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	65	35
8 - 20	65 → 30	35 → 70
20 - 30	30	70
30 - 31	30 → 65	70 → 35
31 - 36	65	35

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Equilibration 60 min with the mobile phase at the initial composition, before each series of injections.

Injection 20 µl of the test solution, reference solutions (a), (b) and (c) and of the solvent mixture as a blank.

Relative retention With reference to propafenone (retention time = about 5 min): impurity B = about 0.8; impurity D = about 2.3; impurity G = about 3.6; impurity C = about 4.1; impurity F = about 5.3.

System suitability Reference solution (c):

— *resolution*: minimum 3.0 between the peaks due to impurity B and propafenone.

Limits:

— *impurities B, C, D, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 0.4 ml of *acetic acid R* and 15.0 ml of *water R*, heating on a water-bath. To the warm solution add 3 ml of *buffer solution pH 3.5 R*. After cooling to room temperature, filter through a sintered-glass filter (40) (*2.1.2*) and rinse with *water R* until 20.0 ml of filtrate is obtained. 12.0 ml of the filtrate complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating during the titration, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

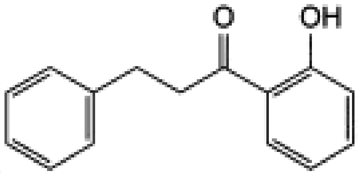
Dissolve 0.300 g in 2 ml of *anhydrous formic acid R*. Add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 37.79 mg of $C_{21}H_{28}ClNO_3$.

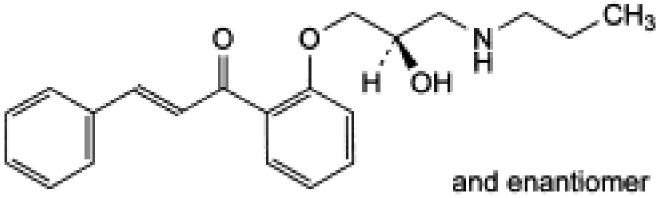
IMPURITIES

Specified impurities B, C, D, F, G.

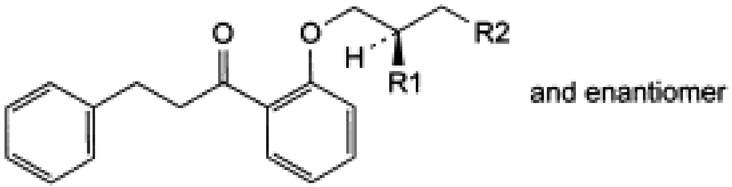
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, E, H.*



A. 1-(2-hydroxyphenyl)-3-phenylpropan-1-one,



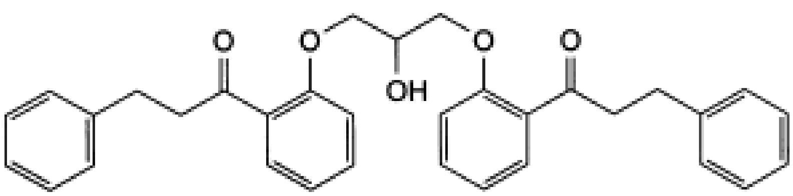
B. (2*E*)-1-[2-[(2*RS*)-2-hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylprop-2-en-1-one,



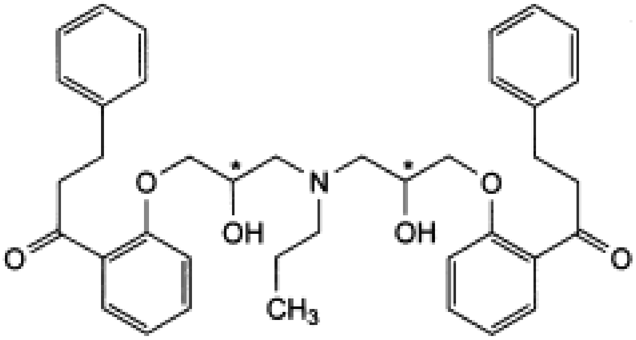
C. R1 + R2 = O: 1-[2-[[(2*RS*)-oxiranyl]methoxy]phenyl]-3-phenylpropan-1-one,

D. R1 = R2 = OH: 1-[2-[(2*RS*)-2,3-dihydroxypropoxy]phenyl]-3-phenylpropan-1-one,

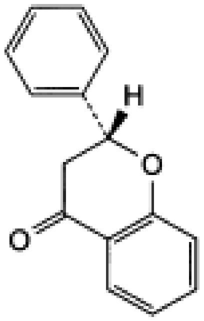
E. R1 = OH, R2 = Cl: 1-[2-[(2*RS*)-3-chloro-2-hydroxypropoxy]phenyl]-3-phenylpropan-1-one,



F. 1,1′-[2-hydroxypropane-1,3-diylbis(oxy-2,1-phenylene)]bis(3-phenylpropan-1-one),



G. 1,1′-[propyliminobis[(2-hydroxypropane-3,1-diyl)oxy-2,1-phenylene]]bis(3-phenylpropan-1-one),



and enantiomer

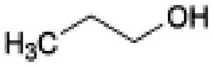
H. (2*RS*)-2-phenyl-2,3-dihydro-4*H*-1-benzopyran-4-one.

Ph Eur

Propanol

General Notices

(Ph Eur monograph 2036)



C_3H_8O 60.1 *71-23-8*

Ph Eur

DEFINITION

Propan-1-ol.

CHARACTERS

Appearance

Clear, colourless liquid.

Solubility

Miscible with water and with ethanol.

IDENTIFICATION

First identification *C, B.*

Second identification *A, B, D.*

A. Refractive index (*2.2.6*): 1.384 to 1.387.

B. Boiling point (*2.2.12*): 96 °C to 98 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of propanol.*

D. To 1.0 ml add 0.10 g of *dinitrobenzoyl chloride R* and 0.05 ml of *sulphuric acid R*. Boil under reflux for 30 min. Evaporate until the excess of propanol is removed, add 5 ml of *heptane R* to the residue and heat to boiling. Filter the hot solution. Wash the crystals formed on cooling with *heptane R* and dry in vacuum (2 kPa, at room temperature for 24 h). The small, colourless, shiny plates melt (*2.2.14*) between 71 °C and 74 °C.

TESTS

Solution S

Dissolve the residue obtained in the test for non-volatile matter in 1 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *water R*.

Appearance

The substance to be examined is clear (*2.2.1*) and colourless (*2.2.2, Method II*). Dilute 2 ml to 10 ml with *water R*. After 5 min, the solution is clear (*2.2.1*).

Acidity or alkalinity

To 10.0 ml of *carbon dioxide-free water R* add 0.1 ml of *phenolphthalein solution R* and *0.01 M sodium hydroxide* until the solution becomes pale pink. After addition of 5.0 ml of the substance to be examined the colour of the solution does not become more intense. If the colour fades, add 0.2 ml of *0.01 M sodium hydroxide*. The solution is pink.

Absorbance (*2.2.25*)

Measure the absorbance between 230 nm and 310 nm using *water R* as the compensation liquid. The absorbance A is not greater than the following values.

Wavelength (nm)	Absorbance *A*
230	0.300
250	0.100
270	0.030
290	0.020
310	0.010

The absorption curve does not show any peaks.

Reducing substances

Place 10.0 ml in a test tube of about 20 mm in diameter in a water bath at 20 °C. Keep protected from actinic light and add 1.0 ml of a freshly prepared 0.16 g/l solution of *potassium permanganate R*. The mixture, maintained at 20 °C, slowly changes its colour from violet to red. After 30 min, the test solution is not less intensely coloured (*2.2.2, Method II*) than 10.0 ml of a reference solution prepared as follows: to 5.5 ml of primary solution yellow, add 13.0 ml of primary solution red and dilute to 100.0 ml with *water R*.

Related substances

Gas chromatography (*2.2.28*).

Test solution The substance to be examined.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *heptane R*. Dilute 1.0 ml of the solution to 10.0 ml with *heptane R*.

Reference solution (b) Mix 0.1 ml of *acetone R* with 0.1 ml of *2-propanol R* and dilute to 100 ml with the test solution.

Column:
— *material*: fused silica;

— *size*: *l* = 30 m, Ø = 0.25 mm;

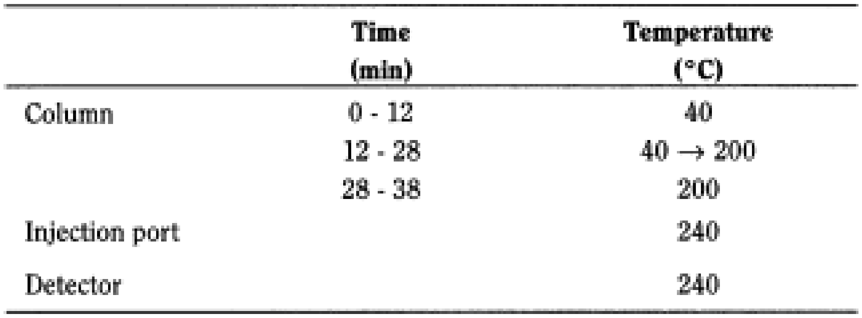
— *stationary phase*: *poly[(cyanopropyl)(phenyl)][dimethyl]siloxane R* (film thickness 1.4 µm).

Carrier gas *helium for chromatography R*.

Linear velocity 25 cm/s.

Split ratio 1:200.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 12	40
	12 - 28	40 → 200
	28 - 38	200
Injection port		240
Detector		240

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity D and impurity E.

Limits:

— *any impurity*: not more than the area of the peak due to propanol in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 3 times the area of the peak due to propanol in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.1 times the area of the peak due to propanol in the chromatogram obtained with reference solution (a) (0.01 per cent).

Non-volatile matter

Maximum 0.004 per cent.

Evaporate 50 ml of the substance to be examined to dryness at 100 °C and dry the residue in an oven at 100-105 °C to constant mass. The residue weighs a maximum of 2 mg. The residue is used for the preparation of solution S.

Water (*2.5.12*)

Maximum 0.2 per cent, determined on 10 g.

STORAGE

Protected from light.

IMPURITIES

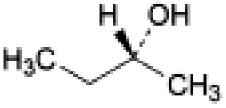
A. CH_3-OH: methanol,

B. ethanol,

C. CH_3-CH_2-CHO: propanal,

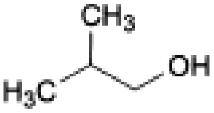
D. acetone,

E. isopropyl alcohol (2-propanol),



and enantiomer

F. butan-2-ol (*sec*-butanol),



G. 2-methylpropan-1-ol (isobutanol),

H. CH_3-$[CH_2]_3$-OH: butan-1-ol (*n*-butanol),

I. CH_3-$[CH_2]_4$-OH: pentan-1-ol (*n*-pentanol),

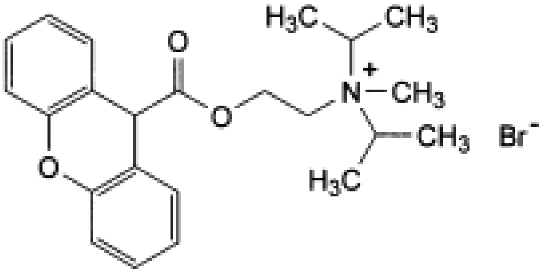
J. CH_3-$[CH_2]_5$-OH: hexan-1-ol (*n*-hexanol).

Ph Eur

Propantheline Bromide

General Notices

(Ph Eur monograph 0857)



$C_{23}H_{30}BrNO_3$ 448.4 *50-34-0*

Action and use

Anticholinergic.

Preparation

Propantheline Tablets

Ph Eur

DEFINITION

N-Methyl-*N*,*N*-bis(1-methylethyl)-2-[(9*H*-xanthen-9-ylcarbonyl)oxy]ethanaminium bromide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, slightly hygroscopic powder.

Solubility

Very soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 60 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *methanol R*.

Spectral range 230-350 nm.

Absorption maxima At 246 nm and 282 nm.

Specific absorbance at the absorption maxima:
— at 246 nm: 115 to 125;

— at 282 nm: 57 to 63.

B. Dissolve 0.2 g in 15 ml of *water R* and add 1 ml of *strong sodium hydroxide solution R*. Boil for 2 min and cool slightly. Add 7.5 ml of *dilute hydrochloric acid R* and filter. Wash the residue with *water R* and recrystallise from *ethanol (50 per cent V/V) R*. Dry at 100-105 °C for 1 h. Dissolve about 10 mg of the residue in 5 ml of *sulphuric acid R*. The solution has an intense yellow colour and shows an intense yellowish-green fluorescence when examined in ultraviolet light at 365 nm.

C. Dissolve 50 mg in 0.1 ml of *water R* in a 25 ml flask and add 1 ml of a saturated solution of *potassium permanganate R*. Attach a fractionating column and a condenser, with the end of the delivery tube immersed in 1 ml of *water R* in a test-tube placed in a bath of iced water. Distil fairly vigorously and continue heating for 1 min after a dry residue has been obtained in the flask. Prepare a blank by introducing into an identical test-tube a volume of *water R* equal to that of the distillate. Place the tubes in a bath of iced water. To each tube, add 0.5 ml of a 20 per cent *V/V* solution of *morpholine R* and 0.5 ml of a freshly prepared 50 g/l solution of sodium *nitroprusside R*. Mix and allow to stand at 0 °C for 5 min, and then at room temperature for 3 min. No blue colour develops in either tube. Add 1 g of *ammonium sulphate R*, mix and allow to stand for 15 min. A stable, intense pink colour develops in the test solution. A brownish-yellow colour develops in the blank.

D. It gives reaction (a) of bromides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*).

Dissolve 0.6 g in *water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, *water R* (40:60 *V/V*).

Test solution (a) Dissolve 6 mg of the substance to be examined in the solvent mixture and dilute to 50 ml with the solvent mixture.

Test solution (b) Dissolve 6 mg of the substance to be examined in 30 ml of the solvent mixture. Add 5 ml of reference solution (b) and dilute to 50 ml with the solvent mixture.

Test solution (c) Dissolve 6 mg of *xanthydrol R1* and 6 mg of the substance to be examined in the solvent mixture, then dilute to 50 ml with the solvent mixture.

Reference solution (a) Dissolve 6 mg of *xanthydrol R1* in the solvent mixture and dilute to 50 ml with the solvent mixture.

Reference solution (b) Dilute 5 ml of reference solution (a) to 50 ml with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mixture of equal volumes of *acetonitrile R* and of a solution containing 28 g/l of *sodium perchlorate R* and 11 g/l of *phosphoric acid R*, adjusted to pH 3.8 with *strong sodium hydroxide solution* R and then with *0.1 M sodium hydroxide*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 206 nm.

Injection 20 µl of test solutions (a), (b), (c) and reference solution (a).

Run time Twice the retention time of propantheline.

System suitability Test solution (c):

— in the chromatogram obtained with test solution (a), there is no peak corresponding to the principal peak in the chromatogram obtained with reference solution (a);

— *resolution*: minimum 8.0 between the peaks due to propantheline and xanthydrol.

Limits Test solution (b):

— *any impurity*: for each impurity, not more than the area of the peak due to xanthydrol (1.0 per cent), and not more than one such peak has an area greater than or equal to 0.5 times the area of the peak due to xanthydrol (0.5 per cent);

— *disregard limit*: disregard any peak with a retention time relative to propantheline of less than 0.2 (bromide); disregard the peak due to xanthydrol.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* corresponds to 44.84 mg of $C_{23}H_{30}BrNO_3$.

STORAGE

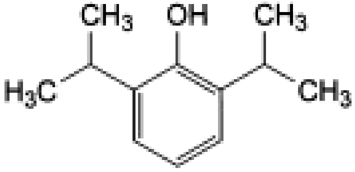
In an airtight container.

Ph Eur

Propofol

General Notices

(Ph Eur monograph 1558)



$C_{12}H_{18}O$ 178.3 *2078-54-8*

Action and use

Intravenous general anaesthetic.

Preparation

Propofol Injection

Ph Eur

DEFINITION

2,6-Bis(1-methylethyl)phenol.

Content

98.0 per cent to 102.0 per cent.

This monograph applies to propofol prepared using distillation for purification.

CHARACTERS

Appearance

Colourless or very light yellow, clear liquid.

Solubility

Very slightly soluble in water, miscible with hexane and with methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison propofol CRS.

TESTS

Refractive index (*2.2.6*): 1.5125 to 1.5145.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 1.00 g of the substance to be examined in *hexane R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 0.240 g of the substance to be examined in *hexane R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 5 µl of the substance to be examined and 15 µl of *propofol impurity J CRS* in *hexane R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 0.1 ml of *propofol for peak identification CRS* (containing impurities E and G) to 1.0 ml with *hexane R*.

Reference solution (c) Dilute 1.0 ml of test solution (a) to 100.0 ml with *hexane R*. Dilute 1.0 ml of this solution to 10.0 ml with *hexane R*.

Reference solution (d) Dissolve 0.240 g of *propofol CRS* in *hexane R* and dilute to 100.0 ml with the same solvent.

Column:
— *size: l* = 0.20 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase *anhydrous ethanol R*, *acetonitrile R*, *hexane R* (1.0:7.5:990 *V/V/V*).

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µl of test solution (a) and reference solutions (a), (b) and (c).

Run time 7 times the retention time of propofol.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities G and E.

Relative retention With reference to propofol (retention time = about 3 min): impurity G = about 0.5; impurity I = about 0.6; impurity B = about 0.7; impurity N = about 2.3; impurity D = about 2.5; impurity P = about 2.9; impurity A = about 3.0; impurity C = about 3.4; impurity E = about 4.0; impurity F = about 5.8; impurity H = about 6.4.

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to impurity J and propofol.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.25; impurity G = 5.0;

— *impurity G*: not more than twice the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurity E*: not more than 0.1 times the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.01 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.05 per cent);

— *total*: not more than 3 times the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *disregard limit*: 0.3 times the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.03 per cent), except for impurity E.

Impurities J, K, L and O

Gas chromatography *(2.2.28)*.

Test solution Dissolve 40.0 mg of the substance to be examined in *methylene chloride R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methylene chloride R*. Dilute 1.0 ml of this solution to 10.0 ml with *methylene chloride R*.

Reference solution (b) Dissolve 5 µl of *propofol impurity J CRS* (corresponding to 5 mg) in *methylene chloride R* and dilute to 100 ml with the same solvent. Dilute 1.0 ml of this solution to 25 ml with *methylene chloride R*.

Reference solution (c) Dissolve 4 mg of *propofol CRS* in reference solution (b) and dilute to 1 ml with the same solution.

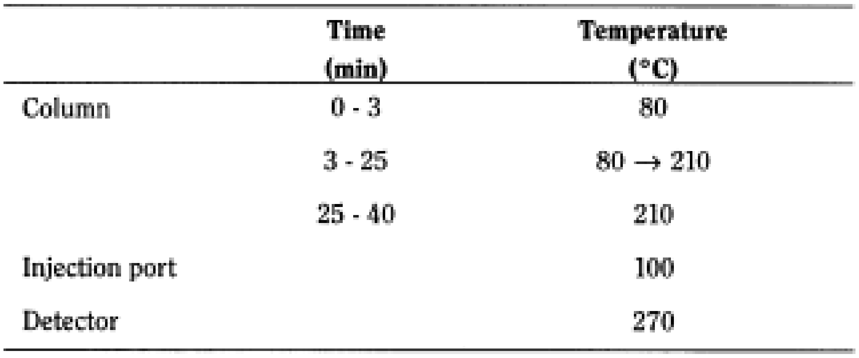
Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *polymethylphenylsiloxane R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.7 ml/min.

Split ratio 1:5.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 3	80
	3 - 25	80 → 210
	25 - 40	210
Injection port		100
Detector		270

Detection Flame ionisation.

Injection 1 µl of the test solution and reference solutions (a) and (c).

Relative retention With reference to propofol (retention time = about 17 min): impurity K = about 0.76; impurity L = about 0.81; impurity J = about 1.01; impurity O = about 1.03.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity J, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to propofol.

Limits:

— *impurities J, K, L, O*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{12}H_{18}O$ using the declared content of *propofol CRS*.

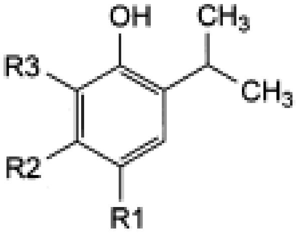
STORAGE

Protected from light under an inert gas.

IMPURITIES

Specified impurities *E, G, J, K, L, O*.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, F, H, I, N, P.*



A. R1 = $CH(CH_3)_2$, R2 = R3 = H: 2,4-bis(1-methylethyl)phenol,

B. R1 = R2 = H, R3 = $C(CH_3)=CH_2$: 2-(1-methylethenyl)-6-(1-methylethyl)phenol,

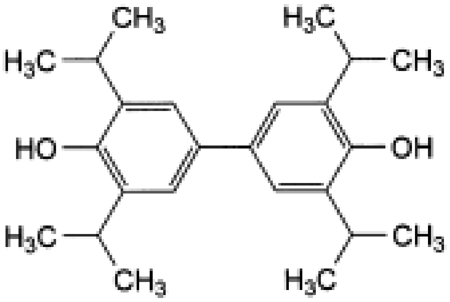
C. R1 = R2 = R3 = H: 2-(1-methylethyl)phenol,

D. R1 = R3 = H, R2 = $CH(CH_3)_2$: 2,5-bis(1-methylethyl)phenol,

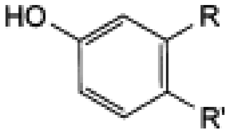
N. R1 = CO_2H, R2 = H, R3 = $CH(CH_3)_2$: 4-hydroxy-3,5-bis(1-methylethyl)benzoic acid,

O. R1 = R2 = H, R3 = CH_2-CH_2-CH_3: 2-(1-methylethyl)-6-propylphenol,

P. R1 = CO-O-$CH(CH_3)_2$, R2 = H, R3 = $CH(CH_3)_2$: 1-methylethyl 4-hydroxy-3,5-bis(1-methylethyl)benzoate,

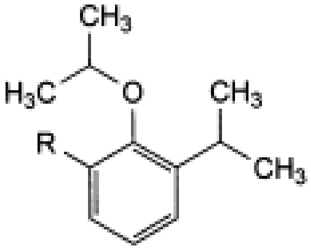


E. 3,3′,5,5′-tetrakis(1-methylethyl)biphenyl-4,4′-diol,



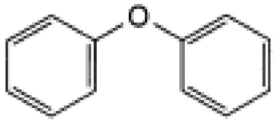
F. R = $CH(CH_3)_2$, R′ = H: 3-(1-methylethyl)phenol,

H. R = H, R′ = $CH(CH_3)_2$: 4-(1-methylethyl)phenol,

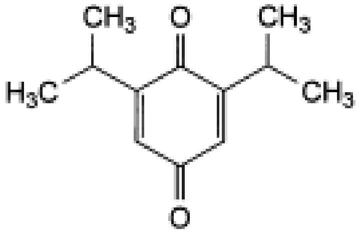


G. R = $CH(CH_3)_2$: 2-(1-methylethoxy)-1,3-bis(1-methylethyl)benzene,

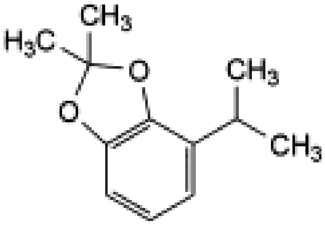
K. R = H: 1-(1-methylethoxy)-2-(1-methylethyl)benzene,



I. oxydibenzene,



J. 2,6-bis(1-methylethyl)benzene-1,4-dione,



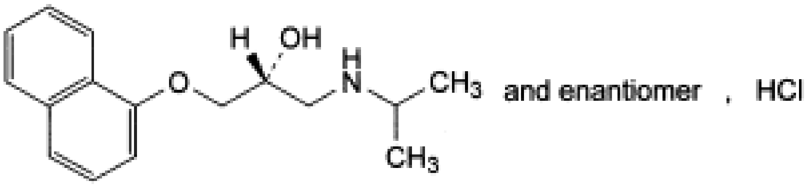
L. 2,2-dimethyl-4-(1-methylethyl)-1,3-benzodioxole.

Ph Eur

Propranolol Hydrochloride

General Notices

(Ph Eur monograph 0568)



$C_{16}H_{21}NO_2,HCl$ 295.8 *318-98-9*

Action and use

Beta-adrenoceptor antagonist.

Preparations

Prolonged-release Propranolol Capsules

Propranolol Injection

Propranolol Tablets

Ph Eur

DEFINITION

(2*RS*)-1-[(1-Methylethyl)amino]-3-(naphthalen-1-yloxy)propan-2-ol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification B, D.

Second identification *A, C, D.*

A. Melting point *(2.2.14)*: 163 °C to 166 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *propranolol hydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in 1 ml of *methanol R*.

Reference solution Dissolve 10 mg of *propranolol hydrochloride CRS* in 1 ml of *methanol R*.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R1*, *methanol R* (1:99 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C until the colour of the spots reaches maximum intensity (10-15 min).

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Dissolve 2.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Acidity or alkalinity

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. Add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M hydrochloric acid*; the solution is red. Add 0.4 ml of *0.01 M sodium hydroxide*; the solution is yellow.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *propranolol hydrochloride for performance test CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
— *size*: *l* = 0.25 m; Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 1.6 g of *sodium laurilsulfate R* and 0.31 g of *tetrabutylammonium dihydrogen phosphate R* in a mixture of 1 ml of *sulphuric acid R*, 450 ml of *water R* and 550 ml of *acetonitrile R*; adjust to pH 3.3 using *dilute sodium hydroxide solution R*.

Flow rate 1.8 ml/min.

Detection Spectrophotometer at 292 nm.

Equilibration For at least 30 min.

Injection 20 µl.

Run time 7 times the retention time of propranolol.

Identification of impurities Use the chromatogram supplied with *propranolol hydrochloride for performance test CRS* to identify the peak due to impurity A.

System suitability Reference solution (a):

— baseline separation is obtained between the peaks due to impurity A and propranolol.

Limits:

— *any impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

Dissolve 1.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) prepared by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of methanol R.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

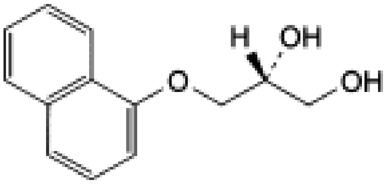
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 25 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

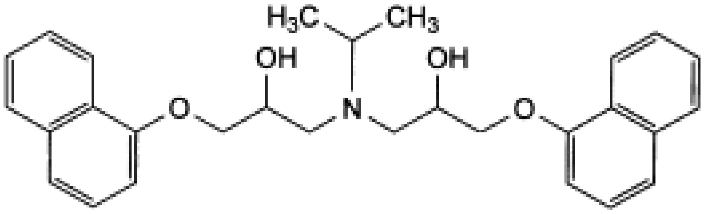
1 ml of *0.1 M sodium hydroxide* is equivalent to 29.58 mg of $C_{16}H_{22}ClNO_2$.

IMPURITIES

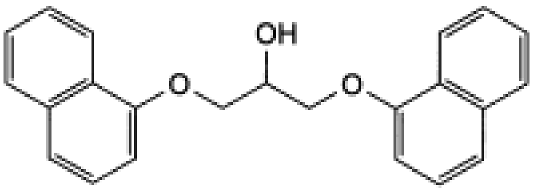


and enantiomer

A. (2*RS*)-3-(naphthalen-1-yloxy)propane-1,2-diol (diol derivative),



B. 1,1′-[(1-methylethyl)imino]bis[3-(naphthalen-1-yloxy)propan-2-ol] (tertiary amine derivative),



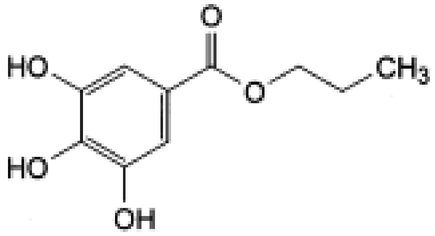
C. 1,3-bis(naphthalen-1-yloxy)propan-2-ol (bis-ether derivative).

Ph Eur

Propyl Gallate

General Notices

(Ph Eur monograph 1039)



$C_{10}H_{12}O_5$ 212.2 *121-79-9*

Action and use

Antioxidant.

Ph Eur

DEFINITION

Propyl gallate contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of propyl 3,4,5-trihydroxybenzoate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very slightly soluble in water, freely soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 148 °C to 151 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *propyl gallate CRS*.

C. Examine the chromatograms obtained in the test for gallic acid. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 10 ml of *water R* by heating to about 70 °C. Cool and add 1 ml of *bismuth subnitrate solution R*. A bright yellow precipitate is formed.

TESTS

Appearance of solution

Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 20 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Gallic acid

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 20 ml with *acetone R*.

Reference solution (a) Dissolve 10 mg of *propyl gallate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *gallic acid R* in *acetone R* and dilute to 20 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *acetone R*.

Reference solution (c) Dilute 0.5 ml of test solution (b) to 5 ml with reference solution (b).

Apply separately to the plate 5 µl of each solution. Develop over a path of 8 cm using a mixture of 10 volumes of *anhydrous formic acid R*, 40 volumes of *ethyl formate R* and 50 volumes of *toluene R*. Allow the plate to dry in air for 10 min and spray with a mixture of 1 volume of *ferric chloride solution R1* and 9 volumes of *ethanol (96 per cent) R*. Any spot due to gallic acid in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated principal spots.

Total chlorine

Mix 0.5 g with 2 g of *calcium carbonate R1*. Dry and ignite at 700 ± 50 °C. Take up the residue with 20 ml of *dilute nitric acid R* and dilute to 30 ml with *water R*. 15 ml of the solution, without further addition of *dilute nitric acid R*, complies with the limit test for chlorides (*2.4.4*) (200 ppm).

Chlorides (*2.4.4*)

To 1.65 g add 50 ml of *water R*. Shake for 5 min. Filter. 15 ml of the filtrate complies with the limit test for chlorides (100 ppm).

Zinc

Not more than 25.0 ppm of Zn, determined by atomic absorption spectrometry (*2.2.23, Method II*).

Test solution To 2.5 ml of the solution obtained in the test for heavy metals, add 2.5 ml of *water R*.

Reference solutions Prepare the reference solutions using *zinc standard solution (10 ppm Zn) R*, diluted as necessary with *water R*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as the source of radiation and an air-acetylene flame.

Heavy metals (*2.4.8*)

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in *methanol R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 200.0 ml with *methanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 275 nm.

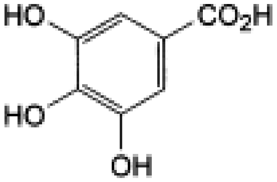
Calculate the content of $C_{10}H_{12}O_5$ taking the specific absorbance to be 503.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*



A. 3,4,5-trihydroxybenzoic acid (gallic acid).

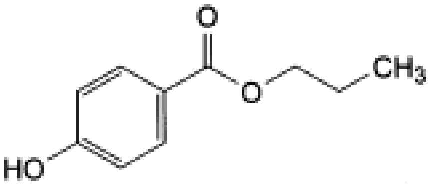
Ph Eur

Propyl Hydroxybenzoate

General Notices

Propylparaben

(Propyl Parahydroxybenzoate, Ph Eur monograph 0431)



$C_{10}H_{12}O_3$ 180.2 *94-13-3*

Action and use

Antimicrobial preservative.

Ph Eur

DEFINITION

Propyl 4-hydroxybenzoate.

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in alcohol and in methanol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 96 °C to 99 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *propyl parahydroxybenzoate CRS.*

C. Examine the chromatograms obtained in the test for related substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

D. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution R*, boil for 30 s and cool (solution A). To a further 10 mg in a similar test-tube add 1 ml of sodium *carbonate solution R*; the substance partly dissolves (solution B). Add at the same time to solution A and solution B 5 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* and mix. Solution B is yellow to orange-brown. Solution A is orange to red, the colour being clearly more intense than any similar colour which may be obtained with solution B.

TESTS

Solution S

Dissolve 1.0 g in *alcohol R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity

To 2 ml of solution S add 3 ml of *alcohol R*, 5 ml of *carbon dioxide-free water R* and 0.1 ml of *bromocresol green solution R*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *acetone R*.

Reference solution (a) Dilute 0.5 ml of test solution (a) to 100 ml with *acetone R*.

Reference solution (b) Dissolve 10 mg of *propyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in 1 ml of test solution (a) and dilute to 10 ml with *acetone R*.

Plate Suitable octadecylsilyl silica gel with a fluorescent indicator having an optimal intensity at 254 nm as the coating substance.

Mobile phase *glacial acetic acid R*, *water R*, *methanol R* (1:30:70 *V/V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated principal spots.

Limits:

— *any impurity*: any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Sulphated ash (*2.4.14*)

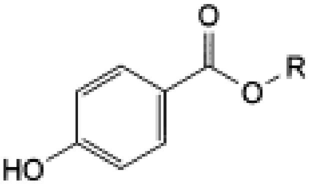
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 1.000 g add 20.0 ml of *1 M sodium hydroxide*. Heat at about 70 °C for 1 h. Cool rapidly in an ice bath. Prepare a blank in the same manner. Carry out the titration on the solutions at room temperature. Titrate the excess sodium hydroxide with *0.5 M sulphuric acid*, continuing the titration until the second point of inflexion and determining the end-point potentiometrically (*2.2.20*).

1 ml of *1 M sodium hydroxide* is equivalent to 180.2 mg of $C_{10}H_{12}O_3$.

IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

B. R = CH_3: methyl 4-hydroxybenzoate,

C. R = CH_2-CH_3: ethyl 4-hydroxybenzoate,

D. R = CH_2-CH_2-CH_2-CH_3: butyl 4-hydroxybenzoate.

Ph Eur

Propylene Glycol Dicaprylocaprate

General Notices

(Ph Eur monograph 2122)

Action and use

Excipient.

Ph Eur

DEFINITION

Propylene glycol diesters of saturated fatty acids, mainly caprylic (octanoic) acid and capric (decanoic) acid, of vegetable origin.

CHARACTERS

Appearance

Almost colourless to light yellow, oily liquid.

Solubility

Practically insoluble in water, soluble in fatty oils and in light petroleum, slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Refractive index (*2.2.6*): 1.439 to 1.442.

B. Relative density (*2.2.5*): 0.910 to 0.930.

C. Viscosity (*2.2.9*): 9 mPa·s to 12 mPa·s.

D. It complies with the test for composition of fatty acids (see Tests).

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acid value (*2.5.1*)

Maximum 0.2.

Hydroxyl value (*2.5.3, Method A*)

Maximum 10.

Iodine value (*2.5.4*)

Maximum 1.0.

Peroxide value (*2.5.5, Method A*)

Maximum 1.0.

Saponification value (*2.5.6*)

320 to 340.

Unsaponifiable matter (*2.5.7*)

Maximum 0.3 per cent, determined on 5.0 g.

Alkaline impurities

Dissolve 2.00 g of the substance to be examined in a mixture of 1.5 ml of *ethanol (96 per cent) R* and 3.0 ml of *ether R*. Add 0.05 ml of *bromophenol blue solution R*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Composition of fatty acids

Gas chromatography (*2.4.22, Method C*). Prepare reference solution (a) as indicated in Table 2.4.22.-2.

Column:

— *material*: fused silica;

— *size: l* = 30 m, Ø = 0.32 mm;

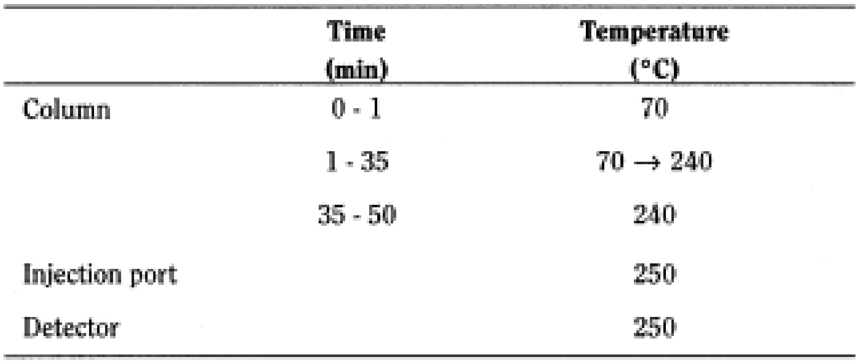
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm),

Carrier gas *helium for chromatography R*.

Flow rate 1.3 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	70
	1 - 35	70 → 240
	35 - 50	240
Injection port		250
Detector		250

Detection Flame ionisation.

Composition of the fatty acid fraction of the substance to be examined:

— *caproic acid*: maximum 2.0 per cent;

- *caprylic acid*: 50.0 per cent to 80.0 per cent;
- *capric acid*: 20.0 per cent to 50.0 per cent;
- *lauric acid*: maximum 3.0 per cent;
- *myristic acid*: maximum 1.0 per cent.

Water (*2.5.12*)

Maximum 0.1 per cent, determined on 5.00 g.

Total ash (*2.4.16*)

Maximum 0.1 per cent, determined on 2.0 g.

STORAGE

Protected from light.

Ph Eur

Propylene Glycol Dilaurate

General Notices

(Ph Eur monograph 2087)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of propylene glycol mono- and diesters of lauric (dodecanoic) acid.

Content

Minimum 70.0 per cent of diesters and maximum 30.0 per cent of monoesters.

CHARACTERS

Appearance

Clear, oily liquid at 20 °C, colourless or slightly yellow.

Solubility

Practically insoluble in water, very soluble in alcohol, in methanol and in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.1 g of the substance to be examined in *methylene chloride R* and dilute to 2 ml with the same solvent.

Reference solution Dissolve 0.1 g of *propylene glycol dilaurate CRS* in *methylene chloride R* and dilute to 2 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R*, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine 6 G R* in *alcohol R*. Examine in ultraviolet light at 365 nm.

Results The spots in the chromatogram obtained with the test solution are similar in position to those in the chromatogram obtained with the reference solution.

B. It complies with the test for composition of fatty acids (see Tests).

C. It complies with the assay (content of diesters).

TESTS

Acid value (*2.5.1*)

Maximum 4.0, determined on 5.00 g.

Iodine value (*2.5.4, Method A*)

Maximum 1.0.

Saponification value (*2.5.6*)

230 to 250.

Composition of fatty acids

Gas chromatography (*2.4.22, Method C*). Use the mixture of calibrating substances in Table 2.4.22.-2.

Composition of the fatty acid fraction of the substance:

- *caprylic acid*: maximum 0.5 per cent;
- *capric acid*: maximum 2.0 per cent;
- *lauric acid*: minimum 95.0 per cent;
- *myristic acid*: maximum 3.0 per cent;
- *palmitic acid*: maximum 1.0 per cent.

Free propylene glycol

Maximum 2.0 per cent, determined as prescribed under Assay.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Size-exclusion chromatography (*2.2.30*).

Stock solution Introduce 0.100 g of *propylene glycol R* into a flask and dilute to 25.0 ml with *tetrahydrofuran R.*

Test solution In a 15 ml flask, weigh 0.200 g (m). Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 ml flasks, introduce respectively 0.25 ml, 0.5 ml, 1.0 ml and 2.5 ml of stock solution and add 5.0 ml of *tetrahydrofuran R*. Weigh each flask and calculate the concentration of propylene glycol in milligrams per gram for each reference solution.

Column:

— *size:* l = 0.6 m, Ø = 7 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R.*

Flow rate 1 ml/min.

Detection Differential refractometer.

Injection 40 µl.

Relative retention With reference to propylene glycol: diesters = about 0.85; monoesters = about 0.90.

Calculations:

— *free propylene glycol* : from the calibration curve obtained with the reference solutions, determine the concentration (*C*) in milligrams per gram in the test solution and calculate the percentage content in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\frac{A}{A + B} \times (100 - D)$$

A = area of the peak due to the monoesters,

B = area of the peak due to the diesters,

D = percentage content of free propylene glycol + percentage content of free fatty acids.

Calculate the percentage content of free fatty acids using the expression:

$$\frac{I_A \times 200}{561.1}$$

I_A = acid value.

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{B}{A + B} \times (100 - D)$$

STORAGE

Protected from moisture.

Ph Eur

Propylene Glycol Monolaurate

General Notices

(Ph Eur monograph 1915)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of propylene glycol mono- and diesters of lauric (dodecanoic) acid.

Content

— propylene glycol monolaurate (type I): 45.0 per cent to 70.0 per cent of monoesters and 30.0 per cent to 55.0 per cent of diesters;

— propylene glycol monolaurate (type II): minimum 90.0 per cent of monoesters and maximum 10.0 per cent of diesters.

CHARACTERS

Appearance

Clear, oily liquid at 20 °C, colourless or slightly yellow.

Solubility

Practically insoluble in water, very soluble in alcohol, in methanol and in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.1 g of the substance to be examined in *methylene chloride R* and dilute to 2 ml with the same solvent.

Reference solution Dissolve 0.1 g of *propylene glycol monolaurate CRS* in *methylene chloride R* and dilute to 2 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase hexane R, *ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine 6 G R* in *alcohol R*. Examine in ultraviolet light at 365 nm.

Results The spots in the chromatogram obtained with the test solution are similar in position to those in the chromatogram obtained with the reference solution.

B. It complies with the test for composition of fatty acids (see Tests).

C. It complies with the assay (content of monoesters).

TESTS

Acid value (*2.5.1*)

Maximum 4.0, determined on 5.00 g.

Iodine value (*2.5.4, Method A*)

Maximum 1.0.

Saponification value (*2.5.6*)

210 to 245 for propylene glycol monolaurate (type I) and 200 to 230 for propylene glycol monolaurate (type II).

Composition of fatty acids

Gas chromatography (*2.4.22, Method C*). Use the mixture of calibrating substances in Table 2.4.22.-2.

Composition of the fatty acid fraction of the substance:

— *caprylic acid*: maximum 0.5 per cent;

— *capric acid*: maximum 2.0 per cent;

— *lauric acid*: minimum 95.0 per cent;

— *myristic acid*: maximum 3.0 per cent;

— *palmitic acid*: maximum 1.0 per cent.

Free propylene glycol

Maximum 5.0 per cent for propylene glycol monolaurate (type I) and maximum 1.0 per cent for propylene glycol monolaurate (type II), determined as prescribed under Assay.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Size-exclusion chromatography (*2.2.30*).

Stock solution Introduce 0.100 g of *propylene glycol R* into a vial and dilute to 25.0 ml with *tetrahydrofuran R*.

Test solution In a 15 ml flask, weigh 0.200 g (*m*). Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 ml flasks, introduce respectively 0.25 ml, 0.5 ml, 1.0 ml and 2.5 ml of stock solution and add 5.0 ml of *tetrahydrofuran R*. In a fifth 15 ml flask, introduce 5.0 ml of stock solution. Weigh each flask and calculate the concentration of propylene glycol in milligrams per gram for each reference solution.

Column:

— *size:* l = 0.6 m, Ø = 7 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 1 ml/min.

Detection Differential refractometer.

Injection 40 µl.

Relative retention With reference to propylene glycol: diesters = about 0.85; monoesters = about 0.90.

Calculations:

— *free propylene glycol* : from the calibration curve obtained with the reference solutions, determine the concentration (*C*) in milligrams per gram in the test solution and calculate the percentage content in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\frac{A}{A+B} \times (100 - D)$$

A = area of the peak due to the monoesters,

B = area of the peak due to the diesters,

D = percentage content of free propylene glycol + percentage content of free fatty acids.

Calculate the percentage content of free fatty acids using the expression:

$$\frac{I_A \times 200}{561.1}$$

I_A = acid value.

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{B}{A+B} \times (100 - D)$$

STORAGE

Protected from moisture.

LABELLING

The label states the type of propylene glycol monolaurate (type I or type II).

Ph Eur

Propylene Glycol Monopalmitostearate

General Notices

Propylene Glycol Monostearate
(Ph Eur monograph 1469)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of propylene glycol mono- and diesters and of stearic (octadecanoic) and palmitic (hexadecanoic) acids, produced by the condensation of propylene glycol and stearic acid 50 of vegetable or animal origin (see *Stearic acid (1474)*).

Content

Minimum of 50.0 per cent of monoesters.

CHARACTERS

Appearance

White or almost white, waxy solid.

Solubility

Practically insoluble in water, soluble in acetone and in hot alcohol.

IDENTIFICATION

A. It complies with the test for melting point (see Tests).

B. It complies for the test for composition of fatty acids (see Tests).

C. It complies with the assay (monoesters content).

TESTS

Melting point (*2.2.15*)

33 °C to 40 °C.

Acid value (*2.5.1*)

Maximum 4.0, determined on 10.0 g.

Iodine value (*2.5.4*)

Maximum 3.0.

Saponification value (*2.5.6*)

170 to 185, determined on 2.0 g.

Composition of fatty acids (*2.4.22, Method A*)

The fatty acid fraction has the following composition:

— *stearic acid*: 40.0 per cent to 60.0 per cent;

— *sum of contents of palmitic acid and stearic acid*: minimum 90.0 per cent.

Free propylene glycol

Maximum 5.0 per cent, determined as prescribed under Assay.

Total ash (*2.4.16*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Size-exclusion chromatography (*2.2.30*).

Test solution In a 15 ml flask, weigh about 0.2 g (*m*), to the nearest 0.1 mg. Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Heat gently, if necessary. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions In four 15 ml flasks, weigh, to the nearest 0.1 mg, about 2.5 mg, 5.0 mg, 10.0 mg and 20.0 mg of *propylene glycol R*. Add 5.0 ml of *tetrahydrofuran R* and shake to dissolve. Weigh the flasks again and calculate the concentration of propylene glycol in milligrams per gram for each reference solution.

Column:

— *size:* *l* = 0.6 m, Ø = 7 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (particle diameter 5 µm, pore size 10 nm).

Mobile phase *tetrahydrofuran R*.

Flow rate 1 ml/min.

Detection Differential refractometer.

Injection 40 µl.

Relative retention With reference to propylene glycol: diesters = about 0.78, monoesters = about 0.84.

Limits:

— *free propylene glycol*: from the calibration curve obtained with the reference solutions, determine the concentration (*C*) in milligrams per gram in the test solution and calculate the percentage content in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\frac{A}{A + B} \times (100 - D)$$

A = area of the peak due to the monoesters,

B = area of the peak due to the diesters,

D = percentage content of free propylene glycol + percentage content of free fatty acids which is determined using the following expression:

$$\frac{I_A \times 270}{561.1}$$

I_A = acid value.

STORAGE

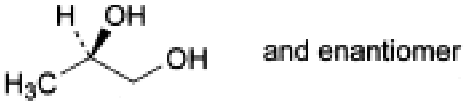
Protected from light.

Ph Eur

Propylene Glycol

General Notices

(Ph Eur monograph 0430)



$C_3H_8O_2$ 76.1 *57-55-6*

Action and use

Excipient.

Ph Eur

DEFINITION

Propylene glycol is (*RS*)-propane-1,2-diol.

CHARACTERS

A viscous, clear, colourless, hygroscopic liquid, miscible with water and with ethanol (96 per cent).

IDENTIFICATION

A. It complies with the test for relative density (see Tests).

B. It complies with the test for refractive index (see Tests).

C. Boiling point (*2.2.12*): 184 °C to 189 °C.

D. To 0.5 ml add 5 ml of *pyridine R* and 2 g of finely ground *nitrobenzoyl chloride R*. Boil for 1 min and pour into 15 ml of cold *water R* with shaking. Filter, wash the precipitate with 20 ml of a saturated solution of *sodium hydrogen carbonate R* and then with *water R* and dry. Dissolve in boiling *ethanol (80 per cent V/V) R* and filter the hot solution. On cooling, crystals are formed which, after drying at 100-105 °C, melt (*2.2.14*) at 121 °C to 128 °C.

TESTS

Appearance

It is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Relative density *(2.2.5)*

1.035 to 1.040.

Refractive index ***(2.2.6)***

1.431 to 1.433.

Acidity

To 10 ml add 40 ml of *water R* and 0.1 ml of *bromothymol blue solution R1*. The solution is greenish-yellow. Not more than 0.05 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Oxidising substances

To 10 ml add 5 ml of *water R*, 2 ml of *potassium iodide solution R* and 2 ml of *dilute sulphuric acid R* and allow to stand in a ground-glass-stoppered flask protected from light for 15 min. Titrate with *0.05 M sodium thiosulphate*, using 1 ml of *starch solution R* as indicator. Not more than 0.2 ml of *0.05 M sodium thiosulphate* is required.

Reducing substances

To 1 ml add 1 ml of *dilute ammonia R1* and heat in a water-bath at 60 °C for 5 min. The solution is not yellow. Immediately add 0.15 ml of *0.1 M silver nitrate* and allow to stand for 5 min. The solution does not change its appearance.

Heavy metals ***(2.4.8)***

Mix 4 ml with 16 ml of *water R*. 12 ml of the solution complies with limit test A for heavy metals (5 ppm m/*V*). Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water ***(2.5.12)***

Not more than 0.2 per cent, determined on 5.00 g by the semi-micro determination of water.

Sulphated ash ***(2.4.14)***

Heat 50 g until it burns and ignite. Allow to cool. Moisten the residue with *sulphuric acid R* and ignite; repeat the operations. The residue weighs not more than 5 mg (0.01 per cent).

STORAGE

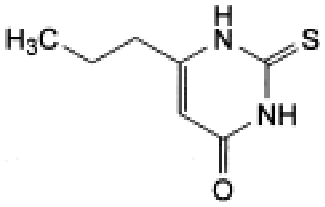
Store in an airtight container .

Ph Eur

Propylthiouracil

General Notices

(Ph Eur monograph 0525)



$C_7H_{10}N_2OS$ 170.2 *51-52-5*

Action and use

Thiourea antithyroid drug.

Preparation

Propylthiouracil Tablets

Ph Eur

DEFINITION

Propylthiouracil contains not less than 98.0 per cent and not more than the equivalent of 100.5 per cent of 2,3-dihydro-6-propyl-2-thioxopyrimidin-4(1*H*)-one, calculated with reference to the dried substance.

CHARACTERS

White or almost white, crystalline powder or crystals, very slightly soluble in water, sparingly soluble in alcohol. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point (*2.2.14*): 217 °C to 221 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *propylthiouracil CRS*. Examine as discs prepared using 1 mg of substance and 0.3 g of *potassium bromide R*.

C. Examine the chromatograms obtained in the test for impurity A and related substances in ultraviolet light at 254 nm before exposure of the plate to iodine vapour. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 20 mg add 8 ml of *bromine water R* and shake for a few minutes. Boil until the mixture is decolourised, allow to cool and filter. To the filtrate add 2 ml of *barium chloride solution R1*. A white precipitate is formed whose colour does not become violet on the addition of 5 ml of *dilute sodium hydroxide solution R*.

TESTS

Impurity A and related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel GF_{254} plate R*.

Test solution (a) Dissolve 0.1 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 10 mg of *propylthiouracil CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 50 mg of *thiourea R* in *methanol R* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 100 ml with *methanol R*.

Reference solution (c) Dilute 1 ml of test solution (a) to 100 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 0.1 volumes of *glacial acetic acid R*, 6 volumes of *2-propanol R* and 50 volumes of *chloroform R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Expose the plate to iodine vapour for 10 min. In the chromatogram obtained with test solution (a), any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.05 per cent) and any spot apart from the principal spot and any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (c) (1.0 per cent).

Heavy metals (*2.4.8*)

1.0 g complies with limit test F for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

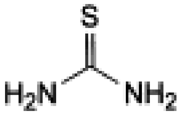
To 0.300 g add 30 ml of *water R* and 30.0 ml of *0.1 M sodium hydroxide*. Boil and shake until dissolution is complete. Add 50 ml of *0.1 M silver nitrate* while stirring, boil gently for 5 min and cool. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). The volume of *0.1 M sodium hydroxide* used is equal to the sum of the volume added initially and the volume used in the final titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 8.511 mg of $C_7H_{10}N_2OS$.

STORAGE

Store protected from light.

IMPURITIES



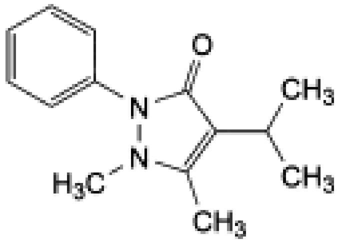
A. thiourea.

Ph Eur

Propyphenazone

General Notices

(Ph Eur monograph 0636)



$C_{14}H_{18}N_2O$ 230.3 *479-92-5*

Action and use

Pyrazolone analgesic.

Ph Eur

DEFINITION

Propyphenazone contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 1,5-dimethyl-4-(1-methylethyl)-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, slightly soluble in water, freely soluble in alcohol and in methylene chloride.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Melting point (*2.2.14*): 102 °C to 106 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *propyphenazone CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 1 ml of solution S (see Tests) add 0.1 ml of *ferric chloride solution R1.* A brownish-red colour appears which becomes yellow on addition of 1 ml of *dilute hydrochloric acid R*.

TESTS

Solution S

Dissolve 2 g in a mixture of equal volumes of *alcohol R* and *carbon dioxide-free water R* and dilute to 50 ml with the same mixture of solvents.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Add 0.2 ml of *0.01 M sodium hydroxide*; the solution becomes pink. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution becomes colourless. Add 0.2 ml of *methyl red solution R*. The solution becomes orange or red.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* HF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.40 g of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 5 ml with *methanol R.*

Reference solution (a) Dissolve 80 mg of *propyphenazone CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 100 ml with *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *butanol R*, 45 volumes of *cyclohexane R* and 45 volumes of *ethyl acetate R*. Dry the plate in a current of hot air for 15 min and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). Spray with a mixture of equal volumes of *potassium ferricyanide solution R* and *ferric chloride solution R1*. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Heavy metals (*2.4.8*)

1.0 g complies with limit test C (10 ppm). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 0.5 g.

ASSAY

Dissolve 0.2000 g in 10 ml of *anhydrous acetic acid R* and add 75 ml of *ethylene chloride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

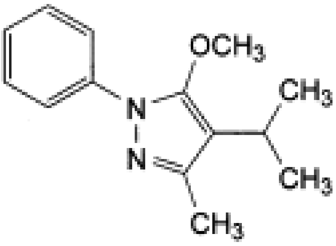
1 ml of *0.1 M perchloric acid* is equivalent to 23.03 mg of $C_{14}H_{18}N_2O$.

STORAGE

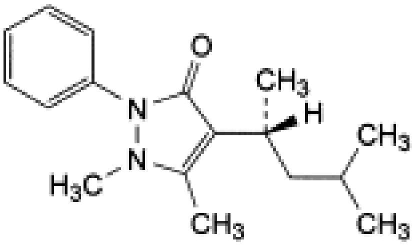
Store protected from light.

IMPURITIES

A. phenazone,



B. 5-methoxy-3-methyl-4-(1-methylethyl)-1-phenyl-1*H*-pyrazole,



and enantiomer

C. 4-[(1*RS*)-1,3-dimethylbutyl]-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one.

Ph Eur

Protamine Hydrochloride

General Notices

(Ph Eur monograph 0686)

Action and use

Antidote to heparin.

Ph Eur

DEFINITION

Protamine hydrochloride consists of the hydrochlorides of basic peptides extracted from the sperm or roe of fish, usually species of *Salmonidae* and *Clupeidae*. It binds with heparin in solution, inhibiting its anticoagulant activity; in the conditions of the assay this binding gives rise to a precipitate. Calculated with reference to the dried substance, 1 mg of protamine hydrochloride precipitates not less than 100 IU of heparin.

PRODUCTION

Protamine hydrochloride is prepared in conditions designed to minimise the risk of microbial contamination.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test:

Abnormal toxicity (*2.6.9*)

Inject into each mouse 0.5 mg dissolved in 0.5 ml of *water for injections R*.

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Soluble in water, practically insoluble in alcohol.

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): - 40 to - 60 (dried substance).

Dissolve 1.000 g in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same solvent.

B. In the conditions of the assay, protamine hydrochloride forms a precipitate.

C. To 0.5 ml of solution S (see Tests) add 4.5 ml of *water R*, 1.0 ml of a 100 g/l solution of *sodium hydroxide R* and 1.0 ml of a 0.2 g/l solution of α-*naphthol R* and mix. Cool the mixture to 5 °C. Add 0.5 ml of *sodium hypobromite solution R*. An intense red colour is produced.

D. Heat 2 ml of solution S in a water-bath at 60 °C and add 0.1 ml of *mercuric sulphate solution R*. Mix. No precipitate is formed. Cool the mixture in iced water. A precipitate is formed.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.50 g in *water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution BY_6 or Y_6 (*2.2.2, Method II*).

To 2.5 ml of solution S add 7.5 ml of *water R*.

Absorbance (*2.2.25*)

Maximum 0.1 between wavelengths of 260 nm to 280 nm.

Dilute 2.5 ml of solution S to 5.0 ml with *water R*.

Chloride

12.3 per cent to 19.0 per cent (dried substance).

Dissolve 0.400 g in 50 ml of *water R*. Add 5 ml of dilute nitric acid R, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R* and shake. Titrate with *0.1 M ammonium thiocyanate* using 2 ml of *ferric ammonium sulphate solution R2* as indicator; shake vigorously when approaching the end-point.

1 ml of *0.1 M silver nitrate* is equivalent to 3.545 mg of chloride (Cl).

Sulphates

Maximum 4.0 per cent (dried substance).

Dissolve 0.500 g in 200 ml of *distilled water R*, add 5.0 ml of *dilute hydrochloric acid R* and heat to boiling. Add dropwise 10 ml of a hot 100 g/l solution of *barium chloride R* while stirring with a glass rod, cover the beaker with a watch glass and allow to stand on a water-bath for 2 h to obtain a coarse granular precipitate. Add 0.1 ml of a 100 g/l solution of *barium chloride R* to the clear supernatant liquid. If a turbidity develops, repeat the precipitation procedure. Transfer the precipitate quantitatively to a previously ignited and tared porcelain crucible and wash with hot *distilled water R* until the addition of *silver nitrate solution R1* to the washings produces no opalescence. Ignite the precipitate at 600 ± 50 °C for 1 h. Allow to cool in a desiccator and weigh.

1 mg of residue is equivalent to 0.412 mg of sulphate (SO_4).

Barium

Maximum 10.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 1.0 g of the substance to be examined in *distilled water R*, add 1 ml of a 250 g/l solution of *caesium chloride R* and 0.2 ml of *hydrochloric acid R* and dilute to 20.0 ml with *distilled water R*.

Reference solution To 1.0 ml of *barium standard solution (50 ppm Ba) R* add 5 ml of a 250 g/l solution of *caesium chloride R* and 1 ml of *hydrochloric acid R* and dilute to 100.0 ml with *distilled water R*.

Source Hollow-cathode lamp.

Wavelength 553.3 nm.

Flame Air-acetylene-nitrous oxide flame of suitable composition.

Iron (*2.4.9*)

Maximum 10 ppm.

Dissolve 1.0 g with heating in *water R* and dilute to 10 ml with the same solvent.

Mercury

Maximum 10 ppm.

Introduce 2.0 g of the substance to be examined into a ground-glass-stoppered 250 ml conical flask and add 20 ml of a mixture of equal volumes of *nitric acid R* and *sulphuric acid R*. Boil under a reflux condenser for 1 h, cool and cautiously dilute with *water R*. Boil until nitrous fumes are no longer seen. Cool the solution, cautiously dilute to 200.0 ml with *water R*, mix and filter. Transfer 50.0 ml of the filtrate to a separating funnel. Shake with successive small portions of *chloroform R* until the chloroform layer remains colourless. Discard the chloroform layers. To the aqueous layer add 25 ml of *dilute sulphuric acid R*, 115 ml of *water R* and 10 ml of a 200 g/l solution of *hydroxylamine hydrochloride R*. Titrate with *dithizone solution R2*; after each addition, shake the mixture 20 times and towards the end of the titration allow to separate and discard the chloroform layer. Titrate until a bluish-green colour is obtained. Calculate the content of mercury using the equivalent in micrograms of mercury per millilitre of titrant determined in the standardisation of the *dithizone solution R2*.

Nitrogen

23.0 per cent to 27.0 per cent (dried substance).

Carry out the determination of nitrogen by sulphuric acid digestion (*2.5.9*), using 10.0 mg and heating for 3-4 h.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Bacterial endotoxins (*2.6.14*)

Less than 7.0 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Test solution (a) Dissolve 15.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Test solution (b) Dilute 2.0 ml of test solution (a) to 3.0 ml with *water R*.

Test solution (c) Dilute 1.0 ml of test solution (a) to 3.0 ml with *water R*.

Use as titrant a 1 in 6 dilution of *heparin sodium BRP* in *water R* (for example, 1.7 ml diluted to 10.0 ml with *water R*). Titrate each test solution in duplicate as follows: introduce an accurately measured volume of the solution to be titrated, for example, 1.5 ml, into the cell of a suitable colorimeter, set the apparatus for measurement at a suitable wavelength (none is critical) in the visible range, add the titrant in small volumes until there is a sharp increase in the absorbance and note the volume of titrant added.

Carry out 3 independent assays. For each individual titration, calculate the number of International Units of heparin in the volume of titrant added at the end-point per milligram of the substance to be examined. Calculate the potency of the substance as the average of the 18 values. Test the linearity of the response by the usual statistical methods. Calculate the 3 standard deviations for the results obtained with each of the 3 test solutions. Calculate the three standard deviations for the results obtained with each of the 3 independent assays. The assay is not valid unless each of the 6 standard deviations is less than 5 per cent of the average result.

STORAGE

In an airtight, tamper-proof container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

Ph Eur

Protamine Sulphate

General Notices

(Ph Eur monograph 0569)

9009-65-8

Action and use

Antidote to heparin.

Preparation

Protamine Sulphate Injection

Ph Eur

DEFINITION

Protamine sulphate consists of the sulphates of basic peptides extracted from the sperm or roe of fish, usually species of *Salmonidae* and *Clupeidae*. It binds with heparin in solution, inhibiting its anticoagulant activity; in the conditions of the assay this binding gives rise to a precipitate. Calculated with reference to the dried substance, 1 mg of protamine sulphate precipitates not less than 100 IU of heparin.

PRODUCTION

Protamine sulphate is prepared in conditions designed to minimise the risk of microbial contamination.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test:

Abnormal toxicity (*2.6.9*)

Inject into each mouse 0.5 mg dissolved in 0.5 ml of *water for injections R*.

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Sparingly soluble in water, practically insoluble in alcohol.

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): - 65 to - 85 (dried substance).

Dissolve 1.000 g in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same solvent.

B. In the conditions of the assay, protamine sulphate forms a precipitate.

C. To 0.5 ml of solution S (see Tests) add 4.5 ml of *water R*, 1.0 ml of a 100 g/l solution of *sodium hydroxide R* and 1.0 ml of a 0.2 g/l solution of α-*naphthol R* and mix. Cool the mixture to 5 °C. Add 0.5 ml of *sodium hypobromite solution R*. An intense red colour is produced.

D. Heat 2 ml of solution S in a water-bath at 60 °C and add 0.1 ml of *mercuric sulphate solution R*. Mix. No precipitate is formed. Cool the mixture in iced water. A precipitate is formed.

E. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

Solution S

Dissolve 0.20 g in *water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution BY_6 or Y_6 (*2.2.2, Method II*).

To 2.5 ml of solution S add 7.5 ml of *water R*.

Absorbance (*2.2.25*)

Maximum 0.1 between wavelengths of 260 nm to 280 nm.

Dilute 2.5 ml of solution S to 5.0 ml with *water R*.

Sulphate

16 per cent to 24 per cent (dried substance).

Dissolve 0.150 g in 15 ml of *distilled water R* in a beaker. Add 5 ml of *dilute hydrochloric acid R*. Heat to boiling and slowly add to the boiling solution 10 ml of a 100 g/l solution of *barium chloride R*. Cover the beaker and heat on a water-bath for 1 h. Filter. Wash the precipitate several times with small quantities of hot *water R*. Dry and ignite the residue at 600 ± 50 °C to constant mass.

1.0 g of residue is equivalent to 0.4117 g of sulphate (SO_4).

Iron (*2.4.9*)

Maximum 10 ppm.

Dissolve 1.0 g with heating in *water R* and dilute to 10 ml with the same solvent.

Mercury

Maximum 10 ppm.

Introduce 2.0 g of the substance to be examined into a 250 ml ground-glass-stoppered conical flask and add 20 ml of a mixture of equal volumes of *nitric acid R* and *sulphuric acid R*. Boil under a reflux condenser for 1 h, cool and cautiously dilute with *water R*. Boil until nitrous fumes are no longer seen. Cool the solution, cautiously dilute to 200.0 ml with *water R*, mix and filter. Transfer 50.0 ml of the filtrate to a separating funnel. Shake with successive small portions of *chloroform R* until the chloroform layer remains colourless. Discard the chloroform layers. To the aqueous layer add 25 ml of *dilute sulphuric acid R*, 115 ml of *water R* and 10 ml of a 200 g/l solution of *hydroxylamine hydrochloride R*. Titrate with *dithizone solution R2*; after each addition, shake the mixture 20 times and towards the end of the titration allow to separate and discard the chloroform layer. Titrate until a bluish-green colour is obtained. Calculate the content of mercury using the equivalent in micrograms of mercury per millilitre of titrant, determined in the standardisation of the *dithizone solution R2*.

Nitrogen

21.0 per cent to 26.0 per cent (dried substance).

Carry out the determination of nitrogen by sulphuric acid digestion (*2.5.9*), using 10.0 mg and heating for 3-4 h.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Bacterial endotoxins (*2.6.14*)

Less than 7.0 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Test solution (a) Dissolve 15.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Test solution (b) Dilute 2.0 ml of test solution (a) to 3.0 ml with *water R*.

Test solution (c) Dilute 1.0 ml of test solution (a) to 3.0 ml with *water R*.

Use as titrant a 1 to 6 dilution of *heparin sodium BRP* in *water R* (for example, 1.7 ml diluted to 10.0 ml with *water R*). Titrate each test solution in duplicate as follows: introduce an accurately measured volume of the solution to be titrated, for example 1.5 ml, into the cell of a suitable colorimeter and set the apparatus for measurement at a suitable wavelength (none is critical) in the visible range. Add the titrant in small volumes until there is a sharp increase in the absorbance and note the volume of titrant added.

Carry out 3 independent assays. For each individual titration, calculate the number of International Units of heparin in the volume of titrant added at the end-point per milligram of the substance to be examined. Calculate the potency of the substance as the average of the 18 values. Test the linearity of the response by the usual statistical methods. Calculate the 3 standard deviations for the results obtained with each of the 3 test solutions. Calculate the 3 standard deviations for the results obtained with each of the 3 independent assays. The assay is not valid unless each of the 6 standard deviations is less than 5 per cent of the average result.

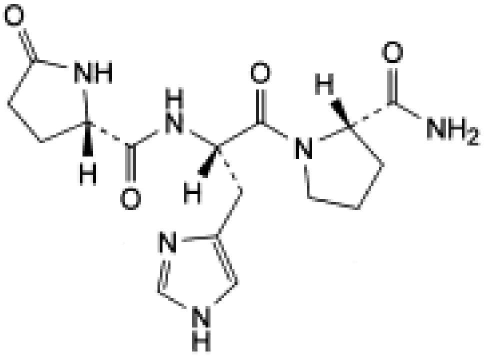
STORAGE

In an airtight, tamper-proof container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

Protirelin

General Notices

(Ph Eur monograph 1144)



$C_{16}H_{22}N_6O_4$ 362.4 *24305-27-9*

Action and use

Thyrotrophin-releasing hormone.

Ph Eur

DEFINITION

5-Oxo-L-prolyl-L-histidyl-L-prolinamide.

Synthetic tripeptide with the same sequence of amino acids as the natural hypothalamic neurohormone, which stimulates the release and synthesis of thyrotropin.

Content

97.0 per cent to 102.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance

White or yellowish-white powder, hygroscopic.

Solubility

Very soluble in water, freely soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison protirelin CRS.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

A 10 g/l solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

- 62 to - 70 (anhydrous and acetic acid-free substance).

Dissolve 10 mg in 1.0 ml of *water R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 5.0 mg of the substance to be examined in mobile phase A and dilute to 5.0 ml with mobile phase A.

Reference solution (a) Dissolve the contents of a vial of *D-His-protirelin CRS* in an appropriate volume of mobile phase A to obtain a concentration of 1 mg/ml. Mix equal volumes of this solution and the test solution.

Reference solution (b) Dilute 0.2 ml of the test solution to 10.0 ml with mobile phase A.

Column:

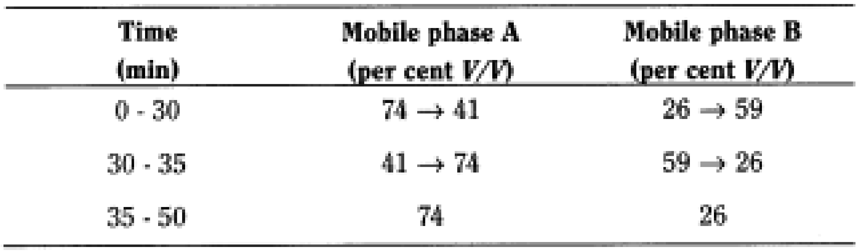
— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 12 nm.

Mobile phase:

— *mobile phase A*: a mixture of 100 ml of *acetonitrile for chromatography R*, 1900 ml of *water R* and 2.0 g of *sodium octanesulphonate R*, containing 2.5 ml/l of *tetraethylammonium hydroxide solution R*; adjust to pH 3.5 with *phosphoric acid R*;

— *mobile phase B*: a mixture of 300 ml of *acetonitrile for chromatography R*, 1700 ml of *water R* and 2.0 g of *sodium octanesulphonate R*, containing 2.5 ml/l of *tetraethylammonium hydroxide solution R*; adjust to pH 3.5 with *phosphoric acid R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	74 → 41	26 → 59
30 - 35	41 → 74	59 → 26
35 - 50	74	26

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl.

Relative retention With reference to protirelin (retention time = about 18 min): impurity C = about 0.2; impurity D = about 0.68; impurity A = about 0.91; impurity B = about 0.95; impurity E = about 1.08.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to impurity A and protirelin;

— *symmetry factor*: 0.9 to 1.2 for the peak due to protirelin.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Acetic acid (*2.5.34*)

Maximum 2.0 per cent.

Test solution. Dissolve 40.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of solvents.

Water (*2.5.12*)

Maximum 7.0 per cent, determined on 0.200 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.7 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Reference solution Dissolve the contents of a vial of *protirelin CRS* in an appropriate volume of mobile phase A to obtain a concentration of 1.0 mg/ml.

Calculate the content of protirelin ($C_{16}H_{22}N_6O_4$) using the peak areas of the chromatograms obtained with the test solution and the reference solution and the declared content of $C_{16}H_{22}N_6O_4$ in *protirelin CRS*.

STORAGE

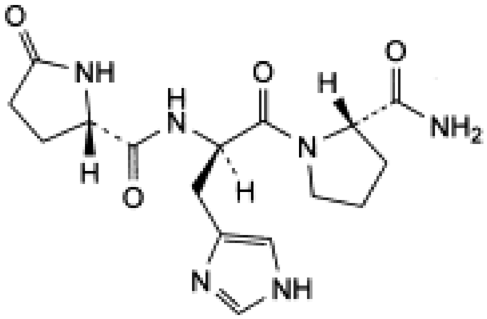
In an airtight container, protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

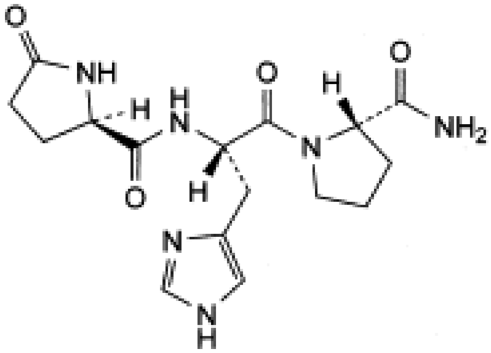
The label states the mass of peptide in the container.

IMPURITIES

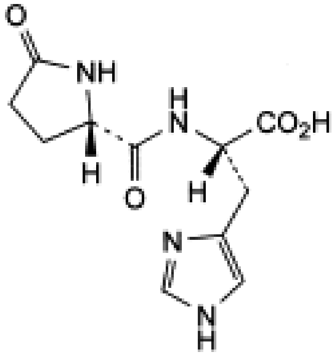
Specified impurities   A, B, C, D, E.



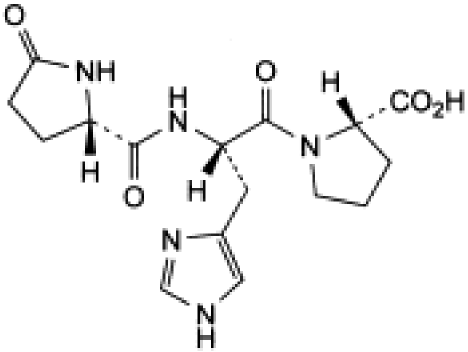
A. 5-oxo-L-prolyl-D-histidyl-L-prolinamide,



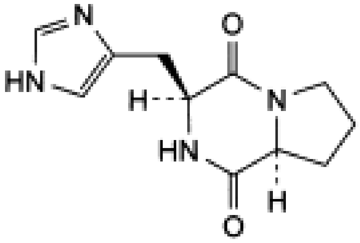
B. 5-oxo-D-prolyl-L-histidyl-L-prolinamide,



C. 5-oxo-L-prolyl-L-histidine,



D. 5-oxo-L-prolyl-L-histidyl-L-proline,

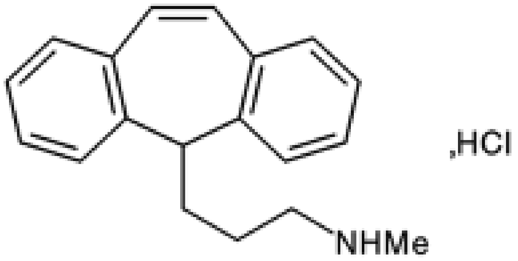


E. (3*S*,8a*S*)-3-(1*H*-imidazol-4-ylmethyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (cyclo(-L-histidyl-L-prolyl-)).

Ph Eur

Protriptyline Hydrochloride

General Notices



$C_{19}H_{21}N,HCl$ 299.8   *1225-55-4*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Protriptyline Tablets

DEFINITION

Protriptyline Hydrochloride is 3-(5*H*-dibenzo[*a,d*]cyclohept-5-yl)propyl(methyl)amine hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{19}H_{21}N,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to yellowish white powder.

Freely soluble in *water* and in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. Dissolve 0.1 g in 10 ml of *water*, make alkaline with 1M *sodium hydroxide*, extract with 5 ml of *chloroform*, dry with *anhydrous sodium sulphate* and evaporate the solvent using a current of nitrogen. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of protriptyline *(RS 301)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 5.0 to 6.5, Appendix V L.

Loss on drying

When dried to constant weight at 60° at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

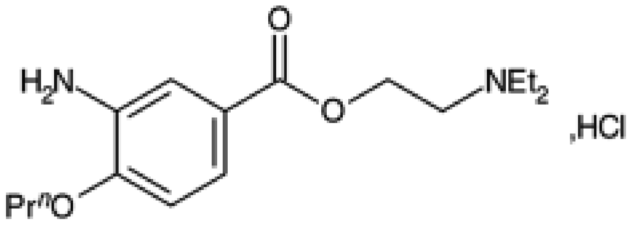
Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.7 g and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 29.98 mg of $C_{19}H_{21}N,HCl$.

Proxymetacaine Hydrochloride

General Notices



$C_{16}H_{26}N_2O_3$,HCl 330.9 *5875-06-9*

Action and use

Local anaesthetic.

Preparation

Proxymetacaine Eye Drops

DEFINITION

Proxymetacaine Hydrochloride is 2-diethylaminoethyl 3-amino-4-propoxybenzoate hydrochloride. It contains not less than 98.0% and not more than 102.0% of $C_{16}H_{26}N_2O_3$,HCl, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Soluble in *water*; very soluble in *absolute ethanol*; practically insoluble in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a 0.002% w/v solution exhibits three maxima, at 231, 268 and 310 nm. The *absorbances* at the maxima at 268 nm and at 310 nm are about 0.58 and about 0.32, respectively.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of proxymetacaine hydrochloride *(RS 303)*.

C. A 5% w/v solution yields the reaction characteristic of *primary aromatic amines* and the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 5.7 to 6.4, Appendix V L.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 75 volumes of *toluene*, 30 volumes of *ethyl acetate* and 5 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 10 µl of each of three solutions of the substance being examined in *methanol* containing (1) 2.0% w/v, (2) 0.020% w/v and (3) 0.010% w/v. After removal of the plate, heat it at 105° for 10 minutes, allow to cool and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Disregard any spot remaining on the line of application.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 80 volumes of *1,4-dioxan*, 20 volumes of *cyclohexane* and 4 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in *methanol* containing (1) 2.0% w/v of the substance being examined and (2) 0.0050% w/v of *3-amino-4-propoxybenzoic acid BPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%). The principal spot remains on or near the line of application.

Loss on drying

When dried at 105° for 3 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.15%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 g, 20 ml of *mercury (II) acetate solution* and *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 16.54 mg of $C_{16}H_{26}N_2O_3,HCl$.

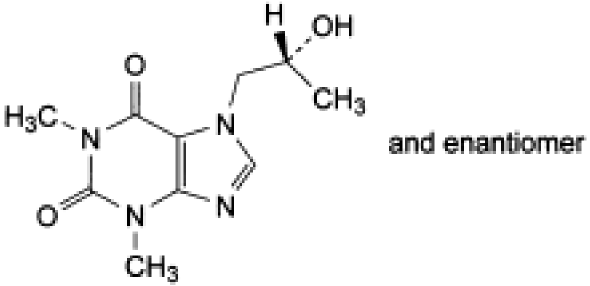
STORAGE

Proxymetacaine Hydrochloride should be protected from light.

Proxyphylline

General Notices

(Ph Eur monograph 0526)



$C_{10}H_{14}N_4O_3$ 238.2   *603-00-9*

Action and use

Non selective phospho-diesterase inhibitor; treatment of reversible airways obstruction.

Ph Eur

DEFINITION

Proxyphylline contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7-[(2*RS*)-2-hydroxypropyl]-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very soluble in water, soluble in alcohol.

IDENTIFICATION

First identification *B, C.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)*: 134 °C to 136 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *proxyphylline CRS*. Examine the substances as discs prepared using 0.5 mg to 1 mg of the substance to be examined in 0.3 g of *potassium bromide R.*

C. Dissolve 1 g in 5 ml of *acetic anhydride R* and boil under a reflux condenser for 15 min. Allow to cool and add 100 ml of a mixture of 20 volumes of *ether R* and 80 volumes of *light petroleum R*. Cool in iced water for at least 20 min, shaking from time to time. Filter, wash the precipitate with a mixture of 20 volumes of *ether R* and 80 volumes of *light petroleum R*, recrystallise from *alcohol R* and dry *in vacuo*. The crystals melt *(2.2.14)* at 87 °C to 92 °C.

D. It gives the reaction of xanthines *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.25 ml of *bromothymol blue solution R1.* The solution is yellow or green. Not more than 0.4 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* HF_{254} *R* as the coating substance.

Test solution Dissolve 0.3 g of the substance to be examined in a mixture of 20 volumes of *water R* and 30 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents. Prepare immediately before use.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *methanol R*.

Reference solution (b) Dilute 0.2 ml of the test solution to 100 ml with *methanol R*.

Reference solution (c) Dissolve 10 mg of *theophylline R* in *methanol R*, add 0.3 ml of the test solution and dilute to 10 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 10 volumes of *ethanol R* and 90 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

Dilute 2.5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (400 ppm).

Heavy metals *(2.4.8)*

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.200 g in 3.0 ml of *anhydrous formic acid R* and add 50.0 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 23.82 mg of $C_{10}H_{14}N_4O_3$.

STORAGE

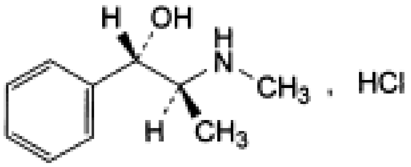
Store protected from light.

Ph Eur

Pseudoephedrine Hydrochloride

General Notices

(Ph Eur monograph 1367)



$C_{10}H_{15}NO,HCl$ 201.7 *345-78-8*

Action and use

Adrenoceptor agonist.

Preparations

Pseudoephedrine Oral Solution

Pseudoephedrine Tablets

Ph Eur

DEFINITION

(1*S*,2*S*)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water and in ethanol (96 per cent), sparingly soluble in methylene chloride.

mp : About 184 °C.

IDENTIFICATION

First identification A, B, D.

Second identification *A, C, D.*

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *pseudoephedrine hydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *pseudoephedrine hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *ephedrine hydrochloride CRS* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate *TLC silica gel plate R*.

Mobile phase *methylene chloride R, concentrated ammonia R, 2-propanol R* (5:15:80 *V/V/V*) .

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 110 °C for 5 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Dilute 2 ml of solution S to 10 ml with *carbon dioxide-free water R*. Add 0.1 ml of *methyl red solution R* and 0.1 ml of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.2 ml of *0.01 M hydrochloric acid* ; the solution is red.

Specific optical rotation *(2.2.7)*

+ 61.0 to + 62.5 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *ephedrine hydrochloride CRS* (impurity A) in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (c) Dissolve 10 mg of *ephedrine hydrochloride CRS* (impurity A) in 5 ml of the test solution and dilute to 100 ml with the mobile phase.

Column:
- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: phenylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 6 volumes of *methanol R* and 94 volumes of an 11.6 g/l solution of *ammonium acetate R* previously adjusted to pH 4.0 with *glacial acetic acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 257 nm.

Injection 20 µl.

Run time 1.5 times the retention time of pseudoephedrine.

Relative retention With reference to pseudoephedrine (retention time = about 18 min): impurity A = about 0.9.

System suitability Reference solution (c):

- *resolution*: minimum 2.0 between the peaks due to impurity A and pseudoephedrine; if necessary, reduce the content of methanol in the mobile phase.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *sum of impurities other than A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.170 g in 30 ml of *ethanol (96 per cent) R*. Add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 20.17 mg of $C_{10}H_{16}ClNO$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A.

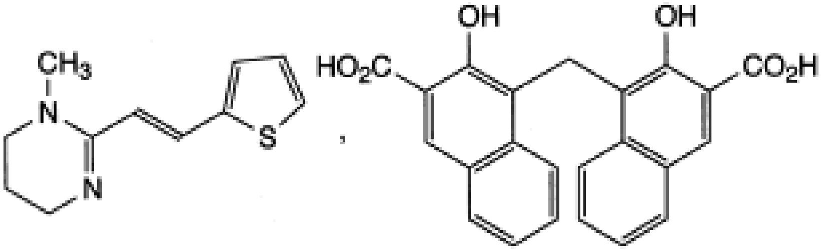
A. ephedrine.

Ph Eur

Pyrantel Embonate

General Notices

(Ph Eur monograph 1680)



$C_{34}H_{30}N_2O_6S$ 594.7 *22204-24-6*

Action and use

Anthelminthic.

Ph Eur

DEFINITION

1-Methyl-2-[(*E*)-2-(thiophen-2-yl)ethenyl]-1,4,5,6-tetrahydropyrimidine hydrogen 4,4′-methylenebis(3-hydroxynaphthalene-2-carboxylate).

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Pale yellow or yellow powder.

Solubility

Pratically insoluble in water, soluble in dimethyl sulphoxide, pratically insoluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison pyrantel embonate CRS.

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and strictly protect from light at all stages.*

Solvent mixture Mix 5 volumes of *glacial acetic R* with 5 volumes of *water R* and add 2 volumes of *diethylamine R* with cooling.

Test solution Dissolve 80 mg in 7 ml of the solvent mixture and dilute to 100.0 ml with *acetonitrile R*.

Reference solution (a) Dissolve 10.0 mg of *pyrantel impurity A CRS* in the solvent mixture, add 2.5 ml of the test solution and dilute to 50.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase Solvent mixture, *acetonitrile for chromatography R* (72:928 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 288 nm.

Injection 20 µl.

Run time 4 times the retention time of pyrantel.

Relative retention With reference to pyrantel (retention time = about 11 min): embonic acid = about 0.5; impurity A = about 1.3; impurity B = about 1.8 (impurity A also gives rise to an embonate peak).

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to pyrantel and impurity A.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 0.4;

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity B*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *sum of impurities other than A and B*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (*2.4.4*)

Maximum 360 ppm.

To 0.46 g add 10 ml of *dilute nitric acid R* and 30 ml of *water R*. Heat on a water-bath for 5 min. Cool, dilute to 50 ml with *water R*, mix well and filter. 15 ml complies with the limit test for chlorides.

Sulphates (*2.4.13*)

Maximum 0.1 per cent.

To 0.50 g add 2.5 ml of *dilute nitric acid R* and dilute to 50 ml with *distilled water R*. Heat on a water-bath for 5 min, shake for 2 min, cool and filter. 15 ml complies with the limit test for sulphates.

Iron (*2.4.9*)

Maximum 75 ppm.

Ignite 0.66 g at 800 ± 50 °C for 2 h. Dissolve the residue in 2.5 ml of *dilute hydrochloric acid R* with gentle heating for 10 min. Cool and dilute to 50 ml with *water R*. 10 ml complies with the limit test for iron.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with limit test D. Prepare the reference solution using 2.0 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 0.450 g add 10 ml of *acetic anhydride R* and 50 ml *glacial acetic acid R*, heat at 50 °C and stir for 10 min. Allow to cool (a clear solution is not obtained). Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

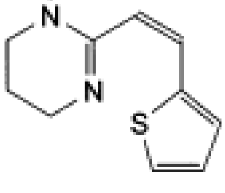
1 ml of *0.1 M perchloric acid* is equivalent to 59.47 mg of $C_{34}H_{30}N_2O_6S$.

STORAGE

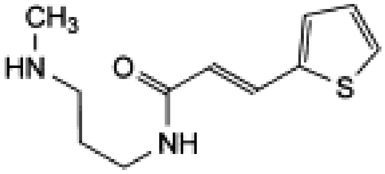
Protected from light.

IMPURITIES

Specified impurities A, B.



A. 1-methyl-2-[(*Z*)-2-(thiophen-2-yl)ethenyl]-1,4,5,6-tetrahydropyrimidine,



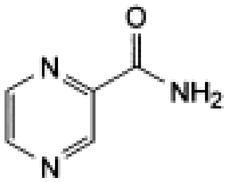
B. (*E*)-*N*-[3-(methylamino)propyl]-3-(thiophen-2-yl)prop-2-enamide.

Ph Eur

Pyrazinamide

General Notices

(Ph Eur monograph 0859)



$C_5H_5N_3O$ 123.1 *98-96-4*

Action and use

Antituberculosis drug.

Preparation

Pyrazinamide Tablets

Ph Eur

DEFINITION

Pyrazinamide contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of pyrazine-2-carboxamide, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, sparingly soluble in water, slightly soluble in alcohol and in methylene chloride.

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Melting point (*2.2.14*): 188 °C to 191 °C.

B. Dissolve 50.0 mg in *water R* and dilute to 100.0 ml with the same solvent (solution (a)). Dilute 1.0 ml of solution (a) to 10.0 ml with *water R*. Examined between 290 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 310 nm. Dilute 2.0 ml of solution (a) to 100.0 ml with *water R*. Examined between 230 nm and 290 nm, the solution shows an absorption maximum at 268 nm. The specific absorbance at the maximum is 640 to 680.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *pyrazinamide CRS*. Examine the substances prepared as discs. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *alcohol R*, evaporate to dryness and record new spectra using the residues.

D. Dissolve 0.1 g in 5 ml of *water R*. Add 1 ml of *ferrous sulphate solution R2*. The solution becomes orange. Add 1 ml of *dilute sodium hydroxide solution R*. The solution becomes dark blue.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 25 ml of solution S add 0.05 ml of *phenolphthalein solution R1* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is red. Add 1.0 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.15 ml of *methyl red solution R*. The solution is red.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a) Dilute 1 ml of the test solution to 50 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*. Dilute 1 ml of the solution to 10 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*.

Reference solution (b) Dissolve 10 mg of *nicotinic acid CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R*, add 1 ml of the test solution and dilute to 10 ml with the same mixture of solvents.

Apply separately to the plate 20 µl of each solution. Develop over a path of 10 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air and examine immediately in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

Heavy metals (*2.4.8*)

1.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*)

Not more than 0.5 per cent, determined on 2.00 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*)

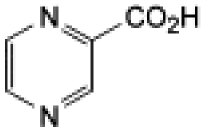
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 12.31 mg of $C_5H_5N_3O$.

IMPURITIES



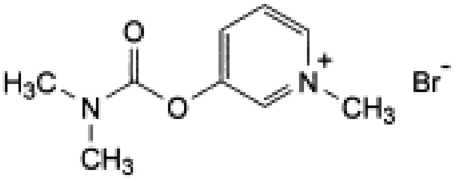
A. pyrazine-2-carboxylic acid.

Ph Eur

Pyridostigmine Bromide

General Notices

(Ph Eur monograph 1255)



$C_9H_{13}BrN_2O_2$ 261.1 *101-26-8*

Action and use

Cholinesterase inhibitor.

Preparation

Pyridostigmine Tablets

Ph Eur

DEFINITION

3-(Dimethylcarbamoyloxy)-1-methylpyridinium bromide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline, deliquescent powder.

Solubility

Very soluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *pyridostigmine bromide CRS.*

B. It gives reaction (a) of bromides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 40 ml of solution S add a few drops of *methyl red solution R*. To 20 ml of this solution add 0.2 ml of *0.02 M sodium hydroxide*. The solution is yellow. To the other 20 ml add 0.2 ml of *0.02 M hydrochloric acid*. The solution is red.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase at about 40 °C. Allow to cool and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 4 mg of *pyridostigmine impurity A CRS* and 4 mg of *pyridostigmine bromide CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Mix 30 volumes of *acetonitrile R* and 70 volumes of a 4.33 g/l solution of *sodium dodecyl sulphate R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 1.1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time Twice the retention time of pyridostigmine.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to pyridostigmine and impurity A.

Limits:

— *impurities A, B*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), at most one such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and at most one further peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.230 g in 10 ml of *anhydrous acetic acid R*. Add 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

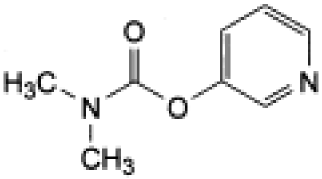
1 ml of *0.1 M perchloric acid* is equivalent to 26.11 mg of $C_9H_{13}BrN_2O_2$.

STORAGE

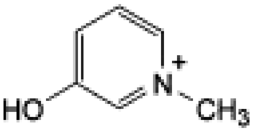
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container, protected from light.

IMPURITIES

Specified impurities A, B.



A. pyridin-3-yl dimethylcarbamate,



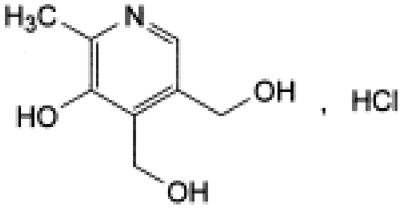
B. 3-hydroxy-1-methylpyridinium.

Ph Eur

Pyridoxine Hydrochloride

General Notices

(Ph Eur monograph 0245)



$C_8H_{11}NO_3,HCl$ 205.6 *58-56-0*

Action and use

Vitamin B_6.

Preparations

Pyridoxine Tablets

Vitamins B and C Injection

When vitamin B6 is prescribed or demanded, Pyridoxine Hydrochloride shall be dispensed or supplied.

Ph Eur

DEFINITION

Pyridoxine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (5-hydroxy-6-methylpyridine-3,4-diyl)dimethanol hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water, slightly soluble in alcohol.

It melts at about 205 °C, with decomposition.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dilute 1.0 ml of solution S (see Tests) to 50.0 ml with *0.1 M hydrochloric acid* (solution A) . Dilute 1.0 ml of solution A to 100.0 ml with *0.1 M hydrochloric acid*. Examined between

250 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 288 nm to 296 nm. The specific absorbance at the maximum is 425 to 445. Dilute 1.0 ml of solution A to 100.0 ml with a mixture of equal volumes of *0.025 M potassium dihydrogen phosphate solution* and *0.025 M disodium hydrogen phosphate* solution (*2.2.3*). Examined between 220 nm and 350 nm, the solution shows 2 absorption maxima, at 248 nm to 256 nm and at 320 nm to 327 nm. The specific absorbances at the maxima are 175 to 195 and 345 to 365, respectively.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *pyridoxine hydrochloride CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Solution S gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 2.4 to 3.0.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel G plate R*.

Test solution (a) Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *water R.*

Reference solution (a) Dissolve 0.10 g of *pyridoxine hydrochloride CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 2.5 ml of test solution (a) to 100 ml with *water R*. Dilute 1 ml of this solution to 10 ml with *water R*.

Apply to the plate 2 µl of each solution. Develop in an unsaturated tank over a path of 15 cm using a mixture of 9 volumes of *concentrated ammonia R*, 13 volumes of *methylene chloride R*, 13 volumes of *tetrahydrofuran R* and 65 volumes of *acetone R*. Allow the plate to dry in air and spray with a 50 g/l solution of *sodium carbonate R* in a mixture of 30 volumes of *alcohol R* and 70 volumes of *water R*. Dry the plate in a current of air, spray with a 1 g/l solution of *dichloroquinonechlorimide R* in *alcohol R* and examine the chromatograms immediately. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent). Disregard any spots remaining on the starting line.

Heavy metals (*2.4.8*)

12 ml of solution S complies with limit test A (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

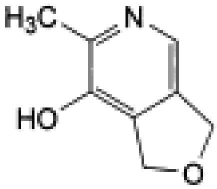
Dissolve 0.150 g in 5 ml of *anhydrous formic acid R*. Add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 20.56 mg of $C_8H_{12}ClNO_3$.

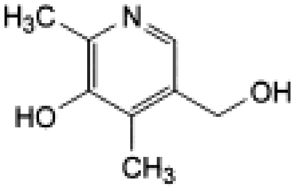
STORAGE

Store protected from light.

IMPURITIES



A. 6-methyl-1,3-dihydrofuro[3,4-*c*]pyridin-7-ol,



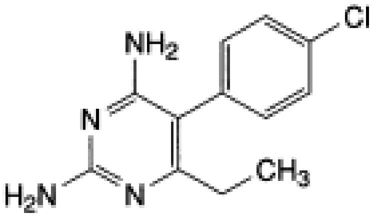
B. 5-(hydroxymethyl)-2,4-dimethylpyridin-3-ol.

Ph Eur

Pyrimethamine

General Notices

(Ph Eur monograph 0288)



$C_{12}H_{13}ClN_4$ 248.7 *58-14-0*

Action and use

Dihydrofolate reductase inhibitor; antiprotozoal (malaria).

Preparation

Pyrimethamine Tablets

Ph Eur

DEFINITION

Pyrimethamine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine, calculated with reference to the dried substance.

CHARACTERS

An almost white, crystalline powder or colourless crystals, practically insoluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification C.

Second identification A, B, D.

A. Melting point (*2.2.14*): 239 °C to 243 °C.

B. Dissolve 0.14 g in *ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*. Dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*. Examined between 250 nm and 300 nm (*2.2.25*), the solution shows an absorption maximum at 272 nm and an absorption minimum at 261 nm. The specific absorbance at the maximum is 310 to 330.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *pyrimethamine CRS*.

D. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Shake 1.0 g with 50 ml of *distilled water R* for 2 min and filter.

Appearance of solution

Prepare the solution immediately before use.

Dissolve 0.25 g in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink. Add 0.4 ml of *0.01 M hydrochloric acid* and 0.05 ml of *methyl red solution R*. The solution is red or orange.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance. *Prepare the solutions immediately before use.*

Test solution (a) Dissolve 0.25 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R* and dilute to 25 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R*.

Reference solution (a) Dissolve 0.1 g of *pyrimethamine CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (b) Dilute 2.5 ml of test solution (a) to 100 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R*. Dilute 1 ml of the solution to 10 ml with a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R*.

Apply to the plate 20 µl of each solution. Develop over a path of 10 cm using a mixture of 4 volumes of *chloroform R*, 8 volumes of *propanol R*, 12 volumes of *glacial acetic acid R* and 76 volumes of *toluene R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Sulphates (*2.4.13*)

15 ml of solution S complies with the limit test for sulphates (80 ppm). Prepare the standard using a mixture of 2.5 ml of *sulphate standard solution (10 ppm SO_4) R* and 12.5 ml of *distilled water R*.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 0.50 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 25 ml of *anhydrous acetic acid R*, heating gently. Cool. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 24.87 mg of $C_{12}H_{13}ClN_4$.

STORAGE

Store protected from light.

Ph Eur

Pyroxylin

General Notices

Cellulose Nitrate

Preparation

Flexible Collodion

DEFINITION

Pyroxylin is a nitrated cellulose obtained by the action of a mixture of nitric and sulphuric acids on wood pulp or cotton linters that have been freed from fatty matter. It must be damped with not less than 25% by weight of Isopropyl Alcohol or of Industrial Methylated Spirit.

CAUTION *Compliance is required with the provisions of relevant legislation relating to the storage of, use of and containers used for Pyroxylin*.

In the following tests, particular care should be exercised when drying pyroxylin. The material so obtained is explosive and sensitive to ignition by impact or friction and it should be handled as carefully as possible.

CHARACTERISTICS

White or almost white, cuboid granules or fibrous material, the latter resembling absorbent cotton but harsher to the touch and more powdery. Both the granules and the fibrous material appear moist. Highly flammable.

Soluble in *glacial acetic acid* and in *acetone*.

IDENTIFICATION

Add *water* to a solution in *acetone*. A white, viscid mass is readily precipitated.

TESTS

Clarity and colour of solution

A 10% w/v solution dissolves at 20° in a mixture of 1 volume of *ethanol (90%)* and 3 volumes of *ether* to produce an almost clear and colourless to pale straw-coloured solution.

Kinematic viscosity

1160 to 2900 mm^2s^{-1} when determined in the following manner. To 20 g, previously dried to constant weight by heating on a water-bath at 80° and allowing to cool in a desiccator over *silica gel*, add 200 ml of a mixture of 19 volumes of *acetone* and 1 volume of *water*. Shake vigorously to prevent the formation of aggregates of partly solvated pyroxylin and rotate suitably to obtain a homogeneous solution. Measure the viscosity of the solution within 48 hours using a falling sphere viscometer complying with British Standard 188: 1977 (Methods for the determination of viscosity of liquids). Fill the fall tube with the solution being examined to about 10 mm above the 220-mm mark, place vertically in the bath and allow to stand for air bubbles to clear and for temperature equilibrium to be attained. Clean the sphere, immerse it in a portion of the liquid being examined maintained at a temperature of 19.9° to 20.1° and when it is at this temperature introduce it, without wiping, into the delivery tube. Observe the time for the lowest part of the sphere to pass through the planes of the tops of the 175-mm mark and the 25-mm mark, using a telescope or other suitable device to avoid errors due to parallax. The average of three readings concordant to within 0.5% is taken as the time of fall. Calculate the kinematic viscosity (ν) in square millimetres per second (mm^2s^{-1}) from the expression:

$$\nu = \frac{d^2 g(\delta - \rho)}{0.18 v \rho} \times 0.867$$

where d = the diameter of the sphere in cm,

δ = density of the sphere in g cm^{-3},

ρ = density of the liquid being tested in g cm^{-3},

v = velocity of fall in cm s^{-1},

g = local acceleration due to gravity in cm s^{-2}.

Nitrogen

11.7 to 12.2%, calculated with reference to the material dried to constant weight by heating on a water-bath at 80° and allowing to cool in a desiccator over silica gel, when determined by the following method. Transfer 0.4 g of the dried substance to a 750 ml round-bottomed flask using 60 ml of *water*, add 20 ml of *hydrogen peroxide solution (20 vol)* and then add slowly, with shaking, 50 ml of a 30% w/w solution of *potassium hydroxide* and 5 ml of *ethanol (96%)*. Agitate slowly for 3 hours or until the substance being examined has dissolved. Add 3 g of *Devarda's alloy* and immediately connect the flask to the spray trap of an ammonia-distillation apparatus, the receiver of which contains 50 ml of 0.1M *hydrochloric acid VS* and 0.15 ml of *methyl red solution*. Allow the reaction to proceed until no further evolution of gas occurs and then heat the contents of the flask to boiling and distil carefully until 30 ml remains in the round-bottomed flask. Rinse the delivery tube into the receiver with *water* and titrate the combined distillate and rinsings with 0.1M *sodium hydroxide VS*. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of acid required to neutralise the ammonia formed. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 1.401 mg of nitrogen.

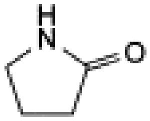
STORAGE

Pyroxylin should be loosely packed, protected from light and stored at a temperature not exceeding 15°, remote from fire. The container should be suitably designed to disrupt should the internal pressure reach or exceed 1400 kPa. The amount of damping fluid must not be allowed to fall below 25% w/w; should this happen, the material should be either rewetted or used immediately for the preparation of Collodion.

Pyrrolidone

General Notices

(Ph Eur monograph 2180)



C_4H_7NO 85.1 *616-45-5*

Ph Eur

DEFINITION

Pyrrolidin-2-one.

CHARACTERS

Appearance: clear, colourless or slightly greyish liquid, or white or almost white crystals, or colourless crystal needles.

Solubility

Miscible with water, with ethanol (96 per cent) and with most common organic solvents.

mp

About 25 °C; the molten substance remains liquid at temperatures below the melting point.

bp

About 245 °C.

IDENTIFICATION

First identification A.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison pyrrolidone CRS.

B. Relative density *(2.2.5)*: 1.112 to 1.115.

C. Refractive index *(2.2.6)*: 1.487 to 1.490.

TESTS

Use the molten substance for all tests.

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than intensity 7 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Alkalinity

To 100 ml of *water R* add 1.0 ml of *bromothymol blue solution R1* and adjust to a green colour with *0.02 M potassium hydroxide* or *0.02 M hydrochloric acid*. To 50 ml of this solution add 20 ml of the substance to be examined and titrate with *0.02 M hydrochloric acid* to the initial colour. Not more than 8.0 ml of *0.02 M hydrochloric acid* is required.

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution The substance to be examined.

Reference solution (a) Dissolve 1 ml of the substance to be examined and 1 ml of *N-methylpyrrolidone R* (impurity C) in *methylene chloride R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 1.1 g of the substance to be examined in *methylene chloride R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 20.0 ml with *methylene chloride R*.

Column:

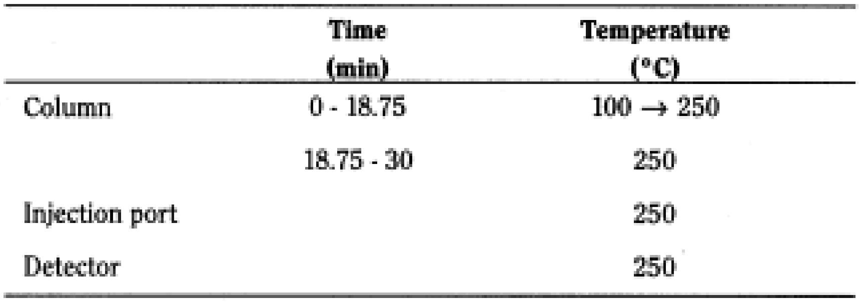
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 5 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 1.3 ml/min.

Split ratio 1:80.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 18.75	100 → 250
	18.75 - 30	250
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 0.1 µl.

Relative retention With reference to pyrrolidone (retention time = about 13 min): impurity B =

about 0.73; impurity A = about 0.76; impurity C = about 0.97.

System suitability   Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to pyrrolidone and impurity C.

Limits:

— *impurities A, B, C*: for each impurity, maximum 0.1 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.3 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 4.0 g in *water R* and dilute to 20.0 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Water *(2.5.32)*

Maximum 0.1 per cent, determined on 1.00 g.

Sulphated ash *(2.4.14)*

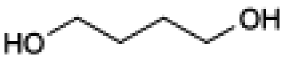
Maximum 0.1 per cent, determined on 1.0 g.

STORAGE

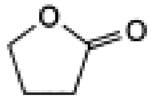
Protected from light.

IMPURITIES

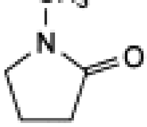
Specified impurities   A, B, C.



A. butane-1,4-diol,



B. dihydrofuran-2(3*H*)-one (γ-butyrolactone),



C. 1-methylpyrrolidin-2-one (*N*-methylpyrrolidone).

Ph Eur

White Soft Paraffin

General Notices

White Petroleum Jelly

(Ph Eur monograph 1799)

Ph Eur

DEFINITION

Purified and wholly or nearly decolorised mixture of semi-solid hydrocarbons, obtained from petroleum. It may contain a suitable antioxidant. White soft paraffin described in this monograph is not suitable for oral use.

CHARACTERS

Appearance

White or almost white, translucent, soft unctuous mass, slightly fluorescent in daylight when melted.

Solubility

Practically insoluble in water, slightly soluble in methylene chloride, practically insoluble in ethanol (96 per cent) and in glycerol.

IDENTIFICATION

First identification *A, B, D.*

Second identification *A, C, D.*

A. The drop point is between 35 °C and 70 °C and does not differ by more than 5 °C from the value stated on the label, according to method *(2.2.17)* with the following modification to fill the cup: heat the substance to be examined at a temperature not exceeding 80 °C, with stirring to ensure uniformity. Warm the metal cup at a temperature not exceeding 80 °C in an oven, remove it from the oven, place on a clean plate or ceramic tile and pour a sufficient quantity of the melted sample into the cup to fill it completely. Allow the filled cup to cool for 30 min on the plate or the ceramic tile and place it in a water bath at 24-26 °C for 30-40 min. Level the surface of the sample with a single stroke of a knife or razor blade, avoiding compression of the sample.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Place about 2 mg on a *sodium chloride R* plate, spread the substance with another *sodium chloride R* plate and remove 1 of the plates.

Comparison Repeat the operations using *white soft paraffin CRS*.

C. Melt 2 g and when a homogeneous phase is obtained, add 2 ml of *water R* and 0.2 ml of *0.05 M iodine*. Shake. Allow to cool. The solid upper layer is violet-pink or brown.

D. Appearance (see Tests).

TESTS

Appearance

The substance is white. Melt 12 g on a water-bath. The melted mass is not more intensely coloured than a mixture of 1 volume of yellow primary solution and 9 volumes of a 10 g/l solution of *hydrochloric acid R (2.2.2, Method II)*.

Acidity or alkalinity

To 10 g add 20 ml of boiling *water R* and shake vigorously for 1 min. Allow to cool and decant. To 10 ml of the aqueous layer add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to red.

Consistency *(2.9.9)*

60 to 300.

Polycyclic aromatic hydrocarbons

Maximum 300 ppm.

Use reagents for ultraviolet spectrophotometry. Dissolve 1.0 g in 50 ml of *hexane R* which has been previously shaken twice with 10 ml of *dimethyl sulphoxide R*. Transfer the solution to a 125 ml separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 20 ml of *dimethyl sulphoxide R*. Shake vigorously for 1 min and allow to stand until 2 clear layers are formed. Transfer the lower layer to a second separating funnel. Repeat the extraction with a further 20 ml of *dimethyl sulphoxide R*. Shake vigorously the combined lower layers with 20 ml of *hexane R* for 1 min. Allow to stand until 2 clear layers are formed. Separate the lower layer and dilute to 50.0 ml with *dimethyl sulphoxide R*. Measure the absorbance *(2.2.25)* over the range 260 nm to 420 nm using a path length of 4 cm and as compensation liquid the clear lower layer obtained by vigorously shaking 10 ml of *dimethyl sulphoxide R* with 25 ml of *hexane R* for 1 min. Prepare a reference solution in *dimethyl sulphoxide R* containing 6.0 mg of *naphthalene R* per litre and measure the absorbance of the solution at the maximum at 278 nm using a path length of 4 cm and *dimethyl sulphoxide R* as compensation liquid. At no wavelength in the range 260 nm to 420 nm does the absorbance of the test solution exceed that of the reference solution at 278 nm.

Sulphated ash *(2.4.14)*

Maximum 0.05 per cent, determined on 2.0 g.

STORAGE

Protected from light.

LABELLING

The label states the nominal drop point.

Ph Eur

Yellow Soft Paraffin

General Notices

Yellow Petroleum Jelly

(Ph Eur monograph 1554)

Action and use

Excipient.

Ph Eur

DEFINITION

Yellow soft paraffin is a purified mixture of semi-solid hydrocarbons, obtained from petroleum. It may contain a suitable antioxidant.

CHARACTERS

A yellow, translucent, unctuous mass, slightly fluorescent in daylight when melted, practically insoluble in water, slightly soluble in methylene chloride, practically insoluble in ethanol (96 per cent) and in glycerol.

IDENTIFICATION

First identification *A, B, D.*

Second identification *A, C, D.*

A. The drop point *(2.2.17)* is 40 °C to 60 °C and does not differ by more than 5 °C from the value stated on the label, with the following modification to fill the cup: heat the substance to be examined at 118-22 °C, with stirring to ensure uniformity, then cool to 100-107 °C. Warm the metal cup at 103-107 °C in an oven, remove it from the oven, place on a clean plate or ceramic tile and pour a sufficient quantity of the melted sample into the cup to fill it completely. Allow the filled cup to cool for 30 min on the ceramic tile and place it in a water-bath at 24-26 °C for a further 30-40 min. Level the surface of the sample with a single stroke of a knife or razor blade, avoiding compression of the sample.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*.

Preparation Place about 2 mg on a *sodium chloride R* plate, spread the substance with another *sodium chloride R* plate and remove 1 of the plates.

Comparison Repeat the operations using *yellow soft paraffin CRS*.

C. Melt 2 g and when a homogeneous phase is obtained, add 2 ml of *water R* and 0.2 ml of *0.05 M iodine*. Shake. Allow to cool. The solid upper layer is violet-pink or brown.

D. Appearance (see Tests).

TESTS

Appearance

The substance is yellow. Melt 12 g on a water-bath. The melted mass is not more intensely coloured than a mixture of 7.6 volumes of yellow primary solution and 2.4 volumes of red primary solution *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 g add 20 ml of boiling *water R* and shake vigorously for 1 min. Allow to cool and decant. To 10 ml of the aqueous layer add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to red.

Consistency *(2.9.9)*

The consistency is 100 to 300.

Polycyclic aromatic hydrocarbons

Use reagents for ultraviolet absorption spectrophotometry. Dissolve 1.0 g in 50 ml of *hexane R* which has been previously shaken twice with one-fifth its volume of *dimethyl sulphoxide R*. Transfer the solution to a 125 ml separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 20 ml of *dimethyl sulphoxide R*. Shake vigorously for 1 min and allow to stand until two clear layers are formed. Transfer the lower layer to a second separating funnel. Repeat the extraction with a further 20 ml of *dimethyl sulphoxide R*. Shake vigorously the combined lower layers with 20 ml of *hexane R* for 1 min. Allow to stand until two clear layers are formed. Separate the lower layer and dilute to 50.0 ml with *dimethyl sulphoxide R*. Measure the absorbance *(2.2.25)* between 260 nm and 420 nm using a path length of 4 cm and using as the compensation liquid the clear lower layer obtained by vigorously shaking 10 ml of *dimethyl sulphoxide R* with 25 ml of *hexane R* for 1 min. Prepare a 9.0 mg/l reference solution of *naphthalene R* in *dimethyl sulphoxide R* and measure the absorbance of this solution at the maximum at 278 nm using a path length of 4 cm and using *dimethyl sulphoxide R* as the compensation liquid. At no wavelength in the range of 260 nm to 420 nm does the absorbance of the test solution exceed that of the reference solution at 278 nm.

Sulphated ash *(2.4.14)*

Not more than 0.05 per cent, determined on 2.0 g.

STORAGE

Store protected from light.

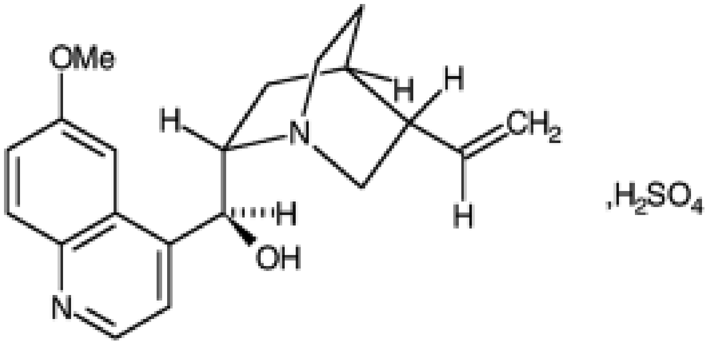
LABELLING

The label states the nominal drop point.

Ph Eur

Quinidine Bisulphate

General Notices



$C_{20}H_{24}N_2O_2,H_2SO_4$ 422.5 *50-54-4*

Action and use

Class I antiarrhythmic.

DEFINITION

Quinidine Bisulphate is (8*R*,9*S*)-6′-methoxycinchonan-9-ol hydrogen sulphate. It contains not less than 98.5% and not more than 101.5% of alkaloid hydrogen sulphates, calculated as $C_{20}H_{24}N_2O_2,H_2SO_4$ with reference to the anhydrous substance.

CHARACTERISTICS

Colourless crystals; odourless or almost odourless.

Freely soluble in *water* and in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 15 volumes of *diethylamine*, 36 volumes of *ether* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 4 µl of each of three solutions in *methanol* containing (1) 1.0% w/v of the substance being examined, (2) 1.0% w/v of *quinidine sulphate EPCRS* and (3) 1.0% w/v each of *quinidine sulphate EPCRS* and *quinine sulphate EPCRS*. After removal of the plate, dry it in a current of air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow it to cool and spray with *iodoplatinate reagent*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Complies with the test for Acidity.

C. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 2.6 to 3.6, Appendix V L.

Specific optical rotation

In a 2% w/v solution in 0.1M *hydrochloric acid*, +246 to +258, Appendix V F, determined using a 2-dm layer and calculated with reference to the anhydrous substance.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 20 mg of the substance being examined, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase. Prepare solutions (2) and (3) in the same manner using *quinine sulphate EPCRS* and *quinidine sulphate EPCRS* respectively in place of the substance being examined. Solution (4) is a mixture of equal volumes of solutions (2) and (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject separately 10 µl of each of solutions (3) and (6). If necessary adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_O being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µl of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinidine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µl of solution (1) and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinidine is not greater than 15%, the content of any related substance eluting before quinidine is not greater than 5% and the content of any other related substance is not greater than 2.5%.

Sulphated ash

Not more than 0.1%, Appendix IX A.

Water

Not more than 5.0% w/w, Appendix IX C. Use 1 g.

Titratable cation

75.3 to 79.6%, calculated with reference to the anhydrous substance, when determined by the following method. To the combined aqueous solutions reserved in the Assay add 0.1 ml of *phenolphthalein solution R1* and titrate with 0.1M *hydrochloric acid VS*. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 16.32 mg of $[C_{20}H_{26}N_2O_2]^{2+}$.

ASSAY

Dissolve 0.45 g in 15 ml of *water*, add 25 ml of 0.1M *sodium hydroxide VS* and extract with three 25 ml quantities of *chloroform.* Wash each chloroform extract successively with the same 20 ml of *water*, combine the aqueous solution and reserve for the test for Titratable cation. Dry the chloroform extracts with *anhydrous sodium sulphate*, evaporate to dryness at a pressure of 2 kPa and dissolve the residue in 50 ml of *anhydrous acetic acid*. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 21.13 mg of $C_{20}H_{24}N_2O_2,H_2SO_4$.

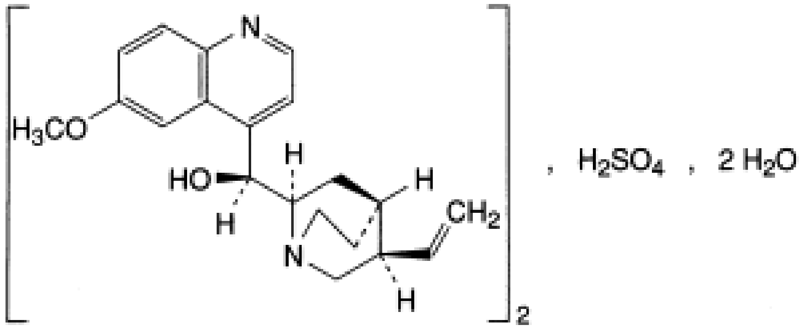
STORAGE

Quinidine Bisulphate should be protected from light.

Quinidine Sulphate

General Notices

(Ph Eur monograph 0017)



$(C_{20}H_{24}N_2O_2)_2H_2SO_4,2H_2O$ 783 *6591-63-5*

Action and use

Class I antiarrhythmic.

Preparation

Quinidine Sulphate Tablets.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of alkaloid monosulphates, expressed as bis[(*S*)-[(2*R*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol] sulphate (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or silky, colourless needles.

Solubility

Slightly soluble in water, soluble in boiling water and in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.10 g of *quinidine sulphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *diethylamine R*, *ether R*, *toluene R* (10:24:40 *V/V/V*).

Application 5 µl.

Development Twice over a path of 15 cm; dry in a current of air for 15 min between the 2 developments.

Drying At 105 °C for 30 min and allow to cool.

Detection Spray with *iodoplatinate reagent R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve about 5 mg in 5 ml of *water R*. Add 0.2 ml of *bromine water R* and 1 ml of *dilute ammonia R2*. A green colour develops.

C. Dissolve 0.1 g in 3 ml of *dilute sulphuric acid R* and dilute to 100 ml with *water R*. When examined in ultraviolet light at 366 nm, an intense blue fluorescence appears which disappears almost completely on addition of 1 ml of *hydrochloric acid R*.

D. Dissolve about 50 mg in 5 ml of hot *water R*, cool, add 1 ml of *silver nitrate solution R1* and stir with a glass rod. After a few minutes, a white precipitate is formed that dissolves on the addition of *dilute nitric acid R*.

E. It gives reaction (a) of sulphates (*2.3.1*).

F. pH (see Tests).

TESTS

Solution S

Dissolve 0.500 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

6.0 to 6.8.

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 275 to + 290 (dried substance), determined on solution S.

Other cinchona alkaloids

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (a) Dissolve 20 mg of *quinine sulphate CRS* (impurity A) in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (b) Dissolve 20 mg of *quinidine sulphate CRS* in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (c) To 1 ml of reference solution (a) add 1 ml of reference solution (b).

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (e) Dissolve 10 mg of *thiourea R* in the mobile phase and dilute to 10 ml with the mobile phase.

Column:

— *size:* *l* = 0.15-0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Dissolve 6.8 g of *potassium dihydrogen phosphate R* and 3.0 g of *hexylamine R* in 700 ml of *water R*, adjust to pH 2.8 with *dilute phosphoric acid R*, add 60 ml of *acetonitrile R* and dilute to 1000 ml with *water R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 250 nm for reference solution (e) and at 316 nm for the other solutions.

Injection 10 µl.

Run time 2.5 times the retention time of quinidine.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurity A and dihydroquinine; use the chromatogram obtained with reference solution (b) to identify the peaks due to quinidine and impurity C; the chromatogram obtained with reference solution (c) shows 4 peaks due to quinidine, impurity A, impurity C and dihydroquinine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

Relative retention with reference to impurity A Dihydroquinine = about 1.4.

Relative retention with reference to quinidine Impurity C = about 1.5.

System suitability:

— *resolution*: minimum 3.0 between the peaks due to impurity A and quinidine and minimum 2.0 between the peaks due to impurities C and A in the chromatogram obtained with reference solution (c);

— *signal-to-noise ratio*: minimum 4 for the principal peak in the chromatogram obtained with reference solution (d);

— *mass distribution ratio*: 3.5 to 4.5 for the peak due to quinidine in the chromatogram obtained with reference solution (b), t_R' being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

- *impurity C*: maximum 15 per cent;
- *any impurity eluted before quinidine* : for each impurity, maximum 5 per cent;
- *any other impurity*: for each impurity, maximum 2.5 per cent;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent).

Boron

Maximum 5 ppm. *Avoid where possible the use of glassware*.

Test solution Dissolve 1.00 g in a mixture of 0.5 ml of *hydrochloric acid R* and 4.0 ml of *water R*.

Reference solution Dissolve 0.572 g of *boric acid R* in *water R* and dilute to 1000.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *water R*. To 1.0 ml of this solution add 3.0 ml of *water R* and 0.5 ml of *hydrochloric acid R*.

Blank solution Add 0.5 ml of *hydrochloric acid R* to 4.0 ml of *water R*.

Add 3.0 ml of a 100 g/l solution of *2-ethylhexane-1,3-diol R* in *methylene chloride R* to the test solution, to the reference solution and to the blank solution, then shake for 1 min. Allow to stand for 6 min. To 1.0 ml of the lower layer, add 2.0 ml of a 3.75 g/l solution of *curcumin R* in *anhydrous acetic acid R* and 0.3 ml of *sulphuric acid R*. Mix and after 20 min add 25.0 ml of *ethanol (96 per cent) R*. Mix. The blank solution is yellow. Any red colour in the test solution is not more intense than that in the reference solution.

Loss on drying (*2.2.32*)

3.0 per cent to 5.0 per cent, determined on 1.000 g by drying in an oven at 130 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, using 0.15 ml of *naphtholbenzein solution R* as indicator.

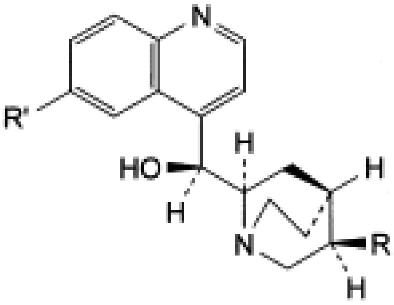
1 ml of *0.1 M perchloric acid* is equivalent to 24.90 mg of $C_{40}H_{50}N_4O_8S$.

STORAGE

Protected from light.

IMPURITIES

A. quinine,



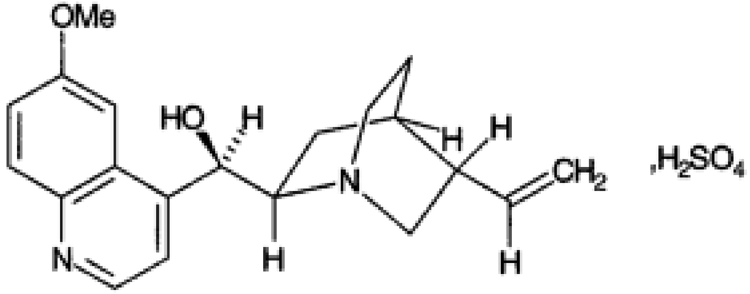
B. R = $CH=CH_2$, R′ = H: (*S*)-[(2*R*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](quinolin-4-yl) methanol (cinchonine),

C. R = C_2H_5, R′ = OCH_3: (*S*)-[(2*R*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (dihydroquinidine).

Ph Eur

Quinine Bisulphate

General Notices



$C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$ 548.6   *549-56-4*

Action and use

Antiprotozoal (malaria).

Preparation

Quinine Bisulphate Tablets

DEFINITION

Quinine Bisulphate is (8*S*,9*R*)-6′-methoxycinchonan-9-ol hydrogen sulphate heptahydrate. It contains not less than 98.5% and not more than 101.5% of alkaloid hydrogen sulphates, calculated as $C_{20}H_{24}N_2O_2,H_2SO_4$ with reference to the anhydrous substance.

CHARACTERISTICS

Colourless crystals or a white, crystalline powder; efflorescent in dry air.

Freely soluble in *water*; sparingly soluble in *ethanol (96%)*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 15 volumes of *diethylamine*, 36 volumes of *ether* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 4 µl of each of three solutions in *methanol* containing (1) 1.0% w/v of the substance being examined, (2) 1.0% w/v of *quinine sulphate EPCRS* and (3) 1.0% w/v each of *quinidine sulphate EPCRS* and *quinine sulphate EPCRS*. After removal of the plate, dry it in a current of air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow it to cool and spray with *iodoplatinate reagent*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Complies with the test for Acidity.

C. Yields the reactions characteristic of sulphates, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 2.8 to 3.4, Appendix V L.

Specific optical rotation

In a 3% w/v solution in 0.1M *hydrochloric acid*, -208 to -216, calculated with reference to the anhydrous substance, Appendix V F.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 20 mg of the substance being examined, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase. Prepare solutions (2) and (3) in the same manner using *quinine sulphate EPCRS* and *quinidine sulphate EPCRS* respectively in place of the substance being examined. Solution (4) is a mixture of equal volumes of solutions (2) and (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject separately 10 µl of each of solutions (3) and (6). If necessary adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_O being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µl of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinidine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µl of solution (1) and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinine is not greater than 10%, the content of any related substances eluting before quinine is not greater than 5% and the content of any other related substances is not greater than 2.5%.

Sulphated ash

Not more than 0.1%, Appendix IX A.

Water

19.0 to 25.0% w/w, Appendix IX C. Use 0.2 g.

Titratable cation

75.3 to 79.6%, calculated with reference to the anhydrous substance, when determined by the following method. Add to the combined aqueous solutions reserved in the Assay 0.1 ml of *phenolphthalein solution R1* and titrate with 0.1M *hydrochloric acid VS*. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 16.32 mg of $[C_{20}H_{26}N_2O_2]^{2+}$.

ASSAY

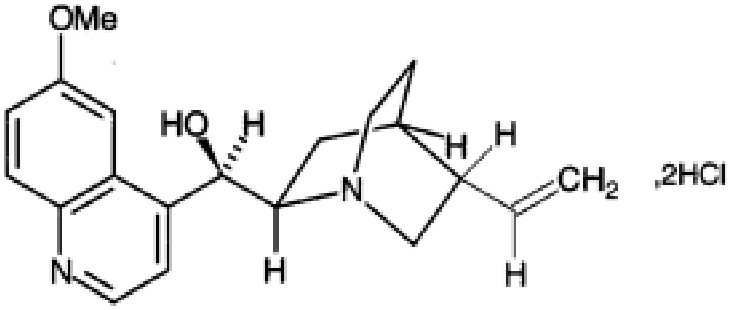
Dissolve 0.45 g in 15 ml of *water*. Add 25 ml of 0.1M *sodium hydroxide VS* and extract with three 25 ml quantities of *chloroform*. Wash the combined chloroform extracts with 20 ml of *water*, combine the aqueous solutions and reserve for the test for Titratable cation. Dry the chloroform extracts with *anhydrous sodium sulphate*, evaporate to dryness at a pressure of 2 kPa and dissolve the residue in 50 ml of *anhydrous acetic acid*. Carry out method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 21.13 mg of $C_{20}H_{24}N_2O_2,H_2SO_4$.

STORAGE

Quinine Bisulphate should be protected from light.

Quinine Dihydrochloride

General Notices



$C_{20}H_{24}N_2O_2,2HCl$ 397.3 *60-93-5*

Action and use

Antiprotozoal (malaria).

Preparation

Quinine Dihydrochloride Intravenous Infusion

DEFINITION

Quinine Dihydrochloride is (8*S*,9*R*)-6′-methoxycinchonan-9-ol dihydrochloride. It contains not less than 99.0% and not more than 101.0% of alkaloid dihydrochlorides, calculated as $C_{20}H_{24}N_2O_2,2HCl$, with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Very soluble in *water*; soluble in *ethanol (96%)*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 15 volumes of *diethylamine*, 36 volumes of *ether* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 4 µl of each of three solutions in *methanol* containing (1) 1.0% w/v of the substance being examined, (2) 1.0% w/v of *quinine sulphate EPCRS* and (3) 1.0% w/v each of *quinidine sulphate EPCRS* and *quinine sulphate EPCRS*. After removal of the plate, dry it in a current of air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow it to cool and spray with *iodoplatinate reagent*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Complies with the test for Acidity.

C. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 3% w/v solution, 2.0 to 3.0, Appendix V L.

Specific optical rotation

In a 3% w/v solution in 0.1M *hydrochloric acid*, -223 to -229 calculated with reference to the dried substance, Appendix V F.

Barium

To 15 ml of a 2.0% w/v solution add 1 ml of 1M *sulphuric acid*. The solution remains clear for at least 15 minutes.

Sulphate

0.125 g complies with *the limit test for sulphates*, Appendix VII (0.12%).

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 20 mg of the substance being examined, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase. Prepare solutions (2) and (3) in the same manner using *quinine sulphate EPCRS* and *quinidine sulphate EPCRS* respectively in place of the substance being examined. Solution (4) is a mixture of equal volumes of solutions (2) and (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject separately 10 µl of each of solutions (3) and (6). If necessary adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_O being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µl of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinidine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µl of solution (1) and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5). The content of dihydroquinine is not greater than 10%, the content of any related substance eluting before quinine is not more than 5% and the content of any other related substance is not more than 2.5%.

Loss on drying

When dried to constant weight at 105°, loses not more than 3.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

Titratable cation

79.7 to 84.2%, calculated with reference to the dried substance, when determined by the following method. Dissolve 0.4 g in 10 ml of *water*, add 40 ml of *methanol* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 16.32 mg of $[C_{20}H_{26}N_2O_2]^{2+}$.

ASSAY

Dissolve 0.3 g in a mixture of 50 ml of *anhydrous acetic acid* and 20 ml of *acetic anhydride*, add 10 ml of *mercury(II) acetate solution* and carry out method I for *non-aqueous titration*, Appendix VIII A, using crystal violet solution as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 19.87 mg of $C_{20}H_{24}N_2O_2$,2HCl.

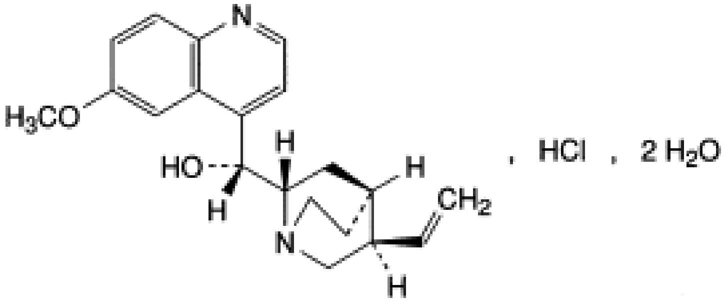
STORAGE

Quinine Dihydrochloride should be protected from light.

Quinine Hydrochloride

General Notices

(Ph Eur monograph 0018)



$C_{20}H_{24}N_2O_2,HCl,2H_2O$ 396.9 *130-89-2*

Action and use

Antiprotozoal (malaria).

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of alkaloid monohydrochlorides, expressed as (*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol hydrochloride (dried substance).

CHARACTERS

Appearance

White or almost white or colourless, fine, silky needles, often in clusters.

Solubility

Soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.10 g of *quinine sulphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *diethylamine R*, *ether R*, *toluene R* (10:24:40 *V/V/V*).

Application 5 µl.

Development Twice over a path of 15 cm; dry in a current of air for 15 min between the 2 developments.

Drying At 105 °C for 30 min and allow to cool.

Detection Spray with *iodoplatinate reagent R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve about 10 mg in *water R* and dilute to 10 ml with the same solvent. To 5 ml of this solution add 0.2 ml of *bromine water R* and 1 ml of *dilute ammonia R2*. A green colour develops.

C. Dissolve 0.1 g in 3 ml of *dilute sulphuric acid R* and dilute to 100 ml with *water R*. When examined in ultraviolet light at 366 nm, an intense blue fluorescence appears which disappears almost completely on the addition of 1 ml of *hydrochloric acid R*.

D. It gives the reactions of chlorides (*2.3.1*).

E. pH (see Tests).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)

6.0 to 6.8.

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

- 245 to - 258 (dried substance).

Dissolve 0.500 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Other cinchona alkaloids

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (a) Dissolve 20 mg of *quinine sulphate CRS* in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (b) Dissolve 20 mg of *quinidine sulphate CRS* (impurity A) in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (c) To 1 ml of reference solution (a) add 1 ml of reference solution (b).

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (e) Dissolve 10 mg of *thiourea R* in the mobile phase and dilute to 10 ml with the mobile phase.

Column:
- *size:* l = 0.15-0.25 m, Ø = 4.6 mm;
- *stationary phase* = *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Dissolve 6.8 g of *potassium dihydrogen phosphate R* and 3.0 g of *hexylamine R* in 700 ml of *water R*, adjust to pH 2.8 with *dilute phosphoric acid R*, add 60 ml of *acetonitrile R* and dilute to 1000 ml with *water R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 250 nm for reference solution (e) and at 316 nm for the other solutions.

Injection 10 µl.

Run time 2.5 times the retention time of quinine.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to quinine and impurity C; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurity A and dihydroquinidine; the chromatogram obtained with reference solution (c) shows 4 peaks due to impurity A, quinine, dihydroquinidine and impurity C, which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

Relative retention with reference to quinine Impurity C = about 1.4.

Relative retention with reference to impurity A Dihydroquinidine = about 1.5.

System suitability:
- *resolution*: minimum 3.0 between the peaks due to quinine and impurity A and minimum 2.0 between the peaks due to dihydroquinidine and quinine in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 4 for the principal peak in the chromatogram obtained with reference solution (d);
- *mass distribution ratio*: 3.5 to 4.5 for the peak due to impurity A in the chromatogram obtained with reference solution (b), t_R' being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:
- *impurity C*: maximum 10 per cent;

— *any impurity eluted before quinine* : for each impurity, maximum 5 per cent;

— *any other impurity*: for each impurity, maximum 2.5 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent).

Sulphates (*2.4.13*)

Maximum 500 ppm, determined on solution S.

Barium

To 15 ml of solution S add 1 ml of *dilute sulphuric acid R*. Allow to stand for 15 min. Any opalescence in the solution is not more intense than that in a mixture of 15 ml of solution S and 1 ml of *distilled water R*.

Loss on drying (*2.2.32*)

6.0 per cent to 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric* acid. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 inflexion points.

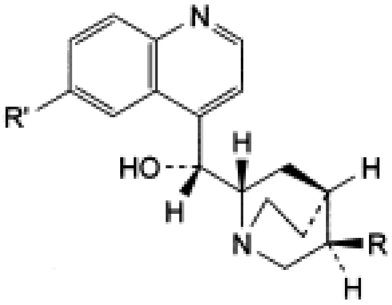
1 ml of *0.1 M sodium hydroxide* is equivalent to 36.09 mg of $C_{20}H_{25}ClN_2O_2$

STORAGE

Protected from light.

IMPURITIES

A. quinidine,



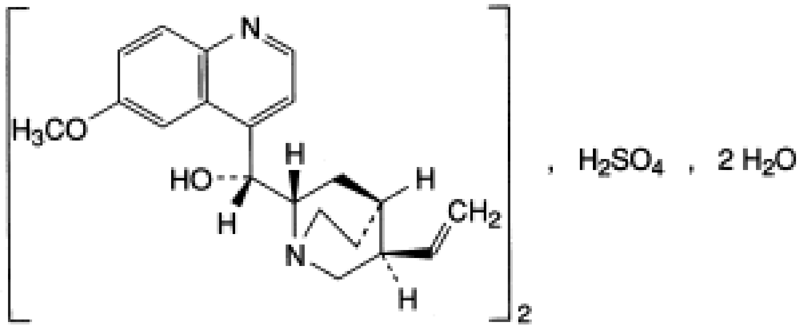
B. R = $CH{=}CH_2$, R′ = H: (*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](quinolin-4-yl) methanol (cinchonidine),

C. R = C_2H_5, R′ = OCH_3: (*R*)-[(2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (dihydroquinine).

Quinine Sulphate

General Notices

(Ph Eur monograph 0019)



$(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$ 783 *6119-70-6*

Action and use

Antiprotozoal (malaria).

Preparation

Quinine Sulphate Tablets

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of alkaloid monohydrochlorides, expressed as (*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol hydrochloride (dried substance).

CHARACTERS

Appearance

White or almost white or colourless, fine, silky needles, often in clusters.

Solubility

Soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.10 g of *quinine sulphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *diethylamine R*, *ether R*, *toluene R* (10:24:40 *V/V/V*).

Application 5 µl.

Development Twice over a path of 15 cm; dry in a current of air for 15 min between the 2 developments.

Drying At 105 °C for 30 min and allow to cool.

Detection Spray with *iodoplatinate reagent R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve about 10 mg in *water R* and dilute to 10 ml with the same solvent. To 5 ml of this solution add 0.2 ml of *bromine water R* and 1 ml of *dilute ammonia R2*. A green colour develops.

C. Dissolve 0.1 g in 3 ml of *dilute sulphuric acid R* and dilute to 100 ml with *water R*. When examined in ultraviolet light at 366 nm, an intense blue fluorescence appears which disappears almost completely on the addition of 1 ml of *hydrochloric acid R*.

D. It gives the reactions of chlorides (*2.3.1*).

E. pH (see Tests).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2*, *Method II*).

pH (*2.2.3*)

6.0 to 6.8.

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

- 245 to - 258 (dried substance).

Dissolve 0.500 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Other cinchona alkaloids

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (a) Dissolve 20 mg of *quinine sulphate CRS* in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (b) Dissolve 20 mg of *quinidine sulphate CRS* (impurity A) in 5 ml of the mobile phase, with gentle heating if necessary, and dilute to 10 ml with the mobile phase.

Reference solution (c) To 1 ml of reference solution (a) add 1 ml of reference solution (b).

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (e) Dissolve 10 mg of *thiourea R* in the mobile phase and dilute to 10 ml with the mobile phase.

Column:

- *size:* l = 0.15-0.25 m, Ø = 4.6 mm;
- *stationary phase* = *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Dissolve 6.8 g of *potassium dihydrogen phosphate R* and 3.0 g of *hexylamine R* in 700 ml of *water R*, adjust to pH 2.8 with *dilute phosphoric acid R*, add 60 ml of *acetonitrile R* and dilute to 1000 ml with *water R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 250 nm for reference solution (e) and at 316 nm for the other solutions.

Injection 10 µl.

Run time 2.5 times the retention time of quinine.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to quinine and impurity C; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurity A and dihydroquinidine; the chromatogram obtained with reference solution (c) shows 4 peaks due to impurity A, quinine, dihydroquinidine and impurity C, which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

Relative retention With reference to quinine: impurity C = about 1.4.

Relative retention With reference to impurity A: dihydroquinidine = about 1.5.

System suitability:

- *resolution*: minimum 3.0 between the peaks due to quinine and impurity A and minimum 2.0 between the peaks due to dihydroquinidine and quinine in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 4 for the principal peak in the chromatogram obtained with reference solution (d);
- *mass distribution ratio*: 3.5 to 4.5 for the peak due to impurity A in the chromatogram obtained with reference solution (b), t_R' being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of

acetonitrile in the mobile phase.

Limits:

- *impurity C*: maximum 10 per cent;
- *any impurity eluted before quinine* : for each impurity, maximum 5 per cent;
- *any other impurity*: for each impurity, maximum 2.5 per cent;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent).

Loss on drying *(2.2.32)*

3.0 per cent to 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

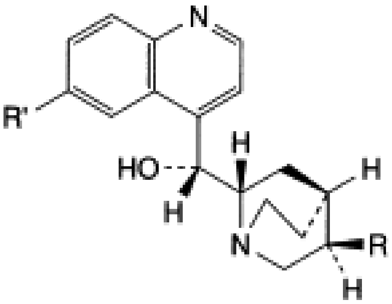
Dissolve 0.300 g in a mixture of 10 ml of *chloroform R* and 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. 1 ml of *0.1 M perchloric acid* is equivalent to 24.90 mg of $C_{40}H_{50}N_4O_8S$.

STORAGE

Protected from light.

IMPURITIES

A. quinidine,



B. R = CH=CH$_2$, R′ = H: (*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](quinolin-4-yl) methanol (cinchonidine),

C. R = C$_2$H$_5$, R′ = OCH$_3$: (*R*)-[(2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (dihydroquinine).

Ph Eur

Products of Recombinant DNA Technology

General Notices

(Ph Eur monograph 0784)

Ph Eur

This monograph provides general requirements for the development and manufacture of products of recombinant DNA technology. These requirements are not necessarily comprehensive in a given case and requirements complementary or additional to those prescribed in this monograph may be imposed in an individual monograph or by the competent authority.

The monograph is not applicable to modified live organisms that are intended to be used directly in man and animals, for example as live vaccines.

DEFINITION

Products of rDNA technology are produced by genetic modification in which DNA coding for the required product is introduced, usually by means of a plasmid or a viral vector, into a suitable micro-organism or cell line, in which that DNA is expressed and translated into protein. The desired product is then recovered by extraction and purification. The cell or micro-organism before harbouring the vector is referred to as the host cell, and the stable association of the two used in the manufacturing process is referred to as the host-vector system.

PRODUCTION

Production is based on a validated seed-lot system using a host-vector combination that has been shown to be suitable to the satisfaction of the competent authority. The seed-lot system uses a master cell bank and a working cell bank derived from the master seed lot of the host-vector combination. A detailed description of cultivation, extraction and purification steps and a definition of the production batch shall be established.

Where products of rDNA technology are manufactured using materials of human or animal origin, the requirements of chapter *5.1.7*. *Viral safety* apply.

The determination of the suitability of the host-vector combination and the validation of the seed-lot system include the following elements.

CLONING AND EXPRESSION

The suitability of the host-vector system, particularly as regards microbiological purity, is demonstrated by:

Characterisation of the host cell, including source, phenotype and genotype, and of the cell-culture media;

Documentation of the strategy for the cloning of the gene and characterisation of the recombinant vector, including:

i. the origin and characterisation of the gene;

ii. nucleotide-sequence analysis of the cloned gene and the flanking control regions of the expression vector; the cloned sequences are kept to a minimum and all relevant expressed sequences are clearly identified and confirmed at the RNA level; the DNA sequence of the cloned gene is normally confirmed at the seed-lot stage, up to and beyond the normal level of population doubling for full-scale fermentation; in certain systems, for example, where multiple copies of the gene are inserted into the genome of a continuous cell line, it may be inappropriate to sequence the cloned gene at the production level; under these circumstances, Southern blot analysis of total cellular DNA or sequence analysis of the messenger RNA (mRNA) may be helpful, particular attention being paid to the characterisation of the expressed protein;

iii. the construction, genetics and structure of the complete expression vector;

Characterisation of the host-vector system, including:

i. mechanism of transfer of the vector into the host cells;

ii. copy number, physical state and stability of the vector inside the host cell;

iii. measures used to promote and control the expression.

CELL-BANK SYSTEM

The master cell bank is a homogeneous suspension of the original cells already transformed by the expression vector containing the desired gene, distributed in equal volumes into individual containers for storage (for example, in liquid nitrogen). In some cases it may be necessary to establish separate master cell banks for the expression vector and the host cells.

The working cell bank is a homogeneous suspension of the cell material derived from the master cell bank(s) at a finite passage level, distributed in equal volumes into individual containers for storage (for example, in liquid nitrogen).

In both cell banks, all containers are treated identically during storage and, once removed from storage, the containers are not returned to the cell stock.

The cell bank may be used for production at a finite passage level or for continuous-culture production.

Production at a finite passage level This cultivation method is defined by a limited number of passages or population doublings which must not be exceeded during production. The maximum number of cell doublings, or passage levels, during which the manufacturing process routinely meets the criteria described below must be stated.

Continuous-culture production By this cultivation method the number of passages or population doublings is not restricted from the beginning of production. Criteria for the harvesting as well as for the termination of production have to be defined by the manufacturer. Monitoring is necessary throughout the life of the culture; the required frequency and type of monitoring will depend on the nature of the production system and the product.

Information is required on the molecular integrity of the gene being expressed and on the phenotypic and genotypic characteristics of the host cell after long-term cultivation. The acceptance of harvests for further processing must be clearly linked to the schedule of monitoring applied and a clear definition of a 'batch' of product for further processing is required.

VALIDATION OF THE CELL BANKS

Validation of the cell banks includes:

i. stability by measuring viability and the retention of the vector;

ii. identity of the cells by phenotypic features;

iii. where appropriate, evidence that the cell banks are free from potentially oncogenic or infective adventitious agents (viral, bacterial, fungal or mycoplasmal); special attention has to be given to viruses that can commonly contaminate the species from which the cell line has been derived; certain cell lines contain endogenous viruses, for example, retroviruses, which may not readily be eliminated; the expression of these organisms, under a variety of conditions known to cause their induction, shall be tested for;

iv. for mammalian cells, details of the tumorigenic potential of the cell bank shall be obtained.

CONTROL OF THE CELLS

The origin, form, storage, use and stability at the anticipated rate of use must be documented in full for all cell banks under conditions of storage and recovery. New cell banks must be fully validated.

VALIDATION OF THE PRODUCTION PROCESS

Extraction and purification

The capacity of each step of the extraction and purification procedure to remove and/or inactivate contaminating substances derived from the host cell or culture medium, including, in particular, virus particles, proteins, nucleic acids and added substances, must be validated.

Validation studies are carried out to demonstrate that the production process routinely meets the following criteria:

— exclusion of extraneous agents from the product; studies including, for example, viruses with relevant physico-chemical features are undertaken, and a reduction capacity for such contaminants at each relevant stage of purification is established;

— adequate removal of vector, host-cell, culture medium and reagent-derived contaminants from the product; the reduction capacity for DNA is established by spiking; the reduction of proteins of animal origin can be determined by immunochemical methods;

— maintenance within stated limits of the yield of product from the culture;

— adequate stability of any intermediate of production and/or manufacturing when it is intended to use intermediate storage during the process.

Characterisation of the substance

The identity, purity, potency and stability of the final bulk product are established initially by carrying out a wide range of chemical, physical, immunochemical and biological tests. Prior to release, each batch of the product is tested by the manufacturer for identity and purity and an appropriate assay is carried out.

Production consistency

Suitable tests for demonstrating the consistency of the production and purification are performed. In particular, the tests include characterisation tests, in-process controls and final-product tests as exemplified below.

AMINO-ACID COMPOSITION

Partial amino-acid sequence analysis The sequence data permit confirmation of the correct *N*-terminal processing and detection of loss of the *C*-terminal amino acids.

Peptide mapping Peptide mapping using chemical and/or enzymatic cleavage of the protein product and analysis by a suitable method such as two-dimensional gel electrophoresis, capillary electrophoresis or liquid chromatography must show no significant difference between the test protein and the reference preparation. Peptide mapping can also be used to demonstrate correct disulphide bonding.

DETERMINATION OF MOLECULAR MASS

Cloned-gene retention The minimum percentage of cells containing the vector or the cloned gene after cultivation is approved by the relevant authority.

Total protein The yield of protein is determined.

Chemical purity The purity of the protein product is analysed in comparison with a reference preparation by a suitable method such as liquid chromatography, capillary electrophoresis or sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Host-cell-derived proteins Host-cell-derived proteins are detected by immunochemical methods, using, for example, polyclonal antisera raised against protein components of the host-vector system used to manufacture the product, unless otherwise prescribed. The following types of procedure may be used: liquid-phase displacement assays (for example, radio-immunoassay), liquid-phase direct-binding assays and direct-binding assays using antigens immobilised on nitrocellulose (or similar) membranes (for example, dot-immunoblot assays, Western blots). General requirements for the validation of immunoassay procedures are given under *2.7.1. Immunochemical Methods*. In addition, immunoassay methods for host-cell contaminants meet the following criteria.

— *Antigen preparations*. Antisera are raised against a preparation of antigens derived from the host organism, into which has been inserted the vector used in the manufacturing process that lacks the specific gene coding for the product. This host cell is cultured, and proteins are extracted, using conditions identical to those used for culture and extraction in the manufacturing process. Partly purified preparations of antigens, using some of the purification steps in the manufacturing process, may also be used for the preparation of antisera.

— *Calibration and standardisation*. Quantitative data are obtained by comparison with dose-response curves obtained using standard preparations of host-derived protein antigens. Since these preparations are mixtures of poorly defined proteins, a standard preparation is prepared and calibrated by a suitable protein determination method. This preparation is stored in a stable state suitable for use over an extended period of time.

— *Antisera*. Antisera contain high-avidity antibodies recognising as many different proteins in the antigen mixture as possible, and do not cross-react with the product.

Host-cell- and vector-derived DNA Residual DNA is detected by hybridisation analysis, using suitably sensitive, sequence-independent analytical techniques or other suitably sensitive analytical techniques.

Hybridisation analysis

DNA in the test sample is denatured to give single-stranded DNA, immobilised on a nitrocellulose or other suitable filter and hybridised with labelled DNA prepared from the host-vector manufacturing system (DNA probes). Although a wide variety of experimental approaches is available, hybridisation methods for measurement of host-vector DNA meet the following criteria.

— *DNA probes*. Purified DNA is obtained from the host-vector system grown under the same conditions as those used in the manufacturing process. Host chromosomal DNA and vector DNA may be separately prepared and used as probes.

— *Calibration and standardisation*. Quantitative data are obtained by comparison with responses obtained using standard preparations. Chromosomal DNA probes and vector DNA probes are used with chromosomal DNA and vector DNA standards, respectively. Standard preparations are calibrated by spectroscopic measurements and stored in a state suitable for use over an extended period of time.

— *Hybridisation conditions*. The stringency of hybridisation conditions is such as to ensure specific hybridisation between probes and standard DNA preparations and the drug substances must not interfere with hybridisation at the concentrations used.

Sequence-independent techniques

Suitable procedures include: detection of sulphonated cytosine residues in single-stranded DNA (where DNA is immobilised on a filter and cytosines are derivatised *in situ*, before detection and quantitation using an antibody directed against the sulphonated group); detection of single-stranded DNA using a fragment of single-stranded DNA bound to a protein and an antibody of this protein. Neither procedure requires the use of specific host or vector DNA as an assay standard. However, the method used must be validated to ensure parallelism with the DNA standard used, linearity of response and non-interference of either the drug substance or excipients of the formulation at the dilutions used in the assay.

IDENTIFICATION, TESTS AND ASSAY

The requirements with which the final product (bulk material or dose form) must comply throughout its period of validity, as well as specific test methods, are stated in the individual monograph.

STORAGE

See the individual monographs.

LABELLING

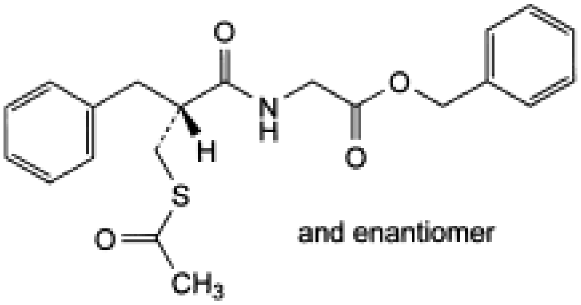
See the individual monographs.

Ph Eur

Racecadotril

General Notices

(Ph Eur monograph 2171)



$C_{21}H_{23}NO_4S$ 385.5 *81110-73-8*

Ph Eur

DEFINITION

Benzyl [[(2*RS*)-2-[(acetylsulfanyl)methyl]-3-phenylpropanoyl]amino]acetate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison racecadotril CRS.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2,* *Method II).*

Dissolve 5.0 g in 10 ml of *acetone R*.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture Mobile phase A, mobile phase B (50:50 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Test solution (b) Dilute 5.0 ml of test solution (a) to 25.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dilute 500 µl of *racecadotril impurity A CRS* in *acetonitrile R* and dilute to 250.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *racecadotril impurity G CRS* in the solvent mixture and dilute to 50 ml with the solvent mixture. To 5 ml of this solution add 1 ml of test solution (b) and dilute to 100 ml with the solvent mixture.

Reference solution (d) Dissolve 50.0 mg of *racecadotril CRS* in the solvent mixture and dilute to 25.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 25.0 ml with the solvent mixture.

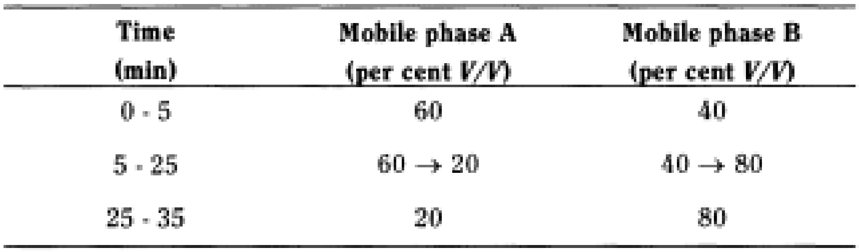
Reference solution (e) Dissolve the contents of a vial of *racecadotril for peak identification CRS* (containing impurities C, E and F) in 1.0 ml of the solvent mixture.

Column:

- *size: l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: dissolve 1.0 g of *potassium dihydrogen phosphate R* in *water R*, adjust to pH 2.5 with *phosphoric acid R* and dilute to 1000 ml with *water R*;
- *mobile phase B: acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	60	40
5 - 25	60 → 20	40 → 80
25 - 35	20	80

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µl of the solvent mixture, test solution (a) and reference solutions (a), (b), (c) and (e).

Identification of impurities Use the chromatogram supplied with *racecadotril for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities C, E and F.

Relative retention With reference to racecadotril (retention time = about 16 min): impurity A = about 0.2; impurity C = about 0.3; impurity E = about 0.5; impurity F = about 0.9.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to impurity G and racecadotril.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.4; impurity E = 0.6; impurity F = 0.7;

— *impurities C, E, F*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

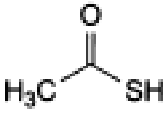
Calculate the percentage content of $C_{21}H_{23}NO_4S$ from the declared content of *racecadotril CRS*.

IMPURITIES

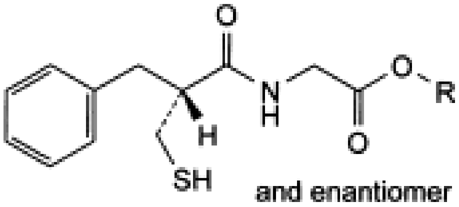
Specified impurities A, C, E, F.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances*

for pharmaceutical use): *B, D, G, H.*

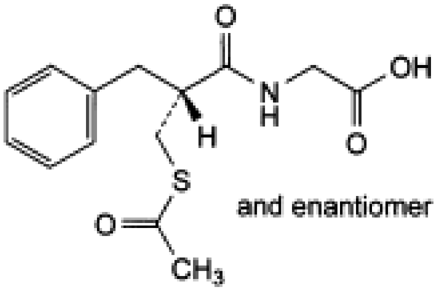


A. ethanethioic acid (thioacetic acid),

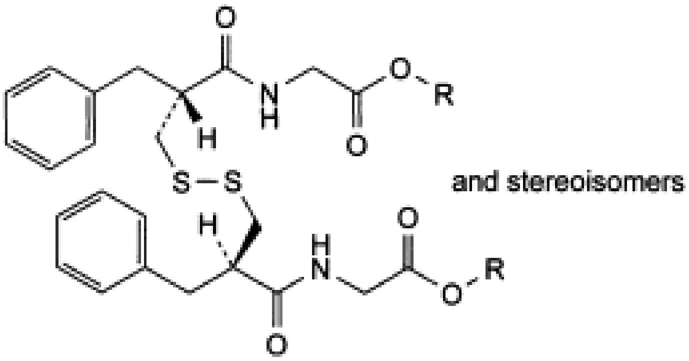

and enantiomer

B. R = H: [[(2*RS*)-2-benzyl-3-sulfanylpropanoyl]amino]acetic acid,

G. R = CH_2-C_6H_5: benzyl [[(2*RS*)-2-benzyl-3-sulfanylpropanoyl]amino]acetate,

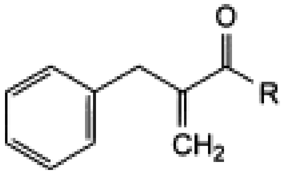

and enantiomer

C. (2*RS*)-2-[(acetylsulfanyl)methyl]-3-phenylpropanoyl]amino]acetic acid,


and stereoisomers

D. R = H: 5,10-dibenzyl-4,11-dioxo-7,8-dithia-3,12-diazatetradecanedioic acid,

H. R = CH_2-C_6H_5: dibenzyl 5,10-dibenzyl-4,11-dioxo-7,8-dithia-3,12-diazatetradecanedioate,



E. R = OH: 2-benzylprop-2-enoic acid (2-benzylacrylic acid),

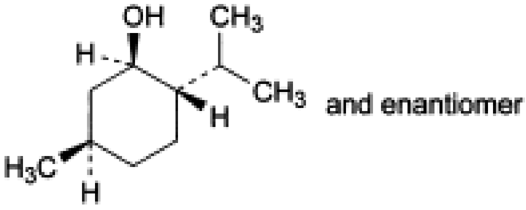
F. R = NH-CH_2-CO-O-CH_2-C_6H_5: benzyl [(2-benzylprop-2-enoyl)amino]acetate.

Ph Eur

Racementhol

General Notices

(Racemic Menthol, Ph Eur monograph 0623)



$C_{10}H_{20}O$ 156.3 *15356-70-4*

Preparation

Menthol and Benzoin Inhalation

Ph Eur

DEFINITION

Mixture of equal parts of (1*RS*,2*SR*,5*RS*)-5-methyl-2-(1-methylethyl)cyclohexanol.

CHARACTERS

Appearance

Free-flowing or agglomerated, crystalline powder or prismatic or acicular, colourless, shiny crystals.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent) and in light petroleum, freely soluble in fatty oils and in liquid paraffin, very slightly soluble in glycerol.

mp: about 34 °C.

IDENTIFICATION

First identification *A, C.*

Second identification *B, D.*

A. Optical rotation (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5

ml with the same solvent.

Reference solution Dissolve 25 mg of *menthol CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air, until the solvents have evaporated.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in position and approximate dimensions to the principal peak in the chromatogram obtained with reference solution (c).

D. Dissolve 0.20 g in 0.5 ml of *anhydrous pyridine R*. Add 3 ml of a 150 g/l solution of *dinitrobenzoyl chloride R* in *anhydrous pyridine R*. Heat on a water-bath for 10 min. Add 7.0 ml of *water R* in small quantities with stirring and allow to stand in iced water for 30 min. A precipitate is formed. Allow to stand and decant the supernatant liquid. Wash the precipitate with 2 quantities, each of 5 ml, of iced *water R*, recrystallise from 10 ml of *acetone R*, wash with *iced acetone R* and dry at 75 °C at a pressure not exceeding 2.7 kPa for 30 min. The crystals melt (*2.2.14*) at 130 °C to 131 °C.

TESTS

Solution S

Dissolve 2.50 g in 10 ml of *ethanol (96 per cent) R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent. Add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Optical rotation (*2.2.7*)

- 0.2° to + 0.2°, determined on solution S.

Related substances

Gas chromatography (*2.2.28*).

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 50.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *methylene chloride R*.

Reference solution (a) Dissolve 40.0 mg of the substance to be examined and 40.0 mg of *isomenthol R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 0.10 ml of test solution (a) to 100.0 ml with *methylene chloride R*.

Reference solution (c) Dissolve 40.0 mg of *menthol CRS* in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Column:
- *material*: glass;
- *size:* *l* = 2.0 m, Ø = 2 mm;
- *stationary phase*: *diatomaceous earth for gas chromatography R* impregnated with 15 per cent *m/m* of *macrogol 1500 R*.

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:
- *column*: 120 °C;
- *injection port*: 150 °C;
- *detector*: 200 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time Twice the retention time of menthol.

System suitability:
- *resolution*: minimum 1.4 between the peaks due to menthol and isomenthol in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits Test solution (a):
- *total*: not more than 1 per cent of the area of the principal peak;
- *disregard limit*: 0.05 per cent of the area of the principal peak.

Residue on evaporation

Maximum 0.05 per cent.

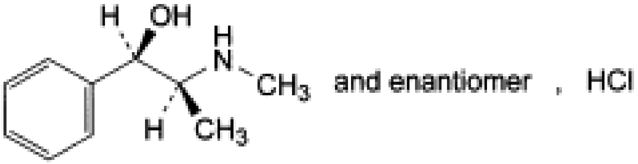
Evaporate 2.00 g on a water-bath and heat in an oven at 100-105 °C for 1 h. The residue weighs not more than 1.0 mg.

Ph Eur

Racephedrine Hydrochloride

General Notices

(Racemic Ephedrine Hydrochloride, Ph Eur monograph 0715)



$C_{10}H_{15}NO,HCl$ 201.7 *134-71-4*

Action and use

Adrenoceptor agonist.

Ph Eur

DEFINITION

Racemic ephedrine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (1*RS*,2*SR*)-2-(methylamino)-1-phenylpropan-1-ol hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, soluble in alcohol.

It melts at about 188 °C.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. It complies with the test for angle of optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *racemic ephedrine hydrochloride CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 ml of solution S (see Tests) add 1 ml of *water R*, 0.2 ml of *copper sulphate solution R* and 1 ml of *strong sodium hydroxide solution R*. A violet colour is produced. Add

2 ml of *ether R* and shake. The ether layer is purple and the aqueous layer is blue.

E. To 5 ml of solution S add 5 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 5.00 g in *distilled water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* and 0.1 ml of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.2 ml of *0.01 M hydrochloric acid*; the solution is red.

Angle of optical rotation (*2.2.7*)

+ 0.2° to - 0.2°, determined on solution S.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 20 mg of racemic ephedrine *hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (a) to 200 ml with *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *chloroform R*, 15 volumes of *concentrated ammonia R* and 80 volumes of *2-propanol R*. Allow the plate to dry in air. Spray with *ninhydrin solution R* and heat at 110 °C for 5 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Disregard any spot of lighter colour than the background.

Sulphates (*2.4.13*)

15 ml of solution S complies with the limit test for sulphates (100 ppm).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.170 g in 30 ml of *alcohol R*. Add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* corresponds to 20.17 mg of $C_{10}H_{16}ClNO$.

STORAGE

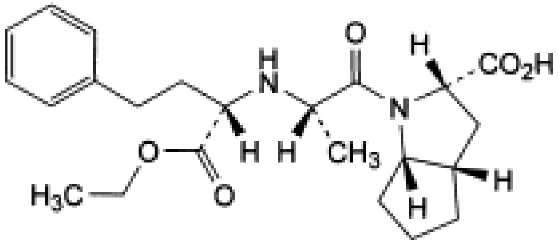
Store protected from light.

Ph Eur

Ramipril

General Notices

(Ph Eur monograph 1368)



$C_{23}H_{32}N_2O_5$ 416.5 *87333-19-5*

Action and use

Angiotensin converting enzyme inhibitor.

Preparations

Ramipril Capsules

Ramipril Tablets

Ph Eur

DEFINITION

(2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, freely soluble in methanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison ramipril CRS.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.1 g in *methanol R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 32.0 to + 38.0 (dried substance).

Dissolve 0.250 g in a mixture of 14 volumes of *hydrochloric acid R1* and 86 volumes of *methanol R* and dilute to 25.0 ml with the same mixture of solvents.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in mobile phase A and dilute to 20.0 ml with mobile phase A.

Reference solution (a) Dissolve 5 mg of *ramipril impurity A CRS*, 5 mg of *ramipril impurity B CRS*, 5 mg of *ramipril impurity C CRS* and 5 mg of *ramipril impurity D CRS* in 5 ml of the test solution, and dilute to 10 ml with mobile phase B.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with mobile phase B. Dilute 5.0 ml of this solution to 50.0 ml with mobile phase B.

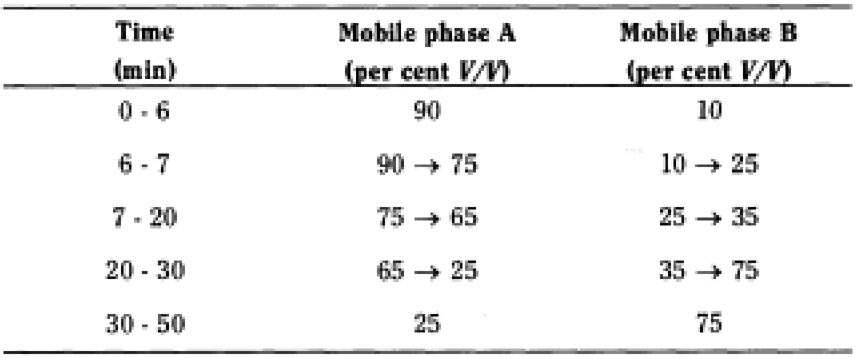
Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with mobile phase B.

Column:

- *size:* l = 0.25 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 65 °C.

Mobile phase:

- *mobile phase A*: dissolve 2.0 g of *sodium perchlorate R* in a mixture of 0.5 ml of *triethylamine R* and 800 ml of *water R*; adjust to pH 3.6 with *phosphoric acid R* and add 200 ml of *acetonitrile R1*;
- *mobile phase B*: dissolve 2.0 g of *sodium perchlorate R* in a mixture of 0.5 ml of *triethylamine R* and 300 ml of *water R*; adjust to pH 2.6 with *phosphoric acid R* and add 700 ml of *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	90	10
6 - 7	90 → 75	10 → 25
7 - 20	75 → 65	25 → 35
20 - 30	65 → 25	35 → 75
30 - 50	25	75

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Equilibration With the mobile phase at the initial composition for at least 35 min; if a suitable baseline cannot be obtained, use another grade of triethylamine.

Injection 10 µl.

Relative retention With reference to ramipril (retention time = about 18 min): impurity A = about 0.8; impurity B = about 1.3; impurity G = about 1.4; impurity C = about 1.5; impurity D = about 1.7; impurity O = about 2.4.

System suitability:

— *resolution*: minimum 3.0 between the peaks due to impurity A and ramipril in the chromatogram obtained with reference solution (a);

— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (c);

— *symmetry factor*: 0.8 to 2.0 for the peak due to ramipril in the chromatogram obtained with the test solution.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 2.4;

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Palladium

Maximum 20.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 0.200 g in a mixture of 0.3 volumes of *nitric acid R* and 99.7 volumes of *water R*, and dilute to 100.0 ml with the same mixture of solvents.

Reference solutions Use solutions containing 0.02 µg, 0.03 µg and 0.05 µg of palladium per millilitre, freshly prepared by dilution of *palladium standard solution (0.5 ppm Pd) R* with a mixture of 0.3 volumes of *nitric acid R* and 99.7 volumes of *water R*.

Modifier solution Dissolve 0.150 g of *magnesium nitrate R* in a mixture of 0.3 volumes of *nitric acid R* and 99.7 volumes of *water R*, and dilute to 100.0 ml with the same mixture of solvents.

Injection 20 µl of the test solution and the reference solution, and 10 µl of the modifier solution.

Source Palladium hollow-cathode lamp using a transmission band preferably of 1 nm and a graphite tube.

Wavelength 247.6 nm.

Loss on drying *(2.2.32)*

Maximum 0.2 per cent, determined on 1.000 g by drying in an oven under high vacuum at 60 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 25 ml of *methanol R* and add 25 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 41.65 mg of $C_{23}H_{32}N_2O_5$.

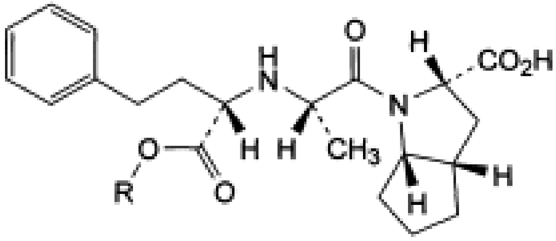
STORAGE

Protected from light.

IMPURITIES

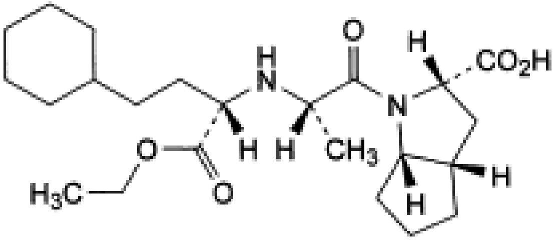
Specified impurities A, B, C, D.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F, G, H, I, J, K, L, M, N, O*.

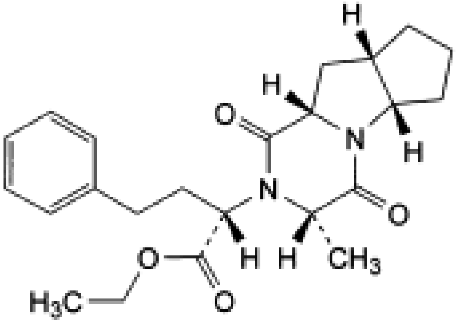


A. R = CH_3: (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(methoxycarbonyl)-3-phenylpropyl]amino] propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (ramipril methyl ester),

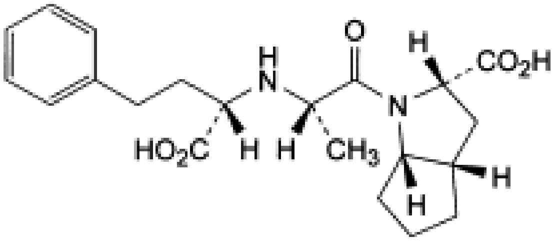
B. R = $CH(CH_3)_2$: (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-[(1-methylethoxy)carbonyl]-3-phenylpropyl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (ramipril isopropyl ester),


H
N
O
H
CO2H
N
O
H H CH3
H3C
O
H
H

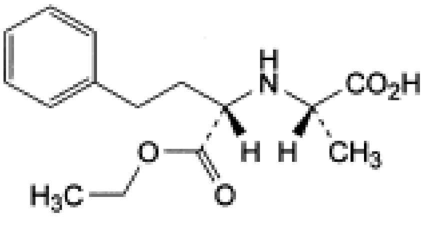
C. (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)-propyl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (hexahydroramipril),


H
H
O
N
H
N
O
O
H H CH3
H3C
O

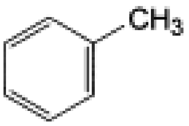
D. ethyl (2*S*)-2-[(3*S*,5a*S*,8a*S*,9a*S*)-3-methyl-1,4-dioxodecahydro-2*H*-cyclopenta[4,5]pyrrolo [1,2-a]pyrazin-2-yl]-4-phenylbutanoate (ramipril diketopiperazine),


H
N
O
H
CO2H
N
HO2C H H CH3
H
H

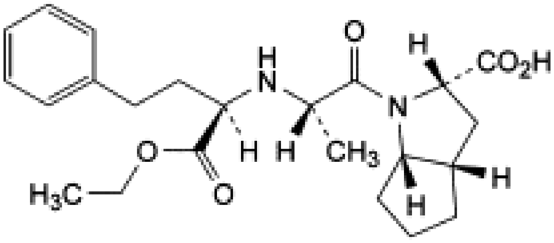
E. (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (ramipril diacid),


H
N
CO2H
O
H H CH3
H3C
O

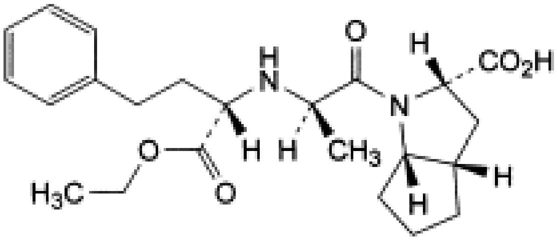
F. (2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoic acid,



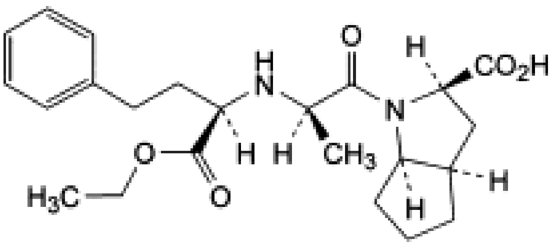
G. methylbenzene (toluene),



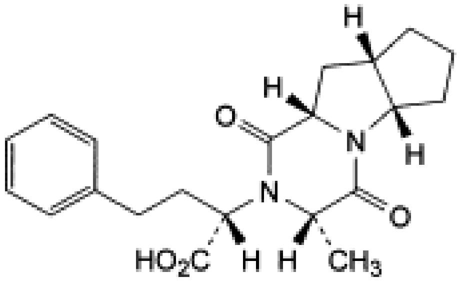
H. (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid ((*R,S,S,S,S*)-epimer of ramipril),



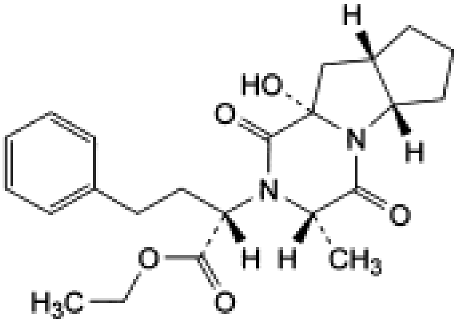
I. (2*S*,3a*S*,6a*S*)-1-[(2*R*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid ((*S,R,S,S,S*)-epimer of ramipril),



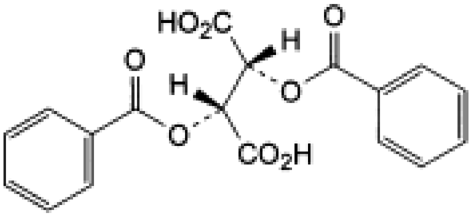
J. (2*R*,3a*R*,6a*R*)-1-[(2*R*)-2-[[(1*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (enantiomer of ramipril),



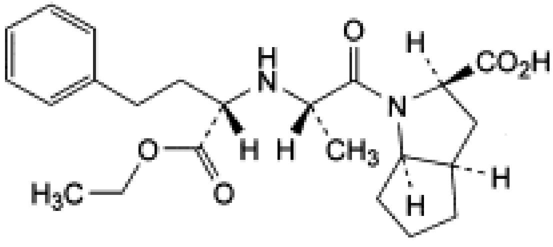
K. (2*S*)-2-[(3*S*,5a*S*,8a*S*,9a*S*)-3-methyl-1,4-dioxodecahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-a] pyrazin-2-yl]-4-phenylbutanoic acid (ramipril diketopiperazine acid),



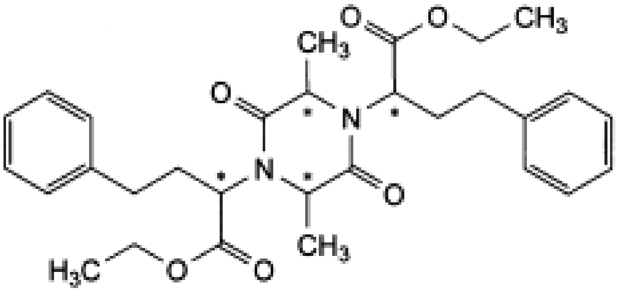
L. ethyl (2*S*)-2-[(3*S*,5a*S*,8a*S*,9a*S*)-9a-hydroxy-3-methyl-1,4-dioxodecahydro-2*H*-cyclopenta [4,5]pyrrolo[1,2-*a*]pyrazin-2-yl]-4-phenylbutanoate (ramipril hydroxydiketopiperazine),



M. (2*R*,3*R*)-2,3-bis(benzoyloxy)butanedioic acid (dibenzoyltartric acid),



N. (2*R*,3a*R*,6a*R*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid ((*S,S,R,R,R*)-isomer of ramipril),

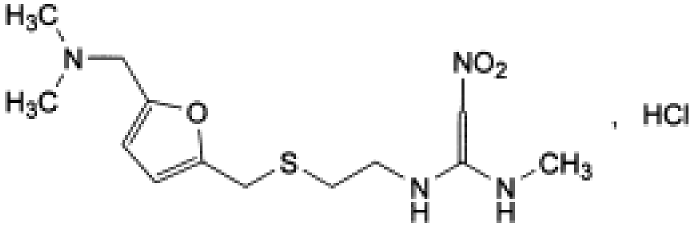


O. diethyl 2,2′-(2,5-dimethyl-3,6-dioxopiperazine-1,4-diyl)bis(4-phenylbutanoate).

Ranitidine Hydrochloride

General Notices

(Ph Eur monograph 0946)



$C_{13}H_{22}N_4O_3S,HCl$ 350.9 *71130-06-8*

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

Preparations

Ranitidine Injection

Ranitidine Oral Solution

Ranitidine Tablets

Ph Eur

DEFINITION

N-[2-[[[5-[(Dimethylamino)methyl]furan-2-yl]methyl]sulphanyl]ethyl]-*N′*-methyl-2-nitroethene-1,1-diamine hydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or pale yellow, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble or slightly soluble in anhydrous ethanol, very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ranitidine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve 10 mg of the substance to be examined and 10 mg of the reference substance separately in 0.5 ml of *methanol R* in an agate mortar. Evaporate to dryness under a stream of *nitrogen R*. Dry the residues under vacuum for 30 min. Add 3 drops of *liquid paraffin R* to the residues and triturate until the mull shows a milky appearance. Compress the mulls between 2 plates transparent to infrared radiation and record new spectra.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 6.0 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Buffer solution

Dissolve 6.8 g of *potassium dihydrogen phosphate R* in 950 ml of *water R*. Adjust to pH 7.1 with *strong sodium hydroxide solution R* and dilute to 1000 ml with *water R*.

Test solution Dissolve 13 mg of the substance to be examined in mobile phase A and dilute to 100 ml with mobile phase A.

Reference solution (a) Dissolve 6.5 mg of *ranitidine for system suitability CRS* (containing impurities A, D and H) in mobile phase A and dilute to 50 ml with mobile phase A.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (c) Dissolve the contents of a vial of *ranitidine impurity J CRS* in 1 ml of test solution.

Column:

— *size:* *l* = 0.1 m, Ø = 4.6 mm;

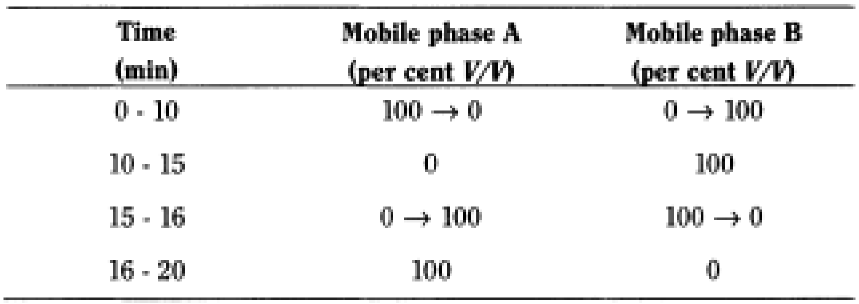
— *stationary phase*: *octadecylsilyl amorphous organosilica polymer R* (3.5 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R*, *buffer solution* (2:98 *V/V*);

— *mobile phase B*: *acetonitrile R*, *buffer solution* (22:78 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100 → 0	0 → 100
10 - 15	0	100
15 - 16	0 → 100	100 → 0
16 - 20	100	0

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl of the test solution, reference solutions (a), (b) and (c) and mobile phase A as a blank.

Relative retention with reference to ranitidine (retention time = about 6.8 min): impurity H = about 0.1; impurity G = about 0.2; impurity F = about 0.4; impurity B = about 0.5; impurity C = about 0.6; impurity E = about 0.7; impurity D = about 0.8; impurity J = about 0.9; impurity I = about 1.3; impurity A = about 1.7.

System suitability:

— *resolution*: minimum 1.5 between the peaks due to impurity J and ranitidine in the chromatogram obtained with reference solution (c);

— the chromatogram obtained with reference solution (a) is similar to the chromatogram supplied with *ranitidine for system suitability CRS*;

— the chromatogram obtained with the blank solution does not show any peak with the same relative retention as the peak due to impurity A in the chromatogram obtained with reference solution (a).

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity J by 2;

— *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities B, C, D, E, F, G, H, I, J*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *any other impurity*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *sum of impurities other than A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the blank.

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution*

(10 ppm Pb) R.

Loss on drying (*2.2.32*)

Maximum 0.75 per cent, determined on 1.000 g by drying under high vacuum at 60 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.280 g in 35 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* is equivalent to 35.09 mg of $C_{13}H_{23}ClN_4O_3S$.

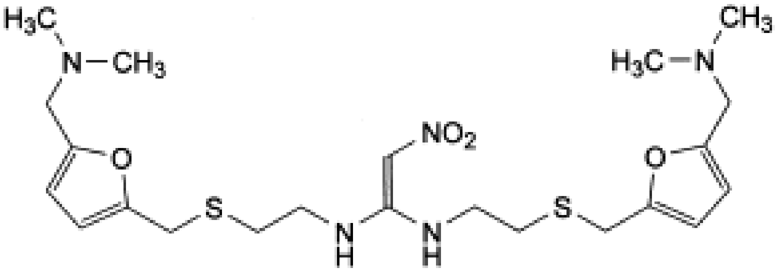
STORAGE

In airtight container , protected from light.

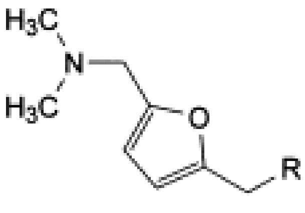
IMPURITIES

Specified impurities   *A, B, C, D, E, F, G, H, I, J*.

Other detectable impurities   (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): K.*



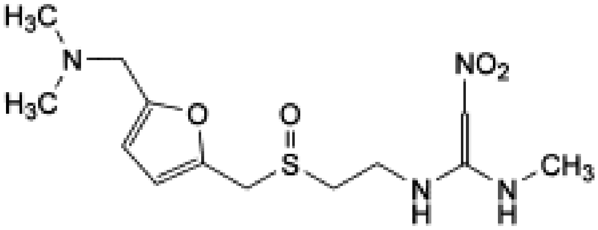
A. *N*,*N*′-bis[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulphanyl]ethyl]-2-nitroethene-1, 1-diamine,



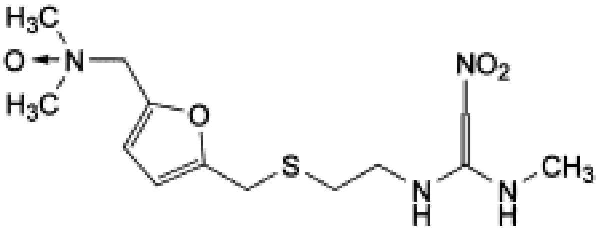
B. R = S-CH_2-CH_2-NH_2: 2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulphanyl] ethanamine,

D. R = $S-CH_2-CH_2-NH-CO-CH_2-NO_2$: *N*-[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl] sulphanyl]ethyl]-2-nitroacetamide,

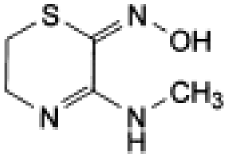
F. R = OH: [5-[(dimethylamino)methyl]furan-2-yl]methanol,



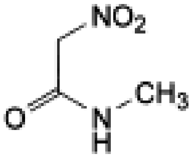
C. *N*-[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulphinyl]ethyl]-*N′*-methyl-2-nitroethene-1,1-diamine,



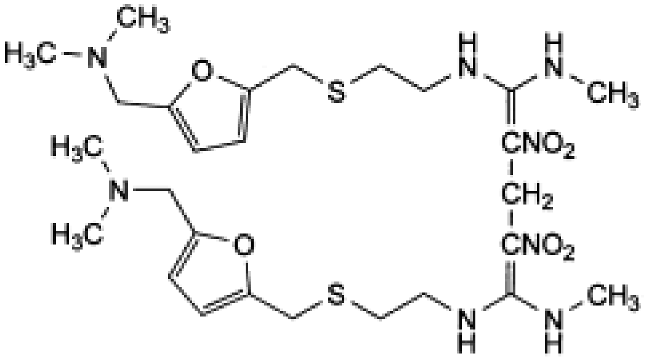
E. *N*-[2-[[[5-[(dimethyloxidoamino)methyl]furan-2-yl]methyl]sulphanyl]ethyl]-*N′*-methyl-2-nitroethene-1,1-diamine,



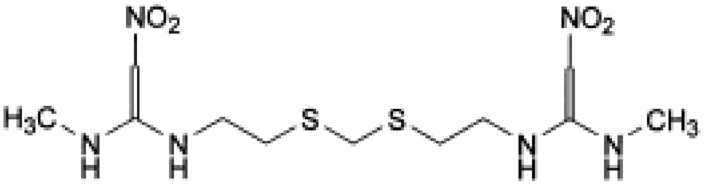
G. 3-(methylamino)-5,6-dihydro-2*H*-1,4-thiazin-2-one-oxime,



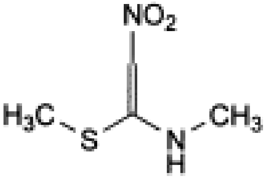
H. *N*-methyl-2-nitroacetamide,



I. 2,2′-methylenebis[*N*-[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulphanyl]ethyl]-*N*′-methyl-2-nitroethene-1,1-diamine],



J. 1,1′-*N*-[methylenebis(sulphanediylethylene)]bis(*N*′-methyl-2-nitroethene-1,1-diamine),



K. *N*-methyl-1-methylthio-2-nitroethenamine.

Ph Eur

Refined Rapeseed Oil

General Notices

(Ph Eur monograph 1369)

Ph Eur

DEFINITION

Fatty oil obtained from the seeds of *Brassica napus* L. and *Brassica campestris* L. by mechanical expression or by extraction. It is then refined. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, light yellow liquid.

Solubility

Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C).

Relative density

About 0.917.

Refractive index

About 1.473.

IDENTIFICATION

Identification of fatty oils by thin-layer chromatography *(2.3.2)*.

Results The chromatogram obtained is similar to the typical chromatogram for rapeseed oil.

TESTS

Acid value *(2.5.1)*

Maximum 0.5, determined on 10.0 g.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0.

Unsaponifiable matter *(2.5.7)*

Maximum 1.5 per cent, determined on 5.0 g.

Alkaline impurities ***(2.4.19)***

It complies with the test.

Composition of fatty acids ***(2.4.22, Method A)***

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

— *palmitic acid*: 2.5 per cent to 6.0 per cent;

— *stearic acid*: maximum 3.0 per cent;

— *oleic acid*: 50.0 per cent to 67.0 per cent;

— *linoleic acid*: 16.0 per cent to 30.0 per cent;

— *linolenic acid*: 6.0 per cent to 14.0 per cent;

— *eicosenoic acid*: maximum 5.0 per cent;

— *erucic acid*: maximum 2.0 per cent.

STORAGE

In an airtight, well-filled container, protected from light.

LABELLING

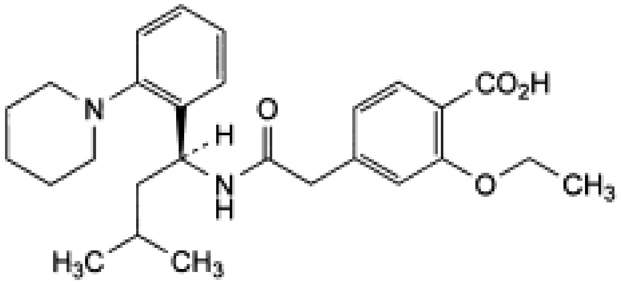
The label states whether the oil is obtained by mechanical expression or by extraction.

Ph Eur

Repaglinide

General Notices

(Ph Eur monograph 2135)



$C_{27}H_{36}N_2O_4$ 452.6 *135062-92-1*

Action and use

Stimulates insulin release; treatment of diabetes mellitus.

Ph Eur

DEFINITION

2-Ethoxy-4-[2-[[(1*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl]amino]-2-oxoethyl]benzoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): + 6.3 to + 7.7.

Dissolve 1.00 g in *methanol R* and dilute to 20.0 ml with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *repaglinide CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Enantiomeric purity

Liquid chromatography (*2.2.29*). *Prepare the solutions in amber flasks and vials*.

Test solution Dissolve 10.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *repaglinide impurity E CRS* in *methanol R* and dilute to 50.0 ml with the same of solvent.

Reference solution (b) Dilute 2.0 ml of reference solution (a) to 100.0 ml with *methanol R*.

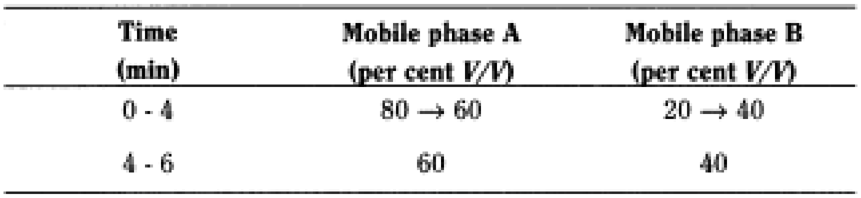
Reference solution (c) Mix 1.0 ml of the test solution and 10 ml of reference solution (a) and dilute to 50.0 ml with methanol R.

Column:
— *size:* *l* = 0.1 m, Ø = 4.0 mm;

— *stationary phase*: *silica gel AGP for chiral chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 1.0 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 4.7 with *dilute sodium hydroxide solution R*;

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	80 → 60	20 → 40
4 - 6	60	40

Equilibration after installation of the column for use Using *water R*, slowly increase the flow rate from 0.2 ml/min to 0.5 ml/min. Maintain the flow rate at 0.5 ml/min for 5 min. The column must be washed for 1 h at a flow rate of 1 ml/min with *water R* and for 1 h with the mobile phase at the initial composition prior to the 1st analysis.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl of the test solution and reference solutions (b) and (c).

Retention time Repaglinide = about 3.3 min; impurity E = about 5.0 min.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to repaglinide and impurity E.

Limit:

— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with *acetonitrile R*. Dilute 2.0 ml of this solution to 100.0 ml with *acetonitrile R*.

Reference solution (b) With the aid of an ultrasonic bath, dissolve the contents of 1 vial of *repaglinide for system suitability CRS* in 2.0 ml of *acetonitrile R*.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

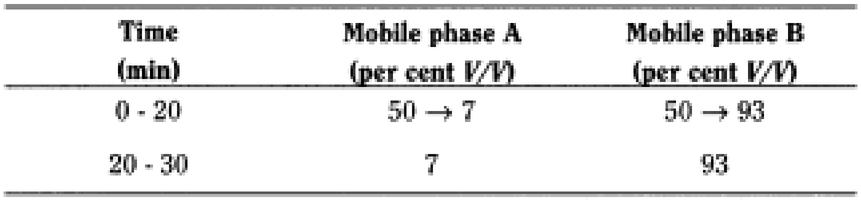
— *stationary phase*: *silica gel for chromatography, alkyl-bonded for use with highly aqueous mobile phases R* (5 µm);

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase* A: 4.0 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.2 with *dilute phosphoric acid R*;

— *mobile phase B*: mobile phase A, *acetonitrile R* (300:700 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	50 → 7	50 → 93
20 - 30	7	93

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl.

Relative retention With reference to repaglinide (retention time = about 10 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.4; impurity D = about 1.5.

System suitability

Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to impurity B and impurity C;

— the chromatogram obtained is similar to the chromatogram supplied with *repaglinide for system suitability CRS*.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity B = 0.7; impurity C = 3.1;

— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.320 g in 10 ml *methanol R* and add 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

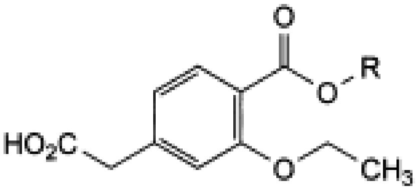
1 ml of *0.1 M perchloric acid* is equivalent to 45.26 mg of $C_{27}H_{36}N_2O_4$.

STORAGE

Protected from light.

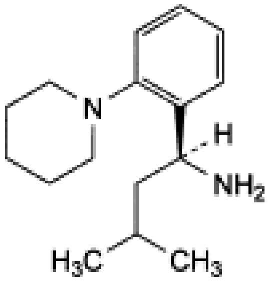
IMPURITIES

Specified impurities *A, B, C, D, E.*

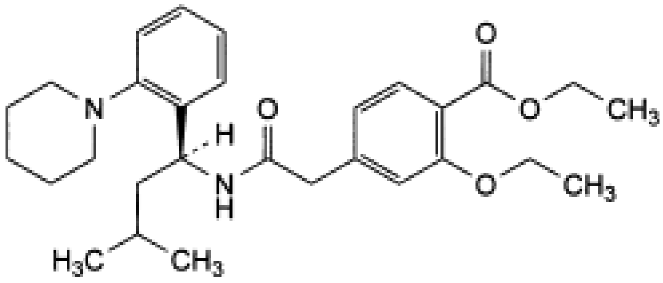


A. R = H: 4-(carboxymethyl)-2-ethoxybenzoic acid,

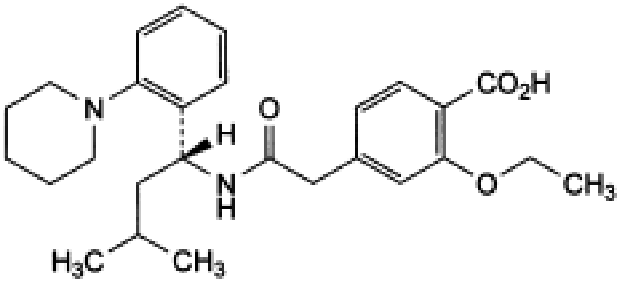
B. R = C_2H_5: [3-ethoxy-4-(ethoxycarbonyl)phenyl]acetic acid,



C. (1*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butan-1-amine,



D. ethyl 2-ethoxy-4-[2-[[(1*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl]amino]-2-oxoethyl] benzoate,



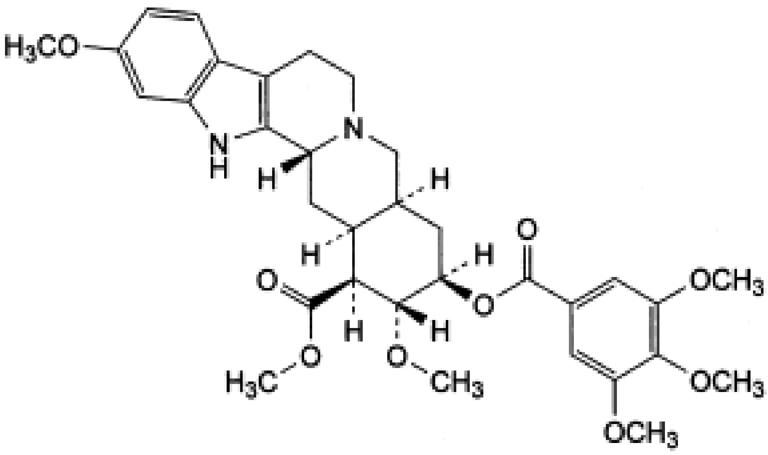
E. 2-ethoxy-4-[2-[[(1*R*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl]amino]-2-oxoethyl]benzoic acid.

Ph Eur

Reserpine

General Notices

(Ph Eur monograph 0528)



$C_{33}H_{40}N_2O_9$ 609 *50-55-5*

Action and use

Rauwolfia alkaloid; treatment of hypertension.

Ph Eur

DEFINITION

Methyl 11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimban-16β-carboxylate.

Content:

- *reserpine*: 98.0 per cent to 102.0 per cent (dried substance);
- *total alkaloids*: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, small crystals or crystalline powder, darkening slowly on exposure to light.

Solubility

Practically insoluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B.*

Second identification *A, C, D, E.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 20.0 mg in *chloroform R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Examine immediately.

Spectral range 230-350 nm.

Absorption maximum At 268 nm.

Specific absorbance at the absorption maximum 265 to 285.

Over the range 288-295 nm, the curve shows a slight absorption minimum followed by a shoulder or a slight absorption maximum; over this range, the specific absorbance is about 170.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *reserpine CRS.*

C. To about 1 mg add 0.1 ml of a 1 g/l solution of *sodium molybdate R* in *sulphuric acid R*. A yellow colour is produced which becomes blue within 2 min.

D. To about 1 mg add 0.2 ml of a freshly prepared 10 g/l solution of *vanillin R* in *hydrochloric acid R*. A pink colour develops within 2 min.

E. Mix about 0.5 mg with 5 mg of *dimethylaminobenzaldehyde R* and 0.2 ml of *glacial acetic acid R* and add 0.2 ml of *sulphuric acid R*. A green colour is produced. Add 1 ml of *glacial acetic acid R*. The colour becomes red.

TESTS

Specific optical rotation *(2.2.7)*

- 116 to - 128 (dried substance).

Dissolve 0.250 g in *chloroform R* and dilute to 25.0 ml with the same solvent. Examine immediately.

Oxidation products

Dissolve 20 mg in *glacial acetic acid R* and dilute to 100.0 ml with the same acid. The absorbance (*2.2.25*) measured immediately at the absorption maximum at 388 nm is not greater than 0.10.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 0.500 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 667 Pa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 0.5 g.

ASSAY

Total alkaloids

Dissolve 0.500 g in a mixture of 6 ml of *acetic anhydride R* and 40 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 60.9 mg of total alkaloids.

Reserpine

Protect the solutions from light. Moisten 25.0 mg with 2 ml of *ethanol (96 per cent) R*, add 2 ml of *0.25 M sulphuric acid* and 10 ml of *ethanol (96 per cent) R*, and warm gently to dissolve. Cool and dilute to 100.0 ml with *ethanol (96 per cent) R*. Dilute 5.0 ml of this solution to 50.0 ml with *ethanol (96 per cent) R*. Prepare a reference solution in the same manner using 25.0 mg of *reserpine CRS*. Place 10.0 ml of each solution separately in 2 boiling-tubes, add 2.0 ml of *0.25 M sulphuric acid* and 2.0 ml of a freshly prepared 3 g/l solution of *sodium nitrite R*. Mix and heat in a water-bath at 55 °C for 35 min. Cool, add 1.0 ml of a freshly prepared 50 g/l solution of *sulphamic acid R* and dilute to 25.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) of each solution at the absorption maximum at 388 nm, using as the compensation liquid 10.0 ml of the same solution prepared at the same time in the same manner, but omitting the sodium nitrite.

Calculate the content of $C_{33}H_{40}N_2O_9$ from the absorbances measured and the concentrations of the solutions.

STORAGE

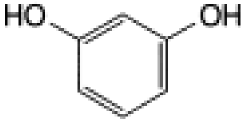
Protected from light.

Ph Eur

Resorcinol

General Notices

(Ph Eur monograph 0290)



$C_6H_6O_2$ 110.1 *108-46-3*

Action and use

Keratolytic.

Ph Eur

DEFINITION

Resorcinol contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of benzene-1,3-diol, calculated with reference to the dried substance.

CHARACTERS

A colourless or slightly pinkish-grey, crystalline powder or crystals, turning red on exposure to light and air, very soluble in water and in alcohol.

IDENTIFICATION

A. Melting point (*2.2.14*): 109 °C to 112 °C.

B. Dissolve 0.1 g in 1 ml of *water R*, add 1 ml of *strong sodium hydroxide solution R* and 0.1 ml of *chloroform R*, heat and allow to cool. An intense, deep-red colour develops which becomes pale yellow on the addition of a slight excess of *hydrochloric acid R*.

C. Thoroughly mix about 10 mg with about 10 mg of *potassium hydrogen phthalate R*, both finely powdered. Heat over a naked flame until an orange-yellow colour is obtained. Cool and add 1 ml of *dilute sodium hydroxide solution R* and 10 ml of *water R* and shake to dissolve. The solution shows an intense green fluorescence.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_5 or R_5 (*2.2.2, Method II*) and remains so when heated in a water-bath for 5 min.

Acidity or alkalinity

To 10 ml of solution S add 0.05 ml of *bromophenol blue solution R2*. Not more than 0.05 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.5 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dilute 0.1 ml of the test solution to 20 ml with *methanol R*.

Apply separately to the plate 2 µl of each solution. Develop over a path of 15 cm using a mixture of 40 volumes of *ethyl acetate R* and 60 volumes of *hexane R*. Allow the plate to dry in air for 15 min and expose it to iodine vapour. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Pyrocatechol

To 2 ml of solution S add 1 ml of *ammonium molybdate solution R2* and mix. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 2 ml of a 0.1 g/l solution of *pyrocatechol R*.

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 1.00 g of powdered substance by drying in a desiccator for 4 h.

Sulphated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in *water R* and dilute to 250.0 ml with the same solvent. To 25.0 ml of the solution in a ground-glass-stoppered flask add 1.0 g of *potassium bromide R*, 50.0 ml of *0.0167 M potassium bromate*, 15 ml of *chloroform R* and 15.0 ml of *hydrochloric acid R1*. Stopper the flask, shake and allow to stand in the dark for 15 min, shaking occasionally. Add 10 ml of a 100 g/l solution of *potassium iodide R*, shake thoroughly, allow to stand for 5 min and titrate with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution R* as indicator.

1 ml of *0.0167 M potassium bromate* is equivalent to 1.835 mg of $C_6H_6O_2$.

STORAGE

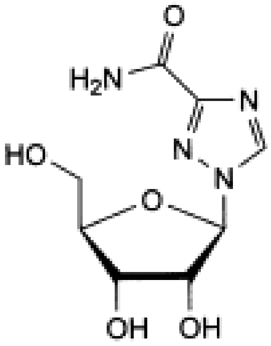
Store protected from light.

Ph Eur

Ribavirin

General Notices

(Ph Eur monograph 2109)



$C_8H_{12}N_4O_5$ 244.2 *36791-04-5*

Action and use

Antiviral (hepatitis C, respiratory syncytial virus).

Preparation

Ribavirin Nebuliser Solution

Ph Eur

DEFINITION

1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), slightly soluble or very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ribavirin CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

pH (*2.2.3*)

4.0 to 6.5.

Dissolve 0.200 g in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Specific optical rotation (*2.2.7*)

- 33 to - 37 (dried substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent. Determine the specific optical rotation within 10 min of preparing the solution.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *ribavirin for system suitability CRS* in 2.0 ml of the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *ribavirin CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Column:

— *size:* l = 0.10 m, Ø = 7.8 mm;

— *stationary phase*: *strong cation-exchange resin R* (9 µm);

— *temperature*: 40 °C.

Mobile phase *water R* adjusted to pH 2.5 with *sulphuric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 207 nm.

Injection 10 µl of test solution (a) and reference solutions (a) and (b).

Run time 11 times the retention time of ribavirin.

Relative retention With reference to ribavirin (retention time = about 4 min): impurity F = about 1.2.

System suitability Reference solution (b):

- *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ribavirin.

Limits:

- *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 4.0 g in 20 ml of *water R*, with heating if necessary. 12 ml of the solution complies with limit test A. Prepare the reference solution using 10 ml of *lead standard solution (2 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_8H_{12}N_4O_5$ using the declared content of *ribavirin CRS*.

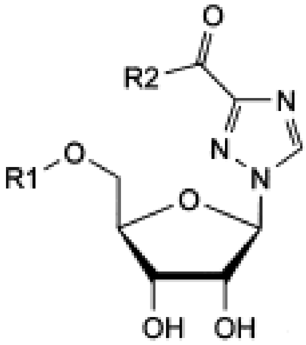
STORAGE

Protected from light.

IMPURITIES

Specified impurities F.

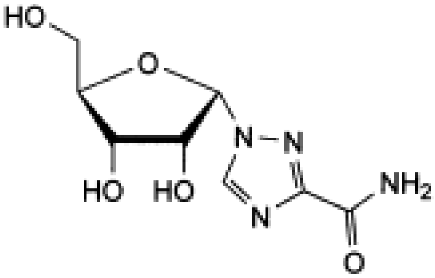
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, D, E, G.*



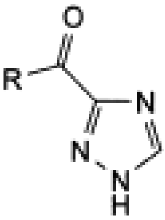
A. R1 = H, R2 = OH: 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid,

E. R1 = CO-C_6H_5, R2 = NH_2: 1-(5-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide (5′-*O*-benzoylribavirin),

F. R1 = CO-CH_3, R2 = NH_2: 1-(5-*O*-acetyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide (5′-*O*-acetylribavirin),

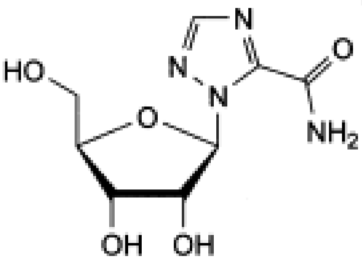


B. 1-α-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide (anomer),



C. R = OH: 1*H*-1,2,4-triazole-3-carboxylic acid,

D. R = NH_2: 1*H*-1,2,4-triazole-3-carboxamide,



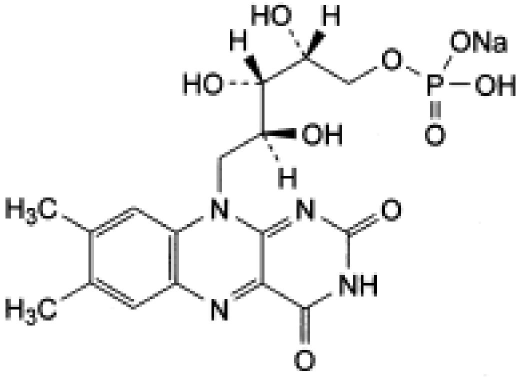
G. 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-5-carboxamide (*N*-isomer).

Ph Eur

Riboflavin Sodium Phosphate

General Notices

(Ph Eur monograph 0786)



$C_{17}H_{20}N_4NaO_9P$ 478.3 *130-40-5*

Action and use

Vitamin B_2.

Preparation

Vitamins B and C Injection

Ph Eur

DEFINITION

Mixture containing riboflavin 5′-(sodium hydrogen phosphate) as the main component and other riboflavin sodium monophosphates.

Content

73.0 per cent to 79.0 per cent of riboflavin ($C_{17}H_{20}N_4O_6$; M_r 376.4) (dried substance).

It contains a variable amount of water.

CHARACTERS

Appearance

Yellow or orange-yellow, crystalline, hygroscopic powder.

Solubility

Soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *phosphate buffer solution pH 7.0 R* and dilute to 100.0 ml with the same buffer solution. Dilute 2.0 ml of this solution to 100.0 ml with *phosphate buffer solution pH 7.0 R*.

Spectral range 230-350 nm.

Absorption maximum At 266 nm.

Specific absorbance at the absorption maximum 580 to 640.

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in position and approximate size to the principal peak in the chromatogram obtained with reference solution (b).

C. Dissolve about 10 mg in *dilute sodium hydroxide solution R* and dilute to 100 ml with the same solution. Expose 1 ml of this solution to ultraviolet light at 254 nm for 5 min, add *sufficient acetic acid R* to make the solution acidic to *blue litmus paper R* and shake with 2 ml of *methylene chloride R*. The lower layer shows yellow fluorescence.

D. To 0.5 g add 10 ml of *nitric acid R* and evaporate the mixture to dryness on a water-bath. Ignite the residue until it becomes white, dissolve the residue in 5 ml of *water R* and filter. The filtrate gives reaction (a) of sodium and reaction (b) of phosphates (*2.3.1*).

TESTS

pH (*2.2.3*)

5.0 to 6.5.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Specific optical rotation (*2.2.7*)

+ 38.0 to + 43.0 (dried substance).

Dissolve 0.300 g in 18.2 ml of *hydrochloric acid R1* and dilute to 25.0 ml with *water R*.

Impurity E

To about 35 mg add 10 ml of *methylene chloride R*, shake for 5 min and filter. The filtrate is not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from actinic light.*

Test solution Dissolve 0.100 g of the substance to be examined in 50 ml of *water R* and dilute to 100.0 ml with the mobile phase. Dilute 8.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 60 mg of *riboflavin CRS* (impurity D) in 1 ml of *hydrochloric acid R* and dilute to 250.0 ml with *water R*. Dilute 4.0 ml of this solution to 100.0 ml with the

mobile phase.

Reference solution (b) Dissolve 0.100 g of *riboflavin sodium phosphate CRS* in 50 ml of *water R* and dilute to 100.0 ml with the mobile phase. Dilute 8.0 ml of this solution to 50.0 ml with the mobile phase.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, 7.35 g/l solution of *potassium dihydrogen phosphate R* (150:850 V/V).

Flow rate 2 ml/min.

Detection Spectrophotometer at 266 nm.

Injection 100 µl.

Run time Until the peak due to riboflavin can be clearly evaluated.

Relative retention With reference to riboflavin 5′-monophosphate (retention time = about 20 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.5; riboflavin 3′-monophosphate = about 0.7; riboflavin 4′-monophosphate = about 0.9; impurity D = about 2.

System suitability Reference solution (b):

- *resolution*: minimum 1.5 between the peaks due to riboflavin 4′-monophosphate and riboflavin 5′-monophosphate.

Calculate the percentage content of free riboflavin (impurity D) and of riboflavin in the form of the diphosphates of riboflavin (impurities A, B, C) from the areas of the peaks in the chromatogram obtained with the test solution and the amount of free riboflavin in reference solution (a).

Limits:
- *impurity D*: maximum 6.0 per cent (dried substance);
- *sum of impurities A, B and C*: maximum 6.0 per cent (dried substance).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Inorganic phosphate

Maximum 1.5 per cent.

Dissolve 0.10 g in *water R* and dilute to 100 ml with the same solvent. To 5 ml of this solution, add 10 ml of *water R*, 5 ml of *buffered copper sulphate solution pH 4.0 R*, 2 ml of a 30 g/l solution of *ammonium molybdate R*, 1 ml of a freshly prepared solution containing 20 g/l of 4-*methylaminophenol sulphate R* and 50 g/l of *sodium metabisulphite R*, and 1 ml of a 3 per cent *V/V* solution of *perchloric acid R*. Dilute to 25.0 ml with *water R* and measure, within 15 min of its preparation, the absorbance (*2.2.25*) of the solution at 800 nm, using as the compensation liquid a solution prepared in the same manner but without the substance to be examined. The absorbance is not greater than that of a solution prepared as follows: to 15 ml of *phosphate standard solution (5 ppm PO_4) R*, add 5 ml of *buffered copper sulphate solution pH 4.0 R*, 2 ml of a 30 g/l solution of *ammonium molybdate R*, 1 ml of a freshly prepared solution containing 20 g/l of *4-methylaminophenol sulphate R* and 50 g/l of *sodium metabisulphite R,* and 1 ml of a 3 per cent *V/V* solution of *perchloric acid R*; dilute to 25.0 ml with *water R*.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

To 2.0 g in a silica crucible add 2 ml of *nitric acid R*, dropwise, followed by 0.25 ml of *sulphuric acid R*. Heat cautiously until white fumes are evolved and ignite. Extract the cooled residue with 2 quantities, each of 2 ml, of *hydrochloric acid R* and evaporate the extracts to dryness. Dissolve the residue in 2 ml of *dilute acetic acid R* and dilute to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)

Maximum 8.0 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa for 5 h.

ASSAY

Carry out the assay protected from light.

Dissolve 0.100 g in 150 ml of *water R*, add 2 ml of *glacial acetic acid R* and dilute to 1000.0 ml with *water R*. To 10.0 ml of this solution add 3.5 ml of a 14 g/l solution of *sodium acetate R* and dilute to 50.0 ml with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 444 nm.

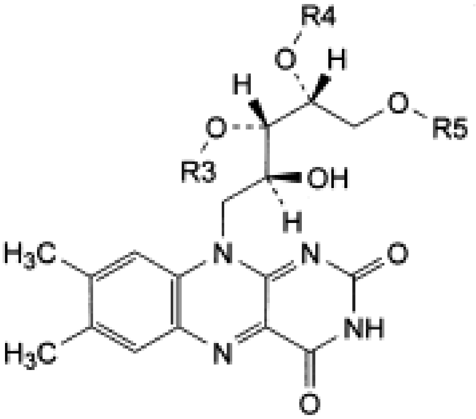
Calculate the content of $C_{17}H_{20}N_4O_6$ taking the specific absorbance to be 328.

STORAGE

In an airtight container , protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*

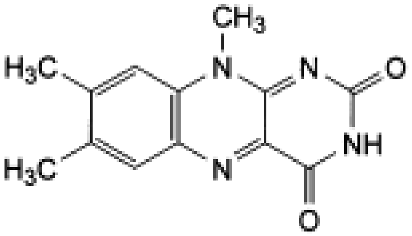


A. R3 = R4 = PO_3H_2, R5 = H: riboflavin 3′,4′-diphosphate,

B. R3 = R5 = PO_3H_2, R4 = H: riboflavin 3′,5′-diphosphate,

C. R3 = H, R4 = R5 = PO_3H_2: riboflavin 4′,5′-diphosphate,

D. R3 = R4 = R5 = H: riboflavin,



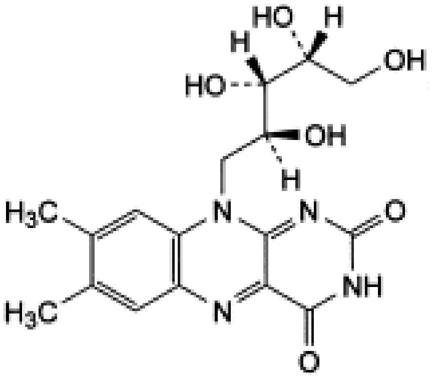
E. 7,8,10-trimethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (lumiflavin).

Ph Eur

Riboflavin

General Notices

(Ph Eur monograph 0292)



$C_{17}H_{20}N_4O_6$ 376.4 *83-88-5*

Action and use

Vitamin B_2.

Ph Eur

DEFINITION

7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(3H,10*H*)-dione.

This monograph applies to riboflavin produced by fermentation.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow or orange-yellow, crystalline powder.

Solubility

Very slightly soluble in water, practically insoluble in ethanol (96 per cent).

Solutions deteriorate on exposure to light, especially in the presence of alkali.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution Suspend 25 mg of the substance to be examined in 10 ml of *water R*, shake for 5 min and filter the suspension to remove the undissolved material.

Reference solution Suspend 25 mg of *riboflavin CRS* in 10 ml of *water R*, shake for 5 min and filter the suspension to remove the undissolved material.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *water R*.

Application As follows, drying in a current of cold air after each individual application:

— *1st application*: 2 µl of *methylene chloride R* then 2 µl of the test solution;

— *2nd application*: 2 µl of *methylene chloride R* then 2 µl of the reference solution.

Development Over a path of 6 cm.

Drying In a current of cold air.

Detection Examine in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 1 mg in 100 ml of *water R*. The solution has, by transmitted light, a pale greenish-yellow colour, and, by reflected light, an intense yellowish-green fluorescence which disappears on the addition of mineral acids or alkalis.

TESTS

Specific optical rotation (*2.2.7*)

- 115 to - 135 (dried substance).

Dissolve 50.0 mg in *0.05 M sodium hydroxide* free from carbonate and dilute to 10.0 ml with the same alkaline solution. Measure the optical rotation within 30 min of dissolution.

Absorbance (*2.2.25*)

Test solution Dilute the final solution prepared for the assay with an equal volume of *water R*.

Absorption maxima At 223 nm, 267 nm, 373 nm and 444 nm.

Absorbance ratios:

— A_{373}/A_{267} = 0.31 to 0.33;

— A_{444}/A_{267} = 0.36 to 0.39.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect* *from light.*

Solution A 13.6 g/l solution of *sodium acetate R*.

Test solution With the aid of ultrasound, dissolve 0.120 g of the substance to be examined in 10 ml of *0.1 M sodium hydroxide* and dilute to 100 ml with solution A.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with solution A. Dilute 1.0 ml of this solution to 100.0 ml with solution A.

Reference solution (b) With the aid of ultrasound, dissolve the contents of a vial of *riboflavin for peak identification CRS* (containing impurities C and D) in 1.0 ml of a mixture of 1 volume of mobile phase B and 9 volumes of mobile phase A.

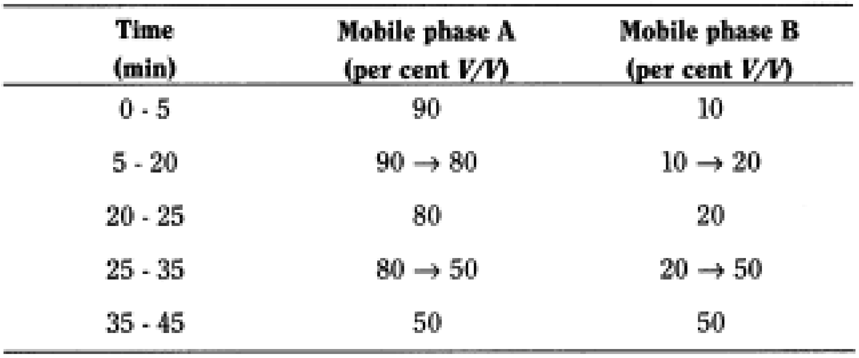
Reference solution (c) In order to prepare in situ impurities A and B, dissolve 10 mg of the substance to be examined in 1 ml of *0.5 M sodium hydroxide*. Expose to daylight for 1.5 h. Add 0.5 ml of *acetic acid R* and dilute to 100 ml with *water R*.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: *phosphoric acid R*, *water R* (1:1000 *V/V*);
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 20	90 → 80	10 → 20
20 - 25	80	20
25 - 35	80 → 50	20 → 50
35 - 45	50	50

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 267 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with riboflavin for *peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities C and D.

Relative retention With reference to riboflavin (retention time = about 16 min): impurity C = about 0.2; impurity D = about 0.5; impurity A = about 1.4; impurity B = about 1.9.

System suitability:

- *resolution*: minimum 5 between the peaks due to impurities A and B in the chromatogram obtained with reference solution (c);
- the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *riboflavin for peak identification CRS*.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.7; impurity B = 1.4; impurity C = 2.3; impurity D = 1.4;

— *impurity A:* not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent);

— *impurities B, C, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit for peaks other than those due to impurity A*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Carry out the assay protected from light.

In a brown-glass 500 ml volumetric flask, suspend 65.0 mg in 5 ml of *water R* ensuring that it is completely wetted and dissolve in 5 ml of *dilute sodium hydroxide solution R*. As soon as dissolution is complete, add 100 ml of *water R* and 2.5 ml of *glacial acetic acid R* and dilute to 500.0 ml with *water R*. Place 20.0 ml of this solution in a 200 ml brown-glass volumetric flask, add 3.5 ml of a 14 g/l solution of *sodium acetate R* and dilute to 200.0 ml with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 444 nm.

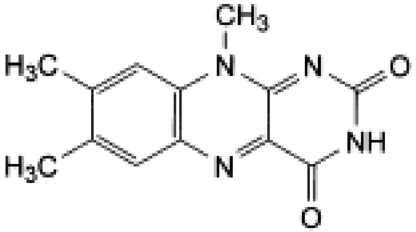
Calculate the content of $C_{17}H_{20}N_4O_6$ taking the specific absorbance to be 328.

STORAGE

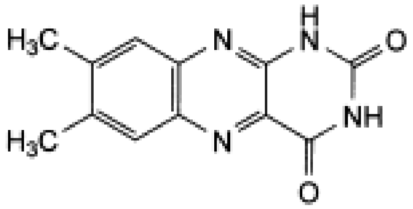
In an airtight container , protected from light.

IMPURITIES

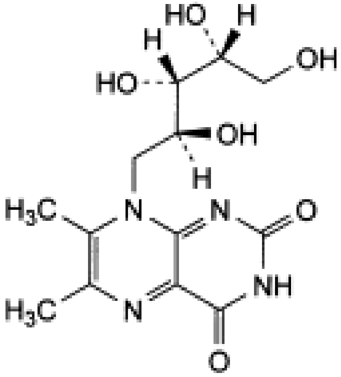
Specified impurities *A, B, C, D.*



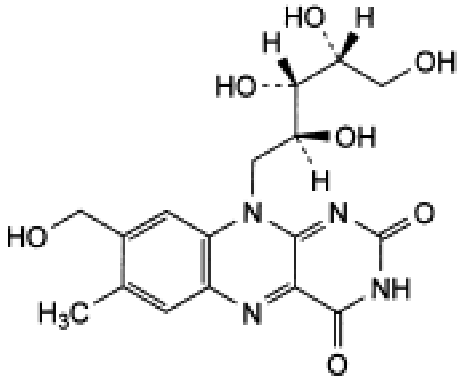
A. 7,8,10-trimethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (lumiflavine),



B. 7,8-dimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione,



C. 6,7-dimethyl-8-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]pteridine-2,4(3*H*,8*H*)-dione,



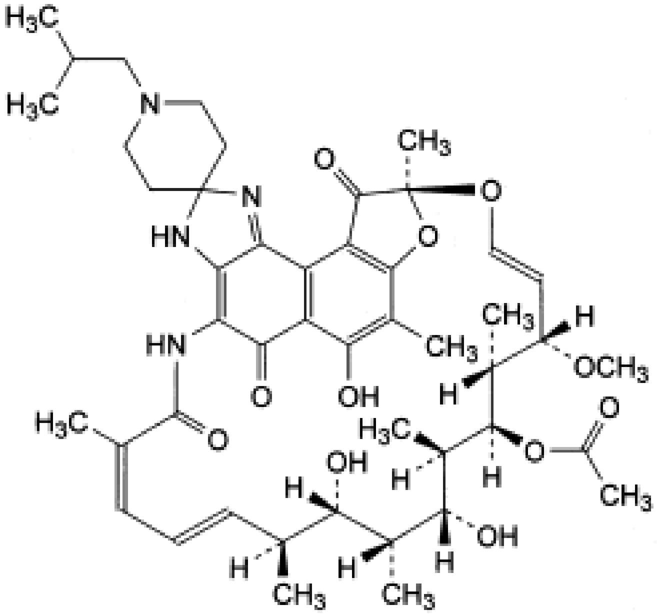
D. 8-(hydroxymethyl)-7-methyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione.

Ph Eur

Rifabutin

General Notices

(Ph Eur monograph 1657)



$C_{46}H_{62}N_4O_{11}$ 847 *72559-06-9*

Action and use

Rifamycin antituberculosis drug.

Ph Eur

DEFINITION

(9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,18,20-trihydroxy-14-methoxy-7,9,15,17,19,21,25-heptamethyl-1′-(2-methylpropyl)-5,10,26-trioxo-3,5,9,10-tetrahydrospiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2′,3′:7,8]naphtho[1,2-*d*]imidazole-2,4′-piperidine]-16-yl acetate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Reddish-violet amorphous powder.

Solubility

Slightly soluble in water, soluble in methanol, slightly soluble in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *rifabutin CRS.*

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.100 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 10 mg of *rifabutin impurity A CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 ml with the same mixture of solvents. Dilute 3 ml of the solution to 100 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetone R*, *light petroleum R* (23:77 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose the plate to iodine vapour for about 5 min, then spray with *potassium iodide and starch solution R* and allow to stand for 5 min.

Limit:

— *impurity A*: any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with the reference solution (0.3 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *rifabutin CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dissolve about 10 mg of *rifabutin CRS* in 2 ml of *methanol R*, add 1 ml of *dilute sodium hydroxide solution R* and allow to stand for about 4 min. Add 1 ml of *dilute hydrochloric acid R* and dilute to 50 ml with the mobile phase.

Column:

— *size*: *l* = 0.110 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix equal volumes of *acetonitrile R* and a 13.6 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 6.5 with *dilute sodium hydroxide solution R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time 2.5 times the retention time of rifabutin.

Relative retention With reference to rifabutin (retention time = about 9 min): impurity E = about 0.5; impurity B = about 0.6; impurity D = about 0.9; impurity C = about 1.3.

System suitability Reference solution (c):

— *resolution*: minimum 2.0 between the second peak of the 3 peaks due to degradation products and the peak due to rifabutin.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent); not more than 1 such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

Maximum 2.5 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.3 per cent, determined on 1.0 g.

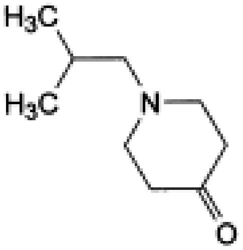
ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

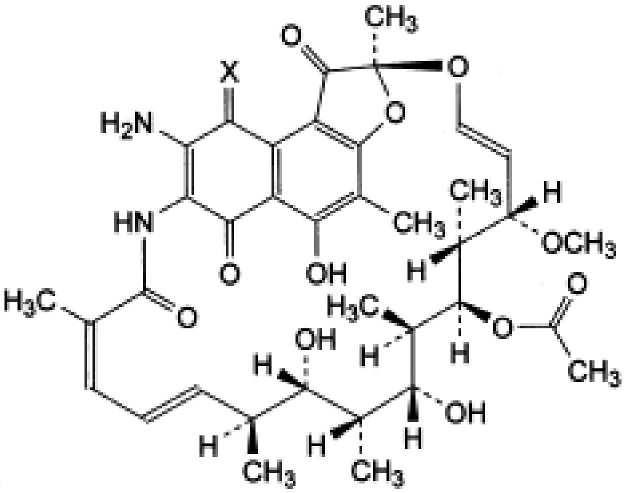
Injection Test solution and reference solution (a).

Calculate the percentage content of ribabutin.

IMPURITIES

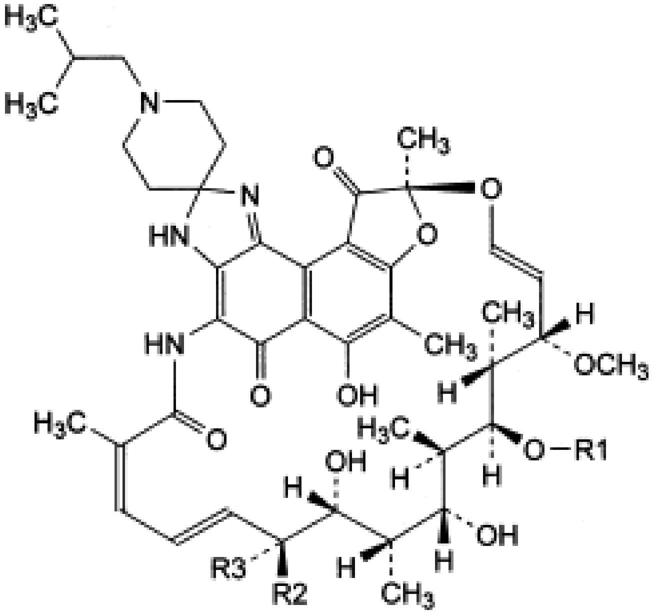


A. 1-(2-methylpropyl)piperidin-4-one,


X
H2N
HN
O
OH
CH3
O
O
CH3
O
OCH3
H
H3C
O
H3C
OH
H
OH
H
CH3
CH3
H
H
CH3
O
CH3

B. X = O: 3-aminorifamycin S,

D. X = NH: 3-amino-4-imidorifamycin S,


H3C
H3C
N
N
HN
HN
O
OH
CH3
O
O
CH3
O
OCH3
H
H3C
O–R1
H3C
OH
H
OH
H
R3
R2
H
CH3
H
H
CH3
O

C. R1 = CO-CH_3, R2 + R3 = CH_2: 21,31-didehydrorifabutin,

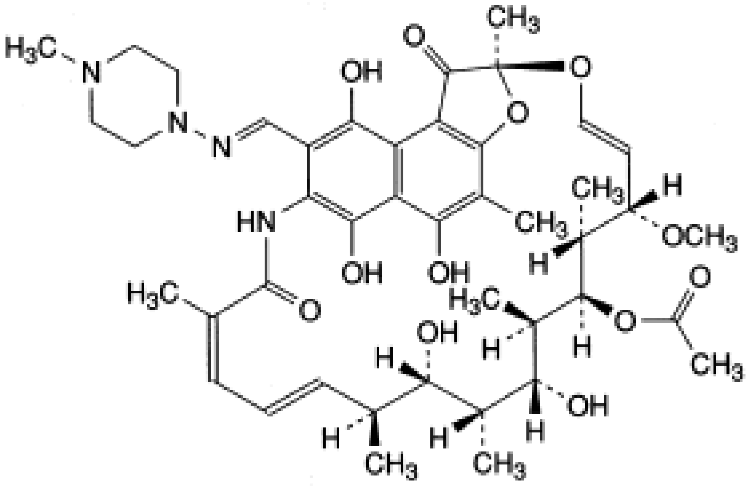
E. R1 = R3 = H, R2 = CH_3: 16-deacetylrifabutin.

Ph Eur

Rifampicin

General Notices

(Ph Eur monograph 0052)



$C_{43}H_{58}N_4O_{12}$ 823 *13292-46-1*

Action and use

Rifamycin antituberculosis drug.

Preparations

Rifampicin Capsules

Rifampicin Oral Suspension

Ph Eur

DEFINITION

(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-5,6,9,17,19-Pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[[(4-methylpiperazin-1-yl)imino]methyl]-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienimino)naphto[2,1-*b*]furan-21-yl acetate.

Semisynthetic antibiotic obtained from rifamycin SV.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Reddish-brown or brownish-red, crystalline powder.

Solubility

Slightly soluble in water, soluble in methanol, slightly soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50 mg in 50 ml of *methanol R*. Dilute 1 ml of this solution to 50 ml with *phosphate buffer solution pH 7.4 R*.

Spectral range 220-500 nm.

Absorption maxima At 237 nm, 254 nm, 334 nm and 475 nm.

Absorbance ratio A_{334}/A_{475} = about 1.75.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Mulls in *liquid paraffin R*.

Comparison *rifampicin CRS.*

C. Suspend about 25 mg in 25 ml of *water R*, shake for 5 min and filter. To 5 ml of the filtrate add 1 ml of a 100 g/l solution of *ammonium persulphate R* in *phosphate buffer solution pH 7.4 R* and shake for a few minutes. The colour changes from orange-yellow to violet-red and no precipitate is formed.

TESTS

pH (*2.2.3*)

4.5 to 6.5 for a 10 g/l suspension in *carbon dioxide-free water R*.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the test solution and the reference solution immediately before use.*

Solvent mixture To 10 volumes of a 210.1 g/l solution of *citric acid R* add 23 volumes of a 136.1 g/l solution of *potassium dihydrogen phosphate R*, 77 volumes of a 174.2 g/l solution of *dipotassium hydrogen phosphate R*, 250 volumes of *acetonitrile R* and 640 volumes of *water R*.

Test solution Dissolve 20.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixure.

Reference solution Dissolve 20.0 mg of *rifampicin quinone CRS* (impurity A) in *acetonitrile R* and dilute to 100.0 ml with the same solvent. To 1.0 ml of this solution add 1.0 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.12 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a solution containing 0.1 per cent *V/V* of *phosphoric acid R*, 1.9 g/l of *sodium perchlorate R*, 5.9 g/l of *citric acid R* and 20.9 g/l of *potassium dihydrogen phosphate R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time Twice the retention time of rifampicin.

System suitability Reference solution:

— *resolution*: minimum 4.0 between the peaks due to rifampicin and impurity A; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *impurity A*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (1.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the peak due to rifampicin in the chromatogram obtained with the reference solution (1.0 per cent);

— *sum of impurities other than A*: not more than 3.5 times the area of the peak due to rifampicin in the chromatogram obtained with the reference solution (3.5 per cent);

— *disregard limit*: 0.05 times the area of the peak due to rifampicin in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (*2.4.8*)

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying at 80 °C at a pressure not exceeding 0.67 kPa for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.100 g in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *phosphate buffer solution pH 7.4 R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 475 nm, using *phosphate buffer solution pH 7.4 R* as the compensation liquid.

Calculate the content of $C_{43}H_{58}N_4O_{12}$, taking the specific absorbance to be 187.

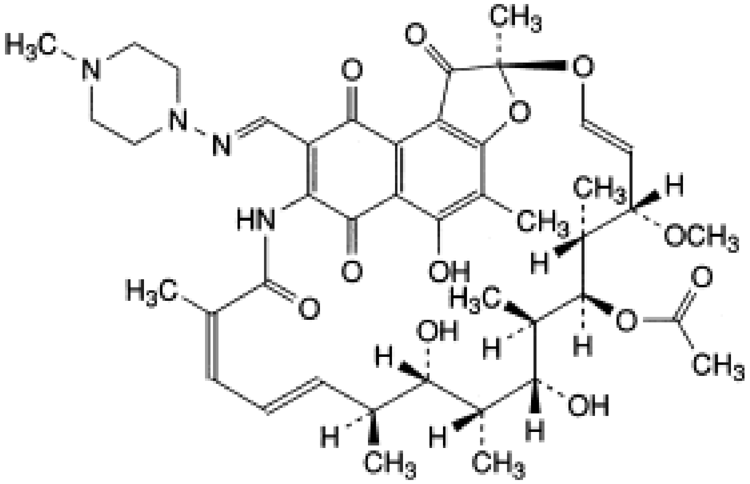
STORAGE

Under nitrogen in an airtight container, protected from light, at a temperature not exceeding 25 °C.

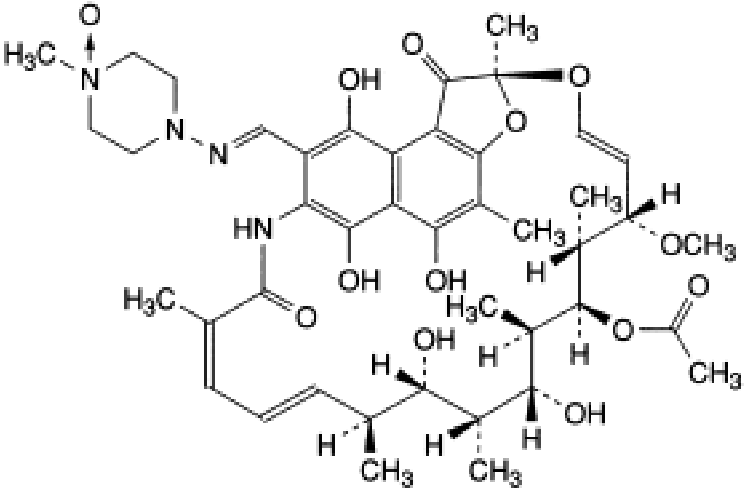
IMPURITIES

Specified impurities   *A.*

Other detectable impurities   (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B.*



A. rifampicin quinone,



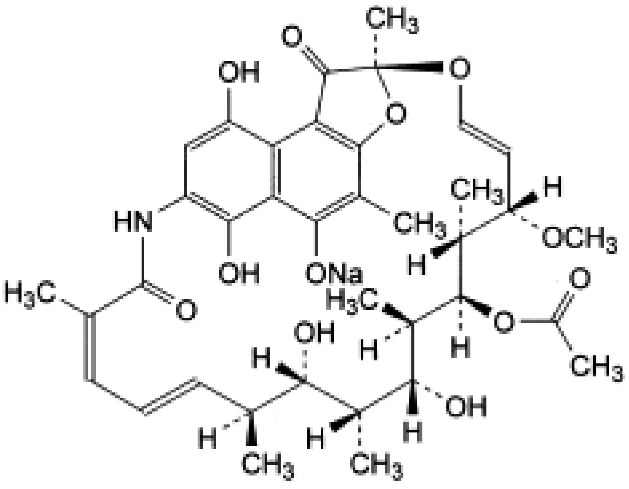
B. rifampicin *N*-oxide.

Ph Eur

Rifamycin Sodium

General Notices

(Ph Eur monograph 0432)



$C_{37}H_{46}NNaO_{12}$ 720 *14897-39-3*

Action and use

Rifamycin antituberculosis drug.

Ph Eur

DEFINITION

Sodium (2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-21-(acetyloxy)-6,9,17,19-tetrahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-*b*]furan-5-olate.

Monosodium salt of rifamycin SV, obtained by chemical transformation of rifamycin B, which is produced during the growth of certain strains of *Amycolatopsis mediterranei*. Rifamycin SV may also be obtained directly from certain A. *mediterranei* mutants.

Potency

Minimum 900 IU/mg (anhydrous substance).

PRODUCTION

It is produced by methods of manufacture designed to minimise or eliminate substances lowering blood pressure.

The manufacturing process is validated to demonstrate that the product, if tested, would comply with the following test.

Abnormal toxicity (*2.6.9*)

Inject into each mouse 4 mg dissolved in 0.5 ml of *water for injections R*.

CHARACTERS

Appearance

Fine or slightly granular, red powder.

Solubility

Soluble in water, freely soluble in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Comparison *rifamycin sodium CRS.*

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*)

6.5 to 8.0.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Absorbance (*2.2.25*)

Dissolve 20.0 mg in 5 ml of *methanol R* and dilute to 100.0 ml with freshly prepared *phosphate buffer solution pH 7.0 R1* to which 1 g/l of *ascorbic acid R* has been added immediately before use. Dilute 5.0 ml of this solution to 50.0 ml with the same phosphate buffer solution containing ascorbic acid. Allow to stand for 30 min. The solution shows an absorption maximum at 445 nm. The specific absorbance at this absorption maximum is 190 to 210 (anhydrous substance).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Solvent mixture Mix 50 volumes of a 3.9 g/l solution of *sodium dihydrogen phosphate R*, adjusted to pH 3.0 with *phosphoric acid R*, and 50 volumes of *acetonitrile R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 10.0 mg of *rifamycin B CRS* (impurity A) and 40.0 mg of *rifamycin S CRS* (impurity B) in the solvent mixture and dilute to 200.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (b) Dissolve 25 mg of the substance to be examined and 8 mg of *rifamycin S CRS* in the solvent mixture and dilute to 250.0 ml with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: mix 10 volumes of *acetonitrile R* and 90 volumes of a 3.9 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 7.5 with dilute sodium hydroxide solution R;
- *mobile phase B*: mix 30 volumes of a 3.9 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R* and 70 volumes of *acetonitrile R*;
- *temperature*: minimum 20 °C;

Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 40	80 → 20	20 → 80
40 - 45	20	80
45 - 47	20 → 80	80 → 20
47 - 55	80	20

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Elution order Impurity A, rifamycin SV, impurity B.

System suitability Reference solution (b):
- *resolution*: minimum 5.0 between the peaks due to rifamycin SV and impurity B.

Limits:
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (2 per cent);
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *sum of impurities other than A and B*: not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (2 per cent);
- *disregard limit*: the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

12.0 per cent to 17.0 per cent, determined on 0.200 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.50 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*).

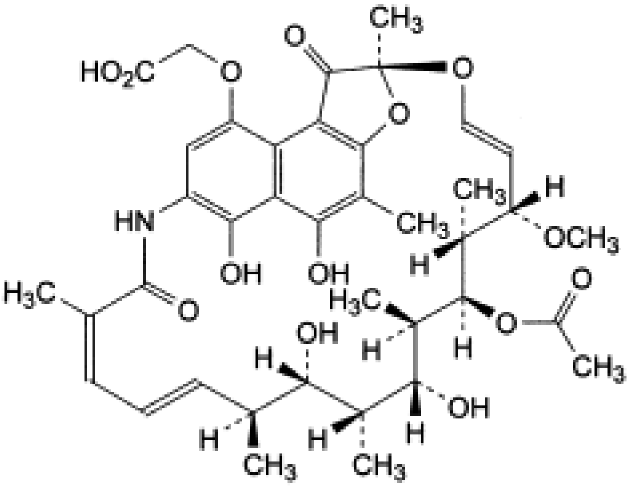
STORAGE

In an airtight container, protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

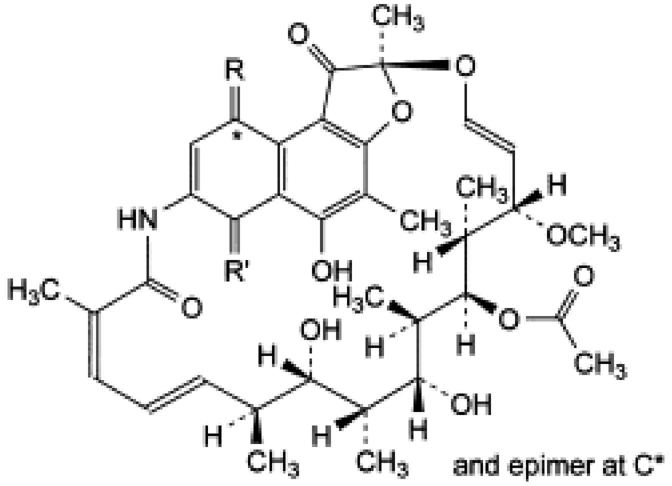
IMPURITIES

Specified impurities *A, B.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C.*



A. rifamycin B,



R
R'
HN
OH
H₃C
O
CH₃
OCH₃
and epimer at C*

B. R = R′ = O: rifamycin S,

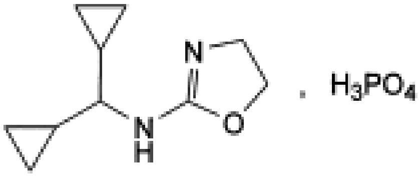
C. -R- = -O-CO-CH$_2$-O-, R′ = O: rifamycin O.

Ph Eur

Rilmenidine Dihydrogen Phosphate

General Notices

(Ph Eur monograph 2020)



$C_{10}H_{19}N_2O_5P$ 278.2 *85409-38-7*

Action and use

Antihypertensive.

Ph Eur

DEFINITION

N-(Dicyclopropylmethyl)-4,5-dihydro-oxazol-2-amine dihydrogen phosphate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, slightly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of rilmenidine dihydrogen phosphate.

B. Dissolve 10 mg in *water R* and dilute to 1 ml with the same solvent. The solution gives reaction (b) of phosphates (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 60.0 mg of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *water R* and dilute 10.0 ml of this solution to 50.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 20.0 ml with *water R*.

Reference solution (c) Dissolve 15.0 mg of *rilmenidine for system suitability CRS* in *water R* and dilute to 5.0 ml with the same solvent.

Column:

— *size*: *l* = 0.15 m, Ø = 3 mm;

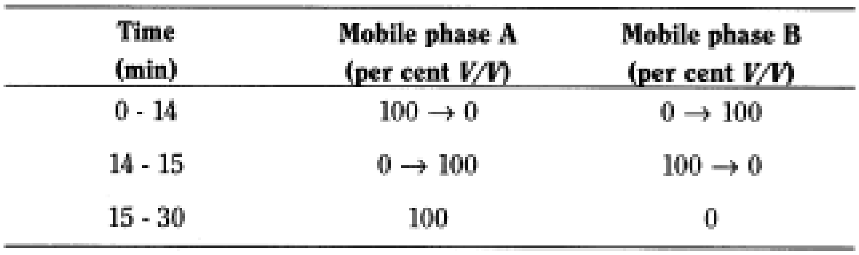
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm and a carbon loading of 25 per cent;

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: dissolve 3 g of *sodium heptanesulphonate R* in *water R* and dilute to 860 ml with the same solvent; add 130 ml of *methanol R2*, 10 ml of *tetrahydrofuran for chromatography R* and 1.0 ml of *phosphoric acid R*;

— *mobile phase B*: dissolve 3 g of *sodium heptanesulphonate R* in *water R* and dilute to 600 ml with the same solvent; add 350 ml of *acetonitrile for chromatography R*, 50 ml of *tetrahydrofuran for chromatography R* and 1.0 ml of *phosphoric acid R,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	100 → 0	0 → 100
14 - 15	0 → 100	100 → 0
15 - 30	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl.

Relative retention With reference to rilmenidine (retention time = about 13 min): impurity A = about 0.6; impurity B = about 0.9; impurity C = about 1.4.

With these conditions the inflexion of the baseline, corresponding to the beginning of the gradient, appears on the recorder after a minimum time t of 5 min. If this is not the case (*t* < 5 min) modify the chromatographic sequence by adding an isocratic elution with 100 per cent of mobile phase A for a time corresponding to (5-*t*) min before the linear gradient.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 3, where H_r = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rilmenidine.

Limits:

- — *any impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- — *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 50 °C over *diphosphorus pentoxide R* for 2 h.

ASSAY

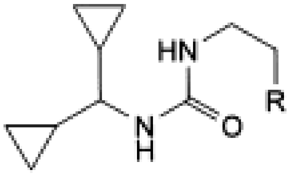
Dissolve 0.200 g in 50 ml of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 27.82 mg of $C_{10}H_{19}N_2O_5P$.

IMPURITIES

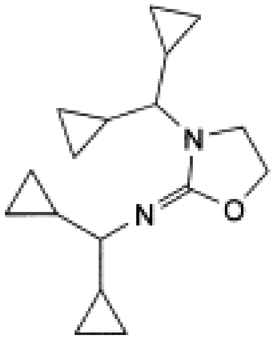
SPECIFIED IMPURITIES

A, B, C.



A. R = OH: 1-(dicyclopropylmethyl)-3-(2-hydroxyethyl)urea,

B. R = Cl: 1-(2-chloroethyl)-3-(dicyclopropylmethyl)urea,



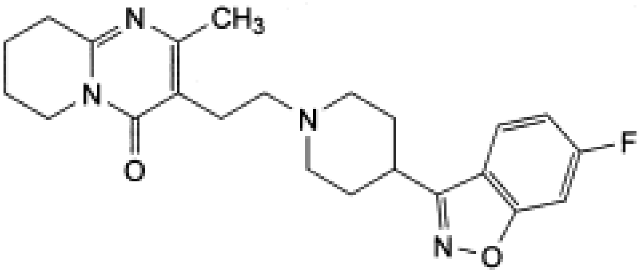
C. *N*,3-bis(dicyclopropylmethyl)oxazolidin-2-imine.

Ph Eur

Risperidone

General Notices

(Ph Eur monograph 1559)



$C_{23}H_{27}FN_4O_2$ 410.5 *106266-06-2*

Action and use

Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent). It dissolves in dilute acid solutions.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *risperidone CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.1 g in a 7.5 g/l solution of *tartaric acid R* and dilute to 100 ml with the same acid solution.

Related substances

Liquid chromatography (*2.2.29*).

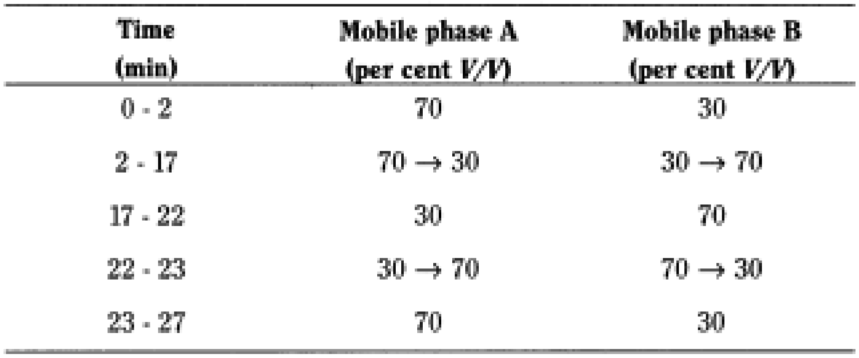
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *risperidone for system suitability CRS* (containing impurities A, B, C, D and E) in *methanol R* and dilute to 1.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 25.0 ml with *methanol R*.

Column:
- *size: l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: 5 g/l solution of *ammonium acetate R*;
- *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	70	30
2 - 17	70 → 30	30 → 70
17 - 22	30	70
22 - 23	30 → 70	70 → 30
23 - 27	70	30

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 10 µl.

Relative retention With reference to risperidone (retention time = about 12 min): impurity A =

about 0.69; impurity B = about 0.75; impurity C = about 0.81; impurity D = about 0.94; impurity H = about 0.96; impurity E = about 1.12; impurity F = about 1.32; impurity I = about 1.60.

System suitability Reference solution (a):

- — *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to risperidone;
- — the chromatogram obtained is similar to the chromatogram supplied with *risperidone for system suitability CRS* .

Limits:

- — *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- — *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- — *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- — *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.160 g in 70 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R* and titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 20.53 mg of $C_{23}H_{27}FN_4O_2$.

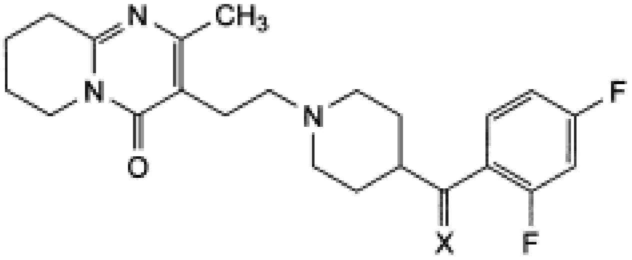
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*

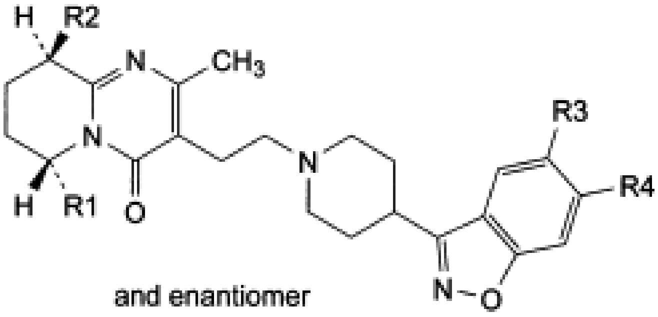
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F, H, I.*



A. X = N-OH: 3-[2-[4-[(*E*)-(2,4-difluorophenyl)(hydroxyimino)methyl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,

B. X = N-OH: 3-[2-[4-[(*Z*)-(2,4-difluorophenyl)(hydroxyimino)methyl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,

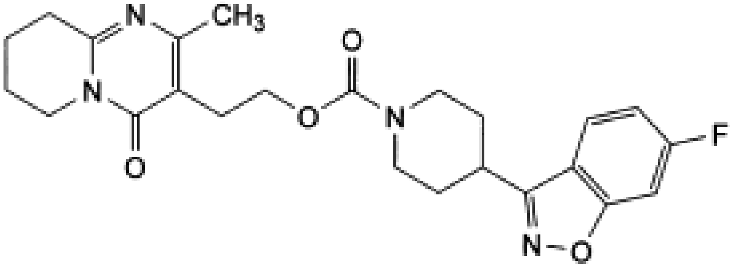
H. X = O: 3-[2-[4-(2,4-difluorobenzoyl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,



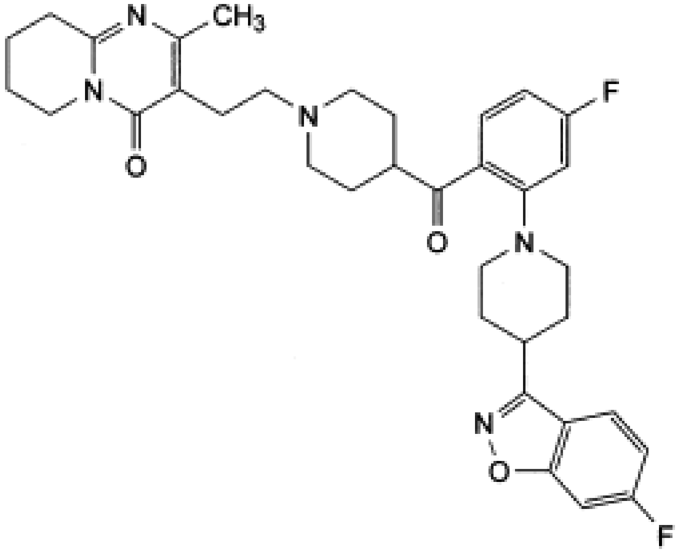
C. R1 = R3 = H, R2 = OH, R4 = F: (9*RS*)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,

D. R1 = R2 = R4 = H, R3 = F: 3-[2-[4-(5-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,

E. R1 = CH_3, R2 = R3 = H, R4 = F: (6*RS*)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2,6-dimethyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-a]pyrimidin-4-one,



F. 2-[2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-a]pyrimidin-3-yl]ethyl 4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-carboxylate,

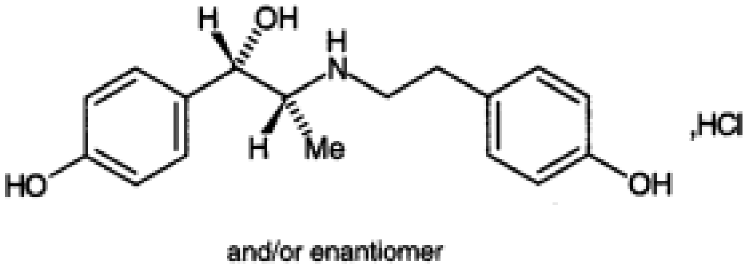


I. 3-[2-[4-[4-fluoro-2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]benzoyl]piperidin-1-yl] ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.

Ph Eur

Ritodrine Hydrochloride

General Notices



$C_{17}H_{21}NO_3,HCl$ 323.8 *23239-51-2*

Action and use

$Beta_2$-adrenoceptor agonist; bronchodilator.

Preparations

Ritodrine Injection

Ritodrine Tablets

DEFINITION

Ritodrine Hydrochloride is *erythro*-1-(*p*-hydroxyphenyl)-2-(4-hydroxyphenethylamino)propan-1-ol hydrochloride. It contains not less than 97.0% and not more than 103.0% of $C_{17}H_{21}NO_3$, HCl, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Freely soluble in *water*; soluble in *absolute ethanol*; practically insoluble in *acetone* and in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of ritodrine hydrochloride *(RS 313)*.

B. A 1% w/v solution yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 2% w/v solution, 4.5 to 6.0, Appendix V L.

Heavy metals

12 ml of a 10.0% w/v solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard (20 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.10% w/v of the substance being examined in the mobile phase. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) dissolve about 20 mg of the substance being examined in 50 ml of the mobile phase, add 5.6 ml of *sulphuric acid* and sufficient of the mobile phase to produce 100 ml, mix and heat at a temperature of 85° for 2 hours. Cool to room temperature, carefully mix 10 ml of the cooled solution with 8 ml of a 10% w/v solution of *sodium hydroxide* and allow to cool (generation of *threo*-diastereoisomer).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of a solution containing 6.6 g of *diammonium hydrogen orthophosphate* and 1.1 g of *sodium heptanesulphonate* in 700 ml of *water* and 300 ml of *methanol*; the mixture adjusted to pH 3.0 with an 85% v/v solution of *orthophosphoric acid* and (c) a detection wavelength of 214 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks (ritodrine and the *threo*-diastereoisomer) is at least 1.5.

In the chromatogram obtained with solution (1), identify any peaks corresponding to tyramine (relative retention time to ritodrine, 0.3), 'hexahydroketone II' (0.65), 'hexahydroketone I' (0.85), *threo*-diastereoisomer (1.15) and 'aminoketone' (2.3). In the chromatogram obtained with solution (1), (a) the area of any peak corresponding to hexahydroketone II is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3% [response relative to ritodrine 0.35]), (b) the areas of any peaks corresponding to tyramine and aminoketone are not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2% each), (c) the area of any peak corresponding to hexahydroketone I is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4% [response relative to ritodrine 0.5]), (d) the area of any peak corresponding to the *threo*-diastereoisomer is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%) and (e) the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Loss on drying

When dried at 105° for 2 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.2%, Appendix IX A.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of the substance being examined in the mobile phase. Solution (2) contains 0.02% w/v of *ritodrine hydrochloride BPCRS* in the mobile phase. For solution (3) dissolve about 20 mg of the substance being examined in 50 ml of the mobile phase, add 5.6 ml of *sulphuric acid* and sufficient of the mobile phase to produce 100 ml, mix and heat at a temperature of 85° for 2 hours. Cool to room temperature, carefully mix 10 ml of the cooled solution with 8 ml of a 10% w/v solution of *sodium hydroxide* and allow to cool (generation of *threo*-diastereoisomer).

The chromatographic procedure described under Related substances may be used.

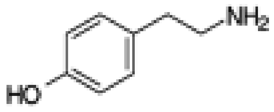
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks (ritodrine and the *threo*-diastereoisomer) is at least 1.5.

Calculate the content of $C_{17}H_{21}NO_3$,HCl in the substance being examined from the chromatograms obtained and from the declared content of $C_{17}H_{21}NO_3$, HCl in *ritodrine hydrochloride BPCRS*.

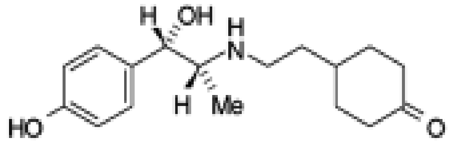
STORAGE

Ritodrine Hydrochloride should be kept in an airtight container.

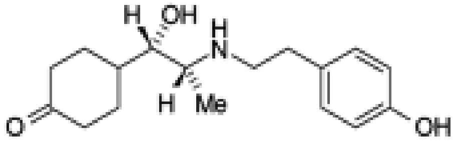
IMPURITIES



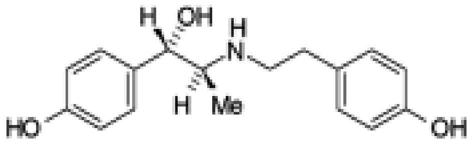
A. tyramine



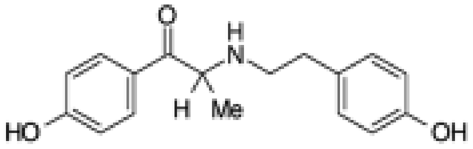
B. *erythro*p-hydroxy-α-1-2-(4-oxocyclo-hexyl)ethylamino]ethylbenzyl alcohol *(hexahydroketone I)*



C. *erythro*1-(4-oxocyclohexyl)-2-[(*p*-hydroxy-phenethyl)amino]propan-1-ol *(hexahydroketone II)*



D. *threo*1-(4-hydroxyphenyl)-2-(4-hydroxy-phenethylamino)propan-1-ol (threo-*diastereoisomer)*

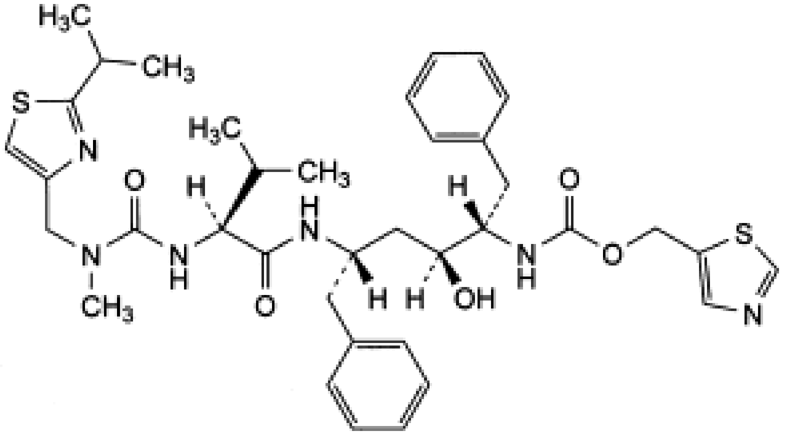


E. *p*-hydroxy-β-[(*p*-hydroxyphenethyl)-amino]propiophenone *(aminoketone)*

Ritonavir

General Notices

(Ph Eur monograph 2136)



$C_{37}H_{48}N_6O_5S_2$ 721 *155213-67-5*

Action and use

Protease inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

Thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

The production method is validated to demonstrate suitable enantiomeric purity of the final product.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methanol and in methylene chloride, very slightly soluble in acetonitrile.

It shows polymorphism *(5.9)*.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison ritonavir CRS.

If the spectra obtained in the solid state show differences dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture Mix equal volumes of *acetonitrile R* and a 4.1 g/l solution of *potassium dihydrogen phosphate R*.

Test solution (a) Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture. Sonicate if necessary.

Test solution (b) Dilute 5.0 ml of test solution (a) to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 5.0 mg of *ritonavir for peak identification CRS* (containing impurities E, F, L, O and T) in the solvent mixture and dilute to 5.0 ml with the solvent mixture. Sonicate if necessary.

Reference solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

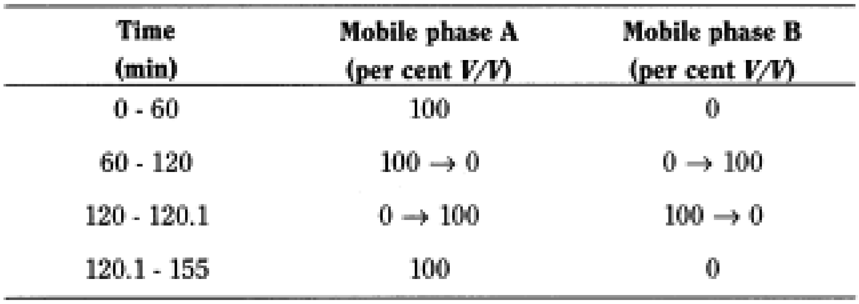
Reference solution (c) Dissolve 10.0 mg of *ritonavir CRS* in the solvent mixture and dilute to 10.0 ml with the solvent mixture. Sonicate if necessary. Dilute 5.0 ml of this solution to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Column:

- — *size:* *l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase: end-capped butylsilyl silica gel for chromatography R* (3 µm);
- — *temperature*: 60 °C.

Mobile phase:

- — *mobile phase A*: mix 5 volumes of *butanol R*, 8 volumes of *tetrahydrofuran R*, 18 volumes of *acetonitrile R* and 69 volumes of a 4.1 g/l solution of *potassium dihydrogen phosphate R* filtered through a 0.45 µm nylon membrane;
- — *mobile phase B*: mix 5 volumes of *butanol R*, 8 volumes of *tetrahydrofuran R*, 40 volumes of a 4.1 g/l solution of *potassium dihydrogen phosphate R* filtered through a 0.45 µm nylon membrane and 47 volumes of *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	100	0
60 - 120	100 → 0	0 → 100
120 - 120.1	0 → 100	100 → 0
120.1 - 155	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 50 µl of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *ritonavir for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities E, F, L, O and T.

Relative retention With reference to ritonavir (retention time = about 34 min): Impurity E = about 0.39; impurity F = about 0.40; impurity L = about 0.8; impurity O = about 1.1; impurity T = about 2.6.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity F = 1.4; impurity L = 1.9; impurity T = 1.4;

— *impurities E, O*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity T*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities F, L*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{37}H_{48}N_6O_5S_2$ from the declared content of *ritonavir CRS*.

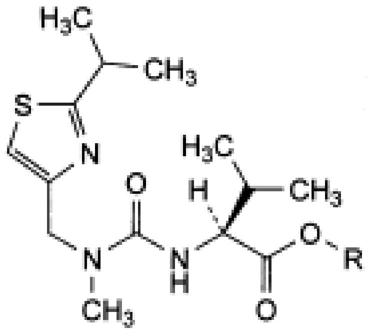
STORAGE

Protected from light.

IMPURITIES

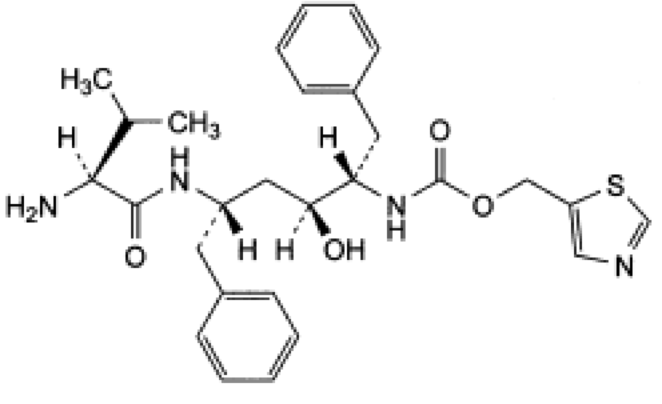
Specified impurities *E, F, L, O, T.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, G, H, I, J, K, M, N, P, Q, R, S, U.*

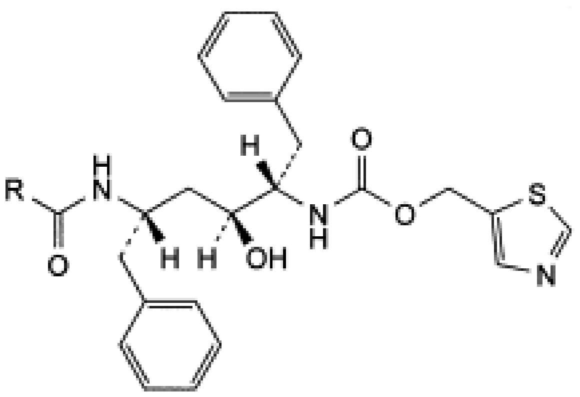


A. R = H: (2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino] butanoic acid,

M. R = CH_2-$CH(CH_3)$2: 2-methylpropyl (2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoate,



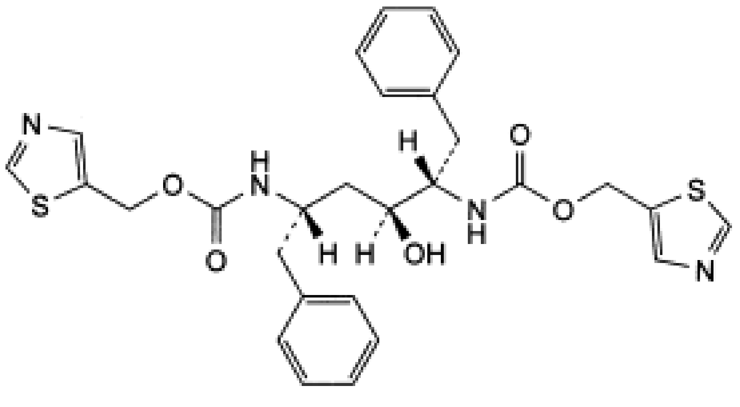
B. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-4-[[(2*S*)-2-amino-3-methylbutanoyl]amino]-1-benzyl-2-hydroxy-5-phenylpentyl]carbamate,



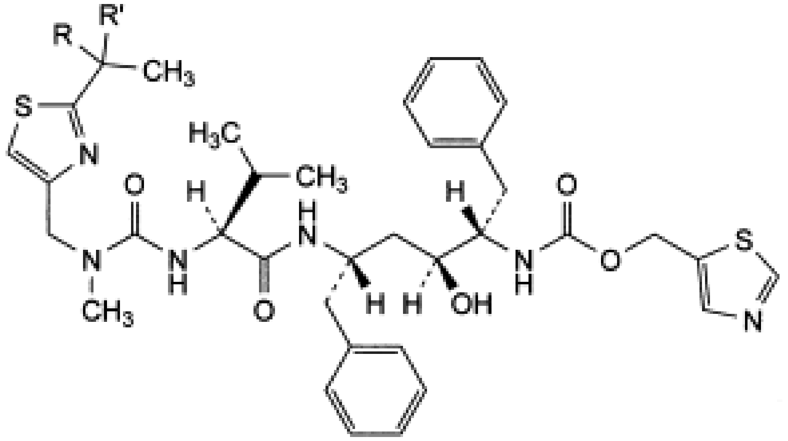
C. R = CH_3: thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-4-(acetylamino)-1-benzyl-2-hydroxy-5-phenylpentyl]carbamate,

J. R = O-$C(CH_3)_3$: thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(1,1-dimethylethoxy)carbonyl] amino]-2-hydroxy-5-phenylpentyl]carbamate,

K. R = O-CH_2-$CH(CH_3)_2$: thiazol-5-ylmethyl (1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2-methylpropoxy)carbonyl]amino]-5-phenylpentyl]carbamate,



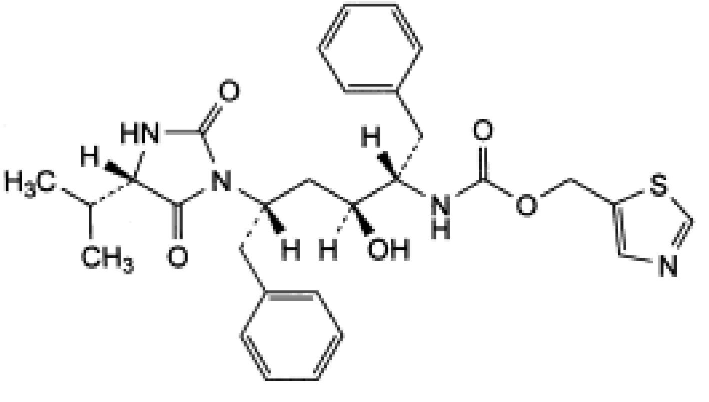
D. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-5-phenyl-4-[[(thiazol-5-ylmethoxy) carbonyl]amino]pentyl]carbamate,



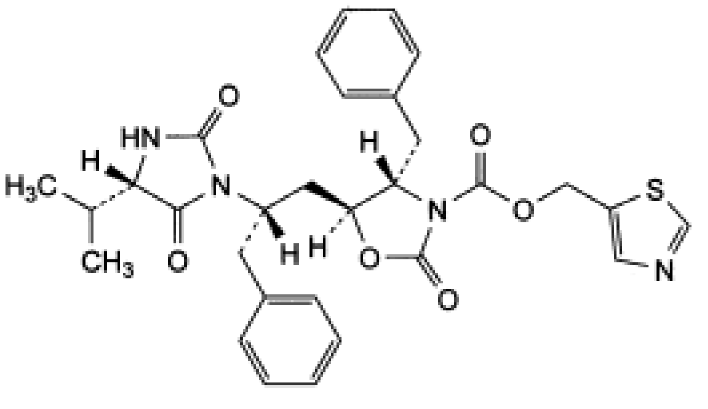
E. R = OH, R′ = CH_3: thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2S)-2-[[[[2-(1-hydroxy-1-methylethyl)thiazol-4-yl]methyl]methylcarbamoyl]amino]-3-methylbutanoyl]amino]-5-phenylpentyl]carbamate,

G. R = OOH, R′ = CH_3: thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-2-[[[[2-(1-hydroperoxy-1-methylethyl)thiazol-4-yl]methyl]methylcarbamoyl]amino]-3-methylbutanoyl]amino]-2-hydroxy-5-phenylpentyl]carbamate,

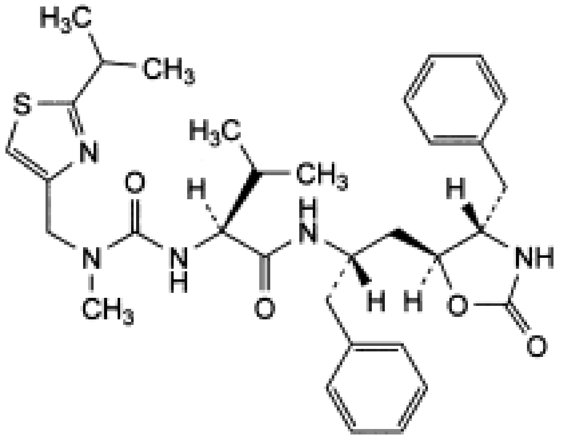
I. R = R′ = H: thiazol-5-ylmethyl [((1*S*,2*S*,4*S*)-1-benzyl-4-[[(2S)-2-[[[[2-ethylthiazol-4-yl]methyl]methylcarbamoyl]amino]-3-methylbutanoyl]amino]-2-hydroxy-5-phenylpentyl]carbamate,



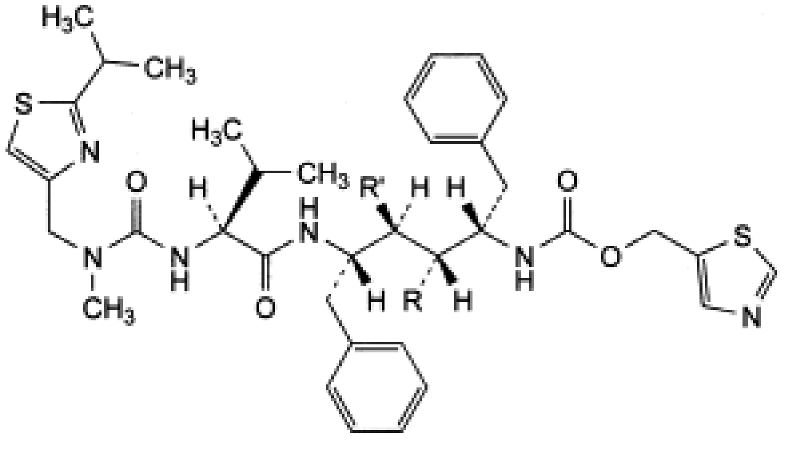
F. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-1-benzyl-2-hydroxy-4-[(4*S*)-4-(1-methylethyl)-2,5-dioxoimidazolidin-1-yl]-5-phenylpentyl]carbamate,



H. thiazol-5-ylmethyl (4*S*,5*S*)-4-benzyl-5-[(2*S*)-2-[(4*S*)-4-(1-methylethyl)-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl]-2-oxooxazolidine-3-carboxylate,



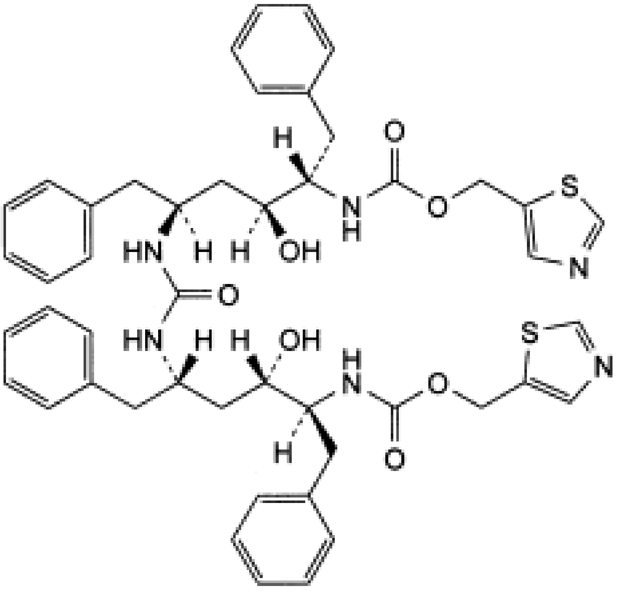
L. (4*S*,5*S*)-4-benzyl-5-[(2*S*)-2-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-3-phenylpropyl]oxazolidin-2-one,



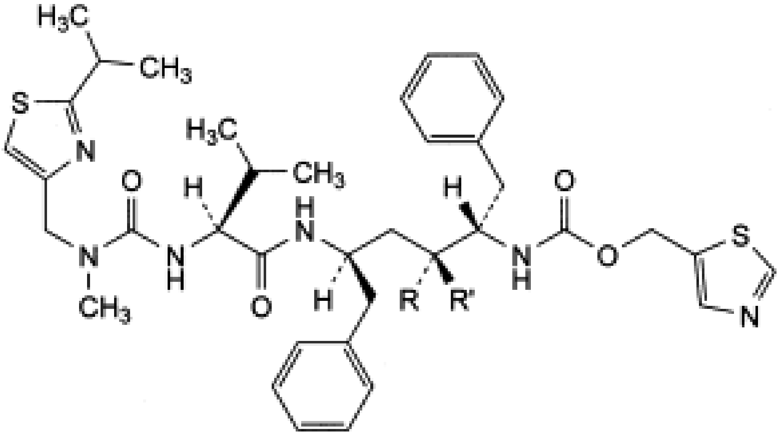
N. R = H, R' = OH: thiazol-5-ylmethyl [(1*S*,3*S*,4*S*)-1-benzyl-3-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate,

O. R = OH, R′ = H: thiazol-5-ylmethyl [(1*S*,2*R*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[

[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate,

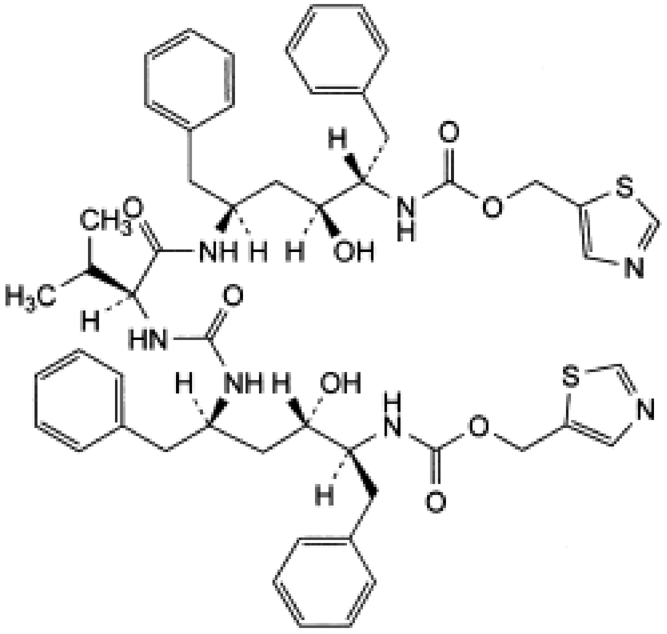


P. bis(thiazol-5-ylmethyl) [carbonylbis[imino[(2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-5,2-diyl]]]dicarbamate,

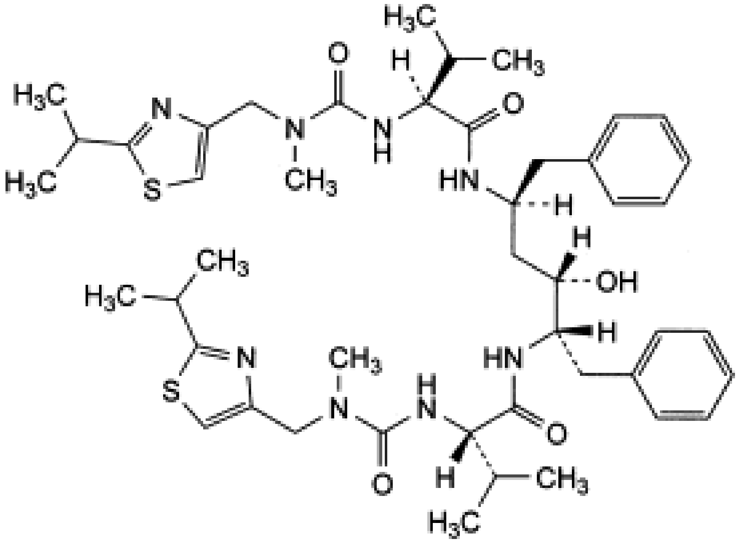


Q. R = OH, R′ = H: thiazol-5-ylmethyl [(1*S*,2*R*,4*R*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate,

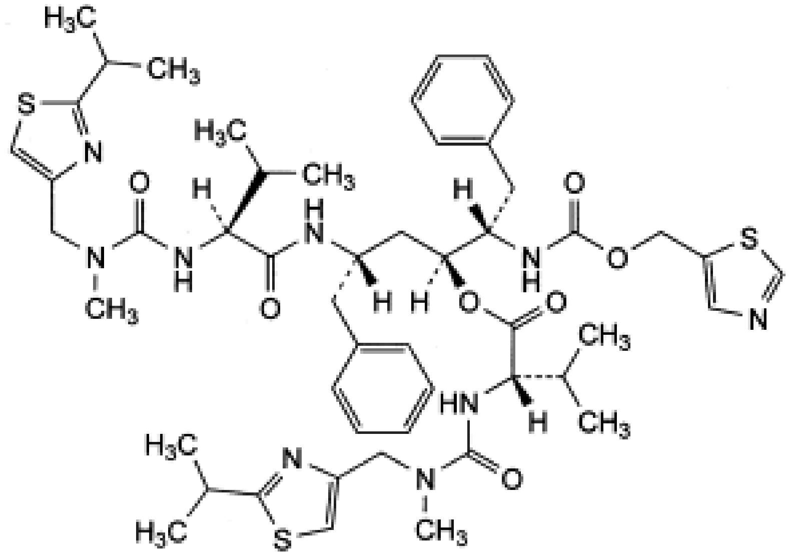
R. R = H, R′ = OH: thiazol-5-ylmethyl [(1*S*,2*S*,4*R*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate,



S. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-2-[[[(1*S*,3*S*,4*S*)-1-benzyl-3-hydroxy-5-phenyl-4-[[(thiazol-5-ylmethoxy)carbonyl]amino]pentyl]carbamoyl]amino]-3-methylbutanoyl]amino]-2-hydroxy-5-phenylpentyl]carbamate,



T. (2*S*)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanamide,



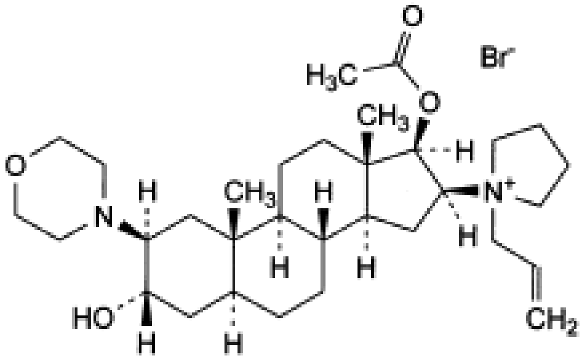
U. (1*S*,3*S*)-3-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-4-phenyl-1-[(1*S*)-2-phenyl-1-[[(thiazol-5-ylmethoxy)carbonyl]amino]ethyl]butyl (2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoate.

Ph Eur

Rocuronium Bromide

General Notices

(Ph Eur monograph 1764)



$C_{32}H_{53}BrN_2O_4$ 610 *119302-91-9*

Action and use

Non-depolarizing neuromuscular blocker.

Ph Eur

DEFINITION

1-[17β-(Acetyloxy)-3α-hydroxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl) pyrrolidinium bromide.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Almost white or pale yellow, slightly hygroscopic powder.

Solubility

Freely soluble in water and in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of rocuronium bromide.*

B. Solution S (see Tests) gives reaction (a) of bromides (*2.3.1*).

TESTS

Solution S

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

+ 28.5 to + 32.0 (anhydrous substance).

Dissolve 0.250 g in a 5.15 g/l solution of *hydrochloric acid R* and dilute to 25.0 ml with the same solution.

pH (*2.2.3*)

8.9 to 9.5 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *water R*, *acetonitrile R1* (10:90 *V/V*).

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *rocuronium for peak identification CRS* (containing impurities A, B, C, F, G and H) in the solvent mixture and dilute to 5 ml with the solvent mixture.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase Mix 100 volumes of a 4.53 g/l solution of *tetramethylammonium hydroxide R* adjusted to pH 7.4 with *phosphoric acid R* and 900 volumes of *acetonitrile R1*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 5 µl of the test solution, reference solutions (a) and (b) and the solvent mixture (blank).

Run time 2.5 times the retention time of rocuronium.

Relative retention With reference to rocuronium (retention time = about 9 min): impurity A =

about 0.20; impurity G = about 0.44; impurity F = about 0.75; impurity B = about 0.80; impurity D = about 0.90; impurity H = about 0.95; impurity C = about 1.20; impurity E = about 1.53.

System suitability Reference solution (b):

— the chromatogram obtained is similar to the chromatogram supplied with *rocuronium for peak identification CRS*.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.47; impurity F = 1.26; impurity G = 0.43; impurity H = 0.35;

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities B, C*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities D, E, F, G, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peaks due to the blank and to bromide ion eluting just before impurity A.

Chlorides

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dissolve 0.824 g of *sodium chloride R* and 0.644 g of *sodium bromide R* in *water R* and dilute to 1000.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with *water R*.

Reference solution (b) Dissolve 0.824 g of *sodium chloride R* in *water R* and dilute to 1000.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *water R*. Dilute 2.0 ml of this solution to 50.0 ml with *water R*.

Blank solution *Water R*.

Precolumn:

— *size:* *l* = 0.05 m, Ø = 4.0 mm;

— *stationary phase*: *anion exchange resin R* (13 µm).

Column:

— *size:* *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *anion exchange resin R* (13 µm).

Mobile phase A solution containing 0.063 g/l of *sodium hydrogen carbonate R* and 0.212 g/l of *anhydrous sodium carbonate R*.

Flow rate 2.0 ml/min.

Detection Conductivity detector set at 100 µS/V and maintained at 30 °C.

Use a self-generating anion suppressor.

Injection 25 µl.

Retention times Chloride = about 1.7 min; bromide = about 2.8 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to chloride and bromide.

Limit:

— *chlorides*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*)

Maximum 4.0 per cent, determined on 0.400 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 40 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

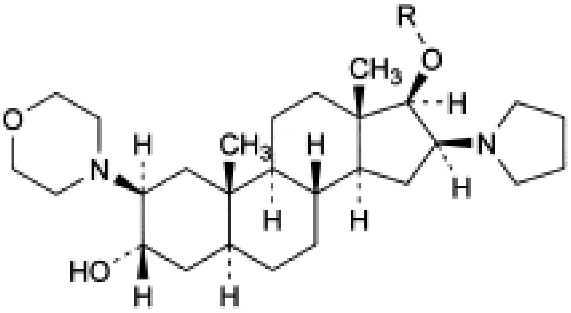
1 ml of *0.1 M perchloric acid* is equivalent to 60.97 mg of $C_{32}H_{53}BrN_2O_4$.

STORAGE

In an airtight container , protected from light.

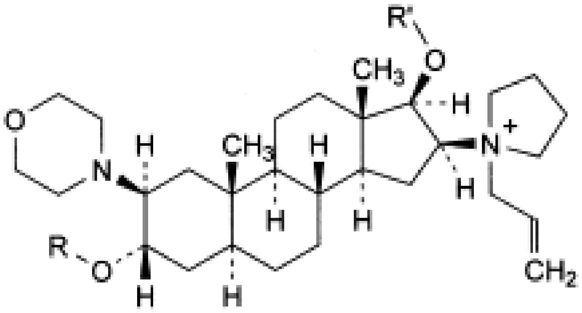
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H.*



A. R = CO-CH_3: 3α-hydroxy-2β-(morpholin-4-yl)-16β-(pyrrolidin-1-yl)-5α-androstan-17β-yl acetate,

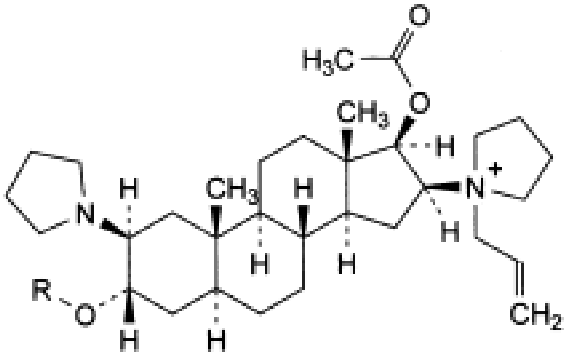
G. R = H: 2β-(morpholin-4-yl)-16β-(pyrrolidin-1-yl)-5α-androstane-3α,17β-diol,



B. R = R′ = CO-CH_3: 1-[3α,17β-bis(acetyloxy)-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,

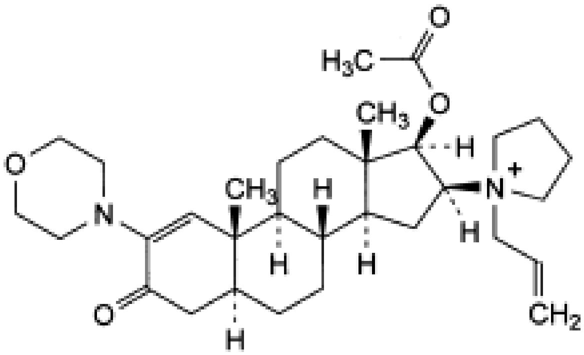
C. R = R′ = H: 1-[3α,17β-dihydroxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl) pyrrolidinium,

D. R = CO-CH_3, R′ = H: 1-[3α-(acetyloxy)-17β-hydroxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,



E. R = H: 1-[17β-(acetyloxy)-3α-hydroxy-2β-(pyrrolidin-1-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,

F. R = CO-CH_3: 1-[3α,17β-bis(acetyloxy)-2β-(pyrrolidin-1-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,



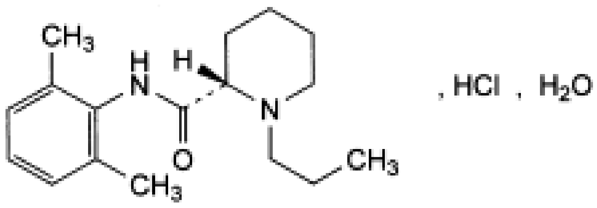
H. 1-[17β-(acetyloxy)-2-(morpholin-4-yl)-3-oxo-5α-androst-1-en-16β-yl]-1-(prop-2-enyl) pyrrolidinium.

Ph Eur

Ropivacaine Hydrochloride Monohydrate

General Notices

(Ph Eur monograph 2335)



$C_{17}H_{26}N_2O,HCl,H_2O$ 328.9 *132112-35-7*

Action and use

Local anaesthetic.

Ph Eur

DEFINITION

(-)-(2*S*)-*N*-(2,6-Dimethylphenyl)-1-propylpiperidine-2-carboxamide hydrochloride monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water and in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION

Carry out either tests A, C, D or tests A, B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison ropivacaine hydrochloride monohydrate CRS.

B. Specific optical rotation *(2.2.7)*: - 64.0 to - 74.0 (anhydrous substance).

Mix 2 ml of a 200 g/l solution of *sodium hydroxide R* and 30 ml of *water R* and dilute to 100.0

ml with *ethanol (96 per cent) R* (solution A). Dissolve 0.500 g of the substance to be examined in solution A and dilute to 50.0 ml with solution A.

C. It gives reaction (a) of chlorides *(2.3.1)*.

D. Enantiomeric purity (see Tests).

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

pH *(2.2.3)*

4.5 to 6.0 for solution S.

Absorbance *(2.2.25)*

Maximum 0.030 at 405 nm and maximum 0.025 at 436 nm, determined on solution S prepared immediately before use, with a path length of 5 cm and using *water R* as the compensation liquid.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 55 mg of the substance to be examined in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *bupivacaine hydrochloride CRS* (impurity A) in the mobile phase and dilute to 5 ml with the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase:* spherical *end-capped octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase Mix 1.3 ml of a 138 g/l solution of *sodium dihydrogen phosphate R* and 32.5 ml of an 89 g/l solution of *disodium hydrogen phosphate R* and dilute to 1000 ml with *water R*; mix equal volumes of this solution (pH 8.0) and *acetonitrile R*.

Flow rate 1.0 ml/min.

Injection 20 µl.

Detection Spectrophotometer at 240 nm.

Run time 2.5 times the retention time of ropivacaine.

Relative retention With reference to ropivacaine (retention time = about 6 min): impurity A = about 1.6.

System suitability Reference solution (b):

— *resolution*: minimum 6.0 between the peaks due to ropivacaine and impurity A.

Limits:

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity H

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution Dissolve 13.0 mg of *2,6-dimethylaniline hydrochloride R* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Retention time Impurity H = about 2-3 min.

Limit:

— *impurity H*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (10 ppm).

Enantiomeric purity

Capillary electrophoresis *(2.2.47)*: use the normalisation procedure.

Test solution Dissolve 50 mg of the substance to be examined in *water R* and dilute to 25 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with *water R*.

Reference solution (b) Dissolve 1.5 mg of the substance to be examined and 1.5 mg of *ropivacaine impurity G CRS* in *water R* and dilute to 100 ml with the same solvent.

Capillary:

— *material*: fused silica;

— *size*: effective length = about 72 cm, total length = 80 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer Prepare a 13.3 g/l solution of *dimethyl-β-cyclodextrin R* in an 11.5 g/l solution of *phosphoric acid R* previously adjusted to pH 3.0 with *triethanolamine R*. The CZE buffer is prepared and filtered through a 0.45 µm membrane filter immediately before use.

Detection Spectrophotometer at 206 nm.

Preconditioning of the capillary Rinse the capillary at 100 kPa with *water R* for 1 min, with

0.1 M sodium hydroxide for 10 min and with *water R* for 3 min. If the capillary is new or dry, increase the sodium hydroxide rinse to 30 min.

Between-run rinsing Rinse the capillary at 100 kPa with *water R* for 1 min, with *0.1 M sodium hydroxide* for 4 min, with *water R* for 1 min and with the CZE buffer for 4 min.

Injection Under pressure (5 kPa) for 5 s.

Migration Apply a field strength of 375 V/cm with an initial ramp of 500 V/s and a positive polarity corresponding to a current of 40-45 µA.

Run time 30 min.

System suitability:

— *resolution*: minimum 3.7 between the peaks due to impurity G (1st peak) and (*S*)-ropivacaine in the electropherogram obtained with reference solution (b); if necessary, increase the dimethyl-β-cyclodextrin concentration in the CZE buffer or vary the pH between 2.9 and 3.1 or lower the temperature;

— *signal-to-noise ratio*: minimum 10 for the principal peak in the electropherogram obtained with reference solution (a).

Limit:

— *impurity G*: maximum 0.5 per cent.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution R* (*100 ppm Pb*) with a mixture of 15 volumes of *water R* and 85 volumes of *methanol R*.

Water *(2.5.12)*

5.0 per cent to 6.0 per cent, determined on 0.100 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 10 ml of *water R* and 40 ml of *ethanol (96 per cent) R*. Add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

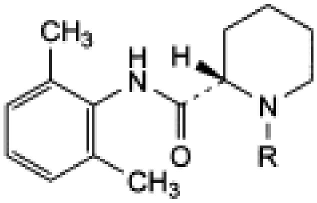
1 ml of *0.1 M sodium hydroxide* is equivalent to 31.09 mg of $C_{17}H_{27}ClN_2O$.

IMPURITIES

Specified impurities A, G, H.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these

impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, E, F*.



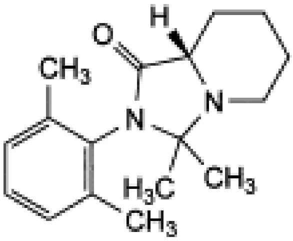
A. R = $[CH_2]_3$-CH_3: (*S*)-bupivacaine,

B. R = H: (-)-(2*S*)-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide,

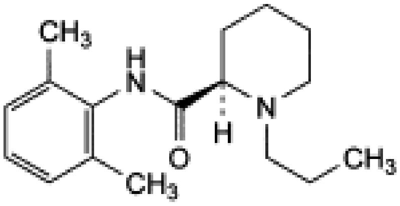
C. R = CH_3: (-)-(2*S*)-*N*-(2,6-dimethylphenyl)-1-methylpiperidine-2-carboxamide ((*S*)-mepivacaine),

D. R = C_2H_5: (-)-(2S)-N-(2,6-dimethylphenyl)-1-ethylpiperidine-2-carboxamide,

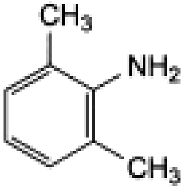
E. R = $CH(CH_3)_2$: (-)-(2*S*)-*N*-(2,6-dimethylphenyl)-1-(1-methylethyl)piperidine-2-carboxamide,



F. (8a*S*)-2-(2,6-dimethylphenyl)-3,3-dimethylhexahydroimidazo[1,5-*a*]pyridin-1(5*H*)-one (acetone adduct),



G. (+)-(2*R*)-*N*-(2,6-dimethylphenyl)-1-propylpiperidine-2-carboxamide ((*R*)-ropivacaine),

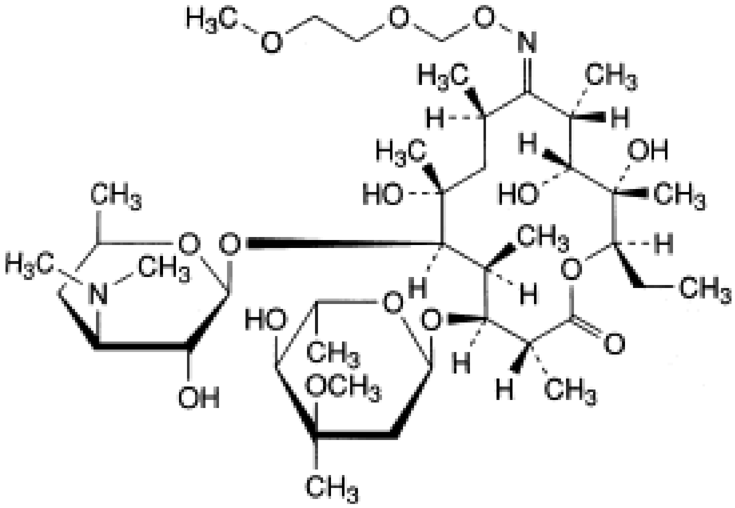


H. 2,6-dimethylaniline.

Roxithromycin

General Notices

(Ph Eur monograph 1146)



$C_{41}H_{76}N_2O_{15}$ 837 80214-83-1

Action and use

Antibacterial.

Ph Eur

DEFINITION

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*)-4-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-10-[(*E*)-[(2-methoxyethoxy)methoxy]imino]-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecan-2-one (erythromycin 9-(*E*)-[*C*-[(2-methoxyethoxy)methyl]oxime]).

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in acetone, in alcohol and in methylene chloride. It is slightly soluble in dilute hydrochloric acid.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison roxithromycin CRS.

If the spectra obtained shows differences, prepare further spectra using 90 g/l solutions in *methylene chloride R*.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.2 g in *methanol R* and dilute to 20 ml with the same solvent.

Specific optical rotation (*2.2.7*)

- 93 to - 96 (anhydrous substance).

Dissolve 0.500 g in *acetone R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solution A Mix 30 volumes of *acetonitrile R* and 70 volumes of a 48.6 g/l solution of *ammonium dihydrogen phosphate R*, adjusted to pH 5.3 with *dilute sodium hydroxide solution R*.

Test solution Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 25.0 ml with solution A.

Reference solution (a) Dissolve 50.0 mg of *roxithromycin CRS* in solution A and dilute to 25.0 ml with solution A.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with solution A.

Reference solution (c) Dissolve 2.0 mg of *roxithromycin for system suitability CRS* in solution A and dilute to 1.0 ml with solution A.

Reference solution (d) Dilute 1.0 ml of *toluene R* to 100.0 ml with *acetonitrile R*. Dilute 0.2 ml of this solution to 200.0 ml with solution A.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

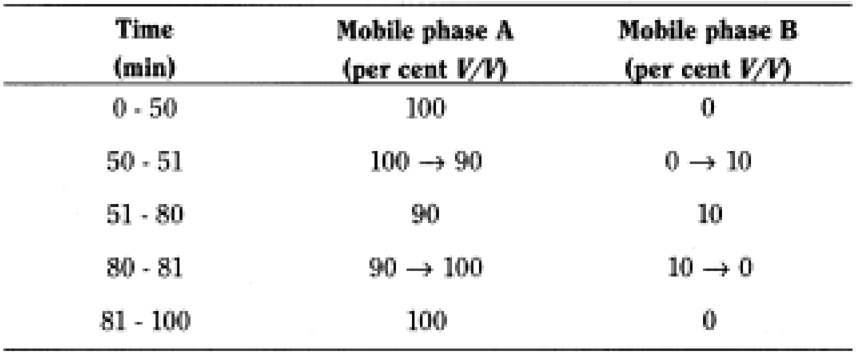
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a 10 nm pore size and a carbon loading of about 19 per cent;

— *temperature*: 15 °C.

Mobile phase:

— *mobile phase A*: mix 26 volumes of *acetonitrile R* and 74 volumes of a 59.7 g/l solution of *ammonium dihydrogen phosphate R*, adjusted to pH 4.3 with *dilute sodium hydroxide solution R*;

— *mobile phase B*: *water R*, *acetonitrile R* (30:70 *V/V*),



Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 50	100	0
50 - 51	100 → 90	0 → 10
51 - 80	90	10
80 - 81	90 → 100	10 → 0
81 - 100	100	0

Flow rate 1.1 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl, using an injector maintained at 8 °C, of the test solution and reference solutions (b), (c) and (d).

Relative retention With reference to roxithromycin (retention time = about 22 min): impurity A = about 0.28; impurity B = about 0.31; impurity C = about 0.33; impurity D = about 0.62; impurity E = about 0.67; impurity F = about 0.83; impurity G = about 1.15; impurity K = about 1.7; impurity H = about 1.85; impurity J = about 2.65; impurity I = about 3.1.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to roxithromycin.

Limits:

— *impurity G:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurities A, B, C, D, E, F, H, I, J*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent). Disregard any peak due to toluene (use reference solution (d) to identify it).

Heavy metals (*2.4.8*)

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with the limit test B. Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*.

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances, with the following modifications.

Column:
— *size*: *l* = 0.25 m.

Mobile phase Mix 307 volumes of *acetonitrile R* and 693 volumes of a 49.1 g/l solution of *ammonium dihydrogen phosphate R* adjusted to pH 5.3 with *dilute sodium hydroxide solution R*.

Flow rate 1.5 ml/min.

Injection Test solution and reference solutions (a) and (c).

Retention time Roxithromycin = about 12 min.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to roxithromycin.

STORAGE

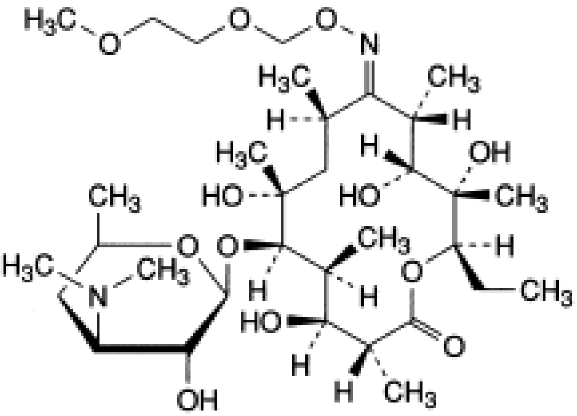
In an airtight container.

IMPURITIES

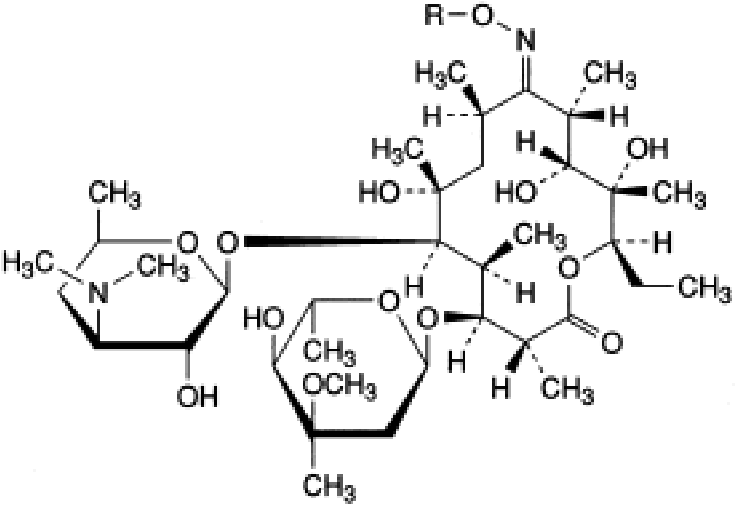
Specified impurities *A, B, C, D, E, F, G, H, I, J.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): K.*

A. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (erythromycin A),



B. 3-*O*-de(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)erythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime],

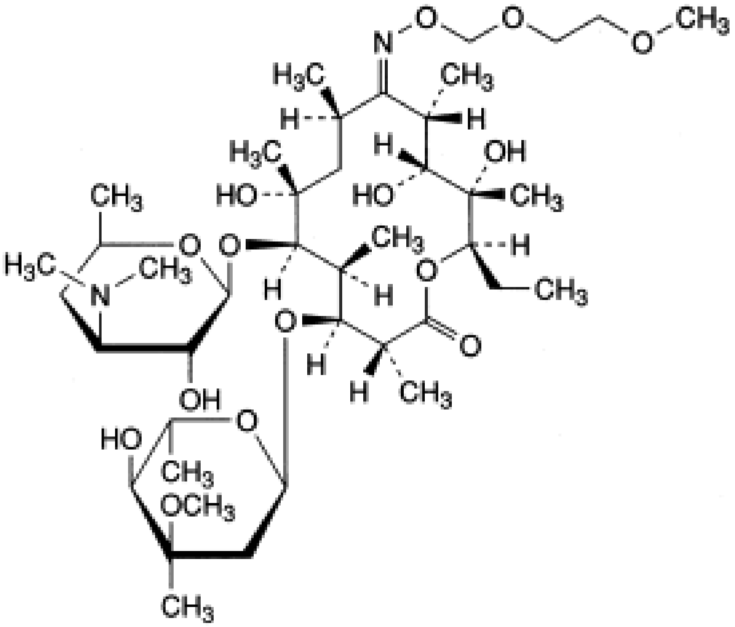


C. R = H: erythromycin 9-(*E*)-oxime,

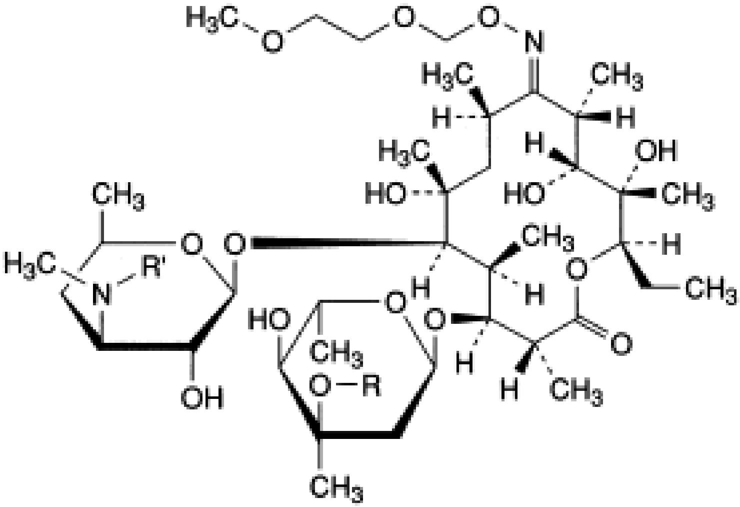
G. R = CH_2-O-CH_2-O-CH_2-CH_2-OCH_3: erythromycin 9-(*E*)-[*O*-[[(2-methoxyethoxy)methoxy] methyl]oxime],

J. R = CH_2-O-CH_2-CH_2Cl: erythromycin 9-(*E*)-[*O*-[(2-chloroethoxy)methyl]oxime],

K. R = CH_2-O-CH_2-CH_2-O-CH_2OH: erythromycin 9-(*E*)-[*O*-[[2-(hydroxymethoxy)ethoxy] methyl]oxime],

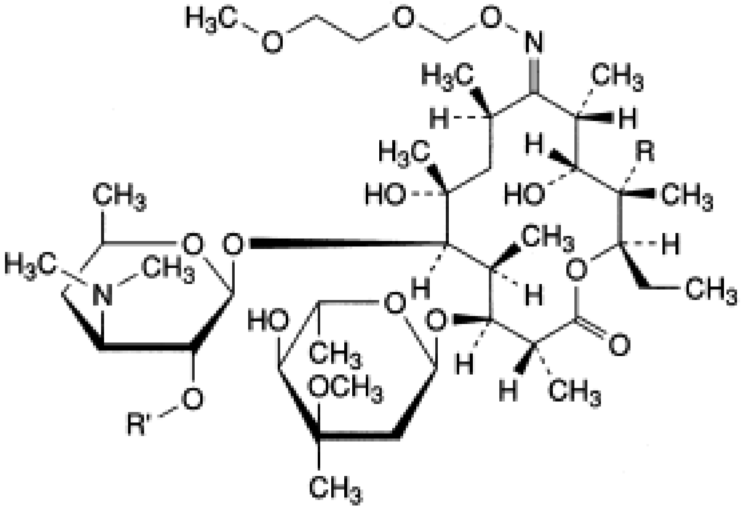


D. erythromycin 9-(*Z*)-[*C*-[(2-methoxyethoxy)methyl]oxime],



E. R = H, R′ = CH_3: 3″-*C*-demethylerythromycin 9-(*E*)-[*C*-[(2-methoxyethoxy)methyl]oxime],

F. R = CH_3, R′ = H: 3′-*N*-demethylerythromycin 9-(*E*)-[*C*-[(2-methoxyethoxy)methyl]oxime],


H$_3$C
O
O
O
N
H$_3$C
CH$_3$
H
H
H$_3$C
H
R
CH$_3$
HO
HO
CH$_3$
CH$_3$
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CH$_3$
H
H$_3$C
N
CH$_3$
H
H
O
HO
O
O
CH$_3$
O
H
OCH$_3$
H
CH$_3$
R'
O
CH$_3$

H. R = R′ = H: 12-deoxyerythromycin 9-(*E*)-[*C*-[(2-methoxyethoxy)methyl]oxime],

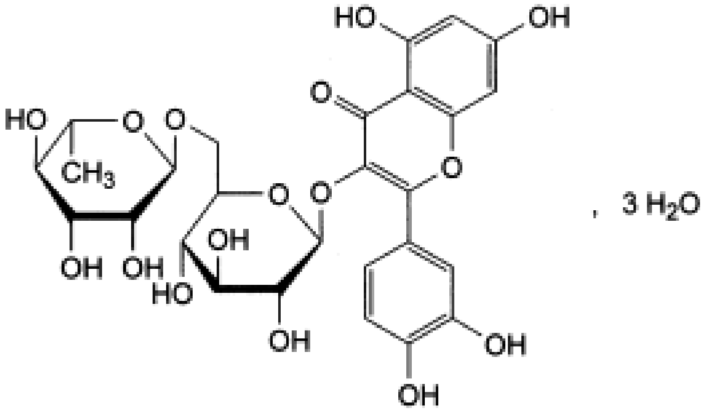
I. R = OH, R′ = CH_2-O-CH_2-CH_2-OCH_3: 2′-*C*-[(2-methoxyethoxy)methyl]erythromycin 9-(*E*)-[*C*-[(2-methoxyethoxy)methyl]oxime].

Ph Eur

Rutoside Trihydrate

General Notices

(Ph Eur monograph 1795)



$C_{27}H_{30}O_{16},3H_2O$ *665* *153-18-4 (anhydrous)*

Action and use

Bioflavinoid.

Ph Eur

DEFINITION

3-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Content

95.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow or greenish-yellow, crystalline powder.

Solubility

Practically insoluble in water, soluble in methanol, sparingly soluble in ethanol, practically insoluble in methylene chloride. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Dissolve 50.0 mg in *methanol R*, dilute to 250.0 ml with the same solvent and filter if necessary. Dilute 5.0 ml of the solution to 50.0 ml with *methanol R*. Examined between 210 nm and 450 nm (*2.2.25*), the solution shows 2 absorption maxima, at 257 nm and 358 nm. The specific absorbance at the maximum at 358 nm is 305 to 330, calculated with reference to the anhydrous substance.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rutoside trihydrate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 25 mg of *rutoside trihydrate CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *butanol R*, *anhydrous acetic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (5:10:10:30:50 *V/V/V/V/V)*.

Application 10 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a mixture of 7.5 ml of a 10 g/l solution of *potassium ferricyanide R* and 2.5 ml of *ferric chloride solution R1* and examine for 10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 10 mg in 5 ml of *alcohol R,* add 1 g of *zinc R* and 2 ml of *hydrochloric acid R1*. A red colour develops.

TESTS

Light absorbing impurities (*2.2.25*)

Maximum 0.10 at wavelengths between 450 nm and 800 nm.

Dissolve 0.200 g in 40 ml of *2-propanol R*. Stir for 15 min, dilute to 50.0 ml with *2-propanol R* and filter.

Substances insoluble in methanol

Maximum 3 per cent.

Shake 2.5 g for 15 min in 50 ml of *methanol R* at 20-25 °C. Filter under reduced pressure through a sintered-glass filter (1.6) (*2.1.2*) previously dried for 15 min at 100-105 °C, allowed to cool in a desiccator and tared. Wash the filter 3 times with 20 ml of *methanol R*. Dry the filter for 30 min at 100-105 °C. Allow to cool and weigh. The residue weighs a maximum of 75 mg.

Related substances

Liquid chromatography (*2.2.29*).

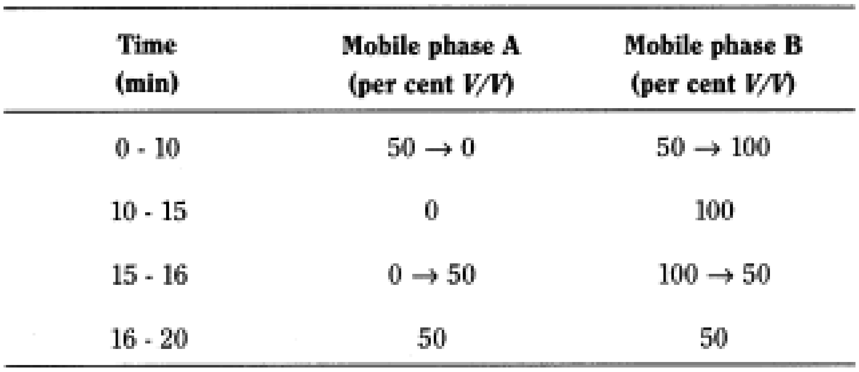
Test solution Dissolve 0.10 g of the substance to be examined in 20 ml of *methanol R* and dilute to 100.0 ml with mobile phase B.

Reference solution (a) Dissolve 10.0 mg of *rutoside trihydrate CRS* in 10.0 ml of *methanol R.*

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 50.0 ml with mobile phase B.

Column:
- *size: l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: mix 5 volumes of *tetrahydrofuran R* with 95 volumes of a 15.6 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B*: mix 40 volumes of *tetrahydrofuran R* with 60 volumes of a 15.6 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	50 → 0	50 → 100
10 - 15	0	100
15 - 16	0 → 50	100 → 50
16 - 20	50	50

Flow rate 1 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Relative retention With reference to rutoside (retention time = about 7 min): impurity B = about 1.1; impurity A = about 1.2; impurity C = about 2.5.

System suitability Reference solution (a):

- *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rutoside.

Limits Locate the impurities by comparison with the chromatogram provided with *rutoside trihydrate CRS.*

- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.8; impurity C = 0.5;
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained

with reference solution (b) (2.0 per cent);

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Water (*2.5.12*)

7.5 per cent to 9.5 per cent, determined on 0.100 g.

Sulphated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

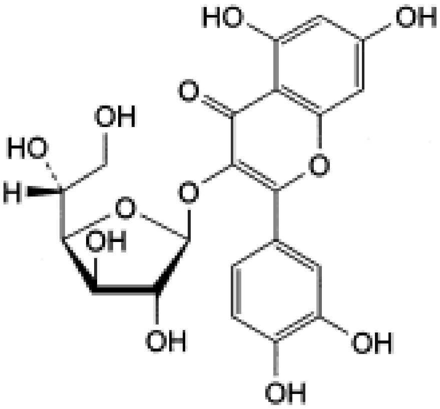
Dissolve 0.200 g in 20 ml of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 30.53 mg of $C_{27}H_{30}O_{16}$.

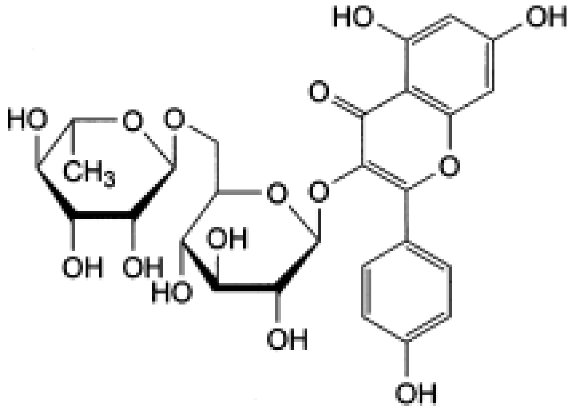
STORAGE

Protected from light.

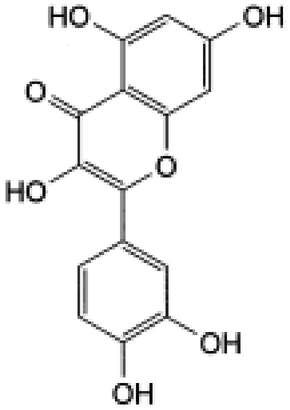
IMPURITIES



A. 2-(3,4-dihydroxyphenyl)-3-(β-D-glucofuranosyloxy)-5,7-dihydroxy-4*H*-1-benzopyran-4-one (isoquercitroside),



B. 3-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (kaempferol 3-rutinoside),



C. 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one (quercetin).

Ph Eur

Synthetic Retinol Concentrate (Oily Form)

General Notices

(Vitamin A Concentrate (Oily Form) Synthetic, Ph Eur monograph 0219)

Action and use

Vitamin A.

Ph Eur

DEFINITION

Oily concentrate prepared from synthetic retinol ester (*0217*) as is or by dilution with a suitable vegetable fatty oil.

Content

95.0 per cent to 110.0 per cent of the vitamin A content stated on the label, which is not less than 500 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance

Yellow or brownish-yellow, oily liquid.

Solubility

Practically insoluble in water, soluble or partly soluble in anhydrous ethanol, miscible with organic solvents.

Partial crystallisation may occur in highly concentrated solutions.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Prepare a solution containing about 3.3 IU of vitamin A per microlitre in *cyclohexane R* containing 1 g/l of *butylhydroxytoluene R*.

Reference solution Prepare a 10 mg/ml solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *cyclohexane R* containing 1 g/l of *butylhydroxytoluene R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R*, *cyclohexane R* (20:80 *V/V*).

Application 3 µl.

Development Immediately, over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:

— the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

Results The composition of the test solution is confirmed by the correspondence of the principal spot or spots with those obtained with the reference solution.

TESTS

Acid value *(2.5.1)*

Maximum 2.0, determined on 2.0 g.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034*) do not apply.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light, air, oxidising agents, oxidation catalysts (e.g. copper , iron), acids and prolonged heat; use freshly prepared solutions. If partial crystallisation has occurred, homogenise the material at a temperature of about 65 ‘C, but avoid prolonged heating.

Carry out the assay according to Method A. If the assay is not shown to be valid, use Method B.

Method A

Ultraviolet absorption spectrophotometry *(2.2.25)*.

Dissolve 25-100 mg, weighed with an accuracy of 0.1 per cent, in 5 ml of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10-15 IU/ml.

Verify that the absorption maximum of the solution lies between 325 nm and 327 nm and measure the absorbances at 300 nm, 326 nm, 350 nm and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio A_{λ}/A_{326} for each wavelength.

If the ratios do not exceed: 0.60 at 300 nm, 0.54 at 350 nm, 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram using the following expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

A_{326} = absorbance at 326 nm,

m = mass of the preparation to be examined, in grams,

V = total volume to which the preparation to be examined is diluted to give 10-15 IU/ml,

1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram.

If one or more of the ratios A_{λ}/A_{326} exceeds the values given, or if the wavelength of the absorption maximum does not lie between 325 nm and 327 nm, use Method B.

Method B

Liquid chromatography *(2.2.29)*.

Test solution (a) Introduce 0.100 g of the preparation to be examined into a 100 ml volumetric flask and dissolve immediately in 5 ml of *pentane R*. Add 40 ml of *0.1 M tetrabutylammonium hydroxide* in *2-propanol*. Swirl gently and let the mixture react for 10 minutes at 60-65 °C for hydrolysis, swirling occasionally. Allow to cool to room temperature, dilute to 100.0 ml with *2-propanol R* containing 1 g/l *butylhydroxytoluene R*, and homogenise carefully to avoid air-bubbles.

Test solution (b) Dilute test solution (a) with *2-propanol R* to a final concentration of 100 IU/ml.

Reference solution (a) Introduce about 0.100 g of *retinol acetate CRS* into a 100 ml volumetric flask and proceed as described for test solution (a).

Reference solution (b) Introduce into a 50 ml volumetric flask 5.0 ml of reference solution (a) and dilute to 50.0 ml with *2-propanol R*. Homogenise carefully to avoid air-bubbles.

Column:
— *size:* l = 0.125 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R*, *methanol R* (5:95 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 325 nm.

Injection 10 µl of test solution (b) and reference solution (b).

Run time 1.5 times the retention time of retinol.

Retention time Retinol = about 3 min.

Calculate the content of vitamin A in International Units per gram using the following expression:

$$\frac{A_1 \times C \times m_2}{A_2 \times m_1}$$

A_1 = area of the peak due to retinol in the chromatogram obtained with test solution (b),

A_2 = area of the peak due to retinol in the chromatogram obtained with reference solution (b),

C = concentration of *retinol acetate CRS* in International Units per gram, determined by method A; the absorption ratios A_{λ}/A_{326} must conform,

m_1 = mass of the substance to be examined in test solution (a), in milligrams,

m_2 = mass of *retinol acetate CRS* in reference solution (a), in milligrams.

STORAGE

In an airtight container , protected from light.

Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

LABELLING

The label states:

— the number of International Units per gram;

— the name of the ester or esters;

— the name of any added stabilisers;

— the method of restoring the solution if partial crystallisation has occurred.

Ph Eur

Synthetic Retinol Concentrate (Powder Form)

General Notices

(Vitamin A Concentrate (Powder Form), Synthetic, Ph Eur monograph 0218)

Action and use

Vitamin A.

Ph Eur

DEFINITION

Powder concentrate obtained by dispersing a synthetic retinol ester (*0217*) in a matrix of *Gelatin (0330)* or *Acacia (0307)* or other suitable material.

Content

95.0 per cent to 115.0 per cent of the vitamin A content stated on the label, which is not less than 250 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance

Yellowish powder usually in the form of particles of almost uniform size.

Solubility

Practically insoluble in water, swells or forms an emulsion, depending on the formulation.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Introduce a quantity of the preparation to be examined containing about the equivalent of 17 000 IU of vitamin A into a 20 ml glass-stoppered test tube. Add about 20 mg of *bromelains R*, 2 ml of *water R* and about 150 µl of *2-propanol R*, swirling gently for 2-5 min in a water-bath at 60-65 °C. Cool to below 30 °C and add 5 ml of *2-propanol R* containing 1 g/l of *butylhydroxytoluene R*. Shake vigorously for 1 min, allow to stand for a few minutes and use the supernatant solution.

Reference solution Prepare a 10 mg/ml solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *2-propanol R* containing 1 g/l of *butylhydroxytoluene R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R*, *cyclohexane R* (20:80 *V/V*).

Application 3 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:

- the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

Results The composition of the test solution is confirmed by the correspondence of the principal spot or spots with those obtained with the reference solution.

TESTS

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light, air, oxidising agents, oxidation catalysts (e.g. copper , iron), acids and prolonged heat.

Liquid chromatography *(2.2.29)*.

Test solution (a) Introduce 0.200 g of the preparation to be examined into a 100 ml volumetric flask. Add 20-30 mg of *bromelains R*, 5.0 ml of *water R* and 0.15 ml of *2-propanol R*. Heat gently in a water-bath at 60 °C for about 5 min, swirling occasionally. Add 40 ml of *0.1 M tetrabutylammonium hydroxide* in *2-propanol* . Swirl gently and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Ensure that all sample material is wetted. Allow to cool to room temperature, dilute to 100.0 ml with *2-propanol R* containing 1 g/l *butylhydroxytoluene R,* and homogenise carefully to avoid air-bubbles. The solution may be turbid.

Test solution (b) Dilute test solution (a) with *2-propanol R* to a final concentration of 100 IU/ml. Filter before injection.

Reference solution (a) Introduce about 0.100 g of *retinol acetate CRS* into a 100 ml volumetric flask and dissolve immediately in 5 ml of *pentane R*. Add 40 ml of *0.1 M tetrabutylammonium hydroxide* in *2-propanol* . Swirl gently and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Allow to cool to room temperature, dilute to 100.0 ml with *2-propanol R* containing 1 g/l *butylhydroxytoluene R*, and homogenise carefully to avoid air-bubbles.

Reference solution (b) Introduce into a 50 ml volumetric flask 5.0 ml of reference solution (a) and dilute to 50.0 ml with *2-propanol R*. Homogenise carefully to avoid air-bubbles.

Column:

- *size:* *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R*, *methanol R* (5:95 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 325 nm.

Injection 10 µl of test solution (b) and reference solution (b).

Run time 1.5 times the retention time of retinol.

Retention time Retinol = about 3 min.

Calculate the content of vitamin A using the following expression:

$$\frac{A_1 \times C \times m_2}{A_2 \times m_1}$$

A_1 = area of the peak due to retinol in the chromatogram obtained with test solution (b),

A_2 = area of the peak due to retinol in the chromatogram obtained with reference solution (b),

C = concentration of *retinol acetate CRS* in International Units per gram, determined by the method below,

m_1 = mass of the substance to be examined in test solution (a), in milligrams,

m_2 = mass of *retinol acetate CRS* in reference solution (a), in milligrams.

The exact concentration of *retinol acetate CRS* is assessed by ultraviolet absorption spectrophotometry (*2.2.25*). Dissolve 25-100 mg of *retinol acetate CRS*, weighed with an accuracy of 0.1 per cent, in 5 ml of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10-15 IU/ml.

Verify that the absorption maximum of the solution lies between 325 nm and 327 nm and measure the absorbances at 300 nm, 326 nm, 350 nm and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio A_λ/A_{326} for each wavelength.

If the ratios do not exceed: 0.60 at 300 nm, 0.54 at 350 nm, 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram using the following expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

A_{326} = absorbance at 326 nm,

m = mass of *retinol acetate CRS*, in grams,

V = total volume to which the *retinol acetate CRS* is diluted to give 10-15 IU/ml,

1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram.

The absorbance ratios A_{λ}/A_{326} must conform.

STORAGE

In an airtight container, protected from light.

Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

LABELLING

The label states:

— the number of International Units per gram;

— the name of the ester or esters;

— the name of the principal excipient or excipients used and the name of any added stabilisers.

Ph Eur

Synthetic Retinol Concentrate, Solubilisate/Emulsion

General Notices

Synthetic Retinol Concentrate (Water-dispersible Form)

(Vitamin A Concentrate (Solubilisate/Emulsion), Synthetic, Ph Eur monograph 0220)

Ph Eur

DEFINITION

Liquid concentrate (water is generally used as solvent) of a synthetic retinol ester (*0217*) and a suitable solubiliser.

Content

95.0 per cent to 115.0 per cent of the vitamin A content stated on the label, which is not less than 100 000 IU/g.

It may contain suitable stabilisers such as antimicrobial preservatives and antioxidants.

CHARACTERS

Appearance

Yellow or yellowish liquid of variable opalescence and viscosity. Highly concentrated solutions may become cloudy at low temperature or take the form of a gel.

A mixture of 1 g with 10 ml of *water R* previously warmed to 50 °C gives, after cooling to 20 °C, a uniform, slightly opalescent and slightly yellow dispersion.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Introduce a quantity of the preparation to be examined containing about the equivalent of 17 000 IU of vitamin A into a 20 ml glass-stoppered test-tube. Add 5 ml of *2-propanol R* containing 1 g/l of *butylhydroxytoluene R* and mix thoroughly.

Reference solution Prepare a 10 mg/ml solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *2-propanol R* containing 1 g/l of *butylhydroxytoluene R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R*, *cyclohexane R* (20:80 *V/V*).

Application 3 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:

- the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

Results The composition of the test solution is confirmed by the correspondence of the principal spot or spots with those obtained with the reference solution.

TESTS

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light, air, oxidising agents, oxidation catalysts (e.g. copper, iron), acids and prolonged heat.

Liquid chromatography *(2.2.29)*.

Test solution (a) Introduce 0.200 g of the preparation to be examined into a 100 ml volumetric flask. Add 40 ml of *0.1 M tetrabutylammonium hydroxide* in *2-propanol*. Swirl into dispersion and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Ensure that all sample material is wetted. Allow to cool to room temperature, dilute to 100.0 ml with *2-propanol R* containing 1 g/l *butylhydroxytoluene R* and homogenise carefully to avoid air-bubbles. Residue of the matrix may make the solution more or less cloudy.

Test solution (b) Dilute test solution (a) with *2-propanol R* to a final concentration of 100 IU/ml. Filter before injection.

Reference solution (a) Introduce about 0.100 g of *retinol acetate CRS* into a 100 ml volumetric flask and dissolve immediately in 5 ml of *pentane R*. Add 40 ml of *0.1 M tetrabutylammonium hydroxide* in *2-propanol*. Swirl gently and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Allow to cool to room temperature, dilute to 100.0 ml with *2-propanol R* containing 1 g/l *butylhydroxytoluene R*, and homogenise carefully to avoid air-bubbles.

Reference solution (b) Introduce into a 50 ml volumetric flask 5.0 ml of reference solution (a) and dilute to 50.0 ml with *2-propanol R*. Homogenise carefully to avoid air-bubbles.

Column:

- *size:* *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R*, *methanol R* (5:95 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 325 nm.

Injection 10 µl of test solution (b) and reference solution (b).

Run time 1.5 times the retention time of retinol.

Retention time Retinol = about 3 min.

Calculate the content of vitamin A using the following expression:

$$\frac{A_1 \times C \times m_2}{A_2 \times m_1}$$

A_1 = area of the peak due to retinol in the chromatogram obtained with test solution (b),

A_2 = area of the peak due to retinol in the chromatogram obtained with reference solution (b),

C = concentration of *retinol acetate CRS* in International Units per gram, determined by the method below,

m_1 = mass of the substance to be examined in test solution (a), in milligrams,

m_2 = mass of *retinol acetate CRS* in reference solution (a), in milligrams.

The exact concentration of *retinol acetate CRS* is assessed by ultraviolet absorption spectrophotometry *(2.2.25)*. Dissolve 25-100 mg of *retinol acetate CRS*, weighed with an accuracy of 0.1 per cent, in 5 ml of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10-15 IU/ml.

Verify that the absorption maximum of the solution lies between 325 nm and 327 nm and measure the absorbances at 300 nm, 326 nm, 350 nm and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio A_λ/A_{326} for each wavelength.

If the ratios do not exceed: 0.60 at 300 nm, 0.54 at 350 nm, 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram using the following expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

A_{326} = absorbance at 326 nm,

m = mass of *retinol acetate CRS*, in grams,

V = total volume to which the *retinol acetate CRS* is diluted to give 10-15 IU/ml,

1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram.

The absorbance ratios A_λ/A_{326} must conform.

STORAGE

In an airtight container, protected from light, at the temperature stated on the label.

Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

LABELLING

The label states:

— the number of International Units per gram;

— the name of the ester or esters;

— the name of the principal solubiliser or solubilisers used and the name of any added stabilisers;

— the storage temperature.

Ph Eur

Anhydrous Sodium Carbonate

General Notices

(Ph Eur monograph 0773)

Na_2CO_3 106.0 *497-19-8*

Ph Eur

DEFINITION

Content

99.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, slightly granular powder, hygroscopic.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in *water R* and dilute to 10 ml with the same solvent. The solution is strongly alkaline *(2.2.4)*.

B. The solution prepared for identification test A gives the reaction of carbonates *(2.3.1)*.

C. The solution prepared for identification test A gives reaction (a) of sodium *(2.3.1)*.

D. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 2.0 g in portions in a mixture of 5 ml of *hydrochloric acid R* and 25 ml of *distilled water R*. Heat to boiling and cool. Add *dilute sodium hydroxide solution R* until the solution is neutral and dilute to 50 ml with *distilled water R*.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method I)*.

Dissolve 2.0 g in 10 ml of *water R*.

Alkali hydroxides and bicarbonates

Dissolve 0.4 g in 20 ml of *water R*. Add 20 ml of *barium chloride solution R1* and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. The solution does not become red. Boil the remainder of the filtrate for 2 min. The solution remains clear *(2.2.1)*.

Chlorides ***(2.4.4)***

Maximum 125 ppm.

Dissolve 0.4 g in *water R*, add 4 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 250 ppm, determined on solution S.

Arsenic ***(2.4.2, Method A)***

Maximum 5 ppm, determined on 5 ml of solution S.

Iron ***(2.4.9)***

Maximum 50 ppm.

Dilute 5 ml of solution S to 10 ml with *water R.*

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying at 300 ± 15 C.

ASSAY

Dissolve 1.000 g in 25 ml of *water R*. Add 0.2 ml of *methyl orange solution R* as indicator. Titrate with *1 M hydrochloric acid* until the colour changes from yellow to red.

1 ml of *1 M hydrochloric acid* is equivalent to 52.99 mg of Na_2CO_3.

STORAGE

In an airtight container.

Ph Eur

Anhydrous Sodium Dihydrogen Phosphate

General Notices

NaH_2PO_4 120.0 *7558-80-7*

Action and use

Excipient.

Preparation

Phosphates Enema

DEFINITION

Anhydrous Sodium Dihydrogen Phosphate contains not less than 98.0% and not more than 100.5% of NaH_2PO_4, calculated with reference to the dried substance.

CHARACTERISTICS

White, slightly deliquescent crystals or granules.

Very soluble in *water*; very slightly soluble in *ethanol (96%)*.

Dissolve 10.0 g in sufficient carbon dioxide-free water *prepared from* distilled water *to produce 100 ml (solution S).*

IDENTIFICATION

A. Solution S is *faintly acid*, Appendix V K.

B. Solution S yields the reactions characteristic of *phosphates*, Appendix VI.

C. Solution S neutralised with a 10% w/v solution of *potassium hydroxide* yields reaction A characteristic of *sodium salts*, Appendix VI.

D. Complies with the test for Loss on drying.

TESTS

Acidity

pH of a mixture of 5 ml of solution S and 5 ml of *carbon dioxide-free water*, 4.2 to 4.5, Appendix V L.

Clarity and colour of solution

Solution S is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Arsenic

0.5 g complies with the *limit test for arsenic*, Appendix VII (2 ppm).

Heavy metals

12 ml of solution S complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (10 ppm).

Iron

10 ml of solution S complies with the *limit test for iron*, Appendix VII (10 ppm).

Chloride

Dilute 2.5 ml of solution S to 15 ml with *water*. The resulting solution complies with the *limit test for chlorides*, Appendix VII (200 ppm).

Sulphate

To 5 ml of solution S add 0.5 ml of *hydrochloric acid* and dilute to 15 ml with *distilled water*. The resulting solution complies with the *limit test for sulphates*, Appendix VII (300 ppm).

Reducing substances

To 5 ml of solution S add 0.25 ml of 0.02M *potassium permanganate* and 5 ml of 1M *sulphuric acid* and heat in a water bath for 5 minutes. The solution retains a slight red colour.

Loss on drying

When dried to constant weight at 130°, loses not more than 1.0% of its weight. Use 1 g.

ASSAY

Dissolve 2 g in 50 ml of *water* and titrate with carbonate-free 1M *sodium hydroxide VS* determining the end point potentiometrically. Each ml of 1M *sodium hydroxide VS* is equivalent to 0.120 g of NaH_2PO_4.

Anhydrous Sodium Sulphate

General Notices

(Ph Eur monograph 0099)

Na_2SO_4 142.0 *7757-82-6*

Action and use

Laxative.

Ph Eur

DEFINITION

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Freely soluble in water.

IDENTIFICATION

A. It gives the reactions of sulphates *(2.3.1)* .

B. It gives the reactions of sodium *(2.3.1)* .

C. It complies with the test for loss on drying (see Tests).

TESTS

Solution S

Dissolve 2.2 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1* . Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides ***(2.4.4)***

Maximum 450 ppm.

5 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides.

Calcium ***(2.4.3)***

Maximum 450 ppm, if intended for use in the manufacture of parenteral dosage forms.

10 ml of solution S diluted to 15 ml with *distilled water R* complies with the limit test for calcium.

Iron ***(2.4.9)***

Maximum 90 ppm, if intended for use in the manufacture of parenteral dosage forms.

5 ml of solution S diluted to 10 ml with *water R* complies with the limit test for iron.

Magnesium

Maximum 200 ppm, if intended for use in the manufacture of parenteral dosage forms.

To 10 ml of solution S add 1 ml of *glycerol (85 per cent) R* , 0.15 ml of *titan yellow solution R* , 0.25 ml of *ammonium oxalate solution R* and 5 ml of *dilute sodium hydroxide solution R* and shake. Any pink colour in the test solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 5 ml of *magnesium standard solution (10 ppm Mg) R* and 5 ml of *water R* .

Heavy metals ***(2.4.8)***

Maximum 45 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R .*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 130 °C.

ASSAY

Dissolve 0.100 g in 40 ml of *water R.* Add a mixture of 0.2 ml of *0.1 M hydrochloric acid* and 80 ml of *methanol R* . Carry out a potentiometric titration *(2.2.20)* , using *0.1 M lead nitrate* and as indicator electrode a lead-selective electrode and as reference electrode a silver-silver chloride electrode.

1 ml of *0.1 M lead nitrate* is equivalent to 14.20 mg of Na_2SO_4.

STORAGE

Store in an airtight container.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Colloidal Anhydrous Silica

General Notices

(Ph Eur monograph 0434)

SiO_2 60.1 *7631-86-9*

Action and use

Excipient.

Ph Eur

DEFINITION

Colloidal anhydrous silica contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of SiO_2, determined on the ignited substance.

CHARACTERS

A light, fine, white or almost white, amorphous powder, with a particle size of about 15 nm, practically insoluble in water and in mineral acids except hydrofluoric acid. It dissolves in hot solutions of alkali hydroxides.

IDENTIFICATION

About 20 mg gives the reaction of silicates *(2.3.1)*.

TESTS

pH *(2.2.3)*

Shake 1.0 g with 30 ml of *carbon dioxide-free water R*. The pH of the suspension is 3.5 to 5.5.

Chlorides *(2.4.4)*

To 1.0 g add a mixture of 20 ml of *dilute nitric acid R* and 30 ml of *water R* and heat on a water-bath for 15 min, shaking frequently. Dilute to 50 ml with *water R* if necessary, filter and cool. 10 ml of the filtrate diluted to 15 ml with *water R* complies with the limit test for chlorides (250 ppm).

Heavy metals *(2.4.8)*

Suspend 2.5 g in sufficient *water R* to produce a semi-fluid slurry. Dry at 140 °C. When the dried substance is white, break up the mass with a glass rod. Add 25 ml of 1 *M hydrochloric acid* and boil gently for 5 min, stirring frequently with the glass rod. Centrifuge for 20 min and filter the supernatant liquid through a membrane filter. To the residue in the centrifuge tube add 3 ml of *dilute hydrochloric acid R* and 9 ml of *water R* and boil. Centrifuge for 20 min and filter the supernatant liquid through the same membrane filter. Wash the residue with small quantities of *water R*, combine the filtrates and washings and dilute to 50 ml with *water R*. To 20 ml of the solution add 50 mg of *ascorbic acid R* and 1 ml of *concentrated ammonia R*. Neutralise with *dilute ammonia R2*. Dilute to 25 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (25 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

Not more than 5.0 per cent, determined on 0.200 g by ignition in a platinum crucible at 900 ± 50 °C for 2 h. Allow to cool in a desiccator before weighing.

ASSAY

To the residue obtained in the test for loss on ignition add 0.2 ml of *sulphuric acid R* and sufficient *alcohol R* to moisten the residue completely. Add 6 ml of *hydrofluoric acid R* and evaporate to dryness on a hot-plate at 95 °C to 105 °C, taking care to avoid loss from sputtering. Wash down the sides of the dish with 6 ml of *hydrofluoric acid R* and evaporate to dryness. Ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh.

The difference between the mass of the final residue and the mass of the residue obtained in the test for loss on ignition gives the amount of SiO_2 in the quantity of the substance to be examined used.

Ph Eur

Colloidal Hydrated Silica

General Notices

(Ph Eur monograph 0738)

SiO_2,xH_2O 60.1 *63231-67-4*

(anhydrous)

Action and use

Excipient.

Ph Eur

DEFINITION

Colloidal hydrated silica contains not less than 98.0 per cent and not more than the equivalent of 100.5 per cent of SiO_2 (M_r 60.1), determined on the ignited substance.

CHARACTERS

A white or almost white, light, fine, amorphous powder, practically insoluble in water and in mineral acids, with the exception of hydrofluoric acid. It dissolves in hot solutions of alkali hydroxides.

IDENTIFICATION

A. About 20 mg gives the reaction of silicates *(2.3.1)*.

B. When heated in an oven at 100 °C to 105 °C for 2 h, it shows a loss of mass not less than 3.0 per cent.

TESTS

Solution S

To 2.5 g add 50 ml of *hydrochloric acid R* and mix. Heat on a water-bath for 30 min, stirring from time to time. Maintain the original volume by adding *dilute hydrochloric acid R*. Evaporate to dryness. Add to the residue a mixture of 8 ml of *dilute hydrochloric acid R* and 24 ml of *water R*. Heat to boiling and filter under reduced pressure through a sintered-glass filter (16) *(2.1.2)*. Wash the residue on the filter with a hot mixture of 3 ml of *dilute hydrochloric acid R* and 9 ml of *water R*. Wash with small quantities of *water R*, combine the filtrate and washings and dilute to 50 ml with *water R*.

pH *(2.2.3)*

Suspend 1.0 g in 30 ml of a 75 g/l solution of *potassium chloride R*. The pH of the suspension is 4.0 to 7.0.

Water-absorption capacity

In a mortar, triturate 5 g with 5 ml of *water R*, added drop by drop. The mixture remains powdery.

Substances soluble in hydrochloric acid

In a platinum dish, evaporate to dryness 10.0 ml of solution S and dry to constant mass at 100 °C to 105 °C. The mass of the residue is not more than 10 mg (2.0 per cent).

Chlorides *(2.4.4)*

Heat 0.5 g with 50 ml of *water R* on a water-bath for 15 min. Dilute to 100 ml with *water R* and centrifuge at 1500 g for 5 min. 10 ml of the supernatant solution diluted to 15 ml with *water R* complies with the limit test for chlorides (0.1 per cent).

Sulphates *(2.4.13)*

Dilute 2 ml of solution S to 100 ml with *distilled water R*. 15 ml of the solution complies with the limit test for sulphates (1 per cent).

Iron *(2.4.9)*

To 2 ml of solution S add 28 ml of *water R*. 10 ml of the solution complies with the limit test for iron (300 ppm).

Heavy metals *(2.4.8)*

To 20 ml of solution S add 50 mg of *hydroxylamine hydrochloride R* and 1 ml of *concentrated ammonia R*. Adjust to pH 3.5 by adding *dilute ammonia R2*, monitoring the pH potentiometrically. Dilute to 25 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (25 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

Not more than 20.0 per cent, determined on 0.200 g in a platinum crucible by heating at 100 °C to 105 °C for 1 h and then at 900 ± 50 °C for 2 h.

ASSAY

To the residue obtained in the test for loss on ignition add 0.2 ml of *sulphuric acid R* and a quantity of *alcohol R* sufficient to moisten the residue completely. Add 6 ml of *hydrofluoric acid R* and evaporate to dryness at 95 C to 105 C, taking care to avoid loss from sputtering. Wash the inside of the dish with 6 ml of *hydrofluoric acid R* and evaporate to dryness again. Ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh. The difference between the mass of the final residue and that of the mass obtained in the test for loss on ignition corresponds to the mass of SiO_2 in the test sample.

Ph Eur

Colloidal Silver for External Use

General Notices

(Ph Eur monograph 2281)

9015-51-4

Action and use

Antibacterial.

Ph Eur

DEFINITION

Colloidal metallic silver containing protein.

Content

70.0 per cent to 80.0 per cent of Ag (dried substance).

CHARACTERS

Appearance

Green or bluish-black metallic shiny flakes or powder, hygroscopic.

Solubility

Freely soluble or soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. To 5 ml of the filtrate obtained in the test for alkalinity (see Tests) add 0.05 ml of *copper sulphate solution R* and 1 ml of *dilute sodium hydroxide solution R*. Shake. A violet colour appears within 15 min.

B. To 1 ml of solution S (see Tests), add 2 ml of *sodium chloride solution R*. A precipitate is formed which dissolves in an exces of water.

C. Ignite 0.05 g of the substance to be examined. Dissolve the residue in 10 ml of *nitric acid R*. The filtrate gives the reaction of silver *(2.3.1)*.

TESTS

Solution S

Dissolve 1.25 g of the substance to be examined in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent. Allow to stand for 5 min then shake vigorously. Filter through a tared sintered-glass filter (16) *(2.1.2)* after 30 min.

Alkalinity

To 40.0 ml of solution S add 10.0 ml of *0.05 M sulphuric acid R* and 2.0 g of *anhydrous sodium sulphate R*. Shake and filter several times if necessary. To 25.0 ml of the clear and colourless solution add 0.1 ml of *phenolphthalein solution R*. Not less than 1.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Silver ions

To 0.50 g of the substance to be examined add 5 ml of *anhydrous ethanol R*. Shake for 1 min, filter and add 2 ml of *hydrochloric acid R* to the filtrate. No precipitate is formed.

Sensitivity to electrolytes

Dissolve 0.1 g of the substance to be examined in 100 ml of *water R*. Transfer a part of the solution into a test tube. When examined viewing horizontally the solution appears clear and reddish-brown. When examined viewing vertically, the solution appears turbid with a greenish-brown fluorescence. To 5 ml of the solution add 5 ml of a solution of 0.50 g/l *sodium chloride R* and mix by shaking for 1 minute. When examined viewing horizontally the solution remains clear and reddish-brown.

Water insoluble substances

Maximum 1.0 per cent.

Wash the residue obtained on the filter during preparation of solution S 5 times with 10 ml of *water R*. Dry the filter to constant mass at 100-105 C. The residue weighs a maximum of 12.5 mg.

Loss on drying *(2.2.32)*

Maximum 8.0 per cent, determined on 0.500 g by drying in an oven at 80 C.

ASSAY

Ignite 0.200 g of the substance to be examined at 650 ± 50 C until the residue is white. Allow to cool, add 10 ml of a mixture of equal volumes of *nitric acid R* and *water R* and boil for 1 min. Transfer the contents of the crucible into a flask and titrate with *0.1 M ammonium thiocyanate* using 50 mg *ferric sulphate R* as indicator, until a reddish-brown colour appears.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 10.79 mg of Ag.

STORAGE

In an airtight container.

Ph Eur

Compressible Sugar

General Notices

Action and use

Excipient.

DEFINITION

Compressible Sugar contains Sucrose and maltodextrin or dried glucose syrup. It contains 95.0 to 98.0% of Sucrose and 2 to 5% of dried glucose syrup or maltodextrin. It may contain a suitable lubricant, invert sugar or suitable colouring matter.

CHARACTERISTICS

Dry free-flowing powder or microcrystalline agglomerates.

Very soluble in *water*.

Dissolve 20 g in 80 ml of water*, dilute to 100 ml with* water *and filter (solution S).*

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of compressible sugar *(RS 401)*.

B. The specific optical rotation of the uninverted solution obtained in the Assay is not less than 62.6 and the acid-inverted solution is laevorotatory.

TESTS

Conductivity

Not more than 35 µS·cm^{-1}, Appendix V O. Dissolve 31.3 g in *carbon dioxide-free water* prepared from *distilled water* and dilute to 100 ml with the same solvent. Measure the conductivity of the solution (C_1), while gently stirring with a magnetic stirrer, and that of the water used for preparing the solution (C_2). The readings must be stable within 1% over a period of 30 seconds. Calculate the conductivity of the solution of the substance being examined from the expression $C_1-0.35C_2$.

Calcium

Add 1 ml of *ammonium oxalate* to 5 ml of solution S. The solution remains clear for not less than 1 minute.

Lead

Not more than 0.5 ppm of Pb when determined by *atomic absorption spectrometry*, Appendix II D, Method II, using an apparatus equipped with a graphite furnace and the following solution. Dissolve 50 mg in 0.5 ml of *lead-free nitric acid* in a polyfluorocarbon-lined digestion bomb and heat at 150° for 5 hours. Allow to cool and dilute to 5 ml with *water*. Measure the absorbance at 283.3 nm, maintaining the drying temperature of the furnace at 110°, the ashing temperature at 600° and the atomising temperature at 2100°.

Chloride

2 ml of solution S diluted to 15 ml with *water* complies with the *limit test for chlorides,* Appendix VII (125 ppm).

Sulphate

7.5 ml of solution S diluted to 15 ml with *water* complies with the *limit test for sulphates*, Appendix VII (100 ppm).

Loss on drying

When dried at 105° for 4 hours, loses 0.25 to 1.0% of its weight. Use 1 g.

ASSAY

To 26 g of the substance being examined, previously dried, add 0.3 ml of a saturated aqueous solution of *lead(II) acetate* and 90 ml of *water*, shake, dilute to 100 ml with *water* and mix. Distribute evenly on the surface of a sheet of medium-fast filter paper 8 g of chromatographic siliceous earth and filter the solution with the aid of vacuum, discarding the first 20 ml of the filtrate. Transfer 25 ml of the filtrate into each of two 50 ml flasks. Slowly add 6 ml of 2M *hydrochloric acid* to one flask while rotating it, add 10 ml of *water*, mix, place the flask in a water bath at 60°, continuously shake for 3 minutes and allow the flask to stand in the water bath for a further 7 minutes. Immediately cool to 20° and dilute the solution with *water* to 50 ml and mix. Cool the contents of the second flask to 20°, dilute with *water* to volume and mix. Maintain both flasks at 20° for 30 minutes. Determine the *specific optical rotation*, Appendix V F, of each solution at 20°. Calculate the percentage of $C_{12}H_{22}O_{11}$ using the expression $100(a_0 - a_i)/88.3$ where a_0 and a_i are the specific optical rotations of the uninverted and acid-inverted solutions respectively.

Dental-type Silica

General Notices

(Ph Eur monograph 1562)

Action and use

Excipient.

Ph Eur

DEFINITION

Amorphous silica (precipitated, gel or obtained by flame hydrolysis).

Content

94.0 per cent to 100.5 per cent of SiO_2 (ignited substance).

CHARACTERS

Appearance

White or almost white, light, fine amorphous powder.

Solubility

Practically insoluble in water and in mineral acids. It dissolves in hydrofluoric acid and hot solutions of alkali hydroxides.

IDENTIFICATION

About 20 mg gives the reaction of silicates *(2.3.1)*.

TESTS

Solution S

To 2.5 g add 50 ml of *hydrochloric acid R* and mix. Heat on a water-bath for 30 min, stirring from time to time. Evaporate to dryness. Add to the residue a mixture of 8 ml of *dilute hydrochloric acid R* and 24 ml of *water R*. Heat to boiling and filter under reduced pressure through a sintered-glass filter (16) *(2.1.2)*. Wash the residue on the filter with a hot mixture of 3 ml of *dilute hydrochloric acid R* and 9 ml of *water R*. Wash with small quantities of *water R*, combine the washings and the filtrate, then dilute to 50 ml with *water R*.

pH *(2.2.3)*

3.2 to 8.9.

Suspend 5 g in a mixture of 5 ml of a 7.46 g/l solution of *potassium chloride R* and 90 ml of *carbon dioxide-free water R*.

Chlorides

Liquid chromatography *(2.2.29)* as described in the test for sulphates.

Retention time Chlorides = about 4 min.

Limit:

— *chlorides*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.3 per cent).

Sulphates

Liquid chromatography *(2.2.29)*.

Test solution To 0.625 g of the substance to be examined add 30 ml of *water R* and boil for 2 h. Allow to cool and quantitatively transfer to a 50 ml graduated flask. Dilute to 50.0 ml with *water R*. Dilute 5.0 ml of the supernatant to 50.0 ml with *water R* and filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution Dissolve 0.50 g of *anhydrous sodium sulphate R* and 0.062 g of *sodium chloride R* in *water R* and dilute to 1000.0 ml with *water R*. Dilute 5.0 ml of this solution to 50.0 ml with *water R*.

Column:

— *material*: non-metallic;

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: suitable anion-exchange resin (30-50 µm).

Mobile phase Dissolve 0.508 g of *sodium carbonate R* and 0.05 g of *sodium hydrogen carbonate R* in *water R*, then dilute to 1000 ml with the same solvent.

Flow rate 1.2 ml/min.

Detection Conductivity detector.

Injection 25 µl.

Retention time Sulphates = about 8 min.

Limit:

— *sulphates*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (4.0 per cent, expressed as sodium sulphate).

Iron *(2.4.9)*

Maximum 400 ppm.

Dilute 2 ml of solution S to 40 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 25 ppm.

To 20 ml of solution S, add 50 mg of *hydroxylamine hydrochloride R* and 1 ml of *concentrated ammonia R*. Adjust to pH 3.5 by adding *dilute ammonia R2*, monitoring the pH potentiometrically. Dilute to 25 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on ignition

Maximum 25.0 per cent, determined on 0.200 g in a platinum crucible by heating at 100-105 °C for 1 h and then at 1000 ± 50 °C for 2 h.

ASSAY

To the residue obtained in the test for loss on ignition add 0.2 ml of *sulphuric acid R* and a quantity of *ethanol (96 per cent) R* sufficient to moisten the residue completely. Add 6 ml of *hydrofluoric acid R* and evaporate to dryness at 95-105 °C, taking care to avoid loss from sputtering. Wash the inside of the crucible with 6 ml of *hydrofluoric acid R* and evaporate to dryness again. Ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh. The difference between the mass of the final residue and that of the mass obtained in the test for loss on ignition corresponds to the mass of SiO_2 in the test sample.

Ph Eur

Dilute Sulphuric Acid

General Notices

DEFINITION

Dilute Sulphuric Acid is prepared by adding 104 g of Sulphuric Acid to 896 g of Purified Water with constant stirring and cooling. It contains not less than 9.5% and not more than 10.5% w/w of H_2SO_4.

Weight per ml

1.062 g to 1.072 g, Appendix V G.

ASSAY

To 10 g add 40 ml of *water* and titrate with 1M *sodium hydroxide VS* using *methyl orange solution* as indicator. Each ml of 1M *sodium hydroxide VS* is equivalent to 49.04 mg of H_2SO_4.

Farmed Salmon Oil

General Notices

(Ph Eur monograph 1910)

Ph Eur

DEFINITION

Purified fatty oil obtained from fresh farmed *Salmo salar*. The positional distribution (β(2)-acyl) is 60-70 per cent for cervonic (docosahexaenoic) acid (C22:6 n-3; DHA), 25-35 per cent for timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA) and 40-55 per cent for moroctic acid (C18:4 n-3).

Content:

— sum of the contents of EPA and DHA (expressed as triglycerides): 10.0 per cent to 28.0 per cent.

Authorised antioxidants in concentrations not exceeding the levels specified by the competent authority may be added.

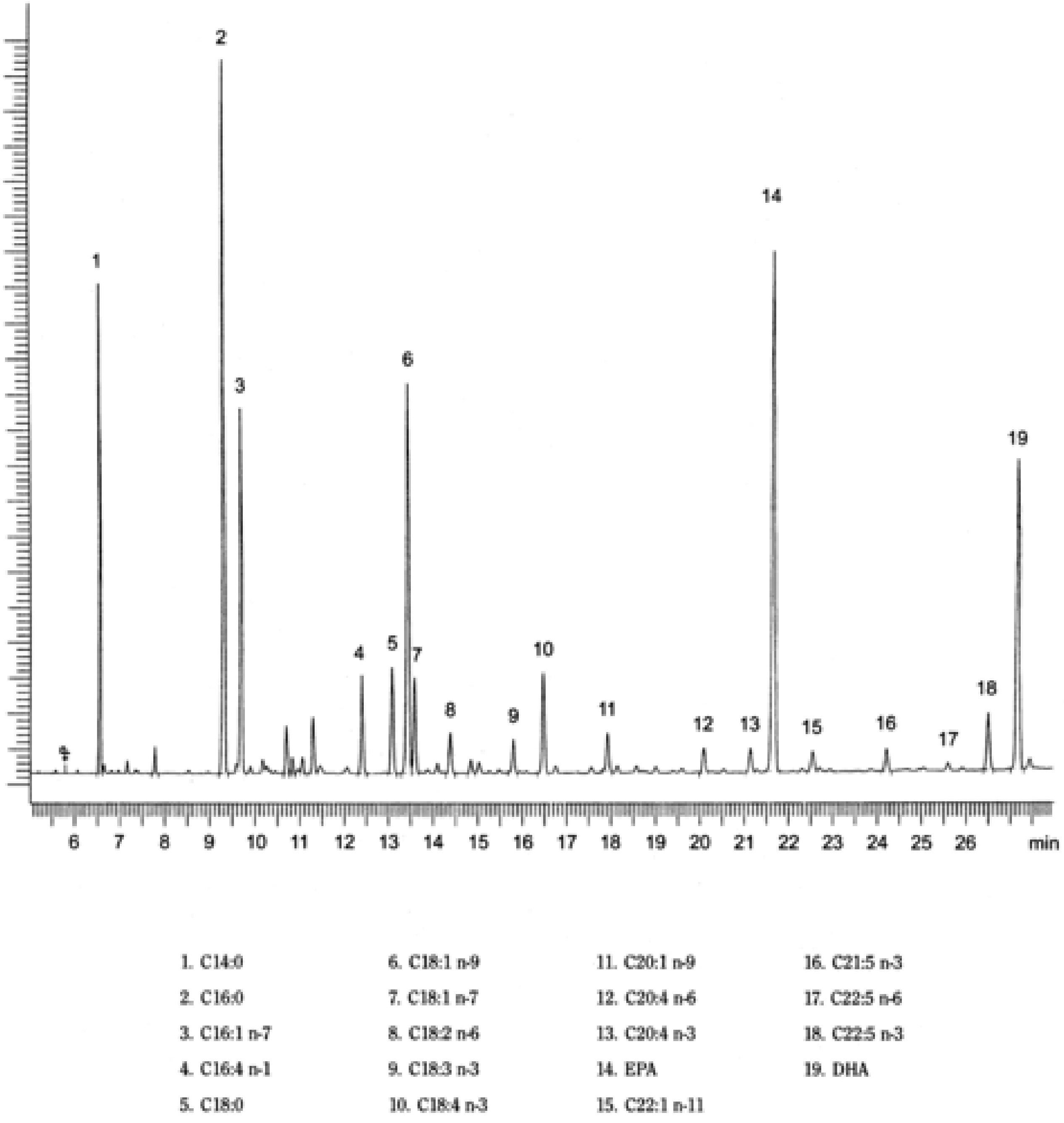


Figure 1910.-1. – *Chromatogram for the composition of fatty acids in farmed salmon oil*

PRODUCTION

The fish shall only be given feed with a composition that is in accordance with the relevant EU or other applicable regulations.

The oil is produced by mechanical expression of fresh raw materials, either from the whole fish, or fish where the fillets have been removed, at a temperature not exceeding 100 °C, and without using solvents. After centrifugation, solid substances may be removed from the oil by cooling and filtering (winterisation).

CHARACTERS

Appearance

Pale pink liquid.

Solubility

Practically insoluble in water, very soluble in acetone and in heptane, slightly soluble in anhydrous ethanol.

IDENTIFICATION

Examine the ^{13}C NMR spectra obtained in the assay for positional distribution (β(2)-acyl) of fatty acids. The spectra contain peaks between 172 ppm and 173 ppm with shifts similar to those in the type spectrum (Figure 1910.-2). The oil to be examined complies with the limits of this assay.

TESTS

Absorbance *(2.2.25)*

Minimum 0.10, measured at the maximum between 470 nm and 480 nm.

Dissolve 5.0 ml in 5.0 ml of *trimethylpentane R*.

Acid value *(2.5.1)*

Maximum 2.0.

Anisidine value *(2.5.36)*

Maximum 10.0.

Peroxide value *(2.5.5, Method A)*

Maximum 5.0.

Unsaponifiable matter *(2.5.7)*

Maximum 1.5 per cent, determined on 5.0 g.

Linoleic acid *(2.4.29)*

Maximum 11.0 per cent.

Identify the peak due to linoleic acid using the chromatogram in Figure 1910.-1. Determine the percentage content by normalisation.

ASSAY

Positional distribution (β(2)-acyl) of fatty acids *(2.2.33)*

Use a high resolution FT-NMR spectrometer operating at minimum 300 MHz.

Test solution Dissolve 190-210 mg of fresh salmon oil in 500 µl of *deuterated chloroform R*. Prepare at least 3 samples and examine within 3 days.

Acquisition of ^{13}C NMR spectra. The following parameters may be used.

— *sweep width*: 200 ppm (- 5 to 195 ppm);

— *irradiation frequency offset*: 95 ppm;

— *time domain*: 64 K;

— *pulse delay*: 2 s;

— *pulse program*: zgig 30 (inverse gated, 30 ° excitation pulse);

— *dummy scans*: 4;

— *number of scans*: 4096.

Processing and plotting The following parameters may be used:

— *size*: 64 K (zero-filling);

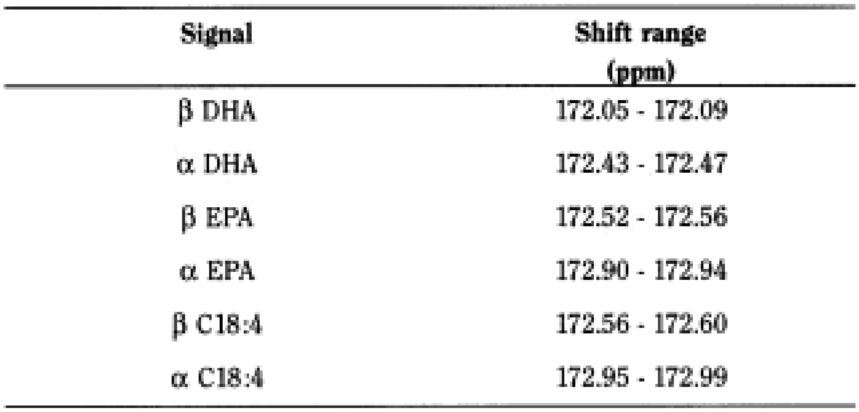
— *window multiplication*: exponential;

— *Lorentzian broadening factor*: 0.2 Hz.

Use the $CDCl_3$ signal for shift referencing. The shift of the central peak of the 1:1:1 triplet is set to 77.16 ppm.

Plot the spectral region δ 171.5-173.5 ppm. Compare the spectrum with the reference spectrum in Figure 1910.-2. The shift values lie within the ranges given in Table 1910.-1.

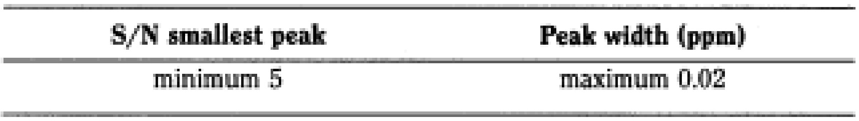
Table 1910.-1 – Shift values



Signal	Shift range (ppm)
β DHA	172.05 - 172.09
α DHA	172.43 - 172.47
β EPA	172.52 - 172.56
α EPA	172.90 - 172.94
β C18:4	172.56 - 172.60
α C18:4	172.95 - 172.99

System suitability Calculate the signal-to-noise ratio for the smallest relevant peak corresponding to α C18:4 signal (in the range δ 172.95-172.99 ppm). Measure the peak width at half-height for the central $CDCl_3$ signal (at δ 77.16 ppm). The system suitability criteria in Table 1910.-2 are fulfilled.

Table 1910.-2 – System suitability criteria



S/N smallest peak	Peak width (ppm)
minimum 5	maximum 0.02

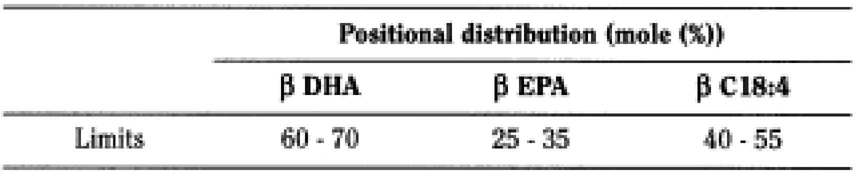
Calculation of positional distribution Calculate the positional distribution (β(2)-acyl) using the expression:

$$\frac{\beta}{\alpha + \beta} \times 100$$

α = peak area of the corresponding α-carbonyl peak,

β = peak area of β-carbonyl peak from C22:6 n-3, C20:5 n-3 or C18:4 n-3, respectively.

Limits:



	Positional distribution (mole (%))		
	β DHA	β EPA	β C18:4
Limits	60 - 70	25 - 35	40 - 55

EPA and DHA ***(2.4.29)***

See Figure 1910.-1.

STORAGE

In an airtight, well-filled container, protected from light, under inert gas.

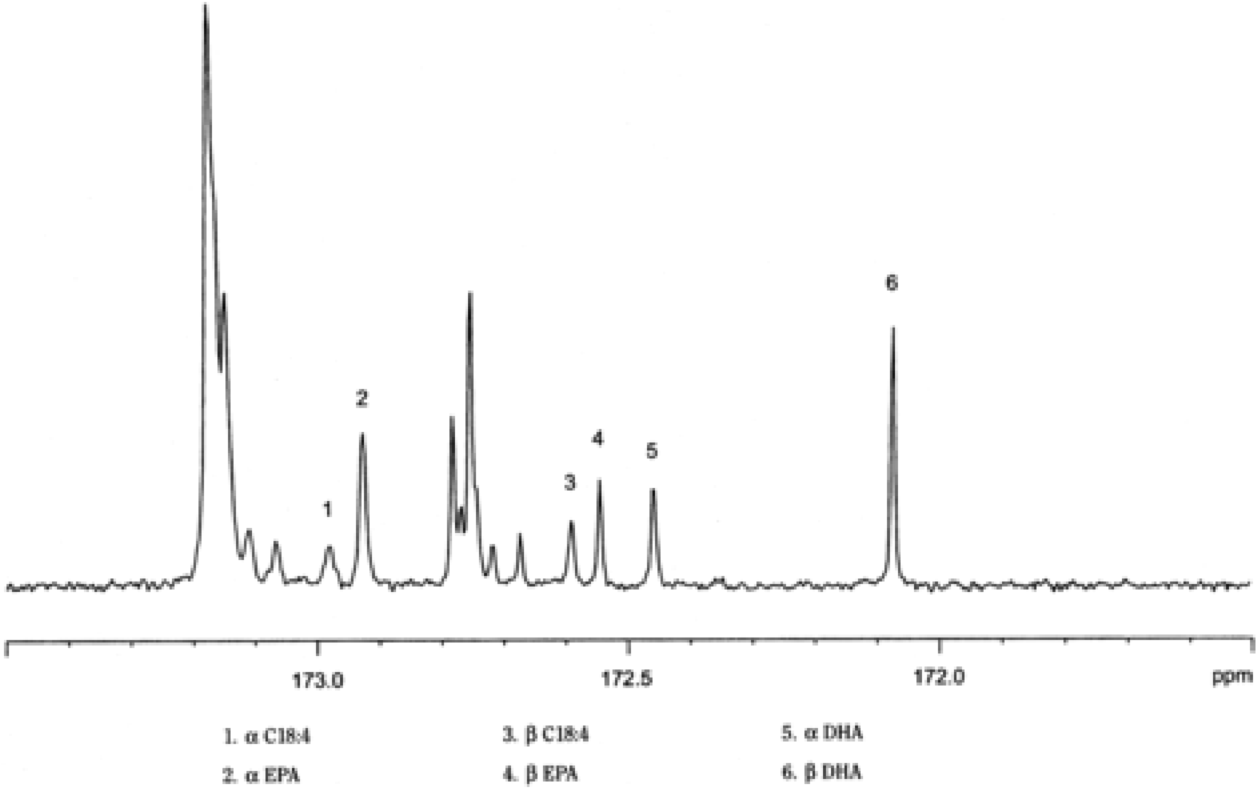
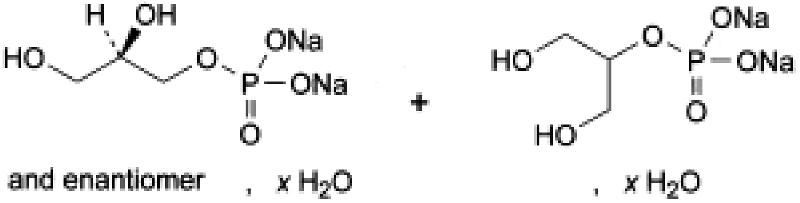


Figure 1910.-2. – *^{13}C NMR spectrum carbonyl region of farmed salmon oil*

Hydrated Sodium Glycerophosphate

General Notices

(Ph Eur monograph 1995)



$C_3H_7Na_2O_6P$, xH_2O 216.0 (anhydrous substance)

Ph Eur

DEFINITION

Mixture of variable proportions of sodium (*2RS*)-2,3-dihydroxypropyl phosphate and sodium 2-hydroxy-1-(hydroxymethyl)ethyl phosphate. The degree of hydration is 4 to 6.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or crystals.

Solubility

Freely soluble in water, practically insoluble in acetone and in alcohol.

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

B. To 0.1 g add 5 ml of *dilute nitric acid R*. Heat to boiling and boil for 1 min. Cool. The solution gives reaction (b) of phosphates (*2.3.1*).

C. In a test-tube fitted with a glass tube, mix 0.1 g with 5 g of *potassium hydrogen sulphate R*. Heat strongly and direct the white vapour into 5 ml of *decolorised fuchsin solution R*. A violet-red colour develops which becomes violet upon heating for 30 min on a water-bath.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Alkalinity

To 10 ml of solution S add 0.2 ml of *phenolphthalein solution R*. Not more than 1.0 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator, (n_2).

Glycerol and alcohol-soluble substances

Maximum 1.0 per cent.

Shake 1.000 g with 25 ml of *alcohol R* for 10 min. Filter. Evaporate the filtrate on a water-bath and dry the residue at 70 °C for 1 h. The residue weighs not more than 10 mg.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R.*

Phosphates *(2.4.11)*

Maximum 0.1 per cent.

Dilute 1 ml of solution S to 10 ml with *water R*. Dilute 1 ml of this solution to 100 ml with *water R.*

Sulphates *(2.4.13)*

Maximum 500 ppm.

Dilute 3 ml of solution S to 15 ml with *water R*.

Iron *(2.4.9)*

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

25.0 per cent to 35.0 per cent, determined on 0.100 g.

ASSAY

Dissolve 0.250 g in 30 ml of *water R*. Titrate with *0.05 M sulphuric acid*, determining the end-point potentiometrically *(2.2.20)*, (n_1).

Calculate the percentage content of sodium glycerophosphate (anhydrous substance) from the expression:

$$\frac{216.0\left(n_1 - \frac{n_2}{4}\right)}{m\,(100 - a)}$$

a = percentage content of water,

n_1 = volume of *0.05 M sulphuric acid* used in the assay, in millilitres,

n_2 = volume of *0.1 M hydrochloric acid* used in the test for alkalinity, in millilitres,

m = mass of the substance to be examined, in grams

Ph Eur

Hydrogenated Soya Oil

General Notices

Hydrogenated Soyabean Oil

(Hydrogenated Soya-bean Oil, Ph Eur monograph 1265)

Action and use

Excipient.

Ph Eur

DEFINITION

Product obtained by refining, bleaching, hydrogenation and deodorisation of oil obtained from seeds of *Glycine soja Sieb*. and Zucc. and *Glycine max* (L.) Merr. (*G. hispida* (Moench) Maxim.). The product consists mainly of triglycerides of palmitic and stearic acids.

CHARACTERS

Appearance

White or almost white mass or powder which melts to a clear, pale yellow liquid when heated.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, in light petroleum (bp: 65-70 °C) after heating and in toluene, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Melting point (see Tests).

B. Composition of fatty acids (see Tests).

TESTS

Melting point *(2.2.15)*

66 °C to 72 °C.

Acid value *(2.5.1)*

Maximum 0.5.

Dissolve 10.0 g in 50 ml of a hot mixture of equal volumes of *ethanol (96 per cent) R* and *toluene R*, previously neutralised with *0.1 M potassium hydroxide* using 0.5 ml of *phenolphthalein solution R1* as indicator. Titrate the solution immediately while still hot.

Peroxide value ***(2.5.5, Method A)***

Maximum 5.0.

Unsaponifiable matter ***(2.5.7)***

Maximum 1.0 per cent, determined on 5.0 g.

Alkaline impurities in fatty oils ***(2.4.19)***

Dissolve 2.0 g with gentle heating in a mixture of 1.5 ml of *ethanol (96 per cent) R* and 3 ml of *toluene R*. Add 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96 per cent) R*. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour to yellow.

Composition of fatty acids ***(2.4.22, Method A)***

Use the mixture of calibrating substances in Table 2.4.22.-3.

Column:
- *material*: fused silica;
- *size*: *l* = 25 m, Ø = 0.25 mm;
- *stationary phase*: *poly(cyanopropyl)siloxane R* (film thickness 0.2 µm).

Carrier gas *helium for chromatography R.*

Flow rate 0.65 ml/min.

Split ratio 1:100.

Temperature:
- *column*: 180 °C for 20 min;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Composition of the fatty-acid fraction of the oil:
- *saturated fatty acids of chain length less than* C_{14}: maximum 0.1 per cent;
- *myristic acid*: maximum 0.5 per cent;
- *palmitic acid*: 9.0 per cent to 16.0 per cent;
- *stearic acid*: 79.0 per cent to 89.0 per cent;
- *oleic acid and isomers*: maximum 4.0 per cent;
- *linoleic acid and isomers*: maximum 1.0 per cent;
- *linolenic acid and isomers*: maximum 0.2 per cent;
- *arachidic acid*: maximum 1.0 per cent;
- *behenic acid*: maximum 1.0 per cent.

Nickel

Maximum 1.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Introduce 5.0 g into a platinum or silica crucible, previously tared after calcination. Cautiously heat and introduce into the substance a wick formed from twisted ashless filter paper. Light the wick. When the substance is alight stop heating. After combustion, ignite in a muffle furnace at about 600 ± 50 °C. Continue the ignition until white ash is obtained. After cooling, take up the residue with 2 quantities, each of 2 ml, of *dilute hydrochloric acid R* and transfer into a 25 ml graduated flask. Add 0.3 ml of *nitric acid R* and dilute to 25.0 ml with *water R* .

Reference solutions Prepare 3 reference solutions by adding 1.0 ml, 2.0 ml and 4.0 ml of *nickel standard solution (0.2 ppm Ni) R* to 2.0 ml of the test solution and diluting to 10.0 ml with *water R* .

Source Nickel hollow-cathode lamp.

Wavelength 232 nm.

Atomisation device Graphite furnace.

Carrier gas *argon R.*

Water ***(2.5.12)***

Maximum 0.3 per cent, determined on 1.000 g.

STORAGE

Protected from light.

Ph Eur

Hydrophobic Colloidal Anhydrous Silica

General Notices

(Ph Eur monograph 2208)

Ph Eur

DEFINITION

Colloidal silicon dioxide partly alkylated for hydrophobation.

Content

99.0 per cent to 101.0 per cent SiO_2 (ignited substance).

CHARACTERS

Appearance

Light, fine, white or almost white, amorphous powder, not wettable by water.

Solubility

Practically insoluble in water and mineral acids except hydrofluoric acid. It dissolves slowly in hot solutions of alkali hydroxides.

IDENTIFICATION

A. About 25 mg ignited at 900 ± 50 °C in a platinum crucible for 2 h gives the reaction of silicates *(2.3.1)*.

B. Water-dispersible fraction (see Tests).

TESTS

Chlorides *(2.4.4)*

Maximum 250 ppm.

To 1.0 g add 30 ml of *methanol R* and 20 ml of *dilute nitric acid R*. Heat on a water-bath for 15 min stirring frequently. Cool, dilute to 50 ml with *water R* and filter. Dilute 10 ml of the filtrate to 15 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 25 ppm.

Suspend 2.5 g in 30 ml of *methanol R*, stir and add 30 ml of *dilute ammonia R1*. With frequent stirring evaporate on a water-bath and dry the residue in an oven at 140 °C. When the dried substance is white, break up the mass with a glass rod. Reduce the residue to a powder and add 15 ml of *methanol R* and 25 ml of *1 M hydrochloric acid*. Boil gently for 5 min, stirring frequently with the glass rod. Centrifuge for 20 min and filter the supernatant liquid through a membrane filter. To the residue in the centrifuge tube add 3 ml of *dilute hydrochloric acid R* and 9 ml of *water R* and boil. Centrifuge for 20 min and filter the supernatant liquid through the same membrane filter. Wash the residue with small quantities of *water R*, combine the filtrates and washings and dilute to 50 ml with *water R*. To 20 ml of this solution add 50 mg of *ascorbic acid R* and 1 ml of *concentrated ammonia R*. Neutralise with *dilute ammonia R2*. Dilute to 25 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water-dispersible fraction

Maximum 3.0 per cent.

Place 0.400 g in a 500 ml separating funnel, add 100 ml of *water R* and shake for 1 min. Allow to stand for 1 h. Allow 90 ml of the aqueous phase to run out dropwise without filtration into a suitable dish dried at 140 °C and cooled in a desiccator. Evaporate to dryness at 140 °C, starting at a low temperature to avoid splashing. Cool in a desiccator. The residue weighs a maximum of 12 mg.

Loss on ignition

Maximum 6.0 per cent, determined on 0.200 g by ignition in a platinum crucible at 900 ± 50 °C for 2 h. It is advisable to place the crucible in a cold oven and then to heat up the oven. Allow to cool in a desiccator before weighing.

ASSAY

To the residue obtained in the test for loss on ignition add sufficient *ethanol (96 per cent) R* to moisten the residue completely and 0.2 ml of *sulphuric acid R*. Add 6 ml of *hydrofluoric acid R* and evaporate to dryness on a hot-plate at about 100 C, taking care to avoid loss from sputtering. Wash down the sides of the platinum crucible with 6 ml of *hydrofluoric acid R* and evaporate to dryness. Ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh.

The difference between the mass of the residue obtained in the test for loss on ignition and the mass of the final residue gives the amount of SiO_2 in the quantity of the substance to be examined.

Ph Eur

Liquid Sorbitol (Crystallising)

General Notices

Sorbitol Solution (70 per cent) (Crystallising)

(Ph Eur monograph 0436)

Action and use

Excipient.

Ph Eur

DEFINITION

Aqueous solution of a hydrogenated, partly hydrolysed starch.

Content

— anhydrous substance: 68.0 per cent *m/m* to 72.0 per cent *m/m*,

— D-glucitol (D-sorbitol, $C_6H_{14}O_6$): 92.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless, syrupy liquid, miscible with water.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. To 7.0 g add 40 ml of *water R* and 6.4 g of *disodium tetraborate R* , allow to stand for 1 h, shaking occasionally, and dilute to 50.0 ml with *water R* . Filter if necessary. The angle of rotation *(2.2.7)* is 0° to + 1.5°.

C. It is a clear, syrupy liquid at a temperature of 25 °C.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dilute 7.0 g to 50 ml with *water R* .

Conductivity ***(2.2.38)***

Maximum 10 µS.cm^{-1} measured on the undiluted liquid sorbitol (crystallising) while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent calculated as glucose equivalent.

To 5.0 g add 6 ml of *water R* , 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine* . With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R* , added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Lead ***(2.4.10)***

Maximum 0.5 ppm.

Nickel ***(2.4.15)***

Maximum 1 ppm.

Water ***(2.5.12)***

28.0 per cent to 32.0 per cent *m/m*, determined on 0.1 g.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution Mix 1.00 g of the substance to be examined with 20 ml of *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 65.0 mg of *sorbitol CRS* in 2 ml of *water R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dissolve 65 mg of *mannitol R* and 65 mg of *sorbitol R* in 2 ml of *water R* and dilute to 5.0 ml with the same solvent.

Column:

— *size*: *l* = 0.3 m, Ø = 7.8 mm,

— *stationary phase*: *strong cation exchange resin (calcium form) R* (9 µm),

— *temperature*: 85 ± 1 °C.

Mobile phase Degassed *water R* .

Flow rate 0.5 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl.

Run time 3 times the retention time of sorbitol.

Relative retention with reference to sorbitol (retention time = about 27 min): mannitol = about 0.8.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to mannitol and to sorbitol.

Calculate the percentage content of D-sorbitol from the areas of the peaks and the declared content of *sorbitol CRS* .

Ph Eur

Liquid Sorbitol (Non-crystallising)

General Notices

Sorbitol Solution (70 per cent) (Non-crystallising)

(Ph Eur monograph 0437)

Action and use

Excipient.

Ph Eur

DEFINITION

Aqueous solution of a hydrogenated, partly hydrolysed starch.

Content

— anhydrous substance: 68.0 per cent *m/m* to 72.0 per cent *m/m*,

— D-glucitol (D-sorbitol, $C_6H_{14}O_6$): 72.0 per cent to 92.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless, syrupy liquid, miscible with water.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. To 7.0 g add 40 ml of *water R* and 6.4 g of *disodium tetraborate R* . Allow to stand for 1 h, shaking occasionally, and dilute to 50.0 ml with *water R* . Filter if necessary. The angle of rotation (2.2.7) is + 1.5° to + 3.5°.

C. It is a clear, syrupy liquid at 25 °C.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dilute 7.0 g to 50 ml with *water R*.

Conductivity ***(2.2.38)***

Maximum 10 µS.cm^{-1} measured on the undiluted liquid sorbitol (non crystallising) while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent calculated as glucose equivalent.

To 5.0 g add 6 ml of *water R*, 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Reducing sugars after hydrolysis

Maximum 9.3 per cent calculated as glucose equivalent.

To 6.0 g add 35 ml of *water R*, 40 ml of *1 M hydrochloric acid* and a few glass beads. Boil under a reflux condenser for 4 h. Cool and neutralise with *dilute sodium hydroxide solution R* using 0.2 ml of *bromothymol blue solution R1* as indicator. Cool and dilute to 100.0 ml with *water R*. To 3.0 ml of the solution add 5 ml of *water R*, 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R*. When the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Not less than 8.0 ml of *0.05 M sodium thiosulphate* is required.

Lead ***(2.4.10)***

Maximum 0.5 ppm.

Nickel ***(2.4.15)***

Maximum 1 ppm.

Water ***(2.5.12)***

28.0 per cent to 32.0 per cent *m/m*, determined on 0.1 g.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Mix 1.00 g of the substance to be examined with 20 ml of *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 55.0 mg of *sorbitol CRS* in 2 ml of *water R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dissolve 55 mg of *mannitol R* and 55 mg of *sorbitol R* in 2 ml of *water R* and dilute to 5.0 ml with the same solvent.

Column:

— *size*: *l* = 0.3 m, Ø = 7.8 mm,

— *stationary phase*: *strong cation exchange resin (calcium form) R* (9 µm),

— *temperature*: 85 ± 1 °C.

Mobile phase Degassed *water R* .

Flow rate 0.5 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl.

Run time 3 times the retention time of sorbitol.

Relative retention with reference to sorbitol (retention time = about 27 min): mannitol = about 0.8.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to mannitol and to sorbitol.

Calculate the percentage content of D-sorbitol from the areas of the peaks and the declared content of *sorbitol CRS* .

Ph Eur

Maize Starch

General Notices

(Ph Eur monograph 0344)

Action and use

Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical countries where these are not available, Tapioca Starch may be supplied or used.

Ph Eur

DEFINITION

Maize starch is obtained from the caryopsis of *Zea mays* L.

CHARACTERS

Appearance

Matt, white to slightly yellowish, very fine powder which creaks when pressed between the fingers.

Solubility

Practically insoluble in cold water and in ethanol (96 per cent).

The presence of granules with cracks or irregularities on the edge is exceptional.

IDENTIFICATION

A. Examined under a microscope, using not less than 20 × magnification and using equal volumes of *glycerol R* and *water R,* it appears as either angular polyhedral granules of irregular sizes with diameters ranging from about 2 µm to about 23 µm or as rounded or spheroidal granules of irregular sizes with diameters ranging from about 25 µm to about 35 µm. The central hilum consists of a distinct cavity or two-to five-rayed cleft and there are no concentric striations. Between crossed nicol prisms, the starch granules show a distinct black cross intersecting at the hilum.

B. Suspend 1 g in 50 ml of *water R,* boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 ml of the mucilage obtained in identification test B add 0.05 ml of *iodine solution R1*

. An orange-red to dark blue colour is produced which disappears on heating.

TESTS

pH ***(2.2.3)***

4.0 to 7.0.

Shake 5.0 g with 25.0 ml of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

Foreign matter

Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.

Oxidising substances ***(2.5.30)***

Maximum 20 ppm, calculated as H_2O_2.

Sulphur dioxide ***(2.5.29)***

Maximum 50 ppm.

Iron ***(2.4.9)***

Maximum 10 ppm.

Shake 1.5 g with 15 ml of *dilute hydrochloric acid R*. Filter. The filtrate complies with the limit test for iron.

Loss on drying ***(2.2.32)***

Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulphated ash ***(2.4.14)***

Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 103 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the test for Escherichia coli *(2.6.13)*.

Ph Eur

Partially Dehydrated Liquid Sorbitol

General Notices

(Sorbitol, Liquid, Partially Dehydrated, Ph Eur monograph 2048)

Action and use

Excipient.

Ph Eur

DEFINITION

Liquid sorbitol, partially dehydrated is obtained by acid-catalysed partial internal dehydration of liquid sorbitol. It contains not less than 68.0 per cent *m/m* and not more than 85.0 per cent *m/m* of anhydrous substances, composed of a mixture of mainly D-sorbitol and 1,4-sorbitan with mannitol, hydrogenated oligo- and disaccharides, and sorbitans.

Content (nominal value)

— 1,4-sorbitan ($C_6H_{12}O_5$): minimum 15.0 per cent (anhydrous substance),

— D-sorbitol ($C_6H_{14}O_6$): minimum 25.0 per cent (anhydrous substance).

The contents of 1,4-sorbitan and D-sorbitol are within 95.0 per cent to 105.0 per cent of the nominal values.

CHARACTERS

Appearance

Clear, colourless, syrupy liquid.

Solubility

Miscible with water, practically insoluble in mineral oils and vegetable oils.

IDENTIFICATION

Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the peaks in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dilute the substance to be examined with *carbon dioxide-free water R* prepared from *distilled water R* to obtain a solution containing 50.0 per cent *m/m* of anhydrous substance.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Conductivity ***(2.2.38)***

Maximum 20 µS·cm^{-1}.

Measure the conductivity of solution S, while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.3 per cent, calculated as glucose (anhydrous substance).

To an amount of the substance to be examined equivalent to 3.3 g of anhydrous substance, add 3 ml of *water R*, 20.0 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min. Maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 ml of *hydrochloric acid R* and 94 ml of *water R*. When the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 2 ml of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Nickel ***(2.4.15)***

Maximum 1 ppm (anhydrous substance).

Water ***(2.5.12)***

15.0 per cent to 32.0 per cent, determined on 0.10 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram and not more than 10^2 fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.400 g of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *sorbitol CRS* and 20.0 mg of *1,4-sorbitan CRS* in *water R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dissolve 0.100 g of *mannitol R* and 0.100 g of *sorbitol R* in *water R* and dilute to 10.0 ml with the same solvent.

Column:

- — *size*: *l* = 0.3 m, Ø = 7.8 mm,
- — *stationary phase*: *strong cation exchange resin (calcium form) R* (9 µm),
- — *temperature*: 80 ± 5 °C.

Mobile phase Degassed *water R* .

Flow rate 0.5 ml/min.

Detection Refractometer maintained at a constant temperature of about 30-35 °C.

Injection 40 µl.

Relative retention with reference to D-sorbitol (retention time = about 25 min): 1,4-sorbitan = about 0.5; mannitol = about 0.8.

System suitability Reference solution (b):

- — *resolution*: minimum 2.0 between the peaks due to mannitol and D-sorbitol.

Calculate the percentage contents of 1,4-sorbitan and D-sorbitol using the chromatogram obtained with reference solution (a) and the declared contents of *1,4-sorbitan CRS* and of *sorbitol CRS* .

LABELLING

The label states the content of D-sorbitol and the content of 1,4-sorbitan (= nominal values).

Ph Eur

Potato Starch

General Notices

(Ph Eur monograph 0355)

Action and use

Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical countries where these are not available, Tapioca Starch may be supplied or used.

Ph Eur

DEFINITION

Potato starch is obtained from the tuber of *Solanum tuberosum* L.

CHARACTERS

Appearance

Very fine, white or almost white powder which creaks when pressed between the fingers.

Solubility

Practically insoluble in cold water and in alcohol.

Potato starch does not contain starch grains of any other origin. It may contain a minute quantity, if any, of tissue fragments of the original plant.

IDENTIFICATION

A. Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R* , it presents granules, either irregularly shaped, ovoid or pear-shaped, usually 30 µm to 100 µm in size but occasionally exceeding 100 µm, or rounded, 10 µm to 35 µm in size. There are occasional compound granules having 2 to 4 components. The ovoid and pear-shaped granules have an eccentric hilum and the rounded granules acentric or slightly eccentric hilum. All granules show clearly visible concentric striations. Between crossed nicol prisms, the granules show a distinct black cross intersecting at the hilum.

B. Suspend 1 g in 50 ml of *water R* , boil for 1 min and cool. A thick, opalescent mucilage is formed.

C. To 1 ml of the mucilage obtained in identification test B, add 0.05 ml of *iodine solution*

R1 . An orange-red to dark blue colour is produced which disappears on heating.

TESTS

pH *(2.2.3)*

5.0 to 8.0.

Shake 5.0 g with 25.0 ml of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

Foreign matter

Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R* , not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.

Oxidising substances *(2.5.30)*

Maximum 20 ppm, calculated as H_2O_2.

Sulphur dioxide *(2.5.29)*

Maximum 50 ppm.

Iron *(2.4.9)*

Maximum 10 ppm.

Shake 1.5 g with 15 ml of *dilute hydrochloric acid R.* Filter. The filtrate complies with the limit test for iron.

Loss on drying *(2.2.32)*

Maximum 20.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulphated ash *(2.4.14)*

Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and not more than 10^2 fungi per gram, determined by plate count. It complies with the test for *Escherichia coli (2.6.13)* .

Ph Eur

Pregelatinised Starch

General Notices

Pregelatinised Maize Starch
(Ph Eur monograph 1267)

When Pregelatinised Starch is prepared from *Zea mays*, the title Pregelatinised Maize Starch may be used.

Ph Eur

DEFINITION

Pregelatinised starch is prepared from *Maize starch* (*0344*), *Potato starch* (*0355*) or *Rice starch* (*0349*) by mechanical processing in the presence of water, with or without heat, to rupture all or part of the starch granules and subsequent drying. It contains no added substances but it may be modified to render it compressible and to improve its flow characteristics.

CHARACTERS

Appearance

White or yellowish-white powder.

It swells in cold water.

IDENTIFICATION

A. Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R* it presents irregular, translucent, white or yellowish-white flakes or pieces with an uneven surface. Under polarised light (between crossed nicol prisms), starch granules with a distinct black cross intersecting at the hilum may be seen.

B. Disperse 0.5 g in 2 ml of *water R* without heating and add 0.05 ml of *iodine solution R1* . A reddish-violet to blue colour is produced.

TESTS

pH *(2.2.3)*

4.5 to 7.0.

Progressively add 3.0 g to 100.0 ml of *carbon dioxide-free water R* , stirring continuously. Determine the pH when a homogeneous solution is obtained.

Oxidising substances ***(2.5.30)***

It complies with the test for oxidising substances. Use a mixture of equal volumes of *water R* and *methanol R* as solvent.

Sulphur dioxide ***(2.5.29)***

Maximum 50 ppm.

Iron ***(2.4.9)***

Maximum 20 ppm.

Dissolve the residue obtained in the test for sulphated ash in 20 ml of *dilute hydrochloric acid R*. Filter. 10 ml of the filtrate complies with the limit test for iron

Foreign matter

Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, not more than traces of matter other than starch granules are present.

Loss on drying ***(2.2.32)***

Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulphated ash ***(2.4.14)***

Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and not more than 10^2 fungi per gram, determined by plate-count. It complies with the test for *Escherichia coli (2.6.13)*.

LABELLING

The type of starch used as starting material is stated.

Ph Eur

Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies

General Notices

(Ph Eur monograph 1483)

Ph Eur

DEFINITION

Products with risk of transmitting agents of animal spongiform encephalopathies are those derived from tissues or secretions of animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge. This monograph applies to all substances or preparations obtained from such animals and to all substances or preparations where products obtained from such animals are included as active substances or excipients or have been used during production, for example as raw or source materials, starting materials or reagents.

PRODUCTION

Production complies with chapter *Minimising the risk of transmitting animal spongiform encephalophathy agents via human and veterinary medicinal products (5.2.8)*.

Ph Eur

Refined Safflower Oil

General Notices

(Ph Eur monograph 2088)

Ph Eur

DEFINITION

Fatty oil obtained from seeds of *Carthamus tinctorius* L. (type I) or from seeds of hybrids of *Carthamus tinctorius* L. (type II), by expression and/or extraction followed by refining. Type II refined safflower oil is rich in oleic (*cis*-9-octadecenoic) acid.

It may contain a suitable antioxidant.

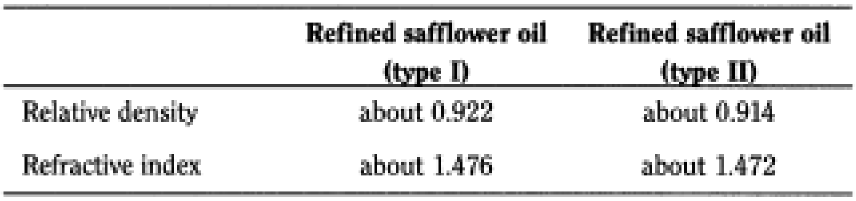
CHARACTERS

Appearance

Clear, viscous, yellow to pale yellow liquid.

Solubility

Miscible with light petroleum (bp: 40-60 °C), practically insoluble in alcohol.



	Refined safflower oil (type I)	Refined safflower oil (type II)
Relative density	about 0.922	about 0.914
Refractive index	about 1.476	about 1.472

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Carry out the identification of fatty oils by thin-layer chromatography *(2.3.2)*. The chromatogram obtained is comparable with the type chromatogram for type I or type II refined safflower oil.

B. It complies with the test for composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 0.5.

Peroxide value ***(2.5.5)***

Maximum 10.0. Maximum 5.0, if intended for the manufacture of parenteral dosage forms.

Unsaponifiable matter ***(2.5.7)***

Maximum 1.5 per cent, determined on 5.0 g.

Alkaline impurities ***(2.4.19)***

It complies with the test for alkaline impurities in fatty oils.

Composition of fatty acids ***(2.4.22, Method A)***

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty acid fraction of type I refined safflower oil:

— *saturated fatty acids of chain length less than C_{14}*: maximum 0.2 per cent;

— *myristic acid*: maximum 0.2 per cent;

— *palmitic acid*: 4.0 per cent to 10.0 per cent;

— *stearic acid*: 1.0 per cent to 5.0 per cent;

— *oleic acid* (equivalent chain length on polyethyleneglycol adipate 18.3): 8.0 per cent to 21.0 per cent;

— *linoleic acid* (equivalent chain length on polyethyleneglycol adipate 18.9): 68.0 per cent to 83.0 per cent;

— *linolenic acid* (equivalent chain length on polyethyleneglycol adipate 19.7): maximum 0.5 per cent;

— *arachidic acid*: maximum 0.5 per cent;

— *eicosenoic acid* (equivalent chain length on polyethyleneglycol adipate 20.3): maximum 0.5 per cent;

— *behenic acid*: maximum 1.0 per cent.

Composition of the fatty acid fraction of type II refined safflower oil:

— *saturated fatty acids of chain length less than C_{14}*: maximum 0.2 per cent;

— *myristic acid*: maximum 0.2 per cent;

— *palmitic acid*: 3.6 per cent to 6.0 per cent;

— *stearic acid*: 1.0 per cent to 5.0 per cent;

— *oleic acid* (equivalent chain length on polyethyleneglycol adipate 18.3): 70.0 per cent to 84.0 per cent;

— *linoleic acid* (equivalent chain length on polyethyleneglycol adipate 18.9): 7.0 per cent to 23.0 per cent;

— *linolenic acid* (equivalent chain length on polyethyleneglycol adipate 19.7): maximum 0.5 per cent;

— *arachidic acid*: maximum 1.0 per cent;

— *eicosenoic acid* (equivalent chain length on polyethyleneglycol adipate 20.3): maximum 1.0 per cent;

- *behenic acid*: maximum 1.2 per cent.

Brassicasterol ***(2.4.23)***

Maximum 0.3 per cent of brassicasterol in the sterol fraction of the oil.

Water ***(2.5.32)***

Maximum 0.1 per cent, determined on 5.00 g, if intended for use in the manufacture of parenteral dosage forms.

STORAGE

In a well-filled, airtight container, protected from light.

LABELLING

The label states:

- where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms;
- the type of oil (type I or type II).

Ph Eur

Refined Sesame Oil

General Notices

Sesame Oil

(Ph Eur monograph 0433)

Ph Eur

DEFINITION

Fatty oil obtained from the ripe seeds of *Sesamum indicum* L. by expression or extraction. It is then refined. Improved colour and odour may be obtained by further refining. It may contain a suitable antioxidant.

CHARACTERS

Appearance

Clear, light yellow liquid, almost colourless.

Solubility

Practically insoluble in ethanol (96 per cent), miscible with light petroleum.

Relative density

About 0.919.

Refractive index

About 1.473.

It solidifies to a butter-like mass at about - 4 °C.

IDENTIFICATION

First identification A.

Second identification B.

A. Composition of triglycerides (see Tests).

B. Carry out the identification of fatty oils by thin-layer chromatography *(2.3.2)*. The chromatogram obtained is similar to the typical chromatogram for sesame oil.

TESTS

Acid value *(2.5.1)*

Maximum 0.5, determined on 10.0 g; maximum 0.3 if intended for use in the manufacture of parenteral dosage forms.

Peroxide value *(2.5.5)*

Maximum 10.0; maximum 5.0 if intended for use in the manufacture of parenteral dosage forms.

Unsaponifiable matter *(2.5.7)*

Maximum 2.0 per cent, determined on 5.0 g.

Alkaline impurities *(2.4.19)*

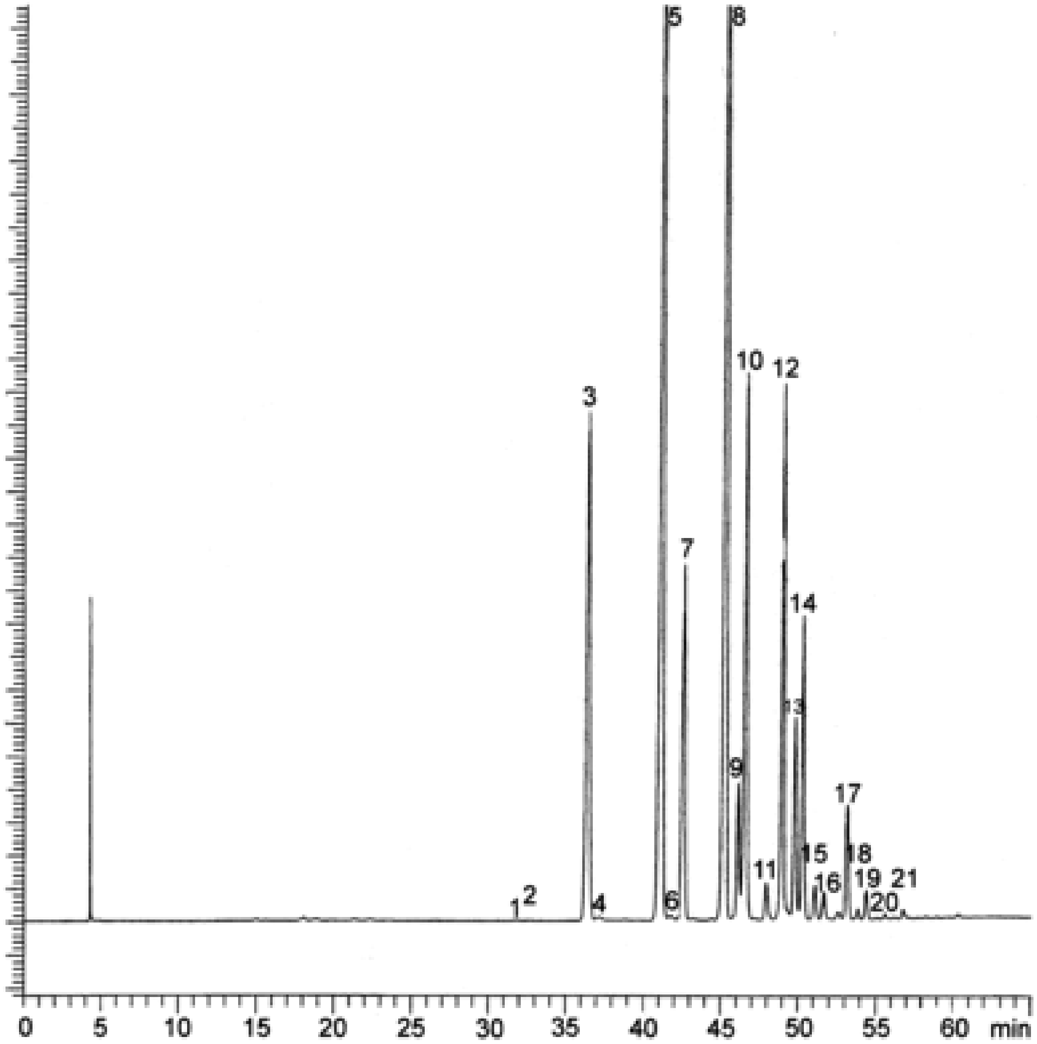
It complies with the test for alkaline impurities in fatty oils.

Cottonseed oil

Mix 5 ml in a test-tube with 5 ml of a mixture of equal volumes of *pentanol R* and a 10 g/l solution of *sulphur R* in *carbon disulphide R*. Warm the mixture carefully until the carbon disulphide is expelled, and immerse the tube to 1/3 of its depth in boiling *saturated sodium chloride solution R*. No reddish colour develops within 15 min.

Composition of triglycerides

Liquid chromatography *(2.2.29)*.



1. LLLn
2. OLnLn
3. LLL
4. OLLn
5. OLL
6. OOLn
7. PLL
8. OOL
9. SLL
10. POL
11. PPL
12. OOO
13. SOL
14. POO
15. PSL
16. PPO
17. SOO
18. PSO
19. SSL
20. PPS
21. SSO

Figure 0433.-1. – ***Typical chromatogram for the composition of triglycerides in refined sesame oil***

Test solution Dilute 50.0 mg of the substance to be examined to 10.0 ml with a mixture of equal volumes of *acetone R* and *methylene chloride R*.

Reference solutions Dissolve 80.0 mg of *triolein R* in a mixture of equal volumes of *acetone R* and *methylene chloride R* and dilute to 50.0 ml with the same mixture of solvents. Prepare 5 reference solutions by dilution of this solution so as to cover concentrations ranging from the disregard limit (0.5 per cent) to the upper limit for OLL (30.0 per cent).

Plot the logarithm of the area of the peak due to triolein against the logarithm of the mass of triolein in the reference solution, in milligrams.

Column 2 columns coupled in series:

— *size of each column*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:
— *mobile phase A*: *acetone R, methylene chloride R, acetonitrile R* (5:15:80 *V/V/V*);

— *mobile phase B*: *acetone R, acetonitrile R, methylene chloride R* (20:20:60 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100 → 75	0 → 25
15 - 25	75	25
25 - 70	75 → 0	25 → 100
70 - 75	0 → 100	100 → 0
75 - 80	100	0

Flow rate 1.0 ml/min.

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:

— *carrier gas*: *nitrogen R*;

— *flow rate*: 0.7 l/min;

— *evaporator temperature*: 85 °C;

— *nebuliser temperature*: 45 °C.

Injection 20 µl.

Identification of peaks Use the chromatograms obtained with the reference solutions to identify the peak due to triolein; identify the other peaks using the chromatogram shown in Figure 0433.-1. The fatty acids are designated as linolenic (Ln), linoleic (L), oleic (O), palmitic (P) and stearic (S).

System suitability Test solution:

— *resolution*: minimum 1.5 between the peaks due to OOO (triolein) and SOL.

Using the calibration curve obtained with the reference solutions, determine the percentage content of each peak with an area greater than that of the peak corresponding to the disregard limit (0.5 per cent). Assuming that the sum of these peak areas is 100 per cent, normalise the percentage content of each of the 8 triglycerides specified below.

Composition of triglycerides:
— *LLL*: 7.0 per cent to 19.0 per cent;

— *OLL*: 13.0 per cent to 30.0 per cent;

— *PLL*: 5.0 per cent to 9.0 per cent;

— *OOL*: 12.0 per cent to 23.0 per cent;

— *POL*: 6.0 per cent to 14.0 per cent;

— *OOO*: 5.0 per cent to 14.0 per cent;

- *SOL*: 2.0 per cent to 8.0 per cent;
- *POO*: 2.0 per cent to 10.0 per cent.

Water ***(2.5.12)***

Maximum 0.05 per cent, determined on 5.0 g, if intended for use in the manufacture of parenteral dosage forms.

STORAGE

In an airtight, well-filled container, protected from light; if intended for use in the manufacture of parenteral dosage forms store under an inert gas in an airtight container.

When the container has been opened, its contents are to be used as soon as possible. Any part of the contents not used at once is protected by an atmosphere of an inert gas.

LABELLING

The label states:

- whether the oil is obtained by expression or extraction;
- where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms;
- where applicable, the name of the inert gas used.

Ph Eur

Refined Soya Oil

General Notices

Refined Soyabean Oil

(Refined Soya-bean Oil, Ph Eur monograph 1473)

Action and use

Excipient.

When Soya Oil, Soyabean Oil or Soya-bean Oil is demanded, Refined Soya Oil shall be supplied.

When intended for use in the manufacture of a parenteral dosage form, Refined Soya Oil complying with the requirement for Water below should be used.

Ph Eur

DEFINITION

Fatty oil obtained from seeds of *Glycine soja* Siebold et Zucc. and *Glycine max* (L.) Merr. (*Glycine hispida* (Moench) Maxim.) by extraction and subsequent refining. It may contain a suitable antioxidant.

CHARACTERS

Appearance

Clear, pale yellow liquid.

Solubility

Miscible with light petroleum (50-70 °C), practically insoluble in ethanol (96 per cent).

Relative density

About 0.922.

Refractive index

About 1.475.

IDENTIFICATION

Identification of fatty oils by thin-layer chromatography *(2.3.2)* .

Results The chromatogram obtained is similar to the typical chromatogram for soya-bean oil.

TESTS

Acid value *(2.5.1)*

Maximum 0.5, determined on 10.0 g.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0, and maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter *(2.5.7)*

Maximum 1.5 per cent, determined on 5.0 g.

Alkaline impurities *(2.4.19)*

It complies with the test.

Composition of fatty acids *(2.4.22, Method A)*

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

— *saturated fatty acids of chain length less than* C_{14} : maximum 0.1 per cent;

— *myristic acid* : maximum 0.2 per cent;

— *palmitic acid* : 9.0 per cent to 13.0 per cent;

— *palmitoleic acid* : maximum 0.3 per cent;

— *stearic acid* : 2.5 per cent to 5.0 per cent;

— *oleic acid* : 17.0 per cent to 30.0 per cent;

— *linoleic acid* : 48.0 per cent to 58.0 per cent;

— *linolenic acid* : 5.0 per cent to 11.0 per cent;

— *arachidic acid*: maximum 1.0 per cent;

— *eicosenoic acid*: maximum 1.0 per cent;

— *behenic acid*: maximum 1.0 per cent.

Brassicasterol *(2.4.23)*

Maximum 0.3 per cent in the sterol fraction of the oil.

Water *(2.5.32)*

Maximum 0.1 per cent, determined on 1.00 g, if intended for use in the manufacture of parenteral preparations. Use a mixture of equal volumes of *decanol R* and *anhydrous methanol R* as the solvent.

STORAGE

In a well-filled container, protected from light, at a temperature not exceeding 25 °C.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Refined Sunflower Oil

General Notices

(Ph Eur monograph 1371)

Ph Eur

DEFINITION

Fatty oil obtained from the seeds of *Helianthus annuus* L. by mechanical expression or by extraction. It is then refined. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, light yellow liquid.

Solubility

Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C).

Relative density

About 0.921.

Refractive index

About 1.474.

IDENTIFICATION

Identification of fatty oils by thin-layer chromatography *(2.3.2)* .

Results The chromatogram obtained is similar to the typical chromatogram for sunflower oil.

TESTS

Acid value *(2.5.1)*

Maximum 0.5, determined on 10.0 g.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0.

Unsaponifiable matter *(2.5.7)*

Maximum 1.5 per cent, determined on 5.0 g.

Alkaline impurities *(2.4.19)*

It complies with the test.

Composition of fatty acids *(2.4.22, Method A)*

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

- *palmitic acid* : 4.0 per cent to 9.0 per cent;
- *stearic acid* : 1.0 per cent to 7.0 per cent;
- *oleic acid* : 14.0 per cent to 40.0 per cent;
- *linoleic acid* : 48.0 per cent to 74.0 per cent.

STORAGE

In an airtight, well-filled container, protected from light.

LABELLING

The label states whether the oil is obtained by mechanical expression or by extraction.

Ph Eur

Rice Starch

General Notices

(Ph Eur monograph 0349)

Action and use

Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical countries where these are not available, Tapioca Starch may be supplied or used.

Ph Eur

DEFINITION

Rice starch is obtained from the caryopsis of *Oryza sativa* L.

CHARACTERS

Appearance

Very fine, whiteor almost white powder, which creaks when pressed between the fingers.

Solubility

Practically insoluble in cold water and in ethanol (96 per cent).

Rice starch does not contain starch grains of any other origin. It may contain traces of, if any, fragments of the endosperm tissue of the fruit.

IDENTIFICATION

A. Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R* , it presents polyhedral, simple grains 1-10 µm, mostly 4-6 µm, in size. These simple grains often gather in ellipsoidal, compound grains 50-100 µm in diameter. The grains have a poorly visible central hilum and there are no concentric striations. Between orthogonally orientated polarising plates or prisms, the starch grains show a distinct black cross intersecting at the hilum.

B. Suspend 1 g in 50 ml of *water R* , boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 ml of the mucilage obtained in identification test B add 0.05 ml of *iodine solution R1*. An orange-red to dark blue colour is produced, which disappears on heating.

TESTS

pH *(2.2.3)*

5.0 to 8.0.

Shake 5.0 g with 25.0 ml of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

Iron *(2.4.9)*

Maximum 10 ppm for the filtrate.

Shake 1.5 g with 15 ml of *dilute hydrochloric acid R*. Filter.

Foreign matter

Examine under a microscope using a mixture of equal volumes of *glycerol R* and *water R*. Not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 1.00 g by drying in an oven at 130 °C for 90 min.

Sulphated ash *(2.4.14)*

Maximum 0.6 per cent, determined on 1.0 g.

Oxidising substances *(2.5.30)*

Maximum 0.002 per cent, calculated as H_2O_2.

Sulphur dioxide *(2.5.29)*

Maximum 50 ppm

Microbial contamination

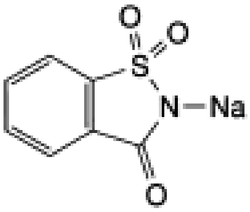
Total viable count *(2.6.12)* not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate-count. It complies with the test for *Escherichia coli (2.6.13)*.

Ph Eur

Saccharin Sodium

General Notices

Soluble Saccharin
(Ph Eur monograph 0787)



$C_7H_4NNaO_3S$ 205.2 *128-44-9*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

2-Sodio-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

It may contain a variable quantity of water.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, efflorescent in dry air.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, E*.

Second identification *A, C, D, E*.

A. Melting point *(2.2.14)*: 226 °C to 230 °C.

To 5 ml of solution S (see Tests) add 3 ml of *dilute hydrochloric acid R*. A white precipitate is formed. Filter and wash with *water R*. Dry the precipitate at 100-105 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs; dry the substances at 100-105 °C before use.

Comparison *Saccharin sodium CRS*.

C. Mix about 10 mg with about 10 mg of *resorcinol R*, add 0.25 ml of *sulphuric acid R* and carefully heat the mixture over a naked flame until a dark green colour is produced. Allow to cool, add 10 ml of *water R* and *dilute sodium hydroxide solution R* until an alkaline reaction is produced. An intense green fluorescence develops.

D. To 0.2 g add 1.5 ml of *dilute sodium hydroxide solution R*, evaporate to dryness and heat the residue carefully until it melts, avoiding carbonisation. Allow to cool, dissolve the mass in about 5 ml of *water R*, add *dilute hydrochloric acid R* until a weak acid reaction is produced and filter, if necessary. To the filtrate add 0.2 ml of *ferric chloride solution R2*. A violet colour develops.

E. Solution S gives reaction (a) of sodium *(2.3.1).*

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 5.0 g in 25 ml of *carbon dioxide-free water R*.

Acidity or alkalinity

To 10 ml of solution S add about 0.05 ml of a 10 g/l solution of *phenolphthalein R* in *ethanol (96 per cent) R*. The solution is not pink. Add 0.1 ml of *sodium hydroxide 0.1 M*. The solution becomes pink.

o- and p-Toluenesulphonamide

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 25 mg of *caffeine R* in *methylene chloride R* and dilute to 100 ml with the same solvent.

Test solution Dissolve 10.0 g of the substance to be examined in 50 ml of *water R*. If necessary adjust the solution to pH 7-8 by addition of *1 M sodium hydroxide* or *1 M hydrochloric acid*. Shake the solution with 4 quantities, each of 50 ml, of *methylene chloride R*. Combine the lower layers, dry over *anhydrous sodium sulphate R* and filter. Wash the filter and the sodium sulphate with 10 ml of *methylene chloride R*. Combine the solution and the washings and evaporate almost to dryness in a water-bath at a temperature not exceeding 40 °C. Using a small quantity of *methylene chloride R*, quantitatively transfer the residue into a suitable 10 ml tube, evaporate to dryness in a current of *nitrogen R* and add 1.0 ml of the internal standard solution.

Blank solution Evaporate 200 ml of *methylene chloride R* to dryness in a water-bath at a temperature not exceeding 40 °C. Dissolve the residue in 1 ml of *methylene chloride R*.

Reference solution Dissolve 20.0 mg of *o-toluenesulphonamide R* and 20.0 mg of *p-toluenesulphonamide R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *methylene chloride R*. Evaporate 5.0 ml of the final solution to dryness in a current of *nitrogen R*. Take up the residue using 1.0 ml of the internal standard solution.

Column:

— *material*: fused silica;

— *size*: *l* = 10 m, Ø = 0.53 mm;

— *stationary phase*: *polymethylphenylsiloxane R* (film thickness 2 µm).

Carrier gas *Nitrogen for chromatography R*.

Flow rate 10 ml/min.

Split ratio 1:2.

Temperature:

— *column*: 180 °C;

— *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection: 1 µl.

Elution order *O*-toluenesulphonamide, *p*-toluenesulphonamide, caffeine.

System suitability:

— *Resolution*: minimum 1.5 between the peaks due to *o*-toluenesulphonamide and *p*-toluenesulphonamide in the chromatogram obtained with the reference solution;

— The chromatogram obtained with the blank solution does not show any peak with the same retention times as the internal standard, *o*-toluenesulphonamide and *p*-toluenesulphonamide.

Limits:

— *o-toluenesulphonamide*: the ratio of its area to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm);

— *p-toluenesulphonamide*: the ratio of its area to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm).

Readily carbonisable substances

Dissolve 0.20 g in 5 ml of *sulphuric acid R* and keep at 48-50 °C for 10 min. When viewed against a white background, the solution is not more intensely coloured than a solution prepared by mixing 0.1 ml of red primary solution, 0.1 ml of blue primary solution and 0.4 ml of yellow primary solution *(2.2.2)* with 4.4 ml of *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 15.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*, with slight heating if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 20.52 mg of $C_7H_4NNaO_3S$.

STORAGE

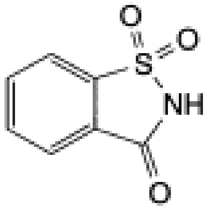
In an airtight container .

Ph Eur

Saccharin

General Notices

(Ph Eur monograph 0947)



$C_7H_5NO_3S$ 183.2 *81-07-2*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

1,2-Benzisothiazol-3(2*H*)-one 1,1-dioxide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Sparingly soluble in boiling water and in ethanol (96 per cent), slightly soluble in cold water. It dissolves in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification C.

Second identification A, B, D, E.

A. A saturated solution, prepared without heating, turns *blue litmus paper R* red.

B. Melting point *(2.2.14)*: 226 °C to 230 °C.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *Saccharin CRS*.

D. Mix about 10 mg with about 10 mg of *resorcinol R*, add 0.25 ml of *sulphuric acid R* and carefully heat the mixture over a naked flame until a dark green colour is produced. Allow to cool, add 10 ml of *water R* and *dilute sodium hydroxide solution R* until an alkaline reaction is produced. An intense green fluorescence develops.

E. To 0.2 g add 1.5 ml of *dilute sodium hydroxide solution R*, evaporate to dryness and heat the residue carefully until it melts, avoiding carbonisation. Allow to cool, dissolve the mass in about 5 ml of *water R*, add *dilute hydrochloric acid R* until a weak acid reaction is produced and filter, if necessary. To the filtrate add 0.2 ml of *ferric chloride solution R2*. A violet colour develops.

TESTS

Solution S

Dissolve 5.0 g in 20 ml of a 200 g/l solution of *sodium acetate R* and dilute to 25 ml with the same solution.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

o- and p-Toluenesulphonamide

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 25 mg of *caffeine R* in *methylene chloride R* and dilute to 100 ml with the same solvent.

Test solution Suspend 10.0 g of the substance to be examined in 20 ml of *water R* and dissolve using 5-6 ml of *strong sodium hydroxide solution R*. If necessary adjust the solution to pH 7-8 with *1 M sodium hydroxide* or *1 M hydrochloric acid* and dilute to 50 ml with *water R*. Shake the solution with 4 quantities, each of 50 ml, of *methylene chloride R*. Combine the lower layers, dry over *anhydrous sodium sulphate R* and filter. Wash the filter and the sodium sulphate with 10 ml of *methylene chloride R*. Combine the solution and the washings and evaporate almost to dryness in a water-bath at a temperature not exceeding 40 °C. Using a small quantity of *methylene chloride R*, quantitatively transfer the residue into a suitable 10 ml tube, evaporate to dryness in a current of nitrogen and dissolve the residue in 1.0 ml of the internal standard solution.

Blank solution Evaporate 200 ml of *methylene chloride R* to dryness in a water-bath at a temperature not exceeding 40 °C. Dissolve the residue in 1 ml of *methylene chloride R*.

Reference solution Dissolve 20.0 mg of *o-toluenesulphonamide R* and 20.0 mg of *p-toluenesulphonamide R* in *methylene chloride R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *methylene chloride R*. Evaporate 5.0 ml of the final solution to dryness in a current of nitrogen. Dissolve the residue in 1.0 ml of the internal standard solution.

Column:
— *material*: fused silica;

— *size*: l = 10 m, Ø = 0.53 mm;

— *stationary phase*: *polymethylphenylsiloxane R* (film thickness 2 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 10 ml/min.

Split ratio 1:2.

Temperature:

— *column*: 180 °C;

— *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µl.

Order of elution *o*-toluenesulphonamide, *p*-toluenesulphonamide, caffeine.

System suitability:

— *resolution*: minimum 1.5 between the peaks due to o-toluenesulphonamide and p-toluenesulphonamide in the chromatogram obtained with the reference solution;

— the chromatogram obtained with the blank solution does not show any peak with the same retention times as the internal standard, *o*-toluenesulphonamide and *p*-toluenesulphonamide.

Limits:

— *o-toluenesulphonamide: the ratio of its area to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm);*

— *p-toluenesulphonamide: the ratio of its area to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm).*

Readily carbonisable substances

Dissolve 0.20 g in 5 ml of *sulphuric acid R* and keep at 48-50 °C for 10 min. When viewed against a white background, the solution is not more intensely coloured than a solution prepared by mixing 0.1 ml of red primary solution, 0.1 ml of blue primary solution and 0.4 ml of yellow primary solution *(2.2.2)* with 4.4 ml of *water R*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 40 ml of *ethanol (96 per cent) R*. Add 40 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, using a 10 g/l solution of *phenolphthalein R* in *ethanol (96 per cent) R* as indicator. Carry out a blank titration.

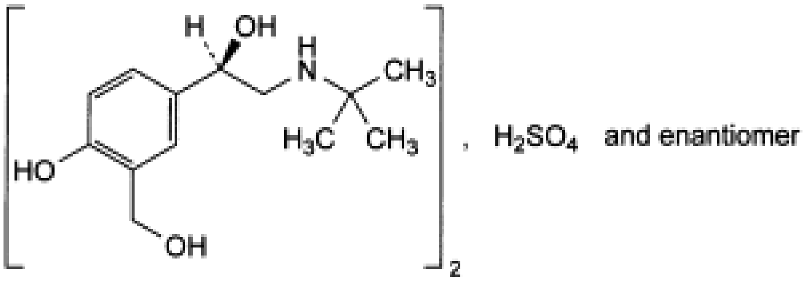
1 ml of *0.1 M sodium hydroxide* is equivalent to 18.32 mg of $C_7H_5NO_3S$.

Ph Eur

Salbutamol Sulphate

General Notices

(Ph Eur monograph 0687)



$(C_{13}H_{21}NO_3)_2H_2SO_4$ 576.7 *51022-70-9*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Preparations

Salbutamol Injection

Salbutamol Nebuliser Solution

Salbutamol Oral Solution

Salbutamol Powder for Inhalation

Salbutamol Pressurised Inhalation

Salbutamol Tablets

Ph Eur

DEFINITION

Bis[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol] sulphate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, practically insoluble or very slightly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E.*

A. Dissolve 80.0 mg in a 10 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with a 10 g/l solution of *hydrochloric acid R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 276 nm. The specific absorbance at the absorption maximum is 55 to 64.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium bromide R*.

Comparison *Salbutamol sulphate CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 12 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 12 mg of *salbutamol sulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *Concentrated ammonia R, water R, ethyl acetate R, 2-propanol R, methyl isobutyl ketone R* (3:18:35:45:50 *V/V/V/V/V*).

Application 1 µl.

Development Over a path of 18 cm.

Drying In air.

Detection Spray with a 1 g/l solution of *methylbenzothiazolone hydrazone hydrochloride R* in a 90 per cent *V/V* solution of *methanol R*, followed by a 20 g/l solution of *potassium ferricyanide R* in a mixture of 1 volume of *concentrated ammonia R1* and 3 volumes of *water R*, followed by a further spraying with a 1 g/l solution of *methylbenzothiazolone hydrazone hydrochloride R* in a 90 per cent *V/V* solution of *methanol R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 50 ml of a 20 g/l solution of *disodium tetraborate R*. Add 1 ml of a 30 g/l solution of *aminopyrazolone R*, 10 ml of *methylene chloride R* and 10 ml of a 20 g/l solution of *potassium ferricyanide R*. Shake and allow to separate. An orange-red colour develops in the methylene chloride layer.

E. It gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 0.250 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.10 ° to + 0.10 °, determined on solution S.

Acidity or alkalinity

To 10 ml of solution S add 0.15 ml of *methyl red solution R* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Impurity J

Maximum 0.2 per cent.

Dissolve 60.0 mg in a 1 g/l solution of *hydrochloric acid R* and dilute to 25.0 ml with the same solvent. The absorbance *(2.2.25)* of the solution measured at 310 nm is not greater than 0.10.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.4 mg of *salbutamol sulphate CRS*, 2.0 mg of *salbutamol impurity B CRS*, 3.0 mg of *salbutamol impurity D CRS*, 3.0 mg of *salbutamol impurity F CRS* and 3.0 mg of *salbutamol impurity G CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of one vial of *salbutamol impurity I CRS* with 1 ml of the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 335 m2/g, a pore size of 10 nm and a carbon loading of 11.7 per cent.

Mobile phase Mix 22 volumes of *acetonitrile R* and 78 volumes of a solution containing 2.87 g/l of *sodium heptanesulphonate R* and 2.5 g/l of *potassium dihydrogen phosphate R* previously adjusted to pH 3.65 with *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 25 times the retention time of salbutamol.

Relative retention With reference to salbutamol (retention time = about 1.9 min): impurity B = about 1.3, impurity A = about 1.7, impurity C = about 2.0, impurity D = about 2.7, impurity H = about 3.0, impurity E = about 3.1, impurity G = about 4.1, impurity F = about 6.2, impurity I = about 23.2.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity I.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to salbutamol and impurity B.

Limits:

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity G*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities A, B, C, E, H, I*: for each impurity, not more than 1.5 times the area of the peak due to salbutamol in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *total*: maximum 1.0 per cent;

— *disregard limit*: 0.05 per cent.

Boron

Maximum 50 ppm.

Test solution To 50 mg of the substance to be examined add 5 ml of a solution containing 13 g/l of *anhydrous sodium carbonate R* and 17 g/l of *potassium carbonate R*. Evaporate to dryness on a water-bath and dry at 120 °C. Ignite the residue rapidly until the organic matter has been destroyed, allow to cool and add 0.5 ml of *water R* and 3.0 ml of a freshly prepared 1.25 g/l solution of *curcumin R* in *glacial acetic acid R*. Warm gently to effect solution, allow to cool and add 3.0 ml of a mixture prepared by adding 5 ml of *sulphuric acid R*, slowly and with stirring, to 5 ml of *glacial acetic acid R*. Mix and allow to stand for 30 min. Dilute to 100.0 ml with *ethanol (96 per cent) R*, filter and use the filtrate.

Reference solution Dissolve 0.572 g of *boric acid R* in 1000.0 ml of *water R*. Dilute 1.0 ml to 100.0 ml with *water R*. To 2.5 ml of the solution add 5 ml of a solution containing 13 g/l of *anhydrous sodium carbonate R* and 17 g/l of *potassium carbonate R*, and treat this mixture in the same manner as the test solution.

Measure the absorbance *(2.2.25)* of the test solution and of the reference solution at the maximum at about 555 nm. The absorbance of the test solution is not greater than that of the reference solution.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 5 ml of *anhydrous formic acid R* and add 35 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

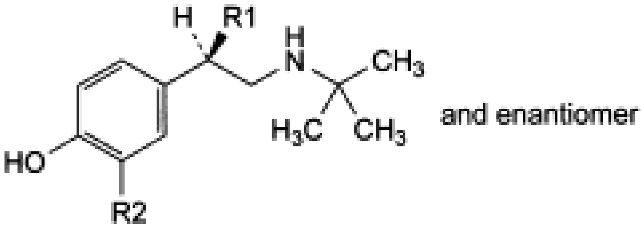
1 ml of *0.1 M perchloric acid* is equivalent to 57.67 mg of $C_{26}H_{44}N_2O_{10}S$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J.

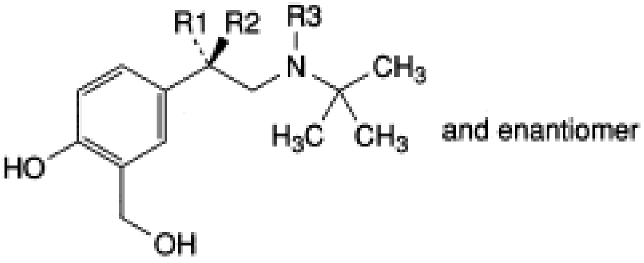


A. R1 = OCH_3, R2 = CH_2OH: [5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-methoxyethyl]-2-hydroxyphenyl]methanol,

B. R1 = OH, R2 = H: (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxyphenyl)ethanol,

C. R1 = OH, R2 = CH_3: (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxy-3-methylphenyl) ethanol,

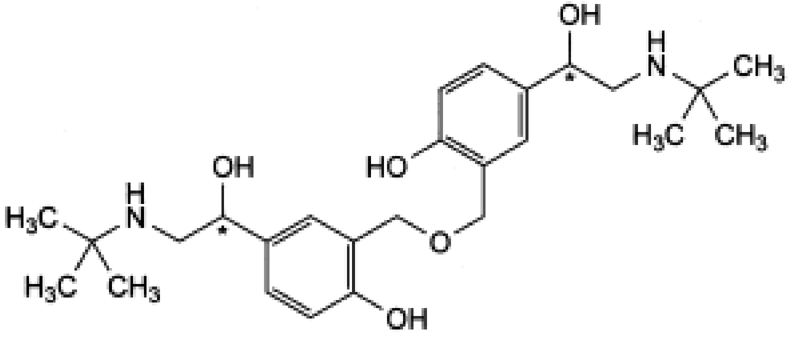
D. R1 = OH, R2 = CHO: 5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde,



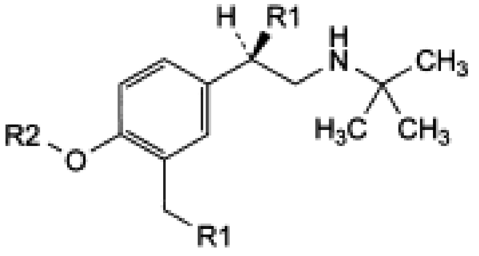
E. R1 = H, R2 = OH, R3 = CH_2-C_6H_5: (1*RS*)-2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol,

G. R1 + R2 = O, R3 = CH_2-C_6H_5: 2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone,

J. R1 + R2 = O, R3 = H: 2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl] ethanone (salbutamone),



F. 1,1′-[oxybis[methylene(4-hydroxy-1,3-phenylene)]]bis[2-[(1,1-dimethylethyl)amino] ethanol],



H. R1 = R2 = H: 4-[2-[(1,1-dimethylethyl)amino]ethyl]-2-methylphenol,

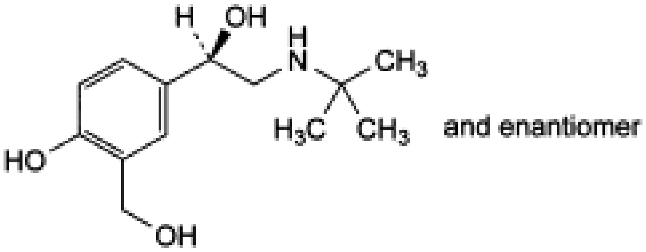
I. R1 = OH, R2 = CH_2-C_6H_5: (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[3-(hydroxymethyl)-4-benzyloxyphenyl]ethanol.

Ph Eur

Salbutamol

General Notices

(Ph Eur monograph 0529)



$C_{13}H_{21}NO_3$ 239.3 *18559-94-9*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Preparation

Salbutamol Pressurised Inhalation

Ph Eur

DEFINITION

(1*RS*)-2-[(1,1-Dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, soluble in ethanol (96 per cent).

mp

About 155 °C, with decomposition.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Dissolve 80.0 mg in a 10 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with a 10 g/l solution of *hydrochloric acid R*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 276 nm. The specific absorbance at the absorption maximum is 66 to 75.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Salbutamol CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 50 ml with the same solvent.

Reference solution Dissolve 10 mg of *salbutamol CRS* in *methanol R* and dilute to 50 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *Concentrated ammonia R, water R, ethyl acetate R, 2-propanol R, methyl isobutyl ketone R* (3:18:35:45:50 *V/V/V/V/V*).

Application 5 µl.

Development Over a path of 18 cm.

Drying In air.

Detection Spray with a 1 g/l solution of *methylbenzothiazolone hydrazone hydrochloride R* in a 90 per cent *V/V* solution of *methanol R*, followed by a 20 g/l solution of *potassium ferricyanide R* in a mixture of 1 volume of *concentrated ammonia R1* and 3 volumes of *water R*, followed by a further spraying with a 1 g/l solution of *methylbenzothiazolone hydrazone hydrochloride R* in a 90 per cent *V/V* solution of *methanol R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 50 ml of a 20 g/l solution of *disodium tetraborate R*. Add 1 ml of a 30 g/l solution of *aminopyrazolone R*, 10 ml of *methylene chloride R* and 10 ml of a 20 g/l solution of *potassium ferricyanide R*. Shake and allow to separate. An orange-red colour develops in the methylene chloride layer.

TESTS

Solution S

Dissolve 0.50 g in *methanol R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *salbutamol CRS*, 2.0 mg of *salbutamol impurity B CRS*, 3.0 mg of *salbutamol impurity D CRS*, 3.0 mg of *salbutamol impurity F CRS* and 3.0 mg of *salbutamol impurity G CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of 1 vial of *salbutamol impurity I CRS* with 1 ml of the mobile phase.

Column:

— *size: l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 335 m2/g, a pore size of 10 nm and a carbon loading of 11.7 per cent.

Mobile phase Mix 22 volumes of *acetonitrile R* and 78 volumes of a solution containing 2.87 g/l of *sodium heptanesulphonate R* and 2.5 g/l of *potassium dihydrogen phosphate R* previously ajusted to pH 3.65 with *dilute phosphoric acid R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 25 times the retention time of salbutamol.

Relative retention With reference to salbutamol (retention time = about 1.9 min): impurity B = about 1.3; impurity A = about 1.7; impurity C = about 2.0; impurity D = about 2.7; impurity H = about 3.0; impurity E = about 3.1; impurity G = about 4.1; impurity F = about 6.2; impurity I = about 23.2.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity I.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to salbutamol and impurity B.

Limits:

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity G*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities A, B, C, E, H, I*: for each impurity, not more than 1.5 times the area of the peak due to salbutamol in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *total*: maximum 1.0 per cent;

— *disregard limit*: 0.05 per cent.

Impurity J

Maximum 0.2 per cent.

Dissolve 50.0 mg in a 1 g/l solution of *hydrochloric acid R* and dilute to 25.0 ml with the same solvent. The absorbance *(2.2.25)* of the solution measured at 310 nm is not greater than 0.10.

Boron

Maximum 50 ppm.

Test solution To 50 mg of the substance to be examined add 5 ml of a solution containing 13 g/l of *anhydrous sodium carbonate R* and 17 g/l of *potassium carbonate R*. Evaporate to dryness on a water-bath and dry at 120 °C. Ignite the residue rapidly until the organic matter has been destroyed, allow to cool and add 0.5 ml of *water R* and 3.0 ml of a freshly prepared 1.25 g/l solution of *curcumin R* in *glacial acetic acid R*. Warm gently to effect solution, allow to cool and add 3.0 ml of a mixture prepared by adding 5 ml of *sulphuric acid R*, slowly and with stirring, to 5 ml of *glacial acetic acid R*. Mix and allow to stand for 30 min. Dilute to 100.0 ml with *ethanol (96 per cent) R*, filter and use the filtrate.

Reference solution Dissolve 0.572 g of *boric acid R* in 1000.0 ml of *water R*. Dilute 1.0 ml to 100.0 ml with *water R*. To 2.5 ml of the solution add 5 ml of a solution containing 13 g/l of *anhydrous sodium carbonate R* and 17 g/l of *potassium carbonate R*, and treat this mixture in the same manner as the test solution.

Measure the absorbance *(2.2.25)* of the test solution and of the reference solution at the maximum at about 555 nm. The absorbance of the test solution is not greater than that of the reference solution.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

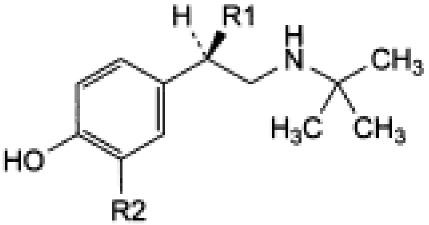
1 ml of *0.1 M perchloric acid* is equivalent to 23.93 mg of $C_{13}H_{21}NO_3$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J.

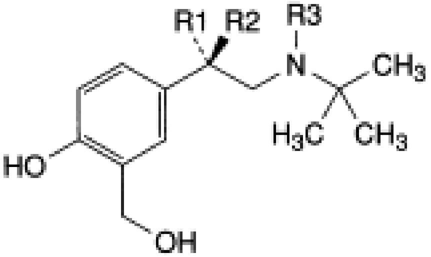


A. R1 = OCH_3, R2 = CH_2OH: [5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-methoxyethyl]-2-hydroxyphenyl]methanol,

B. R1 = OH, R2 = H: (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxyphenyl)ethanol,

C. R1 = OH, R2 = CH_3: (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxy-3-methylphenyl) ethanol,

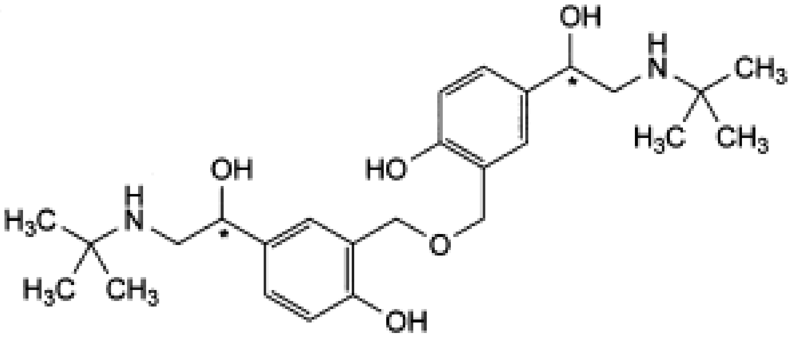
D. R1 = OH, R2 = CHO: 5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde,



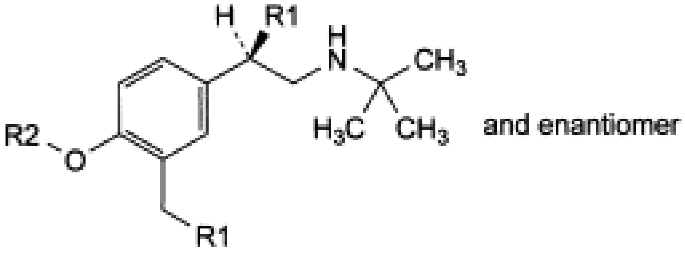
E. R1 = H, R2 = OH, R3 = CH_2-C_6H_5: (1*RS*)-2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol,

G. R1 + R2 = O, R3 = CH_2-C_6H_5: 2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone,

J. R1 + R2 = O, R3 = H: 2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl] ethanone (salbutamone),



F. 1,1′-[oxybis[methylene(4-hydroxy-1,3-phenylene)]]bis[2-[(1,1-dimethylethyl)amino] ethanol],



H
R1
H
N
CH3
H3C
CH3
R2
O
R1
and enantiomer

H. R1 = R2 = H: 4-[2-[(1,1-dimethylethyl)amino]ethyl]-2-methylphenol,

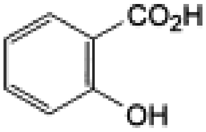
I. R1 = OH, R2 = CH_2-C_6H_5: (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[3-(hydroxymethyl)-4-benzyloxyphenyl]ethanol.

Ph Eur

Salicylic Acid

General Notices

(Ph Eur monograph 0366)



$C_7H_6O_3$ 138.1 *69-72-7*

Action and use

Keratolytic.

Preparations

Salicylic Acid Collodion

Salicylic Acid Ointment

Coal Tar and Salicylic Acid Ointment

Zinc and Salicylic Acid Paste

Ph Eur

DEFINITION

2-Hydroxybenzenecarboxylic acid.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or white or colourless, acicular crystals.

Solubility

Slightly soluble in water, freely soluble in ethanol (96 per cent), sparingly soluble in methylene chloride.

IDENTIFICATION

First identification A, B.

Second identification A, C.

A. Melting point *(2.2.14)*: 158 °C to 161 °C.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Salicylic acid CRS*.

C. Dissolve about 30 mg in 5 ml of *0.05 M sodium hydroxide*, neutralise if necessary and dilute to 20 ml with *water R*. 1 ml of the solution gives reaction (a) of salicylates *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in 50 ml of boiling *distilled water R*, cool and filter.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1 g in 10 ml of *ethanol (96 per cent) R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.50 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 10 mg of *phenol R* (impurity C) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *salicylic acid impurity B CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 50 mg of *4-hydroxybenzoic acid R* (impurity A) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (e) Dilute a mixture of 1.0 ml of each of reference solutions (a), (b) and (c) to 10.0 ml with the mobile phase.

Reference solution (f) Dilute a mixture of 0.1 ml of each of reference solutions (a), (b) and (c) to 10.0 ml with the mobile phase.

Column:

— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: non-deactivated *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *Glacial acetic acid R, methanol R, water R* (1:40:60 *V/V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µl of the test solution and reference solutions (d), (e) and (f).

Relative retention with reference to impurity C: impurity A = about 0.70; impurity B = about 0.90.

System suitability Reference solution (e):

— the 3rd peak in the chromatogram corresponds to the peak due to phenol in the chromatogram obtained with reference solution (d);

— *resolution*: minimum 1.0 between the peaks due to impurities B and C; if necessary, adjust the quantity of acetic acid in the mobile phase.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.1 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.05 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.02 per cent);

— *any other impurity*: for each impurity, not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (f) (0.05 per cent);

— *total*: not more than twice the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.2 per cent);

— *disregard limit*: 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (f).

Chlorides *(2.4.4)*

Maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulphates

Maximum 200 ppm.

Dissolve 1.0 g in 5 ml of *dimethylformamide R* and add 4 ml of *water R*. Mix thoroughly. Add 0.2 ml of *dilute hydrochloric acid R* and 0.5 ml of a 25 per cent *m/m* solution of *barium chloride R*. After 15 min any opalescence in the solution is not more intense than that in a standard prepared as follows: to 2 ml of *sulphate standard solution (100 ppm SO_4) R* add 0.2 ml of *dilute hydrochloric acid R*, 0.5 ml of a 25 per cent *m/m* solution of *barium chloride R*, 3 ml of *water R* and 5 ml of *dimethylformamide R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in 15 ml of *ethanol (96 per cent) R* and add 5 ml of *water R*. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) prepared by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 5 volumes of *water R* and 15 volumes of *ethanol (96 per cent) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.120 g in 30 ml of *ethanol (96 per cent) R* and add 20 ml of *water R*. Titrate with 0.1 M sodium hydroxide, using 0.1 ml of *phenol red solution R* as indicator.

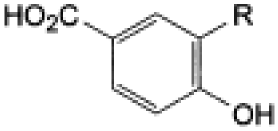
1 ml of *0.1 M sodium hydroxide* is equivalent to 13.81 mg of $C_7H_6O_3$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C.



A. R = H: 4-hydroxybenzoic acid,

B. R = CO_2H: 4-hydroxyisophthalic acid,

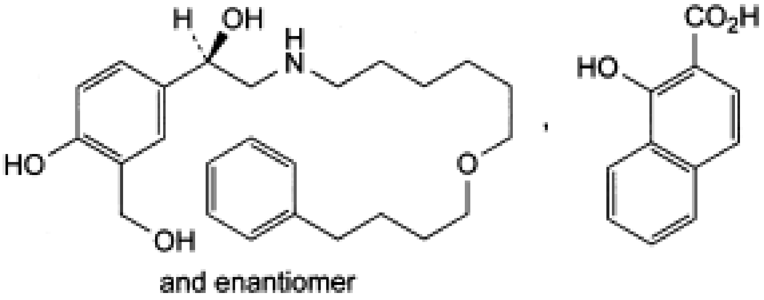
C. phenol.

Ph Eur

Salmeterol Xinafoate

General Notices

(Ph Eur monograph 1765)



$C_{36}H_{45}NO_7$ 604 *94749-08-3*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Ph Eur

DEFINITION

(1*RS*)-1-[4-Hydroxy-3-(Hydroxymethyl)phenyl]-2-[[6-(4-phenylbutoxy)hexyl]amino]ethanol 1-hydroxynaphthalene-2-carboxylate.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in methanol, slightly soluble in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison salmeterol xinafoate CRS.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Protect the solutions from light*.

Solvent mixture *acetonitrile R*, *water R* (50:50 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 11 mg of *salmeterol xinafoate for system suitability CRS* (salmeterol containing impurities E and G) in the solvent mixture and dilute to 2 ml with the solvent mixture.

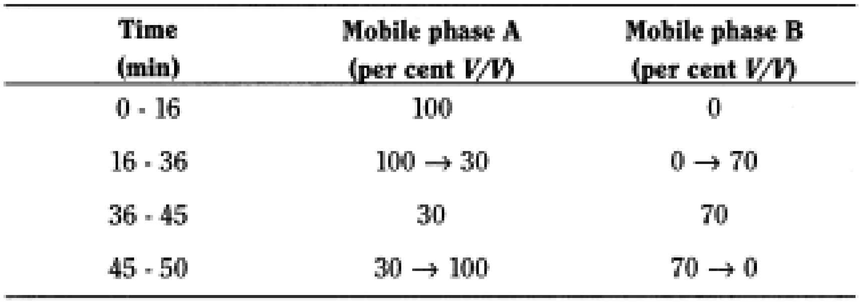
Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- stationary phase: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: mix 24 volumes of a 7.71 g/l solution of *ammonium acetate R* with 24 volumes of a 28.84 g/l solution of *sodium dodecyl sulphate R* and adjust to pH 2.7 with *glacial acetic acid R*; mix with 52 volumes of *acetonitrile R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 16	100	0
16 - 36	100 → 30	0 → 70
36 - 45	30	70
45 - 50	30 → 100	70 → 0

Flow rate 2 ml/min.

Detection Spectrophotometer at 278 nm.

Injection 20 µl; inject the solvent mixture as a blank solution.

Relative retention With reference to salmeterol (retention time = about 13 min): xinafoic acid = about 0.2; impurity A = about 0.3; impurity B = about 0.5; impurity C = about 0.7; impurity D = about 0.8; impurity E = about 0.9; impurity F = about 1.6; impurity G = about 2.7.

System suitability Reference solution (a):

- *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to salmeterol;
- the chromatogram obtained is similar to the chromatogram supplied with *salmeterol xinafoate for system suitability CRS*.

Limits:

- *impurity D*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *impurities A, F, G*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities B, C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than 9 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.9 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to xinafoic acid and any peaks due to the blank.

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 1.000 g.

Dissolve the sample with 30 ml of *anhydrous methanol R*.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29).*

Test solution Dissolve 12.50 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 12.50 mg of *salmeterol xinafoate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1 ml of reference solution (a) described in the test for related substances to 20 ml with the mobile phase.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 24 volumes of a 7.71 g/l solution of *ammonium acetate R* with 24 volumes of a 28.84 g/l solution of *sodium dodecyl sulphate R* and adjust to pH 2.7 with *glacial acetic acid R*. Mix with 52 volumes of *acetonitrile R*.

Flow rate 2 ml/min.

Detection Spectrophotometer at 278 nm.

Injection 20 µl.

Run time Until complete elution of the peak due to salmeterol (about 16 min).

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to salmeterol.

The stationary phase may be regenerated using the gradient described under the test for related substances.

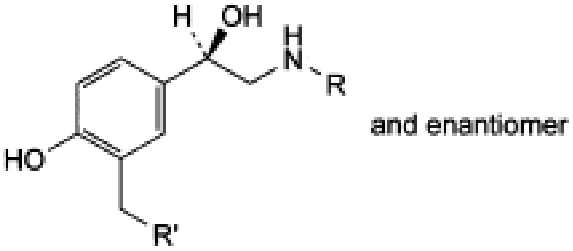
Calculate the percentage content of $C_{36}H_{45}NO_7$ using the chromatogram obtained with reference solution (a) and the declared content of $C_{36}H_{45}NO_7$ in *salmeterol xinafoate CRS*.

STORAGE

Protected from light.

IMPURITIES

Specified impurities: *A, B, C, D, E, F, G*.

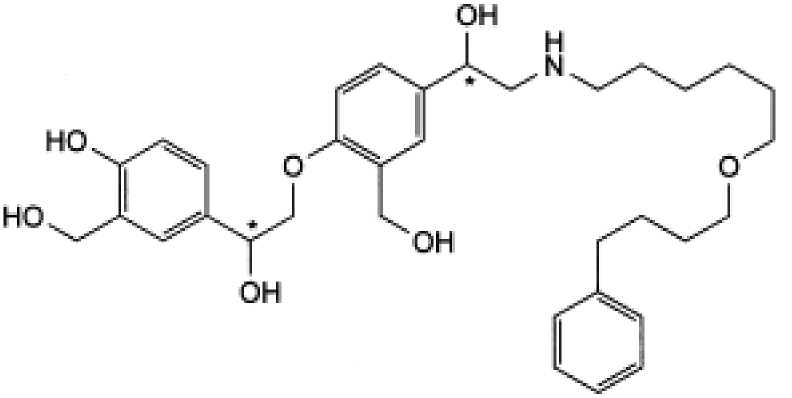


A. $R = [CH_2]_4\text{-}C_6H_5$, $R' = OH$: (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[(4-phenylbutyl)amino]ethanol,

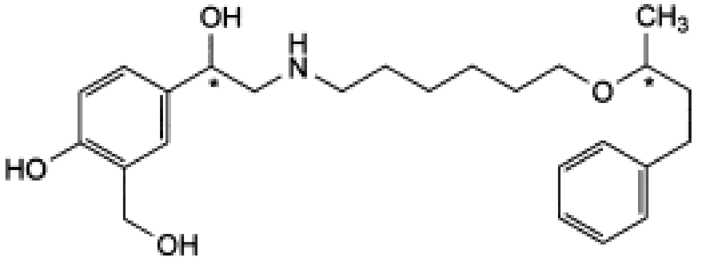
B. $R = [CH_2]_6\text{-}O\text{-}[CH_2]_2\text{-}C_6H_5$, $R' = OH$: (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(2-phenylethoxy)hexyl]amino]ethanol,

C. $R = [CH_2]_6\text{-}O\text{-}[CH_2]_3\text{-}C_6H_5$, $R' = OH$: (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(3-phenylpropoxy)hexyl]amino]ethanol,

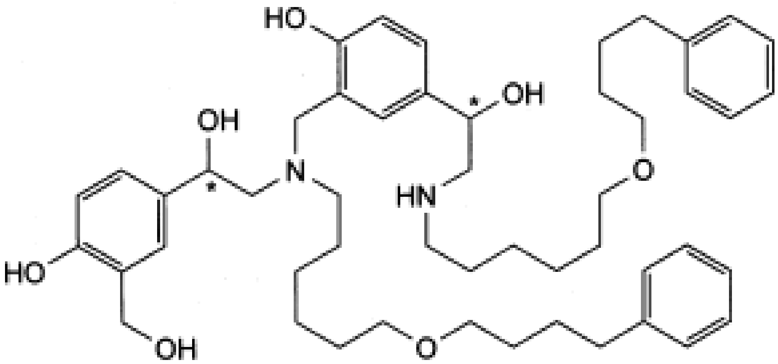
F. $R = [CH_2]_6\text{-}O\text{-}[CH_2]_4\text{-}C_6H_5$, $R' = H$: (1*RS*)-1-(4-hydroxy-3-methylphenyl)-2-[[6-(4-phenylbutoxy)hexyl]amino]ethanol,



D. 1-[4-[2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethoxy]-3-(hydroxymethyl)phenyl]-2-[[6-(3-phenylbutoxy)hexyl]amino]ethanol,


OH
H
N
CH3
O
HO
OH

E. 1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(1-methyl-3-phenylpropoxy)hexyl]amino] ethanol,


HO
OH
OH
N
HN
O
HO
OH
O

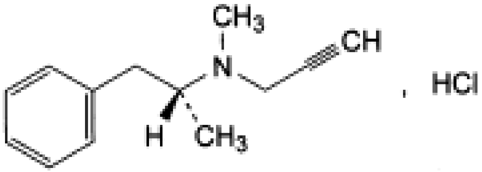
G. 1-[4-hydroxy-3-[[[2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl][6-(4-phenylbutoxy)hexyl]amino]methyl]phenyl]-2-[[6-(4-phenylbutoxy)hexyl]amino]ethanol.

Ph Eur

Selegiline Hydrochloride

General Notices

(Ph Eur monograph 1260)



$C_{13}H_{17}N,HCl$ 223.7 *14611-52-0*

Action and use

Monoamine oxidase type B inhibitor; treatment of Parkinson's disease.

Preparations

Selegiline Oral Solution

Selegiline Tablets

Ph Eur

DEFINITION

N-Methyl-*N*-[(1*R*)-1-methyl-2-phenylethyl]prop-2-yn-1-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, slightly soluble in acetone.

mp: about 143 °C.

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: - 10.0 to - 12.0 (dried substance).

Dissolve 2.000g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs of *potassium chloride R*.

Comparison *Selegiline hydrochloride CRS*.

C. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

3.5 to 4.5.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *selegiline hydrochloride CRS* and 10.0 mg of *butyl parahydroxybenzoate R* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Column:

- size*: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dilute 500 ml of *acetonitrile R* to 1000.0 ml with a butylammonium acetate buffer solution pH 6.5 prepared as follows: dissolve 4 ml of *butylamine R* in 900 ml of *water R*, adjust to pH 6.5 with *acetic acid R* and dilute to 1000.0 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 1.7 times the retention time of selegiline.

System suitability Reference solution (a):

- *resolution*: minimum 3 between the peaks due to selegiline and butyl parahydroxybenzoate.

Limits:

- *impurities A, B, C, D*: for each impurity, not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent); disregard any peak due to chlorides.

Impurity E

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of 1 ml of *2-propanol R* and 10 µl of *butylamine R* and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 8.0 mg of *(RS)-selegiline hydrochloride CRS* in a mixture of 10 µl of *butylamine R* and 1 ml of *2-propanol R* and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 0.5 ml of reference solution (a) to 20.0 ml with the mobile phase.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel OD for chiral separations R*.

Mobile phase *2-propanol R*, *cyclohexane R* (0.2:99.8 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Retention time Impurity E = about 10 min.

System suitability Reference solution (a):
- *resolution*: minimum 1.5 between the peaks due to impurity E and (*R*)-selegiline; if necessary, adjust the concentration of 2-propanol in the mobile phase.

Limit:
- *impurity E*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.5 kPa.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.180 g in 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

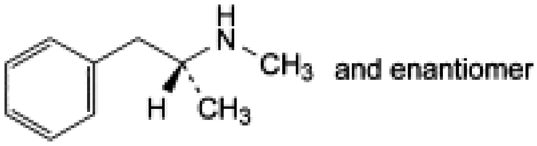
1 ml of *0.1 M perchloric acid* is equivalent to 22.37 mg of $C_{13}H_{18}ClN$.

STORAGE

Protected from light.

IMPURITIES

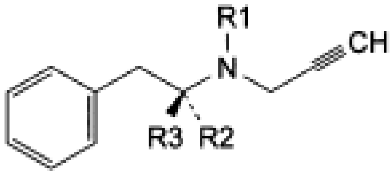
Specified impurities *A, B, C, D, E.*


H
N
CH3
H
CH3
and enantiomer

A. (2*RS*)-*N*-methyl-1-phenylpropan-2-amine [(*RS*)-metamphetamine],

B. (2*R*)-1-phenylpropan-2-amine (amphetamine),

C. (1*RS*,2*SR*)-2-amino-1-phenylpropan-1-ol (phenylpropanolamine),



D. R1 = R3 = H, R2 = CH_3: *N*-[(1*R*)-1-methyl-2-phenylethyl]prop-2-yn-1-amine (demethylselegiline),

E. R1 = R3 = CH_3, R2 = H: *N*-methyl-*N*-[(1*S*)-1-methyl-2-phenylethyl]prop-2-yn-1-amine [(*S*)-selegiline].

Ph Eur

Selenium Sulphide

General Notices

(Selenium Disulphide, Ph Eur monograph 1147)

SeS_2 143.1 *7488-56-4*

Action and use

Used in treatment of dandruff and seborrhoeic dermatitis of the scalp.

Preparation

Selenium Sulphide Scalp Application

Ph Eur

DEFINITION

Content

52.0 per cent to 55.5 per cent of Se.

CHARACTERS

Appearance

Bright orange or reddish-brown powder.

Solubility

Practically insoluble in water.

IDENTIFICATION

A. Gently boil about 50 mg with 5 ml of *nitric acid R* for 30 min. Dilute to 50 ml with *water R* and filter. To 5 ml of the filtrate add 10 ml of *water R* and 5 g of *urea R*. Heat to boiling, cool and add 1.5 ml of *potassium iodide solution R*. A yellow or orange colour is produced which darkens rapidly on standing. This solution is used in identification test B.

B. Allow the coloured solution obtained under identification A to stand for 10 min and filter through *kieselguhr for chromatography R*. 5 ml of the filtrate gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Soluble selenium compounds

Maximum 5 ppm, calculated as Se.

To 10 g add 100 ml of *water R*, mix well, allow to stand for 1 h with frequent shaking and filter.

To 10 ml of the filtrate add 2 ml of a 115 g/l solution of *anhydrous formic acid R*, dilute to 50 ml with *water R* and adjust to pH 2.0-3.0 with an 115 g/l solution of *anhydrous formic acid R*. Add 2 ml of a 5 g/l solution of *3,3′-diaminobenzidine tetrahydrochloride R*. Allow to stand for 45 min and then adjust to pH 6.0-7.0 with *dilute ammonia R1*. Shake the solution for 1 min with 10 ml of *toluene R* and allow the phases to separate. The absorbance *(2.2.25)* of the upper layer measured at 420 nm is not greater than that of a standard prepared at the same time and in the same manner beginning at the words "add 2 ml of an 115 g/l solution of *anhydrous formic acid R*" and using 5 ml of *selenium standard solution (1 ppm Se) R* instead of 10 ml of the filtrate.

ASSAY

To 0.100 g add 25 ml of *fuming nitric acid R* and heat on a water-bath for 1 h; a small insoluble residue may remain. Cool and dilute to 100.0 ml with *water R*. To 25.0 ml of this solution add 50 ml of *water R* and 5 g of *urea R* and heat to boiling. Cool, add 7 ml of *potassium iodide solution R* and 3 ml of *starch solution R*. Titrate immediately with *0.1 M sodium thiosulphate*. Carry out a blank titration.

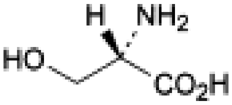
1 ml of *0.1 M sodium thiosulphate* is equivalent to 1.974 mg of Se.

Ph Eur

Serine

General Notices

(Ph Eur monograph 0788)



$C_3H_7NO_3$ 105.1 *56-45-1*

Action and use

Amino acid.

Ph Eur

DEFINITION

Serine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2-amino-3-hydroxypropanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, practically insoluble in alcohol.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *serine CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 1 ml of a 10 g/l solution of the substance to be examined in a test-tube, add 5 ml of a 20 g/l solution of *sodium periodate R*. Heat on a water-bath and collect the vapour on glass wool moistened with *water R* and inserted in the opening of the test tube. After heating for 5 min, transfer the glass wool to a test-tube containing 1 ml of a 15 g/l solution of *chromotropic acid, sodium salt R* and 3 ml of *sulphuric acid R*. Heat on a water-bath for 10 min. A violet-red colour is produced.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 2.50 g in *dilute hydrochloric acid R* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 14.0 to + 16.0, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *serine CRS* in *0.1 M hydrochloric acid* and dilute to 50 ml with the same acid.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *methionine CRS* and 10 mg of *serine CRS* in *0.1 M hydrochloric acid* and dilute to 25 ml with the same acid.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

Dilute 5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals *(2.4.8)*

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Using 0*.1 ml of naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 10.51 mg of $C_3H_7NO_3$.

STORAGE

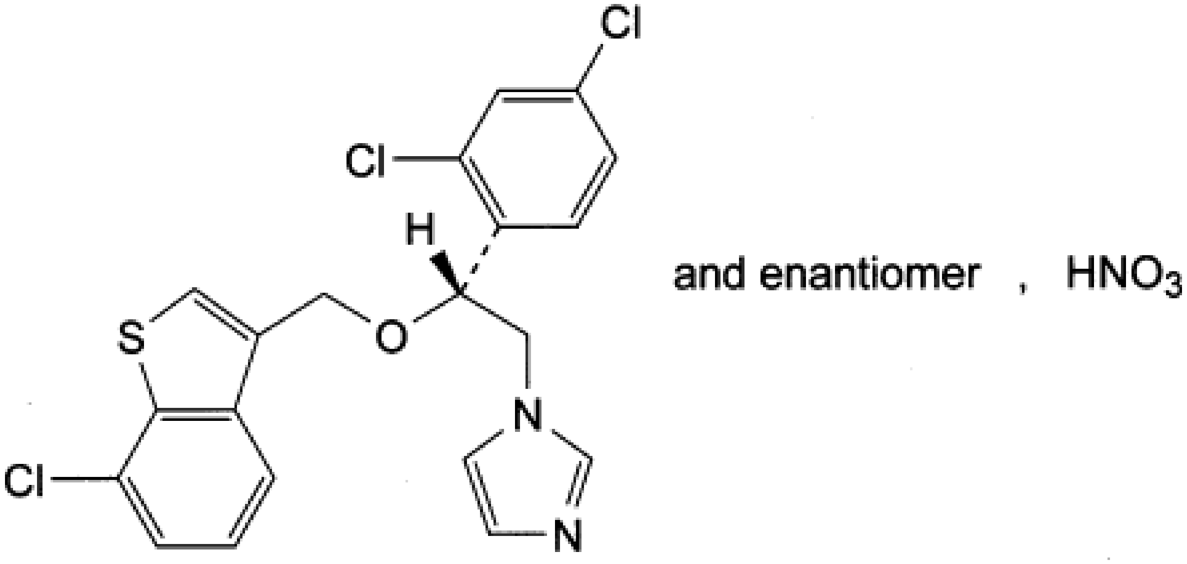
Store protected from light.

Ph Eur

Sertaconazole Nitrate

General Notices

(Ph Eur monograph 1148)



$C_{20}H_{15}Cl_3N_2OS,HNO_3$ 500.8 *99592-39-9*

Action and use

Antifungal.

Ph Eur

DEFINITION

(*RS*)-1-[2-[(7-Chloro-1-benzothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in methanol, sparingly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D, E.*

A. Melting point *(2.2.14)*: 156 °C to 161 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 0.1 g in *methanol R* and dilute to 100 ml with the same solvent. Dilute 10 ml of this solution to 100 ml with *methanol R*.

Spectral range 240-320 nm.

Absorption maxima At 260 nm, 293 nm and 302 nm.

Absorbance ratio A_{302}/A_{293} = 1.16 to 1.28.

C. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dry the substances at 100-105 °C for 2 h and examine as discs of *potassium bromide R*.

Comparison *sertaconazole nitrate CRS.*

D. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *concentrated ammonia R, methanol R* (10:90 *V/V*).

Test solution Dissolve 40 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 40 mg of *sertaconazole nitrate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 20 mg of *miconazole nitrate CRS* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, toluene R, dioxan R* (1:40:60 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of air for 15 min.

Detection Expose to iodine vapour for 30 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

E. About 1 mg gives the reaction of nitrates *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Dissolve 0.1 g in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of s*ertaconazole nitrate CRS* and 5.0 mg of *miconazole nitrate CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *nitrile silica gel for chromatography R1* (10 µm).

Mobile phase *acetonitrile R1, 1.5 g/l solution of sodium dihydrogen phosphate R* (37:63 *V/V*).

Flow rate 1.6 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 1.3 times the retention time of sertaconazole.

Retention time Nitrate ion = about 1 min; miconazole = about 17 min; sertaconazole = about 19 min.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to miconazole and sertaconazole.

Limits:

— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to the nitrate ion.

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 0.50 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of a mixture of equal volumes of *anhydrous acetic acid R* and *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

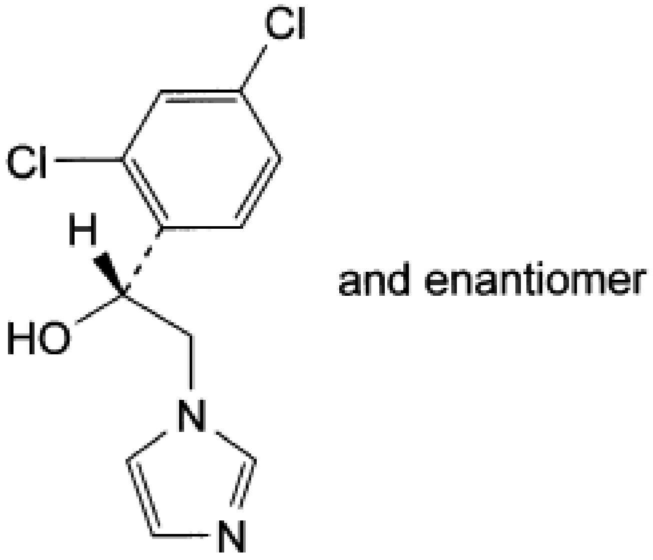
1 ml of *0.1 M perchloric acid* is equivalent to 50.08 mg of $C_{20}H_{16}Cl_3N_3O_4S$.

STORAGE

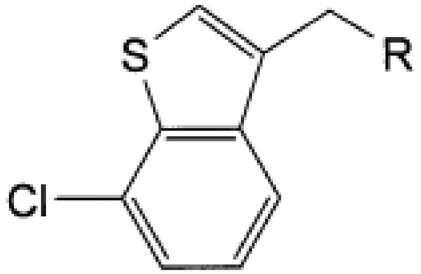
Protected from light.

IMPURITIES

Specified impurities   A, B, C.



A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,



B. R = Br: 3-(bromomethyl)-7-chloro-1-benzothiophen,

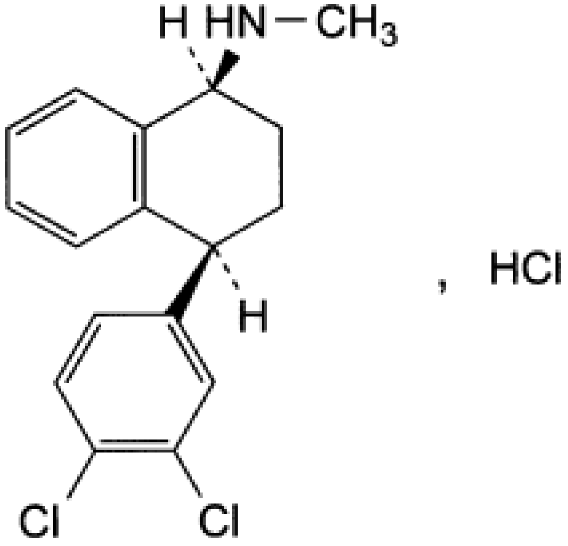
C. R = OH: (7-chloro-1-benzothiophen-3-yl)methanol.

Ph Eur

Sertraline Hydrochloride

General Notices

(Ph Eur monograph 1705)



$C_{17}H_{18}Cl_3N$ 342.7   *79559-97-0*

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

Ph Eur

DEFINITION

(1*S*,4*S*)-4-(3,4-Dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in anhydrous ethanol, slightly soluble in acetone and in isopropanol.

It shows polymorphism *(5.9)*.

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*: + 38.8 to + 43.0 (anhydrous substance), measured at 25 °C.

Solvent mixture Dilute 1 volume of a 103 g/l solution of *hydrochloric acid R* to 20 volumes with *methanol R.*

Dissolve 0.250 g in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison sertraline hydrochloride CRS.

If the spectra obtained in the solid state show differences, record new spectra using 10 g/l solutions in *methylene chloride R*.

C. Dissolve 10 mg in 5 ml of *anhydrous ethanol R* and add 5 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Enantiomeric purity

Liquid chromatography *(2.2.29)*.

Solvent mixture diethylamine R, *hexane R*, *2-propanol R* (1:40:60 *V/V/V*).

Test solution Dissolve 60.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *sertraline for system suitability CRS* (containing impurity G) in 1.0 ml of solvent mixture.

Reference solution (b) Dilute 0.5 ml of the test solution to 100.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel AD for chiral separation R* (5 µm).

Mobile phase Mix 30 volumes of *hexane R* and 70 volumes of a mixture of 1 volume of *diethylamine R*, 25 volumes of *2-propanol R* and 975 volumes of *hexane R.*

Flow rate 0.4 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µl.

Run time 30 min.

Elution order Sertraline, impurity G.

System suitability:

— *resolution*: minimum 1.5 between the peaks due to sertraline and impurity G in the chromatogram obtained with reference solution (a);

- *signal-to-noise ratio*: minimum 10 for the peak due to sertraline in the chromatogram obtained with reference solution (b).

Limit:

- *impurity G*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent).

Impurity E

Thin-layer chromatography *(2.2.27)*.

Solvent mixture *methanol R*, *methylene chloride R* (50:50 *V/V*).

Test solution Dissolve 0.500 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 5.0 mg of *mandelic acid R* (impurity E) in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *mandelic acid R* (impurity E) and 5 mg of the substance to be examined with the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *dilute ammonia R2*, *methanol R*, *methylene chloride R* (15:50:120 *V/V/V*).

Application 50 µl as bands of about 4 cm. Allow to dry.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

- the chromatogram shows 2 clearly separated spots.

Limit:

- *impurity E*: any zone due to impurity E is not more intense than the zone in the chromatogram obtained with reference solution (a) (0.2 per cent).

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Introduce 0.250 g of the substance to be examined into a 15 ml stoppered centrifuge tube, add 2.0 ml of *methanol R* and 0.20 ml of a 25 per cent solution of *potassium carbonate R* and mix in a vortex mixer for 30 s. Add 8.0 ml of *methylene chloride R*, stopper the tube and mix in a vortex mixer for 60 s. Add 1 g of *anhydrous sodium sulphate R*, mix well and then centrifuge for about 5 min.

Reference solution (a) Dissolve the contents of a vial of *sertraline for peak identification CRS* (containing impurities A, B, C and F) in 0.2 ml of *methylene chloride R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methylene chloride R*. Dilute 1.0 ml of this solution to 20.0 ml with *methylene chloride R*.

Column:

- *material*: fused silica;

— *size*: *l* = 30 m, Ø = 0.53 mm;

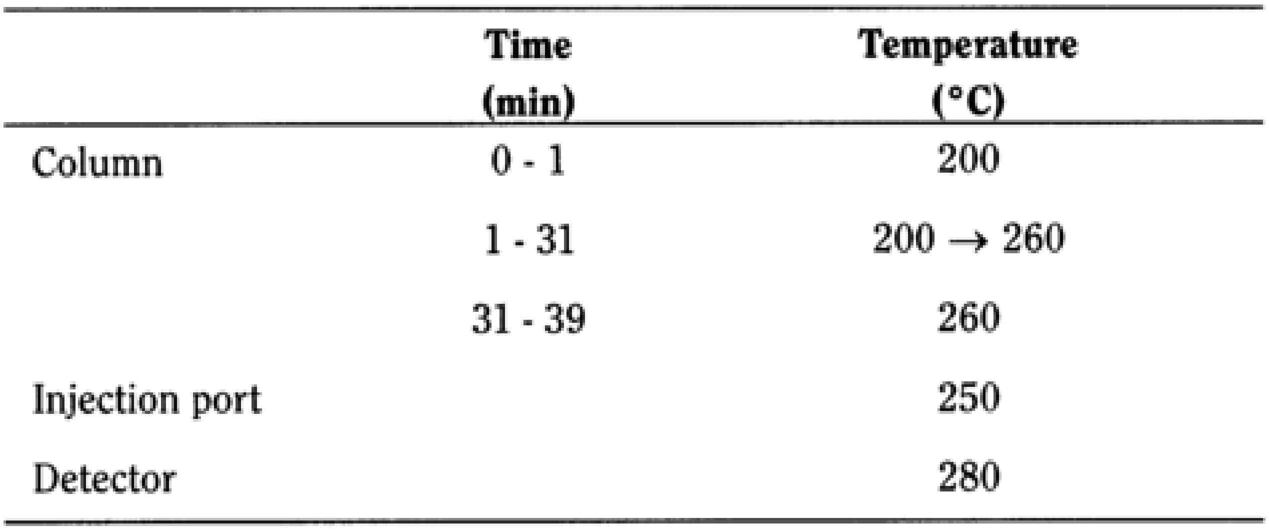
— *stationary phase*: *polymethylphenylsiloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R.*

Flow rate 9 ml/min.

Split ratio 1:10.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	200
	1 - 31	200 → 260
	31 - 39	260
Injection port		250
Detector		280

Detection Flame ionisation.

Injection 1 µl.

Identification of impurities Use the chromatogram supplied with *sertraline for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C and F.

Relative retention With reference to sertraline (retention time = about 24 min): impurity B = about 0.5; impurities C and D = about 0.7; impurity A = about 1.05; impurity F = about 1.1.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 15, where H_r = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to sertraline.

Limits:

— *impurities A, B, F*: for each impurity, maximum 0.2 per cent;

— *sum of impurities C and D*: maximum 0.8 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 1.5 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 20.0 ml with the same solvent. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with *ethanol (96 per cent) R.*

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 2.00 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)*.

Buffer solution To 28.6 ml of *glacial acetic acid R* slowly add, while stirring and cooling, 34.8 ml of *triethylamine R*, and dilute to 100 ml with *water R*. Dilute 10 ml of this solution to 1000 ml with *water R.*

Test solution Dissolve 55.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution Dissolve 55.0 mg of *sertraline hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (4 µm);

— *temperature*: 30 °C.

Mobile phase *methanol R*, buffer solution, *acetonitrile R* (15:40:45 *V/V/V*).

Flow rate 1.8 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time Twice the retention time of sertraline.

Retention time Sertraline = about 1.9 min.

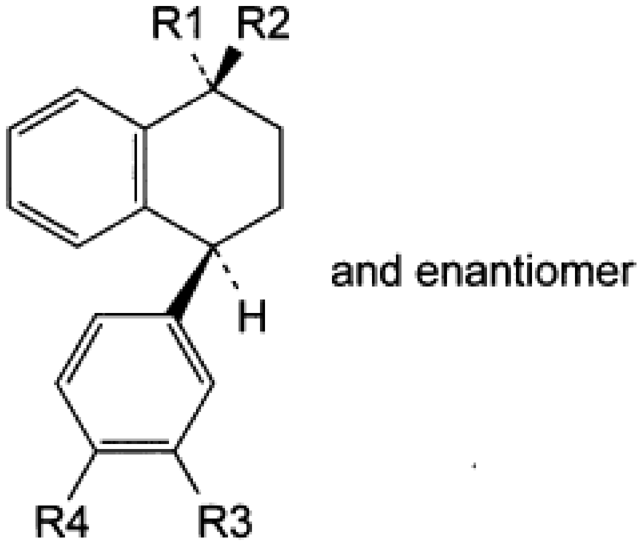
Calculate the percentage content of $C_{17}H_{18}Cl_3N$ from the declared content of *sertraline hydrochloride CRS.*

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F, G.*

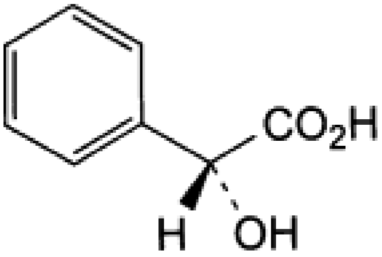


A. R1 = NH-CH_3, R2 = H, R3 = R4 = Cl: (1*RS*,4*SR*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine,

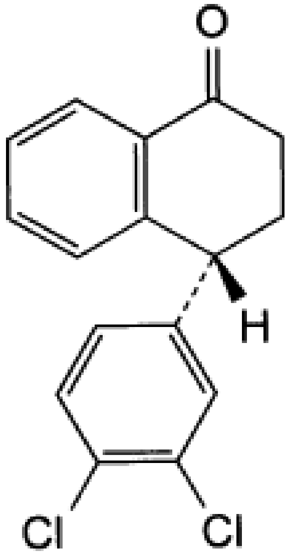
B. R1 = R3 = R4 = H, R2 = NH-CH_3: (1*RS*,4*RS*)-*N*-methyl-4-phenyl-1,2,3,4-tetrahydronaphthalen-1-amine,

C. R1 = R3 = H, R2 = NH-CH_3, R4 = Cl: (1*RS*,4*RS*)-4-(4-chlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine,

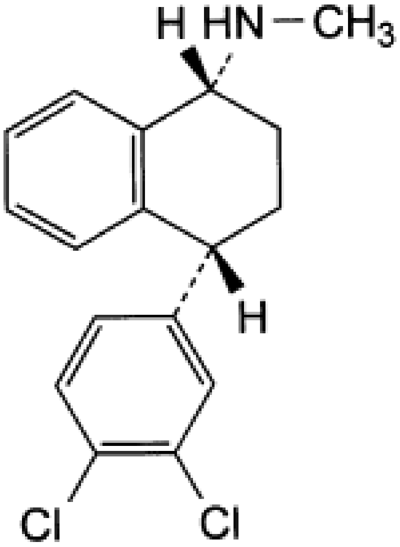
D. R1 = R4 = H, R2 = NH-CH_3, R3 = Cl: (1*RS*,4*RS*)-4-(3-chlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine,



E. (2*R*)-hydroxyphenylacetic acid ((*R*)-mandelic acid),



F. (4*R*)-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-1(2*H*)-one,



G. (1*R*,4*R*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine (sertraline enantiomer).

Ph Eur

Shellac

General Notices

(Ph Eur monograph 1149)

Action and use

Excipient.

Ph Eur

DEFINITION

Purified material obtained from the resinous secretion of the female insect *Kerria lacca* (Kerr) Lindinger (*Laccifer lacca* Kerr). There are 4 types of shellac depending on the nature of the treatment of crude secretion (seedlac): wax-containing shellac, bleached shellac, dewaxed shellac and bleached, dewaxed shellac.

Wax-containing shellac is obtained from seedlac: it is purified by filtration of the molten substance and/or by hot extraction using a suitable solvent.

Bleached shellac is obtained from seedlac by treatment with sodium hypochlorite after dissolution in a suitable alkaline solution, precipitation by dilute acid and drying.

Dewaxed shellac is obtained from wax-containing shellac or seedlac by treatment with a suitable solvent and removal of the insoluble wax by filtering.

Bleached, dewaxed shellac is obtained from wax-containing shellac or seedlac by treatment with sodium hypochlorite after dissolution in a suitable alkaline solution; the insoluble wax is removed by filtration. It is precipitated by dilute acid and dried.

CHARACTERS

Appearance

Brownish-orange or yellow, shining, translucent, hard or brittle, more or less thin flakes (wax-containing shellac and dewaxed shellac), or a creamy white or brownish-yellow powder (bleached shellac and bleached, dewaxed shellac).

Solubility

Practically insoluble in water, gives a more or less opalescent solution (wax containing shellac and bleached shellac) or a clear solution (dewaxed shellac and bleached, dewaxed shellac) in anhydrous ethanol. When warmed it is sparingly soluble or soluble in alkaline solutions.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Heat 0.25 g of the powdered substance (500) *(2.9.12)* on a water-bath with 2 ml of *dilute sodium hydroxide solution R* for 5 min. Cool, add 5 ml of *ethyl acetate R* and slowly, with stirring, 2 ml of *dilute acetic acid R*. Shake and filter the upper layer through *anhydrous sodium sulphate R*.

Reference solution Dissolve 6.0 mg of *aleuritic acid R* in 1.0 ml of *methanol R*, heating slightly if necessary.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetic acid R, methanol R, methylene chloride R, ethyl acetate R* (1:8:32:60 *V/V/V/V*).

Application 10 µl, as bands.

Development Twice over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R*, heat at 100-105 °C for 5-10 min and examine in daylight.

Results The chromatogram obtained with the test solution shows several coloured zones, one of which is similar in position and colour to the zone in the chromatogram obtained with the reference solution. Above this zone the chromatogram obtained with the test solution shows a pink zone and below it several violet zones. Below the zone due to aleuritic acid, there is a light blue zone (shellolic acid) accompanied by zones of the same colour but of lower intensity. Other faint grey and violet zones may be visible.

B. Examine the chromatograms obtained in the test for colophony.

Results For wax-containing shellac, in the chromatogram obtained with the test solution, a more or less strong bluish-grey zone is visible, just above the zone due to thymolphthalein in the chromatogram obtained with the reference solution; for dewaxed shellac, no such zone is visible just above the zone due to thymolphthalein in the chromatogram obtained with the reference solution.

TESTS

Acid value *(2.5.1)*

65 to 95 (dried substance).

Examine 1.00 g of the coarsely ground substance. Determine the end-point potentiometrically *(2.2.20)*.

Colophony

Thin-layer chromatography *(2.2.27)* as described under identification test A with the following modifications.

Test solution Dissolve 50 mg of the powdered substance (500) *(2.9.12)*, with heating, in a mixture of 0.5 ml of *methylene chloride R* and 0.5 ml of *methanol R*.

Reference solution Dissolve 2.0 mg of *thymolphthalein R* in 1.0 ml of *methanol R*.

Detection Examine in ultraviolet light at 254 nm; mark the quenching zones in the chromatogram obtained with the test solution that have similar R_F values to that of the quenching zone due to thymolphthalein in the chromatogram obtained with the reference solution; spray with *anisaldehyde solution R*, heat at 100-105 °C for 5-10 min and examine in daylight.

Results The chromatogram obtained with the reference solution shows a principal zone with a reddish-violet colour (thymolphthalein). None of the quenching zones in the chromatogram obtained with the test solution that have an R_F value similar to the zone due to thymolphthalein in the reference solution show a more or less strong violet or brownish colour (colophony). Disregard any faint violet zone at this level that does not show quenching before spraying and heating.

Arsenic ***(2.4.2, Method A)***

Maximum 3 ppm.

Introduce 0.33 g of the substance to be examined and 5 ml of *sulphuric acid R* into a combustion flask. Carefully add a few millilitres of *strong hydrogen peroxide solution R* and heat to boiling until a clear, colourless solution is obtained. Continue heating to eliminate the water and as much sulphuric acid as possible and dilute to 25 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 2.0 per cent for unbleached shellac and maximum 6.0 per cent for bleached shellac, determined on 1.000 g of the powdered substance (500) *(2.9.12)* by drying in an oven at 40-45 °C for 24 h.

STORAGE

Protected from light. Store bleached shellac and bleached, dewaxed shellac at a temperature not exceeding 15 °C.

LABELLING

The label indicates the type of shellac.

Ph Eur

Silver Nitrate

General Notices

(Ph Eur monograph 0009)

$AgNO_3$ 169.9 *7761-88-8*

Action and use

Antiseptic.

Preparation

Sterile Silver Nitrate Solution

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or transparent, colourless crystals.

Solubility

Very soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. 10 mg gives the reaction of nitrates *(2.3.1)*.

B. 10 mg gives the reaction of silver *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 2 ml of solution S add 0.1 ml of *bromocresol green solution R*. The solution is blue. To 2 ml of solution S add 0.1 ml of *phenol red solution R*. The solution is yellow.

Foreign salts

Maximum 0.3 per cent.

To 30 ml of solution S, add 7.5 ml of *dilute hydrochloric acid R*, shake vigorously, heat for 5 min on a water-bath and filter. Evaporate 20 ml of the filtrate to dryness on a water-bath and dry at 100-105 °C. The residue weighs a maximum of 2 mg.

Aluminium, lead, copper and bismuth

Dissolve 1.0 g in a mixture of 4 ml of *concentrated ammonia R* and 6 ml of *water R*. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

ASSAY

Dissolve 0.300 g in 50 ml of *water R*, add 2 ml of *dilute nitric acid R* and 2 ml of *ferric ammonium sulphate solution R2*. Titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 16.99 mg of $AgNO_3$.

STORAGE

In a non-metallic container, protected from light.

Ph Eur

Simeticone for Oral Use

General Notices

Action and use

Silicon dioxide analogue; defoaming agent.

Preparation

Simeticone Suspension for Infants

When activated dimethicone is prescribed or demanded, Simeticone for Oral Use shall be dispensed or supplied.

DEFINITION

Simeticone for Oral Use contains not less than 4.5% and not more than 8.0% w/w of silica, SiO_2, and not less than 91.0% and not more than 97.0% w/w of Dimeticone 1000. It is prepared by the addition of finely powdered silica to Dimeticone 1000.

CHARACTERISTICS

A viscous, grey, translucent liquid.

Immiscible with *water* and with *ethanol (96%)*; almost completely soluble in *toluene*, leaving a residue of silica.

IDENTIFICATION

A. The *infrared absorption spectrum* of the supernatant liquid obtained by centrifugation, Appendix II A, is concordant with the *reference spectrum* of dimeticone *(RS 381)*.

B. Ignite; dense white fumes are evolved leaving a white residue which is insoluble in *hydrochloric acid*.

TESTS

Acidity

Dissolve 15.0 g in a mixture of 15 ml of *toluene* and 15 ml of *butan-1-ol,* previously neutralised to a 0.5% w/v solution of *bromophenol blue* in *ethanol (96%)*, and titrate with 0.1M *ethanolic potassium hydroxide VS* using the bromophenol blue solution as indicator. Not more than 0.15 ml is required to change the colour of the solution.

Refractive index

Of the supernatant liquid obtained in Identification test A, 1.4050 to 1.4080, Appendix V E.

Viscosity

Of the supernatant liquid obtained in Identification test A, 950 to 1050 $mm^2 s^{-1}$, Appendix V H, Method I, using a U-tube viscometer (size G).

Weight per ml

Of the supernatant liquid obtained in Identification test A, 0.965 to 0.980 g, Appendix V G.

Heavy metals

1.0 g complies with *limit test F for heavy metals*, Appendix VII (20 ppm). Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

ASSAY

For silica

To 1 g add 50 ml of *toluene*, mix well and filter through an ignited silica crucible. Wash the residue thoroughly with *toluene*, dry at 105° and ignite to constant weight at 500°.

For dimeticone

Place 40 mg in a stoppered centrifuge tube, add 20 ml of *toluene* and shake for 20 minutes. Filter and record the *infrared absorption spectrum*, Appendix II A, of a 0.5-mm layer of the filtrate over the range 1330 to 1180 cm^{-1} (7.52 to 8.47 µm). Measure the absorbance of the CH_3Si stretching band at the maximum at 1261 cm^{-1} (7.93 µm). Repeat the operation using *dimeticone EPCRS* in place of the preparation being examined.

Simeticone

General Notices

(Ph Eur monograph 1470)

Action and use

Silicon dioxide analogue; defoaming agent.

Ph Eur

DEFINITION

Simeticone is prepared by incorporation of 4 per cent to 7 per cent silica into poly (dimethylsiloxane) with a degree of polymerisation between 20 and 400.

Content

90.5 per cent to 99.0 per cent of poly(dimethylsiloxane).

PRODUCTION

Poly(dimethylsiloxane) is obtained by hydrolysis and polycondensation of dichlorodimethylsilane and chlorotrimethylsilane and the silica is modified at the surface by incorporation of methylsilyl groups.

CHARACTERS

Appearance

Viscous, greyish-white, opalescent liquid.

Solubility

Practically insoluble in water, very slightly soluble or practically insoluble in anhydrous ethanol, practically insoluble in methanol, partly miscible with ethyl acetate, with methylene chloride, with methyl ethyl ketone and with toluene.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Thin films between plates of *sodium chloride R*.

Absorption maxima At 2964 cm^{-1}, 2905 cm^{-1}, 1412 cm^{-1}, 1260 cm^{-1} and 1020 cm^{-1}.

B. Heat 0.5 g in a test-tube over a small flame until white fumes begin to appear. Invert the tube over a 2nd tube containing 1 ml of a 1 g/l solution of *chromotropic acid, sodium salt R* in *sulphuric acid R* so that the fumes reach the solution. Shake the 2nd tube for about 10 s and heat on a water-bath for 5 min. The solution is violet.

C. The residue obtained in the test for silica under Assay, gives the reaction of silicates *(2.3.1)*.

TESTS

Acidity

To 2.0 g add 25 ml of a mixture of equal volumes of *anhydrous ethanol R* and *ether R* previously neutralised to 0.2 ml of *bromothymol blue solution R1*, and shake. Not more than 3.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to blue.

Defoaming activity

Foaming solution Dissolve 5.0 g of *docusate sodium R* in 1 litre of *water R*, warm to 50 °C if necessary.

Defoaming solution To 50 ml of *methyl ethyl ketone R* add 0.250 g of the substance to be examined, warm to not more than 50 °C with shaking.

Into a 250 ml cylindrical tube about 5 cm in diameter introduce 100 ml of foaming solution and 1 ml of defoaming solution. Close tightly and fix the tube on a suitable oscillating shaker that complies with the following conditions:

- 250-300 oscillations per minute;
- angle of oscillation of about 10 ;
- oscillation radius of about 10 cm.

Shake for 10 s and record the time between the end of the shaking and the instant the 1st portion of foam-free liquid surface appears.

This duration is not longer than 15 s.

Mineral oils

Place 2.0 g in a test-tube and examine in ultraviolet light at 365 nm. The fluorescence is not more intense than that of a solution containing 0.1 ppm of *quinine sulphate R* in *0.005 M sulphuric acid* examined in the same conditions.

Phenylated compounds

The corrected absorbance *(2.2.25)* is not greater than 0.2.

Test solution Dissolve 5.0 g with shaking in 10.0 ml of *cyclohexane R*.

Spectral range 200-350 nm.

Calculate the corrected absorbance using the following expression:

$$B - C$$

B = absorbance at the absorption maximum between 250 nm and 270 nm,

C = absorbance at 300 nm.

Heavy metals

Maximum 5 ppm.

Mix 1.0 g with *methylene chloride R* and dilute to 20 ml with the same solvent. Add 1.0 ml of a freshly prepared 0.02 g/l solution of *dithizone R* in *methylene chloride R*, 0.5 ml of *water R* and 0.5 ml of a mixture of 1 volume of *dilute ammonia R2* and 9 volumes of a 2 g/l solution of *hydroxylamine hydrochloride R*. At the same time, prepare the reference solution as follows: to 20 ml of *methylene chloride R* add 1.0 ml of a freshly prepared 0.02 g/l solution of *dithizone R* in *methylene chloride R*, 0.5 ml of *lead standard solution (10 ppm Pb) R* and 0.5 ml of a mixture of 1 volume of *dilute ammonia R2* and 9 volumes of a 2 g/l solution of hydroxylamine *hydrochloride R*. Immediately shake each solution vigorously for 1 min. Any red colour in the test solution is not more intense than that in the reference solution.

Volatile matter

Maximum 1.0 per cent, determined on 1.00 g by heating in an oven at 150 °C for 2 h. Carry out the test using a dish 60 mm in diameter and 10 mm deep.

ASSAY

Silica

Heat not less than 20.0 mg to 800 °C increasing the temperature by 20 °C/min under a current of *nitrogen R* at a flow rate of 200 ml/min and weigh the residue (silica).

Poly(dimethylsiloxane)

Infrared absorption spectrophotometry *(2.2.24)*.

Test solution Place about 50 mg (E) in a screw-capped 125 ml cylindrical tube, add 25.0 ml of *toluene R*, swirl manually to disperse and add 50 ml of *dilute hydrochloric acid R*, close the tube and place on a vortex mixer; shake for 5 min. Transfer the contents of the tube to a separating funnel, allow to settle and transfer 5 ml of the upper layer to a screw-capped test tube containing 0.5 g of *anhydrous sodium sulphate R*. Cap and shake vigorously manually. Centrifuge to obtain a clear solution.

Reference solution Introduce about 0.20 g of *dimeticone CRS* (poly(dimethylsiloxane)) into 100.0 ml of *toluene R*. Prepare the reference solution in the same way as for the test solution, using 25.0 ml of the dimeticone solution obtained above.

Blank solution Shake 10 ml of *toluene R* with 1 g of *anhydrous sodium sulphate R*. Centrifuge the resulting suspension.

Record the infrared absorption spectra for the test solution and the reference solution in 0.5 mm cells, from 1330 cm^{-1} to 1180 cm^{-1}. Determine the absorbance of the band at 1260 cm^{-1}.

Calculate the percentage content of poly(dimethylsiloxane) using the following expression:

$$\frac{25 \times C \times A_M \times 100}{A_E \times E}$$

A_M = absorbance of the test solution,

A_E = absorbance of the reference solution,

C = concentration of the reference solution, in milligrams per millilitre,

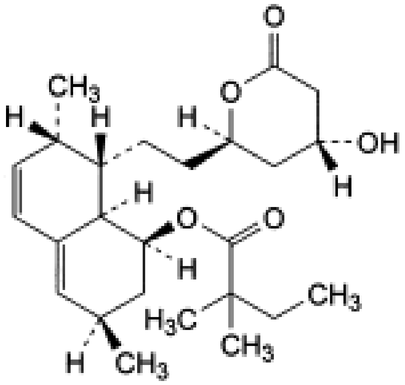
E = mass of the substance to be examined, in milligrams.

Ph Eur

Simvastatin

General Notices

(Ph Eur monograph 1563)



$C_{25}H_{38}O_5$ 418.6 *79902-63-9*

Action and use

HMG Co-A reductase inhibitor; lipid-regulating drug.

Preparation

Simvastatin Tablets

Ph Eur

DEFINITION

Simvastatin contains not less than 97.0 per cent and not more than the equivalent of 102.0 per cent of (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate, calculated with reference to the dried substance. A suitable antioxidant may be added.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, very soluble in methylene chloride, freely soluble in alcohol.

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *simvastatin CRS*. Examine the substances prepared as discs.

TESTS

Appearance of solution

Dissolve 0.200 g in *methanol R* and dilute to 20 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 0.125 g in *acetonitrile R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 285 to + 300, calculated with reference to the dried substance.

Related substances

Examine by liquid chromatography *(2.2.29)* as prescribed under Assay.

Inject 5 µl of reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 20 per cent of the full scale of the recorder. Inject 5 µl of test solution (a) and continue the chromatography for five times the retention time of simvastatin. When the chromatograms are recorded under the prescribed conditions the relative retentions are: impurity A about 0.45, lovastatin (impurity E) and epilovastatin (impurity F) about 0.60, impurity G about 0.80, impurity B about 2.38, impurity C about 2.42 and impurity D about 3.80 (retention time of simvastatin: about 2.6 min). In the chromatogram obtained with test solution (a): the area of the peak due to lovastatin and epilovastatin is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent); the area of any peak apart from the principal peak and the peak due to lovastatin and epilovastatin is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent); the sum of the areas of all peaks, apart from the principal peak and the peak due to lovastatin and epilovastatin, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in a desiccator under high vacuum at 60 °C for 3 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Examine by liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Solvent mixture Prepare a mixture of 40 volumes of a solution of a 1.4 g/l solution of *potassium dihydrogen phosphate R*, adjusted to pH 4.0 with *phosphoric acid R*, and 60 volumes of *acetonitrile R*. Filter.

Test solution (a) Dissolve 75.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Test solution (b) Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 1.0 mg of *simvastatin CRS* and 1.0 mg of *lovastatin CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c) Dissolve 40.0 mg of *simvastatin CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

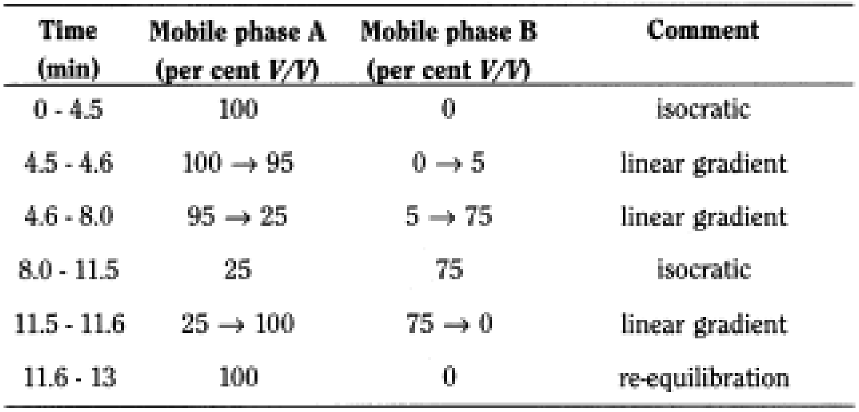
The chromatographic procedure may be carried out using:

— a stainless steel column 0.033 m long and 4.6 mm in internal diameter packed with end-*capped octadecylsilyl silica gel for chromatography R* (3 µm);

— as mobile phase at a flow rate of 3.0 ml/min:

Mobile phase A Mix 50 volumes of *acetonitrile R* and 50 volumes of a 0.1 per cent *V/V* solution of *phosphoric acid R*,

Mobile phase B A 0.1 per cent *V/V* solution of *phosphoric acid R* in *acetonitrile R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 4.5	100	0	isocratic
4.5 - 4.6	100 → 95	0 → 5	linear gradient
4.6 - 8.0	95 → 25	5 → 75	linear gradient
8.0 - 11.5	25	75	isocratic
11.5 - 11.6	25 → 100	75 → 0	linear gradient
11.6 - 13	100	0	re-equilibration

— as detector a spectrophotometer set at 238 nm.

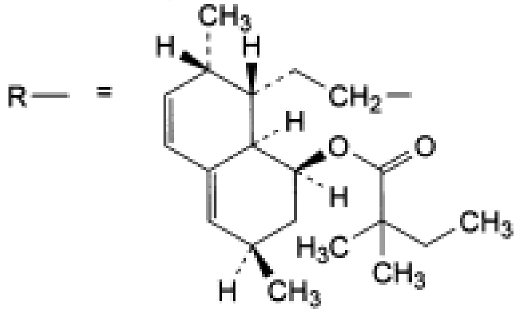
Inject 5 µl of reference solution (a). The test and the assay are not valid unless, in the chromatogram obtained, the resolution between the peak corresponding to lovastatin and epilovastatin and the peak corresponding to simvastatin is at least 5.0. When the chromatograms are recorded under the prescribed conditions the retention times are: lovastatin and epilovastatin about 1.6 min and simvastatin about 2.6 min. Inject 5 µl of reference solution (c). Adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder. Inject 5 µl of test solution (b).

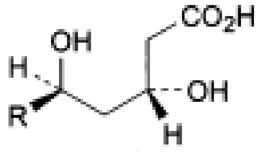
Calculate the content of simvastatin from the peak areas in the chromatograms obtained with test solution (b) and reference solution (c) and the declared content of *simvastatin CRS*.

STORAGE

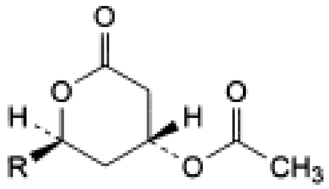
Store under nitrogen, in an airtight container, protected from light.

IMPURITIES

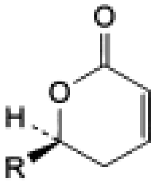




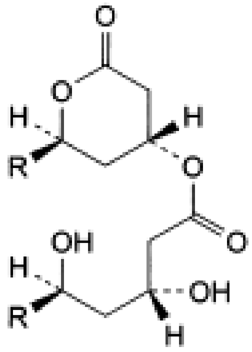
A. (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid (hydroxy acid),



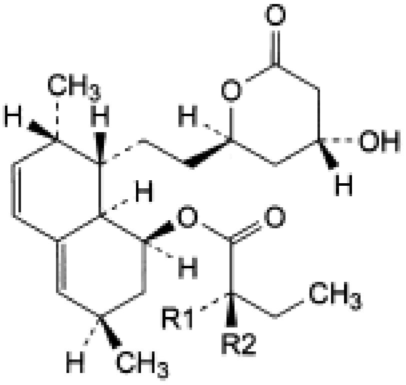
B. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-(acetyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate (acetate ester),



C. (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-dimethyl-8-[2-[(2*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl]-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate (anhydrosimvastatin),

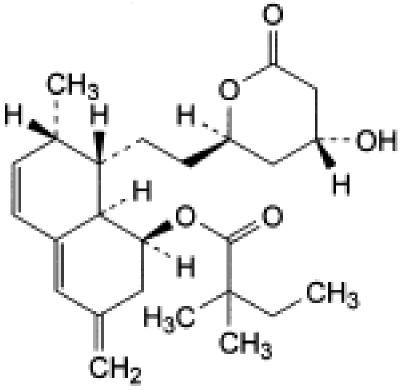


D. (2*R*,4*R*)-2-[[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]-6-oxotetrahydro-2*H*-pyran-4-yl (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate (dimer),



E. R1 = CH$_3$, R2 = H: (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (lovastatin),

F. R1 = H, R2 = CH$_3$: (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*R*)-2-methylbutanoate (epilovastatin),



G. (1*S*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-7-methyl-3-methylene-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

Ph Eur

Soda Lime

General Notices

8006-28-8

Action and use

Used to absorb carbon dioxide.

DEFINITION

Soda Lime is a mixture of sodium hydroxide, or sodium hydroxide and potassium hydroxide, with calcium hydroxide.

CHARACTERISTICS

White or greyish white granules, or it may be coloured with an indicator to show when its absorptive capacity is exhausted. It absorbs about 20% of its weight of carbon dioxide.

Partially soluble in *water*; almost completely soluble in 1M *acetic acid*.

IDENTIFICATION

A. When moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. A solution in 1M *acetic acid* yields reaction C characteristic of *calcium salts*, Appendix VI.

C. A suspension in *water* is strongly alkaline to *litmus paper*.

TESTS

Hardness of granules

Shake 200 g on a sieve no. 2000 for 3 minutes using a mechanical sieve shaker that reproduces in a uniform manner the circular and tapping motion given to sieves in manual use and has a frequency of oscillation of 282 to 288 cycles per minute. Place 50 g of the retained material in a hardness pan 20 cm in diameter having a concave brass bottom, 7.9 mm thick at the circumference, 3.2 mm thick at the centre and with an inside spherical radius of curvature of 109 cm. Add 15 steel balls, 7.9 mm in diameter, and shake on the mechanical sieve shaker for 30 minutes. Remove the steel balls, transfer the contents of the pan to a sieve no. 2000 and again shake on the mechanical sieve shaker for 3 minutes. The material retained by the sieve weighs not less than 37.5 g.

Size of granules

Shake 500 g on a perforated plate of nominal pore size 6.70 mm; not more than 5 g is retained. Then shake on a sieve no. 4750; not more than 50 g is retained. Shake the unretained material on a sieve no. 1400; not more than 20 g passes through. Shake the unretained material on a sieve no. 600; not more than 7.5 g passes through.

Loss on drying

When dried to constant weight at 105°, loses 14.0 to 21.0% of its weight. Use 1 g.

Moisture absorption

Place 10 g in an open glass dish about 50 mm in diameter and 30 mm high in a desiccator over *sulphuric acid (14%)* and allow it to remain for 24 hours. The increase in weight is not more than 7.5%.

Carbon dioxide absorption

The activity is not less than 120 minutes when determined by the following method.

Use a vertically-clamped tube of glass or other suitable transparent material about 25 cm long and 29 to 31 mm internal diameter with closely fitting rubber bungs at each end; the bungs are bored to receive polythene or glass tubing of about 8 mm external diameter, the tubing being flush with the inner ends of the bungs. With the lower bung in position, place sufficient nylon mesh support on top of the bung to produce a bed of mesh about 10 cm deep and press a closely fitting disc of stainless steel gauze of nominal mesh aperture about 500 µm on top of the nylon mesh so that its surface is at right angles to the axis of the tube. Introduce 59.8 to 60.2 g of the substance being examined onto the steel gauze in three portions, tamping lightly after the addition of each portion. Place a second disc of steel gauze on top, followed by a sufficient quantity of nylon mesh such that the soda lime is kept consolidated by slight pressure when the second bung has been inserted. The exit tube is connected to a condenser, consisting of two 50 ml separating funnels, leading to a drying tube packed with *anhydrous calcium chloride* and then to a carbon dioxide analyser sufficiently sensitive to detect 0.2% v/v of carbon dioxide. A katharometer, calibrated for carbon dioxide and preferably used in conjunction with a chart recorder, is suitable.

Using the gas analyser in accordance with the manufacturer's instructions, accurately determine the carbon dioxide content, p, as a percentage v/v, of a nominal 5% carbon dioxide mixture, the balance gas being oxygen, air or nitrogen as appropriate to the type of gas analyser being used. Suitable compressed gas mixtures are available commercially. Assemble the apparatus described above and pass the gas mixture downwards into the absorption tube at a rate of 900 cm^3 per minute until such time that the gas analyser shows the content of carbon dioxide in the effluent gas to have risen to 0.2% v/v. Steps should be taken to vent the effluent gas if an oxygen-carbon dioxide mixture is being used. Record the time taken, t, in minutes. The *activity* of the soda lime is given, in minutes, by the expression $tp/5$.

Sodium *(S)* -Lactate Solution

General Notices

(Ph Eur monograph 2033)

Ph Eur

DEFINITION

Content

Minimum 50.0 per cent *m/m* of sodium (*S*)-2-hydroxypropanoate ($C^3H^5NaO^3$; M_r 112.1); 96.0 per cent to 104.0 per cent of the content of sodium lactate stated on the label, not less than 95.0 per cent of which is the (*S*)-enantiomer.

CHARACTERS

Appearance

Clear, colourless, slightly syrupy liquid.

Solubility

Miscible with water and with alcohol.

IDENTIFICATION

A. To 0.1 ml add 10 ml of *water R*. 5 ml of the solution gives the reaction of lactates *(2.3.1)*.

B. It gives reaction (a) of sodium *(2.3.1)*.

C. It complies with the limits of the assay.

TESTS

Solution S

Dilute a quantity of the substance to be examined corresponding to 40.0 g of sodium lactate to 200 ml with *distilled water R*.

Appearance of solution

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

6.5 to 9.0 for the substance to be examined.

Reducing sugars and sucrose

To 5 ml of the substance to be examined add 2 ml of *dilute sodium hydroxide solution R* and 0.2 ml of *copper sulphate solution R.* The solution is clear and blue and remains so on boiling. Add to the hot solution 4 ml of *hydrochloric acid R.* Boil for 1 min. Add 6 ml of *strong sodium hydroxide solution R* and heat to boiling again. The solution is clear and blue.

Methanol

Gas chromatography *(2.4.24)*.

Limit:

— *methanol*: maximum 50 ppm, calculated with reference to sodium lactate, if intended for use in the manufacture of parenteral dosage forms, dialysis, haemodialysis or haemofiltration solutions.

Chlorides *(2.4.4)*

Maximum 50 ppm calculated with reference to sodium lactate.

Dilute 5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides.

Oxalates and phosphates

To 1 ml of the substance to be examined add 15 ml of *alcohol R* and 2 ml of *calcium chloride solution R*. Heat at 75 °C for 5 min. Any opalescence in the solution is not more intense than that of a standard prepared at the same time and in the same manner using a mixture of 1 ml of the substance to be examined, 15 ml of *alcohol R* and 2 ml of *water R*.

Sulphates *(2.4.13)*

Maximum 100 ppm calculated with reference to sodium lactate.

To 7.5 ml of solution S, add 1.9 ml of *hydrochloric acid R1* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates without addition of 0.5 ml of *acetic acid R*. Acidify the standard solution with 0.05 ml of *hydrochloric acid R1* instead of 0.5 ml of *acetic acid R*.

Aluminium

Maximum 0.1 ppm, if intended for use in the manufacture of parenteral dosage forms, dialysis, haemodialysis or haemofiltration solutions.

Atomic absorption spectrometry *(2.2.23, Method I)*. For the preparation of the solutions, use equipment that is aluminium-free or that will not release aluminium under the conditions of use (glass, polyethylene, etc).

Modifier solution

Dissolve 100.0 g of *ammonium nitrate R* in a mixture of 50 ml of *water R* and 4 ml of *nitric acid R* and dilute to 200 ml with *water R.*

Blank solution Dilute 2.0 ml of the modifier solution to 25.0 ml with *water R*.

Test solution To 1.25 g add 2.0 ml of the modifier solution and dilute to 25.0 ml with *water R*.

Reference solutions Prepare the reference solutions immediately before use (0.010 ppm to 0.050 ppm of aluminium) using *aluminium standard solution (200 ppm Al) R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device A graphite furnace.

Carrier gas argon R.

Conditions The device is equipped with a non-specific absorption correction system. Heat the oven to 120 °C for as many seconds as there are microlitres of solution introduced into the apparatus, then heat at 1000 °C for 30 s and finally at 2700 °C for 6 s.

Barium

To 10 ml of solution S add 10 ml of *calcium sulphate solution R*. Allow to stand for 30 min. Any opalescence *(2.2.1)* in the solution is not more intense than that of a standard prepared at the same time and in the same manner using a mixture of 10 ml of solution S and 10 ml of distilled water R.

Iron *(2.4.9)*

Maximum 10 ppm calculated with reference to sodium lactate.

Dilute 5 ml of solution S to 10 ml with *water R*. The solution complies with the limit test for iron.

Heavy metals *(2.4.8)*

Maximum 10 ppm calculated with reference to sodium lactate.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Bacterial endotoxins *(2.6.14)*

Less than 5 IU/g if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve a quantity of the substance to be examined corresponding to 75.0 mg of sodium lactate in a mixture of 10 ml of *glacial acetic acid R* and 20 ml of *acetic anhydride R*. Allow to stand for 15 min. Add 1 ml of *naphtholbenzein solution R* and titrate *with 0.1 M perchloric acid*.

1 ml of *0.1 M perchloric acid* is equivalent to 11.21 mg of $C_3H_5NaO_3$.

(*S*)-enantiomer

Transfer a quantity of the substance to be examined corresponding to 2.50 g of sodium lactate into a 50 ml volumetric flask, dilute with about 30 ml of *water R* and add 5.0 g of *ammonium molybdate R*. Dissolve and dilute with *water R* to 50.0 ml. Measure the angle of optical rotation *(2.2.7)*. Calculate the percentage content of (*S*)-enantiomer using the expression:

$$50 + \left(24.04 \times \alpha \times \frac{5.0}{m} \times \frac{50}{c}\right)$$

α = angle of optical rotation (absolute value)

m = mass of the substance to be examined, in grams

c = percentage content of $C_3H_5NaO_3$ in the substance to be examined

The complex of sodium (*S*)-lactate formed under these test conditions is leavorotatory.

LABELLING

The label states:

— where applicable, that the substance is suitable for use in the manufacture of dialysis, haemodialysis and haemofiltration solutions;

— where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms;

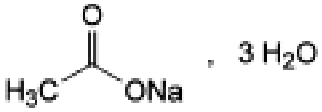
— the declared content of sodium lactate.

Ph Eur

Sodium Acetate Trihydrate

General Notices

(Ph Eur monograph 0411)



$C_2H_3NaO_2,3H_2O$ 136.1 *6131-90-4*

Action and use

Used in solutions for dialysis; excipient.

Ph Eur

DEFINITION

Sodium ethanoate trihydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Colourless crystals.

Solubility

Very soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. 1 ml of solution S (see Tests) gives reaction (b) of acetates *(2.3.1)*.

B. 1 ml of solution S gives reaction (a) of sodium *(2.3.1)*.

C. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to

100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

7.5 to 9.0.

Dilute 5 ml of solution S to 10 ml with *carbon dioxide-free water R*.

Reducing substances

Dissolve 5.0 g in 50 ml of *water R*, then add 5 ml of *dilute sulphuric acid R* and 0.5 ml of *0.002 M potassium permanganate*. The pink colour persists for at least 1 h. Prepare a blank in the same manner but without the substance to be examined.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*.

Aluminium *(2.4.17)*

Maximum 0.2 ppm, if intended for use in the manufacture of dialysis solutions.

Prescribed solution Dissolve 20 g in 100 ml of *water R* and adjust to pH 6.0 by the addition of *1 M hydrochloric acid* (about 10 ml).

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Arsenic *(2.4.2, Method A)*

Maximum 2 ppm, determined on 0.5 g.

Calcium and magnesium

Maximum 50 ppm, calculated as Ca.

To 200 ml of *water R* add 10 ml of *ammonium chloride buffer solution pH 10.0 R*, 0.1 g of *mordant black 11 triturate R*, 2.0 ml of *0.05 M zinc chloride* and, dropwise, *0.02 M sodium edetate* until the colour changes from violet to blue. Add to the solution 10.0 g of the substance to be examined and shake to dissolve. Titrate with *0.02 M sodium edetate* until the blue colour is restored. Not more than 0.65 ml of *0.02 M sodium edetate* is required.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard*

solution (1 ppm Pb) R.

Iron (2.4.9)

Maximum 10 ppm, determined on 10 ml of solution S.

Loss on drying ***(2.2.32)***

39.0 per cent to 40.5 per cent, determined on 1.000 g by drying in an oven at 130 °C. Introduce the substance to be examined into the oven while the latter is cold.

ASSAY

Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R*, add 5 ml of *acetic anhydride R*, mix and allow to stand for 30 min. Using 0.3 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until a green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 8.20 mg of $C_2H_3NaO_2$.

STORAGE

In an airtight container .

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

Ph Eur

Sodium Acid Citrate

General Notices

Disodium Hydrogen Citrate

$C_6H_6Na_2O_7,1½H_2O$ 263.1 *144-33-2*

Action and use

Anticoagulant.

DEFINITION

Sodium Acid Citrate contains not less than 98.0% and not more than 104.0% of $C_6H_6Na_2O_7$, 1½ H_2O.

CHARACTERISTICS

A white powder.

Freely soluble in *water*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION

Yields the reactions characteristic of *sodium salts* and of *citrates*, Appendix VI.

TESTS

Acidity

pH of a 3% w/v solution, 4.9 to 5.2, Appendix V L.

Arsenic

0.50 g dissolved in 25 ml of *water* complies with the *limit test for arsenic*, Appendix VII (2 ppm) .

Heavy metals

Dissolve 1.0 g in 20 ml of *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (20 ppm).

Chloride

Dissolve 1.0 g in 100 ml of *water*. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Oxalate

Dissolve 1.0 g in 4 ml of *water*, add 3 ml of *hydrochloric acid* and 1 g of granulated *zinc* and heat on a water bath for 1 minute. Allow to stand for 2 minutes, decant the liquid into a test tube containing 0.25 ml of a 1% w/v solution of *phenylhydrazine hydrochloride* and heat to boiling. Cool rapidly, transfer to a graduated measuring cylinder, add an equal volume of *hydrochloric acid* and 0.25 ml of *potassium hexacyanoferrate(III) solution*, shake and allow to stand for 30 minutes. Any red colour produced is not more intense than that produced by treating in the same manner 4 ml of a 0.005% w/v solution of *oxalic acid* (150 ppm, calculated as anhydrous oxalic acid).

Sulphate

Dissolve 0.50 g in 57 ml of *water* and add 3 ml of 2M *hydrochloric acid*. 15 ml of the resulting solution complies with the *limit test for sulphates*, Appendix VII (0.12%).

Readily carbonisable substances

Heat 1.0 g, in powder, with 10 ml of *sulphuric acid* for 30 minutes in a water bath protected from light. Not more than a pale brown colour is produced.

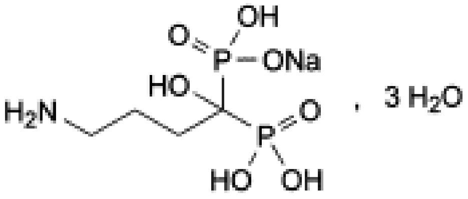
ASSAY

Heat 2 g until carbonised, cool and boil the residue with 50 ml each of *water* and 0.5M *hydrochloric acid VS*. Filter, wash the filter with *water* and titrate the excess of acid in the filtrate and washings with 0.5M *sodium hydroxide VS* using *methyl orange solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 65.78 mg of $C_6H_6Na_2O_7$, $1\frac{1}{2}H_2O$.

Sodium Alendronate

General Notices

(Ph Eur monograph 1564)



$C_4H_{12}NNaO_7P_2,3H_2O$ 325.1 *121268-17-5*

Action and use

Bisphosphonate; treatment of osteoporosis.

Ph Eur

DEFINITION

Sodium alendronate contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (4-amino-1-hydroxybutylidene)bisphosphonic acid monosodium salt, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, soluble in water, very slightly soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *sodium alendronate CRS*. Examine the substances prepared as discs.

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 or BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of is 4.0 to 5.0.

4-aminobutanoic acid

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution

Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 0.10 g of *4-aminobutanoic acid R* in *water R* and dilute to 200 ml with the same solvent.

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with *water R*.

Apply to the plate 5 µl of the test solution and 5 µl of reference solution (b). Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *water R*, 20 volumes of *glacial acetic acid R* and 60 volumes of *butanol R*. Dry the plate in a current of warm air. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spots corresponding to 4-aminobutanoic acid in the chromatogram obtained with the test solution are not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Phosphate and phosphate

Examine the chromatograms obtained in the assay. In the chromatogram obtained with the test solution: the area of any peak corresponding to phosphate is not greater than that of the peak due to phosphate in the chromatogram obtained with reference solution (d) (0.5 per cent); the area of any peak corresponding to phosphite is not greater than that of the peak due to phosphite in the chromatogram obtained with reference solution (d) (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test F for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

16.1 per cent to 17.1 per cent, determined on 1.000 g by drying in an oven at 140 °C to 145 °C.

ASSAY

Examine by liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *sodium alendronate CRS* in *water R* and dilute to 25.0 ml with the same solvent.

Reference solution (b) Dissolve 3.0 g of *phosphoric acid R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *water R*.

Reference solution (c) Dissolve 2.5 g of *phosphorous acid R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *water R*.

Reference solution (d) Mix 2.0 ml of reference solution (b) and 2.0 ml of reference solution (c) and dilute to 50.0 ml with *water R*.

The chromatographic procedure may be carried out using:

— a column 0.15 m long and 4.6 mm in internal diameter packed with *anion exchange resin R1* (7 µm);

— as mobile phase at a flow rate of 1.2 ml/min a solution of 0.2 ml of *anhydrous formic acid R* in 1000 ml of *water R*, adjusted to pH 3.5 with *2 M sodium hydroxide R*;

— as detector a refractometer;

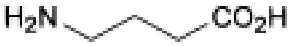
— a 100 µl loop injector,

maintaining the temperature of the column at 35 °C.

Inject reference solution (a) six times. The assay is not valid unless the relative standard deviation of the peak area of sodium alendronate is at most 1.0 per cent. Inject the test solution, reference solution (a) and reference solution (d). The retention time of sodium alendronate is about 16 min and the relative retention times are: phosphate about 1.3 and phosphite about 1.6. Record the chromatograms for twice the retention time of the principal peak in the chromatogram obtained with the test solution.

Calculate the percentage content of $C_4H_{12}NNaO_7P_2$ from the peak areas and the declared content of *sodium alendronate CRS*.

IMPURITIES



A. 4-aminobutanoic acid,

B. phosphate,

C. phosphite.

Ph Eur

Sodium Alginate

General Notices

(Ph Eur monograph 0625)

Action and use

Excipient.

Preparations

Alginate Raft-forming Oral Suspension

Compound Alginate Antacid Oral Suspension

Ph Eur

DEFINITION

Sodium alginate consists mainly of the sodium salt of alginic acid, which is a mixture of polyuronic acids [$(C_6H_8O_6)_n$] composed of residues of D-mannuronic acid and L-guluronic acid. Sodium alginate is obtained mainly from algae belonging to the Phaeophyceae.

CHARACTERS

Appearance

White or pale yellowish-brown powder.

Solubility

Slowly soluble in water forming a viscous, colloidal solution, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 0.2 g with shaking in 20 ml of *water R*. To 5 ml of this solution add 1 ml of *calcium chloride solution R*. A voluminous gelatinous mass is formed.

B. To 10 ml of the solution prepared in identification test A add 1 ml of *dilute sulphuric acid R*. A gelatinous mass is formed.

C. To 5 mg add 5 ml of *water R*, 1 ml of a freshly prepared 10 g/l solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and 5 ml of *hydrochloric acid R*. Boil for 3 min, cool, add 5 ml of *water R*, and shake with 15 ml of *di-isopropyl ether R*. Carry out a blank test. The upper layer obtained with the substance to be examined exhibits a deeper bluish-red colour than that obtained with the blank.

D. It complies with the test for sulphated ash. The residue obtained, dissolved in 2 ml of *water R*, gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 0.10 g in *water R*, with constant stirring, dilute to 30 ml with the same solvent and allow to stand for 1 h.

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Dilute 1 ml of solution S to 10 ml with *water R*.

Chlorides

Maximum 1.0 per cent.

To 2.50 g add 50 ml of *dilute nitric acid R*, shake for 1 h and dilute to 100.0 ml with *dilute nitric acid R*. Filter. To 50.0 ml of the filtrate add 10.0 ml of *0.1 M silver nitrate* and 5 ml of *toluene R*. Titrate with *0.1 M ammonium thiocyanate*, using 2 ml of *ferric ammonium sulphate solution R2* as indicator and shaking vigorously towards the end point.

1 ml of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Calcium

Maximum 1.50 per cent.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 0.10 g in 50 ml of *dilute ammonia R2*, heating on a water-bath. Allow to cool and dilute to 100.0 ml with *distilled water R* (solution (a)). Dilute 3.0 ml of solution (a) to 100.0 ml with *distilled water R*.

Reference solutions Prepare 3 reference solutions in the same manner as the test solution but add 0.75 ml, 1.0 ml and 1.5 ml respectively of *calcium standard solution (100 ppm Ca) R* to the 3.0 ml of solution (a).

Set the zero of the instrument using a mixture of 1.5 volumes of *dilute ammonia R2* and 98.5 volumes of *distilled water R*.

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Air-acetylene flame.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 0.1000 g by drying in an oven at 105 C for 4 h.

Sulphated ash *(2.4.14)*

30.0 per cent to 36.0 per cent (dried substance), determined on 0.1000 g.

Microbial contamination

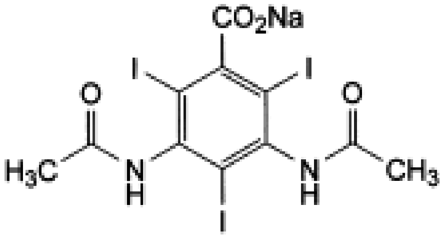
Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate-count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

Ph Eur

Sodium Amidotrizoate

General Notices

(Ph Eur monograph 1150)



$C_{11}H_8I_3N_2NaO_4$ 636 *737-31-5*

Action and use

Iodinated contrast medium.

Preparation

Sodium Amidotrizoate Injection

Ph Eur

DEFINITION

Sodium amidotrizoate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of sodium 3,5-bis(acetylamino)-2,4,6-tri-iodobenzoate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white powder, freely soluble in water, slightly soluble in alcohol, practically insoluble in acetone.

It melts at about 261 C with decomposition.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *sodium amidotrizoate CRS*. Dry both the substance to be examined and the reference substance at 100 C to 105 C for 3 h.

B. Examine the chromatograms obtained in the test for related substances. The principal

spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

C. Heat 50 mg gently in a small porcelain dish over a naked flame. Violet vapour is evolved.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 10 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Dilute 1 ml of solution S to 10 ml with *water R*. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 7.5 to 9.5.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel* GF_{254} *plate R*. *Prepare the solutions in subdued light and develop the chromatograms protected from light*.

Test solution (a) Dissolve 0.50 g of the substance to be examined in a 3 per cent *V/V* solution of *ammonia R* in *methanol R* and dilute to 10 ml with the same solution.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a 3 per cent *V/V* solution of *ammonia R* in *methanol R*.

Reference solution (a) Dilute 1 ml of test solution (b) to 50 ml with a 3 per cent *V/V* solution of *ammonia R* in *methanol R*.

Reference solution (b) Dissolve 50 mg of *sodium amidotrizoate CRS* in a 3 per cent *V/V* solution of *ammonia R* in *methanol R* and dilute to 10 ml with the same solution.

Apply separately to the plate 2 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *anhydrous formic acid R*, 25 volumes of *methyl ethyl ketone R* and 60 volumes of *toluene R*. Allow the plate to dry until the solvents have evaporated and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent).

Free aromatic amines

Maintain the solutions and reagents in iced water, protected from light. To 0.50 g in a 50 ml volumetric flask add 15 ml of *water R*. Shake and add 1 ml of *dilute sodium hydroxide solution R*. Cool in iced water, add 5 ml of a freshly prepared 5 g/l solution of *sodium nitrite R* and 12 ml of *dilute hydrochloric acid R*. Shake gently and allow to stand for exactly 2 min after adding the hydrochloric acid. Add 10 ml of a 20 g/l solution of *ammonium sulphamate R*. Allow to stand for 5 min, shaking frequently, and add 0.15 ml of a 100 g/l solution of α-*naphthol R* in *alcohol R*. Shake and allow to stand for 5 min. Add 3.5 ml of *buffer solution pH 10.9 R*, mix and dilute to 50.0 ml with *water R*. The absorbance *(2.2.25)*, measured within 20 min at 485 nm using as the compensation liquid a solution prepared at the same time and in the same manner but omitting the substance to be examined, is not greater than 0.30.

Free iodine and iodides

Not more than 50 ppm. Dissolve 1.0 g in *distilled water R* and dilute to 10 ml with the same solvent. Add dropwise *dilute nitric acid R* until the precipitation is complete, then add 3 ml of *dilute nitric acid R*. Filter and wash the precipitate with 5 ml of *water R*. Collect the filtrate and washings. Add 1 ml of *strong hydrogen peroxide solution R* and 1 ml of *methylene chloride R*. Shake. The lower layer is not more intensely coloured than a reference solution prepared simultaneously and in the same manner, using a mixture of 5 ml of *iodide standard solution (10 ppm I) R*, 3 ml of *dilute nitric acid R* and 15 ml of *water R*.

Heavy metals *(2.4.8)*

Dilute 4 ml of solution S to 20 ml with *water R*. 12 ml of this solution complies with limit test A for heavy metals (20 ppm). Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Water *(2.5.12)*

Not more than 11.0 per cent, determined on 0.400 g by the semi-micro determination of water.

ASSAY

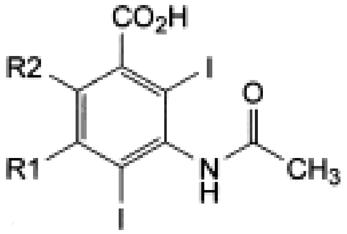
To 0.150 g in a 250 ml round-bottomed flask add 5 ml of *strong sodium hydroxide solution R*, 20 ml of *water R*, 1 g of *zinc powder R* and a few glass beads. Boil under a reflux condenser for 30 min. Allow to cool and rinse the condenser with 20 ml of *water R*, adding the rinsings to the flask. Filter through a sintered-glass filter *(2.1.2)* and wash the filter with several quantities of *water R*. Collect the filtrate and washings. Add 40 ml of *dilute sulphuric acid R* and titrate immediately with *0.1 M silver nitrate*. Determine the end-point potentiometrically *(2.2.20)*, using a suitable electrode system such as silver-mercurous sulphate.

1 ml of *0.1 M silver nitrate* is equivalent to 21.20 mg of $C_{11}H_8I_3N_2NaO_4$.

STORAGE

Store protected from light.

IMPURITIES



A. R1 = NH_2, R2 = I: 3-acetylamino-5-amino-2,4,6-tri-iodobenzoic acid,

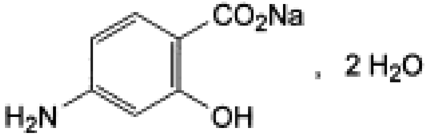
B. R1 = $NHCOCH_3$, R2 = H: 3,5-bis(acetylamino)-2,4-di-iodobenzoic acid.

Ph Eur

Sodium Aminosalicylate Dihydrate

General Notices

(Ph Eur monograph 1993)



$C_7H_6NNaO_3,2H_2O$ 211.2 *6018-19-5*

Action and use

Antituberculous.

Ph Eur

DEFINITION

Sodium 4-amino-2-hydroxybenzoate dihydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder or crystals, slightly hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, E.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of sodium aminosalicylate dihydrate.

B. Introduce 0.3 g into a porcelain crucible. Cautiously heat on a small flame until vapour is evolved. Cover the crucible with a watch glass and collect the white sublimate. The melting point *(2.2.14)* of the sublimate is 120 C to 124 C.

C. To 0.1 ml of solution S (see Tests) add 5 ml of *water R* and 0.1 ml of *ferric chloride solution R1*. A reddish-brown colour develops.

D. 2 ml of solution S gives the reaction of primary aromatic amines *(2.3.1)*.

E. 0.5 ml of solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

The freshly prepared solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_5 (*2.2.2, Method II)*.

Dissolve 2.5 g in *water R* and dilute to 25 ml with the same solvent.

pH *(2.2.3)*

6.5 to 8.5 for solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Use freshly prepared solutions and mobile phases*.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *3-aminophenol R* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dissolve 5.0 mg of *mesalazine CRS* in *water R* and dilute to 100.0 ml with the same solvent. To 10.0 ml of this solution add 1.0 ml of reference solution (a) and dilute to 50.0 ml with *water R*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: spherical *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: dissolve 2.2 g of *perchloric acid R* and 1.0 g of *phosphoric acid R* in *water R* and dilute to 1000.0 ml with the same solvent;

— *mobile phase B*: dissolve 1.7 g of *perchloric acid R* and 1.0 g of *phosphoric acid R* in *acetonitrile R* and dilute to 1000.0 ml with the same solvent,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 30	100 → 40	0 → 60
30 - 35	40 → 100	60 → 0
35 - 45	100	0

Flow rate 1.25 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Relative retention With reference to 4-aminosalicylate (retention time = about 12 min): impurity A = about 0.30; impurity B = about 0.37.

System suitability: Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to impurity A and impurity B.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *any other impurity*: for each impurity, not more than 0.1 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

16.0 per cent to 17.5 per cent, determined on 1.000 g by drying in an oven at 105 C.

Pyrogens *(2.6.8)*

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass, 10 ml of a 20 mg/ml solution of the substance to be examined in *water for injections R*.

ASSAY

Dissolve 0.150 g in 20 ml of *water R*. Add 10 ml of a 500 g/l solution of *sodium bromide R* and 25 ml of *glacial acetic acid R*. Add 5 ml of *0.1 M sodium nitrite* rapidly and continue the titration with the same titrant, determining the end-point potentiometrically *(2.2.20)*.

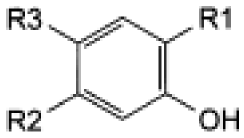
1 ml of *0.1 M sodium nitrite* is equivalent to 17.52 mg of $C_7H_6NNaO_3$.

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities *A, B.*



A. R1 = R3 = H, R2 = NH_2: 3-aminophenol,

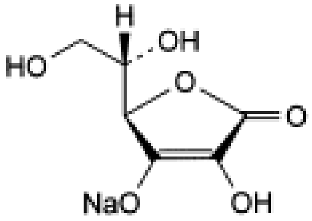
B. R1 = CO_2H, R2 = H, R3 = NH_2: 5-amino-2-hydroxybenzoic acid (mesalazine).

Ph Eur

Sodium Ascorbate

General Notices

(Ph Eur monograph 1791)



$C_6H_7NaO_6$ 198.1 *134-03-2*

Action and use

Excipient.

Ph Eur

DEFINITION

Sodium (2*R*)-2-[(1*S*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder or crystals.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Specific optical rotation *(2.2.7)* (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *Sodium ascorbate CRS*.

C. To 1 ml of solution S (see Tests) add 0.2 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2*. A grey precipitate is formed.

D. 1 ml of solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 or BY_6 *(2.2.2, Method II)*. Examine the colour immediately after preparation of the solution.

pH *(2.2.3)*

7.0 to 8.0 for solution S.

Specific optical rotation *(2.2.7)*

+ 103 to + 108 (dried substance), determined on freshly prepared solution S.

Oxalic acid

Maximum 0.3 per cent.

Test solution Dissolve 0.25 g in 5 ml of *water R*. Add 1 ml of *dilute acetic acid R* and 0.5 ml of *calcium chloride solution R*.

Reference solution Dissolve 70 mg of *oxalic acid R* in *water R* and dilute to 500 ml with the same solvent; to 5 ml of the solution add 1 ml of *dilute acetic acid R* and 0.5 ml of *calcium chloride solution R*.

Allow the solutions to stand for 1 h. Any opalescence in the test solution is not more intense than that in the reference solution.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Benzene *(2.4.24, System A)*

Maximum 2 ppm.

Sulphates *(2.4.13)*

Maximum 150 ppm.

To 10 ml of solution S add 2 ml of *hydrochloric acid R1* and dilute to 15 ml with *distilled water R*.

Copper

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 2.0 g in *0.1 M nitric acid* and dilute to 25.0 ml with the same solvent.

Reference solutions Prepare reference solutions (0.2 ppm, 0.4 ppm and 0.6 ppm) by diluting *copper standard solution (10 ppm Cu) R* with *0.1 M nitric acid*.

Source Copper hollow-cathode lamp.

Wavelength 324.8 nm.

Atomisation device Air-acetylene flame.

Iron

Maximum 2.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 5.0 g in *0.1 M nitric acid* and dilute to 25.0 ml with the same solvent.

Reference solutions Prepare reference solutions (0.2 ppm, 0.4 ppm and 0.6 ppm) by diluting *iron standard solution (20 ppm Fe) R* with *0.1 M nitric acid*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Nickel

Maximum 1.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 10.0 g in *0.1 M nitric acid* and dilute to 25.0 ml with the same solvent.

Reference solutions Prepare reference solutions (0.2 ppm, 0.4 ppm and 0.6 ppm) by diluting *nickel standard solution (10 ppm Ni) R* with *0.1 M nitric acid*.

Source Nickel hollow-cathode lamp.

Wavelength 232.0 nm.

Atomisation device Air-acetylene flame.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.25 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 80 mg in a mixture of 10 ml of *dilute sulphuric acid R* and 80 ml of *carbon dioxide-free water R*. Add 1 ml of *starch solution R*. Titrate with *0.05 M iodine* until a persistent violet-blue colour is obtained.

1 ml of *0.05 M iodine* is equivalent to 9.91 mg of $C_6H_7NaO_6$.

STORAGE

In a non-metallic container, protected from light.

Ph Eur

Sodium Aurothiomalate

General Notices

(Ph Eur monograph 1994)

39377-38-3

Action and use

Used in the treatment of rheumatoid arthritis.

Preparation

Sodium Aurothiomalate Injection

Ph Eur

DEFINITION

Mixture of monosodium and disodium salts of (*2RS*)-2-(aurosulphanyl)butanedioic acid.

CONTENT

— *gold* (Au; A_r 197.0): 44.5 per cent to 46.0 per cent (dried substance);

— *sodium* (Na; A_r 22.99): 10.8 per cent to 11.8 per cent (dried substance).

CHARACTERS

Appearance

Fine, pale yellow, hygroscopic powder.

Solubility

Very soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Dissolve 20 mg in *water R* and dilute to 2 ml with the same solvent. Add 2 ml of *strong hydrogen peroxide solution R* and 1 ml of *sodium hydroxide solution R*. Carefully heat to boiling and boil for 30 s. A precipitate is produced that appears brownish-black by reflected light and bluish-green by transmitted light.

B. To 20 mg add 2 ml of *water R*. The solution gives reaction (a) of sodium *(2.3.1)*.

C. Ignite 100 mg, dissolve the residue in *hydrochloric acid R* and dilute to 10 ml with the same acid. Allow to stand. 5 ml of the clear supernatant gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 1.0 g in *water R* and dilute to 10 ml with the same solvent. Filter, seal in an ampoule and heat at 100 °C for 1 h. Cool and dilute the contents of the ampoule to 100 ml with *water R*. The solution remains clear and is not more intensely coloured than a 0.100 g/l solution of *potassium ferricyanide R*.

pH *(2.2.3)*

6.0 to 7.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *fumaric acid R* and 100.0 mg of *thiomalic acid R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 25.0 ml with *water R*.

Reference solution (b) Dissolve 12.0 mg of *thiomalic acid R* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (c) Dissolve 10.0 mg of *maleic acid R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 25.0 ml with *water R*.

Column:

- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 90 volumes of a 10.5 g/l solution of *phosphoric acid R*, 100 volumes of *methanol R2* and 810 volumes of *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µl.

Run time Twice the retention time of impurity C.

Relative retention With reference to impurity C (retention time = about 8 min): impurity A = about 0.4; impurity B = about 0.6. Aurothiomalate does not elute under the chromatographic conditions described.

System suitability reference solution (a):

- *resolution*: minimum 5.0 between the peaks due to impurities B and C.

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent).

Glycerol

Maximum 8.0 per cent.

Test solution Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 0.80 g of *glycerol R* to 100.0 ml with *water R*.

Reference solution (b) To 2.5 ml of reference solution (a) add 7.5 ml of *water R*.

Reference solution (c) To 5.0 ml of reference solution (a) add 5.0 ml of *water R*.

Reference solution (d) To 7.5 ml of reference solution (a) add 2.5 ml of *water R*.

Blank solution 10 ml of *water R*.

To the test solution, reference solutions (b), (c) and (d) and the blank solution, add 2.5 ml of a freshly prepared 235 g/l solution of *sodium hydroxide R* and mix. Add dropwise in 0.2 ml increments a 38.0 g/l solution of *cupric chloride R*, shaking vigorously after each addition, until the solutions become slightly turbid. Then add 0.2 ml of the 38.0 g/l solution of *cupric chloride R*. Stopper and shake vigorously for 1 min. Dilute to 25.0 ml with *water R* and mix. Centrifuge for 2 min. Measure the absorbance *(2.2.25)* of the supernatant solution of a 1 cm layer at 635 nm. Use the solution prepared from the blank solution as the compensation liquid. Draw a calibration curve and calculate the content of glycerol in the sample.

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 24 h.

ASSAY

Gold

Heat 0.2 g with 10 ml of *sulphuric acid R* and continue to boil gently until a clear, pale yellow liquid is produced. Cool, add about 1 ml of *nitric acid R* dropwise and boil for 1 h. Cool, dilute to 70 ml with *water R*, boil for 5 min and filter. Wash the residue of gold with *water R* at 60 °C. Dry and ignite at a temperature of at least 600 °C for 3 h. Weigh the residue and calculate the percentage content of Au.

Sodium

Evaporate to dryness the filtrate and washings obtained in the assay for gold, moisten with *sulphuric acid R* and ignite at 600 ± 50 °C for 3 h.

1.000 g of residue is equivalent to 0.324 g of Na.

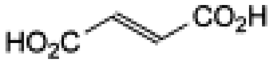
STORAGE

In an airtight container.

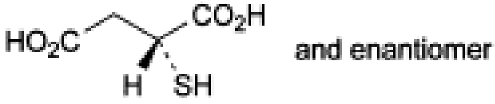
IMPURITIES

Specified impurities: A, B, C.

A. maleic acid,



B. (*2E*)-butenedioic acid (fumaric acid),


and enantiomer

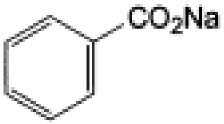
C. (*2RS*)-2-sulphanylbutanedioic acid (thiomalic acid).

Ph Eur

Sodium Benzoate

General Notices

(Ph Eur monograph 0123)



$C_7H_5NaO_2$ 144.1 *532-32-1*

Ph Eur

DEFINITION

Sodium benzenecarboxylate.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline or granular powder or flakes, slightly hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in ethanol (90 per cent *V/V*).

IDENTIFICATION

A. It gives reactions (b) and (c) of benzoates *(2.3.1)*.

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 10 ml of *carbon dioxide-free water R* and 0.2 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Halogenated compounds

Maximum 200 ppm for ionised chlorine and maximum 300 ppm for total chlorine.

All glassware used must be chloride-free and may be prepared by soaking overnight in a 500 g/l solution of nitric acid R, rinsed with water R and stored full of water R. It is recommended that glassware be reserved exclusively for this test.

Test solution To 20.0 ml of solution S add 5 ml of *water R* and dilute to 50.0 ml with *ethanol (96 per cent) R*.

Determination of ionised chlorine
In three 25 ml volumetric flasks, prepare the following solutions.

Solution (a) To 4.0 ml of the test solution add 3 ml of *dilute sodium hydroxide solution R* and 3 ml of *ethanol (96 per cent) R*. This solution is used to prepare solution A.

Solution (b) To 3 ml of *dilute sodium hydroxide solution R* add 2 ml of *water R* and 5 ml of *ethanol (96 per cent) R*. This solution is used to prepare solution B.

Solution (c) To 4.0 ml of *chloride standard solution (8 ppm Cl) R* add 6.0 ml of *water R*. This solution is used to prepare solution C.

In a fourth 25 ml volumetric flask, place 10 ml of *water R*. To each flask add 5 ml of *ferric ammonium sulphate solution R5*, mix and add dropwise and with swirling 2 ml of *nitric acid R* and 5 ml of *mercuric thiocyanate solution R*. Shake. Dilute the contents of each flask to 25.0 ml with *water R* and allow the solutions to stand in a water-bath at 20 °C for 15 min. Measure at 460 nm in a 2 cm cell the absorbance *(2.2.25)* of solution A using solution B as the compensation liquid, and the absorbance of solution C using the solution obtained with 10 ml of *water R* as the compensation liquid. The absorbance of solution A is not greater than that of solution C.

Determination of total chlorine
Solution (a) To 10.0 ml of the test solution add 7.5 ml of *dilute sodium hydroxide solution R* and 0.125 g of *nickel-aluminium alloy R* and heat on a water-bath for 10 min. Allow to cool to room temperature, filter into a 25 ml volumetric flask and wash the filter with 3 quantities, each of 2 ml, of *ethanol (96 per cent) R* (a slight precipitate may form that disappears on acidification). Dilute the filtrate and washings to 25.0 ml with *water R*. This solution is used to prepare solution A.

Solution (b) In the same manner, prepare a similar solution replacing the test solution by a mixture of 5 ml of *ethanol (96 per cent) R* and 5 ml of *water R*. This solution is used to prepare solution B.

Solution (c) To 6.0 ml of *chloride standard solution (8 ppm Cl) R* add 4.0 ml of *water R*. This solution is used to prepare solution C.

In four 25 ml volumetric flasks, place separately 10 ml of solution (a), 10 ml of solution (b), 10 ml of solution (c) and 10 ml of *water R*. To each flask add 5 ml of *ferric ammonium sulphate solution R5*, mix and add dropwise and with swirling 2 ml of *nitric acid R* and 5 ml of *mercuric thiocyanate solution R*. Shake. Dilute the contents of each flask to 25.0 ml with *water R* and allow the solutions to stand in a water-bath at 20 °C for 15 min. Measure at 460 nm in a 2 cm cell the absorbance *(2.2.25)* of solution A using solution B as the compensation liquid, and the absorbance of solution C using the solution obtained with 10 ml of *water R* as the compensation liquid. The absorbance of solution A is not greater than that of solution C.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.250 g in 20 ml of *anhydrous acetic acid R*, heating to 50 °C if necessary. Cool. Using 0.05 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until a green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 14.41 mg of $C_7H_5NaO_2$.

Ph Eur

Sodium Bicarbonate

General Notices

(Sodium Hydrogen Carbonate, Ph Eur monograph 0195)

$NaHCO_3$ 84.0 *144-55-8*

Action and use

Antacid; used in treatment of electrolyte deficiency.

Preparations

Sodium Bicarbonate Ear Drops

Sodium Bicarbonate Eye Lotion

Sodium Bicarbonate Intravenous Infusion

Sodium Bicarbonate Oral Solution

Compound Sodium Bicarbonate Tablets

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

When heated in the dry state or in solution, it gradually changes into sodium carbonate.

IDENTIFICATION

A. To 5 ml of solution S (see Tests) add 0.1 ml of *phenolphthalein solution R*. A pale pink colour is produced. Heat; gas is evolved and the solution becomes red.

B. It gives the reaction of carbonates and bicarbonates *(2.3.1)*.

C. Solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in 90 ml of *carbon dioxide-free water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Carbonates

The pH *(2.2.3)* of freshly prepared solution S is not greater than 8.6.

Chlorides *(2.4.4)*

Maximum 150 ppm.

To 7 ml of solution S add 2 ml of *nitric acid R* and dilute to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 150 ppm.

To a suspension of 1.0 g in 10 ml of *distilled water R* add *hydrochloric acid R* until neutral and about 1 ml in excess. Dilute to 15 ml with *distilled water R*.

Ammonium *(2.4.1)*

Maximum 20 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*. Prepare the standard using a mixture of 5 ml of *water R* and 10 ml of *ammonium standard solution (1 ppm NH_4) R*.

Arsenic *(2.4.2, Method A)*

Maximum 2 ppm, determined on 0.5 g.

Calcium *(2.4.3)*

Maximum 100 ppm.

To a suspension of 1.0 g in 10 ml of *distilled water R* add *hydrochloric acid R* until neutral and dilute to 15 ml with *distilled water R*.

Iron *(2.4.9)*

Maximum 20 ppm.

Dissolve 0.5 g in 5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 2 ml of *hydrochloric acid R* and 18 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

Dissolve 1.500 g in 50 ml of *carbon dioxide-free water R*. Titrate with *1 M hydrochloric acid*, using 0.2 ml of methyl orange solution R as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 84.0 mg of $NaHCO_3$.

Ph Eur

Sodium Bromide

General Notices

(Ph Eur monograph 0190)

NaBr 102.9 *7647-15-6*

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, granular powder or small, colourless, transparent or opaque crystals, slightly hygroscopic.

Solubility

Freely soluble in water, soluble in alcohol.

IDENTIFICATION

A. It gives reaction (a) of bromides *(2.3.1)*.

B. Solution S (see Tests) gives the reactions of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromates

To 10 ml of solution S add 1 ml of *starch solution R*, 0.1 ml of a 100 g/l solution of *potassium iodide R* and 0.25 ml of *0.5 M sulphuric acid* and allow to stand protected from light for 5 min. No blue or violet colour develops.

Chlorides

Maximum 0.6 per cent.

In a conical flask, dissolve 1.000 g in 20 ml of *dilute nitric acid R*. Add 5 ml of *strong hydrogen peroxide solution R* and heat on a water-bath until the solution is completely decolourised. Wash down the sides of the flask with a little *water R* and heat on a water-bath for 15 min. Allow to cool, dilute to 50 ml with *water R* and add 5.0 ml of *0.1 M silver nitrate* and 1 ml of *dibutyl phthalate R*. Shake and titrate with *0.1 M ammonium thiocyanate*, using 5 ml of *ferric ammonium sulphate solution R2* as indicator. Not more than 1.7 ml of *0.1 M silver nitrate* is used. Note the volume of *0.1 M silver nitrate* used (see Assay). Carry out a blank test.

Iodides

To 5 ml of solution S add 0.15 ml of *ferric chloride solution R1* and 2 ml of *methylene chloride R*. Shake and allow to separate. The lower layer is colourless *(2.2.2, Method I)*.

Sulphates *(2.4.13)*

Maximum 100 ppm.

15 ml of solution S complies with the limit test for sulphates.

Iron *(2.4.9)*

Maximum 20 ppm.

5 ml of solution S diluted to 10 ml with *water R* complies with the limit test for iron.

Magnesium and alkaline-earth metals *(2.4.7)*

Maximum 200 ppm, calculated as Ca.

10.0 g complies with the limit test for magnesium and alkaline-earth metals. The volume of *0.01 M sodium edetate* used does not exceed 5.0 ml.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 2.000 g in *water R* and dilute to 100.0 ml with the same solvent. To 10.0 ml of the solution add 50 ml of *water R*, 5 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R*. Shake. Titrate with *0.1 M ammonium thiocyanate*, using 2 ml of *ferric ammonium sulphate solution R2* as indicator and shaking vigorously towards the end-point.

1 ml of *0.1 M silver nitrate* is equivalent to 10.29 mg of NaBr.

Calculate the percentage content of NaBr from the expression:

$$a - 2.902\,b$$

a = percentage content of NaBr and NaCl obtained in the assay and calculated as NaBr,

b = percentage content of Cl in the test for chlorides.

STORAGE

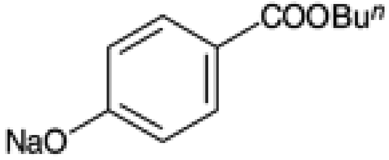
In an airtight container .

Ph Eur

Sodium Butyl Hydroxybenzoate

General Notices

Sodium Butylparaben



$C_{11}H_{13}NaO_3$ 216.2 *36457-20-2*

Action and use

Antimicrobial preservative.

DEFINITION

Sodium Butyl Hydroxybenzoate is the sodium salt of butyl 4-hydroxybenzoate. It contains not less than 99.0% and not more than 102.0% of $C_{11}H_{13}NaO_3$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white powder; hygroscopic.

Freely soluble in *water* and in *ethanol (96%)*.

IDENTIFICATION

A. Dissolve 0.5 g in 5 ml of *water* and acidify to *litmus paper* with *hydrochloric acid*. A white precipitate is produced. Wash the precipitate with *water* and dry. The *infrared absorption spectrum* of the precipitate, Appendix II A, is concordant with the *reference spectrum* of butyl hydroxybenzoate *(RS 036)*.

B. The residue on ignition yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

pH of a 0.1% w/v solution, 9.5 to 10.5, Appendix V L.

Clarity of solution

Dissolve 1.0 g in 10 ml of *water*. The solution is *clear*, Appendix IV A.

Chloride

Dissolve 1.0 g in 100 ml of *water*, add 1 ml of *nitric acid* and filter. 15 ml of the filtrate complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Sulphate

Dissolve 0.50 g in 40 ml of *water*, add 3.5 ml of 2M *hydrochloric acid*, dilute to 60 ml with *water* and filter. 15 ml of the filtrate complies with the *limit test for sulphates*, Appendix VII (0.12%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a plate precoated with silica gel F_{254} the surface of which has been modified with chemically-bonded octadecylsilyl groups (Whatman KC18F plates are suitable) and a mixture of 70 volumes of *methanol*, 30 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute a 2.0% w/v solution of the substance being examined in *water* with an equal volume of *acetone*. For solution (2) dilute 1 volume of solution (1) to 25 volumes with a mixture of equal volumes of *acetone* and *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Water

Not more than 5.0% w/w, Appendix IX C. Use 1 g.

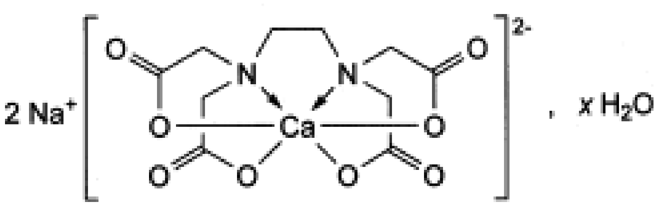
ASSAY

Gently boil 0.1 g under a reflux condenser with 25 ml of 1.25M *sodium hydroxide* for 30 minutes. Allow to cool, add 25 ml of 0.0333M *potassium bromate VS*, 5 ml of a 12.5% w/v solution of *potassium bromide* and 10 ml of *hydrochloric acid* and immediately stopper the flask. Shake for 15 minutes and allow to stand for 15 minutes. Add 25 ml of *dilute potassium iodide solution* and shake vigorously. Titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of potassium bromate required. The volume of 0.0333M *potassium bromate VS* used is equivalent to half of the volume of 0.1M *sodium thiosulphate VS* required for the titration. Each ml of 0.0333M *potassium bromate VS* is equivalent to 7.207 mg of $C_{11}H_{13}NaO_3$.

Sodium Calcium Edetate

General Notices

(Ph Eur monograph 0231)



$C_{10}H_{12}CaN_2Na_2O_8,xH_2O$ 374.3 *62-33-9*
(anhydrous) *(anhydrous)*

Action and use

Chelating agent.

Preparation

Sodium Calcium Edetate Intravenous Infusion

Ph Eur

DEFINITION

Disodium [(ethylenedinitrilo)tetraacetato]calciate(2-).

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

It contains a variable amount of water of crystallisation.

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, C, D.

Second identification *B, C, D*.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *Sodium calcium edetate CRS*.

B. Dissolve 2 g in 10 ml of *water R*, add 6 ml of *lead nitrate solution R*, shake and add 3 ml of *potassium iodide solution R*. No yellow precipitate is formed. Make alkaline to *red litmus paper R* by the addition of *dilute ammonia R2* and add 3 ml of *ammonium oxalate solution R*. A white precipitate is formed.

C. Ignite. The residue gives reaction (b) of calcium *(2.3.1)*.

D. Dissolve 0.5 g in 10 ml of *water R* and add 10 ml of *potassium pyroantimonate solution R*. A white, crystalline precipitate is formed. The formation of the precipitate is accelerated by rubbing the wall of the tube with a glass rod.

TESTS

Solution S

Dissolve 5.0 g in *water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

6.5 to 8.0.

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Impurity A

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light*.

Solvent mixture Dissolve 10.0 g of *ferric sulphate pentahydrate R* in 20 ml of *0.5 M sulphuric acid* and add 780 ml of *water R*. Adjust to pH 2.0 with *1 M sodium hydroxide* and dilute to 1000 ml with *water R*.

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution Dissolve 40.0 mg of *nitrilotriacetic acid R* (impurity A) in the solvent mixture and dilute to 100.0 ml with the solvent mixture. To 1.0 ml of this solution add 0.1 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: spherical *graphitised carbon for chromatography R1* (5 µm) with a specific surface area of 120 m^2/g and a pore size of 25 nm.

Mobile phase Dissolve 50.0 mg of *ferric sulphate pentahydrate R* in 50 ml of *0.5 M sulphuric acid* and add 750 ml of *water R*; adjust to pH 1.5 with *0.5 M sulphuric acid* or *1 M sodium hydroxide*, add 20 ml of *ethylene glycol R* and dilute to 1000 ml with *water R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µl; filter the solutions and inject immediately.

Run time 4 times the retention time of the iron complex of impurity A.

Retention time Iron complex of impurity A = about 5 min; iron complex of edetic acid = about 10 min.

System suitability
Reference solution:

- *resolution*: minimum 7 between the peaks due to the iron complex of impurity A and the iron complex of edetic acid;
- *signal-to-noise ratio*: minimum 50 for the peak due to impurity A.

Limit:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Disodium edentate

Maximum 1.0 per cent.

Dissolve 5.0 g in 250 ml of *water R*. Add 10 ml of *ammonium chloride buffer solution pH 10.0 R* and about 50 mg of *mordant black 11 triturate R*. Not more than 1.5 ml of *0.1 M magnesium chloride* is required to change the colour of the indicator to violet.

Chlorides

Maximum 0.1 per cent.

Dissolve 0.7 g in *water R* and dilute to 20 ml with the same solvent. Add 30 ml of *dilute nitric acid R*, allow to stand for 30 min and filter. Dilute 10 ml of the filtrate to 50 ml with *water R*. Use this solution as the test solution. Prepare the reference solution using 0.40 ml of *0.01 M hydrochloric acid*, add 6 ml of *dilute nitric acid R* and dilute to 50 ml with *water R*. Filter both solutions if necessary. Add 1 ml of *silver nitrate solution R2* to the test solution and to the reference solution and mix. After standing for 5 min protected from light, any opalescence in the test solution is not more intense than that in the reference solution.

Iron *(2.4.9)*

Maximum 80 ppm.

Dilute 2.5 ml of solution S to 10 ml with *water R*. Add 0.25 g of *calcium chloride R* to the test solution and the standard before the addition of the *thioglycollic acid R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

5.0 per cent to 13.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.500 g in *water R* and dilute to 200 ml with the same solvent. To 20.0 ml of this solution, add 80 ml of *water R* and adjust to pH 2 with *dilute nitric acid R*. Titrate with *0.01 M bismuth nitrate*, using 0.1 ml of a 1 g/l solution of *xylenol orange R* as indicator. The colour of the solution changes from yellow to red.

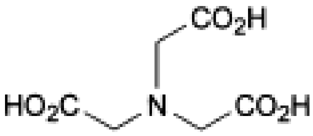
1 ml of *0.01 M bismuth nitrate* is equivalent to 3.74 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

STORAGE

In an airtight container , protected from light.

IMPURITIES

Specified impurities A.



A. nitrilotriacetic acid.

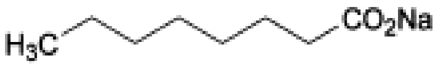
Ph Eur

Sodium Caprylate

General Notices

Sodium Octanoate

(Ph Eur monograph 1471)



$C_8H_{15}NaO_2$ 166.2 *1984-06-1*

Action and use

Excipient.

Ph Eur

DEFINITION

Sodium octanoate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble or freely soluble in water, freely soluble in acetic acid, sparingly soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. To 0.2 ml of solution S (see Tests) add 0.3 ml of *water R*. The solution gives reaction (b) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

8.0 to 10.5 for solution S.

Related substances

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 0.116 g in *water R* and dilute to 5 ml with the same solvent. Add 1 ml of a 2.8 per cent *V/V* solution of *sulphuric acid R* and shake with 10 ml of *ethyl acetate R*. Separate the organic layer and dry over *anhydrous sodium sulphate R*.

Reference solution (a) Dissolve 0.10 g of *caprylic acid CRS* in *ethyl acetate R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of the test solution to 100 ml with *ethyl acetate R*. Dilute 5 ml of this solution to 50 ml with *ethyl acetate R*.

Column:

— *material*: fused silica;

— *size*: *l* = 30 m, Ø = 0.25 mm;

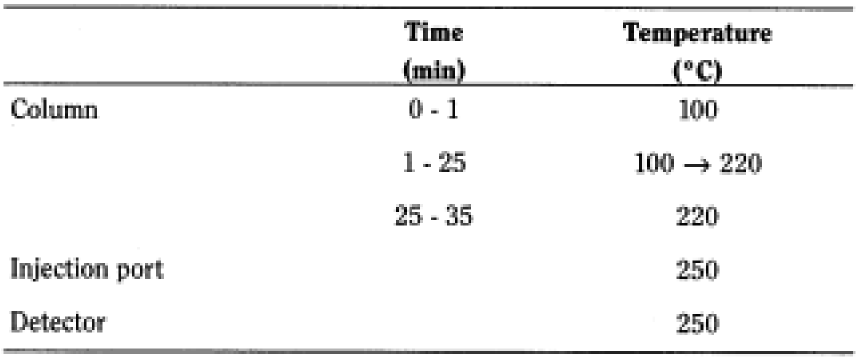
— *stationary phase*: *macrogol 20 000 2-nitroterephthalate R* (film thickness 0.25 µm).

Carrier gas *Helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	100
	1 - 25	100 → 220
	25 - 35	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

— *signal-to-noise ratio*: minimum 5 for the principal peak.

Limits:

— *any impurity*: for each impurity, maximum 0.3 per cent;

— *total*: maximum 0.5 per cent;

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in *glacial acetic acid R* and dilute to 10 ml with the same acid. Add 10 ml of *ethanol (96 per cent) R*. 12 ml of the solution complies with test B. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R* and 9 ml of a mixture of equal volumes of *glacial acetic acid R* and *ethanol (96 per cent) R*.

Water *(2.5.12)*

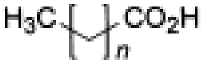
Maximum 3.0 per cent, determined on 1.000 g.

ASSAY

Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 16.62 mg of $C_8H_{15}NaO_2$.

IMPURITIES



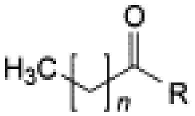
A. *n* = 4: hexanoic acid,

B. *n* = 5: heptanoic acid,

C. *n* = 7: nonanoic acid,

D. *n* = 8: decanoic acid,

E. valproic acid,

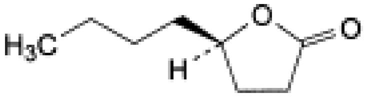


F. R = OCH_3, *n* = 6: methyl octanoate,

G. R = OC_2H_5, *n* = 6: ethyl octanoate,

H. R = OCH_3, *n* = 8: methyl decanoate,

I. R = CH_3, *n* = 8: undecan-2-one,


and enantiomer

J. (*RS*)-5-butyltetrahydrofuran-2-one (γ-hydroxyoctanoic acid lactone).

Ph Eur

Sodium Carbonate Decahydrate

General Notices

(Ph Eur monograph 0191)

$Na_2CO_3,10H_2O$ 286.1 *497-19-8*

Ph Eur

DEFINITION

Content

36.7 per cent to 40.0 per cent of Na_2CO_3.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless, transparent crystals, efflorescent.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in *water R* and dilute to 10 ml with the same solvent. The solution is strongly alkaline *(2.2.4)*.

B. The solution prepared for identification test A gives the reaction of carbonates *(2.3.1)*.

C. The solution prepared for identification test A gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in portions in a mixture of 5 ml of *hydrochloric acid R* and 25 ml of *distilled water R*. Heat the solution to boiling and cool. Add *dilute sodium hydroxide solution R* until the solution is neutral and dilute to 50 ml with *distilled water R*.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y6 *(2.2.2, Method I)*.

Dissolve 4.0 g in 10 ml of *water R*.

Alkali hydroxides and bicarbonates

Dissolve 1.0 g in 20 ml of *water R*, add 20 ml of *barium chloride solution R1* and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. The solution does not become red. Heat the remainder of the filtrate to boiling for 2 min. The solution remains clear *(2.2.1)*.

Chlorides ***(2.4.4)***

Maximum 50 ppm.

Dissolve 1.0 g in *water R*, add 4 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 100 ppm, determined on solution S.

Arsenic ***(2.4.2, Method A)***

Maximum 2 ppm, determined on 5 ml of solution S.

Iron ***(2.4.9)***

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

ASSAY

Dissolve 2.000 g in 25 ml of *water R*. Titrate with *1 M hydrochloric acid*, using 0.2 ml of *methyl orange solution R* as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 52.99 mg of Na_2CO_3.

STORAGE

In an airtight container.

Ph Eur

Sodium Carbonate Monohydrate

General Notices

(Ph Eur monograph 0192)

Na_2CO_3,H_2O 124.0 *5698-11-6*

Ph Eur

DEFINITION

Content

83.0 per cent to 87.5 per cent of Na_2CO_3.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in *water R* and dilute to 10 ml with the same solvent. The solution is strongly alkaline *(2.2.4)*.

B. The solution prepared for identification test A gives the reaction of carbonates *(2.3.1)*.

C. The solution prepared for identification test A gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.0 g in portions in a mixture of 5 ml of *hydrochloric acid R* and 25 ml of *distilled water R*. Heat the solution to boiling and cool. Add *dilute sodium hydroxide solution R* until the solution is neutral and dilute to 50 ml with *distilled water R*.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method I)*.

Dissolve 2.0 g in 10 ml of *water R*.

Alkali hydroxides and bicarbonates

Dissolve 0.4 g in 20 ml of *water R*, add 20 ml of *barium chloride solution R1* and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R*. The solution does not become red. Heat the remainder of the filtrate to boiling for 2 min. The solution remains clear *(2.2.1)*.

Chlorides ***(2.4.4)***

Maximum 125 ppm.

Dissolve 0.4 g in *water R*, add 4 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 250 ppm, determined on solution S.

Arsenic ***(2.4.2, Method A)***

Maximum 5 ppm, determined on 5 ml of solution S.

Iron ***(2.4.9)***

Maximum 50 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

ASSAY

Dissolve 1.000 g in 25 ml of *water R*. Titrate with *1 M hydrochloric acid*, using 0.2 ml of *methyl orange solution R* as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 52.99 mg of Na_2CO_3.

STORAGE

In an airtight container.

Ph Eur

Sodium Cetostearyl Sulphate

General Notices

(Ph Eur monograph 0847)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of sodium cetyl sulphate ($C_{16}H_{33}NaO_4S$; M_r 344.5) and sodium stearyl sulphate ($C_{18}H_{37}NaO_4S$; M_r 372.5). A suitable buffer may be added.

Content:

— *sodium cetostearyl sulphate*: minimum 90.0 per cent (anhydrous substance);

— *sodium cetyl sulphate*: minimum 40.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or pale yellow, amorphous or crystalline powder.

Solubility

Soluble in hot water giving an opalescent solution, practically insoluble in cold water, partly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *B, D, F*.

Second identification *A, C, D, E, F*.

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in 10 ml of *ethanol (70 per cent V/V) R*, heating on a water-bath.

Reference solution Dissolve 50 mg of *sodium cetostearyl sulphate CRS* in 10 ml of *ethanol (70 per cent V/V) R*, heating on a water-bath.

Plate *TLC silanised silica gel plate R*.

Mobile phase *water R*, *acetone R*, *methanol R* (20:40:40 *V/V/V*).

Application 2 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with a 50 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R*. Heat at 120 C until the spots appear (about 3 h).

Results The principal spots in the chromatogram obtained with the test solution are similar in position and colour to the principal spots in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with test solution (c) are similar in retention time to the 2 principal peaks in the chromatogram obtained with the reference solution.

C. Dissolve 0.1 g in 10 ml of *water R* and shake. A foam is formed.

D. It gives a yellow colour to a non-luminous flame.

E. To 0.1 ml of the solution prepared for identification test C add 0.1 ml of a 1 g/l solution of *methylene blue R* and 2 ml of *dilute sulphuric acid R*. Add 2 ml of *methylene chloride R* and shake. The methylene chloride layer has an intense blue colour.

F. Mix about 10 mg with 10 ml of *anhydrous ethanol R*. Heat to boiling on a water-bath, shaking frequently. Filter immediately and evaporate to dryness. Dissolve the residue in 7 ml of *water R*, add 3 ml of *dilute hydrochloric acid R* and evaporate the solution to half its volume. Allow to cool. Filter. To the filtrate add 1 ml of *barium chloride solution R1*. A white, crystalline precipitate is formed.

TESTS

Acidity or alkalinity

Dissolve 0.5 g with heating in a mixture of 10 ml of *water R* and 15 ml of *ethanol (90 per cent V/V) R*. Add 0.1 ml of *phenolphthalein solution R1*. The solution is colourless. Add 0.1 ml of *0.1 M sodium hydroxide*. The solution is red.

Sodium chloride and sodium sulphate

Maximum 8.0 per cent for the sum of the percentage contents.

Sodium chloride

Dissolve 5.00 g in 50 ml of *water R*, add *dilute nitric acid R* dropwise until the solution is neutral to *blue litmus paper R*. Add 2 ml of *potassium chromate solution R* and titrate with *0.1 M silver nitrate*.

1 ml of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Sodium sulphate Dissolve 0.500 g in 20 ml of *water R*, warming gently if necessary, and add 1 ml of a 0.5 g/l solution of *dithizone R* in *acetone R*. If the solution is red, add *1 M nitric acid*, dropwise, until a bluish-green colour is obtained. Add 2.0 ml of *dichloroacetic acid solution R* and 80 ml of *acetone R*. Titrate with *0.01 M lead nitrate* until a persistent orange-red colour is obtained.

1 ml of *0.01 M lead nitrate* is equivalent to 1.420 mg of Na_2SO_4.

Free cetostearyl alcohol

Maximum 4.0 per cent.

From the chromatogram obtained with test solution (a) in the assay, calculate the percentage content of free cetostearyl alcohol in the substance to be examined, using the following expression:

$$S\frac{100 \times m_H}{S_{Ha(corr)} \times m}$$

S = sum of the areas of the peaks due to cetyl alcohol and stearyl alcohol in the chromatogram obtained with test solution (a),

m_H = mass of the internal standard added in the preparation of test solution (a), in milligrams,

$S_{Ha(corr)}$ = corrected area of the peak due to the internal standard in the chromatogram obtained with test solution (a),

m = mass of sodium cetostearyl sulphate used for the preparation of test solution (a), in milligrams.

Water *(2.5.12)*

Maximum 1.5 per cent, determined on 5.00 g.

ASSAY

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.20 g of *heptadecanol CRS* in *anhydrous ethanol R* and dilute to 50 ml with the same solvent.

Test solution (a) Dissolve 0.300 g of the substance to be examined in 50 ml of *anhydrous ethanol R* and add 2 ml of the internal standard solution and 48 ml of *water R*. Shake with 4 quantities, each of 25 ml, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the organic layers. Reserve the hydro-alcoholic layer for the preparation of test solutions (c) and (d). Wash the organic layer with 2 quantities, each of 30 ml, of *water R*. Dry over *anhydrous sodium sulphate R* and filter.

Test solution (b) Dissolve 0.300 g of the substance to be examined in 50 ml of *anhydrous ethanol R* and add 50 ml of *water R*. Shake with 4 quantities, each of 25 ml, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the organic layers, wash with 2 quantities, each of 30 ml, of *water R*. Dry over *anhydrous sodium sulphate R* and filter.

Test solution (c) Transfer 25 ml of the hydro-alcoholic solution obtained in the preparation of test solution (a) to a 200 ml flask that can be fitted with a reflux condenser. Add 20 ml of *hydrochloric acid R* and 10 ml of the internal standard solution. Boil under a reflux condenser for 2 h. Allow to cool and shake with 4 quantities, each of 20 ml, of *pentane R*. Combine the organic layers and wash with 2 quantities, each of 20 ml, of *water R*. Dry over *anhydrous sodium sulphate R* and filter.

Test solution (d) Transfer 25 ml of the hydro-alcoholic solution obtained in the preparation of test solution (a) to a 200 ml flask that can be fitted with a reflux condenser. Add 20 ml of *hydrochloric acid R* and 10 ml of *anhydrous ethanol R*. Boil under a reflux condenser for 2 h. Allow to cool and shake with 4 quantities, each of 20 ml, of *pentane R*. Combine the organic layers and wash with 2 quantities, each of 20 ml, of *water R*. Dry over *anhydrous sodium sulphate R* and filter.

Reference solution Dissolve 50 mg of *cetyl alcohol CRS* and 50 mg of *stearyl alcohol CRS* in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Column:

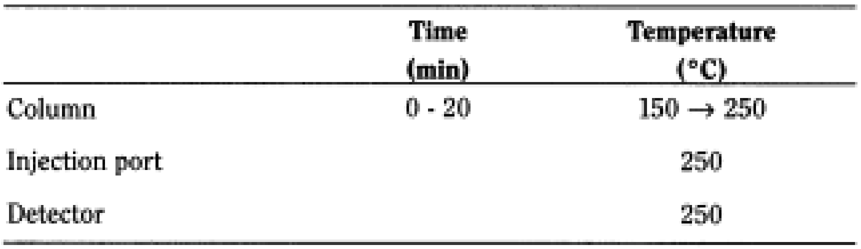
- *material*: fused silica;
- *size:* l = 25 m, Ø = 0.25 mm;
- *stationary phase*: *poly(dimethyl)siloxane R* or another suitable polar phase.

Carrier gas *nitrogen for chromatography R*.

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 20	150 → 250
Injection port		250
Detector		250

Detection Flame ionisation.

Elution order Cetyl alcohol, heptadecanol (internal standard) and stearyl alcohol.

Inject 1 µl of test solution (a) and test solution (b). If the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak due to the internal standard in the chromatogram obtained with test solution (a), calculate the ratio *r* using the following expression:

$$\frac{S_{ci}}{S_i}$$

S_{ci} = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (b),

S_i = area of the peak with the same retention time as the peak due to the internal standard in the chromatogram obtained with test solution (a).

If *r* is less than 300, calculate the corrected area $S_{Ha(corr)}$ of the peak due to the internal standard in the chromatogram obtained with test solution (a) using the following expression:

$$S'_{Ha} - \frac{S_i \times S_c}{S_{ci}}$$

S'_{Ha} = area of the peak due to the internal standard in the chromatogram obtained with test solution (a),

S_c = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (a).

Inject 1 µl of test solution (c) and test solution (d). Carry out the correction for interference in the same manner as for test solution (a) and calculate the corrected area $S_{Hc(corr)}$ of the peak due to the internal standard in the chromatogram obtained with test solution (c).

Inject equal volumes of the reference solution, test solution (c) and test solution (d). Identify the peaks in the chromatograms obtained with the test solutions by comparison of their retention times with those of the peaks in the chromatogram obtained with the reference solution. Determine the area of each peak.

Calculate the percentage content of sodium cetyl sulphate in the substance to be examined, using the following expression:

$$\frac{(A \times 1.421) \times m'_H \times 100}{S_{Hc(corr)} \times m'}$$

A = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (c),

m'_H = mass of the internal standard added in the preparation of test solution (c), in milligrams,

$S_{Hc(corr)}$ = corrected area of the peak due to the internal standard in the chromatogram obtained with test solution (c),

m' = mass of the substance to be examined in test solution (c), in milligrams.

Calculate the percentage content of sodium stearyl sulphate in the substance to be examined, using the following expression:

$$\frac{(B \times 1.377) \times m'_H \times 100}{S_{Hc(corr)} \times m'}$$

B = area of the peak due to stearyl alcohol in the chromatogram obtained with test solution (c).

The percentage content of sodium cetostearyl sulphate corresponds to the sum of the percentage content of sodium cetyl sulphate and the percentage content of sodium stearyl sulphate.

LABELLING

The label states, where appropriate, the name and concentration of any added buffer.

Ph Eur

Sodium Chloride

General Notices

(Ph Eur monograph 0193)

NaCl 58.44 *7647-14-5*

Action and use

Used in treatment of electrolyte deficiency.

Preparations

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution

Oral Rehydration Salts

Potassium Chloride and Sodium Chloride Intravenous Infusion

Potassium Chloride, Sodium Chloride and Glucose Intravenous Infusion

Sodium Chloride Eye Drops

Sodium Chloride Eye Lotion

Sodium Chloride Intravenous Infusion

Sodium Chloride and Glucose Intravenous Infusion

Sodium Chloride Irrigation Solution

Compound Sodium Chloride Mouthwash

Sodium Chloride Oral Solution

Sodium Chloride Solution

Sodium Chloride Tablets

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals or white or almost white pearls.

Solubility

Freely soluble in water, practically insoluble in anhydrous ethanol.

IDENTIFICATION

A. It gives the reactions of chlorides *(2.3.1)*.

B. It gives the reactions of sodium *(2.3.1)*.

TESTS

If the substance is in the form of pearls crush before use.

Solution S

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 20 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides

Maximum 100 ppm.

To 0.5 ml of solution S add 4.0 ml of *water R*, 2.0 ml of *phenol red solution R2* and 1.0 ml of a 0.1 g/l solution of *chloramine R* and mix immediately. After exactly 2 min, add 0.15 ml of 0*.1 M sodium thiosulphate*, mix and dilute to 10.0 ml with *water R*. The absorbance *(2.2.25)* of the solution measured at 590 nm, using *water R* as the compensation liquid, is not greater than that of a standard prepared at the same time and in the same manner, using 5.0 ml of a 3.0 mg/l solution of *potassium bromide R*.

Ferrocyanides

Dissolve 2.0 g in 6 ml of *water R*. Add 0.5 ml of a mixture of 5 ml of a 10 g/l solution of *ferric ammonium sulphate R* in a 2.5 g/l solution of *sulphuric acid R* and 95 ml of a 10 g/l solution of *ferrous sulphate R*. No blue colour develops within 10 min.

Iodides

Moisten 5 g by the dropwise addition of a freshly prepared mixture of 0.15 ml of *sodium nitrite solution R*, 2 ml of *0.5 M sulphuric acid*, 25 ml of *iodide-free starch solution R* and 25 ml of *water R*. After 5 min, examine in daylight. The mixture shows no blue colour.

Nitrites

To 10 ml of solution S add 10 ml of *water R*. The absorbance *(2.2.25)* is not greater than 0.01 at 354 nm.

Phosphates *(2.4.11)*

Maximum 25 ppm.

Dilute 2 ml of solution S to 100 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 200 ppm.

Dilute 7.5 ml of solution S to 30 ml with *distilled water R*.

Aluminium ***(2.4.17)***

Maximum 0.2 ppm, if intended for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions or haemofiltration solutions.

Prescribed solution Dissolve 20.0 g in 100 ml of *water R* and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Arsenic ***(2.4.2, Method A)***

Maximum 1 ppm, determined on 5 ml of solution S.

Barium

To 5 ml of solution S add 5 ml of *distilled water R* and 2 ml of *dilute sulphuric acid R*. After 2 h, any opalescence in the solution is not more intense than that in a mixture of 5 ml of solution S and 7 ml of *distilled water R*.

Iron ***(2.4.9)***

Maximum 2 ppm, determined on solution S.

Prepare the standard using a mixture of 4 ml of *iron standard solution (1 ppm Fe) R* and 6 ml of *water R*.

Magnesium and alkaline-earth metals ***(2.4.7)***

Maximum 100 ppm, calculated as Ca and determined on 10.0 g.

Use 150 mg of *mordant black 11 triturate R*. The volume of *0.01 M sodium edetate* used is not more than 2.5 ml.

Potassium: maximum 5.00×10^2 ppm, if intended for use in the manufacture of parenteral dosage forms or haemodialysis, haemofiltration or peritoneal dialysis solutions.

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 ml with the same solvent.

Reference solutions Dissolve 1.144 g of *potassium chloride R*, previously dried at 100-105 °C for 3 h, in *water R* and dilute to 1000.0 ml with the same solvent (600 µg of K per millilitre). Dilute as required.

Wavelength 766.5 nm.

Heavy metals ***(2.4.8)***

Maximum 5 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Bacterial endotoxins *(2.6.14)*

Less than 5 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Dissolve 50.0 mg in *water R* and dilute to 50 ml with the same solvent. Titrate with *0.1 M silver nitrate* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

LABELLING

The label states:

— where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms;

— where applicable, that the substance is suitable for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions or haemofiltration solutions.

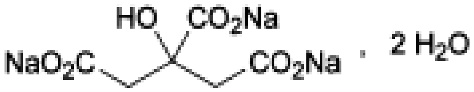
Ph Eur

Sodium Citrate

General Notices

Trisodium Citrate

(Ph Eur monograph 0412)



$C_6H_5Na_3O_7,2H_2O$ 294.1 *6132-04-3*

Action and use

Systemic alkalinising substance.

Preparations

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution

Sodium Citrate Eye Drops

Sodium Citrate Irrigation Solution

Ph Eur

DEFINITION

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or white or almost white, granular crystals, slightly deliquescent in moist air.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. To 1 ml of solution S (see Tests) add 4 ml of *water R*. The solution gives the reaction of citrates *(2.3.1)*.

B. 1 ml of solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Readily carbonisable substances

To 0.20 g of the powdered substance to be examined add 10 ml of *sulphuric acid R* and heat in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly. The solution is not more intensely coloured than reference solution Y_2 or GY_2 *(2.2.2, Method II)*.

Chlorides *(2.4.4)*

Maximum 50 ppm.

Dilute 10 ml of solution S to 15 ml with *water R.*

Oxalates

Maximum 300 ppm.

Dissolve 0.50 g in 4 ml of *water R*, add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules and heat on a water-bath for 1 min. Allow to stand for 2 min, decant the liquid into a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 4 ml of a 50 mg/l solution of *oxalic acid R*.

Sulphates *(2.4.13)*

Maximum 150 ppm.

To 10 ml of solution S add 2 ml of *hydrochloric acid R1* and dilute to 15 ml with *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

11.0 per cent to 13.0 per cent, determined on 0.300 g. After adding the substance to be examined, stir for 15 min before titrating.

Pyrogens ***(2.6.8)***

If intended for use in the manufacture of large-volume parenteral dosage forms, the competent authority may require that it comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 ml of a freshly prepared solution in *water for injections R* containing per millilitre 10.0 mg of the substance to be examined and 7.5 mg of pyrogen-free *calcium chloride R*.

ASSAY

Dissolve 0.150 g in 20 ml of *anhydrous acetic acid R*, heating to about 50 °C. Allow to cool. Titrate with *0.1 M perchloric acid*, using 0.25 ml of *naphtholbenzein solution R* as indicator until a green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 8.602 mg of $C_6H_5Na_3O_7$.

STORAGE

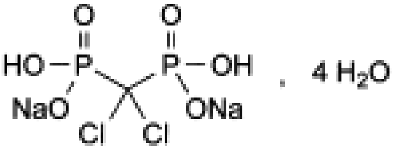
In an airtight container .

Ph Eur

Sodium Clodronate Tetrahydrate

General Notices

(Clodronate Disodium Tetrahydrate, Ph Eur monograph 1777)



$CH_2Cl_2Na_2O_6P_2,4H_2O$ 360.9 *22560-50-5*

Ph Eur

DEFINITION

Disodium (dichloromethylene)bis(hydrogen phosphonate) tetrahydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent), slightly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison clodronate disodium tetrahydrate CRS.

B. Dissolve 0.5 g in 10 ml of *water R*. The solution gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH ***(2.2.3)***

3.0 to 4.5, for solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.125 g of the substance to be examined in 30 ml of *water R*, sonicate for 10 min and dilute to 50.0 ml with *water R* (test stock solution). Dilute 10.0 ml of the test stock solution to 20.0 ml of *water R*.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with *water R*. Dilute 1.0 ml of this solution to 50.0 ml with *water R*.

Reference solution (b) Dissolve 1 mg of *clodronate impurity D CRS* in 10 ml of *water R*, sonicate for 10 min and dilute to 20.0 ml with *water R*. Mix 2.0 ml of this solution with 10.0 ml of the test stock solution and dilute to 20.0 ml with *water R*.

Reference solution (c) Dilute 1.0 ml of a 0.3 g/l solution of *phosphoric acid R* (impurity B) to 100.0 ml with *water R*.

Precolumn:

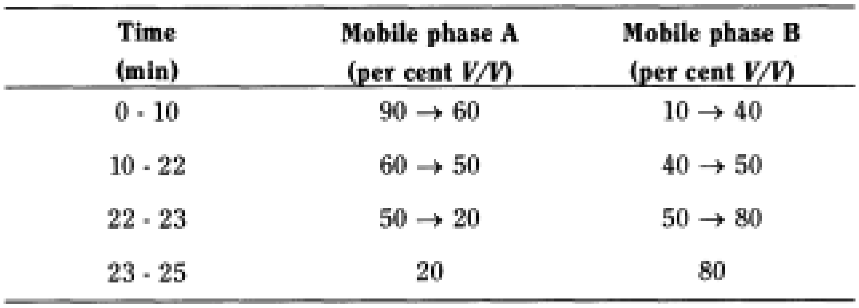
- *size: l* = 0.05 m, Ø = 4 mm;
- *stationary phase: anion exchange resin R*;
- *particle size*: 9 µm.

Column:

- *size: l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: *anion exchange resin R*;
- *particle size*: 9 µm.

Mobile phase:

- *mobile phase A*: 0.21 g/l solution of *sodium hydroxide R* in *carbon dioxide-free water R*; close immediately, mix and use under helium pressure;
- *mobile phase B*: 4.2 g/l solution of *sodium hydroxide R* in *carbon dioxide-free water R*; close immediately, mix and use under helium pressure;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	90 → 60	10 → 40
10 - 22	60 → 50	40 → 50
22 - 23	50 → 20	50 → 80
23 - 25	20	80

Flow rate 1 ml/min.

Detection Conductivity detector. Use a self-regenerating anion suppressor.

Injection 20 µl.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B.

Relative retention With reference to clodronate (retention time = about 13 min): impurities A and B = about 0.7; impurity D = about 1.1.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to clodronate.

Limits:

— *sum of impurities A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

0.5 g complies with test G. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

18.5 per cent to 21.0 per cent, determined on 0.100 g.

ASSAY

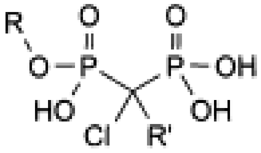
Dissolve 0.140 g in 10 ml of *water R*. Add 10 ml of *concentrated sodium hydroxide solution R* and some glass beads. Boil until the solution is completely decolourised (about 10 min). Cool in an ice-bath and add 30 ml of *water R* and 10 ml of *nitric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M silver nitrate* is equivalent to 14.44 mg of $CH_2Cl_2Na_2O_6P_2$.

IMPURITIES

Specified impurities *A, B.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *D*.



A. R = $CH(CH_3)_2$, R' = Cl: [dichloro[hydroxy(1-methylethoxy)phosphinoyl]methyl]phosphonic acid,

D. R = R' = H: (chloromethylene)bis(phosphonic acid),

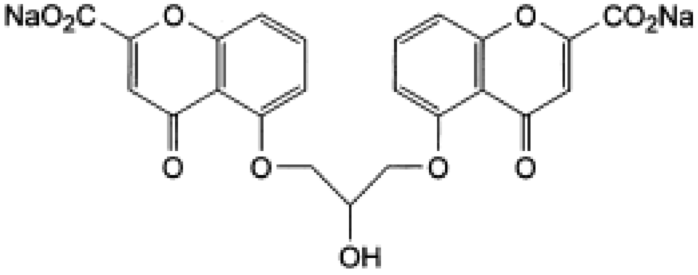
B. phosphoric acid.

Ph Eur

Sodium Cromoglicate

General Notices

(Ph Eur monograph 0562)



$C_{23}H_{14}Na_2O_{11}$ 512.3 *15826-37-6*

Action and use

Prophylaxis of allergic conditions.

Preparations

Sodium Cromoglicate Eye Drops

Sodium Cromoglicate Powder for Inhalation

Ph Eur

DEFINITION

Sodium cromoglicate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of disodium 5,5′-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(4-oxo-4*H*-1-benzopyran-2-carboxylate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, hygroscopic, soluble in water, practically insoluble in alcohol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Dissolve 10.0 mg in *phosphate buffer solution pH 7.4 R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with the same solvent. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows two absorption maxima, at 239 nm and 327 nm. The ratio of the absorbance at the maximum at 327 nm to that at the maximum at 239 nm is 0.25 to 0.30.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *sodium cromoglicate CRS*. Examine the substances prepared as discs.

C. Dissolve about 5 mg in 0.5 ml of *methanol R*. Add 3 ml of a solution in *methanol R* containing 5 g/l of *aminopyrazolone R* and 1 per cent *V/V* of *hydrochloric acid R*. Allow to stand for 5 min. The solution shows an intense yellow colour.

D. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Add 0.2 ml of *0.01 M sodium hydroxide*. The solution is pink. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.25 ml of *methyl red solution R*. The solution is red.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 1 volume of *acetone R*, 4 volumes of *tetrahydrofuran R* and 6 volumes of *water R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 10 mg of *1,3-bis(2-acetyl-3-hydroxyphenoxy)-2-propanol CRS* in *chloroform R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 5 volumes of *glacial acetic acid R*, 50 volumes of *ethyl acetate R* and 50 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot (which remains at the starting point), is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

OXALATE

Dissolve 0.10 g in 20 ml of *water R*, add 5.0 ml of *iron salicylate solution R* and dilute to 50.0 ml with *water R*. Determine the absorbance (*2.2.25*) at 480 nm. The absorbance is not less than that of a standard prepared in the same manner using 0.35 mg of *oxalic acid R* instead of the substance to be examined.

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of

lead standard solution (10 ppm Pb) R.

Loss on drying *(2.2.32)*

Not more than 10.0 per cent, determined on 1.000 g by drying over *diphosphorus pentoxide R* at 105 °C and at a pressure of 300 Pa to 600 Pa.

ASSAY

Dissolve 0.200 g with heating in a mixture of 5 ml of *2-propanol R* and 25 ml of *ethylene glycol R*. Cool and add 30 ml of *dioxan R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 25.62 mg of $C_{23}H_{14}Na_2O_{11}$.

STORAGE

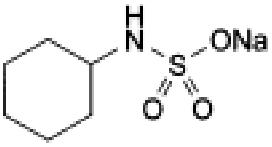
Store in an airtight container, protected from light.

Ph Eur

Sodium Cyclamate

General Notices

(Ph Eur monograph 0774)



$C_6H_{12}NNaO_3S$ 201.2 *139-05-9*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

Sodium *N*-cyclohexylsulphamate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A*, E.

Second identification *B*, C, D, E.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Sodium cyclamate CRS*.

B. Examine the chromatograms obtained in the test for impurity A.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in

position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 1 ml of solution S (see Tests), add 1 ml of *water R* and 2 ml of *silver nitrate solution R1*, then shake. A white, crystalline precipitate is formed.

D. To 1 ml of solution S add 5 ml of *water R*, 2 ml of *dilute hydrochloric acid R* and 4 ml of *barium chloride solution R1* and mix. The solution is clear. Add 2 ml of *sodium nitrite solution R*. A voluminous white precipitate is formed and gas is given off.

E. A mixture of 1 ml of solution S and 1 ml of *water R* gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.5 to 7.5 for solution S.

Absorbance *(2.2.25)*

Maximum 0.10, determined at 270 nm on solution S.

Impurity A

Thin-layer chromatography *(2.2.27)*.

Test solution (a) Solution S.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *water R*.

Reference solution (a) Dissolve 0.10 g of *sodium cyclamate CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *sulphamic acid R* (impurity A) in *water R* and dilute to 100 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *Concentrated ammonia R*, *water R*, *ethyl acetate R*, *propanol R* (10:10:20:70 *V/V/V/V*).

Application 2 µl.

Development Over a path of 12 cm.

Drying In a current of warm air, then heat at 105 C for 5 min.

Detection Spray the hot plate with *strong sodium hypochlorite solution R* diluted to a concentration of 5 g/l of active chlorine. Place in a current of cold air until an area of coating below the points of application gives at most a faint blue colour with a drop of *potassium iodide and starch solution R*; avoid prolonged exposure to cold air. Spray with *potassium iodide and starch solution R* and examine the chromatograms within 5 min.

Limit Test solution (a):

— *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Impurities B, C and D

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 2 µl of *tetradecane R* in *methylene chloride R* and dilute to 100 ml with the same solvent.

Test solution Dissolve 2.00 g of the substance to be examined in 20 ml of *water R*, add 0.5 ml of *strong sodium hydroxide solution R* and shake with 30 ml of *toluene R*. Shake 20 ml of the upper layer with 4 ml of a mixture of equal volumes of *dilute acetic acid R* and *water R*. Separate the lower layer, add 0.5 ml of *strong sodium hydroxide solution R* and 0.5 ml of the internal standard solution and shake. Use the lower layer immediately after separation.

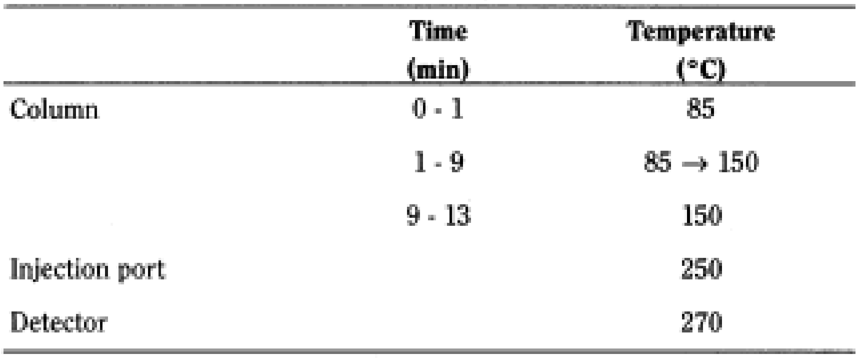
Reference solution Dissolve 10.0 mg (about 12 µl) of *cyclohexylamine R* (impurity C), 1.0 mg (about 1.1 µl) of *dicyclohexylamine R* (impurity D) and 1.0 mg (about 1 µl) of *aniline R* (impurity B) in *water R*, then dilute to 1000 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water R* (solution A). To 20.0 ml of solution A, add 0.5 ml of *strong sodium hydroxide solution R* and extract with 30 ml of *toluene R*. Shake 20 ml of the upper layer with 4 ml of a mixture of equal volumes of *dilute acetic acid R* and *water R*. Separate the lower layer, add 0.5 ml of *strong sodium hydroxide solution R* and 0.5 ml of the internal standard solution and shake. Use the lower layer immediately after separation.

Column:
- *material*: fused silica;
- *size*: *l* = 25 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.51 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.8 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	85
	1 - 9	85 → 150
	9 - 13	150
Injection port		250
Detector		270

Detection Flame ionisation.

Injection 1.5 µl; use a split vent at a flow rate of 20 ml/min.

Relative retention With reference to impurity C (retention time = about 2.3 min): impurity B =

about 1.4; tetradecane = about 4.3; impurity D = about 4.5.

Limits:

- — *impurity C*: maximum 10 ppm;
- — *impurities B, D*: for each impurity, maximum 1 ppm.

Sulphates ***(2.4.13)***

Maximum 0.1 per cent.

Dilute 1.5 ml of solution S to 15 ml with *distilled water R*.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

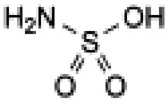
ASSAY

Dissolve without heating 0.150 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

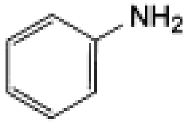
1 ml of *0.1 M perchloric acid* is equivalent to 20.12 mg of $C_6H_{12}NNaO_3S$.

IMPURITIES

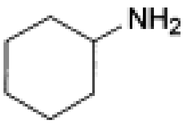
Specified impurities *A, B, C, D*.



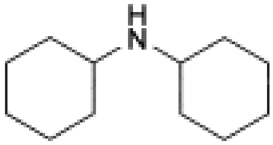
A. sulphamic acid,



B. aniline (phenylamine),



C. cyclohexanamine,



D. *N*-cyclohexylcyclohexanamine.

Ph Eur

Sodium Dihydrogen Phosphate Dihydrate

General Notices

Sodium Acid Phosphate

(Ph Eur monograph 0194)

$NaH_2PO_4,2H_2O$ 156.0 *13472-35-0*

Preparation

Phosphates Enema

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or colourless crystals.

Solubility

Very soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly acid *(2.2.4)*.

B. Solution S gives the reactions of phosphates *(2.3.1)*.

C. Solution S previously neutralised using a 100 g/l solution of *potassium hydroxide R* gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.2 to 4.5.

To 5 ml of solution S add 5 ml of *carbon dioxide-free water R*.

Reducing substances

To 5 ml of solution S add 0.25 ml of *0.02 M potassium permanganate* and 5 ml of *dilute sulphuric acid R* and heat in a water-bath for 5 min. The solution retains a slight red colour.

Chlorides ***(2.4.4)***

Maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates ***(2.4.13)***

Maximum 300 ppm.

To 5 ml of solution S add 0.5 ml of *hydrochloric acid R* and dilute to 15 ml with *distilled water R*.

Arsenic ***(2.4.2, Method A)***

Maximum 2 ppm, determined on 0.5 g.

Iron ***(2.4.9)***

Maximum 10 ppm, determined on solution S.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

21.5 per cent to 24.0 per cent, determined on 0.50 g by drying in an oven at 130 °C.

ASSAY

Dissolve 2.500 g in 40 ml of *water R*. Titrate with carbonate-free *1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *1 M sodium hydroxide* is equivalent to 0.120 g of NaH_2PO_4.

Ph Eur

Sodium Dihydrogen Phosphate Monohydrate

General Notices

NaH_2PO_4,H_2O 138.0 *10049-21-5*

Action and use

Excipient.

Preparation

Phosphates enema

DEFINITION

Sodium Dihydrogen Phosphate Monohydrate contains not less than 98.0% and not more than 100.5% of NaH_2PO_4, calculated with reference to the dried substance.

CHARACTERISTICS

Colourless crystals or a white powder.

Very soluble in *water*; very slightly soluble in *ethanol (96%)*.

Dissolve 10.0 g in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 100 ml (solution S).

IDENTIFICATION

A. Solution S is *faintly acid ,* Appendix V K.

B. Solution S yields the reactions characteristic of *phosphates*, Appendix VI.

C. Solution S neutralised with a 10% w/v solution of *potassium hydroxide* yields reaction A characteristic of *sodium salts*, Appendix VI.

D. Complies with the test for Loss on drying.

TESTS

Acidity

pH of a mixture of 5 ml of solution S and 5 ml of *carbon dioxide-free water* , 4.2 to 4.5, Appendix V L.

Clarity and colour of solution

Solution S is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Arsenic

0.5 g complies with the *limit test for arsenic*, Appendix VII (2 ppm).

Heavy metals

12 ml of solution S complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (10 ppm).

Iron

10 ml of solution S complies with the *limit test for iron*, Appendix VII (10 ppm).

Chloride

Dilute 2.5 ml of solution S to 15 ml with *water*. The resulting solution complies with the *limit test for chlorides*, Appendix VII (200 ppm).

Sulphate

To 5 ml of solution S add 0.5 ml of *hydrochloric acid* and dilute to 15 ml with *distilled water*. The resulting solution complies with the *limit test for sulphates*, Appendix VII (300 ppm).

Reducing substances

To 5 ml of solution S add 0.25 ml of 0.02M *potassium permanganate* and 5 ml of 1M *sulphuric acid* and heat in a water bath for 5 minutes. The solution retains a slight red colour.

Loss on drying

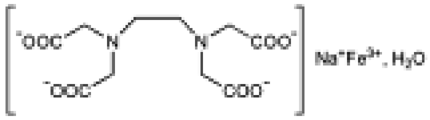
When dried to constant weight at 130°, loses 11.5 to 14.5% of its weight. Use 0.5 g.

ASSAY

Dissolve 2.2 g in 50 ml of *water* and titrate with carbonate-free 1M *sodium hydroxide VS* determining the end point potentiometrically. Each ml of 1M *sodium hydroxide VS* is equivalent to 0.120 g of NaH_2PO_4.

Sodium Feredetate

General Notices



$C_{10}H_{21}N_2O_8FeNa,H_2O$ 385.1 *15708-41-5* (anhydrous)

Action and use

Source of iron.

Preparation

Sodium Feredetate Oral Solution

DEFINITION

Sodium Feredetate is iron(III) sodium ethylenediaminetetra-acetate monohydrate. It contains not less than 98.0% and not more than 102.0% of $C_{10}H_{12}O_8N_2FeNa$, calculated with reference to the dried substance.

CHARACTERISTICS

A yellow or yellowish brown, crystalline powder; hygroscopic.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of sodium feredetate *(RS 378)*.

B. Ignite 0.5 g and allow to cool. Dissolve the residue in 2 ml of *hydrochloric acid*, add sufficient *water* to produce 20 ml and filter. The filtrate yields reaction C characteristic of *iron salts*, Appendix VI. Dilute 1 volume of the filtrate to 10 volumes with *water*; the solution yields reaction B characteristic of *iron salts*, Appendix VI.

C. Dissolve 2 g in 30 ml of *water*, slowly add 6.5 ml of a 20% w/v solution of *potassium hydroxide*, shake and filter the resulting suspension. Evaporate 8 ml of the filtrate to dryness, ignite and dissolve the residue in 4 ml of *water*. The solution yields reaction B characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 4.0 to 6.5, Appendix V L.

Free iron

Not more than 500 ppm when determined by the following method. Dissolve 0.2 g in sufficient *water* to produce 20 ml and filter. Label three tubes A, B and C. Place 5 ml of the filtrate into each of tubes A and B and 4 ml of *water* and 1 ml of an iron standard solution prepared in the following manner in tube C. For the iron standard solution dilute 25 volumes of a 0.1726% w/v solution of *ammonium iron(III) sulphate* in 0.05M *sulphuric acid* to 200 volumes with *water* (25 ppm of Fe(III)). Add 1 ml of a 1.0% w/v solution of *disodium catechol-3,5-disulphonate* into each of tubes A and C and 1 ml of *water* to tube B. Measure the *absorbance* of solution A at 670 nm, Appendix II B, using solution B in the reference cell and of solution C using *water* in the reference cell. The absorbance of solution A is not greater than that of solution C.

Heavy metals

1 g complies with *limit test D for heavy metals*, Appendix VII (20 ppm). Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard solution.

Free sodium edetate

To 4 ml of a 1.0% w/v solution add 2 ml of *ferric iron standard solution (50 ppm)* and 1 ml of a 1.0% w/v solution of *disodium catechol-3,5-disulphonate* and mix. Prepare a standard in the same manner using 4 ml of a 0.010% w/v solution of *disodium edetate* in place of the solution of the substance being examined. Measure the *absorbance* of the solutions at 670 nm, Appendix II B, using *water* in the reference cell. The absorbance of the solution of the substance being examined is not less than that of the standard solution (1%).

Sulphates

Dissolve 2 g in 40 ml of *water*, add 5 ml of a 20% w/v solution of *sodium hydroxide* and sufficient *water* to produce 50 ml, shake and filter. Evaporate 6.2 ml of this solution to dryness and ignite until no trace of carbon remains. Cool and dissolve the residue in 10 ml of *distilled water*. Neutralise the solution with 2M *hydrochloric acid* using *litmus paper* as external indicator and add 2 ml in excess. Boil the solution, cool, dilute to 15 ml with *distilled water* and filter. The filtrate complies with the *limit test for sulphates*, Appendix VII (600 ppm).

Loss on drying

When dried over silica gel at 120° at a pressure not exceeding 2 kPa for 5 hours, loses 4.0 to 13.5% of its weight. Use 1 g.

ASSAY

Dissolve 1 g in 40 ml of *water* in an iodine flask, add 20 ml of *hydrochloric acid* and 3 g of *potassium iodide,* close the flask and allow to stand for 5 minutes. Titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 36.71 mg of $C_{10}H_{12}O_8N_2FeNa$.

STORAGE

Sodium Feredetate should be kept in an airtight container.

Sodium Fluoride

General Notices

(Ph Eur monograph 0514)

NaF 41.99 *7681-49-4*

Action and use

Used in prevention of dental caries.

Preparations

Sodium Fluoride Mouthwash

Sodium Fluoride Oral Drops

Sodium Fluoride Oral Solution

Sodium Fluoride Tablets

Ph Eur

DEFINITION

Content

98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or colourless crystals.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. To 2 ml of solution S (see Tests) add 0.5 ml of *calcium chloride solution R*. A gelatinous white precipitate is formed that dissolves on adding 5 ml of *ferric chloride solution R1*.

B. To about 4 mg add a mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R* and mix. The colour changes from red to yellow.

C. Solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* without heating and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Dissolve 2.5 g of *potassium nitrate R* in 40 ml of solution S and dilute to 50 ml with *carbon dioxide-free water R*. Cool to 0 °C and add 0.2 ml of *phenolphthalein solution R*. If the solution is colourless, not more than 1.0 ml of *0.1 M sodium hydroxide* is required to produce a red colour that persists for at least 15 s. If the solution is red, not more than 0.25 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Chlorides *(2.4.4)*

Maximum 200 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Fluorosilicates

Heat to boiling the neutralised solution obtained in the test for acidity or alkalinity and titrate whilst hot. Not more than 0.75 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to red.

Sulphates *(2.4.13)*

Maximum 200 ppm.

Dissolve 0.25 g in 10 ml of a saturated solution of *boric acid R* in *distilled water R*. Add 5 ml of *distilled water R* and 0.6 ml of *hydrochloric acid R1*. Prepare the standard by mixing 0.6 ml of *hydrochloric acid R1*, 5 ml of *sulphate standard solution (10 ppm SO_4) R* and 10 ml of a saturated solution of *boric acid R* in *distilled water R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 130 °C for 3 h.

ASSAY

Dissolve 0.100 g in *water R* and dilute to 60 ml with the same solvent. Titrate with *0.1 M lanthanum nitrate*, determining the end-point potentiometrically *(2.2.20)* using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode.

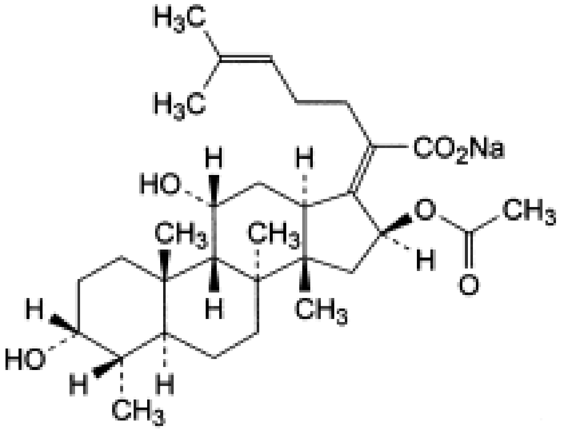
1 ml of *0.1 M lanthanum nitrate* is equivalent to 12.60 mg of NaF.

Ph Eur

Sodium Fusidate

General Notices

(Ph Eur monograph 0848)



$C_{31}H_{47}NaO_6$ 538.7 *751-94-0*

Action and use

Antibacterial.

Preparation

Sodium Fusidate Ointment

Ph Eur

DEFINITION

Sodium (*Z*)-*ent*-16α-(acetyloxy)-3β,11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oate.

Antimicrobial substance produced by the growth of certain strains of *Fusidium coccineum* or by any other means.

Content

97.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, slightly hygroscopic.

Solubility

Freely soluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 0.1 g in 5 ml of *water R*, add 5 ml of *chloroform R* and 0.1 ml of *dilute phosphoric acid R*. Shake vigorously for 1 min, allow to separate and filter the lower layer through absorbent cotton covered with *anhydrous sodium sulphate R*. Repeat the extraction with 2 quantities, each of 5 ml, of *chloroform R* and evaporate the combined extracts to dryness under reduced pressure. Dry the residue over *diphosphorus pentoxide R* under reduced pressure for 2 h, dissolve in 1 ml of *chloroform R*.

Comparison *Ph. Eur. reference spectrum of fusidic acid*.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 24 mg of *diethanolamine fusidate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *Methanol R*, *cyclohexane R*, *glacial acetic acid R*, *chloroform R* (2.5:10:10:80 *V/V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Ignite 1 g. The residue gives reaction (a) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

Dissolve 1.5 g in 10 ml of *water R*.

pH (2.2.3)

7.5 to 9.0.

Dissolve 0.125 g in 10 ml of *carbon dioxide-free water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *3-ketofusidic acid CRS* in 5 ml of the mobile phase. To 1.0 ml of this solution add 0.20 ml of the test solution and dilute to 20.0 ml with the mobile phase.

Reference solution (b) Dilute 20 µl of the test solution to 100.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.125-0.15 m, Ø = 4-5 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *Methanol R*, 10 g/l solution of *phosphoric acid R*, *water R*, *acetonitrile R* (10:20:20:50 *V/V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl.

Run time 3.5 times the retention time of sodium fusidate.

System suitability:

— *resolution:* minimum 2.5 between the peaks due to 3-ketofusidic acid and sodium fusidate in the chromatogram obtained with reference solution (a);

— *signal-to-noise* ratio: minimum 3 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *total*: not more than twice the area of the peak due to sodium fusidate in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.50 g.

ASSAY

Dissolve 0.500 g in 30 ml of *water R* and add 40 ml of *ethanol (96 per cent) R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M hydrochloric acid* is equivalent to 53.87 mg of $C_{31}H_{47}NaO_6$.

STORAGE

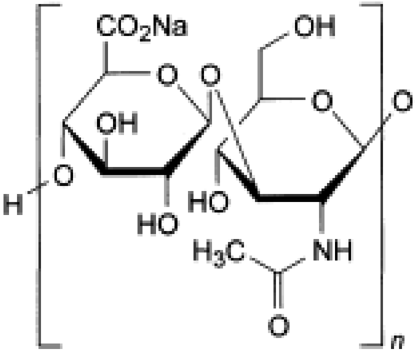
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Sodium Hyaluronate

General Notices

(Ph Eur monograph 1472)



$(C_{14}H_{20}NNaO_{11})_n$ *9067-32-7*

Action and use

High viscosity mucopolysaccharide.

Ph Eur

DEFINITION

Sodium salt of hyaluronic acid, a glycosaminoglycan consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine disaccharide units.

Content

95.0 per cent to 105.0 per cent (dried substance).

Intrinsic viscosity

90 per cent to 120 per cent of the value stated on the label.

PRODUCTION

It is extracted from cocks' combs or obtained by fermentation from *Streptococci*, Lancefield Groups A and C. When produced by fermentation of gram-positive bacteria, the process must be shown to reduce or eliminate pyrogenic or inflammatory components of the cell wall.

CHARACTERS

Appearance

White or almost white, very hygroscopic powder or fibrous aggregate.

Solubility

Sparingly soluble or soluble in water, practically insoluble in acetone and in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison Ph. Eur. reference spectrum of sodium hyaluronate.

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Weigh a quantity of the substance to be examined equivalent to 0.10 g of the dried substance and add 30.0 ml of a 9 g/l solution of *sodium chloride R*. Mix gently on a shaker until dissolved (about 12 h).

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 600 nm is not greater than 0.01.

pH *(2.2.3)*

5.0 to 8.5.

Dissolve the substance to be examined in *carbon dioxide-free water R* to obtain a solution containing a quantity equivalent to 5 mg of the dried substance per millilitre.

Intrinsic viscosity

Sodium hyaluronate is very hygroscopic and must be protected from moisture during weighing.

Buffer solution (*0.15 M sodium chloride in 0.01 M phosphate buffer solution pH 7.0*). Dissolve 0.78 g of *sodium dihydrogen phosphate R* and 4.50 g of *sodium chloride R* in *water R* and dilute to 500.0 ml with the same solvent (solution A). Dissolve 1.79 g of *disodium hydrogen phosphate R* and 4.50 g of *sodium chloride R* in *water R* and dilute to 500.0 ml with the same solvent (solution B). Mix solutions A and B until a pH of 7.0 is reached. Filter through a sintered-glass filter (4) *(2.1.2)*.

Test solution (a) Weigh 0.200 g (m_{0p}) (*NOTE: this value is only indicative and should be adjusted after an initial measurement of the viscosity of test solution (a))* of the substance to be examined and dilute with 50.0 g (m_{0s}) of buffer solution at 4 °C. Mix the solution by shaking at 4 °C during 24 h. Weigh 5.00 g (m_{1p}) of the solution and dilute with 100.0 g (m_{1s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) *(2.1.2)*, and discard the first 10 ml.

Test solution (b) Weigh 30.0 g (m_{2p}) of test solution (a) and dilute with 10.0 g (m_{2s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) *(2.1.2)* and discard the first 10 ml.

Test solution (c) Weigh 20.0 g (m_{3p}) of test solution (a) and dilute with 20.0 g (m_{3s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) *(2.1.2)* and discard the first 10 ml.

Test solution (d) Weigh 10.0 g (m_{4p}) of test solution (a) and dilute with 30.0 g (m_{4s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) *(2.1.2)* and discard the first 10 ml.

Determine the flow-times *(2.2.9)* for the buffer solution (t_0) and for the 4 test solutions (t_1, t_2, t_3 and t_4), at 25.00 ± 0.03 °C. Use an appropriate suspended level viscometer (specifications: viscometer constant about 0.005 mm^2/s^2, kinematic viscosity of 1-5 mm^2/s, internal diameter of tube *R* 0.53 mm, volume of bulb *C* 5.6 ml, internal diameter of tube *N* 2.8-3.2 mm) with a funnel-shaped lower capillary end. Use the same viscometer for all measurements; measure all outflow times in triplicate. The test is not valid unless the results do not differ by more than 0.35 per cent from the mean and if the flow time t_1 is not less than 1.6 and not more than 1.8 times t_0. If this is not the case, adjust the value of m_{0p} and repeat the procedure.

Calculation of the relative viscosities Since the densities of the sodium hyaluronate solutions and of the solvent are almost equal, the relative viscosities η_{ri} (being η_{r1}, η_{r2}, η_{r3} and η_{r4}) can be calculated from the ratio of the flow times for the respective solutions t_i (being t_1, t_2, t_3 and t_4) to the flow time of the solvent t_0, but taking into account the kinetic energy correction factor for the capillary (B = 30 800 s^3), using the following expression:

$$\frac{t_i - \frac{B}{t_i^2}}{t_0 - \frac{B}{t_0^2}}$$

Calculation of the concentrations Calculate the concentration c_1 (expressed in kg/m^3) of sodium hyaluronate in test solution (a) using the following expression:

$$\frac{m_{0p} \times x \times (100 - h) \times m_{1p} \times \rho_{25}}{100 \times 100 \times (m_{0p} + m_{0s}) \times (m_{1p} + m_{1s})}$$

x = percentage content of sodium hyaluronate as determined under Assay;

h = percentage loss on drying;

ρ_{25} = 1005 kg/m^3 (density of the test solution at 25 °C).

Calculate the concentration c_2 (expressed in kg/m^3) of sodium hyaluronate in test solution (b) using the following expression:

$$c_1 \times \frac{m_{2p}}{m_{2s} + m_{2p}}$$

Calculate the concentration c_3 (expressed in kg/m^3) of sodium hyaluronate in test solution (c) using the following expression:

$$c_1 \times \frac{m_{3p}}{m_{3s} + m_{3p}}$$

Calculate the concentration c_4 (expressed in kg/m^3) of sodium hyaluronate in test solution (d) using the following expression:

$$c_1 \times \frac{m_{4p}}{m_{4s} + m_{4p}}$$

Calculation of the intrinsic viscosity Calculate the intrinsic viscosity [η] by linear least-squares regression analysis using the Martin equation:

$$\log\left(\frac{\eta_r - 1}{c}\right) = \log[\eta] + k[\eta]c$$

The decimal antilogarithm of the intercept is the intrinsic viscosity expressed in m^3/kg.

Sulphated glycosaminoglycans

Maximum 1 per cent, if the product is extracted from cocks' combs.

Appropriate safety precautions are to be taken when handling perchloric acid at elevated temperature.

Test solution Introduce a quantity of the substance to be examined equivalent to 50.0 mg of the dried substance into a test-tube 150 mm long and 16 mm in internal diameter and dissolve in 1.0 ml of *perchloric acid R*.

Reference solution Dissolve 0.149 g of *anhydrous sodium sulphate R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water R*. Evaporate 1.0 ml in a test-tube 150 mm long and 16 mm in internal diameter in a heating block at 90-95 °C, and dissolve the residue in 1.0 ml of *perchloric acid R*.

Plug each test-tube with a piece of glass wool. Place the test-tubes in a heating block or a silicone oil bath maintained at 180 °C and heat until clear, colourless solutions are obtained (about 12 h). Remove the test-tubes and cool to room temperature. Add to each test-tube 3.0 ml of a 33.3 g/l solution of *barium chloride R*, cap and shake vigorously. Allow the test-tubes to stand for 30 min. Shake each test-tube once again, and determine the absorbance *(2.2.25)* at 660 nm, using *water R* as a blank.

The absorbance obtained with the test solution is not greater than the absorbance obtained with the reference solution.

Nucleic acids

The absorbance *(2.2.25)* of solution S at 260 nm is maximum 0.5.

Protein

Maximum 0.3 per cent; maximum 0.1 per cent, if intended for use in the manufacture of parenteral preparations.

Test solution (a) Dissolve the substance to be examined in *water R* to obtain a solution containing a quantity equivalent to about 10 mg of the dried substance per millilitre.

Test solution (b) Mix equal volumes of test solution (a) and *water R*.

Reference solutions Prepare a 0.5 mg/ml stock solution of *bovine albumin R* in *water R*. Prepare 5 dilutions of the stock solution containing between 5 µg/ml and 50 µg/ml of *bovine albumin R*.

Add 2.5 ml of freshly prepared *cupri-tartaric solution R3* to test-tubes containing 2.5 ml of *water R* (blank), 2.5 ml of the test solutions (a) or (b) or 2.5 ml of the reference solutions. Mix after each addition. After about 10 min, add to each test-tube 0.50 ml of a mixture of equal volumes of *phosphomolybdotungstic reagent R* and *water R* prepared immediately before use. Mix after each addition. After 30 min, measure the absorbance *(2.2.25)* of each solution at 750 nm against the blank. From the calibration curve obtained with the 5 reference solutions determine the content of protein in the test solutions.

Chlorides *(2.4.4)*

Maximum 0.5 per cent.

Dissolve 67 mg in 100 ml of *water R*.

Iron

Maximum 80.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve a quantity of the substance to be examined equivalent to 0.25 g of the dried substance in 1 ml of *nitric acid R* by heating on a water-bath. Cool and dilute to 10.0 ml with *water R*.

Reference solutions Prepare 2 reference solutions in the same manner as the test solution, adding 1.0 ml and 2.0 ml respectively of *iron standard solution (10 ppm Fe) R* to the dissolved substance to be examined.

Source Iron hollow-cathode lamp using a transmission band of 0.2 nm.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Heavy metals *(2.4.8)*

Maximum 20 ppm; maximum 10 ppm if intended for use in the manufacture of parenteral preparations.

1.0 g complies with test F. Prepare the reference solution using 2.0 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 20.0 per cent, determined on 0.500 g by drying at 100-110 °C over *diphosphorus pentoxide R* for 6 h.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^2 micro-organisms per gram. Use 1 g of the substance to be examined.

Bacterial endotoxins ***(2.6.14)***

Less than 0.5 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins; less than 0.05 IU/mg, if intended for use in the manufacture of intra-ocular preparations or intra-articular preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Determine the glucuronic acid content by reaction with carbazole as described below.

Reagent A Dissolve 0.95 g of *disodium tetraborate R* in 100.0 ml of *sulphuric acid R*.

Reagent B Dissolve 0.125 g of *carbazole R* in 100.0 ml of *anhydrous ethanol R*.

Test solution Prepare this solution in triplicate. Dissolve 0.170 g of the substance to be examined in *water R* and dilute to 100.0 g with the same solvent. Dilute 10.0 g of this solution to 200.0 g with *water R*.

Reference stock solution Dissolve 0.100 g of *D-glucuronic acid R*, previously dried to constant mass in vacuum over *diphosphorus pentoxide R (2.2.32)*, in *water R* and dilute to 100.0 g with the same solvent.

Reference solutions Prepare 5 dilutions of the reference stock solution containing between 6.5 µg/g and 65 µg/g of *D-glucuronic acid R*.

Place 25 test-tubes, numbered 1 to 25, in iced water. Add 1.0 ml of the 5 reference solutions in triplicate to the test-tubes 1 to 15 (reference tubes), 1.0 ml of the 3 test solutions in triplicate to the test-tubes 16 to 24 (sample tubes), and 1.0 ml of *water R* to test-tube 25 (blank). Add to each test-tube 5.0 ml of freshly prepared reagent A, previously cooled in iced water. Tightly close the test-tubes with plastic caps, shake the contents, and place on a water bath for exactly 15 min. Cool in iced water, and add to each test tube 0.20 ml of reagent B. Recap the tubes, shake, and put them again on a water-bath for exactly 15 min. Cool to room temperature and measure the absorbance *(2.2.25)* of the solutions at 530 nm, against the blank.

From the calibration curve obtained with the mean absorbances read for each reference solution, determine the mean concentrations of D-glucuronic acid in the test solutions.

Calculate the percentage content of sodium hyaluronate using the following expression:

$$\frac{c_g}{c_s} \times Z \times \frac{100}{100 - h} \times \frac{401.3}{194.1}$$

c_g = mean of concentrations of D-glucuronic acid in the test solutions, in milligrams per gram;

c_s = mean of concentrations of the substance to be examined in the test solutions, in milligrams per gram;

Z = determined percentage content of $C_6H_{10}O_7$ in *D-glucuronic acid R*;

h = percentage loss on drying;

401.3 = relative molecular mass of the disaccharide fragment;

194.1 = relative molecular mass of glucuronic acid.

STORAGE

In an airtight container, protected from light and humidity. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

- the intrinsic viscosity;
- the origin of the substance;
- the intended use of the substance;
- where applicable, that the substance is suitable for parenteral administration other than intra-articular administration;
- where applicable, that the substance is suitable for parenteral administration, including intra-articular administration;
- where applicable that the material is suitable for intra-ocular use.

Ph Eur

Sodium Hydroxide

General Notices

Caustic Soda

(Ph Eur monograph 0677)

NaOH 40.00 *1310-73-2*

Ph Eur

DEFINITION

Content

97.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline masses, supplied as pellets, sticks or slabs, deliquescent, readily absorbing carbon dioxide.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. pH *(2.2.3)*: minimum 11.0.

Dissolve 0.1 g in 10 ml of *water R*. Dilute 1 ml of the solution to 100 ml with *water R*.

B. 2 ml of solution S (see Tests) gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Carry out the procedure described below with caution. Dissolve 5.0 g in 12 ml of *distilled water R*. Add 17 ml of *hydrochloric acid R1*, adjust to pH 7 with *1 M hydrochloric acid* and dilute to 50 ml with *distilled water R*.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in 10 ml of *water R*.

Carbonates

Maximum 2.0 per cent, calculated as Na_2CO_3 as determined in the assay.

Chlorides *(2.4.4)*

Maximum 50 ppm.

Dissolve 1.0 g in 5 ml of *water R*, acidify the solution with about 4 ml of *nitric acid R* and dilute to 15 ml with *water R*. The solution, without addition of *dilute nitric acid R*, complies with the test.

Sulphates *(2.4.13)*

Maximum 50 ppm.

Dissolve 3.0 g in 6 ml of distilled *water R*, adjust to pH 7 with *hydrochloric acid R* (about 7.5 ml) and dilute to 15 ml with *distilled water R*.

Iron *(2.4.9)*

Maximum 10 ppm, determined on solution S.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

ASSAY

Dissolve 2.000 g in about 80 ml of *carbon dioxide-free water R*. Add 0.3 ml of *phenolphthalein solution R* and titrate with *1 M hydrochloric acid*. Add 0.3 ml of *methyl orange solution R* and continue the titration with *1 M hydrochloric acid*.

1 ml of *1 M hydrochloric acid* used in the 2nd part of the titration is equivalent to 0.1060 g of Na_2CO_3.

1 ml of *1 M hydrochloric acid* used in the combined titrations is equivalent to 40.00 mg of total alkali, calculated as NaOH.

STORAGE

In an airtight, non-metallic container.

Ph Eur

Sodium Iodide

General Notices

(Ph Eur monograph 0196)

NaI 149.9 *7681-82-5*

Preparation

Sodium Iodide Injection

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives the reactions of iodides *(2.3.1).*

B. Solution S gives the reactions of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Alkalinity

To 12.5 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.7 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Iodates

To 10 ml of solution S add 0.25 ml of *iodide-free starch solution R* and 0.2 ml of *dilute sulphuric acid R* and allow to stand protected from light for 2 min. No blue colour develops.

Sulphates *(2.4.13)*

Maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Thiosulphates

To 10 ml of solution S add 0.1 ml of *starch solution R* and 0.1 ml of *0.005 M iodine*. A blue colour is produced.

Iron *(2.4.9)*

Maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 3.0 per cent, determined on 1.00 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 1.300 g in *water R* and dilute to 100.0 ml with the same solvent. To 20.0 ml of the solution add 40 ml of *hydrochloric acid R* and titrate with *0.05 M potassium iodate* until the colour changes from red to yellow. Add 5 ml of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer is decolorised.

1 ml of *0.05 M potassium iodate* is equivalent to 14.99 mg of NaI.

STORAGE

Protected from light.

Ph Eur

Sodium Lactate Solution

General Notices

(Ph Eur monograph 1151)

Action and use

Systemic alkalinising agent.

Ph Eur

DEFINITION

Solution of a mixture of the enantiomers of sodium 2-hydroxypropanoate in approximately equal proportions.

Content

Minimum 50.0 per cent *m/m* of sodium 2-hydroxypropanoate ($C_3H_5NaO_3$; M_r 112.1); 96.0 per cent to 104.0 per cent of the content of sodium lactate stated on the label.

CHARACTERS

Appearance

Clear, colourless, slightly syrupy liquid.

Solubility

Miscible with water and with alcohol.

IDENTIFICATION

A. To 0.1 ml add 10 ml of *water R*. 5 ml of the solution gives the reaction of lactates *(2.3.1)*.

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dilute a quantity of the substance to be examined corresponding to 40.0 g of sodium lactate to 200 ml with *distilled water R*.

Appearance of solution

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

6.5 to 9.0 for the substance to be examined.

Reducing sugars and sucrose

To 5 ml of the substance to be examined add 2 ml of *dilute sodium hydroxide solution R* and 0.2 ml of *copper sulphate solution R*. The solution is clear and blue and remains so on boiling. Add to the hot solution 4 ml of *hydrochloric acid R*. Boil for 1 min. Add 6 ml of *strong sodium hydroxide solution R* and heat to boiling again. The solution is clear and blue.

Methanol

Gas chromatography *(2.4.24)*.

Limit:

— *methanol*: maximum 50 ppm, calculated with reference to sodium lactate, if intended for use in the manufacture of parenteral dosage forms, dialysis, haemodialysis or haemofiltration solutions.

Chlorides *(2.4.4)*

Maximum 50 ppm calculated with reference to sodium lactate.

Dilute 5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides.

Oxalates and phosphates

To 1 ml of the substance to be examined add 15 ml of *alcohol R* and 2 ml of *calcium chloride solution R*. Heat at 75 °C for 5 min. Any opalescence in the solution is not more intense than that of a standard prepared at the same time and in the same manner using a mixture of 1 ml of the substance to be examined, 15 ml of *alcohol R* and 2 ml of *water R*.

Sulphates *(2.4.13)*

Maximum 100 ppm calculated with reference to sodium lactate.

To 7.5 ml of solution S, add 1.9 ml of *hydrochloric acid R1* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates without addition of 0.5 ml of *acetic acid R*. Acidify the standard solution with 0.05 ml of *hydrochloric acid R1* instead of 0.5 ml of *acetic acid R*.

Aluminium

Maximum 0.1 ppm, if intended for use in the manufacture of parenteral dosage forms, dialysis, haemodialysis or haemofiltration solutions.

Atomic absorption spectrometry *(2.2.23, Method I)*. For the preparation of the solutions, use equipment that is aluminium-free or that will not release aluminium under the conditions of use (glass, polyethylene, etc).

Modifier solution Dissolve 100.0 g of *ammonium nitrate R* in a mixture of 50 ml of *water R* and 4 ml of *nitric acid R* and dilute to 200 ml with *water R*.

Blank solution Dilute to 2.0 ml of the modifier solution to 25.0 ml with *water R*.

Test solution To 1.25 g add 2.0 ml of the modifier solution and dilute to 25.0 ml with *water R*.

Reference solutions Prepare the reference solutions immediately before use (0.010 ppm to 0.050 ppm of aluminium) using *aluminium standard solution (200 ppm Al) R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device A graphite furnace.

Carrier gas *argon R*.

Conditions The device is equipped with a non-specific absorption correction system. Heat the oven to 120 °C for as many seconds as there are microlitres of solution introduced into the apparatus, then heat at 1000 °C for 30 s and finally at 2700 °C for 6 s.

Barium

To 10 ml of solution S add 10 ml of *calcium sulphate solution R*. Allow to stand for 30 min. Any opalescence *(2.2.1)* in the solution is not more intense than that of a standard prepared at the same time and in the same manner using a mixture of 10 ml of solution S and 10 ml of *distilled water R*.

Iron *(2.4.9)*

Maximum 10 ppm calculated with reference to sodium lactate.

Dilute 5 ml of solution S to 10 ml with *water R*. The solution complies with the limit test for iron.

Heavy metals *(2.4.8)*

Maximum 10 ppm calculated with reference to sodium lactate.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Bacterial endotoxins *(2.6.14)*

Less than 5 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve a quantity of the substance to be examined corresponding to 75.0 mg of sodium lactate in a mixture of 10 ml of *glacial acetic acid R* and 20 ml of *acetic anhydride R*. Allow to stand for 15 min. Add 1 ml of *naphtholbenzein solution R* and titrate with *0.1 M perchloric acid*.

1 ml of *0.1 M perchloric acid* is equivalent to 11.21 mg of $C_3H_5NaO_3$.

LABELLING

The label states:

- where applicable, that the substance is suitable for use in the manufacture of dialysis, haemodialysis and haemofiltration solutions;
- where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms;
- the declared content of sodium lactate.

Ph Eur

Sodium Lauryl Sulphate

General Notices

Sodium Dodecyl Sulphate

(Sodium Laurilsulfate, Ph Eur monograph 0098)

Action and use

Anionic emulsifying agent.

Ph Eur

DEFINITION

Mixture of sodium alkyl sulphates consisting chiefly of sodium dodecyl sulphate ($C_{12}H_{25}NaO_4S$; Mr 288.4).

Content:

— *sodium alkyl sulphates*: minimum 85.0 per cent, expressed as $C_{12}H_{25}NaO_4S$.

CHARACTERS

Appearance

White or pale yellow, powder or crystals.

Solubility

Freely soluble in water giving an opalescent solution, partly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 0.1 g in 10 ml of *water R* and shake. A copious foam is formed.

B. To 0.1 ml of the solution prepared for identification test A, add 0.1 ml of a 1 g/l solution of *methylene blue R* and 2 ml of *dilute sulphuric acid R.* Add 2 ml of *methylene chloride R* and shake. An intense blue colour develops in the methylene chloride layer.

C. Mix about 10 mg with 10 ml of *anhydrous ethanol R.* Heat to boiling on a water-bath, shaking frequently. Filter immediately and evaporate the ethanol. Dissolve the residue in 8 ml of *water R*, add 3 ml of *dilute hydrochloric acid R,* evaporate the solution to half its volume and allow to cool. Separate the congealed fatty alcohols by filtration. To the filtrate add 1 ml of *barium chloride solution R1*. A white, crystalline precipitate is formed.

D. Ignite 0.5 g. The residue gives reaction (a) of sodium *(2.3.1).*

TESTS

Alkalinity

Dissolve 1.0 g in 100 ml of *carbon dioxide-free water R* and add 0.1 ml of *phenol red solution R*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Non-esterified alcohol

Maximum 4 per cent.

Dissolve 10 g in 100 ml of *water R*, add 100 ml of *ethanol (96 per cent) R* and shake the solution with 3 quantities, each of 50 ml, of *pentane R*, adding *sodium chloride R*, if necessary, to promote separation of the 2 layers. Wash the combined organic layers with 3 quantities, each of 50 ml, of *water R*, dry over *anhydrous sodium sulphate R*, filter and evaporate on a water-bath until the solvent has evaporated. Heat the residue at 105 °C for 15 min and cool. The residue weighs a maximum of 0.4 g.

Sodium chloride and sodium sulphate

Maximum 8.0 per cent for the total percentage content.

Sodium chloride

Dissolve 5.00 g in 50 ml of *water R*, add *dilute nitric acid R* dropwise until the solution is neutral to *blue litmus paper R*, add 2 ml of *potassium chromate solution R* and titrate with *0.1 M silver nitrate*.

1 ml of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Sodium sulphate

Dissolve 0.500 g in 20 ml of *water R*, warming gently if necessary, then add 1 ml of a 0.5 g/l solution of *dithizone R1* in *acetone R*. If the solution is red, add *1 M nitric acid*, dropwise, until the solution becomes bluish-green. Add 2.0 ml of *dichloroacetic acid solution R* and 80 ml of *acetone R*. Titrate with *0.01 M lead nitrate* until a persistent violet-red or orange-red colour is obtained. Carry out a blank titration.

1 ml of *0.01 M lead nitrate* is equivalent to 1.420 mg of Na_2SO_4.

ASSAY

Dissolve 1.15 g in *water R*, warming if necessary, and dilute to 1000.0 ml with the same solvent. To 20.0 ml of the solution add 15 ml of *chloroform R* and 10 ml of *dimidium bromide-sulphan blue mixed solution R*. Titrate with *0.004 M benzethonium chloride*, shaking vigorously and allowing the layers to separate before each addition, until the pink colour of the chloroform layer is completely discharged and a greyish-blue colour is obtained.

1 ml of *0.004 M benzethonium chloride* is equivalent to 1.154 mg of sodium alkyl sulphates, expressed as $C_{12}H_{25}NaO_4S$.

Ph Eur

Sodium Metabisulphite

General Notices

Sodium Pyrosulphite

(Ph Eur monograph 0849)

$Na_2S_2O_5$ 190.1 *7681-57-4*

Action and use

Antioxidant.

Ph Eur

DEFINITION

Sodium metabisulphite also called sodium disulphite.

Content

95.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. pH (see Tests).

B. To 0.4 ml of *iodinated potassium iodide solution R* add 8 ml of *distilled water R* and 1 ml of solution S diluted 1 to 10 in *distilled water R*. The solution is colourless and gives reaction (a) of sulphates *(2.3.1)*.

C. Solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH ***(2.2.3)***

3.5 to 5.0 for solution S.

Thiosulphates

To 5 ml of solution S add 5 ml of *dilute hydrochloric acid R*. The solution remains clear *(2.2.1)* for at least 15 min.

Arsenic ***(2.4.2, Method A)***

Maximum 5 ppm.

Mix 0.20 g with 2 ml of *water R* in a dish. Add, drop by drop, 1.5 ml of *nitric acid R*. Evaporate the mixture to dryness on a water-bath. Heat over a flame until no more vapour is evolved. Take up the residue in 25 ml of *water R*.

Iron ***(2.4.9)***

Maximum 20 ppm, determined on solution S.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

To 40 ml of solution S in a silica crucible, add 10 ml of *hydrochloric acid R* and evaporate to dryness. Dissolve the residue in 19 ml of *water R* and add 1 ml of a 40 g/l solution of *sodium fluoride R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

ASSAY

Dissolve 0.200 g in 50.0 ml of *0.05 M iodine*. Add 5 ml of *dilute hydrochloric acid R*. Titrate with *0.1 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 4.753 mg of $Na_2S_2O_5$.

STORAGE

Protected from light.

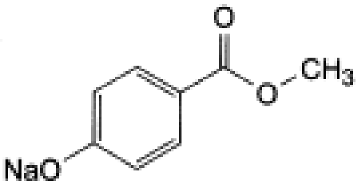
Ph Eur

Sodium Methyl Hydroxybenzoate

General Notices

Sodium Methylparaben

(Sodium Methyl Parahydroxybenzoate, Ph Eur monograph 1262)



$C_8H_7NaO_3$ 174.1 *5026-62-0*

Action and use

Antimicrobial preservative.

Ph Eur

DEFINITION

Sodium methyl parahydroxybenzoate contains not less than 99.0 per cent and not more than the equivalent of 102.0 per cent of sodium 4-(methoxycarbonyl)phenolate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water, sparingly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. Dissolve 0.5 g in 50 ml of *water R*. Immediately add 5 ml of *hydrochloric acid R1.* Filter and wash the precipitate with *water R*. Dry under vacuum at 80 °C for 2 h. The precipitate obtained melts *(2.2.14)* at 125 °C to 128 °C.

B. Examine the precipitate obtained in identification test A by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *methyl parahydroxybenzoate CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (c).

D. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution R*, boil for 30 s and cool. Add 5 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* and mix. An orange to red colour develops.

E. To 1 ml of solution S (see Tests) add 1 ml of *water R*. The solution gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S examined immediately after preparation is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method I)*.

pH *(2.2.3)*

Dilute 1 ml of solution S to 100 ml with *carbon dioxide-free water R*. The pH of the solution is 9.5 to 10.5.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable octadecylsilyl silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 0.100 g in 10 ml of *water R*. Immediately add 2 ml of *hydrochloric acid R* and shake with 50 ml of *ether R*. Evaporate the upper layer to dryness and dissolve the residue to 10 ml with *acetone R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *acetone R*.

Reference solution (a) Dissolve 34.3 mg of *4-hydroxybenzoic acid R* in *acetone R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100 ml with *acetone R.*

Reference solution (c) Dissolve 10 mg of methyl *parahydroxybenzoate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (d) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in 1 ml of test solution (a) and dilute to 10 ml with *acetone R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *glacial acetic acid R*, 30 volumes of *water R* and 70 volumes of *methanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a): any spot due to 4-hydroxybenzoic acid is not more intense than the spot in the chromatogram obtained with reference solution (a) (4 per cent) and any spot, apart from the principal spot and the spot due to 4-hydroxybenzoic acid, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

Chlorides *(2.4.4)*

To 10 ml of solution S, add 30 ml of *water R* and 1 ml of *nitric acid R* and dilute to 50 ml with water R. Shake and filter. Dilute 10 ml of the filtrate to 15 ml with *water R.* The solution complies with the limit test for chlorides (350 ppm). Prepare the standard using 14 ml of *chloride standard solution (5 ppm Cl) R* to which 1 ml of *water R* has been added.

Sulphates *(2.4.13)*

To 25 ml of solution S, add 5 ml of *distilled water R* and 10 ml of *hydrochloric acid R* and dilute to 50 ml with *distilled water R*. Shake and filter. Dilute 10 ml of the filtrate to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Heavy metals *(2.4.8)*

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

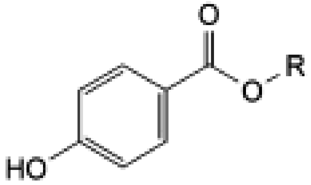
Not more than 5.0 per cent, determined on 0.500 g by the semi-micro determination of water.

ASSAY

Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 17.41 mg of $C_8H_7NaO_3$.

IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

B. R = CH_2-CH_3: ethyl 4-hydroxybenzoate,

C. R = CH_2-CH_2-CH_3: propyl 4-hydroxybenzoate,

D. R = CH_2-CH_2-CH_2-CH_3: butyl 4-hydroxybenzoate.

Ph Eur

Sodium Molybdate Dihydrate

General Notices

(Ph Eur monograph 1565)

$Na_2MoO_4,2H_2O$ 241.9 *10102-40-6*

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or colourless crystals.

Solubility

Freely soluble in water.

IDENTIFICATION

A. Loss on drying (see Tests).

B. Dissolve 0.2 g in 5 ml of a mixture of equal volumes of *nitric acid R* and *water R* and add 0.1 g of *ammonium chloride R*. Add 0.3 ml of *disodium hydrogen phosphate solution R* and heat slowly at 50-60 °C. A yellow precipitate is formed.

C. Dissolve 0.15 g in 2 ml of *water R*, the solution gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Chlorides

Maximum 50 ppm.

To 10 ml of a mixture of equal volumes of *nitric acid R* and *water R* add 10 ml of solution S with shaking. Add 1 ml of *0.1 M silver nitrate*. Any opalescence in the solution is not more intense after 5 min than that of a standard solution prepared at the same time in the same manner with 2 ml of *chloride standard solution (50 ppm Cl) R*.

Phosphates

Maximum 200 ppm.

Dissolve 2.0 g by heating in 13 ml of *water R.* In the still hot solution, dissolve 8.0 g of *ammonium nitrate R1*. Add this solution to 27 ml of a mixture of equal volumes of *nitric acid R* and *water R.* Any yellow colour or opalescence in the solution is not more intense within 3 h than that in a standard solution prepared at the same time as follows: dissolve 1.0 g in 12 ml of *water R* and add 1 ml of *phosphate standard solution (200 ppm* PO_4*) R.*

Ammonium ***(2.4.1, Method B)***

Maximum 10 ppm, determined on 0.10 g.

Prepare the standard using 1 ml of *ammonium standard solution (1 ppm* NH_4*) R*.

Heavy metals

Maximum 10 ppm.

To 10 ml of solution S, add 2 ml of *water R,* 6 ml of a 168 g/l solution of *sodium hydroxide R* and 2 ml of *concentrated ammonia R* (solution A). To 0.5 ml of *thioacetamide reagent R* add a mixture of 15 ml of solution A and 5 ml of *water R*. Any coloration of the solution is not more intense after 2 min than that of a reference solution prepared at the same time as follows: to 0.5 ml of *thioacetamide reagent R* add a mixture of 5 ml of solution A, 1 ml of *lead standard solution (10 ppm Pb) R* and 14 ml of *water R*.

Loss on drying ***(2.2.32)***

14.0 per cent to 16.0 per cent, determined on 1.000 g by drying in an oven at 140 °C for 3 h.

ASSAY

Dissolve 0.100 g in 30 ml of *water R*, add 0.5 g of *hexamethylenetetramine R* and 0.1 ml of a 250 g/l solution of *nitric acid R*. Heat to 60 °C. Titrate with *0.05 M lead nitrate* using *4-(2-pyridylazo)resorcinol monosodium salt R* as indicator.

1 ml of *0.05 M lead nitrate* is equivalent to 10.30 mg of Na_2MoO_4.

Ph Eur

Sodium Nitrite

General Notices

(Ph Eur monograph 1996)

$NaNO_2$ 69.0 *7632-00-0*

Action and use

Used in treatment of cyanide poisoning.

Ph Eur

DEFINITION

Content

98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

Colourless crystals or mass or yellowish rods, hygroscopic.

Solubility

Freely soluble in water, soluble in alcohol.

IDENTIFICATION

A. Dilute 1 ml of solution S1 (see Tests) to 25 ml with *water R*. To 0.1 ml of the solution add 1 ml of *sulphanilic acid solution R1*. Allow to stand for 2-3 min. Add 1 ml of β*-naphthol solution R* and 1 ml of *dilute sodium hydroxide solution R*. An intense red colour develops.

B. To 1 ml of the solution prepared for identification test A add 3 ml of a 20 g/l solution of *phenazone R* and 0.4 ml of *dilute sulphuric acid R.* An intense green colour develops.

C. To 0.15 ml of solution S1, add 0.35 ml of *water R*. The solution gives reaction (b) of sodium *(2.3.1)*.

TESTS

Solution S1

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Solution S2

Dissolve 3 g in *distilled water R*. Cautiously add 10 ml of *nitric acid R* and evaporate to dryness. Dissolve the residue in 10 ml of *distilled water R*, neutralise with *dilute sodium* *hydroxide solution R* and dilute to 30 ml with *distilled water R.*

Appearance of solution

Solution S1 is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S1, add 0.05 ml of *phenol red solution R*. Add 0.1 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.3 ml of *0.01 M hydrochloric acid*. The solution is yellow.

Chlorides *(2.4.4)*

Maximum 50 ppm.

Dilute 10 ml of solution S2 to 15 ml with *water R*. The solution complies with the limit test for chlorides.

Sulphates *(2.4.13)*

Maximum 200 ppm.

Dilute 7.5 ml of solution S2 to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dilute 10 ml of solution S2 to 20 ml with *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo*.

ASSAY

Dissolve 0.400 g in 100.0 ml of *water R*. Introduce 20.0 ml of the solution, while stirring continuously and keeping the tip of the pipette below the surface of the liquid, into a conical flask containing 30.0 ml of *0.1 M cerium sulphate*. Immediately stopper the flask and allow to stand for 2 min. Add 10 ml of a 200 g/l solution of *potassium iodide R* and 2 ml of *starch solution R.*

While stirring continuously, titrate with *0.1 M sodium thiosulphate* until the blue colour disappears. Carry out a blank titration.

1 ml of *0.1 M cerium sulphate* is equivalent to 3.45 mg of $NaNO_2$.

STORAGE

In an airtight container.

Ph Eur

Sodium Nitroprusside

General Notices

(Ph Eur monograph 0565)

$Na_2[Fe(CN)_5NO],2H_2O$ 298.0 *13755-38-9*

Action and use

Vasodilator.

Preparation

Sodium Nitroprusside Intravenous Infusion

Ph Eur

DEFINITION

Sodium pentacyanonitrosylferrate (III) dihydrate.

Content

99.0 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS

Appearance

Reddish-brown powder or crystals.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 0.700 g in *water R* and dilute to 100.0 ml with the same solvent. Examine the solution immediately after preparation.

Spectral range 350-600 nm.

Absorption maximum At 395 nm.

Shoulder At about 510 nm.

Absorption minimum At 370 nm.

Specific absorbance at the absorption maximum 0.65 to 0.80.

B. Dissolve about 20 mg in 2 ml of *water R* and add 0.1 ml of *sodium sulphide solution R*. A deep violet-red colour is produced.

C. Dissolve 50 mg in 1 ml of *water R* and acidify the solution by the addition of *hydrochloric acid R*. Place a drop of the solution in an oxidising flame. A persistent yellow colour is produced.

TESTS

Insoluble matter

Maximum 100 ppm.

Dissolve 10 g without heating in 50 ml of *water R*. Allow to stand for 30 min and filter through a sintered-glass filter (16) *(2.1.2)*. Wash the filter with cold *water R* until the filtrate is colourless. Dry the residue on the filter at 105 °C. The residue weighs a maximum of 1 mg.

Chlorides *(2.4.4)*

Maximum 200 ppm.

In a metallic crucible (nickel) mix 1.0 g with 8 ml of a 200 g/l solution of *sodium hydroxide R*. Heat slowly and evaporate carefully to dryness over a small flame, then heat to a dull red colour for 30 min. Allow to cool and transfer the solid residue with 3 quantities, each of 8 ml, of *dilute sulphuric acid R.* Filter the sulphuric acid extracts on a filter paper and collect the filtrates. Render the filtrate acid to *litmus paper R* by adding, if necessary, a few drops of dilute *sulphuric acid R*. Wash the crucible and the filter paper with 3 quantities, each of 10 ml, of *water R,* add the washings to the main sulphuric acid solution and dilute to 60 ml with *water R*. Mix.

Ferricyanides

Maximum 200 ppm.

Dissolve 1.25 g in *acetate buffer solution pH 4.6 R* and dilute to 50.0 ml with the same buffer solution. Use three 50 ml volumetric flasks (A, B, C). To flask B add 1.0 ml of *ferricyanide standard solution (50 ppm Fe(CN)6) R*. To flasks A and B add 1 ml of a 5 g/l solution of *ferrous ammonium sulphate R*. To the 3 flasks add 10.0 ml of the solution of the substance to be examined. Dilute the contents of each flask to 50.0 ml with *water R*. Allow to stand for 30 min. The absorbance *(2.2.25)* of the solution in flask A measured at 720 nm using the solution in flask C as the compensation liquid is not greater than the absorbance of the solution in flask B measured at 720 nm using the solution in flask A as the compensation liquid.

Ferrocyanides

Maximum 200 ppm.

Dissolve 4.0 g in *water R* and dilute to 100.0 ml with the same solvent. Use three 50 ml volumetric flasks (A, B, C). To flask B add 2.0 ml of *ferrocyanide standard solution (100 ppm* $Fe(CN)_6$*) R*. To flasks A and B add 1 ml of *ferric chloride solution R2*. To the 3 flasks add 25.0 ml of the solution of the substance to be examined. Dilute the contents of each flask to 50.0 ml with *water R*. Allow to stand for 30 min. The absorbance *(2.2.25)* of the solution in flask A measured at 695 nm using the solution in flask C as the compensation liquid is not greater than the absorbance of the solution in flask B measured at 695 nm using the solution in flask A as the compensation liquid.

Sulphates

Maximum 100 ppm.

Test solution Dissolve 3.6 g in 120 ml of *distilled water R,* add with mixing 4 ml of *sulphate standard solution (10 ppm SO_4) R* and 20 ml of a 250 g/l solution of *cupric chloride R* and dilute to 150.0 ml with *distilled water R*. Allow to stand for 16 h and filter or centrifuge until a clear light-blue solution is obtained.

Reference solution To 40 ml of *sulphate standard solution (10 ppm SO_4) R* add 80 ml of *distilled water R* and 12-13 ml of a 250 g/l solution of *cupric chloride R*. Dilute to 150.0 ml with *distilled water R*. The volume of cupric chloride solution added is such that the colour of the final solution matches that of the test solution.

Allow the solutions to stand. Filter both solutions, discarding the first 25 ml of filtrate. To 100 ml of each filtrate, add 0.5 ml of *acetic acid R*. Mix and add 2 ml of a 250 g/l solution of *barium chloride R* and mix again. The test solution is not more opalescent than the reference solution.

Water *(2.5.12)*

9.0 per cent to 15.0 per cent, determined on 0.250 g.

ASSAY

Dissolve 0.250 g in 100 ml of *water R* and add 0.1 ml of *dilute sulphuric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)* with a silver-mercurous sulphate electrode system.

1 ml of *0.1 M silver nitrate* is equivalent to 13.10 mg of $Na_2[Fe(CN)_5(NO)]$.

STORAGE

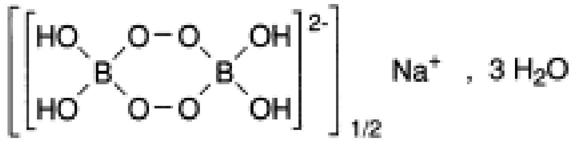
Protected from light.

Ph Eur

Sodium Perborate

General Notices

(Hydrated Sodium Perborate, Ph Eur monograph 1997)



$NaBH_2O_4,3H_2O$ 153.9 *7632-04-4*

Action and use

Antiseptic.

Ph Eur

DEFINITION

Content

96.0 per cent to 103.0 per cent.

CHARACTERS

Appearance

Colourless, prismatic crystals or white or almost white powder, stable in the crystalline form.

Solubility

Sparingly soluble in water, with slow decomposition. It dissolves in dilute mineral acids.

IDENTIFICATION

A. Mix 1 ml of a saturated solution with a mixture of 1 ml of *dilute sulphuric acid R* and 0.2 ml of a 70 g/l solution of *potassium dichromate R*, shake with 2 ml of *ether R* and allow to stand. A blue colour is produced in the ether layer.

B. The mixture obtained by treating about 100 mg with 0.1 ml of *sulphuric acid R* and 5 ml of *methanol R* burns with a greenish flame when ignited.

C. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Chlorides ***(2.4.4)***

Maximum 330 ppm.

Dissolve 0.15 g in 15 ml of *water R*. The solution complies with the limit test for chlorides.

Sulphates ***(2.4.13)***

Maximum 1.2 per cent.

Dissolve 0.13 g in 150 ml of *distilled water R*. 15 ml of the solution complies with the limit test for sulphates.

Iron ***(2.4.9)***

Maximum 20 ppm.

Dissolve 2.5 g in 10 ml of *dilute hydrochloric acid R* with heating, evaporate to dryness, with stirring, and dissolve the residue in 25 ml of hot *water R*. Dilute 5 ml of the obtained solution to 10 ml with *water R*. The solution complies with the limit test for iron.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of the solution obtained in the test for iron complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

ASSAY

Dissolve 0.300 g in 50.0 ml of *water R*. Dilute 10.0 ml of the solution to 50 ml with *water R* and add 10 ml of dilute *sulphuric acid R*. Titrate with *0.02 M potassium permanganate*.

1 ml of *0.02 M potassium permanganate* is equivalent to 7.693 mg of NaH_8BO_7.

STORAGE

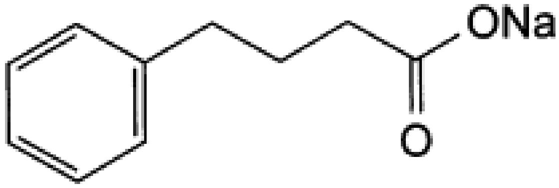
In an airtight container .

Ph Eur

Sodium Phenylbutyrate

General Notices

(Ph Eur monograph 2183)



$C_{10}H_{11}NaO_2$ 186.2 *1716-12-7*

Action and use

Glutamine conjugate; treatment of hyperammonaemia.

Ph Eur

DEFINITION

Sodium 4-phenylbutanoate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Freely soluble in water and in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison sodium phenylbutyrate CRS.

B. Dissolve 0.15 g in 2 ml of *water R*. The solution gives reaction (a) of sodium *(2.3.1)*.

TESTS

pH ***(2.2.3)***

6.5 to 7.5.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Impurity C

Gas chromatography *(2.2.28)*.

Silylation solution To 2 ml of *N,O-bis(trimethylsilyl)trifluoroacetamide R* add 0.04 ml of *chlorotrimethylsilane R* and mix.

Test solution Dissolve 50.0 mg of the substance to be examined in 3 ml of *water R* and add 0.5 ml of *hydrochloric acid R*. Extract with 2 quantities, each of 5 ml, of *methylene chloride R*. Evaporate the combined methylene chloride extracts to dryness in a vial with a screw cap and add 0.5 ml of the silylation solution. Seal the vial and heat at 70 ± 5 °C for 20 min.

Reference solution (a) Dissolve 5.0 mg of *sodium phenylbutyrate impurity C CRS* in *methylene chloride R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 10.0 ml with *methylene chloride R*. Place 1.0 ml of this solution in a vial with a screw cap, evaporate to dryness and add 0.5 ml of the silylation solution. Seal the vial and heat at 70 ± 5 °C for 20 min.

Reference solution (c) Dissolve 10 mg of the substance to be examined in 25 ml of *water R.* To 3 ml of this solution add 0.1 ml of *hydrochloric acid R*. Extract with 2 quantities, each of 5 ml, of *methylene chloride R*. Combine the methylene chloride extracts in a vial with a screw cap and add 2 ml of reference solution (a). Evaporate to dryness and add 0.5 ml of the silylation solution. Seal the vial and heat at 70 ± 5 °C for 20 min.

Column:

— *material*: fused silica;

— *size: l* = 25 m, Ø = 0.25 mm;

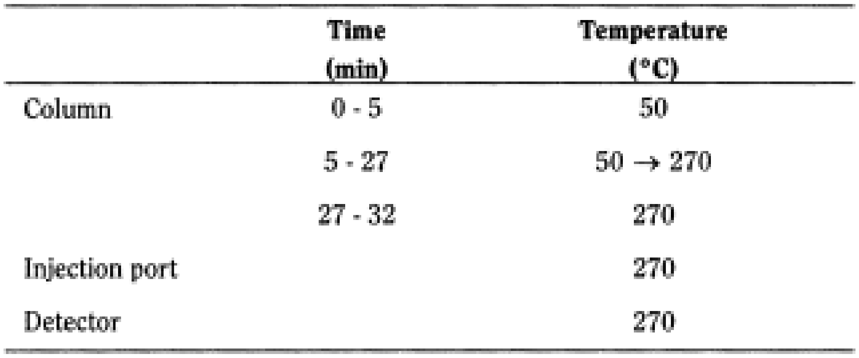
— *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R*.

Flow rate 0.9 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	50
	5 - 27	50 → 270
	27 - 32	270
Injection port		270
Detector		270

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to phenylbutyrate (retention time = about 20 min): impurity C = about 0.98.

System suitability Reference solution (c):

— *resolution*: minimum 3.0 between the peaks due to impurity C and phenylbutyrate.

Limit:
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.20 g of the substance to be examined in 10 ml of *methanol R* and dilute to 50.0 ml with *water R*.

Reference solution (a) Dissolve 4.0 mg of *α-tetralone R* (impurity B) in 10 ml of *methanol R* and dilute to 200.0 ml with the same solvent.

Reference solution (b) Dissolve 0.20 g of the substance to be examined in 10 ml of *methanol R*, add 1 ml of reference solution (a) and dilute to 50 ml with *water R*.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 50.0 ml with *water R.*

Reference solution (d) Dissolve 5.0 mg of *3-benzoylpropionic acid R* (impurity A) in 2.5 ml of *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with *water R.*

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R*, *methanol R*, *water R* (1:49:50 *V/V/V*).

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 245 nm.

Injection 20 µl of the test solution and reference solutions (b), (c) and (d).

Run time Twice the retention time of phenylbutyrate.

Relative retention With reference to phenylbutyrate (retention time = about 17 min): impurity A = about 0.3; impurity B = about 0.7.

System suitability Reference solution (b):

— *resolution*: minimum 6 between the peaks due to impurity B and phenylbutyrate.

Limits:
— *impurity A*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.01 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent);

— *disregard limit of impurities other than B*: 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.03 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in a mixture of 25 volumes of *water R* and 75 volumes of *ethanol (96 per cent) R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 25 volumes of *water R* and 75 volumes of *ethanol (96 per cent) R.*

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 2.00 g.

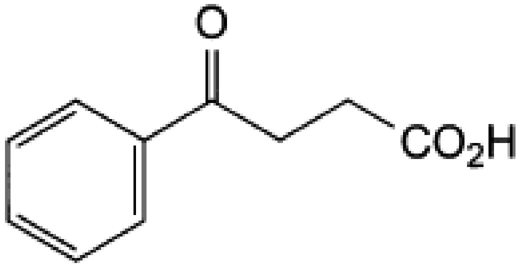
ASSAY

Disperse 0.150 g in 50 ml of *anhydrous acetic acid R*. The opalescence of the solution disappears during the titration. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

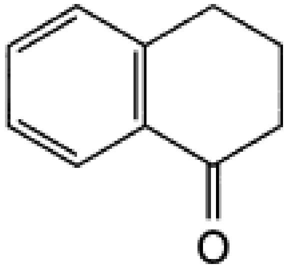
1 ml of *0.1 M perchloric acid* is equivalent to 18.62 mg of $C_{10}H_{11}NaO_2$.

IMPURITIES

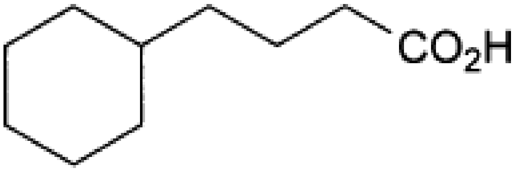
Specified impurities *A, B, C*.



A. 4-oxo-4-phenylbutanoic acid (3-benzoylpropionic acid),



B. 3,4-dihydronaphthalen-1(2*H*)-one (α-tetralone),



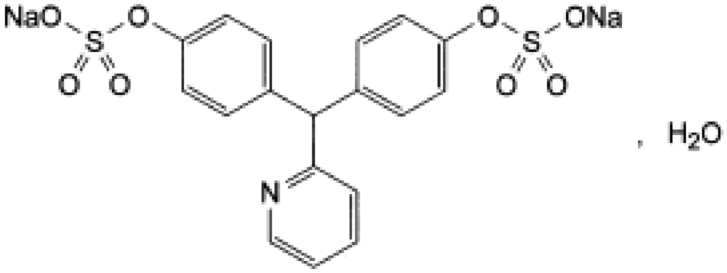
C. 4-cyclohexylbutanoic acid.

Ph Eur

Sodium Picosulfate

General Notices

(Ph Eur monograph 1031)



$C_{18}H_{13}NNa_2O_8S_2,H_2O$ 499.4 *10040-45-6*
(anhydrous)

Action and use

Stimulant laxative.

Preparation

Compound Sodium Picosulfate Powder for Oral Solution

Sodium Picosulfate Oral Solution

Ph Eur

DEFINITION

Sodium picosulfate contains not less than 98.5 per cent and not more than the equivalent of 100.5 per cent of 4,4′-(pyridin-2-ylmethylene)bisphenyl bis(sodium sulphate), calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification *A, E.*

Second identification *B, C, D, E.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *sodium picosulfate CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 5 ml of solution S (see Tests) add 1 ml of *dilute hydrochloric acid R* and heat to boiling. Add 1 ml of *barium chloride solution R1*. A white precipitate is formed.

D. To about 10 mg add 3 ml of *sulphuric acid R* and 0.1 ml of *potassium dichromate solution R1*. A violet colour develops.

E. The solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution GY_7 *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0*.05 ml of phenolphthalein solution R*. The solution is colourless. Not more than 0.25 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R.*

Reference solution (a) Dissolve 20 mg of *sodium picosulfate CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 2 ml of test solution (b) to 100 ml with *methanol R*.

Reference solution (c) Dissolve 0.20 g of the substance to be examined in 2 ml of a 103 g/l solution of *hydrochloric acid R*. Heat rapidly to boiling and maintain boiling for 10 s. Cool in iced water and dilute to 10 ml with *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 2.5 volumes of *anhydrous formic acid R*, 12.5 volumes of *water R*, 25 volumes of *methanol R* and 60 volumes of *ethyl acetate R*. Dry the plate in a current of hot air for 15 min and examine in ultraviolet light at 254 nm. Spray with a 200 g/l solution of *hydrochloric acid R* in *methanol R* and heat at 110 °C for 10 min. Spray the hot plate with a freshly prepared solution containing 50 g/l of *ferric chloride R* and 1 g/l of *potassium ferricyanide R.* Examine the wet plate. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows three clearly separated spots. A fourth spot may be present on the starting-line.

Chlorides *(2.4.4)*

Dilute 5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (400 ppm).

Heavy metals *(2.4.8)*

12 ml of solution S complies with limit test A for heavy metals (20 ppm). Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Water *(2.5.12)*

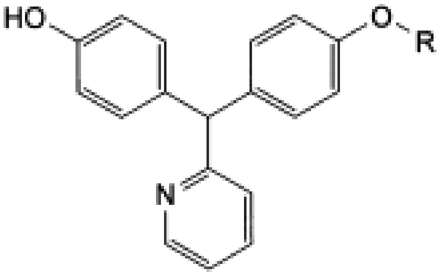
3.0 per cent to 5.0 per cent, determined on 0.500 g by the semi-micro determination of water.

ASSAY

Dissolve 0.400 g in 80 ml of *methanol R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 48.14 mg of $C_{18}H_{13}NNa_2O_8S_2$.

IMPURITIES



A. R = SO_3Na: 4-[(pyridin-2-yl)(4-hydroxyphenyl)methyl]phenyl sodium sulphate,

B. R = H: 4,4′-[(pyridin-2-yl)methylene]bisphenol.

Ph Eur

Sodium Polystyrene Sulphonate

General Notices

(Ph Eur monograph 1909)

Action and use

Used in the treatment of hyperkalaemia.

Ph Eur

DEFINITION

Polystyrene sulphonate resin prepared in the sodium form.

Exchange capacity

2.8 mmol to 3.4 mmol of potassium per gram (dried substance).

Content

9.4 per cent to 11.0 per cent of Na (dried substance).

CHARACTERS

Appearance

Almost white or light brown powder.

Solubility

Practically insoluble in water, in alcohol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs using finely ground substance.

Comparison *Ph. Eur. reference spectrum of sodium polystyrene sulphonate*.

B. Suspend 0.1 g in *water R*, add 2 ml of a 150 g/l solution of *potassium carbonate R*, and heat to boiling. Allow to cool and filter. To the filtrate add 4 ml of *potassium pyroantimonate solution R* and heat to boiling. Allow to cool in iced water and if necessary rub the inside of the test-tube with a glass rod. A dense white precipitate is formed.

TESTS

Styrene

Liquid chromatography *(2.2.29)*.

Test solution Shake 10.0 g of the substance to be examined with 10 ml of *acetone R* for 30 min, centrifuge and use the supernatant liquid.

Reference solution Dissolve 10 mg of *styrene R* in *acetone R* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 100 ml with *acetone R*.

Column:
- *size:* *l* = 0.25 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water R* (1:1 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Limit:
- *styrene*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (1 ppm).

Calcium

Maximum 0.10 per cent.

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution To 1.10 g add 5 ml of *hydrochloric acid R*, heat to boiling, cool and add 10 ml of *water R.* Filter, wash the filter and residue with *water R* and dilute the filtrate and washing to 25.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *calcium standard solution (400 ppm Ca) R*, diluted as necessary with *water R*.

Wavelength 422.7 nm.

Potassium

Maximum 0.10 per cent.

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution To 1.10 g add 5 ml of *hydrochloric acid R*, heat to boiling, cool and add 10 ml of *water R*. Filter, wash the filter and residue with *water R* and dilute the filtrate and washings to 25.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*, diluted as necessary with *water R*.

Wavelength 766.5 nm.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Treat 1.0 g as described in limit test F. After the addition of the *buffer solution pH 3.5 R* and of the *thioacetamide reagent R*, dilute to 50 ml with *water R* and continue as described in limit test E, beginning at the words "mix and allow to stand for 10 min...".

Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying *(2.2.32)*

Maximum 7.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Microbial contamination *(2.6.13)*

Not more than 10^2 enterobacteria and certain other gram-negative bacteria per gram.

ASSAY

Sodium

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution In a platinum crucible moisten 0.90 g with a few drops of *sulphuric acid R*, ignite very gently and allow to cool. Moisten with a few drops of *sulphuric acid R* again, ignite at 800 ± 50 °C until a carbon-free ash is obtained and allow to cool.

Add 20 ml of *water R* to the crucible, warm gently on a water-bath until dissolution, cool, transfer quantitatively to a 100 ml graduated flask and dilute to 100.0 ml with *water R*. Dilute 5 ml of this solution to 1000.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589 nm.

Exchange capacity

Atomic emission spectrometry *(2.2.22, Method I)*.

Solution A 9.533 g/l solution of *potassium chloride R*.

Test solution To 1.6 g of the substance to be examined in a dry 250 ml ground-glass-stoppered flask add 100 ml of solution A, stopper and shake for 15 min. Filter, discard the first 20 ml of the filtrate and dilute 4 ml of the filtrate to 1000 ml with *water R*.

Reference solutions Prepare the reference solutions by diluting 0, 1, 2, 3 and 4 ml of solution A respectively and 4, 3, 2, 1 and 0 ml of a 7.63 g/l solution of *sodium chloride R* to 1000 ml with *water R*.

Wavelength 766.5 nm.

Prepare a calibration curve using the reference solutions and calculate the potassium exchange capacity of the substance to be examined in millimoles per gram taking the concentration of potassium in solution A as 128 mmoles of K per litre.

STORAGE

In an airtight container.

IMPURITIES

Specified impurities *A.*

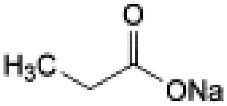
A. styrene.

Ph Eur

Sodium Propionate

General Notices

(Ph Eur monograph 2041)



$C_3H_5NaO_2$ 96.1 *137-40-6*

Action and use

Antifungal.

Ph Eur

DEFINITION

Sodium propanoate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Colourless crystals or, white or almost white powder, slightly hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of sodium propionate.*

B. Dissolve 0.1 g in a mixture of 2 ml of *copper sulphate solution R* and 2 ml of *methylene chloride R*. Shake vigorously and allow to stand. Both the upper and the lower layer show a blue colour.

C. To 5 ml of solution S (see Tests) add 2 ml of *0.1 M silver nitrate*. A white precipitate is formed.

D. Solution S gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 10 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

7.8 to 9.2.

Dilute 1 ml of solution S to 5 ml with *water R*.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.250 g of the substance to be examined in *water R* and dilute to 100 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of the substance to be examined and 10 mg of *sodium acetate R* in *water R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100 ml with *water R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dilute 1 ml of *phosphoric acid R* to 1000 ml with *water R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

System suitability Reference solution (a):

- *resolution*: minimum 5 between the peaks due to sodium acetate and sodium propionate.

Limits:

- *any impurity*: not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than half the area of the principal peak in the chromatrogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit:* 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Readily oxidisable substances

In a ground-glass-stoppered conical flask introduce 10 g of the substance to be examined. Add 100 ml of *water R* and stir to dissolve. Add 25 ml of *sodium hypobromite solution R* and 10 ml of a 200 g/l solution of *sodium acetate R*, stopper the flask and allow to stand for 15 min. Add 10 ml of *potassium iodide solution R* and 20 ml of *hydrochloric acid R* while cooling. Titrate with *0.2 M sodium thiosulphate*, adding 2 ml of *starch solution R* towards the end of the titration. Carry out a blank titration. The difference between the volumes used in the 2 titrations is not greater than 2.2 ml.

Iron *(2.4.9)*

Maximum 10 ppm.

10 ml of solution S complies with the limit test for iron.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by heating in an oven at 105 °C for 3 h.

ASSAY

Dissolve 80.0 mg in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 9.61 mg of $C_3H_5NaO_2$.

STORAGE

In an airtight container.

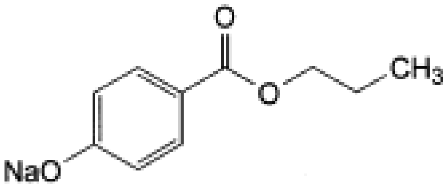
Ph Eur

Sodium Propyl Hydroxybenzoate

General Notices

Sodium Propylparaben

(Sodium Propyl Parahydroxybenzoate, Ph Eur monograph 1263)



$C_{10}H_{11}NaO_3$ 202.2 *35285-69-9*

Action and use

Antimicrobial preservative.

Ph Eur

DEFINITION

Sodium propyl parahydroxybenzoate contains not less than 99.0 per cent and not more than the equivalent of 104.0 per cent of sodium 4-(propoxycarbonyl)phenolate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water, sparingly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification *A, B, E*.

Second identification *A, C, D, E*.

A. Dissolve 0.5 g in 50 ml of *water R*. Immediately add 5 ml of *hydrochloric acid R1*. Filter, and wash the precipitate with *water R*. Dry at 80 °C *in vacuo* for 2 h. The melting point *(2.2.14)* of the precipitate is 96 °C to 99 °C.

B. Examine the precipitate obtained in identification test A by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *propyl parahydroxybenzoate CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (c).

D. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution R*, boil for 30 s and cool. Add 5 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* and mix. An orange to red colour develops.

E. To 1 ml of solution S (see Tests) add 1 ml of *water R*. The solution gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R*, and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S, examined immediately after preparation, is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

Dilute 1 ml of solution S to 100 ml with *carbon dioxide-free water R*. The pH of the solution is 9.5 to 10.5.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using as the coating substance a suitable octadecylsilyl silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10 ml of *water R*. Immediately add 2 ml of *hydrochloric acid R* and shake with 50 ml of *ether R*. Evaporate the upper layer to dryness and take up the residue with 10 ml of *acetone R.*

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *acetone R*.

Reference solution (a) Dissolve 34.3 mg of *4-hydroxybenzoic acid R* in *acetone R* and dilute to 100 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100 ml with *acetone R*.

Reference solution (c) Dissolve 10 mg of *propyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (d) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in 1 ml of test solution (a) and dilute to 10 ml with *acetone R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *glacial acetic acid R*, 30 volumes of *water R* and 70 volumes of *methanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a): any spot due to 4-hydroxybenzoic acid is not more intense than the spot in the chromatogram obtained with reference solution (a) (4 per cent); and any spot, apart from the principal spot and the spot due to 4-hydroxybenzoic acid, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

Chlorides *(2.4.4)*

To 10 ml of solution S add 30 ml of *water R* and 1 ml of *nitric acid R* and dilute to 50 ml with *water R*. Shake and filter. Dilute 10 ml of the filtrate to 15 ml with *water R*. The solution complies with the limit test for chlorides (350 ppm). Prepare the standard using 14 ml of *chloride standard solution (5 ppm Cl) R* to which 1 ml of *water R* has been added.

Sulphates ***(2.4.13)***

To 25 ml of solution S add 5 ml of *distilled water R* and 10 ml of *hydrochloric acid R* and dilute to 50 ml with *distilled water R*. Shake and filter. Dilute 10 ml of the filtrate to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Heavy metals ***(2.4.8)***

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water ***(2.5.12)***

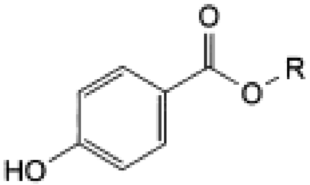
Not more than 5.0 per cent, determined on 0.500 g by the semi-micro determination of water.

ASSAY

Dissolve 0.150 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 20.22 mg of $C_{10}H_{11}NaO_3$.

IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

B. R = CH_3 : methyl 4-hydroxybenzoate,

C. R = CH_2-CH_3: ethyl 4-hydroxybenzoate,

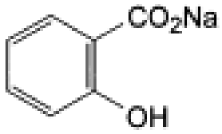
D. R = CH_2-CH_2-CH_2-CH_3: butyl 4-hydroxybenzoate.

Ph Eur

Sodium Salicylate

General Notices

(Ph Eur monograph 0413)



$C_7H_5NaO_3$ 160.1 *54-21-7*

Action and use

Anti-inflammatory; analgesic.

Ph Eur

DEFINITION

Sodium 2-hydroxybenzenecarboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or small, colourless crystals or shiny flakes.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison sodium salicylate CRS.

B. Solution S (see Tests) gives the reactions of salicylates *(2.3.1)*.

C. It gives reaction (b) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II).*

Acidity

To 20 ml of solution S add 0.1 ml of *phenol red solution R*. The solution is yellow. Not more than 2.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to violet-red.

Chlorides *(2.4.4)*

Maximum 200 ppm.

To 5 ml of solution S add 5 ml of *water R* and 10 ml of *dilute nitric acid R* and filter. Dilute 10 ml of the filtrate to 15 ml with *water R.*

Sulphates *(2.4.13)*

Maximum 600 ppm.

Dilute 2.5 ml of solution S to 15 ml with *distilled water R.*

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.6 g in 16 ml of a mixture of 5 volumes of *water R* and 10 volumes of *ethanol (96 per cent) R.* 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 5 volumes of *water R* and 10 volumes of *ethanol (96 per cent) R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.130 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 16.01 mg of $C_7H_5NaO_3$.

STORAGE

In an airtight container, protected from light.

Ph Eur

Sodium Selenite Pentahydrate

General Notices

(Ph Eur monograph 1677)

$Na_2SeO_3,5H_2O$ 263.0 *26970-82-1*

Action and use

Used in treatment of selenium deficiency.

Ph Eur

DEFINITION

Content

98.5 per cent to 101.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 50 mg in 5 ml of a mixture of equal volumes of *dilute hydrochloric acid R* and *water R* and heat to boiling. Add 0.05 g of *ascorbic acid R*; a red precipitate is formed which may become black.

B. Dissolve 50 mg in a mixture of 1 ml of *dilute hydrochloric acid R* and 5 ml of *water R.* Add 1 ml of *barium chloride solution R1*; the solution remains clear.

C. It gives reaction (a) of sodium *(2.3.1)*.

D. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH ***(2.2.3)***

9.8 to 10.8 for solution S.

Chlorides ***(2.4.4)***

Maximum 50 ppm.

To 10 ml of solution S add 2 ml of *nitric acid R* and dilute to 15 ml with *water R*.

Sulphates and selenates ***(2.4.13)***

Maximum 300 ppm (determined as sulphates).

Dissolve 0.5 g in 10 ml of *distilled water R*. Add 0.5 ml of *hydrochloric acid R1* and dilute to 15 ml with *distilled water R*.

Iron

Maximum 50 ppm.

To 2 ml of solution S add 2 ml of a 200 g/l solution of *sulphosalicylic acid R*, 5 ml of *concentrated ammonia R* and dilute to 10 ml with *water R*. The solution is not more intensely coloured than a reference solution prepared in the same manner using 1 ml of *iron standard solution (10 ppm Fe) R*.

ASSAY

Dissolve 0.120 g in 50 ml of *water R*, add 7 ml of *glacial acetic acid R,* 25.0 ml of *0.1 M sodium thiosulphate* and 0.5 g of *potassium iodide R.* Titrate immediately with *0.05 M iodine solution* using *starch solution R* as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 6.575 mg of $Na_2SeO_3,5H_2O$.

STORAGE

In an airtight container.

Ph Eur

Sodium Starch Glycolate (Type A)

General Notices

(Ph Eur monograph 0983)

Action and use

Excipient.

Ph Eur

DEFINITION

Sodium salt of a cross-linked partly *C*-carboxymethylated potato starch.

Content

2.8 per cent to 4.2 per cent of Na (A_r 22.99) (substance washed with ethanol (80 per cent *V/V*) and dried).

CHARACTERS

Appearance

White or almost white, fine, free-flowing powder, very hygroscopic.

Solubility

Practically insoluble in methylene chloride. It gives a translucent suspension in water.

Examined under a microscope it is seen to consist of: granules, irregularly shaped, ovoid or pear-shaped, 30-100 µm in size, or rounded, 10-35 µm in size; compound granules consisting of 2-4 components occur occasionally; the granules have an eccentric hilum and clearly visible concentric striations; between crossed nicol prisms, the granules show a distinct black cross intersecting at the hilum; small crystals are visible at the surface of the granules. The granules show considerable swelling in contact with water.

IDENTIFICATION

A. pH (see Tests).

B. Prepare with shaking and without heating a mixture of 4.0 g of the substance to be examined and 20 ml of *carbon dioxide-free water R.* The mixture has the appearance of a gel. Add 100 ml of *carbon dioxide-free water R* and shake. A suspension forms that settles after standing.

C. To an acidified solution, add *iodinated potassium iodide solution R1*. The solution becomes blue or violet.

D. Solution S2 (see Tests) gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S1

Centrifuge the suspension obtained in identification test B at 2500 g for 10 min. Collect carefully the supernatant liquid.

Solution S2

Place 2.5 g in a silica or platinum crucible and add 2 ml of a 500 g/l solution of *sulphuric acid R*. Heat on a water-bath, then cautiously over a naked flame, raising the temperature progressively, then incinerate in a muffle furnace at 600 ± 25 °C. Continue heating until all black particles have disappeared. Allow to cool, add a few drops of *dilute sulphuric acid R*, heat and incinerate as above. Allow to cool, add a few drops of *ammonium carbonate solution R,* evaporate to dryness and incinerate cautiously. Allow to cool and dissolve the residue in 50 ml of *water R*.

Appearance of solution S1

Solution S1 is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.5 to 7.5.

Disperse 1.0 g in 30 ml of *water R*.

Sodium glycolate

Maximum 2.0 per cent. Carry out the test protected from light.

Test solution Place 0.20 g in a beaker. Add 5 ml of *acetic acid R* and 5 ml of *water R*. Stir until dissolution is complete (about 10 min). Add 50 ml of *acetone R* and 1 g of *sodium chloride R*. Filter through a fast filter paper impregnated with *acetone R*, rinse the beaker and filter with *acetone R*. Combine the filtrate and washings and dilute to 100.0 ml with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant liquid.

Reference solution Dissolve 0.310 g of *glycollic acid R*, previously dried in vacuo over *diphosphorus pentoxide R* at room temperature overnight, in *water R* and dilute to 500.0 ml with the same solvent. To 5.0 ml of this solution add 5 ml of *acetic acid R* and allow to stand for about 30 min. Add 50 ml of *acetone R* and 1 g of *sodium chloride R.* Filter through a fast filter paper impregnated with *acetone R*, rinse the beaker and filter with *acetone R.* Combine the filtrate and washings and dilute to 100.0 ml with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant liquid.

Heat 2.0 ml of the test solution on a water-bath for 20 min. Cool to room temperature and add 20.0 ml of *2,7-dihydroxynaphthalene solution R*. Shake and heat in a water-bath for 20 min. Cool under running water, transfer to a volumetric flask and dilute to 25.0 ml with sulphuric *acid R*, maintaining the flask under running water. Within 10 min, measure the absorbance at 540 nm *(2.2.25)* using *water R* as the compensation liquid. The absorbance of the solution prepared with the test solution is not greater than that of a solution prepared at the same time and in the same manner with 2.0 ml of the reference solution.

Sodium chloride

Maximum 7.0 per cent.

Place 0.500 g in a beaker and suspend in 100 ml of *water R.* Add 1 ml of *nitric acid R.* Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*, using a silver indicator electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Iron *(2.4.9)*

Maximum 20 ppm determined on 10 ml of solution S2.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 1.5 h.

Microbial contamination

It complies with the test for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

Shake 1.000 g with 20 ml of *ethanol (80 per cent V/V) R*, stir for 10 min and filter. Repeat the operation until chloride has been completely extracted and verify the absence of chloride using *silver nitrate solution R2*. Dry the residue at 105 °C to constant mass. To 0.700 g of the dried residue, add 80 ml of *glacial acetic acid R* and heat under a reflux condenser for 2 h. Cool the solution to room temperature. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 2.299 mg of Na.

STORAGE

In an airtight container, protected from light.

Ph Eur

Sodium Starch Glycolate (Type B)

General Notices

(Ph Eur monograph 0984)

Action and use

Excipient.

Ph Eur

DEFINITION

Sodium salt of a cross-linked partly *C*-carboxymethylated potato starch.

Content

2.0 per cent to 3.4 per cent of Na (A_r 22.99) (substance washed with ethanol (80 per cent *V/V*) and dried).

CHARACTERS

Appearance

White or almost white, fine, free-flowing powder, very hygroscopic.

Solubility

Practically insoluble in methylene chloride. It gives a translucent suspension in water.

Examined under a microscope it is seen to consist of: granules, irregularly shaped, ovoid or pear shaped, 30-100 µm in size, or rounded, 10-35 µm in size; compound granules consisting of 2-4 components occur occasionally; the granules have an eccentric hilum and clearly visible concentric striations; between crossed nicol prisms, the granules show a distinct black cross intersecting at the hilum; small crystals are visible at the surface of the granules. The granules show considerable swelling in contact with water.

IDENTIFICATION

A. pH (see Tests).

B. Prepare with shaking and without heating a mixture of 4.0 g of the substance to be examined and 20 ml of *carbon dioxide-free water R*. The mixture has the appearance of a gel. Add 100 ml of *carbon dioxide-free water R* and shake. A suspension forms that settles after standing.

C. To an acidified solution, add *iodinated potassium iodide solution R1*. The solution becomes blue or violet.

D. Solution S2 (see Tests) gives reaction (a) of sodium *(2.3.1).*

TESTS

Solution S1

Centrifuge the suspension obtained in identification test B at 2500 g for 10 min. Collect carefully the supernatant liquid.

Solution S2

Place 2.5 g in a silica or platinum crucible and add 2 ml of a 500 g/l solution of *sulphuric acid R*. Heat on a water-bath, then cautiously over a naked flame, raising the temperature progressively, and then incinerate in a muffle furnace at 600 ± 25 °C. Continue heating until all black particles have disappeared. Allow to cool, add a few drops of *dilute sulphuric acid R* and heat and incinerate as above. Allow to cool, add a few drops of *ammonium carbonate solution R*, evaporate to dryness and incinerate cautiously. Allow to cool and dissolve the residue in 50 ml of *water R*.

Appearance of solution S1

Solution S1 is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

3.0 to 5.0.

Disperse 1.0 g in 30 ml of *water R*.

Sodium glycolate

Maximum 2.0 per cent. Carry out the test protected from light.

Test solution Place 0.20 g in a beaker. Add 5 ml of *acetic acid R* and 5 ml of *water R.* Stir until dissolution is complete (about 10 min). Add 50 ml of *acetone R* and 1 g of *sodium chloride R*. Filter through a fast filter paper impregnated with *acetone R,* rinse the beaker and filter with *acetone R*. Combine the filtrate and washings and dilute to 100.0 ml with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant liquid.

Reference solution Dissolve 0.310 g of *glycollic acid R*, previously dried *in vacuo* over *diphosphorus pentoxide* R at room temperature overnight, in *water R* and dilute to 500.0 ml with the same solvent. To 5.0 ml of this solution add 5 ml of *acetic acid R* and allow to stand for about 30 min. Add 50 ml of *acetone R* and 1 g of *sodium chloride R.* Filter through a fast filter paper impregnated with *acetone R*, rinse the beaker and filter with *acetone R*. Combine the filtrate and washings and dilute to 100.0 ml with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant liquid.

Heat 2.0 ml of the test solution on a water-bath for 20 min. Cool to room temperature and add 20.0 ml of *2,7-dihydroxynaphthalene solution R.* Shake and heat in a water-bath for 20 min. Cool under running water, transfer quantitatively to a volumetric flask and dilute to 25.0 ml with *sulphuric acid R*, maintaining the flasks under running water. Within 10 min, measure the absorbance at 540 nm (*2.2.25*) using *water R* as the compensation liquid. The absorbance of the solution prepared with the test solution is not greater than that of a solution prepared at the same time and in the same manner with 2.0 ml of the reference solution.

Sodium chloride

Maximum 7.0 per cent.

Place 0.500 g in a beaker and suspend in 100 ml of *water R*. Add 1 ml of *nitric acid R*. Titrate with 0.1 M silver nitrate, determining the end-point potentiometrically *(2.2.20)* using a silver indicator electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Iron *(2.4.9)*

Maximum 20 ppm determined on 10 ml of solution S2.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 1.5 h.

Microbial contamination

It complies with the test for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

Shake 1.000 g with 20 ml of *ethanol (80 per cent V/V) R*, stir for 10 min and filter. Repeat the operation until chloride has been completely extracted and verify the absence of chloride using *silver nitrate R2*. Dry the residue at 105 °C to constant mass. To 0.700 g of the dried residue, add 80 ml of *glacial acetic acid R* and heat under a reflux condenser for 2 h. Cool the solution to room temperature. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of 0*.1 M perchloric acid* is equivalent to 2.299 mg of Na.

STORAGE

In an airtight container, protected from light.

Ph Eur

Sodium Starch Glycolate (Type C)

General Notices

(Ph Eur monograph 1566)

Action and use

Excipient.

Ph Eur

DEFINITION

Sodium salt of a partly *O*-carboxymethylated starch, cross-linked by physical dehydration.

Content

2.8 per cent to 5.0 per cent of Na (A_r 22.99) (substance washed with ethanol (80 per cent *V/V*) and dried).

CHARACTERS

Appearance

White or almost white, fine, free-flowing powder, very hygroscopic.

Microscopic examination

It is seen to consist of granules, irregularly shaped, ovoid or pear-shaped, 30-100 µm in size, or rounded, 10-35 µm in size; compound granules consisting of 2-4 components occur occasionally; the granules have an eccentric hilum and clearly visible concentric striations; between crossed nicol prisms, the granules show a distinct black cross intersecting at the hilum; small crystals are visible at the surface of the granules. The granules show considerable swelling in contact with water.

Solubility

Soluble in water, practically insoluble in methylene chloride. It gives a translucent gel-like product in water.

IDENTIFICATION

A. pH (see Tests).

B. Prepare with shaking and without heating a mixture of 4.0 g of the substance to be examined and 20 ml of *carbon dioxide-free water R*. The mixture has the appearance of a gel. Add 100 ml of *carbon dioxide-free water R* and shake: the gel remains stable (difference from types A and B). Keep the gel for the tests for appearance of gel and pH.

C. To 5 ml of the gel obtained in identification test B add 0.05 ml of *iodine solution R1*. A dark blue colour is produced.

D. Solution S (see Tests) gives reaction (a) of sodium *(2.3.1).*

TESTS

Solution S

Place 2.5 g in a silica or platinum crucible and add 2 ml of a 500 g/l solution of *sulphuric acid R*. Heat on a water-bath, then cautiously over a naked flame, raising the temperature progressively, and then incinerate in a muffle furnace at 600 ± 25 °C. Continue heating until all black particles have disappeared. Allow to cool, add a few drops of *sulphuric acid R,* heat and incinerate as above. Allow to cool, add a few drops of *ammonium carbonate solution R*, evaporate to dryness and incinerate cautiously. Allow to cool and dissolve the residue in 50 ml of *water R*.

Appearance of gel

The gel obtained in identification test B is colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.5 to 7.5 for the gel obtained in identification test B.

Sodium glycolate

Maximum 2.0 per cent. Carry out the test protected from light.

Test solution Place 0.20 g in a beaker. Add 5 ml of *acetic acid R* and 5 ml of *water R*. Stir until dissolution is complete (about 10 min). Add 50 ml of *acetone R* and 1 g of *sodium chloride R.* Filter through a fast filter paper impregnated with *acetone R,* rinse the beaker and filter with *acetone R.* Combine the filtrate and washings and dilute to 100.0 ml with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant liquid.

Reference solution Dissolve 0.310 g of *glycollic acid R*, previously dried *in vacuo* over *diphosphorus pentoxide R*, in *water R* and dilute to 500.0 ml with the same solvent. To 5.0 ml of this solution, add 5 ml of *acetic acid R* and allow to stand for about 30 min. Add 50 ml of *acetone R* and 1 g of *sodium chloride R* and dilute to 100.0 ml with *acetone R*.

Heat 2.0 ml of the test solution on a water-bath for 20 min. Cool to room temperature and add 20.0 ml of *2,7-dihydroxynaphthalene solution R*. Shake and heat on a water-bath for 20 min. Cool under running water, transfer to a volumetric flask and dilute to 25.0 ml with sulphuric *acid R*, maintaining the flask under running water. Within 10 min, measure the absorbance *(2.2.25)* at 540 nm using *water R* as the compensation liquid. The absorbance of the solution prepared with the test solution is not greater than that of a solution prepared at the same time and in the same manner with 2.0 ml of the reference solution.

Sodium chloride

Maximum 1 per cent.

Shake 1.00 g with 20 ml of *ethanol (80 per cent V/V) R* for 10 min and filter. Repeat the operation 4 times. Dry the residue to constant mass at 100 °C and set aside for the assay. Combine the filtrates. Evaporate to dryness, take up the residue with *water R* and dilute to 25.0 ml with the same solvent. To 10.0 ml of the solution add 30 ml of *water R* and 5 ml of *dilute nitric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*, using a silver indicator electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Iron *(2.4.9)*

Maximum 20 ppm, determined on solution S.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 7.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Microbial contamination

It complies with the test for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

To 0.500 g of the dried and crushed residue obtained in the test for sodium chloride add 80 ml of *anhydrous acetic acid R* and heat under a reflux condenser for 2 h. Cool the solution to room temperature. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20).* Carry out a blank test.

1 ml of *0.1 M perchloric acid* is equivalent to 2.299 mg of Na.

STORAGE

In an airtight container, protected from light.

Ph Eur

Sodium Stearate

General Notices

(Ph Eur monograph 2058)

822-16-2

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of sodium salts of different fatty acids consisting mainly of stearic (octadecanoic) acid [$C_{17}H_{35}COONa$; M_r 306.5] and palmitic (hexadecanoic) acid [$C_{15}H_{31}COONa$; M_r 278.4].

Content:

— *sodium*: 7.4 per cent to 8.5 per cent (A_r 22.99) (dried substance);

— *stearic acid in the fatty acid fraction*: minimum 40 per cent;

— *sum of stearic acid and palmitic acid in the fatty acid fraction*: minimum 90 per cent.

CHARACTERS

Appearance

White or yellowish, fine powder, greasy to the touch.

Solubility

Slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification C, D.

Second identification A, B, D.

A. Freezing point *(2.2.18)*

Minimum 53 °C for the residue obtained in the preparation of solution S (see Tests).

B. Acid value *(2.5.1)*

195 to 210, determined on 0.200 g of the residue obtained in the preparation of solution S dissolved in 25 ml of the prescribed mixture of solvents.

C. Examine the chromatograms obtained in the assay of stearic acid and palmitic acid.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the 2 principal peaks in the chromatogram obtained with the reference solution.

D. Solution S gives reaction (b) of sodium *(2.3.1)*.

TESTS

Solution S

To 10.0 g add 100 ml of *peroxide-free ether R* and 80 ml of *acetic acid R*. Boil under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 8 ml, of *acetic acid R*. Combine the aqueous layers, wash with 30 ml of *peroxide-free ether R* and dilute to 100 ml with *distilled water R* (solution S). Evaporate the ether layers to dryness on a water-bath and dry the residue at 100-105 °C.

Acidity or alkalinity

Suspend 2.0 g in 50 ml of previously neutralised *ethanol (96 per cent) R.* Heat under reflux to dissolve and add 3 drops of *phenolphthalein solution R*; the solution is colourless. Not less than 0.60 ml and not more than 0.85 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides *(2.4.4)*

Maximum 0.2 per cent.

Dilute 0.25 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 0.3 per cent.

Dilute 0.5 ml of solution S to 15 ml with *distilled water R*.

Nickel

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Place 50.0 mg of the substance to be examined in a polytetrafluoroethylene digestion flask and add 0.5 ml of a mixture of 1 volume of *heavy metal-free hydrochloric acid R* and 5 volumes of *heavy metal-free nitric acid R*. Allow to digest at 170 °C for 5 h. Allow to cool. Dissolve the residue in *water R* and dilute to 5.0 ml with the same solvent.

Reference solutions Prepare the reference solutions using *nickel standard solution (10 ppm Ni) R*, diluted as necessary with *water R*.

Source Nickel hollow-cathode lamp.

Wavelength 232.0 nm.

Atomisation device air-acetylene flame.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent.

In a weighing glass introduce 1.0 g of previously *washed sand R*, dry at 105 °C and weigh. Add 0.500 g of the substance to be examined and 10 ml of *ethanol (96 per cent) R*. Evaporate at 80 °C and dry the residue at 105 °C for 4 h.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli (2.6.13)*.

ASSAY

Sodium

Dissolve 0.250 g with gentle heating in a mixture of 5 ml of *acetic anhydride R* and 20 ml of *anhydrous acetic acid R*. Cool and add 20 ml of *dioxan R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 2.299 mg of Na.

Stearic acid and palmitic acid

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution In a conical flask fitted with a reflux condenser, dissolve 0.10 g of the substance to be examined in 5 ml of *boron trifluoride-methanol solution R.* Boil under a reflux condenser for 10 min. Add 4 ml of *heptane R* through the condenser and boil again under a reflux condenser for 10 min. Allow to cool. Add 20 ml of *saturated sodium chloride solution R.* Shake and allow the layers to separate. Remove about 2 ml of the organic layer and dry over 0.2 g of *anhydrous sodium sulphate R.* Dilute 1.0 ml of the solution to 100.0 ml with *heptane R.*

Reference solution Prepare the reference solution in the same manner as the test solution using 50.0 mg of *palmitic acid CRS* and 50.0 mg of *stearic acid CRS* instead of the substance to be examined.

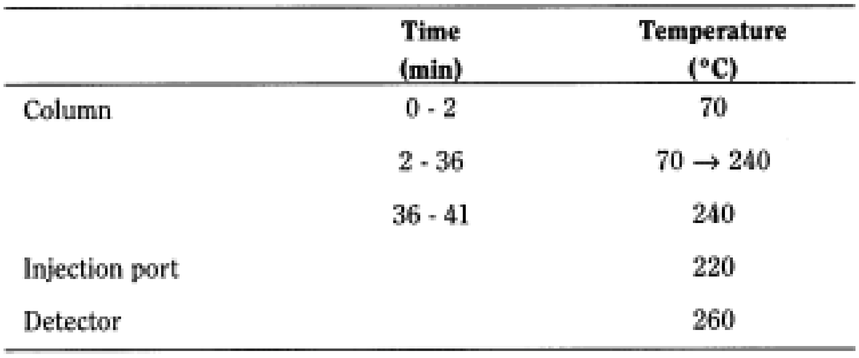
Column:

- *material:* fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.4 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	70
	2 - 36	70 → 240
	36 - 41	240
Injection port		220
Detector		260

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to methyl stearate (retention time = about 40 min): methyl palmitate = about 0.88.

System suitability Reference solution:

— *resolution:* minimum 5.0 between the peaks due to methyl stearate and methyl palmitate.

Calculate the content of stearic acid and palmitic acid.

STORAGE

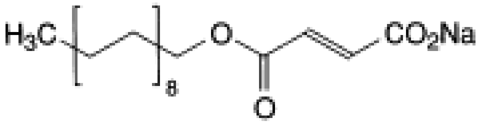
In an airtight container , protected from light.

Ph Eur

Sodium Stearyl Fumarate

General Notices

(Ph Eur monograph 1567)



$C_{22}H_{39}NaO_4$ 390.5 *4070-80-8*

Ph Eur

DEFINITION

Sodium octadecyl (*E*)-butenedioate.

Content

99.0 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, fine powder with agglomerates of flat, circular particles.

Solubility

Practically insoluble in water, slightly soluble in methanol, practically insoluble in acetone and in ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Sodium stearyl fumarate CRS.*

TESTS

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Silylation solution To 2 ml of *N,O- bis(trimethylsilyl)trifluoroacetamide R* add 0.02 ml of *chlorotrimethylsilane R* and mix.

Test solution Introduce 15.0 mg of the substance to be examined in a vial with a screw cap and add 1 ml of the silylation solution. Seal the vial and heat at about 70 °C for 1 h. After the reaction a precipitate remains in the vial; filter the solution through a nylon filter (pore size 0.45 µm).

Reference solution Introduce 1.0 mg of sodium *stearyl maleate CRS* and 1.0 mg of *sodium stearyl fumarate CRS* into a vial with a screw cap and add 1 ml of the silylation solution. Seal the vial and heat at about 70 °C for 1 h.

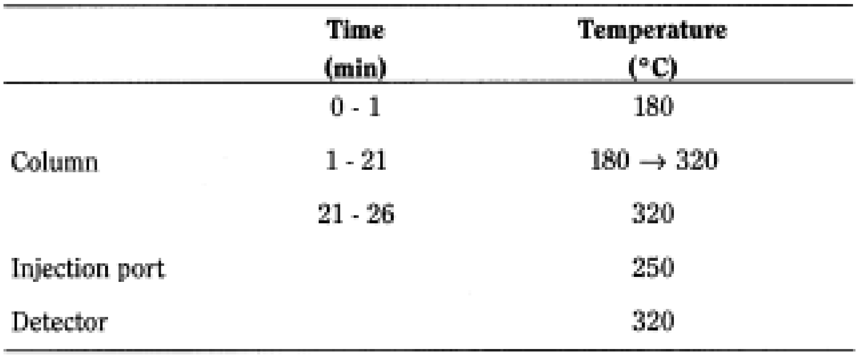
Column:
- *material:* fused silica;
- *size*: *l* = 15 m, Ø = 0.53 mm;
- *stationary phase: poly(dimethyl)siloxane R* (film thickness 0.15 µm).

Carrier gas *helium for chromatography R* .

Flow rate 2 ml/min.

Split ratio 1:25.

Temperature:



	Time (min)	Temperature (°C)
	0 - 1	180
Column	1 - 21	180 → 320
	21 - 26	320
Injection port		250
Detector		320

Detection Flame ionisation.

Injection 2 µl.

Relative retention With reference to stearyl trimethylsilyl fumarate (retention time = about 9 min): stearyl alcohol = 0.30; stearyl trimethylsilyl ether = 0.35; palmityl trimethylsilyl fumarate = 0.80; heptadecyl trimethylsilyl fumarate = 0.85; stearyl trimethylsilyl maleate = 0.90; nonadecyl trimethylsilyl fumarate = 1.05; eicos-11-enyl trimethylsilyl fumarate = 1.15; distearyl fumarate = 2.25.

System suitability:

- *resolution*: minimum 1.5 between the peaks in the chromatogram obtained with the reference solution.

Limits:
- *any impurity:* maximum 0.5 per cent;

— *total*: maximum 5.0 per cent.

Water ***(2.5.12)***

Maximum 5.0 per cent, determined on 0.250 g.

ASSAY

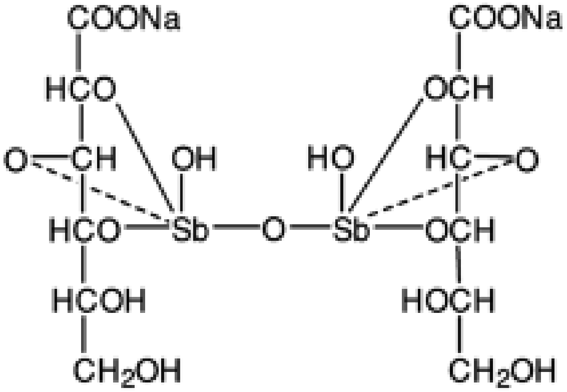
Dissolve 0.250 g, accurately weighed, in 10 ml of *methylene chloride R* and add 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 39.05 mg of $C_{22}H_{39}NaO_4$.

Ph Eur

Sodium Stibogluconate

General Notices



16037-91-5

Action and use

Trivalent ammonium compound; antiprotozoal.

Preparation

Sodium Stibogluconate Injection

DEFINITION

Sodium Stibogluconate is mainly the disodium salt of µ-oxy-bis[gluconato(3-)-*O* ^{2}, *O* ^{3},*O* ^{4}-hydroxoantimony]. It contains not less than 30.0% and not more than 34.0% of antimony(V), calculated with reference to the dried and methanol-free substance.

PRODUCTION

The method of manufacture is such as to ensure consistently controlled reaction stoichiometry in order to yield sodium stibogluconate that is satisfactory with regard to intrinsic toxicity.

CHARACTERISTICS

A colourless, mostly amorphous powder.

Very soluble in *water*; practically insoluble in *ethanol (96%)* and in *ether* .

IDENTIFICATION

A. A solution is dextrorotatory.

B. Pass *hydrogen sulphide* into a 5% w/v solution for several minutes. An orange precipitate is produced.

C. When heated, it chars without melting leaving a residue which yields the reactions characteristic of *antimony compounds* and the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Stability and acidity of solution

Heat a solution containing the equivalent of 10% w/v of antimony(V) in an autoclave at 115.5° and at a pressure of 70 kPa for 30 minutes. The resulting solution is colourless or almost colourless and has a pH of 5.0 to 5.6, Appendix V L.

Antimony(III)

Dissolve 2 g in 30 ml of *water*, add 15 ml of *hydrochloric acid* and titrate with 0.00833M *potassium bromate VS* using *methyl orange solution* as indicator. Not more than 1.3 ml of 0.00833M *potassium bromate VS* is required.

Chloride

Dissolve 2.5 g in 50 ml of *water* and add 2 ml of 2M *nitric acid* and 75 ml of *acetate buffer pH 5.0*. Titrate with 0.1M *silver nitrate VS* determining the end point potentiometrically. Not more than 3.0 ml of 0.1M *silver nitrate VS* is required.

Methanol

Not more than 2.0% w/w when determined by the following method. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 1 ml of a 1.0% v/v solution of *methanol* to 5 ml of a 0.2% v/v solution of *absolute ethanol* (internal standard). For solution (2) add 5 ml of *water* to 0.5 g of the substance being examined and mix with the aid of ultrasound until solution is complete. For solution (3) add 5 ml of a 0.2% v/v solution of the internal standard to 0.5 g of the substance being examined and mix with the aid of ultrasound until solution is complete.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with porous polymer beads (80 to 100 mesh) (Porapak Q and Chromosorb 101 are suitable) and maintained at 130°.

Calculate the percentage w/w of methanol taking 0.792 g as its weight per ml at 20°.

Loss on drying

When dried to constant weight at 130° at a pressure not exceeding 0.7 kPa, loses not more than 15.0% of its weight. Use 1 g.

ASSAY

Dissolve 0.16 g in 30 ml of *hydrochloric acid*, add 70 ml of *orthophosphoric acid* and stir carefully until completely mixed. Titrate with 0.05M *ammonium iron(II) sulphate VS* prepared using *sulphuric acid (1%)* and determining the end point potentiometrically using a platinum electrode and a silver-silver chloride reference electrode. Each ml of 0.05M *ammonium iron(II) sulphate VS* is equivalent to 3.044 mg of antimony(V).

Sodium Sulphite Heptahydrate

General Notices

Sodium Sulphite

(Ph Eur monograph 0776)

$Na_2SO_3,7H_2O$ 252.2 *10102-15-5*

Action and use

Antioxidant.

Ph Eur

DEFINITION

Content

48.0 per cent to 52.5 per cent of Na_2SO_3.

CHARACTERS

Appearance

Colourless crystals.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly alkaline *(2.2.4)* .

B. To 5 ml of solution S add 0.5 ml of *0.05 M iodine* . The solution is colourless and gives reaction (a) of sulphates *(2.3.1)* .

C. Solution S gives reaction (a) of sodium *(2.3.1)* .

D. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 10 g in *water R* and dilute to 100 ml with the same solvent.

Solution S1

To 20.0 g add 25 ml of *water R* . Shake until mostly dissolved, and carefully and progressively, add 15 ml of *hydrochloric acid R* . Heat to boiling. Cool and dilute to 100.0 ml with *water R* .

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method I)*.

Thiosulphates

Maximum 0.05 per cent.

To 4.00 g add 100 ml of *water R* . Shake to dissolve, add 10 ml of *formaldehyde solution R* and 10 ml of *acetic acid R* . Allow to stand for 5 min, then add 0.5 ml of *starch solution R* and titrate with *0.05 M iodine* . Carry out a blank titration. The difference between the volumes used in the titrations is not more than 0.15 ml.

Iron *(2.4.9)*

Maximum 5 ppm, determined on solution S1.

Selenium

Maximum 5 ppm.

To 6.0 g add 10 ml of *formaldehyde solution R* , carefully and progressively add 2 ml of *hydrochloric acid R* . Heat on a water-bath for 20 min. Any pink colour in the solution is not more intense than that of a standard prepared at the same time and in the same manner using 2.0 g of the substance to be examined to which 0.2 ml of *selenium standard solution (100 ppm Se) R* has been added.

Zinc

Maximum 12.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dilute 2.0 ml of solution S1 to 10.0 ml with *water R.*

Reference solutions Prepare the reference solutions using *zinc standard solution (100 ppm Zn) R* , diluting with *water R* .

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame.

Heavy metals *(2.4.8)*

Maximum 5 ppm.

Evaporate 20 ml of solution S1 almost to dryness. Add 10 ml of *water R* , neutralise with *concentrated ammonia R* and dilute to 20 ml with *water R* . 12 ml of this solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R* .

ASSAY

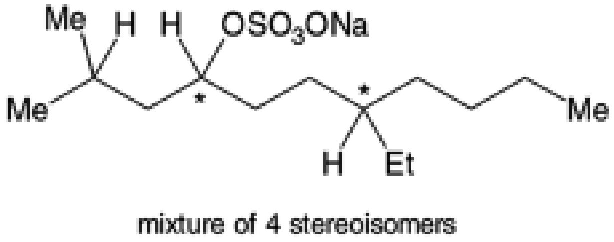
Introduce 0.500 g into a 500 ml conical flask containing 50.0 ml of *0.05 M iodine* . Shake until completely dissolved. Add 1 ml of *starch solution R* and titrate the excess of iodine with *0.1 M sodium thiosulphate* . Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 6.30 mg of Na_2SO_3.

Ph Eur

Sodium Tetradecyl Sulphate Concentrate

General Notices



$C_{14}H_{29}NaO_4S$ 316.4 *139-88-8*

Action and use

Sclerosant.

Preparation

Sodium Tetradecyl Sulphate Injection

DEFINITION

Sodium Tetradecyl Sulphate Concentrate is an aqueous gel containing sodium *all-rac*-4-ethyl-1-isobutyloctyl sulphate. It contains not less than 46.0% w/w and not more than 52.0% w/w of $C_{14}H_{29}NaO_4S$.

CHARACTERISTICS

A clear, colourless gel.

IDENTIFICATION

A. Carry out the method for *gas chromatography* , Appendix III B, using the following solutions. For solution (1) boil 0.2 g under a reflux condenser with 20 ml of 2M *hydrochloric acid* for 15 minutes, allow to cool, add 20 ml of *ethanol (96%)* and extract the mixture with two 10 ml quantities of n- *pentane* . Wash the combined pentane extracts with 20 ml of *water* and dry over *anhydrous sodium sulphate* . Solution (2) contains 0.35% w/v of *decan-1-ol* and 0.7% w/v of *dodecan-1-ol* in n- *pentane* .

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of polyethylene glycol (Carbowax 20M is suitable) and maintained at 120°.

The retention time of the principal peak in the chromatogram obtained with solution (1) is less than the retention time of the peak due to dodecan-1-ol and more than that of the peak due to decan-1-ol in the chromatogram obtained with solution (2).

B. Mix 0.1 ml of a 2% w/v solution with 0.1 ml of a 0.1% w/v solution of *methylene blue* and 2 ml of 1M *sulphuric acid* , add 2 ml of *chloroform* and shake. The chloroform layer is intensely blue.

C. Mix 20 mg with 10 ml of *ethanol (96%)* and heat to boiling on a water bath, shaking frequently. Filter immediately and evaporate the ethanol. Dissolve the residue in 8 ml of *water*, add 3 ml of 2M *hydrochloric acid* , evaporate the solution to half its volume and cool. Filter to remove the congealed fatty alcohols and add 1 ml of 0.25M *barium chloride* to the filtrate. A white, crystalline precipitate is produced.

D. Yields reaction B characteristic of *sodium salts* , Appendix VI.

TESTS

Alkalinity

Dissolve 1 g in 100 ml of *water* and add 0.1 ml of *methyl red solution* ; the solution is yellow. Not more than 0.5 ml of 0.1M *hydrochloric acid VS* is required to change the colour of the solution.

Non-ionic impurities

Not more than 3.0% w/w, with respect to the nominal content of Sodium Tetradecyl Sulphate, when determined by the method for *gas chromatography* , Appendix III B, using the following solutions. Dissolve 75 mg of *dodecan-1-ol* (internal standard) in sufficient n- *hexane* to produce 100 ml (solution A). For solution (1) extract 1 g of the concentrate being examined with 10 ml of solution A followed by two 10 ml quantities of n- *hexane* . Pass 10 ml of the combined extracts at a rate of about 1.5 ml per minute through a column, 1 cm in diameter, packed with 5 g of *basic aluminium oxide* (Brockmann grade II is suitable) and pre-washed with 25 ml of n- *hexane* . Elute with 20 ml of a mixture of equal volumes of n- *hexane* and *ether* , evaporate to dryness using a rotary evaporator and dissolve the residue in 2 ml of n- *hexane* . Prepare solution (2) in the same manner but using 10 ml of n- *hexane* in place of 10 ml of solution A.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of polyethylene glycol (Carbowax 20M is suitable) and maintained at 120°.

Allow the chromatography to proceed for the retention time of the peak due to the internal standard.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than twice the area of the peak due to the internal standard.

Chloride

Dissolve 33 mg in sufficient *water* to produce 100 ml. 15 ml of the resulting solution complies with the *limit test for chlorides* , Appendix VII (1%).

Sulphated ash

10.3 to 13.3%, Appendix IX A.

ASSAY

Dissolve 2.4 g in sufficient *water* to produce 1000 ml. To 20 ml add 15 ml of *chloroform* and 10 ml of *dimidium bromide-sulphan blue mixed solution* and titrate with *0.004 M benzethonium chloride VS*, shaking vigorously and allowing the layers to separate after each addition, until the pink colour of the chloroform layer is completely discharged and a greyish blue colour is produced. Each ml of *0.004 M benzethonium chloride VS* is equivalent to 1.266 mg of $C_{14}H_{29}NaO_4S$.

STORAGE

Sodium Tetradecyl Sulphate Concentrate should be protected from light.

Sodium Thiosulphate

General Notices

(Ph Eur monograph 0414)

$Na_2S_2O_3,5H_2O$ 248.2 *10102-17-7*

Action and use

Used in treatment of cyanide poisoning.

Preparation

Sodium Thiosulphate Injection

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of $Na_2S_2O_3,5H_2O$.

CHARACTERS

Appearance

Transparent, colourless crystals, efflorescent in dry air.

Solubility

Very soluble in water, practically insoluble in ethanol 96 per cent. It dissolves in its water of crystallisation at about 49 °C.

IDENTIFICATION

A. It decolourises iodinated *potassium iodide solution R* .

B. To 0.5 ml of solution S (see Tests) add 0.5 ml of *water R* and 2 ml of *silver nitrate solution R2* . A white precipitate is formed which rapidly becomes yellowish and then black.

C. To 2.5 ml of solution S add 2.5 ml of *water R* and 1 ml of *hydrochloric acid R* . A precipitate of sulphur is formed and gas is evolved which gives a blue colour to *starch iodate paper R* .

D. 1 ml of solution S gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

The freshly prepared solution is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Dissolve 10.0 g in 50 ml of *distilled water R* , add 1 ml of *0.1 M sodium hydroxide* and dilute to 100 ml with the same solvent.

pH *(2.2.3)*

6.0 to 8.4 for the freshly prepared solution S.

Sulphates and sulphites *(2.4.13)*

Maximum 0.2 per cent.

Dilute 2.5 ml of freshly prepared solution S to 10 ml with *distilled water R* . To 3 ml of this solution first add 2 ml of *iodinated potassium iodide solution R* and continue the addition dropwise until a very faint persistent yellow colour appears. Dilute to 15 ml with *distilled water R* .

Sulphides

To 10 ml of solution S add 0.05 ml of a freshly prepared 50 g/l solution of *sodium nitroprusside R* . The solution does not become violet.

Heavy metals

Maximum 10 ppm.

To 10 ml of solution S add 0.05 ml of *sodium sulphide solution R* . After 2 min, any brown colour in the solution is not more intense than that in a reference solution prepared at the same time and in the same manner using 10 ml of *lead standard solution (1 ppm Pb) R.*

ASSAY

Dissolve 0.500 g in 20 ml of *water R* and titrate with *0.05 M iodine,* using 1 ml of *starch solution R,* added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 24.82 mg of $Na_2S_2O_3,5H_2O$.

STORAGE

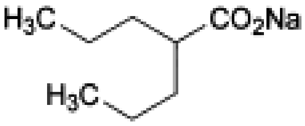
In an airtight container .

Ph Eur

Sodium Valproate

General Notices

(Ph Eur monograph 0678)



$C_8H_{15}NaO_2$ 166.2 *1069-66-5*

Action and use

Antiepileptic.

Preparations

Sodium Valproate Oral Solution

Sodium Valproate Tablets

Gastro-resistant Sodium Valproate Tablets

Ph Eur

DEFINITION

Sodium 2-propylpentanoate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline, hygroscopic powder.

Solubility

Very soluble in water, slightly or freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium valproate CRS.*

If the spectra obtained in the solid state show differences, record new spectra using discs prepared by placing 50 µl of a 100 g/l solution in *methanol R* on a disc of *potassium bromide R* and evaporating the solvent *in vacuo*. Examine immediately.

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time to the principal peak in the chromatogram obtained with reference solution (b).

C. 2 ml of solution S (see Tests) gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 1.25 g in 20 ml of *distilled water R* in a separating funnel, add 5 ml of *dilute nitric acid R* and shake. Allow the mixture to stand for 12 h. Use the lower layer.

Appearance of solution

The solution is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 2.0 g in *water R* and dilute to 10 ml with the same solvent.

Acidity or alkalinity

Dissolve 1.0 g in 10 ml of *water R.* Add 0.1 ml of *phenolphthalein solution R* . Not more than 0.75 ml *of 0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 10 mg of *butyric acid R* in *heptane R* and dilute to 200 ml with the same solvent.

Test solution (a) Dissolve 0.500 g of the substance to be examined in 10 ml of *water R* . Add 5 ml of *dilute sulphuric acid R* and shake with 3 quantities, each of 20 ml, of *heptane R.* Add 10.0 ml of the internal standard solution to the combined upper layers, shake with anhydrous *sodium sulphate R* , filter and evaporate the filtrate, at a temperature not exceeding 30 °C, using a rotary evaporator. Take up the residue with *heptane R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *heptane R* .

Test solution (b) Dissolve 40 mg of the substance to be examined in 100 ml of *water R* . To 10 ml of this solution add 0.5 ml of *dilute sulphuric acid R* and shake with 3 quantities, each of 5 ml, of *heptane R* . Shake with *anhydrous sodium sulphate R,* filter and evaporate the filtrate, at a temperature not exceeding 30 °C, to a volume of about 10 ml, using a rotary evaporator.

Reference solution (a) Dissolve 20 mg of *2-(1-methylethyl)pentanoic acid CRS* (impurity C) in 5.0 ml of test solution (b) and dilute to 10 ml with *heptane R* . Dilute 1 ml of this solution to 10 ml with *heptane R.*

Reference solution (b) Prepare as prescribed for test solution (b), using *sodium valproate CRS* instead of the substance to be examined.

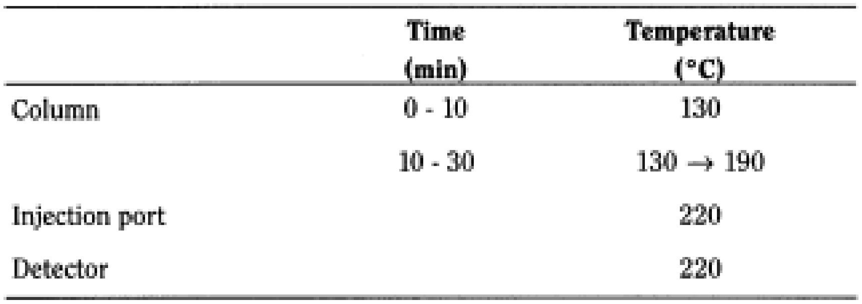
Column:

- — *material*: wide-bore fused silica;
- — *size:* *l* = 30 m, Ø = 0.53 mm;
- — *stationary phase*: *macrogol 20 000 2-nitroterephthalate R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 8 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	130
	10 - 30	130 → 190
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (a):

- — *resolution*: minimum 3.0 between the peaks due to impurity C and valproic acid.

Limits Test solution (a):

- — *any impurity*: for each impurity, not more than the area of the peak due to the internal standard (0.1 per cent);
- — *total:* not more than 3 times the area of the peak due to the internal standard (0.3 per cent);
- — *disregard limit:* 0.1 times the area of the peak due to the internal standard (0.01 per cent).

Chlorides ***(2.4.4)***

Maximum 200 ppm.

To 5 ml of solution S add 10 ml of *water R.*

Sulphates ***(2.4.13)***

Maximum 200 ppm, determined on Solution S.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C

ASSAY

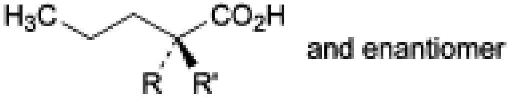
Dissolve 0.1500 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 16.62 mg of $C_8H_{15}NaO_2$.

STORAGE

In an airtight container.

IMPURITIES

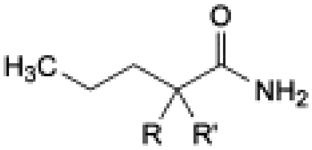


A. R = R′ = H: pentanoic acid (valeric acid),

B. R = H, R′ = CH_2-CH_3: (2*RS*)-2-ethylpentanoic acid,

C. R = H, R′ = $CH(CH_3)_2$: (2*RS*)-2-(1-methylethyl)pentanoic acid,

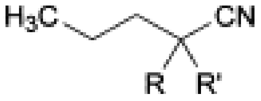
D. R = R′ = CH_2-CH_2-CH_3: 2,2-dipropylpentanoic acid,



E. R = R′ = H: pentanamide (valeramide),

F. R = H, R′ = CH_2-CH_2-CH_3: 2-propylpentanamide,

G. R = R′ = CH_2-CH_2-CH_3: 2,2-dipropylpentanamide,



H. R = R′ = H: pentanenitrile (valeronitrile),

I. R = H, R′ = CH_2-CH_2-CH_3: 2-propylpentanenitrile,

J. R = R′ = CH_2-CH_2-CH_3: 2,2-dipropylpentanenitrile.

Ph Eur

Soft Soap

General Notices

Preparation

Soap Spirit

DEFINITION

Soft Soap is soap made by the interaction of potassium hydroxide or sodium hydroxide with a suitable vegetable oil or oils or with fatty acids derived there from. It yields not less than 44.0% of fatty acids. It may be coloured with chlorophyll or not more than 0.015% of a suitable green soap dye.

CHARACTERISTICS

A yellowish white to green or brown, unctuous substance.

Soluble in *water* and in *ethanol (96%)*.

TESTS

Chlorides and other ethanol-insoluble substances

Dissolve 5 g in 100 ml of hot *ethanol (96%)* previously neutralised to *phenolphthalein solution R1*, filter through a dried and tared filter, wash the residue thoroughly with hot neutralised *ethanol (96%)* and dry to constant weight at 105°. The residue weighs not more than 0.15 g.

Free fatty acid or alkali hydroxide

Boil 250 ml of *ethanol (96%)* to remove carbon dioxide, add 0.5 ml of *phenolphthalein solution R1*, allow to cool to 70° and neutralise, if necessary, with 0.1M *sodium hydroxide VS* or 0.05M *sulphuric acid VS*. To 100 ml of the neutral ethanol add 10 g of the substance being examined and dissolve it as quickly as possible by heating under a reflux condenser. Cool to 70° and, if the solution is not pink, titrate at 70° with 0.1M *sodium hydroxide VS*; not more than 0.2 ml is required. If the solution is pink add, in a thin stream, 5 ml of hot *barium chloride solution* previously neutralised to *phenolphthalein solution R1*, mix thoroughly and titrate with 0.1M *hydrochloric acid VS* until the pink colour disappears; not more than 1.0 ml is required.

Total free alkali

To 100 ml of the neutral ethanol prepared as described in the test for Free fatty acid or alkali hydroxide add 10 g of the substance being examined and dissolve it as quickly as possible by heating under a reflux condenser. Add immediately 3 ml of 0.5M *sulphuric acid VS* and boil under a reflux condenser on a water bath for at least 10 minutes. If the solution is not pink, cool to 70° and titrate with 1M *sodium hydroxide VS* until a pink colour is produced. The volume of 0.5M *sulphuric acid VS* neutralised by the substance being examined is not more than 1.0 ml.

Unsaponifiable matter and unsaponified neutral fat

Dissolve 5 g in 80 ml of a mixture of 50 ml of *ethanol (96%)* and 100 ml of *water*, without heating more than is necessary, and transfer to a separating funnel, washing the vessel with the remaining 70 ml of the mixture. Extract with 100 ml of *ether* while still slightly warm, run off the ethanolic soap layer into a second separating funnel and extract with 50 ml of *ether*. Repeat the extraction with 50 ml of *ether* and pour the three ether extracts into a separating funnel containing 20 ml of *water*. Rotate the separating funnel without violent shaking and, after allowing the liquids to separate, run off the water. Repeat the washing with *water* in the same manner until the separated washings are not more than faintly turbid when acidified. Wash the ether solution twice by shaking vigorously with 20 ml of 0.5M *potassium hydroxide*, each washing with alkali being immediately followed by washing with 20 ml of *water*, shaking vigorously each time. Acidify the last alkali washing after separation and, if the liquid becomes turbid, repeat the washing with 0.5M *potassium hydroxide* and *water* until the alkali washing remains clear on acidification. Finally wash with successive 20 ml quantities of *water* until the washings do not give a pink colour with *phenolphthalein solution R1*. Transfer the ether solution to a tared flask and remove the ether. When nearly all the ether has evaporated, add 3 ml of *acetone*. With the aid of a gentle current of air remove the solvent completely from the flask, which is preferably almost entirely immersed in boiling *water*, held obliquely and rotated. Repeat the last operation until the weight of the residue is constant. The residue weighs not more than 40 mg.

CHARACTERISTICS OF THE FATTY ACIDS OBTAINED IN THE ASSAY

Acid value

Not more than 205, Appendix X B, when determined on 2 to 3 g of the fatty acids, using 0.5M *potassium hydroxide VS* and substituting 2.805 for 5.610 in the formula.

Iodine value

Not less than 83 (*iodine monochloride method*), Appendix X E.

Solidifying point

Not above 31°, Appendix V B, with the following modifications. Where the determination is made at 15° to 20° the 1000 ml beaker and cooling liquid need not be used. Where the room temperature falls outside this range the 1000 ml beaker should contain water maintained at 15° to 20° and the level of this water should not be below the level of the sample in the inner tube.

Transfer about 15 ml of the melted fatty acids to the inner test tube. Before the temperature of the fatty acids drops to a point 10° above their expected solidifying point, begin agitation in a vertical manner at a rate of 100 complete up and down motions per minute, the stirrer moving through a vertical distance of about 38 mm. Continue stirring in this manner until the temperature has remained constant for 30 seconds or has begun to rise within 30 seconds of ceasing to fall. Discontinue stirring immediately and lift the stirrer out of the sample. Observe the rise in temperature; the highest temperature reached after cessation of stirring is the solidifying point of the fatty acids. When reading the thermometer avoid all undue vibration as this will cause the temperature to drop before reaching the maximum.

Resin

Mix 0.5 ml of the melted fatty acids in a test tube with 2 ml of *acetic anhydride*, warm, shake until clear and cool to 15.5°. Transfer one drop of this solution to a white porcelain tile, place one drop of a cold mixture of equal volumes of *sulphuric acid* and *water* adjacent to it and gently bring the drops together with a glass rod. No transient violet colour is produced.

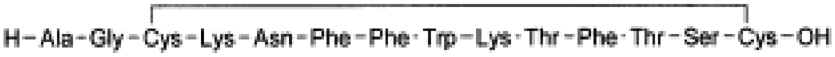
ASSAY

Dissolve 30 g in 100 ml of *water*, transfer to a separating funnel, acidify with 1M *sulphuric acid* and extract with successive quantities of 50, 40 and 30 ml of *ether*. Mix the ether solutions in a separating funnel and wash with *water* until the washings are free from mineral acid. Transfer the ether solution to a tared flask, remove the ether and dry the residue of fatty acids to constant weight at 80°.

Somatostatin

General Notices

(Ph Eur monograph 0949)



H–Ala–Gly–Cys–Lys–Asn–Phe–Phe–Trp–Lys–Thr–Phe–Thr–Ser–Cys–OH

$C_{76}H_{104}N_{18}O_{19}S_2$ 1638 *38916-34-6*

Action and use

Growth hormone release inhibiting hormone.

Ph Eur

DEFINITION

L-Alanylglycyl-L-cysteinyl-L-lysyl-L-asparaginyl-L-phenylalanyl-L-phenylalanyl-L-tryptophyl-L-lysyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-cysteine cyclic (3→14)-disulfide.

Synthetic cyclic tetradecapeptide having the structure of the hypothalamic hormone that inhibits the release of human growth hormone. It contains a variable amount of acetic acid. It is available in the freeze-dried form.

Content

95.0 per cent to 103.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance

White or almost white, amorphous powder.

Solubility

Freely soluble in water and in acetic acid, practically insoluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 1.0 mg of the substance to be examined in 1.0 ml *of water R* .

Reference solution Dissolve the contents of a vial of *somatostatin CRS* in *water R* and dilute with the same solvent to obtain a final concentration of 1 mg/ml.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, pyridine R, water R, butanol R* (10:15:20:45 *V/V/V/V*).

Application 20 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with a 1 g/l solution of *ninhydrin R* and heat in an oven at 110 °C for about 5 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

C. Amino acid analysis *(2.2.56)* . For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking 1/8 of the sum of the number of moles of aspartic acid, alanine, lysine, glycine and phenylalanine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glycine: 0.90 to 1.10; alanine: 0.90 to 1.10; phenylalanine: 2.7 to 3.3; serine: 0.7 to 1.05; threonine: 1.4 to 2.1; half-cystine: 1.4 to 2.1; lysine: 1.8 to 2.2. Not more than traces of other amino acids are present.

TESTS

Specific optical rotation *(2.2.7)*

- 37 to - 47 (anhydrous and acetic acid-free substance).

Dissolve 2.0 mg in 1.0 ml of a 1 per cent *V/V* solution of *glacial acetic acid R* .

Absorbance *(2.2.25)*

Maximum 0.20 at 280 nm (calculated with reference to the peptide content as determined in the assay).

Dissolve 5.0 mg in a 9 g/l solution of *sodium chloride R* and dilute to 100.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* : use the normalisation procedure.

Test solution Dissolve 5.0 mg of the substance to be examined in *water R* and dilute to

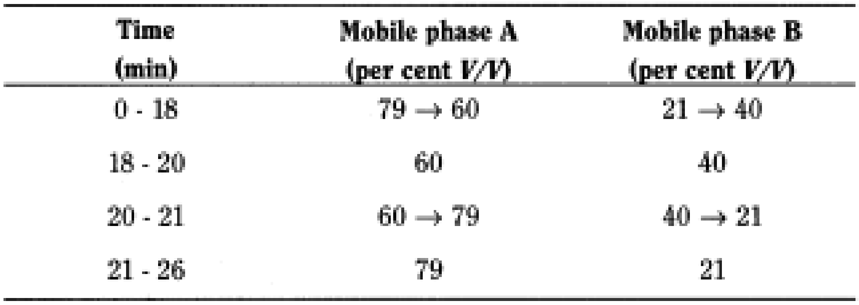
10.0 ml with the same solvent.

Column:

- *size*: *l* = 0.05 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A:* dilute 11 ml of *phosphoric acid R* with *water R* , adjust to pH 2.3 with *triethylamine R* and dilute to 1000 ml with *water R* ;
- *mobile phase B: acetonitrile for chromatography R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 18	79 → 60	21 → 40
18 - 20	60	40
20 - 21	60 → 79	40 → 21
21 - 26	79	21

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 25 µl.

Limits:

- *any impurity*: maximum 1 per cent;
- *total:* maximum 2 per cent;
- *disregard limit*: 0.03 per cent.

Acetic acid *(2.5.34)*

3.0 per cent to 15.0 per cent.

Test solution Dissolve 7.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A, then dilute to 10.0 ml with the same mixture of mobile phases.

Water *(2.5.12)*

Maximum 8.0 per cent, determined on 10.0 mg.

Bacterial endotoxins *(2.6.14)*

Less than 10 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Reference solution Dissolve the contents of a vial of *somatostatin CRS* in *water R* and dilute with the same solvent to obtain a final concentration of 0.5 mg/ml.

Mobile phase mobile phase B, mobile phase A (25:75 *V/V*).

Run time 15 min.

Calculate the content of somastatin ($C_{76}H_{104}N_{18}O_{19}S_2$) from the declared content of $C_{76}H_{104}N_{18}O_{19}S_2$ in *somatostatin CRS*.

STORAGE

In an airtight container protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

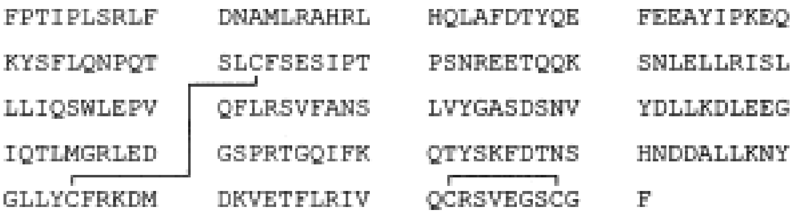
Ph Eur

Somatropin Concentrated Solution

General Notices

Somatropin Bulk Solution

(Ph Eur monograph 0950)


FPTIPLSRLF DNAMLRAHRL HQLAFDTYQE FEEAYIPKEQ
KYSFLQNPQT SLCFSESIPT PSNREETQQK SNLELLRISL
LLIQSWLEPV QFLRSVFANS LVYGASDSNV YDLLKDLEEG
IQTLMGRLED GSPRTGQIFK QTYSKFDTNS HNDDALLKNY
GLLYCFRKDM DKVETFLRIV QCRSVEGSCG F

$C_{990}H_{1528}N_{262}O_{300}S_{7}$ 22,125

Action and use

Growth hormone.

Preparation

Somatropin Injection

Ph Eur

DEFINITION

Solution containing a protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary. It may contain buffer salts and other auxiliary substances.

Content

91.0 per cent to 105.0 per cent of the amount of somatropin stated on the label.

By convention, for the purpose of labelling somatropin preparations, 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_{7}$) is equivalent to 3.0 IU of biological activity.

PRODUCTION

Somatropin concentrated solution is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of at least 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority.

Somatropin concentrated solution complies with the following additional requirements.

Host-cell-derived proteins

The limit is approved by the competent authority.

Host-cell- and vector-derived DNA

The limit is approved by the competent authority.

CHARACTERS

Appearance: clear or slightly turbid, colourless solution.

IDENTIFICATION

A. Capillary electrophoresis *(2.2.47)* as described in the test for charged variants with the following modifications.

Injection Test solution (b); under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

Results In the electropherogram obtained, only 1 principal peak, corresponding to somatropin, is detected: no doubling of this peak is observed.

B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

C. Peptide mapping *(2.2.55)* .

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Dilute the solution to be examined with *0.05 M tris-hydrochloride buffer solution pH 7.5 R* so that it contains 2.0 mg/ml of somatropin and transfer about 1.0 ml to a tube made from a suitable material such as polypropylene. Prepare a 1 mg/ml solution of *trypsin for peptide mapping R* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* and add 30 µl to the solution of the substance to be examined. Cap the tube and place in a water-bath at 37 °C for 4 h. Remove from the water-bath and stop the reaction immediately, for example by freezing. If analysed immediately using an automatic injector, maintain at 2-8 °C.

Note: If a 2 mg/ml somatropin concentration is not obtainable, a similar digest relationship (micrograms of trypsin per milligram of somatropin) may be used.

Reference solution Prepare at the same time and in the same manner as for the test solution, but using *somatropin CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION

Liquid chromatography (*2.2.29*).

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

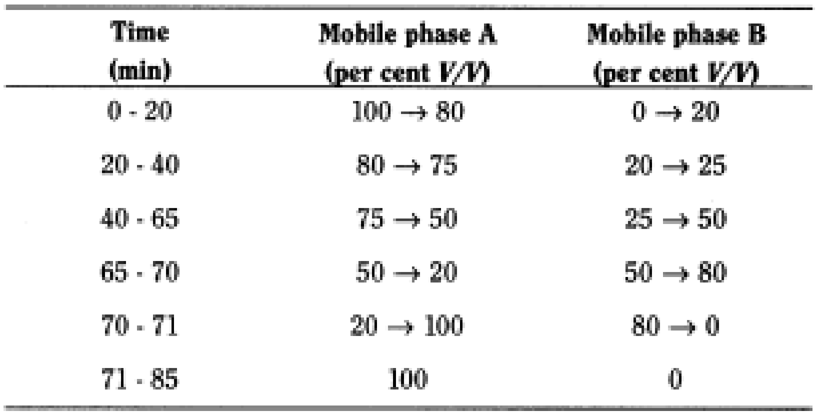
— *stationary phase: octylsilyl silica gel for chromatography R* (5-10 µm) with a pore size of 30 nm;

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: dilute 1 ml of *trifluoroacetic acid R* to 1000 ml with *water R* ;

— *mobile phase B*: to 100 ml of *water R* , add 1 ml of *trifluoroacetic acid R* and dilute to 1000 ml with *acetonitrile for chromatography R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 80	0 → 20
20 - 40	80 → 75	20 → 25
40 - 65	75 → 50	25 → 50
65 - 70	50 → 20	50 → 80
70 - 71	20 → 100	80 → 0
71 - 85	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µl.

System suitability The chromatograms obtained with the test solution and the reference solution are similar to the chromatogram of somatropin digest supplied with *somatropin CRS.*

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Related proteins

Liquid chromatography *(2.2.29)* : use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h. If an automatic injector is used, maintain it at 2-8 °C.*

Test solution Dilute the solution to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, so as to contain 2.0 mg/ml of somatropin. A weaker solution may be prepared, in which case the injection volume is adjusted accordingly.

Reference solution Prepare a solution of *somatropin CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/ml of somatropin.

Resolution solution Dissolve the contents of a vial of *somatropin/desamidosomatropin resolution mixture CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a concentration of 2 mg/ml of somatropin.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: a suitable singly end-capped butylsilyl silica gel, with a granulometry of 5 µm and a porosity of 30 nm; a silica saturation column is placed between the pump and the injector valve;

— *temperature*: 45 °C.

Mobile phase *propanol R* , *0.05 M tris-hydrochloride buffer solution pH 7.5 R* (29:71 *V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 220 nm.

Preconditioning of the column Rinse with 200-500 ml of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* in a 50 per cent *V/V* solution of *acetonitrile R;* repeat as necessary, to improve column performance.

Injection 20 µl.

Relative retention With reference to somatropin (retention time = about 33 min; if necessary adjust the concentration of *propanol R* in the mobile phase): desamidosomatropin = about 0.85.

System suitability Resolution solution:

— *resolution:* minimum 1.0 between the peaks due to desamidosomatropin and somatropin;

— *symmetry factor* : 0.9 to 1.8 for the peak due to somatropin.

Limit:

— *total*: maximum 6.0 per cent.

Dimer and related substances of higher molecular mass

Size-exclusion chromatography *(2.2.30)* : use the normalisation procedure.

Test solution Dilute the solution to be examined in *0.025 M phosphate buffer solution pH 7.0 R* , so as to contain 1.0 mg/ml of somatropin.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/ml.

Resolution solution Place 1 vial of *somatropin CRS* in an oven at 50 °C for a period sufficient to generate 1-2 per cent of dimer (typically 12-24 h). Dissolve its contents in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/ml.

Column:

— *size*: l = 0.30 m, Ø = 7.8 mm;

— *stationary phase*: *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase *2-propanol R*, *0.063 M phosphate buffer solution pH 7.0 R* (3:97 *V/V*); filter and degas.

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl.

Relative retention with reference to somatropin monomer (retention time = 12 min to 17 min): related substances of higher molecular mass = about 0.65; somatropin dimer = about 0.9.

System suitability Resolution solution:

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limit:
— sum of the peaks with retention times less than that of the principal peak: maximum 4.0 per cent.

Charged variants

Capillary electrophoresis *(2.2.47)*.

Test solution (a) Dilute the solution to be examined so as to obtain a concentration of 1 mg/ml of somatropin.

Test solution (b) Mix equal volumes of test solution (a) and the reference solution.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *water R* and dilute with the same solvent to obtain a concentration of 1 mg/ml.

Capillary:
— *material*: uncoated fused silica;

— *size:* effective length = at least 70 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer 13.2 g/l solution of *ammonium phosphate R* adjusted to pH 6.0 with *phosphoric acid R* and filtered.

Detection Spectrophotometer at 200 nm.

Set the autosampler to store the samples at 4 °C during analysis.

Preconditioning of the capillary Rinse with 1 M sodium hydroxide for 20 min, with *water R* for 10 min and with the CZE buffer for 20 min.

Between-run rinsing Rinse with *0.1 M sodium hydroxide* for 2 min and with the CZE buffer for 6 min.

Note: rinsing times may be adapted according to the length of the capillary and the equipment used.

Injection Test solution (a) and the reference solution; under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s. The injection time and pressure may be adapted in order to meet the system suitability criteria.

Migration Apply a field strength of 217 V/cm (20 kV for capillaries of 92 cm total length) for 80 min, using the CZE buffer as the electrolyte in both buffer reservoirs.

Relative migration With reference to somatropin

Deamidated forms = 1.02 to 1.11.

System suitability Reference solution:

— The electropherogram obtained is similar to the electropherogram of somatropin supplied with *somatropin CRS*; 2 peaks (I_1, I_2) eluting prior to the principal peak and at least 2 peaks (I_3, I_4) eluting after the principal peak are clearly visible.

Note: peak I_2 *corresponds to the cleaved form and peak* I_4 *corresponds to the deamidated forms, eluting as a doublet.*

Limits:

— *deamidated forms:* maximum 5.0 per cent;

— *any other impurity*: for each impurity, maximum 2.0 per cent;

— *total:* maximum 10.0 per cent.

Bacterial endotoxins ***(2.6.14)***

Less than 5 IU in the volume that contains 1 mg of somatropin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Size-exclusion chromatography *(2.2.30)* as described in the test for dimer and related substances of higher molecular mass.

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the declared content of $C_{990}H_{1528}N_{262}O_{300}S_7$ in *somatropin CRS*.

STORAGE

In an airtight container at a temperature of - 20 °C. Avoid repeated freezing and thawing. If the solution is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

— the content of somatropin in milligrams per millilitre;

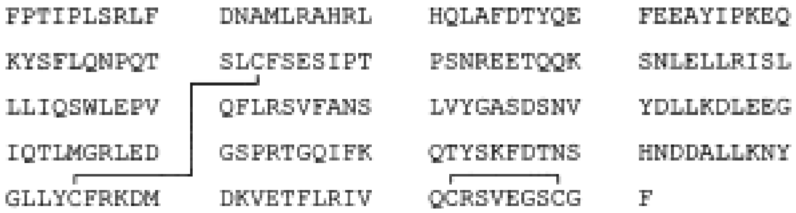
— the name and concentration of any auxiliary substance.

Ph Eur

Somatropin

General Notices

(Ph Eur monograph 0951)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LLIQSWLEPV QFLRSVFANS LVYGASDSNV YDLLKDLEEG
IQTLMGRLED GSPRTGQIFK QTYSKFDTNS HNDDALLKNY
GLLYCFRKDM DKVETFLRIV QCRSVEGSCG F

$C_{990}H_{1528}N_{262}O_{300}S_7$ 22,125 *12629-01-5*

Action and use

Growth hormone.

Preparation

Somatropin Injection

Ph Eur

DEFINITION

Protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary.

Content

91.0 per cent to 105.0 per cent (anhydrous substance).

By convention, for the purpose of labelling somatropin preparations, 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) is equivalent to 3.0 IU of biological activity.

PRODUCTION

Somatropin is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of not less than 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority.

Somatropin complies with the following additional requirements.

Host-cell-derived proteins

The limit is approved by the competent authority.

Host-cell- and vector-derived DNA

The limit is approved by the competent authority.

CHARACTERS

Appearance: white or almost white powder.

IDENTIFICATION

A. Capillary electrophoresis *(2.2.47)* as described in the test for charged variants with the following modifications.

Injection Test solution (b); under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

Results In the electropherogram obtained, only 1 principal peak, corresponding to somatropin, is detected: no doubling of this peak is observed.

B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

C. Peptide mapping *(2.2.55)* .

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a solution containing 2.0 mg/ml of somatropin and transfer about 1.0 ml to a tube made from a suitable material such as polypropylene. Prepare a 1 mg/ml solution of *trypsin for peptide mapping R* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* and add 30 µl to the solution of the substance to be examined. Cap the tube and place in a water-bath at 37 °C for 4 h. Remove from the water-bath and stop the reaction immediately, for example by freezing. If analysed immediately using an automatic injector, maintain at 2-8 °C.

Reference solution Prepare at the same time and in the same manner as for the test solution, but using *somatropin CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION

Liquid chromatography *(2.2.29)* .

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (5-10 µm) with a pore size of

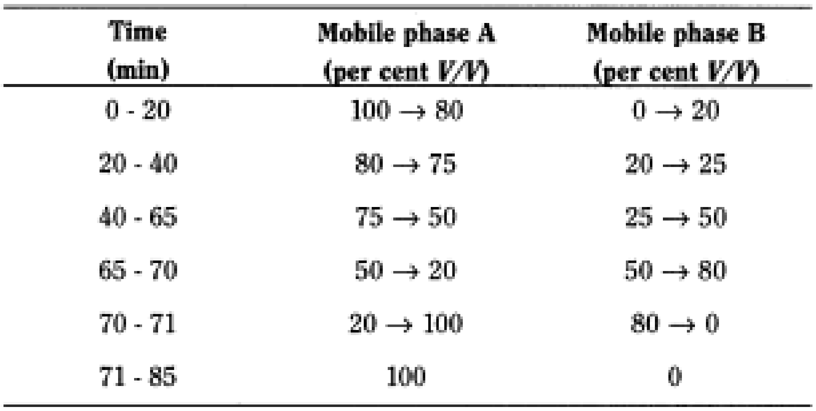
30 nm;

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: dilute 1 ml of *trifluoroacetic acid R* to 1000 ml with *water R;*

— *mobile phase B*: to 100 ml of *water R* , add 1 ml of *trifluoroacetic acid R* and dilute to 1000 ml with *acetonitrile for chromatography R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 80	0 → 20
20 - 40	80 → 75	20 → 25
40 - 65	75 → 50	25 → 50
65 - 70	50 → 20	50 → 80
70 - 71	20 → 100	80 → 0
71 - 85	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µl.

System suitability The chromatograms obtained with the test solution and the reference solution are similar to the chromatogram of somatropin digest supplied with *somatropin CRS.*

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h. If an automatic injector is used, maintain it at 2-8 °C*.

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/ml of somatropin.

Reference solution Prepare a solution of *somatropin CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/ml of somatropin.

Resolution solution Dissolve the contents of a vial of *somatropin/desamidosomatropin resolution mixture CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a concentration of 2 mg/ml of somatropin.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: a suitable singly end-capped butylsilyl silica gel, with a granulometry of 5 µm and a porosity of 30 nm; a silica saturation column is placed between the pump and the injector valve;

— *temperature:* 45 °C.

Mobile phase *propanol R*, *0.05 M tris-hydrochloride buffer solution pH 7.5* R (29:71 *V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 220 nm.

Preconditioning of the column Rinse with 200-500 ml of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* in a 50 per cent *V/V* solution of *acetonitrile R*; repeat as necessary, to improve column performance.

Injection 20 µl.

Relative retention With reference to somatropin (retention time = about 33 min; if necessary adjust the concentration of *propanol R* in the mobile phase): desamidosomatropin = about 0.85.

System suitability Resolution solution:

— *resolution*: minimum 1.0 between the peaks due to desamidosomatropin and somatropin;

— *symmetry factor*: 0.9 to 1.8 for the peak due to somatropin.

Limit:

— *total*: maximum 6.0 per cent.

Dimer and related substances of higher molecular mass

Size-exclusion chromatography *(2.2.30)*: use the normalisation procedure.

Test solution Prepare a solution of the substance to be examined in *0.025 M phosphate buffer solution pH 7.0 R*, containing 1.0 mg/ml of somatropin.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/ml.

Resolution solution Place 1 vial of *somatropin CRS* in an oven at 50 °C for a period sufficient to generate 1-2 per cent of dimer (typically 12-24 h). Dissolve its contents in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/ml.

Column:

— *size: l* = 0.30 m, Ø = 7.8 mm;

— *stationary phase*: *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase *2-propanol R, 0.063 M phosphate buffer solution pH 7.0 R* (3:97 *V/V*); filter and degas.

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl.

Relative retention With reference to somatropin monomer (retention time = 12 min to 17 min): related substances of higher molecular mass = about 0.65; somatropin dimer = about 0.9.

System suitability Resolution solution:

— *peak-to-valley ratio* : minimum 2.5, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limit:

— *sum of the peaks with retention times less than that of the principal peak: maximum 4.0 per cent*.

Charged variants

Capillary electrophoresis *(2.2.47)* .

Test solution (a) Prepare a solution of the substance to be examined containing 1 mg/ml of somatropin.

Test solution (b) Mix equal volumes of test solution (a) and the reference solution.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *water R* and dilute with the same solvent to obtain a concentration of 1 mg/ml.

Capillary:

— *material*: uncoated fused silica;

— *size*: effective length = at least 70 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer 13.2 g/l solution of *ammonium phosphate R* adjusted to pH 6.0 with *phosphoric acid R* and filtered.

Detection Spectrophotometer at 200 nm.

Set the autosampler to store the samples at 4 °C during analysis.

Preconditioning of the capillary Rinse with *1 M sodium hydroxide* for 20 min, with *water R* for 10 min and with the CZE buffer for 20 min.

Between-run rinsing Rinse with *0.1 M sodium hydroxide* for 2 min and with the CZE buffer for 6 min.

Note: rinsing times may be adapted according to the length of the capillary and the equipment used.

Injection Test solution (a) and the reference solution; under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

The injection time and pressure may be adapted in order to meet the system suitability criteria.

Migration Apply a field strength of 217 V/cm (20 kV for capillaries of 92 cm total length) for 80 min, using the CZE buffer as the electrolyte in both buffer reservoirs.

Relative migration with reference to somatropin

deamidated forms = 1.02 to 1.11.

System suitability Reference solution:

— The electropherogram obtained is similar to the electropherogram of somatropin supplied with *somatropin CRS*; 2 peaks (I_1, I_2) eluting prior to the principal peak and at least 2 peaks (I_3, I_4) eluting after the principal peak are clearly visible.

Note: peak I_2 corresponds to the cleaved form and peak I_4 corresponds to the deamidated forms, eluting as a doublet.

Limits:

- *deamidated forms*: maximum 5.0 per cent;
- *any other impurity*: for each impurity, maximum 2.0 per cent;
- *total*: maximum 10.0 per cent.

Water ***(2.5.32)***

Maximum 10.0 per cent.

Bacterial endotoxins ***(2.6.14)***

Less than 5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Size-exclusion chromatography *(2.2.30)* as described in the test for dimer and related substances of higher molecular mass.

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the declared content of $C_{990}H_{1528}N_{262}O_{300}S_7$ in *somatropin CRS*.

STORAGE

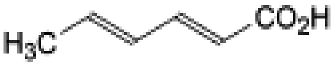
In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

Ph Eur

Sorbic Acid

General Notices

(Ph Eur monograph 0592)



$C_6H_8O_2$ 112.1 *22500-92-1*

Action and use

Antimicrobial preservative.

Ph Eur

DEFINITION

(*E*,*E*)-Hexa-2,4-dienoic acid.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Melting point *(2.2.14)* : 132 °C to 136 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 50.0 mg in *water R* and dilute to 250.0 ml with the same solvent. Dilute 2.0 ml of this solution to 200.0 ml with *0.1 M hydrochloric acid* .

Spectral range 230-350 nm.

Absorption maximum At 264 nm.

Specific absorbance at the absorption maximum 2150 to 2550.

C. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *sorbic acid CRS.*

D. Dissolve 0.2 g in 2 ml of *ethanol (96 per cent) R* and add 0.2 ml of *bromine water R.* The solution is decolorised.

TESTS

Solution S

Dissolve 1.25 g in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Aldehydes

Maximum 0.15 per cent, calculated as C_2H_4O.

Dissolve 1.0 g in a mixture of 30 ml of *water R* and 50 ml of *2-propanol R* , adjust to pH 4 with *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* and dilute to 100 ml with *water R* . To 10 ml of this solution add 1 ml of *decolorised fuchsin solution R* and allow to stand for 30 min. Any colour in the solution is not more intense than that in a standard prepared at the same time by adding 1 ml of *decolorised fuchsin solution R* to a mixture of 1.5 ml of *acetaldehyde standard solution (100 ppm* C_2H_4O*) R*, 4 ml of *2-propanol R* and 4.5 ml of *water R* .

Heavy metals *(2.4.8)*

Maximum 10 ppm.

12 ml of solution S complies with test B. Prepare the reference solution using 5 ml of *lead standard solution (1 ppm Pb) R* and 5 ml of *ethanol (96 per cent) R.*

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 2.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.1000 g in 20 ml of *ethanol (96 per cent) R* . Using 0.2 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 11.21 mg of $C_6H_8O_2$.

STORAGE

Protected from light.

Ph Eur

Sorbitan Laurate

General Notices

(Ph Eur monograph 1040)

1338-39-2

Action and use

Non-ionic surfactant.

When sorbitan monolaurate is demanded, Sorbitan Laurate shall be supplied.

Ph Eur

DEFINITION

Mixture usually obtained by partial esterification of sorbitol and its mono- and di-anhydrides with lauric (dodecanoic) acid.

CHARACTERS

Appearance

Brownish-yellow, viscous liquid.

Solubility

Practically insoluble, but dispersible in water, miscible with alcohol.

Relative density About 0.98.

IDENTIFICATION

A. It complies with the test for hydroxyl value (see Tests).

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 7.0, determined on 5.0 g.

Hydroxyl value ***(2.5.3, Method A)***

330 to 358.

Iodine value ***(2.5.4)***

Maximum 10.

Peroxide value ***(2.5.5)***

Maximum 5.0.

Saponification value ***(2.5.6)***

158 to 170.

Carry out the saponification for 1 h.

Composition of fatty acids

Gas chromatography *(2.4.22, Method C)*.

Prepare reference solution (a) as indicated in tables 2.4.22.-1 and 2.4.22.-2.

Composition of the fatty acid fraction of the substance:
— *caproic acid* : maximum 1.0 per cent;
— *caprylic acid* : maximum 10.0 per cent;
— *capric acid*: maximum 10.0 per cent;
— *lauric acid* : 40.0 per cent to 60.0 per cent;
— *myristic acid* : 14.0 per cent to 25.0 per cent;
— *palmitic acid* : 7.0 per cent to 15.0 per cent;
— *stearic acid* : maximum 7.0 per cent;
— *oleic acid* : maximum 11.0 per cent;
— *linoleic acid* : maximum 3.0 per cent.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 1.5 per cent, determined on 1.00 g.

Total ash ***(2.4.16)***

Maximum 0.5 per cent.

STORAGE

Protected from light.

Ph Eur

Sorbitan Oleate

General Notices

(Ph Eur monograph 1041)

1338-43-8

Action and use

Non-ionic surfactant.

When sorbitan mono-oleate is demanded, Sorbitan Oleate shall be supplied.

Ph Eur

DEFINITION

Mixture usually obtained by esterification of 1 mole of sorbitol and its mono-and di-anhydrides per mole of oleic (*cis*-9-octadecenoic) acid. A suitable antioxidant may be added.

CHARACTERS

Appearance

Brownish-yellow, viscous liquid.

Solubility

Practically insoluble but dispersible in water, soluble in fatty oils producing a hazy solution, miscible with alcohol.

Relative density

About 0.99.

IDENTIFICATION

A. It complies with the test for hydroxyl value (see Tests).

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

Margaric acid Maximum 0.2 per cent for oleic acid of vegetable origin and maximum 4.0 per cent for oleic acid of animal origin.

TESTS

Acid value *(2.5.1)*

Maximum 8.0, determined on 5.0 g.

Hydroxyl value *(2.5.3, Method A)*

190 to 210.

Iodine value *(2.5.4)*

62 to 76.

Peroxide value *(2.5.5)*

Maximum 10.0.

Saponification value *(2.5.6)*

145 to 160.

Carry out the saponification for 1 h.

Composition of fatty acids

Gas chromatography *(2.4.22, Method C)*.

Composition of the fatty acid fraction of the substance:

— *myristic acid* : maximum 5.0 per cent;

— *palmitic acid* : maximum 16.0 per cent;

— *palmitoleic acid* : maximum 8.0 per cent;

— *stearic acid* : maximum 6.0 per cent;

— *oleic acid* : 65.0 per cent to 88.0 per cent;

— *linoleic acid* : maximum 18.0 per cent;

— *linolenic acid* : maximum 4.0 per cent;

— *fatty acids with chain length greater than* C_{18} : maximum 4.0 per cent.

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water *(2.5.12)*

Maximum 1.5 per cent, determined on 1.000 g.

Total ash *(2.4.16)*

Maximum 0.5 per cent, determined on 1.5 g.

STORAGE

Protected from light.

LABELLING

The label states the origin of the oleic acid used (animal or vegetable).

Ph Eur

Sorbitan Palmitate

General Notices

(Ph Eur monograph 1042)

26266-57-9

Action and use

Non-ionic surfactant.

When sorbitan monopalmitate is demanded, Sorbitan Palmitate shall be supplied.

Ph Eur

DEFINITION

Mixture usually obtained by partial esterification of sorbitol and its mono- and di-anhydrides with palmitic (hexadecanoic) acid.

CHARACTERS

Appearance

Yellow or yellowish powder, waxy flakes or hard masses.

Solubility

Practically insoluble in water, soluble in fatty oils, slightly soluble in alcohol.

IDENTIFICATION

A. Melting point *(2.2.15)* 44 °C to 51 °C.

Introduce the melted substance into the glass capillary tubes and allow to stand at a temperature below 10 °C for 24 h.

B. It complies with the test for hydroxyl value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 8.0, determined on 5.0 g.

Hydroxyl value ***(2.5.3, Method A)***

270 to 305.

Peroxide value ***(2.5.5)***

Maximum 5.0.

Saponification value ***(2.5.6)***

140 to 155.

Carry out the saponification for 1 h.

Composition of fatty acids

Gas chromatography *(2.4.22, Method C)*.

Composition of the fatty acid fraction of the substance:

— *palmitic acid* : minimum 92.0 per cent;

— *stearic acid* : maximum 6.0 per cent.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 1.5 per cent, determined on 1.00 g.

Total ash ***(2.4.16)***

Maximum 0.5 per cent.

STORAGE

Protected from light.

Ph Eur

Sorbitan Sesquioleate

General Notices

(Ph Eur monograph 1916)

8007-43-0

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture usually obtained by esterification of 2 moles of sorbitol and its mono- and di-anhydrides per 3 moles of oleic (*cis*-9-octadecenoic) acid. A suitable antioxidant may be added.

CHARACTERS

Appearance

Pale yellow or slightly brownish-yellow paste, which becomes a viscous, oily, brownish-yellow liquid at about 25 °C.

Solubility

Dispersible in water, soluble in fatty oils, slightly soluble in ethanol.

Relative density

About 0.99.

IDENTIFICATION

A. It complies with the test for hydroxyl value (see Tests).

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

Margaric acid Maximum 0.2 per cent for oleic acid of vegetable origin and maximum 4.0 per cent for oleic acid of animal origin.

TESTS

Acid value ***(2.5.1)***

Maximum 16.0, determined on 5.0 g.

Hydroxyl value ***(2.5.3, Method A)***

180 to 215.

Iodine value ***(2.5.4)***

70 to 95.

Peroxide value ***(2.5.5)***

Maximum 10.0.

Saponification value ***(2.5.6)***

145 to 166.

Carry out the saponification for 1 h.

Composition of fatty acids

Gas chromatography *(2.4.22, Method C)*.

Composition of the fatty acid fraction of the substance:

— *myristic acid* : maximum 5.0 per cent;

— *palmitic acid* : maximum 16.0 per cent;

— *palmitoleic acid* : maximum 8.0 per cent;

— *stearic acid* : maximum 6.0 per cent;

— *oleic acid* : 65.0 per cent to 88.0 per cent;

— *linoleic acid* : maximum 18.0 per cent;

— *linolenic acid* : maximum 4.0 per cent;

— *fatty acids with chain length greater than* C_{18} : maximum 4.0 per cent.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 1.5 per cent, determined on 1.000 g.

Total ash ***(2.4.16)***

Maximum 0.5 per cent, determined on 1.5 g.

STORAGE

Protected from light.

LABELLING

The label states the origin of the oleic acid used (animal or vegetable).

Ph Eur

Sorbitan Stearate

General Notices

(Ph Eur monograph 1043)

1338-41-6

Action and use

Non-ionic surfactant.

When sorbitan monostearate is demanded, Sorbitan Stearate shall be supplied.

Ph Eur

DEFINITION

Mixture usually obtained by partial esterification of sorbitol and its mono- and di-anhydrides with *Stearic acid 50 (1474)* or *Stearic acid 70 (1474).*

CHARACTERS

Appearance

Pale yellow, waxy solid.

Solubility

Practically insoluble, but dispersible in water, slightly soluble in alcohol.

IDENTIFICATION

A. Melting point *(2.2.15)* : 50 °C to 60 °C.

Introduce the melted substance into the capillary tubes and allow to stand at a temperature below 10 °C for 24 h.

B. It complies with the test for hydroxyl value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 10.0, determined on 5.0 g.

Hydroxyl value ***(2.5.3, Method A)***

235 to 260.

Peroxide value ***(2.5.5)***

Maximum 5.0.

Saponification value ***(2.5.6)***

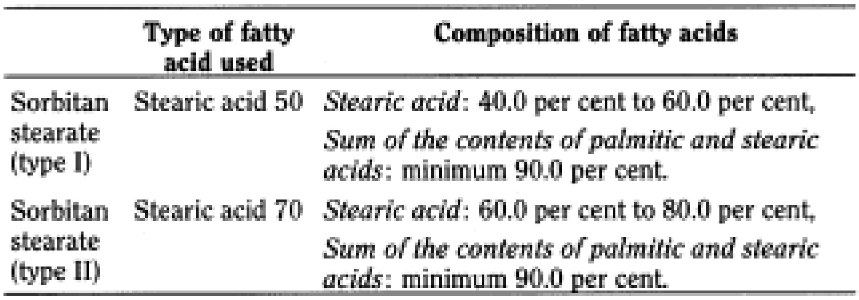
147 to 157.

Carry out the saponification for 1 h.

Composition of fatty acids

Gas chromatography *(2.4.22, Method C)*.

Composition of the fatty acid fraction of the subtance:



	Type of fatty acid used	Composition of fatty acids
Sorbitan stearate (type I)	Stearic acid 50	*Stearic acid*: 40.0 per cent to 60.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.
Sorbitan stearate (type II)	Stearic acid 70	*Stearic acid*: 60.0 per cent to 80.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 1.5 per cent, determined on 1.00 g.

Total ash ***(2.4.16)***

Maximum 0.5 per cent.

STORAGE

Protected from light.

LABELLING

The label states the type of sorbitan stearate.

Sorbitan Trioleate

General Notices

(Ph Eur monograph 1044)

26266-58-0

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture usually obtained by esterification of 1 mole of sorbitol and its mono-anhydride per 3 moles of oleic (*cis*-9-octadecenoic) acid. A suitable antioxidant may be added.

CHARACTERS

Appearance

Pale yellow, light yellowish or brown solid, which becomes a viscous, oily, brownish-yellow liquid at about 25 °C.

Solubility

Practically insoluble but dispersible in water, soluble in fatty oils, slightly soluble in alcohol.

Relative density About 0.98.

IDENTIFICATION

A. It complies with the test for hydroxyl value (see Tests).

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

Margaric acid Maximum 0.2 per cent for oleic acid of vegetable origin and maximum 4.0 per cent for oleic acid of animal origin.

TESTS

Acid value *(2.5.1)*

Maximum 16.0, determined on 5.0 g.

Hydroxyl value ***(2.5.3, Method A)***

55 to 75.

Iodine value ***(2.5.4)***

76 to 90.

Peroxide value ***(2.5.5)***

maximum 10.0.

Saponification value ***(2.5.6)***

170 to 190.

Carry out the saponification for 1 h.

Composition of fatty acids

Gas chromatography *(2.4.22, Method C)*.

Composition of the fatty acid fraction of the substance:
- — *myristic acid* : maximum 5.0 per cent;
- — *palmitic acid* : maximum 16.0 per cent;
- — *palmitoleic acid* : maximum 8.0 per cent;
- — *stearic acid* : maximum 6.0 per cent;
- — *oleic acid* : 65.0 per cent to 88.0 per cent;
- — *linoleic acid* : maximum 18.0 per cent;
- — *linolenic acid* : maximum 4.0 per cent;
- — *fatty acids with chain length greater than* C_{18} : maximum 4.0 per cent.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water ***(2.5.12)***

Maximum 1.5 per cent, determined on 1.000 g.

Total ash ***(2.4.16)***

Maximum 0.5 per cent, determined on 1.5 g.

STORAGE

Protected from light.

LABELLING

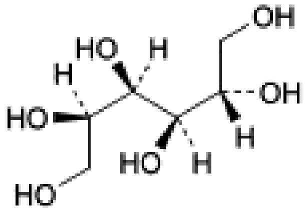
The label states the origin of the oleic acid used (animal or vegetable).

Ph Eur

Sorbitol

General Notices

(Ph Eur monograph 0435)



$C_6H_{14}O_6$ 182.2 *50-70-4*

Action and use

Used for parenteral nutrition.

Ph Eur

DEFINITION

D-Glucitol (D-sorbitol).

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, practically insoluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification A.

Second identification B, C, D.

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. Dissolve 0.5 g with heating in a mixture of 0.5 ml of *pyridine R* and 5 ml of *acetic anhydride R* . After 10 min, pour the solution into 25 ml of *water R* and allow to stand in iced water for 2 h. The precipitate, recrystallised from a small volume of *ethanol (96 per cent) R* and dried *in vacuo*, melts *(2.2.14)* at 98 °C to 104 °C.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *sorbitol CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 25 mg of *mannitol CRS* and 25 mg of *sorbitol CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R* .

Mobile phase *water R* , *ethyl acetate R*, *propanol R* (10:20:70 *V/V/V*).

Application 2 µl.

Development Over a path of 17 cm.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R* ; dry in a current of cold air until the acetone is removed; heat at 100 °C for 15 min; allow to cool and spray with a 2 g/l solution of *sodium periodate R* ; dry in a current of cold air; heat at 100 °C for 15 min.

System suitability Reference solution (b):

— The chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Specific optical rotation *(2.2.7)* : + 4.0 to + 7.0 (anhydrous substance).

Dissolve 5.00 g of the substance to be examined and 6.4 g of *disodium tetraborate R* in 40 ml of *water R* . Allow to stand for 1 h, shaking occasionally, and dilute to 50.0 ml with *water R* . Filter if necessary.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Dissolve 5 g in *water R* and dilute to 50 ml with the same solvent.

Conductivity ***(2.2.38)***

Maximum 20 µS·cm^{-1}.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent, expressed as glucose equivalent.

Dissolve 5.0 g in 6 ml of *water R* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R,* added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Related products

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 5.0 g of the substance to be examined in 20 ml of *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 0.50 g of *sorbitol CRS* in 2 ml of *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with *water R*.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 100.0 ml with *water R*.

Reference solution (d) Dissolve 0.5 g of *sorbitol R* and 0.5 g of *mannitol R* (impurity A) in 5 ml of *water R* and dilute to 10.0 ml with the same solvent.

Column:

- *size*: *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase:* strong cation exchange *resin (calcium form) R* (9 µm);
- *temperature*: 85 ± 1 °C.

Mobile phase Degassed *water R.*

Flow rate 0.5 ml/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µl of the test solution and reference solutions (b), (c) and (d).

Run time 3 times the retention time of sorbitol.

Relative retention With reference to sorbitol (retention time = about 27 min): impurity C = about 0.6; impurity A = about 0.8; impurity B = about 1.1.

System suitability Reference solution (d):

— *resolution*: minimum 2 between the peaks due to impurity A and sorbitol.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Lead ***(2.4.10)***

Maximum 0.5 ppm.

Nickel ***(2.4.15)***

Maximum 1 ppm.

Dissolve the substance to be examined in 150.0 ml of the prescribed mixture of solvents.

Water ***(2.5.12)***

Maximum 1.5 per cent, determined on 1.00 g.

Microbial contamination

If intended for use in the manufacture of parenteral dosage forms, the total viable aerobic count *(2.6.12)* is not more than 10^2 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)* .

Bacterial endotoxins ***(2.6.14)***

Less than 4 IU/g for parenteral dosage forms having a concentration of less than 100 g/l of sorbitol, and less than 2.5 IU/g for parenteral dosage forms having a concentration of 100 g/l or more of sorbitol, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related products with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of D-sorbitol from the declared content of *sorbitol CRS* .

LABELLING

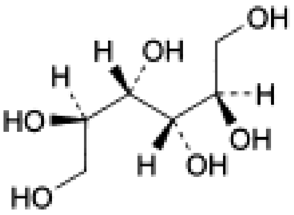
The label states:

— where applicable, the maximum concentration of bacterial endotoxins;

— where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES

A. mannitol,


OH
H
OH
H
H
HO
OH
H
OH
HO

B. iditol,

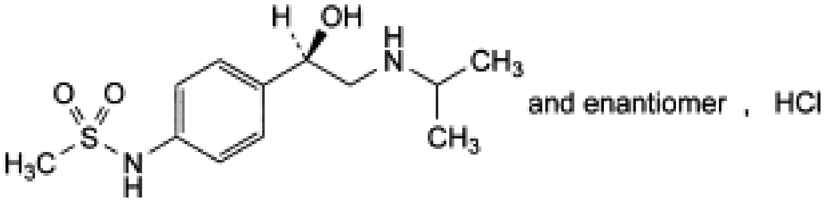
C. maltitol.

Ph Eur

Sotalol Hydrochloride

General Notices

(Ph Eur monograph 2004)



$C_{12}H_{20}N_2O_3S,HCl$ 308.8 *959-24-0*

Action and use

Beta-adrenoceptor antagonist; class II and class III antiarrhythmic.

Ph Eur

DEFINITION

N-[4-[(1*RS*)-1-hydroxy-2-[(1-methylethyl)amino]ethyl]phenyl]methanesulphonamide hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *sotalol hydrochloride CRS.*

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.0 to 5.0.

Dilute 5.0 ml of solution S to 10.0 ml with *carbon dioxide-free water R.*

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dilute 25.0 ml of solution S to 50.0 ml with *water R* .

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 3.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 8.0 mg of *sotalol impurity B CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d) Dilute 1.5 ml of reference solution (b) to 100 ml with the mobile phase. To 1 ml of this solution add 1 ml of reference solution (a).

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 2 g of *sodium octanesulphonate R* in 790 ml of *water R.* Adjust to pH 3.0 with *phosphoric acid R* and add 210 ml of *acetonitrile R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 228 nm.

Injection 10 µl; inject the test solution and reference solutions (a), (c) and (d).

Run time 2.5 times the retention time of sotalol.

System suitability Reference solution (d):

- *resolution*: minimum 4.0 between the peaks due to sotalol and to impurity B.

Limits:

- *impurity B*: not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), and not more than 1 such peak has an area greater than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total of other impurities*: not more than 1.65 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.17 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Palladium

Maximum 0.5 ppm.

Atomic absorption spectrometry *(2.2.23, Method I).*

Test solution Dissolve 1.00 g in a mixture of 0.25 volumes of *nitric acid R*, 0.75 volumes of *hydrochloric acid R* and 99.0 volumes of *water R* and dilute to 20.0 ml with the same mixture of solvents.

Reference solutions Use solutions containing 0.02 µg, 0.03 µg and 0.05 µg of palladium per millilitre, freshly prepared by dilution of *palladium standard solution (0.5 ppm Pd) R* with a mixture of 0.25 volumes of *nitric acid R*, 0.75 volumes of *hydrochloric acid R* and 99.0 volumes of *water R*.

Source Palladium hollow-cathode lamp.

Wavelength 247.6 nm.

Use a graphite tube.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

To 10 ml of solution S add 10 ml of *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating during the titration, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

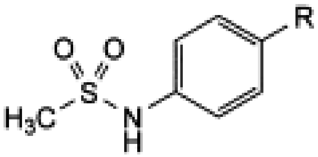
Dissolve 0.250 g in 10 ml of *anhydrous formic acid R*, if necessary with the aid of ultrasound. Add 40 ml of *acetic anhydride R* and titrate immediately with *0.1 M perchloric acid*. Determine the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 30.88 mg of $C_{12}H_{21}ClN_2O_3S$.

STORAGE

Protected from light.

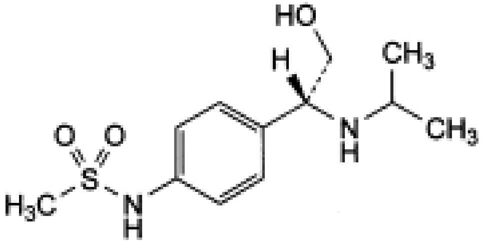
IMPURITIES



A. R = CH_2-CH_2-NH-CH$(CH_3)_2$: *N*-[4-[2-[(1-methylethyl)amino]ethyl]phenyl] methanesulphonamide,

B. R = CO-CH_2-NH-CH$(CH_3)_2$: *N*-[4-[[(1-methylethyl)amino]acetyl]phenyl] methanesulphonamide,

C. R = CHO: *N*-(4-formylphenyl)methanesulphonamide,



and enantiomer

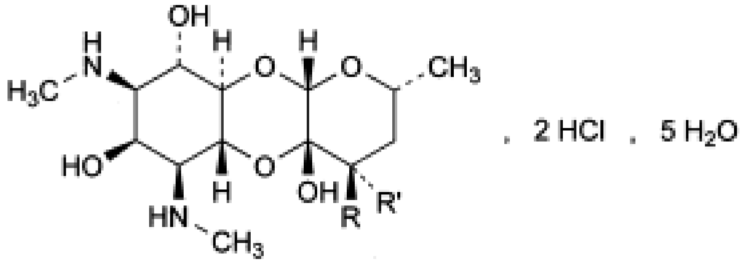
D. *N*-[4-[(1*RS*)-2-hydroxy-1-[(1-methylethyl)amino]ethyl]phenyl]methanesulphonamide.

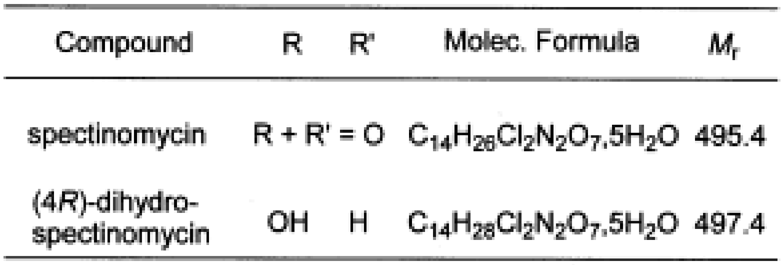
Ph Eur

Spectinomycin Dihydrochloride Pentahydrate

General Notices

(Ph Eur monograph 1152)





Compound	R	R'	Molec. Formula	M_r
spectinomycin	R + R' = O		$C_{14}H_{26}Cl_2N_2O_7,5H_2O$	495.4
(4*R*)-dihydro-spectinomycin	OH	H	$C_{14}H_{28}Cl_2N_2O_7,5H_2O$	497.4

$C_{14}H_{24}N_2O_7,2HCl,5H_2O$ 495.4 *22189-32-8*

Action and use

Aminocyclotol antibacterial.

Ph Eur

DEFINITION

Mixture of (2*R*,4a*R*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)4a,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one dihydrochloride pentahydrate (spectinomycin dihydrochloride pentahydrate) and of (2*R*,4*R*,4a*S*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-2-methyl-6,8-bis(methylamino)decahydro-2*H*-pyrano[2,3-*b*][1,4]benzodioxine-4,4a,7,9-tetrol dihydrochloride pentahydrate ((4*R*)-dihydrospectinomycin dihydrochloride pentahydrate).

It is produced by *Streptomyces spectabilis* or by any other means.

Content:

— *(4R)-dihydrospectinomycin dihydrochloride*: maximum 9.0 per cent (anhydrous substance);

— *sum of the contents of spectinomycin dihydrochloride and (4R)-dihydrospectinomycin dihydrochloride*: 93.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *spectinomycin hydrochloride CRS.*

B. Dilute 1.0 ml of solution S (see Tests) to 10 ml with *water R* . The solution gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution.

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Dilute 2.0 ml of solution S to 20.0 ml with *water R* .

pH *(2.2.3)*

3.8 to 5.6 for solution S.

Specific optical rotation *(2.2.7)*

+ 15.0 to + 21.0 (anhydrous substance), determined on solution S within 20 min of preparation.

Related substances

Liquid chromatography *(2.2.29)*. *In order to avoid formation of anomers, prepare the solutions immediately before use*.

Test solution Dissolve 15.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 3 mg of *spectinomycin for system suitability CRS* in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 3.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: ambient and constant.

Mobile phase Dissolve 4.2 g of *oxalic acid R* and 2.0 ml of *heptafluorobutyric acid R* in *water R* and dilute to 1000 ml with *water R*; adjust to pH 3.2 with *sodium hydroxide solution R,* add 105 ml of *acetonitrile R* and mix; filter through a 0.45 µm filter and degas with *helium for chromatography R* for 10 min.

Flow rate 1.0 ml/min.

Post-column solution *Carbonate-free sodium hydroxide solution R* diluted with *carbon dioxide-free water R* to obtain a final concentration of NaOH of 21 g/l. Degas the solution with *helium for chromatography R* for 10 min before use. Add it pulse-less to the column effluent using a 375 µl polymeric mixing coil.

Post-column flow rate 0.5 ml/min.

Detection Pulsed amperometric detection or equivalent with a gold indicator electrode having preferably a diameter of 1.4 mm or greater, a suitable reference electrode and a stainless steel counter electrode, held at + 0.12 V detection, + 0.70 V oxidation and - 0.60 V reduction potentials respectively, with pulse durations according to the instrument used. Keep the detection cell at ambient and constant temperature. Clean the gold indicator electrode with an eraser and damp precision wipe prior to start-up of the system to enhance the detector sensitivity and increase the signal-to-noise ratio.

Injection 20 µl.

Run time 1.5 times the retention time of spectinomycin.

Identification of impurities Use the chromatogram supplied with *spectinomycin for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, D and E.

Relative retention With reference to spectinomycin (retention time = 11 min to 20 min): impurity A = about 0.5; impurity F = about 0.53; impurity G = about 0.6; impurity D = about 0.7; impurity E = about 0.9; (4R)-dihydrospectinomycin = about 1.3; impurity C = about 1.4.

System suitability Reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to impurity E and spectinomycin.

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.4;
- *impurities A, C, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurities D, E*: for each impurity, not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the

chromatogram obtained with reference solution (b) (1.0 per cent);

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent); disregard the peak due to (4*R*)-dihydrospectinomycin.

Water ***(2.5.12)***

16.0 per cent to 20.0 per cent, determined on 0.100 g.

Sulphated ash ***(2.4.14)***

Maximum 1.0 per cent, determined on 1.0 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.09 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins. Prepare the solutions using a 0.42 per cent *m/m* solution of *sodium hydrogen carbonate R* .

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Test solution Dissolve 40.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent. Allow to stand for not less than 15 h and not more than 72 h (formation of anomers). Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution Dissolve 40.0 mg of *spectinomycin hydrochloride CRS* (containing (4*R*)-dihydrospectinomycin) in *water R* and dilute to 50.0 ml with the same solvent. Allow to stand for the same period of time as the test solution (formation of anomers). Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

System suitability:

— *repeatability*: maximum relative standard deviation of 3.0 per cent for the principal peak after 6 injections of the reference solution.

Calculate the sum of the percentage contents of spectinomycin dihydrochloride and (4*R*)-dihydrospectinomycin dihydrochloride from the declared contents of $C_{14}H_{26}Cl_2N_2O7$ and $C_{14}H_{28}Cl_2N_2O_7$ in *spectinomycin hydrochloride CRS* .

STORAGE

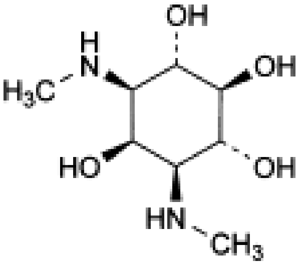
In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

IMPURITIES

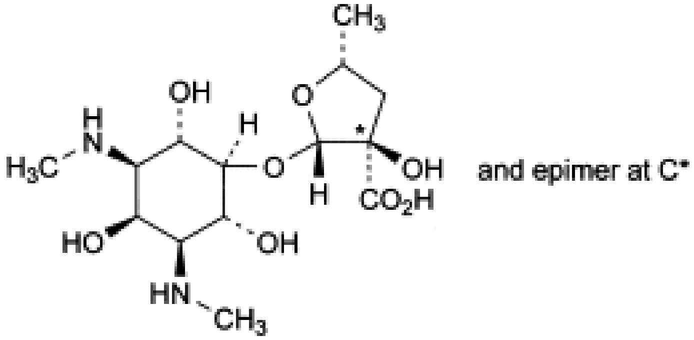
Specified impurities *A, C, D, E, F, G.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these

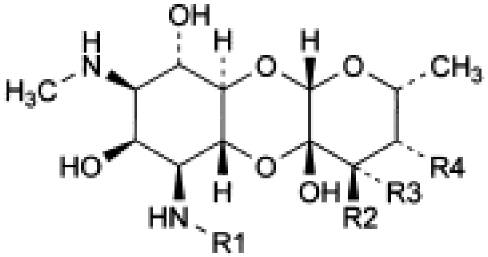
impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B.*



A. 1,3-dideoxy-1,3-bis(methylamino)-*myo*-inositol (actinamine),



B. (2*S*,3*RS*,5*R*)-3-hydroxy-5-methyl-2-[[(1*r*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,4,6-trihydroxy-3,5-bis (methylamino)cyclohexyl]oxy]tetrahydrofuran-3-carboxylic acid (actinospectinoic acid),



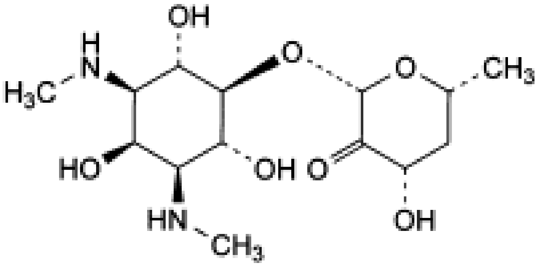
C. R1 = CH_3, R2 = R4 = H, R3 = OH: (2*R*,4*S*,4a*S*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-2-methyl-6, 8-bis(methylamino)decahydro-2*H*-pyrano[2,3-*b*][1,4]benzodioxine-4,4a,7,9-tetrol ((4*S*)-dihydrospectinomycin),

D. R1 = CH_3, R2 = H, R3 = R4 = OH: (2*R*,3*R*,4*S*,4a*S*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-2-methyl-6,8-bis(methylamino)decahydro-2*H*-pyrano[2,3-*b*][1,4]benzodioxine-3,4,4a,7,9-pentol (dihydroxyspectinomycin),

E. R1 = R4 = H, R2 + R3 = O: (2*R*,4a*R*,5a*R*,6*S*,7*R*,8*R*,9*S*,9a*R*,10a*S*)-6-amino-4a,7,9-trihydroxy-2-methyl-8-(methylamino)decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one (*N*-desmethylspectinomycin),

G. R1 = CH_3, R2 + R3 = O, R4 = OH: (2*R*,3*S*,4a*R*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-3,4a,7,9-

tetrahydroxy-2-methyl-6,8-bis(methylamino)decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one (tetrahydroxyspectinomycin),

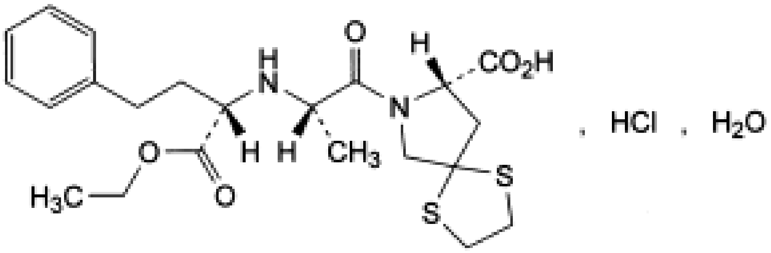


F. (2*S*,4*S*,6*R*)-4-hydroxy-6-methyl-2-[[(1r,2*R*,3*S*,4r,5*R*,6*S*)-2,4,6-trihydroxy-3,5-bis(methylamino)cyclohexyl]oxy]dihydro-2*H*-pyran-3(4*H*)-one (triol spectinomycin).

Spirapril Hydrochloride Monohydrate

General Notices

(Ph Eur monograph 1766)



$C_{22}H_{30}N_2O_5S_2,HCl,H_2O$ 521.1 *94841-17-5*

Action and use

Angiotensin-converting enzyme inhibitor.

Ph Eur

DEFINITION

(8*S*)-7-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid hydrochloride monohydrate.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, fine crystalline powder.

Solubility

Very slightly soluble in water, soluble in methanol, slightly soluble in acetonitrile, practically insoluble in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs of *potassium bromide R.*

Comparison *Spirapril hydrochloride monohydrate CRS* .

C. It gives the reactions of chlorides *(2.3.1)* .

TESTS

Specific optical rotation *(2.2.7)*

- 11.0 to - 13.0 (anhydrous substance).

Dissolve 0.200 g in *dimethylformamide R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Solvent mixture *acetonitrile R1* , *water R* (2:8 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 6.0 mg of *spirapril for system suitability CRS* (spirapril spiked with impurity B and impurity D) in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.125 m, Ø = 4.6 mm;

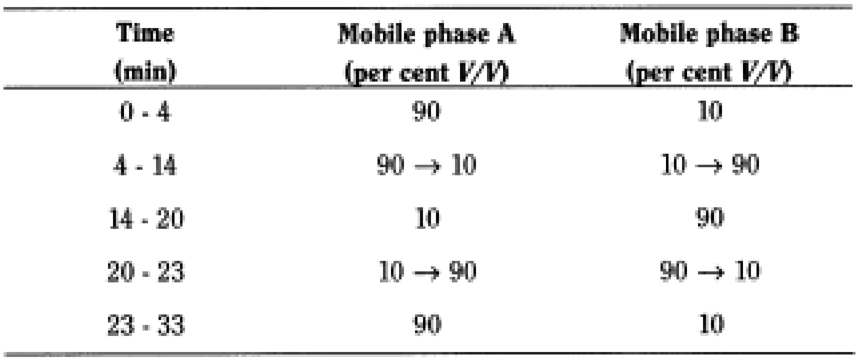
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 70 °C.

Mobile phase:

— *mobile phase A*: dissolve 4.5 g of *tetramethylammonium hydroxide R* in 900 ml of *water R* , add 100 ml of *acetonitrile R1* and adjust to pH 2.2 with *phosphoric acid R* ;

— *mobile phase B*: dissolve 4.5 g of *tetramethylammonium hydroxide R* in 400 ml of *water R* , add 600 ml of *acetonitrile R1* and adjust to pH 2.2 with *phosphoric acid R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	90	10
4 - 14	90 → 10	10 → 90
14 - 20	10	90
20 - 23	10 → 90	90 → 10
23 - 33	90	10

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Relative retention With reference to spirapril (retention time = about 10 min): impurity C = about 0.6; impurity B = about 0.7; impurity A = about 1.26; impurity D = about 1.38.

System suitability Reference solution (a):

— *resolution*: minimum 3.5 between the peaks due to impurity B and spirapril, and minimum 5.5 between the peaks due to spirapril and impurity D.

Limits:

— *impurities A, C*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity B*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity D*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *any other impurity:* for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak due to the blank (solvent mixture).

Water ***(2.5.12)***

3.0 per cent to 4.0 per cent, determined on 0.200 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* .

Solvent mixture Mix equal volumes of *acetonitrile R1* and *water R* .

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *spirapril hydrochloride monohydrate CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (b) Dissolve 6.0 mg of *spirapril for system suitability CRS* (spirapril spiked with impurity B and impurity D) in a mixture of 2 volumes of *acetonitrile R* and 8 volumes of *water R* and dilute to 20 ml with the same mixture of solvents.

Solution A Dissolve 4.5 g of *tetramethylammonium hydroxide R* in 900 ml of *water R* , adjust to pH 1.75 with *phosphoric acid R* and add 100 ml of *acetonitrile R1* .

Solution B Dissolve 4.5 *g of tetramethylammonium hydroxide R in 400 ml of water R, adjust to pH 1.75 with phosphoric acid R* and add 600 ml of *acetonitrile R1* .

Column:

- *size*: *l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 70 °C.

Mobile phase Solution A, solution B (45:55 *V/V*).

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Retention time Spirapril = 1.6 min to 2.9 min; impurity D = about 13 min. Adjust the proportion of solution B in the mobile phase if necessary.

System suitability Reference solution (b):

- *resolution*: minimum 15 between the peaks due to spirapril and impurity D.

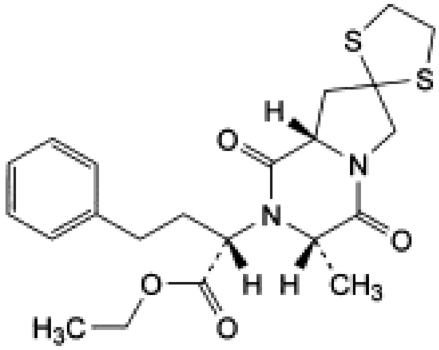
Calculate the percentage content of $C_{22}H_{31}ClN_2O_5S_2$ from the chromatograms obtained with the test solution and reference solution (a) and the declared content of *spirapril hydrochloride monohydrate CRS.*

STORAGE

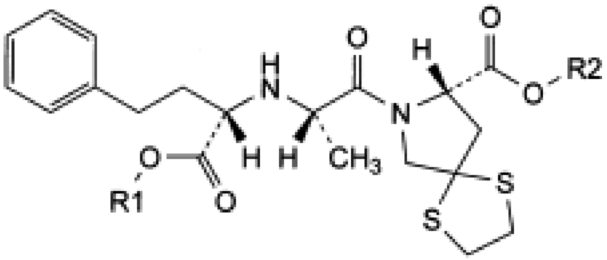
In an airtight container , protected from light.

IMPURITIES

Specified impurities A, B, C, D.

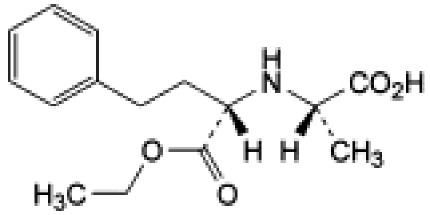


A. ethyl (2*S*)-2-[(3′*S*,8′a*S*)-3′-methyl-1′,4′-dioxo-hexahydrospiro[1,3-dithiolane-2,7′(6′*H*)-pyrrolo[1,2-*a*]pyrazin]-2′-yl]-4-phenylbutanoate,



B. R1 = R2 = H: (8*S*)-7-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid (spiraprilat),

D. R1 = C_2H_5, R2 = $CH(CH_3)_2$: 1-methylethyl (8*S*)-7-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylate,



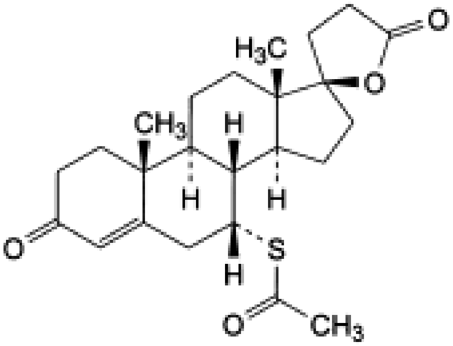
C. (2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoic acid.

Ph Eur

Spironolactone

General Notices

(Ph Eur monograph 0688)



$C_{24}H_{32}O_4S$ 416.6 *52-01-7*

Action and use

Aldosterone receptor antagonist; potassium-sparing diuretic.

Preparation

Spironolactone Tablets

Ph Eur

DEFINITION

Spironolactone contains not less than 97.0 per cent and not more than the equivalent of 102.0 per cent of 7α-(acetylsulfanyl)-3′,4′-dihydrospiro[androst-4-ene-17,2′(5′*H*)-furan]-3,5′-dione, calculated with reference to the dried substance.

CHARACTERS

A white or yellowish-white powder, practically insoluble in water, soluble in alcohol.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the spectrum obtained with *spironolactone CRS* . Examine the substances as 50 g/l solutions in *chloroform R* .

B. Examine by thin-layer chromatography *(2.2.27),* using *silica gel GF*$_{254}$ *R* as the coating substance.

Test solution Dissolve 20 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *spironolactone CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *water R* , 24 volumes of *cyclohexane R* and 75 volumes of *ethyl acetate R* . Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 10 mg add 2 ml of a 50 per cent *V/V* solution of *sulphuric acid R* and shake. An orange solution with an intense yellowish-green fluorescence is produced. Heat the solution gently; the colour becomes deep red and hydrogen sulphide, which blackens *lead acetate paper R,* is evolved. Add the solution to 10 ml of *water R* ; a greenish-yellow solution is produced which shows opalescence or a precipitate.

TESTS

Specific optical rotation *(2.2.7)*

Dissolve 0.100 g in *chloroform R* and dilute to 10.0 ml with the same solvent. The specific optical rotation is - 33 to - 37, calculated with reference to the dried substance.

Related substances

Examine by liquid chromatography *(2.2.29)* .

Test solution Dissolve 62.5 mg of the substance to be examined in 2.5 ml of *tetrahydrofuran R* and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 25.0 mg of *canrenone CRS* in 1 ml of *tetrahydrofuran R* and dilute to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d) Mix 1 ml of the test solution with 1 ml of reference solution (b) and dilute to 100 ml with the mobile phase.

Reference solution (e) Dilute 0.50 ml of reference solution (a) to 10.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.15 m long and 4.6 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm);

— a mixture of 8 volumes of *acetonitrile R*, 18 volumes of *tetrahydrofuran R* and 74 volumes of *water R* as the mobile phase at a flow rate of 1.8 ml/min;

— a variable wavelength spectrophotometer capable of operating at 254 nm and at 283 nm as detector.

Inject separately 20 µl of the test solution and 20 µl of reference solution (a), 20 µl of reference solution (d) and 20 µl of reference solution (e) and record the chromatograms with the detector set for recording at 254 nm. Continue the chromatography for twice the retention time of spironolactone. In the chromatogram obtained with the test solution, the sum of the areas of the peaks, except those corresponding to spironolactone and canrenone, is not greater than the area of the peak corresponding to spironolactone in the chromatogram obtained with reference solution (a) (1.0 per cent). Disregard any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent). Inject 20 µl of the test solution and 20 µl of reference solution (c) and record the chromatogram with the detector set for recording at 283 nm. In the chromatogram obtained with the test solution, the area of any peak corresponding to canrenone is not greater than the area of the peak corresponding to canrenone in the chromatogram obtained with reference solution (c) (1.0 per cent). Calculate the percentage content of canrenone found when recording at 283 nm and the percentage content of the other related substances found when recording at 254 nm. Add together these percentages. The sum is not greater than 1.0 per cent. The test is not valid unless: the chromatogram obtained with reference solution (d) shows peaks, corresponding to canrenone and spironolactone, with a resolution greater than 1.4; the principal peak in the chromatogram obtained with reference solution (e) has a signal-to-noise ratio of at least 6.

Free mercapto compounds

To 2.0 g add 20 ml of *water R*, shake for 1 min and filter. To 10 ml of the filtrate add 0.05 ml of *0.01 M iodine* and 0.1 ml of *starch solution R* and mix. A blue colour develops.

Chromium

To 0.20 g in a platinum crucible add 1 g of *potassium carbonate R* and 0.3 g of *potassium nitrate R*. Heat gently until fused, and ignite at 600 °C to 650 °C until carbon is removed. Cool, dissolve the residue as completely as possible in 10 ml of *water R* with the aid of gentle heat, filter, and dilute to 20 ml with *water R*. To 10 ml of this solution add 0.5 g of *urea R*, and add a 14 per cent *V/V* solution of *sulphuric acid R* until the solution is just acid. When effervescence ceases, add a further 1 ml of the sulphuric acid, dilute to 20 ml with *water R* and add 0.5 ml of *diphenylcarbazide solution R*. The solution is not more intensely coloured than a standard prepared by adding 1 ml of a 14 per cent *V/V* solution of *sulphuric acid R* to 0.50 ml of a freshly prepared 28.3 mg/l solution of *potassium dichromate R*, diluting to 20 ml with *water R* and adding 0.5 ml of *diphenylcarbazide solution R* (50 ppm).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 50.0 mg in *methanol R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *methanol R*. Measure the absorbance *(2.2.25)* of the solution at the maximum at 238 nm.

Calculate the content of $C_{24}H_{32}O_4S$, taking the specific absorbance to be 470.

STORAGE

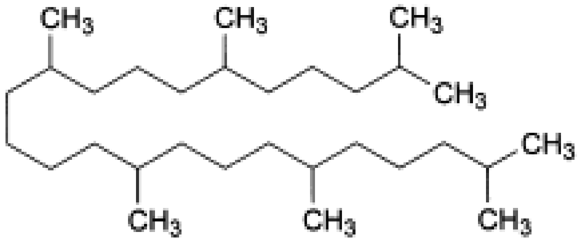
Store protected from light.

Ph Eur

Squalane

General Notices

(Ph Eur monograph 1630)



$C_{30}H_{62}$ 422.8 *111-01-3*

Action and use

Excipient; emollient.

Ph Eur

DEFINITION

2,6,10,15,19,23-Hexamethyltetracosane (perhydrosqualene). It may be of vegetable (unsaponifiable matter of olive oil) or animal (shark liver oil) origin.

Content

96.0 per cent to 103.0 per cent.

CHARACTERS

Appearance

Clear, colourless, oily liquid.

Solubility

Practically insoluble in water, miscible with most fats and oils, freely soluble in acetone and in cyclohexane, practically insoluble in ethanol (96 per cent).

Relative density

About 0.815.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison squalane CRS.

B. Refractive index (see Tests).

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

The chromatogram obtained with squalane of vegetable origin shows a peak corresponding to cyclosqualane (Figure 1630.-1 and Figure 1630.-2).

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Refractive index *(2.2.6)*

1.450 to 1.454.

Acid value *(2.5.1)*

Maximum 0.2.

Iodine value *(2.5.4, Method A)*

Maximum 4.0.

Saponification value *(2.5.6)*

Maximum 3.0.

Nickel *(2.4.31)*

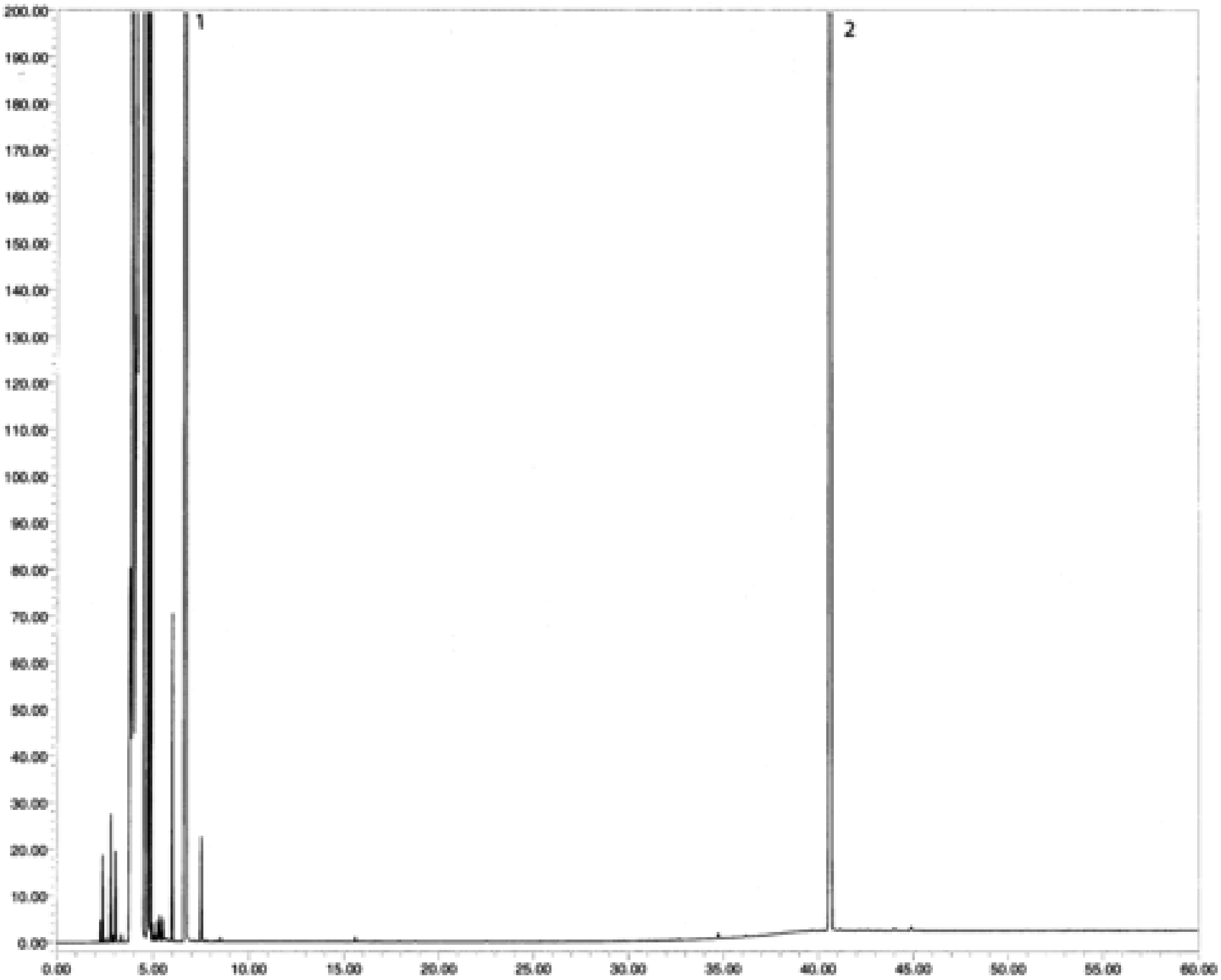
Maximum 1 ppm.

Total ash *(2.4.16)*

Maximum 0.5 per cent, determined on 1.000 g.

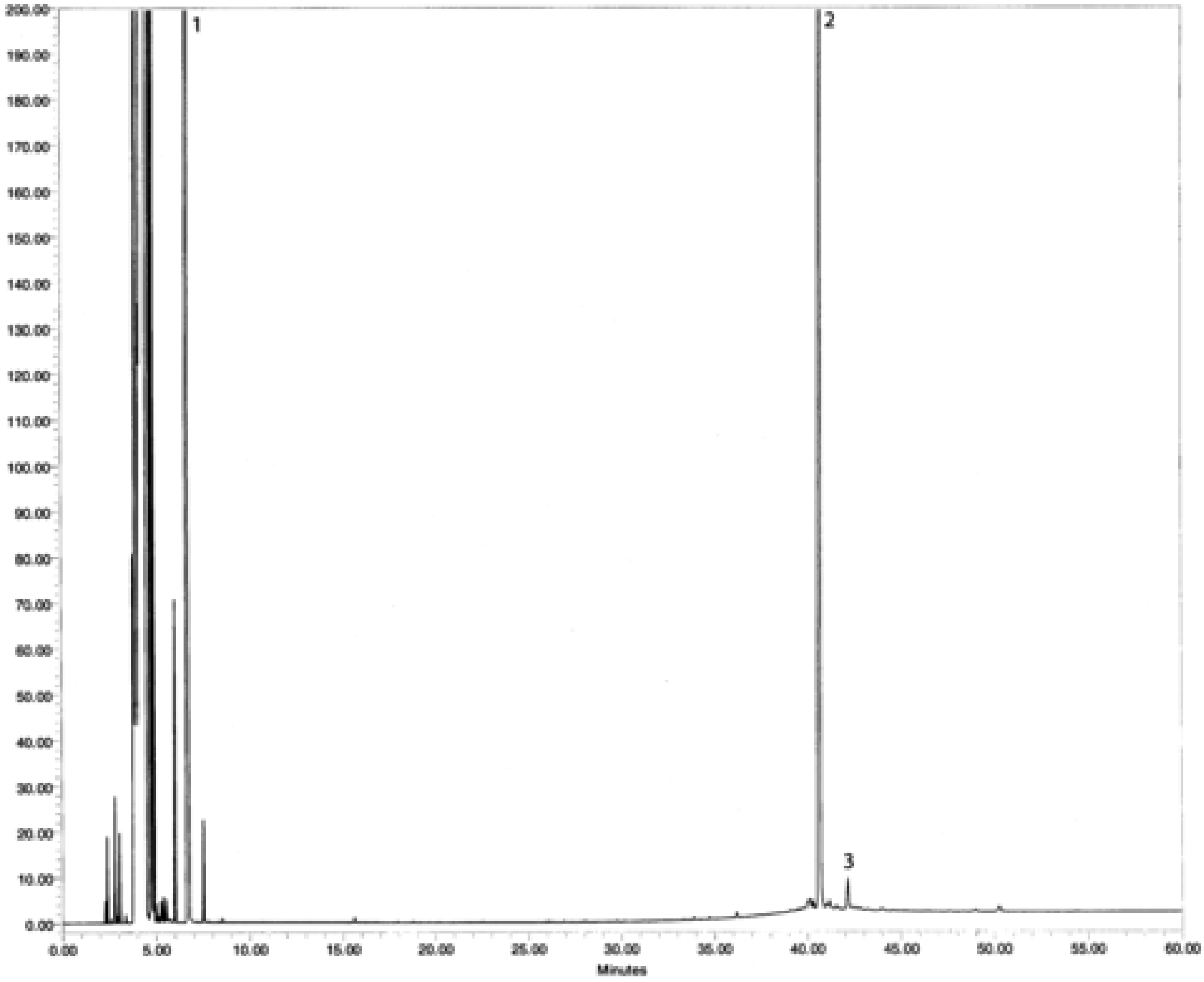
ASSAY

Gas chromatography *(2.2.28)* .



1. DMA 2. squalane

Figure 1630.-1. – *Chromatogram of squalane of animal origin*



1. DMA 2. squalane 3. cyclosqualane

Figure 1630.-2. – *Chromatogram of squalane of vegetable origin*

Internal standard solution To 1.0 ml of *dimethylacetamide R* , add 100.0 ml of *heptane R* .

Test solution Dissolve 0.100 g in the internal standard solution and dilute to 25.0 ml with the same solution.

Reference solution (a) Dissolve 0.100 g of *squalane CRS* in the internal standard solution and dilute to 25.0 ml with the same solution.

Reference solution (b) To 0.1 ml of *methyl erucate R* add 0.100 g of the substance to be examined, dissolve in the internal standard solution and dilute to 25.0 ml with the same solution.

Column:

— *material*: fused silica;

— *size*: *l* = 30 m, Ø = 0.32 mm;

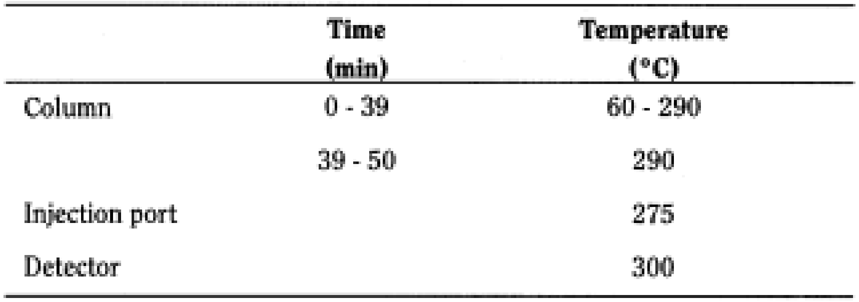
— *stationary phase: poly(dimethyl)siloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1.7 ml/min.

Split ratio 1:12.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 39	60 - 290
	39 - 50	290
Injection port		275
Detector		300

Detection Flame ionisation.

Injection 1 µl.

Relative retentions With reference to squalane (retention time = about 41 min): internal standard = about 0.2; methyl erucate = about 0.9; cyclosqualane = 1.05.

System suitability Reference solution (b):

— *resolution*: minimum 5 between the peaks due to methyl erucate and squalane.

Calculate the percentage content of squalane from the declared content of *squalane CRS* .

LABELLING

The label states the origin of squalane (vegetable or animal).

Ph Eur

Stannous Chloride Dihydrate

General Notices

(Ph Eur monograph 1266)

$SnCl_2,2H_2O$ 225.6 *10025-69-1*

Ph Eur

DEFINITION

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, efflorescent in air.

Solubility

Freely soluble in water (the solution becomes cloudy after standing or on dilution), freely soluble in ethanol (96 per cent). It dissolves in dilute hydrochloric acid.

IDENTIFICATION

A. To 1 ml of solution S1 (see Tests) add 5 ml of *water R* and 0.05 ml of *mercuric chloride solution R* . A blackish-grey precipitate is formed.

B. Dissolve 1.0 g in 3.0 ml of *water R* . Add 0.5 ml of *dilute sodium hydroxide solution R* to the cloudy solution; a yellowish flocculent precipitate is formed. Add 6.5 ml of *water R* . To 1.0 ml of the previously shaken suspension add 1.0 ml of *strong sodium hydroxide solution R* ; the precipitate dissolves and the resulting solution is clear and colourless.

C. Dissolve 10 mg in 2 ml of *dilute nitric acid R* . The solution gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S1

To 0.40 g add 1 ml of *dilute hydrochloric acid R* and dilute to 20 ml with *distilled water R* .

Solution S2

Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 30 ml with the same acid. Heat to boiling. Add 30 ml of *thioacetamide solution R* and boil for 15 min (solution A). Take 5 ml, filter and heat the filtrate to boiling. Add 5 ml of thioacetamide solution R and boil for 15 min. If a precipitate is formed, add the remainder of solution A (solution A′) to the mixture. Add 10 ml of *thioacetamide solution R* and boil. Repeat the series of operations from "Take 5 ml" until a precipitate is no longer formed on addition of *thioacetamide solution R* to the filtrate obtained from the 5 ml of solution A (solution A′, solution A″, etc. respectively). If no precipitate is formed or if no more precipitate is formed combine the solution obtained with the remainder of solution A (solution A′, solution A″, etc. respectively), filter and wash the precipitate with 10 ml of *water R* . Heat the filtrate until the resulting vapour no longer turns a moistened piece of *lead acetate paper R* blackish-grey. Allow to cool and dilute to 50 ml with *water R* .

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 10.0 g in *dilute hydrochloric acid R* and dilute to 20 ml with the same acid.

Substances not precipitated by thioacetamide

Maximum 0.2 per cent.

Evaporate 25 ml of solution S2 to dryness and ignite at 600 ± 50 °C. The residue weighs a maximum of 1 mg.

Sulphates ***(2.4.13)***

Maximum 500 ppm, determined on solution S1.

Iron ***(2.4.9)***

Maximum 100 ppm.

Dilute 5 ml of solution S2 to 10 ml with *water R* .

Heavy metals

Maximum 50 ppm.

Dissolve 1.0 g in 2 ml of a mixture of 1 volume of *nitric acid R* and 3 volumes of hydrochloric *acid R*. Heat on a water-bath until nitrous vapour is no longer evolved. Dissolve the residue in *water R* and dilute to 25 ml with the same solvent. To 5 ml of this solution add 3 ml of *strong sodium hydroxide solution R* and 2 ml of *water R* . Heat until a clear solution is obtained, then cool and add 0.5 ml of *thioacetamide reagent R* . After 2 min, any colour in the solution is not more intense than that of a mixture of 1.0 ml of *lead standard solution (10 ppm Pb) R* , 6 ml of *water R* , 3 ml of *strong sodium hydroxide solution R* and 0.5 ml of *thioacetamide reagent R* .

ASSAY

Dissolve 0.100 g in 50 ml of *water R* , freed from oxygen by purging with carbon dioxide or nitrogen for 15 min. Add 1.5 ml of *hydrochloric acid R1* , 5 g of *sodium potassium tartrate R* , 10 g of *sodium hydrogen carbonate R* and 1 ml of *starch solution R* . Titrate immediately with *0.05 M iodine* . Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 11.28 mg of $SnCl_2,2H_2O$.

STORAGE

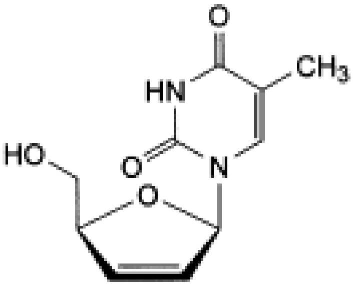
In an airtight container .

Ph Eur

Stavudine

General Notices

(Ph Eur monograph 2130)



$C_{10}H_{12}N_2O_4$ 224.2 *3056-17-5*

Action and use

Nucleoside reverse transciptase inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water, sparingly soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation *(2.2.7)*

- 39.5 to - 45.9 (anhydrous substance).

Dissolve 0.100 g in *water R* and dilute to 10.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *stavudine CRS* .

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R* , evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use or maintain at 2-8 ‘C until use.*

Test solution Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 0.5 ml of the test solution to 100.0 ml with *water R.*

Reference solution (b) Dilute 20 ml of reference solution (a) to 100.0 ml with *water R.*

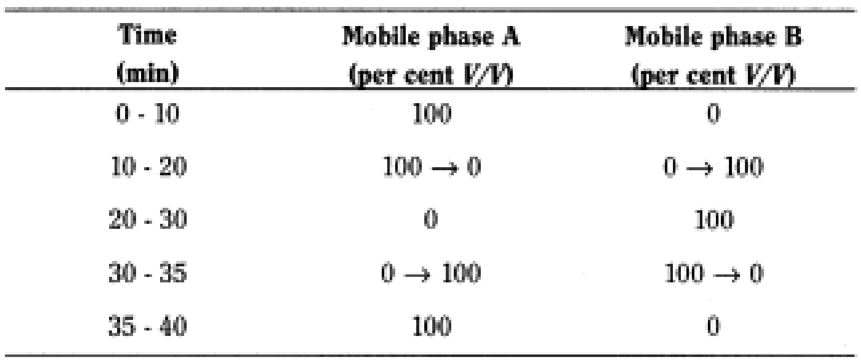
Reference solution (c) Dissolve 5.0 mg of *stavudine for system suitability CRS* (stavudine containing impurities A to H) in *water R* and dilute to 10.0 ml with the same solvent.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: mix 35 volumes of *acetonitrile for chromatography* R and 965 volumes of a 0.77 g/l solution of *ammonium acetate R;*

— *mobile phase B*: mix 250 volumes of *acetonitrile for chromatography R* and 750 volumes of a 0.77 g/l solution of *ammonium acetate R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 20	100 → 0	0 → 100
20 - 30	0	100
30 - 35	0 → 100	100 → 0
35 - 40	100	0

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *stavudine for system suitability CRS* to identify the peaks due to impurities A to H.

Relative retention With reference to stavudine (retention time = 9.5-12.5 min): impurity A = about 0.3; impurity B = about 0.50; impurity C = about 0.53; impurity D = about 0.8; impurity E = about 1.1; impurity F = about 1.4; impurity G = about 1.8; impurity H = about 2.0.

System suitability Reference solution (c):

— *peak-to-valley ratio* : minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and *Hv* = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B; minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and *Hv* = height above the baseline of the lowest point of the curve separating this peak from the peak due to stavudine.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.7;

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use or maintain at 2-8 °C until use.*

Test solution Dissolve 10.0 mg of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 50.0 ml with *water R* .

Reference solution (a) Dissolve 10.0 mg of *stavudine CRS* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 50.0 ml with *water R* .

Reference solution (b) Dissolve 5 mg of *thymine R* and 7.5 mg of *thymidine R* in *water R* and dilute to 100 ml with the same solvent. Dilute 10 ml of the solution to 50 ml with *water R* .

Column:
- *size*: *l* = 0.033 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Mix 5 volumes of *acetonitrile for chromatography R* and 95 volumes of a 0.77 g/l solution of *ammonium acetate R* .

Flow rate 0.7 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 25 µl.

Retention time Stavudine = 2.8 min to 5.0 min.

System suitability:
- *symmetry factor* : maximum 1.6 for the peak due to stavudine in the chromatogram obtained with reference solution (a);
- *resolution:* minimum 3.5 between the peaks due to impurity A and impurity C in the chromatogram obtained with reference solution (b).

Calculate the percentage content of $C_{10}H_{12}N_2O_4$ using the chromatograms obtained with the test solution and reference solution (a) and the declared content of *stavudine CRS.*

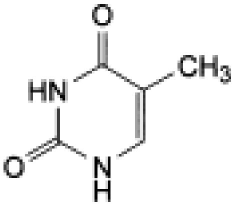
STORAGE

Protected from light and humidity.

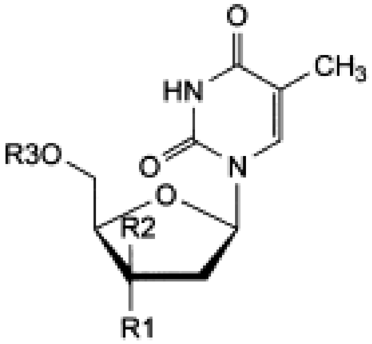
IMPURITIES

Specified impurities *A.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E, F, G, H.*



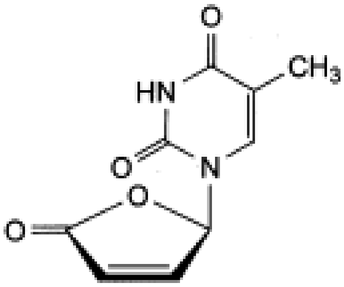
A. 5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine),



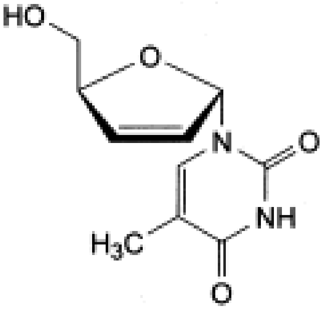
B. R1 = R3 = H, R2 = OH: 1-(2-deoxy-β-D-*threo*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*, 3*H*)-dione (3′-epithymidine),

C. R1 = OH, R2 = R3 = H: 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4 (1*H*,3*H*)-dione (thymidine),

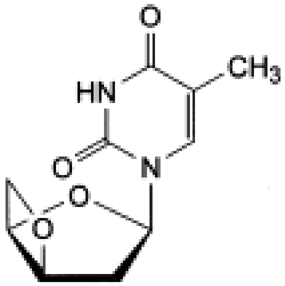
H. R1 = H, R2 = OH, R3 = $CH(CH_3)_2$: 1-[2-deoxy-5-*C*-(1-methylethyl)-β-D-*erythro*-pentofuranosyl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



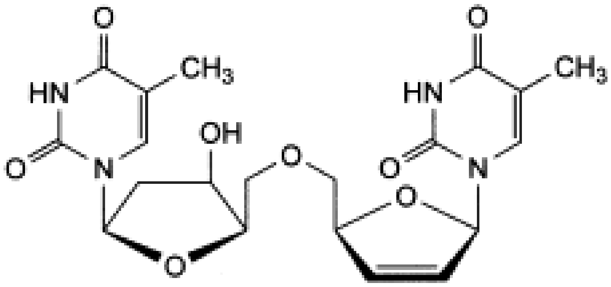
D. 1-[(2*R*)-5-oxo-2,5-dihydrofuran-2-yl]-5-methylpyrimidine2,4(1*H*,3*H*)-dione,



E. 1-(2,3-dideoxy-α-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (stavudine anomer α),



F. 1-(3,5-anhydro-2-deoxy-β-D-*threo*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



G. 5′-*O*-[[(2*S*,5*R*)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-yl)-2,5-dihydrofuran-2-yl]methyl]-3′-epithymidine.

Ph Eur

Stearic Acid

General Notices

(Ph Eur monograph 1474)

Action and use

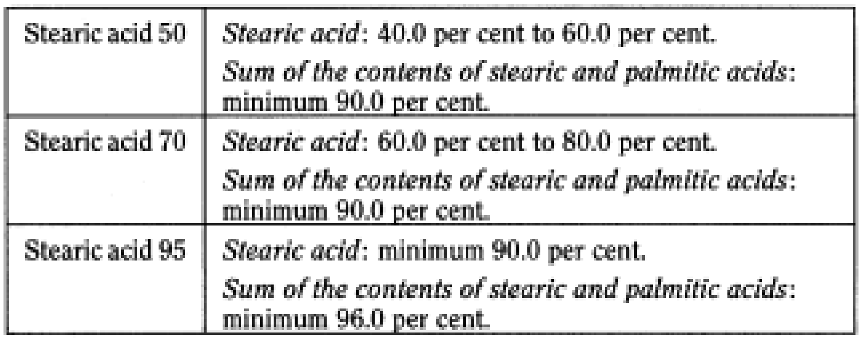
Excipient.

Ph Eur

DEFINITION

Mixture consisting mainly of stearic (octadecanoic) acid ($C_{18}H_{36}O_2$; M_r 284.5) and palmitic (hexadecanoic) acid ($C_{16}H_{32}O_2$; M_r 256.4) obtained from fats or oils of vegetable or animal origin.

Content



Stearic acid 50	*Stearic acid*: 40.0 per cent to 60.0 per cent. *Sum of the contents of stearic and palmitic acids*: minimum 90.0 per cent.
Stearic acid 70	*Stearic acid*: 60.0 per cent to 80.0 per cent. *Sum of the contents of stearic and palmitic acids*: minimum 90.0 per cent.
Stearic acid 95	*Stearic acid*: minimum 90.0 per cent. *Sum of the contents of stearic and palmitic acids*: minimum 96.0 per cent.

CHARACTERS

Appearance

White or almost white, waxy, flaky crystals, white or almost white hard masses or white or yellowish-white powder.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) and in light petroleum (50-70 °C).

IDENTIFICATION

A. It complies with the test for freezing point (see Tests).

B. Acid value *(2.5.1)* : 194 to 212, determined on 0.5 g.

C. Examine the chromatograms obtained in the assay.

Results The retention times of the principal peaks in the chromatogram obtained with the test solution are approximately the same as those of the principal peaks in the chromatogram obtained with the reference solution.

TESTS

Appearance

Heat the substance to be examined to about 75 °C. The resulting liquid is not more intensely coloured than reference solution Y_7 or BY_7 *(2.2.2, Method I)*.

Acidity

Melt 5.0 g, shake for 2 min with 10 ml of hot *carbon dioxide-free water R* , cool slowly and filter. To the filtrate add 0.05 ml of *methyl orange solution R* . No red colour develops.

Iodine value *(2.5.4)*

See Table 1474.-1.

Freezing point *(2.2.18)*

See Table 1474.-1.

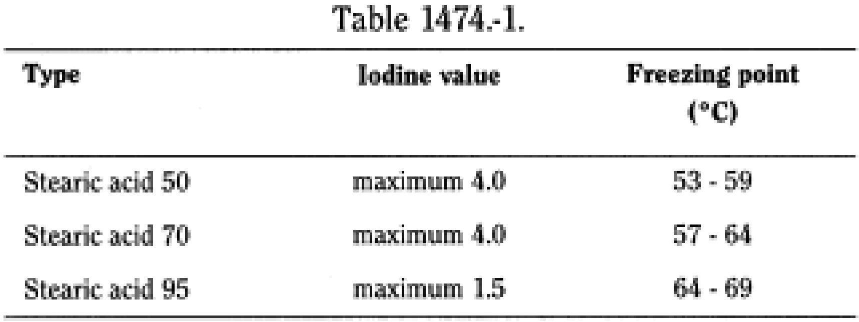


Table 1474.-1.

Type	Iodine value	Freezing point (°C)
Stearic acid 50	maximum 4.0	53 - 59
Stearic acid 70	maximum 4.0	57 - 64
Stearic acid 95	maximum 1.5	64 - 69

Nickel *(2.4.27)*

Maximum 1 ppm.

ASSAY

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution In a conical flask fitted with a reflux condenser, dissolve 0.100 g of the substance to be examined in 5 ml of *boron trifluoride-methanol solution R* . Boil under reflux for 10 min. Add 4.0 ml of *heptane R* through the condenser and boil again under reflux for 10 min. Allow to cool. Add 20 ml of a saturated solution of *sodium chloride R* . Shake and allow the layers to separate. Remove about 2 ml of the organic layer and dry it over 0.2 g of *anhydrous sodium sulphate R* . Dilute 1.0 ml of this solution to 10.0 ml with *heptane R* .

Reference solution Prepare the reference solution in the same manner as the test solution using 50 mg of *palmitic acid CRS* and 50 mg of *stearic acid CRS* instead of the substance to be examined.

Column:

— *material:* fused silica;

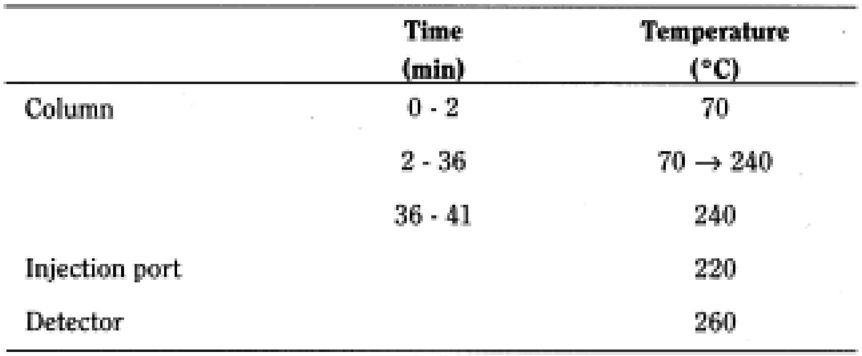
— *size:* *l* = 30 m, Ø = 0.32 mm;

— *stationary phase: macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R* .

Flow rate 2.4 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	70
	2 - 36	70 → 240
	36 - 41	240
Injection port		220
Detector		260

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to methyl stearate: methyl palmitate = about 0.88.

System suitability Reference solution:

— *resolution*: minimum 5.0 between the peaks due to methyl stearate and methyl palmitate.

LABELLING

The label states the type of stearic acid (50, 70, 95).

Ph Eur

Stearoyl Macrogolglycerides

General Notices

(Ph Eur monograph 1268)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols with a mean relative molecular mass between 300 and 4000.

They are obtained by partial alcoholysis of saturated oils containing mainly triglycerides of stearic (octadecanoic) acid, using macrogol, or by esterification of glycerol and macrogol with saturated fatty acids, or by mixture of glycerol esters and condensates of ethylene oxide with the fatty acids of these hydrogenated oils.

The hydroxyl value is within 15 units of the nominal value. The saponification value is within 10 units of the nominal value.

CHARACTERS

Appearance

Pale yellow waxy solid.

Solubility

Dispersible in warm water and in warm liquid paraffin, freely soluble in methylene chloride, soluble in warm anhydrous ethanol.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R* .

Mobile phase *hexane R, ether R* (30:70 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/l solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9 (R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.7), to 1,2-diglycerides (R_{st} 0.6), to monoglycerides (R_{st} 0.1) and to esters of macrogol (R_{st} 0).

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Fatty acid composition (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 2.0, determined on 2.0 g.

Hydroxyl value *(2.5.3, Method A)*

Within 15 units of the nominal value, determined on 1.0 g.

Peroxide value *(2.5.5, Method A)*

Maximum 6.0, determined on 2.0 g.

Saponification value *(2.5.6)*

Within 10 units of the nominal value, determined on 2.0 g.

Alkaline impurities

Into a test-tube introduce 5.0 g and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 ml of a 0.4 g/l solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 ml of *water R* and 10 ml of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 ml of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol

Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 ml of *methylene chloride R.* Heat if necessary. After cooling, add 100 ml of *water R*. Shake and add 25.0 ml of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 ml of a 75 g/l solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 ml of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulphate*. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids

Gas chromatography *(2.4.22, Method A)*.

Composition of the fatty-acid fraction of the substance:

- *lauric acid* : maximum 5.0 per cent;
- *myristic acid* : maximum 5.0 per cent;
- stearic acid and palmitic acid: different nominal amounts and minimum 90.0 per cent for the sum of $C_{18}H_{36}O_2$ and $C_{16}H_{32}O_2$.

Ethylene oxide and dioxan ***(2.4.25)***

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R* .

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.0 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash ***(2.4.16)***

Maximum 0.2 per cent

LABELLING

The label states:

- The nominal hydroxyl value;
- The nominal saponification value;
- The type of the macrogol used (mean relative molecular mass) or the number of moles of ethylene oxide reacted per mole of substance (nominal value).

Ph Eur

Stearyl Alcohol

General Notices

(Ph Eur monograph 0753)

112-92-5

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of solid alcohols, mainly octadecan-1-ol ($C_{18}H_{38}O$; M_r 270.5), of animal or vegetable origin.

Content

Minimum 95.0 per cent of $C_{18}H_{38}O$.

CHARACTERS

Appearance

White or almost white, unctuous flakes, granules or mass.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent). When melted, it is miscible with fatty oils, with liquid paraffin and with melted wool fat.

IDENTIFICATION

Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (b).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_6

(2.2.2, Method II).

Dissolve 0.50 g in 20 ml of boiling *ethanol (96 per cent) R* . Allow to cool.

Melting point ***(2.2.14)***

57 °C to 60 °C.

Acid value ***(2.5.1)***

Maximum 1.0.

Hydroxyl value ***(2.5.3, Method A)***

197 to 217.

Iodine value ***(2.5.4, Method A)***

Maximum 2.0.

Dissolve 2.00 g in *methylene chloride R* , warming if necessary and dilute to 25 ml with the same solvent.

Saponification value ***(2.5.6)***

Maximum 2.0.

ASSAY

Gas chromatography *(2.2.28)*

use the normalisation procedure.

Test solution Dissolve 0.100 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of *cetyl alcohol R* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 50 mg of *stearyl alcohol CRS* in *ethanol (96 per cent) R* and dilute to 5 ml with the same solvent.

Reference solution (c) Mix 1 ml of reference solution (a) and 1 ml of reference solution (b) and dilute to 10 ml with *ethanol (96 per cent) R.*

Column:

— *size: l* = 30 m, Ø = 0.32 mm;

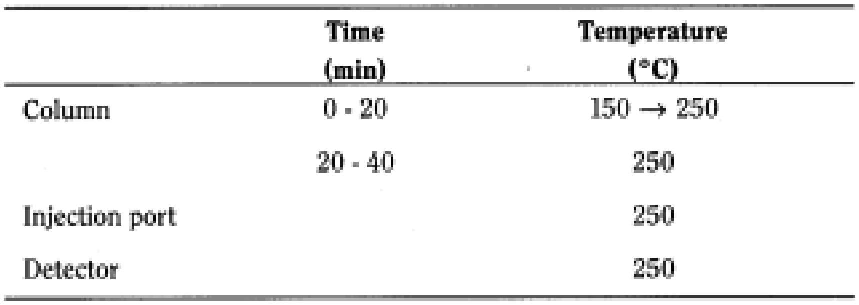
— *stationary phase*: *poly(dimethyl)siloxane R* (1 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 20	150 → 250
	20 - 40	250
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl of the test solution and reference solutions (b) and (c).

System suitability Reference solution (c):

— *resolution*: minimum 5.0 between the peaks due to cetyl alcohol and stearyl alcohol.

Calculate the percentage content of $C_{18}H_{38}O$.

Ph Eur

Streptokinase Concentrated Solution

General Notices

Streptokinase Bulk Solution

(Ph Eur monograph 0356)

Action and use

Tissue-type plasminogen activator; fibrinolytic.

Preparation

Streptokinase Injection

Ph Eur

DEFINITION

Streptokinase concentrated solution is a preparation of a protein obtained from culture filtrates of certain strains of haemolytic *Streptococcus* group C; it has the property of combining with human plasminogen to form plasminogen activator. It may contain buffer salts and other excipients. The potency is not less than 510 IU per microgram of nitrogen.

PRODUCTION

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Abnormal toxicity *(2.6.9)*

Inject into each mouse a quantity of the preparation to be examined (if necessary, dilute with *water for injections R*) equivalent to 50 000 IU of streptokinase activity, the injection lasting 15-20 s.

CHARACTERS

Appearance

Clear, colourless liquid.

IDENTIFICATION

A. Place 0.5 ml of citrated human plasma in a polystyrene tube maintained in a water-bath at 37 °C. Add 0.1 ml of a dilution of the preparation to be examined containing 10 000 IU of streptokinase activity per millilitre in *phosphate buffer solution pH 7.2 R* and 0.1 ml of a solution of *human thrombin R* containing 20 IU/ml in *phosphate buffer solution pH 7.2 R*. Mix immediately. A clot forms and lyses within 30 min. Repeat the procedure using citrated bovine plasma. The clot does not lyse within 60 min.

B. Perform an immunochemical test using double immunodiffusion techniques *(2.7.1)*. Place in the central cavity about 80 µl of goat or rabbit antistreptokinase serum containing about 10 000 units of antistreptokinase activity per millilitre; place in each of the surrounding cavities about 80 µl of a dilution of the preparation to be examined containing 125 000 IU of streptokinase activity per millilitre. Allow the plates to stand in a humidified tank for 24 h. Only one precipitation arc appears and it is well defined.

TESTS

pH *(2.2.3)*

6.8 to 7.5.

Dilute the preparation to be examined in *carbon dioxide-free water R* to obtain a solution containing at least 1000 000 IU of streptokinase activity per millilitre.

Streptodornase

Maximum 10 IU of streptodornase activity per 100 000 IU of streptokinase activity.

Test solution Dilute the preparation to be examined in *imidazole buffer solution pH 6.5 R* to obtain a solution containing 150 000 IU of streptokinase activity per millilitre.

Reference solution Dissolve in *imidazole buffer solution pH 6.5 R* a reference preparation of streptodornase, calibrated in International Units against the International Standard of streptodornase, to obtain a solution containing 20 IU of streptodornase activity per millilitre. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

To each of 8 numbered centrifuge tubes, add 0.5 ml of a 1 g/l solution of *sodium deoxyribonucleate R* in *imidazole buffer solution pH 6.5 R*. To tube number 1 and tube number 2 add 0.25 ml of *imidazole buffer solution pH 6.5 R*, 0.25 ml of the test solution and, immediately, 3.0 ml of perchloric acid (25 g/l $HClO_4$). Mix, centrifuge at about 3000 g for 5 min and measure the absorbances *(2.2.25)* of the supernatant liquids at 260 nm, using as the compensation liquid a mixture of 1.0 ml of *imidazole buffer solution pH 6.5 R* and 3.0 ml of perchloric acid (25 g/l $HClO_4$) (absorbances A_1 and A_2). To the other 6 tubes (numbers 3 to 8) add 0.25 ml, 0.25 ml, 0.125 ml, 0.125 ml, 0 ml and 0 ml respectively of *imidazole buffer solution pH 6.5 R*; add to each tube 0.25 ml of the test solution and 0 ml, 0 ml, 0.125 ml, 0.125 ml, 0.25 ml and 0.25 ml respectively of the reference solution. Mix the contents of each tube and heat at 37 °C for 15 min. To each tube add 3.0 ml of perchloric acid (25 g/l $HClO_4$), mix and centrifuge. Measure the absorbances *(2.2.25)* of the supernatant liquids at 260 nm using the compensation liquid described above (absorbances A_3 to A_8). The absorbances comply with the following requirement:

$$(A_3 + A_4) - (A_1 + A_2) < \frac{(A_5 + A_6 + A_7 + A_8)}{2} - (A_3 + A_4)$$

Streptolysin

In a polystyrene tube, use a quantity of the preparation to be examined equivalent to 500 000 IU of streptokinase activity and dilute to 0.5 ml with a mixture of 1 volume of *phosphate buffer solution pH 7.2 R* and 9 volumes of a 9 g/l solution of *sodium chloride R*. Add 0.4 ml of a 23 g/l solution of *sodium thioglycollate R*. Heat in a water-bath at 37 °C for 10 min. Add 0.1 ml of a solution of a reference preparation of human antistreptolysin O containing 5 IU/ml. Heat at 37 °C for 5 min. Add 1 ml of *rabbit erythrocyte suspension R*. Heat at 37 °C for 30 min. Centrifuge at about 1000 *g*. In the same manner, prepare a polystyrene tube in which the solution of the preparation to be examined has been replaced by 0.5 ml of a mixture of 1 volume of *phosphate buffer solution pH 7.2 R* and 9 volumes of a 9 g/l solution of *sodium chloride R*. Measure the absorbances *(2.2.25)* of the supernatant liquids at 550 nm. The absorbance of the test solution is not more than 50 per cent greater than that of the reference solution.

Related substances

Liquid chromatography *(2.2.29)* : use the normalisation procedure.

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of about 0.5-1 g/l, depending on the chromatographic system used.

Reference solution Dilute 1 volume of *streptokinase for system suitability CRS* with 49 volumes of *water R*.

Column:

— *size: l* = 0.10 m, Ø = 4.6 mm;

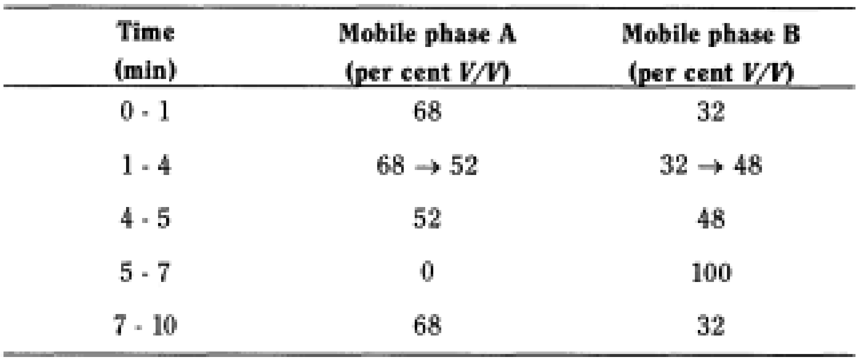
— *stationary phase: styrene-divinylbenzene copolymer R* (10 µm) with a pore size of 200 nm;

— *temperature*: 25 °C.

Mobile phase:

— *mobile phase A*: *trifluoroacetic acid R, water for injections R* (1:1000 *V/V*); degas;

— *mobile phase B*: *trifluoroacetic acid R, acetonitrile for chromatography R* (1:1000 *V/V*); degas;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	68	32
1 - 4	68 → 52	32 → 48
4 - 5	52	48
5 - 7	0	100
7 - 10	68	32

The above conditions may be modified to improve the separation efficiency of the chromatographic system.

Flow rate 5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Retention time Streptokinase = 2.3 min to 2.8 min.

System suitability Reference solution:

— *symmetry factor* : maximum 1.9 for the peak due to streptokinase;

— *peak-to-valley ratio* : minimum 2, where H_p = height above the baseline of the 1st peak eluting after the principal peak and H_v = height above the baseline of the lowest point of the curve separating this peak from the 2nd peak eluting after the principal peak;

— the chromatogram obtained with the reference solution is similar to the chromatogram supplied with *streptokinase for system suitability CRS*.

Limit:
— *total*: maximum 5 per cent.

Bacterial endotoxins ***(2.6.14)***

Less than 0.02 IU per 100 IU of streptokinase activity, if intended for use without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Nitrogen *(2.5.9)*.

Potency

The potency of streptokinase is determined by comparing its capacity to activate plasminogen to form plasmin with the same capacity of a reference preparation of streptokinase calibrated in International Units; the formation of plasmin is determined using a suitable chromogenic substrate.

The International Unit is the activity of a stated amount of the International Standard for streptokinase. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Reference and test solutions Prepare 2 independent series of at least 3 dilutions of each of the preparation to be examined and of the reference preparation of streptokinase in *tris (hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R1*, in the linear range of the assay (a range of 0.5-4.0 IU/ml has been found suitable). Prepare and maintain all solutions at 37 °C.

Substrate solution Mix 1.0 ml of *tris(hydroxymethyl)aminomethane buffer solution pH 7.4 R* with 1.0 ml of *chromophore substrate R3* . Add 5 µl of a 100 g/l solution of *polysorbate 20 R* . Keep at 37 °C in a water-bath. Immediately before commencing the activation assay, add 45 µl of a 1 mg/ml solution of *human plasminogen R* .

Method Analyse each streptokinase dilution, maintained at 37 °C, in duplicate. Initiate the activation reaction by adding 60 µl of each dilution to 40 µl of substrate solution. For blank wells, use 60 µl of *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R1* instead of the reference and test solutions. Allow the reaction to proceed at 37 °C for 20 min and read the absorbance *(2.2.25)* at 405 nm. If a suitable thermostatted plate reader is available, this may be used to monitor the reaction. Alternatively, it may be necessary to stop the reaction after 20 min using 50 µl of a 50 per cent *V/V* solution of *glacial acetic acid R* .

Best results are obtained when the absorbance for the highest streptokinase concentration is between 0.1 and 0.2 (after blank subtraction). If necessary, adjust the time of incubation in order to reach this range of absorbances.

Calculate the regression of the absorbance on log concentrations of the solutions of the preparation to be examined and of the reference preparation of streptokinase and calculate the potency of the preparation to be examined using a suitable statistical method, for example the parallel-line assay *(5.3)*.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

STORAGE

In an airtight container, protected from light and at a temperature of - 20 °C. If the preparation is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

— the number of International Units of streptokinase activity per milligram, calculated with reference to the dried preparation;

— the name and quantity of any added substance;

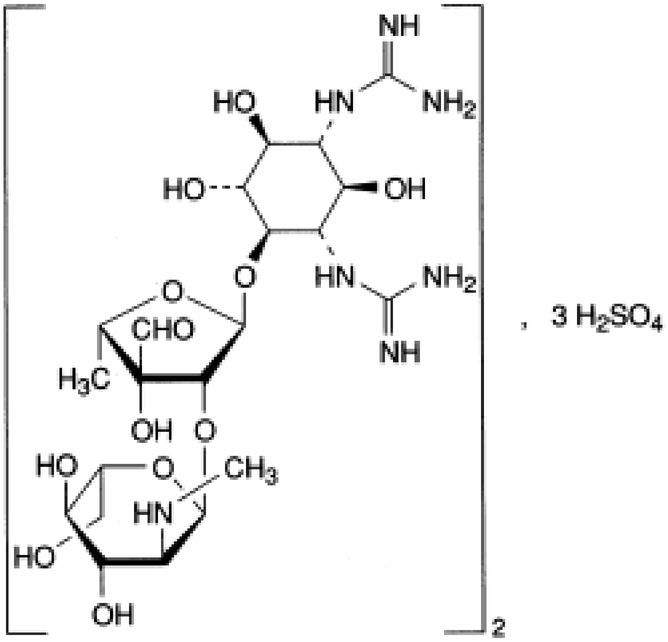
— that the preparation is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Streptomycin Sulphate

General Notices

(Ph Eur monograph 0053)



$(C_{21}H_{39}N_7O_{12})_2,3H_2SO_4$ 1457 *3810-74-0*

Action and use

Aminoglycoside antibacterial; antituberculosis drug.

Preparation

Streptomycin Injection

Ph Eur

DEFINITION

Streptomycin sulphate is bis[*N*,*N*′-bis(aminoiminomethyl)-4-*O*-[5-deoxy-2-*O*-[2-deoxy-2-(methylamino)-α-L-glucopyranosyl]-3-*C*-formyl-α-L-lyxofuranosyl]-D-streptamine] trisulphate, a substance produced by the growth of certain strains of *Streptomyces griseus* or obtained by any other means. Stabilisers may be added. The potency is not less than 720 IU/mg, calculated with reference to the dried substance.

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

The method of manufacture is validated to demonstrate that the product if tested would comply with the following test:

Abnormal toxicity *(2.6.9)*

Inject into each mouse 1 mg of the substance to be examined dissolved in 0.5 ml of *water for injections R.*

CHARACTERS

A white or almost white powder, hygroscopic, very soluble in water, practically insoluble in ethanol.

IDENTIFICATION

A. Examine by thin-layer chromatography *(2.2.27),* using a plate coated with a 0.75 mm layer of the following mixture: mix 0.3 g of *carbomer R* with 240 ml of *water R* and allow to stand, with moderate shaking, for 1 h; adjust to pH 7 by the gradual addition, with continuous shaking, of *dilute sodium hydroxide solution R* and add 30 g of *silica gel H R* .

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *streptomycin sulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *kanamycin monosulphate CRS* , 10 mg of *neomycin sulphate CRS* and 10 mg of *streptomycin sulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm using a 70 g/l solution of *potassium dihydrogen phosphate R* . Dry the plate in a current of warm air, and spray with a mixture of equal volumes of a 2 g/l solution of *1,3-dihydroxynaphthalene R* in *alcohol R* and a 460 g/l solution of *sulphuric acid R* . Heat at 150 °C for 5 min to 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 5 mg to 10 mg in 4 ml of *water R* and add 1 ml of *1 M sodium hydroxide* . Heat in a water-bath for 4 min. Add a slight excess of *dilute hydrochloric acid R* and 0.1 ml of *ferric chloride solution R1* . A violet colour develops.

C. Dissolve 0.1 g in 2 ml of *water R* , add 1 ml of *α-naphthol solution R* and 2 ml of a mixture of equal volumes of *strong sodium hypochlorite solution R* and *water R.* A red colour develops.

D. Dissolve about 10 mg in 5 ml of *water R* and add 1 ml of *1 M hydrochloric acid* . Heat in a water-bath for 2 min. Add 2 ml of a 5 g/l solution of *α-naphthol R* in *1 M sodium hydroxide* and heat in a water-bath for 1 min. A faint yellow colour develops.

E. It gives the reactions of sulphates *(2.3.1)* .

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is not more intensely coloured than intensity 3 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II).* Allow to stand protected from light, at a temperature of about 20 °C for 24 h. Solution S is not more opalescent than reference suspension II *(2.2.1).*

pH *(2.2.3)*

The pH of solution S is 4.5 to 7.0.

Methanol

Examine by gas chromatography *(2.2.28)* .

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Reference solution Dilute 12.0 mg of *methanol R* to 100 ml with *water R* .

The chromatographic procedure may be carried out using:

— a column 1.5 m to 2.0 m long and 2 mm to 4 mm in internal diameter, packed with *ethylvinylbenzene-divinylbenzene copolymer R* (150 µm to 180 µm);

— *nitrogen for chromatography R* as the carrier gas at a constant flow rate of 30 ml to 40 ml per minute;

— a flame-ionisation detector.

Maintain the column at a constant temperature between 120 °C and 140 °C and the injection port and the detector at a temperature at least 50 °C higher than that of the column. Inject the test solution and the reference solution. The area of the peak corresponding to methanol in the chromatogram obtained with the test solution is not greater than the area of the peak in the chromatogram obtained with the reference solution (0.3 per cent).

Streptomycin B

Examine by thin-layer chromatography *(2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in a freshly prepared mixture of 3 volumes of *sulphuric acid R* and 97 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents. Heat under a reflux condenser for 1 h, cool, rinse the condenser with *methanol R* and dilute to 20 ml with the same solvent (10 g/l solution).

Reference solution Dissolve 36 mg of *mannose R* in a freshly prepared mixture of 3 volumes of *sulphuric acid R* and 97 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents. Heat under a reflux condenser for 1 h, cool, rinse the condenser with *methanol R* and dilute to 50 ml with the same solvent. Dilute 5 ml of the solution to 50 ml with *methanol R* (0.3 g/l solution expressed as strepto-mycin B; 1 mg of *mannose R* is equivalent to 4.13 mg of streptomycin B).

Apply separately to the plate 10 µl of each solution. Develop over a path of 13 cm to 15 cm using a mixture of 25 volumes of *glacial acetic acid R,* 25 volumes of *methanol R* and 50 volumes of *toluene R* . Allow the plate to dry in air and spray with a freshly prepared mixture of equal volumes of a 2 g/l solution of *1,3-dihydroxynaphthalene R* in *alcohol R* and a 20 per cent *V/V* solution of *sulphuric acid R* and heat at 110 °C for 5 min. Any spot corresponding to streptomycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (3.0 per cent).

Loss on drying *(2.2.32)*

Not more than 7.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa for 24 h.

Sulphated ash *(2.4.14)*

Not more than 1.0 per cent, determined on 1.000 g.

Sulphate

18.0 per cent to 21.5 per cent of sulphate (SO_4), calculated with reference to the dried substance. Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R* . Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R* . Titrate with *0.1 M sodium edetate* adding 50 ml of *alcohol R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of sulphate (SO_4).

Colorimetric test

Dry the substance to be examined and *streptomycin sulphate CRS* at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa for 24 h. Dissolve 0.100 g of the dried substance to be examined in *water R* and dilute to 100.0 ml with the same solvent. Prepare a reference solution in the same manner using 0.100 g of the dried *streptomycin sulphate CRS* . Place 5.0 ml of each solution separately in two volumetric flasks and in a third flask place 5 ml of *water R.* To each flask add 5.0 ml of *0.2 M sodium hydroxide* and heat for exactly 10 min in a water-bath. Cool in ice for exactly 5 min, add 3 ml of a 15 g/l solution of *ferric ammonium sulphate R* in *0.5 M sulphuric acid* , dilute to 25.0 ml with *water R* and mix. Exactly 20 min after the addition of the ferric ammonium sulphate solution measure the absorbance *(2.2.25)* of the test solution and the reference solution in a 2 cm cell at the maximum at 525 nm, using as compensation liquid the solution prepared from 5 ml of *water R* . The absorbance of the test solution is not less than 90.0 per cent of that of the reference solution.

Bacterial endotoxins ***(2.6.14)***

Less than 0.25 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)* .

STORAGE

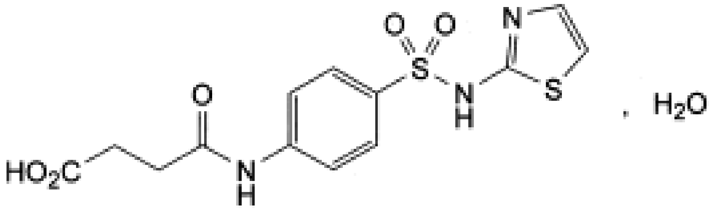
Store in an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

Ph Eur

Succinylsulfathiazole

General Notices

(Ph Eur monograph 0357)



$C_{13}H_{13}N_3O_5S_2,H_2O$ 373.4 *116-43-8*

Action and use

Sulfonamide antibacterial.

Ph Eur

DEFINITION

4-Oxo-4-[[4-(thiazol-2-ylsulphamoyl)phenyl]amino]butanoic acid monohydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Very slightly soluble in water, slightly soluble in acetone and in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification A, D.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison succinylsulfathiazole CRS .

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in hot *water R* , allow to crystallise, dry the crystals carefully between 2 sheets of filter paper and prepare new discs.

B. To 2 g add 10 ml of *water R* and 10 ml of *strong sodium hydroxide solution R* and boil for 10 min. Cool and adjust to pH 3.0 with *hydrochloric acid R1* . Cool, adjust to pH 7.0 with *sodium hydrogen carbonate solution R* and filter. The precipitate, washed with *water R* and dried at 100-105 °C, melts *(2.2.14)* at 196 °C to 204 °C. Introduce the capillary containing the precipitate into the bath at a temperature of 190 °C.

C. Heat 0.1 g in a test-tube over a small flame. Fumes are evolved which blacken *lead acetate paper R* .

D. Place 0.1 g in a borosilicate-glass tube of about 30 ml, add 0.5 g of *hydroquinone R* and 1 ml of *sulphuric acid R* . Heat in a glycerin bath at 135 °C for 10 min, mixing at the beginning of heating to obtain a homogeneous liquid phase. Allow to cool and place in iced water. Add carefully and with shaking 15 ml of *water R* . Add 5 ml of *toluene R* , shake for 5-10 s and allow to stand for 2 min; promote separation of the 2 layers using a stirrer. The upper layer (toluene) is intense pink.

E. Dissolve about 10 mg of the precipitate obtained in identification test B in 200 ml of *0.1 M hydrochloric acid* . 2 ml of this solution gives the reaction of primary aromatic amines *(2.3.1)* with formation of an orange precipitate.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_4 or BY_4 *(2.2.2, Method II)*.

Dissolve 1.0 g in a mixture of 5 ml of *dilute sodium hydroxide solution R* and 15 ml of *water R* .

Acidity

To 2.0 g add 20 ml of *water R* , shake continuously for 30 min and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R* . Not more than 2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Sulfathiazole and other primary aromatic amines

Maximum 0.75 per cent.

Dissolve 20 mg in a mixture of 3.5 ml of *water R* , 6 ml of *dilute hydrochloric acid R* and 25 ml of *ethanol (96 per cent) R,* previously cooled to 15 °C. Place immediately in iced water and add 1 ml of a 2.5 g/l solution of *sodium nitrite R* . Allow to stand for 3 min, add 2.5 ml of a 40 g/l solution of *sulphamic acid R* and allow to stand for 5 min. Add 1 ml of a 4 g/l solution of *naphthylethylenediamine dihydrochloride R* and dilute to 50 ml with *water R* . The absorbance (*2.2.25*) measured at 550 nm is not greater than that of a standard prepared at

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the same time and in the same manner using a mixture of 1.5 ml of a 100 ml aqueous solution containing 10 mg of *sulfathiazole R* and 0.5 ml of *hydrochloric acid R* ; 2 ml of *water R* ; 6 ml of *dilute hydrochloric acid R* ; and 25 ml of *ethanol (96 per cent) R* .

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

4.0 per cent to 5.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 100 ml of a mixture of 1 volume of *hydrochloric acid R* and 2 volumes of *water R* . Heat under a reflux condenser for 1 h. Carry out the determination of primary aromatic amino-nitrogen *(2.5.8*), determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 35.54 mg of $C_{13}H_{13}N_3O_5S_2$.

STORAGE

Protected from light.

Ph Eur

Sucrose Monopalmitate

General Notices

(Ph Eur monograph 2319)

Ph Eur

DEFINITION

Mixture of sucrose monoesters, mainly sucrose monopalmitate, obtained by transesterification of palmitic acid methyl esters of vegetable origin with *Sucrose (0204*). The manufacture of the fatty acid methyl esters includes a distillation step.

It contains variable quantities of mono- and diesters.

Content:

— *monoesters*: minimum 55.0 per cent;

— *diesters*: maximum 40.0 per cent;

— *sum of triesters and polyesters*: maximum 20.0 per cent.

CHARACTERS

Appearance

White or almost white, unctuous powder.

Solubility

Very slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Composition of fatty acids (see Tests).

B. It complies with the limits of the assay.

TESTS

Acid value *(2.5.1)*

Maximum 6.0, determined on 3.00 g.

Use a freshly neutralised mixture of 1 volume of *water R* and 2 volumes of *2-propanol R* as solvent and heat gently.

Composition of fatty acids *(2.4.22, Method C)*

Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty-acid fraction of the substance:

- *lauric acid* : maximum 3.0 per cent;
- *myristic acid* : maximum 3.0 per cent;
- *palmitic acid* : 70.0 per cent to 85.0 per cent;
- *stearic acid* : 10.0 per cent to 25.0 per cent;
- *sum of the contents of palmitic acid and stearic acid* : minimum 90.0 per cent.

Free sucrose

Liquid chromatography *(2.2.29)* .

Solvent mixture *water for chromatography R* , *tetrahydrofuran for chromatography R* (12.5:87.5 *V/V*).

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 4.0 ml with the solvent mixture.

Reference solution (a) Dissolve 5.0 mg of *sucrose CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

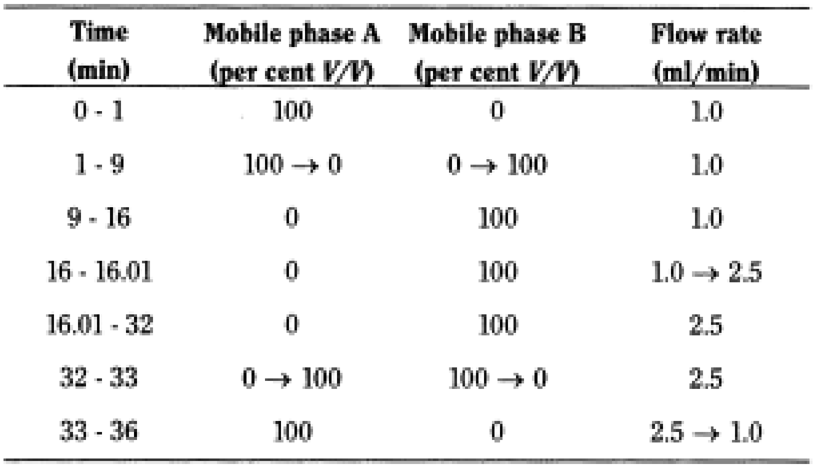
Reference solution (b) In 4 volumetric flasks, introduce respectively 5.0 mg, 10.0 mg, 20.0 mg and 25.0 mg of *sucrose CRS* , dissolve in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: spherical *aminopropylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:

- *mobile phase A*: 0.01 g/l solution of *ammonium acetate R* in *acetonitrile for chromatography R ;*
- *mobile phase B*: 0.01 g/l solution of *ammonium acetate R* in a mixture of 10 volumes of *water for chromatography R* and 90 volumes of *tetrahydrofuran for chromatography R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (ml/min)
0 - 1	100	0	1.0
1 - 9	100 → 0	0 → 100	1.0
9 - 16	0	100	1.0
16 - 16.01	0	100	1.0 → 2.5
16.01 - 32	0	100	2.5
32 - 33	0 → 100	100 → 0	2.5
33 - 36	100	0	2.5 → 1.0

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:

— *carrier gas*: *nitrogen R* ;

— *flow rate*: 1.0 ml/min;

— *evaporator temperature*: 45 °C;

— *nebuliser temperature*: 40 °C.

Injection 20 µl.

Retention time About 26 min.

System suitability Reference solution (a):

— *signal-to-noise ratio* : minimum 10.

Limit Maximum 4.0 per cent.

Water ***(2.5.12)***

Maximum 4.0 per cent, determined on 0.20 g.

Total ash ***(2.4.16)***

Maximum 1.5 per cent.

ASSAY

Size-exclusion chromatography *(2.2.30)* : use the normalisation procedure.

Test solution Dissolve 60.0 mg of the substance to be examined in *tetrahydrofuran R* and dilute to 4.0 ml with the same solvent.

Column:

— *size:* l = 0.6 m, Ø = 7 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R* .

Flow rate 1.2 ml/min.

Detection Differential refractometer.

Injection 20 µl.

Relative retention With reference to monoesters (retention time = about 10 min): diesters = about 0.92; triesters and polyesters = about 0.90.

Calculations:

— *disregard limit*: disregard the peaks having a signal-to-noise ratio less than 10;

— *free fatty acids*: calculate the percentage content (*D*) of free fatty acids, using the following expression:

$$\frac{I_A \times 256}{561.1}$$

I_A = acid value.

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\frac{A \times (100 - D - S - E)}{100}$$

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{B \times (100 - D - S - E)}{100}$$

— *sum of triesters and polyesters*: calculate the sum of the percentage contents of triesters and polyesters using the following expression:

$$\frac{C \times (100 - D - S - E)}{100}$$

A = percentage content of monoesters determined by the normalisation procedure

S = percentage content of free sucrose (see Tests)

E = percentage content of water (see Tests)

B = percentage content of diesters determined by the normalisation procedure

C = sum of the percentage contents of triesters and polyesters determined by the normalisation procedure.

STORAGE

Protected from humidity.

Sucrose Stearate

General Notices

(Ph Eur monograph 2318)

Ph Eur

DEFINITION

Mixture of sucrose esters, mainly sucrose stearate, obtained by transesterification of stearic acid methyl esters of vegetable origin with *sucrose (0204).* The manufacture of the fatty acid methyl esters includes a distillation step.

It contains variable quantities of mono- and diesters.

Content:

Sucrose stearate type I:
— *monoesters*: minimum 50.0 per cent;
— *diesters*: maximum 40.0 per cent;
— *sum of triesters and polyesters*: maximum 25.0 per cent;

Sucrose stearate type II:
— *monoesters*: 20.0 per cent to 45.0 per cent;
— *diesters*: 30.0 per cent to 40.0 per cent;
— *sum of triesters and polyesters*: maximum 30.0 per cent.

CHARACTERS

Appearance

White or almost white, unctuous powder.

Solubility

Very slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Composition of fatty acids (see Tests).

B. It complies with the limits of the assay.

TESTS

Acid value ***(2.5.1)***

Maximum 6.0, determined on 3.00 g.

Use a freshly neutralised mixture of 1 volume of *water R* and 2 volumes of *2-propanol R* as solvent and heat gently.

Composition of fatty acids ***(2.4.22, Method C)***

Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty-acid fraction of the substance:

- *lauric acid* : maximum 3.0 per cent;
- *myristic acid* : maximum 3.0 per cent;
- *palmitic acid* : 25.0 per cent to 40.0 per cent;
- *stearic acid* : 55.0 per cent to 75.0 per cent;
- *sum of the contents of palmitic acid and stearic acid* : minimum 90.0 per cent.

Free sucrose

Liquid chromatography *(2.2.29)* .

Solvent mixture *water for chromatography R* , *tetrahydrofuran for chromatography R* (12.5:87.5 *V/V*).

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 4.0 ml with the solvent mixture.

Reference solution (a) Dissolve 5.0 mg of *sucrose CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

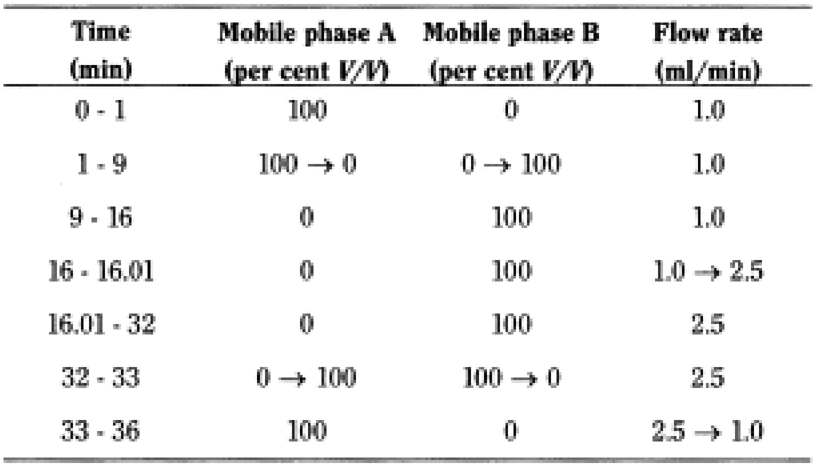
Reference solution (b) In 4 volumetric flasks, introduce respectively 5.0 mg, 10.0 mg, 20.0 mg and 25.0 mg of *sucrose CRS* , dissolve in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: spherical *aminopropylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:

- *mobile phase A*: 0.01 g/l solution of *ammonium acetate R* in *acetonitrile for chromatography R ;*
- *mobile phase B*: 0.01 g/l solution of *ammonium acetate R* in a mixture of 10 volumes of *water for chromatography R* and 90 volumes of *tetrahydrofuran for chromatography R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (ml/min)
0 - 1	100	0	1.0
1 - 9	100 → 0	0 → 100	1.0
9 - 16	0	100	1.0
16 - 16.01	0	100	1.0 → 2.5
16.01 - 32	0	100	2.5
32 - 33	0 → 100	100 → 0	2.5
33 - 36	100	0	2.5 → 1.0

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:

— *carrier gas*: *nitrogen R* ;

— *flow rate*: 1.0 ml/min;

— *evaporator temperature*: 45 °C;

— *nebuliser temperature*: 40 °C.

Injection 20 µl.

Retention time About 26 min.

System suitability Reference solution (a):

— *signal-to-noise ratio* : minimum 10.

Limit Maximum 4.0 per cent.

Water (*2.5.12*)

Maximum 4.0 per cent, determined on 0.20 g.

Total ash (*2.4.16*)

Maximum 1.5 per cent.

ASSAY

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Dissolve 60.0 mg of the substance to be examined in *tetrahydrofuran R* and dilute to 4.0 ml with the same solvent.

Column:

— *size*: *l* = 0.6 m, Ø = 7 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R.*

Flow rate 1.2 ml/min.

Detection Differential refractometer.

Injection 20 µl.

Relative retention With reference to monoesters (retention time = about 10 min): diesters = about 0.92; triesters and polyesters = about 0.90.

Calculations:

— *disregard limit*: disregard the peaks having a signal-to-noise ratio less than 10;

— *free fatty acids*: calculate the percentage content (*D*) of free fatty acids, using the following expression:

$$\frac{I_A \times 284.5}{561.1}$$

I_A = acid value;

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\frac{A \times (100 - D - S - E)}{100}$$

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{B \times (100 - D - S - E)}{100}$$

— *sum of triesters and polyesters*: calculate the sum of the percentage contents of triesters and polyesters using the following expression:

$$\frac{C \times (100 - D - S - E)}{100}$$

A = percentage content of monoesters determined by the normalisation procedure

S = percentage content of free sucrose (see Tests)

E = percentage content of water (see Tests)

B = percentage content of diesters determined by the normalisation procedure

C = sum of the percentage contents of triesters and polyesters determined by the normalisation procedure.

LABELLING

The label states the type of sucrose stearate (type I or II).

STORAGE

Protected from humidity.

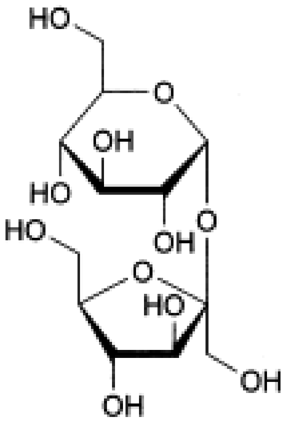
Ph Eur

Sucrose

General Notices

Refined Sugar

(Ph Eur monograph 0204)



$C_{12}H_{22}O_{11}$ 342.3 *57-30-1*

Action and use

Sweetening agent.

Preparation

Syrup

Ph Eur

DEFINITION

β-D-Fructofuranosyl α-D-glucopyranoside.

It contains no additives.

CHARACTERS

Appearance

White or almost white, crystalline powder, or lustrous, colourless or white or almost white crystals.

Solubility

Very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in anhydrous ethanol.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *sucrose CRS.*

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (a) Dissolve 10 mg of *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg each of *fructose CRS* , *glucose CRS* , *lactose CRS* and *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Plate *TLC silica gel G plate R* .

Mobile phase *Cold saturated boric acid solution R* , 60 per cent *V/V* solution of *glacial acetic acid R* , *ethanol R, acetone R, ethyl acetate R* (10:15:20:60:60 *V/V/V/V/V*).

Application 2 µl.

Development In an unsaturated tank over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *alcohol R* . Heat the plate at 130 °C for 10 min.

System suitability The chromatogram obtained with reference solution (b) shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dilute 1 ml of solution S (see Tests) to 100 ml with *water R* . To 5 ml of the solution add 0.15 ml of freshly prepared *copper sulphate solution R* and 2 ml of freshly prepared *dilute sodium hydroxide solution R* . The solution is blue and clear and remains so after boiling. To the hot solution add 4 ml of *dilute hydrochloric acid R* and boil for 1 min. Add 4 ml of *dilute sodium hydroxide solution R .* An orange precipitate is formed immediately.

TESTS

Solution S

Dissolve 50.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)*.

Conductivity *(2.2.38)*

Maximum 35 µS·cm^{-1}.

Dissolve 31.3 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent. Measure the conductivity of the solution (C_1), while gently stirring with a magnetic stirrer, and that of the water used for preparing the solution (C_2). The readings must be stable within 1 per cent over a period of 30 s. Calculate the conductivity of the solution of the substance to be examined from the following expression:

$$C_1 - 0.35\,C_2$$

Specific optical rotation *(2.2.7)*

+ 66.3 to + 67.0.

Dissolve 26.0 g in *water R* and dilute to 100.0 ml with the same solvent.

Colour value

Maximum 45.

Dissolve 50.0 g in 50.0 ml of *water R*. Mix, filter (diameter of pores 0.45 µm) and degas. Measure the absorbance *(2.2.25)* at 420 nm, using a cell of minimum 4 cm (a cell length of 10 cm or more is preferred).

Calculate the colour value using the following expression:

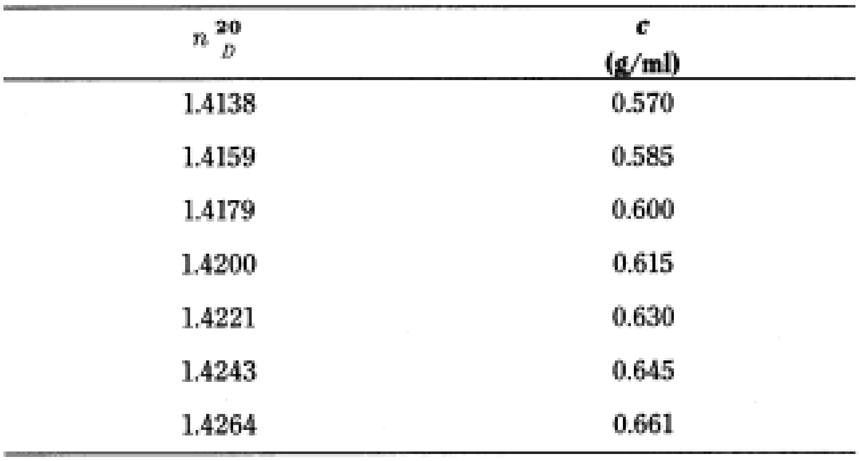
$$\frac{A \times 1000}{b \times c}$$

A = absorbance measured at 420 nm

b = path length in centimetres

c = concentration of the solution, in grams per millilitre, calculated from the refractive index *(2.2.6)* of the solution; use Table 0204.-1 and interpolate the values if necessary

Table 0204.-1



n_D^{20}	c (g/ml)
1.4138	0.570
1.4159	0.585
1.4179	0.600
1.4200	0.615
1.4221	0.630
1.4243	0.645
1.4264	0.661

System suitability:

— *repeatability*: the absolute difference between 2 results is not greater than 3.

Dextrins

If intended for use in the preparation of large-volume infusions, it complies with the test for dextrins. To 2 ml of solution S add 8 ml of *water R* , 0.05 ml of *dilute hydrochloric acid R* and 0.05 ml of *0.05 M iodine* . The solution remains yellow.

Reducing sugars

To 5 ml of solution S in a test-tube about 150 mm long and 16 mm in diameter add 5 ml of *water R* , 1.0 ml of *1 M sodium hydroxide* and 1.0 ml of a 1 g/l solution of *methylene blue R* . Mix and place in a water-bath. After exactly 2 min, take the tube out of the bath and examine the solution immediately. The blue colour does not disappear completely. Ignore any blue colour at the air/solution interface.

Sulphites

Maximum 10 ppm, calculated as SO_2.

Determine the sulphites content by a suitable enzymatic method based on the following reactions. Sulphite is oxidised by sulphite oxidase to sulphate and hydrogen peroxide which in turn is reduced by nicotinamide-adenine dinucleotide-peroxidase in the presence of reduced nicotinamide-adenine dinucleotide (NADH). The amount of NADH oxidised is proportional to the amount of sulphite.

Test solution Dissolve 4.0 g of the substance to be examined in freshly prepared *distilled water R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 4.0 g of the substance to be examined in freshly prepared *distilled water R,* add 0.5 ml of *sulphite standard solution (80 ppm SO_2) R* and dilute to 10.0 ml with freshly prepared *distilled water R* .

Blank solution Freshly prepared *distilled water R* .

Separately introduce 2.0 ml each of the test solution, the reference solution and the blank in

10 mm cuvettes and add the reagents as described in the instructions in the kit for sulphite determination. Measure the absorbance *(2.2.25)* at the absorption maximum at about 340 nm before and at the end of the reaction time and subtract the value obtained with the blank.

The absorbance difference of the test solution is not greater than half the absorbance difference of the reference solution.

Lead

Maximum 0.5 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 1.5 g of the substance to be examined in 1.5 ml of *deionised distilled water R* in a digestion tube. Add 0.75 ml of *lead-free nitric acid R1* and slowly warm to 90-95 °C, avoiding spattering. Heat for about 60 min, add again 0.75 ml of *lead-free nitric acid R1*. Heat until all brown vapours have dissipated and any reddish tint is gone (about 60 min). Cool. Add 0.5 ml of *strong hydrogen peroxide solution R* dropwise and heat at 90-95 °C for 15 min. Cool. Add again 0.5 ml of *strong hydrogen peroxide solution R* dropwise, heat at 90-95 °C for 60 min and cool. Repeat these operations until a clear, light yellow solution is obtained. Dilute to 10.0 ml with deionised *distilled water R* . Store in capped plastic vials.

Reference solutions Prepare 3 reference solutions in the same manner as the test solution but adding 0.5 ml, 1.0 ml and 1.5 ml respectively of *lead standard solution (0.5 ppm Pb) R* in addition to the 1.5 g of the substance to be examined.

Blank solution Prepare a blank in the same manner as for the test solution but without the substance to be examined.

Zero-setting solution *Deionised distilled water R* .

Apparatus Suitable graphite furnace atomic absorption spectrometer equipped with a background compensation system, an autosampler and pyrolytically-coated tubes or with pyrolytic graphite platforms.

Source Hollow-cathode lamp or electrodeless discharge lamp.

Gas:

— *argon R* as the purge gas;

— air as alternate gas during the charring step.

Flow rate To be adapted according to the apparatus; usually between 200 ml/min and 300 ml/min.

Wavelength 283.3 nm.

Method Separately inject 20 µl each of the zero-setting solution, the blank solution, the test solution, the reference solutions, and add immediately 5 µl of *magnesium nitrate solution R1*, which is used as matrix modifier, to each of the solutions. Inject each solution in triplicate.

The ashing and atomisation temperatures and times vary according to the apparatus, the background compensation system, and the graphite tube etc. The following parameters are given for guidance and have to be adjusted.

Heat the furnace progressively to 200 °C and maintain the drying temperature at 200 °C for 30 s, the ashing temperature at 750 ± 50 °C for 40 s after a 40 s temperature rise, and the atomisation temperature at 1800 °C for 10 s (0 s temperature rise). Clean out at 2600 °C for 7 s after a 1 s temperature rise. Use the zero-setting solution to set the instrument zero. Calculate the mean readings of the test solution and of the reference solutions subtracting the mean blank. If necessary, dilute with the zero-setting solution to obtain a reading within the linear range.

System suitability:

— The relative standard deviation of the 3 readings obtained for the triplicate injections of the reference solutions from which the mean blank has been subtracted is not greater than 15 per cent.

Loss on drying *(2.2.32)*

Maximum 0.1 per cent, determined on 2.000 g by drying in an oven at 105 °C for 3 h.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/mg, if intended for use in the preparation of large-volume infusions.

LABELLING

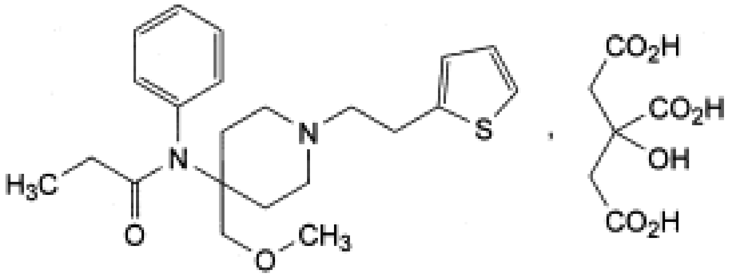
The label states, where applicable, that the substance is suitable for use in the manufacture of large-volume parenteral dosage forms.

Ph Eur

Sufentanil Citrate

General Notices

(Ph Eur monograph 1269)



$C_{22}H_{30}N_2O_2S,C_6H_8O_7$ 578.7 *60561-17-3*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

N-[4-(Methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylpropanamide citrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water and in ethanol (96 per cent), freely soluble in methanol.

mp

about 140 °C, with decomposition.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of sufentanil citrate.*

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Dissolve 0.2 g in *water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) In order to produce impurity E *in situ*, dissolve 10 mg of the substance to be examined in 10.0 ml of *dilute hydrochloric acid R* . Heat on a water-bath under a reflux condenser for 4 h. Add 10.0 ml of *dilute sodium hydroxide solution R* . Evaporate to dryness on a water-bath. Cool and take up the residue in 10 ml of *methanol R* . Filter.

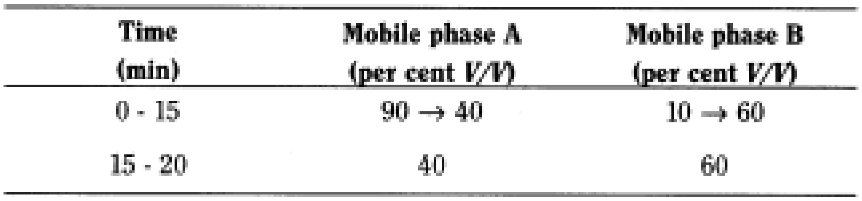
Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R* . Dilute 1.0 ml of this solution to 10.0 ml with *methanol R* .

Column:

- *size: l* = 0.1 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

- *mobile phase A*: 5 g/l solution of *ammonium carbonate R* in a mixture of 10 volumes of *tetrahydrofuran R* and 90 volumes of *water R;*
- *mobile phase B: acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 40	10 → 60
15 - 20	40	60

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Impurity E = about 12 min; sufentanil = about 13 min.

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to impurity E and sufentanil; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient elution.

Limits:

— *impurities A, B, C, D, E, F, G, H, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak with a retention time relative to sufentanil of 0.05 or less.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

ASSAY

Dissolve 0.400 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R* and titrate with *0.1 M perchloric acid* , using 0.2 ml of *naphtholbenzein solution R* as indicator.

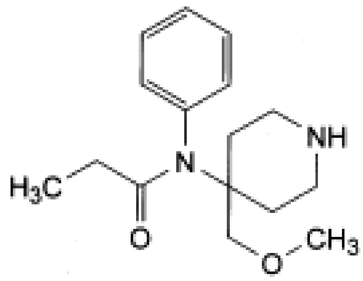
1 ml of *0.1 M perchloric acid* is equivalent to 57.87 mg of $C_{28}H_{38}N_2O_9S$.

STORAGE

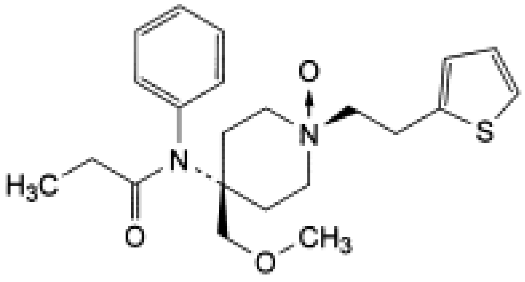
Protected from light.

IMPURITIES

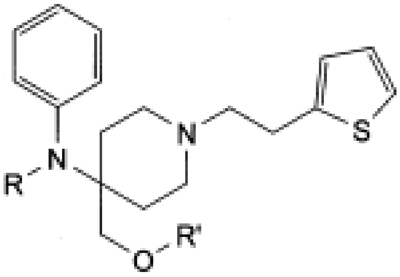
Specified impurities A, B, C, D, E, F, G, H, I.



A. *N*-[4-(methoxymethyl)piperidin-4-yl]-*N*-phenylpropanamide,



B. *cis*-4-(methoxymethyl)-4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidine 1-oxide,



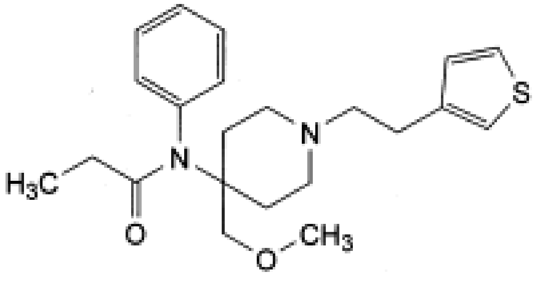
C. R = R′ = H: [4-(phenylamino)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]methanol,

D. R = CO-CH_3, R′ = CH_3: *N*-[4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylacetamide,

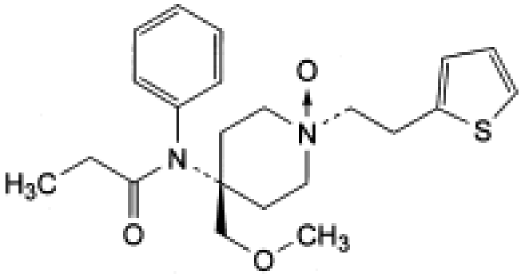
E. R = H, R′ = CH_3: 4-(methoxymethyl)-*N*-phenyl-1-[2-(thiophen-2-yl)ethyl] piperidin-4-amine,

G. R = R′ = CO-CH_2-CH_3: [4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl] piperidin-4-yl] methyl propanoate,

H. R = CO-CH_2-CH_2-CH_3, R′ = CH3: *N*-[4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl] piperidin-4-yl]-*N*-phenylbutanamide,



F. *N*-[4-(methoxymethyl)-1-[2-(thiophen-3-yl)ethyl]piperidin-4-yl]-*N*-phenylpropanamide,



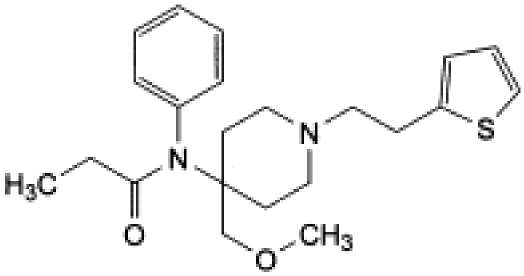
I. *trans*-4-(methoxymethyl)-4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidine 1-oxide.

Ph Eur

Sufentanil

General Notices

(Ph Eur monograph 1569)



$C_{22}H_{30}N_2O_2S$ 386.6 *56030-54-7*

Action and use

Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION

N-[4-(Methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylpropanamide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent) and in methanol.

mp

about 98 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of sufentanil.*

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.10 g in *methanol R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) In order to produce impurity E in situ, dissolve 10 mg of the substance to be examined in 10.0 ml of *dilute hydrochloric acid R* . Heat on a water-bath under a reflux condenser for 4 h. Add 10.0 ml of *dilute sodium hydroxide solution R* . Evaporate to dryness on a water-bath. Cool and take up the residue in 10 ml of *methanol R* . Filter.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R* . Dilute 5.0 ml of this solution to 20.0 ml with *methanol R* .

Column:

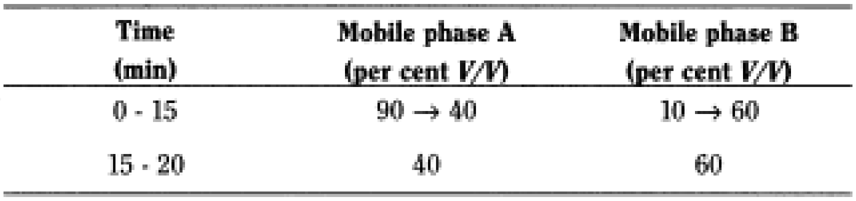
— *size: l* = 0.1 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 5 g/l solution of *ammonium carbonate R* in a mixture of 10 volumes of *tetrahydrofuran R* and 90 volumes of *water R* ;

— *mobile phase B: acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 40	10 → 60
15 - 20	40	60

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Equilibration With *acetonitrile R* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Impurity E = about 12 min; sufentanil = about 13 min.

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to impurity E and sufentanil; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient elution.

Limits:

— *impurities D, F, H:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 2 h.

ASSAY

Dissolve 0.300 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R* and titrate with *0.1 M perchloric acid,* using 0.2 ml of *naphtholbenzein solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 38.66 mg of $C_{22}H_{30}N_2O_2S$.

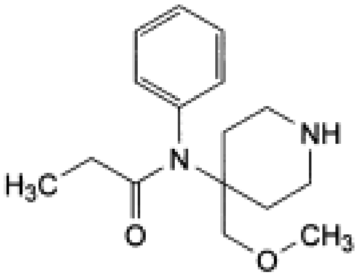
STORAGE

Protected from light.

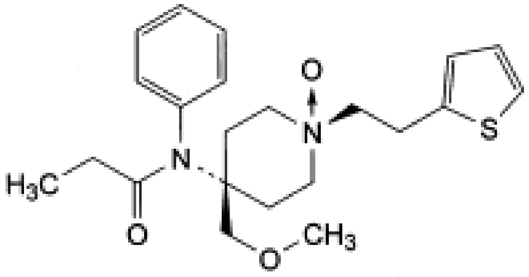
IMPURITIES

Specified impurities *D, F, H.*

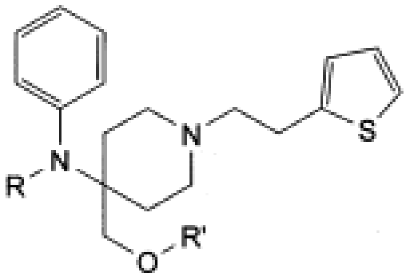
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, E, G, I.*



A. *N*-[4-(methoxymethyl)piperidin-4-yl]-*N*-phenylpropanamide,



B. *cis*-4-(methoxymethyl)-4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidine 1-oxide,



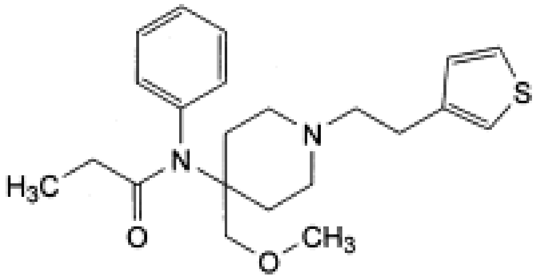
C. R = R′ = H: [4-(phenylamino)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]methanol,

D. R = CO-CH_3, R′ = CH_3: *N*-[4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-N-phenylacetamide,

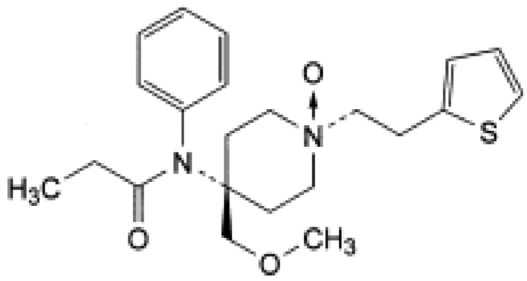
E. R = H, R′ = CH_3: 4-(methoxymethyl)-*N*-phenyl-1-[2-(thiophen-2-yl)ethyl]piperidin-4-amine,

G. R = R′ = CO-CH_2-CH_3: [4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl] methyl propanoate,

H. R = CO-CH_2-CH_2-CH_3, R′ = CH3: *N*-[4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl] piperidin-4-yl]-*N*-phenylbutanamide,



F. *N*-[4-(methoxymethyl)-1-[2-(thiophen-3-yl)ethyl]piperidin-4-yl]-*N*-phenylpropanamide,



I. *trans*-4-(methoxymethyl)-4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidine 1-oxide.

Ph Eur

Sugar Spheres

General Notices

(Ph Eur monograph 1570)

Action and use

Excipient.

Ph Eur

DEFINITION

Sugar spheres contain not more than 92 per cent of sucrose, calculated on the dried basis. The remainder consists of maize starch and may also contain starch hydrolysates and colour additives. The diameter of sugar spheres varies usually from 200 µm to 2000 µm and the upper and lower limits of the size of the sugar spheres are stated on the label.

IDENTIFICATION

A. Examine by thin-layer chromatography *(2.2.27)* , using a *TLC silica gel G plate R* .

Test solution Mix 2 ml of solution S (see Tests) with 3 ml of *methanol R* . Dilute to 20 ml with a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* .

Reference solution (a) Dissolve 10 mg of *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *fructose CRS* , 10 mg of *glucose CRS* , 10 mg of *lactose CRS* and 10 mg of *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Apply to the plate 2 µl of each solution and thoroughly dry the starting points. Develop over a path of 15 cm using a mixture of 10 volumes of *water R* , 15 volumes of *methanol R* , 25 volumes of *anhydrous acetic acid R* and 50 volumes of *ethylene chloride R* , measured accurately as a slight excess of water causes cloudiness of the solution. Dry the plate in a current of warm air. Repeat the development immediately after renewing the mobile phase. Dry the plate in a current of warm air and spray evenly with a 5 g/l solution of *thymol R* in a mixture of 5 volumes of *sulphuric acid R* and 95 volumes of *alcohol R* . Heat at 130 °C for 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 4 clearly separated spots.

B. To water slurry of the insoluble portion obtained (see Assay), add 0.05 ml of *iodine solution R1* . A dark-blue is produced which disappears on heating.

C. To 5 ml of solution S add 0.15 ml of freshly prepared *copper sulphate solution R* and 2 ml of freshly prepared *dilute sodium hydroxide solution R*. The solution is blue and clear and remains so after boiling. To the hot solution add 4 ml of *dilute hydrochloric acid R* and boil for 1 min. Add 4 ml of *dilute sodium hydroxide solution R*. An orange precipitate is formed immediately.

TESTS

Solution S

To 0.5 g in a 100 ml volumetric flask add 80 ml of *water R* and shake until the sucrose is dissolved. Dilute to 100.0 ml with *water R*. Filter under vacuum to obtain a clear solution.

Fineness *(2.9.12)*

Minimum of 90 per cent (*m/m*) of the sugar spheres are between the lower and the upper limits of the size of the sugar spheres stated on the label.

Heavy metals *(2.4.8)*

2.0 g complies with limit test C for heavy metals (5 ppm). Prepare the standard using 1.0 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Not more than 0.2 per cent, determined on 2 g.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

Sucrose content

Weigh 10.000 g of ground sugar spheres in a 100 ml graduated flask and complete to 100.0 ml with *water R*. Stir and decant. Filter under vacuum to obtain a clear solution (the insoluble portion is used for the identification test B). Measure the angle of optical rotation *(2.2.7)* and calculate the sucrose percentage content as follows:

$$\frac{10^6 \times \alpha}{66.5 \times l \times m \times (100 - H)}$$

α = angle of rotation

l = length of the polarimeter tube, in decimetres

m = exact weight of the granules sample, in grams

H = loss on drying

LABELLING

The label states:

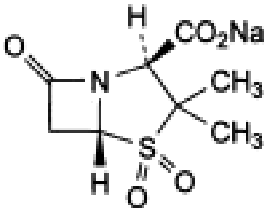
- — the upper and the lower limits of the size of the sugar spheres;
- — any added colour additives;
- — any added starch hydrolysates.

Ph Eur

Sulbactam Sodium

General Notices

(Ph Eur monograph 2209)



$C_8H_{10}NNaO_5S$ 255.2 *69388-84-7*

Action and use

Beta-lactam antibacterial.

Ph Eur

DEFINITION

Sodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Semi-synthetic product derived from a fermentation product.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in ethyl acetate, very slightly soluble in ethanol (96 per cent). It is freely soluble in dilute acids.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *sulbactam sodium CRS.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* .

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent.

Absorbance *(2.2.25)*

Maximum 0.10 at 430 nm.

Dissolve 1.0 g in *water R* and dilute to 100.0 ml with the same solvent.

pH *(2.2.3)*

4.5 to 7.2; if the substance is sterile: 5.2 to 7.2.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 219 to + 233 (anhydrous substance).

Dissolve 0.500 g in *water R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Solution A 2.72 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 4.0 with *dilute phosphoric acid R* .

Solution B Dilute 2 ml of *acetonitrile R1* to 100.0 ml with solution A.

Test solution Suspend 77.0 mg of the substance to be examined in 2 ml of *acetonitrile R1* and sonicate for about 5 min. Dilute to 100.0 ml with solution A.

Reference solution (a) Suspend 70.0 mg of *sulbactam CRS* in 2 ml of *acetonitrile R1* and sonicate for about 5 min. Dilute to 100.0 ml with solution A.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with solution B. Dilute 1.0 ml of this solution to 10.0 ml with solution B.

Reference solution (c) Dissolve 15.0 mg of *6-aminopenicillanic acid R* in solution A and dilute to 50.0 ml with solution A.

Reference solution (d) Mix 1 ml of reference solution (a) and 1 ml of reference solution (c) and dilute to 25.0 ml with solution B.

Reference solution (e) Dissolve 8 mg of *sulbactam for peak identification CRS* (containing impurities A, C, D, E and F) in 1 ml of *acetonitrile R1*, sonicate for about 5 min and dilute to 10 ml with solution B.

Column:

— *size: l* = 0.10 m, Ø = 4.0 mm;

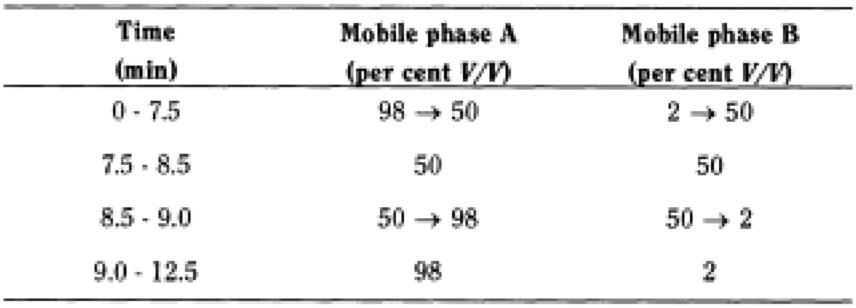
— *stationary phase: octadecylsilyl silica gel for chromatography R* (3.0 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: 5.44 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 4.0 with *dilute phosphoric acid R*;

— *mobile phase B: acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7.5	98 → 50	2 → 50
7.5 - 8.5	50	50
8.5 - 9.0	50 → 98	50 → 2
9.0 - 12.5	98	2

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl of the test solution, solution B and reference solutions (b), (d) and (e).

Relative retention With reference to sulbactam (retention time = about 2.5 min): impurity A = about 0.4; impurity B = about 0.6; impurity C = about 1.6; impurity D = about 2.0; impurity E = about 2.1; impurity F = about 2.5.

Identification of impurities Use the chromatogram supplied with *sulbactam for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities A, C, D, E and F.

System suitability Reference solution (d):

— *resolution*: minimum 7.0 between the peaks due to impurity B and sulbactam.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity B = 0.5; impurity D = 0.5; impurity F = 0.6;

— *impurity A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities B, D, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurities C, E*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

2-Ethylhexanoic acid *(2.4.28)*

Maximum 0.5 per cent *m/m.*

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 gin *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using 10.0 ml of *lead standard solution (2 ppm Pb) R* .

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 1.00 g.

Bacterial endotoxins *(2.6.14, Method A)*

Less than 0.17 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of sulbactam sodium by multiplying the percentage content of sulbactam by 1.094 and using the declared content of *sulbactam CRS*.

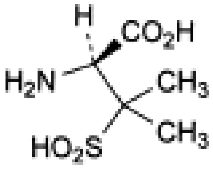
STORAGE

In an airtight container . If the substance is sterile, store in a sterile, airtight, tamper-proof container .

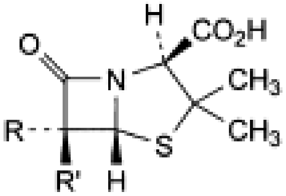
IMPURITIES

Specified impurities *A, B, C, D, E, F.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): G*.



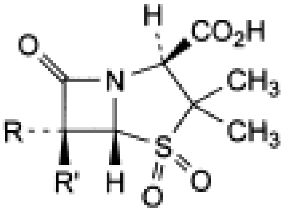
A. (2*S*)-2-amino-3-methyl-3-sulphinobutanoic acid,



B. R = NH_2, R′ = H: (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (6-aminopenicillanic acid),

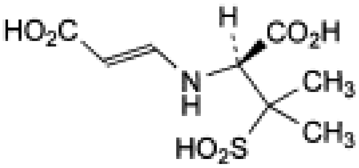
D. R = Br, R′ = H: (2*S*,5*R*,6*R*)-6-bromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (6-bromopenicillanic acid),

F. R = R′ = Br: (2*S*,5*R*)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6,6-dibromopenicillanic acid),



C. R = Br, R′ = H: (2*S*,5*R*,6*R*)-6-bromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid 4,4-dioxide (6-bromopenicillanic acid sulphone),

E. R = R′ = Br: (2*S*,5*R*)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (6,6-dibromopenicillanic acid sulphone),



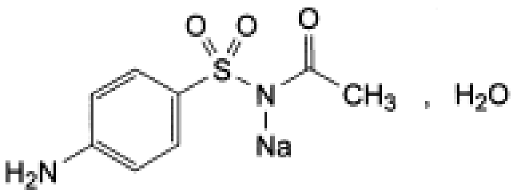
G. (2*E*)-3-[[(1*S*)-1-carboxy-2-methyl-2-sulphinopropyl]amino]prop-2-enoic acid.

Sulfacetamide Sodium

General Notices

Soluble Sulfacetamide

(Ph Eur monograph 0107)



$C_8H_9N_2NaO_3S,H_2O$ 254.2 *6209-17-2*

Action and use

Sulfonamide antibacterial.

Ph Eur

DEFINITION

Sodium acetyl[(4-aminophenyl)sulphonyl]azanide.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in anhydrous ethanol.

IDENTIFICATION

First identification B, F.

Second identification *A, C, E, F.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 0.1 g in *phosphate buffer solution pH 7.0 R* and dilute to 100.0 ml with the same buffer solution. Dilute 1.0 ml of this solution to 100.0 ml with *phosphate buffer solution pH 7.0 R* .

Spectral range 230-350 nm.

Absorption maximum At 255 nm.

Specific absorbance at the absorption maximum 660 to 720 (anhydrous substance).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *sulfacetamide sodium CRS.*

C. Melting point *(2.2.14)* : 181 °C to 185 °C.

Dissolve 1 g in 10 ml of *water R* , add 6 ml of *dilute acetic acid R* and filter. Wash the precipitate with a small quantity of *water R* and dry at 100-105 °C for 4 h.

E. Dissolve about 1 mg of the precipitate obtained in identification C, with heating, in 1 ml of *water R* . The solution gives the reaction of primary aromatic amines *(2.3.1)* with formation of an orange-red precipitate.

F. Solution S (see Tests) gives the reactions of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution GY_4 *(2.2.2, Method II)*.

pH *(2.2.3)*

8.0 to 9.5 for solution S.

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use and carry out the test protected from light.*

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *sulfacetamide sodium CRS* and 5 mg of *sulfanilamide R* (impurity A) in 1.0 ml of the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size:* *l* = 0.125 m, Ø = 4 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, methanol R, water for chromatography R* (1:10:89 *V/V/V*).

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 7 times the retention time of sulfacetamide.

Relative retention With reference to sulfacetamide (retention time = about 5 min): impurity A = about 0.5.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to impurity A and sulfacetamide.

Limits:

— *correction factor*: for the calculation of the content, multiply the peak area of impurity A by 0.5;

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphates ***(2.4.13)***

Maximum 200 ppm.

Dissolve 2.5 g in *distilled water R* and dilute to 25 ml with the same solvent. Add 25 ml of *dilute acetic acid R* , shake for 30 min and filter. 15 ml of the filtrate complies with the limit test for sulphates.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of the filtrate obtained in the test for sulphates complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R* .

Water ***(2.5.12)***

6.0 per cent to 8.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.500 g in a mixture of 50 ml of *water R* and 20 ml of *dilute hydrochloric acid R* .

Cool the solution in a bath of iced water and carry out the determination of primary aromatic amino-nitrogen *(2.5.8)*, determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 23.62 mg of $C_8H_9N_2NaO_3S$.

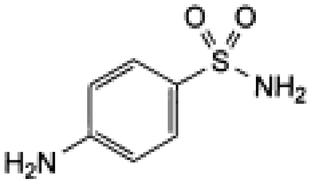
STORAGE

Protected from light.

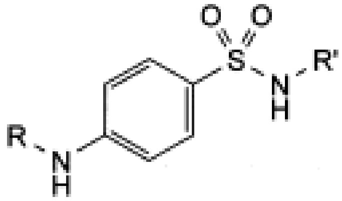
IMPURITIES

Specified impurities A.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D.*



A. sulfanilamide,



B. R = $CO\text{-}CH_3$, R′ = H: *N*-(4-sulphamoylphenyl)acetamide,

C. R = R′ = $CO\text{-}CH_3$: *N*-[[4-(acetylamino)phenyl]sulphonyl]acetamide,

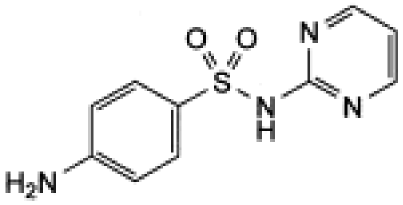
D. dapsone.

Ph Eur

Sulfadiazine

General Notices

(Ph Eur monograph 0294)



$C_{10}H_{10}N_4O_2S$ 250.3 *68-35-9*

Action and use

Sulfonamide antibacterial.

Preparation

Sulfadiazine Injection

Ph Eur

DEFINITION

Sulfadiazine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-pyrimidin-2-ylbenzenesulphonamide, calculated with reference to the dried substance.

CHARACTERS

White, yellowish-white or pinkish-white, crystalline powder or crystals, practically insoluble in water, slightly soluble in acetone, very slightly soluble in alcohol. It dissolves in solutions of alkali hydroxides and in dilute mineral acids.

It melts at about 255 °C, with decomposition.

IDENTIFICATION

First identification A, B.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *sulfadiazine CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (a) corresponds in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place 3 g in a dry tube. Immerse the lower part of the tube, inclined at 45°, in a silicone oil bath and heat to about 270 °C. The substance to be examined decomposes and a white or yellowish-white sublimate is formed which, after recrystallisation from *toluene R* and drying at 100 °C, melts *(2.2.14)* at 123 °C to 127 °C.

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid*. Dilute 1 ml of the solution to 10 ml with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines *(2.3.1).*

TESTS

Appearance of solution

Dissolve 0.8 g in a mixture of 5 ml of *dilute sodium hydroxide solution R* and 5 ml of *water R.* The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 *(2.2.2, Method II)*.

Acidity

To 1.25 g, finely powdered, add 25 ml of *carbon dioxide-free water R*. Heat at about 70 °C for 5 min. Cool in iced water for about 15 min and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution (a) Dissolve 20 mg of the substance to be examined in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5.0 ml with the same mixture of solvents.

Test solution (b) Dissolve 0.10 g of the substance to be examined in 0.5 ml of *concentrated ammonia R* and dilute to 5.0 ml with *methanol R*. If the solution is not clear, heat gently until dissolution is complete.

Reference solution (a) Dissolve 20 mg of *sulfadiazine CRS* in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.25 ml of test solution (a) to 50 ml with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 3 volumes of *dilute ammonia R1*, 5 volumes of *water R*, 40 volumes of *nitromethane R* and 50 volumes of *dioxan R*. Dry the plate at 100 °C to 105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (b), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals ***(2.4.8)***

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 20 ml of *dilute hydrochloric acid R* and 50 ml of *water R* . Cool the solution in iced water. Carry out the determination of primary aromatic amino-nitrogen *(2.5.8)*, determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 25.03 mg of $C_{10}H_{10}N_4O_2S$.

STORAGE

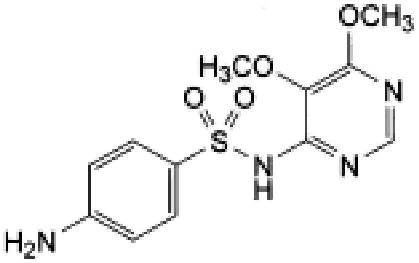
Store protected from light.

Ph Eur

Sulfadoxine

General Notices

(Ph Eur monograph 0740)



$C_{12}H_{14}N_4O_4S$ 310.3 *2447-57-6*

Action and use

Sulfonamide antibacterial.

Ph Eur

DEFINITION

Sulfadoxine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(5,6-dimethoxypyrimidin-4-yl)benzenesulphonamide, calculated with reference to the dried substance.

CHARACTERS

White or yellowish-white crystalline powder or crystals, very slightly soluble in water, slightly soluble in alcohol and in methanol. It dissolves in solutions of alkali hydroxides and in dilute mineral acids.

It melts at about 198 °C, with decomposition.

IDENTIFICATION

First identification *A, C.*

Second identification *B, C, D.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *sulfadoxine CRS* . Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.5 g in 1 ml of a 40 per cent *V/V* solution of *sulphuric acid R*, heating gently. Continue heating until a crystalline precipitate appears (about 2 min). Allow to cool and add 10 ml of *dilute sodium hydroxide solution R.* Cool again. Add 25 ml of *ether R* and shake for 5 min. Separate the ether layer, dry over *anhydrous sodium sulphate R* and filter. Evaporate the solvent by heating in a water-bath. The residue melts *(2.2.14)* at 80 °C to 82 °C or at 90 °C to 92 °C.

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid*. Dilute 1 ml of the solution to 10 ml with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 5 ml of *dilute sodium hydroxide solution R* and 5 ml of *water R*. The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 *(2.2.2, Method II)*.

Acidity

To 1.25 g, finely powdered, add 25 ml of *carbon dioxide-free water R*. Heat at 70 °C for 5 min. Cool in a bath of iced water for about 15 min and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.2 ml *of 0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 5 ml with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R.*

Reference solution (a) Dissolve 20 mg of *sulfadoxine CRS* in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (b) Dilute 2.5 ml of test solution (b) to 100 ml with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 3 volumes of *dilute ammonia R1*, 5 volumes of *water R*, 40 volumes of *nitromethane R* and 50 volumes of *dioxan R*. Dry the plate at 100 °C to 105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals ***(2.4.8)***

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the determination of primary aromatic amino- nitrogen *(2.5.8),* using 0.250 g and determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 31.03 mg of $C_{12}H_{14}N_4O_4S$.

STORAGE

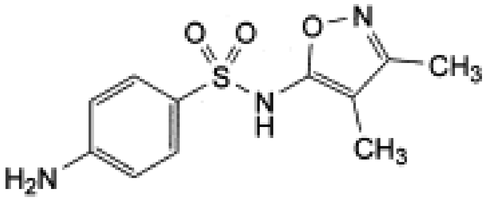
Store protected from light.

Ph Eur

Sulfafurazole

General Notices

(Ph Eur monograph 0741)



$C_{11}H_{13}N_3O_3S$ 267.3 *127-69-5*

Action and use

Sulfonamide antibacterial.

Ph Eur

DEFINITION

Sulfafurazole contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulphonamide, calculated with reference to the dried substance.

CHARACTERS

White or yellowish-white, crystalline powder or crystals, practically insoluble in water, sparingly soluble in alcohol, slightly soluble in methylene chloride. It dissolves in solutions of alkali hydroxides and in dilute mineral acids.

It melts at about 197 °C, with decomposition.

IDENTIFICATION

First identification A, C.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *sulfafurazole CRS* . Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal

spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 0.5 g add 1 ml of a 40 per cent *V/V* solution of *sulphuric acid R* and heat over a low flame to dissolve. Continue heating until a crystalline precipitate appears (about 2 min). Allow to cool and add 10 ml of *dilute sodium hydroxide solution R.* Cool. Shake the solution for 5 min with 25 ml of *ether R.* Separate the ether layer, dry over *anhydrous sodium sulphate R* and filter. Evaporate the solvent by heating on a water-bath. The residue melts *(2.2.14)* at 119 °C to 123 °C.

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid* . Dilute 1 ml of the solution to 10 ml with *water R* . The solution, without further acidification, gives the reaction of primary aromatic amines *(2.3.1)* .

TESTS

Appearance of solution

Dissolve 0.4 g in a mixture of 5 ml of *dilute sodium hydroxide solution R* and 5 ml of *water R* , with gently warming if necessary. The solution is not more intensely coloured than reference solution Y_6 , BY_6 or GY_6 *(2.2.2, Method II)*.

Acidity

To 1.25 g, finely powdered, add 25 ml of *carbon dioxide-free water R* . Heat at 70 °C for 5 min. Cool in iced water for about 15 min and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1* . Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography *(2.2.27)* , using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 5 ml with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* .

Reference solution (a) Dissolve 20 mg of *sulfafurazole CRS* in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.25 ml of test solution (b) to 50 ml with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* .

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R* , 25 volumes of *methanol R* and 75 volumes of *methylene chloride R* . Dry the plate at 100 °C to 105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals ***(2.4.8)***

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *acetone R*. Titrate with *0.1 M tetrabutylammonium hydroxide* using a 4 g/l solution of *thymol blue R* in *methanol R* as indicator.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 26.73 mg of $C_{11}H_{13}N_3O_3S$.

STORAGE

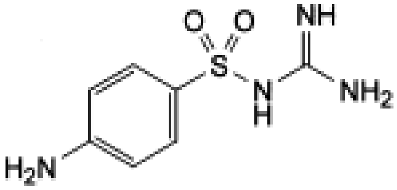
Store protected from light.

Ph Eur

Sulfaguanidine

General Notices

(Ph Eur monograph 1476)



$C_7H_{10}N_4O_2S$ 214.3 *56-67-0*

Action and use

Sulfonamide antibacterial.

Ph Eur

DEFINITION

Sulfaguanidine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (4-aminophenylsulphonyl)guanidine, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, fine crystalline powder, very slightly soluble in water, slightly soluble in acetone, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in dilute solutions of mineral acids.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D, E.*

A. Melting point *(2.2.14)* : 189 °C to 193 °C, determined on the dried substance.

B. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *sulfaguanidine CRS* .

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid* . Dilute 1 ml of the solution to 10 ml with *water R* . The solution, without further acidification, gives the reaction of primary aromatic amines *(2.3.1)* .

E. Suspend 0.1 g in 2 ml of *water R* , add 1 ml of *α-naphthol solution R* and 2 ml of a mixture of equal volumes of *water R* and *strong sodium hypochlorite solution R*. A red colour develops.

TESTS

Solution S

To 2.5 g, add 40 ml of *carbon dioxide-free water R* . Heat at about 70 °C for 5 min. Cool while stirring in iced water for about 15 min, filter and dilute to 50 ml with *carbon dioxide-free water R.*

Acidity

To 20 ml of solution S, add 0.1 ml of *bromothymol blue solution R1* . Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin layer chromatography *(2.2.27)* , using a *TLC silica gel GF$_{254}$ plate R*.

Test solution (a) Dissolve 50 mg of the substance to be examined in *acetone R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 2 ml of test solution (a) to 10 ml with *acetone R.*

Reference solution (a) Dissolve 10 mg of *sulfaguanidine CRS* in *acetone R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 5 ml of test solution (b) to 200 ml with *acetone R.*

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with *acetone R* .

Reference solution (d) Dissolve 10 mg of *sulfanilamide R* in test solution (b) and dilute to 5 ml with the same solution.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *anhydrous formic acid R* , 20 volumes of *methanol R* and 70 volumes of *methylene chloride R* . Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and at most one such spot is more intense that the spot in the chromatogram obtained with reference solution (c) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

Heavy metals *(2.4.8)*

1.0 g complies with test F (20 ppm). Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R* .

Loss on drying ***(2.2.32)***

Not more than 8.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

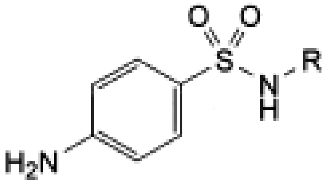
Dissolve 0.175 g in 50 ml of *dilute hydrochloric acid R*. Cool the solution in iced water. Carry out the determination of primary aromatic amino-nitrogen *(2.5.8),* determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 21.42 mg of $C_7H_{10}N_4O_2S$.

STORAGE

Store protected from light.

IMPURITIES



A. R = H: 4-aminobenzenesulphonamide (sulphanilamide),

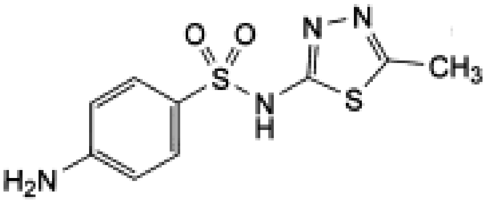
B. R = CO-NH_2: *N*-[(4-aminophenyl)sulphonyl]urea (sulphacarbamide).

Ph Eur

Sulfamethizole

General Notices

(Ph Eur monograph 0637)



$C_9H_{10}N_4O_2S_2$ 270.3 *144-82-1*

Action and use

Sulfonamide antibacterial.

Ph Eur

DEFINITION

Sulfamethizole contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulphonamide, calculated with reference to the dried substance.

CHARACTERS

White or yellowish-white crystalline powder or crystals, very slightly soluble in water, soluble in acetone, sparingly soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides and in dilute mineral acids.

It melts at about 210 °C.

IDENTIFICATION

First identification A, B.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *sulfamethizole CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal

spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 50 mg in 4 ml of *methanol R* and add 0.2 ml of a 40 g/l solution of *copper acetate R*. A flocculent, yellowish-green precipitate is formed, changing to dark green.

D. Dissolve about 5 mg in *1 M hydrochloric acid* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 5 ml of *dilute sodium hydroxide solution R* and 5 ml of *water R*. The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 *(2.2.2, Method II).*

Acidity

To 1.25 g add 25 ml of *carbon dioxide-free water R* and heat at 70 °C for 5 min. Cool for about 15 min in iced water and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF $_{254}$ R* as the coating substance.

Test solution (a) Dissolve 0.30 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *acetone R.*

Reference solution (a) Dissolve 30 mg of *sulfamethizole CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 20 ml with *acetone R.*

Apply to the plate 2 µl of each solution. Develop over a path of 15 cm using a mixture of 15 volumes of *methanol R* and 80 volumes of *chloroform R.* Dry the plate at 100 °C to 105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the determination of primary aromatic amino- nitrogen *(2.5.8)*, using 0.2500 g and determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 27.03 mg of $C_9H_{10}N_4O_2S_2$.

STORAGE

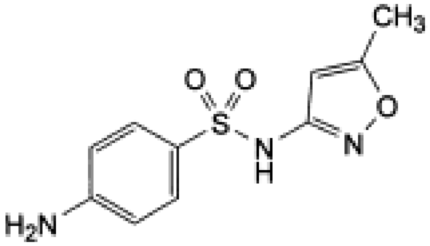
Store protected from light.

Ph Eur

Sulfamethoxazole

General Notices

(Ph Eur monograph 0108)



$C_{10}H_{11}N_3O_3S$ 253.3 *723-46-6*

Action and use

Sulfonamide antibacterial.

Preparations

Co-trimoxazole Intravenous Infusion

Co-trimoxazole Oral Suspension

Paediatric Co-trimoxazole Oral Suspension

Co-trimoxazole Tablets

Dispersible Co-trimoxazole Tablets

Paediatric Co-trimoxazole Tablets

Ph Eur

DEFINITION

4-Amino-*N*-(5-methylisoxazol-3-yl)benzenesulphonamide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of sodium hydroxide and in dilute acids.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)*

169 °C to 172 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *sulfamethoxazole CRS.*

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 20 mg of the substance to be examined in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dissolve 20 mg of *sulfamethoxazole CRS* in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *dilute ammonia R1, water R, nitromethane R, dioxan R* (3:5:41:51 *V/V/V/V*).

Application 5 µl.

Development Over 3/4 of the plate.

Drying At 100-105 °C.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid* . Dilute 1 ml of the solution to 10 ml with *water R* . The solution, without further acidification, gives the reaction of primary aromatic amines *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 , BY_5 or GY_5 *(2.2.2, Method II)*.

Dissolve 1.0 g in a mixture of 5 ml of *dilute sodium hydroxide solution R* and 5 ml of *water R* .

Acidity

To 1.25 g, finely powdered, add 25 ml of *water R* . Heat at 70 °C for 5 min. Cool in iced water for about 15 min and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1* . Not more than 0.3 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in 45 ml of the mobile phase, sonicate at about 45 °C for 10 min, cool and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 1 mg of the substance to be examined and 1 mg of *sulfamethoxazole impurity A CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 1.0 mg of *sulfamethoxazole impurity F CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.0 mm;
- — *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 30 °C.

Mobile phase Mix 35 volumes of *methanol R2* and 65 volumes of a 13.6 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 5.3 with a 20 g/l solution of *potassium hydroxide R* .

Flow rate 0.9 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Run time 3 times the retention time of sulfamethoxazole.

Relative retention With reference to sulfamethoxazole (retention time = about 10 min): impurity D = about 0.3; impurity E = about 0.35; impurity F = about 0.45; impurity C = about 0.5; impurity A = about 1.2; impurity B = about 2.0.

System suitability Reference solution (b):

- — *resolution*: minimum 3.5 between the peaks due to sulfamethoxazole and impurity A.

Limits:
- — *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- — *impurity F:* not more than the area of the principal peak in the chromatogram obtained

with reference solution (c) (0.1 per cent);

— *any other impurity:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit:* 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the assay of primary aromatic amino-nitrogen *(2.5.8)*, using 0.200 g and determining the end-point electrometrically.

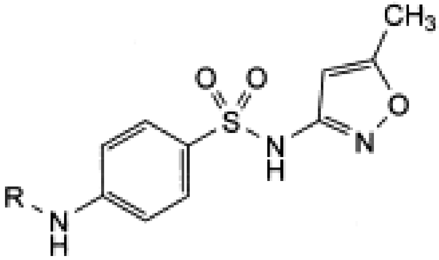
1 ml of *0.1 M sodium nitrite* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

STORAGE

Protected from light.

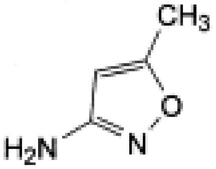
IMPURITIES

Specified impurities   A, B, C, D, E, F.

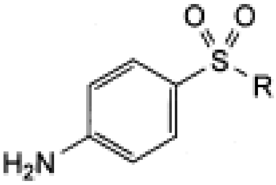


A. R = CO-CH_3: *N*-[4-[(5-methylisoxazol-3-yl)sulphamoyl]phenyl]acetamide,

B. R = SO_2-C_6H_4-*p*NH_2: 4-[[(4-aminophenyl)sulphonyl]amino]-*N*-(5-methylisoxazol-3-yl) benzenesulphonamide,

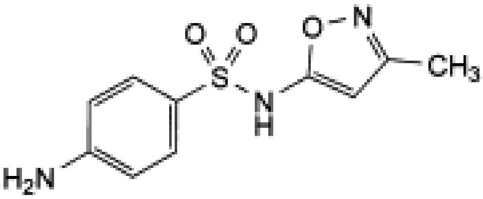


C. 5-methylisoxazol-3-amine,



D. R = OH: 4-aminobenzenesulphonic acid (sulphanilic acid),

E. R = NH_2: 4-aminobenzenesulphonamide (sulphanilamide),



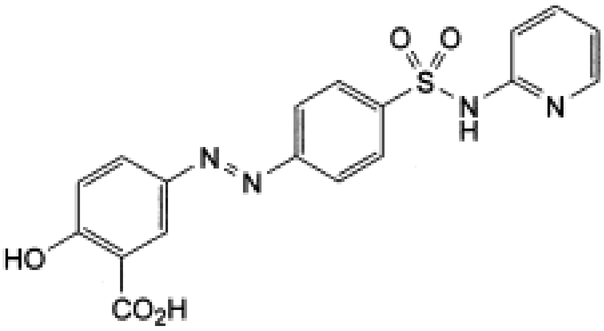
F. 4-amino-*N*-(3-methylisoxazol-5-yl)benzenesulphonamide.

Ph Eur

Sulfasalazine

General Notices

(Ph Eur monograph 0863)



$C_{18}H_{14}N_4O_5S$ 398.4 *599-79-1*

Action and use

Sulfonamide aminosalicylate; treatment of ulcerative colitis.

Preparation

Gastro-resistant Sulfasalazine Tablets

Sulfasalazine Tablets

Ph Eur

DEFINITION

2-Hydroxy-5-[2-[4-(pyridin-2-ylsulphamoyl)phenyl]diazenyl]benzoic acid.

Content

97.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

Bright yellow or brownish-yellow, fine powder.

Solubility

Practically insoluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24*).

Preparation Discs.

Comparison *sulfasalazine CRS.*

TESTS

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 25.0 mg of the substance to be examined in *dilute ammonia R3* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *dilute ammonia R3* .

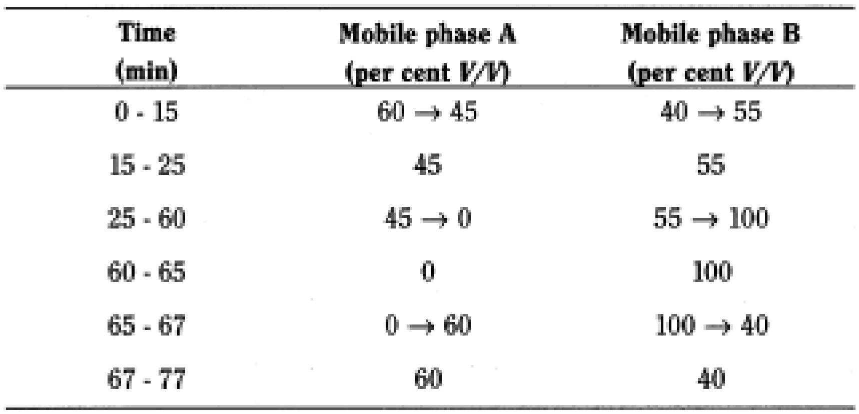
Reference solution (b) Dissolve 1.0 mg of *sulfasalazine derivative for resolution CRS* in 10.0 ml of reference solution (a). Dilute 1.0 ml of this solution to 10.0 ml with reference solution (a).

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A:* in a 1000 ml volumetric flask dissolve 1.13 g of *sodium dihydrogen phosphate R* and 2.5 g of *sodium acetate R* in 900 ml of *water R* ; adjust to pH 4.8 with *glacial acetic acid R* and dilute to 1000 ml with *water R* ;
- *mobile phase B:* mobile phase A, *methanol R* (10:40 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	60 → 45	40 → 55
15 - 25	45	55
25 - 60	45 → 0	55 → 100
60 - 65	0	100
65 - 67	0 → 60	100 → 40
67 - 77	60	40

Flow rate 1 ml/min.

Detection Spectrophotometer at 320 nm.

Injection 20 µl.

Relative retention With reference to sulfasalazine: impurity H = about 0.16; impurity I = about 0.28; impurity C = about 0.80; impurity F = about 0.85; impurity G = about 1.39; impurity E = about 1.63; impurity B = about 1.85; impurity D = about 1.90; impurity A = about 2.00.

System suitability Reference solution (b):

— *resolution*: minimum 3.0 between the peaks due to sulfasalazine and sulfasalazine derivative for resolution.

Limits:

— *impurities A, B, C, D, E, F, G, I:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *total:* not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (4 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak with a retention time less than 6 min (due to impurities H and J).

Impurities H and J

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in *dilute ammonia R3* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *salicylic acid R* (impurity H) and 5.0 mg of *sulfapyridine CRS* (impurity J) in *dilute ammonia R3* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 2.0 ml of reference solution (a) to 100.0 ml with *dilute ammonia R3.*

Column:

— *size*: *l* = 0.25, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mobile phase B (described in the test for related substances), mobile phase A (described in the test for related substances) (30:70 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 300 nm.

Injection 20 µl of the test solution and reference solution (b).

Run time 10 min.

Retention time Impurity H = about 6 min; impurity J = about 7 min.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to impurities H and J.

Limits:

— *impurities H, J*: for each impurity, not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides ***(2.4.4.)***

Maximum 140 ppm.

To 1.25 g add 50 ml of *distilled water R* . Heat at about 70 °C for 5 min. Cool and filter. To 20 ml of the filtrate add 1 ml of *nitric acid R* , allow to stand for 5 min and filter to obtain a clear solution.

Sulphates ***(2.4.13)***

Maximum 400 ppm.

To 20 ml of the filtrate prepared for the test for chlorides add 1 ml of *dilute hydrochloric acid R ,* allow to stand for 5 min and filter.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same solvent. Transfer 5.0 ml of this solution to a 1000 ml volumetric flask containing about 750 ml of *water R* . Add 20.0 ml *of 0.1 M acetic acid* and dilute to 1000.0 ml with *water R* . Prepare a standard solution at the same time and in the same manner using 0.150 g of *sulfasalazine CRS* . Measure the absorbance *(2.2.25)* of the 2 solutions at the absorption maximum at 359 nm.

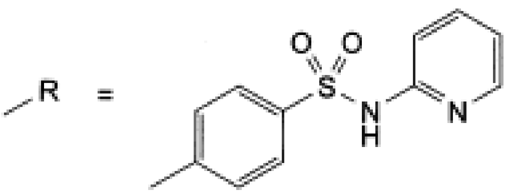
Calculate the content of $C_{18}H_{14}N_4O_5S$ from the absorbances measured and the concentration of the solutions.

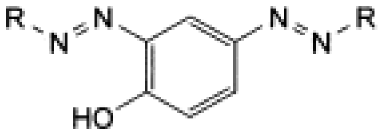
STORAGE

Protected from light.

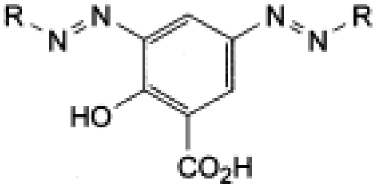
IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J.

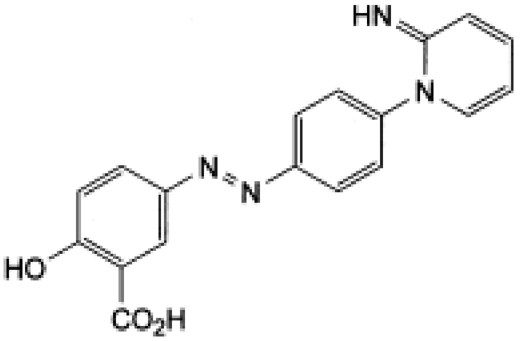




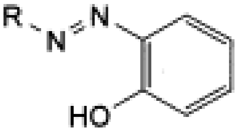
A. 4,4′-[(4-hydroxy-1,3-phenylene)bis(diazenediyl)]bis[*N*-(pyridin-2-yl)benzenesulphonamide],



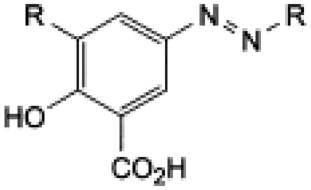
B. 2-hydroxy-3,5-bis[2-[4-(pyridin-2-ylsulphamoyl)phenyl]diazenyl]benzoic acid,



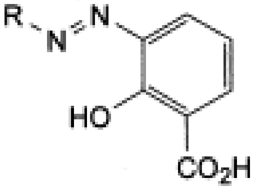
C. 2-hydroxy-5-[2-[4-(2-iminopyridin-1(2*H*)-yl)phenyl]diazenyl]benzoic acid,



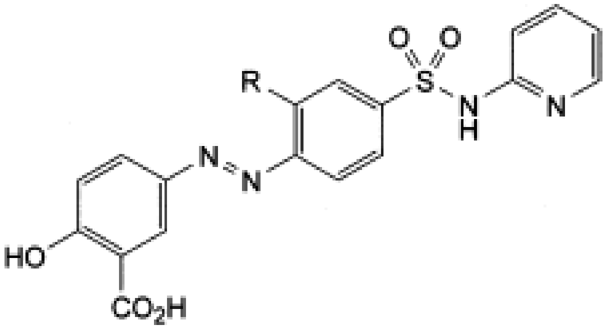
D. 4-[2-(2-hydroxyphenyl)diazenyl]-*N*-(pyridin-2-yl)benzenesulphonamide,



E. 2-hydroxy-4′-(pyridin-2-ylsulphamoyl)-5-[2-[4-(pyridin-2-ylsulphamoyl)phenyl]diazenyl] biphenyl-3-carboxylic acid,

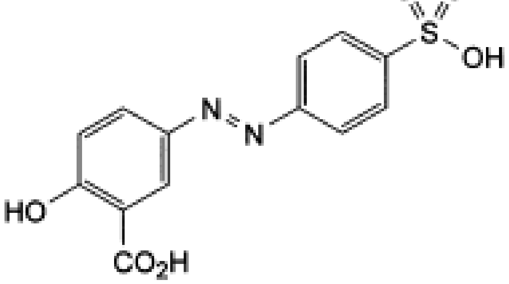


F. 2-hydroxy-3-[2-[4-(pyridin-2-ylsulphamoyl)phenyl]diazenyl]benzoic acid,



G. 5-[2-[4′,5-bis(pyridin-2-ylsulphamoyl)biphenyl-2-yl]diazenyl]-2-hydroxybenzoic acid,

H. salicylic acid,



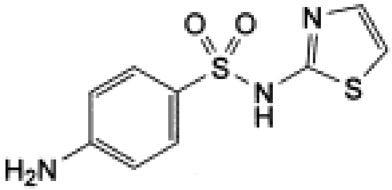
I. 2-hydroxy-5-[2-(4-sulphophenyl)diazenyl]benzoic acid,

J. H_2N-R: 4-amino-*N*-(pyridin-2-yl)benzenesulphonamide (sulfapyridine).

Sulfathiazole

General Notices

(Ph Eur monograph 0742)



$C_9H_9N_3O_2S_2$ 255.3 *72-14-0*

Action and use

Sulfonamide antibacterial.

Ph Eur

DEFINITION

Sulfathiazole contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(thiazol-2-yl)benzenesulphonamide, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, practically insoluble in water, slightly soluble in alcohol, practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides and in dilute mineral acids.

IDENTIFICATION

First identification A, B.

Second identification A, C, D, E.

A. Melting point *(2.2.14)* : 200 °C to 203 °C. Melting may occur at about 175 °C, followed by solidification and a second melting between 200 °C and 203 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *sulfathiazole CRS* . Examine the substances prepared as discs. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *alcohol R* , evaporate to dryness in vacuo and record the spectra again using the residues.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in a mixture of 10 ml of *water R* and 2 ml of *0.1 M sodium hydroxide* and add 0.5 ml of *copper sulphate solution R* . A greyish-blue or purple precipitate is formed.

E. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid* . Dilute 1 ml of the solution to 10 ml with *water R* . The solution, without further addition of acid, gives the reaction of primary aromatic amines *(2.3.1)* .

TESTS

Appearance of solution

Dissolve 1.0 g in 10 ml of *1 M sodium hydroxide* . The solution is clear *(2.2.1)* and not more intensely coloured than reference solution GY_4 *(2.2.2, Method II)*.

Acidity

To 1.0 g add 50 ml of *carbon dioxide-free water R* . Heat to 70 °C for 5 min. Cool rapidly to 20 °C and filter. To 25 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1* . Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography *(2.2.27)* , using *silica gel H R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 5 ml with a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol R* .

Reference solution (a) Dissolve 20 mg of *sulfathiazole CRS* in a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 50 mg of *sulfanilamide R* in a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol R* and dilute to 100 ml with the same mixture of solvents. Dilute 1 ml of this solution to 10 ml with the same mixture of solvents.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 18 volumes of *ammonia R* and 90 volumes of *butanol R* . Dry the plate at 100 °C to 105 °C for 10 min and spray with a 1 g/l solution of *dimethylaminobenzaldehyde R* in *alcohol R* containing 1 per cent *V/V* of *hydrochloric acid R* . Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the determination of primary aromatic amino- nitrogen *(2.5.8)*, using 0.200 g, determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 25.53 mg of $C_9H_9N_3O_2S_2$.

STORAGE

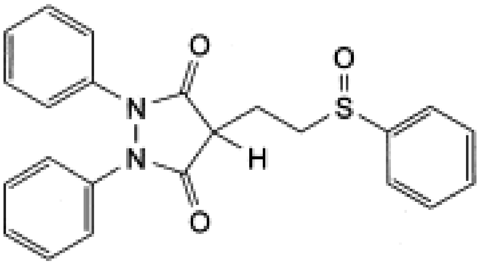
Store protected from light.

Ph Eur

Sulfinpyrazone

General Notices

(Ph Eur monograph 0790)



$C_{23}H_{20}N_2O_3S$ 404.5 *57-96-5*

Action and use

Uricosuric.

Preparation

Sulfinpyrazone Tablets

Ph Eur

DEFINITION

Sulfinpyrazone contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 1,2-diphenyl-4-[2-(phenylsulphinyl)ethyl]pyrazolidine-3,5-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, very slightly soluble in water, sparingly soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Melting point *(2.2.16)* : 131 °C to 135 °C.

B. Dissolve 30.0 mg in *0.01 M sodium hydroxide* and dilute to 100.0 ml with the same alkaline solution. Dilute 1.0 ml of this solution to 20.0 ml with *0.01 M sodium hydroxide*. Examined between 230 nm and 350 nm *(2.2.25)*, the solution shows an absorption maximum at 260 nm. The specific absorbance at the maximum is 530 to 580.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *sulfinpyrazone CRS*. Examine the substances prepared as discs.

D. Dissolve about 10 mg in 3 ml of *acetone R* and add a mixture of 0.2 ml of *ferric chloride solution R2* and 3 ml of *water R*. A red to violet colour develops.

TESTS

Appearance of solution in acetone

Dissolve 1.25 g in *acetone R* and dilute to 25 ml with the same solvent. The solution is clear *(2.2.1)*. The absorbance *(2.2.25)* of the solution, measured at 420 nm with a path-length of 4 cm, is not greater than 0.10.

Appearance of solution in *1 M sodium hydroxide*

Dissolve 1.25 g, heating gently if necessary, in 25 ml of *1 M sodium hydroxide*. The solution is clear *(2.2.1).* The absorbance *(2.2.25)* of the solution, measured at 420 nm with a path-length of 4 cm, is not greater than 0.15.

Related substances

Examine by thin-layer chromatography *(2.2.27),* using a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm. Expose the plate, in a tank, to the vapour from *glacial acetic acid R*. After 10 min, remove the plate and heat at 60 °C for 40 min. Cool and pass a current of nitrogen over the plate for 10 min.

Prepare the solutions immediately before use.

Test solution Dissolve 0.25 g of the substance to be examined in *acetone R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dilute 2 ml of the test solution to 100 ml with *acetone R.* Dilute 1 ml of this solution to 10 ml with *acetone R*.

Reference solution (b) Dissolve 10 mg of *sulfinpyrazone impurity A CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with *acetone R*.

Reference solution (d) Dissolve 10 mg of *sulfinpyrazone impurity B CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (e) Dilute 5 ml of reference solution (d) to 10 ml with *acetone R*.

Apply to the plate, under an atmosphere of nitrogen, 2 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R* and 80 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution: any spots corresponding to sulfinpyrazone impurity A and sulfinpyrazone impurity B are not more intense than the spots in the chromatograms obtained with reference solutions (b) (2.0 per cent) and (d) (2.0 per cent), respectively, and at most one of the spots is more intense than the corresponding spot in the chromatogram obtained with reference solution (c) (1.0 per cent) or (e) (1.0 per cent); any spot apart from the principal spot and the two spots previously cited is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

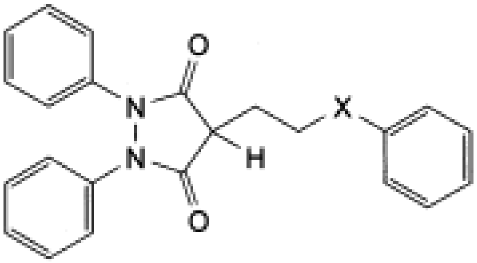
Dissolve 0.300 g in 25 ml of *acetone R*. Add 0.5 ml of *bromothymol blue solution R1.* Titrate with *0.1 M sodium hydroxide* until the colour changes from yellow to blue.

1 ml of *0.1 M sodium hydroxide* is equivalent to 40.45 mg of $C_{23}H_{20}N_2O_3S$.

STORAGE

Store protected from light.

IMPURITIES



A. X = SO_2: 1,2-diphenyl-4-[2-(phenylsulphonyl)ethyl]pyrazolidine-3,5-dione,

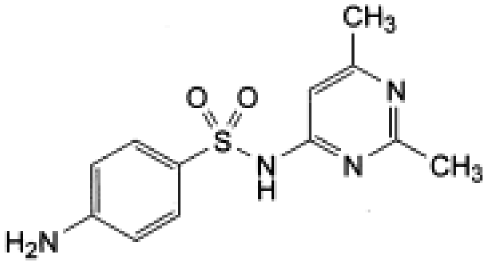
B. X = S: 1,2-diphenyl-4-[2-(phenylsulphanyl)ethyl]pyrazolidine-3,5-dione.

Ph Eur

Sulfisomidine

General Notices

(Ph Eur monograph 0639)



$C_{12}H_{14}N_4O_2S$ 278.3 *515-64-0*

Action and use

Sulfonamide antibacterial.

Ph Eur

DEFINITION

Sulfisomidine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(2,6-dimethylpyrimidin-4-yl)benzenesulphonamide, calculated with reference to the dried substance.

CHARACTERS

White or yellowish-white powder or crystals, very slightly soluble in water, slightly soluble in acetone and in alcohol. It dissolves in dilute solutions of the alkali hydroxides and in dilute mineral acids.

It melts at about 240 °C with decomposition.

IDENTIFICATION

First identification *A, B.*

Second identification *B, C, D.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *sulfisomidine CRS* . Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 50 mg with heating in 4 ml of *methanol R*. Cool and filter. Add to the filtrate 0.2 ml of a 40 g/l *solution of copper acetate R*. The solution becomes yellowish-green.

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid*. Dilute 1 ml of the solution to 10 ml with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines *(2.3.1)*.

TESTS

Appearance of solution

Dissolve 1.0 g in a mixture of 5 ml of dilute *sodium hydroxide solution R* and 5 ml of *water R*. The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 *(2.2.2, Method II)*.

Acidity

To 1.25 g, finely powdered, add 25 ml of *carbon dioxide-free water R*. Heat at 70 °C for 5 min. Cool in a bath of iced water for about 15 min and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1.* Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 5 ml with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Reference solution (a) Dissolve 20 mg of *sulfisomidine CRS* in 3 ml of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.25 ml of test solution (b) to 50 ml with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 3 volumes of *dilute ammonia R1*, 5 volumes of *water R*, 40 volumes of *nitromethane R* and 50 volumes of *dioxan R*. Dry the plate at 100 °C to 105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of

lead standard solution (10 ppm Pb) R.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the determination of primary aromatic amino- nitrogen *(2.5.8)*, using 0.2500 g and determining the end-point electrometrically.

1 ml of *0.1 M sodium nitrite* is equivalent to 27.83 mg of $C_{12}H_{14}N_4O_2S$.

STORAGE

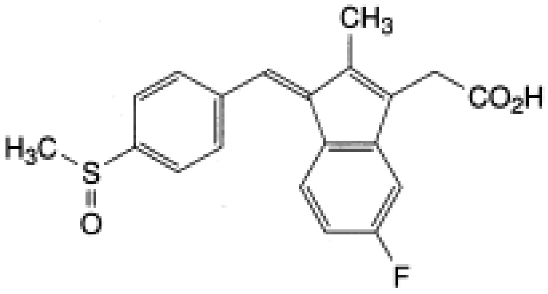
Store protected from light.

Ph Eur

Sulindac

General Notices

(Ph Eur monograph 0864)



$C_{20}H_{17}FO_3S$ 356.4 *38194-50-2*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparation

Sulindac Tablets

Ph Eur

DEFINITION

(*Z*)-[5-Fluoro-2-methyl-1-[4-(methylsulphinyl)benzylidene]-1*H*-inden-3-yl]acetic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Very slightly soluble in water, soluble in methylene chloride, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification C.

Second identification A, B, D, E.

A. Melting point *(2.2.14)* : 182 °C to 186 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 50 mg in a 0.3 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* and dilute to 100 ml with the same acid solution. Dilute 2 ml of this solution to 50 ml with a 0.3 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* .

Spectral range 230-350 nm.

Absorption maxima At 284 nm and 327 nm.

Shoulder At about 258 nm.

Absorbance ratio A_{284}/A_{327} = 1.10 to 1.20.

C. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *sulindac CRS* .

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of hot *methanol R* , evaporate to dryness and record new spectra using the residues.

D. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *sulindac CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *diflunisal CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 2 ml with reference solution (a).

Plate *TLC silica gel GF$_{254}$ plate R.*

Mobile phase *glacial acetic acid R, methylene chloride R, acetone R* (1:49:50 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— The chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

E. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R* , 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R* . Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 20.0 mg of *sulindac CRS* (which has an assigned content of *E*-isomer) in the mobile phase and dilute to 10.0 ml with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: silica gel for chromatography R* (10 µm).

Mobile phase *glacial acetic acid R, ethanol (96 per cent)* R, *ethyl acetate R, ethanol-free chloroform R* (1:4:100:400 *V/V/V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Run time Twice the retention time of sulindac.

Identification of peaks The chromatogram obtained with reference solution (b) shows a principal peak due to sulindac and a peak due to the *E*-isomer.

Relative retention With reference to sulindac: *E*-isomer = about 1.75.

From the chromatograms obtained with the test solution and reference solution (b), determine the percentage content of *E*-isomer, taking into account the assigned content of this isomer in *sulindac CRS* .

Limits:
— *E-isomer*: maximum 0.5 per cent;
— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *methanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

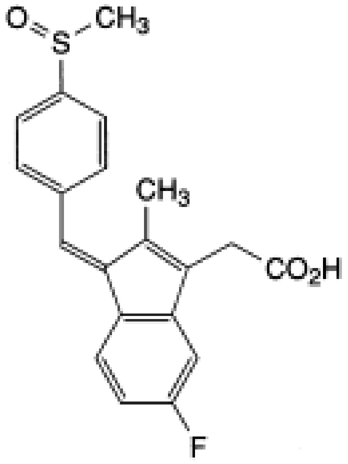
1 ml of *0.1 M sodium hydroxide* is equivalent to 35.64 mg of $C_{20}H_{17}FO_3S$.

STORAGE

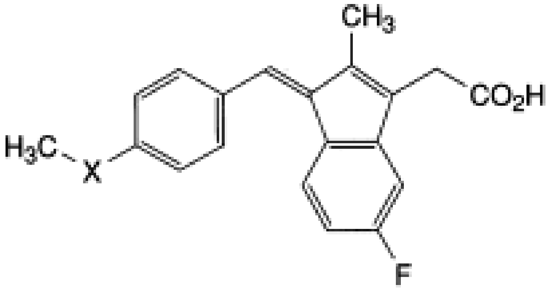
Protected from light.

IMPURITIES

Specified impurities *A, B, C.*



A. (*E*)-[5-fluoro-2-methyl-1-[4-(methylsulphinyl)benzylidene]-1*H*-inden-3-yl]acetic acid,



B. X = SO_2: (*Z*)-[5-fluoro-2-methyl-1-[4-(methylsulphonyl)benzylidene]-1*H*-inden-3-yl]acetic acid,

C. X = S: (*Z*)-[5-fluoro-2-methyl-1-[4-methylsulphanyl)benzylidene]-1*H*-inden-3-yl]acetic acid.

Ph Eur

Sulphur for External Use

General Notices

(Ph Eur monograph 0953)

S 32.07 *7704-34-9*

Action and use

Keratolytic.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

Yellow powder.

Solubility

Practically insoluble in water, soluble in carbon disulphide, slightly soluble in vegetable oils.

mp: about 120 °C.

The size of most of the particles is not greater than 20 µm and that of almost all the particles is not greater than 40 µm.

IDENTIFICATION

A. Heated in the presence of air, it burns with a blue flame, emitting sulphur dioxide which changes the colour of moistened *blue litmus paper R* to red.

B. Heat 0.1 g with 0.5 ml of *bromine water R* until decolourised. Add 5 ml of *water R* and filter. The solution gives reaction (a) of sulphates *(2.3.1).*

TESTS

Solution S

To 5 g add 50 ml of *carbon dioxide-free water R* prepared from *distilled water R.* Allow to stand for 30 min with frequent shaking and filter.

Appearance of solution

Solution S is colourless *(2.2.2, Method II)*.

Odour *(2.3.4)*

It has no perceptible odour of hydrogen sulphide.

Acidity or alkalinity

To 5 ml of solution S add 0.1 ml of *phenolphthalein solution R1.* The solution is colourless. Add 0.2 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.3 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.15 ml of *methyl red solution R*. The solution is orange-red.

Chlorides *(2.4.4)*

Maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulphates *(2.4.13)*

Maximum 100 ppm, determined on solution S.

Sulphides

To 10 ml of solution S add 2 ml of *buffer solution pH 3.5 R* and 1 ml of a freshly prepared 1.6 g/l solution of *lead nitrate R* in *carbon dioxide-free water R*. Shake. After 1 min any colour in the solution is not more intense than that in a reference solution prepared at the same time using 1 ml of *lead standard solution (10 ppm Pb) R*, 9 ml of *carbon dioxide-free water R*, 2 ml of *buffer solution pH 3.5 R* and 1.2 ml of *thioacetamide reagent R*.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Carry out the oxygen-flask method *(2.5.10)*, using 60.0 mg in a 1000 ml combustion flask. Absorb the combustion products in a mixture of 5 ml of *dilute hydrogen peroxide solution R* and 10 ml of *water R.* Heat to boiling, boil gently for 2 min and cool. Using 0.2 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until the colour changes from colourless to red. Carry out a blank titration under the same conditions.

1 ml of *0.1 M sodium hydroxide* is equivalent to 1.603 mg of S.

STORAGE

Protected from light.

Ph Eur

Sulphuric Acid

General Notices

(Ph Eur monograph 1572)

H_2SO_4 98.1 *7664-93-9*

Preparation

Dilute Sulphuric Acid

Ph Eur

DEFINITION

Content

95.0 per cent *m/m* to 100.5 per cent *m/m*.

CHARACTERS

Appearance

Colourless, oily liquid, very hygroscopic.

Solubility

Miscible with water and with ethanol (96 per cent) producing intense heat.

Relative density

About 1.84.

IDENTIFICATION

A. Carefully add 1 ml to 100 ml of *water R*. The solution is strongly acid *(2.2.4)*.

B. The solution obtained in identification test A gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Carefully pour, while cooling, 5 ml into 30 ml of *water R* and dilute to 50 ml with the same solvent.

Chlorides ***(2.4.4)***

Maximum 50 ppm.

Mix carefully, while cooling, 3.3 g with 30 ml of *water R*. Neutralise with *ammonia R* and dilute to 50 ml with *water R*.

Nitrates

Add 5 ml to 5 ml of *water R*. Cool to room temperature and add 0.5 ml of *indigo carmine solution R*. The blue colour persists for at least 1 min.

Arsenic ***(2.4.2, Method A)***

Maximum 1 ppm.

Mix, while cooling, 1 g with 20 ml of *water R* and dilute to 25 ml with the same solvent.

Iron ***(2.4.9)***

Maximum 25 ppm.

Cautiously evaporate 10.0 g and ignite to dull redness. Dissolve the ignition residue in 1 ml of *dilute hydrochloric acid R* with gentle heating and dilute to 25 ml with *water R*. Dilute 1 ml of this solution to 10 ml with *water R.*

Heavy metals ***(2.4.8)***

Maximum 5 ppm.

4.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

ASSAY

Weigh accurately a ground-glass-stoppered flask containing 30 ml of *water R*. Introduce 0.2 ml, cool and weigh again. Titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *1 M sodium hydroxide* is equivalent to 49.04 mg of H_2SO_4.

STORAGE

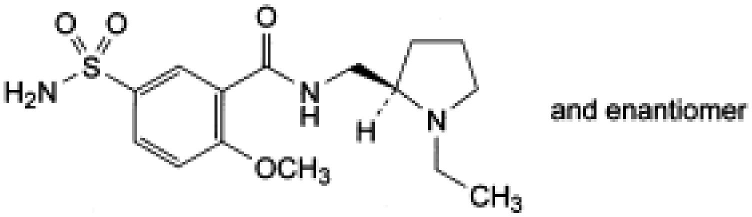
In an airtight container.

Ph Eur

Sulpiride

General Notices

(Ph Eur monograph 1045)



$C_{15}H_{23}N_3O_4S$ 341.4 *15676-16-1*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Sulpiride Tablets

Ph Eur

DEFINITION

(*RS*)-*N*-[(1-Ethylpyrrolidin-2-yl)methyl]-2-methoxy-5-sulphamoylbenzamide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

First identification *A, B*.

Second identification *A, C, D*.

A. Melting point *(2.2.14)* : 177 °C to 181 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *Sulpiride CRS* .

C. Examine the chromatograms obtained in test A for related substances.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 1 mg in a porcelain dish, add 0.5 ml of *sulphuric acid R* and 0.05 ml of *formaldehyde solution R* . Examined in ultraviolet light at 365 nm, the solution shows blue fluorescence.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method I)*.

Dissolve 1.0 g in *dilute acetic acid R* and dilute to 10 ml with the same acid.

Related substances

A. Thin-layer chromatography *(2.2.27)* .

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent. Sonicate until complete dissolution.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R* .

Reference solution (a) Dissolve 20 mg of *sulpiride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *sulpiride impurity A CRS* in *methanol R* and dilute to 25 ml with the same solvent.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10 ml with *methanol R* .

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *Concentrated ammonia R, dioxan R, methanol R, methylene chloride R* (2:10:14:90 *V/V/V/V*).

Application 10 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm for identification test C and then spray with *ninhydrin solution R* ; heat at 100-105 °C for 15 min and examine in daylight.

Limit Test solution (a):

- *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

B. Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 3.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *sulpiride CRS* and 10 mg of *sulpiride impurity B CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm) in spherical microparticles.

Mobile phase Mix 10 volumes of *acetonitrile R* , 10 volumes of *methanol R* and 80 volumes of a solution containing 6.8 g/l of *potassium dihydrogen phosphate R* and 1 g/l of *sodium octanesulphonate R* , adjusted to pH 3.3 using *phosphoric acid R* .

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl.

Run time 2.5 times the retention time of sulpiride.

System suitability Reference solution (b):

- *resolution*: minimum 2.5 between the peaks due to impurity B and sulpiride.

Limit:

- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent).

Chlorides ***(2.4.4)***

Maximum 100 ppm.

Shake 1.0 g with 20 ml of *water R* . Filter through a sintered-glass filter (40) *(2.1.2)* . To 10 ml of the filtrate add 5 ml of *water R* .

Iron ***(2.4.9)***

Maximum 10 ppm.

Ignite 1.0 g in a silica crucible. To the residue add 1 ml of *1 M hydrochloric acid* , 3 ml of *water R* and 0.1 ml of *nitric acid R*. Heat on a water-bath for a few minutes. Place the solution in a test-tube. Rinse the crucible with 4 ml of *water R*. Collect the rinsings in the test-tube and dilute to 10 ml with *water R*.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

1.0 g complies with test C. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

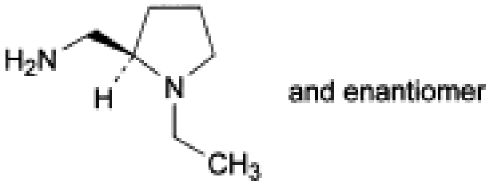
Dissolve 0.250 g in 80 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 34.14 mg of $C_{15}H_{23}N_3O_4S$.

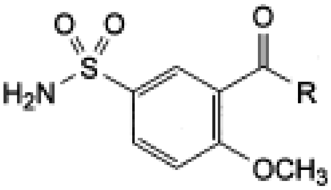
IMPURITIES

Specified impurities A.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E, F, G.*



A. [(2*RS*)-1-ethylpyrrolidin-2-yl]methanamine,

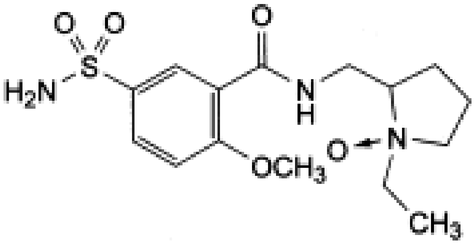


B. R = $O-CH_3$: methyl 2-methoxy-5-sulphamoylbenzoate,

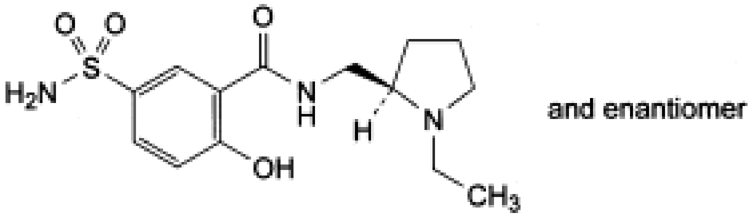
C. R = $O-C_2H_5$: ethyl 2-methoxy-5-sulphamoylbenzoate,

D. R = OH: 2-methoxy-5-sulphamoylbenzoic acid,

E. R = NH_2: 2-methoxy-5-sulphamoylbenzamide,


H2N
OCH3
CH3

F. 1-ethyl-2-[[(2-methoxy-5-sulphamoylbenzoyl)amino]methyl]pyrrolidine 1-oxide,


H2N
OH
CH3
and enantiomer

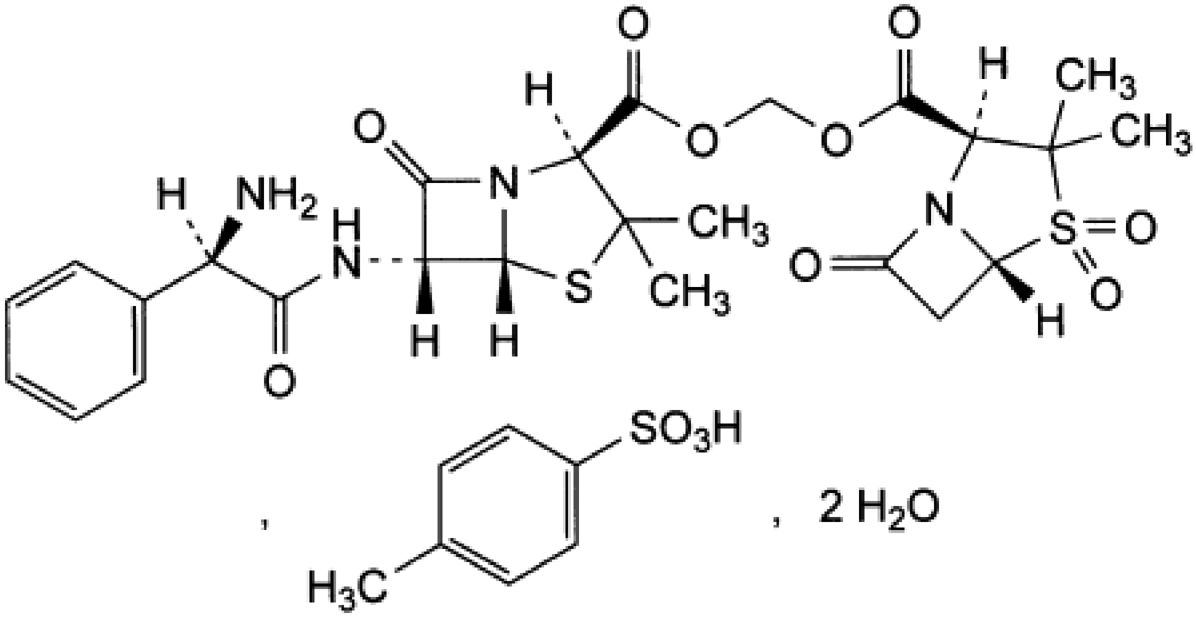
G. (*RS*)-*N*-[(1-ethylpyrrolidin-2-yl)methyl]-2-hydroxy-5-sulphamoylbenzamide.

Ph Eur

Sultamicillin Tosilate Dihydrate

General Notices

(Ph Eur monograph 2212)



$C_{25}H_{30}N_4O_9S_2,C_7H_8O_3S,2H_2O$ 803 *83105-70-8*

Action and use

Beta-lactamase inhibitor.

Ph Eur

DEFINITION

4-Methylbenzenesulphonate of methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-$4\lambda^6$-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate dihydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison sultamicillin tosilate CRS.

TESTS

Specific optical rotation *(2.2.7)*

+ 178 to + 195 (anhydrous substance).

Dissolve 1.000 g in *dimethylformamide R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use or keep at 2-8 ‘C for not more than 6 h.*

Solution A methanol R1, acetonitrile R1 (20:80 *V/V*).

Solution B Dissolve 1.56 g of *sodium dihydrogen phosphate R* in 900 ml of *water R* . Add 7.0 ml of *phosphoric acid R* and dilute to 1000 ml with *water R* .

Blank solution Solution B, solution A (30:70 *V/V*).

Test solution Dissolve 70.0 mg of the substance to be examined in 35 ml of solution A and sonicate for about 1 min. Add 13 ml of solution B, mix and sonicate for about 1 min. Dilute to 50.0 ml with solution B and mix.

Reference solution (a) Dissolve 70.0 mg of *sultamicillin tosilate CRS* in 35 ml of solution A and sonicate for about 1 min. Add 13 ml of solution B, mix and sonicate for about 1 min. Dilute to 50.0 ml with solution B and mix.

Reference solution (b) Suspend 15 mg of the substance to be examined in 20 ml of a 0.4 g/l solution of *sodium hydroxide R* and sonicate in an ultrasonic bath for about 5 min. Add 20 ml of a 0.36 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with *water R* .

Reference solution (c) Dissolve 0.200 g of the substance to be examined in 70.0 ml of solution A and sonicate for about 1 min. Add 25.0 ml of solution B, mix and sonicate for about 1 min. Dilute to 100.0 ml with solution B and mix. Dilute 1.0 ml of this solution to 100.0 ml with the blank solution.

Reference solution (d) Dissolve 32.3 mg of *ampicillin trihydrate CRS* (impurity B) and 7.0 mg of *sulbactam CRS* (impurity A) in *water R* and dilute to 1000 ml with the same solvent.

Column:

— *size: l* = 0.10 m, Ø = 4.6 mm;

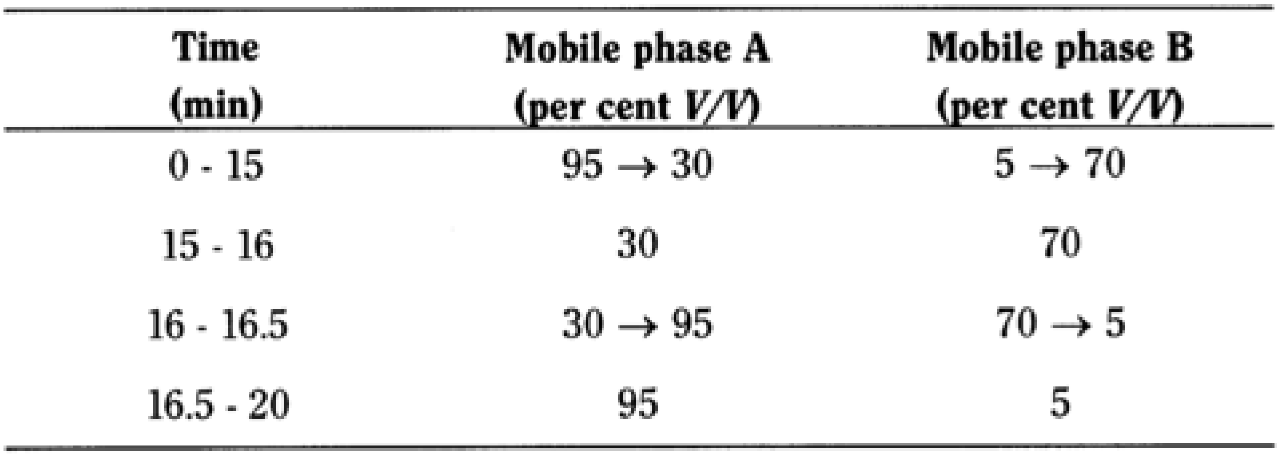
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3.5 µm);

— *temperature*: 25 °C.

Mobile phase:

— *mobile phase A*: 4.68 g/l solution of *sodium dihydrogen phosphate* R adjusted to pH 3.0 with *phosphoric acid R* ;

— *mobile phase B: acetonitrile R1* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	95 → 30	5 → 70
15 - 16	30	70
16 - 16.5	30 → 95	70 → 5
16.5 - 20	95	5

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µl of the blank solution, the test solution and reference solutions (b), (c) and (d).

Relative retention With reference to sultamicillin (retention time = about 9.3 min): impurity A = about 0.41; ampicillin penicilloic acid = about 0.47; tosilate = about 0.50; impurity B = about 0.55; impurity C = about 0.94; impurity D = about 1.09; impurity E = about 1.23; impurity F = about 1.26; impurity G = about 1.42.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to ampicillin penicilloic acid and tosilate and minimum 2.5 between the peaks due to tosilate and impurity B.

Limits:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (2.0 per cent);

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *impurities C, D, E, F, G*: for each impurity, not more than 0.5 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *total*: not more than 4 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (4.0 per cent);

— *disregard limit*: 0.1 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.1 per cent).

Ethyl acetate

Head space gas chromatography *(2.2.28)* .

Test solution Dissolve 0.200 g in 7.0 ml of a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R* .

Reference solution Dissolve 0.200 g of *ethyl acetate R* in 240 ml of a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R* and dilute to 250.0 ml with the same mixture of solvents. Dilute 5.0 ml of this solution to 7.0 ml with a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R* .

Immediately close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous solution.

Column:

— *material*: fused silica;

— *size*: *l* = 50 m, Ø = 0.32 mm;

— *stationary phase*: *poly(dimethyl)siloxane R* (film thickness: 1.8 µm or 3 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 35 cm/s.

Split ratio 1:5.

Static head-space conditions that may be used:

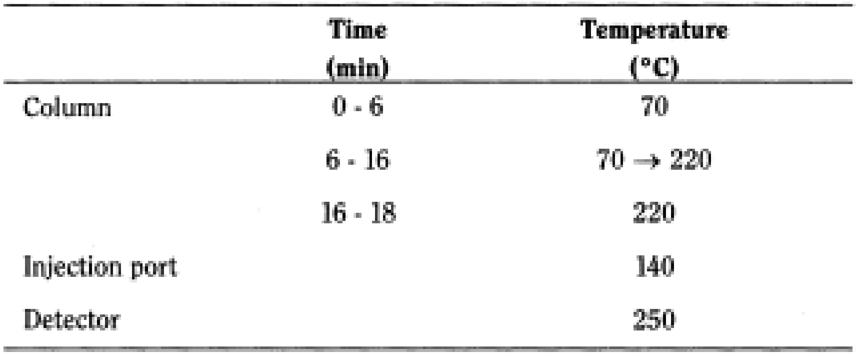
— *equilibration temperature*: 105 °C;

— *equilibration time*: 45 min;

— *transfer-line temperature*: 110 °C;

— *pressurisation time*: 30 s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	70
	6 - 16	70 → 220
	16 - 18	220
Injection port		140
Detector		250

Detection Flame ionisation.

Injection 1 ml.

Relative retention With reference to dimethylformamide (retention time = about 14 min): ethyl acetate = about 0.7.

Limit:

- — *ethyl acetate* : maximum 2.0 per cent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in a mixture of 40 volumes of *methanol R* and 60 volumes of *acetonitrile R* and dilute to 20.0 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 40 volumes of *methanol R* and 60 volumes of *acetonitrile R* .

Water *(2.5.12)*

4.0 per cent to 6.0 per cent, determined on 0.200 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of sultamicillin tosilate ($C_{32}H_{38}N_4O_{12}S_3$) from the declared content of *sultamicillin tosilate CRS*.

STORAGE

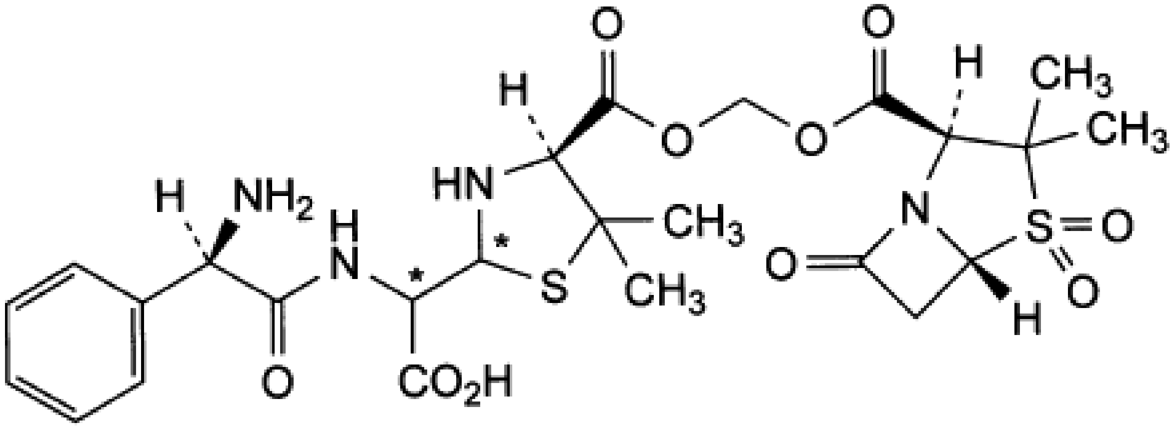
In an airtight container .

IMPURITIES

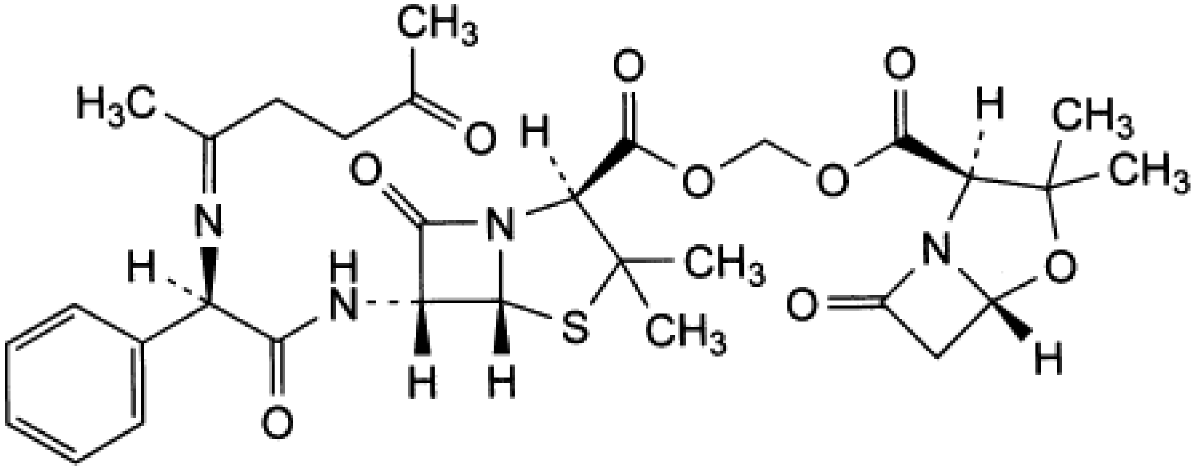
Specified impurities *A, B, C, D, E, F, G.*

A. sulbactam,

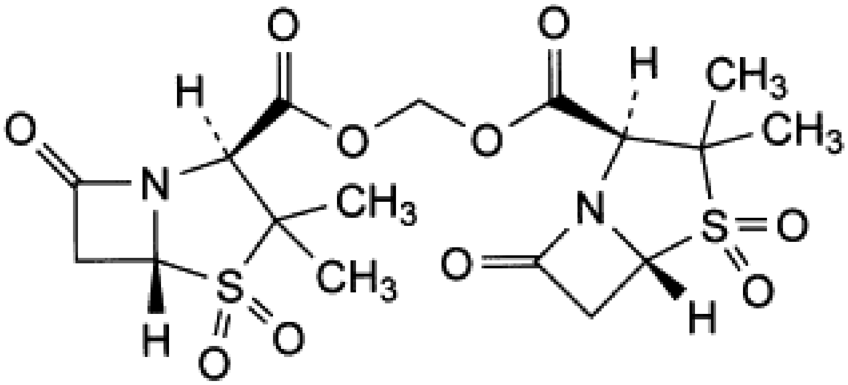
B. ampicillin,



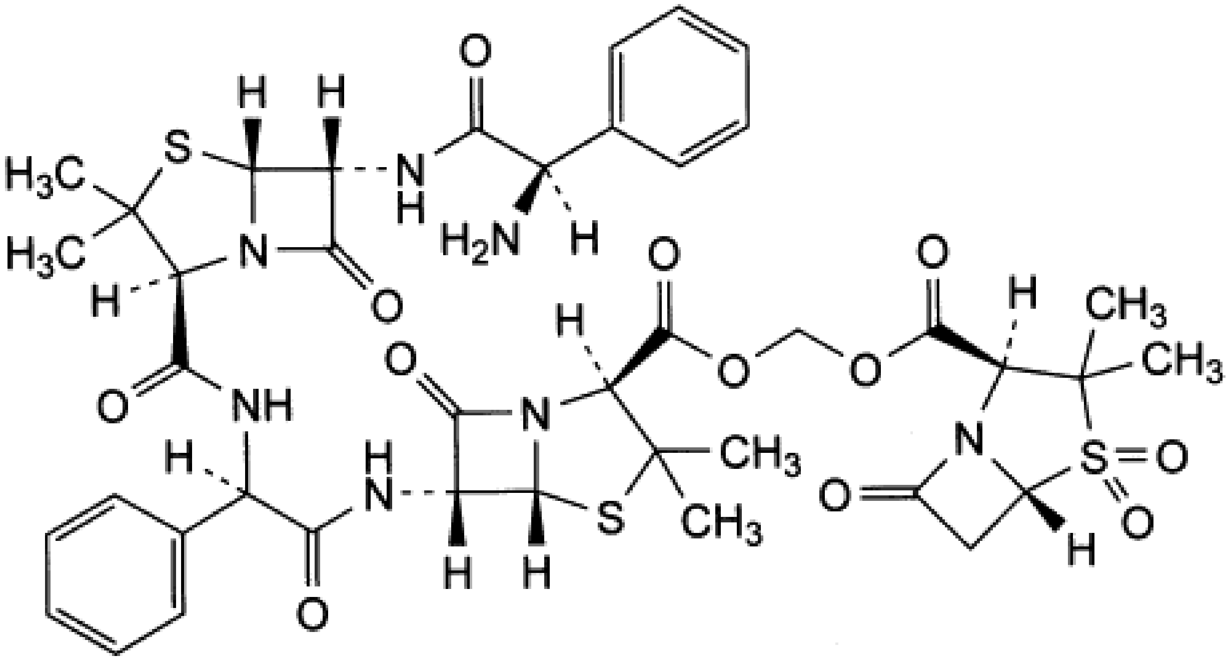
C. [[(*2R*)-aminophenylacetyl]amino][(*4S*)-4-[[[[[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ^6-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetic acid (penicilloic acids of sultamicillin),



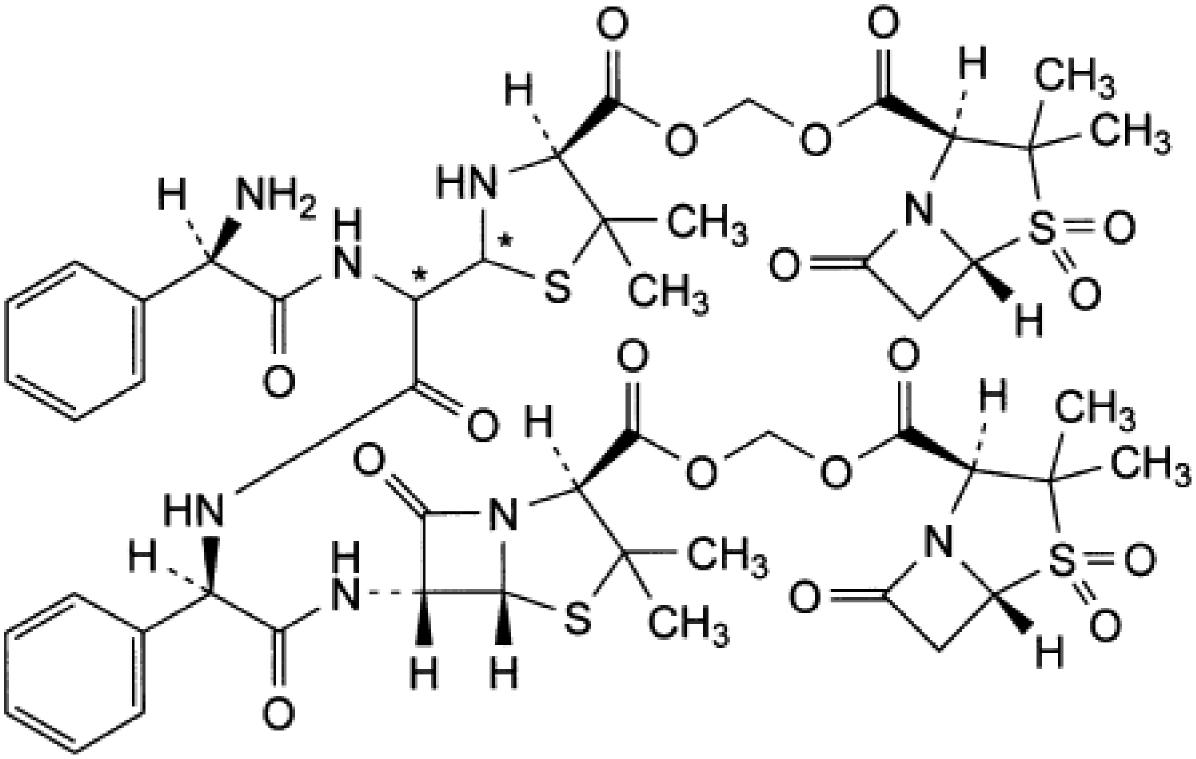
D. methylene (2*S*,5*R*,6*R*)-3,3-dimethyl-6-[[(2*R*)-[(1-methyl-4-oxopentylidene)amino] phenylacetyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate,



E. methylene bis[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ6-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate] (sulbactam methylene ester),



F. methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ6-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (ampicillin sultamicillin amide),



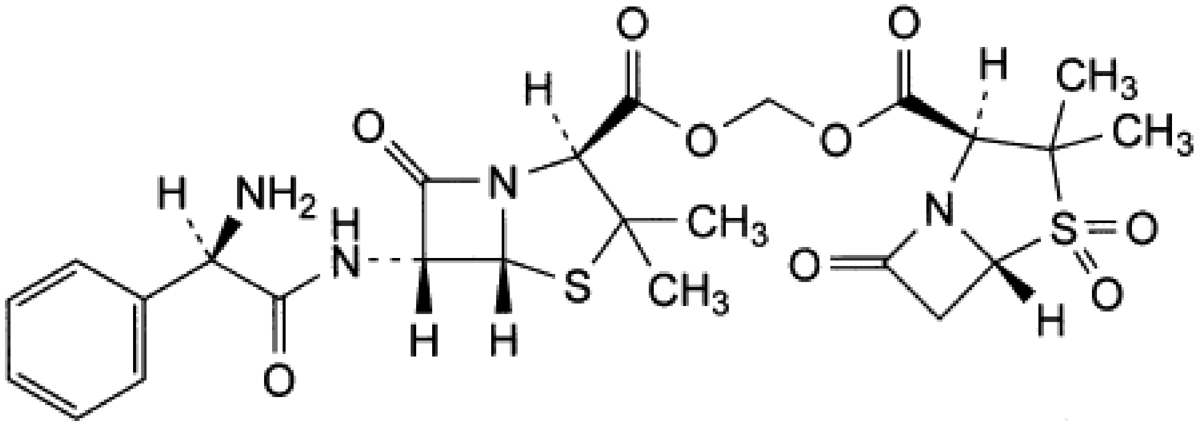
G. methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-[[[[(2*R*)-aminophenylacetyl]amino][(4*S*)-4-[[[[[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ^6-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ^6-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (sultamicillin dimer).

Ph Eur

Sultamicillin

General Notices

(Ph Eur monograph 2211)



$C_{25}H_{30}N_4O_9S_2$ 594.7 *76497-13-7*

Action and use

Beta-lactamase inhibitor.

Ph Eur

DEFINITION

Methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ^6-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic, crystalline powder.

Solubility

Practically insoluble in water, very slightly soluble in methanol, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sultamicillin CRS.*

TESTS

Specific optical rotation (*2.2.7*)

+ 190 to + 210 (anhydrous substance).

Dissolve 0.500 g in *dimethylformamide R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or keep at 2-8 ‘C for not more than 6 h.*

Solution A *methanol R1* , *acetonitrile R1* (20:80 *V/V*).

Solution B Dissolve 1.56 g of *sodium dihydrogen phosphate R* in 900 ml of *water R* . Add 7.0 ml of *phosphoric acid R* and dilute to 1000 ml with *water R.*

Blank solution Solution B, solution A (30:70 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in 35 ml of solution A and sonicate for about 1 min. Add 13 ml of solution B, mix and sonicate for about 1 min. Dilute to 50.0 ml with solution B and mix.

Reference solution (a) Dissolve 70.0 mg of *sultamicillin tosilate CRS* in 35 ml of solution A and sonicate for about 1 min. Add 13 ml of solution B, mix and sonicate for about 1 min. Dilute to 50.0 ml with solution B and mix.

Reference solution (b) Suspend 15 mg of *sultamicillin tosilate CRS* in 20 ml of a 0.4 g/l solution of *sodium hydroxide R* and sonicate in an ultrasonic bath for about 5 min. Add 20 ml of a 0.36 g/l solution of *hydrochloric acid R* and dilute to 100 ml with *water R.*

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the blank solution.

Reference solution (d) Dissolve 17.3 mg of *ampicillin trihydrate CRS* (impurity C) and 15.0 mg of *sulbactam CRS* (impurity A) in *water R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *water R* .

Reference solution (e) Dissolve 5 mg of *sultamicillin for peak identification CRS* (containing impurity G) in 7.0 ml of solution A and sonicate for about 1 min. Dilute to 10.0 ml with solution B, mix and sonicate for about 1 min.

Column:

— *size:* *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3.5 µm);

— *temperature*: 25 °C.

Mobile phase:

— *mobile phase A*: 4.68 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R* ;

— *mobile phase B: acetonitrile R1* ;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	95 → 30	5 → 70
15 - 16	30	70
16 - 16.5	30 → 95	70 → 5
16.5 - 20	95	5

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µl of the blank solution, the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *sultamicillin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peak due to impurity G.

Relative retention With reference to sultamicillin (retention time = about 9.3 min): impurity A = about 0.41; ampicillin penicilloic acid = about 0.47; impurity B = about 0.50; impurity C = about 0.55; impurity D = about 0.94; impurity E = about 1.09; impurity F = about 1.26; impurity G = about 1.42.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to ampicillin penicilloic acid and impurity B and minimum 2.5 between the peaks due to impurities B and C.

Limits:

— *impurity G*: not more than the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram

obtained with reference solution (d) (0.3 per cent);

— *impurities D, E, F*: for each impurity, not more than 0.3 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *any other impurity*: for each impurity, not more than 0.3 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *total*: not more than 3 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (3.0 per cent);

— *disregard limit*: 0.1 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.1 per cent).

Ethyl acetate

Head-space gas chromatography *(2.2.28)* .

Test solution Dissolve 0.200 g in 7.0 ml of a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R.*

Reference solution Dissolve 0.200 g of *ethyl acetate R* in 240 ml of a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R* and dilute to 250.0 ml with the same mixture of solvents. Dilute 5.0 ml of this solution to 7.0 ml with a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R.*

Close the vials immediately with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous solution.

Column:

— *material*: fused silica;

— *size: l* = 50 m, Ø = 0.32 mm;

— *stationary phase: poly(dimethyl)siloxane R* (film thickness: 1.8 µm or 3 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 35 cm/s.

Split ratio 1:5.

Static head-space conditions that may be used:

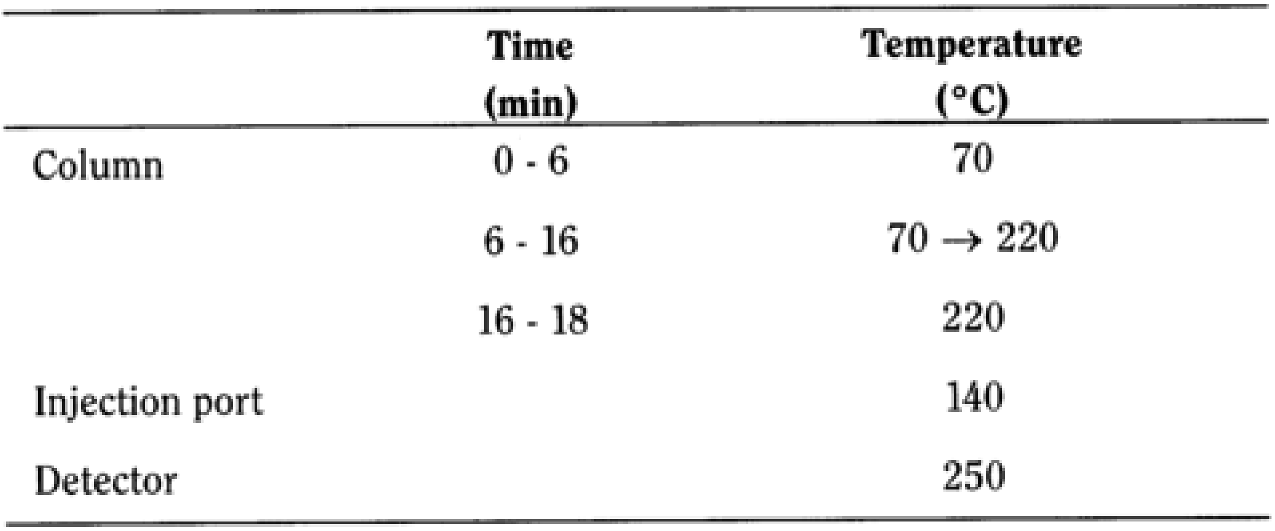
— *equilibration temperature*: 105 °C;

— *equilibration time*: 45 min;

— *transfer-line temperature*: 110 °C;

— *pressurisation time*: 30 s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	70
	6 - 16	70 → 220
	16 - 18	220
Injection port		140
Detector		250

Detection Flame ionisation.

Injection 1 ml.

Relative retention With reference to dimethylformamide (retention time = about 14 min): ethyl acetate = about 0.7.

Limit:
- *ethyl acetate* : maximum 2.5 per cent.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in a mixture of 40 volumes of *methanol R* and 60 volumes of *acetonitrile R* and dilute to 20.0 ml with the same mixture of solvents. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 40 volumes of *methanol R* and 60 volumes of *acetonitrile R.*

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 0.50 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

Calculate the percentage content of sultamicillin ($C_{25}H_{30}N_4O_9S_2$) from the declared content of $C_{25}H_{30}N_4O_9S_2$ in *sultamicillin tosilate CRS* and by multiplying the sultamicillin tosilate content by 0.7752.

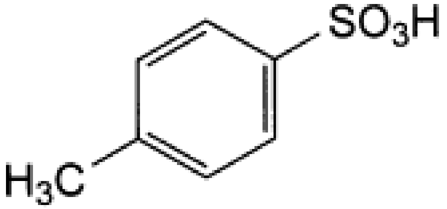
STORAGE

In an airtight container .

IMPURITIES

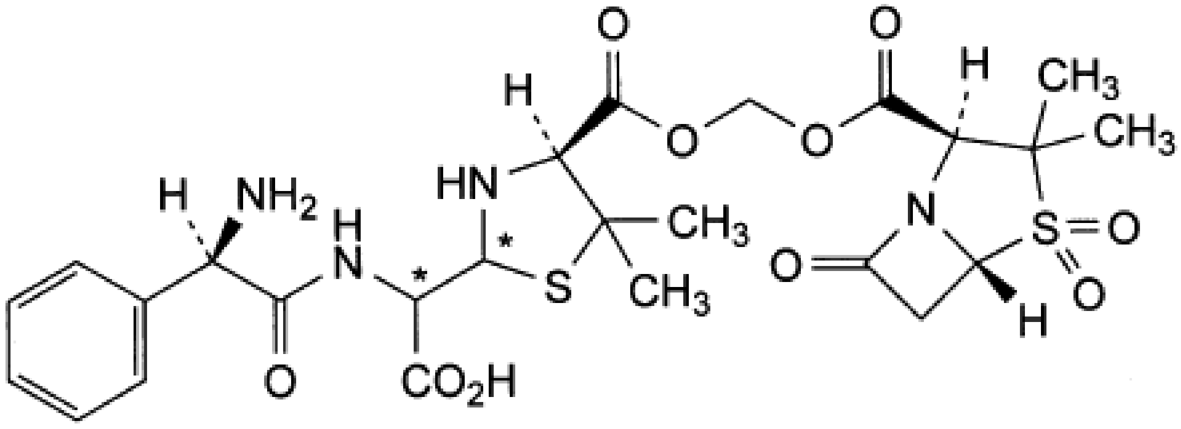
Specified impurities   *A, B, C, D, E, F, G.*

A. sulbactam,

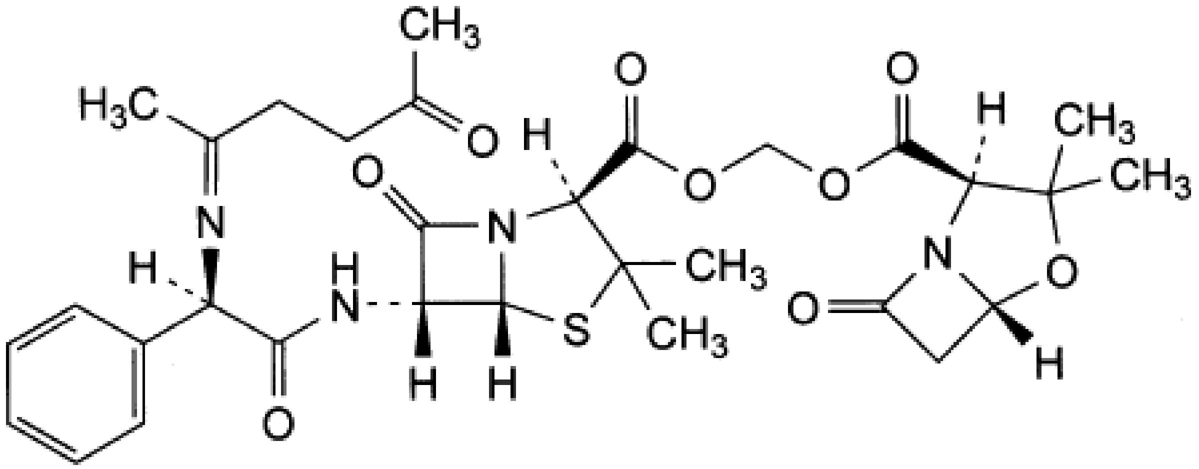


B. 4-methylbenzenesulphonic acid (*p*-toluenesulphonic acid),

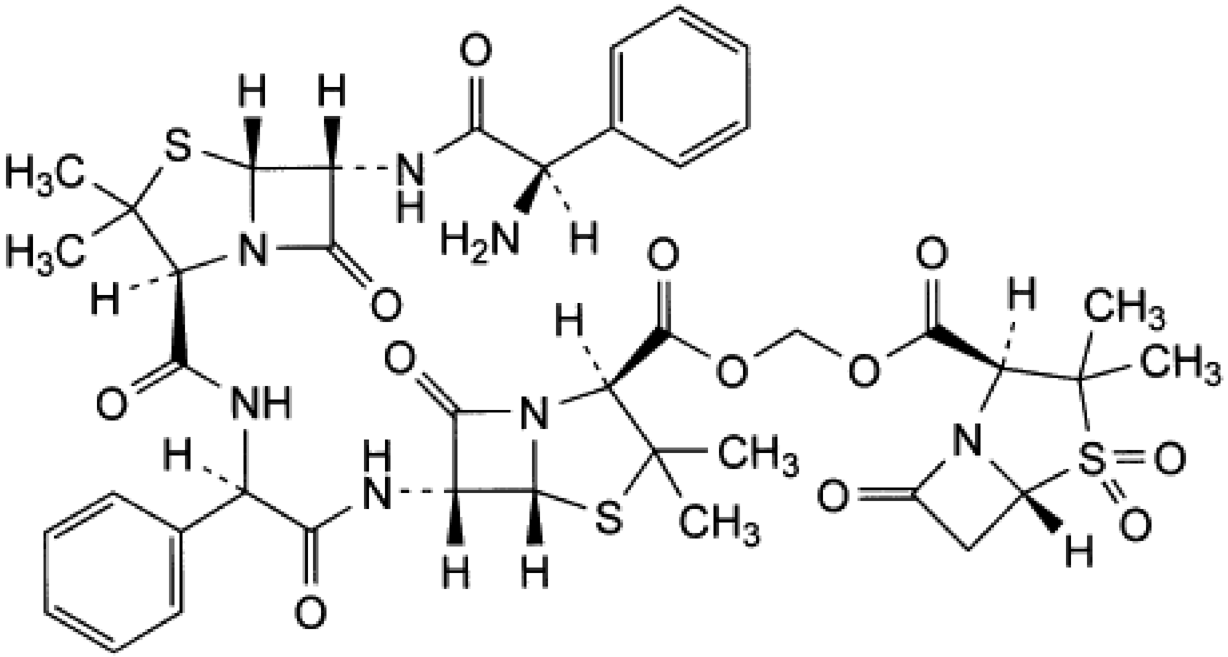
C. ampicillin,


O
O
H
H
CH3
CH3
O
O
HN
H
NH2
H
N
*
N
CH3
S=O
O
*
S
CH3
O
H
O
CO2H

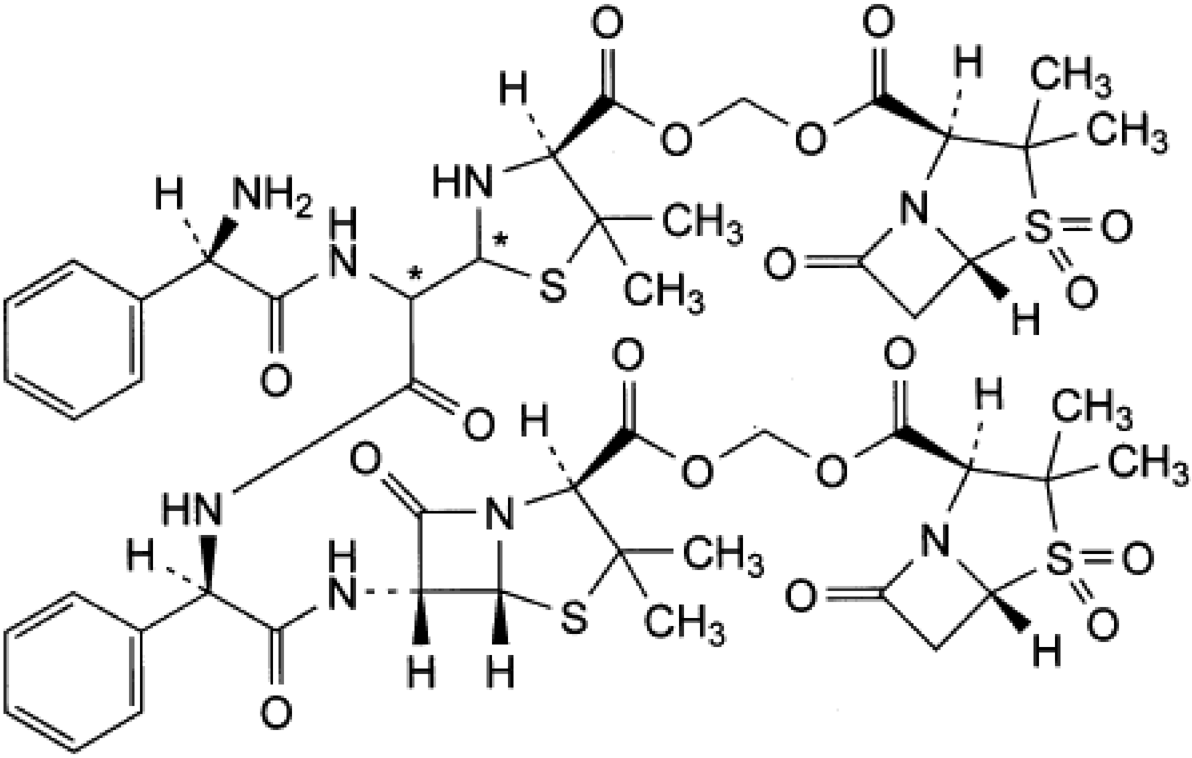
D. [[(2*R*)-aminophenylacetyl]amino][(4*S*)-4-[[[[[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ^6-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetic acid (penicilloic acids of sultamicillin),



E. methylene (2*S*,5*R*,6*R*)-3,3-dimethyl-6-[[(2*R*)-[(1-methyl-4-oxopentylidene)amino] phenylacetyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate,



F. methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ^6-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (ampicillin sultamicillin amide),



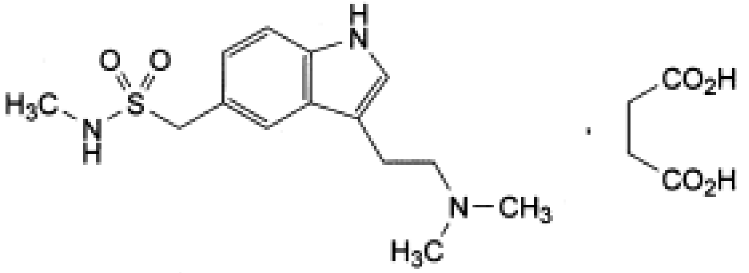
G. methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-[[[(2*R*)-aminophenylacetyl]amino][(4*S*)-4-[[[[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ^{6}-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ^{6}-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (sultamicillin dimer).

Ph Eur

Sumatriptan Succinate

General Notices

(Ph Eur monograph 1573)



$C_{18}H_{27}N_3O_6S$ 413.5   *103628-48-4*

Action and use

Serotonin $5HT_1$ receptor agonist; treatment of migraine.

Preparations

Sumatriptan Injection

Sumatriptan Tablets

Ph Eur

DEFINITION

[3-[2-(Dimethylamino)ethyl]-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide hydrogen butanedioate.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, sparingly soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *sumatriptan succinate CRS.*

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

pH *(2.2.3)*

4.5 to 5.3.

Dilute 2.5 ml of solution S to 10 ml with *carbon dioxide-free water R* .

Absorbance *(2.2.25)*

Maximum 0.10, measured at 440 nm on solution S.

Impurities A and H

Liquid chromatography *(2.2.29).*

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *sumatriptan for system suitability CRS* (containing impurities A and H) in the mobile phase and dilute to 1 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase Mix 10 volumes of a 771 g/l solution of *ammonium acetate R* and 90 volumes of *methanol R* .

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 282 nm.

Injection 20 µl.

Run time 5 times the retention time of sumatriptan.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to sumatriptan and impurity A;

— the chromatogram is similar to the chromatogram supplied with *sumatriptan for system suitability CRS.*

Limits:

— *impurity A*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);

— *impurity H*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent).

Related substances

Liquid chromatography *(2.2.29)* .

Solution A Dissolve 2.925 g of *sodium dihydrogen phosphate R* in 600 ml of *water R* , adjust to pH 6.5 with strong sodium *hydroxide solution R*, dilute to 750 ml with *water R* , add 250 ml of *acetonitrile R* and mix.

Test solution (a) Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Test solution (b) Dissolve 15.0 mg of the substance to be examined in solution A and dilute to 100.0 ml with solution A.

Reference solution (a) Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *sumatriptan impurity mixture CRS* (containing impurities B, C, D and E) in the mobile phase and dilute to 1 ml with the mobile phase.

Reference solution (c) Dissolve 15.0 mg of *sumatriptan succinate CRS* in solution A and dilute to 100.0 ml with solution A.

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography* R (5 µm).

Mobile phase Mix 25 volumes of *acetonitrile R* with 75 volumes of a solution prepared as follows: dissolve 0.970 g of *dibutylamine R* , 0.735 g of *phosphoric acid R* and 2.93 g of *sodium dihydrogen phosphate R* in 750 ml of *water R* , adjust to pH 6.5 with *strong sodium hydroxide solution R* and dilute to 1000 ml with *water R* .

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 282 nm.

Injection 10 µl of test solution (a) and reference solutions (a) and (b).

Run time 4 times the retention time of sumatriptan.

Identification of impurities Use the chromatogram obtained with reference solution (b) and the chromatogram supplied with *sumatriptan impurity mixture CRS* to identify the peaks due to impurities B, C, D and E.

System suitability Reference solution (b):

— *resolution:* minimum 1.5 between the peaks due to sumatriptan and impurity C;

— The chromatogram shows 5 clearly separated peaks.

Limits:

— *impurities B, C, D*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total:* not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{18}H_{27}N_3O_6S$ from the declared content of *sumatriptan succinate CRS.*

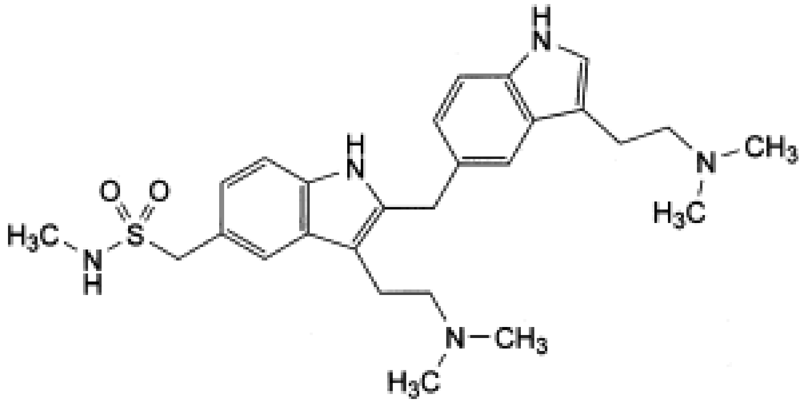
STORAGE

Protected from light.

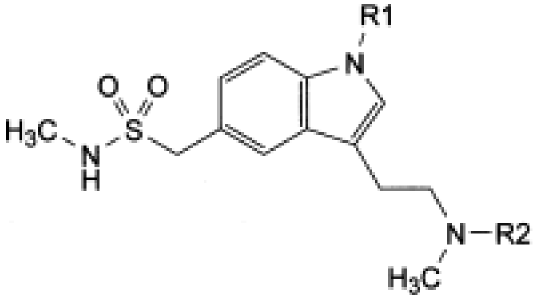
IMPURITIES

Specified impurities A, B, C, D, E, H.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F, G.*

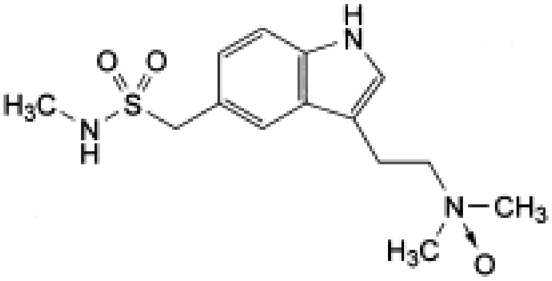


A. [3-[2-(dimethylamino)ethyl]-2-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide,

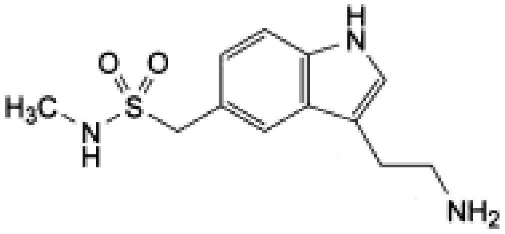


B. R1 = R2 = H: *N*-methyl[3-[2-(methylamino)ethyl]-1*H*-indol-5-yl]methanesulphonamide,

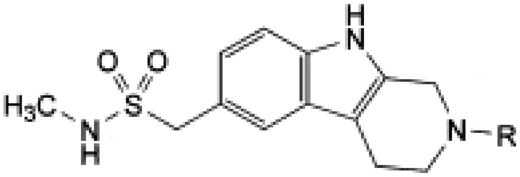
C. R1 = CH_2-OH, R2 = CH_3: [3-[2-(dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide,



D. *N,N*-dimethyl-2-[5-[(methylsulphamoyl)methyl]-1*H*-indol-3-yl]ethanamine N-oxide,

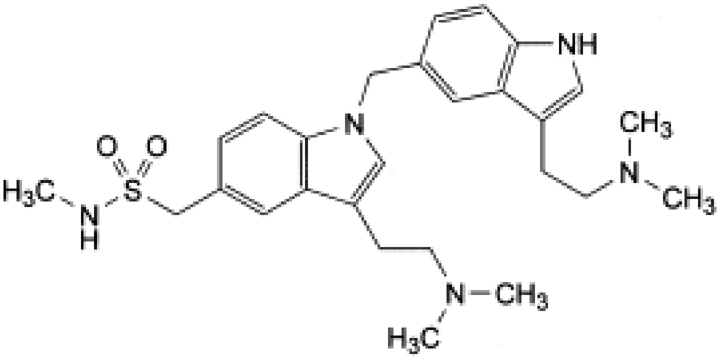


E. [3-(2-aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide,



F. R = H: *N*-methyl(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)methanesulphonamide,

G. R = CH_3: *N*-methyl(2-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl) methanesulphonamide,

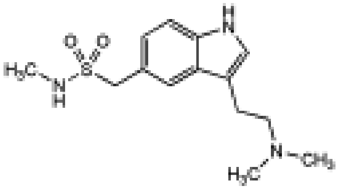


H. [3-[2-(dimethylamino)ethyl]-1-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide.

Ph Eur

Sumatriptan

General Notices



$C_{14}H_{21}N_3O_2S$ 295.4 *103628-46-2*

Serotonin $5HT_1$ receptor agonist; treatment of migraine.

Preparation

Sumatriptan Nasal Spray

DEFINITION

Sumatriptan is 3-(2-dimethylaminoethyl)indol-5-yl-*N*-methylmethanesulfonamide. It contains not less than 97.5% and not more than 102.0% of $C_{14}H_{21}N_3O_2S$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white to pale yellow powder. Very slightly soluble in *water*.

IDENTIFICATION

The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of sumatriptan *(RS 414)* .

TESTS

Impurities A and H

Carry out the method for *liquid chromatography* , Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate* , the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile* (solution A).

(1) 0.2% w/v of the substance being examined in solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute the contents of a vial of *sumatriptan for system suitability EPCRS* to 1 ml with 1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb silica S5W is suitable).

(b) Use isocratic elution using the mobile phase below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for 5 times the retention time of the principal peak.

MOBILE PHASE

A mixture of 10 volumes of 10M *ammonium acetate* and 90 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles that supplied with *sumatriptan for system suitability EPCRS* and the *resolution factor* between impurity A and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.6%; taking into account the correction factor of 0.6 for impurity A);

— the area of any peak due to impurity H is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile* (solution A).

(1) 0.2% w/v of the substance being examined in solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume of the resulting solution to 10 volumes with solution A.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 ml with 0.1M

hydrochloric acid .

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution using the mobile phase below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 75 volumes of a solution containing 0.97 g of *dibutylamine,* 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 ml *water,* adjusted to pH 6.5 with 10M *sodium hydroxide* and diluted to 1000 ml with *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

— the areas of any peaks corresponding to impurities B, C and D are not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

— the area of any peak corresponding to impurity E is not greater than the principal peak in the chromatogram obtained with solution (2) (0.1%);

— the area of any other *secondary peak* is not greater than the principal peak in the chromatogram obtained with solution (2) (0.1%).

The total impurity content in the test for Impurities A and H and the test for Related substances is not greater than 1.5%.

Disregard any peak with an area less than 0.5 times the area of the peak in the chromatogram obtained with solution (2) (0.05%).

Water

Not more than 1.0% w/w, Appendix IX C. Use 1.0 g.

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate* , the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile* (solution A).

(1) 0.01% w/v of the substance being examined in solution A.

(2) 0.014% w/v of *sumatriptan succinate EPCRS* in solution A.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 ml with 0.1M *hydrochloric acid* .

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to sumatriptan and impurity C is at least 1.5.

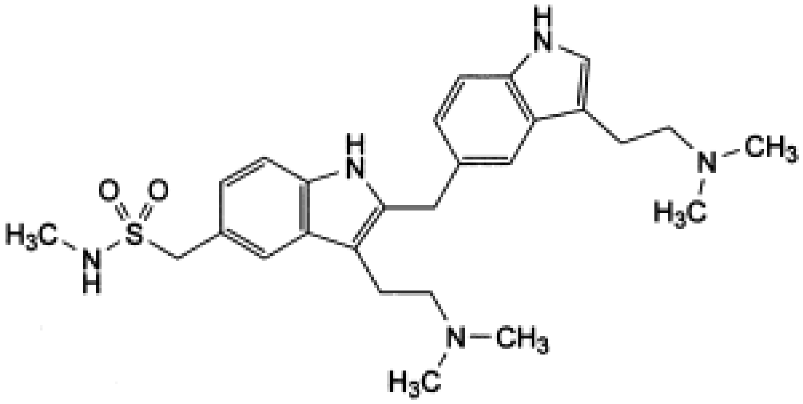
DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{21}N_3O_2S$ using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate EPCRS*. 1 mg of $C_{14}H_{21}N_3O_2S$ is equivalent to 1.4 mg of $C_{14}H_{21}N_3O_2S$, $C_4H_6O_4$.

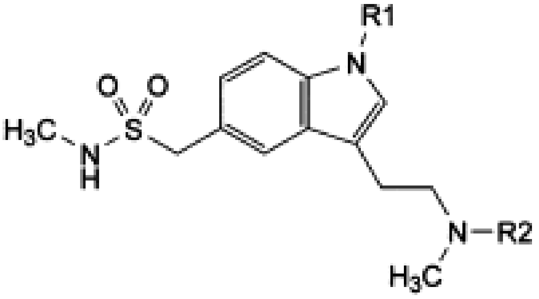
STORAGE

Sumatriptan should be protected from light.

IMPURITIES

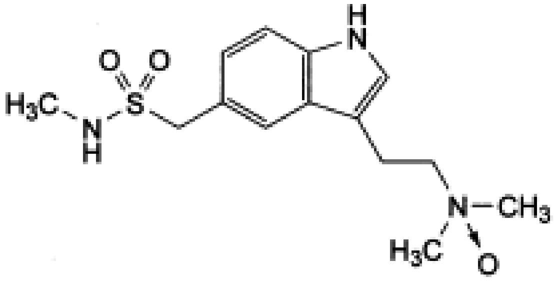


A. [3-[2-(dimethylamino)ethyl]-2-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide,

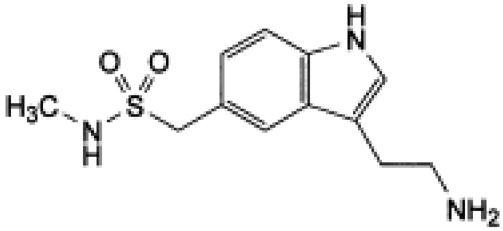


B. R1 = R2 = H: *N*-methyl[3-[2-(methylamino)ethyl]-1*H*-indol-5-yl]methanesulphonamide,

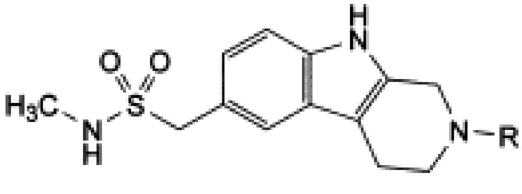
C. R1 = CH_2-OH, R2 = CH_3: [3-[2-(dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide,



D. *N*,*N*-dimethyl-2-[5-[(methylsulphamoyl)methyl]-1*H*-indol-3-yl]ethanamine *N*-oxide,

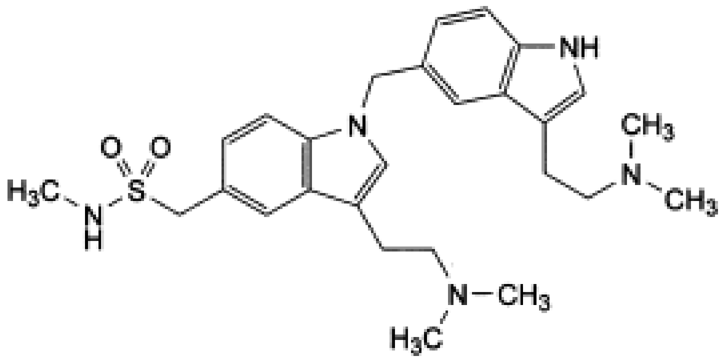


E. [3-(2-aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide,



F. R = H: *N*-methyl(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)methanesulphonamide,

G. R = CH_3: *N*-methyl(2-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl) methanesulphonamide,

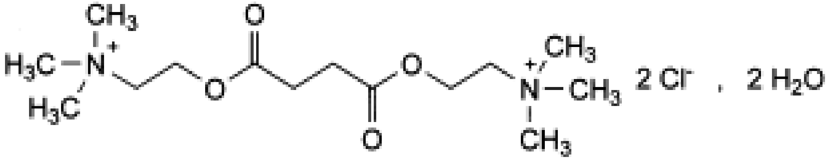


H. [3-[2-(dimethylamino)ethyl]-1-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide.

Suxamethonium Chloride

General Notices

(Ph Eur monograph 0248)



$C_{14}H_{30}Cl_2N_2O_4,2H_2O$ 397.3 *6101-15-1*

Action and use

Depolarizing neuromuscular blocker.

Preparation

Suxamethonium Chloride Injection

Ph Eur

DEFINITION

Suxamethonium chloride contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 2,2′-[butanedioylbis(oxy)]bis(*N*,*N*,*N*-trimethylethanaminium) dichloride, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, hygroscopic, freely soluble in water, slightly soluble in alcohol.

It melts at about 160 °C, determined without previous drying.

IDENTIFICATION

First identification A, D.

Second identification B, C, D.

A. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *suxamethonium chloride CRS* . Examine the substances prepared as discs.

B. To 1 ml of solution S (see Tests) add 9 ml of *water R* , 10 ml of *dilute sulphuric acid R* and 30 ml of *ammonium reineckate solution R.* A pink precipitate is formed. Allow to stand for 30 min, filter, wash with *water R* , with *alcohol R* and then with *ether R* and dry at 80 °C. The melting point *(2.2.14)* of the precipitate is 180 °C to 185 °C.

C. Dissolve about 25 mg in 1 ml of *water R* and add 0.1 ml of a 10 g/l solution of cobalt *chloride R* and 0.1 ml of *potassium ferrocyanide solution R*. A green colour is produced.

D. About 20 mg gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* . Dilute 4 ml of solution S to 10 ml with *water R* . The solution is colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

Dilute 1 ml of solution S to 10 ml with *carbon dioxide-free water R* . The pH of the solution is 4.0 to 5.0.

Choline chloride

Examine by thin-layer chromatography *(2.2.27)* , using *cellulose for chromatography R1* as the coating substance.

Test solution Dissolve 0.4 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.4 g of *suxamethonium chloride CRS* and 2 mg of *choline chloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Apply to the plate 5 µl of each solution. Prepare the mobile phase as follows: shake together for 10 min, 10 volumes of *anhydrous formic acid R* , 40 volumes of *water R* and 50 volumes of *butanol R* ; allow to stand and use the upper layer. Develop over a path of 15 cm. Dry the plate in a current of air and spray with *potassium iodobismuthate solution R.* Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot corresponding to choline chloride in the chromatogram obtained with the reference solution (0.5 per cent). The test is not valid unless the chromatogram obtained with the reference solution shows two clearly separated spots.

Water *(2.5.12)*

8.0 per cent to 10.0 per cent, determined on 0.30 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 18.07 mg of $C_{14}H_{30}Cl_2N_2O_4$.

STORAGE

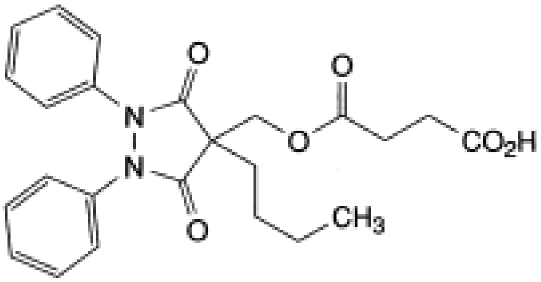
Store in an airtight container, protected from light.

Ph Eur

Suxibuzone

General Notices

(Ph Eur monograph 1574)



$C_{24}H_{26}N_2O_6$ 438.5 *27470-51-5*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

4-[(4-Butyl-3,5-dioxo-1,2-diphenylpyrazolidin-4-yl)methoxy]-4-oxobutanoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, soluble in alcohol, practically insoluble in cyclohexane.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *suxibuzone CRS.*

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1 g in *ethanol R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.10 g of the substance to be examined in *acetonitrile R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dissolve 2.8 mg of *phenylbutazone CRS* (impurity A of suxibuzone), 2.8 mg of *suxibuzone impurity B CRS* and 2.8 mg of *suxibuzone impurity C CRS* in *acetonitrile R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *acetonitrile R* .

Reference solution (b) Dissolve 4 mg of *phenylbutazone CRS* (impurity A of suxibuzone) in *acetonitrile R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *acetonitrile R.*

Reference solution (c) Dissolve 10 mg of *phenylbutazone CRS* (impurity A of suxibuzone) in *acetonitrile R* and dilute to 25.0 ml with the same solvent. Mix 10.0 ml of this solution with 1.0 ml of the test solution and dilute the mixture to 25.0 ml with *acetonitrile R* .

Column:

- *size*: *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 44 volumes of *acetonitrile R* and 56 volumes of a solution prepared as follows: dissolve 6.7 g of *citric acid R* and 2.4 g of *tris(hydroxymethyl)aminomethane R* in 950 ml of *water R* , adjust to pH 3.0 with *citric acid R* and dilute to 1000 ml with *water R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µl.

Relative retention With reference to suxibuzone (retention time = about 7 min): impurity C = 0.7; impurity A = 1.4; impurity B = 3.3.

System suitability Reference solution (c):

- *resolution*: minimum of 2.0 between the peaks due to suxibuzone and to impurity A.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.7 per cent);
- *impurity B*: not more than the area of the corresponding peak in the chromatogram

obtained with reference solution (a) (0.7 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.07 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 60 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in previously *neutralised ethanol R* and dilute to 10 ml with the same solvent. Carry out a potentiometric titration *(2.2.20)* using *0.1 M sodium hydroxide* .

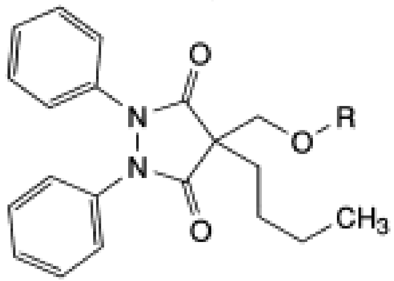
1 ml of *0.1 M sodium hydroxide* is equivalent to 43.85 mg of $C_{24}H_{26}N_2O_6$.

STORAGE

Protected from light.

IMPURITIES

A. phenylbutazone,



B. R = CO-CH_2-CH_2-CO-*O*-CH_2-CH_3: (4-butyl-3,5-dioxo-1,2-diphenylpyrazolidin-4-yl)methyl

ethyl butanedioate,

C. R = H: 4-butyl-4-(hydroxymethyl)-1,2-diphenyl-1,2-dihydro-4*H*-pyrazole-3,5-dione.

Ph Eur

Tapioca Starch

General Notices

Cassava Starch

Action and use

Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical and sub-tropical countries where these are not available, Tapioca Starch may be supplied or used.

DEFINITION

Tapioca Starch is obtained from the rhizomes of *Manihot utilissima* Pohl.

CHARACTERISTICS

Very fine powder which creaks when pressed between the fingers.

Practically insoluble in cold *water* and in *ethanol (96%).*

IDENTIFICATION

A. Principally simple granules, subspherical, muller-shaped or rounded polyhedral; smaller granules 5 to 10 µm, larger granules 20 to 35 µm in diameter; hilum, central, punctate, linear or triradiate; striations, faint, concentric; compound granules, few, of two to three unequal components.

B. Heat to boiling a suspension of 1 g in 50 ml of *water* for 1 minute and cool. A thin, cloudy mucilage is formed.

C. Mix 0.05 ml of *iodine solution R1* with 1 ml of the mucilage obtained in test B. A dark blue colour is produced which disappears on heating and reappears on cooling.

TESTS

Acidity

Add 10 g of the starch to 100 ml of *ethanol (70%)* previously neutralised to 0.5 ml of *phenolphthalein solution* , shake for 1 hour, filter and titrate 50 ml of the filtrate with 0.1M *sodium hydroxide VS* . Not more than 2.0 ml is required to change the colour of the solution.

Foreign matter

Not more than traces of cell membranes and protoplasm are present.

Loss on drying

When dried to constant weight at 100° to 105°, loses not more than 15.0% of its weight. Use 1 g.

Sulphated ash

Not more than 0.6%, Appendix IX A, Method II. Use 1 g.

Microbial contamination

1.0 g is free from *Escherichia coli,* Appendix XVI B1.

STORAGE

Tapioca Starch should be kept in an airtight container.

Wheat Starch

General Notices

(Ph Eur monograph 0359)

Action and use

Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical countries where these are not available, Tapioca Starch may be supplied or used.

Ph Eur

DEFINITION

Wheat starch is obtained from the caryopsis of *Triticum aestivum* L. (*T. vulgare* Vill.).

CHARACTERS

Appearance

Very fine, white or almost white powder which creaks when pressed between the fingers.

Solubility

Practically insoluble in cold water and in alcohol.

Wheat starch does not contain starch grains of any other origin. It may contain a minute quantity, if any, of tissue fragments of the original plant.

IDENTIFICATION

A. Examined under a microscope using equal volumes of *glycerol R* and *water R* , it presents large and small granules, and, very rarely, intermediate sizes. The large granules, 10 µm to 60 µm in diameter, are discoid or, more rarely, reniform when seen face-on. The central hilum and striations are invisible or barely visible and the granules sometimes show cracks on the edges. Seen in profile, the granules are elliptical and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are 2 µm to 10 µm in diameter. Between crossed nicol prisms, the granules show a distinct black cross intersecting at the hilum.

B. Suspend 1 g in 50 ml of *water R* , boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 ml of the mucilage obtained in identification test B, add 0.05 ml of *iodine solution R1*. A dark blue colour is produced which disappears on heating.

TESTS

pH *(2.2.3)*

4.5 to 7.0.

Shake 5.0 g with 25.0 ml of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

Foreign matter

Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.

Total protein

Maximum 0.3 per cent of total protein (corresponding to 0.048 per cent N_2, conversion factor: 6.25), determined on 6.0 g by sulphuric acid digestion *(2.5.9)* modified as follows: wash any adhering particles from the neck into the flask with 25 ml of *sulphuric acid R*; continue the heating until a clear solution is obtained; add 45 ml of *strong sodium hydroxide solution R*.

Oxidising substances *(2.5.30)*

Maximum 20 ppm, calculated as H_2O_2.

Sulphur dioxide *(2.5.29)*

Maximum 50 ppm.

Iron *(2.4.9)*

Maximum 10 ppm.

Shake 1.5 g with 15 ml of *dilute hydrochloric acid R*. Filter. The filtrate complies with the limit test for iron.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulphated ash *(2.4.14)*

Maximum 0.6 per cent, determined on 1.0 g.

MICROBIAL CONTAMINATION

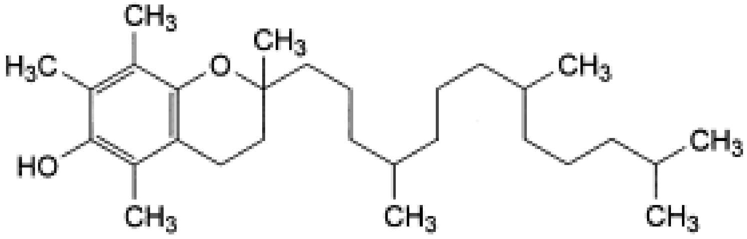
Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and not more than 10^2 fungi per gram, determined by plate count. It complies with the test for *Escherichia coli (2.6.13)*.

Ph Eur

all-*rac* -Alpha-Tocopherol

General Notices

(all-rac-α-Tocopherol, Ph Eur monograph 0692)



$C_{29}H_{50}O_2$ 430.7 *59-02-9*

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

all-*rac*-2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-ol.

Content

96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

Clear, colourless or yellowish-brown, viscous, oily liquid.

Solubility

Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol, in methylene chloride and in fatty oils.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. Optical rotation *(2.2.7)* : - 0.01° to + 0.01°.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *α-tocopherol CRS* .

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 10 mg of the substance to be examined in 2 ml of *cyclohexane R* .

Reference solution Dissolve 10 mg of *α-tocopherol CRS* in 2 ml of *cyclohexane R* .

Plate *TLC silica gel F254 plate R.*

Mobile phase *ether R, cyclohexane R* (20:80 V/V).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10.0 ml of the internal standard solution.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10 ml of *cyclohexane R.*

Reference solution (a) Dissolve 0.100 g of *α-tocopherol CRS* in 10.0 ml of the internal standard solution.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 10 mg of *α-tocopheryl acetate R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of *all-rac-α-tocopherol for peak identification CRS* (containing impurities A and B) in *cyclohexane R* and dilute to 1 ml with the same solvent.

Reference solution (d) Dilute 1.0 ml of test solution (b) to 100.0 ml with *cyclohexane R* . Dilute 1.0 ml of this solution to 10.0 ml with *cyclohexane R.*

Column:

— *material*: fused silica;

— *size*: *l* = 30 m, Ø = 0.25 mm;

— *stationary phase: poly(dimethyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:

— column: 280 °C;

— injection port and detector: 290 °C.

Detection Flame ionisation.

Injection 1 µl of test solution (b) and reference solutions (b), (c) and (d).

Run time Twice the retention time of all-*rac*-α-tocopherol.

Identification of impurities Use the chromatogram supplied with *all-rac-α-tocophero l for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to all-*rac*-α-tocopherol (retention time = about 13 min): squalane = about 0.5; impurity A = about 0.7; impurity B = about 0.8; impurities C and D = about 1.05 (eluting immediately after the all-*rac*-α-tocopherol peak).

System suitability reference solution (b):

— *resolution*: minimum 3.5 between the peaks due to all-rac-α-tocopherol and α-tocopheryl acetate.

Limits:

— *impurity A*: maximum 0.5 per cent;

— *impurity B*: maximum 1.5 per cent;

— *sum of impurities C and D*: maximum 1.0 per cent;

— *any other impurity*: for each impurity, maximum 0.25 per cent;

— *total*: maximum 2.5 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography *(2.2.28)* as described in the test for related substances with the following modification.

Injection Test solution (a) and reference solution (a).

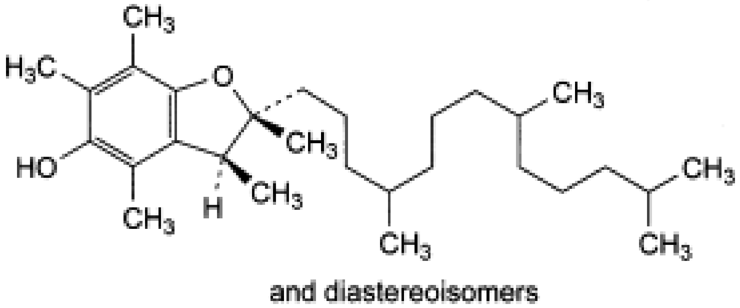
Calculate the percentage content of $C_{29}H_{50}O_2$ from the declared content of α-*tocopherol CRS*.

STORAGE

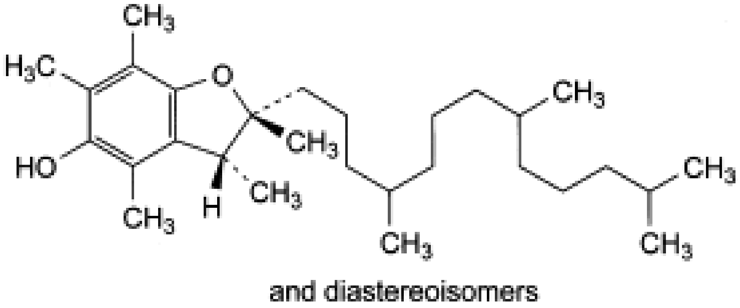
Under an inert gas, protected from light.

IMPURITIES

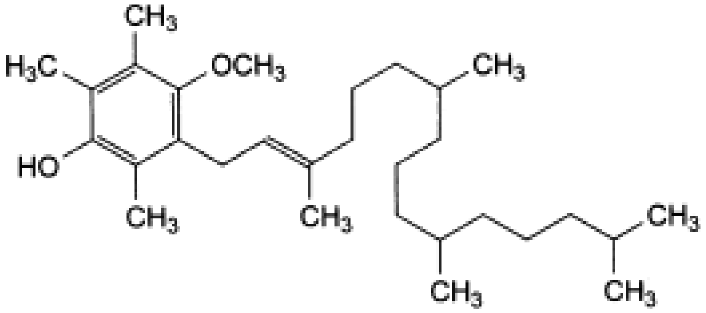
Specified impurities A, B, C, D.



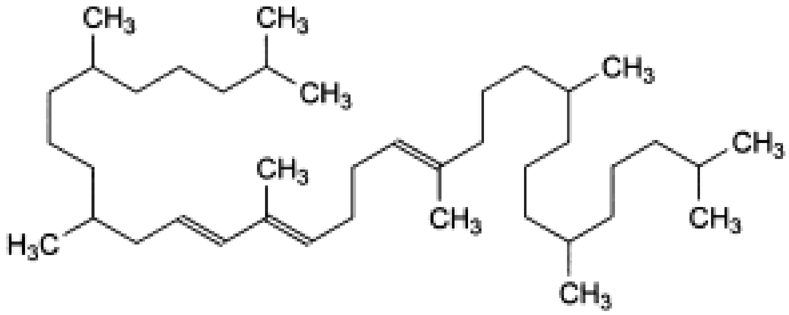
A. all-*rac-trans*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-ol,



B. all-*rac-cis*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-ol.



C. 4-methoxy-2,3,6-trimethyl-5-[(all-*RS*,*E*)-3,7,11,15-tetramethylhexadec-2-enyl]phenol,



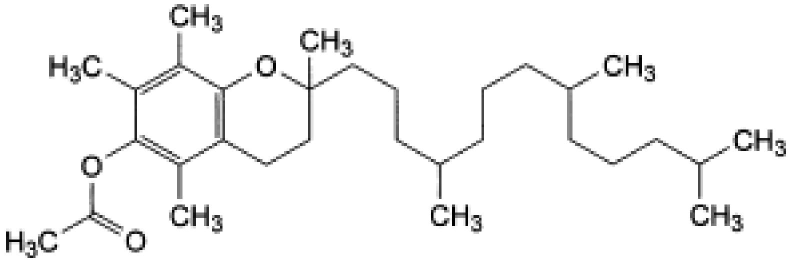
D. (all-*RS*,all-*E*)-2,6,10,14,19,23,27,31-octamethyldotriaconta-12,14,18-triene.

Ph Eur

all-*rac* -Alpha-Tocopheryl Acetate

General Notices

(all-rac-α-Tocopheryl Acetate, Ph Eur monograph 0439)



$C_{31}H_{52}O_3$ 472.7 *7695-91-2*

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

all-*rac*-2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate.

Content

96.5 per cent to 102.0 per cent.

CHARACTERS

Appearance

Clear, colourless or slightly greenish-yellow, viscous, oily liquid.

Solubility

Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol and in fatty oils.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. Optical rotation *(2.2.7)* - 0.01 ° to + 0.01 °.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison α*-tocopheryl acetate CRS* .

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve about 10 mg of the substance to be examined in 2 ml of *cyclohexane R.*

Reference solution Dissolve about 10 mg of α*-tocopheryl acetate CRS* in 2 ml of *cyclohexane R.*

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10.0 ml of the internal standard solution.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10 ml of *cyclohexane R.*

Reference solution (a) Dissolve 0.100 g of α*-tocopheryl acetate CRS* in 10.0 ml of the internal standard solution.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 10 mg of α*-tocopherol R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Reference solution (c) Dissolve 10 mg of *all-rac-*α*-tocopheryl acetate for peak identification CRS* (containing impurities A and B) in *cyclohexane R* and dilute to 1 ml with the same solvent.

Reference solution (d) Dilute 1.0 ml of test solution (b) to 100.0 ml with *cyclohexane R* . Dilute 1.0 ml of this solution to 10.0 ml with *cyclohexane R* .

Column:
- *material*: fused silica;
- *size:* l = 30 m, Ø = 0.25 mm;
- *stationary phase: poly(dimethyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:
- *column:* 280 °C;
- *injection port and detector*: 290 °C.

Detection Flame ionisation.

Injection 1 µl of test solution (b) and reference solutions (a), (b), (c) and (d); inject directly onto the column or via a sufficiently inert, glass-lined injection port using an automatic injection device or other reproducible injection method.

Run time Twice the retention time of all-*rac*-α-tocopheryl acetate.

Identification of impurities Use the chromatogram supplied with *all-rac-α-tocopheryl acet ate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to all-*rac*-α-tocopheryl acetate (retention time = about 15 min): squalane = about 0.4; impurity A = about 0.7; impurity B = about 0.8; impurity C = about 0.9; impurities D and E = about 1.05 (eluting immediately after the all-*rac*-α-tocopheryl acetate peak).

System suitability:
- *resolution:* minimum 3.5 between the peaks due to impurity C and all-*rac*-α-tocopheryl acetate in the chromatogram obtained with reference solution (b);
- In the chromatogram obtained with reference solution (a), the area of the peak due to impurity C is not greater than 0.2 per cent of the area of the peak due to all-*rac*-α-tocopheryl acetate.

Limits:
- *impurities A, C*: for each impurity, maximum 0.5 per cent;
- *impurity B*: maximum 1.5 per cent;
- *sum of impurities D and E*: maximum 1.0 per cent;
- *any other impurity*: for each impurity, maximum 0.25 per cent;

— *total*: maximum 2.5 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography *(2.2.28)* as described in the test for related substances with the following modification.

Injection Test solution (a) and reference solution (a).

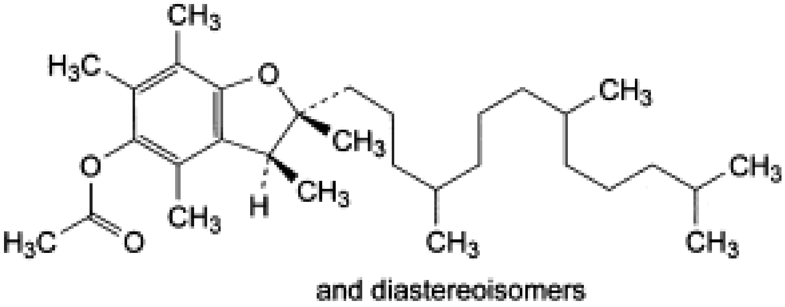
Calculate the percentage content of $C_{31}H_{52}O_3$ from the declared content of α-*tocopheryl acetate CRS.*

STORAGE

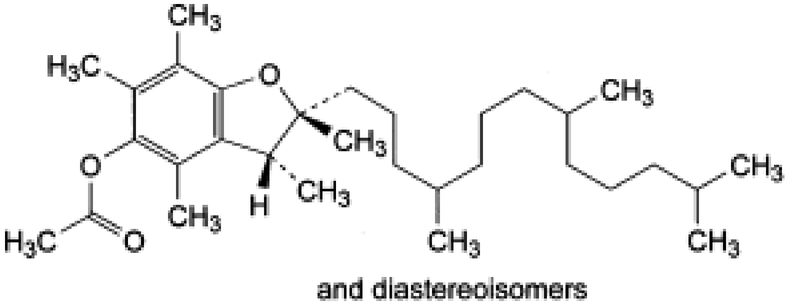
Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*

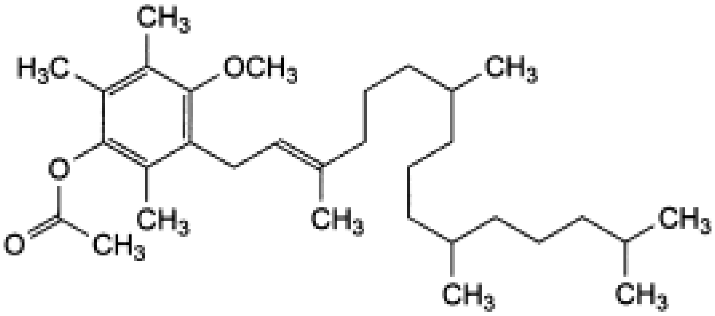


A. all-*rac*-*trans*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-yl acetate,

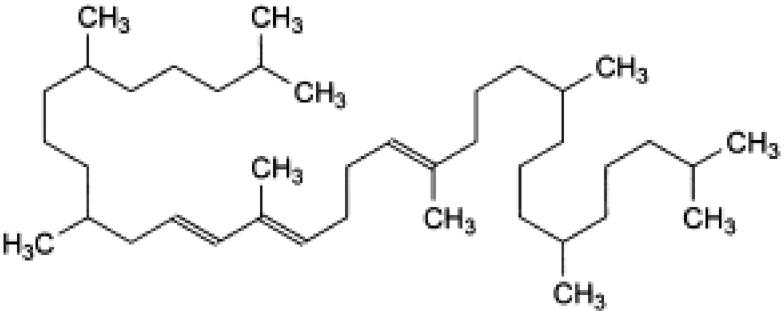


B. all-*rac*-*cis*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-yl acetate,

C. all-*rac*-α-tocopherol,


CH3
H3C
OCH3
CH3
O
O
CH3
CH3
CH3
CH3
CH3
CH3

D. 4-methoxy-2,3,6-trimethyl-5-[(all-*RS*,*E*)-3,7,11,15-tetramethylhexadec-2-enyl]phenyl acetate,


CH3
CH3
CH3
CH3
CH3
CH3
H3C
CH3
CH3
CH3
CH3

E. (all-*RS*,all-*E*)-2,6,10,14,19,23,27,31-octamethyldotriaconta-12,14,18-triene.

Ph Eur

Alpha Tocopheryl Acetate Concentrate (Powder Form)

General Notices

(α-Tocopheryl Acetate Concentrate (Powder Form), Ph Eur monograph 0691)

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

Preparation obtained either by finely dispersing *all-rac-α-Tocopheryl acetate (0439*) in a suitable carrier of suitable quality (for example gelatin, acacia, carbohydrates, lactoproteins or a mixture thereof) or by adsorbing *all-rac-α-Tocopheryl acetat e (0439)* on silicic acid of suitable quality.

Content

90.0 per cent to 115.0 per cent of the α-tocopheryl acetate content stated on the label, which is not less than 25 g per 100 g of concentrate.

CHARACTERS

Appearance

Almost white, yellowish or light brown, small particles.

Solubility

Practically insoluble or swells or forms a dispersion in water, depending on the formulation.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution To a quantity of the preparation to be examined corresponding to 50 mg of α-tocopheryl acetate add 5 ml of *0.01 M hydrochloric acid* and treat with ultrasound at 60 °C. Add 5 ml of *anhydrous ethanol R* and 10 ml of *cyclohexane R,* shake for 1 min and centrifuge for 5 min. Use the upper layer.

Reference solution Dissolve 50 mg of α-*tocopheryl acetate CRS* in *cyclohexane R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F $_{254}$ plate R* .

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography *(2.2.28)* .

Internal standard solution Dissolve 0.20 g of *dotriacontane R* in *hexane R* and dilute to 100.0 ml with the same solvent.

Test solution Weigh accurately a quantity of the preparation to be examined corresponding to about 0.100 g of α-tocopheryl acetate in a 250 ml conical flask. Add 20 ml of *1 M hydrochloric acid* and treat with ultrasound at 70 °C for 20 min. Add 50 ml of *anhydrous ethanol R* and 50.0 ml of the internal standard solution and thoroughly mix the 2 layers for 30 min. Allow to separate, and use the upper layer.

Reference solution Dissolve 0.100 g of *α-tocophery l acetate CRS* in the internal standard solution and dilute to 50.0 ml with the same solution.

Column:
- *material*: silanised glass;
- *size*: *l* = 2.0-3.0 m, Ø = 2.2-4.0 mm;
- *stationary phase*: *diatomaceous earth for gas chromatography R* (125-150 µm or 150-180 µm), silanised with dimethyldichlorosilane and impregnated with 1-5 per cent m/m of *poly(dimethyl)siloxane R;*
- *plug:* silanised glass wool placed at each end of the column.

Carrier gas *nitrogen for chromatography R* .

Flow rate 25-90 ml/min.

Temperature:
- *column*: constant between 245 °C and 280 °C;

— *injection port and detector*: constant between 270 °C and 320 °C.

Detection Flame ionisation.

Injection 1 µl; inject directly onto the column or via an injection port (preferably glass-lined) using an automatic injection device or other reproducible injection method.

System suitability Reference solution:

— *resolution*: minimum 1.4 between the peaks due to dotriacontane and α-tocopheryl acetate. Set the temperature of the column and the flow rate of the carrier gas in such a manner that the required resolution is achieved. Repeat the injection until the response factor (R_F) determined as described below is constant to within ± 2 per cent.

Interference test Weigh accurately a quantity of the substance to be examined corresponding to about 0.100 g of α-tocopheryl acetate in a 250 ml conical flask. Add 20 ml of *1 M hydrochloric acid* and treat with ultrasound at 70 °C for 20 min. Add 50 ml of *anhydrous ethanol R* and 50 ml of *hexane R* and thoroughly mix the 2 layers for 30 min. Allow to separate. Inject 1 µl of the upper layer and record the chromatogram, choosing an attenuation such that the height of the peak due to α-tocopheryl acetate is greater than 50 per cent of the maximum recorder response; during the recording, change the attenuation so that any peak appearing with the same retention time as that of the peak due to dotriacontane is recorded with a sensitivity at least 8 times greater than for the peak due to α-tocopheryl acetate. If a peak with a height of at least 5 mm for a recorder paper width of 250 mm is detected with the same retention time as that of dotriacontane, use the corrected peak area $S'_{D(corr)}$ for the final calculation.

$$S'_{\mathrm{D(corr)}} = S'_{\mathrm{D}} - \frac{S_{\mathrm{I}} \times S'_{\mathrm{T}}}{f \times S_{\mathrm{TI}}}$$

S'_D = area of the peak due to dotriacontane in the chromatogram obtained with the test solution

S_I = area of the peak with the same retention time as that of the peak due to dotriacontane in the chromatogram obtained in the interference test

S'_T = area of the peak due to α-tocopheryl acetate in the chromatogram obtained with the test solution

S_{TI} = area of the peak due to α-tocopheryl acetate in the chromatogram obtained in the interference test

f = factor by which the attenuation was changed

Record the chromatograms choosing an attenuation such that the peak due to α-tocopheryl acetate is greater than 50 per cent of the maximum recorder response.

Measure the areas of the peaks due to α-*tocopheryl acetate CRS* (S_T) and to dotriacontane

(S_D) in the chromatogram obtained with the reference solution and the areas of the peaks due to α-tocopheryl acetate (S'_T) and to dotriacontane (S'_D) in the chromatogram obtained with the test solution.

Determine the response factor (RF) for α-tocopheryl acetate from the areas of the peak due to α-tocopheryl acetate and the peak due to dotriacontane in the chromatogram obtained with the reference solution using the following expression:

$$\frac{S_D \times m_T}{S_T \times m_D}$$

Calculate the percentage content of α-tocopheryl acetate using the following expression:

$$\frac{100 \times S'_T \times m_D \times \mathrm{RF}}{S'_{D(\mathrm{corr.})} \times m}$$

S_D = area of the peak due to dotriacontane in the chromatogram obtained with the reference solution

$S'_{D(\mathrm{corr.})}$ = corrected area of the peak due to dotriacontane in the chromatogram obtained with the test solution

S_T = area of the peak due to α-*tocopheryl acetate CRS* in the chromatogram obtained with the reference solution

S'_T = area of the peak due to α-tocopheryl acetate in the chromatogram obtained with the test solution

m_D = mass of dotriacontane in the test solution and in the reference solution, in milligrams

m_T = mass of α-*tocopheryl acetate CRS* in the reference solution, in milligrams

m = mass of the substance to be examined in the test solution, in milligrams

STORAGE

In an airtight, well-filled container, protected from light.

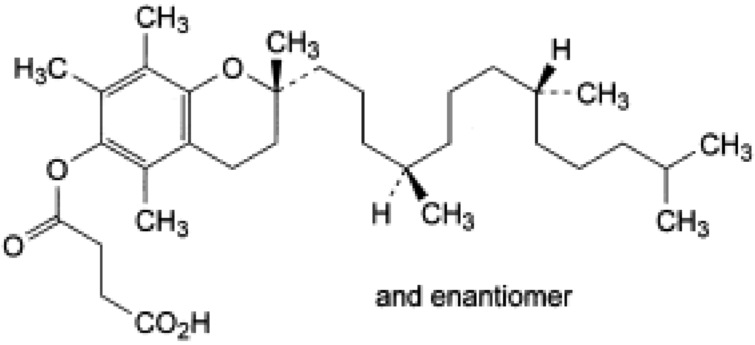
LABELLING

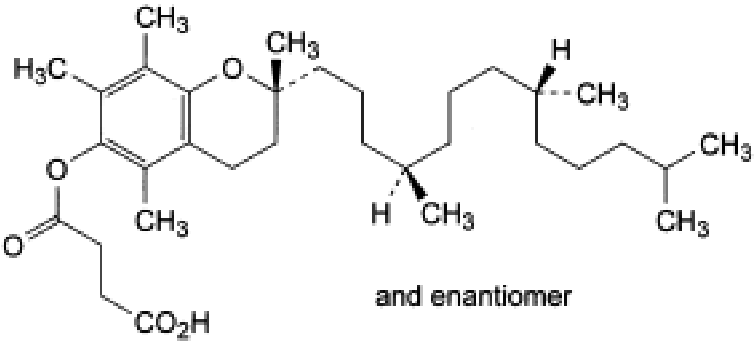
The label states the content of α-tocopheryl acetate, expressed in grams per 100 g of concentrate.

Alpha Tocopheryl Hydrogen Succinate

General Notices

(DL-α-Tocopheryl Hydrogen Succinate, Ph Eur monograph 1258)


CH3
H3C
O
CH3
H
CH3
CH3
O
O
CH3
H
CH3
CH3
CO2H
and enantiomer


CH3
H3C
O
CH3
H
CH3
CH3
O
O
CH3
H
CH3
CH3
CO2H
and enantiomer

$C_{33}H_{54}O_5$ 530.8

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

(2*RS*)-2,5,7,8-Tetramethyl-2-[(4*RS*,8*RS*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl hydrogen succinate.

Content

96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, soluble in acetone and in anhydrous ethanol.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Absorbance (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *RRR-α-tocopheryl hydrogen succinate CRS.*

C. Thin-layer chromatography *(2.2.27)* .

Test solution (a) Dissolve 10 mg of the substance to be examined in 2 ml of *cyclohexane R* .

Test solution (b) In a ground-glass-stoppered tube, dissolve 10 mg of the substance to be examined in 2 ml of *2.5 M alcoholic sulphuric acid R* . Heat on a water-bath for 5 min. Cool and add 2 ml of *water R* and 2 ml of *cyclohexane R* . Shake for 1 min. Use the upper layer.

Reference solution (a) Dissolve 10 mg of *RRR-α-tocopheryl hydrogen succinate CRS* in 2 ml of *cyclohexane R* .

Reference solution (b) Prepare as described for test solution (b), using *RRR-α-tocopheryl hydrogen succinate CRS* instead of the substance to be examined.

Plate *TLC silica gel F_{254} plate R* .

Mobile phase *glacial acetic acid R, ether R, cyclohexane R* (0.2:20:80 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). In the chromatograms obtained with test solution (b) and reference solution (b), there are 2 spots: the spot with the higher R_F value is due to α-tocopherol, the spot with the lower R_F value is due to DL-α-tocopheryl hydrogen succinate and corresponds to the spot obtained with reference solution (a). Depending on the degree of hydrolysis, the lower spot may be weak or even absent.

Detection B Spray with a mixture of 10 volumes of *hydrochloric acid R*, 40 volumes of a 2.5 g/l solution of *ferric chloride R* in *ethanol (96 per cent) R* and 40 volumes of a 10 g/l solution of *phenanthroline hydrochloride R* in *ethanol (96 per cent) R*.

Results B In the chromatograms obtained with test solution (b) and reference solution (b), the spot due to α-tocopherol is orange.

D. Optical rotation (see Tests).

TESTS

Optical rotation *(2.2.7)*

- 0.01 ° to + 0.01 °.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Absorbance *(2.2.25)*.

Solution A Dissolve 0.150 g in *anhydrous ethanol R* and dilute to 100 ml with the same solvent.

Test solution (a) Dilute 10.0 ml of solution A to 100.0 ml with *anhydrous ethanol R*.

Test solution (b) Dilute 20.0 ml of solution A to 50.0 ml with *anhydrous ethanol R*.

Absorption maximum At 284 nm for test solution (a).

Absorption minimum At 254 nm for test solution (b).

Specific absorbance at the absorption maximum 35 to 38 for test solution (a).

Specific absorbance at the absorption minimum 6.0 to 8.0 for test solution (b).

Acid value *(2.5.1)*

101 to 108, determined on 1.00 g.

Free tocopherol

Maximum 1.0 per cent.

Dissolve 0.500 g in 100 ml of *0.25 M alcoholic sulphuric acid R*. Add 20 ml of *water R* and 0.1 ml of a 2.5 g/l solution of *diphenylamine R* in *sulphuric acid R*. Titrate with *0.01 M ammonium and cerium sulphate* until a blue colour is obtained that persists for at least 5 s. Carry out a blank titration.

1 ml of *0.01 M ammonium and cerium sulphate* is equivalent to 2.154 mg of free tocopherol.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

0.50 g complies with test D. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography *(2.2.28)* .

Internal standard solution Dissolve 0.300 g of *dotriacontane R* in *hexane R* and dilute to 100.0 ml with the same solvent.

Test solution Weigh 30.0 mg of the substance to be examined into a 20 ml vial. Add 2.0 ml of *methanol R* , 1.0 ml of *dimethoxypropane R* and 0.1 ml of *hydrochloric acid R* . Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 10.0 ml of the internal standard solution. Vortex into solution.

Reference solution Weigh 30.0 mg of *RRR-α-tocopheryl hydrogen succinate CRS* into a 20 ml vial. Add 2.0 ml of *methanol R* , 1.0 ml of *dimethoxypropane R* and 0.1 ml of *hydrochloric acid R* . Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 10.0 ml of the internal standard solution. Vortex into solution.

Column:

— *material*: fused silica;

— *size*: *l* = 15 m, Ø = 0.32 mm;

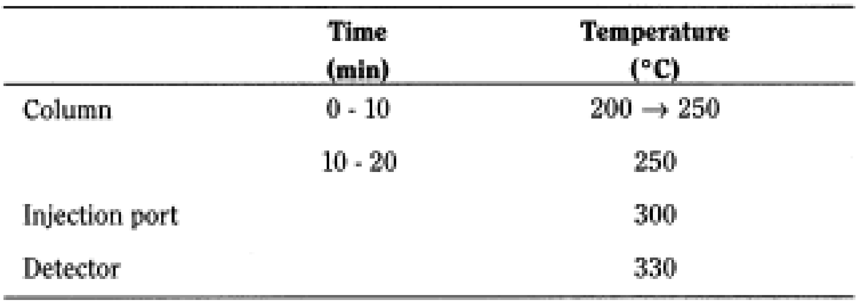
— *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

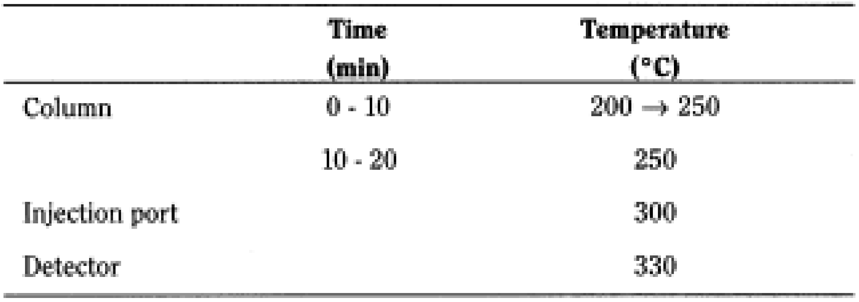
Flow rate 3-6 ml/min.

Split ratio 1:10 to 1:20.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	200 → 250
	10 - 20	250
Injection port		300
Detector		330



	Time (min)	Temperature (°C)
Column	0 - 10	200 → 250
	10 - 20	250
Injection port		300
Detector		330

Detection Flame ionisation.

Injection 1 µl; inject directly onto the column or via a glass-lined injection port using an automatic injection device or some other reproducible injection method.

System suitability Reference solution:

— *resolution*: minimum 12.0 between the peaks due to dotriacontane and DL-α-tocopheryl hydrogen succinate.

Interference test Dissolve 0.100 g of the substance to be examined in *hexane R* and dilute to 50.0 ml with the same solvent. Inject 1 µl of the solution and record the chromatogram. If a peak is detected with the same retention time as that of the peak due to dotriacontane, calculate the area of this peak relative to the peak area of the substance to be examined. If the relative peak area is greater than 0.5 per cent, use the corrected peak area $S'_{D(corr)}$ for the final calculation.

$$S'_{\mathrm{D(corr)}} = S'_{\mathrm{D}} - \frac{S_{\mathrm{I}} \times S'_{\mathrm{T}}}{S_{\mathrm{TI}}}$$

$$S'_{\mathrm{D(corr)}} = S'_{\mathrm{D}} - \frac{S_{\mathrm{I}} \times S'_{\mathrm{T}}}{S_{\mathrm{TI}}}$$

S'_D = area of the peak due to dotriacontane in the chromatogram obtained with the test solution

S_I = area of the peak with the same retention time as that of the peak due to dotriacontane in the chromatogram obtained in the interference test

S'_T = area of the peak due to DL-α-tocopheryl hydrogen succinate in the chromatogram obtained with the test solution

S_{TI} = area of the peak due to DL-α-tocopheryl hydrogen succinate in the chromatogram obtained in the interference test

Measure the areas of the peaks due to *RRR-α-tocopheryl hydrogen succinate CRS* (S_T) and dotriacontane (S_D) in the chromatogram obtained with the reference solution and the areas of the peaks due to DL-α-tocopheryl hydrogen succinate (S'_T) and dotriacontane (S'_D) in the chromatogram obtained with the test solution.

Determine the response factor (R_F) for DL-α-tocopheryl hydrogen succinate from the areas of the peaks due to *RRR-α-tocopheryl hydrogen succinate CRS* and dotriacontane in the chromatogram obtained with the reference solution, using the following expression:

$$\frac{S_D \times m_T}{S_T \times m_D}$$

$$\frac{S_D \times m_T}{S_T \times m_D}$$

Calculate the percentage content of dl-α-tocopheryl hydrogen succinate using the following expression:

$$\frac{100 \times S'_T \times m_D \times \mathrm{RF}}{S'_{D(corr)} \times m}$$

$$\frac{100 \times S'_T \times m_D \times \mathrm{RF}}{S'_{D(corr)} \times m}$$

S_D = area of the peak due to dotriacontane in the chromatogram obtained with the reference solution

$S'_{D(corr)}$ = corrected area of the peak due to dotriacontane in the chromatogram obtained with the test solution

S_T = area of the peak due to *RRR-α-tocopheryl hydrogen succinate CRS* in the chromatogram obtained with the reference solution

S'_T = area of the peak due to DL-α-tocopheryl hydrogen succinate in the chromatogram obtained with the test solution

m_D = mass of dotriacontane in the test solution and in the reference solution, in milligrams

m_T = mass of *RRR-α-tocopheryl hydrogen succinate CRS* in the reference solution, in milligrams

m = mass of the substance to be examined in the test solution, in milligrams

STORAGE

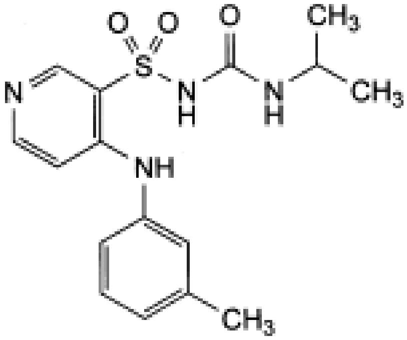
Protected from light.

Ph Eur

Anhydrous Torasemide

General Notices

(Ph Eur monograph 2132)



$C_{16}H_{20}N_4O_3S$ 348.4 *56211-40-6*

Action and use

Thiazide-like diuretic.

Ph Eur

DEFINITION

1-(1-Methylethyl)-3-[[4-[(3-methylphenyl)amino]pyridin-3-yl]sulphonyl]urea.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent). It is sparingly soluble in dilute solutions of alkali hydroxides and slightly soluble in dilute acids.

It shows polymorphism (5.9).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *anhydrous torasemide CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R* , evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography *(2.2.29)* .

Solution A Dissolve 2.7 g of *potassium dihydrogen phosphate R* in 950 ml of *water R* , adjust to pH 3.5 with *phosphoric acid R* and dilute to 1000 ml with *water R* .

Test solution Dissolve 20.0 mg of the substance to be examined in 15 ml of *methanol R* , sonicate for 15 min. Add 22.5 ml of solution A, cool to room temperature and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve the contents of a vial of *torasemide* for *system suitability CRS* (containing impurities A, B, C and D) in 0.5 ml of *methanol R* . Add 0.5 ml of solution A.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:
- *size*: *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase *methanol R* , solution A (40:60 *V/V*).

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 288 nm.

Injection 20 µl.

Run time 2.5 times the retention time of torasemide.

Relative retention With reference to torasemide (retention time = about 10 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.5; impurity D = about 2.3.

System suitability Reference solution (a):

- *resolution*: minimum 3.0 between the peaks due to impurity B and impurity C;
- the chromatogram obtained is similar to the chromatogram supplied with *torasemide for system suitability CRS.*

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 5.1; impurity B = 0.76;

— *impurities A, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with limit test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

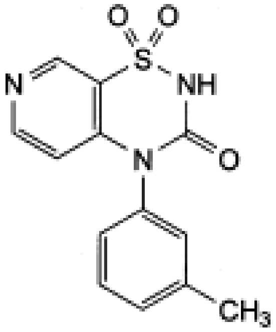
1 ml of *0.1 M perchloric acid* is equivalent to 34.84 mg of $C_{16}H_{20}N_4O_3S$.

STORAGE

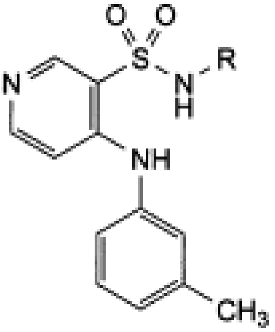
Protected from light.

IMPURITIES

Specified impurities A, B, C, D.



A. 4-(3-methylphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazin-3(4*H*)-one 1,1-dioxide,



B. R = H: 4-[(3-methylphenyl)amino]pyridine-3-sulphonamide,

C. R = CO-NH-C_2H_5: 1-ethyl-3-[[4-[(3-methylphenyl)amino]pyridin-3-yl]sulphonyl]urea,

D. R = CO-NH-$[CH_2]_3$-CH_3: 1-butyl-3-[[4-[(3-methylphenyl)amino]pyridin-3-yl]sulphonyl]urea.

Ph Eur

Coal Tar

General Notices

Action and use

Used in treatment of psoriasis.

Preparations

Coal Tar and Salicylic Acid Ointment

Coal Tar Solution

Strong Coal Tar Solution

Zinc and Coal Tar Paste

DEFINITION

Coal Tar is a product obtained from bituminous coal by destructive distillation at about 1000°.

CHARACTERISTICS

A nearly black, viscous liquid. On exposure to air, the viscosity gradually increases. It burns in air with a luminous, sooty flame. It has a weight per ml of about 1.15 g.

Slightly soluble in *water*; partly soluble in *ethanol*, in *ether* and in volatile oils.

IDENTIFICATION

A. A saturated solution is alkaline to *litmus solution*.

B. Carefully add 0.5 g to 10 ml of *petroleum spirit (boiling range, 40° to 60°)* and allow to stand for 30 minutes. When examined in daylight, the supernatant liquid has a blue fluorescence which becomes more intense when viewed under *ultraviolet light (365 nm)*.

TESTS

Ash

Not more than 2.0%, Appendix XI J.

Medium-chain Triglycerides

General Notices

Fractionated Coconut Oil

(Ph Eur monograph 0868)

Action and use

Excipient.

When Medium-chain Triglycerides are prepared from the endosperm of *Cocos nucifera* L., the title Fractionated Coconut Oil may be used.

Ph Eur

DEFINITION

Mixture of triglycerides of saturated fatty acids, mainly of caprylic (octanoic) acid and of capric (decanoic) acid. Medium-chain triglycerides are obtained from the oil extracted from the hard, dried fraction of the endosperm of *Cocos nucifera* L. or from the dried endosperm of *Elaeis guineensis* Jacq.

Content

Minimum 95.0 per cent of saturated fatty acids with 8 and 10 carbon atoms.

CHARACTERS

Appearance

Colourless or slightly yellowish, oily liquid.

Solubility

Practically insoluble in water, miscible with alcohol, with methylene chloride, with light petroleum and with fatty oils.

IDENTIFICATION

First identification B, C.

Second identification A, D.

A. Heat 3.0 g under a reflux condenser for 30 min with 50 ml of a mixture of equal volumes of *alcohol R* and *2 M alcoholic potassium hydroxide R* . Reserve 10 ml of the mixture for identification test D. To 40 ml of the mixture add 30 ml of *water R* , evaporate the alcohol and acidify the hot solution with 25 ml of *dilute hydrochloric acid R*. After cooling, shake with 50 ml of *peroxide-free ether R*. Wash the ether layer with 3 quantities, each of 10 ml, of *sodium chloride solution R*, dry over *anhydrous sodium sulphate R* and filter. Evaporate the ether and determine the acid value (*2.5.1*) of the residue, using 0.300 g. The acid value is 350 to 390.

B. It complies with the test for saponification value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

D. Evaporate 10 ml of the alcoholic mixture obtained in identification test A to dryness on a water-bath. Transfer the residue into a test-tube, add 0.3 ml of *sulphuric acid R* and close the test-tube with a stopper through which a U-shaped glass tube is inserted. One end of the U-tube is dipped into 3 ml of a 10 g/l solution of *tryptophan R* in a mixture of equal volumes of *sulphuric acid R* and *water R*. Heat the test-tube in a silicone-oil bath at 180 °C for 10 min and collect the liberated fumes in the tryptophan reagent. Heat the tryptophan reagent on a water-bath for 1 min. A violet colour develops.

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution Y_3 *(2.2.2, Method I)*.

Alkaline impurities

Dissolve 2.00 g in a mixture of 1.5 ml of *alcohol R* and 3.0 ml of *ether R*. Add 0.05 ml of *bromophenol blue solution R*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Relative density *(2.2.5)*

0.93 to 0.96.

Refractive index *(2.2.6)*

1.440 to 1.452.

Viscosity *(2.2.9)*

25 mPa·s to 33 mPa·s.

Acid value *(2.5.1)*

Maximum 0.2.

Hydroxyl value *(2.5.3, Method A)*

Maximum 10.

Iodine value *(2.5.4)*

Maximum 1.0.

Peroxide value *(2.5.5, Method A)*

Maximum 1.0.

Saponification value ***(2.5.6)***

310 to 360.

Unsaponifiable matter ***(2.5.7)***

Maximum 0.5 per cent, determined on 5.0 g.

Composition of fatty acids

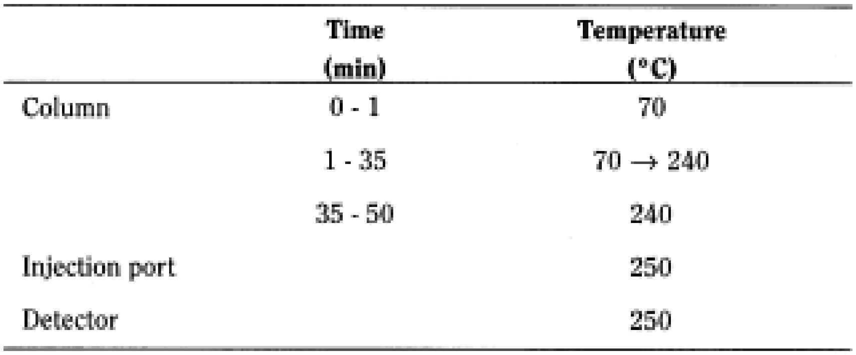
Gas chromatography *(2.4.22, Method C)*

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase: macrogol 20 000 R* (film thickness 0.5 µm),

Carrier gas *helium for chromatography R.*

Flow rate 1.3 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	70
	1 - 35	70 → 240
	35 - 50	240
Injection port		250
Detector		250

Detection Flame ionisation.

Split ratio 1:100.

Composition of the fatty acid fraction of the substance:
- *caproic acid* : maximum 2.0 per cent;
- *caprylic acid* : 50.0 per cent to 80.0 per cent;
- *capric acid*: 20.0 per cent to 50.0 per cent;
- *lauric acid* : maximum 3.0 per cent;
- *myristic acid* : maximum 1.0 per cent.

Chromium

Maximum 0.05 ppm, if intended for use in parenteral nutrition.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 2.0 g of the substance to be examined in *methyl isobutyl ketone R3* and dilute to 10.0 ml with the same solvent.

Solution A Dilute 0.100 ml of *chromium liposoluble standard solution (1000 ppm Cr) R* to 10.0 ml with *methyl isobutyl ketone R3.*

Stock solution Dilute 0.100 ml of solution A to 10.0 ml with *methyl isobutyl ketone R3* .

Reference solutions Prepare 3 reference solutions by dissolving for each 2.0 g of the substance to be examined in the minimum volume of *methyl isobutyl ketone R3* , adding 0.5 ml, 1.0 ml and 2.0 ml, respectively, of stock solution and diluting to 10.0 ml with *methyl isobutyl ketone R3* .

Source Chromium hollow-cathode lamp.

Wavelength 357.8 nm.

Atomic generator Graphite furnace.

Carrier gas *argon R.*

Copper

Maximum 0.1 ppm, if intended for use in parenteral nutrition.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 2.0 g of the substance to be examined in *methyl isobutyl ketone R3* and dilute to 10.0 ml with the same solvent.

Solution A Dilute 0.100 ml of *copper liposoluble standard solution (1000 ppm Cu) R* to 10.0 ml with *methyl isobutyl ketone R3* .

Stock solution Dilute 0.100 ml of solution A to 10.0 ml with *methyl isobutyl ketone R3* .

Reference solutions Prepare 3 reference solutions by dissolving for each 2.0 g of the substance to be examined in the minimum volume of *methyl isobutyl ketone R3* , adding 1.0 ml, 2.0 ml and 4.0 ml, respectively, of stock solution and diluting to 10.0 ml with *methyl isobutyl ketone R3* .

Source Copper hollow-cathode lamp.

Wavelength 324.7 nm.

Atomic generator Graphite furnace.

Carrier gas *argon R* .

Lead

Maximum 0.1 ppm, if intended for use in parenteral nutrition.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 2.0 g of the substance to be examined in *methyl isobutyl ketone R3* and dilute to 10.0 ml with the same solvent.

Solution A Dilute 0.100 ml of *lead liposoluble standard solution (1000 ppm Pb) R* to 10.0 ml with *methyl isobutyl ketone R3* .

Stock solution Dilute 0.100 ml of solution A to 10.0 ml with *methyl isobutyl ketone R3* .

Reference solutions Prepare 3 reference solutions by dissolving for each 2.0 g of the substance to be examined in the minimum volume of *methyl isobutyl ketone R3* , adding 1.0 ml, 2.0 ml and 4.0 ml, respectively, of stock solution and diluting to 10.0 ml with *methyl isobutyl ketone R3.*

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm.

Atomic generator Graphite furnace coated inside with palladium carbide; calcination is carried out in the presence of oxygen at a temperature below 800 °C.

Carrier gas *argon R* .

Nickel

Maximum 0.2 ppm, if intended for use in parenteral nutrition.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 2.0 g of the substance to be examined in *methyl isobutyl ketone R3* and dilute to 10.0 ml with the same solvent.

Solution A Dilute 0.100 ml of *nickel liposoluble standard solution (1000 ppm Ni) R* to 10.0 ml with *methyl isobutyl ketone R3* .

Stock solution Dilute 0.100 ml of solution A to 10.0 ml with *methyl isobutyl ketone R3* .

Reference solutions Prepare 3 reference solutions by dissolving for each 2.0 g of the substance to be examined in the minimum volume of *methyl isobutyl ketone R3* , adding 1.0 ml, 2.0 ml and 4.0 ml, respectively, of stock solution and diluting to 10.0 ml with *methyl isobutyl ketone R3* .

Source Nickel hollow-cathode lamp.

Wavelength 232 nm.

Atomic generator Graphite furnace.

Carrier gas *argon R.*

Tin

Maximum 0.1 ppm, if intended for use in parenteral nutrition.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 2.0 g of the substance to be examined in *methyl isobutyl ketone R3* and dilute to 10.0 ml with the same solvent.

Solution A Dilute 0.100 ml of *tin liposoluble standard solution (1000 ppm Sn) R* to 10.0 ml with *methyl isobutyl ketone R3.*

Stock solution Dilute 0.100 ml of solution A to 10.0 ml with *methyl isobutyl ketone R3.*

Reference solutions Prepare 3 reference solutions by dissolving for each 2.0 g of the substance to be examined in the minimum volume of *methyl isobutyl ketone R3* , adding 1.0 ml, 2.0 ml and 4.0 ml, respectively, of stock solution and diluting to 10.0 ml with *methyl isobutyl ketone R3* .

Source Tin hollow-cathode lamp.

Wavelength 286.3 nm.

Atomic generator Graphite furnace coated inside with palladium carbide.

Carrier gas *argon R.*

Heavy metals (*2.4.8*)

Maximum 10 ppm, if intended for use other than parenteral nutrition.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 0.2 per cent, determined on 10.00 g.

Total ash *(2.4.16)*

Maximum 0.1 per cent, determined on 2.0 g.

STORAGE

In a well-filled container, protected from light.

LABELLING

The label states, where applicable, that the substance is intended for use in parenteral nutrition.

Ph Eur

Purified Talc

General Notices

(Talc, Ph Eur monograph 0438)

14807-96-6

Preparation

Talc Dusting Powder

Ph Eur

DEFINITION

Powdered, selected, natural, hydrated magnesium silicate. Pure talc has the formula $Mg_3Si_4O_{10}(OH)_2$ (M_r 379.3). It may contain variable amounts of associated minerals among which chlorites (hydrated aluminium and magnesium silicates), magnesite (magnesium carbonate), calcite (calcium carbonate) and dolomite (calcium and magnesium carbonate) are predominant.

PRODUCTION

Talc derived from deposits that are known to contain associated asbestos is not suitable for pharmaceutical use. The manufacturer is responsible for demonstrating by the test for amphiboles and serpentines that the product is free from asbestos. The presence of amphiboles and of serpentines is revealed by X-ray diffraction or by infrared spectrophotometry (see A and B). If detected, the specific morphological criteria of asbestos are investigated by a suitable method of optical microscopy to determine whether tremolite asbestos or chrysotile is present, as described below.

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs of *potassium bromide R* .

In the range 740 cm^{-1} to 760 cm^{-1} using scale expansion, any absorption band at 758 ± 1 cm^{-1} may indicate the presence of tremolite or of chlorite. If the absorption band remains after ignition of the substance to be examined at 850 ± 50 °C for at least 30 min, it indicates the presence of the tremolite. In the range 600 cm^{-1} to 650 cm^{-1} using scale expansion, any absorption band or shoulder may indicate the presence of serpentines.

B. X-ray diffraction.

Preparation Place the sample on the sample holder; pack and smooth its surface with a polished glass microscope slide.

Radiation Cu Kα monochromatic, 40 kV, 24-30 mA.

Incident slit 1°.

Detection slit 0.2°.

Goniometer speed 1/10° 2θ/min.

Scanning range 10-13° 2θ and 24-26° 2θ.

Sample Not oriented.

Results The presence of amphiboles is detected by a diffraction peak at 10.5 ± 0.1° 2θ, the presence of serpentines is detected by diffraction peaks at 24.3 ± 0.1° 2θ and at 12.1 ± 0.1° 2θ.

If, by one of the 2 methods, amphiboles and/or serpentine are detected, examine by a suitable method of optical microscopy to determine the asbestos character.

The presence of asbestos is shown if the following 2 criteria are met:

— a range of length to width ratios of 20:1 to 100:1, or higher for fibres longer than 5 µm;

— capability of splitting into very thin fibrils;

And if at least 2 of the following 4 criteria are met:

— parallel fibres occurring in bundles;

— fibre bundles displaying frayed ends;

— fibres in the form of thin needles;

— matted masses of individual fibres and/or fibres showing curvature.

CHARACTERS

Appearance

Light, homogeneous, white or almost white powder, greasy to the touch (non abrasive).

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in dilute solutions of acids and alkali hydroxides.

IDENTIFICATION

First identification *A*.

Second identification *B, C*.

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs of *potassium bromide R* .

Absorption bands At 3677 ± 2 cm-1, 1018 ± 2 cm-1 and 669 ± 2 cm-1.

B. In a platinum crucible, melt a mixture of 0.2 g of *anhydrous sodium carbonate R* and 2.0 g of *potassium carbonate R* . To the melted mass add 0.1 g of the substance to be examined and heat until the mixture is completely melted. Allow to cool and transfer the melted mass into an evaporating dish with 50 ml of hot *water R.* Add *hydrochloric acid R* until effervescence ceases. Add 10 ml of *hydrochloric acid R* and evaporate to dryness on a water-bath. Allow to cool. Add 20 ml of *water R,* heat to boiling and filter (the residue is used for identification test C). To 5 ml of the filtrate add 1 ml of *ammonia R* and 1 ml of *ammonium chloride solution R* and filter. To the filtrate add *1 ml of disodium hydrogen phosphate solution R* . A white, crystalline precipitate is formed.

C. The residue obtained in identification test B gives the reaction of silicates *(2.3.1)* .

TESTS

Solution S1

Weigh 10.0 g into a conical flask fitted with a reflux condenser, add 50 ml of *0.5 M hydrochloric acid* gradually while stirring and heat on a water-bath for 30 min. Allow to cool. Transfer the mixture to a beaker and allow the undissolved material to settle. Filter the supernatant through medium-speed filter paper into a 100 ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the residue and the beaker with 3 quantities, each of 10 ml, of hot *water R* . Wash the filter with 15 ml of hot *water R,* allow the filtrate to cool and dilute to 100.0 ml with the same solvent.

Solution S2

Perchlorates mixed with heavy metals are known to be explosive. Take proper precautions while performing this procedure. Weigh 0.5 g in a 100 ml polytetrafluoroethylene dish, add 5 ml of *hydrochloric acid R* , 5 ml of *lead-free nitric acid R* and 5 ml of *perchloric acid R .* Stir gently then add 35 ml of *hydrofluoric acid R* and evaporate slowly to dryness on a hot plate. To the residue, add 5 ml of *hydrochloric acid R* , cover with a watch-glass, heat to boiling and allow to cool. Rinse the watch-glass and the dish with *water R* . Transfer into a volumetric flask, rinse the dish with *water R* and dilute to 50.0 ml with the same solvent.

Acidity or alkalinity

Boil 2.5 g with 50 ml of *carbon dioxide-free water R* under reflux. Filter *in vacuo*. To 10 ml of the filtrate add 0.1 ml of *bromothymol blue solution R1* ; not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to green. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R1* ; not more than 0.3 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Water-soluble substances

Maximum 0.2 per cent.

To 10.0 g add 50 ml of *carbon dioxide-free water R* , heat to boiling and maintain boiling under a reflux condenser for 30 min. Allow to cool, filter through a medium-speed filter paper and dilute to 50.0 ml with *carbon dioxide-free water R.* Take 25.0 ml of the filtrate, evaporate to dryness and heat at 105 °C for 1 h. The residue weighs a maximum of 10 mg.

Aluminium

Maximum 2.0 per cent.

Atomic absorption spectrometry *(2.2.23, Method I).*

Test solution To 5.0 ml of solution S2 add 10 ml of a 25.34 g/l solution of *caesium chloride R* , 10.0 ml of *hydrochloric acid R* and dilute to 100.0 ml with *water R* .

Reference solutions Into 4 identical volumetric flasks, each containing 10.0 ml of hydrochloric acid R and 10 ml of a 25.34 g/l solution of *caesium chloride R* , introduce respectively 5.0 ml, 10.0 ml, 15.0 ml and 20.0 ml of *aluminium standard solution (100 ppm Al) R* and dilute to 100.0 ml with *water R.*

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Nitrous oxide-acetylene flame.

Calcium

Maximum 0.90 per cent.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution To 5.0 ml of solution S2 add 10.0 ml of *hydrochloric acid R* , 10 ml of *lanthanum chloride solution R* and dilute to 100.0 ml with *water R* .

Reference solutions Into 4 identical volumetric flasks, each containing 10.0 ml of *hydrochloric acid R* and 10 ml of *lanthanum chloride solution R,* introduce respectively 1.0 ml, 2.0 ml, 3.0 ml and 4.0 ml of *calcium standard solution (100 ppm Ca) R1* and dilute to 100.0 ml with *water R.*

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Nitrous oxide-acetylene flame.

Iron

Maximum 0.25 per cent.

Atomic absorption spectrometry *(2.2.23, Method I).*

Test solution To 2.5 ml of solution S1, add 50.0 ml of *0.5 M hydrochloric acid* and dilute to 100.0 ml with *water R.*

Reference solutions Into 4 identical volumetric flasks, each containing 50.0 ml of *0.5 M hydrochloric acid* , introduce respectively 2.0 ml, 2.5 ml, 3.0 ml and 4.0 ml of *iron standard solution (250 ppm Fe) R* and dilute to 100.0 ml with *water R* .

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Correction Deuterium lamp.

Lead

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use solution S1.

Reference solutions Into 4 identical volumetric flasks, each containing 50.0 ml of *0.5 M hydrochloric acid*, introduce respectively 5.0 ml, 7.5 ml, 10.0 ml and 12.5 ml of *lead standard solution (10 ppm Pb) R1* and dilute to 100.0 ml with *water R*.

Source Lead hollow-cathode lamp.

Wavelength 217.0 nm.

Atomisation device Air-acetylene flame.

Magnesium

17.0 per cent to 19.5 per cent.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dilute 0.5 ml of solution S2 to 100.0 ml with *water R*. To 4.0 ml of the solution, add 10.0 ml of *hydrochloric acid R*, 10 ml of *lanthanum chloride solution R* and dilute to 100.0 ml with *water R.*

Reference solutions Into 4 identical volumetric flasks, each containing 10.0 ml of *hydrochloric acid R* and 10 ml of *lanthanum chloride solution R*, introduce respectively 2.5 ml, 3.0 ml, 4.0 ml and 5.0 ml of *magnesium standard solution (10 ppm Mg) R1* and dilute to 100.0 ml with *water R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Air-acetylene flame.

Loss on ignition

Maximum 7.0 per cent, determined on 1.00 g by ignition to constant weight at 1050-1100 °C.

Microbial contamination

If intended for topical administration, the total viable aerobic count *(2.6.12)* is not more than a total of 10^2 bacteria and fungi per gram.

If intended for oral administration, the total viable aerobic count *(2.6.12)* is not more than 10^3 bacteria and not more than 10^2 fungi per gram.

LABELLING

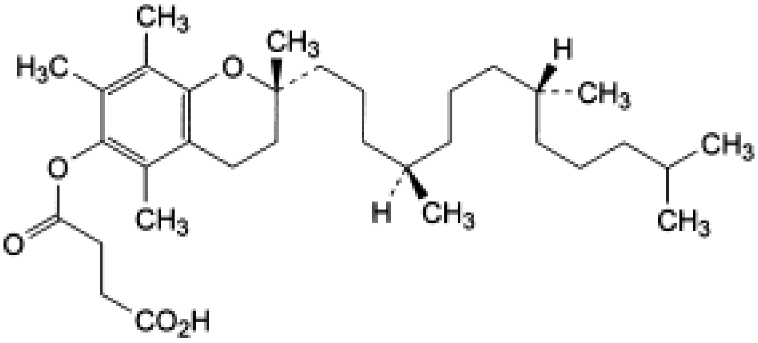
The label states, where applicable, that the substance is suitable for oral or topical administration.

Ph Eur

RRR -Alpha Tocopheryl Hydrogen Succinate

General Notices

(RRR-α-Tocopheryl Hydrogen Succinate, Ph Eur monograph 1259)



$C_{33}H_{54}O_5$ 530.8 *4345-03-3*

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Preparation

Alpha Tocopheryl Succinate Tablets

Ph Eur

DEFINITION

(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl hydrogen succinate.

Content

96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, soluble in acetone and in anhydrous ethanol.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Absorbance (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *RRR*-α-*tocopheryl hydrogen succinate CRS* .

C. Thin-layer chromatography *(2.2.27).*

Test solution (a) Dissolve 10 mg of the substance to be examined in 2 ml of *cyclohexane R* .

Test solution (b) In a ground-glass-stoppered tube, dissolve 10 mg of the substance to be examined in 2 ml of *2.5 M alcoholic sulphuric acid R* . Heat on a water-bath for 5 min. Cool and add 2 ml of *water R* and 2 ml of *cyclohexane R* . Shake for 1 min. Use the upper layer.

Reference solution (a) Dissolve 10 mg of *RRR*-α-*tocopheryl hydrogen succinate CRS* in 2 ml of *cyclohexane R* .

Reference solution (b) Prepare as described for test solution (b), using *RRR*-α-*tocopheryl hydrogen succinate CRS* instead of the substance to be examined.

Plate *TLC silica gel F_{254} plate R* .

Mobile phase *glacial acetic acid R, ether R, cyclohexane R* (0.2:20:80 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In a current of air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). In the chromatograms obtained with test solution (b) and reference solution (b), there are 2 spots: the spot with the higher R_F value is due to α-tocopherol, the spot with the lower R_F value is due to *RRR*-α-tocopheryl hydrogen succinate and corresponds to the spot obtained with reference solution (a). Depending on the degree of hydrolysis, the lower spot may be weak or even absent.

Detection B Spray with a mixture of 10 volumes of *hydrochloric acid R* , 40 volumes of a 2.5 g/l solution of *ferric chloride R* in *ethanol (96 per cent) R* and 40 volumes of a 10 g/l solution of *phenanthroline hydrochloride R* in *ethanol (96 per cent) R* .

Results B In the chromatograms obtained with test solution (b) and reference solution (b), the spot due to α-tocopherol is orange.

D. After saponification, the resulting *RRR*-α-tocopherol is dextrorotatory *(2.2.7)*. The specific optical rotation after oxidation to the quinone form is not less than + 24.

Carry out the test avoiding exposure to actinic light. Transfer 1.0 g to a round bottomed, ground-glass-stoppered, 250 ml flask, dissolve in 30 ml of *anhydrous ethanol R* and heat under reflux for 3 min. While the solution is boiling, add, through the condenser, 20 ml of *2 M alcoholic potassium hydroxide solution R*. Continue heating under reflux for 20 min and, without cooling, add 4.0 ml of *hydrochloric acid R* dropwise through the condenser. Cool, rinse the condenser with 10 ml of *anhydrous ethanol R*, transfer the contents of the flask to a 500 ml separating funnel, and rinse the flask with 4 quantities, each of 25 ml, of *water R* and 4 quantities, each of 25 ml, of *ether R*. Add the rinsings to the separating funnel. Shake vigorously for 2 min, allow the layers to separate and collect each of the 2 layers in individual separating funnels. Shake the aqueous layer with 2 quantities, each of 50 ml, of *ether R* and add these extracts to the main ether extract. Wash the combined ether extracts with 4 quantities, each of 100 ml, of *water R* and discard the washings.

To the ether solution add 40 ml of a 100 g/l solution of *potassium ferricyanide R* in an 8 g/l solution of *sodium hydroxide R* and shake for 3 min. Wash the ether solution with 4 quantities, each of 50 ml, of *water R,* discard the washings and dry the ether layer over *anhydrous sodium sulphate R .* Evaporate the ether on a water-bath under reduced pressure or in an atmosphere of nitrogen until a few millilitres remain, then complete the evaporation removing the last traces of ether without the application of heat. Immediately dissolve the residue in 25.0 ml of *trimethylpentane R* and determine the optical rotation.

Calculate the specific optical rotation of the substance in the test solution using as *c* the number of grams equivalent to α-tocopherol (factor 0.811) in 1000 ml.

TESTS

Absorbance *(2.2.25).*

Solution A Dissolve 0.150 g in *anhydrous ethanol R* and dilute to 100 ml with the same solvent.

Test solution (a) Dilute 10.0 ml of solution A to 100.0 ml with *anhydrous ethanol R*.

Test solution (b) Dilute 20.0 ml of solution A to 50.0 ml with *anhydrous ethanol R*.

Absorption maximum At 284 nm for test solution (a).

Absorption minimum At 254 nm for test solution (b).

Specific absorbance at the absorption maximum 35 to 38 for test solution (a).

Specific absorbance at the absorption minimum 6.0 to 8.0 for test solution (b).

Acid value *(2.5.1)*

101 to 108, determined on 1.00 g.

Free tocopherol

Maximum 1.0 per cent.

Dissolve 0.500 g in 100 ml of *0.25 M alcoholic sulphuric acid R*. Add 20 ml of *water R* and 0.1 ml of a 2.5 g/l solution of *diphenylamine R* in *sulphuric acid R*. Titrate with *0.01 M* *ammonium and cerium sulphate* until a blue colour is obtained that persists for at least 5 s. Carry out a blank titration.

1 ml of *0.01 M ammonium and cerium sulphate* is equivalent to 2.154 mg of free tocopherol.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

0.50 g complies with test D. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography *(2.2.28)* .

Internal standard solution Dissolve 0.300 g of *dotriacontane R* in *hexane R* and dilute to 100.0 ml with the same solvent.

Test solution Weigh 30.0 mg of the substance to be examined into a 20 ml vial. Add 2.0 ml of *methanol R*, 1.0 ml of *dimethoxypropane R* and 0.1 ml of *hydrochloric acid R*. Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 10.0 ml of the internal standard solution. Vortex into solution.

Reference solution Weigh 30.0 mg of *RRR-α-tocopheryl hydrogen succinate CRS* into a 20 ml vial. Add 2.0 ml of *methanol R*, 1.0 ml of *dimethoxypropane R* and 0.1 ml of *hydrochloric acid R*. Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 10.0 ml of the internal standard solution. Vortex into solution.

Column:

— *material:* fused silica;

— *size*: *l* = 15 m, Ø = 0.32 mm;

— *stationary phase: poly(dimethyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *Helium for chromatography R*.

Flow rate 3-6 ml/min.

Split ratio 1:10 to 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	200 → 250
	10 - 20	250
Injection port		300
Detector		330

Detection Flame ionisation.

Injection 1 µl; inject directly onto the column or via a glass-lined injection port using an automatic injection device or some other reproducible injection method.

System suitability Reference solution:

— *resolution*: minimum 12.0 between the peaks due to dotriacontane and *RRR*-α-tocopheryl hydrogen succinate.

Interference test Dissolve 0.100 g of the substance to be examined in *hexane R* and dilute to 50.0 ml with the same solvent. Inject 1 µl of the solution and record the chromatogram. If a peak is detected with the same retention time as that of the peak due to dotriacontane, calculate the area of this peak relative to the peak area of the substance to be examined. If the relative peak area is greater than 0.5 per cent, use the corrected peak area $S'_{D(corr)}$ for the final calculation.

$$S'_{\mathrm{D(corr)}} = S'_{\mathrm{D}} - \frac{S_{\mathrm{I}} \times S'_{\mathrm{T}}}{S_{\mathrm{TI}}}$$

S'_D = area of the peak due to dotriacontane in the chromatogram obtained with the test solution

S_I = area of the peak with the same retention time as that of the peak due to dotriacontane in the chromatogram obtained in the interference test

S'_T = area of the peak due to *RRR*-α-tocopheryl hydrogen succinate in the chromatogram obtained with the test solution

S_{TI} = area of the peak due to *RRR*-α-tocopheryl hydrogen succinate in the chromatogram obtained in the interference test

Measure the areas of the peaks due to *RRR-α-tocopheryl hydrogen succinate CRS* (S_T) and dotriacontane (S_D) in the chromatogram obtained with the reference solution and the areas of the peaks due to *RRR*-α-tocopheryl hydrogen succinate (S'_T) and dotriacontane (S'_D) in the chromatogram obtained with the test solution.

Determine the response factor (RF) for *RRR*-α-tocopheryl hydrogen succinate from the areas of the peaks due to *RRR*-α-*tocopheryl hydrogen succinate CRS* and dotriacontane in the chromatogram obtained with the reference solution, using the following expression:

$$\frac{S_{\mathrm{D}} \times m_{\mathrm{T}}}{S_{\mathrm{T}} \times m_{\mathrm{D}}}$$

Calculate the percentage content of *RRR*-α-tocopheryl hydrogen succinate using the following expression:

$$\frac{100 \times S'_{\mathrm{T}} \times m_{\mathrm{D}} \times \mathrm{RF}}{S'_{\mathrm{D(corr)}} \times m}$$

S_D = area of the peak due to dotriacontane in the chromatogram obtained with the reference solution

$S'_{D(corr)}$ = corrected area of the peak due to dotriacontane in the chromatogram obtained with the test solution

S_T = area of the peak due to *R RR*-α-*tocopheryl hydrogen succinate CRS* in the chromatogram obtained with the reference solution

S'_T = area of the peak due to *RRR*-α-tocopheryl hydrogen succinate in the chromatogram obtained with the test solution

m_D = mass of dotriacontane in the test solution and in the reference solution, in milligrams

m_T = mass of *RRR*-α-*tocopheryl hydrogen succinate CRS* in the reference solution, in milligrams

m = mass of the substance to be examined in the test solution, in milligrams

STORAGE

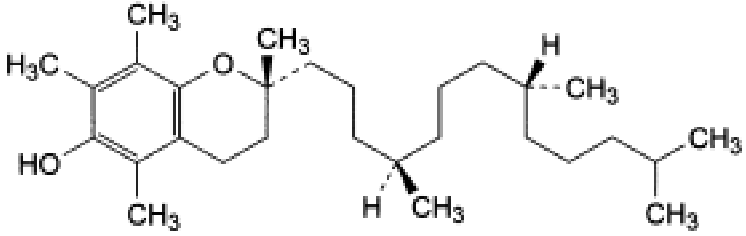
Protected from light.

Ph Eur

RRR -Alpha-Tocopherol

General Notices

(RRR-α-Tocopherol, Ph Eur monograph 1256)



$C_{29}H_{50}O_2$ 430.7 *59-02-9*

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol.

Content

94.5 per cent to 102.0 per cent.

CHARACTERS

Appearance

Clear, colourless or yellowish-brown, viscous, oily liquid.

Solubility

Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol, in methylene chloride and in fatty oils.

IDENTIFICATION

First identification A, B.

Second identification A, C.

A. Optical rotation *(2.2.7)* : + 0.05 ° to + 0.10 °.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *α-tocopherol CRS* .

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 10 mg of the substance to be examined in 2 ml of *cyclohexane R.*

Reference solution Dissolve 10 mg of *α-tocopherol CRS* in 2 ml of *cyclohexane R* .

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10.0 ml of the internal standard solution.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10 ml of *cyclohexane R* .

Reference solution (a) Dissolve 0.100 g of *α-tocopherol CRS* in 10.0 ml of the internal standard solution.

Reference solution (b) Dissolve 10 mg of *α-tocopherol R* and 10 mg of *α-tocopheryl acetate R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Column:

— *material:* fused silica;

— *size:* *l* = 30 m, Ø = 0.25 mm;

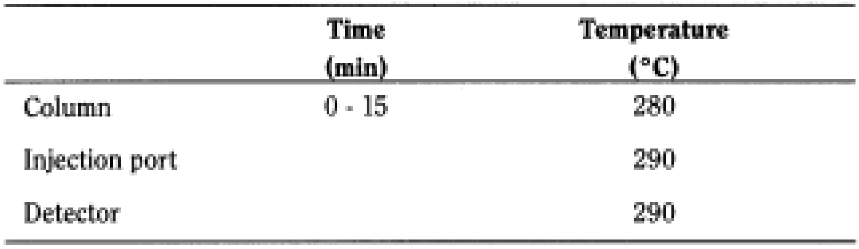
— *stationary phase: poly(dimethyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 15	280
Injection port		290
Detector		290

Detection Flame ionisation.

Injection 1 µl of test solution (b) and reference solution (b).

System suitability Reference solution (b):

— *resolution*: minimum 3.5 between the peaks due to α-tocopherol and α-tocopheryl acetate.

Limits:

— *total*: maximum 4.0 per cent;

— *disregard limit*: 0.1 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography *(2.2.28)* as described in the test for related substances with the following modification.

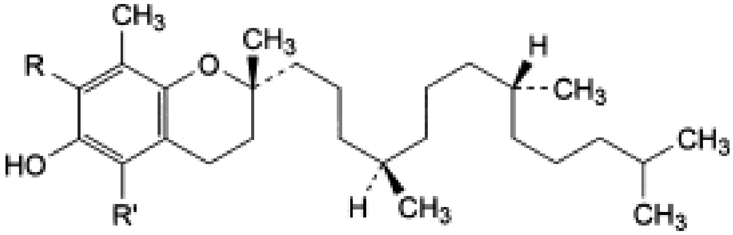
Injection Test solution (a) and reference solution (a).

Calculate the percentage content of $C_{29}H_{50}O_2$ taking into account the declared content of *α-tocopherol CRS.*

STORAGE

Under an inert gas, protected from light.

IMPURITIES



A. R = R′ = H: (2*R*)-2,8-dimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol (*RRR*-δ-tocopherol),

B. R = H, R′ = CH_3: (2*R*)-2,5,8-trimethyl-2-[(4*R*,8*R*)4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol (*RRR*-β-tocopherol),

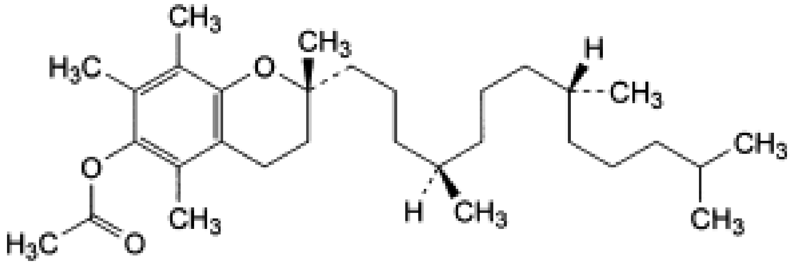
C. R = CH_3, R′ = H: (2*R*)-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol (*RRR*-γ-tocopherol).

Ph Eur

RRR -Alpha-Tocopheryl Acetate

General Notices

(RRR-α-Tocopheryl Acetate, Ph Eur monograph 1257)



$C_{31}H_{52}O_3$ 472.7

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate.

Content

95.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

Clear, colourless or slightly greenish-yellow, viscous, oily liquid.

Solubility

Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol and in fatty oils, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. Optical rotation *(2.2.7)*

+ 0.25° to + 0.35°.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *α-tocopheryl acetate CRS.*

C. Thin-layer chromatography *(2.2.27)* .

Test solution (a) Dissolve 10 mg of the substance to be examined in 2 ml of *cyclohexane R* .

Test solution (b) In a ground-glass-stoppered tube, dissolve about 10 mg of the substance to be examined in 2 ml of *2.5 M alcoholic sulphuric acid R.* Heat on a water-bath for 5 min. Cool and add 2 ml of *water R* and 2 ml of *cyclohexane R* . Shake for 1 min. Use the upper layer.

Reference solution (a) Dissolve 10 mg of *α-tocopheryl acetate CRS* in 2 ml of *cyclohexane R* .

Reference solution (b) Prepare as described for test solution (b), using *α-tocopheryl acetate CRS* instead of the substance to be examined.

Plate *TLC silica gel F_{254} plate R .*

Mobile phase *ether R, cyclohexane R* (20:80 V/V).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). In the chromatograms obtained with test solution (b) and reference solution (b), there may be 2 spots depending on the degree of hydrolysis: the spot with the higher R_F value is due to α-tocopheryl acetate and corresponds to the spot in the chromatogram obtained with reference solution (a); the spot with the lower R_F value is due to α-tocopherol.

TESTS

Related substances

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10.0 ml of the internal standard solution.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10 ml of *cyclohexane R.*

Reference solution (a) Dissolve 0.100 g of α*-tocopheryl acetate CRS* in 10.0 ml of the internal standard solution.

Reference solution (b) Dissolve 10 mg of α*-tocopherol R* and 10 mg of α*-tocopheryl acetate R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Column:

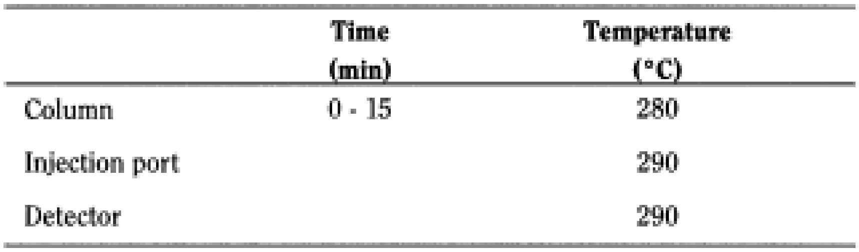
- *material*: fused silica;
- *size*: l = 30 m, Ø = 0.25 mm;
- *stationary phase: poly(dimethyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 15	280
Injection port		290
Detector		290

Detection Flame ionisation.

Injection 1 µl of test solution (b) and reference solutions (a) and (b); inject directly onto the column or via a sufficiently inert, glass-lined injection port using an automatic injection device or other reproducible injection method.

System suitability:

- *resolution*: minimum 3.5 between the peaks due to α-tocopherol and α-tocopheryl acetate in the chromatogram obtained with reference solution (b);
- in the chromatogram obtained with reference solution (a), the area of the peak due to α-tocopherol is not greater than 0.2 per cent of the area of the peak due to α-tocopheryl acetate.

Limits:

- *total*: maximum 4.0 per cent;
- *disregard limit*: 0.1 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography *(2.2.28)* as described in the test for related substances with the following modification.

Injection Test solution (a) and reference solution (a).

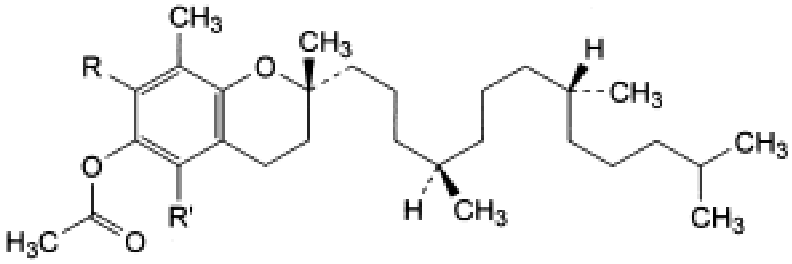
Calculate the percentage content of $C_{31}H_{52}O_3$ taking into account the declared content of *α-tocopheryl acetate CRS*.

STORAGE

Protected from light.

IMPURITIES

A. *RRR*-α-tocopherol,



B. R = H, R′ = CH_3: (2*R*)-2,5,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-β-tocopheryl acetate),

C. R = CH_3, R′ = H: (2*R*)-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-γ-tocopheryl acetate),

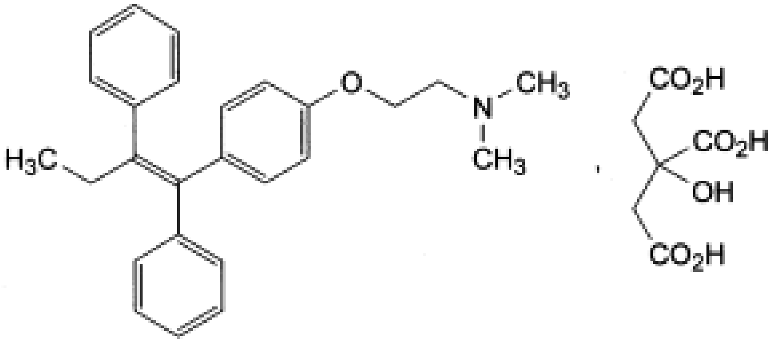
D. R = R′ = H: (2*R*)-2,8-dimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-δ-tocopheryl acetate).

Ph Eur

Tamoxifen Citrate

General Notices

(Ph Eur monograph 1046)



$C_{26}H_{29}NO,C_6H_8O_7$ 563.6 *54965-24-1*

Action and use

Selective estrogen receptor modulator.

Preparation

Tamoxifen Tablets

Ph Eur

DEFINITION

2-[4-[(*Z*)-1,2-Diphenylbut-1-enyl]phenoxy]-*N,N*-dimethylethanamine dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, soluble in methanol, slightly soluble in acetone.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 20 mg in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *methanol R* .

Spectral range 220-350 nm.

Absorption maxima At 237 nm and 275 nm.

Absorption ratio A_{237}/A_{275} = 1.45 to 1.65.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *tamoxifen citrate CRS* .

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R* , evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *tamoxifen citrate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *tamoxifen citrate CRS* and 10 mg of *clomifene citrate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *triethylamine R, toluene R* (10:90 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in

---

position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 4 ml of *pyridine R* with shaking and add 2 ml of *acetic anhydride R* ; a yellow colour is produced. Heat on a water-bath for 2 min; a pink or red colour is produced.

TESTS

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 15.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 15.0 mg of *tamoxifen citrate* for *performance test CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of *water R* containing 0.9 g/l of *sodium dihydrogen phosphate R* and 4.8 g/l of *N,N-dimethyloctylamine R*; adjust to pH 3.0 with *phosphoric acid R* .

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 240 nm.

Equilibration With the mobile phase for about 30 min.

Injection 10 µl.

Run time Twice the retention time of tamoxifen.

System suitability Reference solution (a):

— the chromatogram obtained is similar to the chromatogram supplied with *tamoxifen citrate for performance test CRS* ; there is baseline separation of the peak due to impurity F from the following peak of the principal component;

— *resolution*: minimum 3 between the peaks due to impurities A and F.

Limits:

— *impurities A, B, C, D, E, F, G, H*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *sum of impurities other than A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit:* 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the citrate (retention time = about 2.5 min).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 65 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

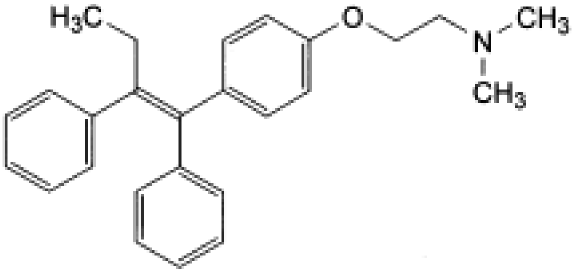
ASSAY

Dissolve 0.400 g in 75 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* using 0.1 ml of *naphtholbenzein solution R* as indicator.

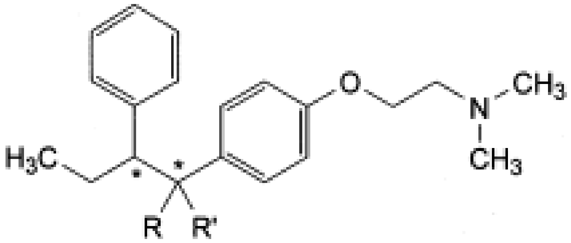
1 ml of *0.1 M perchloric acid* is equivalent to 56.36 mg of $C_{32}H_{37}NO_8$.

IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H.*

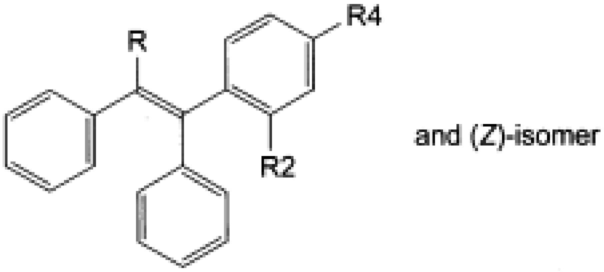


A. 2-[4-[(*E*)-1,2-diphenylbut-1-enyl]phenoxy]-*N,N*-dimethylethanamine ((*E*)-isomer),



B. R = OH, R′ = C_6H_5: 1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenylbutan-1-ol,

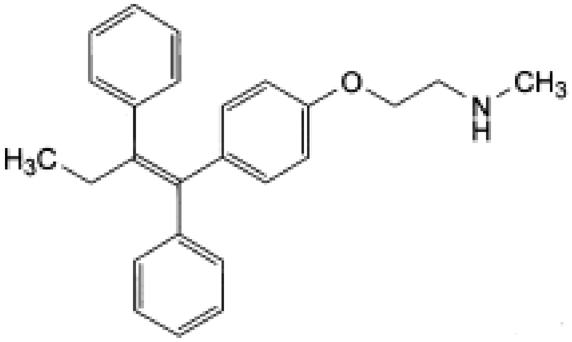
G. R + R′ = *O*: (2*RS*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenylbutan-1-one,



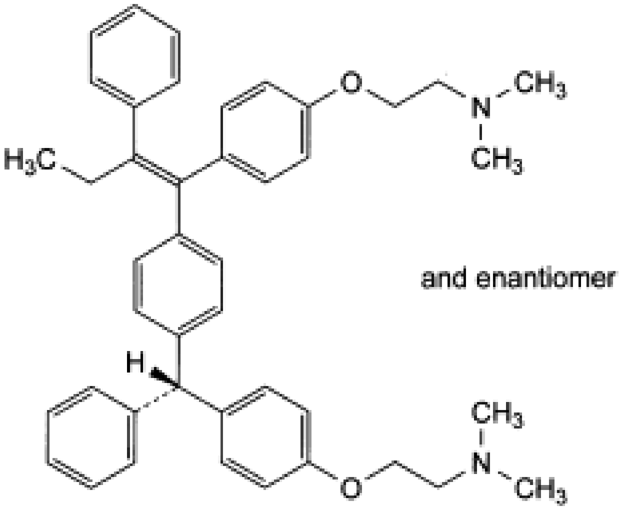
C. R = R2 = H, R4 = *O*-CH_2-CH_2-$N(CH_3)_2$: 2-[4-[(*EZ)*-1,2-diphenylethenyl]phenoxy]-*N,N*-dimethylethanamine,

D. R = CH_3, R2 = H, R4 = *O*-CH_2-CH_2-$N(CH_3)_2$: 2-[4-[(*EZ*)-1,2-diphenylprop-1-enyl]phenoxy]-N,N-dimethylethanamine,

E. R = C_2H_5, R2 = O-CH_2-CH_2-$N(CH_3)_2$, R4 = H: 2-[2-[(*EZ*)-1,2-diphenylbut-1-enyl]phenoxy]-N,N-dimethylethanamine,



F. 2-[4-[(*Z)*-1,2-diphenylbut-1-enyl]phenoxy]-*N*-methylethanamine,


H3C
O
N
CH3
CH3
and enantiomer
H
O
N
CH3
CH3

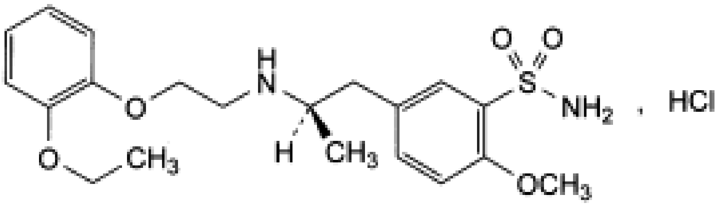
H. 2-[4-[(*Z)*-1-[4-[(*RS*)-[4-[2-(dimethylamino)ethoxy]phenyl]phenylmethyl]phenyl]-2-phenylbut-1-enyl]phenoxy]-*N,N*-dimethylethanamine.

Ph Eur

Tamsulosin Hydrochloride

General Notices

(Ph Eur monograph 2131)



$C_{20}H_{28}N_2O_5S,HCl$ 445.0 *106463-17-6*

Action and use

Alpha-1-adrenoceptor antagonist.

Ph Eur

DEFINITION

5-[(2*R*)-2-[[2-(2-Ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in formic acid, slightly soluble in anhydrous ethanol.

mp

About 230 °C.

IDENTIFICATION

Carry out either tests A, C, D or tests A, B, D.

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *tamsulosin hydrochloride CRS*.

B. Specific optical rotation *(2.2.7)* : - 17.5 to - 20.5 (dried substance).

Dissolve with heating 0.15 g in *water R* and dilute to 20.0 ml with the same solvent.

C. Enantiomeric purity (see Tests).

D. Dissolve with heating 0.75 g in *water R* and dilute to 100.0 ml with the same solvent. Take 5 ml of the solution and cool in an ice-bath. Add 3 ml of *dilute nitric acid R* and shake. Allow to stand at room temperature for 30 min and filter. The filtrate gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Related substances.

A. Impurities eluting before tamsulosin. Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 4 mg of *tamsulosin impurity D CRS* and 4 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 4 mg of *tamsulosin impurity H CRS* and 4 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Dissolve 3.0 g of *sodium hydroxide R* in a mixture of 8.7 ml of *perchloric acid R* and 1.9 litres of *water R* ; adjust to pH 2.0 with *0.5 M sodium hydroxide* and dilute to 2 litres with *water R* ; to 1.4 litres of this solution, add 600 ml of *acetonitrile R* .

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl of the test solution and reference solutions (a) and (b).

Run time 1.5 times the retention time of tamsulosin (retention time = about 6 min).

System suitability Reference solution (b):

— *resolution*: minimum 6 between the peaks due to impurity D and tamsulosin.

Limits:

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

B. Impurities eluting after tamsulosin. Liquid chromatography *(2.2.29)* as described in test A with the following modifications.

Mobile phase Dissolve 3.0 g of *sodium hydroxide R* in a mixture of 8.7 ml of *perchloric acid R* and 1.9 litres of *water R* ; adjust to pH 2.0 with *0.5 M sodium hydroxide* and dilute to 2 litres with *water R* ; add 2 litres of *acetonitrile R* .

Flow rate 1.0 ml/min.

Injection 10 µl of the test solution and reference solutions (a) and (c).

Run time 5 times the retention time of tamsulosin (retention time = about 2.5 min).

System suitability Reference solution (c):

— *resolution*: minimum 2 between the peaks due to tamsulosin and impurity H.

Limits:

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities eluting before tamsulosin in test A and after tamsulosin in test B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Enantiomeric purity

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R* . Dilute 1.0 ml of this solution to 10.0 ml with *methanol R* .

Reference solution (b) Dissolve 5.0 mg of *tamsulosin racemate CRS* in *methanol R* and dilute to 25.0 ml with the same solvent. Dilute 2.0 ml of this solution to 10.0 ml with *methanol R* .

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *silica gel OD for chiral separations R*;

— *temperature*: 40 °C.

Mobile phase d*iethylamine R, methanol R, anhydrous ethanol R, hexane R* (1:150:200:650 *V/V/V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl.

Relative retention With reference to tamsulosin (retention time = about 14 min): impurity G = about 0.8.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to impurity G and tamsulosin.

Limit:

— *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R* .

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

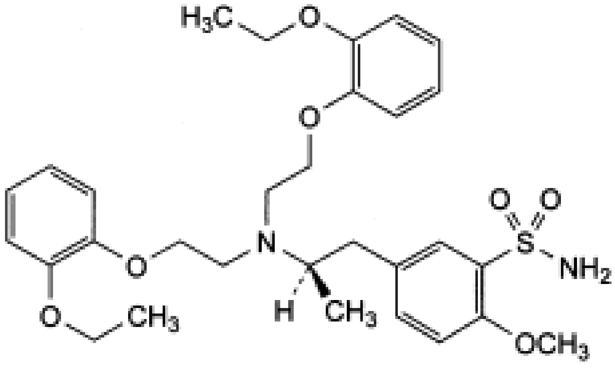
Dissolve 0.350 g in 5.0 ml of *anhydrous formic acid R* , add 75 ml of a mixture of 2 volumes of *acetic anhydride R* and 3 volumes of *glacial acetic acid R* . Titrate immediately with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* . Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 44.50 mg of $C_{20}H_{29}ClN_2O_5S$.

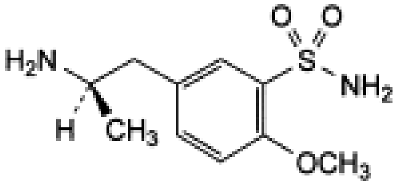
IMPURITIES

Specified impurities G.

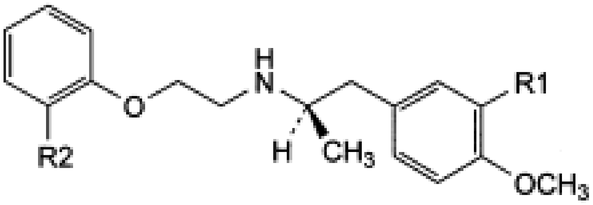
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, D, E, F, H, I.*



A. 5-[(2*R*)-2-[bis[2-(2-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide,



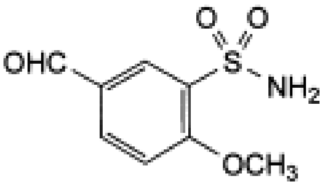
B. 5-[(2*R*)-2-aminopropyl]-2-methoxybenzenesulfonamide,



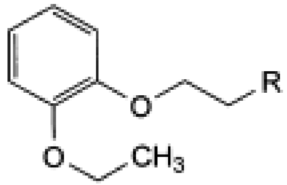
C. R1 = SO_2-NH_2, R2 = H: 2-methoxy-5-[(2*R*)-2-[(2-phenoxyethyl)amino]propyl] benzenesulfonamide,

D. R1 = SO_2-NH_2, R2 = OCH_3: 2-methoxy-5-[(2*R*)-2-[[2-(2-methoxyphenoxy)ethyl]amino] propyl]benzenesulfonamide,

H. R1 = H, R2 = OC_2H_5: (2*R*)-*N*-[2-(2-ethoxyphenoxy)ethyl]-1-(4-methoxyphenyl)propan-2-amine,

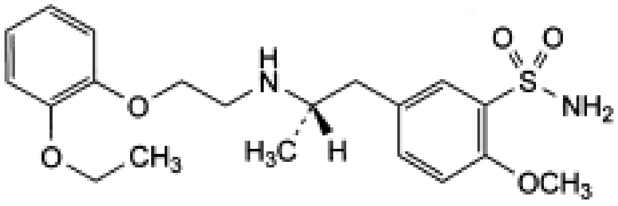


E. 5-formyl-2-methoxybenzenesulfonamide,



F. R = NH_2: 2-(2-ethoxyphenoxy)ethanamine,

I. R = Br: 1-(2-bromoethoxy)-2-ethoxybenzene,



G. 5-[(2*S*)-2-[[2-(2-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide.

Ph Eur

Tannic Acid

General Notices

(Ph Eur monograph 1477)

1401-55-4

Action and use

Astringent.

Ph Eur

DEFINITION

Mixture of esters of glucose with gallic acid and 3-galloylgallic acid.

CHARACTERS

Appearance

Yellowish-white or slightly brown amorphous light powder or shiny plates.

Solubility

Very soluble in water, freely soluble in acetone, in ethanol (96 per cent) and in glycerol (85 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Dilute 0.1 ml of solution S (see Tests) to 5 ml with *water R* . Add 0.1 ml of *ferric chloride solution R1* . A blackish-blue colour is produced which becomes green on the addition of 1 ml of *dilute sulphuric acid R* .

B. To 1 ml of solution S, add 3 ml of a 1 g/l solution of *gelatin R* . The mixture becomes turbid and a flocculent precipitate is formed.

C. Dilute 0.1 ml of solution S to 5 ml with *water R.* Add 0.3 ml of *barium hydroxide solution R* . A greenish-blue precipitate is formed.

TESTS

Solution S

Dissolve 4.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* .

Dextrins, gum, salts, sugars

To 2 ml of solution S, add 2 ml of *ethanol (96 per cent) R* . The solution is clear. Add 1 ml of *ether R* . The solution remains clear for at least 10 min.

Resins

To 5 ml of solution S, add 5 ml of *water R.* The mixture remains clear *(2.2.1)* for at least 15 min.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 0.200 g by drying at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

STORAGE

Protected from light.

Ph Eur

Tar

General Notices

Action and use

Used in treatment of psoriasis.

DEFINITION

Tar is bituminous liquid obtained from the wood of various trees of the family Pinaceae by destructive distillation and is known in commerce as Stockholm Tar.

CHARACTERISTICS

Dark brown or nearly black, semi-liquid; denser than water.

Soluble in *ethanol (90%)*, in *ether* and in fixed and volatile oils.

IDENTIFICATION

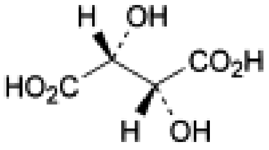
A. The aqueous liquid obtained by shaking 1 g with 20 ml of *water* for 5 minutes is acidic to *litmus paper* .

B. Carefully add 0.5 g to 10 ml of *petroleum spirit (boiling range, 40° to 60°)* and allow to stand for 30 minutes. When examined in daylight no fluorescence is produced.

Tartaric Acid

General Notices

(Ph Eur monograph 0460)



$C_4H_6O_6$ 150.1 *87-69-4*

Ph Eur

DEFINITION

(2*R*,3*R*)-2,3-Dihydroxybutanedioic acid.

Content

99.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is strongly acid *(2.2.4)* .

B. It gives the reactions of tartrates *(2.3.1)* .

TESTS

Solution S

Dissolve 5.0 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

+ 12.0 to + 12.8 (dried substance)

Dissolve 5.00 g in *water R* and dilute to 25.0 ml with the same solvent.

Oxalic acid

Maximum 350 ppm, calculated as anhydrous oxalic acid.

Dissolve 0.80 g in 4 ml of *water R* . Add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules and boil for 1 min. Allow to stand for 2 min. Collect the liquid in a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of a 50 g/l solution of *potassium ferricyanide R* . Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 4 ml of a 0.1 g/l solution of *oxalic acid R* .

Chlorides *(2.4.4)*

Maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R* .

Sulphates *(2.4.13)*

Maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R* .

Calcium *(2.4.3)*

Maximum 200 ppm.

To 5 ml of solution S add 10 ml of a 50 g/l solution of *sodium acetate R* in distilled *water R* .

Heavy metals *(2.4.8)*

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.2 per cent, determined on 1.000 g by drying in an oven at 105 °C

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.650 g in 25 ml of *water R*. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

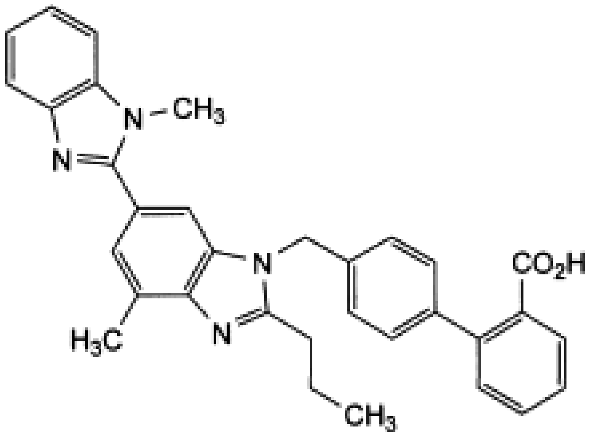
1 ml of *1 M sodium hydroxide* is equivalent to 75.05 mg of $C_4H_6O_6$.

Ph Eur

Telmisartan

General Notices

(Ph Eur monograph 2154)



$C_{33}H_{30}N_4O_2$ 514.6 *144701-48-4*

Action and use

Angiotensin II (AT_1) receptor antagonist.

Ph Eur

DEFINITION

4′-[[4-Methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl] biphenyl-2-carboxylic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in methanol, sparingly soluble in methylene chloride. It dissolves in 1 M sodium hydroxide.

It shows polymorphism *(5.9)* .

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *telmisartan CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in hot *anhydrous ethanol R* , evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution Y_4 *(2.2.2, Method II)*.

Dissolve 0.5 g in *1 M sodium hydroxide* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution To 25 mg of the substance to be examined add about 5 ml of *methanol R* and 100 µl of a 40 g/l solution of *sodium hydroxide R* . Dissolve with the aid of ultrasound and dilute to 50 ml with *methanol R* .

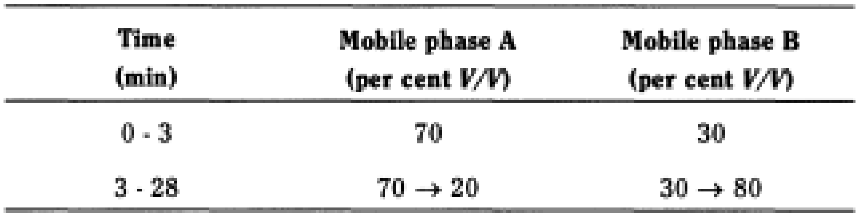
Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with *methanol R* . Dilute 1.0 ml of this solution to 100.0 ml with *methanol R* .

Reference solution (b) Dissolve the contents of a vial of *telmisartan for system suitability CRS* (containing impurities A, B, C, E and F) in 2 ml of *methanol R* .

Reference solution (c) To 5 mg of *telmisartan for peak identification CRS* (containing impurity D) add about 5 ml of *methanol R* and 100 µl of a 40 g/l solution of *sodium hydroxide R* . Dissolve with the aid of ultrasound and dilute to 10 ml with *methanol R* .

Column:
- *size:* *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm;
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: dissolve 2.0 g of *potassium dihydrogen phosphate R* and 3.8 g of *sodium pentanesulphonate monohydrate R1* in *water R* , adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R* ;
- *mobile phase B*: *methanol R2* , *acetonitrile R1* (20:80 *V/V*);



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 3	70	30
3 - 28	70 → 20	30 → 80

Flow rate 1 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *telmisartan for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, E and F; use the chromatogram supplied with *telmisartan for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to telmisartan (retention time = about 12 min): impurity A = about 0.2; impurity E = about 0.6; impurity F = about 0.7; impurity B = about 0.9; impurity C = about 1.5; impurity D = about 1.6.

System suitability Reference solution (b):

— the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *telmisartan for system suitability CRS*;

— *resolution*: minimum 3.0 between the peaks due to impurity B and telmisartan.

Limits:

— *impurities C, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities A, B*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

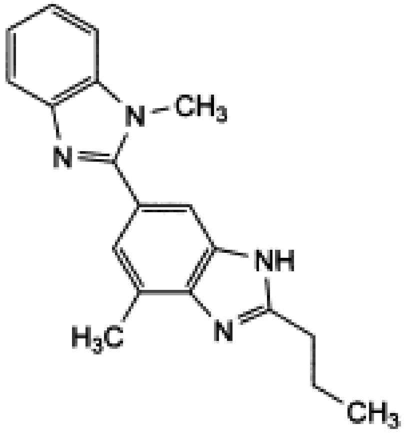
Dissolve 0.190 g in 5 ml of *anhydrous formic acid R* . Add 75 ml of *acetic anhydride R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 25.73 mg of $C_{33}H_{30}N_4O_2$.

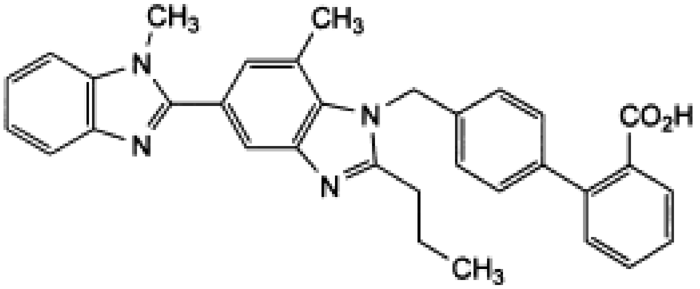
IMPURITIES

Specified impurities *A, B, C, D.*

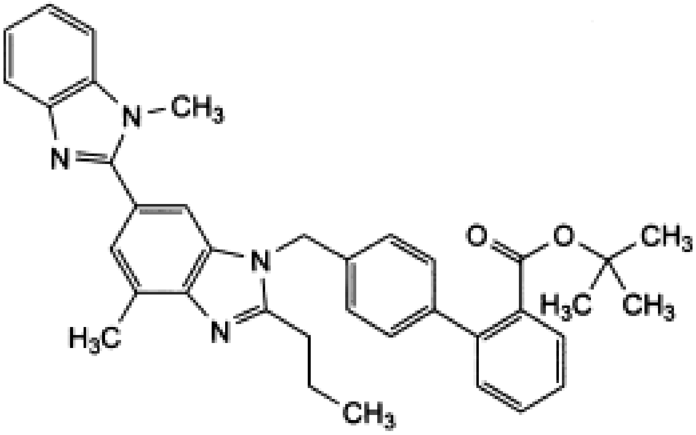
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, F, G, H.*



A. 4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazole,

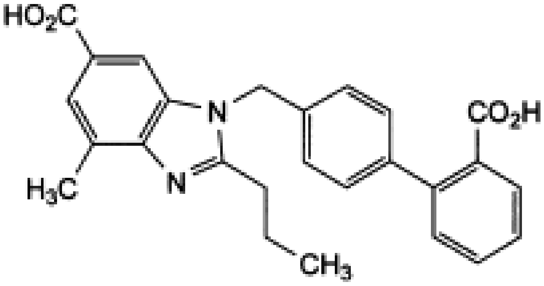


B. 4′-[[7-methyl-5-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl] biphenyl-2-carboxylic acid,

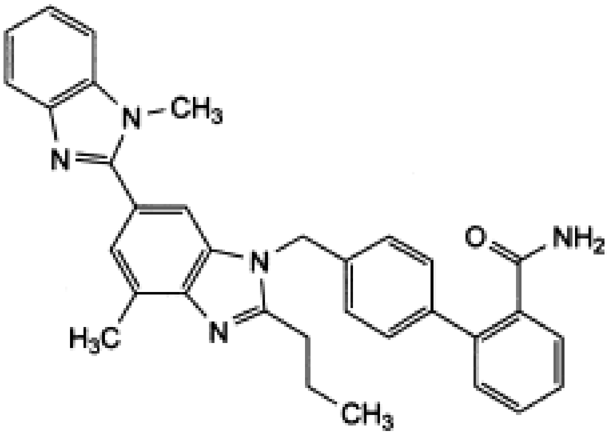


C. 1,1-dimethylethyl 4′-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl]biphenyl-2-carboxylate,

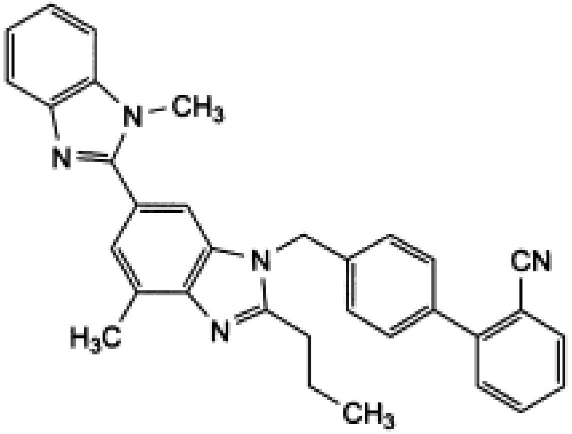
D. unidentified impurity,



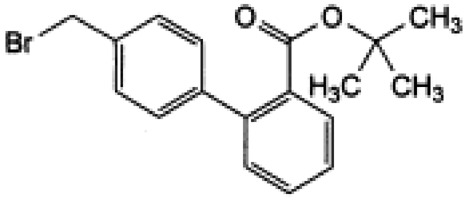
E. 1-[(2′-carboxybiphenyl-4-yl)methyl]-4-methyl-2-propyl-1*H*-benzimidazol-6-carboxylic acid,



F. 4′-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl] biphenyl-2-carboxamide,


N
CH₃
N
N
CN
H₃C
N
CH₃

G. 4′-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl] biphenyl-2-carbonitrile,


Br
O
O
CH₃
H₃C
CH₃

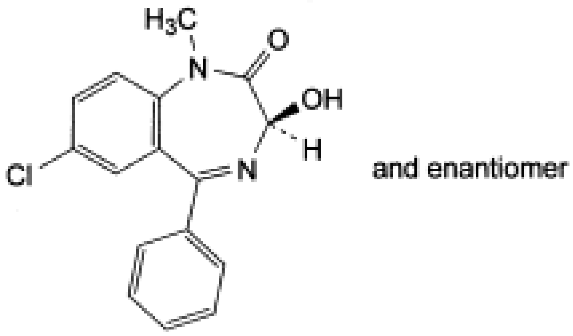
H. 1,1-dimethylethyl 4′-(bromomethyl)biphenyl-2-carboxylate.

Ph Eur

Temazepam

General Notices

(Ph Eur monograph 0954)



$C_{16}H_{13}ClN_2O_2$ 300.7 *846-50-4*

Action and use

Benzodiazepine.

Preparations

Temazepam Oral Solution

Temazepam Tablets

Ph Eur

DEFINITION

(3*RS*)-7-Chloro-3-hydroxy-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison temazepam CRS.

TESTS

Impurity A

Maximum 0.05 per cent.

Dissolve 0.400 g in *methylene chloride R* and dilute to 20.0 ml with the same solvent. The absorbance *(2.2.25)* is not greater than 0.30 at 409 nm.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 1 volume of *water R* and 9 volumes of *methanol R* . Dilute 2.0 ml of this solution to 10.0 ml with a mixture of 1 volume of *water R* and 9 volumes of *methanol R.*

Reference solution (b) Dissolve 1 mg of *oxazepam R* , 1 mg of *temazepam impurity F CRS* and 1 mg of *temazepam impurity G CRS* in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 25 ml with the same mixture of solvents.

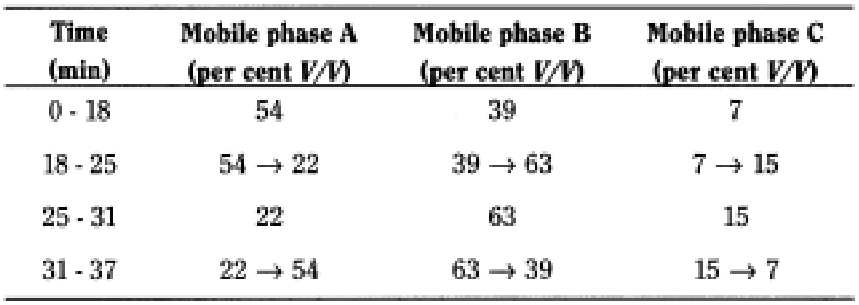
Reference solution (c) Dissolve 1 mg of *temazepam impurity C CRS* and 1 mg of *temazepam impurity D CRS* with a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 25 ml with the same mixture of solvents.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:

- *mobile phase A*: solution containing 4.9 g/l of *sodium dihydrogen phosphate R* and 0.63 g/l of *disodium hydrogen phosphate R* (pH 5.6);
- *mobile phase B: methanol R;*
- *mobile phase C: acetonitrile R;*



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)	Mobile phase C (per cent V/V)
0 - 18	54	39	7
18 - 25	54 → 22	39 → 63	7 → 15
25 - 31	22	63	15
31 - 37	22 → 54	63 → 39	15 → 7

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µl.

Relative retention With reference to temazepam (retention time = about 16 min): impurity E = about 0.55; impurity F = about 0.67; impurity G = about 0.73; impurity B = about 0.8; impurity D = about 1.2; impurity C = about 1.3; impurity A = about 1.5.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity F and impurity G;

— *peak-to-valley ratio* : minimum 1.7, where H_r = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity F = 3.2; impurity G = 3.1;

— *impurities B, C, D, E, F, G:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total:* not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *nitroethane R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* .

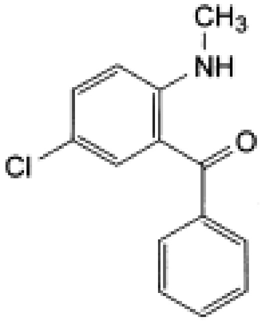
1 ml of *0.1 M perchloric acid* is equivalent to 30.07 mg of $C_{16}H_{13}ClN_2O_2$.

STORAGE

Protected from light.

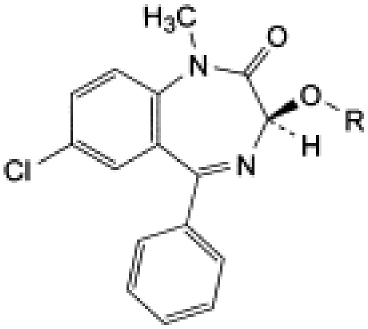
IMPURITIES

Specified impurities   A, B, C, D, E, F, G.



A. [5-chloro-2-(methylamino)phenyl]phenylmethanone,

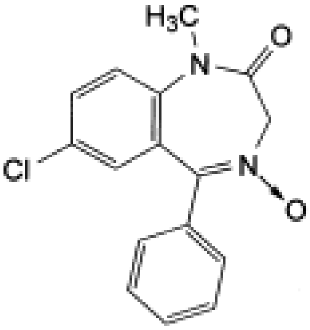
B. oxazepam,



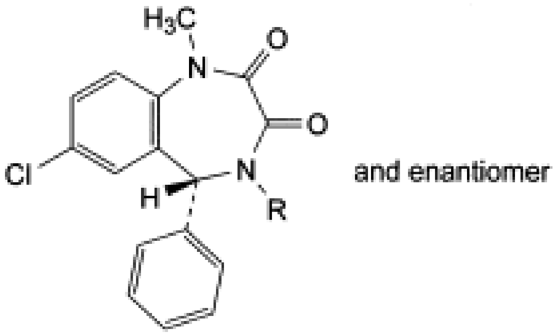
and enantiomer

C. R = CO-CH_3: (3*RS*)-7-chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl acetate,

D. R = CH_3: (3*RS*)-7-chloro-3-methoxy-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



E. 7-chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide,


H$_3$C
N
O
O
Cl
N
H
R
and enantiomer

F. R = H: (5*RS*)-7-chloro-1-methyl-5-phenyl-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione,

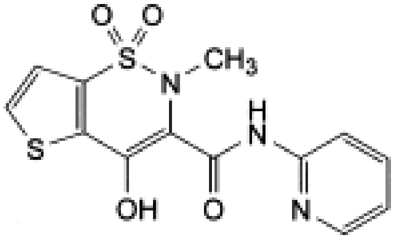
G. R = CH_3: (5*RS*)-7-chloro-1,4-dimethyl-5-phenyl-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione.

Ph Eur

Tenoxicam

General Notices

(Ph Eur monograph 1156)



$C_{13}H_{11}N_3O_4S_2$ 337.4 *59804-37-4*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations

Tenoxicam Injection

Tenoxicam Tablets

Ph Eur

DEFINITION

Tenoxicam contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-thieno[2,3-*e*]1,2-thiazine-3-carboxamide 1,1-dioxide, calculated with reference to the anhydrous substance.

CHARACTERS

A yellow, crystalline powder, practically insoluble in water, sparingly soluble in methylene chloride, very slightly soluble in ethanol. It dissolves in solutions of acids and alkalis.

It shows polymorphism (*5.9*).

IDENTIFICATION

Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *tenoxicam CRS* . If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum quantity of *methylene chloride R,* evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS

Appearance of solution

Dissolve 0.10 g in 20 ml of *methylene chloride R*. The solution is clear *(2.2.1)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution Dissolve 0.4 g in a mixture of 4 volumes of *concentrated ammonia R* and 96 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (a) Dilute 1 ml of the test solution to 20 ml with a mixture of 4 volumes of *concentrated ammonia R* and 96 volumes of *methanol R*. Dilute 1 ml of the solution to 20 ml with the same mixture of solvents.

Reference solution (b) Dissolve 20 mg of *tenoxicam CRS* and 20 mg of *salicylic acid CRS* in a mixture of 4 volumes of *concentrated ammonia R* and 96 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution (c) Dissolve 20 mg of *pyridin-2-amine R* in a mixture of 4 volumes of *concentrated ammonia R* and 96 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents. Dilute 2 ml of the solution to 50 ml with the same mixture of solvents.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm with a mixture of 5 volumes of *anhydrous formic acid R,* 5 volumes of *methanol R,* 20 volumes of *acetone R* and 70 volumes of *methylene chloride R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot corresponding to pyridin-2-amine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent). Any spot in the chromatogram obtained with the test solution apart from the principal spot and any spot corresponding to pyridin-2-amine, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Heavy metals *(2.4.8)*

0.5 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 5 ml of *lead standard solution (2 ppm Pb) R*.

Water *(2.5.12)*

Not more than 0.5 per cent, determined on 1.000 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

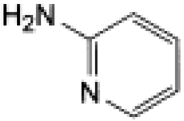
Dissolve 0.250 g in 5 ml of *anhydrous formic acid R* . Add 70 ml of *anhydrous acetic acid R* . Titrate with *0.1 M perchloric acid ,* determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 33.74 mg of $C_{13}H_{11}N_3O_4S_2$.

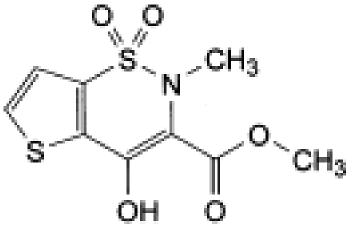
STORAGE

Store protected from light.

IMPURITIES



A. pyridin-2-amine,



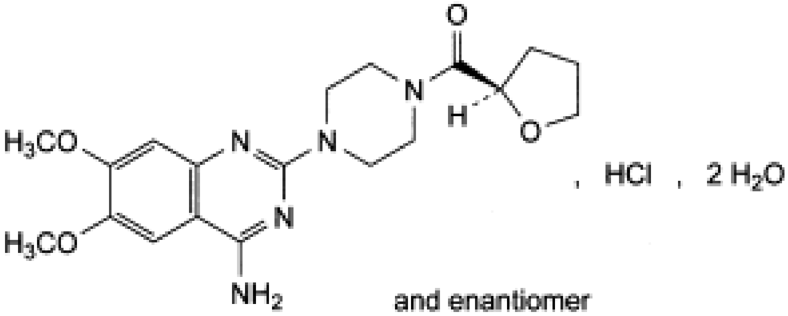
B. methyl 4-hydroxy-2-methyl-2*H*-thieno[2,3-*e*]1,2-thiazine-3-carboxylate 1,1-dioxide.

Ph Eur

Terazosin Hydrochloride Dihydrate

General Notices

(Ph Eur monograph 2021)



$C_{19}H_{25}N_5O_4,HCl,2H_2O$ 459.9 *70024-40-7*

Action and use

Alpha$_1$-adrenoceptor antagonist.

Ph Eur

DEFINITION

1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-4-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine hydrochloride dihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *terazosin hydrochloride dihydrate CRS*.

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Dilute 10 ml of solution S to 20 ml with *water R* .

pH *(2.2.3)*

3.0 to 5.0 for solution S.

Impurities N and O

Liquid chromatography *(2.2.29)* .

Solvent mixture *acetonitrile R1, water R* (20:80 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *terazosin impurity A CRS* and 5.0 mg of *terazosin impurity N CRS* in *acetonitrile R1* using sonication, add 5.0 ml of the test solution and dilute to 50.0 ml with *acetonitrile R1* . Dilute 10.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 10.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 25 °C.

Mobile phase Dissolve 2.80 g of *sodium laurilsulfate R* in 1000.0 ml of *water R* and add 11.0 ml of a solution containing 202.4 g/l of *triethylamine R* and 230.0 g/l of *phosphoric acid R* ; adjust to pH 2.5 with *phosphoric acid R* ; mix 600 volumes of this solution with 400 volumes of *acetonitrile R1* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Run time 4 times the retention time of terazosin.

Relative retention With reference to terazosin (retention time = about 10 min): impurity O = about 0.2; impurity N = about 0.3; impurity A = about 0.4.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurities A and N.

Limits:

— *impurity N*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity O*: not more than the area of the peak due to terazosin in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *terazosin for system suitability CRS* (containing impurities A, B, C, J, K and M) in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *terazosin impurity L CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (d) To 5 mg of *terazosin impurity E CRS*, add 70 ml of *methanol R* and 30 ml of *water R* . Allow to stand for at least 1 h to dissolve the substance. Use sonication if necessary.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 2 volumes of *triethylamine R* , 350 volumes of *acetonitrile R* , and 1650 volumes of a solution containing 6 g/l of *sodium citrate R* and 14.25 g/l of *anhydrous citric acid R* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 245 nm.

Injection 20 µl.

Run time 4 times the retention time of terazosin.

Identification of impurities Use the chromatogram supplied with *terazosin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, J, K and M; use the chromatograms obtained with reference solutions (c) and (d) to identify the peaks due to impurities L and E respectively.

Retention time Terazosin = about 11 min.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurities B and J; if necessary, adjust the proportion of the aqueous component in the mobile phase (an increase in the proportion of the aqueous component increases the retention times);

— the chromatogram obtained is similar to the chromatogram supplied with *terazosin for system suitability CRS*; in case of insufficient separation of the impurities, reduce the amount of triethylamine in the mobile phase.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.7; impurity M = 1.6;

— *impurities A, C, E, K*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity L*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *impurities B, J, M*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm) Pb R*.

Water *(2.5.12)*

7.0 per cent to 8.6 per cent, determined on 0.200 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *methanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 42.39 mg of $C_{19}H_{26}ClN_5O_4$.

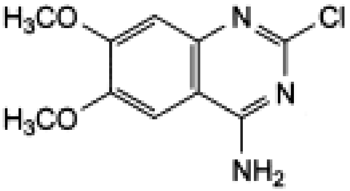
STORAGE

Protected from light.

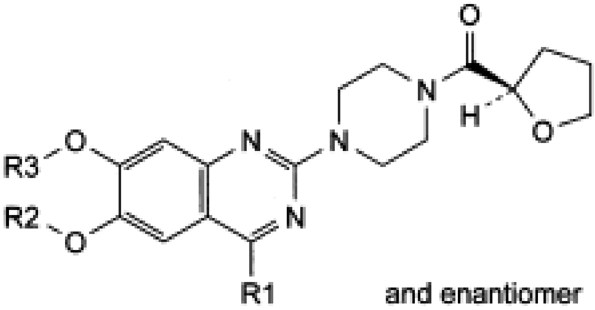
IMPURITIES

Specified impurities *A, B, C, E, J, K, L, M, N, O*.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D, F, G, H, I.*



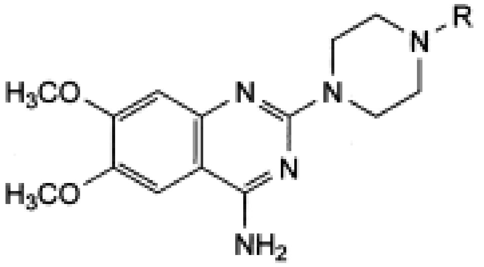
A. 2-chloro-6,7-dimethoxyquinazolin-4-amine,



B. R1 = OH, R2 = R3 = CH_3: 1-(4-hydroxy-6,7-dimethoxyquinazolin-2-yl)-4-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine,

G. R1 = NH_2, R2 = H, R3 = CH_3: 1-(4-amino-6-hydroxy-7-methoxyquinazolin-2-yl)-4-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine,

H. R1 = NH_2, R2 = CH_3, R3 = H: 1-(4-amino-7-hydroxy-6-methoxyquinazolin-2-yl)-4-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine,

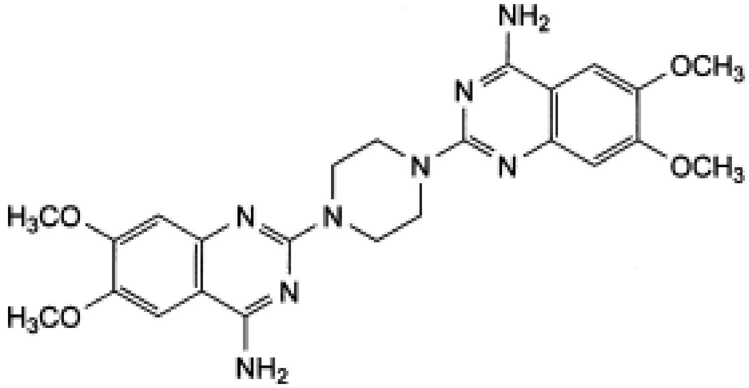


C. R = H: 6,7-dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine,

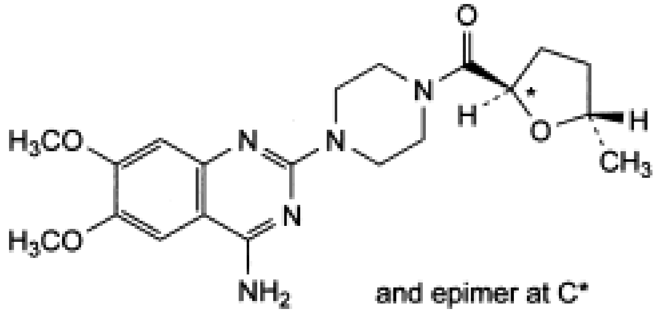
D. R = CHO: 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-formylpiperazine,

F. R = CO-$[CH_2]_4$-OH: 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-(5-hydroxypentanoyl) piperazine,

J. R = CO-CH(OH)-CH_2-CH_2-CH_3: 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-[(2*RS*)-2-hydroxypentanoyl]piperazine,

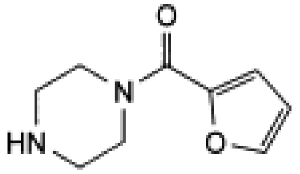


E. 2,2′-(piperazine-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine),

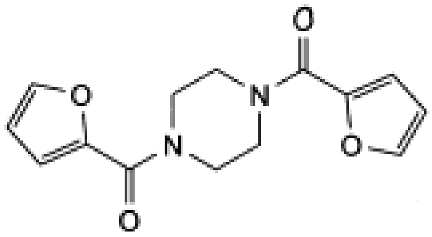


I. 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-[[(2*RS*,5*S*)-5-methyltetrahydrofuran-2-yl] carbonyl]piperazine,

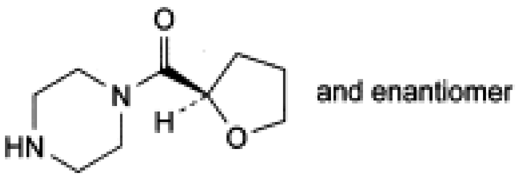
K. prazosin,



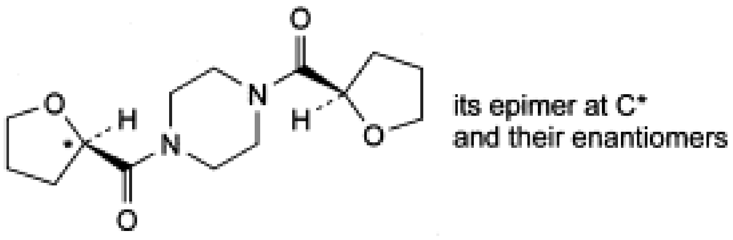
L. 1-(furan-2-ylcarbonyl)piperazine,



M. 1,4-bis(furan-2-ylcarbonyl)piperazine,


and enantiomer

N. 1-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine,


its epimer at C*
and their enantiomers

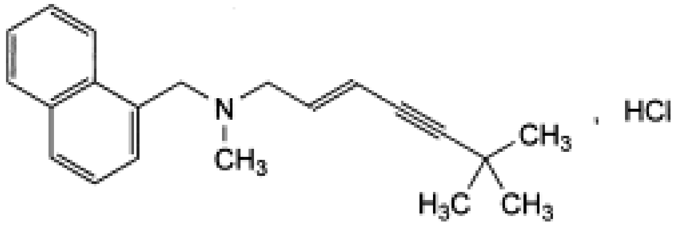
O. 1,4-bis[(tetrahydrofuran-2-yl)carbonyl]piperazine.

Ph Eur

Terbinafine Hydrochloride

General Notices

(Ph Eur monograph 1734)



$C_{21}H_{25}N,HCl$ 327.9 *78628-80-5*

Action and use

Antifungal.

Ph Eur

DEFINITION

(2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly or slightly soluble in water, freely soluble in anhydrous ethanol and in methanol, slightly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *terbinafine hydrochloride CRS.*

B. It gives reaction (a) of chlorides *(2.3.1)* using *anhydrous ethanol R* as solvent.

TESTS

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions protected from light.*

Solvent mixture *methanol R1, water R, acetonitrile R1* (25:25:50 *V/V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase. Expose to ultraviolet light at 254 nm for 1 h.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 50.0 ml with the solvent mixture.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase:* spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 300 ± 60 m2/g and a pore size of 12 nm.

Mobile phase Mix 50 volumes of *acetonitrile R1* , 25 volumes of *methanol R1* and 25 volumes of a 1.74 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 7.5 with *phosphoric acid R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 224 nm.

Injection 20 µl.

Run time Twice the retention time of terbinafine.

Relative retention With reference to terbinafine (retention time = about 31 min): impurity A = about 0.15; impurity C = about 0.8; impurity B = about 0.9; impurity D = about 1.5.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to impurity B and terbinafine.

Limits:

— *impurity B:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *ethanol (96 per cent) R*, add 5 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically *(2.2.20).* Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 32.79 mg of $C_{21}H_{26}ClN$.

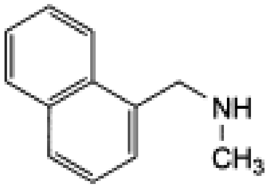
STORAGE

Protected from light.

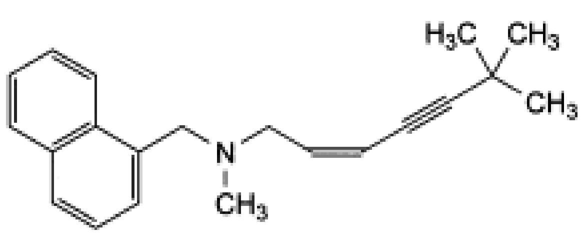
IMPURITIES

Specified impurities *B.*

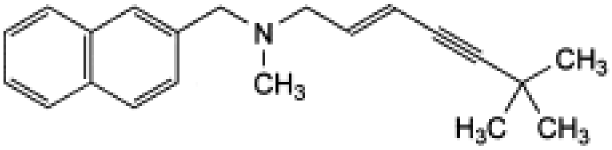
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, C, D.*



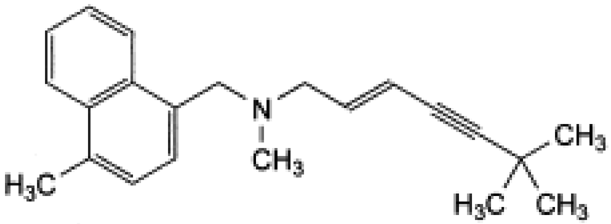
A. *N*-methyl-*C*-(naphthalen-1-yl)methanamine,



B. (2*Z*)-*N*,6,6-trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine (*cis*-terbinafine),



C. (2*E*)-*N*,6,6-trimethyl-*N*-(naphthalen-2-ylmethyl)hept-2-en-4-yn-1-amine (*trans*-isoterbinafine),



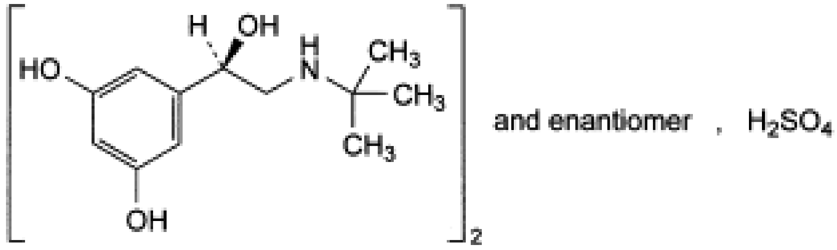
D. (2*E*)-N,6,6-trimethyl-*N*-[(4-methylnaphthalen-1-yl)methyl]hept-2-en-4-yn-1-amine (4-methylterbinafine).

Ph Eur

Terbutaline Sulphate

General Notices

(Ph Eur monograph 0690)



$C_{24}H_{40}N_2O_{10}S$ 548.7 *23031-32-5*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Preparation

Terbutaline Tablets

Ph Eur

DEFINITION

Bis[(1*RS*)-1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl)amino]ethanol] sulphate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison terbutaline sulphate CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *aldehyde-free methanol R*, evaporate to dryness and record new spectra using the residues.

B. 5 ml of solution S (see Tests) gives reaction (a) of sulphates *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 400 nm in a 2 cm cell is not greater than 0.11.

Acidity

To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 1.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Optical rotation *(2.2.7)*

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 75.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 7.5 mg of *terbutaline impurity C CRS* and 22.5 mg of *terbutaline sulphate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm) .

Mobile phase Dissolve 4.23 g of *sodium hexanesulphonate R* in 770 ml of *0.050 M ammonium formate solution* prepared as follows: dissolve 3.15 g of *ammonium formate R* in about 980 ml of *water R*; adjust to pH 3.0 by adding about 8 ml of *anhydrous formic acid R* and dilute to 1000 ml with *water R;* then add 230 ml of *methanol R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 276 nm.

Injection 20 µl.

Run time 6 times the retention time of terbutaline.

Retention time Impurity C = about 9 min; terbutaline = about 11 min.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to impurity C and terbutaline; if necessary adjust the composition of the mobile phase, decrease the content of methanol to increase the retention time.

Limits:

— *impurity C:* not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurities A, B, D:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *sum of impurities other than C:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

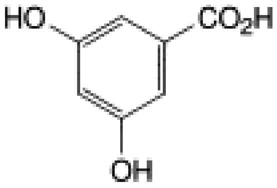
ASSAY

Dissolve 0.400 g in 70 ml of *anhydrous acetic acid R* with heating. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20).*

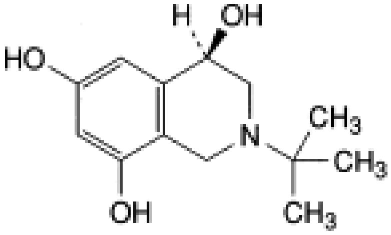
1 ml of *0.1 M perchloric acid* is equivalent to 54.87 mg of $C_{24}H_{40}N_2O_{10}S$.

IMPURITIES

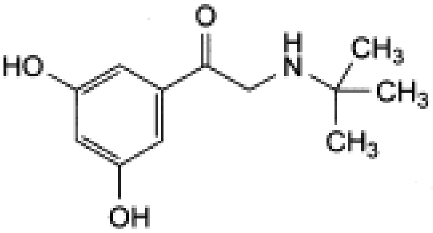
Specified impurities A, B, C, D.



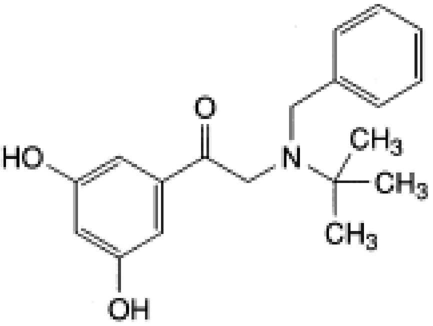
A. 3,5-dihydroxybenzoic acid (α-resorcylic acid),



B. (4*RS*)-2-(1,1-dimethylethyl)-1,2,3,4-tetrahydroisoquinoline-4,6,8-triol,



C. 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl)amino]ethanone,



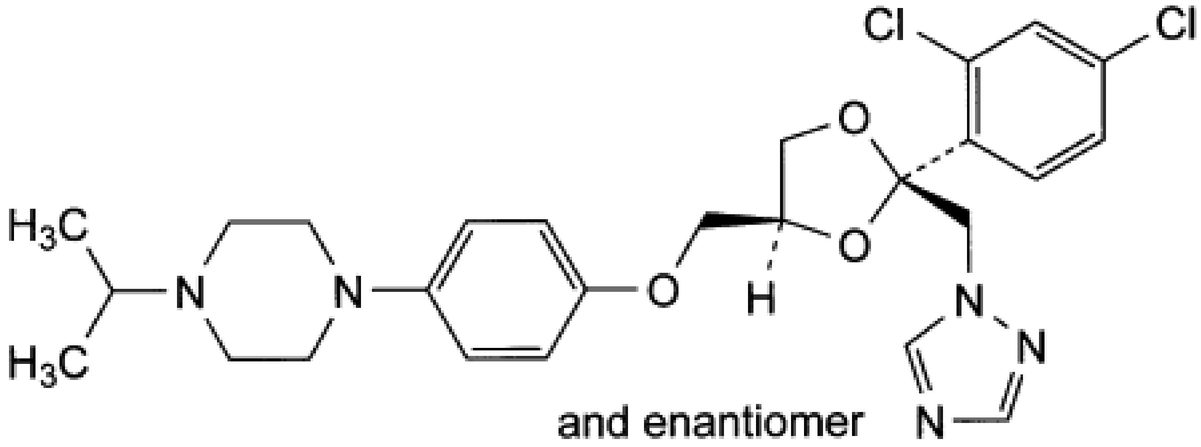
D. 2-[benzyl-(1,1-dimethylethyl)amino]-1-(3,5-dihydroxyphenyl)ethanone.

Ph Eur

Terconazole

General Notices

(Ph Eur monograph 1270)



$C_{26}H_{31}Cl_2N_5O_3$ 532.5 *67915-31-5*

Action and use

Antifungal.

Ph Eur

DEFINITION

1-[4-[[(2*RS*,4*SR*)-2-(2,4-Dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl] methoxy]phenyl]-4-(1-methylethyl)piperazine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, sparingly soluble in ethanol (96 per cent).

It shows polymorphism *(5.9)* .

IDENTIFICATION

First identification A.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison terconazole CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R* , evaporate to dryness in a current of air and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 30 mg of *terconazole CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 30 mg of *terconazole CRS* and 30 mg of *ketoconazole CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate TLC octadecylsilyl silica gel plate R.

Mobile phase ammonium acetate solution R, dioxan R, methanol R (20:40:40 *V/V/V*).

Application 5 µl.

Development In an unsaturated tank over a path of 10 cm.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate R* . Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R* . The solution gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 1.0 g in *methylene chloride R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

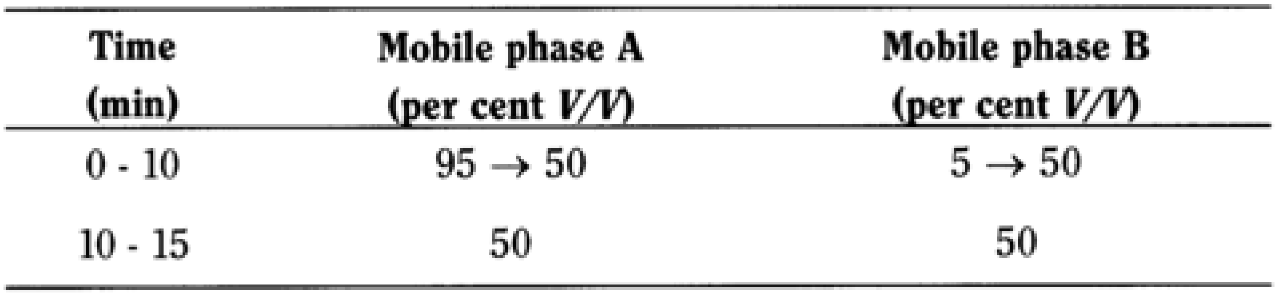
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *terconazole CRS* and 2.0 mg of *ketoconazole CRS* in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol R*.

Column:
— *size: l* = 0.1 m, Ø = 4.6 mm;
— *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: 3.4 g/l solution of *tetrabutylammonium hydrogen sulphate R*;
— *mobile phase B*: *acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	95 → 50	5 → 50
10 - 15	50	50

Flow rate 2 ml/min.

Detection Spectrophotometer at 220 nm.

Equilibration With *acetonitrile R1* for at least 30 min and then with the mobile phase at the initial composition for at least 5 min.

Injection 10 µl; inject *methanol R* as a blank.

Retention time Ketoconazole = about 6 min; terconazole = about 7.5 min.

System suitability Reference solution (a):

— *resolution*: minimum 13 between the peaks due to ketoconazole and terconazole; if necessary, adjust the concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient elution.

Limits:
— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained

with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 70 ml of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically at the 2nd point of inflexion *(2.2.20)*.

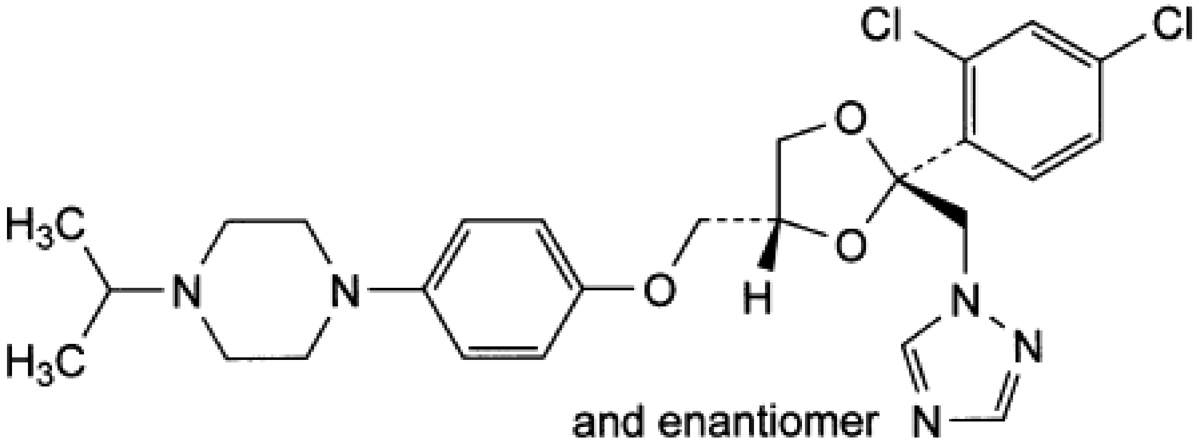
1 ml of *0.1 M perchloric acid* is equivalent to 17.75 mg of $C_{26}H_{31}Cl_2N_5O_3$.

STORAGE

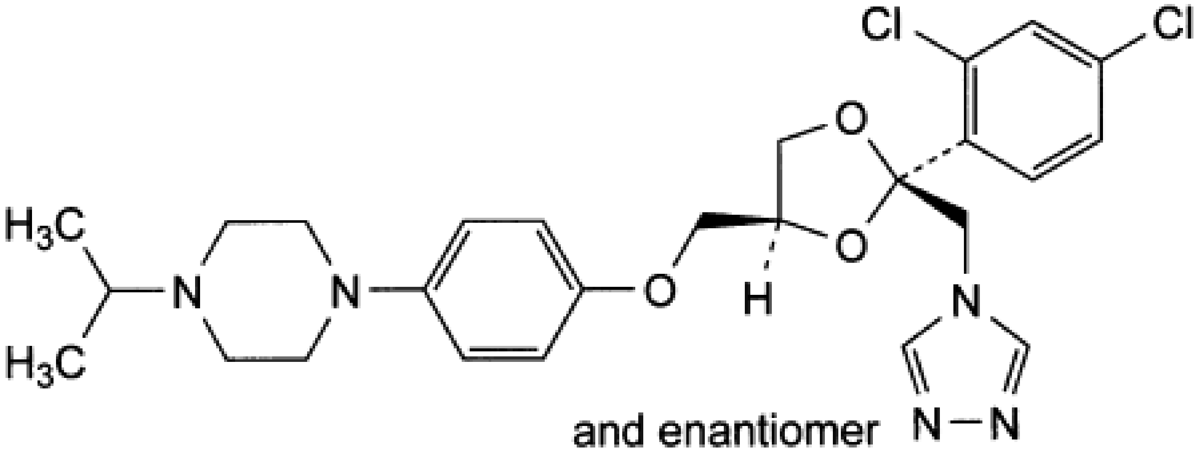
Protected from light.

IMPURITIES

Specified impurities A, B.



A. 1-[4-[[(2*RS*,4*RS*)2-(2,4-dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl] methoxy]phenyl]-4-(1-methylethyl)piperazine,


and enantiomer

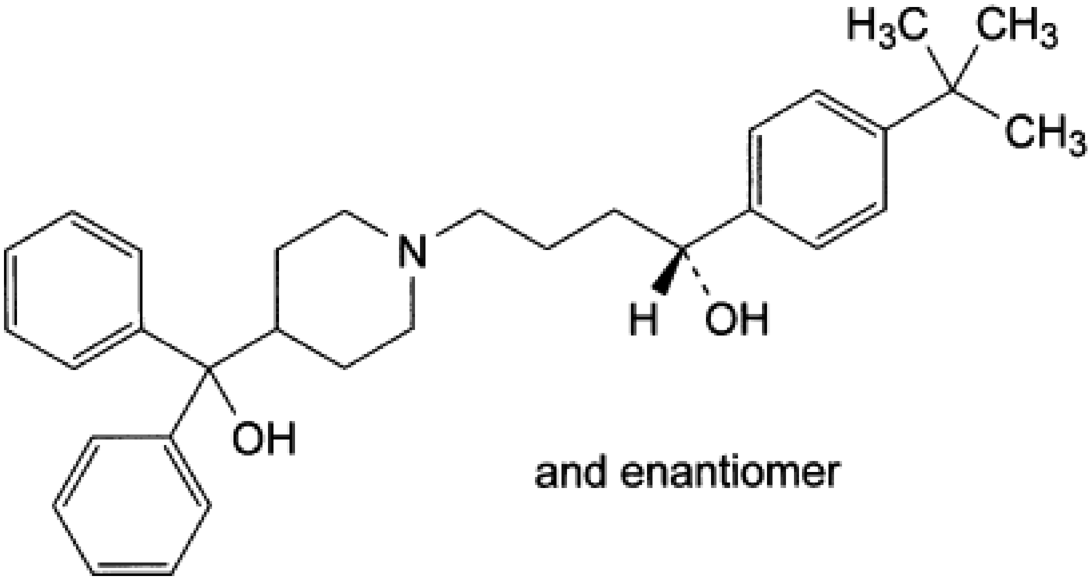
B. 1-[4-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-[(4*H*-1,2,4-triazol-4-yl)methyl]-1,3-dioxolan-4-yl] methoxy]phenyl]-4-(1-methylethyl)piperazine.

Ph Eur

Terfenadine

General Notices

(Ph Eur monograph 0955)



$C_{32}H_{41}NO_2$ 471.7 *50679-08-8*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparations

Terfenadine Oral Suspension

Terfenadine Tablets

Ph Eur

DEFINITION

(1*RS*)-1-[4-(1,1-Dimethylethyl)phenyl]-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butan-1-ol.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in methylene chloride, soluble in methanol. It is very slightly soluble in dilute hydrochloric acid.

It shows polymorphism *(5.9)* .

IDENTIFICATION

First identification *C.*

Second identification *A, B, D.*

A. Melting point *(2.2.14)* : 146 °C to 152 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 50.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent.

Spectral range 230-350 nm.

Absorption maximum At 259 nm.

Shoulders At 253 nm and 270 nm.

Specific absorbance at the absorption maximum 13.5 to 14.9.

C. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *terfenadine CRS.*

D. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *terfenadine CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R .*

Mobile phase *methanol R, methylene chloride R* (10:90 *V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 15 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 15 mg of *terfenadine impurity A CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. To 5.0 ml of this solution, add 5.0 ml of the test solution and dilute to 50.0 ml with the mobile phase.

Reference solution (c) Dilute 10.0 ml of reference solution (a) to 25.0 ml with the mobile phase.

Reference solution (d) Dissolve 0.1 g of *potassium iodide R* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dilute 600 ml of *acetonitrile R1* to 1 litre with *diethylammonium phosphate buffer solution pH 6.0 R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 217 nm.

Injection 20 µl.

Run time 5 times the retention time of terfenadine.

System suitability Reference solution (b):

- *resolution*: minimum 5.0 between the peaks due to terfenadine and impurity A;
- *mass distribution ratio* : minimum 2.0 for the peak due to terfenadine; use *potassium iodide R* as the unretained compound (reference solution (d)).

Limits:

- *impurities A, B, C, D, E, F, G, H, I, J*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.005 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding

0.5 kPa.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

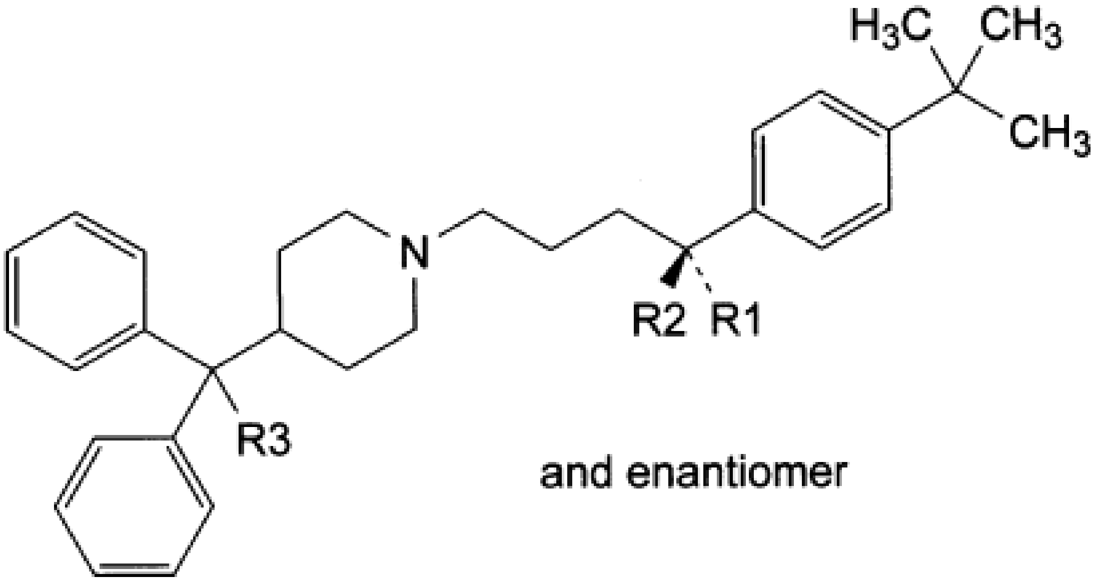
1 ml of *0.1 M perchloric acid* is equivalent to 47.17 mg of $C_{32}H_{41}NO_2$.

STORAGE

Protected from light.

IMPURITIES

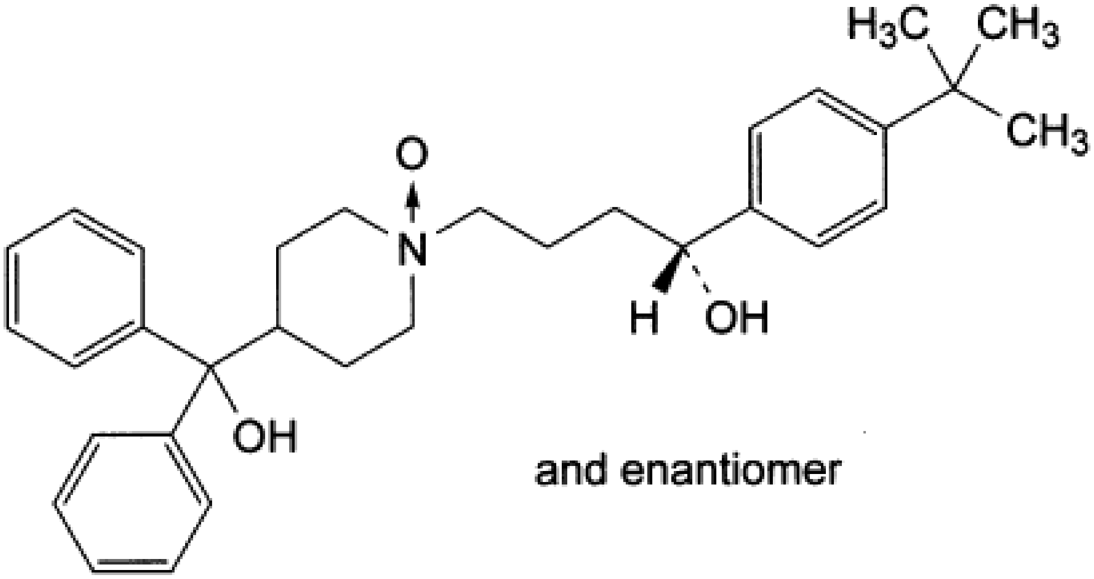
Specified impurities A, B, C, D, E, F, G, H, I, J.



A. R1 + R2 = O, R3 = OH: 1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(hydroxydiphenylmethyl) piperidin-1-yl]butan-1-one,

B. R1 = OH, R2 = R3 = H: (1*RS*)-1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(diphenylmethyl) piperidin-1-yl]butan-1-ol,

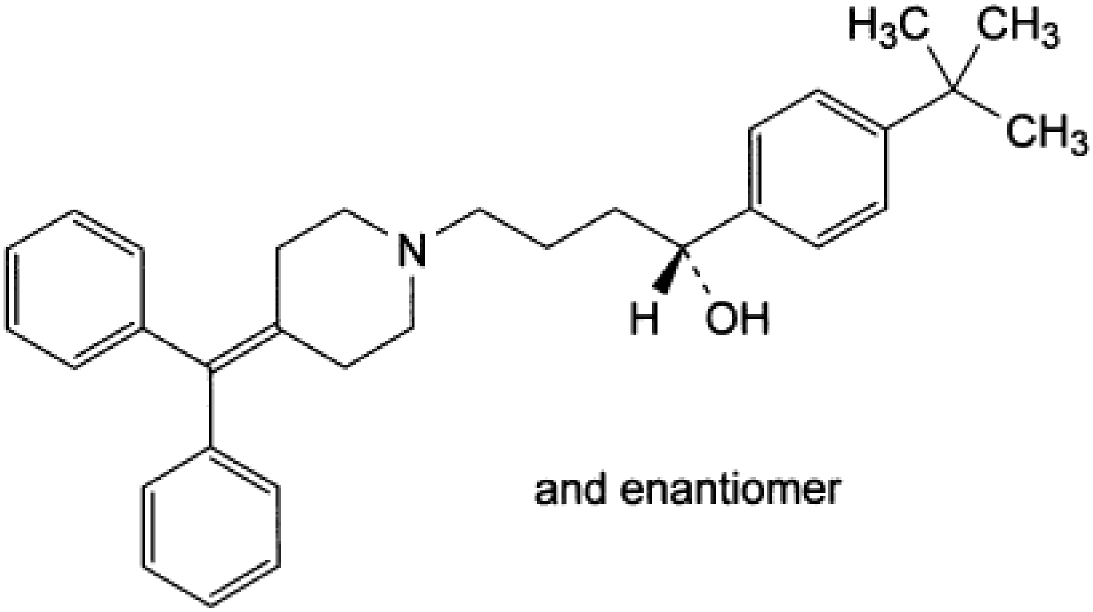
H. R1 = R2 = H, R3 = OH: [1-[4-[4-(1,1-dimethylethyl)phenyl]butyl]piperidin-4-yl] diphenylmethanol,



H₃C
CH₃
CH₃
O
N
H
OH
OH

and enantiomer

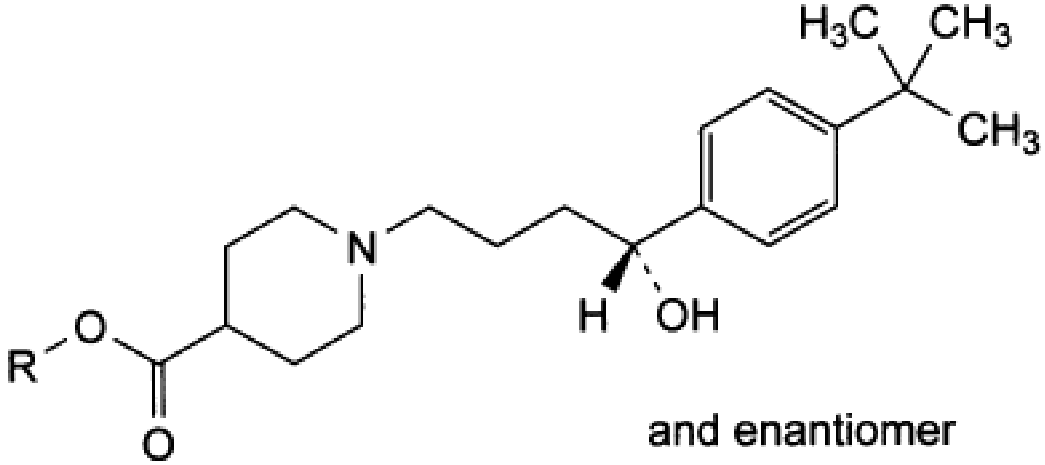
C. 1-[(4*RS*)-4-[4-(1,1-dimethylethyl)phenyl]-4-hydroxybutyl]-4-(hydroxydiphenylmethyl) piperidine 1-oxide,



H₃C
CH₃
CH₃
N
H
OH

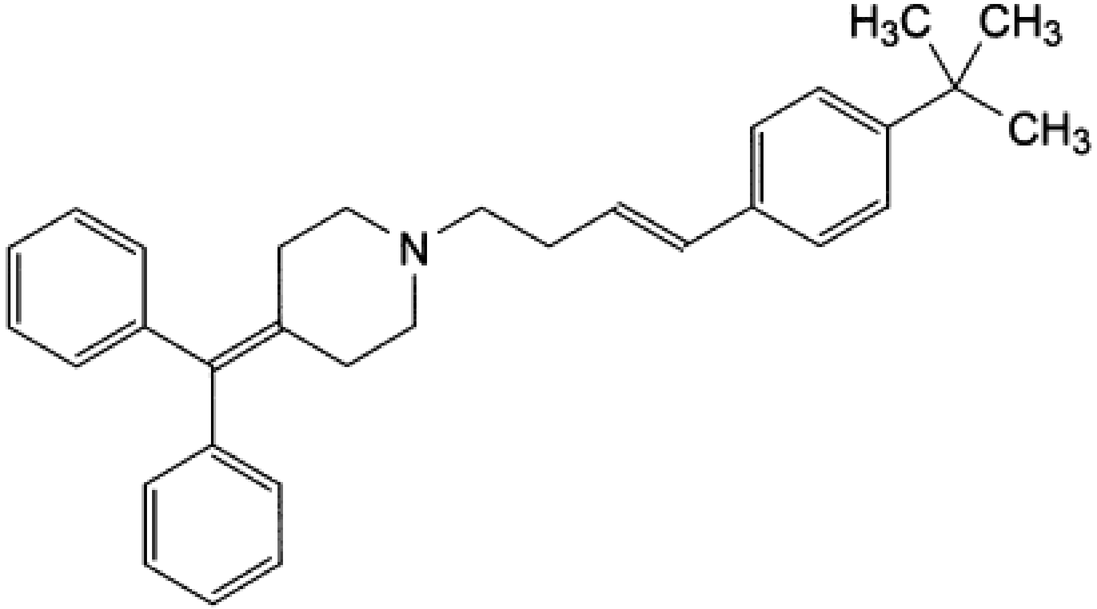
and enantiomer

D. (1*RS*)-1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(diphenylmethylene)piperidin-1-yl]butan-1-ol,

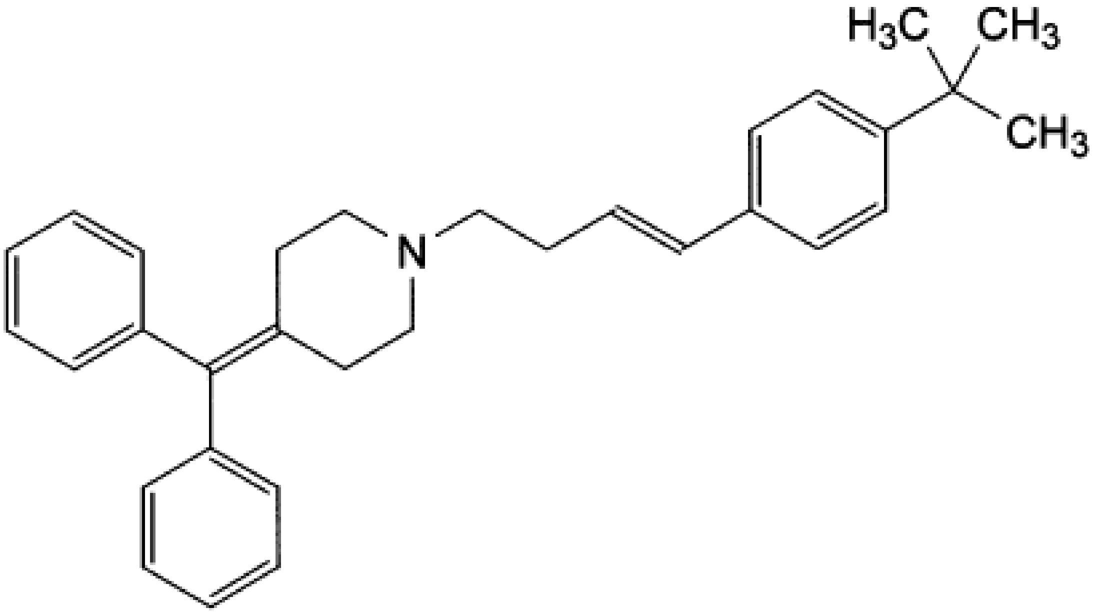

H$_3$C
CH$_3$
CH$_3$
N
H
OH
R
O
O
and enantiomer

E. R = H: 1-[(4*RS*)-4-[4-(1,1-dimethylethyl)phenyl]-4-hydroxybutyl]piperidine-4-carboxylic acid,

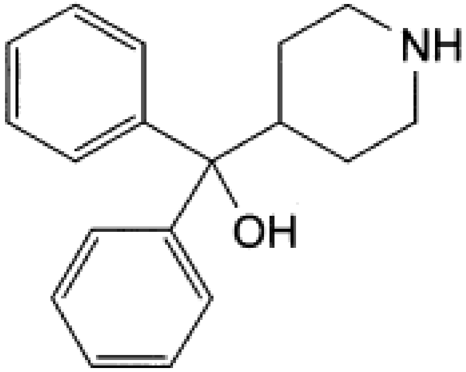
J. R = C_2H_5: ethyl 1-[(4*RS*)-4-[4-(1,1-dimethylethyl)phenyl]-4-hydroxybutyl]piperidine-4-carboxylate,


H$_3$C
CH$_3$
CH$_3$
N

F. 1-[4-[4-(1,1-dimethylethyl)phenyl]but-3-enyl]-4-(diphenylmethylene)piperidine,


H3C
CH3
CH3
N

G. [1-[4-[4-(1,1-dimethylethyl)phenyl]but-3-enyl]piperidin-4-yl]diphenylmethanol,

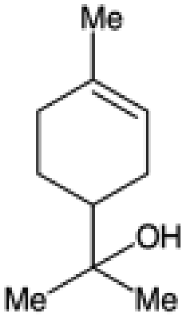


I. diphenyl(piperidin-4-yl)methanol.

Ph Eur

Terpineol

General Notices



$C_{10}H_{18}O$ 154.3 *98-55-5*

DEFINITION

Terpineol is a mixture of structural isomers in which α-terpineol predominates.

CHARACTERISTICS

A colourless, slightly viscous, liquid which may deposit crystals; odour, pleasant and characteristic.

Very slightly soluble in *water*; freely soluble in *ethanol (70%);* soluble in *ether* .

TESTS

Refractive index

1.4800 to 1.4855, Appendix V E.

Weight per ml

0.931 to 0.935 g, Appendix V G.

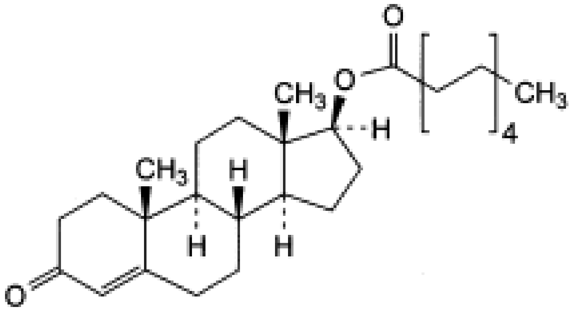
Low-boiling substances

Not more than 4.0% v/v distils below 214°, Appendix V C.

Testosterone Decanoate

General Notices

(Ph Eur monograph 1736)



$C_{29}H_{46}O_3$ 442.7 *5721-91-5*

Action and use

Androgen.

Ph Eur

DEFINITION

3-Oxoandrost-4-en-17β-yl decanoate.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, very soluble in acetone, in methylene chloride and in anhydrous ethanol, freely soluble in fatty oils.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *testosterone decanoate CRS* .

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Dissolve 0.20 g in 20 ml of *methanol R* .

Specific optical rotation *(2.2.7)*

+ 75.0 to + 80.0 (dried substance).

Dissolve 0.200 g in *anhydrous ethanol R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve the contents of a vial of *testosterone decanoate for system suitability CRS* (containing impurities A, B, C, D, E and F) in 1 ml of the mobile phase.

Reference solution (b) Dilute 10.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 20.0 mg of *testosterone decanoate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *water R, acetonitrile R* (5:95 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of testosterone decanoate.

Identification of impurities Use the chromatogram supplied with *testosterone decanoate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E and F.

Relative retention With reference to testosterone decanoate (retention time = about 20 min): impurity A = about 0.2; impurity B = about 0.6; impurities C and G = about 0.79; impurity D = about 0.83; impurity E = about 1.3; impurity F = about 1.7.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurities C and D.

Limits:

— *correction factors*: for the calculation of content, multiply the peak area of impurity A by 0.7;

— *impurities A, B, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *sum of impurities C and G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Free acid

Dissolve 0.65 g in 10 ml of *ethanol (96 per cent) R*, previously neutralised to *bromothymol blue solution R3*, and titrate immediately with *0.01 M sodium hydroxide*, using 0.1 ml of *bromothymol blue solution R3* as indicator. Not more than 0.6 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection 20 µl of the test solution and reference solution (c).

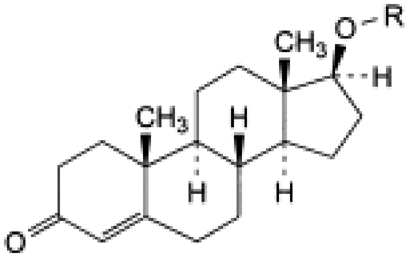
Calculate the percentage content of $C_{29}H_{46}O_3$ from the declared content of *testosterone decanoate CRS*.

STORAGE

At a temperature of 2 °C to 8 °C.

IMPURITIES

Specified impurities *A, B, C, D, E, F, G*.



A. R = H: testosterone,

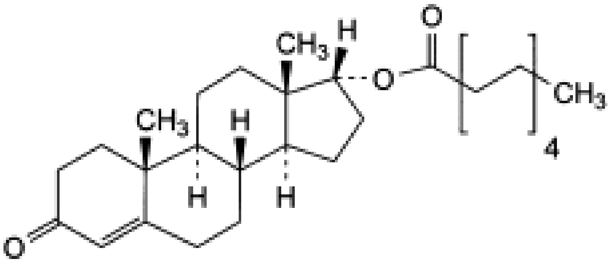
B. R = CO-[CH$_2$]$_6$-CH$_3$: 3-oxoandrost-4-en-17β-yl octanoate (testosterone octanoate),

C. R = CO-[CH$_2$]$_7$-CH$_3$: 3-oxoandrost-4-en-17β-yl nonanoate (testosterone nonanoate),

D. R = CO-[CH$_2$]$_8$-CH=CH$_2$: 3-oxoandrost-4-en-17β-yl undec-10-enoate (testosterone undecylenate),

E. R = CO-[CH$_2$]$_9$-CH$_3$: 3-oxoandrost-4-en-17β-yl undecanoate (testosterone undecanoate),

F. R = CO-[CH$_2$]$_{10}$-CH$_3$: 3-oxoandrost-4-en-17β-yl dodecanoate (testosterone laurate),



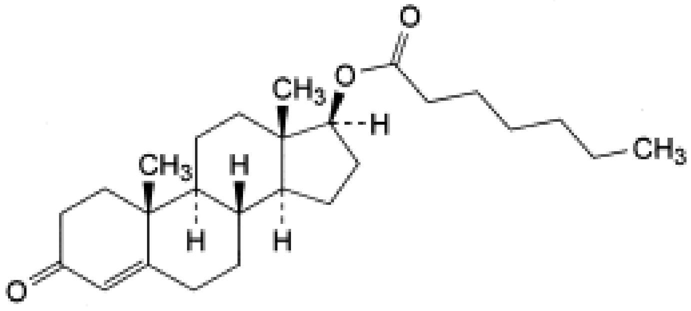
G. 3-oxoandrost-4-en-17α-yl decanoate (epitestosterone decanoate).

Ph Eur

Testosterone Enantate

General Notices

(Ph Eur monograph 1048)



$C_{26}H_{40}O_3$ 400.6 *315-37-7*

Action and use

Androgen.

Ph Eur

DEFINITION

3-Oxoandrost-4-en-17β-yl heptanoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in anhydrous ethanol, freely soluble in fatty oils.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)* : 34 °C to 39 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *testosterone enantate CRS.*

C. Thin-layer chromatography *(2.2.27)* .

Solvent mixture *methanol R, methylene chloride R* (10:90 *V/V*).

Test solution Dissolve 5 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *testosterone enantate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *testosterone enantate CRS* , 5 mg of *testosterone decanoate CRS* and 5 mg of *testosterone isocaproate CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase *water R, acetonitrile R, 2-propanol R* (20:40:60 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air, then at 100 °C for 10 min; allow to cool.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulphuric acid R* ; heat at 120 °C for 10 min; allow to cool and examine in daylight.

Results B The principal spot in the chromatogram obtained with the test solution is green and is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):

— The chromatogram shows 3 clearly separated principal spots by each method of visualisation.

D. To about 25 mg add 2 ml of a 10 g/l solution of *potassium hydroxide R* in *methanol R* and boil under a reflux condenser for 1 h. Cool. Add 10 ml of *water R* . Acidify with dilute *hydrochloric acid R* until *blue litmus paper R* turns red. Filter and wash the precipitate with a small quantity of *water R.* The residue, after drying at 60 °C at a pressure not exceeding 0.7 kPa for 3 h, melts *(2.2.14)* at 150 °C to 153 °C.

TESTS

Specific optical rotation ***(2.2.7)***

+ 77 to + 82 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent.

Impurity A

Maximum 0.16 per cent.

Dissolve 0.50 g in 10 ml of *ethanol (96 per cent) R* previously neutralised to *bromothymol blue solution R3*. Titrate immediately with *0.01 M sodium hydroxide*. Not more than 0.6 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Impurities C and D

Thin-layer chromatography *(2.2.27).*

Solvent mixture *methanol R, methylene chloride R* (10:90 *V/V*).

Test solution Dissolve 0.20 g of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

Reference solution (b) Dissolve 20 mg of *testosterone propionate CRS* in the solvent mixture, add 1 ml of the test solution and dilute to 100 ml with the solvent mixture.

Plate *TLC silica gel G plate R*.

Mobile phase *ethyl acetate R, toluene R* (20:80 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with alcoholic solution of *sulphuric acid R* and heat at 120 °C for 10 min.

System suitability Reference solution (b):

— The chromatogram shows 2 clearly separated principal spots.

Limit:

— *impurities C, D*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (1.0 per cent).

Impurity B

Liquid chromatography (2.2.29).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *testosterone caproate CRS* (impurity B) in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. To 1 ml of this solution add 1 ml of reference solution (a).

Column:
- *size:* *l* = 0.12 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R, acetonitrile R* (30:70 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

System suitability Reference solution (b):

- *relative retention with reference to testosterone enantate*: impurity B = about 0.7.

Limit:
- *impurity B:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa.

ASSAY

Dissolve 50.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *anhydrous ethanol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 241 nm.

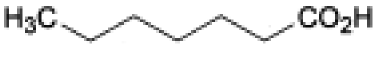
Calculate the content of $C_{26}H_{40}O_3$ taking the specific absorbance to be 422.

STORAGE

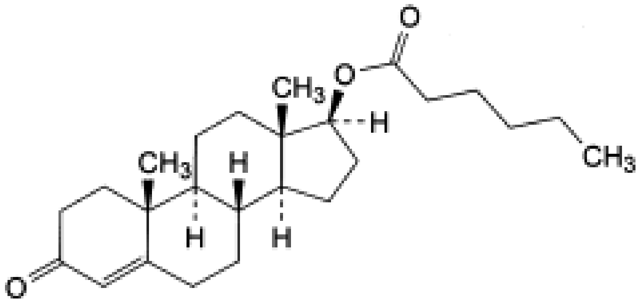
Protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES

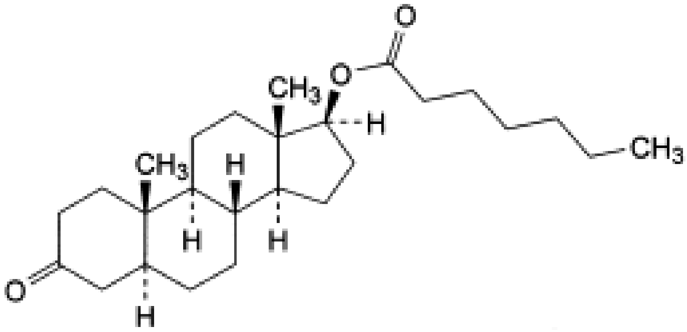
Specified impurities *A, B, C, D.*



A. heptanoic acid,



B. 3-oxoandrost-4-en-17β-yl hexanoate (testosterone caproate),



C. 3-oxo-5α-androstan-17β-yl heptanoate,

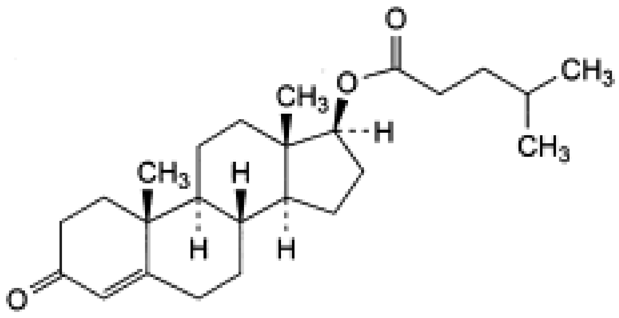
D. 17β-hydroxyandrost-4-en-3-one (testosterone).

Ph Eur

Testosterone Isocaproate

General Notices

(Ph Eur monograph 1737)



$C_{25}H_{38}O_3$ 386.6 *15262-86-9*

Action and use

Androgen.

Ph Eur

DEFINITION

3-Oxoandrost-4-en-17β-yl 4-methylpentanoate.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, very soluble in acetone and in methylene chloride, freely soluble in fatty oils.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *testosterone isocaproate CRS* .

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Dissolve 0.20 g in 20 ml of *methanol R* .

Specific optical rotation *(2.2.7)*

+ 82.0 to + 88.0 (dried substance).

Dissolve 0.200 g in *anhydrous ethanol R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 2 mg of *testosterone isocaproate for system suitability CRS* (containing impurities A, B, C, D, E, F and G) in 10 ml of the mobile phase.

Reference solution (b) Dilute 10.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c) Dissolve 20.0 mg of *testosterone isocaproate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *water R, acetonitrile R* (15:85 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of testosterone isocaproate.

Identification of impurities Use the chromatogram supplied with *testosterone isocaproate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F and G.

Relative retention With reference to testosterone isocaproate (retention time = about 14 min): impurity A = about 0.2; impurity B = about 0.4; impurity C = about 0.5; impurity D = about 0.7; impurity G = about 0.8; impurity E = about 1.1; impurity F = about 1.4.

System suitability Reference solution (a):

— *peak-to-valley ratio* : minimum 2.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to testosterone isocaproate.

Limits:

— *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Free acid

Dissolve 0.44 g in 10 ml of *ethanol (96 per cent) R* , previously neutralised to *bromothymol blue solution R3* , and titrate immediately with *0.01 M sodium hydroxide* , using 0.1 ml of *bromothymol blue solution R3* as indicator. Not more than 0.6 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa.

ASSAY

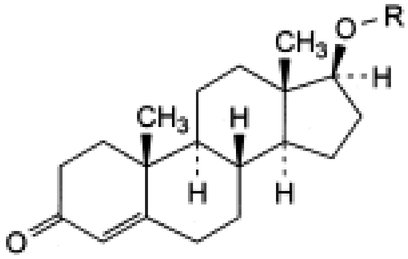
Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection 20 µl of the test solution and reference solution (c).

Calculate the percentage content of $C_{25}H_{38}O_3$ from the declared content of *testosterone isocaproate CRS* .

IMPURITIES

Specified impurities *A, B, C, D, E, F, G*.



A. R = H: testosterone,

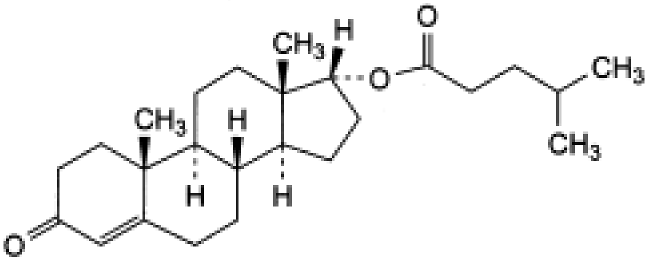
B. R = CO-CH_3: 3-oxoandrost-4-en-17β-yl acetate (testosterone acetate),

C. R = CO-C_2H_5: testosterone propionate,

D. R = CO-CH$(CH_3)_2$: 3-oxoandrost-4-en-17β-yl 2-methylpropanoate (testosterone isobutyrate),

E. R = CO-$[CH_2]_4$-CH3: 3-oxoandrost-4-en-17β-yl hexanoate (testosterone caproate),

F. R = CO-$[CH_2]_5$-CH3: testosterone enantate,



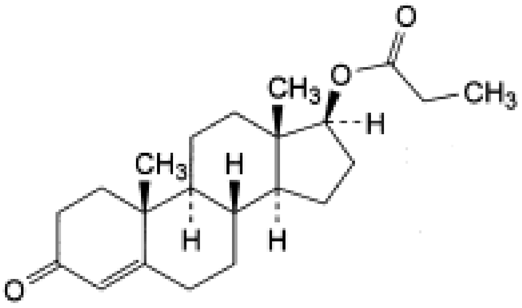
G. 3-oxoandrost-4-en-17α-yl 4-methylpentanoate (epitestosterone isocaproate).

Ph Eur

Testosterone Propionate

General Notices

(Ph Eur monograph 0297)



$C_{22}H_{32}O_3$ 344.5 *57-85-2*

Action and use

Androgen.

Preparations

Testosterone Propionate Injection

Ph Eur

DEFINITION

3-Oxoandrost-4-en-17β-yl propanoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in acetone and in alcohol, soluble in fatty oils.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of testosterone propionate.*

TESTS

Specific optical rotation *(2.2.7)*

+ 84 to + 90 (dried substance).

Dissolve 0.250 g in *ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of the substance to be examined and 2 mg of *testosterone acetate CRS* in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R* .

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography* R (5 µm).

Mobile phase *water R, methanol R* (20:80 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time Twice the retention time of testosterone propionate.

Relative retention With reference to testosterone propionate (retention time = about 9 min): impurity C = about 0.5; impurity A = about 0.7; impurity D = about 0.8; impurity B = about 1.4.

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to testosterone propionate and to impurity A.

Limits:

— *any impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

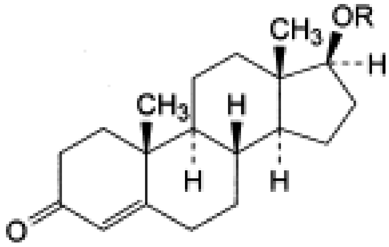
Dissolve 25.0 mg in *ethanol R* and dilute to 250.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *ethanol R* . Measure the absorbance *(2.2.25)* at the maximum at 240 nm.

Calculate the content of $C_{22}H_{32}O_3$ taking the specific absorbance to be 490.

IMPURITIES

Specified impurities A, B, C, D.

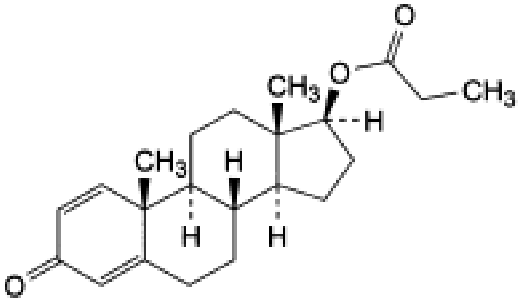
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E.*



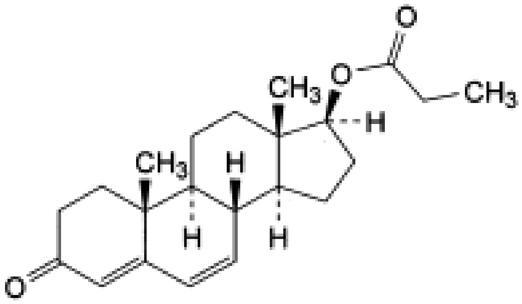
A. R = CO-CH_3: 3-oxoandrost-4-en-17β-yl acetate (testosterone acetate),

B. R = CO-$CH(CH_3)_2$: 3-oxoandrost-4-en-17β-yl 2-methylpropanoate (testosterone isobutyrate),

C. R = H: testosterone,



D. 3-oxoandrosta-1,4-dien-17β-yl propanoate,



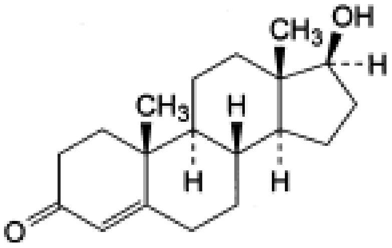
E. 3-oxoandrosta-4,6-dien-17β-yl propanoate.

Ph Eur

Testosterone

General Notices

(Ph Eur monograph 1373)



$C_{19}H_{28}O_2$ 288.4 *58-22-0*

Action and use

Androgen.

Preparation

Testosterone Implants

Ph Eur

DEFINITION

17β-Hydroxyandrost-4-en-3-one.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder, or colourless or yellowish-white crystals.

Solubility

Practically insoluble in water, freely soluble in alcohol and in methylene chloride, practically insoluble in fatty oils.

mp

About 155 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *testosterone CRS.*

TESTS

Specific optical rotation *(2.2.7)*

+ 106 to + 114 (dried substance).

Dissolve 0.250 g in *ethanol R* and dilute to 25.0 ml with the same solvent.

Impurities D and F

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 1 mg of *stanolone R* in *methanol R* and dilute to 10 ml with the same solvent. In 1 ml of this solution, dissolve 10 mg of testosterone for *impurity D identification CRS* (testosterone spiked with about 1 per cent of impurity D).

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R* .

Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10.0 ml with *methanol R* .

Reference solution (d) Dilute 1.0 ml of reference solution (b) to 10.0 ml with *methanol R* .

Plate *TLC silica gel F_{254} plate R* (6-8 µm).

Preconditioning (in the dark)

Add about 5 g of powdered *silver nitrate R* to 100 ml of *methanol R.* Stir the suspension for 30 min. Filter or decant the suspension and immerse the plate in the silver nitrate solution for at least 30 min. Dry at 75 °C for 30 min.

A pre-conditioned plate can be stored in the dark for 5-7 days.

Mobile phase *acetic acid R, ethanol R, dioxan R, methylene chloride R* (1:2:10:90 *V/V/V/V*).

Application 2 µl.

Development In a saturated tank over 3/4 of the plate.

Drying Allow to stand at room temperature and protected from light for 30 min.

Detection Spray with a 200 g/l solution of *toluenesulphonic acid R* in *ethanol R* and heat at 105 °C for 10 min. Examine in ultraviolet light at 365 nm.

System suitability The chromatogram obtained with reference solution (a) shows 3 clearly separated spots; impurity D R_F = about 0.5; testosterone R_F = about 0.65; impurity F R_F = about 0.7.

Limits:

- *impurity D*: any spot due to impurity D is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *impurity F*: any spot due to impurity F is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *testosterone for system suitability CRS* (containing impurities C and I) in 1 ml of *methanol R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R*.

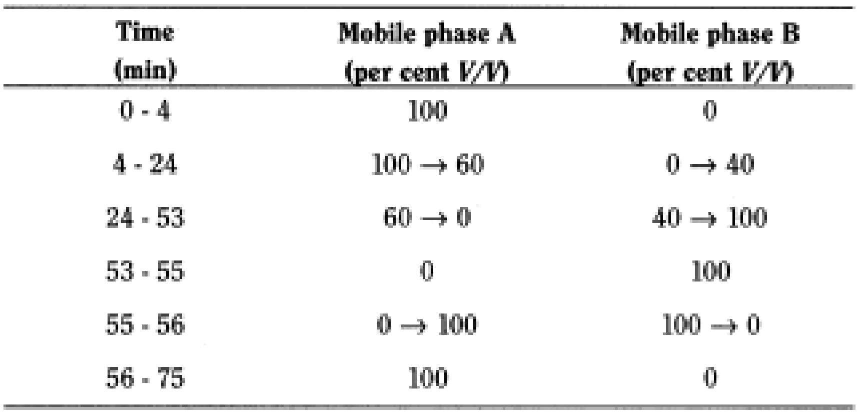
Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10.0 ml with *methanol R*.

Column

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: spherical end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 15 nm;
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A: water for chromatography R, methanol R* (45:55 *V/V*);
- *mobile phase B: methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 24	100 → 60	0 → 40
24 - 53	60 → 0	40 → 100
53 - 55	0	100
55 - 56	0 → 100	100 → 0
56 - 75	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Relative retention With reference to testosterone (retention time = about 18 min): impurity G = about 0.6; impurity H = about 0.8; impurity A = about 0.9; impurity I = about 0.95; impurity C = about 1.2; impurity E = about 1.7; impurity J = about 2.1; impurity B = about 2.5.

System suitability Reference solution (a):

— *resolution*: minimum baseline separation between the peaks due to impurity I and testosterone.

Limits Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C and I:

— *correction factor*: for the calculation of content, multiply the peak area of impurity I by 2.9;

— *impurity C:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurity I:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurities A, B, E, G, H, J*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *total*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

Dissolve 50.0 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml to 100.0 ml with *alcohol R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 241 nm.

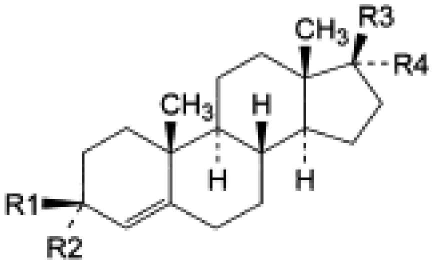
Calculate the content of $C_{19}H_{28}O_2$ taking the specific absorbance to be 569.

STORAGE

Protected from light.

IMPURITIES

Specified impurities A, B, C, D, E, F, G, H, I, J.

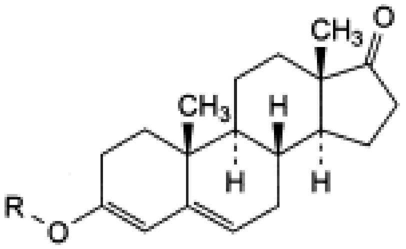

CH3
R3
R4
CH3
H
H
H
R1
R2

A. R1 + R2 = R3 + R4 = O: androst-4-ene-3,17-dione (androstenedione),

C. R1 + R2 = O, R3 = H, R4 = OH: 17α-hydroxyandrost-4-en-3-one (epitestosterone),

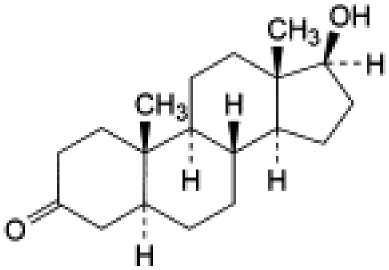
D. R1 = R3 = OH, R2 = R4 = H: androst-4-ene-3β,17β-diol (Δ4-androstenediol),

E. R1 + R2 = O, R3 = $O-CO-CH_3$, R4 = H: 3-oxoandrost-4-en-17β-yl acetate (testosterone acetate),

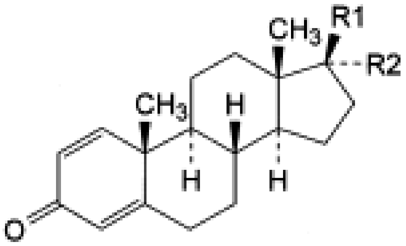

CH3
O
CH3
H
H
H
R
O

B. R = C_2H_5: 3-ethoxyandrosta-3,5-dien-17-one (androstenedione ethylenolether),

J. R = CH_3: 3-methoxyandrosta-3,5-dien-17-one (androstenedione methylenolether),

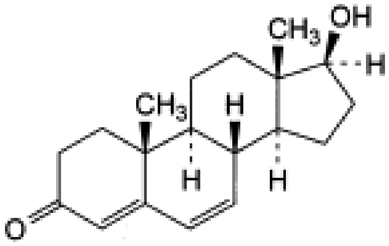

CH3
OH
H
CH3
H
H
H
O
H

F. 17β-hydroxy-5α-androstan-3-one (androstanolone, stanolone),


CH3
R1
R2
CH3
H
H
H
O

G. R1 + R2 = O: androsta-1,4-diene-3,17-dione (androstadienedione).

H. R1 = OH, R2 = H: 17β-hydroxyandrosta-1,4-dien-3-one (boldenone),


CH3
OH
H
CH3
H
H
H
O

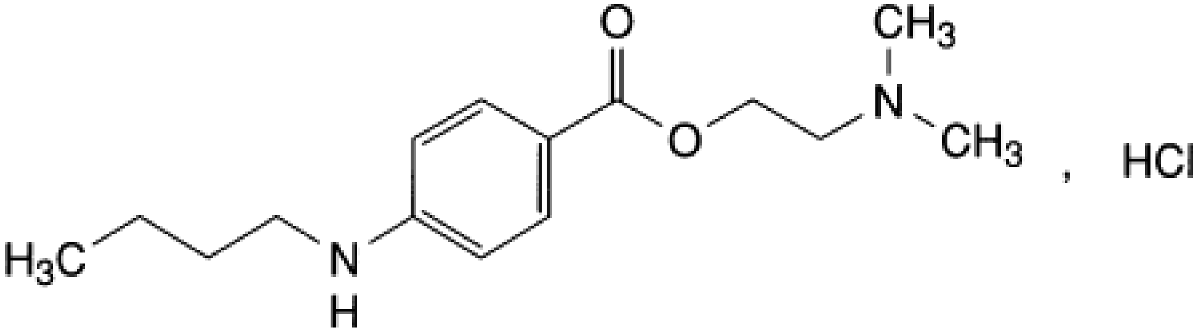
I. 17β-hydroxyandrosta-4,6-dien-3-one (Δ6-testosterone).

Ph Eur

Tetracaine Hydrochloride

General Notices

(Ph Eur monograph 0057)



$C_{15}H_{24}N_2O_2,HCl$ 300.8 *136-47-0*

Action and use

Local anaesthetic.

Preparation

Tetracaine Eye Drops

Ph Eur

DEFINITION

2-(Dimethylamino)ethyl 4-(butylamino)benzoate hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic, crystalline powder.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

It melts at about 148 °C or it may occur in either of 2 other crystalline forms which melt respectively at about 134 °C and 139 °C. Mixtures of these forms melt within the range 134 °C to 147 °C.

IDENTIFICATION

First identification   A, B, D.

Second identification   B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison   tetracaine hydrochloride CRS.

B. To 10 ml of solution S (see Tests) add 1 ml of *ammonium thiocyanate solution R* . A white, crystalline precipitate is formed which, after recrystallisation from *water R* and drying at 80 °C for 2 h, melts *(2.2.14)* at about 131 °C.

C. To about 5 mg add 0.5 ml of *fuming nitric acid R* . Evaporate to dryness on a water-bath, allow to cool and dissolve the residue in 5 ml of *acetone R* . Add 1 ml of *0.1 M alcoholic potassium hydroxide* . A violet colour develops.

D. Solution S gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dilute 2 ml of solution S to 10 ml with *water R.*

pH *(2.2.3)* : 4.5 to 6.5.

Dilute 1 ml of solution S to 10 ml with *carbon dioxide-free water R.*

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use or store them at a temperature of 2 ‘C to 8 ‘C.*

Solvent mixture   *acetonitrile R* , *water R* (20:80 *V/V*).

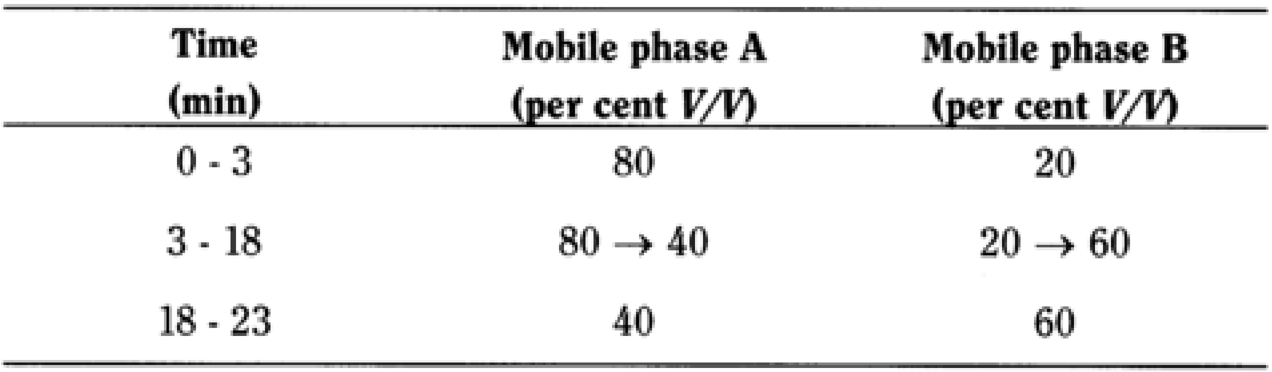
Test solution   Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 50 ml with the solvent mixture.

Reference solution (a)   Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *tetracaine for system suitability CRS* (containing impurities A, B and C) in 2 ml of the solvent mixture.

Column:
- — *size:* *l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 30 °C.

Mobile phase:
- — *mobile phase A*: dissolve 1.36 g of *potassium dihydrogen phosphate R* in *water R* , add 0.5 ml of *phosphoric acid R* and dilute to 1000 ml with *water* R;
- — *mobile phase B: acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	80	20
3 - 18	80 → 40	20 → 60
18 - 23	40	60

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 300 nm.

Injection 10 µl.

Identification of impurities Use the chromatogram supplied with *tetracaine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to tetracaine (retention time = about 8 min): impurity A = about 0.3; impurity B = about 1.7; impurity C = about 2.1.

System suitability Reference solution (b):

- — *resolution*: minimum 5.0 between the peaks due to tetracaine and impurity B.

Limits:
- — *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.6; impurity C = 0.7;
- — *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- — *impurities B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric* acid. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide.* Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 30.08 mg of $C_{15}H_{25}ClN_2O_2$.

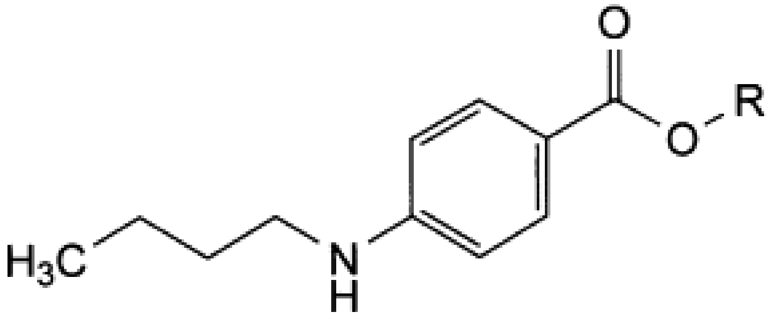
STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities *A, B, C.*

A. 4-aminobenzoic acid,



B. R = H: 4-(butylamino)benzoic acid,

C. R = CH_3: methyl 4-(butylamino)benzoate.

Ph Eur

Tetracosactide

General Notices

(Ph Eur monograph 0644)

H-Ser—Tyr—Ser—Met—Glu—His—Phe—Arg—Trp—Gly—
Lys—Pro—Val—Gly—Lys—Lys—Arg—Arg—Pro—Val—
Lys—Val—Tyr—Pro-OH

$C_{136}H_{210}N_{40}O_{31}S$ 2933 *16960-16-0*

Action and use

Corticotrophic peptide.

Preparations

Tetracosactide Injection

Tetracosactide Zinc Injection

Ph Eur

DEFINITION

Tetracosactide is a synthetic tetracosapeptide in which the sequence of amino acids is the same as that of the first twenty-four residues of human corticotropin. It is available as an acetate and contains water. It increases the rate at which corticoid hormones are secreted by the adrenal glands. The potency is not less than 800 International Units per milligram, calculated with reference to the anhydrous, acetic acid-free substance.

CHARACTERS

A white or yellow, amorphous powder, sparingly soluble in water.

IDENTIFICATION

A. It increases the amount of corticosterone produced by isolated rat adrenal cells in the conditions of the assay.

B. Examine by electrophoresis *(2.2.31)* and thin-layer chromatography *(2.2.27)* to obtain a two-dimensional separation using two plates with *cellulose for chromatography R1* as the coating substance.

Test solution Dissolve 1 mg of the substance to be examined in 0.2 ml of a 15.4 g/l solution of *ammonium acetate R* adjusted to pH 8.2 with *dilute ammonia R2*. Add 10 µl of a 2 g/l solution of *trypsin R*, maintain the mixture at 37 °C to 38 °C for 40 min, heat on a water-bath for 3 min and add 5 µl of *glacial acetic acid R*. Evaporate to dryness at 40 °C at a pressure not exceeding 3 kPa, dry the glassy residue at 40 °C for 1 h and dissolve in 0.1 ml of *glacial acetic acid R*. Dry the solution from the frozen state, dissolve the residue in 0.1 ml of *water R* and dry again from the frozen state. Dry the final residue at 45 °C for 1 h at a pressure not exceeding 3 kPa and dissolve in 50 µl of *water R*.

Reference solution Prepare at the same time and in the same manner as the test solution, using *tetracosactide CRS* instead of the substance to be examined.

Spray the plates with the electrolyte solution which consists of a solution containing 0.2 per cent *V/V* of *glacial acetic acid R* and 0.2 per cent *V/V* of *pyridine R*. Place the filter paper tongues to connect the plates with the appropriate compartment of each trough so that each tongue covers an area 1.5 cm wide at one end of the plate. Close the tank and allow to stand for 30 min. Apply the solutions on the anodic side. Apply to the first plate, at a point about 2.5 cm from each of two adjacent edges, 4 µl of the test solution. Apply to the second plate, at a similar position, 4 µl of the reference solution. Apply to both plates a potential of 280 V for a plate 200 mm long and allow electrophoresis to proceed for 90 min. Allow the plates to dry in air for 30 min and then dry in a current of air at 30 °C for 30 min. Carry out a second separation on each plate by thin-layer chromatography. Develop at right angles to the direction of electrophoresis over a path of 15 cm using a mixture of 8 volumes of *glacial acetic acid R*, 24 volumes of *pyridine R*, 30 volumes of *water R* and 38 volumes of *butanol R*. Dry the plates in a current of air and spray with *ninhydrin solution R1*. The principal spots in the chromatogram obtained with the test solution are similar in position to those in the chromatogram obtained with the reference solution, but their intensity may differ.

TESTS

Specific optical rotation (*2.2.7*)

Dissolve 10.0 mg in 1.0 ml of a mixture of 1 volume of *glacial acetic acid R* and 99 volumes of *water R.* The specific optical rotation is - 99 to - 109, calculated with reference to the anhydrous, acetic acid-free substance.

Absorbance *(2.2.25)*

Dissolve 1.0 mg in *0.1 M hydrochloric acid* and dilute to 5.0 ml with the same acid. Examined between 240 nm and 280 nm, the solution shows an absorption maximum at 276 nm. The absorbance at the maximum is 0.51 to 0.61, calculated with reference to the anhydrous, acetic acid-free substance. The ratio of the absorbance at the maximum at 276 nm to the absorbance at 248 nm is 2.4 to 2.9.

Amino acids

Examine by means of an amino-acid analyser. Standardise the apparatus with a mixture containing equimolar amounts of ammonia, glycine and the L-form of the following amino acids:

Lysine
Histidine
Threonine
Serine
Alanine
Valine
Leucine
Tyrosine

Arginine
Aspartic acid
Glutamic acid
Proline
Methionine
Isoleucine
Phenylalanine

together with half the equimolar amount of L-cystine. For the validation of the method, an appropriate internal standard, such as *DL-norleucine R*, is used.

Test solution Place 1.0 mg of the substance to be examined in a rigorously cleaned hard-glass tube, 100 mm long and 6 mm in internal diameter. Add a suitable amount of a 50 per cent *V/V* solution of *hydrochloric acid R*. Immerse the tube in a freezing mixture at - 5 °C, reduce the pressure to below 133 Pa and seal. Heat at 110 °C to 115 °C for 16 h. Cool, open the tube, transfer the contents to a 10 ml flask with the aid of five quantities, each of 0.2 ml, of *water R* and evaporate to dryness over *potassium hydroxide R* under reduced pressure. Take up the residue in *water R* and evaporate to dryness over *potassium hydroxide R* under reduced pressure; repeat these operations once. Take up the residue in a buffer solution suitable for the amino-acid analyser used and dilute to a suitable volume with the same buffer solution. Apply a suitable volume to the amino-acid analyser.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking that for valine to be equivalent to three. The values fall within the following limits: lysine 3.5 to 4.7; histidine 0.9 to 1.1; arginine 2.7 to 3.3; serine 1.1 to 2.2; glutamic acid 0.9 to 1.1; proline 2.5 to 3.5; glycine 1.8 to 2.2; methionine 0.9 to 1.1; tyrosine 1.7 to 2.2; phenylalanine 0.9 to 1.1. Not more than traces of other amino acids are present, with the exception of tryptophan.

Related peptides

A. Examine by liquid chromatography *(2.2.29)*. Use degassed solvents.

Test solution Dissolve 1.0 mg of the substance to be examined in 1 ml of *water R*.

Reference solution (a) Dissolve 1.0 mg of the substance to be examined in 1 ml of a 1 per cent *V/V* solution of *glacial acetic acid R* and add 50 µl of a mixture of 1 volume of *strong hydrogen peroxide solution R* and 999 volumes of *water R*. Allow to stand for 2 h.

Reference solution (b) Dissolve 1.0 mg of *tetracosactide CRS* in 1 ml of *water R*.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (10 µm);

— as mobile phase at a flow rate of 2.0 ml/min, a mixture of 365 ml of *acetonitrile R*, 10.0 ml of *glacial acetic acid R* and 10.0 g of *ammonium sulphate R*, diluted to 2000 ml with *water R;*

— as detector a spectrophotometer set at 280 nm.

Inject 20 µl of each solution ensuring that the syringe used to inject the test solution is not contaminated with peroxide. The chromatogram obtained with reference solution (a) shows a peak due to tetracosactide corresponding to the principal peak in the chromatogram obtained with the test solution and a peak with a lower retention time, due to tetracosactide sulphoxide, of significantly greater area than any corresponding peak in the chromatogram obtained with the test solution. The test is not valid unless in the chromatogram obtained with reference solution (a), the resolution between the peaks corresponding to tetracosactide and tetracosactide sulphoxide is at least 7. In the chromatogram obtained with the test solution, the area of the peak corresponding to tetracosactide sulphoxide is not greater than 4 per cent of the sum of the areas of all the peaks, disregarding any peaks due to the solvent and the mobile phase.

B. Examine by thin-layer chromatography *(2.2.27)* , using *cellulose for chromatography R* as the coating substance.

Test solution Dissolve 3.0 mg of the substance to be examined in 1.5 ml of a mixture of equal volumes of *dilute acetic acid R* and *water R.*

Reference solution (a) Dilute 0.5 ml of the test solution to 10 ml with a mixture of equal volumes of *dilute acetic acid R* and *water R* .

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with a mixture of equal volumes of *dilute acetic acid R* and *water R.*

Reference solution (c) To 0.5 ml of the test solution add 50 µl of a mixture of 1 volume of *strong hydrogen peroxide solution R* and 999 volumes of *water R* . Allow to stand for 2 h.

Apply to the plate as 1 cm bands 10 µl of each solution. Develop over a path of 15 cm using a mixture of 4 volumes of *glacial acetic acid R* , 24 volumes of *pyridine R* , 30 volumes of *water R* and 42 volumes of *butanol R* . Dry the plate in a current of air and spray with *ninhydrin solution R1* . In the chromatogram obtained with reference solution (c), the band corresponding in position to the principal band in the chromatogram obtained with the test solution is reduced in intensity, and a prominent band, due to tetracosactide sulphoxide, of lower R_F is present. In the chromatogram obtained with the test solution, any band, apart from the principal band and any band due to tetracosactide sulphoxide, is not more intense than the band in the chromatogram obtained with reference solution (a) (5.0 per cent) and at most one such band is more intense than the band in the chromatogram obtained with reference solution (b) (2.5 per cent).

Peptide

Not less than 85.0 per cent of peptide, expressed as $C_{136}H_{21}0N_{40}O_{31}S$, calculated with reference to the anhydrous, acetic acid-free substance.

Examine the chromatograms obtained in test A for related peptides. Calculate the content of $C_{136}H_{210}N_{40}O_{31}S$ from the peak heights or areas in the chromatograms obtained with the test solution and reference solution (b), and the declared content of $C_{136}H_{210}N_{40}O_{31}S$ in *tetracosactide CRS* .

Acetic acid ***(2.5.34)***

8.0 per cent to 13.0 per cent.

Test solution. Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of solvents.

Water ***(2.5.12)***

5.0 per cent to 16.0 per cent, determined on 80.0 mg by the semi-micro determination of water.

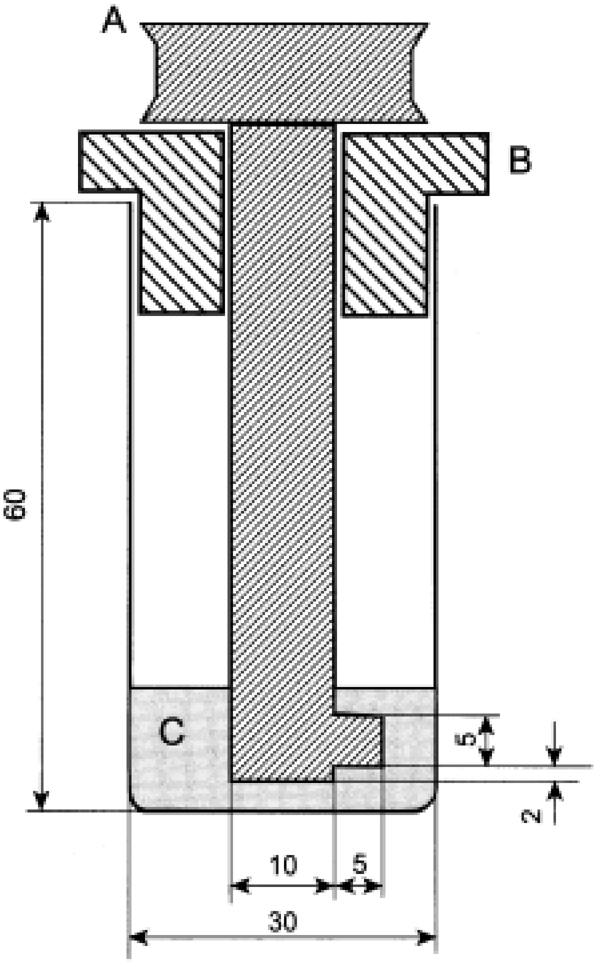
ASSAY

The potency of tetracosactide is estimated by comparing in given conditions its activity in increasing the amount of corticosterone produced by isolated rat adrenal cells with that of the International Reference Preparation of tetracosactide or a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the International Reference Preparation which consists of a quantity of synthetic tetracosactide with mannitol. The equivalence in International Units of the International Reference Preparation is stated by the World Health Organisation.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) of the estimated potency are not less than 64 per cent and not more than 156 per cent of the stated potency.

Use siliconised glassware and rinse well with *water R* before use. Euthanise four male rats, each weighing between 200 g and 400 g, by exsanguination. Remove the adrenal glands, carefully free them of adhering fat and immerse in solution B maintained at 4 °C. Cut each gland into four equal pieces and transfer to a suitable plastic stirring apparatus (see Figure 0644.-1) containing 5 ml of solution C maintained at 37 °C. Disperse the adrenal cells by stirring the mixture at 500 r/min. After 20 min, remove the supernatant liquid, cool to 4 °C, add 5 ml of solution C and repeat the dispersal procedure. Repeat the operation a further three times. Combine the five supernatant liquids, centrifuge at 4 °C in a polyethylene test-tube for 30 min after slow acceleration to 100 g. Suspend the resulting pellet in 8 ml of solution D and centrifuge for 30 min at 100 g. Again suspend the residue in 8 ml of solution D and filter the mixture through nylon gauze with 100 µm pores into a polyethylene beaker and add a suitable volume of solution D to the filtrate (a total volume of 65 ml to 105 ml has been found suitable). Maintain the resulting suspension at 4 °C.



A - pulley and stirring paddle,

B - bearing,

C - incubation medium.

Figure 0644.-1. – *Stirring Apparatus*

DIMENSIONS IN MILLIMETRES

Prepare four independent dilutions, with two-fold dose intervals, from solutions of suitable concentrations of the substance to be examined and of the reference preparation using solution E as diluent. Pipette 0.1 ml of each dilution into each of four polystyrene test-tubes and add 1.0 ml of the cell suspension prepared above to each tube. Incubate at 37 °C for 2 h and then cool to 4 °C. Transfer 1 ml of the contents of each tube to a glass tube containing 1.4 ml of *methylene chloride R* , mix in a vortex mixer for 10 s and centrifuge at 3000 g for 5 min. Transfer 1 ml of each of the methylene chloride layers, avoiding taking up aqueous phase, to a glass tube containing 0.6 ml of a mixture of 15 volumes of *alcohol R* and 35 volumes of *sulphuric acid R* . Mix in a vortex mixer for 10 s and centrifuge at 1500 g for 5 min. Allow each tube to stand for 30 min. Examine by fluorimetry *(2.2.21)* , irradiating the lower layer with an excitant beam of suitable wavelength such as 436 nm or 470 nm and measuring the fluorescence at the maximum between 530 nm and 545 nm. If the solutions are not transferred to spectrophotometric measurement cells, select glass tubes that give fluorescence values for a standard corticosterone which do not differ from each other by more than 5 per cent. Calculate the result of the assay by the usual statistical methods using the linear portion of the log dose-response curve.

Solution A

Sodium chloride R	6.60 g
Potassium chloride R	0.353 g
Sodium hydrogen carbonate R	0.840 g
Potassium dihydrogen phosphate	0.161 g
Magnesium sulphate R	0.291 g
Calcium chloride R	0.373 g
2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethane sulphonic acid R	4.77 g

Dissolve the above ingredients in about 950 ml of *water R*, adjust to pH 7.4 with *1 M sodium hydroxide* and add 60 mg of *benzylpenicillin sodium R* and a quantity of *streptomycin sulphate R* equivalent to 100 mg of streptomycin. Dilute to 1000 ml with *water R.*

Solution B Add 2 g/l of *glucose R* to solution A.

Solution C To solution B add 1 g/l of a preparation of collagenase obtained from Clostridium histolyticum and of a grade suitable for the preparation of dispersed cells.

Solution D Add 5 g/l of *bovine albumin R* to solution B.

Solution E A 9 g/l sterile solution of *sodium chloride R* containing 1 g/l of *bovine albumin R* and adjusted to pH 2.0 with *1 M hydrochloric acid* .

STORAGE

Store under nitrogen, protected from light, at a temperature between 2 °C and 8 °C.

LABELLING

The label states:

— the potency in International Units per milligram;

— the peptide content per container;

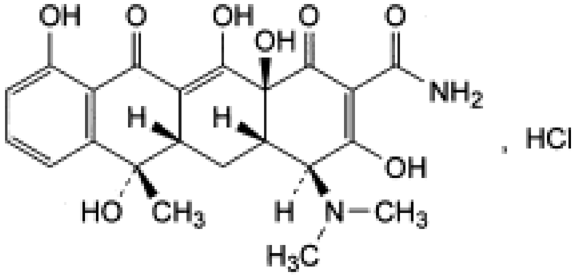
— the storage conditions.

Ph Eur

Tetracycline Hydrochloride

General Notices

(Ph Eur monograph 0210)



$C_{22}H_{24}N_2O_8$,HCl 480.9 *64-75-5*

Action and use

Tetracycline antibacterial.

Preparations

Tetracycline Capsules

Tetracycline Tablets

Ph Eur

DEFINITION

(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by certain strains of *Streptomyces aerofaciens* or obtained by any other means.

Content

95.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone. It dissolves in solutions of alkali hydroxides and carbonates. Solutions in water become turbid on standing, owing to the precipitation of tetracycline.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *tetracycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *tetracycline hydrochloride CRS* , 5 mg of *demeclocycline hydrochloride R* and 5 mg of *oxytetracycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel F* $_{254}$ *plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R,* 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R* .

Application 1 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R* . A violet-red colour develops. Add the solution to 2.5 ml of *water R* . The colour becomes yellow.

C. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

pH *(2.2.3)*

1.8 to 2.8.

Dissolve 0.1 g in 10 ml of *carbon dioxide-free water R* .

Specific optical rotation *(2.2.7)*

- 240 to - 255 (dried substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use*.

Test solution Dissolve 25.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 15.0 mg of *4-epitetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solution (c) Dissolve 10.0 mg of *anhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid.

Reference solution (d) Dissolve 10.0 mg of *4-epianhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solution (e) Mix 1.0 ml of reference solution (a), 2.0 ml of reference solution (b) and 5.0 ml of reference solution (d) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (f) Mix 20.0 ml of reference solution (b), 10.0 ml of reference solution (c) and 5.0 ml of reference solution (d) and dilute to 200.0 ml using *0.01 M hydrochloric acid*.

Reference solution (g) Dilute 1.0 ml of reference solution (c) to 50.0 ml with *0.01 M hydrochloric acid*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: styrene-divinylbenzene copolymer R* (8 µm);

— *temperature*: 60 °C.

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R;* add 100 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 9.0 with *dilute phosphoric acid R*, 200 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 ml with *water R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl; inject the test solution and reference solutions (e), (f) and (g).

System suitability

— *resolution*: minimum 2.5 between the peaks due to impurity A (1st peak) and tetracycline (2nd peak) and minimum 8.0 between the peaks due to tetracycline and impurity D (3rd peak) in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of 2-methyl-2-propanol in the mobile phase;

— *signal-to-noise ratio:* minimum 3 for the principal peak in the chromatogram obtained with reference solution (g);

— *symmetry factor:* maximum 1.25 for the peak due to tetracycline in the chromatogram obtained with reference solution (e).

Limits:

— *impurity A:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (3.0 per cent);

— *impurity B* (eluting on the tail of the principal peak): not more than 0.5 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (1.5 per cent);

— *impurity C:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent);

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent).

Heavy metals (*2.4.8*)

Maximum 50 ppm.

0.5 g complies with test C. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 2.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

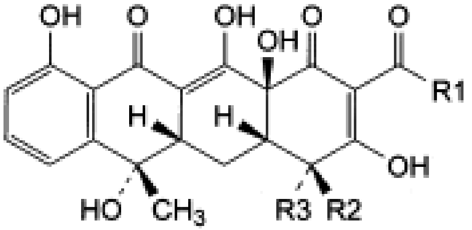
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$.

STORAGE

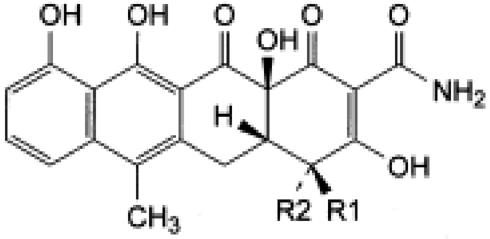
Protected from light. If the substance is sterile, store in a sterile, tamper-proof container.

IMPURITIES



A. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$: (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),

B. R1 = CH_3, R2 = $N(CH_3)_2$. R3 = H: (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyltetracycline),



C. R1 = $N(CH_3)_2$, R2 = H: (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

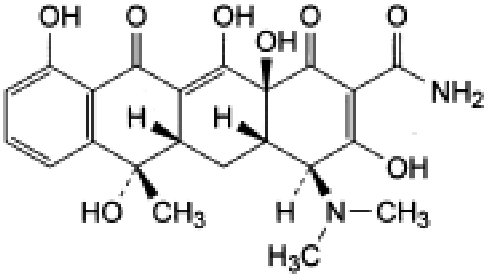
D. R1 = H, R2 = $N(CH_3)_2$: (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline).

Ph Eur

Tetracycline

General Notices

(Ph Eur monograph 0211)



$C_{22}H_{24}N_2O_8$ 444.4 *60-54-8*

Action and use

Tetracycline antibacterial.

Ph Eur

DEFINITION

(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

Substance produced by certain strains of *Streptomyces aerofaciens* or obtained by any other means.

Content

88.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Very slightly soluble in water, soluble in ethanol (96 per cent) and in methanol, sparingly soluble in acetone. It dissolves in dilute acid and alkaline solutions.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *tetracycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *tetracycline hydrochloride CRS* , 5 mg of *demeclocycline hydrochloride R* and 5 mg of *oxytetracycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R*.

Mobile phase Mix 20 volumes of *acetonitrile R* , 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R* .

Application 1 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R* . A violet-red colour develops. Add the solution to 2.5 ml of *water R* . The colour becomes yellow.

C. Dissolve about 10 mg in a mixture of 1 ml of *dilute nitric acid R* and 5 ml of *water R* . Shake and add 1 ml of *silver nitrate solution R2* . Any opalescence in the solution is not more intense than that in a mixture of 1 ml of *dilute nitric acid R* , 5 ml of *water R* and 1 ml of *silver nitrate solution R2* .

TESTS

pH *(2.2.3)*

3.5 to 6.0.

Suspend 0.1 g in 10 ml of *carbon dioxide-free water R* .

Specific optical rotation *(2.2.7)*

- 260 to - 280 (dried substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Related substances

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use*.

Test solution Dissolve 25.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a) Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b) Dissolve 12.5 mg of *4-epitetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solution (c) Dissolve 10.0 mg of *anhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid.

Reference solution (d) Dissolve 10.0 mg of *4-epianhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solution (e) Mix 1.0 ml of reference solution (a), 2.0 ml of reference solution (b) and 5.0 ml of reference solution (d) and dilute to 25.0 ml with *0.01 M hydrochloric acid* .

Reference solution (f) Mix 40.0 ml of reference solution (b), 20.0 ml of reference solution (c) and 5.0 ml of reference solution (d) and dilute to 200.0 ml with *0.01 M hydrochloric acid.*

Reference solution (g) Dilute 1.0 ml of reference solution (c) to 50.0 ml with *0.01 M hydrochloric acid.*

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: styrene-divinylbenzene copolymer R* (8 µm);
- *temperature*: 60 °C.

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R* ; add 100 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 9.0 with dilute *phosphoric acid R* , 200 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R* ; dilute to 1000.0 ml with *water R* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl; inject the test solution and reference solutions (e), (f) and (g).

System suitability

- *resolution*: minimum 2.5 between the peaks due to impurity A (1st peak) and tetracycline (2nd peak) and minimum 8.0 between the peaks due to tetracycline and impurity D (3rd peak) in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of 2-methyl-2-propanol in the mobile phase;
- *signal-to-noise ratio* : minimum 3 for the principal peak in the chromatogram obtained with reference solution (g);
- *symmetry factor* : maximum 1.25 for the peak due to tetracycline in the chromatogram obtained with reference solution (e).

Limits:

— *impurity A:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (5.0 per cent);

— *impurity B* (eluting on the tail of the principal peak): not more than 0.4 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (2.0 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (1.0 per cent);

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent).

Heavy metals ***(2.4.8)***

Maximum 50 ppm.

0.5 g complies with test C. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 13.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

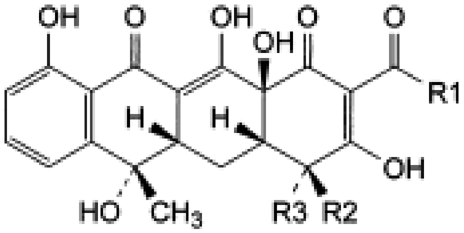
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{24}N_2O_8$.

STORAGE

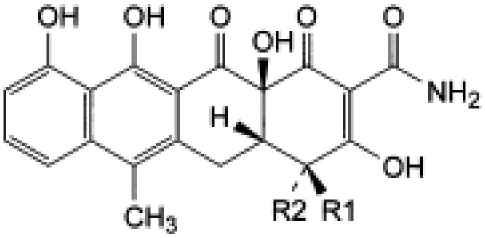
Protected from light.

IMPURITIES



A. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$: (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),

B. R1 = CH_3, R2 = $N(CH_3)_2$, R3 = H: (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyltetracycline),



C. R1 = $N(CH_3)_2$, R2 = H: (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

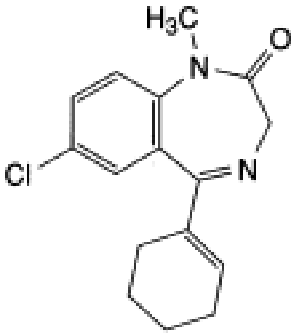
D. R1 = H, R2 = $N(CH_3)_2$: (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline).

Ph Eur

Tetrazepam

General Notices

(Ph Eur monograph 1738)



$C_{16}H_{17}ClN_2O$ 288.8 *10379-14-3*

Action and use

Benzodiazepine; hypnotic.

Ph Eur

DEFINITION

7-Chloro-5-(cyclohex-1-enyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Light yellow or yellow crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in acetonitrile.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of tetrazepam.*

TESTS

Related substances

Liquid chromatography *(2.2.29).*

Test solution Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of the substance to be examined and 5.0 mg of *tetrazepam impurity C CRS* in *acetonitrile R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *acetonitrile R* .

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with *acetonitrile R* . Dilute 1.0 ml of this solution to 10.0 ml with *acetonitrile R* .

Column:

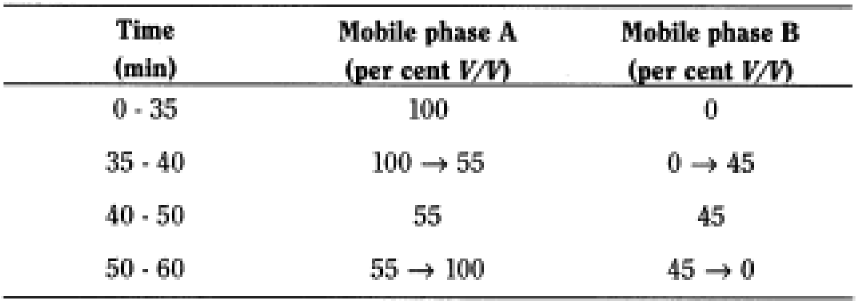
— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 40 volumes of *acetonitrile R* and 60 volumes of a 3.4 g/l solution of *potassium dihydrogen phosphate R* ;

— *mobile phase B: acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100	0
35 - 40	100 → 55	0 → 45
40 - 50	55	45
50 - 60	55 → 100	45 → 0

Flow rate 1.5 ml/min.

Detection A spectrophotometer at 229 nm.

Injection 20 µl.

System suitability Reference solution (a):

— *resolution:* minimum 2.0 between the peaks due to tetrazepam and to impurity C.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides ***(2.4.4)***

Maximum 100 ppm.

Dissolve 0.750 g in 10 ml of *methylene chloride R* and add 15 ml of *water R*. Shake and separate the 2 layers. Dilute 10 ml of the aqueous layer to 15 ml with *water R*. The solution obtained complies with the limit test for chlorides.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

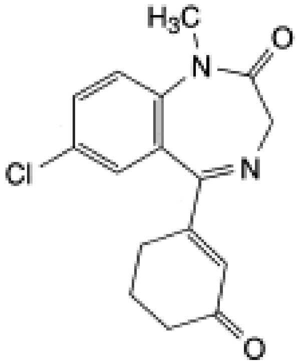
Dissolve 0.230 g in 50.0 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 28.88 mg of $C_{16}H_{17}ClN_2O$.

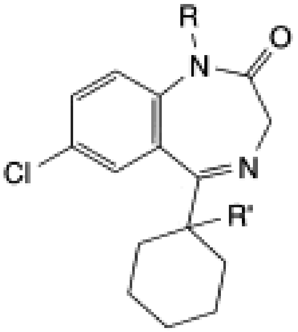
STORAGE

Protected from light.

IMPURITIES



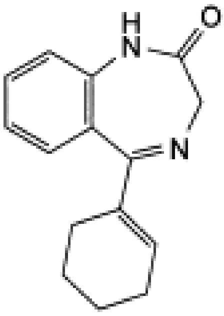
A. 7-chloro-5-(3-oxocyclohex-1-enyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



B. R = R′ = H: 7-chloro-5-cyclohexyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,

C. R = CH_3, R′ = H: 7-chloro-5-cyclohexyl-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,

D. R = CH_3, R′ = Cl: 7-chloro-5-(1-chlorocyclohexyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



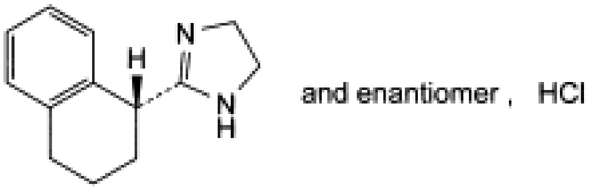
E. 5-(cyclohex-1-enyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Ph Eur

Tetryzoline Hydrochloride

General Notices

(Ph Eur monograph 2101)



$C_{13}H_{17}ClN_2$ 236.7 *522-48-5*

Action and use

Adrenoceptor agonist; decongestant.

Ph Eur

DEFINITION

2-[(1*RS*)-1,2,3,4-Tetrahydronaphthalen-1-yl]-4,5-dihydro-1*H*-imidazole hydrochloride.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, in anhydrous ethanol and in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison tetryzoline hydrochloride CRS.

B. Dissolve 50 mg in 10 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution.

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in *water R* and dilute to 10 ml with the same solvent.

Related substances

Gas chromatography *(2.2.28)*.

Test solution Dissolve 1.0 g of the substance to be examined in a mixture of 25 volumes of *1 M sodium hydroxide* and 75 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 25 volumes of *1 M sodium hydroxide* and 75 volumes of *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with a mixture of 25 volumes of *1 M sodium hydroxide* and 75 volumes of *methanol R*.

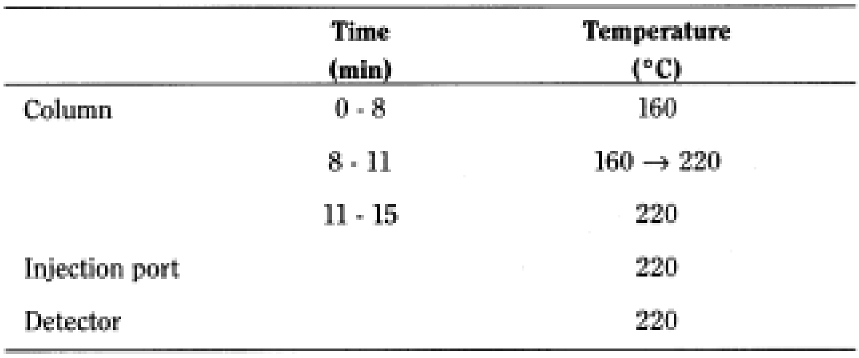
Column:
- *material:* fused silica;
- *size*: *l* = 25 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)siloxane R* (1 µm).

Carrier gas *helium for chromatography R*.

Split ratio 1:40.

Flow rate 2.5 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 8	160
	8 - 11	160 → 220
	11 - 15	220
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to tetryzoline (retention time = about 12 min): impurity A = about 0.5.

System suitability Reference solution:

— *signal-to-noise ratio* : minimum 50 for the principal peak.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent);

— *total:* not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent);

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

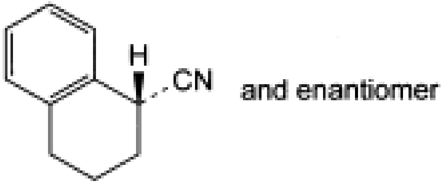
ASSAY

Dissolve 0.200 g in 100 ml of a mixture of 3 volumes of *anhydrous acetic acid R* and 7 volumes of *acetic anhydride R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* . Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 23.67 mg of $C_{13}H_{17}ClN_2$.

IMPURITIES

Specified impurities *A.*



A. (1*RS*)-1,2,3,4-tetrahydronaphthalene-1-carbonitrile (α-cyanotetraline).

Ph Eur

Theobroma Oil

General Notices

Cocoa Butter

DEFINITION

Theobroma Oil is the solid fat obtained from the roasted seeds of *Theobroma cacao* L.

CHARACTERISTICS

A yellowish white, solid fat; odour, slight, agreeable and resembling that of cocoa. Somewhat brittle.

Freely soluble in *ether* and in *petroleum spirit (boiling range, 40° to 60°)*; slightly soluble in *ethanol (96%)* .

TESTS

Acid value

Not more than 4.0, Appendix X B.

Iodine value

35 to 40 (*iodine monochloride method*), Appendix X E.

Melting point

31° to 34°, Appendix V A, Method IV. Prepare the substance being examined in the following manner. Melt about 30 g in an oven at a temperature of 55° to 60° and filter through a suitable dry filter paper, maintaining the temperature between 53° and 60°. Cool with occasional stirring until the temperature falls to between 32° and 34°, stir continuously with a mechanical stirrer until the first signs of cloudiness appear and continue to stir by hand until the substance has the consistence of a paste. Immediately transfer to a vessel previously kept at a temperature of 15° to 22° and allow to stand at this temperature for 24 hours before carrying out the test.

Refractive index

At 40°, 1.456 to 1.458, Appendix V E.

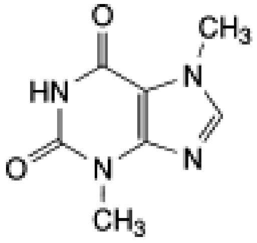
Saponification value

188 to 196, Appendix X G.

Theobromine

General Notices

(Ph Eur monograph 0298)



$C_7H_8N_4O_2$ 180.2 *83-67-0*

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

Ph Eur

DEFINITION

Theobromine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, very slightly soluble in water and in ethanol, slightly soluble in ammonia. It dissolves in dilute solutions of alkali hydroxides and in mineral acids.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *theobromine CRS*.

B. Dissolve about 20 mg in 2 ml of *dilute ammonia R1*, warming slightly, and cool. Add 2 ml of *silver nitrate solution R2*. The solution remains clear. Boil the solution for a few minutes. A white, crystalline precipitate is formed.

C. It gives the reaction of xanthines *(2.3.1)* .

TESTS

Acidity

To 0.4 g add 20 ml of boiling *water R* and boil for 1 min. Allow to cool and filter. Add 0.05 ml of *bromothymol blue solution R1* . The solution is yellow or yellowish-green. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substance

Examine by thin-layer chromatography *(2.2.27)* , using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution To 0.2 g of the finely powdered substance to be examined add 10 ml of a mixture of 4 volumes of *methanol R* and 6 volumes of *chloroform R* . Heat under a reflux condenser on a water-bath for 15 min, shaking occasionally. Cool and filter.

Reference solution Dissolve 5 mg of *theobromine CRS* in a mixture of 4 volumes of *methanol R* and 6 volumes of *chloroform R* and dilute to 50 ml with the same mixture of solvents.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R* , 30 volumes of *acetone R,* 30 volumes of *chloroform R* and 40 volumes of *butanol R* . Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R* .

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 125 ml of boiling *water R* , cool to 50 °C to 60 °C and add 25 ml of *0.1 M silver nitrate* . Using 1 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

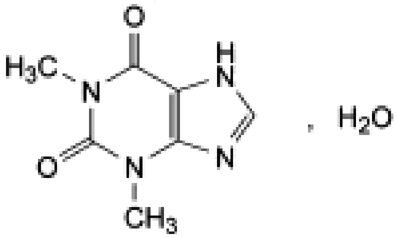
1 ml of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

Ph Eur

Theophylline Hydrate

General Notices

(Theophylline Monohydrate, Ph Eur monograph 0302)



$C_7H_8N_4O_2,H_2O$ 198.2 *5967-84-0*

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

Preparations

Aminophylline Injection

Prolonged-release Theophylline Tablets

Ph Eur

DEFINITION

1,3-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione monohydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in ethanol. It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D, E.*

A. Melting point *(2.2.14)* : 270 °C to 274 °C, determined after drying at 100-105 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Dry the substance to be examined at 100-105 °C before use.

Comparison *Ph. Eur. reference spectrum of theophylline.*

C. Heat 10 mg with 1.0 ml of a 360 g/l solution of *potassium hydroxide R* in a water-bath at 90 °C for 3 min, then add 1.0 ml of *diazotised sulphanilic acid solution R* . A red colour slowly develops. Carry out a blank test.

D. It complies with the test for water (see Tests).

E. It gives the reaction of xanthines *(2.3.1)* .

TESTS

Solution S

Dissolve 0.5 g with heating in *carbon dioxide-free water R* , cool and dilute to 75 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity

To 50 ml of solution S add 0.1 ml of *methyl red solution R* . The solution is red. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *theobromine R* in the mobile phase, add 5 ml of the test solution and dilute to 100 ml with the mobile phase. Dilute 5 ml of this solution to 50 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 7 volumes of *acetonitrile for chromatography R* and 93 volumes of a 1.36 g/l solution of *sodium acetate R* containing 5.0 ml/l of *glacial acetic acid R* .

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 272 nm.

Injection 20 µl.

Run time 3.5 times the retention time of theophylline.

Relative retention With reference to theophylline (retention time = about 6 min): impurity C = about 0.3; impurity B = about 0.4; impurity D = about 0.5; impurity A = about 2.5.

System suitability Reference solution (b):

- *resolution:* minimum 2.0 between the peaks due to theobromine and theophylline.

Limits:

- *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

8.0 per cent to 9.5 per cent, determined on 0.20 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g in 100 ml of *water R* , add 20 ml of *0.1 M silver nitrate* and shake. Add 1 ml of *bromothymol blue solution R1* . Titrate with *0.1 M sodium hydroxide* .

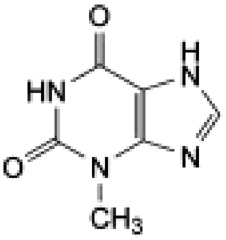
1 ml of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

IMPURITIES

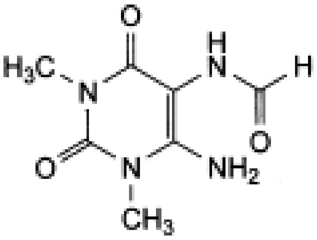
Specified impurities A, B, C, D.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F.*

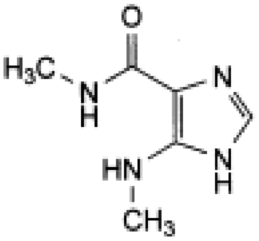
A. caffeine,



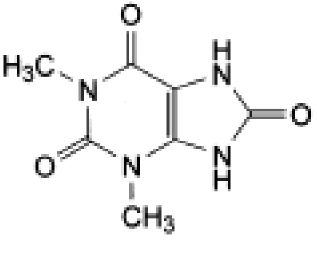
B. 3-methyl-3,7-dihydro-1*H*-purine-2,6-dione,



C. *N*-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide,



D. *N*-methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide,



E. 1,3-dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione,

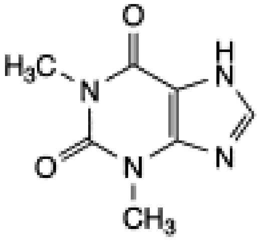
F. etofylline.

Ph Eur

Theophylline

General Notices

(Ph Eur monograph 0299)



$C_7H_8N_4O_2$ 180.2 *58-55-9*

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

Preparations

Aminophylline Injection

Prolonged-release Theophylline Tablets

Ph Eur

DEFINITION

1,3-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in ethanol. It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D, E.*

A. Melting point *(2.2.14)*

270 °C to 274 °C, determined after drying at 100-105 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of theophylline.*

C. Heat 10 mg with 1.0 ml of a 360 g/l solution of *potassium hydroxide R* in a water-bath at 90 °C for 3 min, then add 1.0 ml of *diazotised sulphanilic acid solution R* . A red colour slowly develops. Carry out a blank test.

D. It complies with the test for loss on drying (see Tests).

E. It gives the reaction of xanthines *(2.3.1)* .

TESTS

Solution S

Dissolve 0.5 g with heating in *carbon dioxide-free water R* , cool and dilute to 75 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity

To 50 ml of solution S add 0.1 ml of *methyl red solution R* . The solution is red. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography (2.2.29).

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 10 mg of *theobromine R* in the mobile phase, add 5 ml of the test solution and dilute to 100 ml with the mobile phase. Dilute 5 ml of this solution to 50 ml with the mobile phase.

Column:

- — *size*: *l* = 0.25 m, Ø = 4 mm;
- — *stationary phase: octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 7 volumes of *acetonitrile for chromatography R* and 93 volumes of a 1.36 g/l solution of *sodium acetate R* containing 5.0 ml/l of *glacial acetic acid R*.

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 272 nm.

Injection 20 µl.

Run time 3.5 times the retention time of theophylline.

Relative retention With reference to theophylline (retention time = about 6 min): impurity C = about 0.3; impurity B = about 0.4; impurity D = about 0.5; impurity A = about 2.5.

System suitability Reference solution (b):

- — *resolution*: minimum 2.0 between the peaks due to theobromine and theophylline.

Limits:

- — *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- — *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- — *total:* not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 100 ml of *water R,* add 20 ml of *0.1 M silver nitrate* and shake. Add 1 ml of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide*.

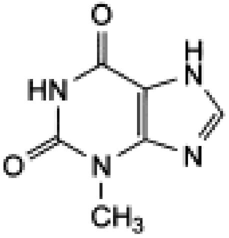
1 ml of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

IMPURITIES

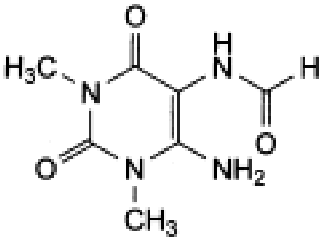
Specified impurities *A, B, C, D.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F.*

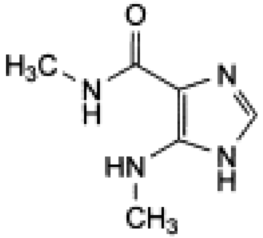
A. caffeine,



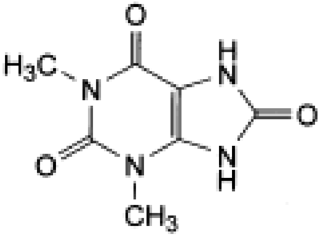
B. 3-methyl-3,7-dihydro-1*H*-purine-2,6-dione,



C. *N*-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide,



D. *N*-methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide,



E. 1,3-dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione,

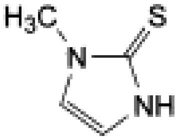
F. etofylline.

Ph Eur

Thiamazole

General Notices

(Ph Eur monograph 1706)



$C_4H_6N_2S$ 114.2 *60-56-0*

Action and use

Thionamide antithyroid.

Ph Eur

DEFINITION

1-Methyl-1,3-dihydro-2*H*-imidazole-2-thione.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or pale brown, crystalline powder.

Solubility

Freely soluble in water, freely soluble in methylene chloride, freely soluble or soluble in ethanol (96 per cent).

IDENTIFICATION

First identification A, C.

Second identification A, B, D.

A. Melting point *(2.2.14)* : 143 °C to 146 °C.

B. Dissolve 25 mg in 10 ml of a 0.28 per cent *V/V* solution of *sulphuric acid R* and dilute to 50.0 ml with the same solution. Dilute 1.0 ml of this solution to 100.0 ml with a 0.28 per cent *V/V* solution of *sulphuric acid R*. Examined between 200 nm and 300 nm *(2.2.25),* the solution shows 2 absorption maxima, at 211 nm and 251 nm. The ratio of the absorbance measured at the absorption maximum at 251 nm to that measured at the absorption maximum at 211 nm is 2.5 to 2.7.

C. Infrared absorption spectrophotometry *(2.2.24).*

Preparation Discs.

Comparison *thiamazole CRS*.

D. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 5.0 mg of the substance to be examined in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *thiamazole CRS* in *methanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dissolve 5.0 mg of 2-*methylimidazole R* in *methanol R* and dilute to 5.0 ml with the same solvent. Dilute 1.0 ml of this solution to 2.0 ml with the test solution.

Plate *TLC silica gel F_{254} plate R* .

Mobile phase *concentrated ammonia R1, 2-propanol R, toluene R* (1:24:75 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— expose the plate to iodine vapour for 30 min; the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

Related substances

Gas chromatography *(2.2.28)* .

Test solution Dissolve 0.100 g of the substance to be examined in *chloroform R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *chloroform R* . Dilute 1.0 ml of this solution to 10.0 ml with *chloroform R.*

Reference solution (b) Dissolve 5.0 mg of *thiamazole impurity A CRS*, 5.0 mg of 1-*methylimidazole R1* and 5.0 mg of *thiamazole impurity C CRS* in *chloroform R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *chloroform R* .

Column:

— *material:* fused silica;

— *size*: l = 30.0 m, Ø = 0.25 mm;

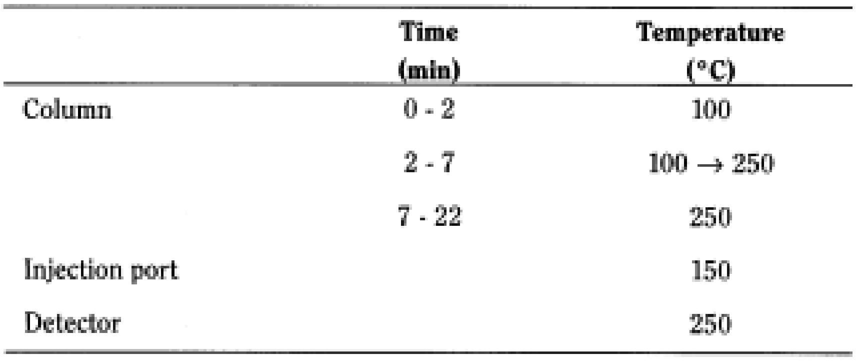
— *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* with special deactivation for basic compounds (film thickness 0.5 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1.5 ml/min.

Split ratio 3:20.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	100
	2 - 7	100 → 250
	7 - 22	250
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Relative retention With reference to thiamazole (retention time = about 6.5 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.7.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity A and impurity B.

Limits

— *impurities A, B, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total:* not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit:* 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

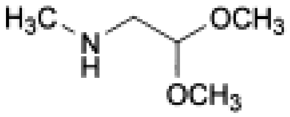
ASSAY

Dissolve 0.250 g in 75 ml of *water R* . Add 15.0 ml of *0.1 M sodium hydroxide* , mix and add with stirring, about 30 ml of *0.1 M silver nitrate* . Continue the titration with *0.1 M sodium hydroxide* , determining the end-point potentiometrically *(2.2.20)* .

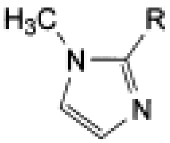
1 ml of *0.1 M sodium hydroxide* is equivalent to 11.42 mg of $C_4H_6N_2S$.

IMPURITIES

Specified impurities *A, B, C.*



A. 2,2-dimethoxy-*N*-methylethanamine,



B. R = H: 1-methyl-1*H*-imidazole,

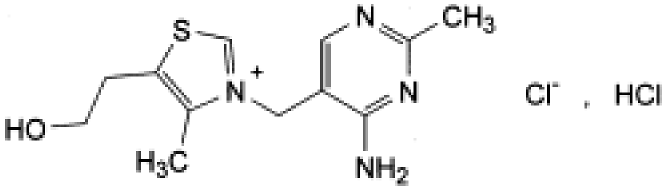
C. R = SCH_3: 1-methyl-2-(methylsulphanyl)-1*H*-imidazole.

Ph Eur

Thiamine Hydrochloride

General Notices

(Ph Eur monograph 0303)



$C_{12}H_{17}ClN_4OS,HCl$ 337.3 *67-03-8*

Action and use

Vitamin B_1.

Preparations

Thiamine Injection

Thiamine Tablets

Vitamins B and C Injection

Ph Eur

DEFINITION

3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, soluble in glycerol, slightly soluble in alcohol.

IDENTIFICATION

First identification *A, C.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *thiamine hydrochloride CRS* .

B. Dissolve about 20 mg in 10 ml of *water R,* add 1 ml of *dilute acetic acid R* and 1.6 ml of *1 M sodium hydroxide* , heat on a water-bath for 30 min and allow to cool. Add 5 ml of *dilute sodium hydroxide solution R* , 10 ml of *potassium ferricyanide solution R* and 10 ml of *butanol R* and shake vigorously for 2 min. The upper alcoholic layer shows an intense light-blue fluorescence, especially in ultraviolet light at 365 nm. Repeat the test using 0.9 ml of *1 M sodium hydroxide* and 0.2 g of *sodium sulphite R* instead of 1.6 ml of *1 M sodium hydroxide* . Practically no fluorescence is seen.

C. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 25 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 or GY_7 *(2.2.2, Method II)*.

Dilute 2.5 ml of solution S to 5 ml with *water R* .

pH *(2.2.3)*

2.7 to 3.3.

Dilute 2.5 ml of solution S to 10 ml with *water R* .

Related substances

Liquid chromatography *(2.2.29)* .

Solution A Add 5 volumes of *glacial acetic acid R* to 95 volumes of *water R* and mix.

Test solution Dissolve 0.35 g of the substance to be examined in 15.0 ml of solution A and dilute to 100.0 ml with *water R* .

Reference solution (a) Dissolve 5 mg of the substance to be examined and 5 mg of *thiamine impurity E CRS* in 4 ml of solution A and dilute to 25.0 ml with *water R* . Dilute 5.0 ml of the solution to 25.0 ml with *water R* .

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with *water R* . Dilute 5.0

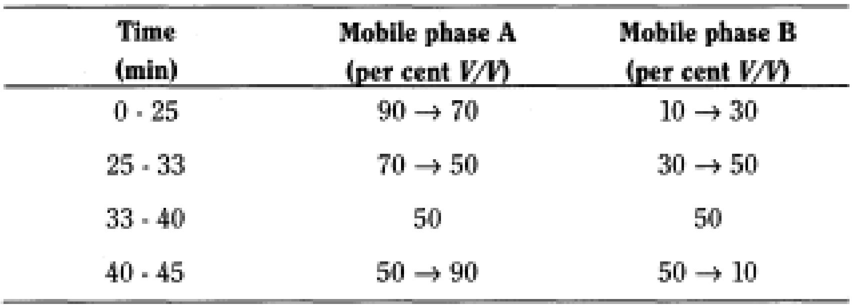
ml of this solution to 25.0 ml with *water R* .

Column:

- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase:* spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m2/g and a pore size of 10 nm;
- *temperature*: 45 °C.

Mobile phase:

- *mobile phase A*: 3.764 g/l solution of *sodium hexanesulphonate R* adjusted to pH 3.1 with *phosphoric acid R;*
- *mobile phase B: methanol R2* ,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	90 → 70	10 → 30
25 - 33	70 → 50	30 → 50
33 - 40	50	50
40 - 45	50 → 90	50 → 10

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 248 nm.

Injection 25 µl.

Relative retention With reference to thiamine (retention time = about 30 min): impurity A = about 0.3; impurity B = about 0.9; impurity C = about 1.2.

System suitability Reference solution (a):

- *resolution*: minimum 1.6 between the peaks due to impurity E and to thiamine.

Limits:

- *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);
- *total:* not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.125 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphates ***(2.4.13)***

Maximum 300 ppm.

5 ml of solution S diluted to 15 ml with *distilled water R* complies with the limit test for sulphates.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 5.0 per cent, determined on 0.40 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.110 g in 5 ml of *anhydrous formic acid R* and add 50 ml of *acetic anhydride R* . Titrate immediately with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* and carrying out the titration within 2 min. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 16.86 mg of $C_{12}H_{18}Cl_2N_4OS$.

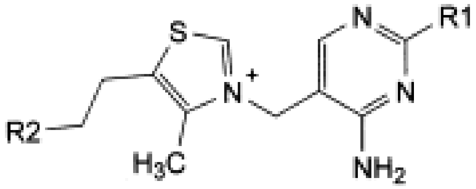
STORAGE

In a non-metallic container, protected from light.

IMPURITIES

Specified impurities *A, B, C.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D, E, F, G, H.*



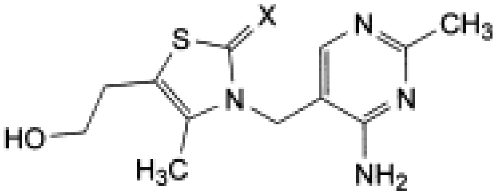
A. R1 = CH_3, R2 = O-SO_3-: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-5-[2-(sulphonatooxy)ethyl]thiazolium (thiamine sulphate ester),

B. R1 = H, R2 = OH: 3-[(4-aminopyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium (desmethylthiamine),

C. R1 = CH_3, R2 = Cl: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-chloroethyl)-4-methylthiazolium (chlorothiamine),

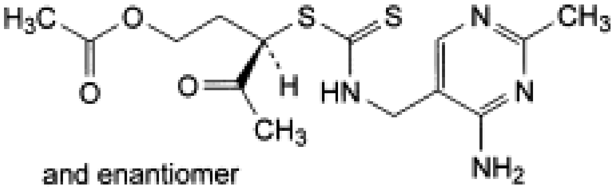
F. R1 = C_2H_5, R2 = OH: 3-[(4-amino-2-ethylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium (ethylthiamine),

G. R1 = CH_3, R2 = O-CO-CH_3: 5-[2-(acetyloxy)ethyl]-3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methylthiazolium (acetylthiamine),


X
S
N
N
CH3
N
HO
H3C
NH2

D. X = O: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazol-2(3*H*)-one (oxothiamine),

E. X = S: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazol-2(3*H*)-thione (thioxothiamine),


H3C
O
S
S
N
CH3
O
O
H
HN
N
CH3
and enantiomer
NH2

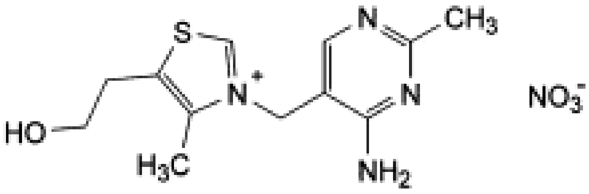
H. (3*RS*)-3-[[[(4-amino-2-methylpyrimidin-5-yl)methyl]thiocarbamoyl]sulphanyl]-4-oxopentyl acetate (ketodithiocarbamate).

Ph Eur

Thiamine Nitrate

General Notices

(Ph Eur monograph 0531)



$C_{12}H_{17}N_5O_4S$ 327.4 *532-43-4*

Action and use

Vitamin B_1.

Ph Eur

DEFINITION

3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium nitrate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or small, colourless crystals.

Solubility

Sparingly soluble in water, freely soluble in boiling water, slightly soluble in alcohol and in methanol.

IDENTIFICATION

First identification *A, C.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of thiamine nitrate.*

B. Dissolve about 20 mg in 10 ml of *water R,* add 1 ml of *dilute acetic acid R* and 1.6 ml of *1 M sodium hydroxide* , heat on a water-bath for 30 min and allow to cool. Add 5 ml of dilute *sodium hydroxide solution R* , 10 ml of *potassium ferricyanide solution R* and 10 ml of *butanol R* and shake vigorously for 2 min. The upper alcoholic layer shows an intense light-blue fluorescence, especially in ultraviolet light at 365 nm. Repeat the test using 0.9 ml of *1 M sodium hydroxide* and 0.2 g of *sodium sulphite R* instead of 1.6 ml of *1 M sodium hydroxide* . Practically no fluorescence is produced.

C. About 5 mg gives the reaction of nitrates *(2.3.1)* .

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

6.8 to 7.6 for solution S.

Related substances

Liquid chromatography *(2.2.29)* .

Solution A Add 5 volumes of *glacial acetic acid R* to 95 volumes of *water R* and mix.

Test solution Dissolve 0.35 g of the substance to be examined in 15.0 ml of solution A and dilute to 100.0 ml with *water R* .

Reference solution (a) Dissolve 5 mg of the substance to be examined and 5 mg of *thiamine impurity E CRS* in 4 ml of solution A and dilute to 25.0 ml with *water R* . Dilute 5.0 ml of the solution to 25.0 ml with *water R* .

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *water R* .

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *spherical end-capped octadecylsilyl silica gel for chromatography R* (4 µm) with a specific surface area of 350 m2/g and a pore size of 10 nm;

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A*: 3.764 g/l solution of *sodium hexanesulphonate R* adjusted to pH 3.1 with *phosphoric acid R* ;

— *mobile phase B*: *methanol R2* ;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	90 → 70	10 → 30
25 - 33	70 → 50	30 → 50
33 - 40	50	50
40 - 45	50 → 90	50 → 10

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 248 nm.

Injection 25 µl.

Relative retention With reference to thiamine (retention time = about 30 min): impurity A = about 0.3; impurity B = about 0.9; impurity C = about 1.2.

System suitability Reference solution (a):

— *resolution*: minimum 1.6 between the peaks due to impurity E and to thiamine.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.140 g in 5 ml of *anhydrous formic acid R* and add 50 ml of *acetic anhydride R*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)* and carrying out the titration within 2 min. Carry out a blank titration.

1.0 ml of *0.1 M perchloric acid* is equivalent to 16.37 mg of $C_{12}H_{17}N_5O_4S$.

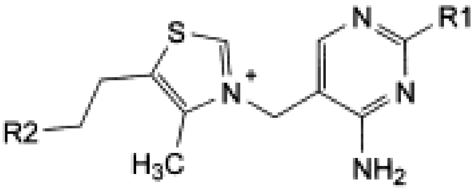
STORAGE

In a non-metallic container, protected from light.

IMPURITIES

Specified impurities   A, B, C.

Other detectable impurities   (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D, E, F, G, H.*



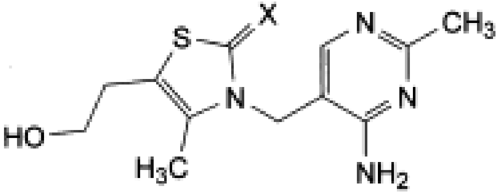
A. R1 = CH_3, R2 = O-SO_3-: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-5-[2-(sulphonatooxy)ethyl]thiazolium (thiamine sulphate ester),

B. R1 = H, R2 = OH: 3-[(4-aminopyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium (desmethylthiamine),

C. R1 = CH_3, R2 = Cl: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-chloroethyl)-4-methylthiazolium (chlorothiamine),

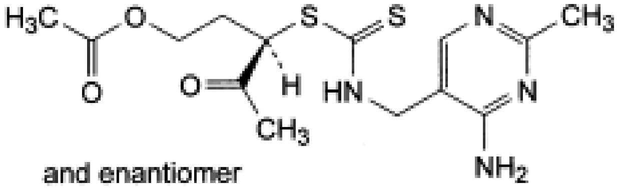
F. R1 = C_2H_5, R2 = OH: 3-[(4-amino-2-ethylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium (ethylthiamine),

G. R1 = CH_3, R2 = O-CO-CH_3: 5-[2-(acetyloxy)ethyl]-3-[(4-amino-2-methylpyrimidin-5-yl) methyl]-4-methylthiazolium (acetylthiamine),



D. X = O: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazol-2 (3*H*)-one (oxothiamine),

E. X = S: 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazol-2 (3*H*)-thione (thioxothiamine),


and enantiomer

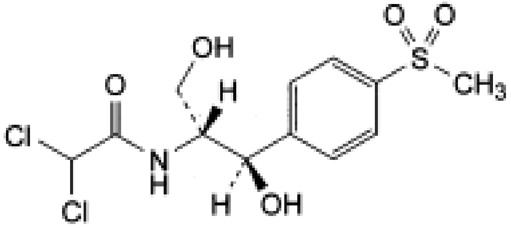
H. (3*RS*)-3-[[[(4-amino-2-methylpyrimidin-5-yl)methyl]thiocarbamoyl]sulphanyl]-4-oxopentyl acetate (ketodithiocarbamate).

Ph Eur

Thiamphenicol

General Notices

(Ph Eur monograph 0109)



$C_{12}H_{15}Cl_2NO_5S$ 356.2 *15318-45-3*

Action and use

Antibacterial.

Ph Eur

DEFINITION

2,2-Dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-[4- (methylsulphonyl)phenyl]ethyl] acetamide.

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

Fine, white or yellowish-white, crystalline powder or crystals.

Solubility

Slightly soluble in water, very soluble in dimethylacetamide, freely soluble in acetonitrile and in dimethylformamide, soluble in methanol, sparingly soluble in acetone and in anhydrous ethanol, slightly soluble in ethyl acetate.

A solution in anhydrous ethanol is dextrorotatory and a solution in dimethylformamide is laevorotatory.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Dry the substance to be examined and the reference substance at 100-105 °C for 2 h; examine as discs of *potassium bromide R.*

Comparison *thiamphenicol CRS.*

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.1 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.1 g of *thiamphenicol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *silica gel* GF_{254} *R* as the coating substance.

Mobile phase *methanol R, ethyl acetate R* (3:97 *V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the spot in the chromatogram obtained with the reference solution.

C. To 50 mg in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R* . Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R.* The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Acidity or alkalinity

Shake 0.1 g with 20 ml of *carbon dioxide-free water R* and add 0.1 ml of *bromothymol blue solution R1 .* Not more than 0.1 ml of *0.02 M hydrochloric acid* or *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation *(2.2.7)*

- 21 to - 24 (dried substance).

Dissolve 1.25 g in *dimethylformamide R* and dilute to 25.0 ml with the same solvent.

Melting point *(2.2.14)*

163 °C to 167 °C.

Absorbance *(2.2.25)* .

Test solution (a) Dissolve 20 mg in *water R*, heating to about 40 °C, and dilute to 100.0 ml with the same solvent.

Test solution (b) Dilute 2.5 ml of test solution (a) to 50.0 ml with *water R*.

Spectral range 240-300 nm for test solution (a); 200-240 nm for test solution (b).

Absorption maxima At 266 nm and 273 nm for test solution (a); at 224 nm for test solution (b).

Specific absorbances at the absorption maxima:

— at 266 nm: 25 to 28 for test solution (a);

— at 273 nm: 21.5 to 23.5 for test solution (a);

— at 224 nm: 370 to 400 for test solution (b).

Chlorides ***(2.4.4)***

Maximum 200 ppm.

Shake 0.5 g with 30 ml of *water R* for 5 min and filter.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

1.0 g complies with test C. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.300 g in 30 ml of *ethanol (96 per cent) R*, add 20 ml of a 500 g/l solution of *potassium hydroxide R,* mix and heat under a reflux condenser for 4 h. Cool, add 100 ml of *water R*, neutralise with *dilute nitric acid R* and add 5 ml of the same acid in excess. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*, using a silver indicator electrode and a mercurous sulphate reference electrode or any other appropriate electrode. Carry out a blank test.

1 ml of *0.1 M silver nitrate* is equivalent to 17.81 mg of $C_{12}H_{15}Cl_2NO_5S$.

STORAGE

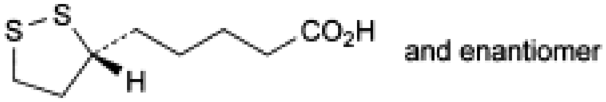
In an airtight container, protected from light.

Ph Eur

Thioctic Acid

General Notices

(Ph Eur monograph 1648)



$C_8H_{14}O_2S_2$ 206.3 *62-46-4*

Action and use

Antioxidant.

Ph Eur

DEFINITION

5-[(3*RS*)-1,2-Dithiolan-3-yl]pentanoic acid.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Very slightly soluble in water, very soluble in dimethylformamide, freely soluble in methanol.

mp

About 61 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *thioctic acid CRS.*

TESTS

Appearance of solution

The solution is clear *(2.2.1)* .

Dissolve 0.50 g in a 20 g/l solution of *sodium hydroxide R* and dilute to 10 ml with the same solution.

Impurity B

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.20 g of the substance to be examined in *dimethylformamide R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of thioctic acid containing *impurity B CRS* in *dimethylformamide R* and dilute to 1.0 ml with the same solvent (1.0 per cent impurity B solution).

Plate *TLC silica gel plate R.*

Mobile phase 25 per cent *V/V* solution of *ammonia R, water R, ethyl acetate R, propanol R* (5:10:40:40 *V/V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying At 50 °C for 20 min.

Detection Expose to iodine vapour for 30 min or until the spots appear.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated principal spots due to impurity B (R_F = 0.0) and thioctic acid (R_F = about 0.3).

Limit:

— *impurity B*: any spot due to impurity B is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (1.0 per cent).

Related substances

Liquid chromatography *(2.2.29)* . *Protect the solutions from light.*

Solvent mixture a mixture of equal volumes of *acetonitrile R1* and a 0.7 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.6 with *phosphoric acid R* .

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *thioctic acid for system suitability CRS* (containing impurity A) in the solvent mixture and dilute to 5 ml with the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c) Dissolve 50.0 mg of *thioctic acid CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Blank solution Solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase Mix 8 volumes of *acetonitrile R1*, 41 volumes of a 0.7 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*, and 51 volumes of *methanol R*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 2.5 times the retention time of thioctic acid.

Relative retention With reference to thioctic acid (retention time = about 6 min): impurity A = about 2.2.

System suitability:
- *resolution:* minimum 6.0 between the peaks due to thioctic acid and impurity A in the chromatogram obtained with reference solution (a);
- *symmetry factor*: maximum 2.0 for the peak due to thioctic acid in the chromatogram obtained with reference solution (c).

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.6;
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.2 per cent, determined on 1.000 g by drying *in vacuo* at 40 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

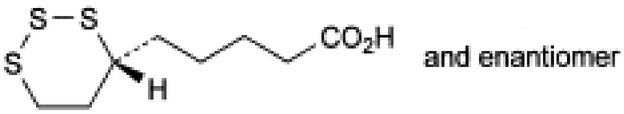
Calculate the percentage content of $C_8H_{14}O_2S_2$ from the peak areas and the declared content of *thioctic acid CRS*.

STORAGE

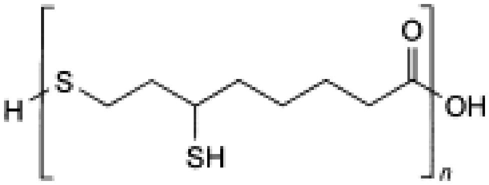
Protected from light.

IMPURITIES

Specified impurities A, B.



A. 5-[(4*RS*)-1,2,3-trithian-4-yl]pentanoic acid,



B. α-hydro-ω-hydroxypoly[sulphanediyl(3-sulphanyl-8-oxooctane-1,8-diyl)] (mixture of thioctic acid polymers).

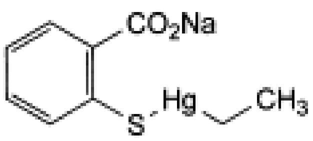
Ph Eur

Thiomersal

General Notices

Thimerosal

(Ph Eur monograph 1625)



$C_9H_9HgNaO_2S$ 404.8 *54-64-8*

Action and use

Antiseptic; adjuvant in vaccine formulations.

Ph Eur

DEFINITION

Sodium ethyl[2-sulphanylbenzoato(2-)-*C*,*S*]mercurate(1-).

Content

97.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble or soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Melting point *(2.2.14)* : 103 °C to 115 °C.

Dissolve 0.5 g in *water R* and dilute to 10 ml with the same solvent. Add 2 ml of *dilute hydrochloric acid R* . A white precipitate is formed. Wash the precipitate with *water R* and dry over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison thiomersal CRS.

C. Treat 50 mg by the oxygen-flask method *(2.5.10)* . Use a mixture of 1 ml of *strong hydrogen peroxide solution R* and 50 ml of *water R* to absorb the combustion products. To the solution add 5 ml of *dilute nitric acid R* . 0.1 ml of this solution gives reaction (a) of mercury *(2.3.1)* . To the remaining part of the solution add 10 ml of *dilute hydrochloric acid R* and filter. 5 ml of the filtrate, without further addition of acid, gives reaction (a) of sulphates *(2.3.1)* .

D. Solution S (see Tests) gives reaction (a) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

6.0 to 8.0.

Dilute 5 ml of solution S to 50 ml with *carbon dioxide-free water R* .

Inorganic mercury compounds

Maximum 0.70 per cent.

Protect the solutions from light throughout the procedure.

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Reference solution Dissolve 95.0 mg of *mercuric chloride R* in *water R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 20.0 ml with *water R* .

Test, reference and blank preparations Label five 10 ml volumetric flasks A, B, C, D and E. Place 5 ml of the test solution in flasks A, B, C and D. To each of the flasks C and D add 0.5 ml of the reference solution. Dilute the contents of flasks A and C to 10 ml with *water R* (blank preparations A and C). Dilute the contents of flasks B and D to 10 ml with a freshly prepared 332 g/l solution of *potassium iodide R* (test preparation B and reference preparation D). Place 5 ml of a 332 g/l solution of *potassium iodide R* in flask E. Dilute to 10 ml with *water R* (blank preparation E).

Measure the absorbance *(2.2.25)* of each solution (A_a, A_b, A_c, A_d and A_e) at 323 nm using *water R* as the compensation liquid. Calculate the content of inorganic mercury compounds, expressed as Hg from the expression:

$$\frac{(A_b - A_a - A_e) \times m_R \times 0.1847}{(A_d - A_c - A_b + A_a) \times m_T}$$

m_R = mass of mercuric chloride in the reference solution in milligrams

m_T = mass of the substance to be examined in milligrams.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 24 h.

ASSAY

Place 0.5 g in a 100 ml long-necked combustion flask, add 5 ml of *sulphuric acid R* and heat gently until charring occurs, continue to heat and add dropwise *strong hydrogen peroxide solution R* until the mixture is colourless. Dilute with *water R*, evaporate until slight fuming occurs, dilute to 10 ml with *water R*, cool down and titrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution R2 as indicator.*

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 20.24 mg of $C_9H_9HgNaO_2S$.

STORAGE

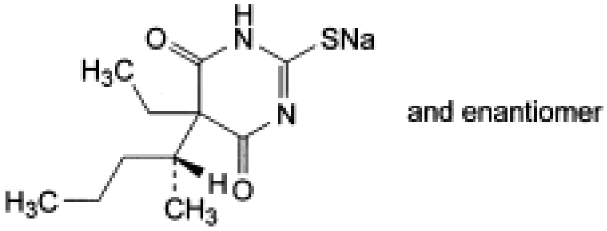
Protected from light.

Ph Eur

Thiopental Sodium

General Notices

(Thiopental Sodium and Sodium Carbonate, Ph Eur monograph 0212)



71-73-8

Action and use

Intravenous barbiturate; general anaesthetic.

Preparation

Thiopental Injection

Ph Eur

DEFINITION

Thiopental sodium and sodium carbonate is a mixture of the sodium derivative of 5-ethyl-5-[(1*RS*)-1-methylbutyl]-2-thioxo-2,3-dihydropyrimidine-4,6(1*H*,5*H*)-dione ($C_{11}H_{17}N_2NaO_2S$; M_r 264.3) and anhydrous sodium carbonate, containing the equivalent of not less than 84.0 per cent and not more than 87.0 per cent of thiopental and not less than 10.2 per cent and not more than 11.2 per cent of Na, both calculated with reference to the dried substance.

CHARACTERS

A yellowish-white powder, hygroscopic, freely soluble in water, partly soluble in ethanol.

IDENTIFICATION

First identification A, B, E.

Second identification A, C, D, E.

A. Acidify 10 ml of solution S (see Tests) with *dilute hydrochloric acid R* . An effervescence is produced. Shake with 20 ml of *ether R*. Separate the ether layer, wash with 10 ml of *water R*, dry over *anhydrous sodium sulphate R* and filter. Evaporate the filtrate to dryness and dry the residue at 100 °C to 105 °C (test residue). Determine the melting point *(2.2.14)* of the test residue. Mix equal parts of the test residue and *thiopental CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 160 °C) is not greater than 2 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing the test residue (see identification test A) with the spectrum obtained with *thiopental CRS*.

C. Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF$_{254}$ R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *water R* and dilute to 100 ml with the same solvent.

Reference solution Dissolve 85 mg of *thiopental CRS* in 10 ml of *dilute sodium hydroxide solution R* and dilute to 100 ml with *water R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 18 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R.* Examine immediately in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates *(2.3.1)*.

E. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution GY_3 *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel GF$_{254}$ R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 100 ml with the same solvent. Disregard any slight residue.

Reference solution Dilute 0.5 ml of the test solution to 100 ml with *water R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine immediately in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent). Disregard any spot at the starting-point.

Chlorides *(2.4.4)*

To 5 ml of solution S add 35 ml of *water R* and 10 ml of *dilute nitric acid R*. Shake with three quantities, each of 25 ml, of *ether R* and discard the ether layers. Eliminate the ether from the aqueous layer by heating on a water-bath.15 ml of the aqueous layer complies with the limit test for chlorides (330 ppm).

Loss on drying *(2.2.32)*

Not more than 2.5 per cent, determined on 0.50 g by drying *in vacuo* at 100 °C for 4 h.

ASSAY

Sodium

Dissolve 0.400 g in 30 ml of *water R*. Add 0.1 ml of *methyl red solution R* and titrate with *0.1 M hydrochloric acid* until a red colour is obtained. Boil gently for 2 min. Cool and, if necessary, continue the titration with *0.1 M hydrochloric acid* until the red colour is again obtained.

1 ml of *0.1 M hydrochloric acid* is equivalent to 2.299 mg of Na.

Thiopental

Dissolve 0.150 g in 5 ml of *water R*. Add 2 ml of *dilute sulphuric acid R* and shake with four quantities, each of 10 ml, of *chloroform R*. Combine the chloroform layers, filter and evaporate the filtrate to dryness on a water-bath. Dissolve the residue in 30 ml of previously neutralised *dimethylformamide R* and add 0.1 ml of a 2 g/l solution of *thymol blue R* in *methanol R*. Titrate immediately with *0.1 M lithium methoxide* until a blue colour is obtained. Protect the solution from atmospheric carbon dioxide during the titration.

1 ml of *0.1 M lithium methoxide* is equivalent to 24.23 mg of $C_{11}H_{18}N_2O_2S$.

STORAGE

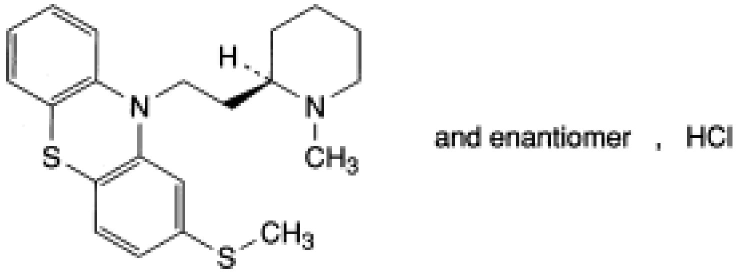
Store in an airtight container, protected from light.

Ph Eur

Thioridazine Hydrochloride

General Notices

(Ph Eur monograph 0586)



$C_{21}H_{26}N_2S_2$,HCl 407.0 *130-61-0*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

10-[2-[(2*RS*)-1-Methylpiperidin-2-yl]ethyl]-2-(methylsulphanyl)-10*H*-phenothiazine hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, soluble in ethanol 96 per cent.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison thioridazine hydrochloride CRS.

B. 0.2 g gives reaction (b) of chlorides *(2.3.1)* .

TESTS

Carry out all operations protected from light.

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II).*

Dissolve 1.0 g in *methanol R* and dilute to 20 ml with the same solvent.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 1.0 g in *methanol R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* . *Carry out the test as quickly as possible and protected from light.*

Test solution Dissolve 20.0 mg of the substance to be examined in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R* . Dilute 2.0 ml of this solution to 100.0 ml with *methanol R* .

Reference solution (b) Dissolve the content of a vial of *thioridazine for system suitability CRS* (containing impurities A, B, C, D and E) in 1.0 ml of *methanol R* .

Column:

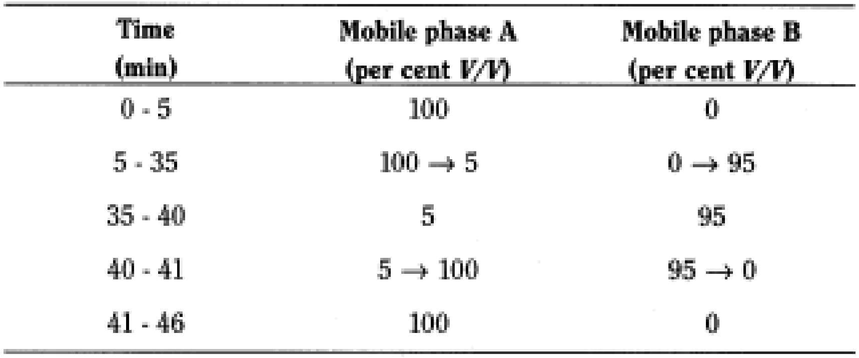
— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) resistant to bases up to pH 11.

Mobile phase:

— *mobile phase A: triethylamine R1, acetonitrile R, water R* (2:400:600 *V/V/V*);

— *mobile phase B: triethylamine R1, acetonitrile R* (2:1000 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 35	100 → 5	0 → 95
35 - 40	5	95
40 - 41	5 → 100	95 → 0
41 - 46	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 25 µl.

Identification of impurities Use the chromatogram supplied with *thioridazine for system suitability CRS* to identify the peaks due to impurities A, B, C, D and E .

Relative retention With reference to thioridazine (retention time = about 30 min): impurity D = about 0.1; impurity A = about 0.3; impurity C = about 0.4; impurity B = about 0.5; impurity E = about 0.6; impurity F = about 0.9.

System suitability Reference solution (b):

— *resolution*: minimum 3.5 between the peaks due to impurities C and B.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.9; impurity B = 2.4; impurity C = 0.5; impurity D = 1.5;

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in 20 ml of *water R* . 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R* .

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 10 ml of *anhydrous acetic acid R* and 60 ml of *acetic anhydride R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 40.70 mg of $C_{21}H_{27}ClN_2S_2$.

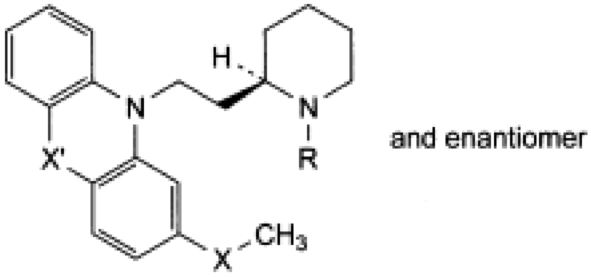
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F.*



A. R = CH_3, X = X′ = SO_2: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphonyl)-10*H*-phenothiazine 5,5-dioxide,

B. R = CH_3, X = SO, X′ = S: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphinyl)-10*H*-phenothiazine,

C. R = CH_3, X = S, X′ = SO: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphanyl)-10*H*-phenothiazine 5-oxide,

D. R = CH_3, X = X′ = SO: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphinyl)-10*H*-phenothiazine 5-oxide,

E. R = CH_3, X = SO_2, X′ = S: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphonyl)-10*H*-phenothiazine,

F. R = H, X = X′ = S: 2-(methylsulphanyl)-10-[2-[(2*RS*)-piperidin-2-yl]ethyl]-10*H*-

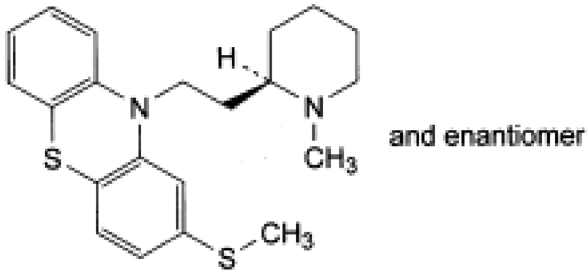
phenothiazine.

Ph Eur

Thioridazine

General Notices

(Ph Eur monograph 2005)



$C_{21}H_{26}N_2S_2$ 370.6 *50-52-2*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

10-[2-[(2*RS*)-1-Methylpiperidin-2-yl]ethyl]-2-(methylsulphanyl)-10*H*-phenothiazine

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, freely soluble in methanol, soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *thioridazine CRS.*

TESTS

Solution S

Dissolve 1.25 g in *methanol R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II) .*

Related substances

Liquid chromatography *(2.2.29). Carry out the test as quickly as possible and protected from light.*

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 100 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with *methanol R* . Dilute 2.0 ml of this solution to 100.0 ml with *methanol R* .

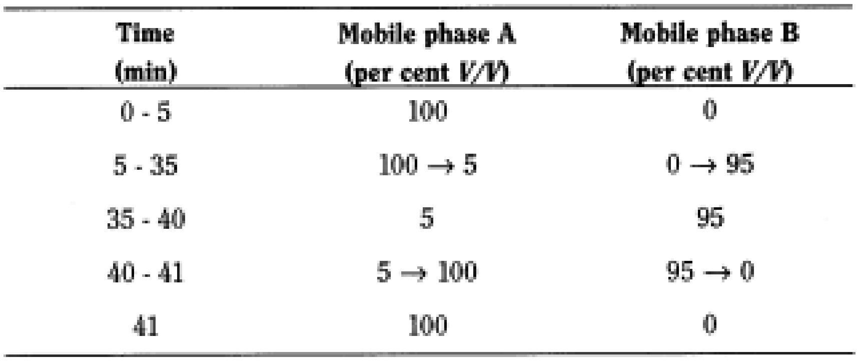
Reference solution (b) Dissolve the contents of a vial of *thioridazine for system suitability CRS* (containing impurities A, B, C, D and E) in 1.0 ml of *methanol R.*

Column:

- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* resistant to bases up to pH 11.

Mobile phase:

- *mobile phase A: triethylamine R1, acetonitrile R, water R* (2:400:600 *V/V/V*);
- *mobile phase B: triethylamine R1, acetonitrile R* (2:1000 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 35	100 → 5	0 → 95
35 - 40	5	95
40 - 41	5 → 100	95 → 0
41	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 25 µl.

Identification of impurities Use the chromatogram supplied with *thioridazine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to thioridazine (retention time = about 30 min): impurity D = about 0.1; impurity A = about 0.3; impurity C = about 0.4; impurity B = about 0.5; impurity E = about 0.6.

System suitability Reference solution (b):

— *resolution*: minimum 3.5 between the peaks due to impurities C and B.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.9; impurity B = 2.4; impurity C = 0.5; impurity D = 1.5;

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g *in vacuo* at 50 °C for 4 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 37.06 mg of $C_{21}H_{26}N_2S_2$.

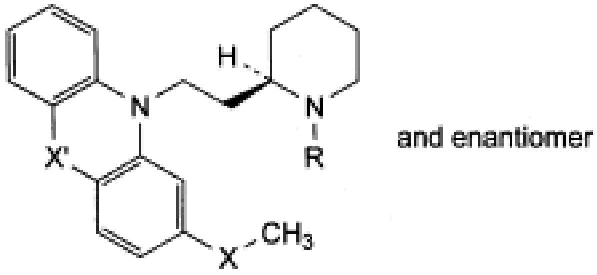
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F.*



A. R = CH_3, X = X′ = SO_2: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphonyl)-10*H*-phenothiazine 5,5-dioxide,

B. R = CH_3, X = SO, X′ = S: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphinyl)-10*H*-phenothiazine (mesoridazine),

C. R = CH_3, X = S, X′ = SO: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphanyl)-10*H*-phenothiazine 5-oxide,

D. R = CH_3, X = X′ = SO: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphinyl)-10*H*-phenothiazine 5-oxide,

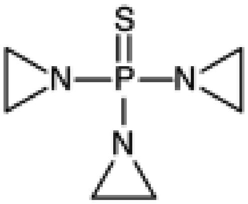
E. R = CH_3, X = SO_2, X′ = S: 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulphonyl)-10*H*-phenothiazine (sulforidazine),

F. R = H, X = X′ = S: 2-(methylsulphanyl)-10-[2-[(2*RS*)-piperidin-2-yl]ethyl]-10*H*-phenothiazine (northioridazine).

Ph Eur

Thiotepa

General Notices



$C_6H_{12}N_3PS$ 189.2 *52-24-4*

Action and use

Cytotoxic alkylating agent.

Preparation

Thiotepa Injection

DEFINITION

Thiotepa is phosphorothioic tri(ethyleneamide). It contains not less than 97.0% and not more than 102.0% of $C_6H_{12}N_3PS$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

Fine, white crystalline flakes.

Freely soluble in *water*, in *chloroform* and in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of thiotepa *(RS 337)*.

B. Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 5 ml of 1.25M *sodium hydroxide* as the absorbing liquid. When the process is complete, dilute to 25 ml with *water*. To 5 ml of the resulting solution add 0.1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of 1M *hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*. The solution becomes turbid.

C. To 2 ml of the solution obtained in test B add 40 ml of *water* and 4 ml of *ammonium molybdate-sulphuric acid solution*, mix, add 0.1 g of L- *ascorbic acid* and boil for 1 minute. A blue colour is produced.

TESTS

Melting point

52° to 57°, Appendix V A.

Clarity of solution

A 2.0% w/v solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. Solutions (1) and (2) are solutions of the substance being examined in *water* containing 0.350% w/v and 0.00035% w/v, respectively. For solution (3) dissolve 10 mg of the substance being examined in 2 ml of *methanol* in a ground-glass-stoppered tube, add 50 µl of a 0.1% v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (generation of methoxythiotepa). Allow the solution to cool and add 1 ml of *methanol*. For solution (4) dissolve 15 mg of the substance being examined in 10 ml of *water*, add 1 g of *sodium chloride*, boil in a water bath for 10 minutes and cool (generation of chloro-adduct).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) 15 volumes of *acetonitrile* and 85 volumes of 0.1M *mixed phosphate buffer pH 7.0* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 215 nm.

The chromatogram obtained with solution (3) shows a peak corresponding to methoxythiotepa with a retention time relative to thiotepa of about 1.3 and the chromatogram obtained with solution (4) shows a peak due to the chloro-adduct with a retention time relative to thiotepa of about 3.75. The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 3.

For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to the 'chloro-adduct' (identified from the peak in the chromatogram obtained with solution (4)) is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Water

Not more than 0.5% w/w, Appendix IX C. Cool the reagents and titration vessel in ice throughout the procedure and use 1.2 g. Complete the procedure as quickly as possible.

ASSAY

Transfer 0.2 g to an iodine flask with the aid of 50 ml of a 20% w/v solution of *sodium thiosulphate* and titrate immediately with 0.1M *hydrochloric acid VS*, using 0.05 ml of *methyl orange solution* as indicator, until a faint red colour persists for 10 seconds. Stopper the flask, allow to stand for 30 minutes and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Subtract the volume of 0.1M *sodium hydroxide VS* used from the volume of 0.1M *hydrochloric acid VS* used. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of hydrochloric acid required. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 6.307 mg of $C_6H_{12}N_3PS$.

STORAGE

Thiotepa should be stored at a temperature of 2° to 8°. At higher temperatures it polymerises and becomes inactive.

IMPURITIES

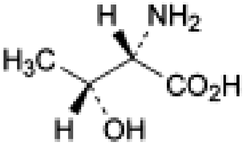
A. 'chloro-adduct'

B. 'hydroxythiotepa (A and B)'.

Threonine

General Notices

(Ph Eur monograph 1049)



$C_4H_9NO_3$ 119.1 *72-19-5*

Action and use

Amino acid.

Ph Eur

DEFINITION

Threonine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*S*,3*R*)-2-amino-3-hydroxybutanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, soluble in water, practically insoluble in alcohol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the spectrum obtained with *threonine CRS* . Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Mix 1 ml of a 2 g/l solution of the substance to be examined with 1 ml of a 20 g/l solution of *sodium periodate R*. Add 0.2 ml of *piperidine R* and 0.1 ml of a 25 g/l solution of *sodium nitroprusside R*. A blue colour develops that changes to yellow after a few minutes.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

pH *(2.2.3)*

The pH of solution S is 5.0 to 6.5.

Specific optical rotation *(2.2.7)*

Dissolve 1.50 g in *water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is - 27.6 to - 29.0, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dilute hydrochloric acid R* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *threonine CRS* in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50 ml with the same acid solution.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c) Dissolve 10 mg of *threonine CRS* and 10 mg of *proline CRS* in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 25 ml with the same acid solution.

Apply to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Chlorides *(2.4.4)*

Dilute 10 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dissolve 0.5 g in *distilled water R* and dilute to 15 ml with the same solvent. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

0.10 g complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.2 ml of *ammonium standard solution (100 ppm* NH_4*) R.*

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1,* shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals *(2.4.8)*

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 5 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 11.91 mg of $C_4H_9NO_3$.

STORAGE

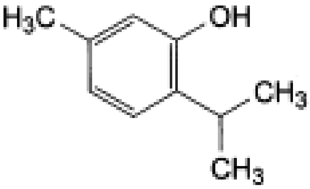
Store protected from light.

Ph Eur

Thymol

General Notices

(Ph Eur monograph 0791)



$C_{10}H_{14}O$ 150.2 *89-83-8*

Ph Eur

DEFINITION

5-Methyl-2-(methylethyl)phenol.

CHARACTERS

Appearance

Colourless crystals.

Solubility

Very slightly soluble in water, very soluble in ethanol (96 per cent), freely soluble in essential oils and in fatty oils, sparingly soluble in glycerol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point *(2.2.14)* : 48 °C to 52 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *thymol CRS.*

C. Dissolve 0.2 g with heating in 2 ml of *dilute sodium hydroxide solution R* and add 0.2 ml of *chloroform R*. Heat on a water-bath. A violet colour develops.

D. Dissolve about 2 mg in 1 ml of *anhydrous acetic acid R*. Add 0.15 ml of *sulphuric acid R* and 0.05 ml of *nitric acid R.* A bluish-green colour develops.

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension IV *(2.2.1)* and not more intensely coloured than reference solution R_6 *(2.2.2, Method II)*.

Dissolve 1.0 g in 10 ml of *dilute sodium hydroxide solution R*.

Acidity

To 1.0 g in a 100 ml glass-stoppered conical flask add 20 ml of *water R.* Boil until dissolution is complete, cool and stopper the flask. Shake vigorously for 1 min. Add a few crystals of the substance to be examined to initiate crystallisation. Shake vigorously for 1 min and filter. To 5 ml of the filtrate add 0.05 ml of *methyl red solution R* and 0.05 ml of *0.01 M sodium hydroxide*. The solution is yellow.

Related substances

Gas chromatography *(2.2.28)*.

Test solution Dissolve 0.100 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dilute 1 ml of the test solution to 100 ml with *ethanol (96 per cent) R* .

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with *ethanol (96 per cent) R .*

Reference solution (c) Dilute 5 ml of reference solution (b) to 10 ml with *ethanol (96 per cent) R .*

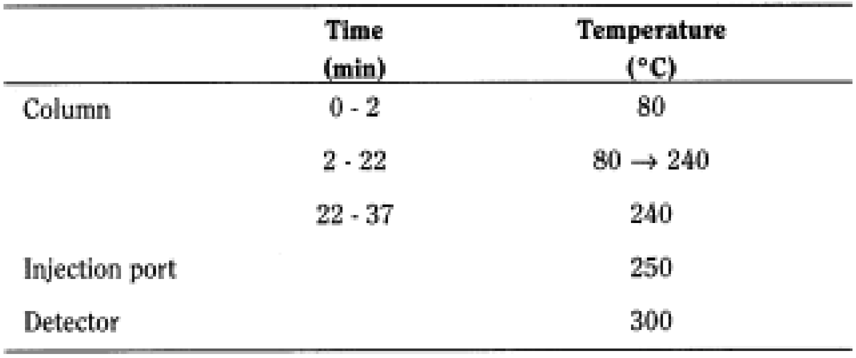
Column:

- *material*: glass or steel;
- *size*: *l* = 4 m, Ø = 2 mm;
- *stationary phase*: diatomaceous earth for *gas chromatography R*, impregnated with a mixture suitable for the separation of free fatty acids.

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	80
	2 - 22	80 → 240
	22 - 37	240
Injection port		250
Detector		300

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (b):

— *signal-to-noise ratio* : minimum 5 for the principal peak.

Limits:

— *total:* not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit:* the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Residue on evaporation

Maximum 0.05 per cent.

Evaporate 2.00 g on a water-bath and heat in an oven at 100-105 °C for 1 h. The residue weighs not more than 1.0 mg.

STORAGE

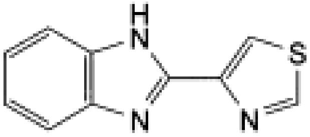
Protected from light.

Ph Eur

Tiabendazole

General Notices

(Ph Eur monograph 0866)



$C_{10}H_7N_3S$ 201.2 *148-79-8*

Action and use

Benzimidazole antihelminthic.

Preparation

Tiabendazole Tablets

Ph Eur

DEFINITION

Tiabendazole contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 2-(thiazol-4-yl)-1*H*-benzimidazole, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, slightly soluble in alcohol and in methylene chloride. It dissolves in dilute mineral acids. It melts at about 300 °C.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Dissolve 25 mg in *0.1 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 2.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid* . Examined between 230 nm and 350 nm *(2.2.25)* , the solution shows two absorption maxima, at 243 nm and 302 nm. The ratio of the absorbance measured at the maximum at 302 nm to that measured at the maximum at 243 nm is 1.8 to 2.1.

B. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the spectrum obtained with *tiabendazole CRS.* Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in *0.1 M hydrochloric acid* and dilute to 5 ml with the same acid. Add 3 mg of *p-phenylenediamine dihydrochloride R* and shake until dissolved. Add 0.1 g of *zinc powder R* , mix, allow to stand for 2 min and add 5 ml of *ferric ammonium sulphate solution R2* . A bluish-violet colour develops.

TESTS

Related substances

Examine by thin-layer chromatography *(2.2.27)* , using *silica gel HF$_{254}$ R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 2 ml of test solution (a) to 20 ml with *methanol R* .

Reference solution (a) Dissolve 20 mg of *tiabendazole CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 10 ml with *methanol R* .

Reference solution (c) Dilute 1 ml of test solution (b) to 25 ml with *methanol R* .

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using a mixture of 2.5 volumes of *water R,* 10 volumes of *acetone R* , 25 volumes of *glacial acetic acid R* and 62.5 volumes of *toluene R* . Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.4 per cent).

o-Phenylenediamine

To 5.0 g in a flask fitted with a ground-glass stopper, add 25 ml of a mixture of 1 volume of *methanol R* and 2 volumes of *water R* . Shake for 3 min. Filter through a sintered-glass filter (16) *(2.1.2)* under reduced pressure. To 10 ml of the filtrate add 0.5 ml of *hydrochloric acid R* and 0.5 ml of *acetylacetone R* and shake until the solution is clear. The solution is not more intensely coloured than reference solution R_7 *(2.2.2, Method I)* (10 ppm).

Heavy metals *(2.4.8)*

1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R* .

Water *(2.5.12)*

Not more than 0.5 per cent, determined on 1.00 g by the semi-micro determination of water.

Sulphated ash ***(2.4.14)***

Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

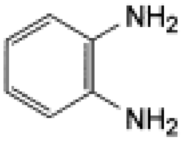
Dissolve 0.150 g in 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 20.12 mg of $C_{10}H_7N_3S$.

STORAGE

Store protected from light.

IMPURITIES



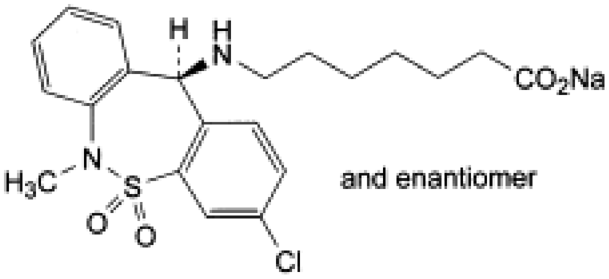
A. benzene-1,2-diamine.

Ph Eur

Tianeptine Sodium

General Notices

(Ph Eur monograph 2022)



$C_{21}H_{24}ClN_2NaO_4S$ 458.9 *66981-73-5*

(base)

Action and use

Antidepressant.

Ph Eur

DEFINITION

Sodium 7-[[(11*RS*)-3-chloro-6-methyl-6,11-dihydrodibenzo[*c,f*][1,2]thiazepin-11-yl]amino] heptanoate *S*,*S*-dioxide.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish powder, very hygroscopic.

Solubility

Freely soluble in water, in methanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of tianeptine sodium.*

B. It gives reaction (a) of sodium *(2.3.1)* .

TESTS

Impurity A

Gas chromatography *(2.2.28)* .

Internal standard solution Dilute 1 ml of *ethyl 5-bromovalerate R* in *ethanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 250.0 ml with *ethanol R* .

Test solution Dissolve 0.1000 g of the substance to be examined in the internal standard solution and dilute to 2.0 ml with the same solution.

Reference solution. Dissolve 10.0 mg of *tianeptine impurity A CRS* in the internal standard solution and dilute to 200.0 ml with the same solution.

Column:

— *material:* fused silica;

— *size*: $l = 25$ m, Ø = 0.25 mm;

— *stationary phase: poly(cyanopropyl)siloxane R* (film thickness 0.2 µm).

Carrier gas *helium for chromatography R* .

Linear velocity 26 cm/s.

Split ratio 1:100.

Temperature:

— *column*: 150 °C;

— *injection port and detector*: 210 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time Twice the retention time of ethyl 5-bromovalerate.

System suitability Reference solution:

— *elution order*: ethanol, ethyl 5-bromovalerate, impurity A;

— *resolution*: minimum 10 between the peaks due to ethyl 5-bromovalerate and impurity A;

— *signal-to-noise ratio* : minimum 20 for the peak due to impurity A.

Limit:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mix 50 volumes of *methanol R* and 50 volumes of *water for chromatography R* .

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Reference solution (b) Dissolve 20.0 mg of *sodium tianeptine for system suitability CRS* in the solvent mixture and dilute to 200.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

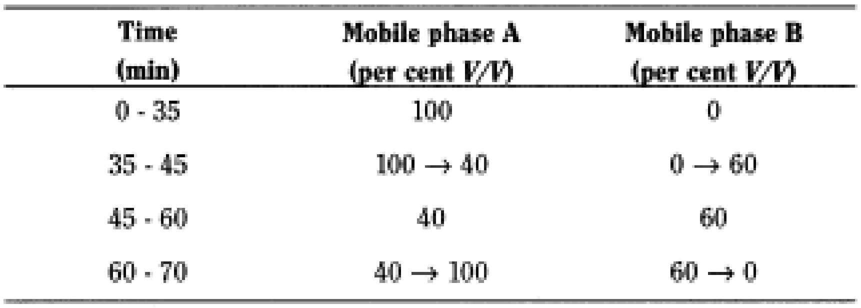
— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm) with a pore size of 0.01 µm;

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: mix 21 volumes of *methanol R1* , 31.5 volumes of *acetonitrile R1* and 47.5 volumes of a 2 g/l solution of *sodium laurilsulfate R* , adjusted to pH 2.5 with *phosphoric acid R;*

— *mobile phase B*: mix 20 volumes of *methanol R1* , 20 volumes of a 2 g/l solution of *sodium laurilsulfate R* , adjusted to pH 2.5 with *phosphoric acid R* and 60 volumes of *acetonitrile R1* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100	0
35 - 45	100 → 40	0 → 60
45 - 60	40	60
60 - 70	40 → 100	60 → 0

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Relative retention With reference to tianeptine (retention time = about 30 min): impurity C = about 0.4; impurity D1 = about 0.6; impurity D2 = about 0.8; impurity E = about 1.1; impurity B = about 1.7.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to tianeptine and impurity E.

Limits:

— *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*

Maximum 5.0 per cent, determined on 0.100 g.

ASSAY

Dissolve 0.165 g in 50 ml of *anhydrous acetic acid R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* .

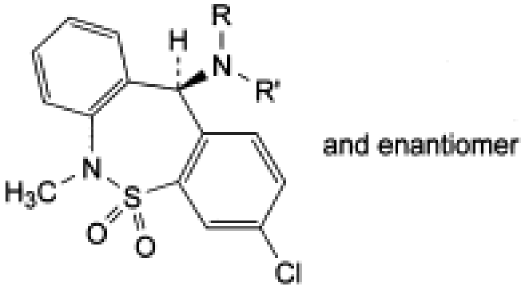
1 ml of *0.1 M perchloric acid* is equivalent to 22.95 mg of $C_{21}H_{24}ClN_2NaO_4S$.

STORAGE

In an airtight container .

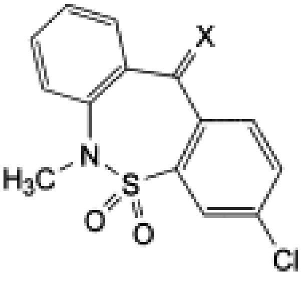
IMPURITIES

A. Br-$[CH_2]_6$-CO-*O*-C_2H_5: ethyl 7-bromoheptanoate,



B. R = H, R′ = $[CH_2]_6$-CO-*O*-C_2H_5: ethyl 7-[[(11*RS*)-3-chloro-6-methyl-6,11-dihydrodibenzo[c, f][1,2]thiazepin-11-yl]amino]heptanoate *S*,*S*-dioxide,

E. R = R′ = $[CH_2]_6$-CO_2H: 7,7′-[[(11*RS*)-3-chloro-6-methyl-6,11-dihydrodibenzo[*c,f*][1,2] thiazepin-11-yl]imino]diheptanoic acid *S*,*S*-dioxide,



C. X = O: 3-chloro-6-methyldibenzo[*c,f*][1,2]thiazepin-11(6*H*)-one *S*,*S*-dioxide,

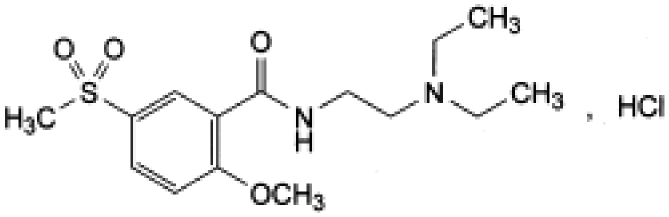
D. X = N-$[CH_2]_6$-CO_2H: 7-[[(11*RS*)-3-chloro-6-methyldibenzo[*c,f*][1,2]thiazepin-11(6*H*)-ylidene]amino]heptanoic acid S,S-dioxide.

Ph Eur

Tiapride Hydrochloride

General Notices

(Ph Eur monograph 1575)



$C_{15}H_{24}N_2O_4S,HCl$ 364.9 *51012-33-0*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

N-[2-(Diethylamino)ethyl]-2-methoxy-5-(methylsulphonyl)benzamide hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, soluble in methanol, slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *tiapride hydrochloride CRS.*

B. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 450 nm is not greater than 0.030.

pH *(2.2.3)*

4.0 to 6.0 for solution S.

Impurity C

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.400 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20.0 mg of *metoclopramide impurity E CRS* (impurity C) in *methanol R* and dilute to 50 ml with the same solvent. Dilute 2.0 ml of this solution to 20 ml with *methanol R* .

Plate *TLC silica gel G plate R* .

Mobile phase *concentrated ammonia R, dioxan R, methanol R, methylene chloride R* (2:10:14:90 *V/V/V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with a 2 g/l solution of *ninhydrin R* in *butanol R* and heat at 100 °C for 15 min.

Limit:

- *impurity C*: any spot due to impurity C is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *tiapride hydrochloride CRS* and 5.0 mg of *tiapride N-oxide CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Dissolve 5.44 g of *potassium dihydrogen phosphate R* and 0.08 g of *sodium octanesulphonate R* in 780 ml of *water R*, adjust to pH 2.7 using *phosphoric acid R* and dilute to 800 ml with *water R ;* add 150 ml of *methanol R* and 50 ml of *acetonitrile R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µl.

Run time 3 times the retention time of tiapride.

Retention time Tiapride = about 9 min; tiapride *N*-oxide = about 13 min.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to tiapride and tiapride N-oxide.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

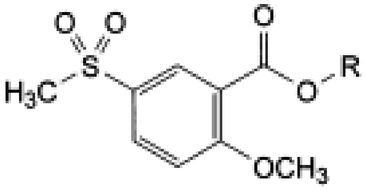
ASSAY

Dissolve 0.300 g in 20 ml of *anhydrous acetic acid R*. Add 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 36.49 mg of $C_{15}H_{25}ClN_2O_4S$.

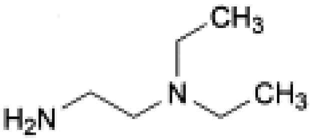
IMPURITIES

Specified impurities   A, B, C.


O
O
O
S
H3C
O
R
OCH3

A. R = CH_3: methyl 2-methoxy-5-(methylsulphonyl)benzoate,

B. R = H: 2-methoxy-5-(methylsulphonyl)benzoic acid,


CH3
N
CH3
H2N

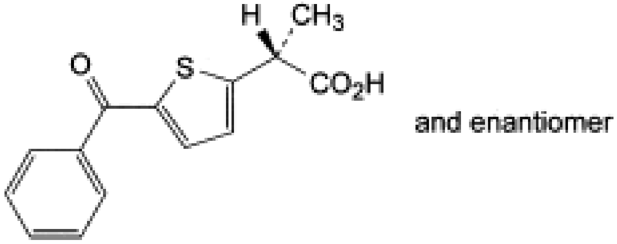
C. *N,N*-diethylethane-1,2-diamine.

Ph Eur

Tiaprofenic Acid

General Notices

(Ph Eur monograph 1157)



$C_{14}H_{12}O_3S$ 260.3 *33005-95-7*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

(2*RS*)-2-(5-Benzoylthiophen-2-yl)propanoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification *C.*

Second identification A, B, D.

A. Melting point *(2.2.14)* : 95 °C to 99 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 25.0 mg in *ethanolic hydrochloric acid R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with *ethanolic hydrochloric acid R* .

Spectral range 220-350 nm.

Absorption maximum At 305 nm.

Shoulder At 262 nm.

Specific absorbance at the absorption maximum 550 to 590.

C. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *tiaprofenic acid CRS.*

D. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 10 mg of *tiaprofenic acid CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *ketoprofen CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 2 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R* .

Mobile phase *acetic acid R, methylene chloride R, acetone R* (1:20:80 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— The chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 20 ml with the same solvent.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 0.50 g in *ethyl acetate R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 10.0 mg of *tiaprofenic acid impurity C CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 2.0 ml with reference solution (c).

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase *Water R*, *glacial acetic acid R*, *hexane R*, *methylene chloride R* (0.25:20:500:500 *V/V/V/V*); add the water to the acetic acid, then hexane and methylene chloride; sonicate the mixture for 2 min. Do not degas with helium during analysis.

Flow rate 1 ml/min.

Detection Spectrophotometer at 250 nm.

Injection 20 µl.

Run time Twice the retention time of tiaprofenic acid.

Relative retention With reference to tiaprofenic acid: impurity A = about 0.19; impurity B = about 0.43; impurity C = about 0.86.

System suitability Reference solution (d):

— *resolution*: minimum 3.0 between the peaks due to impurity C and tiaprofenic acid.

Limits:

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *sum of impurities other than C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 25 ml of *ethanol (96 per cent) R*. Add 25 ml of *water R* and 0.5 ml of *phenolphthalein solution R*. Titrate with *0.1 M sodium hydroxide*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 26.03 mg of $C_{14}H_{12}O_3S$.

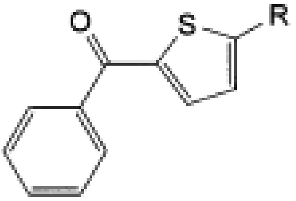
STORAGE

Protected from light.

IMPURITIES

Specified impurities C.

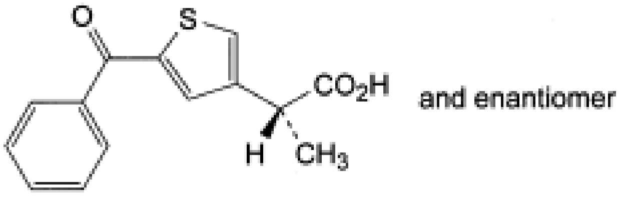
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, D, E, F.*



A. R = C_2H_5: (5-ethylthiophen-2-yl)phenylmethanone,

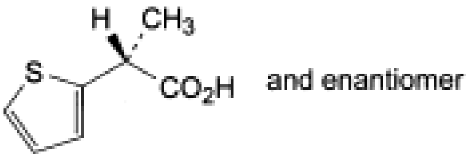
B. R = $CO\text{-}CH_3$: 1-(5-benzoylthiophen-2-yl)ethanone,

F. R = Br: (5-bromothiophen-2-yl)phenylmethanone,



C. (2*RS*)-2-(5-benzoylthiophen-3-yl)propanoic acid,

D. benzoic acid,



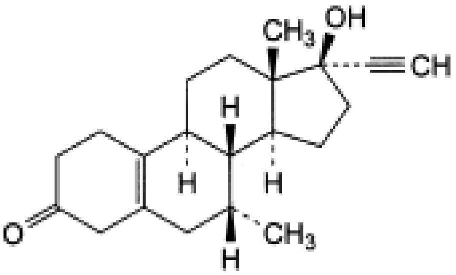
E. (2*RS*)-2-(thiophen-2-yl)propanoic acid.

Ph Eur

Tibolone

General Notices

(Ph Eur monograph 1739)



$C_{21}H_{28}O_2$ 312.5 *5630-53-5*

Action and use

Steroid with estrogenic and progestogenic properties.

Ph Eur

DEFINITION

17-Hydroxy-7α-methyl-19-nor-17α-pregn-5(10)-en-20-yn-3-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or crystals.

Solubility

Practically insoluble in water, soluble in acetone and in methanol.

It shows polymorphism *(5.9)* .

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of tibolone*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined in the minimum volume of *anhydrous ethanol R* , evaporate to dryness on a water-bath and record a new spectrum using the residue.

TESTS

Specific optical rotation *(2.2.7)*

+ 100 to + 106 (dried substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Select a brand of acetonitrile such that the formation of possible artefact peaks, eluting after impurity C at relative retentions 0.6 to 0.8, is avoided.

Solvent mixture *water R, acetonitrile R1* (25:75 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a) Dissolve 4 mg of *tibolone for system suitability CRS* (containing impurities A, B, C, D and E) in 1 ml of the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Column:

— *size*: l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, acetonitrile R1, water R* (8:40:52 *V/V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 5 µl.

Run time 3 times the retention time of tibolone.

Identification of impurities Use the chromatogram supplied with *tibolone for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to tibolone (retention time = about 14 min): impurity A = about 0.22; impurity B = about 0.24; impurity C = about 0.58; impurity D = about 1.12; impurity E = about 2.24.

System suitability Reference solution (a):

— *peak-to-valley ratio* : minimum 2.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to tibolone.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.7; impurity B = 1.5; impurity C = 2.1;

— *impurities A, E*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity B*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *impurity C*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurity D*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 60 ml of *tetrahydrofuran R* . Add 25 ml of a 100 g/l solution of *silver nitrate R* . Titrate with *0.1 M sodium hydroxide* , determining the end-point potentiometrically *(2.2.20)* .

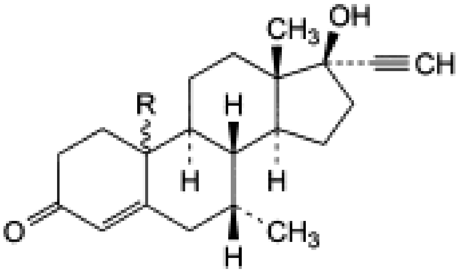
1 ml of *0.1 M sodium hydroxide* is equivalent to 31.25 mg of $C_{21}H_{28}O_2$.

STORAGE

At a temperature of 2 °C to 8 °C.

IMPURITIES

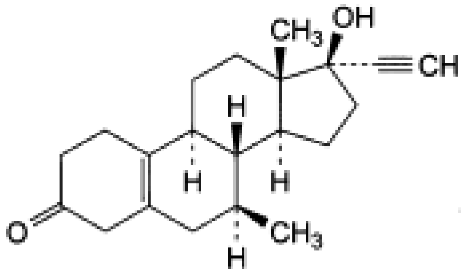
Specified impurities *A, B, C, D, E*.


CH3
OH
CH
R
H
H
H
O
H
CH3

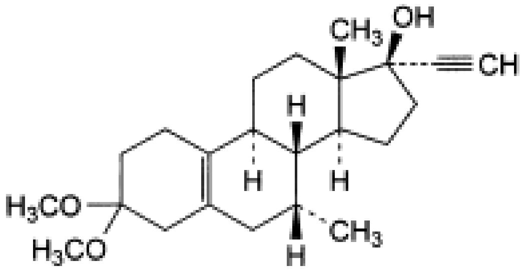
A. R = OH: 10,17-dihydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one,

B. R = O-OH: 10-hydroperoxy-17-hydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one,

C. R = H: 17-hydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one,


CH3
OH
CH
H
H
H
O
CH3
H

D. 17-hydroxy-7β-methyl-19-nor-17α-pregn-5(10)-en-20-yn-3-one,


CH3
OH
CH
H
H
H
H3CO
H3CO
CH3
H

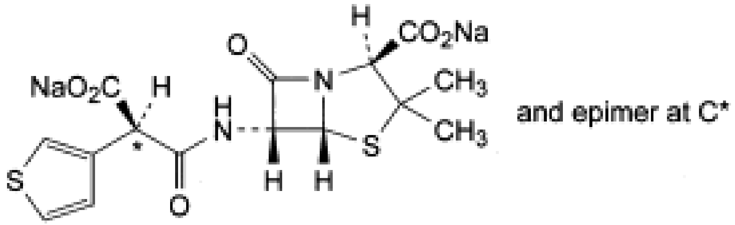
E. 3,3-dimethoxy-7α-methyl-19-nor-17α-pregn-5(10)-en-20-yn-17-ol.

Ph Eur

Ticarcillin Sodium

General Notices

(Ph Eur monograph 0956)



$C_{15}H_{14}N_2Na_2O_6S_2$ 428.4 *4697-14-7*

Action and use

Penicillin antibacterial.

Ph Eur

DEFINITION

Disodium (2*S*,5*R*,6*R*)-6-[[(2*RS*)-2-carboxylato-2-(thiophen-3-yl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content

89.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, hygroscopic powder.

Solubility

Freely soluble in water, soluble in methanol.

IDENTIFICATION

First identification A, D, E.

Second identification B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Dissolve 50 mg of the substance to be examined in 1 ml of *water R* , add 0.1 ml of *hydrochloric acid R1* , swirl and allow to stand in iced water for 10 min. Filter the precipitate and rinse with 2 ml of *water R* . Dissolve in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* . Evaporate the solvent almost to dryness, then dry in an oven at 60 °C for 30 min.

Comparison Repeat the operations using *ticarcillin monosodium CRS* .

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *ticarcillin monosodium CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 25 mg of *carbenicillin sodium CRS* and 25 mg of *ticarcillin monosodium CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silanised silica gel plate R* .

Mobile phase Mix 10 volumes of *acetone R* and 90 volumes of a 154 g/l solution of *ammonium acetate R* , adjusted to pH 5.0 with *glacial acetic acid R* .

Application 1 µl.

Development Over a path of 12 cm.

Drying In a current of hot air.

Detection Expose to iodine vapour.

System suitability Reference solution (b):

— The chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 15 cm long and 15 mm in diameter. Moisten with 0.05 ml of *water R* and add 2 ml of *sulphuric acid-formaldehyde reagent R* . Mix the contents of the tube by swirling; the solution is brown. Place the test-tube in a water-bath for 1 min; a dark reddish-brown colour develops.

D. It gives reaction (a) of sodium *(2.3.1)* .

E. Specific optical rotation (see Tests).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

pH ***(2.2.3)***

5.5 to 7.5 for solution S.

Specific optical rotation ***(2.2.7)***

+ 172 to + 187 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

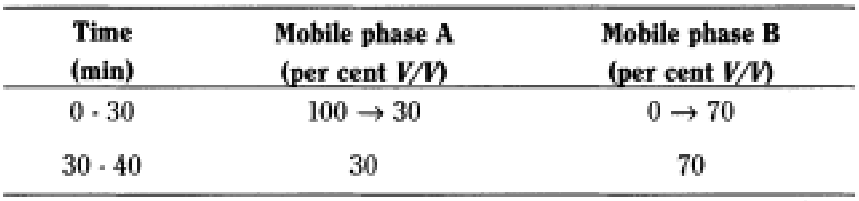
Test solution Dissolve 25.0 mg of the substance to be examined in mobile phase A and dilute to 25.0 ml with mobile phase A.

Reference solution (a) Dissolve 20.0 mg of *ticarcillin impurity A CRS* in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 5.0 ml of this solution to 50.0 ml with mobile phase A.

Reference solution (b) Dilute 1 ml of the test solution to 50 ml with mobile phase A.

Column:
- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: 1.3 g/l solution of *ammonium phosphate R* adjusted to pH 7.0 with *phosphoric acid R* ;
- *mobile phase B*: *methanol R* , mobile phase A (50:50 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	100 → 30	0 → 70
30 - 40	30	70

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the 2 principal peaks (diastereoisomers).

Limits:

- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (4 per cent);
- *any other impurity*: for each impurity, not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

N*,*N*-Dimethylaniline** ***(2.4.26, Method B)

Maximum 20 ppm.

2-Ethylhexanoic acid ***(2.4.28)***

Maximum 0.5 per cent *m/m*.

Water ***(2.5.12)***

Maximum 5.5 per cent, determined on 0.150 g.

Bacterial endotoxins ***(2.6.14)***

Less than 0.05 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution Dissolve 50.0 mg of *ticarcillin monosodium CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *methanol R* and 80 volumes of a 1.3 g/l solution of *ammonium phosphate R* adjusted to pH 7.0 with *phosphoric acid R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

System suitability Reference solution:

- *resolution*: minimum 2.5 between the 2 principal peaks;
- *repeatability*: maximum relative standard deviation of 1.0 per cent for the 2 peaks due to ticarcillin after 6 injections.

Calculate the percentage content of ticarcillin sodium as the sum of the areas of the 2 peaks, multiplying the content of ticarcillin monosodium by 1.054.

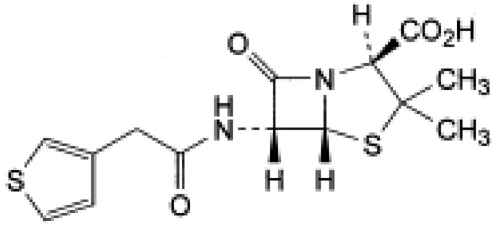
STORAGE

In an airtight container , at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

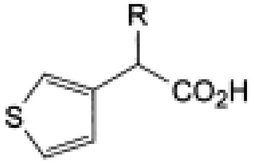
IMPURITIES

Specified impurities *A.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E.*

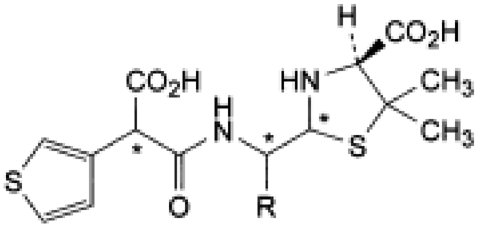


A. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[[(thiophen-3-yl)acetyl]amino]-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (decarboxyticarcillin),



B. R = H: (thiophen-3-yl)acetic acid,

C. R = CO_2H: 2-(thiophen-3-yl)propanedioic acid (3-thienylmalonic acid),



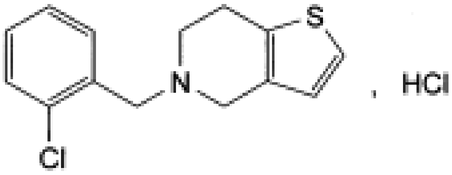
D. R = CO_2H: (4*S*)-2-[carboxy[[2-carboxy-2-(thiophen-3-yl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of ticarcillin),

E. R = H: (4*S*)-2-[[[2-carboxy-2-(thiophen-3-yl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of ticarcillin).

Ticlopidine Hydrochloride

General Notices

(Ph Eur monograph 1050)



$C_{14}H_{14}ClNS,HCl$ 300.2 *53885-35-1*

Action and use

Inhibition of adenosine diphosphate (ADP)-mediated platelet aggregation; antiplatelet drug.

Ph Eur

DEFINITION

5-(2-Chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water and in anhydrous ethanol, very slightly soluble in ethyl acetate.

IDENTIFICATION

First identification B, D.

Second identification A, C, D.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25).*

Test solution (a) Dissolve 40 mg in *water R* and dilute to 100.0 ml with the same solvent.

Test solution (b) Dilute 5.0 ml of test solution (a) to 100.0 ml with *water R* .

Spectral range 250-350 nm for test solution (a); 200-350 nm for test solution (b).

Absorption maxima At 268 nm and 275 nm for test solution (a); at 214 nm and 232 nm for test solution (b).

Absorption ratio A_{268}/A_{275} = 1.1 to 1.2.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *ticlopidine hydrochloride CRS.*

C. Mix about 6 mg of *citric acid R* and 0.3 ml of *acetic anhydride R* . Add about 5 mg of the substance to be examined and heat in a water-bath at 80 °C. A red colour develops.

D. About 20 mg gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 0.5 g in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 20 ml with the same solution.

pH *(2.2.3)*

3.5 to 4.0.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Solvent mixture Mobile phase B, mobile phase A (20:80 *V/V*).

Test solution Dissolve 0.250 g of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

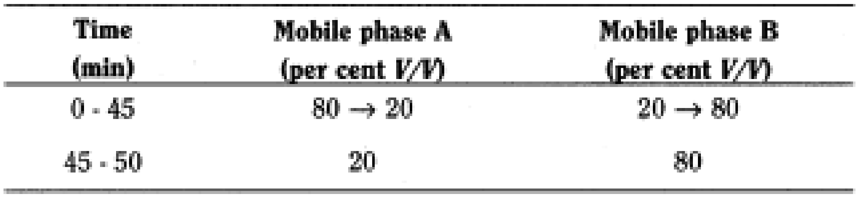
Reference solution Dissolve 5.0 mg of *ticlopidine impurity F CRS* in the solvent mixture. Add 1.00 ml of the test solution and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: 0.95 g/l solution of *sodium pentanesulphonate monohydrate R*,

adjusted to pH 3.4 with a 50 per cent *V/V* solution of *phosphoric acid R* ;

— *mobile phase B: methanol R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 45	80 → 20	20 → 80
45 - 50	20	80

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl; inject the solvent mixture as a blank.

Retention time Ticlopidine = about 15 min.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the peaks due to ticlopidine and impurity F; if necessary, adjust the pH of mobile phase A;

— *signal-to-noise ratio* : minimum 50 for the peak due to ticlopidine.

Limits:

— *impurity F*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the peak due to ticlopidine in the chromatogram obtained with the reference solution (0.05 per cent);

— *total*: not more than the area of the peak due to ticlopidine in the chromatogram obtained with the reference solution (0.1 per cent);

— *disregard limit*: 0.1 times the area of the peak due to ticlopidine in the chromatogram obtained with the reference solution (0.01 per cent).

Formaldehyde

Maximum 20 ppm.

Dissolve 0.200 g in 4.0 ml of *water R* . Add 0.4 ml of *dilute sodium hydroxide solution R* . Centrifuge, filter the supernatant liquid through cotton previously impregnated with *water R* and dilute to 5.0 ml with *water R* . Transfer to a test-tube. Add 5.0 ml of *acetylacetone reagent R1* . Place the test-tube in a water-bath at 40 °C for 40 min. The test solution is not more intensely coloured than a standard prepared at the same time and in the same manner using 5.0 ml of a 0.8 ppm solution of formaldehyde (CH_2O), obtained by dilution of *formaldehyde standard solution (5 ppm* CH_2O*) R* with *water R* . Examine the tubes down their vertical axis.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 2.0 g in a 85 per cent *V/V* solution of *methanol R* and dilute to 20.0 ml with the same solvent. 12 ml of the solution complies with test B. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R* .

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

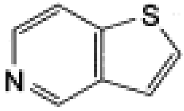
Dissolve 0.150 g in 15 ml of *anhydrous acetic acid R* . Add 35 ml of *acetic anhydride R* . Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 30.02 mg of $C_{14}H_{15}Cl_2NS$.

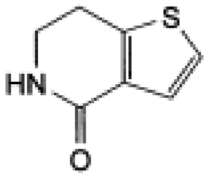
IMPURITIES

Specified impurities F.

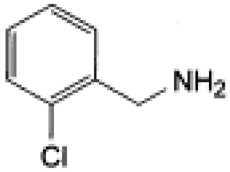
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, D, E, G, H, I, J, K, L.*



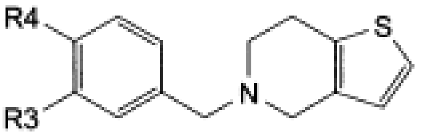
A. thieno[3,2-*c*]pyridine,



B. 6,7-dihydrothieno[3,2-*c*]pyridin-4(5*H*)-one,



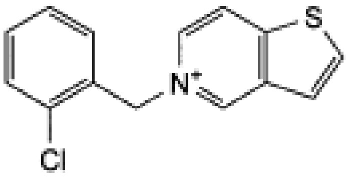
C. (2-chlorophenyl)methanamine,



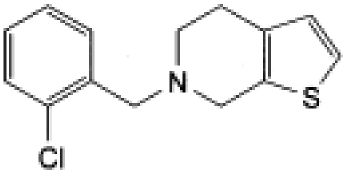
D. R3 = R4 = H: 5-benzyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine,

G. R3 = Cl, R4 = H: 5-(3-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine,

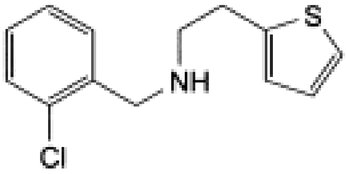
H. R3 = H, R4 = Cl: 5-(4-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine,



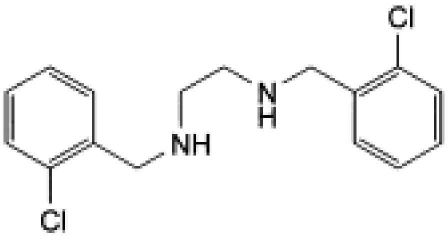
E. 5-(2-chlorobenzyl)thieno[3,2-*c*]pyridinium,



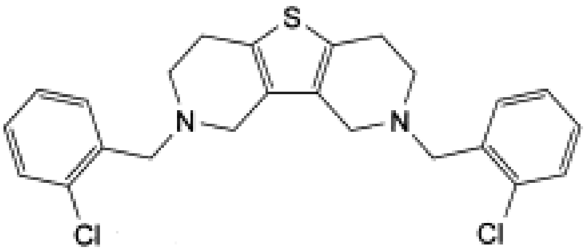
F. 6-(2-chlorobenzyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine,



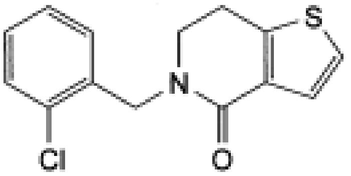
I. *N*-(2-chlorobenzyl)-2-(thiophen-2-yl)ethanamine,



J. *N*,*N′*-bis(2-chlorobenzyl)ethane-1,2-diamine,



K. 2,8-bis(2-chlorobenzyl)-1,2,3,4,6,7,8,9-octahydrothieno[3,2-*c*:4,5-*c′*]dipyridine (bis-ticlopidine),



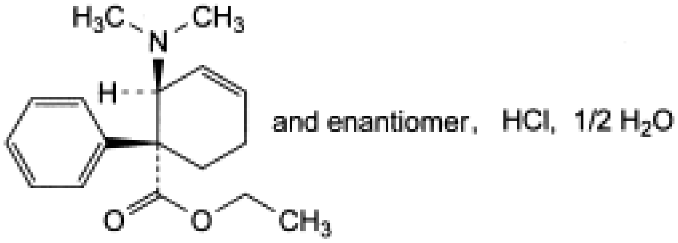
L. 5-(2-chlorobenzyl)-6,7-dihydrothieno[3,2-*c*]pyridin-4(*5H*)-one.

Ph Eur

Tilidine Hydrochloride Hemihydrate

General Notices

(Ph Eur monograph 1767)



$C_{17}H_{23}NO_2,HCl,\frac{1}{2}H_2O$ *318.9 (anhydrous)* *27107-79-5*

Action and use

Opioid Analgesic.

Ph Eur

DEFINITION

Ethyl (1*RS*,2*SR*)-2-(dimethylamino)-1-phenylcyclohex-3-enecarboxylate hydrochloride hemihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

A suitable antioxidant may be added.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very soluble in methylene chloride, freely soluble in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of tilidine hydrochloride hemihydrate.*

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II).*

Acidity or alkalinity

To 20 ml of solution S add 0.2 ml of *0.01 M sodium hydroxide* . The pH is not less than 4.1. Add 0.4 ml of *0.01 M hydrochloric acid* . The pH is not more than 4.3.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dilute 0.5 ml of the test solution to 100.0 ml with *water R* .

Reference solution (b) Dilute 2.0 ml of reference solution (a) to 10.0 ml with *water R* .

Precolumn:

— *size*: *l* = 4 mm, Ø = 4.0 mm;

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix equal volumes of *acetonitrile R* and a 0.98 g/l solution of *ammonium carbonate R* .

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Run time Twice the retention time of tilidine.

Relative retention With reference to tilidine (retention time = about 11 min): impurity D = about 0.4; impurity C = about 0.5; impurity B = about 0.7; impurity A = about 1.5.

Limits:

- *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total:* not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Water *(2.5.12)*

2.5 per cent to 3.1 per cent, determined on 0.300 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/mg, if intented for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.250 g in a mixture of 10 ml of *anhydrous acetic acid R* and 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 30.99 mg of $C_{17}H_{24}ClNO_2$.

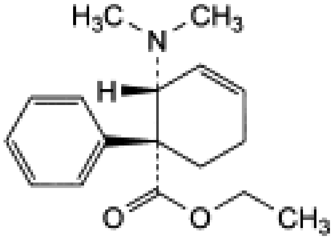
STORAGE

Protected from light.

IMPURITIES

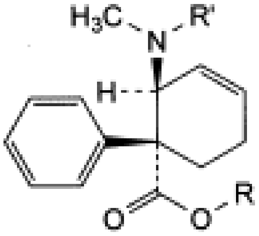
Specified impurities A, B, C.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D.*



and enantiomer

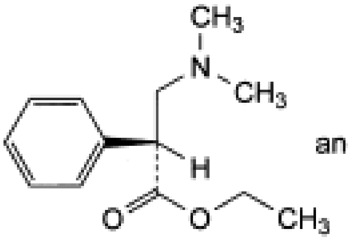
A. ethyl (1*RS*,2*RS*)-2-(dimethylamino)-1-phenylcyclohex-3-enecarboxylate,



and enantiomer

B. R = R′ = CH_3: methyl (1*RS*,2*SR*)-2-(dimethylamino)-1-phenylcyclohex-3-enecarboxylate,

C. R = C_2H_5, R′ = H: ethyl (1*RS*,2*SR*)-2-(methylamino)-1-phenylcyclohex-3-enecarboxylate,



and enantiomer

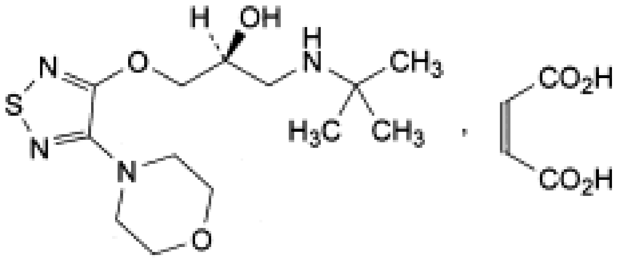
D. ethyl (2*RS*)-3-dimethylamino-2-phenylpropanoate.

Ph Eur

Timolol Maleate

General Notices

(Ph Eur monograph 0572)



$C_{13}H_{24}N_4O_3S,C_4H_4O_4$ 432.5 *26921-17-5*

Action and use

Beta-adrenoceptor antagonist

For Timolol Eye Drops only: treatment of glaucoma.

Preparations

Timolol Eye Drops

Timolol Tablets

Ph Eur

DEFINITION

Timolol maleate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*S*)-1-[(1,1-dimethylethyl)amino]-3-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]propan-2-ol (*Z*)-butenedioate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, soluble in water and in ethanol (96 per cent).

It melts at about 199 °C, with decomposition.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Dissolve 1.000 g in *1 M hydrochloric acid* and dilute to 10.0 ml with the same acid. The specific optical rotation *(2.2.7)* is - 5.7 to - 6.2.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *timolol maleate CRS*.

C. Examine the chromatograms obtained in the test for related substances after exposure to iodine vapour. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Triturate 0.1 g with a mixture of 1 ml of *dilute sodium hydroxide solution R* and 3 ml of *water R*. Shake with 3 quantities, each of 5 ml, of *ether R*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min. No violet-red colour develops. Neutralise the remainder of the aqueous layer with *dilute sulphuric acid R* and add 1 ml of *bromine water R*. Heat on a water-bath for 15 min, then heat to boiling and cool. To 0.2 ml of this solution add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat on a water-bath for 15 min; a violet-red colour develops. Add 0.2 ml of a 100 g/l solution of *potassium bromide R* and heat for 5 min on a water-bath; the colour becomes violet-blue.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_8 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 3.8 to 4.3.

Enantiomeric purity

Examine by liquid chromatography *(2.2.29)*. *Carry out the test protected from actinic light*.

Test solution Dissolve 30.0 mg of the substance to be examined in a mixture of 1 volume of *methylene chloride R* and 3 volumes of *2-propanol R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 30 mg of *timolol maleate CRS* in a mixture of 1 volume of *methylene chloride R* and 3 volumes of *2-propanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 15.0 mg of *(R)-timolol CRS* in a mixture of 1 volume of *methylene chloride R* and 3 volumes of *2-propanol R* and dilute to 10.0 ml with the same mixture of solvents. Dilute 1.0 ml of the solution to 50.0 ml with a mixture of 1 volume of *methylene chloride R* and 3 volumes of *2-propanol R*.

Reference solution (c) Dilute 1 ml of reference solution (a) to 100 ml with a mixture of 1 volume of *methylene chloride R* and 3 volumes *of 2-propanol R.* Mix 1 ml of this solution with 1 ml of reference solution (b).

Reference solution (d) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 1 volume of *methylene chloride R* and 3 volumes of *2-propanol R*.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *silica gel OD for chiral separations R* (5 µm);

— as mobile phase at a flow rate of 1 ml/min a mixture of 2 ml of *diethylamine R*, 40 ml of *2-propanol R* and 960 ml of *hexane R*;

— as detector a spectrophotometer set at 297 nm.

Under these conditions the peak corresponding to the (*R*)-isomer appears first.

Inject 5 µl of reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full scale of the recorder. Inject 5 µl of each solution. The test is not valid unless: in the chromatogram obtained with reference solution (c), the resolution between the peaks corresponding to the (*R*)-enantiomer and to the (*S*)-enantiomer is at least 4.0; the retention times of the principal peaks (corresponding to the (*S*)-enantiomer) in the chromatograms obtained with the test solution and reference solution (a) are identical. In the chromatogram obtained with the test solution, the area of any peak corresponding to the (*R*)-enantiomer is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1 per cent).

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate GF$_{254}$ R.*

Test solution (a) Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *methanol R*.

Reference solution (a) Dissolve 10 mg of *timolol maleate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 10 ml of test solution (b) to 50 ml with *methanol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 20 volumes of *methanol R* and 80 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.4 per cent). Disregard the spot remaining at the starting point. Expose the plate to iodine vapour for 2 h. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.4 per cent). Disregard the spot remaining at the starting point.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

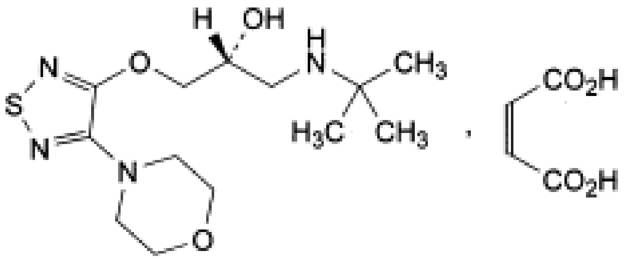
Dissolve 0.350 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 43.25 mg of $C_{17}H_{28}N_4O_7S$.

STORAGE

Store protected from light.

IMPURITIES



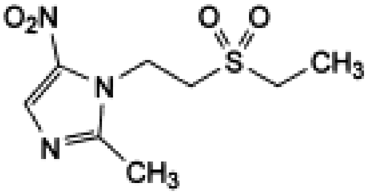
A. (2*R*)-1-[(1,1-dimethylethyl)amino]-3-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]propan-2-ol (*Z*)-butenedioate.

Ph Eur

Tinidazole

General Notices

(Ph Eur monograph 1051)



$C_8H_{13}N_3O_4S$ 247.3 *19387-91-8*

Action and use

Antiprotozoal; antibacterial.

Ph Eur

DEFINITION

1-[2-(Ethylsulphonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Almost white or pale yellow, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone and in methylene chloride, sparingly soluble in methanol.

IDENTIFICATION

First identification A, C.

Second identification A, B, D, E.

A. Melting point *(2.2.14)* : 125 °C to 128 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 10.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *methanol R* .

Spectral range 220-350 nm.

Absorption maximum At 310 nm.

Specific absorbance at the absorption maximum 340 to 360.

C. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *tinidazole CRS.*

D. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 mg of *tinidazole CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel GF*$_{254}$ *plate R.*

Pretreatment Heat at 110 °C for 1 h and allow to cool.

Mobile phase *butanol R, ethyl acetate R* (25:75 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

E. To about 10 mg add about 10 mg of *zinc powder R* , 0.3 ml of *hydrochloric acid R* and 1 ml of *water R* . Heat in a water-bath for 5 min and cool. The solution gives the reaction of primary aromatic amines *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Dissolve 1.0 g in *acetone R* and dilute to 20 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* . *Protect solutions from light*.

Test solution Dissolve 10.0 mg of the substance to be examined in 10.0 ml of *methanol R* and dilute to 100.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *tinidazole impurity A CRS* and 5.0 mg of *tinidazole impurity B CRS* in 10.0 ml of *methanol R* and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 50.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 3.0 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Regular column conditioning by subsequent flushing with 50 ml of *water R*, 100 ml of *methanol R*, 25 ml of *water R* and 100 ml of the mobile phase is recommended.

Mobile phase *acetonitrile R, methanol R, water R* (10:20:70 *V/V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 320 nm.

Injection 20 µl.

Run time 1.5 times the retention time of tinidazole.

Relative retention With reference to tinidazole (retention time = about 6 min): impurity A = about 0.6; impurity B = about 0.7.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 25 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

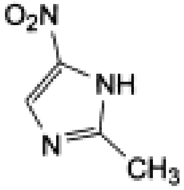
1 ml of *0.1 M perchloric acid* is equivalent to 24.73 mg of $C_8H_{13}N_3O_4S$.

STORAGE

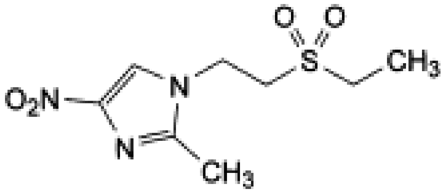
Protected from light.

IMPURITIES

Specified impurities A, B.



A. 2-methyl-5-nitro-1*H*-imidazole,



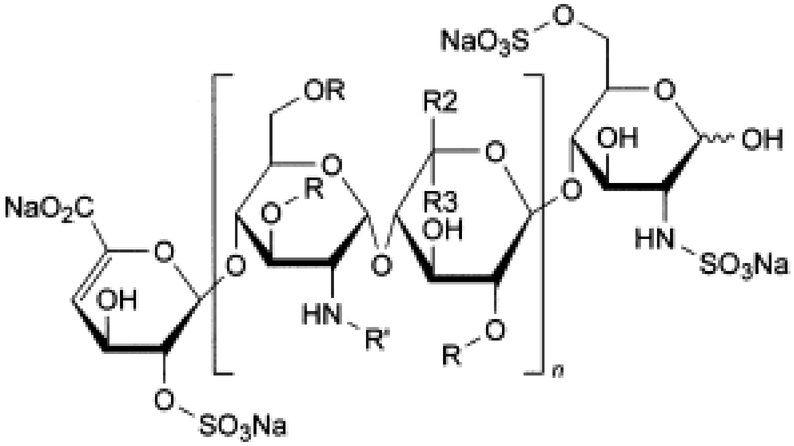
B. 1-[2-(ethylsulphonyl)ethyl]-2-methyl-4-nitro-1*H*-imidazole.

Ph Eur

Tinzaparin Sodium

General Notices

(Ph Eur monograph 1271)



n = 1 to 25 , R = H or SO_3Na , R' = H or SO_3Na or CO-CH_3
R2 = H and R3 = CO_2Na or R2 = CO_2Na and R3 = H

DEF Action and use

Low molecular weight heparin.

Ph Eur

DEFINITION

Tinzaparin sodium is the sodium salt of a low-molecular-mass heparin that is obtained by controlled enzymatic depolymerisation of heparin from porcine intestinal mucosa using heparinase from *Flavobacterium heparinum*. The majority of the components have a 2-*O*-sulpho-4-enepyranosuronic acid structure at the non-reducing end and a 2-*N*,6-*O*-disulpho-D-glucosamine structure at the reducing end of their chain.

Tinzaparin sodium complies with the monograph on Low-molecular-mass heparins (0828) with the modifications and additional requirements below The mass-average relative molecular mass ranges between 5500 and 7500 with a characteristic value of about 6500.

The degree of sulphatation is 1.8 to 2.5 per disaccharide unit.

The potency is not less than 70 IU and not more than 120 IU of anti-factor Xa activity per milligram calculated with reference to the dried substance. The ratio of the anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 2.5.

IDENTIFICATION

Carry out identification test A as described in the monograph for *Low-molecular-mass heparins (0828)* using *tinzaparin sodium CRS* .

Carry out identification test C as described in the monograph for *Low-molecular-mass heparins (0828)*. The following requirements apply.

The mass-average relative molecular mass ranges between 5500 and 7500. The mass percentage of chains lower than 2000 is not more than 10.0 per cent. The mass percentage of chains between 2000 and 8000 ranges between 60.0 and 72.0 per cent. The mass percentage of chains above 8000 ranges between 22.0 and 36.0 per cent.

TESTS

Appearance of solution

Dissolve 1.0 g in 10 ml of *water R* . The solution is clear *(2.2.1)* and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*

Absorbance *(2.2.25)*

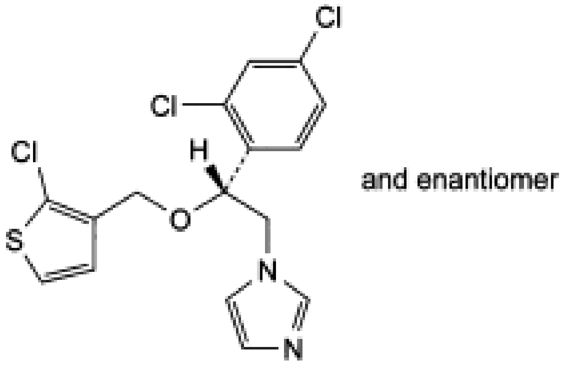
Dissolve 50.0 mg in 100 ml of *0.01 M hydrochloric acid* . The specific absorbance, measured at 231 nm and calculated with reference to the dried substance, is 8.0 to 12.5.

Ph Eur

Tioconazole

General Notices

(Ph Eur monograph 2074)



$C_{16}H_{13}Cl_3N_2OS$ 387.7 *65899-73-2*

Action and use

Antifungal

Preparations

Tioconazole Cream

Tioconazole Nail Solution

Ph Eur

DEFINITION

1-[(2*RS*)-2-[(2-Chlorothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, very soluble in methylene chloride, freely soluble in alcohol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24).*

Comparison Ph. Eur. reference spectrum of tioconazole.

TESTS

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *tioconazole for system suitability CRS* in the mobile phase and dilute to 2.5 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 170 m^2/g, a pore size of 12 nm and a carbon loading of 10 per cent.

Mobile phase Mix 1 volume of a 1.7 g/l solution of *tetrabutylammonium dihydrogen phosphate R* previously adjusted to pH 7.4 with *dilute ammonia R2* and 3 volumes of *methanol R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 218 nm.

Injection 20 µl.

Run time 2.5 times the retention time of tioconazole.

System suitability Reference solution (b):

— *resolution:* minimum 1.0 between the peaks due to impurity B and impurity C (locate impurities A, B and C by comparison with the chromatogram provided with *tioconazole for system suitability CRS*).

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.7; impurity C = 1.7;

— *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 1.00 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 38.77 mg of $C_{16}H_{13}Cl_3N_2OS$.

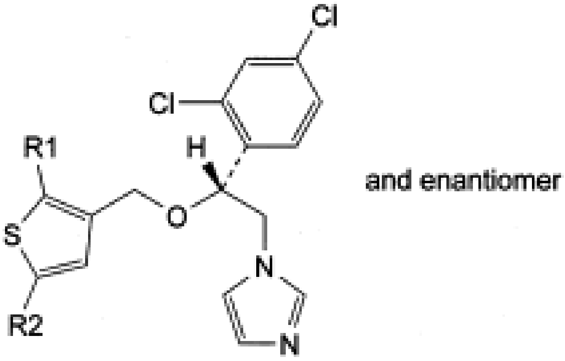
STORAGE

Protected from light.

IMPURITIES

Specified impurities *A, B, C.*

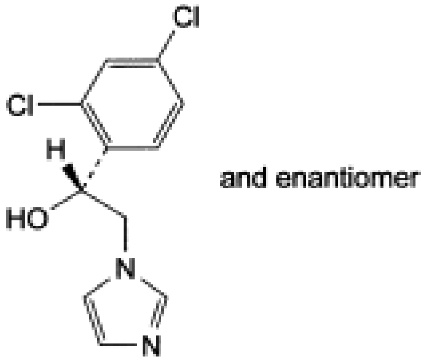
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): D.*



A. R1 = R2 = H: 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[(thiophen-3-yl)methoxy]ethyl]-1*H*-imidazole,

B. R1 = R2 = Cl: 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[(2,5-dichlorothiophen-3-yl)methoxy]ethyl]-1*H*-imidazole,

C. R1 = Cl, R2 = Br: 1-[(2*RS*)-2-[(5-bromo-2-chlorothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

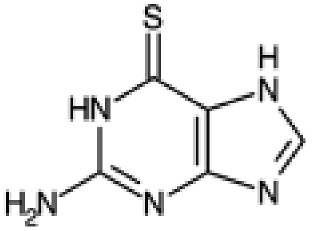

Cl
Cl
H
HO
N
N
and enantiomer

D. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol.

Ph Eur

Tioguanine

General Notices



$C_5H_5N_5S$ 167.2 *154-42-7*

Action and use

Purine analogue; cytostatic.

Preparation

Tioguanine Tablets

DEFINITION

Tioguanine is 2-aminopurine-6(1*H*)-thione. It contains not less than 98.0% and not more than 101.0% of $C_5H_5N_5S$, calculated with reference to the dried substance.

CHARACTERISTICS

A pale yellow, crystalline powder.

Practically insoluble in *water* and in *ethanol (96%)*. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Heat a suitable quantity at 105° at a pressure not exceeding 0.7 kPa for 5 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of tioguanine *(RS 340)*.

B. The *light absorption*, Appendix II B, in the range 230 to 370 nm of a 0.001% w/v solution in 0.1M *hydrochloric acid* exhibits two maxima, at 258 nm and 348 nm. The *absorbance* at 348 nm is about 1.24.

C. Mix 10 mg with 10 mg of *sodium formate* in a test tube and heat gently until melted. A gas is evolved which turns *lead acetate paper* black.

TESTS

Phosphate

To 0.5 g add 2 ml of 5M *sulphuric acid* and heat on a water bath for 5 minutes. Add, drop wise, 5 ml of *nitric acid* and continue heating until a clear solution is obtained. Cool and add 10 ml of *water* and 0.75 ml of a solution prepared by dissolving 8.3 g of *ammonium molybdate* in 40 ml of *water* and adding 33 ml of 5M *sulphuric acid* and sufficient *water* to produce 100 ml. Add 1.0 ml of *strong aminohydroxynaphthalenesulphonic acid solution*, mix and dilute to 25 ml with *water*. Measure the *absorbance* of the resulting solution at 820 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but omitting the substance being examined. The absorbance is not more than that obtained by treating 1.5 ml of *phosphate standard solution (100 ppm* PO_4*)* in the same manner, beginning at the words 'add 10 ml of *water* ...'.

Free sulphur

Dissolve 50 mg in 5 ml of 1M *sodium hydroxide*. The solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 90 volumes of *methanol* and 15 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 2 µl of solution (1) and 5 µl and 1 µl of solution (2). Solution (1) contains 1.0% w/v of the substance being examined in 0.1M *sodium hydroxide*. Solution (2) contains 0.010% w/v of *guanine* in 0.1M *sodium hydroxide*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to guanine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with 5 µl of solution (2). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with 1 µl of solution (2).

Loss on drying

When dried at 105° at a pressure not exceeding 0.7 kPa for 5 hours, loses not more than 1.0% of its weight. Use 1 g.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using a solution prepared by dissolving 0.15 g in 15 ml of *anhydrous formic acid*, adding 50 ml of *butan-2-one* and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 16.72 mg of $C_5H_5N_5S$.

Titanium Dioxide

General Notices

(Ph Eur monograph 0150)

TiO_2 79.9 *13463-67-7*

Action and use

Protective; excipient.

Preparation

Titanium Ointment

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water. It does not dissolve in dilute mineral acids but dissolves slowly in hot concentrated sulphuric acid.

IDENTIFICATION

A. When strongly heated, it becomes pale yellow; the colour disappears on cooling.

B. To 5 ml of solution S2 (see Tests) add 0.1 ml of *strong hydrogen peroxide solution R*. An orange-red colour appears.

C. To 5 ml of solution S2 add 0.5 g of *zinc R* in granules. After 45 min, the mixture has a violet-blue colour.

TESTS

Solution S1

Shake 20.0 g with 30 ml of *hydrochloric acid R* for 1 min. Add 100 ml of *distilled water R* and heat the mixture to boiling. Filter the hot mixture through a hardened filter paper until a clear filtrate is obtained. Wash the filter with 60 ml of *distilled water R* and dilute the combined filtrate and washings to 200 ml with *distilled water R* .

Solution S2

Mix 0.500 g (m g) with 5 g of *anhydrous sodium sulphate R* in a 300 ml long-necked combustion flask. Add 10 ml of *water R* and mix. Add 10 ml of *sulphuric acid R* and boil vigorously, with the usual precautions, until a clear solution is obtained. Cool, add slowly a cooled mixture of 30 ml of *water R* and 10 ml of *sulphuric acid R* , cool again and dilute to 100.0 ml with *water R.*

Appearance of solution

Solution S2 is not more opalescent than reference suspension II *(2.2.1)* and is colourless *(2.2.2, Method II)*.

Acidity or alkalinity

Shake 5.0 g with 50 ml of *carbon dioxide-free water R* for 5 min. Centrifuge or filter until a clear solution is obtained. To 10 ml of the solution add 0.1 ml of *bromothymol blue solution R1* . Not more than 1.0 ml *of 0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Water-soluble substances

Maximum 0.5 per cent.

To 10.0 g add a solution of 0.5 g of *ammonium sulphate R* in 150 ml of *water R* and boil for 5 min. Cool, dilute to 200 ml with *water R* and filter until a clear solution is obtained. Evaporate 100 ml of the solution to dryness in a tared evaporating dish and ignite. The residue weighs a maximum of 25 mg.

Antimony

Maximum 100 ppm.

To 10 ml of solution S2 add 10 ml of *hydrochloric acid R* and 10 ml of *water R.* Cool to 20 °C, if necessary, and add 0.15 ml of *sodium nitrite solution R* . After 5 min, add 5 ml of a 10 g/l solution of *hydroxylamine hydrochloride R* and 10 ml of a freshly prepared 0.1 g/l solution of *rhodamine B R* . Mix thoroughly after each addition. Shake vigorously with 10.0 ml of *toluene R* for 1 min. Allow to separate and centrifuge for 2 min if necessary. Any pink colour in the toluene phase is not more intense than that in the toluene phase of a standard prepared at the same time in the same manner using a mixture of 5.0 ml of *antimony standard solution (1 ppm Sb) R* , 10 ml of *hydrochloric acid R* and 15 ml of a solution containing 0.5 g of *anhydrous sodium sulphate R* and 2 ml of *sulphuric acid R* instead of the mixture of 10 ml of solution S2, 10 ml of *hydrochloric acid R* and 10 ml of *water R* .

Arsenic *(2.4.2, Method A)*

Maximum 5 ppm.

Place 0.50 g in a 250 ml round-bottomed flask, fitted with a thermometer, a funnel with stopcock and a vapour-outlet tube connected to a flask containing 30 ml of *water R*. Add 50 ml of *water R*, 0.5 g of *hydrazine sulphate R*, 0.5 g of *potassium bromide R* and 20 g of *sodium chloride R*. Through the funnel, add dropwise 25 ml of *sulphuric acid R,* heat and maintain the temperature of the liquid at 110-115 °C for 20 min. Collect the vapour in the flask containing 30 ml of *water R*. Dilute to 50 ml with *water R*. 20 ml of the solution complies with the test.

Barium

To 10 ml of solution S1 add 1 ml of *dilute sulphuric acid R*. After 30 min, any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution S1 and 1 ml of *distilled water R*.

Iron

Maximum 200 ppm.

To 8 ml of solution S2 add 4 ml of *water R*. Mix and add 0.05 ml of *bromine water R*. Allow to stand for 5 min and remove the excess of bromine with a current of air. Add 3 ml of *potassium thiocyanate solution R*. Any colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 4 ml of *iron standard solution (2 ppm Fe) R* and 8 ml of a 200 g/l solution of *sulphuric acid R.*

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dilute 10 ml of solution S1 to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

ASSAY

To 300 g of *zinc R* in granules (710) add 300 ml of a 20 g/l solution of *mercuric nitrate R* and 2 ml of *nitric acid R*, shake for 10 min and wash with *water R*. Pack the amalgamated zinc into a glass tube about 400 mm long and about 20 mm in diameter fitted with a tap and a filter plate. Pass through the column 100 ml of *dilute sulphuric acid R* followed by 100 ml of *water R ,* making sure that the amalgam is always covered with liquid. Pass slowly at a rate of about 3 ml/min through the column a mixture of 100 ml of *dilute sulphuric acid R* and 100 ml of *water R* followed by 100 ml of *water R*. Collect the eluate in a 500 ml conical flask containing 50.0 ml of a 150 g/l solution of *ferric ammonium sulphate R* in a mixture of 1 volume of *sulphuric acid R* and 3 volumes of *water R*. Add 0.1 ml of *ferroin R* and titrate immediately with *0.1 M ammonium and cerium nitrate* until a greenish colour is obtained (n1 ml). Pass slowly at a rate of about 3 ml/min through the column a mixture of 50 ml of *dilute sulphuric acid R* and 50 ml of *water R*, followed by 20.0 ml of solution S2, a mixture of 50 ml of *dilute sulphuric acid R* and 50 ml of *water R* and finally 100 ml of *water R.* Collect the eluate in a 500 ml conical flask containing 50.0 ml of a 150 g/l solution of *ferric ammonium sulphate R* in a mixture of 1 volume of *sulphuric acid R* and 3 volumes of *water R.* Rinse the lower end of the column with *water R*, add 0.1 ml of *ferroin R* and titrate immediately with *0.1 M ammonium and cerium nitrate* until a greenish colour is obtained (n2 ml).

Calculate the percentage content of TiO2 using the following expression:

$$\frac{3.99 \times (n_2 - n_1)}{m}$$

m = mass of the substance to be examined used for the preparation of solution S2, in grams

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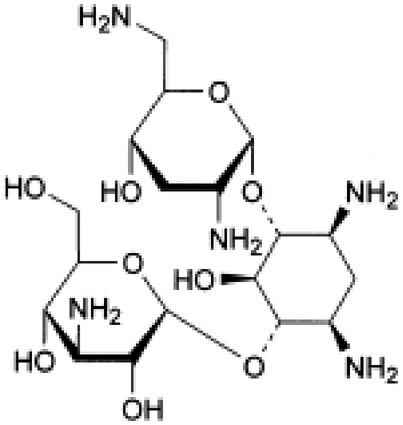
Ph Eur

Tobramycin

General Notices

(Ph Eur monograph 0645)



$C_{18}H_{37}N_5O_9$ 467.5 *32986-56-4*

Action and use

Aminoglycoside antibacterial.

Preparation

Tobramycin Injection

Ph Eur

DEFINITION

4-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxy-6-*O*-(2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl)-L-streptamine.

Substance produced by *Streptomyces tenebrarius* or obtained by any other means.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Nuclear magnetic resonance spectrometry *(2.2.33)* .

Preparation 100 g/l solution in *deuterium oxide R* .

Comparison 100 g/l solution of *tobramycin CRS* in *deuterium oxide R* .

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 20 mg of the substance to be examined in *water R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *tobramycin CRS* in *water R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 4 mg of *neomycin sulphate CRS* and 4 mg of *kanamycin monosulphate CRS* in 1 ml of reference solution (a).

Plate *TLC silica gel plate R.*

Mobile phase *Methylene chloride R, concentrated ammonia R, methanol R* (17:33:50 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Spray with a mixture of equal volumes of a 2 g/l solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and a 460 g/l solution of *sulphuric acid R* ; heat at 105 °C for 5-10 min.

System suitability The chromatogram obtained with reference solution (b) shows 3 major spots which are clearly separated.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 5 mg in 5 ml of *water R* . Add 5 ml of a 1 g/l solution of *ninhydrin R* in *ethanol (96 per cent) R* and heat in a water-bath for 3 min. A violet-blue colour develops.

TESTS

pH *(2.2.3)*

9.0 to 11.0.

Dissolve 1.0 g in 10 ml of *carbon dioxide-free water R*.

Specific optical rotation *(2.2.7)*

+ 138 to + 148 (anhydrous substance).

Dissolve 1.00 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Test solution (b) Dilute 10.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *tobramycin CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (d) Dissolve 10.0 mg of *kanamycin B sulphate CRS* in 20.0 ml of the mobile phase. To 1.0 ml of this solution, add 2.0 ml of reference solution (a) and dilute to 10.0 ml with the mobile phase.

Reference solution (e) Dilute 10.0 ml of reference solution (a) to 25.0 ml with the mobile phase.

Column:

- *size:* l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm;
- *temperature*: 55 °C.

Mobile phase Mixture prepared with *carbon dioxide-free water R* containing 52 g/l of *anhydrous sodium sulphate R*, 1.5 g/l of *sodium octanesulphonate R*, 3 ml/l of *tetrahydrofuran R* stabilised with *butylhydroxytoluene R*, and 50 ml/l of *0.2 M potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *dilute phosphoric acid R*. Degas.

Flow rate 1.0 ml/min.

Post-column solution *Carbonate-free sodium hydroxide solution R* diluted 25-fold with *carbon dioxide-free water R*, which is added pulselessly to the column effluent using a 375 µl polymeric mixing coil.

Flow rate 0.3 ml/min.

Detection Pulsed amperometric detector or equivalent with a gold working electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively + 0.05 V detection, + 0.75 V oxidation and - 0.15 V reduction potentials, with pulse durations according to the instrument used. The temperature of the detector is set at 35 °C.

NOTE: to prevent problems due to salt precipitation, the electrochemical cell can be flushed with water R overnight.

Injection 20 µl using a refrigerated injector (4-8 °C); inject test solution (a) and reference solutions (b), (c) and (d).

Run time 1.5 times the retention time of tobramycin.

Relative retention With reference to tobramycin (retention time = about 18 min): impurity C = about 0.35; impurity B = about 0.40, impurity A = about 0.70.

System suitability:

— *resolution*: minimum 3.0 between the peaks due to impurity A and to tobramycin in the chromatogram obtained with reference solution (d); if necessary, adjust the concentration of sodium octanesulphonate in the mobile phase;

— *signal-to-noise ratio* : minimum 10 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and not more than 1 such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.5 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent).

2-Methyl-1-propanol *(2.4.24, System B)*

Maximum 1.0 per cent m/m.

Water *(2.5.12)*

Maximum 8.0 per cent, determined on 0.30 g.

Sulphated ash *(2.4.14)*

Maximum 0.3 per cent, determined on 1.0 g.

Bacterial endotoxins *(2.6.14)*

Less than 2.0 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modifications.

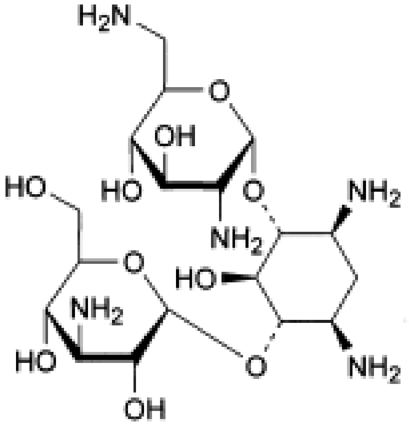
Injection Test solution (b) and reference solution (e).

Calculate the percentage content of tobramycin.

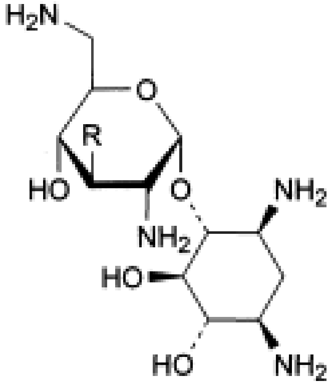
STORAGE

If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES


H2N
O
OH
HO
HO
O
NH2
NH2
O
HO
NH2
HO
NH2
HO
O
NH2
OH

A. 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxy-6-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-L-streptamine (kanamycin B),


H2N
O
R
HO
O
NH2
NH2
HO
HO
NH2

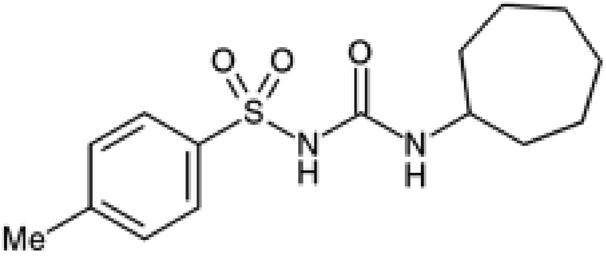
B. R = H: 2-deoxy-4-*O*-(2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl)-D-streptamine (nebramine),

C. R = OH: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (neamine).

Ph Eur

Tolazamide

General Notices



$C_{14}H_{21}N_3O_3S$ 311.4 *1156-19-0*

Action and use

Inhibition of ATP-dependent potassium channels (sulphonylurea); treatment of diabetes mellitus.

Preparation

Tolazamide Tablets

DEFINITION

Tolazamide is 1-perhydroazepin-1-yl-3-tolyl-*p*-sulphonylurea. It contains not less than 98.0% and not more than 101.0% of $C_{14}H_{21}N_3O_3S$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder; odourless or almost odourless.

Very slightly soluble in *water*; freely soluble in *chloroform* ; soluble in *acetone* ; slightly soluble in *ethanol (96%)* .

IDENTIFICATION

A. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of tolazamide *(RS 342)* .

B. The *light absorption* , Appendix II B, in the range 230 to 350 nm of a 0.04% w/v solution in *ethanol (96%)* exhibits maxima at 256, 263 and 275 nm and a shoulder at 268 nm. The *absorbances* at the maxima are about 0.78, about 0.83 and about 0.62, respectively.

C. *Melting point* , about 165°, Appendix V A.

TESTS

Heavy metals

Moisten the residue obtained in the test for Sulphated ash with 1 ml of *hydrochloric acid*, evaporate to dryness and dissolve the residue in 20 ml of *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (20 ppm).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 200 volumes of *chloroform*, 100 volumes of *methanol*, 60 volumes of *cyclohexane* and 23 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in *acetone* containing (1) 2.0% w/v of the substance being examined and (2) 0.010% w/v of *toluene*-p-*sulphonamide*. After removal of the plate, dry it in a current of cold air, heat at 110° for 10 minutes, place the hot plate in a tank of chlorine gas prepared by the addition of *hydrochloric acid* to a 5% w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying

When dried to constant weight at 60° at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.2%, Appendix IX A. Use 1 g.

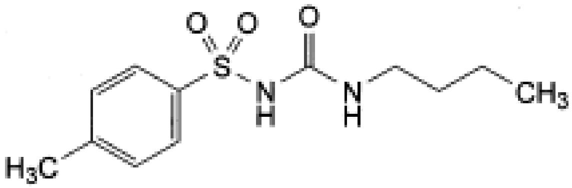
ASSAY

Dissolve 0.5 g in 20 ml of *butan-2-one* with the aid of gentle heat. Allow to cool, add 30 ml of *ethanol (96%)* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 31.14 mg of $C_{14}H_{21}N_3O_3S$.

Tolbutamide

General Notices

(Ph Eur monograph 0304)



$C_{12}H_{18}N_2O_3S$ 270.3 *64-77-7*

Action and use

Inhibition of ATP-dependent potassium channels (sulphonylurea); treatment of diabetes mellitus.

Preparation

Tolbutamide Tablets

Ph Eur

DEFINITION

Tolbutamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 1-butyl-3-[(4-methylphenyl)sulphonyl]urea, calculated with reference to the dried substance.

CHARACTERS

A white or almost white crystalline powder, practically insoluble in water, soluble in acetone and in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D.*

A. Melting point *(2.2.14)* : 126 °C to 130 °C.

B. Dissolve 25.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Examined between 245 nm and 300 nm *(2.2.25)*, the solution shows three absorption maxima, at 258 nm, 263 nm and 275 nm, and a shoulder at 268 nm. Dilute 10.0 ml of the solution to 250.0 ml with *methanol R.* Examined between 220 nm and 235 nm, the solution shows a single absorption maximum, at 228 nm. The specific absorbance at this maximum is 480 to 520.

C. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *tolbutamide CRS*.

D. To 0.2 g add 8 ml of a 500 g/l solution of *sulphuric acid R* and heat under a reflux condenser for 30 min. Allow to cool. Crystals are formed which, after recrystallisation from hot *water R* and drying at 100 °C to 105 °C, melt *(2.2.14)* at 135 °C to 140 °C.

TESTS

Appearance of solution

Dissolve 0.2 g in 5 ml of dilute *sodium hydroxide solution R* and add 5 ml of *water R.* The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

To 2.0 g add 50 ml of *carbon dioxide-free water R* and heat at 70 °C for 5 min. Cool rapidly and filter. The pH of the filtrate is 4.5 to 5.5.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance.

Test solution Dissolve 0.50 g of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 15 mg of *toluenesulphonamide R* in *acetone R* and dilute to 100 ml with the same solvent.

Reference solution (b) To 5 ml of the test solution add 5 ml of reference solution (a).

Apply to the plate 5 µl of the test solution and of reference solution (a) and 10 µl of reference solution (b). Develop over a path of 15 cm using a mixture of 2 volumes of *anhydrous formic acid R*, 8 volumes of *methanol R* and 90 volumes of *chloroform R*. Dry the plate in a current of warm air and heat at 110 °C for 10 min. At the bottom of a chromatography tank, place an evaporating dish containing a 50 g/l solution of *potassium permanganate R* and add an equal volume of *hydrochloric acid R*. Place the plate whilst still hot in the tank and close the tank. Leave the plate in contact with the chlorine vapour for 2 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the points of application gives at most a very faint blue colour with a drop of potassium iodide and *starch solution R*; avoid prolonged exposure to cold air. Spray with *potassium iodide and starch solution R* and allow to stand for 5 min. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.3 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Heavy metals *(2.4.8)*

Dissolve 1.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B for heavy metals (20 ppm). Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R.*

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.2500 g in a mixture of 20 ml of *water R* and 40 ml of *alcohol R* . Titrate with *0.1 M sodium hydroxide* , using *1 ml of phenolphthalein solution R* as indicator.

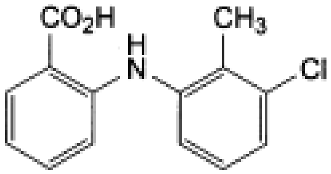
1 ml of *0.1 M sodium hydroxide* is equivalent to 27.03 mg of $C_{12}H_{18}N_2O_3S$.

Ph Eur

Tolfenamic Acid

General Notices

(Ph Eur monograph 2039)



$C_{14}H_{12}ClNO_2$ 261.7 *13710-19-5*

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION

2-[(3-Chloro-2-methylphenyl)amino]benzoic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Practically insoluble in water, soluble in dimethylformamide, sparingly soluble in ethanol and in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

mp

About 213 °C.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Dissolve 20 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 100 ml with the same mixture of solvents. Dilute 5.0 ml of the solution to 50 ml with a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R*. Examined between 250 nm and 380 nm *(2.2.25)*, the solution shows 2 absorption maxima, at 286 nm and 345 nm. The ratio of the absorbance measured at the maximum at 286 nm to that measured at the maximum at 345 nm is 1.2 to 1.4.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *tolfenamic acid CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 25 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 25 mg of *tolfenamic acid CRS* in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Plate *TLC silica gel GF* $_{254}$ *plate R*.

Mobile phase *glacial acetic acid R, dioxan R, toluene R* (1:25:90 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in 5 ml of *ethanol R* and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 25 mg of *2-chlorobenzoic acid R* and 25 mg of *3-chloro-2-methylaniline R* in 5 ml of ethanol R and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *spherical end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 450 m2/g and a pore size of 8 nm.

Mobile phase *glacial acetic acid R*, *water R*, *ethanol R* (2:350:650 *V/V/V*).

Flow rate 0.8 ml/min.

Detection spectrophotometer at 232 nm.

Injection 20 µl.

Run time 3 times the retention time of tolfenamic acid.

Relative retention With reference to tolfenamic acid (retention time = about 15 min): impurity A = about 0.25; impurity B = about 0.34.

System suitability Reference solution (a):

- *resolution*: minimum 2.5 between the peaks due to impurity A and to impurity B.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *impurity B*: not more than half the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Copper

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I).*

Test solution Place 1.00 g of the substance to be examined in a silica crucible, moisten with *sulphuric acid R*, heat cautiously on a flame for 30 min and then progressively to about 650 ± 50 °C. Continue ignition until all black particles have disappeared. Allow to cool, dissolve the residue in *0.1 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solutions Prepare the reference solutions using *copper standard solution (0.1 per cent Cu) R*, diluted as necessary using *0.1 M nitric acid*.

Source Copper hollow-cathode lamp.

Wavelength 324.8 nm.

Flame Air-acetylene.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

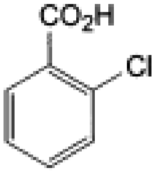
Dissolve 0.200 g with the aid of ultrasound in 100 ml of *ethanol R* . Add 0.1 ml of *phenol red solution R* and titrate with *0.1 M sodium hydroxide* .

1 ml of *0.1 M sodium hydroxide* is equivalent to 26.17 mg of $C_{14}H_{12}ClNO_2$.

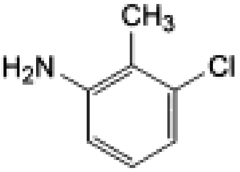
STORAGE

Protected from light.

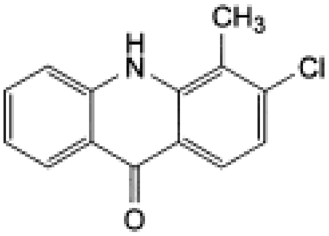
IMPURITIES



A. 2-chlorobenzoic acid,



B. 3-chloro-2-methylaniline,



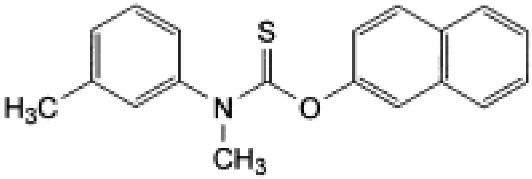
C. 3-chloro-4-methyl-9-oxo-9,10-dihydroacridine.

Ph Eur

Tolnaftate

General Notices

(Ph Eur monograph 1158)



$C_{19}H_{17}NOS$ 307.4 *2398-96-1*

Action and use

Antifungal.

Ph Eur

DEFINITION

Tolnaftate contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of *C*-naphthalen-2-yl methyl(3-methylphenyl)thiocarbamate, calculated with reference to the dried substance.

CHARACTERS

A white or yellowish-white powder, practically insoluble in water, freely soluble in acetone and in methylene chloride, very slightly soluble in alcohol.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)* : 109 °C to 112 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *tolnaftate CRS* . Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Mix about 1 mg with 0.5 ml of *sulphuric acid R* . Add 0.05 ml of *formaldehyde solution R* . A greenish-blue colour develops.

TESTS

Appearance of solution

Dissolve 0.5 g in 10 ml of *acetone R* . The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Related substances

Examine by thin-layer chromatography *(2.2.27)* , using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 2 ml with the same solvent.

Test solution (b) Dilute 0.5 ml of test solution (a) to 10 ml with *acetone R.*

Reference solution (a) Dissolve 25 mg of *tolnaftate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 10 ml with *acetone R* .

Reference solution (c) Dissolve 50 mg of β*-naphthol R* in 1 ml of test solution (a) and dilute to 10 ml with *acetone R* .

Apply separately to the plate 5 µl of each solution. Develop over a path of 12 cm using *toluene R.* Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

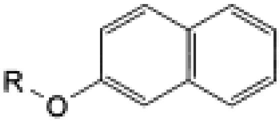
Dissolve 50.0 mg in *methanol R* and dilute to 250.0 ml with the same solvent. Dilute 2.0 ml to 50.0 ml with *methanol R* . Measure the absorbance *(2.2.25)* at the maximum at 257 nm.

Calculate the content of $C_{19}H_{17}NOS$ taking the specific absorbance to be 720.

STORAGE

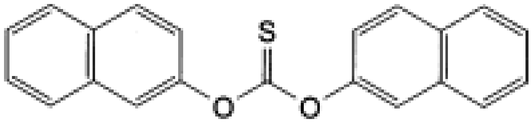
Store protected from light.

IMPURITIES

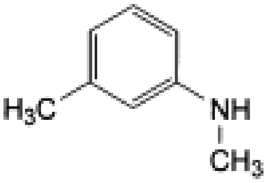


A. R = H: naphthalen-2-ol (β-naphtol),

C. R = CS-Cl: *O*-naphthalen-2-yl chlorothioformate,



B. *O,O*-dinaphtalen-2-yl thiocarbonate,



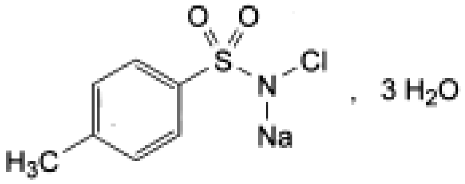
D. *N*,3-dimethylaniline.

Ph Eur

Tosylchloramide Sodium

General Notices

(Ph Eur monograph 0381)



$C_7H_7ClNNaO_2S,3H_2O$ 281.7 *127-65-1*

Action and use

Antiseptic.

Ph Eur

DEFINITION

Sodium *N*-chloro-4-methylbenzene-sulphonimidate trihydrate.

Content

98.0 per cent to 103.0 per cent of $C_7H_7ClNNaO_2S,_3H_2O$.

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) turns *red litmus paper R* blue and then bleaches it.

B. To 10 ml of solution S add 10 ml of *dilute hydrogen peroxide solution R* . A white precipitate is formed which dissolves on heating. Filter the hot solution and allow to cool.

White crystals are formed which, when washed and dried at 100-105 °C, melt *(2.2.14)* at 137 °C to 140 °C.

C. Ignite cautiously 1 g, because of the risk of deflagration. Dissolve the residue in 10 ml of *water R* . The solution gives reaction (a) of chlorides *(2.3.1)* .

D. The solution prepared for identification test C gives reaction (a) of sulphates *(2.3.1)* .

E. The solution prepared for identification test C gives reaction (b) of sodium *(2.3.1)* .

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and is colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

8.0 to 10.0 for solution S.

Ortho compound

To 2 g add 10 ml of *water R* , mix, add 1 g of *sodium metabisulphite R* and heat to boiling. Cool to 0 °C, filter rapidly and wash with 3 quantities, each of 5 ml, of iced *water R* . The precipitate, dried over *diphosphorus pentoxide R* at a pressure not exceeding 600 Pa, melts *(2.2.14)* at a minimum of 134 °C.

Residue insoluble in anhydrous ethanol

Maximum 2 per cent.

Shake 1.00 g with 20 ml of *anhydrous ethanol R* for 30 min, filter on a tared filter, wash any residue with 5 ml of *anhydrous ethanol R* and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

ASSAY

Dissolve 0.125 g in 100 ml of *water R* in a ground-glass-stoppered flask. Add 1 g of *potassium iodide R* and 5 ml of *dilute sulphuric acid R* . Allow to stand for 3 min. Titrate with *0.1 M sodium thiosulphate* , using 1 ml of *starch solution R* as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 14.08 mg of $C_7H_7ClNNaO_2S,_3H_2O$.

STORAGE

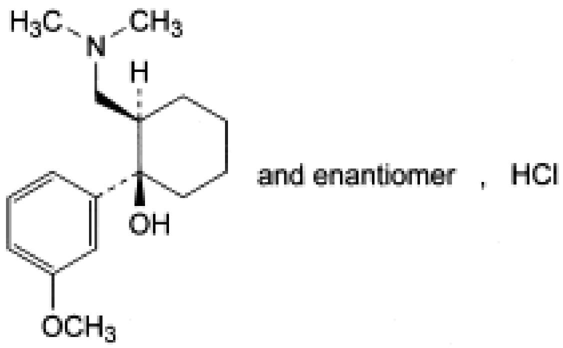
In an airtight container , protected from light.

Ph Eur

Tramadol Hydrochloride

General Notices

(Ph Eur monograph 1681)



$C_{16}H_{26}ClNO_2$ 299.8 *36282-47-0*

Action and use

Opioid receptor agonist; noradrenaline reuptake inhibitor; analgesic.

Preparation

Tramadol Capsules

Ph Eur

DEFINITION

(1*RS*,2*RS*)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, very slightly soluble in acetone.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)* : 180 °C to 184 °C.

B. Infrared absorption spectrophotometry *(2.2.24*).

Comparison *tramadol hydrochloride CRS.*

C. Chromatograms obtained in the test for impurity E.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides *(2.3.1).*

TESTS

Solution S

Dissolve 1.0 g in *water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Acidity

To 10 ml of solution S, add 0.2 ml of *methyl red solution R* and 0.2 ml *of 0.01 M hydrochloric acid* . The solution is red. Not more than 0.4 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°, determined on solution S.

Impurity E

Thin-layer chromatography *(2.2.27)* .

Test solution (a) Dissolve 0.10 g in *methanol R* and dilute to 2 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R* .

Reference solution (a) Dissolve 25 mg of *tramadol hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *tramadol impurity E CRS* in 5 ml of *methanol R* . Dilute 1 ml of the solution to 10 ml with *methanol R* .

Reference solution (c) Dissolve 5 mg of *tramadol impurity A CRS* in 1 ml of reference solution (a).

Plate *TLC silica gel F $_{254}$ plate R*, prewashed with *methanol R*.

Mobile phase *Concentrated ammonia R, 2-propanol R, toluene R* (1:19:80 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate. Saturate the plate for 20 min with *concentrated ammonia R*. For this, add *concentrated ammonia R* to one trough of a twin trough tank. Just before developing, add the mobile phase to the other trough. Place the plate in the chromatographic tank, ensuring that the layer of silica gel is orientated towards the middle of the tank.

Drying In air.

Detection Expose the plate to iodine vapour for 1 h, examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Limit In the chromatogram obtained with test solution (a):

— *impurity E*: any spot corresponding to impurity E is not more intense and not greater than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances

Liquid chromatography *(2.2.29*).

Test solution Dissolve 0.15 g of the substance to be examined in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (a) Dilute 2.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of *tramadol impurity A CRS* in 4.0 ml of the test solution and dilute to 100 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase: base-deactivated end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 295 volumes of *acetonitrile R* and 705 volumes of a mixture of 0.2 ml of *trifluoroacetic acid R* and 100 ml of *water R.*

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µl.

Run time 4 times the retention time of tramadol.

Relative retention With reference to tramadol (retention time = about 5 min): impurity A = about 0.85.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and tramadol.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of this solution complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R* .

Water *(2.5.12)*

Maximum 0.5 per cent, determined on 1.000 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

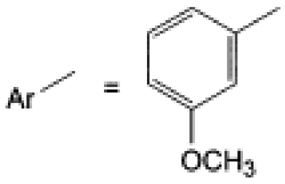
Dissolve 0.180 g in 25 ml of *anhydrous acetic acid R* and add 10 ml of *acetic anhydride R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20).*

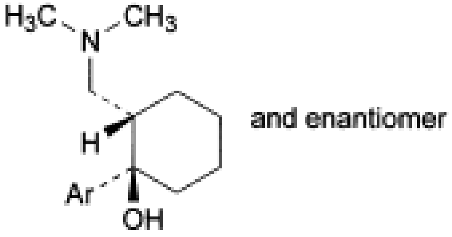
1 ml of *0.1 M perchloric acid* is equivalent to 29.98 mg of $C_{16}H_{26}ClNO_2$.

STORAGE

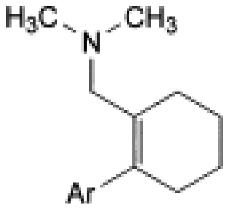
Protected from light.

IMPURITIES

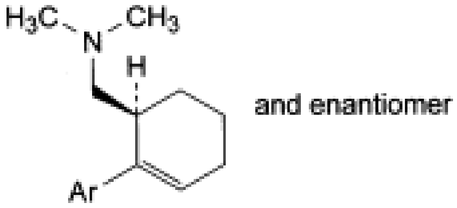



H₃C
N
CH₃
H
Ar
OH
and enantiomer

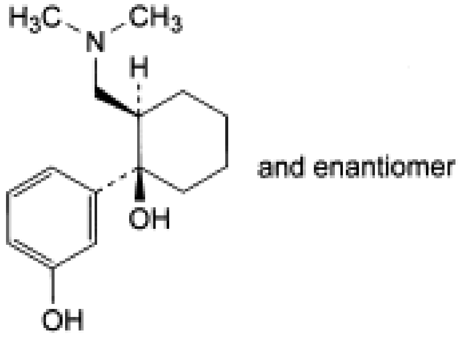
A. (1*RS*,2*SR*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol,



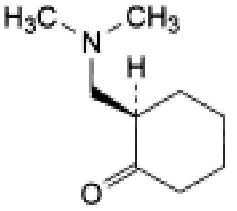
B. [2-(3-methoxyphenyl)cyclohex-1-enyl]-*N*,*N*-dimethylmethanamine,


H₃C
N
CH₃
H
Ar
and enantiomer

C. (1*RS*)-[2-(3-methoxyphenyl)cyclohex-2-enyl]-*N,N*-dimethylmethanamine,


H₃C
N
CH₃
H
OH
OH
and enantiomer

D. (1*RS*,2*RS*)-2-[(dimethylamino)methyl]-1-(3-hydroxyphenyl)cyclohexanol,



and enantiomer

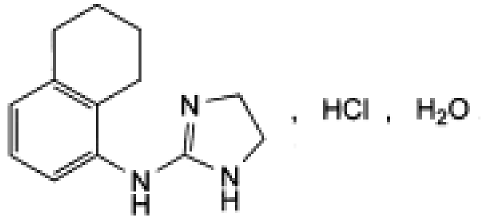
E. (2*RS*)-2-[(dimethylamino)methyl]cyclohexanone.

Ph Eur

Tramazoline Hydrochloride Monohydrate

General Notices

(Ph Eur monograph 1597)



$C_{13}H_{17}N_3,HCl,H_2O$ 269.8 *3715-90-0*

Action and use

Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION

N-(5,6,7,8-Tetrahydronaphthalen-1-yl)-4,5-dihydro-1*H*-imidazol-2-amine hydrochloride monohydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *tramazoline hydrochloride monohydrate CRS.*

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

4.9 to 6.3 for solution S.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 50.0 mg of the substance to be examined in a mixture of 50 volumes of *acetonitrile R* and 50 volumes of *water R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 5.0 mg of *tramazoline impurity A CRS* and 5.0 mg of *tramazoline impurity B CRS* in 5 ml of a mixture of 50 volumes of *acetonitrile R* and 50 volumes of *water R* and add 5 ml of the test solution.

Reference solution (b) Dilute 0.2 ml of reference solution (a) to 100 ml with a mixture of 50 volumes of *acetonitrile R* and 50 volumes of *water R* .

Column:

— *size:* *l* = 0.125 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 2.0 g/l solution of *sodium dodecyl sulphate R* in a mixture of 6 volumes of *2-propanol R* , 42 volumes of *acetonitrile R* and 52 volumes of *water R* .

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µl.

Run time 3 times the retention time of tramazoline.

Relative retention With reference to tramazoline (retention time = about 6.5 min): impurity A = about 0.71; impurity B = about 0.86.

System suitability Reference solution (a):

— the chromatogram obtained shows 3 clearly separated peaks;

— *resolution*: minimum 1.5 between tramazoline and impurity B.

Limits:

- *impurity A:* not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *impurity B*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *any other impurity:* not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *sum of other impurities*: not more than twice the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *disregard limit:* 0.2 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.02 per cent).

Water ***(2.5.12)***

6.2 per cent to 7.2 per cent, determined on 0.500 g.

Sulphated ash ***(2.4.14)***

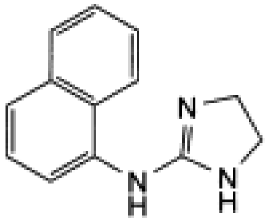
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

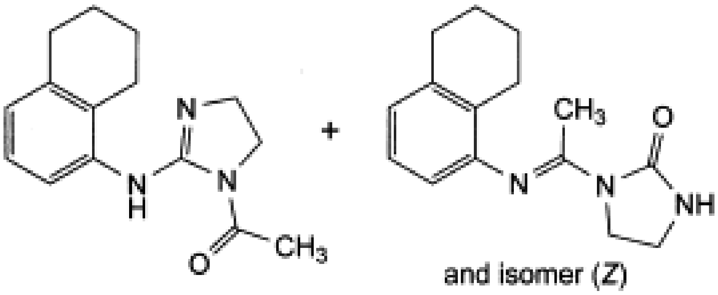
Dissolve 2.000 g in a mixture of 5 ml of *0.1 M hydrochloric acid* and 75 ml of *ethanol (96 per cent) R* . Carry out a potentiometric titration (*2.2.20*) using *1 M sodium hydroxide* . Read the volume added between the 2 points of inflexion.

1 ml of *1 M sodium hydroxide* is equivalent to 251.8 mg of $C_{13}H_{18}ClN_3$.

IMPURITIES



A. *N*-(naphthalen-1-yl)-4,5-dihydro-1*H*-imidazol-2-amine,



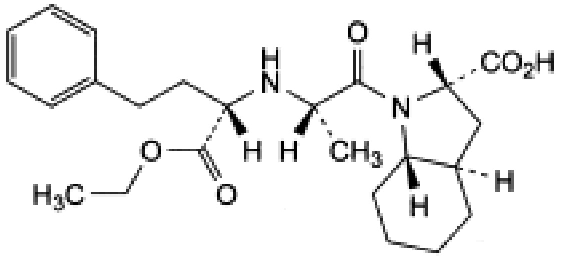
B. mixture of 1-acetyl-*N*-(5,6,7,8-tetrahydronaphthalen-1-yl)-4,5-dihydro-1*H*-imidazol-2-amine and 1-[(*EZ)*-1-[(5,6,7,8-tetrahydronaphthalen-1-yl)imino]ethyl]imidazolidin-2-one.

Ph Eur

Trandolapril

General Notices

(Ph Eur monograph 2245)



$C_{24}H_{34}N_2O_5$ 430.5 *87679-37-6*

Action and use

Angiotensin converting enzyme inhibitor.

Ph Eur

DEFINITION

(2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *trandolapril CRS.*

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

Dissolve 1.0 g in *methanol R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

- 16.5 to - 18.5 (anhydrous substance).

Dissolve 1.0 g in *anhydrous ethanol R* and dilute to 50.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 100.0 ml with mobile phase A.

Reference solution (b) Dissolve 5 mg of *trandolapril impurity C CRS* and 5 mg of *trandolapril impurity D CRS* in mobile phase A and dilute to 5 ml with mobile phase A. Dilute 1 ml of this solution to 20 ml with mobile phase A.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

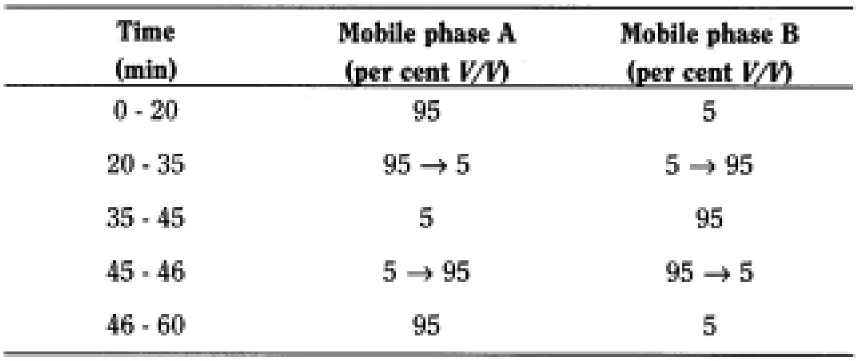
— *stationary phase: end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (3.5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: mix 25 volumes of *acetonitrile R,* and 75 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.5 ± 0.1 with *phosphoric acid R;*

— *mobile phase B*: mix equal volumes of *acetonitrile R* , and a 6.8 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.2 ± 0.1 with *phosphoric acid R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	95	5
20 - 35	95 → 5	5 → 95
35 - 45	5	95
45 - 46	5 → 95	95 → 5
46 - 60	95	5

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Relative retention With reference to trandolapril (retention time = about 14.5 min): impurity C = about 2.1; impurity D = about 2.5.

System suitability Reference solution (b):

— *resolution*: minimum 4 between the peaks due to impurity C and impurity D.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 2.2;

— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity D*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *sum of impurities other than D*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Palladium

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Solvent mixture *nitric acid R, water R* (1:99 *V/V*).

Test solution To the residue of the test for sulphated ash add 3 ml of *hydrochloric acid R* and 1 ml of fuming *nitric acid R* . Cover the crucible with a watch glass and heat at 160-170 °C for 1 h to dissolve the residue. Afterwards continue heating in the open crucible and evaporate the solution. Stop heating before the residue is completely dried, add 1 ml of *nitric acid R* , heat at 160-170 °C for further 10 min, and after cooling dilute to 10.0 ml with *water R* .

Reference solutions Prepare reference solutions containing 0.5 µg, 1.0 µg and 1.5 µg of Pd per millilitre by diluting *palladium standard solution (500 ppm Pd) R* with the solvent mixture.

Source Palladium hollow-cathode lamp.

Wavelength 244.8 nm.

Atomisation device Air-acetylene flame.

Water ***(2.5.32)***

Maximum 0.2 per cent, determined on 1.000 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 2.0 g in a porcelain or quartz crucible.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 43.05 mg of $C_{24}H_{34}N_2O_5$.

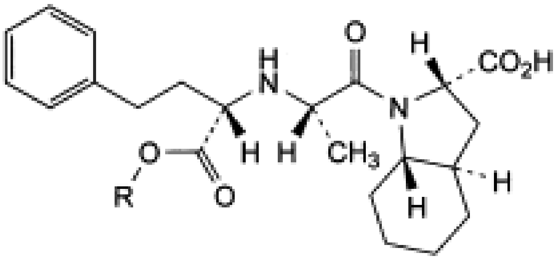
STORAGE

Protected from light.

IMPURITIES

Specified impurities C, D.

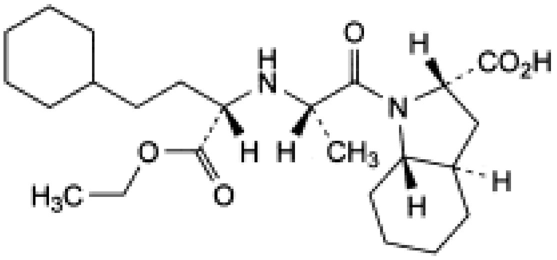
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, E, F.*



A. R = CH_3: (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(methoxycarbonyl)-3-phenylpropyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (methyl ester derivative),

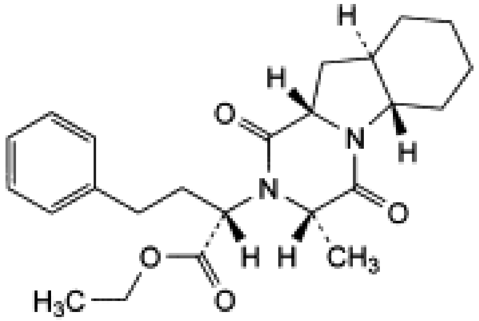
B. R = $CH(CH_3)_2$: (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-[(1-methylethoxy)carbonyl]-3-phenylpropyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (isopropyl ester derivative),

E. R = *H:* (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl] octahydro-1*H*-indole-2-carboxylic acid (trandolaprilate),



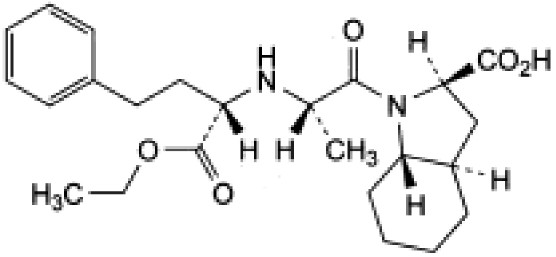
O
H
H
N
CO2H
N
H
H
CH3
O
H
H3C
O
H

C. (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)propyl]amino]propanoyl] octahydro-1*H*-indole-2-carboxylic acid (hexahydrotrandolapril),



H
H
O
N
H
N
O
O
H
H
CH3
H3C
O

D. ethyl (2*S*)-2-[(3*S*,5a*S*,9a*R*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*) -yl]-4-phenylbutanoate (trandolapril diketopiperazine),



O
H
H
N
CO2H
N
O
H
H
CH3
H
H3C
O
H

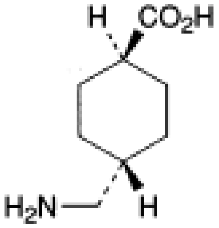
F. (2*R,*3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl] octahydro-1*H*-indole-2-carboxylic acid.

Ph Eur

Tranexamic Acid

General Notices

(Ph Eur monograph 0875)



$C_8H_{15}NO_2$ 157.2 *1197-18-8*

Action and use

Antifibrinolytic.

Preparations

Tranexamic Acid Injection

Tranexamic Acid Tablets

Ph Eur

DEFINITION

trans-4-(Aminomethyl)cyclohexanecarboxylic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in glacial acetic acid, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *tranexamic acid CRS.*

TESTS

pH *(2.2.3)*

7.0 to 8.0.

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.20 g of the substance to be examined in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with *water R* . Dilute 1.0 ml of this solution to 10.0 ml with *water R* .

Reference solution (b) Dissolve 20 mg of *tranexamic acid CRS* (containing impurity C) in water R and dilute to 2 ml with the same solvent.

Reference solution (c) Dissolve 12 mg of 4-*aminomethylbenzoic acid R* (impurity D) in *water R* and dilute to 100 ml with the same solvent. Dilute 1 ml of the solution to 50 ml with *water R* . Dilute 5 ml of this solution to 200 ml with *water R* .

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm or l = 0.25 m, Ø = 6.0 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 11.0 g of *anhydrous sodium dihydrogen phosphate R* in 500 ml of *water R* and add 5 ml of *triethylamine R* and 1.4 g of *sodium laurilsulfate R.* Adjust to pH 2.5 with *dilute phosphoric acid R* and dilute to 600 ml with *water R* . Add 400 ml of *methanol R* and mix.

Flow rate 0.9 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Run time 3 times the retention time of tranexamic acid.

Identification of impurities Use the chromatogram supplied with *tranexamic acid CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to tranexamic acid (retention time = about 13 min):

impurity C = about 1.1; impurity D = about 1.3; impurity B = about 1.5; impurity A = about 2.1.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to tranexamic acid and impurity C.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.2; impurity C = 0.005; impurity D = 0.006;

— *impurity A*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *impurity B:* not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of unspecified impurities*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Halides expressed as chlorides ***(2.4.4)***

Maximum 140 ppm.

Dissolve 1.2 g in *water R* and dilute to 50 ml with the same solvent.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of this solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

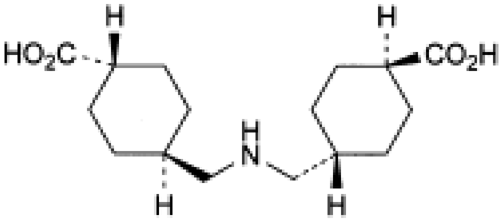
Dissolve 0.140 g in 20 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 15.72 mg of $C_8H_{15}NO_2$.

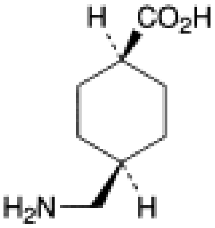
IMPURITIES

Specified impurities A, B.

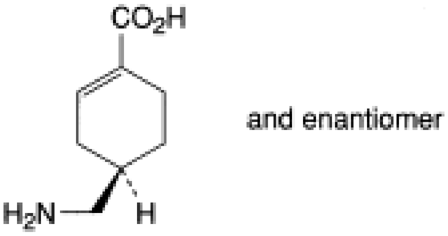
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): C, D.*



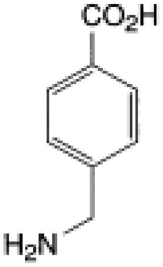
A. *trans*,*trans*-4,4′-(iminodimethylene)di(cyclohexanecarboxylic) acid,



B. *cis*-4-(aminomethyl)cyclohexanecarboxylic acid,



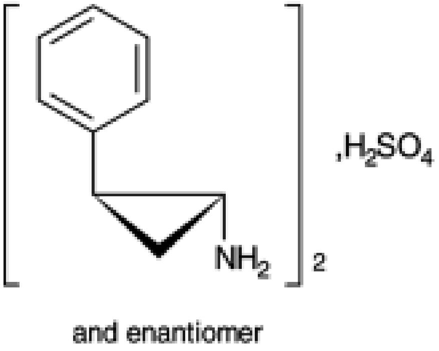
C. (*RS*)-4-(aminomethyl)cyclohex-1-enecarboxylic acid,



D 4-aminomethylbenzoic acid.

Tranylcypromine Sulphate

General Notices



$(C_9H_{11}N)_2,H_2SO_4$ 364.5 *13492-01-8*

Action and use

Monoamine oxidase inhibitor; antidepressant.

Preparation

Tranylcypromine Tablets

DEFINITION

Tranylcypromine Sulphate is (1*RS*,2*SR*)-2-phenylcyclopropylamine sulphate. It contains not less than 98.0% and not more than 101.0% of $(C_9H_{11}N)_2,H_2SO_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Soluble in *water*; very slightly soluble in *ethanol (96%)* and in *ether* .

IDENTIFICATION

A. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of tranylcypromine sulphate *(RS 345)* .

B. Yields the reactions characteristic of *sulphates* , Appendix VI.

TESTS

Related substances

Dissolve 10 mg of *4-chloroaniline* (internal standard) in sufficient 0.1M *hydrochloric acid* to produce 20 ml (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 5 ml of 1M *sodium hydroxide* to 1 ml of solution A, extract with 10 ml of *dichloromethane*, add 1 ml of *trifluoroacetic anhydride* to the dichloromethane extract and allow to stand for 10 minutes. Evaporate the solution at a pressure of 2 kPa using a rotary evaporator and a water bath at 20° and dissolve the residue in 2 ml of *dichloromethane*. For solution (2) dissolve 0.1 g of the substance being examined in 5 ml of *water*, add 1 ml of 5M *sodium hydroxide* and continue as for solution (1), beginning at the words 'extract with...'. For solution (3) dissolve 0.1 g of the substance being examined in 5 ml of *water*, add 1 ml of solution A and 1 ml of 5M *sodium hydroxide* and continue as for solution (1), beginning at the words 'extract with...'.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable) and maintained at 170°.

In the chromatogram obtained with solution (3) the area of any *secondary peak* is not greater than that of the peak due to the trifluoracetyl derivative of 4-chloroaniline (0.5%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

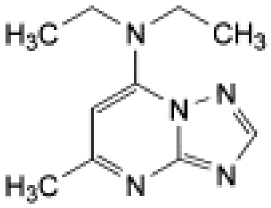
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.3 g and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 36.45 mg of $(C_9H_{11}N)_2,H_2SO_4$.

Trapidil

General Notices

(Ph Eur monograph 1576)



$C_{10}H_{15}N_5$ 205.3 *15421-84-8*

Action and use

Antiplatelet agent; vasodilator.

Ph Eur

DEFINITION

N,N-Diethyl-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, soluble in ethanol and in methylene chloride.

mp

About 102 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *trapidil CRS.*

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 10 ml of solution S add 0.2 ml of *methyl red solution R* and 0.2 ml of *0.01 M hydrochloric acid* . The solution is red. Add 0.4 ml of *0.01 M sodium hydroxide* . The solution is yellow.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *trapidil impurity A CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *trapidil impurity B CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c) Mix equal volumes of reference solution (a) and reference solution (b).

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm),

Mobile phase 50 ml of *methanol R* , 75 ml of *acetonitrile R* and 800 ml of a 1.7 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 2.45 with *phosphoric acid R;* dilute to 1000 ml with *water R* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µl.

Run time 3 times the retention time of trapidil.

System suitability:

— *resolution*: minimum of 4.0 between the peaks due to impurity A and impurity B in the chromatogram obtained with reference solution (c).

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *impurity B:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Chlorides ***(2.4.4)***

Maximum 100 ppm.

Dissolve 0.25 g in 10 ml of *water R* and dilute to 15 ml with *water R*. The solution complies with the limit test for chlorides. Prepare the standard using 5 ml of *chloride standard solution (5 ppm Cl) R.*

Ammonium ***(2.4.1)***

Maximum 20 ppm.

0.50 g complies with limit test A. Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dissolve 2.0 g in 20 ml of *water R*. 12 ml of the solution complies with limit test A. Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

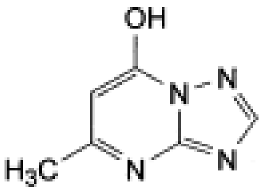
Dissolve 0.180 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 20.53 mg of $C_{10}H_{15}N_5$.

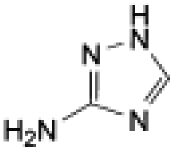
STORAGE

Protected from light.

IMPURITIES



A. 5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol,

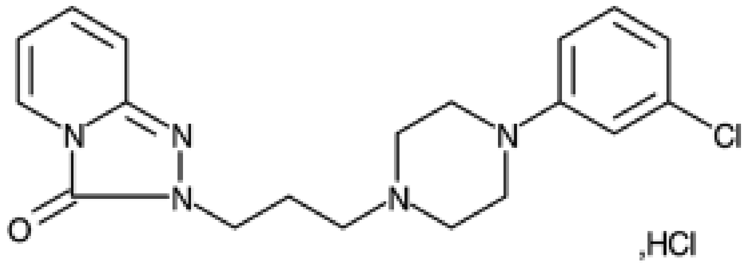


B. 1,2,4-triazol-3-amine.

Ph Eur

Trazodone Hydrochloride

General Notices



$C_{19}H_{22}ClN_5O,HCl$ 408.3 *25332-39-2*

Action and use

Monoamine reuptake inhibitor; antidepressant.

DEFINITION

Trazodone Hydrochlorideis 2-3-[4-(3-chloro)phenylpiperazin-1-yl]propyl-1,2,4triazolo[4,3-*a*] pyridin-3(2*H*)-one hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{19}H_{22}ClN_5O,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline powder.

Soluble in *water*; sparingly soluble in *ethanol (96%)* ; practically insoluble in *ether* .

IDENTIFICATION

A. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of trazodone hydrochloride *(RS 346)* . In the preparation of the disc, avoid excessive grinding when triturating the substance being examined with the *potassium chloride* .

B. Yields the reactions characteristic of *chlorides* , Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 3.9 to 4.5, Appendix V L.

Heavy metals

1.0 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (20 ppm).

3-Chloroaniline

To 10 ml of a 1% w/v solution of the substance being examined in a mixture of equal volumes of *water* and *ethanol* add 2 ml of a freshly prepared 5% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol* and 0.1 ml of *hydrochloric acid*. Shake well and allow to stand for 5 minutes. Any yellow colour produced within 5 minutes of preparation of the solution is not more intense than that produced by treating at the same time and in the same manner 10 ml of a 1 µg per ml solution of *3-chloro-aniline* in a mixture of equal volumes of *water* and *ethanol* beginning at the words 'add 2 ml…' (100 ppm).

Related substances

The combined nominal total content of impurities determined in tests A and B below is not more than 1.0% and no single impurity is more than 0.3%.

A. Carry out the method for *liquid chromatography*, Appendix III D, using three solutions in the mobile phase containing (1) 0.10% w/v of the substance being examined, (2) 0.00050% w/v of the substance being examined and (3) 0.10% w/v of *trazodone hydrochloride impurity standard BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *non-endcapped alkylamide silica gel for chromatography* (5 µm) (Supelco Suplex pKb 100 is suitable) and a pre-column (1 cm × 4.6 mm) packed with the same material placed between the pump and the injector, (b) a mixture of 0.4 volume of *diethylamine*, 350 volumes of *acetonitrile* and 650 volumes of *water* as the mobile phase with a flow rate of 2.0 ml per minute and (c) a detection wavelength of 254 nm. If necessary adjust the proportions of *acetonitrile* and *water* in the mobile phase to obtain a retention time of about 10 minutes for the principal peak. For solution (1) allow the chromatography to proceed for three times the retention time of the principal peak.

The test is not valid unless the chromatogram obtained with solution (3) closely resembles reference chromatogram A supplied with *trazodone hydrochloride impurity standard BPCRS*.

From any *secondary peaks* in the chromatogram obtained with solution (1) calculate the nominal content of each impurity with a retention time of up to and including that of impurity E, identified from reference chromatogram A. Use, as reference, the principal peak in the chromatogram obtained with solution (2). Disregard any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with solution (2) (0.05%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using three solutions in the mobile phase containing (1) 0.10% w/v of the substance being examined, (2) 0.00050% w/v of the substance being examined and (3) 0.10% w/v of *trazodone hydrochloride impurity BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *non-endcapped alkylamide silica gel for chromatography* (5 µm) (Supelco Suplex pKb 100 is suitable) and a pre-column (1 cm × 4.6 mm) packed with the same material placed between the pump and the injector, (b) a mixture of 0.4 volume of *diethylamine* , 320 volumes of *water* and 680 volumes of *acetonitrile* as the mobile phase with a flow rate of 1.7 ml per minute and (c) a detection wavelength of 254 nm. If necessary adjust the proportions of *acetonitrile* and *water* in the mobile phase to obtain a retention time of about 2.5 minutes for the principal peak. For solution (1) allow the chromatography to proceed for at least five times the retention time of the principal peak.

From any *secondary peaks* in the chromatogram obtained with solution (1) calculate the nominal content of each impurity with a retention time longer than that of impurity E identified from reference chromatogram B, supplied with *trazodone hydrochloride impurity standard BPCRS* . Use, as reference, the principal peak in the chromatogram obtained with solution (2) . Disregard any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Loss on drying

When dried to constant weight at 105° at a pressure of 3.5 to 6.5 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash

Not more than 0.2%, Appendix IX A.

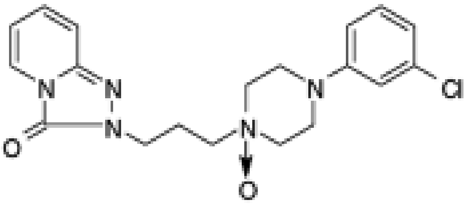
ASSAY

Dissolve 0.3 g in 60 ml of *glacial acetic acid* , add 5 ml of *mercury(* II *) acetate solution* and carry out Method I for *non-aqueous titration* , Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 40.83 mg of $C_{19}H_{22}ClN_5O,HCl$.

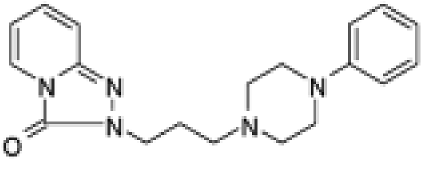
STORAGE

Trazodone Hydrochloride should be kept in an airtight container and protected from light.

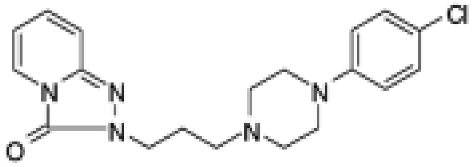
IMPURITIES



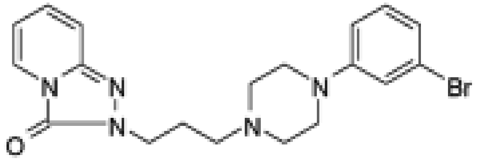
A. 4-(3-chlorophenyl)-1-[3-(3-oxo-2,3-dihydro-1,2,4-triazolo[4,3-*a*]pyridin-2-yl)propyl] piperazine *N* 1-oxide



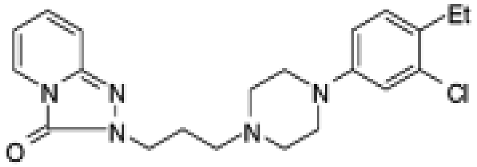
B. 2-[3-(4-phenylpiperazin-1-yl)propyl]-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one



C. 2-3-[4-(4-chlorophenyl)piperazin-1-yl]propyl-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one

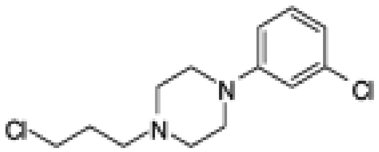


D. 2-3-[4-(3-bromophenyl)piperazin-1-yl]propyl-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one

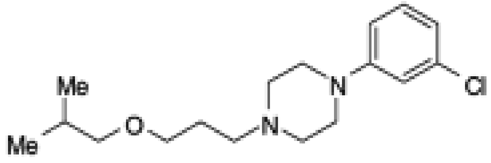


Et
N
N
N
O
N
N
Cl

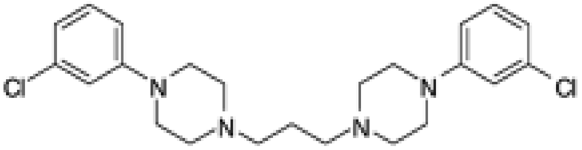
E. 2-3-[4-(3-chloro-4-ethylphenyl)piperazin-1-yl]propyl-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one



F. 1-(3-chloropropyl)-3chlorophenylpiperazine



G. 3-[4-(3-chlorophenyl)piperazin-1-yl]propyl isobutyl ether

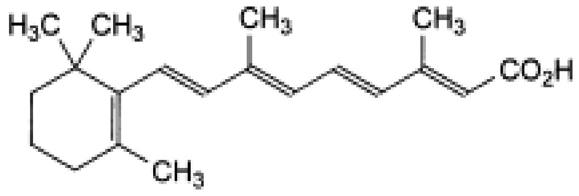


H. 1,3-bis-[4-(3-chlorophenyl)piperazin-1-yl]propane

Tretinoin

General Notices

(Ph Eur monograph 0693)



$C_{20}H_{28}O_{2}$ 300.4 *302-79-4*

Action and use

Vitamin A analogue (retinoid); treatment of acne.

Preparations

Tretinoin Gel

Tretinoin Solution

Ph Eur

DEFINITION

Tretinoin contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid, calculated with reference to the dried substance.

CHARACTERS

A yellow or light orange, crystalline powder, practically insoluble in water, soluble in methylene chloride, slightly soluble in alcohol. It is sensitive to air, heat and light, especially in solution.

It melts at about 182 °C, with decomposition.

Carry out all operations as rapidly as possible and avoid exposure to actinic light; use freshly prepared solutions.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C, D.*

A. Dissolve 75.0 mg in 5 ml of *methylene chloride R* and dilute immediately to 100.0 ml with acidified 2-propanol (prepared by diluting 1 ml of *0.01 M hydrochloric acid* to 1000 ml with *2-propanol R*). Dilute 5.0 ml of this solution to 100.0 ml with the acidified 2-propanol. Dilute 5.0 ml of this latter solution to 50.0 ml with the acidified 2-propanol. Examined between 300 nm and 400 nm *(2.2.25)* , the solution shows a single maximum, at 353 nm. The specific absorbance at the maximum is 1455 to 1545.

B. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the spectrum obtained with *tretinoin CRS* . Examine the substances prepared as discs.

C. Examine by thin-layer chromatography *(2.2.27)* , using a *TLC silica gel GF* $_{254}$ *plate R*.

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *tretinoin CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 2 volumes of *glacial acetic acid R* , 4 volumes of *acetone R* , 40 volumes of *peroxide-free ether R* and 54 volumes of *cyclohexane R.* Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 5 mg in 2 ml of *antimony trichloride solution R* . An intense red colour develops and later becomes violet.

TESTS

Related substances

Examine by liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.100 g of the substance to be examined in *methanol* R and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *isotretinoin CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 25.0 ml with *methanol R.*

Reference solution (c) Mix 1.0 ml of reference solution (a) with 0.5 ml of the test solution and dilute to 25.0 ml with *methanol R* .

Reference solution (d) Dilute 0.5 ml of the test solution to 100.0 ml with *methanol R.*

The chromatographic procedure may be carried out using:

— a stainless steel column 0.15 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (3 µm);

— as mobile phase at a flow rate of 1.0 ml/min a mixture of 5 volumes of *glacial acetic acid R* , 225 volumes of *water R* and 770 volumes of *methanol R* ;

— as detector a spectrophotometer set at 355 nm.

Inject separately 10 µl of reference solutions (b), (c) and (d) and of the test solution. Adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with reference solution (b) is about 70 per cent of the full scale of the recorder. The test is not valid unless the resolution between isotretinoin and tretinoin in the chromatogram obtained with reference solution (c) is at least 2.0. In the chromatogram obtained with the test solution, the area of the peak due to isotretinoin is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the sum of the areas of any peaks, apart from the principal peak and any peak due to isotretinoin, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

Heavy metals ***(2.4.8)***

0.5 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb) R* .

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 70 ml of *acetone R* . Titrate with *0.1 M tetrabutylammonium hydroxide* determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 30.04 mg of $C_{20}H_{28}O_2$.

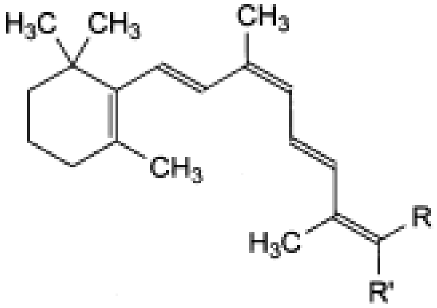
STORAGE

Store in an airtight container, protected from light, at a temperature not exceeding 25 °C.

It is recommended that the contents of an opened container be used as soon as possible and any unused part be protected by an atmosphere of an inert gas.

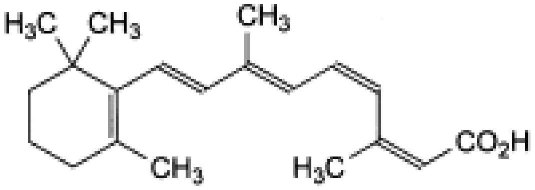
IMPURITIES

A. isotretinoin,



B. R = CO_2H, R′ = H: (2Z,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (9,13-di-*cis*-retinoic acid),

D. R = H, R′ = CO_2H: (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (9-*cis*-retinoic acid),



C. (2*Z*,4*Z*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (11,13-di-*cis*-retinoic acid),

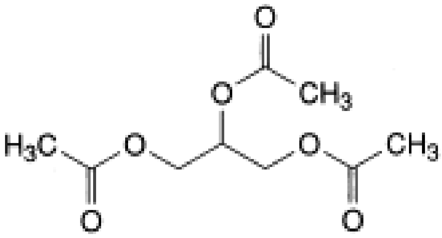
E. oxidation products of tretinoin.

Ph Eur

Triacetin

General Notices

(Ph Eur monograph 1106)



$C_9H_{14}O_6$ 218.2 *102-76-1*

Action and use

Antifungal.

Ph Eur

DEFINITION

Propane-1,2,3-triyl triacetate.

Content

97.0 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless, slightly viscous oily liquid.

Solubility

Soluble in water, miscible with ethanol (96 per cent) and toluene.

bp: about 260 °C.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24).*

Comparison: Ph. Eur. reference spectrum of triacetin.

TESTS

Appearance

It is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity

Dissolve 5.00 g in 25 ml of *anhydrous ethanol R*, previously neutralised to 0.2 ml of *phenolphtalein solution R* and add 0.20 ml of *0.1 M sodium hydroxide*. The pink colour of the mixture persists for 15 s.

Relative density *(2.2.5)*

1.159 to 1.164.

Refractive index *(2.2.6)*

1.429 to 1.432.

Water *(2.5.12)*

Maximum 0.2 per cent, determined on 5.00 g.

ASSAY

Introduce 0.300 g into a 250 ml borosilicate glass flask fitted with a reflux condenser. Add 25.0 ml of *0.5 M alcoholic potassium hydroxide* and a few glass beads. Attach the condenser and heat under reflux for 30 min. Add *1 ml of phenolphthalein solution R1* and titrate immediately with *0.5 M hydrochloric acid*. Carry out a blank test under the same conditions. Calculate the content from the difference in consumption of alkali in the main and the blank procedure.

1 ml of *0.5 M alcoholic potassium* hydroxide is equivalent to 36.37 mg of $C_9H_{14}O_6$.

STORAGE

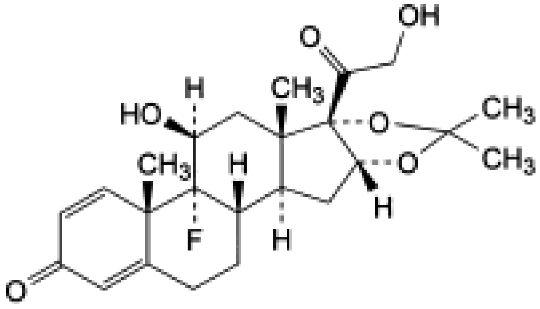
In a well-filled container.

Ph Eur

Triamcinolone Acetonide

General Notices

(Ph Eur monograph 0533)



$C_{24}H_{31}FO_6$ 434.5 *76-25-5*

Action and use

Glucocorticoid.

Preparations

Triamcinolone Cream

Triamcinolone Acetonide Injection

Triamcinolone Ointment

Triamcinolone Dental Paste

Ph Eur

DEFINITION

9-Fluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione.

Content

97.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification A, B.

Second identification C, D.

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison triamcinolone acetonide CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R* and evaporate to dryness. Using the residues, prepare halogen salt discs or mulls in *liquid paraffin R* and record new spectra.

B. Thin-layer chromatography *(2.2.27)* . *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *triamcinolone acetonide CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *triamcinolone hexacetonide CRS in* reference solution (a) and dilute to 10 ml with reference solution (a).

Plate TLC *silica gel* F_{254} *plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R* .

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm, immediately after development.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Thin-layer chromatography *(2.2.27)* . *Prepare the solutions immediately before use and protect from light.*

Test solution (a) Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Test solution (b) In a separating funnel, dissolve 10 mg of the substance to be examined in 1.5 ml of *glacial acetic acid R* , add 0.5 ml of a 20 g/l solution of *chromium trioxide R* and allow to stand for 60 min. Add 5 ml of *water R* , 2 ml of *methylene chloride R* and shake vigorously for 2 min. Allow to separate and use the lower layer.

Reference solution (a) Dissolve 10 mg of *triamcinolone acetonide CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) In a separating funnel, dissolve 10 mg of *triamcinolone acetonide CRS* in 1.5 ml of *glacial acetic acid R* , add 0.5 ml of a 20 g/l solution of *chromium trioxide R* and allow to stand for 60 min. Add 5 ml of *water R* , 2 ml of *methylene chloride R* and shake vigorously for 2 min. Allow to separate and use the lower layer.

Plate TLC *silica gel F $_{254}$ plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R* .

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm, immediately after development.

Results The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spot in the chromatograms obtained with test solution (b) and reference solution (b) has an R_F value distinctly higher than that of the principal spot in the chromatograms obtained with test solution (a) and reference solution (a).

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R* , 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R,* add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution to that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Specific optical rotation *(2.2.7)*

+ 100 to + 107 (anhydrous substance).

Dissolve 0.100 g in *dioxan R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography (2.2.29) Carry out the test protected from light.

Test solution Dissolve 25.0 mg of the substance to be examined in 7 ml of *methanol R* and dilute to 10.0 ml with *water R* .

Reference solution (a) Dissolve 2 mg of *triamcinolone acetonide CRS* and 2 mg of *triamcinolone R* (impurity A) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 525 ml of *methanol R* with 400 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 10 min.

Injection 20 µl.

Run time 3.5 times the retention time of triamcinolone acetonide.

Retention time Impurity A = about 5 min; triamcinolone acetonide = about 17 min.

System suitability Reference solution (a):

- *resolution*: minimum 15 between the peaks due to impurity A and triamcinolone acetonide; if necessary, adjust the concentration of methanol in the mobile phase.

Limits:

- *impurity A:* not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water ***(2.5.12)***

Maximum 2.0 per cent, determined on 0.500 g.

ASSAY

Protect the solutions from light throughout the assay.

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R* . Measure the absorbance *(2.2.25)* at the absorption maximum at 238.5 nm.

Calculate the content of $C_{24}H_{31}FO_6$ taking the specific absorbance to be 355.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A.*

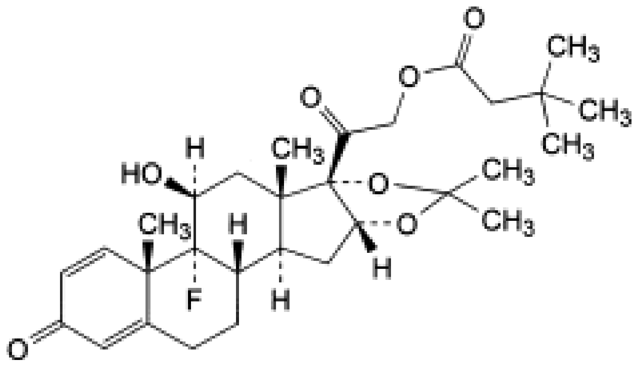
A. triamcinolone.

Ph Eur

Triamcinolone Hexacetonide

General Notices

(Ph Eur monograph 0867)



$C_{30}H_{41}FO_7$ 532.6 *5611-51-8*

Action and use

Glucocorticoid.

Preparation

Triamcinolone Hexacetonide Injection

Ph Eur

DEFINITION

9-Fluoro-11β-hydroxy-16α,17-(1-methylethylidenedioxy)-3,20-dioxopregna-1,4-diene-21-yl 3, 3-dimethylbutanoate.

Content

97.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in anhydrous ethanol and in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *triamcinolone hexacetonide CRS* .

B. Thin-layer chromatography *(2.2.27)* . *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *triamcinolone hexacetonide CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *triamcinolone acetonide CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate *TLC silica gel F_{254} plate R .*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R* .

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm, immediately after development.

System suitability Reference solution (b):

— The chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Specific optical rotation *(2.2.7)*

+ 92 to + 98 (anhydrous substance).

Dissolve 0.100 g in *methylene chloride R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 2 mg of *triamcinolone hexacetonide CRS* and 2 mg of *triamcinolone acetonide CRS* (impurity A) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase In a 1000 ml volumetric flask mix 750 ml of *methanol R* with 200 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate 2 ml/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 10 min.

Injection 20 µl.

Run time 3 times the retention time of triamcinolone hexacetonide.

Retention time Impurity A = about 3 min; triamcinolone hexacetonide = about 12 min.

System suitability Reference solution (a):
- *resolution:* minimum 20.0 between the peaks due to impurity A and triamcinolone hexacetonide; if necessary, adjust the concentration of methanol in the mobile phase.

Limits:
- *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- *disregard limit:* 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

Maximum 2.0 per cent, determined on 0.50 g.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 238 nm.

Calculate the content of $C_{30}H_{41}FO_7$ taking the specific absorbance to be 291.

STORAGE

Protected from light.

IMPURITIES

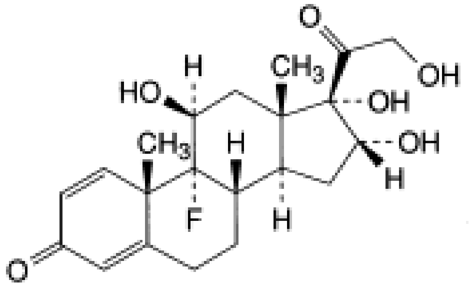
Specified impurities *A.*

A. triamcinolone acetonide.

Triamcinolone

General Notices

(Ph Eur monograph 1376)



$C_{21}H_{27}FO_6$ 394.4 *124-94-7*

Action and use

Glucocorticoid.

Preparation

Triamcinolone Tablets

Ph Eur

DEFINITION

9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione.

Content

97.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in methanol, practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison *triamcinolone CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness, dry the residues at 60 °C at a pressure not exceeding 0.7 kPa and record new spectra using the residues.

B. Thin-layer chromatography *(2.2.27)*. *Prepare the solutions immediately before use and protect from light. Examine the plate under ultraviolet light immediately after development.*

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *triamcinolone CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Reference solution (b) Dissolve 10 mg of *dexamethasone CRS* in reference solution (a) and dilute to 10 ml with the same solution.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of met*hylene chloride R*.

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Specific optical rotation *(2.2.7)*

-65 to + 72 (anhydrous substance).

Dissolve 0.100 g in *dimethylformamide R* and dilute to 10.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 2 mg of *triamcinolone CRS* and 2 mg of *triamcinolone impurity C CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *methanol R* and *water R*.

Blank *methanol R*.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase A mixture prepared as follows: in a 1000 ml volumetric flask mix 525 ml of *methanol R* with 400 ml of *water R* and allow to equilibrate; adjust the volume to 1000 ml with *water R* and mix again.

Flow rate 1 ml/min.

Detection Spectrophotometer set at 238 nm.

Injection 20 µl.

Run time 4.5 times the retention time of triamcinolone.

Retention time Triamcinolone = about 11 min.

System suitability Reference solution (a):

— *resolution*: minimum of 1.8 between the peaks due to triamcinolone and to impurity C.

Limits:

— *any impurity:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent) and not more than 2 such peaks have an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water *(2.5.12)*

Maximum 1.0 per cent, determined on 0.500 g.

ASSAY

Prepare the solutions immediately before use and protect from light.

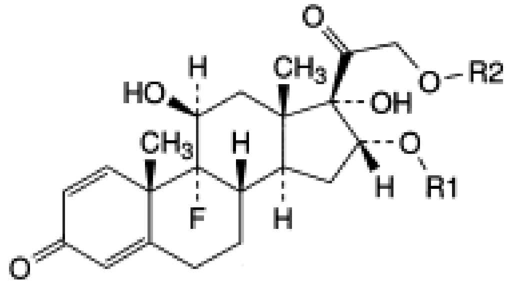
Dissolve 50.0 mg in *alcohol R* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *alcohol R*. Measure the absorbance *(2.2.25)* at the maximum at 238 nm.

Calculate the content of $C_{21}H_{27}FO_6$ taking the specific absorbance to be 389.

STORAGE

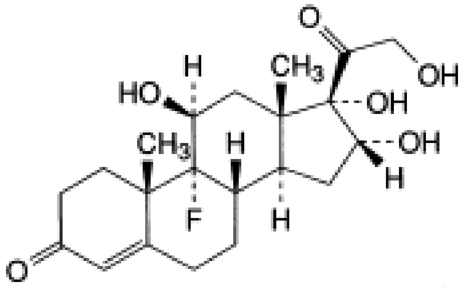
Protected from light.

IMPURITIES


O
H
CH3
HO
OH
O
R2
CH3
H
O
H
R1
F
H
O

A. R1 = R2 = CO-CH_3: 9-fluoro-11β,17-dihydroxy-3,20-dioxopregna-1,4-diene-16α,21-diyl diacetate (triamcinolone 16,21-diacetate),

B. R1 = H, R2 = CO-CH_3: 9-fluoro-11β,16α,17-trihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate (triamcinolone 21-acetate),


O
H
CH3
OH
HO
OH
CH3
H
OH
H
F
H
O

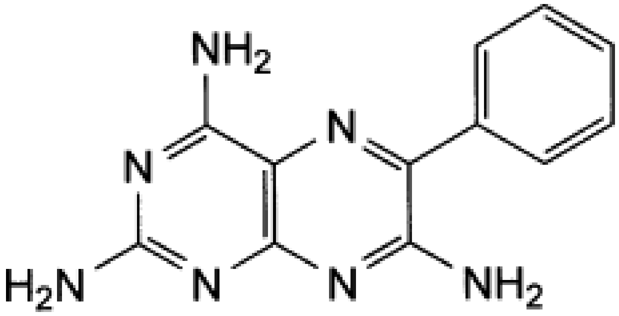
C. 9-fluoro-11β,16α,17,21-tetrahydroxypregn-4-ene-3,20-dione (pretriamcinolone).

Ph Eur

Triamterene

General Notices

(Ph Eur monograph 0058)



$C_{12}H_{11}N_7$ 253.3 *396-01-0*

Action and use

Sodium channel blocker; potassium-sparing diuretic.

Preparations

Co-triamterzide Tablets

Triamterene Capsules

Ph Eur

DEFINITION

6-Phenylpteridine-2,4,7-triamine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Very slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *triamterene CRS.*

TESTS

Acidity

Boil 1.0 g with 20 ml of *water R* for 5 min, cool, filter and wash the filter with 3 quantities, each of 10 ml, of *water R*. Combine the filtrate and washings and add 0.3 ml of *phenolphthalein solution R*. Not more than 1.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Impurity D

Gas chromatography (*2.2.28*).

Internal standard solution Dilute 0.1 ml of *nitrobenzene R* to 100 ml with *methanol R*. Dilute 1 ml of this solution to 50 ml with *methanol R*.

Test solution Introduce 0.800 g of the substance to be examined into a suitable vial, add 5 ml of *dimethyl sulphoxide R* and heat until the sample is dissolved (do not heat to boiling). Allow to cool. Add 5 ml of cold *methanol R* to enhance the precipitation of triamterene. Filter and wash the filter with 5 ml of *methanol R*. Combine the filtrate and washing, add 2.0 ml of the internal standard solution and dilute to 20.0 ml with *methanol R.*

Reference solution Dissolve 20.0 mg of *benzyl cyanide R* (impurity D) in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *methanol R*. To 2.0 ml of this solution add 2.0 ml of the internal standard solution and 5 ml of *dimethyl sulphoxide R* and dilute to 20.0 ml with *methanol R*.

Blank solution Dilute 5 ml of *dimethyl sulphoxide R* to 20 ml with *methanol R.*

Column:
- *material*: fused silica;
- *size: l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 R* (0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:15.

Temperature:
- *column*: 170 °C;
- *injection port*: 210 °C;
- *detector*: 230 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time Twice the retention time of the internal standard.

Relative retention With reference to the internal standard (retention time = about 6 min): impurity D = about 1.6.

System suitability Reference solution:

- *resolution*: *minimum* 2.0 between the peak due to impurity D and the nearest peak due to the solvent (blank solution);
- *signal-to-noise ratio* : minimum 10 for the peak due to impurity D.

Limit:

- *impurity D*: calculate the ratio (*R*) of the area of the peak due to impurity D to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to impurity D to the area of the peak due to the internal standard: this ratio is not greater than *R* (50 ppm).

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *nitrosotriaminopyrimidine CRS* (impurity A) in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *triamterene impurity B CRS* in 0.5 ml of the mobile phase with the aid of ultrasound. To this solution, add 0.5 ml of the test solution.

Column:

- *size: l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *butylamine R* , *acetonitrile R* , *methanol R* , *water R* (2:200:200:600 *V/V/V/V*), adjusted to pH 5.3 with *acetic acid R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 320 nm and at 355 nm.

Injection 50 µl.

Relative retention With reference to triamterene (retention time = about 5 min): impurity A = about 0.6; impurity B = about 0.8; impurity C = about 1.7.

System suitability:

- *resolution*: minimum 1.5 between the peaks due to impurity B and triamterene in the chromatogram obtained with reference solution (c) at 355 nm; if necessary, increase the quantity of *water R* in the mobile phase;

— *signal-to-noise ratio* : minimum *10* for the principal peak in the chromatogram obtained with reference solution (b) at 320 nm.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.8; impurity C = 1.5;

— *impurity A at 320 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (50 ppm);

— *impurities B, C a*t 355 nm: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities at 355 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total at 355 nm*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit at 355 nm*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 5 ml of *anhydrous formic acid R* and add 100 ml of *anhydrous acetic acid R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically (*2.2.20*).

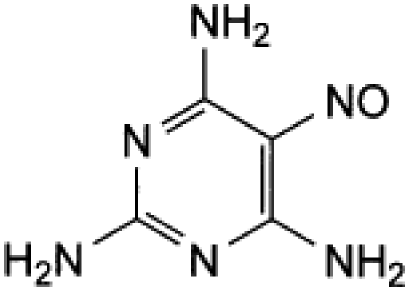
1 ml of *0.1 M perchloric acid* is equivalent to 25.33 mg of $C_{12}H_{11}N_7$.

STORAGE

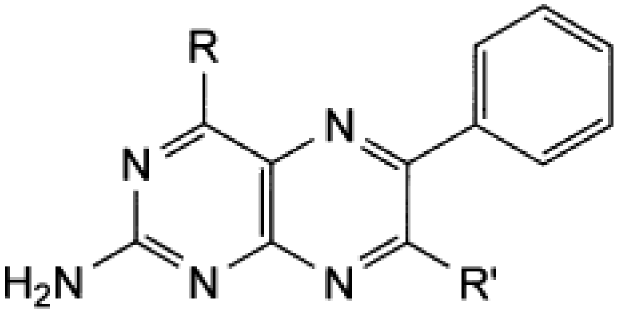
Protected from light.

IMPURITIES

Specified impurities A, B, C, D.

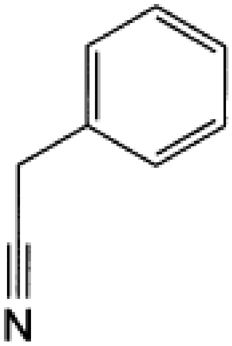


A. 5-nitrosopyrimidine-2,4,6-triamine (nitrosotriaminopyrimidine),



B. R = OH, R′ = NH_2: 2,7-diamino-6-phenylpteridin-4-ol,

C. R = NH_2, R′ = OH: 2,4-diamino-6-phenylpteridin-7-ol,



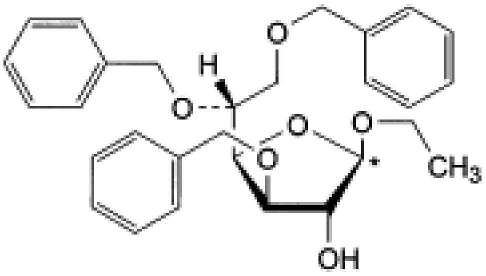
D. phenylacetonitrile (benzyl cyanide).

Ph Eur

Tribenoside

General Notices

(Ph Eur monograph 1740)



and anomer at C*

$C_{29}H_{34}O_6$ 478.6 *10310-32-4*

Action and use

Sclerosing agent.

Ph Eur

DEFINITION

Mixture of α- and β-anomers of ethyl 3,5,6-tri-*O*-benzyl-D-glucofuranoside.

Content

96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

Yellowish to pale yellow, clear, viscous liquid.

Solubility

Practically insoluble in water, very soluble in acetone, in methanol and in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *tribenoside CRS.*

TESTS

Solution S

Dissolve 4.00 g in *methanol R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 420 nm has a maximum of 0.10.

Specific optical rotation *(2.2.7)*

- 31.0 to - 40.0.

Dilute 2.0 ml of solution S to 20.0 ml with *methanol R* .

Related substances

Liquid chromatography *(2.2.29)* .

Test solution (a) Dissolve 1.000 g of the substance to be examined in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 25.0 ml with the same mixture of solvents.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (a) Dilute 25.0 mg of *benzaldehyde R* and 30.0 mg of *tribenoside impurity A CRS* to 100.0 ml with *acetonitrile R.* Introduce 20.0 ml of this solution into a 50 ml volumetric flask, add 2.5 ml of *water R* and dilute to 50.0 ml with *acetonitrile R.*

Reference solution (b) Dissolve 50.0 mg of *tribenoside CRS* in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (c) Dissolve 12.0 mg of *benzyl ether R* in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 100.0 ml with the same mixture of solvents.

Column:

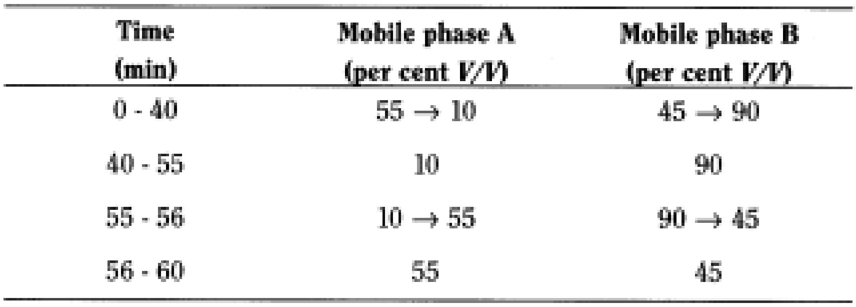
— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: 0.1 per cent *V/V* solution of *phosphoric acid R* ;

— *mobile phase B: acetonitrile R;*



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 40	55 → 10	45 → 90
40 - 55	10	90
55 - 56	10 → 55	90 → 45
56 - 60	55	45

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl; inject test solution (a) and reference solutions (a), (b) and (c).

Relative retentions With reference to the β-anomer of tribenoside (retention time = about 18 min): α-anomer = about 1.1; impurity C = about 0.2; impurity B = about 0.6; impurity D = about 0.8; impurity A = about 1.4.

System suitability Reference solution (b):

— *resolution:* minimum 3.0 between the peaks due to the α-anomer and to the β-anomer of tribenoside.

Limits:

— *impurity A*: not more than 1.7 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *impurity C*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent); if the area of the peak due to impurity C in the chromatogram obtained with the test solution is greater than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.25 per cent), dilute the test solution to obtain an area equal to or smaller than the area of the peak in the chromatogram obtained with reference solution (a); calculate the content of impurity C taking into account the dilution factor;

— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *any other impurity*: not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *total:* not more than 6.7 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *disregard limit*: 0.17 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dilute 5.0 ml of solution S to 20.0 ml with *methanol R*. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with *methanol R*.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

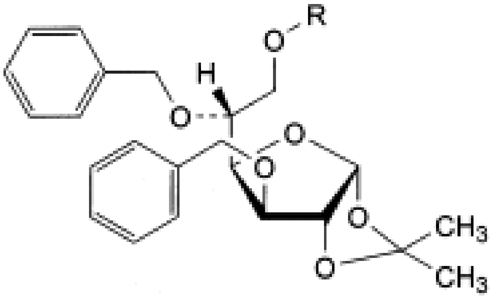
Injection Test solution (b) and reference solution (b).

Calculate the sum of the percentage contents of the α-anomer and the β-anomer of tribenoside.

STORAGE

Under nitrogen, in an airtight container.

IMPURITIES



A. R = CH_2-C_6H_5: 3,5,6-tri-*O*-benzyl-1,2-*O*-(1-methylethylidene)-α-D-glucofuranose,

B. R = H: 3,5-di-*O*-benzyl-1,2-*O*-(1-methylethylidene)-α-D-glucofuranose,

C. C_6H_5-CHO: benzaldehyde,

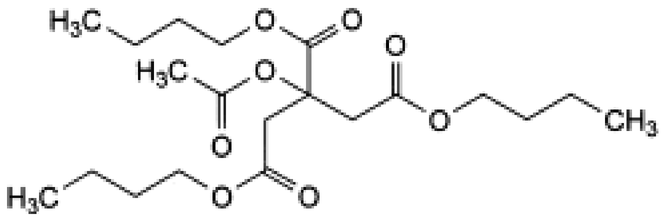
D. C_6H_5-CH_2-O-CH_2-C_6H_5: dibenzyl ether.

Ph Eur

Tributyl Acetylcitrate

General Notices

(Ph Eur monograph 1770)



$C_{20}H_{34}O_8$ 402.5 *77-90-7*

Action and use

Excipient.

Ph Eur

DEFINITION

Tributyl 2-(acetyloxy)propane-1,2,3-tricarboxylate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, oily liquid.

Solubility

Not miscible with water, miscible with alcohol and with methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24).*

Comparison Ph. Eur. reference spectrum of tributyl acetylcitrate.

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II)*.

Acidity

Dilute 10 g with 10 ml of previously neutralised *alcohol R*, add 0.5 ml of *bromothymol blue solution R2*. Not more than 0.3 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Refractive index *(2.2.6)*

1.442 to 1.445.

Related substances

Gas chromatography *(2.2.28)*.

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Reference solution (a) Dissolve 50 mg of the substance to be examined and 50 mg of *tributyl citrate R* in *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *methylene chloride R*. Dilute 1.0 ml of this solution to 25.0 ml with *methylene chloride R*.

Column:
- *material:* fused silica;
- *size:* *l* = 30 m, Ø = 0.53 mm;
- *stationary phase: poly[(cyanopropyl)(methyl)][(phenyl)(methyl)]siloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 36 cm/s.

Split ratio 1:20.

Temperature:
- *column*: 200 °C;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µl.

Run time Twice the retention time of tributyl acetylcitrate.

Relative retention With reference to tributyl acetylcitrate (retention time = abou 26 min): impurity B = about 0.83; impurity A = about 0.87.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to impurity A and tributyl acetylcitrate.

Limits:

- — *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- — *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- — *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- — *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test F. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Water ***(2.5.12)***

Maximum 0.25 per cent, determined on 2.00 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

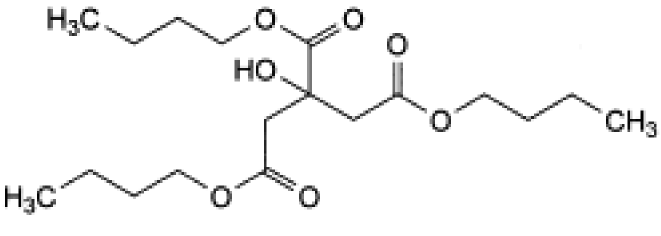
ASSAY

Introduce 1.500 g into a 250 ml borosilicate glass flask. Add 25 ml of *2-propanol R,* 50 ml of *water R* , 25.0 ml of *1 M sodium hydroxide* and a few glass beads. Heat under a reflux condenser for 1 h. Allow to cool. Add 1 ml of *phenolphthalein solution R1* and titrate with *1 M hydrochloric acid* . Carry out a blank titration.

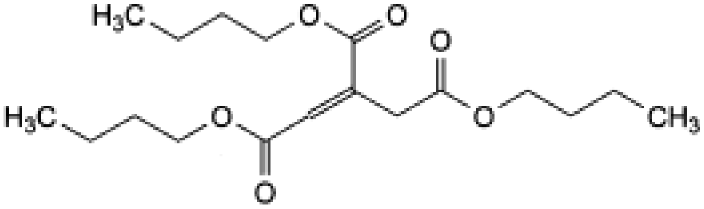
1 ml of *1 M sodium hydroxide* is equivalent to 100.6 mg of $C_{20}H_{34}O_8$.

IMPURITIES

Specified impurities A, B.



A. tributyl 2-hydroxypropane-1,2,3-tricarboxylate (tributyl citrate),


H₃C
O
O
O
H₃C
O
O
O
CH₃

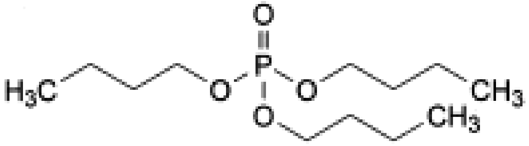
B. tributyl propene-1,2,3-tricarboxylate (tributyl aconitate).

Ph Eur

Tributyl Phosphate

General Notices

(Tri-n-butyl Phosphate, Ph Eur monograph 1682)



$C_{12}H_{27}O_4P$ 266.3 *126-73-8*

Action and use

Excipient.

Ph Eur

CHARACTERS

Appearance

Clear, colourless to pale yellow liquid.

Solubility

Slightly soluble in water, miscible with alcohol.

bp

About 289 °C, with decomposition.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *tri-*n*-butyl phosphate CRS* .

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Acidity

Dissolve 50 ml in 50 ml of *alcohol R* previously adjusted with *0.02 M potassium hydroxide* or *0.02 M hydrochloric acid* to a bluish-green colour, using 0.5 ml of *bromothymol blue solution R1* as indicator. Titrate with *0.02 M potassium hydroxide* to (the initial) bluish-green coloration. Not more than 0.8 ml are required.

Related substances

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution Substance to be examined.

Reference solution Dissolve 10 mg of the substance to be examined and 10 mg of *methyl myristate R* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Column:
- — *material*: fused silica;
- — *size*: *l* = 30 m, Ø = 0.32 mm;
- — *stationary phase*: poly(dimethyl)siloxane R (5 µm).

Carrier gas *helium for chromatography R* .

Linear velocity 32 cm/s.

Split-ratio 65:1.

Temperature:
- — *column*: 250 °C;
- — *injection port and detector*: 250 °C.

Detector Flame ionisation.

Injection 1 µl.

Run time Twice the retention time of tri-*n*-butyl phosphate.

System suitability Reference solution:

- — *resolution*: minimum 10 between the peaks due to tri-*n*-butyl phosphate and methyl myristate.

Limits:
- — *any impurity*: maximum 0.1 per cent;
- — *total*: maximum 0.3 per cent;
- — *disregard limit*: 0.01 per cent.

Chlorides ***(2.4.4)***

Maximum 200 ppm.

Dissolve 0.25 g in 15 ml of *alcohol (70 per cent V/V) R*. The solution complies with the limit test for chlorides. Prepare the reference solution with 10 ml of *chloride standard solution (5 ppm Cl) R* and 5 ml of *ethanol R* .

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in 13 ml of *alcohol R* and dilute to 20.0 ml with *water R*. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (2 ppm Pb) prepared by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 5 volumes of *water R* and 13 volumes of *alcohol R*.

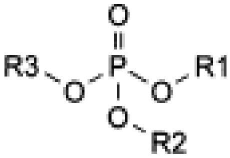
Water *(2.5.32)*

Maximum 0.1 per cent, determined on 1.0 g.

STORAGE

Protected from light.

IMPURITIES



A. R1 = R2 = $[CH_2]_3$-CH_3, R3 = H: dibutyl hydrogen phosphate,

B. R1 = $[CH_2]_3$-CH_3, R2 = R3 = H: butyl dihydrogen phosphate,

D. R1 = R2 = $[CH_2]_3$-CH_3, R3 = CH_3: dibutyl methyl phosphate,

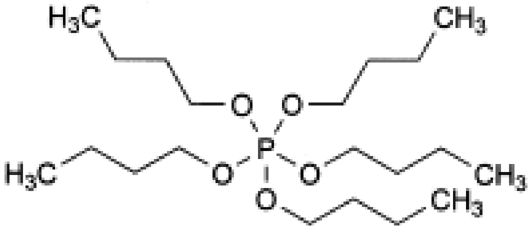
E. R1 = R2 = $[CH_2]_3$-CH_3, R3 = C_2H_5: dibutyl ethyl phosphate,

F. R1 = R2 = $[CH_2]_3$-CH_3, R3 = $[CH_2]_2$-CH_3: dibutyl propyl phosphate,

G. R1 = R2 = $[CH_2]_3$-CH_3, R3 = CH_2-$CH(CH_3)_2$: dibutyl 2-methylpropyl phosphate,

H. R1 = R2 = $[CH_2]_3$-CH_3, R3 = $[CH_2]_4$-CH_3: dibutyl pentyl phosphate,

C. H_3C-$[CH_2]_3$-OH: butan-1-ol,

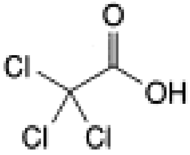


I. pentabutyl phosphate.

Trichloroacetic Acid

General Notices

(Ph Eur monograph 1967)



$C_2HCl_3O_2$ 163.4 *76-03-9*

Ph Eur

DEFINITION

2,2,2-Trichloroacetic acid.

Content

98.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline mass or colourless crystals, very deliquescent.

Solubility

Very soluble in water, in alcohol and in methylene chloride.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of trichloroacetic acid* .

B. To 0.5 ml of solution S (see Tests) add 2 ml of *pyridine R* and 5 ml of *strong sodium hydroxide solution R* . Shake vigorously and heat in a water-bath at 60-70 °C for 5 min. The upper layer shows an intense red colour.

C. Solution S is strongly acidic *(2.2.4)* .

TESTS

Solution S

Dissolve 2.5 g in *water R* and dilute to 25 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Chlorides *(2.4.4)*

Maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R* . The solution complies with the limit test for chlorides.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 20 ml of *water R* . Titrate with *0.1 M sodium hydroxide* , determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M sodium hydroxide* is equivalent to 16.34 mg of $C_2HCl_3O_2$.

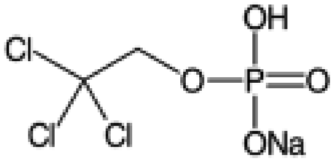
STORAGE

In an airtight container .

Ph Eur

Triclofos Sodium

General Notices



$C_2H_3Cl_3NaO_4P$ 251.4 *7246-20-0*

Action and use

Hypnotic.

Preparation

Triclofos Oral Solution

DEFINITION

Triclofos Sodium is sodium 2,2,2-trichloroethyl hydrogen orthophosphate. It contains not less than 41.3% and not more than 43.2% of Cl and not less than 97.0% and not more than 102.0% of $C_2H_3Cl_3NaO_4P$, both calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder; hygroscopic.

Freely soluble in *water*; slightly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of triclofos sodium *(RS 350)*.

B. Yields the reactions characteristic of *sodium salts*, Appendix VI.

C. Heat 0.1 g with 1 g of *anhydrous sodium carbonate* to a dull red heat and maintain for 10 minutes, cool, extract the residue with *water* and filter. The filtrate yields the reactions characteristic of *chlorides* and of *phosphates*, Appendix VI.

TESTS

Acidity

pH of a 2% w/v solution, 3.0 to 4.5, Appendix V L.

Clarity of solution

A 2.0% w/v solution is *clear*, Appendix IV A.

Heavy metals

A 10.0% w/v solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard (20 ppm).

Chloride

Dissolve 0.1 g in 50 ml of *water*. 15 ml of the solution complies with the *limit test for chlorides*, Appendix VII (0.17%).

Phosphate

Not more than 1.0%, calculated as PO_4, when determined by the following method. Dissolve 25 mg in 10 ml of *water*, add 4 ml of 1M *sulphuric acid*, 1 ml of *ammonium molybdate solution* and 2 ml of *methylaminophenol—sulphite reagent* and allow to stand for 15 minutes. Add sufficient *water* to produce 25 ml, allow to stand for a further 15 minutes and measure the *absorbance* of a 4 cm layer of the resulting solution at 730 nm, Appendix II B. Calculate the content of phosphate from a calibration curve prepared by treating suitable volumes of a 0.00143% w/v solution of *potassium dihydrogen orthophosphate* in the same manner.

Loss on drying

When dried at 100° at a pressure not exceeding 0.7 kPa for 3 hours, loses not more than 5.0% of its weight. Use 1 g.

ASSAY

For Cl

Mix 0.25 g with 1 g of *anhydrous sodium carbonate* in a nickel crucible about 3 cm in diameter, fill the crucible completely with *anhydrous sodium carbonate* and invert into a nickel crucible about 4 cm in diameter; cover the smaller crucible with *anhydrous sodium carbonate*, well pressed down, using about 25 g of *anhydrous sodium carbonate* in all. Heat for 30 minutes at a dull red heat, cool, transfer to a 400 ml beaker, add 150 ml of *water* and boil gently for 10 minutes. Filter through absorbent cotton into a 600 ml beaker, washing the residue thoroughly with hot *water*, until about 400 ml of filtrate has been collected. Cool, cautiously add *nitric acid* until the solution is neutral to *litmus paper* and add 3 ml of *nitric acid* in excess. Add 50 ml of 0.1M *silver nitrate VS*, allow to stand until precipitation is complete, filter, wash the precipitate with *water* and titrate the combined filtrate and washings with 0.1M *ammonium thiocyanate VS* using *ammonium iron(III) sulphate solution R2* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 3.545 mg of Cl.

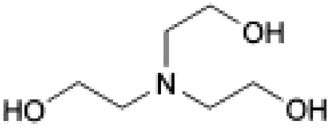
For $C_2H_3Cl_3NaO_4P$

Heat 0.2 g in a Kjeldahl flask with 2 ml of *sulphuric acid* and 2.5 ml of *nitric acid* until brown fumes cease to be evolved, cool, add 1 ml of *nitric acid* and heat again. Continue adding *nitric acid* and heating until brown fumes are no longer evolved and the solution is colourless when cold. Heat until dense, white fumes are evolved, cool, transfer the solution to a flask with the aid of 150 ml of *water*, add 50 ml of *citric—molybdic acid solution* and heat slowly to boiling. Swirling the flask continuously, add 25 ml of *quinoline solution* at first drop wise and then in a steady stream, heat on a water bath for 5 minutes and cool. Filter, wash the precipitate with *water* until free from acid, transfer the precipitate to a flask with the aid of 100 ml of *water*, add 50 ml of 0.5M *sodium hydroxide VS* and shake until dissolved. Titrate the excess of alkali with 0.5M *hydrochloric acid VS* using *phenolphthalein—thymol blue solution* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 4.835 mg of $C_2H_3Cl_3NaO_4P$. Correct the result for the content of phosphate, as determined by the test described above; each mg of PO_4 is equivalent to 2.65 mg of $C_2H_3Cl_3NaO_4P$.

Triethanolamine

General Notices

(Trolamine, Ph Eur monograph 1577)



$C_6H_{15}NO_3$ 149.2 *102-71-6*

Action and use

Pharmaceutical aid.

Ph Eur

DEFINITION

2,2′,2″-Nitrilotriethanol.

Content

99.0 per cent *m/m* to 103.0 per cent *m/m* of total bases (anhydrous substance).

CHARACTERS

Appearance

Clear, viscous, colourless or slightly yellow liquid, very hygroscopic.

Solubility

Miscible with water and with alcohol, soluble in methylene chloride.

IDENTIFICATION

First identification B, C.

Second identification A, B, D.

A. Relative density *(2.2.5):* 1.120 to 1.130.

B. Refractive index *(2.2.6)*: 1.482 to 1.485.

C. Examine the chromatograms obtained in the test for related substances.

Results the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

D. To 1 ml add 0.3 ml of *copper sulphate solution R* . A blue colour develops. Add 2.5 ml of *dilute sodium hydroxide solution R* and heat to boiling. The blue colour remains unchanged.

TESTS

Solution S

Dissolve 12 g in *water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

Related substances

Gas chromatography *(2.2.28)* .

Internal standard solution Dissolve 5.0 g of *3-aminopropanol R* in *water R* and dilute to 100.0 ml with the same solvent.

Test solution Dissolve 10.0 g of the substance to be examined in *water R* . Add 1.0 ml of the internal standard solution and dilute to 100.0 ml with *water R* .

Reference solution (a) Dissolve 1.0 g of *trolamine CRS* in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dissolve 1.0 g of *ethanolamine R* , 5.0 g of *diethanolamine R* and 1.0 g of *trolamine CRS* in *water R* and dilute to 100.0 ml with the same solvent. To 1.0 ml of the solution add 1.0 ml of the internal standard solution and dilute to 100.0 ml with *water R.*

Column:

— *material*: fused silica;

— *size*: *l* = 25 m, Ø = 0.25 mm;

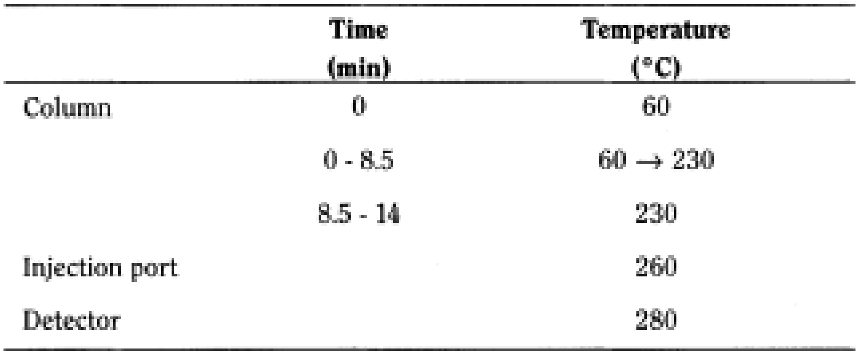
— *stationary phase: poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.50 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1 ml/min.

Split ratio 1:35.

Temperature:



	Time (min)	Temperature (°C)
Column	0	60
	0 - 8.5	60 → 230
	8.5 - 14	230
Injection port		260
Detector		280

Detection Flame ionisation.

Injection 2 µl; if necessary inject a blank.

Elution order Impurity A, 3-aminopropanol, impurity B, trolamine.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to 3-aminopropanol and impurity A.

Limits:

— *impurity A*: calculate the ratio R1 of the area of the peak due to impurity A to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak due to impurity A to the area of the peak due to the internal standard: this ratio is not greater than R1 (0.1 per cent);

— *impurity B*: calculate the ratio R2 of the area of the peak due to impurity B to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak due to impurity B to the area of the peak due to the internal standard: this ratio is not greater than R2 (0.5 per cent);

— *total:* calculate the ratio R3 of the area of the peak due to trolamine to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than 10 times R3 (1.0 per cent);

— *disregard limit:* 0.5 times the ratio of the area of the peak due to trolamine to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity C

Gas chromatography *(2.2.28)*

Carry out the test under a ventilated hood, wear gloves and safety glasses.

Test solution In a suitable distillation apparatus mix 100.0 g of the substance to be examined and 100.0 ml of *ethylene glycol R* and gently distil 10.0 ml under vacuum at a pressure not exceeding 1.3 kPa. From this distillate, distil 1 ml.

Reference solution Dilute 25.0 mg of *N-nitrosodiethanolamine R* to 100.0 ml with *ethylene glycol R* . Dilute 5.0 ml of the solution to 50.0 ml with *ethylene glycol R* . Dilute 5.0 ml of this solution to 50.0 ml with *ethylene glycol R* .

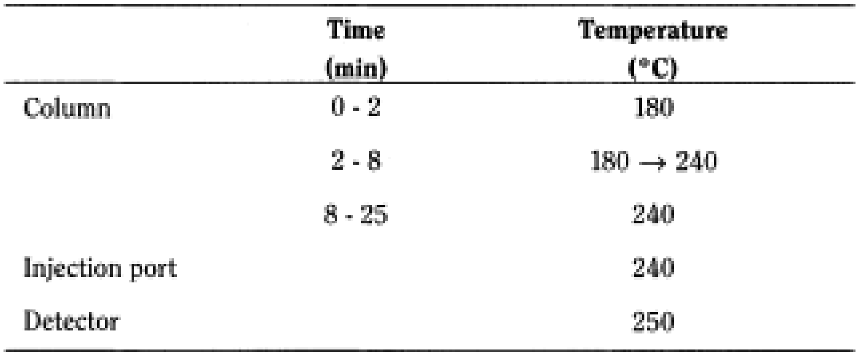
Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 0.75 ml/min.

Split ratio 1:15.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	180
	2 - 8	180 → 240
	8 - 25	240
Injection port		240
Detector		250

Detection Mass selective spectrometer set at 72 m/e or 91 m/e.

Injection 3 µl; if necessary inject a blank between each injection.

Retention times Impurity C = about 20 min.

Limit:
- *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (25 ppb).

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dilute 5 ml of solution S to 30 ml with *water R* . The solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R* .

Water ***(2.5.12)***

Maximum 1.0 per cent, determined on 1.000 g.

Open the titration vessel, introduce the substance to be examined directly into the previously titrated solvent. Stopper the flask immediately.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g. Do not carry out the initial heating on a water-bath.

ASSAY

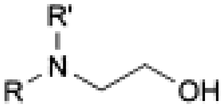
Dissolve 1.200 g in 75 ml of *carbon dioxide-free water R*. Add 0.3 ml of *methyl red solution R*. Titrate with *1 M hydrochloric acid*.

1 ml of *1 M hydrochloric acid* is equivalent to 0.149 g of $C_6H_{15}NO_3$.

STORAGE

In an airtight container, protected from light.

IMPURITIES



A. R = R′ = H: 2-aminoethanol (ethanolamine),

B. R = CH_2-CH_2OH, R′ = H: 2,2′-iminodiethanol (diethanolamine),

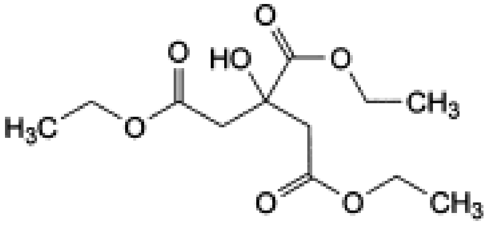
C. R = CH_2-CH_2OH, R′ = NO: 2,2′-(nitrosoimino)diethanol (*N*-nitrosodiethanolamine).

Ph Eur

Triethyl Citrate

General Notices

(Ph Eur monograph 1479)



$C_{12}H_{20}O_7$ 276.3 *77-93-0*

Action and use

Excipient.

Ph Eur

DEFINITION

Triethyl 2-hydroxypropane-1,2,3-tricarboxylate.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, viscous, colourless or almost colourless, hygroscopic liquid.

Solubility

Soluble in water, miscible with ethanol (96 per cent), slightly soluble in fatty oils.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Refractive index (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *Ph. Eur. reference spectrum of triethyl citrate.*

C. It gives the reaction of esters *(2.3.1)* .

D. To 0.5 ml add 5 ml of *ethanol (96 per cent) R* and 4 ml of *dilute sodium hydroxide solution R* . Boil under reflux for about 10 min. 2 ml of the solution gives the reaction of citrates *(2.3.1)* .

TESTS

Appearance

The substance to be examined is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Acidity

Dilute 10 g with 10 ml of previously neutralised *ethanol (96 per cent) R* , add 0.5 ml of *bromothymol blue solution R2* . Not more than 0.3 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Refractive index *(2.2.6)*

1.440 to 1.446.

Related substances

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution Dissolve 1.0 ml of the substance to be examined in *methylene chloride R* and dilute to 50.0 ml with the same solvent.

Reference solution Dissolve 1.0 ml of the substance to be examined and 0.5 ml of *methyl tridecanoate R* in *methylene chloride R,* then dilute to 50.0 ml with the same solvent.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase: poly(dimethyl)siloxane R* (5 µm).

Carrier gas *helium for chromatography R* .

Linear velocity About 26 cm/s.

Split ratio About 1:50.

Temperature:
— *column*: 200 °C;
— *injection port and detector*: 220 °C.

Detection Flame ionisation.

Injection 1.0 µl.

Run time Twice the retention time of triethyl citrate.

Retention time Triethyl citrate = about 13.6 min.

System suitability reference solution

— *resolution*: minimum 1.5 between the peaks due to triethyl citrate and methyl tridecanoate.

Limits:

— *any impurity*: for each impurity, maximum 0.2 per cent;

— *total*: maximum 0.5 per cent;

— *disregard limit*: 0.04 per cent.

Heavy metals ***(2.4.8)***

Maximum 5 ppm.

Dissolve 4.0 g in 8 ml of *ethanol (96 per cent) R* and dilute to 20 ml with *water R* . 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (1 ppm) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of equal volumes of *ethanol (96 per cent) R* and *water R.*

Water ***(2.5.12)***

Maximum 0.25 per cent, determined on 1.000 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

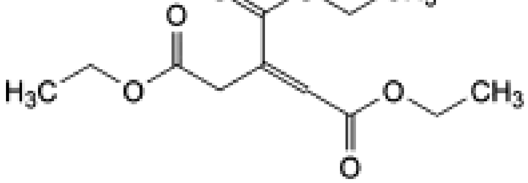
Introduce 1.500 g into a 250 ml borosilicate-glass flask fitted with a reflux condenser. Add 25 ml of *2-propanol R* , 50 ml of *water R* , 25.0 ml of *1 M sodium hydroxide* and a few glass beads. Heat under a reflux condenser for 1 h. Allow to cool. Add 1 ml of *phenolphthalein solution R1* and titrate with *1 M hydrochloric acid*. Carry out a blank titration.

1 ml of *1 M sodium hydroxide* is equivalent to 92.1 mg of $C_{12}H_{20}O_7$.

STORAGE

In an airtight container .

IMPURITIES



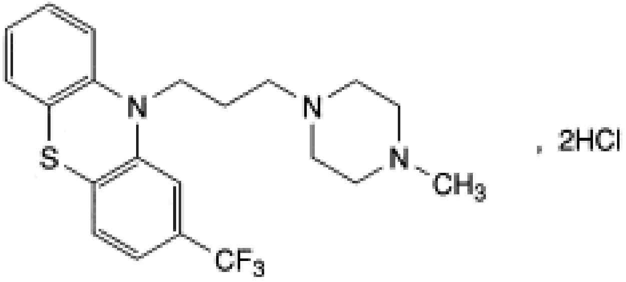
A. triethyl propene-1,2,3-tricarboxylate (triethyl aconitate).

Ph Eur

Trifluoperazine Hydrochloride

General Notices

(Ph Eur monograph 0059)



$C_{21}H_{24}F_3N_3S,2HCl$ 480.4   *440-17-5*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Trifluoperazine Tablets

Ph Eur

DEFINITION

Trifluoperazine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 10-[3-(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)-10*H*-phenothiazine dihydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white to pale yellow, crystalline powder, hygroscopic, freely soluble in water, soluble in alcohol.

It melts at about 242 °C, with decomposition.

IDENTIFICATION

A. *Protect the solutions from bright light and measure the absorbances immediately.* Dissolve 50 mg in *0.1 M hydrochloric acid* and dilute to 500 ml with the same acid.

Examined between 280 nm and 350 nm, the solution shows an absorption maximum *(2.2.25)* at 305 nm. Dilute 5 ml of the solution to 100 ml with *0.1 M hydrochloric acid* . Examined between 230 nm and 280 nm, this solution shows an absorption maximum at 255 nm. The specific absorbance at this maximum is about 650.

B. It complies with the identification test for phenothiazines by thin-layer chromatography *(2.3.3)* : use *trifluoperazine hydrochloride CRS* to prepare the reference solution.

C. Place 0.25 g in a 100 ml separating funnel, add 5 ml of *water R* and 2 ml of *dilute sodium hydroxide solution R* . Shake vigorously with 20 ml of *ether R* . Wash the ether layer with 5 ml of *water R* , add 0.15 g of *maleic acid R* and evaporate the ether. The residue, recrystallised from 30 ml of *alcohol R* and dried, melts *(2.2.14)* at about 192 °C.

D. Dissolve about 0.5 mg in 1 ml of *water R* , add 0.1 ml of *bromine water R* and shake for about 1 min. Add dropwise 1 ml of *sulphuric acid R* with constant, vigorous agitation. A red colour develops.

E. Dissolve about 50 mg in 5 ml of *water R* and add 2 ml of *nitric acid R* . A dark-red colour develops which turns to pale yellow. The solution gives reaction (a) of chlorides *(2.3.1)*.

TESTS

pH *(2.2.3)*

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. The pH of the solution is 1.6 to 2.5.

Related substances

Carry out the test protected from bright light.

Examine by thin-layer chromatography *(2.2.27)* , using a *TLC silica gel GF$_{254}$ plate R*.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents. Prepare immediately before use.

Reference solution Dilute 1 ml of the test solution to 200 ml with a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R* .

Apply to the plate 10 µl of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *acetone R,* 10 volumes of *diethylamine R* and 80 volumes of *cyclohexane R.* Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying *(2.2.32)*

Not more than 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 48.04 mg of $C_{21}H_{26}Cl_2F_3N_3S$.

STORAGE

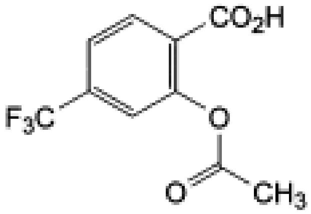
Store in an airtight container, protected from light.

Ph Eur

Triflusal

General Notices

(Ph Eur monograph 1377)



$C_{10}H_7F_3O_4$ 248.2 *322-79-2*

Action and use

Thromboxane synthesis inhibitor; antiplatelet drug.

Ph Eur

DEFINITION

2-(Acetyloxy)-4-(trifluoromethyl)benzoic acid.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

practically insoluble in water, very soluble in ethanol, freely soluble in methylene chloride.

mp

About 118 °C, with decomposition.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *triflusal CRS.*

TESTS

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.20 g of the substance to be examined in *acetonitrile R* and dilute to 20.0 ml with the same solvent. *Prepare the solution immediately before use.*

Reference solution (a) Dissolve 5.0 mg of *triflusal impurity B CRS* in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 25.0 ml with *acetonitrile R* .

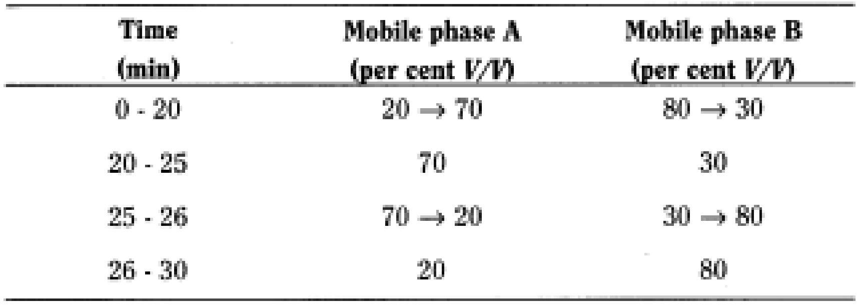
Reference solution (c) Dissolve 2.5 mg of the substance to be examined in *acetonitrile R* , add 5 ml of reference solution (a) and dilute to 10 ml with *acetonitrile R* . Prepare the solution immediately before use.

Column:

- *size:* *l* = 0.15 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (4-5 µm).

Mobile phase:

- *mobile phase A: acetonitrile R* ;
- *mobile phase B:* 0.5 per cent *V/V* solution of *phosphoric acid R,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	20 → 70	80 → 30
20 - 25	70	30
25 - 26	70 → 20	30 → 80
26 - 30	20	80

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 237 nm.

Injection 10 µl of the test solution and reference solutions (b) and (c).

Relative retention With reference to triflusal (retention time = about 13 min): impurity A = about 0.3; impurity B = about 1.2; impurity C = about 1.3; impurity D = about 1.6.

System suitability Reference solution (c):

- *resolution*: minimum 3.0 between the peaks due to triflusal and impurity B.

Limits:

- *impurity B*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *total of impurities other than B*: not more than 0.5 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *disregard limit*: 0.1 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.02 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

Dissolve 2.0 g in 12 ml of *alcohol R* and dilute to 20 ml with *water R* . 12 ml complies with limit test B. Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 2 volumes of *water R* and 3 volumes of *alcohol R* .

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator *in vacuo* over *diphosphorus pentoxide R* .

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.200 g in 50 ml of *ethanol R* . Titrate with *0.1 M sodium hydroxide* , determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M sodium hydroxide* is equivalent to 24.82 mg of $C_{10}H_7F_3O_4$.

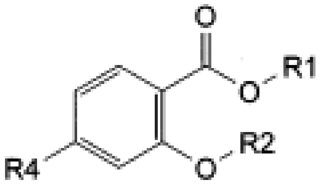
STORAGE

In an airtight container , at a temperature not exceeding 25 °C.

IMPURITIES

Specified impurities *B.*

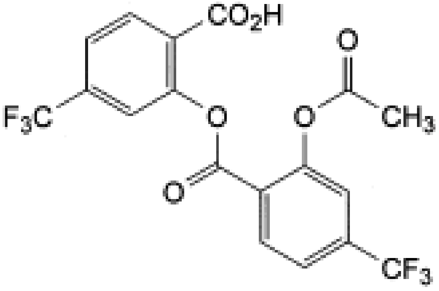
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, C, D.*



A. R1 = H, R2 = CO-CH_3, R4 = CO_2H: 2-(acetyloxy)benzene-1,4-dicarboxylic acid (2-acetoxyterephthalic acid),

B. R1 = R2 = H, R4 = CF_3: 2-hydroxy-4-(trifluoromethyl)benzoic acid (4-(trifluoromethyl) salicylic acid),

C. R1 = R2 = CO-CH_3, R4 = CF_3: acetic 2-(acetyloxy)-4-(trifluoromethyl)benzoic anhydride,



D. 2-[[2-(acetyloxy)-4-(trifluoromethyl)benzoyl]oxy]-4-(trifluoromethyl)benzoic acid.

Ph Eur

Triglycerol Diisostearate

General Notices

(Ph Eur monograph 2032)

Ph Eur

DEFINITION

Mixture of polyglycerol diesters of mainly isostearic acid, obtained by esterification of polyglycerol and isostearic acid. The polyglycerol consists mainly of triglycerol.

CHARACTERS

Appearance

Clear, yellowish, viscous liquid.

Solubility

Practically insoluble in water, miscible with ethanol (96 per cent) and with fatty oils.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Film between 2 plates of *sodium chloride R.*

Comparison *triglycerol diisostearate CRS.*

B. Composition of fatty acids (see Tests).

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_3 *(2.2.2, Method I)*.

Mix 10 ml with 10 ml of *ethanol (96 per cent) R.*

Acid value *(2.5.1)*

Maximum 3.0, determined on 1.0 g.

Hydroxyl value *(2.5.3, Method A)*

180 to 230, determined on 0.25 g.

Iodine value ***(2.5.4, Method B)***

Maximum 3.0.

Peroxide value ***(2.5.5, Method B)***

Maximum 6.0.

Saponification value ***(2.5.6)***

128 to 160.

Composition of fatty acids ***(2.4.22, Method B)***

Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty-acid fraction of the substance:

— *sum of the contents of the fatty acids eluting between palmitic acid and stearic acid* : minimum 60.0 per cent;

— *sum of the contents of myristic acid, palmitic acid and stearic acid* : maximum 11.0 per cent.

Water ***(2.5.12)***

Maximum 0.5 per cent, determined on 2.00 g.

Sulphated ash

Maximum 0.5 per cent, determined on 1.0 g.

Heat a silica crucible to redness for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.00 g of the substance to be examined in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 °C ± 25 °C, until the substance is thoroughly charred. Carry out the test for sulphated ash *(2.4.14)* on the residue obtained, starting with "Moisten the substance to be examined...".

STORAGE

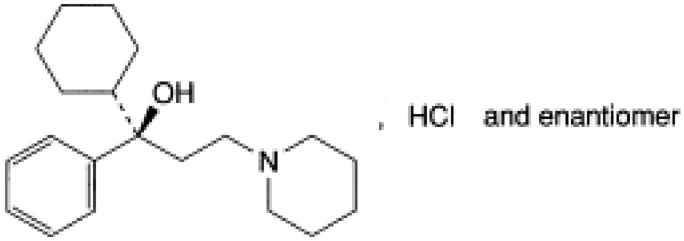
In an airtight container, protected from light.

Ph Eur

Trihexyphenidyl Hydrochloride

General Notices

(Ph Eur monograph 1626)



$C_{20}H_{31}NO,HCl$ 337.9 *52-49-3*

Action and use

Anticholinergic.

Preparation

Trihexyphenidyl Tablets

Ph Eur

DEFINITION

(1*RS*)-1-Cyclohexyl-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in alcohol and in methylene chloride.

mp

About 250 °C, with decomposition.

IDENTIFICATION

First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *trihexyphenidyl hydrochloride CRS* .

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 25 mg of the substance to be examined in a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution Dissolve 25 mg of *trihexyphenidyl hydrochloride CRS* in a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Plate *TLC silica gel G plate R* .

Mobile phase *diethylamine R, hexane R* (5:95 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a 0.1 g/l solution of *chloroplatinic acid R* in *hydrochloric acid R* containing 0.4 per cent *V/V* of *hydriodic acid R* .

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 0.5 g in 5 ml of warm *methanol R* and make just alkaline to *red litmus paper R* with *sodium hydroxide solution R* . A precipitate is formed which, after recrystallisation from *methanol R* , melts *(2.2.14)* at about 113 °C to 115 °C.

D. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

pH *(2.2.3)*

5.2 to 6.2.

Dissolve 0.5 g with heating in 25 ml of *carbon dioxide-free water R.* Cool to room temperature and dilute to 50 ml with *carbon dioxide-free water R.*

Optical rotation *(2.2.7)*

- 0.10° to + 0.10°.

Dissolve 1.25 g in a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R* and dilute to 25.0 ml with the same mixture of solvents.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase. Dilute 10.0 ml to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *trihexyphenidyl impurity A CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d) To 1 ml of reference solution (b), add 1 ml of the test solution and dilute to 100 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 200 ml of *water R* with 0.2 ml of *triethylamine R* . Adjust to pH 4.0 with *phosphoric acid R* and add 800 ml of *acetonitrile R* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Run time 3 times the retention time of trihexyphenidyl.

System suitability Reference solution (d):

— *resolution*: minimum 4.0 between the peaks corresponding to trihexyphenidyl and to impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total*: not more than 0.5 per cent;

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

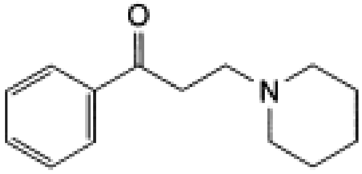
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid* . Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide* . Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 33.79 mg of $C_{20}H_{32}ClNO$.

IMPURITIES



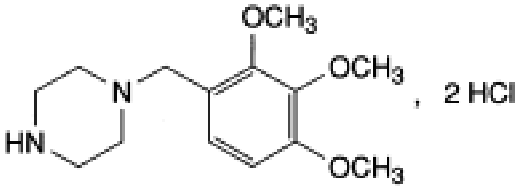
A. 1-phenyl-3-(piperidin-1-yl)propan-1-one.

Ph Eur

Trimetazidine Hydrochloride

General Notices

(Trimetazidine Dihydrochloride, Ph Eur monograph 1741)



$C_{14}H_{22}N_2O_3$,2HCl 339.3 *13171-25-0*

Action and use

Vasodilator.

Ph Eur

DEFINITION

1-(2,3,4-Trimethoxybenzyl)piperazine dihydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, slightly hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in alcohol.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison Ph. Eur. reference spectrum of trimetazidine dihydrochloride.

B. Dissolve 25 mg in 5 ml of *water R* . 2 ml of the solution gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Dissolve 1.0 g in *water R* and dilute to 10 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *trimetazidine for system suitability CRS* in *water R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dilute 2.0 ml of the test solution to 100.0 ml with *water R* . Dilute 5.0 ml of this solution to 100.0 ml with *water R* .

Reference solution (c) Dilute 25.0 ml of reference solution (b) to 50.0 ml with *water R* .

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

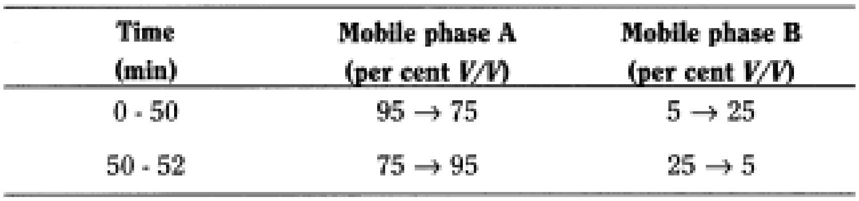
— *stationary phase: spherical octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm;

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: mix 357 volumes of *methanol R* and 643 volumes of a 2.87 g/l solution of *sodium heptanesulphonate R* adjusted to pH 3.0 with *dilute phosphoric acid R* ;

— *mobile phase B*: *methanol R,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	95 → 75	5 → 25
50 - 52	75 → 95	25 → 5

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 240 nm.

Equilibration For at least 1 h with the mobile phase at the initial composition.

Injection 10 µl.

Relative retention With reference to trimetazidine (retention time = about 25 min): impurity D

= about 0.2; impurity C = about 0.4; impurity H = about 0.6; impurities A and I = about 0.9; impurity E = about 0.95; impurity F = about 1.4; impurity B = about 1.8.

System suitability:

— *peak-to-valley ratio* : minimum 3, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the principal peak in the chromatogram obtained with reference solution (a);

— *signal-to-noise ratio* : minimum 10 for the principal peak in the chromatogram obtained with reference solution (c).

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.55; impurity C = 0.37; impurity F = 0.71;

— *impurities A, B, C, D, E, F, H, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Impurity G

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 22.6 mg of *piperazine hydrate R* in *methanol R* and dilute to 100 ml with the same solvent. Dilute 10 ml of the solution to 100 ml with *methanol R* .

Plate *TLC silica gel plate R* .

Mobile phase *Concentrated ammonia R, alcohol R* (20:80 *V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying At 100-105 °C for 30 min.

Detection Spray with *iodoplatinate reagent R* .

Limit:

— *impurity G*: any spot due to impurity G is not more intense than the spot in the chromatogram obtained with the reference solution (0.1 per cent, expressed as anhydrous piperazine).

Loss on drying *(2.2.32)*

Maximum 2.5 per cent, determined on 1.000 g by drying in an oven at 105 °C over *diphosphorus pentoxide R* at a pressure not exceeding 15 kPa.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.120 g in 50.0 ml of *water R*. Add 1 ml of *nitric acid R* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*.

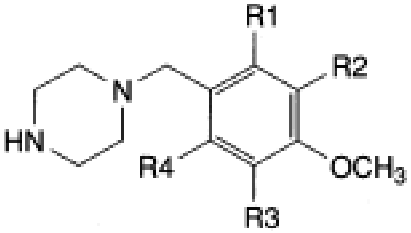
1 ml of *0.1 M silver nitrate* is equivalent to 16.96 mg of $C_{14}H_{24}Cl_2N_2O_3$.

STORAGE

In an airtight container.

IMPURITIES

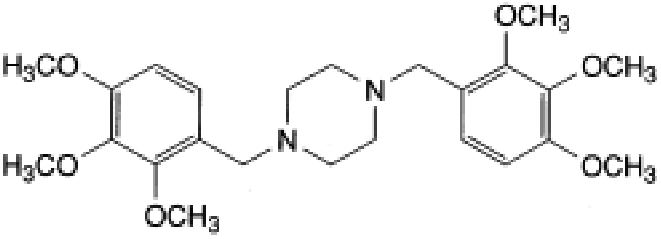
Specified impurities A, B, C, D, E, F, G, H, I.


R1
R2
HN
N
R4
OCH3
R3

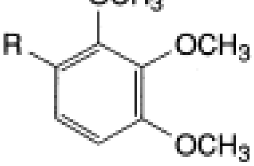
A. R1 = R4 = H, R2 = R3 = OCH_3: 1-(3,4,5-trimethoxybenzyl)piperazine,

E. R1 = R3 = OCH_3, R2 = R4 = H: 1-(2,4,5-trimethoxybenzyl)piperazine,

F. R1 = R4 = OCH_3, R2 = R3 = H: 1-(2,4,6-trimethoxybenzyl)piperazine,


OCH3
H3CO
OCH3
N
N
H3CO
OCH3
OCH3

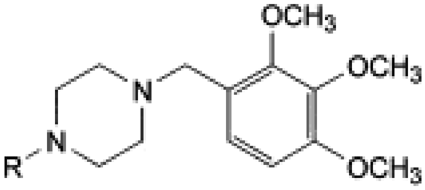
B. 1,4-bis(2,3,4-trimethoxybenzyl)piperazine,


OCH3
R
OCH3
OCH3

C. R = CHO: 2,3,4-trimethoxybenzaldehyde,

D. R = CH_2OH: (2,3,4-trimethoxyphenyl)methanol,

G. piperazine,



H. R = $COOC_2H_5$: ethyl 4-(2,3,4-trimethoxybenzyl)piperazine-1-carboxylate,

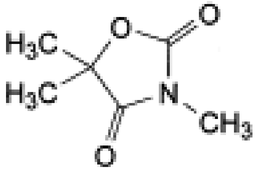
I. R = CH_3: 1-methyl-4-(2,3,4-trimethoxybenzyl)piperazine (*N*-methyltrimetazidine).

Ph Eur

Trimethadione

General Notices

(Ph Eur monograph 0440)



$C_6H_9NO_3$ 143.1 *127-48-0*

Action and use

Antiepileptic.

Ph Eur

DEFINITION

3,5,5-Trimethyloxazolidine-2,4-dione.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Colourless or almost colourless crystals.

Solubility

Soluble in water, very soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Melting point *(2.2.14)* : 45 °C to 47 °C, determined without previous drying.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs prepared using 3 mg of substance per 0.4 g of *potassium bromide R* .

Comparison *trimethadione CRS* .

C. To 2 ml of solution S (see Tests) add 1 ml of *barium hydroxide solution R* . A white precipitate is formed, which dissolves on addition of 1 ml of *dilute hydrochloric acid R.*

D. Dissolve 0.3 g in a mixture of 5 ml of *alcoholic potassium hydroxide solution R* and 5 ml of *ethanol (96 per cent) R* . Allow to stand for 10 min. Add 0.05 ml of *phenolphthalein solution R1* and neutralise exactly with *hydrochloric acid R* . Evaporate to dryness on a water-bath and take up the residue with 4 quantities, each of 5 ml, of *ether R* . Filter the combined ether layers and evaporate to dryness. The residue, recrystallised from 5 ml of *toluene R* and dried, melts *(2.2.14)* at about 80 °C.

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 40 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II).*

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *methyl red solution R* . Not more than 0.1 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.00 g by drying in a desiccator over *anhydrous silica gel R* for 6 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography *(2.2.28)* .

Internal standard solution Dissolve 0.125 g of *decanol R* in *anhydrous ethanol R* and dilute to 25 ml with the same solvent.

Test solution Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 10.0 ml with the same solution.

Reference solution Dissolve 0.100 g of *trimethadione CRS* in the internal standard solution and dilute to 10.0 ml with the same solution.

Column:
- *material:* stainless steel;
- *size*: *l* = 0.75 m, Ø = 3 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (125-150 µm).

Carrier gas *nitrogen for chromatography R* .

Flow rate 20 ml/min.

Temperature:
- *column:* 210 °C,
- *injection port*: 240 °C,
- *detector:* 270 °C.

Detection Flame ionisation.

Injection 1 µl.

Calculate the content of $C_6H_9NO_3$ from the declared content of *trimethadione CRS* .

STORAGE

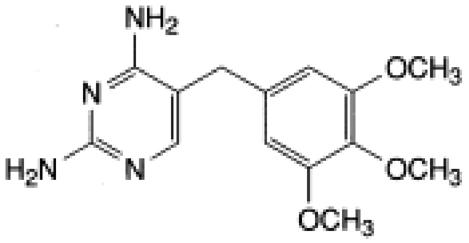
Protected from light.

Ph Eur

Trimethoprim

General Notices

(Ph Eur monograph 0060)



$C_{14}H_{18}N_4O_3$ *290.3* *738-70-5*

Action and use

Dihydrofolate reductase inhibitor; antibacterial.

Preparations

Co-trimoxazole Intravenous Infusion

Co-trimoxazole Oral Suspension

Paediatric Co-trimoxazole Oral Suspension

Co-trimoxazole Tablets

Dispersible Co-trimoxazole Tablets

Paediatric Co-trimoxazole Tablets

Trimethoprim Oral Suspension

Trimethoprim Tablets

Ph Eur

DEFINITION

5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Very slightly soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification *C.*

Second identification *A, B, D.*

A. Melting point *(2.2.14)* : 199 °C to 203 °C.

B. Dissolve about 20 mg in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *0.1 M sodium hydroxide* . Examined between 230 nm and 350 nm *(2.2.25)* , the solution shows an absorption maximum at 287 nm. The specific absorbance at the absorption maximum is 240 to 250.

C. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *trimethoprim CRS* .

D. Dissolve about 25 mg, heating if necessary, in 5 ml of *0.005 M sulphuric acid* and add 2 ml of a 16 g/l solution of *potassium permanganate R* in *0.1 M sodium hydroxide* . Heat to boiling and add to the hot solution 0.4 ml of *formaldehyde R* . Mix, add 1 ml of *0.5 M sulphuric acid* , mix and heat again to boiling. Cool and filter. To the filtrate, add 2 ml of *methylene chloride R* and shake vigorously. The organic layer, examined in ultraviolet light at 365 nm, shows green fluorescence.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 0.5 g in 10 ml of a mixture of 1 volume of *water R,* 4.5 volumes of *methanol R* and 5 volumes of *methylene chloride R* .

Related substances

A. Liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *trimethoprim for system suitability CRS* (containing impurity E) in 1 ml of the mobile phase.

Column:

- *size*: *l* = 0.250 m, Ø = 4.0 mm;
- *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *methanol R* and 70 volumes of a 1.4 g/l solution of *sodium perchlorate R* adjusted to pH 3.6 with *phosphoric acid R* .

Flow rate 1.3 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl loop injector.

Run time 11 times the retention time of trimethoprim.

Relative retention With reference to trimethoprim (retention time = about 5 min): impurity C = about 0.8; impurity E = about 0.9; impurity A = about 1.5; impurity D = about 2.0; impurity G = about 2.1; impurity B = about 2.3; impurity J = about 2.7; impurity F = about 4.0.

System suitability Reference solution (b):

- *resolution*: minimum 2.5 between the peaks due to impurity E and trimethoprim.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.43; impurity E = 0.53; impurity J = 0.66;
- *any impurity*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total:* not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.04 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent); disregard any peak corresponding to impurity H (relative retention = about 10.3).

B. Liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *trimethoprim CRS* and 5.0 mg of *trimethoprim impurity B CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *nitrile silica gel for chromatography R* (5 µm) with a specific surface area of 350 m2/g and a pore diameter of 10 nm.

Mobile phase Dissolve 1.14 g of *sodium hexane sulphonate R* in 600 ml of a 13.6 g/l solution of *potassium dihydrogen phosphate R* ; adjust to pH 3.1 with *phosphoric acid R* and mix with 400 ml of *methanol R.*

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl loop injector.

Run time 6 times the retention time of trimethoprim.

Relative retention With reference to trimethoprim (retention time = about 4 min): impurity H = about 1.8; impurity I = about 4.9.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to trimethoprim and impurity B.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity H = 0.50; impurity I = 0.28;

— *any impurity*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total:* not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.04 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent); disregard any peak due to impurity B (relative retention = about 1.3).

Impurity K

Gas chromatography *(2.2.28)* .

Test solution Dissolve 0.500 g of the substance to be examined in 35.0 ml of *citrate buffer solution pH 5.0 R*, add 10.0 ml of *1,1-dimethylethyl methyl ether R* , shake thoroughly and centrifuge for 10 min. Use the upper layer.

Reference solution Dilute 5.0 ml of *hydrochloric acid R* to 50.0 ml with *water R* , add 12.5 mg of *aniline R* and shake thoroughly. Add 10.0 µl of this solution and 10.0 ml of *1,1-dimethylethyl methyl ether R* to 35.0 ml of *citrate buffer solution pH 5.0 R*, shake thoroughly and centrifuge for 10 min. Use the upper layer.

Column:

— *material:* fused silica;

— *size*: *l* = 30 m, Ø = 0.53 mm;

— *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 3 µm).

Carrier gas *helium for chromatography R* .

Flow rate 12 ml/min.

Temperature:

— *column*: 80 °C;

— *injection port*: 230 °C;

— *detector*: 270 °C.

Detection Nitrogen-phosphorus detector.

Injection 3 µl.

Run time 15 min.

System suitability Reference solution:

— *repeatability*: maximum relative standard deviation of 5.0 per cent after 6 injections.

Limit:

— *impurity K*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (5 ppm).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20)*.

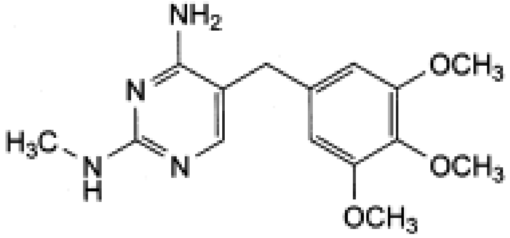
1 ml of *0.1 M perchloric acid* is equivalent to 29.03 mg of $C_{14}H_{18}N_4O_3$.

IMPURITIES

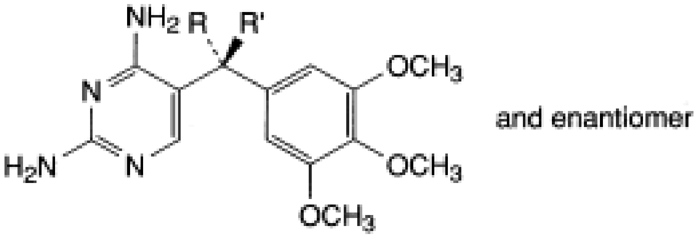
By liquid chromatography A *A, B, C, D, E, F, G, H, J.*

By liquid chromatography B *B, H, I.*

By gas chromatography *K.*

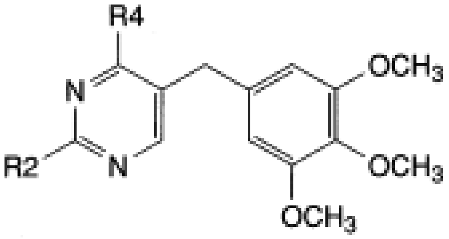


A. *N* ^{2}-methyl-5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diamine,



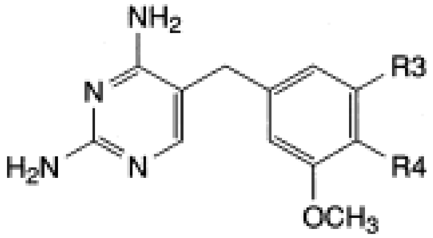
B. R + R′ = O: (2,4-diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl)methanone,

C. R = OH, R′ = H: *(RS)*-(2,4-diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl)methanol,



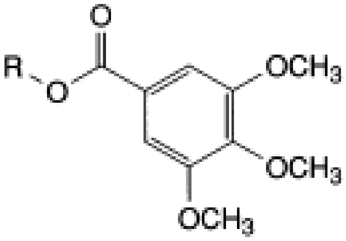
D. R2 = NH_2, R4 = OH: 2-amino-5-(3,4,5-trimethoxybenzyl)pyrimidin-4-ol,

E. R2 = OH, R4 = NH_2: 4-amino-5-(3,4,5-trimethoxybenzyl)pyrimidin-2-ol,



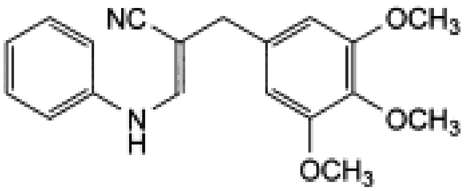
F. R3 = Br, R4 = OCH_3: 5-(3-bromo-4,5-dimethoxybenzyl)pyrimidine-2,4-diamine,

G. R3 = OCH_3, R4 = OC_2H_5: 5-(4-ethoxy-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine,

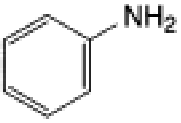


H. R = CH_3: methyl 3,4,5-trimethoxybenzoate,

J. R = H: 3,4,5-trimethoxybenzoic acid,



I. 3-(phenylamino)-2-(3,4,5-trimethoxybenzyl)prop-2-enenitrile,



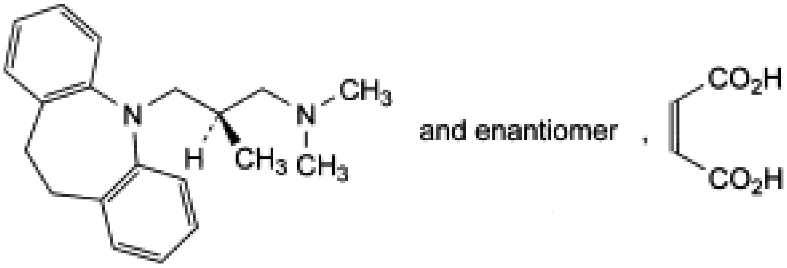
K. aniline.

Ph Eur

Trimipramine Maleate

General Notices

(Ph Eur monograph 0534)



$C_{20}H_{26}N_2,C_4H_4O_4$ 410.5 *521-78-8*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Trimipramine Tablets

Ph Eur

DEFINITION

(2*RS*)-3-(10,11-Dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N,N*,2-trimethylpropan-1-amine (*Z*)-butenedioate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification *A, C.*

Second identification *A, B, D, E.*

A. Melting point *(2.2.14)* : 140 °C to 144 °C.

B. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution Dissolve 40.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of this solution to 100.0 ml with *0.01 M hydrochloric acid* .

Spectral range 230-350 nm.

Absorption maximum At 250 nm.

Shoulder At 270 nm.

Specific absorbance at the absorption maximum 205 to 235.

C. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *trimipramine maleate CRS* .

D. Thin-layer chromatography *(2.2.27). Prepare the solutions immediately before use.*

Test solution Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 20 ml with *methanol R.*

Reference solution Dissolve 25 mg of *trimipramine maleate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R* .

Mobile phase *concentrated ammonia R* , *anhydrous ethanol R* , *toluene R* (0.7:10:90 *V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air for 15 min.

Detection Spray with a 5 g/l solution of *potassium dichromate R* in a mixture of 1 volume of *sulphuric acid R* and 4 volumes of *water R* and examine immediately.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

E. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 56 mg of *maleic acid R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel GF$_{254}$ R*.

Mobile phase *water R, anhydrous formic acid R*, *di-isopropyl ether R* (3:7:90 *V/V/V*).

Application 5 µl as bands of 10 mm.

Development Over 2/3 of the plate.

Drying In a current of air for a few minutes and then at 120 °C for 10 min.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution shows 2 zones: one is on the starting line and the other is similar in position and size to the principal zone in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography *(2.2.29)*. *Carry out the test protected from light.*

Buffer solution pH 7.7 2.64 g/l solution of *ammonium phosphate R* in *water for chromatography R*; adjust to pH 7.7 with *phosphoric acid R*.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of the substance to be examined and 5 mg of *iminodibenzyl R* (impurity F) in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *trimipramine for peak identification CRS* (containing impurities A, B, C, D and E) in 1 ml of *acetonitrile R1*.

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R1*, buffer solution pH 7.7 (38:62 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Run time 3 times the retention time of trimipramine.

Identification of impurities Use the chromatogram supplied with *trimipramine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D and E; doubling of the peak due to impurity E may be observed.

Relative retention With reference to trimipramine (retention time = about 27 min): impurity A = about 0.1; impurity B = about 0.3; impurity C = about 0.4; impurity D = about 0.5; impurity F = about 1.4; impurity E = about 1.5.

System suitability:

— *resolution*: minimum 3.5 between the peaks due to trimipramine and impurity F in the chromatogram obtained with reference solution (a);

— the chromatogram obtained with reference solution (c) is similar to the chromatogram supplied with *trimipramine for peak identification CRS*.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity F by 0.5;

— *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity F*: not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities A, B, C, D*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

2.0 g complies with test C. Prepare the reference solution using 4 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 50 ml of *anhydrous acetic acid R* . Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M perchloric acid* is equivalent to 41.05 mg of $C_{24}H_{30}N_2O_4$.

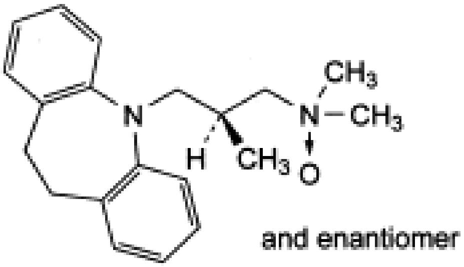
STORAGE

Protected from light.

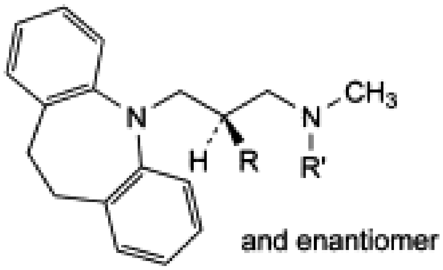
IMPURITIES

Specified impurities A, B, C, D, E, F.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): G.*

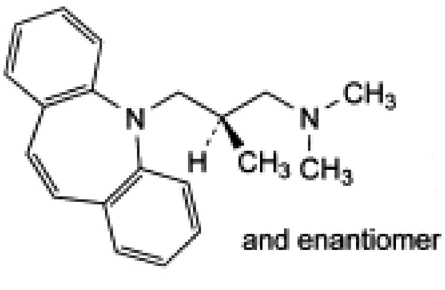

N
CH_3
$N-CH_3$
H
CH_3
O
and enantiomer

A. (2*RS*)-3-(10,11-dihydro-5*H*-dibenzo[*b*,*ı*]azepin-5-yl)-*N,N*,2-trimethylpropan-1-amine N-oxide,

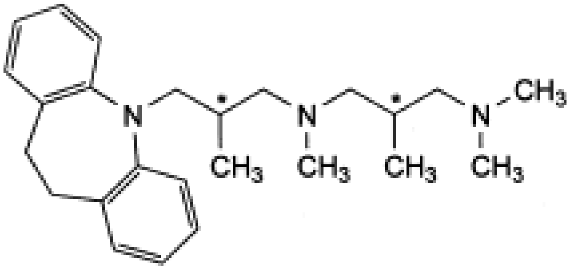

N
N
CH_3
H
R
R'
and enantiomer

B. R = CH_3, R′ = H: (2*RS*)-3-(10,11-dihydro-5*H*-dibenzo[*b*,*ı*]azepin-5-yl)-*N*,2-dimethylpropan-1-amine,

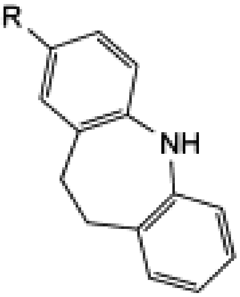
D. R = H, R′ = CH_3: imipramine,


N
N
CH_3
H
CH_3
CH_3
and enantiomer

C. (2*RS*)-3-(5*H*-dibenzo[b,f]azepin-5-yl)-*N,N*,2-trimethylpropan-1-amine,



E. mixture of the stereoisomers of *N*-[3-(10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-2-methylpropyl]-*N,N′ ,N′*,2-tetramethylpropane-1,3-diamine,



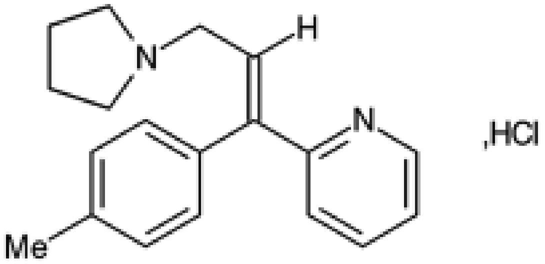
F. R = *H*: 10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine,

G. R = CH_3: 2-methyl-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine.

Ph Eur

Triprolidine Hydrochloride

General Notices



$C_{19}H_{22}N_2,HCl,H_2O$ 332.9 *6138-79-0*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Triprolidine Tablets

DEFINITION

Triprolidine Hydrochloride is (*E*)-2-(3-pyrrolidin-1-yl-1-*p*-tolylprop1-enyl)pyridine hydrochloride monohydrate. It contains not less than 98.5% and not more than 101.0% of $C_{19}H_{22}N_2,HCl$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white, crystalline powder.

Freely soluble in *water*; freely soluble in *ethanol (96%)* and practically insoluble in *ether* .

IDENTIFICATION

A. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of triprolidine hydrochloride *(RS 356)* .

B. Yields reaction A characteristic of *chlorides* , Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of equal volumes of *butan-2-one* and *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions in *methanol* containing (1) 1.0% w/v of the substance being examined, (2) 0.020% w/v of Z- *triprolidine hydrochloride BPCRS* and (3) 0.010% w/v of the substance being examined. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to *Z*-triprolidine is not more intense than the spot in the chromatogram obtained with solution (2) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3).

Sulphated ash

Not more than 0.1%, Appendix IX A.

Water

4.5 to 6.0% w/w, Appendix IX C. Use 0.4 g.

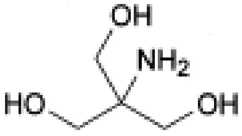
ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 g dissolved in a mixture of 50 ml of *anhydrous acetic acid* and 0.5 ml of *acetic anhydride* and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 15.74 mg of $C_{19}H_{22}N_2$,HCl.

Trometamol

General Notices

(Ph Eur monograph 1053)



$C_4H_{11}NO_3$ 121.1 *77-86-1*

Action and use

Organic amine proton acceptor; alkalinizing agent.

Ph Eur

DEFINITION

Trometamol contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of aminomethylidynetri(methanol), calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, or colourless crystals, freely soluble in water, sparingly soluble in alcohol, very slightly soluble in ethyl acetate.

IDENTIFICATION

First identification B, C.

Second identification A, B, D.

A. Solution S (see Tests) is strongly alkaline *(2.2.4)* .

B. Melting point *(2.2.14)* : 168 °C to 174 °C.

C. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *trometamol CRS* .

D. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of freshly prepared solution S is 10.0 to 11.5.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using *silica gel G R* as the coating substance. Wash the plate with *methanol R* before applying the solutions.

Test solution (a) Dissolve 0.20 g in 1 ml of *water R*, with heating, and dilute to 10 ml with *methanol R*.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 20 mg of *trometamol CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (a) to 100 ml with *methanol R*.

Apply to the plate 10 µl of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of *dilute ammonia R1* and 90 volumes of *2-propanol R*. Dry the plate at 100 °C to 105 °C. Spray with a 5 g/l solution of *potassium permanganate R* in a 10 g/l solution of *sodium carbonate R*. After about 10 min examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

Chlorides *(2.4.4)*

To 10 ml of solution S add 2.5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution complies with the limit test for chlorides (100 ppm).

Heavy metals *(2.4.8)*

Dissolve 2.0 g in 10 ml of *water R*. Neutralise the solution with *hydrochloric acid R1* and dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Iron *(2.4.9)*

Dissolve 1.0 g in *water R* and dilute to 10 ml with the same solvent. The solution complies with the limit test for iron (10 ppm).

Loss on drying *(2.2.32)*

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins ***(2.6.14)***

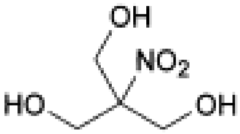
Less than 0.03 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.100 g in 20 ml of *water R* . Add 0.2 ml of *methyl red solution R* . Titrate with *0.1 M hydrochloric acid* until the colour changes from yellow to red.

1 ml of *0.1 M hydrochloric acid* is equivalent to 12.11 mg of $C_4H_{11}NO_3$.

IMPURITIES



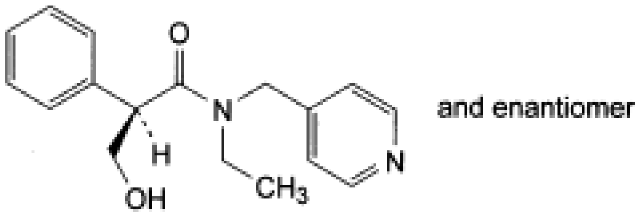
A. nitromethylidynetri(methanol).

Ph Eur

Tropicamide

General Notices

(Ph Eur monograph 1159)



$C_{17}H_{20}N_2O_2$ 284.4 *1508-75-4*

Action and use

Anticholinergic.

Preparation

Tropicamide Eye Drops

Ph Eur

DEFINITION

Tropicamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*)-*N*-ethyl-3-hydroxy-2-phenyl-*N*-(pyridin-4-ylmethyl)propanamide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water, freely soluble in alcohol and in methylene chloride.

IDENTIFICATION

First identification C.

Second identification A, B, D, E.

A. Melting point *(2.2.14)* : 95 °C to 98 °C.

B. Dissolve 20.0 mg in *0.1 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Dilute 2.0 ml of the solution to 20.0 ml *with 0.1 M hydrochloric acid* . Examined between 230 nm and 350 nm *(2.2.25)* , the solution shows an absorption maximum at 254 nm. The specific absorbance at the maximum is 170 to 190.

C. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *tropicamide CRS* . Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the spot in the chromatogram obtained with reference solution (a).

E. Dissolve about 5 mg in 3 ml of a mixture of 9 ml of *acetic anhydride R* , 1 ml of *acetic acid R* and 0.1 g of *citric acid R.* Heat on a water-bath for 5 min to 10 min. A reddish-yellow colour is produced.

TESTS

Appearance of solution

Dissolve 0.1 g in *alcohol R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Optical rotation *(2.2.7)*

Dissolve 2.5 g in *ethanol R* and dilute to 25.0 ml with the same solvent. The angle of optical rotation is - 0.1 ° to + 0.1 °.

Related substances

Examine by thin-layer chromatography *(2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *methylene chloride R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 20 ml *with methylene chloride R* .

Reference solution (a) Dissolve 10 mg of *tropicamide CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dilute 1 ml of test solution (b) to 10 ml with *methylene chloride R* .

Reference solution (c) Dilute 2 ml of reference solution (b) to 5 ml with *methylene chloride R* .

Reference solution (d) Dissolve 20 mg of *4-[(ethylamino)methyl]pyridine R* in *methylene chloride R* and dilute to 20 ml with the same solvent. Dilute 1 ml of the solution and 1 ml of reference solution (a) to 10 ml with *methylene chloride R* .

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 0.5 volumes of *concentrated ammonia R* , 5 volumes of *methanol R* and 95 volumes of *methylene chloride R* . Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots.

Tropic acid

To 10.0 mg add 5 mg of *disodium tetraborate R* and 0.35 ml of a freshly prepared 100 g/l solution of *dimethylaminobenzaldehyde R* in a mixture of 1 volume of *water R* and 9 volumes of *sulphuric acid R.* Heat on a water-bath for 3 min. Cool in ice water and add 5 ml of *acetic anhydride R* . No violet-red colour develops (0.05 per cent).

Chlorides ***(2.4.4)***

Dissolve 1.0 g with heating in 8 ml of *acetic acid R* , cool and dilute to 10 ml with the same acid. Dilute 5 ml of the solution to 15 ml with *water R.* The solution complies with the limit test for chlorides (100 ppm).

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 80 °C at a pressure not exceeding 0.7 kPa for 4 h.

Sulphated ash ***(2.2.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

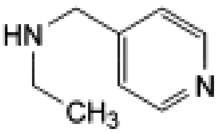
Dissolve 0.200 g in 50 ml of *anhydrous acetic acid R* . Add 0.2 ml of *naphtholbenzein solution R* and titrate with *0.1 M perchloric acid* until the colour changes from orange to green.

1 ml of *0.1 M perchloric acid* is equivalent to 28.44 mg of $C_{17}H_{20}N_2O_2$.

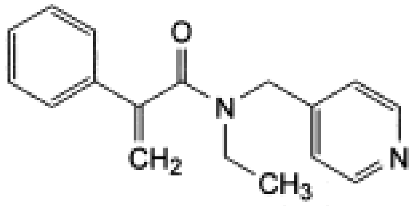
STORAGE

Store protected from light.

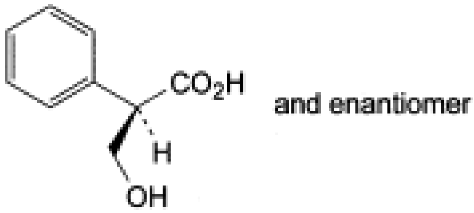
IMPURITIES



A. *N*-(pyridin-4-ylmethyl)ethanamine,



B. *N*-ethyl-2-phenyl-*N*-(pyridin-4-ylmethyl)propenamide,


CO₂H
H
OH
and enantiomer

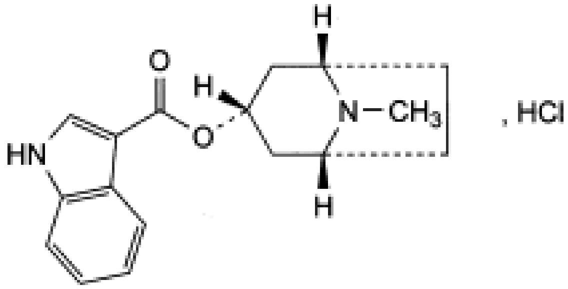
C. (2*RS*)-3-hydroxy-2-phenylpropanoic acid (tropic acid).

Ph Eur

Tropisetron Hydrochloride

General Notices

(Ph Eur monograph 2102)



$C_{17}H_{20}N_2O_2$, HCl 320.8 *105826-92-4*

Action and use

Serotonin $5HT_3$ receptor antagonist; antiemetic.

Ph Eur

DEFINITION

Hydrochloride of (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 1*H*-indole-3-carboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble or soluble in water, sparingly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

IDENTIFICATION

First identification *B, D*.

Second identification *A, C, D*.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution. Dissolve 50 mg in *methanol R* and dilute to 25.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *methanol R* .

Spectral range 220-360 nm.

Absorption maxima At 228 nm and 282 nm.

Absorbance ratio A_{228}/A_{282} = 1.3 to 1.4.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *tropisetron hydrochloride CRS*.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 5 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dissolve 5 mg of *tropisetron hydrochloride CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5 ml with the same mixture of solvents.

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *anhydrous formic acid R, water R, methanol R, methylene chloride R* (2:2:30:70 *V/V/V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In cold air.

Detection A Examine in ultraviolet light at 254 nm.

Detection B Spray first with a solution prepared as follows: dissolve 0.85 g of *bismuth subnitrate R* in a mixture of 10 ml of *acetic acid R* and 40 ml of *water R* ; to 5 ml of this solution add 5 ml of a 400 g/l solution of *potassium iodide R* and dilute to 100 ml with *water R* . Then spray with *strong hydrogen peroxide solution R* .

Results For both detection methods, the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

Dissolve 1.00 g in *water R* and dilute to 20 ml with the same solvent.

Impurity A

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.200 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5.0 ml with the same mixture of solvents.

Reference solution (a) Dissolve 5.0 mg of *tropine CRS* (impurity A) in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 25.0 ml with the same mixture of solvents.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with a mixture of equal volumes of *methanol R* and *methylene chloride R* . To 0.1 ml of this solution add 1.0 ml of reference solution (a).

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *ammonia R, methanol R, methylene chloride R* (5:40:60 *V/V/V*).

Application 10 µl.

Development Over 2/3 of the plate.

Drying In a current of cold air.

Detection Dip the plate in *potassium iodobismuthate solution R1* .

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 20.0 ml with mobile phase A.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b) Dissolve 5.0 mg of *tropisetron impurity B CRS* and 5 mg of *ethyl indole-3-carboxylate CRS* in mobile phase A and dilute to 20.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 50.0 ml with mobile phase A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

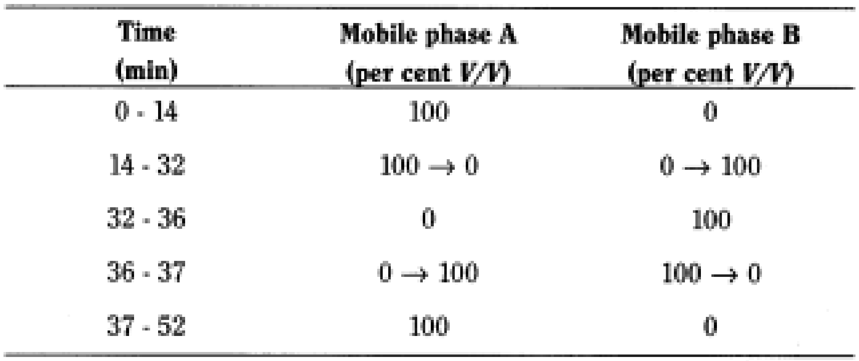
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *triethylamine R, acetonitrile R, water R, methanol R* (0.3:35:400:565 *V/*

V/V/V);

— *mobile phase B*: *triethylamine R, acetonitrile R, water R, methanol R* (0.3:100:100:800 *V/V/V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	100	0
14 - 32	100 → 0	0 → 100
32 - 36	0	100
36 - 37	0 → 100	100 → 0
37 - 52	100	0

Flow rate 2 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

Relative retention With reference to tropisetron (retention time = about 22 min): impurity B = about 0.05; ethyl indole-3-carboxylate = about 0.2.

System suitability Reference solution (b):

— *resolution*: minimum 4 between the peaks due to impurity B and ethyl indole-3-carboxylate.

Limits:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

***N,N*-Dimethylaniline**

Liquid chromatography *(2.2.29)* . *Prepare the solutions immediately before use*.

Test solution Dissolve 250 mg of the substance to be examined in mobile phase A and dilute to 5.0 ml with mobile phase A.

Reference solution Dissolve 10.0 mg of *N,N-dimethylaniline R* in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 100.0 ml with mobile phase A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: *triethylamine R, acetonitrile R, water R, methanol R* (0.3:35:400:565 *V/V/V/V*);
- *mobile phase B*: *triethylamine R, acetonitrile R, water R; methanol R* (0.3:100:100:800 *V/V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 11	100 → 0	0 → 100
11 - 30	0	100
30 - 31	0 → 100	100 → 0
31 - 50	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 248 nm.

Injection 20 µl.

Limit:

- *N,N-dimethylaniline* : not more than the area of the principal peak in the chromatogram obtained with the reference solution (20 ppm).

Loss on drying *(2.2.32)*

Maximum 0.3 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

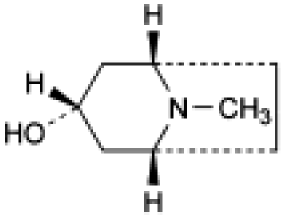
ASSAY

Dissolve 0.250 g in 10 ml of *anhydrous acetic acid R* and add 70 ml of *acetic anhydride R* . Titrate with *0.1 M perchloric acid* , determining the end-point potentiometrically *(2.2.20)* .

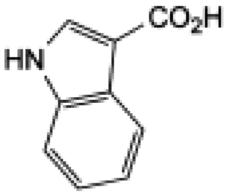
1 ml of *0.1 M perchloric acid* is equivalent to 32.08 mg of $C_{17}H_{21}ClN_2O_2$.

IMPURITIES

Specified impurities *A, B.*



A. (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]octan-3-ol (tropine),



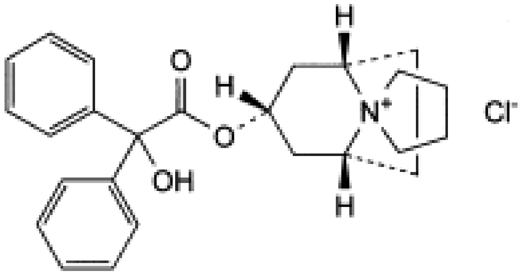
B. 1*H*-indole-3-carboxylic acid.

Ph Eur

Trospium Chloride

General Notices

(Ph Eur monograph 1798)



$C_{25}H_{30}ClNO_3$ 428.0 *10405-02-4*

Action and use

Anticholinergic.

Ph Eur

DEFINITION

(1*R*,3*r*,5*S*)-3-[(Hydroxydiphenylacetyl)oxy]spiro[8-azoniabicyclo[3.2.1]octane-8,1′-pyrrolidinium] chloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *trospium chloride CRS.*

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Solution S

Dissolve 3.0 g in *carbon dioxide-free water R* and dilute to 30 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 *(2.2.2, Method II)*.

pH *(2.2.3)*

5.0 to 7.0.

Dilute 5 ml of solution S to 50 ml with *carbon dioxide-free water R* .

Impurity C

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.20 g of the substance to be examined in 2.0 ml of *methanol R* .

Reference solution (a) Dissolve 1.0 mg of *trospium impurity C CRS* in 2.0 ml of *methanol R* .

Reference solution (b) Dilute 1.0 ml of test solution to 10.0 ml with *methanol R* . To 50 µl of this solution add 1 ml of reference solution (a).

Plate *TLC silica gel plate R* .

Mobile phase *glacial acetic acid R, hydrochloric acid R, acetonitrile R* (1:3.5:45 *V/V/V*).

Application 10 µl as bands.

Development Over 2/3 of the plate.

Drying In a current of warm air until the odour of acetic acid is no longer perceptible.

Detection Spray with *potassium iodobismuthate solution R* and subsequently with a 5 g/l solution of *sodium nitrite R* .

System suitability Reference solution (b):

— the chromatogram shows 2 clearly visible and separated zones.

Limit:

— *impurity C*: any zone due to impurity C is not more intense than the zone in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 6.0 mg of *trospium impurity A CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (b) Dissolve 7.5 mg of *trospium impurity B CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (c) Dilute a mixture of 0.3 ml of the test solution, 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 1 volume of *triethylamine R* and 3 volumes of *phosphoric acid R* with 700 volumes of *water R* and add 300 volumes of *acetonitrile R* .

Flow rate: 1 ml/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µl.

Run time 3 times the retention time of trospium.

Relative retention With reference to trospium (retention time = about 10 min): impurity B = about 0.7; impurity A = about 1.9.

System suitability Reference solution (c):

— *resolution*: minimum 3 between the peaks due to impurity B and trospium.

Limits:

— *impurity A*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *impurity B:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.2 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *total*: not more than twice the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit:* 0.1 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 ml of *water R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically *(2.2.20)*.

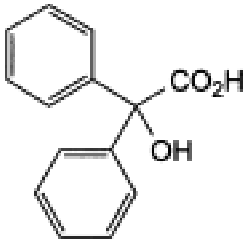
1 ml of *0.1 M silver nitrate* is equivalent to 42.80 mg of $C_{25}H_{30}ClNO_3$.

STORAGE

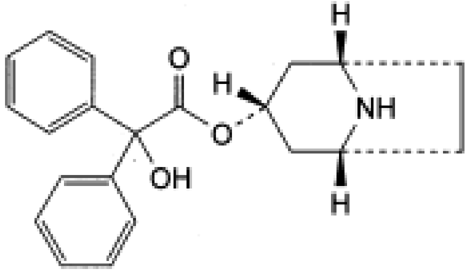
Protected from light.

IMPURITIES

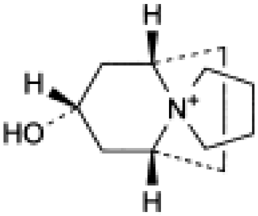
Specified impurities: A, B, C.



A. hydroxydiphenylacetic acid (benzilic acid),



B. (1*R*,3*r*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl hydroxydiphenylacetate,

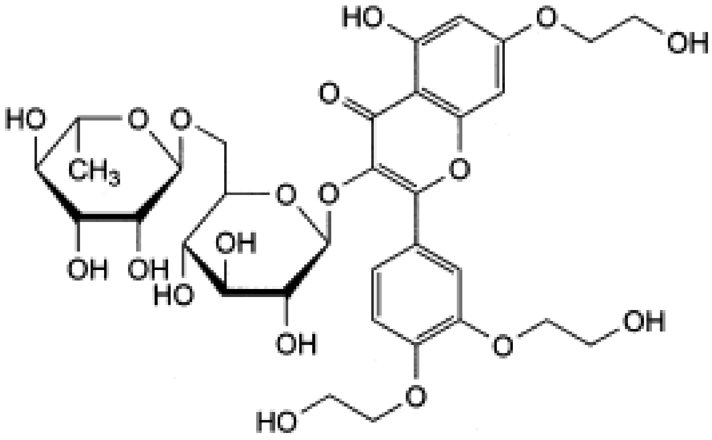


C. (1*R*,3*r*,5*S*)-3-hydroxyspiro[8-azoniabicyclo[3.2.1]octane8,1′-pyrrolidinium].

Troxerutin

General Notices

(Ph Eur monograph 2133)



$C_{33}H_{42}O_{19}$ 743 *7085-55-4*

Action and use

Bioflavonoid.

Ph Eur

DEFINITION

Mixture of *C*-hydroxyethylated derivatives of rutin containing minimum 80 per cent of 2-[3,4-bis(2-hydroxyethoxy)phenyl]-3-[[6-*C*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-7-(2-hydroxyethoxy)-4H-1-benzopyran-4-one (tris(hydroxyethyl)rutin).

Content

95.0 per cent to 105.0 per cent (dried substance).

CHARACTERS

Appearance

Yellowish-green, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent) and practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *troxerutin CRS.*

B. Examine the chromatograms obtained in the test for composition.

Results The principal peak in the chromatogram obtained with the test solution is similar in position and size to the principal peak in the chromatogram obtained with the reference solution (a).

TESTS

Composition

Liquid chromatography *(2.2.29)* : use the normalisation procedure.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase, if necessary using an ultrasonic bath and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *troxerutin CRS* in the mobile phase, if necessary using an ultrasonic bath and dilute to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1 ml of reference solution (a) to 10 ml with the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a 15.6 g/l solution of *sodium dihydrogen phosphate* R adjusted to pH 4.4 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R .*

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 350 nm.

Injection 10 µl.

Run time Twice the retention time of the main compound of troxerutin (tris(hydroxyethyl) rutin).

Relative retention With reference to tris(hydroxyethyl)rutin (retention time = about 25 min): tetrakis(hydroxyethyl)rutin = about 0.5; mono(hydroxyethyl)rutin = about 0.8; bis(hydroxyethyl) rutin = about 1.1.

System suitability Reference solution (a):

— *peak-to-valley ratio* : minimum 2.0, where H_p = height above the baseline of the peak due to bis(hydroxyethyl)rutin and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to tris(hydroxyethyl)rutin;

— *signal-to-noise ratio* : minimum 10 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *principal peak*: minimum 80 per cent;

— *any other peak*: for each peak, maximum 5 per cent, except for 1 peak which can be maximum 10 per cent;

— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (b).

Ethylene oxide

Head-space gas chromatography *(2.2.28)* .

Test solution   To 1.00 g of the substance to be examined in a vial, add 1.0 ml of *water R.* Mix to obtain a homogeneous solution. Heat at 70 °C for 45 min.

Reference solution   To 1.00 g of the substance to be examined in a vial, add 50 µl of *ethylene oxide solution R4* and 950 µl of *water R* and close tightly. Mix to obtain a homogeneous solution. Heat at 70 °C for 45 min.

Column:

— *material*: fused silica;

— *size*: *l* = 30 m, Ø = 0.32 mm;

— *stationary phase: poly(cyanopropyl)(7)(phenyl)(7)methyl(86)siloxane R* (film thickness 1 µm).

Carrier gas   *helium for chromatography R.*

Flow rate   1.1 ml/min.

Static head-space conditions which may be used:

— *equilibration temperature*: 80 °C;

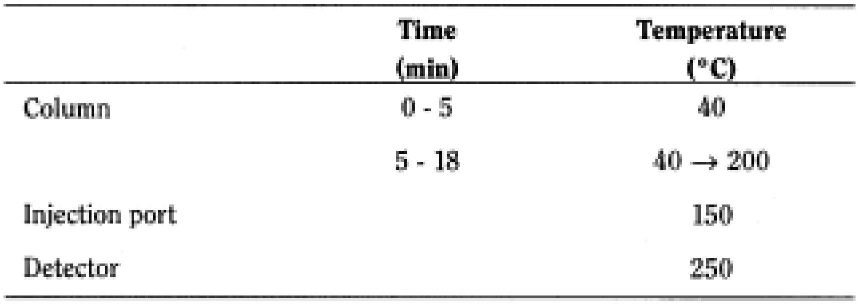
— *equilibration time*: 45 min;

— *transfer line temperature*: 110 °C;

— *pressurisation time*: 2 min;

— *injection time*: 12 s.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	40
	5 - 18	40 → 200
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 1.0 ml.

The peak due to ethylene oxide is identified by injecting solutions of ethylene oxide of increasing concentration.

Determine the content of ethylene oxide (ppm) in the substance to be examined using the following expression:

$$\frac{A_1 \times m_1}{(A_2 \times m_2) - (A_1 \times m_3)}$$

A_1 = area of the peak due to ethylene oxide in the chromatogram obtained with the test solution

A_2 = area of the peak due to ethylene oxide in the chromatogram obtained with the reference solution

m_1 = mass of ethylene oxide in the reference solution, in micrograms

m_2 = mass of the substance to be examined in the test solution, in grams

m_3 = mass of the substance to be examined in the reference solution, in grams

Limit:

— *ethylene oxide* : maximum 1 ppm.

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.0 g complies with test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulphated ash ***(2.4.14)***

Maximum 0.4 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 100.0 ml of *water R* . Dilute 10.0 ml of this solution to 100.0 ml with *water R* . Dilute 10.0 ml to 100.0 ml with *water R.* Measure the absorbance *(2.2.25)* at the absorption maximum at 350 nm.

Calculate the percentage content of $C_{33}H_{42}O_{19}$ taking the specific absorbance to be 250.

STORAGE

In an airtight container , protected from light.

Ph Eur

Trypsin

General Notices

(Ph Eur monograph 0694)

9002-07-7

Action and use

Proteolytic enzyme.

Ph Eur

DEFINITION

Trypsin is a proteolytic enzyme obtained by the activation of trypsinogen extracted from the pancreas of healthy mammals. It has an activity of not less than 0.5 microkatal per milligram, calculated with reference to the dried substance. In solution, it has maximum enzymic activity at pH 8; the activity is reversibly inhibited at pH 3, at which pH it is most stable.

PRODUCTION

The animals from which trypsin is derived must fulfil the requirements for the health of animals suitable for human consumption

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine *(2.6.10)*

Not more than 1 µg of histamine base per 0.2 microkatal of trypsin activity. Use a 10 g/l solution of the substance to be examined in *0.0015 M borate buffer solution pH 8.0 R* inactivated by heating on a water-bath for 30 min. Carry out dilutions with a 9 g/l solution of *sodium chloride R* .

CHARACTERS

A white or almost white, crystalline or amorphous powder, sparingly soluble in water. The amorphous form is hygroscopic.

IDENTIFICATION

A. Dilute 1 ml of solution S (see Tests) to 100 ml with *water R* . In a depression in a white spot-plate, mix 0.1 ml of this solution with 0.2 ml of *tosylarginine methyl ester hydrochloride solution R*. A reddish-violet colour develops within 3 min.

B. Dilute 0.5 ml of solution S to 5 ml with *water R*. Add 0.1 ml of a 20 g/l solution of *tosyl-lysyl-chloromethane hydrochloride R*. Adjust to pH 7.0, shake for 2 h and dilute to 50 ml with *water R*. In one of the depressions of a white spot-plate, mix 0.1 ml of this solution with 0.2 ml of *tosylarginine methyl ester hydrochloride solution R.* No reddish-violet colour develops within 3 min.

TESTS

Solution S

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension III *(2.2.1).*

pH *(2.2.3)*

The pH of solution S is 3.0 to 6.0.

Absorbance (*2.2.25)*

Dissolve 30.0 mg in *0.001 M hydrochloric acid* and dilute to 100.0 ml with the same acid. The solution shows an absorption maximum at 280 nm and a minimum at 250 nm. The specific absorbance at the absorption maximum is 13.5 to 16.5 and at the absorption minimum is not greater than 7.0.

Chymotrypsin

To 1.8 ml of *buffer solution pH 8.0 R* add 7.4 ml of *water R* and 0.5 ml of *0.2 M acetyltyrosine ethyl ester R*. While shaking the solution, add 0.3 ml of solution S and start a timer. After exactly 5 min, measure the pH *(2.2.3)* (test solution). Prepare a reference solution in the same manner, replacing solution S by 0.3 ml of a 0.5 g/l solution of *chymotrypsin BRP* and measure the pH *(2.2.3)* exactly 5 min after adding the chymotrypsin. The pH of the test solution is higher than that of the reference solution.

Loss on drying *(2.2.32)*

Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C at a pressure not exceeding 670 Pa for 2 h.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 micro-organisms per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13).*

ASSAY

The activity of trypsin is determined by comparing the rate at which it hydrolyses *benzoylarginine ethyl ester hydrochloride R* with the rate at which *trypsin BRP* hydrolyses the same substrate in the same conditions.

Apparatus Use a reaction vessel of about 30 ml capacity provided with:

— a device that will maintain a temperature of 25.0 ± 0.1 °C;

— a stirring device (for example, a magnetic stirrer);

— a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration device may be used. For the latter, the burette is graduated in 0.005 ml and the pH meter is provided with a wide-range scale and glass-calomel or glass-silver-silver chloride electrodes.

Test solution Dissolve sufficient of the substance to be examined in *0.001 M hydrochloric acid* and dilute to 25.0 ml with the same acid in order to obtain a solution containing approximately 700 nanokatals per millilitre.

Reference solution Dissolve 25.0 mg of *trypsin BRP* in *0.001 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Store the solutions at 0-5 °C. Warm 1 ml of each solution to about 25 °C over 15 min and use 50 µl of each solution for each titration. Carry out the titration in an atmosphere of nitrogen. Transfer 10.0 ml of *0.0015 M borate buffer solution pH 8.0 R* to the reaction vessel and, while stirring, add 1.0 ml of a freshly prepared 6.86 g/l solution of *benzoylarginine ethyl ester hydrochloride R*. When the temperature is steady at 25.0 ± 0.1 °C (after about 5 min) adjust the pH to exactly 8.0 with *0.1 M sodium hydroxide*. Add 50 µl of the test solution and start a timer. Maintain the pH at 8.0 by the addition of *0.1 M sodium hydroxide*, the tip of the microburette being immersed in the solution; note the volume added every 30 s. Follow the reaction for 8 min. Calculate the volume of *0.1 M sodium hydroxide* used per second. Carry out a titration in the same manner using the reference solution and calculate the volume of *0.1 M sodium hydroxide* used per second.

Calculate the activity in microkatals per milligram using the following expression:

$$\frac{m' \times V}{m \times V'} \times A$$

m = mass of the substance to be examined, in milligrams

m' = mass of *trypsin BRP*, in milligrams

V = volume of *0.1 M sodium hydroxide* used per second by the test solution

V' = volume of *0.1 M sodium hydroxide* used per second by the reference solution

A = activity of *trypsin BRP*, in microkatals per milligram

STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

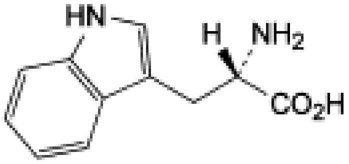
LABELLING

The label states the activity in microkatals per milligram.

Tryptophan

General Notices

(Ph Eur monograph 1272)



$C_{11}H_{12}N_2O_2$ 204.2 *73-22-3*

Ph Eur

DEFINITION

(*S*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline or amorphous powder.

Solubility

Sparingly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

First identification A, B.

Second identification A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *tryptophan CRS.*

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 20 mg in 10 ml of *water R* . Add 5 ml of *dimethylaminobenzaldehyde solution R6* and 2 ml of *hydrochloric acid R1* . Heat on a water-bath. A purple-blue colour develops.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II*).

Dissolve 0.1 g in *1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Specific optical rotation *(2.2.7)*

- 30.0 to - 33.0 (dried substance).

Dissolve 0.25 g in *water R* , heating on a water-bath if necessary, and dilute to 25.0 ml with the same solvent.

Ninhydrin-positive substances

Thin-layer chromatography *(2.2.27)* .

Solvent mixture *glacial acetic acid R, water R* (50:50 *V/V*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *tryptophan CRS* in the solvent mixture and dilute to 50 ml with the solvent mixture.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with the solvent mixture.

Reference solution (c) Dissolve 10 mg of *tryptophan CRS* and 10 mg of *tyrosine CRS* in the solvent mixture and dilute to 25 ml with the solvent mixture.

Plate *TLC silica gel plate R* .

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Limit Test solution (a):

— *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Impurity A and other related substances

Liquid chromatography *(2.2.29)*. *Prepare the standard, test and reference solutions immediately before use.*

Buffer solution pH 2.3 Dissolve 3.90 g of *sodium dihydrogen phosphate R* in 1000 ml of *water R*. Add about 700 ml of a 2.9 g/l solution of *phosphoric acid R* and adjust to pH 2.3 with the same acid solution.

Solvent mixture *acetonitrile R*, *water R* (10:90 *V/V*).

Standard solution Dissolve 10.0 mg of *N-acetyltryptophan R* in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 100.0 ml with the solvent mixture.

Test solution (a) Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Test solution (b) Dissolve 0.10 g of the substance to be examined in the standard solution and dilute to 10.0 ml with the standard solution.

Reference solution (a) Dissolve 1.0 mg of *1,1′-ethylidenebistryptophan CRS* (impurity A) in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 10.0 ml of reference solution (a) to 50.0 ml with the standard solution.

Reference solution (c) Dilute 10.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Reference solution (d) Dissolve 0.10 g of the substance to be examined in reference solution (c) and dilute to 10.0 ml with reference solution (c).

Reference solution (e) Dilute 1.0 ml of reference solution (c) to 10.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

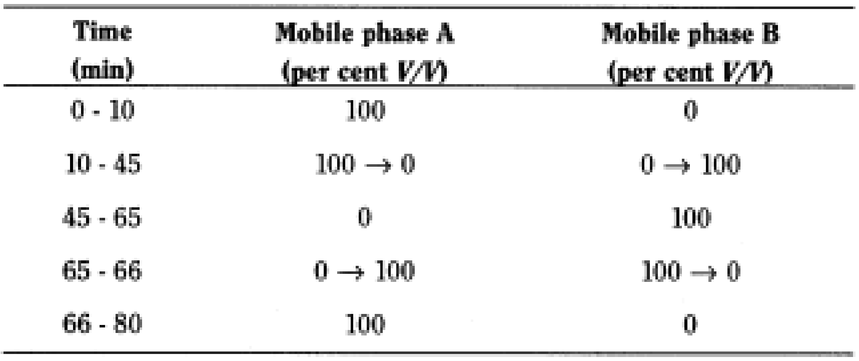
— *stationary phase:* octadecylsilyl silica gel for chromatography R (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R,* buffer solution pH 2.3 (115:885 *V/V*);

— *mobile phase B*: *acetonitrile R*, buffer solution pH 2.3 (350:650 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 45	100 → 0	0 → 100
45 - 65	0	100
65 - 66	0 → 100	100 → 0
66 - 80	100	0

Flow rate 0.7 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solutions (a) and (b) and reference solutions (b), (d) and (e).

Retention time Tryptophan = about 8 min; *N*-acetyltryptophan = about 29 min; impurity A = about 34 min.

System suitability:

— *resolution*: minimum 8.0 between the peaks due to *N*-acetyltryptophan and impurity A in the chromatogram obtained with reference solution (b); if necessary, adjust the time programme for the elution gradient (an increase in the duration of elution with mobile phase A produces longer retention times and a better resolution);

— *signal-to-noise ratio* : minimum 15 for the principal peak in the chromatogram obtained with reference solution (e);

— in the chromatogram obtained with test solution (a) there is no peak with the same retention time as *N*-acetyltryptophan (in such case correct the area of the *N*-acetyltryptophan peak).

Limits Test solution (b):

— *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (10 ppm);

— sum of impurities with a retention time less than that of tryptophan: not more than 0.6 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b) (100 ppm);

— sum of impurities with a retention time greater than that of tryptophan and up to 1.8 times the retention time of *N*-acetyltryptophan: not more than 1.9 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b) (300 ppm);

— *disregard limit*: 0.02 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b); disregard the peak due to *N*-acetyltryptophan.

Chlorides ***(2.4.4)***

Maximum 200 ppm.

Dissolve 0.25 g in 3 ml of *dilute nitric acid R* and dilute to 15 ml with *water R* . The solution, without any further addition of nitric acid, complies with the test.

Sulphates ***(2.4.13)***

maximum 300 ppm.

Dissolve 0.5 g in a mixture of 5 volumes of *dilute hydrochloric acid R* and 25 volumes of *distilled water R,* and dilute to 15 ml with the same mixture of solvents.

Ammonium *(2.4.1, Method B)*

Maximum 200 ppm, determined on 0.10 g.

Prepare the standard using 0.2 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron *(2.4.9)*

Maximum 20 ppm.

In a separating funnel, dissolve 0.50 g in 10 ml of *dilute hydrochloric acid R.* Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. Examine the aqueous layer.

Heavy metals (*2.4.8)*

Maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

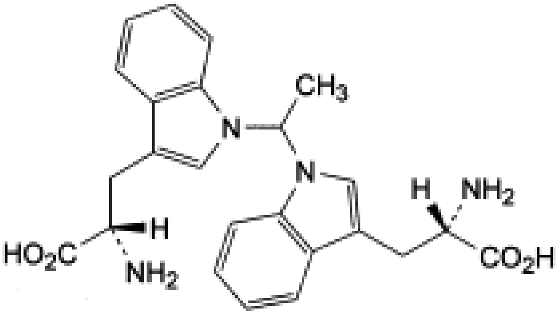
Dissolve 0.150 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid*, using 0.1 ml of *naphtholbenzein solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 20.42 mg of $C1_1H_{12}N_2O_2$.

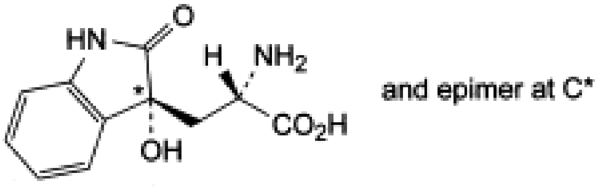
STORAGE

Protected from light.

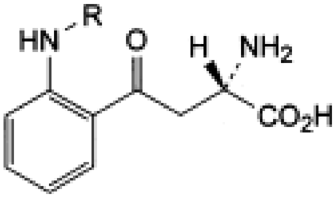
IMPURITIES



A. 3,3′-[ethylidenebis(1*H*-indole-1,3-diyl)]bis[(2*S*)-2-aminopropanoic] acid (1,1′-ethylidenebistryptophan),

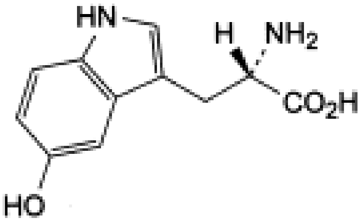


B. (*S*)-2-amino-3-[(3*RS*)-3-hydroxy-2-oxo-2,3-dihydro-1*H*-indol-3-yl]propanoic acid (dioxyindolylalanine),

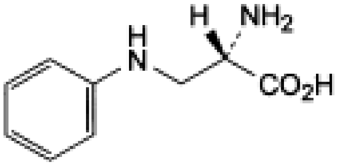


C. R = H: (*S*)-2-amino-4-(2-aminophenyl)-4-oxobutanoic acid (kynurenine),

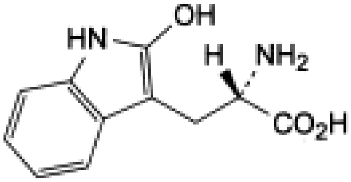
E. R = CHO: (*S*)-2-amino-4-[2-(formylamino)phenyl]-4-oxobutanoic acid (*N*-formylkynurenine),



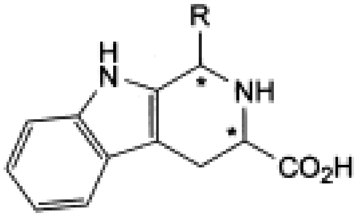
D. (*S*)-2-amino-3-(5-hydroxy-1*H*-indol-3-yl)propanoic acid (5-hydroxytryptophan),



F. (*S*)-2-amino-3-(phenylamino)propanoic acid (3-phenylaminoalanine),

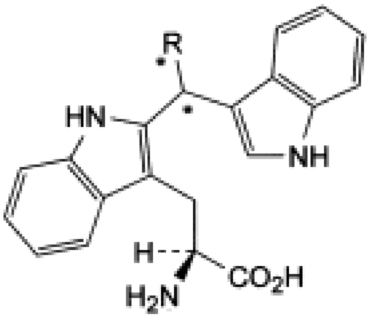


G. (*S*)-2-amino-3-(2-hydroxy-1*H*-indol-3-yl)propanoic acid (2-hydroxytryptophan),



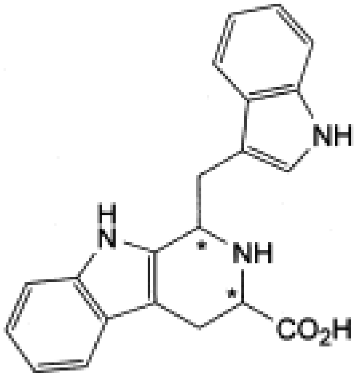
H. R = H: (3*RS*)-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylic acid,

I. R = CH_3: 1-methyl-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylic acid,



J. R = CHOH-CH_2-OH: (*S*)-2-amino-3-[2-[2,3-dihydroxy-1-(1*H*-indol-3-yl)propyl]-1*H*-indol-3-yl]propanoic acid,

K. R = H: (*S*)-2-amino-3-[2-(1*H*-indol-3-ylmethyl)-1*H*-indol-3-yl]propanoic acid,


NH
H
N
NH
CO_2H

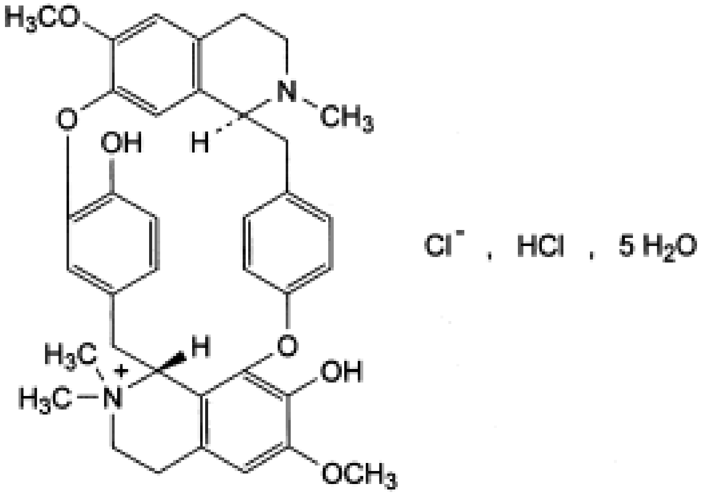
L. 1-(1*H*-indol-3-ylmethyl)-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylic acid.

Ph Eur

Tubocurarine Chloride

General Notices

(Ph Eur monograph 0305)



$C_{37}H_{41}ClN_2O_6,HCl,5H_2O$ 772 *6989-98-6*

Action and use

Non-depolarizing neuromuscular blocker.

Ph Eur

DEFINITION

Tubocurarine chloride contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (3a*R*,16a*S*)-8,23-dihydroxy-24,29-dimethoxy-3,3,16-trimethyl-2,3,3a,4,15,16,16a,17-octahydro-1*H*-11,13:18,21-dietheno-5,9-metheno-14*H*-pyrido[3′,2′:14,15][1,11]dioxacycloicosino[2,3,4-*ij*]isoquinolinium chloride hydrochloride, calculated with reference to the anhydrous substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, soluble in water and in alcohol, practically insoluble in acetone. It dissolves in solutions of alkali hydroxides.

It melts at about 270 °C, with decomposition.

IDENTIFICATION

First identification *B, E.*

Second identification *A, C, D, E, F.*

A. Dissolve 50.0 mg in *water R* and dilute to 1000.0 ml with the same solvent. Examined between 230 nm and 350 nm *(2.2.25)* , the solution shows an absorption maximum at 280 nm and a minimum at 255 nm. The specific absorbance at the maximum is 113 to 123, calculated with reference to the anhydrous substance.

B. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *tubocurarine chloride CRS* .

C. Dissolve about 25 mg in 1 ml of *water R*. Add 0.2 ml of *ferric chloride solution* R2 and heat in a water-bath for 1 min. The solution is green. Carry out a blank test; after heating in a water-bath, the colour is brown.

D. To 1 ml of solution S (see Tests) add 1 ml of nitric acid *solution of mercury R* . A red colour develops slowly.

E. It gives reaction (a) of chlorides *(2.3.1)* .

F. It gives the reaction of alkaloids *(2.3.1)* .

TESTS

Solution S

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

pH *(2.2.3)*

The pH of solution S is 4.0 to 6.0.

Specific optical rotation *(2.2.7)*

+ 210 to + 222, determined on solution S 3 h after preparation and calculated with reference to the anhydrous substance.

Chloroform-soluble substances

Dissolve 0.25 g in 150 ml of *water R* . Add 5 ml of a saturated solution of *sodium bicarbonate R* and shake with three quantities, each of 20 ml, of *chloroform R* . Wash the combined chloroform layers with 10 ml of *water R* , filter the chloroform solution into a tared beaker and wash the filter with two quantities, each of 5 ml, of *chloroform R* . Add the washings to the filtrate. Evaporate to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs not more than 5 mg. The residue does not dissolve in 10 ml of *water R* , but dissolves on the addition of 1 ml of *dilute hydrochloric acid R.*

Related substances

Examine by thin-layer chromatography *(2.2.27)* , using *silica gel G R* as the coating substance.

Test solution Dissolve 0.25 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dilute 1.5 ml of the test solution to 100 ml with *water R.*

Reference solution (b) Dilute 5 ml of reference solution (a) to 10 ml with *water R* .

Apply to the plate 5 µl of each solution. Develop in a non-saturated tank over a path of 15 cm using the lower layer of a mixture of equal volumes of *chloroform R* , *methanol R* and a 125 g/l solution of *trichloroacetic acid R* . Dry the plate in a current of cold air. Spray with a mixture of 1 volume of *potassium ferricyanide solution R* , 1 volume of *water R* and 2 volumes of *ferric chloride solution R1* , prepared immediately before use. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.75 per cent).

Water *(2.5.12)*

9.0 per cent to 12.0 per cent, determined on 0.30 g by the semi-micro determination of water.

Sulphated ash *(2.4.14)*

Not more than 0.25 per cent, determined on 0.2 g.

ASSAY

Dissolve 25.0 mg in *water R* and dilute to 500.0 ml with the same solvent. In the same manner, prepare a reference solution using 25.0 mg of *tubocurarine chloride CRS* . Measure the absorbance *(2.2.25)* at the maximum at 280 nm.

Calculate the content of $C_{37}H_{42}Cl_2N_2O_6$ from the absorbances measured, the concentrations of the solutions and the declared content of anhydrous tubocurarine chloride in *tubocurarine chloride CRS* .

STORAGE

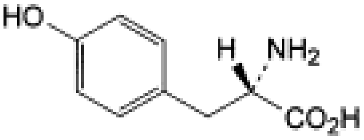
Store in an airtight container .

Ph Eur

Tyrosine

General Notices

(Ph Eur monograph 1161)



$C_9H_{11}NO_3$ 181.2 *60-18-4*

Action and use

Amino acid.

Ph Eur

DEFINITION

Tyrosine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2-amino-3-(4-hydroxyphenyl)propanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white crystalline powder or colourless crystals, very slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification A, B.

Second identification A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *tyrosine CRS* . Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 50 mg add 1 ml of *dilute nitric acid R*. A dark red colour is produced within 15 min.

E. Dissolve about 30 mg in 2 ml of *dilute sodium hydroxide solution R.* Add 3 ml of a freshly prepared mixture of equal volumes of a 100 g/l solution of *sodium nitrite R* and a solution of 0.5 g of *sulphanilic acid R* in a mixture of 6 ml of *hydrochloric acid R1* and 94 ml of *water R*. An orange-red colour is produced.

TESTS

Appearance of solution

Dissolve 0.5 g in *dilute hydrochloric acid R* and dilute to 20 ml with the *same acid.* The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II).*

Specific optical rotation *(2.2.7)*

Dissolve 1.25 g in a mixture of equal volumes of *dilute hydrochloric acid R* and *water R* and dilute to 25.0 ml with the same mixture of solvents. The specific optical rotation is - 11.0 to - 12.3, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel plate R*.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dilute ammonia R2* and dilute to 10 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a) Dissolve 10 mg of *tyrosine CRS* in 1 ml of *dilute ammonia R2* and dilute to 50 ml with *water R*.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R.*

Reference solution (c) Dissolve 10 mg of *tyrosine CRS* and 10 mg of *phenylalanine CRS* in 1 ml of *dilute ammonia R2* and dilute to 25 ml with *water R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of *concentrated ammonia R1* and 70 volumes of *propanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

Dissolve 0.25 g in 3 ml of *dilute nitric acid R* and dilute to 15 ml with *water R.* The solution complies with the limit test for chlorides, without any further addition of nitric acid (200 ppm).

Sulphates *(2.4.13)*

Dissolve with gentle heating, 0.5 g in 5 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium ***(2.4.1)***

0.10 g complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.2 ml of *ammonium standard solution (100 ppm NH_4) R*. Replace the *heavy magnesium oxide R* by 2.0 ml of *strong sodium hydroxide solution R.*

Iron ***(2.4.9)***

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals ***(2.4.8)***

2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 5 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 18.12 mg of $C_9H_{11}NO_3$.

STORAGE

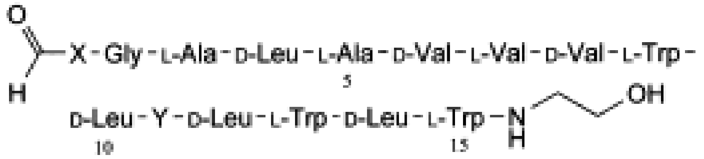
Store protected from light.

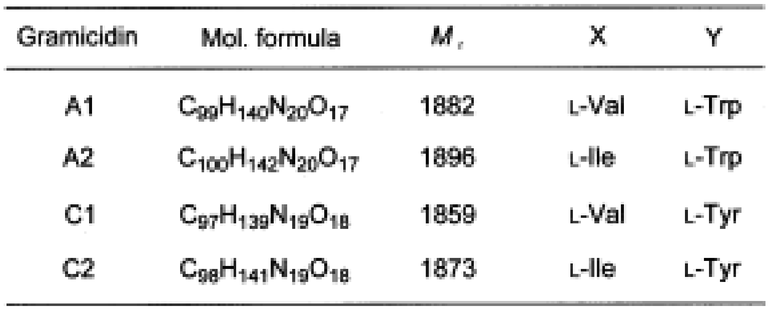
Ph Eur

Tyrothricin

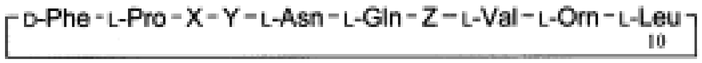
General Notices

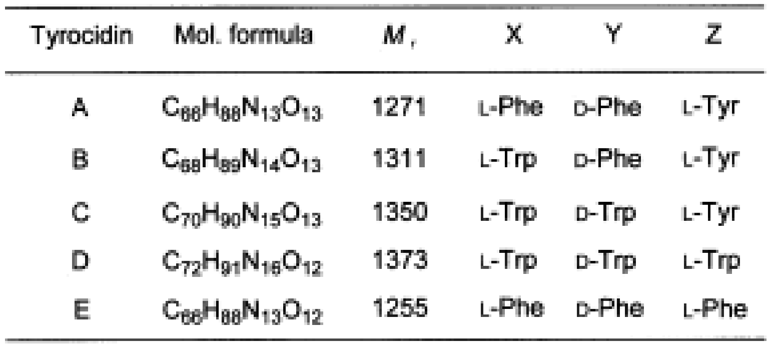
(Ph Eur monograph 1662)





Gramicidin	Mol. formula	M_r	X	Y
A1	$C_{99}H_{140}N_{20}O_{17}$	1882	L-Val	L-Trp
A2	$C_{100}H_{142}N_{20}O_{17}$	1896	L-Ile	L-Trp
C1	$C_{97}H_{139}N_{19}O_{18}$	1859	L-Val	L-Tyr
C2	$C_{98}H_{141}N_{19}O_{18}$	1873	L-Ile	L-Tyr





Tyrocidin	Mol. formula	M_r	X	Y	Z
A	$C_{66}H_{88}N_{13}O_{13}$	1271	L-Phe	D-Phe	L-Tyr
B	$C_{68}H_{89}N_{14}O_{13}$	1311	L-Trp	D-Phe	L-Tyr
C	$C_{70}H_{90}N_{15}O_{13}$	1350	L-Trp	D-Trp	L-Tyr
D	$C_{72}H_{91}N_{16}O_{12}$	1373	L-Trp	D-Trp	L-Trp
E	$C_{66}H_{88}N_{13}O_{12}$	1255	L-Phe	D-Phe	L-Phe

1404-88-2

Action and use

Polypeptide antibacterial.

Ph Eur

DEFINITION

Mixture of antimicrobial linear and cyclic polypeptides, isolated from the fermentation broth of *Brevibacillus brevis* Dubos. It consists mainly of gramicidins and tyrocidins as described above; other related compounds may be present in smaller amounts.

Potency 180 IU/mg to 280 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 5 mg of the substance to be examined in 4.0 ml of *ethanol (96 per cent) R.*

Reference solution Dissolve 5 mg of *tyrothricin CRS* in 4.0 ml of *ethanol (96 per cent) R* .

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *methanol R, butanol R, water R, acetic acid R, butyl acetate R* (2.5:7.5:12:20:40 *V/V/V/V/V*).

Application 1 µl.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spots or groups of principal spots in the chromatogram obtained with the test solution are similar in position and size to the principal spots or groups of principal spots in the chromatogram obtained with the reference solution. The upper group corresponds to gramicidins, the lower group to tyrocidins.

Detection B Spray with *dimethylaminobenzaldehyde solution R2* . Heat the plate in a current of warm air until the spots appear.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated spots or groups of spots.

Results B The principal spots or groups of principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the principal spots or groups of principal spots in the chromatogram obtained with the reference solution. The upper group corresponds to gramicidins, the lower group to tyrocidins.

B. It complies with the test for composition (see Tests).

TESTS

Composition

Liquid chromatography (*2.2.29*)

Use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution Dissolve 5 mg of the substance to be examined in 2 ml of *methanol R* and dilute to 5.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *tyrothricin CRS* in 2 ml of *methanol R* and dilute to 5.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 60 °C.

Mobile phase 0.79 g/l solution of *ammonium sulphate R, methanol R* (25:75 *V/V*).

Flow rate 1.2 ml/min.

Detection spectrophotometer at 280 nm.

Injection 25 µl.

Run time 6 times the retention time of gramicidin A1. Use the chromatogram obtained with reference solution (a) and the chromatogram supplied with *tyrothricin CRS* to identify the peaks due to gramicidin A1, gramicidin A2 and the tyrocidins.

Relative retention With reference to gramicidin A1 (retention time = about 10 min): gramicidin C1 = about 0.8; gramicidin C2 = about 0.9; gramicidin A2 = about 1.1; tyrocidins = about 1.5 to 6.

System suitability Reference solution (a):

— *peak-to-valley ratio* : minimum 3.0, where H_p = height above the baseline of the peak due to gramicidin A2 and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to gramicidin A1.

Composition:

— *sum of gramicidins*: 25 per cent to 50 per cent;

— *sum of tyrocidins*: 50 per cent to 70 per cent;

— *total*: minimum 85 per cent;

— *disregard limit*: the sum of the areas of the peaks due to gramicidins in the chromatogram obtained with reference solution (b).

Loss on drying *(2.2.32)*

Maximum 4.0 per cent, determined on 1.000 g by drying under high vacuum at 60°C for 3 h.

Sulphated ash ***(2.4.14)***

Maximum 1.5 per cent, determined on 1.0 g.

ASSAY

Carry out the microbiological assay of antibiotics *(2.7.2)* using the turbidimetric method. Use *gramicidin CRS* as the reference substance.

Test solution Prepare a solution of tyrothricin containing about the same amount of gramicidin as the corresponding solution of *gramicidin CRS* i.e. 5 times more concentrated.

STORAGE

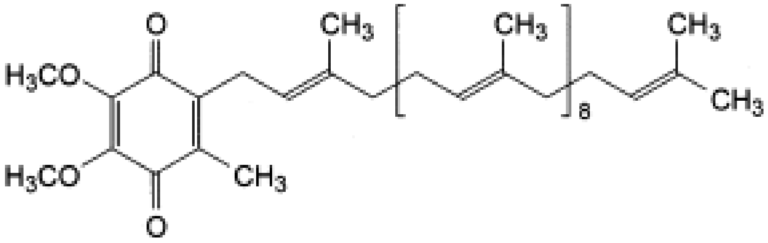
In an airtight container, protected from light.

Ph Eur

Ubidecarenone

General Notices

(Ph Eur monograph 1578)



$C_{59}H_{90}O_4$ 863 *303-98-0*

Action and use

Co-enzyme in mitochondrial electron transport.

Ph Eur

DEFINITION

2-[(all-*E*)-3,7,11,15,19,23,27,31,35,39-Decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaenyl]-5,6-dimethoxy-3-methylbenzene-1,4-dione.

Content

97.0 per cent to 103.0 per cent.

CHARACTERS

Appearance

Yellow or orange, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone, very slightly soluble in ethanol.

It gradually decomposes and darkens on exposure to light.

mp

About 48 °C.

Carry out all operations avoiding exposure to light.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *ubidecarenone CRS.*

B. Examine the chromatograms obtained in the test for related substances.

Results The retention time of the principal peak in the chromatogram obtained with the test solution is similar to that of the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in 25.0 ml of *ethanol R* by heating at about 50 °C for 2 min. Allow to cool.

Reference solution (a) Dissolve 5 mg of *ubidecarenone CRS* in 5 ml of *ethanol R* by heating at about 50 °C for 2 min. Allow to cool.

Reference solution (b) Dissolve 2 mg of *ubidecarenone impurity D CRS* in 2 ml of the test solution by heating at about 50 °C for 2 min. Allow to cool. Dilute 1 ml to 50 ml with *ethanol R* .

Reference solution (c) Dilute 1.0 ml of the test solution to 100.0 ml with *ethanol R* .

Column:

— *size*: l = 0.15 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *ethanol R, methanol R2* (20:80 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µl.

Run time 2 times the retention time of ubidecarenone.

Relative retention With reference to ubidecarenone (retention time = about 12 min): impurity D = about 0.67.

System suitability Reference solution (b):

— *resolution*: minimum 6.5 between the peaks due to impurity D and to ubidecarenone.

Limits:

— *any impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Impurity F

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 25.0 mg of the substance to be examined in 25.0 ml of *hexane R* .

Reference solution (a) Dissolve the contents of a vial of *ubidecarenone for system suitability CRS* in 1 ml of *hexane R.*

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with *hexane R* .

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase: silica gel for chromatography R* (7 µm).

Mobile phase *ethyl acetate R, hexane R* (3:97 *V/V*).

Flow rate 2 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µl.

Run time 1.2 times the retention time of ubidecarenone.

Relative retention With reference to ubidecarenone (retention time = about 10 min): impurity F = about 0.85.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurity F and to ubidecarenone.

Limit:
— *impurity F*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

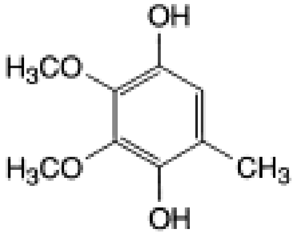
Dissolve 50.0 mg in 1.0 ml of *hexane R* and dilute to 50.0 ml with *ethanol R* . Dilute 2.0 ml of the solution to 50.0 ml with *ethanol R.* Measure the absorbance *(2.2.25)* at the maximum at 275 nm. Calculate the content of $C_{59}H_{90}O_4$ taking the specific absorbance to be 169.

STORAGE

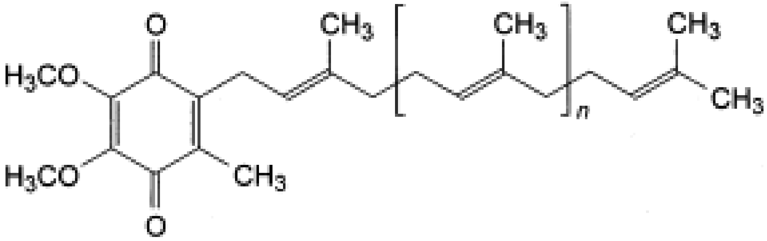
Store in an airtight container , protected from light.

IMPURITIES

Specified impurities *A, B, C, D, E, F.*


OH
H3CO
H3CO
CH3
OH

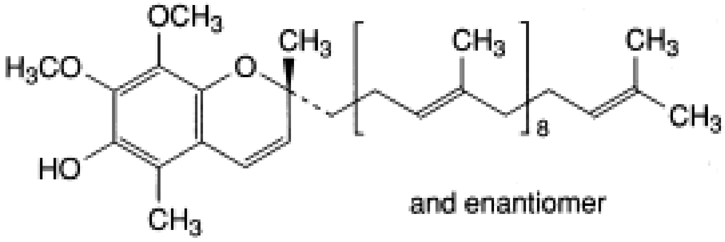
A. 2,3-dimethoxy-5-methylbenzene-1,4-diol,


O
CH3
CH3
CH3
H3CO
n
CH3
H3CO
CH3
O

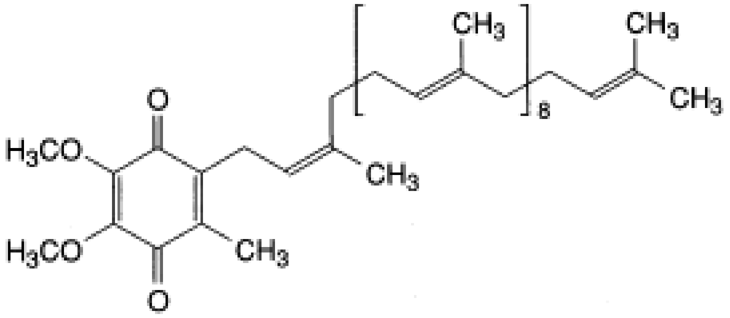
B. *n* = 5: 2-[(all-*E*)-3,7,11,15,19,23,27-heptamethyloctadocosa-2,6,10,14,18,22,26-heptaenyl]-5,6-dimethoxy-3-methylbenzene-1,4-dione (ubiquinone-7),

C. *n* = 6: 5,6-dimethoxy-3-methyl-2-[(all-*E*)-3,7,11,15,19,23,27,31-octamethyldotriaconta-2,6,10,14,18,22,26,30-octaenyl]benzene-1,4-dione (ubiquinone-8),

D. *n* = 7: 5,6-dimethoxy-3-methyl-2-[(all-*E*)-3,7,11,15,19,23,27,31,35-nonamethylhexatriaconta-2,6,10,14,18,22,26,30,34-nonaenyl]benzene-1,4-dione (ubiquinone-9),


OCH3
H3CO
O
CH3
CH3
CH3
8
CH3
HO
CH3
and enantiomer

E. (2*RS*)-7,8-dimethoxy-2,5-dimethyl-2-[(all-*E*)-4,8,12,16,20,24,28,32,36-nonamethylheptatriaconta-3,7,11,15,19,23,27,31,35-nonaenyl]-2*H*-1-benzopyran-6-ol (ubicromenol),



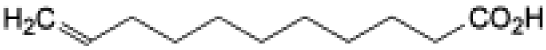
F. 2-[(2*Z*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*,34*E*,38*E*)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl]-5,6-dimethoxy-3-methylbenzene-1,4-dione (ubidecarenone (*Z*)-isomer).

Ph Eur

Undecenoic Acid

General Notices

(Undecylenic Acid, Ph Eur monograph 0461)



$C_{11}H_{20}O_2$ 184.3 *112-38-9*

Ph Eur

DEFINITION

Undec-10-enoic acid.

Content

97.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or very pale yellow, crystalline mass or colourless or pale yellow liquid.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent) and in fatty and essential oils.

IDENTIFICATION

A. Refractive index *(2.2.6)*

1.447 to 1.450, determined at 25 ± 0.5 °C.

B. Freezing point *(2.2.18)* : 21 °C to 24 °C.

C. To 2.0 g add 2 ml of freshly distilled *aniline R* and boil under a reflux condenser for 10 min. Allow to cool and add 30 ml of *ether R*. Shake with 3 quantities, each of 20 ml, of *dilute hydrochloric acid R* and then with 20 ml of *water R.* Evaporate the organic layer to dryness on a water-bath. After recrystallising twice from *ethanol (70 per cent V/V) R* and drying *in vacuo* for 3 h, the residue melts *(2.2.14)* at 66 °C to 68 °C.

D. Dissolve 0.1 g in a mixture of 2 ml of *dilute sulphuric acid R* and 5 ml of *glacial acetic acid R*. Add dropwise 0.25 ml of *potassium permanganate solution R*. The colour of the potassium permanganate is discharged.

TESTS

Peroxide value *(2.5.5, Method A)*

Maximum 10.

Fixed and mineral oils

To 1.0 g add 5 ml of *sodium carbonate solution R* and 25 ml of *water R* and boil for 3 min. The hot solution is not more opalescent than reference suspension II *(2.2.1)*.

Water-soluble acids

To 1.0 g add 20 ml of *water R* heated to 35-45 °C and shake for 2 min. Cool and filter the aqueous layer through a moistened filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution R.* Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Degree of unsaturation

Dissolve 85.0 mg in a mixture of 5 ml of *dilute hydrochloric acid R* and 30 ml of *glacial acetic acid R*. Using 0.05 ml of *indigo carmine solution R1* as indicator, added towards the end of the titration, titrate with *0.0167 M bromide-bromate* until the colour changes from blue to yellow. 8.9 ml to 9.4 ml of *0.0167 M bromide-bromate* is required. Carry out a blank titration.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 0.50 g.

ASSAY

Dissolve 0.750 g in 10 ml of *ethanol (96 per cent) R.* Titrate with *0.5 M sodium hydroxide* using 0.1 ml of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

1 ml of *0.5 M sodium hydroxide* is equivalent to 92.14 mg of $C_{11}H_{20}O_2$.

STORAGE

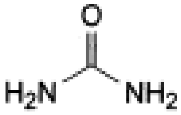
In a non-metallic container, protected from light.

Ph Eur

Urea

General Notices

(Ph Eur monograph 0743)



CH_4N_2O 60.1 *57-13-6*

Action and use

Keratolytic.

Preparation

Urea Cream

Ph Eur

DEFINITION

Carbamide.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or transparent crystals, slightly hygroscopic.

Solubility

Very soluble in water, soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, B.

Second identification *A, C, D.*

A. Melting point *(2.2.14)* : 132 °C to 135 °C.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Preparation Discs.

Comparison *urea CRS.*

C. Dissolve 0.1 g in 1 ml of *water R.* Add 1 ml of *nitric acid R* . A white, crystalline precipitate is formed.

D. Heat 0.5 g in a test tube until it liquefies and the liquid becomes turbid. Cool, dissolve in a mixture of 1 ml of *dilute sodium hydroxide solution R* and 10 ml of *water R* and add 0.05 ml of *copper sulphate solution R* . A reddish-violet colour is produced.

TESTS

Solution S

Dissolve 10.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

To 2.5 ml of solution S add 7.5 ml of *water R* .

Alkalinity

To 2.5 ml of solution S add 7.5 ml of *water R,* 0.1 ml of *methyl red solution R* and 0.4 ml of *0.01 M hydrochloric acid* . The solution is red to orange.

Biuret

Maximum 0.1 per cent.

To 10 ml of solution S add 5 ml of *water R* , 0.5 ml of a 5 g/l solution of *copper sulphate R* and 0.5 ml of *strong sodium hydroxide solution R* . Allow to stand for 5 min. Any reddish-violet colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 10 ml of a 0.2 g/l solution of *biuret R*.

Ammonium ***(2.4.1)***

Maximum 500 ppm, determined on 0.1 ml of solution S.

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

Dilute 10 ml of solution S to 20 ml with *water R* . 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R* .

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.2000 g in *water R* and dilute to 50.0 ml with the same solvent. Introduce 1.0 ml of this solution into a combustion flask. Add 4 g of a powdered mixture of 100 g of *dipotassium sulphate R*, 5 g of *copper sulphate R* and 2.5 g of *selenium R,* and 3 glass beads. Wash any adhering particles from the neck into the flask with 5 ml of *sulphuric acid R*, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulphuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulphuric acid in the neck of the flask; take precautions to prevent the upper part of the flask from becoming overheated. Continue the heating for 30 min. Cool, dissolve the solid material by cautiously adding to the mixture 25 ml of *water R*, cool again and place in a steam-distillation apparatus. Add 30 ml of strong *sodium hydroxide solution R* and distil immediately by passing steam through the mixture. Collect the distillate in 15 ml of a 40 g/l *solution of boric acid R* to which has been added 0.2 ml of *methyl red mixed solution R* and enough *water R* to cover the tip of the condenser. Towards the end of the distillation, lower the receiver so that the tip of the condenser is above the surface of the acid. Take precautions to prevent any water on the outer surface of the condenser from reaching the contents of the receiver. Titrate the distillate with *0.01 M sulphuric acid*.

1 ml of *0.01 M sulphuric acid* is equivalent to 0.6006 mg of CH_4N_2O.

STORAGE

In an airtight container.

Ph Eur

Urofollitropin

General Notices

(Ph Eur monograph 0958)

97048-13-0

Action and use

Follicle-stimulating hormone.

Preparation

Urofollitropin Injection

Ph Eur

DEFINITION

Urofollitropin is a dry preparation containing menopausal gonadotrophin obtained from the urine of post-menopausal women. It has follicle-stimulating activity and no or virtually no luteinising activity. The potency is not less than 90 International Units of follicle-stimulating hormone (hFSH) per milligram. The ratio of units of luteinising hormone (interstitial-cell-stimulating hormone) [hLH(ICSH)] to units of follicle-stimulating hormone is not more than 1/60.

PRODUCTION

It may be prepared by a suitable fractionation procedure followed by immunoaffinity chromatography.

CHARACTERS

Appearance

Almost white or slightly yellowish powder.

Solubility

Soluble in water.

IDENTIFICATION

When administered as prescribed in the assay it causes enlargement of the ovaries of immature female rats.

TESTS

Hepatitis virus antigens

Examined by a suitably sensitive immunochemical method *(2.7.1)* , hepatitis virus antigens are not detected.

HIV antigen

Examined by a suitably sensitive immunochemical method *(2.7.1)* , HIV antigen is not detected.

Residual luteinising activity

The International Units of FSH and LH are the activities contained in stated amounts of the International Standard of human urinary follicle-stimulating hormone and luteinising hormone (interstitial-cell-stimulating hormone) which consists of a mixture of a freeze-dried extract of urine of post-menopausal women with lactose. The equivalence in International Units of the International Standard is stated by the World Health Organisation. Use immature female rats approximately 21 days old and having masses such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to 4 equal groups of at least 6 animals. If sets of 4 litter mates are available, assign one litter mate at random from each set to each group and mark according to litter.

Inject subcutaneously into each rat 50 IU of *serum gonadotrophin R* on the first day and 25 IU of *chorionic gonadotrophin R* on the fourth day, each in 0.5 ml of *phosphate-albumin buffered saline pH 7.2 R.*

Choose 3 doses of the reference preparation such that the smallest dose produces a depletion of the ovarian ascorbic acid content in all the rats and the largest dose does not produce a maximal depletion in all the rats. Use doses in geometric progression; as an initial approximation, total doses of 0.5 IU, 1.0 IU and 2.0 IU may be tried although the dose to be used will depend on the sensitivity of the animals.

Choose a dose of the preparation to be examined expected to contain 60X IU of follicle-stimulating hormone (hFSH), in which X = the number of IU of hLH in the middle dose of the reference preparation.

Dissolve separately the total quantities of the preparation to be examined and of the reference preparation in 1.0 ml of *phosphate-albumin buffered saline pH 7.2 R*. Inject into a tail vein to each separate group of rats 6 days after the injection of chorionic gonadotrophin. Exactly 4 h after the injection, euthanise the rats and remove the ovaries from each animal. Remove any extraneous fluid and tissue from the ovaries and weigh the ovaries immediately.

Treat the combined ovaries from each rat separately, as follows. Crush and homogenise within 2 min in a freshly prepared 25 g/l solution of *metaphosphoric acid R* at a temperature of 4 °C and dilute to 7 ml with the same solution. Allow the homogenate to stand for 30 min at 4 °C and centrifuge at 4 °C at approximately 2500 g. Filter the supernatant liquid, if necessary, through a 0.22 µm filter.

Prepare a fresh solution consisting of a mixture of 2 ml of a 45.3 g/l solution of *sodium acetate R* adjusted to pH 7 with *acetic acid R* , 3 ml of *water R* and 2 ml of dichlorophenolindophenol *standard solution R.* Mix 2 ml of this solution with 2 ml of the clear supernatant liquid. 30 s after mixing, measure the absorbance (*2.2.25*) of the solution at the maximum at about 520 nm. Use as reference a solution with a known content of *ascorbic* *acid CRS* in a 25 g/l solution of *metaphosphoric acid R*, treated by the same process.

Calculate the amount of ascorbic acid from the ascorbic acid standard curve obtained and express in milligrams per 0.1 g of ovary to obtain the ascorbic acid content of the ovaries. Calculate the mean and its variance of the ascorbic acid content of the ovaries of the rats treated with the preparation to be examined.

For each dose-group of the reference preparation, plot the mean ascorbic acid content of the ovaries as a function of the logarithm of the dose and analyse the regression of the ascorbic acid content on the logarithm of the dose injected, using standard methods of analysis (the method of least squares).

The test is not valid unless:

— the slope constant b is significant at the 5 per cent level of significance;

— for the groups treated with the reference preparation, the sum of squares due to linear regression is equal to at least 95 per cent of the total sum of squares of the ascorbic acid content;

— the within-group variance of the ascorbic acid content of the group receiving the preparation to be examined is not significantly different at the 5 per cent level of significance from the within-group variance of the ascorbic acid content of the groups receiving the reference preparation.

The mean ascorbic acid content of the ovaries of the rats treated with the preparation to be examined is not significantly lower than that of the rats treated with the middle dose of the reference preparation (calculated from the regression equation) at the 5 per cent level of significance.

Water ***(2.5.32)***

Maximum 5.0 per cent.

Bacterial endotoxins ***(2.6.14, Method C)***

Less than 0.40 IU per IU of urofollitropin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

The follicle-stimulating activity of urofollitropin is estimated by comparing under given conditions its effect in enlarging the ovaries of immature rats treated with chorionic gonadotrophin with the same effect of the International Standard preparation of human urinary follicle-stimulating hormone and luteinising hormone or of a reference preparation calibrated in International Units. The International Units of FSH and LH are the activities contained in stated amounts of the International Standard of human urinary follicle-stimulating hormone and luteinising hormone (interstitial-cell-stimulating hormone) which consists of a mixture of a freeze-dried extract of urine of post-menopausal women with lactose. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Use immature female rats of the same strain, 19 to 28 days old, differing in age by not more than 3 days and having masses such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to 6 equal groups of at least 5 animals. If sets of 6 litter mates are available, assign one litter mate from each set to each group and mark according to litter.

Choose 3 doses of the reference preparation and 3 doses of the preparation to be examined such that the smallest dose produces a positive response in some of the rats and the largest dose does not produce a maximal response in all the rats. Use doses in geometric progression and as an initial approximation total doses of 1.5 IU, 3.0 IU and 6.0 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation to be examined and of the reference preparation corresponding to the daily doses to be used in sufficient *phosphate-albumin buffered saline pH 7.2 R* such that the daily dose is administered in a volume of about 0.5 ml. The buffer solution shall contain in the daily dose not less than 14 IU of chorionic gonadotrophin to ensure complete luteinisation. Add a suitable antimicrobial preservative such as 4 g/l of phenol or 0.02 g/l of thiomersal. Store the solutions at 5 ± 3 °C.

Inject subcutaneously into each rat the daily dose allocated to its group. Repeat the injection of each dose 24 h and 48 h after the first injection. About 24 h after the last injection, euthanise the rats and remove the ovaries from each animal. Remove any extraneous fluid and tissue from the ovaries and weigh the 2 combined ovaries of each animal immediately. Calculate the results by the usual statistical methods, using the mass of the 2 combined ovaries as the response. (The precision of the assay may be improved by a suitable correction of the organ mass with reference to the mass of the animal from which it was taken; an analysis of covariance may be used).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) of the estimated potency are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE

In an airtight, tamper-proof container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

- the activity expressed in International Units of follicle-stimulating hormone per container;
- the potency expressed in International Units of follicle-stimulating hormone per milligram.

Ph Eur

Urokinase

General Notices

(Ph Eur monograph 0695)

9039-53-6

Action and use

Plasminogen activator; fibrinolytic; enzyme.

Ph Eur

DEFINITION

Urokinase is an enzyme, obtained from human urine, that activates plasminogen. It consists of a mixture of low-molecular-mass (LMM) (M_r 33 000) and high-molecular-mass (HMM) (M_r 54 000) forms, the high-molecular-mass form being predominant. The potency is not less than 70 000 International Units per milligram of protein.

PRODUCTION

It is produced by validated methods of manufacturing designed to minimise or eliminate asoactive substances.

CHARACTERS

A white or almost white, amorphous powder, soluble in water.

IDENTIFICATION

A. Place separately in two haemolysis tubes 0.5 ml of citrated human plasma and 0.5 ml of citrated bovine plasma and maintain in a water-bath at 37 °C. To each tube add 0.1 ml of a solution containing a quantity of the substance to be examined equivalent to 1000 IU/ml in *phosphate buffer solution pH 7.4 R* and 0.1 ml of a solution containing a quantity of *humanthrombin R* equivalent to 20 IU/ml in *phosphate buffer solution pH 7.4 R* . Shake immediately. In both tubes, a clot forms and lyses within 30 min.

B. Carry out identification by a suitable immunodiffusion test.

TESTS

Appearance of solution

Dissolve 10 mg in 10 ml of *water R* . The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Hepatitis B surface antigen

Examine by a suitably sensitive method such as radio-immunoassay. Hepatitis B surface antigen is not detected.

Thromboplastic contaminants

Test solutions Dissolve suitable quantities of the substance to be examined in *barbital buffer solution pH 7.4 R* to obtain solutions with activities of 5000 IU/ml, 2500 IU/ml, 1250 IU/ml, 625 IU/ml and 312 IU/ml.

To each of six haemolysis tubes 1 cm in internal diameter add 0.1 ml of *citrated rabbit plasma R* . Allocate the test solutions one to each of five of the tubes; add to each tube 0.1 ml of the solution allocated to it and to the sixth tube add 0.1 ml of *barbital buffer solution pH 7.4 R* (blank). Incubate the tubes at 25 ± 0.5 °C for 5 min and add 0.1 ml of a 3.675 g/l solution of *calcium chloride R* . Measure with a stop-watch the coagulation time for each tube. Plot the shortening of the recalcification time (clotting time of the blank minus clotting time measured) against log concentration in International Units. Extrapolate the best-fitting straight line through the five points until it reaches the log-concentration axis. The urokinase activity at the intersection point, which represents the limit concentration for coagulant activity (zero coagulant activity), is not less than 150 IU/ml.

Molecular fractions

Examine by size-exclusion chromatography *(2.2.30)* .

Test solution Dissolve about 1 mg of the substance to be examined in 1.0 ml of *0.02 M phosphate buffer pH 8.0 R* . Prepare immediately before use.

The chromatographic procedure may be carried out using:

- — a column 0.9 m long and 16 mm in internal diameter packed with *cross-linked dextran for chromatography R3*;
- — as mobile phase at a flow rate of 6 ml/h a 17.5 g/l solution of *sodium chloride R* in *0.02 M phosphate buffer solution pH 8.0 R* ;

equilibrating the column and operating at 5 °C. Apply the test solution to the head of the column rinsing twice with 0.5 ml portions of the buffer and carry out the elution. The eluate may be collected in fractions of 1 ml. Measure the absorbance *(2.2.25*) of the eluate at the maximum at 280 nm and plot the individual values on a graph. Draw perpendicular lines towards the axis of the abscissae from the minima before the HMM peak, between the HMM and the LMM peaks, and after the LMM peak, thus identifying the fractions to be considered in calculating the HMM/LMM activity ratio. Pool the HMM fractions and, separately, the LMM fractions. Determine separately the urokinase activity in International Units of each of the fraction pools by the method prescribed under Assay. The ratio of the urokinase activity in the HMM fraction pool to that in the LMM fraction pool is not less than 2.0.

Total protein

Determine the nitrogen content, using 10 mg, by the method of sulphuric acid digestion *(2.5.9)* and calculate the quantity of protein by multiplying by 6.25.

Pyrogens *(2.6.8)*

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogen, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1.0 ml of a sterile 9 g/l solution of *sodium chloride R* containing a quantity of the substance to be examined equivalent to 20 000 IU/ml.

ASSAY

The potency of urokinase is determined by comparing its capacity to activate plasminogen to form plasmin with the same capacity of a reference preparation of urokinase calibrated in International Units; the formation of plasmin is measured by the determination of the lysis time of a fibrin clot in given conditions.

The International Unit is the activity contained in a stated amount of the International Reference Preparation, which consists of freeze-dried urokinase with lactose. The equivalence in International Units of the International Reference Preparation is stated by the World Health Organisation.

Unless otherwise prescribed, use *phosphate buffer solution pH 7.4* R containing 30 g/l of *bovine albumin R* for the preparation of the solutions and dilutions used in the assay.

Test solution Prepare a solution of the substance to be examined expected to have an activity of 1000 IU/ml.

Reference solution Prepare a solution of a reference preparation having an activity of 1000 IU/ml.

Keep the test solution and the reference solution in iced water and use within 6 h. Prepare three serial 1.5-fold dilutions of the reference preparation such that the longest clot-lysis time is less than 20 min and the shortest clot-lysis time is greater than 3 min. Prepare three similar dilutions of the test solution. Keep the solutions in iced water and use within 1 h. Use twenty-four tubes 8 mm in diameter. Label the tubes T_1, T_2 and T_3 for the dilutions of the test solution and S_1, S_2 and S_3 for the dilutions of the reference solution, allocating four tubes to each dilution. Place the tubes in iced water. Into each tube, introduce 0.2 ml of the appropriate dilution, 0.2 ml of *phosphate buffer solution pH 7.4 R* containing 30 g/l of bovine *albumin R* and 0.1 ml of a solution of *humanthrombin R* having an activity of not less than 20 IU/ml. Place the tubes in a water-bath at 37 °C and allow to stand for 2 min to attain temperature equilibrium. Using an automatic pipette, introduce into the bottom of the first tube 0.5 ml of a 10 g/l solution of *bovine euglobulins R*, ensuring mixing. At intervals of 5 s, introduce successively into the remaining tubes 0.5 ml of a 10 g/l solution of *bovine euglobulins R*. Using a stop-watch, measure for each tube the time in seconds that elapses between the addition of the euglobulins solution and the lysis of the clot. Plot the logarithms of the lysis times for the substance to be examined and for the reference preparation against the logarithms of the concentration and calculate the activity of the substance to be examined using the usual statistical methods.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits ($P = 0.95$) of the estimated potency are not less than 80 per cent and not more than 125 per cent of the stated potency.

STORAGE

Store in an airtight container, protected from light, at a temperature not exceeding 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

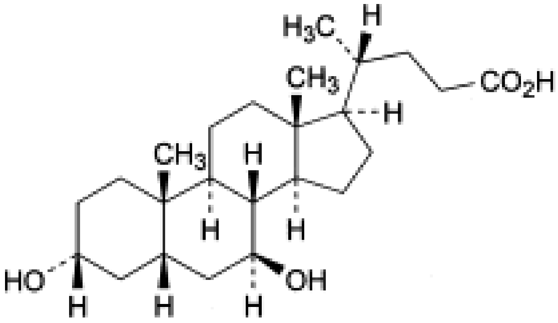
The label states the potency in International Units per milligram of protein.

Ph Eur

Ursodeoxycholic Acid

General Notices

(Ph Eur monograph 1275)



$C_{24}H_{40}O_4$ 392.6 *128-13-2*

Action and use

Bile acid; treatment of gallstones.

Preparations

Ursodeoxycholic Acid Capsules

Ursodeoxycholic Acid Tablets

Ph Eur

DEFINITION

Ursodeoxycholic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3α,7β-dihydroxy-5β-cholan-24-oic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in acetone, practically insoluble in methylene chloride.

It melts at about 202 °C.

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *ursodeoxycholic acid CRS.* Examine the substances as discs prepared using *potassium bromide R*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 1 ml of *sulphuric acid R*. Add 0.1 ml of *formaldehyde solution R* and allow to stand for 5 min. Add 5 ml of *water R*. The suspension obtained is greenish-blue.

TESTS

Specific optical rotation *(2.2.7)*

Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 58.0 to + 62.0, calculated with reference to the dried substance.

Related substances

Examine by thin-layer chromatography *(2.2.27)*, using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 0.40 g of the substance to be examined in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R*.

Reference solution (a) Dissolve 40 mg of *ursodeoxycholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b) Dissolve 20 mg of *lithocholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 10 ml with the same mixture of solvents. Dilute 2 ml of this solution to 100 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R.*

Reference solution (c) Dissolve 20 mg of *chenodeoxycholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 50 ml with the same mixture of solvents.

Reference solution (d) Dissolve 20 mg of *cholic acid CRS* in a mixture of 1 volume of *water R* and 9 volumes of *acetone R* and dilute to 100 ml with the same mixture of solvents.

Reference solution (e) Dilute 0.5 ml of test solution (a) to 20 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R.* Dilute 1 ml of this solution to 10 ml with a mixture of 1 volume of *water R* and 9 volumes of *acetone R*.

Reference solution (f) Dissolve 10 mg of *ursodeoxycholic acid CRS* in reference solution (c) and dilute to 25 ml with the same solution.

Apply 5 µl of each solution to the plate. Develop in an unsaturated tank over a path of 15 cm using a mixture of 1 volume of *glacial acetic acid R*, 30 volumes of *acetone R* and 60 volumes of *methylene chloride R*. Dry the plate at 120 °C for 10 min. Spray the plate immediately with a 47.6 g/l solution of *phosphomolybdic acid R* in a mixture of 1 volume of *sulphuric acid R* and 20 volumes of *glacial acetic acid R* and heat again at 120 °C until blue spots appear on a lighter background. In the chromatogram obtained with test solution (a): any spot corresponding to lithocholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent); any spot corresponding to chenodeoxycholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (1 per cent); any spot corresponding to cholic acid is not more intense than the principal spot in the chromatogram obtained with reference solution (d) (0.5 per cent); any spot, apart from the principal spot and any spots corresponding to lithocholic acid, chenodeoxycholic acid and cholic acid, is not more intense than the principal spot in the chromatogram obtained with reference solution (e) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (f) shows two clearly separated principal spots.

Heavy metals *(2.4.8)*

1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

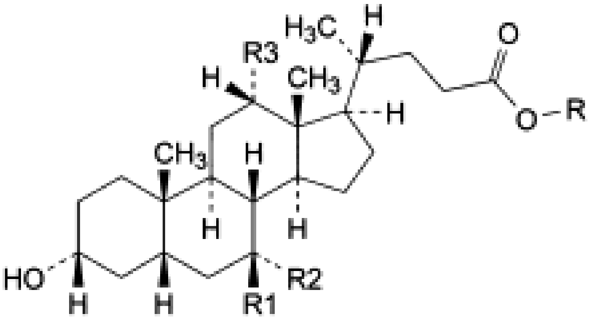
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 50 ml of *ethanol (96 per cent) R*, previously neutralised to 0.2 ml of *phenolphthalein solution R*. Add 50 ml of *water R* and titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 39.26 mg of $C_{24}H_{40}O_4$.

IMPURITIES



A. R = R1 = R3 = H, R2 = OH: chenodeoxycholic acid,

B. R = R1 = H, R2 = R3 = OH: 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (cholic acid),

C. R = R1 = R2 = R3 = H: 3α-hydroxy-5β-cholan-24-oic acid (lithocholic acid),

D. R = R2 = H, R1 = R3 = OH: 3α,7β,12α-trihydroxy-5β-cholan-24-oic acid (ursocholic acid),

E. R = R1 = R2 = H, R3 = OH: 3α,12α-dihydroxy-5β-cholan-24-oic acid (deoxycholic acid),

F. R = R3 = H, R1+R2 = O: 3α-hydroxy-7-oxo-5β-cholan-24-oic acid,

G. R = CH_3, R1 = OH, R2 = R3 = H: methyl 3α,7β-dihydroxy-5β-cholan-24-oate.

Ph Eur

Hydrogenated Vegetable Oil

General Notices

68334-00-9

Action and use

Excipient.

DEFINITION

Hydrogenated Vegetable Oil is a mixture of triglycerides of fatty acids of vegetable origin.

CHARACTERISTICS

An almost white, fine powder at room temperature and a pale yellow, oily liquid above its melting point.

Practically insoluble in *water*; soluble in *hexane* and in hot *propan-2-ol*.

IDENTIFICATION

Complies with the tests for Acid value, Iodine value and Saponification value.

TESTS

Melting point

57° to 70°, Appendix V A.

Acid value

Not more than 4.0, Appendix X B.

Iodine value

Not more than 5, Appendix X E, Method B.

Saponification value

175 to 205, Appendix X G, Method II.

Unsaponifiable matter

Not more than 0.8% w/w, Appendix X H.

Heavy metals

2.0 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Loss on drying

When dried at 105° for 4 hours, loses not more than 0.1% of its weight. Use 5 g.

STORAGE

Hydrogenated Vegetable Oil should be stored at a temperature of 8° to 25°.

Natural Vitamin A Ester Concentrate

General Notices

DEFINITION

Natural Vitamin A Ester Concentrate consists of a natural ester or a mixture of natural esters of retinol or of a solution of the ester or mixture of esters in Arachis Oil or other suitable vegetable oil. It contains in 1 g not less than 485,000 IU of vitamin A and not less than 97.0% of the number of IU of vitamin A stated on the label. It may contain a suitable antioxidant or mixture of antioxidants.

CHARACTERISTICS

A yellow oil or a mixture of oil and crystalline material which yields a homogeneous yellow oil on warming.

Solubility

Practically insoluble in *water*; soluble or partly soluble in *ethanol (96%)*; miscible with *ether* and with petroleum spirit.

IDENTIFICATION

A. Dissolve a quantity containing 10 IU in a mixture of 100 parts of *absolute ethanol* and 1 part of *hydrochloric acid*. The *light absorption*, Appendix II B, of the solution immediately after preparation exhibits a single maximum at 326 nm. Heat the solution in a water bath for 30 seconds and cool rapidly. The *light absorption* in the range 300 to 400 nm exhibits a low maximum or inflection at 332 nm and sharp maxima at 348, 367 and 389 nm.

B. Dissolve a quantity containing 30 IU in 1 ml of *chloroform* and add 10 ml of *antimony trichloride solution*. A transient bright blue colour is produced immediately.

TESTS

Acid value

Not more than 2.0, Appendix X B.

Peroxide value

Place 1 g in a boiling tube (20 cm × 2.5 cm) and dissolve in 20 ml of a mixture of 2 volumes of *glacial acetic acid* and 1 volume of *ethanol-free chloroform*. Add 1 g of finely powdered *potassium iodide* and pass a rapid current of *oxygen-free nitrogen* through the mixture for 1 minute. Stopper the tube loosely, partly immerse in boiling water for 30 seconds and then in water at 80° for 2 minutes, tighten the stopper and cool rapidly. Transfer the contents to a flask containing 25 ml of a freshly prepared 1% w/v solution of *potassium iodide*, rinse the tube with a further 25 ml of the potassium iodide solution, shake the combined solution and rinsings and titrate with 0.01M *sodium thiosulphate VS*. Repeat the procedure without the concentrate. The difference between the titrations does not exceed 1.4 ml.

Retinol

Carry out the method for *descending paper chromatography*, Appendix III E, using a mixture of 70 volumes of *1,4-dioxan*, 15 volumes of *methanol* and 15 volumes of *water* containing 1% w/v of *butylated hydroxyanisole* in the bottom of the tank and as the mobile phase. Saturate the paper with a 10% w/v solution of *liquid paraffin* in *petroleum spirit (boiling range, 40° to 60°)* and dry without the aid of heat. Apply separately to the impregnated paper 5 µl and 10 µl of each of the following freshly prepared solutions. For solution (1) dissolve sufficient of the concentrate in *petroleum spirit (boiling range, 40° to 60°)* to yield a solution containing 16,150 to 17,850 IU per ml. For solution (2) mix a quantity of the concentrate containing not less than 500 IU of vitamin A and not more than 1 g of fat with 30 ml of *absolute ethanol* and 3 ml of a 50% w/w solution of *potassium hydroxide*. Boil gently under a reflux condenser in a current of *oxygen-free nitrogen* for 30 minutes, cool rapidly and add 30 ml of water. Transfer the hydrolysate to a separating funnel using three 50 ml quantities of *ether* and extract the vitamin A by shaking for 1 minute. After complete separation discard the aqueous layer and wash the extract with four 50 ml quantities of *water*, mixing very cautiously during the first two washes to avoid the formation of emulsions. Evaporate the separated extract to about 5 ml and remove the remaining solvent in a current of *oxygen-free nitrogen* without the application of heat. Dissolve the residue in sufficient *petroleum spirit (boiling range, 40° to 60°)* to produce a solution of retinol containing about 340 IU per ml.

Develop until the solvent front approaches the bottom of the paper. Examine the dried paper under *ultraviolet light (365 nm)*. The fluorescence of any spot corresponding to retinol in the chromatograms obtained with solution (1) is not more intense than that of the spot in the corresponding chromatogram obtained with solution (2).

ASSAY

Carry out the test as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper and iron) and acids.

Examine by *ultraviolet absorption spectrophotometry*, Appendix II B (Method A). If method A is found not to be valid, examine by *liquid chromatography*, Appendix III D (Method B).

Method A

Test solution To a quantity of the substance being examined containing 50,000 IU in a round-bottomed flask, add 3 ml of a freshly prepared 50% w/w solution of *potassium hydroxide* and 30 ml of *absolute ethanol*. Boil under a reflux condenser in a current of *nitrogen* for 30 minutes. Cool rapidly and add 30 ml of *water*. Extract with four 50 ml quantities of *ether* discarding the lower layer after complete separation. Wash the combined upper layers with four 50 ml quantities of *water* and evaporate to dryness under a gentle current of *nitrogen* at a temperature not exceeding 30° or in a rotary evaporator at a temperature not exceeding 30° under reduced pressure (water ejector). Dissolve the residue in sufficient *propan-2-ol* to give an expected concentration of vitamin A equivalent to 10 to 15 IU per ml.

Measure the *absorbances*, Appendix II B, of the solution at 300 nm, 310 nm, 325 nm and 334 nm and at the wavelength of maximum absorption with a suitable spectrophotometer in 1-cm specially matched cells, using *propan-2-ol* as the compensation liquid.

Calculate the content of vitamin A, as all-*trans*-retinol, in IU per gram from the expression:

$$A_{325} \times \frac{1830}{100m} \times V$$

where A_{325} = absorbance at 325 nm,

m = mass of the substance to be examined in grams,

V = total volume of solution containing 10 IU to 15 IU of vitamin A per ml,

1830 = conversion factor for the specific absorbance of all-*trans*-retinol in IU.

The above expression can be used only if A_{325} has a value of not greater than $A_{325.\ corr}$ / 0.970 where $A_{325,\ corr}$ is the corrected *absorbance* at 325 nm and is given by the equation:

$A_{325,\ corr} = 6.815A_{325} - 2.555A_{310} - 4.260A_{334}$

where A designates the absorbance at the wavelength indicated by the subscript.

If A_{325} has a value greater than $A_{325,\ corr}$ / 0.970, calculate the content of vitamin A from the expression:

$$A_{325,corr} \times \frac{1830}{100m} \times V$$

The assay is not valid unless:

(a) the wavelength of maximum absorption lies between 323 nm and 327 nm and

(b) the *absorbance* at 300 nm relative to that at 325 nm is at most 0.73.

Method B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare solution (1) as follows. To a quantity of the substance being examined containing 50, 000 IU in a round-bottomed flask, add 5 ml of a freshly prepared 10% w/v solution of *ascorbic acid* and 10 ml of a freshly prepared 80% w/v solution of *potassium hydroxide* and 100 ml of *ethanol (96%)*. Boil under a reflux condenser on a water bath for 15 minutes. Add 100 ml of a 1% w/v solution of *sodium chloride* and cool. Transfer the solution to a 500 ml separating funnel rinsing the round-bottomed flask with about 75 ml of a 1% w/v solution of *sodium chloride* and then with 150 ml of a mixture of equal volumes of *petroleum spirit (boiling range, 40° to 60°)* and *ether*. Shake for 1 minute. When the layers have separated completely, discard the lower layer and wash the upper layer, first with 50 ml of a 3% w/v solution of *potassium hydroxide* in a 10% v/v solution of *ethanol (96%)* and then with three 50 ml quantities of a 1% w/v solution of *sodium chloride*. Filter the upper layer through 5 g of *anhydrous sodium sulphate* on a fast filter paper into a 250 ml flask suitable for a rotary evaporator. Wash the funnel with 10 ml of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30° under reduced pressure (water ejector) and fill with *nitrogen* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen* at a temperature not exceeding 30°. Dissolve the residue in *propan-2-ol*, transfer to a 25 ml volumetric flask and dilute to 25 ml with *propan-2-ol*. Gentle heating in an ultrasonic bath may be required. For solution (2) prepare a solution of *retinyl acetate EPCRS* in *propan-2-ol R1* so that 1 ml contains about 1000 IU of all-*trans*-retinol.

The exact concentration of solution (2) is assessed by ultraviolet absorption spectrophotometry, Appendix II B. Dilute solution (2) with *propan-2-ol R1* to a presumed concentration of 10 IU per ml to 15 IU per ml and measure the *absorbance* at 326 nm in matched 1-cm cells using *propan-2-ol R1* as the compensation liquid.

Calculate the content of vitamin A in IU per ml of solution (2) from the following expression, taking into account the assigned content of *retinyl acetate EPCRS:*

$$A_{326} \times \frac{1900 \times V_2}{100 \times V_1}$$

where A_{326} = *absorbance* at 326 nm,

V_2 = volume of the diluted solution,

V_1 = volume of solution (2) used,

1900 = conversion factor for the specific absorbance of *retinyl acetate EPCRS* in IU.

For solution (3) proceed as described for solution (1) but use 2 ml of solution (2) in place of the substance being examined.

The exact concentration of solution (3) is assessed by *ultraviolet absorption spectrophotometry*, Appendix II B. Dilute solution (3) with *propan-2-ol R1* to a presumed concentration of 10 IU per ml to 15 IU per ml of all-*trans*-retinol and measure the absorbance at 325 nm in matched 1 cm cells using *propan-2-ol R1* as the compensation liquid.

Calculate the content of all-*trans*-retinol in IU per millilitre of solution (3) from the expression:

$$A_{325} \times \frac{1830 \times V_4}{100 \times V_3}$$

where A_{325} = *absorbance* at 325 nm,

V_3 = volume of the diluted solution,

V_4 = volume of solution (3) used,

1830 = conversion factor for the specific absorbance of all-*trans*-retinol in IU.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm), (b) as mobile phase at a flow rate of 1 ml per minute a mixture of 3 volumes of *water* and 97 volumes of *methanol*, (c) a detection wavelength of 325 nm, (d) a 10 µl loop injector and (e) an electronic integrator.

Inject in triplicate solution (1) and solution (3). The retention time of all-*trans*-retinol is 5 minute ± 1 minute.

The assay is not valid unless (a) the chromatogram obtained with solution (1) shows a peak corresponding to that of all-*trans*-retinol in the chromatogram obtained with solution (3), (b) when using the method of standard additions to solution (1) there is greater than 95% recovery of the added *retinyl acetate EPCRS*, and (c) the recovery of all-*trans*-retinol in solution (3) as assessed by direct absorption spectrophotometry is greater than 95%.

Calculate the content of vitamin A using the following expression:

$$A_1 \times \frac{C \times V}{A_2} \times \frac{1}{m}$$

where A_1 = area of the peak corresponding to all-*trans*-retinol in the chromatogram obtained with solution (1),

A_2 = area of the peak corresponding to all-*trans*-retinol in the chromatogram obtained with solution (3),

C = concentration of *retinyl acetate EPCRS* in solution (2) as assessed prior to the saponification in International Units per ml (1000 IU per ml),

V = volume of solution (2) treated,

m = mass of the substance being examined in solution (1).

STORAGE

Vitamin A Ester Concentrate (Natural) should be kept in an airtight container, protected from light and stored at a temperature of 8° to 15°. Once the container has been opened its contents should be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of an inert gas.

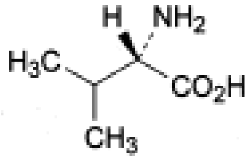
LABELLING

The label states (1) the number of IU (Units) of vitamin A per g; (2) the name of the ester or esters; (3) the name and proportion of the principal excipients; (4) the method of restoring the solution if partial crystallisation has occurred.

Valine

General Notices

(Ph Eur monograph 0796)



$C_5H_{11}NO_2$ 117.1 *72-18-4*

Action and use

Amino acid.

Ph Eur

DEFINITION

Valine contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2-amino-3-methylbutanoic acid, calculated with reference to the dried substance.

CHARACTERS

White or almost white, crystalline powder or colourless crystals, soluble in water, very slightly soluble in alcohol.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry *(2.2.24)* , comparing with the spectrum obtained with *valine CRS* . Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 2.5 g in *water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*

Dissolve 2.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 26.5 to + 29.0, calculated with reference to the dried substance.

Ninhydrin-positive substances

Examine by thin-layer chromatography *(2.2.27)* , using a *TLC silica gel plate R* .

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dilute hydrochloric acid R* and dilute to 10 ml with the same acid.

Test solution (b) Dilute 1 ml of test solution (a) to 50 ml with *water R* .

Reference solution (a) Dissolve 10 mg of *valine CRS* in *0.1 M hydrochloric acid* and dilute to 50 ml with the same acid.

Reference solution (b) Dilute 5 ml of test solution (b) to 20 ml with *water R* .

Reference solution (c) Dissolve 10 mg of *phenylalanine CRS* and 10 mg of *valine CRS* in *0.1 M hydrochloric acid* and dilute to 25 ml with the same acid.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R,* 20 volumes of *water R* and 60 volumes of *butanol R* . Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides *(2.4.4)*

Dilute 10 ml of solution S to 15 ml with *water R* . The solution complies with the limit test for chlorides (200 ppm).

Sulphates *(2.4.13)*

Dissolve 0.5 g in *distilled water R* and dilute to 15 ml with the same solvent. The solution complies with the limit test for sulphates (300 ppm).

Ammonium *(2.4.1)*

50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm* NH_4*) R*.

Iron *(2.4.9)*

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals ***(2.4.8)***

2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying ***(2.2.32)***

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Using 0.1 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 11.71 mg of $C_5H_{11}NO_2$.

STORAGE

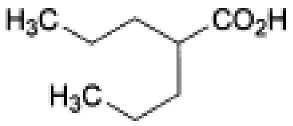
Store protected from light.

Ph Eur

Valproic Acid

General Notices

(Ph Eur monograph 1378)



$C_8H_{16}O_2$ 144.2 *99-66-1*

Action and use

Antiepileptic.

Ph Eur

DEFINITION

2-Propylpentanoic acid.

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

Colourless or very slightly yellow, clear liquid, slightly viscous.

Solubility

Very slightly soluble in water, miscible with ethanol (96 per cent) and with methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification B.

Second identification A, C, D.

A. Refractive index *(2.2.5)* : 1.422 to 1.425.

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *valproic acid CRS* .

C. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 50 mg of *valproic acid CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R* .

Mobile phase *Ether R, methylene chloride R* (50:50 *V/V*).

Application 2 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *bromocresol green solution R* .

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 1 ml add 3 ml of dilute *sodium hydroxide solution R* . Add 3 ml of *water R* and 1 ml of a 100 g/l solution of *cobalt nitrate R* . A violet precipitate is formed. Filter. The precipitate dissolves in *methylene chloride R* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Dissolve 2.0 g in *dilute sodium hydroxide solution R* and dilute to 10 ml with the same alkaline solution.

Related substances

Gas chromatography *(2.2.28)* .

Internal standard solution Dissolve 10 mg of *butyric acid R* in *heptane R* and dilute to 200 ml with the same solvent.

Test solution Dissolve 0.250 g of the substance to be examined in the internal standard solution and dilute to 5.0 ml with the internal standard solution. Dilute 1.0 ml of this solution to 10.0 ml with *heptane R* .

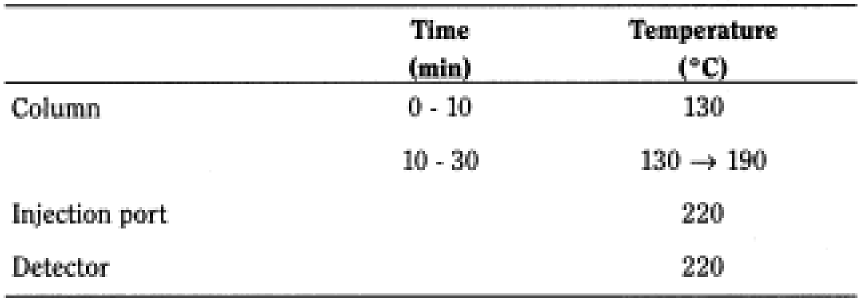
Reference solution Dissolve 20 mg of the substance to be examined and 20 mg of *2-(1-methylethyl)pentanoic acid CRS* (impurity C) in *heptane R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *heptane R* .

Column:
- *material*: wide-bore fused silica;
- *size*: *l* = 30 m, Ø = 0.53 mm;
- *stationary phase: macrogol 20 000 2-nitroterephthalate R* (film thickness 0.5 µm).

Carrier gas *Helium for chromatography R*.

Flow rate 8 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	130
	10 - 30	130 → 190
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution:
- *resolution*: minimum 3.0 between the peaks due to impurity C and valproic acid.

Limits:
- *any impurity*: for each impurity, not more than the area of the peak due to the internal standard (0.1 per cent);
- *total*: not more than 3 times the area of the peak due to the internal standard (0.3 per cent);
- *disregard limit*: 0.1 times the area of the peak due to the internal standard (0.01 per cent) .

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 2.0 g in *ethanol (80 per cent V/V) R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with *ethanol (80 per cent V/V) R.*

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

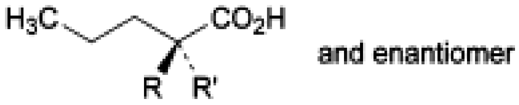
Dissolve 0.100 g in 25 ml of *ethanol (96 per cent) R* . Add 2 ml of *water R* . Titrate with *0.1 M sodium hydroxide* , determining the end-point potentiometrically *(2.2.20)* .

1 ml of *0.1 M sodium hydroxide* is equivalent to 14.42 mg of $C_8H_{16}O_2$.

STORAGE

In an airtight container .

IMPURITIES

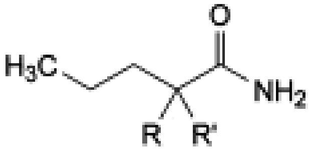


A. R = R′ = H: pentanoic acid (valeric acid),

B. R = H, R′ = CH_2-CH_3: (2*RS*)-2-ethylpentanoic acid,

C. R = H, R′ = $CH(CH_3)_2$: (2*RS*)-2-(1-methylethyl)pentanoic acid,

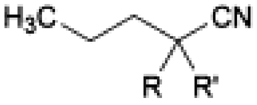
D. R = R′ = CH_2-CH_2-CH_3: 2,2-dipropylpentanoic acid,



E. R = R′ = H: pentanamide (valeramide),

F. R = H, R′ = CH_2-CH_2-CH_3: 2-propylpentanamide,

G. R = R′ = CH_2-CH_2-CH_3: 2,2-dipropylpentanamide,



H. R = R′ = H: pentanenitrile (valeronitrile),

I. R = H, R′ = CH_2-CH_2-CH_3: 2-propylpentanenitrile,

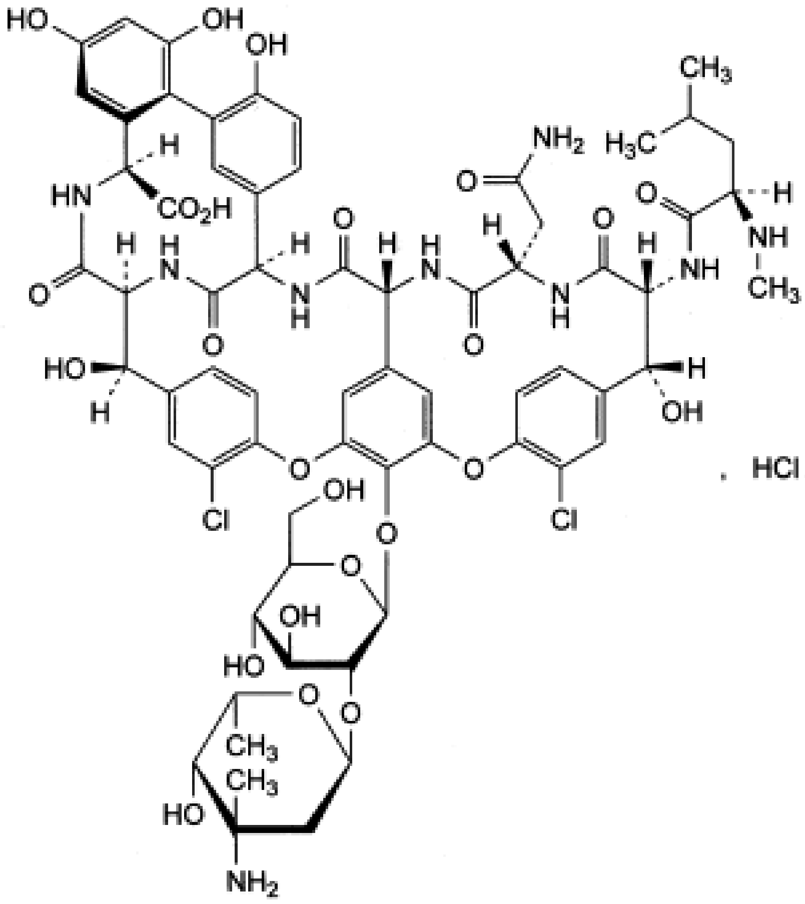
J. R = R′ = CH_2-CH_2-CH_3: 2,2-dipropylpentanenitrile.

Ph Eur

Vancomycin Hydrochloride

General Notices

(Ph Eur monograph 1058)


HO
OH
OH
CH3
NH2
H3C
H
HN
CO2H
O
H
H
H
H
H
H
N
H
NH
O
N
N
NH
CH3
HO
H
OH
O
O
O
O
Cl
Cl
OH
O
O
OH
HO
O
O
CH3
CH3
HO
NH2
. HCl

$C_{66}H_{75}Cl_2N_9O_{24}$,HCl   1486   *1404-93-9*

Action and use

Glycopeptide antibacterial.

Preparations

Vancomycin Intravenous Infusion

Vancomycin Oral Solution

Ph Eur

DEFINITION

Hydrochloride of a mixture of related glycopeptides, consisting principally of the monohydrochloride of (3*S*,6*R*,7*R*,22*R*,23*S*,26*S*,30a*S*a,36*R*,38a*R*)-3-(2-amino-2-oxoethyl)-44-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-10,19-dichloro-7,22,28,30,32-pentahydroxy-6-[[(2*R*)-4-methyl-2-(methylamino)pentanoyl]amino]-2,5,24,38,39-pentaoxo-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*,13*H*-[1,6,9]oxadiazacyclohexadecino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-26-carboxylic acid (vancomycin B).

Substance produced by certain strains of *Amycolatopsis orientalis* or obtained by any other means.

Potency Minimum 1050 IU/mg (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Examine the chromatograms obtained in the test for vancomycin B.

Results The principal peak in the chromatogram obtained with test solution (a) is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. It gives reaction (a) of chlorides *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and its absorbance *(2.2.25)* at 450 nm is not greater than 0.10.

Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent.

pH *(2.2.3)*

2.5 to 4.5.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Vancomycin B

Liquid chromatography *(2.2.29)* . Use the solutions within 4 h of preparation.

Test solution (a) Dissolve 10.0 mg of the substance to be examined in mobile phase A and dilute to 5.0 ml with mobile phase A.

Test solution (b) Dilute 2.0 ml of test solution (a) to 50.0 ml with mobile phase A.

Test solution (c) Dilute 0.5 ml of test solution (b) to 20.0 ml with mobile phase A.

Reference solution Dissolve the contents of a vial of *vancomycin hydrochloride CRS* in *water R* and dilute with the same solvent to obtain a solution containing 0.5 mg/ml. Heat at 65 °C for 24 h. Allow to cool.

Column:

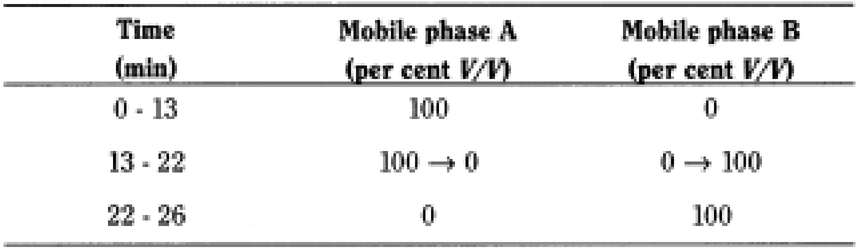
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: to 4 ml of *triethylamine R* add 1996 ml of *water R* and adjust to pH 3.2 with *phosphoric acid R* ; to 920 ml of this solution add 10 ml of *tetrahydrofuran R* and 70 ml of *acetonitrile R* ;

— *mobile phase B*: to 4 ml of *triethylamine R* add 1996 ml of *water R* and adjust to pH 3.2 with *phosphoric acid R* ; to 700 ml of this solution add 10 ml of *tetrahydrofuran R* and 290 ml of *acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 13	100	0
13 - 22	100 → 0	0 → 100
22 - 26	0	100

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

System suitability:

— *resolution*: minimum 5.0 between the 2 principal peaks in the chromatogram obtained with the reference solution;

— *signal-to-noise ratio* : minimum 5 for the principal peak in the chromatogram obtained with test solution (c);

— *symmetry factor* : maximum 1.6 for the peak due to vancomycin in the chromatogram obtained with test solution (b).

Calculate the percentage content of vancomycin B hydrochloride using the following expression:

$$\frac{A_b \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

A_b = area of the peak due to vancomycin B in the chromatogram obtained with test solution (b);

A_t = sum of the areas of the peaks due to impurities in the chromatogram obtained with test solution (a).

Limit:

— *vancomycin B*: minimum 93.0 per cent.

Related substances

Liquid chromatography *(2.2.29)* as described in the test for vancomycin B with the following modifications.

Injection Test solution (a), (b) and (c).

Calculate the percentage content of each impurity using the following expression:

$$\frac{\left(\frac{A_i}{25}\right) \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

A_i = area of the peak due to an impurity in the chromatogram obtained with test solution (a);

A_b = area of the peak due to vancomycin B in the chromatogram obtained with test solution (b);

A_t = sum of the areas of the peaks due to impurities in the chromatogram obtained with test solution (a).

Limits:

— *any impurity*: for each impurity, maximum 4.0 per cent;

— *total*: maximum 7.0 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with test solution (c) (0.1 per cent).

Heavy metals *(2.4.8)*

Maximum 30 ppm.

1.0 g complies with test C. Prepare the reference solution using 3.0 ml of *lead standard solution (10 ppm Pb) R.*

Water *(2.5.12)*

Maximum 5.0 per cent, determined on 0.500 g.

Sulphated ash *(2.4.14)*

Maximum 1.0 per cent, determined on 1.00 g.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

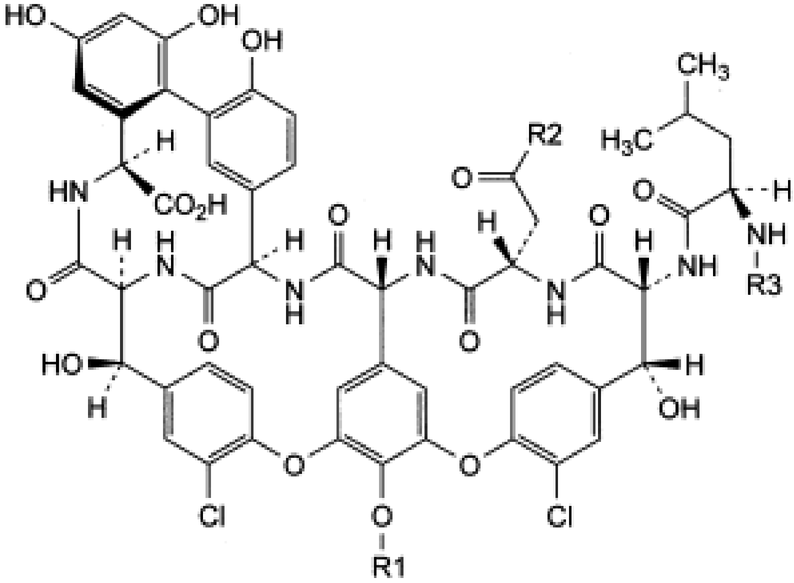
ASSAY

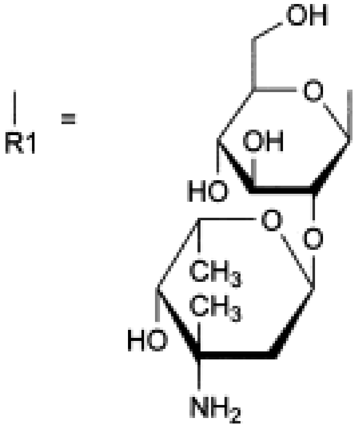
Carry out the microbiological assay of antibiotics *(2.7.2).* Use *vancomycin hydrochloride CRS* as the chemical reference substance.

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

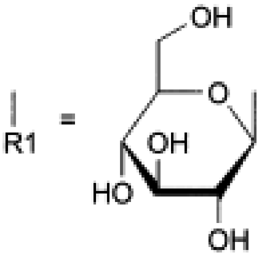
IMPURITIES


HO
OH
OH
H
HN
CO₂H
O
H
H
N
HO
H
H
N
O
H
H
N
R2
O
H
N
H
H
NH
O
CH₃
H₃C
O
H
NH
R3
O
O
O
H
OH
Cl
O
O
Cl
O
R1

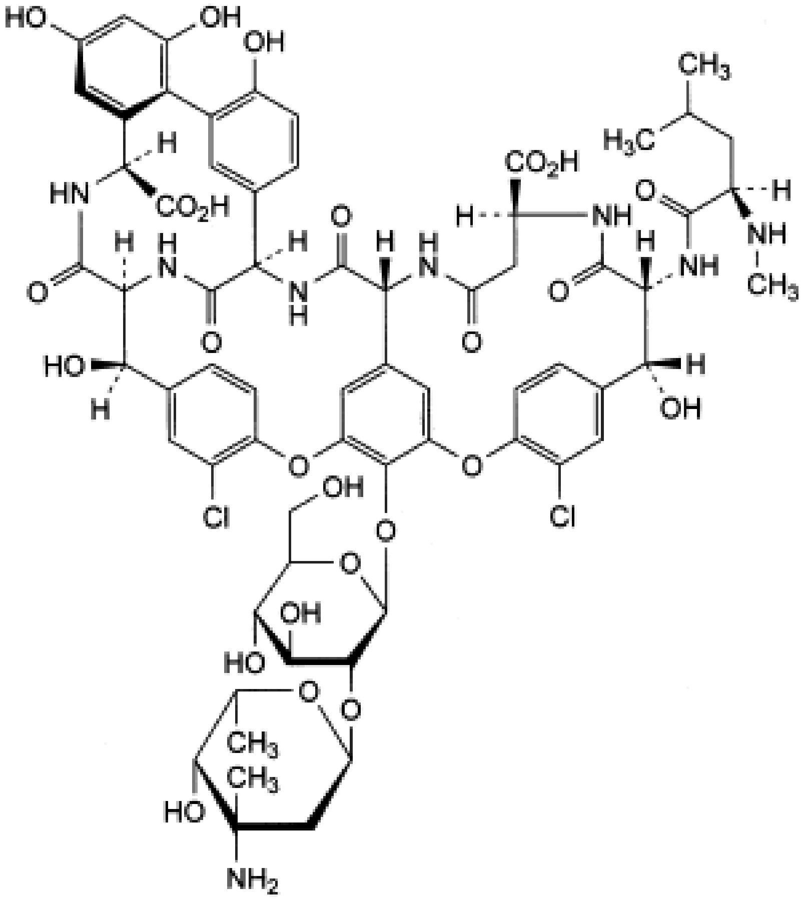

R1 =
OH
O
OH
HO
O
O
CH₃
CH₃
HO
NH₂

A. R2 = NH_2, R3 = H: N-demethylvancomycin B,

C. R1 = H, R2 = NH_2, R3 = CH_3: aglucovancomycin B,


R1 =
OH
O
OH
HO
OH

D. R2 = NH_2, R3 = CH_3: desvancosaminylvancomycin B,



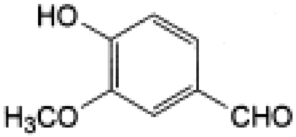
B. (4*S*,7*R*,8*R*,23*R*,24*S*,27*S*,31a*S*a,37*R*,39a*R*)-45-[[2-*C*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-11,20-dichloro-8,23,29,31,33-pentahydroxy-7-[[(2*R*)-4-methyl-2-(methylamino)pentanoyl]amino]-2,6,25,39,40-pentaoxo-1,2,3,4,5,6,7,8,24,25,26,27,37,38,39,39a-hexadecahydro-23*H*-9,12:19,22-dietheno-24,37-(iminomethano)-14,17:32,36-dimetheno-14*H*-[1,6,10]oxadiazacycloheptadecino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-4,27-dicarboxylic acid ([β Asp^3]vancomycin B).

Ph Eur

Vanillin

General Notices

(Ph Eur monograph 0747)



$C_8H_8O_3$ 152.1 *121-33-5*

Ph Eur

DEFINITION

Vanillin contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-hydroxy-3-methoxybenzaldehyde, calculated with reference to the dried substance.

CHARACTERS

White or slightly yellowish, crystalline powder or needles, slightly soluble in water, freely soluble in alcohol and in methanol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification *B.*

Second identification *A, C, D.*

A. Melting point *(2.2.14)* : 81 °C to 84 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the spectrum obtained with *vanillin CRS* . Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances in daylight after spraying. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 5 ml of a saturated solution of the substance to be examined add 0.2 ml of *ferric chloride solution R1* . A blue colour is produced. Heat to 80 °C. The solution becomes brown. On cooling, a white precipitate is formed.

TESTS

Appearance of solution

Dissolve 1.0 g in *alcohol R* and dilute to 20 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II).*

Related substances

Examine by thin-layer chromatography *(2.2.27),* using *silica gel GF $_{254}$ R* as the coating substance.

Test solution (a) Dissolve 0.1 g of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Test solution (b) Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a) Dissolve 10 mg of *vanillin CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dilute 0.5 ml of test solution (a) to 100 ml with *methanol R*.

Apply to the plate 5 µl of each solution. Develop in an unsaturated tank over a path of 10 cm using a mixture of 0.5 volumes of *anhydrous acetic acid R*, 1 volume of *methanol R* and 98.5 volumes of *methylene chloride R*. Dry the plate in a current of cold air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Spray with *dinitrophenylhydrazine-aceto-hydrochloric solution R* and examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Reaction with sulphuric acid Dissolve 50 mg in 5 ml of *sulphuric acid R*. After 5 min, the solution is not more intensely coloured than a mixture of 4.9 ml of yellow primary solution and 0.1 ml of red primary solution or a mixture of 4.9 ml of yellow primary solution and 0.1 ml of blue primary solution *(2.2.2, Method I)*.

Loss on drying *(2.2.32)*

Not more than 1.0 per cent, determined on 1.000 g by drying in a desiccator for 4 h.

Sulphated ash *(2.4.14)*

Not more than 0.05 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.120 g in 20 ml of *alcohol R* and add 60 ml of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 15.21 mg of $C_8H_8O_3$.

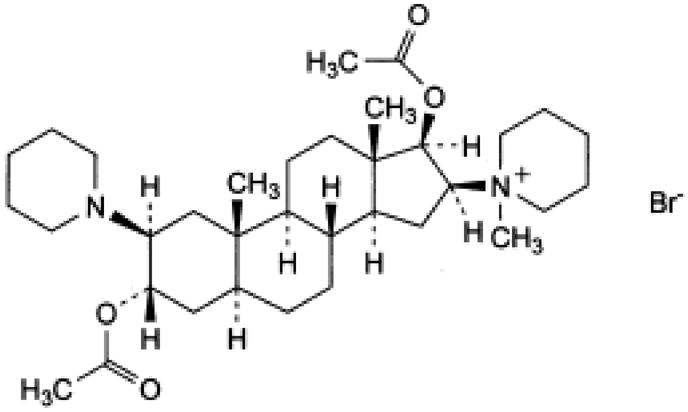
STORAGE

Store protected from light.

Vecuronium Bromide

General Notices

(Ph Eur monograph 1769)



$C_{34}H_{57}BrN_2O_4$ 638 *50700-72-6*

Action and use

Non-depolarizing neuromuscular blocker.

Ph Eur

DEFINITION

1-[3α,17β-Bis(acetyloxy)-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidinium bromide.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white crystals or crystalline powder.

Solubility

Slightly soluble in water, freely soluble in methylene chloride, sparingly soluble in acetonitrile and in anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)* .

Comparison *vecuronium bromide CRS.*

C. It gives reaction (a) of bromides *(2.3.1)* .

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 0.1 g in *water R* and dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*

+ 30.5 to + 35.0 (anhydrous substance).

Dissolve 0.250 g in *0.05 M hydrochloric acid* and dilute to 25.0 ml with the same solvent.

Impurity B

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.10 g of the substance to be examined in *methylene chloride R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of the substance to be examined and 5 mg of *pancuronium bromide CRS* (impurity B) in *methylene chloride R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 5.0 mg of *pancuronium bromide CRS* (impurity B) in *methylene chloride R* and dilute to 100.0 ml with the same solvent.

Stationary phase *TLC silica gel plate R* (2-10 µm).

Mobile phase Dissolve 1 g of *sodium bromide R* in 5 ml of *water R* . Add 85 ml of *2-propanol R* , then 10 ml of *acetonitrile R* .

Application 1 µl.

Development In an unsaturated tank, over 2/3 of the plate.

Drying In air for 30 min.

Detection Spray with a 2.5 g/l solution of *iodine R* in a mixture of equal volumes of *methanol R* and *methylene chloride R* .

System suitability Reference solution (a):

— the chromatogram obtained shows 2 clearly separated spots.

Limit:

— *impurity B*: any spot due to impurity B is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Related substances

Liquid chromatography *(2.2.29).* Use freshly prepared solutions.

Test solution Dissolve 40.0 mg of the substance to be examined in a 0.2 g/l solution of *hydrochloric acid R* in *methanol R* and dilute to 20.0 ml with the same solution.

Reference solution (a) Dissolve 4 mg of *vecuronium for peak identification CRS* (containing impurities A, C, D and E) in a 0.2 g/l solution of *hydrochloric acid R* in *methanol R* and dilute to 2 ml with the same solution.

Reference solution (b) Dilute 5.0 ml of the test solution to 100.0 ml with a 0.2 g/l *solution of hydrochloric acid R* in *methanol R* . Dilute 5.0 ml of this solution to 100.0 ml with a 0.2 g/l *solution of hydrochloric acid R* in *methanol R* .

Reference solution (c) Dilute 10.0 ml of reference solution (b) to 50.0 ml with a 0.2 g/l solution of *hydrochloric acid R* in *methanol R.*

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 50 volumes of a 18.0 g/l solution of *tetramethylammonium hydroxide R* adjusted to pH 6.5 with *phosphoric acid R* , 250 volumes of *methanol R* and 700 volumes of *acetonitrile R.*

Flow rate 2.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl.

Run time 2.5 times the retention time of vecuronium.

Identification of impurities Use the chromatogram supplied with *vecuronium for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, C, D and E. The elution order may vary, but the quantity of each impurity in the CRS is different so that a clear identification of the impurities is possible.

Relative retention With reference to vecuronium (retention time = about 5 min): impurity C = about 0.8; impurity D = about 0.9; impurity E = about 1.2; impurity A = about 1.3.

System suitability Reference solution (a):

— *peak-to-valley ratio* : minimum 2.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the principal peak; if necessary, increase the volume of the buffer solution while simultaneously decreasing the volume of acetonitrile in the mobile phase; do not change the volume of methanol;

— *symmetry factor* : maximum 3.5 for the principal peak.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity C = 1.4;
- *impurities A, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *unspecified impurities*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- *total*: not more than 2.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water ***(2.5.12)***

Maximum 4.0 per cent, determined on 0.300 g.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.450 g in 50 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 63.8 mg of $C_{34}H_{57}BrN_2O_4$.

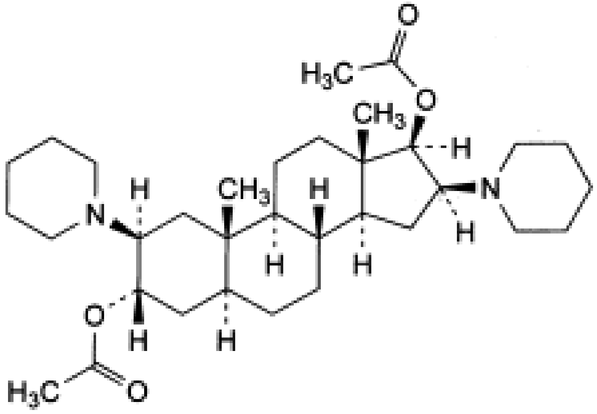
STORAGE

In an airtight container, protected from light and moisture.

IMPURITIES

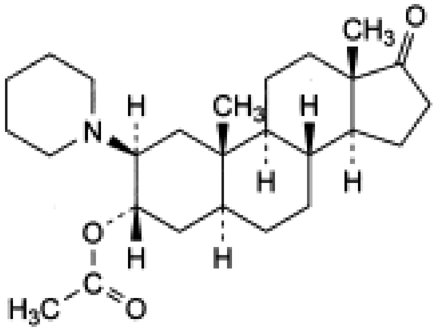
Specified impurities *A, B, C, D, E.*

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): F.*



A. 2β,16β-bis(piperidin-1-yl)-5α-androstane-3α,17β-diyl diacetate,

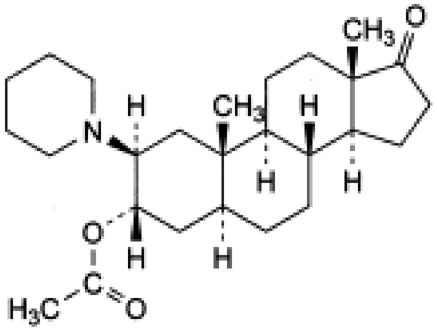
B. pancuronium,



C. R = H, R′ = CO-CH_3: 1-[17β-(acetyloxy)-3α-hydroxy-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidinium,

D. R = R′ = H: 1-[3α,17β-dihydroxy-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidinium,

E. R = CO-CH_3, R′ = H: 1-[3α-(acetyloxy)-17β-hydroxy-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidinium,



F. 2β-(piperidin-1-yl)-17-oxo-5α-androstan-3α-yl acetate.

Ph Eur

Vegetable Fatty Oils

General Notices

(Ph Eur monograph 1579)

Ph Eur

DEFINITION

Vegetable fatty oils are mainly solid or liquid triglycerides of fatty acids. They may contain small amounts of other lipids such as waxes, free fatty acids, partial glycerides or unsaponifiable matters. Vegetable fatty oils are obtained from the seeds, the fruit or the pit/stone/kernel of various plants by expression and/or solvent extraction, then possibly refined and hydrogenated. A suitable antioxidant may be added if necessary.

Virgin oil An oil obtained from raw materials of special quality by mechanical procedures (e.g. by cold expression or centrifugation).

Refined oil An oil obtained by expression and/or solvent extraction, and subsequently either alkali refining (followed by bleaching and any deodorisation) or physical refining.

Hydrogenated oil An oil obtained by expression and/or solvent extraction, and subsequently either alkali refining or physical refining, then possible bleaching, followed by drying, hydrogenation and subsequent bleaching and deodorisation.

Only alkali-refined oils are used in the preparation of parenteral dosage forms.

PRODUCTION

Measures are taken to ensure that the oil complies with the limit for benzo[a]pyrene decided by the competent authority. A limit of 2.0 ppb is set in Commission Regulation (EC) No. 208/2005.

OBTENTION OF A CRUDE OIL

Where the plant has a high oil content, the oil is generally obtained by expression under heating followed by an extraction; where the plant has a low oil content, the oil is generally obtained by direct extraction.

Mechanical procedures

A. Expression

High-pressure screw-pressing It consists of some or all of the following steps: cleaning, drying, dehulling or decorticating, grinding, cooking and flaking.

During *cleaning* the foreign matter is eliminated. *Drying* may be necessary if the seed moisture content is higher than desirable for downstream processing. *Decorticating* is useful to obtain a high-protein meal by reduction of fibre and to reduce impurities in the oil. *Cooking* serves various purposes: completion of the breakdown of oil cells, lowering of the viscosity of the oil, coagulation of the protein in the meal, adjustment of the moisture level, sterilisation of the seed, detoxifying undesirable seed constituents (gossypol for cottonseed) and fixing certain phosphatides in the cake thus lowering subsequent refining losses. The efficacy of the expression process is such that only 3 per cent to 6 per cent of the oil is left in the cake.

Wet screw-pressing The bunches are loaded into cages (for palm fruit) and moved into a horizontal steriliser with application of live steam and heating. The purposes of this steriliser are inactivation of enzymes, loosening of the fruit on the bunch, coagulation of proteins, etc. After heating in a digester, the pulp is fed to a screw-press. The oil is centrifugally clarified and vacuum-dried.

Pre-pressing followed by solvent extraction The same sequence of steps is performed as above. The main function of pre-pressing is to obtain a cake of excellent permeability for the following solvent extraction stage. The extraction is performed either in a percolation-type or in an immersion-type apparatus. The efficacy of the solvent extraction process is such that residual oil levels in meal are generally below 1 per cent.

B. Centrifugation

Centrifugation separates the oily phase from the aqueous phase, which contains water-soluble components and residual solid particles. This operation can be carried out using:

— self-cleaning bowl or disc centrifuges;

— super-decanters, which are horizontal turbines equipped with a cylindrical bowl that tapers slightly at one end and which contains a continuously turning screw that scrapes the sides of the bowl; the screw and the bowl rotate at different speeds; the solid particles are discarded from the tapered end of the bowl and the oil flows out from the other end.

Solvent extraction

Prior to extraction, the following steps are carried out: the seeds are tempered for about a week at a temperature below 24 °C in order to loosen the hull from the seed and allow the seed moisture to attain equilibrium, then the seeds are cleaned, ground, dehulled and flaked. The most widely used solvent is a mixture of mainly *n*-hexane and methylpentanes (bp: 65-70 °C) commonly referred to as 'hexane'. Due to the major fire and explosive risks of this mixture, liquified gases and supercritical gases may also be used.

REFINING

The objective of refining is to remove impurities and contaminants of the oil with the least possible damage to the triglycerides and with minimal loss of oil. The contents of the following substances are reduced:

— free fatty acids, which may cause deterioration of the oil by oxidation, a smoked taste when heated and a sharp flavour (by alkali refining);

— water, which favours the enzymatic hydrolysis reactions (by alkali refining, drying);

— partial glycerides, which may cause foaming and a bitter taste (by neutralisation, washing);

— phosphatides and phosphorous compounds, which have emulsifying properties and may cause deposits, a darkening of the oil when heated, a cloudy appearance and bad organoleptic stability (by alkali refining);

— colouring matters such as chlorophyll (by alkali refining) and carotenoids (by bleaching);

— glycolipids, which may form colloidal solutions with water;

— free hydrocarbons, paraffin, waxes and resinous materials;

— metals (Fe, Cu, Pb, Sn, Pt, Pd, etc.), which are strong oxidation catalysts;

— pigments such as gossypol (in cottonseed oil) or mycotoxins such as aflatoxin (mainly in arachis seeds);

— pesticides;

— oxidation products (aldehydes, peroxides);

— proteins having possible allergic reactions;

— unsaponifiable matters (sterols, tocopherols and other vitamins);

— polycyclic aromatic hydrocarbons.

Alkali refining

It involves the following steps: degumming if necessary, neutralisation using alkali, washing and drying.

Degumming During this step of the refining, i.e. treatment with water and/or phosphoric acid and/or sodium chloride, the phosphatides, phosphorous compounds and metals are eliminated. The use of this step depends on the nature of the oil.

Neutralisation with alkali This step reduces the free-fatty-acid content below 0.1 per cent; the fatty acids are converted into oil-insoluble soaps, also called 'soapstocks'. Other substances may be removed by adsorption on these soaps: mucilaginous substances, phosphatides, oxidation products, colouring matters, etc. All substances that become insoluble in the oil on hydration are removed. Neutralisation with alkali has the disadvantage of saponifying a portion of neutral oil if the neutralisation is not well conducted.

Washing This operation consists in removing the excess of soaps and alkali as well as the remaining traces of metals, phosphatides and other impurities, using hot water.

Drying The remaining water is eliminated under vacuum before any further steps, such as bleaching.

Physical refining

It involves a steam treatment of the oil under high vacuum at a temperature greater than 235 °C. This technique must be applied to oils naturally low in phosphatides and metals (palm, coconut, olive) or from which phosphatides and metals have been removed by an acid treatment using concentrated phosphoric acid followed by an adsorptive treatment with activated bleaching earth (for sunflower, rapeseed, soya-bean). Moreover, it cannot be used for heat-sensitive oils (cottonseed oil), which darken.

Bleaching

The common method of bleaching is by adsorption treatment of the oil, which is generally heated at 90 °C for 30 min under vacuum, with bleaching earth (natural or activated) or carbon (activated or not); synthetic silica adsorbents may also be added. Substances that have not been totally removed during refining are eliminated, for example carotenoids and chlorophyll.

Deodorisation

Deodorisation eliminates odours, volatile substances and any residual extraction solvents; it involves injecting dry vapour into the oil, which is kept under vacuum at a high temperature. Different temperatures are used according to the oil: 200-235 °C for 1.5-3 h or greater than 240 °C for 30 min.

One of the main side reactions is thermic decolourisation due to the destruction of carotenoids when the temperature is greater than 150 °C. This technique provokes a loss of substances that may be distilled (free fatty acids, sterols, tocopherols, part of the refined oil), and may cause *cis-trans* isomerisation of the unsaturated fatty-acid double bonds.

WINTERISATION

Elimination of solids and waxes by filtration at low temperature (also called dewaxing). These solids and waxes could affect the appearance of the oil and cause deposits.

HYDROGENATION

The hydrogenation of the dried and/or bleached oil is performed using a catalyst (e.g. Ni, Pt, Pd), at a temperature of about 100-200 °C under hydrogen pressure. The catalyst is then removed by filtration at 90 °C. The hydrogen must be pure: free of poisons for the catalyst, water-free, and low in carbon dioxide, methane and nitrogen contents. Small amounts of polymers may be obtained. *Trans*-fatty acids are formed during partial hydrogenation.

CHROMATOGRAPHIC PURIFICATION

In high-purity applications, mainly for parenteral uses, the oil may be further purified by passing the oil through a column containing an activated earth. A solvent may sometimes be used to improve the efficiency. High-polarity molecules, such as oxidised materials, acids, alcohols, partial glycerides and free sterols, are preferentially removed.

When the oil is used in the preparation of parenteral dosage forms, the limits set in the monograph for the acid value, the peroxide value and the water content may be different.

LABELLING

The label states:

— where applicable, that the oil was obtained by expression or extraction;

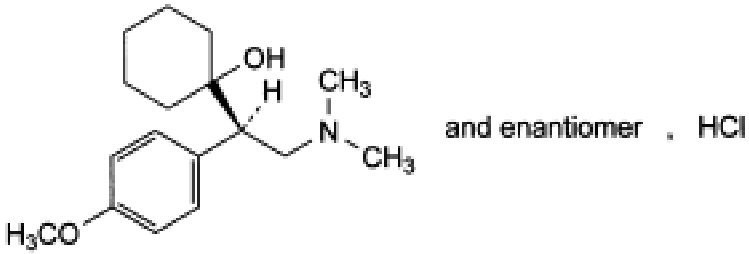
— where applicable, that the oil is suitable for use in the manufacture of parenteral dosage forms.

Ph Eur

Venlafaxine Hydrochloride

General Notices

(Ph Eur monograph 2119)



$C_{17}H_{27}NO_2,HCl$ 313.9 *99300-78-4*

Action and use

Inhibition of 5HT and noradrenaline reuptake; antidepressant.

Ph Eur

DEFINITION

1-[(1*RS*)-2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water and in methanol, soluble in anhydrous ethanol, slightly soluble or practically insoluble in acetone.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison venlafaxine hydrochloride CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *2-propanol R,* evaporate to dryness and record new spectra using the residues.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Acidity or alkalinity

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. Add 0.05 ml of *methyl red solution R* and 0.1 ml of *0.01 M hydrochloric acid*. The solution is pink. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *venlafaxine for system suitability CRS* (containing impurities D and F) in 1.0 ml of the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm.

Mobile phase Mix 510 volumes of *acetonitrile R* and 1490 volumes of a solution prepared as follows: dissolve 17 g of *ammonium dihydrogen phosphate R* in 1490 ml of *water R* and adjust to pH 4.4 using *phosphoric acid R*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µl.

Run time 10 times the retention time of venlafaxine.

Relative retention With reference to venlafaxine (retention time = about 9 min): impurity D = about 0.9; impurity F = about 3.4.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity D and venlafaxine.

Limits:

— *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

Dissolve 1.0 g in 20 ml of water R. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 3 h.

Sulphated ash *(2.4.14*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

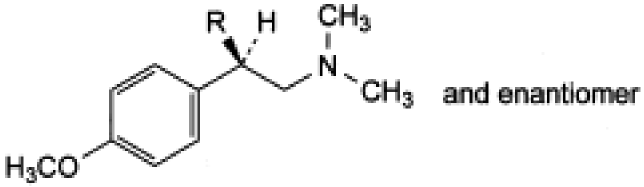
Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (96 per cent) R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.39 mg of $C_{17}H_{28}ClNO_2$.

IMPURITIES

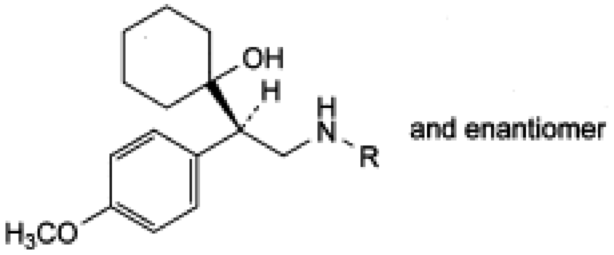
Specified impurities F.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A, B, C, D, E, G, H.*



A. R = H: 2-(4-methoxyphenyl)-*N*-*N*-dimethylethanamine,

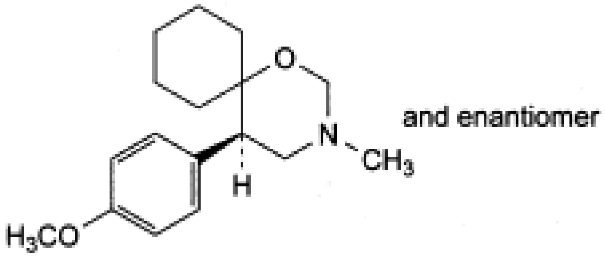
B. R = CO-O-C_2H_5: ethyl (2*RS*)-3-(dimethylamino)-2-(4-methoxyphenyl)propanoate,


and enantiomer

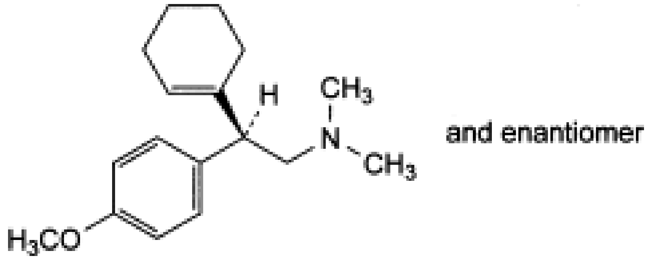
C. R = H: 1-[(1*RS*)-2-amino-1-(4-methoxyphenyl)ethyl]cyclohexanol,

D. R = CH_3: 1-[(1*RS*)-1-(4-methoxyphenyl)-2-(methylamino)ethyl]cyclohexanol,

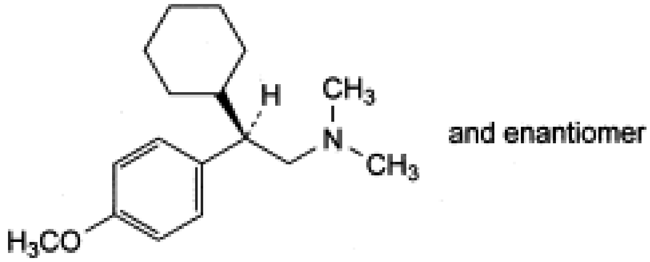
H. R = CH2-CH2-C6H4-p-OCH3: 1-[(1RS)-1-(4-methoxyphenyl)-2-[[2-(4-methoxyphenyl) ethyl]amino]ethyl]cyclohexanol,


and enantiomer

E. (5*RS*)-5-(4-methoxyphenyl)-3-methyl-1-oxa-3-azaspiro[5.5]undecane,


and enantiomer

F. (2*RS*)-2-(cyclohex-1-enyl)-2-(4-methoxyphenyl)-*N,N*-dimethylethanamine,


and enantiomer

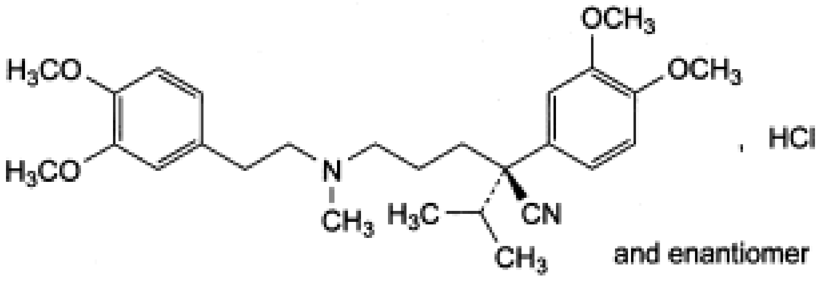
G. (2*RS*)-2-cyclohexyl-2-(4-methoxyphenyl)-*N,N*-dimethylethanamine.

Ph Eur

Verapamil Hydrochloride

General Notices

(Ph Eur monograph 0573)



$C_{27}H_{38}N_2O_4HCl$ 491.1 *152-11-4*

Action and use

Calcium channel blocker.

Preparations

Verapamil Injection

Verapamil Tablets

Prolonged-release Verapamil Tablets

Ph Eur

DEFINITION

(2*RS*)-2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]-2-(1-methylethyl)pentanenitrile hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in methanol, sparingly soluble in ethanol (96 per cent).

mp

About 144 °C.

IDENTIFICATION

First identification *B, D.*

Second identification *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 20.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml of this solution to 50.0 ml with *0.01 M hydrochloric acid*.

Spectral range 210-340 nm.

Absorption maxima At 229 nm and 278 nm.

Shoulder At 282 nm.

Absorbance ratio $A_{278}/A_{229} = 0.35$ to 0.39.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *verapamil hydrochloride CRS.*

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 5 ml with the same solvent.

Reference solution (a) Dissolve 20 mg of *verapamil hydrochloride CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *papaverine hydrochloride CRS* in reference solution (a) and dilute to 5 ml with reference solution (a).

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *diethylamine R, cyclohexane R* (15:85 *V/V*).

Application 5 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (b) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* while gently heating and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.5 to 6.0 for solution S.

Optical rotation *(2.2.7)*

- 0.10 ° to + 0.10 °, determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the initial mobile phase composition and dilute to 10.0 ml with the initial mobile phase composition.

Reference solution (a) Dissolve 5 mg of *verapamil hydrochloride CRS*, 5 mg of *verapamil impurity I CRS* and 5 mg of *verapamil impurity M CRS* in the initial mobile phase composition and dilute to 20 ml with the initial mobile phase composition. Dilute 1 ml of this solution to 10 ml with the initial mobile phase composition.

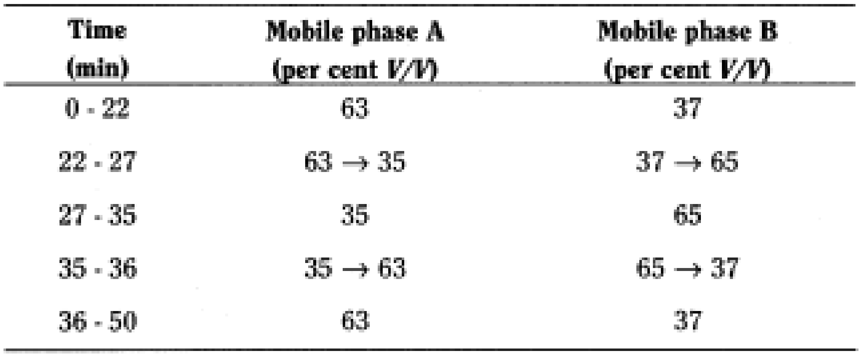
Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the initial mobile phase composition. Dilute 1.0 ml of this solution to 10.0 ml with the initial mobile phase composition.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped palmitamidopropylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: 6.97 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 7.20 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 22	63	37
22 - 27	63 → 35	37 → 65
27 - 35	35	65
35 - 36	35 → 63	65 → 37
36 - 50	63	37

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 278 nm.

Equilibration With the mobile phase at the initial composition for about 60 min.

Injection 10 µl.

Retention time Verapamil = about 16 min; impurity I = about 21 min; impurity M eluting as a doublet = about 32 min.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to verapamil and impurity I;

— impurity M elutes from the column.

Limits:

— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals *(2.4.8)*

Maximum 10 ppm.

1.0 g complies with test C. Prepare the reference solution using 1 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

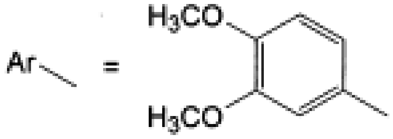
Dissolve 0.400 g in 50 ml of *anhydrous ethanol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20).* Measure the volume added between the 2 points of inflexion.

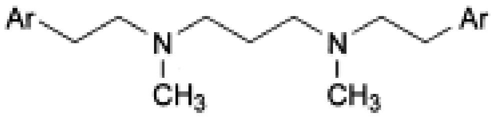
1 ml of *0.1 M sodium hydroxide* is equivalent to 49.11 mg of $C_{27}H_{39}ClN_2O_4$.

STORAGE

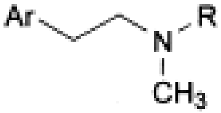
Protected from light.

IMPURITIES


Ar
=
H3CO
H3CO


Ar
N
CH3
N
CH3
Ar

A. *N,N′*-bis[2-(3,4-dimethoxyphenyl)ethyl]-*N,N′*-dimethylpropane-1,3-diamine,

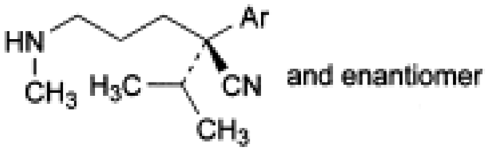


B. R = H: 2-(3,4-dimethoxyphenyl)-*N*-methylethanamine,

C. R = CH_3: 2-(3,4-dimethoxyphenyl)-*N,N*-dimethylethanamine,

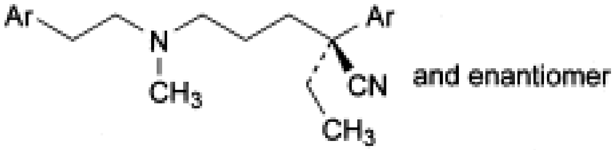
D. R = CH_2-CH_2-CH_2-Cl: 3-chloro-*N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylpropan-1-amine,

E. Ar-CH_2OH: (3,4-dimethoxyphenyl)methanol,

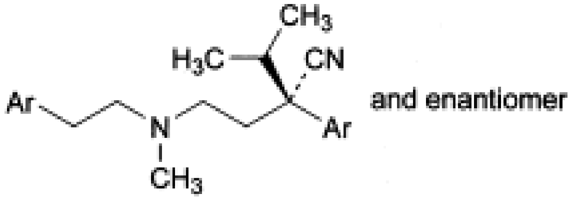

HN
CH3
H3C
CH3
Ar
CN
and enantiomer

F. (2*RS*)-2-(3,4-dimethoxyphenyl)-5-(methylamino)-2-(1-methylethyl)pentanenitrile,

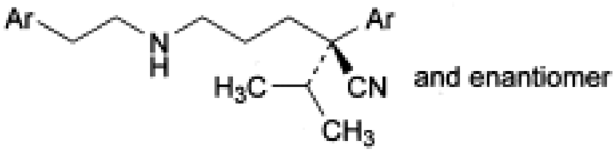
G. Ar-CHO: 3,4-dimethoxybenzaldehyde,


Ar
N
CH3
Ar
CN
CH3
and enantiomer

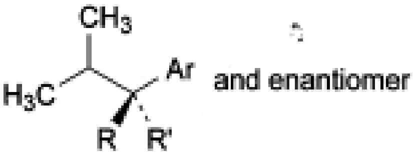
H. (2*RS*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]-2-ethylpentanenitrile,


CH3
H3C
CN
Ar
N
CH3
Ar
and enantiomer

I. (2*RS*)-2-(3,4-dimethoxyphenyl)-2-[2-[[2-(3,4-dimethoxy-phenyl)ethyl](methyl)amino]ethyl]-3-methylbutanenitrile,

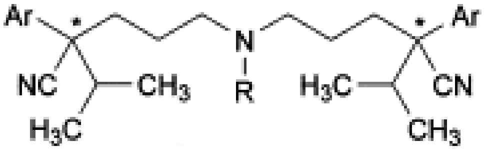

Ar
N
H
Ar
H3C
CN
CH3
and enantiomer

J. (2*RS*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxy-phenyl)ethyl]amino]-2-(1-methylethyl) pentanenitrile (*N*-norverapamil),


CH3
H3C
Ar
and enantiomer
R
R'

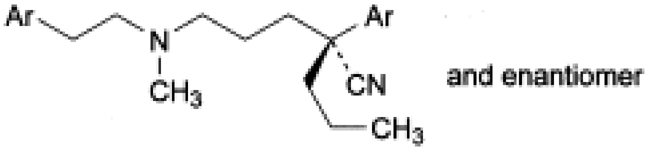
K. R = H, R′ = CN: (2*RS*)-2-(3,4-dimethoxyphenyl)-3-methylbutanenitrile,

L. R+ R′ = O: 1-(3,4-dimethoxyphenyl)-2-methylpropan-1-one,

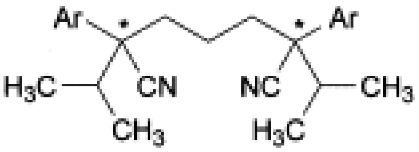

Ar
N
R
Ar
NC
CH3
H3C
CN
H3C
CH3

M. R = CH_2-CH_2-Ar: 5,5′-[[2-(3,4-dimethoxyphenyl)ethyl]imino]bis[2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile],

N. R = CH_3: 5,5′-(methylimino)bis[2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile],


Ar
N
CH3
Ar
CN
CH3
and enantiomer

O. (2*RS*)-2-(3,4-dimethoxyphenyl)-5-[2-[2-(3,4-dimethoxy-phenyl)ethyl](methyl)amino]- 2-propylpentanenitrile,

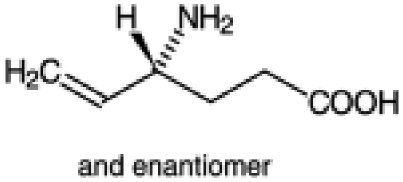

Ar
Ar
H3C
CN
NC
CH3
CH3
H3C

P. 2,6-bis(3,4-dimethoxyphenyl)-2,6-bis(1-methylethyl)-heptane-1,7-dinitrile.

Ph Eur

Vigabatrin

General Notices



$C_6H_{11}NO_2$ 129.2 *60643-86-9*

Action and use

Antiepileptic.

Preparations

Vigabatrin Oral Powder

Vigabatrin Tablets

DEFINITION

Vigabatrin is (*RS*)-4-aminohex-5-enoic acid. It contains not less than 98.0% and not more than 102.0% of $C_6H_{11}NO_2$, calculated with reference to the anhydrous, ethanol-free substance.

CHARACTERISTICS

A white to almost white powder.

Very soluble in *water*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of vigabatrin *(RS 360)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Optical rotation

+0.5° to-0.5°, determined in a 20% w/v solution, Appendix V F.

Related substances

The sum of the impurities determined by methods A and B below is not more than 0.5%.

A. Not more than 0.1% of any single impurity determined by the following method. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. Solution (1) contains 0.4% w/v of the substance being examined. Solution (2) contains 0.0004% w/v of *3-aminopent-4-ene-1,1-dicarboxylic acid BPCRS*. Solution (3) contains 0.0004% w/v of *5-vinyl-2-pyrrolidone BPCRS*. Solution (4) contains 0.0004% w/v of (E)*-4-amino-2-ethylidenebutyric acid hydrochloride BPCRS*. Solution (5) contains 0.002% w/v of *5-vinyl-2-pyrrolidone BPCRS* and 0.4% w/v of *vigabatrin BPCRS*.

The chromatographic procedure may be carried out using (a) two stainless steel columns in series; the first (25 cm × 4.6 mm) packed with particles of silica, the surface of which has been modified with chemically-bonded hexylsilyl groups (5 µm) (Spherisorb C6 is suitable) and the second (25 cm × 4.6 mm) packed with cation exchange resin (10 µm) (Partisil-10 SCX is suitable), (b) as the mobile phase at a flow rate of 1.0 ml per minute, a mixture of 25 volumes of *acetonitrile*, 25 volumes of a phosphate buffer solution prepared by dissolving 58.5 g of *sodium dihydrogen orthophosphate monohydrate* in *water*, adding 23 ml of *orthophosphoric acid* and sufficient *water* to produce 1000 ml, and 950 volumes of *water* and (c) a detection wavelength of 210 nm.

Inject 20 µl of solution (5). When the chromatograms are recorded under the prescribed conditions, the retention times are 5-vinyl-2-pyrrolidone, about 18 minutes and vigabatrin, about 21 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to 5-vinyl-2-pyrrolidone and vigabatrin is at least 1.5; if necessary, adjust the concentration of the mobile phase (reduce the concentration of the phosphate buffer solution to increase the retention time of vigabatrin and increase the concentration of acetonitrile to decrease the retention time of 5-vinyl-2-pyrrolidone).

Inject separately 20 µl of each of solutions (1) to (4). For solution (1) continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the areas of any peaks corresponding to 5-vinyl-2-pyrrolidone and *(E)*-4-amino-2-ethylidenebutyric acid are not greater than the areas of the peaks in the chromatograms obtained with solutions (3) and (4), respectively (0.1% of each). The area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%). Calculate the percentage content of 5-vinyl-2-pyrrolidone and *(E)*-4-amino-2-ethylidenebutyric acid using the areas of the peaks in the chromatograms obtained with solutions (3) and (4) respectively and of any other impurity from the peak in the chromatogram obtained with solution (2) taking, for the purposes of calculation, that this peak is equivalent to 0.1%, and hence determine the sum of the contents of the impurities.

B. Not more than 0.2% of 4-aminobutyric acid when determined by the following method. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add to 1 ml of a 0.20% w/v solution of the substance being examined 2 ml of a solution prepared by dissolving 7.7 g of *boric acid* in *water*, adjusting to pH 7.7 with a 50% w/v solution of *sodium hydroxide* and diluting to 250 ml with *water* and mix. Add 3 ml of a 0.16% w/v solution of *(9-fluorenyl)methyl chloroformate* in *acetone*, mix and allow to stand for 5 minutes. Add 3 ml of *ethyl acetate*, shake vigorously for a few seconds, and allow to separate; use the lower layer within 8 hours of preparation. Prepare solution (2) in the same manner using 1 ml of a solution containing 0.20% w/v of the substance being examined and 0.002% w/v of *4-aminobutyric acid* in place of the solution of the substance being examined.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemically-bonded phenyl groups (5 µm) (Vydac Phenyl is suitable), (b) as the mobile phase at a flow rate of 1.0 ml per minute, a mixture of 25 volumes of *acetonitrile* and 75 volumes of a solution prepared by dissolving 8.2 g of *anhydrous sodium acetate* in *water*, adjusting the pH to 4.2 with *glacial acetic acid* and diluting to 2000 ml with *water* and (c) a detection wavelength of 263 nm.

Inject 25 µl of solution (2). When the chromatogram is recorded under the prescribed conditions, the retention times for the derivatives of the following are (9-fluorenyl)methanol, about 6 minutes; 4-aminobutyric acid, about 9 minutes and vigabatrin, about 14 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to the derivatives of 4-aminobutyric acid and (9-fluoreny)-lmethanol is at least 2; if necessary, adjust the proportion of the components of the mobile phase.

Inject separately 25 µl of each of solutions (1) and (2) and calculate the content of 4-aminobutyric acid in the substance being examined from the chromatograms obtained.

Heavy metals

2.0 g complies with *limit test C for heavy metals*, Appendix VII. Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard (10 ppm).

Ethanol

Not more than 0.6% w/w of ethanol when determined by the following method. Carry out the method for head-space *gas chromatography*, Appendix III B. Solution (1) contains 2 g of the substance being examined in 10 ml of a solution containing 0.0025% w/v of *1,2-dichloroethane* (internal standard) in *water* in a 20 ml headspace vial. Heat the vial at 60° for 30 minutes. Solution (2) contains 0.025% w/v of *absolute ethanol* and 0.0025% w/v of *1,2-dichloro-ethane* in *water*. Use *water* as the blank.

The chromatographic procedure may be carried out using a fused-silica column (60 m × 0.32 mm) coated with a 1.0-µm film of bonded methylsilicone (SPB-1 is suitable) at an initial temperature of 35° for 12 minutes, increasing to 175° at a constant rate of 10° per minute, maintaining the injector at 150° and the detector at 250°. Use *helium* as the carrier gas. Calculate the content of ethanol in the substance being examined from the areas of the peaks in the chromatograms obtained with solutions (1) and (2).

Water

Not more than 0.5% w/w, Appendix IX C. Use 0.3 g dissolved in 50 ml of *anhydrous methanol* .

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

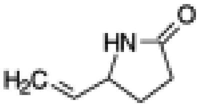
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. Solution (1) contains 0.2% w/v of the substance being examined. Solution (2) contains 0.2% w/v of *vigabatrin BPCRS*. Solution (3) contains 0.002% w/v of *5-vinyl-2-pyrrolidone BPCRS* and 0.2% w/v of *vigabatrin BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with cation exchange resin (10 µm) (Whatman Partisil 10 SCX is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 1000 volumes of a 0.34% w/v solution of *potassium dihydrogen orthophosphate*, the mixture adjusted to pH 2.8 with *orthophosphoric acid* and (c) a detection wavelength of 210 nm.

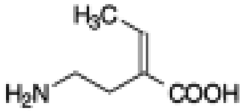
Inject 20 µl of solution (3). When the chromatogram is recorded under the prescribed conditions, the retention times are 5-vinyl-2-pyrrolidone, about 5 minutes and vigabatrin, about 8 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to 5-vinyl-2-pyrrolidone and vigabatrin is at least 1.5.

Inject separately 20 µl of solutions (1) and (2) and calculate the percentage content of vigabatrin from the areas of the peaks using the declared content of $C_6H_{11}NO_2$ in *vigabatrin BPCRS*.

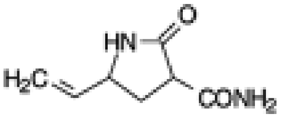
IMPURITIES



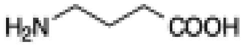
A. 5-vinyl-2-pyrrolidone



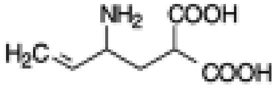
B. (*E*)-4-amino-2-ethylidenebutyric acid



C. 2-oxo-5-vinylpyrrolidine-3-carboxamide



D. 4-aminobutyric acid

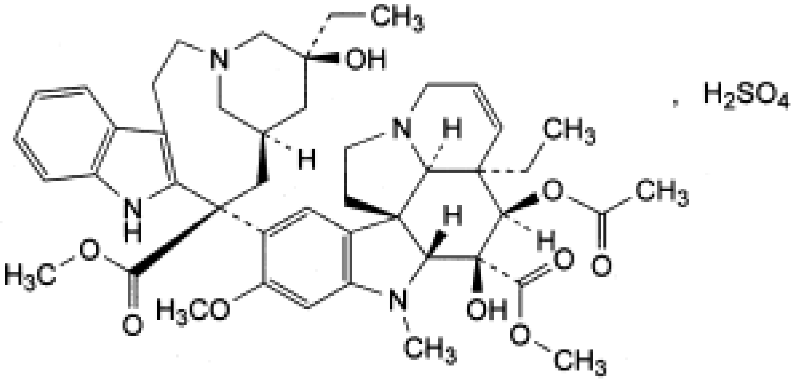


E. 3-aminopent-4-ene-1,1-dicarboxylic acid

Vinblastine Sulphate

General Notices

(Ph Eur monograph 0748)



$C_{46}H_{58}N_4O_9,H_2SO_4$ 909 *143-67-9*

Action and use

Vinca alkaloid cytotoxic.

Preparation

Vinblastine Injection

Ph Eur

DEFINITION

Vinblastine sulphate contains not less than 95.0 per cent and not more than the equivalent of 104.0 per cent of methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate sulphate, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, very hygroscopic, freely soluble in water, practically insoluble in alcohol.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry *(2.2.24),* comparing with the *Ph. Eur. reference spectrum of vinblastine sulphate.*

B. Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution is similar in position and approximate size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 50.0 mg in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method I).*

pH *(2.2.3)*

Dilute 3 ml of solution S to 10 ml with *carbon dioxide-free water R.* The pH of this solution is 3.5 to 5.0.

Related substances

Examine the chromatograms obtained in the assay. In the chromatogram obtained with the test solution, the area of any peak apart from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent). Disregard any peak with an area less than that of the peak in the chromatogram obtained with reference solution (d).

Loss on drying

Not more than 15.0 per cent, determined on 3 mg by thermogravimetry *(2.2.34).* Heat to 200 °C at a rate of 5 °C/min, under a stream of *nitrogen for chromatography R*, at a flow rate of 40 ml/min.

ASSAY

Examine by liquid chromatography *(2.2.29)*.

Keep the solutions in iced water before use.

Test solution Dilute 1.0 ml of solution S (see Tests) to 5.0 ml with *water R*.

Reference solution (a) Dissolve the contents of a vial of *vinblastine sulphate CRS* in 5.0 ml of *water R* to obtain a concentration of 1.0 mg/ml.

Reference solution (b) Dissolve 1.0 mg of *vincristine sulphate CRS* in 1.0 ml of reference solution (a).

Reference solution (c) Dilute 1.0 ml of reference solution (a) to 50.0 ml with *water R*.

Reference solution (d) Dilute 1.0 ml of reference solution (c) to 20.0 ml with *water R.*

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm). Place between the injector and the column a guard column packed with suitable silica gel;

— as mobile phase at a flow rate of 1.0 ml/min a mixture of 38 volumes of a 1.5 per cent *V/V* solution of *diethylamine R* adjusted to pH 7.5 with *phosphoric acid R,* 12 volumes of *acetonitrile R* and 50 volumes of *methanol R*;

— as detector a spectrophotometer set at 262 nm;

— a loop injector.

Inject 10 µl of each solution and record the chromatograms for three times the retention time of the peak corresponding to vinblastine. The assay is not valid unless: in the chromatogram obtained with reference solution (b) the resolution between the peaks corresponding to vincristine and vinblastine is not less than four; the peak in the chromatogram obtained with reference solution (d) has a signal-to-noise ratio not less than five. Calculate the percentage content of $C_{46}H_{60}N_4O_{13}S$ from the area of the principal peak in each of the chromatograms obtained with the test solution and reference solution (a) and from the declared content of *vinblastine sulphate CRS*.

STORAGE

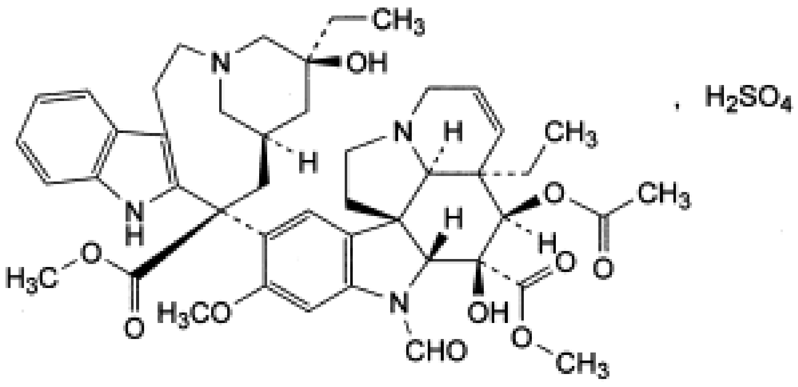
Store in an airtight, glass container, protected from light, at a temperature not exceeding - 20 °C. If the substance is sterile, store in an sterile, airtight, tamper-proof glass container.

Ph Eur

Vincristine Sulphate

General Notices

(Ph Eur monograph 0749)



$C_{46}H_{56}N_4O_{10},H_2SO_4$ 923.1 *2068-78-2*

Action and use

Vinca alkaloid cytotoxic.

Preparation

Vincristine Injection

Ph Eur

DEFINITION

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate sulphate.

Content

95.0 per cent to 104.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder, very hygroscopic.

Solubility

Freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of vincristine sulphate.*

TESTS

Solution S

Dissolve 50.0 mg in *carbon dioxide-free water R* and dilute to 10.0 ml with the same solvent. *Keep the solution in iced water to carry out the test for related substances*.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method I)*.

pH *(2.2.3)*

3.5 to 4.5.

Dilute 2 ml of solution S to 10 ml with *carbon dioxide-free water R*.

Related substances

Liquid chromatography *(2.2.29)*. *Keep the solutions in iced water before use*.

Test solution Dilute 1.0 ml of solution S to 5.0 ml with *water R*.

Reference solution (a) Dissolve the contents of a vial of *vincristine sulphate CRS* in 5.0 ml of *water R* to obtain a concentration of 1.0 mg/ml.

Reference solution (b) Dissolve 1.0 mg of *vinblastine sulphate CRS* in 1.0 ml of reference solution (a).

Reference solution (c) Dilute 1.0 ml of the test solution to 50.0 ml with *water R.*

Reference solution (d) Dilute 1.0 ml of reference solution (c) to 20.0 ml with *water R*.

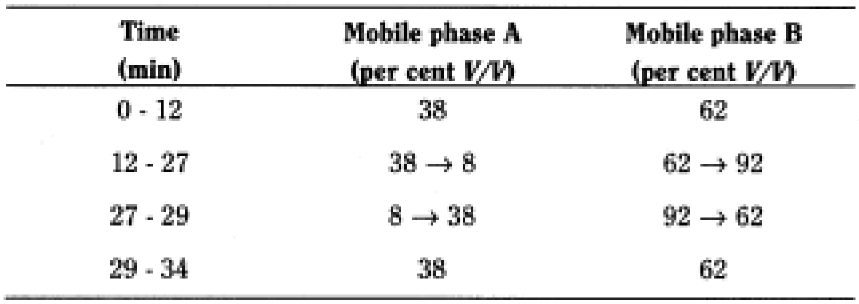
Precolumn:
— *stationary phase*: *octylsilyl silica gel for chromatography R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 1.5 per cent *V/V* solution of *diethylamine R* adjusted to pH 7.5 with *phosphoric acid R*;

— *mobile phase B*: *methanol R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	38	62
12 - 27	38 → 8	62 → 92
27 - 29	8 → 38	92 → 62
29 - 34	38	62

Flow rate 2 ml/min.

Detection Spectrophotometer at 297 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 4 between the peaks due to vincristine and vinblastine.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent);

— *disregard limit*: area of the peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Loss on drying

Maximum 12.0 per cent, determined on 3 mg by thermogravimetry *(2.2.34)*. Heat the substance to be examined to 200 °C increasing the temperature by 5 °C/min, under a current of *nitrogen for chromatography R*, at a flow rate of 40 ml/min.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances, with the following modifications.

Mobile phase Mix 30 volumes of a 1.5 per cent *V/V* solution of *diethylamine R* adjusted to pH 7.5 with *phosphoric acid R* and 70 volumes of *methanol R*.

Flow rate 1.0 ml/min.

Calculate the percentage content of $C_{46}H_{58}N_4O_{14}S$ using the chromatogram obtained with reference solution (a) and the declared content of *vincristine sulphate CRS.*

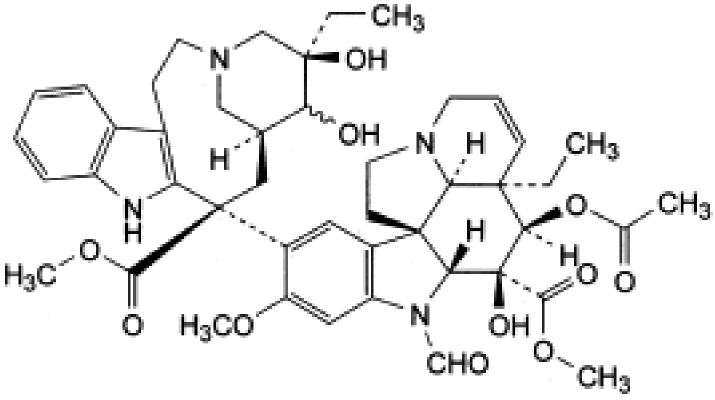
STORAGE

In an airtight, glass container, protected from light, at a temperature not exceeding - 20 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof glass container.

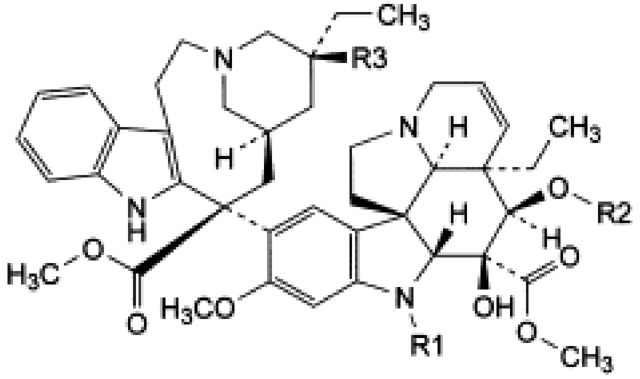
IMPURITIES

Specified impurities A, B, C, D, H.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): E, F, G.*



A. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*R*,7*S*,9*S*)-5-ethyl-5,6-dihydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (3'-hydroxy-VCR),



B. R1 = CHO, R2 = CO-CH_3, R3 = H: methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R,*13a*R)*-4-(acetyloxy)-3a-ethyl-9-[(5*R,*7*S*,9*S*)-5-ethyl-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (4′-deoxyvincristine),

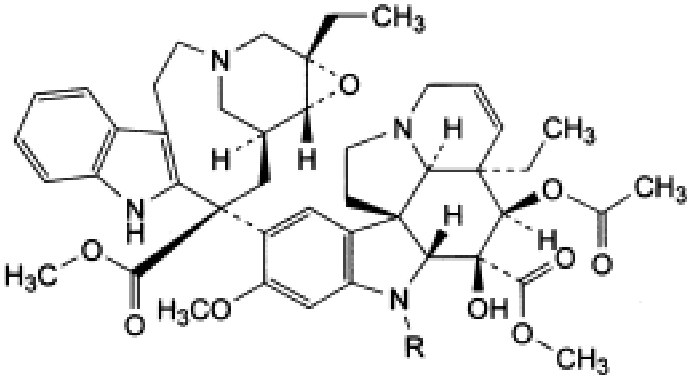
C. R1 = H, R2 = CO-CH_3, R3 = OH: methyl (3a*R*,4*R*,5*S*,5a*R*,10b*S*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (*N*-desmethylvinblastine),

D. R1 = CHO, R2 = H, R3 = OH: methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-4,5-dihydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (deacetylvincristine),

E. R1 = CH_3, R2 = H, R3 = OH: methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-3a-ethyl-9-[(5*S*,7*R*,9*S*)

-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-4,5-dihydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (deacetylvinblastine),

H. R1 = CH_3, R2 = CO-CH_3, R3 = OH: vinblastine,



F. R = CH_3: methyl (3a*R*,4*R*,5*S*,5a*R*,10bR,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(1a*S*,11*S*,13*S*,13a*R*)-1a-ethyl-11-(methoxycarbonyl)-1a,4,5,10,11,12,13,13a-octahydro-2*H*-3,13-methano-oxireno[9,10]azacycloundecino[5,4-*b*]indol-11-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (leurosine),

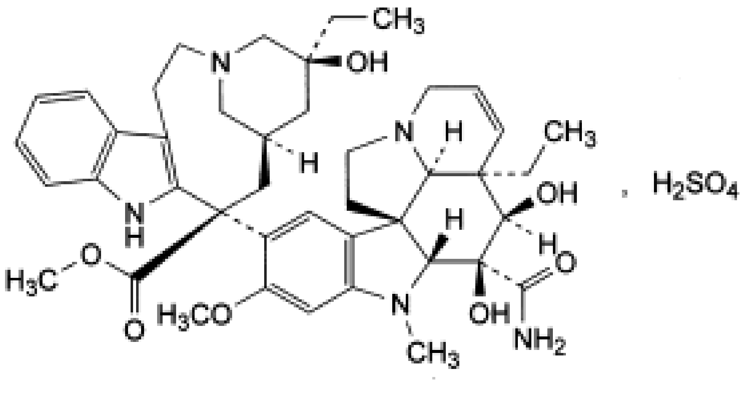
G. R = CHO: methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(1a*S*,11*S*,13*S*,13a*R*)-1a-ethyl-11-(methoxycarbonyl)-1a,4,5,10,11,12,13,13a-octahydro-2*H*-3,13-methano-oxireno[9,10]azacycloundecino[5,4-*b*]indol-11-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (formylleurosine).

Ph Eur

Vindesine Sulphate

General Notices

(Ph Eur monograph 1276)



$C_{43}H_{57}N_5O_{11}S$ 852.0 *59917-39-4*

Action and use

Vinca alkaloid cytotoxic.

Preparation

Vindesine Injection

Ph Eur

DEFINITION

Methyl (5*S*,7*R*,9*S*)-9-[(3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-5-carbamoyl-3a-ethyl-4,5-dihydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazol-9-yl]-5-ethyl-5-hydroxy-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[4,5-*b*]indole-9-carboxylate sulphate.

Content

96.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, amorphous, hygroscopic substance.

Solubility

Freely soluble in water and in methanol, practically insoluble in cyclohexane.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison   Ph. Eur. reference spectrum of vindesine sulphate.

TESTS

Solution S

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method I)*.

pH *(2.2.3)*

3.5 to 5.5 for solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Keep the solutions in iced water before use*.

Test solution   Dissolve 10.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a)   Dilute 1.0 ml of the test solution to 50.0 ml with *water R.*

Reference solution (b)   Dissolve 1.0 mg of *desacetylvinblastine CRS* in *water R*, add 1.0 ml of the test solution and dilute to 50.0 ml with *water R.*

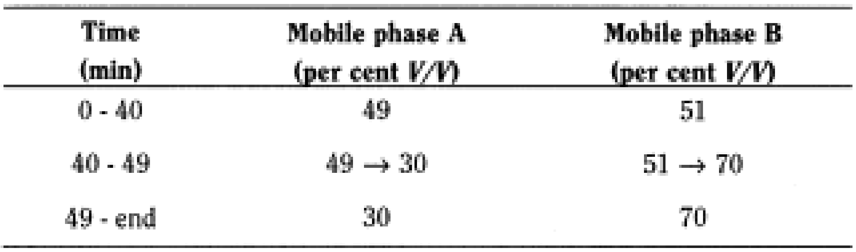
Reference solution (c)   Dilute 1.0 ml of reference solution (a) to 200.0 ml with *water R*.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: 1.5 per cent *V/V solution of diethylamine R* adjusted to pH 7.4 with *phosphoric acid R;*
- *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	49	51
40 - 49	49 → 30	51 → 70
49 - end	30	70

Flow rate   2 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 200 µl.

Run time Twice the retention time of vindesine.

System suitability Reference solution (b)

- the retention time of vindesine is less than 40 min;
- *resolution*: minimum 2.0 between the peaks due to vindesine and desacetylvinblastine;
- *symmetry factor*: maximum 2.0 for the peak due to vindesine.

Limits:

- *impurities A, B, C*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.01 per cent).

Acetonitrile

Gas chromatography (*2.2.28*).

Internal standard solution (a) Dilute 0.500 g of *propanol R* to 100 ml with *water R.*

Internal standard solution (b) Dilute 10.0 ml of internal standard solution (a) to 50.0 ml with *water R*.

Reference solution Dilute 10.0 g of *acetonitrile R* to 1000 ml with *water R*. To 3.0 ml of this solution add 10.0 ml of internal standard solution (a) and dilute to 50.0 ml with *water R*.

Test solution Dissolve 40 mg of the substance to be examined in 1.0 ml of internal standard solution (b).

Column:

- *material*: glass;
- *size*: *l* = 1.25 m, Ø = 3 mm;
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R*.

Carrier gas *Helium for chromatography R*.

Flow rate 60 ml/min.

Temperature:

- *column*: 170 °C;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 3 µl.

System suitability Reference solution:

- *resolution*: minimum 1.5 between the peaks due to acetonitrile and propanol;
- *symmetry factor*: maximum 1.6 for the peak due to acetonitrile.

Limit:

- *acetonitrile*: maximum 1.5 per cent m/m.

Loss on drying

Maximum 10.0 per cent, determined on 9.00 mg by thermogravimetry *(2.2.34)*. Heat to 200 °C at a rate of 5 °C/min, under a stream of *nitrogen for chromatography R* at a flow rate of 40 ml/min.

ASSAY

Liquid chromatography *(2.2.29)*. *Keep the solutions in iced water before use*.

Test solution Dissolve 5.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve and dilute the entire contents of a vial of *vindesine sulphate CRS* with *water R* to yield a concentration of approximately 0.50 mg/ml.

Reference solution (b) Add 1.0 mg of *desacetylvinblastine CRS* to 2.0 ml of reference solution (a).

Column:

- *size*: *l* = 0.15 m, 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 38 volumes of a 1.5 per cent *V/V* solution of *diethylamine R*, previously adjusted to pH 7.4 with *phosphoric acid R*, and 62 volumes of *methanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µl.

System suitability Reference solution (b):

- *resolution*: minimum 1.5 between the peaks due to vindesine and desacetylvinblastine;
- *symmetry factor*: maximum 2.0 for the peak due to vindesine;
- *repeatability*: maximum relative standard deviation of 1.5 per cent for the peak due to vindesine after 5 injections.

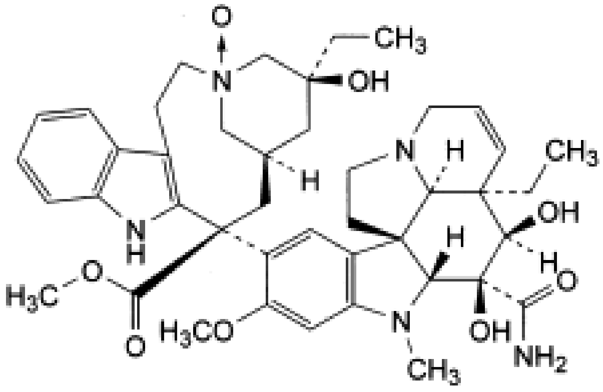
Calculate the percentage content of $C_{43}H_{57}N_5O_{11}S$ from the declared content of *vindesine sulphate CRS.*

STORAGE

In an airtight polypropylene container with a polypropylene cap, at a temperature of - 50 °C or below. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

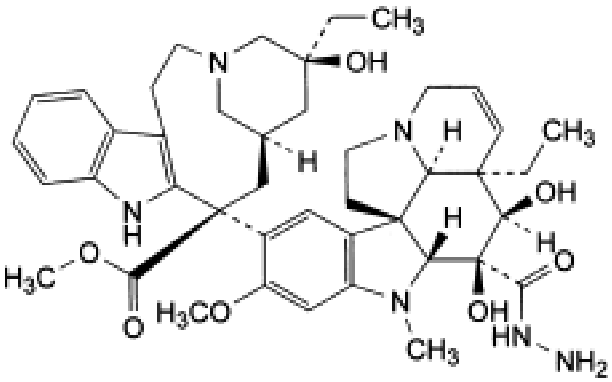
IMPURITIES

Specified impurities *A, B, C.*



A. vindesine 3′-*N*-oxide,

B. vinblastine,



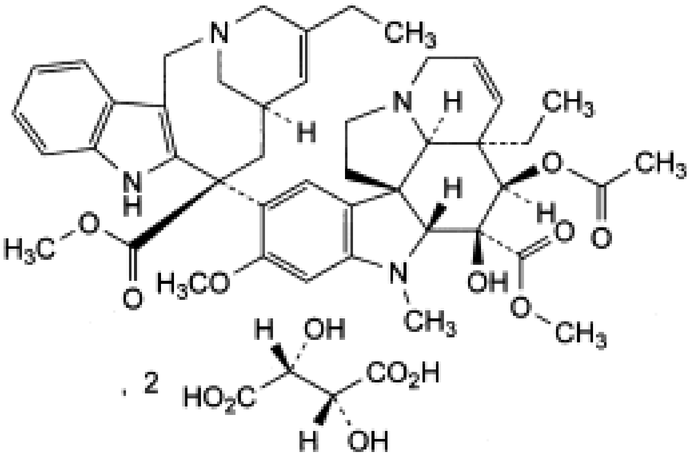
C. desacetylvinblastine hydrazide.

Ph Eur

Vinorelbine Tartrate

General Notices

(Ph Eur monograph 2107)



$C_{45}H_{54}N_4O_8,2C_4H_6O_6$ 1079 *125317-39-7*

Action and use

Vinca alkaloid cytotoxic.

Ph Eur

DEFINITION

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(6*R*,8*S*)-4-ethyl-8-(methoxycarbonyl)-1,3,6,7,8,9-hexahydro-2,6-methano-2*H*-azacyclodecino[4,3-*b*]indol-8-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate dihydrogen bis[(2*R*,3*R*)-2,3-dihydroxybutanedioate].

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Freely soluble in water and in methanol, practically insoluble in hexane.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 10 mg in 5 ml of *water R.* Add 0.5 ml of *sodium hydroxide solution R.* Extract with 5 ml of *methylene chloride R*. Dry the organic layer over *anhydrous sodium sulphate R*, filter and reduce its volume to about 0.5 ml by evaporation and apply to a disc of *potassium bromide R.* Evaporate and record the spectrum.

Comparison *vinorelbine tartrate CRS*, treated as described above.

B. It gives reaction (b) of tartrates *(2.3.1)*.

TESTS

Solution S

Dissolve a quantity equivalent to 0.140 g of the anhydrous substance in *water R* and dilute to 10.0 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 420 nm is not greater than 0.030.

pH *(2.2.3)*

3.3 to 3.8 for solution S.

Related substances

Liquid chromatography *(2.2.29)*: use the normalisation procedure.

Test solution Dissolve 35.0 mg of the substance to be examined in the mobile phase and dilute to 25 ml with the mobile phase.

Reference solution (a) Dissolve 7 mg of *vinorelbine impurity B CRS* in *water R* and dilute to 50 ml with the same solvent. To 1 ml of this solution add 14 mg of *vinorelbine tartrate CRS*, dissolve in *water R* and dilute to 10 ml with the same solvent. Expose this solution for 1 h to a xenon lamp apparatus at a wavelength of 310-880 nm, supplying a dose of 1600 kJ/m2 at a fluence rate of 500 W/m2 in order to generate impurity A.

Reference solution (b) Dilute 1.0 ml of the test solution to 20.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

- — *size*: *l* = 0.15 m, Ø = 3.9 mm;
- — *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 125 m2/g, a pore size of 30 nm and a carbon loading of 7 per cent;
- — *temperature*: 35 ± 5 °C.

Mobile phase Dissolve 1.22 g of *sodium decanesulphonate R* in 620 ml of *methanol R* and dd 380 ml of a 7.80 g/l solution of *sodium dihydrogen phosphate R* previously adjusted to pH 4.2 with dilute *phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 267 nm.

Injection 20 µl.

Run time Twice the retention time of vinorelbine.

Relative retention With reference to vinorelbine (retention time = about 14 min): impurity A = about 0.8; impurity B = about 1.2.

System suitability:

— *peak-to-valley ratio*: minimum 4, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to vinorelbine in the chromatogram obtained with reference solution (a);

— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *impurity A*: maximum 0.3 per cent;

— *any other impurity*: for each impurity, maximum 0.2 per cent;

— *sum of impurities other than A*: maximum 0.7 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b).

Boron

Maximum 50 ppm.

Test solution Dissolve 0.10 g of the substance to be examined in 2 ml of *water R*. Slowly add 10.0 ml of *sulphuric acid R* while cooling in iced water. Stir and allow to warm to room temperature. Add 10.0 ml of a 0.5 g/l solution of *carminic acid R* in *sulphuric acid R*.

Reference solution Dilute 2.5 ml of a 0.572 g/l solution of *boric acid R* to 100.0 ml with *water R*. To 2.0 ml of this solution slowly add 10.0 ml of *sulphuric acid R* while cooling in iced water. Stir and allow to warm to room temperature. Add 10.0 ml of a 0.5 g/l solution of *carminic acid R* in *sulphuric acid R*.

Blank solution To 2.0 ml of *water R* slowly add 10.0 ml of *sulphuric acid R* while cooling in iced water. Stir and allow to warm to room temperature. Add 10.0 ml of a 0.5 g/l solution of *carminic acid R* in *sulphuric acid R*.

After 45 min, measure the absorbance *(2.2.25)* of the test solution and the reference solution, between 560 nm and 650 nm, using the blank solution as compensation liquid. The maximum absorbance value of the test solution is not greater than that of the reference solution.

Fluorides

Maximum 50 ppm.

Potentiometry *(2.2.36, Method I)* using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode.

Test solution Dissolve 0.19 g of the substance to be examined in 20 ml of *water R.* Add 5.0 ml of *total-ionic-strength-adjustment buffer R* and dilute to 50 ml with *water R.*

Reference solutions To 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml and 1.4 ml of *fluoride standard solution (10 ppm F) R*, add 5.0 ml of *total-ionic-strength-adjustment buffer R* and dilute to 50 ml with *water R.*

Introduce the electrodes into the reference solutions and allow to stand for 5 min. Determine the potential difference between the electrodes after 1 min of stabilisation. Using semi-logarithmic paper plot the potential difference obtained for each reference solution as a function of concentration of fluoride. Using exactly the same conditions, determine the potential difference obtained with the test solution and calculate the content of fluoride.

Silver

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 0.500 g of the substance to be examined in 10.0 ml of *water R.*

Reference solutions Prepare the reference solutions using *silver standard solution (5 ppm Ag) R* and diluting with a 6.5 per cent *V/V* solution of *lead-free nitric acid R.*

Source Silver hollow-cathode lamp.

Wavelength 328.1 nm.

Atomisation device Air-acetylene flame.

Water *(2.5.12)*

Maximum 4.0 per cent, determined on 0.250 g.

Bacterial endotoxins *(2.6.14)*

Less than 2 IU/mg (expressed as vinorelbine base), if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.350 g in 40 ml of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 53.96 mg of $C_{53}H_{66}N_4O_20$.

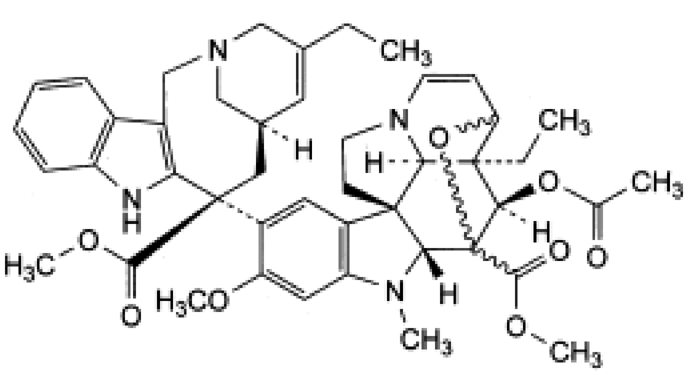
STORAGE

Under an inert gas, protected from light, at a temperature not exceeding - 15 °C.

IMPURITIES

Specified impurities A.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E, F, G, H, I, J.*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CH_3
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O
CH_3
H
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O
CH_3
N
H
H
H_3C
O
O
O
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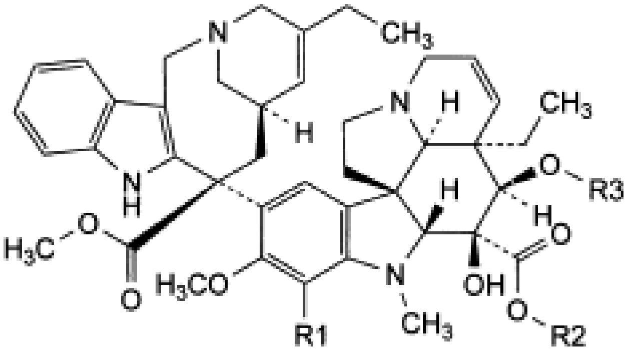
H_3CO
N
H
O
O
CH_3
CH_3

A. methyl (3a*S*,4*R*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3,5-epoxy-3a-ethyl-9-[(6*R*,8*S*)-4-ethyl-8-(methoxycarbonyl)-1,3,6,7,8,9-hexahydro-2,6-methano-2*H*-azacyclodecino[4,3-*b*]indol-8-yl]-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-3*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate,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CH_3
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H
CH_3
H
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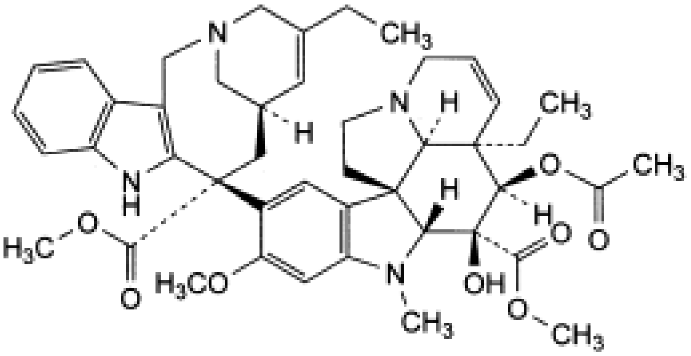
H
H
O
R3
H
H_3C
O
O
O
H_3CO
N
OH
O
R1
CH_3
R2

B. R1 = R3 = H, R2 = CH_3: methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-3a-ethyl-9-[(6*R*,8*S*)-4-ethyl-8-(methoxycarbonyl)-1,3,6,7,8,9-hexahydro-2,6-methano-2*H*-azacyclodecino[4,3-*b*]indol-8-yl]-4,5-dihydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate,

H. R1 = R2 = H, R3 = CO-CH_3: (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(6*R*,8*S*)-4-ethyl-8-(methoxycarbonyl)-1,3,6,7,8,9-hexahydro-2,6-methano-2*H*-azacyclodecino[4,3-*b*]indol-8-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylic acid,

I. R1 = Br, R2 = CH_3, R3 = CO-CH_3: methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-7-bromo-3a-ethyl-9-[(6*R*,8*S*)-4-ethyl-8-(methoxycarbonyl)-1,3,6,7,8,9-hexahydro-2,6-methano-2*H*-azacyclodecino[4,3-*b*]indol-8-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate,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CH_3
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CH_3
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H
H
O
CH_3
H
H_3C
O
O
O
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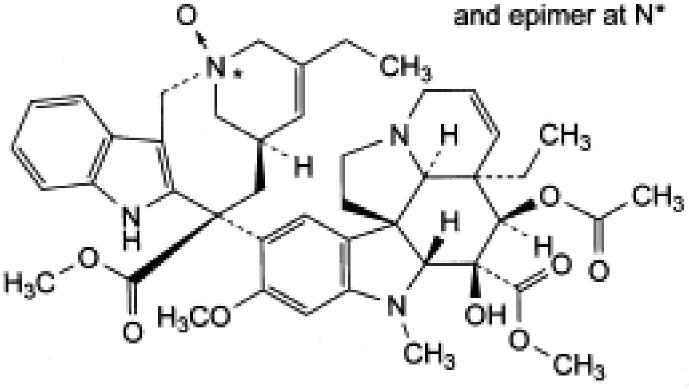
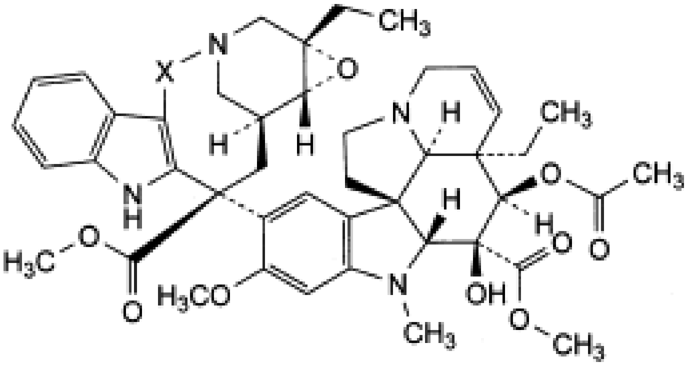
H_3CO
N
OH
O
CH_3
CH_3

C. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(6*R*,8*R*)-4-ethyl-8-(methoxycarbonyl)-1,3,6,7,8,9-hexahydro-2,6-methano-2*H*-azacyclodecino[4,3-*b*]indol-8-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate,



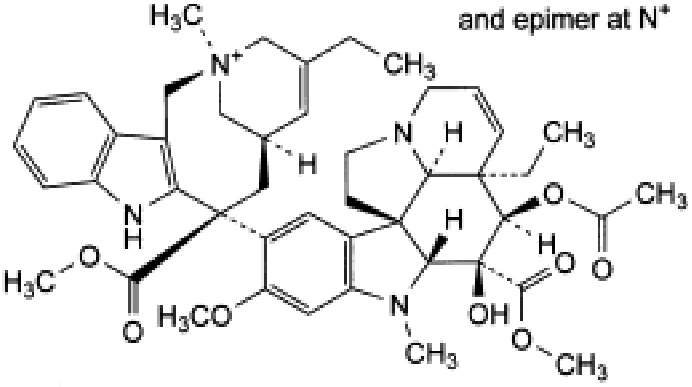
and epimer at N*

D. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(2*RS*,6*R*,8*S*)-4-ethyl-8-(methoxycarbonyl)-2-oxido-1,3,6,7,8,9-hexahydro-2,6-methano-2*H*-azacyclodecino[4,3-*b*]indol-8-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate,



E. X = CH_2-CH_2: methyl (1a*S*,11*S*,13*S*,13a*R*)-11-[(3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-5-hydroxy-8-methoxy-5-(methoxycarbonyl)-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazol-9-yl]-1a-ethyl-1a,4,5,10,11,12,13,13a-octahydro-2*H*-3,13-methanooxireno[9,10]azacycloundecino[5,4-*b*]indole-11-carboxylate (leurosine),

G. X = CH_2: methyl (1a*S*,10*S*,12*S*,12a*R*)-10-[(3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-5-hydroxy-8-methoxy-5-(methoxycarbonyl)-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazol-9-yl]-1a-ethyl-1a,2,4,9,10,11,12,12a-octahydro-3,12-methano-3*H*-oxireno[8,9]azacyclodecino[4,3-*b*]indole-10-carboxylate,


H₃C
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N
H
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CH₃
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H₃C
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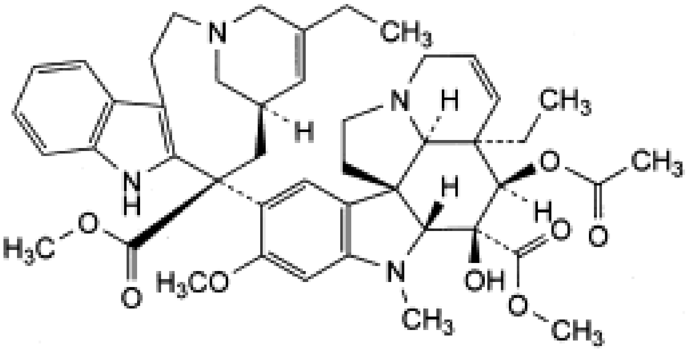
O
O
H₃CO
N
OH
O
CH₃
O
CH₃

and epimer at N⁺

F. (2*RS*,6*R*,8*S*)-8-[(3a*R*,4*R*,5*S*,5a*R,*10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-5-hydroxy-8-methoxy-5-(methoxycarbonyl)-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazol-9-yl]-4-ethyl-8-(methoxycarbonyl)-2-methyl-1,3,6,7,8,9-hexahydro-2,6-methano-2*H*-azacyclodecino[4,3-*b*]indolium,


N
CH₃
N
H
H
CH₃
O
CH₃
N
H
H
H
H₃C
O
H
O
O
H₃CO
N
OH
O
CH₃
O
CH₃

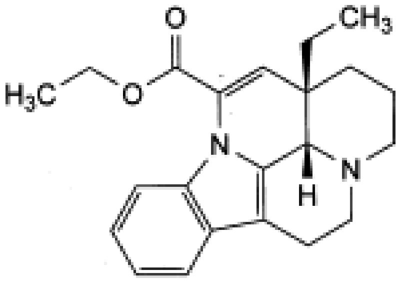
J. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(7*R*,9*S*)-5-ethyl-9-(methoxycarbonyl)-1,4,7,8,9,10-hexahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*] carbazole-5-carboxylate.

Ph Eur

Vinpocetine

General Notices

(Ph Eur monograph 2139)



$C_{22}H_{26}N_2O_2$ 350.5 *42971-09-5*

Action and use

Vasodilator.

Ph Eur

DEFINITION

Ethyl (13a*S*,13b*S*)-13a-ethyl-2,3,5,6,13a,13b-hexahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Practically insoluble in water, soluble in methylene chloride, slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *vinpocetine CRS.*

TESTS

Specific optical rotation *(2.2.7)*

+ 127 to + 134 (dried substance).

Dissolve 0.25 g in *dimethylformamide R* and dilute to 25.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *vinpocetine impurity B CRS*, 6.0 mg of *vinpocetine impurity A CRS*, 5.0 mg of *vinpocetine impurity C CRS* and 5.0 mg of *vinpocetine impurity D CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (c) Dilute 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 20.0 ml with the mobile phase.

Column:

- *size:* *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl end-capped silica gel for chromatography R* (5 µm).

Mobile phase 15.4 g/l solution of *ammonium acetate R*, *acetonitrile R* (45:55 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 15 µl.

Run time 3 times the retention time of vinpocetine.

Relative retention With reference to vinpocetine (retention time = about 16 min): impurity A = about 0.4; impurity D = about 0.68; impurity B = about 0.75; impurity C = about 0.83.

System suitability Reference solution (c):

- *resolution*: minimum 2.0 between the peaks due to impurities B and D.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.6 per cent);
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *impurity C*: not more than 0.6 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the peak due to vinpocetine in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 10 times the area of the peak due to vinpocetine in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the peak due to vinpocetine in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* in an oven at 100 °C for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

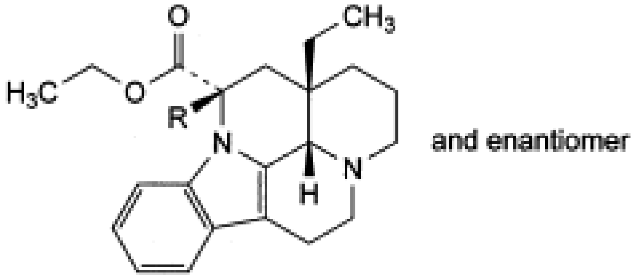
ASSAY

Dissolve 0.300 g in 50 ml of a mixture of equal volumes of *acetic anhydride R* and *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 35.05 mg of $C_{22}H_{26}N_2O_2$.

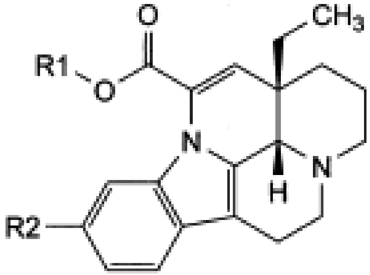
IMPURITIES

Specified impurities *A, B, C, D.*



A. R = OH: ethyl (12*RS*,13a*SR*,13b*SR*)-13a-ethyl-12-hydroxy-2,3,5,6,12,13,13a,13b-octahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate (ethyl vincaminate),

D. R = H: ethyl (12*RS*,13a*RS*,13b*RS*)-13a-ethyl-2,3,5,6,12,13,13a,13b-octahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate (dihydrovinpocetine),



B. R1 = CH_3, R2 = H: methyl (13a*S*,13b*S*)-13a-ethyl-2,3,5,6,13a,13b-hexahydro-1*H*-indolo[3, 2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate (apovincamine),

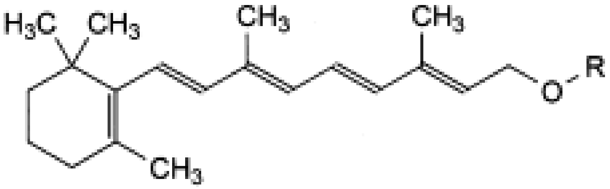
C. R1 = C_2H_5, R2 = OCH_3: ethyl (13a*S*,13b*S*)-13a-ethyl-9-methoxy-2,3,5,6,13a,13b-hexahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate (methoxyvinpocetine).

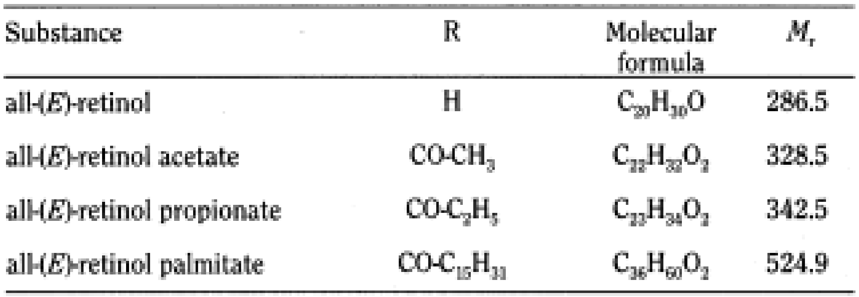
Ph Eur

Vitamin A

General Notices

(Ph Eur monograph 0217)





Substance	R	Molecular formula	M_r
all-(*E*)-retinol	H	$C_{20}H_{30}O$	286.5
all-(*E*)-retinol acetate	$CO\text{-}CH_3$	$C_{22}H_{32}O_2$	328.5
all-(*E*)-retinol propionate	$CO\text{-}C_2H_5$	$C_{23}H_{34}O_2$	342.5
all-(*E*)-retinol palmitate	$CO\text{-}C_{15}H_{31}$	$C_{36}H_{60}O_2$	524.9

In the British Pharmacopoeia, the term 'Retinol' is used within titles for preparations containing synthetic ester(s) and the term 'Vitamin A' within the title for the preparation containing material of natural origin.

Preparation

Paediatric Vitamins A, C and D Oral Drops

Ph Eur

DEFINITION

Vitamin A refers to a number of substances of very similar structure (including (*Z*)-isomers) found in animal tissues and possessing similar activity. The principal and biologically most active substance is all-(*E*)-retinol (all-(*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraen-1-ol; $C_{20}H_{30}O$). Vitamin A is generally used in the form of esters such as the acetate, propionate and palmitate.

Synthetic retinol ester refers to an ester (acetate, propionate or palmitate) or a mixture of synthetic retinol esters.

The activity of vitamin A is expressed in retinol equivalents (R.E.). 1 mg R.E. corresponds to the activity of 1 mg of all-(*E*)-retinol. The activity of the other retinol esters is calculated stoichiometrically, so that 1 mg R.E. of vitamin A corresponds to the activity of:

— 1.147 mg of all-(*E*)-retinol acetate;

— 1.195 mg of all-(*E*)-retinol propionate;

— 1.832 mg of all-(*E*)-retinol palmitate.

International Units (IU) are also used to express the activity of vitamin A. 1 IU of vitamin A is equivalent to the activity of 0.300 µg of all-(*E*)-retinol. The activity of the other retinol esters is calculated stoichiometrically, so that 1 IU of vitamin A is equivalent to the activity of:

— 0.344 µg of all-(*E*)-retinol acetate;

— 0.359 µg of all-(*E*)-retinol propionate;

— 0.550 µg of all-(*E*)-retinol palmitate;

1 mg of retinol equivalent is equivalent to 3333 IU.

CHARACTERS

Appearance

Retinol acetate: pale-yellow crystals (mp: about 60 °C). Once melted retinol acetate tends to yield a supercooled melt.

Retinol propionate: reddish-brown oily liquid.

Retinol palmitate: a fat-like, light yellow solid or a yellow oily liquid, if melted (mp: about 26 °C) .

Solubility

All retinol esters are practically insoluble in water, soluble or partly soluble in anhydrous ethanol and miscible with organic solvents.

Vitamin A and its esters are very sensitive to the action of air, oxidising agents, acids, light and heat.

Carry out the assay and all tests as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper , iron), acids and heat; use freshly prepared solutions.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Prepare a solution containing about 3.3 IU of vitamin A per microlitre in *cyclohexane R* containing 1 g/l of *butylhydroxytoluene R*.

Reference solution Prepare a 10 mg/ml solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *cyclohexane R* containing 1 g/l of *butylhydroxytoluene R*.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 3 µl.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:

— the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

Results The composition of esters is confirmed by the correspondence of the principal spot or spots of the test solution with those obtained with the reference solution.

B. It complies with the test for related substances.

TESTS

Retinol

Thin-layer chromatography *(2.2.27)*.

Test solution Prepare a solution in *cyclohexane R*, stabilised with a solution containing 1 g/l of *butylhydroxytoluene R*, containing about 330 IU of vitamin A per microlitre.

Reference solution Shake 1 ml of the test solution with 20 ml of *0.1 M tetrabutylammonium hydroxide* in *2-propanol* for 2 min and dilute to 100 ml with *cyclohexane R*, stabilised with a solution containg 1 g/l of *butylhydroxytoluene R*.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 3 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:

— in the chromatogram obtained no or only traces of the retinol esters are seen.

Limit Any spot corresponding to retinol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Related substances

Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution The solution described under Activity.

Absorption maximum At 325 nm to 327 nm.

Absorbance ratios:

— A_{300}/A_{326} = maximum 0.60;

— A_{350}/A_{326} = maximum 0.54;

— A_{370}/A_{326} = maximum 0.14.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ACTIVITY

The activity of the substance is determined in order to be taken into account for the production of concentrates.

Dissolve 25-100 mg, weighed with an accuracy of 0.1 per cent, in 5 ml of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10 IU/ml to 15 IU/ml. Measure the absorbance *(2.2.25)* at the absorption maximum at 326 nm. Calculate the activity of vitamin A in International Units per gram from the expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

A_{326} = absorbance at 326 nm

m = mass of the substance to be examined, in grams

V = total volume to which the substance to be examined is diluted to give 10 IU/ml to 15 IU/ml

1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram

STORAGE

In an airtight container, protected from light.

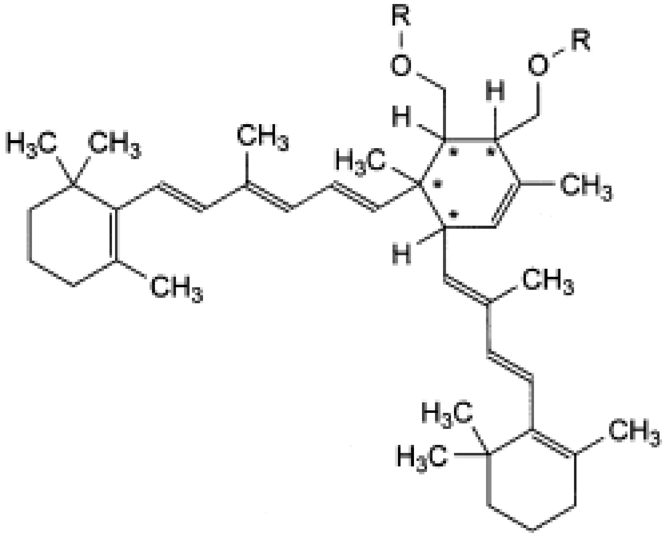
Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

LABELLING

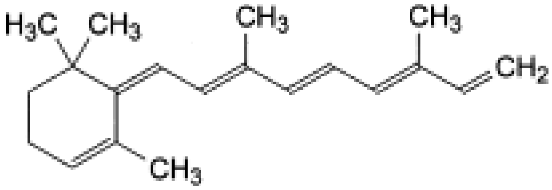
The label states:

- the number of International Units per gram;
- the name of the ester or esters.

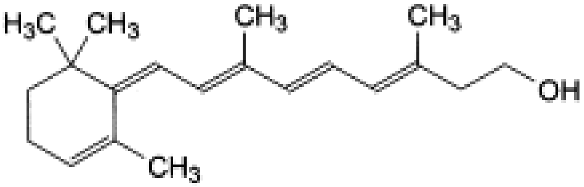
IMPURITIES



A. R = H, CO-CH_3: kitols (Diels-Alder dimers of vitamin A),



B. (3*E*,5*E*,7*E*)-3,7-dimethyl-9-[(1*Z*)-2,6,6-trimethylcyclohex-2-enylidene]nona-1,3,5,7-tetraene (anhydro-vitamin A),



C. (3*E*,5*E*,7*E*)-3,7-dimethyl-9-[(1*Z*)-2,6,6-trimethylcyclohex-2-enylidene]nona-3,5,7-trien-1-ol (*retro*-vitamin A),

D. oxidation products of vitamin A.

Ph Eur

Highly Purified Water

General Notices

(Ph Eur monograph 1927)

H_2O 18.02 *7732-18-5*

Ph Eur

DEFINITION

Water intended for use in the preparation of medicinal products where water of high biological quality is needed, except where *Water for injections (0169)* is required.

PRODUCTION

Highly purified water is obtained from water that complies with the regulations on water intended for human consumption laid down by the competent authority.

Current production methods include for example double-pass reverse osmosis coupled with other suitable techniques such as ultrafiltration and deionisation. Correct operation and maintenance of the system is essential.

During production and subsequent storage, appropriate measures are taken to ensure that the total viable aerobic count is adequately controlled and monitored. Appropriate alert and action limits are set so as to detect adverse trends. Under normal conditions, an appropriate action limit is a total viable aerobic count *(2.6.12)* of 10 micro-organisms per 100 ml when determined by membrane filtration, using agar medium S, at least 200 ml of highly purified water and incubating at 30-35 °C for 5 days.

Total organic carbon *(2.2.44)*

Maximum 0.5 mg/l.

Conductivity

Determine the conductivity off-line or in-line under the following conditions.

EQUIPMENT

Conductivity cell:

- electrodes of a suitable material such as stainless steel;
- *cell constant*: within 2 per cent of the given value determined using a certified reference solution with a conductivity less than 1500 $\mu S \cdot cm^{-1}$.

Conductometer Resolution 0.1 $\mu S \cdot cm^{-1}$ on the lowest range.

System calibration (conductivity cell and conductometer):

- against one or more suitable certified standard solutions;
- accuracy: within 3 per cent of the measured conductivity plus 0.1 $\mu S \cdot cm^{-1}$.

Conductometer calibration by means of precision resistors or equivalent devices after disconnecting the conductivity cell, for all ranges used for conductivity measurement and cell calibration (with an accuracy within 0.1 per cent of the stated value, traceable to the official standard).

If in-line conductivity cells cannot be dismantled, system calibration may be performed against a calibrated conductivity cell placed close to the cell to be calibrated in the water flow.

PROCEDURE

Stage 1

1. Measure the conductivity without temperature compensation, recording simultaneously the temperature. Temperature-compensated measurement may be performed after suitable validation.

2. Using Table 1927.-1, find the closest temperature value that is not greater than the measured temperature. The corresponding conductivity value is the limit at that temperature.

3. If the measured conductivity is not greater than the value in Table 1927.-1, the water to be examined meets the requirements of the test for conductivity. If the conductivity is higher than the value in Table 1927.-1, proceed with stage 2.

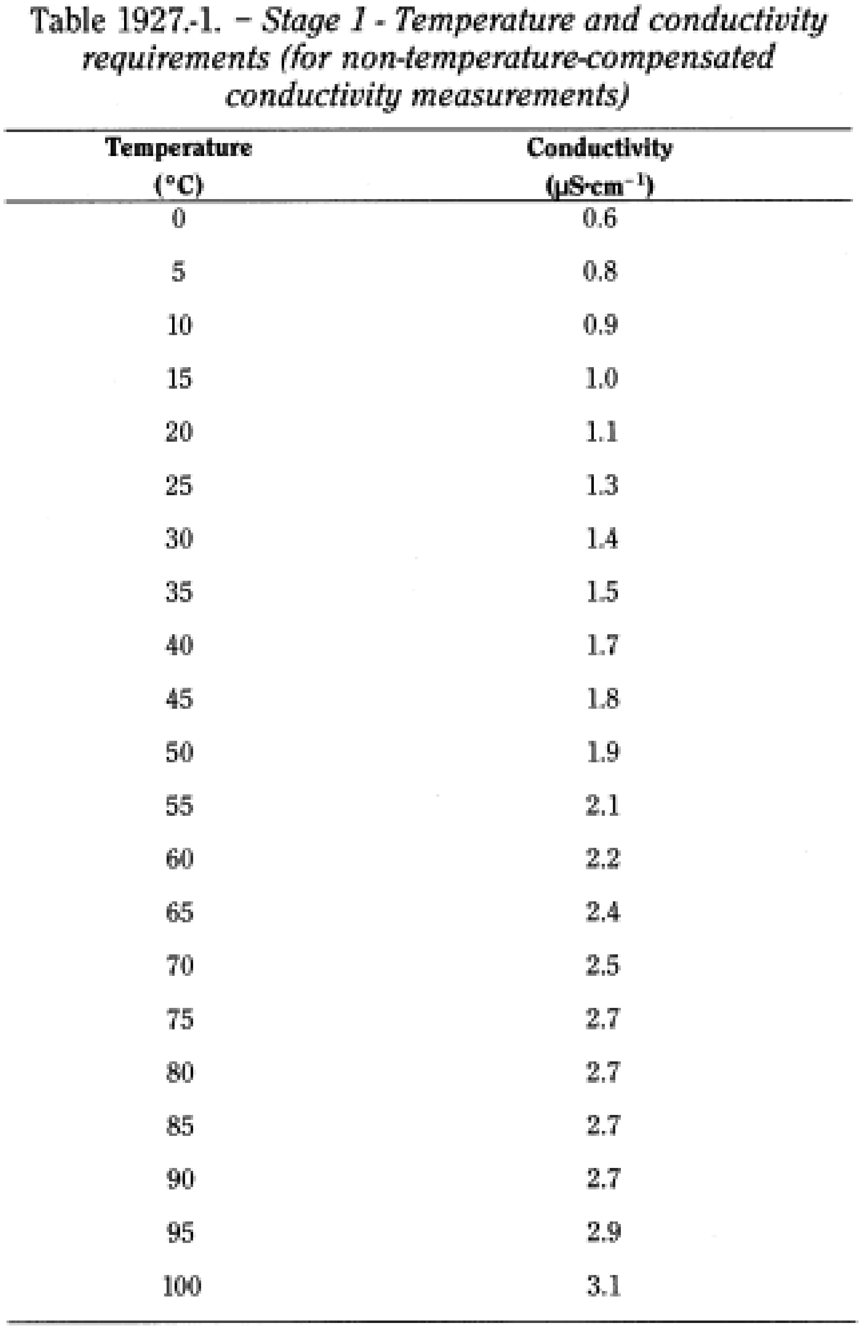


Table 1927.-1. – *Stage 1 - Temperature and conductivity requirements (for non-temperature-compensated conductivity measurements)*

Temperature (°C)	Conductivity (µS·cm^{-1})
0	0.6
5	0.8
10	0.9
15	1.0
20	1.1
25	1.3
30	1.4
35	1.5
40	1.7
45	1.8
50	1.9
55	2.1
60	2.2
65	2.4
70	2.5
75	2.7
80	2.7
85	2.7
90	2.7
95	2.9
100	3.1

Stage 2

4. Transfer a sufficient amount of the water to be examined (100 ml or more) to a suitable container, and stir the test sample. Adjust the temperature, if necessary, and while maintaining it at 25 ± 1 °C, begin vigorously agitating the test sample while periodically observing the conductivity. When the change in conductivity (due to uptake of atmospheric carbon dioxide) is less than 0.1 µS·cm^{-1} per 5 min, note the conductivity.

5. If the conductivity is not greater than 2.1 µS·cm^{-1}, the water to be examined meets the requirements of the test for conductivity. If the conductivity is greater than 2.1 µS·cm^{-1}, proceed with stage 3.

Stage 3

6. Perform this test within approximately 5 min of the conductivity determination in step 5 under stage 2, while maintaining the sample temperature at 25 ± 1 °C. Add a recently prepared saturated solution of *potassium chloride R* to the test sample (0.3 ml per 100 ml of

the test sample), and determine the pH *(2.2.3)* to the nearest 0.1 pH unit.

7. Using Table 1927.-2, determine the conductivity limit at the measured pH value in step 6. If the measured conductivity in step 4 under stage 2 is not greater than the conductivity requirements for the pH determined, the water to be examined meets the requirements of the test for conductivity. If either the measured conductivity is greater than this value or the pH is outside the range of 5.0-7.0, the water to be examined does not meet the requirements of the test for conductivity.

In order to ensure the appropriate quality of the water, validated procedures and in-process monitoring of the electrical conductivity and regular microbial monitoring are applied.

Highly purified water is stored in bulk and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

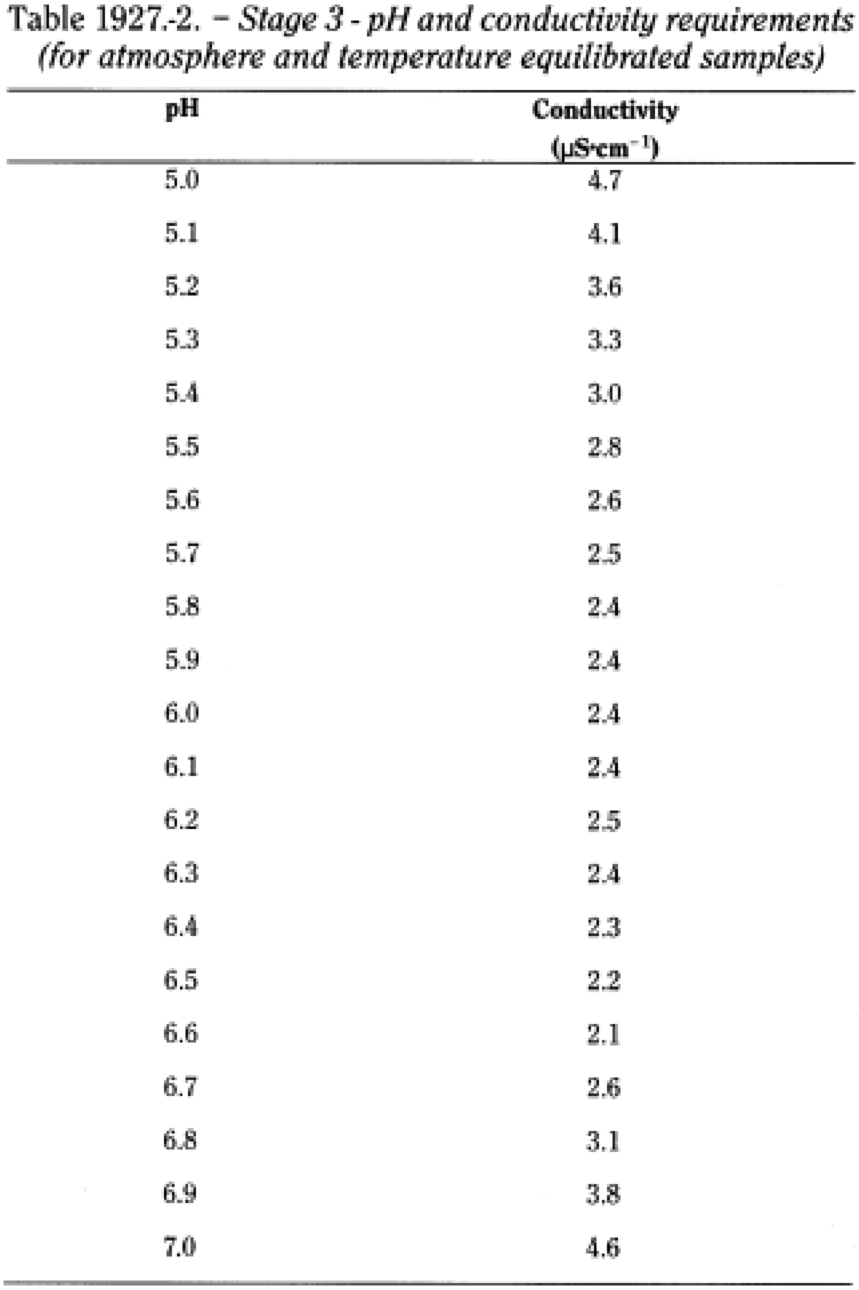


Table 1927.-2. – *Stage 3 - pH and conductivity requirements (for atmosphere and temperature equilibrated samples)*

pH	Conductivity ($\mu S \cdot cm^{-1}$)
5.0	4.7
5.1	4.1
5.2	3.6
5.3	3.3
5.4	3.0
5.5	2.8
5.6	2.6
5.7	2.5
5.8	2.4
5.9	2.4
6.0	2.4
6.1	2.4
6.2	2.5
6.3	2.4
6.4	2.3
6.5	2.2
6.6	2.1
6.7	2.6
6.8	3.1
6.9	3.8
7.0	4.6

CHARACTERS

Appearance

Clear and colourless liquid.

TESTS

Nitrates

Maximum 0.2 ppm.

Place 5 ml in a test-tube immersed in iced water, add 0.4 ml of a 100 g/l solution of *potassium chloride R*, 0.1 ml of *diphenylamine solution R* and, dropwise with shaking, 5 ml of *nitrogen-free sulphuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 ml of *nitrate-free water R* and 0.5 ml of *nitrate standard solution (2 ppm* NO_3*) R*.

Aluminium *(2.4.17)*

Maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution To 400 ml of the water to be examined add 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *distilled water R.*

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 0.1 ppm.

Heat 200 ml in a glass evaporating dish on a water-bath until the volume is reduced to 20 ml. 12 ml of the concentrated solution complies with limit test A. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R.*

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/ml.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

Ph Eur

Hydrogenated Wool Fat

General Notices

(Ph Eur monograph 0969)

Ph Eur

DEFINITION

Mixture of higher aliphatic alcohols and sterols obtained from the direct, high-pressure, high-temperature hydrogenation of *wool fat (0134)* during which the esters and acids present are reduced to the corresponding alcohols. It may contain butylhydroxytoluene.

CHARACTERS

Appearance

White or pale yellow, unctuous substance.

Solubility

Practically insoluble in water, soluble in boiling alcohol and in light petroleum.

IDENTIFICATION

First identification B.

Second identification A, C.

A. It complies with the test for melting point (see Tests).

B. Examine the chromatograms obtained in the test for fatty alcohols and sterols.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the principal peaks in the chromatogram obtained with reference solution (a).

C. Dissolve 50 mg in 5 ml of *methylene chloride R* and add 1 ml of *acetic anhydride R* and 0.1 ml of *sulphuric acid R.* A green colour is produced.

TESTS

Melting point *(2.2.15)*

45 °C to 55 °C. Allow to stand at 20 °C for 16 h.

Acid value *(2.5.1)*

Maximum 1.0, determined on 5.0 g.

Hydroxyl value *(2.5.3, Method A)*

140 to 180.

Saponification value ***(2.5.6)***

Maximum 8.0. Heat under reflux for 4 h.

Fatty alcohols and sterols

Gas chromatography *(2.2.28)*.

Test solution Dissolve 0.25 g of the substance to be examined in 60 ml of *ethanol* R and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dissolve 0.25 g of *hydrogenated wool fat CRS* in 60 ml of *ethanol R* and dilute to 100.0 ml with the same solvent.

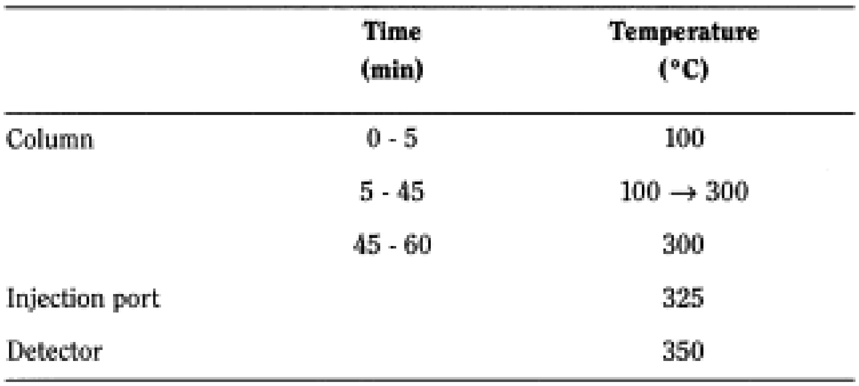
Reference solution (b) Dissolve 50 mg of *cetyl alcohol CRS* and 50 mg of *stearyl alcohol CRS* in 60 ml of *ethanol R* and dilute to 100.0 ml with the same solvent.

Column:

- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase: poly(dimethyl)siloxane R* or another non-polar phase (film thickness: 0.25 µm).

Carrier gas *helium for chromatography R* at a pressure of 100 kPa

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	100
	5 - 45	100 → 300
	45 - 60	300
Injection port		325
Detector		350

Detection Flame ionisation.

Injection 1 µl.

The chromatogram obtained with the test solution does not differ significantly from the chromatogram obtained with reference solution (a) (Figure 0969.-1) and it does not show enhanced peaks with retention times corresponding to cetyl alcohol and stearyl alcohol present in the chromatogram obtained with reference solution (b).

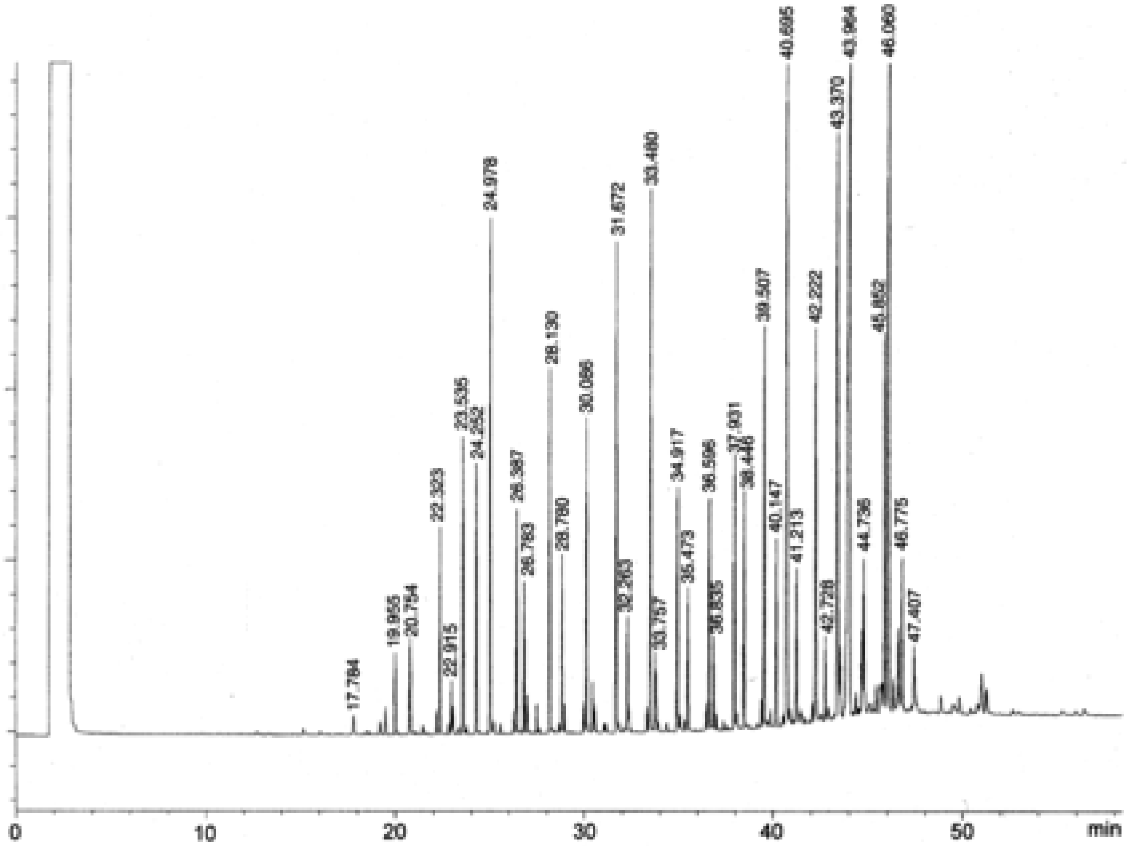


Figure 0969.-1. – *Chromatogram for the test for fatty alcohols and sterols (reference solution (a)) in hydrogenated wool fat*

Heavy metals ***(2.4.8)***

Maximum 10 ppm.

2.0 g complies with limit test C. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 3.0 per cent, determined on 2.000 g by drying in an oven at 105 °C for 1 h.

Total ash ***(2.4.16)***

Maximum 0.1 per cent, determined on 5.0 g.

STORAGE

In a well-filled container, protected from light.

Ph Eur

Hydrous Wool Fat

General Notices

Lanolin

(Ph Eur monograph 0135)

Ph Eur

DEFINITION

Mixture of 75 per cent *m/m* of wool fat and 25 per cent *m/m* of water. It is obtained by the gradual addition of water to melted wool fat with continuous stirring. It may contain not more than 150 ppm of butylhydroxytoluene.

CHARACTERS

Appearance

Pale yellow, unctuous substance.

IDENTIFICATION

A. In a test-tube, dissolve 0.5 g in 5 ml of *chloroform R* and add 1 ml of *acetic anhydride R* and 0.1 ml of *sulphuric acid R.* A green colour develops.

B. Dissolve 50 mg in 5 ml of *chloroform R*, add 5 ml of *sulphuric acid R* and shake. A red colour develops and an intense green fluorescence appears in the lower layer.

TESTS

Water-soluble acid or alkaline substances

Melt 6.7 g on a water-bath and shake vigorously for 2 min with 75 ml of *water R* previously heated to 90-95 °C. Allow to cool and filter through filter paper previously rinsed with *water R.* To 60 ml of the filtrate (which may not be clear) add 0.25 ml of *bromothymol blue solution R1*. Not more than 0.2 ml of *0.02 M hydrochloric acid* or 0.15 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Drop point *(2.2.17)*

38 °C to 44 °C.

To fill the metal cup, melt the residue obtained in the test for wool-fat content on a water-bath, cool to about 50 °C, pour into the cup and allow to stand at 15-20 °C for 24 h.

Water-absorption capacity

Place 10 g of the residue obtained in the test for wool-fat content in a mortar. Add *water R* in portions of 0.2-0.5 ml from a burette, stirring vigorously after each addition to incorporate the *water R*. The end-point is reached when visible droplets remain which cannot be incorporated. Not less than 20 ml of *water R* is absorbed.

Acid value *(2.5.1)*

Maximum 0.8, determined on 5.0 g dissolved in 25 ml of the prescribed mixture of solvents.

Peroxide value *(2.5.5, Method A)*

Maximum 15.

Saponification value *(2.5.6)*

67 to 79, determined on 2.00 g while heating under reflux for 4 h.

Water-soluble oxidisable substances

To 10 ml of the filtrate obtained in the test for water-soluble acid or alkaline substances add 1 ml of *dilute sulphuric acid R* and 0.1 ml of *0.02 M potassium permanganate*. After 10 min, the solution is not completely decolourised.

Butylhydroxytoluene

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.2 g of *methyl decanoate R* in carbon *disulphide R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *carbon disulphide R*.

Test solution (a) Dissolve 1.0 g of the residue obtained in the test for wool-fat content in *carbon disulphide R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 1.0 g of the residue obtained in the test for wool-fat content in *carbon disulphide R*, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *carbon disulphide R*.

Reference solution Dissolve 0.2 g of *butylhydroxytoluene R* in *carbon disulphide R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *carbon disulphide R*. To 1.0 ml of this solution add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *carbon disulphide R*.

Column:

- *size*: *l* = 1.5 m, Ø = 4 mm;
- *stationary phase*: silanised diatomaceous earth *for gas chromatography R* impregnated with 10 per cent m/m of *poly(dimethyl)siloxane R*.

The column is preceded by a column containing silanised glass wool.

Carrier gas *nitrogen for chromatography R.*

Flow rate 40 ml/min.

Temperature:

- *column*: 150 °C;
- *injection port*: 180 °C;
- *detector*: 300 °C.

Detection Flame ionisation.

Limit:

- *butylhydroxytoluene*: maximum 150 ppm.

Paraffins

Maximum 1.0 per cent.

The tap and cotton plugs used must be free from grease. Prepare a column of *anhydrous aluminium oxide* 230 mm long and 20 mm in diameter by adding a slurry of *anhydrous aluminium oxide R* and *light petroleum R1* to a glass tube fitted with a tap and containing *light petroleum R1*. Allow to settle and reduce the depth of the layer of solvent above the column to about 40 mm. Dissolve 3.0 g of the residue obtained in the test for wool-fat content in 50 ml of warm *light petroleum R1*, cool, pass the solution through the column at a rate of 3 ml/min and wash with 250 ml of *light petroleum R1*. Concentrate the combined eluate and washings to low bulk by distillation, evaporate to dryness on a water-bath and heat the residue at 105 °C for periods of 10 min until 2 successive weighings do not differ by more than 1 mg. The residue weighs a maximum of 30 mg.

Chlorides

Maximum 115 ppm.

Boil 1.3 g with 20 ml of *ethanol (90 per cent V/V) R* under a reflux condenser for 5 min. Cool, add 40 ml of *water R* and 0.5 ml of *nitric acid R* and filter. To the filtrate add 0.15 ml of a 10 g/l solution of *silver nitrate R* in *ethanol (90 per cent V/V) R*. Allow to stand for 5 min, protected from light. Any opalescence in the solution is not more intense than that in a standard prepared at the same time by adding 0.15 ml of a 10 g/l solution of *silver nitrate R* in *ethanol (90 per cent V/V) R* to a mixture of 0.2 ml of *0.02 M hydrochloric acid*, 20 ml of *ethanol (90 per cent V/V) R*, 40 ml of *water R* and 0.5 ml of *nitric acid R*.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent.

Ignite 5.0 g and use the residue.

Wool-fat content

72.5 per cent to 77.5 per cent.

In a suitable tared dish containing a glass rod, heat 30.0 g to constant mass on a water-bath, stirring continuously. Weigh the residue.

STORAGE

At a temperature not exceeding 25 °C.

LABELLING

The label states, where applicable, the concentration of added butylhydroxytoluene.

Ph Eur

Purified Water

General Notices

(Ph Eur monograph 0008)

H_2O 18.02 *7732-18-5*

Ph Eur

DEFINITION

Water for the preparation of medicines other than those that are required to be both sterile and apyrogenic, unless otherwise justified and authorised.

PURIFIED WATER IN BULK

PRODUCTION

Purified water in bulk is prepared by distillation, by ion exchange, by reverse osmosis or by any other suitable method from water that complies with the regulations on water intended for human consumption laid down by the competent authority.

During production and subsequent storage, appropriate measures are taken to ensure that the total viable aerobic count is adequately controlled and monitored. Appropriate alert and action limits are set so as to detect adverse trends. Under normal conditions, an appropriate action limit is a total viable aerobic count *(2.6.12)* of 100 micro-organisms per millilitre, determined by membrane filtration, using agar medium S and incubating at 30-35 °C for 5 days. The size of the sample is to be chosen in relation to the expected result.

In addition, the test for total organic carbon *(2.2.44)* with a limit of 0.5 mg/l or alternatively the following test for oxidisable substances is carried out: to 100 ml add 10 ml of *dilute sulphuric acid R* and 0.1 ml of *0.02 M potassium permanganate* and boil for 5 min; the solution remains faintly pink.

Conductivity

Determine the conductivity off-line or in-line under the following conditions.

EQUIPMENT

Conductivity cell:

- — electrodes of a suitable material such as stainless steel;
- — cell constant: within 2 per cent of the given value determined using a certified reference solution with a conductivity less than 1500 µS·cm-1.

Conductometer resolution 0.1 µS·cm-1 on the lowest range.

System calibration (conductivity cell and conductometer)

- — against one or more suitable certified standard solutions;
- — accuracy: within 3 per cent of the measured conductivity plus 0.1 µS·cm-1.

Conductometer calibration by means of precision resistors or equivalent devices, after disconnecting the conductivity cell, for all ranges used for conductivity measurement and cell calibration (with an accuracy within 0.1 per cent of the stated value, traceable to the official standard).

If in-line conductivity cells cannot be dismantled, system calibration may be performed against a calibrated conductivity cell placed close to the cell to be calibrated in the water flow.

PROCEDURE

Measure the conductivity without temperature compensation, recording simultaneously the temperature. Temperature-compensated measurement may be performed after suitable validation.

The water to be examined meets the requirements if the measured conductivity at the recorded temperature is not greater than the value in Table 0008.-1.

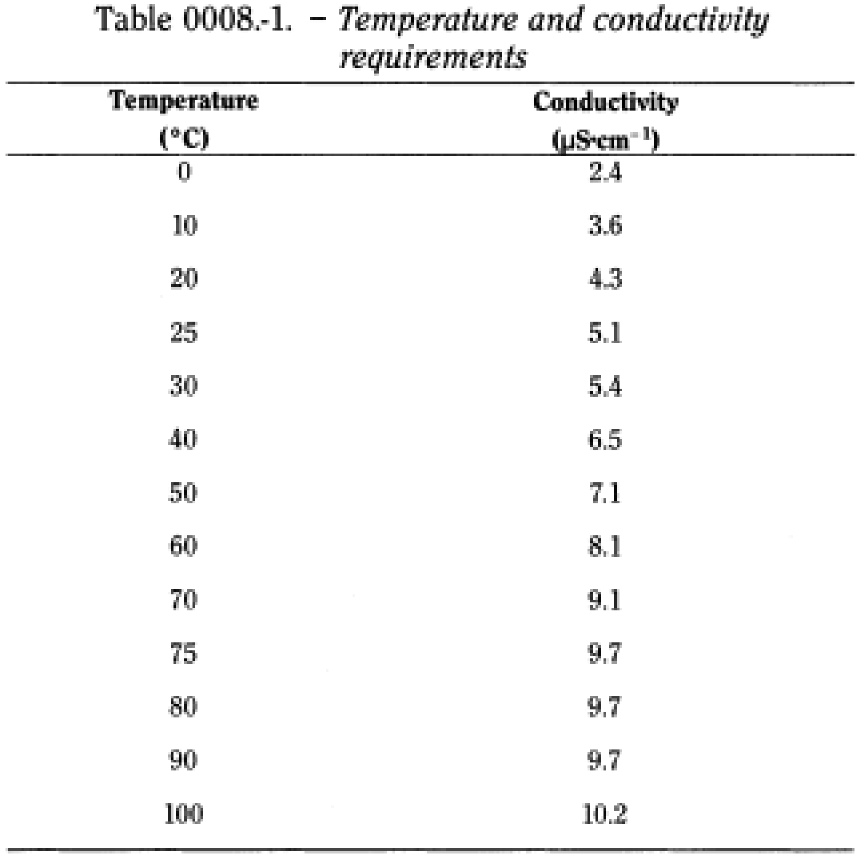


Table 0008.-1. – *Temperature and conductivity requirements*

Temperature (°C)	**Conductivity (µS·cm^{-1})**
0	2.4
10	3.6
20	4.3
25	5.1
30	5.4
40	6.5
50	7.1
60	8.1
70	9.1
75	9.7
80	9.7
90	9.7
100	10.2

For temperatures not listed in Table 0008.-1, calculate the maximal permitted conductivity by interpolation between the next lower and next higher data points in the table.

Purified water in bulk is stored and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

CHARACTERS

Appearance

Clear and colourless liquid.

TESTS

Nitrates

Maximum 0.2 ppm.

Place 5 ml in a test-tube immersed in iced water, add 0.4 ml of a 100 g/l solution of *potassium chloride R*, 0.1 ml of *diphenylamine solution R* and, dropwise with shaking, 5 ml of *nitrogen-free sulphuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 ml of *nitrate-free water R* and 0.5 ml of *nitrate standard solution (2 ppm* NO_3*) R.*

Aluminium *(2.4.17)*

Maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution To 400 ml of the water to be examined add 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *distilled water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R.*

Heavy metals *(2.4.8)*

Maximum 0.1 ppm.

Heat 200 ml in a glass evaporating dish on a water-bath until the volume is reduced to 20 ml. 12 ml of the concentrated solution complies with limit test A. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/ml, if intended for use in the manufacture of dialysis solutions without a further appropriate procedure for removal of bacterial endotoxins.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

PURIFIED WATER IN CONTAINERS

DEFINITION

Purified water in bulk that has been filled and stored in conditions designed to assure the required microbiological quality. It is free from any added substances.

CHARACTERS

Appearance

Clear and colourless liquid.

TESTS

It complies with the tests prescribed in the section on Purified water in bulk and with the following additional tests.

Acidity or alkalinity

To 10 ml, freshly boiled and cooled in a borosilicate glass flask, add 0.05 ml of *methyl red solution R*. The solution is not coloured red.

To 10 ml add 0.1 ml of *bromothymol blue solution R1*. The solution is not coloured blue.

Oxidisable substances

To 100 ml add 10 ml of *dilute sulphuric acid R* and 0.1 ml of *0.02 M potassium permanganate* and boil for 5 min. The solution remains faintly pink.

Chlorides

To 10 ml add 1 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2*. The solution shows no change in appearance for at least 15 min.

Sulphates

To 10 ml add 0.1 ml of *dilute hydrochloric acid R* and 0.1 ml of *barium chloride solution R1*. The solution shows no change in appearance for at least 1 h.

Ammonium

Maximum 0.2 ppm.

To 20 ml add 1 ml of *alkaline potassium tetraiodomercurate solution R*. After 5 min, examine the solution down the vertical axis of the tube. The solution is not more intensely coloured than a standard prepared at the same time by adding 1 ml of *alkaline potassium tetraiodomercurate solution R* to a mixture of 4 ml of *ammonium standard solution (1 ppm* NH_4*) R* and 16 ml of *ammonium-free water R.*

Calcium and magnesium

To 100 ml add 2 ml of *ammonium chloride buffer solution pH 10.0 R*, 50 mg of mordant black *11 triturate R* and 0.5 ml of *0.01 M sodium edetate*. A pure blue colour is produced.

Residue on evaporation

Maximum 0.001 per cent.

Evaporate 100 ml on a water-bath and dry in an oven at 100-105 °C. The residue weighs a maximum of 1 mg.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^2 micro-organisms per millilitre, determined by membrane filtration, using agar medium B.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

Ph Eur

Refined Wheat-germ Oil

General Notices

(Ph Eur monograph 1379)

Ph Eur

DEFINITION

Fatty oil obtained from the germ of the grain of *Triticum aestivum* L. by cold expression or by other suitable mechanical means and/or by extraction. It is then refined. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, light yellow liquid.

Solubility

Practically insoluble in water and in alcohol, miscible with light petroleum (40 °C to 60 °C).

Relative density

About 0.925.

Refractive index

About 1.475.

IDENTIFICATION

A. Identification of fatty oils by thin-layer chromatography *(2.3.2)*. The chromatogram obtained is similar to the type chromatogram for wheat-germ oil.

B. It complies with the test for composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Maximum 0.9. If intended for use in the manufacture of parenteral dosage forms: maximum 0.3.

Peroxide value *(2.5.5)*

Maximum 10.0. If intended for use in the manufacture of parenteral dosage forms: maximum 5.0.

Unsaponifiable matter *(2.5.7)*

Maximum 5.0 per cent, determined on 5.0 g.

Alkaline impurities *(2.4.19)*

It complies with the test for alkaline impurities in fatty oils.

Composition of fatty acids

Gas chromatography *(2.4.22, Method C)*. Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

— *palmitic acid*: 14.0 per cent to 19.0 per cent;

— *stearic acid*: maximum 2.0 per cent;

— *oleic acid*: 12.0 per cent to 23.0 per cent;

— *linoleic acid*: 52.0 per cent to 59.0 per cent;

— *linolenic acid*: 3.0 per cent to 10.0 per cent;

— *eicosenoic acid:* maximum 2.0 per cent.

Brassicasterol *(2.4.23)*

Maximum 0.3 per cent of brassicasterol in the sterol fraction of the oil.

Water *(2.5.32)*

If intended for use in the manufacture of parenteral dosage forms: maximum 0.1 per cent, determined on 5.00 g. Use a mixture of equal volumes of *methanol R* and *methylene chloride R* as solvent.

STORAGE

In an airtight, well-filled container, protected from light.

LABELLING

The label states:

— where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms;

— whether the oil is obtained by mechanical means, by extraction or by a combination of the two.

Ph Eur

Virgin Wheat-germ Oil

General Notices

(Ph Eur monograph 1480)

Ph Eur

DEFINITION

Virgin wheat-germ oil is the fatty oil obtained from the germ of the grain of *Triticum aestivum* L. by cold expression or other suitable mechanical means.

CHARACTERS

A clear, light yellow or golden-yellow liquid, practically insoluble in water and in alcohol, miscible with light petroleum (40 °C to 60 °C).

It has a relative density of about 0.925 and a refractive index of about 1.475.

IDENTIFICATION

A. Carry out the identification of fatty oils by thin-layer chromatography *(2.3.2).* The chromatogram obtained is comparable with the typical chromatogram for wheat-germ oil.

B. It complies with test for composition of fatty acids (see Tests).

TESTS

Acid value *(2.5.1)*

Not more than 20.0, determined on 10.0 g.

Peroxide value *(2.5.5)*

Not more than 15.0.

Unsaponifiable matter *(2.5.7)*

Not more than 5.0 per cent, determined on 5.0 g.

Composition of fatty acids *(2.4.22, Method C)*

The fatty-acid fraction of the oil has the following composition:

— *palmitic acid*: 14.0 per cent to 19.0 per cent;

— *stearic acid*: not more than 2.0 per cent;

— *oleic acid*: 12.0 per cent to 23.0 per cent;

— *linoleic acid*: 52.0 per cent to 59.0 per cent;

- *linolenic acid* : 3.0 per cent to 10.0 per cent;
- *eicosenoic acid*: not more than 2.0 per cent.

Brassicasterol *(2.4.23)*

The sterol fraction of the oil contains not more than 0.3 per cent of brassicasterol.

Water *(2.5.32)*

Not more than 0.1 per cent, determined on 5.00 g by the micro-determination of water. Use a mixture of equal volumes of *methanol R* and *methylene chloride R* as solvent.

STORAGE

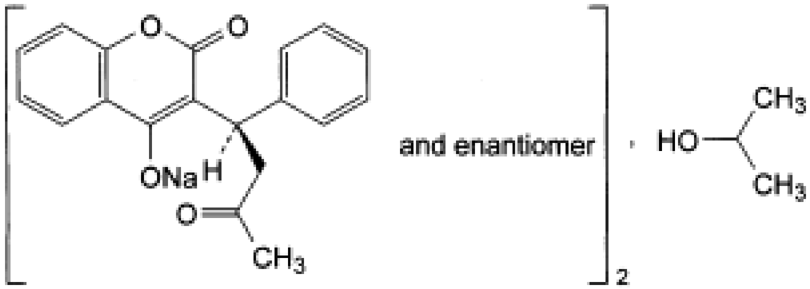
Store in an airtight, well-filled container, protected from light.

Ph Eur

Warfarin Sodium Clathrate

General Notices

(Ph Eur monograph 0699)



Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant (coumarin).

Preparation

Warfarin Tablets

Ph Eur

DEFINITION

Mixture, in the form of a clathrate, of warfarin sodium (sodium 2-oxo-3-[(1*RS*)-3-oxo-1-phenylbutyl]-2*H*-1-benzopyran-4-olate) and propan-2-ol in molecular proportions 2:1 (equivalent to about 92 per cent of warfarin sodium).

Content:

- — *warfarin sodium*: 98.0 per cent to 102.0 per cent (anhydrous and propan-2-ol-free substance);
- — *propan-2-ol* : 8.0 per cent to 8.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), soluble in acetone, very slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *warfarin sodium clathrate CRS.*

B. Dissolve 1 g in 10 ml of *water R*, add 5 ml of *nitric acid R* and filter. To the filtrate add 2 ml of *potassium dichromate solution R1* and shake for 5 min. Allow to stand for 20 min. The solution is greenish-blue when compared with a blank.

C. It gives reaction (b) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1.0 g in *water R* and dilute to 20 ml with the same solvent.

pH *(2.2.3)*

7.6 to 8.6.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *methanol R*, *water R* (25:75 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *4-hydroxycoumarin R* (warfarin impurity B) and 2 mg of *benzalacetone R* (warfarin impurity C) in 25 ml of *methanol R* and dilute to 100 ml with *water R*.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: spherical *nitrile silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *glacial acetic acid R*, acetonitrile R, *water R* (1:25:75 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µl.

Run time Twice the retention time of warfarin.

Relative retention With reference to warfarin (retention time = about 9 min): impurity B = about 0.4; impurity C = about 0.6.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to impurities B and C.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.5; impurity C = 0.4;

— *impurities B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Phenolic ketones

The absorbance (*2.2.25*) is maximum 0.20 measured at 385 nm within 15 min of preparing the solution.

Dissolve 1.25 g in a 20 g/l solution of *sodium hydroxide R* and dilute to 10.0 ml with the same solvent.

Propan-2-ol

Gas chromatography (*2.2.28*).

Internal standard solution Dilute 1.0 ml of *propanol R* to 200.0 ml with *water R*.

Test solution (a) Dissolve 0.250 g of the substance to be examined in *water R* and dilute to 5.0 ml with the same solvent.

Test solution (b) Dissolve 0.50 g of the substance to be examined in the internal standard solution and dilute to 10.0 ml with the internal standard solution.

Reference solution Dilute 0.50 ml of *2-propanol R* to 100.0 ml with the internal standard solution.

Column:

— *size:* *l* = 1.5 m, Ø = 4 mm;

— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (125-150 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 40 ml/min.

Temperature:

— *column*: 150 °C;

— *injection port*: 180 °C;

— *detector*: 200 °C.

Detection Flame ionisation.

Injection The chosen volume of the test solutions and the reference solution.

Calculate the content of propan-2-ol taking its density at 20 °C to be 0.785 g/ml.

Limit:

— *propan-2-ol* : 8.0 per cent to 8.5 per cent.

Water (*2.5.12*)

Maximum 0.3 per cent, determined on 2.500 g.

ASSAY

Dissolve 0.100 g in *0.01 M sodium hydroxide* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M sodium hydroxide*. Dilute 10.0 ml of this solution to 100.0 ml with *0.01 M sodium hydroxide*. Measure the absorbance (*2.2.25*) at the absorption maximum at 308 nm.

Calculate the percentage content of warfarin sodium ($C_{19}H_{15}NaO_4$) taking the specific absorbance to be 431.

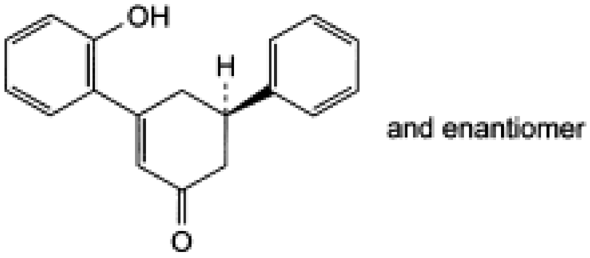
STORAGE

In an airtight container , protected from light.

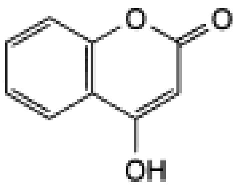
IMPURITIES

Specified impurities *B, C.*

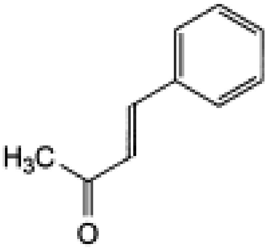
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A.*



A. (5*RS*)-3-(2-hydroxyphenyl)-5-phenylcyclohex-2-enone,



B. 4-hydroxy-2*H*-1-benzopyran-2-one (4-hydroxycoumarin),



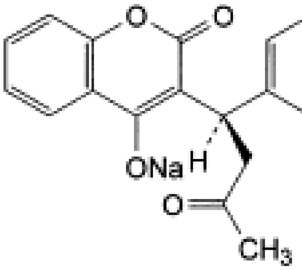
C. (3*E*)-4-phenylbut-3-en-2-one (benzalacetone).

Ph Eur

Warfarin Sodium

General Notices

(Ph Eur monograph 0698)



and enantiomer

$C_{19}H_{15}NaO_4$ 330.3 *129-06-6*

Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant (coumarin).

Preparation

Warfarin Tablets

Ph Eur

DEFINITION

Sodium 2-oxo-3-[(1*RS*)-3-oxo-1-phenylbutyl]-2*H*-1-benzopyran-4-olate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, amorphous powder.

Solubility

Very soluble in water and in ethanol (96 per cent), soluble in acetone, very slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *warfarin sodium CRS*.

B. Dissolve 1 g in 10 ml of *water R*, add 5 ml of *nitric acid R* and filter. To the filtrate add 2 ml of *potassium dichromate solution R1* and shake for 5 min. Allow to stand for 20 min. The solution is not greenish-blue when compared with a blank.

C. It gives reaction (b) of sodium *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1.0 g in *water R* and dilute to 20 ml with the same solvent.

pH *(2.2.3)*

7.6 to 8.6.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Solvent mixture *methanol R*, *water R* (25:75 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *4-hydroxycoumarin R* (impurity B) and 2 mg of *benzalacetone R* (impurity C) in 25 ml of *methanol R* and dilute to 100 ml with *water R.*

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: spherical *nitrile silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *glacial acetic acid R, acetonitrile R, water R* (1:25:75 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µl.

Run time Twice the retention time of warfarin.

Relative retention With reference to warfarin (retention time = about 9 min): impurity B = about 0.4; impurity C = about 0.6.

System suitability Reference solution (a):

— *resolution*: minimum 2.0 between the peaks due to impurities B and C.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.5; impurity C = 0.4;

— *impurities B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Phenolic ketones

Dissolve 1.25 g in a 20 g/l solution of *sodium hydroxide R* and dilute to 10.0 ml with the same solvent. The absorbance *(2.2.25)* is maximum 0.20 measured at 385 nm within 15 min of preparing the solution.

Water ***(2.5.12)***

Maximum 4.0 per cent, determined on 0.750 g.

ASSAY

Dissolve 0.100 g in *0.01 M sodium hydroxide* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M sodium hydroxide*. Dilute 10.0 ml of this solution to 100.0 ml with *0.01 M sodium hydroxide*. Measure the absorbance *(2.2.25)* at the absorption maximum at 308 nm.

Calculate the percentage content of $C_{19}H_{15}NaO_4$ taking the specific absorbance to be 431.

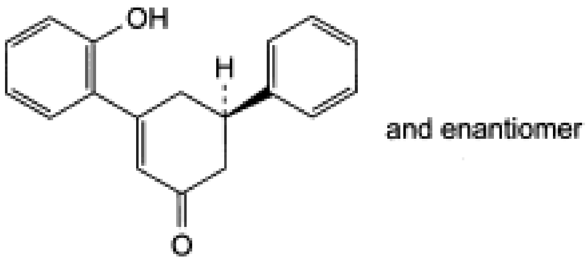
STORAGE

In an airtight container, protected from light.

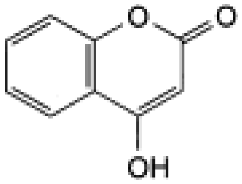
IMPURITIES

Specified impurities B, C.

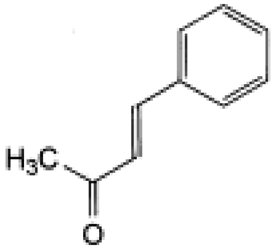
Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): A.*



A. (5*RS*)-3-(2-hydroxyphenyl)-5-phenylcyclohex-2-enone,



B. 4-hydroxy-*2H*-1-benzopyran-2-one (4-hydroxycoumarin),



C. (3*E*)-4-phenylbut-3-en-2-one (benzalacetone).

Ph Eur

Water for Injections

General Notices

(Ph Eur monograph 0169)

H_2O 18.02 *7732-18-5*

Ph Eur

DEFINITION

Water for the preparation of medicines for parenteral administration when water is used as vehicle (water for injections in bulk) and for dissolving or diluting substances or preparations for parenteral administration (sterilised water for injections).

WATER FOR INJECTIONS IN BULK

PRODUCTION

Water for injections in bulk is obtained from water that complies with the regulations on water intended for human consumption laid down by the competent authority or from purified water by distillation in an apparatus of which the parts in contact with the water are of neutral glass, quartz or suitable metal and which is fitted with an effective device to prevent the entrainment of droplets. The correct maintenance of the apparatus is essential. The first portion of the distillate obtained when the apparatus begins to function is discarded and the distillate is collected.

During production and subsequent storage, appropriate measures are taken to ensure that the total viable aerobic count is adequately controlled and monitored. Appropriate alert and action limits are set so as to detect adverse trends. Under normal conditions, an appropriate action limit is a total viable aerobic count *(2.6.12)* of 10 micro-organisms per 100 ml when determined by membrane filtration, using agar medium S, using at least 200 ml of water for injections in bulk and incubating at 30-35 °C for 5 days. For aseptic processing, stricter alert limits may need to be applied.

Total organic carbon *(2.2.44)*

Maximum 0.5 mg/l.

Conductivity

Determine the conductivity off-line or in-line under the following conditions.

EQUIPMENT

Conductivity cell:

— electrodes of a suitable material such as stainless steel;

— cell constant: within 2 per cent of the given value determined using a certified reference solution with a conductivity less than 1500 $\mu S\cdot cm^{-1}$.

Conductometer Resolution 0.1 µS·cm^{-1} on the lowest range.

System calibration (conductivity cell and conductometer):
— against one or more suitable certified standard solutions;

— accuracy: within 3 per cent of the measured conductivity plus 0.1 µS·cm^{-1}.

Conductometer calibration By means of precision resistors or equivalent devices, after disconnecting the conductivity cell, for all ranges used for conductivity measurement and cell calibration (with an accuracy within 0.1 per cent of the stated value, traceable to the official standard).

If in-line conductivity cells cannot be dismantled, system calibration may be performed against a calibrated conductivity cell placed close to the cell to be calibrated in the water flow.

PROCEDURE

Stage 1

1. Measure the conductivity without temperature compensation, recording simultaneously the temperature. Temperature-compensated measurement may be performed after suitable validation.

2. Using Table 0169.-1, find the closest temperature value that is not greater than the measured temperature. The corresponding conductivity value is the limit at that temperature.

3. If the measured conductivity is not greater than the value in Table 0169.-1, the water to be examined meets the requirements of the test for conductivity. If the conductivity is higher than the value in Table 0169.-1, proceed with stage 2.

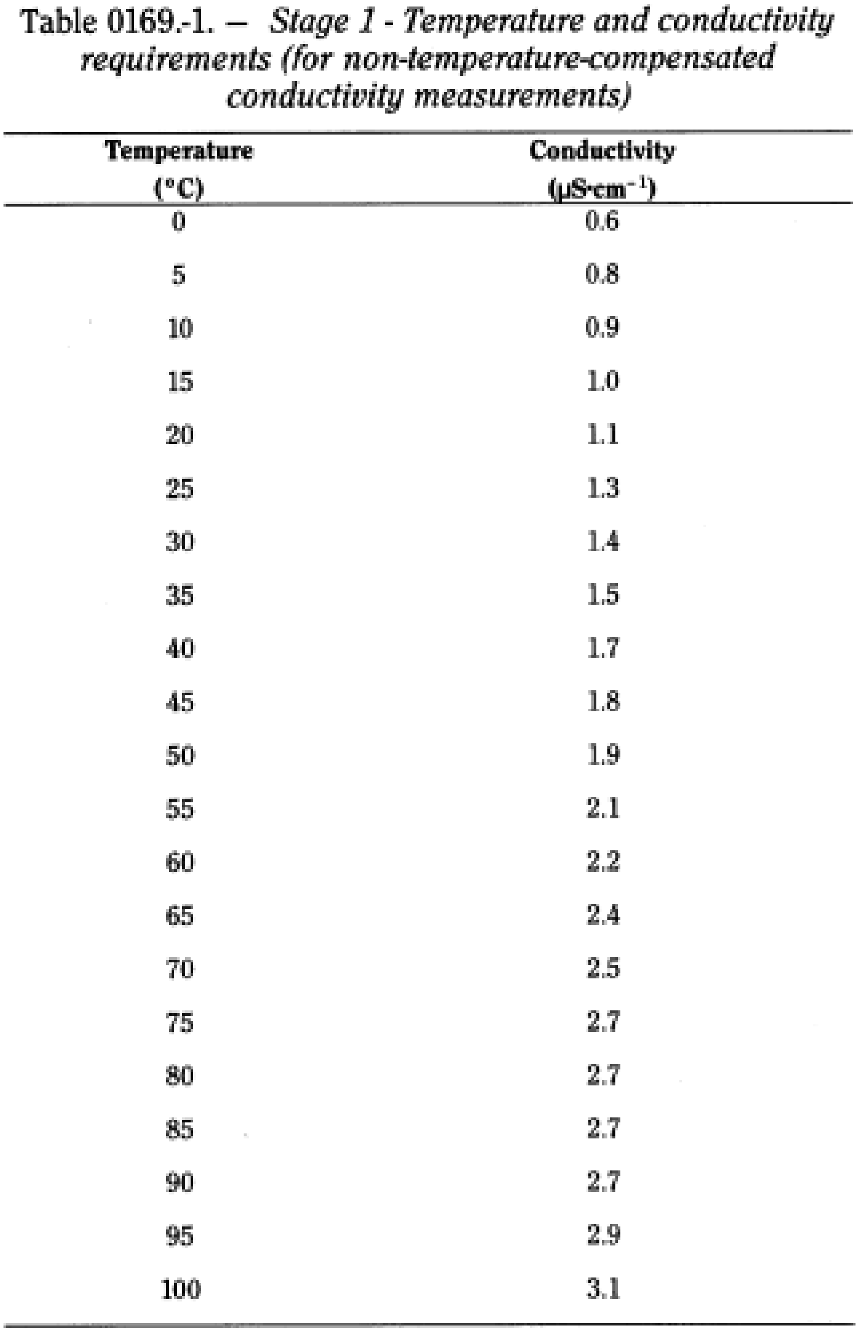


Table 0169.-1. — *Stage 1 - Temperature and conductivity requirements (for non-temperature-compensated conductivity measurements)*

Temperature (°C)	Conductivity (µS·cm^{-1})
0	0.6
5	0.8
10	0.9
15	1.0
20	1.1
25	1.3
30	1.4
35	1.5
40	1.7
45	1.8
50	1.9
55	2.1
60	2.2
65	2.4
70	2.5
75	2.7
80	2.7
85	2.7
90	2.7
95	2.9
100	3.1

Stage 2

4. Transfer a sufficient amount of the water to be examined (100 ml or more) to a suitable container, and stir the test sample. Adjust the temperature, if necessary, and while maintaining it at 25 ± 1 °C, begin vigorously agitating the test sample while periodically observing the conductivity. When the change in conductivity (due to uptake of atmospheric carbon dioxide) is less than 0.1 µS.cm^{-1} per 5 min, note the conductivity.

5. If the conductivity is not greater than 2.1 µS.cm^{-1}, the water to be examined meets the requirements of the test for conductivity. If the conductivity is greater than 2.1 µS.cm^{-1}, proceed with stage 3.

Stage 3

6. Perform this test within approximately 5 min of the conductivity determination in step 5 under stage 2, while maintaining the sample temperature at 25 ± 1 °C. Add a recently prepared saturated solution of *potassium chloride R* to the test sample (0.3 ml per 100 ml of

the test sample), and determine the pH *(2.2.3)* to the nearest 0.1 pH unit.

7. Using Table 0169.-2, determine the conductivity limit at the measured pH value in step 6. If the measured conductivity in step 4 under stage 2 is not greater than the conductivity requirements for the pH determined, the water to be examined meets the requirements of the test for conductivity. If either the measured conductivity is greater than this value or the pH is outside the range of 5.0-7.0, the water to be examined does not meet the requirements of the test for conductivity.

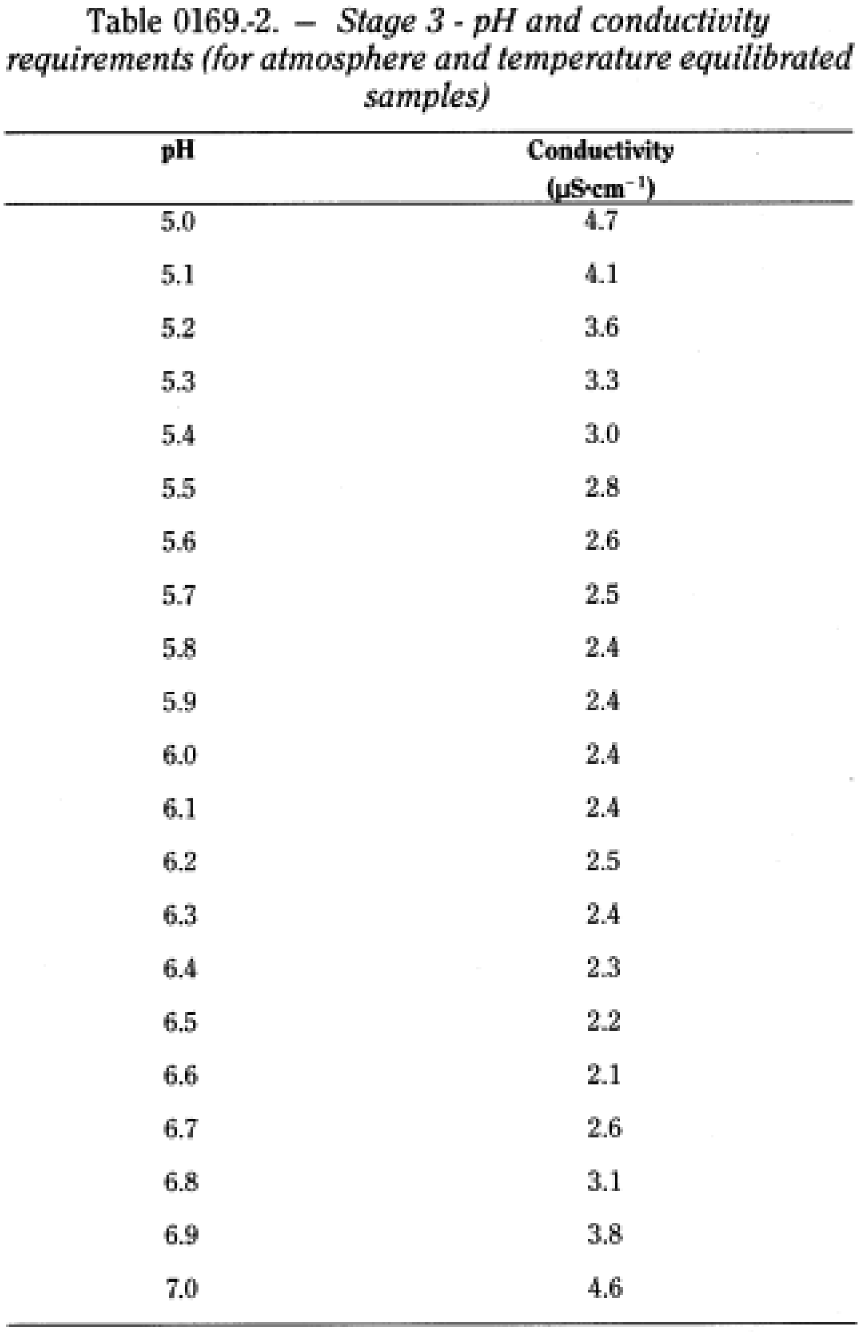


Table 0169.-2. — *Stage 3 - pH and conductivity requirements (for atmosphere and temperature equilibrated samples)*

pH	**Conductivity (µS·cm^{-1})**
5.0	4.7
5.1	4.1
5.2	3.6
5.3	3.3
5.4	3.0
5.5	2.8
5.6	2.6
5.7	2.5
5.8	2.4
5.9	2.4
6.0	2.4
6.1	2.4
6.2	2.5
6.3	2.4
6.4	2.3
6.5	2.2
6.6	2.1
6.7	2.6
6.8	3.1
6.9	3.8
7.0	4.6

In order to ensure the appropriate quality of the water, validated procedures and in-process-monitoring of the electrical conductivity and regular microbial monitoring are applied.

Water for injections in bulk is stored and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

CHARACTERS

Appearance

Clear and colourless liquid.

TESTS

Nitrates

Maximum 0.2 ppm.

Place 5 ml in a test-tube immersed in iced water, add 0.4 ml of a 100 g/l solution of *potassium chloride R,* 0.1 ml of *diphenylamine solution R* and, dropwise with shaking, 5 ml of *nitrogen-free sulphuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 ml of *nitrate-free water R* and 0.5 ml of *nitrate standard solution (2 ppm* NO_3*) R*.

Aluminium *(2.4.17)*

Maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution To 400 ml of the water to be examined add 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *distilled water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Heavy metals *(2.4.8)*

Maximum 0.1 ppm.

Heat 200 ml in a glass evaporating dish on a water-bath until the volume is reduced to 20 ml. 12 ml of the concentrated solution complies with limit test A. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/ml.

STERILISED WATER FOR INJECTIONS

DEFINITION

Water for injections in bulk that has been distributed into suitable containers, closed and sterilised by heat in conditions which ensure that the product still complies with the test for bacterial endotoxins. Sterilised water for injections is free from any added substances.

Examined in suitable conditions of visibility, it is clear and colourless.

Each container contains a sufficient quantity of water for injections to permit the nominal volume to be withdrawn.

TESTS

Acidity or alkalinity

To 20 ml add 0.05 ml of *phenol red solution R.* If the solution is yellow, it becomes red on the addition of 0.1 ml of *0.01 M sodium hydroxide*; if red, it becomes yellow on the addition of 0.15 ml of *0.01 M hydrochloric acid*.

Conductivity

Maximum 25 µS·cm^{-1} for containers with a nominal volume of 10 ml or less; maximum 5 µS·cm^{-1} for containers with a nominal volume greater than 10 ml.

Use equipment and the calibration procedure as defined under Water for injections in bulk, maintaining the sample temperature at 25 ± 1 °C.

Oxidisable substances

Boil 100 ml with 10 ml of *dilute sulphuric acid R*. Add 0.2 ml of *0.02 M potassium permanganate* and boil for 5 min. The solution remains faintly pink.

Chlorides ***(2.4.4)***

Maximum 0.5 ppm for containers with a nominal volume of 100 ml or less.

15 ml complies with the limit test for chlorides. Prepare the standard using a mixture of 1.5 ml of *chloride standard solution (5 ppm Cl) R* and 13.5 ml of *water R*. Examine the solutions down the vertical axes of the tubes.

For containers with a nominal volume greater than 100 ml, use the following test: to 10 ml add 1 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2*. The solution shows no change in appearance for at least 15 min.

Nitrates

Maximum 0.2 ppm.

Place 5 ml in a test-tube immersed in iced water, add 0.4 ml of a 100 g/l solution of *potassium chloride R*, 0.1 ml of *diphenylamine solution R* and, dropwise with shaking, 5 ml of *nitrogen-free sulphuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 ml of *nitrate-free water R* and 0.5 ml of *nitrate standard solution (2 ppm* NO_3*) R*.

Sulphates

To 10 ml add 0.1 ml of *dilute hydrochloric acid R* and 0.1 ml of *barium chloride solution R1*. The solution shows no change in appearance for at least 1 h.

Aluminium ***(2.4.17)***

Maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution To 400 ml of the water to be examined add 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 98 ml of *distilled water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *distilled water R*.

Ammonium

Maximum 0.2 ppm.

To 20 ml add 1 ml of *alkaline potassium tetraiodomercurate solution R*. After 5 min, examine the solution down the vertical axis of the tube. The solution is not more intensely coloured than a standard prepared at the same time by adding 1 ml of *alkaline potassium tetraiodomercurate solution R* to a mixture of 4 ml of *ammonium standard solution (1 ppm* NH_4*) R* and 16 ml of *ammonium-free water R*.

Calcium and magnesium

To 100 ml add 2 ml of *ammonium chloride buffer solution pH 10.0 R*, 50 mg of *mordant black 11 triturate R* and 0.5 ml of *0.01 M sodium edetate*. A pure blue colour is produced.

Heavy metals *(2.4.8)*

Maximum 0.1 ppm.

Heat 200 ml in a glass evaporating dish on a water-bath until the volume is reduced to 20 ml. 12 ml of the concentrated solution complies with limit test A. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Residue on evaporation

Maximum 4 mg (0.004 per cent) for containers with a nominal volume of 10 ml or less; maximum 3 mg (0.003 per cent) for containers with a nominal volume greater than 10 ml.

Evaporate 100 ml to dryness on a water-bath and dry in an oven at 100-105 °C.

Particulate contamination

Sub-visible particles *(2.9.19)*. It complies with test A or test B, as appropriate.

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/ml.

Ph Eur

Wool Alcohols

General Notices

Wool Wax Alcohols

(Ph Eur monograph 0593)

Preparation

Wool Alcohols Ointment

Ph Eur

DEFINITION

Mixture of sterols and higher aliphatic alcohols from wool fat. It may contain not more than 200 ppm of butylhydroxytoluene.

Content

Minimum 30.0 per cent of cholesterol.

CHARACTERS

Appearance

Pale-yellow or brownish-yellow, brittle mass becoming plastic on heating.

Solubility

Practically insoluble in water, soluble in methylene chloride and in boiling ethanol, slightly soluble in alcohol (90 per cent *V/V*).

IDENTIFICATION

Dissolve 50 mg in 5 ml of *methylene chloride R* and add 1 ml of *acetic anhydride R* and 0.1 ml of *sulphuric acid R*. Within a few seconds, a green colour develops.

TESTS

Appearance of solution

To 1.0 g add 10 ml of *light petroleum R1* and shake while warming in a water-bath. The substance dissolves completely. After cooling, the solution is clear *(2.2.1)*.

Alkalinity

Dissolve 2.0 g in 25 ml of hot *alcohol (90 per cent V/V) R* and add 0.5 ml of *phenolphthalein solution R1*. No red colour develops.

Melting point *(2.2.15)*

Minimum 58 °C.

Melt the substance to be examined by heating in a water-bath at a temperature which exceeds the expected melting point by not more than 10 °C; introduce the substance to be examined into the capillary tubes and allow to stand at 15-17 °C for not less than 16 h.

Acid value ***(2.5.1)***

Maximum 2.0.

If necessary, heat in a water-bath under a reflux condenser to dissolve the substance to be examined.

Hydroxyl value ***(2.5.3, Method A)***

120 to 180.

Peroxide value ***(2.5.5, Method A*****)**

Maximum 15.

Take from the substance to be examined wedge-shaped pieces whose base consists of part of the surface. Melt the pieces before carrying out the determination. Before adding 0.5 ml of *saturated potassium iodide solution R*, cool the solution obtained to room temperature.

Saponification value ***(2.5.6)***

Maximum 12.0, determined on 2.00 g. Heat under reflux for 4 h.

Butylhydroxytoluene

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.20 g of methyl *decanoate R* in *carbon disulphide R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *carbon disulphide R*.

Test solution (a) Dissolve 1.0 g of the substance to be examined in *carbon disulphide R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 1.0 g of the substance to be examined in *carbon disulphide R,* add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *carbon disulphide R*.

Reference solution Dissolve 0.20 g of *butylhydroxytoluene R* in *carbon disulphide R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *carbon disulphide R*. To 1.0 ml of this solution add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *carbon disulphide R.*

Precolumn Column filled with silanised glass wool.

Column:

- *size:* l = 1.5 m, Ø = 4 mm;
- *stationary phase: silanised diatomaceous earth for gas chromatography R* impregnated with 10 per cent *m/m* of *poly(dimethyl)siloxane R.*

Carrier gas *nitrogen for chromatography R.*

Flow rate 40 ml/min.

Temperature:

— *column*: 150 °C;

— *injection port*: 180 °C;

— *detector*: 300 °C.

Detection Flame ionisation.

Limit:
— *butylhydroxytoluene*: maximum 200 ppm.

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 2.000 g by drying in an oven at 105 °C.

Total ash ***(2.4.16)***

Maximum 0.1 per cent.

Water-absorption capacity

Place 0.6 g of the substance to be examined and 9.4 g of *white soft paraffin R* in a mortar and melt on a water-bath. Allow to cool and incorporate 20 ml of *water R*, added in portions. Within 24 h no water is released from the almost white, ointment-like emulsion.

ASSAY

Dissolve 0.1000 g in 12 ml of hot *alcohol (90 per cent V/V) R*. Allow to stand for 18 h, filter through a sintered-glass filter (16) *(2.1.2)* and wash the filter with 2 quantities, each of 15 ml, of *alcohol (90 per cent V/V) R*. Combine the filtrate and washings, add 20 ml of a freshly prepared 10 g/l solution of *digitonin R* in *alcohol (90 per cent V/V) R* and heat to about 60 °C. Allow to cool, filter through a sintered-glass filter (16) *(2.1.2)*, wash the residue with 10 ml of *alcohol (90 per cent V/V) R* and dry to constant mass at 100-105 °C.

1 g of residue is equivalent to 0.239 g of cholesterol.

STORAGE

In a well-filled container, protected from light.

LABELLING

The label states, where applicable, the concentration of any added butylhydroxytoluene.

Ph Eur

Wool Fat

General Notices

Anhydrous Lanolin

(Ph Eur monograph 0134)

Ph Eur

DEFINITION

Purified, anhydrous, waxy substance obtained from the wool of sheep (*Ovis aries*). It may contain not more than 200 ppm of butylhydroxytoluene.

CHARACTERS

Appearance

Yellow, unctuous substance. When melted, it is a clear or almost clear, yellow liquid. A solution in light petroleum is opalescent.

Solubility

Practically insoluble in water, slightly soluble in boiling anhydrous ethanol.

It has a characteristic odour.

IDENTIFICATION

A. In a test-tube, dissolve 0.5 g in 5 ml of *methylene chloride R* and add 1 ml of acetic anhydride R and 0.1 ml of *sulphuric acid R*. A green colour develops.

B. Dissolve 50 mg in 5 ml of *methylene chloride R*, add 5 ml of *sulphuric acid R* and shake. A red colour develops and an intense green fluorescence appears in the lower layer when examined in daylight, with illumination from the rear of the observer.

TESTS

Water-soluble acid or alkaline substances

Melt 5.0 g on a water-bath and shake vigorously for 2 min with 75 ml of *water R* previously heated to 90-95 °C. Allow to cool and filter through filter paper previously rinsed with *water R.* To 60 ml of the filtrate (which may not be clear) add 0.25 ml of *bromothymol blue solution R1.* Not more than 0.2 ml of *0.02 M hydrochloric acid* or 0.15 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Drop point *(2.2.17)*

38 °C to 44 °C.

To fill the metal cup, melt the wool fat on a water-bath, cool to about 50 °C, pour into the cup and allow to stand at 15-20 °C for 24 h.

Water-absorption capacity

Place 10 g of molten wool fat in a mortar and allow to cool to room temperature. Weigh the mortar. Add *water R* in portions of 0.2-0.5 ml from a burette, stirring vigorously after each addition to incorporate the *water R*. Instead of a pestle, use a high-density polypropylene cylindrical rod (120 mm long and 10 mm in diameter, for example). The end-point is reached when visible droplets remain which cannot be incorporated. Weigh the mortar again and determine the amount of water absorbed by weight difference. Not less than 20 g of *water R* is absorbed.

Acid value *(2.5.1)*

Maximum 1.0, determined on 5.0 g dissolved in 25 ml of the prescribed mixture of solvents.

Peroxide value *(2.5.5, Method A)*

Maximum 20.

Before adding 0.5 ml of saturated *potassium iodide solution R*, cool the solution obtained to room temperature.

Saponification value *(2.5.6)*

90 to 105, determined on 2.00 g while heating under reflux for 4 h.

Water-soluble oxidisable substances

To 10 ml of the filtrate obtained in the test for water-soluble acid or alkaline substances add 1 ml of *dilute sulphuric acid R* and 0.1 ml of *0.02 M potassium permanganate*. After 10 min, the solution is not completely decolourised.

Butylhydroxytoluene

Gas chromatography *(2.2.28)*.

Internal standard solution Dissolve 0.2 g of *methyl decanoate R* in *carbon disulphide R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *carbon disulphide R.*

Test solution (a) Dissolve 1.0 g of the substance to be examined in *carbon disulphide R* and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 1.0 g of the substance to be examined in *carbon disulphide R*, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *carbon disulphide R*.

Reference solution Dissolve 0.2 g of *butylhydroxytoluene R* in *carbon disulphide R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *carbon disulphide R*. To 1.0 ml of this solution add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *carbon disulphide R.*

Column:

- *size:* l = 1.5 m, Ø = 4 mm;
- *stationary phase*: silanised diatomaceous earth for *gas chromatography R* impregnated with 10 per cent *m/m* of *poly(dimethyl)siloxane R*.

The column is preceded by a column containing silanised glass wool.

Carrier gas nitrogen for chromatography R.

Flow rate 40 ml/min.

Temperature:
- *column*: 150 °C;
- *injection port:* 180 °C;
- *detector*: 300 °C.

Detection Flame ionisation.

Limit:
- *butylhydroxytoluene*: maximum 200 ppm.

Paraffins

Maximum 1.0 per cent.

The tap and cotton plugs used must be free from grease. Prepare a column of anhydrous aluminium oxide 0.23 m long and 20 mm in diameter by adding a slurry of anhydrous *aluminium oxide R* and *light petroleum R1* to a glass tube fitted with a tap and containing *light petroleum R1* (before use, dehydrate the anhydrous aluminium oxide by heating it in an oven at 600 °C for 3 h). Allow to settle and reduce the depth of the layer of solvent above the column to about 40 mm. Dissolve 3.0 g of the substance to be examined in 50 ml of warm *light petroleum R1*, cool, pass the solution through the column at a flow rate of 3 ml/min and wash with 250 ml of *light petroleum R1*. Concentrate the combined eluate and washings to low bulk by distillation, evaporate to dryness on a water-bath and heat the residue at 105 °C for periods of 10 min until 2 successive weighings do not differ by more than 1 mg. The residue weighs a maximum of 30 mg.

Pesticide residues

Maximum 0.05 ppm for each organochlorine pesticide, 0.5 ppm for each other pesticide and 1 ppm for the sum of all the pesticides.

All glassware used is thoroughly washed using phosphate-free detergent as follows. The glassware is immersed in a bath of detergent solution (5 per cent in deionised water) and allowed to soak for 24 h. The detergent is washed off with copious amounts of acetone and hexane for pesticide analysis. It is important to keep glassware specifically for pesticide analyses, it must not be mixed up with glassware used for other applications. The glassware used must be free of chlorinated solvents, plastics and rubber materials, in particular phthalate plasticisers, oxygenated compounds and nitrogenated solvents such as acetonitrile. Use hexane, toluene and acetone for pesticide analysis. Use HPLC grade reagents for ethyl acetate, cyclohexane and water.

The test consists of the isolation of pesticide residues by size-exclusion chromatography *(2.2.30)* followed by solid phase extraction and identification by gas chromatography coupled with an electron capture detector or a thermionic detector.

ISOLATION OF THE PESTICIDE RESIDUES

As detector, use a UV-visible spectrophotometer set at a wavelength of 254 nm to calibrate the chromatographic column for gel permeation.

Calibration is extremely important in gel permeation chromatography to check that the pressure, solvent flow rate, solvent ratio, temperature and column conditions remain constant. The gel permeation column is to be calibrated at regular intervals using a standard mixture prepared as follows: into a 1000 ml volumetric flask, introduce 50.00 g of *maize oil R*, 2.00 g of *di(2-ethylhexyl) phthalate R*, 0.20 g of *methoxychlor R*, 50.0 mg of *perylene R*, 50.0 mg of *naphthalene R*, and 80.0 mg of *sulphur R*. Dilute to 1000.0 ml with a mixture of equal volumes of *cyclohexane R* and *ethyl acetate R.*

To calibrate the column, set the mobile phase at a flow rate of 5 ml/min with a mixture of equal volumes of *cyclohexane R* and *ethyl acetate R*. Inject 5 ml of the standard mixture and record the resulting chromatogram. The retention times for the analytes must not vary by more than ± 5 per cent between calibrations. If the retention time shift is greater than ± 5 per cent, take corrective action. Excessive retention time shifts may be caused by:

— poor laboratory temperature control;

— the pump containing air; this can be verified by measuring the flow rate: collect 25 ml of column eluate in a volumetric flask and record the time (300 ± 5 s);

— a leak in the system.

Changes in pressure, in mobile phase flow rate or in column temperature conditions, as well as column contamination, can affect pesticide retention times and are to be monitored. If the flow rate or column pressure are outside desired bands the guard column or column is to be replaced.

Test solution In a volumetric flask, dissolve 1 g of wool fat, accurately weighed, in a mixture of 1 volume of ethyl acetate *R* and 7 volumes of *cyclohexane R.* Add 1 ml of an internal standard (2 ppm) *either isodrin R* or *ditalimphos R* and dilute to 20 ml. *The internal standard solutions are used to establish that recoveries of the pesticides from the GPC purification stage, evaporation and solid phase extraction stage are at acceptable levels. Recovery levels of the internal standard solutions from the wool fat are determined by comparing the peak areas of the wool fat extracts with peak areas of solutions of the internal standards.*

Guard column:

— *size*: *l* = 0.075 m, Ø = 21.2 mm;

— *stationary phase: styrene-divinylbenzene copolymer R* (5 µm).

Gel permeation column:

— *size*: *l* = 0.3 m, Ø = 21.2 mm;

— *stationary phase: styrene-divinylbenzene copolymer R* (5 µm).

Mobile phase *ethyl acetate R, cyclohexane R* (1:7 *V/V*).

Flow rate 5 ml/min.

Detection Spectrophotometer at 254 nm.

Inject 5 ml of the test solution. Discard the first 95 ml (19 min) of eluate containing the substance to be examined. Collect the next 155 ml of eluate (31 min) containing any pesticide residues in an evaporating vessel.

Place the 155 ml of the eluate collected from the gel permeation chromatography column into an evaporating vessel. Place this vessel in an autoevaporator setting the water-bath temperature at 45 °C and the nitrogen pressure at 55 kPa. Evaporate the eluate down to 0.5 ml.

To prepare the solid phase extraction cartridges take some magnesium silicate for *pesticide residue analysis R* and heat it in a muffle furnace at 700 °C for 4 h to remove moisture and polychlorinated biphenyls. Subsequently allow the magnesium silicate to cool for 2 h and transfer it directly to an oven at 100-105 °C, and allow to stand for 30 min. Transfer the magnesium silicate to a stoppered glass jar and allow to equilibrate for 48 h. This material may be used for up to 2 weeks. After that period the magnesium silicate is to be reactivated, by heating at 600 °C for 2 h in a muffle furnace. Remove the magnesium silicate from the furnace, cool and store in a stoppered glass jar. The magnesium silicate is deactivated by adding 1 per cent of *water R*. After the water has been added, shake the magnesium silicate intermittently over 15 min just prior to use. The deactivated magnesium silicate is suitable for use for up to 1 week. It is essential that only deactivated magnesium silicate is used.

Take a 6 ml empty solid phase extraction cartridge and weigh into the cartridge 1 g of the deactivated magnesium silicate.

At this stage the GPC fraction still contains about 10 per cent of the substance to be examined, so further clean-up is necessary. A separate isolation procedure is carried out a) for organochlorine and synthetic pyrethroid pesticides and b) for organophosphorus pesticides. Place a preconditioned solid phase extraction cartridge containing I g of deactivated *magnesium silicate for pesticide residue analysis R* onto a vacuum manifold.

Condition the cartridge by adding 10 ml of *toluene R* and allowing the solvent to elute through the cartridge. Place the 0.5 ml of the solvent fraction from the evaporating vessel on the preconditioned cartridge. Elute the pesticide fractions from the cartridges using 20 ml of either of the 2 different solvent systems shown below:

a) for determination of the organochlorine and *synthetic pyrethroid pesticides: toluene R*; a very small amount of the substance to be examined is co-eluted;

b) for determination of the organophosphorus pesticides: a mixture of 2 volumes of *acetone R* and 98 volumes of *toluene R*; this solvent system is used to elute all the pesticides including the more polar organophosphorus pesticides; unfortunately, some of the substance to be examined is co-eluted with this solvent system, which can interfere with the electron capture detector.

Collect the eluate from the extraction cartridges in 25 ml glass vials. Quantitatively transfer the eluate to an evaporating vessel, washing the vial with 3 quantities, each of 10 ml, of *hexane R*.

Place the evaporating vessel on the autoevaporator and evaporate the solid phase extraction fractions down to 0.5 ml. The water-bath temperature is set at 45 °C and the nitrogen pressure is 55 kPa.

Examine the residues by gas chromatography *(2.2.28)* using electron capture and thermionic detectors as described below.

Recovery Calculate the recovery correction factor (R_{cf}) of the internal standards *(ditalimphos R or isodrin R)* added to the test solution using the following expression:

$$\frac{A_2}{A_1} \times 100$$

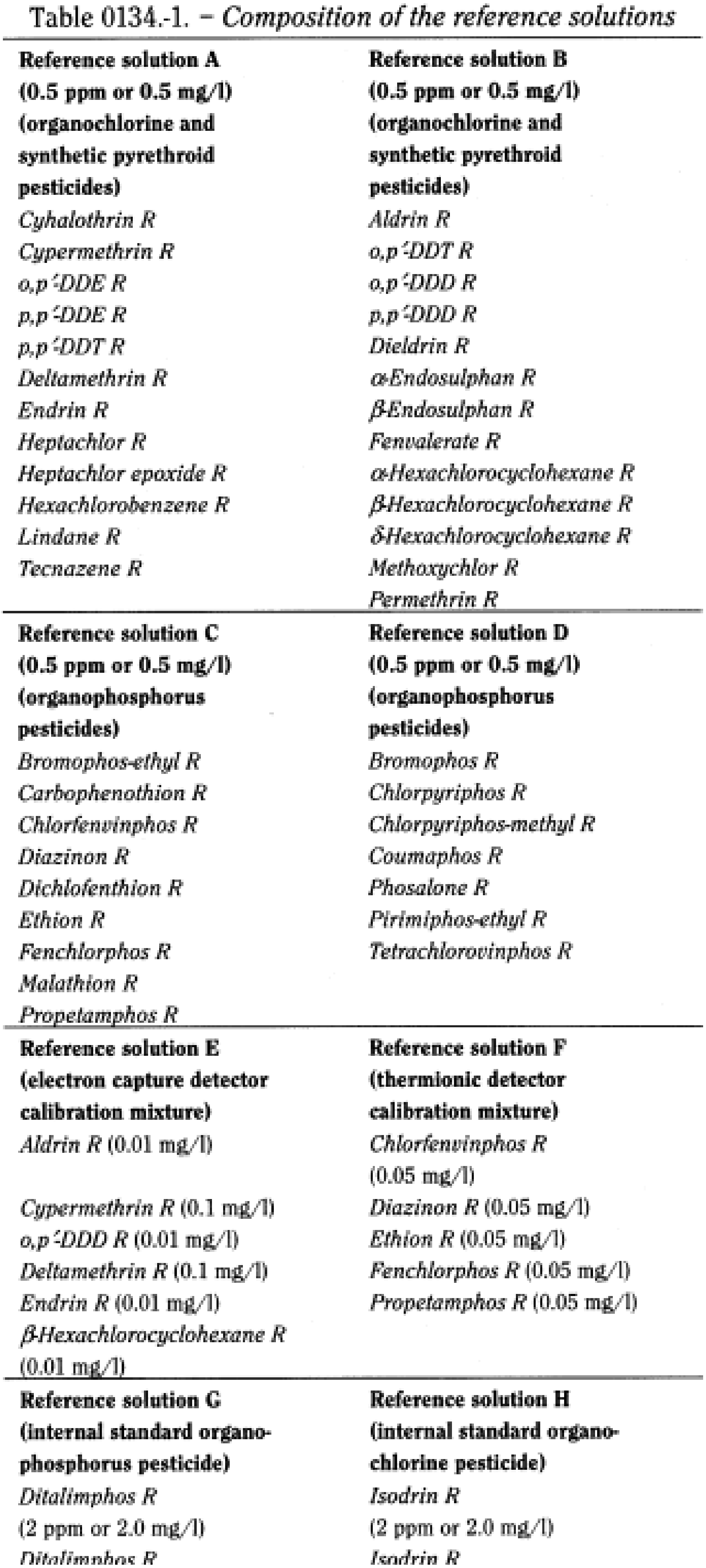
A_1 = peak area of an internal standard 1 ppm in solution

A_2 = peak area of internal standard extracted from the test solution

5 ml of the 20 ml test solution containing 1 ml of 2 ppm internal standard concentrated to 0.5 ml is equivalent to 1 ppm of the internal standard in the solution.

If the recovery of the internal standards falls outside the range of 70 per cent to 110 per cent the test is not valid.

Table 0134.-1. – *Composition of the reference solutions*



Reference solution A (0.5 ppm or 0.5 mg/l) (organochlorine and synthetic pyrethroid pesticides) *Cyhalothrin R* *Cypermethrin R* *o,p′-DDE R* *p,p′-DDE R* *p,p′-DDT R* *Deltamethrin R* *Endrin R* *Heptachlor R* *Heptachlor epoxide R* *Hexachlorobenzene R* *Lindane R* *Tecnazene R*	**Reference solution B (0.5 ppm or 0.5 mg/l) (organochlorine and synthetic pyrethroid pesticides)** *Aldrin R* *o,p′-DDT R* *o,p′-DDD R* *p,p′-DDD R* *Dieldrin R* *α-Endosulphan R* *β-Endosulphan R* *Fenvalerate R* *α-Hexachlorocyclohexane R* *β-Hexachlorocyclohexane R* *δ-Hexachlorocyclohexane R* *Methoxychlor R* *Permethrin R*
Reference solution C (0.5 ppm or 0.5 mg/l) (organophosphorus pesticides) *Bromophos-ethyl R* *Carbophenothion R* *Chlorfenvinphos R* *Diazinon R* *Dichlofenthion R* *Ethion R* *Fenchlorphos R* *Malathion R* *Propetamphos R*	**Reference solution D (0.5 ppm or 0.5 mg/l) (organophosphorus pesticides)** *Bromophos R* *Chlorpyriphos R* *Chlorpyriphos-methyl R* *Coumaphos R* *Phosalone R* *Pirimiphos-ethyl R* *Tetrachlorovinphos R*
Reference solution E (electron capture detector calibration mixture) *Aldrin R* (0.01 mg/l) *Cypermethrin R* (0.1 mg/l) *o,p′-DDD R* (0.01 mg/l) *Deltamethrin R* (0.1 mg/l) *Endrin R* (0.01 mg/l) *β-Hexachlorocyclohexane R* (0.01 mg/l)	**Reference solution F (thermionic detector calibration mixture)** *Chlorfenvinphos R* (0.05 mg/l) *Diazinon R* (0.05 mg/l) *Ethion R* (0.05 mg/l) *Fenchlorphos R* (0.05 mg/l) *Propetamphos R* (0.05 mg/l)
Reference solution G (internal standard organophosphorus pesticide) *Ditalimphos R* (2 ppm or 2.0 mg/l) *Ditalimphos R*	**Reference solution H (internal standard organochlorine pesticide)** *Isodrin R* (2 ppm or 2.0 mg/l) *Isodrin R*

(1 ppm or 1.0 mg/l)	(1 ppm or 1.0 mg/l)

Reference solutions Prepare reference solutions of pesticides using the pesticides standards at a concentration of 0.5 ppm (see composition of reference solutions A to D in Table 0134.-1). Commercially available pesticides may be purchased. The individual standards have a concentration of 10 ppm.

At the same time prepare solutions of pesticides equivalent to the limit of detection of the method (see recommended compositions in Table 0134.-1). These reference solutions are used to optimise the electron capture detector and thermionic detector to achieve the detection limits of the method (reference solutions E and F).

To prepare the reference solutions at the different concentrations use a calibrated pipette and volumetric flasks. To prepare the internal standard solutions G and H use a four-place analytical balance, pipette and volumetric flasks.

IDENTIFICATION AND QUANTIFICATION OF THE PESTICIDE RESIDUES

To identify any pesticide residues compare the chromatograms obtained with chromatograms obtained with reference solutions A to D.

The identity of the pesticides can be confirmed by spiking samples or overlaying chromatograms using an integration package on a computer. The interpretation of pesticides in trace residue analyses is extremely complex. The detectors, particularly the electron capture detector, are prone to interference, both from the substance to be examined itself, and from solvents, reagents and apparatus used in the extraction. These peaks can easily be misinterpreted or quoted as a false positive. Confirmation of pesticides can be achieved by running samples and standards on different capillary columns (see chromatographic systems A or B described below). The peaks can be identified by using the information in Table 0134.-2.

A knowledge of the different responses the pesticides have with the 2 detectors is useful in identification of unknown peaks.

Once the pesticides have been identified, calculate the content of each pesticide using the following expression:

$$C_{\mathrm{P}} = \frac{P_{\mathrm{P}} \times D \times C_{\mathrm{e}}}{P_{\mathrm{e}}} \times \frac{100}{R_{\mathrm{cf}}}$$

C_p = concentration of identified pesticide (ppm)

P_p = peak area of the individual pesticide in the test sample obtained

C_e = concentration of the individual pesticide in the external standard (ppm)

P_e = peak area of the individual pesticide in the external standard

D = dilution factor

R_{cf} = recovery correction factor

The dilution factor (D) can be defined as follows:

$$\frac{V_1}{m \times \frac{V_2}{V_3}}$$

V_1 = volume of sample obtained after the 2nd evaporation stage

m = sample weight

V_2 = GPC injection volume

V_3 = sample volumetric flask volume

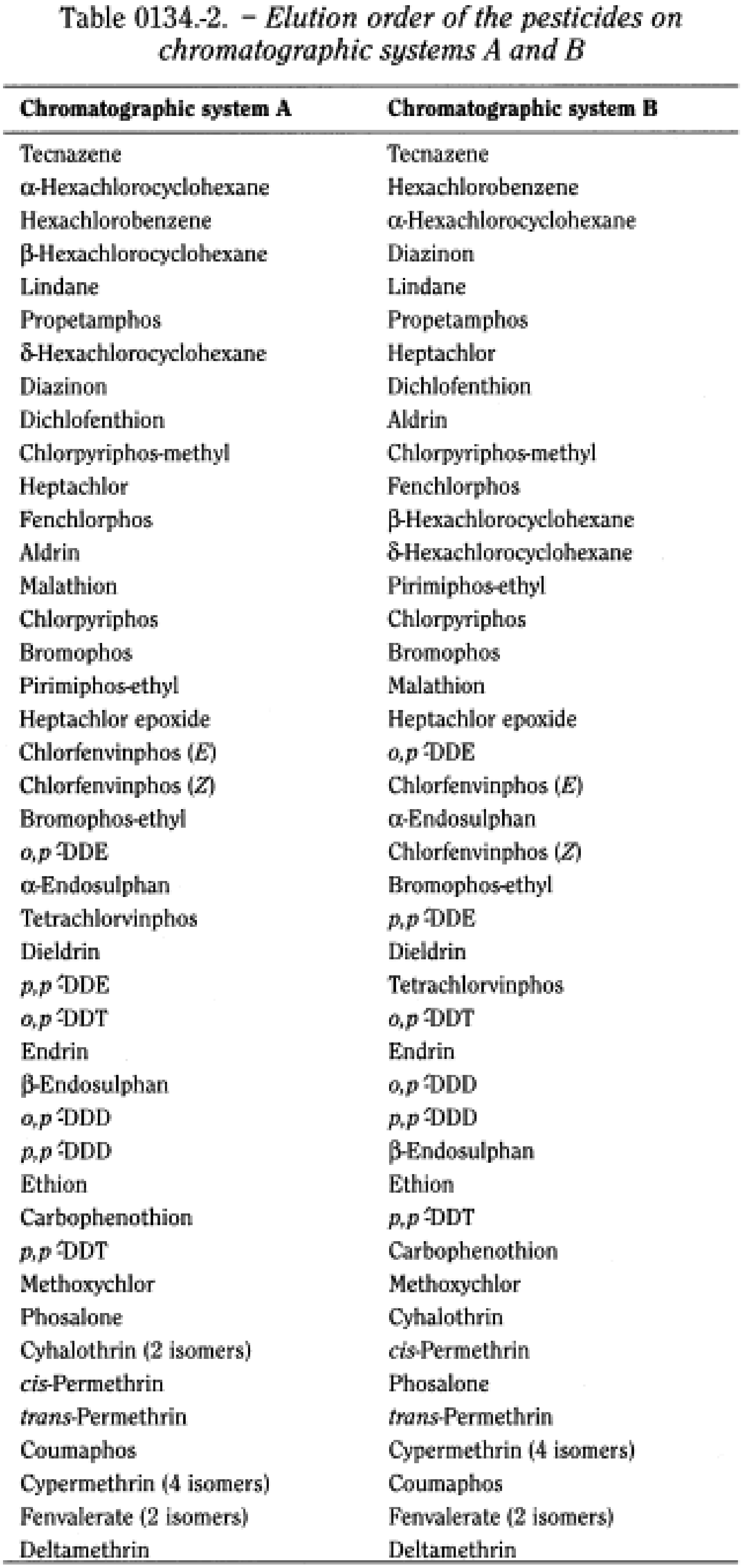


Table 0134.-2. – *Elution order of the pesticides on chromatographic systems A and B*

Chromatographic system A	Chromatographic system B
Tecnazene	Tecnazene
α-Hexachlorocyclohexane	Hexachlorobenzene
Hexachlorobenzene	α-Hexachlorocyclohexane
β-Hexachlorocyclohexane	Diazinon
Lindane	Lindane
Propetamphos	Propetamphos
δ-Hexachlorocyclohexane	Heptachlor
Diazinon	Dichlofenthion
Dichlofenthion	Aldrin
Chlorpyriphos-methyl	Chlorpyriphos-methyl
Heptachlor	Fenchlorphos
Fenchlorphos	β-Hexachlorocyclohexane
Aldrin	δ-Hexachlorocyclohexane
Malathion	Pirimiphos-ethyl
Chlorpyriphos	Chlorpyriphos
Bromophos	Bromophos
Pirimiphos-ethyl	Malathion
Heptachlor epoxide	Heptachlor epoxide
Chlorfenvinphos (*E*)	*o,p*′-DDE
Chlorfenvinphos (*Z*)	Chlorfenvinphos (*E*)
Bromophos-ethyl	α-Endosulphan
o,p′-DDE	Chlorfenvinphos (*Z*)
α-Endosulphan	Bromophos-ethyl
Tetrachlorvinphos	*p,p*′-DDE
Dieldrin	Dieldrin
p,p′-DDE	Tetrachlorvinphos
o,p′-DDT	*o,p*′-DDT
Endrin	Endrin
β-Endosulphan	*o,p*′-DDD
o,p′-DDD	*p,p*′-DDD
p,p′-DDD	β-Endosulphan
Ethion	Ethion
Carbophenothion	*p,p*′-DDT
p,p′-DDT	Carbophenothion
Methoxychlor	Methoxychlor
Phosalone	Cyhalothrin
Cyhalothrin (2 isomers)	*cis*-Permethrin
cis-Permethrin	Phosalone
trans-Permethrin	*trans*-Permethrin
Coumaphos	Cypermethrin (4 isomers)
Cypermethrin (4 isomers)	Coumaphos
Fenvalerate (2 isomers)	Fenvalerate (2 isomers)
Deltamethrin	Deltamethrin

Chromatographic system A:

Guard column:
— *material*: deactivated silica;

— *size*: *l* = 4.5 m, Ø = 0.53 mm.

Column:
— *material*: fused silica;

— *size*: *l* = 60 m, Ø = 0.25 mm;

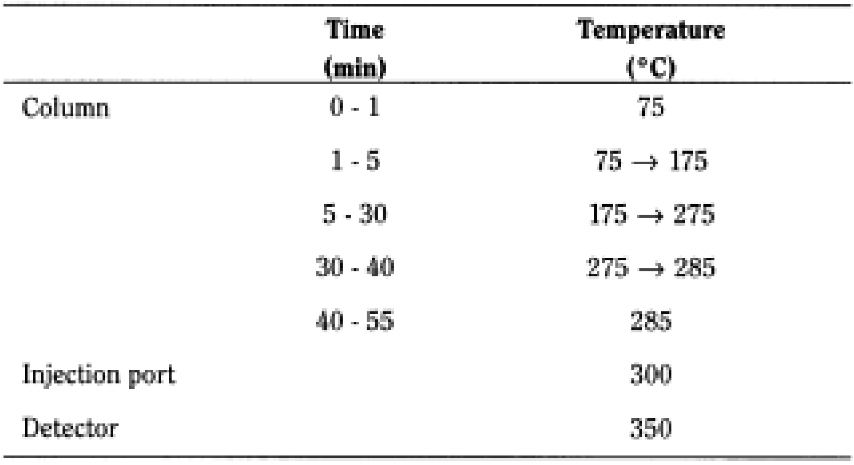
— *stationary phase: poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 25 cm/s.

Pressure 180 kPa.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	75
	1 - 5	75 → 175
	5 - 30	175 → 275
	30 - 40	275 → 285
	40 - 55	285
Injection port		300
Detector		350

Detection Electron capture or thermionic specific detector.

Injection 2 µl.

Chromatographic system B which may be used for confirmation analysis:

Guard column:
— *material*: deactivated silica;

— *size*: *l* = 4.5 m, Ø = 0.53 mm.

Column:
— *material*: fused silica;

— *size*: *l* = 60 m, Ø = 0.25 mm;

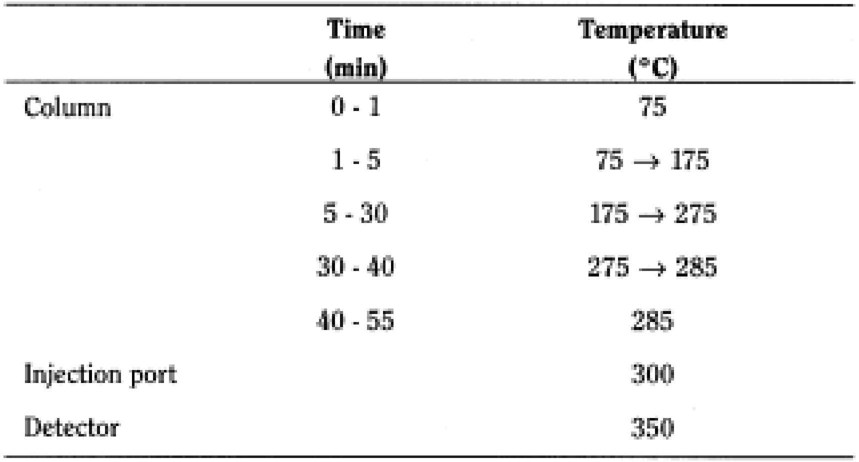
— *stationary phase*: *poly(cyanopropyl)(7)(phenyl)(7)(methyl)(86)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 25 cm/s.

Pressure 180 kPa.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	75
	1 - 5	75 → 175
	5 - 30	175 → 275
	30 - 40	275 → 285
	40 - 55	285
Injection port		300
Detector		350

Detection Electron capture or thermionic specific detector.

Injection 2 µl.

Chlorides

Maximum 150 ppm.

Boil 1.0 g with 20 ml of *ethanol (90 per cent V/V) R* in a round-bottomed flask fitted with a reflux condenser for 5 min. Cool, add 40 ml of *water R* and 0.5 ml of *nitric acid R* and filter. To the filtrate add 0.15 ml of a 10 g/l solution of *silver nitrate R* in *ethanol (90 per cent V/V) R*. Allow to stand for 5 min protected from light. Any opalescence in the solution is not more intense than that in a standard prepared at the same time by adding 0.15 ml of a 10 g/l solution of *silver nitrate R* in *ethanol (90 per cent V/V) R* to a mixture of 0.2 ml of *0.02 M hydrochloric acid*, 20 ml of *ethanol (90 per cent V/V) R*, 40 ml of *water R* and 0.5 ml of *nitric acid R*.

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulphated ash *(2.4.14)*

Maximum 0.15 per cent.

Ignite 5.0 g and use the residue to determine the sulphated ash.

STORAGE

At a temperature not exceeding 25 °C.

LABELLING

The label states, where applicable, the concentration of added butylhydroxytoluene.

Ph Eur

Xanthan Gum

General Notices

(Ph Eur monograph 1277)

Action and use

Excipient.

Ph Eur

DEFINITION

High-molecular-mass anionic polysaccharide produced by fermentation of carbohydrates with *Xanthomonas campestris*. It consists of a principal chain of β(1→4)-linked D-glucose units with trisaccharide side chains, on alternating anhydroglucose units, consisting of 1 glucuronic acid unit included between 2 mannose units. Most of the terminal units contain a pyruvate moiety and the mannose unit adjacent to the principal chain may be acetylated at C-6.

Xanthan gum has a relative molecular mass of approximately 1 × 10^6. It exists as the sodium, potassium or calcium salt.

Content

Minimum 1.5 per cent of pyruvoyl groups ($C_3H_3O_2$; M_r 71.1) (dried substance).

CHARACTERS

Appearance

White or yellowish-white, free-flowing powder.

Solubility

Soluble in water giving a highly viscous solution, practically insoluble in organic solvents.

IDENTIFICATION

A. In a flask, suspend 1 g in 15 ml of *0.1 M hydrochloric acid*. Close the flask with a fermentation bulb containing *barium hydroxide solution R* and heat carefully for 5 min. The barium hydroxide solution shows a white turbidity.

B. To 300 ml of *water R*, previously heated to 80 °C and stirred rapidly with a mechanical stirrer in a 400 ml beaker, add, at the point of maximum agitation, a dry blend of 1.5 g of *carob bean gum R* and 1.5 g of the substance to be examined. Stir until the mixture forms a solution, and then continue stirring for 30 min or longer. Do not allow the water temperature to drop below 60 °C during stirring. Discontinue stirring and allow the mixture to stand for at least 2 h. A firm rubbery gel forms after the temperature drops below 40 °C but no such gel forms in a 1 per cent control solution of the sample prepared in the same manner but omitting the carob bean gum.

TESTS

pH *(2.2.3)*

6.0 to 8.0 for a 10.0 g/l solution.

Viscosity *(2.2.10)*

Minimum 600 mPa·s.

Add 3.0 g within 45-90 s into 250 ml of a 12 g/l solution of *potassium chloride R* in a 500 ml beaker stirring with a low-pitch propeller-type stirrer rotating at 800 r/min. When adding the substance take care that agglomerates are destroyed. Add an additional quantity of 44 ml of *water R*, to rinse any adhering residue from the walls of the beaker. Stir the preparation at 800 r/min for 2 h whilst maintaining the temperature at 24 ± 1 °C. Determine the viscosity within 15 min at 24 ± 1 °C using a rotating viscosimeter set at 60 r/min and equipped with a rotating spindle 12.7 mm in diameter and 1.6 mm high which is attached to a shaft 3.2 mm in diameter. The distance from the top of the cylinder to the lower tip of the shaft being 25.4 mm, and the immersion depth being 50.0 mm.

2-Propanol

Gas chromatography *(2.2.28)*.

Internal standard solution Dilute 0.50 g of *2-methyl-2-propanol R* to 500 ml with *water R*.

Test solution To 200 ml of *water R* in a 1000 ml round-bottomed flask, add 5.0 g of the substance to be examined and 1 ml of a 10 g/l emulsion of *dimeticone R* in *liquid paraffin R*, stopper the flask and shake for 1 h. Distil about 90.0 ml, mix the distillate with 4.0 ml of the internal standard solution and dilute to 100.0 ml with *water R*.

Reference solution Dilute a suitable quantity of *2-propanol R*, accurately weighed, with *water R* to obtain a solution having a known concentration of 2-propanol of about 1 mg/ml. To 4.0 ml of this solution add 4.0 ml of the internal standard solution and dilute to 100.0 ml with *water R*.

Column:
- *size:* *l* = 1.8 m, Ø = 4.0 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (149-177 µm).

Carrier gas *helium for chromatography R.*

Flow rate 30 ml/min.

Temperature:
- *column*: 165 °C;
- *injection port and detector*: 200 °C.

Detection Flame ionisation.

Injection 5 µl.

Relative retention With reference to 2-propanol: 2-methyl-2-propanol = about 1.5.

Limit:
- *2-propanol*: maximum 750 ppm.

Other polysaccharides

Thin-layer chromatography *(2.2.27)*.

Test solution To 10 mg of the substance to be examined in a thick-walled centrifuge test tube add 2 ml of a 230 g/l solution of *trifluoroacetic acid R*, shake vigorously to dissolve the forming gel, stopper the test tube, and heat the mixture at 120 °C for 1 h. Centrifuge the hydrolysate, transfer the clear supernatant liquid carefully into a 50 ml flask, add 10 ml of *water R* and evaporate the solution to dryness under reduced pressure. Take up the residue thus obtained in 10 ml of *water R* and evaporate to dryness under reduced pressure. Wash 3 times with 20 ml of *methanol R* and evaporate under reduced pressure. To the resulting clear film which has no odour of acetic acid, add 0.1 ml of *water R* and 1 ml of *methanol R*. Centrifuge to separate the amorphous precipitate. Dilute the supernatant liquid, if necessary, to 1 ml with *methanol R*.

Reference solution Dissolve 10 mg of *glucose R* and 10 mg of *mannose R* in 2 ml of *water R* and dilute to 10 ml with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase 16 g/l solution of *sodium dihydrogen phosphate R, butanol R, acetone R* (10:40:50 *V/V/V*).

Application 5 µl, as bands.

Development Over a path of 15 cm.

Detection Spray with a solution of 0.5 g of *diphenylamine R* in 25 ml of *methanol R* to which 0.5 ml of *aniline R* and 2.5 ml of *phosphoric acid R* have been added. Heat for 5 min at 120 °C and examine in daylight.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated greyish-brown zones due to glucose and mannose in the middle third.

Results The chromatogram obtained with the test solution shows 2 zones corresponding to the zones due to glucose and mannose in the chromatogram obtained with the reference solution. In addition, 1 weak reddish and 2 faint bluish-grey bands may be visible just above the starting line. 1 or 2 bluish-grey bands may also be seen in the upper quarter of the chromatogram. No other bands are visible.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2.5 h.

Total ash *(2.4.16)*

6.5 per cent to 16.0 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the test for *Escherichia coli* *(2.6.13)*.

ASSAY

Test solution Dissolve a quantity of the substance to be examined corresponding to 120.0 mg of the dried substance in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution Dissolve 45.0 mg of *pyruvic acid R* in *water R* and dilute to 500.0 ml with the same solvent.

Place 10.0 ml of the test solution in a 50 ml round-bottomed flask, add 20.0 ml of *0.1 M hydrochloric acid* and weigh. Boil on a water-bath under a reflux condenser for 3 h. Weigh and adjust to the initial mass with *water R*. In a separating funnel mix 2.0 ml of the solution with 1.0 ml of *dinitrophenylhydrazine-hydrochloric solution R*. Allow to stand for 5 min and add 5.0 ml of *ethyl acetate R*. Shake and allow the solids to settle. Collect the upper layer and shake with 3 quantities, each of 5.0 ml, of *sodium carbonate solution R*. Combine the aqueous layers and dilute to 50.0 ml with *sodium carbonate solution R*. Mix. Treat 10.0 ml of the reference solution at the same time and in the same manner as for the test solution.

Immediately measure the absorbance *(2.2.25)* of the 2 solutions at 375 nm, using *sodium carbonate solution R* as the compensation liquid.

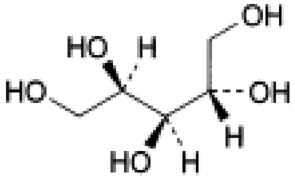
The absorbance of the test solution is not less than that of the reference solution, which corresponds to a content of pyruvic acid of not less than 1.5 per cent.

Ph Eur

Xylitol

General Notices

(Ph Eur monograph 1381)



$C_5H_{12}O_5$ 152.1 *87-99-0*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

Xylitol contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of *meso*-xylitol, calculated with reference to the anhydrous substance.

CHARACTERS

White or almost white, crystalline powder or crystals, very soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification B.

Second identification A, C.

A. Melting point *(2.2.14)*: 92 °C to 96 °C.

B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *xylitol CRS*. Examine the substance prepared as a mull in *liquid paraffin R*.

C. Examine by thin-layer chromatography *(2.2.27)*, using a *TLC silica gel G plate R*.

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 25 mg of *xylitol CRS* in *water R* and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dissolve 25 mg of *mannitol CRS* and 25 mg of *xylitol CRS* in *water R* and dilute to 5 ml with the same solvent.

Apply to the plate 2 µl of each solution. Develop over a path of 17 cm using a mixture of 10 volumes of *water R*, 20 volumes of *ethyl acetate R* and 70 volumes of *propanol R*. Allow the plate to dry in air and spray with *4-aminobenzoic acid solution R*. Dry the plate in a current of cold air until the acetone is removed. Heat the plate at 100 °C for 15 min. Allow to cool and spray with a 2 g/l solution of *sodium periodate R*. Dry the plate in a current of cold air. Heat the plate at 100 °C for 15 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

TESTS

Appearance of solution

Dissolve 2.5 g in *water R* and dilute to 50.0 ml with the same solvent. The solution is not more opalescent than reference suspension IV *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II).*

Conductivity *(2.2.38)*

Not more than 20 $\mu S \cdot cm^{-1}$.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Reducing sugars

Dissolve 5.0 g in 6 ml of *water R* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required (0.2 per cent, calculated as glucose equivalent).

Related substances

Examine by gas chromatography *(2.2.28)* as described under Assay. The sum of the percentage contents of the related substances in the chromatogram obtained with test solution (a) is not greater than 2.0 per cent.

Lead *(2.4.10)*

It complies with the limit test for lead in sugars (0.5 ppm). Dissolve the substance to be examined in 150.0 ml of the prescribed mixture of solvents.

Nickel *(2.4.15)*

It complies with the limit test for nickel in polyols (1 ppm). Dissolve the substance to be examined in 150.0 ml of the prescribed mixture of solvents.

Water *(2.5.12)*

Not more than 1.0 per cent, determined on 1.00 g.

Bacterial endotoxins *(2.6.14)*

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins, less than 4 IU/g for parenteral dosage forms having a concentration of less than 100 g/l of xylitol and less than 2.5 IU/g for parenteral dosage forms having a concentration of 100 g/l or more of xylitol.

ASSAY

Examine by gas chromatography *(2.2.28)*, using erythritol as the internal standard.

Internal standard solution Dissolve 5 mg of *erythritol R* in *water R* and dilute to 25.0 ml with the same solvent.

Test solution (a) Dissolve 5.000 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Test solution (b) Dilute 1.0 ml of test solution (a) to 10.0 ml with *water R*.

Reference solution (a) Dissolve 5.0 mg each of *l-arabinitol CRS*, *galactitol CRS*, *mannitol CRS* and *sorbitol CRS* in *water R* and dilute to 20.0 ml with the same solvent.

Reference solution (b) Dissolve 50.0 mg of *xylitol CRS* in *water R* and dilute to 10.0 ml with the same solvent.

The chromatographic procedure may be carried out using:

— a stainless steel column 2 m long and 2 mm in internal diameter packed with acid washed *silanised diatomaceous earth for gas chromatography R* (150-180 µm) and impregnated with 3 per cent *m/m* of *poly[(cyanopropyl)(methyl)][(phenyl)(methyl)]siloxane R*;

— *nitrogen R* as the carrier gas at a flow rate of 30 ml/min;

— a flame-ionisation detector,

maintaining the temperature of the column at 170 °C for 1 min, then raising the temperature at a rate of 6 °C/min to 200 °C and maintaining the temperature of the injection port and of the detector at 250 °C.

Pipet 1.0 ml of reference solutions (a) and (b) and test solutions (a) and (b) into 4 separate 100 ml round-bottomed flasks. Add 1.0 ml of the internal standard solution separately to the flasks with test solution (a) and reference solution (a) and 5.0 ml of the internal standard solution separately to the flasks with test solution (b) and reference solution (b). Evaporate the respective mixtures to dryness in a water-bath at 60 °C with the aid of a rotary evaporator. Dissolve each dry residue in 1 ml of *anhydrous pyridine R* and add 1 ml of *acetic anhydride R* to each flask and boil each solution under reflux for 1 h to complete acetylation.

Inject 1 µl of each of the solutions obtained from the reference solutions and the test solutions.

When the chromatograms are recorded in the prescribed conditions, the relative retentions calculated with reference to xylitol are: erythritol about 0.3, arabinitol about 0.7, mannitol about 1.8, galactitol about 2.0 and sorbitol about 2.2.

Calculate the percentage content of $C_5H_{12}O_5$ from the expression:

$$\mathrm{T} \times \frac{m_t}{m_v} \times \frac{R_v}{R_t}$$

T = declared content of *xylitol CRS* in percentage

m_t = mass of *xylitol CRS* in 1 ml of reference solution (b), in milligrams

m_v = mass of the substance to be examined in 1 ml of test solution (b), in milligrams

R_t = ratio of the areas of the derivatised xylitol peak to the derivatised erythritol peak in the chromatogram obtained with reference solution (b)

R_v = ratio of the areas of the derivatised xylitol peak to the derivatised erythritol peak in the chromatogram obtained with test solution (b)

Calculate the percentage content of each related substance in the substance to be examined from the expression:

$$100 \times \frac{m_s}{m_u} \times \frac{R_u}{R_s}$$

m_s = mass of the respective component in 1 ml of reference solution (a), in milligrams

m_u = mass of the substance to be examined in 1 ml of test solution (a), in milligrams

R_s = ratio of the areas of the derivatised component peak to the derivatised erythritol peak in the chromatogram obtained with reference solution (a)

R_u = ratio of the areas of the derivatised component peak to the derivatised erythritol peak in the chromatogram obtained with test solution (a)

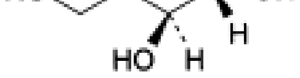
LABELLING

The label states:

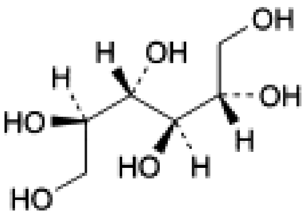
— where applicable, the maximum concentration of bacterial endotoxins;

— where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES



A. L-arabinitol,



B. *meso*-galactitol,

C. mannitol,

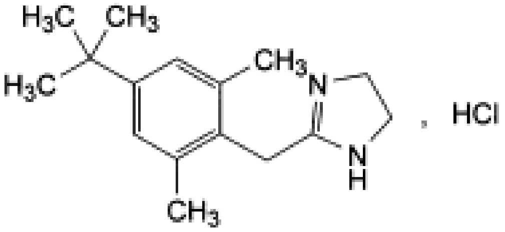
D. sorbitol.

Ph Eur

Xylometazoline Hydrochloride

General Notices

(Ph Eur monograph 1162)



$C_{16}H_{24}N_2$,HCl 280.8 *1218-35-5*

Action and use

Alpha-adrenoceptor agonist.

Preparation

Xylometazoline Nasal Drops

Ph Eur

DEFINITION

2-[4-(1,1-Dimethylethyl)-2,6-dimethylbenzyl]-4,5-dihydro-1*H*-imidazole hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification A, E.

Second identification B, C, D, E.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *xylometazoline hydrochloride CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution Dissolve 20 mg of *xylometazoline hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R, methanol R* (5:100 *V/V*).

Application 5 µl.

Development Over 2/3 of the plate.

Drying In air.

Chlorine treatment At the bottom of a chromatography tank place a beaker containing a mixture of 1 volume of *hydrochloric acid R1*, 1 volume of *water R* and 2 volumes of a 15 g/l solution of *potassium permanganate R*. Close the tank and allow to stand for 15 min. Place the dried plate in the tank and reclose the tank. Leave the plate in contact with the chlorine vapour for 5 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of the coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution R*.

Detection Spray with *potassium iodide and starch solution R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 0.5 mg in 1 ml of *methanol R*. Add 0.5 ml of a freshly prepared 50 g/l solution of *sodium nitroprusside R* and 0.5 ml of a 20 g/l solution of *sodium hydroxide R.* Allow to stand for 10 min and add 1 ml of an 80 g/l solution of *sodium bicarbonate R*. A violet colour develops.

D. Dissolve 0.2 g in 1 ml of *water R*, add 2.5 ml of *ethanol (96 per cent) R* and 2 ml of *1 M sodium hydroxide*. Mix thoroughly and examine in ultraviolet light at 365 nm. The solution shows no fluorescence or at most the same fluorescence as a blank solution prepared in the same manner. The identification is not valid unless a solution prepared in the same manner using *naphazoline hydrochloride CRS* instead of the substance to be examined shows a distinct bluish fluorescence.

E. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Dissolve 2.5 g in *water R* and dilute to 50.0 ml with the same solvent.

Acidity or alkalinity

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent. Add 0.1 ml of *methyl red solution R* and 0.1 ml of *0.01 M hydrochloric acid*. The solution is red. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent. Allow to stand for 1 h before injection.

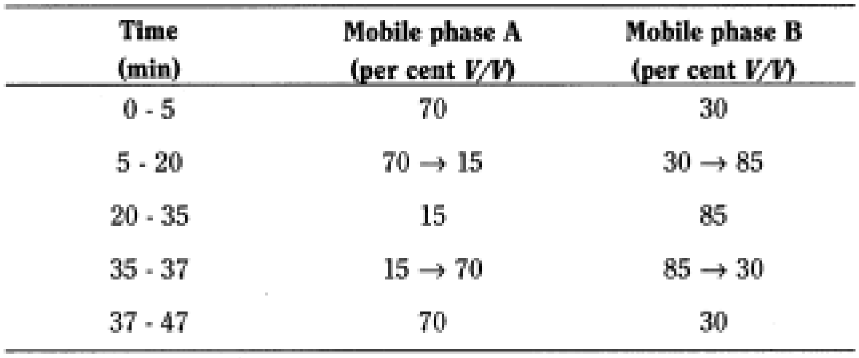
Reference solution (a) Dilute 5.0 ml of the test solution to 100.0 ml with *water R*. Dilute 2.0 ml of this solution to 100.0 ml with *water R*.

Reference solution (b) Dissolve 5.0 mg of *xylometazoline impurity A CRS* and 5 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent. Dilute 10.0 ml of this solution to 50.0 ml with *water R*.

Reference solution (c) Dilute 5.0 ml of reference solution (b) to 50.0 ml with *water R.*

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography with polar incorporated groups R (5 µm).*

Mobile phase:
- *mobile phase A*: 1.36 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B: acetonitrile R1*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	70	30
5 - 20	70 → 15	30 → 85
20 - 35	15	85
35 - 37	15 → 70	85 → 30
37 - 47	70	30

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µl.

Relative retention With reference to xylometazoline (retention time = about 7.2 min): impurity A = about 0.79.

System suitability Reference solution (b):

- *resolution:* minimum 2.5 between the peaks due to impurity A and xylometazoline.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 25 ml of *anhydrous acetic acid R* and add 10 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M perchloric acid* is equivalent to 28.08 mg of $C_{16}H_{25}ClN_2$.

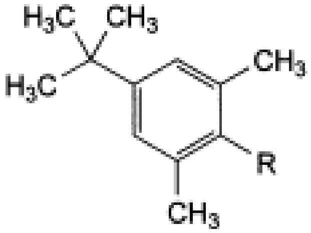
STORAGE

Protected from light.

IMPURITIES

Specified impurities A.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E, F.*



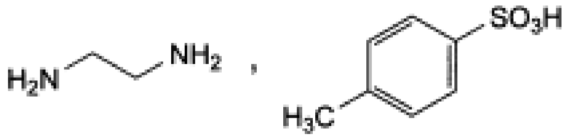
A. R = CH_2-CO-NH-CH_2-CH_2-NH_2: *N*-(2-aminoethyl)-2-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetamide,

B. R = CH_2-Cl: 2-(chloromethyl)-5-(1,1-dimethylethyl)-1,3-dimethylbenzene,

C. R = CH_2-CN: [4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetonitrile,

D. R = H: 1-(1,1-dimethylethyl)-3,5-dimethylbenzene,

F. CH_2-CO_2H: [4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetic acid,



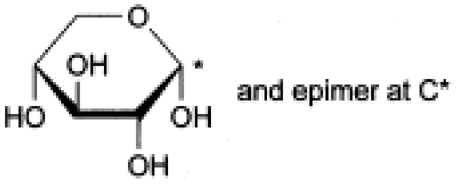
E. ethane-1,2-diamine mono(4-methylbenzenesulphonate).

Ph Eur

Xylose

General Notices

(Ph Eur monograph 1278)



$C_5H_{10}O_5$ 150.1 *58-86-6*

Ph Eur

DEFINITION

(+)-D-Xylopyranose.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless needles.

Solubility

Freely soluble in water, soluble in hot ethanol (96 per cent).

IDENTIFICATION

First identification *A.*

Second identification *B, C.*

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *xylose CRS.*

B. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *water R, methanol R* (2:3 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *xylose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *fructose R*, 10 mg of *glucose R* and 10 mg of *xylose R* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Plate *TLC silica gel plate R*.

Mobile phase *water R*, *methanol R*, *anhydrous acetic acid R*, *ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application 2 µl; thoroughly dry the starting points.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with a 5 g/l solution of *thymol R* in a mixture of 5 volumes of *sulphuric acid R* and 95 volumes of *ethanol (96 per cent) R*. Heat in an oven at 130 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. An orange or red precipitate is formed.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity

To 50 ml of solution S add 0.3 ml of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation *(2.2.7)*

+ 18.5 to + 19.5 (dried substance).

Dissolve 10.0 g in 80 ml of *water R*, add 1 ml of *dilute ammonia R2* and dilute to 100.0 ml with *water R*. Allow to stand for 30 min.

Chlorides *(2.4.4)*

Maximum 330 ppm.

Dilute 1.5 ml of solution S to 15 ml with *water R*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash ***(2.4.14)***

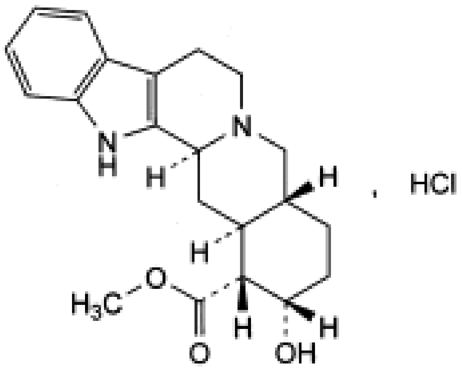
Maximum 0.1 per cent, determined on 1.0 g.

Ph Eur

Yohimbine Hydrochloride

General Notices

(Ph Eur monograph 2172)



$C_{21}H_{26}N_2O_3$,HCl 390.9 *65-19-0*

Ph Eur

DEFINITION

Methyl 17α-hydroxyyohimban-16α-carboxylate hydrochloride.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder.

Solubility

Sparingly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *yohimbine hydrochloride CRS*.

B. It gives reaction (a) of chlorides *(2.3.1)*.

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* with heating, allow to cool to room temperature and dilute to 50.0 ml with the same solvent.

pH *(2.2.3)*

3.5 to 5.5 for solution S.

Specific optical rotation *(2.2.7)*

+ 101.0 to + 105.0 (dried substance), determined on solution S.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions protected from light*.

Test solution Dissolve 10.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *yohimbine hydrochloride CRS* (containing impurities A, F and G) in *methanol R* and dilute to 25.0 ml with the same solvent.

Reference solution (b) Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol R*.

Reference solution (c) Dilute 1.0 ml of reference solution (b) to 10.0 ml with *methanol R*.

Column:

- *size*: l = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (4 µm);
- *temperature*: 40 °C.

Mobile phase Mix 50 ml of a 9.08 g/l solution of *potassium dihydrogen phosphate R*, 100 ml of an 11.88 g/l solution of *disodium hydrogen phosphate dihydrate R*, 285 ml of *acetonitrile R*, 4.0 g of *sodium laurilsulfate R* and 355 ml of *water R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 229 nm.

Injection 10 µl.

Run time 3 times the retention time of yohimbine.

Relative retention With reference to yohimbine (retention time = about 7 min): impurity F = about 0.65; impurity G = about 0.70; impurity A = about 0.75.

System suitability Reference solution (a):

- *peak-to-valley ratio*: minimum 1.3, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A; and minimum 1.3, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:

- *sum of impurities A and G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *impurity F*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.4 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{21}H_{27}ClN_2O_3$ from the declared content of *yohimbine hydrochloride CRS*.

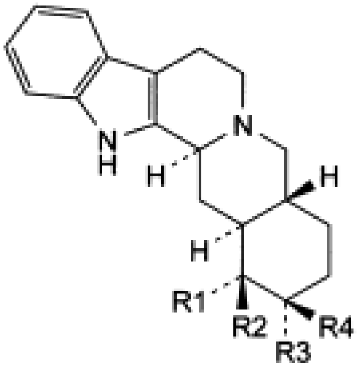
STORAGE

In an airtight container , protected from light.

IMPURITIES

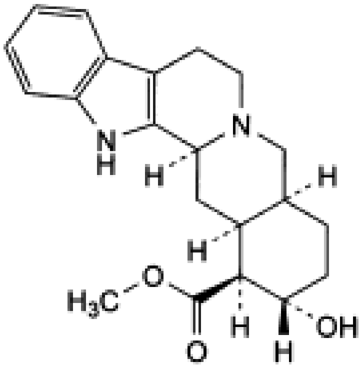
Specified impurities *A, F, G*.

Other detectable impurities (The following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E.*



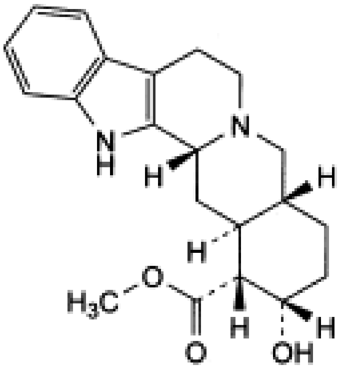
A. R1 = CO-OCH_3, R2 = R3 = H, R4 = OH: methyl 17β-hydroxyyohimban-16α-carboxylate (β-yohimbine),

C. R1 = R4 = H, R2 = CO-OCH_3, R3 = OH: methyl 17α-hydroxyyohimban-16β-carboxylate (corynantheine),



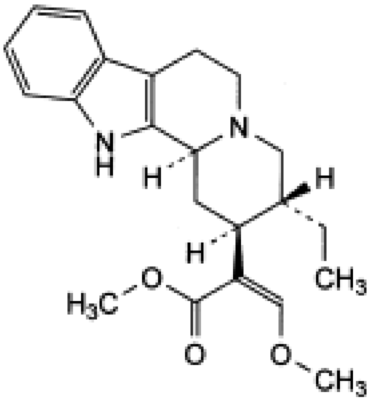
N
H
N
H
H
H
H_3C
O
O
H
OH
H

B. methyl 17α-hydroxy-20α-yohimban-16β-carboxylate (α-yohimbine),



N
H
N
H
H
H
H_3C
O
O
H
H
OH

D. methyl 17α-hydroxy-3β-yohimban-16α-carboxylate (pseudo-yohimbine),



N
H
N
H
H
H
CH_3
H_3C
O
O
O
CH_3

E. methyl (2*Z*)-2-[(2*S*,3*R*,12b*S*)-3-ethyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizin-2-yl]-3-methoxyprop-2-enoate,

F. unknown structure,

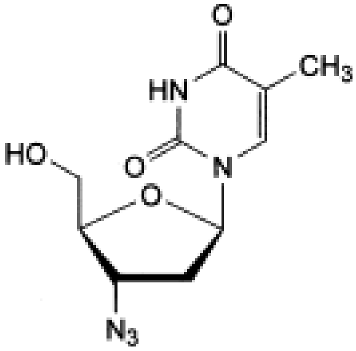
G. unknown structure.

Ph Eur

Zidovudine

General Notices

(Ph Eur monograph 1059)



$C_{10}H_{13}N_5O_4$ 267.2 *30516-87-1*

Action and use

Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

1-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or brownish powder.

Solubility

Sparingly soluble in water, soluble in anhydrous ethanol.

mp

About 124 °C.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *zidovudine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness in a desiccator, under high vacuum over *diphosphorus pentoxide R* and record new spectra using the residues.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_5 *(2.2.2, Method II)*.

Dissolve 0.5 g in 50 ml of *water R*, heating if necessary.

Specific optical rotation *(2.2.7)*

+ 60.5 to + 63.0 (dried substance).

Dissolve 0.50 g in *anhydrous ethanol R* and dilute to 50.0 ml with the same solvent. Carry out the determination at 25 °C.

Related substances

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a) Dissolve 5 mg of *thymine R* (impurity C), 5 mg of *zidovudine impurity A CRS* and 5 mg of *triphenylmethanol R* (impurity D) in *methanol R,* add 0.25 ml of the test solution and dilute to 25 ml with *methanol R*.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 10 ml with *methanol R.*

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *methanol R*, *methylene chloride R* (10:90 *V/V*).

Application 10 µl.

Development Over a path of 12 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Limits:

— *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: any other spot apart from the principal spot and any spot due to impurity C (which is limited by liquid chromatography) is not more intense than the spot due to zidovudine in the chromatogram obtained with reference solution (b) (0.5 per cent).

Detection B Spray with a 10 g/l solution of *vanillin R* in *sulphuric acid R*.

Limit:

— *impurity D*: any spot due to impurity D is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

System suitability Reference solution (a):

— the chromatogram shows 4 clearly separated spots, due to impurity C, impurity A, zidovudine and impurity D, in order of increasing R_F value.

B. Liquid chromatography *(2.2.29)*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b) Dilute 10.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *zidovudine CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *thymine R* (impurity C) in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (c) Dissolve 5 mg of *zidovudine impurity B CRS* in 25.0 ml of reference solution (a) and dilute to 50.0 ml with the mobile phase.

Reference solution (d) Dilute 5.0 ml of reference solution (c) to 50.0 ml with the mobile phase.

Reference solution (e) Dilute 0.25 ml of test solution (a) to 50.0 ml with the mobile phase.

Column:
— *size:* *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, *water R* (20:80 *V/V*).

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 265 nm.

Equilibration With the mobile phase for about 45 min.

Injection 10 µl of test solution (a) and reference solutions (b), (c), (d) and (e).

Run time 1.5 times the retention time of zidovudine.

Elution order Impurity C, zidovudine, impurity B.

System suitability Reference solution (c):

— *resolution*: minimum 1.0 between the peaks due to zidovudine and impurity B.

Limits:
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (2 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (1 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.5 per cent);

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained

with reference solution (e) (3.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Heavy metals ***(2.4.8)***

Maximum 20 ppm.

1.00 g complies with test D. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying ***(2.2.32)***

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash ***(2.4.14)***

Maximum 0.25 per cent, determined on 1.00 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

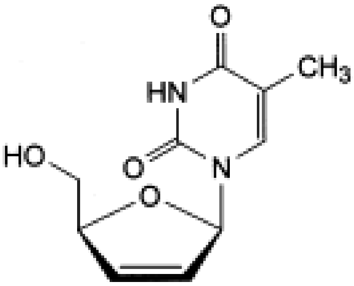
Calculate the content of $C_{10}H_{13}N_5O_4$ from the declared content of *zidovudine CRS*.

STORAGE

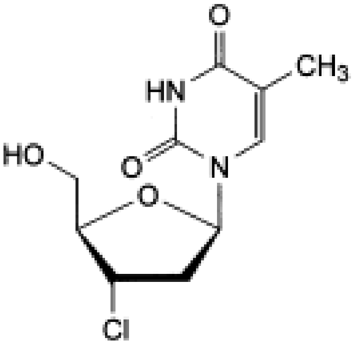
Protected from light.

IMPURITIES

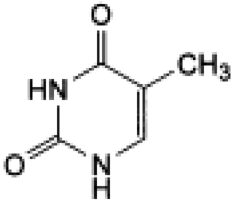
Specified impurities *A, B, C, D.*



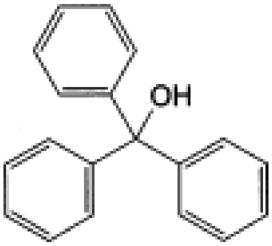
A. 1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



B. 1-(3-chloro-2,3-dideoxy-β-D-erythro-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



C. 5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine),



D. triphenylmethanol.

Ph Eur

Zinc Acetate

General Notices

(Zinc Acetate Dihydrate, Ph Eur monograph 1482)

$C_4H_6O_4Zn,2H_2O$ 219.5 *5970-45-6*

Action and use

Astringent.

Preparation

Erythromycin and Zinc Acetate Lotion

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of $C_4H_6O_4Zn,2H_2O$.

CHARACTERS

Appearance

White or almost white crystalline powder or flakes.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives reaction (a) of acetates *(2.3.1)*.

B. It gives the reaction of zinc *(2.3.1)*.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.8 to 7.0.

Dilute 10 ml of solution S to 20 ml with *carbon dioxide-free water R*.

Reducing substances

Boil for 5 min a mixture of 10 ml of solution S, 90 ml of *water R*, 5 ml of *dilute sulphuric acid R* and 1.5 ml of a 0.3 g/l solution of *potassium permanganate R*. The pink colour of the solution remains.

Chlorides *(2.4.4)*

Maximum 50 ppm.

Dilute 10 ml of solution S with 15 ml of *water R*.

Sulphates *(2.4.13)*

Maximum 100 ppm, determined on solution S.

Aluminium

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 2.50 g in 20 ml of a 200 g/l solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 ml with the same acidic solution.

Reference solutions Prepare the reference solutions using *aluminium standard solution (200 ppm Al) R*, diluted with a 200 g/l solution of *cadmium- and lead-free nitric acid R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Air-acetylene or acetylene-nitrous oxide flame.

Arsenic *(2.4.2, Method A)*

Maximum 2 ppm, determined on 0.5 g.

Cadmium

Maximum 2.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use the solution described in the test for aluminium.

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R*, diluted with a 200 g/l solution of *cadmium- and lead-free nitric acid R*.

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Air-acetylene flame.

Copper

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use the solution described in the test for iron.

Reference solutions Prepare the reference solutions using *copper standard solution (10 ppm Cu) R*, diluted with a 200 g/l solution of *cadmium- and lead-free nitric acid R*.

Source Copper hollow-cathode lamp.

Wavelength 324.8 nm.

Atomisation device Air-acetylene flame.

Iron

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 1.25 g in 20 ml of a 200 g/l solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 ml with the same acid solution.

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, diluted with a 200 g/l solution of *cadmium- and lead-free nitric acid R*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device air-acetylene flame.

Lead

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 5.00 g in 20 ml of a 200 g/l solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 ml with the same acid solution.

Reference solutions Prepare the reference solutions using *lead standard solution (0.1 per cent of Pb) R*, diluting with a 200 g/l solution of cadmium- and *lead-free nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm.

Atomisation device Air-acetylene flame.

ASSAY

Dissolve 0.200 g in 5 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 21.95 mg of $C_4H_6O_4Zn,2H_2O$.

STORAGE

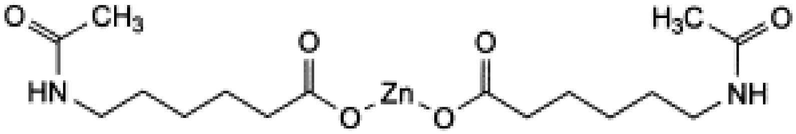
In a non-metallic container.

Ph Eur

Zinc Acexamate

General Notices

(Ph Eur monograph 1279)



$C_{16}H_{28}N_2O_6Zn$ 409.8 *70020-71-2*

Ph Eur

DEFINITION

Zinc 6-(acetylamino)hexanoate.

Content

97.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, practically insoluble in acetone and in ethanol (96 per cent). It dissolves in dilute nitric acid.

mp: about 198 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation discs.

Comparison *zinc acexamate CRS.*

B. 5 ml of solution S (see Tests) gives the reaction of zinc *(2.3.1)*.

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension IV *(2.2.1)* and is colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

5.0 to 7.0 for solution S.

Impurity B

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.30 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 15 mg of *6-aminohexanoic acid R* (impurity B) in *water R* and dilute to 10 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *water R*.

Plate *TLC silica gel plate R*.

Mobile phase *ammonia R, water R, ethanol (96 per cent) R* (2:30:68 *V/V/V*).

Application 5 µl; allow to dry in air.

Development Over a path of 15 cm.

Drying Iin a current of warm air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

Limit:

— *impurity B*: any spot due to impurity B is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances

Liquid chromatography (2.2.29).

Test solution (a) Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 100.0 ml with the same solvent.

Test solution (b) To 20.0 ml of test solution (a), add 20 ml of the mobile phase and 0.4 ml of a 100 g/l solution of *phosphoric acid R*, then dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 40 mg of *N-acetyl-∊-caprolactam R* (impurity C) in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (b) Dilute 5.0 ml of reference solution (a) to 100.0 ml with *water R.*

Reference solution (c) Dissolve 20 mg of *zinc acexamate impurity A CRS* in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (d) Dissolve 40 mg of *∊-caprolactam R* (impurity D) in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*.

Reference solution (e) To 20.0 ml of test solution (a), add 5.0 ml of reference solution (b), 5.0 ml of reference solution (c), 5.0 ml of reference solution (d) and 0.4 ml of a 100 g/l solution of *phosphoric acid R*, then dilute to 50.0 ml with the mobile phase.

Reference solution (f) To 5.0 ml of reference solution (c), add 5.0 ml of reference solution (b) , 5.0 ml of reference solution (d) and 0.4 ml of a 100 g/l solution of *phosphoric acid R*, then dilute to 50.0 ml with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 0.2 volumes of *phosphoric acid R*, 8 volumes of *acetonitrile R* and 92 volumes of *water R*, then adjust to pH 4.5 with dilute *ammonia R1*.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µl of test solution (b) and reference solutions (e) and (f).

Run time 8 times the retention time of zinc acexamate.

Elution order Zinc acexamate, impurity D, impurity A, impurity C.

System suitability Reference solution (e):

— *resolution*: minimum 3.0 between the peaks due to zinc acexamate and impurity D; if necessary, adjust the mobile phase to pH 4.7 with *dilute ammonia R1*.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (2 per cent);

— *impurities C, D*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.1 per cent);

— *sum of impurities other than A*: not more than 5 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (f) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (f) (0.05 per cent).

Arsenic ***(2.4.2, Method A)***

Maximum 2 ppm, determined on 0.5 g.

Cadmium

Maximum 2.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 2.50 g in 20 ml of a 200 g/l solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 ml with the same acidic solution.

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R*, diluting with a 200 g/l solution of *cadmium- and lead-free nitric acid R*.

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Air-acetylene flame.

Iron

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 1.25 g in 20 ml of a 200 g/l solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 ml with the same acidic solution.

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, diluting with a 200 g/l solution of *cadmium- and lead-free nitric acid R*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Lead

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 5.00 g in 20 ml of a 200 g/l solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 ml with the same acidic solution.

Reference solutions Prepare the reference solutions using *lead standard solution (0.1 per cent Pb) R*, diluting with a 200 g/l solution of *cadmium- and lead-free nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm.

Atomisation device Air-acetylene flame.

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.400 g in 10 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc *(2.5.11)*.

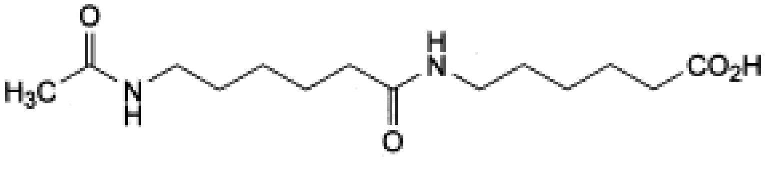
1 ml of *0.1 M sodium edetate* is equivalent to 40.98 mg of $C_{16}H_{28}N_2O_6Zn$.

STORAGE

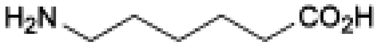
In a non-metallic container.

IMPURITIES

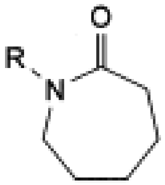
Specified impurities *A, B, C, D.*



A. 6-[[6-(acetylamino)hexanoyl]amino]hexanoic acid,



B. 6-aminohexanoic acid (6-aminocaproic acid),



C. R = CO-CH$_3$: 1-acetylhexahydro-2*H*-azepin-2-one (*N*-acetyl-∈-caprolactam),

D. R = H: hexahydro-2*H*-azepin-2-one (∈–caprolactam).

Ph Eur

Zinc Chloride

General Notices

(Ph Eur monograph 0110)

$ZnCl_2$ 136.3 *7646-85-7*

Ph Eur

DEFINITION

Content

95.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or cast in white or almost white sticks, deliquescent.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent) and in glycerol.

IDENTIFICATION

A. Dissolve 0.5 g in *dilute nitric acid R* and dilute to 10 ml with the same acid. The solution gives reaction (a) of chlorides *(2.3.1)*.

B 5 ml of solution S (see Tests) gives the reaction of zinc *(2.3.1)*.

TESTS

Solution S

To 2.0 g add 38 ml of *carbon dioxide-free water R* prepared from *distilled water R* and add *dilute hydrochloric acid R* dropwise until dissolution is complete. Dilute to 40 ml with *carbon dioxide-free water R* prepared from *distilled water R*.

pH *(2.2.3)*

4.6 to 5.5.

Dissolve 1.0 g in 9 ml of *carbon dioxide-free water R*, ignoring any slight turbidity.

Oxychlorides

Dissolve 1.5 g in 1.5 ml of *carbon dioxide-free water R.* The solution is not more opalescent than reference suspension II *(2.2.1)*. Add 7.5 ml of *ethanol (96 per cent) R*. The solution may become cloudy within 10 min. Any cloudiness disappears on the addition of 0.2 ml of *dilute* *hydrochloric acid R*.

Sulphates *(2.4.13)*

Maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*. Prepare the standard using a mixture of 5 ml of *sulphate standard solution (10 ppm SO_4) R* and 10 ml of *distilled water R*.

Aluminium, calcium, heavy metals, iron, magnesium

To 8 ml of solution S add 2 ml of *concentrated ammonia R* and shake. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II).* Add 1 ml of *disodium hydrogen phosphate solution R.* The solution remains clear for at least 5 min. Add 0.2 ml of *sodium sulphide solution R.* A white precipitate is formed and the supernatant liquid remains colourless.

Ammonium *(2.4.1)*

Maximum 400 ppm.

Dilute 0.5 ml of solution S to 15 ml with *water R*.

ASSAY

Dissolve 0.250 g in 5 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc *(2.5.11).*

1 ml of *0.1 M sodium edetate* is equivalent to 13.63 mg of $ZnCl_2$.

STORAGE

In a non-metallic container.

Ph Eur

Zinc Oxide

General Notices

(Ph Eur monograph 0252)

ZnO 81.4 *1341-13-2*

Action and use

Mild astringent.

Preparations

Zinc Cream

Coal Tar and Zinc Ointment

Zinc Ointment

Zinc and Castor Oil Ointment

Compound Zinc Paste

Zinc and Salicylic Acid Paste

Zinc and Coal Tar Paste

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent (ignited substance).

CHARACTERS

Appearance

Soft, white or faintly yellowish-white, amorphous powder, free from gritty particles.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION

A. It becomes yellow when strongly heated; the yellow colour disappears on cooling.

B. Dissolve 0.1 g in 1.5 ml of *dilute hydrochloric acid R* and dilute to 5 ml with *water R*. The solution gives the reaction of zinc *(2.3.1)*.

TESTS

Alkalinity

Shake 1.0 g with 10 ml of *boiling water R*. Add 0.1 ml of *phenolphthalein solution R* and filter. If the filtrate is red, not more than 0.3 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Carbonates and substances insoluble in acids

Dissolve 1.0 g in 15 ml of *dilute hydrochloric acid R*. It dissolves without effervescence and the solution is not more opalescent than reference suspension II *(2.2.1)* and is colourless (*2.2.2, Method II).*

Arsenic *(2.4.2, Method A)*

Maximum 5 ppm, determined on 0.2 g.

Cadmium

Maximum 10.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 2.0 g in 14 ml of a mixture of equal volumes of *water R* and *cadmium- and lead-free nitric acid R*, boil for 1 min, cool and dilute to 100.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R* and diluting with a 3.5 per cent *V/V* solution of *cadmium- and lead-free nitric acid R*.

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Air-acetylene or air-propane flame.

Iron *(2.4.9)*

Maximum 200 ppm.

Dissolve 50 mg in 1 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. Use in this test 0.5 ml of *thioglycollic acid R*.

Lead

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dissolve 5.0 g in 24 ml of a mixture of equal volumes of *water R* and *cadmium- and lead-free nitric acid R*, boil for 1 min, cool and dilute to 100.0 ml with *water R.*

Reference solutions Prepare the reference solutions using *lead standard solution (0.1 per cent Pb) R* and diluting with a 3.5 per cent *V/V* solution of *cadmium- and lead-free nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm; 217.0 nm may be used depending on the apparatus.

Atomisation device Air-acetylene flame.

Loss on ignition

Maximum 1.0 per cent, determined on 1.00 g by ignition to constant mass at 500 ± 50 °C.

ASSAY

Dissolve 0.150 g in 10 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 8.14 mg of ZnO.

Ph Eur

Zinc Stearate

General Notices

(Ph Eur monograph 0306)

$(C_{17}H_{35}CO_2)_2Zn$ 632 *557-05-1*

Action and use

Excipient.

Ph Eur

DEFINITION

Zinc stearate [$(C_{17}H_{35}COO)_2Zn$; M_r 632] may contain varying proportions of zinc palmitate [$(C_{15}H_{31}COO)_2Zn$; M_r 576.2] and zinc oleate [$(C_{17}H_{33}COO)_2Zn$; M_r 628].

Content

10.0 per cent to 12.0 per cent of Zn.

CHARACTERS

Appearance

Light,white or almost white, amorphous powder, free from gritty particles.

Solubility

Practically insoluble in water and in anhydrous ethanol.

IDENTIFICATION

A. Freezing point *(2.2.18)*: minimum 53 °C, determined on the residue obtained in the preparation of solution S (see Tests).

B. Neutralise 5 ml of solution S to *red litmus paper R* with *strong sodium hydroxide solution R*. The solution gives the reaction of zinc *(2.3.1)*.

TESTS

Solution S

To 5.0 g add 50 ml of *ether R* and 40 ml of a 7.5 per cent *V/V* solution of *cadmium- and lead-free nitric acid R* in *distilled water R*. Heat under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 4 ml, of *distilled water R*. Combine the aqueous layers, wash with 15 ml of *ether R* and heat on a water-bath until ether is completely eliminated. Allow to cool and dilute to 50.0 ml with *distilled water R* (solution S). Evaporate the ether layer to dryness and dry the residue at 105 °C.

Appearance of solution

Solution S is not more intensely coloured than reference solution Y_6 *(2.2.2, Method II).*

Appearance of solution of fatty acids

Dissolve 0.5 g of the residue obtained in the preparation of solution S in 10 ml of *chloroform R*. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_5 *(2.2.2, Method II)*.

Acidity or alkalinity

Shake 1.0 g with 5 ml of *ethanol (96 per cent) R* and add 20 ml of *carbon dioxide-free water R* and 0.1 ml of *phenol red solution R*. Not more than 0.3 ml of *0.1 M hydrochloric acid* or 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Acid value of the fatty acids *(2.5.1)*

195 to 210.

Dissolve 0.20 g of the residue obtained in the preparation of solution S in 25 ml of the prescribed mixture of solvents.

Chlorides *(2.4.4)*

Maximum 250 ppm.

Dilute 2 ml of solution S to 15 ml with *water R.*

Sulphates *(2.4.13)*

Maximum 0.6 per cent.

Dilute 1 ml of solution S to 50 ml with *distilled water R*. Dilute 12.5 ml of this solution to 15 ml with *distilled water R*.

Cadmium

Maximum 5.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Dilute 20.0 ml of solution S to 50.0 ml with a 3.5 per cent *V/V* solution of *cadmium- and lead-free nitric acid R.*

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R* and diluting with a 3.5 per cent *V/V* solution of *cadmium- and lead-free nitric acid R.*

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Air-acetylene or an air-propane flame.

Lead

Maximum 25.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution Solution S.

Reference solutions Prepare the reference solutions using *lead standard solution (0.1 per cent Pb) R* and diluting with a 3.5 per cent *V/V* solution *of cadmium- and lead-free nitric acid R.*

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm. Depending on the apparatus the line at 217.0 nm may be used.

Atomisation device Air-acetylene flame.

ASSAY

To 1.000 g add 50 ml of *dilute acetic acid R* and boil for at least 10 min or until the layer of fatty acids is clear, adding more *water R* as necessary to maintain the original volume. Cool and filter. Wash the filter and the flask with *water R* until the washings are no longer acid to *blue litmus paper R*. Combine the filtrate and washings. Carry out the complexometric titration of zinc *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 6.54 mg of Zn.

Ph Eur

Zinc Sulphate Heptahydrate

General Notices

(Ph Eur monograph 0111)

$ZnSO_4,7H_2O$ 287.5 *7446-20-0*

Action and use

Astringent.

Preparations

Zinc Sulphate Eye Drops

Zinc Sulphate Lotion

Ph Eur

DEFINITION

Content

99.0 per cent to 104.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless, transparent crystals, efflorescent.

Solubility

Very soluble in water, practically insoluble in alcohol.

IDENTIFICATION

A. Solution S (see Tests) gives the reactions of sulphates *(2.3.1)*.

B. Solution S gives the reaction of zinc *(2.3.1)*.

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH ***(2.2.3)***

4.4 to 5.6 for solution S.

Chlorides ***(2.4.4)***

Maximum 300 ppm.

3.3 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides.

Iron ***(2.4.9)***

Maximum 100 ppm.

2 ml of solution S diluted to 10 ml with *water R* complies with the limit test for iron. Use in this test 0.5 ml of *thioglycollic acid R*.

ASSAY

Dissolve 0.200 g in 5 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 28.75 mg of $ZnSO_4,7H_20$.

STORAGE

In a non-metallic, airtight container .

Ph Eur

Zinc Sulphate Hexahydrate

General Notices

(Ph Eur monograph 1683)

$ZnSO_4,6H_2O$ 269.5 *13986-24-8*

Action and use

Astringent.

Ph Eur

DEFINITION

Content

99.0 per cent to 104.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless transparent crystals, efflorescent.

Solubility

Very soluble in water, practically insoluble in alcohol.

IDENTIFICATION

A. Solution S (see Tests) gives the reactions of sulphates *(2.3.1)*.

B. Solution S gives the reaction of zinc *(2.3.1)*.

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.4 to 5.6 for solution S.

Chlorides *(2.4.4)*

Maximum 300 ppm.

3.3 ml of solution S diluted to 15 ml with *water R* complies with the limit test for chlorides.

Iron *(2.4.9)*

Maximum 100 ppm.

2 ml of solution S diluted to 10 ml with *water R* complies with the limit test for iron. Use in this test 0.5 ml of *thioglycollic acid R*.

ASSAY

Dissolve 0.200 g in 5 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 26.95 mg of $ZnSO_4,6H_20$.

STORAGE

In a non-metallic, airtight container .

Ph Eur

Zinc Sulphate Monohydrate

General Notices

(Ph Eur monograph 2159)

$ZnSO_4,H_2O$ 179.5 *7446-19-7*

Action and use

Astringent.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder, or colourless, transparent crystals.

Solubility

Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives the reactions of sulphates *(2.3.1)*.

B. Solution S gives the reaction of zinc *(2.3.1).*

C. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*

4.4 to 5.6 for solution S.

Chlorides *(2.4.4)*

Maximum 300 ppm.

Dilute 3.3 ml of solution S to 15 ml with *water R*.

Iron *(2.4.9)*

Maximum 100 ppm.

Dilute 2 ml of solution S to 10 ml with *water R*. Use 0.5 ml of *thioglycollic acid R* in this test.

ASSAY

Dissolve 0.160 g in 5 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc *(2.5.11)*.

1 ml of *0.1 M sodium edetate* is equivalent to 17.95 mg of $ZnSO_4,H_2O$.

STORAGE

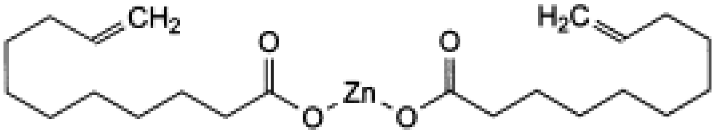
In a non-metallic container.

Ph Eur

Zinc Undecenoate

General Notices

(Zinc Undecylenate, Ph Eur monograph 0539)



$C_{22}H_{38}O_4Zn$ 431.9 *557-08-4*

Action and use

Used topically in treatment of fungal infections.

Ph Eur

DEFINITION

Zinc di(undec-10-enoate).

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, fine powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent).

mp: 116 °C to 121 °C, it may leave a slight solid residue.

IDENTIFICATION

A. To 2.5 g add 10 ml of *water R* and 10 ml of *dilute sulphuric acid R*. Shake with 2 quantities, each of 10 ml, of *ether R*. Reserve the aqueous layer for identification test C. Wash the combined ether layers with *water R* and evaporate to dryness. To the residue add 2 ml of freshly *distilled aniline R* and boil under a reflux condenser for 10 min. Allow to cool and add 30 ml of *ether R*. Shake with 3 quantities, each of 20 ml, of *dilute hydrochloric acid R* and then with 20 ml of *water R*. Evaporate the organic layer to dryness on a water-bath. The residue, after recrystallisation twice from *ethanol (70 per cent V/V) R* and drying *in vacuo* for 3 h, melts *(2.2.14)* at 66 °C to 68 °C.

B. Dissolve 0.1 g in a mixture of 2 ml of *dilute sulphuric acid R* and 5 ml of *glacial acetic acid R*. Add dropwise 0.25 ml of *potassium permanganate solution R*. The colour of the potassium permanganate solution is discharged.

C. A mixture of 1 ml of the aqueous layer obtained in identification test A and 4 ml of *water R* gives the reaction of zinc *(2.3.1)*.

TESTS

Alkalinity

Mix 1.0 g with 5 ml of *ethanol (96 per cent) R* and 0.5 ml of *phenol red solution R*. Add 50 ml of *carbon dioxide-free water R* and examine immediately. No reddish colour appears.

Alkali and alkaline-earth metals

Maximum 2.0 per cent.

To 1.0 g add 25 ml of *water R* and 5 ml of *hydrochloric acid R* and heat to boiling. Filter whilst hot. Wash the filter and the residue with 25 ml of *hot water R*. Combine the filtrate and washings and add *concentrated ammonia R* until alkaline. Add 7.5 ml of *thioacetamide solution R* and heat on a water-bath for 30 min. Filter and wash the precipitate with 2 quantities, each of 10 ml, of *water R*. Combine the filtrate and washings, evaporate to dryness on a water-bath and ignite. The residue weighs a maximum of 20 mg.

Sulphates *(2.4.13)*

Maximum 500 ppm.

To 0.1 g add a mixture of 2 ml of *dilute hydrochloric acid R* and 10 ml of *distilled water R* and heat to boiling. Cool, filter and dilute to 15 ml with *distilled water R.* Prepare the standard using 5 ml of *sulphate standard solution (10 ppm* SO_4*) R* and 10 ml of *distilled water R*.

Loss on drying *(2.2.32)*

Maximum 1.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Degree of unsaturation

Dissolve 0.100 g in a mixture of 5 ml of *dilute hydrochloric acid R* and 30 ml of *glacial acetic acid R*. Using 0.05 ml indigo *carmine solution R1*, added towards the end of the titration as indicator. Titrate with *0.0167 M bromide-bromate* until the colour changes from blue to yellow. 9.1 ml to 9.4 ml of *0.0167 M bromide-bromate* is required. Carry out a blank titration.

ASSAY

To 0.350 g add 25 ml of *dilute acetic acid R* and heat to boiling. Carry out the complexometric titration of zinc *(2.5.11)*.

1 ml of 0*.1 M sodium edetate* is equivalent to 43.19 mg of $C_{22}H_{38}O_4Zn$.

STORAGE

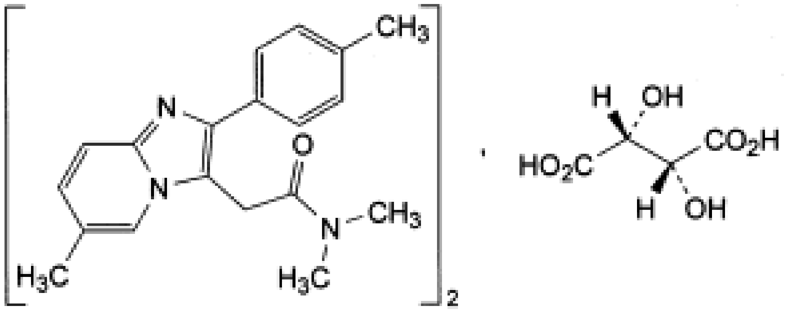
Protected from light.

Ph Eur

Zolpidem Tartrate

General Notices

(Ph Eur monograph 1280)



$C_{42}H_{48}N_6O_8$ *765* *99294-93-6*

Action and use

Non-benzodiazepine hypnotic.

Ph Eur

DEFINITION

Bis[*N*,*N*-dimethyl-2-[6-methyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide] (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content

98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification A, C.

Second identification B, C.

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 0.10 g in 10 ml of *0.1 M hydrochloric acid*. Add 10 ml of *water R*. Add dropwise with stirring 1 ml of *dilute ammonia R2*. Filter and collect the resulting precipitate. Wash the precipitate with *water R* and then dry at 100-105 °C for 2 h. Examine the precipitate as a disc.

Comparison Repeat the operations using 0.10 g of *zolpidem tartrate CRS*.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 50 mg of the substance to be examined in 5 ml of *methanol R*, add 0.1 ml of *diethylamine R* and dilute to 10 ml with *methanol R*.

Reference solution (a) Dissolve 50 mg of *zolpidem tartrate CRS* in 5 ml of *methanol R*, add 0.1 ml of *diethylamine R* and dilute to 10 ml with *methanol R.*

Reference solution (b) Dissolve 50 mg of *flunitrazepam CRS* in 5 ml of *methylene chloride R* and dilute to 10 ml with the same solvent. Mix 1 ml of this solution with 1 ml of reference solution (a).

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *diethylamine R, cyclohexane R, ethyl acetate R* (10:45:45 *V/V/V*).

Application 5 µl.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 0.1 g in 1 ml of *methanol R* heating gently. 0.1 ml of this solution gives reaction (b) of tartrates *(2.3.1)*.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 or BY_6 *(2.2.2, Method II)*. *Prepare the solutions protected from light and carry out the test as rapidly as possible*.

Triturate 0.25 g with 0.125 g of *tartaric acid R*. Dissolve the mixture in 20 ml of *water R* and dilute to 25 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and

dilute to 50.0 ml with the mobile phase.

Reference solution (a) Dissolve 5 mg of *zolpidem impurity A CRS* in the mobile phase and dilute to 50 ml with the mobile phase.

Reference solution (b) Dissolve 5 mg of the substance to be examined in the mobile phase and dilute to 50 ml with the mobile phase. To 10 ml of this solution, add 10 ml of reference solution (a).

Reference solution (c) Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase Mix 18 volumes of *acetonitrile R*, 23 volumes of *methanol R* and 59 volumes of a 5.6 g/l solution of *phosphoric acid R* adjusted to pH 5.5 with *triethylamine R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and zolpidem.

Limits:

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.02 per cent); disregard any peak due to tartaric acid (with a relative retention with reference to zolpidem of 0.16).

Water *(2.5.12)*

Maximum 3.0 per cent, determined on 0.50 g.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

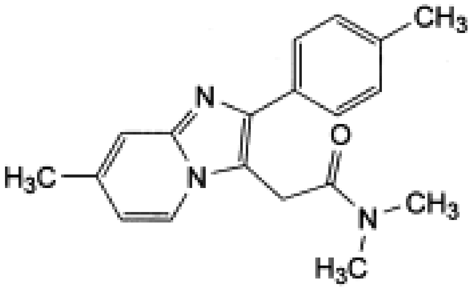
Dissolve 0.300 g in a mixture of 20 ml of *anhydrous acetic acid R* and 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 38.24 mg of $C_{42}H_{48}N_6O_8$.

STORAGE

In an airtight container, protected from light.

IMPURITIES



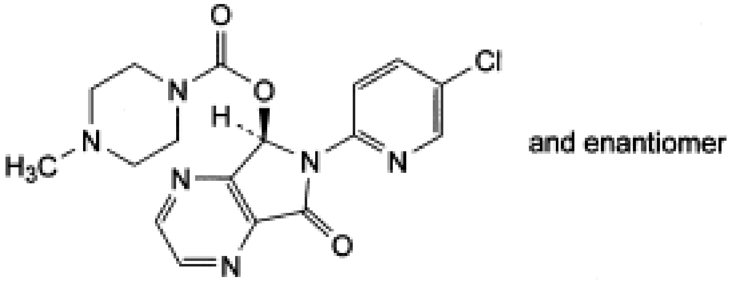
A. *N*,*N*-dimethyl-2-[7-methyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide.

Ph Eur

Zopiclone

General Notices

(Ph Eur monograph 1060)



$C_{17}H_{17}ClN_6O_3$ 388.8 *43200-80-2*

Action and use

Non-benzodiazepine hypnotic.

Preparation

Zopiclone Tablets

Ph Eur

DEFINITION

(5*RS*)-6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate.

Content

98.5 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or slightly yellowish powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, practically insoluble in ethanol (96 per cent). It dissolves in dilute mineral acids.

mp: about 177 °C, with decomposition.

IDENTIFICATION

First identification *B.*

Second identification *A, C.*

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Test solution Dissolve 50.0 mg in a 3.5 g/l solution of *hydrochloric acid R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with a 3.5 g/l solution of *hydrochloric acid R.*

Spectral range 220-350 nm.

Absorption maximum At 303 nm.

Specific absorbance at the absorption maximum 340 to 380.

B. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *zopiclone CRS*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 mg of *zopiclone CRS* in *methylene chloride R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *triethylamine R, acetone R, ethyl acetate R* (2:50:50 *V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 1.0 g in *dimethylformamide R* and dilute to 20.0 ml with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II *(2.2.1)* and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour *(2.2.2, Method II)*.

Optical rotation ***(2.2.7)***

- 0.05° to + 0.05°.

Dilute 10.0 ml of solution S to 50.0 ml with *dimethylformamide R*.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) Dilute 3.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c) Dissolve 4.0 mg of *zopiclone oxide CRS* (impurity A) in the mobile phase and dilute to 10.0 ml with the mobile phase. To 10.0 ml of this solution, add 1.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase Mix 38 volumes of *acetonitrile R* and 62 volumes of a solution containing 8.1 g/l of *sodium laurilsulfate R* and 1.6 g/l of *sodium dihydrogen phosphate R* adjusted to pH 3.5 with a 10 per cent *V/V* solution of *phosphoric acid R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 303 nm.

Injection 20 µl.

Run time 1.5 times the retention time of zopiclone.

Retention time Zopiclone = 27 min to 31 min; if necessary, adjust the concentration of acetonitrile in the mobile phase (increasing the concentration decreases the retention times).

System suitability Reference solution (c):

- *resolution*: minimum 3.0 between the peaks due to impurity A and zopiclone; if necessary, adjust the mobile phase to pH 4.0 with a 10 per cent *V/V* solution of *phosphoric acid R*.

Limits:

- *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and not more than 2 such peaks have an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

2-Propanol

Gas chromatography *(2.2.28).*

Internal standard solution Dilute 5 ml of *ethanol R1* to 100 ml with *ethylene chloride R*. Dilute 1 ml of this solution to 10 ml with *ethylene chloride R*.

Test solution Dissolve 0.25 g of the substance to be examined in *ethylene chloride R*, add

0.5 ml of the internal standard solution and dilute to 5.0 ml with *ethylene chloride R*.

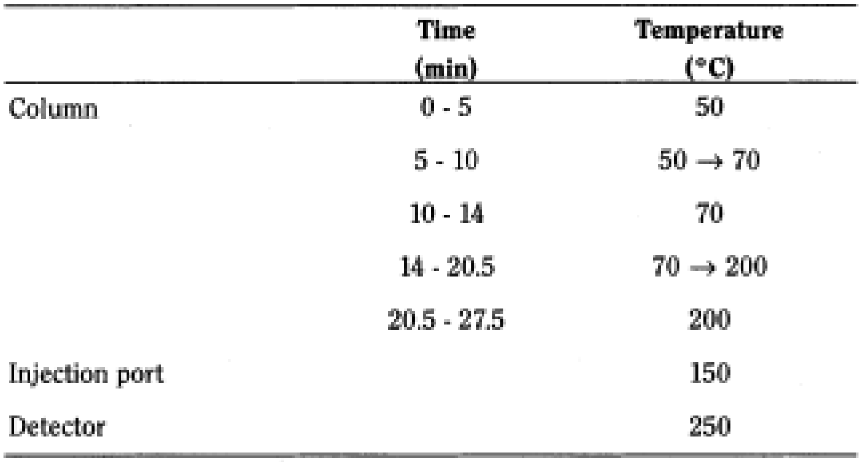
Reference solution Dilute 4.5 ml of *2-propanol R* to 100.0 ml with *ethylene chloride R*. To 1.0 ml of this solution, add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *ethylene chloride R*.

Column:
— *material*: fused silica;
— *size*: *l* = 10 m, Ø = about 0.53 mm;
— *stationary phase: styrene-divinylbenzene copolymer R* (film thickness 20 µm).

Carrier gas *helium for chromatography R.*

Flow rate 4 ml/min.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	50
	5 - 10	50 → 70
	10 - 14	70
	14 - 20.5	70 → 200
	20.5 - 27.5	200
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Calculate the percentage content *m/m* of 2-propanol taking its density to be 0.785 g/ml at 20 °C.

Limit:
— 2-propanol: maximum 0.7 per cent *m/m*.

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 10 ml of anhydrous *acetic acid R* and 40 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically

(2.2.20).

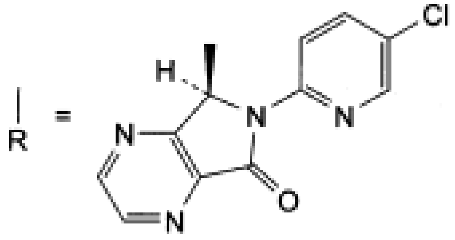
1 ml of *0.1 M perchloric acid* is equivalent to 38.88 mg of $C_{17}H_{17}ClN_6O_3$.

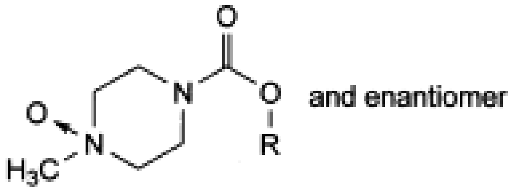
STORAGE

Protected from light.

IMPURITIES

Specified impurities   A, B, C.





A. (5*RS*)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate 4-oxide (zopiclone oxide),

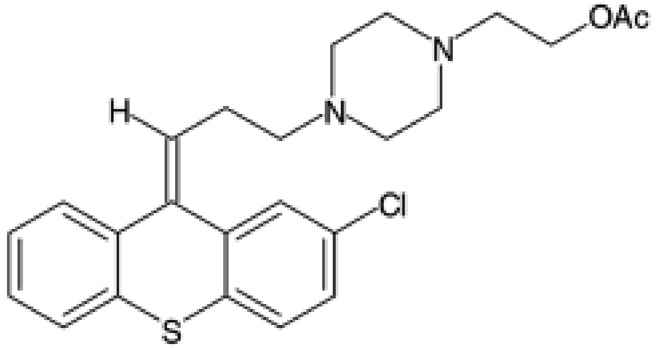
B. R-OH and enantiomer: (7*RS*)-6-(5-chloropyridin-2-yl)-7-hydroxy-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-one,

C. R-H: 6-(5-chloropyridin-2-yl)-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-one.

Ph Eur

Zuclopenthixol Acetate

General Notices



$C_{24}H_{27}ClN_2O_2S$ 443.0 *85721-05-7*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Zuclopenthixol Acetate Injection

DEFINITION

Zuclopenthixol Acetate is (*Z*)-2-4-[3-(2-chlorothioxanthene-9-ylidene)propyl]piperazin-1-ylethyl acetate. It contains not less than 98.0% and not more than 102.0% of $C_{24}H_{27}ClN_2O_2S$, calculated with reference to the dried substance.

CHARACTERISTICS

A yellowish, viscous oil.

Very slightly soluble in *water*; very soluble in *dichloromethane*, in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. The *light absorption* of a 0.0015% w/v solution in *ethanol (96%)*, Appendix II B, in the range 210 to 350 nm exhibits two maxima at 230 and 268 nm. The *absorbances* at the maxima are about 1.18 and 0.51 respectively.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of zuclopenthixol acetate *(RS 363)*.

TESTS

Heavy metals

1.0 g complies with *limit test C for heavy metals*, Appendix VII (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable), a mixture of 10 volumes of *diethylamine*, 40 volumes of *dichloromethane* and 50 volumes of *cyclohexane* as the mobile phase but using an unlined tank. Apply separately to the plate 4 µl of each of the following solutions. Solutions (1) to (3) contain (1) 0.250% w/v, (2) 0.00025% w/v and (3) 0.000125% w/v respectively of the substance being examined in *dichloromethane*. Solution (4) contains 0.00050% w/v of *2-chlorothioxanthone BPCRS* in *dichloromethane*. Solution (5) contains 0.000625% w/v of *zuclopenthixol hydrochloride BPCRS* in *dichloromethane* containing a few drops of *diethylamine*. After removal of the plate, allow it to dry in air, spray with a mixture of equal volumes of *sulphuric acid* and *absolute ethanol*, heat at 110° for 5 minutes and examine under *ultraviolet light (365 nm)* immediately. In the chromatogram obtained with solution (1) any spot corresponding to 2-chlorothioxanthone is not more intense than the spot in the chromatogram obtained with solution (4) (0.2%) and any spot corresponding to zuclopenthixol is not more intense than the spot in the chromatogram obtained with solution (5) (0.25%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.05%).

***trans*-Isomer**

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using three solutions in *dichloromethane* containing (1) 0.040% w/v of the substance being examined, (2) 0.00046% w/v of trans-*clopenthixol acetate dihydrochloride BPCRS* (equivalent to 0.00040% w/v of *trans*-clopenthixol acetate) and (3) 0.020% w/v of the substance being examined and 0.023% w/v of trans-*clopenthixol acetate dihydrochloride BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb S 5W is suitable), (b) a mixture of 0.03 volume of 13.5M *ammonia,* 45 volumes of *dichloromethane*, 45 volumes of *heptane* and 50 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Inject 15 µl of each solution. The test is not valid unless the *resolution factor* between the principal peaks in the chromatogram obtained with solution (3) is at least 2.6.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *trans*-clopenthixol acetate is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, loses not more than 0.4% of its weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

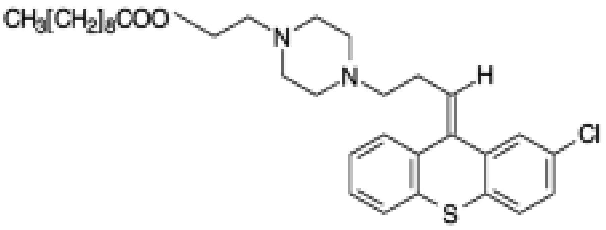
ASSAY

Dissolve 0.2 g in 50 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous* *titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 22.15 mg of $C_{24}H_{27}ClN_2O_2S$.

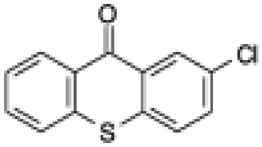
STORAGE

Zuclopenthixol Acetate should be protected from light and stored at a temperature not exceeding -20°.

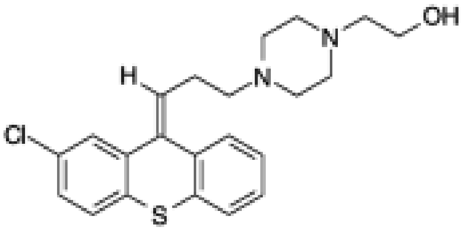
IMPURITIES



A. *trans*-clopenthixol acetate*(*trans*-isomer)*



B. 2-chlorothioxanthone

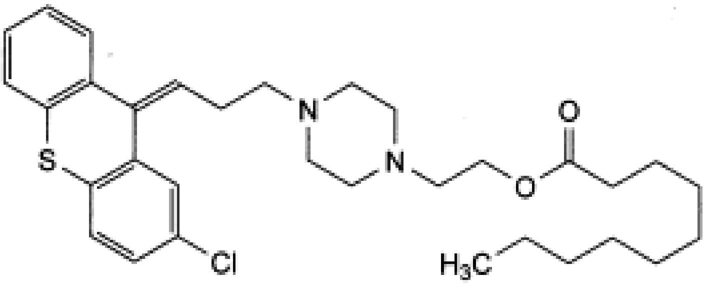


C. zuclopenthixol

Zuclopenthixol Decanoate

General Notices

(Ph Eur monograph 1707)



$C_{32}H_{43}ClN_2O_2S$ 555.2 *64053-00-5*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Zuclopenthixol Decanoate Injection

Ph Eur

DEFINITION

2-[4-[3-[(9*Z*)-2-Chloro-9*H*-thioxanthen-9-ylidene]propyl]piperazin-1-yl]ethyl decanoate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, viscous, oily liquid.

Solubility

Very slightly soluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *Ph. Eur. reference spectrum of zuclopenthixol decanoate*.

TESTS

Appearance of solution

The solution is clear *(2.2.1).*

Using an ultrasonic bath, dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 20.0 ml with the same solvent.

Related substances

Liquid chromatography *(2.2.29)*

Carry out the test protected from light and prepare the solutions immediately before use.

Solution A Dissolve 8.89 g of *docusate sodium R* in *water R*, stirring for about 6-8 h, and dilute to 1000 ml with the same solvent.

Test solution Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R.*

Reference solution (b) Dissolve 5.0 mg of *zuclopenthixol impurity B CRS* in *acetonitrile R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *acetonitrile R.*

Reference solution (c) Dissolve the contents of a vial of *zuclopenthixol for system suitability CRS* (zuclopenthixol decanoate containing impurities A, B and C) in 1 ml of *methanol R.*

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase:* spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 25 volumes of solution A and 75 volumes of *anhydrous ethanol R*, then add 0.1 volumes of *phosphoric acid R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µl.

Run time Twice the retention time of zuclopenthixol decanoate.

Identification of impurities Use the chromatogram supplied with *zuclopenthixol for system suitability CRS* and the chromatograms obtained with reference solutions (b) and (c) to identify the peaks due to impurities A, B and C.

Relative retention With reference to zuclopenthixol decanoate (retention time = about 12 min): impurity C = about 0.4; impurity B = about 0.5; impurity A = about 1.1.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B; and minimum 2.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to zuclopenthixol decanoate.

Limits:

— *impurity A*: not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.3 per cent);

— *impurity B*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity C*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *disregard limit:* 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals *(2.4.8)*

Maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying *(2.2.32)*

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash *(2.4.14)*

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

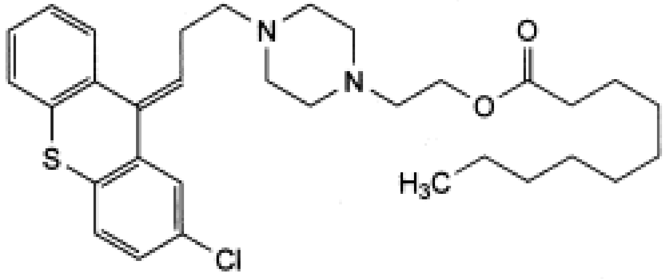
1 ml of *0.1 M perchloric acid* is equivalent to 27.76 mg of $C_{32}H_{43}ClN_2O_2S$.

STORAGE

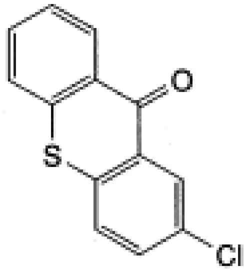
Under an inert gas in an airtight container, protected from light, at a temperature not exceeding - 20 °C.

IMPURITIES

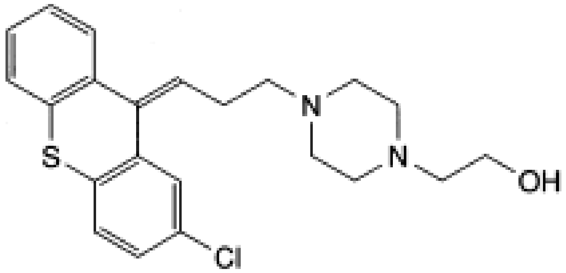
Specified impurities A, B, C.



A. 2-[4-[3-[(9*E*)-2-chloro-9*H*-thioxanthen-9-ylidene]propyl]piperazin-1-yl]ethyl decanoate,



B. 2-chloro-9*H*-thioxanthen-9-one,

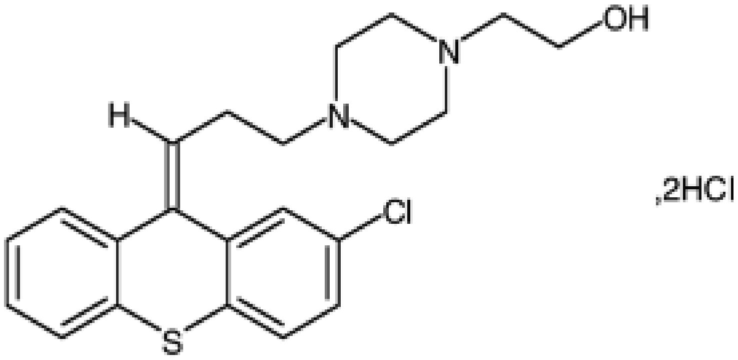


C. 2-[4-[3-[(9*Z*)-2-chloro-9*H*-thioxanthen-9-ylidene]propyl]piperazin-1-yl]ethanol.

Ph Eur

Zuclopenthixol Hydrochloride

General Notices



$C_{22}H_{25}ClN_2OS,2HCl$ 473.9 *633-59-0*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Zuclopenthixol Tablets

DEFINITION

Zuclopenthixol Hydrochloride is (*Z*)-2-4-[3-(2-chlorothioxanthene-9-ylidene)propyl]piperazin-1-ylethanol dihydrochloride. It contains not less than 98.0% and not more than 101.0% of $C_{22}H_{25}ClN_2OS,2HCl$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

An off-white, granular powder.

Very soluble in *water*; sparingly soluble in *ethanol (96%)*; very slightly soluble in *ether*.

IDENTIFICATION

A. The *light absorption* of a 0.0015% w/v solution in *ethanol (96%)*, Appendix II B, in the range 210 to 350 nm exhibits two maxima at 230 and 268 nm. The *absorbances* at the maxima are about 1.05 and 0.42, respectively.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of zuclopenthixol hydrochloride *(RS 365)*.

C. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 2.0 to 3.0, Appendix V L.

Heavy metals

1.0 g complies with *limit test C for heavy metals*, Appendix VII (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a *TLC silica gel* F_{254} *plate* (Merck plates are suitable), a mixture of 10 volumes of *ethyl acetate* and 90 volumes of *cyclohexane* as the mobile phase but using an unlined tank and allowing the mobile phase to ascend 10 cm above the line of application. Apply separately to the plate 4 µl of each of the following solutions. Solutions (1) to (3) contain (1) 2.5% w/v, (2) 0.0025% w/v and (3) 0.00125% w/v respectively of the substance being examined in *methanol* containing a few drops of *diethylamine*. Solution (4) contains 0.0025% w/v of *2-chlorothioxanthone BPCRS* in *methanol*. Solution (5) contains 0.0025% w/v of *9-allyl-2-chlorothioxanthen-9-ol BPCRS* in *methanol*. After removal of the plate, allow it to dry in air, spray with a mixture of equal volumes of *sulphuric acid* and *absolute ethanol*, heat at 110° for 5 minutes and examine under *ultraviolet light (365 nm)* immediately. In the chromatogram obtained with solution (1) any spots corresponding to 2-chlorothioxanthone and to 9-allyl-2-chlorothioxanthenol are not more intense than the spots in the chromatograms obtained with solutions (4) and (5) respectively (0.1% each), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%) and not more than four such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.05%).

Free amine

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2 volumes of *water*, 10 volumes of 13.5M *ammonia*, 20 volumes of *butan-1-ol* and 65 volumes of *acetone* as the mobile phase. Apply separately to the plate 4 µl of each of the following solutions. Solution (1) is a 0.25% w/v solution of the substance being examined in *ethanol (96%)*; add a few drops of *diethylamine* to aid dissolution. For solution (2), dilute 1 volume of solution (1) to 400 volumes with *ethanol (96%)*. After removal of the plate, allow it to dry in air, spray with a mixture of equal volumes of *sulphuric acid* and *absolute ethanol*, heat at 110° for 5 minutes and examine under *ultraviolet light (365 nm)* immediately. In the chromatogram obtained with solution (1) any *secondary spot* of the same colour and at an Rf value lower than that of the principal spot is not more intense than the spot in the chromatogram obtained solution (2) (0.25%).

***trans*-Isomer**

Carry out the method for *liquid chromatography,* Appendix III D, protected from light, using the following solutions. For solution (1) dissolve 10 mg of the substance being examined in 5 ml of *water*, add 25 ml of the mobile phase, shake thoroughly for 1 minute and dilute to 50 ml with the mobile phase; use the upper layer. For solution (2) prepare a 0.004% w/v solution of *trans-clopenthixol hydrochloride BPCRS* in *water*, shake 5 ml of this solution with 25 ml of the mobile phase for 1 minute and dilute to 50 ml with the mobile phase; use the upper layer. For solution (3) dissolve 10 mg of the substance being examined in 5 ml of a 0.004% w/v solution of *trans-clopenthixol hydrochloride BPCRS*, add 25 ml of the mobile phase, shake thoroughly for 1 minute, dilute to 50 ml with the mobile phase and use the upper layer.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb S 5W is suitable), (b) a mixture of 0.2 volume of *water*, 0.3 volume of 13.5M *ammonia,* 15 volumes of *propan-2-ol* and 85 volumes of n-*heptane* as the mobile phase with a flow rate of 1.5 ml per minute and (c) and a detection wavelength of 254 nm.

The test is not valid unless the chromatogram obtained with solution (3) shows a similar resolution to that in the reference chromatogram supplied with *trans-clopenthixol hydrochloride BPCRS.*

In the chromatogram obtained with solution (1) the area of any peak corresponding to *trans*-clopenthixol hydrochloride is not greater than 1.5 times the area of the peak in the chromatogram obtained with solution (2) (3%).

Water

Not more than 2.5%, determined on 0.5 g, Appendix IX C.

Sulphated ash

Not more than 0.1%, Appendix IX A.

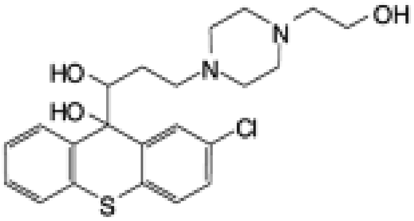
ASSAY

Dissolve 0.5 g in 50 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 23.70 mg of $C_{22}H_{27}Cl_3N_2OS$.

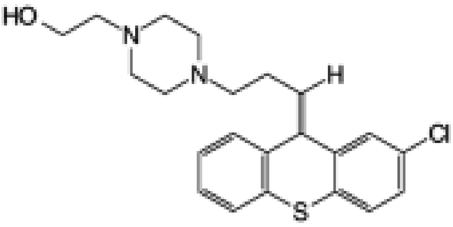
STORAGE

Zuclopenthixol Hydrochloride should be protected from light.

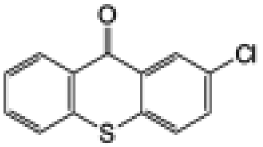
IMPURITIES



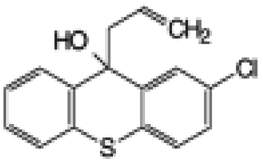
A. 2-chloro-9-(1-hydroxy-3-(4-(2-hydroxyethyl)]piperazin-1-ylpropyl)thioxanthen9-ol,



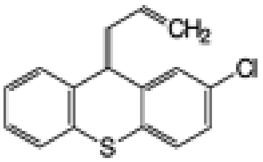
B. *trans*-clopenthixol,



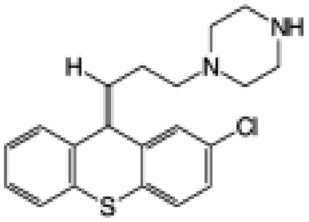
C. 2-chlorothioxanthone,



D. 9-allyl-2-chlorothioxanthen-9-ol,



E. 9-allylidene-2-chlorothioxanthene,



F. 2-chloro-9-(3-piperazin-1-ylpropylidene)thioxanthene.

AROMATIC WATERS

General Notices

DEFINITION

Aromatic Waters are saturated solutions of volatile oils or other aromatic substances in water, usually employed for their flavouring rather than their medicinal properties. Aromatic Waters prepared as described below contain a small amount of Ethanol.

PRODUCTION

Aromatic Waters are normally prepared by diluting a concentrated, ethanolic solution of the aromatic substance with Water.

LABELLING

The label states (1) the date after which the Aromatic Water is not intended to be used; (2) the conditions under which the Aromatic Water should be stored.

Capsules of the British Pharmacopoeia

General Notices

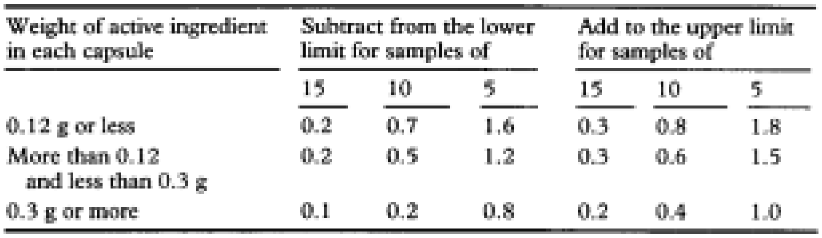
In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those capsules that are the subject of an individual monograph in the British Pharmacopoeia.

When presented as granules the contents of the requisite number of capsules should be mixed and powdered before performing the Assay and tests described in the monograph.

Content of active ingredient in capsules

The range for the content of active ingredient stated in the monograph is based on the requirement that 20 capsules, or such other number as may be indicated in the monograph, are used in the Assay. In the circumstances where 20 capsules cannot be obtained, a smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table I.

Table I



Weight of active ingredient in each capsule	Subtract from the lower limit for samples of			Add to the upper limit for samples of		
	15	10	5	15	10	5
0.12 g or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 and less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5
0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

The requirements of Table I apply when the stated limits are 90 to 110%. For limits other than 90 to 110%, proportionately smaller or larger allowances should be made.

Disintegration

Comply with the *disintegration test for tablets and capsules,* Appendix XII A1, unless otherwise stated in the individual monographs.

For those Hard Capsules or Soft Capsules for which a requirement for Dissolution is included in the individual monograph, the requirement for Disintegration does not apply.

Uniformity of content

Details of the analytical method to be employed for determining the content of active ingredient may be included in certain monographs. Unless otherwise stated in the monograph the limits are as given in test B for *uniformity of content*, Appendix XII C3.

Any capsules that when examined individually show a gross deviation from the prescribed or stated content are not official.

LABELLING

The label states (1) the quantity of the active ingredient contained in each Capsule; (2) the date after which the Capsules are not intended to be used; (3) the conditions under which the Capsules should be stored.

CAPSULES

General Notices

(Ph Eur monograph 0016)

Capsules comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

The requirements of this monograph do not necessarily apply to preparations that are presented as capsules intended for use other than by oral administration. Requirements for such preparations may be found, where appropriate, in other general monographs, for example Rectal preparations (1145) and Vaginal preparations (1164).

DEFINITION

Capsules are solid preparations with hard or soft shells of various shapes and capacities, usually containing a single dose of active substance(s). They are intended for oral administration.

The capsule shells are made of gelatin or other substances, the consistency of which may be adjusted by the addition of substances such as glycerol or sorbitol. Excipients such as surface-active agents, opaque fillers, antimicrobial preservatives, sweeteners, colouring matter authorised by the competent authority and flavouring substances may be added. The capsules may bear surface markings.

The contents of capsules may be solid, liquid or of a paste-like consistency. They consist of one or more active substances with or without excipients such as solvents, diluents, lubricants and disintegrating agents. The contents do not cause deterioration of the shell. The shell, however, is attacked by the digestive fluids and the contents are released.

Where applicable, containers for capsules comply with the requirements of *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Several categories of capsules may be distinguished:

- — hard capsules;
- — soft capsules;
- — gastro-resistant capsules;
- — modified-release capsules;
- — cachets.

PRODUCTION

In the manufacture, packaging, storage and distribution of capsules, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

TESTS

Uniformity of dosage units

Capsules comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, capsules with a content of active substance less than 2 mg or less than 2 per cent of the fill mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those ingredients which correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Capsules comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Dissolution

A suitable test may be carried out to demonstrate the appropriate release of the active substance(s), for example one of the tests described in *Dissolution test for solid dosage forms (2.9.3)*.

Where a dissolution test is prescribed, a disintegration test may not be required.

STORAGE

Store at a temperature not exceeding 30 °C.

LABELLING

The label states the name of any added antimicrobial preservative.

HARD CAPSULES

DEFINITION

Hard capsules have shells consisting of 2 prefabricated, cylindrical sections, each of which has one rounded, closed end and one open end.

PRODUCTION

The active substance(s), usually in solid form (powder or granules), are filled into one of the sections that is then closed by slipping the other section over it. The security of the closure may be strengthened by suitable means.

TESTS

Disintegration

Hard capsules comply with the test for disintegration of tablets and capsules *(2.9.1)*. Use *water R* as the liquid medium. When justified and authorised, *0.1 M hydrochloric acid* or *artificial gastric juice R* may be used as the liquid medium. If the capsules float on the surface of the water, a disc may be added. Operate the apparatus for 30 min, unless otherwise justified and authorised.

SOFT CAPSULES

DEFINITION

Soft capsules have thicker shells than those of hard capsules. The shells consist of a single part and are of various shapes.

PRODUCTION

Soft capsules are usually formed, filled and sealed in one operation, but for extemporaneous use the shell may be prefabricated. The shell material may contain an active substance.

Liquids may be enclosed directly; solids are usually dissolved or dispersed in a suitable vehicle to give a solution or dispersion of a paste-like consistency.

There may be partial migration of the constituents from the capsule contents into the shell and vice versa because of the nature of the materials and the surfaces in contact.

TESTS

Disintegration

Soft capsules comply with the test for disintegration of tablets and capsules *(2.9.1)*. Use *water R* as the liquid medium. When justified and authorised, *0.1 M hydrochloric acid* or *artificial gastric juice R* may be used as the liquid medium. Add a disc to each tube. Liquid active substances dispensed in soft capsules may attack the disc; in such circumstances and where authorised, the disc may be omitted. Operate the apparatus for 30 min, unless otherwise justified and authorised. If the capsules fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 capsules omitting the discs.

MODIFIED-RELEASE CAPSULES

DEFINITION

Modified-release capsules are hard or soft capsules in which the contents or the shell or both contain special excipients or are prepared by a special process designed to modify the rate, the place or the time at which the active substance(s) are released.

Modified-release capsules include prolonged-release capsules and delayed-release capsules.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) .

GASTRO-RESISTANT CAPSULES

DEFINITION

Gastro-resistant capsules are delayed-release capsules that are intended to resist the gastric fluid and to release their active substance or substances in the intestinal fluid. Usually they are prepared by filling capsules with granules or with particles covered with a gastro-resistant coating, or in certain cases, by providing hard or soft capsules with a gastro-resistant shell (enteric capsules).

PRODUCTION

For capsules filled with granules or filled with particles covered with a gastro-resistant coating, a suitable test is carried out to demonstrate the appropriate release of the active substance(s) .

TESTS

Disintegration

For capsules with a gastro-resistant shell carry out the test for disintegration *(2.9.1)* with the following modifications. Use *0.1 M hydrochloric acid* as the liquid medium and operate the apparatus for 2 h, or other such time as may be authorised, without the discs. Examine the state of the capsules. The time of resistance to the acid medium varies according to the formulation of the capsules to be examined. It is typically 2 h to 3 h but even with authorised deviations it must not be less than 1 h. No capsule shows signs of disintegration or rupture permitting the escape of the contents. Replace the acid by *phosphate buffer solution pH 6.8 R*. When justified and authorised, a buffer solution of pH 6.8 with added pancreas powder (for example, 0.35 g of *pancreas powder R* per 100 ml of buffer solution) may be used. Add a disc to each tube. Operate the apparatus for 60 min. If the capsules fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 capsules omitting the discs.

Dissolution

For capsules prepared from granules or particles already covered with a gastro-resistant coating, a suitable test is carried out to demonstrate the appropriate release of the active substance(s), for example the test described in *Dissolution test for solid dosage forms (2.9.3)*.

CACHETS

DEFINITION

Cachets are solid preparations consisting of a hard shell containing a single dose of one or more active substances. The cachet shell is made of unleavened bread usually from rice flour and consists of 2 prefabricated flat cylindrical sections. Before administration, the cachets are immersed in water for a few seconds, placed on the tongue and swallowed with a draught of water.

LABELLING

The label states the method of administration of the cachets.

Ph Eur

Ear Preparations of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any ear drops or ear spray that is the subject of an individual monograph in the British Pharmacopoeia.

EAR DROPS

STORAGE

Ear Drops are supplied in containers of glass or suitable plastic that are fitted with an integral dropper or with a screw cap of suitable materials incorporating a dropper and rubber or plastic teat. Alternatively, such a cap assembly is supplied separately.

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) that the Ear Drops are intended for external use only.

EAR SPRAYS

LABELLING

The label states (1) the instructions for using the spray; (2) the amount of active ingredients delivered by each actuation of the valve; (3) the number of deliveries available from the container; (4) that the Ear Spray is intended for external use only.

EAR PREPARATIONS

General Notices

(Ph Eur monograph 0652)

Ear Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Ear preparations are liquid, semi-solid or solid preparations intended for instillation, for spraying, for insufflation, for application to the auditory meatus or as an ear wash.

Ear preparations usually contain 1 or more active substances in a suitable vehicle. They may contain excipients, for example, to adjust tonicity or viscosity, to adjust or stabilise the pH, to increase the solubility of the active substances, to stabilise the preparation or to provide adequate antimicrobial properties. The excipients do not adversely affect the intended medicinal action of the preparation or, at the concentrations used, cause toxicity or undue local irritation.

Preparations for application to the injured ear, particularly where the eardrum is perforated, or prior to surgery are sterile, free from antimicrobial preservatives and supplied in single-dose containers.

Ear preparations are supplied in multi-dose or single-dose containers, provided, if necessary, with a suitable administration device which may be designed to avoid the introduction of contaminants.

Unless otherwise justified and authorised, aqueous ear preparations supplied in multidose containers contain a suitable antimicrobial preservative at a suitable concentration, except where the preparation itself has adequate antimicrobial properties.

Where applicable, containers for ear preparations comply with the requirements of *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Several categories of ear preparations may be distinguished:

— ear drops and sprays;

— semi-solid ear preparations;

— ear powders;

— ear washes;

— ear tampons.

PRODUCTION

During development of an ear preparation whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in the text on *Efficacy of antimicrobial preservation (5.1.3)*.

During development of ear washes, it must be demonstrated that the nominal content can be withdrawn from the container of preparations presented in single-dose containers.

In the manufacture, packaging, storage and distribution of ear preparations, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

Sterile ear preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products (5.1.1)*.

In the manufacture of ear preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units

Single-dose ear preparations comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose ear preparations with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those ingredients that correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Single-dose ear preparations comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Sterility *(2.6.1)*

Where the label indicates that the ear preparation is sterile, it complies with the test for sterility.

STORAGE

If the preparation is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

— the name of any added antimicrobial preservative;

— where applicable, that the preparation is sterile;

— for multidose containers, the period after opening the container after which the contents must not be used. This period does not exceed 4 weeks, unless otherwise justified and authorised.

EAR DROPS AND EAR SPRAYS

DEFINITION

Ear drops and ear sprays are solutions, emulsions or suspensions of one or more active substances in liquids suitable for application to the auditory meatus without exerting harmful pressure on the eardrum (for example, water, glycols or fatty oils). They may also be placed in the auditory meatus by means of a tampon impregnated with the liquid.

Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment which is readily dispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Ear drops are usually supplied in multidose containers of glass or suitable plastic material that are fitted with an integral dropper or with a screw cap of suitable materials incorporating a dropper and rubber or plastic teat. Alternatively, such a cap assembly is supplied separately. Ear sprays are usually supplied in multi-dose containers fitted with an appropriate applicator. When ear sprays are supplied in pressurised containers, these comply with the requirements of the monograph on *Pressurised pharmaceutical preparations (0523).*

SEMI-SOLID EAR PREPARATIONS

DEFINITION

Semi-solid ear preparations are intended for application to the external auditory meatus, if necessary by means of a tampon impregnated with the preparation.

Semi-solid ear preparations comply with the requirements of the monograph on *Semi-solid preparations for cutaneous application (0132).*

They are supplied in containers fitted with a suitable applicator.

EAR POWDERS

DEFINITION

Ear powders comply with the requirements of the monograph on *Powders for cutaneous application (1166).*

They are supplied in containers fitted with a suitable device for application or insufflation.

EAR WASHES

DEFINITION

Ear washes are preparations intended to cleanse the external auditory meatus. They are usually aqueous solutions with a pH within physiological limits.

Ear washes intended for application to injured parts or prior to a surgical operation are sterile.

EAR TAMPONS

DEFINITION

Ear tampons are intended to be inserted into the external auditory meatus. They comply with the requirements of the monograph on *Medicated tampons (1155).*

Ph Eur

Eye Preparations of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any eye drops, eye lotion or eye ointment that is the subject of an individual monograph in the British Pharmacopoeia.

EYE DROPS

DEFINITION

Definition of particular Eye Drops as a solution or suspension in Purified Water does not preclude the inclusion of suitable additional substances where necessary for the purposes referred to above under the requirements of the European Pharmacopoeia. However if buffering agents are used in preparations intended for use in surgical procedures great care should be taken to ensure that the nature and concentration of the chosen agent are suitable.

Where the active ingredient is susceptible to oxidative degradation appropriate precautions such as the addition of a suitable antioxidant should be taken. If an antioxidant is added care should be taken to ensure compatibility between the antioxidant and the antimicrobial preservative.

PRODUCTION

Methods of sterilisation that may be used in the manufacture of Eye Drops are described in Appendix XVIII.

STORAGE

Eye Drops are supplied in tamper-evident containers. The compatibility of plastic or rubber components should be confirmed before use.

Containers for multi-dose Eye Drops are fitted with an integral dropper or with a sterile screw cap of suitable materials incorporating a dropper and rubber or plastic teat. Alternatively such a cap assembly is supplied, sterilised, separately.

LABELLING

The label states (1) the names and percentages of the active ingredients; (2) the date after which the Eye Drops are not intended to be used; (3) the conditions under which the Eye Drops should be stored.

For multi-dose containers the label states that care should be taken to avoid contamination of the contents during use.

Single-dose containers that because of their size bear only an indication of the active ingredient and the strength of the preparation do so by use of an approved code, Appendix XXI C, together with an expression of the percentage present. When a code is used on the container, the code is also stated on the package.

EYE OINTMENTS

DEFINITION

Eye Ointments of the British Pharmacopoeia that are intended to be used solely or primarily as a suitable eye-ointment basis contain no active ingredient.

PRODUCTION

Methods of sterilisation that may be used in the manufacture of Eye Ointments are described in Appendix XVIII.

In preparing Eye Ointments in tropical or subtropical countries where the prevailing high temperatures otherwise make the basis too soft for convenient use, the proportions of Yellow Soft Paraffin and Liquid Paraffin specified in the individual monograph may be varied, or Hard Paraffin may be added but the proportions of active ingredients must not be changed.

STORAGE

Single-dose containers for Eye Ointments, or the nozzles of tubes, are of such a shape as to facilitate administration without contamination. The former type of container is individually wrapped. Tubes are tamper-evident.

LABELLING

The label states (1) the names and percentages of the active ingredients; (2) the date after which the Eye Ointment is not intended to be used; (3) the conditions under which the Eye Ointment should be stored.

EYE PREPARATIONS

General Notices

(Ph Eur monograph 1163)

Eye Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Eye preparations are sterile liquid, semi-solid or solid preparations intended for administration upon the eyeball and/or to the conjunctiva, or for insertion in the conjunctival sac.

Where applicable, containers for eye preparations comply with the requirements of materials used for the manufacture of containers *(3.1 and subsections)* and containers *(3.2 and subsections)*.

Several categories of eye preparations may be distinguished:

- — eye drops;
- — eye lotions;
- — powders for eye drops and powders for eye lotions;
- — semi-solid eye preparations;
- — ophthalmic inserts.

PRODUCTION

During the development of an eye preparation whose formulation contains an antimicrobial preservative, the necessity for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in chapter *5.1.3*. *Efficacy of antimicrobial preservation*.

Eye preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in chapter *5.1.1*. *Methods of preparation of sterile products.*

In the manufacture of eye preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

During development, it must be demonstrated that the nominal contents can be withdrawn from the container of liquid and semi-solid eye preparations supplied in single-dose containers.

TESTS

Sterility *(2.6.1)*

Eye preparations comply with the test. Applicators supplied separately also comply with the test. Remove the applicator with aseptic precautions from its package and transfer it to a tube of culture medium so that it is completely immersed. Incubate and interpret the results as described in the test.

STORAGE

Unless otherwise justified and authorised, store in a sterile, tamper-proof container.

LABELLING

The label states the name of any added antimicrobial preservative.

EYE DROPS

DEFINITION

Eye drops are sterile aqueous or oily solutions, emulsions or suspensions of one or more active substances intended for instillation into the eye.

Eye drops may contain excipients, for example, to adjust the tonicity or the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance, or to stabilise the preparation. These substances do not adversely affect the intended medicinal action or, at the concentrations used, cause undue local irritation.

Aqueous preparations supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservative chosen must be compatible with the other ingredients of the preparation and must remain effective throughout the period of time during which eye drops are in use.

If eye drops do not contain antimicrobial preservatives they are supplied in single-dose containers or in multidose containers preventing microbial contamination of the contents after opening.

Eye drops intended for use in surgical procedures do not contain antimicrobial preservatives.

Eye drops that are solutions, examined under suitable conditions of visibility, are practically clear and practically free from particles.

Eye drops that are suspensions may show a sediment that is readily redispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Multidose preparations are supplied in containers that allow successive drops of the preparation to be administered. The containers contain at most 10 ml of the preparation, unless otherwise justified and authorised.

TESTS

Particle size

Unless otherwise justified and authorised, eye drops in the form of a suspension comply with the following test: introduce a suitable quantity of the suspension into a counting cell or with a micropipette onto a slide, as appropriate, and scan under a microscope an area corresponding to 10 µg of the solid phase. For practical reasons, it is recommended that the whole sample is first scanned at low magnification (e.g. × 50) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g. × 200 to × 500). For each 10 µg of solid active substance, not more than 20 particles have a maximum dimension greater than 25 µm, and not more than 2 of these particles have a maximum dimension greater than 50 µm. None of the particles has a maximum dimension greater than 90 µm.

LABELLING

The label states, for multidose containers, the period after opening the container after which the contents must not be used. This period does not exceed 4 weeks, unless otherwise justified and authorised.

EYE LOTIONS

DEFINITION

Eye lotions are sterile aqueous solutions intended for use in rinsing or bathing the eye or for impregnating eye dressings.

Eye lotions may contain excipients, for example to adjust the tonicity or the viscosity of the preparation or to adjust or stabilise the pH. These substances do not adversely affect the intended action or, at the concentrations used, cause undue local irritation.

Eye lotions supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservative chosen is compatible with the other ingredients of the preparation and remains effective throughout the period of time during which the eye lotions are in use.

If eye lotions do not contain antimicrobial preservatives, they are supplied in single-dose containers. Eye lotions intended for use in surgical procedures or in first-aid treatment do not contain an antimicrobial preservative and are supplied in single-dose containers.

Eye lotions, examined under suitable conditions of visibility, are practically clear and practically free from particles.

The containers for multidose preparations do not contain more than 200 ml of eye lotion, unless otherwise justified and authorised.

LABELLING

The label states:

— where applicable, that the contents are to be used on one occasion only;

— for multidose containers, the period after opening the container after which the contents must not be used; this period does not exceed 4 weeks, unless otherwise justified and authorised.

POWDERS FOR EYE DROPS AND POWDERS FOR EYE LOTIONS

DEFINITION

Powders for the preparation of eye drops and eye lotions are supplied in a dry, sterile form to be dissolved or suspended in an appropriate liquid vehicle at the time of administration. They may contain excipients to facilitate dissolution or dispersion, to prevent caking, to adjust the tonicity, to adjust or stabilise the pH or to stabilise the preparation.

After dissolution or suspension in the prescribed liquid, they comply with the requirements for eye drops or eye lotions, as appropriate.

TESTS

Uniformity of dosage units *(2.9.40)*

Single-dose powders for eye drops and eye lotions comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose powders for eye drops and eye lotions with a content of active substance less then 2 mg or less than 2 per cent of the total mass comply with test B. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above condition.

Uniformity of mass *(2.9.5)*

Single-dose powders for eye drops and eye lotions comply with the test. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

SEMI-SOLID EYE PREPARATIONS

DEFINITION

Semi-solid eye preparations are sterile ointments, creams or gels intended for application to the conjunctiva or to the eyelids. They contain one or more active substances dissolved or dispersed in a suitable basis. They have a homogeneous appearance.

Semi-solid eye preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132).* The basis is non-irritant to the conjunctiva.

Semi-solid eye preparations are packed in small, sterilised collapsible tubes fitted or provided with a sterilised cannula. The containers contain at most 10 g of the preparation, unless otherwise justified and authorised. The tubes must be well-closed to prevent microbial contamination. Semi-solid eye preparations may also be packed in suitably designed single-dose containers. The containers, or the nozzles of tubes, are of such a shape as to facilitate administration without contamination.

TESTS

Particle size

Semi-solid eye preparations containing dispersed solid particles comply with the following test: spread gently a quantity of the preparation corresponding to at least 10 µg of solid active substance as a thin layer. Scan under a microscope the whole area of the sample. For practical reasons, it is recommended that the whole sample is first scanned at a small magnification (e.g. × 50) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g. × 200 to × 500). For each 10 µg of solid active substance, not more than 20 particles have a maximum dimension greater than 25 µm, and not more than 2 of these particles have a maximum dimension greater than 50 µm. None of the particles has a maximum dimension greater than 90 µm.

LABELLING

The label states, for multidose containers, the period after opening the container after which the contents must not be used. This period does not exceed 4 weeks, unless otherwise justified and authorised.

OPHTHALMIC INSERTS

DEFINITION

Ophthalmic inserts are sterile, solid or semi-solid preparations of suitable size and shape, designed to be inserted in the conjunctival sac, to produce an ocular effect. They generally consist of a reservoir of active substance embedded in a matrix or bounded by a rate-controlling membrane. The active substance, which is more or less soluble in lacrymal liquid, is released over a determined period of time.

Ophthalmic inserts are individually distributed into sterile containers.

PRODUCTION

In the manufacture of ophthalmic inserts, measures are taken to ensure a suitable dissolution behaviour.

TESTS

Uniformity of dosage units *(2.9.40)*

Ophthalmic inserts comply with the test or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Ophthalmic inserts comply, where applicable, with test A.

LABELLING

The label states:

- — where applicable, the total quantity of active substance per insert;
- — where applicable, the dose released per unit time.

Ph Eur

GLOSSARY

General Notices

(Ph Eur monograph 1502)

A glossary of terms relating to formulated preparations is included in the European Pharmacopoeia. The glossary is reproduced below.

Ph Eur

The following introductory text provides definitions and/or explanations of terms that may be found in, or used in association with, the general monographs on dosage forms, but that are not defined within them. Where relevant, reference is made to other equivalent terms that may be found in other publications or contexts.

This glossary is published for information.

Standard Term

Standard Terms for describing the pharmaceutical form of a medicinal product, the routes of administration and the containers used have been established by the European Pharmacopoeia Commission and are provided in a separate publication on Standard Terms.

Active substance

Equivalent terms: active ingredient, drug substance, medicinal substance, active pharmaceutical ingredient.

Vehicle

A vehicle is the carrier, composed of one or more excipients, for the active substance(s) in a liquid preparation.

Basis

A basis is the carrier, composed of one or more excipients, for the active substance(s) in semi-solid and solid preparations.

Conventional-release dosage forms

Conventional-release dosage forms are preparations showing a release of the active substance(s) which is not deliberately modified by a special formulation design and/or manufacturing method. In the case of a solid dosage form, the dissolution profile of the active substance depends essentially on its intrinsic properties. Equivalent term: immediate-release dosage form.

Modified-release dosage forms

Modified-release dosage forms are preparations where the rate and/or place of release of the active substance(s) is different from that of a conventional-release dosage form administered by the same route. This deliberate modification is achieved by a special formulation design and/or manufacturing method. Modified-release dosage forms include prolonged-release, delayed-release and pulsatile-release dosage forms.

Prolonged-release dosage forms

Prolonged-release dosage forms are modified-release dosage forms showing a slower release of the active substance(s) than that of a conventional-release dosage form administered by the same route. Prolonged-release is achieved by a special formulation design and/or manufacturing method. Equivalent term: extended-release dosage form.

Delayed-release dosage forms

Delayed-release dosage forms are modified-release dosage forms showing a release of the active substance(s) which is delayed. Delayed release is achieved by a special formulation design and/or manufacturing method. Delayed-release dosage forms include gastro-resistant preparations as defined in the general monographs on solid oral dosage forms.

Pulsatile-release dosage forms

Pulsatile-release dosage forms are modified-release dosage forms showing a sequential release of the active substance(s). Sequential release is achieved by a special formulation design and/or manufacturing method.

Large-volume parenterals

Infusions and injections supplied in containers with a nominal content of more than 100 ml.

Small-volume parenterals

Infusions and injections supplied in containers with a nominal content of 100 ml or less.

Ph Eur

Granules of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those granules that are the subject of an individual monograph in the British Pharmacopoeia.

LABELLING

For single-dose containers the label states the name and amount of active ingredient per container and for multi-dose containers the label states the name and amount of active ingredient in a suitable quantity by weight.

The label also states (1) the date after which the Granules are not intended to be used; (2) the conditions under which the Granules should be stored; (3) the instructions for using the Granules.

GRANULES

General Notices

(Ph Eur monograph 0499)

Granules comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Requirements for granules to be used for the preparation of oral solutions or suspensions are given in the monograph on Liquid preparations for oral use (0672). Where justified and authorised, the requirements of this monograph do not apply to granules for veterinary use.

DEFINITION

Granules are preparations consisting of solid, dry aggregates of powder particles sufficiently resistant to withstand handling. They are intended for oral administration. Some are swallowed as such, some are chewed and some are dissolved or dispersed in water or another suitable liquid before being administered.

Granules contain one or more active substances with or without excipients and, if necessary, colouring matter authorised by the competent authority and flavouring substances.

Granules are presented as single-dose or multidose preparations. Each dose of a multidose preparation is administered by means of a device suitable for measuring the quantity prescribed. For single-dose granules, each dose is enclosed in an individual container, for example a sachet or a vial.

Where applicable, containers for granules comply with the requirements of *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Several categories of granules may be distinguished:

— effervescent granules,

— coated granules,

— gastro-resistant granules,

— modified-release granules.

PRODUCTION

In the manufacture, packaging, storage and distribution of granules, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

TESTS

Uniformity of dosage units

Single-dose granules comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose granules with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances which correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Single-dose granules except for coated granules comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Uniformity of mass of delivered doses from multidose containers *(2.9.27)*

Granules supplied in multidose containers comply with the test.

STORAGE

If the preparation contains volatile ingredients or the contents have to be protected, store in an airtight container.

EFFERVESCENT GRANULES

DEFINITION

Effervescent granules are uncoated granules generally containing acid substances and carbonates or hydrogen carbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

TESTS

Disintegration

Place one dose of the effervescent granules in a beaker containing 200 ml of *water R* at 15-25 °C; numerous bubbles of gas are evolved. When the evolution of gas around the individual grains ceases, the granules have disintegrated, being either dissolved or dispersed in the water. Repeat the operation on 5 other doses. The preparation complies with the test if each of the 6 doses used disintegrates within 5 min.

STORAGE

In an airtight container.

COATED GRANULES

DEFINITION

Coated granules are usually multidose preparations and consist of granules coated with one or more layers of mixtures of various excipients.

PRODUCTION

The substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs.

TESTS

Dissolution

A suitable test may be carried out to demonstrate the appropriate release of the active substance(s), for example one of the tests described in *Dissolution test for solid dosage forms (2.9.3)*.

MODIFIED-RELEASE GRANULES

DEFINITION

Modified-release granules are coated or uncoated granules which contain special excipients or which are prepared by special procedures, or both, designed to modify the rate, the place or the time at which the active substance or substances are released.

Modified-release granules include prolonged-release granules and delayed-release granules.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) .

TESTS

Dissolution

Carry out a suitable test to demonstrate the appropriate release of the active substance(s), for example the test described in *Dissolution test for solid dosage forms (2.9.3)*.

GASTRO-RESISTANT GRANULES

DEFINITION

Gastro-resistant granules are delayed-release granules that are intended to resist the gastric fluid and to release the active substance(s) in the intestinal fluid. These properties are achieved by covering the granules with a gastro-resistant material (enteric-coated granules) or by other suitable means.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) .

TESTS

Dissolution

Carry out a suitable test to demonstrate the appropriate release of the active substance(s), for example the test described in *Dissolution test for solid dosage forms (2.9.3)*.

INFUSIONS

General Notices

DEFINITION

Infusions are dilute solutions containing the readily-soluble constituents of crude drugs. They are usually prepared by diluting one volume of a concentrated Infusion to ten volumes with Water.

For dispensing purposes, Infusions should be used within 12 hours of their preparation.

LABELLING

The label states (1) the time after which the Infusion is not intended to be used; (2) the conditions under which the Infusion should be stored.

Liquids for Cutaneous Application of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any application, collodion, liniment, lotion or paint that is the subject of an individual monograph in the British Pharmacopoeia and to the solutions listed below.

APPLICATIONS

LABELLING

The label states (1) that the Application is intended for external use only; (2) the date after which the Application is not intended to be used; (3) the conditions under which the Application should be stored; (4) the directions for using the Application; (5) any special precautions associated with the use of the Application.

COLLODIONS

DEFINITION

Collodions are Liquids for Cutaneous Application, usually containing Pyroxylin in a mixture of Ether and Ethanol. When they are allowed to dry, a flexible film is formed at the site of application.

STORAGE

Collodions should be stored remote from fire.

LABELLING

The label states (1) that the Collodion is intended for external use only; (2) the date after which the Collodion is not intended to be used; (3) the conditions under which the Collodion should be stored; (4) the directions for using the Collodion; (5) any special precautions associated with the use of the Collodion.

LINIMENTS

DEFINITION

Liniments are Liquids for Cutaneous Application that are intended to be applied to the unbroken skin with friction.

STORAGE

Certain plastic containers, such as those made from polystyrene, are unsuitable for Liniments.

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) that the

Liniment is intended for external use only; (3) if appropriate, that the contents of the container should be shaken before use; (4) the date after which the Liniment is not intended to be used; (5) the conditions under which the Liniment should be stored; (6) the directions for using the Liniment; (7) any special precautions associated with the use of the Liniment.

LOTIONS

DEFINITION

Lotions are Liquids for Cutaneous Application that are intended to be applied to the unbroken skin without friction.

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) that the Lotion is intended for external use only; (3) that the Lotion should be shaken before use; (4) the date after which the Lotion is not intended to be used; (5) the conditions under which the Lotion should be stored; (6) the directions for using the Lotion; (7) any special precautions associated with the use of the Lotion.

POWDERS FOR LOTIONS

DEFINITION

Powders for Lotions are preparations consisting of solid, loose, dry particles containing one or more active substances, with or without excipients, intended for reconstitution using a suitable solvent prior to cutaneous application. They may contain colouring matter if authorised by the competent authority.

Powders for Lotions are presented for use as single-dose or multi-dose preparations.

TESTS

Uniformity of Content

Unless otherwise prescribed or justified and authorised, single-dose Powders for Lotions with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content, Appendix XII C3. If the preparation has more than one active substance, the requirement applies only to those substances which correspond to the above conditions.

Uniformity of Mass

Single-dose Powders for Lotions comply with the test for uniformity of weight (mass), Appendix XII C1. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

LABELLING

The label states (1) that the Powder for Lotion is intended for reconstitution and external use only; (2) the names and amounts of active ingredients contained in the sealed container; (3) the directions for preparation of the Lotion; (4) the date after which the Powder for Lotion is not intended to be used; (5) the conditions under which the Powder for Lotion should be stored; (6) any special precautions associated with the use of the Powder for Lotion.

NAIL SOLUTIONS

LABELLING

The label states (1) the name and concentration of the active ingredient; (2) that the Nail Solution is intended for external use only; (3) the date after which the Nail Solution is not intended to be used; (4) the conditions under which the Nail Solution should be stored; (5) the directions for using the Nail Solution; (6) any special precautions associated with use of the Nail Solution.

PAINTS

DEFINITION

Paints are solutions or dispersions of one or more active ingredients. They are intended for application to the skin or, in some cases, mucous membranes.

STORAGE

Paints should be kept in airtight containers.

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) the date after which the Paint is not intended to be used; (3) the conditions under which the Paint should be stored; (4) the directions for using the Paint; (5) any special precautions associated with the use of the Paint.

LIQUIDS FOR CUTANEOUS APPLICATION

General Notices

(Liquid Preparations for Cutaneous Application, Ph Eur monograph 0927)

Liquids for Cutaneous Application comply with the requirements of the European Pharmacopoeia monograph for Liquid Preparations for Cutaneous Application. These requirements are reproduced below.

Ph Eur

Where justified and authorised, the requirements of this monograph do not apply to preparations intended for systemic and veterinary use.

DEFINITION

Liquid preparations for cutaneous application are preparations of a variety of viscosities intended for local or transdermal delivery of active ingredients. They are solutions, emulsions or suspensions that may contain 1 or more active substances in a suitable vehicle. They may contain suitable antimicrobial preservatives, antioxidants and other excipients such as stabilisers, emulsifiers and thickeners.

Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment that is readily dispersed on shaking to give a suspension that is sufficiently stable to enable a homogeneous preparation to be delivered.

Where applicable, containers for liquid preparations for cutaneous application comply with the requirements of *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

When liquid preparations for cutaneous application are dispensed in pressurised containers, the containers comply with the requirements of the monograph on *Pressurised pharmaceutical preparations (0523)*.

Preparations specifically intended for use on severely injured skin are sterile.

Several categories of liquid preparations for cutaneous application may be distinguished, for example:

- — shampoos;
- — cutaneous foams.

PRODUCTION

During development of a liquid preparation for cutaneous application whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in the text on *Efficacy of antimicrobial preservation (5.1.3)*.

During development, it must be demonstrated that the nominal content can be withdrawn from the container of liquid preparations for cutaneous application presented in single-dose containers.

In the manufacture, packaging, storage and distribution of liquid preparations for cutaneous application, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

Sterile liquid preparations for cutaneous application are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products (5.1.1)*.

In the manufacture of liquid preparations for cutaneous application containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Sterility *(2.6.1)*

Where the label indicates that the preparation is sterile, it complies with the test for sterility.

STORAGE

If the preparation is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

— the name of any added antimicrobial preservative;

— where applicable, that the preparation is sterile.

SHAMPOOS

DEFINITION

Shampoos are liquid or, occasionally, semi-solid preparations intended for application to the scalp and subsequent washing away with water. Upon rubbing with water they usually form a foam.

They are emulsions, suspensions or solutions. Shampoos normally contain surface active agents.

CUTANEOUS FOAMS

DEFINITION

Cutaneous foams comply with the requirements of the monograph on *Medicated foams (1105).*

Ph Eur

MEDICATED CHEWING GUMS

General Notices

(Ph Eur monograph 1239)

Medicated Chewing Gums comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Medicated chewing gums are solid, single-dose preparations with a base consisting mainly of gum that are intended to be chewed but not swallowed.

They contain one or more active substances which are released by chewing. After dissolution or dispersion of the active substances in saliva, chewing gums are intended to be used for:

— local treatment of mouth diseases,

— systemic delivery after absorption through the buccal mucosa or from the gastrointestinal tract.

PRODUCTION

Medicated chewing gums are made with a tasteless masticatory gum base that consists of natural or synthetic elastomers. They may contain other excipients such as fillers, softeners, sweetening agents, flavouring substances, stabilisers and plasticisers and authorised colouring matter.

Medicated chewing gums are manufactured by compression or by softening or melting the gum bases and adding successively the other substances. In the latter case, chewing gums are then further processed to obtain the desired gum presentation. The medicated chewing gums may be coated, for example, if necessary to protect from humidity and light.

Unless otherwise justified and authorised, a suitable test is carried out to demonstrate the appropriate release of the active substance(s). The method *Dissolution test for medicated chewing gums* (*2.9.25*) may be used to that purpose.

In the manufacture, packaging, storage and distribution of medicated chewing gums, suitable means must be taken to ensure their microbial quality; recommendations related to this aspect are provided in the general chapter on *Microbiological quality of pharmaceutical preparations* (*5.1.4*).

TESTS

Uniformity of dosage units

Medicated chewing gums comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, medicated chewing gums with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A for uniformity of content of single-dose preparations. If the preparation contains more than one active substance, the requirement applies only to those active substances which correspond to the above conditions.

Uniformity of mass (*2.9.5*)

Uncoated medicated chewing gums and, unless otherwise justified and authorised, coated medicated chewing gums comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

STORAGE

Store uncoated medicated chewing gums protected from humidity and light.

Ph Eur

Medicated Foams of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any medicated foam that is the subject of an individual monograph in the British Pharmacopoeia.

LABELLING

The label states (1) the name and concentration of the active ingredient; (2) that the Medicated Foam is intended for external use only; (3) the date after which the Medicated Foam is not intended to be used; (4) the conditions under which the Medicated Foam should be stored; (5) the directions for using the Medicated Foam; (6) any special precautions associated with the use of the Medicated Foam.

MEDICATED FOAMS

General Notices

(Ph Eur monograph 1105)

Medicated Foams comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Additional requirements for medicated foams may be found, where appropriate, in other general monographs, for example on Rectal preparations (1145), Vaginal preparations (1164) and Liquid preparations for cutaneous application (0927).

DEFINITION

Medicated foams are preparations consisting of large volumes of gas dispersed in a liquid generally containing one or more active substances, a surfactant ensuring their formation and various other excipients. Medicated foams are usually intended for application to the skin or mucous membranes.

Medicated foams are usually formed at the time of administration from a liquid preparation in a pressurised container. The container is equipped with a device consisting of a valve and a push button suitable for the delivery of the foam.

Medicated foams intended for use on severely injured skin and on large open wounds are sterile.

Medicated foams supplied in pressurised containers comply with the requirements of the monograph on *Pressurised pharmaceutical preparations (0523)*.

PRODUCTION

Sterile medicated foams are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products (5.1.1)*.

TESTS

Relative foam density

Maintain the container at about 25 °C for at least 24 h. Taking care not to warm the container, fit a rigid tube 70 mm to 100 mm long and about 1 mm in internal diameter onto the push button. Shake the container to homogenise the liquid phase of the contents and dispense 5 ml to 10 ml of foam to waste. Tare a flat-bottomed dish with a volume of about 60 ml and about 35 mm high. Place the end of the rigid tube attached to the push button in the corner of the dish, press the push button and fill the dish uniformly, using a circular motion. After the foam has completely expanded, level off by removing the excess foam with a slide. Weigh. Determine the mass of the same volume of *water R* by filling the same dish with *water R*.

The relative foam density is equivalent to the ratio:

$$\frac{m}{e}$$

m = mass of the test sample of foam, in grams,

e = mass of same volume of *water R*, in grams.

Carry out three measurements. None of the individual values deviate by more than 20 per cent from the mean value.

Duration of expansion

The apparatus (Figure 1105.-1) consists of a 50 ml burette, 15 mm in internal diameter, with 0.1 ml graduations and fitted with a 4 mm single bore stopcock. The graduation corresponding to 30 ml is at least 210 mm from the axis of the stopcock. The lower part of the burette is connected by means of a plastic tube not longer than 50 mm and 4 mm in internal diameter to the foam-generating container equipped with a push button fitted to this connection. Maintain the container at about 25 °C for at least 24 h. Shake the container, taking care not to warm it, to homogenise the liquid phase of the contents and dispense 5 ml to 10 ml of the foam to waste. Connect the push button to the outlet of the burette. Press the button and introduce about 30 ml of foam in a single delivery. Close the stopcock and at the same time start the chronometer and read the volume of foam in the burette. Every 10 s read the growing volume until the maximum volume is reached.

Carry out three measurements. None of the times needed to obtain the maximum volume is more than 5 min.

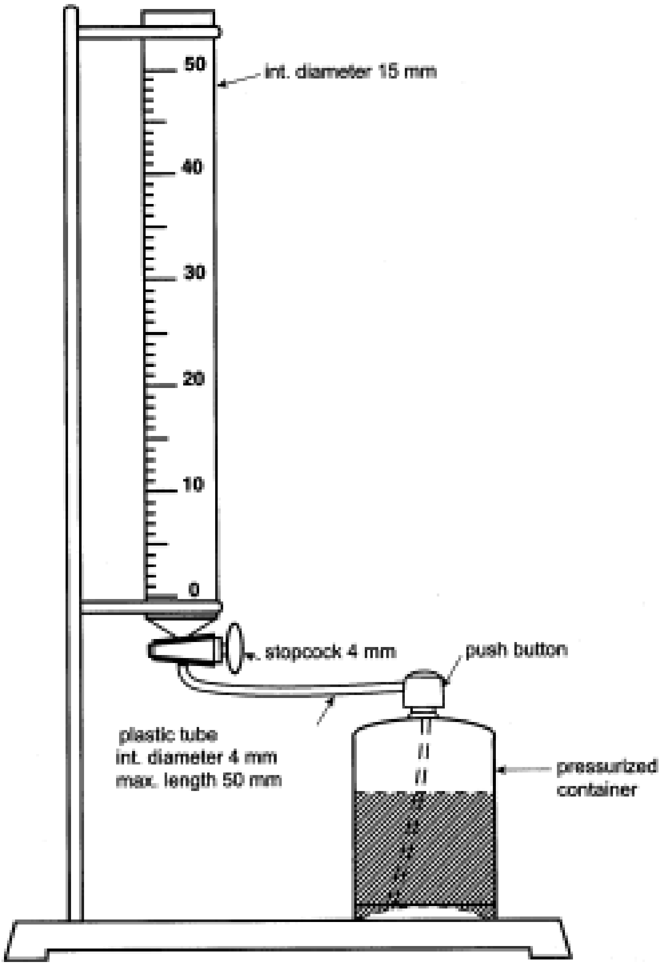

50
40
30
20
10
0
int. diameter 15 mm
stopcock 4 mm
push button
plastic tube
int. diameter 4 mm
max. length 50 mm
pressurized
container

Figure 1105.-1. – *Apparatus for the determination of the duration of expansion*

Sterility ***(2.6.1)***

When the label indicates that the preparation is sterile, it complies with the test for sterility.

LABELLING

The label states, where applicable, that the preparation is sterile.

Ph Eur

MEDICATED TAMPONS

General Notices

(Ph Eur monograph 1155)

Medicated Tampons comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Additional requirements for medicated tampons may be found, where appropriate, in other general monographs, for example Rectal preparations (1145), Vaginal preparations (1164) and Ear preparations (0652).

DEFINITION

Medicated tampons are solid, single-dose preparations intended to be inserted into the body cavities for a limited period of time. They consist of a suitable material such as cellulose, collagen or silicone impregnated with one or more active substances.

PRODUCTION

In the manufacture, packaging, storage and distribution of medicated tampons, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

LABELLING

The label states the quantity of active substance(s) per tampon.

Ph Eur

Nasal Preparations of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any intranasal solution, intranasal suspension, nasal drops, nasal ointment or nasal spray that is the subject of an individual monograph in the British Pharmacopoeia.

INTRANASAL SOLUTIONS AND INTRANASAL SUSPENSIONS

DEFINITION

Intranasal Solutions and Intranasal Suspensions are intended for administration to the nostrils for local or systemic effects. When supplied in a container fitted with a spray mechanism, they may be entitled Nasal Spray.

LABELLING

The label states (1) the name and quantity of the active ingredient; (2) the name and quantity of any added substance; (3) that the preparation is for intranasal administration; (4) the date after which the preparation is not intended to be used; (5) the conditions under which the preparation should be stored.

NASAL DROPS

LABELLING

The label states (1) the name and quantity of the active ingredient; (2) the instructions for using the Nasal Drops; (3) the date after which the Nasal Drops are not intended to be used; (4) the conditions under which the Nasal Drops should be stored.

NASAL PREPARATIONS

General Notices

(Ph Eur monograph 0676)

Nasal Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Nasal preparations are liquid, semi-solid or solid preparations intended for administration to the nasal cavities to obtain a systemic or local effect. They contain one or more active substances. Nasal preparations are as far as possible non-irritating and do not adversely affect the functions of the nasal mucosa and its cilia. Aqueous nasal preparations are usually isotonic and may contain excipients, for example, to adjust the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance, or to stabilise the preparation.

Nasal preparations are supplied in multidose or single-dose containers, provided, if necessary, with a suitable administration device, which may be designed to avoid the introduction of contaminants.

Unless otherwise justified and authorised, aqueous nasal preparations supplied in multidose containers contain a suitable antimicrobial preservative in an appropriate concentration, except where the preparation itself has adequate antimicrobial properties.

Where applicable, the containers comply with the requirements of *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Several categories of nasal preparations may be distinguished:

— nasal drops and liquid nasal sprays;

— nasal powders;

— semi-solid nasal preparations;

— nasal washes;

— nasal sticks.

PRODUCTION

During the development of a nasal preparation whose formulation contains an antimicrobial preservative, the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in the text on *Efficacy of antimicrobial preservation (5.1.3)*.

In the manufacture, packaging, storage and distribution of nasal preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

Sterile nasal preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products (5.1.1)*.

In the manufacture of nasal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Sterility (*2.6.1*)

Where the label states that the preparation is sterile, it complies with the test for sterility.

STORAGE

If the preparation is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

- — the name of any added antimicrobial preservative;
- — where applicable, that the preparation is sterile.

NASAL DROPS AND LIQUID NASAL SPRAYS

DEFINITION

Nasal drops and liquid nasal sprays are solutions, emulsions or suspensions intended for instillation or spraying into the nasal cavities.

Emulsions may show evidence of phase separation but are easily redispersed on shaking. Suspensions may show a sediment, which is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Nasal drops are usually supplied in multidose containers provided with a suitable applicator.

Liquid nasal sprays are supplied in containers with atomising devices or in pressurised containers fitted with a suitable adapter and with or without a metering dose valve, which comply with the requirements of the monograph on *Pressurised pharmaceutical preparations (0523).*

The size of droplets of the spray is such as to localise their deposition in the nasal cavity.

TESTS

Unless otherwise prescribed or justified and authorised, nasal drops supplied in single-dose containers and single doses of metered-dose nasal sprays, both intended for systemic action, comply with the following tests.

NASAL DROPS IN SINGLE DOSE CONTAINERS

Uniformity of dosage units

Nasal drops in single-dose containers comply with the test for uniformity of dosage units

(2.9.40) or, where justified and authorised, with the test for uniformity of mass or uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of mass

Nasal drops that are solutions comply with the following test. Weigh individually the contents of 10 containers emptied as completely as possible, and determine the average mass. Not more than 2 of the individual masses deviate by more than 10 per cent from the average mass, and none deviate by more than 20 per cent.

Uniformity of content *(2.9.6)*

Nasal drops that are suspensions or emulsions comply with the following test. Empty each container as completely as possible and carry out the test on the individual contents. They comply with test B for uniformity of content.

METERED-DOSE NASAL SPRAYS

Uniformity of dosage units

Metered-dose nasal sprays comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the test for uniformity of mass or the test for uniformity of delivered dose shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

In the case of metered-dose nasal sprays that are solutions, proceed as follows. Discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 9 containers. Determine the mass variation *(2.9.40)*.

In the case of metered-dose nasal sprays that are suspensions or emulsions, proceed as follows. Use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomising device. Shake the container for 5 s and discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 s, fire 1 dose of the metered-dose nasal spray into the collecting vessel by actuating the atomising device. Collect the contents of the collecting vessel by successive rinses. Determine the content of active substance in the combined rinses. Repeat the procedure for a further 9 containers. Determine the content uniformity *(2.9.40)*.

Uniformity of mass

Metered-dose nasal sprays that are solutions comply with the following test. Discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 9 containers.

The preparation complies with the test if not more than 2 of the individual values deviate by more than 25 per cent from the average value and none deviate by more than 35 per cent.

Uniformity of delivered dose

Metered-dose nasal sprays that are suspensions or emulsions comply with the following test.

Use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomising device. Shake the container for 5 s and discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 s, fire 1 dose of the metered-dose nasal spray into the collecting vessel by actuating the atomising device. Collect the contents of the collecting vessel by successive rinses. Determine the content of active substance in the combined rinses. Repeat the procedure for a further 9 containers.

Unless otherwise justified and authorised, the preparation complies with the test if not more than 1 of the individual contents is outside the limits of 75 per cent to 125 per cent and none are outside the limits of 65 per cent to 135 per cent of the average content.

If 2 or at most 3 individual contents are outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 20 more containers. The preparation complies with the test if not more than 3 individual contents of the 30 individual contents are outside the limits of 75 per cent to 125 per cent and none are outside the limits of 65 per cent to 135 per cent of the average content.

NASAL POWDERS

DEFINITION

Nasal powders are powders intended for insufflation into the nasal cavity by means of a suitable device.

They comply with the requirements of the monograph on *Powders for cutaneous application (1166).*

The size of the particles is such as to localise their deposition in the nasal cavity and is verified by adequate methods of particle-size determination.

SEMI-SOLID NASAL PREPARATIONS

DEFINITION

Semi-solid nasal preparations comply with the requirements of the monograph on *Semi-solid preparations for cutaneous application (0132)*.

The containers are adapted to deliver the product to the site of application.

NASAL WASHES

DEFINITION

Nasal washes are generally aqueous isotonic solutions intended to cleanse the nasal cavities.

Nasal washes intended for application to injured parts or prior to a surgical operation are sterile.

PRODUCTION

During development, it must be demonstrated that the nominal content can be withdrawn from the container, for nasal washes presented in single-dose containers.

NASAL STICKS

DEFINITION

Nasal sticks comply with the monograph on *Sticks (1154).*

Ph Eur

Oral Liquids of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any elixir, linctus, mixture, oral drops, oral emulsion, oral solution, oral suspension or syrup that is the subject of an individual monograph in the British Pharmacopoeia.

DEFINITION

The vehicle for any particular Oral Liquid should be chosen having regard to the nature of the active ingredient or ingredients and to provide organoleptic characteristics appropriate to the intended use of the preparation.

Oral Liquids other than Oral Emulsions may be supplied as liquids or prepared just before issue for use by dissolving or dispersing granules or powder in the liquid stated on the label.

LABELLING

The label states (1) the date after which the Oral Liquid is not intended to be used; (2) the conditions under which the Oral Liquid should be stored; (3) for Oral Emulsions, Oral Suspensions and, where appropriate, for Mixtures, that the bottle should be shaken before use.

If the Oral Liquid is supplied as granules or powder to be constituted just before issue for use the label states (1) that the contents of the container are granules or powder for the preparation of an Oral Liquid; (2) the strength as the amount of the active ingredient in a suitable dose-volume of the constituted preparation; (3) the directions for preparing the Oral Liquid including the nature and quantity of liquid to be used.

ELIXIRS

DEFINITION

Elixirs are clear, flavoured Oral Liquids containing one or more active ingredients dissolved in a vehicle that usually contains a high proportion of Sucrose or a suitable polyhydric alcohol or alcohols and may also contain Ethanol (96 per cent) or a Dilute Ethanol.

LINCTUSES

DEFINITION

Linctuses are viscous Oral Liquids that may contain one or more active ingredients in solution. The vehicle usually contains a high proportion of Sucrose, other sugars or a suitable polyhydric alcohol or alcohols. Linctuses are intended for use in the treatment or relief of cough, and are sipped and swallowed slowly without the addition of water.

MIXTURES

DEFINITION

Mixtures are Oral Liquids containing one or more active ingredients dissolved, suspended or dispersed in a suitable vehicle. Suspended solids may separate slowly on standing but are easily redispersed on shaking.

ORAL DROPS

DEFINITION

Oral Drops are Oral Liquids that are intended to be administered in small volumes with the aid of a suitable measuring device.

ORAL EMULSIONS

DEFINITION

Oral Emulsions are Oral Liquids containing one or more active ingredients. They are stabilised oil-in-water dispersions, either or both phases of which may contain dissolved solids. Solids may also be suspended in Oral Emulsions.

When issued for use, Oral Emulsions should be supplied in wide-mouthed bottles.

Extemporaneous preparation

In Oral Emulsions prepared according to the formula and directions given for Extemporaneous preparation, the quantity of emulsifying agent specified in individual monographs may be reduced to yield a preparation of suitable consistency provided that by so doing the stability of the preparation is not adversely affected.

ORAL SOLUTIONS

DEFINITION

Oral Solutions are Oral Liquids containing one or more active ingredients dissolved in a suitable vehicle.

ORAL SUSPENSIONS

DEFINITION

Oral Suspensions are Oral Liquids containing one or more active ingredients suspended in a suitable vehicle. Suspended solids may slowly separate on standing but are easily redispersed.

SYRUPS

DEFINITION

Syrups do not contain active ingredients. They are not intended to be administered as such but are used as vehicle ingredients for their flavouring and sweetening properties.

PRODUCTION

Syrups should be recently prepared unless they contain suitable antimicrobial preservatives.

STORAGE

Syrups should be stored at temperatures not exceeding 30^{c}.

LABELLING

The label states (1) the date after which the Syrup is not intended to be used; (2) the conditions under which the Syrup should be stored.

ORAL LIQUIDS

General Notices

(Liquid Preparations for Oral Use, Ph Eur monograph 0672)

Oral Liquids comply with the requirements of the European Pharmacopoeia monograph for Liquid Preparations for Oral Use. These requirements are reproduced below.

Ph Eur

Where justified and authorised, the requirements of this monograph do not apply to liquid preparations for oral use intended for veterinary use.

DEFINITION

Liquid preparations for oral use are usually solutions, emulsions or suspensions containing one or more active substances in a suitable vehicle; they may, however, consist of liquid active substances used as such (oral liquids).

Some preparations for oral use are prepared by dilution of concentrated liquid preparations, or from powders or granules for the preparation of oral solutions or suspensions, for oral drops or for syrups, using a suitable vehicle.

The vehicle for any preparations for oral use is chosen having regard to the nature of the active substance(s) and to provide organoleptic characteristics appropriate to the intended use of the preparation.

Liquid preparations for oral use may contain suitable antimicrobial preservatives, antioxidants and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilising, stabilising, flavouring and sweetening agents and colouring matter, authorised by the competent authority.

Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment, which is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Where applicable, containers for liquid preparations for oral use comply with the requirements of *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Several categories of preparations may be distinguished;

— oral solutions, emulsions and suspensions;

— powders and granules for oral solutions and suspensions;

— oral drops;

— powders for oral drops;

— syrups;

— powders and granules for syrups.

PRODUCTION

During development of a preparation for oral use whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in the text on *Efficacy of antimicrobial preservation (5.1.3)*.

During development, it must be demonstrated that the nominal content can be withdrawn from the container, for liquid preparations for oral use presented in single-dose containers.

In the manufacturing, packaging, storage and distribution of liquid preparations for oral use, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

In the manufacture of liquid preparations for oral use containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units

Solutions, suspensions and emulsions in single-dose containers comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the test for uniformity of content or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose preparations that are suspensions comply with the following test. After shaking, empty each container as completely as possible and carry out the test on the individual contents. They comply with test B for uniformity of content of single-dose preparations.

Uniformity of mass

Single-dose preparations that are solutions or emulsions comply with the following test: weigh individually the contents of 20 containers, emptied as completely as possible, and determine the average mass. Not more than 2 of the individual masses deviate by more than 10 per cent from the average mass and none deviate by more than 20 per cent.

Dose and uniformity of dose of oral drops

Into a suitable graduated cylinder, introduce by means of the dropping device the number of drops usually prescribed for one dose, or introduce by means of the measuring device the usually prescribed quantity. The dropping speed does not exceed 2 drops per second. Weigh the liquid, repeat the addition, weigh again and carry on repeating the addition and weighing until a total of 10 masses are obtained. No single mass deviates by more than 10 per cent from the average mass. The total of 10 masses does not differ by more than 15 per cent from the nominal mass of 10 doses. If necessary, measure the total volume of 10 doses. The volume does not differ by more than 15 per cent from the nominal volume of 10 doses.

Uniformity of mass of delivered doses from multidose containers *(2.9.27)*

Liquid preparations for oral use supplied in multidose containers comply with the test. Oral drops are not subject to the provisions of this test.

LABELLING

The label states the name of any added antimicrobial preservative.

ORAL SOLUTIONS, EMULSIONS AND SUSPENSIONS

DEFINITION

Oral solutions, emulsions and suspensions are supplied in single-dose or multidose containers. Each dose from a multidose container is administered by means of a device suitable for measuring the prescribed volume. The device is usually a spoon or a cup for volumes of 5 ml or multiples thereof or an oral syringe for other volumes.

POWDERS AND GRANULES FOR ORAL SOLUTIONS AND SUSPENSIONS

DEFINITION

Powders and granules for the preparation of oral solutions or suspensions generally conform to the definitions in the monographs on *Oral powders (1165)* or *Granules (0499)* as appropriate. They may contain excipients, in particular to facilitate dispersion or dissolution and to prevent caking.

After dissolution or suspension, they comply with the requirements for oral solutions or oral suspensions, as appropriate.

TESTS

Uniformity of dosage units

Single-dose powders and single-dose granules comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose powders and single-dose granules with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Single-dose powders and single-dose granules comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

LABELLING

The label states:

— the method of preparation of the solution or suspension;

— the conditions and the duration of storage after reconstitution.

ORAL DROPS

DEFINITION

Oral drops are solutions, emulsions or suspensions that are administered in small volumes such as drops by the means of a suitable device.

LABELLING

The label states the number of drops per millilitre of preparation or per gram of preparation if the dose is measured in drops.

POWDERS FOR ORAL DROPS

DEFINITION

Powders for the preparation of oral drops generally conform to the definition of *Oral powders (1165).* They may contain excipients to facilitate dissolution or suspension in the prescribed liquid or to prevent caking.

After dissolution or suspension, they comply with the requirements for oral drops.

TESTS

Uniformity of dosage units

Single-dose powders for oral drops comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose powders for oral drops with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Single-dose powders for oral drops comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

SYRUPS

DEFINITION

Syrups are aqueous preparations characterised by a sweet taste and a viscous consistency. They may contain sucrose at a concentration of at least 45 per cent *m/m*. The sweet taste can also be obtained by using other polyols or sweetening agents. Syrups usually contain aromatic or other flavouring agents. Each dose from a multidose container is administered by means of a device suitable for measuring the prescribed volume. The device is usually a spoon or a cup for volumes of 5 ml or multiples thereof.

LABELLING

The label states the name and concentration of the polyol or sweetening agent.

POWDERS AND GRANULES FOR SYRUPS

DEFINITION

Powders and granules for syrups generally conform to the definitions in the monograph on *Oral powders (1165)* or *Granules (0499)*. They may contain excipients to facilitate dissolution.

After dissolution, they comply with the requirements for syrups.

TESTS

Uniformity of dosage units

Single-dose powders and granules for syrups comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose powders and granules for syrups with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Single-dose powders and granules for syrups comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Ph Eur

Oral Powders of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those oral powders that are the subject of an individual monograph in the British Pharmacopoeia.

LABELLING

For single dose containers the label states the name and quantity of active ingredient per container. For multi-dose containers the label states the name and quantity of active ingredient in a suitable amount by weight.

The label also states (1) the name and proportions of any antimicrobial preservative; (2) the directions for use of the Oral Powder; (3) the date after which the Oral Powder is not intended to be used; (4) the conditions under which the Oral Powder should be stored.

ORAL POWDERS

General Notices

(Ph Eur monograph 1165)

Oral Powders comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Requirements for powders to be used for the preparation of oral solutions or suspensions are given in the monograph for Liquid preparations for oral use (0672). Where justified and authorised, the requirements of this monograph do not apply to oral powders intended for veterinary use.

DEFINITION

Oral powders are preparations consisting of solid, loose, dry particles of varying degrees of fineness. They contain one or more active substances, with or without excipients and, if necessary, colouring matter authorised by the competent authority and flavouring substances. They are generally administered in or with water or another suitable liquid. They may also be swallowed directly. They are presented as single-dose or multidose preparations.

Where applicable, containers for oral powders comply with the requirements of *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Multidose oral powders require the provision of a measuring device capable of delivering the quantity prescribed. Each dose of a single-dose powder is enclosed in an individual container, for example a sachet or a vial.

PRODUCTION

In the manufacture of oral powders, means are taken to ensure a suitable particle size with regard to the intended use.

In the manufacture, packaging, storage and distribution of oral powders, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

TESTS

Uniformity of dosage units

Single-dose oral powders comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose oral powders with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances which correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Single-dose oral powders comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Uniformity of mass of delivered doses from multidose containers *(2.9.27)*

Oral powders supplied in multidose containers comply with the test.

STORAGE

If the preparation contains volatile ingredients, or the contents have to be protected, store in an airtight container.

EFFERVESCENT POWDERS

Effervescent powders are presented as single-dose or multidose preparations and generally contain acid substances and carbonates or hydrogen carbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

STORAGE

In an airtight container.

Ph Eur

Oromucosal Preparations of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any mouthwash, oromucosal solution, oromucosal suspension, oromucosal gel, oromucosal paste, oromucosal spray, sublingual spray, lozenges, pastilles, buccal tablets or sublingual tablets that are the subject of an individual monograph in the British Pharmacopoeia.

MOUTHWASHES

LABELLING

The label states (1) the directions for dilution of the Mouthwash, if appropriate; (2) that large quantities of the Mouthwash should not be swallowed; (3) the date after which the Mouthwash is not intended to be used; (4) the conditions under which it should be stored.

OROMUCOSAL GELS

LABELLING

The label states (1) the directions for use of the Oromucosal Gel; (2) the date after which the Oromucosal Gel is not intended to be used; (3) the conditions under which it should be stored.

OROMUCOSAL PASTES

LABELLING

The label states (1) the directions for use of the Oromucosal Paste; (2) the date after which the Oromucosal Paste is not intended to be used; (3) the conditions under which it should be stored.

OROMUCOSAL SPRAYS

LABELLING

The label states (1) the directions for use of the Oromucosal Spray; (2) the date after which the Oromucosal Spray is not intended to be used; (3) the conditions under which it should be stored.

For Oromucosal Sprays that are presented in metered-dose containers, the label also states (1) the amount of active ingredient per delivered dose; (2) the number of deliveries in the container.

SUBLINGUAL SPRAYS

LABELLING

The label states (1) the amount of active ingredient per delivered dose; (2) the number of deliveries in the container; (3) the directions for use of the Sublingual Spray; (4) the date after which the Sublingual Spray is not intended to be used; (5) the conditions under which it should be stored.

LOZENGES

LABELLING

The label states (1) the directions for use of the Lozenges; (2) the date after which the Lozenges are not intended to be used; (3) the conditions under which they should be stored.

PASTILLES

LABELLING

The label states (1) the directions for use of the Pastilles; (2) the date after which the Pastilles are not intended to be used; (3) the conditions under which they should be stored.

BUCCAL TABLETS

LABELLING

The label states (1) the directions for use of the Buccal Tablets; (2) the date after which the Buccal Tablets are not intended to be used; (3) the conditions under which they should be stored.

SUBLINGUAL TABLETS

LABELLING

The label states (1) the directions for use of the Sublingual Tablets; (2) the date after which the Sublingual Tablets are not intended to be used; (3) the conditions under which they should be stored.

OROMUCOSAL PREPARATIONS

General Notices

(Ph Eur monograph 1807)

Oromucosal Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

This monograph does not apply to dental preparations or to preparations such as chewable tablets (0478), medicated chewing gums (1239), oral lyophilisates and other solid or semi-solid preparations that are intended to be chewed or dispersed in the saliva before being swallowed. Where justified and authorised, this monograph does not apply to preparations for veterinary use.

DEFINITION

Oromucosal preparations are solid, semi-solid or liquid preparations, containing one or more active substances intended for administration to the oral cavity and/or the throat to obtain a local or systemic effect. Preparations intended for a local effect may be designed for application to a specific site within the oral cavity such as the gums (gingival preparations) or the throat (oropharyngeal preparations). Preparations intended for a systemic effect are designed to be absorbed primarily at one or more sites on the oral mucosa (e.g. sublingual preparations). Mucoadhesive preparations are intended to be retained in the oral cavity by adhesion to the mucosal epithelium and may modify systemic drug absorption at the site of application. For many oromucosal preparations, it is likely that some proportion of the active substance(s) will be swallowed and may be absorbed via the gastrointestinal tract.

Oromucosal preparations may contain suitable antimicrobial preservatives and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilising, stabilising, flavouring and sweetening agents. Solid preparations may in addition contain glidants, lubricants and excipients capable of modifying the release of the active substance(s).

Where applicable, containers for oromucosal preparations comply with the requirements for *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Several categories of preparations for oromucosal use may be distinguished:

- — gargles;
- — mouthwashes;
- — gingival solutions;
- — oromucosal solutions and oromucosal suspensions;
- — semi-solid oromucosal preparations (including for example gingival gel, gingival paste, oromucosal gel, oromucosal paste);

— oromucosal drops, oromucosal sprays and sublingual sprays (including oropharyngeal sprays);

— lozenges and pastilles;

— compressed lozenges;

— sublingual tablets and buccal tablets;

— oromucosal capsules;

— mucoadhesive preparations.

PRODUCTION

During the development of an oromucosal preparation containing an antimicrobial preservative, the effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with the criteria for judging the preservative properties of the formulation are provided in *5.1.3 Efficacy of antimicrobial preservation.*

In the manufacture, packaging, storage and distribution of oromucosal preparations, suitable means are taken to ensure their microbiological quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

In the manufacture of semi-solid and liquid oromucosal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units

Single-dose oromucosal preparations comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the test for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose oromucosal preparations with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A (compressed and moulded dosage forms) or test B (capsules) for the uniformity of content of single-dose preparations. If the preparation contains more than one active substance, this requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Solid single-dose oromucosal preparations comply with the test for uniformity of mass. If the test for the uniformity of content is prescribed, or justified and authorised, for all active substances, the test for uniformity of mass is not required.

LABELLING

The label states the name of any added antimicrobial preservative.

GARGLES

DEFINITION

Gargles are aqueous solutions intended for gargling to obtain a local effect. They are not to be swallowed. They are supplied as ready-to-use solutions or concentrated solutions to be diluted. They may also be prepared from powders or tablets to be dissolved in water before use.

Gargles may contain excipients to adjust the pH which, as far as possible, is neutral.

MOUTHWASHES

DEFINITION

Mouthwashes are aqueous solutions intended for use in contact with the mucous membrane of the oral cavity. They are not to be swallowed. They are supplied as ready-to-use solutions or concentrated solutions to be diluted. They may also be prepared from powders or tablets to be dissolved in water before use.

Mouthwashes may contain excipients to adjust the pH which, as far as possible, is neutral.

GINGIVAL SOLUTIONS

DEFINITION

Gingival solutions are intended for administration to the gingivae by means of a suitable applicator.

OROMUCOSAL SOLUTIONS AND OROMUCOSAL SUSPENSIONS

DEFINITION

Oromucosal solutions and oromucosal suspensions are liquid preparations intended for administration to the oral cavity by means of a suitable applicator.

Oromucosal suspensions may show a sediment which is readily dispersible on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

SEMI-SOLID OROMUCOSAL PREPARATIONS

DEFINITION

Semi-solid oromucosal preparations are hydrophilic gels or pastes intended for administration to the oral cavity or to a specific part of the oral cavity such as the gingivae (gingival gel, gingival paste). They may be provided as single-dose preparations.

Semi-solid oromucosal preparations comply with the requirements of the monograph on *Semi-solid preparations for cutaneous use (0132).*

OROMUCOSAL DROPS, OROMUCOSAL SPRAYS AND SUBLINGUAL SPRAYS

DEFINITION

Oromucosal drops, oromucosal sprays and sublingual sprays are solutions, emulsions or suspensions intended for local or systemic effect. They are applied by instillation or spraying into the oral cavity or onto a specific part of the oral cavity such as spraying under the tongue (sublingual spray) or into the throat (oropharyngeal spray).

Emulsions may show evidence of phase separation but are readily redispersed on shaking.

Suspensions may show a sediment which is readily dispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Liquid oromucosal sprays are supplied in containers with atomising devices or in pressurised containers having a suitable adaptor, with or without a metering dose valve, which comply with the requirements of the monograph on *Pressurised pharmaceutical preparations (0523).*

The size of the droplets of the spray is such as to localise their deposition in the oral cavity or the throat as intended.

TESTS

Unless otherwise prescribed or justified and authorised, oromucosal drops supplied in single-dose containers, single doses of metered-dose oromucosal sprays and sublingual sprays, all intended for systemic action, comply with the following tests.

OROMUCOSAL DROPS IN SINGLE-DOSE CONTAINERS

Uniformity of dosage units

Oromucosal drops in single-dose containers comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the test for uniformity of mass or uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of mass

Oromucosal drops that are solutions comply with the following test. Weigh individually the contents of 10 containers emptied as completely as possible, and determine the average mass. Maximum 2 of the individual masses deviate by more than 10 per cent from the average mass and none deviates by more than 20 per cent.

Uniformity of content *(2.9.6)*

Oromucosal drops that are suspensions or emulsions comply with the following test. Empty each container as completely as possible and carry out the test on the individual contents. They comply with test B of uniformity of content.

METERED-DOSE OROMUCOSAL SPRAYS AND SUBLINGUAL SPRAYS

Uniformity of dosage units

Metered-dose oromucosal sprays and sublingual sprays comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the test for uniformity of mass or the test for uniformity of delivered dose shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

In the case of metered-dose oromucosal sprays and sublingual sprays that are solutions, proceed as follows. Discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 9 containers. Determine the mass variation *(2.9.40)*.

In the case of metered-dose oromucosal sprays and sublingual sprays that are suspensions or emulsions proceed as follows. Use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomising device.

Shake the container for 5 s and discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 s, fire 1 dose of the metered-dose spray into the collecting vessel by actuating the atomising device. Collect the contents of the collecting vessel by successive rinses. Determine the content of active substance in the combined rinses. Repeat the procedure for a further 9 containers. Determine the content uniformity *(2.9.40)*.

Uniformity of mass

Metered-dose oromucosal sprays and sublingual sprays that are solutions comply with the following test. Discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 9 containers.

The preparation complies with the test if maximum 2 of the individual values deviate by more than 25 per cent from the average value and none deviates by more than 35 per cent.

Uniformity of delivered dose

Metered-dose oromucosal sprays and sublingual sprays that are suspensions or emulsions comply with the following test. Use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomising device.

Shake the container for 5 s and discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 s, fire 1 dose of the metered-dose spray into the collecting vessel by actuating the atomising device. Collect the contents of the collecting vessel by successive rinses. Determine the content of active substance in the combined rinses. Repeat the procedure for a further 9 containers.

Unless otherwise justified and authorised, the preparation complies with the test if maximum 1 of the individual contents is outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content.

If 2 or maximum 3 individual contents are outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 20 more containers. The preparation complies with the test if maximum 3 individual contents of the 30 individual contents are outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content.

LOZENGES AND PASTILLES

DEFINITION

Lozenges and pastilles are solid, single-dose preparations intended to be sucked to obtain, usually, a local effect in the oral cavity and the throat. They contain one or more active substances, usually in a flavoured and sweetened base, and are intended to dissolve or disintegrate slowly in the mouth when sucked.

Lozenges are hard preparations prepared by moulding. Pastilles are soft, flexible preparations prepared by moulding of mixtures containing natural or synthetic polymers or gums and sweeteners.

COMPRESSED LOZENGES

DEFINITION

Compressed lozenges are solid, single-dose preparations intended to be sucked to obtain a local or systemic effect. They are prepared by compression and are often rhomboid in shape.

Compressed lozenges conform with the general definition of tablets.

PRODUCTION

In the manufacture of compressed lozenges, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by examining the *Friability of uncoated tablets (2.9.7)* and the *Resistance to crushing of tablets (2.9.8)*.

TESTS

Dissolution

For compressed lozenges intended for a systemic effect, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

SUBLINGUAL TABLETS AND BUCCAL TABLETS

DEFINITION

Sublingual tablets and buccal tablets are solid, single-dose preparations to be applied under the tongue or to the buccal cavity, respectively, to obtain a systemic effect. They are prepared by compression of mixtures of powders or granulations into tablets with a shape suited for the intended use.

Sublingual tablets and buccal tablets conform to the general definition of tablets.

PRODUCTION

In the manufacture of sublingual tablets and buccal tablets, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by examining the *Friability of uncoated tablets (2.9.7)* and the *Resistance to crushing of tablets (2.9.8)*.

TESTS

Dissolution

Unless otherwise justified and authorised, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

OROMUCOSAL CAPSULES

DEFINITION

Oromucosal capsules are soft capsules to be chewed or sucked.

MUCOADHESIVE PREPARATIONS

DEFINITION

Mucoadhesive preparations contain one or more active substances intended for systemic absorption through the buccal mucosa over a prolonged period of time. They may be supplied as mucoadhesive buccal tablets or as other mucoadhesive solid or semi-solid preparations.

Mucoadhesive buccal tablets are prepared by compression of mono- or multi-layered tablets. They usually contain hydrophilic polymers, which on wetting with the saliva produce a flexible hydrogel that adheres to the buccal mucosa.

PRODUCTION

In the manufacture of mucoadhesive buccal tablets, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by examining the *Friability of uncoated tablets (2.9.7)* and the *Resistance to crushing of tablets (2.9.8)*.

TESTS

Dissolution

Unless otherwise justified and authorised, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

Ph Eur

Parenteral Preparations of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any injection, intravenous infusion or implant that is the subject of an individual monograph in the British Pharmacopoeia.

DEFINITION

Definition of a particular Parenteral Preparation as a solution, emulsion or suspension in Water for Injections does not preclude the inclusion of suitable excipients where necessary for the purposes referred to in the requirements of the European Pharmacopoeia above. In particular, aqueous Parenteral Preparations for administration by the subcutaneous, intradermal, intramuscular, or, in the case of larger volumes, intravenous route, should if possible be made isotonic with blood by the addition of Sodium Chloride or other suitable substances. However if buffering agents are used in preparations intended for intra-ocular or intracardiac injection, or in preparations that may gain access to the cerebrospinal fluid, great care should be taken to ensure that the nature and concentration of the chosen agent are suitable for the intended route of administration.

Where the active ingredient is susceptible to oxidative degradation appropriate precautions, such as the addition of a suitable antioxidant or storage under oxygen-free nitrogen or other suitable inert gas, should be taken.

PRODUCTION

Methods of sterilisation that may be used in the manufacture of Parenteral Preparations are described in Appendix XVIII.

Where a direction is given to use Water for Injections in the manufacture of a Parenteral Preparation, Water for Injections in bulk is used. Where the use of Water for Injections free from dissolved air or Water for Injections free from dissolved carbon dioxide is specified, water freshly prepared by the process described under Water for Injections is boiled for at least 10 minutes with as little exposure to air as possible, cooled with precautions to exclude air and carbon dioxide, and sterilised by *heating in an autoclave*.

STORAGE

Closures used for containers of oily parenteral preparations should be made of oil-resistant materials.

LABELLING

Where appropriate the label states the strength of the parenteral preparation in terms of the amount of active ingredient in a suitable dose-volume.

The label also states (1) the name of any added substance; (2) the date after which the Parenteral Preparation is not intended to be used; (3) the conditions under which the Parenteral Preparation should be stored.

The label of a single-dose parenteral preparation states that any portion of the contents remaining should be discarded.

INTRAVENOUS INFUSIONS

LABELLING

The label states the nominal volume of the contents.

POWDERS FOR INJECTIONS

Uniformity of weight

When required for the Assay of a powder for injection, determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1.

LABELLING

The label states (1) that, when dissolved or suspended, the contents of the sealed container are intended for parenteral use; (2) the amount of active ingredient contained in the sealed container ; (3) the directions for the preparation of the Injection or Intravenous Infusion.

If a container of the liquid for constituting the parenteral preparation is supplied with the powder for injections, the label of this container states the composition of this liquid.

CONCENTRATED SOLUTIONS FOR INJECTIONS

LABELLING

The label states (1) the name of the concentrated solution; (2) that the solution must be diluted before use; (3) the directions for the preparation of the Injection or Intravenous Infusion.

PARENTERAL PREPARATIONS

General Notices

(Ph Eur monograph 0520)

Parenteral Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

The requirements of this monograph do not necessarily apply to products derived from human blood, to immunological preparations, or radiopharmaceutical preparations. Special requirements may apply to preparations for veterinary use depending on the species of animal for which the preparation is intended.

DEFINITION

Parenteral preparations are sterile preparations intended for administration by injection, infusion or implantation into the human or animal body.

Parenteral preparations may require the use of excipients, for example to make the preparation isotonic with respect to blood, to adjust the pH, to increase solubility, to prevent deterioration of the active substances or to provide adequate antimicrobial properties, but not to adversely affect the intended medicinal action of the preparation or, at the concentrations used, to cause toxicity or undue local irritation.

Containers for parenteral preparations are made as far as possible from materials that are sufficiently transparent to permit the visual inspection of the contents, except for implants and in other justified and authorised cases.

Where applicable, the containers for parenteral preparations comply with the requirements for *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Parenteral preparations are supplied in glass containers *(3.2.1)* or in other containers such as plastic containers *(3.2.2, 3.2.2.1 and 3.2.9)* and prefilled syringes. The tightness of the container is ensured by suitable means. Closures ensure a good seal, prevent the access of micro-organisms and other contaminants and usually permit the withdrawal of a part or the whole of the contents without removal of the closure. The plastic materials or elastomers *(3.2.9)* used to manufacture the closures are sufficiently firm and elastic to allow the passage of a needle with the least possible shedding of particles. Closures for multidose containers are sufficiently elastic to ensure that the puncture is resealed when the needle is withdrawn.

Several categories of parenteral preparations may be distinguished:

— injections,

— infusions,

— concentrates for injections or infusions,

— powders for injections or infusions,

— gels for injections,

— implants.

PRODUCTION

During the development of a parenteral preparation, the formulation for which contains an antimicrobial preservative, the effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided under *Efficacy of antimicrobial preservation (5.1.3)*.

Parenteral preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms. Recommendations on this aspect are provided in the text on *Methods of preparation of sterile products (5.1.1)*.

Water used in the manufacture of parenteral preparations complies with the requirements of water for injections in bulk stated in the monograph on *Water for injections (0169).*

TESTS

Particulate contamination: sub-visible particles (2.9.19)

For preparations for human use, solutions for infusion or solutions for injection comply with the test.

In the case of preparations for subcutaneous or intramuscular injection, higher limits may be appropriate. Radiopharmaceutical preparations are exempt from these requirements. Preparations for which the label states that the product is to be used with a final filter are exempt from these requirements, providing it has been demonstrated that the filter delivers a solution that complies with the test.

For preparations for veterinary use, when supplied in containers with a nominal content of more than 100 ml and when the content is equivalent to a dose of more than 1.4 ml per kilogram of body mass, solutions for infusion or solutions for injection comply with the test for particulate contamination: sub-visible particles.

Sterility *(2.6.1)*

Parenteral preparations comply with the test for sterility.

STORAGE

In a sterile, airtight, tamper-proof container .

LABELLING

The label states:

— the name and concentration of any added antimicrobial preservative,

— where applicable, that the solution is to be used in conjunction with a final filter,

— where applicable, that the preparation is free from bacterial endotoxins or that it is apyrogenic.

INJECTIONS

DEFINITION

Injections are sterile solutions, emulsions or suspensions. They are prepared by dissolving, emulsifying or suspending the active substance(s) and any added excipients in water, in a suitable non-aqueous liquid, that may be non-sterile where justified, or in a mixture of these vehicles.

Solutions for injection, examined under suitable conditions of visibility, are clear and practically free from particles.

Emulsions for injection do not show any evidence of phase separation. Suspensions for injection may show a sediment which is readily dispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be withdrawn.

Multidose preparations Multidose aqueous injections contain a suitable antimicrobial preservative at an appropriate concentration except when the preparation itself has adequate antimicrobial properties. When a preparation for parenteral use is presented in a multidose container , the precautions to be taken for its administration and more particularly for its storage between successive withdrawals are given.

Antimicrobial preservatives Aqueous preparations which are prepared using aseptic precautions and which cannot be terminally sterilised may contain a suitable antimicrobial preservative in an appropriate concentration.

No antimicrobial preservative is added when:

— the volume to be injected in a single dose exceeds 15 ml, unless otherwise justified,

— the preparation is intended for administration by routes where, for medical reasons, an antimicrobial preservative is not acceptable, such as intracisternally, epidurally, intrathecally or by any route giving access to the cerebrospinal fluid, or intra- or retro-ocularly.

Such preparations are presented in single-dose containers.

PRODUCTION

In the manufacture of injections containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

Single-dose preparations The volume of the injection in a single-dose container is sufficient to permit the withdrawal and administration of the nominal dose using a normal technique *(2.9.17)*.

TESTS

Uniformity of dosage units

Single-dose suspensions for injection comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, single-dose suspensions for injection with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A for uniformity of content of single-dose preparations. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Bacterial endotoxins – pyrogens

A test for bacterial endotoxins *(2.6.14)* is carried out or, where justified and authorised, the test for pyrogens *(2.6.8)*. Recommendations on the limits for bacterial endotoxins are given in chapter *2.6.14*.

Preparations for veterinary use When the volume to be injected in a single dose is 15 ml or more and is equivalent to a dose of 0.2 ml or more per kilogram of body mass, the preparation complies with a test for bacterial endotoxins *(2.6.14)* or with the test for pyrogens *(2.6.8)*.

Any preparation Where the label states that the preparation is free from bacterial endotoxins or apyrogenic, respectively, the preparation complies with a test for bacterial endotoxins *(2.6.14)* or with the test for pyrogens *(2.6.8)*, respectively.

INFUSIONS

DEFINITION

Infusions are sterile, aqueous solutions or emulsions with water as the continuous phase. They are usually made isotonic with respect to blood. They are principally intended for administration in large volume. Infusions do not contain any added antimicrobial preservative.

Solutions for infusion, examined under suitable conditions of visibility are clear and practically free from particles.

Emulsions for infusion do not show any evidence of phase separation.

PRODUCTION

In the manufacture of infusions containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

The volume of the infusion in the container is sufficient to permit the withdrawal and administration of the nominal dose using a normal technique *(2.9.17)*.

TESTS

Bacterial endotoxins – pyrogens

They comply with a test for bacterial endotoxins *(2.6.14)* or, where justified and authorised, with the test for pyrogens *(2.6.8)*. For the latter test inject 10 ml per kilogram of body mass into each rabbit, unless otherwise justified and authorised.

CONCENTRATES FOR INJECTIONS OR INFUSIONS

DEFINITION

Concentrates for injections or infusions are sterile solutions intended for injection or infusion after dilution. They are diluted to a prescribed volume with a prescribed liquid before administration. After dilution, they comply with the requirements for injections or for infusions.

TESTS

Bacterial endotoxins – pyrogens

They comply with the requirements prescribed for injections or for infusions, after dilution to a suitable volume.

POWDERS FOR INJECTIONS OR INFUSIONS

DEFINITION

Powders for injections or infusions are solid, sterile substances distributed in their final containers and which, when shaken with the prescribed volume of a prescribed sterile liquid rapidly form either clear and practically particle-free solutions or uniform suspensions. After dissolution or suspension, they comply with the requirements for injections or for infusions.

Freeze-dried products for parenteral use are considered as powders for injections or infusions.

PRODUCTION

The uniformity of content and uniformity of mass of freeze-dried products for parenteral use are ensured by the in-process control of the amount of the solution prior to freeze-drying.

TESTS

Uniformity of dosage units

Powders for injections or infusions comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, powders for injections or infusions with a content of active substance less than 2 mg or less than 2 per cent of the total mass, or with a unit mass equal to or less than 40 mg comply with test A for uniformity of content of single-dose preparations. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Powders for injections or infusions comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Bacterial endotoxins – pyrogens

They comply with the requirements prescribed for injections or for infusions, after dissolution or suspension in a suitable volume of liquid.

LABELLING

The label states the instructions for the preparation of injections and infusions.

GELS FOR INJECTIONS

DEFINITION

Gels for injections are sterile gels with a viscosity suitable to guarantee a modified release of the active substance(s) at the site of injection.

IMPLANTS

DEFINITION

Implants are sterile, solid preparations of a size and shape suitable for parenteral implantation and release of the active substance(s) over an extended period of time. Each dose is provided in a sterile container.

Ph Eur

Preparations for Inhalation of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any inhalation, powder for inhalation, pressurised inhalation or solution for nebulisation that is the subject of an individual monograph in the British Pharmacopoeia.

INHALATIONS

DEFINITION

Inhalations are solutions or dispersions of one or more active ingredients which may contain an inert, suspended, diffusing agent. They are intended to release volatile constituents for inhalation either when placed on a pad or when added to hot, but not boiling, water.

LABELLING

The label on the container states (1) the names and concentrations of the active ingredients; (2) that the Inhalation is not to be taken by mouth; (3) the date after which the Inhalation is not intended to be used; (4) the conditions under which the Inhalation should be stored.

POWDERS FOR INHALATION

LABELLING

The label on the container states (1) the date after which the Powder for Inhalation is not intended to be used; (2) the conditions under which the Powder for Inhalation should be stored.

Where the Powder for Inhalation is supplied in a capsule, the label also states (3) the quantity of the active ingredient contained in each capsule; (4) that the capsules are intended for use in an inhaler and are not to be swallowed.

PRESSURISED INHALATIONS

DEFINITION

Pressurised Inhalations are Pressurised Metered-dose Preparations for Inhalation. They are intended to be inhaled in controlled amounts.

PRODUCTION

The formulation of the inhalation and the components of the delivery device (that is the pressurised container with its integral metering valve and the actuator) should be designed and, where appropriate, the particle size of the active ingredient should be controlled so that, when the pressurised inhalation is used in accordance with the manufacturer's recommendations, an adequate proportion of the active ingredient is made available for inhalation. A proportion of the active ingredient is deposited on the inner surface of the actuator; the amount available for inhalation is therefore less than the amount released by actuation of the valve.

Pressurised Inhalations should be manufactured in conditions designed to minimise microbial and particulate contamination.

Content of active ingredient delivered by actuation of the valve

Remove the pressurised container from the actuator and remove all labels and markings which may be present on the container with a suitable solvent. Dry the container, replace in its actuator, shake for about 30 seconds and prime the metering valve as follows. Discharge once to waste, wait for not less than 5 seconds and discharge again to waste. Remove the pressurised container from its actuator, clean the valve stem (internally and externally) and the valve ferrule by washing with a suitable solvent. Dry the complete valve assembly using an air line fitted with an appropriate narrow jet to ensure that all solvent is removed from the inside of the valve stem.

Place a stainless steel base plate that has three legs and a central circular indentation with a hole about 1.5 mm in diameter in a small vessel suitable for shaking and add the volume of solvent specified in the monograph. The size of the vessel is such that when the pressurised inhalation is discharged into the specified volume of solvent as described below the discharge takes place not less than 25 mm below the surface of the solvent.

Shake the pressurised container for about 30 seconds and place it inverted in the vessel. Discharge 10 deliveries below the surface of the solvent actuating the valve at intervals of not less than 5 seconds, maintaining the pressurised container in the vertical plane and discharging the pressurised inhalation through the hole in the centre of the base plate. (It may be necessary because of the nature of the formulation to shake the pressurised container between each actuation of the valve; where this is the case shaking should be carried out without removing the pressurised container from its inverted position in the vessel.) Remove the pressurised container, wash it with the specified solvent and dilute the combined solution and washings to the volume specified in the monograph. Determine the amount of active ingredient by the method described under the Assay and calculate the amount delivered from each actuation of the valve. The result lies within the range for the content of active ingredient stated in the monograph.

STORAGE

Pressurised Inhalations are supplied in suitable containers fitted with an appropriate metering valve that forms an integral part of the container. Metal containers comply with the relevant requirements of BS 3914:1991 (Non-refillable metal aerosol dispensers of 50 ml to 1400 ml capacity and up to 85 mm diameter). The containers are usually supplied with an appropriate actuator.

LABELLING

The label states (1) the name of the active ingredient or ingredients; (2) the amount of active ingredient or ingredients delivered by each actuation of the valve and the number of deliveries available from the container; (3) the instructions for using the Pressurised Inhalation; (4) the date after which the Pressurised Inhalation is not intended to be used; (5) the conditions under which the Pressurised Inhalation should be stored; (6) any special precautions associated with the use of the Pressurised Inhalation.

SOLUTIONS FOR NEBULISATION

LABELLING

The label states (1) the date after which the solution for nebulisation is not intended to be used; (2) the conditions under which the solution for nebulisation should be stored.

PREPARATIONS FOR INHALATION

General Notices

(Ph Eur monograph 0671)

Preparations for Inhalation comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Preparations for inhalation are liquid or solid preparations intended for administration as vapours or aerosols to the lung in order to obtain a local or systemic effect. They contain one or more active substances which may be dissolved or dispersed in a suitable vehicle.

Preparations for inhalation may, depending on the type of preparation, contain propellants, cosolvents, diluents, antimicrobial preservatives, solubilising and stabilising agents, etc. These excipients do not adversely affect the functions of the mucosa of the respiratory tract or its cilia.

Preparations for inhalation are supplied in multidose or single-dose containers. When supplied in pressurised containers, they comply with the requirements of the monograph on *Pressurised pharmaceutical preparations (0523*).

Preparations intended to be administered as aerosols (dispersions of solid or liquid particles in a gas) are administered by one of the following devices:

— nebuliser,

— pressurised metered-dose inhaler,

— powder inhaler.

PRODUCTION

During the development of a preparation for inhalation which contains an antimicrobial preservative, the effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with the criteria for judging the preservative properties of the formulation are described in the text on *Efficacy of antimicrobial preservation (5.1.3)*.

The size of aerosol particles to be inhaled is controlled so that a significant fraction is deposited in the lung. The fine-particle characteristics of preparations for inhalation are determined by the method for *Aerodynamic assessment of fine particles (2.9.18)*.

In assessing the uniformity of delivered dose of a multidose inhaler, it is not sufficient to test a single inhaler. Manufacturers must substitute procedures which take both inter- and intra-inhaler dose uniformity into account. A suitable procedure based on the intra-inhaler test would be to collect each of the specified doses at the beginning, middle and end of the number of doses stated on the label from separate inhalers.

Pressurised metered-dose inhalers are tested for leakage. All inhalers are tested for extraneous particulate contamination.

LABELLING

For metered-dose preparations the label states:

— the delivered dose, except for preparations for which the dose has been established as a metered-dose or as a predispensed-dose,

— where applicable, the number of deliveries from the inhaler to provide the minimum recommended dose,

— the number of deliveries per inhaler.

The label states, where applicable, the name of any added antimicrobial preservative.

LIQUID PREPARATIONS FOR INHALATION

3 categories of liquid preparations for inhalation may be distinguished:

A. preparations intended to be converted into vapour,

B. liquid preparations for nebulisation,

C. pressurised metered-dose preparations for inhalation.

Liquid preparations for inhalation are solutions or dispersions.

Dispersions are readily dispersible on shaking and they remain sufficiently stable to enable the correct dose to be delivered. Suitable excipients may be used.

A. PREPARATIONS INTENDED TO BE CONVERTED INTO VAPOUR

DEFINITION

Preparations intended to be converted into vapour are solutions, dispersions or solid preparations. They are usually added to hot water and the vapour generated is inhaled.

B. LIQUID PREPARATIONS FOR NEBULISATION

DEFINITION

Liquid preparations for inhalation intended to be converted into aerosols by continuously operating nebulisers or metered-dose nebulisers are solutions, suspensions or emulsions. Suitable cosolvents or solubilisers may be used to increase the solubility of the active substances.

Liquid preparations for nebulisation in concentrated form for use in continuously operating nebulisers are diluted to the prescribed volume with the prescribed liquid before use. Liquids for nebulisation may also be prepared from powders.

The pH of the liquid preparations for use in continuously operating nebulisers is not lower than 3 and not higher than 8.5.

Suspensions and emulsions are readily dispersible on shaking and they remain sufficiently stable to enable the correct dose to be delivered.

Aqueous preparations for nebulisation supplied in multidose containers may contain a suitable antimicrobial preservative at a suitable concentration except where the preparation itself has adequate antimicrobial properties.

Continuously operating nebulisers are devices that convert liquids into aerosols by high-pressure gases, ultrasonic vibration or other methods. They allow the dose to be inhaled at an appropriate rate and particle size which ensures deposition of the preparation in the lungs.

Metered-dose nebulisers are devices that convert liquids into aerosols by high-pressure gases, ultrasonic vibration or other methods. The volume of liquid to be nebulised is metered so that the aerosol dose can be inhaled with one breath.

C. PRESSURISED METERED-DOSE PREPARATIONS FOR INHALATION

DEFINITION

Pressurised metered-dose preparations for inhalation are solutions, suspensions or emulsions supplied in containers equipped with a metering valve and which are held under pressure with suitable propellants or suitable mixtures of liquefied propellants, which can act also as solvents. Suitable cosolvents, solubilisers and stabilisers may be added.

The delivered dose is the dose delivered from the inhaler to the patient. For some preparations, the dose has been established as a metered dose. The metered dose is determined by adding the amount deposited on the inhaler to the delivered dose. It may also be determined directly.

TESTS

For breath-operated pressurised metered-dose inhalers, the test conditions described below may need to be modified to ensure that breath actuation occurs for the inhaler under test.

Uniformity of delivered dose

Containers usually operate in a valve-down position. For containers that operate in a valve-up position, an equivalent test is applied using methods that ensure the complete collection of the delivered dose. In all cases, prepare the inhaler as directed in the instructions to the patient.

The dose collection apparatus must be capable of quantitatively capturing the delivered dose.

The following apparatus (Figure 0671.-1) and procedure may be used.

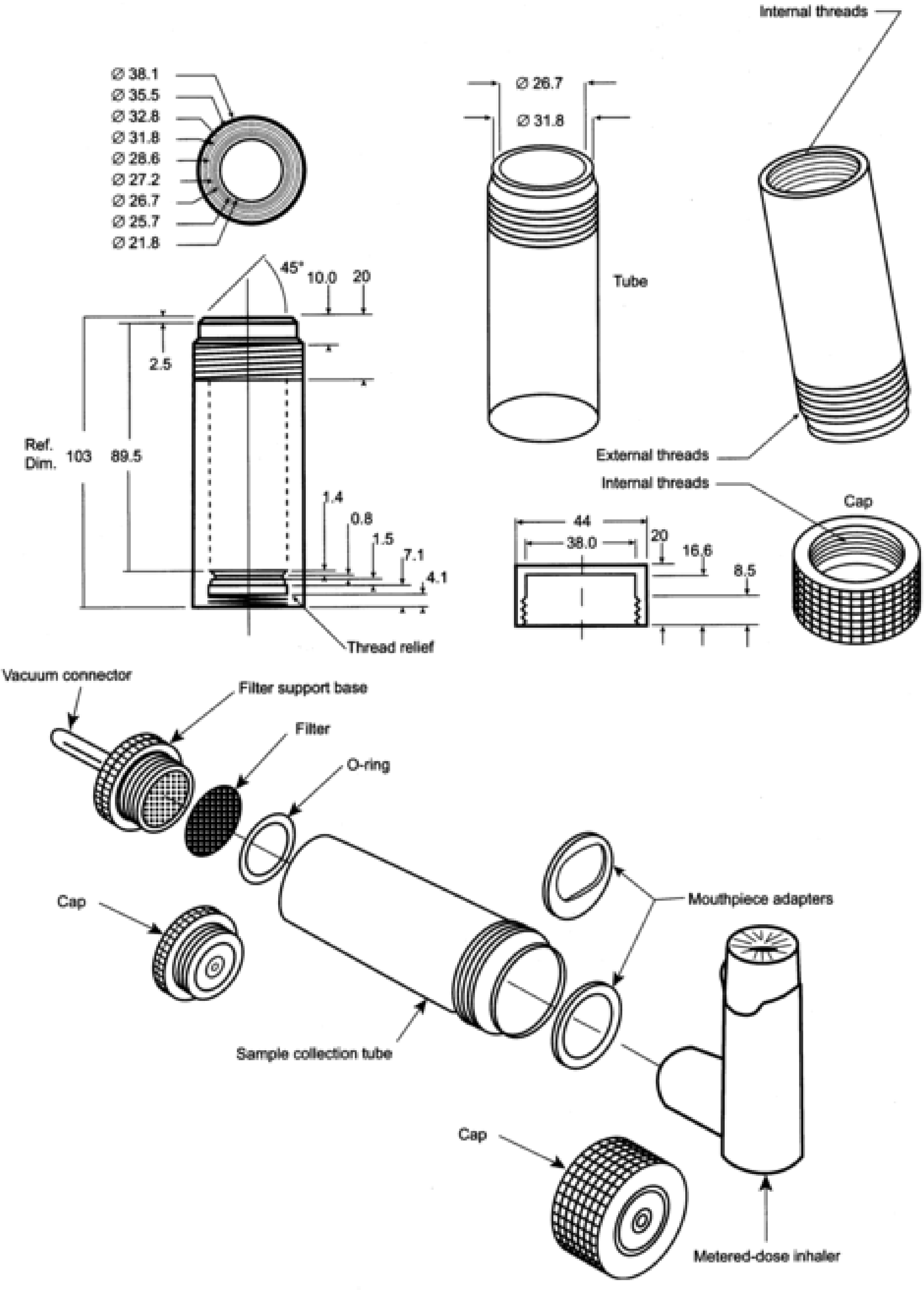

Ø 38.1
Ø 35.5
Ø 32.8
Ø 31.8
Ø 28.6
Ø 27.2
Ø 26.7
Ø 25.7
Ø 21.8
45°
10.0
20
2.5
Ref. Dim.
103
89.5
1.4
0.8
1.5
7.1
4.1
Thread relief
Ø 26.7
Ø 31.8
Tube
Internal threads
External threads
Internal threads
Cap
44
38.0
20
16.6
8.5
Vacuum connector
Filter support base
Filter
O-ring
Cap
Mouthpiece adapters
Sample collection tube
Cap
Metered-dose inhaler

Figure 0671.-1. – *Dose collection apparatus for pressurised metered-dose inhalers*
Dimensions in millimetres

The apparatus consists of a filter-support base with an open-mesh filter-support, such as a stainless steel screen, a collection tube that is clamped or screwed to the filter-support base, and a mouthpiece adapter to ensure an airtight seal between the collection tube and the mouthpiece. Use a mouthpiece adapter which ensures that the front face of the inhaler mouthpiece is flush with the front face or the 2.5 mm indented shoulder of the sample collection tube, as appropriate. The vacuum connector is connected to a system comprising a vacuum source and a flow regulator. The source should be adjusted to draw air through the complete assembly, including the filter and the inhaler to be tested, at 28.3 litres/min (± 5 per cent). Air should be drawn continuously through the apparatus to avoid loss of the active substance into the atmosphere. The filter-support base is designed to accommodate 25 mm diameter filter disks. The filter disk and other materials used in the construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter. One end of the collection tube is designed to hold the filter disk tightly against the filter-support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler.

Unless otherwise prescribed in the instructions to the patient, shake the inhaler for 5 s and discharge one delivery to waste. Fire the inverted inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 2 doses.

Discharge the device to waste, waiting not less than 5 s between actuations until (n/2)+1 deliveries remain, where n is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the device to waste, waiting not less than 5 s between actuations until 3 doses remain. Collect these 3 doses using the procedure described above.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the average value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

Fine particle dose

Using an apparatus and procedure described in *Aerodynamic assessment of fine particles (2.9.18 - Apparatus C, D or E)*, calculate the fine particle dose.

Number of deliveries per inhaler

Take one inhaler and discharge the contents to waste, actuating the valve at intervals of not less than 5 s. The total number of deliveries so discharged from the inhaler is not less than the number stated on the label (this test may be combined with the test for uniformity of delivered dose).

POWDERS FOR INHALATION

DEFINITION

Powders for inhalation are presented as single-dose powders or multidose powders. To facilitate their use, active substances may be combined with a suitable carrier. They are generally administered by powder inhalers. For pre-metered inhalers, the inhaler is loaded with powders pre-dispensed in capsules or other suitable pharmaceutical forms. For inhalers using a powder reservoir, the dose is created by a metering mechanism within the inhaler.

The delivered dose is the dose delivered from the inhaler. For some preparations, the dose has been established as a metered dose or as a predispensed dose. The metered dose is determined by adding the amount deposited on the inhaler to the delivered dose. It may also be determined directly.

TESTS

Uniformity of delivered dose

In all cases, prepare the inhaler as directed in the instructions to the patient. The dose collection apparatus must be capable of quantitatively capturing the delivered dose. A dose collection apparatus similar to that described for the evaluation of pressurised metered-dose inhalers may be used provided that the dimensions of the tube and the filter can accommodate the measured flow rate. A suitable tube is defined in Table 0671.-1. Connect the tube to a flow system according to the scheme specified in Figure 0671.-2 and Table 0671.-1.

Unless otherwise stated, determine the test flow rate and duration using the dose collection tube, the associated flow system, a suitable differential pressure meter and a suitable volumetric flowmeter, calibrated for the flow leaving the meter, according to the following procedure.

Prepare the inhaler for use and connect it to the inlet of the apparatus using a mouthpiece adapter to ensure an airtight seal. Use a mouthpiece adapter which ensures that the front face of the inhaler mouthpiece is flush with the front face of the sample collection tube. Connect one port of a differential pressure meter to the pressure reading point, P1, in Figure 0671.-2 and let the other be open to the atmosphere. Switch on the pump, open the 2-way solenoid valve and adjust the flow control valve until the pressure drop across the inhaler is 4.0 kPa (40.8 cm H_2O) as indicated by the differential pressure meter. Remove the inhaler from the mouthpiece adapter and without touching the flow control valve, connect a flowmeter to the inlet of the sampling apparatus. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

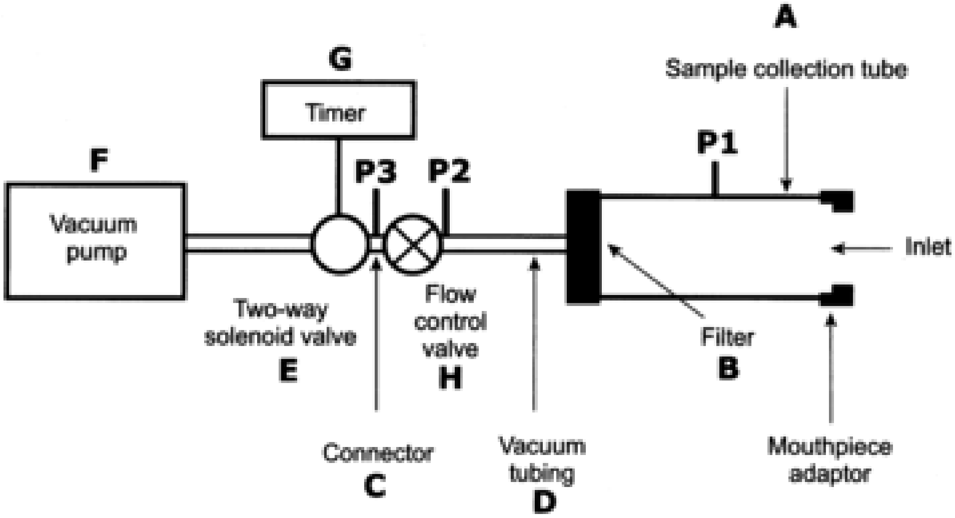

A
Sample collection tube
G
Timer
F
Vacuum pump
P3
P2
P1
Inlet
Two-way solenoid valve
E
Flow control valve
H
Filter
B
Connector
C
Vacuum tubing
D
Mouthpiece adaptor

Figure 0671.-2. – ***Apparatus suitable for measuring the uniformity of delivered dose for powder inhalers***

P_0 = atmospheric pressure,

ΔP = pressure drop over the meter.

If the flow rate is above 100 litres/min adjust the flow control valve to obtain a flow rate of 100 litres/min (± 5 per cent). Note the volumetric airflow rate exiting the meter and define this as the test flow rate, Q_{out}, in litres per minute. Define the test flow duration, T, in seconds so that a volume of 4 litres of air is drawn from the mouthpiece of the inhaler at the test flow rate, Q_{out}.

Ensure that critical flow occurs in the flow control valve by the following procedure; with the inhaler in place and the test flow rate Q_{out}, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Figure 0671.-2). A ratio P3/P2 of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

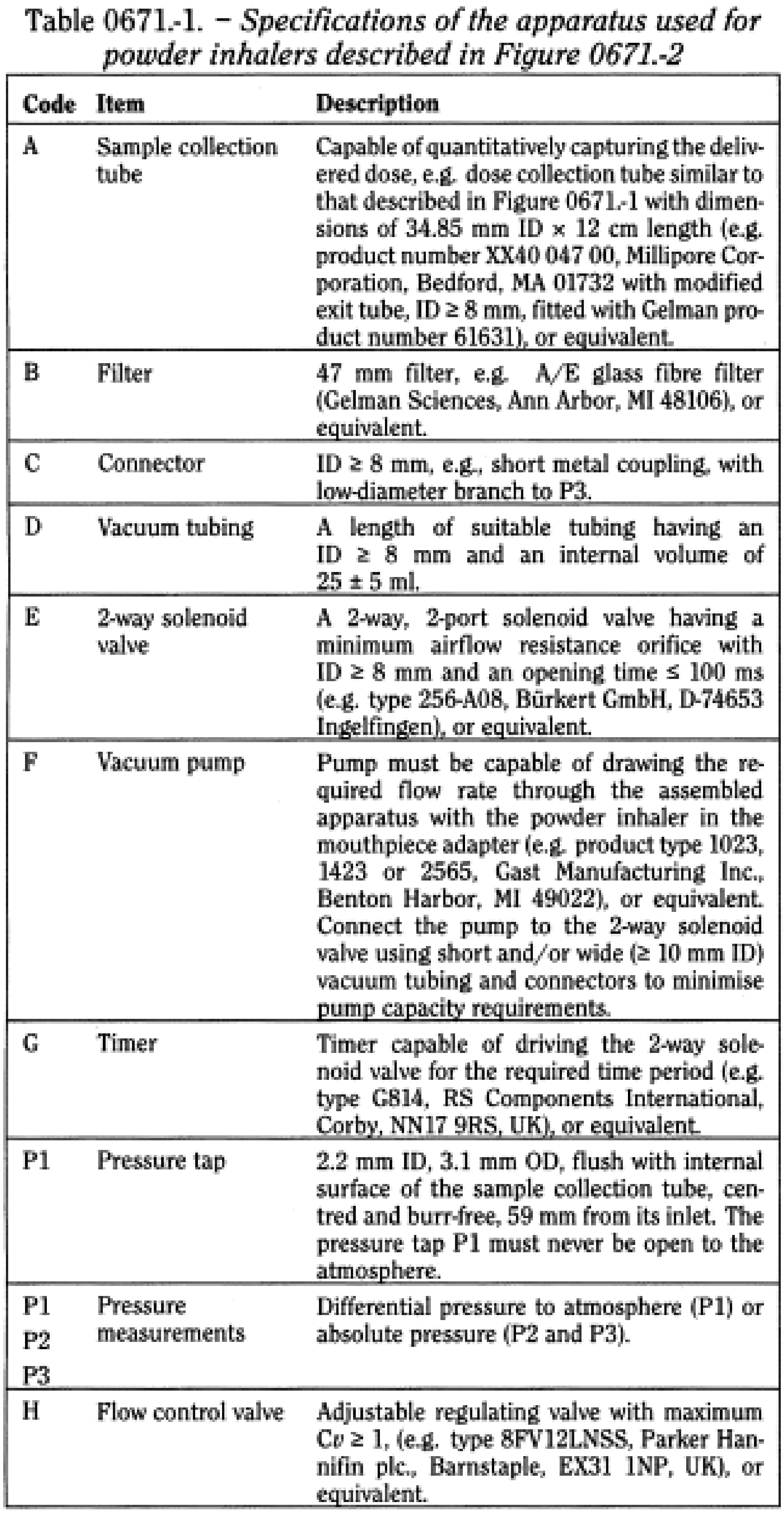


Table 0671.-1. – *Specifications of the apparatus used for powder inhalers described in Figure 0671.-2*

Code	Item	Description
A	Sample collection tube	Capable of quantitatively capturing the delivered dose, e.g. dose collection tube similar to that described in Figure 0671.-1 with dimensions of 34.85 mm ID × 12 cm length (e.g. product number XX40 047 00, Millipore Corporation, Bedford, MA 01732 with modified exit tube, ID ≥ 8 mm, fitted with Gelman product number 61631), or equivalent.
B	Filter	47 mm filter, e.g. A/E glass fibre filter (Gelman Sciences, Ann Arbor, MI 48106), or equivalent.
C	Connector	ID ≥ 8 mm, e.g., short metal coupling, with low-diameter branch to P3.
D	Vacuum tubing	A length of suitable tubing having an ID ≥ 8 mm and an internal volume of 25 ± 5 ml.
E	2-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with ID ≥ 8 mm and an opening time ≤ 100 ms (e.g. type 256-A08, Bürkert GmbH, D-74653 Ingelfingen), or equivalent.
F	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the powder inhaler in the mouthpiece adapter (e.g. product type 1023, 1423 or 2565, Gast Manufacturing Inc., Benton Harbor, MI 49022), or equivalent. Connect the pump to the 2-way solenoid valve using short and/or wide (≥ 10 mm ID) vacuum tubing and connectors to minimise pump capacity requirements.
G	Timer	Timer capable of driving the 2-way solenoid valve for the required time period (e.g. type G814, RS Components International, Corby, NN17 9RS, UK), or equivalent.
P1	Pressure tap	2.2 mm ID, 3.1 mm OD, flush with internal surface of the sample collection tube, centred and burr-free, 59 mm from its inlet. The pressure tap P1 must never be open to the atmosphere.
P1 P2 P3	Pressure measurements	Differential pressure to atmosphere (P1) or absolute pressure (P2 and P3).
H	Flow control valve	Adjustable regulating valve with maximum C*v* ≥ 1, (e.g. type 8FV12LNSS, Parker Hannifin plc., Barnstaple, EX31 1NP, UK), or equivalent.

Predispensed systems Prepare the inhaler as directed in the instructions to the patient and connect it to the apparatus using an adapter which ensures a good seal. Draw air through the inhaler using the predetermined conditions. Repeat the procedure until the number of deliveries which constitute the minimum recommended dose have been sampled.

Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 9 doses.

Reservoir systems Prepare the inhaler as directed in the instructions to the patient and connect it to the apparatus using an adapter which ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries which constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 2 doses.

Discharge the device to waste until (*n*/2)+1 deliveries remain, where n is the number of deliveries stated on the label. If necessary, store the inhaler to discharge electrostatic charges. Collect 4 doses using the procedure described above.

Discharge the device to waste until 3 doses remain. If necessary, store the inhaler to discharge electrostatic charges. Collect 3 doses using the procedure described above.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

Results The preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the average value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

In justified and authorised cases, these ranges may be extended but no value should be greater than 150 per cent or less than 50 per cent of the average value.

Fine particle dose

Using the apparatus and procedure described in *Aerodynamic assessment of fine particles (2.9.18 - Apparatus C, D or E)*, calculate the fine particle dose.

Number of deliveries per inhaler for multidose inhalers

Discharge doses from the inhaler until empty, at the predetermined flow rate. Record the deliveries discharged. The total number of doses delivered is not less than the number stated on the label (this test may be combined with the test for uniformity of delivered dose).

Ph Eur

Preparations for Irrigation of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those irrigation solutions that are the subject of an individual monograph in the British Pharmacopoeia.

Water for Irrigation

When the term 'Water for Irrigation' is used within the individual monographs of the British Pharmacopoeia, it has the meaning defined in the requirements of the European Pharmacopoeia above.

IRRIGATION SOLUTIONS

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) the date after which the Irrigation Solution is not intended to be used; (3) the conditions under which the irrigation solution should be stored.

PREPARATIONS FOR IRRIGATION

General Notices

(Ph Eur monograph 1116)

Preparations for Irrigation comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Preparations for irrigation are sterile, aqueous, large-volume preparations intended to be used for irrigation of body cavities, wounds and surfaces, for example during surgical procedures.

Preparations for irrigation are either solutions prepared by dissolving one or more active substances, electrolytes or osmotically active substances in water complying with the requirements for *Water for injections (0169)* or they consist of such water alone. In the latter case, the preparation may be labelled as 'water for irrigation'. Irrigation solutions are usually adjusted to make the preparation isotonic with respect to blood.

Examined in suitable conditions of visibility, preparations for irrigation are clear and practically free from particles.

Preparations for irrigation are supplied in single-dose containers. The containers and closures comply with the requirements for containers for preparations for parenteral use (*3.2.1* and *3.2.2*), but the administration port of the container is incompatible with intravenous administration equipment and does not allow the preparation for irrigation to be administered with such equipment.

PRODUCTION

Preparations for irrigation are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products* (*5.1.1*).

During development, it must be demonstrated that the nominal content can be withdrawn from the container.

TESTS

Sterility (*2.6.1*)

Preparations for irrigation comply with the test for sterility.

Bacterial endotoxins (*2.6.14*)

Less than 0.5 IU/ml.

Pyrogens (*2.6.8*)

Preparations for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 ml of the preparation, unless otherwise justified and authorised.

LABELLING

The label states:

— that the preparation is not to be used for injection;

— that the preparation is to be used on one occasion only and that any unused portion of the preparation is to be discarded.

Ph Eur

PRESSURISED PHARMACEUTICAL PREPARATIONS

General Notices

(Ph Eur monograph 0523)

Pressurised Pharmaceutical Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Additional requirements for preparations presented in pressurised containers may be found, where appropriate, in other general monographs, for example Preparations for inhalation (0671), Liquid preparations for cutaneous application (0927), Powders for cutaneous application (1166), Nasal preparations (0676) and Ear preparations (0652).

DEFINITION

Pressurised pharmaceutical preparations are presented in special containers under pressure of a gas and contain one or more active substances. The preparations are released from the container, upon actuation of an appropriate valve, in the form of an aerosol (dispersion of solid or liquid particles in a gas, the size of the particles being adapted to the intended use) or of a liquid or semisolid jet such as a foam. The pressure for the release is generated by suitable propellants.

The preparations consist of a solution, an emulsion or a suspension and are intended for local application to the skin or to mucous membranes of various body orifices, or for inhalation. Suitable excipients may also be used, for example solvents, solubilisers, emulsifying agents, suspending agents and lubricants for the valve to prevent clogging.

Propellants The propellants are either gases liquefied under pressure or compressed gases or low-boiling liquids. Liquefied gases are, for example, fluorinated hydrocarbons and low-molecular-mass hydrocarbons (such as propane and butane). Compressed gases are, for example, carbon dioxide, nitrogen and nitrous oxide.

Mixtures of these propellants may be used to obtain optimal solution properties and desirable pressure, delivery and spray characteristics.

Containers The containers are tight and resistant to the internal pressure and may be made of metal, glass, plastic or combinations of these materials. They are compatible with their contents. Glass containers are protected with a plastic coating.

Spraying device The valve keeps the container tightly closed when not in use and regulates the delivery of the contents during use. The spray characteristics are influenced by the type of spraying device, in particular by the dimensions, number and location of orifices. Some valves provide a continuous release, others ("metering dose valves") deliver a defined quantity of product upon each valve actuation.

The various valve materials in contact with the contents are compatible with them.

Requirements for pressurised pharmaceutical preparations

Pressurised preparations are provided with a delivery device appropriate for the intended application.

Special requirements may be necessary for the selection of propellants, for particle size and the single-dose delivered by the metering valves.

LABELLING

The label states:

— the method of use,

— any precautions to be taken,

— for a container with a metering dose valve, the amount of active substance in a unit-spray.

Ph Eur

Rectal Preparations of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any enema, rectal solution or suppositories that is the subject of an individual monograph in the British Pharmacopoeia.

ENEMAS

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) that the preparation should not be swallowed; (3) the date after which the Enema is not intended to be used; (4) the conditions under which the Enema should be stored; (5) the directions for using the Enema.

RECTAL SOLUTIONS

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) that the preparation should not be swallowed; (3) the date after which the Rectal Solution is not intended to be used; (4) the conditions under which the Rectal Solution should be stored; (5) the directions for using the Rectal Solution.

SUPPOSITORIES

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) that the preparation should not be swallowed; (3) the date after which the Suppositories are not intended to be used; (4) the conditions under which the Suppositories should be stored.

RECTAL PREPARATIONS

General Notices

(Ph Eur monograph 1145)

Rectal Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Rectal preparations are intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostic purposes.

Where applicable, containers for rectal preparations comply with the requirements for materials used for the manufacture of containers *(3.1 and subsections)* and containers *(3.2 and subsections)*.

Several categories of rectal preparations may be distinguished:

— suppositories;

— rectal capsules;

— rectal solutions, emulsions and suspensions;

— powders and tablets for rectal solutions and suspensions;

— semi-solid rectal preparations;

— rectal foams;

— rectal tampons.

PRODUCTION

During the development of a rectal preparation whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in chapter *5.1.3 Efficacy of antimicrobial preservation.*

During development, it must be demonstrated that the nominal contents can be withdrawn from the container of liquid and semi-solid rectal preparations presented in single-dose containers.

In the manufacture, packaging, storage and distribution of rectal preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in chapter *5.1.4 Microbiological quality of pharmaceutical preparations.*

In the manufacture of semi-solid and liquid rectal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units *(2.9.40)*

Liquid and semi-solid single-dose rectal preparations comply with the test. Solid single-dose rectal preparations comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, solid single-dose rectal preparations with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A (tablets) or test B (suppositories, rectal capsules). If the preparation contains more than one active substance, this requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Solid single-dose rectal preparations comply with the test. If the test for uniformity of content is prescribed for all active substances, the test for uniformity of mass is not required.

Dissolution

A suitable test may be required to demonstrate the appropriate release of the active substance(s) from solid single-dose rectal preparations, for example *2.9.42 Dissolution test for lipophilic solid dosage forms.*

Where a dissolution test is prescribed, a disintegration test may not be required.

LABELLING

The label states the name of any added antimicrobial preservative.

SUPPOSITORIES

DEFINITION

Suppositories are solid, single-dose preparations. The shape, volume and consistency of suppositories are suitable for rectal administration.

They contain 1 or more active substances dispersed or dissolved in a suitable basis that may be soluble or dispersible in water or may melt at body temperature. Excipients such as diluents, adsorbents, surface-active agents, lubricants, antimicrobial preservatives and colouring matter, authorised by the competent authority, may be added if necessary.

PRODUCTION

Suppositories are prepared by compression or moulding. If necessary, the active substance (s) are previously ground and sieved through a suitable sieve. When prepared by moulding, the medicated mass, sufficiently liquefied by heating, is poured into suitable moulds. The suppository solidifies on cooling. Various excipients are available for this process, such as hard fat, macrogols, cocoa butter, and various gelatinous mixtures consisting of, for example, gelatin, water and glycerol. The determination of the softening time of lipophilic suppositories *(2.9.22)* is carried out.

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from suppositories intended for modified release or for prolonged local action.

In the manufacture of suppositories containing dispersed active substances, measures are taken to ensure a suitable and controlled particle size.

TESTS

Disintegration *(2.9.2)*

Unless intended for modified release or for prolonged local action, they comply with the test. For suppositories with a fatty base, examine after 30 min, and for suppositories with a water-soluble base, examine after 60 min, unless otherwise justified and authorised.

RECTAL CAPSULES

DEFINITION

Rectal capsules (shell suppositories) are solid, single-dose preparations generally similar to soft capsules as defined in the monograph *Capsules (0016)* except that they may have lubricating coatings. They are of elongated shape, are smooth and have a uniform external appearance.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from rectal capsules intended for modified release or for prolonged local action.

TESTS

Disintegration *(2.9.2)*

Unless intended for modified release or for prolonged local action, they comply with the test. Examine the state of the capsules after 30 min, unless otherwise justified and authorised.

RECTAL SOLUTIONS, EMULSIONS AND SUSPENSIONS

DEFINITION

Rectal solutions, emulsions and suspensions are liquid preparations intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostic purposes.

Rectal solutions, emulsions and suspensions are supplied in single-dose containers and contain 1 or more active substances dissolved or dispersed in water, glycerol or macrogols or other suitable solvents. Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment that is readily dispersible on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Rectal solutions, emulsions and suspensions may contain excipients, for example to adjust the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance(s) or to stabilise the preparation. These substances do not adversely affect the intended medical action or, at the concentrations used, cause undue local irritation.

Rectal solutions, emulsions and suspensions are supplied in containers containing a volume in the range of 2.5 ml to 2000 ml. The container is adapted to deliver the preparation to the rectum or is accompanied by a suitable applicator.

POWDERS AND TABLETS FOR RECTAL SOLUTIONS AND SUSPENSIONS

DEFINITION

Powders and tablets intended for the preparation of rectal solutions or suspensions are single-dose preparations that are dissolved or dispersed in water or other suitable solvents at the time of administration. They may contain excipients to facilitate dissolution or dispersion or to prevent aggregation of the particles.

After dissolution or suspension, they comply with the requirements for rectal solutions or rectal suspensions, as appropriate.

TESTS

Disintegration *(2.9.1)*

Tablets for rectal solutions or suspensions comply with the test, but using *water R* at 15-25 °C and 6 tablets. Examine the state of the tablets after 3 min. The tablets comply with the test if all 6 have disintegrated.

LABELLING

The label states:

- — the method of preparation of the rectal solution or suspension;
- — the conditions and duration of storage of the solution or suspension after constitution.

SEMI-SOLID RECTAL PREPARATIONS

DEFINITION

Semi-solid rectal preparations are ointments, creams or gels.

They are often supplied as single-dose preparations in containers provided with a suitable applicator.

Semi-solid rectal preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132).*

RECTAL FOAMS

DEFINITION

Rectal foams comply with the requirements of the monograph *Medicated foams (1105)*.

RECTAL TAMPONS

DEFINITION

Rectal tampons are solid, single-dose preparations intended to be inserted into the lower part of the rectum for a limited time.

They comply with the requirements of the monograph *Medicated tampons (1155).*

Ph Eur

SPIRITS

General Notices

DEFINITION

Spirits are solutions of one or more substances in Ethanol (96 per cent) or a Dilute Ethanol. They may contain a proportion of Water.

STORAGE

Spirits should be kept in well-closed containers of glass or other suitable materials.

LABELLING

The label states (1) the date after which the Spirit is not intended to be used; (2) the conditions under which the Spirit should be stored.

STICKS

General Notices

(Ph Eur monograph 1154)

Sticks comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

Additional requirements for sticks may be found, where appropriate, in other general monographs, for example Nasal preparations (0676).

DEFINITION

Sticks are solid preparations intended for local application. They are rod-shaped or conical preparations consisting of one or more active substances alone or which are dissolved or dispersed in a suitable basis which may dissolve or melt at body temperature.

Urethral sticks and sticks for insertion into wounds are sterile.

PRODUCTION

In the manufacture, packaging, storage and distribution of sticks, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations* (*5.1.4*).

Urethral sticks and other sterile sticks are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products* (*5.1.1*).

In the manufacture of sticks means are taken to ensure that the preparation complies with a test for mass uniformity or, where appropriate, a test for uniformity of content.

TESTS

Sterility (*2.6.1*)

Urethral sticks and sticks for insertion into wounds comply with the test for sterility.

LABELLING

The label states:

— the quantity of active substance(s) per stick,

— for urethral sticks and sticks to be inserted into wounds that they are sterile.

Ph Eur

Tablets of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those tablets that are the subject of an individual monograph in the British Pharmacopoeia.

DEFINITION

Tablets of the British Pharmacopoeia may contain flavouring only when indicated in the individual monograph.

Tablets that are the subject of individual monographs in the British Pharmacopoeia may be uncoated, compression-coated, film-coated or sugar-coated unless otherwise indicated in the monograph. Tablets for which the monograph states 'They are coated' are compression-coated or film-coated or sugar-coated. Tablets may be made gastro-resistant by enteric-coating or by other means only where this is specifically indicated in the monograph.

When presented as coated formulations, where justified and authorised, it may be necessary to remove the coating before performing a test described in a monograph. Removal of a coating is not permitted where it affects the functionality of the product, for example in dissolution or disintegration tests.

In preparing Tablets for which Chocolate Basis is specified, the active ingredient may be incorporated with a mixture of 15 parts of non-alkalinised cocoa powder of commerce, 15 parts of Sucrose and 70 parts of Lactose.

Content of active ingredient of tablets

The range for the content of active ingredient stated in the monograph is based on the requirement that 20 tablets, or such other number as may be indicated in the monograph, are used in the Assay. In circumstances where 20 tablets cannot be obtained, a smaller number, which must not be less than five, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table I.

The requirements of Table I apply when the stated limits are 90 to 110%. For limits other than 90 to 110%, proportionately smaller or larger allowances should be made.

Table I

Weight of active ingredients in each tablet	Subtract from the lower limit for samples of			Add to the upper limit for samples of		
	15	10	5	15	10	5
0.12 g or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 g and less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5

0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

Disintegration

Comply with the *disintegration test for tablets and capsules,* Appendix XII A1, unless otherwise stated in the individual monographs.

For those Uncoated or Coated Tablets for which a requirement for Dissolution is included in the individual monograph, the requirement for Disintegration does not apply.

Uniformity of content

Details of the analytical method to be employed for determining the content of active ingredient may be included in certain monographs. Unless otherwise stated in the monograph the limits are as given in test A for *Uniformity of content*, Appendix XII C3.

Any tablets that when examined individually show a gross deviation from the prescribed or stated content are not official.

LABELLING

The label states (1) the quantity of the active ingredient contained in each tablet; (2) 'enteric-coated' for tablets coated in this manner; (3) the date after which the Tablets are not intended to be used; (4) the conditions under which the Tablets should be stored.

TABLETS

General Notices

(Ph Eur monograph 0478)

Tablets comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

The requirements of this monograph do not necessarily apply to preparations that are presented as tablets intended for use other than by oral administration. Requirements for such preparations may be found, where appropriate, in other general monographs; for example Rectal preparations (1145), Vaginal preparations (1164) and Oromucosal preparations (1807). This monograph does not apply to lozenges, oral pastes and oral gums. Where justified and authorised, the requirements of this monograph do not apply to tablets for veterinary use.

DEFINITION

Tablets are solid preparations each containing a single dose of one or more active substances. They are obtained by compressing uniform volumes of particles or by another suitable manufacturing technique, such as extrusion, moulding or freeze-drying (lyophilisation). Tablets are intended for oral administration. Some are swallowed whole, some after being chewed, some are dissolved or dispersed in water before being administered and some are retained in the mouth where the active substance is liberated.

The particles consist of one or more active substances with or without excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the preparation in the digestive tract, colouring matter authorised by the competent authority and flavouring substances.

Tablets are usually straight, circular solid cylinders, the end surfaces of which are flat or convex and the edges of which may be bevelled. They may have break-marks and may bear a symbol or other markings. Tablets may be coated.

Where applicable, containers for tablets comply with the requirements for materials used for the manufacture of containers *(3.1 and subsections)* and containers *(3.2 and subsections).*

Several categories of tablets for oral use may be distinguished:

- — uncoated tablets;
- — coated tablets;
- — effervescent tablets;
- — soluble tablets;
- — dispersible tablets;
- — orodispersible tablets;

— gastro-resistant tablets;

— modified-release tablets;

— tablets for use in the mouth;

— oral lyophilisates.

PRODUCTION

Tablets are usually prepared by compressing uniform volumes of particles or particle aggregates produced by granulation methods. In the manufacture of tablets, means are taken to ensure that they possess a suitable mechanical strength to avoid crumbling or breaking on handling or subsequent processing. This may be demonstrated using the tests described in chapters *2.9.7*. *Friability of uncoated tablets* and *2.9.8*. *Resistance to crushing of tablets*. Chewable tablets are prepared to ensure that they are easily crushed by chewing.

Subdivision of tablets Tablets may bear a break-mark or break-marks and may be subdivided in parts, either to ease the intake of the medicinal product or to comply with the posology. In the latter case, subdivision must be assessed and authorised by the competent authority. In order to ensure that the patient will receive the intended dose, the efficacy of the break-mark(s) must be assessed during the development of the product, in respect of uniformity of mass of the subdivided parts. Each authorised dose must be tested using the following test.

Take 30 tablets at random, break them by hand and, from all the parts obtained from 1 tablet, take 1 part for the test and reject the other part(s). Weigh each of the 30 parts individually and calculate the average mass. The tablets comply with the test if not more than 1 individual mass is outside the limits of 85 per cent to 115 per cent of the average mass. The tablets fail to comply with the test if more than 1 individual mass is outside these limits, or if 1 individual mass is outside the limits of 75 per cent to 125 per cent of the average mass.

In the manufacture, packaging, storage and distribution of tablets, suitable means are taken to ensure their microbiological quality; recommendations on this aspect are provided in chapter *5.1.4*. *Microbiological quality of pharmaceutical preparations*.

TESTS

Uniformity of dosage units *(2.9.40)*

Tablets comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, tablets with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A. If the preparation has more than 1 active substance, the requirement applies only to those substances that correspond to the above conditions.

Unless otherwise justified and authorised, coated tablets other than film-coated tablets comply with test A irrespective of their content of active substance(s).

Uniformity of mass *(2.9.5)*

Uncoated tablets and, unless otherwise justified and authorised, film-coated tablets comply with the test. If the test for uniformity of content is prescribed or justified and authorised for all the active substances, the test for uniformity of mass is not required.

Dissolution

A suitable test may be carried out to demonstrate the appropriate release of the active substance(s), for example one of the tests described in chapter *2.9.3. Dissolution test for solid dosage forms.*

Where a dissolution test is prescribed, a disintegration test may not be required.

UNCOATED TABLETS

DEFINITION

Uncoated tablets include single-layer tablets resulting from a single compression of particles and multi-layer tablets consisting of concentric or parallel layers obtained by successive compression of particles of different composition. The excipients used are not specifically intended to modify the release of the active substance in the digestive fluids.

Uncoated tablets conform to the general definition of tablets. A broken section, when examined under a lens, shows either a relatively uniform texture (single-layer tablets) or a stratified texture (multi-layer tablets) but no signs of coating.

TESTS

Disintegration *(2.9.1)*

Uncoated tablets comply with the test. Use *water R* as the liquid. Add a disc to each tube. Operate the apparatus for 15 min, unless otherwise justified and authorised, and examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets omitting the discs.

Chewable tablets are not required to comply with the test.

COATED TABLETS

DEFINITION

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as natural or synthetic resins, gums, gelatin, inactive and insoluble fillers, sugars, plasticisers, polyols, waxes, colouring matter authorised by the competent authority and sometimes flavouring substances and active substances. The substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. When the coating is a very thin polymeric coating, the tablets are known as film-coated tablets.

Coated tablets have a smooth surface, which is often coloured and may be polished; a broken section, when examined under a lens, shows a core surrounded by one or more continuous layers with a different texture.

PRODUCTION

Where justified, uniformity of mass or uniformity of content of coated tablets other than film-coated tablets may be ensured by control of the cores.

TESTS

Disintegration *(2.9.1)*

Coated tablets other than film-coated tablets comply with the test. Use *water R* as the liquid. Add a disc to each tube. Operate the apparatus for 60 min, unless otherwise justified and authorised, and examine the state of the tablets. If any of the tablets has not disintegrated, repeat the test on a further 6 tablets, replacing *water R* with *0.1 M hydrochloric acid*.

Film-coated tablets comply with the disintegration test prescribed above except that the apparatus is operated for 30 min, unless otherwise justified and authorised.

If coated tablets or film-coated tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets omitting the discs.

Chewable coated tablets are not required to comply with the test.

EFFERVESCENT TABLETS

DEFINITION

Effervescent tablets are uncoated tablets generally containing acid substances and carbonates or hydrogen carbonates, which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

TESTS

Disintegration

Place 1 tablet in a beaker containing 200 ml of *water R* at 15-25 °C; numerous bubbles of gas are evolved. When the evolution of gas around the tablet or its fragments ceases the tablet has disintegrated, being either dissolved or dispersed in the water so that no agglomerates of particles remain. Repeat the operation on 5 other tablets. The tablets comply with the test if each of the 6 tablets used disintegrates in the manner prescribed within 5 min, unless otherwise justified and authorised.

SOLUBLE TABLETS

DEFINITION

Soluble tablets are uncoated or film-coated tablets. They are intended to be dissolved in water before administration. The solution produced may be slightly opalescent due to the added excipients used in the manufacture of the tablets.

TESTS

Disintegration *(2.9.1)*

Soluble tablets disintegrate within 3 min, using *water R* at 15-25 °C.

DISPERSIBLE TABLETS

DEFINITION

Dispersible tablets are uncoated or film-coated tablets intended to be dispersed in water before administration, giving a homogeneous dispersion.

TESTS

Disintegration *(2.9.1)*

Dispersible tablets disintegrate within 3 min, using *water R* at 15-25 °C.

Fineness of dispersion

Place 2 tablets in 100 ml of *water R* and stir until completely dispersed. A smooth dispersion is produced, which passes through a sieve screen with a nominal mesh aperture of 710 µm.

ORODISPERSIBLE TABLETS

DEFINITION

Orodispersible tablets are uncoated tablets intended to be placed in the mouth where they disperse rapidly before being swallowed.

TESTS

Disintegration *(2.9.1)*

Orodispersible tablets disintegrate within 3 min.

MODIFIED-RELEASE TABLETS

DEFINITION

Modified-release tablets are coated or uncoated tablets that contain special excipients or are prepared by special procedures, or both, designed to modify the rate, the place or the time at which the active substance(s) are released.

Modified-release tablets include prolonged-release tablets, delayed-release tablets and pulsatile-release tablets.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) .

GASTRO-RESISTANT TABLETS

DEFINITION

Gastro-resistant tablets are delayed-release tablets that are intended to resist the gastric fluid and to release their active substance(s) in the intestinal fluid. Usually they are prepared from granules or particles already covered with a gastro-resistant coating or in certain cases by covering tablets with a gastro-resistant coating (enteric-coated tablets).

Tablets covered with a gastro-resistant coating conform to the definition of coated tablets.

PRODUCTION

For tablets prepared from granules or particles already covered with a gastro-resistant coating, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

TESTS

Disintegration *(2.9.1)*

For tablets covered with a gastro-resistant coating, carry out the test with the following modifications. Use *0.1 M hydrochloric acid* as the liquid. Operate the apparatus for 2 h, or another such time as may be justified and authorised, without the discs and examine the state of the tablets. The time of resistance to the acid medium varies according to the formulation of the tablets to be examined. It is typically 2 h to 3 h but even with authorised deviations is not less than 1 h. No tablet shows signs of either disintegration (apart from fragments of coating) or cracks that would allow the escape of the contents. Replace the acid by *phosphate buffer solution pH 6.8 R* and add a disc to each tube. Operate the apparatus for 60 min and examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets omitting the discs.

Dissolution

For tablets prepared from granules or particles already covered with a gastro-resistant coating, a suitable test is carried out to demonstrate the appropriate release of the active substance(s), for example the test described in chapter *2.9.3*. *Dissolution test for solid dosage forms.*

TABLETS FOR USE IN THE MOUTH

DEFINITION

Tablets for use in the mouth are usually uncoated tablets. They are formulated to effect a slow release and local action of the active substance(s) or the release and absorption of the active substance(s) at a defined part of the mouth. They comply with the requirements of the monograph *Oromucosal preparations (1807).*

ORAL LYOPHILISATES

DEFINITION

Oral lyophilisates are solid preparations intended either to be placed in the mouth or to be dispersed (or dissolved) in water before administration.

PRODUCTION

Oral lyophilisates are obtained by freeze-drying (lyophilisation), involving division into single doses, freezing, sublimation and drying of usually aqueous, liquid or semi-solid preparations.

TESTS

Disintegration

Place 1 oral lyophilisate in a beaker containing 200 ml of *water R* at 15-25 °C. It disintegrates within 3 min. Repeat the test on 5 other oral lyophilisates. They comply with the test if all 6 have disintegrated.

Water *(2.5.12)*

Oral lyophilisates comply with the test; the limits are approved by the competent authority.

Ph Eur

Topical Powders of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those dusting powders that are the subject of an individual monograph in the British Pharmacopoeia.

DUSTING POWDERS

DEFINITION

Dusting Powders are finely divided powders that are intended to be applied to the skin for therapeutic, prophylactic or lubricant purposes.

STORAGE

Dusting Powders should be stored in a dry place.

LABELLING

The label states (1) the date after which the Dusting Powder is not intended to be used; (2) the conditions under which the Dusting Powder should be stored.

TOPICAL POWDERS

General Notices

POWDERS FOR CUTANEOUS APPLICATION

(Ph Eur monograph 1166)

Topical Powders comply with the requirements of the European Pharmacopoeia monograph for Powders for Cutaneous Application. These requirements are reproduced below.

Ph Eur

Where justified and authorised, the requirements of this monograph do not apply to powders for cutaneous application intended for veterinary use.

DEFINITION

Powders for cutaneous application are preparations consisting of solid, loose, dry particles of varying degrees of fineness. They contain one or more active substances, with or without excipients and, if necessary, colouring matter authorised by the competent authority.

Powders for cutaneous application are presented as single-dose powders or multidose powders. They are free from grittiness. Powders specifically intended for use on large open wounds or on severely injured skin are sterile.

Multidose powders for cutaneous application may be dispensed in sifter-top containers, containers equipped with a mechanical spraying device or in pressurised containers.

Powders dispensed in pressurised containers comply with the requirements of *Pressurised pharmaceutical preparations (0523).*

Where applicable, containers for powders comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

PRODUCTION

In the manufacture of powders for cutaneous application, measures are taken to ensure a suitable particle size with regard to the intended use.

In the manufacture, packaging, storage and distribution of powders for cutaneous application, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations* (5.1.4).

Sterile powders for cutaneous application are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products* (*5.1.1*).

TESTS

Fineness

If prescribed, the fineness of a powder is determined by the sieve test (*2.9.12*) or another appropriate method.

Uniformity of dosage units

Single-dose powders for cutaneous application comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*)

Unless otherwise prescribed or justified and authorised, single-dose powders for cutaneous application with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances which correspond to the above conditions.

Uniformity of mass (*2.9.5*)

Single-dose powders for cutaneous application comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Sterility (*2.6.1*)

Where the label indicates that the preparation is sterile, it complies with the test for sterility.

LABELLING

The label states:

— that the preparation is for external use,

— where applicable, that the preparation is sterile.

Ph Eur

Topical Semi-solid Preparations of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to any cream, gel, ointment, paste or poultice that is the subject of an individual monograph in the British Pharmacopoeia. Eye ointments are described in the monograph on Eye Preparations.

CREAMS

DEFINITION

Creams are formulated to provide preparations that are essentially miscible with the skin secretion. They are intended to be applied to the skin or certain mucous membranes for protective, therapeutic or prophylactic purposes especially where an occlusive effect is not necessary.

Creams should not normally be diluted. However, should dilution be necessary care should be taken, in particular, to prevent microbial contamination. The appropriate diluent should be used and heating should be avoided during mixing. Excessive dilution may affect the stability of some creams. If diluted, creams should normally be used within two weeks of their preparation.

STORAGE

Creams should not be allowed to freeze.

LABELLING

The label states (1) the date after which the Cream is not intended to be used; (2) the conditions under which the Cream should be stored.

GELS

STORAGE

Gels should not be allowed to freeze.

LABELLING

The label states (1) the date after which the Gel is not intended to be used; (2) the conditions under which the Gel should be stored.

OINTMENTS

DEFINITION

Ointments are formulated to provide preparations that are immiscible, miscible or emulsifiable with the skin secretion. Hydrophobic ointments and water-emulsifying ointments are intended to be applied to the skin or certain mucous membranes for emollient, protective, therapeutic or prophylactic purposes where a degree of occlusion is desired. Hydrophilic ointments are miscible with the skin secretion and are less emollient as a consequence.

In tropical and subtropical countries varying quantities of Arachis Oil, White Beeswax, Wool Fat, Hard Paraffin, White Soft Paraffin, Yellow Soft Paraffin or Liquid Paraffin may be used in the preparation of the Ointments of the Pharmacopoeia when prevailing high temperatures otherwise make them too soft for convenient use, but the specified proportions of the active ingredients must be maintained.

Ointments should not normally be diluted. However, should dilution be necessary care should be taken in the selection of diluents to avoid risk of incompatibility or instability.

LABELLING

The label states (1) the date after which the Ointment is not intended to be used; (2) the conditions under which the Ointment should be stored.

PASTES

DEFINITION

Pastes are usually intended to be applied to small, localised areas of the skin.

LABELLING

The label states (1) the date after which the Paste is not intended to be used; (2) the conditions under which the Paste should be stored.

TOPICAL SEMI-SOLID PREPARATIONS

General Notices

(Semi-solid Preparations for Cutaneous Application, Ph Eur monograph 0132)

Topical Semi-solid Preparations comply with the requirements of the European Pharmacopoeia monograph for Semi-solid Preparations for Cutaneous Application. These requirements are reproduced below.

Ph Eur

The requirements of this monograph apply to all semi-solid preparations for cutaneous application. Where appropriate, additional requirements specific to semi-solid preparations intended to be applied to particular surfaces or mucous membranes may be found in other general monographs, for example Ear preparations (0652), Nasal preparations (0676), Rectal preparations (1145), Eye preparations (1163) and Vaginal preparations (1164).

DEFINITION

Semi-solid preparations for cutaneous application are intended for local or transdermal delivery of active substances, or for their emollient or protective action. They are of homogeneous appearance.

Semi-solid preparations for cutaneous application consist of a simple or compound basis in which, usually, 1 or more active substances are dissolved or dispersed. According to its composition, the basis may influence the activity of the preparation.

The basis may consist of natural or synthetic substances and may be single phase or multiphase. According to the nature of the basis, the preparation may have hydrophilic or hydrophobic properties; it may contain suitable excipients such as antimicrobial preservatives, antioxidants, stabilisers, emulsifiers, thickeners and penetration enhancers.

Semi-solid preparations for cutaneous application intended for use on severely injured skin are sterile.

Where applicable, containers for semi-solid preparations for cutaneous application comply with the requirements for *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*.

Several categories of semi-solid preparations for cutaneous application may be distinguished:

- ointments;
- creams;
- gels;
- pastes;
- poultices;
- medicated plasters.

According to their structure, ointments, creams and gels generally show viscoelastic behaviour and are non-Newtonian in character e.g. plastic, pseudoplastic or thixotropic type flow at high shear rates. Pastes frequently exhibit dilatancy.

PRODUCTION

During development of semi-solid preparations for cutaneous application whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in *Efficacy of antimicrobial preservation (5.1.3)*. In the manufacture, packaging, storage and distribution of semi-solid preparations for cutaneous application, suitable steps are taken to ensure their microbiological quality; recommendations on this are provided in *Microbiological Quality of Pharmaceutical Preparations (5.1.4)*. Sterile semi-solid preparations for cutaneous application are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this are provided in *Methods of Preparation of Sterile Products (5.1.1)*.

During development, it must be demonstrated that the nominal content can be withdrawn from the container of semi-solid preparations for cutaneous application presented in single-dose containers.

In the manufacture of semi-solid preparations for cutaneous application, suitable measures are taken to ensure that the defined rheological properties are fulfilled. Where appropriate, the following non-mandatory tests may be carried out: measurement of consistency by penetrometry *(2.9.9)*, viscosity (apparent viscosity) *(2.2.10)* and a suitable test to demonstrate the appropriate release of the active substance(s).

In the manufacture of semi-solid preparations for cutaneous application containing 1 or more active substances that are not dissolved in the basis (e.g. emulsions or suspensions), measures are taken to ensure appropriate homogeneity of the preparation to be delivered.

In the manufacture of semi-solid preparations for cutaneous application containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Sterility *(2.6.1)*

Where the label indicates that the preparation is sterile, it complies with the test for sterility.

STORAGE

If the preparation contains water or other volatile ingredients, store in an airtight container. If the preparation is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

— the name of any added antimicrobial preservative;

— where applicable, that the preparation is sterile.

OINTMENTS

DEFINITION

An ointment consists of a single-phase basis in which solids or liquids may be dispersed.

Hydrophobic Ointments Hydrophobic ointments can absorb only small amounts of water. Typical bases used for their formulation are hard, liquid and light liquid paraffins, vegetable oils, animal fats, synthetic glycerides, waxes and liquid polyalkylsiloxanes.

Water-emulsifying Ointments Water-emulsifying ointments can absorb larger amounts of water and thereby produce water-in-oil or oil-in-water emulsions depending on the nature of the emulsifiers: water-in-oil emulsifying agents such as wool alcohols, sorbitan esters, monoglycerides and fatty alcohols, or oil-in-water emulsifying agents such as sulphated fatty alcohols, polysorbates, macrogol cetostearyl ether or esters of fatty acids with macrogols may be used for this purpose. Their bases are those of the hydrophobic ointments.

Hydrophilic Ointments Hydrophilic ointments are preparations having bases that are miscible with water. The bases usually consist of mixtures of liquid and solid macrogols (polyethylene glycols). They may contain appropriate amounts of water.

CREAMS

DEFINITION

Creams are multiphase preparations consisting of a lipophilic phase and an aqueous phase.

Lipophilic Creams Lipophilic creams have as the continuous phase the lipophilic phase. They contain water-in-oil emulsifying agents such as wool alcohols, sorbitan esters and monoglycerides.

Hydrophilic Creams Hydrophilic creams have as the continuous phase the aqueous phase. They contain oil-in-water emulsifying agents such as sodium or trolamine soaps, sulphated fatty alcohols, polysorbates and polyoxyl fatty acid and fatty alcohol esters combined, if necessary, with water-in-oil emulsifying agents.

GELS

DEFINITION

Gels consist of liquids gelled by means of suitable gelling agents.

Lipophilic Gels Lipophilic gels (oleogels) are preparations whose bases usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminium or zinc soaps.

Hydrophilic Gels Hydrophilic gels (hydrogels) are preparations whose bases usually consist of water, glycerol or propylene glycol gelled with suitable gelling agents such as starch, cellulose derivatives, carbomers and magnesium-aluminium silicates.

PASTES

DEFINITION

Pastes are semi-solid preparations for cutaneous application containing large proportions of solids finely dispersed in the basis.

POULTICES

DEFINITION

Poultices consist of a hydrophilic heat-retentive basis in which solid or liquid active substances are dispersed. They are usually spread thickly on a suitable dressing and heated before application to the skin.

MEDICATED PLASTERS

DEFINITION

Medicated plasters are flexible preparations containing 1 or more active substances. They are intended to be applied to the skin. They are designed to maintain the active substance(s) in close contact with the skin such that these may be absorbed slowly, or act as protective or keratolytic agents.

Medicated plasters consist of an adhesive basis, which may be coloured, containing 1 or more active substances, spread as a uniform layer on an appropriate support made of natural or synthetic material. It is not irritant or sensitising to the skin. The adhesive layer is covered by a suitable protective liner, which is removed before applying the plaster to the skin. When removed, the protective liner does not detach the preparation from the outer, supporting layer.

Medicated plasters are presented in a range of sizes directly adapted to their intended use or as larger sheets to be cut before use. Medicated plasters adhere firmly to the skin when gentle pressure is applied and can be peeled off without causing appreciable injury to the skin or detachment of the preparation from the outer, supporting layer.

TESTS

Dissolution

A suitable test may be required to demonstrate the appropriate release of the active substance(s), for example one of the tests described in *Dissolution test for transdermal* patches *(2.9.4)*.

Ph Eur

Transdermal Patches of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those preparations that are the subject of an individual monograph in the British Pharmacopoeia.

LABELLING

The label states (1) the date after which the Transdermal Patches are not intended to be used; (2) the conditions under which the Transdermal Patches should be stored; (3) the directions for using the Transdermal Patches.

TRANSDERMAL PATCHES

General Notices

(Ph Eur monograph 1011)

Transdermal Patches comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Transdermal patches are flexible pharmaceutical preparations of varying sizes, containing one or more active substances. They are intended to be applied to the unbroken skin in order to deliver the active substance(s) to the systemic circulation after passing through the skin barrier.

Transdermal patches normally consist of an outer covering which supports a preparation which contains the active substance(s). The transdermal patches are covered on the site of the release surface of the preparation by a protective liner, which is removed before applying the patch to the skin.

The outer covering is a backing sheet impermeable to the active substance(s) and normally impermeable to water, designed to support and protect the preparation. The outer covering may have the same dimensions as the preparation or it may be larger. In the latter case the overlapping border of the outer covering is covered by pressure-sensitive adhesive substances which assure the adhesion of the patch to the skin.

The preparation contains the active substance(s) together with excipients such as stabilisers, solubilisers or substances intended to modify the release rate or to enhance transdermal absorption. It may be a single layer or multi-layer solid or semi-solid matrix, and in this case it is the composition and structure of the matrix which determines the diffusion pattern of the active substance(s) to the skin. The matrix may contain pressure-sensitive adhesives which assure the adhesion of the preparation to the skin. The preparation may exist as a semi-solid reservoir one side of which is a membrane which may control the release and the diffusion of the active substance(s) from the preparation. The pressure-sensitive adhesive substances may, in this case, be applied to some or all parts of the membrane, or only around the border of the membrane of the outer covering.

When applied to the dried, clean and unbroken skin, the transdermal patch adheres firmly to the skin by gentle pressure of the hand or the fingers and can be peeled off without causing appreciable injury to the skin or detachment of the preparation from the outer covering. The patch must not be irritant or sensitising to the skin, even after repeated applications.

The protective liner generally consists of a sheet of plastic or metal material. When removed, the protective liner does not detach the preparation (matrix or reservoir) or the adhesive from the patch.

Transdermal patches are normally individually enclosed in sealed sachets.

PRODUCTION

In the manufacture, packaging, storage and distribution of transdermal patches suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on *Microbiological quality of pharmaceutical preparations (5.1.4)*.

TESTS

Uniformity of dosage units

Transdermal patches comply with the test for uniformity of dosage units *(2.9.40)* or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, transdermal patches comply with test C for uniformity of content of single-dose preparations.

Dissolution

A suitable test may be required to demonstrate the appropriate release of the active substance(s), for example one of the tests described in *Dissolution test for transdermal patches (2.9.4)*. The disc assembly method, the cell method or the rotating cylinder method may be used, as suitable, according to the composition, dimensions and shape of the patch.

A membrane may be used. It can be of various materials, such as inert porous cellulose or silicones, and must not affect the release kinetics of the active substance(s) from the patch. Furthermore, it must be free of substances that may interfere with its performance (for example grease). The membrane may be suitably treated before the tests, for example, by maintaining it in the medium to be used in the test for 24 h. Apply the membrane above the releasing surface of the patch, avoiding the formation of air bubbles.

The test conditions and the requirements are to be authorised by the competent authority.

STORAGE

Store at room temperature, unless otherwise indicated.

LABELLING

The label states, where applicable, the total quantity of active substance(s) per patch, the dose released per unit time and the area of the releasing surface.

Ph Eur

Unlicensed Medicines

General Notices

This monograph describes the minimum quality standards required for Unlicensed Medicines for human use. The statements in this monograph are intended to be read in conjunction with individual monographs for unlicensed medicines or formulated preparations in the Pharmacopoeia, together with relevant General Notices, Appendices and Supplementary Chapters.

Individual monographs are intended to apply throughout the period for which the formulation is expected to be satisfactory for use.

DEFINITION

An unlicensed medicine is one which is prepared, at the request of an authorised healthcare professional, to address patient medicinal requirements, unmet by current licensed medicines, according to the Medicines Act 1968 (see Supplementary Chapter V). Such products will be manufactured under a manufacturing specials licence or prepared extemporaneously under the supervision of a pharmacist.

SCOPE

This general monograph applies to those dosage forms that are routinely manufactured or prepared as unlicensed medicines and are usually presented as conventional-release formulations. These include:

— Capsules

— Liquids for Cutaneous Application

— Ear Drops and Lotions

— Eye Drops

— Preparations for Irrigation

— Nasal Preparations

— Oral Liquids

— Oral Powders

— Parenteral Preparations

— Rectal Preparations

— Tablets

— Topical Semi-solid Preparations

— Vaginal Preparations

PRODUCTION

The production of unlicensed medicines should only be undertaken by competent staff, within suitable facilities and using equipment appropriate for the scale of manufacture and the specific dosage form.

In the UK, batch manufacture should be undertaken in facilities holding a UK Manufacturers Specials Licence in compliance with the standards of Good Manufacturing Practice.

LABELLING

The following requirements are applicable to unlicensed medicines manufactured or prepared in accordance with medicines legislation. They are not intended to apply to repackaging and assembly activities. The requirements were previously included as guidance in Supplementary Chapter V of the British Pharmacopoeia 2007.

Best practice guidance on the labelling and packaging of medicines advises that certain items of information are deemed critical for the safe use of the medicine (see MHRA Guidance Note No. 25: Best Practice Guidance on Labelling and Packaging of Medicines). These critical items of information, which should be located together on the pack and appear in the same field of view, are: name, strength, route of administration, dosage and warnings (highlighted in bold).

1. **The common name of the product.**
2. **A statement of the active ingredients expressed qualitatively and quantitatively per dosage unit or for a given volume or weight.**
3. **Route of administration.**
4. **Instructions for use, including any special warnings.**
5. The pharmaceutical form.
6. The contents of the container by weight, volume or by number of doses.
7. Excipients of known effect. For injectable, topical (including inhalation products) and ophthalmic medicines, all excipients.
8. 'Keep out of reach and sight of children'. [Note 1]
9. The expiry date expressed in unambiguous terms (dd/mm/yy).
10. Any special storage precautions.
11. The manufacturer's ML number, where appropriate.
12. The manufacturer's name and address.
13. The batch number.
14. Statutory warnings required by SI 1994/3144 for particular actives, eg aspirin, paracetamol, etc.

For small containers certain details may be omitted, but the label should contain, as a minimum, the following information:

1. **The common name of the product.**
2. **A statement of the active ingredients expressed qualitatively and quantitatively per dosage unit or for a given volume or weight.**
3. **Route of administration.**

6. The contents of the container by weight, volume or by number of doses.

9. The expiry date expressed in unambiguous terms (dd/mm/yy).

13. The batch number.

In such cases, the label for the outer packaging should contain all the relevant label information.

Note 1: This is a statutory requirement for relevant medicinal products.

MEDICINAL SUBSTANCES AND EXCIPIENTS

Where such a monograph is available, the medicinal substance and any excipients must comply with the specific monograph requirements of the Pharmacopoeia.

The medicinal substance and any excipients must also comply with the General Monograph for Substances for Pharmaceutical Use and, where appropriate, the provisions of Supplementary Chapter IV J on the Control of Impurities in Substances for Pharmaceutical Use and the General Monograph for Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies.

FORMULATED PREPARATIONS

Unlicensed medicines must comply with the requirements of the General Monograph for Unlicensed Medicines and with the requirements of the relevant General Monograph for the specific dosage form. Where a BP monograph for a formulated preparation is available, the product must comply. In addition, where specified, they must comply with the following tests.

ORAL LIQUIDS

PRODUCTION

In the manufacturing, packaging and storage of oral liquids, suitable measures are taken to ensure their microbial quality complies with Pharmacopoeial requirements throughout shelf-life; recommendations for microbial limits are provided in the section on *Microbiological Quality of Pharmaceutical Preparations (Appendix XVI D)*. If necessary, an antimicrobial preservative may be added unless the formulation is required to be preservative-free.

During the preparation, storage and use of preservative-free oral liquids, due consideration should be given to the additional risks of microbial contamination that exist for such preparations. Numbers of viable organisms in starting materials and primary packaging materials should be minimised. Storage conditions of the finished product should prevent contamination and inhibit microbiological proliferation. The product shelf life during storage and use should be based on the risk and probability that the product may fail to meet the requirements of Appendix XVI D.

ORAL SUSPENSIONS

Dissolution

Carry out the test for dissolution described under *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Unless otherwise stated in the individual monograph use 900 ml of an appropriate dissolution medium (as suggested in Supplementary Chapter I E, Table 2.9.3.-5) and rotate the paddle at 50 rpm.

Shake the container containing the oral suspension being examined for 30 seconds and accurately remove 5 ml at a depth of 1 cm below the meniscus. Introduce the dose to the media in the dissolution vessel. If sink conditions cannot be obtained, testing a partial dose of the suspension (10% to 20% of the usual dose) is preferable to using a surfactant.

Operate the apparatus at the specified rate. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the dissolution medium and the top of the rotating paddle, not less than 1 cm from the vessel wall. Where multiple sampling times are specified, replace the aliquots withdrawn for analysis with equal volumes of fresh dissolution medium at 37° or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test and verify the temperature of the medium at suitable times. Perform the analysis using a suitable assay method. Repeat the test with a further five additional doses.

Unless otherwise specified in the individual monograph, the value of Q is 75% of the stated amount at 45 minutes.

Homogeneity of Suspension

Allow a suitable volume of the oral suspension being examined to settle, undisturbed, for 24 hours. Shake the container for 30 seconds and accurately remove one dose (usually 5 to 10 ml) at a depth of 1 cm below the meniscus. Shake the container again for 10 seconds and remove another dose. Repeat this procedure until 10 doses of the suspension have been removed. Assay the 10 doses individually according to the method specified in the individual monograph.

The preparation complies with the test if each individual dose is between 85% and 115% of the average dose. The preparation fails to comply with the test if more than one individual dose is outside these limits or if one individual dose is outside the limits of 75% to 125% of the average content.

Vaginal Preparations of the British Pharmacopoeia

General Notices

In addition to the above requirements of the European Pharmacopoeia, the following statements apply to those pessaries that are the subject of an individual monograph in the British Pharmacopoeia.

PESSARIES

LABELLING

The label states (1) the names and concentrations of the active ingredients; (2) that the preparation should not be swallowed; (3) the date after which the Pessaries are not intended to be used; (4) the conditions under which the Pessaries should be stored.

VAGINAL PREPARATIONS

General Notices

(Ph Eur monograph 1164)

Ph Eur

DEFINITION

Vaginal preparations are liquid, semi-solid or solid preparations intended for administration to the vagina usually in order to obtain a local effect. They contain 1 or more active substances in a suitable basis.

Where appropriate, containers for vaginal preparations comply with the requirements for materials used for the manufacture of containers *(3.1 and subsections)* and containers *(3.2 and subsections)*.

Several categories of vaginal preparations may be distinguished:

— pessaries;

— vaginal tablets;

— vaginal capsules;

— vaginal solutions, emulsions and suspensions;

— tablets for vaginal solutions and suspensions;

— semi-solid vaginal preparations;

— vaginal foams;

— medicated vaginal tampons.

PRODUCTION

During development, it must be demonstrated that the nominal contents can be withdrawn from the container of liquid and semi-solid vaginal preparations presented in single-dose containers.

In the manufacturing, packaging, storage and distribution of vaginal preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in chapter *5.1.4*. *Microbiological quality of pharmaceutical preparations*.

TESTS

Uniformity of dosage units *(2.9.40)*

Liquid and semi-solid single-dose vaginal preparations comply with the test. Solid single-dose vaginal preparations comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content *(2.9.6)*

Unless otherwise prescribed or justified and authorised, solid single-dose vaginal preparations with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A (vaginal tablets) or test B (pessaries, vaginal capsules). If the preparation has more than one active substance, the requirement applies only to those substances which correspond to the above conditions.

Uniformity of mass *(2.9.5)*

Solid single-dose vaginal preparations comply with the test. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Dissolution

A suitable test may be carried out to demonstrate the appropriate release of the active substance(s) from solid single-dose vaginal preparations, for example one of the tests described in chapter *2.9.3*. *Dissolution test for solid dosage forms* or in 2.9.42. *Dissolution test for lipophilic solid dosage forms.*

When a dissolution test is prescribed, a disintegration test may not be required.

PESSARIES

DEFINITION

Pessaries are solid, single-dose preparations. They have various shapes, usually ovoid, with a volume and consistency suitable for insertion into the vagina. They contain 1 or more active substances dispersed or dissolved in a suitable basis that may be soluble or dispersible in water or may melt at body temperature. Excipients such as diluents, adsorbents, surface-active agents, lubricants, antimicrobial preservatives and colouring matter authorised by the competent authority may be added, if necessary.

PRODUCTION

Pessaries are usually prepared by moulding. Where appropriate in the manufacture of pessaries, measures are taken to ensure a suitable and controlled particle size of the active substance(s). If necessary, the active substance(s) are previously ground and sieved through a suitable sieve.

When prepared by moulding, the medicated mass, sufficiently liquefied by heating, is poured into suitable moulds. The pessary solidifies on cooling. Various excipients are available for this process, such as hard fat, macrogols, cocoa butter, and various gelatinous mixtures consisting, for example, of gelatin, water and glycerol.

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from pessaries intended for prolonged local action.

TESTS

Disintegration *(2.9.2)*

Unless intended for prolonged local action, they comply with the test. Examine the state of the pessaries after 60 min, unless otherwise justified and authorised.

VAGINAL TABLETS

DEFINITION

Vaginal tablets are solid, single-dose preparations. They generally conform to the definitions of uncoated or film-coated tablets given in the monograph *Tablets (0478).*

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from vaginal tablets intended for prolonged local action.

TESTS

Disintegration ***(2.9.2)***

Unless intended for prolonged local action, they comply with the test (special method for vaginal tablets). Examine the state of the tablets after 30 min, unless otherwise justified and authorised.

VAGINAL CAPSULES

DEFINITION

Vaginal capsules (shell pessaries) are solid, single-dose preparations. They are generally similar to soft capsules as defined in the monograph *Capsules (0016),* differing only in their shape and size. Vaginal capsules have various shapes, usually ovoid. They are smooth and have a uniform external appearance.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of the active substance(s) from vaginal capsules intended for prolonged local action.

TESTS

Disintegration ***(2.9.2)***

Unless intended for prolonged local action, they comply with the test. Examine the state of the capsules after 30 min, unless otherwise justified and authorised.

VAGINAL SOLUTIONS, EMULSIONS AND SUSPENSIONS

DEFINITION

Vaginal solutions, emulsions and suspensions are liquid preparations intended for a local effect, for irrigation or for diagnostic purposes. They may contain excipients, for example to adjust the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance(s) or to stabilise the preparation. The excipients do not adversely affect the intended medical action or, at the concentrations used, cause undue local irritation.

Vaginal emulsions may show evidence of phase separation but are readily redispersed on shaking. Vaginal suspensions may show a sediment that is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable a homogeneous preparation to be delivered.

They are supplied in single-dose containers. The container is adapted to deliver the preparation to the vagina or it is accompanied by a suitable applicator.

PRODUCTION

In the manufacture of vaginal suspensions measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TABLETS FOR VAGINAL SOLUTIONS AND SUSPENSIONS

DEFINITION

Tablets intended for the preparation of vaginal solutions and suspensions are single-dose preparations that are dissolved or dispersed in water at the time of administration. They may contain excipients to facilitate dissolution or dispersion or to prevent caking.

Apart from the test for disintegration, tablets for vaginal solutions or suspensions conform with the definition for *Tablets (0478).*

After dissolution or dispersion, they comply with the requirements for vaginal solutions or vaginal suspensions, as appropriate.

TESTS

Disintegration *(2.9.1)*

Tablets for vaginal solutions or suspensions comply with the test, but using *water R* at 15-25 °C and 6 tablets. Examine the state of the tablets after 3 min. The tablets comply with the test if all 6 have disintegrated.

LABELLING

The label states:

- — the method of preparation of the vaginal solution or suspension;
- — the conditions and duration of storage of the solution or suspension after constitution.

SEMI-SOLID VAGINAL PREPARATIONS

DEFINITION

Semi-solid vaginal preparations are ointments, creams or gels.

They are often supplied in single-dose containers. The container is provided with a suitable applicator.

Semi-solid vaginal preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132).*

VAGINAL FOAMS

DEFINITION

Vaginal foams comply with the requirements of the monograph *Medicated foams (1105).*

MEDICATED VAGINAL TAMPONS

DEFINITION

Medicated vaginal tampons are solid, single-dose preparations intended to be inserted in the vagina for a limited time.

They comply with the requirements of the monograph *Medicated tampons (1155).*

Ph Eur

Albumin Solution

General Notices

Albumin; Human Albumin

(Human Albumin Solution, Ph Eur monograph 0255)

Ph Eur

DEFINITION

Human albumin solution is an aqueous solution of protein obtained from plasma that complies with the requirements of the monograph on *Plasma for fractionation, human (0853).*

PRODUCTION

Separation of the albumin is carried out under controlled conditions, particularly of pH, ionic strength and temperature so that in the final product not less than 95 per cent of the total protein is albumin. Human albumin solution is prepared as a concentrated solution containing 150-250 g/l of total protein or as an isotonic solution containing 35-50 g/l of total protein. A suitable stabiliser against the effects of heat, such as sodium caprylate (sodium octanoate) or *N*-acetyltryptophan or a combination of these 2, at a suitable concentration, may be added but no antimicrobial preservative is added at any stage during preparation. The solution is passed through a bacteria-retentive filter and distributed aseptically into sterile containers which are then closed so as to prevent contamination. The solution in its final container is heated to 60 ± 1.0 °C and maintained at this temperature for not less than 10 h. The containers are then incubated at 30-32 °C for not less than 14 days or at 20-25 °C for not less than 4 weeks and examined visually for evidence of microbial contamination.

CHARACTERS

A clear, slightly viscous liquid; it is almost colourless, yellow, amber or green.

IDENTIFICATION

Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and the preparation to be examined, both diluted to contain 10 g/l of protein. The main component of the preparation to be examined corresponds to the main component of normal human serum. The preparation may show the presence of small quantities of other plasma proteins.

TESTS

pH (*2.2.3*)

6.7 to 7.3.

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing 10 g/l of protein.

Total protein

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the quantity of protein by multiplying by 6.25. The preparation contains not less than 95 per cent and not more than 105 per cent of the quantity of protein stated on the label.

Protein composition

Zone electrophoresis (*2.2.31*).

Use strips of suitable cellulose acetate gel or agarose gel as the supporting medium and *barbital buffer solution pH 8.6 R1* as the electrolyte solution.

If cellulose acetate is the supporting material, the method described below can be used. If agarose gels are used, and because they are normally part of an automated system, the manufacturer's instructions are followed instead.

Test solution Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to a protein concentration of 20 g/l.

Reference solution Dilute *human albumin for electrophoresis BRP* with a 9 g/l solution of *sodium chloride R* to a protein concentration of 20 g/l.

To a strip apply 2.5 µl of the test solution as a 10 mm band or apply 0.25 µl per millimetre if a narrower strip is used. To another strip, apply in the same manner the same volume of the reference solution. Apply a suitable electric field such that the most rapid band migrates at least 30 mm. Treat the strips with *amido black 10B solution R* for 5 min. Decolorise with a mixture of 10 volumes of *glacial acetic acid R* and 90 volumes of *methanol R* until the background is just free of colour. Develop the transparency of the strips with a mixture of 19 volumes of *glacial acetic acid R* and 81 volumes of *methanol R.* Measure the absorbance of the bands at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strip.

System suitability In the electropherogram obtained with the reference solution on cellulose acetate or on agarose gels, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Results In the electropherogram obtained with the test solution on cellulose acetate or on agarose gels, not more than 5 per cent of the protein has a mobility different from that of the principal band.

Molecular size distribution

Liquid chromatography (*2.2.29*).

Test solution Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to a concentration suitable for the chromatographic system used. A concentration in the range of 4-12 g/l and injection of 50-600 µg of protein are usually suitable.

Column:

— *size*: *l* = 0.6 m, Ø = 7.5 mm, or l = 0.3 m, Ø = 7.8 mm;

— *stationary phase*: *hydrophilic silica gel for chromatography R*, of a grade suitable for fractionation of globular proteins with relative molecular masses in the range 10 000 to 500

000.

Mobile phase Dissolve 4.873 g of *disodium hydrogen phosphate dihydrate R*, 1.741 g of *sodium dihydrogen phosphate monohydrate R*, 11.688 g of *sodium chloride R* and 50 mg of *sodium azide R* in 1 litre of *water R*.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 280 nm.

The peak due to polymers and aggregates is located in the part of the chromatogram representing the void volume. Disregard the peak due to the stabiliser. The area of the peak due to polymers and aggregates is not greater than 10 per cent of the total area of the chromatogram (corresponding to about 5 per cent of polymers and aggregates).

Haem

Dilute the preparation to be examined using a 9 g/l solution of *sodium chloride R* to obtain a solution containing 10 g/l of protein. The absorbance (*2.2.25*) of the solution measured at 403 nm using *water R* as the compensation liquid is not greater than 0.15.

Prekallikrein activator (*2.6.15*)

Maximum 35 IU/ml.

Aluminium

Maximum 2.0 × 102 µg/l.

Atomic absorption spectrometry (*2.2.23, Method I or II*).

Use a furnace as atomic generator.

Use plastic containers for preparation of the solutions and use plastic equipment where possible. Wash glassware (or equipment) in nitric acid (200 g/l HNO_3) before use.

Test solution Use the preparation to be examined, diluted if necessary.

Reference solutions Prepare at least 3 reference solutions in a range spanning the expected aluminium concentration of the preparation to be examined, for example by diluting *aluminium standard solution (10 ppm Al) R* with a 1 g/l solution of *octoxinol 10 R*.

Monitor solution Add *aluminium standard solution (10 ppm Al) R* or a suitable certified reference material to the test solution in a sufficient amount to increase the aluminium concentration by 20 µg/l.

Blank solution 1 g/l solution of *octoxinol 10 R*.

Wavelength 309.3 nm or other suitable wavelength.

Slit width 0.5 nm.

Tube Pyrolytically coated, with integrated platform.

Background corrector Off.

Atomisation device Furnace; fire between readings.

The operating conditions in Table 0255.-1 are cited as an example of conditions found suitable for a given apparatus; they may be modified to obtain optimum conditions.

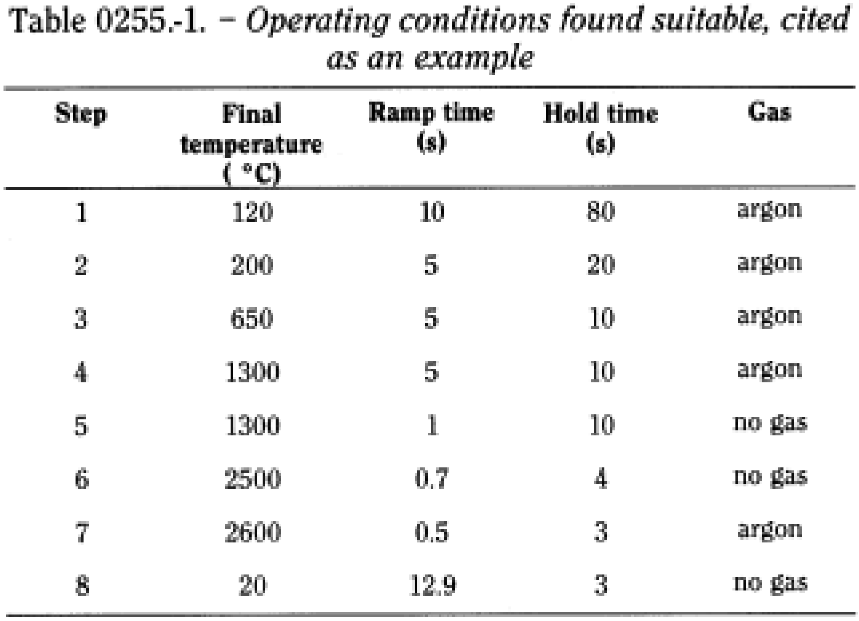


Table 0255.-1. – *Operating conditions found suitable, cited as an example*

Step	Final temperature (°C)	Ramp time (s)	Hold time (s)	Gas
1	120	10	80	argon
2	200	5	20	argon
3	650	5	10	argon
4	1300	5	10	argon
5	1300	1	10	no gas
6	2500	0.7	4	no gas
7	2600	0.5	3	argon
8	20	12.9	3	no gas

Injection Each of the following solutions 3 times: blank solution, reference solutions, test solution and monitor solution.

System suitability:

- — the recovery of aluminium added in preparation of the monitor solution is within the range 80-120 per cent.

Prepare a calibration curve from the mean of the readings obtained with the reference solutions and determine the aluminium content of the preparation to be examined using the calibration curve.

Potassium

Maximum 0.05 mmol of K per gram of protein.

Atomic emission spectrometry (*2.2.22, Method I*).

Wavelength 766.5 nm.

Sodium

Maximum 1.60 × 10^2 mmol/l and not less than 95 per cent and not more than 105 per cent of the content of Na stated on the label.

Atomic emission spectrometry (*2.2.22, Method I*).

Wavelength 589 nm.

Sterility (*2.6.1*)

It complies with the test for sterility.

Pyrogens (*2.6.8*)

It complies with the test for pyrogens. For a solution containing 35-50 g/l of protein, inject per kilogram of the rabbit's mass 10 ml of the preparation to be examined. For a solution containing 150-250 g/l of protein, inject per kilogram of the rabbit's mass 5 ml of the preparation to be examined.

STORAGE

Protected from light.

LABELLING

The label states:

— the name of the preparation;

— the volume of the preparation;

— the content of protein expressed in grams per litre;

— the content of sodium expressed in millimoles per litre;

— that the product is not to be used if it is cloudy or if a deposit has formed;

— the name and concentration of any added substance (for example stabiliser).

Ph Eur

Anticoagulant and Preservative Solutions for Blood

General Notices

(Anticoagulant and Preservative Solutions for Human Blood, Ph Eur monograph 0209)

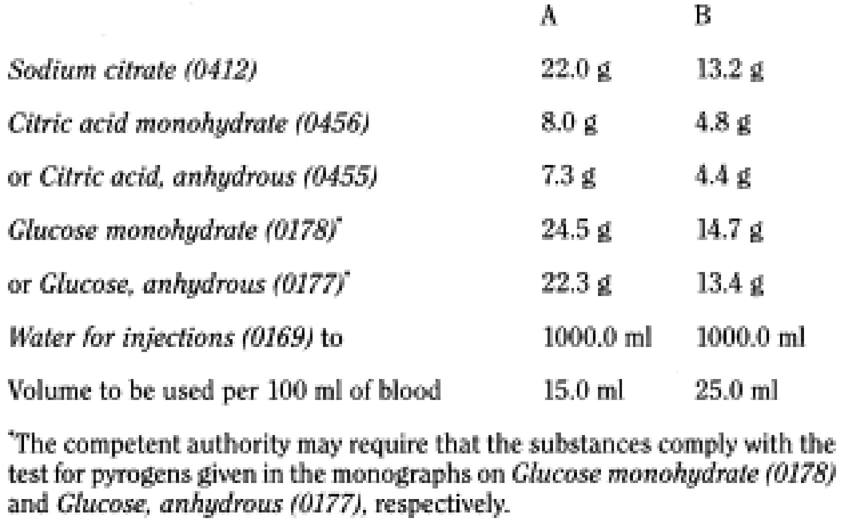
Ph Eur

DEFINITION

Anticoagulant and preservative solutions for human blood are sterile and pyrogen-free solutions prepared with water for injections, filtered, distributed in the final containers and sterilised. The content of sodium citrate ($C_6H_5Na_3O_7,2H_2O$), glucose monohydrate ($C_6H_{12}O_6,H_2O$) or anhydrous glucose ($C_6H_{12}O_6$) and sodium dihydrogen phosphate dihydrate ($NaH_2PO_4,2H_2O$) is not less than 95.0 per cent and not more than 105.0 per cent of that stated in the formulae below. The content of citric acid monohydrate ($C_6H_8O_7,H_2O$) or anhydrous citric acid ($C_6H_8O_7$) is not less than 90.0 per cent and not more than 110.0 per cent of that stated in the formulae below. Subject to agreement by the competent authority, other substances, such as red-cell preservatives, may be included in the formula provided that their name and concentration are stated on the label.

Anticoagulant and preservative solutions for human blood are presented in airtight, tamper-proof containers of glass (*3.2.1*) or plastic (*3.2.3*).

ANTICOAGULANT ACID-CITRATE-GLUCOSE SOLUTIONS (ACD)



	A	B
Sodium citrate (0412)	22.0 g	13.2 g
Citric acid monohydrate (0456)	8.0 g	4.8 g
or *Citric acid, anhydrous (0455)*	7.3 g	4.4 g
*Glucose monohydrate (0178)**	24.5 g	14.7 g
or *Glucose, anhydrous (0177)**	22.3 g	13.4 g
Water for injections (0169) to	1000.0 ml	1000.0 ml
Volume to be used per 100 ml of blood	15.0 ml	25.0 ml

*The competent authority may require that the substances comply with the test for pyrogens given in the monographs on *Glucose monohydrate (0178)* and *Glucose, anhydrous (0177)*, respectively.

CHARACTERS

A colourless or faintly yellow, clear liquid, practically free from particles.

IDENTIFICATION

A. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dilute 2 ml of the solution to be examined (for formula A) or 3 ml (for formula B) to 100 ml with a mixture of 2 volumes of *water R* and 3 volumes of *methanol R*.

Reference solution (a) Dissolve 10 mg of *glucose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg each of *glucose CRS*, *lactose CRS*, *fructose CRS* and *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Apply separately to the plate 2 µl of each solution and thoroughly dry the starting points. Develop over a path of 15 cm using a mixture of 10 volumes of *water R*, 15 volumes of *methanol R*, 25 volumes of *anhydrous acetic acid R* and 50 volumes of *ethylene chloride R*. The volumes of solvents have to be measured accurately since a slight excess of water produces cloudiness. Dry the plate in a current of warm air. Repeat the development immediately, after renewing the mobile phase. Dry the plate in a current of warm air and spray evenly with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *alcohol R*. Heat at 130 °C for 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 4 clearly separated spots.

B. To 2 ml add 5 ml of *cupri-citric solution R*. Heat to boiling. An orange precipitate is formed and the solution becomes yellow.

C. To 2 ml (for formula A) add 3 ml of *water R* or to 4 ml (for formula B) add 1 ml of *water R*. The solution gives the reaction of citrates (*2.3.1*).

D. 0.5 ml gives reaction (b) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*)

The pH of the solution to be examined is 4.7 to 5.3.

Hydroxymethylfurfural

To 2.0 ml add 5.0 ml of a 100 g/l solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R* and 1.0 ml of a 5 g/l solution of *barbituric acid R*. The absorbance (*2.2.25*), determined at 550 nm after allowing the mixture to stand for 2 min to 3 min, is not greater than that of a standard prepared at the same time in the same manner using 2.0 ml of a solution containing 5 ppm of *hydroxymethylfurfural R* for formula A or 3 ppm of *hydroxymethylfurfural R* for formula B.

Sterility (*2.6.1*)

They comply with the test for sterility.

Pyrogens (*2.6.8*)

They comply with the test for pyrogens. Dilute with a pyrogen-free, 9 g/l solution of *sodium chloride R* to obtain a solution containing approximately 5 g/l of sodium citrate. Inject 10 ml of the diluted solution per kilogram of the rabbit's mass.

ASSAY

Citric acid

To 10.0 ml (for formula A) or to 20.0 ml (for formula B) add 0.1 ml of *phenolphthalein solution R1*. Titrate with *0.2 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.2 M sodium hydroxide* is equivalent to 14.01 mg of $C_6H_8O_7,H_2O$ or to 12.81 mg of $C_6H_8O_7$.

Sodium citrate

Prepare a chromatography column 0.10 m long and 10 mm in internal diameter and filled with *strongly acidic ion-exchange resin R* (300 µm to 840 µm). Maintain a 1 cm layer of liquid above the resin at all times. Wash the column with 50 ml of de-ionised *water R* at a flow rate of 12-14 ml/min.

Dilute 10.0 ml of the solution to be examined (for formula A) or 15.0 ml (for formula B) to about 40 ml with de-ionised *water R* in a beaker and transfer to the column reservoir, washing the beaker 3 times with a few millilitres of de-ionised *water R*. Allow the solution to run through the column at a flow rate of 12-14 ml/min and collect the eluate. Wash the column with 2 quantities, each of 30 ml, and with one quantity of 50 ml, of de-ionised *water R*. The column can be used for 3 successive determinations before regeneration with 3 times its volume of *dilute hydrochloric acid R*. Titrate the combined eluate and washings (about 150 ml) with *0.2 M sodium hydroxide*, using 0.1 ml of *phenolphthalein solution R1* as indicator.

Calculate the content of sodium citrate in grams per litre from the following expressions:

For formula A: $1.961n - 1.40C$

or $1.961n - 1.53C'$

For formula B: $1.307n - 1.40C$

or $1.307n - 1.53C'$

n = number of millilitres of *0.2 M sodium hydroxide* used in the titration,

C = content of citric acid monohydrate in grams per litre determined as prescribed above,

C' = content of anhydrous citric acid in grams per litre determined as prescribed above.

Reducing sugars

Dilute 5.0 ml (for formula A) or 10.0 ml (for formula B) to 100.0 ml with *water R*. Introduce 25.0 ml of the solution into a 250 ml conical flask with ground-glass neck and add 25.0 ml of *cupri-citric solution R1*. Add a few pieces of porous material, attach a reflux condenser, heat so that boiling begins within 2 min and boil for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 ml of *water R*. Add 25 ml of a 25 per cent *m/m* solution of *sulphuric acid R* with caution and in small quantities. Titrate with *0.1 M sodium thiosulphate* using 0.5 ml of *starch solution R*, added towards the end of the titration, as indicator (n_1 ml). Carry out a blank titration using 25.0 ml of *water R* (n_2 ml).

Calculate the content of reducing sugars as anhydrous glucose or as glucose monohydrate, as appropriate, from the Table 0209.-1.

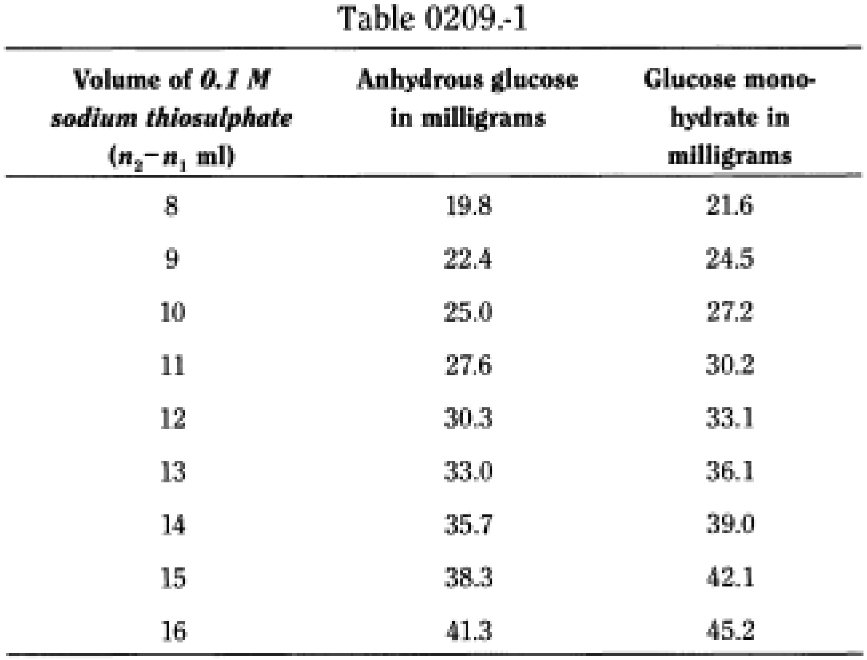


Table 0209.-1

Volume of *0.1 M sodium thiosulphate* (n_2-n_1 ml)	Anhydrous glucose in milligrams	Glucose monohydrate in milligrams
8	19.8	21.6
9	22.4	24.5
10	25.0	27.2
11	27.6	30.2
12	30.3	33.1
13	33.0	36.1
14	35.7	39.0
15	38.3	42.1
16	41.3	45.2

STORAGE

Store in an airtight, tamper-proof container, protected from light.

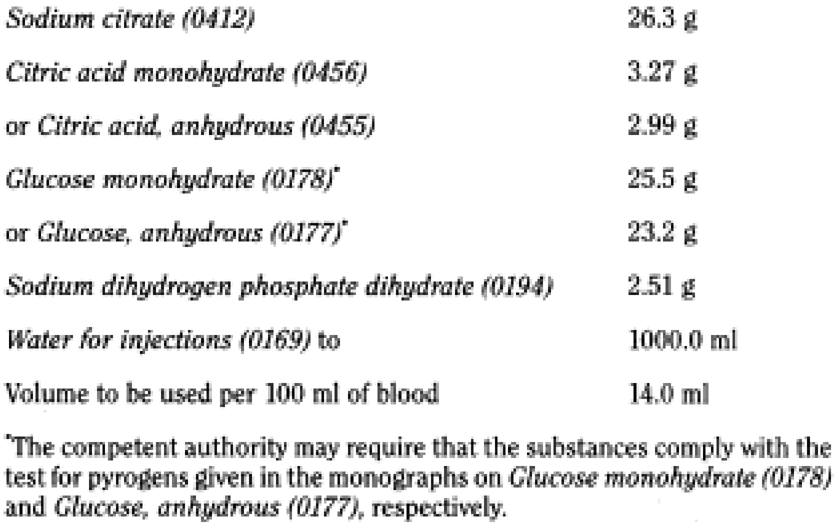
LABELLING

The label states:

— the composition and volume of the solution,

— the maximum amount of blood to be collected in the container.

ANTICOAGULANT CITRATE-PHOSPHATE-GLUCOSE SOLUTION (CPD)



Sodium citrate (0412)	26.3 g
Citric acid monohydrate (0456)	3.27 g
or *Citric acid, anhydrous (0455)*	2.99 g
Glucose monohydrate (0178)[*]	25.5 g
or *Glucose, anhydrous (0177)*[*]	23.2 g
Sodium dihydrogen phosphate dihydrate (0194)	2.51 g
Water for injections (0169) to	1000.0 ml
Volume to be used per 100 ml of blood	14.0 ml

[*] The competent authority may require that the substances comply with the test for pyrogens given in the monographs on *Glucose monohydrate (0178)* and *Glucose, anhydrous (0177)*, respectively.

CHARACTERS

A colourless or faintly yellow, clear liquid, practically free from particles.

IDENTIFICATION

A. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dilute 2 ml of the solution to be examined to 100 ml with a mixture of 2 volumes of *water R* and 3 volumes of *methanol R.*

Reference solution (a) Dissolve 10 mg of *glucose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg each of *glucose CRS*, *lactose CRS*, *fructose CRS* and *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 ml with the same mixture of solvents.

Apply separately to the plate 2 µl of each solution and thoroughly dry the starting points. Develop over a path of 15 cm using a mixture of 10 volumes of *water R,* 15 volumes of *methanol R*, 25 volumes of *anhydrous acetic acid R* and 50 volumes of *ethylene chloride R*. The volumes of solvents have to be measured accurately since a slight excess of water produces cloudiness. Dry the plate in a current of warm air. Repeat the development immediately, after renewing the mobile phase. Dry the plate in a current of warm air and spray evenly with a solution of 0.5 g of *thymol R* in a mixture of 5 ml of *sulphuric acid R* and 95 ml of *alcohol R*. Heat at 130 °C for 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 4 clearly separated spots.

B. To 2 ml add 5 ml of *cupri-citric solution R*. Heat to boiling. An orange precipitate is formed and the solution becomes yellow.

C. To 2 ml add 3 ml of *water R*. The solution gives the reaction of citrates (*2.3.1*).

D. 1 ml gives reaction (b) of phosphates (*2.3.1*).

E. 0.5 ml gives reaction (b) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*)

The pH of the solution is 5.3 to 5.9.

Hydroxymethylfurfural

To 2.0 ml add 5.0 ml of a 100 g/l solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R* and 1.0 ml of a 5 g/l solution of *barbituric acid R*. The absorbance (*2.2.25*), determined at 550 nm after allowing the mixture to stand for 2 min to 3 min, is not greater than that of a standard prepared at the same time in the same manner using 2.0 ml of a solution containing 5 ppm of *hydroxymethylfurfural R*.

Sterility (*2.6.1*)

They comply with the test for sterility.

Pyrogens (*2.6.8*)

They comply with the test for pyrogens. Dilute with a pyrogen-free, 9 g/l solution of *sodium chloride R* to obtain a solution containing approximately 5 g/l of sodium citrate. Inject 10 ml of the diluted solution per kilogram of the rabbit's mass.

ASSAY

Sodium dihydrogen phosphate

Dilute 10.0 ml to 100.0 ml with *water R*. To 10.0 ml of this solution add 10.0 ml of *nitro-vanado-molybdic reagent R*. Mix and allow to stand at 20 °C to 25 °C for 30 min. At the same time and in the same manner, prepare a reference solution using 10.0 ml of a standard solution containing 0.219 g of *potassium dihydrogen phosphate R* per litre. Measure the absorbance (*2.2.25*) of the 2 solutions at 450 nm using as the compensation liquid a solution prepared in the same manner using 10 ml of *water R*. Calculate the content of sodium dihydrogen phosphate dihydrate (*P*) in grams per litre from the expression:

$$\frac{11.46 \times C \times A_1}{A_2}$$

C = concentration of *potassium dihydrogen phosphate R* in the standard solution in grams per litre,

A_1 = absorbance of the test solution,

A_2 = absorbance of the reference solution.

Citric acid

To 20.0 ml add 0.1 ml of *phenolphthalein solution R1* and titrate with *0.2 M sodium hydroxide*.

Calculate the content of citric acid monohydrate (C), or anhydrous citric acid (C'), in grams per litre from the equations:

$$C = 0.7005n - 0.4490P$$

$$C' = 0.6404n - 0.4105P$$

n = number of millilitres of *0.2 M sodium hydroxide* used in the titration,

P = content of sodium dihydrogen phosphate dihydrate in grams per litre determined as prescribed above.

Sodium citrate

Prepare a chromatography column 0.10 m long and 10 mm in internal diameter and filled with *strongly acidic ion-exchange resin R* (300 µm to 840 µm). Maintain a 1 cm layer of liquid above the resin at all times. Wash the column with 50 ml of de-ionised *water R* at a flow rate of 12-14 ml/min.

Dilute 10.0 ml of the solution to be examined to about 40 ml with de-ionised *water R* in a beaker and transfer to the column reservoir, washing the beaker 3 times with a few millilitres of de-ionised *water R.* Allow the solution to run through the column at a flow rate of 12-14 ml/min and collect the eluate. Wash the column with 2 quantities, each of 30 ml, and with one quantity of 50 ml, of de-ionised *water R.* The column can be used for 3 successive determinations before regeneration with 3 times its volume of *dilute hydrochloric acid R*. Titrate the combined eluate and washings (about 150 ml) with *0.2 M sodium hydroxide*, using 0.1 ml of *phenolphthalein solution R1* as indicator.

Calculate the content of sodium citrate in grams per litre from the following expressions:

$$1.961n - 1.257P - 1.40C$$

$$1.961n - 1.257P - 1.53C'$$

n = number of millilitres *of 0.2 M sodium hydroxide* used in the titration,

P = content of sodium dihydrogen phosphate dihydrate in grams per litre determined as prescribed above,

C = content of citric acid monohydrate in grams per litre determined as prescribed above

C' = content of anhydrous citric acid in grams per litre determined as prescribed above.

Reducing sugars

Dilute 5.0 ml to 100.0 ml with *water R*. Introduce 25.0 ml of the solution into a 250 ml conical flask with ground-glass neck and add 25.0 ml of *cupri-citric solution R1*. Add a few pieces of porous material, attach a reflux condenser, heat so that boiling begins within 2 min and boil for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 ml of *water R*. Add 25 ml of a 25 per cent m/m solution of *sulphuric acid R* with caution and in small quantities. Titrate with *0.1 M sodium thiosulphate* using 0.5 ml of *starch solution R*, added towards the end of the titration, as indicator (n_1 ml). Carry out a blank titration using 25.0 ml of *water R* (n_2 ml).

Calculate the content of reducing sugars as anhydrous glucose or as glucose monohydrate, as appropriate, from the Table 0209.-1.

STORAGE

Store in an airtight, tamper-proof container , protected from light.

LABELLING

The label states:

— the composition and volume of the solution,

— the maximum amount of blood to be collected in the container.

Ph Eur

Anti-D (Rh_0) Immunoglobulin

General Notices

(Human Anti-D Immunoglobulin, Ph Eur monograph 0557)

Ph Eur

DEFINITION

Human anti-D immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It contains specific antibodies against erythrocyte D-antigen and may also contain small quantities of other blood-group antibodies. *Human normal immunoglobulin (0338)* and/or *Human albumin solution (0255)* may be added.

It complies with the monograph *Human normal immunoglobulin (0338)*, except for the minimum number of donors and the minimum total protein content.

The test for anti-D antibodies *(2.6.26)* prescribed in the monograph *Human normal immunoglobulin (0338)* is not carried out, since it is replaced by the assay of human anti-D immunoglobulin *(2.7.13)* as prescribed below under Potency.

For products prepared by a method that eliminates immunoglobulins with specificities other than anti-D, where authorised, the test for antibodies to hepatitis B surface antigen is not required.

PRODUCTION

Human anti-D immunoglobulin is preferably obtained from the plasma of donors with a sufficient titre of previously acquired anti-D antibodies. Where necessary, in order to ensure an adequate supply of human anti-D immunoglobulin, it is obtained from plasma derived from donors immunised with D-positive erythrocytes that are compatible in relevant blood group systems in order to avoid formation of undesirable antibodies.

ERYTHROCYTE DONORS

Erythrocyte donors comply with the requirements for donors prescribed in the monograph *Human plasma for fractionation (0853)*.

IMMUNISATION

Immunisation of the plasma donor is carried out under proper medical supervision. Recommendations concerning donor immunisation, including testing of erythrocyte donors, have been formulated by the World Health Organisation (*Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives*, WHO Technical Report Series, No. 840, 1994 or subsequent revision).

POOLED PLASMA

To limit the potential B19 virus burden in plasma pools used for the manufacture of anti-D immunoglobulin, the plasma pool is tested for B19 virus using validated nucleic acid amplification techniques *(2.6.21)*.

B19 virus DNA

Maximum 10.0 IU/µl.

A positive control with 10.0 IU of B19 virus DNA per microlitre and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors.

B19 virus DNA for NAT testing BRP is suitable for use as a positive control.

If *Human normal immunoglobulin (0338)* and/or *Human albumin solution (0255)* are added to the preparation, the plasma pool or pools from which they are derived comply with the above requirement for B19 virus DNA.

POTENCY

Carry out the assay of human anti-D immunoglobulin *(2.7.13, Method A)*. The estimated potency is not less than 90 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Method B or C *(2.7.13)* may be used for potency determination if a satisfactory correlation with the results obtained by Method A has been established for the particular product.

STORAGE

See *Human normal immunoglobulin (0338)*.

LABELLING

See *Human normal immunoglobulin (0338)*.

The label states the number of International Units per container.

Ph Eur

Anti-D Immunoglobulin for Intravenous Use

General Notices

(Human Anti-D Immunoglobulin for Intravenous Administration, Ph Eur monograph 1527)

Ph Eur

DEFINITION

Human anti-D immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. It contains specific antibodies against erythrocyte D-antigen and may also contain small quantities of other blood-group antibodies. *Human normal immunoglobulin for intravenous administration (0918)* and/or *Human albumin solution (0255)* may be added.

It complies with the monograph *Human normal immunoglobulin for intravenous administration (0918)* , except for the minimum number of donors, the minimum total protein content, the limit for osmolality and the limit for prekallikrein activator.

The test for anti-D antibodies (*2.6.26*) prescribed in the monograph *Human normal immunoglobulin for intravenous administration (0918)* is not carried out, since it is replaced by the assay of human anti-D immunoglobulin (*2.7.13*) as prescribed below under Potency.

For products prepared by a method that eliminates immunoglobulins with specificities other than anti-D: where authorised, the test for antibodies to hepatitis B surface antigen is not required; a suitable test for Fc function is carried out instead of that described in chapter *2.7.9*, which is not applicable to such a product.

PRODUCTION

Human anti-D immunoglobulin is preferably obtained from the plasma of donors with a sufficient titre of previously acquired anti-D antibodies. Where necessary, in order to ensure an adequate supply of human anti-D immunoglobulin, it is obtained from plasma derived from donors immunised with D-positive erythrocytes that are compatible in relevant blood group systems in order to avoid formation of undesirable antibodies.

ERYTHROCYTE DONORS

Erythrocyte donors comply with the requirements for donors prescribed in the monograph *Human plasma for fractionation (0853) .*

IMMUNISATION

Immunisation of the plasma donor is carried out under proper medical supervision. Recommendations concerning donor immunisation, including testing of erythrocyte donors, have been formulated by the World Health Organisation (*Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives*, WHO Technical Report Series, No. 840, 1994 or subsequent revision).

POOLED PLASMA

To limit the potential B19 virus burden in plasma pools used for the manufacture of anti-D immunoglobulin, the plasma pool is tested for B19 virus using validated nucleic acid amplification techniques (*2.6.21*).

B19 virus DNA

Maximum 10.0 IU/µl.

A positive control with 10.0 IU of B19 virus DNA per microlitre and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors.

B19 virus DNA for NAT testing BRP is suitable for use as a positive control.

If *Human normal immunoglobulin for intravenous administration (0918)* and/or *Human albumin solution (0255)* are added to the preparation, the plasma pool or pools from which they are derived comply with the above requirement for B19 virus DNA.

POTENCY

Carry out the assay of human anti-D immunoglobulin (*2.7.13*, Method A). The estimated potency is not less than 90 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Method B or C (*2.7.13*) may be used for potency determination if a satisfactory correlation with the results obtained by Method A has been established for the particular product.

STORAGE

See *Human normal immunoglobulin for intravenous administration (0918)*.

LABELLING

See *Human normal immunoglobulin for intravenous administration (0918).*

The label states the number of International Units per container.

Ph Eur

Antithrombin III Concentrate

General Notices

(Human Antithrombin III Concentrate, Ph Eur monograph 0878)

Action and use

Anticoagulant factor.

Ph Eur

DEFINITION

Human antithrombin III concentrate is a preparation of a glycoprotein fraction obtained from human plasma that inactivates thrombin in the presence of an excess of heparin. It is obtained from plasma that complies with the requirements of the monograph on *Human plasma for fractionation (0853).*

When reconstituted in the volume of solvent stated on the label, the potency is not less than 25 IU of antithrombin III per millilitre.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and any residues are such as not to compromise the safety of the preparation for patients.

The antithrombin III is purified and concentrated and a suitable stabiliser may be added. The specific activity is not less than 3 IU of antithrombin III per milligram of total protein, excluding albumin. The antithrombin III concentrate is passed through a bacteria-retentive filter, distributed aseptically into its final, sterile containers and immediately frozen. It is then freeze-dried and the containers are closed under vacuum or in an atmosphere of inert gas. No antimicrobial preservative is added at any stage of production.

VALIDATION TEST

It shall be demonstrated that the manufacturing process yields a product that consistently complies with the following test.

Heparin-binding fraction Examine by agarose gel electrophoresis (*2.2.31*). Prepare a 10 g/l solution of *agarose for electrophoresis R* containing 15 IU of *heparin R* per millilitre in *barbital buffer solution pH 8.4 R*. Pour 5 ml of this solution onto a glass plate 5 cm square. Cool at 4 °C for 30 min. Cut 2 wells 2 mm in diameter 1 cm and 4 cm from the side of the plate and 1 cm from the cathode. Introduce into one well 5 µl of the preparation to be examined, diluted to an activity of about 1 IU of antithrombin III per millilitre. Introduce into the other well 5 µl of a solution of a marker dye such as *bromophenol blue R*. Allow the electrophoresis to proceed at 4 °C, using a constant electric field of 7 V/cm, until the dye reaches the anode.

Cut across the agarose gel 1.5 cm from that side of the plate on which the preparation to be examined was applied and remove the larger portion of the gel leaving a band 1.5 cm wide containing the material to be examined. Replace the removed portion with an even layer consisting of 3.5 ml of a 10 g/l solution of *agarose for electrophoresis R* in *barbital buffer solution pH 8.4 R,* containing a rabbit anti-human antithrombin III antiserum at a suitable concentration, previously determined, to give adequate peak heights of at least 1.5 cm. Place the plate with the original gel at the cathode so that a second electrophoretic migration can occur at right angles to the first. Allow this second electrophoresis to proceed using a constant electric field of 2 V/cm for 16 h. Cover the plates with filter paper and several layers of thick lint soaked in a 9 g/l solution of *sodium chloride R* and compress for 2 h, renewing the saline several times. Rinse with *water R*, dry the plates and stain with *acid blue 92 solution R*.

Calculate the fraction of antithrombin III bound to heparin, which is the peak closest to the anode, with respect to the total amount of antithrombin III, by measuring the area defined by the 2 precipitation peaks.

The fraction of antithrombin III able to bind to heparin is not less than 60 per cent.

CHARACTERS

A white or almost white, hygroscopic, friable solid or a powder.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, the tests (except those for solubility, total protein and water), and the assay.

IDENTIFICATION

It complies with the limits of the assay.

TESTS

pH (*2.2.3*)

6.0 to 7.5.

Solubility. It dissolves completely under gentle swirling within 10 min in the volume of the solvent stated on the label, forming a clear or slightly turbid, colourless solution.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Total protein

If necessary, dilute an accurately measured volume of the preparation to be examined with *water R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of the solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the amount of protein by multiplying the result by 6.25.

Heparin (*2.7.5*)

Maximum 0.1 IU of heparin activity per International Unit of antithrombin III activity. It is necessary to validate the method for assay of heparin for each specific preparation to be examined to allow for interference by antithrombin III.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

Pyrogens (*2.6.8*)

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume of the preparation to be examined equivalent to 50 IU of antithrombin III, calculated from the activity stated on the label.

ASSAY

Assay of human antithrombin III (*2.7.17*).

The estimated potency is not less than 80 per cent and not more than 120 per cent of the potency stated on the label. The confidence limits ($P = 0.95$) are not less than 90 per cent and not more than 110 per cent of the estimated potency.

STORAGE

Protected from light, in an airtight container.

LABELLING

The label states:

— the content of antithrombin III expressed in International Units per container.

— the name and volume of solvent to be used to reconstitute the preparation,

— where applicable, the amount of albumin present as a stabiliser.

Ph Eur

Dried Factor IX Fraction

General Notices

(Human Coagulation Factor IX, Ph Eur monograph 1223)

Action and use

Coagulation factor IX substitute.

Ph Eur

DEFINITION

Human coagulation factor IX is a plasma protein fraction containing coagulation factor IX, prepared by a method that effectively separates factor IX from other prothrombin complex factors (factors II, VII and X). It is obtained from human plasma that complies with the monograph on *Human plasma for fractionation (0853).*

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of factor IX per millilitre.

PRODUCTION

The method of preparation is designed to maintain functional integrity of factor IX, to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 50 IU of factor IX per milligram of total protein, before the addition of any protein stabiliser.

The factor IX fraction is dissolved in a suitable liquid. Heparin, antithrombin and other auxiliary substances such as a stabiliser may be included. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

CONSISTENCY OF THE METHOD OF PRODUCTION

The consistency of the method of production is evaluated by suitable analytical procedures that are determined during process development and which normally include:

— assay of factor IX,

— determination of activated coagulation factors,

— determination of activities of factors II, VII and X which shall be shown to be not more than 5 per cent of the activity of factor IX.

CHARACTERS

A white or pale yellow, hygroscopic powder or friable solid.

Reconstitute the preparation to be examined as stated on the label, immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

It complies with the limits of the assay.

TESTS

pH (*2.2.3*)

6.5 to 7.5.

Solubility

To a container of the preparation to be examined add the volume of the liquid stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 min, giving a clear or slightly opalescent, colourless solution.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Total protein

If necessary, dilute an accurately measured volume of the preparation to be examined with a 9 g/l solution of *sodium chloride R*, to obtain a solution which may be expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of that solution, in a round-bottomed centrifuge tube, add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the amount of protein by multiplying the result by 6.25.

For some products, especially those without a protein stabiliser such as albumin, this method may not be applicable. Another validated method for protein determination must therefore be performed.

Activated coagulation factors (*2.6.22*)

If necessary, dilute the preparation to be examined to contain 20 IU of factor IX per millilitre. For each of the dilutions the coagulation time is not less than 150 s.

Heparin

If heparin has been added during preparation, determine the amount by the assay of heparin in coagulation factor concentrates (*2.7.12*). The preparation to be examined contains not more than the amount of heparin stated on the label and in any case not more than 0.5 IU of heparin per International Unit of factor IX.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

Pyrogens (*2.6.8*)

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume equivalent to not less than 50 IU of factor IX.

ASSAY

Assay of human blood coagulation factor IX (*2.7.11*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

STORAGE

In an airtight container , protected from light.

LABELLING

The label states:

— the number of International Units of factor IX per container,

— the amount of protein per container,

— the name and quantity of any added substances including, where applicable, heparin,

— the name and volume of the liquid to be used for reconstitution,

— that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Ph Eur

Dried Factor VII Fraction

General Notices

(Human Coagulation Factor VII, Ph Eur monograph 1224)

Action and use

Coagulation factor VII substitute.

Ph Eur

DEFINITION

Human coagulation factor VII is a plasma protein fraction that contains the single-chain glycoprotein factor VII and may also contain small amounts of the activated form, the two-chain derivative factor VIIa. It may also contain coagulation factors II, IX and X and protein C and protein S. It is obtained from human plasma that complies with the monograph on *Human plasma for fractionation (0853).*

The potency of the preparation, reconstituted as stated on the label, is not less than 15 IU of factor VII per millilitre.

PRODUCTION

The method of preparation is designed to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 2 IU of factor VII per milligram of total protein, before the addition of any protein stabiliser.

The factor VII fraction is dissolved in a suitable liquid. Heparin, antithrombin and other auxiliary substances such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

CONSISTENCY OF THE METHOD OF PRODUCTION

The consistency of the method of production with respect to the activities of factors II, IX and X of the preparation, expressed in International Units relative to the activity of factor VII, shall be demonstrated.

The consistency of the method of production with respect to the activity of factor VIIa of the preparation shall be demonstrated. The activity of factor VIIa may be determined, for example, using a recombinant soluble tissue factor that does not activate factor VII but possesses a cofactor function specific for factor VIIa; after incubation of a mixture of the recombinant soluble tissue factor with phospholipids reagent and the dilution of the test sample in factor VII-deficient plasma, calcium chloride is added and the clotting time determined; the clotting time is inversely related to the factor VIIa activity of the test sample.

CHARACTERS

A hygroscopic powder or friable solid that may be white or almost white, pale yellow, green or blue.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

It complies with the limits of the assay.

TESTS

Solubility

To a container of the preparation to be examined add the volume of liquid stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 min, giving a clear or slightly opalescent solution that may be coloured.

pH (*2.2.3*)

6.5 to 7.5.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Total protein

If necessary, dilute an accurately measured volume of the reconstituted preparation with a 9 g/l solution of *sodium chloride R* to obtain a solution expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of the solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the amount of protein by multiplying the result by 6.25.

Activated coagulation factors (*2.6.22*)

For each of the dilutions, the coagulation time is not less than 150 s.

Heparin

If heparin has been added during preparation, determine the amount present by the assay of heparin in coagulation factor concentrates (*2.7.12*). The preparation to be examined contains not more than the amount of heparin stated on the label and in any case not more than 0.5 IU of heparin per International Unit of factor VII.

Thrombin

If the preparation to be examined contains heparin, determine the amount present as described in the test for heparin and neutralise the heparin by addition of *protamine sulphate*

R (10 µg of protamine sulphate neutralises 1 IU of heparin). In each of 2 test-tubes, mix equal volumes of the reconstituted preparation and a 3 g/l solution of *fibrinogen R*. Keep one of the tubes at 37 °C for 6 h and the other at room temperature for 24 h. In a third tube, mix a volume of the fibrinogen solution with an equal volume of a solution of *human thrombin R* (1 IU/ml) and place the tube in a water-bath at 37 °C. No coagulation occurs in the tubes containing the preparation to be examined. Coagulation occurs within 30 s in the tube containing thrombin.

Factor II

Carry out the assay of human coagulation factor II (*2.7.18*).

The estimated content is not more than 125 per cent of the stated content. The confidence limits (*P* = 0.95) are not less than 90 per cent and not more than 111 per cent of the estimated potency.

Factor IX

Carry out the assay of human coagulation factor IX (*2.7.11*).

The estimated content is not more than 125 per cent of the stated content. The confidence limits (*P* = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

Factor X

Carry out the assay of human coagulation factor X (*2.7.19*).

The estimated content is not more than 125 per cent of the stated content. The confidence limits (*P* = 0.95) are not less than 90 per cent and not more than 111 per cent of the estimated potency.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near-infrared spectrometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

Pyrogens (*2.6.8*)

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume equivalent to not less than 30 IU of factor VII.

ASSAY

Assay of human coagulation factor VII (*2.7.10*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (*P* = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states:

— the number of International Units of factor VII per container,

— the maximum content of International Units of factor II, factor IX and factor X per container,

— the amount of protein per container,

— the name and quantity of any added substances, including where applicable, heparin,

— the name and volume of the liquid to be used for reconstitution,

— that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Ph Eur

Dried Factor VIII (rDNA)

General Notices

(Human Coagulation Factor VIII (rDNA), Ph Eur monograph 1643)

Action and use

Coagulation factor VIII substitute.

Ph Eur

DEFINITION

Human coagulation factor VIII (rDNA) is a freeze-dried preparation of glycoproteins having the same activity as coagulation factor VIII in human plasma. It acts as a cofactor of the activation of factor X in the presence of factor IXa, phospholipids and calcium ions.

Human coagulation factor VIII circulates in plasma mainly as a two-chain glycosylated protein with 1 heavy (relative molecular mass of about 200 000) and 1 light (relative molecular mass 80 000) chain held together by divalent metal ions. Human coagulation factor VIII (rDNA) is prepared as full-length factor VIII (octocog alfa), or as a shortened two-chain structure (relative molecular mass 90 000 and 80 000), in which the B-domain has been deleted from the heavy chain (moroctocog alfa).

Full-length human rDNA coagulation factor VIII contains 25 potential *N*-glycosylation sites, 19 in the B domain of the heavy chain, 3 in the remaining part of the heavy chain (relative molecular mass 90 000) and 3 in the light chain (relative molecular mass 80 000). The different products are characterised by their molecular size and post-translational modification and/or other modifications.

PRODUCTION

Human coagulation factor VIII (rDNA) is produced by recombinant DNA technology in mammalian cell culture. It is produced under conditions designed to minimise microbial contamination.

Purified bulk factor VIII (rDNA) may contain added human albumin and/or other stabilising agents, as well as other auxiliary substances to provide, for example, correct pH and osmolality.

The specific activity is not less than 2000 IU of factor VIII:C per milligram of total protein before the addition of any protein stabiliser, and varies depending on purity and the type of modification of molecular structure of factor VIII.

The quality of the bulk preparation is controlled using one or more manufacturer's reference preparations as reference.

MANUFACTURER'S REFERENCE PREPARATIONS

During development, reference preparations are established for subsequent verification of batch consistency during production, and for control of bulk and final preparation. They are derived from representative batches of purified bulk factor VIII (rDNA) that are extensively characterised by tests including those described below and whose procoagulant and other relevant functional properties have been ascertained and compared, wherever possible, with the International Standard for factor VIII concentrate. The reference preparations are suitably characterised for their intended purpose and are stored in suitably sized aliquots under conditions ensuring their stability.

PURIFIED BULK FACTOR VIII (RDNA)

The purified bulk complies with a suitable combination of the following tests for characterisation of integrity of the factor VIII (rDNA). Where any substance added during preparation of the purified bulk interferes with a test, the test is carried out before addition of that substance. Where applicable, the characterisation tests may alternatively be carried out on the finished product.

Specific biological activity or ratio of factor VIII activity to factor VIII antigen

Carry out the assay of human coagulation factor VIII (*2.7.4*). The protein content, or where a protein stabiliser is present, the factor VIII antigen content, is determined by a suitable method and the specific biological activity or the ratio of factor VIII activity to factor VIII antigen is calculated.

Protein composition

The protein composition is determined by a selection of appropriate characterisation techniques which may include peptide mapping, Western blots, HPLC, gel electrophoresis, capillary electrophoresis, mass spectrometry or other techniques to monitor integrity and purity. The protein composition is comparable to that of the manufacturer's reference preparation.

Molecular size distribution

Using size-exclusion chromatography (*2.2.30*), the molecular size distribution is comparable to that of the manufacturer's reference preparation.

Peptide mapping (*2.2.55*)

There is no significant difference between the test protein and the manufacturer's reference preparation.

Carbohydrates/sialic acid

To monitor batch-to-batch consistency, the monosaccharide content and the degree of sialylation or the oligosaccharide profile are monitored and correspond to those of the manufacturer's reference preparation.

FINAL LOT

It complies with the requirements under Identification, Tests and Assay.

Excipients

80 per cent to 120 per cent of the stated content, determined by a suitable method, where applicable.

CHARACTERS

Appearance

White or slightly yellow powder or friable mass.

IDENTIFICATION

A. It complies with the limits of the assay.

B. The distribution of characteristic peptide bands corresponds with that of the manufacturer's reference preparation (SDS-PAGE or Western blot).

TESTS

Reconstitute the preparation as stated on the label immediately before carrying out the tests (except those for solubility and water) and assay.

Solubility

It dissolves within 5 min at 20-25 °C, giving a clear or slightly opalescent solution.

pH (*2.2.3*)

6.5 to 7.5.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

Bacterial endotoxins (*2.6.14*)

Less than 3 IU in the volume that contains 100 IU of factor VIII activity.

ASSAY

Carry out the assay of human coagulation factor VIII (*2.7.4*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

STORAGE

Protected from light.

LABELLING

The label states:

— the factor VIII content in International Units,

- the name and amount of any excipient,
- the composition and volume of the liquid to be used for reconstitution.

Ph Eur

Dried Factor VIII Fraction

General Notices

(Human Coagulation Factor VIII, Ph Eur monograph 0275)

Action and use

Coagulation factor VIII substitute.

Ph Eur

DEFINITION

Human coagulation factor VIII is a preparation of a plasma protein fraction that contains the glycoprotein coagulation factor VIII together with varying amounts of von Willebrand factor, depending on the method of preparation. It is prepared from human plasma that complies with the monograph on *Human plasma for fractionation (0853).*

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of factor VIII:C per millilitre.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for the inactivation of viruses, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 1 IU of factor VIII:C per milligram of total protein before the addition of any protein stabiliser.

The factor VIII fraction is dissolved in a suitable liquid. Excipients such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

VALIDATION STUDIES

Products stated to have von Willebrand factor activity For products intended for treatment of von Willebrand's disease it shall be demonstrated that the manufacturing process yields a product with a consistent composition with respect to von Willebrand factor. This composition may be characterised in a number of ways. For example, the number and the relative amount of the different multimers may be determined by sodium dodecyl sulphate (SDS) agarose gel electrophoresis (about 1 per cent agarose) with or without Western blot analysis, using a normal human plasma pool as reference; visualisation of the multimeric pattern may be performed using an immunoenzymatic technique and quantitative evaluation may be carried out by densitometric analysis or by other suitable methods.

Products that show flakes or particles after reconstitution for use If a few small flakes or particles remain when the preparation is reconstituted, it shall be demonstrated during validation studies that the potency is not significantly affected after passage of the preparation through the filter provided.

CHARACTERS

Appearance

White or pale yellow, hygroscopic powder or friable solid.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

It complies with the limits of the assay.

TESTS

Solubility

To a container of the preparation to be examined add the volume of the solvent stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 min, giving a clear or slightly opalescent, colourless or slightly yellow solution.

Where the label states that the product may show a few small flakes or particles after reconstitution, reconstitute the preparation as described on the label and pass it through the filter provided: the filtered solution is clear or slightly opalescent.

pH (*2.2.3*)

6.5 to 7.5.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Total protein

If necessary, dilute an accurately measured volume of the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. Place 2.0 ml of this solution in a round-bottomed centrifuge tube and add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the amount of protein by multiplying the result by 6.25. *For some products, especially those without a protein stabiliser such as albumin, this method may not be applicable and another validated method for protein determination must therefore be performed.*

Anti-A and anti-B haemagglutinins (*2.6.20*)

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to contain 3 IU of factor VIII:C per millilitre. The 1 to 64 dilutions do not show agglutination.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

Pyrogens (*2.6.8*)

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume of the preparation to be examined equivalent to not less than 50 IU of factor VIII:C.

ASSAY

Factor VIII (*2.7.4*)

The estimated potency is not less than 80 per cent and not more than 120 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

von Willebrand factor (*2.7.21*)

Carry out the assay of human von Willebrand factor for preparations intended for the treatment of von Willebrand's disease.

The estimated potency is not less than 60 per cent and not more than 140 per cent of the stated potency.

Pending the availability of an International Standard for von Willebrand factor concentrate calibrated for use in the collagen-binding assay, only the ristocetin cofactor assay may be used.

STORAGE

In an airtight container , protected from light.

LABELLING

The label states:

— the number of International Units of factor VIII:C and, where applicable, of von Willebrand factor in the container;

— the amount of protein in the container;

— the name and quantity of any added substance;

— the name and volume of the liquid to be used for reconstitution;

— where applicable, that the preparation may show the presence of a few small flakes or particles after reconstitution;

— that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Ph Eur

Dried Factor XI Fraction

General Notices

(Human Coagulation Factor XI, Ph Eur monograph 1644)

Action and use

Coagulation factor XI substitute.

Ph Eur

DEFINITION

Human coagulation factor XI is a plasma protein fraction containing coagulation factor XI. It is obtained from *Human plasma for fractionation (0853).*

When reconstituted as stated on the label, the potency is not less than 50 units of factor XI per millilitre.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and any residues are such as not to compromise the safety of the preparation for patients.

After preparation, the factor XI fraction is dissolved in a suitable liquid. Heparin, C_1-esterase inhibitor and antithrombin III may be added. The solution is distributed into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under nitrogen. No antimicrobial preservative is added.

CHARACTERS

A white or almost white powder or friable solid.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

The assay serves also to identify the preparation.

TESTS

Solubility

To a container of the preparation to be examined, add the volume of liquid stated on the label at room temperature. The preparation dissolves completely with gentle swirling within 10 min.

pH (*2.2.3*)

6.8 to 7.4.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Total protein

If necessary, dilute an accurately measured volume of the preparation to be examined with a 9 g/l solution of *sodium chloride R*, to obtain a solution that may be expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of that solution, in a round-bottomed centrifuge tube, add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R.* Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the amount of protein by multiplying the result by 6.25.

Activated coagulation factors (*2.6.22*)

For each of the dilutions, the coagulation time is not less than 150 s.

Heparin (*2.7.12*)

If heparin has been added, the preparation to be examined contains not more than the amount of heparin stated on the label and in any case not more than 0.5 IU of heparin per unit of factor XI.

Antithrombin III (*2.7.17*)

If antithrombin III has been added, the preparation to be examined contains not more than the amount of antithrombin III stated on the label.

C_1-esterase inhibitor

If C_1-esterase inhibitor has been added, the preparation to be examined contains not more than the amount of C_1-esterase inhibitor stated on the label.

The C_1-esterase inhibitor content of the preparation to be examined is determined by comparing its ability to inhibit C_1-esterase with the same ability of a reference preparation consisting of human normal plasma. 1 unit of C_1-esterase is equal to the activity of 1 ml of human normal plasma. Varying quantities of the preparation to be examined are mixed with an excess of C_1-esterase and the remaining C_1-esterase activity is determined using a suitable chromogenic substrate.

Method Reconstitute the preparation as stated on the label. Prepare an appropriate series of 3 or 4 independent dilutions from 1 unit/ml of factor XI, for both the preparation to be examined and the reference preparation, using a solution containing 9 g/l of *sodium chloride R* and either 10 g/l of *human albumin R* or 10 g/l of *bovine albumin R*. Warm all solutions to 37 °C in a water bath for 1-2 min before use. Place a suitable amount of C_1-esterase solution in tubes or in microtitre plate wells and incubate at 37 °C. Add a suitable amount of one of the dilutions of the reference preparation or of the preparation to be examined and incubate at 37 °C for 5 min. Add a suitable amount of a suitable chromogenic substrate such as methoxycarbonyl-L-lysyl(∈-benzyloxycarbonyl)-glycyl-L-arginine 4-nitroanilide. Read the rate of increase of absorbance (ΔA/min) at 405 nm. Carry out a blank test using *tris* *(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R* instead of the C_1-esterase and the substrate.

Calculate the C_1-esterase inhibitor content using the usual statistical methods (for example, *5.3.*).

Anti-A and anti-B haemagglutinins (*2.6.20*)

The 1 to 64 dilutions do not show agglutination.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

The reconstituted preparation complies with the test for sterility.

Pyrogens (*2.6.8*)

The reconstituted preparation complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume of the reconstituted preparation equivalent to 100 units of factor XI, calculated from the activity stated on the label.

ASSAY

Carry out the assay of human coagulation factor XI (*2.7.22*).

The estimated potency is not less than 80 per cent and not more than 120 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

STORAGE

Protected from light, at a temperature of 2 °C to 8 °C.

LABELLING

The label states:

- — the number of units per container,
- — the maximum amount of protein per container,
- — where applicable, the amount of heparin per container,
- — where applicable, the amount of antithrombin III per container,
- — where applicable, the amount of C_1-esterase inhibitor per container,
- — the name and volume of the liquid to be used for reconstitution.

Ph Eur

Dried Fibrinogen

General Notices

(Human Fibrinogen, Ph Eur monograph 0024)

Ph Eur

DEFINITION

Human fibrinogen contains the soluble constituent of human plasma that is transformed to fibrin on the addition of thrombin. It is obtained from *Human plasma for fractionation (0853)*. The preparation may contain excipients such as salts, buffers and stabilisers.

When dissolved in the volume of the solvent stated on the label, the solution contains not less than 10 g/l of fibrinogen.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and any residues are such as not to compromise the safety of the preparation for patients.

No antibiotic is added to the plasma used and no antimicrobial preservative is included in the preparation. The preparation is freeze-dried.

The method of preparation is such as to obtain fibrinogen with a specific activity (fibrinogen content with respect to total protein content) not less than 80 per cent. The fibrinogen content is determined by a suitable method such as that described under Assay, and the total protein content is determined by a suitable method such as that described under Total protein in *Human albumin solution (0255).* If a protein stabiliser (for example, human albumin) is added to the preparation, the requirement for specific activity applies to the fibrinogen before addition of the stabiliser. Albumin may also be obtained with fibrinogen during fractionation, in which case a specific determination of albumin is carried out by a suitable immunochemical method (*2.7.1*) and the quantity of albumin determined is subtracted from the total protein content for the calculation of the specific activity.

CHARACTERS

A white or pale yellow, hygroscopic powder or friable solid.

IDENTIFICATION

It complies with the limits of the assay.

TESTS

pH (*2.2.3*)

6.5 to 7.5.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Solubility

Add the volume of solvent stated on the label to the contents of one container. The preparation dissolves within 30 min at 20-25 °C, forming an almost colourless, slightly opalescent solution.

Stability of solution

Allow the reconstituted solution to stand at 20-25 °C. No gel formation appears within 60 min of reconstitution.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

The reconstituted preparation complies with the test for sterility.

Pyrogens (*2.6.8*)

The reconstituted preparation complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume of the reconstituted preparation equivalent to not less than 30 mg of fibrinogen, calculated from the quantity stated on the label.

ASSAY

Mix 0.2 ml of the reconstituted preparation with 2 ml of a suitable buffer solution (pH 6.6-6.8) containing sufficient thrombin (approximately 3 IU/ml) and calcium (0.05 mol/l). Maintain at 37 °C for 20 min, separate the precipitate by centrifugation (5000 g, 20 min) and wash thoroughly with a 9 g/l solution of *sodium chloride R*. Determine the nitrogen content by sulphuric acid digestion (*2.5.9*) and calculate the fibrinogen (clottable protein) content by multiplying the result by 6.0. The content is not less than 70 per cent and not more than 130 per cent of the amount of fibrinogen stated on the label.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states:

- — the amount of fibrinogen in the container;
- — the name and volume of the solvent to be used to reconstitute the preparation;
- — where applicable, the name and quantity of protein stabiliser in the preparation.

Ph Eur

Dried Prothrombin Complex

General Notices

(Human Prothrombin Complex, Ph Eur monograph 0554)

Action and use

Coagulation factor IX substitute. Preparations with appropriate activity may be used to correct deficiencies of coagulation factors II or X.

Ph Eur

DEFINITION

Human prothrombin complex is a plasma protein fraction containing blood coagulation factor IX together with variable amounts of coagulation factors II, VII and X; the presence and proportion of these additional factors depends on the method of fractionation. It is obtained from human plasma that complies with the monograph on *Human plasma for fractionation (0853).*

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of factor IX per millilitre.

If the content of any of the factors is stated as a single value, the estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency; if the content of any of the factors is stated as a range, the estimated potency is not less than the lower limit and not greater than the upper limit of the stated range.

PRODUCTION

The method of preparation is designed to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 0.6 IU of factor IX per milligram of total protein, before the addition of any protein stabiliser.

The prothrombin complex fraction is dissolved in a suitable liquid. Heparin, antithrombin and other auxiliary substances such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

CHARACTERS

A white or slightly coloured powder or friable solid, very hygroscopic.

Reconstitute the preparation to be examined as stated on the label immediately before *carrying out the identification, tests (except those for solubility and water) and assay.*

IDENTIFICATION

It complies with the limits of the assays for factor IX and for factor II and, where applicable, those for factors VII and X.

TESTS

Solubility

To a container of the preparation to be examined add the volume of the liquid stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 min, giving a clear solution that may be coloured.

pH (*2.2.3*)

6.5 to 7.5.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Total protein

If necessary, dilute an accurately measured volume of the reconstituted preparation with a 9 g/l solution of *sodium chloride R* to obtain a solution expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of the solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the amount of protein by multiplying the result by 6.25.

Activated coagulation factors (*2.6.22*)

If necessary, dilute the preparation to be examined to contain 20 IU of factor IX per millilitre. For each of the dilutions, the coagulation time is not less than 150 s.

Heparin

If heparin has been added during preparation, determine the amount present by the assay of heparin in coagulation factor concentrates (*2.7.12*). The preparation to be examined contains not more than the amount of heparin stated on the label and in all cases not more than 0.5 IU of heparin per International Unit of factor IX.

Thrombin

If the preparation to be examined contains heparin, determine the amount present as described in the test for heparin and neutralise it by addition of *protamine sulphate R* (10 µg of protamine sulphate neutralises 1 IU of heparin). In each of 2 test tubes, mix equal volumes of the reconstituted preparation and a 3 g/l solution of *fibrinogen R*. Keep one of the tubes at 37 °C for 6 h and the other at room temperature for 24 h. In a 3rd tube, mix a volume of the fibrinogen solution with an equal volume of a solution of *human thrombin R* (1 IU/ml) and place the tube in a water-bath at 37 °C. No coagulation occurs in the tubes containing the preparation to be examined. Coagulation occurs within 30 s in the tube containing thrombin.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near-infrared spectrometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

Pyrogens (*2.6.8*)

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume of the reconstituted preparation equivalent to not less than 30 IU of factor IX.

ASSAY

Factor IX

Carry out the assay of human coagulation factor IX (*2.7.11*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence interval ($P = 0.95$) of the estimated potency is not greater than 80 per cent to 125 per cent.

Factor II

Carry out the assay of human coagulation factor II (*2.7.18*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence interval ($P = 0.95$) of the estimated potency is not greater than 90 per cent to 111 per cent.

The estimated factor II potency is not less than 70 per cent and not more than 165 per cent of the estimated factor IX potency.

Factor VII

If the label states that the preparation contains factor VII, carry out the assay of human coagulation factor VII (*2.7.10*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence interval ($P = 0.95$) of the estimated potency is not greater than 80 per cent to 125 per cent.

Factor X

If the label states that the preparation contains factor X, carry out the assay of human coagulation factor X (*2.7.19*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence interval ($P = 0.95$) of the estimated potency is not greater than 90 per cent to 111 per cent.

STORAGE

In an airtight container , protected from light.

LABELLING

The label states:

— the number of International Units of factor IX, and the number or range of International Units of factor II per container;

— where applicable, the number or range of International Units of factor VII and factor X per container;

— the amount of protein per container;

— the name and quantity of any added substances, including, where applicable, heparin and antithrombin;

— the name and quantity of the liquid to be used for reconstitution;

— that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Ph Eur

Fibrin Sealant Kit

General Notices

(Ph Eur monograph 0903)

Ph Eur

DEFINITION

Fibrin sealant kit is essentially composed of 2 components, namely fibrinogen concentrate (component 1), a protein fraction containing human fibrinogen and a preparation containing human thrombin (component 2). A fibrin clot is rapidly formed when the 2 thawed or reconstituted components are mixed. Other ingredients (for example, human coagulation factor XIII, a fibrinolysis inhibitor or calcium ions) and stabilisers (for example, *Human albumin solution (0255)*) may be added. No antimicrobial preservative is added.

Human constituents are obtained from plasma that complies with the requirements of the monograph on *Human plasma for fractionation (0853).* No antibiotic is added to the plasma used.

When thawed or reconstituted as stated on the label, component 1 contains not less than 40 g/l of clottable protein; the thrombin activity of component 2 varies over a wide range (approximately 4-1000 IU/ml).

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and any residues are such as not to compromise the safety of the preparation for patients.

Constituents or mixtures of constituents are passed through a bacteria-retentive filter and distributed aseptically into sterile containers. Containers of freeze-dried constituents are closed under vacuum or filled with oxygen-free nitrogen or other suitable inert gas before being closed. In either case, they are closed so as to exclude micro-organisms.

If the human coagulation factor XIII content in component 1 is greater than 10 units/ml, the assay of factor XIII is carried out.

CHARACTERS

Freeze-dried constituents are hygroscopic, white or pale yellow powders or friable solids. Frozen constituents are colourless or pale yellow, opaque solids. Liquid constituents are colourless or pale yellow.

For the freeze-dried or frozen constituents, reconstitute or thaw as stated on the label immediately before carrying out the identification and the tests, except those for solubility and water.

COMPONENT 1 (FIBRINOGEN CONCENTRATE)

IDENTIFICATION

A. It complies with the limits of the assay of fibrinogen.

B. It complies with the limits of the assay of factor XIII (where applicable).

TESTS

pH (*2.2.3*)

6.5 to 8.0.

Solubility

Freeze-dried concentrates dissolve within 20 min in the volume of solvent for reconstitution and at the temperature stated on the label, forming an almost colourless, clear or slightly turbid solution.

Stability of solution

No gel formation appears at room temperature during 120 min following thawing or reconstitution.

Water

Determined by a suitable method, such as the semi-microdetermination (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

ASSAY

Fibrinogen (clottable protein)

The estimated content in milligrams of clottable protein is not less than 70 per cent and not more than 130 per cent of the content stated on the label.

Mix 0.2 ml of the reconstituted preparation with 2 ml of a suitable buffer solution (pH 6.6-7.4) containing sufficient *human thrombin R* (approximately 3 IU/ml) and calcium (0.05 mol/l). Maintain at 37 °C for 20 min, separate the precipitate by centrifugation (5000 *g*, 20 min), wash thoroughly with a 9 g/l solution of *sodium chloride R* and determine the protein as nitrogen by sulphuric acid digestion (*2.5.9*). Calculate the protein content by multiplying the result by 6.0. If for a particular preparation this method cannot be applied, use another validated method for determination of fibrinogen.

Factor XIII

Where the label indicates that the human coagulation factor XIII activity is greater than 10 units/ml, the estimated activity is not less than 80 per cent and not more than 120 per cent of the activity stated on the label.

Make at least 3 suitable dilutions of thawed or reconstituted component 1 and of human normal plasma (reference preparation) using as diluent coagulation factor XIII deficient plasma or another suitable diluent. Add to each dilution suitable amounts of the following reagents:

— activator reagent, containing bovine or human thrombin, a suitable buffer, calcium chloride and a suitable inhibitor such as Gly-Pro-Arg-Pro-Ala-NH_2 which inhibits clotting of the sample but does not prevent coagulation factor XIII activation by thrombin,

— detection reagent, containing a suitable factor XIIIa-specific peptide substrate, such as Leu-Gly-Pro-Gly-Glu-Ser-Lys-Val-Ile-Gly-NH_2 and glycine ethyl ester as 2^{nd} substrate in a suitable buffer solution,

— NADH reagent, containing glutamate dehydrogenase, α-ketoglutarate and NADH in a suitable buffer solution.

After mixing, the absorbance changes (ΔA/min) are measured at a wavelength of 340 nm, after the linear phase of the reaction is reached.

1 unit of factor XIII is equal to the activity of 1 ml of human normal plasma.

Calculate the activity of the test preparation by the usual statistical methods (*5.3*, for example) . The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated activity.

COMPONENT 2 (THROMBIN PREPARATION)

IDENTIFICATION

It complies with the limits of the assay of thrombin.

TESTS

pH (*2.2.3*)

5.0 to 8.0.

Solubility

Freeze-dried preparations dissolve within 5 min in the volume of solvent for reconstitution stated on the label, forming a colourless, clear or slightly turbid solution.

Water

Determined by a suitable method, such as the semi-microdetermination (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

ASSAY

Thrombin

The estimated activity is not less than 80 per cent and not more than 125 per cent of the activity stated on the label.

If necessary, dilute the reconstituted preparation to be examined to approximately 2-20 IU of thrombin per millilitre using as diluent a suitable buffer pH 7.3-7.5, such as *imidazole buffer solution pH 7.3 R* containing 10 g/l of *human albumin R* or *bovine albumin R*. To a suitable volume of the dilution, add a suitable volume of fibrinogen solution (1 g/l of clottable protein) warmed to 37 °C and start measurement of the clotting time immediately. Repeat the procedure with each of at least 3 dilutions, in the range stated above, of a reference preparation of thrombin, calibrated in International Units. Calculate the activity of the test preparation by the usual statistical methods (*5.3*, for example). The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated activity.

STORAGE

Protected from light and, for freeze-dried components, in an airtight container.

LABELLING

The label states:

- the amount of fibrinogen (milligrams of clottable protein), thrombin (International Units) per container, and coagulation factor XIII, if this is greater than 10 units/ml,
- where applicable, the name and volume of solvent to be used to reconstitute the components.

Ph Eur

Hepatitis A Immunoglobulin

General Notices

(Human Hepatitis A Immunoglobulin, Ph Eur monograph 0769)

Action and use

Ph Eur

DEFINITION

Human hepatitis A immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from selected donors having antibodies against hepatitis A virus. *Human normal immunoglobulin (0338)* may be added.

It complies with the monograph on *Human normal immunoglobulin (0338),* except for the minimum number of donors and the minimum total protein content.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (*2.7.1*).

The International Unit is the activity contained in a stated amount of the International Standard for anti-hepatitis A immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Human hepatitis A immunoglobulin BRP is calibrated in International Units by comparison with the International Standard.

The stated potency is not less than 600 IU/ml. The estimated potency is not less than the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

STORAGE

See *Human normal immunoglobulin (0338).*

LABELLING

See *Human normal immunoglobulin (0338).*

The label states the number of International Units per container.

Ph Eur

Hepatitis B Immunoglobulin for Intravenous Use

General Notices

(Human Hepatitis B Immunoglobulin for Intravenous Administration, Ph Eur monograph 1016)

Ph Eur

DEFINITION

Human hepatitis B immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. It is obtained from plasma from selected and/or immunised donors having antibodies against hepatitis B surface antigen. *Human normal immunoglobulin for intravenous administration (0918)* may be added.

It complies with the monograph on *Human normal immunoglobulinum for intravenous administration (0918)*, except for the minimum number of donors, the minimum total protein content and the limit for osmolality.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay (*2.7.1*) of suitable sensitivity and specificity.

The International Unit is the activity contained in a stated amount of the International Reference Preparation of hepatitis B immunoglobulin. The equivalence in International Units of the International Reference Preparation is stated by the World Health Organisation.

The stated potency is not less than 50 IU/ml. The estimated potency is not less than the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

STORAGE

See *Human normal immunoglobulin for intravenous administration (0918) .*

LABELLING

See *Human normal immunoglobulin for intravenous administration (0918) .*

The label states the minimum number of International Units of hepatitis B immunoglobulin per container.

Ph Eur

Hepatitis B Immunoglobulin

General Notices

(Human Hepatitis B Immunoglobulin, Ph Eur monograph 0722)

Action and use

Ph Eur

DEFINITION

Human hepatitis B immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from selected and/or immunised donors having antibodies against hepatitis B surface antigen. *Human normal immunoglobulin (0338)* may be added.

It complies with the monograph on *Human normal immunoglobulin (0338),* except for the minimum number of donors and the minimum total protein content.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (*2.7.1*).

The International Unit is the activity contained in a stated amount of the International Reference Preparation of hepatitis B immunoglobulin. The equivalence in International Units of the International Reference Preparation is stated by the World Health Organisation.

The stated potency is not less than 100 IU/ml. The estimated potency is not less than the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

STORAGE

See *Human normal immunoglobulin (0338).*

LABELLING

See *Human normal immunoglobulin (0338).*

The label states the number of International Units per container.

Ph Eur

Human α-1-proteinase Inhibitor

General Notices

(Ph Eur monograph 2387)

Ph Eur

DEFINITION

Human α-1-proteinase inhibitor is a plasma protein fraction containing mainly human α-1-proteinase inhibitor (also known as human α-1-antitrypsin or α-1-antiproteinase). Human α-1-proteinase inhibitor is a glycoprotein existing in isoforms with different isoelectric points and is the most abundant multifunctional serine proteinase inhibitor in human plasma. It is obtained from human plasma that complies with the monograph *Human plasma for fractionation (0853)*, using a suitable fractionation process and further purification steps. Other plasma proteins may be present.

PRODUCTION

GENERAL PROVISIONS

The method of preparation includes steps that have been shown to remove or to inactivate known agents of infection. The subsequent purification procedure must be validated to demonstrate that the concentration of any substances used for inactivation of viruses during production is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 0.35 mg of active human α-1-proteinase inhibitor per milligram of total protein. Ratio of human α-1 proteinase inhibitor activity to human α-1-proteinase inhibitor antigen is not less than 0.7.

Buffering and other auxiliary substances such as a stabiliser may be included. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter and distributed aseptically into the final containers. The product may be freeze-dried.

CONSISTENCY OF THE METHOD OF PRODUCTION

The consistency of the method of production, including demonstration that the manufacturing process yields a product with a consistent composition and maintains the functional integrity of human α-1-proteinase inhibitor, is evaluated by suitable analytical procedures that are determined during process development, and which include:

— assay of human α-1-proteinase inhibitor activity;

— determination of specific human α-1-proteinase inhibitor activity, expressed as the ratio of active human α-1-proteinase inhibitor to total protein;

— characterisation of isoform composition and protein structure by suitable methods such as isoelectric focusing *(2.2.54)*, spectrometric methods (for example, mass spectrometry) or capillary electrophoresis *(2.2.47)*;

— determination of the ratio of human α-1-proteinase inhibitor activity to human α-1-proteinase inhibitor antigen;

— characterisation of accompanying plasma proteins that might be present, by a set of suitable methods such as SDS-PAGE, cellulose acetate electrophoresis or capillary zone electrophoresis *(2.2.31)* and quantitative determination of relevant accompanying plasma proteins;

— determination of molecular-size distribution, used to quantify the polymeric forms of human α-1-proteinase inhibitor; consideration is given to the potential presence of accompanying proteins that might affect the results.

CHARACTERS

Appearance

Freeze-dried products are hygroscopic, white or pale yellow or pale brown powders or friable solids; liquid products are clear or slightly opalescent, colourless or pale yellow or pale green or pale brown.

If the preparation to be examined is freeze-dried, reconstitute it as stated on the label immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

The assay of human α-1-proteinase inhibitor activity serves to identify the preparation.

TESTS

pH *(2.2.3)*

6.5 to 7.8.

Solubility

To a container of the preparation to be examined add the volume of the liquid stated on the label at room temperature. The preparation dissolves completely when reconstituted according to the instructions for use, giving a clear, colourless or pale green or pale yellow or pale brown solution.

Osmolality *(2.2.35)*

Minimum 210 mosmol/kg.

Total protein

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion *(2.5.9)* and calculate the protein content by multiplying by 6.25.

Water

Determined by a suitable method, such as the semi-micro determination of water *(2.5.12)*, loss on drying *(2.2.32)* or near-infrared spectrophotometry *(2.2.40)*, the water content is within the limits approved by the competent authority.

Sterility *(2.6.1)*

It complies with the test.

Pyrogens *(2.6.8)*

It complies with the test. Inject per kilogram of the rabbit's mass a volume equivalent to not less than 60 mg of human α-1-proteinase inhibitor.

ASSAY

Carry out the assay of human α-1-proteinase inhibitor *(2.7.32)*. The estimated potency is not less than 80 per cent and not more than 120 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

STORAGE

Unless otherwise justified and authorised, in an airtight and sterile container, at a temperature not exceeding 25 °C.

LABELLING

The label states:

- the potency of active (functional) human α-1-proteinase inhibitor per container;
- the name and quantity of any added substances;
- the quantity of protein per container;
- where applicable, the name and volume of the liquid to be used for reconstitution;
- that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Ph Eur

Human Haematopoietic Stem Cells

General Notices

(Ph Eur monograph 2323)

Ph Eur

This monograph provides a standard for the preparation and control of human haematopoietic stem cells for use in therapy. It does not exclude the use of alternative preparation and control methods that are acceptable to the competent authority.

DEFINITION

Human haematopoietic stem cells are primitive multipotent cells capable of self-renewal as well as differentiation and maturation into all haematopoietic lineages. They are found in small numbers in bone marrow, in the mononuclear cell fraction of circulating blood and in umbilical cord blood. The preparation also contains haematopoietic progenitor cells, which are capable of differentiation but not self-renewal. The numbers of haematopoietic stem cells and haematopoietic progenitor cells are correlated.

This monograph applies to haematopoietic stem cells that have not undergone expansion or genetic modification, and that are intended to provide a successful engraftment leading to a permanent restoration of all lineages of blood cell production to a sufficient level and function in a recipient whose haematopoiesis has been compromised by, for example, disease or high doses of chemotherapy and/or radiation therapy, or has to be replaced in certain congenital diseases. The infused haematopoietic stem cells can originate from the recipient (autologous) or from another individual (allogeneic).

Haematopoietic stem cells are recognised by their ability to reconstitute human haematopoiesis *in vivo*. They also have the capacity to differentiate into colony-forming cells, which are able to give rise to colonies in the presence of various growth factors. The membrane marker CD34 is commonly used for the successful isolation/purification of haematopoietic stem cells from crude preparations and as an indicator of haematopoietic stem cell content in routine quality control.

PRODUCTION

DONORS

Where allogeneic cells are used, they are derived from carefully selected donors in accordance with donor selection criteria. Directive 2004/23/EC of the European Union deals with the criteria for donor selection.

COLLECTION

Peripheral blood stem cells These are collected by cytapheresis after mobilisation from the bone marrow by administration of growth factors and/or treatment of autologous donors with cytotoxic substances. The cells may be processed to select a population of interest and may be cryopreserved.

Bone marrow Bone marrow is harvested by aspirating the cells from the cavities of hollow bones, then removing bone fragments by filtration and, if necessary, separating the buffy coat cells after centrifugation or with commercial kits based on the cytapheresis principle. The cells may be processed to select a population of interest and may be cryopreserved.

Umbilical cord blood Placental blood haematopoietic cells are collected from placentae via the vein of the umbilical cord. The cells are then cryopreserved.

CRYOPRESERVATION

Cryopreservation allows storage for long periods. The cells are suspended in a validated medium containing a suitable cryoprotectant (for example, dimethyl sulphoxide) and macromolecules (for example, autologous plasma/albumin) and are frozen in cryobags in a manner designed to maintain viability of the cells by controlled cooling according to a validated method. They are stored at a temperature of - 140 °C or lower. Where cryobags are stored under other conditions of temperature and duration, the functionality of the preparation must be validated. Cryobags from donors that test positive for any infectious disease marker must be stored in such a way as to avoid cross-contamination.

SUBSTANCES USED IN PRODUCTION

The quality of substances used in production may be critical with respect to the quality, safety and efficacy of the final product, particularly for substances of biological origin. This is of particular importance for:

— proteins, including enzymes and antibodies;

— cryopreservation reagents;

— purification reagents.

Quality assurance

All substances must be produced within a recognised quality assurance system using suitable production facilities.

Quality specifications

A suitable quality specification must be presented for each substance, including notably:

— identity;

— potency (where applicable);

— purity;

— determination of bacterial endotoxins *(2.6.14)* (where applicable);

— microbiological quality (total viable count, tests for specified micro-organisms);

— sterility *(2.6.1)* (where applicable).

Viral safety

The requirements of chapter *5.1.7*. apply.

Transmissible spongiform encephalopathies

A risk assessment of the product with respect to transmissible spongiform encephalopathies is carried out, and suitable measures are taken to minimise any such risk *(5.2.8)*.

Water

Water used in the preparation of cellular products complies with the relevant monograph (*Water for injections (0169), Water, highly purified (1927), Purified water (0008)*). Water incorporated into the final product complies with the section on Water for injections in bulk in the monograph *Water for injections (0169)*, and in addition is sterile.

TESTS

Target specifications are established for the different tests, but these are not used as rigid acceptance criteria.

Tests carried out include the following (further tests, such as purging, cell depletion, allogeneic application, may be necessary depending on any treatment applied to the cells and on the intended recipient):

Nucleated cell count

Nucleated cells are counted using a validated automated apparatus (automated haematology analyser) or by a manual method (counting chamber).

Viability

Viability is assessed for products that are not infused within 24 h of collection. It is determined either using a validated automated apparatus (for example, by flow cytometry *(2.7.24)* with nucleic acid intercalating agents such as propidium iodide or 7-aminoactinomycin D) or by a manual method (for example, visual observation of trypan blue staining).

CD34+ cell count

For peripheral blood stem cells, CD34+ cell count is determined using a validated automated apparatus to analyse cells labelled with anti-CD34 antibodies. The apparatus and method employed must be able to determine the number of CD34+ cells with a sensitivity, accuracy and reproducibility comparable with those of immunophenotyping *(2.7.23)*, where cells are labelled using anti-CD34 and anti-CD45 antibodies conjugated to a fluorochrome and analysed by flow cytometry *(2.7.24)*.

Colony-forming cell (CFC) assay

Proliferative capacity is established by a suitable assay. The test is not necessarily carried out on each unit. The correlation between the dose of CD34 and the number of CFCs in a given situation (pathology, packaging, mobilisation) is determined. The CFC assay is carried out periodically; whenever a change that could affect the quality of CD34+ cells is made to the protocol for packaging or mobilisation, it is carried out on a suitable number of units.

Microbiological control

Examine as prescribed in general method *2.6.27. Microbiological control of cellular products*. Where justified, the product may be released before completion of the test.

Ph Eur

Measles Immunoglobulin

General Notices

(Human Measles Immunoglobulin, Ph Eur monograph 0397)

Ph Eur

DEFINITION

Human measles immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma containing specific antibodies against the measles virus. *Human normal immunoglobulin (0338)* may be added.

It complies with the monograph on *Human normal immunoglobulin (0338),* except for the minimum number of donors and the minimum total protein content.

POTENCY

The potency of the liquid preparation and of the freeze-dried preparation after reconstitution as stated on the label is not less than 50 IU per millilitre of neutralising antibody against measles virus.

The potency is determined by comparing the antibody titres of the immunoglobulin to be examined and of a reference preparation calibrated in International Units, using a challenge dose of measles virus in a suitable cell culture system. A method of equal sensitivity and precision may be used providing that the competent authority is satisfied that it correlates with neutralising activity for the measles virus by comparison with the reference preparation.

The International Unit is the specific neutralising activity for measles virus contained in a stated amount of the International Standard for human anti-measles serum. The equivalence in International Units of the International Reference Preparation is stated by the World Health Organisation.

Prepare serial two-fold dilutions of the immunoglobulin to be examined and of the reference preparation. Mix each dilution with an equal volume of a suspension of measles virus containing about 100 $CCID_{50}$ in 0.1 ml and incubate protected from light at 37 °C for 2 h. Using not fewer than six cell cultures per mixture, inoculate 0.2 ml of each mixture into each of the cell cultures allocated to that mixture and incubate for not less than 10 days. Examine the cultures for viral activity and compare the dilution containing the smallest quantity of the immunoglobulin which neutralises the virus with that of the corresponding dilution of the reference preparation.

Calculate the potency of the immunoglobulin to be examined in International Units per millilitre of neutralising antibody against measles virus.

STORAGE

See *Human normal immunoglobulin (0338).*

LABELLING

See *Human normal immunoglobulin (0338).*

The label states the number of International Units per container.

Ph Eur

Normal Immunoglobulin for Intravenous Use

General Notices

(Human Normal Immunoglobulin for Intravenous Administration, Ph Eur monograph 0918)

Ph Eur

DEFINITION

Human normal immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G (IgG). Other proteins may be present. Human normal immunoglobulin for intravenous administration contains the IgG antibodies of normal subjects. This monograph does not apply to products intentionally prepared to contain fragments or chemically modified IgG.

Human normal immunoglobulin for intravenous administration is obtained from plasma that complies with the requirements of the monograph on *Human plasma for fractionation (0853).* No antibiotic is added to the plasma used.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the immunoglobulin.

The product shall have been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated when administered intravenously.

Human normal immunoglobulin for intravenous administration is prepared from pooled material from not fewer than 1000 donors by a method that has been shown to yield a product that:

— does not transmit infection;

— at an immunoglobulin concentration of 50 g/l, contains antibodies for at least 2 of which (1 viral and 1 bacterial) an International Standard or Reference Preparation is available, the concentration of such antibodies being at least 3 times that in the initial pooled material;

— has a defined distribution of immunoglobulin G subclasses;

— complies with the test for Fc function of immunoglobulin (*2.7.9*).

Human normal immunoglobulin for intravenous administration is prepared as a stabilised solution or as a freeze-dried preparation. A stabiliser may be added. In both cases the preparation is passed through a bacteria-retentive filter. The preparation may subsequently be freeze-dried and the containers closed under vacuum or under an inert gas. No antimicrobial preservative is added either during fractionation or at the stage of the final bulk solution.

The stability of the preparation is demonstrated by suitable tests carried out during development studies.

CHARACTERS

The liquid preparation is clear or slightly opalescent and colourless or pale yellow. The freeze-dried preparation is a hygroscopic, white or slightly yellow powder or solid friable mass.

For the freeze-dried preparation, reconstitute as stated on the label immediately before carrying out the identification and the tests, except those for solubility and water.

IDENTIFICATION

Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and the preparation to be examined, both diluted to contain 10 g/l of protein. The main component of the preparation to be examined corresponds to the IgG component of normal human serum. The preparation to be examined may show the presence of small quantities of other plasma proteins; if human albumin has been added as a stabiliser, it may be seen as a major component.

TESTS

Solubility

For the freeze-dried preparation, add the volume of the liquid stated on the label. The preparation dissolves completely within 30 min at 20-25 °C.

pH (*2.2.3*)

4.0 to 7.4.

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing 10 g/l of protein.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Total protein

Minimum 30 g/l and between 90 per cent and 110 per cent of the quantity of protein stated on the label.

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the centrifugation residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the content of protein by multiplying the result by 6.25.

Protein composition

Zone electrophoresis (*2.2.31*).

Use strips of suitable cellulose acetate gel or suitable agarose gel as the supporting medium and *barbital buffer solution pH 8.6 R1* as the electrolyte solution.

If cellulose acetate is the supporting material, the method described below can be used. If agarose gels are used, and because they are normally part of an automated system, the manufacturer's instructions are followed instead.

Test solution Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to an immunoglobulin concentration of 30 g/l.

Reference solution Reconstitute *human immunoglobulin for electrophoresis BRP* and dilute with a 9 g/l solution of *sodium chloride R* to a protein concentration of 30 g/l.

To a strip apply 4.0 µl of the test solution as a 10 mm band or apply 0.4 µl per millimetre if a narrower strip is used. To another strip apply in the same manner the same volume of the reference solution. Apply a suitable electric field such that the albumin band of normal human serum applied on a control strip migrates at least 30 mm. Stain the strips with *amido black 10B solution R* for 5 min. Decolourise with a mixture of 10 volumes of *glacial acetic acid R* and 90 volumes of *methanol R* so that the background is just free of colour. Develop the transparency of the strips with a mixture of 19 volumes of *glacial acetic acid R* and 81 volumes of *methanol R*. Measure the absorbance of the bands at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strip.

System suitability In the electropherogram obtained with the reference solution on cellulose acetate or on agarose gels, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Results In the electropherogram obtained with the test solution on cellulose acetate or on agarose gels, not more than 5 per cent of protein has a mobility different from that of the principal band. This limit is not applicable if albumin has been added to the preparation as a stabiliser; for such preparations, a test for protein composition is carried out during manufacture before addition of the stabiliser.

Distribution of molecular size

Liquid chromatography (*2.2.29*).

Test solution Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to a concentration suitable for the chromatographic system used. A concentration in the range of 4-12 g/l and injection of 50-600 µg of protein are usually suitable.

Reference solution Dilute *human immunoglobulin (molecular size) BRP* with a 9 g/l solution of *sodium chloride R* to the same protein concentration as the test solution.

Column:

- *size: l* = 0.6 m, Ø = 7.5 mm, or l = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins with relative molecular masses in the range 10 000 to 500 000.

Mobile phase Dissolve 4.873 g of *disodium hydrogen phosphate dihydrate R*, 1.741 g of *sodium dihydrogen phosphate monohydrate R*, 11.688 g of *sodium chloride R* and 50 mg of *sodium azide R* in 1 litre of *water R*.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 280 nm.

In the chromatogram obtained with the reference solution, the principal peak corresponds to the IgG monomer and there is a peak corresponding to the dimer with a relative retention to the principal peak of about 0.85. Identify the peaks in the chromatogram obtained with the test solution by comparison with the chromatogram obtained with the reference solution; any peak with a retention time shorter than that of the dimer corresponds to polymers and aggregates.

Results In the chromatogram obtained with the test solution:

— *relative retention*: for the monomer and for the dimer, the relative retention to the corresponding peak in the chromatogram obtained with the reference solution is 1 ± 0.02;

— *peak area*: the sum of the peak areas of the monomer and the dimer represent not less than 90 per cent of the total area of the chromatogram and the sum of the peak areas of polymers and aggregates represents not more than 3 per cent of the total area of the chromatogram. This requirement does not apply to products where albumin has been added as a stabiliser; for products stabilised with albumin, a test for distribution of molecular size is carried out during manufacture before addition of the stabiliser.

Anticomplementary activity (*2.6.17*)

The consumption of complement is not greater than 50 per cent (1 CH_{50} per milligram of immunoglobulin).

Prekallikrein activator (*2.6.15*)

Maximum 35 IU/ml, calculated with reference to a dilution of the preparation to be examined containing 30 g/l of immunoglobulin.

Anti-A and anti-B haemagglutinins (*2.6.20*)

Carry out the tests for anti-A and anti-B haemagglutinins. If the preparation to be examined contains more than 30 g/l of immunoglobulin, dilute to this concentration before preparing the dilutions to be used in the test. The 1 to 64 dilutions do not show agglutination.

Anti-D antibodies (*2.6.26*)

It complies with the test for anti-D antibodies in human immunoglobulin for intravenous administration.

Antibody to hepatitis B surface antigen

Minimum 0.5 IU/g of immunoglobulin, determined by a suitable immunochemical method (*2.7.1*).

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

Pyrogens (*2.6.8*)

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume equivalent to 0.5 g of immunoglobulin but not more than 10 ml per kilogram of body mass.

STORAGE

For the liquid preparation, store in a colourless glass container, protected from light, at the temperature stated on the label. For the freeze-dried preparation, store in an airtight colourless glass container, protected from light, at a temperature not exceeding 25 °C.

LABELLING

The label states:

- — for liquid preparations, the volume of the preparation in the container and the protein content expressed in grams per litre;
- — for freeze-dried preparations, the quantity of protein in the container;
- — the amount of immunoglobulin in the container;
- — the route of administration;
- — for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added;
- — the distribution of subclasses of immunoglobulin G present in the preparation;
- — where applicable, the amount of albumin added as a stabiliser;
- — the maximum content of immunoglobulin A.

Ph Eur

Normal Immunoglobulin

General Notices

Normal Immunoglobulin Injection

(Human Normal Immunoglobulin, Ph Eur monograph 0338)

Ph Eur

DEFINITION

Human normal immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G (IgG). Other proteins may be present. Human normal immunoglobulin contains the IgG antibodies of normal subjects. It is intended for intramuscular or subcutaneous administration.

Human normal immunoglobulin is obtained from plasma that complies with the requirements of the monograph on *Human plasma for fractionation (0853)*. No antibiotic is added to the plasma used.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the immunoglobulin.

The product shall have been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated when administered intramuscularly or subcutaneously.

Human normal immunoglobulin is prepared from pooled material from at least 1000 donors by a method that has been shown to yield a product that:

— does not transmit infection;

— at a protein concentration of 160 g/l, contains antibodies for at least 2 of which (1 viral and 1 bacterial) an International Standard or Reference Preparation is available, the concentration of such antibodies being at least 10 times that in the initial pooled material.

If the human normal immunoglobulin is intended for subcutaneous administration, the production method shall have been shown to consistently yield products that comply with the test for Fc function of immunoglobulin *(2.7.9)*.

Human normal immunoglobulin is prepared as a stabilised solution, for example in a 9 g/l solution of sodium chloride, a 22.5 g/l solution of glycine or, if the preparation is to be freeze-dried, a 60 g/l solution of glycine. Multidose preparations contain an antimicrobial preservative. Single-dose preparations do not contain an antimicrobial preservative. Any antimicrobial preservative or stabilising agent used shall have been shown to have no deleterious effect on the final product in the amount present. The solution is passed through a bacteria-retentive filter. The preparation may subsequently be freeze-dried and the containers closed under vacuum or under an inert gas.

The stability of the preparation is demonstrated by suitable tests carried out during development studies.

CHARACTERS

The liquid preparation is clear and pale-yellow or light-brown; during storage it may show formation of slight turbidity or a small amount of particulate matter. The freeze-dried preparation is a hygroscopic, white or slightly yellow powder or solid, friable mass.

For the freeze-dried preparation, reconstitute as stated on the label immediately before carrying out the identification and the tests, except those for solubility and water.

IDENTIFICATION

Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and the preparation to be examined, both diluted to a protein concentration of 10 g/l. The main component of the preparation to be examined corresponds to the IgG component of normal human serum. The solution may show the presence of small quantities of other plasma proteins.

TESTS

Solubility

For the freeze-dried preparation, add the volume of the liquid stated on the label. The preparation dissolves completely within 20 min at 20-25 °C.

pH *(2.2.3)*

5.0 to 7.2.

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to a protein concentration of 10 g/l.

Total protein

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion *(2.5.9)* and calculate the content of protein by multiplying the result by 6.25. The preparation has a protein concentration of not less than 100 g/l and not more than 180 g/l and contains not less than 90 per cent and not more than 110 per cent of the quantity of protein stated on the label.

Protein composition

Examine by zone electrophoresis *(2.2.31)*.

Use strips of suitable cellulose acetate gel or suitable agarose gel as the supporting medium and *barbital buffer solution pH 8.6 R1* as the electrolyte solution.

If cellulose acetate is the supporting material, the method described below can be used. If agarose gels are used, and because they are normally part of an automated system, the manufacturer's instructions are followed instead.

Test solution Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to a protein concentration of 50 g/l.

Reference solution Reconstitute *human immunoglobulin for electrophoresis BRP* and dilute with a 9 g/l solution of *sodium chloride R* to a protein concentration of 50 g/l.

To a strip apply 2.5 µl of the test solution as a 10 mm band or apply 0.25 µl per millimetre if a narrower strip is used. To another strip apply in the same manner the same volume of the reference solution. Apply a suitable electric field such that the albumin band of normal human serum applied on a control strip migrates at least 30 mm. Stain the strip with *amido black 10B solution R* for 5 min. Decolourise with a mixture of 10 volumes of *glacial acetic acid R* and 90 volumes of *methanol R* so that the background is just free of colour. Develop the transparency of the strips with a mixture of 19 volumes of *glacial acetic acid R* and 81 volumes of *methanol R*. Measure the absorbance of the bands at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strip.

System suitability In the electropherogram obtained with the reference solution on cellulose acetate or on agarose gels, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Results In the electropherogram obtained with the test solution on cellulose acetate or on agarose gels, not more than 10 per cent of protein has a mobility different from that of the principal band.

Distribution of molecular size

Liquid chromatography *(2.2.29)*.

Test solution Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to a concentration suitable for the chromatographic system used. A concentration in the range of 4-12 g/l and injection of 50-600 µg of protein are usually suitable.

Reference solution Dilute *human immunoglobulin (molecular size) BRP* with a 9 g/l solution of *sodium chloride R* to the same protein concentration as the test solution.

Column:

— *size:* l = 0.6 m, Ø = 7.5 mm [or l = 0.3 m, Ø = 7.8 mm];

— *stationary phase: hydrophilic silica gel for chromatography R*, of a grade suitable for fractionation of globular proteins with relative molecular masses in the range 10 000 to 500 000.

Mobile phase Dissolve 4.873 g of *disodium hydrogen phosphate dihydrate R*, 1.741 g of *sodium dihydrogen phosphate monohydrate R*, 11.688 g of *sodium chloride R* and 50 mg of *sodium azide R* in 1 litre of *water R*.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 280 nm.

In the chromatogram obtained with the reference solution, the principal peak corresponds to the IgG monomer and there is a peak corresponding to the dimer with a relative retention to the principal peak of about 0.85. Identify the peaks in the chromatogram obtained with the test solution by comparison with the chromatogram obtained with the reference solution; any peak with a retention time shorter than that of the dimer corresponds to polymers and aggregates. The preparation to be examined complies with the test if, in the chromatogram obtained with the test solution:

— *relative retention*: for the monomer and for the dimer, the relative retention to the corresponding peak in the chromatogram obtained with the reference solution is 1 ± 0.02;

— *peak area*: the sum of the peak areas of the monomer and the dimer represent not less than 85 per cent of the total area of the chromatogram and the sum of the peak areas of polymers and aggregates represents not more than 10 per cent of the total area of the chromatogram.

Anti-A and anti-B haemagglutinins *(2.6.20)*

If human normal immunoglobulin is intended for subcutaneous administration, carry out the tests for anti-A and anti-B haemagglutinins. Dilute the preparation to be examined to an immunoglobulin concentration of 30 g/l before preparing the dilutions to be used in the test. The 64-fold dilutions do not show agglutination.

Anti-D antibodies *(2.6.26)*

If human normal immunoglobulin is intended for subcutaneous administration, it complies with the test for anti-D antibodies in human immunoglobulin for intravenous administration.

Antibody to hepatitis B surface antigen

Not less than 0.5 IU/g of immunoglobulin, determined by a suitable immunochemical method *(2.7.1)*.

Antibody to hepatitis A virus

If intended for use in the prophylaxis of hepatitis A, it complies with the following additional requirement. Determine the antibody content by comparison with a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity *(2.7.1)*.

The International Unit is the activity contained in a stated amount of the International Standard for anti-hepatitis A immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Human hepatitis A immunoglobulin BRP is calibrated in International Units by comparison with the International Standard.

The stated potency is not less than 100 IU/ml. The estimated potency is not less than the stated potency. The confidence limits ($P = 0.95$) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

Water

Determined by a suitable method, such as the semi-micro determination of water *(2.5.12)*, loss on drying *(2.2.32)* or near infrared spectrophotometry *(2.2.40)*, the water content is within the limits approved by the competent authority.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

It complies with the test for pyrogens. Inject 1 ml per kilogram of the rabbit's mass.

STORAGE

For the liquid preparation, in a colourless glass container, protected from light. For the freeze-dried preparation, in an airtight colourless glass container, protected from light.

LABELLING

The label states:

— for liquid preparations, the volume of the preparation in the container and the protein content expressed in grams per litre;

— for freeze-dried preparations, the quantity of protein in the container;

— the route of administration;

— for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added;

— where applicable, that the preparation is suitable for use in the prophylaxis of hepatitis A infection;

— where applicable, the anti-hepatitis A virus activity in International Units per millilitre;

— where applicable, the name and amount of antimicrobial preservative in the preparation.

Ph Eur

Plasma (Pooled and Treated for Virus Inactivation)

General Notices

(Human Plasma (Pooled and Treated for Virus Inactivation), Ph Eur monograph 1646)

Ph Eur

DEFINITION

Human plasma (pooled and treated for virus inactivation) is a frozen or freeze-dried, sterile, non-pyrogenic preparation obtained from human plasma derived from donors belonging to the same ABO blood group. The preparation is thawed or reconstituted before use to give a solution for infusion.

The human plasma used complies with the monograph on *Human plasma for fractionation (0853)*.

PRODUCTION

The units of plasma to be used are cooled to - 30 °C or lower within 6 h of separation of cells and always within 24 h of collection.

The pool is prepared by mixing units of plasma belonging to the same ABO blood group.

The pool of plasma is tested for hepatitis B surface antigen (HBsAg) and for HIV antibodies using test methods of suitable sensitivity and specificity; the pool must give negative results in these tests.

Hepatitis C virus RNA

The plasma pool is tested using a validated nucleic acid amplification technique *(2.6.21)*. A positive control with 100 IU of hepatitis C virus RNA per millilitre and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors. The pool complies with the test if it is found non-reactive for hepatitis C virus RNA.

Hepatitis C virus RNA for NAT testing BRP is suitable for use as a positive control.

To limit the potential burden of B19 virus in plasma pools, the plasma pool is also tested for B19 virus using a validated nucleic acid amplification technique *(2.6.21)*.

B19 virus DNA

The plasma pool contains not more than 10.0 IU/µl.

A positive control with 10.0 IU of B19 virus DNA per microlitre and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors.

B19 virus DNA for NAT testing BRP is suitable for use as a positive control.

The method of preparation is designed to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to inactivate known agents of infection; if substances are used for the inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

Inactivation process

The solvent-detergent process, which is one of the methods used to inactivate enveloped viruses, uses treatment with a combination of tributyl phosphate and octoxinol 10; these reagents are subsequently removed by oil extraction or by solid phase extraction so that the amount in the final product is less than 2 µg/ml for tributyl phosphate and less than 5 µg/ml for octoxinol 10.

No antimicrobial preservative is added.

The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen; it may subsequently be freeze-dried.

Plastic containers comply with the requirements for sterile plastic containers for human blood and blood components *(3.2.3)*.

Glass containers comply with the requirements for glass containers for pharmaceutical use *(3.2.1)*.

CHARACTERS

The frozen preparation, after thawing, is a clear or slightly opalescent liquid free from solid and gelatinous particles. The freeze-dried preparation is an almost white or slightly yellow powder or friable solid.

Thaw or reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests and assay.

IDENTIFICATION

A. Examine by electrophoresis *(2.2.31)* comparing with normal human plasma. The electropherograms show the same bands.

B. It complies with the test for anti-A and anti-B haemagglutinins (see Tests).

TESTS

pH *(2.2.3)*

6.5 to 7.6.

Osmolality *(2.2.35)*

Minimum 240 mosmol/kg.

Total protein

Minimum 45 g/l.

Dilute with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. Place 2.0 ml of this solution in a round-bottomed centrifuge tube and add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free* *sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion *(2.5.9)* and calculate the quantity of protein by multiplying the result by 6.25.

Activated coagulation factors *(2.6.22)*

It complies with the test for activated coagulation factors. Carry out the test with 0.1 ml of the preparation to be examined instead of 10-fold and 100-fold dilutions. The coagulation time for the preparation to be examined is not less than 150 s.

Anti-A and anti-B haemagglutinins *(2.6.20)*

The presence of haemagglutinins (anti-A or anti-B) corresponds to the blood group stated on the label.

Hepatitis A virus antibodies

Minimum 2 IU/ml, determined by a suitable immunochemical method *(2.7.1)*.

Human hepatitis A immunoglobulin BRP is suitable for use as a reference preparation.

Irregular erythrocyte antibodies

The preparation to be examined does not show the presence of irregular erythrocyte antibodies when examined without dilution by an indirect antiglobulin test.

Citrate

Liquid chromatography *(2.2.29)*.

Test solution Dilute the preparation to be examined with an equal volume of a 9 g/l solution of *sodium chloride R*. Filter the solution using a filter with 0.45 µm pores.

Reference solution Dissolve 0.300 g of *sodium citrate R* in *water R* and dilute to 100.0 ml with the same solvent.

Column:
- *size:* l = 0.3 m, Ø = 7.8 mm;
- *stationary phase: cation exchange resin R* (9 µm).

Mobile phase 0.51 g/l solution of *sulphuric acid R*.

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 215 nm.

Equilibration 15 min.

Injection 10 µl.

Retention time Citrate = about 10 min.

Limit:
- *citrate*: maximum 25 mmol/l.

Calcium

Maximum 5.0 mmol/l.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Source Calcium hollow-cathode lamp using a transmission band preferably of 0.5 nm.

Wavelength 622 nm.

Atomisation device Air-acetylene or acetylene-propane flame.

Potassium

Maximum 5.0 mmol/l.

Atomic emission spectrometry *(2.2.22, Method I)*.

Wavelength 766.5 nm.

Sodium

Maximum 2.00×10^2 mmol/l.

Atomic emission spectrometry *(2.2.22, Method I)*.

Wavelength 589 nm.

Water

Determined by a suitable method, such as the semi-micro determination of water *(2.5.12)*, loss on drying *(2.2.32)* or near-infrared spectrometry *(2.2.40)*, the water content is within the limits approved by the competent authority (freeze-dried product).

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

It complies with the test for pyrogens. Inject 3 ml per kilogram of the rabbit's mass.

ASSAY

Factor VIII

Carry out the assay of human coagulation factor VIII *(2.7.4)* using a reference plasma calibrated against the International Standard for blood coagulation factor VIII in plasma.

The estimated potency is not less than 0.5 IU/ml. The confidence limits (*P* = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Factor V

Carry out the assay of human coagulation factor V described below using a reference plasma calibrated against the International Standard for blood coagulation factor V in plasma.

Using *imidazole buffer solution pH 7.3 R*, prepare at least 3 two-fold dilutions of the preparation to be examined, preferably in duplicate, from 1 in 10 to 1 in 40. Test each dilution as follows: mix 1 volume of *plasma substrate deficient in factor V R*, 1 volume of the dilution to be examined, 1 volume of *thromboplastin R* and 1 volume of a 3.5 g/l solution of *calcium chloride R*; measure the coagulation times, i.e. the interval between the moment at which the calcium chloride solution is added and the 1st indication of the formation of fibrin, which may be observed visually or by means of a suitable apparatus.

In the same manner, determine the coagulation time of 4 twofold dilutions (1 in 10 to 1 in 80) of human normal plasma in *imidazole buffer solution pH 7.3 R*. 1 unit of factor V is equal to the activity of 1 ml of human normal plasma. Human normal plasma is prepared by pooling plasma units from not fewer than 30 donors and stored at - 30 °C or lower.

Check the validity of the assay and calculate the potency of the test preparation by the usual statistical methods for a parallel-line assay (for example, *5.3*).

The estimated potency is not less than 0.5 IU/ml. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Factor XI

Carry out the assay of human coagulation factor XI *(2.7.22)* using a reference plasma calibrated against the International Standard for blood coagulation factor XI in plasma.

The estimated potency is not less than 0.5 IU/ml. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

Protein C

Carry out the assay of human protein C *(2.7.30)* using a reference plasma calibrated against the International Standard for human protein C in plasma.

The estimated potency is not less than 0.7 IU/ml. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Protein S

Carry out the assay of human protein S *(2.7.31)* using a reference plasma calibrated against the International Standard for human protein S in plasma.

The estimated potency is within the limits approved for the particular product. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Plasmin inhibitor (α_2-antiplasmin)

Carry out the assay of human plasmin inhibitor *(2.7.25)* using a reference plasma calibrated against a suitable reference standard for human plasmin inhibitor (α_2-antiplasmin) in plasma.

The estimated potency is not less than 0.2 units/ml. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

LABELLING

The label states:

- the ABO blood group;
- the method used for virus inactivation.

Ph Eur

Plasma for Fractionation

General Notices

(Human Plasma for Fractionation, Ph Eur monograph 0853)

Ph Eur

DEFINITION

Human plasma for fractionation is the liquid part of human blood remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant, or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure; it is intended for the manufacture of plasma-derived products.

PRODUCTION

DONORS

Only a carefully selected, healthy donor who, as far as can be ascertained after medical examination, laboratory blood tests and a study of the donor's medical history, is free from detectable agents of infection transmissible by plasma-derived products may be used. Recommendations in this field are made by the Council of Europe [*Recommendation No. R (95) 15 on the preparation, use and quality assurance of blood components*, or subsequent revision]; a directive of the European Union also deals with the matter: *Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components*.

Immunisation of donors

Immunisation of donors to obtain immunoglobulins with specific activities may be carried out when sufficient supplies of material of suitable quality cannot be obtained from naturally immunised donors. Recommendations for such immunisations are formulated by the World Health Organisation (*Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives*, WHO Technical Report Series, No. 840, 1994 or subsequent revision).

Records

Records of donors and donations made are kept in such a way that, while maintaining the required degree of confidentiality concerning the donor's identity, the origin of each donation in a plasma pool and the results of the corresponding acceptance procedures and laboratory tests can be traced.

Laboratory tests

Laboratory tests are carried out for each donation to detect the following viral markers:

1. antibodies against human immunodeficiency virus 1 (anti-HIV-1);

2. antibodies against human immunodeficiency virus 2 (anti-HIV-2);
3. hepatitis B surface antigen (HBsAg);
4. antibodies against hepatitis C virus (anti-HCV).

The test methods used are of suitable sensitivity and specificity and comply with the regulations in force. If a repeat-reactive result is found in any of these tests, the donation is not accepted.

INDIVIDUAL PLASMA UNITS

The plasma is prepared by a method that removes cells and cell debris as completely as possible. Whether prepared from whole blood or by plasmapheresis, the plasma is separated from the cells by a method designed to prevent the introduction of micro-organisms. No antibacterial or antifungal agent is added to the plasma. The containers comply with the requirements for glass containers *(3.2.1)* or for plastic containers for blood and blood components *(3.2.3)*. The containers are closed so as to prevent any possibility of contamination.

If 2 or more units are pooled prior to freezing, the operations are carried out using sterile connecting devices or under aseptic conditions and using containers that have not previously been used.

When obtained by plasmapheresis or from whole blood (after separation from cellular elements), plasma intended for the recovery of proteins that are labile in plasma is frozen within 24 h of collection by cooling rapidly in conditions validated to ensure that a temperature of - 25 °C or below is attained at the core of each plasma unit within 12 h of placing in the freezing apparatus.

When obtained by plasmapheresis, plasma intended solely for the recovery of proteins that are not labile in plasma is frozen by cooling rapidly in a chamber at - 20 °C or below as soon as possible and at the latest within 24 h of collection.

When obtained from whole blood, plasma intended solely for the recovery of proteins that are not labile in plasma is separated from cellular elements and frozen in a chamber at - 20 °C or below as soon as possible and at the latest within 72 h of collection.

It is not intended that the determination of total protein and factor VIII shown below be carried out on each unit of plasma. They are rather given as guidelines for good manufacturing practice, the test for factor VIII being relevant for plasma intended for use in the preparation of concentrates of labile proteins.

The total protein content of a unit of plasma depends on the serum protein content of the donor and the degree of dilution inherent in the donation procedure. When plasma is obtained from a suitable donor and using the intended proportion of anticoagulant solution, a total protein content complying with the limit of 50 g/l is obtained. If a volume of blood or plasma smaller than intended is collected into the anticoagulant solution, the resulting plasma is not necessarily unsuitable for pooling for fractionation. The aim of good manufacturing practice must be to achieve the prescribed limit for all normal donations.

Preservation of factor VIII in the donation depends on the collection procedure and the subsequent handling of the blood and plasma. With good practice, 0.7 IU/ml can usually be achieved, but units of plasma with a lower activity may still be suitable for use in the production of coagulation factor concentrates. The aim of good manufacturing practice is to conserve labile proteins as much as possible.

Total protein

Carry out the test using a pool of not fewer than 10 units. Dilute the pool with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion *(2.5.9)* and calculate the protein content by multiplying the quantity of nitrogen by 6.25. The total protein content is not less than 50 g/l.

Factor VIII

Carry out the test using a pool of not fewer than 10 units. Thaw the samples to be examined, if necessary, at 37 °C. Carry out the assay of factor VIII *(2.7.4)*, using a reference plasma calibrated against the International Standard for human coagulation factor VIII in plasma. The activity is not less than 0.7 IU/ml.

STORAGE AND TRANSPORT

Frozen plasma is stored and transported in conditions designed to maintain the temperature at or below - 20 °C; for accidental reasons, the storage temperature may rise above - 20 °C on one or more occasions during storage and transport but the plasma is nevertheless considered suitable for fractionation if all the following conditions are fulfilled:

— the total period of time during which the temperature exceeds - 20 °C does not exceed 72 h;

— the temperature does not exceed - 15 °C on more than one occasion;

— the temperature at no time exceeds - 5 °C.

POOLED PLASMA

During the manufacture of plasma products, the first homogeneous pool of plasma (for example, after removal of cryoprecipitate) is tested for HBsAg and for HIV antibodies using test methods of suitable sensitivity and specificity; the pool must give negative results in these tests.

The plasma pool is also tested for hepatitis C virus RNA using a validated nucleic acid amplification technique *(2.6.21)*. A positive control with 100 IU/ml of hepatitis C virus RNA and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors. The plasma pool complies with the test if it is found non-reactive for hepatitis C virus RNA.

Hepatitis C virus RNA for NAT testing BRP is suitable for use as a positive control.

CHARACTERS

Before freezing, a clear to slightly turbid liquid without visible signs of haemolysis; it may vary in colour from light yellow to green.

LABELLING

The label enables each individual unit to be traced to a specific donor.

Ph Eur

Rabies Immunoglobulin

General Notices

(Human Rabies Immunoglobulin, Ph Eur monograph 0723)

Ph Eur

DEFINITION

Human rabies immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from donors immunised against rabies. It contains specific antibodies neutralising the rabies virus. *Human normal immunoglobulin (0338)* may be added.

It complies with the monograph on *Human normal immunoglobulin (0338),* except for the minimum number of donors and the minimum total protein content.

POTENCY

The potency is determined by comparing the dose of immunoglobulin required to neutralise the infectivity of a rabies virus suspension with the dose of a reference preparation, calibrated in International Units, required to produce the same degree of neutralisation (*2.7.1*). The test is performed in sensitive cell cultures and the presence of unneutralised virus is revealed by immunofluorescence.

The International Unit is the specific neutralising activity for rabies virus in a stated amount of the International Standard for anti-rabies immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Human rabies immunoglobulin BRP is calibrated in International Units by comparison with the International Standard.

Carry out the test in suitable sensitive cells. It is usual to use the BHK 21 cell line, grown in the medium described below, between the 18th and 30th passage levels counted from the ATCC seed lot. Harvest the cells after 2 to 4 days of growth, treat with trypsin and prepare a suspension containing 500 000 cells per millilitre (cell suspension). 10 min before using this suspension add 10 µg of *diethylamino-ethyldextran R* per millilitre, if necessary, to increase the sensitivity of the cells.

Use a fixed virus strain grown in sensitive cells, such as the CVS strain of rabies virus adapted to growth in the BHK 21 cell line (seed virus suspension). Estimate the titre of the seed virus suspension as follows.

Prepare a series of dilutions of the viral suspension. In the chambers of cell-culture slides (8 chambers per slide), place 0.1 ml of each dilution and 0.1 ml of medium and add 0.2 ml of the cell suspension. Incubate in an atmosphere of carbon dioxide at 37 °C for 24 h. Carry out fixation, immunofluorescence staining and evaluation as described below. Determine the end-point titre of the seed virus suspension and prepare the working virus dilution corresponding to 100 $CCID_{50}$ per 0.1 ml.

For each assay, check the amount of virus used by performing a control titration: from the dilution corresponding to 100 $CCID_{50}$ per 0.1 ml, make three tenfold dilutions. Add 0.1 ml of each dilution to four chambers containing 0.1 ml of medium and add 0.2 ml of the cell suspension. The test is not valid unless the titre lies between 30 $CCID_{50}$ and 300 $CCID_{50}$.

Dilute the reference preparation to a concentration of 2 IU/ml using non-supplemented culture medium (stock reference dilution, stored below - 80 °C). Prepare two suitable predilutions (1:8 and 1:10) of the stock reference dilution so that the dilution of the reference preparation that reduces the number of fluorescent fields by 50 per cent lies within the four dilutions of the cell-culture slide. Add 0.1 ml of the medium to each chamber, except the first in each of two rows, to which add respectively 0.2 ml of the two predilutions of the stock reference dilution transferring successively 0.1 ml to the other chambers.

Dilute the preparation to be examined 1 in 100 using non-supplemented medium (stock immunoglobulin dilution) - to reduce to a minimum errors due to viscosity of the undiluted preparation - and make three suitable predilutions so that the dilution of the preparation to be examined that reduces the number of fluorescent fields by 50 per cent lies within the four dilutions of the cell-culture slide. Add 0.1 ml of the medium to all the chambers except the first in each of three rows, to which add respectively 0.2 ml of the three predilutions of the stock immunoglobulin dilution. Prepare a series of twofold dilutions transferring successively 0.1 ml to the other chambers.

To all the chambers containing the dilutions of the reference preparation and the dilutions of the preparation to be examined, add 0.1 ml of the virus suspension corresponding to 100 $CCID_{50}$ per 0.1 ml (working virus dilution), shake manually, allow to stand in an atmosphere of carbon dioxide at 37 °C for 90 min, add 0.2 ml of the cell suspension, shake manually and allow to stand in an atmosphere of carbon dioxide at 37 °C for 24 h.

After 24 h, discard the medium and remove the plastic walls. Wash the cell monolayer with *phosphate buffered saline pH 7.4 R* and then with a mixture of 20 volumes of *water R* and 80 volumes of *acetone R* and fix in a mixture of 20 volumes of *water R* and 80 volumes of *acetone R* at - 20 °C for 3 min. Spread on the slides *fluorescein-conjugated rabies antiserum R* ready for use. Allow to stand in an atmosphere with a high level of moisture at 37 °C for 30 min. Wash with *phosphate buffered saline pH 7.4 R* and dry. Examine twenty fields in each chamber at a magnification of 250 ×, using a microscope equipped for fluorescence readings. Note the number of fields with at least one fluorescent cell. Check the test dose used in the virus titration slide and determine the dilution of the reference preparation and the dilution of the preparation to be examined that reduce the number of fluorescent fields by 50 per cent, calculating the two or three dilutions together using probit analysis. The test is not valid unless the statistical analysis shows a significant slope of the dose-response curve and no evidence of deviation from linearity or parallelism.

The stated potency is not less than 150 IU/ml. The estimated potency is not less than the stated potency and is not greater than twice the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

CULTURE MEDIUM FOR GROWTH OF BHK 21 CELLS

Commercially available media that have a slightly different composition from that shown below may also be used.

Sodium chloride	6.4 g
Potassium chloride	0.40 g
Calcium chloride, anhydrous	0.20 g

Magnesium sulphate, heptahydrate	0.20 g
Sodium dihydrogen phosphate, monohydrate	0.124 g
Glucose monohydrate	4.5 g
Ferric nitrate, nonahydrate	0.10 mg
L-Arginine hydrochloride	42.0 mg
L-Cystine	24.0 mg
L-Histidine	16.0 mg
L-Isoleucine	52.0 mg
L-Leucine	52.0 mg
L-Lysine hydrochloride	74.0 mg
L-Phenylalanine	33.0 mg
L-Threonine	48.0 mg
L-Tryptophan	8.0 mg
L-Tyrosine	36.0 mg
L-Valine	47.0 mg
L-Methionine	15.0 mg
L-Glutamine	0.292 g
i-Inositol	3.60 mg
Choline chloride	2.0 mg
Folic acid	2.0 mg
Nicotinamide	2.0 mg
Calcium pantothenate	2.0 mg
Pyridoxal hydrochloride	2.0 mg
Thiamine hydrochloride	2.0 mg
Riboflavine	0.2 mg
Phenol red	15.0 mg
Sodium hydrogen carbonate	2.75 g

Water to	1000 ml

The medium is supplemented with:

Foetal calf serum (heated at 56 °C for 30 min)	10 per cent
Tryptose phosphate broth	10 per cent
Benzylpenicillin sodium	60 mg/l
Streptomycin	0.1 g/l

STORAGE

See *Human normal immunoglobulin (0338).*

LABELLING

See *Human normal immunoglobulin (0338).*

The label states the number of International Units per container.

Ph Eur

Rubella Immunoglobulin

General Notices

(Human Rubella Immunoglobulin, Ph Eur monograph 0617)

Ph Eur

DEFINITION

Human rubella immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma containing specific antibodies against rubella virus. *Human normal immunoglobulin (0338)* may be added.

It complies with the monograph on *Human normal immunoglobulin (0338),* except for the minimum number of donors and the minimum total protein content.

POTENCY

The potency is determined by comparing the activity of the preparation to be examined in a suitable haemagglutination-inhibition test with that of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the International Standard for anti-rubella immunoglobulin. The equivalence in International Units of the International Reference Preparation is stated by the World Health Organisation.

The estimated potency is not less than 4500 IU/ml. The confidence limits ($P = 0.95$) of the estimate of potency are not less than 50 per cent and not more than 200 per cent of the stated potency.

STORAGE

See *Human normal immunoglobulin (0338).*

LABELLING

See *Human normal immunoglobulin (0338).*

The label states the number of International Units per millilitre.

Ph Eur

Tetanus Immunoglobulin

General Notices

(Human Tetanus Immunoglobulin, Ph Eur monograph 0398)

Ph Eur

DEFINITION

Human tetanus immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma containing specific antibodies against the toxin of *Clostridium tetani*. *Human normal immunoglobulin (0338)* may be added.

It complies with the monograph on *Human normal immunoglobulin (0338),* except for the minimum number of donors and the minimum total protein content.

PRODUCTION

During development, a satisfactory relationship shall be established between the potency determined by immunoassay as described under Potency and that determined by means of the following test for toxin-neutralising capacity in mice.

Toxin-neutralising capacity in mice The potency is determined by comparing the quantity necessary to protect mice against the paralytic effects of a fixed quantity of tetanus toxin with the quantity of a reference preparation of human tetanus immunoglobulin, calibrated in International Units, necessary to give the same protection.

The International Unit of antitoxin is the specific neutralising activity for tetanus toxin contained in a stated amount of the International Standard, which consists of freeze-dried human immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Human tetanus immunoglobulin BRP is calibrated in International Units by comparison with the International Standard.

Selection of animals Use mice weighing 16 g to 20 g.

Preparation of the test toxin Prepare the test toxin by a suitable method from the sterile filtrate of a culture in liquid medium of *C. tetani*. The two methods shown below are given as examples and any other suitable method may be used.

(1) To the filtrate of an approximately 9-day culture add 1 to 2 volumes of *glycerol R* and store the mixture in the liquid state at a temperature slightly below 0 °C.

(2) Precipitate the toxin by addition to the filtrate of *ammonium sulphate R*, dry the precipitate *in vacuo* over *diphosphorus pentoxide R*, reduce to a powder and store dry, either in sealed ampoules or *in vacuo* over *diphosphorus pentoxide R*.

Determination of test dose of toxin (Lp/10 dose) Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.5 IU of antitoxin per millilitre. If the test toxin is stored dry, reconstitute it using a suitable liquid. Prepare mixtures of the solution of the reference preparation and the test toxin such that each contains 2.0 ml of the solution of the reference preparation, one of a graded series of volumes of the test toxin and sufficient of a suitable liquid to bring the volume to 5.0 ml. Allow the mixtures to stand, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.5 ml subcutaneously into each mouse. Observe the mice for 96 h. Mice that become paralysed may be euthanised. The test dose of toxin is the quantity in 0.5 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, paralysis in all six mice injected with the mixture, within the observation period.

Determination of potency of the immunoglobulin Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.5 IU of antitoxin per millilitre. Prepare a solution of the test toxin in a suitable liquid such that it contains five test doses per millilitre. Prepare mixtures of the solution of the test toxin and the immunoglobulin to be examined such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the immunoglobulin to be examined and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (2.0 ml) that contains 1 IU and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand, protected from light, for 60 min. Using six mice for each mixture, inject subcutaneously a dose of 0.5 ml into each mouse. Observe the mice for 96 h. Mice that become paralysed may be euthanised. The mixture that contains the largest volume of immunoglobulin that fails to protect the mice from paralysis contains 1 IU. This quantity is used to calculate the potency of the immunoglobulin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the reference preparation show paralysis and all those injected with mixtures containing more do not.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (*2.7.1*).

The International Unit is the activity contained in a stated amount of the International Standard for anti-tetanus immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Human tetanus immunoglobulin BRP is calibrated in International Units by comparison with the International Standard.

The stated potency is not less than 100 IU of tetanus antitoxin per millilitre. The estimated potency is not less than the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

STORAGE

See *Human normal immunoglobulin (0338).*

LABELLING

See *Human normal immunoglobulin (0338).*

The label states the number of International Units per container.

Varicella Immunoglobulin for Intravenous Use

General Notices

(Human Varicella Immunoglobulin for Intravenous Administration, Ph Eur monograph 1528)

Ph Eur

DEFINITION

Human varicella immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. It is obtained from plasma from selected donors having antibodies against human herpesvirus 3 (varicella-zoster virus 1) . *Human normal immunoglobulin for intravenous administration (0918)* may be added.

It complies with the monograph on *Human normal immunoglobulin for intravenous administration (0918)*, except for the minimum number of donors, the minimum total protein content and the limit for osmolality.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (*2.7.1*).

The International Unit is the activity contained in a stated amount of the International Standard for anti varicella-zoster immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

The stated potency is not less than 25 IU/ml. The estimated potency is not less than the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

STORAGE

See *Human normal immunoglobulin for intravenous administration (0918).*

LABELLING

See *Human normal immunoglobulin for intravenous administration (0918).*

The label states the number of International Units per container.

Ph Eur

Varicella Immunoglobulin

General Notices

(Human Varicella Immunoglobulin, Ph Eur monograph 0724)

Ph Eur

DEFINITION

Human varicella immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from selected donors having antibodies against *Herpesvirus varicellae*. *Human normal immunoglobulin (0338)* may be added.

It complies with the monograph on *Human normal immunoglobulin (0338)* except for the minimum number of donors, the minimum total protein content and, where authorised, the test for antibody to hepatitis B surface antigen.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (*2.7.1*).

The International Unit is the activity contained in a stated amount of the International Standard for anti varicella-zoster. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

The stated potency is not less than 100 IU/ml. The estimated potency is not less than the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

STORAGE

See *Human normal immunoglobulin (0338).*

LABELLING

See *Human normal immunoglobulin (0338).*

The label states the number of International Units per container.

Ph Eur

von Willebrand Factor

General Notices

(Human von Willebrand factor, Ph Eur monograph 2298)

Ph Eur

DEFINITION

Human von Willebrand factor is a preparation of a plasma protein fraction that contains the glycoprotein von Willebrand factor with varying amounts of coagulation factor VIII, depending on the method of preparation. It is prepared from human plasma that complies with the monograph on *Human plasma for fractionation (0853).*

This monograph applies to preparations formulated according to the von Willebrand factor activity.

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of von Willebrand factor per millilitre.

PRODUCTION

The method of preparation includes steps that have been shown to remove or to inactivate known agents of infection; if substances are used for the inactivation of viruses, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 1 IU of von Willebrand factor per milligram of total protein before the addition of any protein stabiliser.

The von Willebrand factor fraction is dissolved in a suitable liquid. Excipients such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

VALIDATION STUDIES

Composition It shall be demonstrated that the manufacturing process yields a product having a consistent composition with respect to von Willebrand factor, factor VIII and the proportions of von Willebrand factor and factor VIII.

von Willebrand factor multimers

The distribution of the different von Willebrand factor multimers is determined by a suitable method such as sodium dodecyl sulphate (SDS) agarose gel electrophoresis with or without Western blot analysis, using a suitable normal human plasma as standard. Visualisation of the multimeric pattern may be performed using, for example, an immunoenzymatic technique and quantitative evaluation may be carried out by densitometric analysis.

von Willebrand factor activity (*2.7.21*)

The von Willebrand factor activity is estimated by determining the ristocetin cofactor activity and by one or more other suitable assays such as determination of collagen-binding activity using a suitable reference preparation.

von Willebrand factor activity/antigen ratio

Consistency of the manufacturing process with respect to the ratio of von Willebrand factor activity to von Willebrand factor antigen content is demonstrated.

Products that show particles after reconstitution If a few particles remain when the preparation is reconstituted, it shall be demonstrated during validation studies that the potency is not significantly affected after passage of the preparation through the filter to be provided with the preparation.

CHARACTERS

Appearance

White or pale yellow, hygroscopic powder or friable solid.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

The assay for von Willebrand factor serves also to identify the preparation.

TESTS

Solubility

To a container of the preparation to be examined add the volume of the solvent stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 min, giving a clear or slightly opalescent, colourless or slightly yellow solution.

In addition, where the label states that the product may show a few particles after reconstitution, reconstitute the preparation as described on the label and pass it through the filter provided: the filtered solution is clear or slightly opalescent.

pH (*2.2.3*)

6.5 to 7.5.

Osmolality (*2.2.35*)

Minimum 240 mosmol/kg.

Total protein

If necessary, dilute an accurately measured volume of the preparation to be examined with a 9 g/l solution of *sodium chloride R*, to obtain a solution that may be expected to contain about 15 mg of protein in 2 ml. Place 2.0 ml of this solution in a round-bottomed centrifuge tube and add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the amount of protein by multiplying the result by 6.25. *For some products, especially those without a protein stabiliser, this method may not be applicable. Another validated method for protein determination must therefore be performed.*

Anti-A and anti-B haemagglutinins (*2.6.20*)

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to contain 6 IU of von Willebrand factor activity per millilitre. The 1 to 64 dilutions do not show agglutination.

Water

Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*)

It complies with the test for sterility.

Pyrogens (*2.6.8*)

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume of the preparation to be examined equivalent to not less than 100 IU of von Willebrand factor activity.

ASSAY

von Willebrand factor (*2.7.21*)

The estimated potency is not less than 80 per cent and not more than 120 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

Pending the availability of an International Standard for von Willebrand factor concentrate calibrated for use in the collagen-binding assay, only the ristocetin cofactor assay may be used.

Factor VIII (*2.7.4*)

The assay is carried out where the factor VIII content is greater than 10 IU of factor VIII per 100 IU of von Willebrand factor activity. The estimated potency is not less than 60 per cent and not more than 140 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

STORAGE

In an airtight container , protected from light.

LABELLING

The label states:

— the number of International Units of von Willebrand factor in the container;

— the number of International Units of factor VIII in the container, or that the content of factor VIII is less than or equal to 10 IU of factor VIII per 100 IU of von Willebrand factor activity;

— the amount of protein in the container;

— the name and quantity of any added substance;

— the name and volume of the liquid to be used for reconstitution;

— where applicable, that the preparation may show the presence of a few particles after reconstitution;

— that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Ph Eur

Old Tuberculin

General Notices

(Tuberculin for Human Use, Old, Ph Eur monograph 0152)

Ph Eur

DEFINITION

Old tuberculin for human use consists of a filtrate, concentrated by heating, containing the soluble products of the culture and lysis of one or more strains of *Mycobacterium bovis* and/or *Mycobacterium tuberculosis* that is capable of demonstrating a delayed hypersensitivity in an animal sensitised to micro-organisms of the same species.

Old tuberculin for human use in concentrated form is a transparent, viscous, yellow or brown liquid.

PRODUCTION

GENERAL PROVISIONS

The production of old tuberculin is based on a seed-lot system. The production method shall have been shown to yield consistently old tuberculin of adequate potency and safety in man. A batch of old tuberculin, calibrated in International Units by the method described under Assay and for which adequate clinical information is available as to its activity in man, is set aside to serve as a reference preparation.

The International Unit is the activity of a stated quantity of the International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

SEED LOTS

The strains of mycobacteria used shall be identified by historical records that include information on their origin and subsequent manipulation.

The working seed lots used to inoculate the media for the production of a concentrated harvest shall not have undergone more than 4 subcultures from the master seed lot.

Only seed lots that comply with the following requirements may be used for propagation.

Identification

The species of mycobacterium of the master and working seed lots is identified.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium. The working seed lot complies with the test for sterility except for the presence of mycobacteria.

PROPAGATION AND HARVEST

The bacteria are grown in a liquid medium which may be a glycerolated broth or a synthetic medium. Growth must be typical for the strain. The culture is inactivated by a suitable method, such as treatment in an autoclave (121 °C for not less than 30 min) or in flowing steam at a temperature not less than 100 °C for at least 1 h. The culture liquid, from which the micro-organisms may or may not have been separated by filtration, is concentrated by evaporation, usually to one-tenth of its initial volume. The preparation is free from live mycobacteria. The concentrated harvest is shown to comply with the test for mycobacteria *(2.6.2)* before addition of any antimicrobial preservative or other substance that might interfere with the test. Phenol (5 g/l) or another suitable antimicrobial preservative that does not give rise to false positive reactions may be added.

Only a concentrated harvest that complies with the following requirements may be used in the preparation of the final bulk tuberculin.

pH *(2.2.3)*

The pH of the concentrated harvest is 6.5 to 8.

Glycerol

Where applicable, determine the glycerol content of the concentrated harvest. The amount is within the limits approved for the particular product.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than 85 per cent and not more than 115 per cent of the intended amount. If phenol has been used in the preparation, the concentration is not more than 5 g/l *(2.5.15)*.

Sensitisation

Carry out the test described under Tests.

Sterility *(2.6.1)*

The concentrated harvest complies with the test for sterility, carried out using 10 ml for each medium.

Potency

Determine the potency as described under Assay.

FINAL BULK TUBERCULIN

The concentrated harvest is diluted aseptically.

Only a final bulk tuberculin that complies with the following requirement may be used in the preparation of the final lot.

Sterility *(2.6.1)*

The final bulk tuberculin complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

The final bulk tuberculin is distributed aseptically into sterile containers which are then closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

The following tests may be omitted on the final lot if they have been carried out at the stages indicated:

— live mycobacteria: concentrated harvest;

— sensitisation: concentrated harvest;

— toxicity: concentrated harvest or final bulk tuberculin;

— antimicrobial preservative: final bulk tuberculin.

IDENTIFICATION

Inject increasing doses of the preparation to be examined intradermally into healthy, white or pale-coloured guinea-pigs, specifically sensitised (for example, as described under Assay). A reaction varying from erythema to necrosis is produced at the site of the injection. Similar injections administered to non-sensitised guinea-pigs do not stimulate a reaction. The assay may also serve as identification.

TESTS

Old tuberculin for human use in concentrated form (≥ 100 000 IU/ml) complies with each of the tests prescribed below; the diluted product complies with the tests for antimicrobial preservative and sterility.

Toxicity

Inject a quantity equivalent to 50 000 IU subcutaneously into each of two healthy guinea-pigs weighing 250 g to 350 g and which have not been subjected to any treatment likely to interfere with the test. Observe the animals for 7 days. No adverse effect is produced.

Sensitisation

Use 3 guinea-pigs that have not been subjected to any treatment likely to interfere with the test. On 3 occasions at intervals of 5 days, inject intradermally into each guinea-pig about 500 IU of the preparation to be examined in a volume of 0.1 ml. 2 to 3 weeks after the third injection, administer the same dose intradermally to the same animals and to a control group of 3 guinea-pigs of the same mass that have not previously received injections of tuberculin. After 24 h to 72 h, the reactions in the 2 groups of animals are not substantially different.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than the minimum amount shown to be effective and not more than 115 per cent of the amount stated on the label. If phenol has been used in the preparation, the concentration is not more than 5 g/l *(2.5.15)*.

Live mycobacteria *(2.6.2)*

It complies with the test for mycobacteria.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

The potency of old tuberculin is determined by comparing the reactions produced by the intradermal injection of increasing doses of the preparation to be examined into sensitised guinea-pigs with the reactions produced by known concentrations of the reference preparation.

Prepare a suspension containing a suitable amount (0.1 mg to 0.4 mg/ml) of heat-inactivated, dried mycobacteria in mineral oil with or without emulsifier; use mycobacteria of a strain of the same species as that used in the preparation to be examined. Sensitise not fewer than 6 pale-coloured guinea-pigs weighing not less than 300 g by injecting intramuscularly or intradermally a total of about 0.5 ml of the suspension, divided between several sites if necessary. Carry out the test after the period of time required for optimal sensitisation which is usually 4 to 8 weeks after sensitisation. Depilate the flanks of the animals so that it is possible to make at least three injections on each side and not more than a total of 12 injection points per animal. Use at least three different doses of the reference preparation and at least 3 different doses of the preparation to be examined. For both preparations, use doses such that the highest dose is about 10 times the lowest dose. Choose the doses such that when they are injected the lesions produced have a diameter of not less than 8 mm and not more than 25 mm. In any given test, the order of the dilutions injected at each point is chosen at random in a Latin square design. Inject each dose intradermally in a constant volume of 0.1 ml or 0.2 ml. Measure the diameters of the lesions 24 h to 48 h later and calculate the results of the test by the usual statistical methods, assuming that the diameters of the lesions are directly proportional to the logarithm of the concentration of the preparation.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE

Store protected from light.

LABELLING

The label states:

— the number of International Units per millilitre;

— the species of mycobacterium used to prepare the product;

— the name and quantity of any antimicrobial preservative or other substance added to the preparation;

— the expiry date;

— where applicable, that old tuberculin is not to be injected in its concentrated form but diluted so as to administer not more than 100 IU per dose.

Ph Eur

Tuberculin Purified Protein Derivative

General Notices

Tuberculin P.P.D.

(Tuberculin Purified Protein Derivative for Human Use, Ph Eur monograph 0151)

Ph Eur

DEFINITION

Tuberculin purified protein derivative (tuberculin PPD) for human use is a preparation obtained by precipitation from the heated products of the culture and lysis of *Mycobacterium bovis* and/or *Mycobacterium tuberculosis* and capable of demonstrating a delayed hypersensitivity in an animal sensitised to micro-organisms of the same species.

Tuberculin PPD is a colourless or pale-yellow liquid; the diluted preparation may be a freeze-dried powder which upon dissolution gives a colourless or pale-yellow liquid.

PRODUCTION

GENERAL PROVISIONS

The production of tuberculin PPD is based on a seed-lot system. The production method shall have been shown to yield consistently tuberculin PPD of adequate potency and safety in man. A batch of tuberculin PPD, calibrated in International Units by method A described under Assay and for which adequate clinical information is available as to its activity in man, is set aside to serve as a reference preparation.

The International Unit is the activity of a stated quantity of the International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

SEED LOTS

The strains of mycobacteria used shall be identified by historical records that include information on their origin and subsequent manipulation.

The working seed lots used to inoculate the media for production of a concentrated harvest shall not have undergone more than 4 subcultures from the master seed lot.

Only seed lots that comply with the following requirements may be used for propagation.

Identification

The species of mycobacterium of the master and working seed lots is identified.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium. The working seed lot complies with the test for sterility except for the presence of mycobacteria.

PROPAGATION AND HARVEST

The bacteria are grown in a liquid synthetic medium. Growth must be typical for the strain. The culture is inactivated by a suitable method such as treatment in an autoclave (121 °C for not less than 30 min) or in flowing steam at a temperature not less than 100 °C for at least 1 h and filtered. The active fraction of the filtrate, consisting mainly of protein, is isolated by precipitation, washed and re-dissolved. The preparation is free from mycobacteria. The concentrated harvest is shown to comply with the test for mycobacteria *(2.6.2)* before addition of any antimicrobial preservative or other substance that might interfere with the test. Phenol (5 g/l) or another suitable antimicrobial preservative that does not give rise to false positive reactions may be added; a suitable stabiliser intended to prevent adsorption on glass or plastic surfaces may be added. The concentrated harvest may be freeze-dried. Phenol is not added to preparations that are to be freeze-dried.

Only a concentrated harvest that complies with the following requirements may be used in the preparation of the final bulk tuberculin PPD.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than 85 per cent and not more than 115 per cent of the intended amount. If phenol has been used in the preparation, the concentration is not more than 5 g/l *(2.5.15)*.

Sensitisation

Carry out the test described under Tests.

Sterility *(2.6.1)*

The concentrated harvest, reconstituted if necessary, complies with the test for sterility, carried out using 10 ml for each medium.

Potency

Determine the potency as described under Assay.

FINAL BULK TUBERCULIN PPD

The concentrated harvest is diluted aseptically, after reconstitution if necessary.

Only a final bulk tuberculin PPD that complies with the following requirement may be used in the preparation of the final lot.

Sterility *(2.6.1)*

The final bulk tuberculin PPD complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

The final bulk tuberculin PPD is distributed aseptically into sterile containers which are then closed so as to prevent contamination. It may be freeze-dried.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

The following tests may be omitted on the final lot if they have been carried out at the stages indicated:

- — live mycobacteria: concentrated harvest
- — sensitisation: concentrated harvest
- — toxicity: concentrated harvest or final bulk tuberculin PPD
- — antimicrobial preservative: final bulk tuberculin PPD.

IDENTIFICATION

Inject increasing doses of the preparation to be examined intradermally into healthy, white or pale-coloured guinea-pigs, specifically sensitised (for example as described under Assay). A reaction varying from erythema to necrosis is produced at the site of the injection. Similar injections administered to non-sensitised guinea-pigs do not stimulate a reaction. The assay may also serve as identification.

TESTS

Tuberculin purified protein derivative for human use in concentrated form (≥ 100 000 IU/ml) complies with each of the tests prescribed below; the diluted product complies with the tests for pH, antimicrobial preservative and sterility.

pH *(2.2.3)*

The pH of the preparation, reconstituted if necessary as stated on the label, is 6.5 to 7.5.

Toxicity

Inject subcutaneously 50 000 IU of the preparation to be examined into each of two healthy guinea-pigs weighing 250 g to 350 g and which have not been subjected to any treatment likely to interfere with the test. Observe the animals for 7 days. No adverse effect is produced.

Sensitisation

Use 3 guinea-pigs that have not been subjected to any treatment likely to interfere with the test. On 3 occasions at intervals of 5 days, inject intradermally into each guinea-pig about 500 IU of the preparation to be examined in a volume of 0.1 ml. 2 to 3 weeks after the third injection, administer the same dose intradermally to the same animals and to a control group of three guinea-pigs of the same mass that have not previously received injections of tuberculin. After 24 h to 72 h, the reactions in the 2 groups of animals are not substantially different.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than the minimum amount shown to be effective and not more than 115 per cent of the amount stated on the label. If phenol has been used in the preparation, the concentration is not more than 5 g/l *(2.5.15)*.

Live mycobacteria *(2.6.2)*

It complies with the test for mycobacteria.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

Use method A or, where the preparation contains 1 IU to 2 IU, use method B.

METHOD A

The potency of tuberculin PPD is determined by comparing the reactions produced by the intradermal injection of increasing doses of the preparation to be examined into sensitised guinea-pigs with the reactions produced by known concentrations of the reference preparation.

Prepare a suspension containing a suitable amount (0.1 mg/ml to 0.4 mg/ml) of heat-inactivated, dried mycobacteria in mineral oil with or without emulsifier; use mycobacteria of a strain of the same species as that used in the preparation to be examined. Sensitise not fewer than six pale-coloured guinea-pigs weighing not less than 300 g by injecting intramuscularly or intradermally a total of about 0.5 ml of the suspension, divided between several sites if necessary. Carry out the test after the period of time required for optimal sensitisation which is usually 4 to 8 weeks after sensitisation. Depilate the flanks of the animals so that it is possible to make at least 3 injections on each side but not more than a total of 12 injection points per animal. Prepare dilutions of the preparation to be examined and of the reference preparation using isotonic phosphate-buffered saline (pH 6.5 to 7.5) containing 50 mg/l of *polysorbate 80 R*. If the preparation to be examined is freeze-dried and does not contain a stabiliser, reconstitute it using the liquid described above. Use at least 3 different doses of the reference preparation and at least 3 different doses of the preparation to be examined. For both preparations, use doses such that the highest dose is about 10 times the lowest dose. Choose the doses such that when they are injected the lesions produced have a diameter of not less than 8 mm and not more than 25 mm. In any given test, the order of the dilutions injected at each point is chosen at random in a Latin square design. Inject each dose intradermally in a constant volume of 0.1 ml or 0.2 ml. Measure the diameters of the lesions 24 h to 48 h later and calculate the results of the test by the usual statistical methods, assuming that the diameters of the lesions are directly proportional to the logarithm of the concentration of the preparation.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 64 per cent and not more than 156 per cent of the stated potency.

METHOD B

The potency of tuberculin PPD is determined by comparing the reactions produced by the intradermal injection of the preparation to be examined into sensitised guinea-pigs with the reactions produced by known concentrations of the reference preparation.

Prepare a suspension in mineral oil with or without emulsifier and containing a suitable amount (0.1 mg/ml to 0.4 mg/ml) of heat-inactivated, dried mycobacteria; use mycobacteria of a strain of the same species as that used in the preparation to be examined. Sensitise not fewer than 6 pale-coloured guinea-pigs weighing not less than 300 g by injecting intramuscularly or intradermally a total of about 0.5 ml of the suspension, divided between several sites if necessary. Carry out the test after the period of time required for optimal sensitisation which is usually 4 to 8 weeks after sensitisation. Depilate the flanks of the animals so that it is possible to make at least 3 injections on each side but not more than a total of 12 injection points per animal. Prepare dilutions of the reference preparation using isotonic phosphate-buffered saline (pH 6.5 to 7.5) containing 50 mg/l of *polysorbate 80 R.* Use at least 3 different doses of the reference preparation such that the highest dose is about 10 times the lowest dose and the median dose is the same as that of the preparation to be examined. In any given test, the order of the dilutions injected at each point is chosen at random in a Latin square design. Inject the preparation to be examined and each dilution of the reference preparation intradermally in a constant volume of 0.1 ml or 0.2 ml. Measure the diameters of the lesions 24 h to 48 h later and calculate the results of the test by the usual statistical methods, assuming that the areas of the lesions are directly proportional to the logarithm of the concentration of the preparation to be examined. (This dose relationship applies to this assay and not necessarily to other test systems.)

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE

Store protected from light.

LABELLING

The label states:

— the number of International Units per container;

— the species of mycobacteria used to prepare the product;

— the name and quantity of any antimicrobial preservative or other substance added to the preparation;

— the expiry date;

— for freeze-dried products, a statement that the product is to be reconstituted using the liquid provided by the manufacturer;

— where applicable, that tuberculin PPD is not to be injected in its concentrated form but diluted so as to administer not more than 100 IU per dose.

If the package does not contain a leaflet warning that the inhalation of concentrated tuberculin PPD may produce toxic effects, this warning must be shown on the label on the container together with a statement that the powder must be handled with care.

Ph Eur

Agnus Castus Fruit

General Notices

(Ph Eur monograph 2147)

Ph Eur

DEFINITION

Whole, ripe, dried fruit of *Vitex agnus-castus* L.

Content

Minimum 0.08 per cent of casticin ($C_{19}H_{18}O_8$; M_r 374.3) (dried drug).

IDENTIFICATION

A. Agnus castus fruit is oval to almost globular, with a diameter of up to 5 mm. The persistent calyx is greenish-grey, finely pubescent, ends in 4-5 short teeth and envelops 2/3 to 3/4 of the surface of the fruit. The blackish-brown fruit consists of a pericarp that becomes progressively sclerous up to the endocarp. The style scar is often visible. Some of the fruits may retain a stalk, about 1 mm long. A transverse section of the fruit shows 4 locules, each containing an elongated seed.

B. Reduce to a powder (355) *(2.9.12)*. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the outer epidermis of the calyx composed of polygonal cells densely covered with short, bent or undulate, uni-, bi- or tri-cellular uniseriate covering trichomes; cells of the epicarp with thick walls and well-marked, large pits; isolated glandular trichomes with a unicellular stalk and a uni- or multi-cellular head; layers of parenchyma from the outer part of the mesocarp, some containing brown pigment, others extending into septa; fragments from the inner part of the mesocarp composed of thin-walled, pitted, sclerenchymatous cells and of typical isodiametric sclerous cells with very thick, deeply grooved walls and a narrow, stellate lumen; small brown cells of the endocarp; fragments of the testa containing areas of fairly large, thin-walled lignified cells with reticulate bands of thickening; numerous fragments of the endosperm composed of thin-walled parenchymatous cells containing aleurone grains and oil droplets.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* . Heat in a water-bath at 60 °C for 10 min. Allow to cool and filter.

Reference solution Dissolve 0.5 mg of *aucubin R* and 1 mg of *agnuside R* in *methanol R* and dilute to 1.0 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R* (5-40 µm) [or *TLC silica gel* F_{254} *plate R* (2-10 µm)].

Mobile phase *water R, methanol R, ethyl acetate R* (8:15:77 *V/V/V*).

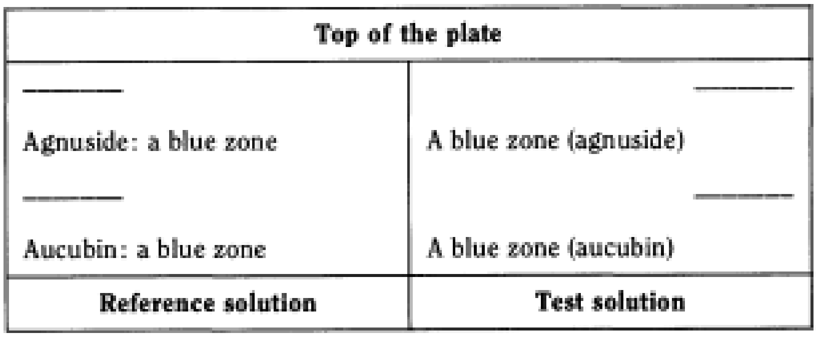
Application 10 µl [or 8 µl] as bands.

Development Over a path of 8 cm [or 5 cm].

Drying In air.

Detection Spray with *formic acid R* and heat at 120 °C for 10 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Agnuside: a blue zone	A blue zone (agnuside)
———	———
Aucubin: a blue zone	A blue zone (aucubin)
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 3.0 per cent.

Other species of *Vitex*, in particular *Vitex negundo*

No fruit of other species with a much greater diameter is present.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

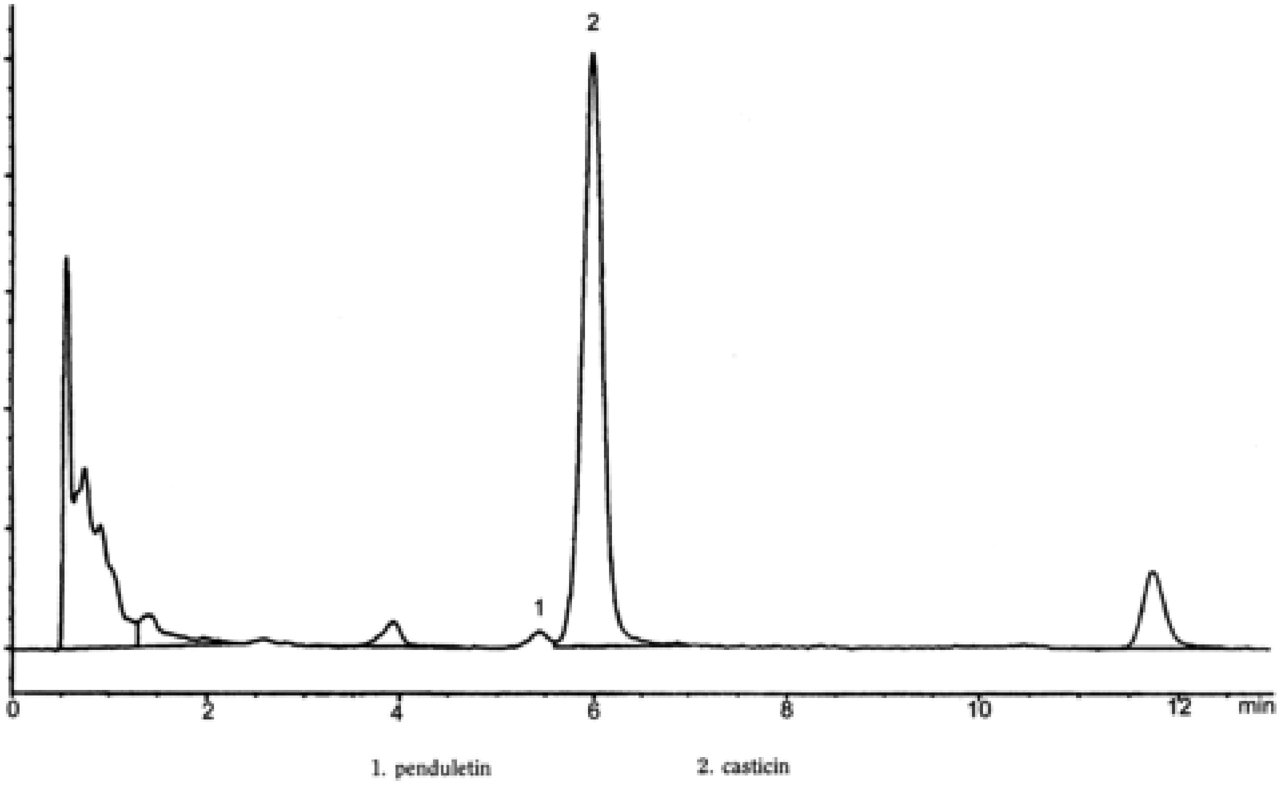


Figure 2147.-1. – *Chromatogram for the assay of casticin in Agnus castus fruit: test solution*

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution Extract 1.000 g of the powdered drug (355) *(2.9.12)* with 40 ml of *methanol R* for 2 min using a suitable-speed homogeniser. Collect the supernatant liquid and filter into a 250 ml flask. Repeat the extraction with a further 40 ml of *methanol R* , collecting the supernatant liquid and filtering as before. Rinse the residue carefully with a small quantity of *methanol R* . Combine the methanol extracts and rinsings and evaporate to dryness *in vacuo* in a water-bath at not more than 30 °C. With the aid of ultrasound, dissolve the residue obtained in *methanol R* and dilute to 20.0 ml with the same solvent. Filter the solution through a membrane filter (0.45 µm). Dilute 1.0 ml to 10.0 ml with *methanol R* .

Reference solution Dissolve 100.0 mg of *agnus castus fruit standardised dry extract CRS* in 20.0 ml of *methanol R* with the aid of ultrasound for 20 min, then dilute to 25.0 ml with the same solvent. Filter the solution through a membrane filter (0.45 µm).

Column:

— *size: l* = 0.125 m, Ø = 3.0 mm;

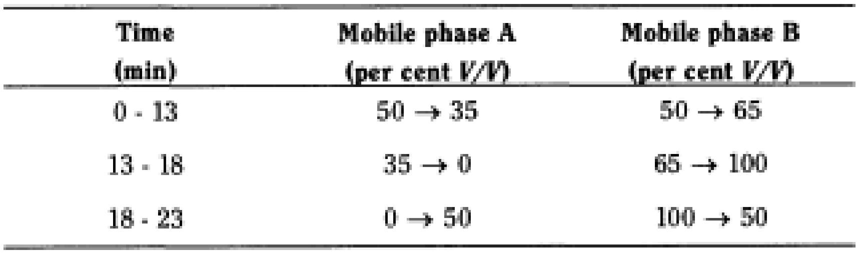
— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 25 °C.

Mobile phase:

— *mobile phase A:* 5.88 g/l solution of *phosphoric acid R* ;

— *mobile phase B: methanol R* ;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 13	50 → 35	50 → 65
13 - 18	35 → 0	65 → 100
18 - 23	0 → 50	100 → 50

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 348 nm.

Injection 10 µl.

System suitability Test solution:

— *resolution*: minimum 1.5 between the peaks due to penduletin and casticin (see Figure 2147.-1).

Calculate the percentage content of casticin using the following expression:

$$\frac{F_1 \times m_2 \times p_1 \times 8}{F_2 \times m_1}$$

F_1 = area of the peak due to casticin in the chromatogram obtained with the test solution;

F_2 = area of the peak due to casticin in the chromatogram obtained with the reference solution;

m_1 = mass of the drug used to prepare the test solution, in grams;

m_2 = mass of *agnus castus fruit standardised dry extract CRS* used to prepare the reference solution, in grams;

p_1 = percentage content of casticin in *agnus castus fruit standardised dry extract CRS*.

Ph Eur

Agrimony

General Notices

(Ph Eur monograph 1587)

Ph Eur

DEFINITION

Dried flowering tops of *Agrimonia eupatoria* L.

Content

Minimum 2.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The stem is green or, more usually, reddish, cylindrical and infrequently branched. It is covered with long, erect or tangled hairs. The leaves are compound imparipennate with 3 or 6 opposite pairs of leaflets, with 2 or 3 smaller leaflets between. The leaflets are deeply dentate to serrate, dark green on the upper surface, greyish and densely tormentose on the lower face. The flowers are small and form a terminal spike. They are pentamerous and borne in the axils of hairy bracts, the calyces closely surrounded by numerous terminal hooked spires, which occur on the rim of the hairy receptacle. The petals are free, yellow and deciduous. Fruit-bearing obconical receptacles, with deep furrows and hooked bristles, are usually present at the base of the inflorescence.

B. Reduce to a powder (355) *(2.9.12)*. The powder is yellowish-green to grey. Examine under a microscope using *chloral hydrate solution R* . The powder shows numerous straight of bent, unicellular, long thick-walled (about 500 µm) trichomes finely warty and sometimes spiraly marked; fragments of parenchyma with prisms and cluster crystals of calcium oxalate; fragments of leaf epidermis with sinuous walls, those of the lower epidermis with abundant stomata, mostly anomocytic but occasionally anisocytic; ovoid to subspherical-pollen grains, with 3 pores and a smooth exine; fragments of glandular trichomes with a multicellular uniseriate stalk and a spherical unicellular or quadricellular head; groups of fibres and spiral and bardered-fitted vessels from the stem.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 2.0 g of the powdered drug (355) *(2.9.12)* add 20 ml of *methanol R* . Heat with shaking at 40 °C for 10 min. Filter.

Reference solution Dissolve 1.0 mg of *rutin R* and 1.0 mg of *isoquercitroside R* in 2 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, water R, ethyl acetate R* (10:10:80 *V/V/V*).

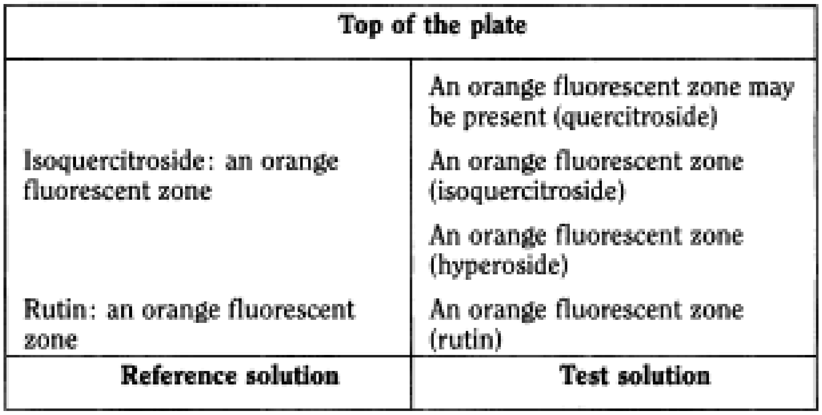
Application 10 µl, as bands.

Development Over a path of 12 cm.

Drying At 100-105 °C.

Detection Spray the still warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for 30 min. Examine in ultraviolet light at 365 nm

Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions.



Top of the plate	
	An orange fluorescent zone may be present (quercitroside)
Isoquercitroside: an orange fluorescent zone	An orange fluorescent zone (isoquercitroside)
	An orange fluorescent zone (hyperoside)
Rutin: an orange fluorescent zone	An orange fluorescent zone (rutin)
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs *(2.8.14)* . Use a 1.000 g of powdered drug (180) *(2.9.12)*.

Ph Eur

Alchemilla

General Notices

(Ph Eur monograph 1387)

Ph Eur

DEFINITION

Whole or cut, dried, flowering, aerial parts of *Alchemilla vulgaris* L. *sensu latiore*.

Content

Minimum 6.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The greyish-green, partly brownish-green, radical leaves which are the main part of the drug are reniform or slightly semicircular with a diameter generally up to 8 cm, seldom up to 11 cm and have 7 to 9, or 11 lobes and a long petiole. The smaller, cauline leaves, which have a pair of large stipules at the base, have 5-9 lobes and a shorter petiole or they are sessile. The leaves are densely pubescent especially on the lower surface and have a coarsely serrated margin. Young leaves are folded with a whitish-silvery pubescence; older leaves are slightly pubescent and have a finely meshed venation, prominent on the lower surface. The greyish-green or yellowish-green petiole is pubescent, about 1 mm in diameter, with an adaxial groove. The apetalous flowers are yellowish-green or light green and about 3 mm in diameter. The calyx is double with 4 small segments of the epicalyx alternating with 4 larger sepals, subacute or triangular. They are 4 short stamens and a single carpel with a capitate stigma. The greyish-green or yellowish-green stem is pubescent, more or less longitudinally wrinkled and hollow.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: unicellular, narrow trichomes up to 1 mm long partly tortuous, acuminate, and bluntly pointed at the apex, with thick lignified walls, somewhat enlarged and pitted at the base; fragments of leaves with 2 layers of palisade parenchyma, the upper layer of which is 2-3 times longer than the lower layer and with spongy parenchyma, containing scattered cluster crystals of calcium oxalate, up to 25 µm in diameter; leaf fragments in surface view with sinuous or wavy epidermal cells, the anticlinal walls unevenly thickened and beaded, anomocytic stomata *(2.8.3)* ; groups of vascular tissue and lignified fibres from the petioles and stems, the vessels spirally thickened or with bordered pits; occasional thin-walled conical trichomes, about 300 µm long; thin-walled parenchyma containing cluster crystals of calcium oxalate; spherical pollen grains, about 15 µm in diameter, with 3 distinct pores and a granular exine; occasional fragments of the ovary wall with cells containing a single crystal of calcium oxalate.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 0.5 g of the powdered drug (355) *(2.9.12)* add 5 ml of *methanol R* and heat in a water-bath at 70 °C under a reflux condenser for 5 min. Cool and filter.

Reference solution Dissolve 1.0 mg of *caffeic acid R* and 1.0 mg of *chlorogenic acid R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, water R, ethyl acetate R* (8:8:84 *V/V/V*).

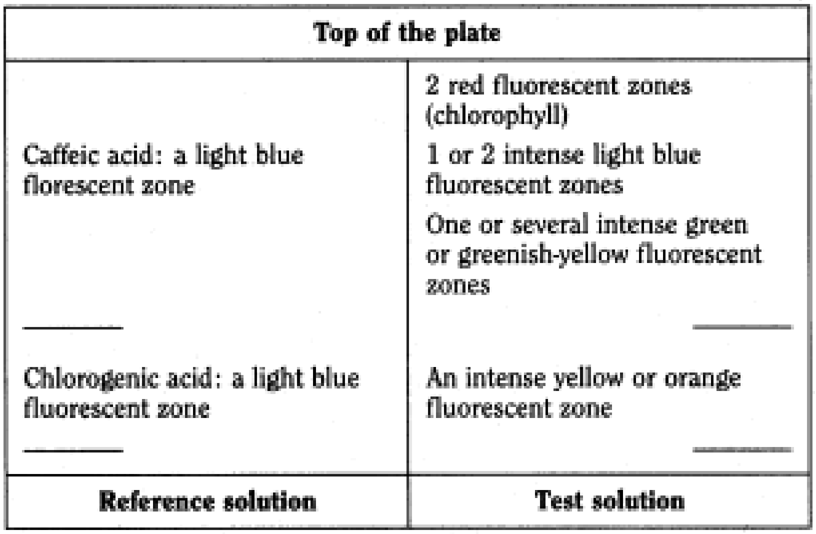
Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 5 min.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* . Subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R* . Allow to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	2 red fluorescent zones (chlorophyll)
Caffeic acid: a light blue florescent zone	1 or 2 intense light blue fluorescent zones
	One or several intense green or greenish-yellow fluorescent zones
_______	_______
Chlorogenic acid: a light blue fluorescent zone	An intense yellow or orange fluorescent zone
_______	_______
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs *(2.8.14)* . Use 0.50 g of the powdered drug (355) *(2.9.12)*.

Ph Eur

Alexandrian Senna Fruit

General Notices

(Alexandrian Senna Pods, Ph Eur monograph 0207)

Preparations

Senna Liquid Extract

Standardised Senna Granules

Senna Tablets

When Powdered Alexandrian Senna Fruit is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried fruit of *Cassia senna* L. (*C. acutifolia* Delile).

Content

Minimum 3.4 per cent of hydroxyanthracene glycosides, expressed as sennoside B ($C_{42}H_{38}O_{20}$; M_r 863) (dried drug).

CHARACTERS

Slight odour.

IDENTIFICATION

A. Flattened reniform pods, green or greenish-brown with brown patches at the positions corresponding to the seeds, usually 40-50 mm long and at least 20 mm wide. At one end is a stylar point and at the other a short stalk. The pods contain 6-7 flattened and obovate seeds, green or pale brown, with a continuous network of prominent ridges on the testa.

B. Reduce to a powder (355) *(2.9.12)*. The powder is brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: epicarp with polygonal cells and a small number of conical warty trichomes and occasional anomocytic or paracytic stomata *(2.8.3)*; fibres in 2 crossed layers accompanied by a crystal sheath of calcium oxalate prisms; characteristic palisade cells in the seed and stratified cells in the endosperm; clusters and prisms of calcium oxalate.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.5 g of the powdered drug (180) *(2.9.12)* add 5 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* and heat to boiling. Centrifuge and use the supernatant liquid.

Reference solution Dissolve 10 mg of *senna extract CRS* in 1 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* (a slight residue remains).

Plate *TLC silica gel G plate R*.

Mobile phase *Glacial acetic acid R, water R, ethyl acetate R, propanol R* (1:30:40:40 *V/V/V/V*).

Application 10 µl, as bands of 20 mm by 2 mm.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 20 per cent *V/V* solution of *nitric acid R* and heat at 120 °C for 10 min; allow to cool and spray with a 50 g/l solution of *potassium hydroxide R* in *ethanol (50 per cent V/V) R* until the zones appear.

Results The principal zones in the chromatogram obtained with the test solution are similar in position (sennosides B, A, D and C in order of increasing R_F value), colour and size to the principal zones in the chromatogram obtained with the reference solution; between the zones due to sennosides D and C, a red zone due to rhein-8-glucoside may be visible; the zones due to sennosides D and C are faint in the chromatogram obtained with the test solution.

D. Place about 25 mg of the powdered drug (180) *(2.9.12)* in a conical flask and add 50 ml of *water R* and 2 ml of *hydrochloric acid R*. Heat in a water-bath for 15 min, cool and shake with 40 ml of *ether R*. Separate the ether layer, dry over *anhydrous sodium sulphate R*, evaporate 5 ml to dryness and to the cooled residue add 5 ml of *dilute ammonia R1*. A yellow or orange colour develops. Heat on a water-bath for 2 min. A reddish-violet colour develops.

TESTS

Foreign matter *(2.8.2)*

Maximum 1 per cent.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 9.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 2.0 per cent.

ASSAY

Carry out the assay protected from bright light. Place 0.150 g of the powdered drug (180) *(2.9.12)* in a 100 ml flask. Add 30.0 ml of *water R*, mix, weigh and place in a water-bath. Heat under a reflux condenser for 15 min. Allow to cool, weigh and adjust to the original mass with *water R*. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150 ml separating funnel. Add 0.1 ml of *dilute hydrochloric acid R* and shake with 3 quantities, each of 15 ml, of *chloroform R*. Allow to separate and discard the chloroform layer. Add 0.10 g of *sodium* *hydrogen carbonate R* and shake for 3 min. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100 ml round-bottomed flask with a ground-glass neck. Add 20 ml of *ferric chloride solution R1* and mix. Place the flask in a water-bath so that the water level is above that of the liquid in the flask, and heat under a reflux condenser for 20 min. Add 1 ml of *hydrochloric acid R* and heat for a further 20 min, with frequent shaking, to dissolve the precipitate. Cool, transfer the mixture to a separating funnel and shake with 3 quantities, each of 25 ml, of *ether R* previously used to rinse the flask. Combine the 3 ether layers and wash with 2 quantities, each of 15 ml, of *water R*. Transfer the ether layers to a volumetric flask and dilute to 100.0 ml with *ether R*. Evaporate 10.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 515 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of hydroxyanthracene glycosides, expressed as sennoside B, using the following expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of sennoside B to be 240.

A = absorbance at 515 nm;

m = mass of the substance to be examined, in grams.

STORAGE

Protected from moisture.

Ph Eur

Angelica Root

General Notices

(Ph Eur monograph 1857)

Ph Eur

DEFINITION

Whole or cut, carefully dried rhizome and root of *Angelica archangelica* L. (*Archangelica officinalis Haffm.*).

Content

Minimum 2.0 ml/kg of essential oil (dried drug).

CHARACTERS

Bitter taste.

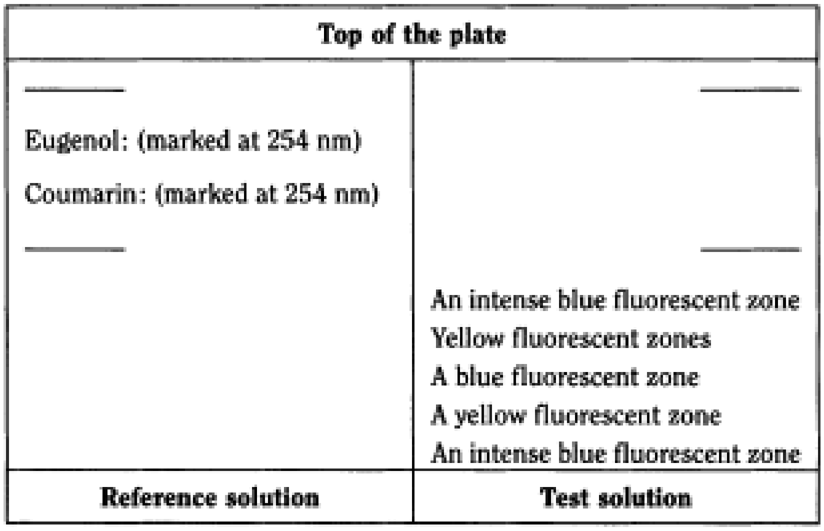
IDENTIFICATION

A. The rhizome is greyish-brown or reddish-brown, with transversely annulated thickenings. The base bears greyish-brown or reddish-brown, cylindrical, longitudinally furrowed, occasionally branched roots often with incompletely encircling, transverse ridges. The apex sometimes shows remnants of stem and leaf bases. The fracture is uneven. The transversely cut surface shows a greyish-white, spongy, distinctly radiate bark, in which the secretory channels are visible as brown spots, and a bright yellow or greyish-yellow wood which, in the rhizome, surrounds the greyish or brownish-white pith.

B. Reduce to a powder (355) *(2.9.12)*. The powder is brownish-white. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of cork consisting of several layers of thin-walled greyish-brown or reddish-brown cells; fragments of large, yellowish-brown secretory channels; fragments of medullary rays 2 or 4 cells wide; fragments of xylem with medullary rays and radially arranged, lignified vessels with reticulate thickenings. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R* . The powder shows numerous, simple starch granules 2-4 µm in diameter.

C. Examine the chromatograms obtained in the test for lovage root.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
Eugenol: (marked at 254 nm)	
Coumarin: (marked at 254 nm)	
———	———
	An intense blue fluorescent zone
	Yellow fluorescent zones
	A blue fluorescent zone
	A yellow fluorescent zone
	An intense blue fluorescent zone
Reference solution	**Test solution**

TESTS

Lovage root

Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the freshly powdered drug (355) *(2.9.12)* add 5 ml of *methanol R* and boil for 30 s. Cool and filter.

Reference solution Dissolve 5 mg of *coumarin R* and 25 µl of *eugenol R* in 10 ml of *methanol R* .

Plate *TLC silica gel F* $_{254}$ *plate R* .

Mobile phase *methylene chloride R* , *toluene R* (50:50 *V/V*).

Application 20 µl, as bands.

Development Twice over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm. Mark the quenching zones due to coumarin and eugenol on the chromatogram obtained with the reference solution. Examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows no pale blue or white fluorescent zone between the zones of coumarin and eugenol in the chromatogram obtained with the reference solution.

Foreign matter *(2.8.2)*

Maximum 5 per cent of leaf bases and stem bases, maximum 5 per cent of discoloured pieces and maximum 1 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 2.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Reduce the drug to a powder (500) *(2.9.12)* and immediately use 40.0 g for the determination. Use a 2 litre round-bottomed flask, 10 drops of *liquid paraffin R*, 500 ml of *water R* as distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 4 h.

Ph Eur

Angelica Sinensis Root for use in THM

General Notices

DEFINITION

Angelica Sinensis Root for use in THM is the dried whole root of *Angelica sinensis* (Oliv.) Diels. (*Angelica polymorpha* Maxim. var. *sinensis* Oliv.). The dried root consists of the top (uppermost part), main body and small lateral roots (tails).

It is collected in late autumn, removed from rootlets and dried.

It contains not less than 0.1% of *Z*-ligustilide ($C_{12}H_{14}O_2$), calculated with reference to the dried material.

IDENTIFICATION

A. The whole root is yellowish-brown to brown, up to 25 cm long, irregularly cylindrical with 3 to 5 or more branch roots arising from the lower end. The upper part is 1.5 to 5 cm in diameter, annulated on the surface and rounded at the apex which may show purple or yellowish-green remains of stems and leaves; the surface of the remainder of the main root is strongly longitudinally wrinkled and has pale, transverse lenticels; the branch roots are 0.3 to 1 cm in diameter in the upper part, twisted and tapering towards the base, the outer surface is strongly striated and has few rootlet scars.

B. Reduce to a powder (355). The powder is pale yellowish to buff. Examine under a microscope using *chloral hydrate solution* . The powder shows brown fragments of cork composed of thin-walled cells; abundant thin-walled parenchyma from the secondary cortex, phloem and medullary rays, some of the phloem cells fusiform with slightly thickened walls; lignified vessels in groups of 2 or 3 associated with small celled and pitted xylem parenchyma; the vessels are up to 80 µm in diameter and have reticulate or scalariform thickening. Examine under a microscope using 50% v/v of *glycerol* . The powder shows small groups of single starch granules, spherical to ovoid, up to about 8 µm in diameter.

C. Carry out the method for *thin-layer chromatography* , Appendix III A, using the following solutions.

(1) Add 4 ml of *heptane* to 1.0 g of the powdered drug, mix with the aid of ultrasound for 5 minutes and filter (use a 0.22 µm membrane filter).

(2) 0.1% w/v of *linoleic acid* in *methanol* .

(3) 0.1% w/v of *ferulic acid* in *methanol* .

(4) 0.1% w/v of Z-*ligustilide CRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel* F_{254} precoated plate (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µl of solution (1) and 5 µl of solutions (2) to (4), as bands.

(d) Develop the plate to 15 cm.

(e) Remove the plate, allow to dry in a stream of warm air for 5 minutes or until the solvents are completely removed. Examine under *ultraviolet light (254 nm)*. Spray the plate with *methanolic sulphuric acid (5%),* heat at 105º for 3 minutes and examine in daylight.

MOBILE PHASE

1 volume of *formic acid*, 10 volumes of *ethyl acetate* and 90 volumes of *toluene*.

SYSTEM SUITABILITY

When examined under *ultraviolet light (254 nm)* the violet band with an Rf value of approximately 0.7 the chromatogram obtained with solution (4) corresponds in colour and position to that in the chromatogram obtained with solution (1). A band with an Rf value of approximately 0.23 in the chromatogram obtained with solution (3) corresponds in position to a band in the chromatogram obtained with solution (1). Other bands may be present in the chromatogram obtained with solution (1).

CONFIRMATION

When sprayed with *methanolic sulphuric acid (5%)* the chromatogram obtained with solution (1) shows three spots with similar Rf values to the spots in the chromatograms obtained with solutions (2), (3) and (4). Other spots may be present in the chromatogram obtained with solution (1).

TESTS

Lovage root (*Levisticum officinale*)

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

(1) Extract approximately 20 g of the coarsely powdered drug in a 500 ml round-bottomed flask by hydrodistillation for 2 to 3 hours in 200 ml of *water*, collecting the distillate in suitable glassware. Extract the oily drops on top of the distillate with 5 ml of *toluene*.

(2) 0.2% w/v each of *coumarin* and *eugenol* in *toluene*.

(3) 0.1% w/v of Z-*ligustilide CRS* in a*cetonitrile*.

(4) 0.1% w/v of *benzyl alcohol* in *toluene*.

(5) 0.1% w/v of *(-)-carvone* in *toluene*.

(6) 0.1% w/v of *octanoic acid* in *toluene*.

(7) 0.1% w/v of *3-propylidenephthalide* in *toluene*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (50 m × 0.32 mm) bonded with a film (1.05 µm) of 5% phenyl/95% dimethylpolysiloxane (HP 5 is suitable).

(b) Use *helium* as the carrier gas at 1 ml per minute.

(c) Use an oven maintained at an initial temperature of 40° increasing linearly to 220° at a rate of 5° per minute, then maintained at 220°.

(d) Use a split injection system having a split ratio of 20:1 maintained at 250°.

(e) Use a flame ionisation detector maintained at a temperature of 250°.

(f) Inject 1 µl of each solution.

(g) Record the chromatograms for a sufficient length of time to elute all the peaks in the chromatogram obtained with solution (1) (55 minutes may be suitable).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the resolution between coumarin (eluting at approximately 34 minutes) and eugenol (eluting at approximately 37 minutes) is at least 3.0.

CONFIRMATION

In the chromatogram obtained with solution (1):

— there are no peaks corresponding to the principal peaks in the chromatograms obtained with solutions (4), (5), (6) and (7);

— there is a peak corresponding to the principal peak in the chromatogram obtained with solution (3).

Loss on drying

When dried at 100° to 105° for 2 hours, loses not more than 12.0% of its weight. Use 1 g.

Total ash

Not more than 7.0%, Appendix XI J, Method II.

Acid-insoluble ash

Not more than 2.0%, Appendix XI K.

Ethanol-soluble extractive

Not less than 45%, Appendix XI B1.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Finely powder not less than 5.0 g of the drug being examined. Transfer 0.5 g of the

powder into a 25 ml volumetric flask and add 20 ml of *methanol* , place in an ultrasonic bath (maintained at a low temperature by adding ice to the bath) for 100 minutes, equilibrate to ambient temperature and dilute to volume with *methanol* . Centrifuge the solution at 5000 rpm for 5 minutes or until a clear supernatant is obtained. Filter through a 0.45-µm filter.

(2) 0.025% w/v of Z-*ligustilide CRS* in *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 ml per minute.

(d) Use a detection wavelength of 350 nm.

(e) Inject 10 µl of each solution.

MOBILE PHASE

A mixture of 8 volumes of *water* and 12 volumes of *acetonitrile* .

SYSTEM SUITABILITY

Inject solution (2) not less than five times. The test is not valid unless the relative standard deviation of the peak areas of the *Z*-ligustilide peak is not more than 3.0%, the relative standard deviation of the retention times of the *Z*-ligustilide peak is not more than 3.0%. The *column efficiency* , determined on the *Z*-ligustilide peak, is not less than 5000 theoretical plates. The *symmetry factor* , determined on the *Z*-ligustilide peak, is not more than 1.3.

Inject solution (1). The test is not valid unless the *resolution factor* between the *Z*-ligustilide peak and the closest peak (relative retention about 0.9 with respect to *Z*-ligustilide) is not less than 1.5.

DETERMINATION OF CONTENT

Using the retention time and the peak area from the chromatograms obtained with solution (2) , locate and integrate the peak due to *Z*-ligustilide in the chromatogram obtained with solution (1).

Calculate the content of *Z*-ligustilide in the sample using the declared content of *Z*-ligustilide ($C_{12}H_{14}O_2$) in Z-*ligustilide CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100-d}$$

A_1 = Area of the peak due to *Z*-ligustilide in the chromatogram obtained with solution (1) .

A_2 = Area of the peak due to *Z*-ligustilide in the chromatogram obtained with solution (2) .

m_1 = Weight of the drug being examined in mg.

m_2 = Weight of Z-*ligustilide CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of $C_{12}H_{14}O_2$ in Z-*ligustilide CRS .*

d = Percentage loss on drying of the herbal drug being examined.

STORAGE

Angelica Sinensis Root for use in THM should be protected from moisture.

Anise Oil

General Notices

Aniseed Oil

(Ph Eur monograph 0804)

Preparation

Concentrated Anise Water

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the dry ripe fruits of *Pimpinella anisum* L.

CHARACTERS

Appearance

Clear, colourless or pale yellow liquid.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 1 g of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 µl of *linalol R* , 30 µl of *anisaldehyde R* and 200 µl of *anethole R* in *toluene R* and dilute to 15 ml with the same solvent. Dilute 1 ml of this solution to 5 ml with *toluene R* .

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *ethyl acetate R* , *toluene R* (7:93 *V/V*).

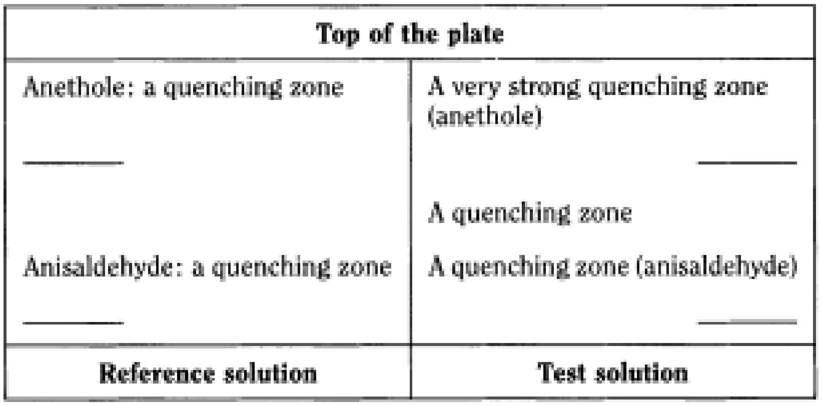
Application 5 µl as bands of 10 mm (for normal TLC plates) or 2 µl as bands of 10 mm (for fine particle size plates).

Development Over a path of 15 cm (for normal TLC plates) or over a path of 6 cm (for fine particle size plates).

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

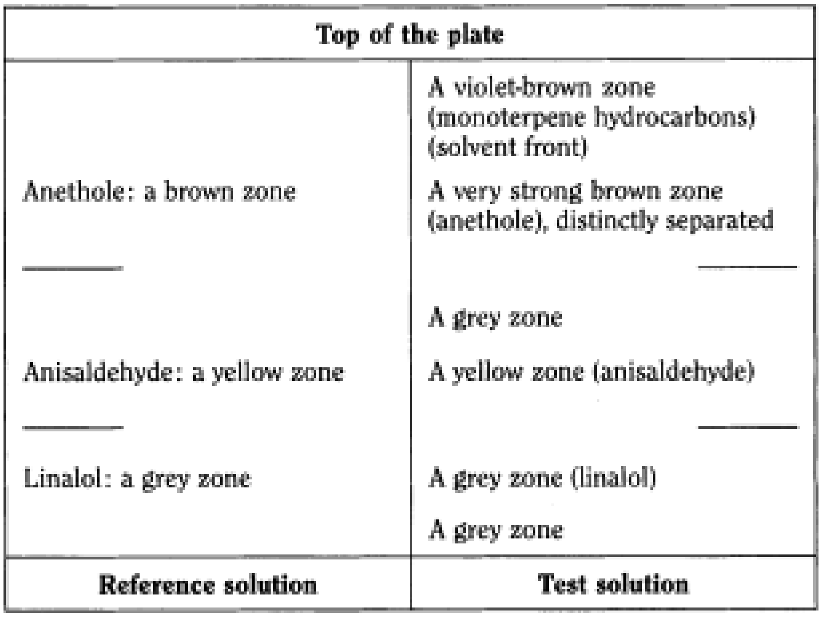
Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Anethole: a quenching zone	A very strong quenching zone (anethole)
———	———
	A quenching zone
Anisaldehyde: a quenching zone	A quenching zone (anisaldehyde)
———	———
Reference solution	**Test solution**

Detection B Spray with *methyl 4-acetylbenzoate reagent R* and heat at 100-105 °C for 10 min; examine the still hot plate in daylight within 5 min.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A violet-brown zone (monoterpene hydrocarbons) (solvent front)
Anethole: a brown zone	A very strong brown zone (anethole), distinctly separated
———	———
	A grey zone
Anisaldehyde: a yellow zone	A yellow zone (anisaldehyde)
———	———
Linalol: a grey zone	A grey zone (linalol)
	A grey zone
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density ***(2.2.5)***

0.980 to 0.990.

Refractive index ***(2.2.6)***

1.552 to 1.561.

Freezing point ***(2.2.18)***

15 °C to 19 °C.

Fenchone

Gas chromatography *(2.2.28)* as described in the test for chromatographic profile with the following modifications.

Test solution Dissolve 400 µl of the substance to be examined in 2.0 ml of *hexane R* .

Reference solution (a) Dilute 10 µl of *fenchone R* to 1.2 g with *hexane R* .

Reference solution (b) Dilute 100 µl of reference solution (a) to 100 ml with *hexane R* .

System suitability Reference solution (b):

— *signal-to-noise ratio* : minimum 10 for the principal peak.

Limit:

— *fenchone* : maximum 0.01 per cent.

Foeniculin

Gas chromatography *(2.2.28)* as described in the test for chromatographic profile with the following modifications.

Test solution The substance to be examined.

Reference solution (a) Dilute 10 mg of the test solution to 1.000 g with *hexane R* . Dilute 0.5 ml of this solution to 100 ml with *hexane R* .

Reference solution (b) *Foeniculin for peak identification CRS.*

System suitability:

— the chromatogram obtained with reference solution (b) is similar to the chromatogram provided with *foeniculin for peak identification CRS* ,

— *signal-to-noise ratio* : minimum 10 for the principal peak in the chromatogram obtained with reference solution (a).

Limit Locate the peak due to foeniculin by comparison with the chromatogram provided with *foeniculin for peak identification CRS.*

— *foeniculin*: maximum 0.01 per cent.

Fatty oils and resinified essential oils ***(2.8.7)***

It complies with the test for fatty oils and resinified essential oils.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution Dissolve 200 µl of the substance to be examined in 1.0 ml of *hexane R* .

Reference solution To 1.0 ml of *hexane R* , add 20 µl of *linalol R* , 20 µl of *estragole R* , 20 µl of α-*terpineol R* , 60 µl of *anethole R* and 30 µl of *anisaldehyde R* .

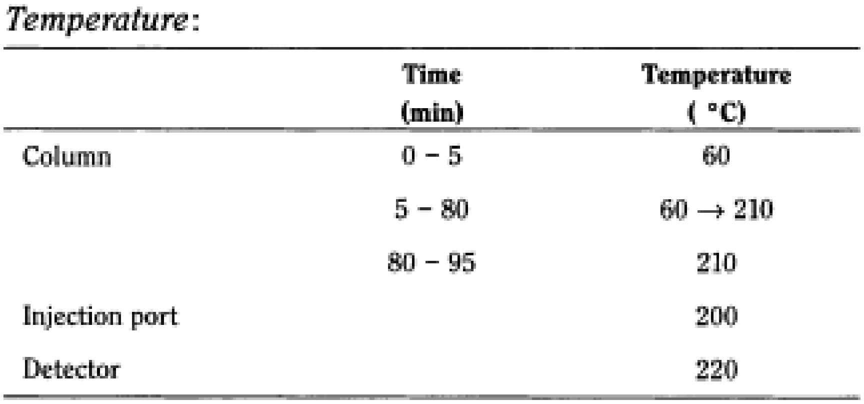
Column:
- *material*: fused silica,
- *size*: *l* = 30 m, Ø = 0.25 mm,
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1.0 ml/min.

Split ratio 1:100.

Temperature:



Temperature:

	Time (min)	Temperature (°C)
Column	0 – 5	60
	5 – 80	60 → 210
	80 – 95	210
Injection port		200
Detector		220

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

- *resolution*: minimum 1.5 between the peaks due to estragole and α-terpineol.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution and locate *cis*-anethole and pseudoisoeugenyl 2-methylbutyrate using the chromatogram shown in Figure 0804.-1 (disregard any peak due to hexane).

Determine the percentage content of these components. The percentages are within the following ranges:

- *linalol* : less than 1.5 per cent,
- *estragole* : 0.5 per cent to 5.0 per cent,
- α-*terpineol* : less than 1.2 per cent,

— *cis-anethole* : 0.1 per cent to 0.4 per cent,

— *trans-anethole* : 87 per cent to 94 per cent,

— *anisaldehyde* : 0.1 per cent to 1.4 per cent,

— *pseudoisoeugenyl 2-methylbutyrate*: 0.3 per cent to 2.0 per cent.

STORAGE

In a well-filled, airtight container, protected from light and at a temperature not exceeding 25 °C.

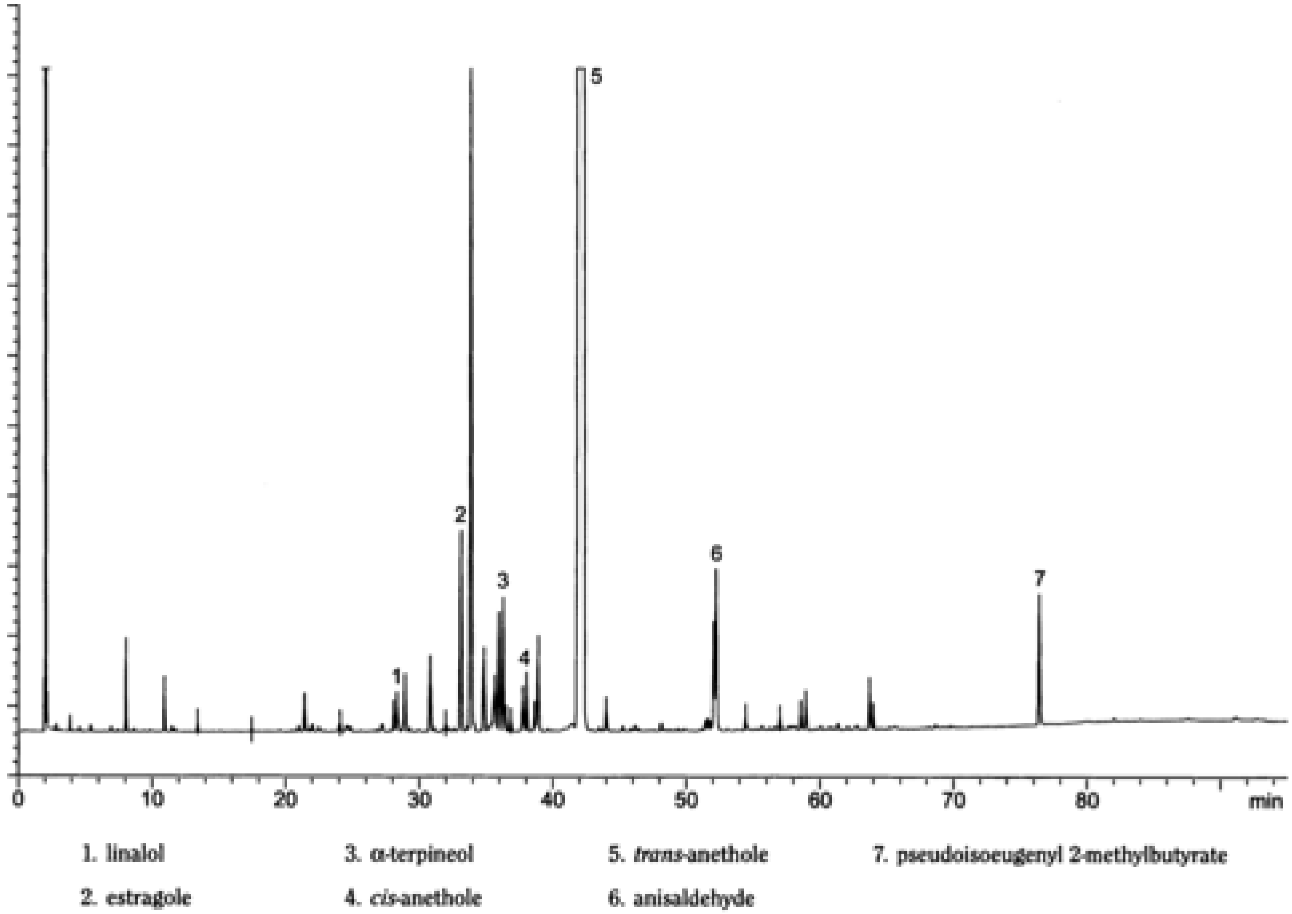


Figure 0804.-1. – *Chromatogram for the test for chromatographic profile of anise oil*

Ph Eur

Aniseed

General Notices

Anise

(Ph Eur monograph 0262)

When Powdered Aniseed is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Whole dry cremocarp of *Pimpinella anisum* L.

Content

Minimum 20 ml/kg of essential oil.

CHARACTERS

Reminiscent odour of anethole.

The fruit is a cremocarp and generally entire; a small fragment of the thin, rigid, slightly curved pedicel is frequently attached.

IDENTIFICATION

A. The cremocarp is ovoid or pyriform and slightly compressed laterally, yellowish-green or greenish-grey, 3-5 mm long and up to 3 mm wide, surmounted by a stylopod with 2 short, reflexed stylar points. The mericarps are attached by their tops to the carpophore with a plane commissural surface and a convex dorsal surface, the latter being covered with short, warty trichomes visible using a lens; the fruit shows 5 primary ridges, running longitudinally, comprising 3 dorsal ridges and 2 lateral ridges, non-prominent, and lighter in colour.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-yellow or brownish-green. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: whole or broken trichomes, mostly unicellular, sometimes curved, with blunt apex and warty cuticle; fragments of epidermis with striated cuticle, occasional anomocytic stomata *(2.8.3)* ; fragments of numerous narrow, branched vittae, fragments of endosperm containing aleurone grains and micro-rosettes of calcium oxalate; oblong sclereids from the commissural zone and bundles of sclerenchymatous fibres from the carpophore and the pedicel. Starch is absent.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Shake 0.10 g of the powdered drug (1500) with 2 ml of *methylene chloride R* for 15 min. Filter and carefully evaporate the filtrate to dryness on a water-bath at 60 °C. Dissolve the residue in 0.5 ml of *toluene R* .

Reference solution Dissolve 3 µl of *anethole R* and 40 µl of *olive oil R* in 1 ml of *toluene R* .

Plate *TLC silica gel GF* $_{254}$ *plate R*.

Mobile phase *toluene R* .

Application 2 µl and 3 µl of the test solution and 1 µl, 2 µl and 3 µl of the reference solution, at 2 cm intervals.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatograms show a quenching zone (anethole) in the central part against a light background.

Detection B Spray with a freshly prepared 200 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R* , using 10 ml for a plate 200 mm square, and heat at 120 °C for 5 min.

Results B The spots due to anethole appear blue against a yellow background. In the chromatogram obtained with 2 µl of the test solution, the spot due to anethole is intermediate in size between the corresponding spots in the chromatograms obtained with 1 µl and 3 µl of the reference solution. The chromatograms obtained with the test solution show in the lower third a blue spot (triglycerides) similar in position to the spot in the lower third of the chromatograms obtained with the reference solution (triglycerides of olive oil).

TESTS

Water *(2.2.13)*

Maximum 70 ml/kg, determined on 20.0 g of the powdered drug.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 2.5 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)* . Use 10.0 g of the drug reduced to a coarse powder immediately before determination, a 250 ml round-bottomed flask, 100 ml of *water R* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 2.5-3.5 ml/min for 2 h.

Arnica Flower

General Notices

(Ph Eur monograph 1391)

Ph Eur

DEFINITION

Whole or partially broken, dried flower-heads of *Arnica montana* L.

Content

Minimum 0.40 per cent *m/m* of total sesquiterpene lactones, expressed as dihydrohelenalin tiglate (dried drug).

CHARACTERS

Aromatic odour.

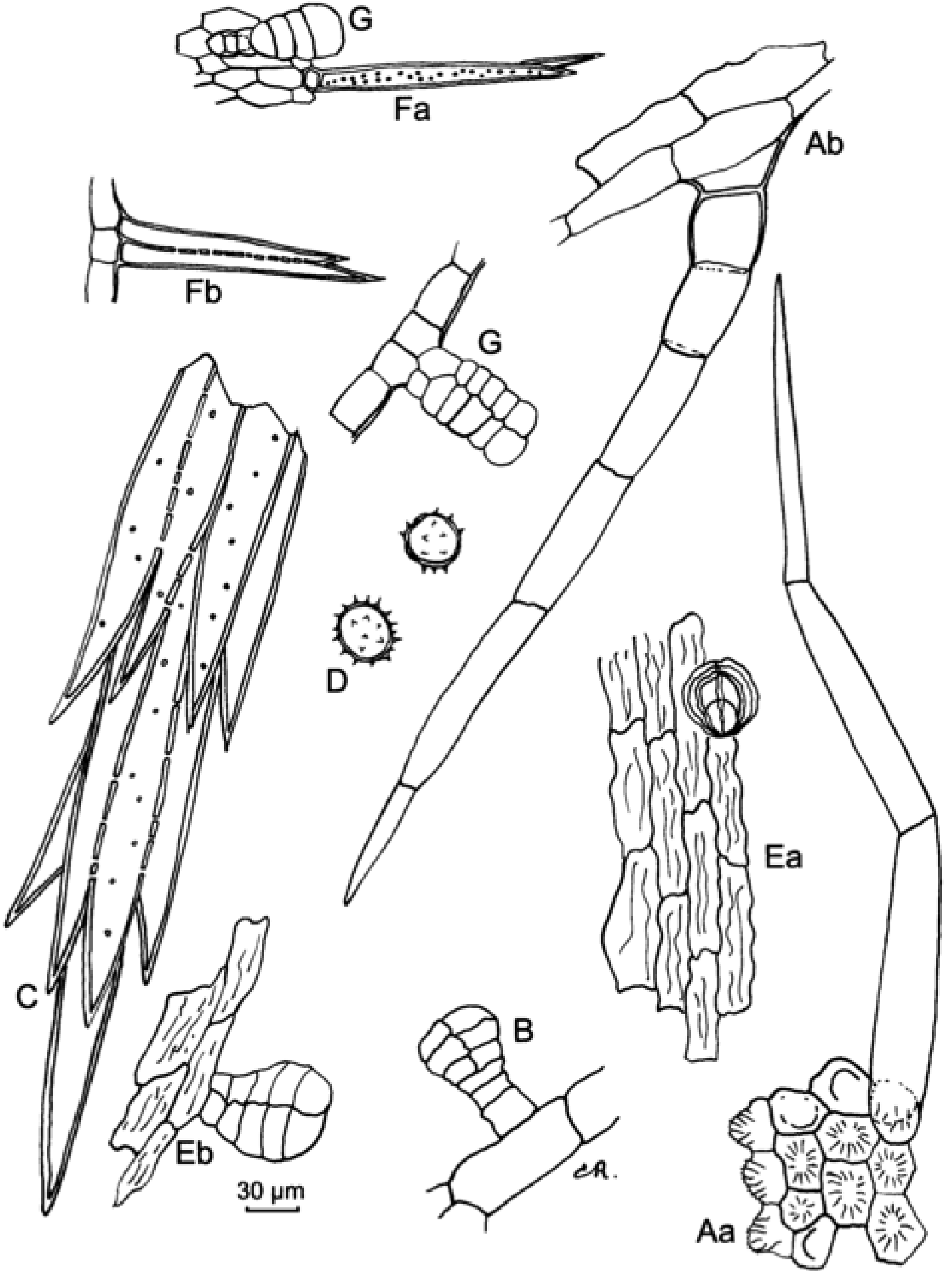
The capitulum, when spread out, is about 20 mm in diameter and about 15 mm deep, and has a peduncle 2-3 cm long. The involucre consists of 18-24 elongated lanceolate bracts, with acute apices, arranged in 1-2 rows: the bracts, about 8-10 mm long, are green with yellowish-green external hairs visible under a lens. The receptacle, about 6 mm in diameter, is convex, alveolate and covered with hairs. Its periphery bears about 20 ligulate florets 20-30 mm long; the disc bears a greater number of tubular florets about 15 mm long. The ovary, 4-8 mm long, is crowned by a pappus of whitish bristles 4-8 mm long. Some brown achenes, crowned or not by a pappus, may be present.

IDENTIFICATION

A. The involucre consists of elongated oval bracts with acute apices; the margin is ciliated. The ligulate floret has a reduced calyx crowned by fine, shiny, whitish bristles, bearing small coarse trichomes. The orange-yellow corolla bears 7-10 parallel veins and ends in 3 small lobes. The stamens, with free anthers, are incompletely developed. The narrow, brown ovary bears a stigma divided into 2 branches curving outwards. The tubular floret is actinomorphic. The ovary and the calyx are similar to those of the ligulate floret. The short corolla has 5 reflexed triangular lobes; the 5 fertile stamens are fused at the anthers.

B. Separate the capitulum into its different parts. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: the epidermises of the bracts of the involucre have stomata and trichomes, more abundant on the outer (abaxial) surface. There are several different types of trichomes: uniseriate multicellular covering trichomes, varying in length from 50-500 µm, particularly abundant on the margins of the bract; secretory trichomes with uni- or biseriate multicellular stalks and with multicellular, globular heads, about 300 µm long, abundant on the outer surface of the bract;

secretory trichomes with uniseriate multicellular stalks and with multicellular, globular heads, about 80 µm long, abundant on the inner surface of the bract. The epidermis of the ligulate corolla consists of lobed or elongated cells, a few stomata and trichomes of different types: covering trichomes, with very sharp ends, whose length may exceed 500 µm, consisting of 1-3 proximal cells with thickened walls and 2-4 distal cells with thin walls; secretory trichomes with biseriate multicellular heads; secretory trichomes with multicellular stalks and multicellular globular heads. The ligule ends in rounded papillose cells. The epidermis of the ovary is covered with trichomes: secretory trichomes with short stalks and multicellular globular heads; twinned covering trichomes usually consisting of 2 longitudinally united cells, with common punctuated walls; their ends are sharp and sometimes bifid. The epidermises of the calyx consist of elongated cells bearing short, unicellular, covering trichomes pointing towards the upper end of the bristle. The pollen grains have a diameter of about 30 µm, are rounded, with a spiny exine, and have 3 germinal pores.


G
Fa
Ab
Fb
G
D
Ea
C
B
Eb
30 µm
cA.
Aa

A. Epidermis of the ligulate corolla with covering trichome

E. Epidermis of the corolla with striated cuticle and biseriate in surface view (Aa) and in side view (Ab)

B. Secretory trichome with multicellular head

C. Bristles of the calyx

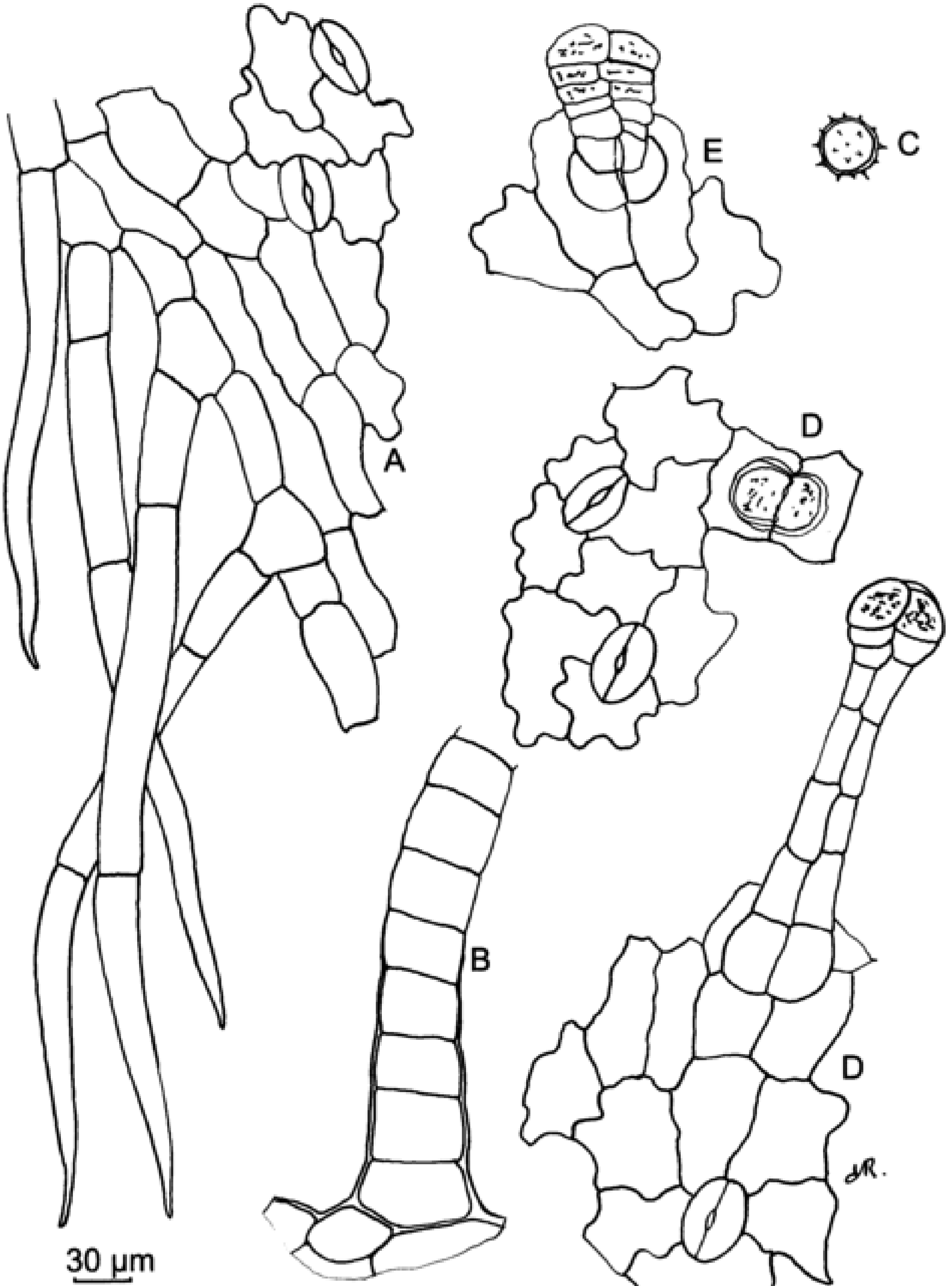
D. Pollen grain

secretory trichome in surface view (Ea) and in side view (Eb)

F. Covering trichome of the ovary in surface view (Fa) and in side view (Fb)

G. Secretory trichome of the ovary

Figure 1391.-1. – *Illustration of powdered herbal drug of arnica flower (see Identification B)*


E
C
A
D
D
B
30 µm
JR.

A. Epidermis of the bracts of the involucre with covering trichomes and stomata

B. Multicellular stalk of covering trichome

C. Pollen grain

D. Epidermis of bracts of the involucre with stomata and biseriate secretory trichome

E. Secretory trichome

Figure 1391.-2. – *Illustration of powdered herbal drug of arnica flower (see Identification B)*

C. Examine the chromatograms obtained in the test for Calendula officinalis L. - *Heterotheca inuloides* Cass.

Results The chromatogram obtained with the test solution shows, in the middle, a fluorescent blue zone corresponding to the zone due to chlorogenic acid in the chromatogram obtained with the reference solution; it shows, above this zone, 3 fluorescent yellowish-brown or orange-yellow zones, and above these 3 zones a fluorescent greenish-yellow zone due to astragalin. The zone located below the astragalin zone is due to isoquercitroside; the zone located just below this zone is due to luteolin-7-glucoside. It also shows a fluorescent greenish-blue zone below the zone due to caffeic acid in the chromatogram obtained with the reference solution.

TESTS

Foreign matter (*2.8.2*)

Maximum 5.0 per cent.

***Calendula officinalis* L. - *Heterotheca inuloides* Cass**

Thin-layer chromatography (*2.2.27*).

Test solution To 2.00 g of the powdered drug (710) (*2.9.12*) add 10 ml of *methanol R*. Heat in a water-bath at 60 °C for 5 min with shaking. Cool and filter.

Reference solution Dissolve 2.0 mg of *caffeic acid R*, 2.0 mg of *chlorogenic acid R* and 5.0 mg of *rutin R* in *methanol R* and dilute to 30 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application 15 µl, as bands.

Development Over a path of 15 cm.

Drying In air for a few minutes.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol*

R, and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Heat at 100-105 °C for 5 min. Allow to dry in air and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the lower part an orange-yellow fluorescent zone due to rutin, in the middle part a fluorescent zone due to chlorogenic acid and in the upper part a light bluish fluorescent zone due to caffeic acid. The chromatogram obtained with the test solution does not show a fluorescent orange-yellow zone corresponding to the zone due to rutin in the chromatogram obtained with the reference solution, nor does it show a zone below this.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 10.0 per cent.

ASSAY

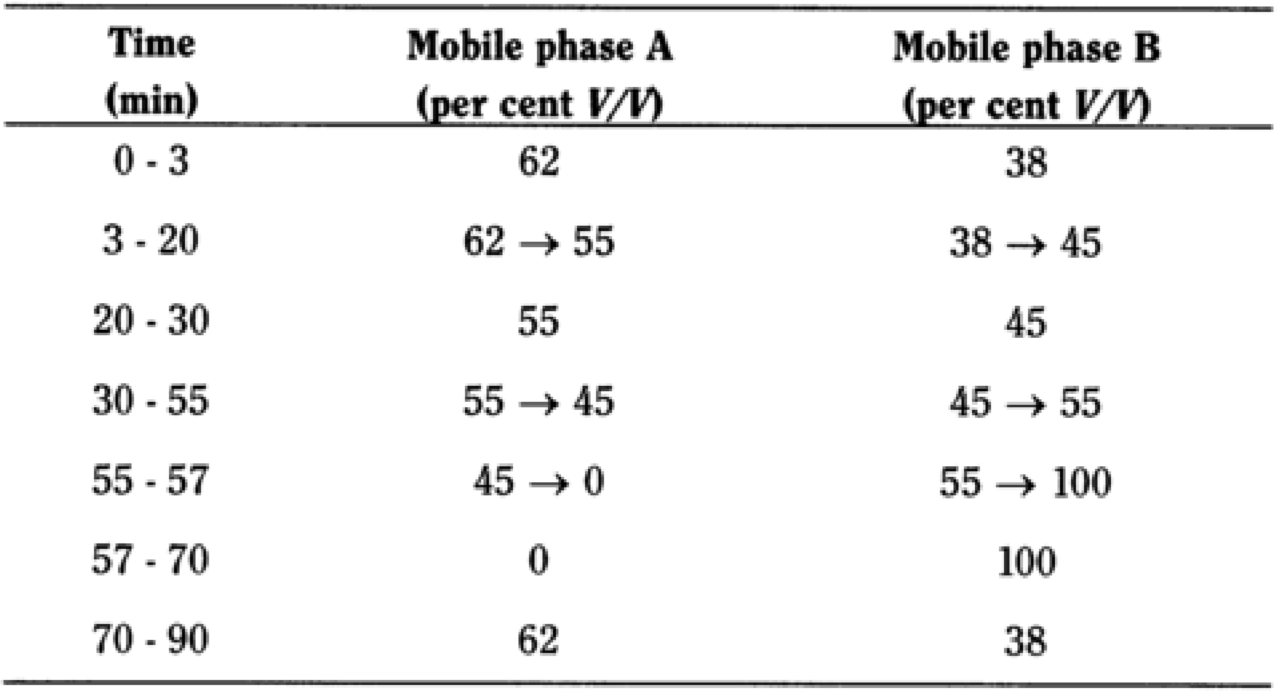
Liquid chromatography (*2.2.29*).

Internal standard solution Dissolve immediately before use 0.010 g of *santonin R*, accurately weighed in 10.0 ml of *methanol R*.

Test solution Introduce 1.00 g of the powdered drug (355) (*2.9.12*) into a 250 ml round-bottomed flask, add 50 ml of a mixture of equal volumes of *methanol R* and *water R* and heat under a reflux condenser in a water-bath at 50-60 °C for 30 min, shaking frequently. Allow to cool and filter through a paper filter. Add the paper filter, cut into pieces, to the residue in the round-bottomed flask, add 50 ml of a mixture of equal volumes of *methanol R* and *water R* and heat under a reflux condenser in a water-bath at 50-60 °C for 30 min, shaking frequently. Repeat this procedure twice. To the combined filtrate add 3.00 ml of the internal standard solution and evaporate to 18 ml under reduced pressure. Rinse the round-bottomed flask with *water R* and dilute, with the washings, to 20.0 ml. Transfer the solution to a chromatography column about 0.15 m long and about 30 mm in internal diameter containing 15 g of *kieselguhr for chromatography R*. Allow to stand for 20 min. Elute with 200 ml of a mixture of equal volumes of *ethyl acetate R* and *methylene chloride R*. Evaporate the eluate to dryness in a 250 ml round-bottomed flask. Dissolve the residue in 10.0 ml of *methanol R* and add 10.0 ml of *water R*. Add 7.0 g of *neutral aluminium oxide R*, shake for 120 s, centrifuge at 5000 g for 10 min and filter through a paper filter. Evaporate 10.0 ml of the filtrate to dryness. Dissolve the residue in 3.0 ml of a mixture of equal volumes of *methanol R* and *water R* and filter.

Column:
- — *size*: *l* = 0.12 m, Ø = 4 mm;
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:
- — *mobile phase A*: *water R*;
- — *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	62	38
3 - 20	62 → 55	38 → 45
20 - 30	55	45
30 - 55	55 → 45	45 → 55
55 - 57	45 → 0	55 → 100
57 - 70	0	100
70 - 90	62	38

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 225 nm.

Injection A 20 µl loop injector.

Calculate the percentage content of total sesquiterpene lactones, expressed as dihydrohelenalin tiglate, using the following expression:

$$\frac{S_{\mathrm{LS}} \times C \times V \times 1.187 \times 100}{S_{\mathrm{S}} \times m \times 1000}$$

S_{LS} = area of all peaks due to sesquiterpene lactones appearing after the santonin peak in the chromatogram obtained with the test solution

S_{S} = area of the peak due to santonin in the chromatogram obtained with the test solution

m = mass of the drug to be examined, in grams

C = concentration of santonin in the internal standard solution used for the test solution, in milligrams per millilitre

V = volume of the internal standard solution used for the test solution, in millilitres

1.187 = peak correlation factor between dihydrohelenalin tiglate and santonin.

Ph Eur

Arnica Tincture

General Notices

(Ph Eur monograph 1809)

Ph Eur

DEFINITION

Tincture produced from *Arnica flower (1391)*.

Content

Minimum 0.04 per cent of sesquiterpene lactones expressed as dihydrohelenalin tiglate ($C_{20}H_{26}O_5$; M_r 346.42).

PRODUCTION

The tincture is produced from 1 part of the drug and 10 parts of ethanol (60 per cent *V/V* to 70 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance

Yellowish-brown liquid.

IDENTIFICATION

Examine the chromatograms obtained in the test for *Calendula officinalis - Heterotheca inuloides.*

Chromatogram obtained with the test solution:

— in the middle, a fluorescent blue zone corresponding to the zone due to chlorogenic –acid in the chromatogram obtained with the reference solution;

— above this zone, 3 fluorescent yellowish-brown to orange-yellow zones, and above these 3 zones a fluorescent greenish-yellow zone corresponding to astragalin; the zone located below the astragalin zone corresponds to isoquercitrin; the zone located just below this zone corresponds to luteolin-7-glucoside;

— a fluorescent greenish-blue zone below the zone due to caffeic acid in the chromatogram obtained with the reference solution.

TESTS

Calendula officinalis - *Heterotheca inuloides*

Thin-layer chromatography (*2.2.27*).

Test solution The tincture to be examined.

Reference solution Dissolve 2.0 mg of *caffeic acid R*, 2.0 mg of *chlorogenic acid R* and 5.0 mg of *rutin R* in *methanol R* and dilute to 30.0 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application 30 µl [or 8 µl] as bands.

Development Over a path of 15 cm [or 8 cm].

Drying At 80-105 °C.

Detection Spray the plate whilst still hot with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Heat 5 min at 100 - 105 °C. Allow the plate to dry in air and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the lower part an orange-yellow fluorescent zone (rutin), in the middle part a fluorescent zone due to chlorogenic acid and in the upper part a light bluish fluorescent zone (caffeic acid). The chromatogram obtained with the test solution does not show any fluorescent orange-yellow zone corresponding to rutin in the chromatogram obtained with the reference solution and no zone below the zone corresponding to rutin.

Ethanol *(2.9.10)*

The final alcohol concentration is not less than 90 per cent of that of the initial extraction solvent.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

Dry residue *(2.8.16)*

Minimum of 1.7 per cent.

ASSAY

Liquid chromatography *(2.2.29)* .

Internal standard solution Dissolve immediately before use 0.010 g accurately weighed of *santonin R* and 0.02 g of *butyl 4-hydroxybenzoate R* in 10.0 ml of *methanol R*.

Test solution In a round-bottomed flask introduce 5.00 g of the tincture to be examined, add 2.00 ml of internal standard solution and 3 g of *anhydrous aluminium oxide R*, shake for 120 s and filter through a paper filter. Rinse the round-bottomed flask and filter with 5 ml of a mixture of equal volumes of *methanol R* and *water R* and filter. Evaporate the filtrate to dryness. Dissolve the residue in 2.0 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R* and filter by a membrane filter (porosity: 0.45 µm).

Reference solution Dissolve 0.02 g of *methyl 4-hydroxybenzoate R* and 0.02 g of *ethyl 4-hydroxybenzoate R* in *methanol R* and dilute to 10.0 ml with the same solvent.

Column:

— *size: l* = 0.12 m, Ø = 4 mm,

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 20 °C.

Mobile phase:

— *mobile phase A*: *water R* ;

— *mobile phase B*: *methanol R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	62	38
3 - 20	62 → 55	38 → 45
20 - 30	55	45
30 - 55	55 → 45	45 → 55

Flow rate 1.2 ml/min.

Detector Spectrophotometer at 225 nm.

Injection 20 µl.

Relative retention With reference to santonin (retention time = about 9.5 min): butyl 4-hydroxybenzoate = about 4.6.

System suitability Reference solution:

— *resolution*: minimum 5 between the peaks due to methyl 4-hydroxybenzoate and ethyl 4-hydroxybenzoate.

Calculate the percentage of lactone sesquiterpenes, expressed as dihydrohelenalin tiglate from the expression:

$$\frac{F_1 \times C \times V \times 1.187}{F_2 \times m \times 10}$$

F_1 = area of all peaks appearing between the peaks due to santonin and butyl 4-hydroxybenzoate in the chromatogram obtained with the test solution,

F_2 = area of the peak due to santonin in the chromatogram obtained with the test solution,

m = mass of the tincture to be examined, in grams,

C = concentration of santonin in the internal standard solution used for the test solution in milligrams per millilitre,

V = volume of internal standard solution used for the test solution, in millilitres,

1.187 = peak correlation factor between dihydrohelenalin tiglate and santonin.

Ph Eur

Artichoke Leaf

General Notices

(Ph Eur monograph 1866)

Ph Eur

DEFINITION

Whole or cut, dried leaf of *Cynara scolymus* L.

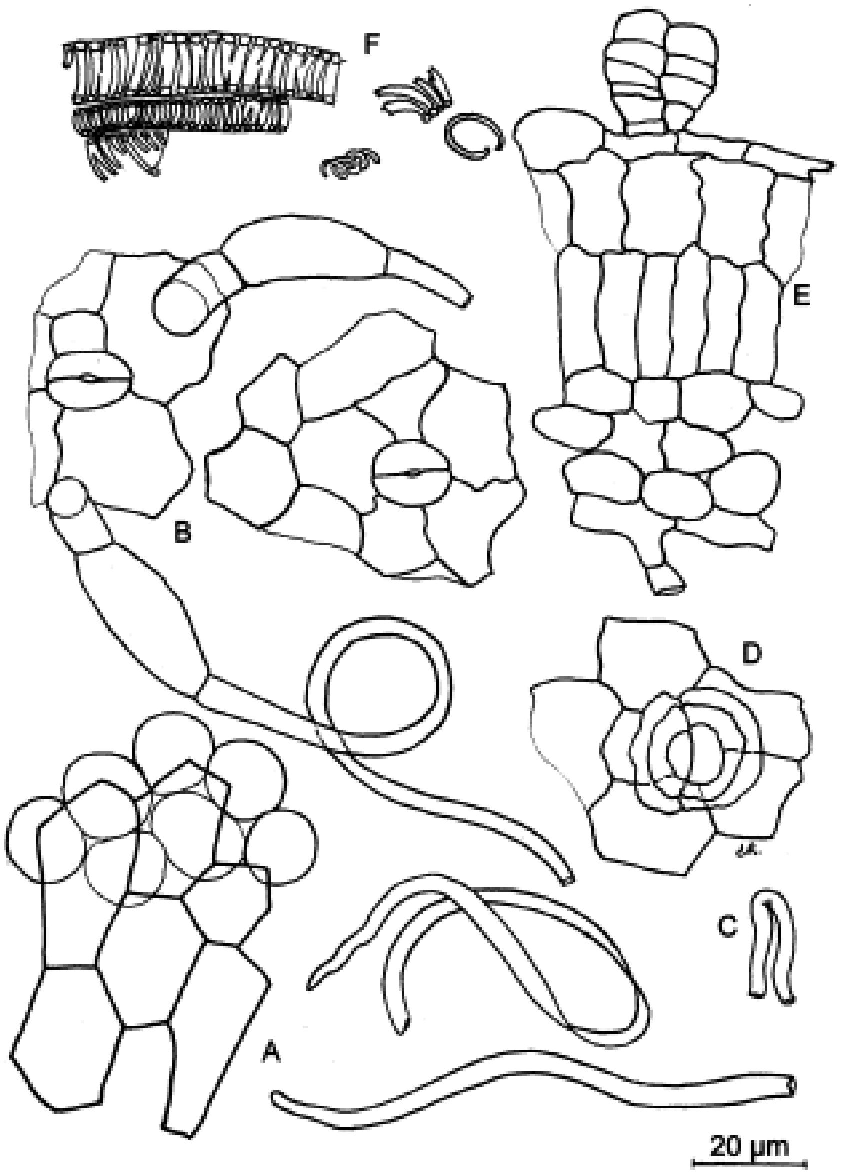
Content

Minimum 0.8 per cent of chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3) (dried drug).

IDENTIFICATION

A. The entire leaf may be up to 70 cm long and 30 cm wide. The lamina is deeply lobed in the upper part to within 1-2 cm of the petiole on either side, in the lower part the leaf becomes pinnate; all the segments have markedly dentate margins and taper at the apex. Spines are absent. The upper surface of the lamina is green with a fine covering of whitish hairs, the lower surface is pale green or white and densely tomentose with long, tangled hairs. The petiole and main veins are flat on the upper surface, prominently raised and longitudinally ridged on the lower surface, with conspicuous hairs on both surfaces.

B. Reduce to a powder (1000) *(2.9.12)*. The powder is greenish-grey. Examine under a microscope using *chloral hydrate solution R* . The powder shows fragments of the epidermis of the lamina, those of the upper epidermis composed of cells with straight or slightly sinuous walls, those of the lower epidermis more sinuous-walled; abundant anomocytic stomata *(2.8.3)* on both surfaces; multicellular, uniseriate covering trichomes in felted masses, the majority with a short stalk composed of several cells and a very long, narrow and frequently curled terminal cell, others consisting of 4-6 cylindrical cells; very occasional glandular trichomes with a short stalk and a biseriate or uniseriate head; abundant fragments of vascular tissue from the petiole and veins.



A. Upper epidermis of lamina in surface view

B. Lower epidermis of lamina in surface view showing stomata and covering trichomes

C. Fragment of covering trichomes

D. Multicelluar, biseriate, glandular trichome in surface view

E. Section view of lamina showing a glandular trichome

F. Fragments of vascular tissue

Figure 1866.-1. – *Illustration for the identification of artichoke leaf (see Identification B)*

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 2.0 g of the powdered drug (1000) *(2.9.12)* add 20 ml of *ethanol (60 per cent V/V) R*. Allow to stand for 2 h with occasional stirring. Filter.

Reference solution Dissolve 5 mg of *luteolin-7-glucoside R* and 5 mg of *chlorogenic acid CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *acetic acid R* , *anhydrous formic acid R* , *water R* , *ethyl acetate R* (11:11:27:100 *V/V/V/V).*

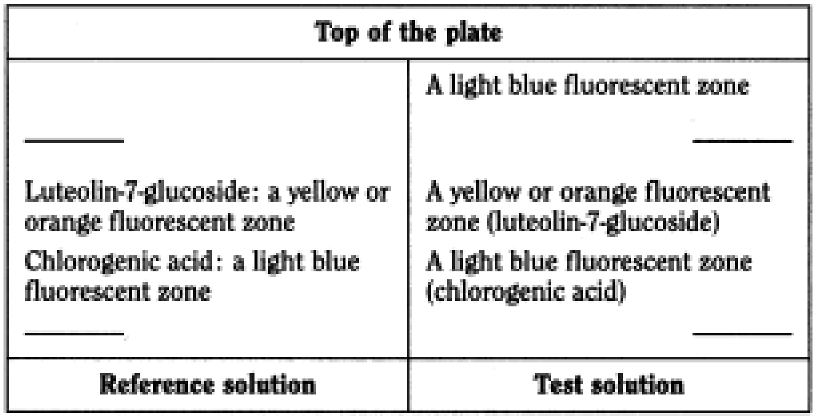
Application 10 µl [or 2 µl] as bands.

Development Over a path of 13 cm [or 6 cm].

Drying In air.

Detection Heat the plate at 100 °C for 5 min; spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* followed by a 50 g/l solution of *macrogol 400 R* in *methanol R* ; examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A light blue fluorescent zone
———	———
Luteolin-7-glucoside: a yellow or orange fluorescent zone	A yellow or orange fluorescent zone (luteolin-7-glucoside)
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
———	———
Reference solution	**Test solution**

TESTS

Total ash *(2.4.16)*

Maximum 20.0 per cent.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (710) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution To 0.500 g of the powdered drug (1000) *(2.9.12)* add 50.0 ml of *methanol R* and heat under a reflux condenser on a water-bath at 70 °C for 1 h. Centrifuge and transfer the supernatant to a 200 ml volumetric flask. Repeat the procedure and dilute to 200.0 ml with *water R* .

Reference solution Dissolve 5.0 mg of *chlorogenic acid CRS* in 50.0 ml of *methanol R* . Transfer 5.0 ml of this solution to a volumetric flask, add 5 ml of *methanol R* and dilute to 20.0 ml with *water R* .

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

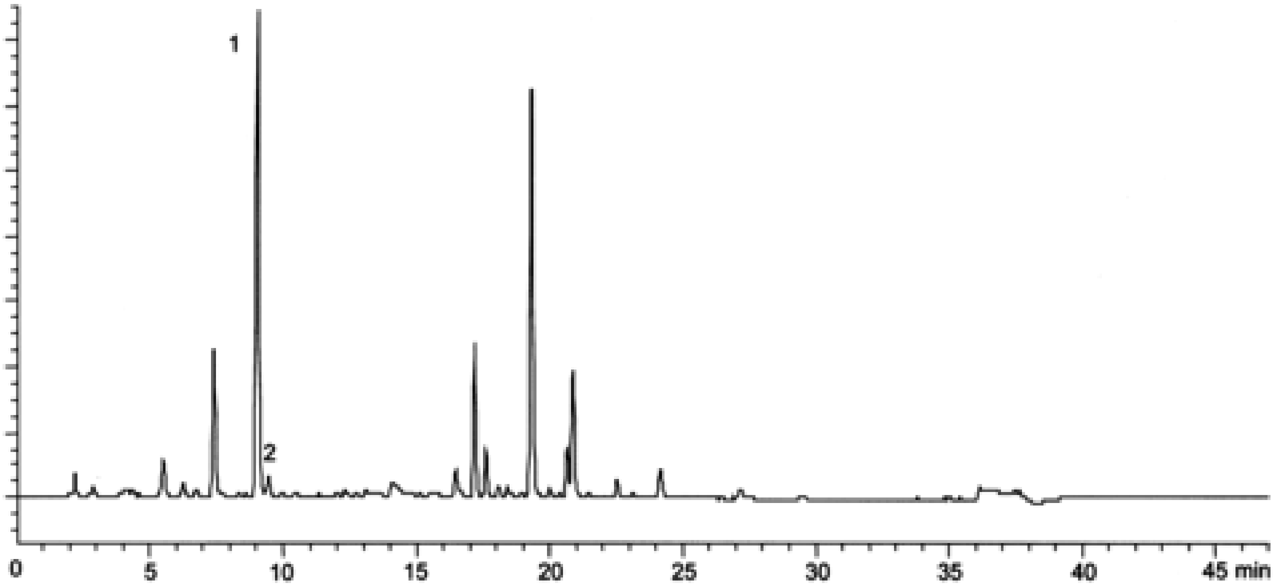


Figure 1866.-2. – *Chromatogram for the assay of artichoke leaf: test solution*

Mobile phase:

- *mobile phase A*: *phosphoric acid R*, *water R* (0.5:99.5 *V/V*);
- *mobile phase B*: *phosphoric acid R*, *acetonitrile R* (0.5:99.5 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	92	8
1 - 20	92 → 75	8 → 25
20 - 33	75	25
33 - 35	75 → 0	25 → 100
35 - 37	0 → 92	100 → 8
37 - 47	92	8

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 330 nm.

Injection 25 µl.

System suitability Test solution:

- the chromatogram obtained is similar to the chromatogram shown in Figure 1866.-2;

— *resolution*: minimum 2.0 between the peak due to chlorogenic acid and the subsequent peak (peak 2).

Calculate the percentage content of chlorogenic acid using the following expression:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1}$$

A_1 = area of the peak due to chlorogenic acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;

m_1 = mass of the drug to be examined in the test solution, in grams;

m_2 = mass of *chlorogenic acid CRS* in the reference solution, in grams;

p = percentage content of chlorogenic acid in *chlorogenic acid CRS*.

Ph Eur

Ash Leaf

General Notices

(Ph Eur monograph 1600)

Ph Eur

DEFINITION

Dried leaf of *Fraxinus excelsior* L. or *Fraxinus oxyphylla* M. Bieb.

Content

Minimum 2.5 per cent of total hydroxycinnamic acid derivatives, expressed as chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3) (dried drug).

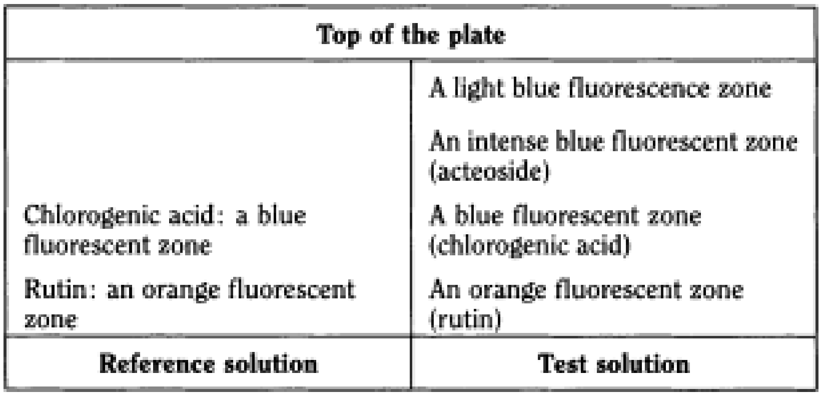
IDENTIFICATION

A. The leaf consists of leaflets which are sometimes detached and separated from the rachis. The leaflet is about 6 cm long and 3 cm wide. Each leaflet is subsessile or shortly petiolate, oblong, lanceolate, somewhat unequal at the base, acuminate at the apex, with fine acute teeth on the margins, the upper surface is dark green and the lower surface is greyish-green. The midrib and secondary veins are whitish and prominent on the lower surface.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green. Examined under a microscope, using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the lamina in surface view, with the lower epidermis showing numerous anomocytic stomata *(2.8.3)* and some of the cells of the upper epidermis with cuticular striations; occasional uniseriate, conical covering trichomes composed of 1 or 2 cells with thick walls and a striated cuticle; rare peltate glands with a unicellular stalk and a shield-like glandular head composed of 8 radiating cells; groups of fibres and fragments of vascular tissue from the veins.

C. Examine the chromatograms obtained in the test for *Fraxinus ornus* L.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	A light blue fluorescence zone
	An intense blue fluorescent zone (acteoside)
Chlorogenic acid: a blue fluorescent zone	A blue fluorescent zone (chlorogenic acid)
Rutin: an orange fluorescent zone	An orange fluorescent zone (rutin)
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 3.0 per cent of stems and maximum 2.0 per cent of other foreign matter.

Fraxinus ornus L

Thin-layer chromatography *(2.2.27)* .

Test solution To 1 g of the powdered drug (355) *(2.9.12)* add 20 ml of *methanol R* . Heat with shaking at 40 °C for 10 min. Filter.

Reference solution Dissolve 5.0 mg of *rutin R* and 5.0 mg of *chlorogenic acid R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R* .

Mobile phase *anhydrous formic acid R* , *water R* , *ethyl acetate R* (10:10:80 *V/V/V*).

Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a solution containing 10 g/l of *diphenylboric acid aminoethyl ester R* and 50 g/l of *macrogol 400 R* in *methanol R* . Examine in ultraviolet light at 365 nm.

Results In the case of a substitution by F. *ornus*, the chromatogram obtained with the test solution does not show the zones of acteoside, chlorogenic acid and rutin.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

ASSAY

Test solution (a) To 0.300 g of the powdered drug (355) *(2.9.12)* add 95 ml of *ethanol (50* *per cent V/V) R*. Boil in a water-bath under a reflux condenser for 30 min. Allow to cool and filter. Rinse the filter with 5 ml of *ethanol (50 per cent V/V) R*. Combine the filtrate and the rinsings in a volumetric flask and dilute to 100.0 ml with *ethanol (50 per cent V/V) R*.

Test solution (b) To 1.0 ml of test solution (a) in a test tube, add 2 ml of *0.5 M hydrochloric acid* , 2 ml of a solution prepared by dissolving 10 g of *sodium nitrite R* and 10 g of *sodium molybdate R* in 100 ml of *water R* , then add 2 ml of *dilute sodium hydroxide solution R* and dilute to 10.0 ml with *water R* ; mix.

Immediately measure the absorbance *(2.2.25)* of test solution (b) at 525 nm, using as compensation liquid a solution prepared as follows: mix 1.0 ml of test solution (a), 2 ml of *0.5 M hydrochloric acid* , 2 ml of *dilute sodium hydroxide solution R* and dilute to 10.0 ml with *water R* .

Calculate the percentage content of total hydroxycinnamic acid derivatives, expressed as chlorogenic acid, from the following expression:

$$\frac{A \times 5.3}{m}$$

taking the specific absorbance of chlorogenic acid to be 188.

A = absorbance at 525 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Astragalus Root for use in THM

General Notices

DEFINITION

Astragalus Root for use in THM is the dried root of *Astragalus propinquus.* Schischk. (synonyms: *Astragalus mongholicus* Bunge; *Astragalus membranaceus* (Fisch.) Bunge.)

It is collected in spring and autumn, separated from the rootlets and rootstock and dried.

IDENTIFICATION

A. The whole root is cylindrical, sometimes branched, up to 30 to 90 cm long and about 2 to 3 cm in diameter at the widest part, buds or the remains of shoots may be present at the crown, near which the root is frequently twisted. The outer surface is pale yellowish to greyish brown, faintly striated longitudinally with irregular transverse ridges. The fracture is difficult, fibrous and starchy. The smoothed transverse surface is cream to yellow and shows an outer region separated by darker cambium ring from the radiate xylem; on the larger pieces there is a central hollow surrounded by dark brown fragments of disintegrating tissue.

B. Reduce to a powder. The powder is yellowish-white. Examine under a microscope using *chloral hydrate solution* . The fibres are in bundles or scattered, 8 to 30 µm in diameter, thick-walled, with longitudinal fissures on the surface, the primary walls often separated from the secondary walls, both ends often broken to tassel-like, or slightly truncated. Bordered-pitted vessels colourless or orange, bordered pits arranged closely. Stone cells occasionally visible, rounded, oblong or irregular, slightly thick-walled. Examine under a microscope using 50% v/v of *glycerol* in *water*. Starch granules usually simple, ovoid, 5 to 20 µm in diameter, with a visible linear or punctuate hilum.

C. Carry out the method for *thin-layer chromatography* , Appendix III A, using the following solutions.

(1) Place 6 g of the powdered drug in a Soxhlet extractor, add 75 ml of *methanol* , heat under reflux for 7 hours, cool and filter.

(2) 0.3% w/v each of *ferulic acid* and *linoleic acid* in *methanol.*

(3) 0.1% w/v each of *astragaloside I CRS, astragaloside II CRS* and *astragaloside IV CRS* in *methanol* .

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution, as bands.

(d) Develop the plate to 15 cm [or 7 cm].

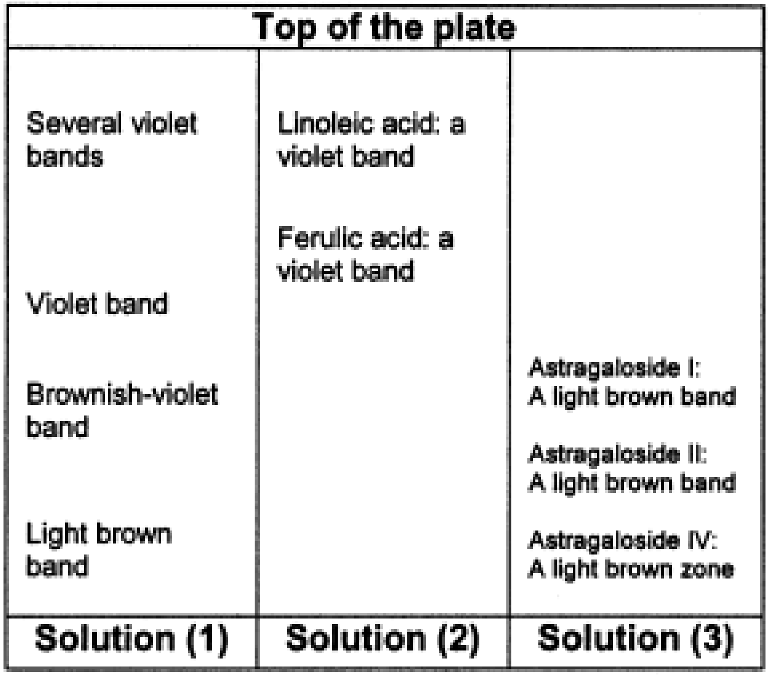
(e) Remove the plate and allow it to dry in cold air for 5 minutes. Examine under *ultraviolet light (254 nm)*. Spray the plate with *anisaldehyde solution*, heat at 100° to 105° for 5 minutes and examine in daylight.

MOBILE PHASE

A mixture of 30 volumes of *water*, 41 volumes of *methanol* and 200 volumes of *ethyl acetate.*

SYSTEM SUITABILITY

The test is not valid unless, when examined under daylight after spraying with *anisaldehyde solution*, the chromatogram obtained with solution (2) shows two clearly separated bands and the chromatogram obtained with solution (3) shows three clearly separated bands.



Top of the plate		
Several violet bands	Linoleic acid: a violet band	
	Ferulic acid: a violet band	
Violet band		
Brownish-violet band		Astragaloside I: A light brown band
		Astragaloside II: A light brown band
Light brown band		Astragaloside IV: A light brown zone
Solution (1)	**Solution (2)**	**Solution (3)**

CONFIRMATION

When examined under daylight after spraying with *anisaldehyde solution* the chromatogram obtained with solution (1) shows a band corresponding to astragaloside IV and several other bands as shown above. Other bands may be present.

When examined under *ultraviolet light (254 nm)* the chromatogram obtained with solution (1) shows violet coloured bands with Rf values of approximately 0.4 and 0.8 and no band corresponding to ferulic acid as shown below. Other bands may be present. The chromatogram obtained with solution (2) shows a single violet band with an Rf value of approximately 0.7 (ferulic acid). The chromatogram obtained with solution (3) shows no bands.

Top of the plate		
Two violet bands		
	Ferulic acid: a violet band	
Two violet bands		
Solution (1)	**Solution (2)**	**Solution (3)**

TESTS

Hedysarum root

Macroscopical *Hedysarum polybotrys* root, a known adulterant of Astragalus Root for use in THM, has much smaller and more branched roots, with many branches arising from near the crown; the outer surface is dark reddish-brown.

Thin-layer chromatography In Identification test C, the chromatogram obtained with solution (1) shows no bands due to *astragaloside I CRS* and *astragaloside IV CRS* when examined under daylight after spraying with *anisaldehyde solution* .

Top of the plate			
Several violet bands	Several violet bands	Linoleic acid: a violet band	
		Ferulic acid: a violet band	
Violet band	Violet band		
Brownish-violet band			Astragaloside I: A light brown band
			Astragaloside II: A light brown band
Light brown band			Astragaloside IV: A light brown zone
Astragalus	***Hedysarum***	**Solution (2)**	**Solution (3)**
Solution (1)			

Acid-insoluble ash

Not more than 1.0%, Appendix XI K .

Total ash

Not more than 5.0%, Appendix XI J, Method II.

Loss on drying

When dried for 2 hours at 100° to 105°, loses not more than 10.0% of its weight. Use 1 g.

Water-soluble extractive

Not less than 30%, Appendix XI B2.

STORAGE

Astragalus Root for use in THM should be protected from moisture. Exposure to sunlight for as little as 30 minutes can bleach the yellow colour.

Barbados Aloes

General Notices

Curaçao Aloes

(Ph Eur monograph 0257)

Preparation

Standardised Aloes Dry Extract

Ph Eur

DEFINITION

Concentrated and dried juice of the leaves of *Aloe barbadensis* Miller.

Content

Minimum 28.0 per cent of hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4) (dried drug).

CHARACTERS

Appearance

Dark brown masses, slightly shiny or opaque with a conchoidal fracture, or brown powder.

Solubility

Partly soluble in boiling water, soluble in hot ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution To 0.25 g of the powdered drug add 20 ml of *methanol R* and heat to boiling in a water-bath. Shake for a few minutes and decant the solution. Store at about 4 °C and use within 24 h.

Reference solution Dissolve 25 mg of *barbaloin R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *water R, methanol R, ethyl acetate R* (13:17:100 *V/V/V*).

Application 10 µl, as bands of 20 mm by maximum 3 mm.

Development Over a path of 10 cm.

Drying In air.

Detection A Spray with a 100 g/l solution of *potassium hydroxide R* in *methanol R* and examine in ultraviolet light at 365 nm.

Results A The chromatogram obtained with the test solution shows in the central part a yellow fluorescent zone (barbaloin) similar in position to the zone due to barbaloin in the chromatogram obtained with the reference solution and in the lower part a light blue fluorescent zone (aloesine).

Detection B Heat at 110 °C for 5 min.

Results B In the chromatogram obtained with the test solution, a violet fluorescent zone appears just below the zone due to barbaloin.

B. Shake 1 g of the powdered drug with 100 ml of boiling *water R*. Cool, add 1 g of *talc R* and filter. To 10 ml of the filtrate add 0.25 g of *disodium tetraborate R* and heat to dissolve. Pour 2 ml of this solution into 20 ml of *water R*. Yellowish-green fluorescence appears which is particularly marked in ultraviolet light at 365 nm.

C. To 5 ml of the filtrate obtained in identification test B add 1 ml of freshly prepared *bromine water R*. A brownish-yellow precipitate is formed and the supernatant liquid is violet.

TESTS

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 2.0 per cent.

ASSAY

Carry out the assay protected from bright light.

Introduce 0.300 g of powdered drug (180) *(2.9.12)* into a 250 ml conical flask. Moisten with 2 ml of *methanol R*, add 5 ml of *water R* warmed to about 60 °C, mix, then add a further 75 ml of *water R* at about 60 °C and shake for 30 min. Cool, filter into a volumetric flask, rinse the conical flask and filter with 20 ml of *water R*, add the rinsings to the volumetric flask and dilute to 1000.0 ml with *water R*. Transfer 10.0 ml of this solution to a 100 ml round-bottomed flask containing 1 ml of a 600 g/l solution of *ferric chloride R* and 6 ml of *hydrochloric acid R*. Heat in a water-bath under a reflux condenser for 4 h, with the water level above that of the liquid in the flask. Allow to cool, transfer the solution to a separating funnel, rinse the flask successively with 4 ml of *water R*, 4 ml of *1 M sodium hydroxide* and 4 ml of *water R* and add the rinsings to the separating funnel. Shake the contents of the separating funnel with 3 quantities, each of 20 ml, of *ether R*. Wash the combined ether layers with 2 quantities, each of 10 ml, of *water R*. Discard the washings and dilute the organic phase to 100.0 ml with *ether R*. Evaporate 20.0 ml of the solution carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 512 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of hydroxyanthracene derivatives, as barbaloin, from the following expression:

$$\frac{A \times 19.6}{m}$$

i.e. taking the specific absorbance of barbaloin to be 255.

A = absorbance at 512 nm,

m = mass of the substance to be examined, in grams.

STORAGE

In an airtight container .

Ph Eur

Bearberry Leaf

General Notices

(Ph Eur monograph 1054)

Ph Eur

DEFINITION

Whole or cut, dried leaf of *Arctostaphylos uva-ursi* (L.) Spreng.

Content

Minimum 7.0 per cent of anhydrous arbutin ($C_{12}H_{16}O_7$; M_r 272.3) (dried drug).

IDENTIFICATION

A. The leaf, shiny and dark green on the adaxial surface, lighter on the abaxial surface, is normally 7-30 mm long and 5-12 mm wide. The entire leaf is obovate with smooth margins, somewhat reflexed downwards, narrowing at the base into a short petiole. The leaf is obtuse or retuse at its apex. The lamina is thick and coriaceous. The venation, pinnate and finely reticulate, is clearly visible on both surfaces. The adaxial surface is marked with sunken veinlets, giving it a characteristic grainy appearance. Only the young leaf has ciliated margins. Old leaves are glabrous.

B. Reduce to a powder (355) (*2.9.12*). The powder is green to greenish-grey or yellowish-green. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of epidermises, which, seen in surface view, show polygonal cells covered by a thick smooth cuticle, and with straight, thick and irregularly pitted walls; anomocytic stomata (*2.8.3*), surrounded by 5-11 subsidiary cells and scars of hair bases only on the abaxial epidermis; fragments of palisade parenchyma, with 3 or 4 layers of cells of unequal lengths, and spongy parenchyma; groups of lignified fibres from the pericycle, with rows of cells containing prisms of calcium oxalate; occasional conical, unicellular covering trichomes.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 0.5 g of the powdered drug (355) (*2.9.12*) add 5 ml of a mixture of equal volumes of *methanol R* and *water R* , and heat under a reflux condenser for 10 min. Filter whilst hot. Wash the flask and the filter with a mixture of equal volumes of *methanol R* and *water R* and dilute to 5 ml with the same mixture of solvents.

Reference solution Dissolve 25 mg of *arbutin R* , 25 mg of *gallic acid R* and 25 mg of *hydroquinone R* in *methanol R* and dilute to 10.0 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *anhydrous formic acid R* , *water R* , *ethyl acetate R* (6:6:88 *V/V/V*).

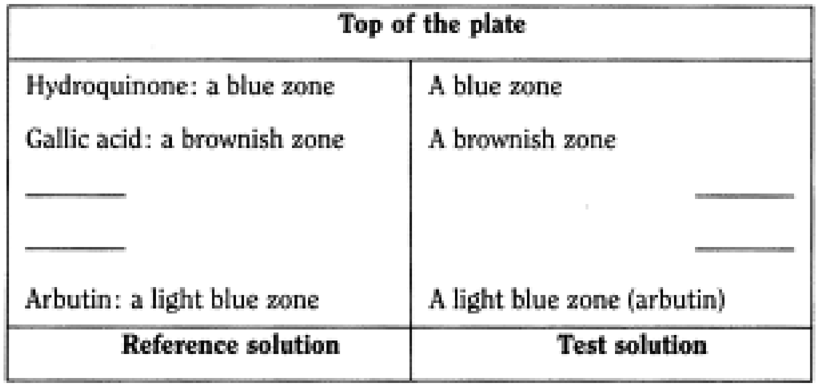
Application 10 µl of the reference solution and 20 µl of the test solution, as bands.

Development Over a path of 15 cm.

Drying At 105-110 °C until the mobile phase has evaporated.

Detection Spray with a 10 g/l solution of *dichloroquinonechlorimide R* in *methanol R* , then spray with a 20 g/l solution of *anhydrous sodium carbonate R* .

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, 2 or 3 blue bands and several brown or brownish-grey bands may be present in the chromatogram obtained with the test solution.



Top of the plate	
Hydroquinone: a blue zone	A blue zone
Gallic acid: a brownish zone	A brownish zone
_______	_______
_______	_______
Arbutin: a light blue zone	A light blue zone (arbutin)
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 5 per cent of stems and maximum 3 per cent of other foreign matter.

Leaves of different colour

Maximum 10 per cent, determined in the same manner as foreign matter (*2.8.2*).

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 5.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution In a 100 ml flask with a ground-glass neck, place 0.800 g of the powdered drug (250) (*2.9.12*). Add 20 ml of *water R* and heat under a reflux condenser on a water-bath for 30 min. Allow to cool and filter the liquid through a plug of absorbent cotton. Add the absorbent cotton to the residue in the 100 ml flask and extract with 20 ml of *water R* under a reflux condenser on a water-bath for 30 min. Allow to cool and filter through a paper filter. Combine the filtrates and dilute to 50.0 ml with *water R*. Filter the liquid through a paper filter. Discard the first 10 ml of the filtrate.

Reference solution (a) Dissolve 50.0 mg of *arbutin CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 2.5 mg of *hydroquinone R* in the mobile phase and dilute to 10.0 ml with the mobile phase. To 5.0 ml of this solution add 2.5 ml of reference solution (a) and dilute to 10.0 ml with the mobile phase.

Column:
— *size*: $l = 0.25$ m, Ø = 4 mm;
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm) .

Mobile phase *methanol R*, *water R* (10:90 *V/V*).

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

System suitability:
— *resolution*: minimum 4.0 between the peaks due to arbutin and hydroquinone in the chromatogram obtained with reference solution (b).

Calculate the percentage content of arbutin using the following expression:

$$\frac{F_1 \times m_2 \times p}{F_2 \times m_1}$$

F_1 = area of the peak due to arbutin in the chromatogram obtained with the test solution

F_2 = area of the peak due to arbutin in the chromatogram obtained with the reference solution

m_1 = mass of the drug to be examined used to prepare the test solution, in grams

m_2 = mass of *arbutin CRS* used to prepare the reference solution, in grams

p = percentage content of arbutin in *arbutin CRS*.

Ph Eur

Belladonna Leaf

General Notices

Belladonna Herb

(Ph Eur monograph 0221)

Preparations

Prepared Belladonna

Standardised Belladonna Leaf Dry Extract

Belladonna Tincture

When Belladonna Herb, Belladonna Leaf or Powdered Belladonna Herb is prescribed, Prepared Belladonna shall be supplied.

Ph Eur

DEFINITION

Dried leaf or dried leaf and flowering, and occasionally fruit-bearing tops *of Atropa belladonna L.*

Content

Minimum 0.30 per cent of total alkaloids, expressed as hyoscyamine ($C_{17}H_{23}NO_3$; M_r 289.4) (dried drug). The alkaloids consist mainly of hyoscyamine together with small quantities of hyoscine (scopolamine).

CHARACTERS

Slightly nauseous odour.

IDENTIFICATION

A. The leaves are green or brownish-green, slightly darker on the upper surface, often crumpled and rolled and partly matted together in the drug. The leaf is petiolate and the base of the lamina is acute and decurrent and the margin entire. The flowering stems are flattened and bear at each node a pair of leaves unequal in size, in the axils of which occur singly the flowers or occasionally fruits. The flowers have a gamosepalous calyx and campanulate corolla. The fruits are globular berries, green or brownish-black and surrounded by the persistent calyx with widely spread lobes.

B. Reduce to a powder (355) *(2.9.12)*. The powder is dark green. Examine under a microscope, using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of leaf lamina showing sinuous-walled epidermal cells, a striated cuticle; stomata more frequent on the lower epidermis (anisocytic and also some anomocytic) *(2.8.3)* ; multicellular uniseriate covering trichomes with smooth cuticle, glandular trichomes with unicellular heads and multicellular, uniseriate stalks or with multicellular heads and unicellular stalks; parenchyma cells including rounded cells containing microsphenoidal crystals of calcium oxalate; annular and spirally thickened vessels. The powdered drug may also show: fibres and reticulately thickened vessels from the stems; subspherical pollen grains, 40-50 µm in diameter, with 3 germinal pores, 3 furrows and an extensively pitted exine; fragments of the corolla, with a papillose epidermis or bearing numerous covering or glandular trichomes of the types previously described; brownish-yellow seed fragments containing irregularly sclerified and pitted cells of the testa.

C. Shake 1 g of powdered drug (180) *(2.9.12)* with 10 ml of *0.05 M sulphuric acid* for 2 min. Filter and add to the filtrate 1 ml of *concentrated ammonia R* and 5 ml of *water R* . Shake cautiously with 15 ml of *ether R* , avoiding formation of an emulsion. Separate the ether layer and dry over *anhydrous sodium sulphate R* . Filter and evaporate the ether in a porcelain dish. Add 0.5 ml of *fuming nitric acid R* and evaporate to dryness on a water-bath. Add 10 ml of *acetone R* and, dropwise, a 30 g/l solution of *potassium hydroxide R* in *ethanol (96 per cent) R* . A deep violet colour develops.

D. Examine the chromatograms obtained in the chromatography test.

Results The principal zones in the chromatograms obtained with the test solution are similar in position, colour and size to the principal zones in the chromatograms obtained with the same volume of the reference solution.

TESTS

Chromatography.

Thin-layer chromatography *(2.2.27)* .

Test solution To 0.6 g of powdered drug (180) *(2.9.12)* add 15 ml of *0.05 M sulphuric acid* , shake for 15 min and filter. Wash the filter with *0.05 M sulphuric acid* until 20 ml of filtrate is obtained. To the filtrate add 1 ml of *concentrated ammonia R* and shake with 2 quantities, each of 10 ml, of *peroxide-free ether R* . If necessary, separate by centrifugation. Dry the combined ether layers over *anhydrous sodium sulphate R* , filter and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 ml of *methanol R* .

Reference solution Dissolve 50 mg of *hyoscyamine sulphate R* in 9 ml of *methanol R* . Dissolve 15 mg of *hyoscine hydrobromide R* in 10 ml of *methanol R* . Mix 1.8 ml of the hyoscine hydrobromide solution and 8 ml of the hyoscyamine sulphate solution.

Plate *TLC silica gel G plate R.*

Mobile phase *Concentrated ammonia R, water R, acetone R* (3:7:90 *V/V/V*).

Application 10 µl and 20 µl, as bands of 20 mm by 3 mm leaving 1 cm between the bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 15 min, allow to cool.

Detection A Spray with *potassium iodobismuthate solution R2* , using about 10 ml for a plate 200 mm square, until the orange or brown zones become visible against a yellow background.

Results A The zones in the chromatograms obtained with the test solution are similar in position (hyoscyamine in the lower third, hyoscine in the upper third of the chromatogram) and colour to the bands in the chromatograms obtained with the reference solution. The zones in the chromatograms obtained with the test solution are at least equal in size to the corresponding zones in the chromatogram obtained with the same volume of the reference solution. Faint secondary zones may appear, particularly in the middle of the chromatogram obtained with 20 µl of the test solution or near the starting point in the chromatogram obtained with 10 µl of the test solution.

Detection B Spray with *sodium nitrite solution R* until the coating is transparent and examine after 15 min.

Results B The zones due to hyoscyamine in the chromatograms obtained with the test solution and the reference solution change from brown to reddish-brown but not to greyish-blue (atropine) and any secondary zones disappear.

Foreign matter ***(2.8.2)***

Maximum 3 per cent of stems with a diameter greater than 5 mm.

Total ash ***(2.4.16)***

Maximum 16.0 per cent.

Ash insoluble in hydrochloric acid ***(2.8.1)***

Maximum 4.0 per cent.

ASSAY

a) Determine the loss on drying *(2.2.32)* on 2.000 g of powdered drug (180) *(2.9.12)*, by drying in an oven at 105 °C.

b) Moisten 10.00 g of powdered drug (180) *(2.9.12)* with a mixture of 5 ml of *ammonia R*, 10 ml of *ethanol (96 per cent) R* and 30 ml of *peroxide-free ether R* and mix thoroughly. Transfer the mixture to a suitable percolator, if necessary with the aid of the extracting mixture. Allow to macerate for 4 h and percolate with a mixture of 1 volume of *chloroform R* and 3 volumes of *peroxide-free ether R* until the alkaloids are completely extracted. Evaporate to dryness a few millilitres of the liquid flowing from the percolator, dissolve the residue in *0.25 M sulphuric acid* and verify the absence of alkaloids using *potassium tetraiodomercurate solution R*. Concentrate the percolate to about 50 ml by distilling on a water-bath and transfer it to a separating funnel, rinsing with *peroxide-free ether R*. Add a quantity of *peroxide-free ether R* equal to at least 2.1 times the volume of the percolate to produce a liquid of a density well below that of water. Shake the solution with no fewer than 3 quantities, each of 20 ml, of *0.25 M sulphuric acid*, separate the 2 layers by centrifugation if necessary and transfer the acid layers to a 2nd separating funnel. Make the acid layer alkaline with *ammonia R* and shake with 3 quantities, each of 30 ml, of *chloroform R*. Combine the chloroform layers, add 4 g of *anhydrous sodium sulphate R* and allow to stand for 30 min with occasional shaking. Decant the chloroform and wash the sodium sulphate with 3 quantities, each of 10 ml, of *chloroform R.* Add the washings to the chloroform extract, evaporate to dryness on a water-bath and heat in an oven at 100-105 °C for 15 min. Dissolve the residue in a few millilitres of *chloroform R*, add 20.0 ml of *0.01 M sulphuric acid* and remove the chloroform by evaporation on a water-bath. Titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red mixed solution R* as indicator.

Calculate the percentage content of total alkaloids, expressed as hyoscyamine, using the following expression:

$$\frac{57.88 \times (20 - n)}{(100 - d) \times m}$$

d = loss on drying as a percentage

n = volume of *0.02 M sodium hydroxide* , in millilitres;

m = mass of drug, in grams

Ph Eur

Belladonna Tincture

General Notices

(Standardised Belladonna Leaf Tincture, Ph Eur monograph 1812)

Ph Eur

DEFINITION

Tincture produced from *Belladonna leaf (0221).*

Content

0.027 per cent to 0.033 per cent of total alkaloids, calculated as hyoscyamine ($C_{17}H_{23}NO_3$; M_r 289.4). The alkaloids consist mainly of hyoscyamine together with small quantities of hyoscine.

PRODUCTION

The tincture is produced from 1 part of the powdered drug (355) (*2.9.12*) and 10 parts of ethanol (70 per cent *V/V*) by a suitable procedure.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Evaporate to dryness 10.0 ml of the tincture to be examined in a water-bath at 40 °C under reduced pressure. Dissolve the residue in 1.0 ml of *methanol R* .

Reference solution Dissolve 1.0 mg of *chlorogenic acid R* and 2.5 mg of *rutin R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R* , *water R* , *methyl ethyl ketone R, ethyl acetate R* (10:10:30:50 *V/V/V/V*).

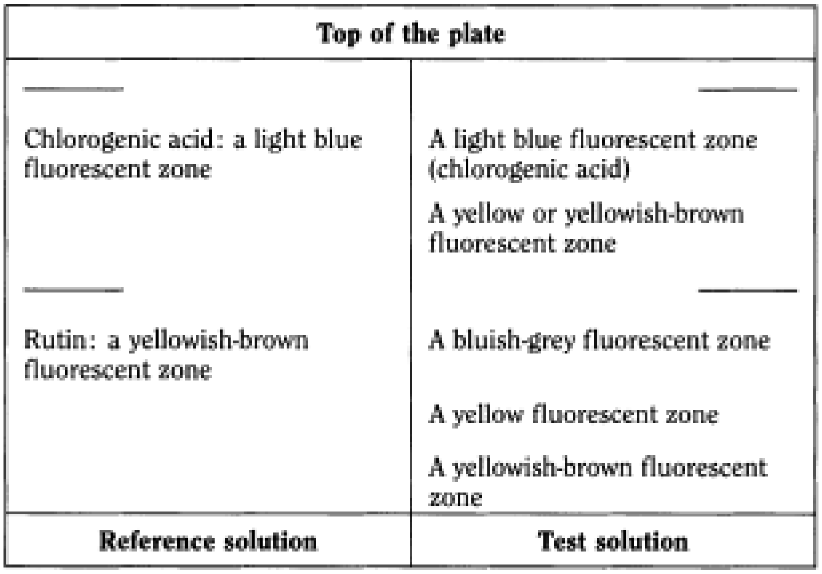
Application 40 µl, as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* ; subsequently spray the plate with a 50 g/l solution of *macrogol 400 R* in *methanol R* ; allow the plate to dry in air for 30 min and examine in ultraviolet light at 365 nm.

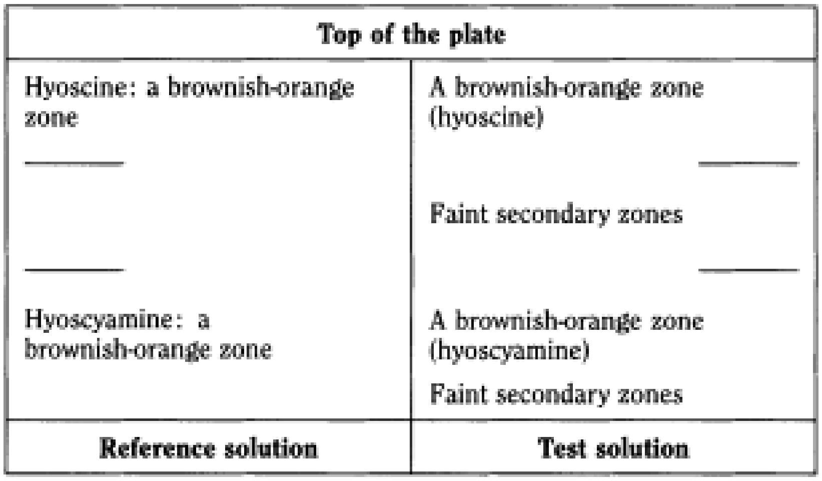
Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
	A yellow or yellowish-brown fluorescent zone
———	———
Rutin: a yellowish-brown fluorescent zone	A bluish-grey fluorescent zone
	A yellow fluorescent zone
	A yellowish-brown fluorescent zone
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for atropine, detection A.

Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Faint secondary zones may appear, particularly in the middle of the chromatogram obtained with 40 µl of the test solution or near the starting point in the chromatogram obtained with 20 µl of the test solution.



Top of the plate	
Hyoscine: a brownish-orange zone	A brownish-orange zone (hyoscine)
———	———
	Faint secondary zones
———	———
Hyoscyamine: a brownish-orange zone	A brownish-orange zone (hyoscyamine)
	Faint secondary zones
Reference solution	**Test solution**

TESTS

Atropine

Thin-layer chromatography (*2.2.27*).

Test solution To 15.0 ml of the tincture to be examined add 15 ml of *0.05 M sulphuric acid* . Filter. Add 1 ml of *concentrated ammonia R* to the filtrate and shake with 2 quantities, each of 10 ml, of *peroxide-free ether R* . Separate by centrifugation if necessary. Dry the combined ether layers over *anhydrous sodium sulphate R*. Filter and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 ml of *methanol R*.

Reference solution Dissolve 50 mg of *hyoscyamine sulphate R* in 9 ml of *methanol R*. Dissolve 15 mg of *hyoscine hydrobromide R* in 10 ml of *methanol R*. Mix 1.8 ml of the hyoscine hydrobromide solution and 8 ml of the hyoscyamine sulphate solution.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R*, *water R*, *acetone R* (3:7:90 *V/V/V*).

Application 20 µl and 40 µl of each solution, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 15 min.

Detection A Spray with *potassium iodobismuthate solution R2*.

Detection B Spray with *sodium nitrite solution R* until the plate is transparent. Examine after 15 min.

Results B The zones due to hyoscyamine in the chromatograms obtained with the test solution and the reference solution change from brownish-orange to reddish-brown but not to greyish-blue (atropine) and any secondary zones disappear.

Ethanol (*2.9.10*)

64 per cent *V/V* to 69 per cent *V/V.*

ASSAY

Evaporate 50.0 g of the tincture to be examined to a volume of about 10 ml. Transfer quantitatively to a separating funnel, with the minimum volume of *alcohol (70 per cent V/V) R*. Add 5 ml of *ammonia R* and 15 ml of *water R*. Shake with not fewer than 3 quantities each of 40 ml of a mixture of 1 volume of *methylene chloride R* and 3 volumes of *peroxide-free ether R*, carefully to avoid emulsion, until the alkaloids are completely extracted. Combine the organic layers and concentrate the solution to a volume of about 50 ml by distilling on a water-bath. Transfer the resulting solution quantitatively to a separating funnel, rinsing with *peroxide-free ether R*. Add a quantity of *peroxide-free ether R* equal to at least 2.1 times the volume of the solution to produce a layer having a density well below that of water. Shake the resulting solution with not fewer than 3 quantities each of 20 ml of *0.25 M sulphuric acid* until the alkaloids are completely extracted. Separate the layers by centrifugation if necessary and transfer the layers to a separating funnel. Make the combined layers alkaline with *ammonia R* and shake with not fewer than 3 quantities each of 30 ml of *methylene chloride R* until the alkaloids are completely extracted. Combine the organic layers, add 4 g of *anhydrous sodium sulphate R* and allow to stand for 30 min with occasional shaking. Decant the methylene chloride and filter. Wash the sodium sulphate with 3 quantities each of 10 ml of *methylene chloride R*. Combine the organic extracts, evaporate to dryness on a water-bath. Heat the residue in an oven at 100-105 °C for 15 min. Dissolve the residue in a few millilitres of *methylene chloride R*, evaporate to dryness on a water-bath and heat the residue in an oven at 100-105 °C for 15 min again. Dissolve the residue in a few millilitres of *methylene chloride R*. Add 20.0 ml of *0.01 M sulphuric acid* and remove the methylene chloride by evaporation on a water-bath. Titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red mixed solution R* as indicator.

Calculate the percentage content of total alkaloids, expressed as hyoscyamine, from the expression:

$$\frac{57.88 \times (20 - n)}{100 \times m}$$

n = volume of *0.02 M sodium hydroxide* used, in millilitres,

m = mass of drug used, in grams.

Ph Eur

Birch Leaf

General Notices

(Ph Eur monograph 1174)

Ph Eur

DEFINITION

Whole or fragmented dried leaves of *Betula pendula* Roth and/or *Betula pubescens* Ehrh. as well as hybrids of both species.

Content

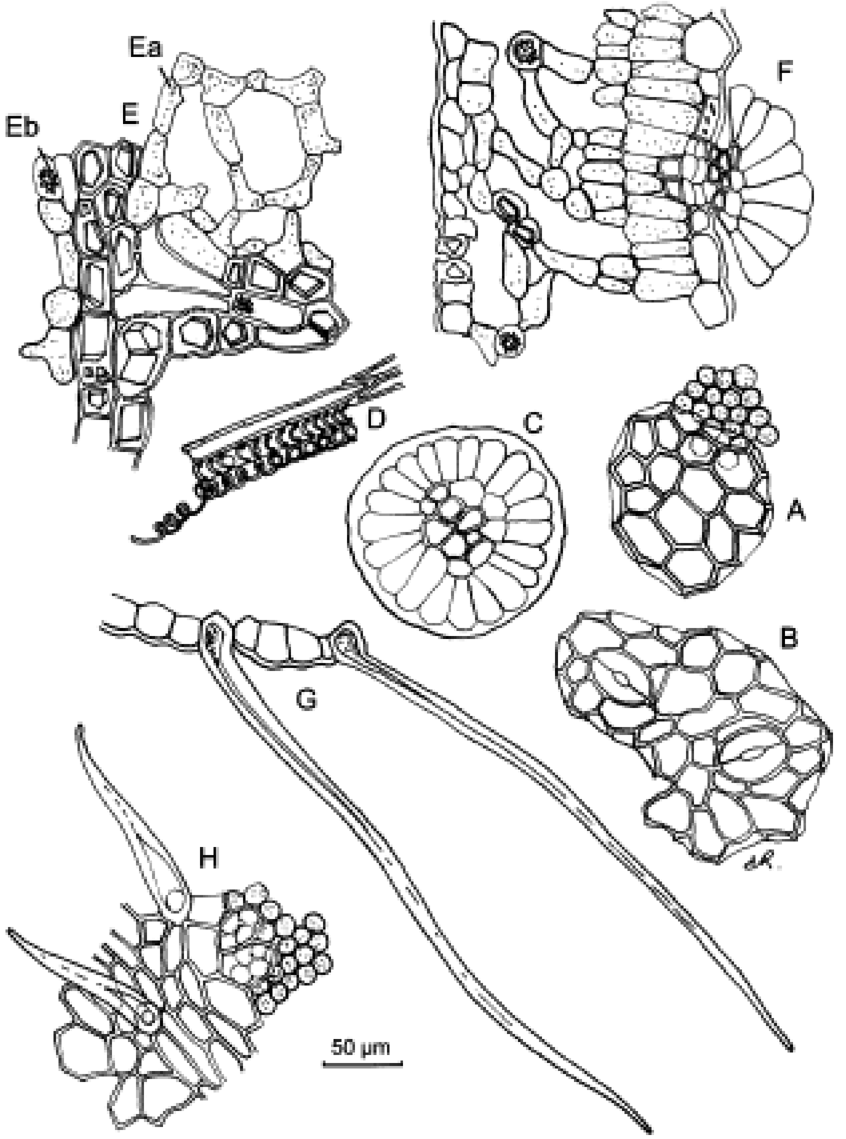
Minimum 1.5 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The leaves of both species are dark green on the adaxial surface and lighter greenish-grey on the abaxial surface; they show a characteristic dense reticulate venation. The veins are light brown or almost white.

The leaves of *Betula pendula* are glabrous and show closely spaced glandular pits on both surfaces. The leaves of *Betula pendula* are 3-7 cm long and 2-5 cm wide; the petiole is long and the doubly dentate lamina is triangular or rhomboid and broadly cuneate or truncate at the base. The angle on each side is unrounded or slightly rounded, and the apex is long and acuminate.

The leaves of *Betula pubescens* show few glandular trichomes and are slightly pubescent on both surfaces. The abaxial surface shows small bundles of yellowish-grey trichomes at the branch points of the veins. The leaves of *Betula pubescens* are slightly smaller, oval or rhomboid and more rounded. They are more roughly and more regularly dentate. The apex is neither long nor acuminate.



A. Upper epidermis of the lamina accompanied by palisade parenchyma

B. Lower epidermis of the lamina with anomocytic stomata

C. Peltate gland, in surface view

D. Xylem vessels accompanied by sclerenchyma fibres

E. Crystal sheaths containing prisms of calcium oxalate, spongy parenchyma (Ea) and cells containing cluster crystals of calcium oxalate (Eb)

F. Transverse section of the lamina, showing a peltate gland in side view

G. Covering trichomes on the margin of the lamina (*B. pubescens*)

H. Covering trichomes on the upper epidermis of the lamina (*B. pubescens*)

Figure 1174.-1. – *Illustration of powdered herbal drug of birch leaf (see Identification B)*

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-grey. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous fragments of lamina with straight-walled epidermal cells and cells of the lower epidermis surrounding anomocytic stomata *(2.8.3)* . Peltate large glands usually measuring 100-120 µm are found on the upper and lower epidermises. The mesophyll fragments contain calcium oxalate crystals. Fragments of radial vascular bundles and sclerenchyma fibres are accompanied by crystal sheaths. If *Betula pubescens* is present, the powder also contains unicellular covering trichomes with very thick walls, about 80-600 µm long, usually 100-200 µm.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* . Heat in a water-bath at 60 °C for 5 min. Cool and filter the solution.

Reference solution Dissolve 1 mg of *caffeic acid R* and 1 mg of *chlorogenic acid R* , 2.5 mg of *hyperoside R* and 2.5 mg of *rutin R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R* .

Mobile phase *anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In a current of warm air.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* ; subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R* ; allow to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows 3 zones in its lower half: in increasing order of R_F a yellowish-brown fluorescent zone (rutin), a light blue fluorescent zone (chlorogenic acid) and a yellowish-brown fluorescent zone (hyperoside), and in its upper third, a light blue fluorescent zone (caffeic acid). The chromatogram obtained with the test solution shows 3 zones similar in position and fluorescence to the zones due to rutin, chlorogenic acid and hyperoside in the chromatogram obtained with the reference solution. The zone due to rutin is very faint and the zone due to hyperoside is intense. It also shows other yellowish-brown faint fluorescence zones between the zones due to caffeic acid and chlorogenic acid in the chromatogram obtained with the reference solution. Near the solvent front, the red fluorescent zone due to chlorophylls is visible. In the chromatogram obtained with the test solution, between this zone and the zone due to caffeic acid in the chromatogram obtained with the reference solution, there is a brownish-yellow zone due to quercetin.

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent of fragments of female catkins and maximum 3 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of powered drug (355) by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

ASSAY

Stock solution In a 100 ml round-bottomed flask introduce 0.200 g of the powdered drug (355) *(2.9.12)*, 1 ml of a 5 g/l solution of *hexamethylenetetramine R* , 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1* . Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton in a 100 ml flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R* , each time boiling under a reflux condenser for 10 min. Allow to cool to room temperature, filter the liquid through a plug of absorbent cotton then through a filter-paper in the volumetric flask, and dilute to 100.0 ml with *acetone R* by rinsing of the flask and filter. Introduce 20.0 ml of the solution into a separating funnel, add 20 ml of *water R* and extract the mixture with 1 quantity of 15 ml and then 3 quantities, each of 10 ml, of *ethyl acetate R* . Combine the ethyl acetate extracts in a separating funnel, rinse with 2 quantities, each of 50 ml, of *water R* , and filter the extract over 10 g of *anhydrous sodium sulphate R* into a 50 ml volumetric flask and dilute to 50.0 ml with *ethyl acetate R* .

Test solution To 10.0 ml of the stock solution add 1 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R* .

Compensation liquid Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R* .

Measure the absorbance *(2.2.25)* of the test solution after 30 min, by comparison with the compensation liquid at 425 nm.

Calculate the percentage content of flavonoids, expressed as hyperoside, using the following expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of hyperoside to be 500.

A = absorbance at 425 nm;

m = mass of the substance to be examined, in grams.

Ph Eur

Bistort Rhizome

General Notices

(Ph Eur monograph 2384)

Ph Eur

DEFINITION

Whole or fragmented, dried rhizome of *Persicaria bistorta* (L.) Samp. (syn. *Polygonum bistorta* L.) without adventitious roots.

Content

Minimum 3.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

CHARACTERS

The whole rhizome is up to 13 cm long and 2.5 cm in diameter. The remnants of the roots are not longer than 1 cm and are about 1 mm in diameter.

IDENTIFICATION

A. The whole rhizome, reddish-brown or blackish-brown, is thick, twisted, and turned back on itself. Its outer surface shows transverse striations and blackish spots. It is flattened and somewhat depressed on the upper surface, convex on the lower surface. It shows adventitious root scars on the surface. The fracture, pinkish-beige, shows an elliptical zone of whitish pits corresponding to the vessels. The drug may also be obtained as more-or-less cylindrical fragments about 0.3 cm in diameter and up to 1 cm long, with a reddish-brown outer surface, marked by adventitious root scars and a pinkish-beige fracture.

B. Reduce to a powder (355) *(2.9.12)*. The powder is reddish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of parenchyma; very numerous calcium oxalate cluster crystals either free or inside parenchyma cells; a few reticulate lignified vessels; rare cork fragments. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R* . The powder shows rounded to ovoid starch granules, simple, about 10 µm in diameter.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of a mixture of equal volumes of *water R* and *methanol R* , heat on a water-bath at about 65 °C for 30 min and filter.

Reference solution Dissolve 5 mg of *fructose R* and 5 mg of *catechin R* in 5 ml of *methanol R* .

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *water R, anhydrous formic acid R, ethyl acetate R* (5:10:85 *V/V/V*).

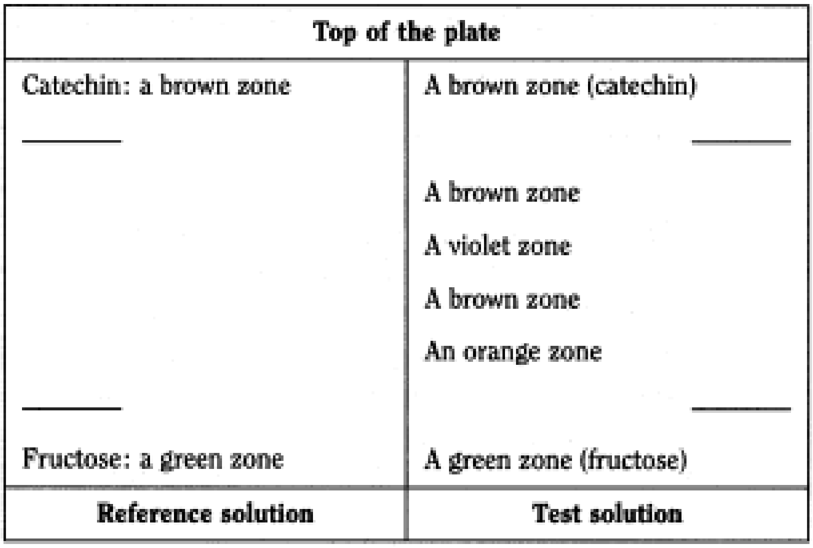
Application 2 µl as bands.

Development Over a path of 7 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Catechin: a brown zone	A brown zone (catechin)
_______	_______
	A brown zone
	A violet zone
	A brown zone
	An orange zone
_______	_______
Fructose: a green zone	A green zone (fructose)
Reference solution	**Test solution**

TESTS

Paris polyphylla* or *Paris quadrifolia

Examine the powdered drug (355) *(2.9.12)* under a microscope using *chloral hydrate solution R* . The presence of raphides of calcium oxalate, free or in bundles, indicates adulteration by the rhizome of *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz or *Paris polyphylla* Smith var. *chinensis* (Franch.) Hara or *Paris quadrifolia* L.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 9.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 1.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs *(2.8.14)* . Use 1.000 g of the powdered drug (180) *(2.9.12)*.

Ph Eur

Bitter Fennel

General Notices

(Ph Eur monograph 0824)

Ph Eur

DEFINITION

Dry cremocarps and mericarps of *Foeniculum vulgare* Miller sp. *vulgare* var. *vulgare*.

Content

— *essential oil*: minimum 40 ml/kg (anhydrous drug);

— *anethole* : minimum 60.0 per cent in the essential oil;

— *fenchone* : minimum 15.0 per cent in the essential oil.

CHARACTERS

Bitter fennel is greenish-brown, brown or green.

IDENTIFICATION

A. The fruit of bitter fennel is a cremocarp, of almost cylindrical shape with a rounded base and a narrower summit crowned with a large stylopod. It is generally 3-12 mm long and 3-4 mm wide. The mericarps, usually free, are glabrous. Each bears 5 prominent slightly carenated ridges. When cut transversely, 4 vittae on the dorsal surface and 2 on the commissural surface may be seen with a lens.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-brown or greyish-yellow. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: yellow fragments of wide secretory canals, often made up of yellowish-brown-walled polygonal secretory cells, frequently associated with a layer of thin-walled transversely elongated cells 2-9 µm wide, having a parquetry arrangement; reticulate parenchyma of the mesocarp; numerous fibre bundles from the ridges, often accompanied by narrow spiral vessels; very numerous endosperm fragments containing aleurone grains and very small calcium oxalate microrosette crystals, as well as some fibre bundles from the carpophore.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Shake 0.3 g of the freshly powdered drug (1400) *(2.9.12)* with 5.0 ml of *methylene chloride R* for 15 min. Filter and carefully evaporate the filtrate to dryness on a water-bath at 60 °C. Dissolve the residue in 0.5 ml of *toluene R* .

Reference solution Dissolve 50 µl of *anethole R* and 10 µl of *fenchone R* in 5.0 ml of *hexane R* .

Plate *TLC silica gel GF$_{254}$ plate R*.

Mobile phase *hexane R* , *toluene R* (20:80 *V/V*).

Application 10 µl, as bands of 20 mm by 3 mm.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatograms show in the central part a quenching zone due to anethole.

Detection B Spray with *sulphuric acid R* and heat at 140 °C for 5-10 min until a yellow zone due to fenchone appears in the lower third of the chromatograms.

Results B Anethole appears as a violet band in the central part. The chromatogram obtained with the test solution also shows a reddish-brown zone in its upper third (terpenes).

TESTS

Estragole

Gas chromatography (2.2.28) Use the normalisation procedure.

Test solution Dilute the mixture of essential oil and *xylene R* obtained in the determination of essential oil to 5.0 ml with *xylene R* , by rinsing the apparatus.

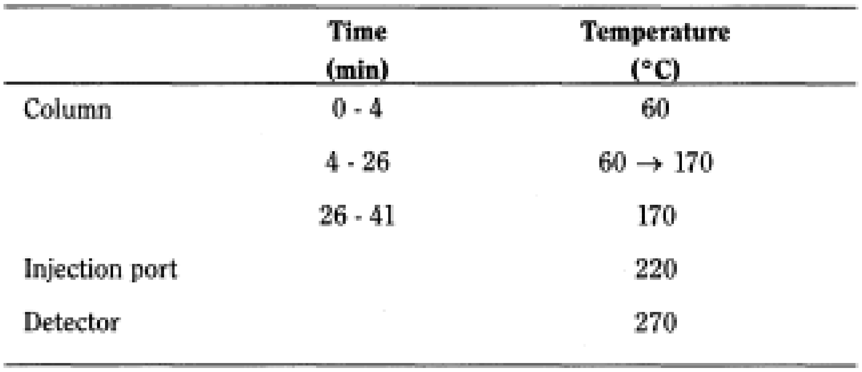
Reference solution Dissolve 5 mg of *estragole R* in 0.5 ml of *xylene R* .

Column:
- *size: l* = 30-60 m, Ø = 0.3 mm;
- *stationary phase*: *macrogol 20 000 R* .

Carrier gas *nitrogen for chromatography R* .

Flow rate 0.40 ml/min.

Split ratio 1:200.



	Time (min)	Temperature (°C)
Column	0 - 4	60
	4 - 26	60 → 170
	26 - 41	170
Injection port		220
Detector		270

Detection Flame ionisation.

Injection 1 µl.

Limits:

— *estragole* : maximum 5.0 per cent in the essential oil obtained in the assay.

Foreign matter ***(2.8.2)***

Maximum 1.5 per cent of peduncles and maximum 1.5 per cent of other foreign matter.

Water ***(2.2.13)***

Maximum 80 ml/kg, determined on 20.0 g of the powdered drug (710) *(2.9.12)*.

Total ash ***(2.4.16)***

Maximum 10.0 per cent.

ASSAY

Essential oil

Carry out the determination of essential oils in herbal drugs *(2.8.12)* . Use a 500 ml round-bottomed flask and 200 ml of *water R* as the distillation liquid. Reduce the drug to a coarse powder (1400) *(2.9.12)* and immediately use 5.0 g for the determination. Introduce 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

Anethole and fenchone

Gas chromatography *(2.2.28)* as described in the test for estragole with the following modifications.

Reference solution Dissolve 5 mg of *fenchone R* and 5 mg of *anethole R* in 0.5 ml of *xylene R* .

Elution order The order indicated in the composition of the reference solution. Record the retention times of these substances.

STORAGE

Protected from moisture.

Ph Eur

Bitter-Fennel Fruit Oil

General Notices

(Ph Eur monograph 1826)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the ripe fruits of *Foeniculum vulgare* Miller, ssp. *vulgare* var. *vulgare*.

Content:

- — fenchone: 12.0 per cent to 25.0 per cent,
- — *trans*-anethole: 55.0 per cent to 75.0 per cent.

CHARACTERS

Appearance

Clear, colourless or pale yellow liquid.

It has a characteristic odour.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.1 ml of the oil to be examined in 5 ml of *toluene R* .

Reference solution Dissolve 10 µl of *fenchone R* and 80 µl of *anethole R* in 5 ml of *toluene R* .

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R* , *toluene R* (5:95 *V/V*).

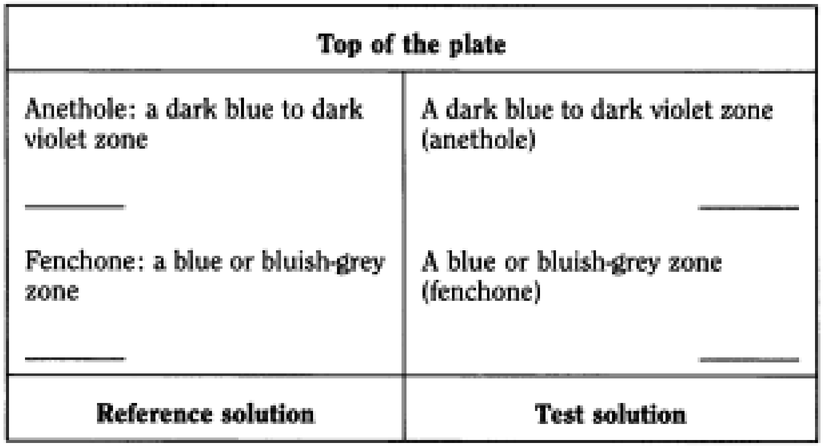
Application 10 µl as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a freshly prepared 200 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R* and heat at 150 °C for 15 min; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Anethole: a dark blue to dark violet zone	A dark blue to dark violet zone (anethole)
Fenchone: a blue or bluish-grey zone	A blue or bluish-grey zone (fenchone)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

0.961 to 0.975.

Refractive index *(2.2.6)*

1.528 to 1.539.

Optical rotation *(2.2.7)*

+ 10.0° to + 24.0°.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution Dissolve 0.20 ml of the oil to be examined in *heptane R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 20 µl of *α-pinene R*, 20 µl of *limonene R*, 50 µl of *fenchone R*, 20 µl of *estragole R*, 100 µl of *anethole R* and 20 µl of *anisaldehyde R* in *heptane R* and dilute to 10.0 ml with the same solvent.

Column:
- *material:* fused silica,
- *size:* l = 60 m, Ø = 0.25 mm,

— *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1 ml/min.

Split ratio 1:200.

Temperature:

	Time (min)	Temperature (°C)
Column	0 – 4	60
	4 – 26	60 → 170
	26 – 41	170
Injection port		220
Detector		270

Detection Flame ionisation.

Injection 1.0 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 5.0 between the peaks due to estragole and *trans*-anethole.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution on the chromatogram obtained with the test solution and locate *cis*-anethole using Figure 1826.-1. (Disregard the peak due to heptane).

Determine the percentage content of each of these components. The percentages are within the following ranges:

— *α-pinene* : 1.0 per cent to 10.0 per cent,

— *limonene* : 0.9 per cent to 5.0 per cent,

— *fenchone* : 12.0 per cent to 25.0 per cent,

— *estragole* : maximum 6.0 per cent,

— *cis-anethole* : maximum 0.5 per cent,

— *trans-anethole* : 55.0 per cent to 75.0 per cent,

— *anisaldehyde* : maximum 2.0 per cent.

The ratio of α-pinene content to limonene content is greater than 1.0.

STORAGE

In a well-filled, airtight container , protected from light and at a temperature not exceeding 25 °C.

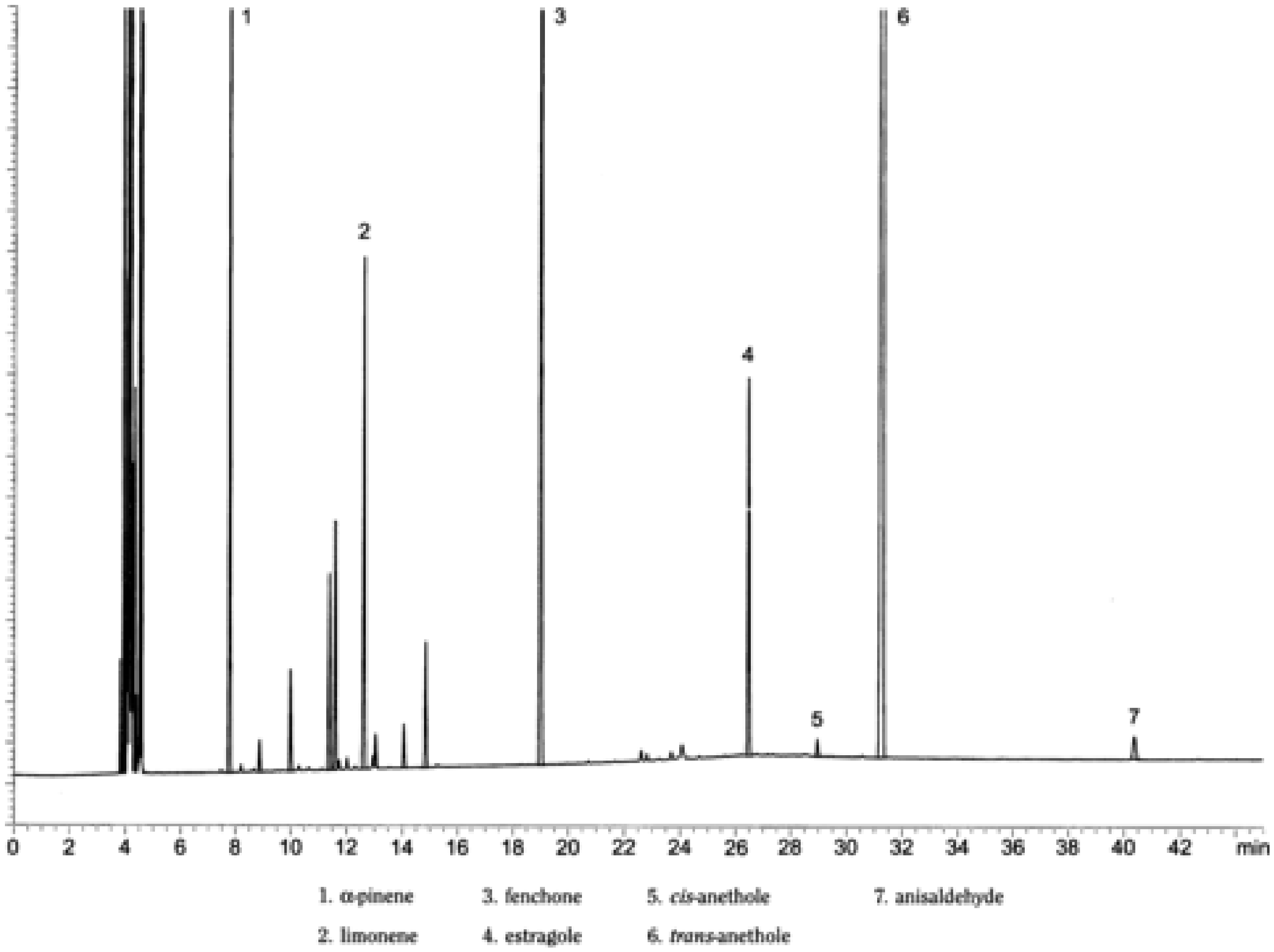


Figure 1826.-1. – *Chromatogram for the test for chromatographic profile of bitter-fennel fruit oil*

Ph Eur

Bitter-Orange Flower

General Notices

(Ph Eur monograph 1810)

Ph Eur

DEFINITION

Whole, dried, unopened flower of *Citrus aurantium* L. ssp. *aurantium* (C. *aurantium* L. ssp. *amara* Engl.).

Content

Minimum 8.0 per cent of total flavonoids, expressed as naringin ($C_{27}H_{32}O_{14}$; M_r 580.5) (dried drug).

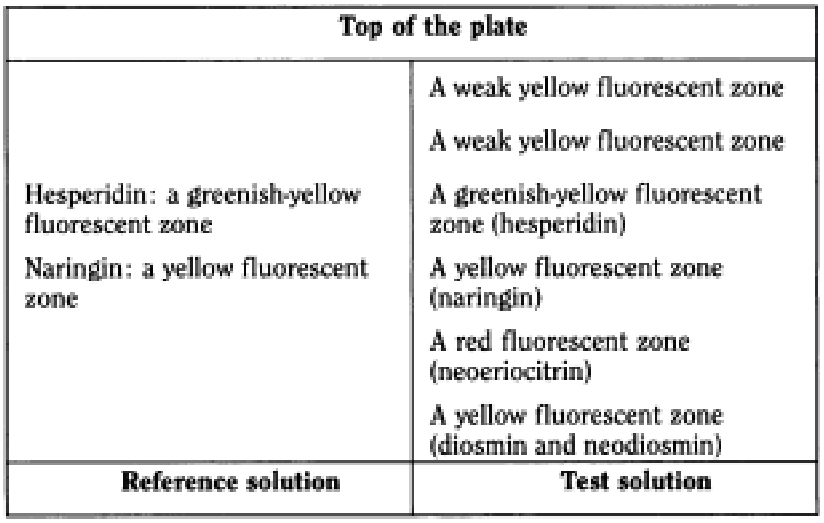
IDENTIFICATION

A. The flower buds are white or yellowish-white and may reach up to 25 mm in length. The dialypetalous corolla is composed of 5 thick, oblong and concave petals dotted with oil glands visible under a hand lens; the short, yellowish-green persistent gamosepalous calyx has 5 spreading sepals, connate at the base and forming a star-shaped structure attached to the yellowish-green peduncle which is about 5 mm to 10 mm long. The flower buds contain at least 20 stamens with yellow anthers and with filaments fused at the base into groups of 4 or 5; the ovary is superior, brownish-black and spherical, consists of 8 to 10 multi-ovular loculi and is surrounded at the base by an annular granular hypogynous disc; the thick, cylindrical style ends in a capitate stigma.

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows numerous spherical pollen grains, with a finely pitted exine and 3 to 5 germinal pores; fragments of the epidermis of the sepals with unicellular trichomes and with large prism crystals of calcium oxalate in the underlying mesophyll; fragments of the epidermis of the petals with a distinctly striated cuticle; fragments of large schizolysigenous oil glands which measure up to 100 µm in diameter, numerous anomocytic stomata (*2.8.3*). Examine under a microscope using a 300 g/l solution of *potassium hydroxide R*. The powder shows yellow crystals of hesperidin.

C. Examine the chromatograms obtained in the test for sweet-orange flower.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
	A weak yellow fluorescent zone
	A weak yellow fluorescent zone
Hesperidin: a greenish-yellow fluorescent zone	A greenish-yellow fluorescent zone (hesperidin)
Naringin: a yellow fluorescent zone	A yellow fluorescent zone (naringin)
	A red fluorescent zone (neoeriocitrin)
	A yellow fluorescent zone (diosmin and neodiosmin)
Reference solution	**Test solution**

TESTS

Sweet-orange flower

Thin-layer chromatography (*2.2.27*).

Test solution To 0.5 g of the powdered drug (355) (*2.9.12*), add 5 ml of *methanol R*. Heat with stirring at 40 °C for 10 min. Filter.

Reference solution Dissolve 3.0 mg of *naringin R* and 3.0 mg of *hesperidin R* in 10 ml of *methanol R*.

Plate TLC silica gel plate R.

Mobile phase water R, *anhydrous formic acid R*, *ethyl acetate R* (10:15:75 *V/V/V*).

Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air, then heat in an oven at 110-120 °C for 5 min.

Detection Spray the hot plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. After at least 1 h, examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows a yellow zone similar in position to the zone of naringin in the chromatogram obtained with the reference solution and immediately below it a red zone (neoeriocitrin).

Loss on drying (*2.2.32*)

Maximum 11.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C.

Total ash (*2.4.16*)

Maximum 10.0 per cent.

ASSAY

Stock solution To 0.175 g of the powdered drug (355) (*2.9.12*) add 95 ml of *alcohol (50 per cent V/V) R*. Heat on a water-bath under a reflux condenser for 30 min. Allow to cool and filter through a sintered-glass filter (*2.1.2*). Rinse the filter with 5 ml of *alcohol (50 per cent V/V) R*. Combine the filtrate and the rinsings in a volumetric flask and dilute to 100.0 ml with *alcohol (50 per cent V/V) R*.

Test solution Into a test tube (10 mm × 180 mm) introduce 0.150 g of powdered (250) (*2.9.12*) *magnesium R*, a magnetic stirring bar 25 mm long and 2.00 ml of the stock solution. Maintain the test tube upright, centrifuge at 125 g and carefully add dropwise, especially at the beginning, 2.0 ml of *hydrochloric acid R*, and then 6.0 ml of *alcohol (50 per cent V/V) R*. Stopper the tube and mix by inverting.

Compensation solution Into a second tube, introduce 2.00 ml of the stock solution and carefully add dropwise, especially at the beginning, 2.0 ml of *hydrochloric acid R* and then 6.0 ml of *alcohol (50 per cent V/V) R*.

After 10 min, measure the absorbance (*2.2.25*) of the test solution at 530 nm.

Calculate the percentage content of total flavonoids, expressed as naringin, from the expression:

$$\frac{A \times 9.62}{m}$$

i.e. taking the value of the specific absorbance of the reaction product of naringin to be 52.

A = absorbance at 530 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Black Currant

General Notices

Preparation

Black Currant Syrup

DEFINITION

Black Currant consists of the fresh ripe fruits of *Ribes nigrum* L., together with their pedicels and rachides.

CHARACTERISTICS

Odour, strong and characteristic.

Macroscopical Berries: globose, ranging in diameter from about 7 to 15 mm, occurring in pendulous racemes; epicarp shiny black externally, enclosing a yellowish green translucent pulp containing numerous flattened ovoid seeds, about 2.5 mm long, 1.25 mm wide and 1 mm thick; berry crowned with withered remains of five-cleft calyx; pedicels thin, up to about 10 mm long, attached to a rachis of variable length.

Microscopical Epicarp: glands yellow, disc-shaped, roughly circular or broadly elliptical, varying in diameter from about 140 to 240 µm, each consisting of a single layer of cells attached in the centre to the epicarp by means of a short, multiseriate stalk. Calyx: trichomes unicellular, blunt-ended with thin, crooked walls, about 10 to 14 µm wide and averaging about 350 µm in length. Seed: testa with pigment layer composed of small cells with horseshoe-shaped wall thickenings as seen in cross section, each cell containing one or two prismatic crystals of calcium oxalate; endosperm cells with irregularly thickened walls.

Black Horehound

General Notices

(Ph Eur monograph 1858)

Ph Eur

DEFINITION

Dried flowering tops of *Ballota nigra* L.

Content

Minimum 1.5 per cent of total *ortho*-dihydroxycinnamic acid derivatives, expressed as acteoside ($C_{29}H_{36}O_{15}$; M_r 624.6) (dried drug).

IDENTIFICATION

A. The stems are conspicuously four-angled, longitudinally striated, dark green or reddish-brown and more or less pubescent. The leaves are greyish-green, petiolate, the lamina ovate or orbicular, 2-4 cm wide, the margin irregularly crenate, and cuneate or cordate at the base; both surfaces are covered with abundant whitish hairs; the venation is pinnate, prominent on the lower surface, slightly depressed on the upper. The flowers are sessile or very shortly pedicellate, the calyx is infundibuliform, densely pubescent, with 10 prominent ribs and 5 subequal, broadly ovate teeth; the corolla, with a tube slightly shorter than the calyx tube, is purple and bilabiate, the upper lip pubescent on the outer surface and the lower lip with 3 lobes, the middle of which is notched.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green and slightly flocculent. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous long, uniseriate, multicellular covering trichomes consisting of 4 or more cells, thickened and swollen at the junctions, with slightly lignified and pitted walls; fewer glandular trichomes, some with a unicellular or multicellular stalk and a globose, uni- or bicellular head, others with a unicellular stalk and a multicellular head; fragments of the leaf epidermis with sinuous walls, those from the lower epidermis with numerous stomata, some diacytic *(2.8.3)* but the majority anomocytic; epidermis of the corolla composed of polygonal cells, those of the inner epidermis papillose; pollen grains subspherical with 3 pores and a smooth exine; groups of collenchyma and lignified, spirally thickened and bordered pitted vessels, from the stem.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 2 g of the powdered drug (355) *(2.9.12)* add 100 ml of *methanol R* . Heat on a water-bath under a reflux condenser for 30 min. Allow to cool. Filter. Evaporate the filtrate under reduced pressure until a volume of about 10 ml is obtained.

Reference solution Dissolve 2.5 mg of *rutin R* and 1 mg of *chlorogenic acid R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, glacial acetic acid R, water R, ethyl acetate* R (7.5:7.5:18:67 *V/V/V/V*).

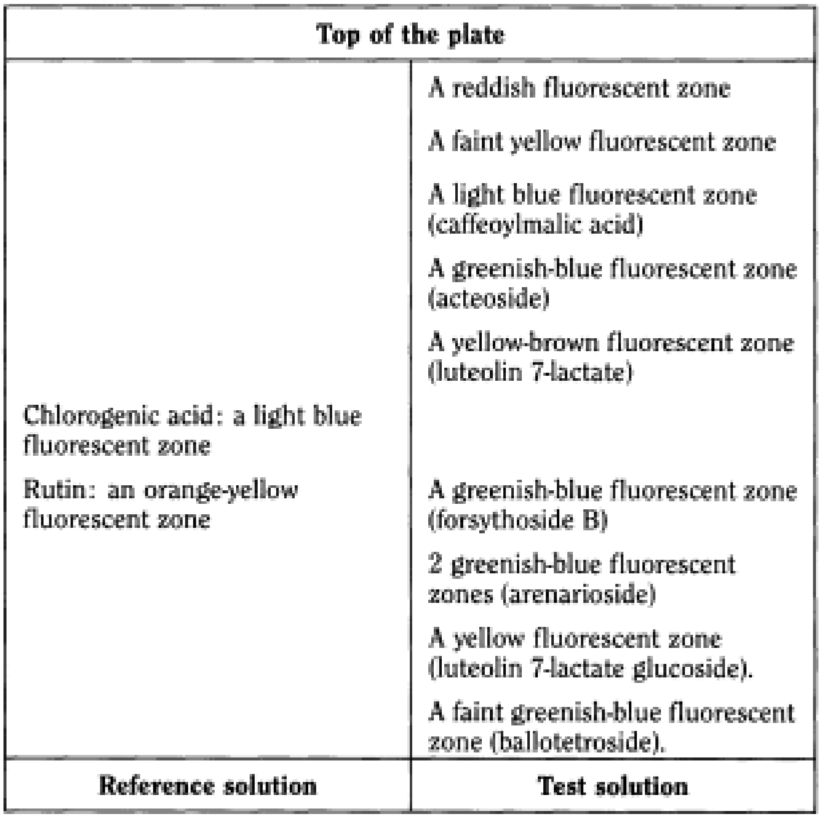
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a solution containing 10 g/l of *diphenylboric acid aminoethyl ester R* and 50 g/l of *macrogol 400 R in methanol R* . Allow to dry in a current of warm air. Examine in ultraviolet light at 365 nm after 30 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A reddish fluorescent zone
	A faint yellow fluorescent zone
	A light blue fluorescent zone (caffeoylmalic acid)
	A greenish-blue fluorescent zone (acteoside)
	A yellow-brown fluorescent zone (luteolin 7-lactate)
Chlorogenic acid: a light blue fluorescent zone	
Rutin: an orange-yellow fluorescent zone	A greenish-blue fluorescent zone (forsythoside B)
	2 greenish-blue fluorescent zones (arenarioside)
	A yellow fluorescent zone (luteolin 7-lactate glucoside).
	A faint greenish-blue fluorescent zone (ballotetroside).
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 13.0 per cent.

ASSAY

Stock solution Place 1.000 g of the powdered drug (355) *(2.9.12)* in a flask. Add 90 ml of *ethanol (50 per cent V/V) R*. Heat under a reflux condenser on a water-bath for 30 min. Allow to cool and filter, collecting the filtrate in a 100 ml volumetric flask. Rinse the flask and the filter with 10 ml of *ethanol (50 per cent V/V) R*. Add the rinsings to the filtrate and dilute to 100.0 ml with *ethanol (50 per cent V/V) R.*

Test solution Into a 10 ml volumetric flask, introduce successively, with shaking after each addition, 1.0 ml of the stock solution, 2 ml of *0.5 M hydrochloric acid* , 2 ml of a solution containing 100 g/l of sodium nitrite R and 100 g/l of *sodium molybdate R* , 2 ml of *dilute sodium hydroxide solution R* and dilute to 10.0 ml with *water R* .

Compensation liquid Into a 10 ml volumetric flask, introduce 1.0 ml of the stock solution, 2 ml of *0.5 M hydrochloric acid* and 2 ml of *dilute sodium hydroxide solution R* , and dilute to 10.0 ml with *water R* .

Measure immediately the absorbance *(2.2.25)* of the test solution at 525 nm, by comparison with the compensation liquid.

Calculate the percentage content of total *ortho*-dihydroxycinnamic acid derivatives, expressed as acteoside, using the following expression:

$$\frac{A \times 1000}{185 \times m}$$

i.e. taking the specific absorbance of acteoside to be 185.

A = absorbance at 525 nm

m = mass of the substance to be examined, in grams

Ph Eur

Bogbean Leaf

General Notices

(Ph Eur monograph 1605)

Ph Eur

DEFINITION

Dried, entire or fragmented leaf of *Menyanthes trifoliata* L.

CHARACTERS

Very bitter and persistant taste.

IDENTIFICATION

A. The leaf is long-petiolated, trifoliate, with long sheaths from the base; the petiole is up to 5 mm in diameter and strongly striated longitudinally. The lamina is divided into equal leaflets, sessile, obovate up to 10 cm long and up to 5 cm wide, with an entire, occasionally sinuous margin with brownish or reddish hydathodes and a spathulate base; it is glabrous, dark green on the upper surface and paler green on the lower surface, with a wide, whitish, finely striated prominent midrib.

B. Reduce to a powder (355) *(2.9.12)*. The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R* . The powder shows fragments of upper epidermis with polyhedral cells and thin wavy walls; fragments of lower epidermis with sinuous walls; anomocytic stomata *(2.8.3),* on both surfaces, with the subsidiary cells showing radiating striations; epidermal cells from the veins straight walled and papillose; fragments of mesophyll parenchyma with large intercellular spaces (aerenchyma); irregular cells with rare sclereids; fragments of spiral or annular vessels.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* . Heat, with stirring, in a water-bath at 60 °C for 5 min. Allow to cool and filter. Evaporate to dryness under reduced pressure in a water-bath at 60 °C. Dissolve the residue in 2.0 ml of *methanol R* .

Reference solution Dissolve 5 mg of *loganin R* in 15 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *water R, methanol R, ethyl acetate R* (8:15:77 *V/V/V*).

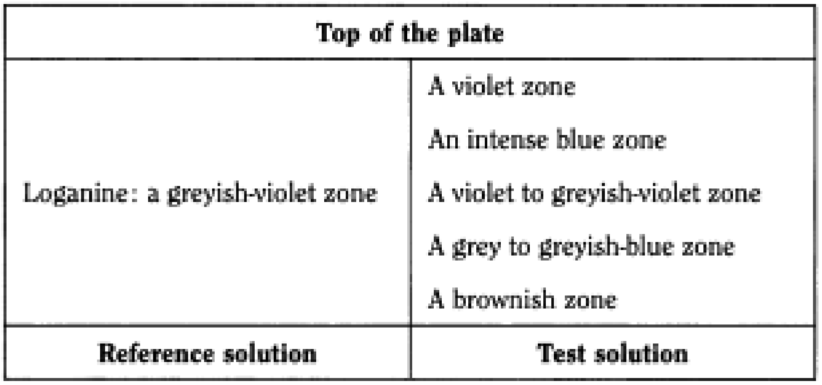
Application 30 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with vanillin *reagent R*. Heat in an oven at 100-105 °C for 10 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	A violet zone
	An intense blue zone
Loganine: a greyish-violet zone	A violet to greyish-violet zone
	A grey to greyish-blue zone
	A brownish zone
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

Bitterness value *(2.8.15)*

Minimum 3000.

Ph Eur

Boldo Leaf Dry Extract

General Notices

(Ph Eur monograph 1816)

Ph Eur

DEFINITION

Extract produced from *Boldo leaf (1396).*

Content:

— *for aqueous extracts*: minimum 0.5 per cent of total alkaloids, expressed as boldine ($C_{19}H_{21}NO_4$; M_r 327.4) (dried extract);

— *for hydroalcoholic extracts*: minimum 1.0 per cent of total alkaloids, expressed as boldine ($C_{19}H_{21}NO_4$; M_r 327.4) (dried extract).

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using either hot water at not less than 65 °C or a hydroalcoholic solvent equivalent in strength to ethanol (45-75 per cent *V/V*).

CHARACTERS

Appearance

Brown or greenish-brown, hygroscopic powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)* .

Test solution To 0.5 g of the extract to be examined add 1 ml of *hydrochloric acid R* and 20 ml of *water R.* Sonicate for 10 min. Transfer the liquid to a separating funnel and make alkaline with 2 ml of *dilute ammonia R1* . Shake with 2 quantities, each of 20 ml, of *methylene chloride R.* Evaporate the combined organic layers to dryness. Dissolve the residue in 1 ml of *methanol R* .

Reference solution Dissolve 2 mg of *boldine R* and 10 mg of *hyoscine hydrobromide R* in 5 ml of *methanol R.*

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *diethylamine R* , *methanol R* , *toluene R* (10:10:80 *V/V/V*).

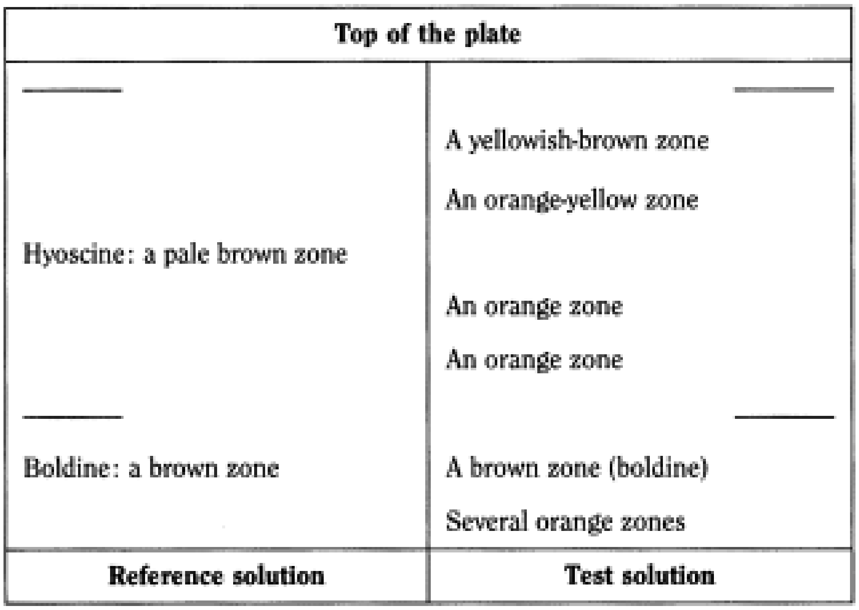
Application 20 µl [or 3 µl], as bands of 15 mm [or 8 mm].

Development Over a path of 15 cm [or 6 cm].

Drying In air.

Detection Spray with *potassium iodobismuthate solution R2* , allow to dry in air for 5 min and spray with *sodium nitrite solution R*; examine in daylight after 30 min.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
	A yellowish-brown zone
	An orange-yellow zone
Hyoscine: a pale brown zone	
	An orange zone
	An orange zone
———	———
Boldine: a brown zone	A brown zone (boldine)
	Several orange zones
Reference solution	**Test solution**

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution To 1.000 g of the extract to be examined add 50 ml of *dilute hydrochloric acid R* and sonicate for 10 min. Transfer to a separating funnel and wash with 10 ml of a mixture of equal volumes of *ethyl acetate R* and *hexane R* . Adjust the aqueous phase to pH 9.5 with *dilute ammonia R1* . After cooling, shake successively with 100 ml, 50 ml, and a further 50 ml of *methylene chloride R* , taking care not to form an emulsion. Evaporate the combined lower layers to dryness under reduced pressure. Dissolve the residue in the mobile phase and transfer the solution to a volumetric flask. Rinse and dilute to 10.0 ml with the mobile phase.

Reference solution Dissolve 12.0 mg of *boldine CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Solution A Mix 0.2 ml of *diethylamine R* with 99.8 ml of *acetonitrile R* .

Solution B Mix 0.2 ml of *diethylamine R* with 99.8 ml of *water R* and adjust to pH 3 with *anhydrous formic acid R* .

Mobile phase Solution A, solution B (16:84 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 304 nm.

Injection 20 µl.

Relative retention With reference to boldine (retention time = about 6 min): isoboldine = about 0.9; isocorydine *N*-oxide = about 1.8; laurotetanine = about 2.2; isocorydine = about 2.8; *N*-methyllaurotetanine = about 3.2. Additional peaks may be present.

System suitability Test solution:

— *resolution*: minimum 1.0 between the peaks due to isoboldine and boldine.

Calculate the percentage content of total alkaloids, expressed as boldine, using the following expression:

$$\frac{(\sum A_1) \times m_2 \times p}{A_2 \times m_1 \times 10}$$

ΣA_1 = sum of the areas of the peaks due to the 6 alkaloids identified in the chromatogram obtained with the test solution

A_2 = area of the peak due to boldine in the chromatogram obtained with the reference solution

m_1 = mass of the extract to be examined used to prepare the test solution, in grams

m_2 = mass of *boldine CRS* used to prepare the reference solution, in grams

p = percentage content of boldine in *boldine CRS* .

Ph Eur

Boldo Leaf

General Notices

(Ph Eur monograph 1396)

Ph Eur

DEFINITION

Whole or fragmented dried leaf of *Peumus boldus* Molina.

Content

Minimum 0.1 per cent of total alkaloids, expressed as boldine ($C_{19}H_{21}NO_4$; M_r 327.4) (anhydrous drug).

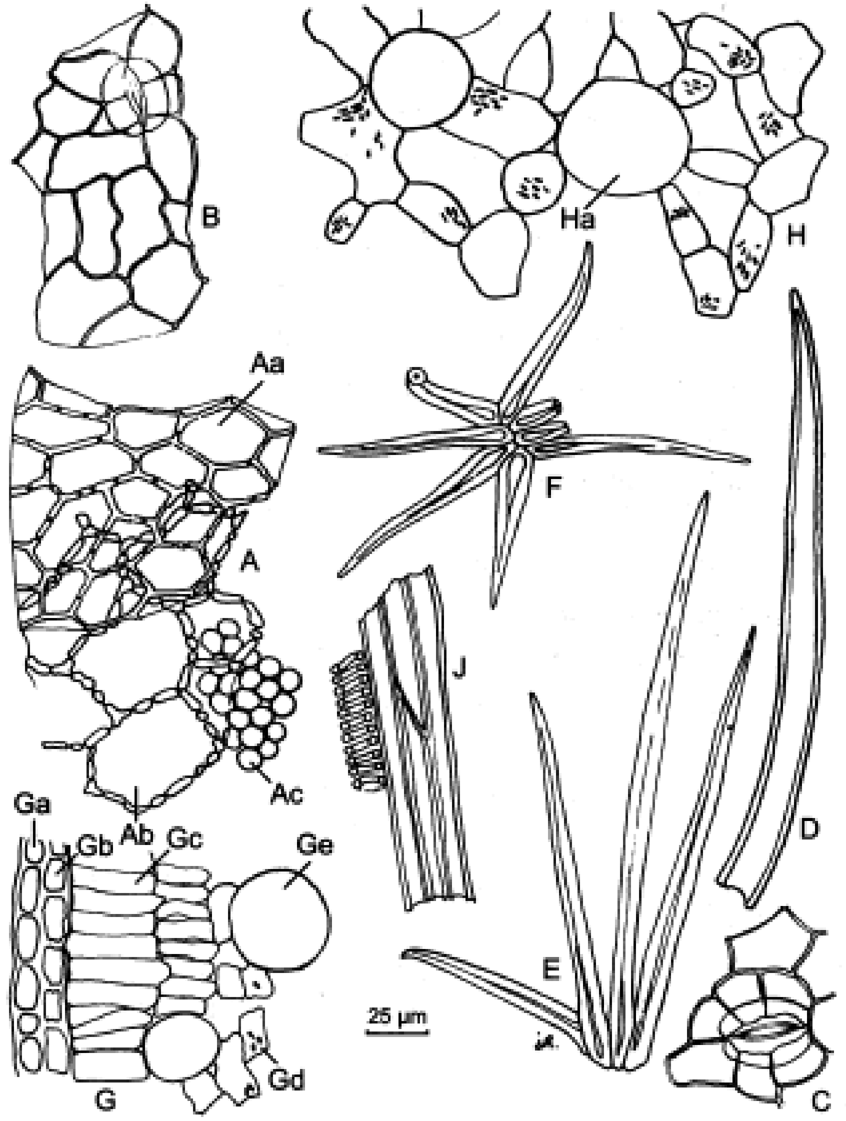
CHARACTERS

Characteristic odour, especially when rubbed.

IDENTIFICATION

A. The leaf is oval or elliptical usually 5 cm long with a short petiole, an obtuse or slightly emarginate or mucronate apex and an equal and rounded base; the margin is entire and slightly undulate and the thickened edges are more or less revolute. The lamina is greyish-green, thick, tough and brittle. The upper surface is rough with numerous prominent small protuberances and a depressed venation. The lower surface is finely pubescent, with the protuberances less well-marked, and a prominent, pinnate venation.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green. Examine under a microscope using *chloral hydrate solution R* . The powder shows fragments of the upper epidermis and underlying hypodermis with straight or slightly sinuous thickened and beaded walls, those of the lower epidermis with numerous stomata surrounded by 4-7 subsidiary cells; solitary, bifurcated or stellate clustered unicellular covering trichomes with more or less thickened and lignified walls; fragments of the lamina showing a two-layered palisade; debris of the spongy mesophyll including numerous large, rounded oil cells and parenchyma containing fine needle-shaped crystals; thick-walled fibres and lignified, pitted parenchymatous cells associated with vascular tissue from the veins.



A. Fragment of the lamina, in surface view, showing the upper epidermis (Aa), hypodermis with thickened and beaded walls (Ab), and palisade parenchyma (Ac)

B and C. Lower epidermis with stomata surrounded by 4-7 subsidiary cells

D. Unicellular covering trichome, solitary

E and F. Unicellular covering trichomes, stellate clustered

G. Fragment of the lamina, in transverse section, showing the upper epidermis (Ga), hypodermis (Gb), palisade parenchyma (Gc) and spongy parenchyma (Gd) containing oil cells (Ge)

H. Spongy parenchyma containing fine needle-shaped crystals and oil cells (Ha)

J. Vascular tissue with fibres

Figure 1396.-1. – *Illustration of powdered herbal drug of boldo leaf (see Identification B)*

C. Thin-layer chromatography *(2.2.27)* .

Test solution Mix 1.5 g of the powdered drug (355) *(2.9.12)* and 5 ml of *methanol R* and sonicate for 10 min. Filter the supernatant through a 3 cm × 0.5 cm column of *cellulose for chromatography R1*. Use the first 1 ml of the eluate as the test solution.

Reference solution Dissolve 2 mg of *boldine R* and 10 mg of *hyoscine hydrobromide R* in 5 ml of *methanol R*.

Plate TLC silica gel plate R (5-40 µm) [or TLC silica gel plate R (2-10 µm)].

Mobile phase *diethylamine R*, *methanol R*, *toluene R* (10:10:80 *V/V/V*).

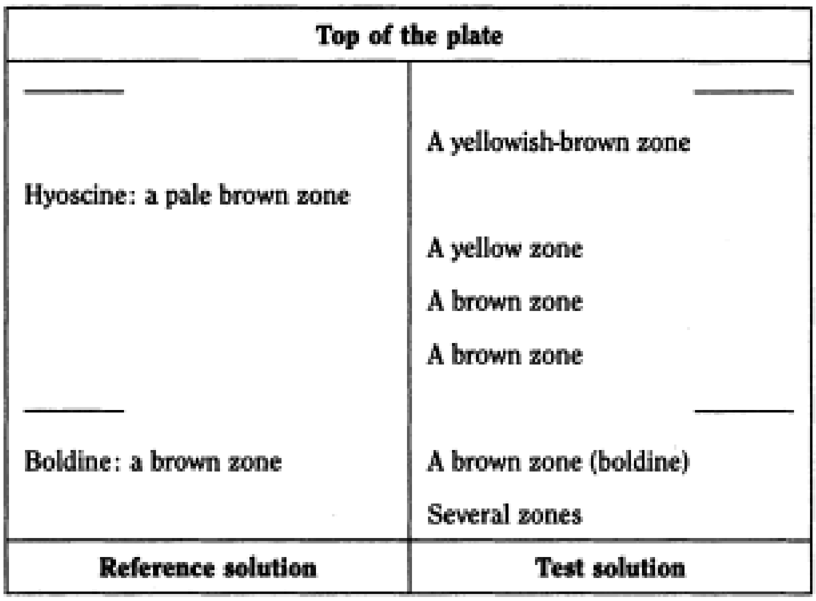
Application 40 µl [or 6 µl] of the test solution and 20 µl [or 2 µl] of the reference solution, as bands of 15 mm [or 8 mm].

Development Over a path of 15 cm [or 6 cm].

Drying In air.

Detection Spray with *potassium iodobismuthate solution R2*, dry for 5 min in air and spray with *sodium nitrite solution R*; examine in daylight after 30 min.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
_______	_______
	A yellowish-brown zone
Hyoscine: a pale brown zone	
	A yellow zone
	A brown zone
	A brown zone
_______	_______
Boldine: a brown zone	A brown zone (boldine)
	Several zones
Reference solution	**Test solution**

TESTS

Essential oil *(2.8.12)*

Maximum 40 ml/kg (anhydrous drug).

Use 10.0 g of the freshly fragmented drug, a 1000 ml flask and 300 ml of *water R* as the distillation liquid. Distil at a rate of 2-3 ml/min for 3 h.

Foreign matter *(2.8.2)*

Maximum 4 per cent of twigs and maximum 2 per cent of other foreign matter.

Water *(2.2.13)*

maximum 100 ml/kg, determined by distillation of 20.0 g of the powdered drug (355) *(2.9.12)*.

Total ash *(2.4.16)*

Maximum 13.0 per cent.

ASSAY

Alkaloids

Liquid chromatography *(2.2.29).*

Test solution To 1.000 g of the powdered drug (355) *(2.9.12)* add 50 ml of *dilute hydrochloric acid R* . Shake in a water-bath at 80 °C for 30 min. Filter, take up the residue with 50 ml of *dilute hydrochloric acid R* and shake in a water-bath at 80 °C for 30 min. Filter and repeat the operation once on the residue obtained. Filter. Combine the cooled filtrates and shake with 100 ml of a mixture of equal volumes of *ethyl acetate R* and *hexane R* . Discard the organic layer. Adjust the aqueous layer to pH 9.5 with *dilute ammonia R1* . Shake successively with 100 ml, 50 ml and 50 ml of *methylene chloride R* . Combine the lower layers and evaporate to dryness under reduced pressure. Dissolve the residue in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution Dissolve 12 mg of *boldine CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Solution A Mix 0.2 ml of *diethylamine R* and 99.8 ml of *acetonitrile R* .

Solution B Mix 0.2 ml of *diethylamine R* and 99.8 ml of *water R* and adjust to pH 3 with *anhydrous formic acid R .*

Mobile phase Solution A, solution B (16:84 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 304 nm.

Injection 20 µl.

Relative retention With reference to boldine (retention time = about 6 min): isoboldine = about 0.9; isocorydine *N*-oxide = about 1.8; laurotetanine = about 2.2; isocorydine = about 2.8; *N*-methyllaurotetanine = about 3.2. Additional peaks may be present.

System suitability Test solution:

— *resolution*: minimum 1 between the peaks due to isoboldine and boldine.

Calculate the percentage content of total alkaloids expressed as boldine using the following expression:

$$\frac{(\sum A_1) \times m_2 \times p}{A_2 \times m_1 \times 100}$$

m_1 = mass of the drug to be examined, in grams

m_2 = mass of *boldine CRS* in the reference solution, in grams

ΣA_1 = sum of the areas of the peaks due to the 6 alkaloids identified in the chromatogram obtained with the test solution

A_2 = of the peak due to boldine in the chromatogram obtained with the reference solution area;

p = percentage content of boldine in *boldine CRS*

Ph Eur

Buckwheat Herb

General Notices

(Ph Eur monograph 2184)

Ph Eur

DEFINITION

Whole or cut aerial parts of *Fagopyrum esculentum* Moench, collected in the early flowering period prior to fruiting and dried immediately.

Content

Minimum 4.0 per cent of rutin ($C_{27}H_{30}O_{16}$,$3H_2O$; M_r 665) (dried drug).

IDENTIFICATION

A. The stem is cylindrical, hollow, finely ridged longitudinally, about 2-6 mm in diameter, brownish-green or reddish, with few branches and thickened at the internodes; the leaves are arranged spirally and have membranous, sheathing stipules; the surface is glabrous except in the region of the stipules, where short, white hairs may occur. The leaves are dark green, paler on the lower surface, up to 7 cm wide and 11 cm long, saggitate or cordate, almost pentagonal with 2 widely rounded lobes; the lower leaves are petiolate, the upper leaves sessile or amplexicaul; the lamina is glabrous and the margin finely sinuate and fringed with minute, reddish-brown projections; similar projections occur on the veins on the upper surface. The inflorescence is a cymose panicle, the individual flowers 1-2 mm long and 6 mm in diameter with 5 free, white or reddish petals.

B. Reduce to a powder (355) *(2.9.12)*. The powder is dark green with a few brown, pink or whitish particles. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the epidermis of the stem and leaf lamina in surface view, those of the stem composed of elongated cells showing striations on the outer walls, those of the lamina polygonal with numerous anomocytic stomata; occasional fragments of the leaf margins and the epidermis over the veins with ovoid to rounded papilla-like projections, often reddish, with thickened and striated walls; abundant cluster crystals of calcium oxalate, 25-100 µm in diameter, and smaller prismatic crystals, occurring scattered and in the mesophyll of the leaf, also in longitudinal files in the parenchyma of the stem; fragments of lignified tissue with bordered pitted or reticulate vessels and thin-walled, pitted fibres; occasional fragments of the corolla with papillose epidermis; pollen spherical, about 50 µm in diameter, with a pitted exine and 3 furrows.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 0.5 g of the powdered drug (355) *(2.9.12)* add 5.0 ml of *methanol R* and heat in a water-bath at 60 °C under a reflux condenser for 10 min. Cool and filter.

Reference solution Dissolve 10 mg of *hyperoside R* and 10 mg of *rutin R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R, water R, ethyl acetate R* (1:1:8 *V/V/V*).

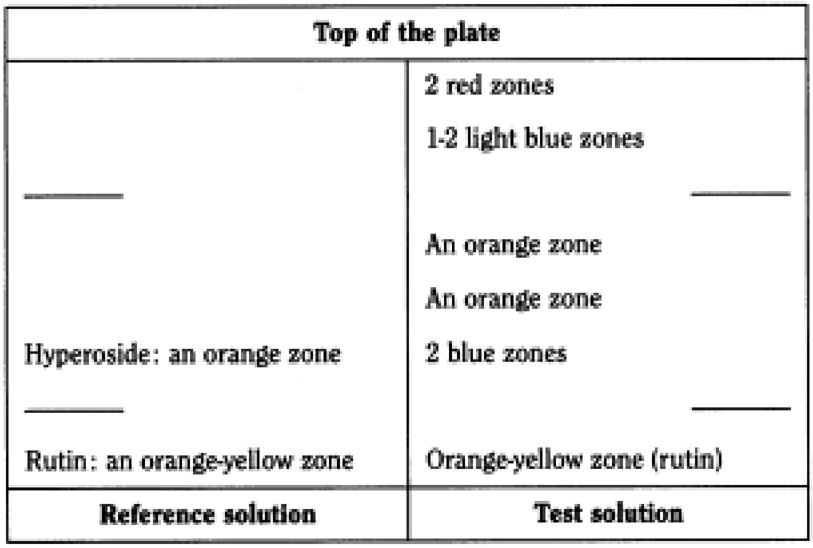
Application 20 µl [or 5 µl] as bands.

Development Over a path of 10 cm [or 6 cm].

Drying At 100-105 °C.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	2 red zones
	1-2 light blue zones
———	———
	An orange zone
	An orange zone
Hyperoside: an orange zone	2 blue zones
———	———
Rutin: an orange-yellow zone	Orange-yellow zone (rutin)
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 15.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution To 0.500 g of the powdered drug (355) *(2.9.12)*, add 30 ml of an 80 per cent *V/V* solution of *methanol R*. Heat the mixture under a reflux condenser in a water-bath at 60 °C for 30 min, then extract the mixture in an ultrasonic bath for 15 min. Allow to cool, dilute to 50.0 ml with an 80 per cent *V/V* solution of *methanol R* and filter.

Reference solution (a) Dissolve 25.0 mg of *rutoside trihydrate CRS* in an 80 per cent *V/V* solution of *methanol R* and dilute to 50.0 ml with the same solvent.

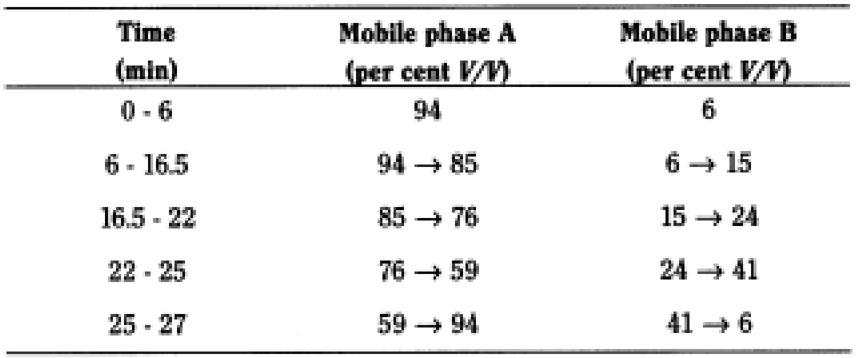
Reference solution (b) Dissolve 20.0 mg of *troxerutin R* and 5.0 mg of *quercitrin R* in an 80 per cent *V/V* solution of *methanol R* and dilute to 50.0 ml with the same solvent.

Column:

- *size: l* = 0.125 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: mix 50 volumes of *acetonitrile R* and 950 volumes of *water R* adjusted to pH 2 with *phosphoric acid R;*
- *mobile phase B*: mix 95 volumes of *water R* adjusted to pH 2 with *phosphoric acid* R and 905 volumes of *acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	94	6
6 - 16.5	94 → 85	6 → 15
16.5 - 22	85 → 76	15 → 24
22 - 25	76 → 59	24 → 41
25 - 27	59 → 94	41 → 6

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 350 nm.

Injection 10 µl.

System suitability Reference solution (b):

- *elution order*: when the chromatogram is recorded in the prescribed conditions, the components elute in the order indicated in the composition of reference solution (b);
- *resolution*: minimum 3 between the peaks due to troxerutin and quercitrin.

Using the retention time from the chromatogram obtained with reference solution (a), locate rutin on the chromatogram obtained with the test solution.

Calculate the percentage content of rutin from the following expression:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1} \times \frac{100}{100 - d}$$

A_1 = area of the peak due to rutin in the chromatogram obtained with the test solution;

A_2 = area of the peak due to rutoside trihydrate in the chromatogram obtained with reference solution (a);

M_1 = mass of the drug to be examined, in grams;

M_2 = mass of *rutoside trihydrate CRS*, in grams;

p = purity of *rutoside trihydrate CRS*, in per cent;

d = loss on drying, in per cent.

Ph Eur

Butcher's Broom

General Notices

(Ph Eur monograph 1847)

Ph Eur

DEFINITION

Dried, whole or fragmented underground parts of *Ruscus aculeatus* L.

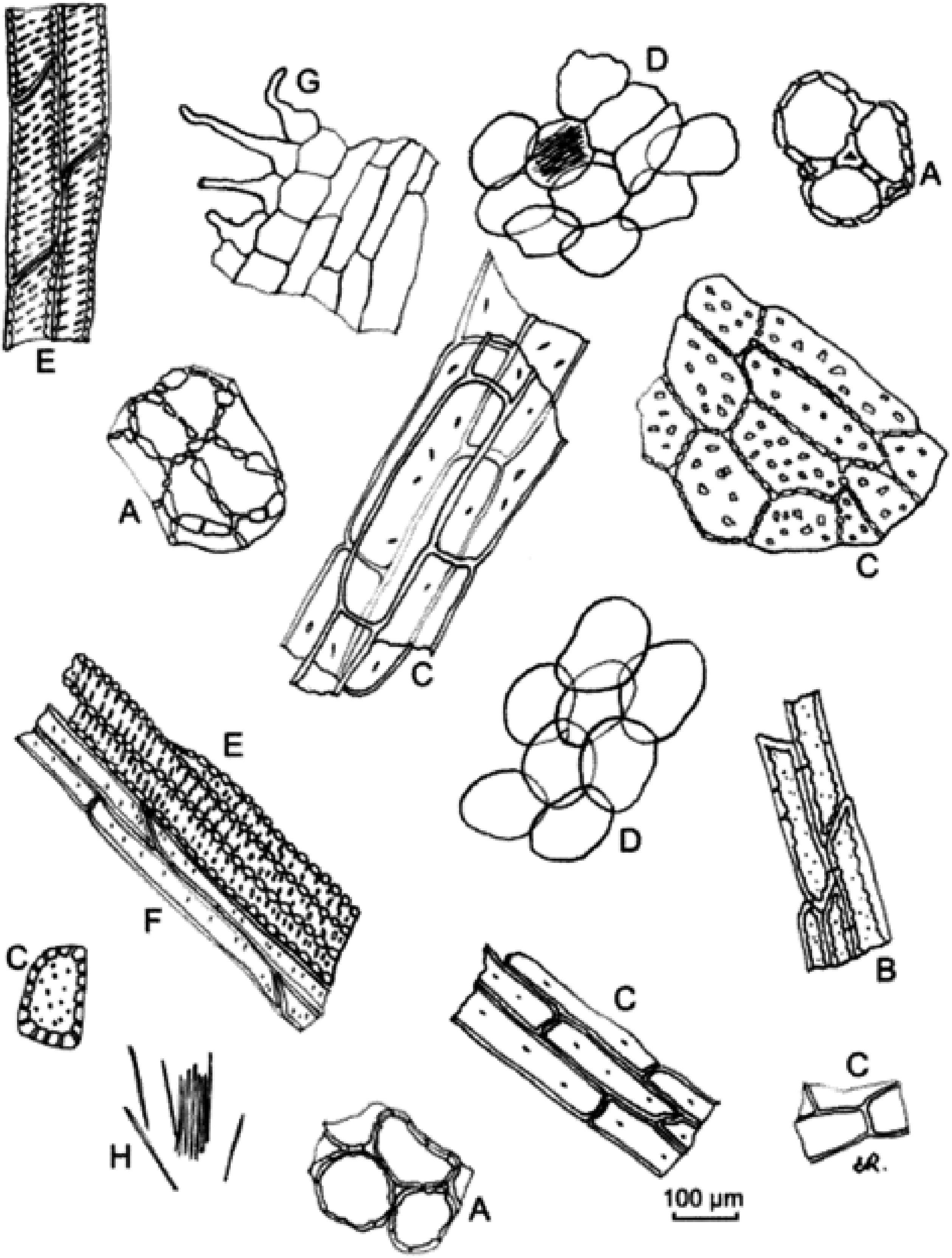
Content

Minimum 1.0 per cent of total sapogenins, expressed as ruscogenins [mixture of neoruscogenin ($C_{27}H_{40}O_4$; M_r 428.6) and ruscogenin ($C_{27}H_{42}O_4$; M_r 430.6)] (dried drug).

IDENTIFICATION

A. The rhizome consists of yellowish, branched, articulated, somewhat knotty pieces, cylindrical or subconical, about 5-10 cm long and about 5 mm thick. The surface is marked with thin annulations about 1-3 mm wide, separated from one another; rounded scars of the aerial stems are present on the upper surface. On the lower surface numerous roots, or their scars, occur; the roots are about 2 mm in diameter and similar in colour to the rhizome. The outer layer is easily detached, revealing a yellowish-white, very hard central cylinder.

B. Reduce to a powder (355) (*2.9.12*). The powder is yellowish. Examine under a microscope using *chloral hydrate solution R* . The powder shows groups of sclereids of the rhizome, variously-shaped cells, ranging from rounded to elongated or rectangular; the walls are moderately thickened and distinctly beaded, with large, rounded to oval pits. Fragments of the endodermis composed of a single layer of irregularly-thickened cells. Groups of rounded parenchymatous cells, thickened at the corners, with small, triangular intercellular spaces; thin-walled parenchyma containing raphides of calcium oxalate. Groups of thick-walled fibres and small vessels, up to about 50 µm in diameter, the walls showing numerous small, slit-shaped pits.


E
G
D
A
E
A
C
C
E
D
F
B
C
C
H
A
C
100 µm

A. Thick-walled parenchymatous cells

B. Endodermis fragment

F. Thick-walled fibres

G. Dermal tissue of the root

C. Variously-shaped sclereid cells

D. Parenchyma, some cells of which contain raphides of calcium oxalate

E. Small vessels sometimes accompanied by fibres (F)

H. Raphides of calcium oxalate

I. Thin-walled parenchyma

Figure 1847.-1. – *Illustration of powdered herbal drug of butcher's broom (see Identification B)*

C. Thin-layer chromatography (*2.2.27*)

Test solution Introduce 1.0 g of the powdered drug (355) (*2.9.12*) and 50 ml of *dilute hydrochloric acid R* into a 100 ml flask with a ground-glass neck. Heat on a water-bath under a reflux condenser for 40 min. Allow to cool and extract the unfiltered mixture with 3 quantities, each of 25 ml, of *methylene chloride R* . Combine the organic solutions and dry over *anhydrous sodium sulphate R* . Filter and evaporate to dryness. Dissolve the residue in 5 ml of *methanol R.*

Reference solution Dissolve 1 mg of *ruscogenins CRS* and 1 mg of *stigmasterol R* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *methanol R* , *methylene chloride R* (7:93 *V/V*).

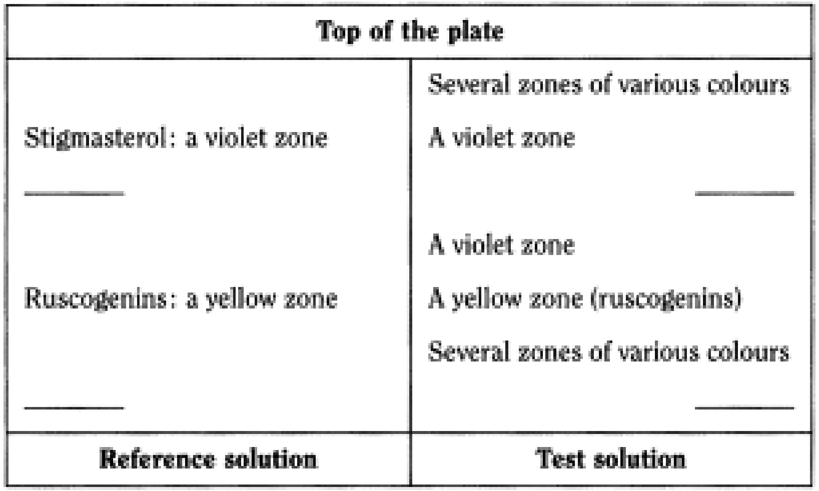
Application 10 µl as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *vanillin reagent R* , dry the plate in an oven at 100-105 °C for 1 min and examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other weak zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	Several zones of various colours
Stigmasterol: a violet zone	A violet zone
_______	_______
	A violet zone
Ruscogenins: a yellow zone	A yellow zone (ruscogenins)
	Several zones of various colours
_______	_______
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 5 per cent.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 12.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 5.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution To 2.000 g of the powdered drug (355) (*2.9.12*), add 60 ml of *anhydrous ethanol R* , 15 ml of *water R* and 0.2 g of *potassium hydroxide R* . Extract under reflux on a water-bath for 4 h. Allow to cool and filter into a 100 ml volumetric flask. Rinse the extraction flask and the residue in the filter with 3 quantities, each of 10 ml, of *anhydrous ethanol R* and add the rinsings to the volumetric flask. Dilute to 100.0 ml with *anhydrous ethanol R* . Introduce 25.0 ml of the solution into a round-bottomed flask fitted to a rotary evaporator and evaporate to dryness. Dissolve the residue in 10 ml of *butanol R* , add 3 ml of *hydrochloric acid R1* and 8 ml of *water R* . Heat under reflux on a water-bath for 1 h. Allow to cool and transfer the liquid into a separating funnel, rinse the round-bottomed flask with 2 quantities, each of 10 ml, of *butanol R* . Add the rinsings to the separating funnel. Extract with 3 quantities, each of 20 ml, of *butanol R* saturated with *water R.* Combine the butanolic extracts and evaporate to dryness using a rotary evaporator. Dissolve the residue in 20 ml of *methanol R* and transfer to a 100 ml volumetric flask. Rinse the extraction flask with 2 quantities, each of 20 ml then 10 ml of *methanol R* and add the rinsings to the volumetric flask. Dilute to 100 ml with *methanol R.*

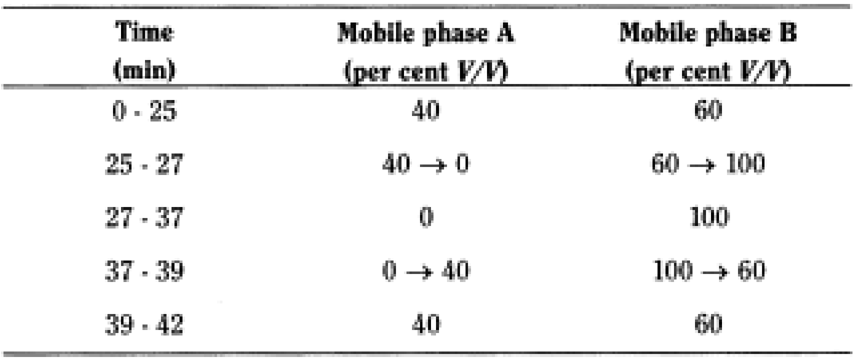
Reference solution Dissolve 5.0 mg of *ruscogenins CRS* in 100 ml of *methanol R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: *water R;*
- *mobile phase B*: *acetonitrile for chromatography R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	40	60
25 - 27	40 → 0	60 → 100
27 - 37	0	100
37 - 39	0 → 40	100 → 60
39 - 42	40	60

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 203 nm.

Injection 20 µl.

Retention time With reference to neoruscogenin (retention time = about 16 min): ruscogenin = about 1.2.

System suitability Reference solution:

- *resolution*: minimum 1.5 between the peaks due to neoruscogenin and ruscogenin.

Calculate the percentage content of sapogenins expressed as ruscogenins (neoruscogenin and ruscogenin) in the test solution by comparing the areas of the peaks in the chromatograms obtained with the test solution and the reference solution.

Ph Eur

Calendula Flower

General Notices

(Ph Eur monograph 1297)

Ph Eur

DEFINITION

Whole or cut, dried, and fully opened flowers that have been detached from the receptacle of the cultivated, double-flowered varieties of *Calendula officinalis* L.

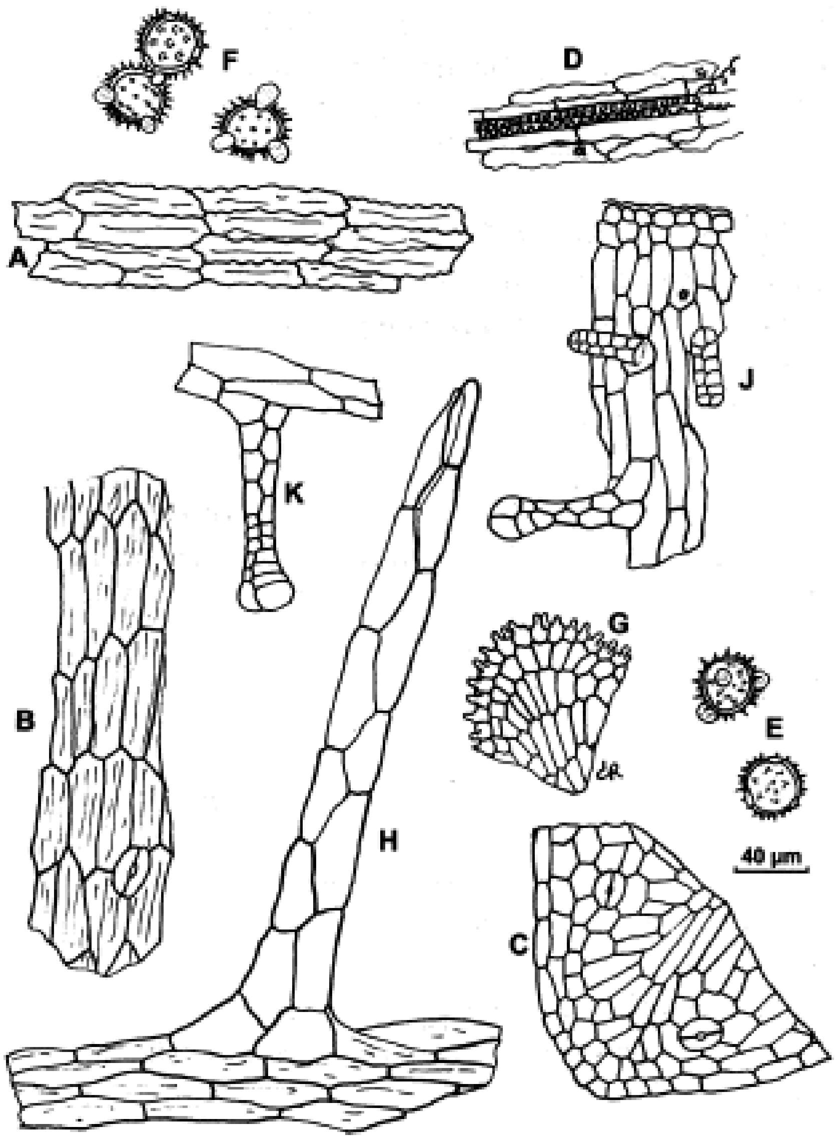
Content

Minimum 0.4 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The ligulate florets consist of a yellow or orange-yellow ligule, about 3-5 mm wide and about 7 mm in the middle part, with a 3-toothed apex and a hairy, partly sickle-shaped, yellowish-brown or orange-brown tube with a projecting style and a bifid stigma occasionally with a partly bent yellowish-brown or orange-brown ovary. The tubular florets, about 5 mm long, are present and consist of the yellow, orange-red or reddish-violet 5-lobed corolla and the yellowish-brown or orange-brown tube, hairy in its lower part, mostly with a partly bent yellowish-brown or orange-brown ovary.

B. Reduce to a powder (355) (*2.9.12*). The powder is yellowish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the corollas containing light yellow oil droplets, some with fairly large anomocytic stomata (*2.8.3*), others containing prisms and very small cluster crystals of calcium oxalate; covering trichomes biseriate, multicellular and conical, and glandular trichomes with a uniseriate or biseriate, multicellular stalk and a large, ovoid, biseriate and multicellular head; spherical pollen grains up to about 40 µm in diameter with a sharply spiny exine and 3 germinal pores; occasional fragments of the stigmas with short, bulbous papillae.



A and B. Epidermis of the corolla

C. Epidermis at the apex of the corolla showing anomocytic stomata

D. Fragments of vessel tissue attached to parenchyma cells containing cluster crystals of calcium oxalate

E and F. Pollen grains

G. Fragment of the stigma

H. Covering trichome (usually only fragments observed)

J. Cells from the base of the corolla and glandular trichomes

K. Glandular trichome

Figure 1297.-1. – *Illustration of powdered herbal drug of calendula flower (see Identification B)*

C. Thin-layer chromatography *(2.2.27)* .

Test solution Mix 1.0 g of the powdered drug (500) (*2.9.12*) and 10 ml of *methanol R* and heat on a water-bath under a reflux condenser for 10 min. Cool and filter.

Reference solution Dissolve 1.0 mg of *caffeic acid R* , 1.0 mg of *chlorogenic acid R* and

2.5 mg of *rutin R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, water R, ethyl acetate R* (10:10:80 *V/V/V*).

Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C.

Detection Spray the still-warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R in methanol R* and then spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the lower part a yellowish-brown fluorescent zone (rutin), in the middle part a light bluish fluorescent zone (chlorogenic acid) and in the upper part a light bluish fluorescent zone (caffeic acid). The chromatogram obtained with the test solution shows a yellowish-brown fluorescent zone corresponding in position to the zone due to rutin in the chromatogram obtained with the reference solution, below and directly above it, it shows a yellowish-green fluorescent zone and a light bluish fluorescent zone corresponding to the zone due to chlorogenic acid in the chromatogram obtained with the reference solution, a yellowish-green fluorescent zone above it and a light bluish fluorescent zone shortly below the zone due to caffeic acid in the chromatogram obtained with the reference solution. Further zones are present.

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of bracts and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (500) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

ASSAY

Stock solution Into a 100 ml round-bottomed flask introduce 0.800 g of the powdered drug (500) *(2.9.12)*, 1 ml of a 5 g/l solution of *hexamethylenetetramine R*, 20 ml of *acetone R* and 7 ml of *hydrochloric acid R1*. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a 100 ml volumetric flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R*, each time boiling under a reflux condenser for 10 min. Allow to cool to room temperature, filter the liquid through a plug of absorbent cotton, then filter the combined acetone solution through a filter-paper into the volumetric flask, and dilute to 100.0 ml with *acetone R* by rinsing the flask and filter. Introduce 20.0 ml of this solution into a separating funnel, add 20 ml of *water R* and extract the mixture with 1 quantity of 15 ml and then with 3 quantities, each of 10 ml, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, rinse with 2 quantities, each of 50 ml, of *water R,* filter the extract over 10 g of *anhydrous sodium sulphate R* into a 50 ml volumetric flask and dilute to 50.0 ml with *ethyl acetate R* .

Test solution To 10.0 ml of the stock solution add 1 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R* .

Compensation liquid Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

After 30 min, measure the absorbance *(2.2.25)* of the test solution at 425 nm, by comparison with the compensation liquid.

Calculate the percentage content of flavonoids, expressed as hyperoside, using the following expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of hyperoside to be 500.

A = absorbance at 425 nm

m = mass of the drug to be examined, in grams

Ph Eur

Cape Aloes

General Notices

(Ph Eur monograph 0258)

Preparation

Standardised Aloes Dry Extract

Ph Eur

DEFINITION

Concentrated and dried juice of the leaves of various species of *Aloe*, mainly *Aloe ferox* Miller and its hybrids.

Content

Minimum 18.0 per cent of hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4) (dried drug).

CHARACTERS

Appearance

Dark brown masses tinged with green and having a shiny conchoidal fracture, or greenish-brown powder.

Solubility

Partly soluble in boiling water, soluble in hot ethanol (96 per cent).

IDENTIFICATION

A. Examine the chromatograms obtained in the test for Barbados aloes.

Results The chromatogram obtained with the test solution shows in the central part a yellow fluorescent zone (barbaloin) similar in position to the zone due to barbaloin in the chromatogram obtained with the reference solution and in the lower part 2 yellow fluorescent zones (aloinosides A and B) and 1 blue fluorescent zone (aloesine).

B. Shake 1 g of the powdered drug with 100 ml of boiling *water R* . Cool, add 1 g of *talc R* and filter. To 10 ml of the filtrate add 0.25 g of *disodium tetraborate R* and heat to dissolve. Pour 2 ml of the solution into 20 ml of *water R* . A yellowish-green fluorescence appears which is particularly marked in ultraviolet light at 365 nm.

C. To 5 ml of the filtrate obtained in identification test B add 1 ml of freshly prepared bromine *water R* . A yellow precipitate is formed. The supernatant liquid is not violet.

TESTS

Barbados aloes

Thin-layer chromatography *(2.2.27)* .

Test solution To 0.25 g of the powdered drug add 20 ml of *methanol R* and heat to boiling in a water-bath. Shake for a few minutes and decant the solution. Store at about 4 °C and use within 24 h.

Reference solution Dissolve 25 mg of *barbaloin R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, methanol R, ethyl acetate R* (13:17:100 *V/V/V*).

Application 10 µl, as bands of 20 mm by maximum 3 mm.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 100 g/l solution of *potassium hydroxide R* in *methanol R* . Heat at 110 °C for 5 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows no violet fluorescent zone just below the zone due to barbaloin.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 2.0 per cent.

ASSAY

Carry out the assay protected from bright light.

Introduce 0.400 g of powdered drug (180) *(2.9.12)* into a 250 ml conical flask. Moisten with 2 ml of *methanol R* , add 5 ml of *water R* warmed to about 60 °C, mix, then add a further 75 ml of *water R* at about 60 °C and shake for 30 min. Cool, filter into a volumetric flask, rinse the conical flask and filter with 20 ml of *water R* , add the rinsings to the volumetric flask and dilute to 1000.0 ml with *water R* . Transfer 10.0 ml of this solution to a 100 ml round-bottomed flask containing 1 ml of a 600 g/l solution of *ferric chloride R* and 6 ml of *hydrochloric acid R* . Heat in a water-bath under a reflux condenser for 4 h, with the water level above that of the liquid in the flask. Allow to cool, transfer the solution to a separating funnel, rinse the flask successively with 4 ml of *water R* , 4 ml of *1 M sodium hydroxide* and 4 ml of *water R* and add the rinsings to the separating funnel. Shake the contents of the separating funnel with 3 quantities, each of 20 ml, of *ether R* . Wash the combined ether layers with 2 quantities, each of 10 ml, of *water R* . Discard the washings and dilute the organic phase to 100.0 ml with *ether R* . Evaporate 20.0 ml of the solution carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 512 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of barbaloin from the following expression:

$$\frac{A \times 19.6}{m}$$

i.e. taking the specific absorbance of hydroxyanthracene derivatives, as barbaloin, to be 255.

A = absorbance at 512 nm,

m = mass of the substance to be examined in grams.

STORAGE

In an airtight container.

Ph Eur

Capsicum

General Notices

(Ph Eur monograph 1859)

Ph Eur

DEFINITION

Dried ripe fruits of *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small-fruited varieties of *Capsicum frutescens* L.

Content

Minimum 0.4 per cent of total capsaicinoids, expressed as capsaicin ($C_{18}H_{27}NO_3$; M_r 305.4) (dried drug).

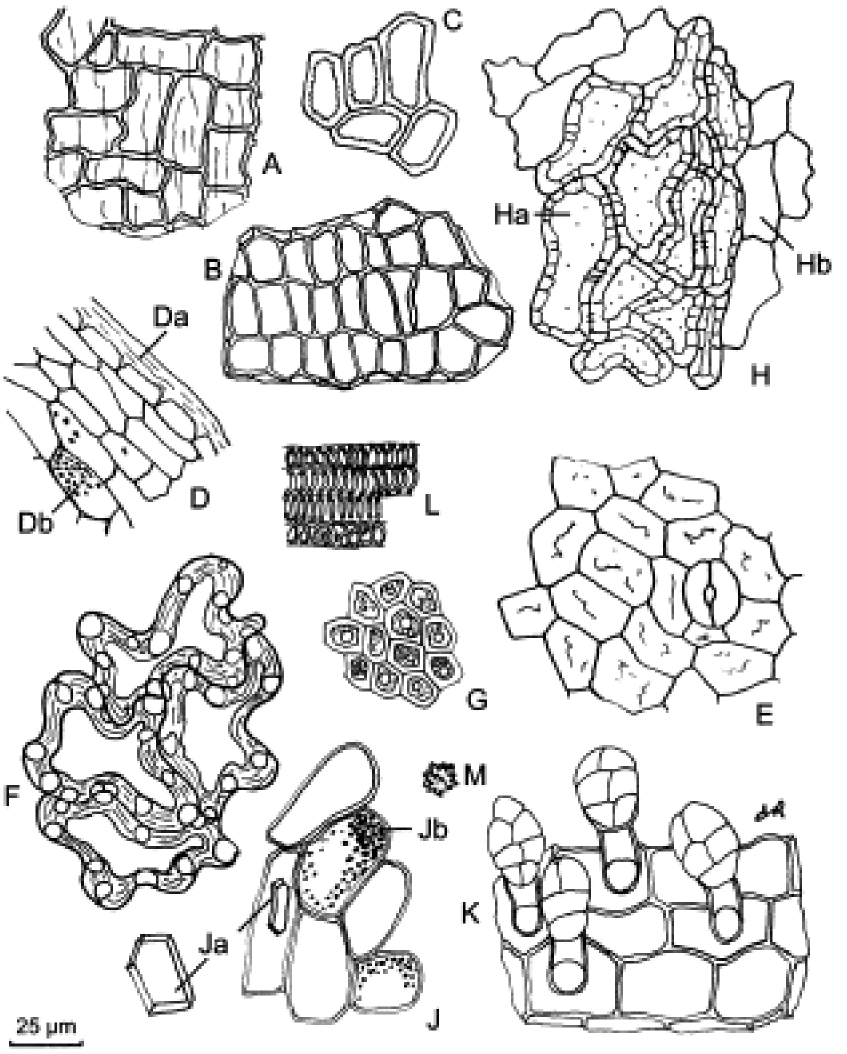
CHARACTERS

Extremely pungent taste.

IDENTIFICATION

A. The fruit is yellowish-orange or reddish-brown, oblong conical with an obtuse apex, about 1-3 cm long and up to 1 cm in diameter at the widest part, occasionally attached to a 5-toothed inferior calyx and a straight pedicel. Pericarp somewhat shrivelled, glabrous, enclosing about 10-20 flat, reniform seeds 3-4 mm long, either loose or attached to a reddish dissepiment.

B. Reduce to a powder (355) *(2.9.12)*. The powder is orange. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the pericarp having an outer epicarp with cells often arranged in rows of 5 to 7, cuticle uniformly striated, parenchymatous cells frequently containing droplets of red oil, occasionally containing microsphenoidal crystals of calcium oxalate, endocarp with characteristic island groups of sclerenchymatous cells, the groups being separated by thin-walled parenchymatous cells; fragments of the seeds having an episperm composed of large, greenish-yellow, sinuous-walled sclereids with thin outer walls and strongly and unevenly thickened radial and inner walls which are conspicuously pitted, endosperm parenchymatous cells with drops of fixed oil and aleurone grains 3-6 µm in diameter; occasional fragments from the calyx having an outer epidermis with anisocytic stomata *(2.8.3)* , inner epidermis with many trichomes but no stomata, trichomes glandular, with uniseriate stalks and multicellular heads, mesophyll with many idioblasts containing microsphenoidal crystals of calcium oxalate.



A, B, C. Epicarp: cells with striated cuticle (A); cells arranged in rows (B) and cells close to the peduncle (C)

D. Epicarp in transverse section showing cuticle (Da), accompanied by parenchyma containing microcrystals of calcium oxalate (Db)

E. Outer epidermis of the calyx with anisocytic stoma

F. Episperm

G. Endosperm with aleurone grains

H. Fragment of endocarp in transverse section with sclerenchymatous cells (Ha) and thin-walled parenchymatous cells (Hb)

J. Mesophyll of the calyx with prisms (Ja), microcrystals (Jb) and cluster crystals of calcium oxalate

K. Inner epidermis of the calyx with glandular trichomes

L. Vessels

M. Calcium oxalate cluster

Figure 1859.-1. – *Illustration of powdered herbal drug of capsicum (see identification B)*

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 0.50 g of the powdered drug (500) *(2.9.12)* add 5.0 ml of *ether R* , shake for 5 min and filter.

Reference solution Dissolve 2 mg of *capsaicin R* and 2 mg of *dihydrocapsaicin R* in 5.0 ml of *ether R* .

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *water R, methanol R* (20:80 *V/V*).

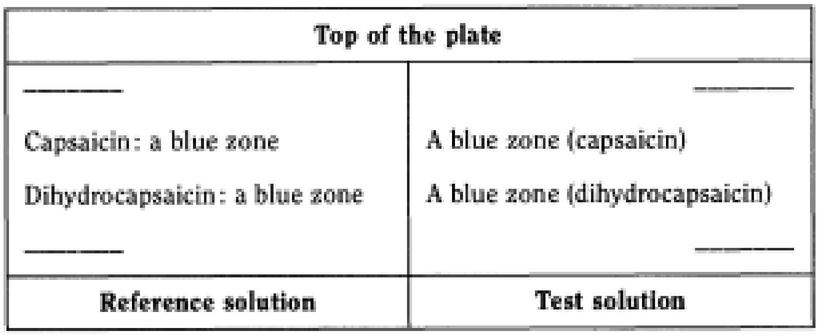
Application 20 µl as bands.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with a 5 g/l solution of *dichloroquinonechlorimide R* in *methanol R* . Expose to ammonia vapour until blue zones appear. Examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Capsaicin: a blue zone	A blue zone (capsaicin)
Dihydrocapsaicin: a blue zone	A blue zone (dihydrocapsaicin)
———	———
Reference solution	**Test solution**

TESTS

Nonivamide

Liquid chromatography *(2.2.29)* .

Test solution To 2.5 g of the powdered drug (500) *(2.9.12)* add 100 ml of *methanol R* . Allow to macerate for 30 min. Place in an ultrasonic bath for 15 min. Filter into a 100 ml volumetric flask, rinse the flask and filter with *methanol R* , then dilute to 100.0 ml with the same solvent.

Reference solution Dissolve 20.0 mg of *capsaicin CRS* and 4.0 mg of *nonivamide CRS* in *methanol R* and dilute to 100.0 ml with the same solvent.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: phenylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a 1 g/l solution of *phosphoric acid R* .

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl.

Elution order nordihydrocapsaicin, nonivamide, capsaicin, dihydrocapsaicin.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to nonivamide and capsaicin.

Calculate the percentage content of nonivamide using the following expression:

$$\frac{F_1 \times m_2 \times p_1}{F_2 \times m_1}$$

F_1 = area of the peak corresponding to nonivamide in the chromatogram obtained with the test solution;

F_2 = area of the peak corresponding to nonivamide in the chromatogram obtained with the reference solution;

m_1 = mass of the drug to be examined, in grams;

m_2 = mass of *nonivamide CRS* used to prepare the reference solution, in grams;

p_1 = percentage content of nonivamide in *nonivamide CRS*.

Limit:

— *nonivamide*: maximum 5.0 per cent of the total capsaicinoid content.

Foreign matter ***(2.8.2)***

Fruits of *C. annuum* L. var. *longum* (Sendtn.) are absent.

Loss on drying ***(2.2.32)***

Maximum 11.0 per cent, determined on 1.000 g of the powdered drug (500) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 10.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for nonivamide.

Calculate the percentage content of total capsaicinoids, expressed as capsaicin, using the following expression:

$$\frac{(F_3 + F_5 + F_6) \times m_4 \times p_2}{F_4 \times m_3}$$

F_3 = area of the peak corresponding to capsaicin in the chromatogram obtained with the test solution;

F_4 = area of the peak corresponding to capsaicin in the chromatogram obtained with the reference solution;

F_5 = area of the peak corresponding to dihydrocapsaicin in the chromatogram obtained with the test solution;

F_6 = area of the peak corresponding to nordihydrocapsaicin in the chromatogram obtained with the test solution;

m_3 = mass of the drug to be examined, in grams;

m_4 = mass of *capsaicin CRS* used to prepare the reference solution, in grams;

p_2 = percentage content of capsaicin in *capsaicin CRS*.

Ph Eur

Caraway Oil

General Notices

(Ph Eur monograph 1817)

Ph Eur

DEFINITION

Oil obtained by steam distillation from the dry fruits of *Carum carvi* L.

CHARACTERS

Appearance

Clear, colourless or yellow liquid.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 40 µl of the substance to be examined in 1.0 ml of *toluene R* .

Reference solution Dissolve 10 µl of *carvone R* and 5 µl of *carveol R* in 1.0 ml of *toluene R* .

Plate *TLC silica gel F* $_{254}$ *plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

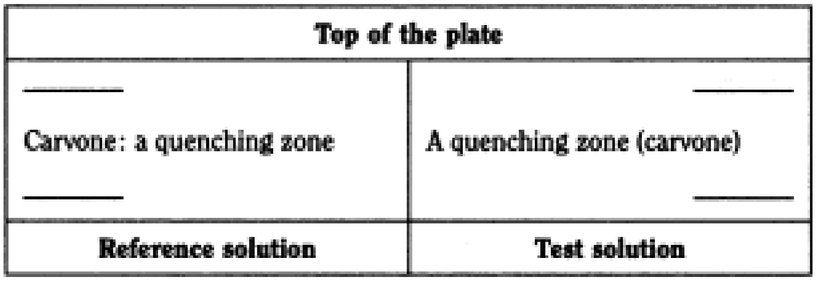
Application 10 µl [or 2 µl] as bands.

Development Over a path of 10 cm [or 5 cm].

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

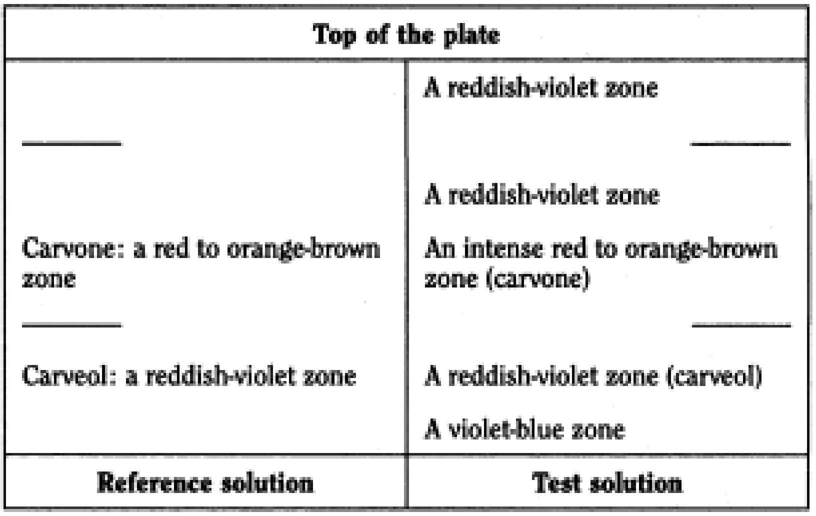
Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Carvone: a quenching zone	A quenching zone (carvone)
———	———
Reference solution	**Test solution**

Detection B Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine immediately in daylight.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, several zones of weak intensity are present, particularly in the lower third, in the chromatogram obtained with the test solution.



Top of the plate	
	A reddish-violet zone
———	———
	A reddish-violet zone
Carvone: a red to orange-brown zone	An intense red to orange-brown zone (carvone)
———	———
Carveol: a reddish-violet zone	A reddish-violet zone (carveol)
	A violet-blue zone
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

0.904 to 0.920.

Refractive index *(2.2.6)*

1.484 to 1.490.

Optical rotation *(2.2.7)*

+ 65 ° to + 81 °.

Acid value *(2.5.1)*

Maximum 1.0, determined on 5.00 g.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution Dissolve 0.200 g of the substance to be examined in *heptane R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 5 µl of β*-myrcene R* , 80 µl of *limonene R* , 5 µl of *dihydrocarvone R* , 100 µl of *carvone R* and 5 µl of *carveol R* in *heptane R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dissolve 10 µl of *carvone R in heptane R* and dilute to 10 ml with the same solvent. Dilute 0.1 ml of this solution to 10 ml with *heptane R.*

Column:

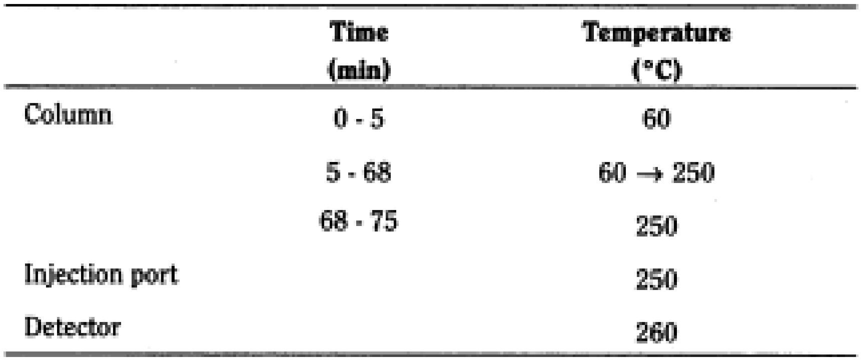
- *material*: fused silica,
- *size*: *l* = 30 m, Ø = 0.53 mm,
- *stationary phase: macrogol 20 000 R* , (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.5 ml/min.

Split ratio 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	60
	5 - 68	60 → 250
	68 - 75	250
Injection port		250
Detector		260

Detection Flame ionisation.

Injection 1.0 µl.

Elution order Order indicated in the composition of reference solution (a). Record the retention times of these substances.

System suitability Reference solution (a):

- *resolution*: minimum 4.5 between the peaks due to β-myrcene and limonene.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Limits:

- *β-myrcene* : 0.1 per cent to 1.0 per cent,
- *limonene* : 30.0 per cent to 45.0 per cent,
- *trans-dihydrocarvone*: maximum 2.5 per cent,
- *carvone* : 50.0 per cent to 65.0 per cent,
- *trans-carveol* : maximum 2.5 per cent.
- *disregard limit*: the area of the peak in the chromatogram obtained with reference solution (b).

Chiral purity

Gas chromatography *(2.2.28)* .

Test solution Dissolve 20 mg of the substance to be examined in *heptane R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 10 mg of (-)- *carvone R* and 10 mg of *carvone R1* in *heptane R* and dilute to 10.0 ml with the same solvent.

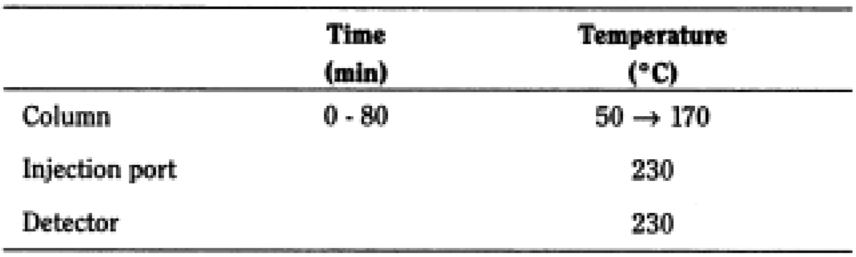
Column:
- *material*: fused silica,
- *size*: *l* = 30 m, Ø = 0.25 mm,
- *stationary phase: modified β-cyclodextrine for chiral chromatography R1* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 2.0 ml/min.

Split ratio 1:30.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 80	50 → 170
Injection port		230
Detector		230

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution:

- *resolution*: minimum 2.4 between the peaks due to (-)-carvone (1st peak) and *carvone R1* (2nd peak).

Calculate the percentage content of the (-)-carvone from the following expression:

$$\frac{A_1}{A_1 + A_2} \times 100$$

A_1 = area of the peak due to (-)-carvone,

A_2 = area of the peak due to carvone R1.

Limit:

- (-)- *carvone* : maximum 1 per cent.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Caraway

General Notices

(Caraway Fruit, Ph Eur monograph 1080)

When Powdered Caraway is prescribed or demanded, material complying with the appropriate requirements below and containing not less than 2.5% v/w (25 ml/kg) of essential oil shall be dispensed or supplied.

Ph Eur

DEFINITION

Whole, dry mericarp of *Carum carvi* L.

Content

Minimum 30 ml/kg of essential oil (anhydrous drug).

CHARACTERS

Odour reminiscent of carvone.

IDENTIFICATION

A. The fruit is a cremocarp of almost cylindrical shape. It is generally 3-6.5 mm long and 1-1.5 mm wide. The mericarps, usually free, are greyish-brown or brown, glabrous, mostly sickle-shaped, with both ends sharply terminated. Each bears 5 prominent narrow ridges. When cut transversely the profile shows an almost regular pentagon and 4 vittae on the dorsal surface and 2 on the commissural surface may be seen with a lens.

B. Reduce to a powder (355) *(2.9.12).* The powder is yellowish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the secretory cells composed of yellowish-brown or brown, thin-walled, polygonal secretory cells, frequently associated with a layer of thin-walled, transversely elongated cells, 8-12 µm wide; fragments of the epicarp with thick-walled cells and occasional anomocytic stomata *(2.8.3);* numerous endosperm fragments containing aleurone grains, droplets of fatty oil and microcrystals of calcium oxalate in rosette formation; spiral vessels accompanied by sclerenchymatous fibres; rarely some fibre bundles from the carpophore; groups of rectangular to sub-rectangular sclereids from the mesocarp with moderately thickened and pitted walls may be present.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Shake 0.5 g of the powdered drug (710) *(2.9.12)* with 5.0 ml of *ethyl acetate R* for 2-3 min. Filter over 2 g of *anhydrous sodium sulphate R.*

Reference solution Dissolve 2 µl of *carvone R* and 5 µl of *olive oil R* in 1.0 ml of *ethyl acetate R* .

Plate *TLC silica gel plate R* .

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatograms obtained with the test solution and with the reference solution show a quenching zone (carvone) in the central part against a light background.

Detection B Spray with *anisaldehyde solution R* and, while observing, heat at 100-105 °C for 2-4 min; examine in daylight.

Results B The zones due to carvone are dark orange-brown; the chromatogram obtained with the test solution shows above the zone due to carvone a violet zone similar in position and colour to the zone due to triglycerides of olive oil in the chromatogram obtained with the reference solution; the chromatogram obtained with the test solution shows close to the solvent front a weak violet zone due to terpene hydrocarbons and in the lower part some weak, mostly violet-greyish and brownish zones.

TESTS

Water *(2.2.13)*

Maximum 100 ml/kg, determined on 10.0 g of powdered drug.

Total ash *(2.4.16)*

Maximum 7.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)* . Use 10.0 g of drug reduced to a powder (710) *(2.9.12)* immediately before the determination, a 500 ml round-bottomed flask, 200 ml of *water R* as the distillation liquid, and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 90 min.

Ph Eur

Cardamom Fruit

General Notices

In making preparations of Cardamom, only the seed is used. The seed is removed from the fruit, immediately powdered or bruised and used immediately in making the preparation. Cardamom seed, after removal from the fruit, should not be stored.

DEFINITION

Cardamom Fruit consists of the dried, nearly ripe fruit of *Elettaria cardamomum* Maton var. *minuscula* Burkill.

CHARACTERISTICS

Odour and taste of the seeds, strongly aromatic.

Macroscopical Fruit: a trilocular inferior capsule, up to about 2 cm long, ovoid or oblong, dull green to pale buff, plump or slightly shrunken, obtusely triangular in cross section, nearly smooth or longitudinally striated. Seeds in each loculus in two rows, forming an adherent mass attached to the axile placenta. Seed: pale to dark reddish brown, about 4 mm long and 3 mm broad, irregularly angular, marked with six to eight transverse wrinkles, with a longitudinal channel containing the raphe, each seed enveloped by a colourless, membranous aril. Transversely cut surface of seed showing a brown testa, white starchy perisperm, grooved on one side, yellowish endosperm and a paler embryo.

Microscopical Seed: aril composed of flattened, thin-walled, parenchymatous cells. Testa composed of the following layers: (i) outer epidermis of thick-walled, narrow, axially elongated cells; (ii) a layer of collapsed parenchyma subjacent to the outer epidermis; (iii) a single layer (two or three layers near the raphe) of large, thin-walled, rectangular cells containing volatile oil; (iv) two or three layers of parenchyma; (v) layers of thin-walled, flattened cells; (vi) distinctive sclerenchymatous layer of closely packed brown, thick-walled cells, each with a bowl-shaped cavity in the upper part containing a warty silica body; (vii) inner layer consisting of flattened cells. Perisperm: cells thin-walled, packed with numerous starch granules up to 6 µm in diameter and, in a small cavity, one to seven prisms of calcium oxalate about 10 to 30 µm long. Endosperm parenchymatous, thin-walled, with a granular hyaline mass of protein in each cell. Embryo: cells small, containing aleurone grains.

TESTS

Foreign matter

Of the fruit, not more than 1.0%; of the separated seeds, not more than 3.0%, Appendix XI D.

Volatile oil

In the seeds, not less than 4.0% v/w, Appendix XI E, Method I. Use 20 g of the unground seeds and distil for 5 hours.

Acid-insoluble ash

Of the seeds, not more than 3.5%, Appendix XI K.

Ash

Of the seeds, not more than 6.0%, Appendix XI J.

Cardamom Oil

General Notices

Preparations

Aromatic Cardamom Tincture

Compound Cardamom Tincture

DEFINITION

Cardamom Oil is obtained by distillation from crushed Cardamom Fruit.

CHARACTERISTICS

A clear, colourless or pale yellow liquid, visibly free from water; odour, that of Cardamom Fruit.

TESTS

Ester value

90 to 156, Appendix X C.

Optical rotation

+20° to +40°, Appendix V F.

Refractive index

1.461 to 1.467, Appendix V E.

Solubility in ethanol

Soluble, at 20°, in 6 volumes of *ethanol (70%)*, Appendix X M.

Weight per ml

0.917 to 0.940 g, Appendix V G.

STORAGE

Cardamom Oil should be kept in a well-filled container and protected from light.

Cascara Dry Extract

General Notices

DEFINITION

Cascara Dry Extract is prepared by extracting Cascara with Purified Water and removing the solvent. It contains not less than 13.5% of hydroxyanthracene derivatives, of which not less than 40% consists of cascarosides, both calculated as cascaroside A with reference to the dried substance.

The extract complies with the requirements stated under Extracts and with the following requirements.

TESTS

Frangula

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *ethyl acetate*, 17 volumes of *methanol* and 13 volumes of *water* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions prepared in the following manner. For solution (1) boil a quantity containing the equivalent of 32 mg of total hydroxyanthracene derivatives with 5 ml of *ethanol (70%)*, cool and centrifuge. Decant the supernatant liquid and use within 30 minutes. For solution (2) dissolve 20 mg of *barbaloin* in 10 ml of *ethanol (70%)*. After removal of the plate, dry it in a current of air until the solvent has evaporated, spray immediately with a 0.1% w/v solution of N,N-*dimethyl*-p-*nitrosoaniline* in *pyridine*, then spray with a 5% w/v solution of *potassium hydroxide* in *ethanol (50%)* and heat at 105° for 15 minutes. In the chromatogram obtained with solution (1) no spot is observed with an Rf value of 1.7 to 1.8 relative to the principal spot in the chromatogram obtained with solution (2).

Loss on drying

Not more than 7.0%.

ASSAY

Carry out the following procedure protected from light. To 0.5 g add 80 ml of *ethanol (70%)*, shake, allow to stand overnight, add sufficient *ethanol (70%)* to produce 100 ml, shake and filter. Transfer 10 ml of the filtrate to a separating funnel, add 0.1 ml of 1M *hydrochloric acid* and shake with two 20 ml quantities of *carbon tetrachloride*. Wash the combined carbon tetrachloride layers with 5 ml of *water* and add the washings to the aqueous solution. Extract the aqueous solution with four 30 ml quantities of water-saturated ethyl acetate, freshly prepared by shaking 150 ml of *ethyl acetate* with 15 ml of *water* for 3 minutes and allowing to separate. Use the combined ethyl acetate extracts for the determination of hydroxyanthracene glycosides other than cascarosides and the aqueous solution for the determination of cascarosides.

For hydroxyanthracene glycosides other than cascarosides

Remove the solvent from the combined ethyl acetate extracts and evaporate just to dryness. Dissolve the residue in 0.5 ml of *methanol*, transfer to a flask with the aid of warm *water*, cool and add sufficient *water* to produce 50 ml. Add 20 ml to a mixture of 2 g of *iron(III) chloride hexahydrate* and 12 ml of *hydrochloric acid* and heat under a reflux condenser in a water bath for 4 hours, maintaining the level of the water above that of the liquid in the flask. Allow to cool, transfer to a separating funnel, wash the flask successively with 4 ml of 1M *sodium hydroxide* and 4 ml of *water* and add the washings to the separating funnel. Extract with three 30 ml quantities of *carbon tetrachloride*, wash the combined carbon tetrachloride layers with two 10 ml quantities of *water*, discard the washings and add sufficient *carbon tetrachloride* to produce 100 ml. Evaporate 20 ml to dryness on a water bath, dissolve the residue in 10 ml of a 0.5% w/v solution of *magnesium acetate* in *methanol* and measure the *absorbance* of the resulting solution at 440 nm and at 515 nm, Appendix II B, using *methanol* in the reference cell.

Calculate the content of hydroxyanthracene glycosides, as cascaroside A, from the absorbance at 515 nm taking 169 as the value of A (1%, 1 cm). The result of the assay is not valid unless the ratio of the absorbance at 515 nm to that at 440 nm is at least 2.6.

For cascarosides

To the aqueous solution reserved from the preliminary extraction add sufficient *water* to produce 50 ml and carry out the Assay for hydroxyanthracene glycosides other than cascarosides, beginning at the words 'Add 20 ml ...'. Calculate the content of cascarosides, as cascaroside A, from the *absorbance* at 515 nm, Appendix II B, taking 169 as the value of A (1%, 1 cm). The result of the assay is not valid unless the ratio of the absorbance at 515 nm to that at 440 nm is at least 2.7.

LABELLING

The label states the percentage of total hydroxyanthracene derivatives.

Cascara

General Notices

(Ph Eur monograph 0105)

Preparation

Cascara Dry Extract

When Powdered Cascara is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried, whole or fragmented bark of *Rhamnus purshianus* D.C. (*Frangula purshiana* (D.C.) A. Gray ex J. C. Cooper).

Content

Minimum 8.0 per cent of hydroxyanthracene glycosides of which minimum 60 per cent consists of cascarosides, both expressed as cascaroside A ($C_{27}H_{32}O_{14}$; M_r 580.5) (dried drug).

IDENTIFICATION

A. The bark occurs in slightly channelled or nearly flat pieces, usually 1-5 mm in thickness, usually varying greatly in length and width. The outer surface is grey or dark greyish-brown and shows occasional lenticels that are orientated transversally. It is usually more or less completely covered by a whitish coat of lichens, epiphytic moss and foliaceous liverwort. The inner surface is yellow or reddish-brown or almost black with fine longitudinal striations; it turns red when treated with alkali. The yellow fracture is short and granular in the outer part and somewhat fibrous at the inner part.

B. Reduce to a powder (355) *(2.9.12)*. The powder is yellowish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: bundles of partly lignified phloem fibres accompanied by crystal sheaths containing prisms of calcium oxalate; groups of sclereids accompanied by crystal sheaths; cluster crystals of calcium oxalate; some parenchymatous cells contain a yellow substance which becomes deep red when treated with alkali; cork cells and, frequently, epiphytes; the latter may be liverworts, entire or in fragments, having a lamina one-cell thick without a midrib and composed of isodiametric cells, or leaves of mosses, having a lamina one-cell thick composed of elongated cells and possessing a midrib several cells thick.

C. Examine the chromatograms obtained in test A for "Other species of *Rhamnus*;

anthrones".

Results The chromatogram obtained with the test solution shows several reddish-brown zones with different intensities: there are 4 faint zones, 3 being situated at about the mid-point of the chromatogram and 1 in the lower third and there is a strong zone in the upper third of the chromatogram. Examine in ultraviolet light at 365 nm. The chromatogram obtained with the test solution shows several zones with the same fluorescence, situated above and particularly below (cascarosides) that due to barbaloin in the chromatogram obtained with the reference solution.

D. Heat 0.2 g of the powdered drug (180) *(2.9.12)* with 50 ml of *water R* on a water-bath for 15 min. Allow to cool and filter. To 10 ml of the filtrate add 20 ml of *hydrochloric acid R1* and heat on a water-bath for 15 min. Allow to cool, transfer to a separating funnel and shake with 3 quantities, each of 20 ml, of *ether R*. Reserve the aqueous layer (solution A). Combine the 3 ether extracts and shake with 10 ml of *dilute ammonia R2*. The aqueous layer becomes reddish-violet. Transfer solution A to a small flask, add 5 g of *ferric chloride R* and heat on a water-bath for 30 min. Allow to cool, transfer to a separating funnel and shake with 15 ml of *ether R*. Wash the ether layer with 10 ml of *water R*, discard the water and shake the ether layer with 5 ml of *dilute ammonia R2*. A red colour develops in the aqueous layer.

TESTS

Other species of *Rhamnus*; anthrones

Thin-layer chromatography *(2.2.27)*.

Test solution To 0.5 g of the powdered drug (180) *(2.9.12)* add 5 ml of *ethanol (70 per cent V/V) R* and heat to boiling. Cool and centrifuge. Decant the supernatant solution immediately and use within 30 min.

Reference solution Dissolve 20 mg of *barbaloin R* in *ethanol (70 per cent V/V) R* and dilute to 10 ml with the same solvent.

Plates *TLC silica gel plate R* (2 plates).

Mobile phase *water R, methanol R, ethyl acetate R* (13:17:100 *V/V/V*).

A. Application: 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air for 5 min.

Detection Spray with about 10 ml of a 50 g/l solution of *potassium hydroxide R* in *ethanol (50 per cent V/V) R* and heat at 100-105 °C for 15 min. Examine immediately after heating.

Results The chromatogram obtained with the reference solution shows, in the central part, a reddish-brown zone due to barbaloin. Examine in ultraviolet light at 365 nm. The zone due to barbaloin shows intense yellowish-brown fluorescence. In the chromatogram obtained with the test solution, no zone with orange-brown fluorescence is seen between the zone due to barbaloin and the zones due to cascarosides.

B. Application: 10 µl of the test solution, as a band.

Development Over a path of 10 cm.

Drying In air for maximum 5 min.

Detection Spray immediately with a 5 g/l solution of *nitrotetrazolium blue R* in *methanol R*. Examine immediately.

Results No violet or greyish-blue zones appear.

Foreign matter ***(2.8.2)***

Maximum 1 per cent.

Loss on drying ***(2.2.32)***

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (180) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 7.0 per cent.

ASSAY

Carry out the assay protected from bright light in 24 h.

Stir 1.00 g of the powdered drug (180) *(2.9.12)* into 100 ml of boiling *water R* and continue boiling and stirring for 5 min. Allow to cool, dilute to 100.0 ml with *water R*, shake, filter and discard the first 20 ml of filtrate. Transfer 10.0 ml of the filtrate to a separating funnel, add 0.1 ml of *1 M hydrochloric acid* and shake with 2 quantities, each of 20 ml, of a mixture of 1 volume of *ether R* and 3 volumes of *hexane R*. Wash the combined organic extracts with 5 ml of *water R*, discard the organic layer and return the rinsings to the aqueous layer. Shake the combined aqueous layers with 4 quantities, each of 30 ml, of *ethyl acetate R* freshly saturated with *water R* (to 150 ml of *ethyl acetate R* add 15 ml of *water R*, shake for 3 min and allow to stand) on each occasion allowing separation to take place until the organic layer is clear. Combine the ethyl acetate extracts. Use the aqueous layer for the assay for cascarosides and the organic layer for the assay for hydroxyanthracene glycosides other than cascarosides.

Hydroxyanthracene glycosides other than cascarosides

Transfer the organic layer to a suitable flask and remove the solvent by distillation, evaporating almost to dryness. Dissolve the residue in 0.3-0.5 ml of *methanol R* and transfer to a volumetric flask, rinsing the 1st flask with warm *water R* and adding the rinsings to the methanolic solution. Allow to cool and dilute to 50.0 ml with *water R*. Transfer 20.0 ml of this solution to a 100 ml round-bottomed flask with a ground-glass neck and containing 2 g of *ferric chloride R* and 12 ml of *hydrochloric acid R*. Attach a reflux condenser and place the flask in a water-bath so that the level of the water is above that of the liquid in the flask and heat for 4 h. Allow to cool, transfer the solution to a separating funnel and rinse the flask successively with 3-4 ml of *1 M sodium hydroxide* and 3-4 ml of *water R*, adding the rinsings to the separating funnel. Shake the contents of the separating funnel with 3 quantities, each of 30 ml, of a mixture of 1 volume of *ether R* and 3 volumes of *hexane R*. Wash the combined organic layers with 2 quantities, each of 10 ml, of *water R* and discard the rinsings. Dilute the organic phase to 100.0 ml with the mixture of ether and hexane. Take 20.0 ml, evaporate carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 440 nm and 515 nm using *methanol R* as the compensation liquid. If the ratio of the absorbance at 515 nm to that at 440 nm is less than 2.4, the assay is invalid.

Calculate the percentage content of hydroxyanthracene glycosides other than cascarosides, expressed as cascaroside A, using the following expression:

$$\frac{A \times 6.95}{m}$$

i.e. taking the specific absorbance to be 180.

A = absorbance at 515 nm,

m = mass of the substance to be examined, in grams.

Cascarosides

Dilute the aqueous layer to 50.0 ml with *water R* . Treat 20.0 ml of this solution as described above in the assay of hydroxyanthracene glycosides other than cascarosides. Measure the absorbance *(2.2.25)* of the test solution at 440 nm and 515 nm. If the ratio of the absorbance at 515 nm to that at 440 nm is less than 2.4, the assay is invalid.

Calculate the percentage content of cascarosides, expressed as cascaroside A, using the following expression:

$$\frac{A \times 6.95}{m}$$

i.e. taking the specific absorbance to be 180.

A = absorbance at 515 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Cassia Oil

General Notices

(Ph Eur monograph 1496)

8007-80-5

Ph Eur

DEFINITION

Essential oil obtained by steam distillation of the leaves and young branches of *Cinnamomum cassia* Blume (*C. aromaticum* Nees).

CHARACTERS

Appearance

Clear, mobile, yellow or reddish-brown liquid.

Characteristic odour reminiscent of cinnamic aldehyde.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.5 ml of the oil to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 µl of *trans-cinnamic aldehyde R* , 10 µl of *eugenol R* and 50 mg of *coumarin R* in *acetone R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *methanol R, toluene R* (10:90 *V/V*).

Application 10 µl as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 365 nm.

Results A The zone of blue fluorescence in the chromatogram obtained with the test solution is similar in position and colour to the zone in the chromatogram obtained with the reference solution (coumarin).

Detection B Spray with *anisaldehyde solution R* ; examine in daylight while heating at 100-105 °C for 5-10 min.

Results B The chromatogram obtained with the reference solution shows in its upper part a violet zone (eugenol) and above this zone a greenish-blue zone (*trans*-cinnamic aldehyde). The chromatogram obtained with the test solution shows a zone similar in position and colour to the zone due to *trans*-cinnamic aldehyde in the chromatogram obtained with the reference solution and may show a very faint zone due to eugenol. Other faint zones are present.

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution. Eugenol may be absent from the chromatogram obtained with the test solution.

TESTS

Relative density *(2.2.5)*

1.052 to 1.070.

Refractive index *(2.2.6)*

1.600 to 1.614.

Optical rotation *(2.2.7)*

- 1 ° to + 1 °.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution The oil to be examined.

Reference solution Dissolve 100 µl of *trans-cinnamic aldehyde R* , 10 µl of *cinnamyl acetate R* , 10 µl of *eugenol R* , 10 µl of *trans-2-methoxycinnamaldehyde R* and 20 mg of *coumarin R* in 1 ml of *acetone R* .

Column:

— *material*: fused silica;

— *size: l* = 60 m, Ø = about 0.25 mm;

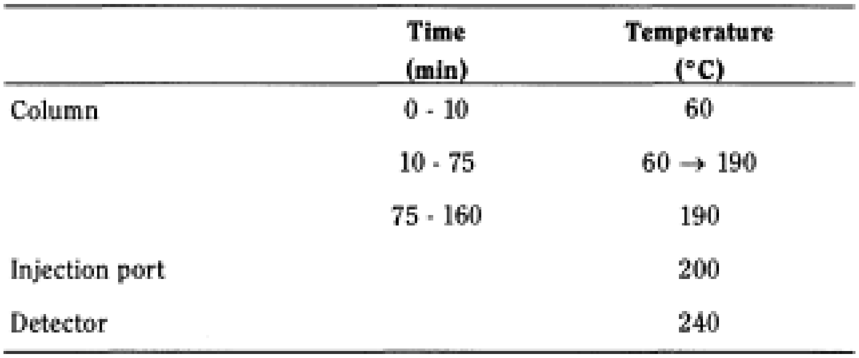
— *stationary phase*: bonded *macrogol 20 000 R* .

Carrier gas *helium for chromatography R.*

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 75	60 → 190
	75 - 160	190
Injection port		200
Detector		240

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of the reference solution, depending on the operating conditions and the state of the column, coumarin may elute before or after trans-2-methoxycinnamaldehyde; record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to trans-2-methoxycinnamaldehyde and coumarin.

Identification of components Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of each of these components. The percentages are within the following ranges:

— *trans-cinnamic aldehyde* : 70 per cent to 90 per cent;

— *cinnamyl acetate* : 1.0 per cent to 6.0 per cent;

— *eugenol* : less than 0.5 per cent;

— *trans-2-methoxycinnamaldehyde* : 3.0 per cent to 15 per cent;

— *coumarin* : 1.5 per cent to 4.0 per cent.

STORAGE

Protected from heat.

Ph Eur

Centaury

General Notices

(Ph Eur monograph 1301)

Ph Eur

DEFINITION

Whole or fragmented dried flowering aerial parts of *Centaurium erythraea* Rafn s. l. including C. *majus* (H. et L.) Zeltner and C. *suffruticosum* (Griseb.) Ronn. (syn.: *Erythraea centaurium* Persoon; C. *umbellatum* Gilibert; C. *minus* Gars.).

CHARACTERS

Bitter taste.

IDENTIFICATION

A. The hollow cylindrical, light green to dark brown stem has longitudinal ridges, and is branched only in its upper part. The sessile leaves are entire, decussately arranged, and have an ovate to lanceolate lamina, up to about 3 cm long. Both surfaces are glabrous and green to brownish-green. The inflorescence is diaxially branched. The tubular calyx is green and has 5 lanceolate, acuminate teeth. The corolla consists of a whitish tube divided into 5 elongated lanceolate pink to reddish lobes, about 5-8 mm long. 5 stamens are present attached to the top of the corolla tube. The ovary is superior and has a short style, a broad bifid stigma and numerous ovules. Cylindrical capsules, about 7-10 mm long, with small brown markedly rough seeds are frequently present.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-yellow or brownish. Examine under a microscope, using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments from the stem with lignified groups of fibres associated with narrow vessels, tracheidal vessels occasional vessels with spiral thickening; pitted parenchyma of the pith and medullary rays; fragments of leaf lamina with sinuous epidermal cells and striated cuticle, especially over the margins and surrounding the stomata; numerous stomata, mainly anisocytic *(2.8.3)* ; fragments of the palisade mesophyll, each cell containing a single prism crystal or, less frequently, a cluster crystal of calcium oxalate; fragments of calyx and corolla, those of the calyx with straight-walled epidermal cells, those of the inner epidermis of the corolla with obtuse papillae and radially striated cuticle; parts of the endothecium with reticulate or ridge-shaped wall thickenings; triangularly rounded or elliptical, yellow pollen grains, about 30 µm in diameter, with a distinctly pitted exine and 3 germinal pores; fragments of the wall of the fruit capsule composed of crossed layers of fusiform cells; oil droplets from the seeds, fragments of the epidermis of the testa showing large, brown reticulations and a pitted surface.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 25 ml of *methanol R,* shake for 15 min and filter. Evaporate the filtrate to dryness under reduced pressure and at a temperature not exceeding 50 °C. Take up the residue with small quantities of *methanol R* so as to obtain 5 ml of solution, which may contain a sediment.

Reference solution Dissolve 1 mg of *rutin R* and 1 mg of *swertiamarin R* in *methanol R* and dilute to 1 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R (5-40 µm) [or TLC silica gel F_{254} plate* R (2-10 µm)].

Mobile phase *water R, anhydrous formic acid R, ethyl formate R* (4:8:88 *V/V/V*).

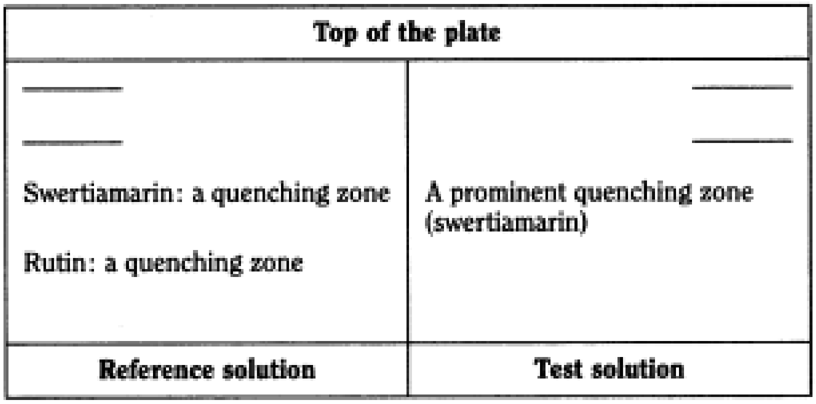
Application 10 µl [or 5 µl] as bands.

Development In an unsaturated tank over a path of 12 cm [or 6 cm].

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

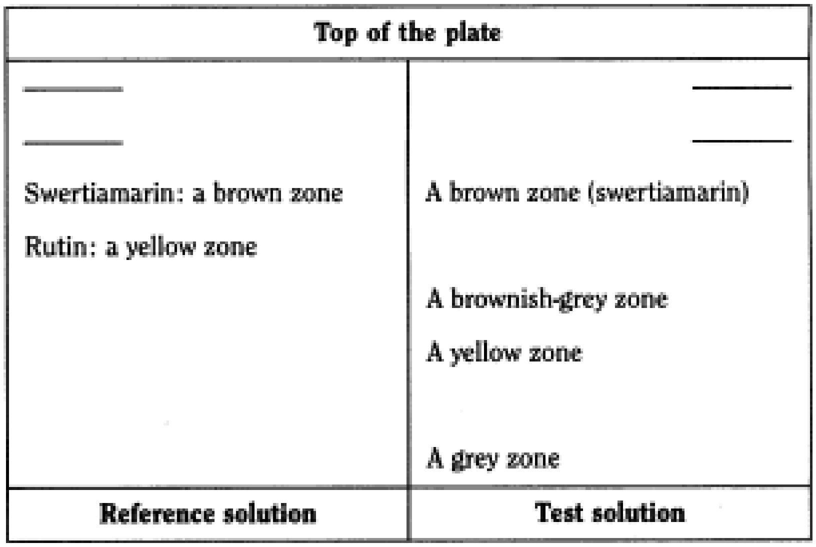
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other less intense quenching zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
_______	_______
_______	_______
Swertiamarin: a quenching zone	A prominent quenching zone (swertiamarin)
Rutin: a quenching zone	
Reference solution	**Test solution**

Detection B Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine in daylight.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other less intense coloured zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
———	———
Swertiamarin: a brown zone	A brown zone (swertiamarin)
Rutin: a yellow zone	
	A brownish-grey zone
	A yellow zone
	A grey zone
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent.

Bitterness value *(2.8.15)*

Minimum 2000.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 6.0 per cent.

Ph Eur

Centella

General Notices

(Ph Eur monograph 1498)

Ph Eur

DEFINITION

Dried, fragmented aerial parts of *Centella asiatica* (L.) Urban.

Content

Minimum 6.0 per cent of total triterpenoid derivatives, expressed as asiaticoside ($C_{48}H_{78}O_{19}$; M_r 959.15) (dried drug).

CHARACTERS

The leaves are very variable in size; the petiole is usually 5-10, sometimes 15, times longer than the lamina, which is 10-40 mm long and 20-40 mm, sometimes up to 70 mm, wide.

IDENTIFICATION

A. The leaves are alternate, sometimes grouped together at the nodes, reniform or orbicular or oblong-elliptic and have palmate nervation, usually with 7 veins, and a crenate margin. Young leaves show a few trichomes on the lower surface while adult leaves are glabrous. The inflorescence, if present, is a single umbel which usually consists of 3 flowers, rarely 2 or 4; the flowers are very small (about 2 mm) pentamerous and have an inferior ovary; the fruit, a brownish-grey, orbicular cremocarp, up to 5 mm long, is very flattened laterally and has 7-9 prominent curved ridges.

B. Reduce the drug to a powder (355) *(2.9.12)*. The powder is greenish-grey. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous fragments of leaf epidermis with polygonal cells having an irregularly striated cuticle, and paracytic stomata *(2.8.3)* that are more numerous in the lower epidermis; fragments of petiole epidermis with elongated cells; uniseriate, long, flexuous unicellular covering trichomes, occasionally multicellular; young leaves; spiral vessels; resiniferous canals; calcium oxalate prisms and macles up to 40 µm in diameter; bundles of narrow septate fibres from the stem; fragments of the fruit: layers of wide cells in a parquetry arrangement, annular vessels, parenchyma cells containing simple or compound starch granules.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 5.0 g of the powdered drug (355) *(2.9.12)* add 50 ml of *ethanol (30 per cent V/V) R*; heat to boiling under a reflux condenser and centrifuge.

Reference solution Dissolve 5 mg of *asiaticoside R in methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R* .

Mobile phase *acetic acid R, formic acid R, water R, ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C; examine in daylight.

Results The chromatograms obtained with the reference solution and the test solution show in the lower third a greenish-blue zone (asiaticoside). The chromatogram obtained with the test solution shows also below this zone a violet zone (madecassoside); near the solvent front it shows a light blue zone (asiatic acid) and just below a pinkish-violet zone (madecassic acid) ; in the lower half it shows brown, grey and brownish-green zones between the starting point and the zone due to madecassoside, and other brownish-yellow or light yellow zones above the zone due to asiaticoside.

TESTS

Foreign matter *(2.8.2)*

Maximum 7 per cent, of which maximum 5 per cent of underground organs and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

ASSAY

Liquid chromatography *(2.2.29).*

Test solution Place 5.0 g of the powdered drug (355) *(2.9.12)* in a cellulose fingerstall in a continuous extraction apparatus (Soxhlet type). Add 100 ml of *methanol R* and heat for 8 h. Cool and dilute the extract to 100.0 ml with *methanol R* . Filter through a 0.45 µm filter. Dilute 2.0 ml of the filtrate to 20.0 ml with *methanol R* .

Reference solution Dissolve 20.0 mg of *asiaticoside R in methanol R* , if necessary using sonication, and dilute to 20.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *methanol R* .

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Phase mobile:

— *mobile phase A: acetonitrile for chromatography R* ;

— *mobile phase B*: dilute 3 ml of *phosphoric acid R* to 1000 ml with *water R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 65	22	78
65 - 66	55	45
66 - 76	95	5
76 - 85	22	78

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 200 nm.

Injection 20 µl.

Relative retention with reference to the solvent: madecassoside = about 5.8; asiaticoside = about 8.1; madecassic acid = about 17.6; Asiatic acid = about 21.7.

Calculate the response factor R_F of asiaticoside using the following expression:

$$\frac{A_1 \times V_1 \times 100}{m_1 \times HPLC_\mathrm{P}}$$

A_1 = area of the peak due to asiaticoside in the chromatogram obtained with the reference solution;

V_1 = volume of the reference solution, in millilitres

m_1 = mass of asiaticoside in the reference solution, in milligrams;

$HPLC_P$ = purity determined for asiaticoside.

Calculate the mean response factor for asiaticoside using the following expression:

$$\frac{\sum_{i=1}^{N} \mathrm{RF}_i}{N}$$

$\sum_{i=1}^{N} \mathrm{RF}_i$ = sum of response factors of asiaticoside for the chromatograms obtained with the reference solution;

N = number of injections of reference solution ($N = 4$, at least).

Calculate the percentage content of total triterpenoid derivatives, expressed as asiaticoside, using the following expression:

$$\frac{V}{m}\left[\frac{A + (B \times 1.017) + (C \times 0.526) + (D \times 0.509)}{\overline{\mathrm{RF}}}\right]$$

V = volume of the test solution, in millilitres;

m = mass of the substance to be examined in the test solution, in milligrams;

A = area of the peak due to asiaticoside in the chromatogram obtained with the test solution;

B = area of the peak due to madecassoside in the chromatogram obtained with the test solution;

C = area of the peak due to madecassic acid in the chromatogram obtained with the test solution;

D = area of the peak due to asiatic acid in the chromatogram obtained with the test solution;

$\overline{\mathrm{RF}}$ = mean response factor of asiaticoside.

Ph Eur

Ceylon Cinnamon Bark Oil

General Notices

Cinnamon Oil

(Ph Eur monograph 1501)

Preparation

Concentrated Cinnamon Water

Ph Eur

DEFINITION

Essential oil obtained by steam distillation of the bark of the shoots of *Cinnamomum zeylanicum* Nees (*C. Verum* J.S. Presl.).

CHARACTERS

Appearance

Clear, mobile, light yellow liquid becoming reddish over time.

It has a characteristic odour reminiscent of cinnamic aldehyde.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 1 ml of the essential oil to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 µl of *trans-cinnamic aldehyde R* , 10 µl of *eugenol R* , 10 µl of *linalol R* and 10 µl of β*-caryophyllene R* in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *methanol R, toluene R* (10:90 *V/V*).

Application 10 µl as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* ; examine in daylight while heating at 100-105 °C for 5-10 min.

Results The zones in the chromatogram obtained with the test solution are similar in position and colour to those in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution. Safrole, coumarin and cineole may be absent from the chromatogram obtained with the test solution.

TESTS

Relative density *(2.2.5)*

1.000 to 1.030.

Refractive index *(2.2.6)*

1.572 to 1.591.

Optical rotation *(2.2.7)*

- 2° to + 1°.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution The essential oil to be examined.

Reference solution Dissolve 10 µl of *cineole R* , 10 µl of *linalol R* , 10 µl of *β-caryophyllene R* , 10 µl of *safrole R*, 100 µl of *trans-cinnamic aldehyde R* , 10 µl of *eugenol R* , 20 mg of *coumarin R* , 10 µl of *trans-2-methoxycinnamaldehyde R* and 10 µl of *benzyl benzoate R* in 1 ml of *acetone R* .

Column:

— *material*: fused silica;

— *size:* *l* = 60 m, Ø = 0.25 mm;

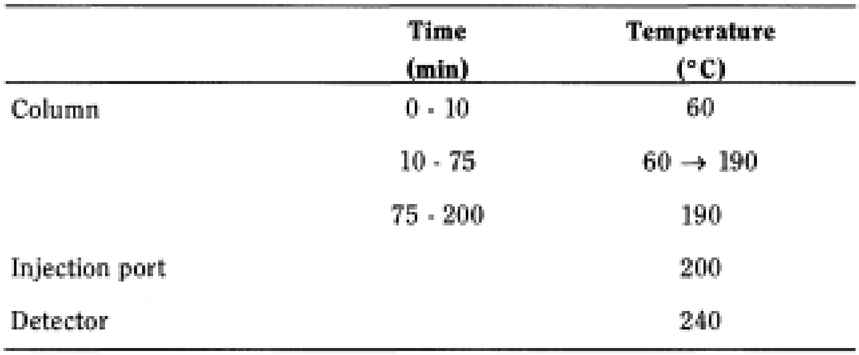
— *stationary phase*: bonded *macrogol 20 000 R* .

Carrier gas *helium for chromatography R.*

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 75	60 → 190
	75 - 200	190
Injection port		200
Detector		240

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of the reference solution; depending on the operating conditions and the state of the column, coumarin may elute before or after trans-2-methoxycinnamaldehyde; record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to linalol and β-caryophyllene.

Identification of components Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of each of these components. The percentages are within the following ranges:

— *cineole* : less than 3.0 per cent;

— *linalol* : 1.0 per cent to 6.0 per cent;

— *β-caryophyllene* : 1.0 per cent to 4.0 per cent;

— *safrole*: less than 0.5 per cent;

— *trans-cinnamic aldehyde* : 55 per cent to 75 per cent;

— *eugenol* : less than 7.5 per cent;

— *coumarin* : less than 0.5 per cent;

— *trans-2-methoxycinnamaldehyde* : 0.1 per cent to 1.0 per cent;

— *benzyl benzoate* : less than 1.0 per cent.

STORAGE

Protected from heat.

Ph Eur

Ceylon Cinnamon Leaf Oil

General Notices

(Ph Eur monograph 1608)

Ph Eur

DEFINITION

Oil obtained by steam distillation of the leaves of *Cinnamomum verum* J.S. Presl.

CHARACTERS

Clear, mobile, reddish-brown to dark brown liquid, with a characteristic odour reminiscent of eugenol.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dilute 1 ml of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution Dilute about 50 µl of *trans-cinnamic aldehyde R* , 10 µl of *eugenol R* , 10 µl of *linalol R* and 10 µl of β*-caryophyllene R* in *alcohol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R* .

Mobile phase *methanol R, toluene R* (10:90 *V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* . Examine in day light while heating at 100-105 °C for 5-10 min.

Results The zones in the chromatogram obtained with the test solution are similar in position and colour to those in the chromatogram obtained with the reference solution. The zone due to *trans*-cinnamic aldehyde may be very faint or absent.

B. Examine the chromatogram obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution. The peaks corresponding to cineole, safrole, trans-cinnamic aldehyde, cinnamyl acetate and coumarin may be absent in the chromatogram obtained with the test solution.

TESTS

Relative density *(2.2.5)*

1.030 to 1.059.

Refractive index *(2.2.6)*

1.527 to 1.540.

Optical rotation *(2.2.7)*

- 2.5° to + 2.0°.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution The substance to be examined.

Reference solution Dissolve 10 µl of *cineole R* , 10 µl of *linalol R* , 10 µl of β*-caryophyllene R* , 10 µl of *safrole R*, 10 µl of *trans-cinnamic aldehyde R* , 10 µl of *cinnamyl acetate R* , 100 µl of *eugenol R* and 10 mg of *coumarin R* in 1 ml of *acetone R.*

Column:

— *material*: fused silica,

— *size: l* = 60 m, Ø = 0.25 mm,

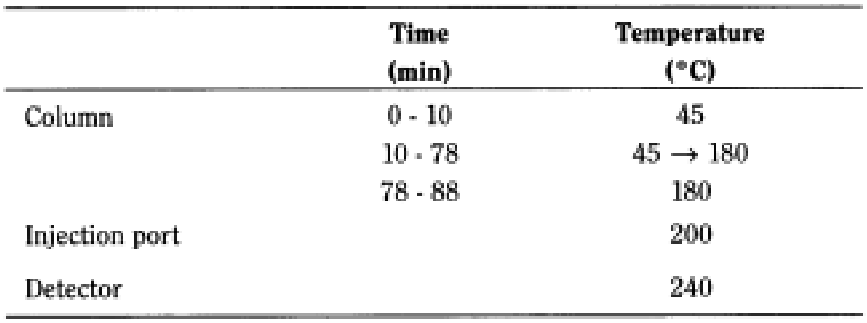
— *stationary phase: macrogol 20 000 R.*

Carrier gas *helium for chromatography R* .

Flow rate 1.5 ml/min.

Split ratio 1/100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	45
	10 - 78	45 → 180
	78 - 88	180
Injection port		200
Detector		240

Detection Flame ionisation.

Injection 0.2 µl.

Elution order The order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

- *resolution*: minimum of 1.5 between the peaks due to linalol and β-caryophyllene.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of these components.

The percentages are within the following ranges:

- *cineole* : less than 1.0 per cent,
- *linalol* : 1.5 per cent to 3.5 per cent,
- β-*caryophyllene* : 1.5 per cent to 7.0 per cent,
- *safrole*: less than 3.0 per cent,
- *trans-cinnamic aldehyde* : less than 3.0 per cent,
- *cinnamyl acetate* : less than 2.0 per cent,
- *eugenol :* 70 per cent to 85 per cent,
- *coumarin:* less than 1.0 per cent.

STORAGE

In a well-filled, airtight container , protected from light and heat.

Ph Eur

Chamomile Flowers

General Notices

(Roman Chamomile Flower, Ph Eur monograph 0380)

Ph Eur

DEFINITION

Dried flower-heads of the cultivated double variety of *Chamaemelum nobile* (L.) All. (*Anthemis nobilis* L.).

Content

Minimum 7 ml/kg of essential oil (dried drug).

CHARACTERS

The flower-heads are white or yellowish-grey, composed of solitary hemispherical capitula, made up of a solid conical receptacle bearing the florets, each subtended by a transparent small palea.

Strong and characteristic odour.

IDENTIFICATION

A. The capitula have a diameter of 8-20 mm; the receptacle is solid; the base of the receptacle is surrounded by an involucre consisting of 2-3 rows of compact and imbricated bracts with scarious margins. Most florets are ligulate, but a few pale yellow tubular florets occur in the central region. Ligulate florets are white, dull, lanceolate and reflexed with a dark brown, inferior ovary, a filiform style and a bifid stigma; tubular florets have a five-toothed corolla tube, 5 syngenesious, epipetalous stamens and a gynoecium similar to that of the ligulate florets.

B. Separate the capitulum into its different parts. Examine under a microscope using *chloral hydrate solution R.* All parts of the flower-heads are covered with numerous small yellow glistening glandular trichomes. The involucral bracts and paleae have epidermal cells in longitudinal rows, sclerified at the base and they are covered with conical trichomes, about 500 µm long, each composed of 3-4 very short base cells and a long, bent, terminal cell about 20 µm wide. The corolla of the ligulate flowers consists of papillary cells with cuticular striations. The ovaries of both kinds of florets have at their base a sclerous ring consisting of a single row of cells. The receptacle and the ovaries contain small clusters of calcium oxalate. The pollen grains have a diameter of about 35 µm and are rounded or triangular with 3 germinal pores and a spiny exine.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 0.5 g of the powdered drug (710) *(2.9.12)* add 10 ml of *methanol R* and heat with shaking in a water-bath at 60 °C for 5 min. Allow to cool and filter.

Reference solution Dissolve 2.5 mg of *apigenin R* and 2.5 mg of *apigenin-7-glucoside R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R, water R, butanol R* (17:17:66 *V/V/V*).

Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 5 min.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, using about 10 ml for a plate 200 mm square; subsequently spray with the same volume of a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow to stand for about 30 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the upper third a yellowish-green fluorescent zone (apigenin) and in the middle third a yellowish fluorescent zone (apigenin-7-glucoside). The chromatogram obtained with the test solution shows a yellowish-green fluorescent zone and a yellowish fluorescent zone similar in position and fluorescence to the zones due to apigenin and apigenin-7-glucoside in the chromatogram obtained with the reference solution; above the apigenin-7-glucoside zone there is a brownish fluorescent zone (luteolin); immediately below the apigenin-7-glucoside zone there is a light brownish fluorescent zone (apiin); immediately below the apiin zone there is a bright blue fluorescent zone and below this zone a bright blue fluorescent zone; other faint zones may be present.

TESTS

Diameter of the flower-heads: maximum 3 per cent of flower-heads have a diameter smaller than 8 mm.

Deteriorated flower-heads: brown or darkened flower-heads are absent.

Loss on drying *(2.2.32)*

Maximum 11.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 8.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use 20.0 g of whole drug, a 500 ml round-bottomed flask, 250 ml of *water R* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 3-3.5 ml/min for 3 h.

Ph Eur

Cinchona Bark

General Notices

Cinchona; Red Cinchona Bark

(Ph Eur monograph 0174)

When Powdered Cinchona is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Whole or cut, dried bark of *Cinchona pubescens* Vahl (*Cinchona succirubra* Pav.), of *Cinchona calisaya* Wedd., of *Cinchona ledgeriana* Moens ex Trimen or of their varieties or hybrids.

Content

Minimum 6.5 per cent of total alkaloids, of which 30 per cent to 60 per cent consists of quinine-type alkaloids (dried drug).

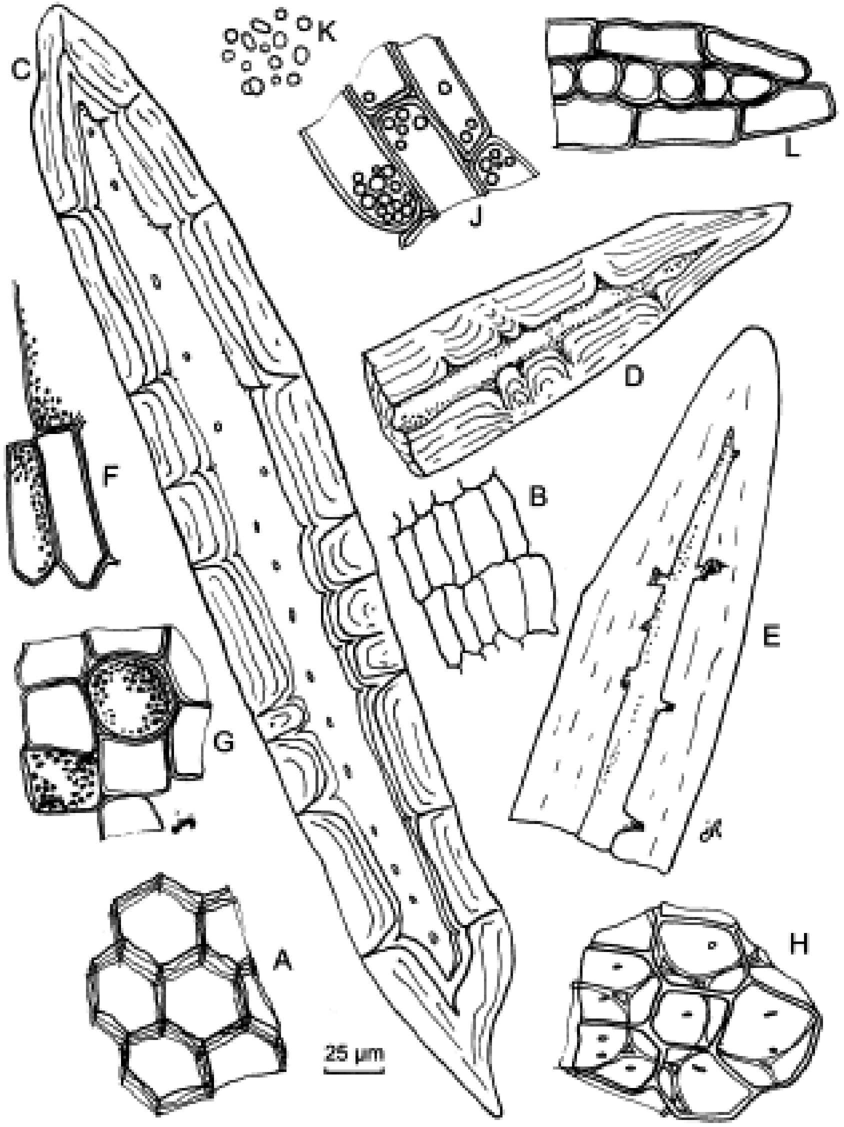
CHARACTERS

Intense bitter, somewhat astringent taste.

IDENTIFICATION

A. The stem and branch bark is supplied in quilled or curved pieces 2-6 mm thick. The outer surface is dull brownish-grey or grey and frequently bears lichens; it is usually rough, marked with transverse fissures and longitudinally furrowed or wrinkled; exfoliation of the outer surface occurs in some varieties. The inner surface is striated and deep reddish-brown; the fracture is short in the outer part and fibrous in the inner part.

B. Reduce to a powder (355) *(2.9.12)*. The powder is reddish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: thin-walled cork cells filled with reddish-brown contents; yellow, spindle-shaped striated phloem fibres up to 90 µm in diameter and up to 1300 µm in length, very thick-walled with an uneven lumen and with conspicuous, funnel-shaped pits; parenchymatous idioblasts filled with microprisms of calcium oxalate; clusters of thin-walled phloem parenchyma cells. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R* . The powder shows a few starch granules 6-10 µm in diameter; mostly simple but occasionally with 2 or 3 components.



A, B. Cork cells in surface view (A) and in transverse section (B)

C, D, E. Phloem fibres

F, G. Parenchymatous idioblasts filled with microprisms of calcium oxalate

H. Parenchymatous cells

J. Parenchymatous cells containing starch granules

K. Starch granules

L. Phloem parenchyma with medullary ray in tangential section

Figure 0174.-1. – *Illustration of powdered herbal drug of cinchona bark (see Identification B)*

C. Thin-layer chromatography *(2.2.27)* .

Test solution   To 0.10 g of the powdered drug (180) *(2.9.12)* in a test-tube add 0.1 ml of *concentrated ammonia R* and 5 ml of *methylene chloride R* . Shake vigorously occasionally during 30 min and filter. Evaporate the filtrate to dryness on a water-bath and dissolve the residue in 1 ml of *anhydrous ethanol R* .

Reference solution Dissolve 17.5 mg of *quinine R*, 2.5 mg of *quinidine R*, 10 mg of *cinchonine R* and 10 mg of *cinchonidine R* in 5 ml of *anhydrous ethanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *diethylamine R, ethyl acetate R, toluene R* (10:20:70 *V/V/V*).

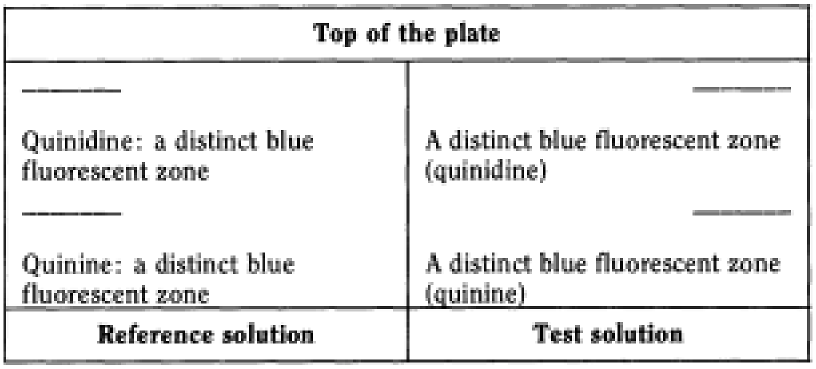
Application 10 µl, as bands.

Development Twice over a path of 15 cm.

Drying At 100-105 °C, then allow to cool.

Detection A Spray with *anhydrous formic acid R* and allow to dry in air; examine in ultraviolet light at 365 nm.

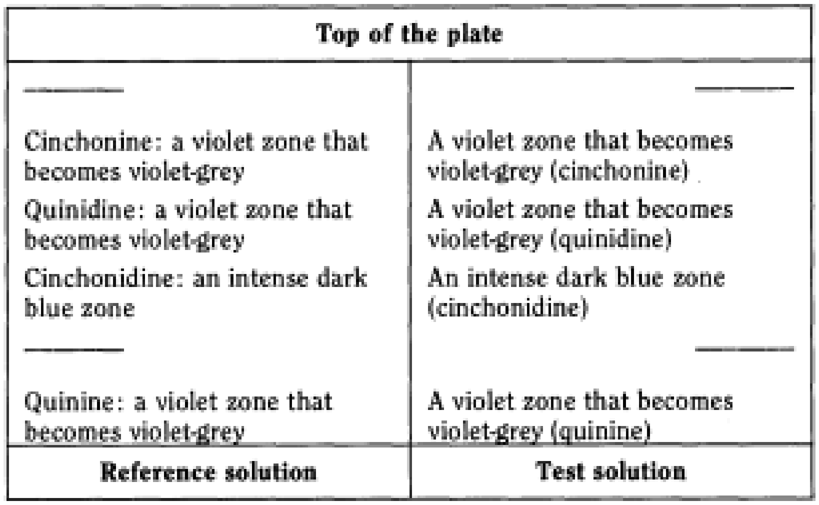
Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones are present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Quinidine: a distinct blue fluorescent zone	A distinct blue fluorescent zone (quinidine)
———	———
Quinine: a distinct blue fluorescent zone	A distinct blue fluorescent zone (quinine)
Reference solution	**Test solution**

Detection B Spray with *iodoplatinate reagent R*.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Cinchonine: a violet zone that becomes violet-grey	A violet zone that becomes violet-grey (cinchonine)
Quinidine: a violet zone that becomes violet-grey	A violet zone that becomes violet-grey (quinidine)
Cinchonidine: an intense dark blue zone	An intense dark blue zone (cinchonidine)
———	———
Quinine: a violet zone that becomes violet-grey	A violet zone that becomes violet-grey (quinine)
Reference solution	**Test solution**

TESTS

Total ash ***(2.4.16)***

Maximum 6.0 per cent.

Loss on drying ***(2.2.32)***

Maximum 10 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

ASSAY

Test solution In a 250 ml conical flask mix 1.000 g of the powdered drug (180) *(2.9.12)* with 10 ml of *water R* and 7 ml of *dilute hydrochloric acid R* . Heat in a water-bath for 30 min, allow to cool and add 25 ml of *methylene chloride R* , 50 ml of *ether R* and 5 ml of a 200 g/l solution of *sodium hydroxide R* . Shake the mixture repeatedly for 30 min, add 3 g of *powdered tragacanth R* and shake until the mixture becomes clear. Filter through a plug of absorbent cotton and rinse the flask and the cotton with 5 quantities, each of 20 ml, of a mixture of 1 volume of *methylene chloride R* and 2 volumes of *ether R* . Combine the filtrate and washings, evaporate to dryness and dissolve the residue in 10.0 ml of *anhydrous ethanol R* . Evaporate 5.0 ml of this solution to dryness, dissolve the residue in *0.1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

Reference solutions Dissolve separately 30.0 mg of *quinine R* and 30.0 mg of *cinchonine R* in *0.1 M hydrochloric acid* and dilute each solution to 1000.0 ml with the same acid.

Measure the absorbances *(2.2.25)* of the 3 solutions at 316 nm and 348 nm using *0.1 M hydrochloric acid* as the compensation liquid.

Calculate the percentage content of alkaloids using the following equations:

$$x = \frac{[A_{316} \times A_{348c}] - [A_{316c} \times A_{348}]}{[A_{316q} \times A_{348c}] - [A_{316c} \times A_{348q}]} \times \frac{100}{m} \times \frac{2}{1000}$$

$$y = \frac{[A_{316} \times A_{348q}] - [A_{316q} \times A_{348}]}{[A_{316c} \times A_{348q}] - [A_{316q} \times A_{348c}]} \times \frac{100}{m} \times \frac{2}{1000}$$

m = mass of the drug used, in grams;

x = percentage content of quinine-type alkaloids;

y = percentage content of cinchonine-type alkaloids;

A_{316} = absorbance of the test solution at 316 nm;

A_{348} = absorbance of the test solution at 348 nm;

A_{316c} = absorbance of the reference solution containing cinchonine at 316 nm, corrected to a concentration of 1 mg/1000 ml;

A_{316q} = absorbance of the reference solution containing quinine at 316 nm, corrected to a concentration of 1 mg/1000 ml;

A_{348c} = absorbance of the reference solution containing cinchonine at 348 nm, corrected to a concentration of 1 mg/1000 ml;

A_{348q} = absorbance of the reference solution containing quinine at 348 nm, corrected to a concentration of 1 mg/1000 ml.

Calculate the content of total alkaloids ($x + y$), and calculate the relative content of quinine-type alkaloids using the following expression:

$$\frac{100x}{x + y}$$

Ph Eur

Cinchona Liquid Extract, Standardised

General Notices

(Ph Eur monograph 1818)

Ph Eur

DEFINITION

Liquid extract produced from *Cinchona bark (0174).*

Content

Minimum 4.0 per cent and maximum 5.0 per cent of total alkaloids, of which 30 per cent to 60 per cent are alkaloids of the quinine type ($C_{20}H_{24}N_2O_2$; M_r 324.4).

PRODUCTION

Standardised cinchona liquid extract is produced from the herbal drug by an appropriate procedure using:

— ethanol (30 per cent *V/V* to 90 per cent *V/V*), or;

— a mixture of diluted hydrochloric acid, ethanol (96 per cent *V/V*), glycerol, water (1:2:5:20 *V/V).*

CHARACTERS

Appearance

Brownish-red liquid.

It has a bitter, astringent taste.

IDENTIFICATION

Thin-layer chromatography (2.2.27).

Test solution Dilute 1 ml of the extract to be examined in 1 ml of *anhydrous ethanol R*.

Reference solution Dissolve 2.5 mg of *quinidine R*, 10 mg of *cinchonidine R*, 10 mg of *cinchonine R* and 17.5 mg of *quinine R* in 5 ml of *anhydrous ethanol R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *diethylamine R*, *ethyl acetate R*, *toluene R* (10:20:70 *V/V/V*).

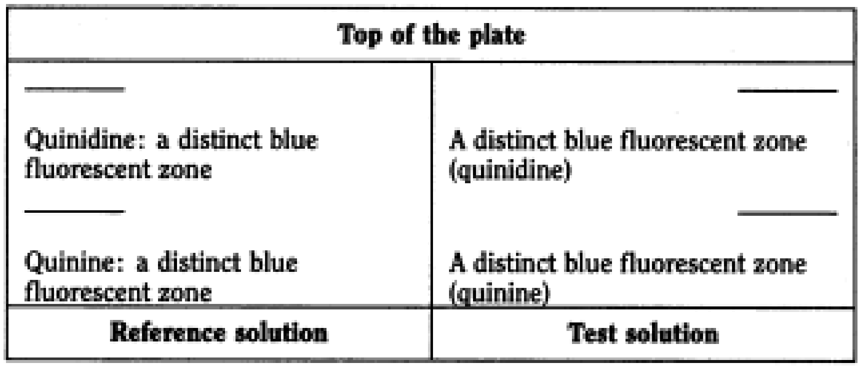
Application 10 µl [or 2 µl] as bands.

Development Twice over a path of 15 cm [or 6 cm].

Drying At 100-105 °C then allow to cool.

Detection A Spray with a 50 g/l solution of *anhydrous formic acid R* and allow to dry in air; examine in ultraviolet light at 365 nm.

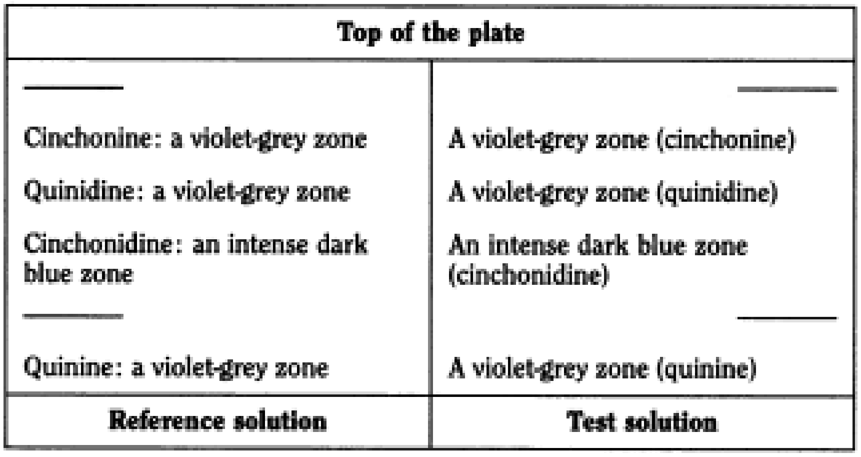
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Quinidine: a distinct blue fluorescent zone	A distinct blue fluorescent zone (quinidine)
———	———
Quinine: a distinct blue fluorescent zone	A distinct blue fluorescent zone (quinine)
Reference solution	**Test solution**

Detection B Spray with *iodoplatinate reagent R* .

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Cinchonine: a violet-grey zone	A violet-grey zone (cinchonine)
Quinidine: a violet-grey zone	A violet-grey zone (quinidine)
Cinchonidine: an intense dark blue zone	An intense dark blue zone (cinchonidine)
———	———
Quinine: a violet-grey zone	A violet-grey zone (quinine)
Reference solution	**Test solution**

TESTS

Ethanol *(2.9.10)*

95 per cent to 105 per cent of the content stated on the label.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

Dry residue ***(2.8.16)***

Minimum 12.0 per cent for glycerol-free standardised cinchona liquid extract and minimum 30.0 per cent for glycerol-containing standardised cinchona extract, determined on 2.0 g.

ASSAY

Test solution In a 250 ml conical flask, mix about 1.000 g of the extract to be examined with 10 ml of *water R* and 7 ml of *dilute hydrochloric acid R*. Heat in a water-bath for 30 min, allow to cool and add 25 ml of *methylene chloride R*, 50 ml of *ether R* and 5 ml of a 200 g/l solution of *sodium hydroxide R*. Shake the mixture frequently for 30 min, add 3 g of powdered *tragacanth R* and shake until the mixture becomes clear. Filter through a plug of absorbent cotton, rinse the flask and the cotton with 5 quantities, each of 20 ml, of a mixture of 1 volume of *methylene chloride R* and 2 volumes of *ether R*. Combine the filtrate and washings, evaporate to dryness and dissolve the residue in 10.0 ml of *ethanol (96 per cent) R*. Evaporate 5.0 ml of this solution to dryness, dissolve the residue in *0.1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

Reference solution (a) Dissolve 30.0 mg of *cinchonine R* in *0.1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

Reference solution (b) Dissolve 30.0 mg of *quinine R* in *0.1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

Measure the absorbances (*2.2.25*) of the 3 solutions at 316 nm and 348 nm, using *0.1 M hydrochloric acid* as the compensation liquid.

Calculate the percentage content of alkaloids from the following equations:

$$n_1 = \frac{[A_1 \times A_{2a}] - [A_{1a} \times A_2]}{[A_{1b} \times A_{2a}] - [A_{1a} \times A_{2b}]} \times \frac{100}{m} \times \frac{2}{1000}$$

$$n_2 = \frac{[A_1 \times A_{2b}] - [A_{1b} \times A_2]}{[A_{1a} \times A_{2b}] - [A_{1b} \times A_{2a}]} \times \frac{100}{m} \times \frac{2}{1000}$$

m = mass of the liquid extract to be examined in grams

n_1 = percentage content of quinine-type alkaloids

n_2 = percentage content of cinchonine-type alkaloids

A_1 = absorbance of the test solution at 316 nm

A_2 = absorbance of the test solution at 348 nm

A_{1a} = absorbance of reference solution (a) at 316 nm, corrected to a concentration of 1 mg/1000 ml

A_{1b} = absorbance of reference solution (b) at 316 nm, corrected to a concentration of 1 mg/1000 ml

A_{2a} = absorbance of reference solution (a) at 348 nm, corrected to a concentration of 1 mg/1000 ml

A_{2b} = absorbance of reference solution (b) at 348 nm, corrected to a concentration of 1 mg/1000 ml

Calculate the content of total alkaloids ($n_1 + n_2$), and the relative content of quinine-type alkaloids, from the following expression:

$$\frac{n_1 \times 100}{n_1 + n_2}$$

LABELLING

The label states the solvent composition used for the production.

Ph Eur

Cinnamon Tincture

General Notices

(Ph Eur monograph 1819)

Ph Eur

DEFINITION

Tincture produced from *Cinnamon (0387)*.

PRODUCTION

The tincture is produced from 1 part of the drug and 5 parts of ethanol (70 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance

Clear, brownish-red liquid, with a characteristic odour.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)* .

Test solution Place 10 ml of the tincture to be examined, 10 ml of *saturated sodium chloride solution R* and 5 ml of *toluene R* in a ground glass-stoppered tube. Shake for 2 min and centrifuge for 10 min. Use the organic layer.

Reference solution Dissolve 5 µl of *eugenol R* , 25 µl of *trans-cinnamic aldehyde R* and 5 µl of *trans-2-methoxycinnamaldehyde R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *methylene chloride R.*

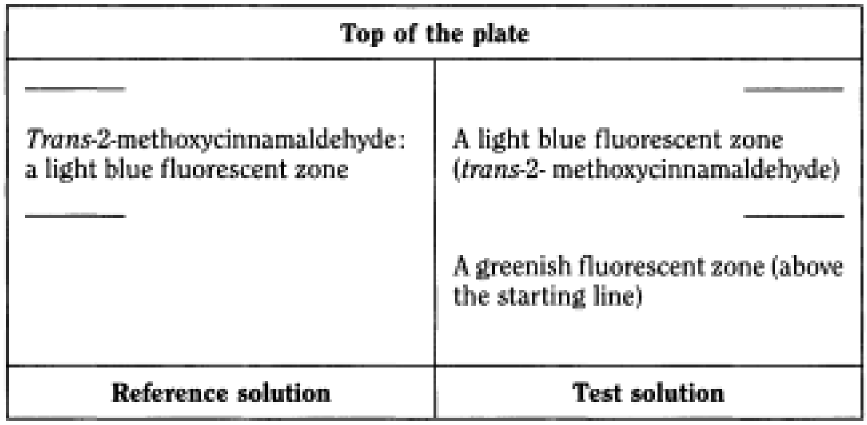
Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 365 nm.

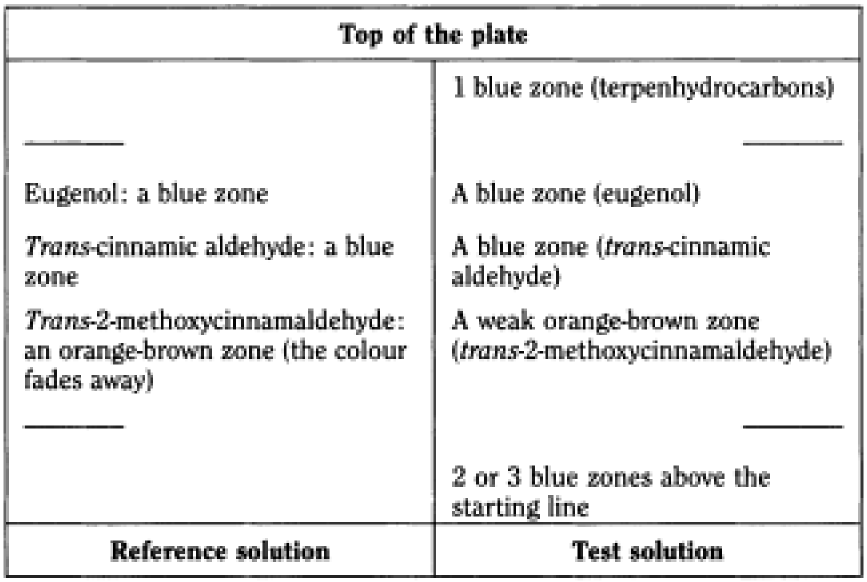
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
Trans-2-methoxycinnamaldehyde: a light blue fluorescent zone	A light blue fluorescent zone (*trans*-2- methoxycinnamaldehyde)
———	———
	A greenish fluorescent zone (above the starting line)
Reference solution	**Test solution**

Detection B Spray with a 200 g/l solution of *phosphomolybdic acid R* in *ethanol R* . Examine in daylight while heating at 100-105 °C for 5-10 min.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	1 blue zone (terpenhydrocarbons)
———	———
Eugenol: a blue zone	A blue zone (eugenol)
Trans-cinnamic aldehyde: a blue zone	A blue zone (*trans*-cinnamic aldehyde)
Trans-2-methoxycinnamaldehyde: an orange-brown zone (the colour fades away)	A weak orange-brown zone (*trans*-2-methoxycinnamaldehyde)
———	———
	2 or 3 blue zones above the starting line
Reference solution	**Test solution**

TESTS

Ethanol *(2.9.10)*

64 per cent *V/V* to 70 per cent *V/V*.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

Dry residue *(2.8.16)*

Minimum 1.5 per cent *m/m*, determined on 5.0 g.

Cinnamon

General Notices

Cinnamon Bark; Ceylon Cinnamon

(Ph Eur monograph 0387)

When Powdered Cinnamon is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and containing not less than 1.0% v/w (10 ml/kg) of essential oil shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried bark, freed from the outer cork and the underlying parenchyma, and shoots grown on cut stock of *Cinnamomum zeylanicum* Nees.

Content

Minimum 12 ml/kg of essential oil.

CHARACTERS

Characteristic, aromatic odour.

IDENTIFICATION

A. The bark is about 0.2-0.8 mm thick and occurs in closely-packed compound quills made up of single or double quills. The outer surface is smooth, yellowish-brown with faint scars marking the position of leaves and axillary buds and has fine, whitish and wavy longitudinal striations. The inner surface is slightly darker and longitudinally striated. The fracture is short and fibrous.

B Reduce to a powder (355) *(2.9.12)*. The powder is yellowish or reddish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: groups of rounded sclereids with pitted, channelled and moderately thickened walls; numerous colourless single fibres, often whole with narrow lumen and thickened, lignified walls and few pits; small acicular crystals of calcium oxalate. Cork fragments are absent or very rare. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R* ; the powder shows abundant starch granules.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Shake 0.1 g of powdered drug (500) (*2.9.12*) with 2 ml of *methylene chloride R* for 15 min. Filter and evaporate the filtrate carefully almost to dryness on a water-bath.

Dissolve the residue in 0.4 ml of *toluene R*.

Reference solution Dissolve 50 µl of *cinnamic aldehyde R* and 10 µl *of eugenol R* in *toluene R*, then dilute to 10 ml with the same solvent.

Plate *TLC silica gel GF $_{254}$ plate R*.

Mobile phase *methylene chloride R*.

Application 10 µl as bands of 20 mm by 3 mm.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm and mark the quenching zones, then, examine in ultraviolet light at 365 nm and mark the fluorescent zones.

Results A In ultraviolet light at 254 nm, the chromatograms obtained with the test solution and the reference solution show a quenching zone due to cinnamaldehyde in the median part and just above it a weaker quenching zone due to eugenol. In ultraviolet light at 365 nm, the chromatogram obtained with the test solution shows a zone of light blue fluorescence due to *o*-methoxy-cinnamaldehyde just below the zone due to cinnamaldehyde.

Detection B Spray with *phloroglucinol solution R*.

Results B The zone due to cinnamaldehyde is yellowish-brown and the zone due to *o*-methoxycinn-amaldehyde is violet.

TESTS

Total ash *(2.4.16)*

Maximum 6.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use a 500 ml flask, 200 ml of *0.1 M hydrochloric acid* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Powder the drug (710) *(2.9.12)* and proceed immediately with the determination, using 20.0 g. Distil at a rate of 2.5-3.5 ml/min for 3 h.

Ph Eur

Citronella Oil

General Notices

(Ph Eur monograph 1609)

Ph Eur

DEFINITION

Oil obtained by steam distillation from the fresh or partially dried aerial parts of *Cymbopogon winterianus* Jowitt.

CHARACTERS

Pale yellow to brown-yellow liquid, with a very strong odour of citronellal.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dilute 0.1 g of citronella oil in 10.0 ml of *alcohol R* .

Reference solution Dilute 20 µl of *citronellal R* in 10.0 ml of *alcohol R* .

Plate *TLC silica gel plate R* .

Mobile phase *ethyl acetate R, toluene R* (10:90 *V/V*).

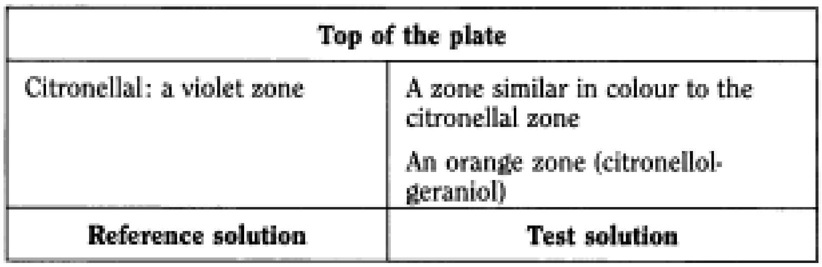
Application 5 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min. Examine in ultraviolet light at 365 nm.

Result See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
Citronellal: a violet zone	A zone similar in colour to the citronellal zone An orange zone (citronellol-geraniol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution. Neral and geranial may be absent in the chromatogram obtained with the test solution.

TESTS

Relative density *(2.2.5)*

0.881 to 0.895.

Refractive index *(2.2.6)*

1.463 to 1.475.

Optical rotation *(2.2.7)*

- 4° to + 1.5°.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution The substance to be examined.

Reference solution Dilute 25 µl of *limonene R*, 100 µl of *citronellal R*, 25 µl of *citronellyl acetate R*, 25 µl of *citral R*, 25 µl of *geranyl acetate R*, 25 µl of *citronellol R* and 100 µl of *geraniol R* in 5 ml of *hexane R.*

Column:

— *material*: fused silica,

— *size*: *l* = 60 m, Ø = 0.25 mm,

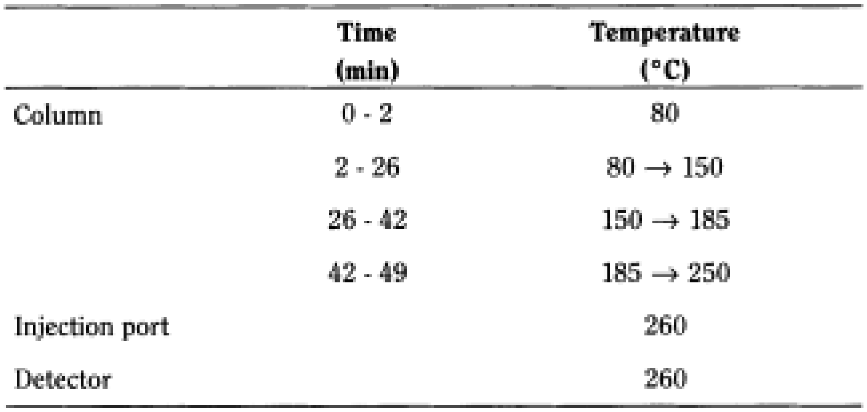
— *stationary phase: macrogol 20 000 R* (0.2 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.0 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	80
	2 - 26	80 → 150
	26 - 42	150 → 185
	42 - 49	185 → 250
Injection port		260
Detector		260

Detection Flame ionisation.

Injection 1 µl of the reference solution, 0.2 µl of the test solution.

Elution order The order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum of 1.2 between the peaks due to geranyl acetate and citronellol.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of each of these components.

The percentages are within the following values:

— *limonene* : 1.0 per cent to 5.0 per cent,

— *citronellal*: 30.0 per cent to 45.0 per cent,

— *citronellyl acetate*: 2.0 per cent to 4.0 per cent,

— *neral:* less than 2.0 per cent,

— *geranial*: less than 2.0 per cent,

— *geranyl acetate* : 3.0 per cent to 8.0 per cent,

— *citronellol*: 9.0 per cent to 15.0 per cent,

— *geraniol* : 20.0 per cent to 25.0 per cent.

STORAGE

In a well-filled container, protected from light.

Ph Eur

Clove Oil

General Notices

(Ph Eur monograph 1091)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the dried flower buds of *Syzygium aromaticum* (L.) Merill et L. M. Perry (*Eugenia caryophyllus* (C. Spreng.) Bull. et Harr.).

CHARACTERS

Appearance

Clear, yellow liquid, which becomes brown when exposed to air.

Solubility

Miscible with methylene chloride, with toluene and with fatty oils.

IDENTIFICATION

First identification *B,*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 20 µl of the substance to be examined in 2.0 ml of *toluene R.*

Reference solution Dissolve 15 µl of *eugenol R* and 15 µl of *acetyleugenol R* in 2.0 ml of *toluene R.*

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *toluene R.*

Application 20 µl of the test solution and 15 µl of the reference solution, as bands.

Development Twice in an unsaturated tank over a path of 10 cm; allow to stand for 5 min between the 2 developments.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm and mark the quenching zones.

Results A The chromatogram obtained with the test solution shows in the middle part a quenching zone (eugenol) that is similar in position to the quenching zone in the chromatogram obtained with the reference solution; just below, there is a weak quenching zone (acetyleugenol) that is similar in position to the zone of acetyleugenol in the chromatogram obtained with the reference solution.

Detection B Spray with *anisaldehyde solution R* and examine in daylight while heating at 100-105 °C for 5-10 min.

Results B The zone due to eugenol in the chromatograms obtained with the test and reference solutions is strong brownish-violet and the zone due to acetyleugenol in the chromatogram obtained with the test solution is faint violet-blue; in the chromatogram obtained with the test solution there are other coloured zones, particularly a faint red zone in the lower part and a reddish-violet zone (β-caryophyllene) in the upper part.

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The 3 principal peaks in chromatogram obtained with the test solution are similar in retention time to the 3 principal peaks in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

1.030 to 1.063.

Refractive index *(2.2.6)*

1.528 to 1.537.

Optical rotation *(2.2.7)*

0° to - 2°.

Fatty oils and resinified essential oils *(2.8.7)*

It complies with the test.

Solubility in alcohol *(2.8.10)*

1.0 ml is soluble in 2.0 ml and more of *ethanol (70 per cent V/V) R*.

Chromatographic profile.

Gas chromatography *(2.2.28)*. Use the normalisation procedure.

Test solution Dissolve 0.2 g of the substance to be examined in 10 g of *hexane R*.

Reference solution Dissolve 7 mg of β-*caryophyllene R*, 80 mg of *eugenol* R and 4 mg of *acetyleugenol R* in 10 g of *hexane R*.

Column:

— *material*: fused silica;

— *size*: *l* = 60 m, Ø = about 0.25 mm;

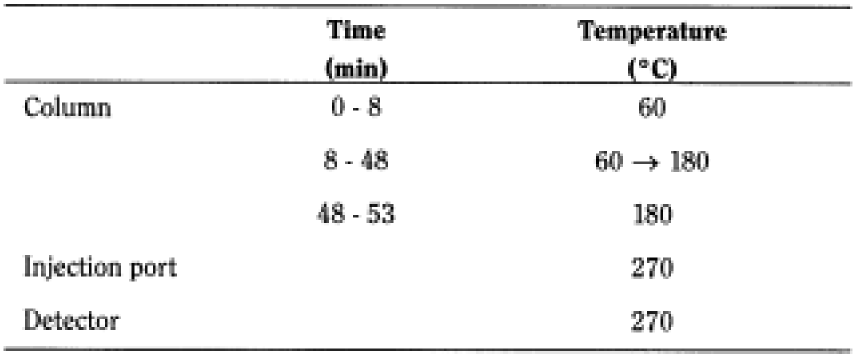
— *stationary phase*: *macrogol 20 000 R*.

Carrier gas *helium for chromatography R.*

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 8	60
	8 - 48	60 → 180
	48 - 53	180
Injection port		270
Detector		270

Detection Flame ionisation.

Injection 1.0 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to eugenol and acetyleugenol;

— *number of theoretical plates* : minimum 30 000, calculated for the peak due to β-caryophyllene at 110 °C.

Identification of components Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution on the chromatogram obtained with the test solution.

Determine the percentage content of each of these components. The limits are within the following ranges:

— β-*caryophyllene* : 5.0 per cent to 14.0 per cent;

— *eugenol* : 75.0 per cent to 88.0 per cent;

— *acetyleugenol* : 4.0 per cent to 15.0 per cent.

STORAGE

Protected from heat.

Ph Eur

Clove

General Notices

(Ph Eur monograph 0376)

When Powdered Clove is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter and containing not less than 12.0% v/w (120 ml/kg) of essential oil shall be dispensed or supplied.

Ph Eur

DEFINITION

Whole flower buds of *Syzygium aromaticum* (L.) Merill et L. M. Perry (*Eugenia caryophyllus* (C. Spreng.) Bull. et Harr.) dried until they become reddish-brown.

Content

Minimum 150 ml/kg of essential oil.

CHARACTERS

Characteristic, aromatic odour.

IDENTIFICATION

A. The flower bud is reddish-brown and consists of a quadrangular stalked portion, the hypanthium, 10-12 mm long and 2-3 mm in diameter, surmounted by 4 divergent lobes of sepals which surround a globular head 4-6 mm in diameter. A bilocular ovary containing numerous ovules is situated in the upper part of the hypanthium. The head is globular and dome-shaped, composed of 4 imbricated petals that enclose numerous incurved stamens and a short, erect style with a nectary disc at the base. The hypanthium exudes essential oil when indented with the finger-nail.

B. Reduce to a powder (355) *(2.9.12)*. The powder is dark brown and has the odour and taste of the unground drug. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the hypanthium showing the epidermis and underlying parenchyma containing large oil glands; short fibres occurring singly or in small groups, with thickened, lignified walls and few pits; abundant fragments of parenchyma containing cluster crystals of calcium oxalate; numerous triangular pollen grains about 15 µm in diameter with 3 pores in the angles. Starch granules are absent.

C. Thin-layer chromatography *(2.2.27).*

Test solution Shake 0.1 g of the powdered drug (500) *(2.9.12)* with 2 ml of *methylene* *chloride R* for 15 min. Filter and carefully evaporate the filtrate to dryness on a water-bath. Dissolve the residue in 2 ml of *toluene R* .

Reference solution Dissolve 20 µl of *eugenol R* in 2 ml of *toluene R* .

Plate *TLC silica gel GF $_{254}$ plate R.*

Mobile phase *toluene R.*

Application 10 µl of the reference solution and 20 µl of the test solution, as bands of 20 mm by 3 mm.

Development Twice, in an unsaturated tank over a path of 10 cm; allow the plate to stand for 5 min between the 2 developments.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm and mark the quenching zones.

Results A In the chromatogram obtained with the test solution there is in the median part a quenching zone due to eugenol similar in position to the quenching zone in the chromatogram obtained with the reference solution and there may be a weak quenching zone due to acetyleugenol just below the zone due to eugenol.

Detection B Spray with *anisaldehyde solution R* using 10 ml for a plate 200 mm square and heat at 100-105 °C for 5-10 min. Examine in daylight.

Results B The zones due to eugenol in the chromatograms obtained with the test and reference solutions are strong brownish-violet and the zone due to acetyleugenol in the chromatogram obtained with the test solution is faint violet-blue. In the chromatogram obtained with the test solution there are other coloured zones, particularly a faint red zone in the lower part and a reddish-violet zone due to caryophyllene in the upper part.

TESTS

Foreign matter *(2.8.2)*

Maximum 6 per cent of peduncles, petioles and fruits, maximum 2 per cent of deteriorated cloves and maximum 0.5 per cent of other foreign matter.

Total ash *(2.4.16)*

Maximum 7.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)* . Use a 250 ml flask, 100 ml of *water R* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Grind 5.0 g of the drug with 5.0 g of *diatomaceous earth R* to form a fine, homogeneous powder and proceed immediately with the determination using 4.0 g of the mixture. Distil at a rate of 2.5-3.5 ml/min for 2 h.

Ph Eur

Cochineal

General Notices

DEFINITION

Cochineal is the dried female insect, *Dactylopius coccus* Costa, containing eggs and larvae.

CHARACTERISTICS

Odour, characteristic.

Macroscopical Purplish black or purplish grey; about 3.5 to 5.5 mm long and 3 to 4.5 mm wide, plano-convex and somewhat oval in outline; the convex dorsal surface is transversely wrinkled and shows about 11 segments; the flat or slightly concave ventral surface carries upon the anterior part two seven-jointed straight antennae, three pairs of short legs, each terminating in a single claw, and a mouth from which projects the remains of a long filiform proboscis; these appendages are frequently more or less broken. Easily reduced to powder, which is dark red or puce.

Microscopical Scattered irregularly over the whole dermis are numerous solitary and grouped, short, tubular wax glands; within each insect are found numerous larvae, which are characterised by their proboscides appearing as two circular coils.

TESTS

Colour value

To 0.5 g in *moderately fine powder* add 60 ml of *phosphate buffer pH 8.0* and heat on a water bath for 30 minutes. Cool, add sufficient *phosphate buffer pH 8.0* to produce 100 ml and filter. Dilute 5 ml of the filtrate to 100 ml with *phosphate buffer pH 8.0*. The *absorbance* of the resulting solution at the maximum at 530 nm is not less than 0.25, Appendix II B.

Foreign matter

Complies with the test for *foreign matter*, Appendix XI D.

Water-insoluble matter

When the insects are placed in *water*, no insoluble powder separates.

Ash

Not more than 7.0%, Appendix XI J.

Microbial contamination

1 g is free from *Escherichia coli*; 10 g is free from Salmonella, Appendix XVI B1.

Cola

General Notices

(Ph Eur monograph 1504)

Ph Eur

DEFINITION

Whole or fragmented dried seeds, freed from the testa, of *Cola nitida* (Vent.) Schott et Endl. (*C. vera* K. Schum.) and its varieties, as well as of *Cola acuminata* (P. Beauv.) Schott et Endl. (*Sterculia acuminata* P. Beauv.).

Content

Minimum 1.5 per cent of caffeine (M_r 194.2) (dried drug).

IDENTIFICATION

A. The kernels have an oblong, somewhat obtuse, sub-tetragonal shape, with deformations resulting from mutual pressure inside the fruit; they vary in size and mass, ranging from 5-15 g; the outside is hard, smooth and very dark brown, the inside is more reddish-brown. In C. *nitida* and its varieties, the kernels are divided in 2 parts, almost plano-convex, corresponding to the cotyledons and usually occurring separated in the commercial drug; the cotyledons are 3-4 cm long, 2-2.5 cm wide and 1-2 cm thick. In C. *acuminata*, the cotyledons are smaller and divided into 4-6 irregular parts.

B. Reduce to a powder (355) *(2.9.12)*. The powder is reddish-brown. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows the following diagnostic characters: numerous ovoid or reniform starch granules, 5-25 µm in size, with concentric striations and a stellate, slightly eccentric hilum; fragments of cotyledon tissue showing large, thick-walled, reddish polygonal cells filled with starch granules; occasional fragments of the external epidermis of the cotyledons.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 5 ml of *ethanol (60 per cent V/V) R*. Shake mechanically at 40 °C for 30 min and filter.

Reference solution (a) Dissolve 25 mg of *caffeine R* in 10 ml of *ethanol (60 per cent V/V) R*.

Reference solution (b) Dissolve 50 mg of *theobromine R* in 10 ml of the mobile phase. Filter.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *water R, methanol R, ethyl acetate R* (10:13:77 *V/V/V*).

Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying In air for 5 min.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatogram obtained with the test solution shows 2 principal quenching zones which are similar in position to the zones in the chromatograms obtained with reference solutions (a) and (b).

Detection B Spray with a mixture of equal volumes of *ethanol (96 per cent) R* and *hydrochloric acid R* and then with a solution prepared immediately before use by dissolving 1 g of *iodine R* and 1 g of *potassium iodide R* in 100 ml of *ethanol (96 per cent) R*.

Results B The chromatogram obtained with the test solution shows a reddish-brown principal zone similar in position and colour to the zone in the chromatogram obtained with reference solution (a).

TESTS

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 2.00 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 9.0 per cent.

ASSAY

Liquid chromatography (*2.2.29)* .

Test solution To 1.00 g (m1) of the powdered drug (355) *(2.9.12)*, add 50 ml of *methanol R*. Heat under a reflux condenser on a water-bath for 30 min. Allow to cool and filter. Rinse the filter with 10 ml of *methanol R*. Take up the residue with 50 ml of *methanol R*. Proceed as before. Combine the filtrates and the washings in a 200.0 ml volumetric flask and dilute to 200.0 ml with *methanol R*. Transfer 20.0 ml of this solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with the mobile phase, transfer to a 50.0 ml volumetric flask and dilute to 50.0 ml with the mobile phase.

Reference solution In a 100.0 ml volumetric flask, dissolve 30.0 mg (m_2) of *caffeine CRS* and 15.0 mg of *theobromine R* in the mobile phase and dilute to 100.0 ml with the mobile phase. Transfer 10.0 ml of this solution to a 100.0 ml volumetric flask and dilute to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, water R* (25:75 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 272 nm.

Injection The chosen volume of each solution; loop injector.

System suitability Reference solution:

— *resolution*: minimum 2.5 between the peaks due to caffeine and theobromine. If necessary, adjust the volume of *water R* in the mobile phase.

Calculate the caffeine content using the following expression:

$$\frac{m_2 \times A_1 \times 50}{m_1 \times A_2}$$

A_1 = area of the peak due to caffeine in the chromatogram obtained with the test solution,

A_2 = area of the peak due to caffeine in the chromatogram obtained with the reference solution,

m_1 = mass of the drug to be examined in the test solution, in grams,

m_2 = mass of *caffeine CRS* in the reference solution, in grams.

Ph Eur

Colophony

General Notices

(Ph Eur monograph 1862)

Ph Eur

DEFINITION

Residue remaining after distillation of the volatile oil from the oleoresin obtained from various species of *Pinus*.

IDENTIFICATION

A. Translucent, pale yellow to brownish-yellow, angular, irregularly-shaped, brittle, glassy pieces of different sizes the surfaces of which bear conchoidal markings.

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 1 g in 10 ml of *methanol R* by gently warming.

Reference solution Dissolve 10 mg of *thymol R* and 10 mg of *linalol R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R* .

Mobile phase *methylene chloride* R.

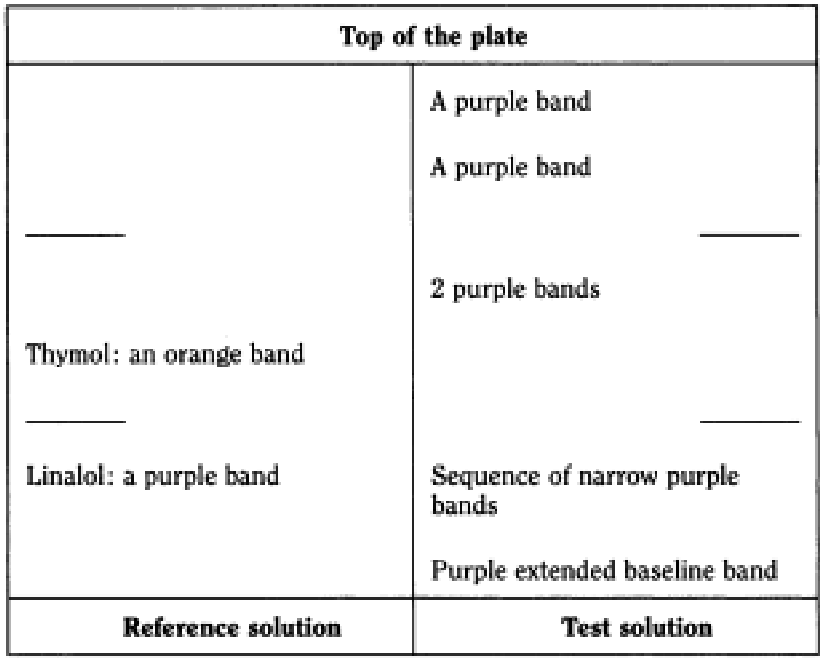
Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other coloured zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	A purple band
	A purple band
———	———
	2 purple bands
Thymol: an orange band	
———	———
Linalol: a purple band	Sequence of narrow purple bands
	Purple extended baseline band
Reference solution	**Test solution**

TESTS

Acid value *(2.5.1)*

145 to 180, determined on 1.0 g.

Total ash *(2.4.16)*

Maximum 0.2 per cent.

STORAGE

Do not reduce to a powder.

Ph Eur

Compound Gentian Infusion

General Notices

DEFINITION

Concentrated Compound Gentian Infusion	100 ml
Water	Sufficient to produce 1000 ml

The infusion complies with the requirements stated under Infusions.

CONCENTRATED COMPOUND GENTIAN INFUSION

DEFINITION

Gentian, cut small and bruised	125 g
Dried Bitter-orange Peel, cut small	125 g
Dried Lemon Peel, cut small	125 g
Ethanol (25 per cent)	1200 ml

Extemporaneous preparation

The following directions apply.

Macerate the Gentian, the Dried Bitter-orange Peel and the Dried Lemon Peel in a covered vessel for 48 hours with 1000 ml of the Ethanol (25 per cent); express the liquid. To the pressed marc add 200 ml of the Ethanol (25 per cent), macerate for 24 hours, press and add the liquid to the product of the first pressing. Allow to stand for not less than 14 days; filter.

TESTS

Ethanol content

20 to 24% v/v, Appendix VIII F.

Total solids

Not less than 9.5% w/v, Appendix XI A.

Coriander Oil

General Notices

(Ph Eur monograph 1820)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the fruits of *Coriandrum sativum* L.

CHARACTERS

Appearance

Clear, colourless or pale yellow liquid.

It has a characteristic spicy odour.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Examine by thin-layer chromatography (2.2.27).

Test solution Dissolve 10 µl of the substance to be examined in 1.0 ml of *toluene R* .

Reference solution Dissolve 10 µl *linalol R* and 2 µl of *geranyl acetate R* in 1.0 ml of *toluene R.*

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

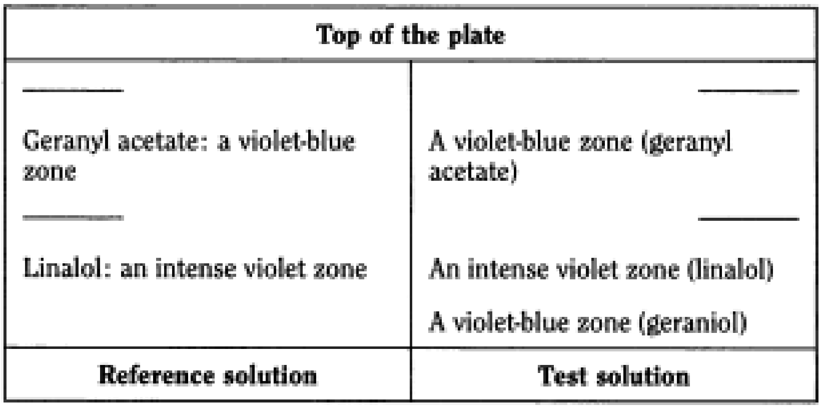
Application 10 µl as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 10-15 min. Examine immediately in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
Geranyl acetate: a violet-blue zone	A violet-blue zone (geranyl acetate)
———	———
Linalol: an intense violet zone	An intense violet zone (linalol)
	A violet-blue zone (geraniol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

0.860 to 0.880.

Refractive index *(2.2.6)*

1.462 to 1.470.

Optical rotation *(2.2.7)*

+ 7° to + 13°.

Acid value *(2.5.1)*

Maximum 3.0, determined on 5.00 g of the substance to be examined.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution The substance to be examined.

Reference solution (a) Dissolve 10 µl of *α-pinene R* , 10 µl of *limonene R* , 10 µl of *γ-terpinene R* , 10 µl of *p-cymene R* , 10 mg of *camphor* R, 20 µl of *linalol R,* 10 µl of *α-terpineol R* , 10 µl of *geranyl acetate R* and 10 µl of *geraniol R* in 1 ml of *hexane R* .

Reference solution (b) Dissolve 5 µl of *geraniol R* in *hexane R* and dilute to 10 ml with the same solvent.

Column:

— *material:* fused silica;

— *size*: *l* = 60 m, Ø = 0.25 mm;

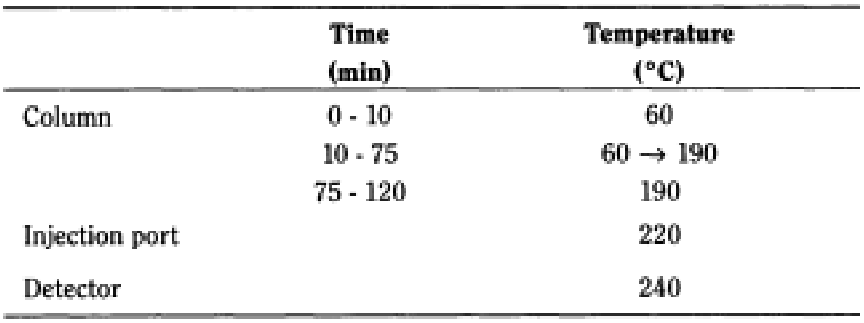
— *stationary phase: macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1 ml/min.

Split ratio 1:65.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 75	60 → 190
	75 - 120	190
Injection port		220
Detector		240

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of reference solution (a). Record the retention times of these substances.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to linalol and camphor.

Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution.

Determine the percentage content of each of these components. The percentages are within the following ranges:

— *α-pinene* : 3.0 per cent to 7.0 per cent;

— *limonene* : 1.5 per cent to 5.0 per cent;

— *γ-terpinene* : 1.5 per cent to 8.0 per cent;

— *p-cymene* : 0.5 per cent to 4.0 per cent;

— *camphor* : 3.0 per cent to 6.0 per cent;

— *linalol* : 65.0 per cent to 78.0 per cent;

— *α-terpineol* : 0.1 per cent to 1.5 per cent;

— *geranyl acetate* : 0.5 per cent to 4.0 per cent;

— *geraniol* : 0.5 per cent to 3.0 per cent;

— *disregard limit*: area of the peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chiral purity

Gas chromatography *(2.2.28)* .

Test solution Dissolve 0.02 g of the substance to be examined in *pentane R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 µl of *linalol R* and 5 mg of *borneol R* in *pentane R* and dilute to 10 ml with the same solvent.

Column:

— *material*: fused silica;

— *size*: *l* = 25 m, Ø = 0.25 mm;

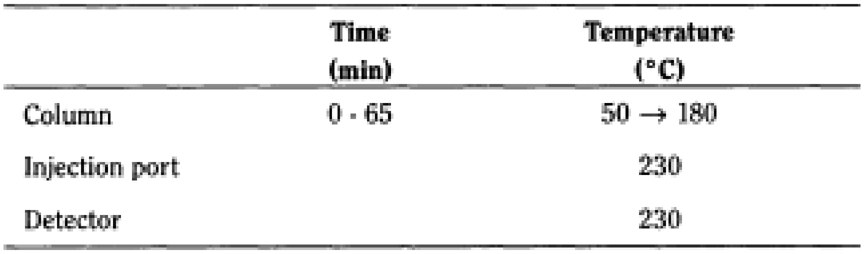
— *stationary phase*: *modified* β*-cyclodextrin* for *chiral chromatography R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* .

Flow rate 1.3 ml/min.

Split ratio 1:30.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 65	50 → 180
Injection port		230
Detector		230

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution:

— *resolution*: minimum 5.5 between the peaks due to (*R*)-linalol (1st peak) and (*S*)-linalol (2nd peak) and minimum 2.9 between the peaks due to (*S*)-linalol and borneol (3rd peak).

Limit Calculate the percentage content of (*R*)-linalol from the expression:

$$\frac{A_R}{A_S + A_R} \times 100$$

A_S = area of the peak due to (*S*)-linalol,

A_R = area of the peak due to (*R*)-linalol.

— (*R*)- *linalol* : maximum 14 per cent.

STORAGE

In a well-filled, airtight container , protected from light and at a temperature not exceeding 25 °C.

Coriander

General Notices

(Ph Eur monograph 1304)

When Powdered Coriander is prescribed or demanded, material complying with the appropriate requirements below but containing not less than 0.2% v/w of essential oil shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried cremocarp of *Coriandrum sativum* L.

Content

Minimum 3 ml/kg of essential oil (dried drug).

CHARACTERS

The cremocarp is brown or light brown and is more or less spherical, about 1.5-5 mm in diameter, or oval form 2-6 mm long.

IDENTIFICATION

A. The mericarps are usually tightly connected. The cremocarp is glabrous and has 10 wavy, slightly raised primary ridges and 8 straight, more prominent secondary ridges. The stylopod crowns the apex. The mericarps are concave on the internal surface. A small fragment of the pedicel may be present.

B. Reduce to a powder (355) *(2.9.12)*. The powder is brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous oil droplets; fragments of endosperm with small thick-walled regular cells containing microcrystals and microrosettes of calcium oxalate and oil droplets; fragments of endocarp with very narrow cells having a parquetry arrangement and usually associated with a layer of thin-walled rectangular sclereids of the mesocarp; fragments from the sclerenchymatous layer of the mesocarp with short, strongly thickened, pitted, fusiform cells occurring in layers with the cells of adjacent layers approximately at right angles to one another; fragments of parenchyma with small, thick-walled cells; occasional fragments of vascular bundles.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Shake 0.50 g of the freshly powdered drug (355) *(2.9.12)* with 5.0 ml of *hexane R* for 2-3 min and filter over 2 g of *anhydrous sodium sulphate R* .

Reference solution Dissolve 15 µl of *linalol R* and 25 µl of *olive oil R* in 5.0 ml of *hexane R* immediately before use.

Plate *TLC silica gel plate R.*

Mobile phase e*thyl acetate R, toluene R* (5:95 *V/V*).

Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Twice over a path of 10 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and examine in daylight while heating at 100-105 °C for 5-10 min.

Results The chromatogram obtained with the reference solution shows in the lower half a violet or greyish-violet zone (linalol) and in the upper half a bluish-violet zone (triglycerides). The chromatogram obtained with the test solution shows zones similar in position and colour to the zones in the chromatogram obtained with the reference solution. Several violet-grey or brownish zones, including the zone due to geraniol, are between the starting point and the zone due to linalol in the chromatogram obtained with the reference solution. It may also show several faint violet-grey zones between the zone due to triglycerides and that due to linalol in the chromatogram obtained with the reference solution.

TESTS

Foreign matter *(2.8.2)*

It complies with the test. None of the cremocarps show perforations due to animals.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 8.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)* . Use a 500 ml round-bottomed flask, 200 ml of *water R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Reduce the drug to a coarse powder and immediately use 30.0 g for the determination. Distil at a rate of 2-3 ml/min for 2 h.

Ph Eur

Couch Grass Rhizome

General Notices

(Ph Eur monograph 1306)

Ph Eur

DEFINITION

Whole or cut, washed and dried rhizome of *Agropyron repens* (L.) Beauv. (*Elymus repens* (L.) Gould); the adventitious roots are removed.

IDENTIFICATION

A. The shiny yellowish, light brown or yellowish-brown pieces of the rhizome are 2 mm to 3 mm thick and longitudinally furrowed. At the nodes are the remains of very thin, more or less branched roots and whitish or brownish scale-like leaves; the internodes, up to 6 cm long, are furrowed and hollow inside. The transverse section of the nodes shows a yellowish medulla.

B. Reduce to a powder (355) *(2.9.12)*. The powder is whitish-yellow. Examine under a microscope, using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the epidermis covered with a thick cuticle and composed of rectangular and elongated thick-walled cells with pitted slightly wavy walls which alternate with small rounded to almost square cells; U-shaped thickened endodermic cells; numerous fragments of moderately thickened fibres and groups of vessels with slit-shaped pits or with spiral and annular thickening.

TESTS

Cynodon dactylon, Imperata cylindrical

Examine under a microscope using *iodine solution R1* . No blue starch grains are visible.

Foreign matter *(2.8.2)*

Maximum 15 per cent of greyish-black pieces of rhizome in the cut drug.

Water-soluble extractive

Minimum 25 per cent.

To 5.0 g of the powdered drug (355) (*2.9.12*) add 200 ml of boiling *water R* . Allow to stand for 10 min, shaking occasionally. Allow to cool, dilute to 200.0 ml with *water R* and filter. Evaporate 20.0 ml of the filtrate to dryness on a water-bath. Dry the residue in an oven at 100-105 °C. The residue weighs a minimum of 0.125 g.

Loss on drying ***(2.2.32)***

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 5.0 per cent.

Ash insoluble in hydrochloric acid ***(2.8.1)***

Maximum 1.5 per cent.

Ph Eur

Dementholised Mint Oil

General Notices

(Partly Dementholised Mint Oil, Ph Eur monograph 1838)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the fresh, flowering aerial parts, recently gathered from *Mentha canadensis* L. (syn. *M. arvensis* L. var. *glabrata* (Benth) Fern., *M. arvensis* var. *piperascens* Malinv. ex Holmes), followed by partial separation of menthol by crystallisation.

CHARACTERS

Appearance

Colourless or pale yellow to greenish-yellow liquid.

It has a characteristic odour.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.1 ml of the substance to be examined in 1.0 ml of *toluene R*.

Reference solution Dissolve 4 µl of *carvone R*, 4 µl of *pulegone R*, 10 µl of *menthyl acetate R*, 20 µl of *cineole R and* 50 mg of *menthol R* in 5 ml of *toluene R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

Application 10 µl, as bands.

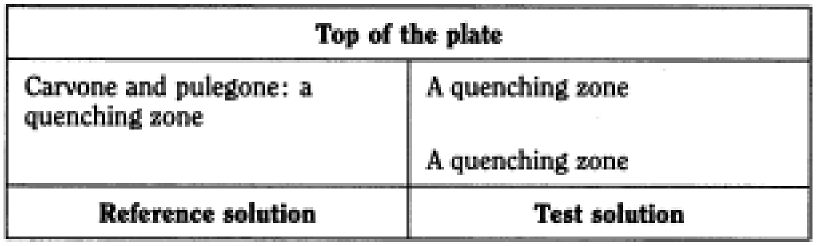
Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A

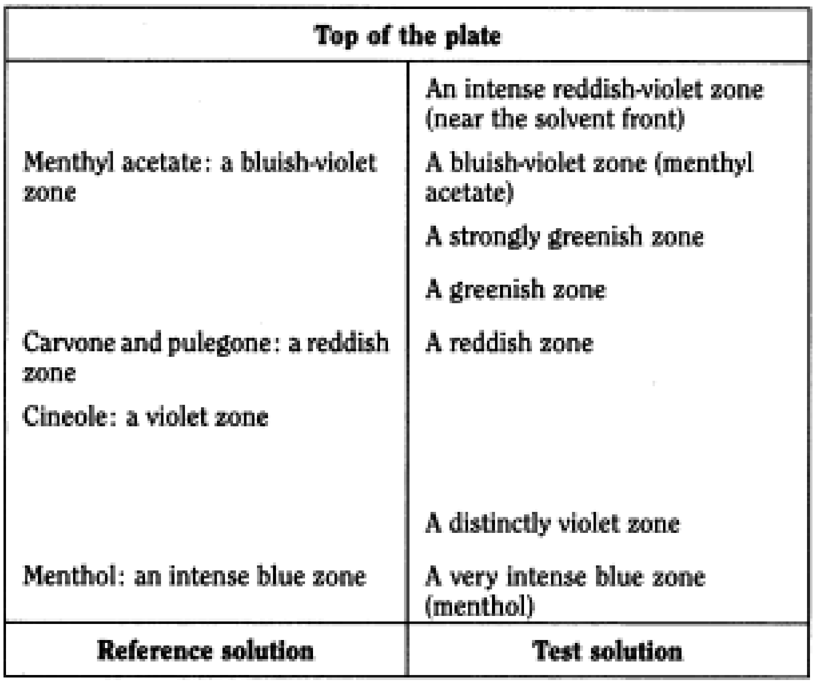
See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, a quenching zone may be present in the upper third of the chromatogram obtained with the test solution.



Top of the plate	
Carvone and pulegone: a quenching zone	A quenching zone
	A quenching zone
Reference solution	**Test solution**

Detection B Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine immediately in daylight.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, the zone due to cineole in the reference solution is absent in the chromatogram obtained with the test solution. No yellowish-brown zone below the intense reddish-violet zone is present in the chromatogram obtained with the test solution.



Top of the plate	
	An intense reddish-violet zone (near the solvent front)
Menthyl acetate: a bluish-violet zone	A bluish-violet zone (menthyl acetate)
	A strongly greenish zone
	A greenish zone
Carvone and pulegone: a reddish zone	A reddish zone
Cineole: a violet zone	
	A distinctly violet zone
Menthol: an intense blue zone	A very intense blue zone (menthol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are approximately similar in retention time to those in the chromatogram obtained with the reference solution. Carvone may be absent from the chromatogram obtained with the test solution.

TESTS

Relative density ***(2.2.5)***

0.888 to 0.910.

Refractive index ***(2.2.6)***

1.456 to 1.470.

Optical rotation ***(2.2.7)***

- 16.0° to - 34.0°.

Acid value ***(2.5.1)***

Maximum 1.0, determined on 5.00 g of the substance to be examined dissolved in 50 ml of the prescribed mixture of solvents.

Chromatographic profile

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 0.20 g of the substance to be examined in *hexane R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 10 mg of *limonene R*, 20 mg of *cineole R*, 40 mg of *menthone R*, 10 mg of *isomenthone R*, 40 mg of *menthyl acetate R*, 20 mg of *isopulegol R*, 60 mg of *menthol R*, 20 mg of *pulegone R* and 10 mg of *carvone R* in *hexane R* and dilute to 10.0 ml with the same solvent.

Column:

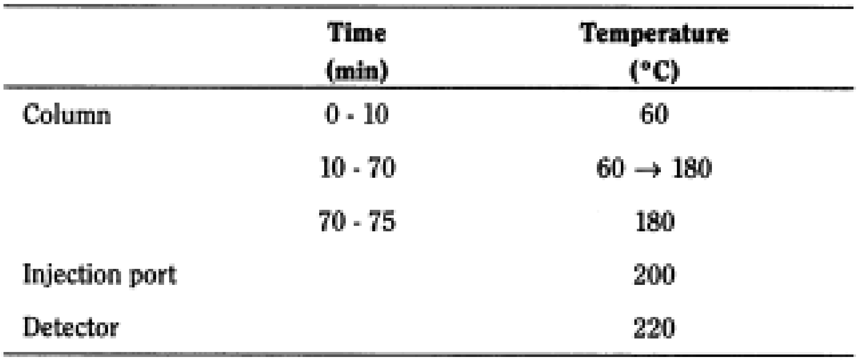
- *material*: fused silica,
- *size*: *l* = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.25-0.53 mm,
- *stationary phase*: *macrogol 20 000 R*.

Carrier gas *helium for chromatography R.*

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 70	60 → 180
	70 - 75	180
Injection port		200
Detector		220

Detection Flame ionisation.

Injection 1.0 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to limonene and cineole.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of these components. The percentages are within the following ranges:

— *limonene*: 1.5 per cent to 7.0 per cent,

— *cineole*: maximum 1.5 per cent,

— *menthone*: 17.0 per cent to 35.0 per cent,

— *isomenthone*: 5.0 per cent to 13.0 per cent,

— *menthyl acetate*: 1.5 per cent to 7.0 per cent,

— *isopulegol*: 1.0 per cent to 3.0 per cent,

— *menthol*: 30.0 per cent to 50.0 per cent,

— *pulegone*: maximum 2.5 per cent,

— *carvone*: maximum 2.0 per cent.

The ratio of cineole content to limonene content is less than 1.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Devil's Claw Dry Extract

General Notices

(Ph Eur monograph 1871)

Ph Eur

DEFINITION

Dry extract obtained from *Devil's claw root (1095)*.

Content

Minimum 1.5 per cent of harpagoside ($C_{24}H_{30}O_{11}$; M_r 494.5) (dried extract).

PRODUCTION

The extract is produced from the herbal drug by an appropriate procedure using either water or a hydroalcoholic solvent that is at most equivalent in strength to ethanol (95 per cent *V/V*).

CHARACTERS

Appearance

Light brown powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the extract to be examined add 10 ml of *methanol R* and heat in a water-bath at 60 °C for 10 min. Cool and filter.

Reference solution Dissolve 1.0 mg of *harpagoside R* and 2.5 mg of *fructose R* in 1.0 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *water R, methanol R, ethyl acetate R* (8:15:77 *V/V/V*).

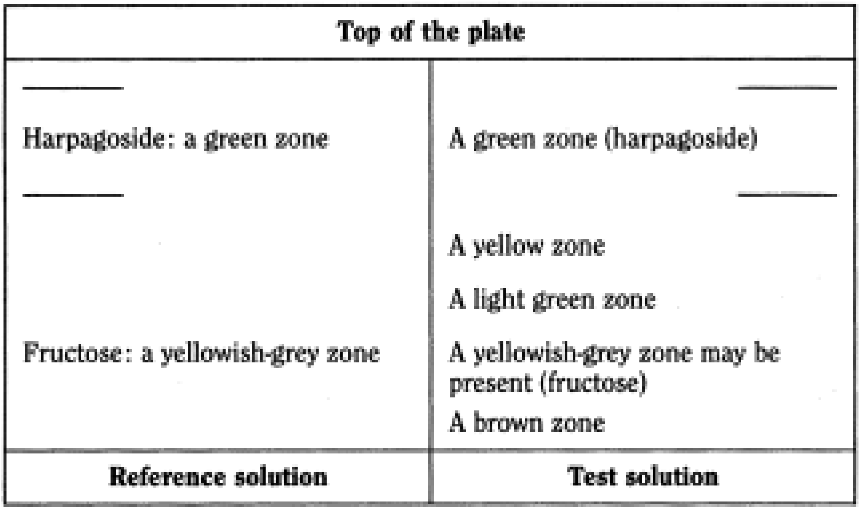
Application 20 µl as bands.

Development Over a path of 10 cm.

Drying In a current of warm air.

Detection Spray with a 10 g/l solution of *phloroglucinol R* in *ethanol (96 per cent) R* and then with *hydrochloric acid R* ; heat at 80 °C for 5-10 min and examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Harpagoside: a green zone	A green zone (harpagoside)
———	———
	A yellow zone
	A light green zone
Fructose: a yellowish-grey zone	A yellowish-grey zone may be present (fructose)
	A brown zone
Reference solution	**Test solution**

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution Introduce 0.350 g of the extract to be examined into a 100 ml volumetric flask, add 90 ml of *methanol R* and sonicate for 20 min. Cool to room temperature, dilute to 100.0 ml with *methanol R* and filter through a membrane filter (0.2 µm).

Reference solution Dissolve the contents of 1 vial of *harpagoside CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Column:

— *size: l* = 0.10 m, Ø = 4.0 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, water R* (50:50 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 278 nm.

Injection 10 µl.

Run time 3 times the retention time of harpagoside.

Retention time Harpagoside = about 7 min.

Calculate the percentage content of harpagoside using the following expression:

$$\frac{A_1 \times m_2 \times 1000}{A_2 \times m_1}$$

A_1 = area of the peak due to harpagoside in the chromatogram obtained with the test solution;

A_2 = area of the peak due to harpagoside in the chromatogram obtained with the reference solution;

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of harpagoside contained in 1 vial of *harpagoside CRS*, in grams.

Ph Eur

Devil's Claw

General Notices

Harpagophytum

(Devil's Claw Root, Ph Eur monograph 1095)

Ph Eur

DEFINITION

Devil's claw root consists of the cut and dried, tuberous secondary roots of *Harpagophytum procumbens* DC. and/or *Harpagophytum zeyheri* Decne.

Content

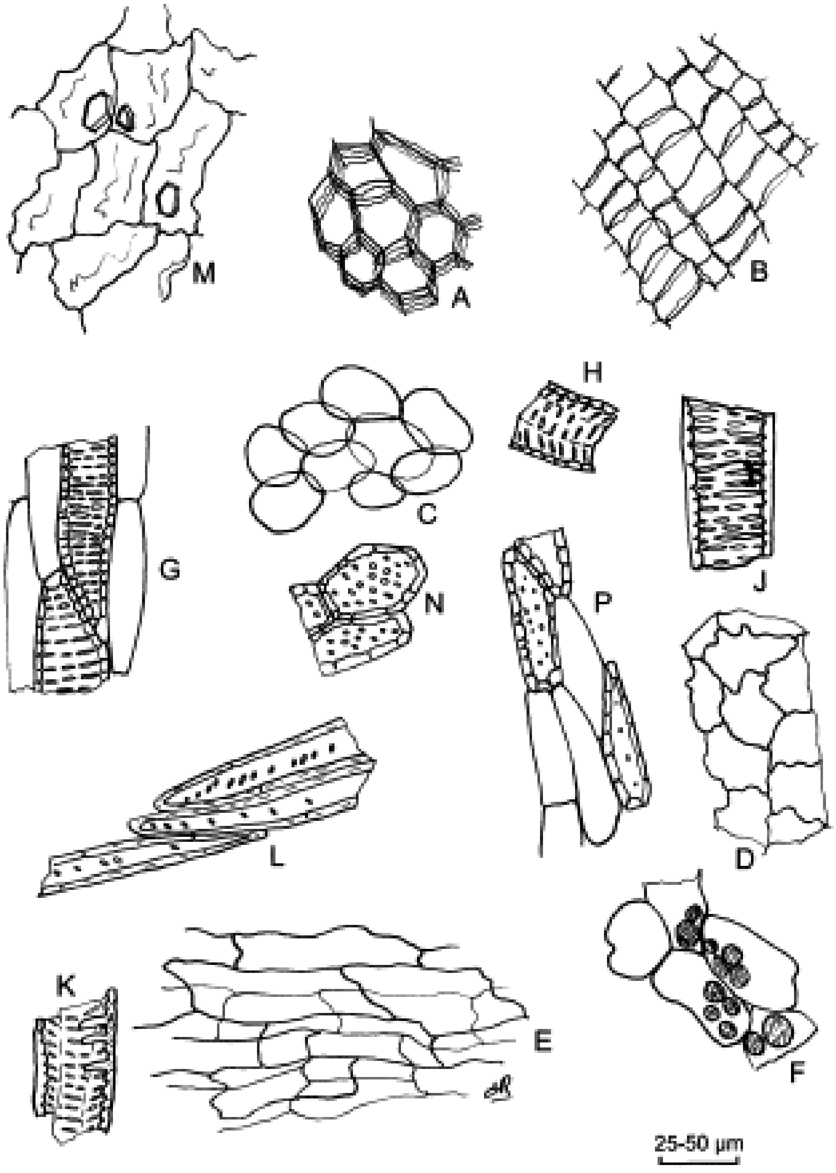
Minimum 1.2 per cent of harpagoside ($C_{24}H_{30}O_{11}$; M_r 494.5) (dried drug).

CHARACTERS

Devil's claw root is greyish-brown to dark brown.

IDENTIFICATION

A. It consists of thick, fan-shaped or rounded slices or of roughly crushed discs. The darker outer surface is traversed by tortuous longitudinal wrinkles. The paler cut surface shows a dark cambial zone and xylem bundles distinctly aligned in radial rows. The central cylinder shows fine concentric striations. Seen under a lens, the cut surface presents yellow to brownish-red granules.



A, B. Fragments of cork layer in surface view (A) and in transverse section (B)

C, D, E, F. Fragments of cortical parenchyma; fragment containing yellow droplets (F)

G, H, J, K. Vessels with reticulate or pitted thickenings

L. Lignified parenchyma from the central cylinder

M. Parenchyma containing prisms of calcium oxalate

N, P. Rectangular or polygonal sclereids

Figure 1095.-1. – *Illustration of powdered herbal drug of devil's claw root (see identification B)*

B. Reduce to a powder (355) *(2.9.12)*. The powder is brownish-yellow. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of cork layer consisting of yellowish-brown, thin-walled cells; fragments of cortical parenchyma consisting of large, thin-walled cells, sometimes containing reddish-brown granular inclusions and isolated yellow droplets; fragments of reticulately thickened or pitted vessels and fragments of lignified parenchyma, sometimes associated with vessels from the central cylinder; prism crystals and rare small needles of calcium oxalate in the parenchyma. The powder may show rectangular or polygonal sclereids with dark reddish-brown contents. With a solution of phloroglucinol in hydrochloric acid, the parenchyma turns green.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Heat 1.0 g of the powdered drug (355) *(2.9.12)* with 10 ml of *methanol R* on a water-bath at 60 °C for 10 min. Filter and reduce the filtrate to about 2 ml under reduced pressure at a temperature not exceeding 40 °C.

Reference solution Dissolve 1 mg of *harpagoside R* and 2.5 mg of *fructose R* in 1 ml of *methanol R* .

Plate *TLC silica gel plate* R (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *water R, methanol R, ethyl acetate R* (8:15:77 *V/V/V*).

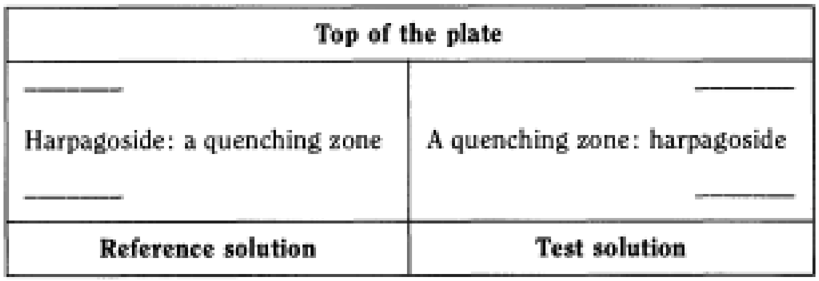
Application 20 µl [or 5 µl], as bands.

Development Over a path of 10 cm [or 7.5 cm].

Drying In a current of warm air.

Detection A Examine in ultraviolet light at 254 nm.

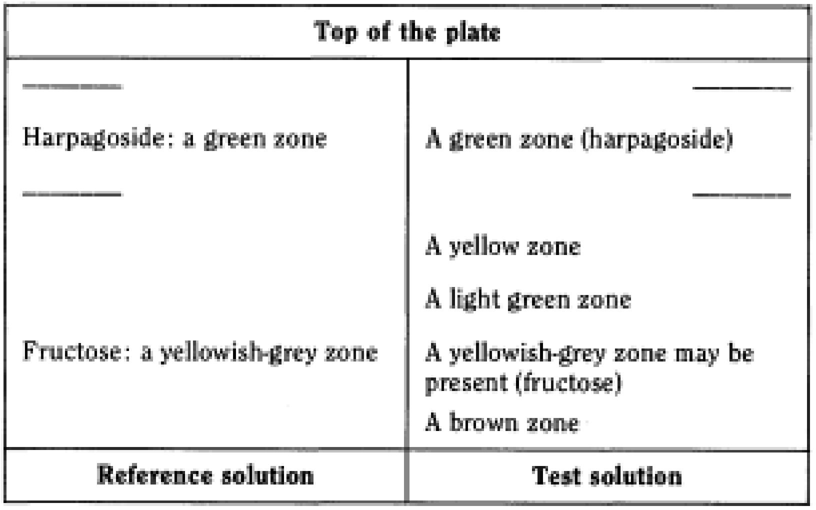
Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. The chromatogram obtained with the test solution shows other distinct zones, mainly above the zone due to harpagoside. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
_______	_______
Harpagoside: a quenching zone	A quenching zone: harpagoside
_______	_______
Reference solution	**Test solution**

Detection B Spray with a 10 g/l solution of *phloroglucinol R* in *ethanol (96 per cent) R* and then with *hydrochloric acid R* ; heat at 80 °C for 5-10 min and examine in daylight.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. The chromatogram obtained with the test solution also shows several yellow to brown zones above the zone due to harpagoside. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Harpagoside: a green zone	A green zone (harpagoside)
———	———
	A yellow zone
	A light green zone
Fructose: a yellowish-grey zone	A yellowish-grey zone may be present (fructose)
	A brown zone
Reference solution	**Test solution**

TESTS

Starch

Examine the powdered drug (355) *(2.9.12)* under a microscope using *water R* . Add *iodine solution R1* . No blue colour develops.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution To 0.500 g of the powdered drug (355) *(2.9.12)* add 100.0 ml of *methanol R* . Shake for 4 h and filter through a membrane filter (nominal pore size: 0.45 µm).

Reference solution Dissolve the contents of a vial of *harpagoside CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Column:
— *size: l* = 0.10 m, Ø = 4.0 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, water R* (50:50 *V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 278 nm.

Injection 10 µl.

Run time 3 times the retention time of harpagoside.

Retention time harpagoside = about 7 min.

Calculate the percentage content of harpagoside using the following expression:

$$\frac{m_2 \times A_1 \times 1000}{A_2 \times m_1}$$

A_1 = area of the peak due to harpagoside in the chromatogram obtained with the test solution;

A_2 = area of the peak due to harpagoside in the chromatogram obtained with the reference solution;

m_1 = mass of the drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *harpagoside CRS* in the reference solution, in grams.

Ph Eur

Digitalis Leaf

General Notices

Digitalis

(Ph Eur monograph 0117)

When Powdered Digitalis is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried leaf of *Digitalis purpurea L*.

Content

Minimum 0.3 per cent of cardenolic glycosides, expressed as digitoxin (M_r 765) (dried drug).

CHARACTERS

Faint but characteristic odour.

The whole leaf is about 10-40 cm long and 4-15 cm wide. The lamina is ovate lanceolate or broadly ovate. The winged petiole is from 1/4 as long as to equal in length to the lamina.

IDENTIFICATION

A. The leaf is brittle and often occurs broken. The upper surface is green and the lower surface is greyish-green. The apex is subacute and the margin is irregularly crenate, dentate or serrate. The base is decurrent. The venation is pinnate, the lateral veins being prominent especially on the lower surface, leaving the midrib at about 45° and anastomosing near the margin; a veinlet terminates in each tooth of the margin and the lower veins run down the winged petiole. The upper surface is rugose and pubescent; the lower surface shows a network of raised veinlets and is densely pubescent.

B. Reduce to a powder (355) *(2.9.12)*. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: epidermal cells with anticlinal walls which are straight or slightly sinuous on the upper surface and markedly sinuous on the lower surface; the cuticle is smooth. Trichomes are of 2 types: uniseriate, bluntly pointed non-glandular, usually of 3-5 cells, often with 1 or more collapsed cells, walls mostly finely warty or faintly striated; glandular trichomes usually with a unicellular, sometimes a multicellular uniseriate stalk and a unicellular or bicellular or exceptionally tetracellular head. Anomocytic stomata *(2.8.3)* are absent or very rare on the upper surface, numerous on the lower surface. Calcium oxalate crystals and sclerenchyma are absent.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (180) *(2.9.12)* add a mixture of 20 ml of *ethanol (50 per cent V/V) R* and 10 ml of *lead acetate solution R* . Boil for 2 min, allow to cool and centrifuge. Shake the supernatant solution with 2 quantities, each of 15 ml, of *chloroform R* ; separate the 2 layers by centrifugation if necessary. Dry the chloroform layers over *anhydrous sodium sulphate R* and filter. Evaporate 10 ml of the solution to dryness on a water-bath and dissolve the residue in 1 ml of a mixture of equal volumes of *chloroform R* and *methanol R* .

Reference solution Dissolve 5 mg of *purpureaglycoside A CRS*, 2 mg of *purpureaglycoside B CRS*, 5 mg of *digitoxin R* and 2 mg of *gitoxin R* in a mixture of equal volumes of *chloroform R* and *methanol R* , then dilute to 10 ml with the same mixture of solvents.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, methanol R, ethyl acetate R* (7.5:10:75 *V/V/V*).

Application 20 µl, as bands of 2 cm by 0.3 cm.

Development Over a path of 10 cm.

Drying Until the solvents have evaporated.

Detection Spray with a mixture of 2 volumes of a 10 g/l solution of *chloramine R* and 8 volumes of a 250 g/l solution of *trichloroacetic acid R* in *ethanol (96 per cent) R* , then heat at 100-105 °C for 10 min. Examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows a zone of light blue fluorescence in the lower part of the chromatogram, due to purpureaglycoside B, and just above it, a zone of brownish-yellow fluorescence due to purpureaglycoside A. A zone of light blue fluorescence, due to gitoxin, appears in the middle of the chromatogram and above it a zone of brownish-yellow fluorescence, due to digitoxin. The zones in the chromatogram obtained with the test solution are similar in position, colour and size to the zones in the chromatogram obtained with the reference solution. Other zones of fluorescence may also appear in the chromatogram obtained with the test solution.

D. Evaporate 5 ml of the chloroformic solution obtained in identification test C to dryness on a water-bath. To the residue add 2 ml of *dinitrobenzoic acid solution R* and 1 ml of *1 M sodium hydroxide* . A reddish-violet colour develops within 5 min.

E. Evaporate 5 ml of the chloroformic solution obtained in identification test C to dryness on a water-bath. To the residue add 3 ml of *xanthydrol solution R* and heat on a water-bath for 3 min. A red colour develops.

TESTS

Foreign matter *(2.8.2)*

There are no leaves with few or no trichomes and epidermal cells showing, in surface view, beaded anticlinal walls (*Digitalis lanata*).

Loss on drying *(2.2.32)*

Maximum 6.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 5.0 per cent.

ASSAY

Shake 0.250 g of the powdered drug (180) *(2.9.12)* with 50.0 ml of *water R* for 1 h. Add 5.0 ml of a 150 g/l solution of *lead acetate R* , shake and after a few minutes add 7.5 ml of a 40 g/l solution of *disodium hydrogen phosphate R* . Filter through a pleated paper filter. Heat 50.0 ml of the filtrate with 5 ml of hydrochloric acid (150 g/l HCl) under a reflux condenser on a water-bath for 1 h. Transfer to a separating funnel, rinse the flask with 2 quantities, each of 5 ml, of *water R* and shake with 3 quantities, each of 25 ml, of *chloroform R* . Dry the combined chloroform layers over *anhydrous sodium sulphate R* and dilute to 100.0 ml with *chloroform R* . Evaporate 40.0 ml of the chloroformic solution to dryness, dissolve the residue in 7 ml of *ethanol (50 per cent V/V) R*, add 2 ml of *dinitrobenzoic acid solution R* and 1 ml of *1 M sodium hydroxide* . At the same time prepare a reference solution as follows. Dissolve 50.0 mg of *digitoxin CRS* in *ethanol (96 per cent) R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *ethanol (96 per cent) R* . To 5.0 ml of the resulting solution add 25 ml of *water R* and 3 ml of hydrochloric acid (150 g/l HCl). Heat the solution under a reflux condenser on a water-bath for 1 h and complete the preparation as described above. Measure the absorbance *(2.2.25)* of the 2 solutions at 540 nm several times during the first 12 min until the maximum is reached, using as the compensation liquid a mixture of 7 ml of *ethanol (50 per cent V/V) R*, 2 ml of *dinitrobenzoic acid solution R* and 1 ml of *1 M sodium hydroxide* .

From the absorbances measured and the concentrations of the solutions, calculate the content of cardenolic glycosides, expressed as digitoxin.

STORAGE

Protected from moisture.

Ph Eur

Dill Oil

General Notices

DEFINITION

Dill Oil is obtained by distillation from the dried ripe fruits of *Anethum graveolens* L.

CHARACTERISTICS

A clear, colourless or pale yellow liquid, visibly free from water; odour, characteristic of the crushed fruit.

TESTS

Optical rotation

+70° to +80°, Appendix V F.

Refractive index

1.481 to 1.492, Appendix V E.

Solubility in ethanol

Soluble, at 20°, in 1 volume or more of *ethanol (90%)* and in 10 volumes or more of *ethanol (80%)*, Appendix X M.

Weight per ml

0.895 to 0.910 g, Appendix V G.

Content of carvone

43.0 to 63.0% w/w when determined by the following method. To 1.5 g in a glass-stoppered tube (approximately 150 mm × 25 mm) add 10 ml of a solution prepared in the following manner. Dissolve 7.0 g of *hydroxylamine hydrochloride* in 90 ml of *ethanol (90%)*, warming gently if necessary, add 1.6 ml of *dimethyl yellow solution* and sufficient 1M *potassium hydroxide* in *ethanol (90%)* to produce a pure yellow colour and dilute to 100 ml with *ethanol (90%)*. Titrate with 1M *potassium hydroxide in ethanol (90%) VS* until the red colour changes to yellow. Place the tube in a water bath at 75° to 80° and, at 5-minute intervals, neutralise with 1M *potassium hydroxide in ethanol (90%) VS* ; after 40 minutes complete the titration to the full yellow colour of the indicator. This procedure gives an approximate value for the carvone content of the oil. Repeat the procedure, using as the colour standard for the end point of the titration the titrated liquid of the first determination with the addition of 0.5 ml of 1M *potassium hydroxide in ethanol (90%) VS* . Calculate the content of carvone from the second determination. Each ml of 1M *potassium hydroxide in ethanol (90%) VS* is equivalent to 151.4 mg of carvone, $C_{10}H_{14}O$.

STORAGE

Dill Oil should be kept in a well-filled container and protected from light. It darkens in colour on storage.

Dog Rose

General Notices

(Ph Eur monograph 1510)

Ph Eur

DEFINITION

Rose hips made up by the receptacle and the remains of the dried sepals of *Rosa canina* L., *R. pendulina* L. and other *Rosa* species, with the achenes removed.

Content

Minimum 0.3 per cent of ascorbic acid ($C_6H_8O_6$; M_r 176.1) (dried drug).

IDENTIFICATION

A. It consists of fragments of the fleshy, hollow, urceolate receptacle, bearing the remains of the reduced sepals, light pink or orange-pink, the convex outer surface shiny and strongly wrinkled; bearing on its lighter inner surface abundant bristle-like hairs.

B. Reduce to a powder (355) *(2.9.12)*. The powder is orange-yellow. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous fragments of receptacle, the outer epidermis with orange-yellow contents and a thick cuticle, the inner epidermis composed of thin-walled cells containing cluster crystals and occasional prisms of calcium oxalate; scattered lignified cells, isodiametric, with thickened and pitted walls forming the trichome bases; abundant long, unicellar trichomes, up to 2 mm long and 30-45 µm thick, tapering towards each end, walls heavily thickened and with a waxy cuticle which may show fissures in a spiral arrangement; numerous oily orange-yellow globules.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 5 g of the powdered drug (355) *(2.9.12)* add 25 ml of *ethanol (96 per cent) R* . Shake for 30 min and filter.

Reference solution Dissolve 10 mg of *ascorbic acid R* in 5.0 ml of *ethanol (60 per cent V/V) R*.

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *acetic acid R, acetone R, methanol R, toluene R* (5:5:20:70 *V/V/V/V*).

Application 20 µl of the test solution and 2 µl of the reference solution.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatogram obtained with the test solution shows a quenching zone similar in position to the principal zone in the chromatogram obtained with the reference solution.

Detection B Spray with a 0.2 g/l solution of *dichlorophenolindophenol, sodium salt R* in *ethanol (96 per cent) R*. Examine in daylight.

Results B The chromatogram obtained with the test solution shows a white zone on a pink background similar in position and colour to the principal zone in the chromatogram obtained with the reference solution. The chromatogram also shows an intense orange-yellow zone near the solvent front and a yellow zone in the upper third (carotenoids).

TESTS

Foreign matter *(2.8.2)*

Maximum 1 per cent.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 7.0 per cent.

ASSAY

Test solution In a round-bottomed flask, weigh 0.500 g of the freshly powdered drug (710) *(2.9.12)*. Add a solution of 1.0 g of *oxalic acid R* in 50.0 ml of *methanol R*. Boil under a reflux condenser for 10 min, and cool in iced water until the temperature reaches 15-20 °C. Filter. Transfer 2.0 ml of the filtrate to a 50 ml conical flask. Add successively, with gentle shaking after each addition, 2.0 ml of *dichlorophenolindophenol standard solution R* and then, exactly 60 s later, 0.5 ml of a 100 g/l solution of *thiourea R* in *ethanol (50 per cent V/V) R* and 0.7 ml of *dinitrophenylhydrazine-sulphuric acid solution R*. Heat under a reflux condenser at 50 °C for 75 min, and place immediately in iced water for 5 min. Add dropwise 5.0 ml of a mixture of 12 ml of *water R* and 50 ml of *sulphuric acid R*, taking care to carry out the addition over a period of minimum 90 s and maximum 120 s while maintaining vigorous stirring in iced water. Allow to stand for 30 min at room temperature and measure the absorbance *(2.2.25)* at 520 nm using solution A as compensation liquid.

Solution A Treat 2.0 ml of the filtrate obtained during the preparation of the test solution as described but adding the *dinitrophenylhydrazine-sulphuric acid solution R* just before the absorbance is measured.

Reference solution Dissolve 40.0 mg of *ascorbic acid R* in a freshly prepared 20 g/l solution of *oxalic acid R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with a freshly prepared 20 g/l solution of *oxalic acid R* in *methanol R*. Treat 2.0 ml of the solution as described above for the filtrate obtained during the preparation of the test solution. Measure the absorbance *(2.2.25)* at 520 nm using solution B as the compensation liquid.

Solution B Treat 2.0 ml of the reference solution as described above for solution A.

Calculate the percentage content of ascorbic acid from the following expression:

$$\frac{2.5 \times A_1 \times m_2}{A_2 \times m_1}$$

A_1 = absorbance of the test solution;

A_2 = absorbance of the reference solution;

m_1 = mass of the substance to be examined, in grams;

m_2 = mass of ascorbic acid used, in grams.

Ph Eur

Dried Bilberry

General Notices

(Dried Bilberry Fruit, Ph Eur monograph 1588)

Ph Eur

DEFINITION

Dried ripe fruit of *Vaccinium myrtillus* L.

Content

Minimum 1.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

CHARACTERS

Sweet and slightly astringent taste.

IDENTIFICATION

A. Dried bilberry is a dark blue, subglobular, shrunken berry about 5 mm in diameter, with a scar at the lower end and surmounted by the persistent calyx, which appears as a circular fold and the remains of the style. The deep violet, fleshy mesocarp contains numerous small, brown, ovoid seeds.

B. Reduce to a powder (355) *(2.9.12)*. The powder is violet-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows: violet-pink sclereids from the endocarp and the mesocarp, usually aggregated, with thick, channelled walls; reddish-brown fragments of the epicarp consisting of polygonal cells with moderately thickened walls; brownish-yellow fragments of the outer seed testa made up of elongated cells with U-shaped thickened walls; clusters and prisms crystals of various size of calcium oxalate.

C. Thin-layer chromatography *(2.2.27)*

Test solution To 2 g of the powdered drug (355) *(2.9.12)* add 20 ml of *methanol R* . Shake for 15 min and filter.

Reference solution Dissolve 5 mg of *chrysanthemin R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, water R, butanol R* (16:19:65 *V/V/V*).

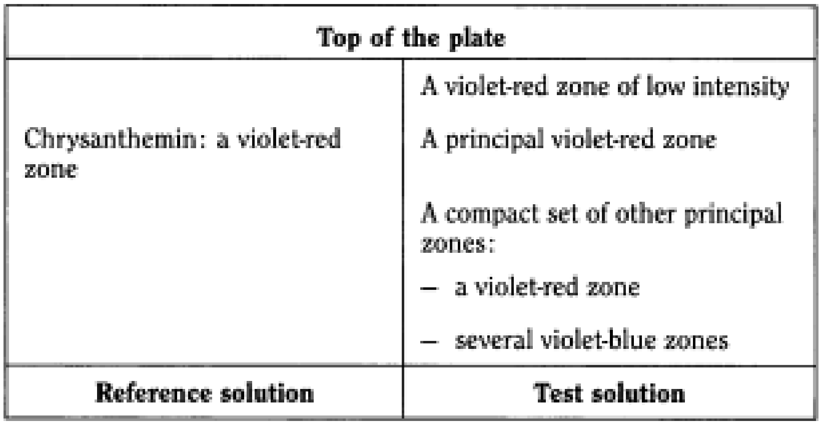
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions.



Top of the plate	
	A violet-red zone of low intensity
Chrysanthemin: a violet-red zone	A principal violet-red zone
	A compact set of other principal zones: – a violet-red zone – several violet-blue zones
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs *(2.8.14)* . Use 1.500 g of the powdered drug (355) *(2.9.12)*.

Ph Eur

Dried Bitter-Orange Peel

General Notices

(Bitter-Orange Epicarp and Mesocarp, Ph Eur monograph 1603)

Preparations

Orange Peel Infusion

Orange Tincture

Ph Eur

DEFINITION

Dried epicarp and mesocarp of the ripe fruit of *Citrus aurantium* L. ssp. *aurantium* (*C. aurantium* L. ssp. *amara* Engl.) partly freed from the white spongy tissue of the mesocarp and endocarp.

Content

Minimum 20 ml/kg of essential oil (anhydrous drug).

CHARACTERS

Aromatic odour and spicy bitter taste.

IDENTIFICATION

A. The drug consists of elliptical to irregular pieces 5 cm to 8 cm long, 3 cm to 5 cm broad and about 3 mm thick. The outer surface is yellowish to reddish-brown and distinctly punctate, the inner surface is yellowish to brownish-white.

B. Reduce to a powder (355) (*2.9.12*). The powder is light brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows small polygonal cells with slightly thickened anticlinal walls, filled with orange-red chromatophores, and very occasional anomocytic stomata (*2.8.3*); fragments of the hypodermis showing collenchymatous thickening; groups of parenchyma with each cell containing a prism crystal of calcium oxalate; fragments of lysigenous oil glands; parenchyma containing crystals of hesperidin which dissolve in a 20 g/l *potassium hydroxide R* solution giving a yellow colour.

C. Thin-layer chromatography (*2.2.27*).

Test solution. To 1.0 g of the powdered drug (710) (*2.9.12*) add 10 ml of *methanol R* and heat in a water-bath at 65 °C for 5 min shaking frequently. Allow to cool and filter.

Reference solution Dissolve 1.0 mg of *naringin R* and 1.0 mg of *caffeic acid R* in 1 ml of *methanol R*.

Plate TLC silica gel plate R.

Mobile phase *water R*, *anhydrous formic acid R*, *ethyl acetate R* (10:15:75 *V/V/V*).

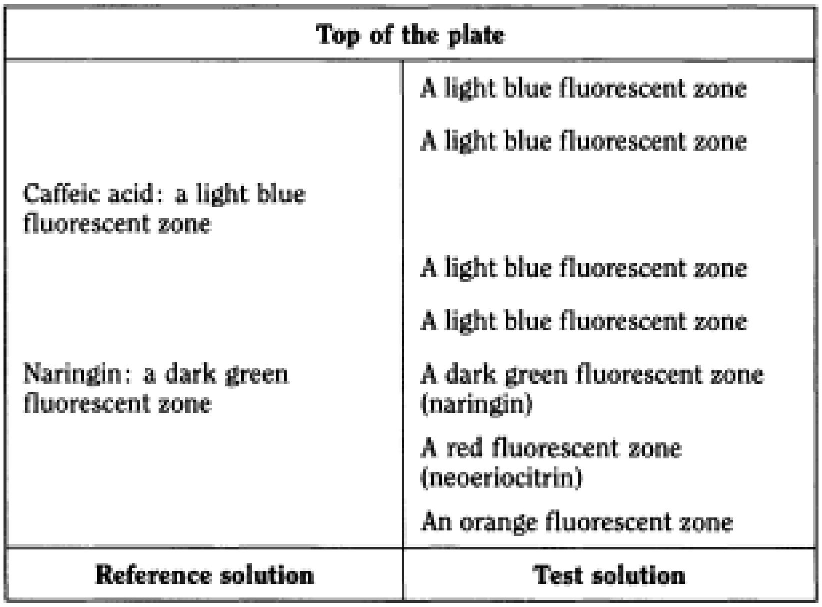
Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying In air, and heat in an oven at 110-120 °C for 5 min.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. After at least 1 h, examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions. Furthermore, other fluorescent zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	A light blue fluorescent zone
	A light blue fluorescent zone
Caffeic acid: a light blue fluorescent zone	
	A light blue fluorescent zone
	A light blue fluorescent zone
Naringin: a dark green fluorescent zone	A dark green fluorescent zone (naringin)
	A red fluorescent zone (neoeriocitrin)
	An orange fluorescent zone
Reference solution	**Test solution**

TESTS

Water (*2.2.13*)

Maximum 10.0 per cent, determined on 20.0 g of powdered drug (355) (*2.9.12*) by distillation.

Total ash (*2.4.16*)

Maximum 7.0 per cent

Extractable matter

Minimum 6.0 per cent.

To 2.000 g of the powdered drug (250) (*2.9.12*) add a mixture of 3 ml of *water R* and 7 ml of *alcohol R* and extract for 2 h, shaking frequently. Filter, evaporate 2.000 g of the filtrate to dryness on a water-bath and dry in an oven at 100-105 °C for 3 h. Allow to cool in a dessicator over *diphosphorus pentoxide R* and weigh. The residue weighs a minimum of 120 mg.

ASSAY

Carry out the determination of essential oil in herbal drugs (*2.8.12*). Use a 500 ml round-bottomed flask, 200 ml of *water R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Reduce the drug to a powder (710) (*2.9.12*) and immediately use 15.0 g for the determination. Distil at a rate of 2-3 ml/min for 90 min.

Ph Eur

Dried Lemon Peel

General Notices

DEFINITION

Dried Lemon Peel is the dried outer part of the pericarp of the ripe, or nearly ripe, fruit of *Citrus limon* (L.) Burm. f.

CHARACTERISTICS

It has the macroscopical and microscopical characters described under Identification tests A and B.

IDENTIFICATION

A. Dried Lemon Peel consists of strips or pieces, showing a marked thickening of the epicarp around the calyx. The outer surface is yellow and somewhat rough from the presence of numerous minute pits, each corresponding to an oil gland; the inner surface with only a small remnant of white, spongy pericarp. Fracture, short.

B. Prepare thin cross sections from material softened by soaking in *water* and examine under a microscope using *chloral hydrate solution*. The sections show a yellow epidermis composed of small, thin-walled cells and an underlying parenchyma with numerous large oil glands and scattered small strands of vascular tissue; prismatic crystals of calcium oxalate occur throughout the parenchyma and are particularly abundant in the layers adjacent to the epidermis.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* and a mixture of 10 volumes of *anhydrous formic acid*, 10 volumes of *water*, 30 volumes of *butan-2-one* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, as bands, 20 µl of each of the following solutions. For solution (1) add 1 g of freshly cut peel to 10 ml of *methanol* and heat in a water-bath at 65° for 5 minutes, shaking frequently. Allow to cool and filter. Solution (2) contains 0.01% w/v each of *caffeic acid* and *naringin* and 0.025% w/v of *rutin* in *methanol*. Dry the plate at 105° and spray the warm plate with a 1% w/v solution of *diphenylboric acid aminoethyl ester* in *methanol* and then with a 5% w/v solution of *polyethylene glycol 400* in *methanol*. Allow the plate to stand for 30 minutes and examine under *ultraviolet light (365 nm).* The chromatogram obtained with solution (1) exhibits a yellowish-brown fluorescent zone corresponding in colour and position to the zone for rutin in the chromatogram obtained with solution (2); above it an intense red fluorescent zone and further above a very weak greenish fluorescent zone corresponding in colour and position to the zone for naringin in the chromatogram obtained with solution (2). Other coloured zones are present in the chromatogram obtained with solution (1).

TESTS

Volatile oil

Not less than 2.0% v/w (1.7% w/w), Appendix XI E, using 300 ml of *water* as the distillation liquid and no xylene in the graduated tube. Use 20 g, soaked in *water* and macerated in a suitable blender, and distil for 3 hours.

Dwarf Pine Oil

General Notices

(Ph Eur monograph 2377)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation of the fresh leaves and twigs of *Pinus mugo* Turra. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, colourless or pale yellow liquid.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography (*2.2.27*).

Test solution Dilute 1 ml of the substance to be examined to 10 ml with *toluene R.*

Reference solution Dissolve 10 mg of *borneol R* and 10 µl of *bornyl acetate R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

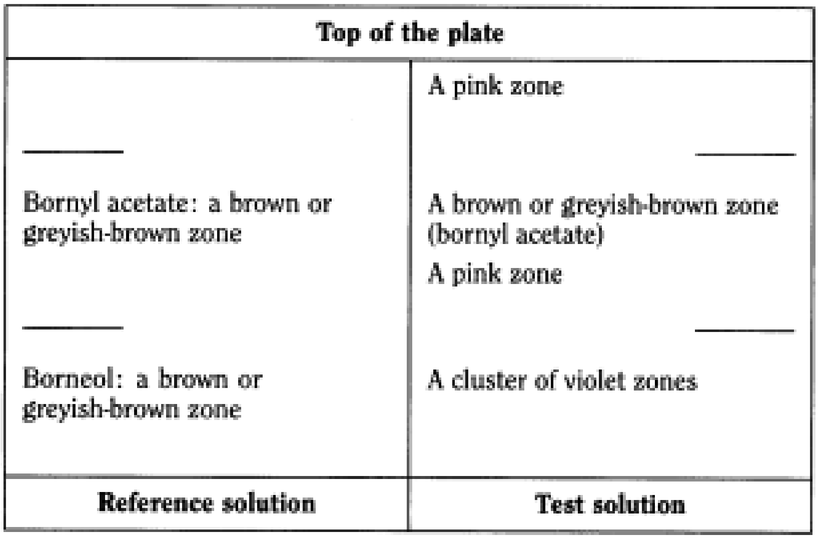
Application 10 µl [or 2 µl], as bands.

Development Over a path of 15 cm [or 6 cm].

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A pink zone
———	———
Bornyl acetate: a brown or greyish-brown zone	A brown or greyish-brown zone (bornyl acetate) A pink zone
———	———
Borneol: a brown or greyish-brown zone	A cluster of violet zones
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with reference solution (a).

TESTS

Relative density (*2.2.5*)

0.857 to 0.868.

Refractive index (*2.2.*6)

1.474 to 1.480.

Optical rotation (*2.2.7*)

- 7° to - 15°.

Acid value (*2.5.1*)

Maximum 1.0.

Peroxide value (*2.5.5*)

Maximum 20.

Fatty oils and resinified oils (*2.8.7*)

It complies with the test.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dilute 200 µl of the substance to be examined to 10.0 ml with *heptane R*.

Reference solution (a) Dilute 30 µl of α-*pinene R*, 5 mg of *camphene R*, 10 µl of β-*pinene R*, 20 µl of *car-3-ene R*, 5 µl of β-*myrcene R*, 10 µl of *limonene R*, 5 µl of *p-cymene R*, 10 µl of *terpinolene R*, 5 µl of *bornyl acetate R* and 5 µl of β-*caryophyllene R* in *heptane R* and dilute to 5 ml with the same solvent.

Reference solution (b) Dissolve 5 mg of *camphene R* in *heptane R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *heptane R*.

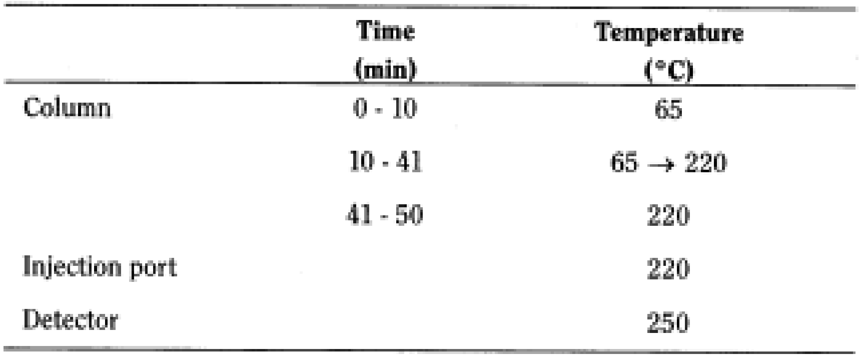
Column:
- *material*: fused silica;
- *size: l* = 60 m, Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	65
	10 - 41	65 → 220
	41 - 50	220
Injection port		220
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Elution order Order indicated in the composition of reference solution (a); record the retention times of these substances.

System suitability Reference solution (a):
- *resolution*: minimum 1.5 between the peaks due to car-3-ene and β-myrcene.

Identification of components: using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution; the peak due to β-phellandrene is eluted after the peak due to limonene with a relative retention of about 1.03 with reference to limonene.

Determine the percentage content of each of these components. The limits are within the following ranges:
- α-*pinene*: 10.0 per cent to 30.0 per cent;
- *camphene*: maximum 2.0 per cent;
- β-*pinene*: 3.0 per cent to 14.0 per cent;
- *car-3-ene*: 10.0 per cent to 20.0 per cent;
- β-*myrcene*: 3.0 per cent to 12.0 per cent;

— *limonene*: 8.0 per cent to 14.0 per cent;

— β-*phellandrene*: 10.0 per cent to 19.0 per cent;

— *p-cymene*: maximum 2.5 per cent;

— *terpinolene*: maximum 8.0 per cent;

— *bornyl acetate*: 0.5 per cent to 5.0 per cent;

— β-*caryophyllene*: 0.5 per cent to 5.0 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

STORAGE

In an inert, well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Echinacea Angustifolia Root

General Notices

(Narrow-leaved Coneflower Root, Ph Eur monograph 1821)

Ph Eur

DEFINITION

Dried, whole or cut underground parts of *Echinacea angustifolia* (D.C.).

Content

Minimum 0.5 per cent of echinacoside ($C_{35}H_{46}O_{20}$; M_r 786.5) (dried drug).

IDENTIFICATION

First identification A, B, C.

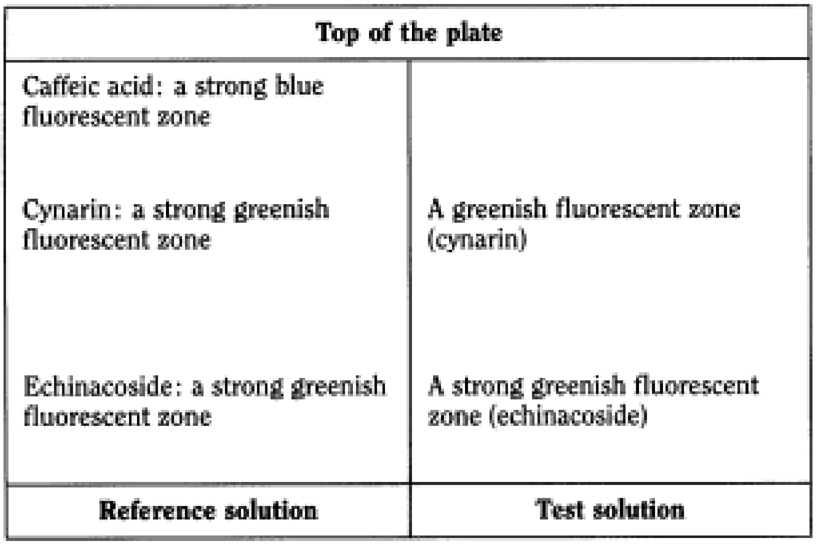
Second identification A, B, D.

A. The root crown is up to about 30 mm in diameter and shows only a few stem bases. The roots are not very numerous, up to about 15 mm in diameter, cylindrical or slightly tapering and sometimes spirally twisted, the outer surface is pale brown to yellowish-brown. The fracture is short, dark brown with a radiate structure.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: narrow lignified fibres (up to about 800 µm in length and 50 µm in diameter) joined together in long bundles surrounded by phytomelanin deposits; lignified reticulately or scalariformly thickened vessels (up to about 60 µm in diameter); abundant sclereids occuring singly or, more usually, in groups of 2 to 10, mostly elongated to rectangular, (up to about 150 µm in length and 40 µm wide), with intercellular spaces filled with phytomelanin deposit; fragments of oleoresin canal (80-150 µm in diameter) with yellowish-orange to reddish-brown content; groups of squarish to rectangular cells, about 30-45 µm from the outer layers of the roots; abundant fine-walled pitted parenchyma with sphaerocrystalline masses of inulin.

C. Examine the chromatograms obtained in the test for *Echinacea purpurea*.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint dark blue fluorescent zones may be present between the zones of echinacoside and cynarin in the chromatogram obtained with the test solution.



Top of the plate	
Caffeic acid: a strong blue fluorescent zone	
Cynarin: a strong greenish fluorescent zone	A greenish fluorescent zone (cynarin)
Echinacoside: a strong greenish fluorescent zone	A strong greenish fluorescent zone (echinacoside)
Reference solution	**Test solution**

D. Examine the chromatograms obtained in the assay.

Results The chromatogram obtained with the test solution shows 1 major peak due to echinacoside and a minor peak due to cynarin. Peaks due to caffeic acid, caftaric acid and chlorogenic acid are minor peaks or may be absent.

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent.

Echinacea purpurea

Thin layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* , treat in an ultrasonic bath for 5 min. Centrifuge and use the supernatant solution.

Reference solution Dissolve 1 mg of *echinacoside R* , 1 mg of *cynarin R* and 0.5 mg of *caffeic acid R* in 5.0 ml of *methanol R* .

Plate *TLC silica gel F $_{254}$ plate R* (5-40 µm) [or *TLC silica gel F $_{254}$ plate R* (2-10 µm)].

Mobile phase *Anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (3:3:9:15 *V/V/V/V*).

Application 25 µl [or 5 µl] of the test solution and 10 µl [or 2 µl] of the reference solution as bands.

Development Over a path of 15 cm [or 5 cm].

Drying In a stream of cold air for about 10 min followed by 2 min at 100-105 °C.

Detection Treat the hot plate using a 5 g/l solution of *diphenylboric acid aminoethyl ester R* in *ethyl acetate R* ; examine in ultraviolet light at 365 nm after 30 min.

Results The chromatogram obtained with the test solution shows no greenish fluorescent zone just below the zone due to caffeic acid in the chromatogram obtained with the reference solution and no greenish fluorescent zone below the zone due to cynarin in the chromatogram obtained with the reference solution. In the chromatogram obtained with the test solution no zones apart from faint dark blue fluorescent zones are visible between the zones due to echinacoside and cynarin.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 9.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

ASSAY

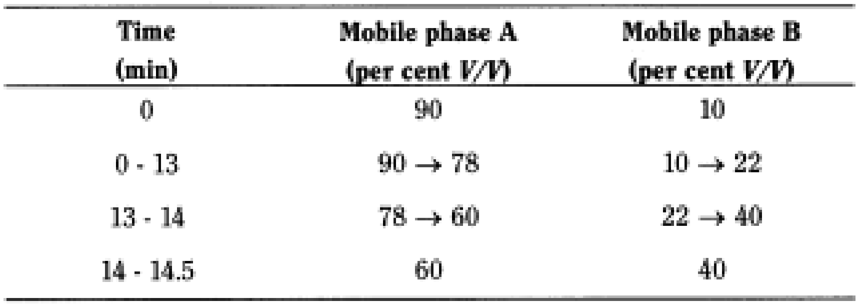
Liquid chromatography *(2.2.29)* .

Test solution In a 100 ml volumetric flask place 0.500 g of powdered drug (355) *(2.9.12)* and add 80 ml of *ethanol (70 per cent V/V) R*. Treat in an ultrasonic bath for 15 min and dilute to 100.0 ml with *ethanol (70 per cent V/V) R*. Mix the suspension and allow to stand for a few minutes so that visible solids settle. Filter a suitable proportion of the solution through a membrane filter (nominal pore size 0.45 µm) before injection.

Reference solution Dissolve 10.0 mg of *chlorogenic acid CRS* and 10.0 mg of *caffeic acid R* in *ethanol (70 per cent V/V) R*, sonicate for 15 min and dilute to 10.0 ml with the same solvent. Dilute 4.0 ml of this solution to 100.0 ml with *ethanol (70 per cent V/V) R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 35 °C.

Mobile phase:
— *mobile phase A*: *phosphoric acid R, water R* (1:999 *V/V*);
— *mobile phase B*: *acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0	90	10
0 - 13	90 → 78	10 → 22
13 - 14	78 → 60	22 → 40
14 - 14.5	60	40

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 330 nm.

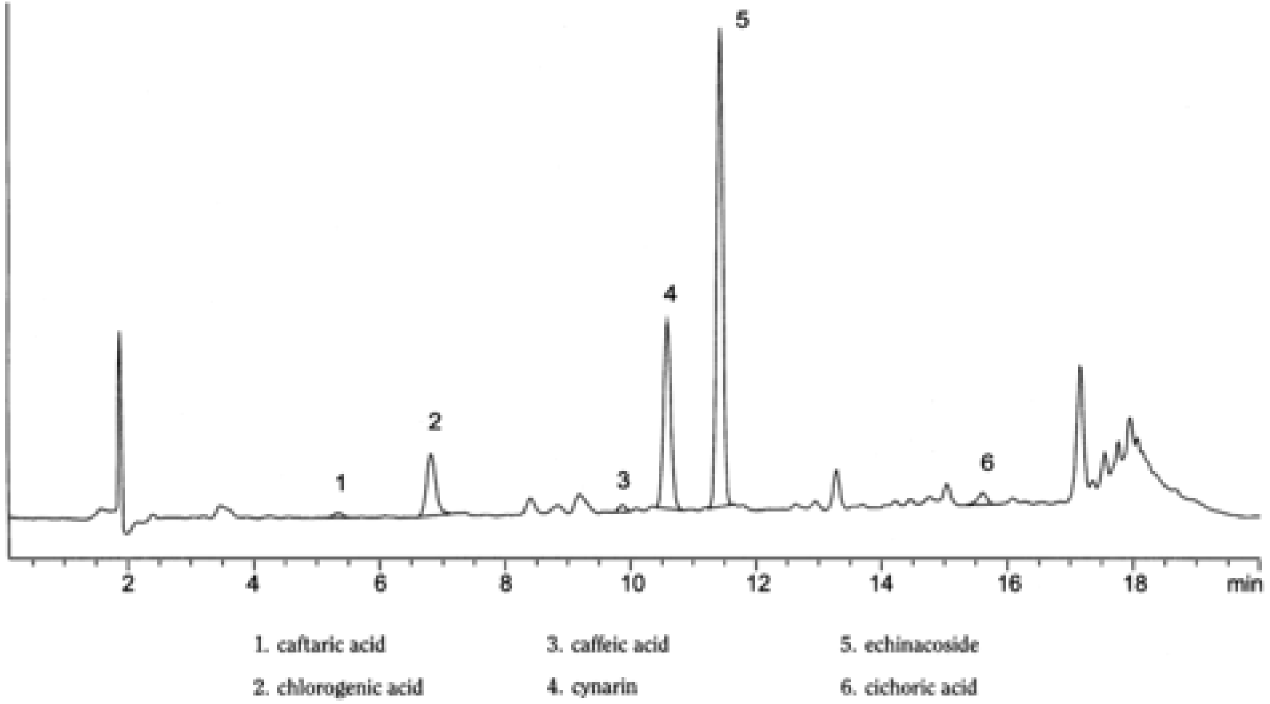


Figure 1821.-1. - *Chromatogram for the assay of echinacoside in narrow-leaved coneflower root*

Injection 10 µl.

Relative retention With reference to chlorogenic acid: caftaric acid = about 0.8; caffeic acid = about 1.5; cynarin = about 1.6; echinacoside = about 1.7; cichoric acid = about 2.3.

System suitability Reference solution:

— *resolution*: minimum 10 between the peaks due to caffeic acid and chlorogenic acid.

Locate the peaks due to caffeic acid and chlorogenic acid using the chromatogram obtained with the reference solution. Locate the peaks due to echinacoside and cynarin using the chromatogram in Figure 1821.–1.

Calculate the percentage content of echinacoside from the following expression:

$$\frac{A_1 \times C_2 \times 100 \times 2.221}{A_2 \times C_1}$$

A_1 = area of the peak due to echinacoside in the chromatogram obtained with the test solution;

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;

C_1 = concentration of the test solution, in milligrams per millilitre;

C_2 = concentration of chlorogenic acid in the reference solution, in milligrams per millilitre;

2.221 = peak correlation factor between chlorogenic acid and echinacoside.

STORAGE

Store uncomminuted.

Ph Eur

Echinacea Pallida Root

General Notices

(Pale Coneflower Root, Ph Eur monograph 1822)

Ph Eur

DEFINITION

Dried, whole or cut underground parts of *Echinacea pallida* Nutt.

Content

Minimum 0.5 per cent of echinacoside ($C_{35}H_{46}O_{20}$; M_r 786.5) (dried drug).

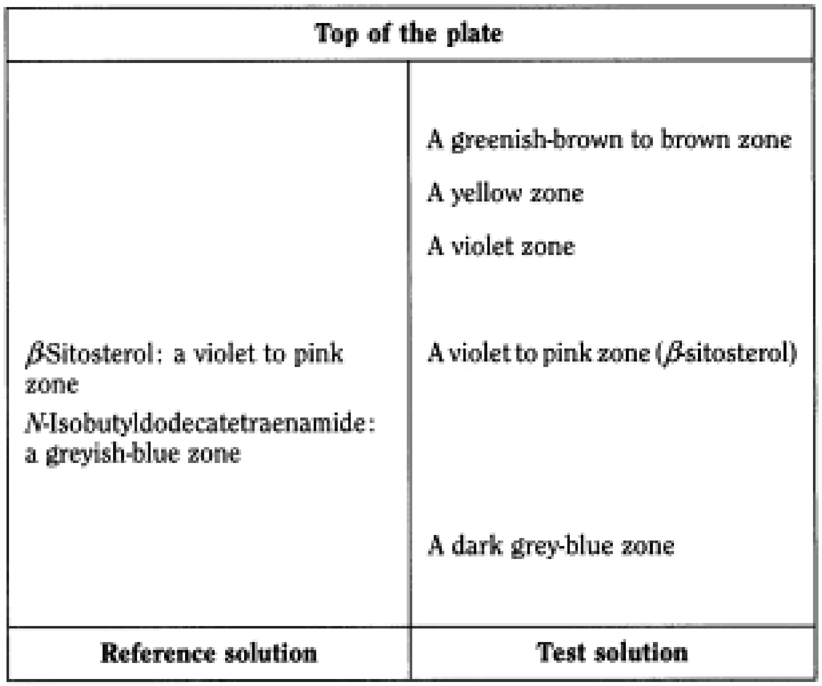
IDENTIFICATION

A. The rhizome and roots are 4-20 mm in diameter, cylindrical and sometimes spirally twisted, longitudinally wrinkled or deeply furrowed; the outer surface is reddish-brown to greyish-brown.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-brown to light yellow. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: short lignified fibres (100-300 µm in length, up to about 80 µm in diameter) occurring singly or joined together in long bundles, sometimes with phytomelanin deposits; lignified reticulately or scalariformly thickened vessels (up to about 70 µm in diameter); abundant sclereids, occurring singly or in small groups of less than 10, varying considerably in shape from rounded to rectangular or irregular, sometimes much elongated and fibre-like and measuring up to 400 µm in length; all the sclereids have associated black, phytomelanin deposits; fragments of oleoresin canals (up to 240 µm in diameter) with yellowish-orange content; groups of squarish to rectangular cells of the outer layers (about 40 × 80 µm); abundant thin-walled pitted parenchyma with sphaerocrystalline masses of inulin.

C. Examine the chromatograms obtained in the test for other *Echinacea* species and *Parthenium integrifolium*.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. The chromatogram obtained with the test solution may also show a weak zone close to the solvent front.



Top of the plate	
	A greenish-brown to brown zone
	A yellow zone
	A violet zone
β-Sitosterol: a violet to pink zone	A violet to pink zone (*β*-sitosterol)
N-Isobutyldodecatetraenamide: a greyish-blue zone	
	A dark grey-blue zone
Reference solution	**Test solution**

D. Examine the chromatograms obtained in the assay.

Results The major peak in the chromatogram obtained with the test solution is due to echinacoside. Peaks due to caftaric acid, caffeic acid, cynarin, chlorogenic acid and cichoric acid are minor peaks or may be absent.

TESTS

Foreign matter ***(2.8.2)***

Maximum 3 per cent.

Other *Echinacea* species and *Parthenium integrifolium*

Thin layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methylene chloride R* and sonicate for 5 min. Centrifuge and use the supernatant solution.

Reference solution Dissolve 1 mg of β-*sitosterol R* and a volume of *N-isobutyldodecatetraenamide solution R* corresponding to 1 mg of *N-isobutyldodecatetraenamide R* in *methanol R* and dilute to 5.0 ml with the same solvent.

Plate *TLC silica gel* F_{254} *plate R* (5-40 µm) [or *TLC silica gel* F_{254} *plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R, cyclohexane R, ethyl acetate R, toluene R* (0.9:3:6:24 *V/V/V/V*)..

Application 25 µl [or 5 µl] of the test solution and 10 µl [or 4 µl] of the reference solution as bands.

Development Over a path of 15 cm [or 5 cm].

Drying In a stream of cold air for about 10 min.

Detection Treat the plate using *anisaldehyde solution R* and heat at 105 °C for 3 min; examine in daylight.

Results The chromatogram obtained with the test solution shows no greyish-blue zone at the position of *N*-isobutyldodecatetraenamide in the chromatogram obtained with the reference solution, and no blue zone at the position of the violet zone due to β-sitosterol in the chromatogram obtained with the reference solution.

Loss on drying ***(2.2.32)***

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 7.0 per cent.

Ash insoluble in hydrochloric acid ***(2.8.1)***

Maximum 2.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution In a 100 ml volumetric flask place 0.500 g of the powdered drug (355) *(2.9.12)* and add 80 ml of *ethanol (70 per cent V/V) R*. Sonicate for 15 min and dilute to 100.0 ml with *ethanol (70 per cent V/V) R*. Mix the suspension and allow to stand for a few minutes to allow visible solids to settle. Filter a suitable proportion of the solution through a membrane filter (nominal pore size 0.45 µm) before injection.

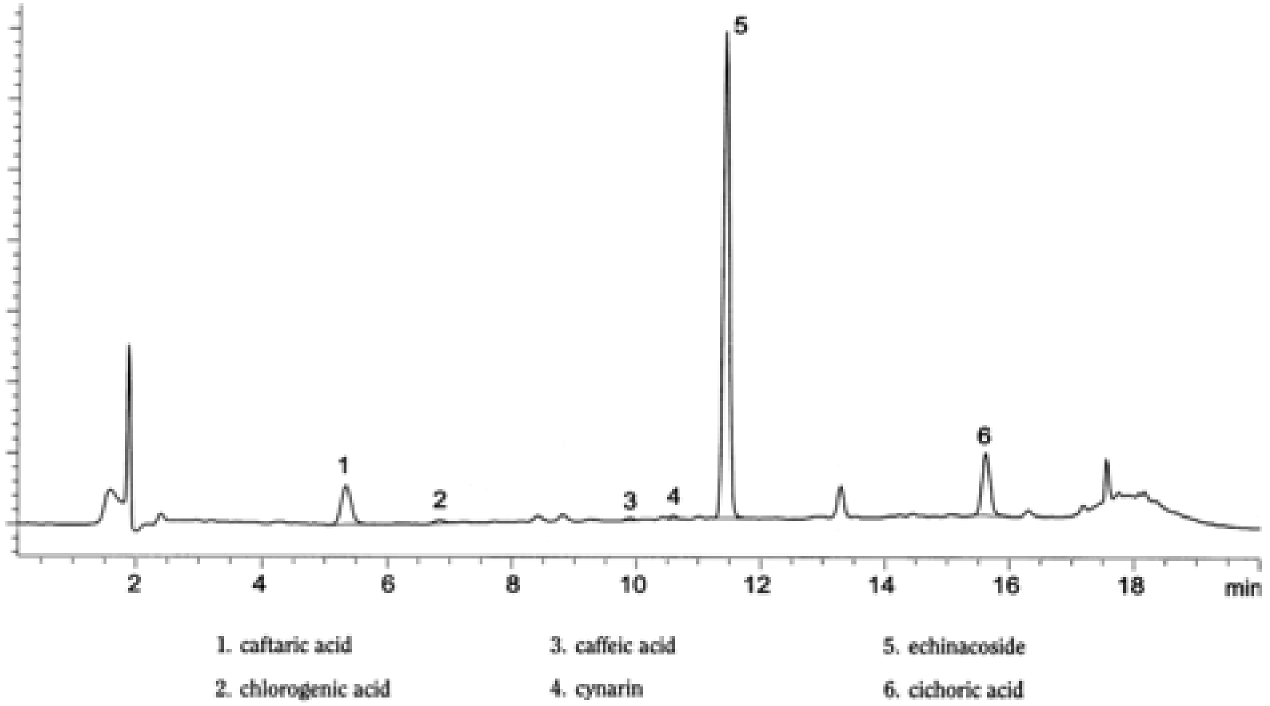


Figure 1822.-1. – *Chromatogram for the assay of echinacoside in pale coneflower root*

Reference solution Dissolve 10.0 mg of *chlorogenic acid CRS* and 10.0 mg of *caffeic acid R* in *ethanol (70 per cent V/V) R*, sonicate for 15 min and dilute to 10.0 ml with the same solvent. Dilute 4.0 ml of this solution to 100.0 ml with *ethanol (70 per cent V/V) R*.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

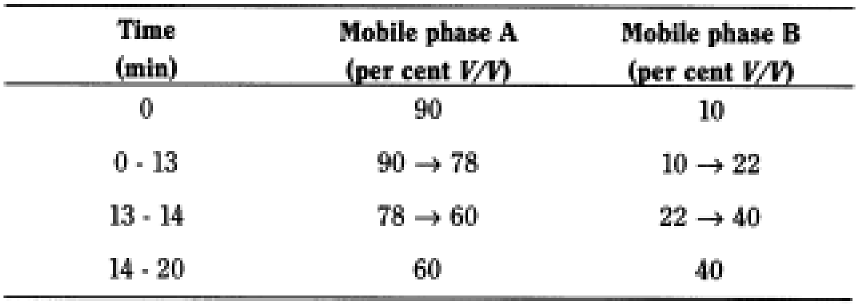
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: *phosphoric acid R, water R* (1:999 *V/V*);

— *mobile phase B*: *acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0	90	10
0 - 13	90 → 78	10 → 22
13 - 14	78 → 60	22 → 40
14 - 20	60	40

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 330 nm.

Injection 10 µl.

Relative retention With reference to chlorogenic acid (retention time = about 7 min): caftaric acid = about 0.8; caffeic acid = about 1.5; cynarin = about 1.6; echinacoside = about 1.7; cichoric acid = about 2.3.

System suitability Reference solution:

— *resolution*: minimum 10 between the peaks due to caffeic acid and chlorogenic acid.

Locate the peaks due to caffeic acid and chlorogenic acid using the chromatogram obtained with the reference solution. Locate the peaks due to echinacoside and cynarin using the chromatogram in Figure 1822.–1.

Calculate the percentage content of echinacoside from the following expression:

$$\frac{A_1 \times C_2 \times 100 \times 2.221}{A_2 \times C_1}$$

A_1 = area of the peak due to echinacoside in the chromatogram obtained with the test solution;

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;

C_1 = concentration of the test solution, in milligrams per millilitre;

C_2 = concentration of chlorogenic acid in the reference solution, in milligrams per millilitre;

2.221 = peak correlation factor between chlorogenic acid and echinacoside.

STORAGE

Uncomminuted.

Ph Eur

Echinacea Purpurea Herb

General Notices

(Purple Coneflower Herb, Ph Eur monograph 1823)

Ph Eur

DEFINITION

Dried, whole or cut flowering aerial parts of *Echinacea purpurea* (L.) Moench.

Content

Minimum 0.1 per cent for the sum of caftaric acid ($C_{13}H_{12}O_9$; M_r 312.2) and cichoric acid ($C_{22}H_{18}O_{12}$; M_r 474.3) (dried drug).

IDENTIFICATION

First identification A, B, C.

Second identification A, B, D.

A. The herbaceous perennial plant is 60-150 cm, rarely up to 180 cm high. The stem is green to red, upright and slightly branched. The leaves are alternate, ovate to ovate-lanceolate, irregularly serrate, rugose on both surfaces, dark green with prominent light green veins; the lamina is thick and shiny. The involucral bracts of the large capitulum are arranged in 2 or 3 rows. The solid receptacle is slightly convex. Each of the outer violet ligulate florets (4-6 cm) and of the inner violet-pink tubular florets is attached to a reddish acute and coriaceous bract, which overtops the tubular florets. The calyx is reduced to a very short crown, one of the sepals is up to 1 mm long.

B. Reduce to a powder (355) *(2.9.12)*. The powder is green. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: whitish-green groups of fibres, 150-200 µm in length, 10-15 µm in diameter, sometimes with black deposits; fragments of leaves in surface view showing anomocytic or anisocytic stomata *(2.8.3)* (about 35-40 µm in length); uniseriate covering trichomes or fragments thereof consisting mainly of 3 or 4 thick-walled cells of which the apical cell is markedly longer than the others; fragments of leaves with rosette-like arranged epidermal cells around the base of the covering trichomes; uniseriate glandular trichomes composed of very thin-walled cells; pitted parenchymatous cells from the pith of the stem as well as pitted elongated cells from the mesocarp of the achenes; fragments of parenchyma from the seeds with oil droplets; fragments of the epidermis of ligulate florets composed of red to violet papillous cells; spheroidal pollen grains, 30-40 µm in diameter, with a spiny exine.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* and sonicate for 5 min. Centrifuge and use the supernatant solution.

Reference solution Dissolve 0.5 mg of *caffeic acid R* and 0.5 mg of *chlorogenic acid R* in 5.0 ml of *methanol R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *Anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (3:3:9:15 *V/V/V/V*).

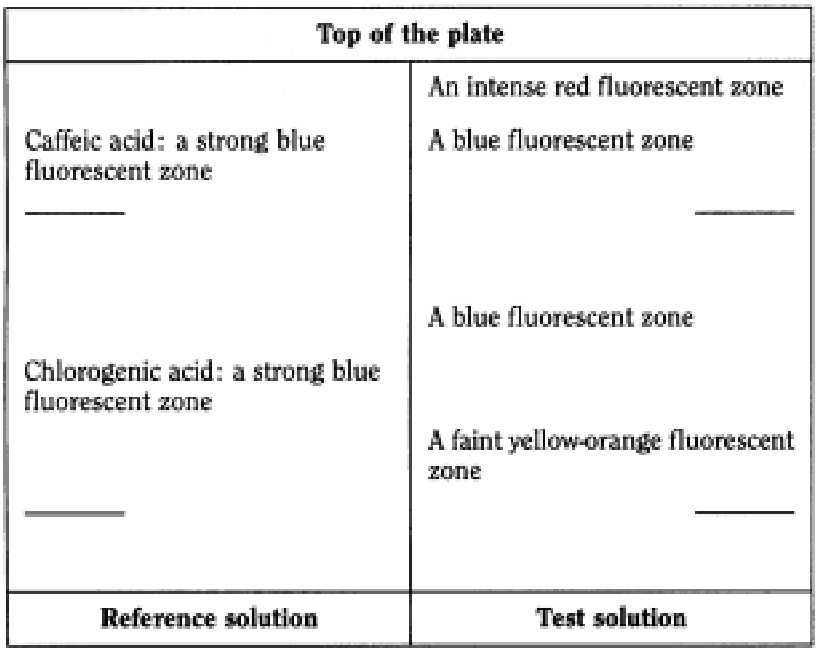
Application 25 µl [or 5 µl] of the test solution and 10 µl [or 2 µl] of the reference solution, as bands.

Development Over a path of 15 cm [or 5 cm].

Drying In a stream of cold air for about 10 min, then at 100 °C for 2 min.

Detection Spray the still-warm plate with a 5 g/l solution of *diphenylboric acid aminoethyl ester R* in *ethyl acetate R*; after 30 min, examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint blue fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	An intense red fluorescent zone
Caffeic acid: a strong blue fluorescent zone	A blue fluorescent zone
———	———
	A blue fluorescent zone
Chlorogenic acid: a strong blue fluorescent zone	
	A faint yellow-orange fluorescent zone
———	———
Reference solution	**Test solution**

D. Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution is due to cichoric acid and a smaller peak is due to caftaric acid. Peaks due to caffeic acid and chlorogenic acid are minor or may be absent.

TESTS

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 12.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution In a 100 ml volumetric flask place 0.500 g of the powdered drug (355) *(2.9.12)* and add 80 ml of *ethanol (70 per cent V/V) R*. Sonicate for 15 min and dilute to 100.0 ml with *ethanol (70 per cent V/V) R*. Mix the suspension and allow to stand for a few minutes to allow visible solids to settle.

Reference solution Dissolve 10.0 mg of *chlorogenic acid CRS* and 10.0 mg of *caffeic acid R* in *ethanol (70 per cent V/V) R*, sonicate for 15 min and dilute to 10.0 ml with the same solvent. Dilute 4.0 ml of this solution to 100.0 ml with *ethanol (70 per cent V/V) R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

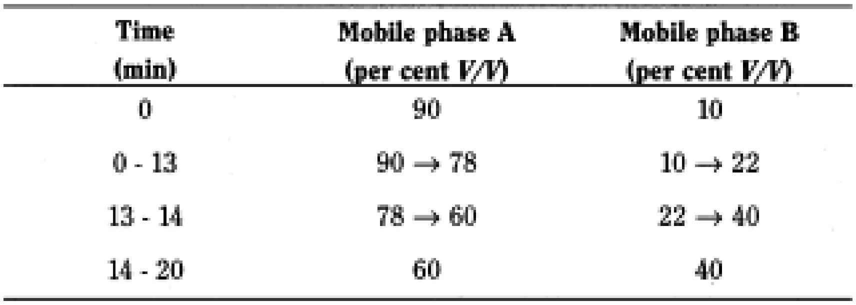
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: *phosphoric acid R, water R* (1:999 *V/V*);

— *mobile phase B*: *acetonitrile R* ;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0	90	10
0 - 13	90 → 78	10 → 22
13 - 14	78 → 60	22 → 40
14 - 20	60	40

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 330 nm.

Injection 10 µl.

Relative retention With reference to chlorogenic acid (retention time = about 7 min): caftaric acid = about 0.8; caffeic acid = about 1.5; cynarin = about 1.6; echinacoside = about 1.7; cichoric acid = about 2.3.

System suitability Reference solution:

— *resolution*: minimum 5 between the peaks due to caffeic acid and chlorogenic acid.

Locate the peaks due to caffeic acid and chlorogenic acid using the chromatogram obtained with the reference solution. Locate the peaks due to caftaric acid and cichoric acid using the chromatogram in Figure 1823.-1.

Calculate the percentage content of caftaric acid using the following expression:

$$\frac{A_1 \times C_2 \times 100 \times 0.881}{A_2 \times C_1}$$

Calculate the percentage content of cichoric acid using the following expression:

$$\frac{A_3 \times C_2 \times 100 \times 0.695}{A_2 \times C_1}$$

A_1 = area of the peak due to caftaric acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;

A_3 = area of the peak due to cichoric acid in the chromatogram obtained with the test solution;

C_1 = concentration of the test solution, in milligrams per millilitre;

C_2 = concentration of chlorogenic acid in the reference solution, in milligrams per millilitre;

0.695 = peak correlation factor based upon the liquid chromatography response observed;

0.881 = peak correlation factor between caftaric acid and chlorogenic acid.

STORAGE

Uncomminuted.

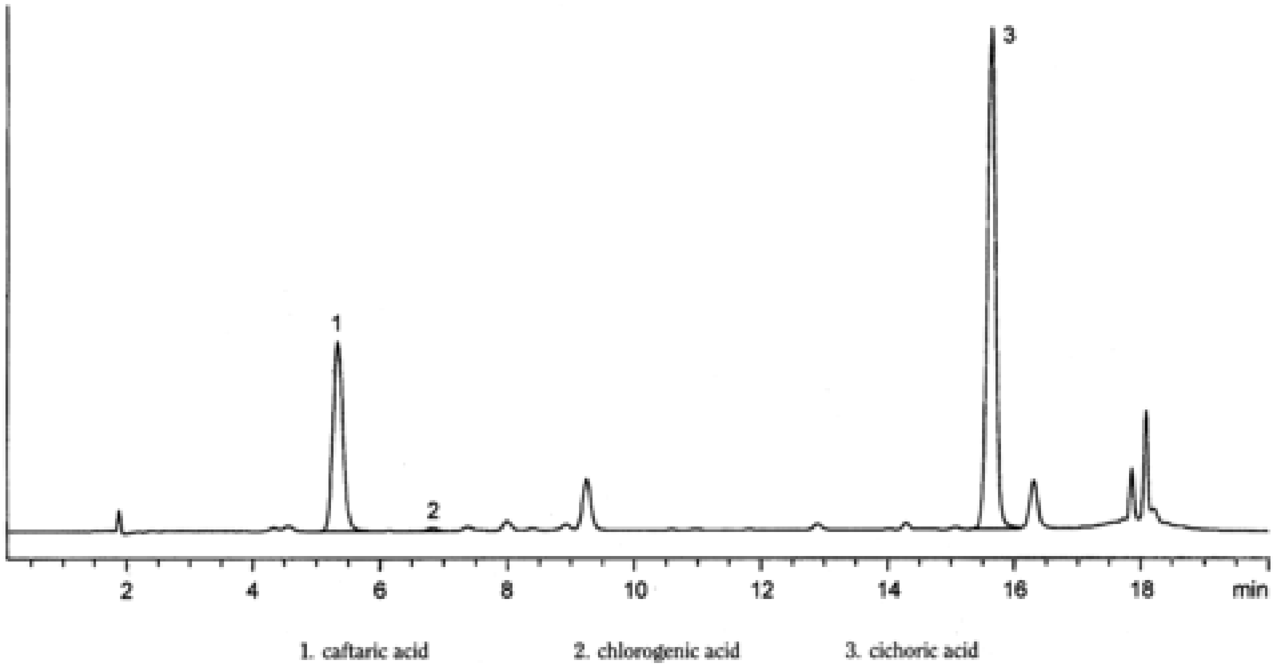


Figure 1823.-1. – *Chromatogram for the assay of caftaric acid and cichoric acid in purple coneflower herb*

Ph Eur

Echinacea Purpurea Root

General Notices

(Purple Coneflower Root, Ph Eur monograph 1824)

Ph Eur

DEFINITION

Dried, whole or cut underground parts of *Echinacea purpurea* (L.) Moench.

Content

Minimum 0.5 per cent for the sum of caftaric acid ($C_{13}H_{12}O_9$; M_r 312.2) and cichoric acid ($C_{22}H_{18}O_{12}$; M_r 474.3) (dried drug).

IDENTIFICATION

First identification *A, B, C, E.*

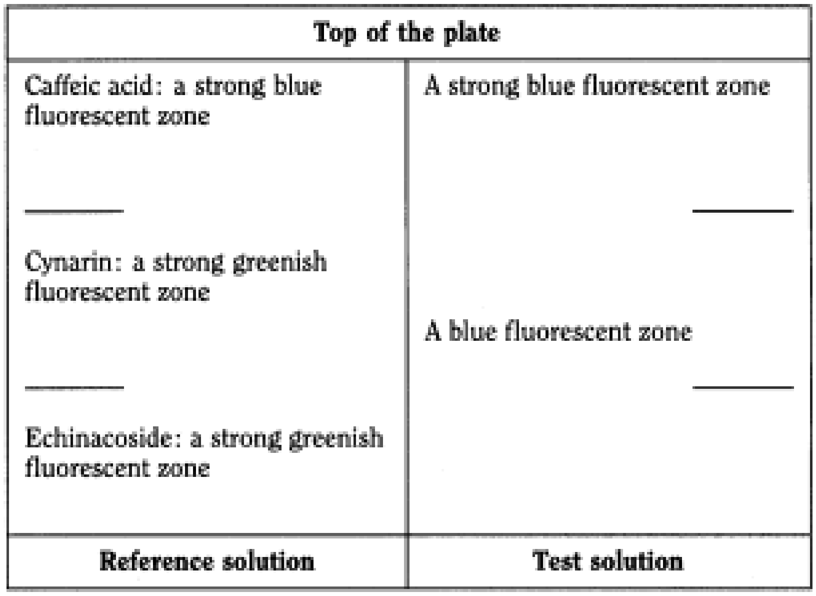
Second identification *A, B, D, E.*

A. The rhizome is up to 15 cm long, branched, reddish-brown to dark brown on the surface and carries many stem bases; the inside is fibrous and white. The numerous roots are spirally twisted, light to dark brown and show a fine cross structuring on the surface.

B. Reduce to a powder (355) *(2.9.12)*. The powder is light yellow to pinkish-beige. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous light-brown spindle-shaped fibres that are joined together in long bundles without black deposits; rare sclereids from the rhizomes and roots, usually occuring singly, those from the rhizomes being isodiametric, about 60 µm in diameter, with black deposits, those from the roots being 50-120 µm in length with no black deposits; secretory cavities up to 180 µm in diameter with yellow oil droplets; squarish to rectangular cells of the outer layers, some with reddish walls; bordered-pitted vessels from the rhizome, 30-40 µm in diameter.

C. Examine the chromatogram obtained in the test for other *Echinacea* species and *Parthenium integrifolium*.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, faint greenish fluorescent zones may be present just below the zone situated in the middle of the chromatogram obtained with the test solution.



Top of the plate	
Caffeic acid: a strong blue fluorescent zone	A strong blue fluorescent zone
_______	_______
Cynarin: a strong greenish fluorescent zone	A blue fluorescent zone
_______	_______
Echinacoside: a strong greenish fluorescent zone	
Reference solution	**Test solution**

D. Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution is due to cichoric acid and a smaller peak is due to caftaric acid. Peaks due to caffeic acid and chlorogenic acid are minor or may be absent.

E. Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methylene chloride R* and sonicate for 5 min. Centrifuge and use the supernatant solution.

Reference solution
Dissolve 1 mg of β*-sitosterol R* and a volume of *N-isobutyldodecatetraenamide solution R* corresponding to 1 mg of *N-isobutyldodecatetraenamide* R in 5.0 ml of *methanol R* .

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *Anhydrous formic acid R, cyclohexane R, ethyl acetate R, toluene R* (0.9:3:6:24 *V/V/V/V*).

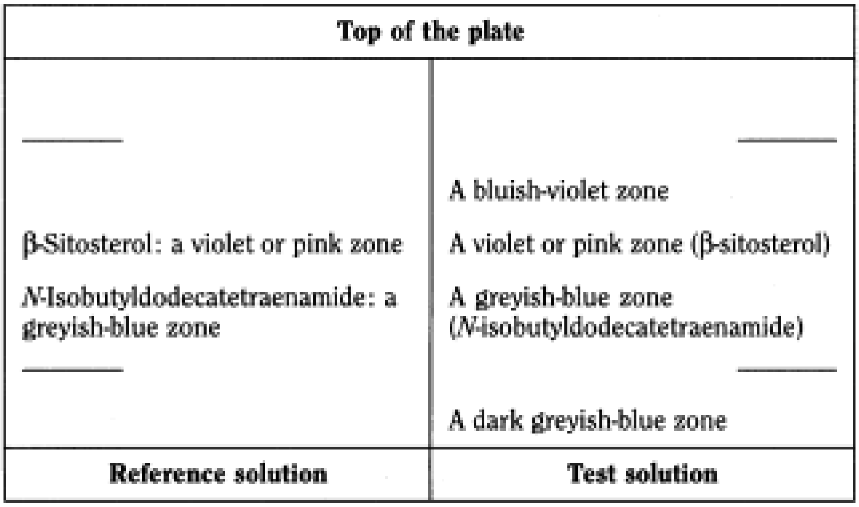
Application 25 µl [or 5 µl], as bands.

Development Over a path of about 15 cm [or 5 cm].

Drying In a stream of cold air for about 10 min.

Detection Dip the plate into *anisaldehyde solution R* for 1 s and heat at 100-105 °C for 3 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
	A bluish-violet zone
β-Sitosterol: a violet or pink zone	A violet or pink zone (β-sitosterol)
N-Isobutyldodecatetraenamide: a greyish-blue zone	A greyish-blue zone (*N*-isobutyldodecatetraenamide)
———	———
	A dark greyish-blue zone
Reference solution	**Test solution**

TESTS

Other *Echinacea* species and *Parthenium integrifolium*

Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* and sonicate for 5 min. Centrifuge and use the supernatant solution.

Reference solution Dissolve 1 mg of *echinacoside R* , 1 mg of *cynarin R* and 0.5 mg of *caffeic acid R* in 5.0 ml of *methanol R* .

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (3:3:9:15 *V/V/V/V*).

Application 10 µl [or 5 µl] of the test solution and 5 µl [or 2 µl] of the reference solution, as bands.

Development Over a path of 10 cm [or 5 cm].

Drying In a stream of cold air for about 10 min, then at 105 °C for 2 min.

Detection Spray the still-warm plate with a 5 g/l solution of *diphenylboric acid aminoethyl ester R* in *ethyl acetate R* ; after 30 min, examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows no greenish fluorescent zone corresponding to the zone due to echinacoside in the chromatogram obtained with the reference solution, and no greenish fluorescent zone corresponding to the zone due to cynarin in the chromatogram obtained with the reference solution. No other zones apart from very faint dark blue fluorescent zones are seen in the lower half of the chromatogram of the test solution.

Foreign matter *(2.8.2)*

Maximum 3 per cent.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 9.0 per cent.

Ash insoluble in hydrochloric acid ***(2.8.1)***

Maximum 2.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)* .

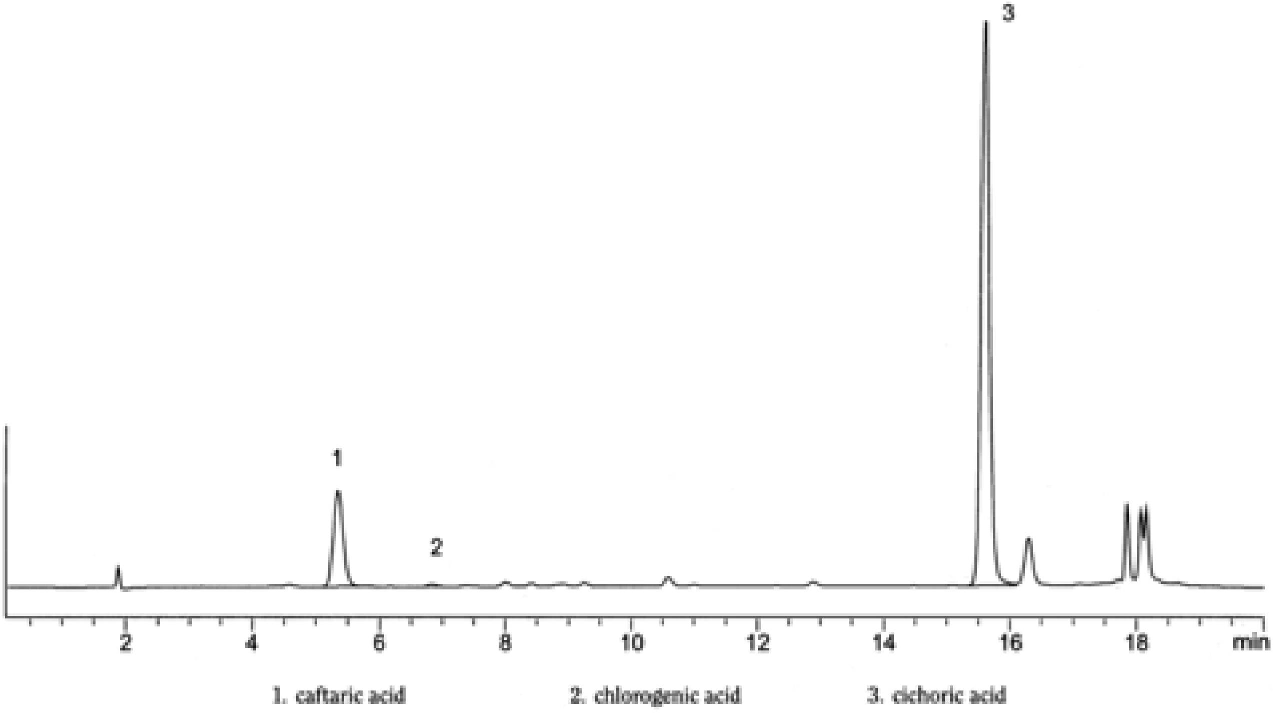


Figure 1824.-1. – *Chromatogram for the assay of caftaric acid and cichoric acid in purple coneflower root*

Test solution In a 100 ml volumetric flask place 0.500 g of the powdered drug (355) *(2.9.12)* and add 80 ml of *ethanol (70 per cent V/V) R*. Sonicate for 15 min and dilute to 100.0 ml with *ethanol (70 per cent V/V) R*. Mix the suspension and allow to stand for a few minutes to allow visible solids to settle.

Reference solution Dissolve 10.0 mg of *chlorogenic acid CRS* and 10.0 mg of *caffeic acid R* in *ethanol (70 per cent V/V) R*, sonicate for 15 min and dilute to 10.0 ml with the same solvent. Dilute 4.0 ml of this solution to 100.0 ml with *ethanol (70 per cent V/V) R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: *phosphoric acid R, water R* (1:999 *V/V*);

— *mobile phase B*: *acetonitrile R* ;

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0	90	10
0 - 13	90 → 78	10 → 22
13 - 14	78 → 60	22 → 40
14 - 20	60	40

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 330 nm.

Injection 10 µl.

Relative retention With reference to chlorogenic acid (retention time = about 7 min): caftaric acid = about 0.8; caffeic acid = about 1.5; cynarin = about 1.6; echinacoside = about 1.7; cichoric acid = about 2.3.

System suitability Reference solution:

— *resolution*: minimum 5 between the peaks due to caffeic acid and chlorogenic acid.

Locate the peaks due to caffeic acid and chlorogenic acid using the chromatogram obtained with the reference solution. Locate the peaks due to caftaric acid and cichoric acid using the chromatogram in Figure 1824.-1.

Calculate the percentage content of caftaric acid using the following expression:

$$\frac{A_1 \times C_2 \times 100 \times 0.881}{A_2 \times C_1}$$

Calculate the percentage content of cichoric acid using the following expression:

$$\frac{A_3 \times C_2 \times 100 \times 0.695}{A_2 \times C_1}$$

A_1 = area of the peak due to caftaric acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;

A_3 = area of the peak due to cichoric acid in the chromatogram obtained with the test solution;

C_1 = concentration of the dried drug in the test solution, in milligrams per millilitre;

C_2 = concentration of chlorogenic acid in the reference solution, in milligrams per millilitre;

0.695 = peak correlation factor based upon the liquid chromatography response observed;

0.881 = peak correlation factor between caftaric acid and chlorogenic acid.

STORAGE

Uncomminuted.

Ph Eur

Elder Flower

General Notices

(Ph Eur monograph 1217)

Ph Eur

DEFINITION

Dried flowers of *Sambucus nigra* L.

Content

Minimum 0.80 per cent of flavonoids, expressed as isoquercitroside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The flower, about 5 mm in diameter, has 3 small bracts (hand lens) and may have a peduncle. The 5-toothed calyx is small; the corolla is light yellow, with 5 broadly oval petals fused at their bases into a tube. The filaments of the 5 yellow stamens alternate with the petals. The corolla is often isolated or attached to the stamens, to which it is fused at the base. The ovary is inferior with 3 locules and it bears a short style with 3 obtuse stigmata.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-yellow. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous spherical, sometimes ellipsoidal, pollen grains about 30 µm in diameter, with 3 germinal pores and very finely pitted exine; calyx epidermal cells with a striated cuticle and occasional unicellular marginal teeth from the basal region; corolla fragments with numerous small globules of volatile oil, those of the upper epidermis with slightly thickened and beaded walls and a striated cuticle; mesophyll cells of petals and sepals with idioblasts containing numerous sandy crystals of calcium oxalate.

C. Examine the chromatograms obtained in the test for *Sambucus ebulus*.

Detection In ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows an intense light blue fluorescent zone due to chlorogenic acid, an orange fluorescent zone due to rutin as well as an orange fluorescent zone due to isoquercitrin, which appears slightly above the zone due to hyperoside in the chromatogram obtained with the reference solution. A greenish-blue fluorescent zone appears in the chromatogram obtained with the test solution a little below the caffeic acid zone in the chromatogram obtained with the reference solution. Additional faint fluorescent zones may be present. In daylight, only the orange fluorescent zones due to rutin and isoquercitroside in the chromatogram obtained with the test solution are clearly visible.

TESTS

Foreign matter *(2.8.2)*

Maximum 8 per cent of fragments of coarse pedicels and other foreign matter and maximum 15 per cent of discoloured, brown flowers. Carry out the determination on 10 g.

Sambucus ebulus L

Thin-layer chromatography *(2.2.27)* .

Test solution To 0.5 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* and heat in a water bath at 65 °C for 5 min, shaking frequently. Allow to cool and filter. Dilute the filtrate to 10 ml with *methanol R* .

Reference solution Dissolve 1 mg of *caffeic acid R* , 1 mg of *chlorogenic acid R* , 2.5 mg of *hyperoside R* and 2.5 mg of *rutin R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (10:10:30:50 *V/V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray the plate whilst hot with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* ; subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R* . Allow to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the lower half, with increasing R_F values, the orange fluorescent zone due to rutin, the light blue fluorescent zone due to chlorogenic acid and the orange-yellow or orange-brown fluorescent zone due to hyperoside. The upper third presents a greenish-blue fluorescent zone due to caffeic acid. The chromatogram obtained with the test solution does not show a pink zone below the zone due to rutin in the chromatogram obtained with the reference solution.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

ASSAY

Stock solution In a 100 ml round-bottomed flask, introduce 0.600 g of the powdered drug (355) *(2.9.12)*, add 1 ml of a 5 g/l solution of *hexamethylenetetramine R* , 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1* . Boil the mixture under a reflux condenser for 30 min. Filter the mixture through a plug of absorbent cotton into a flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R*

, each time boiling under a reflux condenser for 10 min. Allow to cool, filter each extract through the plug of absorbent cotton into the flask. After cooling, filter the combined acetone extracts through a filter paper into a volumetric flask, dilute to 100.0 ml with *acetone R* by rinsing the flask and the filter paper. Introduce 20.0 ml of this solution into a separating funnel, add 20 ml of *water R* and shake the mixture with 1 quantity of 15 ml and then 3 quantities, each of 10 ml, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, wash with 2 quantities, each of 50 ml, of *water R*, and filter the extracts over 10 g of *anhydrous sodium sulphate R* into a volumetric flask and dilute to 50.0 ml with *ethyl acetate R*.

Test solution To 10.0 ml of the stock solution add 1 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation liquid Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

After 30 min, measure the absorbance *(2.2.25)* of the test solution at 425 nm, by comparison with the compensation liquid.

Calculate the percentage content of flavonoids, expressed as isoquercitroside, using the following expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of isoquercitroside to be 500.

A = absorbance at 425 nm,

m = mass of the drug to be examined, in grams.

STORAGE

Store protected from light.

Ph Eur

Eleutherococcus

General Notices

Siberian Ginseng

(Ph Eur monograph 1419)

Ph Eur

DEFINITION

Dried, whole or cut underground organs of *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim.

Content

Minimum 0.08 per cent for the sum of eleutheroside B (M_r 372.4) and eleutheroside E (M_r 742.7).

IDENTIFICATION

A. The rhizome is knotty, of irregular cylindrical shape, 1.5 cm to 4.0 cm in diameter; the surface is rugged, longitudinally wrinkled and greyish-brown to blackish-brown; the bark, about 2 mm thick, closely adheres to the xylem; the heartwood is light brown and the sapwood is pale yellow; the fracture shows short thin fibres in the bark and is coarsely fibrous, especially in the internal part of the xylem. The lower surface bears numerous cylindrical and knotty roots, 3.5 cm to 15 cm long and 0.3 cm to 1.5 cm in diameter; with a smooth, greyish-brown to blackish-brown surface; the bark is about 0.5 mm thick, closely adhering to the pale yellow xylem; the fracture is slightly fibrous; in places where the outer layer has been removed, the outer surface is yellowish-brown.

B. Reduce to a powder (355) *(2.9.12)*. The powder is yellowish-brown. Examine under a microscope, using *chloral hydrate solution R* . The powder shows numerous groups of thick-walled, lignified fibres; fragments of reticulate and bordered pitted vessels with a wide lumen; groups of secretory canals, up to 20 µm in diameter with brown contents; parenchymatous cells containing cluster crystals of calcium oxalate 10 µm to 50 µm in diameter. Examine under a microscope, using a 50 per cent *V/V* solution of *glycerol R* . The powder shows small starch granules, rounded to slightly angular in outline, single compounds or with 2 or 3 components.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *alcohol (50 per cent V/V) R* and boil under reflux for 1 h. Cool and filter. Evaporate the filtrate to dryness on a water-bath. Dissolve the residue in 2.5 ml of a mixture of 5 volumes of *water R* and 20 volumes of *alcohol (50 per cent V/V) R* and filter.

Reference solution Dissolve 2.0 mg of *esculin R* and 2.0 mg of *catalpol R* in 20 ml of a mixture of 2 volumes of water R and 8 volumes of *alcohol (50 per cent V/V) R* .

Plate *TLC silica gel plate R.*

Mobile phase *water R, methanol R, methylene chloride R* (4:30:70 *V/V/V*).

Application 20 µl, as bands.

Development Over a path of 10 cm.

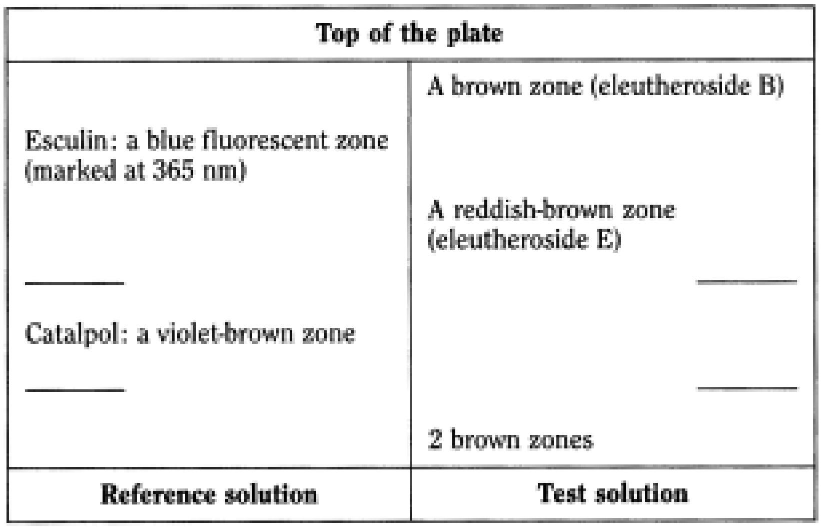
Drying In air.

Detection A Examine in ultraviolet light at 365 nm.

Results A The chromatogram obtained with the reference solution shows in the upper half a blue fluorescent zone (esculin).

Detection B Spray with *anisaldehyde solution R* and examine in daylight while heating at 100-105 °C for 5-10 min.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	A brown zone (eleutheroside B)
Esculin: a blue fluorescent zone (marked at 365 nm)	
	A reddish-brown zone (eleutheroside E)
_______	_______
Catalpol: a violet-brown zone	
_______	_______
	2 brown zones
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 8.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution To 0.500 g of the powdered drug (355) *(2.9.12)* in a 100 ml round-bottomed flask, add 30 ml of a mixture of equal volumes of *alcohol R* and *water R* . Heat in a water-bath at 60 °C for 30 min. Allow to cool and filter through a sintered-glass filter *(2.1.2)* . Collect the liquid in a 250 ml round-bottomed flask. Repeat this operation twice, using the residue obtained in the filtration step instead of the powdered drug. Add both fractions of supernatant liquid to the 250 ml round-bottomed flask. Evaporate under reduced pressure until about 10 ml of supernatant liquid is left in the flask. Transfer the supernatant liquid quantitatively to a 20.0 ml volumetric flask and dilute to 20.0 ml with a mixture of equal volumes of *alcohol R* and *water R* . Filter through a nylon filter (pore size 0.45 µm).

Reference solution (a) Dissolve 10 mg of *ferulic acid R* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 20.0 ml with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *caffeic acid R* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 20.0 ml with the same mixture of solvents.

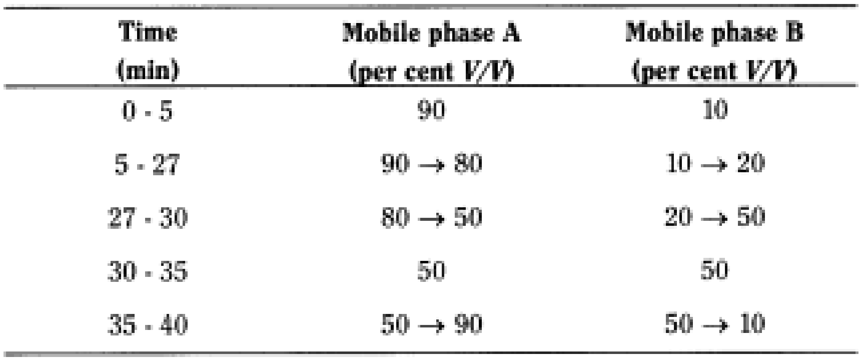
Reference solution (c) Transfer 1 ml of reference solution (a) to a 25 ml volumetric flask and dilute to 25.0 ml with a mixture of equal volumes of *methanol R* and *water R* . Filter through a nylon filter (pore size 0.45 µm).

Reference solution (d) Transfer 1 ml of reference solution (a) and 1 ml of reference solution (b) in a mixture of equal volumes of *methanol R* and *water R* and dilute to 25.0 ml with the same mixture of solvents. Filter through a nylon filter (pore size 0.45 µm).

Precolumn:
— *size: l* = 4 mm, Ø = 4.6 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *phosphoric acid R, water R* (0.5:99.5 *V/V*),
— *mobile phase B*: *acetonitrile for chromatography R,*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 27	90 → 80	10 → 20
27 - 30	80 → 50	20 → 50
30 - 35	50	50
35 - 40	50 → 90	50 → 10

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of the test solution and reference solutions (c) and (d).

Retention time Eleutheroside B = about 10 min; eleutheroside E = about 22 min.

Locate the peaks due to eleutheroside B and eleutheroside E using the UV spectra shown in Figures 1419.-1 and 1419.-2.

System suitability Reference solution (d):

— *resolution*: minimum 15 between the peaks due to caffeic acid and ferulic acid.

Calculate the total percentage content of eleutheroside B and eleutheroside E from the expression:

$$\frac{(A_{\mathrm{B}} \times C \times 0.73 \times 2)}{(A_{\mathrm{R}} \times m)} + \frac{(A_{\mathrm{E}} \times C \times 1.90 \times 2)}{(A_{\mathrm{R}} \times m)}$$

A_{B} = area of the peak due to eleutheroside B in the chromatogram obtained with the test solution,

A_{E} = area of the peak due to eleutheroside E in the chromatogram obtained with the test solution,

A_{R} = area of the peak due to ferulic acid in the chromatogram obtained with reference solution (c),

C = concentration of ferulic acid in reference solution (c), in micrograms per millilitre,

m = mass of the drug to be examined, in milligrams.

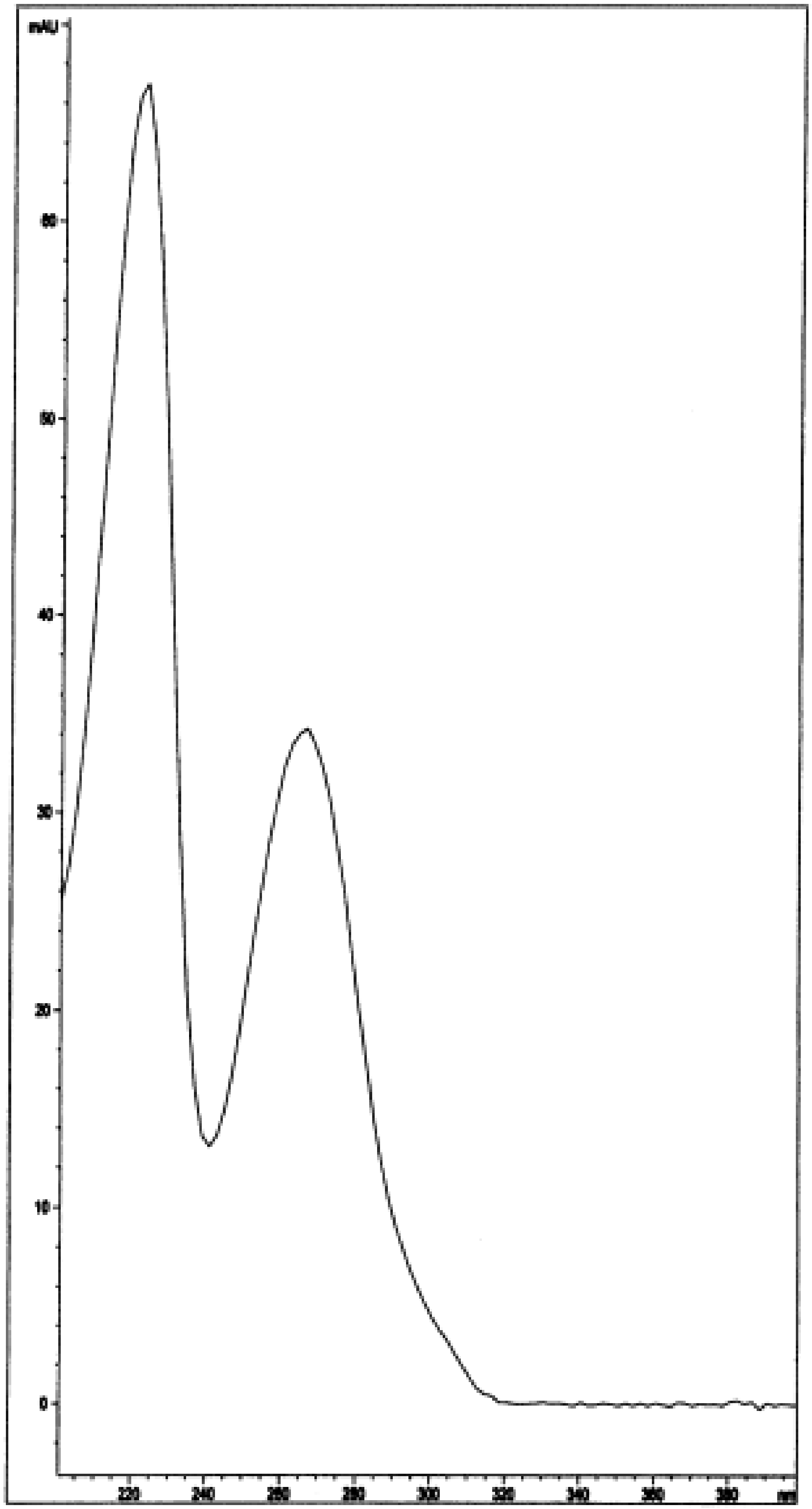


Figure 1419.-1. – *UV spectrum of eleutheroside B for the assay of eleutherococcus*

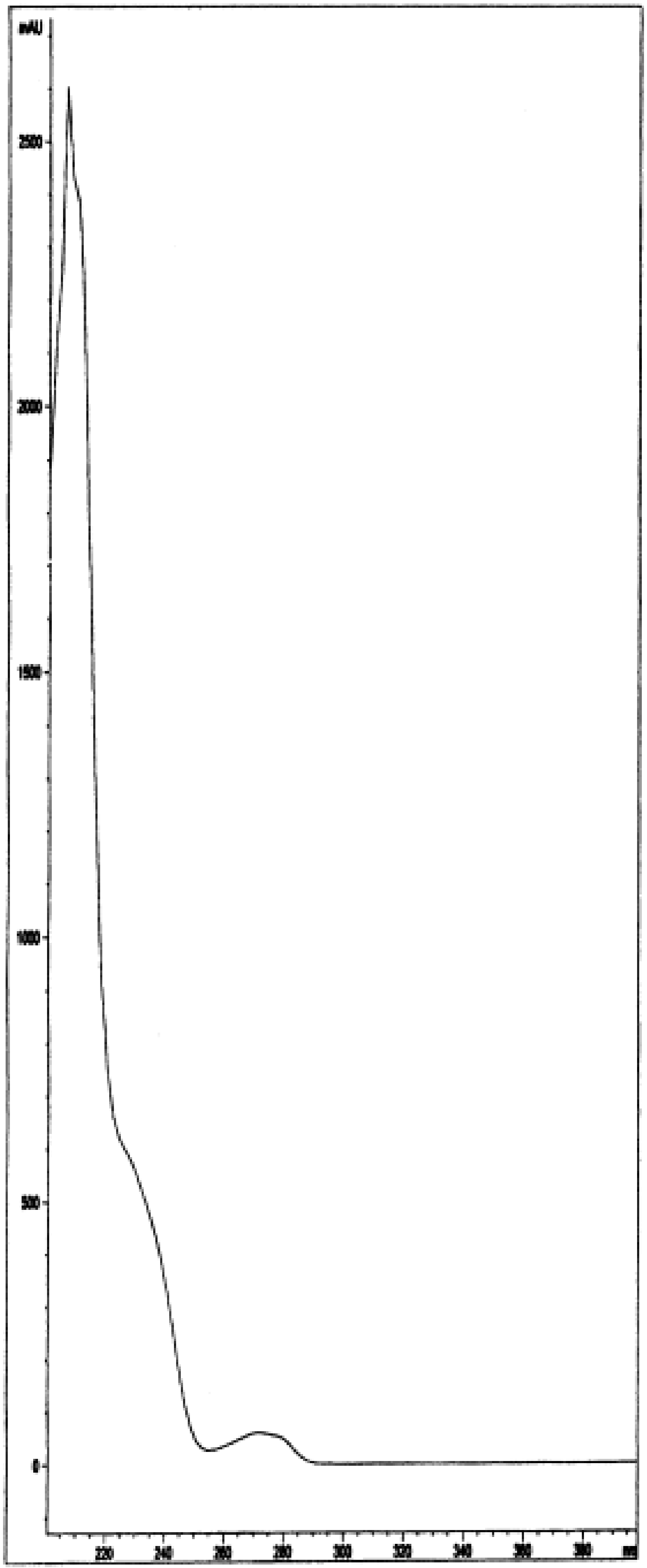


Figure 1419.-2. – *UV spectrum of eleutheroside E for the*

assay of eleutherococcus

Ph Eur

Essential Oils

General Notices

(Ph Eur monograph 2098)

Ph Eur

The statements in this monograph are intended to be read in conjunction with individual monographs on essential oils in the European Pharmacopoeia. Application of the monograph to other essential oils may be decided by the competent authority.

DEFINITION

Odorous product, usually of complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or a suitable mechanical process without heating. Essential oils are usually separated from the aqueous phase by a physical process that does not significantly affect their composition.

Essential oils may be subjected to a suitable subsequent treatment. Thus an essential oil may be commercially known as being deterpenated, desesquiterpenated, rectified or 'x'-free.

— A *deterpenated essential oil* is an essential oil from which monoterpene hydrocarbons have been removed, partially or totally.

— A *deterpenated and desesquiterpenated essential oil* is an essential oil from which mono- and sesquiterpene hydrocarbons have been removed, partially or totally.

— A *rectified essential oil* is an essential oil that has been subjected to fractional distillation to remove certain constituents or modify the content.

— An *x-free essential oil* is an essential oil that has been subjected to partial or complete removal of one or more constituents.

PRODUCTION

Depending on the monograph, the plant raw material may be fresh, wilted, dried, whole, broken or ground.

Steam distillation The essential oil is produced by the passage of steam through the plant raw material in a suitable apparatus. The steam may be introduced from an external source or generated by boiling water below the raw material or by boiling water in which the raw material is immersed. The steam and oil vapours are condensed. The water and essential oil are separated by decantation.

Dry distillation The essential oil is produced by high-temperature heating of stems or barks in a suitable apparatus without the addition of water or steam.

Mechanical process The essential oil, usually known as 'cold-pressed', is produced by a mechanical process without any heating. It is mainly applied to *Citrus* fruit and involves expression of the oil from the pericarp and subsequent separation by physical means.

In certain cases, a suitable antioxidant may be added to the essential oil.

CHARACTERS

The appearance and the odour of the essential oil is determined.

IDENTIFICATION

Essential oils are identified by their gas chromatographic profile, or failing this, by any other test that may be required (for example, a test by thin-layer chromatography).

TESTS

GENERAL TESTS

The essential oil complies with the prescribed limits for the following tests.

Relative density ***(2.2.5)***

Refractive index ***(2.2.6)***

Optical rotation ***(2.2.7)***

Fatty oils and resinified essential oils ***(2.8.7)***

SUPPLEMENTARY TESTS

If necessary, the essential oil complies with the prescribed limits for the following tests.

Freezing point ***(2.2.18)***

Acid value ***(2.5.1)***

Peroxide value ***(2.5.5)***

Foreign esters ***(2.8.6)***

Residue on evaporation ***(2.8.9)***

Water ***(2.8.5)***

Solubility in alcohol ***(2.8.10)***

Falsification

If appropriate, a test for one or more falsifications may be carried out by thin-layer chromatography *(2.2.27)* , by gas chromatography *(2.2.28)* using a chiral column if necessary, or by any other suitable method.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

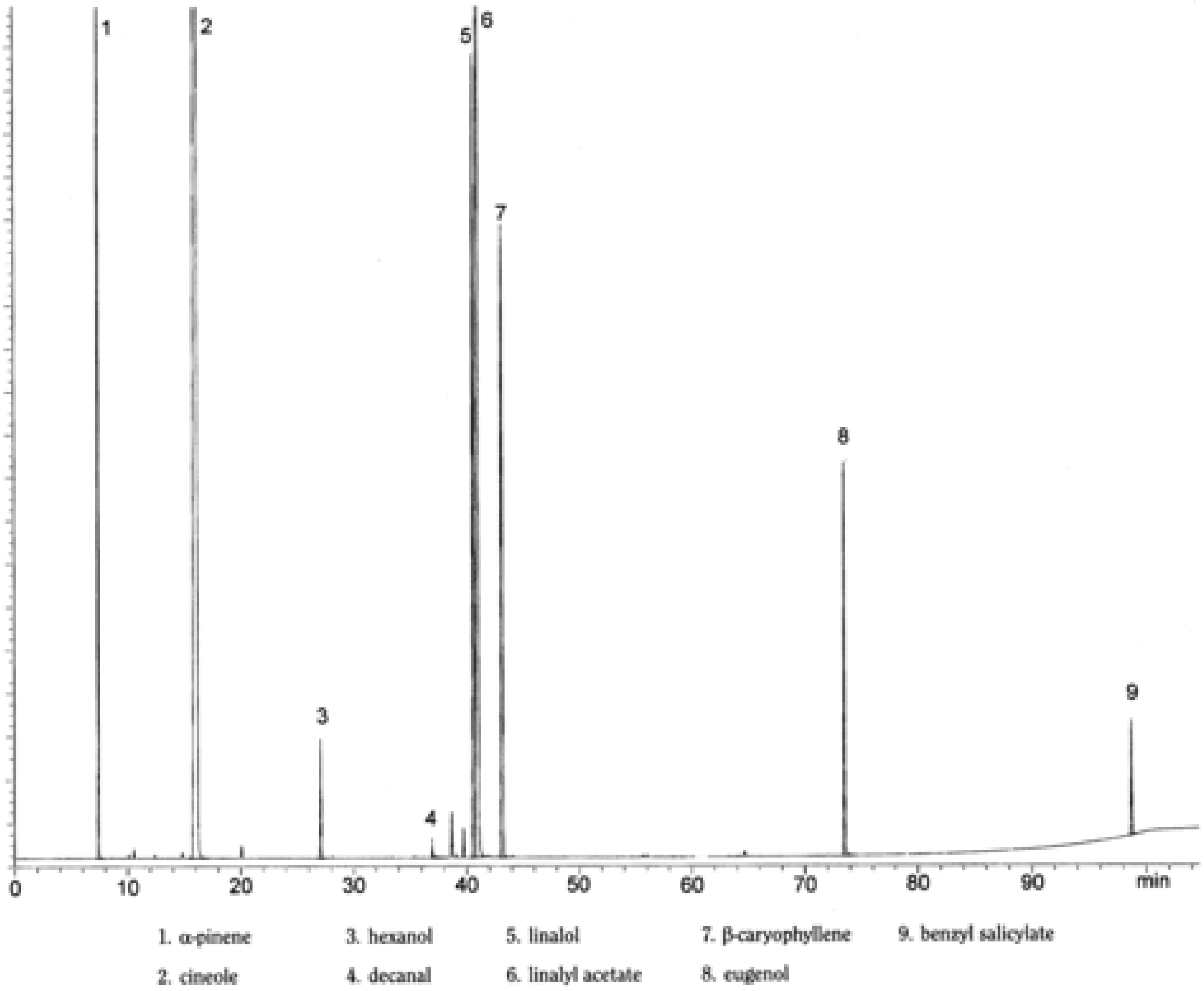


Figure 2098.-1. – *Chromatogram for the test for chromatographic profile of essential oils*

In addition to the system suitability test given in the specific monograph, it is necessary to check the suitability of the chromatographic system using the following test, which is to be carried out periodically within the framework of performance qualification.

The chromatogram shown in Figure 2098.-1 is given as an example.

Reference solution *essential oil CRS*. If necessary, the reference solution can be diluted with *heptane R* .

Column:

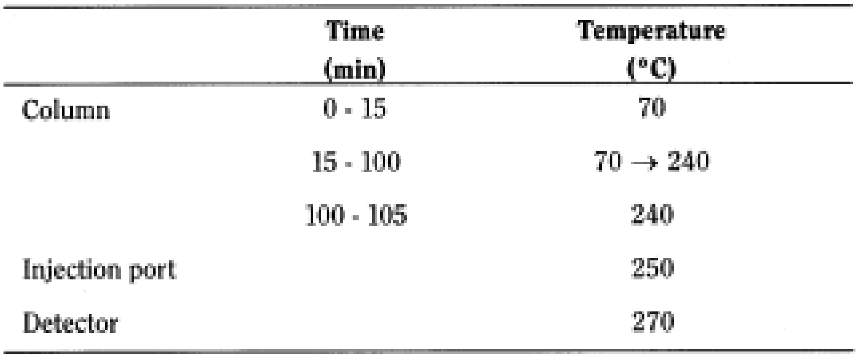
- *material*: fused silica;
- *size: l* = 60 m, Ø = 0.25 mm;
- *stationary phase: macrogol 20 000 R* (0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.5 ml/min.

Split ratio 1:500. The split ratio/injection volume can be adjusted in order to fit the specific equipment used, provided that the column load stays the same.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 15	70
	15 - 100	70 → 240
	100 - 105	240
Injection port		250
Detector		270

Detection Flame ionisation.

Injection 1 µl.

Identification of components Use the chromatogram supplied with *essential oil CRS*.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to linalol and linalyl acetate;

— *signal-to-noise ratio* : minimum 100 for the peak due to decanal;

— *limits*: the percentage content of each of the 9 components is within the limits stated on the leaflet provided with *essential oil CRS*.

STORAGE

In a well-filled, airtight container, protected from light.

LABELLING

The label states:

— the scientific name of the plant raw material used;

— where applicable, the type and/or the chemotype of the essential oil;

— where applicable, the method of production;

— where applicable, the name and concentration of any added antioxidant;

— where applicable, additional processing steps that are not specified under Definition.

Ph Eur

Eucalyptus Leaf

General Notices

(Ph Eur monograph 1320)

Ph Eur

DEFINITION

Whole or cut dried leaves of older branches of *Eucalyptus globulus* Labill.

Content

Minimum 20 ml/kg of essential oil for the whole drug (anhydrous drug) and minimum 15 ml/kg of essential oil for the cut drug (anhydrous drug).

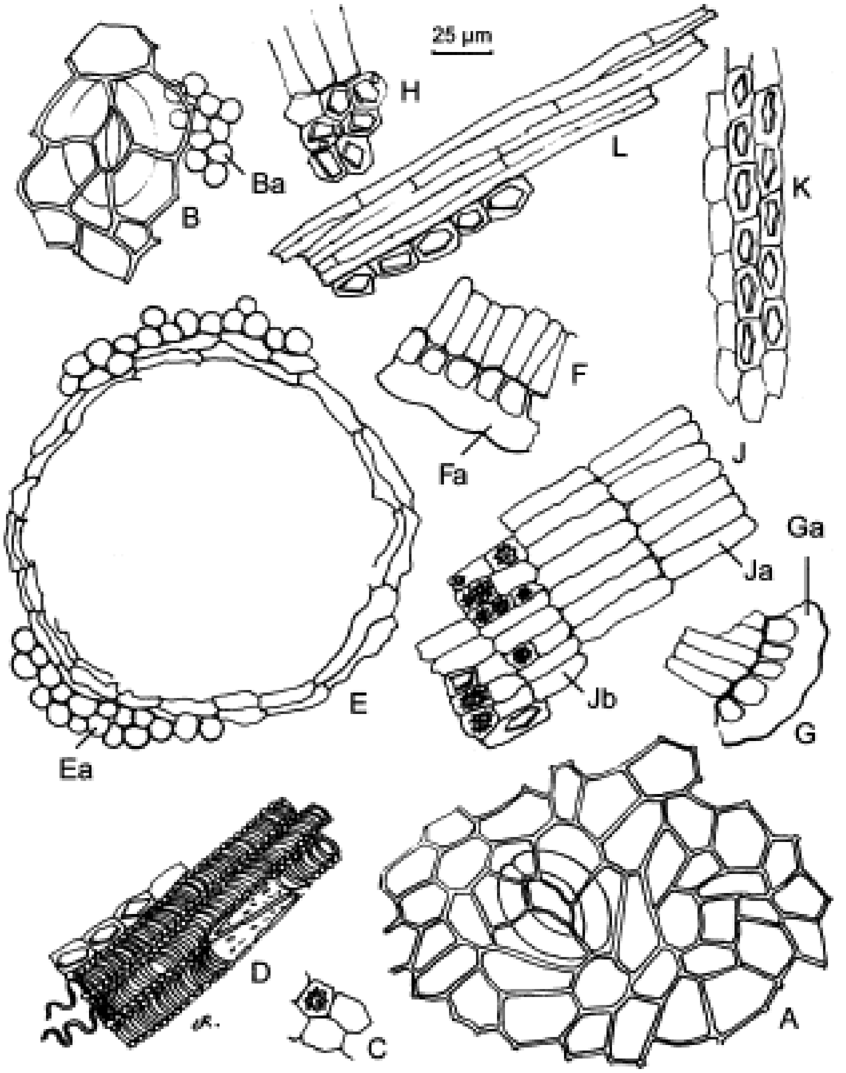
CHARACTERS

Aromatic odour of cineole.

IDENTIFICATION

A. The leaves which are mainly greyish-green and relatively thick are elongated, elliptical and slightly sickle-shaped and usually up to 25 cm in length, and up to 5 cm in width. The petiole is twisted, strongly wrinkled and is 2-3 cm, rarely 5 cm, in length. The coriaceous, stiff leaves are entire and glabrous and have a yellowish-green mid rib. Lateral veins anastomose near the margin to a continuous line. The margin is even and somewhat thickened. On both surfaces are minute, irregularly distributed, warty dark brown spots. Small oil glands may be seen in transmitted light.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green. Examine under a microscope, using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of glabrous lamina with small thick-walled epidermal cells bearing a thick cuticle, numerous anomocytic stomata *(2.8.3)* of more than 80 µm in diameter and occasionally groups of brown cork cells, 300 µm in diameter and brownish-black in their centre; fragments of isobilateral mesophyll with 2-3 layers of palisade parenchyma on each side and in the centre several layers of spongy mesophyll with elongated cells with the same orientation as the palisade cells and containing prisms and cluster crystals of calcium oxalate; fragments of mesophyll containing large schizogenous oil glands.



A. Thick-walled epidermal cells and anomocytic stomata, in surface view

B. Thick-walled epidermal cells and anomocytic stomata, with attached palisade parenchyma (Ba), in surface view

C. Parenchyma cells with cluster crystal of calcium oxalate

D. Vascular tissue

E. Schizogenous oil gland with attached palisade parenchyma (Ea)

F and G. Epidermis covered by a thick cuticle (Fa and Ga), in transverse section

H and J. Palisade parenchyma (Ja) with attached spongy mesophyll (Jb) containing prisms and cluster crystals of calcium oxalate

K. Cells containing prisms of calcium oxalate

L. Fibres

Figure 1320.-1. – ***Illustration of powdered herbal drug of eucalyptus leaf (see Identification B)***

C. Thin-layer chromatography *(2.2.27)* .

Test solution Shake 0.5 g of the freshly powdered drug (355) *(2.9.12)* with 5 ml of *toluene R* for 2-3 min and filter over about 2 g of *anhydrous sodium sulphate R* .

Reference solution Dissolve 50 µl of *cineole R* in *toluene R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R* .

Mobile phase *ethyl acetate R* , *toluene R* (10:90 V/V).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* . Examine in daylight while heating at 100-105 °C for 5-10 min.

Results The chromatogram obtained with the reference solution shows in the middle a zone due to cineole. The main zone in the chromatogram obtained with the test solution is similar in position and colour to the zone due to cineole in the chromatogram obtained with the reference solution, it also shows an intense violet zone (hydrocarbons) near the solvent front and there may also be other fainter zones.

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent of dark and brown leaves, maximum 5 per cent of stems and maximum 2 per cent of other foreign matter. Cordate or ovate sessile leaves of young branches, with numerous glands on both sides, visible as points in transmitted light, are not present. Determine by using 30 g of the drug to be examined.

Water *(2.2.13)*

Maximum 100 ml/kg, determined on 20.0 g of powdered drug (355) *(2.9.12)*.

Total ash *(2.4.16)*

Maximum 6.0 per cent.

ASSAY

Carry out the determination of essential oil in herbal drugs *(2.8.12)* . Use 10.0 g of the drug, cut immediately before determination, a 500 ml round-bottomed flask, 200 ml of *water R* and 100 ml of *glycerol R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

Ph Eur

Eucalyptus Oil

General Notices

(Ph Eur monograph 0390)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branchlets of various species of *Eucalyptus* rich in 1,8-cineole. The species mainly used are *Eucalyptus globulus* Labill., *Eucalyptus polybractea* R.T. Baker and *Eucalyptus smithii* R.T. Baker.

CHARACTERS

Appearance

Colourless or pale yellow liquid.

Aromatic and camphoraceous odour, pungent and camphoraceous taste.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.1 g of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 µl of *cineole R* in *toluene R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R, toluene R* (10:90 *V/V*).

Application 10 µl as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and examine in daylight while heating at 100-105 °C for 5-10 min.

Results The chromatogram obtained with the reference solution shows in the middle part a zone due to cineole; the chromatogram obtained with the test solution shows a main zone similar in position and colour to the zone due to cineole in the chromatogram obtained with the reference solution. Other weaker zones may be present.

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The chromatogram obtained with the test solution shows 5 principal peaks similar in retention time to the 5 principal peaks in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

0.906 to 0.927.

Refractive index *(2.2.6)*

1.458 to 1.470.

Optical rotation *(2.2.7)*

0° to + 10°.

Solubility in alcohol *(2.8.10)*

It is soluble in 5 volumes of *ethanol (70 per cent V/V) R*.

Aldehydes

To 10 ml in a ground-glass-stoppered tube 25 mm in diameter and 150 mm long, add 5 ml of *toluene R* and 4 ml of *alcoholic hydroxylamine solution R*. Shake vigorously and titrate immediately with *0.5 M potassium hydroxide in alcohol (60 per cent V/V)* until the red colour changes to yellow. Continue the titration with shaking; the end-point is reached when the pure yellow colour of the indicator is permanent in the lower layer after shaking vigorously for 2 min and allowing separation to take place. The reaction is complete in about 15 min. Repeat the titration using a further 10 ml of the substance to be examined and, as a reference solution for the end-point, the titrated liquid from the 1st determination to which has been added 0.5 ml of *0.5 M potassium hydroxide in alcohol (60 per cent V/V)*. Not more than 2.0 ml of *0.5 M potassium hydroxide in alcohol (60 per cent V/V)* is required in the 2nd titration.

Chromatographic profile

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution The substance to be examined.

Reference solution Dissolve 80 µl of *α-pinene R*, 10 µl of *β-pinene R*, 10 µl of *sabinene R*, 10 µl of *α-phellandrene R*, 10 µl of *limonene R*, 0.8 ml of *cineole R* and 10 mg of *camphor R* in 10 ml of *acetone R*.

Column:

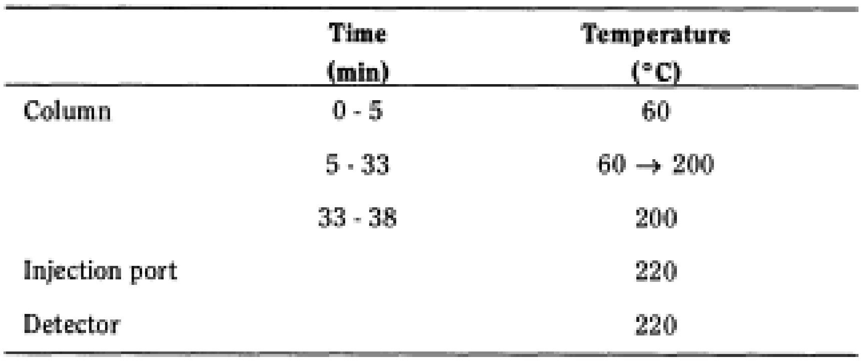
- *material*: fused silica;
- *size: l* = 60 m, Ø = about 0.25 mm;
- *stationary phase*: bonded *macrogol 20 000 R*.

Carrier gas *helium for chromatography R.*

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	60
	5 - 33	60 → 200
	33 - 38	200
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 0.5 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to limonene and cineole;

— *number of theoretical plates* : minimum 30 000, calculated for the peak due to limonene at 110 °C.

Identification of components Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of each of these components. The percentages are within the following ranges:

— α-*pinene* : traces to 9.0 per cent;

— β-*pinene* : less than 1.5 per cent;

— *sabinene* : less than 0.3 per cent;

— α-*phellandrene* : less than 1.5 per cent;

— *limonene* : traces to 12.0 per cent;

— *1,8-cineole* : minimum 70.0 per cent;

— *camphor* : less than 0.1 per cent.

STORAGE

At a temperature not exceeding 25 °C.

European Goldenrod

General Notices

(Ph Eur monograph 1893)

Ph Eur

DEFINITION

Whole or cut, dried, flowering aerial parts of *Solidago virgaurea* L.

Content

Minimum 0.5 per cent and maximum 1.5 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

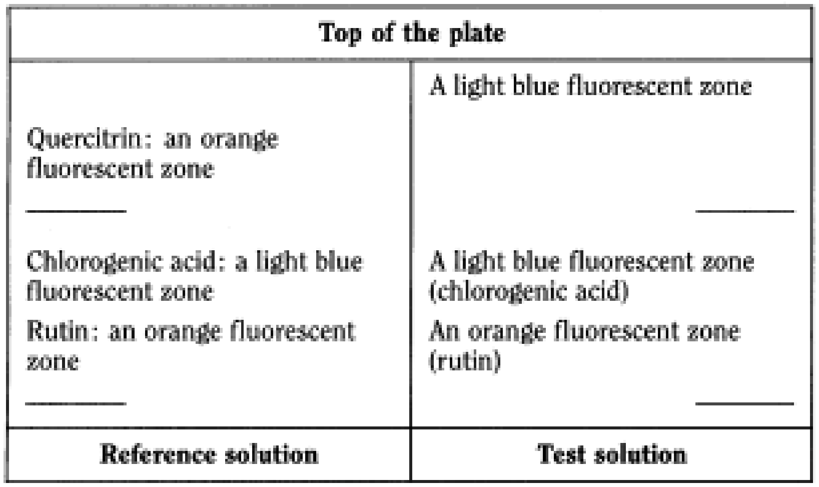
A. The stem is cylindrical, striated, the lower part often reddish-violet, sometimes entirely glabrous or pubescent with short, bent, apically directed hairs. The basal leaves are obovate to oblanceolate, with a serrate margin, and taper at the base into a long, winged petiole; the cauline leaves are alternate, smaller than the basal leaves and more elliptical in outline, with an entire or slightly toothed margin; they are sessile or with only a short petiole. Both surfaces of the leaves are glabrous or only slightly pubescent with a prominent reticulate venation on the lower surface. The capitula form a tightly packed panicle. At the base of the pedicels there are 2, small, linear bracts with scarious margins. The involucre consists of 2-4 rows of loosely-arranged, imbricate bracts, each bract greenish-yellow with a smooth and shiny inner surface, the outer surface hairy or glabrous, with a scarious margin. Each capitulum contains from 6-12 widely separated female ray florets, about twice as long as the bracts, and about 10-30 hermaphrodite, tubular florets. All florets are yellow. The brown, inferior ovary tapers towards the base and has a ribbed surface, covered with scattered hairs; it is surmounted by a whitish pappus composed of smooth or rough, bristly hairs.

B. Reduce to a powder (355) *(2.9.12)*. The powder is light green. Examine under a microscope using *chloral hydrate solution R*. The powder shows fragments of the leaf in surface view, those of the upper epidermis composed of polygonal cells with straight, beaded walls and distinct cuticular striations, those of the lower epidermis more sinuous with fewer striations and numerous anomocytic stomata *(2.8.3)*; occasional leaf fragments showing cells containing small, isolated cluster crystals of calcium oxalate; uniseriate, conical covering trichomes from the leaves and the bracts with up to about 10 cells, some of the shorter trichomes showing a terminal cell extended and pennant-like; occasional glandular trichomes with a 1 or 2 celled stalk and a unicellular, elongated head; rare paired, covering trichomes from the ovary with a distinctly pitted central wall and a bifid apex; abundant pappus hairs and their fragments, multiseriate with the marginal cells overlapping outwards; groups of fibres and vascular tissue from the stems; fragments of the epidermis of the petals with striated cuticle and occasional long, biseriate glandular trichomes; pollen grains spherical, with 3 pores and a spiny exine.

C. Thin-layer chromatography *(2.2.27)* as described in the test for *Solidago gigantea* and

Solidago canadensis.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A light blue fluorescent zone
Quercitrin: an orange fluorescent zone	
———	———
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
Rutin: an orange fluorescent zone	An orange fluorescent zone (rutin)
———	———
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of brown coloured matter and maximum 5 per cent of other foreign matter.

Solidago gigantea and *Solidago Canadensis*

Thin-layer chromatography *(2.2.27)*.

Test solution To 0.75 g of the powdered drug (355) *(2.9.12)* add 5 ml of *methanol R* and heat on a water-bath under a reflux condenser for 10 min. Cool and filter.

Reference solution Dissolve 1.0 mg of *chlorogenic acid R*, 2.5 mg of *quercitrin R* and 2.5 mg of *rutin R* in 10 ml of *methanol R*.

Plate TLC silica gel plate R.

Mobile phase anhydrous formic acid R, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (6:6:18:30 *V/V/V/V*).

Application 20 µl as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray the plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Examine in ultraviolet light at 365 nm after 30 min.

Results The chromatogram obtained with the test solution shows no strong orange fluorescent zone similar in position to the zone of quercitrin in the chromatogram obtained with the reference solution.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 8.0 per cent.

ASSAY

Stock solution In a 100 ml round-bottomed flask, place 0.200 g of the powdered drug (355) *(2.9.12)*, add 1 ml of a 5 g/l solution of *hexamethylenetetramine R*, 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1*. Boil the mixture in a water-bath under a reflux condenser for 30 min. Filter the liquid through a small plug of absorbent cotton into a 100 ml flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R*, each time boiling under a reflux condenser for 10 min. Allow to cool. Filter the combined acetone extracts through filter paper, dilute to 100.0 ml with *acetone R*, rinsing the volumetric flask and the filter paper with acetone. Introduce 20.0 ml of the solution into a suitable separating funnel, add 20 ml of *water R* and shake the mixture with 1 quantity of 15 ml and then with 3 quantities, each of 10 ml, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, wash twice with 50 ml of *water R* and filter the extracts over 10 g of *anhydrous sodium sulphate R* into a volumetric flask. Dilute to 50.0 ml with *ethyl acetate R*, rinsing the separating funnel and the sodium sulphate.

Test solution To 10.0 ml of the stock solution add 1.0 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation liquid Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

After 30 min, measure the absorbance *(2.2.25)* of the test solution at 425 nm by comparison with the compensation liquid.

Calculate the percentage content of flavonoids, expressed as hyperoside, from the following expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of hyperoside to be 500.

A = measured absorbance at 425 nm,

m = mass of the drug to be examined, in grams.

Ph Eur

EXTRACTS

General Notices

(Ph Eur monograph 0765)

Extracts comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Extracts are preparations of liquid (liquid extracts and tinctures), semi-solid (soft extracts and oleoresins) or solid (dry extracts) consistency, obtained from herbal drugs or animal matter, which are usually in a dry state.

Where medicinal products are manufactured using extracts of animal origin, the requirements of chapter *5.1.7*. *Viral safety* apply.

Different types of extract may be distinguished. Standardised extracts are adjusted within an acceptable tolerance to a given content of constituents with known therapeutic activity; standardisation is achieved by adjustment of the extract with inert material or by blending batches of extracts. Quantified extracts are adjusted to a defined range of constituents; adjustments are made by blending batches of extracts. Other extracts are essentially defined by their production process (state of the herbal drug or animal matter to be extracted, solvent, extraction conditions) and their specifications.

PRODUCTION

Extracts are prepared by suitable methods using ethanol or other suitable solvents. Different batches of the herbal drug or animal matter may be blended prior to extraction. The herbal drug or animal matter to be extracted may undergo a preliminary treatment, for example, inactivation of enzymes, grinding or defatting. In addition, unwanted matter may be removed after extraction.

Herbal drugs, animal matter and organic solvents used for the preparation of extracts comply with any relevant monograph of the Pharmacopoeia. For soft and dry extracts where the organic solvent is removed by evaporation, recovered or recycled solvent may be used, provided that the recovery procedures are controlled and monitored to ensure that solvents meet appropriate standards before re-use or admixture with other approved materials. Water used for the preparation of extracts is of a suitable quality. Except for the test for bacterial endotoxins, water complying with the section on Purified water in bulk in the monograph on *Purified water (0008)* is suitable. Potable water may be suitable if it complies with a defined specification that allows the consistent production of a suitable extract.

Where applicable, concentration to the intended consistency is carried out using suitable methods, usually under reduced pressure and at a temperature at which deterioration of the constituents is reduced to a minimum. Essential oils that have been separated during processing may be restored to the extracts at an appropriate stage in the manufacturing process. Suitable excipients may be added at various stages of the manufacturing process, for example to improve technological qualities such as homogeneity or consistency. Suitable stabilisers and antimicrobial preservatives may also be added.

Extraction with a given solvent leads to typical proportions of characterised constituents in the extractable matter; during production of standardised and quantified extracts, purification procedures may be applied that increase these proportions with respect to the expected values; such extracts are referred to as 'refined'.

IDENTIFICATION

Extracts are identified using a suitable method.

TESTS

Where applicable, as a result of analysis of the herbal drug or animal matter used for production and in view of the production process, tests for microbiological quality (*5.1.4*), heavy metals, aflatoxins and pesticide residues (*2.8.13*) in the extracts may be necessary.

ASSAY

Wherever possible, extracts are assayed by a suitable method.

LABELLING

The label states:

— the herbal drug or animal matter used;

— whether the extract is liquid, soft or dry, or whether it is a tincture;

— for standardised extracts, the content of constituents with known therapeutic activity;

— for quantified extracts, the content of constituents (markers) used for quantification;

— the ratio of the starting material to the genuine extract (extract without excipients) (DER);

— the solvent or solvents used for extraction;

— where applicable, that a fresh herbal drug or fresh animal matter has been used;

— where applicable, that the extract is 'refined';

— the name and amount of any excipient used including stabilisers and antimicrobial reservatives;

— where applicable, the percentage of dry residue.

LIQUID EXTRACTS – EXTRACTA FLUIDA

DEFINITION

Liquid extracts are liquid preparations of which, in general, 1 part by mass or volume is equivalent to 1 part by mass of the dried herbal drug or animal matter. These preparations are adjusted, if necessary, so that they satisfy the requirements for content of solvent, and, where applicable, for constituents.

PRODUCTION

Liquid extracts are prepared by using ethanol of a suitable concentration or water to extract the herbal drug or animal matter, or by dissolving a soft or dry extract (which has been produced using the same strength of extraction solvent as is used in preparing the liquid extract by direct extraction) of the herbal drug or animal matter in either ethanol of a suitable concentration or water. Liquid extracts may be filtered, if necessary.

A slight sediment may form on standing, which is acceptable as long as the composition of the liquid extract is not changed significantly.

TESTS

Relative density *(2.2.5)*

Where applicable, the liquid extract complies with the limits prescribed in the monograph.

Ethanol *(2.9.10)*

For alcoholic liquid extracts, carry out the determination of ethanol content. The ethanol content complies with that prescribed.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol for alcoholic liquid extracts, unless otherwise prescribed.

Dry residue *(2.8.16)*

Where applicable, the liquid extract complies with the limits prescribed in the monograph, corrected if necessary, taking into account any excipient used.

STORAGE

Protected from light.

LABELLING

The label states in addition to the requirements listed above:

— where applicable, the ethanol content in per cent *V/V* in the final extract.

TINCTURES – TINCTURAE

DEFINITION

Tinctures are liquid preparations that are usually obtained using either 1 part of herbal drug or animal matter and 10 parts of extraction solvent, or 1 part of herbal drug or animal matter and 5 parts of extraction solvent.

PRODUCTION

Tinctures are prepared by maceration or percolation (outline methodology is given below) using only ethanol of a suitable concentration for extraction of the herbal drug or animal matter, or by dissolving a soft or dry extract (which has been produced using the same strength of extraction solvent as is used in preparing the tincture by direct extraction) of the herbal drug or animal matter in ethanol of a suitable concentration. Tinctures are filtered, if necessary.

Tinctures are usually clear. A slight sediment may form on standing, which is acceptable as long as the composition of the tincture is not changed significantly.

Production by maceration

Unless otherwise prescribed, reduce the herbal drug or animal matter to be extracted to pieces of suitable size, mix thoroughly with the prescribed extraction solvent and allow to stand in a closed container for an appropriate time. The residue is separated from the extraction solvent and, if necessary, pressed out. In the latter case, the 2 liquids obtained are combined.

Production by percolation

If necessary, reduce the herbal drug or animal matter to be extracted to pieces of suitable size. Mix thoroughly with a portion of the prescribed extraction solvent and allow to stand for an appropriate time. Transfer to a percolator and allow the percolate to flow at room temperature slowly making sure that the herbal drug or animal matter to be extracted is always covered with the remaining extraction solvent. The residue may be pressed out and the expressed liquid combined with the percolate.

TESTS

Relative density *(2.2.5)*

Where applicable, the tincture complies with the limits prescribed in the monograph.

Ethanol *(2.9.10)*

The ethanol content complies with that prescribed.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol, unless otherwise prescribed.

Dry residue *(2.8.16)*

Where applicable, the tincture complies with the limits prescribed in the monograph, corrected if necessary, taking into account any excipient used.

STORAGE

Protected from light.

LABELLING

The label states in addition to the requirements listed above:

— for tinctures other than standardised and quantified tinctures, the ratio of starting material to extraction liquid or of starting material to final tincture;

— the ethanol content in per cent *V/V* in the final tincture.

SOFT EXTRACTS – EXTRACTA SPISSA

DEFINITION

Soft extracts are semi-solid preparations obtained by evaporation or partial evaporation of the solvent used for extraction.

TESTS

Dry residue ***(2.8.16)***

The soft extract complies with the limits prescribed in the monograph.

Solvents

Residual solvents are controlled as described in chapter *5.4*, unless otherwise prescribed or justified and authorised.

STORAGE

Protected from light.

OLEORESINS – OLEORESINA

DEFINITION

Oleoresins are semi-solid extracts composed of a resin in solution in an essential and/or fatty oil and are obtained by evaporation of the solvent(s) used for their production.

This monograph applies to oleoresins produced by extraction and not to natural oleoresins.

TESTS

Water ***(2.2.13)***

The oleoresin complies with the limits prescribed in the monograph.

Solvents

Residual solvents are controlled as described in chapter *5.4*, unless otherwise prescribed or justified and authorised.

STORAGE

In an airtight container , protected from light.

DRY EXTRACTS – EXTRACTA SICCA

DEFINITION

Dry extracts are solid preparations obtained by evaporation of the solvent used for their production. Dry extracts have a loss on drying of not greater than 5 per cent *m/m*, unless a loss on drying with a different limit or a test on water is prescribed in the monograph.

TESTS

Water *(2.2.13)*

Where applicable, the dry extract complies with the limits prescribed in the monograph.

Loss on drying *(2.8.17)*

Where applicable, the dry extract complies with the limits prescribed in the monograph.

Solvents

Residual solvents are controlled as described in chapter *5.4*, unless otherwise prescribed or justified and authorised.

STORAGE

In an airtight container , protected from light.

Ph Eur

Fenugreek

General Notices

(Ph Eur monograph 1323)

Ph Eur

DEFINITION

Dried, ripe seeds of *Trigonella foenum-graecum* L.

CHARACTERS

Strong characteristic aromatic odour.

IDENTIFICATION

A. The seed is hard, flattened, brown or reddish-brown and more or less rhomboidal with rounded edges. It is 3-5 mm long, 2-3 mm wide and 1.5-2 mm thick. The widest surfaces are marked by a groove that divides the seed into 2 unequal parts. The smaller part contains the radicle; the larger part contains the cotyledons.

B. Reduce to a powder (355) *(2.9.12)*. The powder is yellowish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of the testa in sectional view with thick cuticle covering lageniform epidermal cells, with an underlying hypodermis of large cells, narrower at the upper end and constricted in the middle, with bar-like thickenings of the radial walls; yellowish-brown fragments of the epidermis in surface view, composed of small, polygonal cells with thickened and pitted walls, frequently associated with the hypodermal cells, circular in outline with thickened and closely beaded walls; fragments of the hypodermis viewed from below, composed of polygonal cells whose bar-like thickenings extend to the upper and lower walls; parenchyma of the testa with elongated, rectangular cells with slightly thickened and beaded walls; fragments of endosperm with irregularly thickened, sometimes elongated cells, containing mucilage.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Place 1.0 g of the powdered drug (710) *(2.9.12)* in a 25 ml conical flask and add 5.0 ml of *methanol R* . Heat in a water-bath at 65 °C for 5 min. Cool and filter.

Reference solution Dissolve 3.0 mg of *trigonelline hydrochloride R* in 1.0 ml of *methanol R* .

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *water R* , *methanol R* (30:70 *V/V*).

Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatogram obtained with the test solution shows in its lower half a quenching zone similar in position and fluorescence to the zone in the chromatogram obtained with the reference solution.

Detection B Spray with *potassium iodobismuthate solution R2*.

Results B The chromatogram obtained with the test solution shows an intense orange-red zone similar in position and colour to the zone in the chromatogram obtained with the reference solution. It also shows in its upper half, a broad light brownish-yellow zone (triglycerides).

TESTS

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Minimum 5.0 per cent.

Swelling index *(2.8.4)*

Minimum 6, determined on the powdered drug (710) *(2.9.12)*.

Ph Eur

Feverfew

General Notices

(Ph Eur monograph 1516)

Ph Eur

DEFINITION

Dried, whole or fragmented aerial parts of *Tanacetum parthenium* (L.) Schultz Bip.

Content

Minimum 0.20 per cent of parthenolide ($C_{15}H_{20}O_3$; M_r 248.3) (dried drug).

CHARACTERS

Camphoraceous odour.

IDENTIFICATION

A. The leafy, more or less branched stem has a diameter of up to 5 mm; it is almost quadrangular, channelled longitudinally and slightly pubescent. The leaves are ovate, 2-5 cm long, sometimes up to 10 cm, yellowish-green, petiolate and alternate. They are pinnate or bipinnate, deeply divided into 5-9 segments, each with a coarsely crenate margin and an obtuse apex. Both surfaces are somewhat pubescent and the midrib is prominent on the lower surface. When present, the flowering heads are 12-22 mm in diameter with long pedicels; they are clustered into broad corymbs consisting of 5-30 flower-heads. The hemispherical involucre is 6-8 mm wide and consists of many overlapping bracts, which are rather narrow, obtuse and scarious and have membranous margins. The central flowers are yellow, hermaphrodite, tube-shaped with 5 teeth and have 5 stamens inserted in the corolla; the filaments of the stamens are separate from each other but the anthers are fused into a tube through which passes the style, bearing 2 stigmatic branches. The peripheral flowers are female and have a white, three-toothed ligule, 2-7 mm long. The fruit is an achene, 1.2-1.5 mm long, brown when ripe, with 5-10 white longitudinal ribs. It is glandular and bears a short, crenate, membranous crown.

B. Reduce to a powder (355) *(2.9.12)*. The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous large, multicellular, uniseriate covering trichomes consisting of a rhomboidal basal cell, 3-5 smaller, thick-walled rectangular cells and a very long, flat, slender terminal cell, often curved at a right angle to the axis of the basal cell; glandular trichomes with a short, biseriate, 2-4 celled stalk and a biseriate head of 4 cells around which the cuticle forms a bladder-like covering; epidermal cells with very sinuous, anticlinal walls, a striated cuticle and anomocytic stomata *(2.8.3)* ; numerous spirally and annularly thickened vessels; stratified parenchyma and collenchyma. Fragments of disc florets containing pale yellow amorphous masses and small rosette crystals of calcium oxalate may be present; spherical pollen grains about 25 µm in diameter, with 3 pores and a spiny exine may be present.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 1 g of the powdered drug (355) *(2.9.12)* add 20 ml of *methanol R* . Heat in a water-bath at 60 °C for 15 min. Allow to cool and filter. Evaporate to dryness under reduced pressure and dissolve the residue in 2 ml of *methanol R* .

Reference solution Dissolve 5 mg of *parthenolide R* in *methanol R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R* .

Mobile phase *acetone R* , *toluene R* (15:85 *V/V*).

Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 5 g/l solution of *vanillin R* in a mixture of 20 volumes of *anhydrous ethanol R* and 80 volumes of *sulphuric acid R* . Examine in daylight after 5 min.

Results The chromatogram obtained with the test solution shows in its central part a blue principal zone that is similar in position, colour and size to the principal zone in the chromatogram obtained with the reference solution, and somewhat below the principal zone a 2nd blue zone may be present; 1 or 2 blue zones are also present in its lower third; other violet zones may be present.

TESTS

Foreign matter *(2.8.2)*

Maximum 10.0 per cent of stem with a diameter greater than 5 mm and maximum 2.0 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)* .

Test solution Completely reduce about 50 g of the drug to be examined to a powder (355) *(2.9.12)*. After homogenisation, introduce 1.00 g of the powdered drug into a flask and add 40 ml of *methanol R* . Heat in a water-bath at 60 °C for 10 min. Allow to cool and filter. Rinse the filter with 15 ml of *methanol R* . Take up the residue with 40 ml of *methanol R* . Repeat the operation. Collect the filtrates and rinsings and evaporate to dryness under reduced pressure.

Take up the residue with *methanol R* and dilute to 20.0 ml with the same solvent. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase. Filter (0.45 µm).

Reference solution Dissolve 5.0 mg of *parthenolide R* in *methanol R* and dilute to 10.0 ml with the same solvent. Dilute 2.0 ml of this solution to 50.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R* , *water R* (40:60 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Retention time Parthenolide = about 11.5 min.

Calculate the percentage content of parthenolide using the following expression:

$$\frac{A_1 \times m_2 \times 40}{A_2 \times m_1}$$

A_1 = area of the peak due to parthenolide in the chromatogram obtained with the test solution;

A_2 = area of the peak due to parthenolide in the chromatogram obtained with the reference solution;

m_1 = mass of the drug to be examined in the test solution, in grams;

m_2 = mass of parthenolide in the reference solution, in grams.

Ph Eur

Fig

General Notices

DEFINITION

The sun-dried succulent fruit of *Ficus carica* L.

CHARACTERISTICS

Odour, pleasantly fruity; taste, sweet.

Macroscopical Fruit compound: soft, fleshy, brown or yellowish brown, sometimes covered with a saccharine efflorescence; at the summit a small opening surrounded by scales and at the base a short, stalk-like prolongation; fruit up to about 5 cm in length and breadth, consisting of a hollow receptacle bearing on the inner surface numerous drupelets, each containing a stone about 1.5 to 2.0 mm long; seed containing endosperm and a curved embryo.

Microscopical Receptacle: epidermal cells polyhedral, stomata raised, trichomes unicellular, thick walled, of varying length up to about 300 µm; hypodermis composed of rounded polyhedral cells, some containing small rosette crystals of calcium oxalate; aerenchyma made up of large, irregular cells, forming the greater part of the receptacle, containing large rosette crystals of calcium oxalate and interspersed with numerous latex tubes, about 30 to 50 µm wide, and slender vascular bundles. Pericarp: epicarp consisting of radially elongated cells with mucilaginous outer walls; mesocarp of delicate, often disorganised cells; endocarp of radially elongated sclereids with pitted walls. Endosperm and embryo: small cells containing aleurone grains and fixed oil; starch absent.

Water-soluble extractive

Not less than 60.0% when determined by the following method. To 25 g, minced, add 500 ml of *water*, boil under a reflux condenser for 1 hour, cool and filter. To 20 ml of the filtrate add 20 g of washed and ignited sand, evaporate to dryness in a tared, flat bottomed shallow dish and dry the residue to constant weight at 100°. Calculate the water-soluble extractive by subtracting the weight of sand from the weight of the residue obtained.

STORAGE

Figs should be stored in a dry place.

Frangula Bark

General Notices

(Ph Eur monograph 0025)

Preparation

Standardised Frangula Bark Dry Extract

When Powdered Frangula Bark is prescribed or demanded, material complying with the requirements below, with the exception of Identification test A and the test for Foreign matter, shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried, whole or fragmented bark of the stems and branches of *Rhamnus frangula* L. (*Frangula alnus* Miller).

Content

Minimum 7.0 per cent of glucofrangulins, expressed as glucofrangulin A ($C_{27}H_{30}O_{14}$; M_r 578.5) (dried drug).

IDENTIFICATION

A. The bark occurs in curved, almost flat or rolled fragments or in single or double quilled pieces usually 0.5-2 mm thick and variable in length and width. The greyish-brown or dark brown outer surface is wrinkled longitudinally and covered with numerous greyish, transversely elongated lenticels; when the outer layers are removed, a dark red layer is exposed. The orange-brown or reddish-brown inner surface is smooth and bears fine longitudinal striations; it becomes red when treated with alkali. The fracture is short, fibrous in the inner part.

B. Reduce to a powder (355) *(2.9.12)*. The powder is yellowish or reddish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: numerous phloem fibres, partially lignified, in groups with crystal sheaths containing calcium oxalate prisms; reddish-brown fragments of cork; fragments of parenchyma containing calcium oxalate cluster crystals. Sclereids are absent.

C. Examine the chromatogram obtained in test B for "Other species of *Rhamnus*; anthrones" in daylight.

Results The chromatogram obtained with the test solution shows 2 orange-brown zones (glucofrangulins) in the lower third and 2-4 red zones (frangulins, not always clearly separated, and above them frangula-emodin) in the upper third.

D. To about 50 mg of the powdered drug (180) *(2.9.12)* add 25 ml of *dilute hydrochloric acid R* and heat the mixture on a water-bath for 15 min. Allow to cool, shake with 20 ml of *ether R* and discard the aqueous layer. Shake the ether layer with 10 ml of *dilute ammonia R1*. The aqueous layer becomes reddish-violet.

TESTS

Other species of *Rhamnus*; anthrones

Thin-layer chromatography *(2.2.27)*.

Test solution To 0.5 g of the powdered drug (180) *(2.9.12)* add 5 ml of *ethanol (70 per cent V/V) R* and heat to boiling. Cool and centrifuge. Decant the supernatant solution immediately and use within 30 min.

Reference solution Dissolve 20 mg of *barbaloin R* in *ethanol (70 per cent V/V) R* and dilute to 10 ml with the same solvent.

Plates *TLC silica gel plate R* (2 plates).

Mobile phase *water R*, *methanol R*, *ethyl acetate R* (13:17:100 *V/V/V*).

A. *Application*: 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air for 5 min.

Detection Spray with a 50 g/l solution of *potassium hydroxide R* in *ethanol (50 per cent V/V) R*, and heat at 100-105 °C for 15 min. Examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows a brownish-yellow zone due to barbaloin in the central part. The chromatogram obtained with the test solution shows no zones of intense yellow fluorescence and no zone of orange or reddish fluorescence similar in position to the zone due to barbaloin in the chromatogram obtained with the reference solution.

B. *Application*: 10 µl of the test solution, as a band.

Development Over a path of 10 cm.

Drying In air for maximum 5 min.

Detection Spray immediately with a 5 g/l solution of *nitrotetrazolium blue R* in *methanol R*. Examine immediately.

Results No violet or greyish-blue zones appear.

Foreign matter *(2.8.2)*

Maximum 1 per cent.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 6.0 per cent.

ASSAY

Carry out the assay protected from bright light.

In a tared, round-bottomed flask with a ground-glass neck, weigh 0.250 g of powdered drug (180) *(2.9.12)*. Add 25.0 ml of a 70 per cent *V/V* solution of *methanol R*; mix and weigh. Heat in a water-bath under a reflux condenser for 15 min. Allow to cool, weigh and adjust to the original mass with a 70 per cent *V/V* solution of *methanol R*. Filter and transfer 5.0 ml of the filtrate to a separating funnel. Add 50 ml of *water R* and 0.1 ml of *hydrochloric acid R*. Shake with 5 quantities, each of 20 ml, of *light petroleum R*. Allow the layers to separate and transfer the aqueous layer to a 100 ml volumetric flask. Combine the light petroleum layers and wash with 2 quantities, each of 15 ml, of *water R*. Use this water for washing the separating funnel and add it to the aqueous solution in the volumetric flask. Add 5 ml of a 50 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with *water R*. Discard the light petroleum layer. Transfer 40.0 ml of the aqueous solution to a 200 ml round-bottomed flask with a ground-glass neck. Add 20 ml of a 200 g/l solution of *ferric chloride R* and heat under a reflux condenser for 20 min in a water-bath with the water level above that of the liquid in the flask. Add 2 ml of *hydrochloric acid R* and continue heating for 20 min, shaking frequently, until the precipitate is dissolved. Allow to cool, transfer the mixture to a separating funnel and shake with 3 quantities, each of 25 ml, of *ether R*, previously used to rinse the flask. Combine the ether extracts and wash with 2 quantities, each of 15 ml, of *water R*. Transfer the ether layer to a volumetric flask and dilute to 100.0 ml with *ether R*. Evaporate 20.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a 5 g/l solution *of magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 515 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of glucofrangulins, expressed as glucofrangulin A, using the following expression:

$$\frac{A \times 3.06}{m}$$

i.e. taking the specific absorbance of glucofrangulin A to be 204.

A = absorbance at 515 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Fresh Bilberry Fruit Dry Extract, Refined and Standardised

General Notices

(Ph Eur monograph 2394)

Ph Eur

DEFINITION

Refined and standardised dry extract produced from *Bilberry fruit, fresh (1602)*.

Content

32.4 per cent to 39.6 per cent of anthocyanins, expressed as cyanidin 3-*O*-glucoside chloride [chrysanthemin ($C_{21}H_{21}ClO_{11}$; M_r 484.8)] (dried extract).

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using ethanol (96 per cent *V/V*) or methanol (100 per cent). Refinement may be performed by ion-exchange chromatography.

CHARACTERS

Appearance

Dark reddish-violet, amorphous, hygroscopic powder.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.10 g of the extract to be examined in 25 ml of *methanol R* . Stir for 15 min and filter.

Reference solution Dissolve 2 mg of *chrysanthemin R* and 2 mg of *myrtillin R* in 5 ml of *methanol R* .

Plate TLC plate coated with *cellulose for chromatography R* (5-40 µm) [or TLC plate coated with *cellulose for chromatography R* (2-10 µm)].

Mobile phase:
— *mobile phase A*: *hydrochloric acid R, acetic acid R, water R* (3:15:82 *V/V/V*);

— *mobile phase B*: *water R, acetic acid R* (40:60 *V/V*).

Application 10 µl [or 2 µl] as bands of 10 mm [or 6 mm].

Development A Over a path of 10 cm [or 6 cm] with mobile phase A.

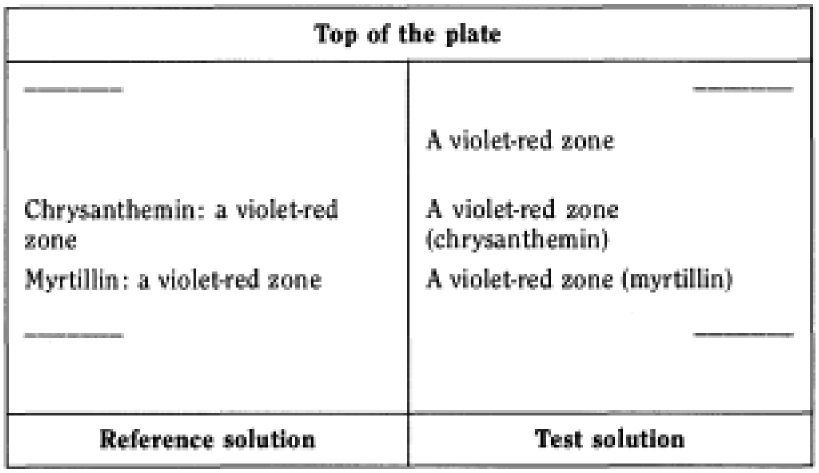
Drying A In warm air.

Development B Over a path of 10 cm [or 6 cm] with mobile phase B.

Drying B In air.

Detection Examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
	A violet-red zone
Chrysanthemin: a violet-red zone	A violet-red zone (chrysanthemin)
Myrtillin: a violet-red zone	A violet-red zone (myrtillin)
———	———
Reference solution	**Test solution**

B. Liquid chromatography *(2.2.29)* as described in the test for total anthocyanidins.

The characteristic anthocyanin peaks (peaks 1-8, 10-15 and 17) in the chromatogram obtained with the test solution are similar in their retention times to those in the chromatogram obtained with reference solution (b).

TESTS

Loss on drying *(2.8.17)*

Maximum 4.5 per cent.

Total ash *(2.4.16)*

Maximum 2.0 per cent.

Total anthocyanidins

Liquid chromatography *(2.2.29)* . *Maintain the solutions at 4 ‘C*.

Solvent mixture *hydrochloric acid R, methanol R* (2:98 *V/V*).

Test solution Dissolve 125.0 mg of the extract to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 20.0 ml with *dilute* *phosphoric acid R* .

Reference solution (a) Dissolve 10.0 mg of *cyanidin chloride CRS* in the solvent mixture and dilute to 25.0 ml with the solvent mixture. Dilute 2.0 ml of this solution to 100.0 ml with *dilute phosphoric acid R* .

Reference solution (b) Dissolve 125.0 mg of *bilberry dry extract CRS* in the solvent mixture and dilute to 25.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 20.0 ml with *dilute phosphoric acid R* .

Column:

— *size: l* = 0.250 m, Ø = 4.6 mm;

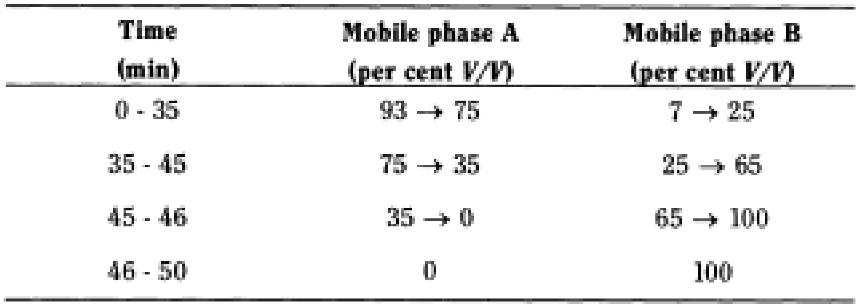
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: *anhydrous formic acid R, water R* (8.5:91.5 *V/V*);

— *mobile phase B*: *anhydrous formic acid R, acetonitrile R, methanol R, water R* , (8.5:22.5:22.5:41.5 *V/V/V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	93 → 75	7 → 25
35 - 45	75 → 35	25 → 65
45 - 46	35 → 0	65 → 100
46 - 50	0	100

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 535 nm.

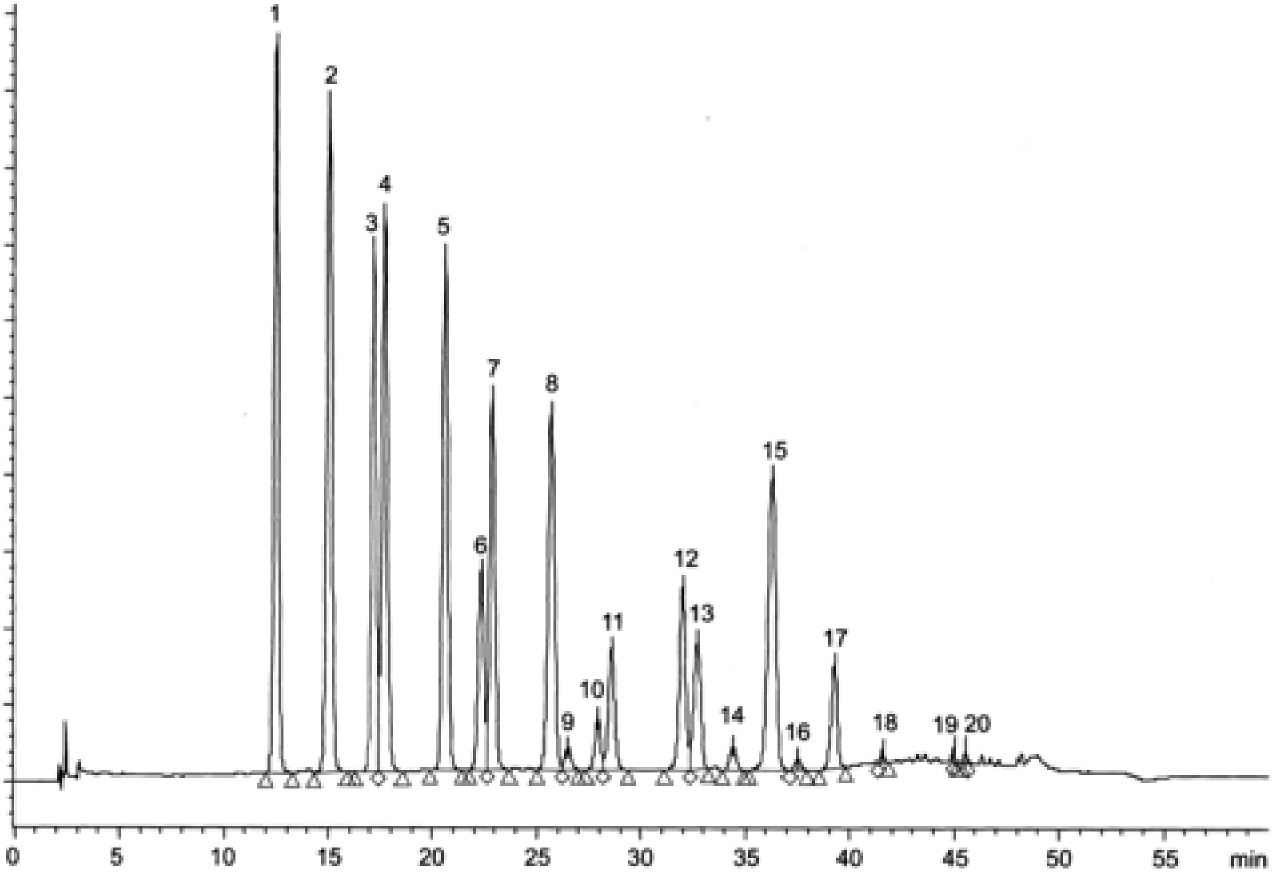
Injection 10 µl.

Identification of peaks Use the chromatogram supplied with *bilberry dry extract CRS* and the chromatograms obtained with reference solutions (a) and (b) to identify the peaks due to the anthocyanins and the anthocyanidins.

Retention times The retention times and the elution order of the peaks are similar to those shown in the chromatogram (Figure 2394.-1).

System suitability Reference solution (b):

— *peak-to-valley ratio* : minimum 2.0, where H_c = height above the baseline of the peak due to cyanidin 3-*O*-galactoside (peak 3) and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to delphinidin 3-*O*-arabinoside (peak 4).



1. delphinidin 3-*O*-galactoside chloride
2. myrtillin (delphinidin 3-*O*-glucoside chloride)
3. cyanidin 3-*O*-galactoside chloride
4. delphinidin 3-*O*-arabinoside chloride
5. chrysanthemin (cyanidin 3-*O*-glucoside chloride)
6. petunidin 3-*O*-galactoside chloride
7. cyanidin 3-*O*-arabinoside chloride
8. petunidin 3-*O*-glucoside chloride
9. delphinidin chloride
10. peonidin 3-*O*-galactoside chloride
11. petunidin 3-*O*-arabinoside chloride
12. peonidin 3-*O*-glucoside chloride
13. malvidin 3-*O*-galactoside chloride
14. peonidin 3-*O*-arabinoside chloride
15. malvidin 3-*O*-glucoside chloride
16. cyanidin chloride
17. malvidin 3-*O*-arabinoside chloride
18. petunidin chloride
19. peonidin chloride
20. malvidin chloride

Figure 2394.-1. – *Chromatogram for the assay of refined and standardised fresh bilberry fruit dry extract*

Calculate the percentage content of total anthocyanidins, expressed as cyanidin chloride, using the following expression:

$$\frac{A_1 \times m_2 \times 100 \times p}{m_1 \times A_2 \times 1250}$$

A_1 = sum of the areas of the peaks due to the anthocyanidins (peaks 9, 16, 18-20) in the chromatogram obtained with the test solution;

A_2 = area of the peak due to cyanidin chloride (peak 16) in the chromatogram obtained with reference solution (a);

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of *cyanidin chloride CRS* used to prepare reference solution (a), in grams;

p = percentage content of cyanidin chloride in *cyanidin chloride CRS*.

Limits Not more than 1.0 per cent of total anthocyanidins, expressed as cyanidin chloride.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for total anthocyanidins with the following modification.

Injection Test solution and reference solution (b).

Calculate the percentage content of total anthocyanins expressed as cyanidin 3-*O*-glucoside chloride, using the following expression:

$$\frac{A_1 \times m_2 \times p}{m_1 \times A_2}$$

A_1 = sum of the areas of the peaks due to the anthocyanins (peaks 1-8, 10-15 and 17) in the chromatogram obtained with the test solution;

A_2 = area of the peak due to cyanidin 3-*O*-glucoside chloride (peak 5) in the chromatogram obtained with reference solution (b);

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of *bilberry dry extract CRS* used to prepare reference solution (b), in grams;

p = percentage content of cyanidin 3-*O*-glucoside chloride in *bilberry dry extract CRS*.

Ph Eur

Fresh Bilberry

General Notices

(Fresh Bilberry Fruit, Ph Eur monograph 1602)

Ph Eur

DEFINITION

Fresh or frozen, ripe fruit of *Vaccinium myrtillus* L.

Content

Minimum 0.30 per cent of anthocyanins, expressed as cyanidin 3-*O*-glucoside chloride (chrysanthemin, $C_{21}H_{21}ClO_{11}$; M_r 484.8) (dried drug).

CHARACTERS

Sweet and slightly astringent taste.

IDENTIFICATION

A. The fresh fruit is a blackish-blue globular berry about 5 mm in diameter. Its lower end shows a scar or, rarely, a fragment of the pedicel. The upper end is flattened and surmounted by the remains of the persistent style and of the calyx, which appears as a circular fold. The violet, fleshy mesocarp includes 4 to 5 locules containing numerous small, brown, ovoid seeds.

B. The crushed fresh fruit is violet-red. Examine under a microscope using *chloral hydrate solution R*. It shows violet-pink sclereids from the endocarp and the mesocarp, usually aggregated, with thick, channelled walls; reddish-brown fragments of the epicarp consisting of polygonal cells with moderately thickened walls; brownish-yellow fragments of the outer layer of the testa composed of elongated cells with U-shaped thickened walls; cluster crystals of calcium oxalate.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 5 g of the freshly crushed drug, add 20 ml of *methanol R*. Stir for 15 min and filter.

Reference solution Dissolve 5 mg of *chrysanthemin R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R*, *water R*, *butanol R* (16:19:65 *V/V/V*).

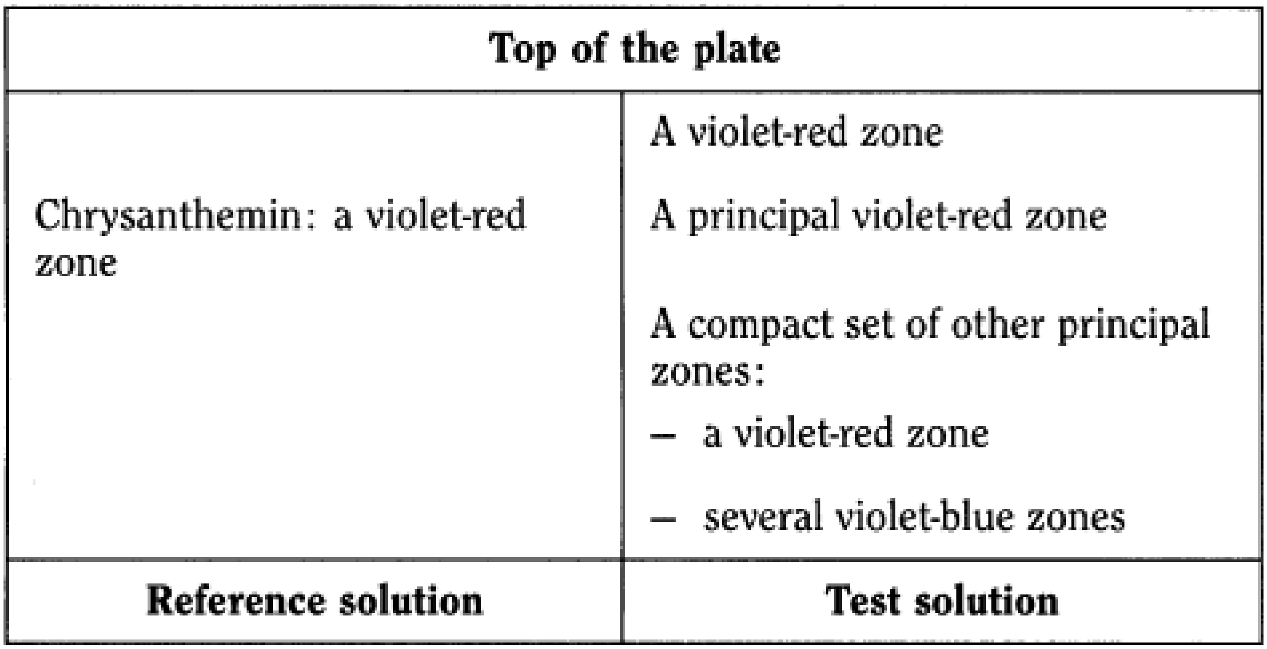
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
	A violet-red zone
Chrysanthemin: a violet-red zone	A principal violet-red zone
	A compact set of other principal zones: – a violet-red zone – several violet-blue zones
Reference solution	**Test solution**

TESTS

Total ash (*2.4.16*)

Maximum 0.6 per cent.

Loss on drying (*2.2.32*)

80.0 per cent to 90.0 per cent, determined on 5.000 g of the freshly crushed drug by drying in an oven at 105 °C.

ASSAY

Crush 50 g extemporaneously. To about 5.00 g of the crushed, accurately weighed drug, add 95 ml of *methanol R* . Stir mechanically for 30 min. Filter into a 100.0 ml volumetric flask. Rinse the filter and dilute to 100.0 ml with *methanol R* . Prepare a 50-fold dilution of this solution in a 0.1 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* .

Measure the absorbance (*2.2.25*) of the solution at 528 nm, using a 0.1 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* as the compensation liquid.

Calculate the percentage content of anthocyanins, expressed as cyanidin 3-*O*-glucoside chloride, using the following expression:

$$\frac{A \times 5000}{718 \times m}$$

718 = specific absorbance of cyanidin 3-*O*-glucoside chloride at 528 nm

A = absorbance at 528 nm

m = mass of the substance to be examined in grams.

STORAGE

When frozen, store at or below - 18 °C.

Ph Eur

Fumitory

General Notices

(Ph Eur monograph 1869)

Ph Eur

DEFINITION

Whole or fragmented, dried aerial parts of *Fumaria officinalis* L. harvested in full bloom.

Content

Minimum 0.40 per cent of total alkaloids, expressed as protopine ($C_{20}H_{19}NO_5$; M_r 353.4) (dried drug).

IDENTIFICATION

A. The hollow, angular stem is light green or greenish-brown. The leaves are alternate, bipinnatisect with 2 or 3 leaf segments, the ultimate lobes lanceolate to obovate; they are greenish-blue and glabrous on both surfaces. The flowers are small and occur in loose racemes; each has a short pedicel and is subtended by a leafy bract; they are pink to purplish-red, dark purple to brown at the apex; the calyx is short, composed of 2 petalloid sepals and the corolla is tubular with 4 petals, the upper petal slightly spurred; there are 6 stamens united by their filaments into 2 groups of 3. The greenish-brown, indehiscent fruits are globular or keel-shaped, truncated or slightly emarginate at the apex, and each contains a small brown seed.

B. Reduce to a powder (355) *(2.9.12)*. The powder is green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the leaf lamina in surface view with the upper epidermis composed of irregularly polygonal cells, some of which contain masses of very small sandy crystals; the cells of the lower epidermis having more wavy walls; anomocytic stomata *(2.8.3)* on both surfaces; marginal cells at the apex of the lamina elongated to form blunt papillae; groups of lignified fibres and reticulate and bordered-pitted vessels from the stem; fragments of the epidermis of the petals composed of polygonal cells with sinuous to wavy anticlinical walls and no papillae; pollen grains spherical, about 30 µm in diameter, with a pitted exine and 6 large pores; polygonal cells of the epicarp with a thick, warty cuticle.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 2 g of the powdered drug (355) *(2.9.12)* add 15 ml of *0.05 M sulphuric acid* and stir for 15 min. Filter. Dilute the filtrate to 20 ml with *0.05 M sulphuric acid* . Add 1 ml of *concentrated ammonia R* and 10 ml of *ethyl acetate R*. Stir and centrifuge. Collect the upper organic layer. Repeat the extraction in the same manner. Collect the organic layers and dry over *anhydrous sodium sulphate R*. Evaporate to dryness under reduced pressure. Take up the residue with *0.5 ml of methanol R*.

Reference solution Dissolve 5 mg of *protopine hydrochloride R* and 5 mg of *quinine R* in 10 ml of *methanol R.*

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R*, *ethanol (96 per cent) R*, *acetone R*, *toluene R* (2:6:40:52 *V/V/V/V*).

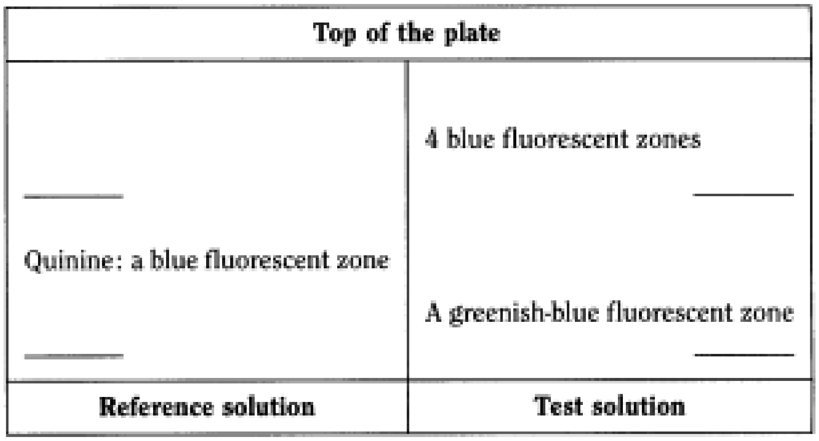
Application 30 µl as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 365 nm.

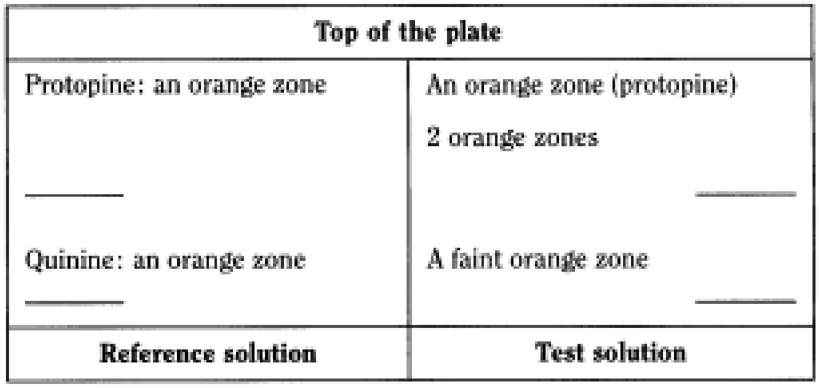
Results A See below the sequence of zones present in the chromatogram obtained with the reference solution and the test solution. Furthermore, other blue fluorescent zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	4 blue fluorescent zones
_______	_______
Quinine: a blue fluorescent zone	
	A greenish-blue fluorescent zone
_______	_______
Reference solution	**Test solution**

Detection B Spray with a mixture of *potassium iodobismuthate solution R2*, *acetic acid R* and *water R* (1:2:10 *V/V/V*) until orange zones appear against a yellow background.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other less intense orange zones are present in the chromatogram obtained with the test solution.



Top of the plate	
Protopine: an orange zone	An orange zone (protopine)
	2 orange zones
_______	_______
Quinine: an orange zone	A faint orange zone
_______	_______
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 15.0 per cent.

ASSAY

To 5.000 g of the powdered drug (355) *(2.9.12)* add 5 ml of *dilute ammonia R1* and 50 ml of *ethyl acetate R*. Shake for 15 min. Filter. Repeat the procedure in the same manner and combine the filtrates. Evaporate the filtrates to dryness under reduced pressure. Dissolve the residue by sonication for 10 min in 50 ml of *0.05 M sulphuric acid*. Filter. Dilute the filtrate to 100 ml with *0.05 M sulphuric acid*. Adjust to pH 9-10 with *concentrated ammonia R* and then add 50 ml of *ethyl acetate R*. Shake gently. Collect the upper organic layer, after centrifugation if necessary. Repeat the procedure in the same manner. Combine the organic layers and dry over *anhydrous sodium sulphate R*. Evaporate to dryness under reduced pressure. Take up the residue with 100 ml of *anhydrous acetic acid R*. Titrate with *0.02 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.02 M perchloric acid* is equivalent to 7.068 mg of protopine.

Calculate the percentage content of total alkaloids expressed as protopine from the following expression:

$$\frac{n \times 706.8}{m}$$

n = volume of *0.02 M perchloric acid* used, in millilitres,

m = mass of the drug to be examined, in milligrams.

Ph Eur

Garlic Powder

General Notices

(Ph Eur monograph 1216)

Ph Eur

DEFINITION

Bulbs of *Allium sativum* L., cut, freeze-dried or dried at a temperature not exceeding 65 °C and powdered.

Content

Minimum 0.45 per cent of allicin ($C_6H_{10}OS_2$; M_r 162.3) (dried drug).

CHARACTERS

Appearance

Light yellowish powder.

IDENTIFICATION

A. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: numerous fragments of parenchyma and groups of spiral or annular vessels accompanied by thin-walled parenchyma.

B. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of garlic powder add 5.0 ml of *methanol R*, shake for 60 s and filter.

Reference solution Dissolve 5 mg of *alanine R* in 10 ml of *water R* and dilute to 20 ml with *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R*, *propanol R*, *water R*, *anhydrous ethanol R* (20:20:20:40 *V/V/V/V*).

Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 2 g/l solution of *ninhydrin R* in a mixture of 5 volumes of *glacial acetic acid R* and 95 volumes of *butanol R* and heat at 105-110 °C for 5-10 min; examine in daylight.

Results The chromatogram obtained with the reference solution shows a violet zone (alanine) in its central third. The chromatogram obtained with the test solution shows a violet or brownish-red zone similar in position to that in the chromatogram obtained with the reference solution and corresponding to alliin; above and below this zone are other, generally fainter, violet zones.

TESTS

Starch

Examine the powdered drug under a microscope using *water R*. Add *iodine solution R1*. No blue colour develops.

Loss on drying *(2.2.32)*

Maximum 7.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

ASSAY

Liquid chromatography *(2.2.29) Carry out the assay as quickly as possible*.

Internal standard solution Dissolve 20.0 mg of *butyl parahydroxybenzoate CRS* in 100.0 ml of a mixture of equal volumes of *methanol R* and *water R*.

Test solution To 0.800 g of garlic powder add 20.0 ml of *water R* and homogenise the mixture in an ultrasonic bath at 4 °C for 5 min. Allow to stand at room temperature for 30 min. Then centrifuge for 30 min. Dilute 10.0 ml of the supernatant to 25.0 ml with a mixture of 40 volumes of a 1 per cent *V/V* solution of *anhydrous formic acid R* and 60 volumes of *methanol R* (stock solution). Shake and centrifuge for 5 min. Place 0.50 ml of the internal standard solution in a volumetric flask and dilute to 10.0 ml with the stock solution.

Precolumn:

- *size:* l = 20 mm, Ø = 4 mm,
- *stationary phase*: silanised *octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

- *size:* l = 0.25 m, Ø = 4 mm,
- *stationary phase*: silanised *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of a 1 per cent *V/V* solution of *anhydrous formic acid R* and 60 volumes of *methanol R*.

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 254 nm.

Injection Loop injector, 1 µl of the internal standard solution and 10 µl of the test solution.

Calculate the percentage of allicin using the following expression:

$$\frac{S_1 \times m_2 \times 22.75}{S_2 \times m_1}$$

S_1 = area of the peak due to allicin (principal peak) in the chromatogram obtained with the test solution,

S_2 = area of the peak due to butyl parahydroxybenzoate in the chromatogram obtained with the test solution,

m_1 = mass of the drug to be examined, in grams,

m_2 = mass of butyl parahydroxybenzoate in 100.0 ml of the internal standard solution, in grams. 1 mg of butylparahydroxybenzoate corresponds to 8.65 mg of allicin.

Ph Eur

Gentian Tincture

General Notices

(Ph Eur monograph 1870)

Ph Eur

DEFINITION

Tincture produced from *Gentian root (0392)* .

PRODUCTION

The tincture is produced from 1 part of the comminuted drug and 5 parts of ethanol (70 per cent *V/V*) by a suitable procedure.

CHARACTERS

Appearance

Yellowish-brown or reddish-brown liquid.

It has a strong bitter taste.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution The tincture to be examined.

Reference solution Dissolve 5 mg of *phenazone R* and 5 mg of *hyperoside R* in 10 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R* .

Mobile phase *water R*, *anhydrous formic acid R*, *ethyl formate R* (4:8:88 *V/V/V*).

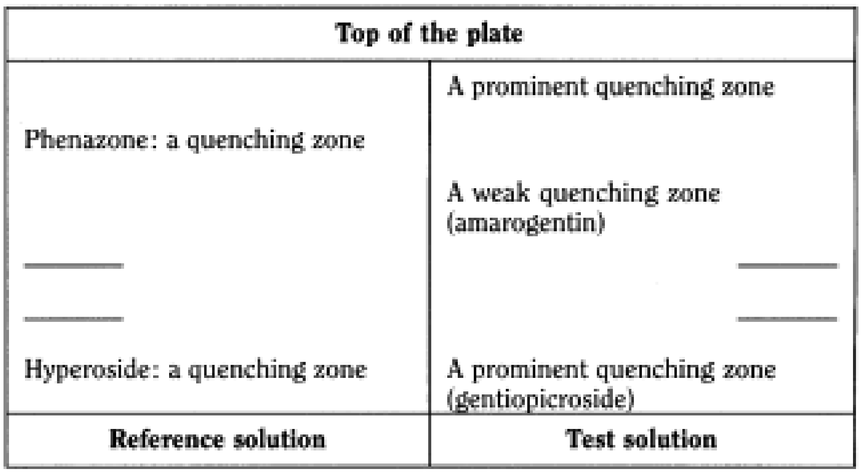
Application 20 µl, as bands.

Development Over a path of 8 cm, in an unsaturated tank.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

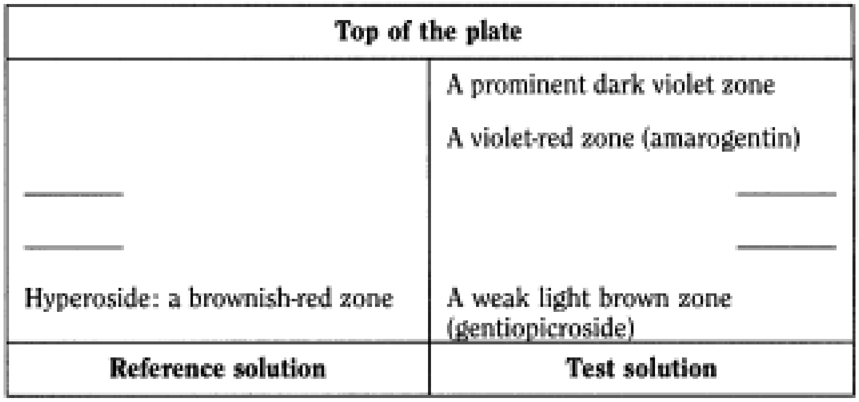
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A prominent quenching zone
Phenazone: a quenching zone	
	A weak quenching zone (amarogentin)
_______	_______
_______	_______
Hyperoside: a quenching zone	A prominent quenching zone (gentiopicroside)
Reference solution	**Test solution**

Detection B Spray with a 10 per cent *V/V* solution of *potassium hydroxide R* in *methanol R* and then with a freshly prepared 2 g/l solution of *fast blue B salt R* in a mixture of *ethanol R* and *water R* (50:50 *V/V*). Examine in daylight.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A prominent dark violet zone
	A violet-red zone (amarogentin)
_______	_______
_______	_______
Hyperoside: a brownish-red zone	A weak light brown zone (gentiopicroside)
Reference solution	**Test solution**

TESTS

Ethanol content (*2.9.10*)

62 per cent *V/V* to 67 per cent *V/V*.

Bitterness value (*2.8.15*)

Minimum 1000.

Dry residue (*2.8.16*)

Minimum 5.0 per cent *m/m*, determined on 3.00 g.

Ph Eur

Gentian

General Notices

(Gentian Root, Ph Eur monograph 0392)

Preparation

Compound Gentian Infusion

When Powdered Gentian is prescribed or demanded, material complying with the requirements below with the exception of Identification test A shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried, fragmented underground organs of *Gentiana lutea* L.

CHARACTERS

Characteristic odour.

Strong and persistent bitter taste.

Gentian root occurs as single or branched subcylindrical pieces of various lengths and usually 10-40 mm thick but occasionally up to 80 mm thick at the crown.

IDENTIFICATION

A. The surface is brownish-grey, and the colour of a transverse section is yellowish or reddish-yellow, but not reddish-brown. The root is longitudinally wrinkled and bears occasional rootlet scars. The branches of the rhizome frequently bear a terminal bud and are always encircled by closely arranged leaf scars. The rhizome and root are brittle when dry and break with a short fracture but they absorb moisture readily to become flexible. The smoothed, transversely cut surface shows a bark, occupying about one-third of the radius, separated by the well-marked cambium from an indistinctly radiate and mainly parenchymatous xylem.

B. Reduce to a powder (355) *(2.9.12)*. The powder is light brown or yellowish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the subero-phellodermic layer, consisting of thin-walled yellowish-brown cork cells and thick-walled collenchyma (phello-derm); fragments of cortical and ligneous parenchymatous cells with moderately thickened walls containing droplets of oil and small prisms and minute needles of calcium oxalate; fragments of lignified vessels with spiral or reticulate thickening.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 25 ml of *methanol R*, shake for 15 min and filter. Evaporate the filtrate to dryness under reduced pressure, at a temperature not exceeding 50 °C. Take up the residue with small quantities of *methanol R* so as to obtain 5 ml of a solution, which may contain a sediment.

Reference solution Dissolve 5 mg of *phenazone R* and 5 mg of *hyperoside R* in 10 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *water R*, *anhydrous formic acid R*, *ethyl formate R* (4:8:88 *V/V/V*).

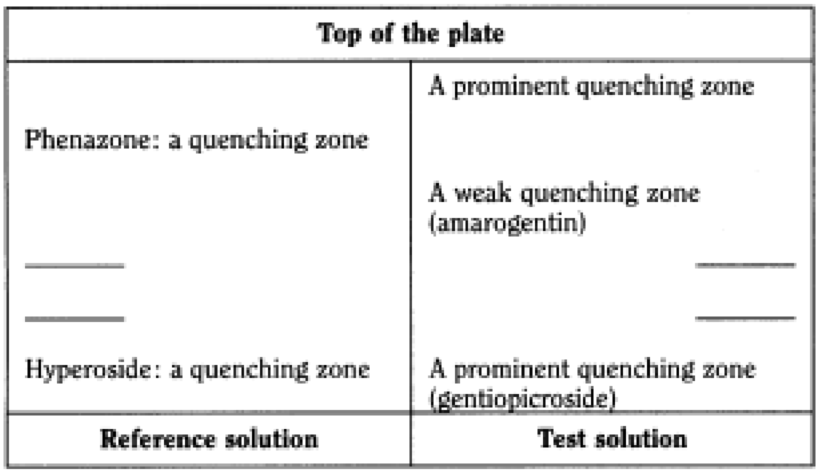
Application 20 µl, as bands.

Development In an unsaturated tank, over a path of 8 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

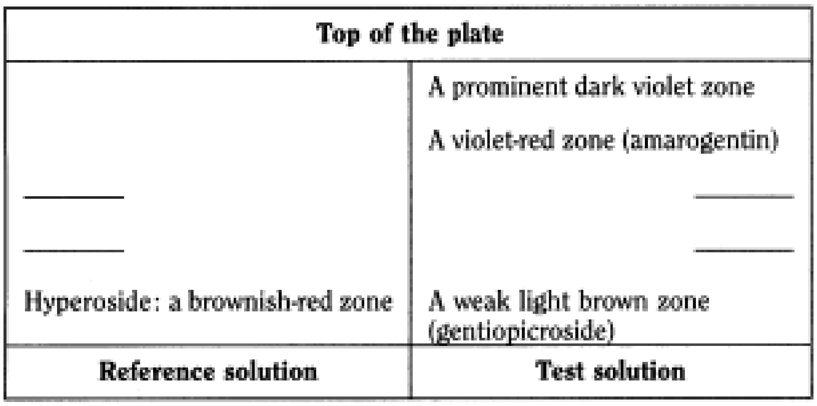
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A prominent quenching zone
Phenazone: a quenching zone	
	A weak quenching zone (amarogentin)
_______	_______
_______	_______
Hyperoside: a quenching zone	A prominent quenching zone (gentiopicroside)
Reference solution	**Test solution**

Detection B Spray with a 100 g/l solution of *potassium hydroxide R* in *methanol R* and then with a freshly prepared 2 g/l solution of *fast blue B salt R* in a mixture of 50 volumes of *anhydrous ethanol R* and 50 volumes of *water R*. Examine in daylight.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A prominent dark violet zone
	A violet-red zone (amarogentin)
_______	_______
_______	_______
Hyperoside: a brownish-red zone	A weak light brown zone (gentiopicroside)
Reference solution	**Test solution**

TESTS

Other species of *Gentiana*

Examine the chromatograms obtained in identification test C, detection B.

Results The chromatogram obtained with the test solution does not show violet zones immediately above the zone due to amarogentine.

Total ash *(2.4.16)*

Maximum 6.0 per cent.

Bitterness value *(2.8.15)*

Minimum 10 000.

Water-soluble extractive

Minimum 33 per cent.

To 5.0 g of powdered drug (710) *(2.9.12)* add 200 ml of boiling *water R*. Allow to stand for 10 min, shaking occasionally. Allow to cool, dilute to 200.0 ml with *water R* and filter. Evaporate 20.0 ml of the filtrate to dryness on a water-bath. Dry the residue in an oven at 100-105 °C. The residue weighs a minimum of 0.165 g.

Ph Eur

Ginger

General Notices

(Ph Eur monograph 1522)

Preparation

Strong Ginger Tincture

Ginger may be known in commerce as unbleached ginger. When Powdered Ginger is prescribed or demanded, material complying with the appropriate requirements below shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried, whole or cut rhizome of *Zingiber officinale* Roscoe, with the cork removed, either completely or from the wide flat surfaces only.

Content

Minimum 15 ml/kg of essential oil (anhydrous drug).

CHARACTERS

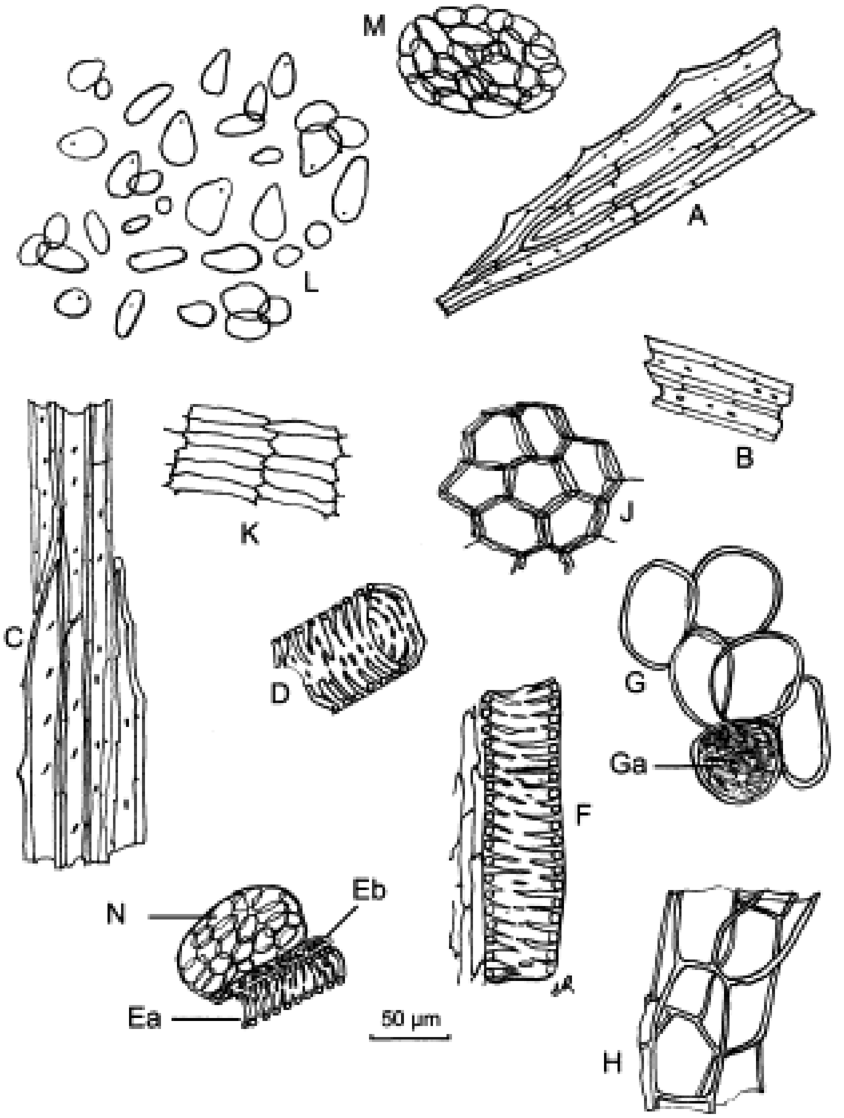
Characteristic aromatic odour.

Spicy and burning taste.

IDENTIFICATION

A. The rhizome is laterally compressed, bearing short, flattened, obovate oblique branches on the upper side, each sometimes having a depressed scar at the apex; the whole rhizomes are about 5-10 cm long, 1.5-3 cm or 4 cm wide and 1-1.5 cm thick, sometimes split longitudinally. The scraped rhizome with a light-brown external surface shows longitudinal striations and occasional loose fibres; the outer surface of the unscraped rhizome varies from pale to dark brown and is more or less covered with cork which shows conspicuous, narrow, longitudinal and transverse ridges; the cork readily exfoliates from the lateral surfaces but persists between the branches. The fracture is short and starchy with projecting fibres. The smoothed transversely cut surface exhibits a narrow cortex separated by an endodermis from a much wider stele; it shows numerous, scattered, fibrovascular bundles and abundant scattered oleoresin cells with yellow contents. The unscraped rhizome shows, in addition, an outer layer of dark brown cork.

B. Reduce to a powder (355) *(2.9.12)*. The powder is pale yellow or brownish. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: groups of large, thin-walled, septate fibres, with one wall frequently dentate; fairly large vessels with reticulate thickening and often accompanied by narrow, thin-walled cells containing brown pigment; abundant thin-walled parenchyma of the ground tissue, some cells containing brown oleoresin; fragments of brown cork, usually seen in surface view. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows abundant starch granules, simple, flattened, oblong or oval or irregular, up to about 50 µm long and 25 µm wide, with a small point hilum situated at the narrower end; occasional granules show faint, transverse striations.



A, B, C. Groups of septate fibres, sometimes with dentate walls

D, E, F. Vessels with reticulate thickening (Ea), sometimes accompanied by cells with brown contents (Eb)

G, H. Parenchyma with cells containing oleoresin (Ga)

J, K. Fragments of cork, in surface view (J) and in transverse section (K)

L, M, N. Starch granules, free (L) and in parenchymatous cells (M, N)

Figure 1522.-1. – *Illustration of powdered herbal drug of ginger (see Identification B)*

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (710) *(2.9.12)* add 5 ml of *methanol R*. Shake for 15 min and filter.

Reference solution Dissolve 10 µl of *citral R* and 10 mg of *resorcinol R* in 10 ml of *methanol R*. Prepare the solution immediately before use.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R, ether R* (40:60 *V/V*).

Application 20 µl, as bands.

Development In an unsaturated tank, over a path of 15 cm.

Drying In air.

Detection Spray with a 10 g/l solution of *vanillin R* in *sulphuric acid R* and examine in daylight while heating at 100-105 °C for 10 min.

Results The chromatogram obtained with the reference solution shows in the lower half an intense red zone (resorcinol) and in the upper half 2 violet zones (citral). The chromatogram obtained with the test solution shows below the zone due to resorcinol in the chromatogram obtained with the reference solution 2 intense violet zones (gingerols) and in the middle, between the zones due to resorcinol and citral in the chromatogram obtained with the reference solution, 2 other less intense violet zones (shogaols). Other zones may be present.

TESTS

Water *(2.2.13)*

Maximum 100 ml/kg, determined by distillation on 20.0 g of the powdered drug (710) *(2.9.12)*.

Total ash *(2.4.16)*

Maximum 6.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use 20.0 g of the freshly, coarsely powdered drug, a 1000 ml round-bottomed flask, 10 drops of *liquid paraffin R* or other antifoam, 500 ml of *water R* as distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 4 h.

Ph Eur

Ginkgo Dry Extract, Refined and Quantified

General Notices

(Ph Eur monograph 1827)

Ph Eur

DEFINITION

Refined and quantified dry extract produced from *Ginkgo leaf (1828).*

Content:

— *flavonoids, expressed as flavone glycosides (M_r 756.7):* 22.0 per cent to 27.0 per cent (dried extract);

— *bilobalide*: 2.6 per cent to 3.2 per cent (dried extract);

— *ginkgolides A, B and C*: 2.8 per cent to 3.4 per cent (dried extract);

— *ginkgolic acids*: maximum 5 ppm (dried extract).

PRODUCTION

The extract is produced from the herbal drug by an appropriate procedure using organic solvents and their mixtures with water, physical separation steps as well as other suitable processes.

CHARACTERS

Appearance

Bright yellow-brown, powder or friable mass.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 20.0 mg of the extract to be examined in 10 ml of a mixture of 2 volumes of *water R* and 8 volumes of *methanol R.*

Reference solution Dissolve 1.0 mg of *chlorogenic acid R* and 3.0 mg of *rutin R* in 20 ml of *methanol R.*

Plate *TLC silica gel plate R* (5-40 µm) or [*TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R*, *glacial acetic acid R*, *water R*, *ethyl acetate R* (7.5:7.5:17.5:67.5 *V/V/V/V*).

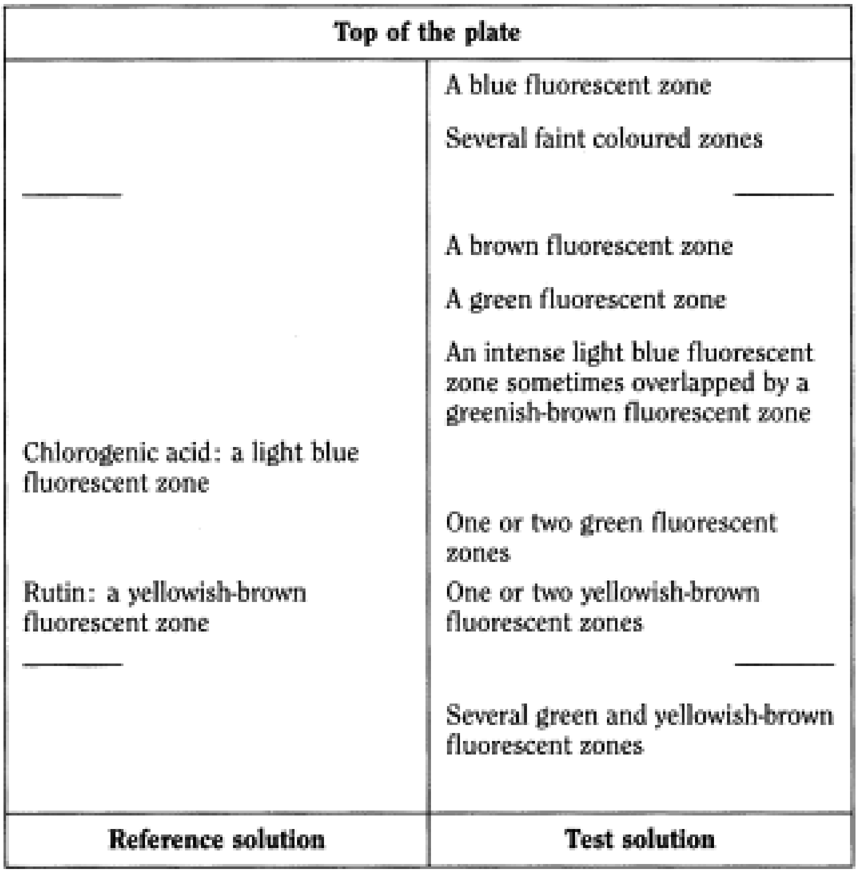
Application 20 µl [or 5 µl], as bands.

Development Over a path of 17 cm [or 6 cm].

Drying At 100-105 °C.

Detection Spray the plate whilst still hot with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, then spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow to dry in air for about 30 min and examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other, weaker fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A blue fluorescent zone
	Several faint coloured zones
_______	_______
	A brown fluorescent zone
	A green fluorescent zone
	An intense light blue fluorescent zone sometimes overlapped by a greenish-brown fluorescent zone
Chlorogenic acid: a light blue fluorescent zone	
	One or two green fluorescent zones
Rutin: a yellowish-brown fluorescent zone	One or two yellowish-brown fluorescent zones
_______	_______
	Several green and yellowish-brown fluorescent zones
Reference solution	**Test solution**

ASSAY

Flavonoids

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.200 g of the extract to be examined in 20 ml of *methanol R*. Add 15.0 ml of *dilute hydrochloric acid R* and 5 ml of *water R* and dilute to 50.0 ml with *methanol R*. Transfer 10.0 ml of this solution into a 10 ml brown-glass vial. Close the vial with a tight rubber membrane stopper and secure with an aluminium crimped cap. Heat on a water-bath for 25 min. Allow to cool to 20 °C.

Reference solution Dissolve 10.0 mg of *quercetin dihydrate CRS* in 20 ml of *methanol R.* Add 15.0 ml of *dilute hydrochloric acid R* and 5 ml of *water R* and dilute to 50.0 ml with *methanol R.*

Column:

— *size:* *l* = 0.125 m, Ø = 4 mm;

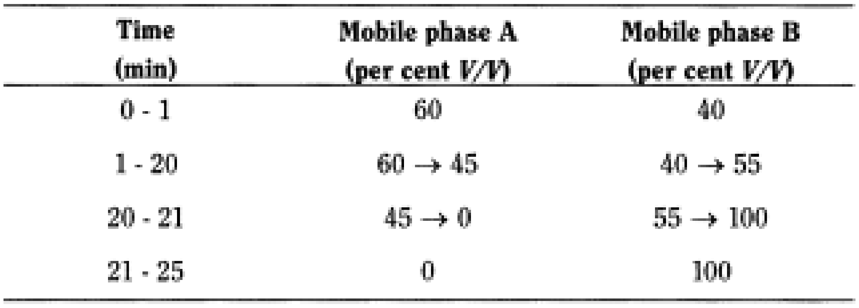
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 25 °C.

Mobile phase:

— *mobile phase A*: 0.3 g/l solution of *phosphoric acid R* adjusted to pH 2.0;

— *mobile phase B*: *methanol R;*



Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 1	60	40
1 - 20	60 → 45	40 → 55
20 - 21	45 → 0	55 → 100
21 - 25	0	100

Flow rate 1.0 ml/min.

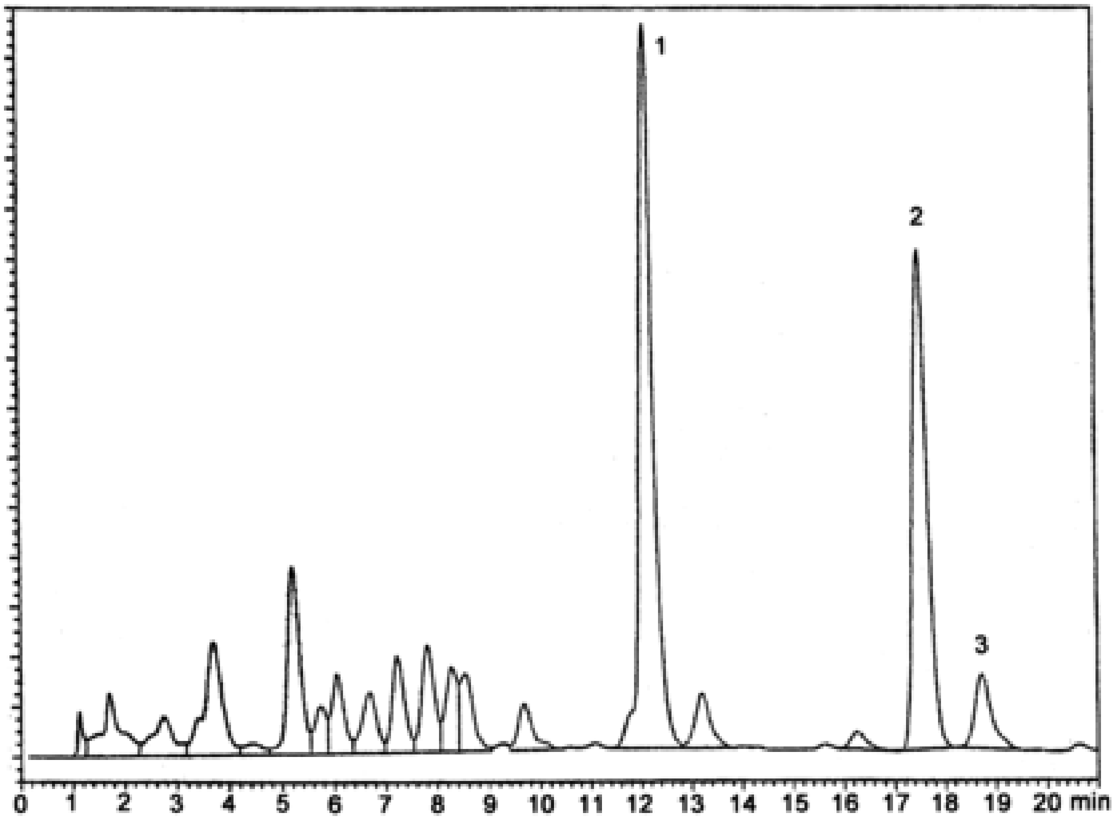
Detector Spectrophotometer at 370 nm.

Injection 10 µl.

Relative retention With reference to quercetin (retention time = about 12.5 min): kaempferol = about 1.4; isorhamnetin = about 1.5.

System suitability Test solution:

— *resolution*: minimum 1.5 between the peaks due to kaempferol and isorhamnetin.



1. quercetin 2. kaempferol 3. isorhamnetin

Figure 1827.-1. – *Chromatogram for the assay of flavonoids in refined and quantified ginkgo dry extract*

Determine the sum of the areas including all the peaks from the peak due to quercetin to the peak due to isorhamnetin in the chromatogram obtained with the test solution (see Figure 1827.-1).

Calculate the percentage content of flavonoids, expressed as flavone glycosides, using the following expression:

$$\frac{F_1 \times m_1 \times 2.514 \times p}{F_2 \times m_2}$$

F_1 = sum of the areas of all the peaks from the peak due to quercetin to the peak due to isorhamnetin in the chromatogram obtained with the test solution

F_2 = area of the peak due to quercetin in the chromatogram obtained with the reference solution

m_1 = mass of *quercetin dihydrate CRS* in the reference solution, in grams

m_2 = mass of the extract to be examined used to prepare the test solution, in grams

p = percentage content of anhydrous quercetin in *quercetin dihydrate CRS*.

Terpene lactones

Liquid chromatography (*2.2.29*).

Test solution Place 0.120 g of the extract to be examined in a 25 ml beaker and dissolve it in 10 ml of *phosphate buffer solution pH 5.8 R* by stirring. Transfer the solution into a chromatography column, about 0.15 m long and about 30 mm in internal diameter, containing 15 g of *kieselguhr for chromatography R*. Wash the beaker with 2 quantities, each of 5 ml, of *phosphate buffer solution pH 5.8 R* and transfer the washings to the chromatography column. Allow to stand for 15 min. Elute with 100 ml of *ethyl acetate R*. Evaporate the eluate to dryness at a pressure not exceeding 4 kPa in a water-bath at 50 °C. The residue of solvent is eliminated by an air-current. Take up the residue in 2.5 ml of the mobile phase.

Reference solution (a) Dissolve 30.0 mg of *benzyl alcohol CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Place 0.120 g of the *ginkgo dry extract for peak identification CRS* in a 25 ml beaker and dissolve it in 10 ml of *phosphate buffer solution pH 5.8 R* by stirring, then proceed as described for the test solution.

Column:
- *size: l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 25 °C.

Mobile phase *tetrahydrofuran R*, *methanol R, water R* (10:20:75 *V/V/V*).

Flow rate 1.0 ml/min.

Detection Refractometer maintained at 35 °C.

Injection 100 µl.

Identification of peaks Use the chromatogram supplied with *ginkgo dry extract for peak identification CRS* and the chromatogram obtained with the reference solution (b) to identify the peaks due to bilobalide and ginkgolides A, B and C.

System suitability:
- the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *ginkgo dry extract for peak identification CRS.*

Calculate the percentage content of bilobalide, using the following expression:

$$\frac{F_1 \times m_1 \times p \times 0.025 \times 1.20}{F_5 \times m_2}$$

Calculate the percentage content of ginkgolide A, using the following expression:

$$\frac{F_2 \times m_1 \times p \times 0.025 \times 1.22}{F_5 \times m_2}$$

Calculate the percentage content of ginkgolide B, using the following expression:

$$\frac{F_3 \times m_1 \times p \times 0.025 \times 1.19}{F_5 \times m_2}$$

Calculate the percentage content of ginkgolide C, using the following expression:

$$\frac{F_4 \times m_1 \times p \times 0.025 \times 1.27}{F_5 \times m_2}$$

F_1 = area of the peak due to bilobalide in the chromatogram obtained with the test solution

F_2 = area of the peak due to ginkgolide A in the chromatogram obtained with the test solution

F_3 = area of the peak due to ginkgolide B in the chromatogram obtained with the test solution

F_4 = area of the peak due to ginkgolide C in the chromatogram obtained with the test solution

F_5 = area of the peak due to benzyl alcohol in the chromatogram obtained with reference solution (a)

m_1 = mass of *benzyl alcohol CRS* in reference solution (a), in grams

m_2 = mass of the extract to be examined used to prepare the test solution, in grams

p = percentage content of benzyl alcohol in *benzyl alcohol CRS*

Calculate the percentage content of the sum of ginkgolides A, B and C, using the following expression:

$$G_A + G_B + G_C$$

G_A = percentage content of ginkgolide A

G_B = percentage content of ginkgolide B

G_C = percentage content of ginkgolide C.

Ginkgolic acids

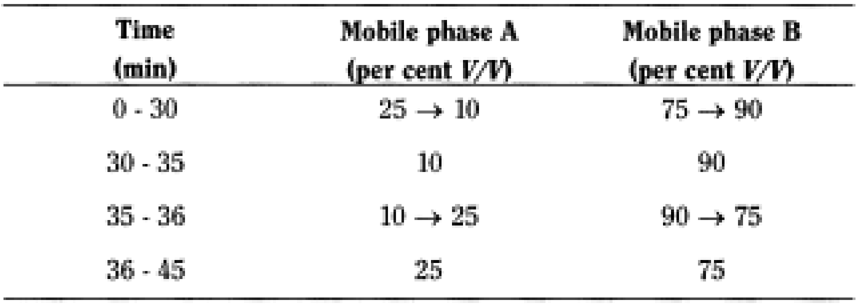
Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.500 g of the powdered extract to be examined in 8 ml of *methanol R*, sonicating if necessary, and dilute to 10.0 ml with the same solvent. Centrifuge if necessary.

Reference solution Dissolve 10.0 mg of *ginkgolic acids CRS* in 8 ml of *methanol R*, sonicating if necessary, and dilute to 10.0 ml with the same solvent. Dilute 2.0 ml of this solution to 10.0 ml with *methanol R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: dilute 0.1 ml of *trifluoroacetic acid R* to 1000 ml with *water R*;
- *mobile phase B*: dilute 0.1 ml of *trifluoroacetic acid R* to 1000 ml with *acetonitrile R;*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	25 → 10	75 → 90
30 - 35	10	90
35 - 36	10 → 25	90 → 75
36 - 45	25	75

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µl.

Identification of components Use the chromatogram supplied with *ginkgolic acids CRS* and the chromatogram obtained with the test solution to identify the peaks due to ginkgolic acids C13, C15 and C17.

System suitability Reference solution:

- *resolution*: minimum 2.0 between the peaks due to ginkgolic acids C13 and C15;
- *symmetry factor*: 0.8 to 2.0 for the peaks due to ginkgolic acids C13, C15 and C17.

Calculate the content in ppm of ginkgolic acids expressed as ginkgolic acid C17, using the following expression:

$$\frac{A_1 \times m_2 \times p \times 2000}{A_2 \times m_1}$$

A_1 = sum of the areas of the peaks due to the ginkgolic acids C13, C15 and C17 in the chromatogram obtained with the test solution

A_2 = area of the peak due to ginkgolic acid C17 in the chromatogram obtained with the reference solution

m_1 = mass of the extract to be examined used to prepare the test solution, in grams

m_2 = mass of *ginkgolic acids CRS* used to prepare the reference solution, in grams

p = percentage content of ginkgolic acid C17 in *ginkgolic acids CRS.*

Ph Eur

Ginkgo Leaf

General Notices

(Ph Eur monograph 1828)

Ph Eur

DEFINITION

Whole or fragmented, dried leaf of *Ginkgo biloba* L.

Content

Not less than 0.5 per cent of flavonoids, calculated as flavone glycosides (M_r 757) (dried drug) .

CHARACTERS

Greyish or yellowish-green or yellowish-brown.

IDENTIFICATION

A. The upper surface of ginkgo leaf is slightly darker than the lower surface. The petioles of the leaf are about 4 cm to 9 cm long. The lamina is about 4 cm to 10 cm wide, fan-shaped, usually bilobate or sometimes undivided. Both surfaces are smooth, and the venation dichotomous, the veins appearing to radiate from the base; they are equally prominent on both surfaces. The distal margin is incised, irregularly and to different degrees, and irregularly lobate or emarginate. The lateral margins are entire and taper towards the base.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish or yellowish-green or yellowish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows irregularly-shaped fragments of the lamina in surface view, the upper epidermis consisting of elongated cells with irregularly sinuous walls, the lower epidermal cells smaller, with a finely striated cuticle and each cell shortly papillose; stomata about 60 µm, large, deeply sunken with 6 to 8 subsidiary cells, are more numerous in the lower epidermis; abundant large cluster crystals of calcium oxalate of various sizes in the mesophyll; fragments of fibro-vascular tissue from the petiole and veins.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 2.0 g of the powdered drug (710) *(2.9.12)* add 10 ml of *methanol R*. Heat in a water-bath at 65 °C for 10 min. Shake frequently. Allow to cool to room temperature and filter.

Reference solution Dissolve 1.0 mg of *chlorogenic acid R* and 3.0 mg of *rutin R* in 20 ml of *methanol R.*

Plate TLC silica gel plate R.

Mobile phase anhydrous formic acid R, *glacial acetic acid R*, *water R*, *ethyl acetate R* (7.5:7.5:17.5:67.5 *V/V/V/V*).

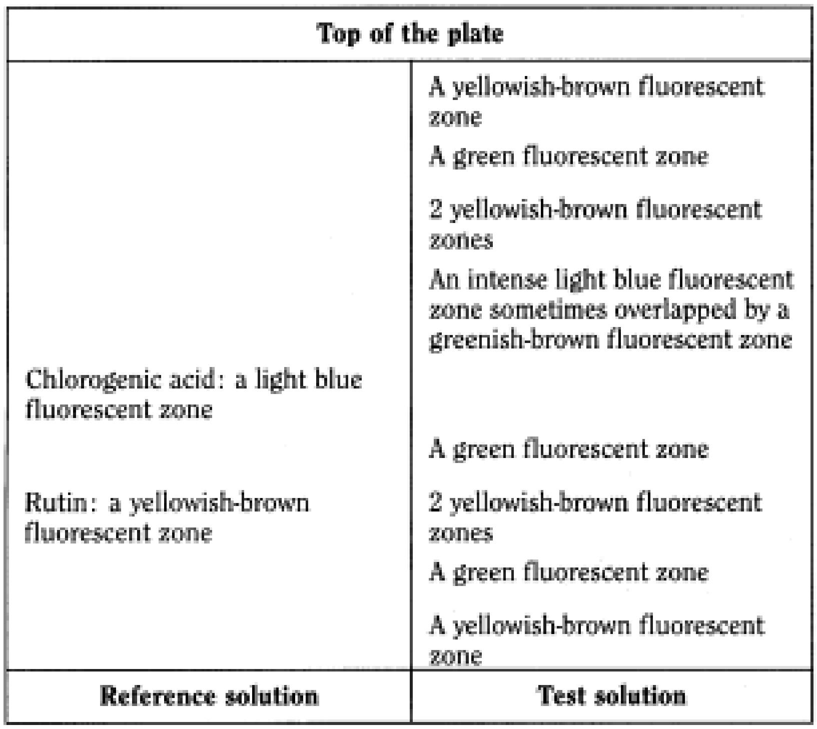
Application 20 µl, as bands.

Development Over a path of 17 cm.

Drying At 100-105 °C.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Subsequently spray with the same volume of a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions. Furthermore, other weaker fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A yellowish-brown fluorescent zone
	A green fluorescent zone
	2 yellowish-brown fluorescent zones
	An intense light blue fluorescent zone sometimes overlapped by a greenish-brown fluorescent zone
Chlorogenic acid: a light blue fluorescent zone	
	A green fluorescent zone
Rutin: a yellowish-brown fluorescent zone	2 yellowish-brown fluorescent zones
	A green fluorescent zone
	A yellowish-brown fluorescent zone
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of stems and 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 11.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 11.0 per cent.

ASSAY

Flavonoids

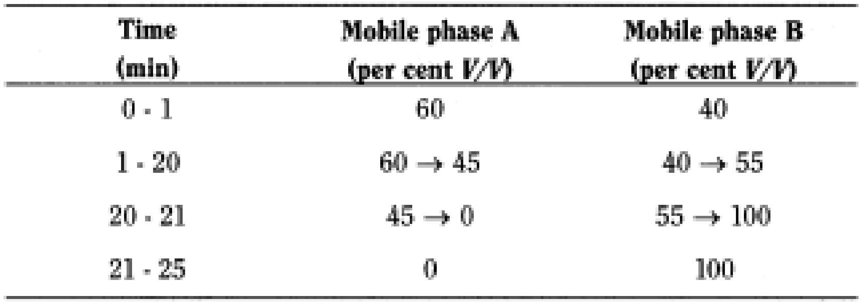
Liquid chromatography *(2.2.29)*.

Test solution Heat 2.500 g of the powdered drug (710) *(2.9.12)* in 50 ml of a 60 per cent *V/V* solution of *acetone R* under a reflux condenser for 30 min. Filter and collect the filtrate. Extract the drug residue a second time in the same manner, using 40 ml of a 60 per cent *V/V* solution of *acetone R* and filter. Collect the filtrates and dilute to 100.0 ml with a 60 per cent *V/V* solution of *acetone R*. Evaporate 50.0 ml of the solution to eliminate the acetone and transfer to a 50.0 ml vial, rinsing with 30 ml of *methanol R*. Add 4.4 ml of *hydrochloric acid R1*, dilute to 50.0 ml with *water R* and centrifuge. Place 10 ml of the supernatant liquid in a 10 ml brown-glass vial. Close with a rubber seal and an aluminium cap and heat on a water-bath for 25 min. Allow to cool to room temperature.

Reference solution Dissolve 10.0 mg of *quercetin dihydrate R* in 20 ml of *methanol R*. Add 15.0 ml of *dilute hydrochloric acid R* and 5 ml of *water R* and dilute to 50.0 ml with *methanol R*.

Column:
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *size*: *l* = 0.125 m, Ø = 4 mm,
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: 0.3 g/l solution of *phosphoric acid R* adjusted to pH 2.0,
- *mobile phase B*: *methanol R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	60	40
1 - 20	60 → 45	40 → 55
20 - 21	45 → 0	55 → 100
21 - 25	0	100

Flow rate 1.0 ml/min.

Detector Spectrophotometer at 370 nm.

Injection 10 µl.

Relative retention With reference to quercetin (retention time = about 12.5 min): kaempferol = about 1.4; isorhamnetin = about 1.5.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to kaempferol and to isorhamnetin.

Do not take into account peaks eluting before the quercetin peak or after the isorhamnetin peak in the chromatogram obtained with the test solution.

Calculate the percentage content of flavonoids, expressed as flavone glycosides, from the expression:

$$2 \times \frac{F_1 \times m_1 \times 2.514 \times p}{F_2 \times m_2}$$

F_1 = sum of the areas of all the considered peaks in the chromatogram obtained with the test solution,

F_2 = area of the peak corresponding to quercetin in the chromatogram obtained with the reference solution,

m_1 = mass of quercetin used to prepare the reference solution, in grams,

m_2 = mass of the drug to be examined used to prepare the test solution, in grams,

p = percentage content of anhydrous quercetin in *quercetin dihydrate R*.

Ph Eur

Ginseng

General Notices

(Ph Eur monograph 1523)

Ph Eur

DEFINITION

Whole or cut dried root, designated white ginseng; treated with steam and then dried, designated red ginseng, of *Panax ginseng* C. A. Meyer.

Content

Minimum 0.40 per cent for the sum of ginsenosides Rg1 ($C_{42}H_{72}O_{14},2H_2O$; M_r 837) and Rb1 ($C_{54}H_{92}O_{23},3H_2O$; M_r 1163) (dried drug).

IDENTIFICATION

A. The principal root is fusiform or cylindrical, sometimes branched, up to about 20 cm long and 2.5 cm in diameter, and may be curved or markedly re-curved. The surface is pale yellow to cream in white ginseng, brownish-red in red ginseng and shows longitudinal ridges. Stem scars may be seen at the crown. The fracture is short. The transversely-cut surface shows a wide outer zone with scattered orange-red resin canals and a finely radiate inner region. The rootlets, numerous in the lower part of white ginseng, are normally absent in red ginseng.

B. Reduce to a powder (355) *(2.9.12)*. The powder is light yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following characters: abundant fragments of thin-walled parenchymatous cells and fragments of large secretory canals containing yellowish-brown resin, non-lignified tracheids and partially-lignified vessels with spiral or reticulate thickening, isolated or in groups; scattered cluster crystals of calcium oxalate. Examine under a microscope using a mixture of equal volumes of *glycerol R* and *water R*. The starch granules are very abundant, simple or 2 or 3 compound, and range from 1 µm to 10 µm in diameter. In red ginseng the starch granules are often deformed and destroyed by treating with steam, or may be absent.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Boil 1.0 g of the powdered drug (355) *(2.9.12)* under a reflux condenser with 10 ml of a 70 per cent *V/V* solution of *methanol R* for 15 min. Filter after cooling and dilute to 10.0 ml with *methanol R*.

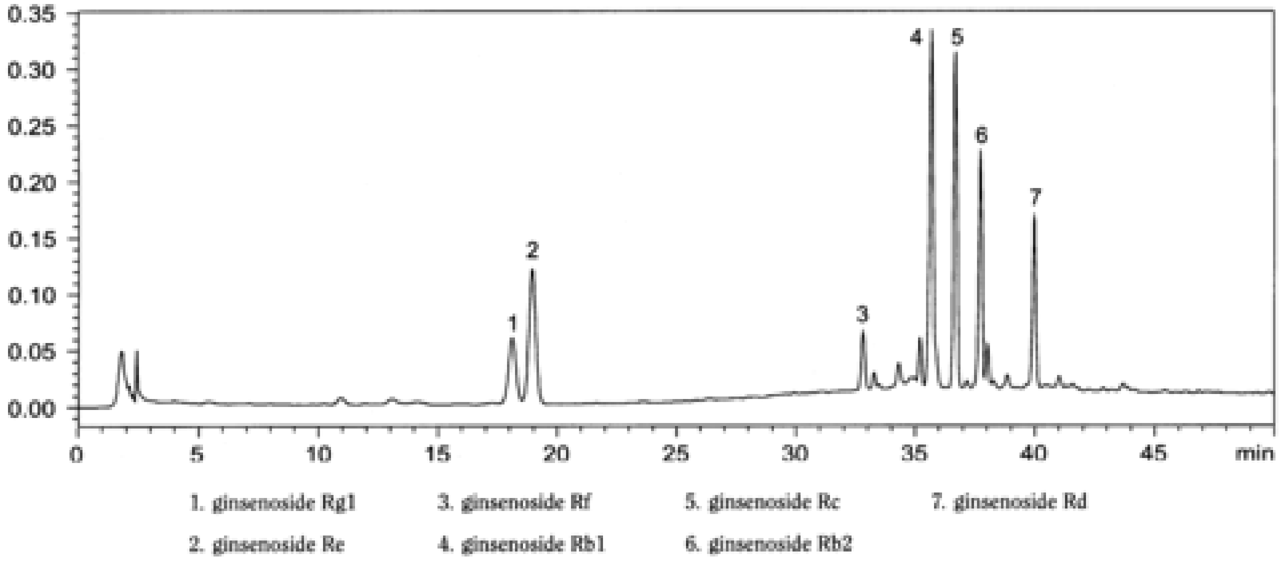


Figure 1523.-1. – *Chromatogram for the assay of ginseng: test solution*

Reference solution Dissolve 5.0 mg of *aescin R* and 5.0 mg of *arbutin R* in 1 ml of *methanol R.*

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *ethyl acetate R*, *water R*, *butanol R* (25:50:100 *V/V/V*), allow the mixture to separate for 10 min. Use the upper layer.

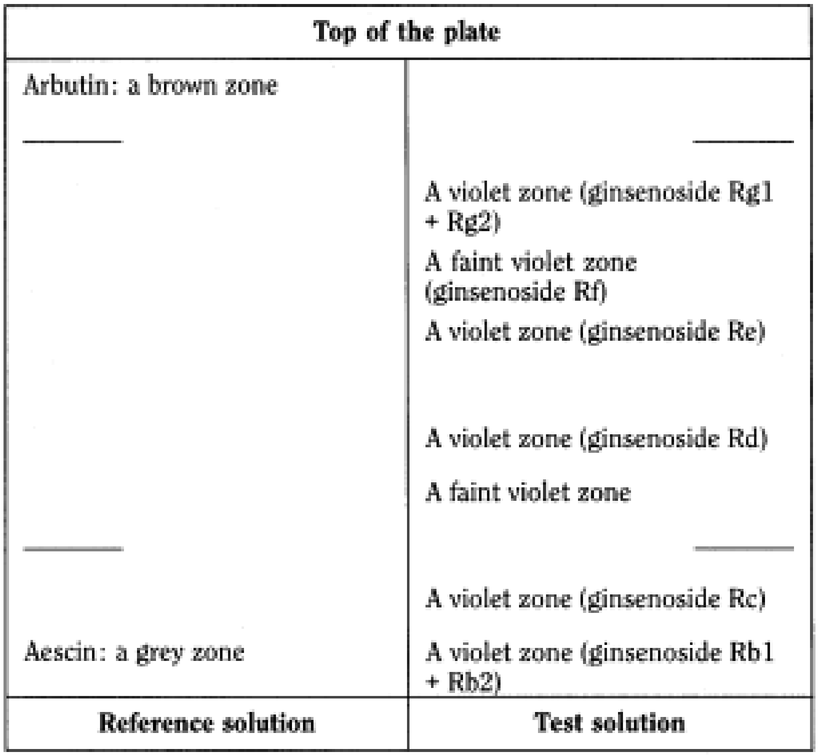
Application 20 µl [or 4 µl] as bands.

Development Over 10 cm [or 5 cm] in an unsaturated tank.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 105-110 °C for 5-10 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
Arbutin: a brown zone	
———	———
	A violet zone (ginsenoside Rg1 + Rg2)
	A faint violet zone (ginsenoside Rf)
	A violet zone (ginsenoside Re)
	A violet zone (ginsenoside Rd)
	A faint violet zone
———	———
	A violet zone (ginsenoside Rc)
Aescin: a grey zone	A violet zone (ginsenoside Rb1 + Rb2)
Reference solution	**Test solution**

TESTS

Panax quinquefolium

Examine the chromatograms obtained in the assay. The chromatogram obtained with the test solution shows a peak due to ginsenoside Rf (see Figure 1523.-1). In the case of a substitution by *Panax quinquefolium* no peak due to ginsenoside Rf is present.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 7.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 1.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Reduce about 50 g to a powder (355) *(2.9.12)*. Place 1.00 g of the powdered drug and 70 ml of a 50 per cent *V/V* solution of *methanol R* in a 250 ml round-bottomed flask. After adding a few grains of pumice, boil on a water-bath under a reflux condenser for 1 h. After cooling, centrifuge and collect the supernatant liquid. Treat the residue as described above. Mix the collected liquids and evaporate to dryness under reduced pressure at a temperature not exceeding 60 °C. Take up the residue with 20.0 ml of a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*. Dilute 2.0 ml of the solution to 10.0 ml with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*. Filter through a suitable membrane filter (0.45 µm) before injection.

Reference solution Dissolve 3.0 mg of *ginsenoside Rg1 R*, 3.0 mg of *ginsenoside Re R*, 3.0 mg of *ginsenoside Rf R* and 3.0 mg of *ginsenoside Rb1 R* in *methanol R* and dilute to 10.0 ml with the same solvent.

Column:
— *size: l* = 0.125 m, Ø = 4.6 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
— *temperature:* 35 °C.

Mobile phase:
— *mobile phase A*: *water R* adjusted to pH 2 with *phosphoric acid R*,
— *mobile phase B*: *acetonitrile R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	80	20
8 - 40	80 → 60	20 → 40
40 - 45	60 → 40	40 → 60
45 - 47	40 → 0	60 → 100
47 - 52	0	100
52 - 55	0 → 80	100 → 20

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 203 nm.

Equilibration time 20 min.

Injection 20 µl.

Elution order Order indicated in the composition of the reference solution; record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.0 between the peaks due to ginsenoside Rg1 and ginsenoside Re.

Locate the peaks due to ginsenoside Rb1 and ginsenoside Rg1 in the chromatogram obtained with the test solution.

Calculate the percentage content of ginsenosides Rb1 and Rg1 from the expression:

$$\frac{A_1 \times m_2 \times p_1}{A_3 \times m_1 \times 100} + \frac{A_2 \times m_3 \times p_2}{A_4 \times m_1 \times 100}$$

A_1 = area of the peak due to ginsenoside Rb1 in the chromatogram obtained with the test solution,

A_2 = area of the peak due to ginsenoside Rg1 in the chromatogram obtained with the test solution,

A_3 = area of the peak due to ginsenoside Rb1 in the chromatogram obtained with the reference solution,

A_4 = area of the peak due to ginsenoside Rg1 in the chromatogram obtained with the reference solution,

m_1 = mass of the drug to be examined, in grams,

m_2 = mass of ginsenoside Rb1 in the reference solution, in milligrams,

m_3 = mass of ginsenoside Rg1 in the reference solution, in milligrams,

p_1 = percentage content of ginsenoside Rb1 in the reagent,

p_2 = percentage content of ginsenoside Rg1 in the reagent.

Ph Eur

Goldenrod

General Notices

(Ph Eur monograph 1892)

Ph Eur

DEFINITION

Whole or cut, dried, flowering aerial parts of *Solidago gigantea* Ait or *Solidago canadensis* L., their varieties or hybrids and/or mixtures of these.

Content

Minimum 2.5 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The stems are greenish-yellow or greenish-brown, partly tinted reddish, roundish, more or less conspicuously grooved, glabrous and smooth in the lower part, slightly or densely pubescent in the upper part. They are solid with a whitish pith.

The leaves are green, sessile, lanceolate, with a serrate margin, 8-12 cm long and about 1-3 cm wide, the upper surface is green and more or less glabrous, the lower surface is greyish-green and pubescent, especially on the veins. The inflorescence consists of a number of unilateral, curved racemes which together form a pyramidal panicle at the end of the stems.

Each capitulum has an involucre composed of linear-lanceolate, imbricated yellowish-green bracts, surrounding a single row of yellow ligulate florets about the same length as the involucre; yellow, radially arranged tubular florets, as long as, or longer, than the ligulate florets; a brownish inferior ovary surmounted by a white pappus of silky hairs.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows pappus bristles and their fragments, consisting of multiseriate trichomes composed of elongated cells with the tips free from the surface and forming pointed projections over the entire length; fragments of the leaf mesophyll with vascular bundles accompanied by secretory cells; fragments of the leaf epidermis with sinuous to wavy-walled cells and stomata of the anomocytic type *(2.8.3)*; uniseriate covering trichomes with up to 5 or 6 cells, some whip-like with a thicker-walled terminal cell; fragments of the style with long, slender papillae; fragments of the stem with reticulate and spiral vessels; pollen grains, with 3 germinal pores and a spiny exine; numerous whisk-shaped hairs, a few isolated twin-hairs from the ovary, absence of multicellular trichomes with a terminal cell bent at a right angle.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.75 g of the powdered drug (355) *(2.9.12)* add 5 ml of *methanol R* and boil in a water-bath under a reflux condenser for 10 min. Cool and filter.

Reference solution Dissolve 1.0 mg of *chlorogenic acid R*, 2.5 mg of *quercitrin R* and 2.5 mg of *rutin R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (6:6:18:30 *V/V/V/V*).

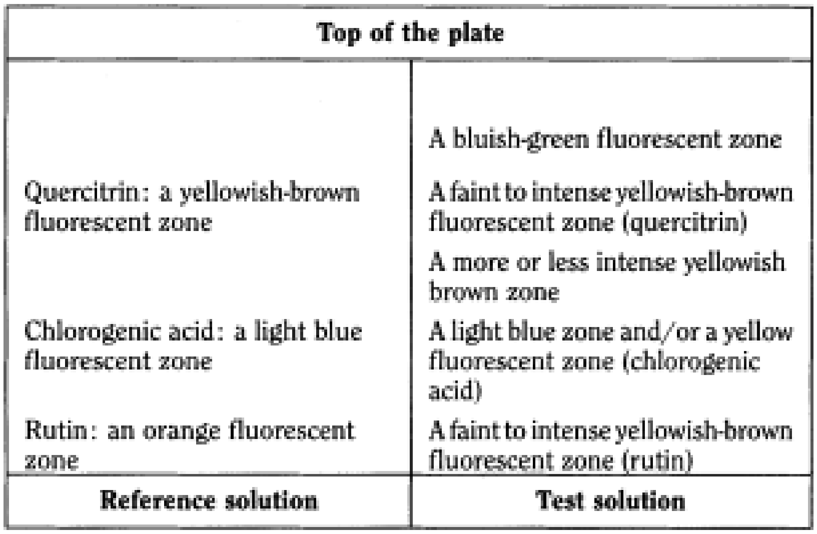
Application 20 µl of the test solution and 10 µl of the reference solution as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow to stand for 30 min. Examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A bluish-green fluorescent zone
Quercitrin: a yellowish-brown fluorescent zone	A faint to intense yellowish-brown fluorescent zone (quercitrin)
	A more or less intense yellowish brown zone
Chlorogenic acid: a light blue fluorescent zone	A light blue zone and/or a yellow fluorescent zone (chlorogenic acid)
Rutin: an orange fluorescent zone	A faint to intense yellowish-brown fluorescent zone (rutin)
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of brownish parts and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10 per cent, determined on 0.500 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 7.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 1.0 per cent.

ASSAY

Stock solution In a 100 ml round-bottomed flask, introduce 0.200 g of the powdered drug (250) *(2.9.12)*, add 1 ml of a 5 g/l solution of *hexamethylenetetramine R*, 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1*. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a small plug of absorbent cotton into a 100 ml flask. Add the absorbent cotton to the residue in the round-bottomed flask, extract with 2 quantities, each of 20 ml of *acetone R*, each time boiling under a reflux condenser for 10 min. Allow to cool. Filter the combined acetone extracts through a filter paper into a volumetric flask. Rinse the flask and the filter paper and dilute to 100.0 ml with *acetone R*. Introduce 20.0 ml of the solution into a separating funnel, add 20 ml of *water R* and shake the mixture with 1 quantity of 15 ml and then 3 quantities, each of 10 ml, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, wash twice with 50 ml of *water R* and filter the extracts over 10 g of *anhydrous sodium sulphate R* into a volumetric flask. Dilute to 50.0 ml with *ethyl acetate R*, rinsing the separating funnel and the sodium sulphate.

Test solution To 10.0 ml of the stock solution add 1.0 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation solution Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Measure the absorbance of the test solution *(2.2.25)* at 425 nm after 30 min by comparison with the compensation solution.

Calculate the percentage content of flavonoids, expressed as hyperoside, from the expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the value of the specific absorbance of hyperoside to be 500.

A = absorbance measured at 425 nm,

m = mass of the drug to be examined, in grams.

Ph Eur

Goldenseal Root

General Notices

(Goldenseal Rhizome, Ph Eur monograph 1831)

Ph Eur

DEFINITION

Whole or cut, dried rhizome and root of *Hydrastis canadensis* L.

Content:

— *hydrastine* ($C_{21}H_{21}NO_6$; M_r 383.4): minimum 2.5 per cent (dried drug);

— *berberine* ($C_{20}H_{19}NO_5$; M_r 353.4): minimum 3.0 per cent (dried drug).

IDENTIFICATION

A. The rhizome is tortuous and knotty, about 5 cm long and 5-10 mm thick. The surface is yellowish or brownish-grey, irregularly wrinkled, and bears the remains of numerous slender, wiry roots; stem bases and scale leaves occur on the upper surface. The fracture is short and resinous. The transversely-cut surface is yellowish-brown and shows a fairly wide bark, a ring of from about 12 to 20 widely separated xylem bundles and a large, central pith.

B. Reduce to a powder (180) *(2.9.12)*. The powder is greenish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: abundant thin-walled fragments of parenchyma; occasional fragments of yellowish-brown cork from the rhizome and roots; groups of small vessels with conspicuous perforations in the oblique end walls and with simple or bordered, slit-shaped pits; infrequent groups of thin-walled, pitted fibres, usually found associated with the vessels; numerous ovoid or spherical, orange-brown granular masses. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows abundant starch granules, mostly simple but sometimes compound with up to 4 components; the granules are small, spherical or ovoid, up to about 10 µm in diameter, occasionally with a small, rounded or slit-shaped hilum.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 250 mg of powdered drug (180) *(2.9.12)* add 4 ml of a mixture of 20 ml of *water R* and 80 ml of *methanol R*. Sonicate for 10 min and filter. Wash the residue with 2 quantities, each of 2 ml, of *methanol R*. Combine the solutions and dilute to 20 ml with *methanol R*.

Reference solution Dissolve 5 mg of *hydrastine hydrochloride R* and 5 mg of *berberine chloride R* in 20 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R*, *water R*, *ethyl acetate R* (10:10:80 *V/V/V*).

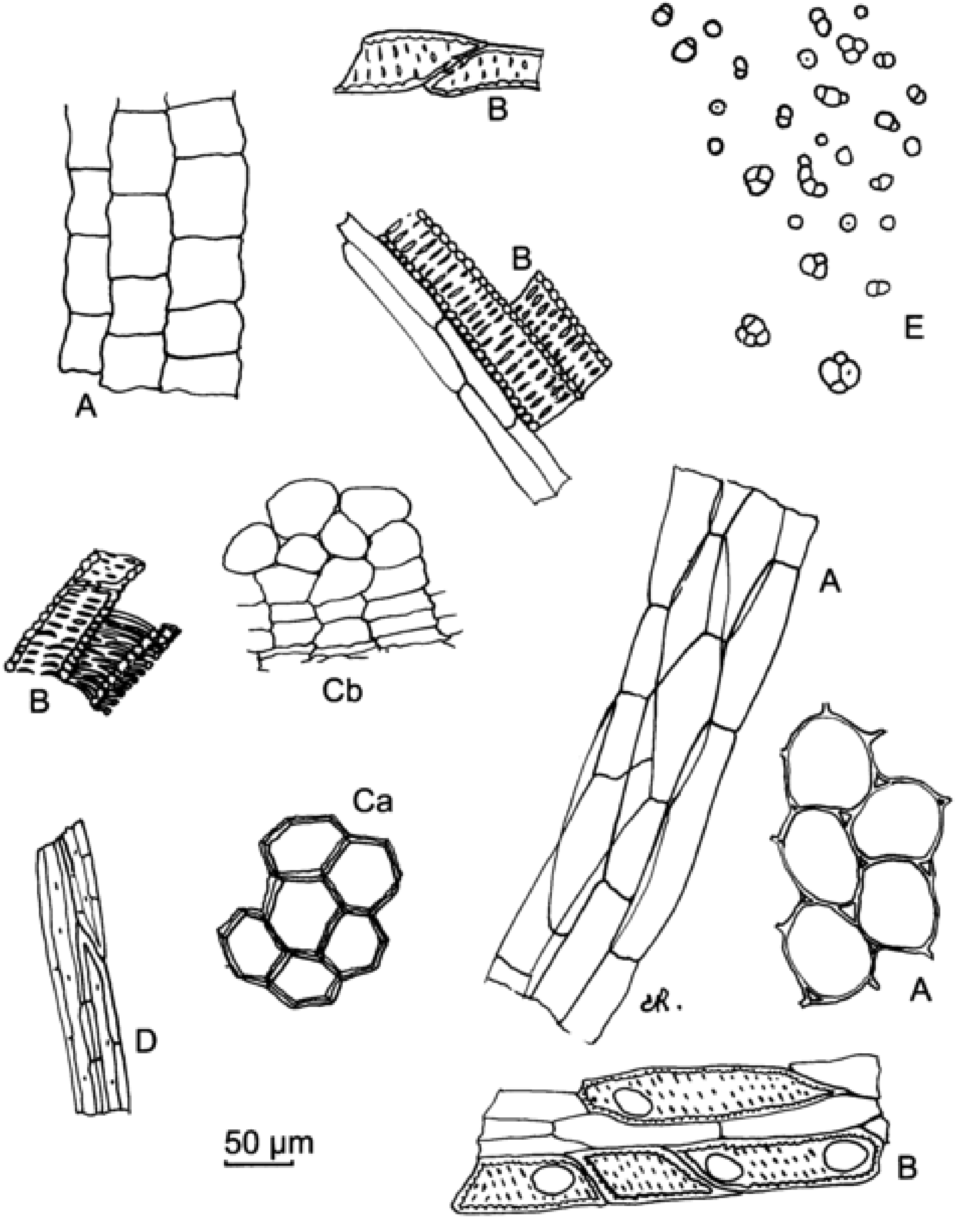
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.


B
E
B
A
A
B
Cb
Ca
A
D
A
50 µm
B

A. Thin-walled parenchyma cells

B. Vessels

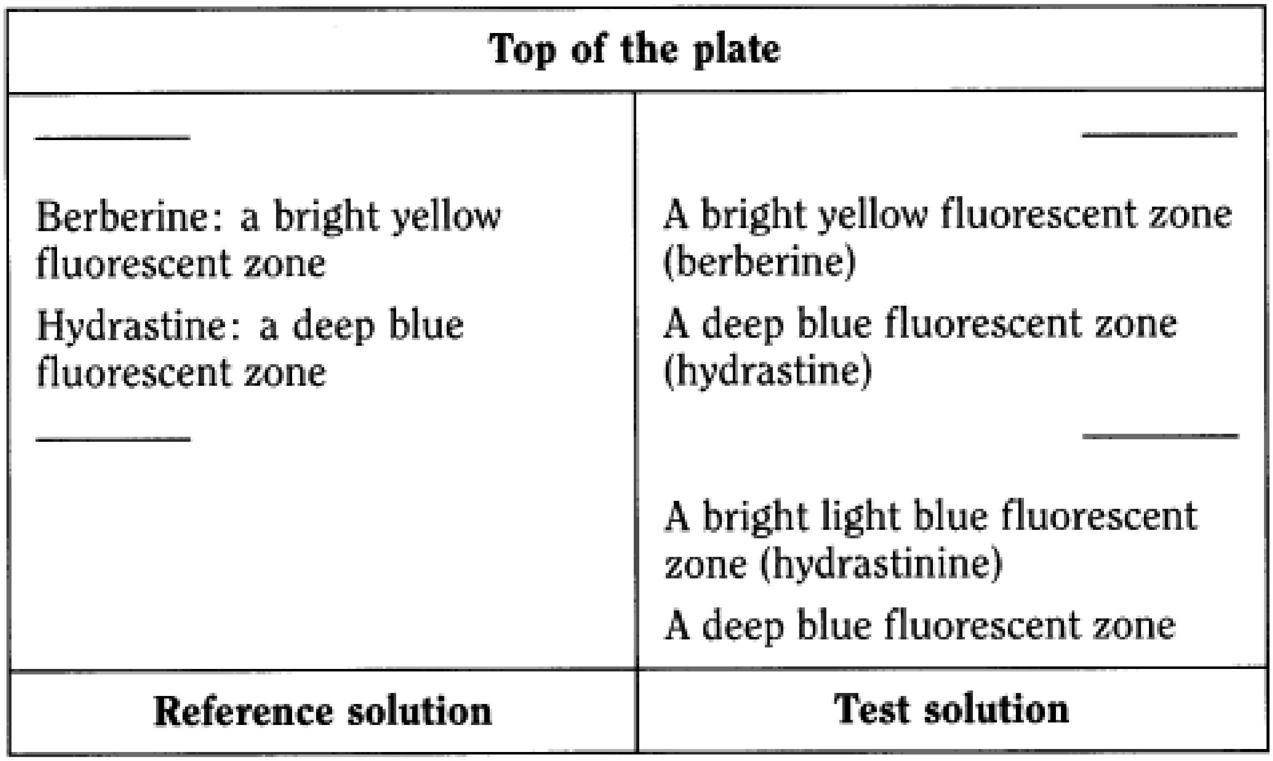
C. Fragment of cork from the

D. Pitted fibres

E. Starch granules

rhizome and roots in surface view
(Ca) and in side view (Cb)

Figure 1831.-1. – *Illustration of powdered herbal drug of goldenseal rhizome (see Identification B)*



Top of the plate	
———	———
Berberine: a bright yellow fluorescent zone	A bright yellow fluorescent zone (berberine)
Hydrastine: a deep blue fluorescent zone	A deep blue fluorescent zone (hydrastine)
———	———
	A bright light blue fluorescent zone (hydrastinine)
	A deep blue fluorescent zone
Reference solution	**Test solution**

TESTS

Loss on drying ***(2.2.32)***

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (180) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 8.0 per cent.

Ash insoluble in hydrochloric acid ***(2.8.1)***

Maximum 4.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution To 1.000 g of the powdered drug (355) *(2.9.12)* in a 100 ml round-bottomed flask, add 50 ml of a 1 per cent *V/V* solution of *concentrated ammonia R* in *ethanol (96 per cent) R* and boil the mixture under a reflux condenser for 30 min. Allow to cool to room temperature and filter the liquid through a plug of absorbent cotton into a flask. Add the plug of absorbent cotton to the residue in the round-bottomed flask and repeat the extraction with a further 2 quantities, each of 30 ml, of a 1 per cent *V/V* solution of *concentrated ammonia R* in *ethanol (96 per cent) R*, each time boiling under a reflux condenser for 10 min and filtering through a plug of absorbent cotton in the same flask as previously. Filter the combined filtrates through a filter paper into a 250 ml round-bottomed flask, and rinse flask and filter with 20 ml of a 1 per cent *V/V* solution of *concentrated ammonia R* in *ethanol (96 per cent) R.* Evaporate the filtrate to dryness *in vacuo* in a water-bath at 55 °C. Dissolve the residue in 50.0 ml of the mobile phase. Dilute 10.0 ml of this solution to 250.0 ml with the mobile phase.

Reference solution Dissolve 10 mg of *hydrastine hydrochloride R* and 10 mg of *berberine chloride R* in *methanol R* and dilute to 100.0 ml with the same solvent.

Column:
- *size*: *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 9.93 g of *potassium dihydrogen phosphate R* in 730 ml of *water R*, add 270 ml of *acetonitrile R* and mix.

Flow rate 1.2 ml/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µl.

System suitability Reference solution:

- *elution order*: order indicated in the composition of the reference solution; record the retention times of these substances;
- *resolution*: minimum 1.5 between the peaks due to hydrastine and berberine.

Using the retention times determined from the chromatogram obtained with the reference solution, locate in the chromatogram obtained with the test solution the components of the reference solution.

Calculate the percentage content of each alkaloid (hydrastine and berberine) using the following expression:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1} \times 12.5$$

A_1 = area of the peak due to hydrastine or berberine in the chromatogram obtained with the test solution

A_2 = area of the peak due to hydrastine or berberine in the chromatogram obtained with the reference solution

m_1 = mass of the drug to be examined, in grams

m_2 = mass of hydrastine hydrochloride or berberine chloride in the reference solution, in grams

p = percentage content of hydrastine in *hydrastine hydrochloride R* or berberine in *berberine chloride R*.

Ph Eur

Greater Burnet Root

General Notices

(Ph Eur monograph 2385)

Ph Eur

DEFINITION

Whole or fragmented, dried underground parts of *Sanguisorba officinalis* L. without rootlets.

Content

Minimum 5.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

CHARACTERS

The adventitious roots are about 5-25 cm long and up to 2 cm in diameter.

IDENTIFICATION

A. The whole drug consists of the rhizome, often ramified, thick, short, fusiform or cylindrical and the adventitious roots whose surface is reddish-brown or blackish-brown, with longitudinal striations, sometimes with transverse fissures, and showing rootlet scars.

It may also be found as more or less cylindrical fragments up to 2 cm long or elliptical or irregular discs. The fracture is light-coloured and very fibrous.

B. Reduce to a powder (355) *(2.9.12)*. The powder is light yellowish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous, whole or fragmented phloem fibres, usually isolated, narrow, sometimes more than 500 µm long and often rough-walled; calcium oxalate cluster crystals, free or inside parenchyma cells; a few reticulate lignified vessels; rare cork fragments. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R* . The powder shows rounded or ovoid starch granules, single or in groups of 2-4; the diameter of a component granule may reach 30 µm. Some starch granules are found in the parenchyma cells or in cells of the medullary rays.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 2.0 g of the powdered drug (355) *(2.9.12)* add 50 ml of *water R* and boil under a reflux condenser for 30 min. Cool the solution and centrifuge for 10 min. Shake the supernatant with 2 quantities, each of 15 ml, of *di-isopropyl ether R* saturated with *hydrochloric acid R* . Combine the ether layers. Evaporate to dryness and dissolve the residue in 1.0 ml of *methanol R* . Filter through a polypropylene syringe filter (nominal pore size 0.45 µm).

Reference solution Dissolve 5 mg of *gallic acid R* and 20 mg of *resorcinol R* in 20 ml of *methanol R*.

Plate *TLC silica gel F $_{254}$ plate R* (5-40 µm) [or *TLC silica gel F $_{254}$ plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R*, *ethyl acetate R*, *toluene R* (10:30:60 *V/V/V*).

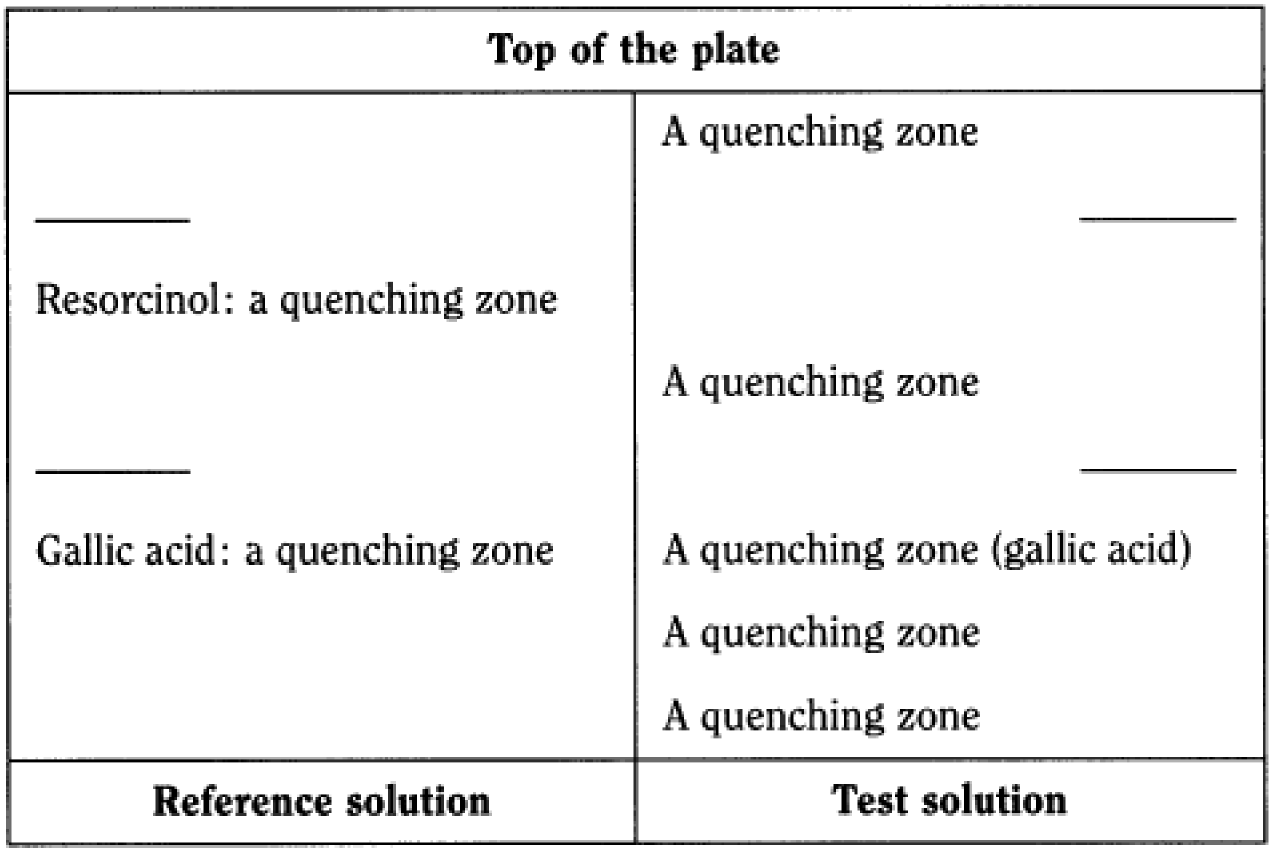
Application 10 µl [or 4 µl] as bands.

Development Over a path of 10 cm [or 6 cm].

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

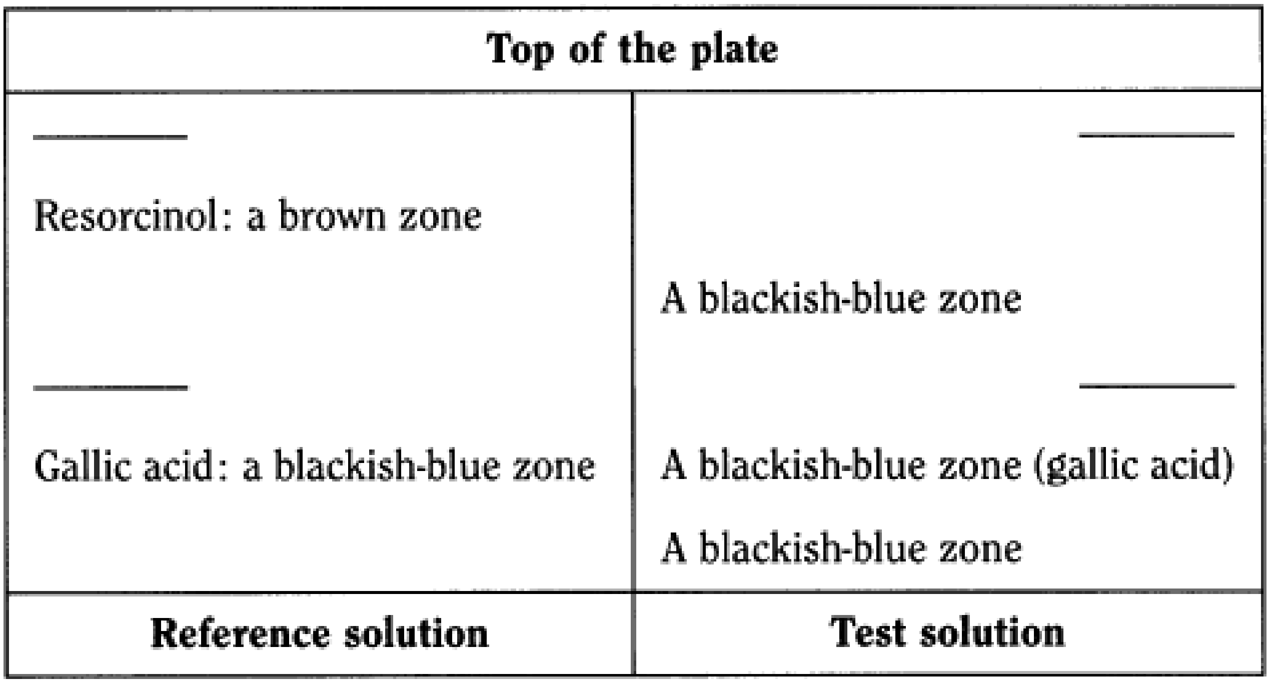
Results A See below the sequence of quenching zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint quenching zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A quenching zone
________	________
Resorcinol: a quenching zone	
	A quenching zone
________	________
Gallic acid: a quenching zone	A quenching zone (gallic acid)
	A quenching zone
	A quenching zone
Reference solution	**Test solution**

Detection B Spray with a 10 g/l solution of *ferric chloride R* in *anhydrous ethanol R* and heat at 100-105 °C for 15 min; examine in daylight.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
_______	_______
Resorcinol: a brown zone	
	A blackish-blue zone
_______	_______
Gallic acid: a blackish-blue zone	A blackish-blue zone (gallic acid)
	A blackish-blue zone
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 2.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs *(2.8.14)* . Use 0.500 g of the powdered drug (180) *(2.9.12)*.

Ph Eur

Greater Celandine

General Notices

(Ph Eur monograph 1861)

Ph Eur

DEFINITION

Dried, whole or cut aerial parts of *Chelidonium majus* L. collected during flowering.

Content

Minimum 0.6 per cent of total alkaloids, expressed as chelidonine ($C_{20}H_{19}NO_5$; M_r 353.4) (dried drug).

IDENTIFICATION

A. The stems are rounded, ribbed, yellowish or greenish-brown, somewhat pubescent, about 3-7 mm in diameter, hollow and mostly collapsed. The leaves are thin, irregularly pinnate, the leaflets ovate to oblong with coarsely dentate margins, the terminal leaflet often three-lobed; the adaxial surface is bluish-green and glabrous, the abaxial surface paler and pubescent, especially on the veins. The flowers have 2 deeply concavo-convex sepals, readily removed, and 4 yellow, broadly ovate, spreading petals about 8-10 mm long; the stamens are numerous, yellow, and a short style arises from a superior ovary; long, capsular, immature fruits are rarely present.

B. Reduce to a powder (355) *(2.9.12)*. The powder is dark greyish-green or brownish-green. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: numerous fragments of leaves in surface view, the epidermal cells with sinuous walls; anomocytic stomata *(2.8.3)* occur on the abaxial surface only; covering trichomes long, uniseriate, with thin walls and usually fragmented; vascular tissue from the leaves and stems with groups of fibres, pitted and spirally thickened vessels and associated latex tubes with yellowish-brown contents; occasional fragments of the corolla with thin-walled, partly papillose cells containing numerous pale yellow droplets of oil; spherical pollen grains about 30-40 µm in diameter with 3 pores and a finely pitted exine.

C. Thin-layer chromatography *(2.2.27)* .

Test solution To 0.4 g of the powdered drug (710) *(2.9.12)* add 50 ml of *dilute acetic acid R* . Boil in a water-bath under a reflux condenser for 30 min. Cool and filter. To the filtrate add *concentrated ammonia R* until a strong alkaline reaction is produced. Shake with 30 ml of *methylene chloride R* . Dry the organic layer over *anhydrous sodium sulphate R* , filter and evaporate *in vacuo* to dryness. Dissolve the residue in 1.0 ml of *methanol R.*

Reference solution Dissolve 2 mg of *papaverine hydrochloride R* and 2 mg of *methyl red R* in 10 ml of *ethanol (96 per cent) R*.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, water R, propanol R* (1:9:90 *V/V/V*).

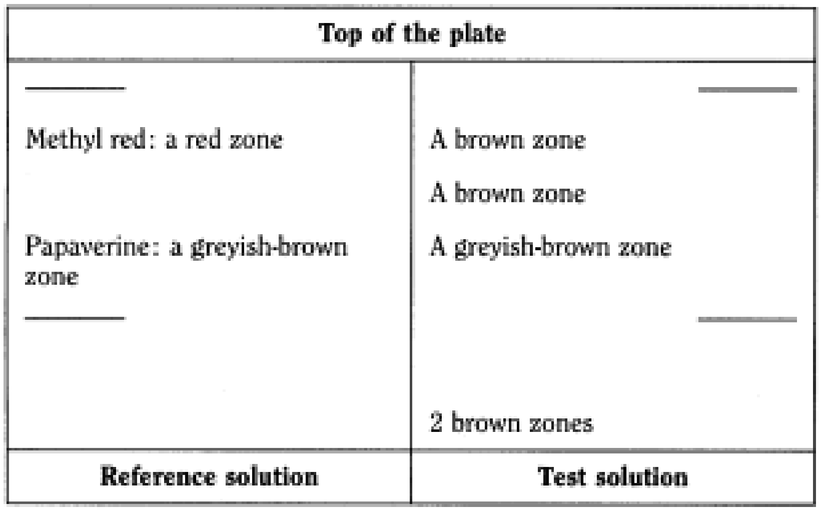
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *potassium iodobismuthate solution R* and dry in air; spray with *sodium nitrite solution R* and allow to dry in air; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other weaker zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Methyl red: a red zone	A brown zone
	A brown zone
Papaverine: a greyish-brown zone	A greyish-brown zone
———	———
	2 brown zones
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 10.0 per cent.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 13.0 per cent.

ASSAY

Test solution To 0.750 g of the powdered drug (710) *(2.9.12)*, add 200 ml of *dilute acetic acid R* and heat on a water-bath for 30 min, shaking frequently. Cool and dilute to 250.0 ml with *dilute acetic acid R*. Filter. Discard the first 20 ml of the filtrate. To 30.0 ml of the filtrate add 6.0 ml of *concentrated ammonia R* and 100.0 ml of *methylene chloride R*. Shake for 30 min. Separate the organic layer, place 50.0 ml in a 100 ml round-bottomed flask and evaporate to dryness *in vacuo* at a temperature not exceeding 40 °C. Dissolve the residue in about 2-3 ml of *ethanol (96 per cent) R*, warming slightly. Transfer the solution to a 25 ml volumetric flask by rinsing the round-bottomed flask with *dilute sulphuric acid R* and dilute to 25.0 ml with the same solvent. To 5.0 ml of the solution, add 5.0 ml of a 10 g/l solution of *chromotropic acid, sodium salt R* in *sulphuric acid R* in a 25 ml volumetric flask, stopper the flask and mix carefully. Dilute to 25.0 ml with *sulphuric acid R* and stopper the flask.

Compensation liquid Prepare at the same time and in the same manner as for the test solution: place in a 25 ml volumetric flask 5.0 ml of *dilute sulphuric acid R* and 5.0 ml of a 10 g/l solution of *chromotropic acid*, *sodium salt R in sulphuric acid R*, stopper the flask and mix carefully. Dilute to 25.0 ml with *sulphuric acid R* and stopper the flask.

Place both solutions on a water-bath for 10 min. Cool to about 20 °C and dilute if necessary to 25.0 ml with *sulphuric acid R*. Measure the absorbance *(2.2.25)* of the test solution at 570 nm by comparison with the compensation liquid.

Calculate the percentage content of total alkaloids, expressed as chelidonine, using the following expression:

$$\frac{A \times 2.23}{m}$$

i.e. taking the specific absorbance of chelidonine to be 933.

A = absorbance at 570 nm

m = mass of the substance to be examined, in grams

Ph Eur

Hamamelis Leaf

General Notices

(Ph Eur monograph 0909)

Ph Eur

DEFINITION

Whole or cut, dried leaf of *Hamamelis virginiana* L.

Content

Minimum 3 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The leaf is green or greenish-brown, often broken, crumpled and compressed into more or less compact masses. The lamina is broadly ovate or obovate; the base is oblique and asymmetric and the apex is acute or, rarely, obtuse. The margins of the lamina are roughly crenate or dentate. The venation is pinnate and prominent on the abaxial surface. Usually, 4-6 pairs of secondary veins are attached to the main vein, emerging at an acute angle and curving gently to the marginal points where there are fine veins often at right angles to the secondary veins.

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of adaxial epidermis with wavy anticlinal walls; abaxial epidermis with stomata mainly paracytic (*2.8.3*); star-shaped covering trichomes, either entire or broken, composed of 4-12 unicellular branches that are united by their bases, elongated, conical and curved, usually up to 250 µm long, thick-walled and with a clearly visible lumen whose contents are often brown; fibres are lignified and thick-walled, isolated or in groups, and accompanied by a sheath of prismatic calcium oxalate crystals; small, cylindrical parenchymatous cells of palisade; irregular-shaped cells of spongy mesophyll; sclereids, frequently enlarged at 1 or both ends, 150-180 µm long, whole or fragmented; fragments of annular or spiral vessels; isolated prisms of calcium oxalate.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *ethanol (60 per cent V/V) R*, shake for 15 min and filter.

Reference solution (a) Dissolve 30 mg of *tannic acid R* in 5 ml of *ethanol (60 per cent V/V) R.*

Reference solution (b) Dissolve 5 mg of *gallic acid R* in 5 ml of *ethanol (60 per cent V/V) R.*

Plate TLC silica gel G plate R.

Mobile phase anhydrous formic acid R, *water R, ethyl formate R* (10:10:80 *V/V/V*).

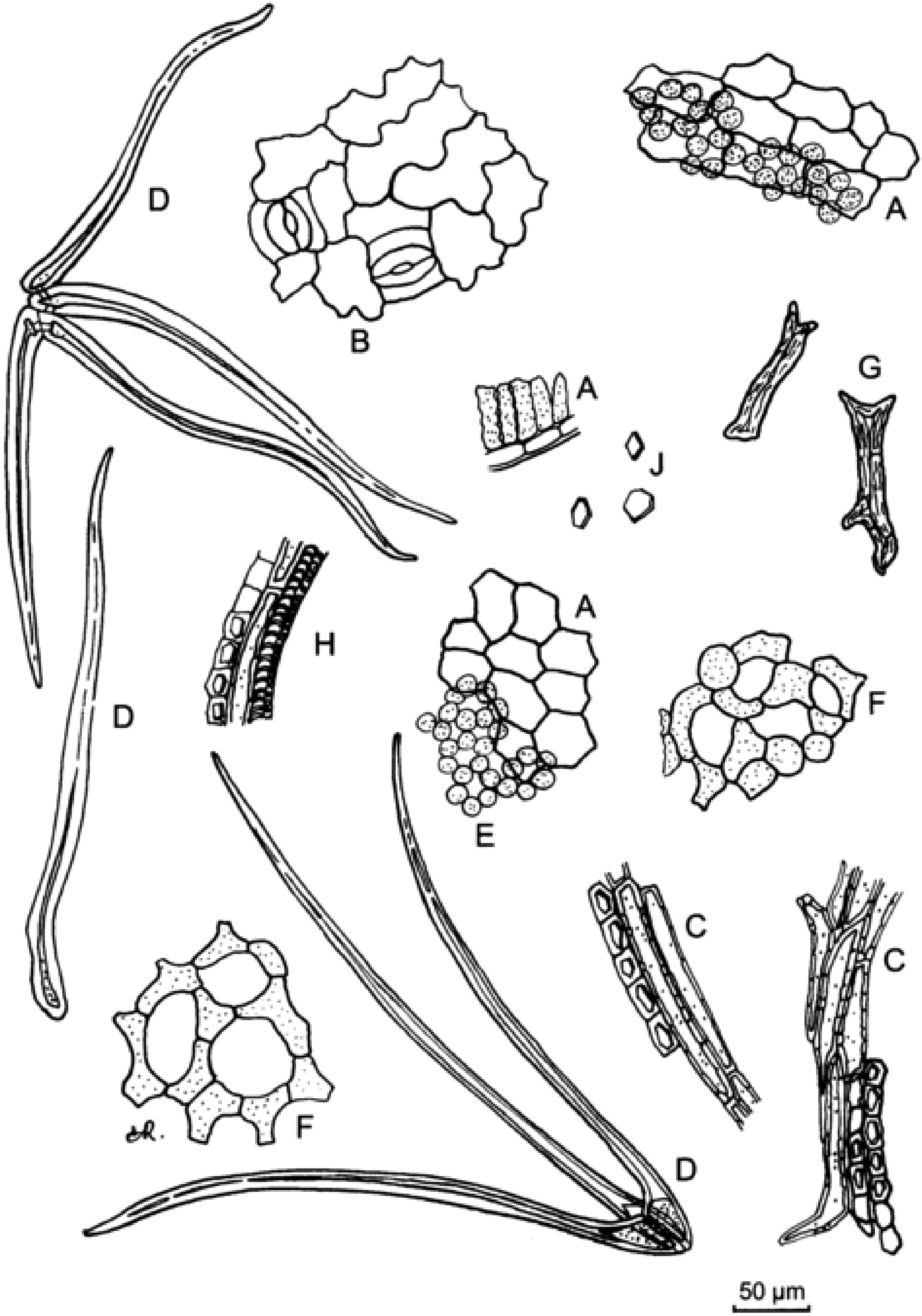
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 10 min, then allow to cool.

Detection Spray with *ferric chloride solution R2* until bluish-grey zones (phenolic compounds) appear.

Results The chromatogram obtained with the test solution shows in its lower third a principal zone similar in position to the principal zone in the chromatogram obtained with reference solution (a) and, in its upper part, a narrow zone similar in position to the principal zone in the chromatogram obtained with reference solution (b). The chromatogram obtained with the test solution shows, in addition, several slightly coloured zones in the central part.


D
B
A
A
G
J
A
H
D
F
E
C
C
F
D
50 µm

A. Adaxial epidermis and palisade parenchyma

B. Abaxial epidermis

C. Lignified fibres with sheath of prismatic calcium oxalate crystals

D. Star-shaped covering trichomes and free covering trichome

E. Palisade parenchyma

F. Spongy mesophyll

G. Sclereid

H. Vessels accompanied by fibres and by sheaths made up of calcium oxalate tubes

J. Isolated prisms of calcium oxalate

Figure 0909.-1. – *Illustration of powdered herbal drug of hamamelis leaf (see Identification B)*

TESTS

Foreign matter (*2.8.2*)

Maximum 7 per cent of stems and maximum 2 per cent of other foreign matter, determined on 50 g.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 2.000 g of powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 4 h.

Total ash (*2.4.16*)

Maximum 7.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 2.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs (*2.8.14*). Use 0.750 g of the powdered drug (180) (*2.9.12*).

Ph Eur

Hawthorn Berries

General Notices

(Ph Eur monograph 1220)

Ph Eur

DEFINITION

Dried false fruits of *Crataegus monogyna* Jacq. (Lindm.), or *Crataegus laevigata* (Poir.) D.C. (synonym: *Crataegus oxyacantha* L.) or their hybrids or a mixture of these false fruits.

Content

Minimum 1.0 per cent of procyanidins, expressed as cyanidin chloride ($C_{15}H_{11}ClO_6$; M_r 322.7) (dried drug).

CHARACTERS

Sweet mucilaginous taste.

IDENTIFICATION

A. The false fruit of *Crataegus monogyna* is obovate or globular, generally 6-10 mm long and 4-8 mm wide, reddish-brown or dark red. The surface is pitted or, more rarely, reticulated. The upper end of the fruit is crowned by the remains of 5 reflexed sepals surrounding a small sunken disc with a shallow, raised rim. The remains of the style occur in the centre of the disc with tufts of stiff, colourless hairs at the base. At the lower end of the fruit is a short length of pedicel or, more frequently, a small pale circular scar where the pedicel was attached. The receptacle is fleshy and encloses a yellowish-brown, ovoid fruit with a hard, thick wall containing a single, elongated, pale brown, smooth and shiny seed.

The false fruit of *Crataegus laevigata* is up to 13 mm long. It contains 2-3 stony fruits, ventrally flattened, with short hairs at the top. Frequently, in the centre of the disc of the false fruit occur the remains of the 2 styles.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-red. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: covering trichomes from inside the disc which are long, unicellular, frequently bent, tapering to a point, with smooth, much thickened and lignified walls; parenchymatous receptacle fragments, the outer layer with red colouring matter, some cells of the inner layers containing small cluster crystals of calcium oxalate; occasional fragments including groups of sclereids and vascular strands with associated files of cells containing prisms of calcium oxalate; pericarp fragments consisting of large thick-walled sclereids with numerous pits, some of which are conspicuously branched; a few fragments of the testa having an epidermal layer composed of hexagonal, mucilaginous cells beneath which is a yellowish-brown pigment layer containing numerous elongated prisms of calcium oxalate; thin-walled parenchyma of the endosperm and cotyledons containing aleurone grains and globules of fixed oil.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* and heat in a water bath at 65 °C for 5 min, shaking frequently. Allow to cool to room temperature and filter. Dilute the filtrate to 10 ml with *methanol R*.

Reference solution Dissolve 2 mg of *chlorogenic acid R*, 2 mg of *caffeic acid R*, 5 mg of *hyperoside R* and 5 mg of *rutin R* in 20 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application 30 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray whilst hot with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the lower half, in order of increasing R_F values, a yellowish-brown fluorescent zone (rutin), a light blue fluorescent zone (chlorogenic acid) and a yellowish-brown fluorescent zone (hyperoside); in the upper third appears a light blue fluorescent zone (caffeic acid). The chromatogram obtained with the test solution shows 3 zones similar in position and fluorescence to the zones due to chlorogenic acid, hyperoside and caffeic acid in the chromatogram obtained with the reference solution, and 3 weak reddish fluorescent zones, one corresponding to the zone due to rutin in the chromatogram obtained with the reference solution and both of the others located above the zone due to hyperoside. Below and above the zone due to caffeic acid some light blue zones appear.

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of deteriorated false fruit and maximum 2 per cent of other foreign matter. It does not contain fruits of other *Crataegus species* (*C. nigra* Waldst. et Kit., *C. pentagyna* Waldst. et Kit. ex Willd. and C. *azarolus* L.) which are characterised by the presence of more than 3 hard stones.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

ASSAY

To 2.50 g of the powdered drug (355) *(2.9.12)* add 30 ml of *ethanol (70 per cent V/V) R*. Heat under a reflux condenser for 30 min and filter. Wash the residue with 10.0 ml of *ethanol (70 per cent V/V) R*. Add to the filtrate 15.0 ml of *hydrochloric acid R1* and 10.0 ml of *water R*.

Heat under a reflux condenser for 80 min. Allow to cool, filter and wash the residue with *ethanol (70 per cent V/V) R* until the filtrate is colourless. Dilute the filtrate to 250.0 ml with *ethanol (70 per cent V/V) R*. Evaporate 50.0 ml of this solution in a round-bottomed flask to about 3 ml and transfer to a separating funnel. Rinse the round-bottomed flask sequentially with 10 ml and 5 ml of *water R* and transfer to the separating funnel. Shake the combined solution with 3 quantities, each of 15 ml, of *butanol R*. Combine the organic layers and dilute to 100.0 ml with *butanol R*.

Measure the absorbance *(2.2.25)* of the solution at 545 nm.

Calculate the percentage content of procyanidins, expressed as cyanidin chloride, using the following expression:

$$\frac{A \times 500}{75 \times m}$$

i.e. taking the specific absorbance of cyanidin chloride to be 75.

A = absorbance at 545 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Hawthorn Leaf and Flower Dry Extract

General Notices

(Ph Eur monograph 1865)

Ph Eur

DEFINITION

Dry extract produced from *Hawthorn leaf and flower (1432)*.

Content:

— for aqueous extracts: minimum 2.5 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried extract);

— for hydroalcoholic extracts: minimum 6.0 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried extract).

PRODUCTION

The extract is produced from the drug by a suitable procedure using either water or a hydroalcoholic solvent equivalent in strength to a minimum of 45 per cent *V/V* ethanol.

CHARACTERS

Appearance

Light brown or greenish-brown powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Suspend 0.2 g of the extract to be examined in 20 ml of *alcohol (70 per cent V/V) R* and filter.

Reference solution Dissolve 1 mg of *chlorogenic acid R*, 2.5 mg of *hyperoside R* and 2.5 mg of *rutin R* in 10 ml of *methanol R*.

Plate TLC silica gel plate R.

Mobile phase anhydrous formic acid R, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

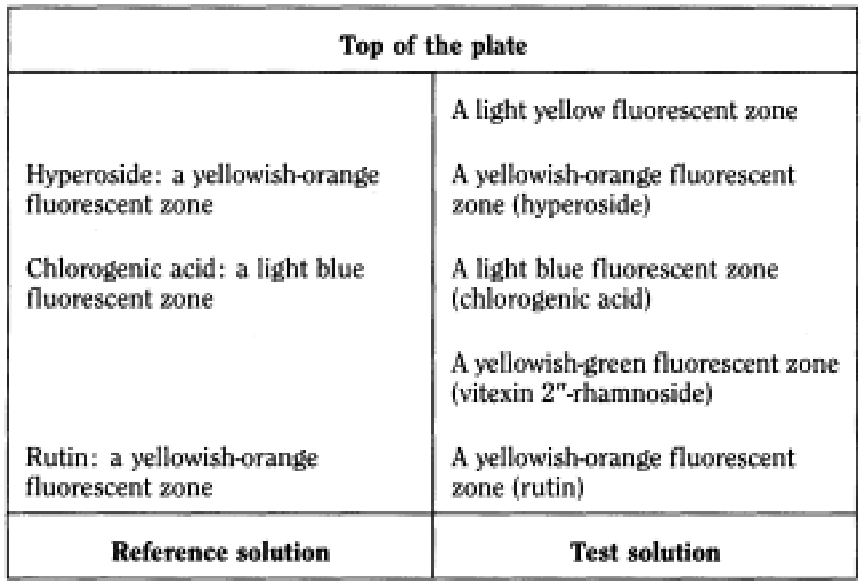
Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray the plate whilst hot with a 10 g/l solution of *diphenylboric acid aminoethyl* *ester R* in *methanol R*; subsequently spray the plate with a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow the plate to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A light yellow fluorescent zone
Hyperoside: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (hyperoside)
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
	A yellowish-green fluorescent zone (vitexin 2″-rhamnoside)
Rutin: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (rutin)
Reference solution	**Test solution**

TESTS

Methanol *(2.9.11)*

Maximum 0.05 per cent *V/V*.

Loss on drying *(2.2.32)*

Maximum 6.0 per cent, determined on 0.500 g of the extract to be examined by drying in an oven at 105 °C for 2 h.

ASSAY

Stock solution Dissolve 0.100 g of the extract to be examined in *alcohol (60 per cent V/V) R* and dilute to 100.0 ml with the same solvent.

Test solution Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Dissolve the residue in 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the 25 ml volumetric flask. Add 10.0 ml of a solution containing 25.0 g/l of *boric acid R* and 20.0 g/l of *oxalic acid R* in *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Compensation liquid Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Dissolve the residue in 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the 25 ml volumetric flask. Add 10.0 ml of *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

After 30 min measure the absorbance *(2.2.25)* of the test solution at 410 nm by comparison with the compensation liquid.

Calculate the percentage content of total flavonoids, calculated as hyperoside, from the expression:

$$\frac{A \times 1.235}{m}$$

i.e. taking the specific absorbance to be 405.

A = absorbance at 410 nm,

m = mass of the extract to be examined, in grams.

Ph Eur

Hawthorn Leaf and Flower

General Notices

(Ph Eur monograph 1432)

Preparation

Hawthorn Leaf and Flower Dry Extract

Ph Eur

DEFINITION

Whole or cut, dried flower-bearing branches of *Crataegus monogyna* Jacq. (Lindm.), *C. laevigata* (Poiret) D.C. (*C. oxyacanthoides* Thuill.) or their hybrids or, more rarely, other European *Crataegus* species including *C. pentagyna* Waldst. et Kit. ex Willd., *C. nigra* Waldst. et Kit., *C. azarolus* L.

Content

Minimum 1.5 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The stems are dark brown, woody, 1-2.5 mm in diameter, bearing alternate, petiolate leaves with small, often deciduous stipules and corymbs of numerous small white flowers. The leaves are more or less deeply lobed with slightly serrate or almost entire margins; those of *C. laevigata* are pinnately lobed or pinnatifid with 3, 5 or 7 obtuse lobes, those of *C. monogyna* pinnatisect with 3 or 5 acute lobes; the adaxial surface is dark green or brownish-green, the abaxial surface is lighter greyish-green and shows a prominent, dense, reticulate venation. The leaves of *C. laevigata*, *C. monogyna* and *C. pentagyna* are glabrous or bear only isolated trichomes, those of *C. azarolus* and *C. nigra* are densely pubescent. The flowers have a brownish-green tubular calyx composed of 5 free, reflexed sepals, a corolla composed of 5 free, yellowish-white or brownish, rounded or broadly ovate and shortly unguiculate petals and numerous stamens. The ovary is fused to the calyx and consists of 1-5 carpels, each with a long style and containing a single ovule; in *C. monogyna* there is 1 carpel, in *C. laevigata* 2 or 3, in *C. azarolus* 2 or 3, or sometimes only 1, in *C. pentagyna* 5 or, rarely, 4.

B. Reduce to a powder (355) *(2.9.12)*. The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: unicellular covering trichomes, usually with a thick wall and wide lumen, almost straight or slightly curved, pitted at the base; fragments of leaf epidermis with cells which have sinuous or polygonal anticlinal walls and with large anomocytic stomata *(2.8.3)* surrounded by 4-7 subsidiary cells; parenchymatous cells of the mesophyll containing calcium oxalate clusters, usually measuring 10-20 µm, those associated with the veins containing groups of small prism crystals; fragments of petals showing rounded polygonal epidermal cells, strongly papillose, with thick walls, the cuticle of which clearly shows wavy striations; fragments of anthers showing endothecium with an arched and regularly thickened margin; fragments of stems containing collenchymatous cells, bordered pitted vessels and groups of lignified sclerenchymatous fibres with narrow lumina; numerous spherical to elliptical or triangular pollen grains up to 45 µm in diameter, with 3 germinal pores and a faintly granular exine.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* and heat in a water-bath at 65 °C under a reflux condenser for 5 min. Cool and filter.

Reference solution Dissolve 1.0 mg of *chlorogenic acid R* and 2.5 mg of *hyperoside R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

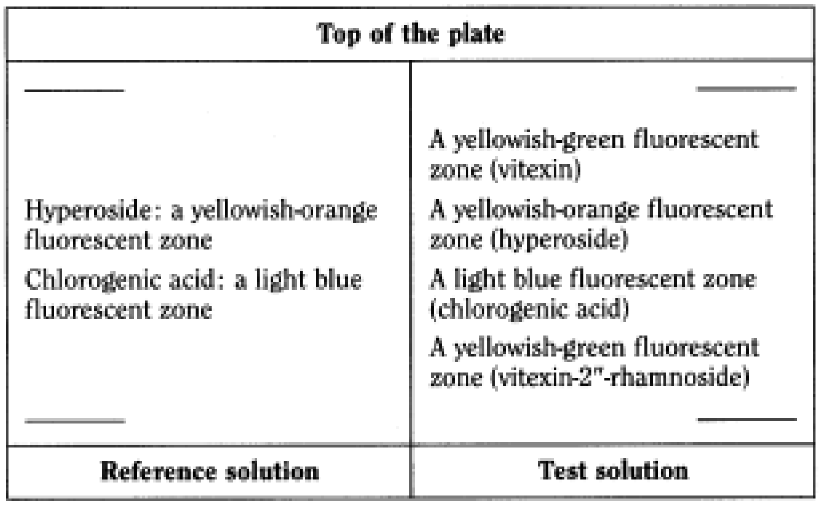
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow to dry in air for about 30 min and examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A yellowish-green fluorescent zone (vitexin)
Hyperoside: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (hyperoside)
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
	A yellowish-green fluorescent zone (vitexin-2″-rhamnoside)
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 8 per cent of lignified branches with a diameter greater than 2.5 mm and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 10.0 per cent.

ASSAY

Stock solution Into a 200 ml flask introduce 0.400 g of the powdered drug (250) *(2.9.12)* and 40 ml of *ethanol (60 per cent V/V) R*. Heat in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter through a plug of absorbent cotton into a 100 ml volumetric flask. Transfer the absorbent cotton with the drug residue back into the 200 ml flask, add 40 ml of *ethanol (60 per cent V/V) R* and heat again in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter into the same 100 ml volumetric flask. Rinse the 200 ml flask with a further quantity of *ethanol (60 per cent V/V) R*, filter and transfer to the same 100 ml volumetric flask. Dilute to 100.0 ml with *ethanol (60 per cent V/V) R* and filter.

Test solution Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the same 25 ml volumetric flask. Add 10.0 ml of a solution containing 25.0 g/l of *boric acid R* and 20.0 g/l of *oxalic acid R* in *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R.*

Compensation liquid Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the same 25 ml volumetric flask. Add 10.0 ml of *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Measure the absorbance *(2.2.25)* of the test solution after 30 min at 410 nm, by comparison with the compensation liquid.

Calculate the percentage content of total flavonoids, expressed as hyperoside, using the following expression:

$$\frac{A \times 1.235}{m}$$

i.e. taking the value of the specific absorbance of hyperoside to be 405.

A = absorbance at 410 nm,

m = mass of the drug to be examined, in grams.

Ph Eur

Herbal Drug Preparations

General Notices

(Ph Eur monograph 1434)

Herbal Drug Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Herbal drug preparations are obtained by subjecting herbal drugs to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal drugs, tinctures, extracts, essential oils, expressed juices and processed exudates.

Herbal teas comply with the monograph on *Herbal teas (1435)* .

Instant herbal teas consist of powder or granules of one or more herbal drug preparation(s) intended for the preparation of an oral solution immediately before use.

Ph Eur

Herbal Drugs

General Notices

(Ph Eur monograph 1433)

Ph Eur

DEFINITION

Herbal drugs are mainly whole, fragmented, or cut plants, parts of plants, algae, fungi or lichen, in an unprocessed state, usually in dried form but sometimes fresh. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal drugs. Herbal drugs are precisely defined by the botanical scientific name according to the binominal system (genus, species, variety and author).

PRODUCTION

Herbal drugs are obtained from cultivated or wild plants. Suitable collection, cultivation, harvesting, drying, fragmentation and storage conditions are essential to guarantee the quality of herbal drugs.

Herbal drugs are, as far as possible, free from impurities such as soil, dust, dirt and other contaminants such as fungal, insect and other animal contaminations. They are not rotten.

If a decontaminating treatment has been used, it is necessary to demonstrate that the constituents of the plant are not affected and that no harmful residues remain. The use of ethylene oxide is prohibited for the decontamination of herbal drugs.

IDENTIFICATION

Herbal drugs are identified using their macroscopic and microscopic descriptions and any further tests that may be required (for example, thin-layer chromatography).

TESTS

Foreign matter *(2.8.2)*

Carry out a test for foreign matter, unless otherwise prescribed or justified and authorised. The content of foreign matter is not more than 2 per cent *m/m*, unless otherwise prescribed or justified and authorised. A specific appropriate test may apply to herbal drugs liable to be adulterated.

Loss on drying *(2.2.32)*

Carry out a test for loss on drying, unless otherwise prescribed or justified and authorised.

Water *(2.2.13)*

A determination of water may be carried out instead of a test for loss on drying for herbal drugs with a high essential-oil content.

Pesticides ***(2.8.13)***

Herbal drugs comply with the requirements for pesticide residues. The requirements take into account the nature of the plant, where necessary the preparation in which the plant might be used, and where available the knowledge of the complete record of treatment of the batch of the plant.

Microbial contamination

Recommendations on the microbiological quality of herbal medicinal products consisting solely of one or more herbal drugs are given in the text *5.1.4*. *Microbiological quality of pharmaceutical preparations.*

Where necessary, herbal drugs comply with other tests, such as the following, for example.

Total ash ***(2.4.16)***

Ash insoluble in hydrochloric acid ***(2.8.1)***

Extractable matter

Swelling index ***(2.8.4)***

Bitterness value ***(2.8.15)***

Heavy metals

The risk of contamination of herbal drugs by heavy metals must be considered. If an individual monograph does not prescribe a test for heavy metals or specific elements, such a test or tests may be required if justified.

Aflatoxins ***(2.8.18)***

Where necessary, limits for aflatoxins may be required.

Radioactive contamination

In some specific circumstances, the risk of radioactive contamination is to be considered.

ASSAY

Unless otherwise prescribed or justified and authorised, herbal drugs are assayed by an appropriate method.

STORAGE

Protected from light.

Ph Eur

Herbal Teas

General Notices

(Ph Eur monograph 1435)

Herbal Teas comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

Herbal teas consist exclusively of one or more herbal drugs intended for oral aqueous preparations by means of decoction, infusion or maceration. The preparation is prepared immediately before use.

Herbal teas are usually supplied in bulk form or in sachets.

The herbal drugs used comply with the appropriate individual European Pharmacopoeia monographs or in their absence to the general monograph on *Herbal drugs (1433)* .

Recommendations on the microbiological quality of herbal teas *(5.1.4. – Category 4)* take into account the prescribed preparation method (use of boiling or non-boiling water).

IDENTIFICATION

The identity of herbal drugs present in herbal teas is checked by botanical examinations.

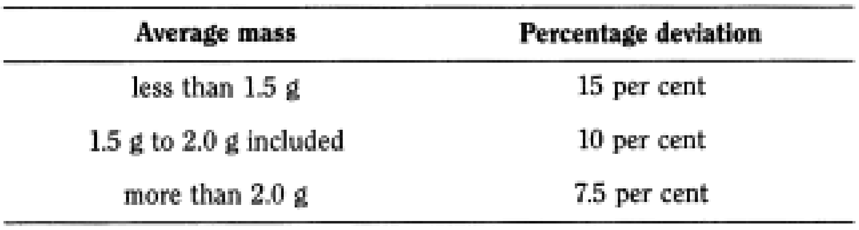
TESTS

The proportion of herbal drugs present in herbal teas is checked by appropriate methods.

Herbal teas in sachets comply with the following test:

Uniformity of mass

Determine the average mass of twenty randomly chosen units as follows: weigh a single full sachet of herbal tea, open it without losing any fragments. Empty it completely using a brush. Weigh the empty sachet and calculate the mass of the contents by subtraction. Repeat the operation on the nineteen remaining sachets. Unless otherwise justified not more than two of the twenty individual masses of the contents deviate from the average mass of the contents by more than the percentage deviation shown in the table below and none deviates by more than twice that percentage.



Average mass	Percentage deviation
less than 1.5 g	15 per cent
1.5 g to 2.0 g included	10 per cent
more than 2.0 g	7.5 per cent

STORAGE

Store protected from light.

Ph Eur

Hop Strobile

General Notices

(Ph Eur monograph 1222)

Ph Eur

DEFINITION

Dried, generally whole, female inflorescence of *Humulus lupulus* L.

CHARACTERS

Characteristic, aromatic odour.

IDENTIFICATION

A. Hop strobiles are generally isolated and 2-5 cm long, petiolate, ovoid, made up of many oval, greenish-yellow, sessile, membranous, overlapping bracts. The external bracts are flattened and symmetrical. The internal bracts are longer and asymmetrical at the base because of a fold generally encircling an induviate fruit (achene). The ovary or rarely the fruit, the base of the bracts and especially the induvial fold, are covered with small orange-yellow glands.

B. Reduce to a powder (355) (*2.9.12*). The powder is greenish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of bracts and bracteoles covered by polygonal, irregular epidermal cells with wavy walls; unicellular, conical, straight or curved covering trichomes with thin, smooth walls; rare anomocytic stomata (*2.8.3*); glandular trichomes, usually free, with bicellular biseriate stalks and heads consisting of 8 small cells; fragments of mesophyll containing small calcium oxalate cluster crystals; many characteristic orange-yellow glandular trichomes with short, bicellular biseriate stalks, bearing a part widening into a cup, 150-250 µm in diameter, made up of a hemispherical layer of secretory cells with a cuticle that has been detached and distended by the accumulation of oleoresinous secretions; fragments of elongated sclerenchymatous cells of the testa with thick walls showing striations and numerous pits.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the freshly powdered drug (355) (*2.9.12*) add 10 ml of a mixture of 3 volumes of *water R* and 7 volumes of *methanol R*; shake for 15 min and filter.

Reference solution Dissolve 1.0 mg of *Sudan orange R*, 2.0 mg of *curcumin R* and 2.0 mg of *dimethylaminobenzaldehyde R* in 20 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous acetic acid R*, *ethyl acetate R*, *cyclohexane R* (2:38:60 *V/V/V*).

Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

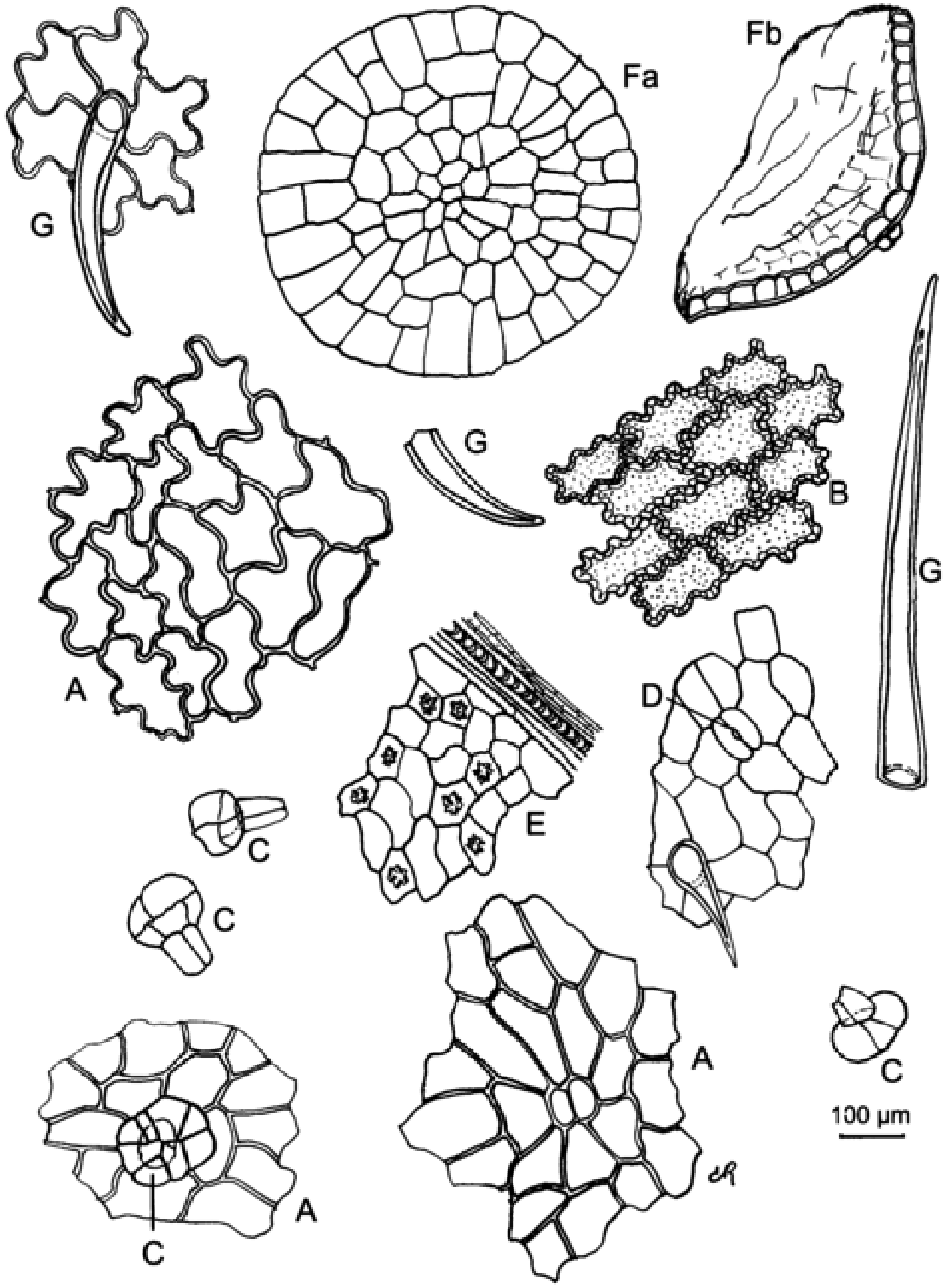
Results A The chromatogram obtained with the reference solution shows 3 quenching zones; in the lower quarter is the faint zone due to curcumin, somewhat below the middle is the zone due to dimethylaminobenzaldehyde and above the zone due to Sudan orange. The chromatogram obtained with the test solution shows a number of quenching zones similar in position to the zones in the chromatogram obtained with the reference solution: at about the level of the zone due to curcumin is a faint zone due to xanthohumol, near the level of the zone due to dimethylaminobenzaldehyde are zones due to humulones, and near the level of the zone due to Sudan orange are zones due to lupulones.

Detection B Examine in ultraviolet light at 365 nm.

Results B In the chromatogram obtained with the test solution the zones due to lupulones show blue fluorescence, the zones due to humulones show brown fluorescence and the zone due to xanthohumol shows dark brown fluorescence.

Detection C Spray with *dilute phosphomolybdotungstic reagent R*; expose to ammonia vapour and examine in daylight.

Results C In the chromatogram obtained with the test solution, the zones due to humulones and to lupulones are bluish-grey and the zone due to xanthohumol is greenish-grey. In the chromatogram obtained with the reference solution, the zones are bluish-grey or brownish-grey.


G
Fa
Fb
G
B
G
A
D
E
C
C
A
C
A
C
100 µm
cR

A. Epidermis of bracts and bracteoles

E. Mesophyll containing calcium oxalate cluster crystals and vessels

B. Sclerenchymatous cells of the testa

C. Multicellular glandular trichome

D. Anomocytic stoma

F. Glandular trichome in surface view (Fa) and in side view (Fb)

G. Covering trichome

Figure 1222.-1. – *Illustration of powdered herbal drug of hop strobile (see Identification B)*

TESTS

Matter extractable by ethanol (70 per cent *V/V*)

Minimum 25.0 per cent.

To 10.0 g of the powdered drug (355) (*2.9.12*) add 300 ml of *ethanol (70 per cent V/V) R* and heat for 10 min on a water bath under a reflux condenser. Allow to cool, filter and discard the first 10 ml of the filtrate. Evaporate 30.0 ml of the filtrate to dryness on a water-bath and dry in an oven at 100-105 °C for 2 h. The residue weighs a minimum of 0.250 g.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 12.0 per cent.

Ph Eur

Horsetail

General Notices

(Equisetum Stem, Ph Eur monograph 1825)

Ph Eur

DEFINITION

Whole or cut, dried sterile aerial parts of *Equisetum arvense* L.

Content

Minimum 0.3 per cent of total flavonoids expressed as isoquercitroside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

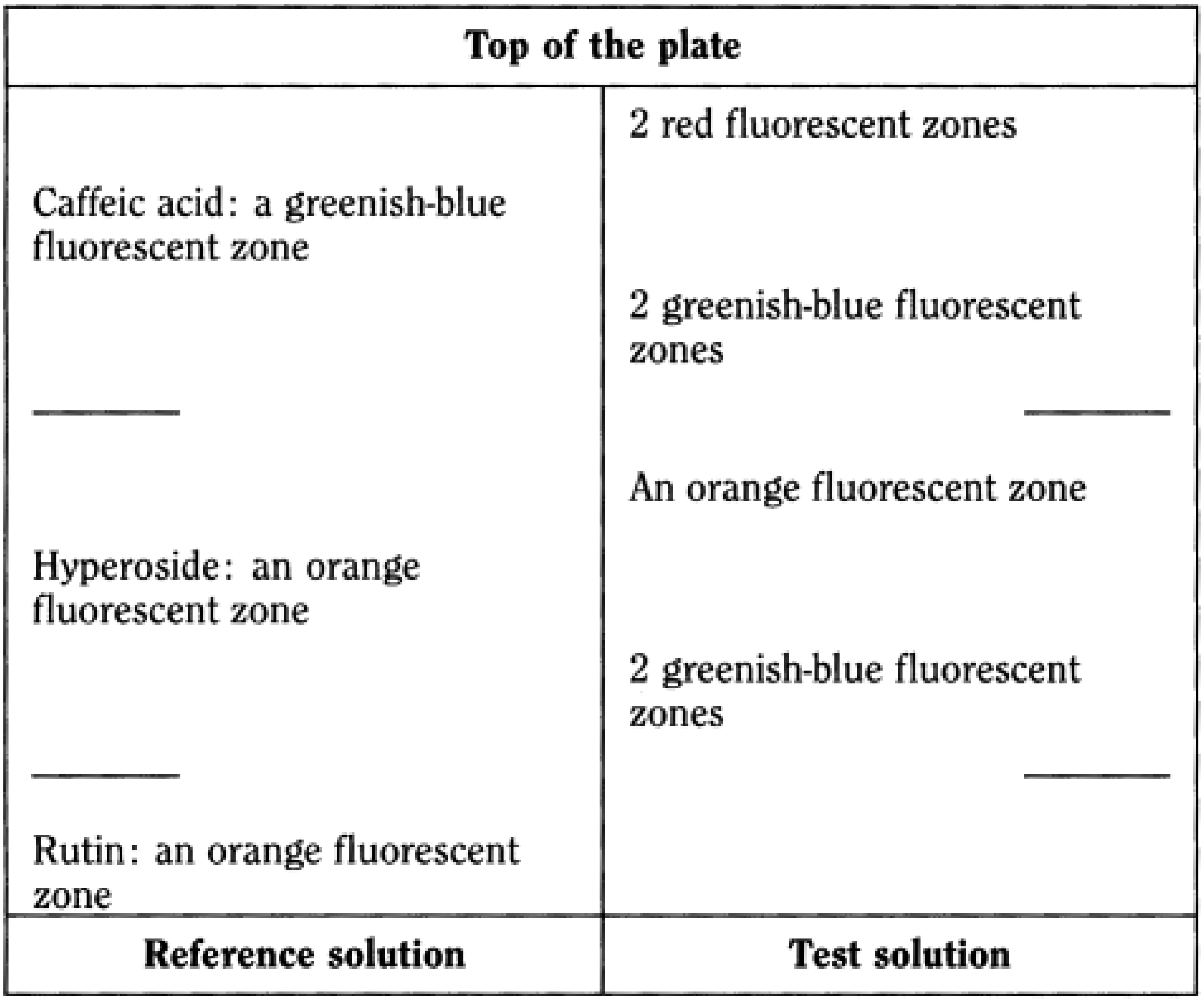
IDENTIFICATION

A. It consists of fragments of grooved stems and linear leaves, light green to greenish-grey. They are rough to the touch, brittle and crunchy when crushed. The main stems are about 0.8 mm to 4.5 mm in diameter, hollow, jointed at the nodes which occur at intervals of about 1.5 cm to 4.5 cm; distinct vertical grooves are present on the internodes, ranging in number from 4 to 14 or more. Verticils of widely spaced and erect branches, usually simple, each about 1 mm thick with 2 to 4 longitudinal grooves, occur at the nodes. The leaves are small, linear, verticillate at each node, concrescent at the base, they form a toothed sheath around the stem; with the number of teeth corresponding to the number of grooves on the stem. Each tooth, often brown, is lanceolate-triangular. The lowest internode of each branch is longer than the sheath of the stem it belongs to.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the epidermis in surface view, composed of rectangular cells with wavy walls and paracytic stomata *(2.8.3)* with the 2 subsidiary cells covering the guard cells and having conspicuous radial ridges; in transverse sectional view the epidermis is crenate, with the protuberances formed from the contiguous walls of 2 adjacent, U-shaped cells. Fragments of large-celled parenchyma and groups of long, non-lignified fibres with narrow lumens; scattered small, lignified vessels with spiral or annular thickening.

C. Examine the chromatograms obtained in the test for other *Equisetum* species and hybrids.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	2 red fluorescent zones
Caffeic acid: a greenish-blue fluorescent zone	
	2 greenish-blue fluorescent zones
———	———
	An orange fluorescent zone
Hyperoside: an orange fluorescent zone	
	2 greenish-blue fluorescent zones
———	———
Rutin: an orange fluorescent zone	
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of stems from other *Equisetum* species and hybrids and maximum 2 per cent of other foreign matter.

Other *Equisetum* species and hybrids

Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R*. Heat in a water-bath at 60 °C for 10 min with occasional shaking. Allow to cool. Filter.

Reference solution Dissolve 1.0 mg of *caffeic acid R*, 2.5 mg of *hyperoside R* and 2.5 mg of *rutin R* in 10 ml of *methanol R*.

Plate TLC silica gel plate R.

Mobile phase anhydrous formic acid R, *glacial acetic acid R*, *water R*, *ethyl acetate R* (7.5:7.5:18:67 *V/V/V/V*).

Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Then spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for 30 min. Examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows no yellow or greenish-yellow fluorescent zone shortly above the starting line.

Loss on drying *(2.2.32)*

Maximum 10 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Ash insoluble in hydrochloric acid *(2.8.1)*

Minimum 3.0 per cent and maximum 15.0 per cent.

Total ash *(2.4.16)*

Minimum 12.0 per cent and maximum 27.0 per cent.

ASSAY

Stock solution In a 100 ml round-bottomed flask, introduce 0.800 g of the powdered drug (355) *(2.9.12)*, add 1 ml of a 5 g/l solution of *hexamethylenetetramine R*, 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1*. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R*, each time boiling under a reflux condenser for 10 min. Allow to cool and filter each extract through a plug of absorbent cotton into the flask. After cooling, filter the combined acetone extracts through a filter paper into a volumetric flask and dilute to 100.0 ml with *acetone R* by rinsing the flask and the filter paper. Introduce 20.0 ml of the solution into a separating funnel, add 20 ml of *water R* and shake the mixture with 1 quantity of 15 ml and then 3 quantities, each of 10 ml, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, wash with 2 quantities, each of 50 ml, of *water R*, and filter the extracts over 10 g of *anhydrous sodium sulphate R* into a volumetric flask. Dilute to 50.0 ml with *ethyl acetate R*.

Test solution To 10.0 ml of the stock solution add 1 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation solution Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Measure the absorbance *(2.2.25)* of the test solution after 30 min, by comparison with the compensation solution at 425 nm. Calculate the percentage content of flavonoids, calculated as isoquercitroside, from the expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of isoquercitroside to be 500,

A = absorbance at 425 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Iceland Moss

General Notices

(Ph Eur monograph 1439)

Ph Eur

DEFINITION

Whole or cut, dried thallus of *Cetraria islandica* (L.) Acharius s.l.

IDENTIFICATION

A. The thallus, up to 15 cm long, is irregularly dichotomous and consists of glabrous, groove-shaped or almost flat, stiff, brittle bands, 0.3-1.5 cm wide and about 0.5 mm thick, sometimes serrated with the margin appearing ciliated (pycnidia). The upper surface is greenish or greenish-brown, the lower surface is greyish-white or light brownish and shows whitish, depressed spots (so-called respiratory cavities). On the apices of the terminal lobes, very rarely, there are brown, discoid apothecia.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-brown. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: numerous fragments of the pseudoparenchyma consisting of narrow-lumened, thick-walled hyphae from the marginal layer and wide-lumened hyphae from the adjacent layer consisting of loosely entwined hyphae, in which, in the medullary zone, greenish or brownish algae cells up to 15 µm in diameter, are embedded; occasionally marginal fragments of the thallus with tube-like or cylindrical spermogonia, up to about 160 µm wide and up to about 400 µm long.

C. To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *water R* and boil for 2-3 min. The greyish-brown solution forms a gel after cooling which gives a blue colour with *iodine solution R.*

D. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 5 ml of *acetone R* and heat in a water-bath under a reflux condenser for 2-3 min. Cool and filter.

Reference solution Dissolve 5 mg of *anethole R* and 5 mg of *caffeic acid R* in 2 ml of *acetone R.*

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *acetone R*, *methanol R*, *anhydrous formic acid R*, *toluene R* (5:5:10:80 *V/V/V/V*).

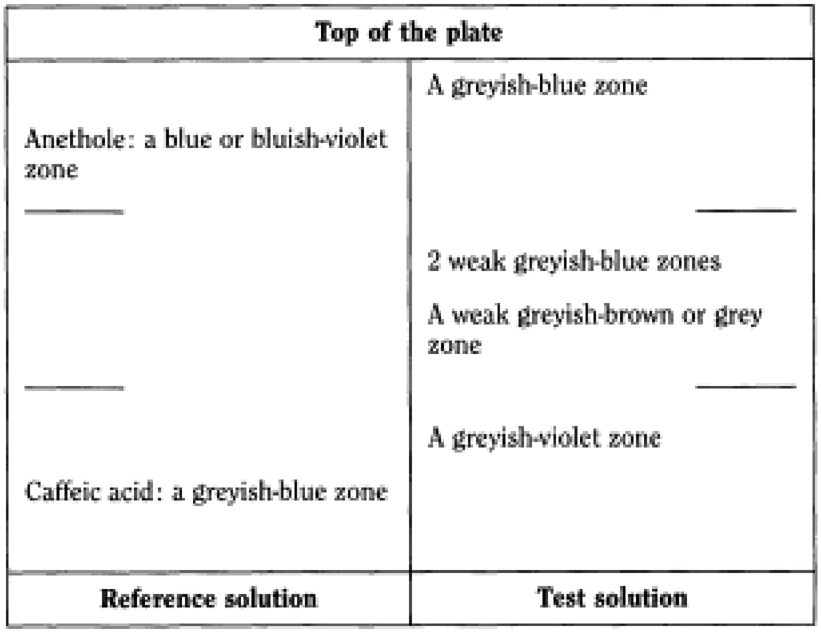
Application 20 µl [or 4 µl] of the test solution and 10 µl [or 2 µl] of the reference solution, as bands.

Development Over a path of 10 cm [or 6 cm].

Drying In air.

Detection Spray with *anisaldehyde solution R*. Heat at 100-105 °C for 5-10 min and examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A greyish-blue zone
Anethole: a blue or bluish-violet zone	
———	———
	2 weak greyish-blue zones
	A weak greyish-brown or grey zone
———	———
	A greyish-violet zone
Caffeic acid: a greyish-blue zone	
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 3.0 per cent.

Swelling value *(2.8.4)*

Minimum 4.5, determined on the powdered drug (355) *(2.9.12)*.

Ph Eur

Indian Frankincense

General Notices

(Ph Eur monograph 2310)

Ph Eur

DEFINITION

Air-dried gum-resin exudate, obtained by incision in the stem or branches of *Boswellia serrata* Roxb. ex Colebr.

Content

- *11-keto-β-boswellic acid* ($C_{30}H_{46}O_4$; M_r 470.7): minimum 1.0 per cent (dried drug);
- *acetyl-11-keto-β-boswellic acid* ($C_{32}H_{48}O_5$; M_r 512.7): minimum 1.0 per cent (dried drug).

IDENTIFICATION

A. Indian frankincense consists of translucent, roundish or irregularly shaped, variable size pieces of up to 3 cm. They are yellowish or reddish-brown. Their surface is covered with grey dust. The fracture is dull or slightly glossy.

B. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 90 ml of *methanol R* and sonicate for 10 min. Shake the mixture vigorously 3 or 4 times during this procedure. Dilute to 100 ml with *methanol R*. Centrifuge and use the clear supernatant solution.

Reference solution Dissolve 2 mg of *11-keto-β-boswellic acid R* and 2 mg of *acetyl-11-keto-β-boswellic acid R* in 20 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F_{254} plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R*, *heptane R*, *ethyl acetate R*, *toluene R* (3:10:20:80 *V/V/V/V*).

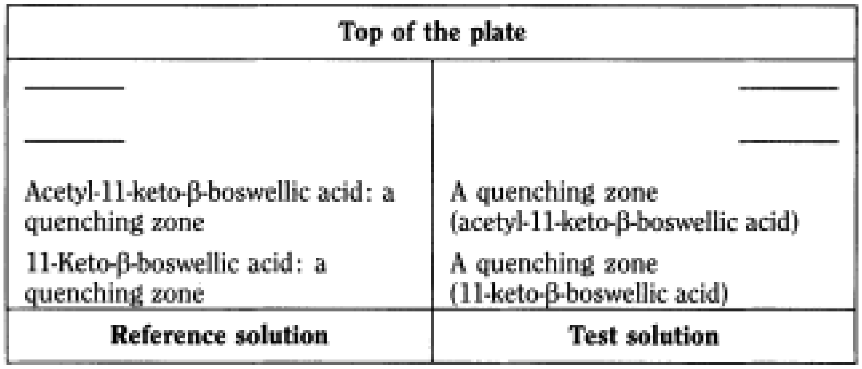
Application 10 µl [or 3 µl] as bands.

Development Over a path of 8 cm [or 5 cm].

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. The zones due to 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid in the test solution are of approximately equivalent intensity. Furthermore, other weak quenching zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
———	———
Acetyl-11-keto-β-boswellic acid: a quenching zone	A quenching zone (acetyl-11-keto-β-boswellic acid)
11-Keto-β-boswellic acid: a quenching zone	A quenching zone (11-keto-β-boswellic acid)
Reference solution	**Test solution**

TESTS

Loss on drying *(2.2.32)*

Maximum 8.0 per cent, determined on 1.000 g of powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 3 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

ASSAY

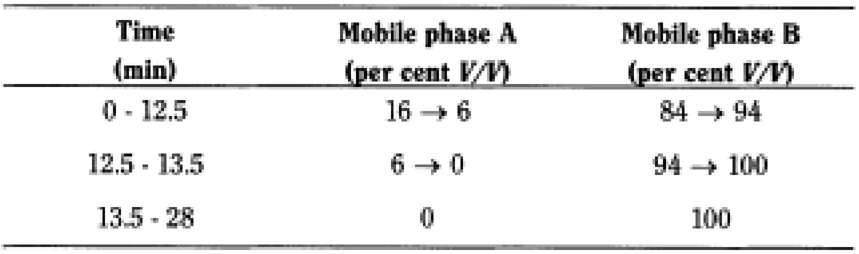
Liquid chromatography *(2.2.29)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 90 ml of *methanol R* and sonicate for 10 min. Shake the mixture vigorously 3 or 4 times during this procedure. Dilute to 100.0 ml with *methanol R*. Centrifuge for 5 min. Dilute 1.0 ml of the clear solution to 10.0 ml with a mixture of 16 volumes of mobile phase A and 84 volumes of mobile phase B.

Reference solution Dissolve 1.0 mg of *11-keto-β-boswellic acid R* and 1.0 mg of *acetyl-11-keto-β-boswellic acid R* in 20.0 ml of *methanol R*. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *phosphoric acid R*, *water R* (0.1:99.9 *V/V*);
- *phase mobile B*: *phosphoric acid R*, *acetonitrile R* (0.1:99.9 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12.5	16 → 6	84 → 94
12.5 - 13.5	6 → 0	94 → 100
13.5 - 28	0	100

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 250 nm.

Injection 20 µl.

Retention time 11-keto-β-boswellic acid = about 8 min; acetyl-11-keto-β-boswellic acid = about 12 min.

System suitability Reference solution:

— *resolution*: minimum 6.0 between the peaks due to 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid.

Calculate the percentage content of 11-keto-β-boswellic acid using the following expression:

$$\frac{A_1 \times m_1 \times 5 \times p_1}{A_2 \times m}$$

A_1 = area of the peak due to 11-keto-β-boswellic acid in the chromatogram obtained with the test solution,

A_2 = = area of the peak due to 11-keto-β-boswellic acid in the chromatogram obtained with the reference solution,

m = mass of the substance to be examined, in grams,

m_1 = mass of *11-keto-β-boswellic acid R* in the reference solution, in grams,

p_1 = percentage content of 11-keto-β-boswellic acid in *11-keto-β-boswellic acid R*.

Calculate the percentage content of acetyl-11-keto-β-boswellic acid using the following expression:

$$\frac{A_3 \times m_2 \times 5 \times p_2}{A_4 \times m}$$

A_3 = area of the peak due to acetyl-11-keto-β-boswellic acid in the chromatogram obtained with the test solution,

A_4 = area of the peak due to acetyl-11-keto-β-boswellic acid in the chromatogram obtained with the reference solution

m = mass of the substance to be examined, in grams

m_2 = mass of *acetyl-11-keto-β-boswellic acid R* in the reference solution, in grams

p_2 = percentage content of acetyl-11-keto-β-boswellic acid in *acetyl-11-keto-β-boswellic acid R.*

Ph Eur

Indian Squill

General Notices

Preparation

Squill Oxymel

When Powdered Indian Squill is prescribed or demanded, material complying with the appropriate requirements below shall be dispensed or supplied.

DEFINITION

Indian Squill consists of the bulb of *Drimia indica* (Roxb.) J P Jessop, collected soon after the plant has flowered, divested of dry, outer membranous coats and usually cut longitudinally into slices and dried.

CHARACTERISTICS

Odourless or almost odourless.

Macroscopical

Curved or irregularly shaped strips, about 10 to 50 mm long, 3 to 10 mm wide and 1 to 3 mm thick, frequently tapering towards the ends, occasionally grouped three or four together and attached to a portion of the axis; ridged in the direction of their length and varying in colour from pale yellowish brown to buff; brittle when dry, but tough and flexible when exposed to air.

Microscopical

Epidermis: cells tetrahedral to hexahedral, thin-walled, three to five times longer than wide, having a thick, striated cuticle; stomata rare, *anomocytic*, Appendix XI H, circular in outline, 40 to 42 µm in diameter; mesophyll of thin-walled polygonal cells containing mucilage, some cells also containing bundles of acicular crystals of calcium oxalate, 20 to 900 µm in length; vascular bundles collateral, scattered throughout the mesophyll; xylem vessels with spiral and annular wall thickening; trichomes and starch absent.

IDENTIFICATION

The mucilage contained in the cells of the mesophyll is stained red with *alkaline corallin solution* and reddish purple with 0.01M *iodine*.

TESTS

Ash

Not more than 6.0%, Appendix XI J.

STORAGE

Indian Squill should be stored in a dry place.

Ipecacuanha Liquid Extract

General Notices

DEFINITION

Ipecacuanha Liquid Extract is prepared from Ipecacuanha by a method stated under the general monograph for Extracts. It contains not less than 1.90% and not more than 2.10% of total alkaloids, calculated as emetine, $C_{29}H_{40}N_2O_4$.

EXTEMPORANEOUS PREPARATION

Prepare by extracting Ipecacuanha with Ethanol (80 per cent) according to the following formula and directions.

Ipecacuanha in *fine powder*	1000 g
Ethanol (80 per cent)	A sufficient quantity

Exhaust the Ipecacuanha by *percolation*, Appendix XI F, with Ethanol (80 per cent), reserving the first 750 ml of the percolate. Remove the ethanol from the remainder of the percolate by evaporation under reduced pressure at a temperature not exceeding 60° and dissolve the residual extract in the reserved portion. Determine the proportion of alkaloids in the liquid thus obtained by the Assay described below. To the remainder of the liquid add sufficient Ethanol (80%) to produce an Ipecacuanha Liquid Extract containing 2% w/v of total alkaloids calculated as emetine. Allow to stand for not less than 24 hours; filter.

The extract complies with the requirements stated under Extracts and with the following requirements.

TESTS

Ethanol content

63 to 69% v/v, Appendix VIII F, Method III.

Relative density

0.910 to 0.960, Appendix V G.

Dry residue

The requirement for Dry residue does not apply to Ipecacuanha Liquid Extract.

ASSAY

To 5 ml in a separating funnel add 20 ml of *water*, 5 ml of 1M *sulphuric acid* and 10 ml of *chloroform* and shake well. Transfer the chloroform extract to a second separating funnel containing a mixture of 4 ml of *ethanol (96%)* and 20 ml of 0.05M *sulphuric acid*, shake, allow to separate and discard the chloroform layer. Continue the extraction of the liquid in the first separating funnel with two further 10 ml quantities of *chloroform*, transferring the chloroform solution each time to the second separating funnel and washing as before. Transfer the acidic liquid from the second separating funnel to the first separating funnel, make distinctly alkaline with 5M *ammonia* and shake with successive quantities of *chloroform* until *complete extraction* of the alkaloids is effected, Appendix XI G, washing each chloroform solution with the same 10 ml of *water* contained in a third separating funnel. Remove the chloroform, add to the residue 2 ml of *ethanol (96%)*, evaporate to dryness and dry for 5 minutes at 80° in a current of air. Dissolve the residue in 2 ml of *ethanol (96%)*, previously neutralised to *methyl red solution*, add 10 ml of 0.05M *sulphuric acid VS* and titrate with 0.1M *sodium hydroxide VS* using *methyl red mixed solution* as indicator. Each ml of 0.05M *sulphuric acid VS* is equivalent to 24.03 mg of total alkaloids, calculated as emetine, $C_{29}H_{40}N_2O_4$.

Ipecacuanha

General Notices

(Ipecacuanha Root, Ph Eur monograph 0094)

Preparations

Prepared Ipecacuanha

Ipecacuanha Liquid Extract

Ph Eur

DEFINITION

Fragmented and dried underground organs of *Cephaelis ipecacuanha* (Brot.) A. Rich., known as Matto Grosso ipecacuanha, or of *Cephaelis acuminata* Karsten, known as Costa Rica ipecacuanha, or of a mixture of both species. The principal alkaloids are emetine and cephaeline.

Content

Minimum 2.0 per cent of total alkaloids, expressed as emetine ($C_{29}H_{40}N_2O_4$; M_r 480.7) (dried drug).

CHARACTERS

Slight odour.

IDENTIFICATION

A. *C. ipecacuanha*. The root occurs as somewhat tortuous pieces, dark reddish-brown or very dark brown, seldom more than 15 cm long or 6 mm thick, closely annulated externally, having rounded ridges completely encircling the root; the fracture is short in the bark and splintery in the wood. The transversely cut surface shows a wide greyish bark and a small uniformly dense wood. The rhizome occurs as short lengths usually attached to roots, cylindrical, up to 2 mm in diameter, finely wrinkled longitudinally and with pith occupying approximately one-sixth of the whole diameter.

C. acuminata. The root in general resembles the root of *C. ipecacuanha*, but differs in the following particulars: it is often up to 9 mm thick; the external surface is greyish-brown or reddish-brown with transverse ridges at intervals of usually 1-3 mm, the ridges being about 0.5-1 mm wide, extending about half-way round the circumference and fading at the extremities into the general surface level.

B. Reduce to a powder (355) *(2.9.12)*. The powder is light grey or yellowish-brown. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: parenchymatous cells, raphides of calcium oxalate up to 80 µm in length either in bundles or scattered throughout the powder; fragments of tracheids and vessels usually 10-20 µm in diameter, with bordered pits; larger vessels and sclereids from the rhizome. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows simple or two- to eight-compound starch granules contained in parenchymatous cells, the simple granules being up to 15 µm in diameter in *C. ipecacuanha* and up to 22 µm in diameter in *C. acuminata*.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.1 g of the powdered drug (180) *(2.9.12)* in a test-tube add 0.05 ml of *concentrated ammonia R* and 5 ml of *ether R* and stir the mixture vigorously with a glass rod. Allow to stand for 30 min and filter.

Reference solution Dissolve 2.5 mg of *emetine hydrochloride CRS* and 3 mg of *cephaeline hydrochloride CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R, methanol R, ethyl acetate R, toluene R* (2:15:18:65 *V/V/V/V*).

Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Spray with a 5 g/l solution of *iodine R* in *ethanol (96 per cent) R* and heat at 60 °C for 10 min. Examine in daylight.

Results A The chromatograms obtained with the test solution and with the reference solution show in the lower part a yellow zone due to emetine and below a light brown zone due to cephaeline.

Detection B Examine in ultraviolet light at 365 nm.

Results B The zone due to emetine shows an intense yellow fluorescence and that due to cephaeline a light blue fluorescence. The chromatogram obtained with the test solution shows also faint fluorescent zones.

With *C acuminata* the principal zones in the chromatogram obtained with the test solution are similar in position, fluorescence and size to the zones in the chromatogram obtained with the reference solution.

With *C ipecacuanha* the only difference is that the zone due to cephaeline in the chromatogram obtained with the test solution is much smaller than the corresponding zone in the chromatogram obtained with the reference solution.

TESTS

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of powdered drug (180) *(2.9.12)* by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

ASSAY

To 7.5 g of the powdered drug (180) *(2.9.12)* in a dry flask, add 100 ml of *ether R* and shake for 5 min. Add 5 ml of *dilute ammonia R1*, shake for 1 h. Add 5 ml of *water R* and shake vigorously. Decant the ether layer into a flask through a plug of cotton. Wash the residue in the flask with 2 quantities, each of 25 ml, of *ether R*, decanting each portion through the same plug of cotton. Combine the ether solutions and eliminate the ether by distillation. Dissolve the residue in 2 ml of *ethanol (90 per cent V/V) R*, evaporate to dryness and heat at 100 °C for 5 min. Dissolve the residue in 5 ml of previously neutralised *ethanol (90 per cent V/V) R*, warming on a water-bath. Add 15.0 ml of *0.1 M hydrochloric acid* and titrate the excess acid with *0.1 M sodium hydroxide* using 0.5 ml of *methyl red mixed solution R* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 24.03 mg of total alkaloids, expressed as emetine.

STORAGE

Protected from moisture.

Ph Eur

Ispaghula Husk

General Notices

(Ph Eur monograph 1334)

Preparations

Ispaghula Husk Oral Powder

Ispaghula Husk Granules

Ispaghula Husk Effervescent Granules

Ph Eur

DEFINITION

Episperm and collapsed adjacent layers removed from the seeds of *Plantago ovata* Forssk. (*P. ispaghula* Roxb.).

IDENTIFICATION

A. The husk consists of pinkish-beige fragments or flakes up to about 2 mm long and 1 mm wide, some showing a light brown spot corresponding to the location of the embryo before it was removed from the seed.

B. Reduce to a powder (355) *(2.9.12)*. The powder is pale yellow. Examine under a microscope using *lactic reagent R*. The powder shows the following diagnostic characters: mainly fragments of the episperm with polygonal cells filled with mucilage; fragments of the inner layers of the testa with brownish thin-walled cells often associated with the outer layers of the endosperm. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows occasional starch granules, single or in groups of 2-4, measuring 3-25 µm in diameter.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 10 mg of the powdered drug (355) *(2.9.12)* in a thick-walled centrifuge tube, add 2 ml of a 230 g/l solution of *trifluoroacetic acid R* and shake vigorously. Stopper the test tube and heat at 120 °C for 1 h. Centrifuge the hydrolysate, transfer the clear supernatant liquid into a 50 ml flask, add 10 ml of *water R* and evaporate to dryness under reduced pressure. Take up the residue in 10 ml of *water R* and evaporate again to dryness under reduced pressure. Take up the residue with 2 ml of *methanol R*.

Reference solution (a) Dissolve 10 mg of *arabinose R* in a small quantity of *water R* and dilute to 10 ml with *methanol R*.

Reference solution (b) Dissolve 10 mg of *xylose R* in a small quantity of *water R* and dilute to 10 ml with *methanol R.*

Reference solution (c) Dissolve 10 mg of *galactose R* in a small quantity of *water R* and dilute to 10 ml with *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *water R, acetonitrile R* (15:85 *V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Detection Spray with *aminohippuric acid reagent R* and heat at 120 °C for 5 min; examine in daylight.

Results The chromatogram obtained with the test solution shows 2 orange-pink zones (arabinose and xylose) and a yellow zone (galactose) similar in position and colour to the zones in the chromatograms obtained with the reference solutions.

TESTS

Foreign matter *(2.8.2)*

Carry out the determination using 5.0 g.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 4.0 per cent.

Swelling index (*2.8.4)*

Minimum 40, determined on 0.1 g of the powdered drug (355) *(2.9.12)*.

Ph Eur

Ispaghula Seed

General Notices

(Ph Eur monograph 1333)

Ph Eur

DEFINITION

Dried ripe seeds of *Plantago ovata* Forssk. (*P. ispaghula* Roxb.).

IDENTIFICATION

A. Ispaghula seed is pinkish-beige, smooth, boat-shaped and curved. It is 1.5 mm to 3.5 mm long, 1.5 mm to 2 mm wide and 1 mm to 1.5 mm thick. The concave surface shows in the centre a light coloured spot corresponding to the hilum. The convex surface shows a light brown spot corresponding to the location of the embryo and takes up about one quarter of the length of the seed.

B. Reduce to a powder (355) *(2.9.12)*. The powder is pale brown. Examine under a microscope using *lactic reagent R*. The powder shows mainly fragments of the episperm with polygonal cells filled with mucilage; fragments of the inner layers of the testa with brownish thin-walled cells often associated with the outer layers of the endosperm; fragments of the endosperm with cells with thick cellulose walls containing aleurone grains and oil droplets; a few fragments of embryo with thin-walled cells. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows starch granules, single or in groups of 2 to 4 and measuring 3 µm to 25 µm in diameter.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 50 mg of the powdered drug (355) *(2.9.12)* in a thick-walled centrifuge tube add 2 ml of a 230 g/l solution of *trifluoroacetic acid R,* and shake vigorously. Stopper the test tube and heat the mixture at 120 °C for 1 h. Centrifuge the hydrolysate, transfer the clear supernatant liquid into a 50 ml flask, add 10 ml of *water R* and evaporate the solution to dryness under reduced pressure. Take up the residue in 10 ml of *water R* and evaporate again to dryness under reduced pressure. Take up the residue in 2 ml of *methanol R*.

Reference solution (a) Dissolve 10 mg of *arabinose R* in a small quantity of *water R* and dilute to 10 ml with *methanol R*.

Reference solution (b) Dissolve 10 mg of *xylose R* in a small quantity of *water R* and dilute to 10 ml with *methanol R*.

Reference solution (c) Dissolve 10 mg of *galactose R* in a small quantity of *water R* and dilute to 10 ml with *methanol R.*

Plate TLC silica gel plate R

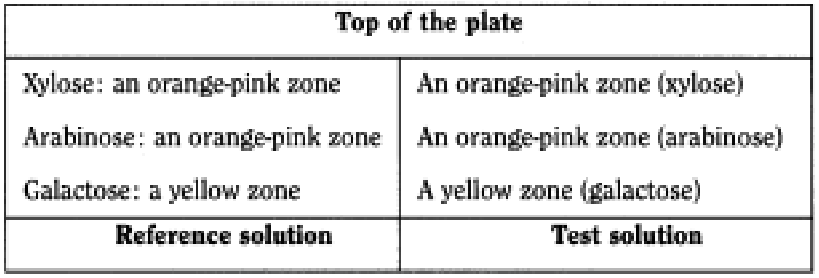
Mobile phase water R, acetonitrile R (15:85 *V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Detection Spray with *aminohippuric acid reagent R* and heat at 120 °C for 5 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference and the test solutions.



Top of the plate	
Xylose: an orange-pink zone	An orange-pink zone (xylose)
Arabinose: an orange-pink zone	An orange-pink zone (arabinose)
Galactose: a yellow zone	A yellow zone (galactose)
Reference solution	**Test solution**

TESTS

Foreign matter ***(2.8.2)***

Carry out the determination using 10.0 g.

Swelling index ***(2.8.4)***

Minimum 9.

Loss on drying ***(2.2.32)***

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 4.0 per cent.

Ph Eur

Ivy Leaf

General Notices

(Ph Eur monograph 2148)

Ph Eur

DEFINITION

Whole or cut, dried leaves of *Hedera helix L*., collected in spring.

Content

Minimum 3.0 per cent of hederacoside C ($C_{59}H_{96}O_{26}$; M_r 1221) (dried drug).

IDENTIFICATION

A. Whole leaves are coriaceous, 4-10 cm in length and width, cordate at the base. The lamina is palmately 3-5 lobed, the lobes more or less triangular with entire margins. The upper surface is dark green with a paler, radiate venation, the lower surface more greyish-green and the venation is distinctly raised. The petioles are long, cylindrical, about 2 mm in diameter and grooved longitudinally. Scattered white hairs occur on the petioles and on the surfaces of younger leaves, the older leaves are glabrous. Occasional entire, ovate-rhombic to lanceolate leaves 3-8 cm long from the flowering stems may be present.

B. Reduce to a powder (355) *(2.9.12)*. The powder is green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the lamina in surface view showing the cells of both the upper and lower epidermises with thickened, sinuous to wavy anticlinal walls and a thick cuticle; numerous stomata occur in the lower epidermis only, they are mostly anomocytic but occasionally anisocytic *(2.8.3)* and some of the surrounding cells may show faint cuticular striations; scattered stellate covering trichomes may be present, composed of 4-8 unicellular branches joined at the base; fragments in sectional view show 1-3 (usually 2) layers of palisade cells and a very porous spongy mesophyll containing occasional large mucilage cells; cluster crystals of calcium oxalate, about 40 µm in diameter, occur throughout the mesophyll; groups of lignified fibro-vascular tissue from the veins.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Extract 0.50 g of the powdered drug (355) *(2.9.12)* under a reflux condenser in a water bath at 60 °C with 5 ml of *methanol R* for 30 min. Cool and filter.

Reference solution Dissolve 1.0 mg of *hederacoside C R* and 1.0 mg of α-*hederin R* in 1.0 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, methanol R, acetone R, ethyl acetate R* (4:20:20:30 *V/V/V/V*).

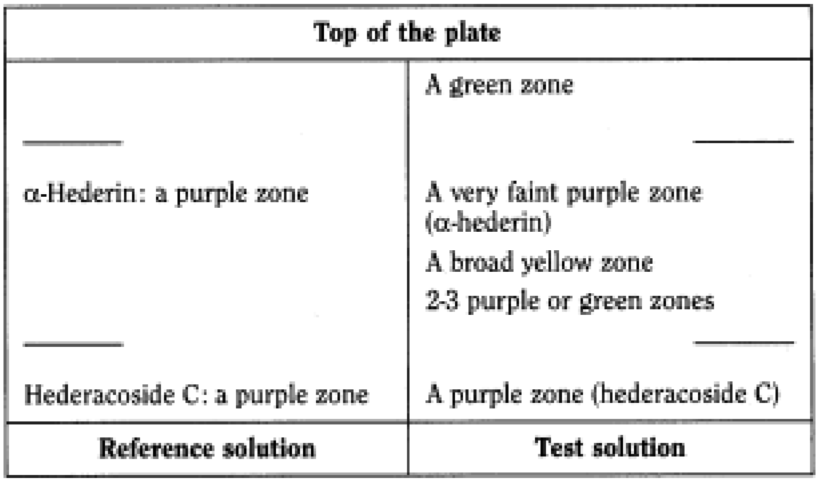
Application 20 µl, as bands of 15 mm.

Development Over a path of 12 cm.

Drying At 100-105 °C.

Detection Spray with *alcoholic solution of sulphuric acid R*. Heat at 110 °C for 10 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A green zone
———	———
α-Hederin: a purple zone	A very faint purple zone (α-hederin)
	A broad yellow zone
	2-3 purple or green zones
———	———
Hederacoside C: a purple zone	A purple zone (hederacoside C)
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 10 per cent of discoloured matter, maximum 10 per cent of stems, and maximum 2 per cent other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution To 1.00 g of the powdered drug (355) *(2.9.12)* in a 250 ml round bottomed flask add 50 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R*. Heat under a reflux condenser in a water-bath at 80 °C for 1 h. Cool and filter through cotton into a 100 ml volumetric flask. The cotton together with the residue is again extracted with 30 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R* under reflux for 30 min. Filter and combine the filtrates. Rinse the round bottomed flask and the cotton with a mixture of 20 volumes of *water R* and 80 volumes of *methanol R* and use this mixture of solvents to dilute the contents of the volumetric flask to exactly 100.0 ml. Filter through a suitable membrane before use.

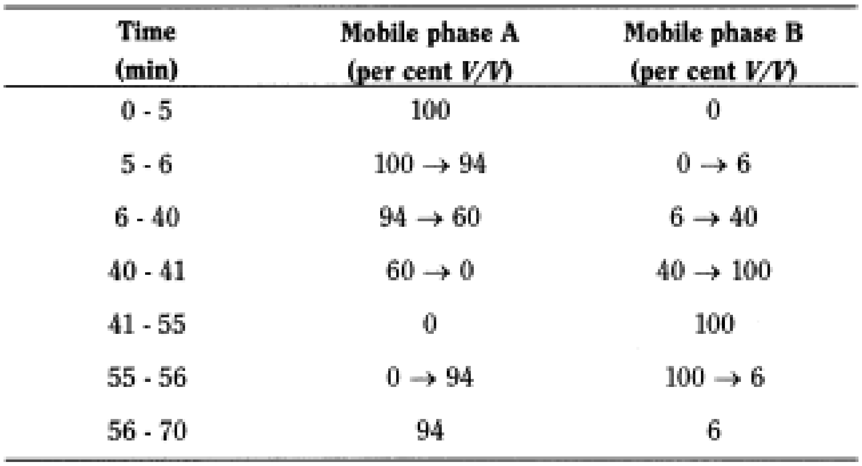
Reference solution Dissolve an amount of *ivy leaf standardised tincture CRS* corresponding to 3.0 mg of hederacoside C in *methanol R* and dilute to 5.0 ml with the same solvent.

Column:
— *size*: *l* = 0.125 m, Ø = 4 mm;

— *stationary phase: suitable octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: mix 140 volumes of *acetonitrile R* and 880 volumes of *water R* and adjust to pH 2.0 with *phosphoric acid R*;

— *mobile phase B*: *phosphoric acid R, acetonitrile R* (2:998 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 6	100 → 94	0 → 6
6 - 40	94 → 60	6 → 40
40 - 41	60 → 0	40 → 100
41 - 55	0	100
55 - 56	0 → 94	100 → 6
56 - 70	94	6

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl.

System suitability Reference solution:

— *retention time*: hederacoside C = about 20 min.

If necessary, adjust the time intervals of the gradient.

Calculate the percentage content of hederacoside C with reference to the dried drug using the following expression:

$$\frac{F_1 \times m_2 \times 400}{F_2 \times m_1 \times (100 - d)}$$

F_1 = area of the peak due to hederacoside C in the chromatogram obtained with the test solution

F_2 = area of the peak due to hederacoside C in the chromatogram obtained with the reference solution

d = loss on drying, in per cent;

m_1 = mass of drug, in grams

m_2 = mass of hederacoside C in the reference solution, in grams

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Ph Eur

Java Tea

General Notices

(Ph Eur monograph 1229)

Ph Eur

DEFINITION

Fragmented, dried leaves and tops of stems of *Orthosiphon stamineus* Benth. (*O. aristatus* Miq.; *O. spicatus* Bak.).

Content

Minimum 0.05 per cent of sinensetin ($C_{20}H_{20}O_7$; M_r 372.4) (dried drug).

IDENTIFICATION

A. The leaves are friable, up to 7.5 cm in length and 2.5 cm in width. The petiole is short. The lamina is oval to lanceolate, the apex acuminate and the base cuneate. The abaxial surface of the leaves is light greyish-green and the adaxial surface is dark green to brownish-green. The venation is pinnate with few secondary veins. Examined under a lens (× 10), the secondary veins, after running parallel to the midrib, diverge at an acute angle. The margin is irregularly and roughly dentate, sometimes crenate and the abaxial surface is slightly curved. The petioles are thin, quadrangular, 4 mm to 8 mm long and, like the primary venation, usually violet-coloured. Occasionally, inflorescences in clusters of bluish-white to violet flowers, not yet opened, are found.

B. Reduce to a powder (355) *(2.9.12)*. The powder is dark green. Examine under a microscope using *chloral hydrate solution R*. The powder shows fragments of epidermis, with cells with sinuous outlines bearing unicellular or bicellular conical covering trichomes and articulated uniseriate trichomes up to 450 µm long, consisting of 3 to 8 cells with thick pitted walls; capitate trichomes with unicellular or bicellular heads; secretory trichomes with unicellular stalks and usually tetracellular heads; diacytic stomata *(2.8.3)*, which are more numerous on the lower epidermis.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Shake 1 g of the powdered drug (710) *(2.9.12)* for 5 min with 10 ml of *methanol R* in a water-bath at 60 °C and filter the cooled solution.

Reference solution Dissolve 1 mg of *sinensetin R* in *methanol R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *methanol R*, *ethyl acetate R*, *toluene R* (5:40:55 *V/V/V*).

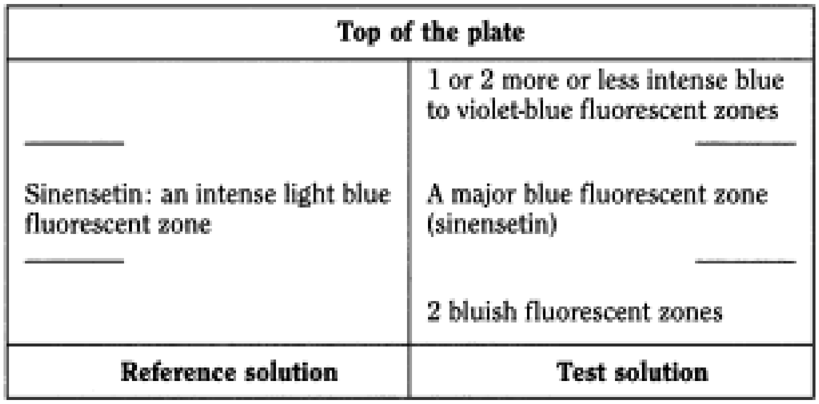
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatogram obtained with the reference solution and the test solution. Furthermore, red fluorescent zones are present in the lower third and near the solvent front of the chromatogram obtained with the test solution.



Top of the plate	
	1 or 2 more or less intense blue to violet-blue fluorescent zones
Sinensetin: an intense light blue fluorescent zone	A major blue fluorescent zone (sinensetin)
	2 bluish fluorescent zones
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of stems with a diameter greater than 1 mm; maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 11.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.5 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Heat 2.5 g of the powdered drug (355) *(2.9.12)* and 100 ml of *methylene chloride R* on a water-bath for 30 min with stirring. Filter. Collect the filtrate and repeat the operation twice, in the same manner, on the filtration residue. Combine the filtrates. Evaporate the solvent under reduced pressure. Dissolve the residue in 25.0 ml of the mobile phase, using an ultrasonic bath if necessary. Filter the solution through a nitrocellulose filter with a pore size of 0.45 µm.

Reference solution Dissolve 5 mg (m_2) of *sinensetin R* in 80 ml of the mobile phase using an ultrasonic bath if necessary and dilute to 100.0 ml with the mobile phase.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *tetrahydrofuran R, acetic acid R, water R, methanol R* (5:8:42:45 *V/V/V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 258 nm.

Injection 20 µl.

Calculate the percentage content of sinensetin from the expression:

$$\frac{m_2 \times F_1 \times 25}{m_1 \times F_2}$$

F_1 = area of the peak corresponding to sinensetin in the chromatogram obtained with the test solution;

F_2 = area of the peak corresponding to sinensetin in the chromatogram obtained with the reference solution;

m_1 = mass of the drug to be examined, in grams;

m_2 = mass of sinensetin in the reference solution, in grams.

Ph Eur

Javanese Turmeric

General Notices

(Ph Eur monograph 1441)

Ph Eur

DEFINITION

Dried rhizome, cut in slices, of *Curcuma xanthorrhiza* Roxb. (*C. xanthorrhiza* D. Dietrich).

Content:

— *essential oil*: minimum 50 ml/kg (anhydrous drug);

— *dicinnamoyl methane derivatives, expressed as curcumin* ($C_{21}H_{20}O_6$; M_r 368.4): minimum 1.0 per cent (anhydrous drug).

CHARACTERS

Aromatic odour.

IDENTIFICATION

A. Orange-yellow or yellowish-brown or greyish-brown slices, mostly peeled 1.5-6 mm thick and 15-50 mm, more rarely up to 70 mm, in diameter. Fragments of the brownish-grey cork are sporadically present. The transverse surface is yellow with dark spots in the paler centre. The fracture is short and finely grained.

B. Reduce to a powder (355) *(2.9.12)*. The powder is reddish-brown. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of colourless parenchyma with orange-yellow or yellowish-brown secretory cells; fragments of reticulate and other vessels; rare fragments of cork and epidermis and fragments of thick-walled unicellular acute trichomes. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows numerous stratified, ovoid or irregular starch granules, about 30-50 µm long and about 10-30 µm wide, with an eccentric hilum and marked, concentric striations.

C. Thin-layer chromatography *(2.2.27)* as described in the test for *Curcuma domestica* with the following modifications.

Detection spray with a freshly prepared 0.4 g/l solution of *dichloroquinonechlorimide R* in 2-*propanol R*. Expose to ammonia vapour until the zone due to thymol becomes bluish-violet.

Results The chromatogram obtained with the reference solution shows almost in the middle a bluish-violet zone (thymol) and in the lower part a yellow zone (fluorescein). The chromatogram obtained with the test solution shows a blue zone (xanthorrhizol) slightly above the zone due to thymol in the chromatogram obtained with the reference solution and 2 yellowish-brown or brown zones (curcumin and demethoxycurcumin) between the zones due to thymol and fluorescein in the chromatogram obtained with the reference solution.

TESTS

Curcuma domestica

Thin-layer chromatography (*2.2.27*).

Test solution Shake 0.5 g of the freshly powdered drug (500) (*2.9.12*) with 5 ml of *methanol R* for 30 min and filter.

Reference solution Dissolve 5 mg of *fluorescein R* and 10 mg of *thymol R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R, toluene R* (20:80 *V/V*).

Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a mixture of 1 volume of *sulphuric acid R* and 9 volumes of *acetic anhydride R*. Examine in ultraviolet light at 365 nm.

Results In the chromatogram obtained with the test solution, no yellowish-red fluorescent zone (bisdemethoxycurcumin) appears slightly above the greenish-blue fluorescent zone due to fluorescein in the chromatogram obtained with the reference solution.

Water *(2.2.13)*

Maximum 120 ml/kg, determined on 20.0 g of the powdered drug (500) *(2.9.12)*.

Total ash *(2.4.16)*

Maximum 8.0 per cent.

ASSAY

Essential oil

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use a 500 ml round-bottomed flask, 200 ml of *water R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Reduce the drug to a powder (500) *(2.9.12)* and immediately use 5.0 g for the determination. Distil at a rate of 3-4 ml/min for 3 h.

Dicinnamoyl methane derivatives

To 0.100 g of the powdered drug (180) *(2.9.12)* add 60 ml of *glacial acetic acid R* and heat in a water-bath at 90 °C for 60 min. Add 2.0 g of *boric acid R* and 2.0 g of *oxalic acid R* and heat in a water-bath at 90 °C for 10 min. Allow to cool, dilute to 100.0 ml with *glacial acetic acid R* and shake. Dilute 5.0 ml of the clear supernatant to 50.0 ml with *glacial acetic acid R*. Measure the absorbance *(2.2.25)* at 530 nm, using *glacial acetic acid R* as the compensation liquid.

Calculate the percentage content of dicinnamoyl methane derivatives, expressed as curcumin, using the following expression:

$$\frac{A \times 0.426}{m}$$

i.e. taking the specific absorbance of curcumin to be 2350.

A = absorbance at 530 nm

m = mass of the drug to be examined, in grams

Ph Eur

Juniper

General Notices

(Ph Eur monograph 1532)

Ph Eur

DEFINITION

Dried ripe cone berry of *Juniperus communis* L.

Content

Minimum 10 ml/kg of essential oil (anhydrous drug).

CHARACTERS

Strongly aromatic odour, especially if crushed.

IDENTIFICATION

A. The berry-shaped cone is globular, up to 10 mm in diameter, and violet-brown or blackish-brown, frequently with a bluish bloom. It consists of 3 fleshy scales. The apex has a three-rayed closed cleft and 3 not very clearly defined projections. A remnant of peduncle is frequently attached at the base. The fleshy part is crumbly and brownish. It contains 3 or, more rarely, 2 small, elongated, extremely hard seeds that have 3 sharp edges and are slightly rounded at the back, acuminate at the apex. The seeds are fused with the fleshy part of the cone berry in the lower part on the outside of their bases. Very large, oval oil glands containing sticky resin lie at the outer surface of the seeds.

B. Reduce to a powder (355) *(2.9.12)*. The powder is brown. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of epidermis of the cone berry wall containing cells with thick, pitted, colourless walls and brown glandular content, occasionnally with anomocytic stomata *(2.8.3)*; fragments of the three-rayed apical cleft of the cone berry with spaces and epidermal cells interlocked by papillous outgrowths; fragments of the hypodermis with collenchymatous thickened cells; fragments of the mesocarp consisting of large thin-walled parenchymatous cells, usually rounded, with large intercellular spaces and irregular, large, usually scarcely pitted, yellow idioblasts (barrel cells); fragments of schizogenous oil cells; fragments of the testa with thick-walled, pitted, colourless sclereids containing one or several prism crystals of calcium oxalate; fragments of the endosperm and embryonic tissue with thin-walled cells containing fatty oil and aleurone grains.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Dilute the oil-xylene mixture obtained in the assay to 5.0 ml with *hexane R*.

Reference solution Dissolve 4.0 mg of *guaiazulene R* and 50 µl of *cineole R* in 10 ml of *hexane R*.

Plate *TLC silica gel plate R*.

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray the plate with *anisaldehyde solution R*. Heat at 100-105 °C for 5-10 min and examine in daylight.

Results The chromatogram obtained with the reference solution shows a red zone (guaiazulene) in the upper half and a brownish-violet or greyish-violet zone (cineole) in the lower half. The chromatogram obtained with the test solution shows a strong violet zone (mono- and sesquiterpenes) similar in position to the zone due to guaiazulene in the chromatogram obtained with the reference solution, a reddish-violet zone a little above the zone due to cineole in the chromatogram obtained with the reference solution, a greyish-violet zone (terpinen-4-ol) a little below the zone due to cineole in the chromatogram obtained with the reference solution, and just below that a blue zone. A faint violet zone may be present in a similar position to the zone due to cineole. Further zones are present.

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of unripe or discoloured cone berries and maximum 2 per cent of other foreign matter.

Water (*2.2.13)*

Maximum 120 ml/kg, determined on 20.0 g of the crushed drug.

Total ash *(2.4.16)*

Maximum 4.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use 20.0 g of drug crushed immediately before the assay, a 500 ml round-bottomed flask, 200 ml of *water R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Distil at a rate of 3-4 ml/min for 90 min.

Ph Eur

Kelp

General Notices

Bladderwrack; Fucus

(Ph Eur monograph 1426)

Ph Eur

DEFINITION

Fragmented dried thallus of *Fucus vesiculosus* L. or *F. serratus* L. or *Ascophyllum nodosum* Le Jolis.

Content

Minimum 0.03 per cent and maximum 0.2 per cent of total iodine (A_r 126.9) (dried drug).

CHARACTERS

Salty and mucilaginous taste.

Unpleasant marine odour.

IDENTIFICATION

A. The drug consists of fragments with a corneous consistency, blackish-brown to greenish-brown, sometimes covered with whitish efflorescence. The thallus consists of a ribbon-like blade, branching dichotomously with prominent central ribs (pseudoveins). *F. vesiculosus* typically shows a foliose blade with smooth edges and bears occasional ovoid, single or paired, air vesicles. The ends of certain branches are of ovoid shape and a little widened. They bear numerous reproductive organs (conceptacles). *F. serratus* has a foliose blade with a serrate margin and no vesicles, the branches bearing conceptacles are less swollen. The thallus of *A. nodosum* is irregularly branched, without pseudo-midrib. It shows single ovoid air vesicles; the falciform conceptacles are located at the end of small branches.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows fragments of surface tissue with regular isodiametric cells with brown contents, and fragments of deep tissue with colourless, elongated cells arranged in long filaments with large mucilaginous spaces between them. Thick-walled cells in files and in closely packed groups, from the pseudovein, are sometimes visible.

C. To 1 g of the powdered drug (355) *(2.9.12)* add 20 ml of a 2 per cent *V/V* solution of *hydrochloric acid R*. Shake vigorously and filter. Wash the residue with 10 ml of *water R* and filter. To the residue add 10 ml of a 200 g/l solution of *sodium carbonate R*. Shake and centrifuge. Collect the supernatant liquid. Adjust to pH 1.5 using *sulphuric acid R*. A white, flocculent precipitate is slowly formed.

TESTS

Arsenic *(2.4.27)*

Maximum 90 ppm.

Cadmium *(2.4.27)*

Maximum 4 ppm.

Lead *(2.4.27)*

Maximum 5 ppm.

Mercury *(2.4.27)*

Maximum 0.1 ppm.

Swelling index *(2.8.4)*

Minimum 6.

Loss on drying *(2.2.32)*

Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 105 °C, for 2 h.

Total ash *(2.4.16)*

Maximum 24 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

ASSAY

Total iodine

To 1.000 g of the powdered drug, in a tall silica crucible, add 5 ml of *water R* and 5 g of *potassium hydroxide R*. Stir with a magnesium rod. Heat on a water bath. Add 1 g of *potassium carbonate R*. Mix, add the tip of the magnesium rod with the residues of the drug and dry, first on a water-bath then over an open flame. Incinerate raising the temperature progressively to not more than 600 °C. Allow to cool. Add 20 ml of *water R* and heat gently to boiling, stirring with a glass rod. Filter the hot mixture through an unpleated filter, into a conical flask. Rinse the residue with 4 quantities, each of 20 ml, of hot *water R*. Rinse the filter and the crucible with 50 ml of hot *water R*. Combine the solutions. Allow to cool. Neutralise with *dilute sulphuric acid R* in the presence of *methyl orange solution R*. Add 3 ml of *dilute sulphuric acid R* and 1 ml of *bromine water R*. The solution is yellow. After 5 min add 0.6 ml of a 50 g/l solution of *phenol R*. The solution is clear. Acidify with 5 ml of *phosphoric acid R* and add 0.2 g of *potassium iodide R.* Allow to stand for 5 min protected from light. Add 1 ml of *starch solution R* and titrate with *0.01 M sodium thiosulphate*.

1 ml of *0.01 M sodium thiosulphate* is equivalent to 0.2115 mg of iodine.

LABELLING

The label states the species of kelp present.

Ph Eur

Knotgrass

General Notices

(Ph Eur monograph 1885)

Ph Eur

DEFINITION

Whole or cut dried flowering aerial parts of *Polygonum aviculare* L. *s.l.*

Content

Minimum 0.30 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The stem is 0.5 mm to 2 mm thick, branched, with nodes, cylindrical or slightly angular and longitudinally striated. It bears sessile or shortly petiolate, glabrous entire leaves, which differ widely in shape and size. The sheath-like stipules (ochrea) are lacerate and silvery. The small axillary flowers have 5 greenish-white perianth segments, the tips of which are often coloured red. The fruits are 2 mm to 4 mm, brown to black triangular nuts, usually punctate or striate.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the leaf epidermis with polygonal to sinuous cell walls and numerous anisocytic stomata *(2.8.3)*, with a striated cuticle; fragments of leaves and stems containing numerous calcium oxalate clusters, some of them very large; groups of thick-walled fibres from the hypodermis of the stem; globular pollen grains with smooth exine and 3 germinal pores; occasional brown fragments of the exocarp composed of cells with thick sinuous walls. Examine under a microscope using a 675 g/l solution of *potassium hydroxide R*. Heat gently. The epidermis of the leaves and a few cells of the mesophyll stain red to reddish-violet. Examine under a microscope using a 0.1 g/l solution of *ferric chloride R*. Leaf fragments are stained almost black.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R*. Heat the mixture under a reflux condenser, in a water-bath for 10 min. Cool and filter.

Reference solution Dissolve 1 mg of *caffeic acid R*, 2.5 mg of *hyperoside R* and 1 mg of *chlorogenic acid R* in 10 ml of *methanol R.*

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R, glacial acetic acid R, water R, ethyl acetate R* (7:7:14:72 *V/V/V/V*).

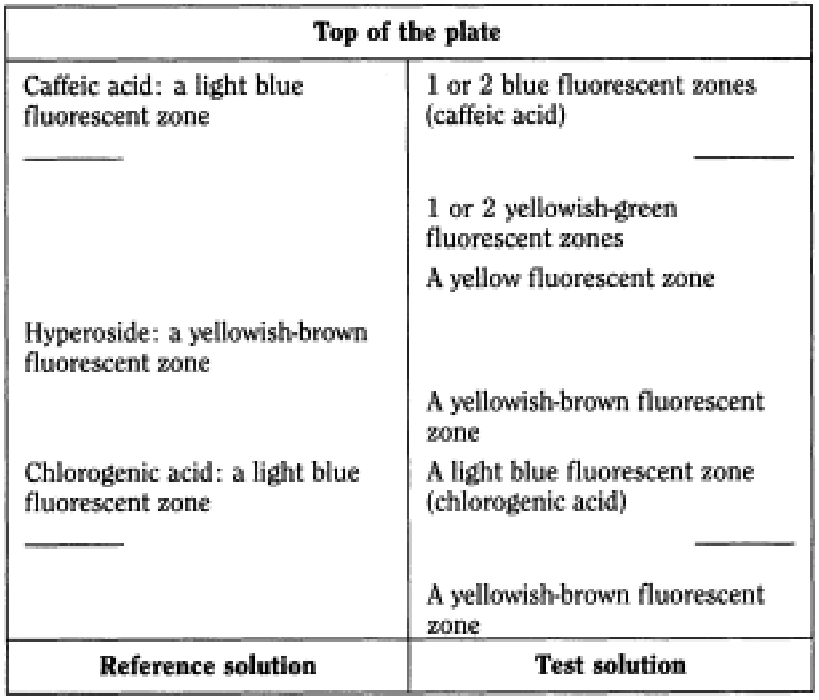
Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Results See below the sequence of the fluorescent zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones are present in the chromatogram obtained with the test solution.



Top of the plate	
Caffeic acid: a light blue fluorescent zone	1 or 2 blue fluorescent zones (caffeic acid)
———	———
	1 or 2 yellowish-green fluorescent zones
	A yellow fluorescent zone
Hyperoside: a yellowish-brown fluorescent zone	
	A yellowish-brown fluorescent zone
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
———	———
	A yellowish-brown fluorescent zone
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 2 per cent of roots and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of powdered drug (710) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

ASSAY

Stock solution In a 100 ml round-bottomed flask, place 0.800 g of the powdered drug (355) *(2.9.12)*, add 1 ml of a 5 g/l solution of *hexamethylenetetramine R*, 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1*. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R,* each time boiling under a reflux condenser for 10 min. Allow to cool, filter each extract through the plug of absorbent cotton into the flask. Filter the combined acetone extracts through a filter paper into a volumetric flask, dilute to 100.0 ml with *acetone R* rinsing the flask and the filter paper. Introduce 20.0 ml of the solution into a separating funnel, add 20 ml of *water R* and shake the mixture with 1 quantity of 15 ml and then 3 quantities, each of 10 ml of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel and wash with 2 quantities, each of 50 ml, of *water R*. Filter the extracts over 10 g of *anhydrous sodium sulphate R* into a 50 ml volume tric flask and dilute to volume with *ethyl acetate R*.

Test solution To 10.0 ml of the stock solution add 1 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation liquid Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Measure the absorbance *(2.2.25)* of the test solution after 30 min by comparison with the compensation liquid at 425 nm. Calculate the percentage content of flavonoids, calculated as hyperoside, from the expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of hyperoside to be 500.

A = absorbance at 425 nm

m = mass of the drug to be examined, in grams

Ph Eur

Lavender Flower

General Notices

(Ph Eur monograph 1534)

Ph Eur

DEFINITION

Dried flower *of Lavandula angustifolia* P. Mill. (*L. officinalis* Chaix).

Content

Minimum 13 ml/kg of essential oil (anhydrous drug).

CHARACTERS

Strongly aromatic odour.

IDENTIFICATION

First identification A, B, D.

Second identification A, B, C.

A. The flower has a short peduncle and consists of a bluish-grey tubular calyx divided distally into 4 very short teeth and a small rounded lobe, a blue bilabial corolla with the upper lip bifid and the lower lip trilobate and 4 didynamous stamens with ovoid anthers.

B. Reduce to a powder (355) (*2.9.12*). The powder is bluish-grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: covering trichomes bifurcating at one or more levels; secretory trichomes with short stalks and eight-celled heads of the *Labiatae* type; secretory trichomes with unicellular or multicellular stalks and unicellular heads; secretory trichomes with long uneven stalks and unicellular heads, separated from the peduncle by an intermediary cell with a smooth cuticle; certain such trichomes show a crown of small spheroid cells just below the insertion point of the intermediary cell on the peduncle; fragments of warty epidermis from the inner surface of the petals; fragments of calyx epidermis with sinuous-walled cells and containing prismatic crystals of calcium oxalate; spherical pollen grains which have a diameter of about 45 µm and an exine with 6 slit-like germinal pores and 6 ribbon-like groins radiating from the poles.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 0.5 g of the powdered drug (355) (*2.9.12*) add 5 ml of *hexane R*, shake for 5 min and filter.

Reference solution Dissolve 10 µl of *linalol R* and 10 µl of *linalyl acetate R* in 5 ml of *hexane R*.

Plate TLC silica gel plate R.

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R*. Heat at 100-105 °C for 5-10 min and examine in daylight.

Results The chromatogram obtained with the reference solution shows in the lower third a greyish-blue zone (linalol) and in the middle third a greyish-blue zone (linalyl acetate). The chromatogram obtained with the test solution shows the zones due to linalol and linalyl acetate and in the middle, between these zones, a redish-violet zone (epoxydihydrocaryophyllene). Further zones are also present.

D. Examine the chromatograms obtained in the test for other species and varieties of lavender.

Results The 5 principal peaks in the chromatogram obtained with the reference solution are similar in retention time to peaks in the chromatogram obtained with the test solution. Among them are mainly linalol and linalyl acetate peaks.

TESTS

Foreign matter (*2.8.2*)

Maximum 3 per cent of stems and maximum 2 per cent of other foreign matter.

Other species and varieties of lavender

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dilute 0.2 ml of the essential oil-xylene mixture obtained in the assay to 5 ml with *hexane R*, add 1 g of *anhydrous sodium sulphate R*, shake and use the supernatant liquid.

Reference solution Dissolve 0.1 g of *limonene R*, 0.2 g of *cineole R*, 0.05 g of *camphor R*, 0.4 g of *linalol R*, 0.6 g of *linalyl acetate R* and 0.2 g of α-*terpineol R* in 100 ml of *hexane R*.

Column:

— *material*: fused silica;

— *size*: *l* = 60 m, Ø = 0.25 mm;

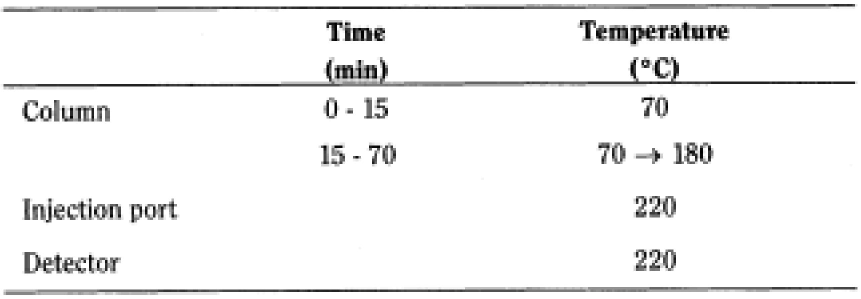
— *stationary phase*: *macrogol 20 000 R*.

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 15	70
	15 - 70	70 → 180
Injection port		220
Detector		220

Detection Flame ionisation.

Injection The same volume of each solution.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to limonene and cineol;

— *number of theoretical plates*: minimum 30 000, calculated for the peak due to limonene at 110 °C.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the 6 components of the reference solution in the chromatogram obtained with the test solution. Disregard the peaks due to hexane and xylene.

Limit:
— *camphor* : maximum 1 per cent.

Water (*2.2.13*)

Maximum 100 ml/kg, determined on 20.0 g.

Total ash (*2.4.16*)

Maximum 9.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs (*2.8.12*). Use 20.0 g of drug, a 1000 ml round-bottomed flask, 500 ml of *water R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

Ph Eur

Lavender Oil

General Notices

(Ph Eur monograph 1338)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the flowering tops of *Lavandula angustifolia* Miller (*Lavandula officinalis* Chaix).

CHARACTERS

Appearance

Colourless or pale yellow, clear liquid.

It has a characteristic odour.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 µl of the substance to be examined in 1 ml of *toluene R*.

Reference solution Dissolve 10 µl of *linalol R* and 10 µl of *linalyl acetate R* in 1 ml of *toluene R*.

Plate TLC silica gel plate R.

Mobile phase ethyl acetate R, *toluene R* (5:95 *V/V*).

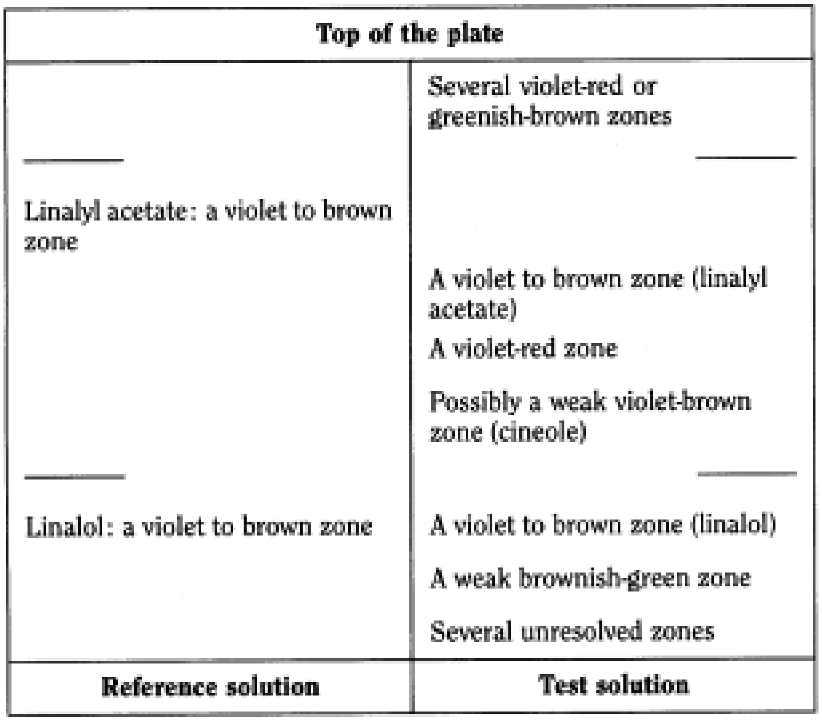
Application 10 µl as bands.

Development Twice, 5 min apart, over a path of 10 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine immediately in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other violet-red or greenish-brown zones are present in the chromatogram obtained with the test solution above the zone of linalyl acetate up to the solvent front.



Top of the plate	
	Several violet-red or greenish-brown zones
———	———
Linalyl acetate: a violet to brown zone	
	A violet to brown zone (linalyl acetate)
	A violet-red zone
	Possibly a weak violet-brown zone (cineole)
———	———
Linalol: a violet to brown zone	A violet to brown zone (linalol)
	A weak brownish-green zone
	Several unresolved zones
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with reference solution (a).

TESTS

Relative density (*2.2.5*)

0.878 to 0.892.

Refractive index (*2.2.6*)

1.455 to 1.466.

Optical rotation (*2.2.7*)

- 12.5 ° to - 7.0 °.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g of the substance to be examined dissolved in 50 ml of the prescribed mixture of solvents.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution The substance to be examined.

Reference solution (a) Dissolve 0.1 g of *limonene R*, 0.2 g of *cineole R*, 0.2 g of *3-octanone R*, 0.05 g of *camphor R*, 0.4 g of *linalol R*, 0.6 g of *linalyl acetate R*, 0.2 g of *terpinen-4-ol R*, 0.1 g of *lavandulyl acetate R*, 0.2 g of *lavandulol R* and 0.2 g of α-*terpineol R* in 5 ml of *hexane R*.

Reference solution (b) Dissolve 5 mg of *3-octanone R* in *hexane R* and dilute to 10 ml with the same solvent.

Column:

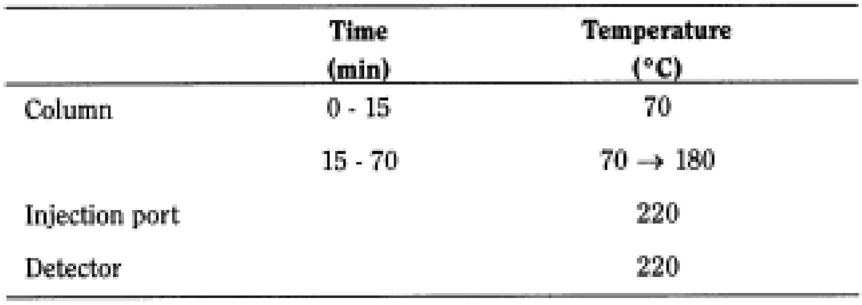
- — *material*: fused silica;
- — *size: l* = 60 m , Ø = 0.25 mm;
- — *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 15	70
	15 - 70	70 → 180
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of reference solution (a). Record the retention times of these substances.

System suitability Reference solution (a):

- — *resolution*: minimum 1.4 between the peaks due to terpinen-4-ol and lavandulyl acetate.

Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution.

Determine the percentage content of each of these components. The percentages are within the following ranges:

- — *limonene*: less than 1.0 per cent;
- — *cineole*: less than 2.5 per cent;
- — *3-octanone*: 0.1 per cent to 2.5 per cent;
- — *camphor* : less than 1.2 per cent;
- — *linalol* : 20.0 per cent to 45.0 per cent;
- — *linalyl acetate*: 25.0 per cent to 46.0 per cent;
- — *terpinen-4-ol* : 0.1 per cent to 6.0 per cent;
- — *lavandulyl acetate*: more than 0.2 per cent;

— *lavandulol*: more than 0.1 per cent;

— α-*terpineol*: less than 2.0 per cent.

— *disregard limit*: area of the peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chiral purity

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.02 g of the substance to be examined in *pentane R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 µl of *linalol R*, add 10 µl of *linalyl acetate R* and 5 mg of *borneol R* in *pentane R* and dilute to 10 ml with the same solvent.

Column:

— *material*: fused silica;

— *size: l* = 25 m, Ø = 0.25 mm;

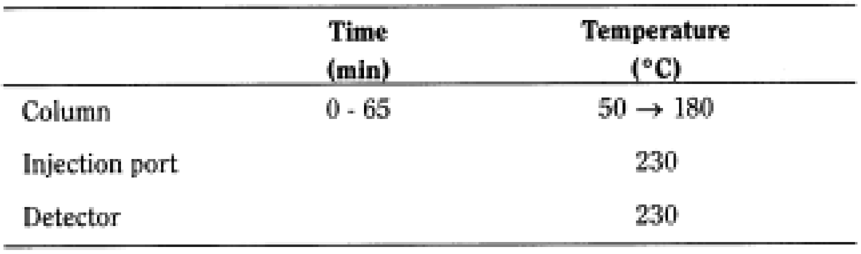
— *stationary phase*: *modified* β*-cyclodextrin for chiral chromatography R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.3 ml/min.

Split ratio 1:30.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 65	50 → 180
Injection port		230
Detector		230

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution:

— *resolution*: minimum 5.5 between the peaks due to (*R*)-linalol (1st peak) and (*S*)-linalol (2nd peak), minimum 2.9 between the peaks due to (*S*)-linalol and borneol (3rd peak) and minimum 2.7 between the peaks due to (*R*)-linalyl acetate (4th peak) and (*S*)-linalyl acetate (5th peak).

Calculate the percentage content of the specified (*S*)-enantiomers from the following expression:

$$\frac{A_S}{A_S + A_R} \times 100$$

A_S = area of the peak due to the corresponding (*S*)-enantiomer,

A_R = area of the peak due to the corresponding (*R*)-enantiomer.

Limits:

- *(S)-linalol*: maximum 12 per cent;
- *(S)-linalyl acetate*: maximum 1 per cent.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Lemon Balm

General Notices

(Melissa Leaf, Ph Eur monograph 1447)

Ph Eur

DEFINITION

Dried leaf of *Melissa officinalis* L.

Content

Minimum 4.0 per cent of total hydroxycinnamic derivatives, expressed as rosmarinic acid ($C_{18}H_{16}O_8$; M_r 360.3) (dried drug).

CHARACTERS

Odour reminiscent of lemon.

IDENTIFICATION

A. Melissa leaf has a petiole of varying length and is oval, cordate and up to about 8 cm long and 5 cm wide. The lamina is thin and the under surface has a conspicuous, raised, reticulate venation; the margins are roughly dentate or crenate. The upper surface is bright green and the lower surface is lighter in colour.

B. Reduce to a powder (355) (*2.9.12*). The powder is greenish. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the leaf epidermis with sinuous walls; short, straight, unicellular, conical covering trichomes with a finely striated cuticle; multicellular, uniseriate covering trichomes with pointed ends and thick, warty cuticles; eight-celled secretory trichomes of lamiaceous type; secretory trichomes with unicellular to tricellular stalks and unicellular or, more rarely bicellular, heads; diacytic stomata (*2.8.3*), on the lower surface only.

C. Thin-layer chromatography (*2.2.27*).

Test solution Place 2.0 g of the powdered drug (355) (*2.9.12*) in a 250 ml round-bottomed flask and add 100 ml of *water R*. Distil for 1 h using the apparatus for the determination of essential oils in herbal drugs *(2.8.12)* and 0.5 ml of *xylene R* in the graduated tube. After distillation transfer the organic phase to a 1 ml volumetric flask rinsing the graduated tube of the apparatus with the aid of a small portion of *xylene R* and dilute to 1.0 ml with the same solvent.

Reference solution Dissolve 1.0 µl of *citronellal R* and 10.0 µl of *citral R* in 25 ml of *xylene R*.

Plate *TLC silica gel plate R*.

Mobile phase *ethyl acetate R*, *hexane R* (10:90 *V/V*).

Application 10 µl of the reference solution and 20 µl of the test solution, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R*. Examine in daylight while heating at 100-105 °C for 10-15 min.

Results The chromatogram obtained with the reference solution shows in the lower third a greyish-violet or bluish-violet double zone (citral) and above it a grey or greyish-violet zone (citronellal). The chromatogram obtained with the test solution shows zones similar in position and colour to the zones in the chromatogram obtained with the reference solution and between these zones a reddish-violet zone (epoxycaryophyllene). Further zones may be present.

TESTS

Foreign matter *(2.8.2)*

Maximum 10 per cent of stems with a diameter greater than 1 mm and maximum 2 per cent of other foreign matter, determined on 20 g.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

ASSAY

Stock solution To 0.200 g of the powdered drug (355) (*2.9.12*) add 190 ml of *ethanol (50 per cent V/V) R*. Boil in a water-bath under a reflux condenser for 30 min. Allow to cool and filter. Rinse the filter with 10 ml of *ethanol (50 per cent V/V) R.* Combine the filtrate and the rinsings in a volumetric flask and dilute to 200.0 ml with *ethanol (50 per cent V/V) R*.

Test solution To 1.0 ml of the stock solution in a test-tube add 2 ml of *0.5 M hydrochloric acid*, 2 ml of a solution prepared by dissolving 10 g of *sodium nitrite R* and 10 g of *sodium molybdate R* in 100 ml of *water R* and then add 2 ml of *dilute sodium hydroxide solution R* and dilute to 10.0 ml with *water R*; mix.

Compensation liquid In another test-tube place 1.0 ml of the stock solution, 2 ml of *0.5 M hydrochloric acid*, 2 ml of *dilute sodium hydroxide solution R* and dilute to 10.0 ml with *water R*.

Immediately measure the absorbance (*2.2.25*) of the test solution at 505 nm by comparison with the compensation liquid.

Calculate the percentage content of total hydroxycinnamic derivatives, expressed as rosmarinic acid, using the following expression:

$$\frac{A \times 5}{m}$$

i.e. taking the specific absorbance of rosmarinic acid to be 400.

A = absorbance at 505 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Lemon Oil

General Notices

(Ph Eur monograph 0620)

Ph Eur

DEFINITION

Essential oil obtained by suitable mechanical means, without the aid of heat, from the fresh peel of *Citrus limon* (L.) Burman fil.

CHARACTERS

Appearance: clear, mobile, pale yellow to greenish-yellow liquid with a characteristic odour. It may become cloudy at low temperatures.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography (*2.2.27*).

Test solution Mix 1 ml of the substance to be examined in 1 ml of *toluene R*.

Reference solution Dissolve 10 mg of *citropten R* and 50 µl of *citral R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel GF$_{254}$ plate R*.

Mobile phase *ethyl acetate R*, *toluene R* (15:85 *V/V*).

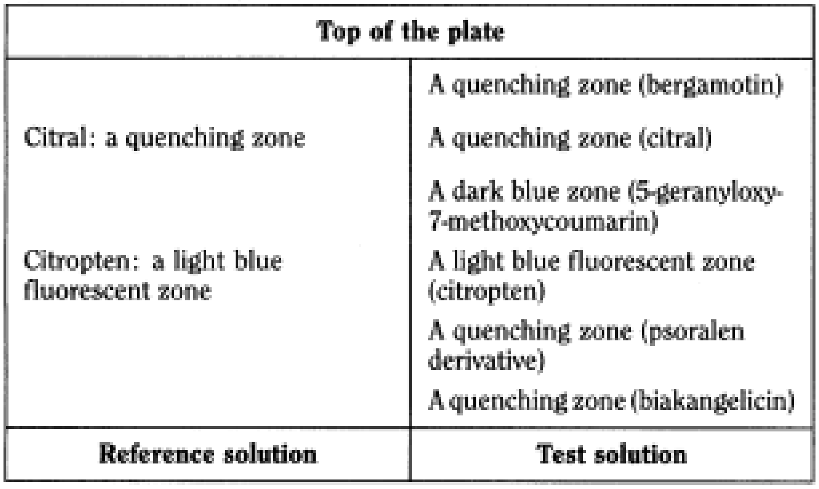
Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

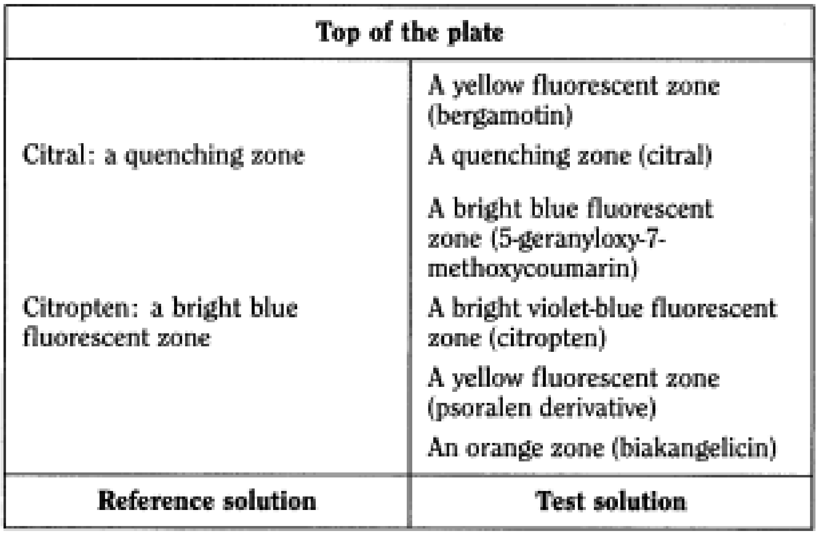
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
	A quenching zone (bergamotin)
Citral: a quenching zone	A quenching zone (citral)
	A dark blue zone (5-geranyloxy-7-methoxycoumarin)
Citropten: a light blue fluorescent zone	A light blue fluorescent zone (citropten)
	A quenching zone (psoralen derivative)
	A quenching zone (biakangelicin)
Reference solution	**Test solution**

Detection B Examine in ultraviolet light at 365 nm.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
	A yellow fluorescent zone (bergamotin)
Citral: a quenching zone	A quenching zone (citral)
	A bright blue fluorescent zone (5-geranyloxy-7-methoxycoumarin)
Citropten: a bright blue fluorescent zone	A bright violet-blue fluorescent zone (citropten)
	A yellow fluorescent zone (psoralen derivative)
	An orange zone (biakangelicin)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density (*2.2.5*)

0.850 to 0.858.

Refractive index (*2.2.6*)

1.473 to 1.476.

Optical rotation (*2.2.7*)

+ 57 ° to + 70 °.

Absorbance (*2.2.25*)

Dissolve 0.250 g of the substance to be examined in *alcohol R*, mix and dilute to 100.0 ml with the same solvent. Measure the absorbance over the range 260 nm to 400 nm. If a manual instrument is used, measure the absorbance at 5 nm intervals from 260 nm to about 12 nm before the expected absorption maximum, then at 3 nm intervals for 3 readings and at 1 nm intervals to about 5 nm beyond the maximum and finally at 10 nm intervals to 400 nm. Plot a curve representing the absorption spectrum with the absorbances as ordinates and the wavelengths as abscissae. Draw as a baseline the tangent between *A* and *B* (Figure 0620.-1). The absorption maximum *C* is situated at 315 ± 3 nm. From C draw a line perpendicular to the axis of abscissae and intersecting *AB* at *D*. Deduct the absorbance corresponding to point *D* from that corresponding to point *C*. The value *C* - *D* is 0.20 to 0.96 and for Italian-type lemon oil it is not less than 0.45.

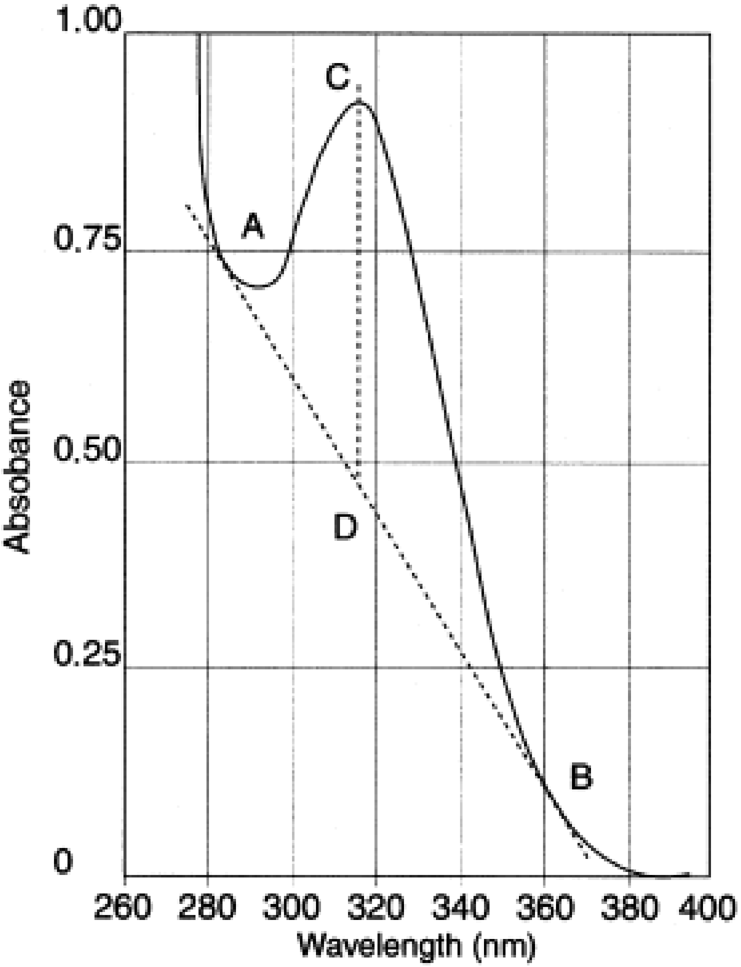


Figure 0620.-1. – *Typical spectrum of lemon oil for the test for absorbance*

Fatty oils and resinified essential oils (*2.8.7*)

It complies with the test for fatty oils and resinified essential oils.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution The substance to be examined.

Reference solution Dissolve 20 µl of β*-pinene R*, 10 µl of *sabinene R*, 100 µl of *limonene R*, 10 µl of γ*-terpinene R*, 5 µl of β*-caryophyllene R*, 20 µl of *citral R*, 5 µl of α*-terpineol R*, 5 µl of *neryl acetate R* and 5 µl of *geranyl acetate R* in 1 ml of *acetone R*.

Column:

— *material*: fused silica;

— *size:* *l* = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.25-0.53 mm;

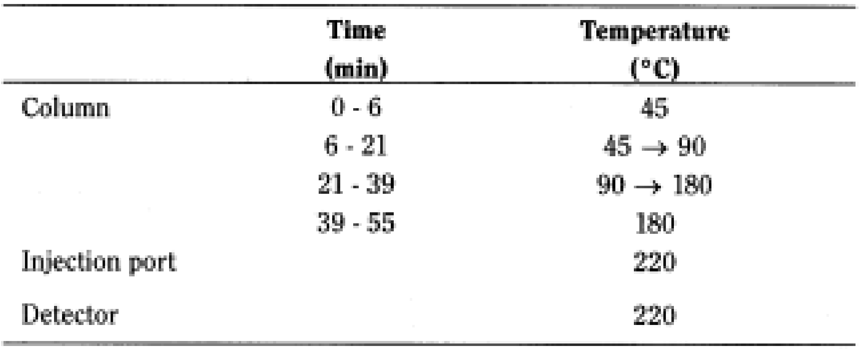
— *stationary phase*: *macrogol 20 000 R*.

Carrier gas *helium for chromatography R*.

Flow rate 1.0 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	45
	6 - 21	45 → 90
	21 - 39	90 → 180
	39 - 55	180
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 0.5 µl of the reference solution and 0.2 µl of the test solution.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to β-pinene and sabinene and minimum 1.5 between the peaks due to geranial and geranyl acetate.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of these components. The percentages are within the following ranges:

— β*-pinene*: 7.0 per cent to 17.0 per cent;

— *sabinene*: 1.0 per cent to 3.0 per cent;

— *limonene*: 56.0 per cent to 78.0 per cent;

— γ-*terpinene*: 6.0 per cent to 12.0 per cent;

— β-*caryophyllene*: maximum 0.5 per cent;

— *neral*: 0.3 per cent to 1.5 per cent;

— α-*terpineol*: maximum 0.6 per cent;

— *neryl acetate*: 0.2 per cent to 0.9 per cent;

— *geranial*: 0.5 per cent to 2.3 per cent;

— *geranyl acetate*: 0.1 per cent to 0.8 per cent.

Residue on evaporation (*2.8.9*)

1.8 per cent to 3.6 per cent after heating on the water-bath for 4 h.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

LABELLING

The label states, where applicable, that the contents are Italian-type lemon oil.

Ph Eur

Lemon Verbena Leaf

General Notices

(Ph Eur monograph 1834)

Ph Eur

DEFINITION

Whole or fragmented, dried leaves of *Aloysia citriodora Palau* (syn. *Aloysia triphylla* (L'Hér.) Kuntze; *Verbena triphylla* L'Hér.; *Lippia citriodora* Kunth.).

Content:

— *acteoside* ($C_{29}H_{36}O_{15}$; M_r 625): minimum 2.5 per cent, expressed as ferulic acid (dried drug);

— *essential oil*: minimum 3.0 ml/kg for the whole drug and minimum 2.0 ml/kg for the fragmented drug (dried drug).

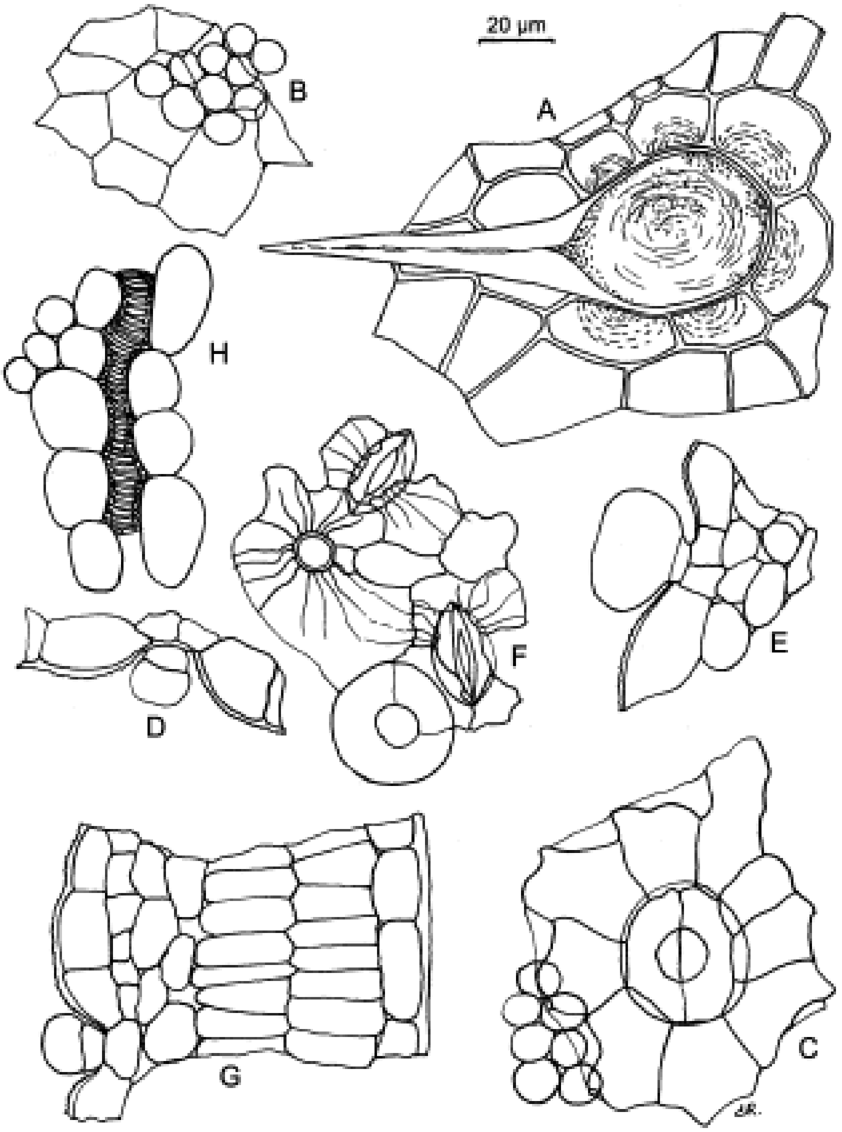
CHARACTERS

After grinding, it has a characteristic odour reminiscent of lemon.

IDENTIFICATION

A. The leaves are simple with short petioles. They are narrow, lanceolate, and about 4 times longer than they are wide. The entire, slightly undulating margins are curled towards the upper surface. The upper surface is dark green and rough to the touch; the lower surface is paler green and shows a prominent midrib with secondary veins running to the margins.

B. Reduce to a powder (355) (*2.9.12*). The powder is light green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the lamina in surface view, those of the upper epidermis being composed of polygonal cells with numerous short, unicellular, thick-walled cystolithic trichomes, each arising from a rosette of cells at the base, stomata are absent; the cells of the lower epidermis are more irregular and somewhat sinuous in outline and anomocytic stomata (*2.8.3*) are abundant, also present are numerous subsessile glandular trichomes with a globular head; fragments of parenchyma of the mesophyll and groups of lignified tissue from the veins.



A. Upper epidermis with cystolithic trichome, with subsidiary cells containing calcium concretons

B-C. Surface view of upper epidermis, with palisade parenchyma and glandular trichome (C)

D-E. Transverse section of glandular trichomes

F. Surface view of lower epidermis with striated cuticule, anomocytic stomata and glandular trichome

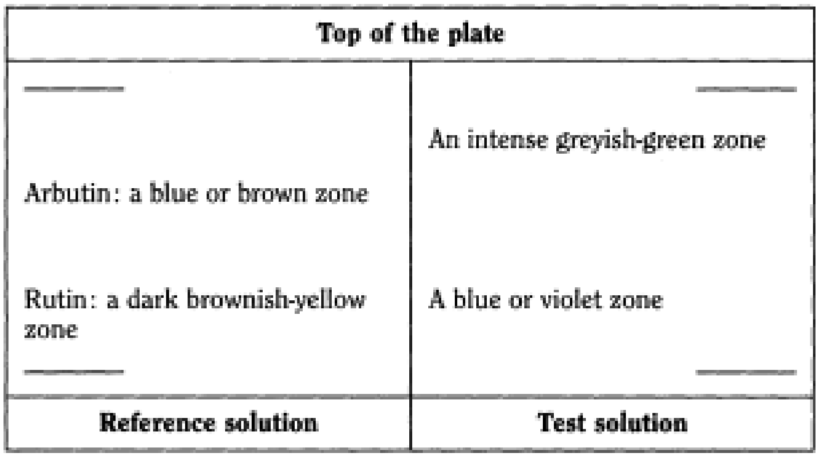
G. Transverse section of the lamina

H. Parenchyma of the mesophyll with a lignified vein

Figure 1834.-1. – *Illustration of powdered herbal drug of lemon verbena leaf (see Identification B)*

C. Examine the chromatograms obtained in the test for *Verbena officinalis*.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution. Zones may be present in the chromatogram obtained with the test solution below the zone due to rutin in the chromatogram obtained with the reference solution.



Top of the plate	
———	———
	An intense greyish-green zone
Arbutin: a blue or brown zone	
Rutin: a dark brownish-yellow zone	A blue or violet zone
———	———
Reference solution	**Test solution**

TESTS

Verbena officinalis

Thin-layer chromatography (*2.2.27*).

Test solution To 0.50 g of the powdered drug (710) (*2.9.12*) add 5 ml of *methanol R*. Heat in a water-bath at 60 °C for 10 min. Cool and filter.

Reference solution Dissolve 10 mg of *arbutin R* and 10 mg of *rutin R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase anhydrous formic acid R, *glacial acetic acid R*, *water R*, *ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application 20 µl [or 5 µl], as bands.

Development Over a path of about 12 cm [or 6 cm].

Drying In air.

Detection Spray with *anisaldehyde solution R* and dry at 100-105 °C for about 10 min; examine in daylight.

Results The chromatogram obtained with the test solution shows no brownish-grey zone at a position between that of arbutin and rutin in the chromatogram obtained with the reference solution.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (710) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 13.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 3.5 per cent.

ASSAY

Acteoside

Liquid chromatography (*2.2.29*).

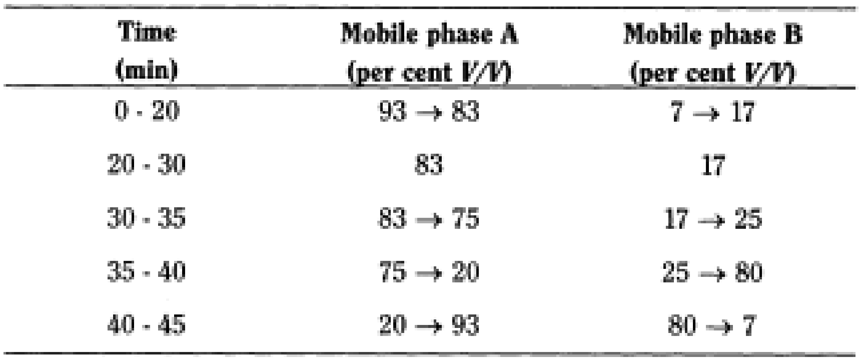
Test solution To 1.00 g of the powdered drug (710) (*2.9.12*) add 50.0 ml of the reference solution and stir for 2 h with a magnetic stirrer. Centrifuge for 15 min and pass the supernatant through a membrane filter (pore size 0.45 µm).

Reference solution Dissolve 10.0 mg of *ferulic acid CRS* in *ethanol (60 per cent V/V) R* and dilute to 100.0 ml with the same solvent.

Precolumn:
- *size:* *l* = 0.01 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Column:
- *size:* *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 20 °C.

Mobile phase:
- *mobile phase A*: 0.3 per cent *V/V* solution of *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	93 → 83	7 → 17
20 - 30	83	17
30 - 35	83 → 75	17 → 25
35 - 40	75 → 20	25 → 80
40 - 45	20 → 93	80 → 7

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 330 nm.

Injection 20 µl.

System suitability Test solution:
- *resolution*: minimum 3.5 between the peaks due to ferulic acid and acteoside.

Calculate the percentage content of acteoside, expressed as ferulic acid, using the following expression:

$$\frac{A_1 \times m_2 \times p \times 5 \times 3.1}{A_2 \times m_1}$$

A_1 = area of the peak due to acteoside in the chromatogram obtained with the test solution;

A_2 = area of the peak due to ferulic acid in the chromatogram obtained with the reference solution;

m_1 = mass of the drug in the test solution, in grams;

m_2 = mass of *ferulic acid CRS* in the reference solution, in grams;

p = percentage content of ferulic acid in *ferulic acid CRS*;

3.1 = correlation factor between acteoside and ferulic acid.

***Essential oil* (*2.8.12*)**

Introduce 25.0 g of the freshly crushed drug into a 1000 ml flask and add 500 ml of a 10 g/l solution of *sodium chloride R* as the distillation liquid. Use 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 3.0-3.5 ml/min for 3 h.

Ph Eur

Lime Flower

General Notices

(Ph Eur monograph 0957)

Ph Eur

DEFINITION

Whole, dried inflorescence of *Tilia cordata* Miller, of *Tilia platyphyllos* Scop., of *Tilia × vulgaris* Heyne or a mixture of these.

CHARACTERS

Faint aromatic odour.

Faint, sweet and mucilaginous taste.

IDENTIFICATION

A. The inflorescence is yellowish-green. The main axis of the inflorescence bears a linguiform bract, membranous, yellowish-green, practically glabrous, the central vein of which is joined for up to about half of its length with the peduncle. The inflorescence usually consists of 2-7 flowers, occasionally up to 16. The sepals are detached easily from the perianth; they are up to 6 mm long, their abaxial surface is usually glabrous, their adaxial surface and their borders are strongly pubescent. The 5 spatulate, thin petals are yellowish-white, up to 8 mm long. They show fine venation and their borders only are sometimes covered with isolated trichomes. The numerous stamens are free and usually constitute 5 groups. The superior ovary has a pistil with a somewhat 5-lobate stigma.

B. Separate the inflorescence into its different parts. Examine under a microscope using *chloral hydrate solution R*. The adaxial epidermis of the bract shows cells with straight or slightly sinuous anticlinal walls; the abaxial epidermis shows cells with wavy-sinuous anticlinal walls and anomocytic stomata (*2.8.3*). Isolated cells in the mesophyll contain small calcium oxalate cluster crystals. The parenchyma of the sepals shows, particularly near the veins, numerous mucilaginous cells and cells containing small calcium oxalate clusters. The adaxial epidermis of sepals bears bent, thick-walled covering trichomes, unicellular or stellate with up to 5 cells. The epidermal cells of the petals show straight anticlinal walls with a striated cuticle without stomata. The parenchyma of the petals shows small calcium oxalate clusters and especially in its acuminate part mucilaginous cells. The pollen grains have a diameter of about 30-40 µm and are oval or slightly triangular with 3 germinal pores and a finely granulated exine. The ovary is glabrous or densely covered with trichomes, often very twisted, unicellular or stellate with 2-4 branches.

C. Thin-layer chromatography (*2.2.27*).

Test solution Shake 1.0 g of the powdered drug (355) (*2.9.12*) with 10 ml of *methanol R* in a water-bath at 65 °C for 5 min. Allow to cool and filter.

Reference solution Dissolve 2.0 mg of *caffeic acid R*, 5 mg of *hyperoside R* and 5 mg of *rutin*

R in 10 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Then spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow to dry for about 30 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in order of increasing R_F value yellowish-orange or brownish-orange fluorescent zones due to rutin and hyperoside and a greenish-blue fluorescent zone due to caffeic acid. In the chromatogram obtained with the test solution, the main zone shows brownish-yellow or orange fluorescence. This zone is situated just above the zone due to hyperoside in the chromatogram obtained with the reference solution. In daylight, this zone stands out from the other zones as the main zone. At the R_F level of rutin there is also a brownish-yellow fluorescent zone. Below this zone, 2 yellow fluorescent zones may be present. Between the zones due to rutin and hyperoside, orange and yellow fluorescent zones are visible. Between the zones due to hyperoside and caffeic acid, up to 5 yellow or orange fluorescent zones are present. Immediately below the zone due to caffeic acid is a a blue fluorescent zone.

TESTS

Foreign matter (*2.8.2*)

Maximum 2 per cent, determined on 30 g. There are no inflorescences with a bract bearing at the abaxial face stellate, five- to eight-rayed trichomes and flowers having an apparent double corolla by transformation of five stamens into petal-like staminoids and having a pistil which is not lobular nor indented. Hexamerous flowers occur only occasionally (*Tilia americana* L., *Tilia tomentosa* Moench).

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 8.0 per cent.

Ph Eur

Linseed

General Notices

(Ph Eur monograph 0095)

When Powdered Linseed is prescribed or demanded, material complying with the appropriate requirements below shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried, ripe seeds of *Linum usitatissimum* L.

IDENTIFICATION

A. The linseed has a flattened, elongated ovoid shape. The testa is dark reddish-brown or yellow, smooth and shiny. The seeds are 4-6 mm long, 2-3 mm wide and 1.5-2 mm thick; one end is rounded and the other end forms an oblique point near which the hilum appears as a slight depression. When viewed with a lens, the surface of the seed-coat is seen to be minutely pitted. Inside the testa a narrow, whitish endosperm and an embryo composed of 2 large, flattened, yellowish and oily cotyledons are present; the radicle points towards the hilum.

B. Reduce to a powder (355) (*2.9.12*). The powder is greasy to the touch. Examine under a microscope using *chloral hydrate solution R*. The powder consists of fragments of the outer epidermal cells of the testa filled with mucillage; collenchymatously thickened sub-epidermal layer seen in surface view as rounded cells with distinct triangular intercellular spaces often attached to the sclerenchymatous layer composed of elongated cells, some with strongly thickened and pitted walls; thin-walled pitted cells of the hyaline layer often remaining attached to the elongated sclereids and crossing them at approximately right angles; pigmented cells of the inner epidermis of the testa composed of moderately thickened polygonal cells filled with orange-brown pigment; parenchyma of the endosperm and cotyledons containing aleurone grains and fatty oil.

TESTS

Foreign matter (*2.8.2*)

Maximum 10 per cent of seeds with a dull coat and maximum 1.5 per cent of other foreign matter.

Swelling index (*2.8.4*)

Minimum 4.

Cadmium (*2.4.27*)

Maximum 0.5 ppm.

Loss on drying (*2.2.32*)

Maximum 8.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 5.0 per cent.

Ph Eur

Liquorice Dry Extract for Flavouring Purposes

General Notices

(Ph Eur monograph 2378)

Ph Eur

DEFINITION

Dry extract produced from *Liquorice root (0277).*

Content

5.0 per cent to 7.0 per cent of 18β-glycyrrhizic acid ($C_{42}H_{62}O_{16}$; M_r 823) (dried extract).

PRODUCTION

The extract is produced from the cut herbal drug by a suitable procedure using water.

CHARACTERS

Appearance

Yellowish-brown or brown powder.

Very sweet taste.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Solvent mixture *ethyl acetate R*, *methanol R* (50:50 *V/V*).

Test solution To 0.30 g of the extract to be examined add 30 ml of *hydrochloric acid R1* and boil on a water-bath under a reflux condenser for 60 min. After cooling, extract the mixture with 2 quantities, each of 20 ml, of *ethyl acetate R*. Combine the organic layers and filter through a filter covered with *anhydrous sodium sulphate R*. Evaporate the filtrate to dryness *in vacuo* and dissolve the residue in 2.0 ml of the solvent mixture.

Reference solution Dissolve 5.0 mg of *glycyrrhetic acid R* and 5.0 mg of *thymol R* in 5.0 ml of the solvent mixture.

Plate *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F_{254} plate R* (2-10 µm)].

Mobile phase *concentrated ammonia R*, *water R*, *ethanol (96 per cent) R*, *ethyl acetate R* (1:9:25:65 *V/V/V/V*).

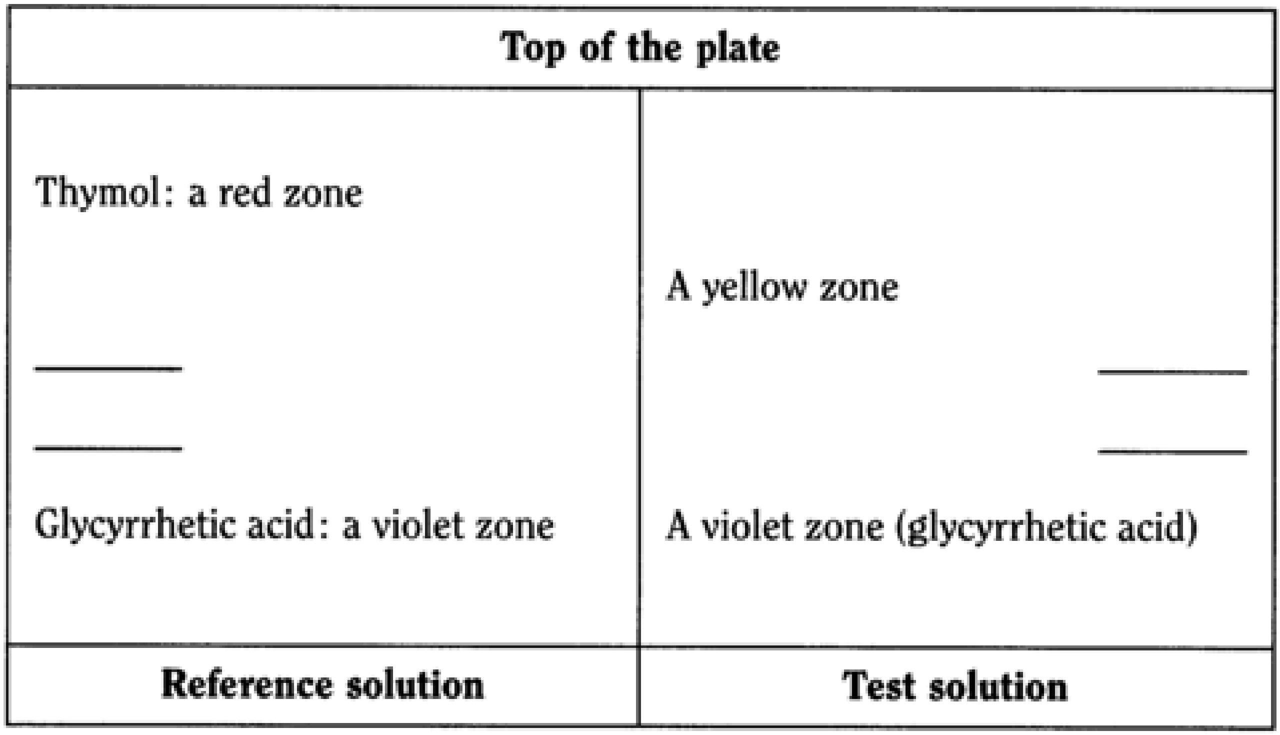
Application 20 µl [or 10 µl], as bands.

Development Over a path of 15 cm [or 7 cm].

Drying In air for 5 min.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Thymol: a red zone	
	A yellow zone
———	———
———	———
Glycyrrhetic acid: a violet zone	A violet zone (glycyrrhetic acid)
Reference solution	**Test solution**

TESTS

Loss on drying ***(2.8.17)***

Maximum 7.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Solvent mixture *water R*, *methanol R* (20:80 *V/V*).

Test solution Place 0.200 g of the extract to be examined in a 150 ml ground-glass conical flask. Add 100.0 ml of the solvent mixture and sonicate for 2 min. Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution Dissolve 50.0 mg of *monoammonium glycyrrhizate CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

- *size:* *l* = 0.10 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R*, *acetonitrile R*, *water R* (6:30:64 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Run time 3 times the retention time of 18β-glycyrrhizic acid.

Retention time 18β-glycyrrhizic acid = about 9 min.

Identification of peaks Use the chromatogram supplied with *monoammonium glycyrrhizate CRS* and the chromatogram obtained with the reference solution to identify the peaks due to 18β-glycyrrhizic acid and 18α-glycyrrhizic acid.

System suitability Reference solution:

— the chromatogram obtained with the reference solution is similar to the chromatogram supplied with *monoammonium glycyrrhizate CRS*;

— *resolution*: minimum 2.0 between the peaks due to 18β-glycyrrhizic acid and 18α-glycyrrhizic acid.

Calculate the percentage content of 18β-glycyrrhizic acid, using the following expression:

$$\frac{A_1 \times m_2 \times p \times 0.979}{A_2 \times m_1 \times 5}$$

A_1 = area of the peak due to 18β-glycyrrhizic acid in the chromatogram obtained with the test solution

A_2 = area of the peak due to 18β-glycyrrhizic acid in the chromatogram obtained with the reference solution

m_1 = mass of the extract to be examined used to prepare the test solution, in grams

m_2 = mass of *monoammonium glycyrrhizate CRS* used to prepare the reference solution, in grams

p = percentage content of 18β-glycyrrhizic acid in *monoammonium glycyrrhizate CRS*

0.979 = peak correlation factor between glycyrrhizic acid and monoammonium glycyrrhizate.

Ph Eur

Liquorice Liquid Extract

General Notices

DEFINITION

Liquorice Liquid Extract is prepared by extracting Liquorice with Purified Water and adding sufficient Ethanol (90 per cent) to give an ethanol content of 18% v/v in the final extract.

Extemporaneous preparation

The following formula and directions apply.

Liquorice, unpeeled, in *coarse powder*	1000 g
Purified Water	A sufficient quantity
Ethanol (90 per cent)	A sufficient quantity

Exhaust the Liquorice with Purified Water by *percolation*, Appendix XI F. Boil the percolate for 5 minutes and set aside for not less than 12 hours. Decant the clear liquid, filter the remainder, mix the two liquids and evaporate until the *weight per ml* of the liquid is 1.198 g, Appendix V G. Add to this liquid, when cold, one quarter of its volume of Ethanol (90 per cent) . Allow to stand for not less than 4 weeks; filter.

The extract complies with the requirements stated under Extracts and with the following requirements.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a suspension of *silica gel* GF_{254} in a 0.25% v/v solution of *orthophosphoric acid* to prepare the plate and a mixture of 5 volumes of *methanol* and 95 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of solution (1) and 5 µl of solution (2). For solution (1) extract 5 ml of the liquid extract with 20 ml of *chloroform*. Heat 2.5 ml of the aqueous layer with 30 ml of 0.5M *sulphuric acid* under a reflux condenser for 1 hour, cool and extract with two 20 ml quantities of *chloroform*. Dry the combined chloroform extracts over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness; dissolve the residue in 4 ml of a mixture of equal volumes of *chloroform* and *methanol*. For solution (2) dissolve 10 mg of *glycyrrhetinic acid* in 2 ml of a mixture of equal volumes of *chloroform* and *methanol*. After removal of the plate, examine it under *ultraviolet light (254 nm)*. The chromatogram obtained with solution (1) exhibits a dark spot corresponding to the principal spot in the chromatogram obtained with solution (2).

TESTS

Ethanol content

16 to 20% v/v, Appendix VIII F, Method III.

Ammonia

Place 5 ml in a distillation apparatus of about 500 ml capacity fitted with an efficient splash-head and a delivery tube that dips below the surface of a mixture of 10 ml of 0.05M *sulphuric acid VS* and 50 ml of *water* contained in the receiver. Add 200 ml of *water*, a little *pumice powder* and 30 ml of a solution prepared by dissolving 1.43 g of *potassium dihydrogen orthophosphate* and 9.1 g of *dipotassium hydrogen orthophosphate* in 100 ml of *water* and distil until about 175 ml of distillate has been collected. Titrate the excess of acid with 0.1M *sodium hydroxide VS* using *methyl red solution* as indicator. Not less than 5 ml of 0.1M *sodium hydroxide VS* is required.

Dry residue

40 to 45% w/v.

Relative density

1.125 to 1.140, Appendix V G.

Liquorice Root for use in THM

General Notices

Liquorice Root for use in use in Traditional Chinese Medicines

DEFINITION

Liquorice Root for use in THM is the dried unpeeled root and rhizome of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat. or *Glycyrrhiza glabra* L.

It is collected in spring and autumn, separated from the rootlets and dried in the sun.

It contains not less than 2.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$), calculated with reference to the dried material.

IDENTIFICATION

A. The root has few branches. Its bark is brownish-grey or brown with longitudinal striations and bears traces of lateral roots. The cylindrical stolons are 1-2 cm in diameter; their external appearance is similar to that of the root but there are occasional small buds. The fracture of the root and the stolon is granular and fibrous. The cork layer is thin; the secondary phloem region is thick and light yellow with radial striations. The yellow xylem cylinder is compact, with a radiate structure. The stolon has a central pith, which is absent from the root.

B. Reduce to a powder. The powder is yellowish-brown. Examine under a microscope using *chloral hydrate solution*. The powder shows abundant fibres occurring in groups, each group surrounded by a calcium oxalate prism crystal sheath, the individual fibres with very thick, partially lignified walls and few small pits; lignified bordered-pitted vessels, singly or in small groups and sometimes accompanied by lignified parenchymatous cells with moderately thickened and pitted walls, some of the individual vessels are very large, with pit apertures much elongated and the borders difficult to discern; prism crystals of calcium oxalate up to about 35 µm long occur scattered and in parenchymatous tissue; fragments of cork composed of thin-walled, slightly lignified polygonal cells. Examine under a microscope using 50% w/w glycerol in water. The powder shows abundant starch granules, mostly simple, spherical to ovoid, 3 µm to 20 µm in diameter.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 16 ml of water and 4 ml of *hydrochloric acid R1* to 0.5 g of the powdered drug, heat on a water-bath under reflux for 30 minutes, cool and filter. Dry the filter paper and residue in a flask at 105oC for 60 minutes, add 20 ml of ether, heat on a water-bath at 40ºC under reflux for 5 minutes, cool and filter. Evaporate the filtrate to dryness and dissolve the residue in 5 ml of ether.

(2) 0.1% w/v each of *glycyrrhetic acid* and *thymol* in ether.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel* GF_{254} (Merck 10 x 20 cm plates are suitable).

(b) Mobile phase is a mixture of 1 volume of *concentrated ammonia*, 9 volumes of water, 25 volumes of *ethanol* (96%) and 65 volumes of *ethyl acetate*.

(c) Apply as a band, an application volume of 10 µl for each solution.

(d) Develop to 15 cm.

(e) Remove the plate and allow it to dry in air for 5 minutes. Examine under *ultraviolet light (254 nm)*. Spray the plate with *anisaldehyde solution* and heat at 100° to 105° for 10 minutes and examine in daylight.

SYSTEM SUITABILITY

Under ultra-violet light, the chromatogram obtained with solution (2) shows in the lower half a quenching zone due to glycyrrhetic acid. When sprayed, the chromatogram obtained with solution (2) shows in the lower half the violet zone of glycyrrhetic acid and in the upper third the red zone of thymol.

VALIDITY

The chromatogram obtained with solution (1) shows in the lower half a violet zone corresponding to the zone of glycyrrhetic acid in the chromatogram obtained with solution (2) and a yellow zone (isoliquiridigenine) in the upper third under the zone of thymol in the chromatogram obtained with solution (2). Further zones may be present.

TESTS

Total Ash

Not more than 7.0%, Appendix XI J, Method II.

Acid-insoluble ash

Not more than 2.0%, Appendix XI K, Method II.

Loss on drying

When dried for 2 hours at 100° to 105°, loses not more than 12.0%. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix 1 g of the powdered drug being examined with 100 ml of 0.8% w/v of *ammonia*, place in an ultrasonic bath for 30 minutes, centrifuge a part of the supernatant layer, dilute 1 ml to 5 ml with 0.8 % w/v of *ammonia* and filter through a 0.45-µm filter.

(2) 0.0065% w/v of *monoammonium glycyrrhizate EPCRS* in 0.8% w/v of *ammonia*.

(3) 0.013% w/v of *monoammonium glycyrrhizate EPCRS* in 0.8% w/v of *ammonia*.

(4) 0.0195% w/v of *monoammonium glycyrrhizate EPCRS* in 0.8% w/v of *ammonia*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS SS is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute

(d) Ambient column temperature.

(e) Detection wavelength of 254 nm.

(f) Injection volume of 10 µl for each solution.

MOBILE PHASE

A mixture of 6 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 64 volumes of *water.*

SYSTEM SUITABILITY

The assay is not valid unless (a) the *column efficiency,* determined on the peak due to glycyrrhizic acid in the chromatogram obtained with solution (2), is at least 30,000 theoretical plates per metre and (b) the *symmetry factor* of the peak is not more than 1.3.

DETERMINATION OF CONTENT

Inject solution (4). Adjust the sensitivity of the system so that the height of the peaks are at least 50% of the full scale of the recorder. Inject solutions (2), (3) and (4) and determine the peak areas. Prepare a calibration curve with the concentration of the solutions (g per 100 ml) as the abscissa and the corresponding areas as the ordinate. Inject solution (1). Using the retention time and the peak area from the chromatograms obtained with solutions (2), (3) and (4), locate and integrate the peak due to glycyrrhizic acid in the chromatogram obtained with solution (1).

Calculate the percentage content of glycyrrhizic acid from the following expression:

$$A \times \frac{5}{m} \times B \times \frac{822}{840}$$

A = concentration of monoammonium glycyrrhizate in solution (1) determined from the calibration curve, in g per 100 ml,

B = declared percentage content of *monoammonium glycyrrhizate EPCRS*,

M = weight of the substance being examined in grams,

822 = molecular weight of glycyrrhizic acid,

840 = molecular weight of monoammonium glycyrrhizate (without any water of crystallisation).

STORAGE

Liquorice Root for use in THM should be protected from moisture.

Liquorice

General Notices

(Liquorice Root, Ph Eur monograph 0277)

Preparation

Liquorice Liquid Extract

When Powdered Liquorice is prescribed or demanded, material complying with the appropriate requirements below shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried unpeeled or peeled, whole or cut root and stolons of *Glycyrrhiza glabra* L. and/or of *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch.

Content

Minimum 4.0 per cent of glycyrrhizic acid ($C_{42}H_{62}O_{16}$; M_r 823) (dried drug).

IDENTIFICATION

A. The root has few branches. Its bark is brownish-grey or brown with longitudinal striations and bears traces of lateral roots. The cylindrical stolons are 1-2 cm in diameter; their external appearance is similar to that of the root but there are occasional small buds. The fracture of the root and the stolon is granular and fibrous. The cork layer is thin; the secondary phloem region is thick and light yellow with radial striations. The yellow xylem cylinder is compact, with a radiate structure. The stolon has a central pith, which is absent from the root. The external part of the bark is absent from the peeled root.

B. Reduce to a powder (355) (*2.9.12*). The powder is light yellow or faintly greyish. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of yellow thick-walled fibres, 700-1200 µm long and 10-20 µm wide with a punctiform lumen, often accompanied by crystal sheaths containing prisms of calcium oxalate 10-35 µm long and 2-5 µm wide. The walls of the large vessels are yellow, 5-10 µm thick, lignified and have numerous bordered pits with a slit-shaped aperture; fragments of cork consisting of thin-walled cells and isolated prisms of calcium oxalate occur as well as fragments of parenchymatous tissue. Fragments of cork are absent from the peeled root. Examine under a microscope using a mixture of equal volumes of *glycerol R* and *water R*. The powder shows the following diagnostic characters: simple, round or oval starch granules, 2-20 µm in diameter.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 0.50 g of the powdered drug (180) (*2.9.12*) in a 50 ml round-bottomed flask add 16.0 ml of *water R* and 4.0 ml of *hydrochloric acid R1* and heat on a water-bath under a reflux condenser for 30 min. Cool and filter. Dry the filter and the round-bottomed flask at 105

°C for 60 min. Place the filter in the round-bottomed flask, add 20.0 ml of *ether R* and heat in a water-bath at 40 °C under a reflux condenser for 5 min. Cool and filter. Evaporate the filtrate to dryness. Dissolve the residue in 5.0 ml of *ether R*.

Reference solution Dissolve 5.0 mg of *glycyrrhetic acid R* and 5.0 mg of *thymol R* in 5.0 ml of *ether R*.

Plate *TLC silica gel* F_{254} *plate R*.

Mobile phase *concentrated ammonia R*, *water R*, *ethanol (96 per cent) R*, *ethyl acetate R* (1:9:25:65 *V/V/V/V*).

Application 10 µl.

Development Over a path of 15 cm.

Drying In air for 5 min.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatograms obtained with the test solution and with the reference solution show in the lower half a quenching zone due to glycyrrhetic acid.

Detection B Spray with *anisaldehyde solution R*, and heat at 100-105 °C for 5-10 min; examine in daylight.

Results B The chromatogram obtained with the reference solution shows in the lower half a violet zone due to glycyrrhetic acid and in the upper third a red zone due to thymol. The chromatogram obtained with the test solution shows in the lower half a violet zone corresponding to the zone of glycyrrhetic acid in the chromatogram obtained with the reference solution and a yellow zone (isoliquiridigenine) in the upper third under the zone of thymol in the chromatogram obtained with the reference solution. Further zones may be present.

TESTS

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 10.0 per cent for the unpeeled drug and maximum 6.0 per cent for the peeled drug.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 2.0 per cent for the unpeeled drug and maximum 0.5 per cent for the peeled drug.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Place 1.000 g of the powdered drug (180) (*2.9.12*) in a 150 ml ground glass conical flask. Add 100.0 ml of an 8 g/l solution of *ammonia R* and treat in a ultrasonic bath for 30 min. Centrifuge a part of the solution and dilute 1.0 ml of the supernatant layer to 5.0 ml with an 8 g/l solution of *ammonia R*. Filter the solution through a filter (0.45 µm) and use the filtrate as the test solution.

Solution A Dissolve 0.130 g of *monoammonium glycyrrhizate CRS* in an 8 g/l solution of *ammonia R* and dilute to 100.0 ml with the same solvent.

Reference solution (a) Dilute 5.0 ml of solution A to 100.0 ml with an 8 g/l solution of *ammonia R*.

Reference solution (b) Dilute 10.0 ml of solution A to 100.0 ml with an 8 g/l solution of *ammonia R*.

Reference solution (c) Dilute 15.0 ml of solution A to 100.0 ml with an 8 g/l solution of *ammonia R*.

Column:

— *size:* l = 0.10 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R*, *acetonitrile R*, *water R* (6:30:64 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Establish a calibration curve with the concentration of the reference solutions (g/100 ml) as the abscissa and the corresponding areas as the ordinate.

Using the retention time and the peak area determined from the chromatograms obtained with the reference solutions, locate and integrate the peak due to glycyrrhizic acid in the chromatogram obtained with the test solution.

Calculate the percentage content of glycyrrhizic acid from the following expression:

$$A \times \frac{5}{m} \times B \times \frac{822}{840}$$

A = concentration of monoammonium glycyrrhizate in the test solution determined from the calibration curve, in g/100 ml;

B = declared percentage content of *monoammonium glycyrrhizate CRS;*

m = mass of the drug, in grams;

822 = molecular weight of glycyrrhizic acid;

840 = molecular weight of the monoammonium glycyrrhizate (without any water of crystallisation).

LABELLING

The label states whether the drug is peeled or unpeeled.

Ph Eur

Loosestrife

General Notices

(Ph Eur monograph 1537)

Ph Eur

DEFINITION

Dried flowering tops, whole or cut, of *Lythrum salicaria* L.

Content

Minimum 5.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The stems are rigid, four-angled, branching at the top, brownish-green, longitudinally wrinkled and pubescent. The leaves are opposite, decussate, rarely verticillate in threes and sometimes alternate at the inflorescence which forms a long terminal spike. The leaves are sessile, lanceolate and cordate at the base, 5-15 cm long and 1-2.5 cm wide, pubescent on the lower surface; the subsidiary veins form arcs that anastomose near the leaf margin. The flowers have a pubescent, tubular, persistent gamosepalous calyx, 4-8 mm long, consisting of 6 sepals bearing 6 small, triangular teeth alternating with 6 large acute teeth at least half as long as the tube; a polypetalous corolla consisting of 6 violet-pink petals, each expanded at the top with a wavy outline and narrowing at the base. The androecium consists of 2 verticils of 6 stamens (one verticil with short, barely emerging stamens, the other with long stamens extending well out of the corolla). The fruit, if formed, is a small capsule included in the persistent calyx.

B. Reduce to a powder (355) (*2.9.12*). The powder is greenish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: unicellular or bicellular, uniseriate, thick-walled, finely pitted covering trichomes from the lower epidermis of the stem and leaf; numerous uniseriate, unicellular or bicellular, thin-walled, finely pitted covering trichomes from the calyx; transparent violet-pink fragments from the petals; numerous cluster crystals of calcium oxalate; pollen grains with 3 pores and a thin and slightly granular exine; fragments of the upper epidermis with large polygonal cells and sinuous walls; fragments of the lower epidermis with smaller polygonal cells and anomocytic stomata (*2.8.3*).

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methanol R* and heat in a water-bath at 65 °C for 5 min with frequent shaking. Cool and filter. Dilute the filtrate to 10 ml with *methanol R*.

Reference solution Dissolve 0.5 mg of *chlorogenic acid R*, 1 mg of *hyperoside R*, 1 mg of *rutin R* and 1 mg of *vitexin R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous acetic acid R*, *anhydrous formic acid R*, *water R*, *ethyl acetate R* (7.5:7.5:18:67 *V/V/V/V*).

Application 10 µl as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray the plate while still warm with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the lower third a yellowish-brown fluorescent zone due to rutin and in the middle third a light blue fluorescent zone due to chlorogenic acid, above it a yellowish-brown fluorescent zone due to hyperoside and a green fluorescent zone due to vitexin. The chromatogram obtained with the test solution shows a bright green fluorescent zone slightly above the zone due to rutin in the chromatogram obtained with the reference solution, a yellow fluorescent zone similar in position to the zone due to chlorogenic acid in the chromatogram obtained with the reference solution, a yellow fluorescent zone similar in position to the zone due to hyperoside in the chromatogram obtained with the reference solution and a bright green fluorescent zone corresponding to the zone due to vitexin in the chromatogram obtained with the reference solution.

TESTS

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C.

Total ash (*2.4.16*)

Maximum 7.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs (*2.8.14*). Use 0.750 g of the powdered drug (180) (*2.9.12*).

Ph Eur

Lovage Root

General Notices

(Ph Eur monograph 1233)

Ph Eur

DEFINITION

Whole or cut, dried rhizome and root of *Levisticum officinale* Koch.

Content

Minimum 4.0 ml/kg of essential oil for the whole drug and minimum 3.0 ml/kg of essential oil for the cut drug (dried drug).

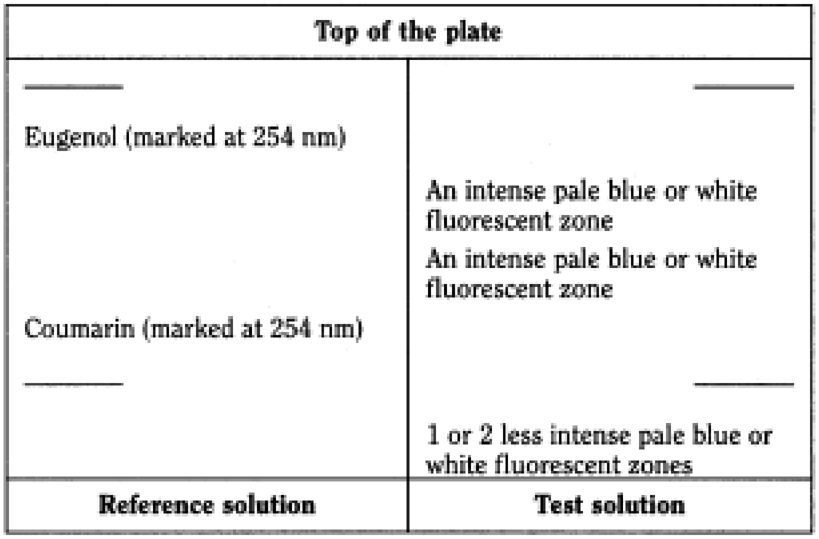
IDENTIFICATION

A. The rhizome and the large roots are often split longitudinally. The rhizome is short, up to 5 cm in diameter, light greyish-brown or yellowish-brown, simple or with several protuberances; the roots, showing little ramification, are the same colour as the rhizome; they are usually up to 1.5 cm thick and up to about 25 cm long; the fracture is usually smooth and shows a very wide yellowish-white bark and a narrow brownish-yellow wood.

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: cork cells, polygonal or rounded in surface view, with brown contents; abundant parenchyma, mostly thin-walled and rounded but some with thicker walls; groups of small, reticulately thickened vessels embedded in small-celled, unlignified parenchyma; fragments of larger vessels with reticulate thickening, up to 125 µm in diameter; fragments of secretory canals up to 180 µm wide. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows starch granules, simple, rounded or ovoid, up to about 12 µm, and numerous larger, compound granules, many with several components.

C. Examine the chromatograms obtained in the test for angelica root.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
Eugenol (marked at 254 nm)	
	An intense pale blue or white fluorescent zone
	An intense pale blue or white fluorescent zone
Coumarin (marked at 254 nm)	
———	———
	1 or 2 less intense pale blue or white fluorescent zones
Reference solution	**Test solution**

TESTS

Angelica root

Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the freshly powdered drug (355) (*2.9.12*) add 5 ml of *methanol R* and boil for 30 s. Cool and filter.

Reference solution Dissolve 5 mg of *coumarin R* and 25 µl of *eugenol R* in 10 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *methylene chloride R*, *toluene R* (50:50 *V/V*).

Application 20 µl, as bands.

Development Twice over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm. Mark the quenching zones due to coumarin and eugenol on the chromatogram obtained with the reference solution. Examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows no blue or yellow fluorescent zones in the lower third.

Foreign matter (*2.8.2*)

Maximum 3 per cent, determined on 50 g.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 8.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 2.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs (*2.8.12*). Use a 2 litre flask, 10 drops of *liquid paraffin R*, 500 ml of *water R* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Reduce the drug to a powder (500) (*2.9.12*) and immediately use 40.0 g for the determination. Distil at a rate of 2-3 ml/min for 4 h.

Ph Eur

Mallow Flower

General Notices

(Ph Eur monograph 1541)

Ph Eur

DEFINITION

Whole or fragmented dried flower of *Malva sylvestris* L. or its cultivated varieties.

IDENTIFICATION

A. The flower consists of an epicalyx with 3 oblong or elliptical-lanceolate parts that are shorter than those of the calyx and situated immediately below it; a calyx with 5 pubescent triangular lobes, gamosepalous at the base; a corolla 3-4 times longer than the calyx with 5 wedge-shaped, notched petals fused to the staminal tube at their base; numerous stamens, the filaments of which fuse into a staminal tube covered by small star-shaped trichomes and occasional simple trichomes visible using a lens; numerous wrinkled carpels, glabrous or sometimes pubescent, enclosed in the staminal tube and arranged into a circle around a central style ending with numerous filiform stigmas. In cultivated varieties, the epicalyx is 3-7 partite, the calyx 5-8 partite and the corolla 5-10 partite.

B. Reduce to a powder (355) (*2.9.12*). The powder is bluish-grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: unicellular, thick-walled, stiff trichomes, up to 2 mm in length; small unicellular covering trichomes, somewhat curved, either isolated or in small star-shaped groups of 2-6; capitate glandular trichomes with multicellular heads; mesophyll fragments with vessels accompanied by cluster crystals of calcium oxalate; spherical pollen grains, about 150 µm in diameter, with a roughly spiny exine.

When mounted with *ethanol (96 per cent) R*, numerous elongated cells containing mucilage are seen in the petal fragments.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1 g of the powdered drug (355) (*2.9.12*) add 10 ml of *ethanol (60 per cent V/V) R*. Stir for 15 min and filter.

Reference solution A 0.5 g/l solution of *quinaldine red R* in *ethanol (96 per cent) R*.

Plate *TLC silica gel plate R.*

Mobile phase *acetic acid R*, *water R*, *butanol R* (15:30:60 *V/V/V*).

Application 10 µl of the test solution and 5 µl of the reference solution, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in daylight.

Results The chromatogram obtained with the reference solution shows an orange-red zone in the upper part of the middle third. The chromatogram obtained with the test solution shows, below the zone in the chromatogram obtained with the reference solution, 2 violet zones in the middle third; the principal zone (6″-malonyl malvin) is situated just below the other violet zone (malvin).

TESTS

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C.

Total ash (*2.4.16*)

Maximum 14.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 2.0 per cent.

Swelling index (*2.8.4*)

Minimum 15, determined on 0.2 g of the powdered drug (710) (*2.9.12*) humidified with 0.5 ml of *anhydrous ethanol R*.

Ph Eur

Mandarin Oil

General Notices

(Ph Eur monograph 2355)

Ph Eur

DEFINITION

Essential oil obtained without heating, by suitable mechanical treatment, from the peel of the fresh fruit of *Citrus reticulata* Blanco.

CHARACTERS

Appearance

Greenish, or yellow to reddish orange liquid showing blue fluorescence.

It has a characteristic odour.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dilute 0.1 ml of the substance to be examined to 1 ml with *toluene R*.

Reference solution Dissolve 2 µl of *methyl N-methylanthranilate R*, 4 mg of *guaiazulene R* and 10 mg of α-*terpineol R* in 10 ml of *toluene R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *ethyl acetate* R, *toluene R* (15:85 *V/V*).

Application 10 µl [or 2 µl] as bands.

Development Over a path of 15 cm [or 6 cm].

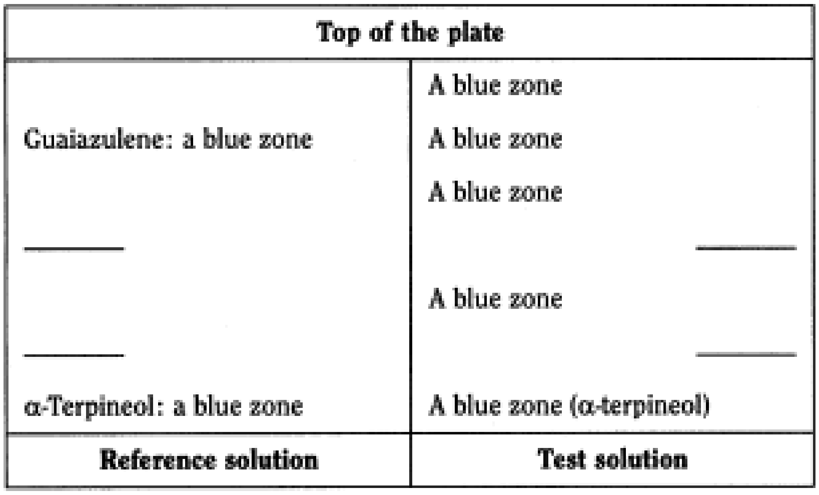
Drying In air.

Detection A Examine in ultraviolet light at 365 nm.

Results A The intense blue fluorescent zone in the chromatogram obtained with the test solution is similar in position and fluorescence to the zone due to methyl *N*-methylanthranilate in the chromatogram obtained with the reference solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.

Detection B Spray with a 200 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R* and heat at 100 °C for 10 min; examine in daylight.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A blue zone
Guaiazulene: a blue zone	A blue zone
	A blue zone
_______	_______
	A blue zone
_______	_______
α-Terpineol: a blue zone	A blue zone (α-terpineol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density (*2.2.5*)

0.848 to 0.855.

Refractive index (*2.2.6*)

1.474 to 1.478.

Optical rotation (*2.2.7*)

+ 64 ° to + 75 °.

Fatty oils and resinified essential oils (*2.8.7*)

It complies with the test.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dilute 0.20 g of the substance to be examined to 10.0 ml with *heptane R*.

Reference solution (a) Dilute 5 µl of α-*pinene R*, 5 µl of *sabinene R*, 5 µl of β-*pinene R*, 5 µl of β-*myrcene R*, 5 µl of *p-cymene R*, 70 µl of *limonene R*, 20 µl of γ-*terpinene R* and 5 µl of *methyl N-methylanthranilate R* to 5.0 ml with *heptane R*.

Reference solution (b) Dissolve 5 µl of *limonene R* in 50 ml of *heptane R*. Dilute 0.5 ml of this solution to 5.0 ml with *heptane R*.

Column:

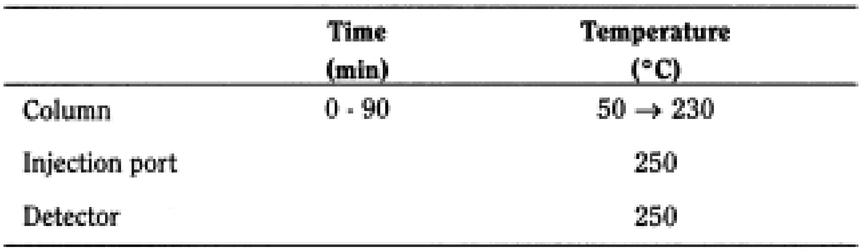
- *material*: fused silica;
- *size:* l = 60 m, Ø = 0.25 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.4 ml/min.

Split ratio 1:70.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 90	50 → 230
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Elution order Order indicated in the composition of reference solution (a); record the retention times of these substances.

System suitability Reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to sabinene and β-pinene and minimum 1.5 between the peaks due to *p*-cymene and limonene.

Identification of components Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution. Disregard the peak due to heptane.

Determine the percentage content of each of these components. The limits are within the following ranges:

- α-*pinene*: 1.6 per cent to 3.0 per cent;
- *sabinene*: less than 0.3 per cent;
- β-*pinene*: 1.2 per cent to 2.0 per cent;
- β-*myrcene*: 1.5 per cent to 2.0 per cent;
- *p-cymene*: less than 1.0 per cent;
- *limonene*: 65.0 per cent to 75.0 per cent;
- γ-*terpinene*: 16.0 per cent to 22.0 per cent;
- *methyl N-methylanthranilate*: 0.30 per cent to 0.60 per cent;
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (b).

Residue on evaporation (*2.8.9*)

1.6 per cent to 4.0 per cent, determined after heating on a water-bath for 4 h.

STORAGE

In a well-filled, airtight container , protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Marshmallow Leaf

General Notices

(Ph Eur monograph 1856)

Ph Eur

DEFINITION

Whole or cut dried leaf of *Althaea officinalis* L.

IDENTIFICATION

A. The leaves have long petioles and are about 7-10 cm long; the lamina is cordate or ovate with 3-5 shallow lobes and crenate or dentate margins; the venation is palmate. The petioles and both surfaces of the lamina are greyish-green and densely pubescent. Rarely, fragments of the inflorescence or immature fruits may be present.

B. Reduce to a powder (355) (*2.9.12*). The powder is greyish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: numerous long, rigid, unicellular covering trichomes with thick walls, pointed at the apex, angular and pitted at the base where they are sometimes still united to form stellate structures with up to 8 components; few secretory trichomes with unicellular stalks and globular, multicellular heads; fragments of the leaf epidermis with anomocytic or paracytic stomata (*2.8.3*); cluster crystals of calcium oxalate, isolated or in the parenchyma of the mesophyll; fragments of veins with small, spiral or annular vessels. Examine under a microscope using *ruthenium red solution R*. The powder shows groups of parenchyma containing mucilage which stains orange-red.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methanol R*. Heat in a water-bath under a reflux condenser for 5 min. Cool and filter. Distil the filtrate under reduced pressure until the total volume is about 2 ml.

Reference solution Dissolve 2.5 mg of *quercitrin R* and 2.5 mg of *chlorogenic acid R* in 10 ml of *methanol R*.

Plate TLC silica gel plate R.

Mobile phase anhydrous formic acid R, *glacial acetic acid R*, *water R, ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application 10 µl, as bands.

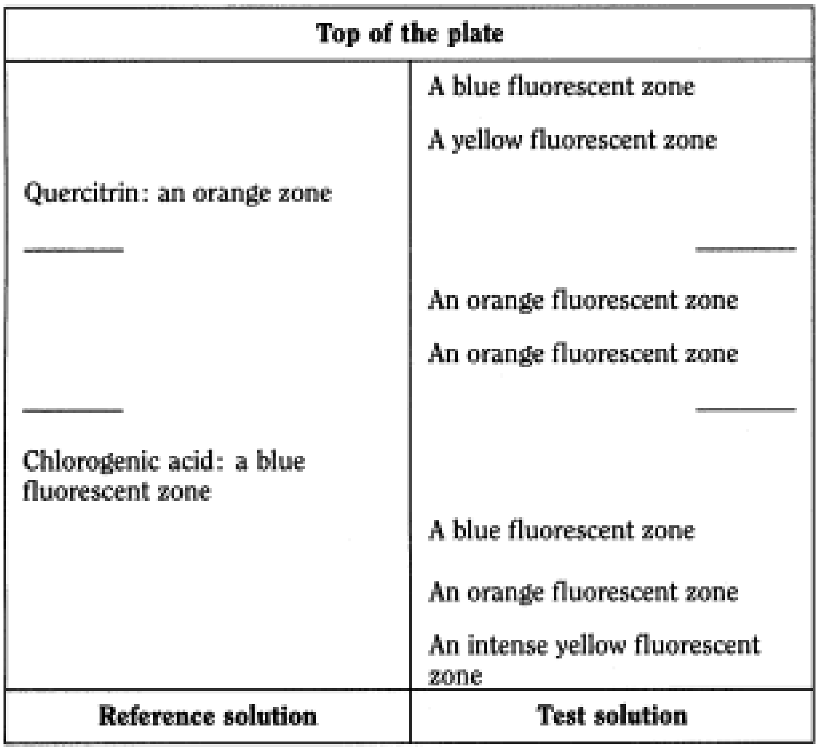
Development Over a path of 15 cm.

Drying At 100–105 °C.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow to dry in air for 30 min.

Examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A blue fluorescent zone
	A yellow fluorescent zone
Quercitrin: an orange zone	
———	———
	An orange fluorescent zone
	An orange fluorescent zone
———	———
Chlorogenic acid: a blue fluorescent zone	
	A blue fluorescent zone
	An orange fluorescent zone
	An intense yellow fluorescent zone
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 4 per cent of leaves infected by *Puccinia malvacearum*, showing red spots and maximum 2 per cent of other foreign matter.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 18.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 2.0 per cent.

Swelling index (*2.8.4*)

Minimum 12, determined on 0.2 g of the powdered drug (355) (*2.9.12*).

Ph Eur

Marshmallow Root

General Notices

(Ph Eur monograph 1126)

Ph Eur

DEFINITION

Peeled or unpeeled, whole or cut, dried root of *Althaea officinalis* L.

IDENTIFICATION

A. The unpeeled, non-fragmented drug consists of cylindrical, slightly twisted roots, up to 2 cm thick, with deep longitudinal furrows. The outer surface is greyish-brown and bears numerous rootlet scars. The fracture is fibrous externally, rugged and granular internally. The section shows a more or less thick, whitish bark with brownish periderm, separated by the well-marked, brownish cambium from a white xylem. The stratified structure of the bark and the radiate structure of xylem become more distinct when moistened.

The peeled drug has a greyish-white, finely fibrous outer surface. Cork and external cortical parenchyma are absent.

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-grey (unpeeled root) or whitish (peeled root). Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of colourless, mainly unlignified, thick-walled fibres with pointed or split ends; fragments of bordered, pitted or scalariformly thickened vessels; cluster crystals of calcium oxalate about 20-35 µm, mostly 25-30 µm in size; parenchymatous cells containing mucilage; fragments of cork with thin-walled, tabular cells in the unpeeled root. Examine under a microscope using *water R*. The powder shows numerous starch granules about 3-25 µm in size occasionally with a longitudinal hilum. The starch grains are mostly simple, a few being 2-4 compound.

TESTS

Foreign matter (*2.8.2*)

Maximum 2 per cent of brown deteriorated drug.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of powdered drug (710) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 6.0 per cent for the peeled root and maximum 8.0 per cent for the unpeeled root.

Swelling index (*2.8.4*)

Minimum 10, determined on the powdered drug (710) (*2.9.12*).

Ph Eur

Mastic

General Notices

(Ph Eur monograph 1876)

Ph Eur

DEFINITION

Dried resinous exudate obtained from stems and branches of *Pistacia lentiscus* L. var. *latifolius* Coss.

Content

Minimum 10 ml/kg of essential oil (anhydrous drug).

IDENTIFICATION

A. Small light yellow to greenish-yellow, non-uniform, spherical or pyriform, clear or opaque, hard glassy fragments.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1 g of the substance to be examined in 10 ml of *methylene chloride R* and filter after 1-2 min.

Reference solution Dissolve 25 mg of *eugenol R* and 25 mg of *borneol R* in 3 ml of *methylene chloride R*.

Plate TLC silica gel plate R.

Mobile phase light petroleum R, *toluene R* (5:95 *V/V*).

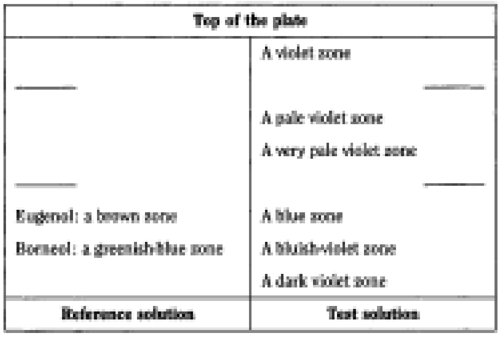
Application 1 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *vanillin reagent R* and heat at 100-105 °C for 5 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones of various colours may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A violet zone
———	———
	A pale violet zone
	A very pale violet zone
———	———
Eugenol: a brown zone	A blue zone
Borneol: a greenish/blue zone	A bluish-violet zone
	A dark violet zone
Reference solution	**Test solution**

TESTS

Acid value (*2.5.1*)

50 to 70, determined on 1.0 g.

Water (*2.2.13*)

Maximum 10 ml/kg, determined on 25.0 g of the drug reduced to a coarse powder (1400) (*2.9.12*).

Total ash (*2.4.16*)

Maximum 0.5 per cent.

ASSAY

Essential oil (*2.8.12*)

Use a 500 ml round-bottomed flask and 200 ml of *water R* as the distillation liquid. Reduce the drug to a coarse powder (1400) (*2.9.12*) and immediately use 20.0 g for the determination. Introduce 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

STORAGE

Do not powder.

Ph Eur

Matricaria Flowers

General Notices

(Matricaria Flower, Ph Eur monograph 0404)

Ph Eur

DEFINITION

Dried capitula of *Matricaria recutita* L. (*Chamomilla recutita* (L.) Rauschert).

Content:

— *blue essential oil*: minimum 4 ml/kg (dried drug);

— *total apigenin 7-glucoside* ($C_{21}H_{20}O_{10}$): minimum 0.25 per cent (dried drug).

IDENTIFICATION

A. Capitula, when spread out, consisting of an involucre made up of many bracts arranged in 1-3 rows; an elongated-conical receptacle, occasionally hemispherical (young capitula); 12-20 marginal ligulate florets with a white ligule; several dozen yellow central tubular florets. The involucre bracts are ovate or lanceolate, with a brownish-grey scarious margin. The receptacle is hollow, without paleae. The corolla of the ligulate florets has a brownish-yellow tube at the base extending to form a white, elongated-oval ligule. The inferior ovary is dark brown, ovoid or spherical, and has a long style and bifid stigma. The tubular florets are yellow and have a five-toothed corolla tube, 5 syngenesious, epipetalous stamens and a gynoecium similar to that of the ligulate florets.

B. Separate the capitulum into its different parts. Examine under a microscope using *chloral hydrate solution R*. The bracts have a margin composed of thin-walled cells and a central region composed of elongated sclereids with occasional stomata (*2.8.3*). The inner epidermis of the corolla of the ligulate florets, in surface view, consisting of thin-walled, polygonal cells, slightly papillose, those of the outer epidermis markedly sinuous and strongly striated; corolla of the tubular florets with longitudinally elongated epidermal cells, and with small groups of papillae near the apex of the lobes. Glandular trichomes each consisting of a short stalk and a head of 2-3 tiers of 2 cells each occur on the outer surfaces of the bracts and on the corollas of both types of florets. The ovaries have a sclerous ring at the base and the wall is composed of vertical bands of thin-walled, longitudinally elongated cells with numerous glandular trichomes, alternating with fusiform groups of small, radially elongated cells containing mucilage. The cells at the apex of the stigmas are extended to form rounded papillae. Numerous small, cluster crystals of calcium oxalate occur in the inner tissues of the ovaries and the anther lobes. Pollen grains spherical to triangular, about 30 µm in diameter with 3 pores and a spiny exine.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dilute 50 µl of essential oil obtained in the assay of essential oil in 1 ml of *xylene R*.

Reference solution Dissolve 2 µl of *chamazulene R*, 5 µl of *(−)-α-bisabolol R* and 10 mg of *bornyl acetate R* in 5 ml of *toluene R*.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

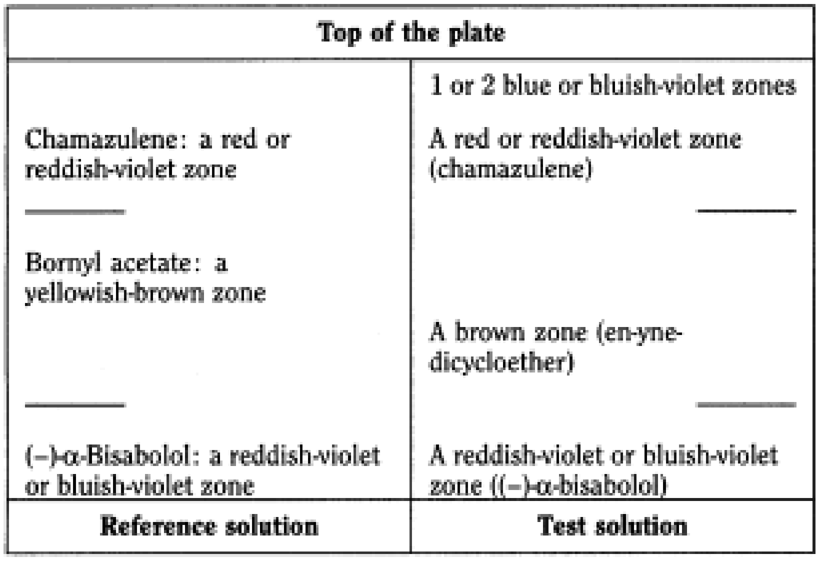
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine immediately in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	1 or 2 blue or bluish-violet zones
Chamazulene: a red or reddish-violet zone	A red or reddish-violet zone (chamazulene)
Bornyl acetate: a yellowish-brown zone	
	A brown zone (en-yne-dicycloether)
(−)-α-Bisabolol: a reddish-violet or bluish-violet zone	A reddish-violet or bluish-violet zone ((−)-α-bisabolol)
Reference solution	**Test solution**

TESTS

Broken drug

Maximum 25 per cent, determined on 20.0 g, passes through a sieve (710) (*2.9.12*).

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 13.0 per cent.

ASSAY

Essential oil (*2.8.12*)

Use 30 g of whole drug, a 1000 ml flask, 300 ml of *water R* as distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 3-4 ml/min for 4 h. Towards the end of this period, stop the flow of water to the condenser assembly but continue distilling until the blue, steam-volatile components have reached the lower end of the condenser. Immediately re-start the flow of water to the condenser assembly to avoid warming the separation space. Stop the distillation after a further 10 min.

Total apigenin 7-glucoside

Liquid chromatography (*2.2.29*).

Test solution Reduce 40 g of the drug to a powder (500) (*2.9.12*). Place 2.00 g of the powdered drug in a 500 ml round-bottomed flask. Add 200 ml of *ethanol (96 per cent) R*. Heat the mixture under a reflux condenser on a water-bath for 15 min. Cool and filter. Rinse the filter and the residue with a few millilitres of *ethanol (96 per cent) R*. To the filtrate add 10 ml of freshly prepared *dilute sodium hydroxide solution R* and heat the mixture under a reflux condenser on a water-bath for about 1 h. Cool. Dilute to 250.0 ml with *ethanol (96 per cent) R*. To 50.0 ml of the solution add 0.5 g of *citric acid R*. Shake for 5 min and filter. Dilute 5.0 ml of this solution to 10.0 ml with the mobile phase (initial mixture).

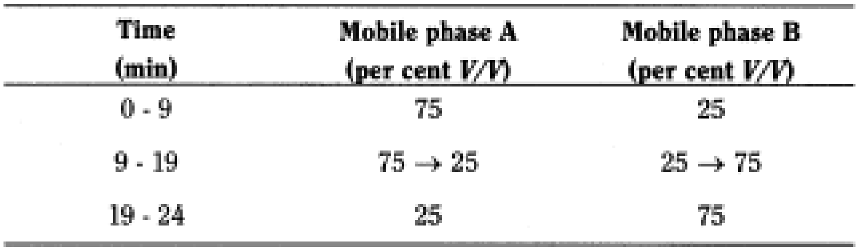
Reference solution (a) Dissolve 10.0 mg of *apigenin 7-glucoside R* in 100.0 ml of *methanol R*. Dilute 25.0 ml of this solution to 200.0 ml with the mobile phase (initial mixture).

Reference solution (b) Dissolve 10.0 mg of *5,7-dihydroxy-4-methylcoumarin R* in 100.0 ml of *methanol R*. Dilute 25.0 ml of this solution to 100.0 ml of the mobile phase (initial mixture). To 4.0 ml of this solution add 4.0 ml of reference solution (a) and dilute to 10.0 ml with the mobile phase (initial mixture).

Precolumn:
- *size: l* = 8 mm, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *phosphoric acid R*, *water R* (0.5:99.5 *V/V*);
- *mobile phase B*: *phosphoric acid R, acetonitrile R* (0.5:99.5 *V/V*).



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 9	75	25
9 - 19	75 → 25	25 → 75
19 - 24	25	75

Flow rate 1 ml/min.

Detection Spectrophotometer at 340 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 1.8 between the peaks due to apigenin 7-glucoside and 5,7-dihydroxy-4-methylcoumarin.

Calculate the percentage content of total apigenin 7-glucoside using the following expression:

$$\frac{A_1 \times m_2}{A_2 \times m_1} \times P \times 0.625$$

A_1 = area of the peak due to apigenin 7-glucoside in the chromatogram obtained with the test solution;

A_2 = area of the peak due to apigenin 7-glucoside in the chromatogram obtained with the reference solution;

m_1 = mass of the drug in the test solution, in grams;

m_2 = mass of *apigenin 7-glucoside R* in reference solution (a), in grams;

P = percentage content of apigenin 7-glucoside in the reagent.

Ph Eur

Matricaria Liquid Extract

General Notices

(Ph Eur monograph 1544)

Ph Eur

DEFINITION

Liquid extract produced from *Matricaria flower (0404)*.

Content

Minimum 0.30 per cent of blue residual oil.

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure for liquid extracts using a mixture of 2.5 volumes of a 10 per cent *m/m* solution of ammonia (NH_3), 47.5 volumes of water and 50 volumes of ethanol (96 per cent).

CHARACTERS

Appearance

Brownish, clear liquid.

Intense characteristic odour and characteristic bitter taste.

Solubility

Miscible with water and with ethanol (96 per cent) with development of turbidity, soluble in ethanol (50 per cent *V/V*).

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution Place 10 ml of the extract to be examined in a separating funnel and shake with 2 quantities, each of 10 ml, of *pentane R*. Combine the pentane layers, dry over 2 g of *anhydrous sodium sulphate R* and filter. Evaporate the filtrate to dryness on a water-bath and dissolve the residue in 0.5 ml of *toluene R*.

Reference solution Dissolve 4 mg of *guaiazulene R*, 20 mg of *(-)-α-bisabolol R* and 20 mg of *bornyl acetate R* in 10 ml of *toluene R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

Application 10 µl as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatogram obtained with the test solution shows several quenching zones, of which 2 main zones are in the middle third (en-yne-dicycloether).

Detection B Examine in ultraviolet light at 365 nm.

Results B The chromatogram obtained with the test solution shows in the middle part an intense blue fluorescent zone (herniarin).

Detection C Spray with *anisaldehyde solution R* and examine in daylight while heating at 100-105 °C for 5-10 min.

Results C The chromatogram obtained with the reference solution shows in the lower third a reddish-violet or bluish-violet zone ((-)-α-bisabolol), in the middle third a yellowish-brown or greyish-green zone (bornyl acetate) and in the upper third a red or reddish-violet zone (guaiazulene). The chromatogram obtained with the test solution shows in the lower third yellowish-brown or greenish-yellow and violet zones and a reddish-violet or bluish-violet zone due to (-)-α-bisabolol in the chromatogram obtained with the reference solution; a brownish zone (en-yne-dicycloether) similar in position to the zone due to bornyl acetate in the chromatogram obtained with the reference solution; a red or reddish-violet zone (chamazulene) corresponding to guaiazulene in the chromatogram obtained with the reference solution and immediately above it 1 or 2 blue or bluish-violet zones; further weak zones may be present in the chromatogram obtained with the test solution.

B. Thin-layer chromatography *(2.2.27)*.

Test solution The extract to be examined.

Reference solution Dissolve 1.0 mg of *chlorogenic acid R*, 2.5 mg of *hyperoside R* and 2.5 mg of *rutin R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R*, *glacial acetic acid R*, *water R*, *ethyl acetate R* (7.5:7.5:18:67 *V/V/V/V*).

Application 10 µl as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*; subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow to dry in air for about 30 min and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the middle part a light blue fluorescent zone (chlorogenic acid), below it a yellowish-brown fluorescent zone (rutin) and above it a yellowish-brown fluorescent zone (hyperoside). The chromatogram obtained with the test solution shows a yellowish-brown fluorescent zone corresponding to the zone of rutin in the chromatogram obtained with the reference solution, a light blue fluorescent zone corresponding to the zone of chlorogenic acid in the chromatogram obtained with the reference solution, a yellowish-brown fluorescent zone similar in position to the zone of hyperoside in the chromatogram obtained with the reference solution; it also shows above the yellowish-brown fluorescent zone a green fluorescent zone, then several bluish or greenish fluorescent zones and near the solvent front a yellowish fluorescent zone.

TESTS

Ethanol *(2.9.10)*

38 per cent *V/V* to 53 per cent *V/V*.

Dry residue *(2.8.16)*

Minimum 12.0 per cent.

ASSAY

Place 20.0 g in a 1000 ml round-bottomed flask, add 300 ml of *water R* and distil until 200 ml has been collected in a flask. Transfer the distillate into a separating funnel. Dissolve 65 g of *sodium chloride R* in the distillate and shake with 3 quantities, each of 30 ml, of *pentane R* previously used to rinse the reflux condenser and the flask. Combine the pentane layers, dry over 2 g of *anhydrous sodium sulphate R* and filter into a tared 100 ml round-bottomed flask which has been dried in a desiccator for 3 h. Rinse the anhydrous sodium sulphate and the filter with 2 quantities, each of 20 ml, of *pentane R*. Evaporate the pentane in a water-bath at 45 °C. The residue of pentane is eliminated in a current of air for 3 min. Dry the flask in a desiccator for 3 h and weigh. The residual oil is blue (chamazulene).

Ph Eur

Matricaria Oil

General Notices

(Ph Eur monograph 1836)

Ph Eur

DEFINITION

Blue essential oil obtained by steam distillation from the fresh or dried flower-heads or flowering tops of *Matricaria recutita* L. (*Chamomilla recutita* L. Rauschert). There are 2 types of matricaria oil which are characterised as rich in bisabolol oxides, or rich in (-)-α-bisabolol.

CHARACTERS

Appearance

Clear, intensely blue, viscous liquid.

It has an intense characteristic odour.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 µl of the substance to be examined in 1.0 ml of *toluene R*.

Reference solution Dissolve 2 mg of *guaiazulene R*, 5 µl of *(-)-α-bisabolol R* and 10 mg of *bornyl acetate R* in 5.0 ml of *toluene R*.

Plate *TLC silica gel plate R*.

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

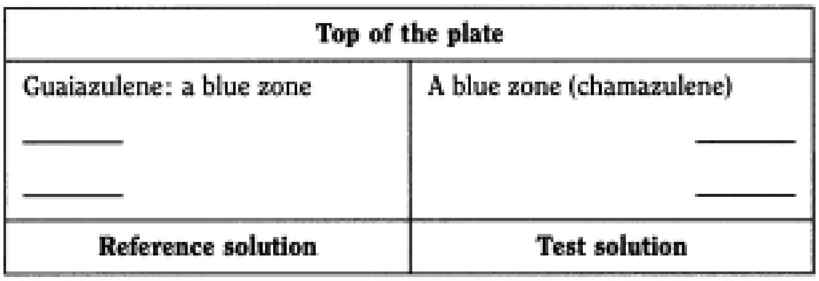
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in daylight.

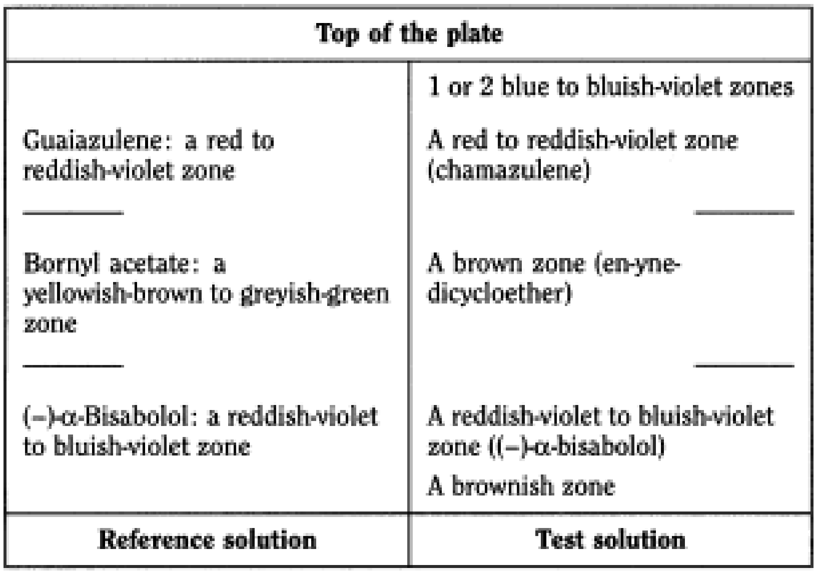
Results A See below for the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
Guaiazulene: a blue zone	A blue zone (chamazulene)
_______	_______
_______	_______
Reference solution	**Test solution**

Detection B Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine immediately in daylight.

Results B See below for the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, yellowish-brown to greenish-yellow zones (lower third), violet zones (lower third) and further weak zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	1 or 2 blue to bluish-violet zones
Guaiazulene: a red to reddish-violet zone	A red to reddish-violet zone (chamazulene)
_______	_______
Bornyl acetate: a yellowish-brown to greyish-green zone	A brown zone (en-yne-dicycloether)
_______	_______
(−)-α-Bisabolol: a reddish-violet to bluish-violet zone	A reddish-violet to bluish-violet zone ((−)-α-bisabolol) A brownish zone
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks due to (-)-α-bisabolol and chamazulene in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

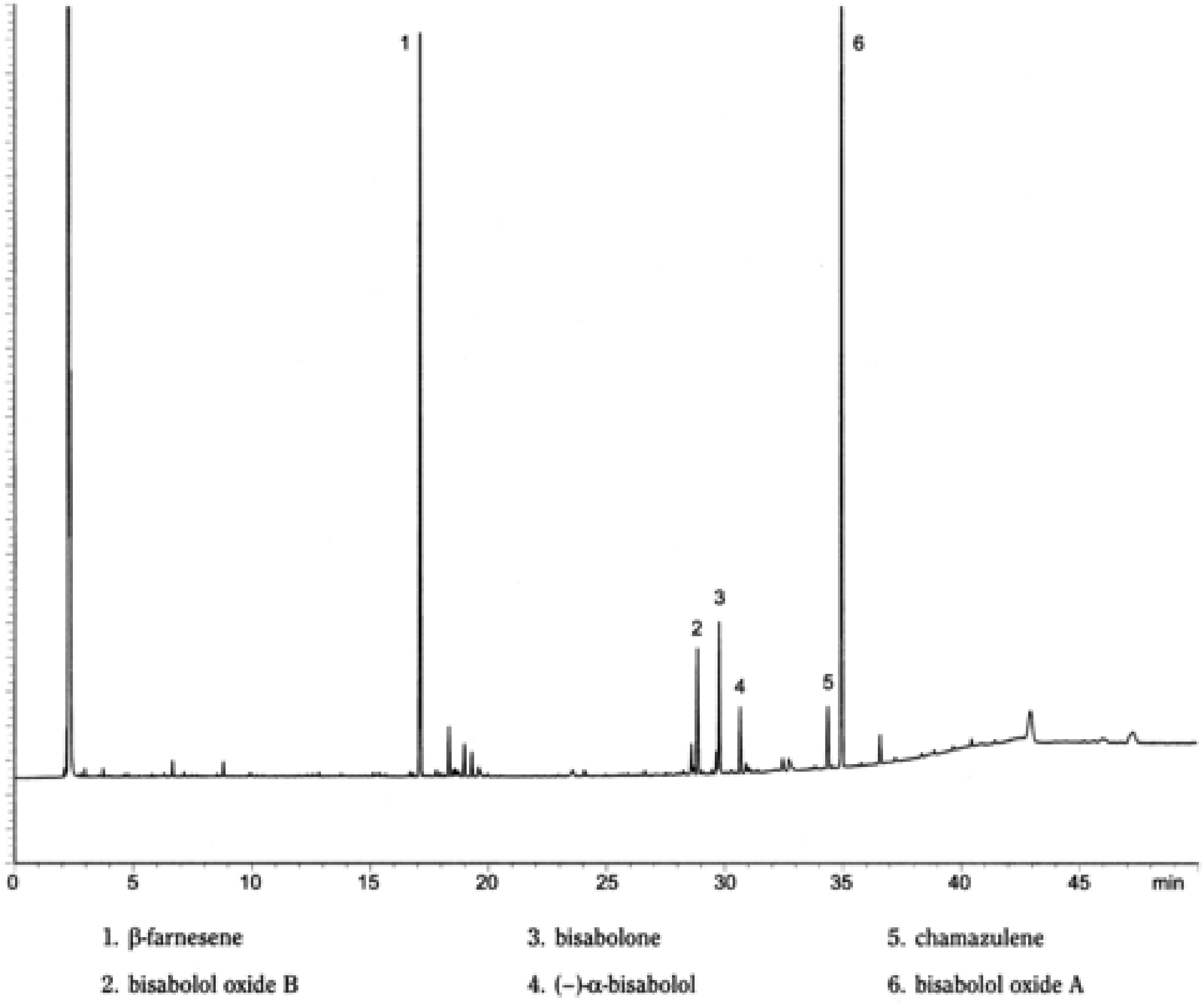
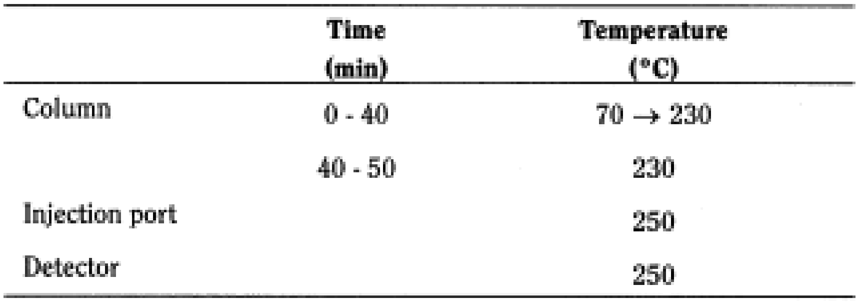


Figure 1836.-1. − *Chromatogram of matricaria oil rich in bisabolol oxides*



	Time (min)	Temperature (°C)
Column	0 - 40	70 → 230
	40 - 50	230
Injection port		250
Detector		250

Test solution Dissolve 20 µl of the oil to be examined in *cyclohexane R* and dilute to 5.0 ml with the same solvent.

Reference solution Dissolve 20 µl of *(-)-α-bisabolol R*, 5 mg of *chamazulene R* and 6 mg of *guaiazulene R* in *cyclohexane R* and dilute to 5.0 ml with the same solvent.

Column:
— *material*: fused silica;

— *size*: *l* = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.25-0.53 mm, when using a column longer than 30 m, an adjustment of the temperature programme may be necessary;

— *stationary phase*: *macrogol 20 000 R*.

Carrier gas *helium for chromatography R*.

Flow rate 1-2 ml/min.

Split ratio 1:100.

Temperature:

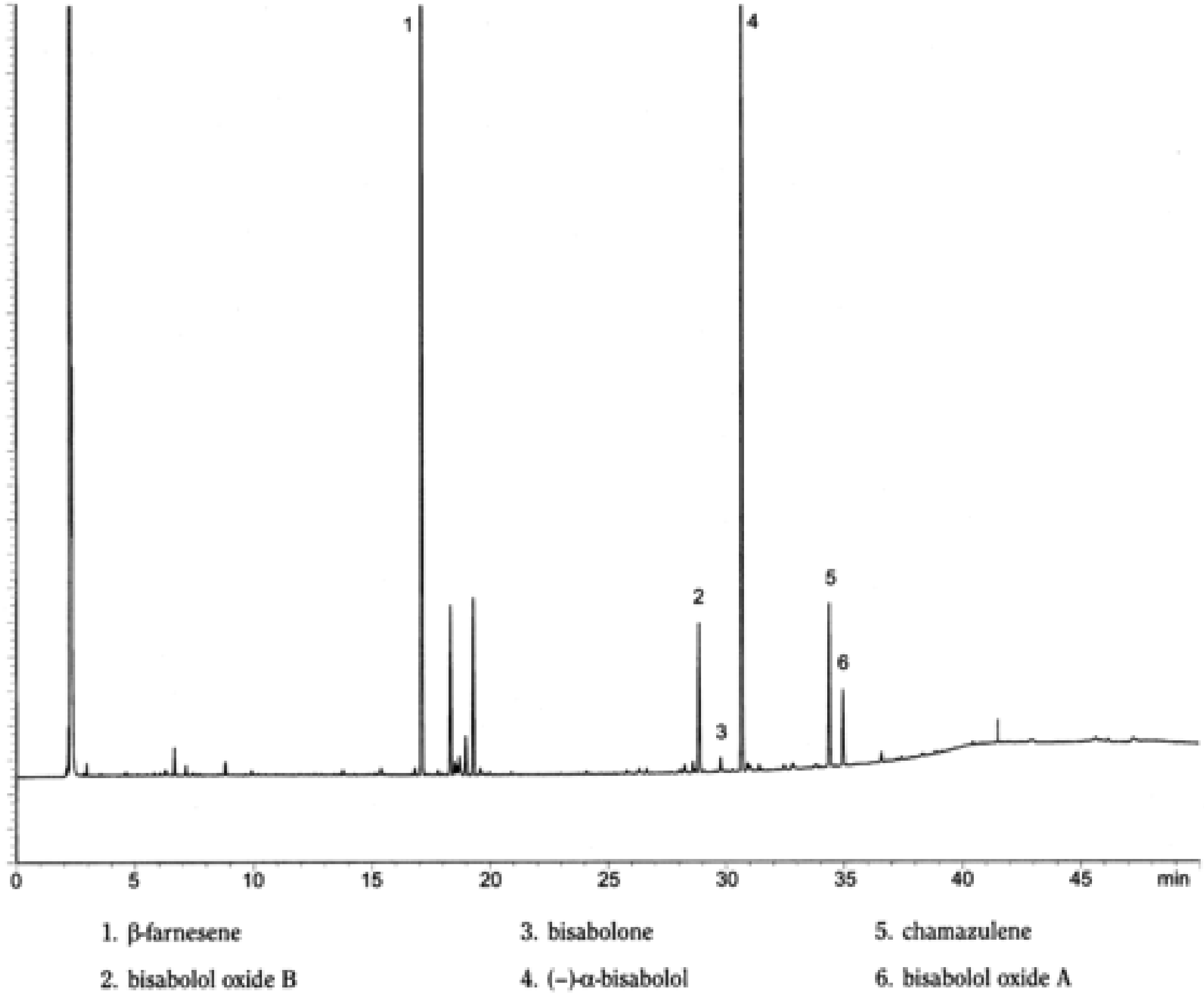


Figure 1836.-2. – *Chromatogram of matricaria oil rich in (−)-α-bisabolol*

Detection Flame ionisation.

Injection 1.0 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

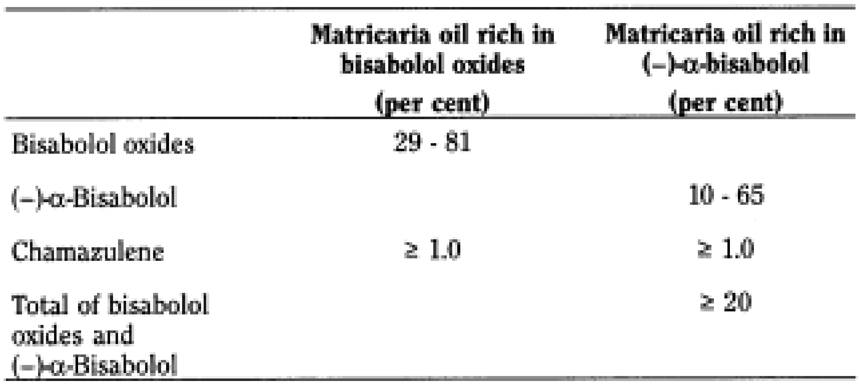
Relative retention With reference to chamazulene (retention time = about 34.4 min): βfarnesene = about 0.5; bisabolol oxide B = about 0.8; bisabolone = about 0.87; (-)-α-bisabolol = about 0.9; bisabolol oxide A = about 1.02.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to chamazulene and guaiazulene.

Using the retention times determined from the chromatogram obtained with the reference solution, locate (-)-α-bisabolol and chamazulene in the chromatogram obtained with the test solution; locate bisabolol oxides (bisabolol oxide B, bisabolone and bisabolol oxide A) using Figures 1836.-1 and 1836.-2 (disregard the peak due to cyclohexane). The chromatogram obtained with the test solution does not show a peak with the retention time of guaiazulene.

Determine the percentage content of the components. The limits are within the following ranges.



	Matricaria oil rich in bisabolol oxides (per cent)	**Matricaria oil rich in (–)-α-bisabolol (per cent)**
Bisabolol oxides	29 - 81	
(–)-α-Bisabolol		10 - 65
Chamazulene	≥ 1.0	≥ 1.0
Total of bisabolol oxides and (–)-α-Bisabolol		≥ 20

STORAGE

In a well-filled, airtight container, protected from light at a temperature not exceeding 25 °C.

LABELLING

The label states the type of matricaria oil (rich in bisabolol oxides or rich in (-)-α-bisabolol.

Ph Eur

Meadowsweet

General Notices

(Ph Eur monograph 1868)

Ph Eur

DEFINITION

Whole or cut, dried flowering tops of *Filipendula ulmaria* (L.) Maxim. (= *Spiraea ulmaria* L.).

Content

Minimum 1 ml/kg of steam-volatile substances (dried drug).

CHARACTERS

Aromatic odour of methyl salicylate, after crushing.

IDENTIFICATION

A. The stem, up to 5 mm in diameter, is greenish-brown, stiff, angular, hollow except at the apex, and has regular, straight, longitudinal furrows. The petiolate leaf, compound imparipinnate, has 2 reddish-brown angular stipules. It consists of 3 to 9 pairs of leaflets, unevenly dentate, some of which are small and fan-shaped. The leaflets are dark green and glabrous on the upper surface, tomentose and lighter, sometimes silvery on the lower surface. The terminal leaflet, the largest, is divided into 3 segments. The veins are prominent and brown on the lower surface. The inflorescence is complex and composed of very numerous flowers arranged in irregular cymose panicles. The flowers are creamish-white and about 3 mm to 6 mm in diameter; the calyx consists of 5 dark green, reflexed and hairy sepals fused at the base to a concave receptacle; the 5 free petals, which are readily detached, are pale yellow, obovate and distinctly narrowed at the base; the stamens are numerous with rounded anthers and they extend beyond the petals; the gynoecium consists of about 4 to 6 carpels, each with a short style and a globular stigma; the carpels become twisted together spirally to form yellowish-brown fruits with a helicoidal twist. Unopened flower buds are frequently present. If the fruit is present, it has a helicoidal twist and contains brownish seeds.

B. Reduce to a powder (355) (*2.9.12*). The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows unicellular covering trichomes, some thin-walled, very long and flexuous, with pointed ends, others shorter, thick-walled, conical and thickened at the base; occasional clavate glandular trichomes with a 1- to 3-celled, uniseriate stalk and a multicellular head containing dense brown contents; fragments of the leaves and sepals with sinuous to wavy epidermal cells, anomocytic stomata (*2.8.3*) on the lower surface only and cluster crystals of calcium oxalate in the mesophyll; thin-walled epidermal cells of the petals, some showing rounded papillae; numerous spherical pollen grains with 3 pores and a faintly pitted exine; fragments of the fibrous layer of the anthers with stellate thickenings; groups of small-celled parenchyma from the ovaries containing prism crystals of calcium oxalate; fragments of vascular tissue with spiral and annular vessels from the leaves and stems.

C. Thin-layer chromatography (*2.2.27*).

Test solution Xylene solution obtained in the assay.

Reference solution Dissolve 0.1 ml of *methyl salicylate R* and 0.1 ml of *salicylaldehyde R* in *xylene R* and dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *hexane R*, *toluene R* (50:50 *V/V*).

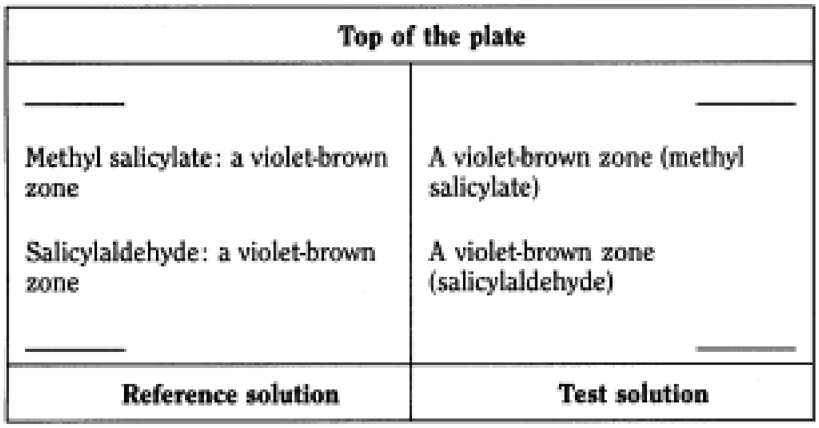
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray the plate with 3 ml of *ferric chloride solution R3* and examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
Methyl salicylate: a violet-brown zone	A violet-brown zone (methyl salicylate)
Salicylaldehyde: a violet-brown zone	A violet-brown zone (salicylaldehyde)
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 5.0 per cent of stems with a diameter greater than 5 mm and maximum 2.0 per cent of foreign matter.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 7.0 per cent.

ASSAY

Examine according to the method described for the determination of essential oils in herbal drugs (*2.8.12*). Use 50.0 g, a 1000 ml flask and 300 ml of *dilute hydrochloric acid R* as distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

Ph Eur

Melilot

General Notices

(Ph Eur monograph 2120)

Ph Eur

DEFINITION

Whole or cut, dried aerial parts of *Melilotus officinalis* (L.) Lam.

Content

Minimum 0.3 per cent of coumarin ($C_9H_6O_2$; M_r 146.1) (dried drug).

IDENTIFICATION

A. The stem is green, cylindrical, glabrous and finely ridged. The leaves are alternate, petiolate and trifoliate with 2 lanceolate stipules; the leaflets are up to about 3 cm long and 20 mm wide, elongated to ovate with a finely dentate margin, acute at the apex and base; the upper surface is dark green and glabrous, the lower surface paler green with short, fine hairs, especially at the base. The inflorescence is racemose with numerous pale yellow flowers, about 7 mm long, each having a hairy calyx with 5 deeply-divided, unequal teeth, and a papilionate corolla. The fruit is an indehiscent pod, often persistent within the calyx, yellowish-brown, short and tapering at the apex; the surface is glabrous and transversely wrinkled.

B. Reduce to a powder (355 *(2.9.12)*). The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the leaf lamina in surface view showing unevenly thickened, slightly sinuous epidermal cells and numerous stomata, mostly anomocytic with 3-6 sudsidiary cells; uniseriate covering trichomes with 2 short, smooth-walled based cells and a long terminal cell, bent at right angles, with a thick wall and a warty cuticle; occasional glandular trichomes with a short, 2-3 celled stalk and an ovoid, biseriate head with 4 indistinct cells; scattered fragments of the petals showing papillae; fragments of vascular tissue from the stems with associated unlignified septate fibres containing prism crystals of calcium oxalate; fibrous layer of the anther; spherical to ovoid pollen grains about 25 µm long with 3 pores and smooth exine.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.3 g of the powdered drug (355) *(2.9.12)* add 3 ml of *methanol R*. Heat on a water-bath at 60 °C for 1 min. Filter.

Reference solution Dissolve 50 mg of *coumarin CRS* and 20 mg of *o-coumaric acid R* in 50 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase Upper phase of the following mixture: *dilute acetic acid R*, *ether R*, *toluene R* (10:50:50 *V/V/V*).

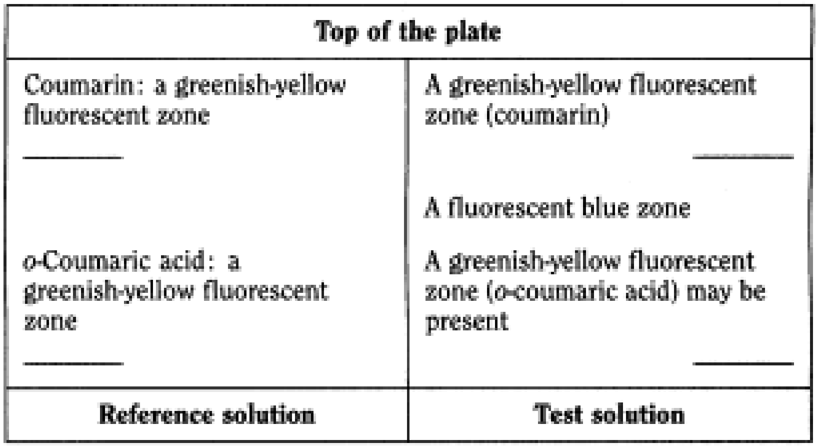
Application 25 µl as bands of 10 mm.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with *2 M alcoholic potassium hydroxide R*. Examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones of various colours may be present in the chromatogram obtained with the test solution.



Top of the plate	
Coumarin: a greenish-yellow fluorescent zone	A greenish-yellow fluorescent zone (coumarin)
	A fluorescent blue zone
o-Coumaric acid: a greenish-yellow fluorescent zone	A greenish-yellow fluorescent zone (*o*-coumaric acid) may be present
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 2 per cent of stems with a diameter greater than 3 mm and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Completely reduce about 50 g to a powder (500) *(2.9.12)*. To 5.00 g of the powdered drug add 90 ml of *methanol R* and boil under a reflux condenser for 30 min. Allow to cool. Filter under vacuum through a fibre-glass filter. Take up the residue and the fragmented filter with 90 ml of *methanol R*. Treat in the same manner as before. Combine the filtrates and dilute to 250.0 ml with *methanol R*.

Reference solution Dissolve 25.0 mg of *coumarin CRS* in *methanol R* and dilute to 250.0 ml with the same solvent.

Column:

— *size:* l = 0.25 m, Ø = 4 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, 5 g/l solution of *phosphoric acid R* (22:78 *V/V*).

Flow rate 1.7 ml/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µl.

System suitability:

— *retention time*: coumarin = about 7.8 min.

Calculate the percentage content of coumarin from the following expression:

$$\frac{A_1 \times m_2}{A_2 \times m_1}$$

A_1 = area of the peak due to coumarin in the chromatogram obtained with the test solution,

A_2 = area of the peak due to coumarin in the chromatogram obtained with reference solution,

m_1 = mass of the drug to be examined, in grams,

m_2 = mass of *coumarin CRS* in the reference solution, in grams.

Ph Eur

Milk-thistle Fruit

General Notices

(Ph Eur monograph 1860)

Ph Eur

DEFINITION

Mature fruit, devoid of the pappus, of *Silybum marianum* L. Gaertner.

Content

Minimum 1.5 per cent of silymarin, expressed as silibinin ($C_{25}H_{22}O_{10}$; M_r 482.4) (dried drug).

CHARACTERS

No rancid odour.

IDENTIFICATION

A. The achene is strongly compressed, elongate-obovate, about 6-8 mm long, 3 mm broad and 1.5 mm thick; the outer surface is smooth and shiny with a grey or pale brown ground colour variably streaked dark brown longitudinally to give an overall pale greyish or brown colour; the fruit is tapering at the base and crowned at the apex with a glistening, pale yellow extension forming a collar about 1 mm high surrounding the remains of the style. Cut transversely, the fruit shows a narrow, brown outer area and 2 large, dense, white oily cotyledons.

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-yellow with darker specks. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the epicarp composed of colourless cells, polygonal in surface view, the lumen appearing fairly large or as a small slit, depending on the orientation; groups of parenchymatous cells from the pigment layer, some of them containing colouring matter which appears bright red; very abundant groups of large sclereids from the testa with bright yellow pitted walls and a narrow lumen; occasionally fragments of small-celled parenchyma with pitted and beaded walls; abundant thin-walled parenchymatous cells from the cotyledons containing oil globules and scattered cluster crystals of calcium oxalate; a few larger, prismatic crystals of calcium oxalate.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of powdered drug (500) (*2.9.12*) add 10 ml of *methanol R*. Heat under reflux in a water-bath at 70 °C for 5 min. Cool and filter. Evaporate the filtrate to dryness and dissolve the residue in 1.0 ml of *methanol R*.

Reference solution Dissolve 2 mg of *silibinin R* and 5 mg of *taxifolin R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R*, *acetone R*, *methylene chloride R* (8.5:16.5:75 *V/V/V*) .

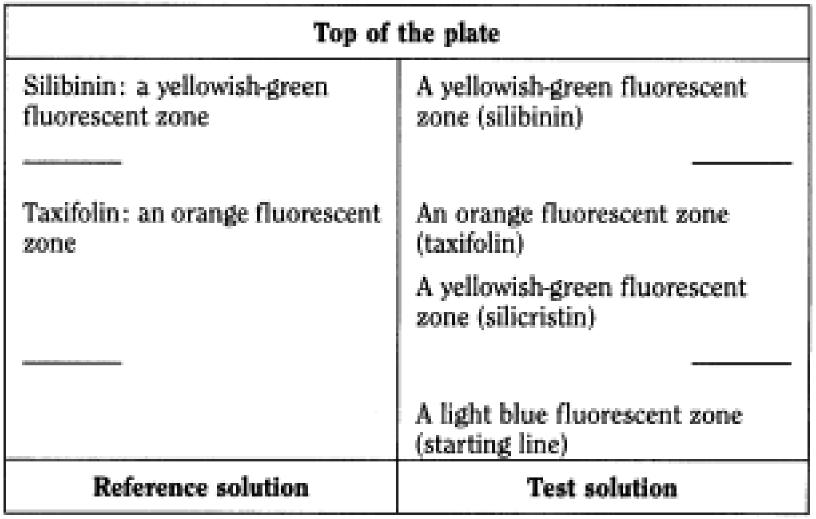
Application 30 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow to dry for 30 min and examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other orange and yellowish-green fluorescent zones are present between the zones of silibinin and taxifolin in the chromatogram obtained with the test solution.



Top of the plate	
Silibinin: a yellowish-green fluorescent zone	A yellowish-green fluorescent zone (silibinin)
———	———
Taxifolin: an orange fluorescent zone	An orange fluorescent zone (taxifolin) A yellowish-green fluorescent zone (silicristin)
———	———
	A light blue fluorescent zone (starting line)
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*)

Maximum 8.0 per cent, determined on 1.000 g of the powdered drug (500) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 8.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Place 5.00 g of the powdered drug (500) (*2.9.12*) in a continuous-extraction apparatus. Add 100 ml of *light petroleum R* and heat in a water-bath for 8 h. Allow the defatted drug to dry at room temperature. In a continuous-extraction apparatus, extract the latter with 100 ml of *methanol R* in a water-bath for 5 h. Evaporate the methanolic extract *in vacuo* to a volume of about 30 ml. Filter into a 50 ml volumetric flask, rinsing the extraction flask and the filter, and diluting to 50.0 ml with *methanol R*. Dilute 5.0 ml of this solution to

50.0 ml with *methanol R*.

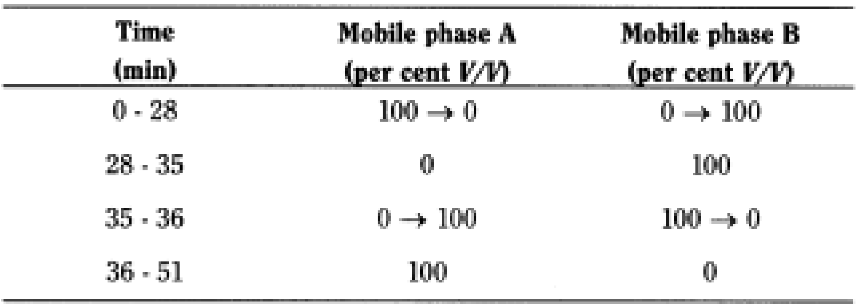
Reference solution Dissolve a quantity of *milk thistle standardised dry extract CRS* equivalent to 5.0 mg of silibinin (m_1 g) in *methanol R* and dilute to 50.0 ml with the same solvent.

Column:

- *size*: *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: *phosphoric acid R*; *methanol R*, *water R* (0.5:35:65 *V/V/V*);
- *mobile phase B*: *phosphoric acid R*; *methanol R*, *water R* (0.5:50:50 *V/V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 28	100 → 0	0 → 100
28 - 35	0	100
35 - 36	0 → 100	100 → 0
36 - 51	100	0

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 288 nm.

Injection 10 µl.

Retention time Silibinin B = about 30 min. If necessary, adjust the time periods of the gradient.

System suitability Reference solution:

- *resolution*: minimum 1.8 between the peaks due to silibinin A and silibinin B;
- the chromatogram obtained is similar to the chromatogram supplied with *milk thistle standardised dry extract CRS*.

Locate silicristin, silidianin, silibinin A, silibinin B, isosilibinin A and isosilibinin B by comparison with the chromatogram supplied with *milk thistle standardised dry extract CRS*. In the chromatogram obtained with the test solution the peak due to silidianin may vary in size, be absent or be present as the principal peak. Determine the area of the peaks due to silicristin, silidianin, silibinin A, silibinin B, isosilibinin A and isosilibinin B.

Calculate the percentage content of silymarin, calculated as silibinin, using the following expression:

$$\frac{(A1 + A2 + A3 + A4 + A5 + A6) \times m_1 \times p \times 1000}{(A7 + A8) \times m_2 \times (100 - d)}$$

A1 area of the peak due to silicristin in the chromatogram obtained with the test solution,

A2 = area of the peak due to silidianin in the chromatogram obtained with the test solution,

A3 = area of the peak due to silibinin A in the chromatogram obtained with the test solution,

A4 = area of the peak due to silibinin B in the chromatogram obtained with the test solution,

A5 = area of the peak due to isosilibinin A in the chromatogram obtained with the test solution,

A6 = area of the peak due to isosilibinin B in the chromatogram obtained with the test solution,

A7 = area of the peak due to silibinin A in the chromatogram obtained with the reference solution,

A8 = area of the peak due to silibinin B in the chromatogram obtained with the reference solution,

m_1 = mass of *milk thistle standardised dry extract CRS* used to prepare the reference solution, in grams,

m_2 = mass of the drug to be examined, in grams,

p = combined percentage content of silibinin A and silibinin B in *milk thistle standardised dry extract CRS,*

d = percentage loss on drying of the drug.

Ph Eur

Motherwort

General Notices

(Ph Eur monograph 1833)

Ph Eur

DEFINITION

Whole or cut, dried flowering aerial parts of *Leonurus cardiaca* L.

Content

Minimum 0.2 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The stem pieces are hairy, longitudinally striated, quadrangular, hollow, and up to about 10 mm wide; they bear opposite and decussate, petiolate leaves and, in the axils of the upper leaves, about 6 to 12 small flowers, arranged in sessile whorls forming a long leafy spike. The lower leaves are ovate-orbicular, palmately 3 to 5-lobed, rarely 7-lobed, the lobes irregularly dentate. The upper leaves are entire or slightly trifid, lanceolate with a serrate margin and cuneate at the base. The upper surface of the leaves is green with scattered hairs, the lower surface is paler green, densely pubescent and shows a prominent palmate and reticulate venation. The flowers have a funnel-shaped calyx, 3 mm to 5 mm long with 5 stiff, recurved teeth; the corolla is 2-lipped, the upper lip pink and pubescent on the outer surface, the lower lip white with purplish spots; stamens 4, densely pubescent.

B. Reduce to a powder (355) (*2.9.12*). The powder is green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the leaf lamina with a one-layered palisade mesophyll reaching almost to the centre and a loosely arranged spongy parenchyma; fragments of leaf epidermis; upper epidermal cells with straight anticlinal walls and a striated cuticle; lower epidermal cells with sinuous anticlinal walls; stomata of the diacytic type (*2.8.3*) more numerous on the lower surface; glandular trichomes with a short unicellular stalk and a globular head composed of 8, sometimes up to 16 cells or with a unicellular head; covering trichomes conical, uniseriate, up to about 300 µm long, occasionally up to 1500 µm, composed of from 2 to 8 cells with slight swellings at the junctions and a warty or striated cuticle; fragments of the calyx containing small, cluster crystals of calcium oxalate; spherical pollen grains, about 25 µm to 30 µm in diameter, with 3 pores and 3 furrows and a smooth exine; thick-walled, lignified fibres and spirally and annularly thickened vessels from the stem; occasional brown fragments of pericarp with single crystals of calcium oxalate.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 0.5 g of the powdered drug (355) (*2.9.12*) add 5 ml of *methanol R*. Heat in a water-bath at 65 °C for 5 min with shaking. Cool and filter.

Reference solution Dissolve 5 mg of *naphthol yellow S R* and 2.0 mg of *catalpol R* in 5.0 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R*, *water R*, *ethyl acetate R* (20:20:60 *V/V/V*).

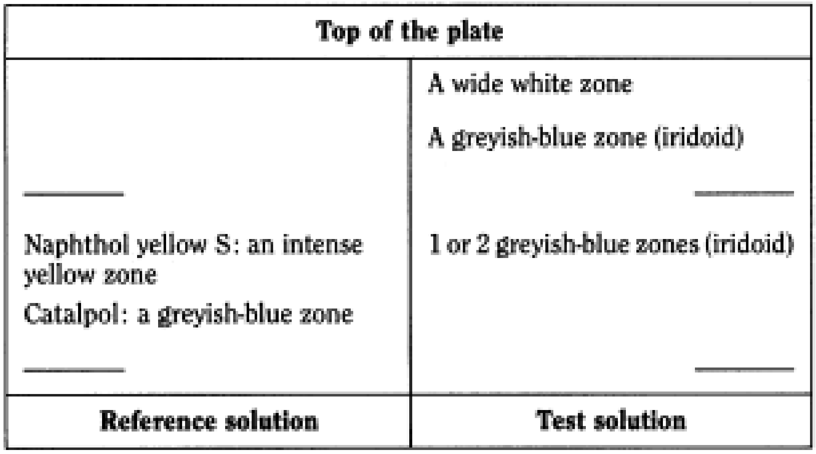
Application 20 µl as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *dimethylaminobenzaldehyde solution R2*, using about 5 ml for a plate 200 mm square; heat at 100-105 °C for 10 min until the spots appear; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, further weak greyish-blue zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A wide white zone A greyish-blue zone (iridoid)
———	———
Naphthol yellow S: an intense yellow zone Catalpol: a greyish-blue zone	1 or 2 greyish-blue zones (iridoid)
———	———
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 2 per cent of brown or yellow leaves and maximum 2 per cent of other foreign matter.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 12.0 per cent.

ASSAY

Stock solution In a 100 ml round-bottomed flask, place 1.00 g of the powdered drug (355) (*2.9.12*), add 1 ml of a 5 g/l solution of *hexamethylenetetramine R*, 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1*. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R*, each time boiling under a reflux condenser for 10 min. Allow to cool, filter each extract through the plug of absorbent cotton into the flask. After cooling, filter the combined acetone extracts through a paper filter into a volumetric flask, dilute to 100.0 ml with *acetone R* by rinsing the flask and the paper filter. Introduce 20.0 ml of the solution into a separating funnel, add 20 ml of *water R* and shake the mixture with 1 quantity of 15 ml and then with 3 quantities, each of 10 ml, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, wash with 2 quantities, each of 50 ml, of *water R*, and filter the extracts over 10 g of *anhydrous sodium sulphate R* into a volumetric flask and dilute to 50.0 ml with *ethyl acetate R*.

Test solution To 10.0 ml of the stock solution add 1 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation liquid Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Measure the absorbance (*2.2.25*) of the test solution after 30 min, by comparison with the compensation liquid at 425 nm. Calculate the percentage content of flavonoids, calculated as hyperoside, from the expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of hyperoside to be 500.

A = absorbance at 425 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Mullein Flower

General Notices

(Ph Eur monograph 1853)

Ph Eur

DEFINITION

Dried flower, reduced to the corolla and the androecium, of *Verbascum thapsus* L., V. *densiflorum Bertol*. (*V. thapsiforme* Schrad), and *V. phlomoides* L.

IDENTIFICATION

A. The corolla of *V. thapsus* is about 20 mm in diameter, pale yellow, yellow to brown, funnel-shaped with 5 slightly unequal and spreading lobes. The corolla lobes are densely hairy on the outer surface, glabrous on the inner surface, with a fine network of light brown veins. There are 5 stamens, alternating with the petal lobes, 2 of these are long, with glabrous filaments, the other 3 shorter, with densely tormentose filaments. The anthers are attached transversely. In *V. phlomoides* the corolla is up to about 30 mm in diameter, bright yellow to orange, and the anthers are obliquely attached to the filaments. The corolla of *V. densiflorum*, about 30 mm in diameter, is almost flat and deeply divided into 5 slightly unequal lobes, with rounded apices.

B. Reduce to a powder (355) (*2.9.12*). The powder is yellow or yellowish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows many covering trichomes from the corolla, whole and fragmented; there are pluricellular, of the candelabra type with a central uniseriate axis from which whorls of branch cells arise at the position of the cross walls and at the apex. The covering trichomes from the stamen filaments are unicellular, long, thin-walled and tubular, sometimes with a club-shaped tip; they have a distinctly granular or striated surface. Numerous pollen grains, ovoid with a finely granular exine with 3 pores. Fragments of the fibrous layer of the anther with thickened walls giving a characteristic star-shaped appearance. Yellow fragments of the petals in the surface view, the epidermal cells polygonal and isodiametric; fragments of the mesophyll consisting of irregular parenchymatous cells and scattered spiral vessels.

C. Thin-layer chromatography (*2.2.27*).

Test solution Heat 1.0 g of the powdered drug (355) (*2.9.12*) in 10 ml of *methanol R* in a water-bath at 60 °C for 5 min, with stirring. Cool and filter.

Reference solution Dissolve 1 mg of *caffeic acid R*, 2.5 mg of *hyperoside R* and 2.5 mg of *rutin R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *water R*, *anhydrous formic acid R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

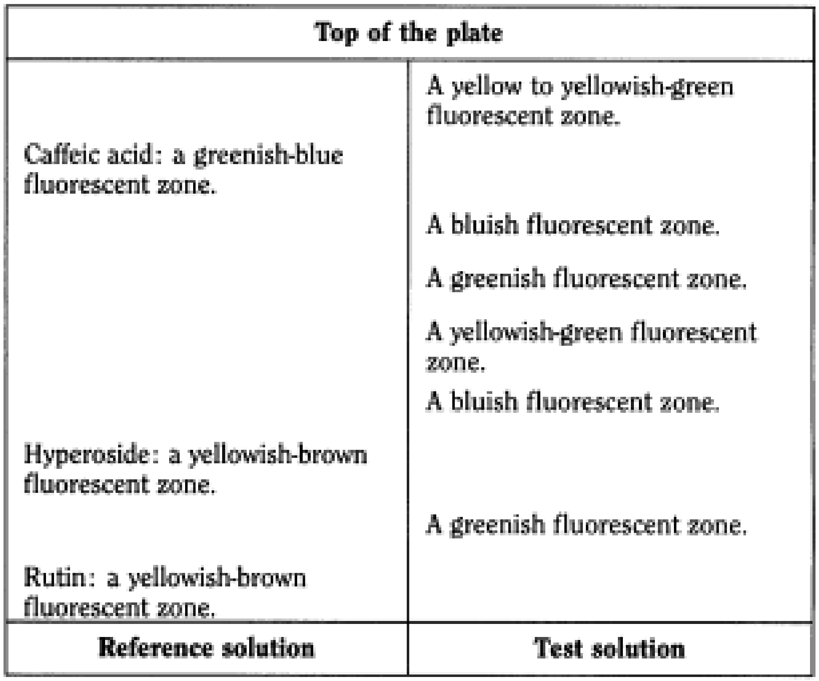
Application 10 µl of the reference solution and 30 µl of the test solution, as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A yellow to yellowish-green fluorescent zone.
Caffeic acid: a greenish-blue fluorescent zone.	
	A bluish fluorescent zone.
	A greenish fluorescent zone.
	A yellowish-green fluorescent zone.
	A bluish fluorescent zone.
Hyperoside: a yellowish-brown fluorescent zone.	
	A greenish fluorescent zone.
Rutin: a yellowish-brown fluorescent zone.	
Reference solution	**Test solution**

D. Boil 1.0 g of the powdered drug (355) (*2.9.12*) with 15 ml of *water R* for 1 min. Filter. Add 1 ml of *hydrochloric acid R* and boil for 1 min. A greenish-blue colour develops and, after a few minutes, cloudiness appears and then a blackish precipitate (iridoids).

TESTS

Foreign matter (*2.8.2*)

Maximum 5 per cent of brown petals and maximum 2 per cent of fragments of the calyx and other foreign matter, determined on 20 g.

Swelling index (*2.8.4*)

Minimum 9, determined on the powdered drug (710) (*2.9.12*), humidified with 2 ml of *alcohol R.*

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (710) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 6.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 2.0 per cent.

STORAGE

In an airtight container .

Ph Eur

Myrrh Tincture

General Notices

(Ph Eur monograph 1877)

Ph Eur

DEFINITION

Tincture produced from *Myrrh (1349)*.

PRODUCTION

The tincture is produced from 1 part of the drug and 5 parts of ethanol (90 per cent *V/V*) by a suitable procedure.

CHARACTERS

Clear yellowish-brown or orange-brown liquid.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution Dilute 5 ml of the tincture to be examined to 10 ml with *alcohol R*.

Reference solution Dissolve 10 mg of *thymol R* and 40 µl of *anethole R* in 10 ml of *ether R*.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (2:98 *V/V*).

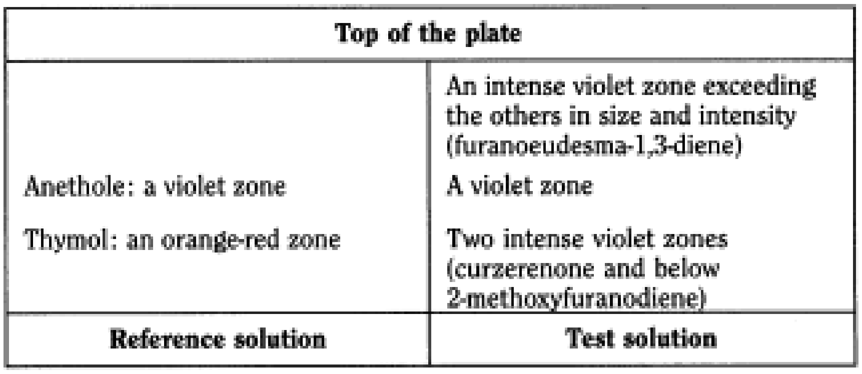
Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and examine in daylight whilst heating at 100-105 °C for 10 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones mostly violet, are present in the chromatogram obtained with the test solution.



Top of the plate	
	An intense violet zone exceeding the others in size and intensity (furanoeudesma-1,3-diene)
Anethole: a violet zone	A violet zone
Thymol: an orange-red zone	Two intense violet zones (curzerenone and below 2-methoxyfuranodiene)
Reference solution	**Test solution**

TESTS

Ethanol content *(2.9.10)*

82 per cent *V/V* to 88 per cent *V/V*.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

Dry residue *(2.8.16)*

Minimum 4.0 per cent *m/m*.

STORAGE

Plastic containers are not recommended.

Ph Eur

Myrrh

General Notices

(Ph Eur monograph 1349)

Ph Eur

DEFINITION

Gum-resin, hardened in air, obtained by incision or produced by spontaneous exudation from the stem and branches of *Commiphora molmo* Engler and/or other species of *Commiphora*.

CHARACTERS

Bitter taste.

IDENTIFICATION

A. The light or dark orange-brown, irregular or roundish grains or pieces of different size show components of various colours. Their surface is mostly covered with grey or yellowish-brown dust.

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-yellow or reddish-brown. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: a few tissue fragments from the original plants including: reddish-brown cork fragments; single or grouped polyhedral or elongated stone cells with partly strongly thickened, pitted and lignified walls with a brownish content; fragments of thin-walled parenchyma and sclerenchymatous fibres; irregular prismatic or polyhedral crystals of calcium oxalate, about 10-25 µm in size.

C. Examine the chromatograms obtained in the test for *Commiphora mukul*.

Detection Spray with *anisaldehyde solution R*, and examine in daylight while heating at 100-105 °C for 10 min.

Results The chromatogram obtained with the reference solution shows in the lower third an orange-red zone (thymol) and in the middle third a violet zone (anethole). The chromatogram obtained with the test solution shows an intense violet zone (furanoeudesma-1,3-diene), exceeding the other zones in size and intensity, above the zone of anethole in the chromatogram obtained with the reference solution; a violet zone similar in position to the zone of anethole in the chromatogram obtained with the reference solution; 2 intense violet zones similar in position to the zone of thymol in the chromatogram obtained with the reference solution, the upper one due to curzerenone and the lower one to 2-methoxyfuranodiene. Further mostly violet zones are present in the chromatogram obtained with the test solution.

TESTS

Commiphora mukul

Thin-layer chromatography (*2.2.27*).

Test solution To 0.5 g of the powdered drug (355) (*2.9.12*) add 5.0 ml of *ethanol (96 per cent) R* and warm the mixture on a water-bath for 2-3 min. Cool and filter.

Reference solution Dissolve 10 mg of *thymol R* and 40 µl of *anethole R* in 10 ml of *ethanol (96 per cent) R*.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (2:98 *V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows no blue or violet fluorescent zones in the lower third of the chromatogram.

Matter insoluble in ethanol

Maximum 70 per cent.

Place 1.00 g of the powdered drug (250) (*2.9.12*) in a flask. Add 30 ml of *ethanol (96 per cent) R* and shake vigorously for 10 min. Filter the supernatant through a tared sintered-glass filter (16) (*2.1.2*) avoiding the transfer of sediment from the flask. Repeat the extraction with 2 quantities, each of 20 ml, of *ethanol (96 per cent) R.* Quantitatively transfer the sediment to the filter by rinsing the flask with *ethanol (96 per cent) R*. Dry the filter and the residue in an oven at 100-105 °C and weigh.

Loss on drying (*2.2.32*)

Maximum 15.0 per cent, determined on 1.000 g of powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 7.0 per cent.

Ph Eur

Neroli Oil

General Notices

(Ph Eur monograph 1175)

Ph Eur

DEFINITION

Neroli oil is obtained by steam distillation from the fresh flowers of *Citrus aurantium* L. subsp. *aurantium* L. (*C. aurantium* L. subsp. *amara* Engl.).

CHARACTERS

Appearance

Clear, pale-yellow or dark-yellow liquid.

It has a characteristic odour.

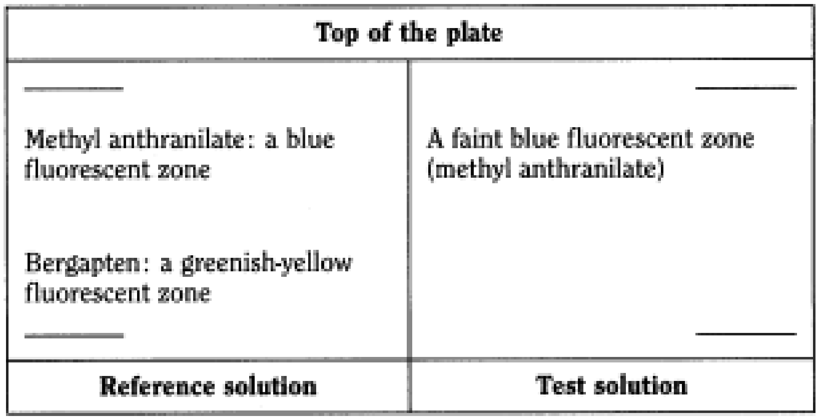
IDENTIFICATION

First identification B.

Second identification A.

A. Examine the chromatograms obtained in the test for bergapten.

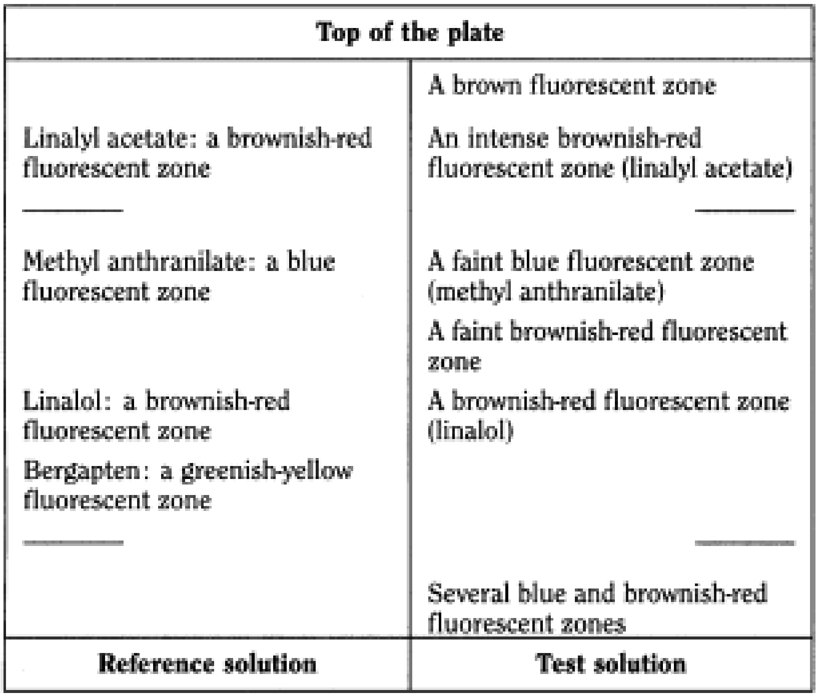
Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore other zones may be present in the chromatograms obtained with the test solution.



Top of the plate	
_______	_______
Methyl anthranilate: a blue fluorescent zone	A faint blue fluorescent zone (methyl anthranilate)
Bergapten: a greenish-yellow fluorescent zone	
_______	_______
Reference solution	**Test solution**

Detection B Spray with *anisaldehyde solution R*; heat at 100-105 °C for 10 min; examine the chromatograms in ultraviolet light at 365 nm.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. In the chromatogram obtained with the test solution the zone due to linalol is more intense than the zone due to linalyl acetate.



Top of the plate	
	A brown fluorescent zone
Linalyl acetate: a brownish-red fluorescent zone	An intense brownish-red fluorescent zone (linalyl acetate)
_______	_______
Methyl anthranilate: a blue fluorescent zone	A faint blue fluorescent zone (methyl anthranilate)
	A faint brownish-red fluorescent zone
Linalol: a brownish-red fluorescent zone	A brownish-red fluorescent zone (linalol)
Bergapten: a greenish-yellow fluorescent zone	
_______	_______
	Several blue and brownish-red fluorescent zones
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time to the principal peaks in the chromatogram obtained with the reference solution.

TESTS

Relative density (*2.2.5*)

0.863 to 0.880.

Refractive index (*2.2.6*)

1.464 to 1.474.

Optical rotation (*2.2.7*)

+ 1.5° to + 11.5°.

Acid value (*2.5.1*)

Maximum 2.0.

Bergapten

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.1 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5.0 ml with the same solvent.

Reference solution Dissolve 2 µl of *methyl anthranilate R*, 10 µl of *linalyl acetate R*, 20 µl of *linalol R* and 5 mg of *bergapten R* in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *ethyl acetate R*, *toluene R* (15:85 *V/V*).

Application 10 µl [or 2 µl] as bands.

Development Over a path of 15 cm [or 8 cm].

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution does not show a zone corresponding to the zone due to bergapten in the chromatogram obtained with the reference solution.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution The substance to be examined.

Reference solution (a) Dissolve 20 µl of β-*pinene R*, 5 mg of *sabinene R*, 40 µl of *limonene R*, 40 µl of *linalol R*, 20 µl of *linalyl acetate R*, 5 mg of α-*terpineol R*, 5 µl of *neryl acetate R*, 5 µl of *geranyl acetate R*, 5 µl of *trans-nerolidol R*, 5 µl of *methyl anthranilate R* and 5 µl (*E,E)-farnesol R* in 2 ml of *heptane R*.

Reference solution (b) Dissolve 5 µl of *methyl anthranilate R* in *heptane R* and dilute to 10 ml with the same solvent.

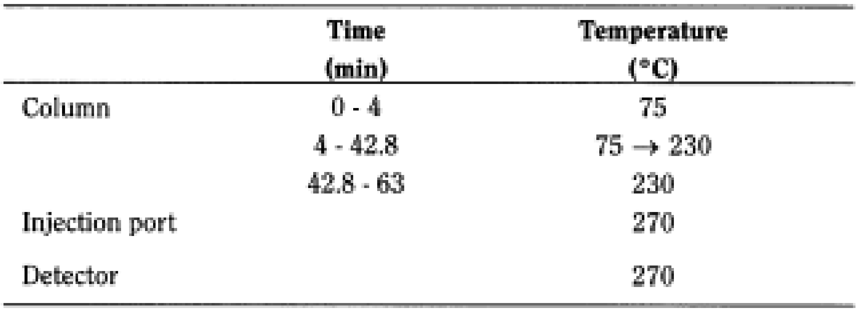
Column:
- *material*: fused silica;
- *size: l* = 60 m, Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 4	75
	4 - 42.8	75 → 230
	42.8 - 63	230
Injection port		270
Detector		270

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of reference solution (a). Record the retention times of these substances.

System suitability Reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to β-pinene and sabinene.

Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution.

Limits:

- β*-pinene*: 7.0 per cent to 17.0 per cent;
- *limonene*: 9.0 per cent to 18.0 per cent;
- *linalol*: 28.0 per cent to 44.0 per cent;
- *linalyl acetate*: 2.0 per cent to 15.0 per cent;
- α*-terpineol*: 2.0 per cent to 5.5 per cent;
- *neryl acetate*: maximum 2.5 per cent;
- *geranyl acetate*: 1.0 per cent to 5.0 per cent;
- *trans-nerolidol*: 1.0 per cent to 5.0 per cent;
- *methyl anthranilate*: 0.1 per cent to 1.0 per cent;
- *(E,E)-farnesol*: 0.8 per cent to 4.0 per cent;
- *disregard limit*: area of the peak in the chromatogram obtained with reference solution (b) .

Chiral purity

Gas chromatography (*2.2.28*).

Test solution Dissolve 20 mg of the substance to be examined in *pentane R* and dilute to 10.0 ml with the same solvent.

Reference solution To 10 µl of *linalol R* add 10 µl of *linalyl acetate R*. Dilute to 10.0 ml with *pentane R*.

Column:

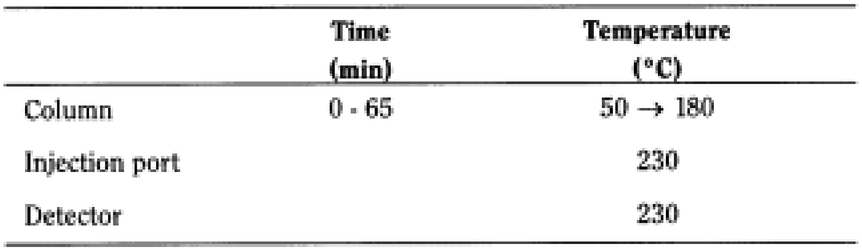
- *material*: fused silica;
- *size: l* = 25 m, Ø = 0.25 mm;
- *stationary phase*: *modified* β*-cyclodextrin for chiral chromatography R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.3 ml/min.

Split ratio 1:30.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 65	50 → 180
Injection port		230
Detector		230

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution:

— *resolution*: minimum 5.5 between the peaks due to (*R*)(–)-linalol (1^{st} peak) and (*S*)(+)-linalol (2^{nd} peak); minimum 2.7 between the peaks due to (*R*)(–)-linalyl acetate (3^{rd} peak) and (*S*)(+)-linalyl acetate 4^{th} peak).

Calculate the percentage content of the specified (*S*)-enantiomers from the following expression:

$$\frac{A_1}{A_1 + A_2} \times 100$$

A_1 = area of the corresponding (*S*)-enantiomer,

A_2 = area of the corresponding (*R*)-enantiomer.

Limits:

— (*S*)(+)-linalol: maximum 30 per cent;

— (*S*)(+)-linalyl acetate: maximum 5 per cent.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Nettle Leaf

General Notices

(Ph Eur monograph 1897)

Ph Eur

DEFINITION

Whole or cut dried leaves of *Urtica dioica* L., *Urtica urens* L. or a mixture of the 2 species.

Content

Minimum 0.3 per cent for the sum of caffeoylmalic acid and chlorogenic acid expressed as chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3) (dried drug).

IDENTIFICATION

A. The leaves are dark green, dark greyish-green or brownish-green on the upper surface, paler on the lower surface; scattered stinging hairs occur on both surfaces, also small covering trichomes which are more numerous along the margins and on the veins on the lower surface. The lamina is strongly shrunken, ovate to oblong, up to 100 mm long and 50 mm wide, with a coarsely serrate margin and a cordate to rounded base. The venation is reticulate and distinctly prominent on the lower surface. The petiole is green or brownish-green, rounded or flattened, about 1 mm wide, longitudinally furrowed and twisted; it bears stinging hairs and covering trichomes.

B. Reduce to a powder (355) (*2.9.12*). The powder is green to greyish-green. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: unicellular stinging hairs, up to 2 mm long, composed of an elongated tapering cell with a slightly swollen stinging tip which readily breaks off, arising from a raised, multicellular base; unicellular, straight or slightly curved covering trichomes, up to 700 µm long, enlarged at the base; small glandular trichomes (35-65 µm) with a stalk consisting of 1 or 2 cells and a head consisting of 2 or 4 cells; sporadic small fragments of leaves showing sinuous, wavy walled epidermal cells, anomocytic stomata (*2.8.3*) and abundant large cystoliths containing dense granular masses of calcium carbonate; small cluster crystals of calcium oxalate occur in the spongy mesophyll; occasional small groups of pitted vessels from the stem.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1 g of the powdered drug (355) (*2.9.12*), add 10 ml of *methanol R*. Boil under a reflux condenser for 15 min. Cool and filter. Evaporate to dryness *in vacuo* at 40 °C. Dissolve the residue in 2 ml of *methanol R*.

Reference solution Dissolve 2 mg of *chlorogenic acid R* and 1 mg of *scopoletin R* in 20 ml of *methanol R*.

Plate TLC silica gel plate R.

Mobile phase anhydrous formic acid R, water R, *methanol R*, *ethyl acetate R* (2.5:4:4:50 *V/*

V/V/V).

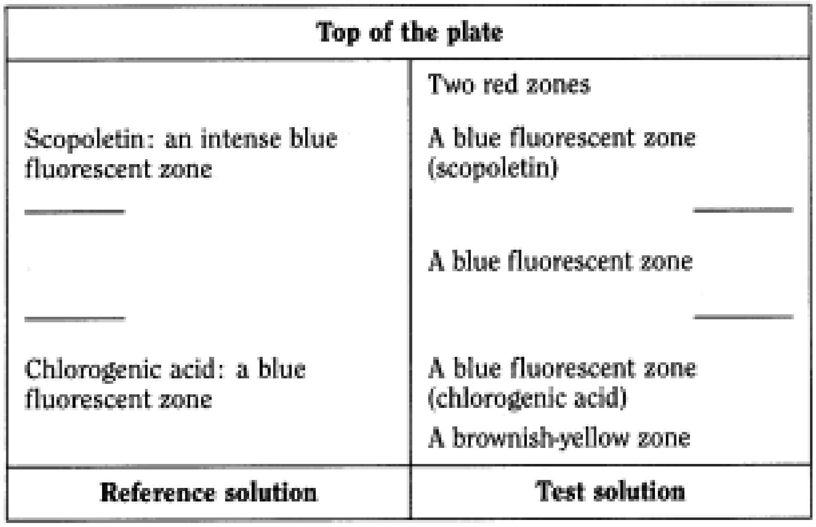
Application 10 µl, as bands.

Development Over a path of 8 cm.

Drying In air.

Detection Heat at 100 °C for 5 min. Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatogram obtained with the reference solution and the test solution. Furthermore, faint blue or yellow fluorescent zones may be present in the lower half of the chromatogram obtained with the test solution.



Top of the plate	
	Two red zones
Scopoletin: an intense blue fluorescent zone	A blue fluorescent zone (scopoletin)
	A blue fluorescent zone
Chlorogenic acid: a blue fluorescent zone	A blue fluorescent zone (chlorogenic acid)
	A brownish-yellow zone
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 5 per cent of stems and maximum 5 per cent of other foreign matter (including inflorescences).

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 20.0 per cent.

Ash insoluble in hydrochloric acid (2.8.1)

Maximum 4.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Internal standard solution Dissolve 20.0 mg of *p-coumaric acid R* in a 40 per cent *V/V* solution of *methanol R* and dilute to 200.0 ml with the same solution.

Test solution To 0.200 g of the powdered drug (355) (*2.9.12*) add 25.0 ml of the internal standard solution. Extract for 30 min in an ultrasonic bath at 40 °C and filter.

Reference solution Dissolve 10.0 mg of *chlorogenic acid CRS* in 100.0 ml of the internal standard solution.

Precolumn:

- *size*: *l* = 4 mm, Ø = 4 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

- *size*: *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 25 °C.

Mobile phase:

- *mobile phase A*: a mixture of 15 volumes of *methanol R* and 85 volumes of *water R* adjusted to pH 2.0 with *dilute phosphoric acid R*;
- *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	100	0
1 - 25	100 → 85	0 → 15
25 - 35	85	15
35 - 36	85 → 0	15 → 100
36 - 37	0 → 100	100 → 0
37 - 41	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 330 nm.

Injection 20 µl of the reference solution and the test solution.

Relative retention With reference to *p*-coumaric acid (retention time = about 24 min): chlorogenic acid = about 0.53; caffeoylmalic acid = about 1.19.

Calculate the percentage content of chlorogenic acid (C_A) or of caffeoylmalic acid (C_B) from the following expression:

$$\frac{A_1 \times A_4 \times C_1 \times 2500}{A_2 \times A_3 \times m_1}$$

A_1 = area of the peak due to caffeoylmalic acid or to chlorogenic acid in the chromatogram obtained with the test solution,

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution,

A_3 = area of the peak due to *p*-coumaric acid in the chromatogram obtained with the test solution,

A_4 = area of the peak due to *p*-coumaric acid in the chromatogram obtained with the reference solution,

m_1 = mass of the drug to be examined, in milligrams,

C_1 = content of chlorogenic acid in the reference solution, in milligrams per millilitre.

Calculate the total percentage content $C_A + C_B$.

Ph Eur

Notoginseng Root

General Notices

(Ph Eur monograph 2383)

Ph Eur

DEFINITION

Whole or fragmented taproot, without secondary roots, of *Panax pseudoginseng* Wall. var. *notoginseng* (Burk.) Hoo et Tseng [*Panax notoginseng* (Burk.) F.H. Chen ex C.Y. Wu et K.M. Feng] treated with steam and dried.

Content

Minimum 3.8 per cent for the sum of ginsenosides Rg1 ($C_{42}H_{72}O_{14},2H_2O$; M_r 837) and Rb1 ($C_{54}H_{92}O_{23},3H_2O$; M_r 1163) (dried drug).

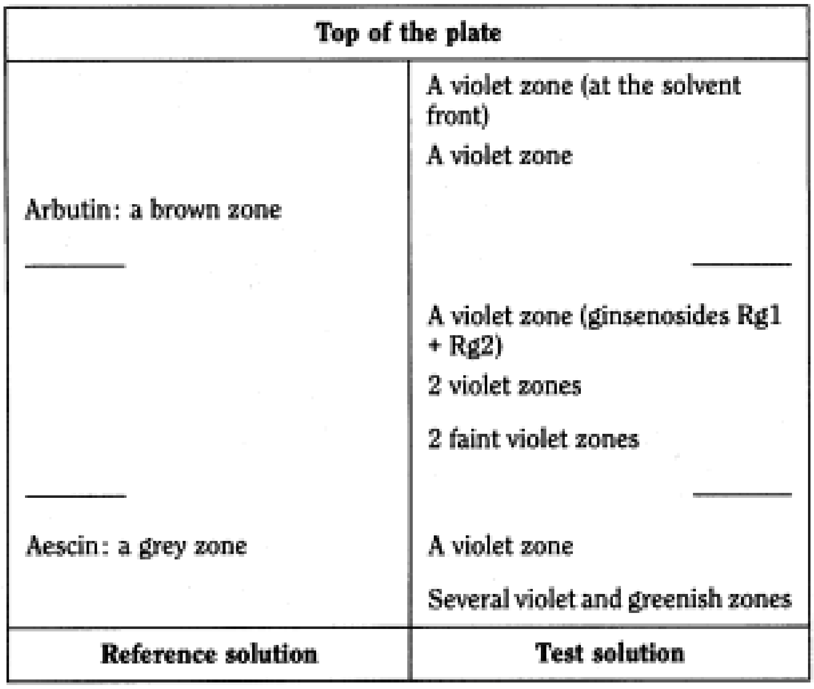
IDENTIFICATION

A. The primary root is conical, subconical or cylindrical, up to 6 cm long and 4 cm in diameter. The outer surface, showing shallow transverse striations and secondary root scars, is brownish-grey or yellowish-grey. The aerial stem scar is surrounded by warty protuberances at the crown. The texture of the root is compact. The fracture is smooth, shiny, brownish-grey and shows a yellowish-grey ring (cambial zone) and many radial striations.

B. Reduce to a powder (355) *(2.9.12)*. The powder is light yellowish-grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: abundant fragments of thin-walled parenchymatous cells; fragments of secretory canals containing yellowish-brown resin; rare lignified vessels about 30 µm in diameter, reticulate or pitted; rare cork fragments. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The starch granules, often deformed, are very abundant, single or in groups of 2-3, and 1-10 µm in diameter.

C. Examine the chromatogram obtained in the test for *Panax ginseng* or *Panax quinquefolium*.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A violet zone (at the solvent front)
	A violet zone
Arbutin: a brown zone	
_______	_______
	A violet zone (ginsenosides Rg1 + Rg2)
	2 violet zones
	2 faint violet zones
_______	_______
Aescin: a grey zone	A violet zone
	Several violet and greenish zones
Reference solution	**Test solution**

TESTS

Panax ginseng* or *Panax quinquefolium

Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of a 70 per cent *V/V* solution of *methanol R* and boil under a reflux condenser for 15 min. Filter after cooling and dilute to 10.0 ml with *methanol R*.

Reference solution Dissolve 5.0 mg of *aescin R* and 5.0 mg of *arbutin R* in 1 ml of *methanol R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *ethyl acetate R, water R, butanol R* (25:50:100 *V/V/V*); allow to stand for 10 min and use the upper layer.

Application 20 µl, as bands of 15 mm [or 4 µl of the test solution and 2 µl of the reference solution, as bands of 8 mm].

Development In an unsaturated tank, over a path of 10 cm [or 5 cm].

Drying In air for 30 min.

Detection Spray with *anisaldehyde solution R* and heat at 105-110 °C for 5-10 min; examine in daylight.

Results In the chromatogram obtained with the test solution, the absence of a violet zone immediately above the zone due to arbutin in the chromatogram obtained with the reference solution suggests the presence of *Panax ginseng*; in the chromatogram obtained with the test solution, the presence of a brown zone immediately below the violet zone due to the ginsenosides Rg1 + Rg2 suggests the presence of *Panax quinquefolium*.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 6.0 per cent.

Ash insoluble in hydrochloric acid ***(2.8.1)***

Maximum 1.0 per cent.

ASSAY

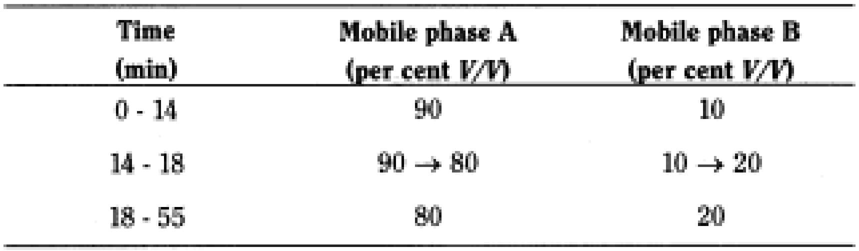
Liquid chromatography *(2.2.29)*.

Test solution Reduce about 50 g to a powder (355) *(2.9.12)*. Place 0.250 g of the powdered drug and 70 ml of a 50 per cent *V/V* solution of *methanol R* in a 250 ml round-bottomed flask. After adding a few grains of pumice, boil on a water-bath under a reflux condenser for 1 h. After cooling, centrifuge and collect the supernatant liquid. Treat the residue as described above. Mix the collected liquids and evaporate to dryness under reduced pressure at a temperature not exceeding 60 °C. Take up the residue with 10.0 ml of a buffer solution, adjusted to pH 4.5, containing 3.5 g of *sodium dihydrogen phosphate R* and 7.2 g of *potassium dihydrogen phosphate R* in 1000 ml of *water R* (solution A). Wash a cartridge containing about 0.36 g of *octadecylsilyl silica gel for chromatography R* with 5 ml of *methanol R* followed by 20 ml of *water for chromatography R*. Apply 5.0 ml of solution A to the cartridge. Elute with 20 ml of *water for chromatography R*, followed by 15 ml of a 30 per cent *V/V* solution of *methanol R*. Discard the eluates after confirming that no ginsenosides are present, otherwise repeat the assay with another type of cartridge. Elute the cartridge with 20 ml of *methanol R* and evaporate the eluate to dryness. Take up the residue with 5.0 ml of *methanol R*.

Reference solution Dissolve 3.0 mg of *ginsenoside Rb1 R*, 3.0 mg of *ginsenoside Rg1 R* and 3.0 mg of *ginsenoside Rf R* in *methanol R* and dilute to 5.0 ml with the same solvent.

Column:
- *size:* *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase: aminopropylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: *acetonitrile R*;
- *mobile phase B*: *water for chromatography R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	90	10
14 - 18	90 → 80	10 → 20
18 - 55	80	20

Flow rate 2 ml/min.

Detection Spectrophotometer at 203 nm.

Injection   20 µl.

System suitability   Reference solution:

— *resolution*: minimum 3.0 between the peaks due to ginsenosides Rf and Rg1.

Calculate the sum of the percentage contents of ginsenosides Rb1 and Rg1 using the following expression:

$$\frac{A_1 \times m_2 \times 2 \times p_1}{m_1 \times A_3} + \frac{A_2 \times m_3 \times 2 \times p_2}{m_1 \times A_4}$$

A_1 = area of the peak due to ginsenoside Rb1 in the chromatogram obtained with the test solution;

A_2 = area of the peak due to ginsenoside Rg1 in the chromatogram obtained with the test solution;

A_3 = area of the peak due to ginsenoside Rb1 in the chromatogram obtained with the reference solution;

A_4 = area of the peak due to ginsenoside Rg1 in the chromatogram obtained with the reference solution;

m_1 = mass of the dried drug to be examined, in grams;

m_2 = mass of *ginsenoside Rb1 R* in the reference solution, in grams;

m_3 = mass of *ginsenoside Rg1 R* in the reference solution, in grams;

p_1 = percentage content of ginsenoside Rb1 in *ginsenoside Rb1 R*;

p_2 = percentage content of ginsenoside Rg1 in *ginsenoside Rg1 R*

Ph Eur

Nutmeg Oil

General Notices

(Ph Eur monograph 1552)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation of the dried and crushed kernels of *Myristica fragrans* Houtt.

CHARACTERS

Appearance

Colourless or pale yellow liquid.

Spicy odour.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 1 ml of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 20 µl of *myristicine R* in 10 ml of *toluene R*.

Plate TLC silica gel plate R.

Mobile phase ethyl acetate R, toluene R (5:95 *V/V*).

Application 10 µl as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *vanillin reagent R*, heat at 100-105 °C for 10 min and examine in daylight.

Results The chromatogram obtained with the reference solution shows in the upper third a pink or reddish-brown zone (myristicine); the chromatogram obtained with the test solution shows a series of zones of which 1 is similar in position and colour to the zone in the chromatogram obtained with the reference solution; above this zone a brownish zone (safrole) and a violet zone (hydrocarbons) are present; below the myristicine zone, 5 blue zones of variable intensity are present.

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

0.885 to 0.905.

Refractive index *(2.2.6)*

1.475 to 1.485.

Optical rotation *(2.2.7)*

+ 8° to + 18°.

Chromatographic profile

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution The substance to be examined.

Reference solution Dissolve 15 µl of α-*pinene R*, 15 µl of β-*pinene R*, 15 µl of *sabinene R*, 5 µl of *car-3-ene R*, 5 µl of *limonene R*, 5 µl of γ-*terpinene R*, 5 µl of *terpinen-4-ol R*, 5 µl of *safrole R* and 10 µl of *myristicine R* in 1 ml of *hexane R*.

Column:

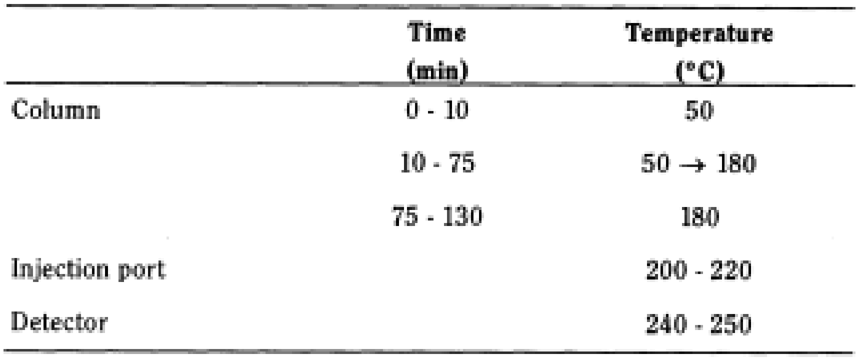
- *material*: fused silica;
- *size: l* = 25-60 m, Ø = about 0.3 mm;
- *stationary phase: bonded macrogol 20 000 R*.

Carrier gas *helium for chromatography R.*

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	50
	10 - 75	50 → 180
	75 - 130	180
Injection port		200 - 220
Detector		240 - 250

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of the reference solution; record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to β-pinene and sabinene.

Identification of components Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of each of these components. The percentages are within the following ranges:

— α-*pinene*: 15 per cent to 28 per cent;

— β-*pinene*: 13 per cent to 18 per cent;

— *sabinene*: 14 per cent to 29 per cent;

— *car-3-ene*: 0.5 per cent to 2.0 per cent;

— *limonene*: 2.0 per cent to 7.0 per cent;

— γ-*terpinene*: 2.0 per cent to 6.0 per cent;

— *terpinen-4-ol* : 2.0 per cent to 6.0 per cent;

— *safrole*: less than 2.5 per cent;

— *myristicine*: 5.0 per cent to 12.0 per cent.

STORAGE

Protected from heat.

Ph Eur

Oak Bark

General Notices

(Ph Eur monograph 1887)

Ph Eur

DEFINITION

Cut and dried bark from the fresh young branches of *Quercus robur* L., *Q. petraea* (Matt.) Liebl. and *Q. pubescens* Willd.

Content

Minimum 3.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The bark occurs in channelled or quilled pieces, not more than 3 mm thick. The outer surface is light grey or greenish-grey, rather smooth, with occasional lenticels. The inner surface is dull brown or reddish-brown and has slightly raised longitudinal striations about 0.5-1 mm wide. The fracture is splintery and fibrous.

B. Reduce to a powder (355) (*2.9.12*). The powder is light brown or reddish-brown and fibrous. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: groups of thick-walled fibres surrounded by a moderately thickened parenchymatous sheath containing prism crystals of calcium oxalate; fragments of cork composed of thin-walled tabular cells filled with brownish or reddish contents; abundant sclereids, isolated and in groups, some large with thick, stratified walls and branching pits, others smaller and thinner-walled with simple pits, often with dense brown contents; fragments of parenchyma containing cluster crystals of calcium oxalate; occasional fragments of sieve tissue, thin-walled, some showing sieve areas on the oblique end-walls.

C. To 1 g of the powdered drug (710) (*2.9.12*) add 10 ml of *ethanol (30 per cent V/V) R* and heat the mixture under a reflux condenser on a water-bath for 30 min. Cool and filter. To 1 ml of this solution add 2 ml of a 10 g/l solution of *vanillin R* in *hydrochloric acid R*. A red colour develops.

TESTS

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (710) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 8.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs (*2.8.14*). Use 0.700 g of the powdered drug (710) (*2.9.12*).

Ph Eur

Olive Leaf

General Notices

(Ph Eur monograph 1878)

Ph Eur

DEFINITION

Dried leaf of *Olea europaea* L.

Content

Minimum 5.0 per cent of oleuropein ($C_{25}H_{32}O_{13}$; M_r 540.5) (dried drug).

IDENTIFICATION

A. The leaf is simple, thick and coriaceous, lanceolate to obovate, 30-50 mm long and 10-15 mm wide, with a mucronate apex and tapering at the base to a short petiole; the margins are entire and reflexed abaxially. The upper surface is greyish-green, smooth and shiny, the lower surface paler and pubescent, particularly along the midrib and main lateral veins.

B. Reduce to a powder (355) (*2.9.12*). The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the epidermis in surface view with small, thick-walled polygonal cells and, in the lower epidermis only, small anomocytic stomata (*2.8.3*); fragments of the lamina in sectional view showing a thick cuticle, a palisade composed of 3 layers of cells and a small-celled spongy parenchyma; numerous sclereids, very thick-walled and mostly fibre-like with blunt or, occasionally, forked ends, isolated or associated with the parenchyma of the mesophyll; abundant, very large peltate trichomes, with a central unicellular stalk from which radiate some 10 to 30 thin-walled cells which become free from the adjoining cells at the margin of the shield, given an uneven, jagged appearance.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methanol R*. Boil under a reflux condenser for 15 min. Cool and filter.

Reference solution Dissolve 10 mg of *oleuropein R* and 1 mg of *rutin R* in 1 ml of *methanol R.*

Plate *TLC silica gel plate R*.

Mobile phase *water R*, *methanol R*, *methylene chloride R* (1.5:15:85 *V/V/V*).

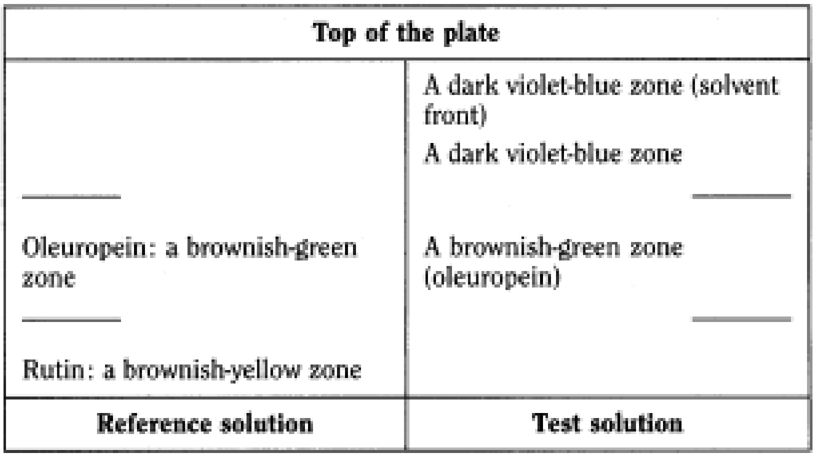
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *vanillin reagent R* and heat at 100-105 °C for 5 min; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A dark violet-blue zone (solvent front) A dark violet-blue zone
———	———
Oleuropein: a brownish-green zone	A brownish-green zone (oleuropein)
———	———
Rutin: a brownish-yellow zone	
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 9.0 per cent.

ASSAY

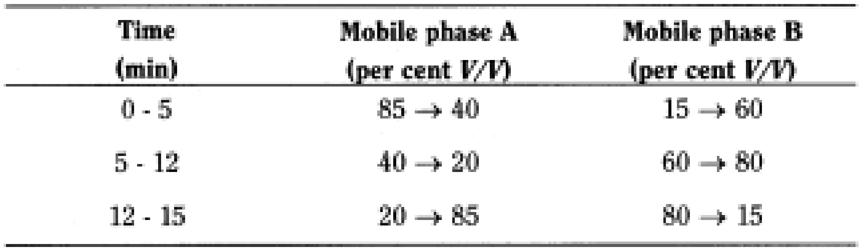
Liquid chromatography (*2.2.29*).

Test solution In a flask, place 1.000 g of the powdered drug (355) (*2.9.12*) and add 50 ml of *methanol R*. Heat in a water-bath at 60 °C for 30 min with shaking. Allow to cool and filter into a 100 ml volumetric flask. Rinse the flask and the filter with *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with *water R*.

Reference solution Dissolve 5.0 mg of *oleuropein CRS* in 5.0 ml of *methanol R*. Dilute 1.0 ml of this solution to 25.0 ml with *water R.*

Column:
— *size:* *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: dilute 1.0 ml of *glacial acetic acid R* to 100 ml with *water R*;
— *mobile phase B*: *methanol R*;



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 5	85 → 40	15 → 60
5 - 12	40 → 20	60 → 80
12 - 15	20 → 85	80 → 15

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Retention time Oleuropein = about 9 min.

Calculate the percentage content of oleuropein using the following expression:

$$\frac{A_1 \times m_2 \times p \times 8}{A_2 \times m_1}$$

A_1 = area of the peak due to oleuropein in the chromatogram obtained with the test solution,

A_2 = area of the peak due to oleuropein in the chromatogram obtained with the reference solution,

m_1 = mass of the drug to be examined, in grams,

m_2 = mass of *oleuropein CRS* in the reference solution, in grams,

p = percentage content of oleuropein in *oleuropein CRS*.

Ph Eur

Opium Tincture

General Notices

DEFINITION

Opium, sliced	200 g
Ethanol (90 per cent)	A sufficient quantity
Purified Water	A sufficient quantity

Extemporaneous preparation

The following directions apply.

Pour 500 ml of boiling Purified Water on to the Opium and allow to stand for 6 hours; add 500 ml of Ethanol (90 per cent), mix thoroughly and allow to stand in a covered vessel for 24 hours; strain, press the marc, mix the liquids and allow to stand for not less than 24 hours; filter.

Determine the concentration of morphine, calculated as anhydrous morphine, in the tincture so prepared by the Assay. To the remainder of the liquid add sufficient of a mixture of equal volumes of Ethanol (90 per cent) and Purified Water to produce an Opium Tincture containing 1% w/v of anhydrous morphine.

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

Content of anhydrous morphine, $C_{17}H_{19}NO_3$

0.925 to 1.075% w/v.

TESTS

Ethanol content

41 to 46% v/v, Appendix VIII F, Method III.

Relative density

0.898 to 0.969, Appendix V G.

ASSAY

Dilute 5 ml to 100 ml with *ethanol* (*45%*). To 10 ml of the resulting solution add 5 ml of *water* and 1 ml of 5M *ammonia* and extract with 30 ml of a mixture of equal volumes of *ethanol* (*96%*) and *chloroform* and then with two 22.5 ml quantities of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*), washing each extract with the same 20 ml of a mixture of equal volumes of *ethanol* (*96%*) and *water*. Evaporate the combined extracts just to dryness, extract the residue with two 5 ml quantities of *calcium hydroxide solution*, filter and wash the filter with 10 ml of *calcium hydroxide solution*. To the combined filtrate and washings add 0.1 g of *ammonium sulphate*, extract with two 10 ml quantities of *ethanol-free chloroform*, wash the combined extracts with 10 ml of *water* and discard the chloroform solution. To the combined alkaline liquid and aqueous washings add 10 ml of 1M *hydrochloric acid*, heat on a water bath to remove any chloroform, cool and dilute to 100 ml with *water*. To 10 ml of this solution add 10 ml of 0.1M *hydrochloric acid* and 8 ml of a freshly prepared 1.0% w/v solution of *sodium nitrite*, allow to stand for 15 minutes, add 12 ml of 5M *ammonia*, dilute to 50 ml with *water* and measure the *absorbance* of a 4-cm layer of the resulting solution at the maximum at 442 nm, Appendix II B, using in the reference cell a solution prepared at the same time and in the same manner but using 8 ml of *water* in place of the solution of sodium nitrite. Calculate the content of $C_{17}H_{19}NO_3$ taking 124 as the value of A(1%, 1 cm) at the maximum at 442 nm.

Opium

General Notices

(Raw Opium, Ph Eur monograph 0777)

Preparation

Opium Tincture

Ph Eur

DEFINITION

Raw opium is intended only as starting material for the manufacture of galenical preparations. It is not dispensed as such.

Air-dried latex obtained by incision from the unripe capsules of *Papaver somniferum* L.

Content:

— *morphine* ($C_{17}H_{19}NO_3$; M_r 285.3): minimum 10.0 per cent (dried drug);

— *codeine* ($C_{18}H_{21}NO_3$; M_r 299.4): minimum 2.0 per cent (dried drug).

CHARACTERS

Characteristic odour.

Appearance

Blackish-brown masses of various sizes, which tend to be soft and shiny and, after drying, become hard and brittle.

IDENTIFICATION

Strip off any covering, cut the substance to be examined into thin slices, dry at about 60 °C for 48 h, if necessary, and reduce to a powder (500) (2.9.12).

A. Examined under a microscope, a suspension of raw opium in a 20 g/l solution of *potassium hydroxide R* shows the following diagnostic characters: granules of latex agglomerated in irregular masses, and light-brown elongated filaments. Some fragments of vessels and rather elongated, refringent crystals are also visible, as well as a smaller number of round pollen grains and fragments of elongated fibres. Hairs of various lengths with sharp points and a few grains of starch introduced during the handling of the latex may be present. Fragments of epicarp consisting of polygonal cells with thick walls defining a stellate lumen may also be present.

B. Thin-layer chromatography (*2.2.27*).

Test solution Triturate 0.10 g of the powdered drug with 5 ml of *ethanol (70 per cent V/V) R*, add 3 ml of *ethanol (70 per cent V/V) R*, transfer to a 25 ml conical flask and heat in a water-bath at 50-60 °C with stirring for 30 min. Cool, filter, wash the filter with *ethanol (70 per cent*

V/V) R and dilute the filtrate to 10 ml with the same solvent.

Reference solution Dissolve 2.0 mg of *papaverine hydrochloride R*, 12.0 mg of *codeine phosphate R*, 12.0 mg of *noscapine hydrochloride R* and 25.0 mg of *morphine hydrochloride R* in *ethanol (70 per cent V/V) R* and dilute to 25.0 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R*, *ethanol (96 per cent) R*, *acetone R*, *toluene R* (2:6:40:40 *V/V/V/V*); use a freshly prepared mixture.

Application 20 µl, as bands of 20 mm by 3 mm.

Development Over a path of 15 cm.

Drying At 100-105 °C for 15 min, then allow to cool.

Detection Spray with *potassium iodobismuthate solution R2* and then with a 4 g/l solution of *sulphuric acid R*.

Results The chromatogram obtained with the reference solution shows in the lower part an orange-red or red zone (morphine) with a similarly coloured zone (codeine) above it, and in the upper part an orange-red or red zone (papaverine) with a similarly coloured zone (noscapine) above that; the chromatogram obtained with the test solution shows orange-red or red zones corresponding to those in the chromatogram obtained with the reference solution, and may also show a dark red zone (thebaine) situated between those due to codeine and papaverine.

C. To 1.0 g of the powdered drug add 5 ml of *water R*, shake for 5 min and filter. To the filtrate add 0.25 ml of *ferric chloride solution R2*. A red colour develops that does not disappear upon the addition of 0.5 ml of *dilute hydrochloric acid R*.

TESTS

Thebaine

Liquid chromatography (*2.2.29*).

Test solution Suspend 1.00 g of the substance to be examined, cut into thin slices, in 50 ml of *ethanol (50 per cent V/V) R*, mix with the aid of ultrasound for 1 h, allow to cool and dilute to 100.0 ml with the same solvent. Allow to stand. To 10.0 ml of the supernatant liquid add 5 ml of *ammonium chloride buffer solution pH 9.5 R*, dilute to 25.0 ml with *water R* and mix. Transfer 20.0 ml of this solution to a chromatography column about 0.15 m long and about 30 mm in internal diameter containing 15 g of *kieselguhr for chromatography R*. Allow to stand for 15 min. Elute with 2 quantities, each of 40 ml, of a mixture of 15 volumes of *2-propanol R* and 85 volumes of *methylene chloride R*. Evaporate the eluate to dryness *in vacuo* at 40 °C. Transfer the residue to a volumetric flask with the aid of the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution Dissolve 25.0 mg of *thebaine R* in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Precolumn:

— *size:* *l* = 4 mm, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Column:

— *size:* *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.0 g of *sodium heptanesulphonate monohydrate R* in 420 ml of *water R,* adjust to pH 3.2 with phosphoric acid (4.9 g/l H_3PO_4) (about 5 ml) and add 180 ml of *acetonitrile R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection A suitable volume with a loop injector.

System suitability Reference solution:

— *number of theoretical plates*: minimum 3000;

— *mass distribution ratio*: minimum 3.0 for the peak due to thebaine.

Calculate the percentage content of the alkaloid using the following expression:

$$\frac{m_1 \times A_2 \times 625}{m_2 \times A_1 \times 5} \times \frac{100}{100 - h}$$

m_1 = mass of the alkaloid used to prepare the reference solution, in grams,

m_2 = mass of the substance to be examined used to prepare the test solution, in grams

A_1 = area of the peak due to the alkaloid in the chromatogram obtained with the reference solution,

A_2 = area of the peak due to the alkaloid in the chromatogram obtained with the test solution,

h = percentage loss on drying.

Limit:

— *thebaine*: maximum 3.0 per cent (dried drug).

Loss on drying (*2.2.32*)

Maximum 15.0 per cent, determined on 1.000 g of the substance to be examined cut into thin slices, by drying in an oven at 105 °C for 4 h.

Total ash (*2.4.16*)

Maximum 6.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for thebaine with the following modifications.

Reference solution Dissolve 0.100 g of *morphine hydrochloride R* and 25.0 mg of *codeine R* in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

System suitability Reference solution:

- *resolution*: minimum 2.5 between the peaks due to morphine and codeine; if necessary, adjust the volume of acetonitrile in the mobile phase;
- *repeatability*: maximum relative standard deviation of 1.0 per cent for the area of the peak due to morphine after 6 injections.

Calculate the percentage content of morphine and codeine from the expression given in the test for thebaine.

For the calculation, 1 mg of *morphine hydrochloride R* is equivalent to 0.759 mg of morphine, and 1 mg of *codeine R* is equivalent to 0.943 mg of codeine.

Ph Eur

Orange Oil

General Notices

DEFINITION

Orange Oil is obtained by mechanical means from the fresh peel of the sweet orange, *Citrus sinensis* (L.) Osbeck.

CHARACTERISTICS

A yellow to yellowish brown liquid, visibly free from water; odour, that of orange.

TESTS

Optical rotation

+94° to +99°, Appendix V F. On distillation, the first 10% of the distillate has an optical rotation the same as, or only slightly lower than, the original oil.

Refractive index

1.472 to 1.476, Appendix V E.

Residue on evaporation

1.0 to 5.0% when determined by the method for *residue on evaporation of volatile oils*, Appendix X M. Use 2 g and heat for 4 hours.

Solubility in ethanol

Soluble at 20°, in 7 parts of *ethanol (90%)*, Appendix X M. A bright solution is rarely obtained due to the presence of waxy non-volatile substances.

Weight per ml

0.842 to 0.848 g, Appendix V G.

Content of aldehydes

Not less than 1.0% w/w, calculated as decanal, $C_{10}H_{20}O$. Carry out the method for the *determination of aldehydes*, Appendix X K, using 10 g, omitting the *toluene* and using a volume, not less than 7 ml, of *alcoholic hydroxylamine solution* that exceeds by 1 to 2 ml the volume of 0.5M *potassium hydroxide in ethanol (60%) VS* required. Each ml of 0.5M *potassium hydroxide in ethanol (60%) VS* is equivalent to 78.76 mg of $C_{10}H_{20}O$.

STORAGE

Orange Oil should be kept in a well-filled container and protected from light.

Orange Peel Infusion

General Notices

DEFINITION

Concentrated Orange Peel Infusion	100 ml
Water	Sufficient to produce 1000 ml

The infusion complies with the requirements stated under Infusions.

CONCENTRATED ORANGE PEEL INFUSION

DEFINITION

Dried Bitter-Orange Peel, cut small	500 g
Ethanol (25 per cent)	1350 ml

Extemporaneous preparation

The following directions apply.

Macerate the Dried Bitter-Orange Peel in a covered vessel for 48 hours with 1000 ml of the Ethanol (25 per cent) and press out the liquid. To the pressed marc add 350 ml of the Ethanol (25 per cent), macerate for 24 hours, press and add the liquid to the product of the first pressing. Allow to stand for not less than 14 days and filter.

TESTS

Ethanol content

18 to 23% v/v, Appendix VIII F.

Total solids

10 to 15% w/v, Appendix XI A. Use 1 ml.

Weight per ml

1.01 to 1.04 g, Appendix V G.

Orange Tincture

General Notices

(Bitter-Orange Epicarp and Mesocarp Tincture, Ph Eur monograph 1604)

Ph Eur

DEFINITION

Tincture produced from *Bitter-orange epicarp and mesocarp (1603).*

PRODUCTION

The tincture is produced from 1 part of the freshly powdered drug (2000) (*2.9.12*) and 5 parts of *alcohol (70 per cent V/V/V)* by an appropriate procedure.

CHARACTERS

Liquid with a bitter taste.

IDENTIFICATION

Examine by thin-layer chromatography (*2.2.27*).

Test solution The tincture to be examined.

Reference solution Dissolve 1.0 mg of *naringin R* and 1.0 mg of *caffeic acid R* in 1 ml of *methanol R*.

Plate TLC silica gel plate R.

Mobile phase water R, *anhydrous formic acid R*, *ethyl acetate R* (10:15:75 *V/V/V*).

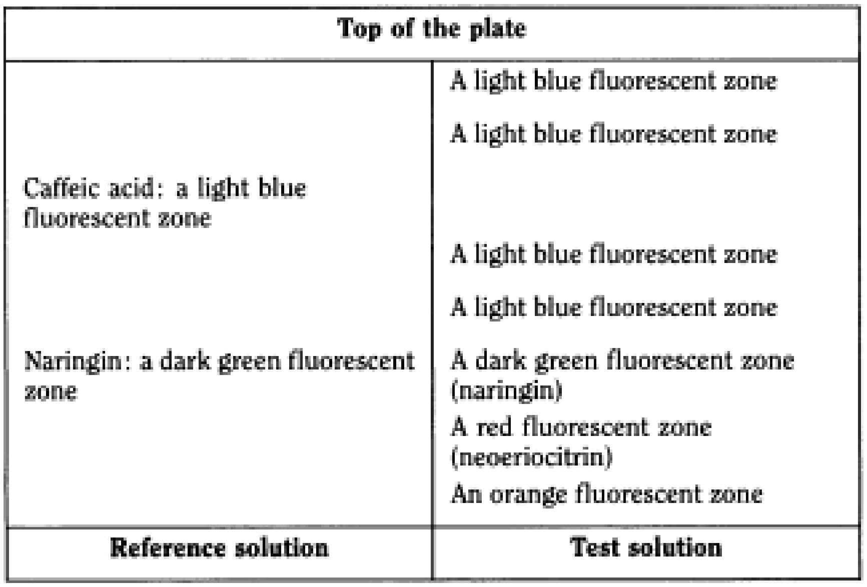
Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying In air, and heat in an oven at 110-120 °C for 5 min.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. After 1 h, examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	A light blue fluorescent zone
	A light blue fluorescent zone
Caffeic acid: a light blue fluorescent zone	
	A light blue fluorescent zone
	A light blue fluorescent zone
Naringin: a dark green fluorescent zone	A dark green fluorescent zone (naringin)
	A red fluorescent zone (neoeriocitrin)
	An orange fluorescent zone
Reference solution	**Test solution**

TESTS

Ethanol content (*2.9.10*)

63 per cent to 67 per cent *V/V*.

Methanol and 2-propanol (*2.9.11*)

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

Dry residue: minimum 6.0 per cent *m/m*, determined on 2.00 g of tincture to be examined.

Ph Eur

Oregano

General Notices

(Ph Eur monograph 1880)

Ph Eur

DEFINITION

Dried leaves and flowers separated from the stems of *Origanum onites* L. or *Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw., or a mixture of both species.

Content

— *essential oil*: minimum 25 ml/kg (anhydrous drug);

— *sum of the contents of carvacrol and thymol* (both $C_{10}H_{14}O$; M_r 150.2): minimum 60 per cent in the essential oil.

IDENTIFICATION

A. *O. onites*. The leaf is yellowish-green, usually 4-22 mm long and 3-14 mm wide. It has a long or short petiole or is sessile. The lamina is ovate, elliptic or ovate-lanceolate. Margins are entire or serrate, the apex is acute or obtuse. The veins are yellowish and conspicuous on the adaxial surface. Flowers are solitary or seen as broken parts of the corymb. The calyx is bract-like and inconspicuous. The corolla is white, on top of inflorescenses or single flowers, or inconspicuous. The bracts are imbricate and green like the leaves. The drug contains yellowish or yellowish-brown stem parts.

O. vulgare (subsp. *hirtum*). The leaf is green and usually 3-28 mm long and 2.5-19 mm wide. It is petiolate or sessile. The lamina is ovate or ovate-eliptic. The margins are entire or serrate, the apex is acute or obtuse. Flowers are rare, found as broken parts of the corymbs. Bracts are greenish-yellow and imbricate. The calyx is corolla-like and inconspicuous. The corolla is white, on top of inflorescenses, slightly conspicuous or inconspicuous.

B. Reduce to a powder (355) (*2.9.12*). The powder is green (*O. vulgare*) to yellowish-green (*O. onites*). Examine under a microscope using *chloral hydrate solution R*. The covering trichomes are of lamiaceous type or short, unicellular and rarely conical; conical trichomes shaped as pointed teeth are more abundant in *O. vulgare*. Covering trichomes are thick-walled in *O. vulgare*. Covering trichomes contain prismatic crystals in *O. onites*, minute needles in O. *vulgare*. The cuticle on covering trichomes is smooth; warty in *O. vulgare*. The epidermises of the leaves have cells with walls that are sinuous and the stomata are of diacytic type (*2.8.3*); in *O. vulgare* cells of the upper epidermis are beaded; secretory trichomes with 8-16 cells (12 in *O. vulgare*); glandular trichomes are numerous in *O. onites*, rare in *O. vulgare*. They have a unicellular head and unicellular, bicellular or tricellular (bicellular or tricellular in *O. vulgare*) stalk; pollen grains are smooth, spherical and more abundant in *O. onites*.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (355) (*2.9.12*) add 5 ml of *methylene chloride R* and shake for 3 min, then filter through about 2 g of *anhydrous sodium sulphate R*.

Reference solution Dissolve 1 mg of *thymol R* and 10 µl of *carvacrol R* in 10 ml of *methylene chloride R*.

Plate *TLC silica gel plate R.*

Mobile phase *methylene chloride R.*

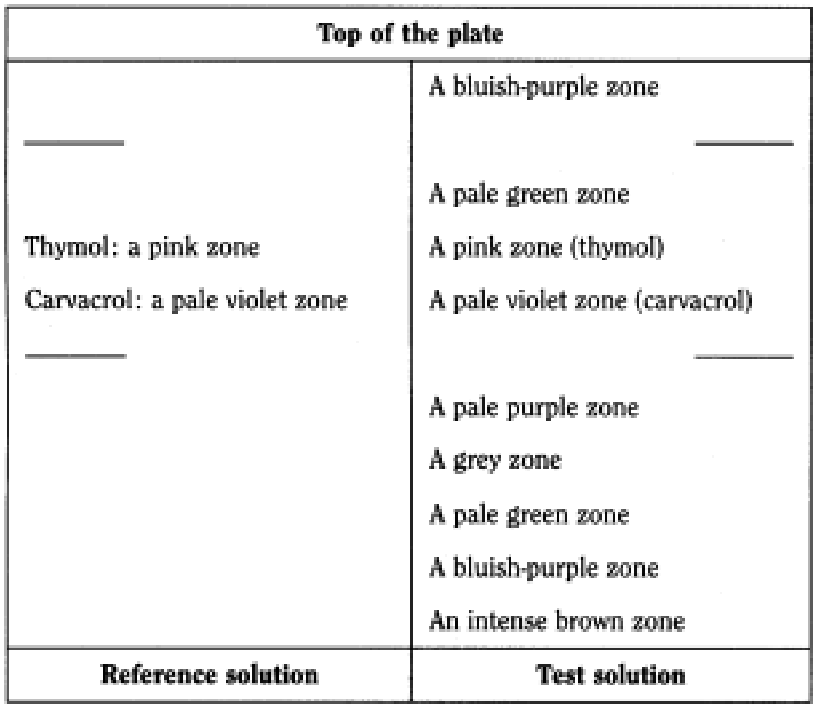
Application 20 µl as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* using 10 ml for a plate 200 mm square and heat at 100-105 °C for 10 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the lower third and upper part of the chromatogram obtained with the test solution.



Top of the plate	
	A bluish-purple zone
———	———
	A pale green zone
Thymol: a pink zone	A pink zone (thymol)
Carvacrol: a pale violet zone	A pale violet zone (carvacrol)
———	———
	A pale purple zone
	A grey zone
	A pale green zone
	A bluish-purple zone
	An intense brown zone
Reference solution	**Test solution**

TESTS

Water (*2.2.13*)

Maximum 120 ml/kg, determined on 20.0 g of the powdered drug (355) (*2.9.12*).

Total ash (*2.4.16*)

Maximum 15.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 4.0 per cent.

ASSAY

Essential oil (*2.8.12*)

Use 30.0 g of the drug to be examined, a 1000 ml round-bottomed flask and 400 ml of *water R* as the distillation liquid. Distil at a rate of 2-3 ml/min for 2 h without *xylene R* in the graduated tube.

Carvacrol and thymol

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Filter the essential oil obtained in the assay of essential oil over a small amount of *anhydrous sodium sulphate R* and dilute to 5.0 ml with *hexane R* by rinsing the apparatus and the anhydrous sodium sulphate.

Reference solution Dissolve 0.20 g of *thymol R* and 50 mg of *carvacrol R* in *hexane R* and dilute to 5.0 ml with the same solvent.

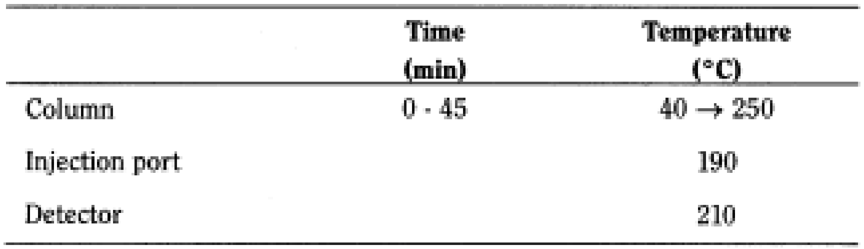
Column:
- — *material*: fused silica;
- — *size: l* = 60 m, Ø = 0.25 mm;
- — *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *nitrogen for chromatography R* or *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 45	40 → 250
Injection port		190
Detector		210

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of the reference solution; record the retention times of these substances.

System suitability Reference solution:

- — *resolution*: minimum of 1.5 between the peaks due to thymol and carvacrol.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of the sum of carvacrol and thymol.

Passion Flower Dry Extract

General Notices

(Ph Eur monograph 1882)

Ph Eur

DEFINITION

Dry extract produced from *Passion flower (1459).*

Content

Minimum 2.0 per cent of flavonoids, expressed as vitexin ($C_{21}H_{20}O_{10}$; M_r 432.4) (dried extract) .

PRODUCTION

The extract is produced from the herbal drug and ethanol (40 per cent *V/V* to 90 per cent *V/V*), methanol (60 per cent *V/V*) or acetone (40 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance: greenish-brown amorphous powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution To 0.25 g of the extract to be examined add *methanol R*. Shake, filter and dilute to 5 ml with *methanol R.*

Reference solution Dissolve 2.0 mg of *hyperoside R* and 2.0 mg of *rutin R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application 10 µl [or 5 µl] as bands.

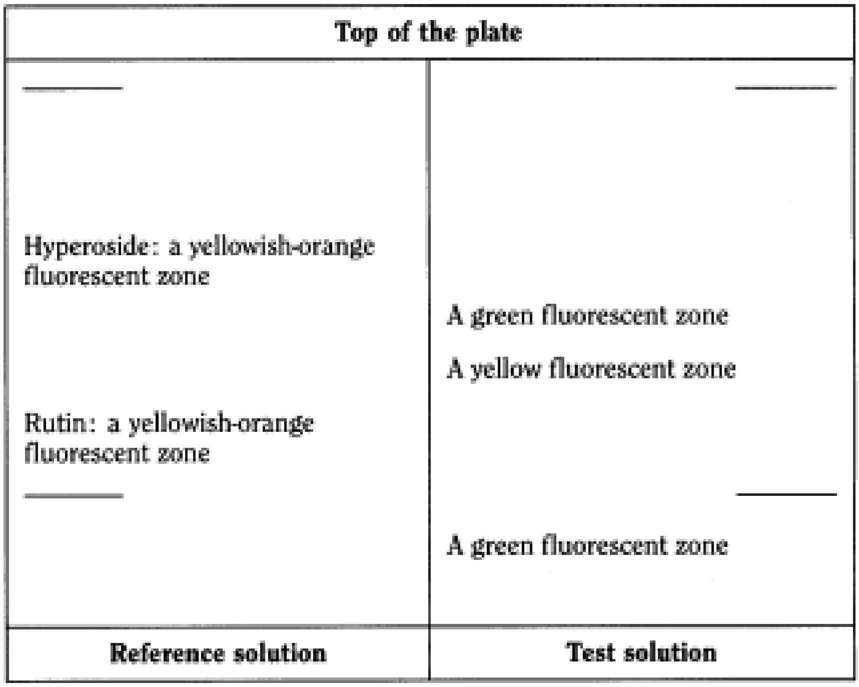
Development Over a path of 15 cm [or 5 cm].

Drying At 100-105 °C.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Other fluorescent zones may be present in the

chromatogram obtained with the test solution.



Top of the plate	
———	———
Hyperoside: a yellowish-orange fluorescent zone	
	A green fluorescent zone
	A yellow fluorescent zone
Rutin: a yellowish-orange fluorescent zone	
———	———
	A green fluorescent zone
Reference solution	**Test solution**

TESTS

Loss on drying *(2.8.17)*

Maximum 5.0 per cent, determined on 0.500 g.

ASSAY

Stock solution To 50 mg of the extract to be examined add *ethanol (60 per cent V/V) R*. Shake, filter and dilute to 100.0 ml with *ethanol (60 per cent V/V) R*.

Test solution Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the 25 ml volumetric flask. Add 10.0 ml of a solution containing 25.0 g/l of *boric acid R* and 20.0 g/l of *oxalic acid R* in *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Compensation liquid Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the 25 ml volumetric flask. Add 10.0 ml of *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

After 30 min, measure the absorbance (*2.2.25*) of the test solution at 401 nm.

Calculate the percentage content of total flavonoids, expressed as vitexin, from the following expression:

$$\frac{A \times 0.8}{m}$$

i.e. taking the specific absorbance of vitexin to be 628.

A = absorbance at 401 nm,

m = mass of the extract to be examined, in grams.

Ph Eur

Passion Flower

General Notices

Passiflora

(Ph Eur monograph 1459)

Ph Eur

DEFINITION

Fragmented or cut, dried aerial parts of *Passiflora incarnata L*. It may also contain flowers and/or fruits.

Content

Minimum 1.5 per cent of total flavonoids, expressed as vitexin ($C_{21}H_{20}O_{10}$; M_r 432.4) (dried drug).

IDENTIFICATION

A. The green or greenish-grey or brownish stem is ligneous, hollow, longitudinally striated, glabrous or very slightly pubescent, with a diameter that is generally less than 8 mm. The green or greenish-brown leaves are alternate, finely dentate and pubescent, deeply divided into 3 acute lobes of which the central lobe is the largest. The midrib is much more prominent on the lower surface. The petiole is pubescent and bears 2 dark nectaries near the lamina. The tendrils are very numerous and grow from the axils of the leaves; they are fine, smooth, round and terminated in cylindrical spirals. The radiate flowers, if present, have 3 small bracts and a corolla consisting of 5 white, elongated petals with several rows of filiform, petaloid appendices. If present, the greenish or brownish fruit is flattened and oval; it contains several flattened, brownish-yellow, pitted seeds.

B. Reduce to a powder (355) (*2.9.12*). The powder is light green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the leaf epidermis with sinuous walls and anomocytic stomata (*2.8.3*); numerous cluster crystals of calcium oxalate isolated or aligned along the veins; many isolated or grouped fibres from the stems associated with pitted vessels and tracheids; uniseriate trichomes with 1-3 thin-walled cells, straight or slightly curved, ending in a point or sometimes a hook. In addition, the powder shows, if flowers are present, papillose epidermises of the petals and appendages and pollen grains with a reticulate exine; and if mature fruits are present, scattered brown tannin cells and brownish-yellow, pitted fragments of the testa.

C. Examine the chromatograms obtained in the test for other species of *Passiflora*.

Results The chromatogram obtained with the test solution shows below the zone due to rutin in the chromatogram obtained with the reference solution a zone of intense yellow fluorescence, above it a zone of green fluorescence (diglycosylflavone), below the zone due to hyperoside in the chromatogram obtained with the reference solution a zone of yellow fluorescence (iso-orientin) and above a zone of green fluorescence (isovitexin), above the zone due to hyperoside in the chromatogram obtained with the reference solution a zone of brownish-yellow fluorescence (orientin) and above it a zone of green fluorescence (vitexin). These latter 2 zones may be absent. Further zones may be present.

TESTS

Other species of *Passiflora*

Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (355) (*2.9.12*) add 5 ml of *methanol R*. Heat to boiling under a reflux condenser for 10 min. Cool and filter.

Reference solution Dissolve with heating 2.0 mg of *rutin R* and 2.0 mg of *hyperoside R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *Anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow to dry in air for 30 min. Examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the lower third a zone of yellowish-brown fluorescence due to rutin and in the middle third a zone of yellowish-brown fluorescence due to hyperoside. The chromatogram obtained with the test solution shows no intense zones of greenish-yellow or orange-yellow fluorescence between the zone due to diglycosylflavones and that due to iso-orientin (P. *coerulea* and P. *edulis*).

Total ash (*2.4.16*)

Maximum 13.0 per cent.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

ASSAY

Stock solution In a 100 ml round-bottomed flask, introduce 0.200 g of the powdered drug (250) (*2.9.12*) and add 40 ml of *ethanol (60 per cent V/V) R*. Heat in a water-bath at 60 °C under a reflux condenser for 30 min while shaking frequently. Allow to cool and filter the mixture through a plug of absorbent cotton in a 100 ml flask. Transfer the absorbent cotton with the drug residue into the round-bottomed flask. Add 40 ml of *ethanol (60 per cent V/V) R* and heat again in a water-bath at 60 °C under reflux for 10 min. Allow to cool and filter the mixture and the first filtrate from the 100 ml flask through a filter paper into the 100 ml volumetric flask. Dilute to 100 ml with the same solvent, while rinsing the flask, round-bottomed flask and filter.

Test solution

Introduce 5.0 ml of stock solution into a flask. Evaporate to dryness under reduced pressure and take up the residue with 10 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. Add 10 ml of a solution consisting of 25 g/l of *boric acid R* and 20 g/l of oxalic acid in *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Compensation liquid

Introduce 5.0 ml of the stock solution into a second flask. Evaporate to dryness under reduced pressure and take up the residue with 10 ml of a mixture of 10 volumes of *methano R* and 100 volumes of *glacial acetic acid R*. Add 10 ml of *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

After 30 min, measure the absorbance (*2.2.25*) of the test solution at 401 nm, by comparison with the compensation liquid.

Calculate the percentage content of total flavonoids, expressed as vitexin, using the following expression:

i.e. taking the specific absorbance of vitexin to be 628.

$$\frac{A \times 0.8}{m}$$

A = absorbance at 401 nm

M = mass of the drug to be examined, in grams.

Ph Eur

Pelargonium Root

General Notices

(Ph Eur monograph 2264)

Ph Eur

DEFINITION

Dried, usually fragmented, underground organs of *Pelargonium sidoides* DC and/or *Pelargonium reniforme* Curt.

Content

Minimum 2.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The root is covered with dark, partly reddish-brown, longitudinally fissured bark. The transverse section shows, underneath the cork layer, yellow or white wood, which clearly shows partly brownish medullary rays.

B. Reduce to a powder (355 (*2.9.12*)). The powder is brownish-red. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: multilayer cork cells consisting of almost uniform, rectangular cells; fragments of parenchyma underneath the cork containing sclereids with a wide lumen; numerous calcium oxalate cluster crystals. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows simple starch granules without striations or cracks.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 0.5 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methanol R*, shake for 15 min and filter.

Reference solution Dissolve 1 mg of *scopoletin R* and 2 mg of *esculin R* in 20 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F254 plate* R (2-10 µm)].

Mobile phase *water R*, *methanol R*, *ethyl acetate R* (10:14:76 *V/V/V*).

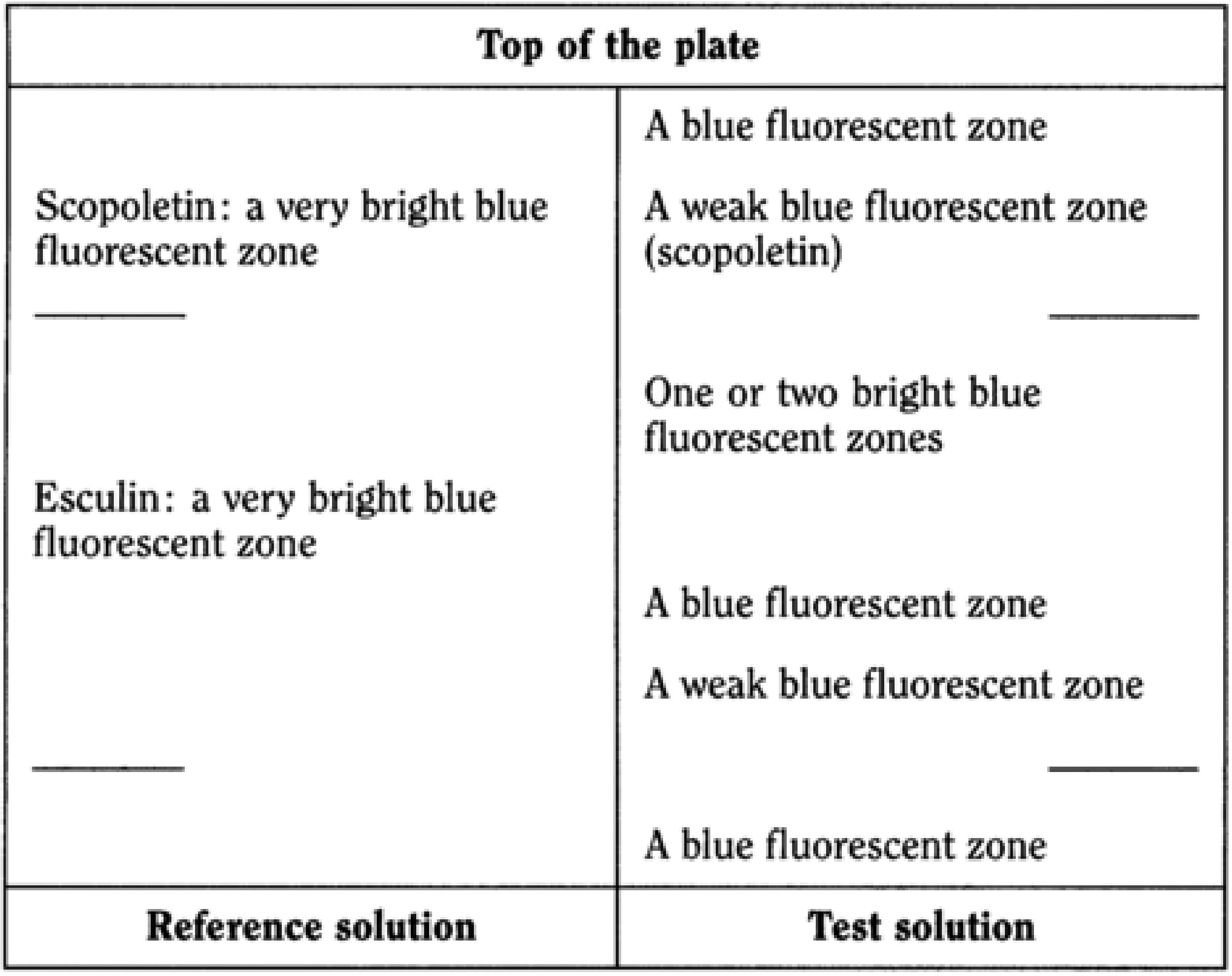
Application 10 µl [or 5 µl] as bands.

Development Over a path of 10 cm [or 6 cm].

Drying In air.

Detection Spray with *alcoholic potassium hydroxide solution R*. Examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other blue fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A blue fluorescent zone
Scopoletin: a very bright blue fluorescent zone	A weak blue fluorescent zone (scopoletin)
_______	_______
	One or two bright blue fluorescent zones
Esculin: a very bright blue fluorescent zone	
	A blue fluorescent zone
	A weak blue fluorescent zone
_______	_______
	A blue fluorescent zone
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C.

Total ash (*2.4.16*)

Maximum 12.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 3.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs (2.8.14). Use 0.750 g of the powdered drug (180) (*2.9.12*).

Ph Eur

Peppermint Leaf

General Notices

(Ph Eur monograph 0406)

Ph Eur

DEFINITION

Whole or cut dried leaves of *Mentha* × *piperita* L.

Content

Minimum 12 ml/kg of essential oil for the whole drug and minimum 9 ml/kg of essential oil for the cut drug.

CHARACTERS

Characteristic and penetrating odour.

Characteristic aromatic taste.

Peppermint leaf is green or brownish-green, with brownish-violet veins in some varieties. The petioles are green or brownish-violet.

IDENTIFICATION

A. The leaf is entire, broken or cut, thin, fragile and often crumpled; the entire leaf is 3-9 cm long and 1-3 cm wide. The lamina is oval or lanceolate, the apex acuminate, the margin sharply dentate and the base asymmetrical. Venation is pinnate, prominent on the lower surface, with lateral veins leaving the midrib at about 45°. The lower surface is slightly pubescent and secretory trichomes are visible under a lens (6×) as bright yellowish points. The petiole is grooved, usually up to 1 mm in diameter and 0.5-1 cm long.

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: leaf-tissue fragments with cells of the epidermis having sinuous-wavy walls and the cuticle striated over the veins and diacytic stomata (2.8.3) predominantly present on the lower epidermis; epidermis fragments from near the leaf margin with isodiametric cells straighter-walled showing distinct beading and pitting in anticlinal walls; covering trichomes short, conical, unicellular or bicellular, or elongated, uniseriate with 3-8 cells with striated cuticle; glandular trichomes of 2 types: (a) unicellular base with small, rounded unicellular head 15-25 µm in diameter; (b) unicellular base with enlarged oval head 55-70 µm in diameter composed of 8 radiating cells; dorsiventral mesophyll fragment with a single palisade layer and 4-6 layers of spongy parenchyma; yellowish crystals of menthol under the cuticle of secretory cells. Calcium oxalate crystals are absent.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 0.2 g of the recently powdered drug add 2 ml of *methylene chloride R*, shake for a few minutes and filter. Evaporate the filtrate to dryness at about 40 °C and dissolve the residue in 0.1 ml of *toluene R*.

Reference solution Dissolve 50 mg of *menthol R*, 20 µl of *cineole R*, 10 mg of *thymol R* and 10 µl of *menthyl acetate R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel* GF_{254} *plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

Application 10 µl of the reference solution and 20 µl of the test solution, as bands.

Development Over a path of 15 cm.

Drying In air until the solvent has evaporated.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatogram obtained with the test solution may show a light quenching zone situated just below the level of the zone due to thymol in the chromatogram obtained with the reference solution (carvone, pulegone).

Detection B Spray with *anisaldehyde solution R* and examine in daylight while heating for 5-10 min at 100-105 °C.

Results B The chromatogram obtained with the reference solution shows, in order of increasing R_F value: in the lower third a deep-blue or violet zone (menthol); a violet-blue or brown zone (cineole); a pink zone (thymol); and a bluish-violet zone (menthyl acetate). The chromatogram obtained with the test solution shows: a zone due to menthol (the most intense); a faint zone due to cineole; at R_F values between those of the cineole and thymol zones in the chromatogram obtained with the reference solution, it may show light pink, or bluish-grey or greyish-green zones (carvone, pulegone, isomenthone); in the middle of the chromatogram, a bluish-violet zone (menthyl acetate) and just below it a greenish-blue zone (menthone) appear; an intense reddish-violet zone (hydrocarbons) appears near the solvent front; other less intensely coloured zones also appear.

TESTS

Foreign matter (*2.8.2*)

Maximum 5 per cent stems, whose diameter is not greater than 1.5 mm; maximum 2 per cent foreign elements; not more than 8 per cent of the leaves show brown stains due to *Puccinia menthae*.

Carry out the determination using 10 g of the drug.

Water (*2.2.13*)

Maximum 110 ml/kg, determined on 20.0 g.

Total ash (*2.4.16*)

Maximum 15.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 1.5 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs (*2.8.12*). Use 20.0 g of crushed drug, a 500 ml flask, 200 ml of *water R* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 3-4 ml/min for 2 h.

Ph Eur

Peppermint Oil

General Notices

(Ph Eur monograph 0405)

Preparations

Gastro-resistant Peppermint Oil Capsules

Concentrated Peppermint Emulsion

Peppermint Spirit

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the fresh aerial parts of the flowering plant of *Mentha* × *piperita* L.

CHARACTERS

Appearance

A colourless, pale yellow or pale greenish-yellow liquid.

It has a characteristic odour and taste followed by a sensation of cold.

Solubility

Miscible with alcohol and with methylene chloride.

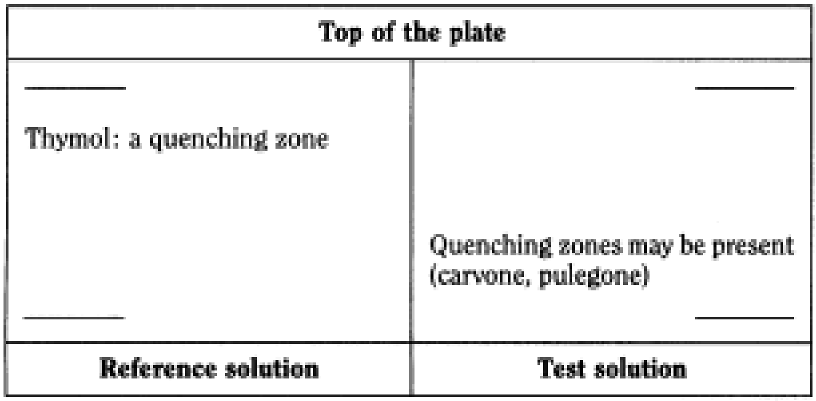
IDENTIFICATION

First identification B.

Second identification A.

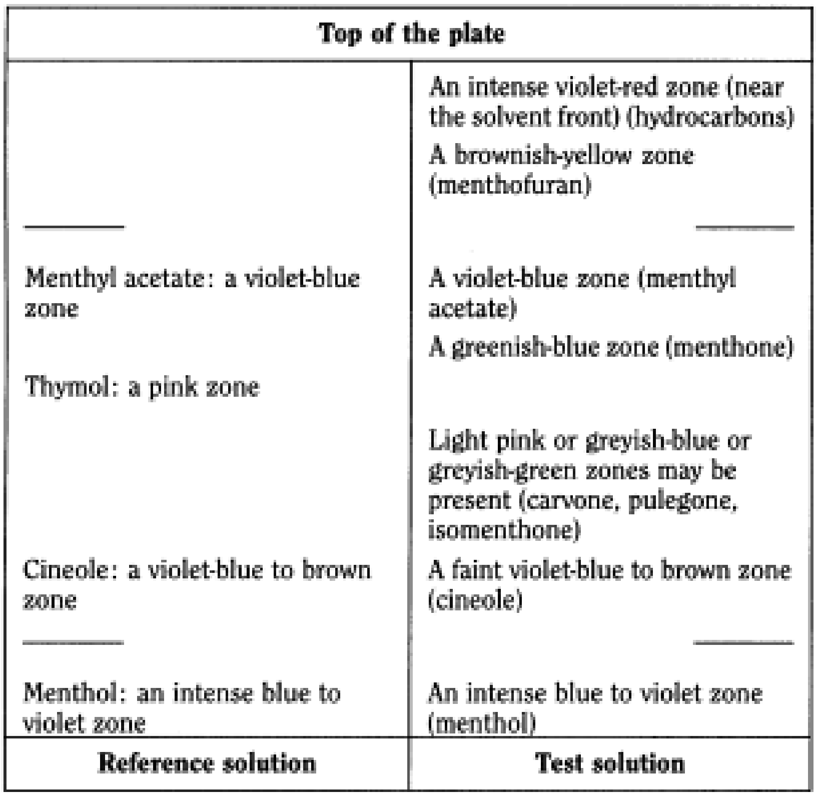
A. Examine the chromatograms obtained in the test for mint oil.

Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
Thymol: a quenching zone	
	Quenching zones may be present (carvone, pulegone)
———	———
Reference solution	**Test solution**

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other less intensely coloured zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	An intense violet-red zone (near the solvent front) (hydrocarbons)
	A brownish-yellow zone (menthofuran)
———	———
Menthyl acetate: a violet-blue zone	A violet-blue zone (menthyl acetate)
	A greenish-blue zone (menthone)
Thymol: a pink zone	
	Light pink or greyish-blue or greyish-green zones may be present (carvone, pulegone, isomenthone)
Cineole: a violet-blue to brown zone	A faint violet-blue to brown zone (cineole)
———	———
Menthol: an intense blue to violet zone	An intense blue to violet zone (menthol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution. Carvone and pulegone may be present in the chromatogram obtained with the test solution.

TESTS

Relative density (*2.2.5*)

0.900 to 0.916.

Refractive index (*2.2.6*)

1.457 to 1.467.

Optical rotation (*2.2.7*)

- 10° to - 30°.

Acid value (*2.5.1)*

Maximum 1.4, determined on 5.0 g diluted in 50 ml of the prescribed mixture of solvents.

Fatty oils and resinified essential oils (*2.8.7*)

It complies with the test for fatty oils and resinified essential oils.

Mint oil

A. Thin-layer chromatography (*2.2.27*).

Test solution Mix 0.1 g of the substance to be examined with *toluene R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *menthol R*, 20 µl of *cineole R*, 10 mg of *thymol R* and 10 µl of *menthyl acetate R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

Application 10 µl of the reference solution and 20 µl of the test solution, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 mm.

Detection B Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine immediately in daylight.

Result B The chromatogram obtained with the test solution shows no blue zone between the zones due to cineole and menthol.

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The chromatogram obtained with the test solution does not show a peak with the retention time of isopulegol that has an area of more than 0.2 per cent of the total area.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Mix 0.20 g of the substance to be examined with *hexane R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 10 µl of *limonene R,* 20 µl of *cineole R*, 40 µl of *menthone R*, 10 µl of *menthofuran R*, 10 µl of *isomenthone R*, 40 µl of *menthyl acetate R*, 20 µl of *isopulegol R*, 60 mg of *menthol R*, 20 µl of *pulegone R*, 10 µl of *piperitone R* and 10 µl of *carvone R* in *hexane R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dissolve 5 µl of *isopulegol R* in *hexane R* and dilute to 10 ml with the same solvent. Dilute 0.1 ml to 5 ml with *hexane R*.

Column:

— *material*: fused silica;

— *size: l* = 60 m, Ø = 0.25 mm;

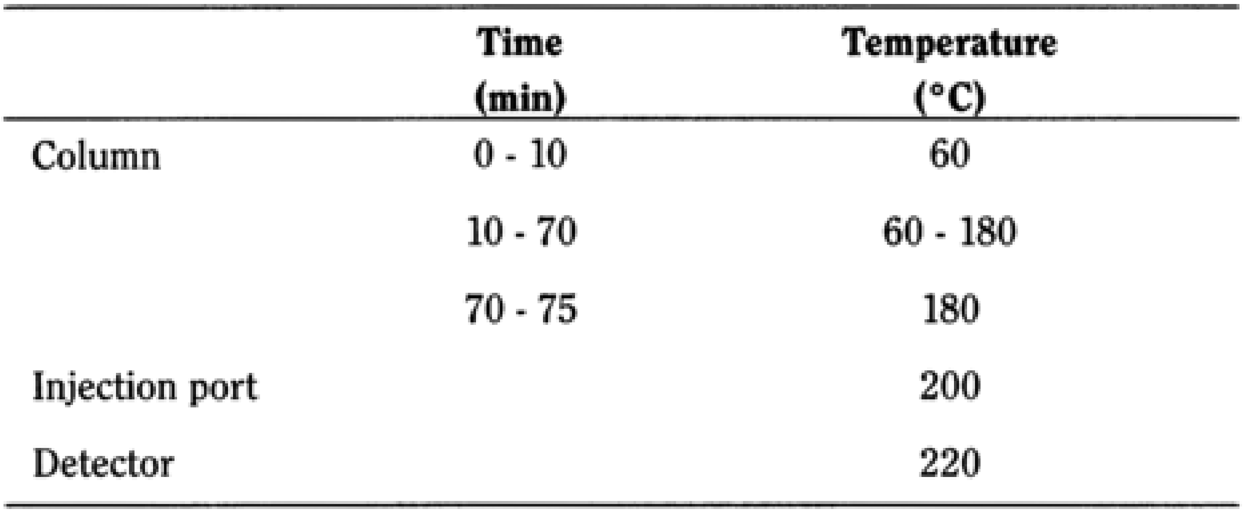
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 70	60 - 180
	70 - 75	180
Injection port		200
Detector		220

Detection Flame ionisation.

Injection 1 µl.

Elution order Order indicated in the composition of reference solution (a); record the retention times of these substances.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to limonene and cineole and minimum 1.5 between the peaks due to piperitone and carvone.

Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of the reference solution in the chromatogram obtained with the test solution (disregard the peak due to hexane).

Determine the percentage content of the components. The limits are within the following ranges:

— *limonene*: 1.0 per cent to 5.0 per cent;

— *cineole*: 3.5 per cent to 14.0 per cent;

— *menthone*: 14.0 per cent to 32.0 per cent;

— *menthofuran*: 1.0 per cent to 9.0 per cent;

— *isomenthone*: 1.5 per cent to 10.0 per cent;

— *menthyl acetate*: 2.8 per cent to 10.0 per cent;

— *isopulegol*: maximum 0.2 per cent;

— *menthol*: 30.0 per cent to 55.0 per cent;

— *pulegone*: maximum 4.0 per cent;

— *carvone*: maximum 1.0 per cent;

— *disregard limit*: peak area obtained with reference solution (b) (0.05 per cent).

The ratio of cineole content to limonene content is not less than 2.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Peru Balsam

General Notices

(Ph Eur monograph 0754)

Ph Eur

DEFINITION

Balsam obtained from the scorched and wounded trunk of *Myroxylon balsamum* (L.) Harms var. *pereirae* (Royle) Harms.

Content

45.0 per cent m/m to 70.0 per cent *m/m* of esters, mainly benzyl benzoate and benzyl cinnamate.

CHARACTERS

Appearance

Dark brown, viscous liquid which is transparent and yellowish-brown when viewed in a thin layer; it is not sticky, non-drying and does not form threads.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol, not miscible with fatty oils, except for castor oil.

IDENTIFICATION

A. Dissolve 0.20 g in 10 ml of *ethanol (96 per cent) R* . Add 0.2 ml of *ferric chloride solution R1* . A green or yellowish-green colour develops.

B. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.5 g of the substance to be examined in 10 ml of *ethyl acetate R* .

Reference solution Dissolve 4 mg of *thymol R* , 30 mg of *benzyl cinnamate R* and 80 µl of *benzyl benzoate R* in 5 ml of *ethyl acetate R* .

Plate *TLC silica gel GF$_{254}$ plate R.*

Mobile phase *glacial acetic acid R, ethyl acetate R, hexane R* (0.5:10:90 *V/V/V*).

Application 10 µl, as bands of 20 mm by 3 mm.

Development Twice over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm and mark the quenching zones.

Results A The chromatogram obtained with the reference solution shows in the upper third 2 quenching zones, the higher one due to benzyl benzoate and the lower one due to benzyl cinnamate. The chromatogram obtained with the test solution shows 2 quenching zones at the same levels and of approximately the same size.

Detection B Spray with a freshly prepared 200 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R*, using 10 ml for a plate 200 mm square and examine in daylight while heating at 100-105 °C for 5-10 min.

Results B The zones due to benzyl benzoate and benzyl cinnamate are blue against a yellow background. The chromatogram obtained with the reference solution shows at about the middle a violet-grey zone (thymol). In the chromatogram obtained with the test solution, a blue zone (nerolidol) is seen just below the level of the zone due to thymol in the chromatogram obtained with the reference solution. Just below the zone due to nerolidol, no blue zone is seen corresponding to a quenching zone seen when examined in ultraviolet light at 254 nm (colophony). In the upper and lower part of the chromatogram obtained with the test solution, other faint blue zones may be seen.

TESTS

Relative density *(2.2.5)*

1.14 to 1.17.

Saponification value *(2.5.6)*

230 to 255, determined on the residue obtained in the assay.

Artificial balsams

Shake 0.20 g with 6 ml of *light petroleum R1*. The light petroleum solution is clear and colourless and the whole of the insoluble parts of the balsam stick to the wall of the test-tube.

Fatty oils

Shake 1 g with 3 ml of a 1000 g/l solution of *chloral hydrate R*. The resulting solution is as clear as the 1000 g/l solution of *chloral hydrate R*.

Turpentine

Evaporate to dryness 4 ml of the solution obtained in the test for artificial balsams. The residue has no odour of turpentine.

ASSAY

To 2.50 g in a separating funnel add 7.5 ml of *dilute sodium hydroxide solution R* and 40 ml of *peroxide-free ether R* and shake vigorously for 10 min. Separate the lower layer and shake it with 3 quantities, each of 15 ml, of *peroxide-free ether R*. Combine the ether layers, dry over 10 g of *anhydrous sodium sulphate R* and filter. Wash the sodium sulphate with 2 quantities, each of 10 ml, of *peroxide-free ether R*. Combine the ether layers and evaporate to dryness. Dry the residue (esters) at 100-105 °C for 30 min and weigh.

STORAGE

Protected from light.

Ph Eur

Pine Silvestris Oil

General Notices

(Ph Eur monograph 1842)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation of the fresh leaves and branches of *Pinus sylvestris* L. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, colourless or pale yellow liquid.

Characteristic odour.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dilute 1 ml of the substance to be examined to 10 ml with *toluene R*.

Reference solution Dissolve 10 mg of *borneol R* and 10 µl of *bornyl acetate R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

Application 10 µl [or 2 µl] as bands.

Development Over a path of 15 cm [or 6 cm].

Drying In air.

Detection Treat with *anisaldehyde solution R*, heat at 100-105 °C for 5-10 min and examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore other faint zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	A pink zone (hydrocarbons)
———	———
Bornyl acetate: a brown or grey-brown zone	A brown or grey-brown zone (bornyl acetate)
	A pink zone
———	———
Borneol: a brown or grey-brown zone	A cluster of violet zones
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with reference solution (a).

TESTS

Relative density (*2.2.5*)

0.855 to 0.875.

Refractive index (*2.2.6*)

1.465 to 1.480.

Optical rotation (*2.2.7*)

- 9° to - 30°.

Acid value (*2.5.1*)

Maximum 1.0.

Peroxide value (*2.5.5*)

Maximum 20.

Fatty oils and resinified oils (*2.8.7*)

It complies with the test.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution The substance to be examined.

Reference solution (a) Dissolve 30 µl of α-*pinene R*, 10 mg of *camphene R*, 20 µl of β-*pinene R*, 10 µl of *car-3-ene R*, 10 µl of β-*myrcene R*, 20 µl of *limonene R*, 10 µl of *p-cymene R*, 10 µl of *terpinolene R*, 10 µl of *bornyl acetate R* and 10 µl of β-*caryophyllene R* in 1 ml of *heptane R*.

Reference solution (b) Dissolve 10 mg of *camphene R* in *heptane R* and dilute to 2 ml with the same solvent. Dilute 0.1 ml of the solution to 1 ml with *heptane R*.

Column:

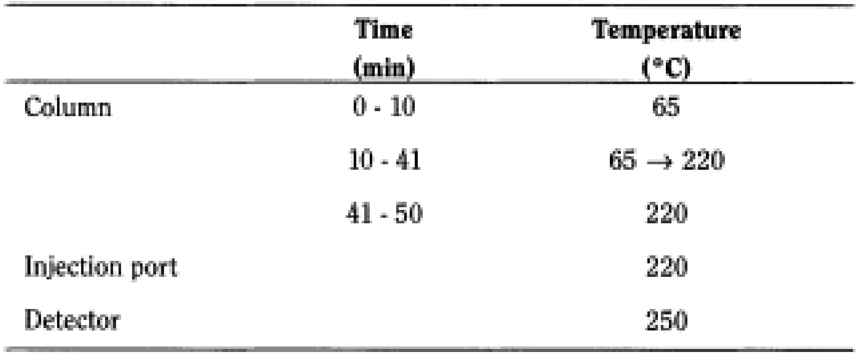
- — *material*: fused silica;
- — *size:* l = 60 m, Ø = 0.22 mm;
- — *stationary phase*: *macrogol 20 000 R* (0.2 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	65
	10 - 41	65 → 220
	41 - 50	220
Injection port		220
Detector		250

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the preparation of reference solution (a). Record the retention times of these substances.

System suitability Reference solution (a):

- — *resolution*: minimum 1.5 between the peaks due to car-3-ene and β-myrcene.

Identification of components Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution. The peak due to β-phellandrene is eluted after the peak due to limonene with a relative retention of about 1.03 with reference to limonene.

Determine the percentage content of these components. The limits are within the following ranges:

— α-*pinene*: 32.0 per cent to 60.0 per cent;

— *camphene*: 0.5 per cent to 2.0 per cent;

— β-*pinene*: 5.0 per cent to 22.0 per cent;

— *car-3-ene*: 6.0 per cent to 18.0 per cent;

— β-*myrcene*: 1.5 per cent to 10.0 per cent;

— *limonene*: 7.0 per cent to 12.0 per cent;

— β-*phellandrene*: maximum 2.5 per cent;

— *p-cymene*: maximum 2.0 per cent;

— *terpinolene*: maximum 4.0 per cent;

— *bornyl acetate*: 1.0 per cent to 4.0 per cent;

— β-*caryophyllene*: 1.0 per cent to 6.0 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b).

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Pinus Pinaster Type Turpentine Oil

General Notices

(Ph Eur monograph 1627)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation, followed by rectification at a temperature below 180 °C, from the oleoresin obtained by tapping *Pinus pinaster* Aiton. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, colourless or pale yellow liquid.

It has a characteristic odour.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)*.

Test solution Mix 1 ml of the substance to be examined with *toluene R* and dilute to 10 ml with the same solvent.

Reference solution Mix 10 µl of β-*pinene R* and 10 µl of *linalol R* with *toluene R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

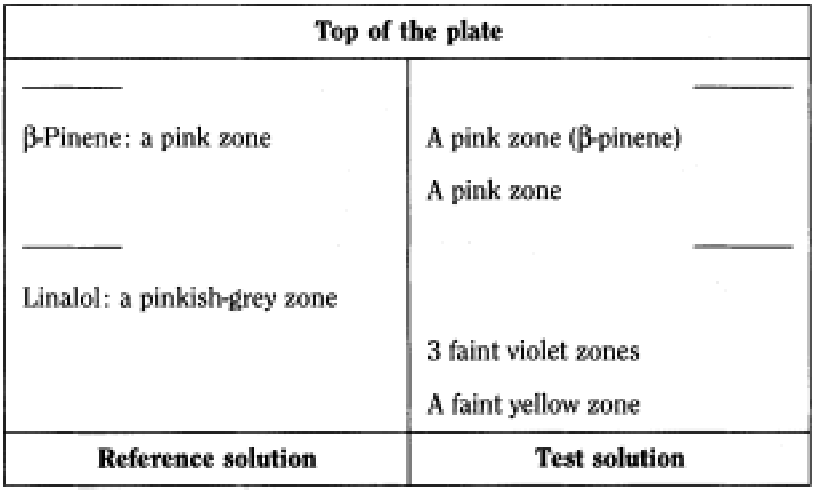
Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
β-Pinene: a pink zone	A pink zone (β-pinene)
	A pink zone
———	———
Linalol: a pinkish-grey zone	
	3 faint violet zones
	A faint yellow zone
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

0.856 to 0.872.

Refractive index *(2.2.6)*

1.465 to 1.475.

Optical rotation *(2.2.7)*

- 40° to - 28°.

Acid value *(2.5.1)*

Maximum 1.0.

Peroxide value *(2.5.5, Method B)*

Maximum 20.

Fatty oils and resinified essential oils *(2.8.7)*

It complies with the test for fatty oils and resinified essential oils.

Chromatographic profile

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution The substance to be examined.

Reference solution (a) Dissolve 30 µl of α-*pinene R,* 10 mg of *camphene R*, 20 µl of β-*pinene R*, 10 µl of *car-3-ene R*, 10 µl of β-*myrcene R*, 20 µl of *limonene R,* 10 µl of *longifolene R*, 10 µl of β-*caryophyllene R* and 10 mg of *caryophyllene oxide R* in 1 ml of *hexane R*.

Reference solution (b) Dissolve 5 µl of β-*caryophyllene R* in *hexane R* and dilute to 1 ml with the same solvent. Dilute 0.1 ml to 1 ml with *hexane R*.

Column:

— *material*: fused silica;

— *size*: *l* = 60 m, Ø = 0.25 mm;

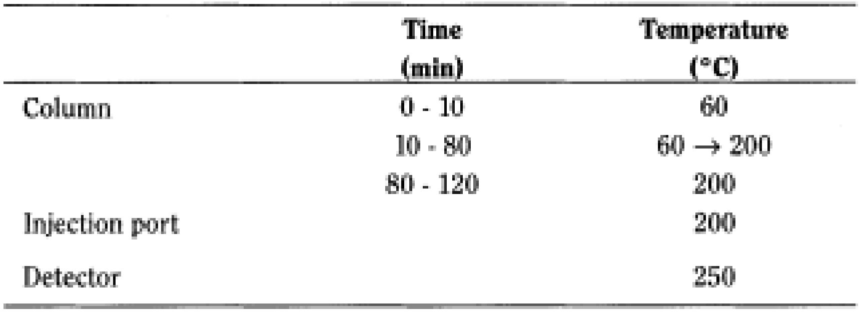
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.0 ml/min.

Split ratio 1:63.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 80	60 → 200
	80 - 120	200
Injection port		200
Detector		250

Detection Flame ionisation.

Injection 0.5 µl.

Elution order Order indicated in the composition of the reference solution (a); record the retention times of these substances.

System suitability:

— *resolution*: minimum 1.5 between the peaks due to car-3-ene and β-myrcene in the chromatogram obtained with reference solution (a).

Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution.

Determine the percentage content of these components. The limits are within the following ranges:

— α-*pinene*: 70.0 per cent to 85.0 per cent;

— *camphene*: 0.5 per cent to 1.5 per cent;

— β-*pinene*: 11.0 per cent to 20.0 per cent;

— *car-3-ene*: maximum 1.0 per cent;

— β-*myrcene*: 0.4 per cent to 1.5 per cent;

— *limonene*: 1.0 per cent to 7.0 per cent;

— *longifolene*: 0.2 per cent to 2.5 per cent;

— β-*caryophyllene*: 0.1 per cent to 3.0 per cent;

— *caryophyllene oxide*: maximum 1.0 per cent;

— *disregard limit:* area of the peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Residue on evaporation ***(2.8.9)***

Maximum 2.5 per cent.

STORAGE

In a well-filled, airtight container , protected from light and at a temperature not exceeding 25 °C.

Ph Eur

Plantain

General Notices

(Ribwort Plantain, Ph Eur monograph 1884)

Ph Eur

DEFINITION

Whole or fragmented, dried leaf and scape of *Plantago lanceolata* L. *s.l.*

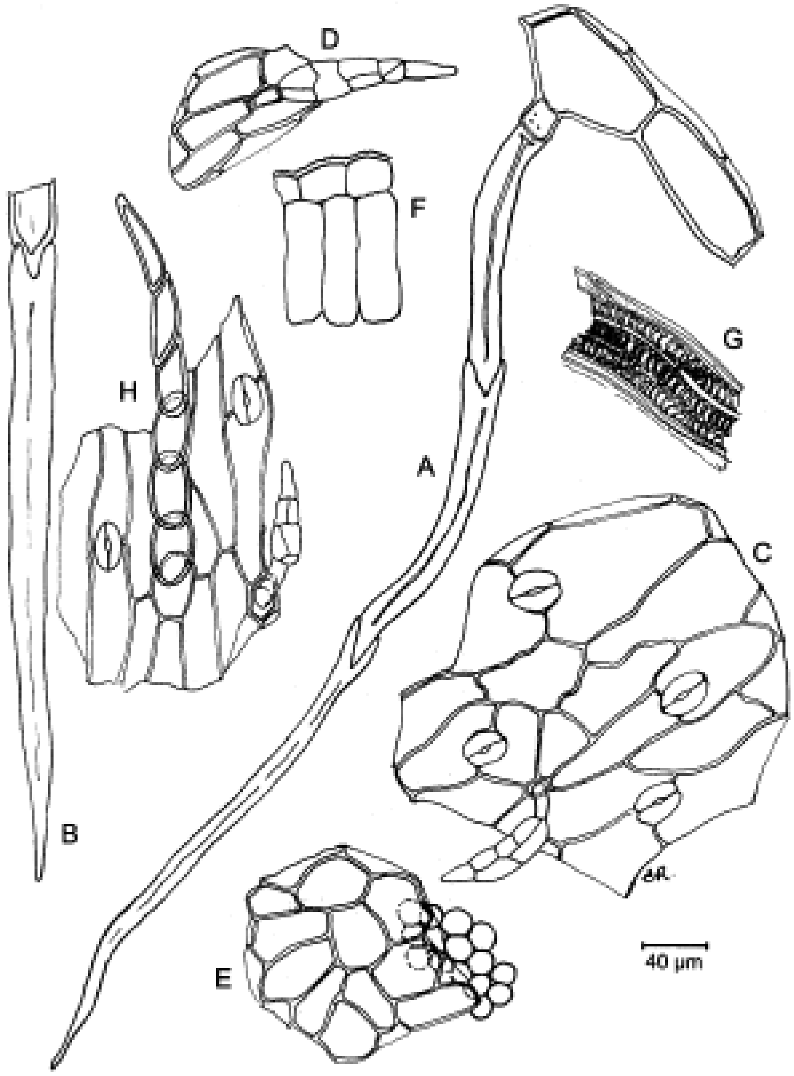
Content

Minimum 1.5 per cent of total *ortho*-dihydroxycinnamic acid derivatives expressed as acteoside ($C_{29}H_{36}O_{15}$; M_r 624.6) (dried drug).

IDENTIFICATION

A. The leaf is up to 30 cm long and 4 cm wide, yellowish-green to brownish-green, with a prominent, whitish-green, almost parallel venation on the abaxial surface. It consists of a lanceolate lamina narrowing at the base into a channelled petiole. The margin is indistinctly dentate and often undulate. It has 3, 5 or 7 primary veins, nearly equal in length and running almost parallel. Hairs may be almost absent, sparsely scattered or sometimes abundant, especially on the lower surface and over the veins. The scape is brownish-green, longer than the leaves, 3-4 mm in diameter and is deeply grooved longitudinally, with 5-7 conspicuous ribs. The surface is usually covered with fine hairs.

B. Reduce to a powder (355) (*2.9.12*). The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of epidermis, composed of cells with irregularly sinuous anticlinal walls, the fragments from the scape with thickened outer walls and a coarsely ridged cuticle; stomata mostly of the diacytic type (2.8.3) and sometimes anomocytic; the multicellular, uniseriate, conical covering trichomes are highly characteristic, with a basal cell larger than the other epidermal cells followed by a short cell supporting 2 or more elongated cells with the lumen narrow and variable, occluded at intervals corresponding to slight swellings in the trichome and giving a jointed appearance; the terminal cell has an acute apex and a filiform lumen; the glandular trichomes have a unicellular cylindrical stalk and a multicellular, elongated, conical head consisting of several rows of small cells and a single terminal cell; dense groups of lignified fibro-vascular tissue with narrow, spirally and annularly thickened vessels and slender, moderately thickened fibres.



A. Covering trichome of the leaf (usually only fragments observed)

B. Fragment of covering trichome of the leaf

C. Lower epidermis of lamina with glandular trichome

D. Glandular trichome

E. Upper epidermis of lamina with palisade parenchyma, in surface view

F. Upper epidermis and palisade parenchyma, in section view

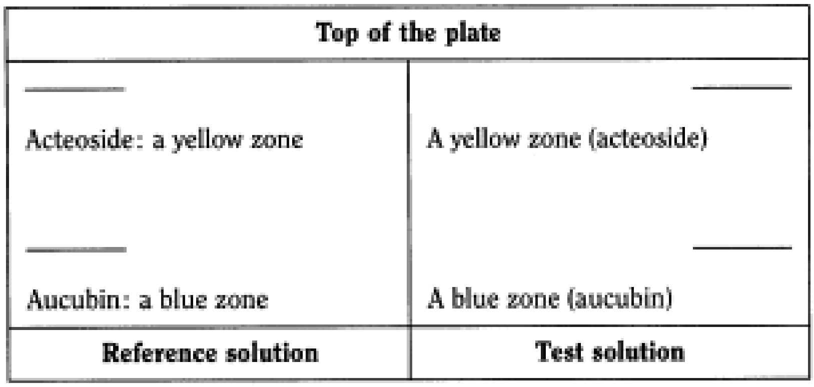
G. Vessels

H. Epidermis from the scape

Figure 1884.-1. – *Illustration of powdered herbal drug of ribwort plantain (see Identification B)*

C. Examine the chromatograms obtained in the test for Digitalis lanata leaves.

Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatograms obtained with the test solution.



Top of the plate	
———	———
Acteoside: a yellow zone	A yellow zone (acteoside)
———	———
Aucubin: a blue zone	A blue zone (aucubin)
Reference solution	**Test solution**

TESTS

***Digitalis lanata* leaves**

Thin layer chromatography (*2.2.27*).

Solvent mixture *water R*, *methanol R* (30:70 *V/V*).

Test solution Use a freshly prepared solution. To 1 g of the powdered drug (355) (*2.9.12*) in a 25 ml flask, add 10 ml of the solvent mixture and shake for 30 min. Filter, rinse the flask and filter with 2 quantities, each of 5 ml, of the solvent mixture. Dilute to 25 ml with the solvent mixture.

Reference solution Dissolve 1 mg of *acteoside R* and 1 mg of *aucubin R* in 1 ml of the solvent mixture.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetic acid R*, *anhydrous formic acid R*, *water R*, *ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application 10 µl as bands.

Development Over a path of 8 cm; heat immediately after development at about 120 °C for 5-10 min.

Detection A Examine in daylight.

Detection B Examine in ultraviolet light at 365 nm.

Results B The chromatogram obtained with the test solution shows no bright blue fluorescent zone just below the reddish-brown fluorescent zone corresponding to aucubin in the chromatogram obtained with the reference solution.

Foreign matter (***2.8.2***)

Maximum 5 per cent of leaves of different colour and maximum 2 per cent of other foreign matter.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 14.0 per cent.

ASSAY

Stock solution In a flask, place 1.000 g of the powdered drug (355) (*2.9.12*) and add 90 ml of *ethanol (50 per cent V/V) R*. Boil in a water-bath under a reflux condenser for 30 min. Allow to cool and filter into a 100 ml volumetric flask. Rinse the flask and the filter with 10 ml of *ethanol (50 per cent V/V) R*. Combine the filtrate and the rinsings and dilute to 100.0 ml with *ethanol (50 per cent V/V) R*.

Test solution To a 10 ml volumetric flask add, mixing after each addition, 1.0 ml of the stock solution, 2 ml of *0.5 M hydrochloric acid*, 2 ml of a solution prepared by dissolving 10 g of *sodium nitrite R* and 10 g of *sodium molybdate R* in 100 ml of *water R*, and 2 ml of dilute sodium hydroxide *solution R*. Dilute to 10.0 ml with *water R*.

Immediately measure the absorbance (*2.2.25*) of the test solution at 525 nm using as compensation liquid a solution prepared as follows: to a 10 ml volumetric flask add 1.0 ml of the stock solution, 2 ml of *0.5 M hydrochloric acid* and 2 ml of *dilute sodium hydroxide solution R*, and dilute to 10.0 ml with *water R*.

Calculate the percentage content of total ortho-dihydroxycinnamic acid derivatives, expressed as acteoside, using the following expression:

$$\frac{A \times 1000}{185 \times m}$$

i.e. taking the specific absorbance to be 185 for acteoside at 525 nm.

A = absorbance of the test solution at 525 nm,

m = mass of the substance to be examined, in grams.

Ph Eur

Podophyllum Resin

General Notices

Podophyllin

Preparation

Compound Podophyllin Paint

DEFINITION

Podophyllum Resin is the resin obtained from rhizomes and roots of *Podophyllum hexandrum* Royle (*P. emodi* Wall.). It contains not less than 50.0% of total aryltetralin lignans, calculated as podophyllotoxin.

CHARACTERISTICS

An amorphous powder varying in colour from light brown to greenish yellow, or brownish grey masses.

Partly soluble in hot *water*, from which it is precipitated on cooling, in *ether* and in 5M *ammonia*.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* GF_{254} *plate* and a mixture of 1 volume of *methanol* and 25 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, as bands, 10 µl of each of the following solutions in *methanol* containing (1) 1% w/v of the substance being examined, (2) 0.5% w/v of *podophyllotoxin* and (3) 0.1% w/v of *phenazone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The chromatogram obtained with solution (1) exhibits quenching zones corresponding in position to the principal quenching zones in the chromatograms obtained with solutions (2) and (3). Other quenching zones may be present in the chromatogram obtained with solution (1). Spray the plate with *methanolic sulphuric acid (50%)* and heat at 130° for 10 minutes. The chromatogram obtained with solution (1) exhibits a purplish zone (podophyllotoxin) corresponding in position and colour to the principal zone in solution (2) and a purplish zone (4′-demethylpodophyllotoxin) corresponding in position to the quenching zone found in solution (3). Other coloured zones may be present in the chromatogram obtained with solution (1).

TESTS

Matter insoluble in ethanol (96%)

Shake 1 g, finely powdered, with 20 ml of *ethanol (96%)* for 5 minutes, filter through a sintered-glass crucible (ISO 4793, porosity grade 2, is suitable), wash the filter with *ethanol (96%)* and dry at 105°. The residue weighs not more than 25 mg.

Matter insoluble in 5M ammonia

Shake 0.5 g, finely powdered, with 30 ml of 5M *ammonia* for 30 minutes at about 20°; filter through a sintered-glass crucible (ISO 4793, porosity grade 2, is suitable) and wash the flask and filter with 30 ml of *water*, the time taken for filtering and washing being not more than 10 minutes. Dry the filter and residue to constant weight at 105°. The residue weighs not less than 0.18 g and not more than 0.30 g.

Loss on drying

When dried to constant weight at 105°, loses not more than 5.0% of its weight. Use 1 g.

Sulphated ash

Not more than 1.0%, Appendix IX A.

ASSAY

Dissolve 0.5 g in sufficient *ethanol (96%)* to produce 100 ml. To 10 ml of this solution in a separating funnel add 190 ml of *water* and extract with six 30 ml quantities of *dichloromethane*. Combine the dichloromethane layers, extract with 10 ml of 0.2M *sodium hydroxide* followed by five 10 ml quantities of *water* and wash each of the six aqueous layers separately with the same 20 ml quantity of *dichloromethane*. Combine the dichloromethane solutions, filter through absorbent cotton and evaporate the filtrate to dryness. Dissolve the residue in sufficient *ethanol (96%)* to produce 100 ml, dilute 10 ml of this solution to 50 ml with *ethanol (96%)* and measure the *absorbance* of the resulting solution at the maximum at 292 nm, Appendix II B. Calculate the content of total aryltetralin lignans expressed as podophyllotoxin, taking 105.4 as the value of A (1%, 1 cm) at the maximum at 292 nm.

STORAGE

Podophyllum Resin should be protected from light. On exposure to light, or to temperatures above 25°, it becomes darker in colour.

LABELLING

The label states the botanical source.

Prepared Belladonna

General Notices

Prepared Belladonna Herb

(Ph Eur monograph 0222)

Ph Eur

DEFINITION

Belladonna leaf powder (180) *(2.9.12)* adjusted, if necessary, by adding powdered lactose or belladonna leaf powder with a lower alkaloidal content.

Content

0.28 per cent to 0.32 per cent of total alkaloids, expressed as hyoscyamine (M_r 289.4) (dried drug).

CHARACTERS

Slightly nauseous odour.

IDENTIFICATION

A. The powder is dark green. Examine under a microscope, using *chloral hydrate solution R* . The powder shows the following diagnostic characters: fragments of leaf lamina showing sinuous-walled epidermal cells, a striated cuticle and numerous stomata predominantly present on the lower epidermis (anisocytic and also some anomocytic) *(2.8.3)* ; multicellular uniseriate covering trichomes with smooth cuticle, glandular trichomes with unicellular heads and multicellular, uniseriate stalks or with multicellular heads and unicellular stalks; parenchyma cells including rounded cells containing microsphenoidal crystals of calcium oxalate; annular and spirally thickened vessels. The powdered drug may also show the following: fibres and reticulately thickened vessels from the stems; subspherical pollen grains, 40-50 µm in diameter, with 3 germinal pores, 3 furrows and an extensively pitted exine; fragments of the corolla, with a papillose epidermis or bearing numerous covering or glandular trichomes of the types previously described; brownish-yellow seed fragments containing irregularly sclerified and pitted cells of the testa. Examined in *glycerol (85 per cent) R* , the powder may be seen to contain lactose crystals.

B. Shake 1 g with 10 ml of *0.05 M sulphuric acid* for 2 min. Filter and add to the filtrate 1 ml of *concentrated ammonia R* and 5 ml of *water R* . Shake cautiously with 15 ml of *ether R* , avoiding formation of an emulsion. Separate the ether layer and dry over *anhydrous sodium sulphate R* . Filter and evaporate the ether in a porcelain dish. Add 0.5 ml of *fuming nitric acid R* and evaporate to dryness on a water-bath. Add 10 ml of *acetone R* and, dropwise, a 30 g/l solution of *potassium hydroxide R* in *ethanol (96 per cent) R* . A deep violet colour develops.

C. Examine the chromatograms obtained in the test Chromatography.

Results The principal zones in the chromatograms obtained with the test solution are similar in position, colour and size to the principal zones in the chromatogram obtained with the same volume of the reference solution.

TESTS

Chromatography

Thin-layer chromatography *(2.2.27)* .

Test solution To 0.6 g of the drug to be examined add 15 ml of *0.05 M sulphuric acid* , shake for 15 min and filter. Wash the filter with *0.05 M sulphuric acid* until 20 ml of filtrate is obtained. To the filtrate add 1 ml of *concentrated ammonia R* and shake with 2 quantities, each of 10 ml, of *peroxide-free ether R* . If necessary, separate by centrifugation. Dry the combined ether layers over *anhydrous sodium sulphate R* , filter, and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 ml of *methanol R* .

Reference solution Dissolve 50 mg of *hyoscyamine sulphate R* in 9 ml of *methanol R* . Dissolve 15 mg of *hyoscine hydrobromide R* in 10 ml of *methanol R* . Mix 1.8 ml of the hyoscine hydrobromide solution and 8 ml of the hyoscyamine sulphate solution.

Plate TLC silica gel G plate R.

Mobile phase concentrated ammonia R, water R, acetone R (3:7:90 *V/V/V*).

Application 10 µl and 20 µl of each solution, as bands of 20 mm by 3 mm, leaving 1 cm between each band.

Development Over a path of 10 cm.

Drying At 100-105 °C for 15 min; allow to cool.

Detection A Spray with *potassium iodobismuthate solution R2* , using about 10 ml for a plate 200 mm square, until orange or brown zones become visible against a yellow background.

Results A The zones in the chromatograms obtained with the test solution are similar in position (hyoscyamine in the lower third, hyoscine in the upper third) and colour to those in the chromatograms obtained with the reference solution; the zones in the chromatograms obtained with the test solution are at least equal in size to the corresponding zones in the chromatogram obtained with the same volume of the reference solution; faint secondary zones may appear, particularly in the middle of the chromatogram obtained with 20 µl of the test solution or near the starting-point in the chromatogram obtained with 10 µl of the test solution.

Detection B Spray with *sodium nitrite solution R* until the coating is transparent and examine after 15 min.

Results B The zones due to hyoscyamine in the chromatograms obtained with the test solution and the reference solution change from brown to reddish-brown but not to greyish-blue (atropine), and any secondary zones disappear.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 16.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 4.0 per cent.

ASSAY

a) Determine the loss on drying *(2.2.32)* on 2.000 g by drying in an oven at 105 °C.

b) Moisten 10.00 g with a mixture of 5 ml of *ammonia R*, 10 ml of *ethanol (96 per cent) R* and 30 ml of *peroxide-free ether R* and mix thoroughly. Transfer the mixture to a suitable percolator, if necessary with the aid of the extracting mixture. Allow to macerate for 4 h and percolate with a mixture of 1 volume of *chloroform R* and 3 volumes of *peroxide-free ether R* until the alkaloids are completely extracted. Evaporate to dryness a few millilitres of the liquid flowing from the percolator, dissolve the residue in *0.25 M sulphuric acid* and verify the absence of alkaloids using *potassium tetraiodomercurate solution R*. Concentrate the percolate to about 50 ml by distilling on a water-bath and transfer it to a separating funnel, rinsing with *peroxide-free ether R*. Add a quantity of *peroxide-free ether R* equal to at least 2.1 times the volume of the percolate to produce a liquid of a density well below that of water. Shake the solution with no fewer than 3 quantities, each of 20 ml, of *0.25 M sulphuric acid*, separate the 2 layers by centrifugation if necessary and transfer the acid layers to a 2nd separating funnel. Make the acid layer alkaline with *ammonia R* and shake with 3 quantities, each of 30 ml, of *chloroform R*. Combine the chloroform layers, add 4 g of *anhydrous sodium sulphate R* and allow to stand for 30 min with occasional shaking. Decant the chloroform and wash the sodium sulphate with 3 quantities, each of 10 ml, of *chloroform R*. Add the washings to the chloroform extract, evaporate to dryness on a water-bath and heat in an oven at 100-105 °C for 15 min. Dissolve the residue in a few millilitres of *chloroform R*, add 20.0 ml of *0.01 M sulphuric acid* and remove the chloroform by evaporation on a water-bath. Titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red mixed solution R* as indicator.

Calculate the percentage content of total alkaloids, expressed as hyoscyamine, using the following expression:

$$\frac{57.88 \times (20 - n)}{(100 - d) \times m}$$

d = loss on drying as a percentage;

n = volume of *0.02 M sodium hydroxide* used, in millilitres;

m = mass of drug used, in grams.

STORAGE

In an airtight container.

Ph Eur

Prepared Ipecacuanha

General Notices

(Ph Eur monograph 0093)

Ph Eur

DEFINITION

Ipecacuanha root powder (180) *(2.9.12)* adjusted, if necessary, by the addition of powdered lactose or ipecacuanha root powder with a lower alkaloidal content.

Content

1.9 per cent to 2.1 per cent of total alkaloids, expressed as emetine ($C_{29}H_{40}N_2O_4$; M_r 480.7) (dried drug).

CHARACTERS

Appearance

Light grey or yellowish-brown powder.

Slight odour.

IDENTIFICATION

A. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: parenchymatous cells, raphides of calcium oxalate up to 80 µm in length either in bundles or scattered throughout the powder; fragments of tracheids and vessels usually 10-20 µm in diameter, with bordered pits; larger vessels and sclereids from the rhizome. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows simple or 2-8-compound starch granules contained in parenchymatous cells, the simple granules being up to 15 µm in diameter in *Cephaelis ipecacuanha* and up to 22 µm in diameter in *C. acuminata*. Examined in *glycerol (85 per cent) R*, it may be seen to contain lactose crystals.

B. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.1 g of the drug to be examined in a test-tube add 0.05 ml of *concentrated ammonia R* and 5 ml of *ether R* and stir the mixture vigorously with a glass rod. Allow to stand for 30 min and filter.

Reference solution Dissolve 2.5 mg of *emetine hydrochloride CRS* and 3 mg of *cephaeline hydrochloride CRS* in *methanol R* and dilute to 20 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R*, *methanol R*, *ethyl acetate R*, *toluene R* (2:15:18:65 *V/V/V/V*).

Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Spray with a 5 g/l solution of *iodine R* in *ethanol (96 per cent) R*; heat at 60 °C for 10 min and examine in daylight.

Results A The chromatograms obtained with the test solution and the reference solution show in the lower part a yellow zone due to emetine and below it a light brown zone due to cephaeline.

Detection B Examine in ultraviolet light at 365 nm.

Results B The zone due to emetine shows an intense yellow fluorescence and that due to cephaeline a light blue fluorescence. The chromatogram obtained with the test solution also shows faint fluorescent zones.

With prepared *C. acuminata*, the principal zones in the chromatogram obtained with the test solution are similar in position, fluorescence and size to the zones in the chromatogram obtained with the reference solution.

With prepared *C. ipecacuanha*, the only difference is that the zone due to cephaeline in the chromatogram obtained with the test solution is much smaller than the corresponding zone in the chromatogram obtained with the reference solution.

TESTS

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

ASSAY

To 7.5 g in a dry flask, add 100 ml of *ether R* and shake for 5 min. Add 5 ml of *dilute ammonia R1*, shake for 1 h, add 5 ml of *water R* and shake vigorously. Decant the ether layer into a flask through a plug of cotton. Wash the residue in the flask with 2 quantities, each of 25 ml, of *ether R*, decanting each portion through the same plug of cotton. Combine the ether solutions and eliminate the ether by distillation. Dissolve the residue in 2 ml of *ethanol (90 per cent V/V) R*, evaporate the ethanol to dryness and heat at 100 °C for 5 min. Dissolve the residue in 5 ml of previously neutralised *ethanol (90 per cent V/V) R*, warming on a water-bath, add 15.0 ml of *0.1 M hydrochloric acid* and titrate the excess acid with *0.1 M sodium hydroxide* using 0.5 ml of *methyl red mixed solution R* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 24.03 mg of total alkaloids, expressed as emetine.

STORAGE

In an airtight container.

Prepared Opium

General Notices

(Ph Eur monograph 1840)

Ph Eur

DEFINITION

Raw opium powdered (180) (*2.9.12*), and dried at a temperature not exceeding 70 °C.

Content:

— *morphine* ($C_{17}H_{19}NO_3$; M_r 285.3): 9.8 per cent to 10.2 per cent (drug dried at 100-105 °C for 4 h);

— *codeine* ($C_{18}H_{21}NO_3$; M_r 299.4): minimum 1.0 per cent (drug dried at 100-105 °C for 4 h).

Content adjusted if necessary by adding a suitable excipient or raw opium powder.

CHARACTERS

Appearance

Yellowish-brown or dark brown powder.

IDENTIFICATION

A. Examine under a microscope using a 20 g/l solution of *potassium hydroxide R*. It is seen to consist of granules of latex agglomerated in irregular masses, and of light brown elongated filaments. Some fragments of vessels and rather elongated, refringent crystals are also visible, as well as a smaller number of round pollen grains and fragments of elongated fibres. Hairs of various lengths with sharp points and fragments of epicarp consisting of polygonal cells with thick walls defining a stellate lumen may be present. Examine under a microscope using *glycerol (85 per cent) R*. Particles of excipient and a few grains of starch introduced during the handling of the latex may be seen.

B. Thin-layer chromatography (*2.2.27*).

Test solution Triturate 0.10 g of the drug to be examined with 5 ml of *ethanol (70 per cent V/V) R*, rinse with 3 ml of *ethanol (70 per cent V/V) R*, transfer to a 25 ml conical flask. Heat in a water-bath at 50-60 °C with stirring for 30 min. Cool, filter, wash the filter with *ethanol (70 per cent V/V) R* and dilute the filtrate to 10 ml with the same solvent.

Reference solution Dissolve 2.0 mg of *papaverine hydrochloride R*, 12.0 mg of *codeine phosphate R*, 12.0 mg of *noscapine hydrochloride R* and 25.0 mg of *morphine hydrochloride R* in *ethanol (70 per cent V/V) R* and dilute to 25.0 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R*, *ethanol (96 per cent) R*, *acetone R*, *toluene R* (2:6:40:40 *V/V/V/V*). Use a freshly prepared mixture.

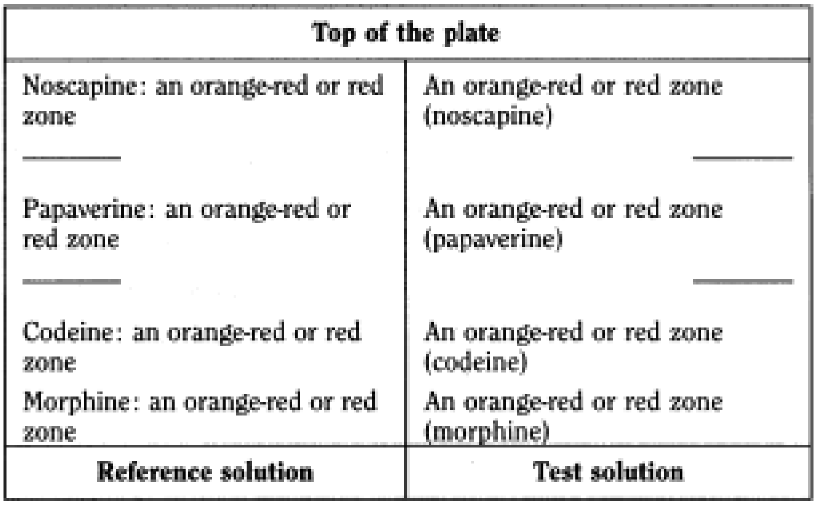
Application 20 µl, as bands of 20 mm by 3 mm.

Development Over a path of 15 cm.

Drying At 100-105 °C for 15 min.

Detection Allow to cool and spray with *potassium iodobismuthate solution R2* and then with a 4 g/l solution of *sulphuric acid R*, examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, a dark red zone (thebaine) situated between the codeine zone and the papaverine zone may be present in the chromatogram obtained with the test solution.



Top of the plate	
Noscapine: an orange-red or red zone	An orange-red or red zone (noscapine)
Papaverine: an orange-red or red zone	An orange-red or red zone (papaverine)
Codeine: an orange-red or red zone	An orange-red or red zone (codeine)
Morphine: an orange-red or red zone	An orange-red or red zone (morphine)
Reference solution	**Test solution**

C. To 1.0 g of the drug to be examined add 5 ml of *water R*, shake for 5 min and filter. To the filtrate add 0.25 ml of *ferric chloride solution R2*. A red colour develops which does not disappear on the addition of 0.5 ml of *dilute hydrochloric acid R*.

TESTS

Thebaine

Liquid chromatography (*2.2.29*).

Test solution Suspend 1.00 g of the drug to be examined in 50 ml of *ethanol (50 per cent V/V) R*, mix using sonication for 1 h, allow to cool and dilute to 100.0 ml with the same solvent. Allow to stand. To 10.0 ml of the supernatant liquid, add 5 ml of *ammonium chloride buffer solution pH 9.5 R*, dilute to 25.0 ml with *water R* and mix. Transfer 20.0 ml of the solution to a chromatography column about 0.15 m long and about 30 mm in internal diameter containing 15 g of *kieselguhr for chromatography R*. Allow to stand for 15 min. Elute with 2 quantities, each of 40 ml, of a mixture of 15 volumes of *2-propanol R* and 85 volumes of *methylene chloride R*. Evaporate the eluate to dryness *in vacuo* at 40 °C. Transfer the residue to a volumetric flask with the aid of the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution Dissolve 25.0 mg of *thebaine R* in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Precolumn:
— *size:* *l* = 4 mm, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Column:
— *size:* *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.0 g of sodium *heptanesulphonate monohydrate R* in 420 ml of *water R*, adjust to pH 3.2 with phosphoric acid (4.9 g/l H_3PO_4) (about 5 ml) and add 180 ml of *acetonitrile R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection A suitable volume with a loop injector.

System suitability Reference solution:

— *mass distribution ratio*: minimum 3.0 for the peak due to thebaine.

Calculate the percentage content of alkaloid from the expression:

$$\frac{m_1 \times A_2 \times 125}{m_2 \times A_1} \times \frac{100}{100 - h}$$

m_1 = mass of the alkaloid in the reference solution, in grams,

m_2 = mass of the substance to be examined in the test solution, in grams,

A_1 = area of the peak due to the alkaloid in the chromatogram obtained with the reference solution,

A_2 = area of the peak due to the alkaloid in the chromatogram obtained with the test solution,

h = percentage loss on drying.

Limit:
— *thebaine*: maximum 3.0 per cent (dried drug).

Loss on drying (*2.2.32*)

Maximum 8.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Total ash (*2.4.16*)

Maximum 6.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for thebaine with the following modifications.

Reference solution Dissolve 0.100 g of *morphine hydrochloride R* and 25.0 mg of *codeine R* in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

System suitability Reference solution:

— *resolution*: minimum 2.5 between the peaks due to morphine and codeine; if necessary, adjust the volume of acetonitrile in the mobile phase;

— *repeatability*: maximum relative standard deviation of 1.0 per cent for the peak area due to morphine, determined on 6 replicate injections.

Calculate the percentage content of morphine and codeine from the expression given in the test for thebaine. For the calculation, 1 mg of *morphine hydrochloride R* is taken to be equivalent to 0.759 mg of morphine and 1 mg of *codeine R* is taken to be equivalent to 0.943 mg of codeine.

Ph Eur

Prepared Stramonium

General Notices

(Ph Eur monograph 0247)

Ph Eur

DEFINITION

Stramonium leaf powder (180) *(2.9.12)* adjusted, if necessary, by the addition of powdered lactose or stramonium leaf of lower content of total alkaloids.

Content

0.23 per cent to 0.27 per cent of total alkaloids, expressed as hyoscyamine ($C_{17}H_{23}NO_3$; M_r 289.4) (dried drug).

CHARACTERS

Appearance

Greyish-green powder.

Unpleasant odour.

IDENTIFICATION

A. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of leaf lamina showing epidermal cells with slightly wavy anticlinal walls and smooth cuticle; stomata are more frequent on the lower epidermis (anisocytic and anomocytic) *(2.8.3)*; covering trichomes are conical, uniseriate with 3-5 cells and warty walls; glandular trichomes are short and clavate with heads formed by 2-7 cells; dorsiventral mesophyll, with a single layer of palisade cells and a spongy parenchyma containing cluster crystals of calcium oxalate; annularly and spirally thickened vessels. The powdered drug may also show the following diagnostic characters: fibres and reticulately thickened vessels from the stems; subspherical pollen grains usually about 60-80 µm in diameter with 3 germinal pores and nearly smooth exine; fragments of the corolla with papillose epidermis; seed fragments containing yellowish-brown, sinuous, thick-walled sclereids of testa; occasional prisms and microsphenoidal crystals of calcium oxalate. Examined in *glycerol (85 per cent) R*, it may be seen to contain lactose crystals.

B. Examine the chromatograms obtained in the Chromatography test.

Results The principal zones in the chromatogram obtained with the test solution are similar in position, colour and size to the principal zones in the chromatogram obtained with the same volume of the reference solution.

C. Shake 1 g with 10 ml of *0.05 M sulphuric acid* for 2 min. Filter and add to the filtrate 1 ml of *concentrated ammonia R* and 5 ml of *water R*. Shake cautiously with 15 ml of *peroxide-free ether R*, avoiding the formation of an emulsion. Separate the ether layer and dry over *anhydrous sodium sulphate R*. Filter and evaporate the ether in a porcelain dish. Add 0.5 ml of *nitric acid R* and evaporate to dryness on a water-bath. Add 10 ml of *acetone R* and, dropwise, a 30 g/l solution of *potassium hydroxide R* in *ethanol (96 per cent) R*. A deep violet colour develops.

TESTS

Chromatography

Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the drug to be examined add 10 ml of *0.05 M sulphuric acid*, shake for 15 min and filter. Wash the filter with *0.05 M sulphuric acid* until 25 ml of filtrate is obtained. To the filtrate add 1 ml of *concentrated ammonia R* and shake with 2 quantities, each of 10 ml, of *peroxide-free ether R*. If necessary, separate by centrifugation. Dry the combined ether layers over *anhydrous sodium sulphate R*, filter and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 ml of *methanol R*.

Reference solution Dissolve 50 mg of *hyoscyamine sulphate R* in 9 ml of *methanol R*. Dissolve 15 mg of *hyoscine hydrobromide R* in 10 ml of *methanol R*. Mix 3.8 ml of the hyoscyamine sulphate solution and 4.2 ml of the hyoscine hydrobromide solution and dilute to 10 ml with *methanol R*.

Plate TLC silica gel G plate R.

Mobile phase concentrated ammonia R, water R, acetone R (3:7:90 *V/V/V*).

Application 10 µl and 20 µl of each solution as bands of 20 mm by 3 mm, leaving 1 cm between the bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 15 min and allow to cool.

Detection A Spray with *potassium iodobismuthate solution R2*, using about 10 ml for a plate 200 mm square, until the orange or brown zones become visible against a yellow background.

Results A The zones in the chromatograms obtained with the test solution are similar in position (hyoscyamine in the lower third, hyoscine in the upper third of the chromatogram) and colour to those in the chromatograms obtained with the reference solution. The zones in the chromatograms obtained with the test solution are at least equal in size to the corresponding zones in the chromatogram obtained with the same volume of the reference solution. Faint secondary zones may appear, particularly in the middle of the chromatogram obtained with 20 µl of the test solution or near the starting point in the chromatogram obtained with 10 µl of the test solution.

Detection B Spray with *sodium nitrite solution R* until the coating is transparent; examine after 15 min.

Results B The zones due to hyoscyamine in the chromatograms obtained with the test solution and the reference solution change from brown to reddish-brown but not to greyish-blue (atropine) and any secondary zones disappear.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Total ash *(2.4.16)*

Maximum 20.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 4.0 per cent.

ASSAY

a) Determine the loss on drying *(2.2.32)* on 2.000 g by drying in an oven at 105 °C.

b) Moisten 10.0 g with a mixture of 5 ml of *ammonia R*, 10 ml of *ethanol (96 per cent) R* and 30 ml of *peroxide-free ether R* and mix thoroughly. Transfer the mixture to a suitable percolator, if necessary with the aid of the extracting mixture. Allow to macerate for 4 h and percolate with a mixture of 1 volume of *chloroform R* and 3 volumes of *peroxide-free ether R* until the alkaloids are completely extracted. Evaporate to dryness a few millilitres of the liquid flowing from the percolator, dissolve the residue in *0.25 M sulphuric acid* and verify the absence of alkaloids using *potassium tetraiodomercurate solution R*. Concentrate the percolate to about 50 ml by distilling on a water-bath and transfer it to a separating funnel, rinsing with *peroxide-free ether R*. Add a quantity of *peroxide-free ether R* equal to at least 2.1 times the volume of the percolate to produce a liquid of a density well below that of water. Shake the solution with no fewer than 3 quantities, each of 20 ml, of *0.25 M sulphuric acid*, separate the 2 layers by centrifugation if necessary and transfer the acid layers to a 2nd separating funnel. Make the acid layer alkaline with *ammonia R* and shake with 3 quantities, each of 30 ml, of *chloroform R*. Combine the chloroform layers, add 4 g of *anhydrous sodium sulphate R* and allow to stand for 30 min with occasional shaking. Decant the chloroform and wash the sodium sulphate with 3 quantities, each of 10 ml, of *chloroform R*. Add the washings to the chloroform extract, evaporate to dryness on a water-bath and heat in an oven at 100-105 °C for 15 min. Dissolve the residue in a few millilitres of *chloroform R*, add 20.0 ml of *0.01 M sulphuric acid* and remove the chloroform by evaporation on a water-bath. Titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red mixed solution R* as indicator.

Calculate the percentage content of total alkaloids, expressed as hyoscyamine, using the following expression:

$$\frac{57.88\,(20 - n)}{(100 - d)\,m}$$

d = loss on drying, as a percentage;

n = volume of *0.02 M sodium hydroxide*, in millilitres;

m = mass of drug, in grams.

STORAGE

In an airtight container.

Ph Eur

Primula Root

General Notices

(Ph Eur monograph 1364)

Ph Eur

DEFINITION

Whole or cut, dried rhizome and root of *Primula veris* L. or *Primula elatior* (L.) Hill.

CHARACTERS

Bitter taste.

IDENTIFICATION

A. The coarsely torose, greyish-brown rhizome is straight or slightly curved, about 1-5 cm long and about 2-4 mm thick. The rhizome crown often bears the remains of stems and leaves. Attached to the rhizome are numerous brittle roots, about 1 mm thick and usually 6-8 cm long. The root of *P. elatior* is light brown or reddish-brown, that of *P. veris* light yellow or yellowish-white. The fracture is smooth.

B. Reduce to a powder (355) (*2.9.12*). The powder is greyish-brown. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of parenchyma from the root bark, medulla and bark of the rhizome consisting of rounded cells with thickened and pitted walls; brownish fragments from the surface tissue showing root hairs; vessels with reticulate thickening. Yellowish-green groups of strongly pitted stone cells are characteristic of the presence of *P. elatior*. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows single or compound starch grains of various size and shape.

C. Thin-layer chromatography (*2.2.27*) as described in the test for *Vincetoxicum hirundinaria medicus* root with the following modifications.

Detection Spray with *anisaldehyde solution R*. Heat at 100-105 °C for 5-10 min. Examine in daylight.

Results The main zone (aescin) in the chromatogram obtained with the reference solution is bluish-violet and is situated near the boundary between the lower and middle thirds. The chromatogram obtained with the test solution shows 1-2 strong dark violet zones a little below the zone due to aescin in the chromatogram obtained with the reference solution; further pale violet, yellowish or brownish-green zones may be visible.

TESTS

Vincetoxicum hirundinaria medicus root

Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (500) (*2.9.12*) add 10 ml of *ethanol (70 per cent V/V) R* and heat under a reflux condenser for 15 min. Cool and filter.

Reference solution Dissolve 10 mg of *aescin R* in 1.0 ml of *ethanol (70 per cent V/V) R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase The upper phase of a mixture of 10 volumes of *glacial acetic acid R*, 40 volumes of *water R* and 50 volumes of *butanol R*.

Application 20 µl, as bands.

Development Over a path of 12 cm.

Drying In an oven at 100-105 °C.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatograms obtained with the reference solution and the test solution show a quenching zone (aescin) near the boundary between the lower and the middle thirds. Mark this zone.

Detection B Examine in ultraviolet light at 365 nm.

Results B In the chromatogram obtained with the test solution no zones of light-blue or greenish fluorescence occur below the main zone due to aescin in the chromatogram obtained with the reference solution.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 9.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Maximum 3.0 per cent.

Ph Eur

Processed Angelica Sinensis Root for use in THMP

General Notices

DEFINITION

Processed Angelica Sinensis Root for use in THMP is the smoked, sliced and dried root of Angelica Sinensis Root for use in THM. The dried root consists of the top (upper most part), main body and small lateral roots (tails). It contains not less than 0.1% of *Z*-ligustilide ($C_{12}H_{14}O_2$), calculated with reference to the dried material.

PRODUCTION

The root is collected in late autumn, removed from rootlet and soil, slightly dried and tied up in small bundles, placed on a shelf and smoke dried. The smoked dried roots may be covered with a moist cloth or sprayed with a gentle mist to allow the roots to soften to facilitate slicing. The roots may be cut transversely or longitudinally to form individual pieces or, 2 to 3 may be pressed together to form one large piece before slicing, "palm slices" (characteristic molar shaped pieces). The sliced roots are dried.

IDENTIFICATION

A. Transversely cut slices are about 3 to 4 mm thick and up to about 2 cm in diameter and the cut surface shows a narrow outer cork layer and a fairly wide, pale brown bark separated by a dark brown cambium from the pale, yellowish-white and radiate central xylem; brown dotted secretory cavities occur throughout the bark and xylem. Longitudinally cut slices form irregular strips up to about 4 cm long and 5 mm thick. Longitudinally cut slices from the compressed roots occur as very smooth, soft and pliable pieces less than 1 mm thick; they are typically molar-shaped, up to about 5 cm wide at the crown and up to 10 cm long. They are light to mid yellow with brown edges and streaks indicating the composite structure from which they are obtained.

B. Reduce to a powder (355). The powder is pale yellowish to buff. Examine under a microscope using *chloral hydrate solution* . The powder shows brown fragments of cork composed of thin-walled cells; abundant thin-walled parenchyma from the secondary cortex, phloem and medullary rays, some of the phloem cells fusiform with slightly thickened walls; lignified vessels in groups of 2 or 3 associated with small celled and pitted xylem parenchyma; the vessels are up to 80 µm in diameter and have reticulate or scalariform thickening. Examine under a microscope using 50% v/v of *glycerol* . The powder shows small groups of single starch granules, spherical to ovoid, up to about 8 µm in diameter.

C. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Add 4 ml of *heptane* to 1.0 g of the powdered drug, sonicate for 5 minutes and filter (use a 0.22 µm membrane filter).

(2) 0.1% w/v of *linoleic acid* in *methanol* .

(3) 0.1% w/v of *ferulic acid* in *methanol* .

(4) 0.1% w/v of Z-*ligustilide CRS* in *methanol* .

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F* $_{254}$ precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Develop to 15 cm.

(d) Apply 10 µl of solution (1) and 5 µl of solutions (2) to (4) as bands.

(e) Remove the plate, allow to dry in a stream of warm air for 5 minutes or until the solvents are completely removed. Examine under *ultraviolet light (254 nm).* Spray the plate with *methanolic sulphuric acid (5%),* heat at 105º for 3 minutes and examine in daylight.

MOBILE PHASE

1 volume of *formic acid* , 10 volumes of *ethyl acetate* and 90 volumes of *toluene* .

SYSTEM SUITABILITY

When examined under ultraviolet light the violet band with an Rf value of approximately 0.7 in the chromatogram obtained with solution (4) corresponds in colour and position to that in the chromatogram obtained with solution (1). A band with an Rf value of approximately 0.23 in the chromatogram obtained with solution (3) corresponds in position to a band in the chromatogram obtained with solution (1). Other bands may be present in the chromatogram obtained with solution (1).

When sprayed with *methanolic sulphuric acid (5%)* the chromatogram obtained with solution (1) shows three spots with similar Rf values to the spots in the chromatograms obtained with solution (2), (3) and (4). Other spots may be present in the chromatogram obtained with solution (1).

TESTS

Lovage root (*Levisticum officinale*)

Carry out the method for *gas chromatography* , Appendix III B, using the following solutions.

(1) Extract approximately 20 g of the coarsely powdered drug in a 500 ml round-bottomed flask by hydrodistillation for 2 to 3 hours in 200 ml of *water*, collecting the distillate in suitable glassware. Extract the oily drops on top of the distillate with 5 ml of *toluene* .

(2) 0.2% w/v each of *coumarin* and *eugenol* in *toluene* .

(3) 0.1% w/v of Z-*ligustilide CRS* in a*cetonitrile*.

(4) 0.1% w/v of *benzyl alcohol* in *toluene* .

(5) 0.1% w/v of *(-)-carvone* in *toluene* .

(6) 0.1% w/v of *octanoic acid* in *toluene* .

(7) 0.1% w/v of *3-propylidenephthalide* in *toluene* .

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (50 m × 0.32 mm) bonded with a film (1.05 µm) of 5% phenyl/95% dimethylpolysiloxane (HP 5 is suitable).

(b) Use *helium* as the carrier gas at a flow rate of 1 ml per minute.

(c) Use an oven maintained at an initial temperature of 40° increasing linearly to 220° at a rate of 5° per minute, then maintained at 220°.

(d) Use a split injection system having a split ratio of 20:1 maintained at 250°.

(e) Use a flame ionisation detector maintained at a temperature of 250°.

(f) Inject 1 µl of each solution.

Record the chromatograms for a sufficient length of time to elute all the peaks in the chromatogram obtained with solution (1) (55 minutes may be suitable).

SYSTEM SUITABILITY

The test is not valid unless two clearly separated peaks, eluting at approximately 34 minutes (coumarin) and 37 minutes (eugenol), are observed in the chromatogram obtained with solution (2).

CONFIRMATION

In the chromatogram obtained with solution (1):

there are no peaks corresponding to the principal peaks obtained in the chromatograms obtained with solutions (4), (5), (6) and (7);

there is a peak corresponding to the principal peak in the chromatogram obtained with solution (3).

Loss on drying

When dried at 100° to 105° for 2 hours, loses not more than 10.0 % of its weight. Use 1 g.

Total ash

Not more than 7.0%, Appendix XI J, Method II.

Acid-insoluble ash

Not more than 2.0%, Appendix XI K.

Ethanol-soluble extractive

Not less than 45%, Appendix XI B1.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Finely powder not less than 5.0 g of the drug being examined. Transfer 0.5 g of the powder into a 25 ml volumetric flask and add 20 ml of *methanol* , place in an ultrasonic bath (maintained at a low temperature by adding ice to the bath) for 100 minutes, equilibrate to ambient temperature and dilute to volume with *methanol* . Centrifuge the solution at 5000 rpm for 5 minutes or until a clear supernatant is obtained. Filter through a 0.45-µm filter.

(2) 0.025% w/v of Z-*ligustilide CRS* in *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution using the mobile phase below.

(c) Use a flow rate of 1.0 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 350 nm.

(f) Inject 10 µl of each solution.

MOBILE PHASE

A mixture of 8 volumes of *water* and 12 volumes of *acetonitrile* .

SYSTEM SUITABILITY

Inject solution (2) not less than five times. The test is not valid unless the relative standard deviation of the peak areas of the *Z*-ligustilide peak is not more than 3.0%, the relative standard deviation of the retention times of the *Z*-ligustilide peak is not more than 3.0%. The *column efficiency* , determined on the *Z*-ligustilide peak, is not less than 5000 theoretical plates. The *symmetry factor* , determined on the *Z*-ligustilide peak, is not more than 1.3.

Inject solution (1). The test is not valid unless the *resolution factor* between the *Z*-ligustilide peak and the closest peak (relative retention about 0.9 with respect to *Z*-ligustilide) is not less than 1.5.

DETERMINATION OF CONTENT

Using the retention time and the peak area from the chromatograms obtained with solution (2) , locate and integrate the peak due to *Z*-ligustilide in the chromatogram obtained with solution (1).

Calculate the content of *Z*-ligustilide in the sample using the declared content of *Z*-ligustilide in *Z-ligustilide CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100 - d}$$

A_1 = Area of the peak due to *Z*-ligustilide in the chromatogram obtained with solution (1) .

A_2 = Area of the peak due to *Z*-ligustilide in the chromatogram obtained with solution (2) .

m_1 = Weight of the drug being examined in mg.

m_2 = Weight of Z-*ligustilide CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of $C_{12}H_{14}O_2$ in Z-*ligustilide CRS .*

d = Percentage loss on drying of the herbal drug being examined.

STORAGE

Processed Angelica Sinensis Root for use in TMHP should be protected from moisture.

Processed Astragalus Root for use in THMP

General Notices

DEFINITION

Processed Astragalus Root for use in THMP is Astragalus Root for use in THM that has been dried and sliced.

PRODUCTION

Astragalus Root for use in THM is washed clean, softened thoroughly and sliced transversely or longitudinally. The longitudinally cut pieces are usually prepared by soaking and pressing or pounding to form spatulate slices.

IDENTIFICATION

A. The transverse or obliquely cut slices are up to about 0.5 cm thick and about 2.5 cm in diameter; fragments of larger pieces are frequently present. The transverse surface varies from cream to yellow and shows an outer zone occupying about a third of the diameter, separated by a darker cambium region from the finely radiate xylem. The central region is dark brown and in the larger pieces this has broken down to form a central hollow surrounded by fragments of disintegrating tissue.

The pounded and pressed longitudinal slices, which resemble tongue depressors, are up to 10 cm or more long by 1.5 to 3 cm wide and about 0.4 cm thick; typically they are rounded at one end and distinctly fibrous at the other end. The surface is yellow and faintly ridged longitudinally, and a number of pieces have the remains of the brown outer layers of the root on the side edges.

B. Reduce to a powder. The powder is yellowish-white. Examine under a microscope using chloral hydrate solution. The fibres are in bundles or scattered, 8 to 30 µm in diameter, thick-walled, with longitudinal fissures on the surface, the primary walls often separated from the secondary walls, both ends often broken to tassel-like, or slightly truncated. Bordered-pitted vessels colourless or orange, bordered pits arranged closely. Stone cells occasionally visible, rounded, oblong or irregular, slightly thick-walled. Examine under a microscope using 50% v/v of *glycerol* in *water*. Starch granules usually simple, ovoid, 5 to 20 µm in diameter, with a visible linear or punctuate hilum.

C. Carry out the method for *thin-layer chromatography* , Appendix III A, using the following solutions.

(1) Place 6 g of the powdered drug in a Soxhlet extractor, add 75 ml of *methanol* , heat under reflux for 7 hours, cool and filter.

(2) 0.3% w/v of *ferulic acid* and 0.3% w/v of *linoleic acid* in *methanol*.

(3) 0.1% w/v each of *astragaloside I CRS, astragaloside II CRS* and *astragaloside IV CRS* in *methanol* .

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel 60 F_{254} [or high-performance silica gel 60 F_{254} (Merck silica gel 60 F_{254} HPTLC plates are suitable)].

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution, as bands

(d) Develop the plate to 15 cm [or 7 cm].

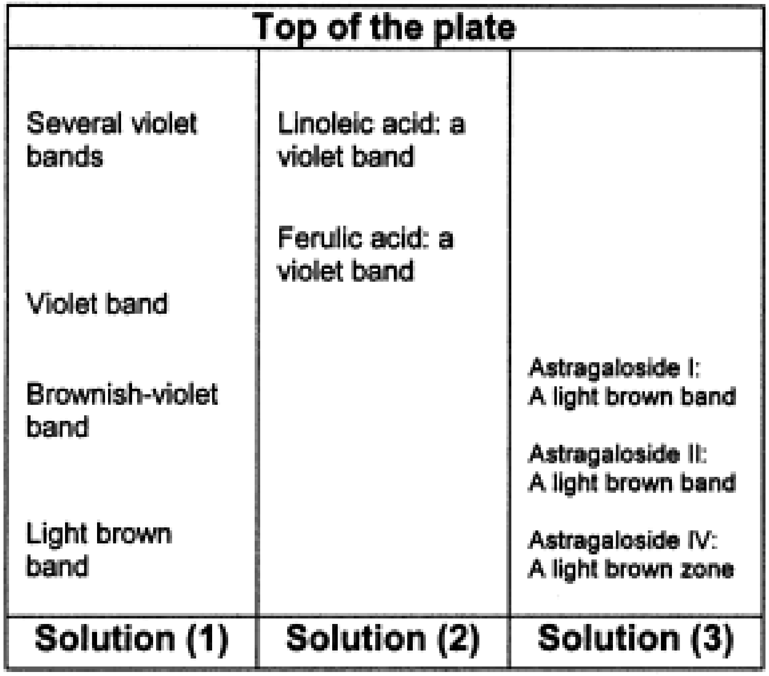
(e) Remove the plate and allow it to dry in cold air for 5 minutes. Examine under *ultraviolet light (254 nm)* . Spray the plate with *anisaldehyde solution* , heat at 100° to 105° for 5 minutes and examine in daylight.

MOBILE PHASE

A mixture of 30 volumes of *water*, 41 volumes of *methanol* and 200 volumes of *ethyl acetate.*

SYSTEM SUITABILITY

The test is not valid unless, when examined under daylight after spraying with *anisaldehyde solution* , the chromatogram obtained with solution (2) shows two clearly separated bands and the chromatogram obtained with solution (3) shows three clearly separated bands.

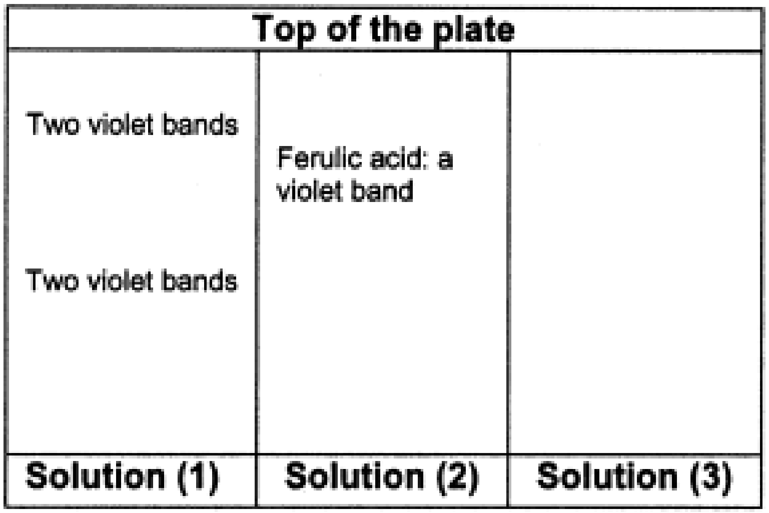


Top of the plate		
Several violet bands	Linoleic acid: a violet band	
	Ferulic acid: a violet band	
Violet band		
Brownish-violet band		Astragaloside I: A light brown band
		Astragaloside II: A light brown band
Light brown band		Astragaloside IV: A light brown zone
Solution (1)	**Solution (2)**	**Solution (3)**

CONFIRMATION

When examined under daylight after spraying with *anisaldehyde solution* the chromatogram obtained with solution (1) shows a band corresponding to astragaloside IV and several other bands as shown above. Other bands may be present.

When examined under *ultraviolet light (254 nm)* the chromatogram obtained with solution (1) shows violet coloured bands with Rf values of approximately 0.4 and 0.8 and no band corresponding to ferulic acid as shown below. Other bands may be present. The chromatogram obtained with solution (2) shows a single violet band with an Rf value of approximately 0.7 (ferulic acid). The chromatogram obtained with solution (3) shows no bands.



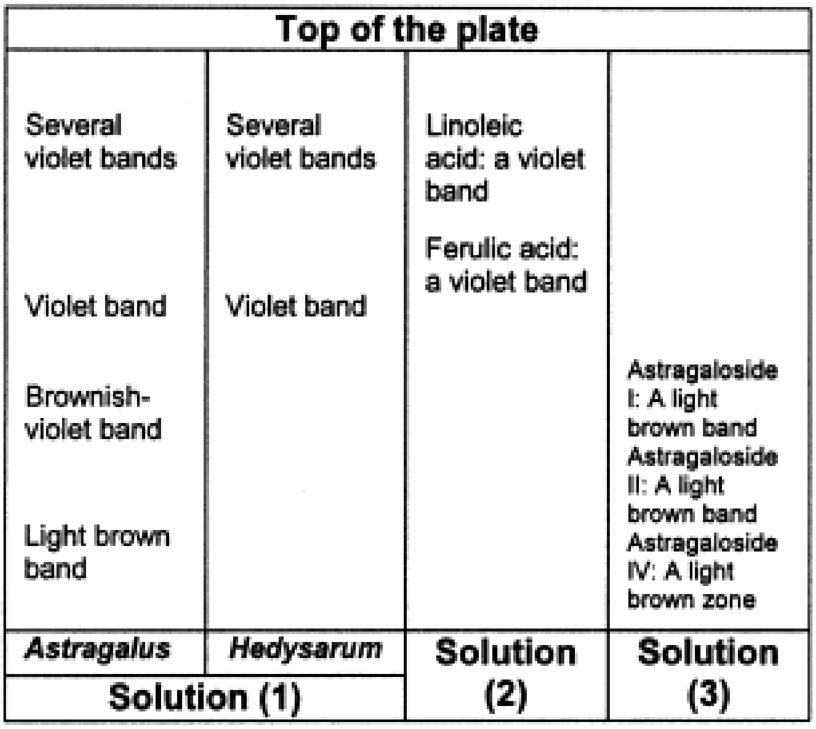
Top of the plate		
Two violet bands		
	Ferulic acid: a violet band	
Two violet bands		
Solution (1)	**Solution (2)**	**Solution (3)**

TESTS

Hedysarum root

Macroscopical *Hedysarum polybotrys* root, a known adulterant of Astragalus Root for use in THM has much smaller and more branched roots, with many branches arising from near the crown, also the outer surface is dark reddish-brown.

Thin-layer chromatography In Identification test C, the chromatogram obtained with solution (1) shows no bands due to *astragaloside I CRS* and *astragaloside IV CRS* when examined under daylight after spraying with *anisaldehyde solution* .



Top of the plate			
Several violet bands	Several violet bands	Linoleic acid: a violet band	
Violet band	Violet band	Ferulic acid: a violet band	
Brownish-violet band			Astragaloside I: A light brown band
			Astragaloside II: A light brown band
Light brown band			Astragaloside IV: A light brown zone
Astragalus	***Hedysarum***	**Solution (2)**	**Solution (3)**
Solution (1)			

Acid-insoluble ash

Not more than 1.0%, Appendix XI K, Method I.

Total ash

Not more than 5.0%, Appendix XI J, Method II.

Loss on drying

When dried for 2 hours at 100° to 105°, loses not more than 10.0% of its weight. Use 1 g.

Water-soluble extractive

Not less than 30%, Appendix XI B2.

STORAGE

Processed Astragalus Root for use in THMP should be protected from moisture. Exposure to sunlight for as little as 30 minutes can bleach the yellow colour.

Processed Liquorice Root for use in THMP

General Notices

DEFINITION

Processed Liquorice Root for use in THMP is the processed Liquorice Root for use in THM.

It contains not less than 2.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$) calculated with reference to the dried material.

PRODUCTION

Liquorice Root for use in THM is cleaned, softened thoroughly, sliced transversely or longitudinally to form uniform pieces and dried.

IDENTIFICATION

A. The transversely-cut pieces are 0.5 to 2.5 cm in diameter, irregularly circular to ovoid, up to about 3 mm thick. The outer surface is dark reddish-brown and longitudinally wrinkled. The transverse surface is cream to yellow and shows a thin layer of cork and a pale brown cambium line separating the radiate phloem region from the distinctly radiate xylem. In pieces cut from the rhizome there is a central, whitish pith; in those cut from the roots the radiate xylem continues to the centre.

The longitudinally-cut pieces have been sliced obliquely and usually include a small portion of the outer surface at the tapering ends; they are about 6 to 8 cm long, 1 to 1.5 cm wide and 3 mm thick. The outer surface is reddish-brown and longitudinally wrinkled with scattered transverse ridges; the smooth cut surface is cream to yellow, faintly fibrous and shows a distinct cambium; on some of the pieces the vessel cavities are visible in the central region.

B. Reduce to a powder. The powder is yellowish-brown. Examine under a microscope using chloral hydrate solution. The powder shows abundant fibres occurring in groups, each group surrounded by a calcium oxalate prism crystal sheath, the individual fibres have very thick, partially lignified walls and few small pits; lignified bordered-pitted vessels, singly or in small groups and sometimes accompanied by lignified parenchymatous cells with moderately thickened and pitted walls, some of the individual vessels are very large, with pit apertures much elongated and the borders difficult to discern; prism crystals of calcium oxalate up to about 35 µm long occur scattered and in parenchymatous tissue; fragments of cork composed of thin-walled, slightly lignified polygonal cells. Examine under a microscope using 50% w/w glycerol in water. The powder shows abundant starch granules, mostly simple, spherical to ovoid, 3 µm to 20 µm in diameter.

C. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Add 16 ml of *water* and 4 ml of *hydrochloric acid (25%)* to 0.5 g of the powdered drug, heat on a water-bath under reflux for 30 minutes, cool and filter. Dry the filter paper and residue in a flask at 105° for 60 minutes, add 20 ml of *ether*, heat on a water-bath at 40° under reflux for 5 minutes, cool and filter. Evaporate the filtrate to dryness and dissolve the residue in 5 ml of *ether*.

(2) 0.1% w/v each of *glycyrrhetic acid* and *thymol* in *ether*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel* $GF_{254.}$ (Merck 10 x 20 cm plates are suitable).

(b) Mobile phase is a mixture of 1 volume of *concentrated ammonia*, 9 volumes of *water*, 25 volumes of *ethanol (96%)* and 65 volumes of *ethyl acetate.*

(c) Apply as a band, an application volume of 10 µl for each solution.

(d) Develop to 15 cm.

(e) Remove the plate and allow it to dry in air for 5 minutes. Examine under *ultraviolet light (254 nm)*. Spray the plate with *anisaldehyde solution* and heat at 100° to 105° for 10 minutes and examine in daylight.

SYSTEM SUITABILITY

Under ultra-violet light, the chromatogram obtained with solution (2) shows in the lower half a quenching zone due to glycyrrhetic acid. When sprayed, the chromatogram obtained with solution (2) shows in the lower half the violet zone of glycyrrhetic acid and in the upper third the red zone of thymol.

VALIDITY

The chromatogram obtained with solution (1) shows in the lower half of violet zone corresponding to the zone of glycyrrhetic acid in the chromatogram obtained with solution (2) and a yellow zone (isoliquiridigenine) in the upper third under the zone of thymol in the chromatogram obtained with solution (2). Further zones may be present.

TESTS

Total Ash

Not more than 5.0 %, Appendix XI J, Method II.

Acid-insoluble ash

Not more than 1.0%, Appendix XI K, Method II.

Loss on drying

When dried for 2 hours at 100° to 105°, loses not more than 10.0% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound for 30 minutes 1 g of the powdered drug being examined with 100 ml of 0.8% w/v of *ammonia*, centrifuge a part of the supernatant layer, dilute 1 ml of this solution to 5 ml with 0.8 % w/v of *ammonia* and filter through a 0.45-µm filter.

(2) Contains 0.0065% w/v of *monoammonium glycyrrhizate EPCRS* in 0.8% w/v of *ammonia*.

(3) Contains 0.013% w/v of *monoammonium glycyrrhizate EPCRS* in 0.8% w/v of *ammonia*.

(4) Contains 0.0195% w/v of *monoammonium glycyrrhizate EPCRS* in 0.8% w/v of *ammonia*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS SS is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 254 nm.

(f) Injection volume of 10 µl for each solution.

MOBILE PHASE

A mixture of 6 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 64 volumes of *water*.

SYSTEM SUITABILITY

The assay is not valid unless (a) the *column efficiency,* determined on the peak due to glycyrrhizic acid in the chromatogram obtained with solution (2), is at least 30,000 theoretical plates per metre and (b) the *symmetry factor* of the peak is not more than 1.3.

DETERMINATION OF CONTENT

Inject solution (4). Adjust the sensitivity of the system so that the height of the peaks are at least 50% of the full scale of the recorder. Inject solutions (2), (3) and (4) and determine the peak areas. Prepare a calibration curve with the concentration of the solutions (g per 100 ml) as the abscissa and the corresponding areas as the ordinate. Inject solution (1). Using the retention time and the peak area from the chromatograms obtained with solutions (2), (3) and (4), locate and integrate the peak due to glycyrrhizic acid in the chromatogram obtained with solution (1).

Calculate the percentage content of glycyrrhizic acid from the following expression:

$$A \times \frac{5}{m} \times B \times \frac{822}{840}$$

A = concentration of monoammonium glycyrrhizate in the solution (1) determined from the calibration curve, in g per 100 ml,

B = declared percentage content of *monoammonium glycyrrhizate EPCRS*,

M = weight of the substance being examined in grams,

822 = molecular weight of glycyrrhizic acid,

840 = molecular weight of the monoammonium glycyrrhizate (without any water of crystallisation).

STORAGE

Processed Liquorice Root for use in THMP should be protected from moisture.

Processed Salvia Miltiorrhiza Rhizome and Root for use in THMP

General Notices

DEFINITION

Processed Salvia Miltiorrhiza Rhizome and Root for use in THMP is Salvia Miltiorrhiza Rhizome and Root for use in THM that has been processed. It contains not less than 0.04% of tanshinone II$_A$ ($C_{19}H_{18}O_3$), not less than 0.17% of rosmarinic acid ($C_{18}H_{16}O_8$) and not less than 3.0% of salvianolic acid B ($C_{36}H_{36}O_{16}$), calculated with reference to the dried material.

PRODUCTION

It is collected in spring or autumn, separated from soil, washed clean, softened thoroughly, sliced longitudinally or transversely and dried. It may be stir baked with wine.

IDENTIFICATION

A. The longitudinally-sliced pieces are up to about 5 cm long, 1.5 cm wide and 1 to 2 mm thick; those cut from the thinner roots show the dark brown striated cork covering one longitudinal surface and the yellowish to cream inner tissues on the other; slices cut from the thicker rhizomes are usually cut obliquely so that parts of the outer and inner tissues are included on both longitudinal surfaces. The transversely-cut slices are irregularly elliptical to nearly circular, 4 to 12 mm wide and 2 to 3 mm thick; the outer surface is dark brown and uneven; the smoothed transverse surface shows the outer layers about 1 to 2 mm wide separated by a darker line from the yellowish white, radiate vascular tissue; some pieces show a small, light brown central pith.

B. Reduce to a powder (355). The powder is reddish-brown. Examine under a microscope using chloral hydrate solution. The powder shows a surface view of cork cells almost rectangular or polygonal, containing yellowish-brown pigment, 12 to 151 µm in diameter. Parenchymatous cells in cortex squarish or polygonal, containing reddish-brown pigmental sediments. Xylem fibres usually in bundles, long fusiform, with oblique or criss-cross striations, 11 to 60 µm in diameter, vivid yellow when examined under a polarizing microscope. Numerous mainly bordered or reticulated vessels, 3 to 120 µm in diameter.

C. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Place 2 g of the powdered drug (355) in a cellulose fingerstall in a continuous extraction apparatus (Soxhlet type). Add 75 ml of *methanol* and heat for 1 hour. Evaporate the extract to 20 ml, cool and filter if necessary.

(2) 0.1% w/v each of *tanshinone II$_A$ CRS*, *rosmarinic acid CRS* and *salvianolic acid B CRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F$_{254}$*.

(b) Use the mobile phase described below.

(c) Apply as bands 8 µl of solution (1) and 5 µl of solution (2).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (366 nm)*.

MOBILE PHASE

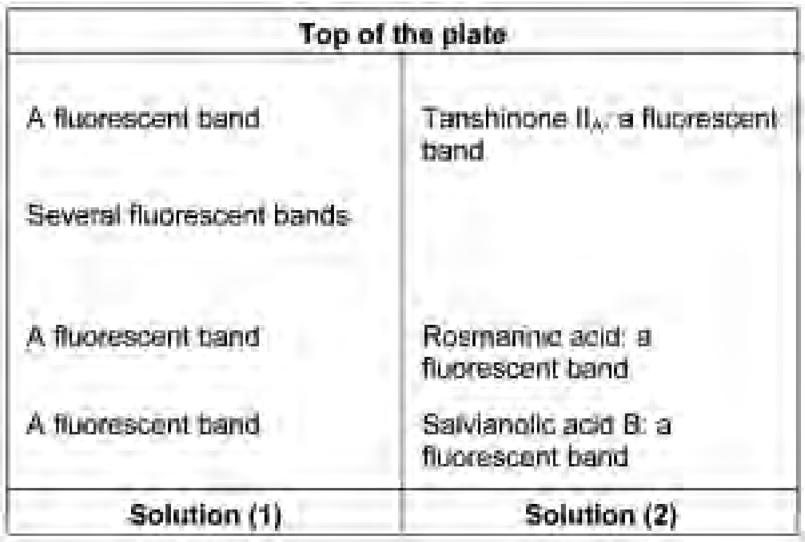
A mixture of 10 volumes of *water*, 13.5 volumes of *methanol* and 100 volumes of *ethyl acetate.*

SYSTEM SUITABILITY

The chromatogram obtained with solution (2) shows three clearly separated bands.

CONFIRMATION

The blue fluorescent bands with Rf values of approximately 0.7 (tanshinone II_A), 0.2 (rosmarinic acid) and 0.06 (salvianolic acid B) in the chromatogram obtained with solution (1) correspond in colour and position to those in the chromatogram obtained with solution (2). Other bands may be present in the chromatogram obtained with solution (1) as shown below.



Top of the plate	
A fluorescent band	Tanshinone II_A: a fluorescent band
Several fluorescent bands	
A fluorescent band	Rosmarinic acid: a fluorescent band
A fluorescent band	Salvianolic acid B: a fluorescent band
Solution (1)	**Solution (2)**

TESTS

Total ash

Not more than 10%, Appendix XI J.

Acid-insoluble ash

Not more than 2.0%, Appendix XI K.

Loss on drying

When dried for 2 hours at 105°, loses not more than 12.0% of its weight. Use 1 g.

ASSAY

For tanshinone II_A

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely powder about 5.0 g of the herbal drug being examined. Transfer 0.5 g of the powder into a 25 ml volumetric flask and add 20 ml of the mobile phase given below. Shake and mix with the aid of ultrasound for 30 minutes, shaking intermittently. Add the mobile phase to give a total volume of 25 ml. Filter through a 0.45-µm filter.

(2) 0.002% w/v of *tanshinone* II_A *CRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.01 M *sodium octyl sulphonate* in a mixture of 25 volumes of *water* and 75 volumes of *methanol* and adjust to pH 5.0 with *acetic acid*.

SYSTEM SUITABILITY

The test is not valid, unless in the chromatogram obtained with solution (2):

— the *symmetry factor* of the peak due to tanshinone II_A is less than 1.2;

— the number of *theoretical plates* is not less than 4500.

DETERMINATION OF CONTENT

Using the retention time and peak area from the chromatograms obtained with solution (2), locate and integrate the peak due to tanshinone II_A in the chromatogram obtained with solution (1).

Calculate the content of tanshinone II_A in the sample using the declared content of tanshinone II_A ($C_{19}H_{18}O_3$) in *tanshinone* II_A *CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100-d}$$

A_1 = Area of the peak due to tanshinone II_A in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to tanshinone II_A in the chromatogram obtained with solution (2).

m_1 = Weight of the drug in mg.

m_2 = Weight of *tanshinone* II_A *CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of tanshinone II_A in *tanshinone* II_A *CRS*.

d = Percentage loss on drying of the herbal drug being examined.

For rosmarinic acid and salvianolic acid B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Finely powder about 5.0 g of the herbal drug being examined. Transfer 0.5 g of the powder into a 25-ml volumetric flask and add 20 ml of the mobile phase given below. Shake and mix with ultrasound for 30 minutes, shaking intermittently. Add the mobile phase to give a total volume of 25 ml. Filter through a 0.45-µm filter.

(2) 0.003% w/v each of *rosmarinic acid CRS* and *ferulic acid* in *water.*

(3) 0.06% w/v of *salvianolic acid B CRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 330 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

22 volumes of *acetonitrile* and 78 volumes of 0.4% v/v of *formic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two main peaks, rosmarinic acid and ferulic acid, is at least 5.0.

DETERMINATION OF CONTENT

Rosmarinic acid Using the retention time and peak area from the chromatogram obtained with solution (2), locate and integrate the peak due to rosmarinic acid in the chromatogram obtained with solution (1).

Calculate the content of rosmarinic acid in the sample using the declared content of rosmarinic acid ($C_{18}H_{16}O_8$) in *rosmarinic acid CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100 - d}$$

A_1 = Area of the peak due to rosmarinic acid in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to rosmarinic acid in the chromatogram obtained with solution (2).

m_1 = Weight of the drug in mg.

m_2 = Weight of *rosmarinic acid CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of rosmarinic acid in *rosmarinic acid CRS.*

d = Percentage loss on drying of the herbal drug being examined.

Salvianolic acid B Using the retention time and peak area from the chromatogram obtained with solution (3), locate and integrate the peak due to salvianolic acid B in the chromatogram obtained with solution (1).

Calculate the content of salvianolic acid B in the sample using the declared content of salvianolic acid B ($C_{36}H_{36}O_{16}$) in *salvianolic acid B CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100 - d}$$

A_1 = Area of the peak due to salvianolic acid in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to salvianolic acid in the chromatogram obtained with solution (3).

m_1 = Weight of the drug in mg.

m_2 = Weight of *salvianolic acid B CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of salvianolic acid B in *salvianolic acid B CRS.*

d = Percentage loss on drying of the herbal drug being examined.

STORAGE

Processed Salvia Miltiorrhiza Rhizome and Root for use in THMP should be protected from moisture.

Processed White Peony Root for use in THMP

General Notices

DEFINITION

Processed White Peony Root for use in THMP is White Peony Root for use in THM that has been the boiled, peeled and dried. It contains not less than 1.6% of paeoniflorin ($C_{23}H_{28}O_{11}$), calculated with reference to the dried material.

PRODUCTION

It is collected in summer and autumn, washed clean, with the two ends and rootlets removed, either peeled after boiling in water or boiled in water after peeling and dried in the sun. The dried root is washed and soaked thoroughly prior to being sliced transversely or longitudinally and dried.

IDENTIFICATION

A. The root slices are greyish-white to pale brown, darker at the edges, and up to about 2 mm thick. They are smooth and compact with a short and even fracture. Those that have been cut transversely occur in oval pieces up to about 4.5 cm in diameter and the cut surface shows a distinctly radiate xylem with narrow lines of vascular tissue separated by wide medullary rays. The longitudinally cut slices are up to 10 cm long and 1.5 cm wide and the cut surface shows raised strands of vascular tissue running longitudinally.

B. Reduce to a powder. The powder is pale yellow. Examine under a microscope using *chloral hydrate solution*. The powder shows abundant rounded, rectangular or elongated parenchymatous cells which occur singly or in groups, some with pitted or slightly beaded walls; many of the cells contain pale yellow or pinkish masses of gelatinised starch which are also found scattered throughout the powder; single cluster crystals of calcium oxalate, 11 to 35 µm in diameter, isolated or packed in rows in parenchymatous cells, sometimes with several crystals in each cell; fragments of lignified vessels, 20 to 65 µm in diameter, usually reticulately thickened but occasionally bordered-pitted; fibres occur rarely, usually accompanying the vessel fragments, they are 15 to 40 µm in diameter, with thickened, slightly lignified and frequently pitted walls.

C. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

D. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake 0.5 g of the powdered root with 10 ml of *ethanol* for 5 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of ethanol.

(2) 0.1% w/v each of *paeoniflorin BPCRS* and *4′-hydroxyacetophenone BPCRS* in *ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 precoated plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution, as a band.

(d) Develop the plate to 15 cm.

(e) Dry the plate in air. Spray with a 5% w/v solution of *vanillin* in *sulphuric acid*, heat at 105º for 5 minutes and examine immediately.

MOBILE PHASE

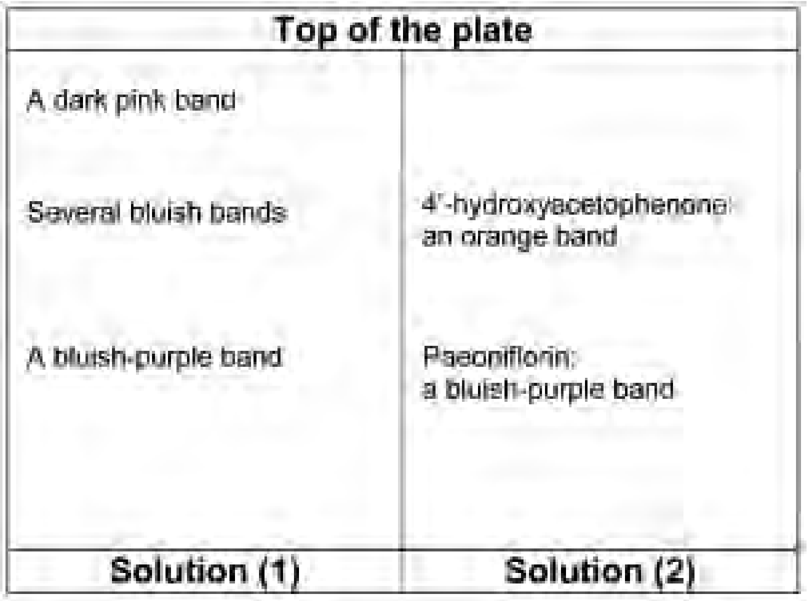
A mixture of 0.2 volumes of *formic acid*, 5 volumes of *ethyl acetate*, 10 volumes of *methanol* and 40 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), two clearly separated spots are observed.

CONFIRMATION

The bluish-purple spot with an Rf value of approximately 0.4 in the chromatogram obtained with solution (1) corresponds in colour and position to that in the chromatogram obtained with solution (2). Other spots are present in the chromatogram obtained with solution (1) as shown below.



Top of the plate	
A dark pink band	
Several bluish bands	4'-hydroxyacetophenone: an orange band
A bluish-purple band	Paeoniflorin: a bluish-purple band
Solution (1)	**Solution (2)**

TESTS

Tree Peony

In the Assay, the chromatogram obtained with solution (1) shows no peak with a relative retention of approximately 1.2 with reference to paeoniflorin.

Loss on drying

When dried for 3 hours at 100º to 105º, loses not more than 12.0% of its weight. Use 1 g.

Acid-insoluble ash

Not more than 0.5%, Appendix XI K.

Ash

Not more than 6.5%, Appendix XI J.

Cadmium

Maximum 3 ppm, Appendix VII.

Lead

Maximum 5 ppm, Appendix VII.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in 0.05M *potassium dihydrogen orthophosphate* (solution A).

(1) Finely powder not less than 5 g of the herbal drug being examined. Mix 0.15 g of the powdered drug with 3 ml of *methanol* and place in an ultrasonic bath for 30 minutes. Dilute to 10 ml with solution A, mix, filter through a 0.45-µm filter and use the filtrate.

(2) Dissolve a quantity of *paeoniflorin BPCRS* in *methanol* and dilute with solution A to produce a solution containing 0.05% w/v in 3 volumes of methanol and 7 volumes of solution A and mix.

(3) Dissolve a quantity of *4′-hydroxyacetophenone BPCRS* in solution (2) to produce a solution containing 0.05% w/v of *4′-hydroxyacetophenone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µl of each solution.

MOBILE PHASE

A mixture of 30 volumes of *methanol* and 70 volumes of solution A.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the number of *theoretical plates* for each peak due to paeoniflorin is at least 3000, the *symmetry factor* for the peak due to paeoniflorin is not more than 1.3 and the *resolution factor* between the two peaks is not less than 1.7.

DETERMINATION OF CONTENT

Using the retention time and the peak area from the chromatograms obtained with solution (2), locate and integrate the peak due to paeoniflorin in the chromatogram obtained with solution (1).

Calculate the content of $C_{23}H_{28}O_{11}$ in the sample, using the declared content of paeoniflorin ($C_{23}H_{28}O_{11}$) in *paeoniflorin BPCRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100-d}$$

A_1 = Area of the peak due to paeoniflorin in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to paeoniflorin in the chromatogram obtained with solution (2).

m_1 = Weight of the drug being examined in mg.

m_2 = Weight of *paeoniflorin BPCRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of $C_{23}H_{28}O_{11}$ in *paeoniflorin BPCRS.*

d = Percentage loss on drying of the herbal drug being examined.

STORAGE

Processed White Peony Root for use in THMP should be protected from moisture.

Psyllium Seed

General Notices

(Ph Eur monograph 0858)

Ph Eur

DEFINITION

Ripe, whole, dry seeds of *Plantago afra* L. (*Plantago psyllium* L.) or *Plantago indica* L. (*Plantago arenaria* Waldstein and Kitaibel).

CHARACTERS

Sweet taste.

IDENTIFICATION

P. afra seeds are light brown to very dark brown but never black, smooth and shiny having an elliptical oblong shape. They are 2-3 mm long and 0.8-1.0 mm wide, one end being wider than the other. Towards the middle of the dorsal surface there is a fairly marked transverse constriction of light colour. On the ventral surface, there is a linear lighter-coloured groove in the middle of which is a clear spot corresponding to the hilum and bounded by swollen edges.

P. indica seeds are almost identical to the seeds of *P. afra*, but a little less shiny; they are 2-3 mm long and have a maximum diameter of 1.5 mm.

TESTS

Swelling index (*2.8.4*)

Minimum 10.

Foreign matter (*2.8.2*)

Maximum 1.0 per cent, determined on 10.0 g of the drug, including greenish unripe seeds. Psyllium seed does not contain seeds having a dark central spot on the groove (*Plantago lanceolata* L. and *P. major* L.) or seeds with brownish-grey or pinkish outer coats (*P. ovata* Forssk. and *P. sempervirens* Crantz).

Loss on drying (*2.2.32*)

Maximum 14.0 per cent, determined on 1.000 g of drug by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 4.0 per cent.

STORAGE

Store protected from moisture.

Ph Eur

Pygeum Bark

General Notices

(Pygeum Africanum Bark, Ph Eur monograph 1886)

Ph Eur

DEFINITION

Whole or cut, dried bark of the stems and branches of *Prunus africana* (Hook f.) Kalkm. (syn. *Pygeum africanum* Hook f.).

IDENTIFICATION

A. The dark brown to reddish-brown bark occurs in curved, hard, irregular pieces. The outer surface has a wrinkled dark reddish-brown cork with areas of adhering lichen. The reddish-brown to dark brown inner surface bears longitudinal striations. It may also occur in rolled fragments with a fibrous fracture.

B. Reduce to a powder (355) (*2.9.12*). The powder is reddish-brown. Examine under a microscope using *chloral hydrate solution R*. The powdered drug shows thick-walled sclereids, solitary or in groups; calcium oxalate cluster crystals of different size; numerous lignified fibres, thick-walled and with narrow lumen, some of them solitary and most in groups with forked ends; fragments of pigmented polygonal cells of reddish-brown colour; fragments of cork. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*, the powder shows some isolated small starch grains that stain bluish-black against *iodine solution R1*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Extract 15.0 g of powdered drug (250) (*2.9.12*) with *methylene chloride R* for 30 min in a continuous extraction apparatus (Soxhlet type). Filter. Evaporate the solvent to dryness under reduced pressure. Dissolve the residue in 1 ml of *methylene chloride R*.

Reference solution Dissolve 20 mg of *β-sitosterol R* and 20 mg of *ursolic acid R* in 10 ml of a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Plate *TLC silica gel plate R*.

Mobile phase *methanol R*, *methylene chloride R* (10:90 *V/V*).

Application 10 µl, as 1 cm bands.

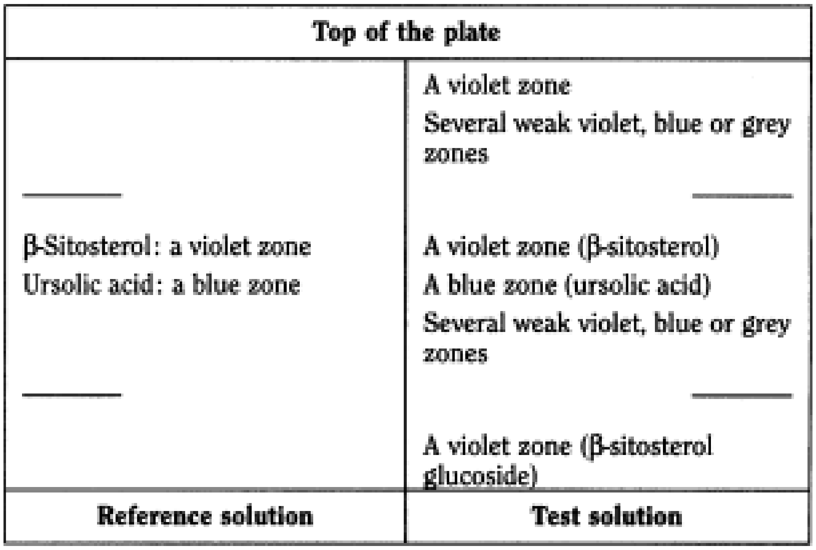
Development Over a path of 15 cm.

Drying In air.

Detection Spray with *vanillin reagent R*. Heat the plate at 100-105 °C for 10 min and allow to cool; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the

chromatogram obtained with the test solution.



Top of the plate	
	A violet zone Several weak violet, blue or grey zones
———	———
β-Sitosterol: a violet zone Ursolic acid: a blue zone	A violet zone (β-sitosterol) A blue zone (ursolic acid) Several weak violet, blue or grey zones
———	———
	A violet zone (β-sitosterol glucoside)
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 3.0 per cent.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 10.0 per cent.

Extractable matter

Minimum 0.5 per cent.

Extract 20.0 g of the powdered drug (250) (*2.9.12*) with *methylene chloride R* for 4 h in a continuous extraction apparatus (Soxhlet type). Evaporate the solution to dryness on a water-bath *in vacuo* and then dry the residue at 80 °C for 2 h. The residue weighs a minimum of 0.10 g.

Ph Eur

Quantified Hawthorn Leaf and Flower Liquid Extract

General Notices

(Ph Eur monograph 1864)

Ph Eur

DEFINITION

Quantified liquid extract produced from *Hawthorn leaf with flower (1432)*.

Content

0.8 per cent to 3.0 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4).

PRODUCTION

The extract is produced from the herbal drug and ethanol (30 per cent *V/V* to 70 per cent *V/V*) by an appropriate procedure.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Dilute 1.0 g in *methanol R* and dilute to 5 ml with the same solvent. Shake and filter.

Reference solution Dissolve 1.0 mg of *chlorogenic acid R* and 2.5 mg of *hyperoside R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R*, *water R*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 *V/V/V/V*).

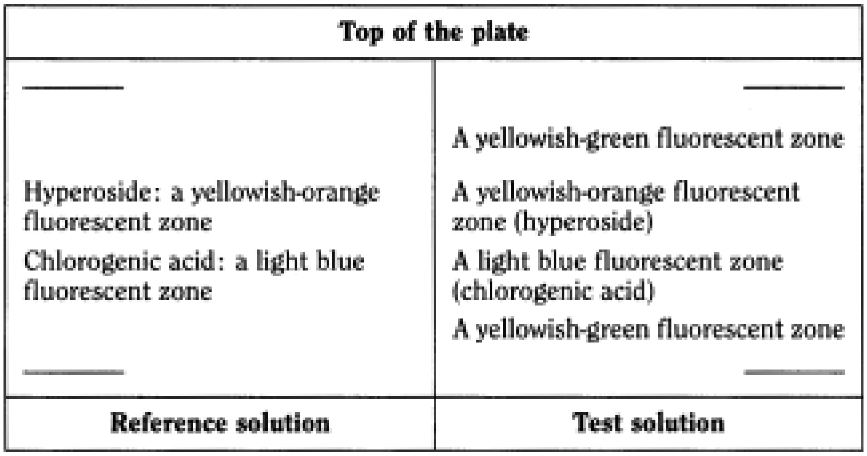
Application 20 µl [or 5 µl] as bands.

Development Over a path of 15 cm [or 6 cm].

Drying At 100-105 °C.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester* R in *methanol R*. Subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
	A yellowish-green fluorescent zone
Hyperoside: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (hyperoside)
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
	A yellowish-green fluorescent zone
———	———
Reference solution	**Test solution**

TESTS

Ethanol *(2.9.10)*

95 per cent *V/V* to 105 per cent *V/V* of the quantity stated on the label.

ASSAY

Stock solution Dilute about 0.400 g, accurately weighed, in *ethanol (60 per cent V/V) R* and dilute to 100.0 ml with the same solvent.

Test solution Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the 25 ml volumetric flask. Add 10.0 ml of a solution containing 25.0 g/l of *boric acid R* and 20.0 g/l of *oxalic acid R* in anhydrous *formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Compensation liquid Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the 25 ml volumetric flask. Add 10.0 ml of *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

After 30 min measure the absorbance *(2.2.25)* of the test solution at 410 nm.

Calculate the percentage content of total flavonoids, expressed as hyperoside, from the following expression:

$$\frac{A \times 1.235}{m}$$

i.e. taking the value of the specific absorbance of hyperoside to be 405.

A = absorbance at 410 nm,

m = mass of the extract to be examined, in grams.

Ph Eur

Quillaia Liquid Extract

General Notices

DEFINITION

Quillaia Liquid Extract is prepared by extracting Quillaia with Ethanol (45 per cent).

Extemporaneous preparation

The following formula and directions apply.

Quillaia, in *moderately fine powder*	1000 g
Ethanol (45 per cent)	A sufficient quantity

Exhaust the Quillaia in *moderately fine powder* with Ethanol (45 per cent) by *percolation*, Appendix XI F, and reserve the first 850 ml of percolate. Evaporate the subsequent percolate to the consistence of a soft extract, dissolve it in the reserved portion and add sufficient Ethanol (45 per cent) to produce 1000 ml. Allow to stand for not less than 24 hours; filter.

The extract complies with the requirements stated under Extracts and with the following requirements.

TESTS

Ethanol content

28 to 34% v/v, Appendix VIII F, Method III.

Dry residue

20 to 30% w/v.

Relative density

1.02 to 1.06, Appendix V G.

Quillaia Tincture

General Notices

DEFINITION

Quillaia Liquid Extract	50 ml
Ethanol (45 per cent)	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Mix, allow to stand for not less than 12 hours and filter.

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

TESTS

Ethanol content

43 to 45% v/v, Appendix VIII F, Method III.

Dry residue

1.0 to 1.5% w/v. Use 10 ml.

Relative density

0.940 to 0.955, Appendix V G.

Quillaia

General Notices

Quillaia Bark

Preparation

Quillaia Liquid Extract

When Powdered Quillaia is prescribed or demanded, material complying with the appropriate requirements below shall be dispensed or supplied.

DEFINITION

Quillaia is the dried inner part of the bark of *Quillaja saponaria* Molina and of other species of *Quillaja*.

IDENTIFICATION

A. Pieces flat, up to about 1 metre long, 10 to 20 cm broad and 3 to 10 mm, usually 6 mm, thick. Outer surface brownish white or pale reddish brown, longitudinally striated or coarsely reticulated, with occasional blackish brown patches of adherent outer bark; inner surface yellowish white, smooth and very hard; fracture splintery and laminated, the broken surface showing numerous large prisms of calcium oxalate as glistening points. Smoothed transversely cut surface appearing chequered, with delicate radial lines representing medullary rays and tangential lines formed by alternating tangential bands of fibrous and non-fibrous phloem.

B. Outer bark, when present, consisting of reddish brown cork cells alternating with bands of brown parenchyma containing numerous groups of phloem fibres and large prisms of calcium oxalate. Inner bark consisting of alternating bands of tortuous fibres, irregularly enlarged at intervals, about 500 to 1000 µm long and 20 to 50 µm wide and of sieve tissue mixed with parenchyma. Medullary rays mostly three to four, but sometimes up to six cells wide, with occasional pitted, subrectangular sclereids adjacent to the bundles of phloem fibres. Starch granules 5 to 20 µm, usually about 10 µm, in diameter and prisms of calcium oxalate usually 50 to 170 µm long and up to 30 µm wide present in the parenchymatous cells.

TESTS

Extractive soluble in ethanol (45%)

Not less than 22.0%, Appendix XI B1.

Acid-insoluble ash

Not more than 1.0%, Appendix XI K.

Foreign matter

Complies with the test for *foreign matter*, Appendix XI D.

Red Poppy Petals

General Notices

(Ph Eur monograph 1881)

Ph Eur

DEFINITION

Dried, whole or fragmented petals of *Papaver rhoeas* L.

IDENTIFICATION

A. The petal is dark red or dark violet-brown, very thin, floppy, wrinkled, often crumpled into a ball and velvety to the touch. It is broadly ovate with an entire margin, about 6 cm long and 4-6 cm wide, narrowing at the base where there is a black spot. The vascular bundles radiate from the base and they anastomose in a continuous arc, all at the same short distance from the margin.

B. Reduce to a powder (355) (*2.9.12*). Examine under a microscope using *chloral hydrate solution R*. The powder has an intense reddish-pink colour and shows the following diagnostic characters: fragments of epidermis composed of elongated, sinuous-walled cells with small, rounded, anomocytic stomata (*2.8.3*), numerous vascular bundles with spiral vessels embedded in the parenchyma; occasional fragments of the fibrous layer of the anthers; rounded pollen grains, about 30 µm in diameter, with 3 pores and a finely verrucose exine.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *ethanol (60 per cent V/V) R*. Stir for 15 min. Filter through a filter paper.

Reference solution Dissolve 1 mg of *quinaldine red R* and 1 mg of *sulphan blue R* in 2 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R*, *water R*, *butanol R* (10:12:40 *V/V/V*).

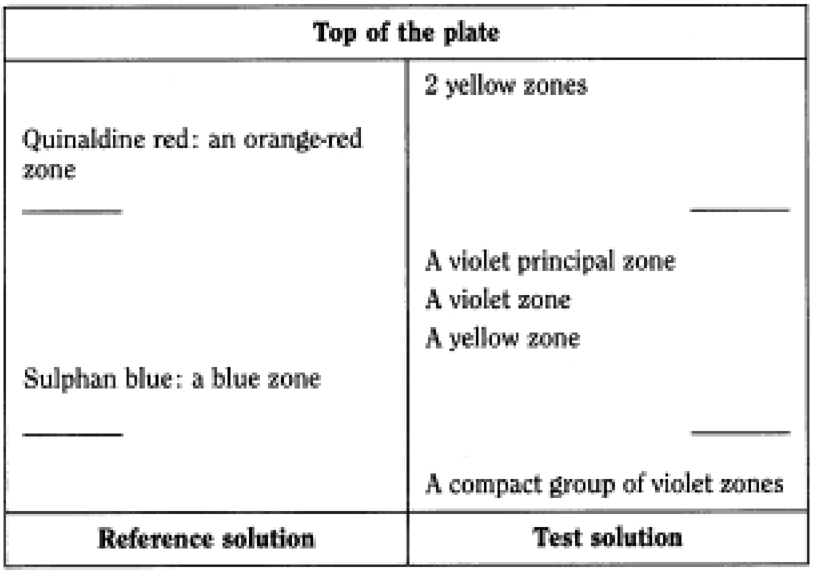
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other bands may be present in the chromatogram obtained with the test solution.



Top of the plate	
	2 yellow zones
Quinaldine red: an orange-red zone	
———	———
	A violet principal zone A violet zone A yellow zone
Sulphan blue: a blue zone	
———	———
	A compact group of violet zones
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 2.0 per cent of capsules and maximum 1.0 per cent of other foreign matter.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 11.0 per cent.

Colouring intensity

Place 1.0 g of the powdered drug (355) (*2.9.12*) in a 250 ml flask and add 100 ml of *ethanol (30 per cent V/V) R.* Allow to macerate for 4 h with frequent stirring. Filter and discard the first 10 ml. Transfer 10.0 ml of the filtrate to a 100 ml volumetric flask and add 2 ml of *hydrochloric acid R*. Dilute to 100.0 ml with *ethanol (30 per cent V/V) R*. Allow to stand for 10 min. The absorbance (*2.2.25*) measured at 523 nm using *ethanol (30 per cent V/V) R* as the compensation liquid is not less than 0.6.

Ph Eur

Refined and Quantified Capsicum Oleoresin

General Notices

(Ph Eur monograph 2336)

Ph Eur

DEFINITION

Refined and quantified oleoresin produced from *Capsicum (1859)* .

Content

6.5 per cent to 8.0 per cent *m/m* of total capsaicinoids, expressed as capsaicin ($C_{18}H_{27}NO_3$; M_r 305.4).

PRODUCTION

The oleoresin is produced from the herbal drug and ethanol (minimum 90 per cent *V/V*) or methanol (minimum 90 per cent *V/V*), by an appropriate procedure.

CHARACTERS

Appearance

Red or brown mobile extract.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 50 mg of the oleoresin to be examined in 5 ml of *ether R* .

Reference solution Dissolve 2 mg of *capsaicin R* and 2 mg of *dihydrocapsaicin R* in 5 ml of *ether R* .

Plate *TLC octadecylsilyl silica gel plate R* (5-40 µm) [or *TLC octadecylsilyl silica gel plate R* (2-10 µm)].

Mobile phase *water R, methanol R* (20:80 *V/V*).

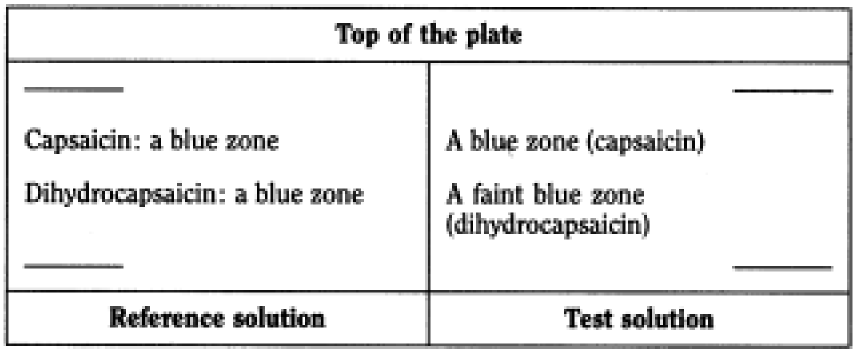
Application 20 µl [or 2 µl] as bands of 15 mm [or 8 mm].

Development Over a path of 12 cm [or 6 cm].

Drying In air.

Detection Spray with a 0.25 g/l solution of *dichloroquinonechlorimide R* in *ethyl acetate R* , expose to ammonia vapour until blue zones appear, then examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Capsaicin: a blue zone	A blue zone (capsaicin)
Dihydrocapsaicin: a blue zone	A faint blue zone (dihydrocapsaicin)
———	———
Reference solution	**Test solution**

TESTS

Nonivamide

Liquid chromatography *(2.2.29)* .

Test solution Dissolve 0.300 g of the oleoresin to be examined in 60 ml of *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution Dissolve 20.0 mg of *capsaicin CRS* and 4.0 mg of *nonivamide CRS* in 100.0 ml of *methanol R* .

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *phenylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R* , 1 g/l solution of *phosphoric acid R* (40:60 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl.

Elution order Nordihydrocapsaicin, nonivamide, capsaicin, dihydrocapsaicin.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to nonivamide and capsaicin.

Calculate the percentage content of nonivamide using the following expression:

$$\frac{F_1 \times m_2 \times p_1}{F_2 \times m_1}$$

F_1 = area of the peak due to nonivamide in the chromatogram obtained with the test solution;

F_2 = area of the peak due to nonivamide in the chromatogram obtained with the reference solution;

m_1 = mass of the oleoresin to be examined, in grams;

m_2 = mass of *nonivamide CRS* in the reference solution, in grams;

p_1 = percentage content of nonivamide in *nonivamide CRS*.

Limit:

— *nonivamide*: maximum 5.0 per cent of the total capsaicinoid content.

Water ***(2.5.12)***

Maximum 8.0 per cent, determined on 5.00 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for nonivamide.

Calculate the percentage content of total capsaicinoids, expressed as capsaicin, using the following expression:

$$\frac{(F_3 + F_5 + F_6) \times m_4 \times p_2}{F_4 \times m_3}$$

F_3 = area of the peak due to capsaicin in the chromatogram obtained with the test solution;

F_4 = area of the peak due to capsaicin in the chromatogram obtained with the reference solution;

F_5 = area of the peak due to dihydrocapsaicin in the chromatogram obtained with the test solution;

F_6 = area of the peak due to nordihydrocapsaicin in the chromatogram obtained with the test solution;

m_3 = mass of the oleoresin to be examined, in grams;

m_4 = mass of *capsaicin CRS* in the reference solution, in grams;

p_2 = percentage content of capsaicin in *capsaicin CRS*.

Ph Eur

Refined and Standardised Milk Thistle Dry Extract

General Notices

(Ph Eur monograph 2071)

Ph Eur

DEFINITION

Dry extract, refined and standardised, produced from *Milk-thistle fruit (1860)*.

Content

90 per cent to 110 per cent of the nominal content of silymarin, expressed as silibinin ($C_{25}H_{22}O_{10}$; M_r 482.4), stated on the label. The nominal content of silymarin is within the range 30 per cent *m/m* to 65 per cent *m/m* (dried extract).

The content of silymarin corresponds to:

- — *sum of the contents of silicristin and silidianin* (both $C_{25}H_{22}O_{10}$; M_r 482.4): 20 per cent to 45 per cent, calculated with reference to total silymarin;
- — *sum of the contents of silibinin A and silibinin B* (both $C_{25}H_{22}O_{10}$; M_r 482.4): 40 per cent to 65 per cent, calculated with reference to total silymarin;
- — *sum of the contents of isosilibinin A and isosilibinin B* (both $C_{25}H_{22}O_{10}$; M_r 482.4): 10 per cent to 20 per cent, calculated with reference to total silymarin.

PRODUCTION

The extract is produced from the herbal drug by an appropriate procedure, using one or more of the following solvents:

- — ethyl acetate;
- — acetone or mixture of acetone and water;
- — ethanol or mixture of ethanol and water;
- — methanol or mixture of methanol and water.

CHARACTERS

Appearance

Yellowish-brown, amorphous powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.250 g of the extract to be examined in 5 ml of *methanol R*.

Reference solution Dissolve 2 mg of *silibinin R* and 5 mg of *taxifolin R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R, acetone R, methylene chloride R* (8.5:16.5:75 *V/V/V*) .

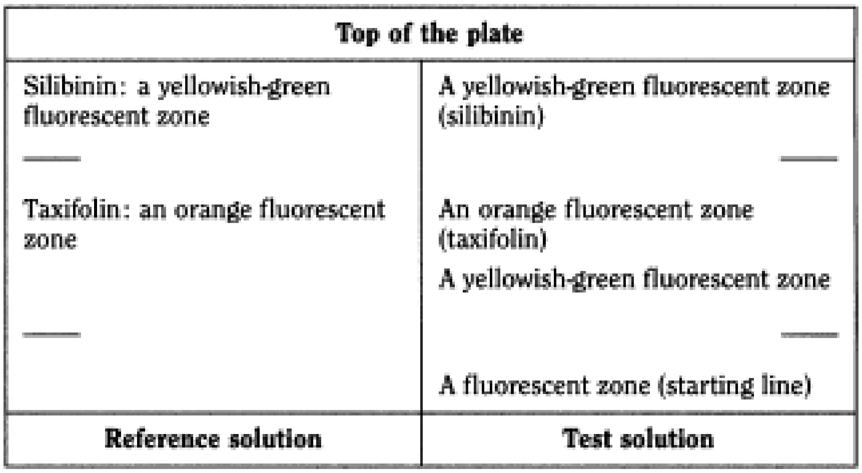
Application 10 µl [or 8 µl] of the test solution and 10 µl [or 2 µl] of the reference solution, as bands.

Development Over a path of 10 cm [or 6 cm].

Drying At 100-105 °C.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and subsequently spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 30 min and examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other yellowish-green fluorescent zones may be present between the zones due to silibinin and taxifolin in the chromatogram obtained with the test solution.



Top of the plate	
Silibinin: a yellowish-green fluorescent zone	A yellowish-green fluorescent zone (silibinin)
——	——
Taxifolin: an orange fluorescent zone	An orange fluorescent zone (taxifolin)
	A yellowish-green fluorescent zone
——	——
	A fluorescent zone (starting line)
Reference solution	**Test solution**

TESTS

Loss on drying *(2.8.17)*

Maximum 5.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 60.0 mg of the extract to be examined in *methanol R* and dilute to 100.0 ml with the same solvent.

Reference solution Dissolve a quantity of *milk thistle standardised dry extract CRS* corresponding to 10.0 mg (m_1 g) of silibinin in *methanol R* and dilute to 100.0 ml with the same solvent.

Column:

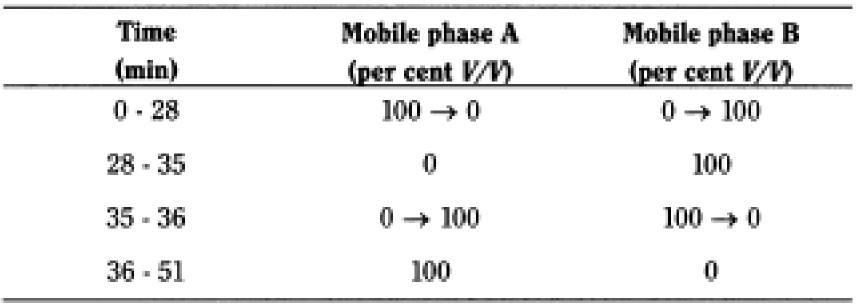
— *size:* l = 0.125 m, Ø = 4 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A: phosphoric acid R, methanol R, water R* (0.5:35:65 *V/V/V*);

— *mobile phase B: phosphoric acid R, methanol R, water R* (0.5:50:50 *V/V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 28	100 → 0	0 → 100
28 - 35	0	100
35 - 36	0 → 100	100 → 0
36 - 51	100	0

Flow rate 0.8 ml/min.

Detection Spectrophotometer at 288 nm.

Injection 10 µl.

Retention time Silibinin B = about 30 min; if necessary, adjust the time periods of the gradient.

System suitability Reference solution:

— *resolution*: minimum 1.8 between the peaks due to silibinin A and silibinin B.

— the chromatogram obtained with the reference solution is similar to the chromatogram supplied with *milk thistle standardised dry extract CRS*.

Locate the peaks due to silicristin, silidianin, silibinin A, silibinin B, isosilibinin A and isosilibinin B using the chromatogram supplied with *milk thistle standardised dry extract CRS*. In the chromatogram obtained with the test solution the peak due to silidianin may vary in size or be absent.

Calculate the percentage content of total silymarin, expressed as silibinin, using the following expression:

$$\frac{(F_1 + F_2 + F_3 + F_4 + F_5 + F_6) \times m_1}{(F_7 + F_8) \times m_2}$$

Calculate the percentage content of the sum of silicristin and silidianin, with reference to total silymarin, using the following expression:

$$\frac{(F_1 + F_2) \times 100}{F_1 + F_2 + F_3 + F_4 + F_5 + F_6}$$

Calculate the percentage content of the sum of silibinin A and silibinin B, with reference to total silymarin, using the following expression:

$$\frac{(F_3 + F_4) \times 100}{F_1 + F_2 + F_3 + F_4 + F_5 + F_6}$$

Calculate the percentage content of the sum of isosilibinin A and isosilibinin B, with reference to total silymarin, using the following expression:

$$\frac{(F_5 + F_6) \times 100}{F_1 + F_2 + F_3 + F_4 + F_5 + F_6}$$

F_1 = area of the peak due to silicristin in the chromatogram obtained with the test solution;

F_2 = area of the peak due to silidianin in the chromatogram obtained with the test solution;

F_3 = area of the peak due to silibinin A in the chromatogram obtained with the test solution;

F_4 = area of the peak due to silibinin B in the chromatogram obtained with the test solution;

F_5 = area of the peak due to isosilibinin A in the chromatogram obtained with the test solution;

F_6 = area of the peak due to isosilibinin B in the chromatogram obtained with the test solution;

F_7 = area of the peak due to silibinin A in the chromatogram obtained with the reference solution;

F_8 = area of the peak due to silibinin B in the chromatogram obtained with the reference solution;

M_1 = mass of silibinin in the reference solution, in grams;

M_2 = mass of the extract to be examined, in grams.

Ph Eur

Restharrow Root

General Notices

(Ph Eur monograph 1879)

Ph Eur

DEFINITION

Whole or cut, dried root of *Ononis spinosa* L.

IDENTIFICATION

A. The root is more or less flattened, twisted and branched, deeply wrinkled, brown and grooved longitudinally. The transversely cut surface shows a thin bark and a xylem cylinder with a conspicuously radiate structure. The fracture of the root is short and fibrous.

B. Reduce to a powder (355) (*2.9.12*). The powder is light brown or brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: brown fragments of cork composed of thin-walled polygonal cells; groups of thick-walled narrow fibres, often accompanied by a parenchymatous crystal sheath containing prisms of calcium oxalate; fragments of vessels with numerous small bordered pits; parenchymatous cells with single prisms of calcium oxalate. Examine under a microscope using a mixture of equal volumes of *glycerol R* and *water R*. The powder shows numerous simple, round starch granules, 5-10 µm in diameter.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (180) (*2.9.12*) add 15.0 ml of *methanol R* and boil under a reflux condenser for 30 min. Cool and filter.

Reference solution Dissolve 10 mg of *resorcinol R* and 50 mg of *vanillin R* in 10 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethanol (96 per cent) R*, *methylene chloride R*, *toluene R* (10:45:45 *V/V/V*).

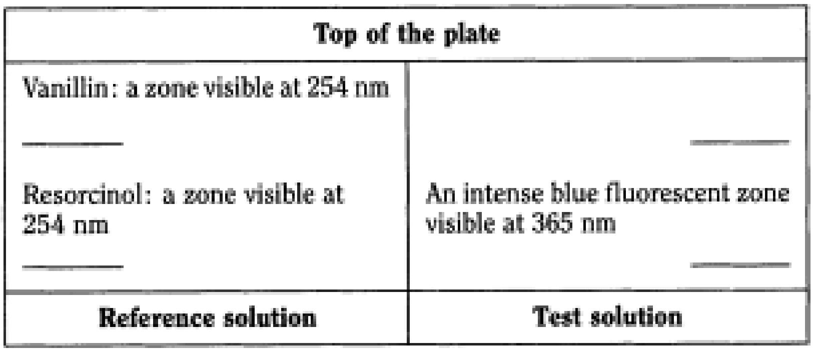
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm and 365 nm.

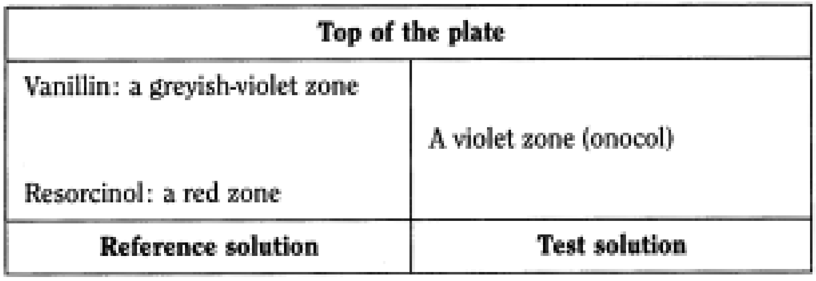
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones are present in the middle third of the chromatogram obtained with the test solution.



Top of the plate	
Vanillin: a zone visible at 254 nm	
———	———
Resorcinol: a zone visible at 254 nm	An intense blue fluorescent zone visible at 365 nm
———	———
Reference solution	**Test solution**

Detection B Spray with *anisaldehyde solution R*. Heat at 100-105 °C for 5-10 min. Examine in daylight.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
Vanillin: a greyish-violet zone	
	A violet zone (onocol)
Resorcinol: a red zone	
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 8.0 per cent.

Extractable matter

Minimum 15.0 per cent.

To 2.00 g of the powdered drug (250) (*2.9.12*) add a mixture of 8 g of *water R* and 12 g of *ethanol (96 per cent) R* and allow to macerate for 2 h, shaking frequently. Filter, evaporate 5 g of the filtrate to dryness on a water-bath and dry in an oven at 100-105 °C for 2 h. The residue weighs a minimum of 75 mg.

Ph Eur

Rhatany Root

General Notices

Krameria
(Ph Eur monograph 0289)

Preparation

Rhatany Tincture

When Powdered Rhatany Root is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried, usually fragmented, underground organs of *Krameria triandra* Ruiz and Pavon, known as Peruvian rhatany.

Content

Minimum 5.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The taproot is dark reddish-brown and has a thick, knotty crown. The secondary roots are the same colour and nearly straight or somewhat tortuous. The bark is rugged or scaly in the older pieces and smooth with sharp, transverse fissures in the younger pieces; it separates readily from the wood. The fracture is fibrous in the bark and splintery in the wood. The smooth, transversely cut surface shows a dark brownish-red bark about one third of the radius in thickness; a dense, pale reddish-brown and finely porous wood is present with numerous fine medullary rays; the central heartwood is often darker.

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-red. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: cork cells containing dark brown phlobaphenes; fragments of unlignified phloem fibres, usually 12-30 µm in diameter with moderately thick walls; phloem parenchyma cells in files containing prisms and microcrystals of calcium oxalate; fragments of vessels usually 20-60 µm in diameter with bordered pits; fragments of tracheids up to 20 µm wide with slit-shaped pits. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows rounded starch granules, simple or 2- to 4-compound, an individual granule measuring up to 30 µm in diameter and some granules being found in the cells of the medullary rays and in the parenchyma.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of a mixture of 3 volumes of *water R* and 7 volumes of *ethanol (96 per cent) R*, shake for 10 min and filter. To the filtrate add 10 ml of *light petroleum R* and shake. Separate the light petroleum layer, add 2 g of *anhydrous sodium sulphate R*, shake and filter. Evaporate the filtrate to dryness. Dissolve the residue in 0.5 ml of *methanol R*.

Reference solution Dissolve 5.0 mg of *Sudan red G R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (2:98 *V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 5 g/l solution of *fast blue B salt R*. Allow to dry in air and spray with *0.1 M ethanolic sodium hydroxide*; examine in daylight.

Results The chromatogram obtained with the reference solution shows in the lower third a red zone due to Sudan red G. The chromatogram obtained with the test solution shows a violet zone due to rhatany phenol I similar in position to the zone of Sudan red G in the chromatogram obtained with the reference solution, below it the brownish zone due to rhatany phenol II and below this the bluish-grey zone due to rhatany phenol III. Further zones may be present.

TESTS

Foreign matter (*2.8.2*)

Maximum 2 per cent of foreign matter and maximum 5 per cent of fragments of crown or root exceeding 25 mm in diameter. Root without bark may be present in very small quantities.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C.

Total ash (*2.4.16*)

Maximum 5.5 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs (*2.8.14*). Use 0.750 g of powdered drug (180) (*2.9.12*).

Ph Eur

Rhatany Tincture

General Notices

(Ph Eur monograph 1888)

Ph Eur

DEFINITION

Tincture produced from *Rhatany root (0289).*

Content

Minimum 1.0 per cent *m/m* of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1).

PRODUCTION

The tincture is produced from 1 part of the drug and 5 parts of ethanol (70 per cent *V/V*) by a suitable procedure.

CHARACTERS

Appearance

Reddish-brown liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution To 5 ml of the tincture to be examined, add 10 ml of *light petroleum R* and shake. Separate the light petroleum layer, add 2 g of *anhydrous sodium sulphate R*, shake and filter. Evaporate the filtrate to dryness. Dissolve the residue in 0.5 ml of *methylene chloride R*.

Reference solution Dissolve 5 mg of *thymol R* and 10 mg of *dichlorophenolindophenol, sodium salt R* in 10 ml of *alcohol (60 per cent V/V) R*.

Plate *TLC silica gel plate R*.

Mobile phase *methylene chloride R*.

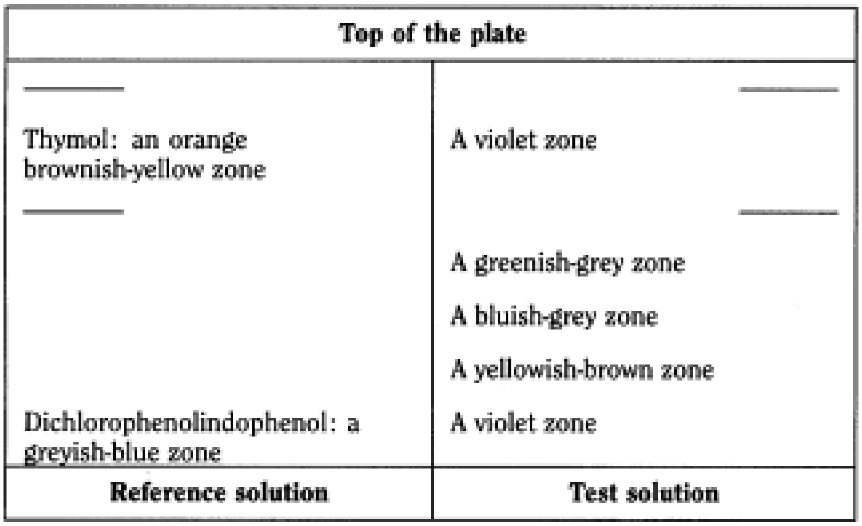
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 5 g/l solution of *fast blue B salt R*; allow the plate to dry in air and spray with *0.1 M ethanolic sodium hydroxide*; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Thymol: an orange brownish-yellow zone	A violet zone
———	———
	A greenish-grey zone
	A bluish-grey zone
	A yellowish-brown zone
Dichlorophenolindophenol: a greyish-blue zone	A violet zone
Reference solution	**Test solution**

TESTS

Ethanol *(2.9.10)*

63 per cent *V/V* to 67 per cent *V/V*.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

ASSAY

Carry out the determination of tannins in herbal drugs *(2.8.14)* using 2.500 g of the tincture to be examined.

Ph Eur

Rhubarb

General Notices

(Ph Eur monograph 0291)

Preparation

Compound Rhubarb Tincture

When Powdered Rhubarb is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Rhubarb consists of the whole or cut, dried underground parts of *Rheum palmatum* L. or of *Rheum officinale* Baillon or of hybrids of these two species or of a mixture. The underground parts are often divided; the stem and most of the bark with the rootlets are removed. It contains not less than 2.2 per cent of hydroxyanthracene derivatives, expressed as rhein ($C_{15}H_8O_6$, M_r 284.2), calculated with reference to the dried drug.

CHARACTERS

Characteristic, aromatic odour.

IDENTIFICATION

A. The appearance is variable: disc-shaped pieces up to 10 cm in diameter and 1 cm to 5 cm in thickness; cylindrical pieces; oval or planoconvex pieces. The surface has a pinkish tinge and is usually covered with a layer of brownish-yellow powder. It shows, especially after moistening, a reticulum of darker lines. This structure causes the marbled appearance of the drug. The fracture is granular. The transverse section of the rhizome shows a narrow outer zone of radiating brownish-red lines. These medullary rays are crossed perpendicularly by a dark cambial ring. Inside this zone is a ring of small star-spot formations of anomalous vascular bundles. The root shows a more radiate structure.

B. Reduce to a powder (355) (*2.9.12*). The powder is orange to brownish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: large calcium oxalate cluster crystals, which may measure more than 100 µm, and their fragments; reticulately thickened non-lignified vessels measuring up to 175 µm. Numerous groups of rounded or polygonal, thin-walled parenchyma cells. Sclereids and fibres are absent. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows simple, rounded or compound (2 to 4) starch granules with a star-shaped hilum.

C. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution Heat 50 mg of the powdered drug (180) (*2.9.12*) in a water-bath for 15 min with a mixture of 1 ml of *hydrochloric acid R* and 30 ml of *water R*. Allow to cool and shake the liquid with 25 ml of *ether R*. Dry the ether layer over *anhydrous sodium sulphate R* and filter. Evaporate the ether layer to dryness and dissolve the residue in 0.5 ml of *ether R*.

Reference solution Dissolve 5 mg of *emodin R* in 5 ml of *ether R*.

Apply separately to the plate as bands 20 µl of each solution. Develop over a path of 10 cm using a mixture of 1 volume of *anhydrous formic acid R*, 25 volumes of *ethyl acetate R* and 75 volumes of *light petroleum R*. Allow the plate to dry in air and examine in ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows in its central part a zone of orange fluorescence (emodin). The chromatogram obtained with the test solution shows: a zone due to emodin; above the emodin zone, two zones of similar fluorescence (physcione and chrysophanol, in order of increasing R_F value); below the emodin zone, also two zones of similar fluorescence (rhein and aloe-emodin, in order of decreasing R_F value). Spray with a 100 g/l solution of *potassium hydroxide R* in *methanol R*. All the zones become red to violet.

D. To about 50 mg of the powdered drug (180) (*2.9.12*) add 25 ml of *dilute hydrochloric acid R* and heat the mixture on a water-bath for 15 min. Allow to cool, shake with 20 ml of *ether R* and discard the aqueous layer. Shake the ether layer with 10 ml of *dilute ammonia R1*. The aqueous layer becomes red to violet.

TESTS

Rheum rhaponticum

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution To 0.2 g of the powdered drug (180) (*2.9.12*) add 2 ml of *methanol R* and boil for 5 min under a reflux condenser. Allow to cool and filter. Use the filtrate as the test solution.

Reference solution Dissolve 10 mg of *rhaponticin R* in 10 ml of *methanol R*.

Apply separately to the plate, as bands not more than 20 mm by 3 mm, 20 µl of each solution. Develop over a path of 12 cm using a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R*. Allow the plate to dry in air and spray with *phosphomolybdic acid solution R*. The chromatogram obtained with the test solution does not show a blue zone near the starting-line (rhaponticin) corresponding to the zone in the chromatogram obtained with the reference solution.

Loss on drying (*2.2.32*)

Not more than 12.0 per cent, determined on 1.000 g of the powdered drug (180) (*2.9.12*) by drying in an oven at 105 °C.

Total ash (*2.4.16*)

Not more than 12.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*)

Not more than 2.0 per cent.

ASSAY

Carry out the assay protected from bright light.

Introduce 0.100 g of the powdered drug (180) (*2.9.12*) into a 100 ml flask. Add 30.0 ml of *water R*, mix and weigh. Heat in a water-bath under a reflux condenser for 15 min. Allow to cool, add 50 mg of *sodium hydrogen carbonate R*, weigh and adjust to the original mass with *water R*. Centrifuge and transfer 10.0 ml of the liquid to a 100 ml round-bottomed flask with a ground-glass neck. Add 20 ml of *ferric chloride solution R1* and mix. Heat under a reflux condenser on a water-bath for 20 min, add 1 ml of *hydrochloric acid R* and heat for a further 20 min, shaking frequently. Cool, transfer to a separating funnel and shake with three quantities, each of 25 ml, of *ether R* previously used to rinse the flask. Combine the ether extracts and wash with two quantities, each of 15 ml, of *water R*. Filter the ether extracts through a plug of absorbent cotton into a volumetric flask and dilute to 100.0 ml with *ether R*. Evaporate 10.0 ml carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance (*2.2.25*) at 515 nm, using *methanol R* as the compensation liquid.

Calculate the percentage content of rhein from the expression:

$$\frac{A \times 0.64}{m}$$

i.e. taking the specific absorbance of rhein to be 468, calculated on the basis of the specific absorbance of barbaloin.

A = absorbance at 515 nm;

m = mass of the drug used, in grams.

Ph Eur

Roselle

General Notices

(Ph Eur monograph 1623)

Ph Eur

DEFINITION

Whole or cut dried calyces and epicalyces of *Hibiscus sabdariffa* L. collected during fruiting.

Content

Minimum 13.5 per cent of acids, expressed as citric acid ($C_6H_8O_7$; M_r 192.1) (dried drug).

CHARACTERS

Acidic taste.

IDENTIFICATION

A. The calyx is joined in the lower half to form an urceolate structure, the upper half dividing to form 5 long acuminate recurved tips. The tips have a prominent, slightly protruding midrib and a large, thick nectary gland about 1 mm in diameter. The epicalyx consists of 8-12 small, obovate leaflets, which are adnate to the base of the calyx. The calyx and epicalyx are fleshy, dry, easily fragmented and bright red or deep purple, somewhat lighter at the base of the inner side.

B. Reduce to a powder (355) (*2.9.12*). The powder is red or purplish-red. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: predominantly red fragments of the parenchyma containing numerous crystal clusters of calcium oxalate and, sporadically, mucilage-filled cavities, sometimes associated with polygonal epidermal cells and anisocytic stomata (*2.8.3*); numerous fragments of vascular bundles with spiral and reticulate vessels; sclerenchymatous fibres with a wide lumen; rarely, rectangular, pitted parenchymatous cells; fragments of unicellular, smooth, bent covering trichomes and occasional glandular trichomes; rounded pollen grains with a spiny exine.

C. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *ethanol (60 per cent V/V) R*. Shake for 15 min and filter.

Reference solution Dissolve 2.5 mg of *quinaldine red R* and 2.5 mg of *sulphan blue R* in 10 ml of *methanol R.*

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R*, *water R*, *butanol R* (10:12:40 *V/V/V*).

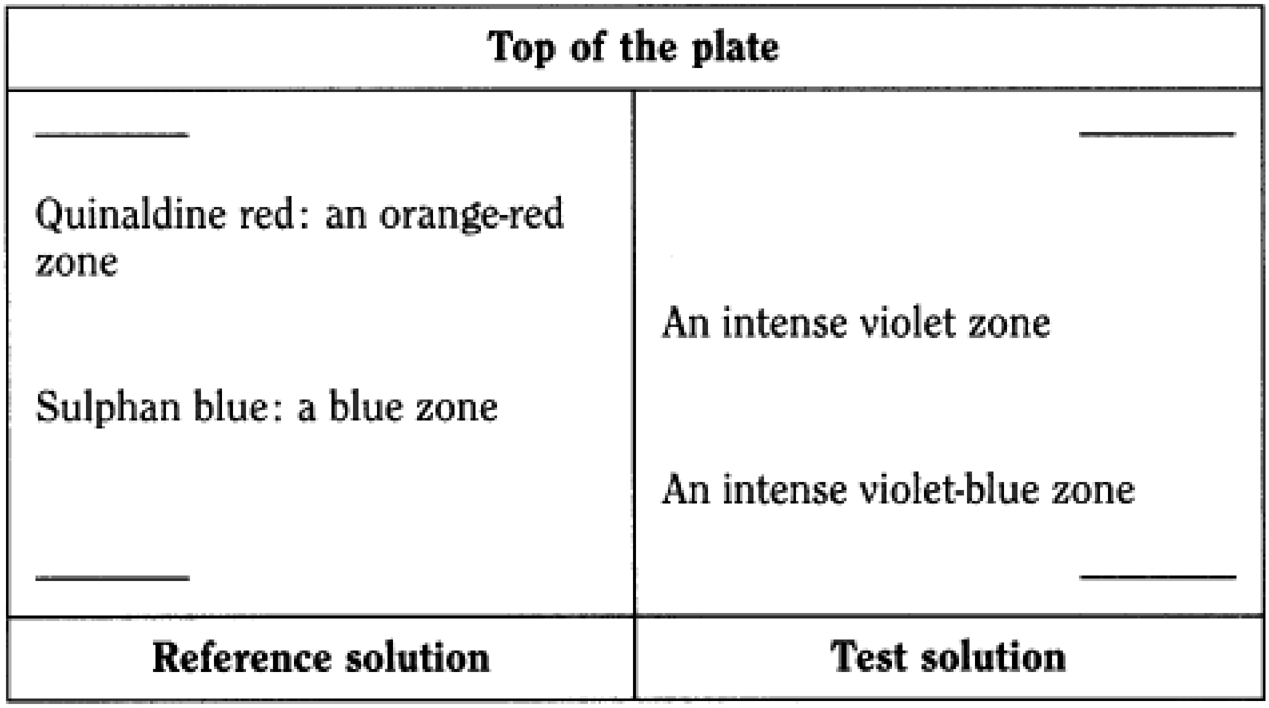
Application 5 µl as bands of 10 mm [or 2 µl as bands of 8 mm].

Development Over a path of 10 cm [or 6 cm].

Drying In air.

Detection Examine immediately in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
_______	_______
Quinaldine red: an orange-red zone	
	An intense violet zone
Sulphan blue: a blue zone	
	An intense violet-blue zone
_______	_______
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 2 per cent of fragments of fruits (red funicles and parts of the 5-caverned capsule with yellowish-grey pericarp, whose thin walls consist of several layers of differently directed fibres; flattened, reniform seeds with a dotted surface).

Loss on drying (*2.2.32*)

Maximum 11.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 10.0 per cent.

Colouring intensity

Reduce 100 g to a coarse powder (1400) (*2.9.12*) and homogenise. Reduce about 10 g of this mixture to a powder (355) (*2.9.12*). To 1.0 g of the powdered drug (355) (*2.9.12*) add 25 ml of boiling *water R* in a 100 ml flask and heat for 15 min on a water-bath with frequent shaking. Filter the hot mixture into a 50 ml graduated flask; rinse successively the 100 ml flask and the filter with 3 quantities, each of 5 ml, of warm *water R*. After cooling, dilute to 50 ml with *water R*. Dilute 5 ml of this solution to 50 ml with *water R*. Measure the absorbance (*2.2.25*) at 520 nm using *water R* as the compensation liquid. The absorbance is not less than 0.350 for the whole drug and not less than 0.250 for the cut drug.

ASSAY

Shake 1.000 g of the powdered drug (355) (*2.9.12*) with 100 ml of *carbon dioxide-free water R* for 15 min. Filter. To 50.0 ml of the filtrate add 100 ml of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide* to pH 7.0, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* is equivalent to 6.4 mg of citric acid.

Ph Eur

Rosemary Leaf

General Notices

(Ph Eur monograph 1560)

Ph Eur

DEFINITION

Whole, dried leaf of *Rosmarinus officinalis* L.

Content:

— minimum 12 ml/kg of essential oil (anhydrous drug);

— minimum 3 per cent of total hydroxycinnamic derivatives, expressed as rosmarinic acid ($C_{18}H_{16}O_8$; M_r 360) (anhydrous drug).

CHARACTERS

Strongly aromatic odour.

IDENTIFICATION

A. The leaves are sessile, tough, linear to linear-lanceolate, 1 cm to 4 cm long and 2 mm to 4 mm wide, with recurved edges. The upper surface is dark green, glabrous and grainy, the lower surface is greyish-green and densely tomentose with a prominent midrib.

B. Reduce to a powder (355) (*2.9.12*). The powder is greyish-green to yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows fragments of lower epidermis with straight to sinuous-walled cells and numerous diacytic stomata (*2.8.3*); fragments of the upper epidermis with straight-walled cells, slightly thickened and pitted, and an underlying hypodermis composed of large, irregular cells with thickened and beaded anticlinal walls; fragments in sectional view showing the hypodermal cells extending across the lamina at intervals, separating the one or two-layered palisade into large, crescent-shaped areas; numerous multicellular, extensively branched, covering trichomes of the lower epidermis and rare conical covering trichomes of the upper epidermis; glandular trichomes of 2 types, the majority with a short, unicellular stalk and a radiate head composed of 8 cells, others, less abundant, with a unicellular stalk and a spherical, unicellular or bicellular head.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 µl of the oil obtained in the assay in 1 ml of *hexane R*.

Reference solution Dissolve 5 mg of *borneol R*, 5 mg of *bornyl acetate R* and 10 µl of *cineole R* in 1 ml of *hexane R*.

Plate *TLC silica gel G plate R*.

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

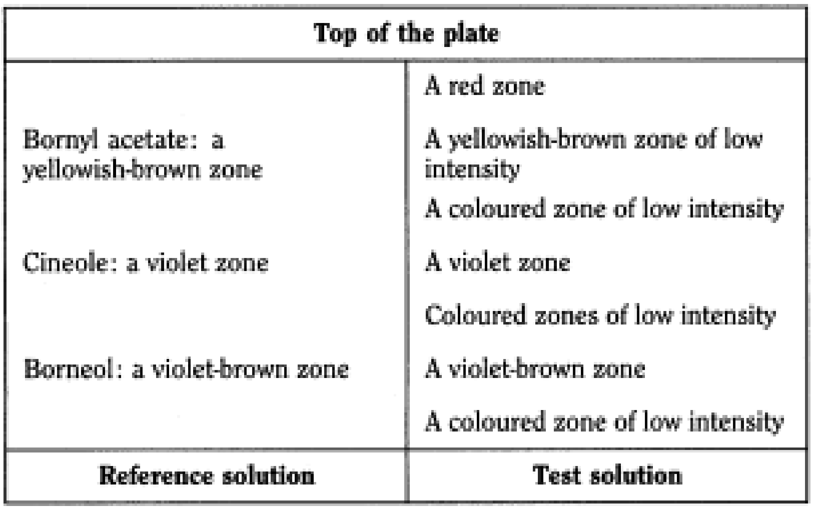
Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R*. Heat at 100-105 °C for 10 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
	A red zone
Bornyl acetate: a yellowish-brown zone	A yellowish-brown zone of low intensity
	A coloured zone of low intensity
Cineole: a violet zone	A violet zone
	Coloured zones of low intensity
Borneol: a violet-brown zone	A violet-brown zone
	A coloured zone of low intensity
Reference solution	**Test solution**

D. Thin-layer chromatography (*2.2.27*).

Test solution Grind 1.0 g of the drug in 10 ml of *methanol R* and filter.

Reference solution Dissolve 5.0 mg of *rosmarinic acid R* and 1.0 mg of *caffeic acid R* in 10 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R*, *acetone R*, *methylene chloride R* (8.5:25:85 *V/V/V*).

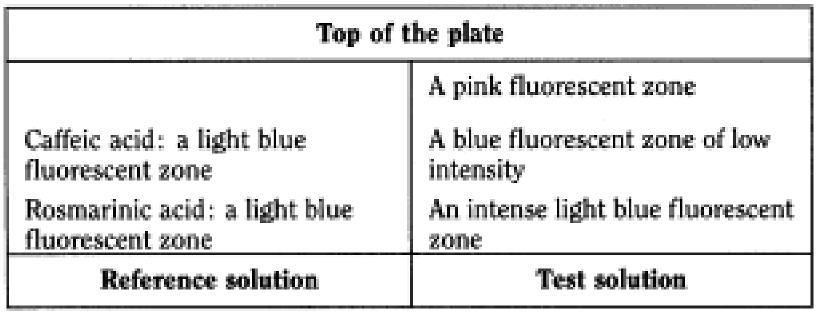
Application 10 µl of the test solution and 20 µl of the reference solution, as bands.

Development Over a path of 8 cm.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
	A pink fluorescent zone
Caffeic acid: a light blue fluorescent zone	A blue fluorescent zone of low intensity
Rosmarinic acid: a light blue fluorescent zone	An intense light blue fluorescent zone
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*)

Maximum 5 per cent of stems and maximum 2 per cent of other foreign matter.

Water (*2.2.13*)

Maximum 100 ml/kg, determined on 20.0 g of the powdered drug (355) (*2.9.12*).

Total ash (*2.4.16*)

Maximum 9.0 per cent.

ASSAY

Total hydroxycinnamic derivatives

Stock solution To 0.200 g of the powdered drug (355) (*2.9.12*) add 80 ml of *alcohol (50 per cent V/V) R*. Boil in a water-bath under a reflux condenser for 30 min. Allow to cool and filter. Rinse the filter with 10 ml of *alcohol (50 per cent V/V) R*. Combine the filtrate and the rinsings in a volumetric flask and dilute to 100.0 ml with *alcohol (50 per cent V/V) R*.

Test solution To 1.0 ml of the stock solution add 2 ml of *0.5 M hydrochloric acid*, 2 ml of a solution prepared by dissolving 10 g of *sodium nitrite R* and 10 g of *sodium molybdate R* in 100 ml of *water R* and then add 2 ml of *dilute sodium hydroxide solution R* and dilute to 10.0 ml with *water R*; mix.

Compensation solution Dilute 1.0 ml of the stock solution to 10.0 ml with *water R*.

Measure immediately the absorbance (*2.2.25*) of the test solution at 505 nm.

Calculate the percentage content of total hydroxycinnamic derivatives, expressed as rosmarinic acid, from the expression:

$$\frac{A \times 2.5}{m}$$

i.e. taking the specific absorbance of rosmarinic acid to be 400.

A = absorbance of the test solution at 505 nm,

m = mass of the substance to be examined, in grams.

Essential oil (*2.8.12*)

Use 25.0 g of the crushed drug, a 1000 ml flask and 300 ml of *water R* as the distillation liquid. Distil at a rate of 2-3 ml/min for 3 h.

Ph Eur

Rosemary Oil

General Notices

(Ph Eur monograph 1846)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the flowering aerial parts of *Rosmarinus officinalis* L.

CHARACTERS

Appearance

Clear, mobile, colourless to pale yellow liquid with a characteristic odour.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.5 ml of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 50 mg of *borneol R*, 50 mg of *bornyl acetate R* and 100 µl of *cineole R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase ethyl acetate R, *toluene R* (5:95 *V/V*).

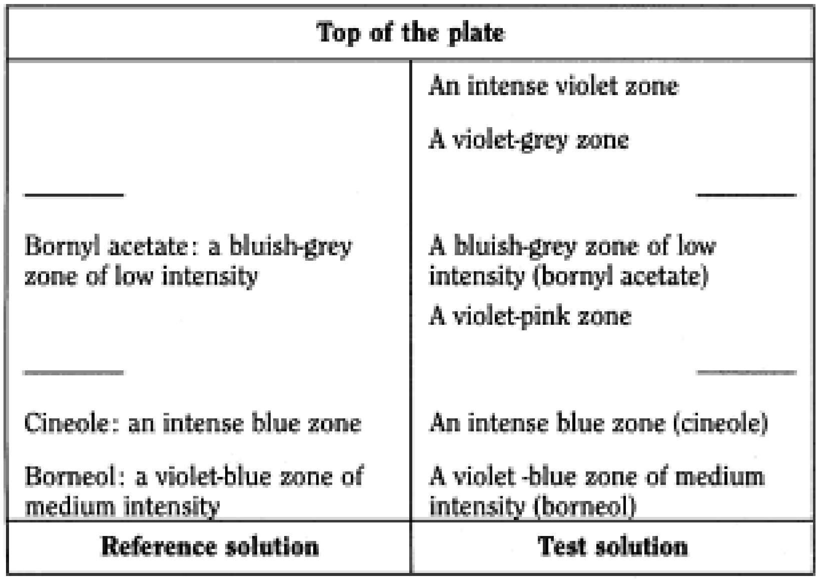
Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray the plate with *vanillin reagent R* and heat the plate at 100-105 °C for 10 min. Examine immediately in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, several violet-blue to violet-grey zones of medium intensity (terpene alcohols) are present in the lower third of the chromatogram obtained with the test solution.



Top of the plate	
	An intense violet zone
	A violet-grey zone
———	———
Bornyl acetate: a bluish-grey zone of low intensity	A bluish-grey zone of low intensity (bornyl acetate)
	A violet-pink zone
———	———
Cineole: an intense blue zone	An intense blue zone (cineole)
Borneol: a violet-blue zone of medium intensity	A violet -blue zone of medium intensity (borneol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density (*2.2.5*)

0.895 to 0.920.

Refractive index (*2.2.6*)

1.464 to 1.473.

Optical rotation (*2.2.7*)

- 5° to + 8°.

Acid value (*2.5.1*)

Maximum 1.0.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.20 ml of the substance to be examined in *hexane R* and dilute to 10.0 ml with the same solvent.

Reference solution Dissolve 20 µl of *α-pinene R*, 10 mg of *camphene R*, 20 µl of *β-pinene R*, 10 µl of *β-myrcene R*, 20 µl of *limonene R*, 50 µl of *cineole R*, 10 µl of *p-cymene R*, 50 mg of *camphor R*, 30 mg of *bornyl acetate R*, 10 mg of *α-terpineol R*, 10 mg of *borneol R* and 10 µl of *verbenone R* in *hexane R* and dilute to 10.0 ml with the same solvent.

Column:

— *material*: fused silica,

— *size:* l = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.25-0.53 mm,

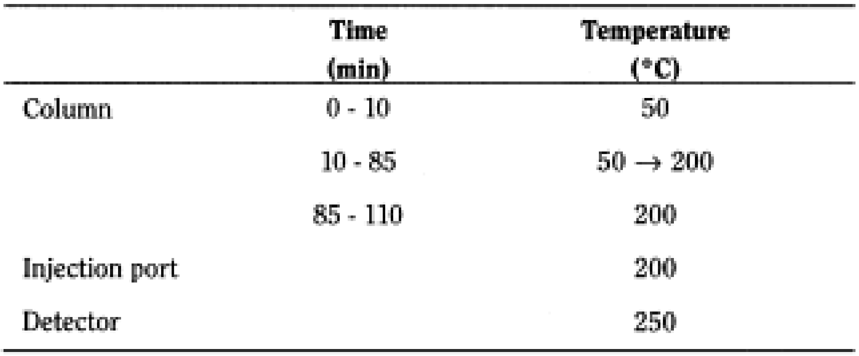
— *stationary phase*: *macrogol 20 000 R*.

Carrier gas *helium for chromatography R*.

Flow rate 1 ml/min.

Split ratio 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	50
	10 - 85	50 → 200
	85 - 110	200
Injection port		200
Detector		250

Detection Flame ionisation.

Injection 1 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to limonene and cineole and minimum 1.5 between the peaks due to α-terpineol and borneol.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of these components.

For rosemary oil, Spanish type, the percentages are within the following ranges:

— α-*pinene*: 18 per cent to 26 per cent,

— *camphene*: 8.0 per cent to 12.0 per cent,

— β-*pinene*: 2.0 per cent to 6.0 per cent,

— β-*myrcene*: 1.5 per cent to 5.0 per cent,

— *limonene*: 2.5 per cent to 5.0 per cent,

— *cineole*: 16.0 per cent to 25.0 per cent,

— *p-cymene*: 1.0 per cent to 2.2 per cent,

— *camphor* : 13.0 per cent to 21.0 per cent,

— *bornyl acetate*: 0.5 per cent to 2.5 per cent,

— α-*terpineol*: 1.0 per cent to 3.5 per cent,

— *borneol*: 2.0 per cent to 4.5 per cent,

— *verbenone*: 0.7 per cent to 2.5 per cent.

For rosemary oil, Moroccan and Tunisian type, the percentages are within the following ranges:

— α-*pinene*: 9.0 per cent to 14.0 per cent,

— *camphene*: 2.5 per cent to 6.0 per cent,

— β-*pinene*: 4.0 per cent to 9.0 per cent,

— β-*myrcene*: 1.0 per cent to 2.0 per cent,

— *limonene*: 1.5 per cent to 4.0 per cent,

— *cineole*: 38.0 per cent to 55.0 per cent,

— *p-cymene*: 0.8 per cent to 2.5 per cent,

— *camphor*: 5.0 per cent to 15.0 per cent,

— *bornyl acetate*: 0.1 per cent to 1.5 per cent,

— α-*terpineol*: 1.0 per cent to 2.6 per cent,

— *borneol*: 1.5 per cent to 5.0 per cent,

— *verbenone*: maximum 0.4 per cent.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

LABELLING

The label states that the content is Spanish type or Moroccan and Tunisian type.

Ph Eur

Safflower Flower

General Notices

(Ph Eur monograph 2386)

Ph Eur

DEFINITION

Dried flower of *Carthamus tinctorius* L.

Content

Minimum 1.0 per cent of total flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The orange-yellow or reddish-orange, tubular, gametalous, actinomorphic florets are separate from the capitulum. Each consists of a long, filiform tube, about 1 cm long divided into 5 equal, narrow, lanceolate lobes, about 0.5 cm long. From the opening of the tube emerges the hollow cylinder formed by the fused yellow anthers, in which the filiform style persists, thickened near the apex.

B. Reduce to a powder (355) *(2.9.12)*. The powder is orange-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows fragments of the corolla tube with epidermis consisting of elongated, thin-walled polygonal cells; fragments of the lobes of the corolla showing at their apices a large number of small, rounded, very prominent papillae; fragments of parenchyma containing vascular bundles surrounded by secretory canals with reddish-brown contents; fragments of anthers consisting of irregularly shaped cells whose walls show thickenings in characteristic bands; fragments of the style, whose lower part consists of elongated cells and which ends in a stigma, bristling with rather long, conical, confluent papillae; rounded or elliptical triporate pollen grains up to 60 µm in diameter with an echinulate exine; calcium oxalate prisms, either isolated or present in parenchyma cells.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R*. Sonicate for 10 min and centrifuge.

Reference solution Dissolve 1 mg of *rutin R* and 5 mg of *quercetin dihydrate R* in 50 ml of *methanol R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R*, *acetic acid R*, *water R*, *ethyl acetate R* (11:11:27:100 *V/V/V/V*).

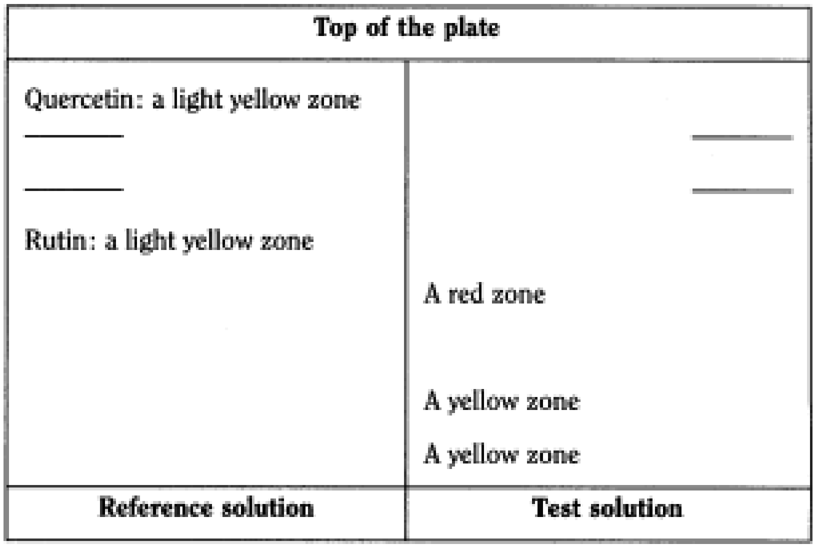
Application 25 µl as bands of 15 mm [or 10 µl as bands of 8 mm].

Development Over a path of 12 cm [or 7 cm].

Drying In air.

Detection A Examine in daylight.

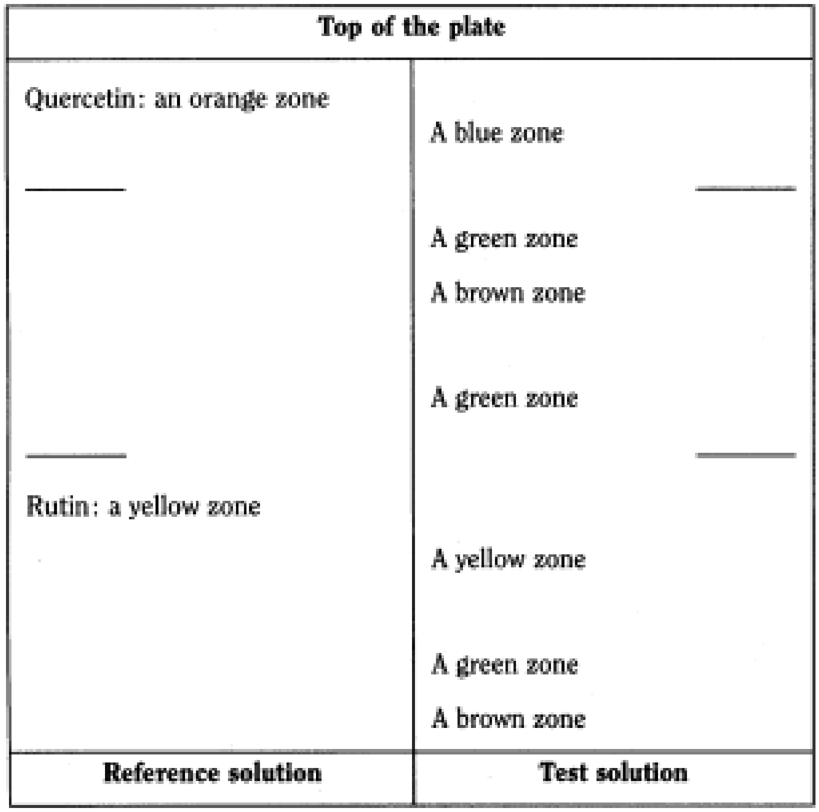
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Quercetin: a light yellow zone	
———	———
———	———
Rutin: a light yellow zone	
	A red zone
	A yellow zone
	A yellow zone
Reference solution	**Test solution**

Detection B Heat the plate at 100 °C for 3 min; spray the plate whilst still hot with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow the plate to dry in air for about 30 min; examine in ultraviolet light at 365 nm.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Quercetin: an orange zone	
	A blue zone
———	———
	A green zone
	A brown zone
	A green zone
———	———
Rutin: a yellow zone	
	A yellow zone
	A green zone
	A brown zone
Reference solution	**Test solution**

TESTS

Absorbance *(2.2.25)*.

A. *Yellow pigment*: macerate 0.1 g of the powdered drug (355) *(2.9.12)* in 150 ml of *water R*, stir for 1 h, filter through a sintered-glass filter (40) *(2.1.2)* and dilute to 500.0 ml, washing the residue, with *water R*. The absorbance is not less than 0.40 at 401 nm.

B. *Red pigment*: to 0.25 g of the powdered drug (355) *(2.9.12)* add 50 ml of a mixture of 20 volumes of *water R* and 80 volumes of *acetone R*. Heat on a water-bath at 50 °C for 90 min. Allow to cool, filter through a sintered-glass filter (40) *(2.1.2)* and dilute to 100.0 ml, washing the residue with a mixture of 20 volumes of *water R* and 80 volumes of *acetone R*. The absorbance is not less than 0.40 at 518 nm.

Loss on drying *(2.2.32)*

Maximum 11.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash (2.4.16)

Maximum 10.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

ASSAY

Solution A Place 0.250 g of the powdered drug (180) *(2.9.12)* in a 250 ml flask and add 95 ml of *methanol R*. Heat under a reflux condenser on a water-bath for 30 min. Allow to cool and filter. Rinse the filter with 5 ml of *methanol R*. Combine the filtrate and the rinsing solution in a volumetric flask and dilute to 100.0 ml with *methanol R*.

Test solution Place 5.0 ml of solution A in a volumetric flask and dilute to 20.0 ml with a 20 g/l solution of *aluminium chloride R* in *methanol R*.

Compensation solution Place 5.0 ml of solution A in a volumetric flask and dilute to 20.0 ml with *methanol R*.

After exactly 15 min, measure the absorbance *(2.2.25)* of the test solution at 420 nm by comparison with the compensation solution. Calculate the percentage content of total flavonoids, expressed as hyperoside, using the following expression:

$$\frac{A}{4 \times m}$$

taking the specific absorbance of hyperoside at 420 nm to be 400.

A = absorbance of the test solution, at 420 nm;

m = mass of the substance to be examined, in grams.

Ph Eur

Sage Leaf

General Notices

(Sage Leaf (Salvia officinalis), Ph Eur monograph 1370)

Ph Eur

DEFINITION

Whole or cut dried leaves of *Salvia officinalis* L.

Content

Minimum 15 ml/kg of essential oil for the whole drug and minimum 10 ml/kg of essential oil for the cut drug (anhydrous drug).

CHARACTERS

Sage leaf (*Salvia officinalis*) oil is rich in thujone.

IDENTIFICATION

A. The lamina of whole sage leaf (*Salvia officinalis*) is about 2 cm to 10 cm long and 1 cm to 2 cm wide, oblong-ovate, elliptical. The margin is finely crenate to smooth. The apex is rounded or subacute and the base is shrunken at the petiole and rounded or cordate. The upper surface is greenish-grey and finely granular; the lower surface is white and pubescent and shows a dense network of raised veinlets.

B. Reduce to a powder (355) *(2.9.12)*. The powder is light grey to brownish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: very numerous articulated and bent trichomes with narrow elongated cells and a very thick cell at the base as well as fragments of these trichomes; fragments of the upper epidermis with pitted, somewhat polygonal cells; fragments of the lower epidermis with sinuous cells and numerous diacytic stomata *(2.8.3)*; rare single glandular trichomes with a uni- or bicellular head and a stalk consisting of 1 to 4 cells; abundant glandular trichomes with a unicellular stalk and a head composed of 8 radiating cells with a raised common cuticle.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Shake 0.5 g of the freshly powdered drug (355) *(2.9.12)* with 5 ml of *ethanol R* for 5 min.

Reference solution Dissolve 20 µl of *thujone R* and 25 µl of *cineole R* in 20 ml of *ethanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

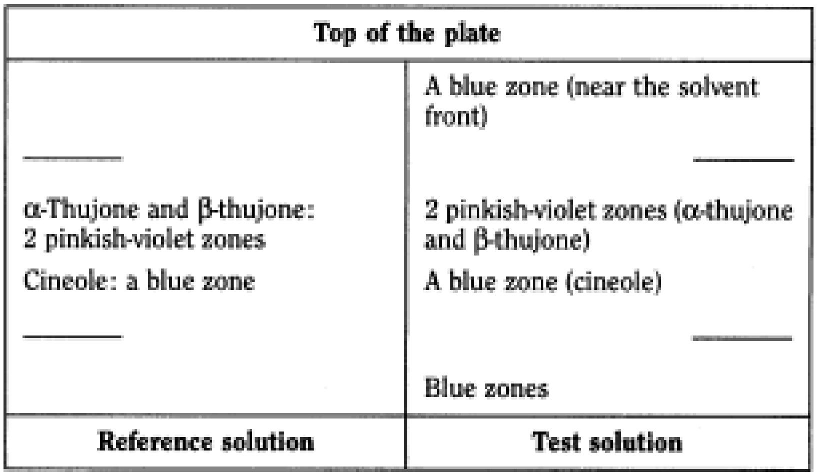
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray the plate with a 200 g/l solution of *phosphomolybdic acid R* in *ethanol R* and heat at 100-105 °C for 10 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	A blue zone (near the solvent front)
_______	_______
α-Thujone and β-thujone: 2 pinkish-violet zones	2 pinkish-violet zones (α-thujone and β-thujone)
Cineole: a blue zone	A blue zone (cineole)
_______	_______
	Blue zones
Reference solution	**Test solution**

TESTS

Foreign matter ***(2.8.2)***

Maximum 3 per cent of stems and maximum 2 per cent of other foreign matter.

Water ***(2.2.13)***

Maximum 100 ml/kg, determined on 20.0 g.

Total ash ***(2.4.16)***

Maximum 10.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use 20.0 g of the substance to be examined, cut, if necessary, immediately before the assay, a 500 ml flask, 250 ml of *water R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

Ph Eur

Sage Oil

General Notices

(Clary Sage Oil, Ph Eur monograph 1850)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the fresh or dried flowering stems of *Salvia sclarea* L.

CHARACTERS

Appearance

Colourless to brownish-yellow liquid, usually pale yellow, with a characteristic odour.

IDENTIFICATION

First identification *B*.

Second identification *A*.

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 1 ml of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 60 µl of *linalol R*, 200 µl of *linalyl acetate R* and 60 µl of α-*terpineol R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *Ethyl acetate R, toluene R* (5:95 *V/V*).

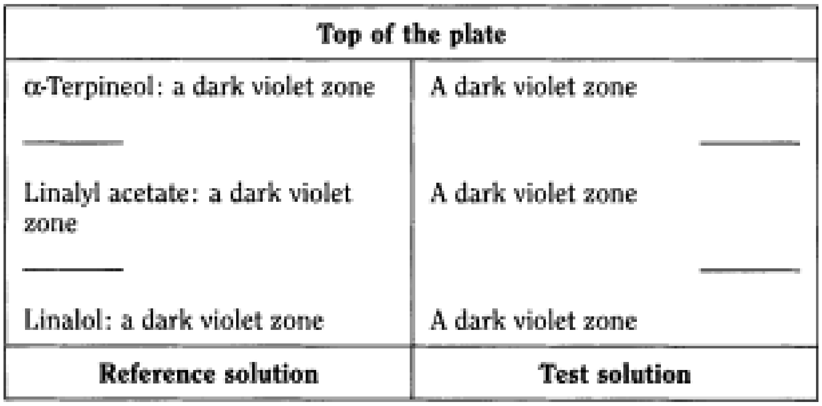
Application 5 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *vanillin reagent R* and heat at 100-105 °C for 5-10 min; examine in daylight within 5 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones are present in the chromatogram obtained with the test solution.



Top of the plate	
α-Terpineol: a dark violet zone	A dark violet zone
———	———
Linalyl acetate: a dark violet zone	A dark violet zone
———	———
Linalol: a dark violet zone	A dark violet zone
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The chromatogram obtained with the test solution shows 5 peaks similar in position to the 5 peaks in the chromatogram obtained with the reference solution. The 2 peaks corresponding to α- and β-thujone may be absent.

TESTS

Relative density *(2.2.5)*

0.890 to 0.908.

Refractive index *(2.2.6)*

1.456 to 1.466.

Optical rotation *(2.2.7)*

- 26 ° to - 10 °.

Acid value *(2.5.1)*

Maximum 1.0.

Chromatographic profile

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution. The substance to be examined.

Reference solution To 1 g of *hexane R*, add 5 µl of *thujone R*, 5 µl of *linalol R*, 100 µl of *linalyl acetate R*, 10 µl of α-*terpineol R* and 25 mg (± 20 per cent) of *sclareol R*. Mix thoroughly by stirring.

Column:

— *material*: fused silica;

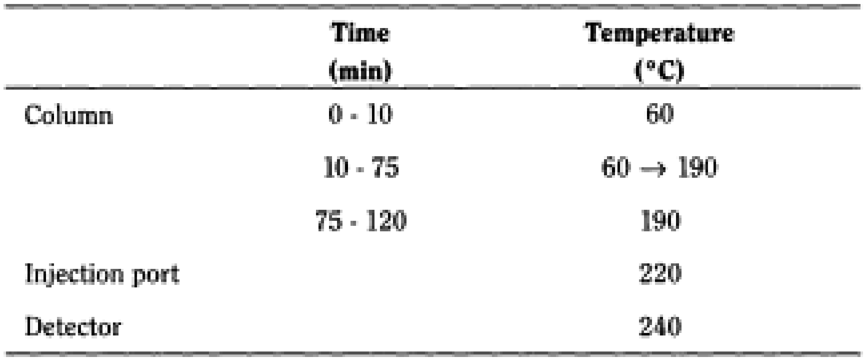
— *size*: l = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.25-0.53 mm;

— *stationary phase*: *macrogol 20 000 R*.

Carrier gas *Helium for chromatography R*.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 75	60 → 190
	75 - 120	190
Injection port		220
Detector		240

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

- *resolution*: minimum 1.5 between the peaks due to linalol and linalyl acetate,

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution (disregard any peak due to hexane). *Thujone R* is a mixture of α- and β-thujone. α-Thujone elutes before β-thujone under the described conditions.

Determine the percentage content of each of these components.

Also determine the percentage content of germacrene-D. The germacrene-D peak can be identified in the chromatogram obtained with the test solution by its relative retention of 1.23 with reference to linalol under the described operating conditions.

The percentages are within the following ranges:

- α- and β*-thujone*: less than 0.2 per cent;
- *linalol*: 6.5 per cent to 24 per cent;
- *inalyl acetate*: 56 per cent to 78 per cent;
- α*-terpineol*: less than 5.0 per cent;
- *germacrene-D*: 1.0 per cent to 12 per cent;
- *sclareol*: 0.4 per cent to 2.6 per cent.

STORAGE

In an airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Sage Tincture

General Notices

(Ph Eur monograph 1889)

Ph Eur

DEFINITION

Tincture produced from *Sage leaf (Salvia officinalis) (1370).*

Content

Minimum 0.1 per cent *m/m* of essential oil.

PRODUCTION

The tincture is produced from 1 part of comminuted drug and 10 parts of ethanol (70 per cent *V/V*) by a suitable procedure.

CHARACTERS

Appearance

Brownish liquid with a characteristic odour.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution The tincture to be examined.

Reference solution Dissolve 20 µl of *thujone R* and 25 µl of *cineole R* in 20 ml of *ethanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (5:95 *V/V*).

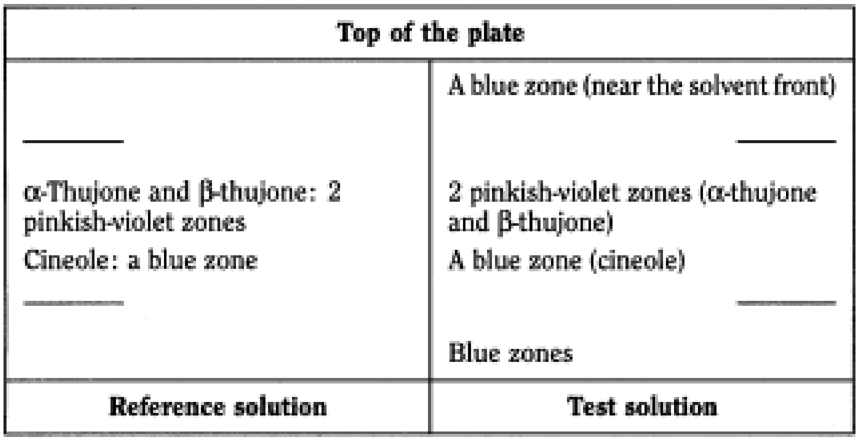
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 200 g/l solution of *phosphomolybdic acid R* in *ethanol R* and heat at 100-105 °C for 10 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
	A blue zone (near the solvent front)
_______	_______
α-Thujone and β-thujone: 2 pinkish-violet zones Cineole: a blue zone	2 pinkish-violet zones (α-thujone and β-thujone) A blue zone (cineole)
_______	_______
	Blue zones
Reference solution	**Test solution**

TESTS

Ethanol content *(2.9.10)*

64 per cent *V/V* to 69 per cent *V/V*.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent of 2-propanol.

Dry residue *(2.8.16)*

Minimum 2.0 per cent *m/m*, determined on 3.00 g.

ASSAY

In a 500 ml round-bottomed flask, place 30.0 g of the tincture and add 100 ml of *water R*. Distil, using a descending condenser, into a separating funnel which has been marked beforehand at 50 ml. Stop the distillation process as soon as the distillate reaches the 50 ml mark. Rinse the condenser with 10 ml of *pentane R*. Dissolve in the distillate sufficient *sodium chloride R* to produce a saturated solution. Shake with 3 quantities, each of 20 ml, of *pentane R.* Dry the combined pentane layers, including the pentane from rinsing the condenser, over *anhydrous sodium sulphate R* and filter through a plug of absorbent cotton into a weighed 100 ml round-bottomed flask. Wash the sodium sulphate several times with small quantities of *pentane R*. Remove the pentane carefully at a temperature not exceeding 40 °C. Dry the residue in a desiccator over *diphosphorus pentoxide R* and hard paraffin at atmospheric pressure and at room temperature for 2 h. Weigh the residue (essential oil).

Ph Eur

Salvia Miltiorrhiza Rhizome and Root for use in THM

General Notices

DEFINITION

Salvia Miltiorrhiza Rhizome and Root for use in THM consists of the dried root and rhizome of *Salvia miltiorrhiza* Bunge. It contains not less than 0.12% of tanshinone II$_A$ ($C_{19}H_{18}O_3$), not less than 0.17% per cent of rosmarinic acid ($C_{18}H_{16}O_8$) and not less than 3.0% of salvianolic acid B ($C_{36}H_{36}O_{16}$), calculated with reference to the dried material.

It is collected in spring or autumn, separated from soil and dried.

IDENTIFICATION

A. The rhizomes are short and stout, sometimes with the remains of the aerial stem at the apex, and give rise to a number of long, cylindrical, slightly curved and occasionally branched roots up to about 20 cm long and about 0.5 to 0.8 cm in diameter; the external surface is dark reddish brown, rough with longitudinal striations, and the roots bear numerous long, very thin and wiry rootlets along their length. The fracture is short or slightly uneven and the smoothed transverse surface shows a narrow brown outer region surrounding a yellowish-white central area with conspicuous medullary rays.

B. Reduce to a powder (355). The powder is reddish-brown. Examine under a microscope using chloral hydrate solution. The powder shows a surface view of cork cells almost rectangular or polygonal, containing yellowish-brown pigment, 12 to 151 µm in diameter. Parenchymatous cells in cortex squarish or polygonal, containing reddish-brown pigmental sediments. Xylem fibres usually in bundles, long fusiform, with oblique or criss-cross striations, 11 to 60 µm in diameter, vivid yellow when examined under a polarizing microscope. Numerous mainly bordered or reticulated vessels, 3 to 120 µm in diameter.

C. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Place 2 g of the powdered drug (355) in a cellulose thimble in a continuous extraction apparatus (Soxhlet type). Add 75 ml of *methanol* and heat for 1 hour. Evaporate the extract to 20 ml, cool and filter if necessary.

(2) 0.1% w/v each of *tanshinone II$_A$ CRS*, *rosmarinic acid CRS* and *salvianolic acid B CRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase described below.

(c) Apply as bands 8 µl of solution (1) and 5 µl of solution (2).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (366 nm)*.

MOBILE PHASE

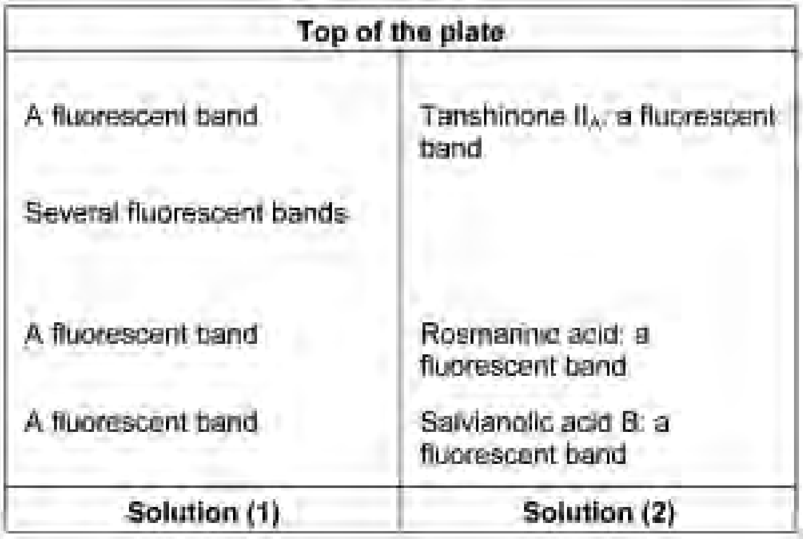
A mixture of 10 volumes of *water*, 13.5 volumes of *methanol* and 100 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The chromatogram obtained with solution (2) shows three clearly separated bands.

CONFIRMATION

The blue fluorescent bands with Rf values of approximately 0.7 (tanshinone II_A), 0.2 (rosmarinic acid) and 0.06 (salvianolic acid B) in the chromatogram obtained with solution (1) correspond in colour and position to those in the chromatogram obtained with solution (2). Other bands may be present in the chromatogram obtained with solution (1) as shown below.



Top of the plate	
A fluorescent band	Tanshinone II_A: a fluorescent band
Several fluorescent bands	
A fluorescent band	Rosmarinic acid: a fluorescent band
A fluorescent band	Salvianolic acid B: a fluorescent band
Solution (1)	**Solution (2)**

TESTS

Total ash

Not more than 10%, Appendix XI J.

Acid-insoluble ash

Not more than 2.0%, Appendix XI K.

Loss on drying

When dried for 2 hours at 105°, loses not more than 12.0% of its weight. Use 1 g.

ASSAY

For tanshinone II_A

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely powder about 5.0 g of the herbal drug being examined. Transfer 0.5 g of the powder into a 25-ml volumetric flask and add 20 ml of the mobile phase given below. Shake and mix with the aid of ultrasound for 30 minutes, shaking intermittently. Add the mobile phase to give a total volume of 25 ml. Filter through a 0.45-µm filter.

(2) 0.002% w/v of *tanshinone II_A CRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 270 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.01 M *sodium octyl sulphonate* in a mixture of 25 volumes of *water* and 75 volumes of *methanol* and adjust to pH 5.0 with *acetic acid*.

SYSTEM SUITABILITY

The test is not valid, unless in the chromatogram obtained with solution (2):

— the *symmetry factor* of the peak due to tanshinone II_A is less than 1.2;

— the number of *theoretical plates* is not less than 4500.

DETERMINATION OF CONTENT

Using the retention time and peak area from the chromatograms obtained with solution (2), locate and integrate the peak due to tanshinone II_A in the chromatogram obtained with solution (1).

Calculate the content of tanshinone II_A in the sample using the declared content of tanshinone II_A ($C_{19}H_{18}O_3$) in *tanshinone II_A CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100-d}$$

A_1 = Area of the peak due to tanshinone II_A in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to tanshinone II_A in the chromatogram obtained with solution (2).

m_1 = Weight of the drug in mg.

m_2 = Weight of *tanshinone II_A CRS* in mg.

V_1 = Dilution volume of solution.(1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of tanshinone II_A in *tanshinone II_A CRS.*

d = Percentage loss on drying of the herbal drug being examined.

For rosmarinic acid and salvianolic acid B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Finely powder about 5.0 g of the herbal drug being examined. Transfer 0.5 g of the powder into a 25-ml volumetric flask and add 20 ml of the mobile phase given below. Shake and mix with the aid of ultrasound for 30 minutes, shaking intermittently. Add the mobile phase to give a total volume of 25 ml. Filter through a 0.45-µm filter.

(2) 0.003% w/v each of *rosmarinic acid CRS* and *ferulic acid* in *water.*

(3) 0.06% w/v of *salvianolic acid B CRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 330 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

22 volumes of *acetonitrile* and 78 volumes of 0.4% v/v *formic acid*.

SYSTEM SUITABILITY

The test is not valid, unless in the chromatogram obtained with solution (2), the *resolution factor* between the two main peaks, rosmarinic acid and ferulic acid, is at least 5.0.

DETERMINATION OF CONTENT

Rosmarinic acid Using the retention time and peak area from the chromatogram obtained with solution (2), locate and integrate the peak due to rosmarinic acid in the chromatogram obtained with solution (1).

Calculate the content of rosmarinic acid in the sample using the declared content of rosmarinic acid ($C_{18}H_{16}O_8$) in *rosmarinic acid CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m} \times p \times \frac{100}{100-d}$$

A_1 = Area of the peak due to rosmarinic acid in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to rosmarinic acid in the chromatogram obtained with solution (2).

m_1 = Weight of the drug in mg.

m_2 = Weight of *rosmarinic acid CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of rosmarinic acid in *rosmarinic acid CRS.*

d = Percentage loss on drying of the herbal drug being examined.

Salvianolic acid B Using the retention time and peak area from the chromatogram obtained with solution (3), locate and integrate the peak due to salvianolic acid B in the chromatogram obtained with solution (1).

Calculate the content of salvianolic acid B in the sample using the declared content of salvianolic acid B ($C_{36}H_{36}O_{16}$) in *salvianolic acid B CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100-d}$$

A_1 = Area of the peak due to salvianolic acid in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to salvianolic acid in the chromatogram obtained with solution (3).

m_1 = Weight of the drug in mg.

m_2 = Weight of *salvianolic acid B CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of salvianolic acid B in *salvianolic acid B CRS.*

d = Percentage loss on drying of the herbal drug being examined.

STORAGE

Salvia Miltiorrhiza Rhizome and Root for use in THM should be protected from moisture.

Saw Palmetto Fruit

General Notices

(Ph Eur monograph 1848)

Ph Eur

DEFINITION

Dried ripe fruit of *Serenoa repens* (Bartram) Small. (*Sabal serrulata* (Michaux) Nichols).

Content

Minimum 11.0 per cent of total fatty acids (dried drug).

CHARACTERS

Characteristic, strong, unpleasant but not rancid odour.

IDENTIFICATION

First identification A, B, D.

Second identification A, B, C.

A. The fruit is an ovoid to subspherical drupe, with a dark brown to blackish, roughly wrinkled surface and more or less coppery sheen, up to 2.5 cm long and 1.5 cm in diameter. The apex sometimes bears the remains of the style and tubular calyx, with 3 teeth, and the base bears a small depression with the scar of the stalk. The epicarp and underlying mesocarp form a thin fragile layer, which partially peels off, revealing the thin, hard, pale brown endocarp, which is fibrous and easily separable. The seed is irregularly spherical to ovoid, up to 12 mm long and 8 mm in diameter, with a hard, smooth or finely pitted surface which is reddish-brown with a paler, raised and membranous area over the raphe and micropyle; cut transversely, the seed has a thin testa, narrow perisperm and a large area of dense, horny, greyish-white endosperm, with the embryo positioned to one side.

B. Reduce to a powder (710) *(2.9.12)*. The powder is reddish or blackish-brown and oily. Examine under a microscope using *chloral hydrate solution R*. The powder shows fragments of epicarp composed of several layers of thin-walled, reddish-brown, pigmented, polyhedral cells (10 µm to 40 µm) which are strongly cuticularised; those of the outer layers are much smaller than those of the inner layers. Parenchyma cells of the mesocarp may be large and filled with oil droplets, or smaller and containing nodules of silica. Groups of xylem tissue of the mesocarp show small lignified, annular or spirally thickened vessels. Stone cells of the mesocarp (20 µm to 200 µm) may be found scattered, usually singly but sometimes in small groups, the walls are moderately thickened, distinctly striated and finely pitted. Fragments of endocarp contain groups of elongated sclereids about 300 µm long, with strongly thickened walls and numerous pits. The seed testa consists of small, thin-walled cells with brownish contents and underlying sclereids; albumen cells are thick-walled with large conspicuous pits and contain aleurone grains and fixed oil.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.5 g of the powdered drug (710) *(2.9.12)*, add 20 ml of *alcohol R* and stir for 15 min. Filter.

Reference solution Dissolve 4 mg of β-*amyrin R* and 10 mg of β-*sitosterol R* in 10 ml of *alcohol R*.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *Acetic acid R, ethyl acetate R, toluene R* (1:30:70 *V/V/V*).

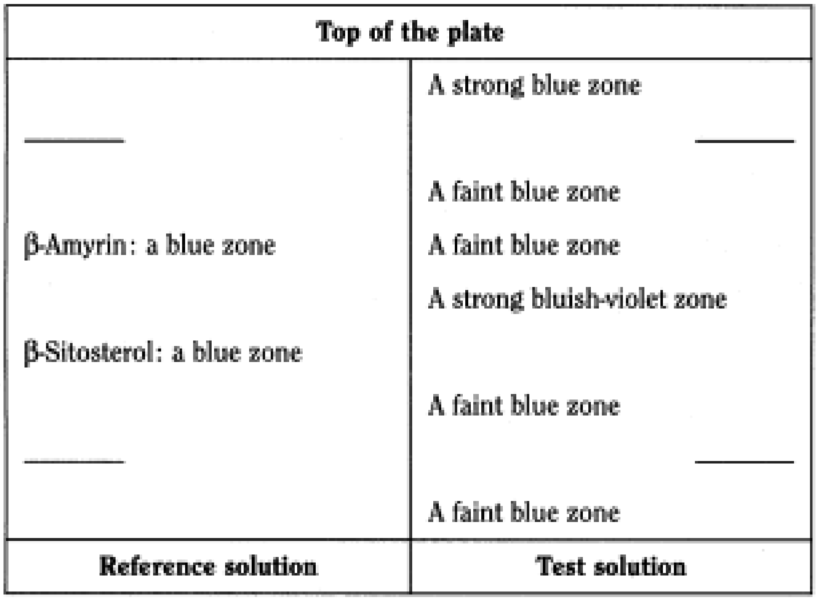
Application 8 µl of the test solution and 2 µl of the reference solution, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R*; dry the plate at 100-105 °C for 5-10 min; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones are present, especially in the lower third, in the chromatogram obtained with the test solution.



Top of the plate	
	A strong blue zone
———	———
	A faint blue zone
β-Amyrin: a blue zone	A faint blue zone
	A strong bluish-violet zone
β-Sitosterol: a blue zone	
	A faint blue zone
———	———
	A faint blue zone
Reference solution	**Test solution**

D. Examine the chromatograms obtained in the assay of total fatty acids.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to the characteristic peaks in the chromatogram obtained with the reference solution. The principal peak is due to lauric acid.

TESTS

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (710) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash ***(2.4.16)***

Maximum 5.0 per cent.

ASSAY

Total fatty acids

Gas chromatography *(2.2.28).*

Internal standard solution Dissolve 0.47 g of *methyl pelargonate R* and 0.47 g of *methyl margarate R* in 20.0 ml of *dimethylformamide R* and dilute to 100.0 ml with the same solvent.

Test solution Reduce 50 g of the drug to a powder (200). Place 4.0 g of the powdered drug in a 100 ml volumetric flask. Add 60.0 ml of *dimethylformamide R*. Mix using sonication for 15 min and then shake for 30 min. Dilute to 100.0 ml with *dimethylformamide R.* Allow to stand for a few minutes and filter. To 20.0 ml of this solution add 4.0 ml of the internal standard solution and dilute to 25.0 ml with *dimethylformamide R*. To 0.4 ml of this solution add 0.6 ml of an 18.84 g/l solution of *trimethylsulphonium hydroxide R* in *methanol R* and mix.

Reference solution Dissolve 32.0 mg of *caproic acid R*, 62.0 mg of *caprylic acid R*, 68.0 mg of *capric acid R*, 0.699 g of *lauric acid R*, 0.267 g of *myristic acid R*, 10.0 mg of *palmitoleic acid R*, 0.217 g of *palmitic acid R*, 0.115 g of *linoleic acid R*, 18.0 mg of *linolenic acid R*, 0.870 g of *oleic acid R* and 49.0 mg of *stearic acid R* in *dimethylformamide R* and dilute to 10.0 ml with the same solvent. To 1.0 ml of the solution add 4.0 ml of the internal standard solution and dilute to 25.0 ml with *dimethylformamide R*. To 0.4 ml of this solution add 0.6 ml of an 18.84 g/l solution of *trimethylsulphonium hydroxide R* in *methanol R* and mix.

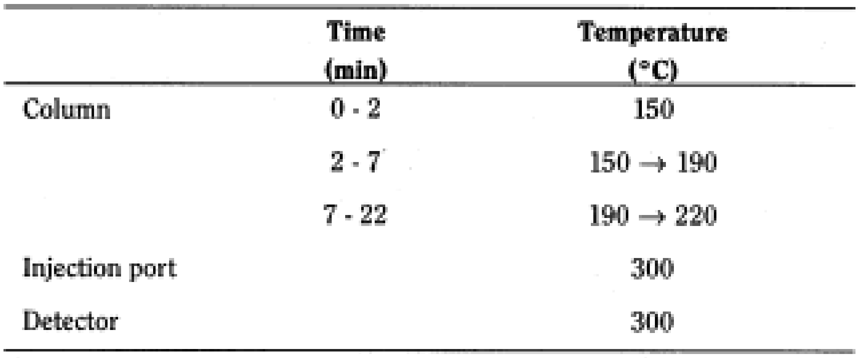
Column:
- *material*: fused silica;
- *size*: *l* = 25 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.20-0.53 mm;
- *stationary phase*: *poly(dimethyl)siloxane R*.

Carrier gas *Helium for chromatography R*.

Flow rate 0.5 ml/min.

Split ratio 1:40.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 2	150
	2 - 7	150 → 190
	7 - 22	190 → 220
Injection port		300
Detector		300

Detection Flame ionisation.

Injection 1.0 µl.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution on the chromatogram obtained with the test solution.

Use the expression below to determine the percentage content of the different fatty acids. Determine the content of caproic acid, caprylic acid, capric acid and lauric acid using methyl pelargonate as the internal standard. Determine the content of myristic acid, palmitoleic acid, palmitic acid, linoleic acid, linolenic acid, oleic acid and stearic acid using methyl margarate as the internal standard. The peak area of lauric acid is not less than 20 per cent of the total area of the peaks.

$$\frac{A_1}{A_3} \times \frac{A_2}{A_4} \times \frac{m_2}{m_1} \times p \times 0.5$$

A_1 = area of the peak due to the considered derivatised fatty acid in the chromatogram obtained with the test solution,

A_2 = area of the peak due to methyl pelargonate or methyl margarate in the chromatogram obtained with the reference solution,

A_3 = area of the peak due to methyl pelargonate or methyl margarate in the chromatogram obtained with the test solution,

A_4 = area of the peak due to the considered derivatised fatty acid in the chromatogram obtained with the reference solution,

m_1 = mass of the test sample, in grams,

m_2 = mass of the considered fatty acid in the reference solution, in grams,

p = percentage purity of the considered fatty acid in the reference solution.

Ph Eur

Senega Root

General Notices

Senega

(Ph Eur monograph 0202)

When Powdered Senega Root is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried and usually fragmented root and root crown of *Polygala senega* L. or of certain other closely related species or of a mixture of these *Polygala* species.

CHARACTERS

Faint, sweet odour, slightly rancid or reminiscent of methyl salicylate.

Reduced to a powder, it is irritant and sternutatory. Shaken with water, the powder produces a copious froth.

IDENTIFICATION

A. The root crown is greyish-brown and wider than the root; it forms an irregular head consisting of numerous remains of stems and tightly packed purplish-brown buds. The taproot is brown or yellow, occasionally branched, sometimes flexuous, usually tortuous and without secondary roots, except in the Japanese varieties and species, which contain numerous fibrous rootlets. The diameter is usually 1-8 mm at the crown, gradually tapering to the tip; the surface is transversely and longitudinally striated and often shows a more or less distinct decurrent, elongated spiral keel. The fracture is short and shows a yellowish cortex of varying thickness surrounding a paler central woody area somewhat circular or irregular in shape depending on the species.

B. Examine under a microscope using *chloral hydrate solution R*. The transverse section of the root shows the following diagnostic characters: cork formed from several layers of thin-walled cells, phelloderm of slightly collenchymatous cells containing droplets of oil; the phloem and xylem arrangement is usually normal, especially near the crown but where a keel is present this is formed by increased development of phloem; other anomalous secondary development sometimes occurs, resulting in the formation of 1 or 2 large wedge-shaped rays in the phloem and xylem, the parenchymatous cells of which contain droplets of oil. The xylem is usually central and consists of vessels up to 60 µm in diameter associated with numerous thin-walled tracheids and a few small lignified parenchymatous cells.

C. Reduce to a powder (355) *(2.9.12)*. The powder is light brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: longitudinal fragments of lignified tissue made up of pitted tracheids and somewhat larger vessels with numerous bordered pits or with reticulate thickening; yellowish parenchyma and collenchymatous cells containing droplets of oil; occasional fragments of cork, and of epidermal tissue with stomata and unicellular trichomes from the bud scales. Crystals and stone cells are absent.

D. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *ethanol (70 per cent V/V) R*, boil under a reflux condenser for 15 min, filter and allow to cool.

Reference solution Dissolve 10 mg of *aescin R* in *ethanol (70 per cent V/V) R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase The upper layer of a mixture of 10 volumes of *glacial acetic acid R*, 40 volumes of *water R* and 50 volumes of *butanol R*.

Application 10 µl of the test solution and 10 µl and 40 µl of the reference solution, as bands of 20 mm by 3 mm.

Development Over a path of 12 cm.

Drying At 100-105 °C.

Detection A Spray with about 10 ml of *anisaldehyde solution R* for a plate 200 mm square and heat again at 100-105 °C until red zones due to saponosides appear in the chromatogram obtained with the test solution.

Results A In the chromatogram obtained with the test solution, 3-5 red zones appear in the lower and middle parts, similar in position to the grey-violet zones due to aescin in the chromatogram obtained with the reference solution.

Detection B Spray with about 10 ml of a 200 g/l solution of *phosphomolybdic acid R* in *anhydrous ethanol R* and heat at 100-105 °C until the zones due to saponosides become blue.

Results B The intensity and size of the zones in the chromatogram obtained with the test solution are between those of the 2 bands due to aescin in the chromatograms obtained with 10 µl and 40 µl of the reference solution.

TESTS

Total ash *(2.4.16)*

Maximum 6.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

STORAGE

Store protected from humidity.

Ph Eur

Senna Leaf

General Notices

(Ph Eur monograph 0206)

Preparation

Standardised Senna Leaf Dry Extract

When Powdered Senna Leaf is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried leaflets of *Cassia senna* L. (*C. acutifolia* Delile), known as Alexandrian or Khartoum senna, or *Cassia angustifolia* Vahl, known as Tinnevelly senna, or a mixture of the 2 species.

Content

Minimum 2.5 per cent of hydroxyanthracene glycosides, expressed as sennoside B ($C_{42}H_{38}O_{20}$; M_r 863) (dried drug).

CHARACTERS

Slight characteristic odour.

IDENTIFICATION

A. *C. senna* occurs as greyish-green or brownish-green, thin, fragile leaflets, lanceolate, mucronate, asymmetrical at the base, usually 15-40 mm long and 5-15 mm wide, the maximum width being at a point slightly below the centre; the lamina is slightly undulant with both surfaces covered with fine, short trichomes. Pinnate venation is visible mainly on the lower surface, with lateral veins leaving the midrib at an angle of about 60° and anastomosing to form a ridge near the margin.

Stomatal index (2.8.3) 10-12.5-15.

C. angustifolia occurs as yellowish-green or brownish-green leaflets, elongated and lanceolate, slightly asymmetrical at the base, usually 20-50 mm long and 7-20 mm wide at the centre. Both surfaces are smooth with a very small number of short trichomes and are frequently marked with transverse or oblique lines.

Stomatal index (2.8.3) 14-17.5-20

B. Reduce to a powder (355) *(2.9.12)*. The powder is light green or greenish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: polygonal epidermal cells showing paracytic stomata *(2.8.3)*; unicellular trichomes, conical in shape, with warted walls, isolated or attached to fragments of epidermis; fibres with a crystal sheath of prismatic crystals of calcium oxalate; cluster crystals isolated or in fragments of parenchyma.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.5 g of the powdered drug (180) *(2.9.12)* add 5 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* and heat to boiling. Centrifuge and use the supernatant liquid.

Reference solution Dissolve 10 mg of *senna extract CRS* in 1 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* (a slight residue remains).

Plate *TLC silica gel G plate R*.

Mobile phase *glacial acetic acid R, water R, ethyl acetate R, propanol R* (1:30:40:40 *V/V/V/V*).

Application 10 µl, as bands of 20 mm by 2 mm.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 20 per cent *V/V* solution of *nitric acid R* and heat at 120 °C for 10 min. Allow to cool and spray with a 50 g/l solution of *potassium hydroxide R* in *alcohol (50 per cent V/V) R* until the zones appear.

Results The principal zones in the chromatogram obtained with the test solution are similar in position (sennosides B, A, D and C in the order of increasing R_F value), colour and size to the principal zones in the chromatogram obtained with the reference solution. Between the zones due to sennosides D and C a red zone due to rhein-8-glucoside may be visible.

D. Place about 25 mg of the powdered drug (180) *(2.9.12)* in a conical flask and add 50 ml of *water R* and 2 ml of *hydrochloric acid R*. Heat in a water-bath for 15 min, cool and shake with 40 ml of *ether R*. Separate the ether layer, dry over *anhydrous sodium sulphate R*, evaporate 5 ml to dryness and to the cooled residue add 5 ml of *dilute ammonia R1*. A yellow or orange colour develops. Heat on a water-bath for 2 min. A reddish-violet colour develops.

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent of foreign organs and maximum 1 per cent of foreign elements.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 2.5 per cent.

ASSAY

Carry out the assay protected from bright light. Place 0.150 g of the powdered drug (180) *(2.9.12)* in a 100 ml flask. Add 30.0 ml of *water R*, mix, weigh and place in a water-bath. Heat under a reflux condenser for 15 min. Allow to cool, weigh and adjust to the original mass with *water R*. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150 ml separating funnel. Add 0.1 ml of *dilute hydrochloric acid R* and shake with 3 quantities, each of 15 ml, of *chloroform R*. Allow to separate and discard the chloroform layer. Add 0.10 g of *sodium hydrogen carbonate R* and shake for 3 min. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100 ml round-bottomed flask with a ground-glass neck. Add 20 ml of *ferric chloride solution R1* and mix. Heat for 20 min in a water-bath under a reflux condenser with the water level above that of the liquid in the flask; add 1 ml of *hydrochloric acid R* and heat for a further 20 min, with frequent shaking, to dissolve the precipitate. Cool, transfer the mixture to a separating funnel and shake with 3 quantities, each of 25 ml, of *ether R* previously used to rinse the flask. Combine the 3 ether layers and wash with 2 quantities, each of 15 ml, of *water R*. Transfer the ether layer to a volumetric flask and dilute to 100.0 ml with *ether R*. Evaporate 10.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 515 nm, using *methanol R* as the compensation liquid.

Calculate the percentage content of hydroxyanthracene glycosides, expressed as sennoside B, using the following expression:

$$\frac{A \times 1.25}{m}$$

i. e. taking the specific absorbance of sennoside B to be 240.

A = absorbance at 515 nm,

m = mass of the substance to be examined, in grams.

STORAGE

Protected from moisture.

Ph Eur

Siam Benzoin Tincture

General Notices

(Ph Eur monograph 2157

Ph Eur

DEFINITION

Tincture produced from *Siam benzoin (2158).*

Content

Minimum 5.0 per cent *m/m* of total acids, calculated as benzoic acid ($C_7H_6O_2$; M_r 122.1).

PRODUCTION

The tincture is produced from 1 part of the drug and 5 parts of ethanol (75 per cent *V/V* to 96 per cent *V/V*) by a suitable procedure.

CHARACTERS

Appearance

Orange-yellow liquid.

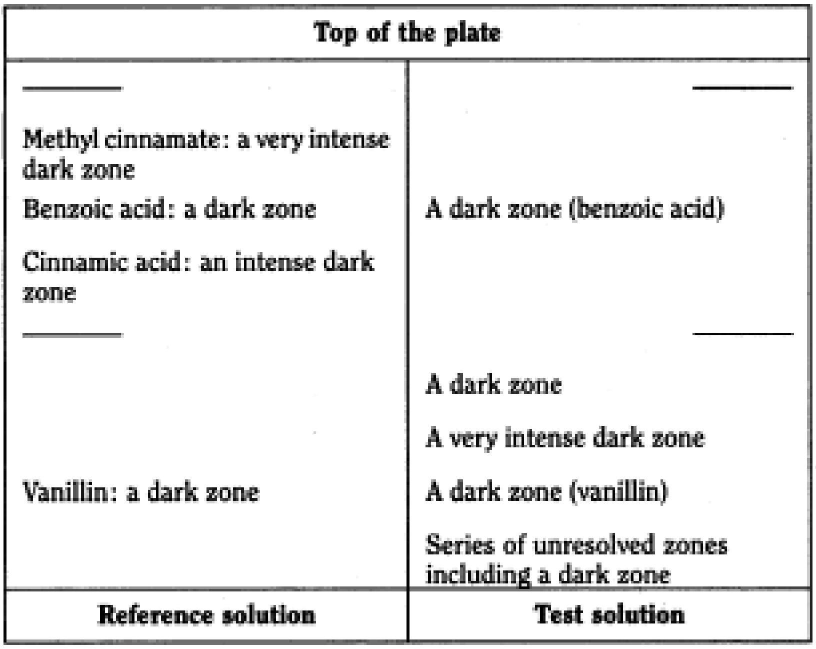
It has a characteristic odour of vanillin.

IDENTIFICATION

A. Place 10 ml in a test tube; add 0.5 ml of a 50 g/l solution of *ferric chloride R* in *ethanol (96 per cent) R* . A green colour is produced.

B. Examine the chromatograms obtained in the test for Sumatra benzoin tincture.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Methyl cinnamate: a very intense dark zone	
Benzoic acid: a dark zone	A dark zone (benzoic acid)
Cinnamic acid: an intense dark zone	
———	———
	A dark zone
	A very intense dark zone
Vanillin: a dark zone	A dark zone (vanillin)
	Series of unresolved zones including a dark zone
Reference solution	**Test solution**

TESTS

Sumatra benzoin tincture

Thin-layer chromatography (*2.2.27*).

Test solution The tincture to be examined.

Reference solution Dissolve 20 mg of *benzoic acid R*, 10 mg of *trans-cinnamic acid R*, 4 mg of *vanillin R* and 20 mg of *methyl cinnamate R* in 20 ml of ethanol of the same concentration as that used for the production of the tincture.

Plate *TLC silica gel* F_{254} *plate R* (5-40 µm) [or *TLC silica gel* F_{254} *plate R* (2-10 µm)].

Mobile phase *glacial acetic acid R*, *di-isopropyl ether R*, *hexane R* (10:40:60 *V/V/V*).

Application 20 µl [or 8 µl] as bands.

Development Over a path of 12 cm [or 6 cm].

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution does not show any zone in the same position as the zones due to cinnamic acid and methyl cinnamate in the chromatogram obtained with the reference solution.

Ethanol (*2.9.10*)

95 per cent to 105 per cent of the content stated on the label.

ASSAY

Place 3.50 g in a 250 ml borosilicate glass flask and add 15.0 ml of *0.5 M alcoholic potassium hydroxide*. Boil under a reflux condenser on a water-bath for 30 min. Allow to cool and rinse the condenser with 20 ml of *ethanol (96 per cent) R*. Titrate the excess of potassium hydroxide with *1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 61.05 mg of benzoic acid ($C_7H_6O_2$).

Ph Eur

Siam Benzoin

General Notices

(Ph Eur monograph 2158)

Ph Eur

DEFINITION

Resin obtained by incising the trunk of *Styrax tonkinensis* (Pierre) Craib ex Hartwich.

Content

45.0 per cent to 55.0 per cent of total acids, calculated as benzoic acid ($C_7H_6O_2$; M_r 122.1) (dried drug).

CHARACTERS

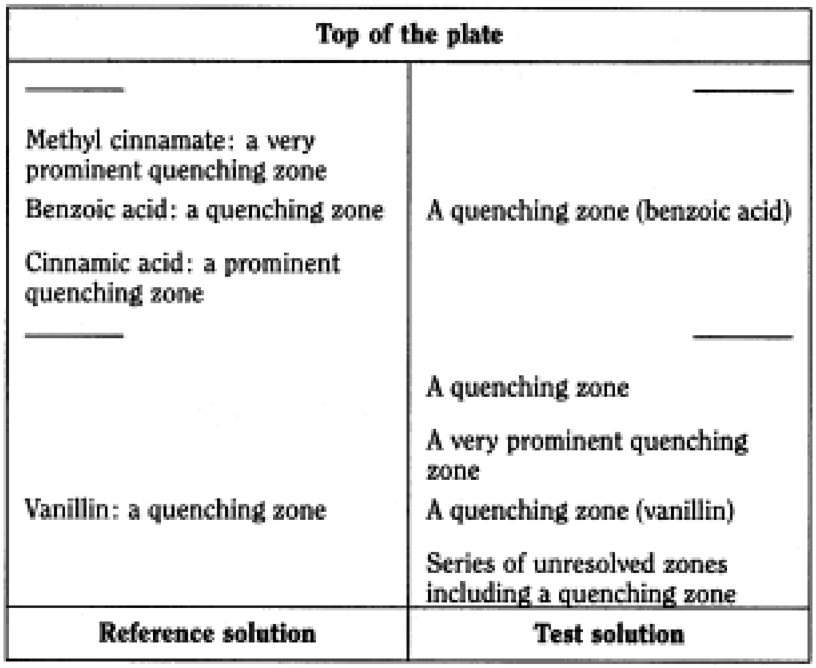
Characteristic odour of vanillin.

IDENTIFICATION

A. Siam benzoin occurs as opaque, granular, rounded or ovoid masses (tears), varying in size from a few millimeters up to 3 cm, separated or sometimes agglomerated together by a reddish-brown, transparent resin. Individual tears are yellowish-white to reddish externally with a waxy, whitish fracture which becomes reddish on exposure to air.

B. Examine the chromatograms obtained in test B for *Styrax benzoin* .

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Methyl cinnamate: a very prominent quenching zone	
Benzoic acid: a quenching zone	A quenching zone (benzoic acid)
Cinnamic acid: a prominent quenching zone	
———	———
	A quenching zone
	A very prominent quenching zone
Vanillin: a quenching zone	A quenching zone (vanillin)
	Series of unresolved zones including a quenching zone
Reference solution	**Test solution**

TESTS

Styrax benzoin

A. To 0.2 g of the finely powdered drug add 10 ml of *ethanol (96 per cent) R*. Shake vigorously until almost completely dissolved and filter. Place 5 ml of the filtrate in a test-tube and add 0.5 ml of a 50 g/l solution of *ferric chloride R* in *ethanol (96 per cent) R.* A green colour is produced. No yellow colour is produced.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Sonicate 0.2 g of the finely powdered drug in 5 ml of *ethanol (96 per cent) R* and filter. Collect the filtrate.

Reference solution Dissolve 20 mg of *benzoic acid R*, 10 mg of *trans-cinnamic acid R*, 4 mg of *vanillin R* and 20 mg of *methyl cinnamate R* in 10 ml of *ethanol (96 per cent) R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *glacial acetic acid R, di-isopropyl ether R, hexane R* (10:40:60 *V/V/V*).

Application 10 µl as bands.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution shows no zone in the same position as the zone due to cinnamic acid in the chromatogram obtained with the reference solution.

Matter insoluble in ethanol

Maximum 5 per cent.

To 2 g of the powdered drug add 25 ml of *ethanol (90 per cent V/V) R*. Boil until almost completely dissolved. Filter through a previously tared sintered-glass filter (16) *(2.1.2)* and wash with 3 quantities, each of 5 ml, of boiling *ethanol (90 per cent V/V) R*. Heat the glass filter and its contents in an oven at 100-105 °C for 2 h. Weigh after cooling.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 2.00 g of the coarsely powdered drug by drying *in vacuo* for 4 h.

Total ash *(2.4.16)*

Maximum 2.0 per cent.

ASSAY

Place 0.750 g of the finely powdered drug in a 250 ml borosilicate glass flask and add 15.0 ml of *0.5 M alcoholic potassium hydroxide*. Boil under a reflux condenser on a water-bath for 30 min. Allow to cool and rinse the condenser with 20 ml of *ethanol (96 per cent) R*. Titrate the excess of potassium hydroxide with *0.5 M hydrochloric acid*. Determine the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 61.05 mg of benzoic acid ($C_7H_6O_2$).

Ph Eur

Spanish Sage Oil

General Notices

(Ph Eur monograph 1849)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the aerial parts of *Salvia lavandulifolia* Vahl, collected at the flowering stage.

CHARACTERS

Appearance

Clear, colourless or pale yellow, mobile liquid.

Camphor-like odour.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.1 ml of the oil to be examined in 10 ml of *toluene R*.

Reference solution Dissolve 20 µl of *thujone R* and 30 µl of *cineole R* in 10 ml of *toluene R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

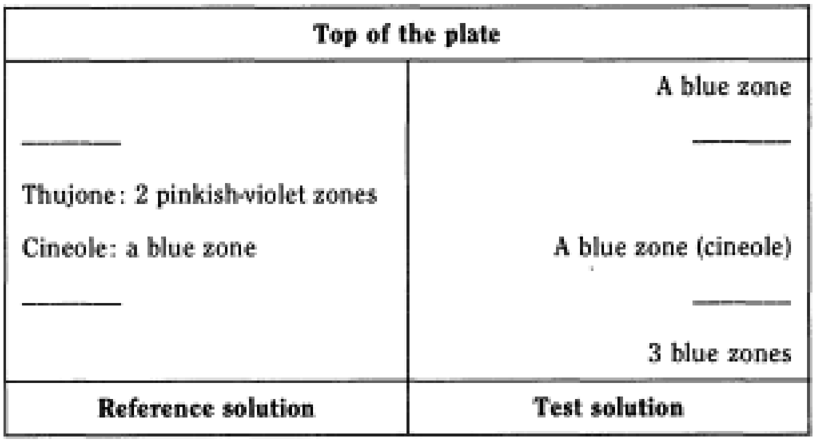
Application 10 µl [or 3 µl] as bands of 10 mm [or 6 mm].

Development Over a path of 15 cm [or 6 cm].

Drying In air.

Detection Spray with a freshly prepared 200 g/l solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R* and heat at 105 °C for 10 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A blue zone
———	———
Thujone: 2 pinkish-violet zones	
Cineole: a blue zone	A blue zone (cineole)
———	———
	3 blue zones
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with reference solution (a).

TESTS

Relative density ***(2.2.5)***

0.907 to 0.932.

Refractive index ***(2.2.6)***

1.465 to 1.473.

Optical rotation ***(2.2.7)***

+ 7° to + 17°.

Acid value ***(2.5.1)***

Maximum 2.0, determined on 5.00 g.

Solubility in alcohol ***(2.8.10)***

1 volume is soluble in 2 volumes and more of *ethanol (80 per cent V/V) R*.

Chromatographic profile

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 0.200 g of the oil to be examined in *heptane R* and dilute to 10.0 ml with the same solvent.

Reference solution (a) Dissolve 0.200 g of *Spanish sage oil for peak identification CRS* in *heptane R* and dilute to 10.0 ml with the same solvent.

Reference solution (b) Dissolve 5 µl of *limonene R* in *heptane R* and dilute to 50.0 ml with the same solvent. Dilute 0.5 ml of this solution to 5.0 ml with *heptane R*.

Column:
- *material*: fused silica;
- *size: l* = 60 m, Ø = 0.25 mm;

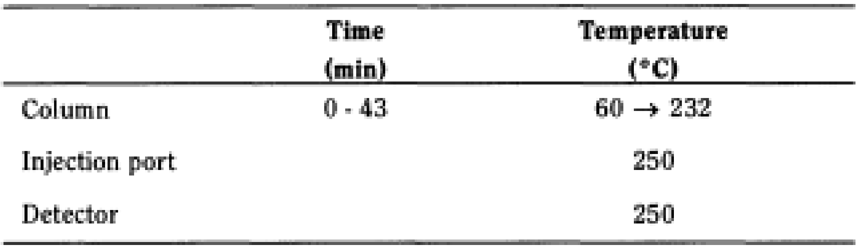
— *stationary phase: macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 ml/min.

Split ratio 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 43	60 → 232
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µl.

System suitability Reference solution (a):

- the chromatogram obtained is similar to the chromatogram supplied with *Spanish sage oil for peak identification CRS*;
- *resolution*: minimum 1.5 between the peaks due to limonene and 1,8-cineole and minimum 1.5 between the peaks due to α-terpinyl acetate and borneol.

Use the chromatogram supplied with *Spanish sage oil for peak identification CRS* and the chromatogram obtained with reference solution (a) to locate the peaks due to α-pinene, sabinene, limonene, 1,8-cineole, thujone, camphor, linalol, linalyl acetate, terpinen-4-ol, sabinyl acetate, α-terpinyl acetate and borneol.

Determine the percentage content of each of these components. The percentages are within the following ranges:

— α-*pinene*: 4.0 per cent to 11.0 per cent;

— *sabinene*: 0.1 per cent to 3.5 per cent;

— *limonene*: 2.0 per cent to 6.5 per cent;

— *1,8-cineole*: 10.0 per cent to 30.5 per cent;

— *thujone*: less than 0.5 per cent;

— *camphor*: 11.0 per cent to 36.0 per cent;

— *linalol*: 0.3 per cent to 4.0 per cent;

— *linalyl acetate*: less than 5.0 per cent;

— *terpinen-4-ol*: less than 2.0 per cent;

— *sabinyl acetate*: 0.5 per cent to 9.0 per cent;

— α-*terpinyl acetate*: 0.5 per cent to 9.0 per cent;

— *borneol*: 1.0 per cent to 7.0 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

STORAGE

At a temperature not exceeding 25 °C.

Ph Eur

Spearmint Oil

General Notices

DEFINITION

Spearmint Oil is obtained by distillation from fresh flowering plants of *Mentha spicata* L. and *Mentha* × *cardiaca* (Gray) Bak.

CHARACTERISTICS

A clear, colourless, pale yellow or greenish yellow liquid when recently distilled, but becoming darker and viscous on keeping; visibly free from water; odour, that of spearmint.

IDENTIFICATION

Examine the chromatograms obtained in the test for Chromatographic profile. The retention times of the principal peaks in the chromatogram obtained with solution (1) are similar to those of the principal peaks in the chromatogram obtained with solution (2).

TESTS

Optical rotation

American-type oil, –45° to –60°; Chinese-type oil, –50° to –62°; Appendix V F.

Refractive index

1.484 to 1.491, Appendix V E.

Solubility in ethanol

Soluble, at 20°, in 1 part of *ethanol (80%)*, Appendix X M. The solution may become cloudy when diluted.

Weight per ml

American-type oil, 0.917 to 0.934 g; Chinese-type oil, 0.935 to 0.952 g, Appendix V G.

Chromatographic profile

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Solution (1) is the substance being examined. For solution (2) mix carefully 0.1 g of *limonene*, 0.2 g of *cineole*, 0.4 g of *menthone*, 0.1 g of (+)-*isomenthone*, 0.4 g of *menthyl acetate*, 0.2 g of *pulegone*, 0.6 g of *menthol* and 0.1 g of *carvone* with 1 g of *hexane*.

The chromatographic procedure may be carried out using (a) a glass capillary column (25 m to 60 m × about 0.25 mm) coated with *polyethylene glycol 20,000* as bonded phase (Carbowax 20M is suitable) and (b) *helium* as the carrier gas at a flow rate of 1.5 ml per minute. Maintain the temperature of the column at 55° for 6 minutes then increase it at the rate of 4° per minute to 180°; keep the injection port temperature at 220° and the detector at 230°.

Inject 0.1 µl of solution (2). When the chromatograms are recorded in the prescribed conditions, the components elute in the order indicated in the composition of the reference solution. Record the retention times of these substances. The test is not valid unless the number of *theoretical plates* calculated from the limonene peak is at least 30,000 and the *resolution factor* between the peaks corresponding to limonene and cineole is at least 1.5.

Inject 0.1 µl of solution (1). Using the retention times determined from the chromatogram obtained with solution (2) locate the components of the reference solution on the chromatogram obtained with solution (1) (disregard the peak due to hexane). Determine the percentage content of the components by *normalisation*. The percentages are within the following ranges:

Limonene 2.0 to 25.0%.

Cineole less than 2.5%.

Menthone less than 2.5%.

Isomenthone less than 1.0%.

Menthyl acetate less than 1.0%.

Pulegone less than 0.5%.

Menthol less than 2.0%.

Carvone Not less than 55.0%.

STORAGE

Spearmint Oil should be kept in a well-filled container and protected from light.

LABELLING

The label states whether the oil is American-type oil or Chinese-type oil.

Squill Liquid Extract

General Notices

DEFINITION

Squill Liquid Extract is prepared by extracting Squill with Ethanol (70 per cent).

Extemporaneous preparation

The following formula and directions apply.

Squill, in *coarse powder*	1000 g
Ethanol (70 per cent)	A sufficient quantity

Exhaust the Squill, in *coarse powder*, with Ethanol (70 per cent) by *percolation*, Appendix XI F. Reserve the first 850 ml of the percolate; evaporate the subsequent percolate to the consistence of a soft extract and dissolve it in the reserved portion. Add sufficient Ethanol (70 per cent) to produce 1000 ml and filter.

The extract complies with the requirements stated under Extracts and with the following requirements.

TESTS

Ethanol content

34 to 50% v/v, Appendix VIII F, Method III.

Dry residue

40 to 55% w/v.

Relative density

1.00 to 1.14, Appendix V G.

Squill

General Notices

Preparations

Squill Liquid Extract

Squill Oxymel

When Powdered Squill is prescribed or demanded, material complying with the appropriate requirements below shall be dispensed or supplied.

DEFINITION

Squill consists of the bulb of *Drimia maritima* (L.) Stearn, collected soon after the plant has flowered, divested of its dry, outer, membranous coats, cut into transverse slices and dried. It is known in commerce as white squill.

IDENTIFICATION

A. Transverse slices, about 5 to 8 mm thick, occurring as straight or curved triangular pieces about 5 to 50 mm long and 3 to 8 mm wide at mid-point, tapering towards each end, yellowish white, texture horny, somewhat translucent, breaking with an almost glassy fracture when quite dry, but readily absorbing moisture when exposed to the air and becoming tough and flexible; transversely cut surface showing a single row of prominent, vascular bundles near the concave edge and numerous smaller bundles scattered throughout the mesophyll.

B. Epidermis: cells polygonal and axially elongated, 1 to 2 times longer than wide, cuticle thick, stratified; stomata very rare, *anomocytic*, Appendix XI H, and nearly circular in outline, about 50 to 60 µm in diameter; mesophyll of colourless, thin-walled parenchyma containing very occasional starch granules, many cells containing bundles of acicular crystals of calcium oxalate embedded in mucilage, crystals up to about 1 mm long and about 1 to 15 µm wide; other cells containing sinistrin; vascular bundles collateral, scattered throughout the mesophyll; xylem vessels with spiral and annular wall thickening; trichomes absent.

C. The mucilage contained in the cells of the mesophyll is stained red with alkaline *corallin* solution but produces no red colour with *ruthenium red* solution and no purple colour with 0.01M *iodine*.

TESTS

Acid-insoluble ash

Not more than 1.5%, Appendix XI K, Method I.

Extractive soluble in ethanol (60%)

Not less than 68.0%, Appendix XI B1. Use material that has been dried for 1 hour at 105° and powdered.

STORAGE

Squill should be stored in a dry place.

St John's Wort

General Notices

Hypericum
(Ph Eur monograph 1438)

Ph Eur

DEFINITION

Whole or cut, dried flowering tops of *Hypericum perforatum* L., harvested during flowering time.

Content

Minimum 0.08 per cent of total hypericins, expressed as hypericin ($C_{30}H_{16}O_8$; M_r 504.4) (dried drug).

IDENTIFICATION

A. The branched and bare stem shows 2 more-or-less prominent longitudinal ridges. The leaves are opposite, sessile, exstipulate, oblong-oval and 15-30 mm long; present on the leaf margins are glands which appear as black dots and over all the surface of the leaves many small, strongly translucent excretory glands which are visible in transmitted light. The flowers are regular and form corymbose clusters at the apex of the stem. They have 5 green, acute sepals, with black secretory glands on the margins; 5 orange-yellow petals, also with black secretory glands on the margins; 3 staminal blades, each divided into many orange-yellow stamens and 3 carpels surmounted by red styles.

The drug may also show the following: immature and ripe fruits and seeds. Immature fruits are green or yellowish, seeds are whitish. Occasional ripe fruits may be present; these are dry trilocular capsules containing numerous seeds, brown, broad or small-ovate, 5-10 mm long, with broad linear or punctiform glands, irregularly striated ducts, conducting secretions. Ripe seeds are 1-1.3 mm long, cylindrical or trigonous, shortly pointed at both ends, brown or almost black, minutely pitted longitudinally.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of polygonal cells of the epidermis with thickened and beaded walls and paracytic or anomocytic stomata *(2.8.3)*; fragments of the leaf and sepal with large oil glands and red pigment cells; thin-walled, elongated cells of the petal epidermis with straight or wavy anticlinal walls; tracheids and tracheidal vessels with pitted walls and groups of thick-walled fibres; fragments of rectangular, lignified and pitted parenchyma; fibrous layer of the anther and elongated, thin-walled cells of the filament with a striated cuticle; numerous pollen grains with 3 pores and a smooth exine, occur singly or in dense groups, and calcium oxalate cluster crystals.

The powder may also show the following diagnostic characters: fragments of the fruit, exocarp solid with rounded polygonal-cells, endocarp with blunt, thick-walled fibres; fragments of the seed testa, whitish or brown with thick-walled hexagonal or rounded polygonal cells;

fragments of the nutritive tissue and embryo, abundant oil droplets.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Stir 0.5 g of the powdered drug (500) *(2.9.12)* in 10 ml of *methanol R* in a water-bath at 60 °C for 10 min and filter.

Reference solution Dissolve 5 mg of *rutin R* and 5 mg of *hyperoside R* in *methanol R*, then dilute to 5 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R*, *water R*, *ethyl acetate R* (6:9:90 *V/V/V*).

Application 10 µl of the test solution and 5 µl of the reference solution, as bands of 10 mm.

Development Over a path of 10 cm.

Drying At 100-105 °C for 10 min.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. After about 30 min, examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the reference solution shows in the lower third the zone due to rutin and above it the zone due to hyperoside, both with yellow-orange fluorescence. The chromatogram obtained with the test solution shows in the lower third the reddish-orange fluorescent zones of rutin and hyperoside and in the lower part of the upper third the zone of pseudohypericin and above it the zone of hypericin, both with red fluorescence. Other yellow or blue fluorescent zones are visible.

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent of stems with a diameter greater than 5 mm and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (500) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 7.0 per cent.

ASSAY

Test solution In a 100 ml round-bottomed flask, introduce 0.800 g of the powdered drug (500) *(2.9.12)*, 60 ml of a mixture of 20 volumes of *water R* and 80 volumes of *tetrahydrofuran R* and a magnetic stirrer. Boil the mixture in a water-bath at 70 °C under a reflux condenser for 30 min. Centrifuge (2 min at 700 g) and decant the supernatant into a 250 ml flask. Take up the residue with 60 ml of a mixture of 20 volumes of *water R* and 80 volumes of *tetrahydrofuran R*. Heat again under a reflux condenser for 30 min. Centrifuge (2 min at 700 g) and decant the supernatant. Combine the extracts and evaporate to dryness. Take up the residue with 15 ml of *methanol R* with the help of ultrasound and transfer to a 25 ml measuring flask. Rinse the 250 ml flask with *methanol R* and dilute to 25.0 ml with the same solvent. Centrifuge again, filter 10 ml through a syringe filter (0.2 µm). Discard the first 2 millilitres of the filtrate. Introduce 5.0 ml of the filtrate into a measuring flask and dilute to 25.0 ml with *methanol R*.

Compensation liquid *methanol R*.

Measure the absorbance *(2.2.25)* at 590 nm of the test solution, by comparison with the compensation liquid.

Calculate the percentage content of total hypericins, expressed as hypericin, using the following expression:

$$\frac{A \times 125}{m \times 870}$$

i.e. taking the specific absorbance of hypericin to be 870.

A = absorbance at 590 nm,

m = mass of the drug to be examined, in grams.

Ph Eur

St. John's Wort Dry Extract, Quantified

General Notices

(Ph Eur monograph 1874)

Ph Eur

DEFINITION

Quantified dry extract obtained from *St. John's wort (1438)*.

Content:

— *total hypericins, expressed as hypericin* ($C_{30}H_{16}O_8$; M_r 504.5): 0.10 per cent to 0.30 per cent (dried extract);

— *flavonoids, expressed as rutin* ($C_{27}H_{30}O_{16}$; M_r 610.5): minimum 6.0 per cent (dried extract);

— *hyperforin* ($C_{35}H_{52}O_4$; M_r 536.8): maximum 6.0 per cent (dried extract) and not more than the content stated on the label.

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using ethanol (50-80 per cent *V/V*) or methanol (50-80 per cent *V/V*).

CHARACTERS

Appearance

Brownish-grey powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Disperse 0.25 g of the extract to be examined in 5 ml of *methanol R*.

Reference solution Dissolve 5 mg of *rutin R* and 5 mg of *hyperoside R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *anhydrous formic acid R, water R, ethyl acetate R* (6:9:90 *V/V/V*).

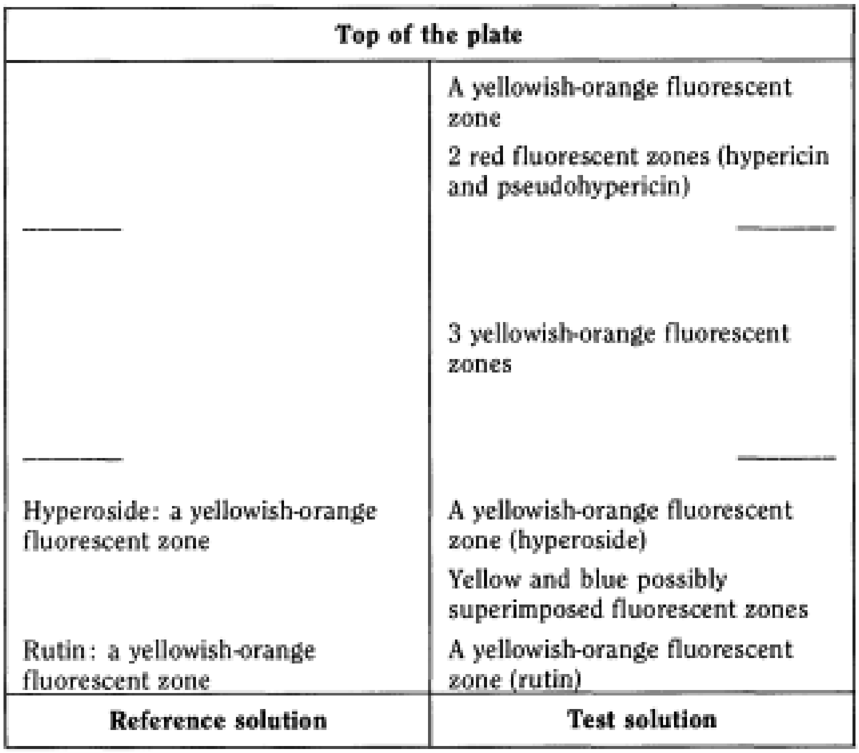
Application 10 µl [or 5 µl] as bands of 10 mm [or 8 mm].

Development Over a path of 10 cm [or 7.5 cm] .

Drying At 100-105 °C for 10 min.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Examine after about 30 min in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A yellowish-orange fluorescent zone 2 red fluorescent zones (hypericin and pseudohypericin)
———	———
	3 yellowish-orange fluorescent zones
———	———
Hyperoside: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (hyperoside) Yellow and blue possibly superimposed fluorescent zones
Rutin: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (rutin)
Reference solution	**Test solution**

ASSAY

Total hypericins

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 70.0 mg of the extract to be examined in 25.0 ml of *methanol R*. Sonicate and centrifuge the solution. Expose the solution to a xenon lamp at about 765 W/m^2 for 8 min.

Reference solution Dissolve a quantity of *St. John's wort standardised dry extract CRS* corresponding to 0.15 mg of hypericin in 25.0 ml of *methanol R*. Sonicate and centrifuge. Expose the solution to a xenon lamp at about 765 W/m^2 for 8 min.

Column:

— *size:* *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 39 volumes of *ethyl acetate R*, 41 volumes of a 15.6 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 2 with *phosphoric acid R* and 160 volumes of *methanol R*.

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 590 nm.

Injection 20 µl.

Run time 15 min.

Identification of peaks Use the chromatogram supplied with *St. John's wort standardised dry extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to pseudohypericin and hypericin.

System suitability Reference solution:

— the chromatogram obtained is similar to the chromatogram supplied with *St. John's wort standardised dry extract CRS*;

— *resolution*: minimum 2 between the peaks due to pseudohypericin and hypericin.

Calculate the percentage content of total hypericins, expressed as hypericin, using the following expression:

$$\frac{(A_1 + A_2) \times m_2 \times p}{A_3 \times m_1}$$

A_1 = area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

A_2 = area of the peak due to hypericin in the chromatogram obtained with the test solution;

A_3 = area of the peak due to hypericin in the chromatogram obtained with the reference solution;

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of *St. John's wort standardised dry extract CRS* used to prepare the reference solution, in grams;

p = percentage content of hypericin in *St. John's wort standardised dry extract CRS*.

Hyperforin and flavonoids

Liquid chromatography *(2.2.29)*. *Carry out the assay protected from light*.

Solvent mixture *water R, methanol R* (20:80 *V/V*).

Test solution Dissolve 75.0 mg of the extract to be examined in 20.0 ml of the solvent mixture. Sonicate and centrifuge.

Reference solution (a) Dissolve 20.0 mg of *rutoside trihydrate CRS* in 200.0 ml of the solvent mixture.

Reference solution (b) Dissolve 75.0 mg of *St. John's wort standardised dry extract CRS* in

20.0 ml of the solvent mixture. Sonicate and centrifuge.

Column:

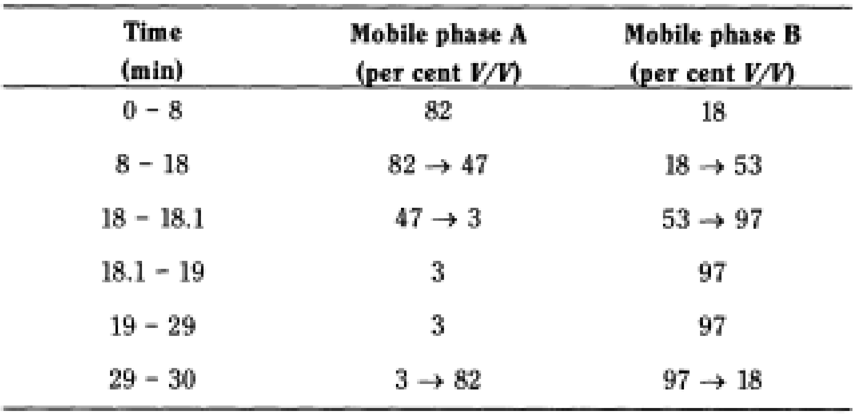
— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A: phosphoric acid R, water R* (3:1000 *V/V*);

— *mobile phase B: phosphoric acid R, acetonitrile R* (3:1000 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	82	18
8 - 18	82 → 47	18 → 53
18 - 18.1	47 → 3	53 → 97
18.1 - 19	3	97
19 - 29	3	97
29 - 30	3 → 82	97 → 18

Detection Spectrophotometer at 360 nm, then at 275 nm after the elution of biapigenin (about 22 min).

Injection 10 µl.

Identification of peaks Use the chromatogram supplied with *St. John's wort standardised dry extract CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to rutin, hyperoside, isoquercitroside, quercitroside, quercetin, biapigenin, hyperforin and adhyperforin.

System suitability Reference solution (b):

— the chromatogram obtained is similar to the chromatogram supplied with *St. John's wort standardised dry extract CRS*;

— *resolution*: minimum 2.0 between the peaks due to rutin and hyperoside, and minimum 2.0 between the peaks due to hyperforin and adhyperforin.

Calculate the percentage content of hyperforin using the following expression:

$$\frac{A_4 \times m_4 \times p \times 2.3}{A_5 \times m_3 \times 10}$$

A_4 = area of the peak due to hyperforin in the chromatogram obtained with the test solution;

A_5 = area of the peak due to rutin in the chromatogram obtained with reference solution (a);

m_3 = mass of the extract to be examined used to prepare the test solution, in grams;

m_4 = mass of *rutoside trihydrate CRS* used to prepare the reference solution, in grams;

2.3 = correction factor for hyperforin with respect to rutin;

p = percentage content of rutin in *rutoside trihydrate CRS*.

Calculate the percentage content of flavonoids, expressed as rutin, using the following expression:

$$\frac{m_4 \times p \times (A_6 + A_7 + A_8 + A_9 + A_{10} + A_{11})}{m_3 \times A_5 \times 10}$$

A_5 = area of the peak due to rutin in the chromatogram obtained with reference solution (a);

A_6 = area of the peak due to rutin in the chromatogram obtained with the test solution;

A_7 = area of the peak due to hyperoside in the chromatogram obtained with the test solution;

A_8 = area of the peak due to isoquercitroside in the chromatogram obtained with the test solution;

A_9 = area of the peak due to quercitroside in the chromatogram obtained with the test solution;

A_{10} = area of the peak due to quercetin in the chromatogram obtained with the test solution;

A_{11} = area of the peak due to biapigenin in the chromatogram obtained with the test solution;

m_3 = mass of the extract to be examined used to prepare the test solution, in grams;

m_4 = mass of *rutoside trihydrate CRS* used to prepare the reference solution, in grams;

p = percentage content of rutin in *rutoside trihydrate CRS*

LABELLING

The label states the content of hyperforin.

Ph Eur

Standardised Aloes Dry Extract

General Notices

(Ph Eur monograph 0259)

Ph Eur

DEFINITION

Standardised dry extract prepared from Barbados aloes or Cape aloes, or a mixture of both.

Content

19.0 per cent to 21.0 per cent of hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4) adjusted, if necessary (dried extract).

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using boiling water.

CHARACTERS

Appearance

Brown or yellowish-brown powder.

Solubility

Sparingly soluble in boiling water.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution To 0.25 g of the extract to be examined add 20 ml of *methanol R* and heat to boiling in a water-bath. Shake for a few minutes and decant the solution. Store at about 4 °C and use within 24 h.

Reference solution Dissolve 25 mg of *barbaloin R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, methanol R, ethyl acetate R* (13:17:100 *V/V/V*).

Application 10 µl as bands of 20 mm by not more than 3 mm.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 100 g/l solution of *potassium hydroxide R* in *methanol R* and examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows, in the central part, a zone of yellow fluorescence (barbaloin) similar in position to the zone due to barbaloin in the chromatogram obtained with the reference solution and in the lower part, a zone of light blue fluorescence (aloesine). In the lower part of the chromatogram obtained with the test solution 2 zones of yellow fluorescence (aloinosides A and B) (Cape aloes) and a zone of violet fluorescence just below the zone due to barbaloin (Barbados aloes) may be present.

B. Shake 1 g with 100 ml of boiling *water R* . Cool, add 1 g of *talc R* and filter. To 10 ml of the filtrate add 0.25 g of *disodium tetraborate R* and heat to dissolve. Pour 2 ml of this solution into 20 ml of *water R* . A yellowish-green fluorescence appears which is particularly marked in ultraviolet light at 365 nm.

TESTS

Loss on drying *(2.8.17)*

Maximum 4.0 per cent m/m.

Total ash *(2.4.16)*

Maximum 2.0 per cent.

ASSAY

Carry out the assay protected from bright light.

Introduce 0.400 g into a 250 ml conical flask. Moisten with 2 ml of *methanol R* , add 5 ml of *water R* warmed to about 60 °C, mix, add a further 75 ml of *water R* at about 60 °C and shake for 30 min. Cool, filter into a volumetric flask, rinse the conical flask and the filter with 20 ml of *water R* , add the rinsings to the volumetric flask and dilute to 1000.0 ml with *water R* . Transfer 10.0 ml of this solution to a 100 ml round-bottomed flask containing 1 ml of a 600 g/l solution of *ferric chloride R* and 6 ml of *hydrochloric acid R* . Heat in a water-bath under a reflux condenser for 4 h, with the water level above that of the liquid in the flask. Allow to cool, transfer the solution to a separating funnel, rinse the flask successively with 4 ml of *water R* , 4 ml of *1 M sodium hydroxide* and 4 ml of *water R* , and add the rinsings to the separating funnel. Shake the contents of the separating funnel with 3 quantities, each of 20 ml, of *ether R* . Wash the combined ether layers with 2 quantities, each of 10 ml, of *water R* . Discard the washings and dilute the organic layer to 100.0 ml with *ether R* . Evaporate 20.0 ml carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R* . Measure the absorbance *(2.2.25)* at 512 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of hydroxyanthracene derivatives, expressed as barbaloin, using the following expression:

$$\frac{A \times 19.6}{m}$$

i.e. taking the specific absorbance of barbaloin to be 255.

A = absorbance at 512 nm;

m = mass of the substance to be examined, in grams.

Ph Eur

Standardised Belladonna Leaf Dry Extract

General Notices

(Ph Eur monograph 1294)

Ph Eur

DEFINITION

Standardised dry extract obtained from *Belladonna leaf (0221)*.

Content

0.95 per cent to 1.05 per cent of total alkaloids, expressed as hyoscyamine ($C_{17}H_{23}NO_3$; M_r 289.4) (dried extract).

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using ethanol (70 per cent *V/V*).

CHARACTERS

Appearance

Brown or greenish, hygroscopic powder.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)* .

Test solution To 1 g of the extract to be examined add 5.0 ml of *methanol R* . Shake for 2 min and filter.

Reference solution Dissolve 1.0 mg of *chlorogenic acid R* and 2.5 mg of *rutin R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application 20 µl as bands.

Development Over a path of 15 cm.

Drying At 100-105 °C.

Detection Spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, then spray with a 50 g/l solution of *macrogol 400 R* in *methanol R*; allow to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results The chromatograms obtained with the reference solution and the test solution show in the central part a light blue fluorescent zone (chlorogenic acid) and in the lower part a yellowish-brown fluorescent zone (rutin); furthermore, the chromatogram obtained with the test solution shows a little above the start a yellowish-brown fluorescent zone and directly above that a yellow fluorescent zone, and a yellow or yellowish-brown fluorescent zone between the zone due to rutin and the zone due to chlorogenic acid. Further zones may be present.

B. Examine the chromatograms obtained in the test for atropine.

Results The principal zones in the chromatogram obtained with the test solution are similar in position and colour to the principal zones in the chromatogram obtained with the reference solution.

TESTS

Atropine

Thin-layer chromatography *(2.2.27)*.

Test solution To 0.20 g of the extract to be examined add 10.0 ml of *0.05 M sulphuric acid*, shake for 2 min and filter. Add 1.0 ml of *concentrated ammonia R* and shake with 2 quantities, each of 10 ml, of *peroxide-free ether R*. If necessary, separate by centrifugation. Dry the combined ether layers over about 2 g of *anhydrous sodium sulphate R*, filter and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 ml of *methanol R*.

Reference solution Dissolve 50 mg of *hyoscyamine sulphate R* in 9 ml of *methanol R*. Dissolve 15 mg of *hyoscine hydrobromide R* in 10 ml of *methanol R*. Mix 1.8 ml of the hyoscine hydrobromide solution and 8 ml of the hyoscyamine sulphate solution.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, water R, acetone R* (3:7:90 *V/V/V*).

Application 20 µl as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 15 min; allow to cool.

Detection A Spray with *potassium iodobismuthate solution R2*, until orange or brown zones become visible against a yellow background.

Results A The zones in the chromatogram obtained with the test solution are similar in position (hyoscyamine in the lower third, hyoscine in the upper third) and colour to those in the chromatogram obtained with the reference solution. Other faint zones may be present in the chromatogram obtained with the test solution.

Detection B Spray with *sodium nitrite solution R* until the coating is transparent and examine after 15 min.

Results B The zones due to hyoscyamine in the chromatograms obtained with the test solution and the reference solution change from orange or brown to reddish-brown but not to greyish-blue (atropine).

Loss on drying *(2.8.17)*

Maximum 5.0 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 micro-organisms per gram of which not more than 10^2 fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and for *Salmonella (2.6.13)* .

ASSAY

At each extraction stage it is necessary to check that the alkaloids have been completely extracted. If the extraction is into the organic phase this is done by evaporating to dryness a few millilitres of the last organic layer, dissolving the residue in *0.25 M sulphuric acid* and verifying the absence of alkaloids using *potassium tetraiodomercurate solution R* . If the extraction is into the acid aqueous phase, this is done by taking a few millilitres of the last acid aqueous phase and verifying the absence of alkaloids using *potassium tetraiodomercurate solution R* .

Disperse 3.00 g in a mixture of 5 ml of *ammonia R* and 15 ml of *water R* . Shake with no fewer than 3 quantities, each of 40 ml, of a mixture of 1 volume of *methylene chloride R* and 3 volumes of *peroxide-free ether R* until the alkaloids are completely extracted. Concentrate the combined organic layers to about 50 ml by distilling on a water-bath and transfer the resulting liquid to a separating funnel, rinsing with *peroxide-free ether R* . Add a quantity of *peroxide-free ether R* equal to at least 2.1 times the volume of the liquid to produce a layer having a density well below that of water. Shake the resulting solution with no fewer than 3 quantities, each of 20 ml, of *0.25 M sulphuric acid* until the alkaloids are completely extracted. Separate the layers by centrifugation, if necessary, and transfer the acid layers to a 2nd separating funnel. Make the combined acid layers alkaline with *ammonia R* and shake with no fewer than 3 quantities, each of 30 ml, of *methylene chloride R* until the alkaloids are completely extracted. Combine the organic layers, add 4 g of *anhydrous sodium sulphate R* and allow to stand for 30 min with occasional shaking. Decant the methylene chloride and wash the sodium sulphate with 3 quantities, each of 10 ml, of *methylene chloride R* . Combine the organic extracts and evaporate to dryness on a water-bath. Heat the residue in an oven at 100-105 °C for 15 min. Dissolve the residue in a few millilitres of *methylene chloride R* , evaporate to dryness on a water-bath and again heat the residue in an oven at 100-105 °C for 15 min. Dissolve the residue in a few millilitres of *methylene chloride R* , add 20.0 ml of *0.01 M sulphuric acid* and remove the methylene chloride by evaporation on a water-bath. Titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red mixed solution R* as indicator.

Calculate the percentage content of total alkaloids, expressed as hyoscyamine, using the following expression:

$$\frac{57.88 \times (20 - n)}{100 \times m}$$

n = volume of *0.02 M sodium hydroxide* used, in millilitres;

m = mass of drug used, in grams.

Ph Eur

Standardised Capsicum Tincture

General Notices

(Ph Eur monograph 2337)

Ph Eur

DEFINITION

Standardised tincture produced from *Capsicum (1859)* or *Refined and quantified capsicum oleoresin (2336)*.

Content

90 per cent to 110 per cent of the nominal content of total capsaicinoids, expressed as capsaicin ($C_{18}H_{27}NO_3$; M_r 305.4), stated on the label, which is between 0.020 per cent *m/m* and 0.060 per cent *m/m*.

PRODUCTION

The tincture is produced from the herbal drug or oleoresin and ethanol (70 per cent *V/V* to 85 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance

Yellowish-orange or reddish-orange liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)* .

Test solution Shake 10 ml of the tincture to be examined with 10 ml of *hexane R* . Allow to separate and use the lower layer.

Reference solution Dissolve 1 mg of *capsaicin R* and 1 mg of *dihydrocapsaicin R* in 5 ml of *ether R* .

Plate *TLC octadecylsilyl silica gel plate R* (5-40 µm) [or *TLC octadecylsilyl silica gel plate R* (2-10 µm)].

Mobile phase *water R, methanol R* (20:80 *V/V*).

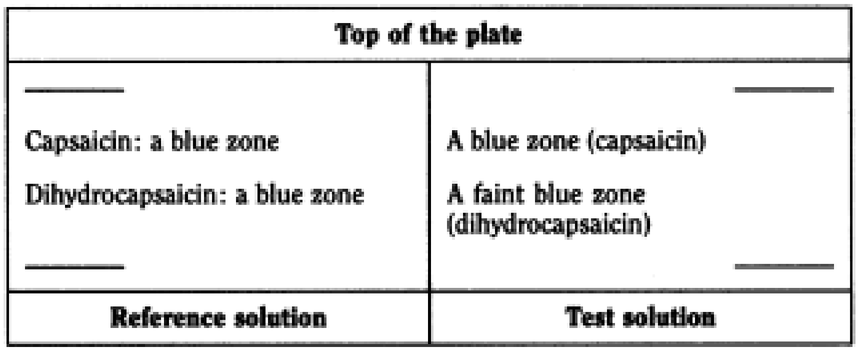
Application 20 µl [or 2 µl] as bands of 15 mm [or 8 mm].

Development Over a path of 12 cm [or 6 cm].

Drying In air.

Detection Spray with a 0.25 g/l solution of *dichloroquinonechlorimide R* in *ethyl acetate R*, expose to ammonia vapour until blue zones appear, then examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Capsaicin: a blue zone	A blue zone (capsaicin)
Dihydrocapsaicin: a blue zone	A faint blue zone (dihydrocapsaicin)
Reference solution	**Test solution**

TESTS

Nonivamide

Liquid chromatography *(2.2.29)*.

Test solution Dilute 50.0 g of the tincture to be examined to 100.0 ml with *methanol R*.

Reference solution Dissolve 20.0 mg of *capsaicin CRS* and 4.0 mg of *nonivamide CRS* in 100.0 ml of *methanol R*.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: phenylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R*, 1 g/l solution of *phosphoric acid R* (40:60 *V/V*).

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µl.

Elution order Nordihydrocapsaicin, nonivamide, capsaicin, dihydrocapsaicin.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to nonivamide and capsaicin.

Calculate the percentage content of nonivamide using the following expression:

$$\frac{F_1 \times m_2 \times p_1}{F_2 \times m_1}$$

F_1 = area of the peak due to nonivamide in the chromatogram obtained with the test solution;

F_2 = area of the peak due to nonivamide in the chromatogram obtained with the reference solution;

m_1 = mass of the tincture to be examined, in grams;

m_2 = mass of *nonivamide CRS* in the reference solution, in grams;

p_1 = percentage content of nonivamide in *nonivamide CRS*.

Limit:
— *nonivamide*: maximum 5.0 per cent of the total capsaicinoid content.

Ethanol *(2.9.10)*

95 per cent to 105 per cent of the content stated on the label.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for nonivamide.

Calculate the percentage content of total capsaicinoids, expressed as capsaicin, using the following expression:

$$\frac{(F_3 + F_5 + F_6) \times m_4 \times p_2}{F_4 \times m_3}$$

F_3 = area of the peak due to capsaicin in the chromatogram obtained with the test solution;

F_4 = area of the peak due to capsaicin in the chromatogram obtained with the reference solution;

F_5 = area of the peak due to dihydrocapsaicin in the chromatogram obtained with the test solution;

F_6 = area of the peak due to nordihydrocapsaicin in the chromatogram obtained with the test solution;

m_3 = mass of the tincture to be examined, in grams;

m_4 = mass of *capsaicin CRS* in the reference solution, in grams;

p_2 = percentage content of capsaicin in *capsaicin CRS*.

Ph Eur

Standardised Cascara Dry Extract

General Notices

(Ph Eur monograph 1844)

Ph Eur

DEFINITION

Standardised dry extract obtained from *Cascara (0105)* .

Content

90 per cent to 110 per cent of the nominal content of hydroxyanthracene glycosides, expressed as cascaroside A ($C_{27}H_{32}O_{14}$; M_r 580.5), stated on the label; minimum 60 per cent of the hydroxyanthracene glycosides are cascarosides, expressed as cascaroside A. The nominal content of hydroxyanthracene glycosides is within the range 8.0 per cent to 25.0 per cent *m/m* (dried extract).

PRODUCTION

The extract is produced from the herbal drug by an appropriate procedure using either boiling water or a hydroalcoholic solvent at least equivalent in strength to ethanol (60 per cent *V/V*).

CHARACTERS

Appearance

Brown, free-flowing powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)* .

Test solution To 0.2 g of the extract to be examined add 5 ml of *ethanol (70 per cent V/V) R* and heat to boiling. Cool and centrifuge. Decant the supernatant solution immediately and use within 30 min.

Reference solution Dissolve 20 mg of *barbaloin R* and 2 mg of *emodin R* in *ethanol (70 per cent V/V) R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *water R, methanol R* , *ethyl acetate R* (13:17:100 *V/V/V*).

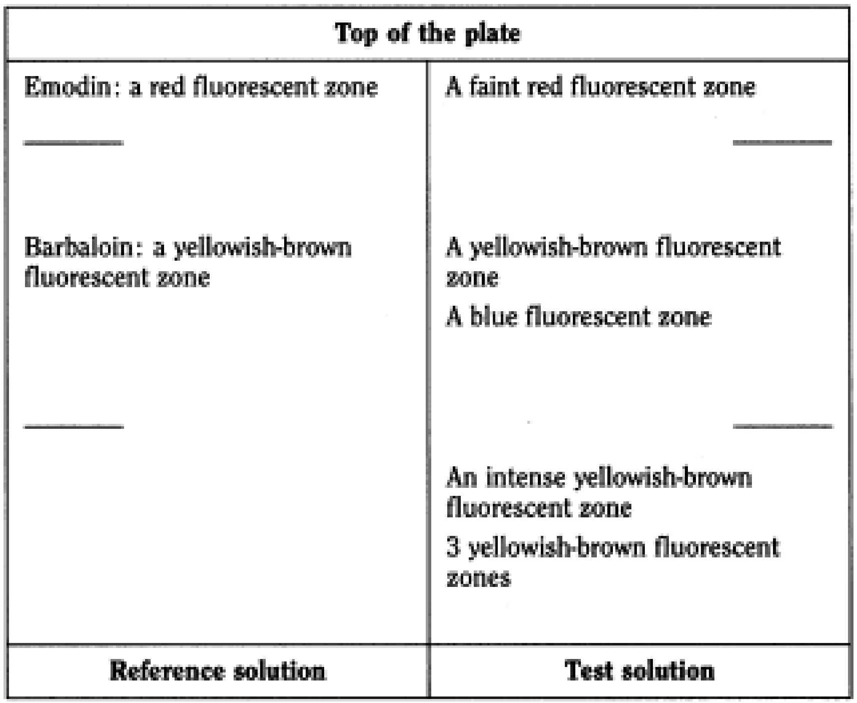
Application 10 µl [or 2 µl] as bands.

Development Over a path of 10 cm [or 6 cm].

Drying In air for 5 min.

Detection Spray with a 50 g/l solution of *potassium hydroxide R* in *ethanol (50 per cent V/V) R* and heat to 100-105 °C for 15 min; examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Emodin: a red fluorescent zone	A faint red fluorescent zone
———	———
Barbaloin: a yellowish-brown fluorescent zone	A yellowish-brown fluorescent zone A blue fluorescent zone
———	———
	An intense yellowish-brown fluorescent zone 3 yellowish-brown fluorescent zones
Reference solution	**Test solution**

TESTS

Loss on drying *(2.8.17)*

Maximum 5.0 per cent.

ASSAY

Carry out the assay within 24 h, protected from bright light.

To 0.500 g of the extract to be examined add 80 ml of *ethanol (70 per cent V/V) R*. Shake, and allow to stand in the dark for at least 8 h. Dilute to 100.0 ml with *ethanol (70 per cent V/V) R*. Shake and filter, discarding the first 20 ml of filtrate. Transfer 10.0 ml of the filtrate to a separating funnel, add 0.1 ml of *1 M hydrochloric acid* and shake with 2 quantities, each of 20 ml, of a mixture of 1 volume of *ether R* and 3 volumes of *hexane R*. Wash the combined organic extracts with 5 ml of *water R*. Discard the organic layer and return the rinsings to the hydroalcoholic layer. Shake with 4 quantities, each of 30 ml, of *ethyl acetate R* freshly saturated with *water R* (prepared as follows: to 150 ml of *ethyl acetate R* add 15 ml of *water R*, shake for 3 min and allow to stand), on each occasion allowing the layers to separate until the organic layer is clear. Combine the ethyl acetate extracts. Use the aqueous layer for the assay of cascarosides and the organic layer for the assay of hydroxyanthracene glycosides other than cascarosides.

Hydroxyanthracene glycosides other than cascarosides

Transfer the organic layer to a round-bottomed flask and remove the solvent by distillation, evaporating almost to dryness. Dissolve the residue in 0.5 ml of *methanol R*, add 10 ml of *water R* at 40 °C and transfer to a 50 ml volumetric flask, rinsing the round-bottomed flask with *water R* at 40 °C and adding the rinsings to the hydromethanolic solution. Allow to cool and dilute to 50.0 ml with *water R*. Transfer 20.0 ml of the solution to a 100 ml round-bottomed flask with a ground-glass neck containing 2 g of *ferric chloride R* and 12 ml of *hydrochloric acid R*. Attach a reflux condenser and place the flask in a water-bath so that the level of the water is above that of the liquid in the flask and heat for 4 h. Allow to cool, transfer the solution to a separating funnel and rinse the flask successively with 4 ml of *1 M sodium hydroxide* and 4 ml of *water R*, adding the rinsings to the separating funnel. Shake the contents of the separating funnel with 3 quantities, each of 30 ml, of a mixture of 1 volume of *ether R* and 3 volumes of *hexane R*. Wash the combined organic layers with 2 quantities, each of 10 ml, of *water R* and discard the rinsings. Dilute the organic layer to 100.0 ml with a mixture of 1 volume of *ether R* and 3 volumes of *hexane R*. Take 20.0 ml of the solution, evaporate carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 440 nm and 515 nm, using *methanol R* as the compensation liquid. If the ratio of the absorbance at 515 nm to that at 440 nm is less than 2.4, the assay is invalid.

Calculate the percentage content of hydroxyanthracene glycosides other than cascarosides, expressed as cascaroside A, using the following expression:

$$\frac{A \times 6.95}{m}$$

i.e. taking the specific absorbance to be 180.

A = absorbance at 515 nm;

m = mass of the substance to be examined, in grams.

Cascarosides

Dilute the aqueous layer to 50.0 ml with *water R*. Treat 20.0 ml of this solution as described above in the assay of hydroxyanthracene glycosides other than cascarosides. Measure the absorbance *(2.2.25)* at 440 nm and 515 nm. If the ratio of the absorbance at 515 nm to that at 440 nm is less than 2.7, the assay is invalid.

Calculate the percentage content of cascarosides, expressed as cascaroside A, using the following expression:

$$\frac{A \times 6.95}{m}$$

i.e. taking the specific absorbance to be 180.

A = absorbance at 515 nm;

m = mass of the substance to be examined, in grams.

LABELLING

The label states the nominal content of hydroxyanthracene glycosides, expressed as cascaroside A.

Ph Eur

Standardised Frangula Bark Dry Extract

General Notices

(Ph Eur monograph 1214)

Ph Eur

DEFINITION

Standardised dry extract obtained from *Frangula bark (0025)*.

Content

15.0 per cent to 30.0 per cent of glucofrangulins, expressed as glucofrangulin A ($C_{27}H_{30}O_{14}$; M_r 578.5) (dried extract); the measured content does not deviate from that stated on the label by more than ± 10 per cent.

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using ethanol (50-80 per cent *V/V*).

CHARACTERS

Appearance

Yellowish-brown, fine powder.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.05 g of the extract to be examined add 5 ml of *ethanol (70 per cent V/V) R* and heat to boiling. Cool and centrifuge. Decant the supernatant solution immediately and use within 30 min.

Reference solution Dissolve 20 mg of *barbaloin R* in *ethanol (70 per cent V/V) R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase water R, methanol R, ethyl acetate R (13:17:100 *V/V/V*).

Application 10 µl as bands.

Development Over a path of 10 cm.

Drying In air for 5 min.

Detection Spray with a 50 g/l solution of *potassium hydroxide R* in *ethanol (50 per cent V/V) R* and heat at 100-105 °C for 15 min; examine immediately after heating.

Results The chromatogram obtained with the reference solution shows in the median third a reddish-brown zone due to barbaloin. The chromatogram obtained with the test solution shows 2 orange-brown zones (glucofrangulins) in the lower third and 2-4 red zones (frangulins, not always clearly separated, and above them frangula-emodin) in the upper third.

B. To about 25 mg add 25 ml of *dilute hydrochloric acid R* and heat the mixture on a water-bath for 15 min. Allow to cool, shake with 20 ml of *ether R* and discard the aqueous layer. Shake the ether layer with 10 ml of *dilute ammonia R1*. The aqueous layer becomes reddish-violet.

TESTS

Loss on drying *(2.8.17)*

Maximum 5.0 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 per gram of which not more than 10^2 fungi per gram, determined by plate count. It complies with the test for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

Carry out the assay protected from bright light.

In a tared round-bottomed flask with a ground-glass neck, weigh 0.100 g. Add 25.0 ml of a 70 per cent *V/V* solution of *methanol R*, mix and weigh again. Heat the flask in a water-bath under a reflux condenser at 70 °C for 15 min. Allow to cool, weigh and adjust to the original mass with a 70 per cent *V/V* solution of *methanol R*. Filter and transfer 5.0 ml of the filtrate to a separating funnel. Add 50 ml of *water R* and 0.1 ml of *hydrochloric acid R*. Shake with 5 quantities, each of 20 ml, of *light petroleum R1*. Allow the layers to separate and transfer the aqueous layer to a 100 ml volumetric flask. Combine the light petroleum layers and wash with 2 quantities, each of 15 ml, of *water R*. Use this water for washing the separating funnel and add it to the aqueous solution in the volumetric flask. Add 5 ml of a 50 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with *water R*. Discard the light petroleum layer. Transfer 40.0 ml of the aqueous solution to a 200 ml round-bottomed flask with a ground-glass neck. Add 20 ml of a 200 g/l solution of *ferric chloride R* and heat under a reflux condenser for 20 min in a water-bath with the water level above that of the liquid in the flask. Add 2 ml of *hydrochloric acid R* and continue heating for 20 min, shaking frequently, until the precipitate is dissolved. Allow to cool, transfer the mixture to a separating funnel and shake with 3 quantities, each of 25 ml, of *ether R*, previously used to rinse the flask. Combine the ether extracts and wash with 2 quantities, each of 15 ml, of *water R*. Transfer the ether layer to a volumetric flask and dilute to 100.0 ml with *ether R*. Evaporate 20.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 515 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of glucofrangulins, expressed as glucofrangulin A, using the following expression:

$$\frac{A \times 3.06}{m}$$

i.e. taking the specific absorbance of glucofrangulin A to be 204, calculated on the basis of the specific absorbance of barbaloin.

A = absorbance at 515 nm;

m = mass of the preparation to be examined, in grams.

LABELLING

The label states the content of glucofrangulins.

Ph Eur

Standardised Ipecacuanha Liquid Extract

General Notices

(Ph Eur monograph 1875)

Ph Eur

DEFINITION

Standardised liquid extract produced from *Ipecacuanha root (0094).*

Content

1.80 per cent to 2.20 per cent of total alkaloids, calculated as emetine ($C_{29}H_{40}N_2O_4$; M_r 480.7) .

PRODUCTION

The extract is produced from the herbal drug and ethanol (60 to 80 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance

Dark brown liquid.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution Dilute 5.0 ml of the extract to be examined to 50 ml with *ethanol (70 per cent V/V) R*. To 2.0 ml of this solution add 2 ml of *water R* and 0.1 ml of *concentrated ammonia R*. Add 10 ml of *ether R* and shake. Separate the upper layer, dry it over about 2 g of *anhydrous sodium sulphate R* and filter.

Reference solution Dissolve 2.5 mg of *emetine hydrochloride CRS* and 3 mg of *cephaeline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase concentrated ammonia R, *methanol R*, *ethyl acetate R*, *toluene R* (2:15:18:65 *V/V/V/V*).

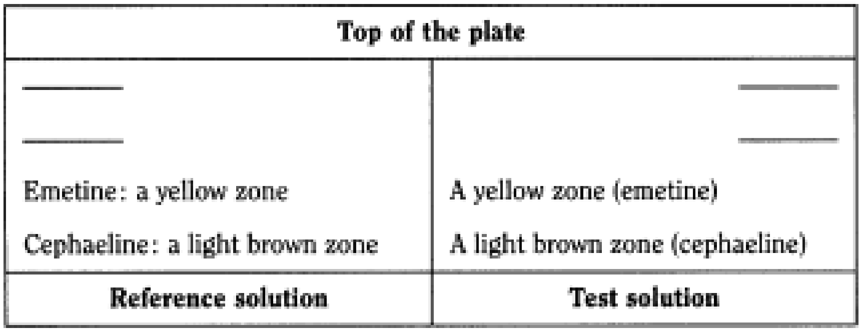
Application 10 µl as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Spray with a 5 g/l solution of *iodine R* in *ethanol (96 per cent) R*. Heat at 60 °C for 10 min and allow to cool for 30 min. Examine in daylight.

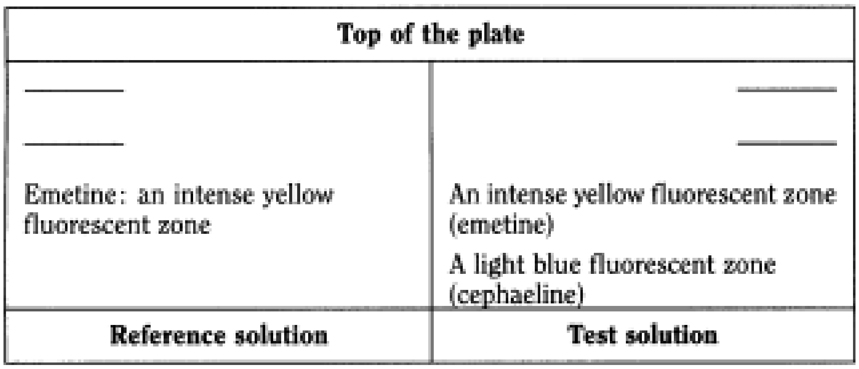
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
———	———
Emetine: a yellow zone	A yellow zone (emetine)
Cephaeline: a light brown zone	A light brown zone (cephaeline)
Reference solution	**Test solution**

Detection B Examine the plate in ultraviolet light at 365 nm.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint fluorescent zones are present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
———	———
Emetine: an intense yellow fluorescent zone	An intense yellow fluorescent zone (emetine)
	A light blue fluorescent zone (cephaeline)
Reference solution	**Test solution**

With a liquid extract from *Cephaelis acuminata* root, the zones of emetine and cephaeline in the chromatogram obtained with the test solution are of similar size.

With a liquid extract from *Cephaelis ipecacuanha* root, the zone of emetine is much larger than the zone of cephaeline in the chromatogram obtained with the test solution.

TESTS

Ethanol (*2.9.10*)

95 per cent to 105 per cent of the quantity stated on the label.

ASSAY

Dilute 1.00 g of the extract to be examined to 10 ml with *ethanol (70 per cent V/V) R* and transfer to a chromatography column about 0.2 m long and about 15 mm in internal diameter, containing 8 g of *basic aluminium oxide R*, using a glass rod. After infiltration into the aluminium oxide layer, rinse the flask, glass rod and internal wall of the column with 3 quantities, each of 2 ml, of *ethanol (70 per cent V/V) R*. Elute in portions with 40 ml of *ethanol (70 per cent V/V) R*. Avoid disturbance or drying of the surface of the aluminium oxide layer. Collect the whole of the eluate. Evaporate the eluate on a water-bath to about 10 ml. Allow to cool. Add 10.0 ml of *0.02 M hydrochloric acid* and 20 ml of *carbon dioxide-free water R*. Titrate the excess acid with *0.02 M sodium hydroxide* using 0.15 ml of *methyl red mixed solution R* as indicator.

Perform a blank assay by replacing the extract to be examined with 10.0 ml of alcohol of the strength stated on the label.

1 ml of *0.02 M hydrochloric acid* is equivalent to 4.807 mg of total alkaloids, calculated as emetine.

Ph Eur

Standardised Ipecacuanha Tincture

General Notices

(Ph Eur monograph 1530)

Ph Eur

DEFINITION

Tincture produced from *Ipecacuanha root (0094).*

Content

0.18 per cent (*m/m*) to 0.22 per cent (*m/m*) of total alkaloids, calculated as emetine ($C_{29}H_{40}N_2O_4$; M_r 480.7).

PRODUCTION

The tincture is produced from the herbal drug and ethanol (70 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance

Yellowish-brown liquid.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution To 2.0 ml of the tincture to be examined add 2 ml of *water R* and 0.1 ml of *concentrated ammonia R*. Add 10 ml of *ether R* and shake. Separate the ether layer, dry it over about 2 g of *anhydrous sodium sulphate R* and filter.

Reference solution Dissolve 2.5 mg of *emetine hydrochloride CRS* and 3 mg of *cephaeline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase concentrated ammonia R, *methanol R*, *ethyl acetate R*, *toluene R* (2:15:18:65 *V/V/V/V*).

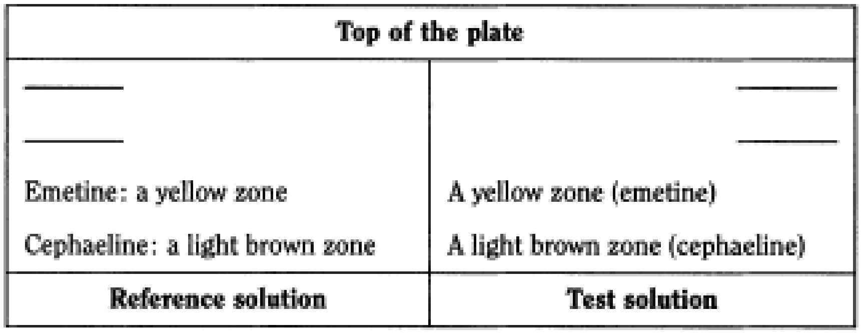
Application 10 µl as bands.

Development Over a path of 10 cm.

Drying In air.

Detection A Spray with a 5 g/l solution of *iodine R* in *ethanol (96 per cent) R* and heat at 60 °C for 10 min. Examine in daylight.

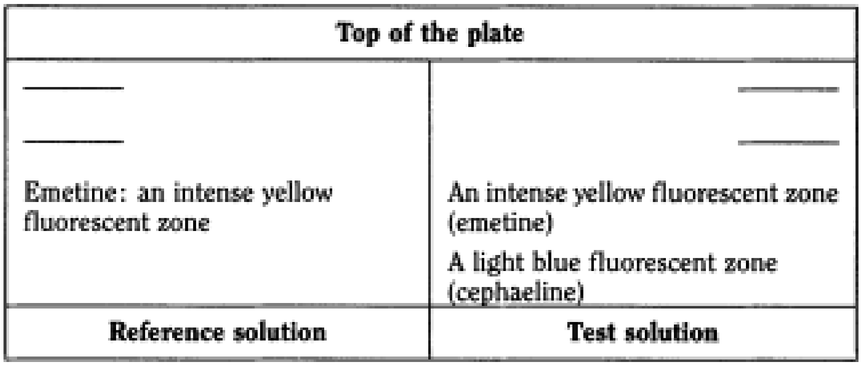
Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
———	———
Emetine: a yellow zone	A yellow zone (emetine)
Cephaeline: a light brown zone	A light brown zone (cephaeline)
Reference solution	**Test solution**

Detection B Examine the plate in ultraviolet light at 365 nm.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint fluorescent zones are present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
———	———
Emetine: an intense yellow fluorescent zone	An intense yellow fluorescent zone (emetine)
	A light blue fluorescent zone (cephaeline)
Reference solution	**Test solution**

With a tincture from *Cephaelis acuminata* root, the zones of emetine and cephaeline in the chromatogram obtained with the test solution are similar in size.

With a tincture from *Cephaelis ipecacuanha* root, the zone of emetine is much larger than the zone of cephaeline in the chromatogram obtained with the test solution.

TESTS

Ethanol *(2.9.10)*

95 per cent to 105 per cent of the quantity stated on the label.

ASSAY

Transfer 10.00 g of the tincture to be examined to a chromatography column about 0.2 m long and about 15 mm in internal diameter, filled with 8 g of *basic aluminium oxide R*. After infiltration into the aluminium oxide layer rinse the internal wall of the column with 3 quantities, each of 2 ml, of *ethanol (70 per cent V/V) R*. Elute in portions, with 40 ml of *ethanol (70 per cent V/V) R*. Avoid whirling or drying of the surface of the aluminium oxide layer. Collect the whole of the eluate. Evaporate the eluate on a water-bath to about 10 ml. Allow to cool. Add 10.0 ml of *0.02 M hydrochloric acid* and 20 ml of *carbon dioxide-free water R*. Titrate the excess acid with *0.02 M sodium hydroxide* using 0.15 ml of *methyl red mixed solution R* as indicator.

Perform a blank assay replacing the tincture to be examined with 10.0 ml of alcohol of the strength stated on the label.

1 ml of *0.02 M hydrochloric acid* is equivalent to 4.807 mg of total alkaloids, calculated as emetine.

Ph Eur

Standardised Liquorice Ethanolic Liquid Extract

General Notices

(Ph Eur monograph 1536)

Ph Eur

DEFINITION

Standardised ethanolic liquid extract produced from *Liquorice root (0277)*.

Content

3.0 per cent to 5.0 per cent of glycyrrhizic acid ($C_{42}H_{62}O_{16}$; M_r 823).

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure for liquid extracts using ethanol (70 per cent *V/V*).

CHARACTERS

Appearance

Dark brown, clear liquid.

It has a faint characteristic odour and a sweet taste.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Place 1.0 g of the extract to be examined in a 50 ml round-bottomed flask, add 16.0 ml of *water R* and 4.0 ml of *hydrochloric acid R1* and heat on a water-bath under a reflux condenser for 30 min. Allow to cool and filter. Dry the filter and the round-bottomed flask at 105 °C for 60 min. Transfer the filter to the round-bottomed flask, add 20 ml of *ether R* and heat in a water-bath at 40 °C under a reflux condenser for 5 min. Allow to cool and filter. Evaporate the filtrate to dryness and dissolve the residue in 5.0 ml of *ether R*.

Reference solution Dissolve 5.0 mg of *glycyrrhetic acid R* and 5.0 mg of *thymol R* in 5 ml of *ether R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, water R, ethanol (96 per cent) R, ethyl acetate R* (1:9:25:65 *V/V/V/V*).

Application 10 µl as bands.

Development Over a path of 15 cm.

Drying In air for 5 min.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatograms obtained with the test solution and the reference solution show in the lower half a quenching zone due to glycyrrhetic acid.

Detection B Spray with *anisaldehyde solution R*; heat at 100-105 °C for 5-10 min and examine in daylight.

Results B The chromatogram obtained with the reference solution shows in the lower half a violet zone (glycyrrhetic acid), and in the upper third a red zone (thymol); the chromatogram obtained with the test solution shows in the lower half a violet zone corresponding to glycyrrhetic acid in the chromatogram obtained with the reference solution, and in the upper third, below the zone of thymol in the chromatogram obtained with the reference solution, a yellow zone due to isoliquiritigenin; further zones are present.

TESTS

Ethanol *(2.9.10)*

52 per cent *V/V* to 65 per cent *V/V*.

Methanol and 2-propanol *(2.9.11)*

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

ASSAY

Liquid chromatography *(2.2.29)*.

Solvent mixture *dilute ammonia R1, water R* (8:92 *V/V*).

Test solution Dilute 1.000 g of the extract to be examined to 100 ml with the solvent mixture and centrifuge. Dilute 2.0 ml of the supernatant to 10.0 ml with the solvent mixture.

Stock solution Dissolve 0.130 g of *monoammonium glycyrrhizate CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a) Dilute 5.0 ml of the stock solution to 100.0 ml with the solvent mixture.

Reference solution (b) Dilute 10.0 ml of the stock solution to 100.0 ml with the solvent mixture.

Reference solution (c) Dilute 15.0 ml of the stock solution to 100.0 ml with the solvent mixture.

Column:
- *size:* *l* = 0.10 m, Ø = 4 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, acetonitrile R, water R* (6:30:64 *V/V/V*).

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µl.

Establish a calibration curve with the concentrations of the reference solutions (g/100 ml) as the abscissa and the corresponding peak areas as the ordinate.

Using the retention times and the peak areas determined from the chromatograms obtained with the reference solutions, locate and integrate the peak due to glycyrrhizic acid in the chromatogram obtained with the test solution.

Calculate the percentage content of glycyrrhizic acid using the following expression:

$$A \times \frac{5}{m} \times B \times \frac{822}{840}$$

A = concentration of monoammonium glycyrrhizate in the test solution, determined from the calibration curve, in g/100 ml;

B = declared percentage content of *monoammonium glycyrrhizate CRS*;

m = mass of the extract to be examined, in grams;

822 = molecular mass of glycyrrhizic acid;

840 = molecular mass of monoammonium glycyrrhizate (without any water of crystallisation)

Ph Eur

Standardised Opium Dry Extract

General Notices

(Ph Eur monograph 1839)

Ph Eur

DEFINITION

Standardised dry extract produced from *Raw opium (0777).*

Content:

- *morphine* ($C_{17}H_{19}NO_3$; M_r 285.3): 19.6 per cent to 20.4 per cent (dried extract);
- *codeine* ($C_{18}H_{21}NO_3$; M_r 299.4): minimum 2.0 per cent (dried extract).

Content adjusted if necessary by adding a suitable excipient (e.g. lactose, dextrin).

PRODUCTION

It is produced from the drug and water by a suitable procedure.

CHARACTERS

Appearance

Brown, amorphous powder.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Triturate 0.05 g of the extract to be examined with 5 ml of *ethanol (70 per cent V/V) R*. Transfer to a 25 ml conical flask. Rinse with 3 ml of *ethanol (70 per cent V/V) R* and transfer to the same 25 ml conical flask. Heat in a water-bath at 50-60 °C, with stirring, for 30 min. Cool, filter, wash the filter with *ethanol (70 per cent V/V) R* and dilute the combined filtrate and washings to 10 ml with the same solvent.

Reference solution Dissolve 5 mg of *morphine hydrochloride R* in the solution prepared as follows and dilute to 5 ml with the same solution: dissolve 2 mg of *papaverine hydrochloride R*, 12 mg of *codeine phosphate R* and 12 mg of *noscapine hydrochloride R* in *ethanol (70 per cent V/V) R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *concentrated ammonia R*, *ethanol (96 per cent) R*, *acetone R*, *toluene R* (2:6:40:40 *V/V/V/V*). Use a freshly prepared mixture.

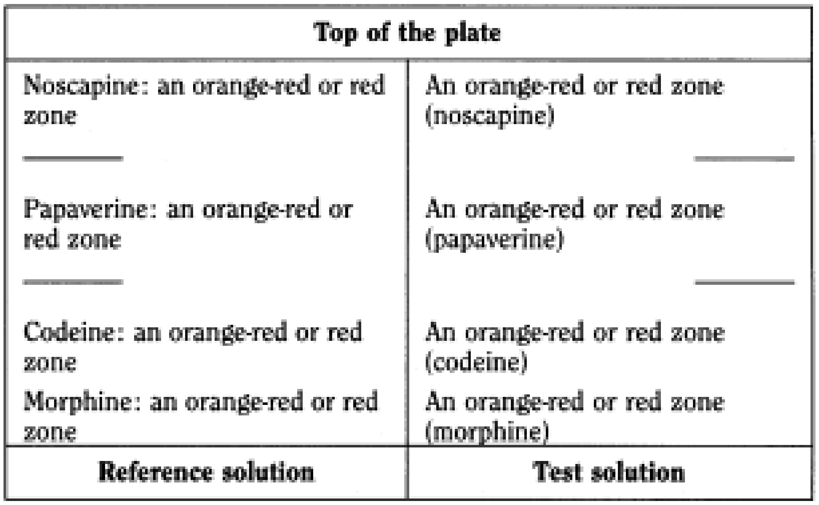
Application 20 µl [or 6 µl] as bands.

Development Over a path of 15 cm [or 8 cm].

Drying At 100-105 °C for 15 min.

Detection Allow to cool and spray with *potassium iodobismuthate solution R2* and then with a 4 g/l solution of *sulphuric acid R*; examine in daylight.

Results See below the sequences of zones present in the chromatograms obtained with the reference solution and the test solution. A dark red zone (thebaine) situated between the zone due to codeine and the zone due to papaverine may be present in the chromatogram obtained with the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Noscapine: an orange-red or red zone	An orange-red or red zone (noscapine)
Papaverine: an orange-red or red zone	An orange-red or red zone (papaverine)
Codeine: an orange-red or red zone	An orange-red or red zone (codeine)
Morphine: an orange-red or red zone	An orange-red or red zone (morphine)
Reference solution	**Test solution**

B. To 0.5 g of the extract to be examined add 5 ml of *water R*, shake for 5 min and filter. To the filtrate add 0.25 ml of *ferric chloride solution R2.* A red colour develops, which does not disappear on the addition of 0.5 ml of *dilute hydrochloric acid R*.

TESTS

Thebaine

Liquid chromatography (*2.2.29*).

Test solution Suspend 0.500 g of the extract to be examined in 50 ml of *ethanol (50 per cent V/V) R*, mix with the aid of ultrasound for 1 h, allow to cool and dilute to 100.0 ml with the same solvent. Allow to stand. To 10.0 ml of the supernatant liquid, add 5 ml of *ammonium chloride buffer solution pH 9.5 R,* dilute to 25.0 ml with *water R* and mix. Transfer 20.0 ml of this solution to a chromatography column about 0.15 m long and about 30 mm in internal diameter containing 15 g of *kieselguhr for chromatography R*. Allow to stand for 15 min. Elute with 2 quantities, each of 40 ml, of a mixture of 15 volumes of *2-propanol R* and 85 volumes of *methylene chloride R*. Evaporate the eluate to dryness *in vacuo* at 40 °C. Transfer the residue to a volumetric flask with the aid of the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *thebaine CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 12.0 mg of *morphine hydrochloride CRS* in the mobile phase and dilute to 15.0 ml with the mobile phase (solution A). Dissolve 10.0 mg of *codeine CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. To 10.0 ml of this solution add 10.0 ml of solution A and mix.

Precolumn:
— *size:* *l* = 4 mm, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Column:
— *size:* *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.0 g of *sodium heptanesulphonate monohydrate R* in 420 ml of *water R*, adjust to pH 3.2 with a 4.9 g/l solution of *phosphoric acid R* and add 180 ml of *acetonitrile R*.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

System suitability:
— *resolution*: minimum 2.5 between the peaks due to morphine and codeine in the chromatogram obtained with reference solution (b);

— *mass distribution ratio*: minimum 3.0 for the peak due to thebaine in the chromatogram obtained with reference solution (a).

Calculate the percentage content of thebaine using the following expression:

$$\frac{A_1 \times m_2 \times F \times p}{A_2 \times m_1}$$

A_1 = area of the peak due to the relevant alkaloid in the chromatogram obtained with the test solution,

A_2 = area of the peak due to the relevant alkaloid in the chromatogram obtained with the reference solution,

m_1 = mass of the extract to be examined in the test solution, in grams,

m_2 = mass of the relevant alkaloid in the reference solution, in grams,

p = percentage content of the alkaloid in the relevant alkaloid CRS,

F = 6.250 for the determination of thebaine.

Limit:
— *thebaine*: maximum 6.0 per cent (dried extract).

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for thebaine with the following modifications.

Injection Test solution and reference solution (b).

System suitability:

— *repeatability*: maximum relative standard deviation of 1.0 per cent for the area of the peak due to morphine after 6 injections of reference solution (b).

Calculate the percentage content of morphine and codeine from the expression given in the test for thebaine, assigning *F* as 10.417 for morphine and as 3.125 for codeine.

Ph Eur

Standardised Opium Tincture

General Notices

(Ph Eur monograph 1841)

Ph Eur

DEFINITION

Standardised tincture produced from *Raw opium (0777)*.

Content:

— *morphine* ($C_{17}H_{19}NO_3$; M_r 285.3): 0.95 per cent to 1.05 per cent;

— *codeine* ($C_{18}H_{21}NO_3$; M_r 299.4): minimum 0.1 per cent.

PRODUCTION

It is produced from the drug and equal volumes of ethanol (70 per cent *V/V*) and water by an appropriate procedure.

CHARACTERS

Appearance: reddish-brown liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Dilute 1.0 ml of the tincture to be examined to 10 ml with *ethanol (70 per cent V/V) R*.

Reference solution Dissolve 5 mg of *morphine hydrochloride R* in the solution prepared as follows and dilute to 5 ml with the same solution: dissolve 2 mg of *papaverine hydrochloride R*, 12 mg of *codeine phosphate R* and 12 mg of *noscapine hydrochloride R* in *ethanol (70 per cent V/V) R* and dilute to 25 ml with the same solvent.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *concentrated ammonia R*, *ethanol (96 per cent) R*, *acetone R*, *toluene R* (2:6:40:40 *V/V/V/V*). Use a freshly prepared mixture.

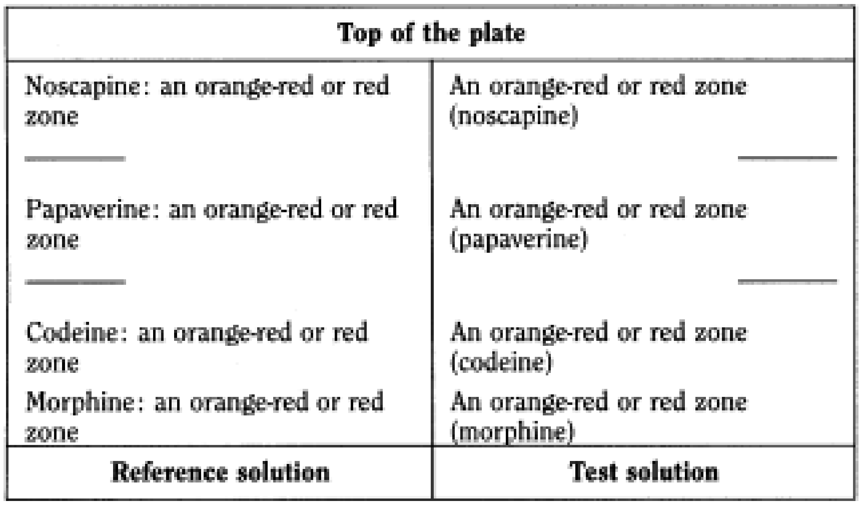
Application 20 µl [or 6 µl] as bands.

Development Over a path of 15 cm [or 8 cm].

Drying At 100-105 °C for 15 min.

Detection: allow to cool and spray the plate with *potassium iodobismuthate solution R2* and then with a 4 g/l solution of *sulphuric acid R*; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. A dark red zone (thebaine) situated between the zone due to codeine and the zone due to papaverine may be present in the chromatogram obtained with the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Noscapine: an orange-red or red zone	An orange-red or red zone (noscapine)
Papaverine: an orange-red or red zone	An orange-red or red zone (papaverine)
Codeine: an orange-red or red zone	An orange-red or red zone (codeine)
Morphine: an orange-red or red zone	An orange-red or red zone (morphine)
Reference solution	**Test solution**

TESTS

Ethanol *(2.9.10)*

31 per cent *V/V* to 34 per cent *V/V*.

Thebaine

Liquid chromatography *(2.2.29)*.

Test solution Dilute 2.000 g of the tincture to be examined to 25.0 ml with *ethanol (50 per cent V/V) R*. To 10.0 ml of the solution add 5 ml of *ammonium chloride buffer solution pH 9.5 R*, dilute to 25.0 ml with *water R* and mix. Transfer 20.0 ml of this solution to a chromatography column about 0.15 m long and about 30 mm in internal diameter containing 15 g of *kieselguhr for chromatography R*. Allow to stand for 15 min. Elute with 2 quantities, each of 40 ml, of a mixture of 15 volumes of *2-propanol R* and 85 volumes of *methylene chloride R*. Evaporate the eluate to dryness *in vacuo* at 40 °C. Transfer the residue to a volumetric flask with the aid of the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *thebaine CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b) Dissolve 12.0 mg of *morphine hydrochloride CRS* in the mobile phase and dilute to 15.0 ml with the mobile phase (solution A). Dissolve 10.0 mg of *codeine CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. To 10.0 ml of this solution add 10.0 ml of solution A and mix.

Precolumn:
— *size:* l = 4 mm, Ø = 4.0 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Column:

— *size:* l = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.0 g of *sodium heptanesulphonate monohydrate R* in 420 ml of *water R*, adjust to pH 3.2 with a 4.9 g/l solution of *phosphoric acid R* and add 180 ml of *acetonitrile R.*

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µl.

System suitability Reference solution (b):

— *resolution*: minimum 2.5 between the peaks due to morphine and codeine.

Calculate the percentage content of thebaine using the following expression:

$$\frac{A_1 \times m_2 \times F \times p}{A_2 \times m_1}$$

A_1 = area of the peak due to the relevant alkaloid in the chromatogram obtained with the test solution

A_2 = area of the peak due to the relevant alkaloid in the chromatogram obtained with the reference solution

m_1 = mass of the tincture to be examined in the test solution, in grams

m_2 = mass of the relevant alkaloid in the reference solution, in grams

p = percentage content of the alkaloid in the relevant alkaloid CRS

F = 1.563 for the determination of thebaine.

Limit:
— *thebaine*: maximum 0.3 per cent.

Dry residue *(2.8.16)*

Minimum 4.0 per cent *m/m*, determined on 3.00 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for thebaine with the following modifications.

Injection Test solution and reference solution (b).

System suitability:
— *repeatability*: maximum relative standard deviation of 1.0 per cent for the area of the peak due to morphine after 6 injections of reference solution (b).

Calculate the percentage content of morphine and codeine from the expression given in the test for thebaine, assigning *F* as 2.604 for morphine and 0.781 for codeine.

Ph Eur

Standardised Senna Leaf Dry Extract

General Notices

(Ph Eur monograph 1261)

Ph Eur

DEFINITION

Standardised dry extract produced from *Senna leaf (0206)*.

Content

5.5 per cent to 8.0 per cent of hydroxyanthracene glycosides, expressed as sennoside B ($C_{42}H_{38}O_{20}$; M_r 863) (dried extract). The measured content does not deviate from the value stated on the label by more than ± 10 per cent.

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using ethanol (50-80 per cent *V/V*).

CHARACTERS

Appearance

Brownish or brown powder.

IDENTIFICATION

A. Thin-layer chromatography *(2.2.27)*.

Solvent mixture *ethanol (96 per cent) R*, *water R* (50:50 *V/V*).

Test solution To 0.1 g of the extract to be examined add 5 ml of the solvent mixture and heat to boiling. Cool and centrifuge. Use the supernatant liquid.

Reference solution Dissolve 10 mg of *senna extract CRS* in 1 ml of the solvent mixture (a slight residue remains).

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R*, *water R*, *ethyl acetate R*, *1-propanol R* (1:30:40:40 *V/V/V/V*).

Application 10 µl as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 20 per cent *V/V* solution of *nitric acid R* and heat at 120 °C for 10 min; allow to cool and spray with a 50 g/l solution of *potassium hydroxide R* in *ethanol (50 per cent V/V) R* until the zones appear.

Results The principal zones in the chromatogram obtained with the test solution are similar in position, colour and size to the principal zones in the chromatogram obtained with the reference solution. The chromatograms show in the lower third a prominent brown zone due to sennoside B and above it a yellow zone followed by another prominent brown zone due to sennoside A. In the upper half of the chromatograms are visible, in order of increasing R_F value, a prominent reddish-brown zone and an orange-brown zone followed by a faint pink zone and 2 yellow zones. Close to the solvent front a dark pink zone appears, which may be followed by several faint zones.

B. Place about 25 mg of the extract to be examined in a conical flask and add 50 ml of *water R* and 2 ml of *hydrochloric acid R*. Heat in a water-bath for 15 min, cool and shake with 40 ml of *ether R*. Separate the ether layer, dry over *anhydrous sodium sulphate R*, evaporate 5 ml to dryness and to the cooled residue add 5 ml of *dilute ammonia R1*. A yellow or orange colour develops. Heat on a water-bath for 2 min. A reddish-violet colour develops.

TESTS

Loss on drying *(2.8.17)*

Maximum 5.0 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 micro-organisms per gram, of which not more than 10^2 fungi per gram, determined by plate-count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

ASSAY

Carry out the assay protected from bright light.

Place 0.150 g of the extract to be examined in a 100 ml flask, dissolve in *water R* and dilute to 100.0 ml with the same solvent. Filter the solution, discard the first 10 ml of the filtrate. Transfer 20.0 ml of the filtrate to a 150 ml separating funnel. Add 0.1 ml of *dilute hydrochloric acid R* and shake with 3 quantities, each of 15 ml, of *ether R*. Allow the layers to separate and discard the ether layer. Add 0.10 g of *sodium hydrogen carbonate R* to the aqueous layer and shake for 3 min. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100 ml round-bottomed flask with a ground-glass neck. Add 20 ml of *ferric chloride solution R1* and mix. Heat for 20 min under a reflux condenser in a water-bath with the water level above that of the liquid in the flask; add 3 ml of *hydrochloric acid R* and heat for a further 30 min with frequent shaking to dissolve the precipitate. Cool, transfer the mixture to a separating funnel and shake with 3 quantities, each of 25 ml, of *ether R* previously used to rinse the flask. Combine the ether layers and wash with 2 quantities, each of 15 ml, of *water R*. Transfer the ether layers to a volumetric flask and dilute to 100.0 ml with *ether R*. Evaporate 10.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a 5.0 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 515 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of hydroxyanthracene glycosides expressed as sennoside B using the following expression:

$$\frac{A \times 4.167}{m}$$

i.e. taking the specific absorbance of sennoside B to be 240.

A = absorbance at 515 nm;

m = mass of the herbal drug to be examined, in grams.

LABELLING

The label states the content of hydroxyanthracene glycosides.

Ph Eur

Star Anise Oil

General Notices

(Ph Eur monograph 2108)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the dry ripe fruits of *Illicium verum* Hook. fil.

CHARACTERS

Appearance

Clear, colourless or pale yellow liquid.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 1 g of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 10 µl of *linalol R* , 30 µl of *anisaldehyde R* and 200 µl of *anethole R* and in *toluene R* and dilute to 15 ml with the same solvent. Dilute 1 ml of this solution to 5 ml with *toluene R* .

Plate *TLC silica gel* F_{254} *plate R* .

Mobile phase *ethyl acetate R* , *toluene R* (7:93 *V/V*).

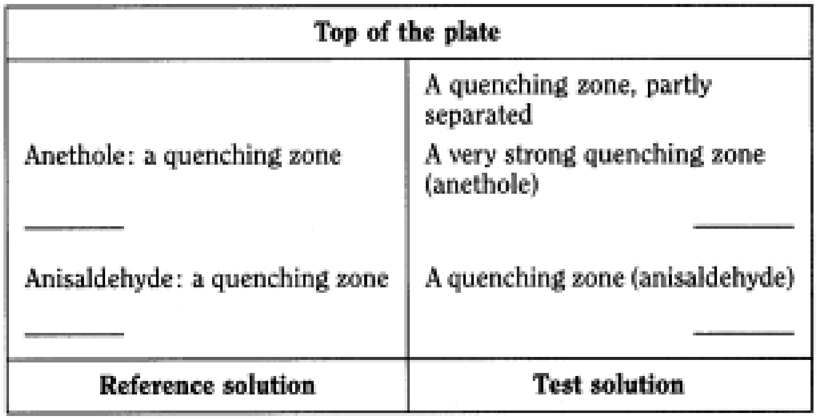
Application 5 µl as bands of 10 mm (for normal TLC plates) or 2 µl as bands of 10 mm (for fine particle TLC plates).

Development Over a path of 15 cm (for normal TLC plates) or over a path of 6 cm (for fine particle size plates).

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

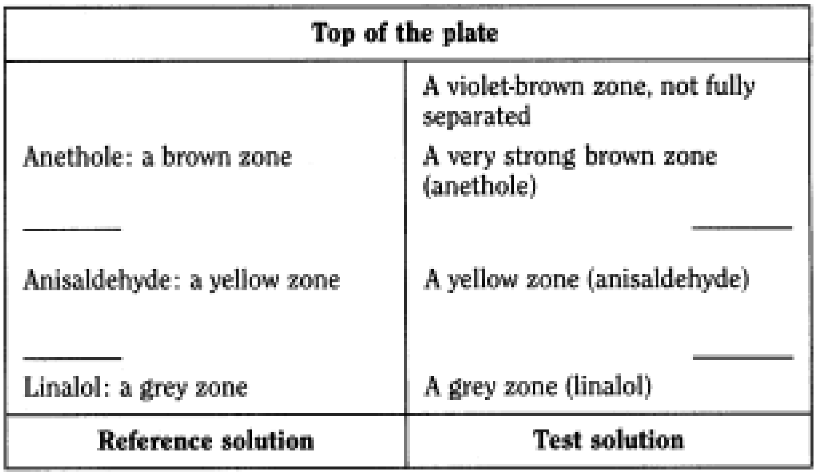
Results A See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A quenching zone, partly separated
Anethole: a quenching zone	A very strong quenching zone (anethole)
_______	_______
Anisaldehyde: a quenching zone	A quenching zone (anisaldehyde)
_______	_______
Reference solution	**Test solution**

Detection B Spray with *methyl 4-acetylbenzoate reagent R* and heat at 100-105 °C for 10 min; examine the still hot plate in daylight within 10 min.

Results B See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A violet-brown zone, not fully separated
Anethole: a brown zone	A very strong brown zone (anethole)
_______	_______
Anisaldehyde: a yellow zone	A yellow zone (anisaldehyde)
_______	_______
Linalol: a grey zone	A grey zone (linalol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density ***(2.2.5)***

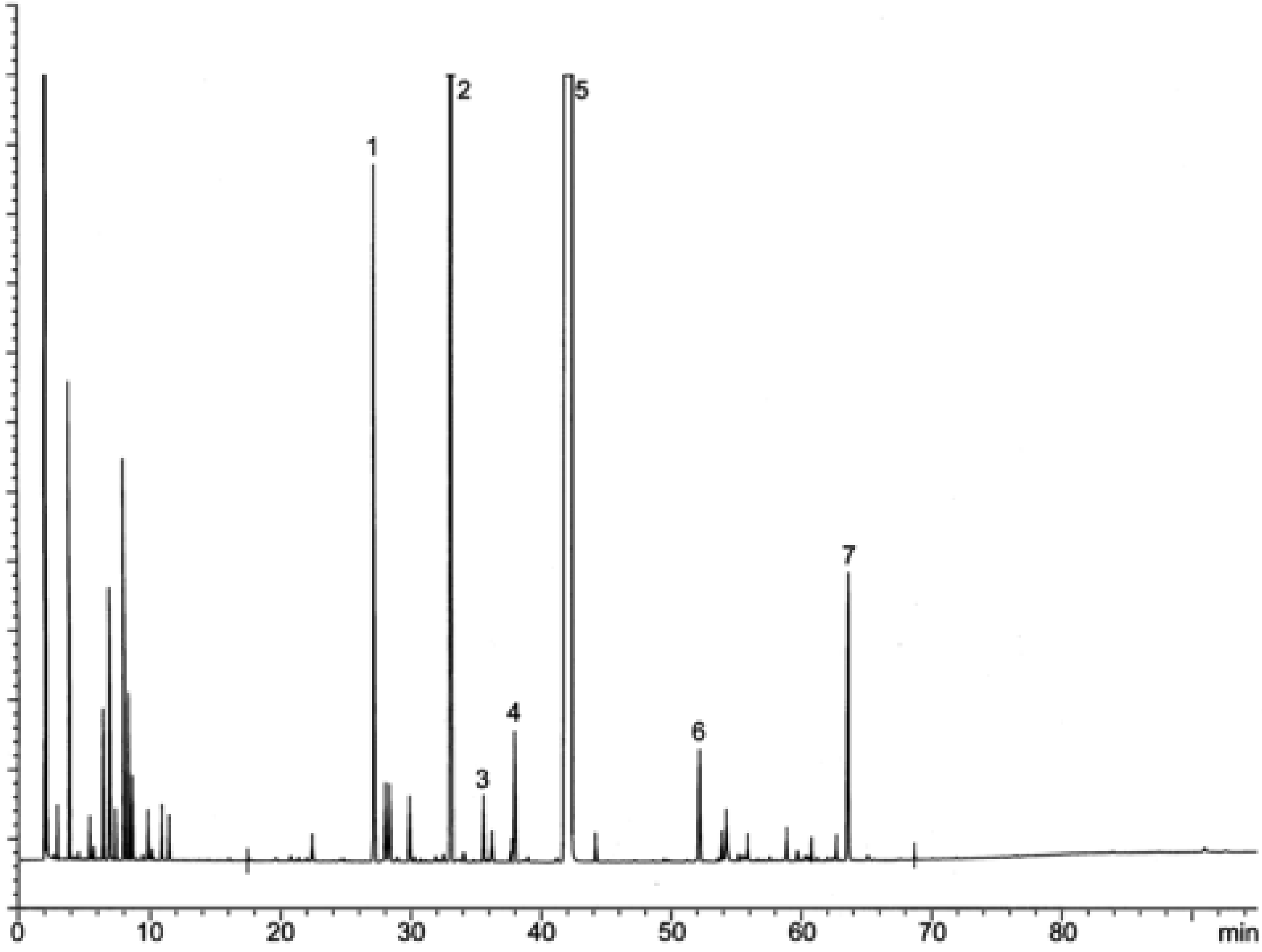
0.979 to 0.985.

Refractive index ***(2.2.6)***

1.553 to 1.556.

Freezing point ***(2.2.18)***

15 °C to 19 °C.



1. linalol
2. estragole
3. α-terpineol
4. *cis*-anethole
5. *trans*-anethole
6. anisaldehyde
7. foeniculin

Figure 2108.-1. – *Chromatogram for the test for chromatographic profile of star anise oil*

Fenchone

Gas chromatography *(2.2.28)* as described in the test for chromatographic profile with the following modifications.

Test solution Dissolve 400 µl of the substance to be examined in 2.0 ml of *hexane R* .

Reference solution (a) Dilute 10 µl of *fenchone R* to 1.2 g with *hexane R* .

Reference solution (b) Dilute 100 µl of reference solution (a) to 100 ml with *hexane R* .

System suitability Reference solution (b):

— *signal-to-noise ratio* : minimum 10 for the principal peak.

Limit:
— *fenchone* : maximum 0.01 per cent.

Pseudoisoeugenyl 2-methylbutyrate

Gas chromatography *(2.2.28)* as described in the test for chromatographic profile with the following modifications.

Test solution The substance to be examined.

Reference solution (a) Dilute 10 mg of the test solution to 1.000 g with *hexane R*. Dilute 0.5 ml of this solution to 100 ml with *hexane R*.

Reference solution (b) *Pseudoisoeugenyl 2-methylbutyrate for peak identification CRS*.

System suitability:

— the chromatogram obtained with reference solution (b) is similar to the chromatogram provided with *pseudoisoeugenyl 2-methylbutyrate for peak identification CRS*.

— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (a).

Limit Locate the peak due to pseudoisoeugenyl 2-methylbutyrate by comparison with the chromatogram provided with *pseudoisoeugenyl 2-methylbutyrate for peak identification CRS*.

— *pseudoisoeugenyl 2-methylbutyrate*: maximum 0.01 per cent.

Fatty oils and resinified essential oils ***(2.8.7)***

It complies with the test for fatty oils and resinified essential oils.

Chromatographic profile

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 200 µl of the substance to be examined in 1.0 ml of *hexane R*.

Reference solution To 1.0 ml of *hexane R*, add 20 µl of *linalol R*, 20 µl of *estragole R*, 20 µl of *α-terpineol R*, 60 µl of *anethole R* and 30 µl of *anisaldehyde R*.

Column:

— *material*: fused silica,

— *size:* *l* = 30 m, Ø = 0.25 mm,

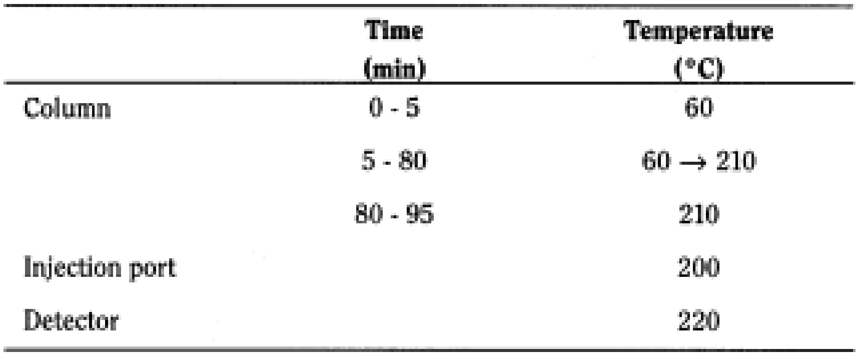
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.0 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	60
	5 - 80	60 → 210
	80 - 95	210
Injection port		200
Detector		220

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of the reference solution; record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to estragole and α-terpineol.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution and locate *cis*-anethole and foeniculin using the chromatogram shown in Figure 2108.-1 (disregard any peak due to hexane).

Determine the percentage content of these components. The percentages are within the following ranges:

— *linalol* : 0.2 per cent to 2.5 per cent,

— *estragole* : 0.5 per cent to 6.0 per cent,

— α-*terpineol* : less than 0.3 per cent,

— *cis-anethole* : 0.1 per cent to 0.5 per cent,

— *trans-anethole* : 86 per cent to 93 per cent,

— *anisaldehyde* : 0.1 per cent to 0.5 per cent,

— *foeniculin*: 0.1 per cent to 3.0 per cent.

STORAGE

In a well-filled, airtight container, protected from light and at a temperature not exceeding 25 °C.

Ph Eur

Star Anise

General Notices

(Ph Eur monograph 1153)

Ph Eur

DEFINITION

Dried composite fruit of *Illicium verum* Hooker fil.

Content:

— minimum 70 ml/kg of essential oil (anhydrous drug),

— minimum 86.0 per cent of *trans*-anethole in the essential oil.

CHARACTERS

The fruit carpels are brown.

Odour of anethole.

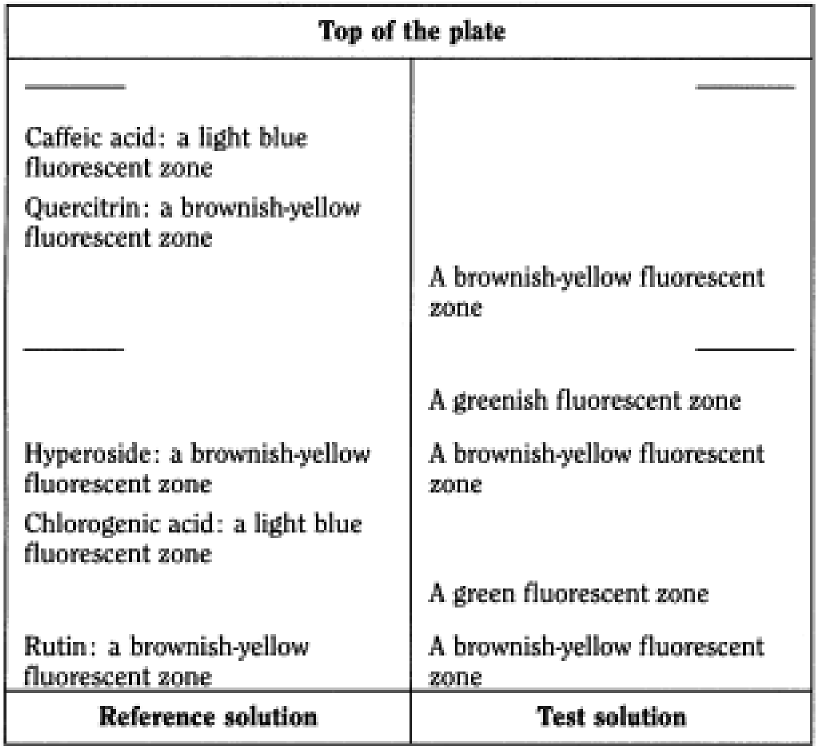
IDENTIFICATION

A. The fruit generally consists of 8 developed, one-seeded follicles, each 12-22 mm long and 6-12 mm high, radially arranged around a short, central, blunt-ending columella. In some fruits 1 or 2 follicles may be missing, but their position is clearly visible. Each follicle is boat-shaped or boot-shaped, with a greyish-brown dorsal surface showing rough markings and lateral surfaces bearing scars from the neighbouring follicles. One or more follicles are split open along the ventral suture, exposing a single, lenticular, shiny, reddish-brown seed about 8 mm in diameter. The markings on the dorsal surface are not visible from the ventral surface. Some of the follicles (1-3) may be imperfectly developed. Isolated follicles, pedicels and seeds may be present.

B. Reduce to a powder (355) *(2.9.12)*. The powder is reddish-brown. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: brown epicarpal cells, polygonal in surface view, with a strongly striated cuticle and occasional anomocytic stomata *(2.8.3)* ; fragments of the endocarp with long palisade-like cells; fragments of the mesocarp with large parenchymatous cells, vessels, oil-containing cells and groups of stone cells; fragments of the seed testa with palisade-like, sclerified, strongly pitted, yellow cells up to 200 µm long; fragments of the columella and the fruit stalk with strongly and irregularly thickened, star-shaped stone cells about 400 µm long and 150 µm wide; rhomboidal or rectangular crystals of calcium oxalate.

C. Examine the chromatograms obtained in test B for *Illicium anisatum* (= *I. religiosum*) and certain other *Illicium* spp.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other weaker zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Caffeic acid: a light blue fluorescent zone	
Quercitrin: a brownish-yellow fluorescent zone	
	A brownish-yellow fluorescent zone
———	———
	A greenish fluorescent zone
Hyperoside: a brownish-yellow fluorescent zone	A brownish-yellow fluorescent zone
Chlorogenic acid: a light blue fluorescent zone	
	A green fluorescent zone
Rutin: a brownish-yellow fluorescent zone	A brownish-yellow fluorescent zone
Reference solution	**Test solution**

TESTS

Illicium anisatum **(=** ***I. religiosum*****) and certain other** ***Illicium*** **spp.**

A. Adulteration with *Illicium anisatum* or certain other *Illicium* spp. is indicated by the presence of fruits mainly consisting of more than 8 follicles; fruits either smaller than 2.5 cm or greater than 3.5 cm; follicles with the suture edged with a thickening extending to the neighbouring follicle, or with dorsal markings visible from the ventral surface; follicles somewhat undulate and ending in a fine beak or a small, ventrally turned hook; follicles with a profile fitting into a rectangle; pedicels more than 5 cm long; seedless fruits; seeds either very flat or almost spherical.

B. Thin-layer chromatography *(2.2.27)* .

Test solution To 2.0 g of the powdered drug (355) *(2.9.12)* add 10 ml of *methanol R* and heat under a reflux condenser in a water-bath at 60 °C for 5 min. Allow to cool and filter.

Reference solution Dissolve 1 mg of *caffeic acid R* , 1 mg of *chlorogenic acid R* , 2.5 mg of *quercitrin R* , 2.5 mg of *rutin R* and 2.5 mg of *hyperoside R* in 10 ml of *methanol R* .

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *anhydrous formic acid R* , *glacial acetic acid R* , *water R* , *ethyl acetate R* (11:11:26:100 *V/V/V/V*).

Application 5 µl as bands.

Development Over a path of 6 cm.

Drying In a current of warm air.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*; after 30 min, examine in ultraviolet light at 365 nm.

Results The chromatogram obtained with the test solution shows no brownish-yellow fluorescent zone at or above the position of the zone due to quercitrin in the chromatogram obtained with the reference solution. No yellow fluorescent zone is seen at or above the position of the zone due to caffeic acid in the chromatogram obtained with the reference solution. No brownish-yellow fluorescent zone is seen directly above the zone due to hyperoside in the chromatogram obtained with the reference solution.

Water *(2.2.13)*

Maximum 100 ml/kg, determined by distillation on 20.0 g of the powdered drug (355) *(2.9.12)*.

Total ash *(2.4.16)*

Maximum 4.0 per cent.

ASSAY

Essential oil

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use a 250 ml round-bottomed flask and 100 ml of *water R* as the distillation liquid. Immediately before the determination, reduce 50.0 g of the drug to a coarse powder (1400) *(2.9.12)* and mix. Further reduce about 10.0 g of this mixture to a finer powder (710) *(2.9.12)*. Use 2.50 g of the powder for the determination. Introduce 0.50 ml of *xylene R* into the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

trans-Anethole

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution Dilute the mixture of essential oil and *xylene R* obtained in the assay of essential oil to 5.0 ml with *xylene R* by rinsing the apparatus.

Reference solution To 1.0 ml of *xylene R* add 20 µl of *estragole R*, 20 mg of α-*terpineol R* and 60 µl of *anethole R*.

Column:

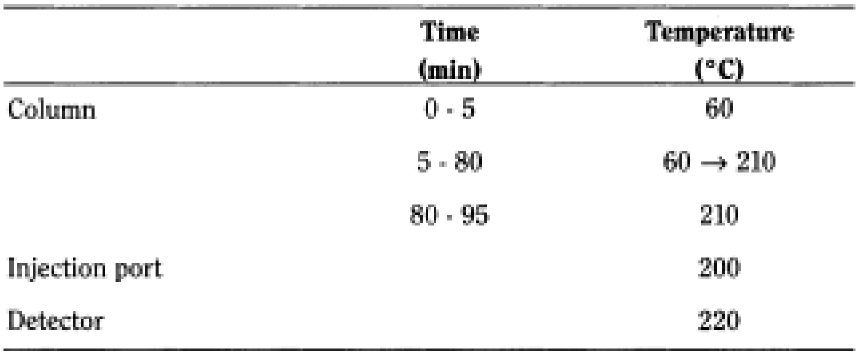
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *macrogol 20 000 R*.

Carrier gas *helium for chromatography R*.

Flow rate 1.0 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 5	60
	5 - 80	60 → 210
	80 - 95	210
Injection port		200
Detector		220

Detection Flame ionisation.

Injection 1 µl.

Elution order Order indicated in the preparation of the reference solution.

System suitability Reference solution:

— *resolution*: minimum 5 between the peaks due to estragole and α-terpineol.

Use the retention times from the chromatogram obtained with the reference solution locate the components of the reference solution in the chromatogram obtained with the test solution.

Calculate the percentage content of *trans*-anethole. Disregard any peak due to the solvent or with an area less than 0.05 per cent of the area of the principal peak in the chromatogram obtained with the test solution.

Ph Eur

Sterculia

General Notices

Sterculia Gum; Karaya Gum

Preparation

Sterculia Granules

When Powdered Sterculia is prescribed or demanded, material complying with the appropriate requirements below and containing not less than 10.0% of volatile acid shall be dispensed or supplied.

DEFINITION

Sterculia is the gum obtained from *Sterculia urens* Roxb. and other species of *Sterculia*.

CHARACTERISTICS

It has the macroscopical and microscopical characters described under Identification tests A and B.

Sparingly soluble in *water*, but swells into a homogeneous, adhesive, gelatinous mass. Practically insoluble in *ethanol (96%)*.

IDENTIFICATION

A. Irregular or vermiform pieces, about 5 to 20 mm thick; greyish white with a brown or pink tinge; surface striated.

B. When powdered and mounted in *ethanol (96%)* it appears as small, transparent, angular particles of various sizes and shapes; the particles lose their sharp edges when *water* is added and each gradually swells until a large, indefinite, almost structureless mass results; when mounted in *ruthenium red solution* the particles are stained red; no blue coloured particles (starch) are visible when mounted in *iodine solution R1*.

C. Add 1 g to 80 ml of *water* and allow to stand for 24 hours, shaking occasionally. A tacky and viscous granular mucilage is produced. Retain the mucilage for use in test D.

D. Boil 4 ml of the mucilage obtained in test C with 0.5 ml of *hydrochloric acid*, add 1 ml of 5M *sodium hydroxide*, filter, add 3 ml of *cupri-tartaric solution R1* to the filtrate and heat. A red precipitate is produced.

E. Warm 0.5 g with 2 ml of 5M *sodium hydroxide*. A brown colour is produced.

TESTS

Acid-insoluble ash

Not more than 1.0%, Appendix XI K.

Foreign matter

Complies with the *test for foreign matter*, Appendix XI D.

Ash

Not more than 7.0%, Appendix XI J.

Volatile acid

Not less than 14.0%, calculated as acetic acid, $C_2H_4O_2$, when determined by the following method. To 1 g contained in a 700 ml Kjeldahl flask add 100 ml of *water* and 5 ml of *orthophosphoric acid*, allow to stand for several hours, or until the gum is completely swollen, and boil gently under a reflux condenser for 2 hours. Steam distil until 800 ml of distillate is obtained and the acid residue measures about 20 ml and titrate the distillate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of alkali required to neutralise the volatile acid. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 6.005 mg of volatile acid, calculated as $C_2H_4O_2$.

Microbial contamination

1.0 g is free from *Escherichia coli*, Appendix XVI B1.

STORAGE

Sterculia should be stored in a dry place.

Stramonium Leaf

General Notices

(Ph Eur monograph 0246)

Preparation

Prepared Stramonium

When Stramonium Leaf or Powdered Stramonium Leaf is prescribed, Prepared Stramonium shall be dispensed.

Ph Eur

DEFINITION

Dried leaf or dried leaf and flowering, and occasionally fruit-bearing, tops of *Datura stramonium* L. and its varieties.

Content

Minimum 0.25 per cent of total alkaloids, expressed as hyoscyamine ($C_{17}H_{23}NO_3$; M_r 289.4) (dried drug). The alkaloids consist mainly of hyoscyamine with varying proportions of hyoscine (scopolamine).

CHARACTERS

Unpleasant odour.

IDENTIFICATION

A. The leaves are dark brownish-green or dark greyish-green, often much twisted and shrunken during drying, thin and brittle, ovate or triangular-ovate, dentately lobed with an acuminate apex and often unequal at the base. Young leaves are pubescent on the veins, older leaves are nearly glabrous. Stems are green or purplish-green, slender, curved and twisted, wrinkled longitudinally and sometimes wrinkled transversely, branched dichasially, with a single flower or an immature fruit in the fork. Flowers, on short pedicels, have a gamosepalous calyx with 5 lobes and trumpet-shaped brownish-white or purplish corolla. The fruit is a capsule, usually covered with numerous short, stiff emergences; seeds are brown or black with a minutely pitted testa.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green. Examine under a microscope using *chloral hydrate solution R.* The powder shows the following diagnostic characters: fragments of leaf lamina showing epidermal cells with slightly wavy anticlinal walls and smooth cuticle; stomata are more frequent on the lower epidermis (anisocytic and anomocytic) *(2.8.3)*; covering trichomes are conical, uniseriate with 3-5 cells and warty walls; glandular trichomes are short and clavate with heads formed by 2-7 cells; dorsiventral mesophyll, with a single layer of palisade cells and a spongy parenchyma containing cluster crystals of calcium oxalate; annularly and spirally thickened vessels. The powdered drug may also show: fibres and reticulately thickened vessels from the stems; subspherical pollen grains about 60-80 µm in diameter with 3 germinal pores and nearly smooth exine; fragments of the corolla with a papillose epidermis; seed fragments containing yellowish-brown, sinuous, thick-walled sclereids of testa; occasional prisms and microsphenoidal crystals of calcium oxalate.

C. Examine the chromatograms obtained in the Chromatography test.

Results The principal zones in the chromatograms obtained with the test solution are similar in position, colour and size to the principal zones in the chromatogram obtained with the same volume of the reference solution.

D. Shake 1 g of the powdered drug (180) *(2.9.12)* with 10 ml of *0.05 M sulphuric acid* for 2 min. Filter and add to the filtrate 1 ml of *concentrated ammonia R* and 5 ml of *water R*. Shake cautiously with 15 ml of *peroxide-free ether R,* avoiding the formation of an emulsion. Separate the ether layer and dry over *anhydrous sodium sulphate R*. Filter and evaporate the ether in a porcelain dish. Add 0.5 ml of *nitric acid R* and evaporate to dryness on a water-bath. Add 10 ml of *acetone R* and, dropwise, a 30 g/l solution of *potassium hydroxide R* in *ethanol (96 per cent) R*. A deep violet colour develops.

TESTS

Chromatography

Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (180) (*2.9.12*) add 10 ml of *0.05 M sulphuric acid,* shake for 15 min and filter. Wash the filter *with 0.05 M sulphuric acid* until 25 ml of filtrate is obtained. To the filtrate add 1 ml of *concentrated ammonia R* and shake with 2 quantities, each of 10 ml, of *peroxide-free ether R*. If necessary, separate by centrifugation. Dry the combined ether layers over *anhydrous sodium sulphate R,* filter and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 ml of *methanol R*.

Reference solution Dissolve 50 mg of *hyoscyamine sulphate R* in 9 ml of *methanol R*. Dissolve 15 mg of *hyoscine hydrobromide R* in 10 ml of *methanol R*. Mix 3.8 ml of the hyoscyamine sulphate solution and 4.2 ml of the hyoscine hydrobromide solution and dilute to 10 ml with *methanol R*.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R, water R, acetone R* (3:7:90 *V/V/V*).

Application 10 µl and 20 µl, as bands of 20 mm by 3 mm, leaving 1 cm between the bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 15 min and allow to cool.

Detection A Spray with *potassium iodobismuthate solution R2*, using about 10 ml for a plate 200 mm square, until the orange or brown zones become visible against a yellow background.

Results A The zones in the chromatograms obtained with the test solution are similar in position (hyoscyamine in the lower third, hyoscine in the upper third of the chromatogram) and colour to those in the chromatograms obtained with the reference solution. The zones in the chromatograms obtained with the test solution are at least equal in size to the corresponding zones in the chromatogram obtained with the same volume of the reference solution. Faint secondary zones may appear, particularly in the middle of the chromatogram obtained with 20 µl of the test solution or near the starting point in the chromatogram obtained with 10 µl of the test solution.

Detection B Spray with *sodium nitrite solution R* until the coating is transparent; examine after 15 min.

Results B The zones due to hyoscyamine in the chromatograms obtained with the test solution and the reference solution change from brown to reddish-brown but not to greyish-blue (atropine) and any secondary zones disappear.

Foreign matter *(2.8.2)*

Maximum 3 per cent stems with a diameter greater than 5 mm.

Total ash *(2.4.16)*

Maximum 20.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 4.0 per cent.

ASSAY

a) Determine the loss on drying *(2.2.32)* on 2.000 g of the powdered drug (180) *(2.9.12)* by drying in an oven at 105 °C.

b) Moisten 10.0 g of the powdered drug (180) *(2.9.12)* with a mixture of 5 ml of *ammonia R*, 10 ml of *ethanol (96 per cent) R* and 30 ml of *peroxide-free ether R* and mix thoroughly. Transfer the mixture to a suitable percolator, if necessary with the aid of the extracting mixture. Allow to macerate for 4 h and percolate with a mixture of 1 volume of *chloroform R* and 3 volumes of *peroxide-free ether R* until the alkaloids are completely extracted. Evaporate to dryness a few millilitres of the liquid flowing from the percolator, dissolve the residue in *0.25 M sulphuric acid* and verify the absence of alkaloids using *potassium tetraiodomercurate solution R*. Concentrate the percolate to about 50 ml by distilling on a water-bath and transfer it to a separating funnel, rinsing with *peroxide-free ether R*. Add a quantity of *peroxide-free ether R* equal to at least 2.1 times the volume of the percolate to produce a liquid of a density well below that of water. Shake the solution with no fewer than 3 quantities, each of 20 ml, of *0.25 M sulphuric acid*, separate the 2 layers by centrifugation if necessary and transfer the acid layers to a second separating funnel. Make the acid layer alkaline with *ammonia R* and shake with 3 quantities, each of 30 ml, of *chloroform R.* Combine the chloroform layers, add 4 g of *anhydrous sodium sulphate R* and allow to stand for 30 min with occasional shaking. Decant the chloroform and wash the anhydrous sodium sulphate with 3 quantities, each of 10 ml, of *chloroform R.* Add the washings to the chloroform extract, evaporate to dryness on a water-bath and heat in an oven at 100-105 °C for 15 min. Dissolve the residue in a few millilitres of *chloroform R*, add 20.0 ml of *0.01 M sulphuric acid* and remove the chloroform by evaporation on a water-bath. Titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red mixed solution R* as indicator.

Calculate the percentage content of total alkaloids, expressed as hyoscyamine, using the following expression:

$$\frac{57.88\,(20-n)}{(100-d)\,m}$$

d = loss on drying, as a percentage

n = volume of *0.02 M sodium hydroxide*, in millilitres

m = mass of drug, in grams

STORAGE

Protected from moisture.

Ph Eur

Strong Ginger Tincture

General Notices

Ginger Essence

DEFINITION

Ginger, in moderately coarse powder	500 g
Ethanol (90 per cent)	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Prepare by *percolation*, Appendix XI F.
The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

TESTS

Ethanol content

80 to 88% v/v, Appendix VIII F, Method III.

Dry residue

2.0 to 3.0% w/v.

Relative density

0.832 to 0.846, Appendix V G.

Sumatra Benzoin Tincture

General Notices

(Ph Eur monograph 1813)

Ph Eur

DEFINITION

Tincture produced from *Sumatra benzoin (1814)*.

Content

Minimum 4.0 per cent *m/m* of total acids, calculated as benzoic acid ($C_7H_6O_2$; M_r 122.1).

PRODUCTION

The tincture is produced from 1 part of the drug and 5 parts of ethanol (75 per cent *V/V* to 96 per cent *V/V*) by a suitable procedure.

CHARACTERS

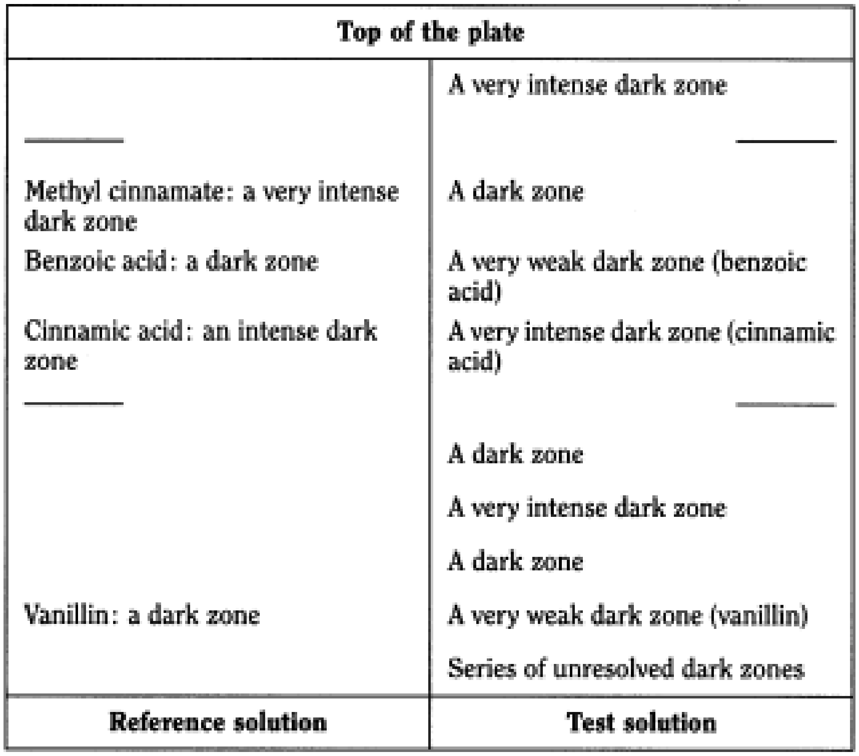
Appearance

Orange-yellow liquid.

IDENTIFICATION

Examine the chromatograms obtained in the test for Siam benzoin tincture.

Results See below the sequence of quenching zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint quenching zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A very intense dark zone
———	———
Methyl cinnamate: a very intense dark zone	A dark zone
Benzoic acid: a dark zone	A very weak dark zone (benzoic acid)
Cinnamic acid: an intense dark zone	A very intense dark zone (cinnamic acid)
———	———
	A dark zone
	A very intense dark zone
	A dark zone
Vanillin: a dark zone	A very weak dark zone (vanillin)
	Series of unresolved dark zones
Reference solution	**Test solution**

TESTS

Siam benzoin tincture

Thin-layer chromatography *(2.2.27)* .

Test solution The tincture to be examined.

Reference solution Dissolve 20 mg of *benzoic acid R* , 10 mg of *trans-cinnamic acid R* , 4 mg of *vanillin R* and 20 mg of *methyl cinnamate R* in 20 ml of ethanol of the same concentration as that used for the production of the tincture.

Plate *TLC silica gel* F_{254} *plate R* (5-40 µm) [or *TLC silica gel* F_{254} *plate R* (2-10 µm)].

Mobile phase *glacial acetic acid R, di-isopropyl ether R, hexane R* (10:40:60 *V/V/V*).

Application 20 µl [or 8 µl] as bands.

Development Over a path of 12 cm [or 6 cm].

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution does not show zones due to benzoic acid and vanillin that are more intense than the corresponding zones in the chromatogram obtained with the reference solution.

Ethanol *(2.9.10)*

95 per cent to 105 per cent of the content stated on the label.

ASSAY

Place 3.50 g in a 250 ml borosilicate glass flask and add 15.0 ml of *0.5 M alcoholic potassium hydroxide* . Boil under a reflux condenser on a water-bath for 30 min. Allow to cool and rinse the condenser with 20 ml of *ethanol (96 per cent) R* . Titrate the excess of potassium hydroxide with *1 M hydrochloric acid* , determining the end-point potentiometrically *(2.2.20)* . Carry out a blank titration.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 61.05 mg of benzoic acid ($C_7H_6O_2$).

Ph Eur

Sumatra Benzoin

General Notices

(Ph Eur monograph 1814)

Benzoin

Preparations

Benzoin Inhalation

Compound Benzoin Tincture

Sumatra Benzoin Tincture

Ph Eur

DEFINITION

Resin obtained by incising the trunk of *Styrax benzoin* Dryander.

Content

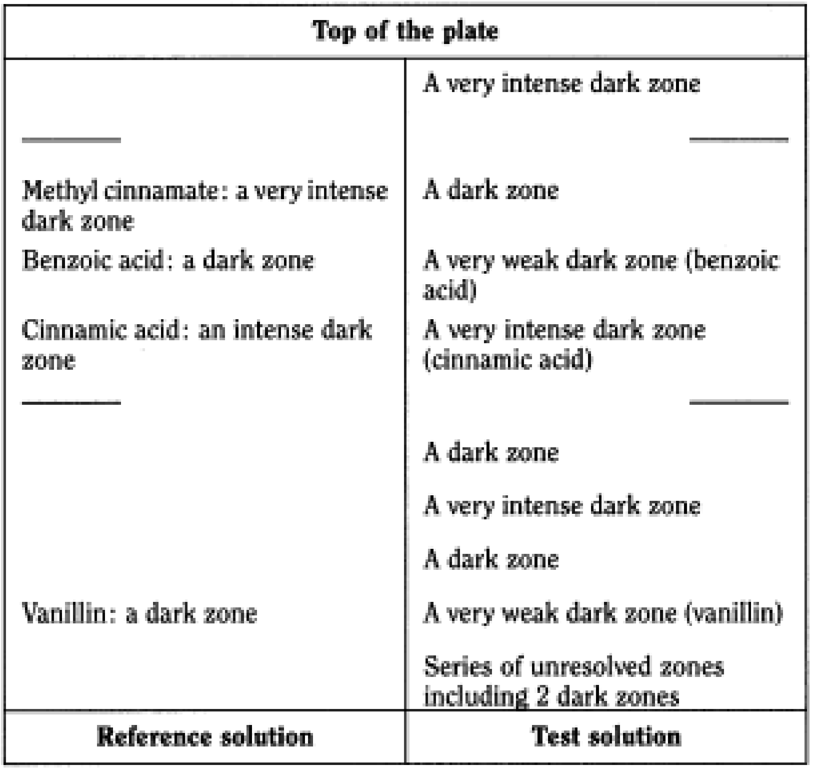
25.0 per cent to 50.0 per cent of total acids, calculated as benzoic acid ($C_7H_6O_2$; M_r 122.1) (dried drug).

IDENTIFICATION

A. Sumatra benzoin occurs as creamy white, rounded to ovoid tears, which may be embedded in a dull greyish-brown or reddish-brown matrix. It is hard and brittle and the fractured surface is dull and uneven.

B. Examine the chromatograms obtained in test B for *Styrax tonkinensis*.

Results See below the sequence of quenching zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint quenching zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A very intense dark zone
———	———
Methyl cinnamate: a very intense dark zone	A dark zone
Benzoic acid: a dark zone	A very weak dark zone (benzoic acid)
Cinnamic acid: an intense dark zone	A very intense dark zone (cinnamic acid)
———	———
	A dark zone
	A very intense dark zone
	A dark zone
Vanillin: a dark zone	A very weak dark zone (vanillin)
	Series of unresolved zones including 2 dark zones
Reference solution	**Test solution**

TESTS

Dammar gum

Thin-layer chromatography *(2.2.27)* .

Test solution Dissolve 0.2 g of the drug to be examined with gentle heating in 10 ml of *ethanol (90 per cent V/V) R* and centrifuge.

Plate *TLC aluminium oxide G plate R* .

Mobile phase *Light petroleum R4, ether R* (40:60 *V/V*).

Application 5 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5 min.

Results The chromatogram obtained does not show any prominent spot with an R_F between 0.4 and 1.0.

Styrax tonkinensis

A. To 0.2 g of the finely powdered drug add 10 ml of *ethanol (96 per cent) R* . Shake vigorously until almost completely dissolved and filter. Place 5 ml of the filtrate in a test-tube and add 0.5 ml of a 50 g/l solution of *ferric chloride R* in *ethanol (96 per cent) R* . A yellowish, slightly green colour is produced.

B. Thin-layer chromatography *(2.2.27)* .

Test solution Sonicate 0.2 g of the finely powdered drug in 5 ml of *ethanol (96 per cent) R* and filter. Collect the filtrate.

Reference solution Dissolve 20 mg of *benzoic acid R*, 10 mg of *trans-cinnamic acid R*, 4 mg of *vanillin R* and 20 mg of *methyl cinnamate R* in 10 ml of *ethanol (96 per cent) R*.

Plate *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F_{254} plate R* (2-10 µm)].

Mobile phase *glacial acetic acid R*, *di-isopropyl ether R*, *hexane R* (10:40:60 *V/V/V*).

Application 10 µl [or 2 µl] as bands.

Development Over a path of 12 cm [or 5 cm].

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution shows 2 faint zones in the same positions as the dark zones due to benzoic acid and vanillin in the chromatogram obtained with the reference solution.

Matter insoluble in ethanol

Maximum 20.0 per cent.

To 2.0 g of the powdered drug add 25 ml of *ethanol (90 per cent V/V) R*. Boil until almost completely dissolved. Filter through a tared sintered-glass filter (16) *(2.1.2)* and wash with 3 quantities, each of 5 ml, of boiling *ethanol (90 per cent V/V) R*. Heat the glass filter and its contents in an oven at 100-105 °C for 2 h. Allow to cool and weigh.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 2.000 g of the coarsely powdered drug by drying *in vacuo* for 4 h.

Total ash *(2.4.16)*

Maximum 2.0 per cent.

ASSAY

Place 0.750 g of the finely powdered drug in a 250 ml borosilicate glass flask and add 15.0 ml of *0.5 M alcoholic potassium hydroxide*. Boil under a reflux condenser on a water-bath for 30 min. Allow to cool and rinse the condenser with 20 ml of *ethanol (96 per cent) R*. Titrate the excess of potassium hydroxide with *0.5 M hydrochloric acid*, determining the end-point potentiometrically *(2.2.20)*. Carry out a blank titration.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 61.05 mg of benzoic acid ($C_7H_6O_2$).

Ph Eur

Sweet Fennel

General Notices

(Ph Eur monograph 0825)

Ph Eur

DEFINITION

Dry cremocarps and mericarps of *Foeniculum vulgare* Miller sp. *vulgare* var. *dulce* (Miller) Thellung.

Content:

— *essential oil*: minimum 20 ml/kg (anhydrous drug);

— *anethole* : minimum 80.0 per cent in the essential oil.

CHARACTERS

Sweet fennel is pale green or pale yellowish-brown.

IDENTIFICATION

A. The fruit of sweet fennel is a cremocarp of almost cylindrical shape with a rounded base and a narrowed summit crowned with a large stylopod. It is generally 3-12 mm long and 3-4 mm wide. The mericarps, usually free, are glabrous. Each bears 5 prominent, slightly carenated ridges. When cut transversely, 4 vittae on the dorsal surface and 2 on the commissural surface may be seen with a lens.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-brown or greyish-yellow. Examine under a microscope using *chloral hydrate solution R* . The powder shows the following diagnostic characters: yellow fragments of wide secretory canals, often made up of yellowish-brown-walled polygonal secretory cells, frequently associated with a layer of thin-walled transversely elongated cells 2-9 µm wide, having a parquetry arrangement; reticulate parenchyma of the mesocarp; numerous fibre bundles from the ridges, often accompanied by narrow spiral vessels; very numerous endosperm fragments containing aleurone grains and very small calcium oxalate microrosette crystals, as well as some fibre bundles from the carpophore.

C. Thin-layer chromatography *(2.2.27)* .

Test solution Shake 0.3 g of the freshly powdered drug (1400) *(2.9.12)* with 5.0 ml of *methylene chloride R* for 15 min. Filter and carefully evaporate the filtrate to dryness on a water-bath at 60 °C. Dissolve the residue in 0.5 ml of *toluene R* .

Reference solution Dissolve 60 µl of *anethole R* in 5.0 ml of *hexane R* .

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *hexane R* , *toluene R* (20:80 *V/V*).

Application 10 µl, as bands of 20 mm by 3 mm.

Development Over a path of 10 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The chromatograms show in the central part a quenching zone due to anethole.

Detection B Spray with *sulphuric acid R* and heat at 140 °C for 5 min; examine in daylight.

Results B The chromatograms show in the central part a violet band due to anethole; the chromatogram obtained with the test solution also shows a reddish-brown zone in the upper third (terpenes).

TESTS

Estragole and fenchone

Gas chromatography *(2.2.28)* : use the normalisation procedure.

Test solution Dilute the mixture of essential oil and *xylene R* obtained in the assay of essential oil to 5.0 ml with *xylene R* , by rinsing the apparatus.

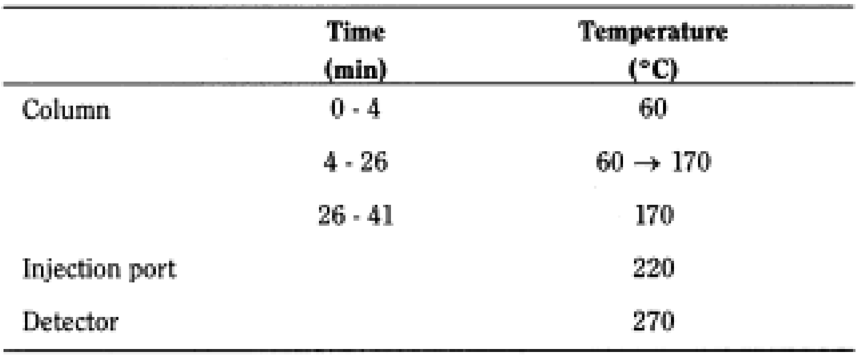
Reference solution Dissolve 5 mg of *estragole R* and 5 mg of *fenchone R* in 0.5 ml of *xylene R* .

Column:
— *size*: *l* = 30-60 m, Ø = 0.3 mm;
— *stationary phase*: *macrogol 20 000 R* .

Carrier gas *nitrogen for chromatography R.*

Flow rate 0.40 ml/min.

Split ratio 1:200.



	Time (min)	Temperature (°C)
Column	0 - 4	60
	4 - 26	60 → 170
	26 - 41	170
Injection port		220
Detector		270

Detection Flame ionisation.

Injection 1 µl.

Limits:

- *estragole* : maximum 10.0 per cent in the essential oil;
- *fenchone* : maximum 7.5 per cent in the essential oil.

Foreign matter ***(2.8.2)***

Maximum 1.5 per cent of peduncles and maximum 1.5 per cent of other foreign matter.

Water ***(2.2.13)***

Maximum 80 ml/kg, determined on 20.0 g of the powdered drug (710) *(2.9.12)*.

Total ash ***(2.4.16)***

Maximum 10.0 per cent.

ASSAY

Essential oil

Carry out the determination of essential oils in herbal drugs *(2.8.12)* . Use a 500 ml round-bottomed flask and 200 ml of *water R* as the distillation liquid. Reduce the drug to a coarse powder (1400) *(2.9.12)* and immediately use 10.0 g for the determination. Introduce 0.50 ml of *xylene R* into the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

Anethole

Gas chromatography *(2.2.28)* as described in the test for estragole and fenchone with the following modification.

Reference solution Dissolve 5 mg of *anethole R* in 0.5 ml of *xylene R* .

STORAGE

Protected from moisture.

Ph Eur

Sweet Orange Oil

General Notices

(Ph Eur monograph 1811)

Ph Eur

DEFINITION

Essential oil obtained without heating, by suitable mechanical treatment from the fresh peel of the fruit of *Citrus sinensis* (L.) Osbeck (*Citrus aurantium* L. var. *dulcis* L.). A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, pale yellow to orange, mobile liquid, which may become cloudy when chilled.

It has a characteristic odour of fresh orange peel.

IDENTIFICATION

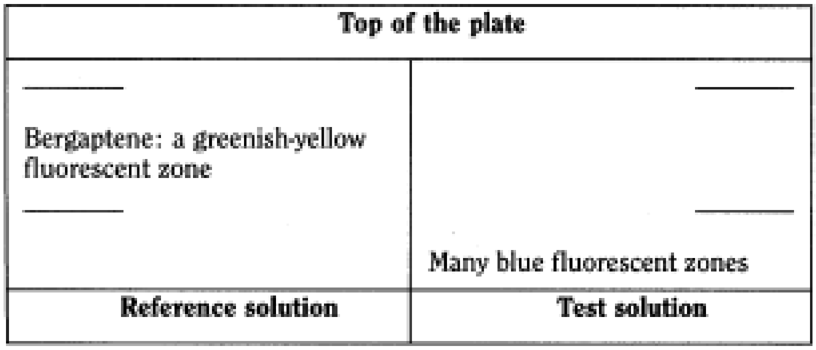
First identification *B.*

Second identification *A.*

A. Thin-layer chromatography (*2.2.27*).

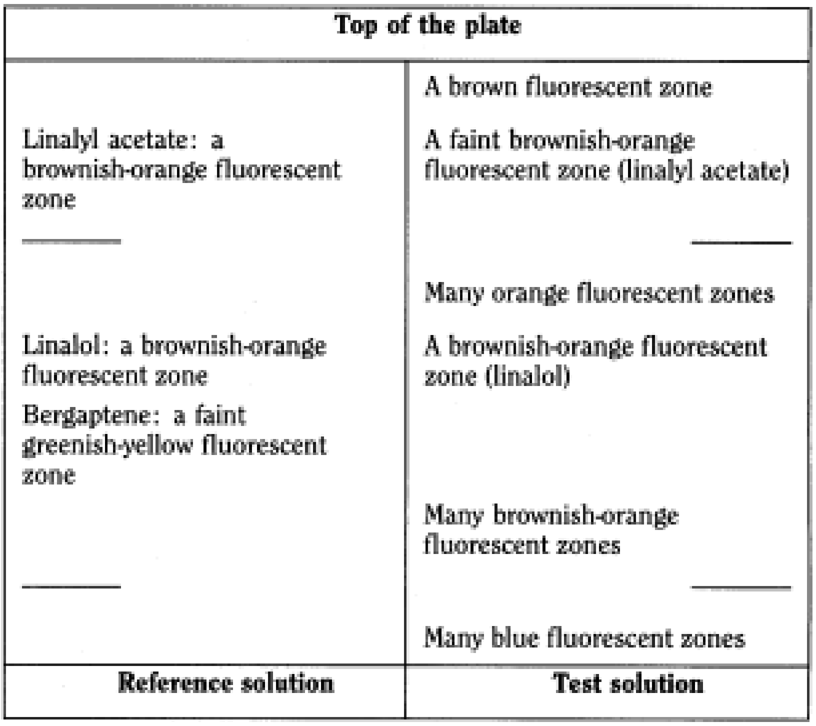
Examine the chromatograms obtained in the test for bergapten.

Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
_______	_______
Bergaptene: a greenish-yellow fluorescent zone	
_______	_______
	Many blue fluorescent zones
Reference solution	**Test solution**

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
	A brown fluorescent zone
Linalyl acetate: a brownish-orange fluorescent zone	A faint brownish-orange fluorescent zone (linalyl acetate)
_______	_______
	Many orange fluorescent zones
Linalol: a brownish-orange fluorescent zone	A brownish-orange fluorescent zone (linalol)
Bergaptene: a faint greenish-yellow fluorescent zone	
	Many brownish-orange fluorescent zones
_______	_______
	Many blue fluorescent zones
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density (*2.2.5*)

0.842 to 0.850.

Refractive index (*2.2.6*)

1.470 to 1.476.

Optical rotation (*2.2.7*)

+ 94 ° to + 99 °.

Peroxide value (*2.5.5, Method B*)

Maximum 20.

Fatty oils and resinified essential oils (*2.8.7*)

It complies with the test for fatty oils and resinified essential oils.

Bergaptene

Thin-layer chromatography (*2.2.27*).

Test solution Dilute 0.2 ml of the substance to be examined in 1 ml of *alcohol R*.

Reference solution Dissolve 2 mg of *bergaptene R*, 10 µl *of linalol R* and 20 µl of *linalyl acetate R* in 10 ml of *alcohol R*.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R*, *toluene R* (15:85 *V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 365 nm.

Results A The chromatogram obtained with the test solution shows no greenish-yellow fluorescent zone present in the chromatogram obtained with the reference solution.

Detection B Spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min; examine the plate in ultraviolet light at 365 nm.

Chromatographic profile

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dilute 300 µl of the substance to be examined to 1 ml with *acetone R*.

Reference solution (a) Dilute 10 µl of α-*pinene R*, 10 µl of β-*pinene R*, 10 µl of *sabinene R*, 20 µl of β-*myrcene R*, 800 µl of *limonene R*, 10 µl of octanal, 10 µl of *decanal R*, 10 µl of *linalol R*, 10 µl of *citral R* (composed of neral and geranial) and 10 µl of *valencene R* in 1 ml of *acetone R*.

Reference solution (b) Dissolve 5 µl of β-*pinene R* in 10 ml of *acetone R*. Dilute 0.5 ml to 10 ml with *acetone R*.

Column:

— *material*: fused silica;

— *size: l* = 30 m, Ø = 0.53 mm;

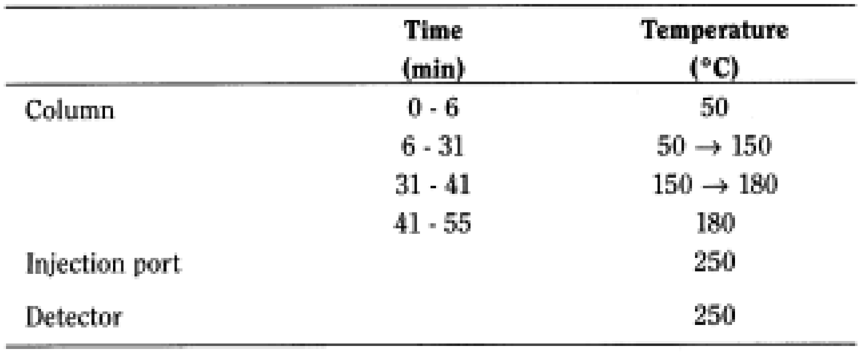
— *stationary phase*: *macrogol 20 000 R* (film thickness 1 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 6	50
	6 - 31	50 → 150
	31 - 41	150 → 180
	41 - 55	180
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 0.5 µl.

Elution order Order indicated in the composition of reference solution (a). Record the retention times of these substances.

System suitability Reference solution (a)

- *resolution*: minimum 3.9 between the peaks due to β-pinene and sabinene and minimum 1.5 between the peaks due to valencene and geranial.

Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution.

Determine the percentage content of these components. The limits are within the following ranges:

- α-*pinene*: 0.4 per cent to 0.6 per cent;
- β-*pinene*: 0.02 per cent to 0.3 per cent;
- *sabinene*: 0.2 per cent to 1.1 per cent;
- β-*myrcene*: 1.7 per cent to 2.5 per cent;
- *limonene*: 92.0 per cent to 97.0 per cent;
- *octanal*: 0.1 per cent to 0.4 per cent;
- *decanal*: 0.1 per cent to 0.4 per cent;
- *linalol*: 0.2 per cent to 0.7 per cent;
- *neral*: 0.02 per cent to 0.10 per cent;
- *valencene*: 0.02 per cent to 0.5 per cent;
- *geranial*: 0.03 per cent to 0.20 per cent.

Disregard limit: area of the peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Residue on evaporation

1.0 per cent to 5.0 per cent.

Evaporate 5.0 g to dryness on a water-bath and dry at 100-105 °C for 4 h.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Tea Tree Oil

General Notices

Melaleuca Oil

(Ph Eur monograph 1837)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the foliage and terminal branchlets of *Melaleuca alternifolia* (Maiden and Betch) Cheel, *M. linariifolia* Smith, *M. dissitiflora* F. Mueller and/or other species of *Melaleuca*.

CHARACTERS

Appearance

Clear, mobile, colourless to pale yellow liquid with a characteristic odour.

IDENTIFICATION

First identification *B.*

Second identification *A.*

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.1 ml of the substance to be examined in 5 ml of *heptane R.*

Reference solution Dissolve 30 µl of *cineole R*, 60 µl of *terpinen-4-ol R* and 10 mg of α-*terpineol R* in 10 ml of *heptane R*.

Plate *TLC silica gel plate R*.

Mobile phase *ethyl acetate R, heptane R* (20:80 *V/V*).

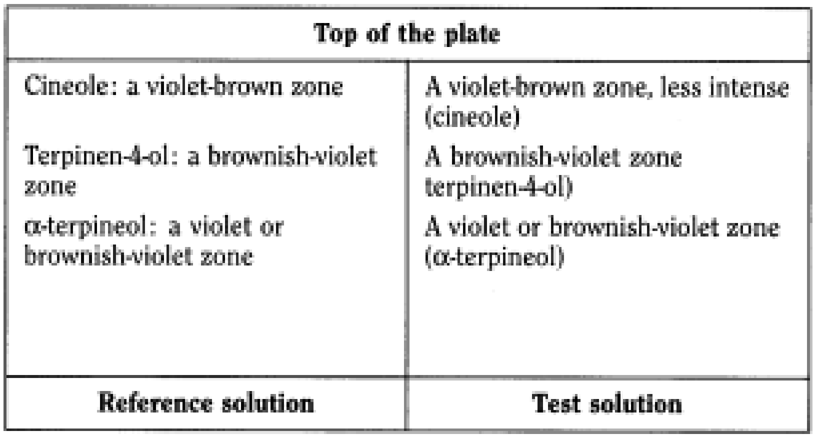
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R*. Heat at 100-105 °C for 5-10 min while observing. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the chromatogram obtained with the test solution.



Top of the plate	
Cineole: a violet-brown zone	A violet-brown zone, less intense (cineole)
Terpinen-4-ol: a brownish-violet zone	A brownish-violet zone terpinen-4-ol)
α-terpineol: a violet or brownish-violet zone	A violet or brownish-violet zone (α-terpineol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

0.885 to 0.906.

Refractive index *(2.2.6)*

1.475 to 1.482.

Optical rotation *(2.2.7)*

+ 5° to + 15°.

Chromatographic profile

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Dissolve 0.15 ml of the substance to be examined in 10 ml of *hexane R*.

Reference solution Dissolve 5 µl of α-*pinene R,* 5 µl of *sabinene R*, 15 µl of α-*terpinene R,* 5 µl of *limonene R*, 5 µl of *cineole R,* 30 µl of γ-*terpinene R*, 5 µl of p-cymene R, 5 µl of *terpinolene R*, 60 µl of *terpinen-4-ol R*, 5 µl of *aromadendrene R* and 5 mg of α-*terpineol R* in 10 ml of *hexane R*.

Column:

— *material*: fused silica;

— *size*: *l* = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.25-0.53 mm;

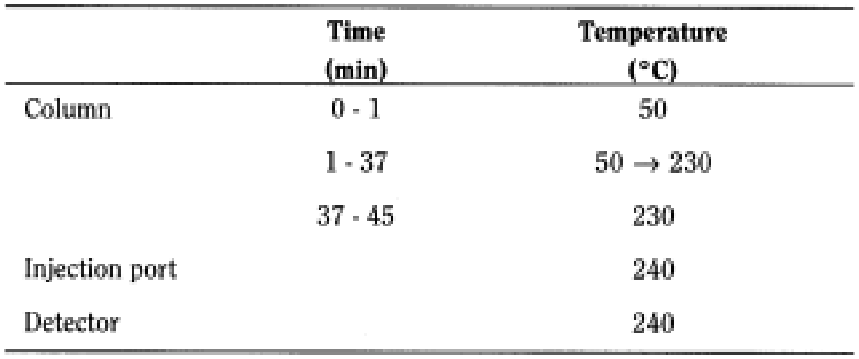
— *stationary phase: macrogol 20 000 R*.

Carrier gas *helium for chromatography R*.

Flow rate 1.3 ml/min.

Split ratio 1:50.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 1	50
	1 - 37	50 → 230
	37 - 45	230
Injection port		240
Detector		240

Detection Flame ionisation.

Injection 1 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 2.7 between the peaks due to terpinen-4-ol and aromadendrene.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution. Disregard the peak due to hexane.

Determine the percentage content of these components. The percentages are within the following ranges:

— α-*pinene*: 1.0 per cent to 6.0 per cent;

— *sabinene*: less than 3.5 per cent;

— α-*terpinene*: 5.0 per cent to 13.0 per cent;

— *limonene*: 0.5 per cent to 4.0 per cent;

— *cineole*: less than 15.0 per cent;

— γ-*terpinene*: 10.0 per cent to 28.0 per cent;

— *p-cymene*: 0.5 per cent to 12.0 per cent;

— *terpinolene*: 1.5 per cent to 5.0 per cent;

— *terpinen-4-ol* : minimum 30.0 per cent;

— *aromadendrene*: less than 7.0 per cent;

— α-*terpineol* : 1.5 per cent to 8.0 per cent.

STORAGE

In an airtight, well-filled container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Terminalia Arjuna Stem Bark for use in THMP

General Notices

DEFINITION

Terminalia Arjuna Stem Bark for use in THMP consists of cut dried bark of the stems of *Terminalia arjuna* W. and A. It contains not less than 6% of tannins, expressed as pyrogallol, calculated with reference to the dried drug.

IDENTIFICATION

A. Irregularly flattened or slightly curved or recurved pieces, up to about 8 cm long, 4 cm wide and 1 cm thick; outer surface uneven, dark brown or sometimes mottled greyish-brown, smooth or, more frequently, irregularly striated longitudinally with occasional transverse ridges; inner surface pink to reddish brown with longitudinal striations and occasional paler brown patches. Fracture short and starchy in the inner part, the outer part frequently laminated.

B. Reduce to a powder (355). The powder is reddish-brown. Examine under a microscope using *chloral hydrate solution*. The powder shows a variety of parenchymatous cells, some thin-walled, square or round and others yellowish, polygonal and thick-walled. Rectangular or polygonal, pitted, thin-walled, light brown, lightly lignified cells from the cork layer or outer areas of the cortex are present.

Fibres occur singly and in small or large groups, individual cells narrowing to highly pointed ends, and possibly showing wavy invaginations of the walls where surrounding cells have become detached; degree of lignification varies; walls are yellowish brown, some pitted, others not. Single fibres may be complete, but those in groups are usually fragmented, individual cells being straight or noticeably curved in places. Rounded cells of the medullary rays, which are one cell wide, intersperse the fibres.

Small, calcium oxalate cluster crystals occur scattered throughout as well as being found within parenchymatous cells, some forming a crystal sheath alongside the fibres. Other crystals are very large and less well defined, and usually free.

Examine under a microscope using 50% v/v of *glycerol*. Starch granules are frequent, but not abundant, mainly free, but some in parenchymatous cells. They are small, simple, round, oval or irregular in shape, and occasionally in 2 to 3 compound granules, without visible hila. More or less frequent scattered lumps of brown pigment may be found sometimes in parenchymatous cells.

C. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Shake 1.0 g of the powdered drug with 10 ml of *absolute ethanol*, centrifuge at 3000 rpm for 5 minutes and filter (Whatman GF/C is suitable).

(2) 0.01% w/v each of *arjunolic acid* and *gallic acid* in *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating high performance silica gel (Merck silica gel 60 HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply as bands 8 µl of each solution.

(d) Develop the plate to 8 cm.

(e) Remove the plate and allow it to dry in air for 5 minutes. Spray the plate with *anisaldehyde solution*, heat at 100° to 105° for 5 minutes and examine in daylight.

MOBILE PHASE

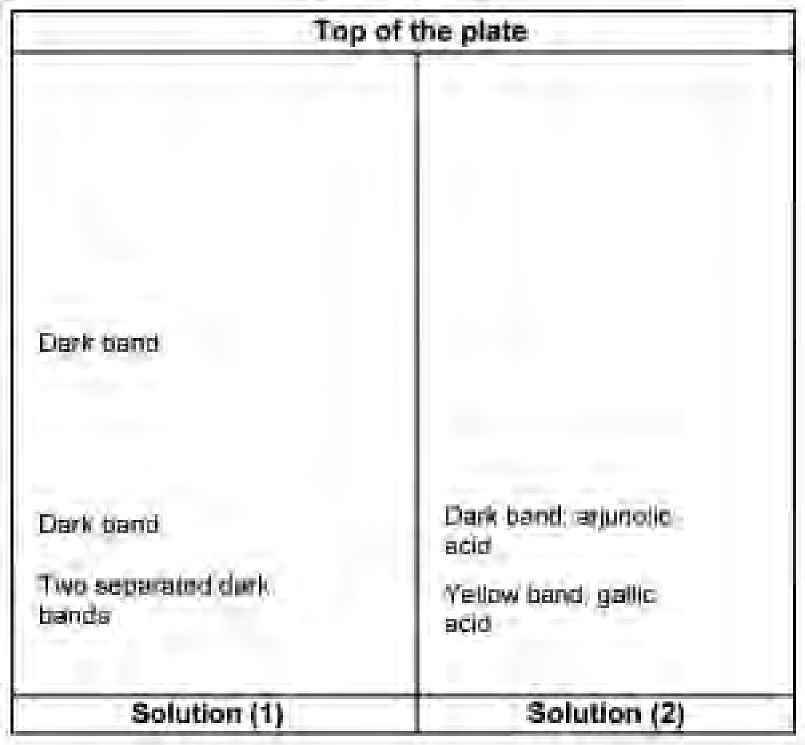
A mixture of 15 volumes of *ethyl acetate,* 15 volumes of *formic acid* and 70 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated bands.

CONFIRMATION

The chromatogram obtained with solution (1) shows a band with an Rf value of approximately 0.30 corresponding in colour and position to the band obtained for arjunolic acid in solution (2) ; two clearly separated dark bands with an Rf value of approximately 0.2; a band with an Rf value of 0.63 is present. Other bands may be present.



Top of the plate	
Dark band	
Dark band	Dark band: arjunolic acid
Two separated dark bands	Yellow band, gallic acid
Solution (1)	**Solution (2)**

TESTS

Ash

Not more than 25%, Appendix XI J.

Loss on drying

When dried for 2 hours at 100° to 105°, loses not more than 10% of its weight. Use 1 g.

Water soluble extractive

Not less than 20%, Appendix XI B2.

ASSAY

Carry out the determination of *tannins in herbal drugs*, Appendix XI M. Use 1.0 g of powdered drug.

Terminalia Belerica Fruit for use in THMP

General Notices

DEFINITION

Terminalia Belerica Fruit for use in THMP consists of pericarp of dried ripe fruits of *Terminalia belerica* Roxb. It contains not less than 10% of tannins, expressed as pyrogallol, calculated with reference to the dried drug.

IDENTIFICATION

A. The dried fruits are spherical to subspherical, about 3 to 5 cm in diameter, slightly depressed at the upper end and more or less tapering to the scar of the pedicel at the lower end. The surface is brown to yellowish brown with a grey velvety sheen, irregularly wrinkled and sometimes with faint, incomplete, longitudinal ridges. Cut transversely, the fruit shows the pericarp about 4 to 5 mm thick enclosing a very hard, yellowish-white seed.

B. Reduce to a powder (355). The powder is light-brown. Examine under a microscope using *chloral hydrate solution*. The powder shows many free, unicellular, straight or slightly bent trichomes from the epicarp. A variety of thick–walled, heavily pitted, lignified fibro-sclereids of elongated and spherical shapes occur in large and small groups; occasional medium sized reticulate vessels; heavily pitted, lignified parenchymatous cells and others with reticulate thickenings; many lignified, pitted, thin walled sclereids and groups of fragmented, thick-walled, pitted, fibres are present. Rarely oil globules, starch granules, and calcium oxalate crystals may be found in the parenchymatous cells from the embryo. Examine under a microscope using 50% v/v of *glycerol*. The powder shows minute, either single or 2 to 4 compound starch granules, some scattered, but mainly filling parenchymatous cells. Some have slit or stellate hila; simple granules are often not perfectly spherical. Larger calcium oxalate crystals, with fewer small ones, are scattered or in parenchymatous cells.

C. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Add 10 ml of *absolute ethanol* to 1.0 g of the powdered drug, centrifuge at 3000 rpm for 5 minutes and filter (Whatman GF/C is suitable).

(2) 0.01% w/v each of *arjunolic acid*, *gallic acid* and *ellagic acid* in *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating high performance silica gel F_{254} (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply as bands 8 µl of each solution.

(d) Develop the plate to 8 cm.

(e) Remove the plate and allow it to dry in air for 5 minutes. Examine under *ultraviolet light (254 nm)*. Spray the plate with *anisaldehyde solution*, heat at 100° to 105° for 5 minutes and examine in daylight.

MOBILE PHASE

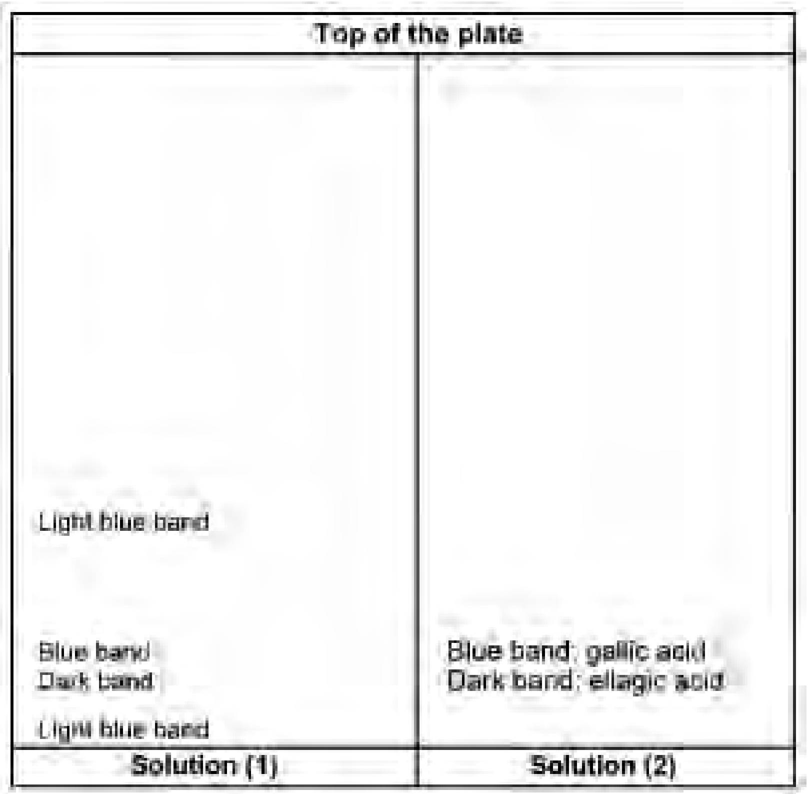
A mixture of 15 volumes of *ethyl acetate,* 15 volumes of *formic acid* and 70 volumes of *toluene*.

SYSTEM SUITABILITY

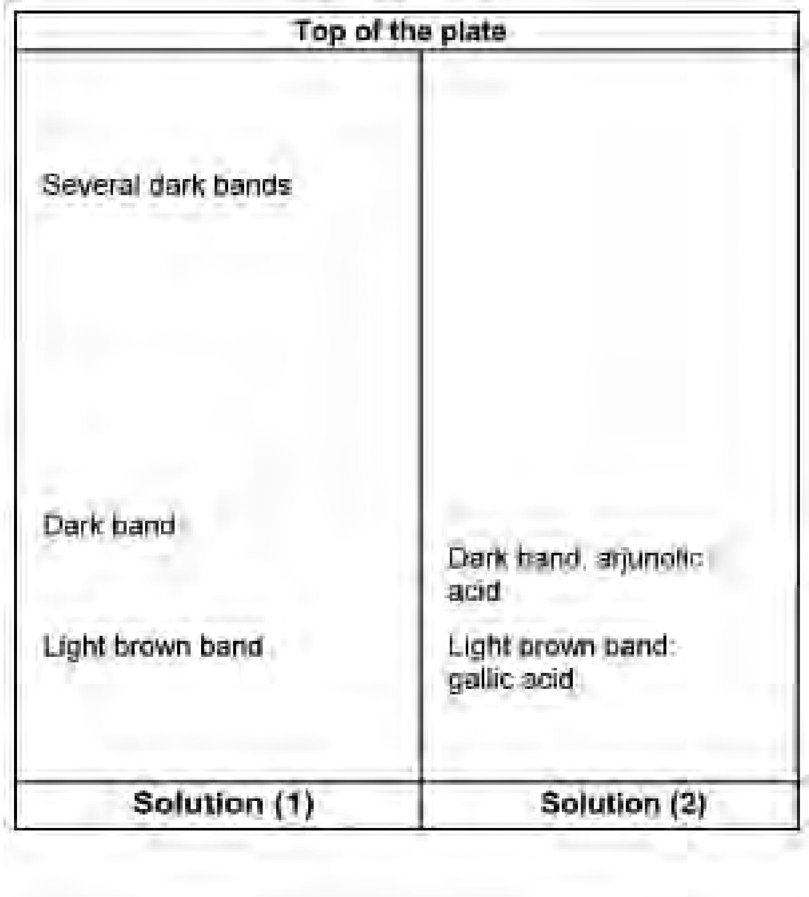
The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated bands under both *ultraviolet light (254 nm)* and daylight.

CONFIRMATION

Under *ultraviolet light (254 nm)* the chromatogram obtained with solution (1) shows bands with Rf values of approximately 0.11 and 0.15 corresponding in colour and position to the bands obtained with ellagic acid and gallic acid in solution (2) and light blue bands with Rf values of approximately 0.04 and 0.36. Other bands may be present.


Top of the plate
Light blue band
Blue band
Dark band
Light blue band
Blue band; gallic acid
Dark band; ellagic acid
Solution (1)
Solution (2)

Under daylight after spraying with *anisaldehyde solution* the chromatogram obtained with solution (1) shows a band with an Rf value of approximately 0.15 corresponding in colour and position to the band obtained gallic acid in solution (2); a dark band with an Rf value of 0.36; several dark bands in the upper part of the plate.



Top of the plate	
Several dark bands	
Dark band	
	Dark band, arjunolic acid
Light brown band	Light brown band: gallic acid
Solution (1)	**Solution (2)**

TESTS

Ash

Not more than 7%, Appendix XI J.

Loss on drying

When dried for 2 hours at 100° to 105°, loses not more than 5.0% of its weight. Use 1 g.

Water soluble extractive

Not less than 45%, Appendix XI B2.

ASSAY

Carry out the determination of *tannins in herbal drugs*, Appendix XI M. Use 1.0 g of powdered drug.

Terminalia Chebula Fruit for use in THMP

General Notices

DEFINITION

Terminalia Chebula Fruit for use in THMP consists of pericarp of mature fruits of *Terminalia chebula* Retz. It contains not less than 20% of tannins, expressed as pyrogallol, calculated with reference to the dried drug.

IDENTIFICATION

A. The dried fruits are sub-globular to ovoid, 3 to 4 cm long and 1.5 to 2 cm wide, bluntly pointed at the tip and tapering towards the base. The surface is yellowish to greenish, sometimes brown, shiny and more or less wrinkled and has distinct longitudinal ridges. Cut transversely, the fruit shows the pericarp about 3 to 4 mm thick, non-adherent to the very hard, creamy-white seed.

B. Reduce to a powder (355). The powder is yellowish brown. Examine under a microscope using *chloral hydrate solution*. The powder shows round, oval or elongated, thin-walled parenchymatous cells in groups. Occasional narrow-walled, unpitted, lightly lignified fibres occur in small or larger groups, some forming wave-like arrangements. Large groups of fragmented, heavily lignified and pitted fibres also occur. A variety of thick–walled, heavily pitted, lignified fibro-sclereids of elongated, rectangular, and irregular shapes occur in large and small groups. Fewer pitted, lignified parenchyma, or thinner-walled, less lignified and pitted sclereids, with broad lumens, are also found. There are very occasional small, spiral, lignified vessel fragments. Small, greenish, thick-walled polygonal epicarp cells are seen in surface view. Parenchymatous cells with reticulate thickenings across the surface are rare, as are others without such thickenings, but containing oil globules. Examine under a microscope using 50% v/v of *glycerol*. The powder shows minute, either single or 2 to 4 compound granules, some scattered, but mainly filling parenchymatous cells. Some have slit or stellate hila; simple granules are often not perfectly spherical. Larger calcium oxalate crystals, with fewer small ones, are scattered or in parenchymatous cells.

C. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) To 1.0 g of the powdered drug, add 10 ml of *absolute ethanol*, centrifuge at 3000 rpm for 5 minutes and filter (Whatman GF/C is suitable).

(2) 0.01% w/v each of *arjunolic acid*, *gallic acid* and *ellagic acid* in *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating high performance silica gel F_{254} (Merck silica gel 60 F_{254} HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply as bands 8 µl of each solution.

(d) Develop the plate to 8 cm.

(e) Remove the plate and allow it to dry in air for 5 minutes. Examine under *ultraviolet light*

(254 nm). Spray the plate with *anisaldehyde solution*, heat at 100° to 105° for 5 minutes and examine in daylight.

MOBILE PHASE

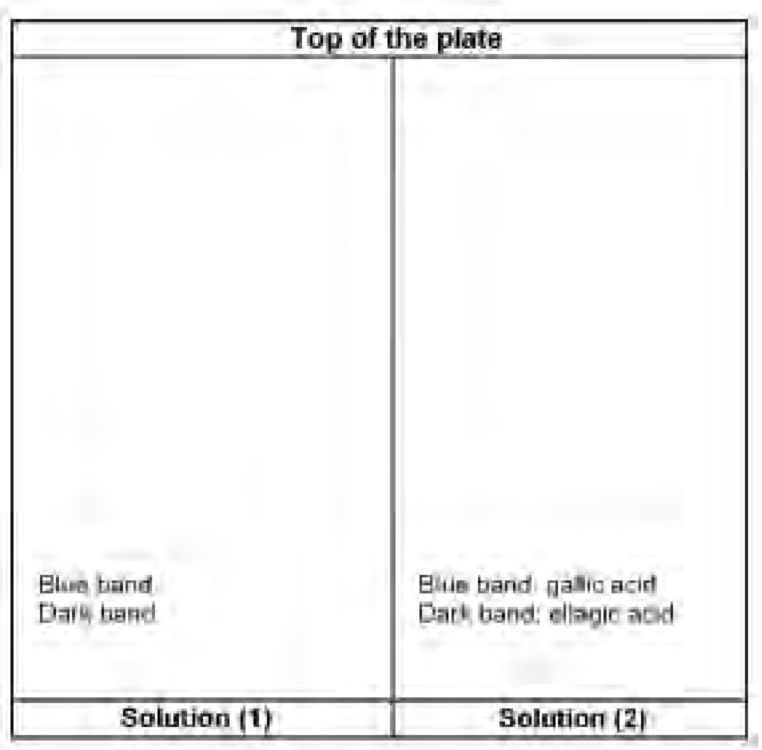
A mixture of 15 volumes of *ethyl acetate,* 15 volumes of *formic acid* and 70 volumes of *toluene*.

SYSTEM SUITABILITY

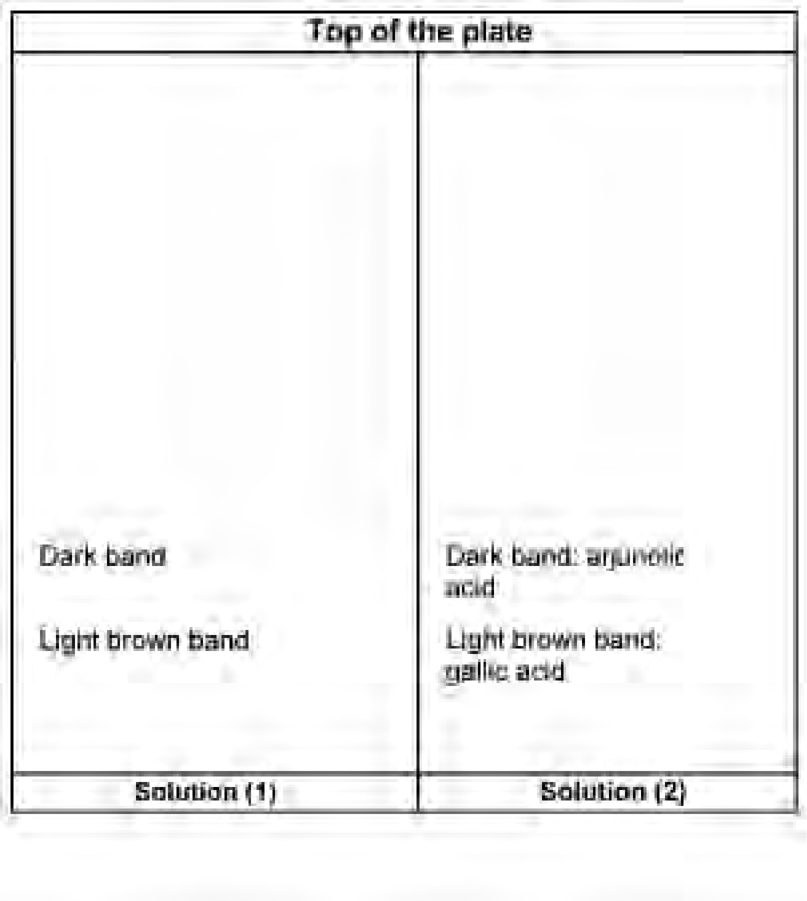
The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated bands under both *ultraviolet light (254 nm)* and daylight.

VALIDITY

When examined under *ultraviolet light (254 nm)* the chromatogram obtained with solution (1) shows bands with Rf values of approximately 0.11 and 0.15 corresponding in colour and position to the bands obtained with gallic acid and ellagic acid in solution (2).



When examined under daylight after spraying with *anisaldehyde solution* the chromatogram obtained with solution (1) shows a band with an Rf value of approximately 0.15 corresponding in colour and position to the band obtained for gallic acid in solution (2) and a dark band with an Rf value of approximately 0.30. There may be some faint brown bands with Rf values of approximately 0.40 and 0.70.


Top of the plate
Dark band
Light brown band
Dark band: arjunolic acid
Light brown band: gallic acid
Solution (1)
Solution (2)

TESTS

Ash

Not more than 5%, Appendix XI J.

Loss on drying

When dried for 2 hours at 100° to 105°, loses not more than 10% of its weight. Use 1 g.

Water soluble extractive

Not less than 50%, Appendix XI B2.

ASSAY

Carry out the determination of *tannins in herbal drugs*, Appendix XI M. Use 1.0 g of powdered drug.

Terpeneless Lemon Oil

General Notices

Preparations

Lemon Spirit

Compound Orange Spirit

DEFINITION

Terpeneless Lemon Oil may be prepared by concentrating Lemon Oil under reduced pressure until most of the terpenes have been removed or by solvent partition. It contains not less than 40% w/w of aldehydes calculated as citral, $C_{10}H_{16}O$.

CHARACTERISTICS

A clear, colourless or pale yellow liquid, visibly free from water; odour and taste, those of lemon.

TESTS

Optical rotation

–5° to +2°, Appendix V F.

Refractive index

1.475 to 1.485, Appendix V E.

Solubility in ethanol

Soluble, at 20°, in 1 volume of *ethanol (80%)*, Appendix X M.

Weight per ml

0.880 to 0.895 g, Appendix V G.

ASSAY

Carry out the method for *determination of aldehydes*, Appendix X K, using 1 g, omitting the *toluene* and using a volume, not less than 7 ml, of *alcoholic hydroxylamine solution* that exceeds by 1 to 2 ml the volume of 0.5M *potassium hydroxide in ethanol (60%) VS* required. Each ml of 0.5M *potassium hydroxide in ethanol (60%) VS* is equivalent to 76.73 mg of $C_{10}H_{16}O$.

STORAGE

Terpeneless Lemon Oil should be kept in a well-filled container and protected from light.

Terpeneless Orange Oil

General Notices

Preparation

Compound Orange Spirit

DEFINITION

Terpeneless Orange Oil may be prepared by concentrating orange oil under reduced pressure until most of the terpenes have been removed or by solvent partition.

CHARACTERISTICS

A clear, yellow or orange-yellow liquid, visibly free from water.

TESTS

Optical rotation

Not more than +60°, Appendix V F.

Refractive index

1.461 to 1.473, Appendix V E.

Solubility in ethanol

Soluble, at 20°, in 1 part of *ethanol (90%)*, Appendix X M.

Weight per ml

0.855 to 0.880 g, Appendix V G.

Content of aldehydes

Not less than 18% w/w, calculated as decanal, $C_{10}H_{20}O$. Carry out the method for the *determination of aldehydes*, Appendix X K, using 1.5 g, omitting the *toluene* and using a volume, not less than 7 ml, of *alcoholic hydroxylamine solution* that exceeds by 1 to 2 ml the volume of 0.5M *potassium hydroxide in ethanol (60%) VS* required. Each ml of 0.5M *potassium hydroxide in ethanol (60%) VS* is equivalent to 78.76 mg of $C_{10}H_{20}O$.

STORAGE

Terpeneless Orange Oil should be kept in a well-filled container and protected from light.

Three-lobed Sage Leaf

General Notices

(Ph Eur monograph 1561)

Ph Eur

DEFINITION

Whole or cut, dried leaves of *Salvia fructicosa* Mill. (*S. triloba* L. fil).

Content

Minimum 18 ml/kg of essential oil in the whole drug (anhydrous drug) and minimum 12 ml/kg of essential oil in the cut drug (anhydrous drug).

CHARACTERS

Spicy odour when ground, similar to eucalyptus oil.

IDENTIFICATION

A. The lamina of the whole three-lobed sage leaf is about 8-50 mm long and about 4-20 mm wide, and oblong-ovate or lanceolate. The margin is finely crenate and undulate but indistinct owing to the dense hairy covering on both surfaces. The base is obtuse and sometimes bears 1 or 2 more or less developed lobes. The upper surface is grey-tomentose pubescent, the lower surface is densely white-tomentose pubescent; the venation is indistinct. The densely white-tomentose pubescent petiole is about 1 mm in diameter.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green and tomentose. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: very numerous, whole or fragmented, covering and glandular trichomes, scattered and attached to fragments of the epidermises; covering trichomes articulated, uniseriate, thick-walled and bluntly tapering, those on the upper epidermis straight, those on the lower epidermis longer, tortuous and more densely packed; glandular trichomes, some with a unicellular or bicellular head and a stalk consisting of from 1-4 cells, the majority having a short, unicellular stalk and a head composed of 8 radiating cells with a raised common cuticle; the upper epidermis with pitted and beaded cells, somewhat polygonal, with a few diacytic stomata *(2.8.3)*; the lower epidermis with sinuous or wavy-walled cells and numerous diacytic stomata.

C. Examine the chromatogram obtained in the test for thujone.

Results The chromatogram obtained with the test solution shows a blue zone due to cineole, equal or greater in size and intensity to the zone in the chromatogram obtained with the reference solution. Further zones are present.

TESTS

Thujone

Thin-layer chromatography *(2.2.27)*.

Test solution Shake 0.3 g of the freshly powdered drug (355) *(2.9.12)* with 5.0 ml of *anhydrous ethanol R* for 5 min.

Reference solution Dissolve 20 µl of *thujone R* and 25 µl of *cineole R* in 20 ml of *anhydrous ethanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *Ethyl acetate R, toluene R* (5:95 *V/V*).

Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 200 g/l solution of *phosphomolybdic acid R* in *anhydrous ethanol R* and heat at 100-105 °C for 10 min. Examine in daylight.

Results The chromatogram obtained with the reference solution shows in the middle part a blue zone (cineole) and in the upper part a pink-blue zone (thujone). The chromatogram obtained with the test solution shows no zone or a very faint pink-blue zone due to thujone.

Foreign matter ***(2.8.2)***

Maximum 8 per cent of stems and maximum 2 per cent of other foreign matter.

Water ***(2.2.13)***

Maximum 100 ml/kg, determined on 20.0 g.

Total ash ***(2.4.16)***

Maximum 10.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use 20.0 g of drug, if necessary cut immediately before the assay, a 500 ml flask, 250 ml of *water R* as the distillation liquid. Add 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

Ph Eur

Thyme Oil

General Notices

(Ph Eur monograph 1374)

Ph Eur

DEFINITION

Essential oil obtained by steam distillation from the fresh flowering aerial parts of *Thymus vulgaris* L., *T. zygis* Loefl. ex L. or a mixture of both species.

CHARACTERS

Appearance

Clear, yellow or very dark reddish-brown, mobile liquid with a characteristic, aromatic, spicy odour, reminiscent of thymol.

Solubility

Miscible with ethanol and with light petroleum.

IDENTIFICATION

First identification B.

Second identification A.

A. Thin-layer chromatography *(2.2.27)*.

Test solution Dissolve 0.2 g of the substance to be examined in *pentane R* and dilute to 10 ml with the same solvent.

Reference solution Dissolve 0.15 g of *thymol R*, 25 mg of α-*terpineol R*, 40 µl of *linalol R* and 10 µl of *carvacrol R* in *pentane R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase ethyl acetate R, *toluene R* (5:95 *V/V*).

Application 20 µl, as bands.

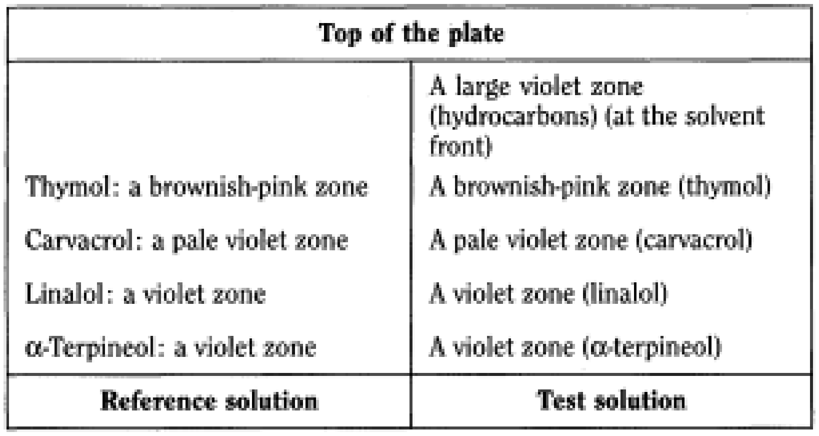
Development Over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat the plate at 100-105 °C for 5-10 min while observing. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other bands may be present in the

chromatogram obtained with the test solution.



Top of the plate	
	A large violet zone (hydrocarbons) (at the solvent front)
Thymol: a brownish-pink zone	A brownish-pink zone (thymol)
Carvacrol: a pale violet zone	A pale violet zone (carvacrol)
Linalol: a violet zone	A violet zone (linalol)
α-Terpineol: a violet zone	A violet zone (α-terpineol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density *(2.2.5)*

0.915 to 0.935.

Refractive index *(2.2.6)*

1.490 to 1.505.

Chromatographic profile

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution The substance to be examined.

Reference solution Dissolve 0.15 g of β-*myrcene R*, 0.1 g of γ-*terpinene R*, 0.1 g of *p-cymene R*, 0.1 g of *linalol R*, 0.2 g of *terpinen-4-ol R*, 0.2 g of *thymol R* and 50 mg of *carvacrol R* in 5 ml of *hexane R.*

Column:

— *material*: fused silica;

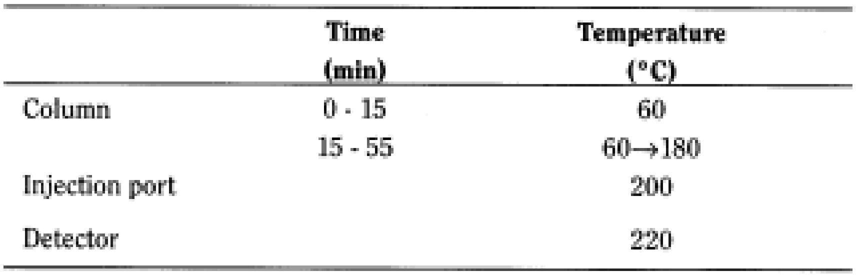
— *size*: *l* = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.25-0.53 mm;

— *stationary phase: macrogol 20 000 R*.

Carrier gas *helium for chromatography R*.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 15	60
	15 - 55	60→180
Injection port		200
Detector		220

Detection Flame ionisation.

Injection 0.2 µl.

Elution orde Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to thymol and carvacrol;

— *number of theoretical plates*: minimum 30 000, calculated for the peak due to p-cymene at 80 °C.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution on the chromatogram obtained with the test solution. Disregard the peak due to hexane.

Determine the percentage content of these components. The limits are within the following ranges:

— β-*myrcene*: 1.0 per cent to 3.0 per cent;

— γ-*terpinene*: 5.0 per cent to 10.0 per cent;

— *p-cymene*: 15.0 per cent to 28.0 per cent;

— *linalol*: 4.0 per cent to 6.5 per cent;

— *terpinen-4-ol*: 0.2 per cent to 2.5 per cent;

— *thymol*: 36.0 per cent to 55.0 per cent;

— *carvacrol*: 1.0 per cent to 4.0 per cent.

STORAGE

In a well-filled, airtight container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Thyme

General Notices

(Ph Eur monograph 0865)

Ph Eur

DEFINITION

Whole leaves and flowers separated from the previously dried stems of *Thymus vulgaris* L. or *Thymus zygis* L. or a mixture of both species.

Content:

— *essential oil*: minimum 12 ml/kg (anhydrous drug);

— *sum of the contents of thymol and carvacrol* (both $C_{10}H_{14}O$; M_r 150.2): minimum 40 per cent in the essential oil.

CHARACTERS

Strong aromatic odour reminiscent of thymol.

IDENTIFICATION

A. The leaf of *Thymus vulgaris* is usually 4 mm to 12 mm long and up to 3 mm wide, sessile or with a very short petiole. The lamina is tough, entire, lanceolate to ovate, covered on both surfaces by a grey or greenish-grey indumentum; the edges are markedly rolled up towards the abaxial surface. The midrib is depressed on the adaxial surface and is very prominent on the abaxial surface. The calyx is green, often with violet spots and is tubular; at the end are 2 lips of which the upper one is bent back and at the end has 3 lobes, the lower is longer and has 2 hairy teeth. After flowering, the calyx tube is closed by a crown of long, stiff hairs. The corolla, about twice as long as the calyx, is usually brownish in the dry state and is slightly bilabiate.

The leaf of *Thymus zygis* is usually 1.7 mm to 6.5 mm long and 0.4 mm to 1.2 mm wide; it is acicular to linear-lanceolate and the edges are markedly rolled towards the abaxial surface. Both surfaces of the lamina are green to greenish-grey and the midrib is sometimes violet; the edges, in particular at the base, have long, white hairs. The dried flowers are very similar to those of *Thymus vulgaris*.

B. Reduce to a powder (355) *(2.9.12)*. The powder of both species is greyish-green or greenish-brown. Examine under a microscope using *chloral hydrate solution R.* The epidermises of the leaves have cells with anticlinal walls which are sinuous and beaded and the stomata are diacytic *(2.8.3)*; numerous secretory trichomes made up of 12 secretory cells, the cuticle of which is generally raised by the secretion to form a globular to ovoid bladder-like covering; the glandular trichomes have a unicellular stalk and a globular to ovoid head; the covering trichomes of the adaxial surface are common to both species; they have warty walls and are shaped as pointed teeth; the warty covering trichomes of the abaxial surface are of many types: unicellular, straight or slightly curved, and bicellular or tricellular, and often elbow-shaped (*Thymus vulgaris*); bicellular or tricellular, more or less straight (*Thymus zygis*). Fragments of calyx are covered by numerous, uniseriate trichomes with 5 or 6 cells and with a weakly striated cuticle. Fragments of the corolla have numerous uniseriate covering trichomes, often collapsed, and secretory trichomes generally with 12 cells. Pollen grains are relatively rare, spherical and smooth with 6 germinal slit-like pores, measuring about 35 µm in diameter. The powder of *Thymus zygis* also contains numerous thick bundles of fibres from the main veins and from fragments of stems.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 5 ml of *methylene chloride R* and shake for 3 min, filter through about 2 g of *anhydrous sodium sulphate R*.

Reference solution Dissolve 5 mg of *thymol R* and 10 µl of *carvacrol R* in 10 ml of *methylene chloride R.*

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methylene chloride R*.

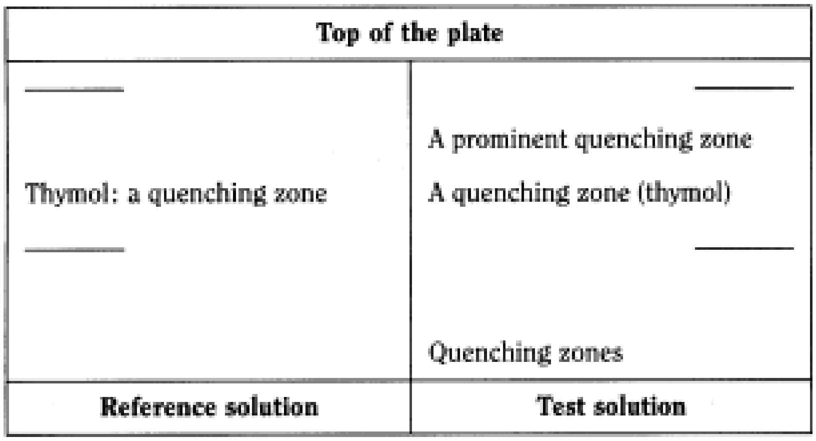
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

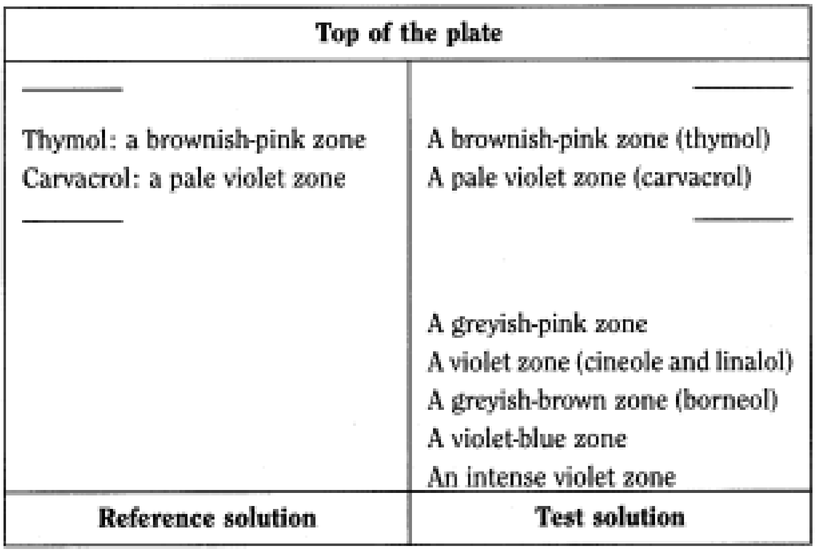
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
_______	_______
	A prominent quenching zone
Thymol: a quenching zone	A quenching zone (thymol)
_______	_______
	Quenching zones
Reference solution	**Test solution**

Detection B Spray with *anisaldehyde solution R* using 10 ml for a plate 200 mm square and heat at 100-105 °C for 10 min.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the lower third of the chromatogram obtained with the test solution. The intensity of the zones due to thymol and carvacrol depends upon the species examined.



Top of the plate	
———	———
Thymol: a brownish-pink zone	A brownish-pink zone (thymol)
Carvacrol: a pale violet zone	A pale violet zone (carvacrol)
———	———
	A greyish-pink zone
	A violet zone (cineole and linalol)
	A greyish-brown zone (borneol)
	A violet-blue zone
	An intense violet zone
Reference solution	**Test solution**

D. Examine the chromatograms obtained in the assay for thymol and carvacrol.

Results The characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Foreign matter *(2.8.2)*

Maximum 10 per cent of stems and maximum 2 per cent of other foreign matter. Stems must not be more than 1 mm in diameter and 15 mm in length. Leaves with long trichomes at their base and with weakly pubescent other parts (*Thymus serpyllum* L.) are absent.

Water *(2.2.13)*

Maximum 100 ml/kg, determined on 20.0 g of the powdered drug (355) *(2.9.12)*.

Total ash *(2.4.16)*

Maximum 15.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

ASSAY

Essential oil *(2.8.12)*

Use 30.0 g of the drug, a 1000 ml round-bottomed flash and 400 ml of *water R* as the distillation liquid. Distil at a rate of 2-3 ml/min for 2 h without *xylene R* in the graduated tube.

Thymol and carvacrol

Gas chromatography *(2.2.28)*: use the normalisation procedure.

Test solution Filter the essential oil obtained in the determination of essential oil over a small amount of *anhydrous sodium sulphate R* and dilute to 5.0 ml with *hexane R* by rinsing the apparatus and the anhydrous sodium sulphate.

Reference solution Dissolve 0.20 g of *thymol R* and 50 mg of *carvacrol R* in *hexane R* and dilute to 5.0 ml with the same solvent.

Column:

— *material*: fused silica;

— *size: l* = 30-60 m, Ø = 0.25 mm;

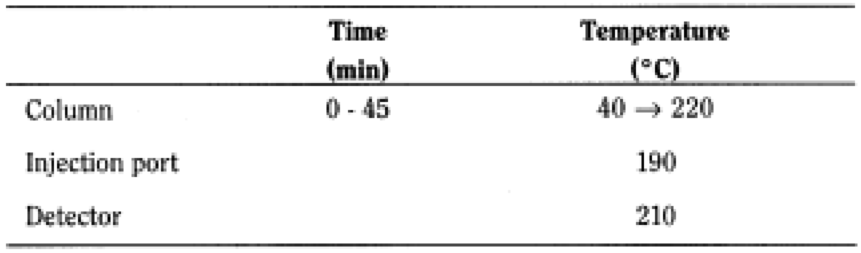
— *stationary phase: macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *nitrogen for chromatography R* or *helium for chromatography R*.

Flow rate 1-2 ml/min.

Split ratio 1:100.

Temperature:



	Time (min)	Temperature (°C)
Column	0 - 45	40 → 220
Injection port		190
Detector		210

Detection Flame ionisation.

Injection 0.2 µl.

Elution order Order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to thymol and carvacrol.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

Determine the percentage content of thymol and carvacrol. Disregard the peak due to hexane.

Ph Eur

Tinctures of the British Pharmacopoeia

General Notices

In addition to the requirements for Tinctures of the European Pharmacopoeia (stated under Extracts), the following statements apply to those tinctures that are the subject of an individual monograph in the British Pharmacopoeia.

DEFINITION

Certain preparations of the British Pharmacopoeia entitled Tinctures do not conform strictly to the definition of the European Pharmacopoeia and consequently application of some of the above requirements is inappropriate. Any necessary exceptions are stated in the relevant individual monographs.

Tinnevelly Senna Fruit

General Notices

(Tinnevelly Senna Pods, Ph Eur monograph 0208)

Preparations

Senna Liquid Extract

Senna Tablets

When Powdered Tinnevelly Senna Fruit is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried fruit of *Cassia angustifolia* Vahl.

Content

Minimum 2.2 per cent of hydroxyanthracene glycosides, expressed as sennoside B ($C_{42}H_{38}O_{20}$; M_r 863) (dried drug).

CHARACTERS

Slight odour.

IDENTIFICATION

A. Flattened, slightly reniform pods, yellowish-brown or brown with dark brown patches at the positions corresponding to the seeds, usually 35-60 mm long and 14-18 mm wide. At one end is a stylar point and at the other a short stalk. The pods contain 5-8 flattened and obovate seeds, green or pale brown, with incomplete, wavy, transverse ridges on the testa.

B. Reduce to a powder (355) *(2.9.12)*. The powder is brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: epicarp with polygonal cells and a small number of conical warty trichomes and occasional anomocytic or paracytic stomata *(2.8.3)*; fibres in 2 crossed layers accompanied by a crystal sheath of calcium oxalate prisms; characteristic palisade cells in the seed and stratified cells in the endosperm; clusters and prisms of calcium oxalate.

C. Thin-layer chromatography *(2.2.27).*

Test solution To 0.5 g of the powdered drug (180) *(2.9.12)* add 5 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* and heat to boiling. Centrifuge and use the supernatant liquid.

Reference solution Dissolve 10 mg of *senna extract CRS* in 1 ml of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* (a slight residue remains).

Plate *TLC silica gel G plate R*.

Mobile phase *Glacial acetic acid R*, *water R*, *ethyl acetate R*, *propanol R* (1:30:40:40 *V/V/V/V*).

Application 10 µl, as bands of 20 mm by 2 mm.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 20 per cent *V/V* solution of *nitric acid R* and heat at 120 °C for 10 min; allow to cool and spray with a 50 g/l solution of *potassium hydroxide R* in *ethanol (50 per cent V/V) R* until the zones appear.

Results The principal zones in the chromatogram obtained with the test solution are similar in position (sennosides B, A, D and C in the order of increasing R_F value), colour and size to the principal zones in the chromatogram obtained with the reference solution. Between the zones due to sennosides D and C a red zone due to rhein-8-glucoside may be visible. The zones due to sennosides D and C are faint in the chromatogram obtained with the test solution.

D. Place about 25 mg of the powdered drug (180) (*2.9.12*) in a conical flask and add 50 ml of *water R* and 2 ml of *hydrochloric acid R*. Heat in a water-bath for 15 min, cool and shake with 40 ml of *ether R*. Separate the ether layer, dry over *anhydrous sodium sulphate R*, evaporate 5 ml to dryness and to the cooled residue add 5 ml of *dilute ammonia R1*. A yellow or orange colour develops. Heat on a water-bath for 2 min. A reddish-violet colour develops.

TESTS

Foreign matter *(2.8.2)*

Maximum 1 per cent.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 9.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 2.0 per cent.

ASSAY

Carry out the assay protected from bright light.

Place 0.150 g of the powdered drug (180) *(2.9.12)* in a 100 ml flask. Add 30.0 ml of *water R*, mix, weigh and place in a water-bath. Heat under a reflux condenser for 15 min. Allow to cool, weigh and adjust to the original mass with *water R*. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150 ml separating funnel. Add 0.1 ml of *dilute hydrochloric acid R* and shake with 3 quantities, each of 15 ml, of *chloroform R*. Allow to separate and discard the chloroform layer. Add 0.10 g of *sodium hydrogen carbonate R* and shake for 3 min. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100 ml round-bottomed flask with a ground-glass neck. Add 20 ml of *ferric chloride solution R1* and mix. Heat for 20 min in a water-bath under a reflux condenser, with the water level above that of the liquid in the flask; add 1 ml of *hydrochloric acid R* and heat for a further 20 min, with frequent shaking, to dissolve the precipitate. Cool, transfer the mixture to a separating funnel and shake with 3 quantities, each of 25 ml, of *ether R* previously used to rinse the flask. Combine the 3 ether layers and wash with 2 quantities, each of 15 ml, of *water R*. Transfer the ether layer to a volumetric flask and dilute to 100.0 ml with *ether R*. Carefully evaporate 10.0 ml to dryness and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate R* in *methanol R*. Measure the absorbance *(2.2.25)* at 515 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of hydroxyanthracene glycosides, expressed as sennoside B, using the following expression:

$$\frac{A \times 1.25}{m}$$

taking the specific absorbance of sennoside B to be 240.

A = absorbance at 515 nm;

m = mass of the substance to be examined, in grams.

STORAGE

Protected from moisture.

Ph Eur

Tolu Balsam

General Notices

(Ph Eur monograph 1596)

Ph Eur

DEFINITION

Oleo-resin obtained from the trunk of *Myroxylon balsamum* (L.) Harms var. *balsamum*.

Content

25.0 per cent to 50.0 per cent of free or combined acids, expressed as cinnamic acid ($C_9H_8O_2$; M_r 148.2) (dried drug).

CHARACTERS

Appearance

Hard, friable, brownish to reddish-brown mass; thin fragments are brownish-yellow when examined against the light.

Reminiscent odour of vanillin.

Solubility

Practically insoluble in water, very soluble to freely soluble in alcohol, practically insoluble in light petroleum.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)* .

Test solution Stir 0.40 g of the fragmented drug with 10 ml of *methylene chloride R* for 5 min and filter.

Reference solution Dissolve 50 mg of *benzyl cinnamate R* in *methylene chloride R,* add 50 µl of *benzyl benzoate R* and dilute to 10 ml with *methylene chloride R* .

Plate *TLC silica gel G plate R* .

Mobile phase *Light petroleum R, toluene R* (5:95 *V/V*).

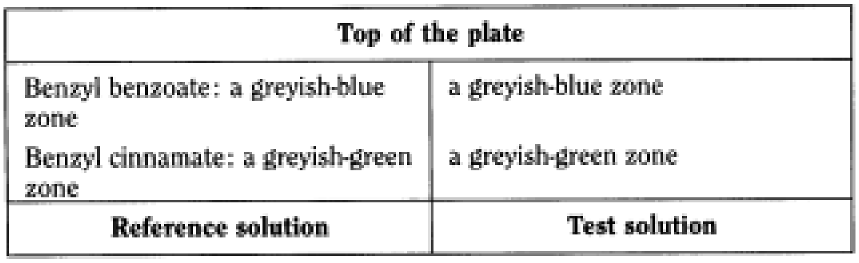
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *vanillin reagent R* and heat at 100-105 °C for 5 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the test and reference solutions. Furthermore, other coloured zones are present in the chromatogram obtained with the test solution.



Top of the plate	
Benzyl benzoate: a greyish-blue zone	a greyish-blue zone
Benzyl cinnamate: a greyish-green zone	a greyish-green zone
Reference solution	**Test solution**

TESTS

Acid value

100 to 160.

Dissolve 0.5 g of the fragmented drug in 50 ml of *alcohol R*. Add 0.5 ml of *acid blue 93 solution R* and 5.0 ml of *0.5 M alcoholic potassium hydroxide*. Stir vigorously and titrate with *0.5 M hydrochloric acid* until the colour changes from brownish-red to blackish-green (n1 ml of *0.5 M hydrochloric acid*). Carry out a blank test in the same manner (n_2 ml of *0.5 M hydrochloric acid*). Calculate the acid value in the same manner as the saponification value *(2.5.6).*

Matter insoluble in alcohol

Maximum 5 per cent.

Boil 2.0 g of the fragmented drug with 25 ml of *alcohol (90 per cent V/V) R* and filter. Wash the residue with *alcohol (90 per cent V/V) R*, boiling until completely extracted, then dry the residue at 100-105 °C. Weigh the residue.

Loss on drying *(2.2.32)*

Maximum 5.0 per cent, determined on 2.000 g of the fragmented drug by spreading on a flat evaporating dish 9 cm in diameter and allowing to dry *in vacuo* for 4 h.

Total ash *(2.4.16)*

Maximum 0.3 per cent.

ASSAY

Boil 1.500 g under a reflux condenser with 25 ml of *0.5 M alcoholic potassium hydroxide* for 1 h. Evaporate the ethanol and heat the residue with 50 ml of *water R* until the substance is homogeneously distributed. After cooling, add 80 ml of *water R* and a solution of 1.5 g of *magnesium sulphate R* in 50 ml of *water R*. Mix, and allow to stand for 10 min. Filter through a pleated filter paper and wash the residue with 20 ml of *water R*. Combine the filtrate and the washings, acidify with *hydrochloric acid R* and extract with 4 quantities, each of 40 ml, of *ether R*. Discard the aqueous layer. Combine the organic extracts and wash with 2 quantities, each of 20 ml, and with 3 quantities, each of 10 ml, of a 50 g/l solution of sodium bicarbonate *R*. Discard the ether layer. Combine the aqueous extracts, acidify with *hydrochloric acid R* and stir once with 30 ml, twice with 20 ml and once with 10 ml of *methylene chloride R*. Dry the combined methylene chloride extracts over *anhydrous sodium sulphate R*. Filter through a pleated filter and wash the residue with 10 ml of *methylene chloride R*. Reduce the combined methylene chloride extracts to 10 ml by distillation and eliminate the remaining methylene chloride in a current of air. Dissolve the residue with heating in 10 ml of *alcohol R* previously neutralised to *phenol red solution R*. After cooling, titrate with *0.1 M sodium hydroxide*, using the same indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 14.82 mg of total acids, expressed as cinnamic acid.

STORAGE

Do not store in powdered form.

Ph Eur

Tormentil Tincture

General Notices

(Ph Eur monograph 1895)

Ph Eur

DEFINITION

Tincture produced from *Tormentil (1478).*

Content

Minimum 1.5 per cent *m/m* of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1).

PRODUCTION

The tincture is produced from 1 part of comminuted drug and 5 parts of ethanol (70 per cent *V/V*) by a suitable procedure.

CHARACTERS

Red or reddish-brown liquid.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution Mix 1.0 ml of the tincture to be examined with 1.0 ml of *alcohol (70 per cent V/V) R*.

Reference solution Dissolve 1.0 mg of *catechin R* in 1.0 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *ether R*, *glacial acetic acid R*, *hexane R*, *ethyl acetate R* (20:20:20:40 *V/V/V/V*).

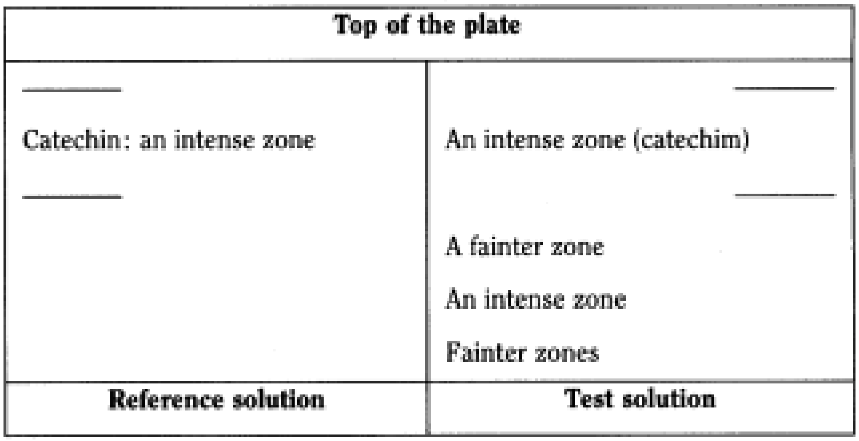
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air for 10-15 min.

Detection Spray with a freshly prepared 5 g/l solution of *fast blue B salt R*. Reddish zones appear. Expose the plate to ammonia vapour, the zones become more intense, turning reddish-brown. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
Catechin: an intense zone	An intense zone (catechim)
———	———
	A fainter zone
	An intense zone
	Fainter zones
Reference solution	**Test solution**

TESTS

Ethanol content (*2.9.10*)

64 per cent *V/V* to 69 per cent *V/V*.

Methanol and 2-propanol (*2.9.11*)

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

ASSAY

Carry out the determination of tannins in herbal drugs *(2.8.14)*. Use 2.50 g of the tincture to be examined.

Ph Eur

Tormentil

General Notices

(Ph Eur monograph 1478)

Ph Eur

DEFINITION

Whole or cut, dried rhizome, freed from the roots, of *Potentilla erecta* (L.) Raeusch. (*P. tormentilla Stokes*).

Content

Minimum 7 per cent of tannins, expressed as pyrogallol ($C_6 H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The rhizome is cylindrically spindle-shaped, with a very irregular appearance, often forming, twisted, knotty tubers, up to 10 cm long and 1 cm to 2 cm thick, very hard and scarcely branched. The surface is brown to reddish-brown, rugose and has remains of roots and transversely elongated depressed whitish scars from the stems. At the top of the rhizome the remains of numerous aerial stems may be present. The fracture is short and granular, dark red to brownish-yellow.

B. Reduce to a powder (355) *(2.9.12)*. The powder is reddish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: coarsely serrate cluster crystals of calcium oxalate, up to 60 µm in diameter; fragments of thin-walled parenchyma containing reddish-brown tannin; groups of narrow, bordered-pitted vessels with lateral pores; thick-walled and pitted, polygonal parenchyma; groups and fragments of sclerenchymatous thick-walled fibres; occasional fragments of cork with thin-walled, brown, tabular cells. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows spherical or elliptical starch granules, up to about 20 µm in length.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.5 g of the powdered drug (355) *(2.9.12)* add 10 ml of *water R*, shake for 10 min and filter. Shake the filtrate with 2 quantities, each of 10 ml, of *ethyl acetate R* and filter the combined upper phases over 6 g of *anhydrous sodium sulphate R*. Evaporate the filtrate to dryness under reduced pressure and dissolve the residue in 1.0 ml of *ethyl acetate R.*

Reference solution Dissolve 1.0 mg of *catechin R* in 1.0 ml of *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R*, *ether R, hexane R*, *ethyl acetate R* (20:20:20:40 *V/V/V/V*) .

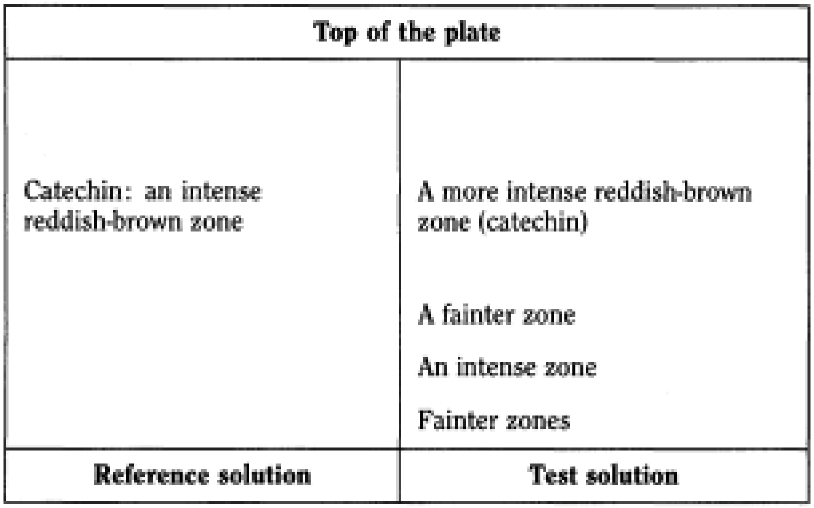
Application 10 µl, as bands.

Development Over a path of 10 cm.

Drying In air for 10-15 min.

Detection Spray with a freshly prepared 5 g/l solution of *fast blue B salt R*. Reddish zones appear. Expose the plate to ammonia vapour, the zones become more intense turning reddish-brown. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fainter zones are present in the chromatogram obtained with the test solution.



Top of the plate	
Catechin: an intense reddish-brown zone	A more intense reddish-brown zone (catechin)
	A fainter zone
	An intense zone
	Fainter zones
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent of root and stems as well as rhizomes with black fracture and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs *(2.8.14)*. Use 0.500 g of the powdered drug (180) *(2.9.12)*.

Ph Eur

Tragacanth

General Notices

(Ph Eur monograph 0532)

When Powdered Tragacanth is prescribed or demanded, material complying with the requirements below with the exception of Identification test A shall be dispensed or supplied.

Ph Eur

DEFINITION

Air-hardened, gummy exudate, flowing naturally or obtained by incision from the trunk and branches of *Astragalus gummifer* Labill. and certain other species of *Astragalus* from western Asia.

IDENTIFICATION

A. Tragacanth occurs in thin, flattened, ribbon-like, white or pale yellow, translucent strips, about 30 mm long and 10 mm wide and up to 1 mm thick, more or less curved, horny, with a short fracture; the surface is marked by fine longitudinal striae and concentric transverse ridges. It may also contain pieces similar in shape but somewhat thicker, more opaque and more difficult to fracture.

B. Reduce to a powder (355) *(2.9.12)*. The powder is white or almost white and forms a mucilaginous gel with about 10 times its mass of *water R*. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows in the gummy mass numerous stratified cellular membranes that turn slowly violet when treated with *iodinated zinc chloride solution R*. The gummy mass includes starch grains, isolated or in small groups, usually rounded in shape and sometimes deformed, with diameters varying between 4 µm and 10 µm, occasionally up to 20 µm, and a central hilum visible between crossed nicol prisms.

C. Examine the chromatograms obtained in the test for acacia.

Results The chromatogram obtained with the test solution shows 3 zones due to galactose, arabinose and xylose. A faint yellowish zone at the solvent front and a greyish-green zone between the zones due to galactose and arabinose may be present.

D. Moisten 0.5 g of the powdered drug (355) *(2.9.12)* with 1 ml of *ethanol (96 per cent) R* and add gradually, while shaking, 50 ml of *water R* until a homogeneous mucilage is obtained. To 5 ml of the mucilage add 5 ml of *water R* and 2 ml of *barium hydroxide solution R*. A slight flocculent precipitate is formed. Heat on a water-bath for 10 min. An intense yellow colour develops.

TESTS

Acacia

Thin-layer chromatography *(2.2.27)*.

Test solution To 100 mg of the powdered drug (355) *(2.9.12)* in a thick-walled centrifuge test-tube, add 2 ml of a 100 g/l solution of *trifluoroacetic acid R*, shake vigorously to dissolve the forming gel, stopper the test-tube and heat the mixture at 120 °C for 1 h. Centrifuge the resulting hydrolysate, transfer the clear supernatant carefully into a 50 ml flask, add 10 ml of *water R* and evaporate the solution to dryness under reduced pressure. To the resulting clear film add 0.1 ml of *water R* and 0.9 ml of *methanol R*. Centrifuge to separate the amorphous precipitate, collect the supernatant and, if necessary, dilute to 1 ml with *methanol R*.

Reference solution Dissolve 10 mg of *arabinose R*, 10 mg of *galactose R*, 10 mg of *rhamnose R* and 10 mg of *xylose R* in 1 ml of *water R* and dilute to 10 ml with *methanol R*.

Plate TLC silica gel plate R.

Mobile phase 16 g/l solution of *sodium dihydrogen phosphate R, butanol R, acetone R* (10:40:50 *V/V/V*).

Application 10 µl as bands.

Development A Over a path of 10 cm.

Drying A In a current of warm air for a few minutes.

Development B Over a path of 15 cm using the same mobile phase.

Drying B At 110 °C for 10 min.

Detection Spray with *anisaldehyde solution R* and dry at 110 °C for 10 min.

Results The chromatogram obtained with the reference solution shows 4 clearly separated coloured zones due to galactose (greyish-green or green), arabinose (yellowish-green), xylose (greenish-grey or yellowish-grey) and rhamnose (yellowish-green), in order of increasing R_F value; the chromatogram obtained with the test solution does not show a yellowish-green zone corresponding to the zone of rhamnose in the chromatogram obtained with the reference solution.

Methylcellulose

Examine the chromatograms obtained in the test for acacia.

Results The chromatogram obtained with the test solution does not show a red zone near the solvent front.

Sterculia gum

A. Place 0.2 g of the powdered drug (355) *(2.9.12)* in a 10 ml ground-glass-stoppered cylinder graduated in 0.1 ml. Add 10 ml of *ethanol (60 per cent V/V) R* and shake. Any gel formed occupies not more than 1.5 ml.

B. To 1.0 g of the powdered drug (355) *(2.9.12)* add 100 ml of *water R* and shake. Add 0.1 ml of *methyl red solution R*. Not more than 5.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Foreign matter

Maximum 1.0 per cent.

Place 2.0 g of the powdered drug (355) *(2.9.12)* in a 250 ml round-bottomed flask and add 95 ml of *methanol R*. Swirl to moisten the powder and add 60 ml of *hydrochloric acid R1*. Add a few glass beads about 4 mm in diameter and heat on a water-bath under a reflux condenser for 3 h, shaking occasionally. Remove the glass beads and filter the hot suspension *in vacuo* through a sintered-glass filter (160) *(2.1.2)*. Rinse the flask with a small quantity of *water R* and pass the rinsings through the filter. Wash the residue on the filter with about 40 ml of *methanol R* and dry to constant mass at 110 °C (about 1 h). Allow to cool in a desiccator and weigh. The residue weighs a maximum of 20 mg.

Flow time

Minimum 10 s, or minimum 50 s if the substance to be examined is to be used for the preparation of emulsions.

Place 1.0 g of the powdered drug (125-250) *(2.9.12)* in a 1000 ml round-bottomed flask with a ground-glass stopper, add 8.0 ml of *ethanol (96 per cent) R* and close the flask. Disperse the suspension over the inner surface of the flask by shaking, taking care not to wet the stopper. Open the flask and add as a single portion 72.0 ml of *water R*. Stopper the flask and shake vigorously for 3 min. Allow to stand for 24 h and shake vigorously again for 3 min. Eliminate air bubbles by applying vacuum above the mucilage for 5 min. Transfer the mucilage to a 50 ml cylinder. Dip in the mucilage a piece of glass tubing 200 mm long and 6.0 mm in internal diameter and graduated at 20 mm and 120 mm from the lower end; the tubing must not be rinsed with surface-active substances. When the mucilage has reached the upper mark, close the tube with a finger. Withdraw the closed tube, remove the finger and measure with a stop-watch the time needed for the meniscus to reach the lower graduation. Carry out this operation 4 times and determine the average value of the last 3 determinations.

Total ash *(2.4.16)*

Maximum 4.0 per cent.

Microbial contamination

Total viable aerobic count *(2.6.12)* not more than 10^4 micro-organisms per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella (2.6.13)*.

LABELLING

The label states whether or not the contents are suitable for preparing emulsions.

Ph Eur

Turpentine Oil

General Notices

Preparation

White Liniment

DEFINITION

Turpentine Oil is obtained by distillation from the oleoresin obtained from various species of *Pinus* and rectified.

CHARACTERISTICS

A clear, bright, colourless liquid, visibly free from water; odour, characteristic.

TESTS

Refractive index

1.467 to 1.477, Appendix V E.

Solubility in ethanol

Soluble, at 20°, in 7 volumes of *ethanol (90%)* and in 3 volumes of *ethanol (96%)*, Appendix X M.

Weight per ml

0.855 to 0.868 g, Appendix V G.

Residue on evaporation

Not more than 1.0% when determined by the method for *residue on evaporation of volatile oils*, Appendix X M. Use 2 g and heat for 4 hours.

STORAGE

Turpentine Oil should be kept in a well-filled container and protected from light.

Valerian Dry Hydroalcoholic Extract

General Notices

(Ph Eur monograph 1898)

Ph Eur

DEFINITION

Extract produced from *Valerian root (0453)*.

Content

Minimum 0.25 per cent *m/m* of sesquiterpenic acids, expressed as valerenic acid ($C_{15}H_{22}O_2$; M_r 234.3) (dried extract).

PRODUCTION

The extract is produced from the drug using ethanol (45 to 80 per cent *V/V*) or methanol (40 to 55 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance

Brown, hygroscopic powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Suspend 1 g of the extract to be examined in 10 ml of *methanol R* and sonicate for 10 min. Filter the supernatant through a membrane filter (0.45 µm). Use the filtrate as the test solution.

Reference solution Dissolve 5 mg of *acetoxyvalerenic acid R* and 5 mg of *valerenic acid R* in 20 ml of *methanol R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *glacial acetic acid R, ethyl acetate R, cyclohexane R* (2:38:60 *V/V/V*).

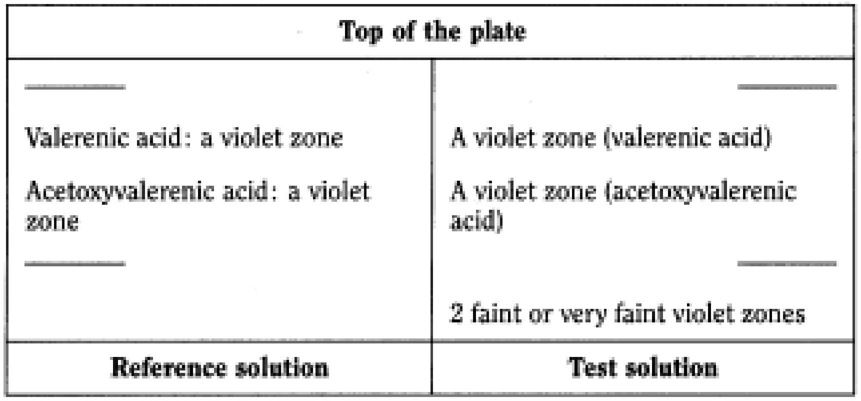
Application 20 µl [or 5 µl] as bands.

Development Over a path of 10 cm [or 6 cm].

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other violet zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Valerenic acid: a violet zone	A violet zone (valerenic acid)
Acetoxyvalerenic acid: a violet zone	A violet zone (acetoxyvalerenic acid)
———	———
	2 faint or very faint violet zones
Reference solution	**Test solution**

TESTS

Loss on drying *(2.8.17)*

Maximum 6.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution Suspend 1.00 g of the extract to be examined in 50.0 ml of *methanol R1*, sonicate for 10 min and filter.

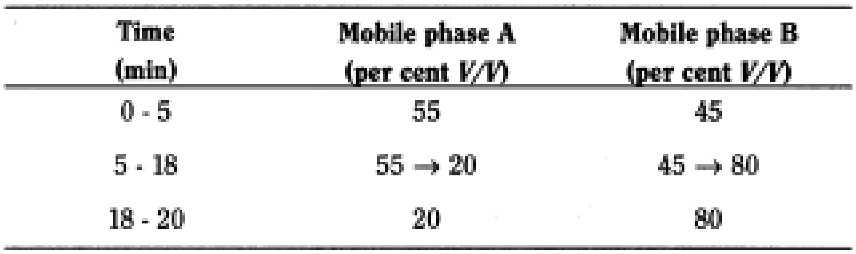
Reference solution Dissolve an amount of *valerian standardised dry extract CRS* corresponding to 1.0 mg of valerenic acid in *methanol R1* and dilute to 10.0 ml with the same solvent.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A: acetonitrile R1*, 5 g/l solution of *phosphoric acid R* (20:80 *V/V*);
- *mobile phase B*: 5 g/l solution of *phosphoric acid R, acetonitrile R1* (20:80 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	55	45
5 - 18	55 → 20	45 → 80
18 - 20	20	80

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Peak identification Use the chromatogram supplied with *valerian standardised dry extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to acetoxyvalerenic acid and valerenic acid.

System suitability Reference solution:

— *relative retention* with reference to valerenic acid (retention time = about 21 min): acetoxyvalerenic acid = about 0.5.

Calculate the percentage content of sesquiterpenic acids, expressed as valerenic acid, using the following expression:

$$\frac{(A_1 + A_2) \times m_2 \times p \times 5}{A_3 \times m_1}$$

A_1 = area of the peak due to acetoxyvalerenic acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to valerenic acid in the chromatogram obtained with the test solution;

A_3 = area of the peak due to valerenic acid in the chromatogram obtained with the reference solution;

m_1 = mass of the dried extract to be examined, in grams;

m_2 = mass of *valerian standardised dry extract CRS* used to prepare the reference solution, in grams;

p = percentage content of valerenic acid in *valerian standardised dry extract CRS*.

Ph Eur

Valerian Tincture

General Notices

(Ph Eur monograph 1899)

Ph Eur

DEFINITION

Tincture produced from *Valerian root (0453)*.

Content

Minimum 0.015 per cent *m/m* of sesquiterpenic acids, expressed as valerenic acid ($C_{15}H_{22}O_2$; M_r 234.3).

PRODUCTION

The tincture is produced from 1 part of the drug and 5 parts of ethanol (60 to 80 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance

Brown liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Dilute 5 ml of the tincture to be examined with 5 ml of *ethanol (70 per cent V/V) R*.

Reference solution Dissolve 5 mg of *acetoxyvalerenic acid R* and 5 mg of *valerenic acid R* in 20 ml of *methanol R*.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *glacial acetic acid R, ethyl acetate R, cyclohexane R* (2:38:60 *V/V/V*).

Application 20 µl [or 5 µl] as bands.

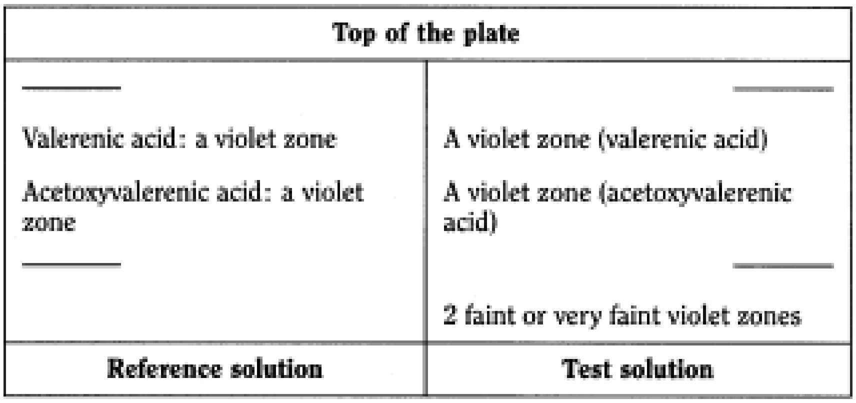
Development Over a path of 10 cm [or 6 cm].

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other violet zones may be present in the

chromatogram obtained with the test solution.



Top of the plate	
Valerenic acid: a violet zone	A violet zone (valerenic acid)
Acetoxyvalerenic acid: a violet zone	A violet zone (acetoxyvalerenic acid)
	2 faint or very faint violet zones
Reference solution	**Test solution**

TESTS

Ethanol *(2.9.10)*

95 per cent to 105 per cent of the quantity stated on the label.

ASSAY

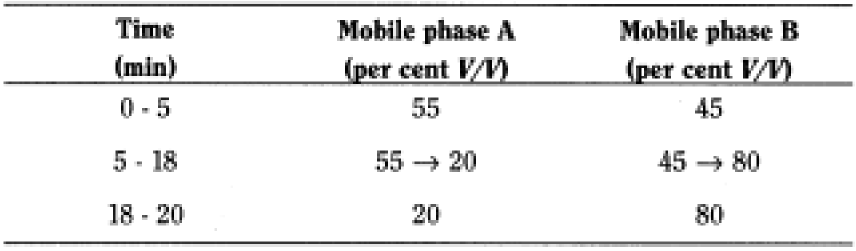
Liquid chromatography *(2.2.29)*.

Test solution Dilute 10.0 g of the tincture to be examined to 50.0 ml with *methanol R1*.

Reference solution Dissolve an amount of *valerian standardised dry extract CRS* corresponding to 1.0 mg of valerenic acid in *methanol R1* and dilute to 10.0 ml with the same solvent.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A: acetonitrile R1*, 5 g/l solution of *phosphoric acid R* (20:80 *V/V*);
- *mobile phase B*: 5 g/l solution of *phosphoric acid R*, *acetonitrile R1* (20:80 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	55	45
5 - 18	55 → 20	45 → 80
18 - 20	20	80

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Peak identification Use the chromatogram supplied with *valerian standardised dry extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to acetoxyvalerenic acid and valerenic acid.

System suitability Reference solution:

— *relative retention* with reference to valerenic acid (retention time = about 20 min): acetoxyvalerenic acid = about 0.5.

Calculate the percentage content of sesquiterpenic acids, expressed as valerenic acid, using the following expression:

$$\frac{(A_1 + A_2) \times m_2 \times p \times 5}{A_3 \times m_1}$$

A_1 = area of the peak due to acetoxyvalerenic acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to valerenic acid in the chromatogram obtained with the test solution;

A_3 = area of the peak due to valerenic acid in the chromatogram obtained with the reference solution;

m_1 = mass of the tincture to be examined, in grams;

m_2 = mass of *valerian standardised dry extract CRS* used to prepare the reference solution, in grams;

p = percentage content of valerenic acid in *valerian standardised dry extract CRS*.

Ph Eur

Valerian

General Notices

(Valerian Root, Ph Eur monograph 0453)

When Powdered Valerian is prescribed or demanded, material complying with the appropriate requirements below shall be dispensed or supplied.

Ph Eur

DEFINITION

Dried, whole or fragmented underground parts of *Valeriana officinalis* L. *s.l.*, including the rhizome surrounded by the roots and stolons.

Content:

— *whole or fragmented drug*;
  — *essential oil*: minimum 4 ml/kg (dried drug);
  — *sesquiterpenic acids*: minimum 0.17 per cent *m/m*, expressed as valerenic acid ($C_{15}H_{22}O_2$; M_r 234.3) (dried drug);

— *cut drug*;
  — *essential oil*: minimum 3 ml/kg (dried drug);
  — *sesquiterpenic acids*: minimum 0.10 per cent m/m expressed as valerenic acid ($C_{15}H_{22}O_2$; M_r 234.3) (dried drug).

IDENTIFICATION

A. The rhizome is yellowish-grey or pale brownish-grey, obconical to cylindrical, up to about 50 mm long and 30 mm in diameter; the base is elongated or compressed, usually entirely covered by numerous roots. The apex usually exhibits a cup-shaped scar from the aerial parts; stem bases are rarely present. When cut longitudinally, the pith exhibits a central cavity transversed by septa. The roots are numerous, almost cylindrical, of the same colour as the rhizome, 1-3 mm in diameter and sometimes more than 100 mm long. A few filiform fragile secondary roots are present. The fracture is short. The stolons show prominent nodes separated by longitudinally striated internodes, each 20-50 mm long, with a fibrous fracture.

B. Reduce to a powder (355) *(2.9.12).* The powder is pale yellowish-grey or pale greyish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: cells containing a pale brown resin or droplets of essential oil; groups of small, rectangular sclereids with thick walls and a narrow, channelled branched lumen; occasional groups of larger, thinner-walled sclereids from the stem bases; lignified, reticulately-thickened vessels, singly or in small groups; thin-walled, elongated cells of the piliferous layer, some with root hairs; occasional fragments of cork. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows abundant starch granules, mainly compound with up to 4-6 components but frequently separated to form single granules, rounded to irregular and up to about 15 µm in diameter; most of the granules show a rather indistinct cleft or radiate hilum.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Suspend 1 g of the powdered drug (355) *(2.9.12)* in 10 ml of *methanol R* and sonicate for 10 min. Filter the supernatant through a membrane filter (0.45 µm). Use the filtrate as the test solution.

Reference solution Dissolve 5 mg of *acetoxyvalerenic acid R* and 5 mg of *valerenic acid R* in 20 ml of *methanol R*.

Plate *TLC silica gel plate R (5-40 µm) [or TLC silica gel plate R* (2-10 µm)].

Mobile phase *glacial acetic acid R, ethyl acetate R, cyclohexane R* (2:38:60 *V/V/V*).

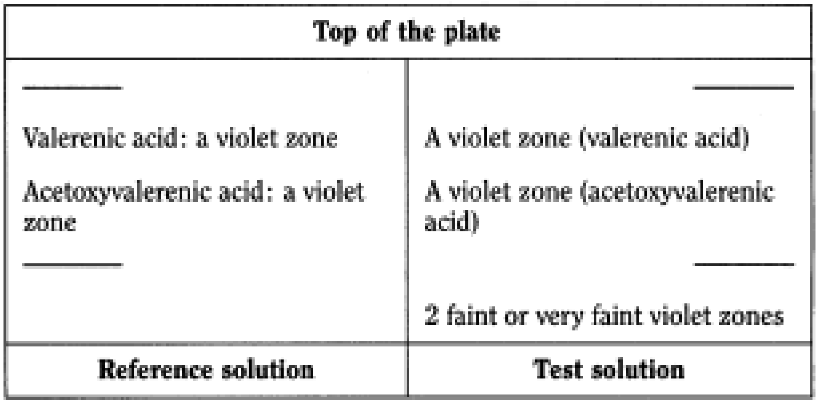
Application 20 µl [or 5 µl] as bands.

Development Over a path of 10 cm [or 6 cm].

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min; examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other violet zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
Valerenic acid: a violet zone	A violet zone (valerenic acid)
Acetoxyvalerenic acid: a violet zone	A violet zone (acetoxyvalerenic acid)
———	———
	2 faint or very faint violet zones
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of stem bases and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of well homogenised powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 5.0 per cent.

ASSAY

Essential oil *(2.8.12)*

Use 40.0 g of freshly powdered drug (500) *(2.9.12)*, a 2000 ml flask, 500 ml of *water R* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 3-4 ml/min for 4 h.

Sesquiterpenic acids

Liquid chromatography *(2.2.29)*.

Test solution Place 1.50 g of the powdered drug (710) (2.9.12) in a 100 ml round-bottomed flask with a ground-glass neck. Add 20 ml of *methanol R1.* Mix and heat on a water-bath under a reflux condenser for 30 min. Allow to cool and filter. Place the filter with the residue in the 100 ml round-bottomed flask. Add 20 ml of *methanol R1* and heat on a water-bath under the reflux condenser for 15 min. Allow to cool and filter. Combine the filtrates and dilute to 50.0 ml with *methanol R1*, rinsing the round-bottomed flask and the filter.

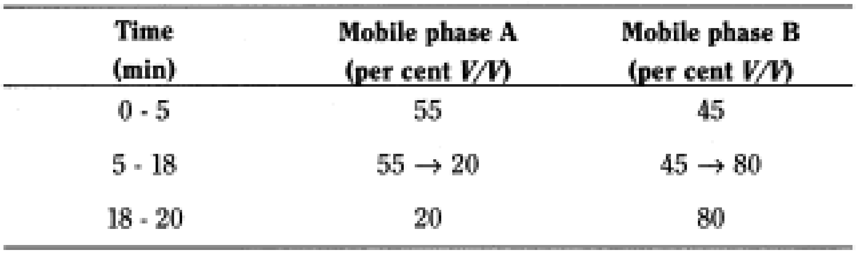
Reference solution Dissolve an amount of *valerian standardised dry extract CRS* corresponding to 1.0 mg of valerenic acid in *methanol R1* and dilute to 10.0 ml with the same solvent.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *acetonitrile R1*, 5 g/l solution of *phosphoric acid R* (20:80 *V/V*);

— *mobile phase B*: 5 g/l solution of *phosphoric acid R, acetonitrile R1* (20:80 *V/V*);



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	55	45
5 - 18	55 → 20	45 → 80
18 - 20	20	80

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl.

Peak identification Use the chromatogram supplied with *valerian standardised dry extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to acetoxyvalerenic acid and valerenic acid.

System suitability Reference solution:

— *relative retention* with reference to valerenic acid (retention time = about 21 min): acetoxyvalerenic acid = about 0.5.

Calculate the percentage content of sesquiterpenic acids, expressed as valerenic acid, using the following expression:

$$\frac{(A_1 + A_2) \times m_2 \times p \times 5}{A_3 \times m_1}$$

A_1 = area of the peak due to acetoxyvalerenic acid in the chromatogram obtained with the test solution

A_2 = area of the peak due to valerenic acid in the chromatogram obtained with the test solution

A_3 = area of the peak due to valerenic acid in the chromatogram obtained with the reference solution

m_1 = mass of the drug to be examined, in grams

m_2 = mass of *valerian standardised dry extract CRS*, used to prepare the reference solution, in grams

p = percentage content of valerenic acid in *valerian standardised dry extract CRS.*

Ph Eur

Verbena Herb

General Notices

(Ph Eur monograph 1854)

Ph Eur

DEFINITION

Whole or fragmented, dried aerial parts of *Verbena officinalis* L. collected during flowering.

Content

Minimum 1.5 per cent of verbenalin ($C_{17}H_{24}O_{10}$; M_r 388.4) (dried drug).

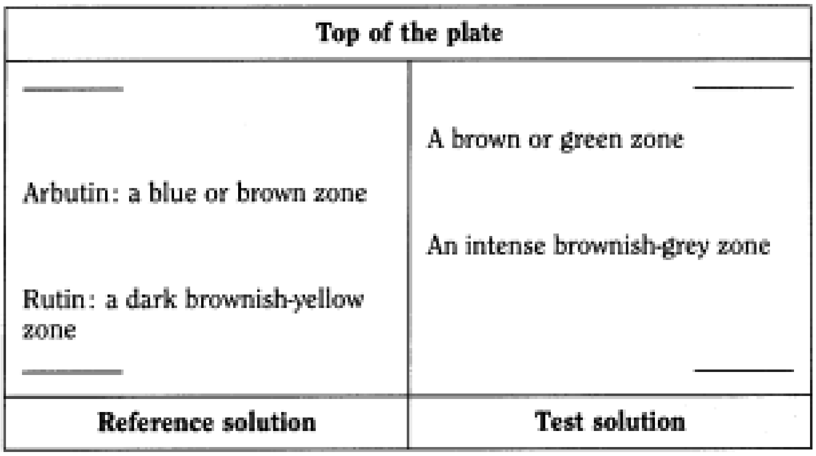
IDENTIFICATION

A. The stem is greenish-brown, quadrangular, longitudinally grooved and roughly hairy, especially on the angles. The larger leaves are petiolate and deeply pinnately lobed, with bluntly dentate margins, the smaller leaves are sessile, not lobed, with crenate to dentate margins; the surfaces are rough and covered with bristly hairs, particularly over the veins which are prominent on the lower surface. The flowers are numerous, arranged in a slender pike in the axils of leaf-like bracts; the tubular calyx has 5 acutely pointed lobes with the pale pink to lilac corolla forming a tube about twice as long as the calyx.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: long, polygonal to rectangular epidermal cells of the stems with thickened outer walls; fragments of the leaves in surface view showing sinuous-walled epidermal cells with anomocytic or anisocytic stomata *(2.8.3)*, more numerous on the lower epidermis; covering trichomes short, unicellular, thick-walled, up to 500 µm long, wide at the base and arising from the centre of a single ring of domed, spherical epidermal cells; occasional glandular trichomes of 2 types: (a) multicellular stalk with a flattened, 4- to 8-celled head about 25 µm in diameter and (b) unicellular stalk and an enlarged ovate head about 65 µm in diameter composed of 8 radiating cells; fragments of the fibrous layer of the anther with well-marked thickenings; triangular to ovoid or rounded pollen grains about 30 µm in diameter, with 3 pores and a smooth exine; abundant groups of fibres, bordered-pitted vessels and parenchymatous tissue from the stems.

C. Examine the chromatograms obtained in test B for Aloysia citrodora.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
	A brown or green zone
Arbutin: a blue or brown zone	
	An intense brownish-grey zone
Rutin: a dark brownish-yellow zone	
———	———
Reference solution	**Test solution**

TESTS

Aloysia citrodora

A. A lemon-like odour indicates the presence of *Aloysia citrodora*.

B. Thin-layer chromatography *(2.2.27)*.

Test solution To 0.50 g of the powdered drug (710) *(2.9.12)* add 5 ml of *methanol R*. Heat in a water-bath at 60 °C for 10 min. Cool and filter.

Reference solution Dissolve 10 mg of *arbutin R* and 10 mg of *rutin R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase anhydrous formic acid R, glacial acetic acid R, water R, ethyl acetate R (11:11:27:100 *V/V/V/V*).

Application 20 µl [or 5 µl] as bands.

Development Over a path of 12 cm [or 6 cm].

Drying In air.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for about 10 min. Examine in daylight.

Results The chromatogram obtained with the test solution shows no intense blue or violet zone approximately at the position of rutin in the chromatogram obtained with the reference solution.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (710) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 2.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

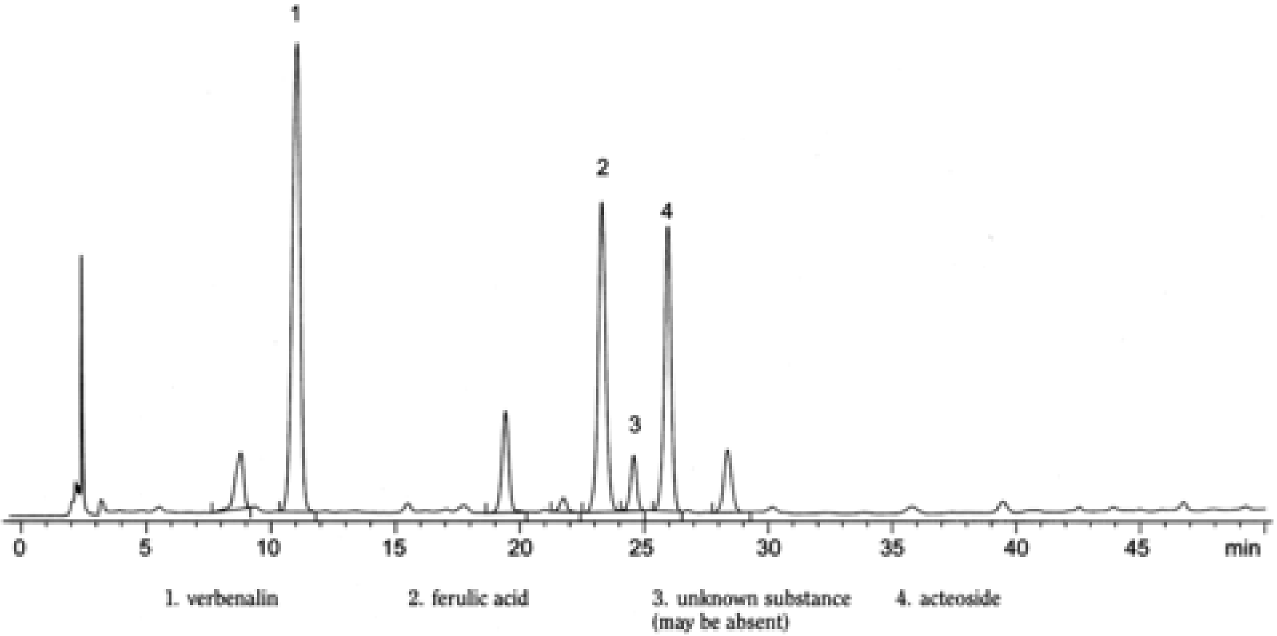


Figure 1854.-1. – *Chromatogram for the assay of verbena herb: test solution*

Internal standard solution Dissolve 10.0 mg of *ferulic acid R* in *ethanol (60 per cent V/V) R* and dilute to 100.0 ml with the same solvent.

Test solution To 1.00 g of the powdered drug (710) *(2.9.12)* add 50.0 ml of the internal standard solution and stir with a magnetic stirrer for 2 h. Centrifuge for 15 min and filter the supernatant using a membrane filter of 0.45 µm pore size.

Reference solution Dissolve the contents of a vial of *verbenalin CRS* in the internal standard solution and dilute to 5.0 ml with the same solution.

Precolumn:
— *size*: *l* = 0.01 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

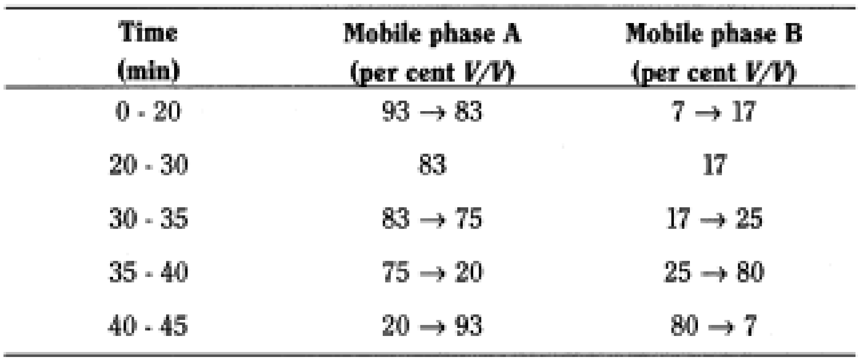
Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 20 °C.

Mobile phase:
— *mobile phase A*: 0.3 per cent *V/V* solution of *phosphoric acid R;*

— *mobile phase B*: *acetonitrile R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	93 → 83	7 → 17
20 - 30	83	17
30 - 35	83 → 75	17 → 25
35 - 40	75 → 20	25 → 80
40 - 45	20 → 93	80 → 7

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µl.

System suitability Test solution:

— the chromatogram obtained is similar to the chromatogram shown in Figure 1854.-1;

— *resolution*: minimum 3.5 between the peaks due to ferulic acid and acteoside.

Calculate the percentage content of verbenalin from the following expression:

$$\frac{A_1 \times A_4 \times m_2 \times 10}{A_2 \times A_3 \times m_1}$$

A_1 = area of the peak due to verbenalin in the chromatogram obtained with the test solution;

A_2 = area of the peak due to verbenalin in the chromatogram obtained with the reference solution;

A_3 = area of the peak due to ferulic acid in the chromatogram obtained with the test solution;

A_4 = area of the peak due to ferulic acid in the chromatogram obtained with the reference solution;

m_1 = mass of the drug used to prepare the test solution, in grams;

m_2 = mass of verbenalin in the reference solution, in grams.

Ph Eur

Weak Ginger Tincture

General Notices

DEFINITION

Strong Ginger Tincture	200 ml
Ethanol (90 per cent)	Sufficient to produce 1000 ml

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

TESTS

Ethanol content

86 to 90% v/v, Appendix VIII F, Method III.

Dry residue

Not less than 0.4% w/v. Use 10 ml.

Relative density

0.825 to 0.835, Appendix V G.

White Horehound

General Notices

(Ph Eur monograph 1835)

Ph Eur

DEFINITION

Whole or fragmented dried flowering aerial parts of *Marrubium vulgare* L.

Content

Minimum 0.7 per cent of marrubiin ($C_{20}H_{28}O_4$; M_r 332.4) (dried drug).

CHARACTERS

Bitter taste.

IDENTIFICATION

A. The stems are up to 50 cm long, quadrangular, up to 7 mm wide, young stems are densely covered with whitish downy hairs, older stems are greenish-grey and less hairy. The lower leaves are broadly ovate to almost orbicular, upper leaves less broadly ovate, both petiolate; lamina 1.5-4 cm long, 1-3.5 cm wide, apex sub-acute, base tapering or somewhat cordate, margin dentate to crenate, petiole up to 3 cm long; venation pinnate, prominent on the lower surface, distinctly depressed on the upper surface. Both leaf surfaces are densely covered with fine, white, woolly hairs, older leaves having fewer hairs on the dark greyish-green upper surface. The flowers are small, sessile in dense axillary clusters. The calyx is 5 mm long, persistent, with 5 long and 5 short, alternating, hooked, recurved fringing spines; throat of calyx with an internal ring of long silky hairs; corolla 7 mm long, dull white, 4-lobed, upper lobe 2-lipped, lower-lobe 3-lipped; 4 short stamens; style with bifid stigma.

B. Reduce to a powder (710) *(2.9.12)*. The powder is greyish-green. Examine under a microscope under *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of leaves with sinuous, polygonal epidermal cells, diacytic stomata *(2.8.3)*, more numerous on the lower surface and cells of the mesophyll with small needles and cluster crystals of calcium oxalate; covering trichomes very numerous, twisted or coiled, 100-200 µm long, unicellular or multicellular and unseriate with 2-6 cells, enlarged at the joints; stellate trichomes of 2 types, one with 15-20 branches arising from a short unicellular stalk and the other with fewer branches arising from a sessile base; 8-celled secretory trichomes of lamiaceous type; glandular trichomes with 1 or 2 celled stalk and 1 to 4 celled head; the covering trichomes on the inner surface of the calyx are up to 1000 µm long with 2 to 3 cells, strongly thickened at the swollen joint and with the upper cell elongated; pollen grains spherical, about 25 µm in diameter with smooth exine; fragments of vascular tissue from the stems and veins.

C. Thin-layer chromatography *(2.2.27)*.

Test solution (a) To 1.0 g of the powdered drug (710) *(2.9.12)* add 2 ml of *dilute hydrochloric* *acid R* and 8 ml of *methanol R*. Heat under a reflux condenser for 30 min, cool and filter.

Test solution (b) To 1.0 g of the powdered drug (710) *(2.9.12)* add 10 ml of *methanol R*. Heat under a reflux condenser for 30 min, cool and filter.

Reference solution Dissolve 10 mg of *cholesterol R* and 10 mg of *guaiazulene R* in 10 ml of *methanol R.*

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *methanol R*, *toluene R* (5:95 *V/V*).

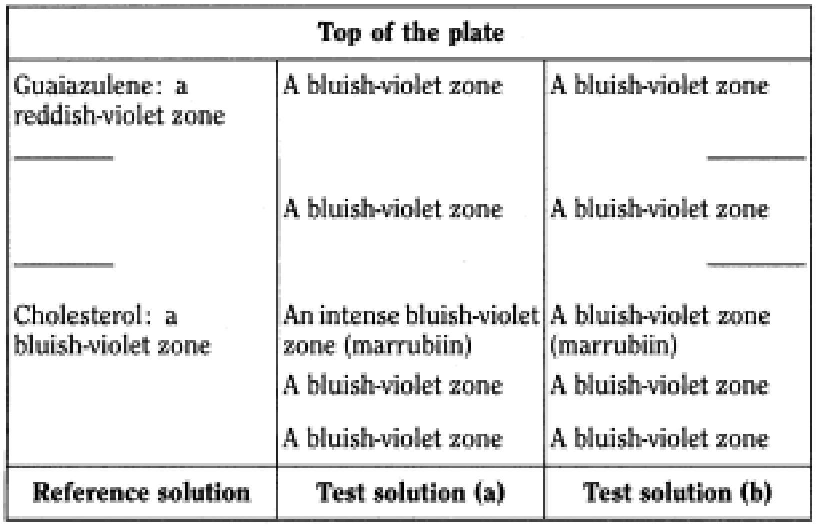
Application 20 µl [or 5 µl] of test solutions (a) and (b) and 10 µl [or 2 µl] of the reference solution, as bands.

Development Over a path of 10 cm [or 6 cm].

Drying In air.

Detection Spray with a 5 g/l solution of *vanillin R* in a mixture of 20 volumes of *ethanol (96 per cent) R* and 80 volumes of *sulphuric acid R* and examine in daylight immediately after heating at 130 °C for 5-10 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and test solutions (a) and (b). Further zones in the chromatograms obtained with test solutions (a) and (b) may be present. The zone due to marrubiin in the chromatogram obtained with test solution (a) is more intense than that in the chromatogram obtained with test solution (b). During extraction with hydrochloric acid and methanol, conversion of pre-marrubiin to marrubiin takes place which leads to an increase in intensity of the zone.



Top of the plate		
Guaiazulene: a reddish-violet zone	A bluish-violet zone	A bluish-violet zone
———		———
	A bluish-violet zone	A bluish-violet zone
———		———
Cholesterol: a bluish-violet zone	An intense bluish-violet zone (marrubiin)	A bluish-violet zone (marrubiin)
	A bluish-violet zone	A bluish-violet zone
	A bluish-violet zone	A bluish-violet zone
Reference solution	**Test solution (a)**	**Test solution (b)**

TESTS

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (710) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 15.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

ASSAY

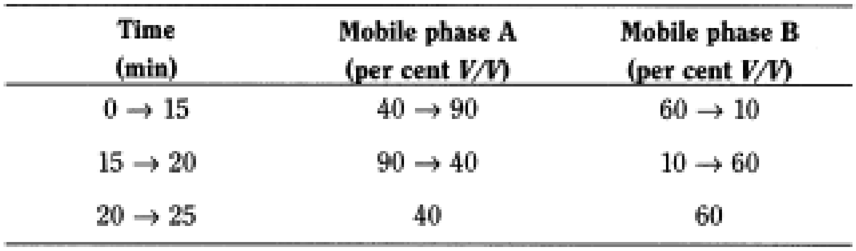
Liquid chromatography *(2.2.29)*.

Test solution Reduce 50 g of the drug to a powder (250) *(2.9.12)* and homogenise. To 1.00 g of the powdered drug in a 50 ml round-bottomed flask add 15 ml of a mixture of 2 volumes of *dilute hydrochloric acid R* and 8 volumes of *methanol R.* Heat in a water bath at 80 °C under a reflux condenser for 30 min. Allow to cool at room temperature and filter through a plug of adsorbent cotton into a 25 ml volumetric flask. Dilute to 25.0 ml with *methanol R* by rinsing the round-bottomed flask and the filter.

Reference solution Dissolve 2.0 mg of *marrubiin R* in 10.0 ml of *methanol R*.

Column:
- *size:* *l* = 0.25 m, Ø = 4 mm,
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *acetonitrile R*,
- *mobile phase B*: dilute 0.5 ml of *phosphoric acid R* to 1000 ml with *water R*,



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 → 15	40 → 90	60 → 10
15 → 20	90 → 40	10 → 60
20 → 25	40	60

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 217 nm.

Injection 20 µl.

Locate the peak due to marrubiin by comparison with the chromatogram obtained with the reference solution.

Calculate the percentage content of marrubiin from the following expression:

$$\frac{A_1 \times m_2 \times p \times 2.5}{A_2 \times m_1}$$

A_1 = area of the peak due to marrubiin in the chromatogram obtained with the test solution,

A_2 = area of the peak due to marrubiin in the chromatogram obtained with the reference solution,

m_1 = mass of the drug to be examined, in milligrams,

m_2 = mass of *marrubiin R*, in milligrams,

p = percentage content of marrubiin in *marrubiin R*.

Ph Eur

White Peony Root for use in THM

General Notices

DEFINITION

White Peony Root for use in THM is the dried whole root of *Paeonia lactiflora* Pallas (*Paeonia albiflora* Pallas; *Paeonia edulis* Salisb.; *Paeonia officinalis* Thunb.). It contains not less than 1.6% of paeoniflorin ($C_{23}H_{28}O_{11}$), calculated with reference to the dried material.

It is collected in summer and autumn, washed clean, with the two ends and rootlet removed and dried.

IDENTIFICATION

A. Cylindrical, straight or slightly curved, two ends truncate, 5 to 20 cm long, 1 to 2.5 cm in diameter. Externally light brown to reddish brown, with longitudinal wrinkles, rootlet scars and with laterally elongated lenticels. Texture compact, easily broken, fracture relatively even, internally whitish or pale brownish-red. Cambium ring distinct and rays radial.

B. Reduce to a powder. The powder is pale yellow. Examine under a microscope using *chloral hydrate solution*. The powder shows abundant rounded, rectangular or elongated parenchymatous cells which occur singly or in groups, some with pitted or slightly beaded walls; single cluster crystals of calcium oxalate, 11 to 35 µm in diameter, isolated or packed in rows in parenchymatous cells, sometimes with several crystals in each cell; fragments of lignified vessels, 20 to 65 µm in diameter, usually reticulately thickened but occasionally bordered-pitted; fibres occur rarely, usually accompanying the vessel fragments, they are 15 to 40 µm in diameter, with thickened, slightly lignified and frequently pitted walls. Examine under a microscope using a 50% v/v solution of *glycerol* in *water*. Many of the parenchymatous cells contain masses of starch grains which may also be found scattered throughout the powder.

C. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

D. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake 0.5 g of the powdered root with 10 ml of *ethanol* for 5 minutes, filter and evaporate the filtrate to dryness and dissolve the residue in 1 ml of *absolute ethanol*.

(2) 0.1% w/v each of *paeoniflorin BPCRS* and *4′-hydroxyacetophenone BPCRS* in *ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 precoated plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µl for each solution, as a band.

(d) Develop the plate to 15 cm.

(e) Dry the plate in air. Spray with a 5% w/v solution of *vanillin* in *sulphuric acid*, heat at 105º for 5 minutes and examine immediately.

MOBILE PHASE

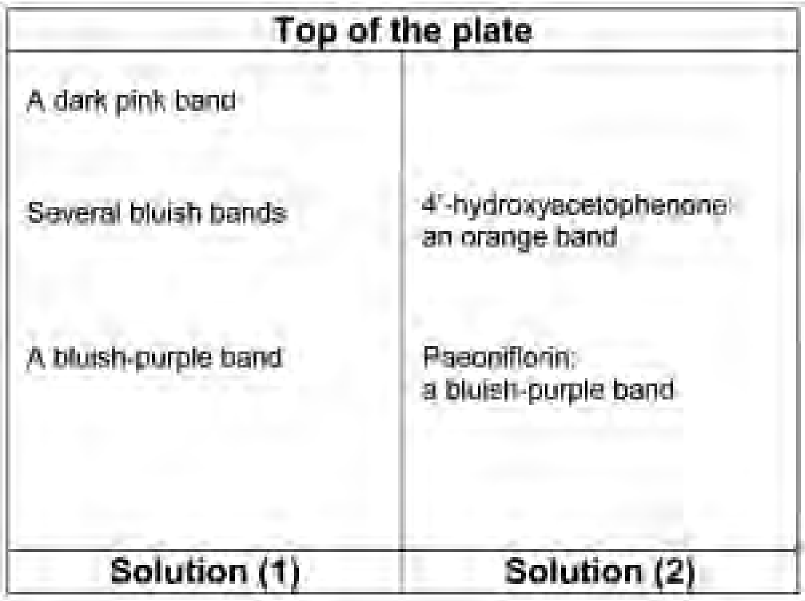
A mixture of 0.2 volumes of *formic acid*, 5 volumes of *ethyl acetate*, 10 volumes of *methanol* and 40 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), two clearly separated bands are observed.

CONFIRMATION

The bluish-purple band with an Rf value of approximately 0.4 in the chromatogram obtained with solution (1) corresponds in colour and position to that in the chromatogram obtained with solution (2). Other bands are present in the chromatogram obtained with solution (1) as shown below.



Top of the plate	
A dark pink band	
Several bluish bands	4'-hydroxyacetophenone: an orange band
A bluish-purple band	Paeoniflorin: a bluish-purple band
Solution (1)	Solution (2)

TESTS

Tree Peony

In the Assay, the chromatogram obtained with solution (1) shows no peak with a relative retention of approximately 1.2 with reference to paeoniflorin.

Loss on drying

When dried for 3 hours at 100º to 105º, loses not more than 12.0% of its weight. Use 1 g.

Acid-insoluble ash

Not more than 0.5%, Appendix XI K.

Ash

Not more than 6.5%, Appendix XI J.

Cadmium

Maximum 3 ppm, Appendix VII.

Lead

Maximum 5 ppm, Appendix VII.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in 0.05M *potassium dihydrogen orthophosphate* (solution A).

(1) Finely powder not less than 5 g of the herbal drug being examined. Mix 0.15 g of the powdered drug with 3 ml of *methanol* and place in an ultrasonic bath for 30 minutes. Dilute to 10 ml with solution A, mix, filter through a 0.45-µm filter and use the filtrate.

(2) Dissolve a quantity of *paeoniflorin BPCRS* in *methanol* and dilute with solution A to produce a solution containing 0.05% w/v in 3 volumes of *methanol* and 7 volumes of solution A and mix.

(3) Dissolve a quantity of *4′-hydroxyacetophenone BPCRS* in solution (2) to produce a solution containing 0.05% w/v of 4′-hydroxyacetophenone.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 10 µl of each solution.

MOBILE PHASE

A mixture of 30 volumes of *methanol* and 70 volumes of solution A.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the number of *theoretical plates* for the peak due to paeoniflorin is at least 3000, the *symmetry factor* for the peak due to paeoniflorin is not more than 1.3 and the *resolution factor* between the two peaks is not less than 1.7.

DETERMINATION OF CONTENT

Using the retention time and the peak area from the chromatograms obtained with solution (2) , locate and integrate the peak due to paeoniflorin in the chromatogram obtained with solution (1).

Calculate the content of $C_{23}H_{28}O_{11}$ in the sample, using the declared content of paeoniflorin ($C_{23}H_{28}O_{11}$) in *paeoniflorin BPCRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100 - d}$$

A_1 = Area of the peak due to paeoniflorin in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to paeoniflorin in the chromatogram obtained with solution (2).

m_1 = Weight of the drug being examined in mg.

m_2 = Weight of *paeoniflorin BPCRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content of $C_{23}H_{28}O_{11}$ in *paeoniflorin BPCRS.*

d = Percentage loss on drying of the herbal drug being examined.

STORAGE

White Peony Root for use in THM should be protected from moisture.

Wild Pansy

General Notices

(Wild Pansy (Flowering Aerial Parts), Ph Eur monograph 1855)

Ph Eur

DEFINITION

Dried flowering aerial parts of *Viola arvensis* Murray and/or *Viola Tricolor* L.

Content

Minimum 1.5 per cent of flavonoids, expressed as violanthin ($C_{27}H_{30}O_{14}$; M_r 578.5) (dried drug).

IDENTIFICATION

A. The stem is angular and hollow. The leaves are oval, petiolate, with a cordate base or elongated and obtuse, with lyrate stipules, divided in the middle. The flowers, with a long peduncle, are zygomorphic, with 5 oval, lanceolate sepals, an appendage pointed outwards and 5 petals of which the lower one bears a spur; in *Viola arvensis*, the petals are shorter than the calyx, the lower petal is cream coloured, with black lines, the 4 upper petals may be cream coloured or violet blue; in *Viola Tricolor*, the petals are longer than the calyx and violet coloured, more or less tinged with yellow. The androecium consisting of 5 stamens bears at the apex a membranous connective appendage with 2 spurs. The trilocular ovary shows a short style and globular stigmata. The fruit are navicular capsules, three-lobed, yellowish brown, 5 mm to 10 mm long. The pale yellow, pyriform seeds are about 1 mm long, bearing a caruncle.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greenish. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the epidermis of the leaves in surface view with wavy-walled cells and anomocytic stomata *(2.8.3)*; conical unicellular covering trichomes, widened at the base and sharply pointed at the apex, with a striated cuticle; glandular trichomes with a multicellular head, and a short, multicellular stalk in the indentations of the leaf margins; cluster crystals of calcium oxalate, sometimes included in parenchyma; fragments of the corolla with wavy-walled epidermal cells, those from the mid-region papillose and with some extended to form flask or bottle-shaped projections, those from the base of the petals with covering trichomes up to about 300 µm long with characteristic hump-like swellings along their length; spherical or polyhedral pollen grains, 60 µm to 80 µm in diameter, with finely pitted exines and 5 pores (*Viola arvensis*) or 4 pores (*Viola Tricolor*); occasional fragments of spiral and reticulate vessels and groups of fibres from the stem.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Heat in a water-bath at 65 °C for 5 min, with frequent stirring, 2.0 g of the powdered drug (355) *(2.9.12)* in 10 ml of *alcohol (70 per cent V/V) R*. Cool and filter.

Reference solution Dissolve 2.5 mg of *rutin R*, 2.5 mg of *hyperoside R* and 1.0 mg of *caffeic* *acid R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *anhydrous formic acid R*, *acetic acid R*, *water R*, *ethyl acetate R* (11:11:27:100 *V/V/V/V*).

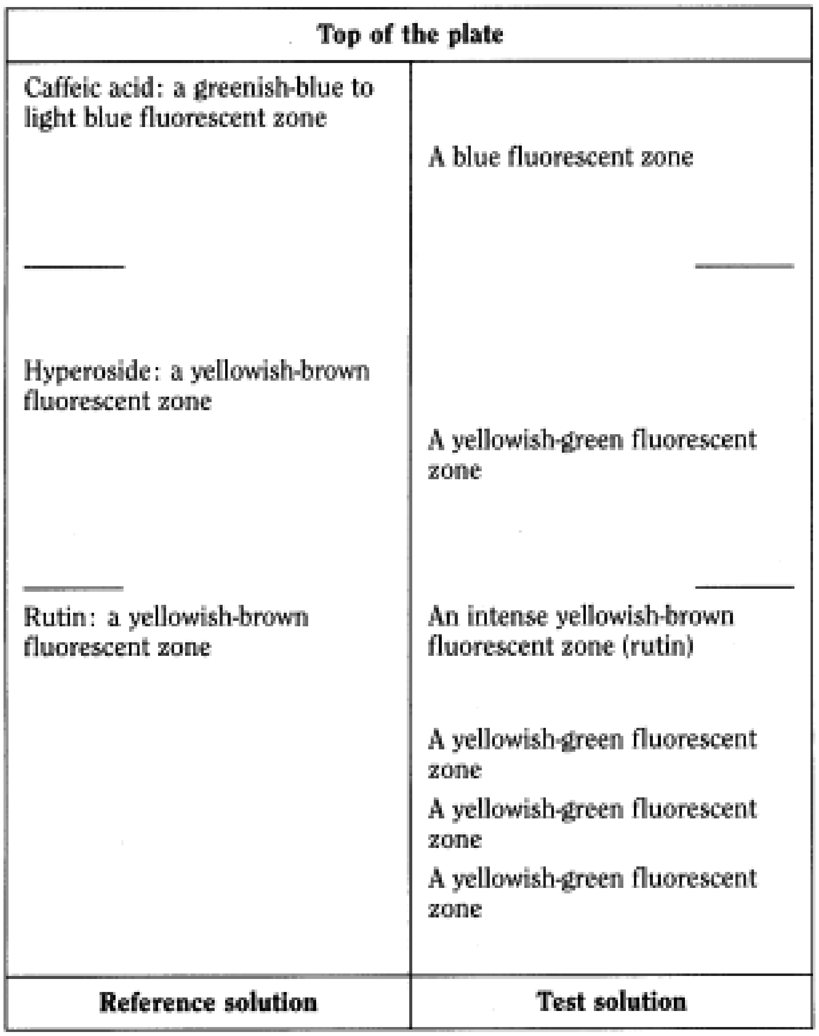
Application 10 µl, as bands.

Development Over a path of 12 cm.

Drying At 100-105 °C.

Detection Spray with a solution containing 10 g/l of *diphenylboric acid aminoethyl ester R* and 50 g/l of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for 30 min. Examine in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
Caffeic acid: a greenish-blue to light blue fluorescent zone	A blue fluorescent zone
_______	_______
Hyperoside: a yellowish-brown fluorescent zone	A yellowish-green fluorescent zone
_______	_______
Rutin: a yellowish-brown fluorescent zone	An intense yellowish-brown fluorescent zone (rutin)
	A yellowish-green fluorescent zone
	A yellowish-green fluorescent zone
	A yellowish-green fluorescent zone
Reference solution	**Test solution**

TESTS

Foreign matter *(2.8.2)*

Maximum 3 per cent.

Swelling index (*2.8.4*)

Minimum 9, determined on the powdered drug (355) *(2.9.12)*.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 15.0 per cent.

ASSAY

Stock solution In a 200 ml flask, introduce 0.300 g of the powdered drug (250) *(2.9.12)* and 40 ml of *alcohol (60 per cent V/V) R*. Heat in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter through a plug of absorbent cotton into a 100 ml volumetric flask. Transfer the absorbent cotton with the drug residue back into the 200 ml flask, add 40 ml of *alcohol (60 per cent V/V) R* and heat again in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter into the same 100 ml volumetric flask as used previously. Rinse the 200 ml flask with a further quantity of *alcohol (60 per cent V/V) R*, filter and transfer to the same 100 ml volumetric flask. Dilute to volume with *alcohol (60 per cent V/V) R* and filter.

Test solution Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the same 25 ml volumetric flask as used previously. Add 10.0 ml of a solution containing 25.0 g/l of *boric acid R* and 20.0 g/l of *oxalic acid R* in *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Compensation liquid Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the same 25 ml volumetric flask as used previously. Add 10.0 ml of *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Measure the absorbance *(2.2.25)* of the test solution at 405 nm after 30 min.

Calculate the percentage content of total flavonoids, expressed as violanthin from the expression:

$$\frac{A \times 1.25}{m}$$

taking the specific absorbance of violanthin to be 400.

A = measured absorbance at 405 nm

M = mass of the drug to be examined, in grams

Ph Eur

Wild Thyme

General Notices

(Ph Eur monograph 1891)

Ph Eur

DEFINITION

Whole or cut, dried, flowering aerial parts of *Thymus serpyllum* L.s.l.

Content

Minimum 3.0 ml/kg of essential oil (dried drug).

IDENTIFICATION

A. The stem is much branched, up to about 1.5 mm in diameter, cylindrical or indistinctly quadrangular, green, reddish or purplish, the older stems brown and woody, the younger stems pubescent. The leaves are opposite, 3 mm to 12 mm long and up to 4 mm wide, elliptical to ovate-lanceolate with an obtuse apex, cuneate and shortly petiolate at the base; the margin is entire and markedly ciliate, especially near the base; both surfaces are more or less glabrous but distinctly punctate. The inflorescence is composed of about 6 to 12 flowers in rounded to ovoid, terminal heads. The calyx is tubular, two-lipped with the upper lip dividing to form 3 teeth, the lower lip with 2 teeth, edged with long hairs; inner surfaces strongly pubescent, the hairs forming a closed tube after flowering. The corolla is purplish-violet to red, two-lipped, the lower lip with 3 lobes, upper lip notched, inner surface strongly pubescent; stamens 4, epipetalous, projecting from the corolla tube.

B. Reduce to a powder (355) *(2.9.12)*. The powder is greyish-green to brownish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the leaf epidermises with sinuous, slightly thickened anticlinical walls and stomata of the diacytic type *(2.8.3)*; numerous covering trichomes on both epidermises and along the leaf margins, the majority short, conical, unicellular, with thickened and warty walls, fewer long, uniseriate, composed of up to 8 cells, slightly swollen at the joints, with moderately thickened walls; abundant glandular trichomes, mostly multicellular with a small, rounded, unicellular stalk and a large globular head composed of a number of indistinct, radiating cells containing brown secretion, others smaller, capitate, with unicellular stalk and a unicellular, globoid or ovoid head; purplish-violet fragments of the corolla, the outer epidermis with numerous covering and glandular trichomes, inner epidermis papillose; pollen grains spherical to elliptical, 30 µm to 40 µm in diameter, with a finely grained exine and 6 germinal pores.

C. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of the powdered drug (355) *(2.9.12)* add 5 ml of *methylene chloride R* and shake for 3 min. Filter through about 2 g of *anhydrous sodium sulphate R*.

Reference solution Dissolve 5 mg of *thymol R* and 10 µl of *carvacrol R* in 10 ml of *methylene chloride R.*

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *methylene chloride R.*

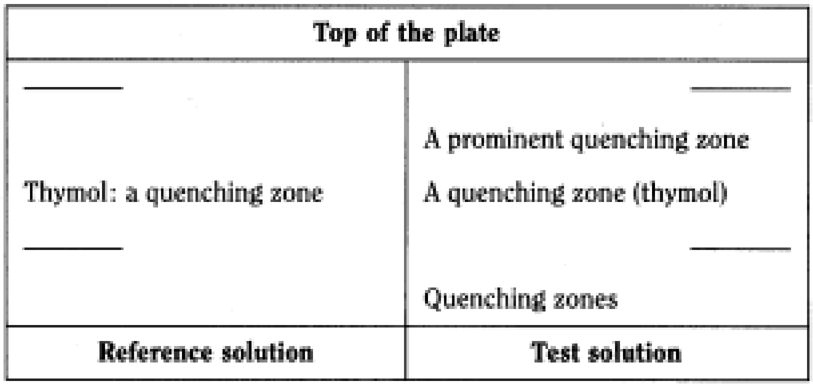
Application 20 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

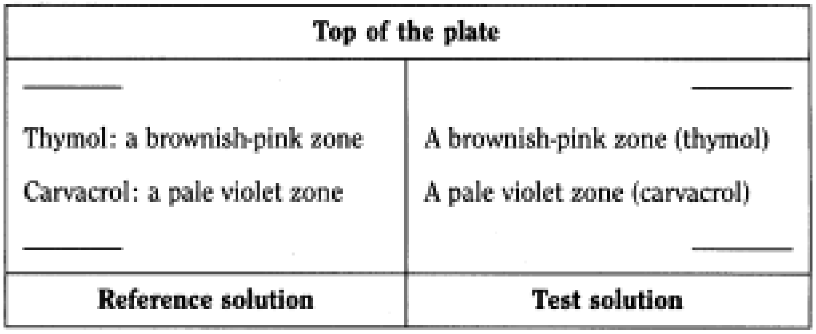
Results A See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
	A prominent quenching zone
Thymol: a quenching zone	A quenching zone (thymol)
———	———
	Quenching zones
Reference solution	**Test solution**

Detection B Spray with *anisaldehyde solution R* using 10 ml for a plate 200 mm square and heat at 100-105 °C for 10 min.

Results B See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the lower third of the chromatogram obtained with the test solution. The intensity of the zones due to thymol and carvacrol depends upon the sample examined (chemotypes).



Top of the plate	
———	———
Thymol: a brownish-pink zone	A brownish-pink zone (thymol)
Carvacrol: a pale violet zone	A pale violet zone (carvacrol)
———	———
Reference solution	**Test solution**

TESTS

Foreign matter ***(2.8.2)***

Maximum 3 per cent, determined on 30 g.

Foreign matter may also consist of acicular to linear-lanceolate leaves with a strongly bent

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margin, the adaxial surface showing covering trichomes shaped as pointed teeth with warty walls, the abaxial surface showing many types of warty covering trichomes: unicellular, straight or slightly curved, bicellular or tricellular, often elbow-shaped, and bicellular or tricellular, more or less straight (*Thymus vulgaris, Thymus zygis*).

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16)*

Maximum 10.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 3.0 per cent.

ASSAY

Carry out the determination of essential oils in herbal drugs (2.8.12). Use 50.0 g of the cut drug, a 1000 ml round-bottomed flask and 500 ml of *water R* as the distillation liquid. Distil at a rate of 2-3 ml/min for 2 h without *xylene R* in the graduated tube.

Ph Eur

Willow Bark Dry Extract

General Notices

(Ph Eur monograph 2312)

Ph Eur

DEFINITION

Dry extract produced from *Willow bark (1583).*

Content

Minimum 5.0 per cent of total salicylic derivatives, expressed as salicin ($C_{13}H_{18}O_7$; M_r 286.3) (dried extract).

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using either water or a hydroalcoholic solvent equivalent in strength to a maximum of 80 per cent *V/V* ethanol.

CHARACTERS

Appearance

Yellowish-brown amorphous powder.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution (a) To 0.200 g of the extract to be examined add 5 ml of *methanol R*. Sonicate for 5 min, filter and dilute to 10 ml with *methanol R.*

Test solution (b) To 5.0 ml of test solution (a) add 1.0 ml of a 50 g/l solution of *anhydrous sodium carbonate R* and heat in a water-bath at about 60 °C for 10 min. Cool and filter if necessary.

Reference solution Dissolve 2.0 mg of *salicin R* and 2.0 mg of *chlorogenic acid R* in 1.0 ml of *methanol R.*

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *water R*, *methanol R*, *ethyl acetate R* (8:15:77 *V/V/V*).

Application 10 µl [or 2 µl] as bands.

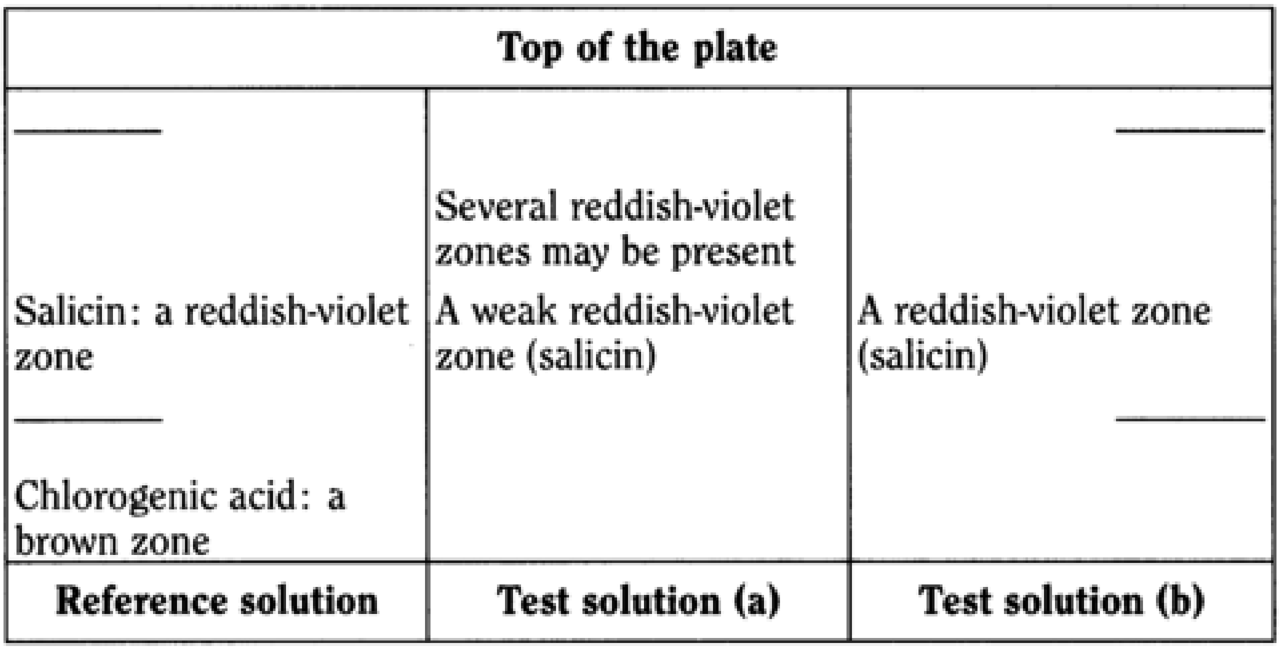
Development Over a path of 15 cm [or 6 cm].

Drying In a current of warm air.

Detection Spray with a mixture of 5 volumes of *sulphuric acid R* and 95 volumes of *methanol*

R. Heat at 100-105 °C for 5 min and examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and test solutions (a) and (b). Furthermore, other zones may be present in the chromatogram obtained with test solutions (a) and (b).



Top of the plate		
———		———
	Several reddish-violet zones may be present	
Salicin: a reddish-violet zone	A weak reddish-violet zone (salicin)	A reddish-violet zone (salicin)
———		———
Chlorogenic acid: a brown zone		
Reference solution	**Test solution (a)**	**Test solution (b)**

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution To 0.300 g of the extract to be examined add 40 ml of *methanol R* and 40.0 ml of *0.1 M sodium hydroxide*. Heat in a water-bath at about 60 °C under a reflux condenser, with frequent shaking, for about 1 h. After cooling, add 4.0 ml of *1 M hydrochloric acid*. Filter the suspension into a 100 ml volumetric flask, then wash and dilute to 100.0 ml with a mixture of equal volumes of *water R* and *methanol R*. Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution Dissolve 5.0 mg of *picein R* in 25.0 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R* (solution A). Dissolve 15.0 mg of *salicin CRS* in 25 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R*. Add 5.0 ml of solution A and dilute to 50.0 ml with *water R.*

Column:

— *size: l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: *tetrahydrofuran R*, 0.5 per cent *V/V* solution of *phosphoric acid R* (1.8:98.2 *V/V*);

— *mobile phase B*: *tetrahydrofuran R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 17	100 → 90	0 → 10
17 - 23	90	10
23 - 25	90 → 100	10 → 0
25 - 40	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µl.

Retention time Salicin = about 6.4 min;

picein = about 7.7 min.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to salicin and picein.

Calculate the percentage content of total salicylic derivatives, expressed as salicin, from the following expression:

$$\frac{A_1 \times m_2 \times p \times 2}{A_2 \times m_1}$$

A_1 = area of the peak due to salicin in the chromatogram obtained with the test solution

A_2 = area of the peak due to salicin in the chromatogram obtained with the reference solution;

m_1 = mass of the extract to be examined used to prepare the test solution, in grams

m_2 = mass of *salicin CRS* used to prepare the reference solution, in grams

p = percentage content of salicin in *salicin CRS.*

Ph Eur

Willow Bark

General Notices

(Ph Eur monograph 1583)

Ph Eur

DEFINITION

Whole or fragmented dried bark of young branches or whole dried pieces of current-year twigs of various species of genus *Salix* including *S. purpurea* L., *S. daphnoides* Vill. and *S. fragilis* L.

Content

Minimum 1.5 per cent of total salicylic derivatives, expressed as salicin ($C_{13}H_{18}O_7$; M_r 286.3) (dried drug).

IDENTIFICATION

A. The bark is 1-2 mm thick and occurs in flexible, elongated, quilled or curved pieces. The outer surface is smooth or slightly wrinkled longitudinally and greenish-yellow or brownish-grey. The inner surface is smooth or finely striated longitudinally and white, pale yellow or reddish-brown, depending on the species. The fracture is short in the outer part and coarsely fibrous in the inner region. The diameter of current-year twigs is not greater than 10 mm. The wood is white or pale yellow.

B. Reduce to a powder (355) (*2.9.12*). The powder is pale yellow, greenish-yellow or light brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: bundles of narrow fibres, up to about 600 µm long, with very thick walls and surrounded by a crystal sheath containing prism crystals of calcium oxalate; parenchyma of the cortex with thick, pitted and deeply beaded walls, and containing large cluster crystals of calcium oxalate; uniseriate medullary rays; thickened cork cells. Groups of brownish collenchyma from the bud may be present. Twigs show, additionally, fragments of lignified fibres and vessels.

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methanol R.* Heat in a water-bath at about 50 °C, with frequent shaking, for 10 min. Cool and filter.

Test solution (b) To 5.0 ml of test solution (a) add 1.0 ml of a 50 g/l solution of *anhydrous sodium carbonate R* and heat in a water-bath at about 60 °C for 10 min. Cool and filter if necessary.

Reference solution Dissolve 2 mg of *salicin R* and 2 mg of *chlorogenic acid R* in 1.0 ml of *methanol R.*

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *water R*, *methanol R*, *ethyl acetate R* (8:15:77 *V/V/V*).

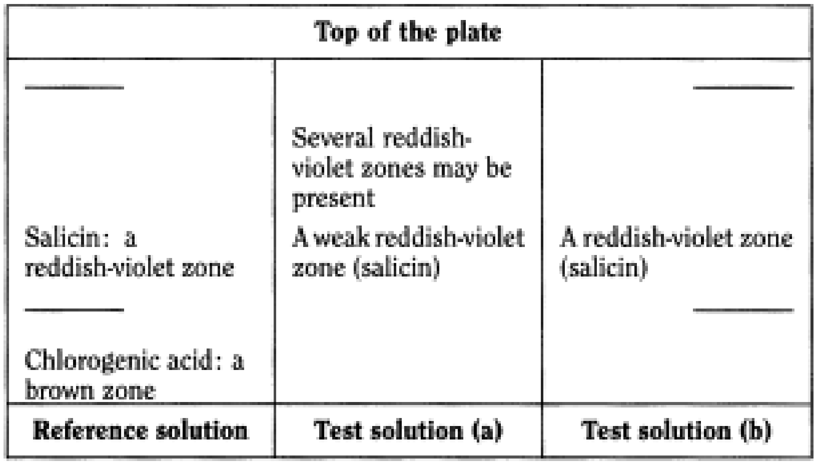
Application 10 µl [or 2 µl], as bands.

Development Over a path of 15 cm [or 6 cm].

Drying In a current of warm air.

Detection Spray with a mixture of 5 volumes of *sulphuric acid R* and 95 volumes of *methanol R*. Heat at 100-105 °C for 5 min and examine in daylight.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and test solutions (a) and (b). Furthermore, other zones may be present in the chromatograms obtained with test solutions (a) and (b).



Top of the plate		
———		———
	Several reddish-violet zones may be present	
Salicin: a reddish-violet zone	A weak reddish-violet zone (salicin)	A reddish-violet zone (salicin)
———		———
Chlorogenic acid: a brown zone		
Reference solution	**Test solution (a)**	**Test solution (b)**

TESTS

Foreign matter (*2.8.2*)

Maximum 3 per cent of twigs with a diameter greater than 10 mm and maximum 2 per cent of other foreign matter.

Loss on drying (*2.2.32*)

Maximum 11 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 10 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution To 1.000 g of the powdered drug (355) (*2.9.12*) add 40 ml of *methanol R* and 40.0 ml of a 4.2 g/l solution of *sodium hydroxide R*. Heat in a water-bath at about 60 °C under a reflux condenser, with frequent shaking, for about 1 h. After cooling, add 4.0 ml of a 103.0 g/l solution of *hydrochloric acid R*. Filter the suspension into a 100 ml volumetric flask, wash and dilute to 100.0 ml with a mixture of 50 volumes of *methanol R* and 50 volumes of *water R.* Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution Dissolve 5.0 mg of *picein R* in 25.0 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R* (solution A). Dissolve 15.0 mg of *salicin CRS* in 25 ml of a mixture of 20 volumes of *water R* and 80 volumes of *methanol R*; add 5.0 ml of solution A and dilute to 50.0 ml with *water R*.

Column:

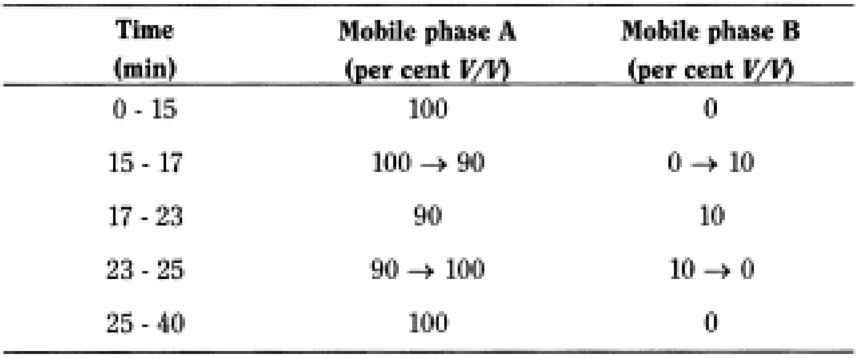
— *size:* l = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A*: *tetrahydrofuran R*, 0.5 per cent *V/V* solution of *phosphoric acid R* (1.8:98.2 *V/V*);

— *mobile phase B*: *tetrahydrofuran R*;



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 17	100 → 90	0 → 10
17 - 23	90	10
23 - 25	90 → 100	10 → 0
25 - 40	100	0

Flow rate 1.0 ml/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µl.

Retention time Salicin = about 6.4 min; picein = about 7.7 min.

System suitability Reference solution:

— *resolution*: minimum 1.5 between the peaks due to salicin and picein.

Calculate the percentage content of total salicylic derivatives, expressed as salicin, using the following expression:

$$\frac{A_1 \times m_2 \times p \times 2}{A_2 \times m_1}$$

A_1 = area of the peak due to salicin in the chromatogram obtained with the test solution;

A_2 = area of the peak due to salicin in the chromatogram obtained with the reference solution;

m_1 = mass of the drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *salicin CRS* used to prepare the reference solution, in grams;

p = percentage content of salicin in *salicin CRS*.

Ph Eur

Withania Somnifera Root for use in THMP

General Notices

DEFINITION

Withania Somnifera Root for use in THMP consists of the dried mature roots of *Withania somnifera* (L.) Dunal.

It contains not less than 0.01% withaferin A ($C_{28}H_{38}O_6$) and not less than 0.01% withanolide A ($C_{29} H_{42}O_7$).

PRODUCTION

It is collected in winter, washed, dried and cut into short pieces.

IDENTIFICATION

A. The drug is cut into short lengths of up to 8 cm and varies in diameter from 2 mm to 1 cm. The outer surface is a pale greyish-brown to somewhat darker brown in larger specimens. The root breaks with a short fracture to show a whitish interior. It consists of a mixture of broader root and small portions of narrower rhizome, often at the transition zone. The cut surface of the root may show a distinction between the xylem and other tissues marked by a faint yellow-green cambial ring.

B. Reduce to a powder (355). Examine under a microscope using *chloral hydrate solution*. Cork cells in surface view polygonal, in sectional view rectangular, thin-walled, yellowish brown, rare, often broken. Parenchymatous cells in fragmented groups elongated, rectangular, or oval to round, filled with starch; occasionally pitted, lightly lignified, possibly alongside vascular fragments; square to round cells of medullary rays crossing xylem elements at right angles. Single or small groups of round or oval lignified, pitted parenchyma cells. Fragments of fibres, vessels and tracheids with surrounding parenchymatous cells. Occasional fragments of spiral, scalariform or pitted vessels with broad lumen. Other tracheids and vessels heavily lignified, reticulate or bordered pitted, single or in small groups. Fibres often accompanying vessels, thick walled, heavily pitted, and lignified; others less pitted and lignified, thin walled, in groups of two or three, or single. Medullary rays, one or two cells wide, of round cells between fibres or vascular elements. Microcrystals of calcium oxalate scattered or occasionally in idioblasts. Examine under a microscope using a 50% v/v solution of *glycerol*. Starch, abundant, of simple and 2 to 4 compound, round to oval granules, some with point, stellate or mainly cleft hila.

C. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Shake 0.5 g of freshly powdered (355) drug with 1 ml of *dilute ammonia R4*, add 10 ml of *methanol*, sonicate for 10 seconds, heat on a water-bath for 3 minutes, cool, filter, evaporate the filtrate to dryness at 60° and dissolve the dried residue in 1 ml of *methanol*. Filter through a 0.45 µm filter.

(2) 0.1% w/v each of *withaferin A CRS, withanolide B CRS* and *β-sitosterol* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254}* precoated high performance plate (Merck silica gel F_{254} HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 2 µl of each of solution, as 6 mm bands.

(d) Develop the plate to 8 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).* Immerse the plate in 5% v/v of *methanolic sulphuric acid* for 1 second, allow to dry in air, heat at 110° for 2 minutes and examine immediately in daylight. Examine the derivatised plate under *ultraviolet light* (*366 nm)*.

MOBILE PHASE

A mixture of 5 volumes of *anhydrous formic acid*, 15 volumes of *ethyl acetate* and 50 volumes of *toluene.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows three clearly separated bands.

CONFIRMATION

The bands with Rf values of approximately 0.1 (withaferin A), 0.26 (withanolide B) and 0.57 (β-sitosterol) in the chromatogram obtained with solution (1) correspond in colour and position to those in the chromatogram obtained with solution (2).

TESTS

Absence of *Withania coagulans*

In Identification test C the derivatised plate under *ultraviolet light* (*366 nm)* shows no orange band with an Rf of approximately 0.2 in the chromatogram obtained with solution (1).

Ash

Not more than 7.0%, Appendix XI J*.*

Acid-insoluble ash

Not more than 1.0%, Appendix XI K*.*

Loss on drying

When dried for 2 hours at 105°, loses not more than 12.0% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Extract 1.0 mg of the powdered drug with 3.0 ml of *methanol* with the aid of ultrasound for 10 minutes, centrifuge at 3000 rpm for 5 minutes and retain the supernatant extract. The extraction is repeated a further two times as described. Combine the three supernatant extracts. Adjust the total volume of the combined extracts to 10.0 ml, dilute to 20.0 ml with *methanol* and filter through a 0.45-µm filter.

(2) 0.02% w/v each of *withaferin A CRS* and *withanolide A CRS* and 0.01% w/v of

withanolide B CRS in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with dodecylsilyl silica gel for chromatography (4 µm) (Phenomenex Synergi Max-RP 80A is suitable).

(b) Use gradient elution and the mobile phases described below. Equilibrate the column with a mixture of 65% Mobile phase A and 35% Mobile phase B for at least 15 minutes.

(c) Use a flow rate of 1 ml per minute.

(d) Use a column temperature of 50º.

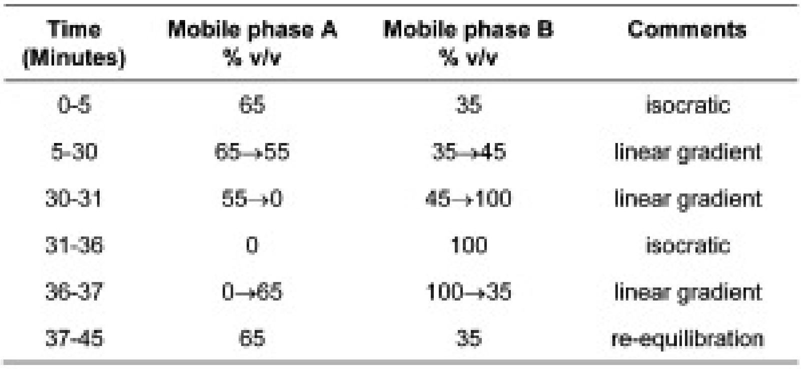
(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

Mobile phase A *water.*

Mobile phase B A mixture of *methanol* and *ethanol (96%)* in equal volumes.



Time (Minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0-5	65	35	isocratic
5-30	65→55	35→45	linear gradient
30-31	55→0	45→100	linear gradient
31-36	0	100	isocratic
36-37	0→65	100→35	linear gradient
37-45	65	35	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the first two main peaks, of withaferin A and withanolide A is at least 5.0 and the *symmetry factor* for both peaks is less than 1.3.

DETERMINATION OF CONTENT

Withaferin A Using the retention time and peak area of the peak due to withaferin A in the chromatogram obtained with solution (2), locate and integrate the peak due to withaferin A in the chromatogram obtained with solution (1).

Calculate the content of withaferin A in the sample using the declared content withaferin A ($C_{28}H_{38}O_6$) in *withaferin A CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100 - d}$$

A_1 = Area of the peak due to withaferin A in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to withaferin A in the chromatogram obtained with solution (2).

m_1 = Weight of the drug being examined in mg.

m_2 = Weight of *withaferin A CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content withaferin A in *withaferin A CRS.*

d = Percentage loss on drying of the herbal drug being examined.

Withanolide A Using the retention time and peak area of the peak due to withanolide A in the chromatogram obtained with solution (2), locate and integrate the peak due to withanolide A in the chromatogram obtained with solution (1).

Calculate the content of withanolide A in the sample using the declared content withanolide A ($C_{29}H_{42}O_7$) in *withanolide A CRS* and the following expression:

$$\frac{A_1}{A_2} \times \frac{m_2}{V_2} \times \frac{V_1}{m_1} \times p \times \frac{100}{100 - d}$$

A_1 = Area of the peak due to withanolide A in the chromatogram obtained with solution (1).

A_2 = Area of the peak due to withanolide A in the chromatogram obtained with solution (2).

m_1 = Weight of the drug in mg.

m_2 = Weight of *withanolide A CRS* in mg.

V_1 = Dilution volume of solution (1) in ml.

V_2 = Dilution volume of solution (2) in ml.

p = Percentage content withanolide A in *withanolide A CRS*.

d = Percentage loss on drying of the herbal drug being examined.

In the chromatogram obtained with solution (2), locate and integrate the peak due to withanolide A in the chromatogram obtained with solution (1).

STORAGE

Withania Somnifera Root for use in THMP should be protected from moisture.

Wormwood

General Notices

(Ph Eur monograph 1380)

Ph Eur

DEFINITION

Basal leaves or slightly leafy, flowering tops, or mixture of these dried, whole or cut organs of *Artemisia absinthium* L.

Content

Minimum 2 ml/kg of essential oil (dried drug).

IDENTIFICATION

A. The leaves are greyish or greenish, densely tomentose on both surfaces. The basal leaves, with long petioles, have triangular or oval bipinnatisect or tripinnatisect lamina, with rounded or lanceolate segments. The cauline leaves are less segmented and the apical leaves are lanceolate. The stem of the flower-bearing region is greenish-grey, tomentose, up to 2.5 mm in diameter and usually with 5 flattened longitudinal grooves. The capitula are arranged as loose, axillary panicles, inserted at the level of the lanceolate to slightly pinnatisect leaves; they are spherical to flattened hemispherical, 2-4 mm in diameter and consist of a grey, tomentose involucre, the outer bracts linear, inner layer ovate, blunt at the apices with scarious margins, a receptacle with very long paleae up to 1 mm or more long, numerous yellow, tubular, hermaphroditic florets about 2 mm long and few yellow, ray florets.

B. Reduce to a powder (355) (*2.9.12)*. The powder is greenish-grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: many T-shaped trichomes with a short uniseriate stalk consisting of 1-5 small cells, perpendicularly capped by a very long, undulating terminal cell tapering at the ends; fragments of epidermises with sinuous or wavy walls, anomocytic stomata *(2.8.3)* and secretory trichomes each with a short, biseriate, 2 celled stalk and a biseriate head with 2-4 cells; fragments of the tubular and ray florets, some containing small cluster crystals of calcium oxalate; numerous paleae each composed of a small cell forming a stalk and a very long, cylindrical and thin-walled terminal cell about 1-1.5 mm long; spheroidal pollen grains, about 30 µm in diameter, with 3 pores and a finely warty exine; groups of fibres, small vessels with spiral and annular thickening, larger vessels with bordered pits and parenchyma with moderately thickened and pitted walls, from the stem.

C. Thin-layer chromatography *(2.2.27)*.

Test solution Place 2 g of the powdered drug (355) *(2.9.12)* in 50 ml of *boiling water R* and allow to stand for 5 min, shaking the flask several times. After cooling, add 5 ml of a 100 g/l solution of *lead acetate R*. Mix and filter. Rinse the flask and the residue on the filter with 20 ml of *water R*. Shake the filter with 50 ml of *methylene chloride R*. Separate the organic layer, dry over *anhydrous sodium sulphate R*, filter and evaporate the filtrate to dryness on a water-bath. Dissolve the residue in 0.5 ml of *ethanol (96 per cent) R*.

Reference solution Dissolve 2 mg of *methyl red R* and 2 mg of *resorcinol R* in 10.0 ml of *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *acetone R, glacial acetic acid R, toluene R, methylene chloride R* (10:10:30:50 *V/V/V/V*).

Application 10 µl, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection A Spray with *acetic anhydride-sulphuric acid solution R* and examine in daylight.

Results A The chromatogram obtained with the test solution shows a blue zone due to artabsin shortly above a red zone due to methyl red in the chromatogram obtained with the reference solution.

Detection B Examine in daylight while heating at 100-105 °C for 5 min.

Results B The chromatogram obtained with the reference solution shows in the middle third a red zone due to methyl red and below it a light pink zone due to resorcinol. The chromatogram obtained with the test solution shows an intense red or brownish-red zone due to absinthin with a similar R_F value to that of the zone due to resorcinol in the chromatogram obtained with the reference solution. Other zones are visible, but less intense than that due to absinthin.

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of stems with a diameter greater than 4 mm and maximum 2 per cent of other foreign matter.

Bitterness value *(2.8.15)*

Minimum 10 000.

Loss on drying *(2.2.32)*

Maximum 10.0 per cent, determined on 1.000 g of powdered drug (355) *(2.9.12)* by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 12.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 1.0 per cent.

ASSAY

Carry out the determination of essential oil in herbal drugs *(2.8.12)*. Use 50.0 g of the cut drug, a 1000 ml round-bottomed flask and 500 ml of *water R* as the distillation liquid. Add 0.5 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for not less than 3 h.

Ph Eur

Yarrow

General Notices

(Ph Eur monograph 1382)

Ph Eur

DEFINITION

Whole or cut, dried flowering tops of *Achillea millefolium* L.

Content:

— *essential oil*: minimum 2 ml/kg (dried drug);

— *proazulenes, expressed as chamazulene* ($C_{14}H_{16}$; M_r 184.3): minimum 0.02 per cent (dried drug).

IDENTIFICATION

A. The leaves are green or greyish-green, faintly pubescent on the upper surface and more pubescent on the lower surface, 2-3 pinnately divided with linear lobes and a finely pointed whitish tip. The capitula are arranged in a corymb at the end of the stem. Each capitulum, 3-5 mm in diameter, consists of the receptacle, usually 4-5 ligulate ray-florets and 3-20 tubular disk-florets. The involucre consists of 3 rows of imbricate lanceolate, pubescent green bracts arranged with a brownish or whitish, membranous margin. The receptacle is slightly convex and, in the axillae of paleae, bears a ligulate ray floret with a three-lobed, whitish or reddish ligule and tubular disk-florets with a radial, five-lobed, yellowish or light brownish corolla. The pubescent green, partly brown or violet stems are longitudinally furrowed, up to 3 mm thick with a light-coloured medulla.

B. Reduce to a powder (355) *(2.9.12)*. The powder is green or greyish-green. Examine under a microscope, using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the stems, leaves and bracts bearing very rare glandular trichomes with a short stalk and a head formed of 2 rows of 3-5 cells enclosed in a bladder-like membrane and uniseriate covering trichomes consisting of 4-6 small, more or less isodiametric cells at the base and a thick-walled, often somewhat tortuous terminal cell, about 400 µm to greater than 1000 µm long; fragments of the ligulate corolla with papillary epidermal cells; small-celled parenchyma from the corolla tubes containing cluster crystals of calcium oxalate; groups of lignified and pitted cells from the bracts; spherical pollen grains, about 30 µm in diameter, with 3 germinal pores and spiny exine; groups of sclerenchymatous fibres and small vessels with spiral or annular thickening, from the stem.

C. To 2.0 g of the powdered drug (710) *(2.9.12)* add 25 ml of *ethyl acetate R*, shake for 5 min and filter. Evaporate to dryness on a water-bath and dissolve the residue in 0.5 ml of *toluene R* (solution A). To 0.1 ml of this solution add 2.5 ml of *dimethylaminobenzaldehyde solution R8* and heat on a water-bath for 2 min. Allow to cool. Add 5 ml of *light petroleum R* and shake the mixture vigorously. The aqueous layer shows a blue or greenish-blue colour.

D. Thin-layer chromatography *(2.2.27)*.

Test solution Use solution A prepared in identification test C.

Reference solution Dissolve 10 mg of *cineole R* and 10 mg of *guaiazulene R* in 20 ml of *toluene R*.

Plate *TLC silica gel plate R*.

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R*. Heat at 100-105 °C for 5-10 min and examine in daylight.

Results The chromatogram obtained with the reference solution shows in the upper part a red zone (guaiazulene) and in the middle part a blue or greyish-blue zone (cineole). The chromatogram obtained with the test solution shows a violet zone a little above the zone due to guaiazulene in the chromatogram obtained with the reference solution; below this zone a reddish-violet zone; below which, 1-2 not clearly separated greyish-violet or greyish zones (which changes to greenish-grey after a few hours) and a reddish-violet zone a little above the zone due to cineole in the chromatogram obtained with the reference solution. Further faint zones are present.

TESTS

Foreign matter *(2.8.2)*

Maximum 5 per cent of stems with a diameter greater than 3 mm and maximum 2 per cent of other foreign matter.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 0.500 g of powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 10.0 per cent.

Ash insoluble in hydrochloric acid *(2.8.1)*

Maximum 2.5 per cent.

ASSAY

Essential oil

Carry out the determination of essential oils in herbal drugs *(2.8.12)*. Use 20.0 g of cut drug, a 1000 ml round-bottomed flask and 500 ml of a mixture of 1 volume of *water R* and 9 volumes of *ethylene glycol R* as the distillation liquid. Add 0.2 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

Stop cooling at the end of distillation and continue distilling until the blue, steam-volatile components have reached the lower end of the cooler. Immediately start cooling again, taking care to avoid warming the separation space. Stop the distillation after 5 min. Replace the 1000 ml round-bottomed flask by a 250 ml round-bottomed flask containing a mixture of 0.4 ml of *xylene R* and 50 ml of *water R*. Distil for 15 min. After 10 min read the total volume. To determine the blank value, use 0.2 ml of *xylene R* in the graduated tube and distil a mixture of 0.4 ml of *xylene R* and 50 ml of *water R* for 15 min.

Proazulenes

To ensure that as little water as possible is transferred, transfer the blue essential oil-xylene mixture obtained in the assay of essential oil into a 50 ml volumetric flask with the aid of small portions of *xylene R*, rinsing the graduated tube of the apparatus with *xylene R* and dilute to 50.0 ml with the same solvent. Measure the absorbance *(2.2.25)* at 608 nm using *xylene R* as the compensation liquid.

Calculate the percentage content of proazulenes, expressed as chamazulene, using the following expression:

$$\frac{A \times 2.1}{m}$$

i.e. taking the specific absorbance of chamazulene to be 23.8.

A = absorbance at 608 nm

m = mass of the drug to be examined, in grams.

Ph Eur

Apomorphine Hydrochloride for Homoeopathic Preparations

General Notices

Apomorphinum Muriaticum for Homoeopathic Preparations

DEFINITION

Apomorphine Hydrochloride for Homoeopathic Preparations contains Apomorphine Hydrochloride.

PRODUCTION OF STOCK

The first trituration of Apomorphine Hydrochloride for Homoeopathic Preparations is prepared using a suitable quantity of Lactose or Anhydrous Lactose as the vehicle and a validated trituration method that ensures homogeneity is achieved. The vehicle complies with the statement under Vehicles in the monograph for Homoeopathic Preparations.

Content of apomorphine hydrochloride, $C_{17}H_{17}NO_2$,HCl

The first decimal trituration contains 9.5% to 10.5% of $C_{17}H_{17}NO_2$,HCl (dried substance).

CHARACTERISTICS

The first decimal trituration is a white powder.

IDENTIFICATION

Dissolve 2.5 g of the substance being examined without heating in *water* and dilute to 25 ml with the same solvent (solution S).

A. To 5 ml of solution S add a few millitres of *sodium hydrogen carbonate solution* until a permanent, white precipitate is formed. The precipitate slowly becomes a greenish colour. Add 0.25 ml of *0.05M iodine* and shake. The precipitate becomes a greyish-green colour. Collect the precipitate. The precipitate dissolves in *ether* giving a purple solution, dissolves in *dichloromethane chloride* giving a violet-blue solution and dissolves in *alcohol* giving a blue solution.

B. To 2 ml of solution S add 0.1 ml of *nitric acid*, mix and filter. The filtrate yields reaction A characteristic of *chlorides,* Appendix VI.

C. Dissolve 0.25 g of the substance being examined in 5 ml of *water*. Add 5 ml of *ammonia* and heat in a water-bath at 80° for 10 minutes. A red colour develops.

ASSAY

Disperse 2.5 g of the substance being examined in a mixture of 5.0 ml of 0.01M *hydrochloric acid* and 50 ml of *ethanol (96%)*. Carry out the method for *potentiometric titration*, Appendix VIII B, using 0.1M *sodium hydroxide*. Measure the titrant between the first 2 points of inflexion. Each ml of 0.1M *sodium hydroxide* is equivalent to 30.38 mg of $C_{17}H_{17}NO_2$,HCl.

Arsenious Trioxide for Homoeopathic Preparations

General Notices

(Ph Eur monograph 1599)

As_2O_3 197.8 *1327-53-3*

Ph Eur

DEFINITION

Content

99.5 per cent to 100.5 per cent of As_2O_3.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble to sparingly soluble in water. It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Dissolve 20 mg in 1 ml of *dilute hydrochloric acid R*, add 4 ml of *water R* and 0.1 ml of *sodium sulphide solution R*. The resulting yellow precipitate is soluble in *dilute ammonia R1.*

B. Dissolve 20 mg in 1 ml of *hydrochloric acid R1*, add 5 ml of *hypophosphorous reagent R* and heat for 15 min on a water-bath. A black precipitate develops.

TESTS

Appearance of solution

A 100 g/l solution in *dilute ammonia R1* is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Sulphides

Dissolve 1.0 g in 10.0 ml of *dilute sodium hydroxide solution R*. Add 0.05 ml of *lead acetate solution R*. Any colour in the test solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 10.0 ml of a 0.015 g/l solution of *sodium sulphide R* in *dilute sodium hydroxide solution R* and 0.05 ml of *lead acetate solution R* (20 ppm).

ASSAY

Dissolve 40.0 mg in a mixture of 10 ml of *water R* and 10 ml of *dilute sodium hydroxide* *solution R*. Add 10 ml of *dilute hydrochloric acid R* and 3 g of *sodium hydrogen carbonate R* and mix. Add 1 ml of *starch solution R* and titrate with *0.05 M iodine*.

1 ml of *0.05 M iodine* is equivalent to 4.946 mg of As_2O_3.

Ph Eur

Artemisia Cina for Homoeopathic Preparations

General Notices

DEFINITION

The dried, unexpanded flower heads of *Artemisia cina* O.C.Berg et C.F. Schmidt.

IDENTIFICATION

A. The dried, unexpanded and slightly shiny capitula are 2 to 4 mm long and 0.5 to 1.5 mm wide, acuminate, yellow-green to slightly brownish with 3 to 6 small hermaphrodite disk flowers in various stages of development; these are enclosed by 12 to 20 bracts arranged like roof tiles. The outer, smaller bracts in the lower area of the cylindrically formed inflorescence base, are keel-shaped, broad elliptic with a keel-shaped midrib frequently appearing at the outer margin; the inner, superior bracts are tongue-shaped, linear elongated and flat; both are apically rounded.

B. Reduce to a powder and examine under a microscope using *chloral hydrate solution*. Abundant fragments of the involucral bracts in surface view. Fragments from the margins are usually only one or two cells thick and are composed of very thin-walled, elongated cells. Towards the central region the bracts become thicker and small, thin-walled palisade cells can be seen underlying the epidermis; on the inner epidermis in this region the cells become somewhat less elongated and the walls show slight beading. The cells of the outer epidermis near the central region are irregularly polygonal in outline and fairly numerous anomocytic stomata are present. Glandular trichomes occur on the bracts and are particularly abundant on the outer epidermis near the central region. Some of the fragments from near the base of the thicker region of the bracts show the presence of very small cluster crystals of calcium oxalate in the underlying tissues.

Very occasional fragments of the bracts occur in sectional view and these show the presence of a fairly thick cuticle and, in fragments from near the central region, a one- or two-layered palisade.

Groups of sclereids from the central region of the bracts whose individual cells vary in shape but are usually considerably elongated; the ends are square or bluntly tapering or, occasionally, somewhat enlarged; the walls are strongly thickened and have scattered pits. Small groups of these sclereids are occasionally found attached to fragments of the epidermis of the bracts.

The covering trichomes occur on the central region of the bracts but they are nearly always found detached; they are not very numerous; they are unicellular and usually very thin-walled although slight thickening may occur in the basal region; some of these trichomes are very long and they are frequently found in groups forming loosely felted, cottony masses. The glandular trichomes are very abundant; they occur on the bracts and are also frequently found detached; each has a short, biseriate stalk, usually composed of two cells and a biseriate head with two or four cells; around each head the cuticle is raised to form a bladder-like covering.

Abundant pollen grains, which are fairly small, spherical, with three pores and three furrows; the exine is finely warted. A large number of immature pollen grains are present, forming elongated, closely packed masses.

Fragments of the fibrous layer of the anthers composed of very thin-walled, rather indistinct cells; the rods of thickening on the walls are very thin and appear as small, elongated beads in surface view.

Fragments of the epidermis of the pedicel in surface view show small rectangular cells with slightly and unevenly thickened walls; the underlying palisade cells are small, thin-walled and closely packed.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 10 ml of *ethanol* (90%) to 1 g of the coarsely powdered drug, stir for one hour and filter.

(2) Dissolve 10 mg each of *santonin* and 10 mg of *cineole* in 10 ml of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H.*

(b) Mobile phase is a mixture of 5 volumes of *glacial acetic acid* and 45 volumes of *hexane* and 50 volumes *ethyl acetate*.

(c) Application volume 10 µl for each solution.

(d) Develop to 10 cm.

(e) Remove the plate, allow to dry in air, spray with *ethanolic phosphomolybdic acid solution*, heat at 100º to 105º for about 5 minutes and examine in daylight.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows the grey-blue santonin zone just between the lower third and middle third and the grey-blue cineole zone in the upper third.

VALIDITY

The chromatogram obtained with solution (1) shows a grey-blue zone below the santonin zone of solution (2), a strong grey-blue zone correlating with that of santonin in solution (2) and one or two grey-blue zones just above it; one or two grey-blue zones between santonin and cineole zones and a strong grey-blue zone at the height of the cineole zone.

TESTS

Foreign matter

Not more than 5% of sections of stem and pieces of narrow-linear hairy leaves; not more than 2% of other foreign matter, Appendix XI D.

Ash

Not more than 11.0%, Appendix XI J, Method II.

Loss on drying

Not more than 10.0%, Appendix IX D.

MOTHER TINCTURE

The mother tincture complies with the requirements stated under Mother Tinctures for Homoeopathic Preparations and with the following requirements.

PRODUCTION

The mother tincture of *Artemisia cina* O.C. Berg et C.F. Schmidt is prepared from the powdered drug using *Method 4a* described in the monograph for Methods of Preparation of Homoeopathic Stocks and Potentisation. Use 86% w/w (90% v/v) of *ethanol*.

CHARACTERISTICS

The mother tincture is a golden yellow to greenish liquid.

IDENTIFICATION

The mother tincture complies with Identification test C above using the mother tincture as solution (1).

TESTS

Ethanol

40% to 46% w/w (47% to 54% v/v), Appendix VIII F.

Dry residue

Not less than 1.8% w/w, Appendix XI P.

Relative density

0.835 to 0.855, Appendix V G.

Barium Chloride Dihydrate for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2142)

$BaCl_2,2H_2O$ 244.3 *10326-27-9*

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of $BaCl_2,2H_2O$.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, very slightly soluble or practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 0.1 g in 1 ml of *water R*. Add 0.3 ml of *dilute sulphuric acid R*. A white precipitate is formed; it is insoluble in *dilute hydrochloric acid R* and in *dilute nitric acid R*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *water R* and dilute to 100 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

ASSAY

Dissolve 0.200 g in 100 ml of *water R*. Add 100 ml of *methanol R*, 10 ml of *concentrated ammonia R* and 2 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to colourless.

1 ml of *0.1 M sodium edetate* is equivalent to 24.43 mg of $BaCl_2,2H_2O$.

Ph Eur

Cadmium Sulphate Hydrate for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2143)

$CdSO_4,8/3H_2O$ 256.5 *10124-36-4*

Ph Eur

DEFINITION

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives reaction (a) of sulphates (*2.3.1*).

B. To 2 ml of solution S (see Tests) add 2 ml of *sodium sulphide solution R*. A yellow precipitate is formed.

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 ml of solution S add 0.3 ml of *methyl orange solution R*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Nitrates

Maximum 100 ppm.

Dissolve 1.0 g in *water R* and dilute to 20.0 ml with the same solvent. To 1.0 ml of this solution add 0.2 ml of a 10 g/l solution of *sulphanilic acid R* in *acetic acid R* and 0.2 ml of a recently prepared 3 g/l solution of *naphthylamine R* in *acetic acid R*. Add a turning of *zinc R*. A pink colour is produced within 5 min. It is not more intense than that of a mixture of 0.5 ml of *nitrate standard solution (10 ppm* NO_3*) R* and 0.5 ml of *water R*, prepared at the same time.

Zinc sulphate, alkaline-earth sulphates, rare-earth sulphates

Dissolve 1.0 g in 17 ml of *water R*. Add 0.5 ml of *hydrochloric acid R* and 1 g of *thioacetamide R.* Heat in a water-bath for 10 min. Dilute to 20.0 ml with *water R* and filter. Evaporate 10.0 ml of this solution to dryness in an oven. Ignite the residue at about 800 ± 50 °C to constant mass. The residue weighs a maximum of 2 mg.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 5 ml of solution S.

Water (*2.5.12*)

16.0 per cent to 20.0 per cent, determined on 80 mg. Shake for 10 min before carrying out the determination.

ASSAY

Dissolve 0.200 g in 50 ml of *water R*. Add 10 ml of *ammonium chloride buffer solution pH 10.0 R* and 50 mg of *mordant black 11 triturate R1*. Titrate with *0.1 M sodium edetate* until the colour changes from red to green.

1 ml of *0.1 M sodium edetate* is equivalent to 20.85 mg of $CdSO_4$.

Ph Eur

Calcium Iodide Tetrahydrate for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2144)

$CaI_2,4H_2O$ 366.0 *13640-62-5*

Ph Eur

DEFINITION

Content

97.0 per cent to 102.0 per cent of CaI_2 (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, very hygroscopic.

Solubility

Very soluble to freely soluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of calcium (*2.3.1*).

B. Solution S (see Tests) gives reaction (b) of iodides (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_5 (*2.2.2, Method II*).

Free iodine, iodates

To 5 ml of solution S add 2 ml of *methylene chloride R.* Shake and allow to stand. The organic layer is colourless (*2.2.2, Method I*) (free iodine). Add 0.2 ml of *dilute sulphuric acid R*. Shake and allow to stand. The organic layer remains colourless (*2.2.2, Method I*) (iodates).

Sulphates (*2.4.13*)

Maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*)

Maximum 10 ppm, determined on 10 ml of solution S.

Heavy metals (*2.4.8*)

Maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Water (*2.5.12*)

18.0 per cent to 22.0 per cent, determined on 0.100 g.

ASSAY

Dissolve 0.300 g in 50 ml of *water R*. Add 5 ml of *dilute nitric acid R* and 25.0 ml of *0.1 M silver nitrate*. Shake. Add 2 ml of *ferric ammonium sulphate solution R2* and titrate with *0.1 M ammonium thiocyanate* until the colour changes to reddish-yellow.

1 ml of *0.1 M silver nitrate* is equivalent to 14.70 mg of CaI_2.

STORAGE

In an airtight container .

Ph Eur

Calcium Phosphate for Homoeopathic Preparations

General Notices

Calcium Phosphoricum for Homoeopathic Preparations

DEFINITION

Calcium Phosphate for Homoeopathic Preparations contains Calcium Phosphate.

PRODUCTION OF STOCK

The first trituration of Calcium Phosphate for Homoeopathic Preparations is prepared using a suitable quantity of a vehicle, such as Lactose, Anhydrous Lactose or Sucrose, and a validated method for trituration that ensures homogeneity is achieved. The vehicle complies with the statement under Vehicles in the monograph for Homoeopathic Preparations.

Content of calcium Ca

The first decimal trituration contains 3.5% to 4.0% of Ca.

CHARACTERISTICS

The first decimal trituration is a white powder.

IDENTIFICATION

Wash 5 g of the first decimal trituration of the substance being examined with three 10-ml quantities of *water.* The dried residue complies with the following tests.

A. Dissolve 0.1 g of the dried residue in 5 ml of a 25% v/v solution of *nitric acid .* The resulting solution yields reaction B of *phosphates*, Appendix VI.

B. The dried residue yields reaction B characteristic of *calcium salts*, Appendix VI. Filter before adding *potassium ferrocyanide solution.*

C. The dried residue complies with the limits of the Assay.

D. If the preparation includes Lactose as the vehicle, it complies with the following test. Dissolve 0.25 g in 5 ml of *water*. Add 5 ml of *ammonia* and heat in a water-bath at 80º for 10 minutes. A red colour develops.

E. If the preparation includes Sucrose as the vehicle, it complies with the following test. Dissolve 5.0 g in *carbon dioxide-free water* and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 100 ml with *water*. To 5 ml of the solution add 0.15 ml of freshly prepared *copper sulphate solution* and 2 ml of freshly prepared *dilute sodium hydroxide solution*. The solution is blue and clear and remains so after boiling. To the hot solution add 4 ml of *dilute hydrochloric acid* and boil for 1 minute. Add 4 ml of *dilute sodium hydroxide solution.* An orange precipitate is formed immediately.

ASSAY

Dissolve 0.2 g of the residue in a mixture of 1.0 ml of hydrochloric acid R1 and 5 ml of water. Add 25.0 ml of 0.1M disodium edetate and dilute to 200 ml with water. Adjust to about pH 10 with concentrated ammonia. Add 10 ml of ammonia buffer pH 10.0 and a few milligrams of mordant black 11 triturate. Titrate the excess disodium edetate with 0.1M zinc sulphate until the colour changes from blue to violet. Each ml of 0.1M sodium hydroxide is equivalent to 4.008 mg of Ca.

Common Stinging Nettle for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2030)

Ph Eur

DEFINITION

Whole, fresh, flowering plant of *Urtica dioica* L.

CHARACTERS

The plant causes an itching, burning sensation on the skin.

IDENTIFICATION

A. Common stinging nettle is perennial. The taproot sends out creeping subterranean rhizomes, more or less 4-angled in transverse section, from which extend adventious secondary roots and very numerous brownish hairy rootlets. The stipes are erect, generally unbranched, 3-5 mm in diameter and 0.3-1.5 m high, rarely up to 2.5 m high, 4-angled, greyish-green and covered in short hairs and stinging hairs.

The decussate leaves are 30-150 mm long and 20-80 mm wide. The petiole is hispid and usually slightly less than one-third the length of the lamina. The leaf blade is ovate, acuminate, cordate or rounded at the base, and coarsely dentate; the apical tooth is distinctly larger than the lateral teeth. The upper side of the leaves is dark green and usually matt, both sides bear short serried hairs intermingled with long stinging hairs. The 2 stipules are linear-subulate and free. The inflorescences growing from the leaf axils are complex, the flowers unisexual, and, particularly in male plants, generally distinctly longer than the petiole. After shedding their pollen, male inflorescences are erect at an oblique angle or horizontal; female inflorescences are pendent when the fruit is ripe. All flowers have long stalks. The perianth of the male flowers is divided half-way down into equal green lobes, widest at their base, with short bristles and stinging hairs at the margins. The stamens are equal and opposite to the perianth segments, each with a long, whitish filament that curves inwards before pollen is shed and spreads out afterwards. The ovary is rudimentary, button or cup-shaped. The perianth of the female flowers is downy or bristly on the outside and consists of outer, and 2 inner segments; the inner segments are about twice the length of the outer ones. The hypogynous, ovate, unilocular ovary bears a large capitate stigma with a brush-like shock of hair. As the one-seeded fruit grows ripe, the 2 inner segments of the perianth fold around it like wings.

B. It complies with the test for *Urtica urens* (see Tests).

TESTS

Urtica urens

The margin of the lamina is not serrate with teeth twice as long as wide. The clusters of flowers in the axils are longer than the petiole of the leaf. Unisexual, apetalous flowers are not together on the same plant and in the same cluster.

Foreign matter *(2.8.2)*

Maximum 5 per cent.

Loss on drying *(2.2.32)*

Minimum 65.0 per cent, determined on 5.0 g of finely cut drug by drying in an oven at 105 °C for 2 h, if performed to demonstrate the freshness of the drug.

MOTHER TINCTURE

The mother tincture complies with the requirements of the general monograph on *Mother tinctures for homoeopathic preparations (2029).*

PRODUCTION

The mother tincture of *Urtica dioica* L. is prepared by maceration using alcohol of a suitable concentration.

CHARACTERS

Appearance

Greenish-brown or orange-brown liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution The mother tincture to be examined.

Reference solution Dissolve 10 mg of *phenylalanine R* and 10 mg of *serine R* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 10 ml with the same mixture of solvents.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R*, *water R*, *acetone R*, *butanol R* (10:20:35:35 *V/V/V/V*).

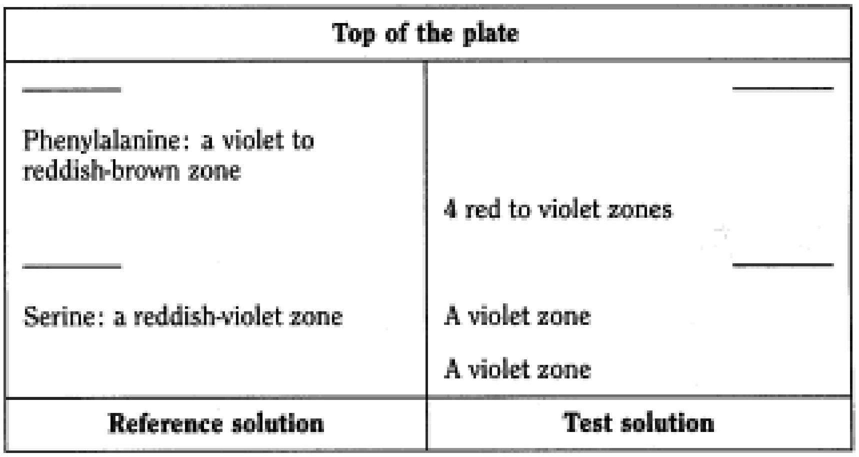
Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a 1 g/l solution of *ninhydrin R* in *alcohol R*. Heat the plate at 105-110 °C for 5-10 min then examine in daylight within 10 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.



Top of the plate	
———	———
Phenylalanine: a violet to reddish-brown zone	
	4 red to violet zones
———	———
Serine: a reddish-violet zone	A violet zone
	A violet zone
Reference solution	**Test solution**

TESTS

Relative density *(2.2.5)*

0.930 to 0.950.

Ethanol *(2.9.10)*

40 per cent *V/V* to 56 per cent *V/V*.

Methanol *(2.9.11)*

Maximum 0.10 per cent *V/V.*

Dry residue *(2.8.16)*

Minimum 1.1 per cent.

Ph Eur

Copper Acetate Monohydrate for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2146)

$Cu(C_2H_3O_2)_2,H_2O$ 199.7 *6046-93-1*

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of $Cu(C_2H_3O_2)_2,H_2O$.

CHARACTERS

Appearance

Geenish-blue crystals or green powder.

Solubility

Soluble in water, slightly soluble or very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives reaction (a) of acetates (*2.3.1*).

B. Dissolve 0.1 g in 10 ml of *water R* and add *dilute ammonia R1* dropwise. A dark blue colour is produced.

TESTS

Solution S

Dissolve 3.0 g in a mixture of 40 ml of *distilled water R* and 0.6 ml of *glacial acetic acid R*, with heating at 70 °C. Cool and dilute to 45 ml with *distilled water R*.

Appearance of solution

Solution S is clear (*2.2.1*).

Impurities not precipitating with hydrogen sulphide: maximum 0.1 per cent, calculated as sulphates.

To 2.000 g add 92 ml of *water R* and 8.0 ml of *dilute sulphuric acid R*. Heat to 70 °C. Pass a current of *hydrogen sulphide R* until there is no longer precipitation of copper sulphide. Allow to cool and stand, then filter. Evaporate to dryness 50.0 ml of the filtrate in a crucible. Ignite the residue at about 600 ± 50 °C to constant mass.

Chlorides (*2.4.4*)

Maximum 50 ppm, determined on 15 ml of solution S.

Sulphates (*2.4.13*)

Maximum 150 ppm, determined on 15 ml of solution S.

Iron (*2.4.9*)

Maximum 20 ppm.

Dissolve 0.500 g in 10 ml of *water R*. Transfer to a separating funnel. Add 20 ml of *hydrochloric acid R1* and 10 ml of *methyl isobutyl ketone R*. Shake vigorously for 3 min. Allow to stand. Transfer the organic layer to a second separating funnel and add 10 ml of *water R.* Shake vigorously for 3 min. Allow to stand. The aqueous layer complies with the limit test for iron.

Nickel

Maximum 10 ppm.

To the residue obtained in the test for impurities not precipitating with hydrogen sulphide, add 2.0 ml of *hydrochloric acid R* and 1.0 ml of *sulphuric acid R*. Evaporate to dryness. Dissolve the residue in a mixture of 3.0 ml of *dilute sulphuric acid R* and 17.0 ml of *water R*. To 4.0 ml of this solution add 4.0 ml of *water R*, 5.0 ml of *bromine water R*, 7.0 ml of *dilute ammonia R1* and 3.0 ml of a 10 g/l solution of *dimethylglyoxime R* in *ethanol (90 per cent V/V) R*. This solution is not more intensely coloured within 1 min than a solution prepared as follows: mix 4.0 ml of a 1 ppm solution of nickel (Ni) prepared from *nickel standard solution (10 ppm Ni) R*, 4.0 ml of *water R* and 5.0 ml of *bromine water R*; carefully add 7.0 ml of *dilute ammonia R1* and 3.0 ml of a 10 g/l solution of *dimethylglyoxime R* in *ethanol (90 per cent V/V) R.*

ASSAY

Dissolve 0.400 g in *water R* and dilute to 50 ml with the same solvent. Add 6.0 ml of *glacial acetic acid R*, 10.0 g of *potassium iodide R* and 1 ml of *starch solution R*. Titrate with *0.1 M sodium thiosulphate.*

1 ml of *0.1 M sodium thiosulphate* is equivalent to 19.97 mg of $Cu(C_2H_3O_2)_2,H_2O$.

Ph Eur

Copper for Homoeopathic Preparations

General Notices

Copper for Homoeopathic Use

(Ph Eur monograph 1610)

Cu 63.5 *7440-50-8*

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of Cu.

CHARACTERS

Appearance

Reddish-brown powder.

Solubility

Practically insoluble in water, soluble in hydrochloric acid and in nitric acid, practically insoluble in alcohol.

IDENTIFICATION

A. To 2 ml of solution S (see Tests) add 0.5 ml of *potassium ferrocyanide solution R*. A reddish-brown precipitate is formed.

B. To 5 ml of solution S add 0.6 ml of *ammonia R*. A blue precipitate is formed. Add 2 ml of *ammonia R*. The precipitate disappears; the solution has an intense blue colour.

TESTS

Solution S

Dissolve 2.0 g in 10 ml of *nitric acid R*. After nitrous fumes are no longer evolved, dilute to 60 ml with *distilled water R*.

Acidity or alkalinity

To 5.0 g add 20 ml of *carbon dioxide-free water R*. Boil for 1 min. Cool. Filter and dilute to 25.0 ml with *carbon dioxide free water R*. To 10 ml of the solution add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides *(2.4.4)*

Maximum 100 ppm.

15 ml of solution S complies with the limit test for chlorides.

Sulphates *(2.4.13)*

Maximum 300 ppm.

15 ml of solution S complies with the limit test for sulphates.

Iron

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 1.00 g in 5 ml of *nitric acid R* and dilute to 50.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, diluted as necessary with a 1 per cent *V/V* solution of *nitric acid R*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Flame Air-acetylene.

Lead

Maximum 1.00×10^2 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use the test solution prepared for the test for iron.

Reference solutions Prepare the reference solutions using *lead standard solution (0.1 per cent Pb) R*, diluted as necessary with a 1 per cent *V/V* solution of *nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm.

Flame Air-acetylene.

Zinc

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Use the test solution prepared for the test for iron.

Reference solutions Prepare the reference solutions using *zinc standard solution (100 ppm Zn) R*, diluted as necessary with a 1 per cent *V/V* solution of *nitric acid R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Flame Air-acetylene.

ASSAY

Dissolve 0.100 g in 5 ml of *nitric acid R*. Heat to expel the nitrous fumes. Add 200 ml of *water*

R and neutralise *(2.2.3)* with *dilute ammonia R1*. Add 1 g of *ammonium chloride R* and 3 mg of *murexide R*. Titrate with *0.1 M sodium edetate* until the colour changes from green to violet.

1 ml of *0.1 M sodium edetate* is equivalent to 6.354 mg of Cu.

Ph Eur

Garlic for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2023)

Ph Eur

DEFINITION

Fresh bulb of *Allium sativum* L.

CHARACTERS

It has a characteristic odour after cutting.

IDENTIFICATION

The bulb is generally 3 cm to 5 cm broad and almost spherical; the flat base bears the remnants of numerous short greyish-brown adventitious roots. The bulb consists of about 10 daughter bulbs (cloves) arranged roughly in a circle around a central axis. Individual daughter bulbs are 1 cm to 3 cm long, laterally compressed and convex on the dorsal side. Each daughter bulb has a tough, white or reddish skin around a fleshy tubular leaf, investing a more or less rounded elongated cone of leaf primordia and vegetative apex.

TESTS

Water *(2.2.13)*

Minimum 55.0 per cent, determined on 10.0 g of the finely cut drug, if performed to demonstrate the freshness of the drug.

MOTHER TINCTURE

The mother tincture complies with the requirements of the general monograph on *Mother tinctures for homoeopathic preparations (2029).*

PRODUCTION

The mother tincture of *Allium sativum* L. is prepared by maceration of the cut drug using alcohol of a suitable concentration.

CHARACTERS

Appearance

Brownish-yellow liquid.

It has a peculiar and unpleasant aromatic odour.

IDENTIFICATION

A. To 2 ml of the mother tincture to be examined, add 0.2 ml of *dilute sodium hydroxide solution R*. A yellowish-white precipitate develops.

B. Thin-layer chromatography *(2.2.27)*.

Test solution Extract 5 ml of the mother tincture to be examined with 2 quantities, each of 10 ml, of *ether R*. Combine the ether layers and dry over *anhydrous sodium sulphate R*. Filter and evaporate the filtrate in a water-bath at low temperature. Dissolve the residue in 0.4 ml of *methanol R*.

Reference solution Dissolve 10 mg of *resorcinol R*, 10 mg of *thymol R* and 30 mg of *gallic acid R* in 10 ml of *methanol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous formic acid R*, *toluene R*, *di-isopropyl ether R* (10:40:50 *V/V/V*).

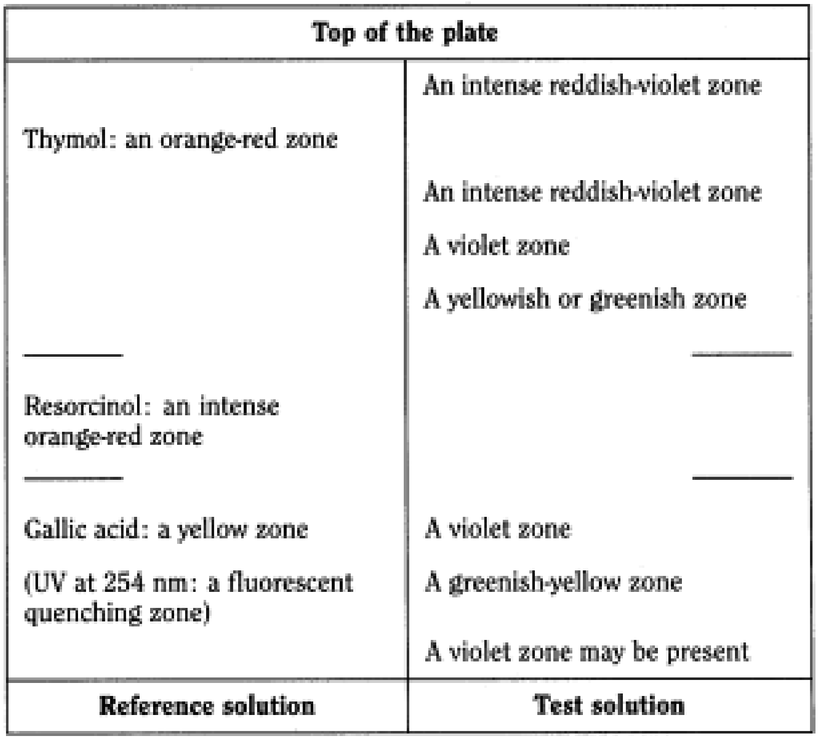
Application 40 µl of the test solution and 10 µl of the reference solution.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm and identify gallic acid; spray with *anisaldehyde solution R*, heat to 105-110 °C for 5-10 min. Examine in daylight within 10 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Other zones may also be visible in the chromatogram obtained with the test solution.



Top of the plate	
	An intense reddish-violet zone
Thymol: an orange-red zone	
	An intense reddish-violet zone
	A violet zone
	A yellowish or greenish zone
_______	_______
Resorcinol: an intense orange-red zone	
_______	_______
Gallic acid: a yellow zone	A violet zone
(UV at 254 nm: a fluorescent quenching zone)	A greenish-yellow zone
	A violet zone may be present
Reference solution	**Test solution**

TESTS

Relative density ***(2.2.5)***

0.885 to 0.960.

Ethanol ***(2.9.10)***

50 per cent *V/V* to 70 per cent *V/V*.

Dry residue ***(2.8.16)***

Minimum 4.0 per cent.

STORAGE

In an airtight container.

Ph Eur

Hedera Helix for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2092)

Ph Eur

DEFINITION

Fresh, young, fully developed but not yet lignified branch of *Hedera helix* L., harvested immediately before or at the beginning of flowering.

IDENTIFICATION

The fresh, young branches of *Hedera helix* L. are thin and flexible, climbing; they cling to their support by stem-roots. The leaves are alternate, simple and petiolate. The petiole shows a cylindrical section. The upper surface of the leaves is glabrous and shiny, darker than the lower surface. The lamina is usually divided into 3 to 5 more or less deeply cut lobes on sterile branches; it is oval, with a pointed apex on fertile branches. The inflorescences are arranged in a simple semi-globular corymb and grouped in terminal clusters. The pedicels of the umbel are covered in whitish hairs. Each flower shows 5 small teeth formed by the upper part of the sepals and 5 petals covered in very small inverted hairs.

TESTS

Foreign matter *(2.8.2)*

If required by the competent authority, maximum 5 per cent.

Loss on drying *(2.2.32)*

If required by the competent authority, minimum 50 per cent, determined on 5.0 g of the finely cut drug by drying in an oven at 105 °C for 2 h.

MOTHER TINCTURE

The mother tincture complies with the requirements of the general monograph on *Mother tinctures for homoeopathic preparations (2029).*

PRODUCTION

The mother tincture of *Hedera helix* L. is prepared by maceration using ethanol of a suitable concentration.

Content

Minimum 0.15 per cent *m/m* of hederacoside C ($C_{59}H_{96}O_{26}$; M_r 1221).

CHARACTERS

Appearance

Dark greenish-brown liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution The mother tincture to be examined.

Reference solution Dissolve 1 mg of α-*hederin R* and 1 mg of *hederacoside C R* in *methanol R* and dilute to 2 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase glacial acetic acid R, *water R*, *butanol R* (1:1:4 *V/V/V*).

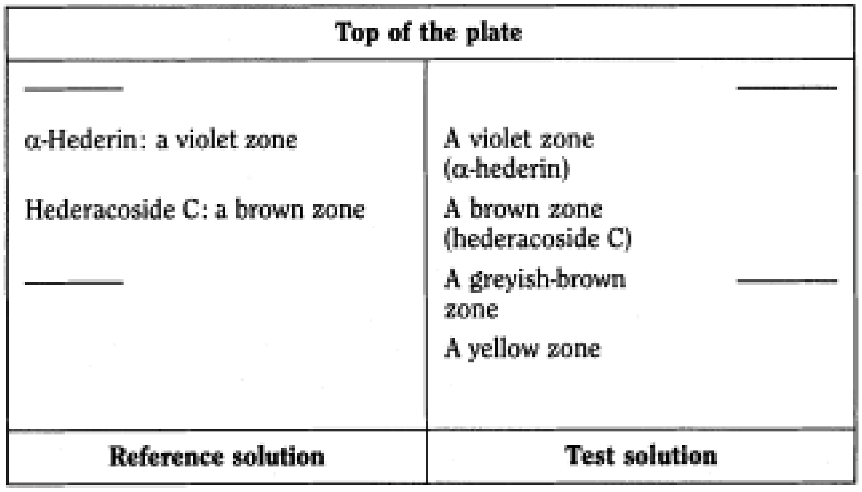
Application 20 µl, as bands.

Development Over half of the plate.

Drying In air.

Detection Spray with a 10 per cent *V/V* solution of *sulphuric acid R* in *methanol R* and heat at 100-105 °C for 10 min. Examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Other faint zones may also be present in the chromatogram obtained with the test solution.



Top of the plate	
———	———
α-Hederin: a violet zone	A violet zone (α-hederin)
Hederacoside C: a brown zone	A brown zone (hederacoside C)
———	A greyish-brown zone ———
	A yellow zone
Reference solution	**Test solution**

TESTS

Relative density *(2.2.5)*

0.890 to 0.925.

Ethanol *(2.9.10)*

60 per cent *V/V* to 70 per cent *V/V*.

Dry residue *(2.8.16)*

Minimum 2.0 per cent.

ASSAY

Liquid chromatography *(2.2.29)*.

Test solution In a 20.0 ml volumetric flask, dilute 3.000 g of the mother tincture to be examined to 20.0 ml with the mobile phase.

Reference solution In a 50.0 ml volumetric flask, dissolve 20.0 mg of *hederacoside C R* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Column:

— *size:* l = 0.25 m, Ø = 4 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *water R* adjusted to pH 3 with *phosphoric acid R* and 65 volumes of *methanol R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µl.

Retention time Hederacoside C = about 8 min.

Calculate the percentage content *m/m* of hederacoside C from the following expression:

$$\frac{A_1 \times m_2 \times C \times 40}{A_2 \times m_1}$$

A_1 = area of the peak due to hederacoside C in the chromatogram obtained with the test solution,

A_2 = area of the peak due to hederacoside C in the chromatogram obtained with the reference solution,

m_1 = mass of the mother tincture in the test solution, in grams,

m_2 = mass of *hederacoside C R* in the reference solution, in grams,

C = percentage content of *hederacoside C R*.

Ph Eur

Herbal Drugs for Homoeopathic Preparations

General Notices

Herbal Drugs for Homoeopathic Use

(Ph Eur monograph 2045)

Ph Eur

DEFINITION

Herbal drugs for homoeopathic preparations are mainly whole, fragmented or cut, plants, parts of plants including algae, fungi or lichens in an unprocessed state, usually in fresh form. The state, fresh or dried, in which the drug is used, is defined in the individual monograph of the European Pharmacopoeia or, in the absence, in the individual monograph of a national Pharmacopoeia. In the absence of such a monograph, the state in which the herbal drug is used has to be defined. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal drugs for homoeopathic preparations. Herbal drugs for homoeopathic preparations are precisely defined by the botanical scientific name of the source species according to the binomial system (genus, species, variety and author).

PRODUCTION

Herbal drugs for homoeopathic preparations are obtained from cultivated or wild plants. Suitable cultivation, harvesting, collection, sorting, drying, fragmentation and storage conditions are essential to guarantee the quality of herbal drugs for homoeopathic preparations.

Herbal drugs for homoeopathic preparations are, as far as possible, free from impurities such as soil, dust, dirt and other contaminants such as fungal, insect and other animal contaminants. They do not present signs of decay.

If a decontaminating treatment has been used, it is necessary to demonstrate that the constituents of the plant are not affected and that no harmful residues remain. The use of ethylene oxide is prohibited for the decontamination of herbal drugs for homoeopathic preparations.

Fresh herbal drugs are processed as rapidly as possible after harvesting. Where justified and authorised for transportation or storage purposes, fresh plant material may be deep-frozen; it may also be kept in ethanol (96 per cent *V/V*) or in ethanol of a suitable concentration, provided the whole material including the storage medium is used for processing.

Adequate measures have to be taken in order to ensure that the microbiological quality of homoeopathic preparations containing 1 or more herbal drugs comply with the recommendations given in the text on *Microbiological quality of pharmaceutical preparations* (*5.1.4*).

IDENTIFICATION

Herbal drugs for homoeopathic preparations are identified using their macroscopic and, where necessary, microscopic descriptions and any further tests that may be required (for example, thin-layer chromatography).

TESTS

When a fresh plant is used as a starting material for the manufacture of homoeopathic preparations, the content of foreign matter is as low as possible; if necessary, the maximum content of foreign matter is indicated in the individual monographs. When a dried plant is used as a starting material for the manufacture of homoeopathic preparations, a test for foreign matter (*2.8.2*) is carried out, unless otherwise prescribed in the individual monographs.

A specific appropriate test may apply to herbal drugs for homoeopathic preparations liable to be falsified.

If appropriate, the herbal drugs for homoeopathic preparations comply with other tests, for example, total ash (*2.4.16*) and bitterness value (*2.8.15*).

The test for loss on drying (*2.2.32*) is carried out on dried herbal drugs for homoeopathic preparations. A determination of water (*2.2.13*) is carried out on herbal drugs for homoeopathic preparations with a high essential oil content. The water content of fresh herbal drugs for homoeopathic preparations may be determined by an appropriate method.

Herbal drugs for homoeopathic preparations comply with the requirements for pesticide residues (*2.8.13*). The requirements take into account the nature of the plant, where necessary the preparation in which the plant might be used, and where available the knowledge of the complete record of treatment of the batch of the plant. The content of pesticide residues may be determined by the method described in the annex to the general method.

The risk of contamination of herbal drugs for homoeopathic preparations by heavy metals must be considered. If an individual monograph does not prescribe limits for heavy metals or specific elements, such limits may be required if justified.

Limits for aflatoxins may be required.

In some specific circumstances, the risk of radioactive contamination is to be considered.

ASSAY

Where applicable, herbal drugs for homoeopathic preparations are assayed by an appropriate method.

STORAGE

Store dried herbal drugs protected from light.

Ph Eur

Homoeopathic Preparations

General Notices

(Ph Eur monograph 1038)

Ph Eur

DEFINITION

Homoeopathic preparations are prepared from substances, products or preparations called stocks, in accordance with a homoeopathic manufacturing procedure. A homoeopathic preparation is usually designated by the Latin name of the stock, followed by an indication of the degree of dilution.

Raw materials

Raw materials for the production of homoeopathic preparations may be of natural or synthetic origin.

For raw materials of zoological or human origin, adequate measures are taken to minimise the risk of agents of infection, including viruses *(5.1.7)*, in the homoeopathic preparations. For this purpose, it is demonstrated that:

— the method of production includes a step or steps that have been shown to remove or inactivate agents of infection;

— where applicable, raw materials of zoological origin comply with the monograph *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)*;

— where applicable, the animals and the tissues used to obtain the raw materials comply with the health requirements of the competent authorities for animals for human consumption;

— for materials of human origin, the donor follows the recommendations applicable to human blood donors and to donated blood (see *Human plasma for fractionation (0853)*), unless otherwise justified and authorised.

A raw material of botanical, zoological or human origin may be used either in the fresh state or in the dried state. Where appropriate, fresh material may be kept deep-frozen. Raw materials of botanical origin comply with the requirements of the monograph *Herbal drugs for homoeopathic preparations (2045)*.

Where justified and authorised for transportation or storage purposes, fresh plant material may be kept in ethanol (96 per cent *V/V*) or in alcohol of a suitable concentration, provided the whole material including the storage medium is used for processing.

Raw materials comply with any requirements of the relevant monographs of the European Pharmacopoeia.

Vehicles

Vehicles are excipients used for the preparation of certain stocks or for the potentisation process. They may include, for example: purified water, alcohol of a suitable concentration, glycerol and lactose.

Vehicles comply with any requirements of the relevant monographs of the European Pharmacopoeia.

Stocks

Stocks are substances, products or preparations used as starting materials for the production of homoeopathic preparations. A stock is usually one of the following: a mother tincture or a glycerol macerate, for raw materials of botanical, zoological or human origin, or the substance itself, for raw materials of chemical or mineral origin.

Mother tinctures comply with the requirements of the monograph *Mother tinctures for homoeopathic preparations (2029)*.

Glycerol macerates are liquid preparations obtained from raw materials of botanical, zoological or human origin by using glycerol or a mixture of glycerol and either alcohol of a suitable concentration or a solution of sodium chloride of a suitable concentration.

Potentisation

Dilutions and triturations are obtained from stocks by a process of potentisation in accordance with a homoeopathic manufacturing procedure: this means successive dilutions and succussions, or successive appropriate triturations, or a combination of the 2 processes.

The potentisation steps are usually one of the following:

— 1 part of the stock plus 9 parts of the vehicle; they may be designated as 'D', 'DH' or 'X' (decimal);

— 1 part of the stock plus 99 parts of the vehicle; they may be designated as 'C' or 'CH' (centesimal).

The number of potentisation steps defines the degree of dilution; for example, 'D3', '3 DH' or '3X' means 3 decimal potentisation steps, and 'C3', '3 CH' or '3C' means 3 centesimal potentisation steps.

'LM-' (or 'Q-') potencies are manufactured according to a specific procedure.

Dosage forms

A dosage form of a homoeopathic preparation complies with any relevant dosage form monograph in the European Pharmacopoeia, and with the following:

— for the purpose of dosage forms for homoeopathic use, 'active substances' are considered to be 'dilutions or triturations of homoeopathic stocks';

— these dosage forms are prepared using appropriate excipients;

— the test for uniformity of content is normally not appropriate; however, in certain circumstances, it is required.

Homoeopathic dosage form pillule
Pillules for homoeopathic use are solid preparations obtained from sucrose, lactose or other suitable excipients. They may be prepared by impregnation of preformed pillules with a dilution or dilutions of homoeopathic stocks or by progressive addition of these excipients and the addition of a dilution or dilutions of homoeopathic stocks. They are intended for oral or sublingual use.

Homoeopathic dosage form tablet

Tablets for homoeopathic use are solid preparations obtained from sucrose, lactose or other suitable excipients according to the monograph *Tablets (0478)*. They may either be prepared by compressing one or more solid active substances with the excipients or by impregnating preformed tablets with a dilution or dilutions of homoeopathic stocks. The preformed tablets for impregnation are obtained from sucrose, lactose or other suitable excipients according to the monograph *Tablets (0478)*. They are intended for oral or sublingual use.

Ph Eur

Honey Bee for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2024)

Ph Eur

DEFINITION

Live worker honey bee (*Apis mellifera* L.).

CHARACTERS

Characters described under Identification.

PRODUCTION

If the bee has been exposed to treatment to prevent or cure diseases, appropriate steps are taken to ensure that the levels of residues are as low as possible.

IDENTIFICATION

The body of a honey bee is about 15 mm long, black, with a silky sheen, and covered with red hairs with a touch of grey. The broad tibiae are without spines. The posterior margins of the segments and legs are brown, with gradual transition to orange-red. The claws are two-membered, the maxillary palps single-membered. On the hind legs are baskets or scoops invested with bristles. The wings have 3 complete cubital cells, with the radial cell twice as long as it is wide; the 3 cells on the lower margin and the 3 middle cells are closed. A duct connects the barbed sting with the poison sac.

MOTHER TINCTURE

The mother tincture complies with the requirements of the general monograph on *Mother tinctures for homoeopathic preparations (2029).*

PRODUCTION

The mother tincture of *Apis mellifera* L. is prepared by maceration using alcohol of a suitable concentration.

CHARACTERS

Pale yellow liquid that may darken on storage.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution The mother tincture to be examined.

Reference solution Dissolve 12 mg of *4-aminobutanoic acid R*, 12 mg of *leucine R* and 12 mg of *proline R* in 5 ml of *water R* and dilute to 50 ml with *alcohol R.*

Plate *TLC silica gel plate R.*

Mobile phase *water R*, *ethanol R* (17:63 *V/V*).

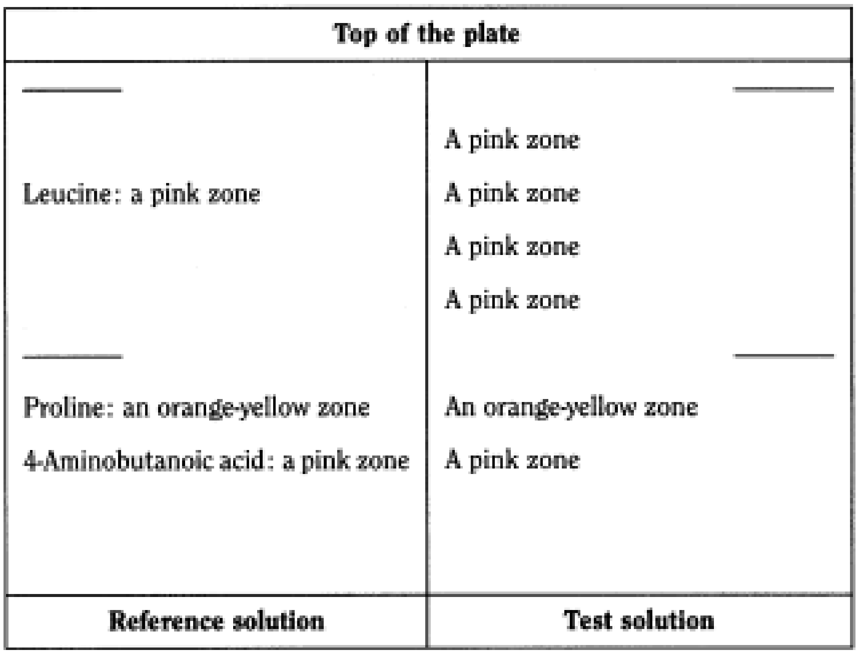
Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 10 min; examine in daylight.

Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions. Other zones may also be visible.



Top of the plate	
———	———
	A pink zone
Leucine: a pink zone	A pink zone
	A pink zone
	A pink zone
———	———
Proline: an orange-yellow zone	An orange-yellow zone
4-Aminobutanoic acid: a pink zone	A pink zone
Reference solution	**Test solution**

TESTS

Relative density *(2.2.5)*

0.890 to 0.910.

Ethanol *(2.9.10)*

60 per cent *V/V* to 70 per cent *V/V*.

Dry residue *(2.8.16)*

Minimum 0.30 per cent.

Ph Eur

Hydrated Iron(II) and Iron(III) Phosphate for Homoeopathic Preparations

General Notices

DEFINITION

Hydrated Iron(II) and Iron(III) Phosphate for Homoeopathic Preparations contains a mixture of hydrated iron(II) phosphate and iron(III) phosphate and some hydrated oxides of iron. It contains not less than 16.0% of Fe^{2+}, equivalent to not less than 47.9% of $Fe_3(PO_4)_{2,}8H_2O$.

CHARACTERISTICS

A slate blue amorphous powder.

Insoluble in *water;* soluble in *hydrochloric acid*.

IDENTIFICATION

Dissolve 0.5 g in 5 ml of *dilute hydrochloric acid* with warming. Dilute the resulting solution to 35 ml with *water* and filter if necessary (solution S).

A. Solution S yields the reactions characteristic of *iron and iron salts*, Appendix VI.

B. Solution S yields the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Heavy metals

Dissolve 1.0 g of the substance being examined in 20 ml of *hydrochloric acid*. Extract the solution using five 20-ml quantities of a mixture of 100 ml of freshly distilled *methyl isobutyl ketone* with 1 ml of *hydrochloric acid R1*. Allow to stand, separate the aqueous layer and evaporate to half its volume, allow to cool and dilute to 35 ml with *water*. Neutralise 7.5 ml of this solution to *litmus paper* using *dilute ammonia R1* and dilute to 15 ml with *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII (70 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Sulphates

Dissolve 0.25 g of the substance being examined in *water*. Add 3 ml of *dilute hydrochloric acid* and dilute to 15 ml with *water*. The resulting solution complies with the *limit test for sulphates*, Appendix VII.

ASSAY

Dissolve 0.3 g of the substance being examined in 3 ml of *orthophosphoric acid* and 10 ml of a 14% v/v solution of *sulphuric acid* in *water*. Add 100 ml of *water* and titrate with 0.1M *potassium permanganate*. Each ml of 0.1M *potassium permanganate VS* is equivalent to 27.925 mg of Fe^{2+}.

PRODUCTION OF STOCK

The first decimal trituration of Hydrated Iron(II) and Iron(III) Phosphate for Homoeopathic Preparations is prepared using a suitable quantity of Lactose or Anhydrous Lactose as the vehicle and a validated trituration method that ensures homogeneity is achieved. The vehicle complies with the statement under Vehicles in the monograph for Homoeopathic Preparations.

Content of hydrated iron(II) and iron(III) phosphate

The first decimal trituration contains 4.5% to 5.0% of $Fe_3(PO_4)_2, 8H_2O$.

CHARACTERISTICS

The first decimal trituration is a light grey powder.

IDENTIFICATION

A. Yields the reactions characteristic of *iron and iron salts*, Appendix VI.

B. Yields the reactions characteristic of *phosphates*, Appendix VI.

C. Dissolve 0.25 g in 5 ml of *water*. Add 5 ml of *ammonia* and heat in a water-bath at 80º for 10 minutes. A red colour develops.

ASSAY

Dissolve 3.0 g of the first decimal trituration in 3 ml of *orthophosphoric acid* and 10 ml of a 14% v/v solution of *sulphuric acid* in *water*. Add 100 ml of *water* and titrate with 0.1M *potassium permanganate*. Each ml of 0.1M *potassium permanganate VS* is equivalent to 27.925 mg of Fe^{2+}.

STORAGE

Hydrated Iron(II) and Iron(III) Phosphate for Homoeopathic Preparations should be protected from light.

Hydrated Iron(III) Phosphate for Homoeopathic Preparations

General Notices

222.8 *10045-86-0*

DEFINITION

Hydrated Iron(III) Phosphate for Homoeopathic Preparations contains hydrated iron(III) phosphate. It contains not less than 96.0% and not more than 106.5% of $FePO_4,4H_2O$.

CHARACTERISTICS

A yellow to pale ochre powder.

Insoluble in *water;* soluble in dilute mineral acids.

IDENTIFICATION

Dissolve 0.5 g of the substance being examined in 5 ml of *dilute hydrochloric acid*, with warming. Dilute the resulting solution to 35 ml with *water* and filter if necessary (solution S).

A. Solution S yields reactions B and C characteristic of *iron and iron salts*, Appendix VI.

B. Solution S yields reaction B characteristic of *phosphates*, Appendix VI.

TESTS

Clarity of solution

Solution S is *clear*, Appendix IV A, Method II.

Chloride

To 0.05 g of the substance being examined add 1 ml of *dilute nitric acid*. Heat, dilute with 14 ml of *water* and filter. The filtrate complies with the *limit test for chlorides*, Appendix VII (0.1%) .

Heavy metals

Dissolve 1.0 g of the substance being examined in 20 ml of *hydrochloric acid* if necessary with heating. Extract the solution using five 20-ml quantities of a mixture of 100 ml of freshly distilled *methyl isobutyl ketone* and 1 ml of *hydrochloric acid R1.* Allow to stand, separate the aqueous layer and evaporate to half its volume, allow to cool and dilute to 35 ml with *water*. Neutralise 7.5 ml of this solution to *litmus paper* using *dilute ammonia R1* and dilute to 15 ml with *water*. 12 ml of the resulting solution complies with *limit test heavy metals*, Appendix VII, Method A (70 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Loss on drying

When dried to constant weight at 200º, loses not less than 28% and not more than 33% of its weight, Appendix IX D. Use 1 g.

ASSAY

Dissolve 0.45 g in 3 ml of *hydrochloric acid R1* in an iodine flask, add 10 ml of *water* and 6.0 g of *potassium iodide,* close the flask and allow to stand protected from light for 30 minutes. Add 100 ml of *water* and 1 ml of *starch solution* and titrate with 0.1M *sodium thiosulphate VS*. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 22.29 mg of $FePO_4,4H_2O$.

PRODUCTION OF STOCK

The first decimal trituration of Hydrated Iron(III) Phosphate for Homoeopathic Preparations is prepared using a suitable quantity of Lactose or Anhydrous Lactose as the vehicle and a validated trituration method that ensures homogeneity is achieved. The vehicle complies with the statement under Vehicles in the monograph for Homoeopathic Preparations.

Content of hydrated iron(III) phosphate $FePO_4$, $4H_2O$

The first decimal trituration contains 9.0% to 11.0% of $FePO_4$, $4H_2O$.

CHARACTERISTICS

The first decimal trituration is a yellowish powder.

IDENTIFICATION

Dissolve, with warming, 1.5 g of the first decimal trituration in a mixture of 1.5 ml of *dilute hydrochloric acid* and 9 ml of *water* (solution S1).

A. Solution S1 yields reactions B and C characteristic of *iron and iron salts*, Appendix VI.

B. Solution S1 yields reaction B characteristic of *phosphates*, Appendix VI.

C. Dissolve 0.25 g of the substance being examined in 5 ml of *water*. Add 5 ml of *ammonia* and heat in a water-bath at 80º for 10 minutes. A red colour develops.

ASSAY

Dissolve 4.0 g of the first decimal trituration in 3 ml of *hydrochloric acid R1* in an iodine flask, add 10 ml of *water* and 8.0 g of *potassium iodide,* close the flask and allow to stand protected from light for 30 minutes. Add 100 ml of *water* and 1 ml of *starch solution* and titrate with 0.1M *sodium thiosulphate VS*. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 22.29 mg of $FePO_4,4H_2O$.

STORAGE

Hydrated Iron(III) Phosphate for Homoeopathic Preparations should be protected from light.

Hyoscyamus for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2091)

Ph Eur

DEFINITION

Whole, fresh flowering plant of *Hyoscyamus niger* L.

IDENTIFICATION

Hyoscyamus is an annual or biennial plant, with a well developed taproot. The robust, erect stem is hollow and subcylindrical and up to 80 cm long. The soft, viscid, dull dark-green leaves are densely pubescent on both surfaces, especially on the veins. The lower leaves are petiolate and are arranged in a rosette; the lower cauline leaves are semi-amplexicaul and the upper ones are completely amplexicaul. The lamina, up to 25 cm long, is oblong to ovate with 2 to 5 broadly dentate lobes on each side. The midrib is well developed. The secondary veins arise at a wide angle from the midrib and terminate in the apices of the lobes. The flowering tops are densely pubescent and form a short drooping cluster. Each flower arises in the axils of a large bract. The gamosepalous calyx is covered with dense cotton-like hairs and has 5 triangular-ovate lobes, each ending in a short point that becomes spiny. The gamopetalous corolla, with 5 nearly equal lobes, is yellowish and with a delicate, brown to blackish-violet venation. The fruit, sometimes present at the base of the inflorescences, is a pyxis distinctly swollen at the base.

TESTS

Foreign matter *(2.8.2)*

If required by the competent authority, maximum 5 per cent.

Loss on drying *(2.2.32)*

If required by the competent authority, minimum 50 per cent, determined on 5.0 g of the finely cut drug by drying in an oven at 105 °C for 2 h.

Hyoscyamus albus L.

The presence of middle and upper leaves with a petiole and of fruits barely swollen at the base indicates adulteration by *Hyoscyamus albus* L.

MOTHER TINCTURE

The mother tincture complies with the requirements of the general monograph on *Mother tinctures for homoeopathic preparations (2029).*

PRODUCTION

The mother tincture of *Hyoscyamus niger* L. is prepared by maceration of the drug, using ethanol of a suitable concentration.

Content

0.002 per cent m/m to 0.01 per cent *m/m* of total alkaloids, expressed as hyoscyamine ($C_{17}H_{23}NO_3$; M_r 289.4).

CHARACTERS

Appearance

Dark greenish-brown liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution Evaporate 10 ml of the mother tincture to be examined in a water-bath at 40 °C, under reduced pressure. Take up the residue with 1 ml of *ammonia R*, and shake with 2 quantities, each of 10 ml, of *ether R*. Combine the ether layers, dry over *anhydrous sodium sulphate R* and filter. Evaporate on a water-bath and dissolve the residue in 0.50 ml of *methanol R.*

Reference solution (a) Dissolve 50 mg of *hyoscyamine sulphate R* in 10 ml of *methanol R* (solution A). Dissolve 15 mg of *hyoscine hydrobromide R* in 10 ml of *methanol R* (solution B). Mix 4 ml of solution A and 2 ml of solution B and dilute to 10 ml with *methanol R*.

Reference solution (b) Dissolve 20 mg of *atropine sulphate R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase concentrated ammonia R, *water R*, *acetone R* (3:7:90 *V/V/V*).

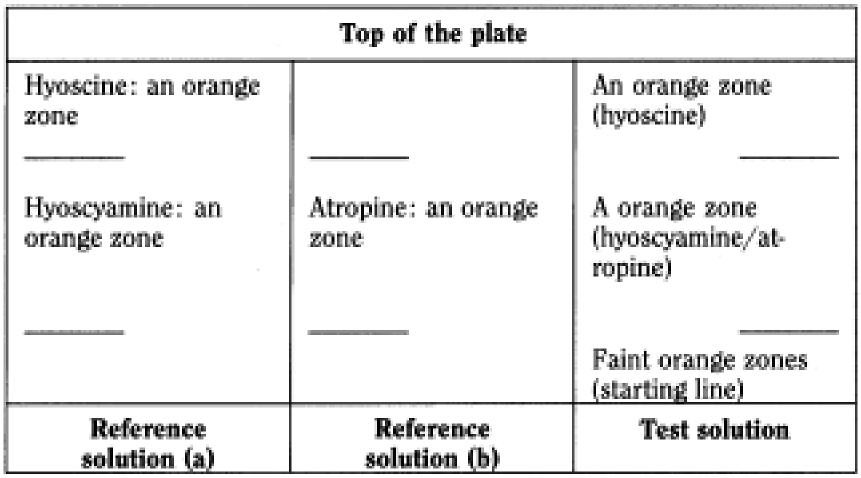
Application 20 µl, as bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 15 min.

Detection A Spray with *dilute potassium iodobismuthate solution R* until orange zones become visible. Examine in daylight.

Results A See below the sequence of the zones present in the chromatograms obtained with the reference solutions and the test solution. Other faint zones may be present in the chromatogram obtained with the test solution.



Top of the plate		
Hyoscine: an orange zone		An orange zone (hyoscine)
Hyoscyamine: an orange zone	Atropine: an orange zone	A orange zone (hyoscyamine/atropine)
		Faint orange zones (starting line)
Reference solution (a)	**Reference solution (b)**	**Test solution**

Detection B Subsequently spray with *sodium nitrite solution R* until the yellow background disappears. Examine in daylight after 15 min.

Results B See test for atropine.

TESTS

Relative density *(2.2.5)*

0.930 to 0.960.

Atropine

Examine the chromatograms obtained in the test for identification.

Results The zone due to hyoscyamine in the chromatogram obtained with the test solution changes from orange to reddish-brown but not to greyish-blue (atropine).

Ethanol *(2.9.10)*

40 per cent *V/V* to 50 per cent *V/V*.

Dry residue *(2.8.16)*

Minimum 1.2 per cent.

ASSAY

Evaporate 100.0 g of the mother tincture to be examined, at a low temperature under reduced pressure, until a residue of about 10 g is obtained. Quantitatively transfer the residue to a separating funnel using a few millilitres of *ethanol (70 per cent V/V) R*. Add 5 ml of *concentrated ammonia R* and 25 ml of *water R*. Extract with successive fractions of a mixture of 1 volume of *chloroform R* and 3 volumes of *peroxide-free ether R* until the alkaloids are completely extracted. Evaporate to dryness a few millilitres of the last organic fraction. Take up the residue in *0.25 M sulphuric acid* and verify the absence of alkaloids using *potassium tetraiodomercurate solution R*. Combine the organic layers and extract several times with *0.25 M sulphuric acid*. Separate the layers by centrifugation if necessary and transfer the acid layers to a second separating funnel. Make the acid layer alkaline with *ammonia R* and shake with at least 3 quantities, each of 30 ml, of *chloroform R*. Combine the chloroform layers, add 4 g of *anhydrous sodium sulphate R* and allow to stand for 30 min with occasional shaking. Decant the chloroform and wash the anhydrous sodium sulphate with 3 quantities, each of 10 ml, of *chloroform R*. Combine the chloroform fractions, evaporate to dryness on a water-bath and dry in an oven at 100-105 °C for 15 min. Dissolve the residue in a few millilitres of *chloroform R,* add 10.0 ml of *0.005 M sulphuric acid* and remove the chloroform by evaporation on a water-bath. Titrate the excess of acid with *0.01 M sodium hydroxide* using *methyl red mixed solution R* as indicator.

Calculate the percentage content *m/m* of total alkaloids, expressed as hyoscyamine, from the expression:

$$\frac{0.2894\,(10 - n)}{m}$$

n = volume of *0.01 M sodium hydroxide* used, in millilitres,

m = mass of the mother tincture used, in grams.

Ph Eur

Hypericum for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2028)

Ph Eur

DEFINITION

Whole, fresh plant of *Hypericum perforatum* L., at the beginning of the flowering period.

IDENTIFICATION

The perennial plant consists of a spindle-shaped root and a branched rhizome, giving rise to long, decumbent runners. The cylindrical, erect stem is woody at the base, 0.2 m to 1 m long, branched in the upper part, with 2 raised longitudinal lines.

The leaves are opposite, sessile, exstipulate, oblong-oval and 15 mm to 30 mm long. The leaf margins show black glandular dots, and many small translucent oil glands are present on the entire surface and are visible by transmitted light.

The flowers are regular and form corymbose clusters at the apex of the stem. They have 5 green, lanceolate sepals with acuminate apices, and black oil glands near the entire margins; 5 orange-yellow petals, much longer than the sepals, with black oil glands near the terminal margins only; 3 staminal blades, each divided into many orange-yellow stamens and 3 carpels surmounted by red styles. Each petal is asymmetrically linear-ovate in shape, with one of the margin entire and the other dentate.

TESTS

Foreign matter (*2.8.2*)

Maximum 4 per cent of fruits and maximum 1 per cent of other foreign matter.

Loss on drying (*2.2.32*)

If performed to demonstrate the freshness of the drug, minimum 55 per cent, determined on 5.0 g of finely cut drug by drying in an oven at 105 °C.

MOTHER TINCTURE

The mother tincture complies with the requirements of the general monograph on *Mother tinctures for homoeopathic preparations (2029).*

PRODUCTION

The mother tincture of *Hypericum perforatum* L. is prepared by maceration using alcohol of a suitable concentration.

CHARACTERS

Dark red to brownish red liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)*.

Test solution The mother tincture to be examined.

Reference solution Dissolve 5 mg of *rutin R*, 1 mg of *hypericin R* and 5 mg of *hyperoside R* in *methanol R* and dilute to 5 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase anhydrous formic acid R, *water R*, *ethyl acetate R* (6:9:90 *V/V/V*).

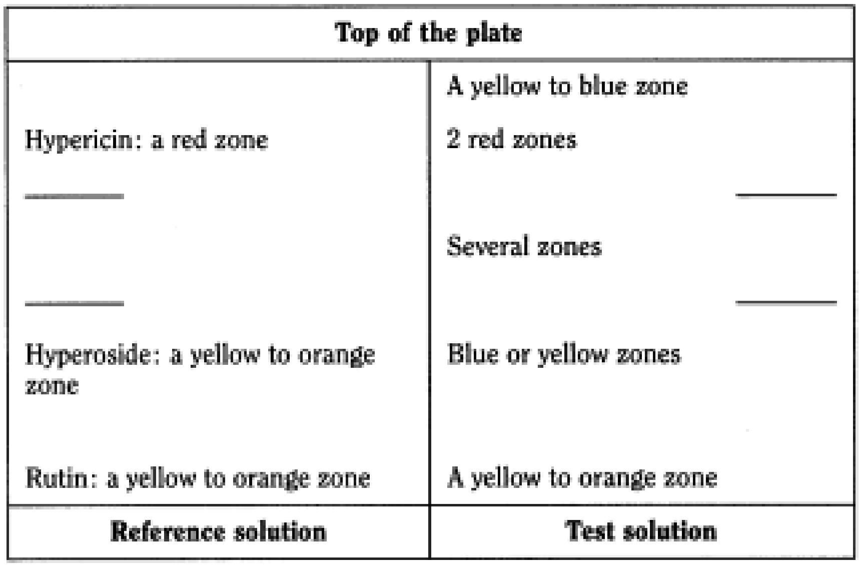
Application 10 µl of the test solution and 5 µl of the reference solution, as 10 mm bands.

Development Over a path of 10 cm.

Drying At 100-105 °C for 10 min.

Detection Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then a 50 g/l solution of *macrogol 400 R* in *methanol R*. Examine the plates after 30 min in ultraviolet light at 365 nm.

Results See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. In the chromatogram obtained with the test solution, the zone due to rutin may be weak or even absent. The chromatogram obtained with the test solution shows a group of zones that may be blue or yellow, with a R_F similar to that of the zone due to hyperoside in the chromatogram obtained with the reference solution. Other weak zones may also be visible.



Top of the plate	
	A yellow to blue zone
Hypericin: a red zone	2 red zones
_______	_______
	Several zones
_______	_______
Hyperoside: a yellow to orange zone	Blue or yellow zones
Rutin: a yellow to orange zone	A yellow to orange zone
Reference solution	**Test solution**

TESTS

Relative density *(2.2.5)*

0.900 to 0.920.

Ethanol ***(2.9.10)***

60 per cent *V/V* to 75 per cent *V/V*.

Dry residue ***(2.8.16)***

Minimum 1.3 per cent.

Ph Eur

Iron for Homoeopathic Preparations

General Notices

Iron for Homoeopathic Use

(Ph Eur monograph 2026)

Fe 55.85 *7439-89-6*

Ph Eur

DEFINITION

Obtained by reduction or sublimation as a fine blackish-grey powder.

Content

97.5 per cent to 101.0 per cent.

CHARACTERS

Appearance

Fine, blackish-grey powder, without metallic lustre.

Solubility

Practically insoluble in water and in alcohol. It dissolves with heating in dilute mineral acids.

IDENTIFICATION

Dissolve 50 mg in 2 ml of *dilute sulphuric acid R* and dilute to 10 ml with *water R*. The solution gives reaction (a) of iron *(2.3.1)*.

TESTS

Solution S

To 10.0 g add 40 ml of *water R*. Boil for 1 min. Cool, filter and dilute to 50.0 ml with *water R*.

Alkalinity

To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.1 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Substances insoluble in hydrochloric acid

Dissolve 2.00 g in 40 ml of *hydrochloric acid R*. Heat on a water-bath. As soon as fumes are no longer evolved, filter through a sintered-glass filter (16) *(2.1.2)*. Rinse with *water R*. Dry the residue in an oven at 100-105 °C for 1 h. The residue weighs a maximum of 20 mg (1.0 per cent).

Substances soluble in water

Evaporate 10.0 ml of solution S on a water-bath and dry at 100-105 °C for 1 h. The residue weighs a maximum of 2 mg (0.1 per cent).

Chlorides ***(2.4.4)***

Maximum 50 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides.

Sulphides and phosphides

In a 100 ml conical flask carefully mix 1.0 g with 10 ml of *dilute hydrochloric acid R*. Within 30 s *lead acetate paper R* moistened with *water R* and placed over the mouth of the flask is not coloured more intensely than light brown by the resulting fumes.

Arsenic ***(2.4.2)***

Maximum 5 ppm.

Boil 0.2 g in 25 ml of *dilute hydrochloric acid R* until completely dissolved. The solution complies with limit test A.

Copper

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dissolve 1.00 g in a mixture of 60 ml of *dilute hydrochloric acid R* and 10 ml of *dilute hydrogen peroxide solution R*. Reduce to a volume of 5 ml and dilute to 50.0 ml with *water R*.

Reference solutions Prepare the reference solutions using *copper standard solution (0.1 per cent Cu) R*, diluted as necessary with a 1 per cent *V/V* solution of *hydrochloric acid R.*

Source Copper hollow-cathode lamp.

Wavelength 324.8 nm.

Flame Air-acetylene.

Lead

Maximum 50.0 ppm.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution In a separating funnel, place 20 ml of the test solution prepared for the test for copper. Add 25 ml of *lead-free hydrochloric acid R*. Stir with 3 quantities, each of 25 ml, of *di-isopropyl ether R*. Collect the aqueous layer. Add 0.10 g of *sodium sulphate decahydrate R*. Evaporate to dryness. Take up the residue with 1 ml of *lead-free nitric acid R* and dilute to 20 ml with *water R.*

Reference solutions Prepare the reference solutions using *lead standard solution (0.1 per cent Pb) R*, diluted as necessary with a 10 per cent *V/V* solution of *nitric acid R* containing 5 g/l of *sodium sulphate decahydrate R.*

Source Lead hollow-cathode lamp.

Wavelength 217 nm.

Flame Air-acetylene.

ASSAY

Stir for 10 min 0.100 g in a hot solution of 1.25 g of *copper sulphate R* in 20 ml of *water R* in a 100 ml conical flask with a ground-glass stopper. Filter rapidly and wash the filter. Combine the filtrate and the washings, acidify with *dilute sulphuric acid R* and titrate with *0.02 M potassium permanganate* until a pink colour is obtained.

1 ml of *0.02 M potassium permanganate* is equivalent to 5.585 mg of Fe.

LABELLING

The label indicates whether the iron for homoeopathic preparations is obtained by reduction or sublimation.

Ph Eur

Methods of Preparation of Homoeopathic Stocks and Potentisation

General Notices

(Ph Eur monograph 2371)

Ph Eur

Homoeopathic stocks are prepared, using suitable methods, from raw materials that comply with the requirements of the monograph *Homoeopathic preparations (1038)*. The methods described below, combined with established methods for potentisation, are examples of methods, but other methods described in an official national pharmacopoeia of a Member State may equally be used.

Where material of animal origin is to be used, particular reference is made to the requirements concerning the use of raw material of zoological or human origin in the monograph *Homoeopathic preparations (1038).*

In the preparation of liquid dilutions, the ethanol of the concentration prescribed in the method may, if necessary, be replaced by ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)] or ethanol (15 per cent *m/m*) [ethanol (18 per cent *V/V*)].

When the individual monograph allows that the mother tincture be prepared from more than one plant species, the mother tincture can be prepared from the specified parts of an individual plant species or from any mixture thereof.

Unless otherwise stated, mother tinctures are prepared by maceration. Maceration lasts not less than 10 days and not more than 30 days.

Maceration may be replaced by long maceration (maximum 60 days) or very long maceration (maximum 180 days), provided it is demonstrated that the quality of the resulting mother tincture is the same as that of the mother tincture prepared by maceration.

Unless otherwise stated in the individual monograph, the term 'part(s)' denotes 'mass part(s)'. Unless otherwise stated in the method, the maximum temperature for the preparation is 25 C.

METHOD 1A

Method 1a is used for fresh herbal drugs containing generally more than 70 per cent of expressed juice and no essential oil or resin or mucilage. Mother tinctures prepared according to Method 1a are mixtures of equal parts of expressed juices and ethanol (86 per cent *m/m*) [ethanol (90 per cent *V/V*)].

Express the comminuted herbal drug. Immediately mix the expressed juice with an equal mass of ethanol (86 per cent *m/m*) [ethanol (90 per cent *V/V*)]. Allow to stand in a closed container at a temperature not exceeding 20 °C for not less than 5 days, then filter.

Adjustment to any value specified in the individual monograph

Determine the percentage dry residue (*2.8.16*) or, where prescribed, the percentage assay content of the above-mentioned filtrate. Calculate the amount (A_1), in kilograms, of ethanol

(43 per cent *m/m*) [ethanol (50 per cent *V/V*)] required, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of filtrate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the filtrate.

Mix the filtrate with the calculated amount of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)]. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The 1st 'decimal' dilution (D1) is made from:

— 2 parts of the mother tincture;

— 8 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

The 2nd decimal dilution (D2) is made from:

— 1 part of the 1st 'decimal' dilution;

— 9 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

Subsequent decimal dilutions are produced as stated for D2.

The 1st 'centesimal' dilution (C1) is made from:

— 2 parts of the mother tincture;

— 98 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

The 2nd centesimal dilution (C2) is made from:

— 1 part of the 1st 'centesimal' dilution;

— 99 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

Subsequent centesimal dilutions are produced as stated for C2.

METHOD 1B

Method 1b is used where the latex of a herbal drug is to be processed.

Mother tinctures prepared according to Method 1b are mixtures of fresh plant latex with ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)]. Mix the fresh latex with 2 parts by mass of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)] and filter.

Adjustment to any value specified in the individual monograph

Determine the percentage dry residue (*2.8.16*) or, where prescribed, the percentage assay content of the above-mentioned filtrate. Calculate the amount (A_1), in kilograms, of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)] required, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of filtrate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the filtrate.

Mix the filtrate with the calculated amount of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)]. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The 1st 'decimal' dilution (D1) is made from:

— 3 parts of the mother tincture;

— 7 parts of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)].

The 2nd decimal dilution (D2) is made from:

— 1 part of the 1st 'decimal' dilution;

— 9 parts of ethanol (15 per cent *m/m*) [ethanol (18 per cent *V/V*)].

Subsequent decimal dilutions are produced as stated for D2.

METHOD 2A

Method 2a is used for fresh herbal drugs containing generally less than 70 per cent of expressed juice and more than 60 per cent moisture (loss on drying) and no essential oil or resin.

Mother tinctures prepared according to Method 2a (ethanol content approximately 43 per cent *m/m* or 50 per cent *V/V*) are prepared by maceration as described below.

Comminute the herbal drug Take a sample and determine the loss on drying (*2.2.32*). Unless otherwise prescribed, determine the loss on drying on 2.00-5.00 g of comminuted raw material in a flat-bottomed tared vessel, 45-55 mm in diameter, that has been previously dried as indicated for the raw material. Dry the raw material at 100-105 °C for 2 h then allow to cool in a desiccator.

To the comminuted herbal drug immediately add not less than half the mass of ethanol (86 per cent *m/m*) [ethanol (90 per cent *V/V*)] and store in well-closed containers at a temperature not exceeding 20 °C.

Use the following expression to calculate the amount (A_2), in kilograms, of ethanol (86 per cent *m/m*) [ethanol (90 per cent *V/V*)] required for the mass (m) of raw material, then subtract the amount of ethanol (86 per cent *m/m*) [ethanol (90 per cent *V/V*)] already added and add the difference to the mixture.

$$\frac{m \times T}{100}$$

m = mass of raw material, in kilograms.

T = percentage loss on drying of the sample.

Allow to stand at a temperature not exceeding 20 °C for not less than 10 days, swirling from time to time, then express the mixture and filter the resulting liquid.

Adjustment to any value specified in the individual monograph

Determine the percentage dry residue (*2.8.16*) or, where prescribed, the percentage assay content of the above-mentioned filtrate. Calculate the amount (A_1), in kilograms, of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)] required, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of filtrate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the filtrate.

Mix the filtrate with the calculated amount of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)]. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The 1st 'decimal' dilution (D1) is made from:

— 2 parts of the mother tincture;

— 8 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

The 2nd decimal dilution (D2) is made from:

— 1 part of the 1st 'decimal' dilution;

— 9 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

Subsequent decimal dilutions are produced as stated for D2.

The 1st 'centesimal' dilution (C1) is made from:

— 2 parts of the mother tincture;

— 98 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

The 2nd centesimal dilution (C2) is made from:

— 1 part of the 1st 'centesimal' dilution;

— 99 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

Subsequent centesimal dilutions are produced as stated for C2.

METHOD 2B

Method 2b is used for fresh herbal drugs containing generally less than 70 per cent of expressed juice and more than 60 per cent moisture (loss on drying) and no essential oil or resin.

Mother tinctures prepared according to Method 2b (ethanol content approximately 30 per cent *m/m* or 36 per cent *V/V*) are prepared by maceration as described below.

Comminute the herbal drug Take a sample and determine the loss on drying (*2.2.32*). Unless otherwise prescribed, determine the loss on drying on 2.00-5.00 g of comminuted raw material in a flat-bottomed tared vessel, 45-55 mm in diameter, that has been previously dried as indicated for the raw material. Dry the raw material at 100-105 °C for 2 h then allow to cool in a desiccator.

To the comminuted herbal drug immediately add not less than half the mass of ethanol (62 per cent *m/m*) [ethanol (70 per cent *V/V*)] and store in well-closed containers at a temperature not exceeding 20 °C.

Use the following expression to calculate the amount (A_2), in kilograms, of ethanol (62 per cent *m/m*) [ethanol (70 per cent *V/V*)] required for the mass (m) of raw material, then subtract the amount of ethanol (62 per cent *m/m*) [ethanol (70 per cent *V/V*)] already added and add the difference to the mixture.

$$\frac{m \times T}{100}$$

m = mass of raw material, in kilograms.

T = percentage loss on drying of the sample.

Allow to stand at a temperature not exceeding 20 °C for not less than 10 days, swirling from time to time, then express the mixture and filter the resulting liquid.

Adjustment to any value specified in the individual monograph

Determine the percentage dry residue (*2.8.16)* or, where prescribed, the percentage assay content of the above-mentioned filtrate. Calculate the amount (A_1), in kilograms, of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)] required, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of filtrate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the filtrate.

Mix the filtrate with the calculated amount of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)]. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The 1st 'decimal' dilution (D1) is made from:

— 2 parts of the mother tincture;

— 8 parts of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)].

The 2nd decimal dilution (D2) is made from:

— 1 part of the 1st 'decimal' dilution;

— 9 parts of ethanol 15 per cent (*m/m*) [ethanol (18 per cent *V/V*)].

Subsequent decimal dilutions are produced as stated for D2.

METHOD 3A

Method 3a is used for fresh herbal drugs containing essential oil or resin, or generally less than 60 per cent moisture (loss on drying).

Mother tinctures prepared according to Method 3a (ethanol content approximately 60 per cent *m/m* or 68 per cent *V/V*) are prepared by maceration as described below.

Comminute the herbal drug Take a sample and determine the loss on drying (*2.2.32*). Unless otherwise prescribed, determine the loss on drying on 2.00-5.00 g of comminuted raw material in a flat-bottomed tared vessel, 45-55 mm in diameter, that has been previously dried as indicated for the raw material. Dry the raw material at 100-105 °C for 2 h then allow to cool in a desiccator.

To the comminuted herbal drug immediately add not less than half the mass of ethanol (86 per cent *m/m*) [ethanol (90 per cent *V/V*)] and store in well-closed containers at a temperature not exceeding 20 °C.

Use the following expression to calculate the amount (A_3), in kilograms, of ethanol (86 per cent *m/m*) [ethanol (90 per cent *V/V*)] required for the mass (m) of raw material, then subtract the amount of ethanol (86 per cent *m/m*) [ethanol (90 per cent *V/V*)] already added and add the difference to the mixture.

$$\frac{2 \times m \times T}{100}$$

m = mass of raw material, in kilograms.

T = percentage loss on drying of the sample.

Allow to stand at a temperature not exceeding 20 °C for not less than 10 days, swirling from time to time, then express the mixture and filter the resulting liquid.

Adjustment to any value specified in the individual monograph

Determine the percentage dry residue (*2.8.16*) or, where prescribed, the percentage assay content of the above-mentioned filtrate. Calculate the amount (A_1), in kilograms, of ethanol (62 per cent *m/m*) [ethanol (70 per cent *V/V*)] required, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of filtrate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the filtrate.

Mix the filtrate with the calculated amount of ethanol (62 per cent *m/m*) [ethanol (70 per cent *V/V*)]. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The 1st 'decimal' dilution (D1) is made from:

— 3 parts of the mother tincture;

— 7 parts of ethanol (62 per cent *m/m*) [ethanol (70 per cent *V/V*)].

The 2nd decimal dilution (D2) is made from:

— 1 part of the 1st 'decimal' dilution;

— 9 parts of ethanol (62 per cent *m/m*) [ethanol (70 per cent *V/V*)].

Subsequent dilutions are produced as stated for D2. Use ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)] for dilutions from D4 onwards.

The 1st 'centesimal' dilution (C1) is made from:

— 3 parts of the mother tincture;

— 97 parts of ethanol (62 per cent *m/m*) [ethanol (70 per cent *V/V*)].

The 2nd 'centesimal' dilution (C2) is made from:

— 1 part of the 1st 'centesimal' dilution;

— 99 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

Subsequent dilutions are produced as stated for C2.

METHOD 3B

Method 3b is used for fresh herbal drugs containing essential oils or resins or generally less than 60 per cent moisture (loss on drying).

Mother tinctures prepared according to Method 3b (ethanol content approximately 43 per cent *m/m* or 50 per cent *V/V*) are prepared by maceration as described below.

Comminute the herbal drug. Take a sample and determine the loss on drying (*2.2.32*). Unless otherwise prescribed, determine the loss on drying on 2.00-5.00 g of comminuted raw material in a flat-bottomed tared vessel, 45-55 mm in diameter, that has been previously dried as indicated for the raw material. Dry the raw material at 100-105 °C for 2 h then allow to cool in a desiccator.

To the comminuted herbal drug immediately add not less than half the mass of ethanol (73 per cent *m/m*) [ethanol (80 per cent *V/V*)] and store in well-closed containers at a temperature not exceeding 20 °C.

Use the following expression to calculate the amount (A_3), in kilograms, of ethanol (73 per cent *m/m*) [ethanol (80 per cent *V/V*)] required for the mass (m) of raw material, then subtract the amount of ethanol (73 per cent *m/m*) [ethanol (80 per cent *V/V*)] already added and add the difference to the mixture.

$$\frac{2 \times m \times T}{100}$$

m = mass of raw material, in kilograms.

T = percentage loss on drying of the sample.

Allow to stand at a temperature not exceeding 20 °C for not less than 10 days, swirling from time to time, then express the mixture and filter the resulting liquid.

Adjustment to any value specified in the individual monograph

Determine the percentage dry residue (*2.8.16*) or, where prescribed, the percentage assay content of the above-mentioned filtrate. Calculate the amount (A_1), in kilograms, of ethanol (43 per cent *m/m*) [ethanol 50 per cent *V/V*)] required, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of filtrate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the filtrate.

Mix the filtrate with the calculated amount of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)]. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The 1st 'decimal' dilution (D1) is made from:

— 3 parts of the mother tincture;

— 7 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)].

The 2nd decimal dilution (D2) is made from:

— 1 part of the 1st 'decimal' dilution;

— 9 parts of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)].

The 3rd decimal dilution (D3) is made from:

— 1 part of the 2nd decimal dilution;

— 9 parts of ethanol (15 per cent *m/m*) [ethanol (19 per cent *V/V*)].

Subsequent decimal dilutions are produced as stated for D3.

METHOD 3C

Method 3c is used for fresh herbal drugs containing generally less than 60 per cent moisture (loss on drying).

Mother tinctures prepared according to Method 3c (ethanol content approximately 30 per cent *m/m* or 36 per cent *V/V*) are prepared by maceration as described below.

Comminute the herbal drug Take a sample and determine the loss on drying (*2.2.32*). Unless otherwise prescribed, determine the loss on drying on 2.00-5.00 g of comminuted raw material in a flat-bottomed tared vessel, 45-55 mm in diameter, that has been previously dried as indicated for the raw material. Dry the raw material at 100-105 °C for 2 h then allow to cool in a desiccator.

To the comminuted herbal drug immediately add not less than half the mass of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)] and store in well-closed containers at a temperature not exceeding 20 °C.

Use the following expression to calculate the amount (A_3), in kilograms, of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)] required for the mass (m) of raw material, then subtract the amount of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)] already added and add the difference to the mixture.

$$\frac{2 \times m \times T}{100}$$

m = mass of raw material, in kilograms.

T = percentage loss on drying of the sample.

Allow to stand at a temperature not exceeding 20 °C for not less than 10 days, swirling from time to time, then express the mixture and filter the resulting liquid.

Adjustment to any value specified in the individual monograph

Determine the percentage dry residue (*2.8.16*) or, where prescribed, the percentage assay content of the above-mentioned filtrate. Calculate the amount (A_1), in kilograms, of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)] required, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of filtrate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the filtrate.

Mix the filtrate with the calculated amount of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)]. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The 1st 'decimal' dilution (D1) is made from:

— 3 parts of the mother tincture;

— 7 parts of ethanol (30 per cent *m/m*) [ethanol (36 per cent *V/V*)].

The 2nd decimal dilution (D2) is made from:

— 1 part of the 1st 'decimal' dilution;

— 9 parts of ethanol (15 per cent *m/m*) [ethanol (18 per cent *V/V*)].

Subsequent decimal dilutions are produced as stated for D2.

METHOD 4A

Method 4a is generally used for dried herbal drugs.

Mother tinctures prepared according to Method 4a are prepared by maceration or percolation as described below, using 1 part of dried herbal drug and 10 parts of ethanol of the appropriate concentration (anhydrous, 94 per cent *m/m* - 96 per cent *V/V*, 86 per cent *m/m* - 90 per cent *V/V*, 73 per cent *m/m* - 80 per cent *V/V*, 62 per cent *m/m* - 70 per cent *V/V*, 43 per cent *m/m* - 50 per cent *V/V*, 30 per cent *m/m* - 36 per cent *V/V*, 15 per cent *m/m* - 18 per cent *V/V*), unless otherwise prescribed in the individual monograph.

Production by maceration Unless otherwise prescribed, comminute the herbal drug, mix thoroughly with ethanol of the appropriate concentration and allow to stand in a closed container for an appropriate time. Separate the residue from the ethanol and, if necessary, press out. In the latter case, combine the 2 liquids obtained.

Production by percolation If necessary, comminute the herbal drug. Mix thoroughly with a portion of ethanol of the appropriate concentration and allow to stand for an appropriate time.

Transfer to a percolator and allow the percolate to flow slowly, at room temperature, making sure that the herbal drug to be extracted is always covered with the remaining ethanol. The residue may be pressed out and the expressed liquid combined with the percolate.

If adjustment to a given concentration is necessary, calculate the amount (A_1), in kilograms, of ethanol of the appropriate concentration required to obtain the concentration specified or used for production, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of percolate or macerate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the percolate or macerate.

Mix the macerate or percolate with the calculated amount of ethanol of the appropriate concentration. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The mother tincture corresponds to the 1st decimal solution (Ø = D1).

The 2nd decimal dilution (D2) is made from:

— 1 part of the mother tincture (D1);

— 9 parts of ethanol of the same concentration.

The 3rd decimal dilution (D3) is made from:

— 1 part of the 2nd decimal dilution;

— 9 parts of ethanol of the same concentration.

Unless a different ethanol concentration is specified, use ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)] for subsequent decimal dilutions from D4 onwards and proceed as stated for D3.

The 1st 'centesimal' dilution (C1) is made from:

— 10 parts of the mother tincture (D1);

— 90 parts of ethanol of the same concentration.

The 2nd centesimal dilution (C2) is made from:

— 1 part of the 1st 'centesimal' dilution;

— 99 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)], unless a different ethanol concentration is specified.

Subsequent centesimal dilutions are produced as stated for C2.

METHOD 4B

Method 4b is generally used for animal matter.

Mother tinctures prepared according to Method 4b are prepared by maceration or percolation as described below, using 1 part of animal matter and 10 parts of ethanol of the appropriate concentration (anhydrous, 94 per cent *m/m* - 96 per cent *V/V*, 86 per cent *m/m* - 90 per cent *V/V*, 73 per cent *m/m* - 80 per cent *V/V*, 62 per cent *m/m* - 70 per cent *V/V*, 43 per cent *m/m* - 50 per cent *V/V*, 30 per cent *m/m* - 36 per cent *V/V*, 15 per cent *m/m* - 18 per cent *V/V*), unless otherwise prescribed in the individual monograph.

Production by maceration Unless otherwise prescribed, comminute the animal matter, mix thoroughly with ethanol of the appropriate concentration and allow to stand in a closed container for an appropriate time. Separate the residue from the ethanol and, if necessary, press out. In the latter case, combine the 2 liquids obtained.

Production by percolation If necessary, comminute the animal matter. Mix thoroughly with a portion of ethanol of the appropriate concentration and allow to stand for an appropriate time. Transfer to a percolator and allow the percolate to flow slowly at room temperature, making sure that the animal matter to be extracted is always covered with the remaining ethanol. The residue may be pressed out and the expressed liquid combined with the percolate.

If adjustment to a given concentration is necessary, calculate the amount (A_1), in kilograms, of ethanol of the appropriate concentration required to obtain the concentration specified or used for production, using the following expression:

$$\frac{m \times (N_x - N_0)}{N_0}$$

m = mass of percolate or macerate, in kilograms.

N_0 = percentage dry residue or percentage assay content as required in the individual monograph.

N_x = percentage dry residue or percentage assay content of the percolate or macerate.

Mix the macerate or percolate with the calculated amount of ethanol of the appropriate concentration. Allow to stand at a temperature not exceeding 20 °C for not less than 5 days, then filter if necessary.

Potentisation

The mother tincture corresponds to the 1st decimal solution (Ø = D1).

The 2nd decimal dilution (D2) is made from:

— 1 part of the mother tincture (D1);

— 9 parts of ethanol of the same concentration.

The 3rd decimal dilution (D3) is made from:

— 1 part of the 2nd decimal dilution;

— 9 parts of ethanol of the same concentration.

Unless a different ethanol concentration is specified, use ethanol (43 per cent *m/m*) [ethanol

(50 per cent *V/V*)] for subsequent decimal dilutions from D4 onwards and proceed as stated for D3.

The 1st 'centesimal' dilution (C1) is made from:

- — 10 parts of the mother tincture (D1);
- — 90 parts of ethanol of the same concentration.

The 2nd centesimal dilution (C2) is made from:

- — 1 part of the 1st 'centesimal' dilution;
- — 99 parts of ethanol (43 per cent *m/m*) [ethanol (50 per cent *V/V*)], unless a different ethanol concentration is specified.

Subsequent centesimal dilutions are produced as stated for C2.

METHOD 4C

Method 4c is generally used for herbal drugs. The state of the herbal drug, fresh or dried, is specified in the individual monograph.

Mother tinctures prepared according to Method 4c are prepared by maceration.

Comminute appropriately the herbal drug Take a sample and determine the loss on drying at 100-105 °C for 2 h (*2.2.32*) or the water content (*2.2.13*). Taking this value into account, calculate and add to the herbal drug the quantities of ethanol of the appropriate concentration required to produce, unless otherwise prescribed, a 1 in 10 mother tincture (1:10 mother tincture) with a suitable ethanol content. Allow to macerate for at least 10 days, with sufficient shaking.

Separate the residue from the ethanol and strain at a pressure of about 10^7 Pa. Allow the combined liquids to stand for 48 h and filter. For mother tinctures with a required assay content, adjustment may be carried out, if necessary, by adding ethanol of the same concentration as used for the preparation of the tincture.

Potentisation

The 1st decimal dilution (D1) is made from:

- — 1 part of the mother tincture;
- — 9 parts of ethanol of the appropriate concentration.

The 2nd decimal dilution (D2) is made from:

- — 1 part of the 1st decimal dilution;
- — 9 parts of ethanol of the appropriate concentration.

Subsequent decimal dilutions are produced as stated for D2, using ethanol of the appropriate concentration.

The 1st centesimal dilution (C1) is made from:

- — 1 part of the mother tincture;
- — 99 parts of ethanol of the appropriate concentration.

The 2nd centesimal dilution (C2) is made from:

- — 1 part of the 1st centesimal dilution;
- — 99 parts of ethanol of the appropriate concentration.

Subsequent centesimal dilutions are produced as stated for C2, using ethanol of the appropriate concentration.

METHOD 4D

Method 4d is generally used for animal matter.

Mother tinctures prepared according to Method 4d are prepared by maceration.

The mass ratio of raw material to mother tincture is usually 1 to 20. To the raw material, appropriately comminuted, add the quantity of ethanol of the appropriate concentration required to produce a 1 in 20 mother tincture. Allow to macerate for at least 10 days, with sufficient shaking. Decant and filter. Allow to stand for 48 h and filter again.

Potentisation

The 1st decimal dilution (D1) is made from:

— 1 part of the mother tincture;

— 9 parts of ethanol of the appropriate concentration.

The 2nd decimal dilution (D2) is made from:

— 1 part of the 1st decimal dilution;

— 9 parts of ethanol of the appropriate concentration.

Subsequent decimal dilutions are produced as stated for D2, using ethanol of the appropriate concentration.

The 1st centesimal dilution (C1) is made from:

— 1 part of the mother tincture;

— 99 parts of ethanol of the appropriate concentration.

The 2nd centesimal dilution (C2) is made from:

— 1 part of the 1st centesimal dilution;

— 99 parts of ethanol of the appropriate concentration.

Subsequent centesimal dilutions are produced as stated for C2, using ethanol of the appropriate concentration.

Ph Eur

Mother Tinctures for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2029)

Ph Eur

DEFINITION

Mother tinctures for homoeopathic preparations are liquid preparations obtained by the solvent action of a suitable vehicle upon raw materials. The raw materials are usually in the fresh form but may be dried. Mother tinctures for homoeopathic preparations may also be obtained from plant juices, with, or without the addition of a vehicle. For some preparations, the matter to be extracted may undergo a preliminary treatment.

PRODUCTION

Mother tinctures for homoeopathic preparations are prepared by maceration, digestion, infusion, decoction, fermentation or as described in the individual monographs, usually using alcohol of suitable concentration.

Mother tinctures for homoeopathic preparations are obtained using a fixed proportion of raw material to solvent, taking the moisture content of the raw material into account, unless otherwise justified and authorised.

If fresh plants are used, suitable procedures are used to ensure freshness. The competent authorities may require that the freshness is demonstrated by means of a suitable test.

Mother tinctures for homoeopathic preparations are usually clear. A slight sediment may form on standing and that is acceptable as long as the composition of the tincture is not changed significantly.

The manufacturing process is defined so that it is reproducible.

Production by maceration

Unless otherwise prescribed, reduce the matter to be extracted to pieces of suitable size, mix thoroughly and extract according to the prescribed extraction method with the prescribed extraction solvent. Allow to stand in a closed vessel for the prescribed time. The residue is separated from the extraction solvent and, if necessary, pressed out. In the latter case, the 2 liquids obtained are combined.

Adjustment of the contents

Adjustment of the content of constituents may be carried out if necessary, either by adding the extraction solvent of suitable concentration, or by adding another mother tincture for homoeopathic preparations of the vegetable or animal matter used for the preparation.

IDENTIFICATION

Where applicable, at least 1 chromatographic identification test is carried out.

TESTS

The limits in an individual monograph are set to include official methods of production. Specific limits will apply to each defined method of production.

If the test for relative density is carried out, the test for ethanol need not be carried out, and vice versa.

Relative density (*2.2.5*)

The mother tincture for homoeopathic preparations complies with the limits prescribed in the monograph.

Ethanol (*2.9.10*)

The ethanol content complies with that prescribed in the monograph.

Methanol and 2-propanol (*2.9.11*)

Maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol, unless otherwise prescribed.

Dry residue (*2.8.16*)

Where applicable, the mother tincture for homoeopathic preparations complies with the limits prescribed in the monograph.

Pesticides (*2.8.13*)

Where applicable, the mother tincture for homoeopathic preparations complies with the test. This requirement is met if the herbal drug has been shown to comply with the test.

ASSAY

Where applicable, an assay with quantitative limits is performed.

STORAGE

Protected from light. A maximum storage temperature may be specified.

LABELLING

The label states:

— that the product is a mother tincture for homoeopathic preparations (designated as ″TM″ or ″Ø″),

— the name of the raw material using the Latin title of the European Pharmacopoeia monograph where one exists,

— the method of preparation,

— the ethanol content or other solvent content, in per cent *V/V*, in the mother tincture,

— the ratio of raw material to mother tincture,

— where applicable, the storage conditions.

Ph Eur

Oriental Cashew for Homoeopathic Preparations

General Notices

(Ph Eur monograph 2094)

Ph Eur

DEFINITION

Dried fruit of *Semecarpus anacardium* L. (*Anacardium orientale* L.).

Content

Minimum 6.0 per cent *m/m* of total phenol derivatives expressed as eugenol ($C_{10}H_{12}O_2$; M_r 164.2) (dried drug).

IDENTIFICATION

A. The dried fruit is oval and more or less heart-shaped; about 2 cm long, nearly 2 cm wide and 0.5 cm thick. Its surface is smooth, shiny and blackish. A transverse section shows a rather well developed, tough pericarp riddled with rather wide lacunae containing an abundant thick reddish-brown juice. The pericarp covers a white kernel under a reddish skin. The fruit may include the blackish, fleshy, wrinkled, cupuliferous receptacle.

B. Thin-layer chromatography *(2.2.27)*.

Test solution To 1.0 g of suitably cut drug, add 10 ml of *ethanol (90 per cent V/V) R*. Heat under reflux on a water-bath at 60 °C for 15 min. Allow to cool and filter.

Reference solution Dissolve 5 mg of *gallic acid R* and 5 mg of *caffeic acid R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate TLC silica gel plate R.

Mobile phase *methanol R*, *toluene R* (15:85 *V/V*).

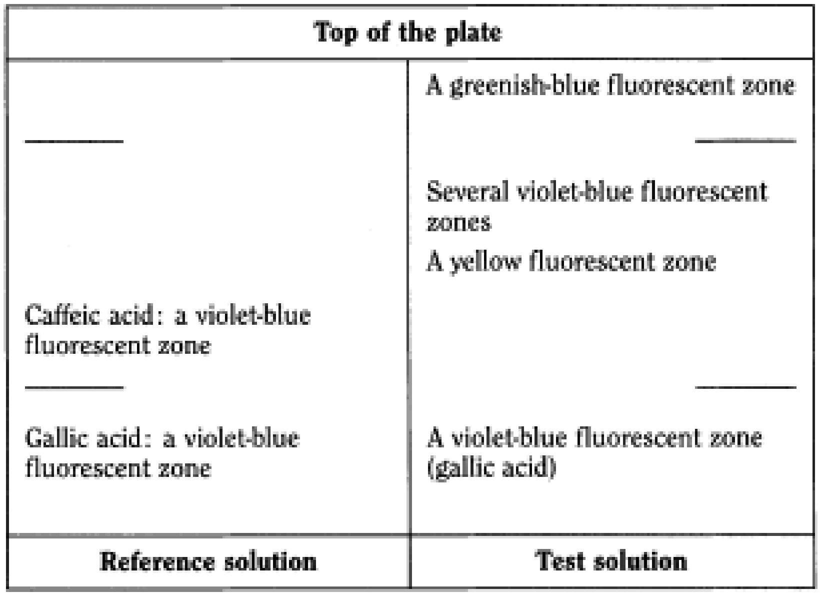
Application 20 µl of the test solution and 10 µl of the reference solution, as bands.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a solution containing 10 g/l of *diphenylboric acid aminoethyl ester R* and 50 g/l of *macrogol 400 R* in *methanol R*. Examine in ultraviolet light at 365 nm.

Results See below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fainter zones may be present in the chromatogram obtained with the test solution.



Top of the plate	
	A greenish-blue fluorescent zone
———	———
	Several violet-blue fluorescent zones A yellow fluorescent zone
Caffeic acid: a violet-blue fluorescent zone	
———	———
Gallic acid: a violet-blue fluorescent zone	A violet-blue fluorescent zone (gallic acid)
Reference solution	**Test solution**

TESTS

Anacardium occidentale L.

Fruits of *Anacardium occidentale* L. are not present. These are up to 35 mm long, 30 mm large, 20 mm thick, light brown and distinctly kidney-shaped. The pericarp is smooth or slightly crinkled with dark marbling in places.

Loss on drying *(2.2.32)*

Maximum 12.0 per cent, determined on 1.000 g of the finely divided drug by drying in an oven at 105 °C for 2 h.

Total ash *(2.4.16)*

Maximum 5.0 per cent.

ASSAY

Total phenol derivatives

Absorption spectrophotometry *(2.2.25)*.

Stock solution Place 4.500 g of the crushed drug in a flask. Add 200 ml of *ethanol (90 per cent V/V) R*. Boil in a water-bath under reflux for 4 h. Cool the flask. Quantitatively transfer into a volumetric flask. Dilute to 250.0 ml with *ethanol (90 per cent V/V) R*. Filter the liquid through a paper filter 125 mm in diameter. Discard the first 50 ml of the filtrate. Dilute 5.0 ml of filtrate to 50.0 ml with *ethanol (90 per cent V/V) R* and shake. Dilute 5.0 ml of this solution to 10.0 ml with *ethanol (90 per cent V/V) R* and shake.

Test solution To 2.0 ml of stock solution add 1.0 ml of *phosphomolybdotungstic reagent R* and 10 ml of *water R*, mix and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. Wait exactly 3 min then filter the solution through a fibre-glass filter with a 1 µm mesh aperture, discarding the first 5 ml.

Reference solution Dissolve 80.0 mg of *eugenol R* in *ethanol (90 per cent V/V) R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with *ethanol (90 per cent V/V) R*. To 2.0 ml of this solution add 1.0 ml of *phosphomolybdotungstic reagent R* and 10 ml of *water R*, mix and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. Wait exactly 3 min then filter the solution through a fibre-glass filter with a 1 µm mesh aperture, discarding the first 5 ml.

Measure the absorbance *(2.2.25)* of the test solution and the reference solution at 755 nm after 30 min using *water R* as compensation liquid.

Calculate the percentage content *m/m* of total phenol derivatives, expressed as eugenol, from the following expression:

$$\frac{A_1 \times m_2 \times 400}{A_2 \times m_1}$$

A_1 = absorbance of the test solution,

A_2 = absorbance of the reference solution,

m_1 = mass of the drug to be examined, in milligrams,

m_2 = mass of eugenol in the reference solution, in milligrams.

MOTHER TINCTURE

The mother tincture complies with the requirements of the general monograph on *Mother tinctures for homoeopathic preparations (2029).*

DEFINITION

The mother tincture of oriental cashew is prepared by maceration using ethanol of a suitable concentration from the dried fruit of *Semecarpus anacardium* L. (*Anacardium orientale* L.).

Content

0.5 per cent *m/m* to 1.0 per cent *m/m* of total phenol derivatives expressed as eugenol.

CHARACTERS

Appearance

Yellowish-brown or reddish-brown liquid.

IDENTIFICATION

Thin-layer chromatography *(2.2.27)* as described under Identification B of the drug with the following modification.

Test solution The tincture to be examined.

Results See identification B for the drug.

TESTS

Relative density ***(2.2.5)***

0.815 to 0.845.

Ethanol ***(2.9.10)***

85 per cent *V/V* to 95 per cent *V/V*.

Dry residue ***(2.8.16)***

Minimum 1.50 per cent *m/m*.

ASSAY

Total phenol derivatives

Absorption spectrophotometry *(2.2.25)* as described in the assay of the drug to be examined with the following modifications.

Stock solution Place 8.000 g of the mother tincture to be examined in a volumetric flask and dilute to 250.0 ml with *ethanol (90 per cent V/V) R*. Dilute 5.0 ml of this solution to 20.0 ml with *ethanol (90 per cent V/V) R*.

Test solution To 2.0 ml of stock solution add 1.0 ml of *phosphomolybdotungstic reagent R* and 10 ml of *water R*, mix and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. Wait exactly 3 min then filter the solution through a fibre-glass filter with a 1 µm mesh aperture, discarding the first 5 ml.

Reference solution Dissolve 80.0 mg of *eugenol R* in *ethanol (90 per cent V/V) R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with *ethanol (90 per cent V/V) R*. To 2.0 ml of this solution add 1.0 ml of *phosphomolybdotungstic reagent R* and 10 ml of *water R*, mix and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. Wait exactly 3 min then filter the solution through a fibre-glass filter with a 1 µm mesh aperture, discarding the first 5 ml.

Measure the absorbance *(2.2.25)* of the test solution and the reference solution at 755 nm after 30 min, using *water R* as compensation liquid.

Calculate the percentage content *m/m* of total phenol derivatives expressed as eugenol, using the following expression:

$$\frac{A_1 \times m_2 \times 80}{A_2 \times m_1}$$

A_1 = absorbance of the test solution,

A_2 = absorbance of the reference solution,

m_1 = mass of the mother tincture to be examined, in milligrams,

m_2 = mass of eugenol in the reference solution, in milligrams.

Ph Eur

Saffron for Homoeopathic Preparations

General Notices

Saffron for Homoeopathic Use
(Ph Eur monograph 1624)

Ph Eur

DEFINITION

Dried stigmas of *Crocus sativus* L. usually joined by the base to a short style.

CHARACTERS

Characteristic, aromatic odour.

IDENTIFICATION

A. The dark brick-red stigmas, when dry, are 20 mm to 40 mm long and after soaking with water, about 35 mm to 50 mm long. The tubes, gradually widening at the top, are incised on one side, the upper margin is open and finely crenated. The style connecting the 3 stigmas is pale yellow and not more than 5 mm long.

B. Examine under a microscope using *chloral hydrate solution R.* It shows the following diagnostic characters: elongated epidermal cells, frequently with a short, central papilla; in water they release a yellow colouring matter; the upper border of the stigma has finger-shaped papillae, up to 150 µm long; between them are single, globular pollen grains, about 100 µm wide, with a finely pitted exine, vascular bundles with small spirally thickened vessels and no fibres.

C. Carefully crush pieces of the drug to coarse particles and moisten with 0.2 ml of *phosphomolybdic acid solution R*. The particles turn blue within 1-2 min or they have a blue areole around them.

D. Examine by thin-layer chromatography (*2.2.27*).

Test solution Carefully crush 0.1 g of the drug with a glass rod and moisten with 0.2 ml of *water R*. After 3 min add 5 ml of *methanol R*, allow to stand for 20 min, protected from light, and filter through a plug of glass wool.

Reference solution Dissolve 5 mg of *naphthol yellow R* in 5 ml of *methanol R* and add a solution of 5 mg of *Sudan red G R* in 5 ml of *methylene chloride R*.

Plate *TLC silica gel* F_{254} *plate R.*

Mobile phase *water R*, *2-propanol R*, *ethyl acetate R* (10:25:65 *V/V/V*).

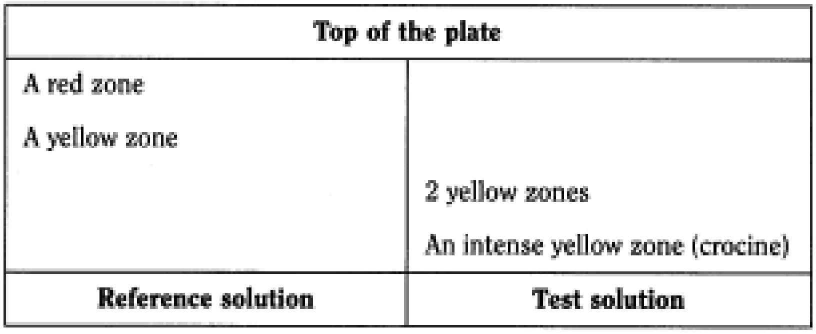
Application 10 µl of the test solution and 5 µl of the reference solution as bands.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in daylight.

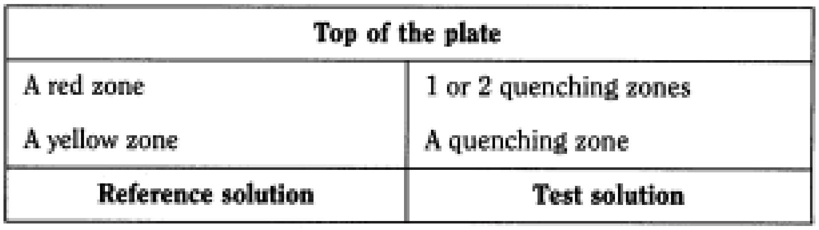
Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions.



Top of the plate	
A red zone	
A yellow zone	
	2 yellow zones
	An intense yellow zone (crocine)
Reference solution	**Test solution**

Detection In ultraviolet light at 254 nm.

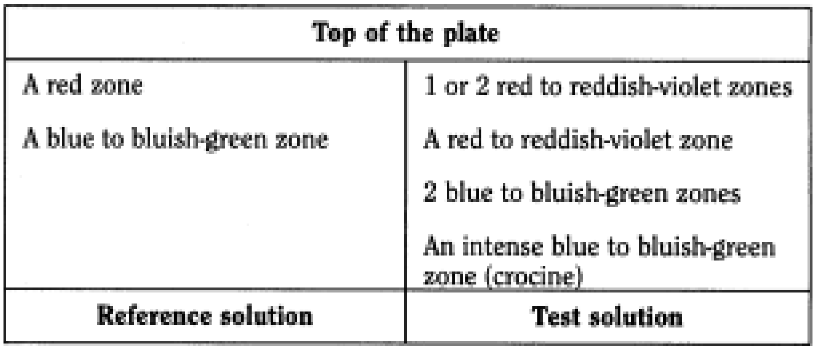
Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions.



Top of the plate	
A red zone	1 or 2 quenching zones
A yellow zone	A quenching zone
Reference solution	**Test solution**

Detection Spray with *anisaldehyde solution R* and examine in daylight while heating at 100-105 °C for 5-10 min.

Results See below the sequence of the zones present in the chromatograms obtained with the reference and test solutions.



Top of the plate	
A red zone	1 or 2 red to reddish-violet zones
A blue to bluish-green zone	A red to reddish-violet zone
	2 blue to bluish-green zones
	An intense blue to bluish-green zone (crocine)
Reference solution	**Test solution**

E. Dilute 0.1 ml of the test solution (see Identification test D) with 1 ml of *methanol R*. Deposit 0.1 ml of this solution on a filter paper, allow to dry and spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Examine in ultraviolet light at 365 nm. The spot shows an intense orange-yellow fluorescence.

TESTS

Colouring intensity

Introduce 0.10 g into a 5 ml volumetric flask and add to 5.0 ml with *distilled water R.* Close the flask and shake every 30 min for 8 h. Then allow to stand for 16 h. Dilute 1.0 ml to 500.0 ml with *distilled water R*. The absorbance (*2.2.25*) measured at 440 nm using *distilled water R* as the compensation liquid, is not less than 0.44.

Foreign matter

Examine the drug microscopically. No parts with rough walls, no crystals and no pollen grains containing 3 germinal pores are present.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 0.200 g by drying in an oven at 105 °C.

Total ash (*2.4.16*)

Maximum 7.0 per cent, determined on the residue obtained in the test for loss on drying.

Ph Eur

Symphytum Officinale Root for Homoeopathic Preparations

General Notices

DEFINITION

Symphytum Officinale Root for Homoeopathic Preparations is the fresh root of *Symphytum officinale* L.

IDENTIFICATION

The strong, spirally-formed rootstock with a smooth, black-brown cortex, subdivides at the base. It is about 5 to 8 cm in diameter and 17 to 30 cm long, surmounted by several tops, close together, consisting of the black residues of the previous year's rosettes of leaves between the current year's new growths. A ring of 10 to 15 horizontally-running, secondary, glabrous smooth roots grows from the base of the rosettes, the roots often reaching a length of more than 50 cm and a diameter of approximately 1.5 cm. The middle portion of the rootstock, which is more or less glabrous, branches at the lower end into several clearly separated, straight downwards pointing, smooth roots, each about 1.5 cm thick and bearing a few secondary rootlets 1 to 2 cm long.

The rootstock and the thick secondary roots are non-lignified, succulent and break off easily. In the yellowish-white, glassy, slightly differentiated cross section, there is a very thin, black rhizodermis, and an easily detachable layer of cortex, 5-8 mm thick. Within this is a single dark pigmented vascular ring. The cut surface and particularly the richly exuding mucilagenous substance turn yellow to red-brown on exposure.

TESTS

Foreign matter

Not more than 2%, Appendix XI D.

MOTHER TINCTURE

The mother tincture complies with the requirements stated under Mother Tinctures for Homoeopathic Preparations and with the following requirements.

PRODUCTION

The mother tincture of *Symphytum officinale* L., is prepared from the herbal drug using *Method 2a* described in the monograph for Methods of Preparation of Homoeopathic Stocks and Potentisation. Use 86% w/w (90% v/v) of *ethanol* .

CHARACTERISTICS

The mother tincture is a brown liquid.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions

(1) The mother tincture.

(2) 0.1% w/v solution of *allantoin* in *ethanol (45%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel G.

(b) Mobile phase is a mixture of 10 volumes of *anhydrous formic acid*, 10 volumes of *water* and 80 volumes of *ethyl acetate.*

(c) Application volumes of 20 µl for solution (1) and 10 µl for solution (2); applied as bands.

(d) Develop to 10 cm.

(e) Remove the plate, allow it to dry in air and examine under *ultraviolet light (365 nm).*

(f) Spray the chromatograms with a 5% (w/v) solution of *dimethylaminobenzaldehyde* in *hydrochloric acid* and examine in daylight.

VALIDITY

Under *ultraviolet light (365 nm)* the chromatogram of solution (1) shows two bluish spots at Rf 0.25 and Rf 0.55 and one light-green spot at the solvent front.

After spraying, the chromatogram of solution (2) shows the yellow allantoin spot at Rf 0.35. The chromatogram of the solution (1) shows one yellow spot with the same Rf of 0.35 as that for allantoin.

TESTS

Ethanol

35% to 45% w/w (42% to 53% v/v), Appendix VIII F

Dry residue

Not less than 1.5% w/w, Appendix XI P.

Relative density

0.920 to 0.970, Appendix V G.

Symphytum Officinale Root, Ethanol. Decoctum for Homoeopathic Preparations

General Notices

DEFINITION

The fresh root of *Symphytum officinale* L.

IDENTIFICATION

The strong, spirally-formed rootstock with a smooth, black-brown cortex, subdivides at the base. It is about 5 to 8 cm in diameter and 17 to 30 cm long, surmounted by several tops, close together, consisting of the black residues of the previous year's rosettes of leaves between the current year's new growths. A ring of 10 to 15 horizontally-running, secondary, glabrous smooth roots, grows from the base of the rosettes, the roots often reaching a length of more than 50 cm and a diameter of approximately 1.5 cm. The middle portion of the rootstock, which is more or less glabrous, branches at the lower end into several clearly separated, straight downwards pointing, smooth roots, each about 1.5 cm thick and bearing a few secondary rootlets 1 to 2 cm long.

The rootstock and the thick secondary roots are non-lignified, succulent and break off easily. In the yellowish-white, glassy, slightly differentiated cross section, there is a very thin, black rhizodermis, and an easily detachable layer of cortex, 5-8 mm thick. Within this is a single dark pigmented vascular ring. The cut surface and particularly the richly exuding mucilagenous substance turn yellow to red-brown on exposure.

TESTS

Foreign matter

Not more than 2%, Appendix XI D.

MOTHER TINCTURE

The mother tincture complies with the requirements stated under Mother Tinctures for Homoeopathic Preparations and with the following requirements.

PRODUCTION

The mother tincture of *Symphytum officinale* L. is prepared by decoction of the herbal drug, using 43% w/w (50% v/v) of *ethanol* .

CHARACTERISTICS

The mother tincture is a brown liquid.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) The mother tincture.

(2) 0.1% w/v solution of *allantoin* in *ethanol (45%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Mobile phase is a mixture of 10 volumes of *anhydrous formic acid ,* 10 volumes of *water* and 80 volumes of *ethyl acetate.*

(c) Application volumes of 20 µl for solution (1) and 10 µl for solution (2); applied as bands.

(d) Develop to 10 cm.

(e) Remove the plate, allow it to dry in air and examine under *ultraviolet light (365 nm).*

(f) Spray the plate with a 5% (w/v) solution of *dimethylaminobenzaldehyde* in *hydrochloric acid* and examine in daylight.

VALIDITY

Under *ultraviolet light (365 nm)*, the chromatogram obtained with solution (1) shows two bluish spots at Rf 0.25 and Rf 0.55 and one light-green spot at the solvent front.

After spraying, the chromatogram obtained with solution (2) shows a yellow spot at Rf 0.35 (allantoin). The chromatogram obtained with solution (1) shows one yellow spot with the same Rf of 0.35 as that for allantoin.

TESTS

Ethanol

25% to 35% w/w (31% to 42% v/v), Appendix VIII F

Dry residue

Not less than 3.0% w/w, Appendix XI P.

Relative density

0.973 to 0.982, Appendix V G.

Toxicodendron Quercifolium for Homoeopathic Preparations

General Notices

Rhus toxicodendron

DEFINITION

Toxicodendron Quercifolium for Homoeopathic Preparations is the fresh, young, not yet lignified shoots, with leaves, of *Toxicodendron quercifolium* (Michx.) Greene.

CAUTION *The shoots contain a yellowish-white milky sap that is a strong cutaneous irritant and darkens the skin. Contact with the skin and mucous membranes is to be avoided.*

IDENTIFICATION

The thin branches have a downy to cottony indumentum. At their ends and in the axils of the alternate leaves they may bear pointed buds covered in brown, woolly hairs. The tripinnate, fairly tough leaves have 15 to 20 cm long stems.The pinnatifid leaves are ovate to slightly rhombic and of varying size. The middle, larger lamina is long-stemmed, 7 to 20 cm long and 5 to 11 cm wide. The two smaller lateral pinnatifid leaves are short stemmed, up to 16 cm long and up to 9 cm wide. The pinnatifid laminae are sometimes asymetrical, entire or 3 to 7-lobate, indentate or broadly crenate. The laminae, particularly asymmetrical in the apical area, have a blunt end, are pointed or drawn out into a longer point. The base of the laminae are rounded or wedge shaped. The leaves are darker green on the upper side, light green on the underside, diffuse, but more hairy particularly on the veins. Flowers are absent.

MOTHER TINCTURE

The mother tincture complies with the requirements stated under Mother Tinctures for Homoeopathic Preparations and with the following requirements.

PRODUCTION

The mother tincture of *Toxicodendron quercifolium* (Michx.) Greene, is prepared from the herbal drug using *Method 2a* described in the monograph for Methods of Preparation of Homoeopathic Stocks and Potentisation. Use 86% w/w (90% v/v) of *ethanol*.

CHARACTERISTICS

The mother tincture is a yellowish-brown to reddish-brown liquid.

IDENTIFICATION

A. To 1 ml of the mother tincture add 1 granule of *zinc,* a few turnings of *magnesium* and 1 ml of *hydrochloric acid*. A dark red colour is produced which can be extracted with *tert-pentyl alcohol*.

B. Carry out the method for *thin-layer chromatography*, Appendix IIIA, using the following solutions.

(1) Mother tincture.

(2) Dissolve 20 mg of *arbutin* and 10 mg of *gallic acid* in 10 ml of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Mobile phase is a mixture of 10 volumes of *anhydrous formic acid*, 10 volumes of *water* and 80 volumes of *ethyl acetate*.

(c) Develop to 10 cm.

(d) Application volume of 10 µl for each solution.

(e) Remove the plate, dry in air, spray with a 0.5% w/v solution of *fast blue B salt* in *water* and after brief intermediate drying spray with 0.1M *alcoholic sodium hydroxide*. Examine in daylight.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows two well separated zones.

VALIDITY

The chromatogram obtained with solution (1) shows the following reddish-brown zones: two or three zones, which lie close together at a position between those given by arbutin and gallic acid with solution (2) and two further pronounced zones at about the same level as that given by gallic acid with solution (2). Other zones may be present in the chromatogram obtained with solution (1).

TESTS

Ethanol

36% to 46% w/w (43% to 54% v/v), Appendix VIII F.

Dry residue

Not less than 3.5% w/w, Appendix XI P.

Relative density

0.945 to 0.965, Appendix V G.

Urtica Urens Herb for Homoeopathic Preparations

General Notices

Urtica urens, Herba

DEFINITION

Fresh leaves and flowers of *Urtica urens* L.

CHARACTERISTICS

The plant produces an itchy, burning sensation.

IDENTIFICATION

Plant Annual.

Leaves Decussate with diffusely haired petiole, which in the lower leaves is mostly as long as the lamina; ovate to elliptic, 1 to 5 cm long and 1 to 4 cm wide lamina with incised serrated leaf margin, blunt to wedge-shaped at the base, acuminate towards the tip. The leaves are dark-green above, slightly shiny, lighter on the underside; diffusely haired above, on the underside occasionally only the vein is covered with hairs apart. The leaves are covered solely with stinging hairs. The two stipules on each side are lance-shaped and smooth-edged.

Flowers The complicated inflorescences consist of numerous female and only a few male flowers; these grow from the leaf axils and are about 1.5 cm, occasionally 2 cm long and thus mostly shorter than the petiole of their leaf. The perigonium of the male flowers is split into four light green lacinula of equal size. Each one of the four stamens situated in front of the perigonium segments has one long, firstly incurved then widening filament before the disbursement of the anther. The perigonium of the female flowers consists of two outer, very short segments and two inner, long segments, ciliated at the edge, glabrous or with a few stiff hairs. The superior ovary is narrow, ovoid and has a stigma with a brushlike fascicle. The ripe fruits are monospermic and are enclosed by the two inner perigonium segments.

TESTS

Foreign matter

Not more than 2%, Appendix XI D.

MOTHER TINCTURE

The mother tincture complies with the requirements stated under Mother Tinctures for Homoeopathic Preparations and with the following requirements.

PRODUCTION

The mother tincture of *Urtica urens* L., is prepared from the herbal drug using *Method 2b* described in the monograph for Methods of Preparation of Homoeopathic Stocks and

Potentisation. Use 62% w/w (70% v/v) of *ethanol*.

CHARACTERISTICS

The mother tincture is a green to brownish-green liquid.

IDENTIFICATION

A. To 1 ml of *potassium hydroxide solution* add 1 ml of the mother tincture and heat to boiling. *Red litmus paper* held over the mouth of the test tube turns blue.

B. Add 1 ml of *hydrochloric acid* and a few crystals of *resorcinol* to 1 ml of the mother tincture. Heat to boiling. A red colour is produced.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Mother tincture.

(2) Dissolve 10 mg of *leucine* and 10 mg of *threonine* in 10 ml of *ethanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Mobile phase is a mixture of 10 volumes of *glacial acetic acid*, 20 volumes of *water*, 35 volumes of *acetone*, and 35 volumes of *butan-1-ol*.

(c) Application volume of 20 µl for each solution.

(d) Develop to 15 cm.

(e) Remove the plate, dry in a current of warm air or in an oven at 100º to 105º. Spray the chromatogram with a 0.5% w/v solution of *ninhydrin* in *butan-1-ol*. Heat for 10 minutes at 100º to 105º and examine in daylight.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with the solution (2) shows two clearly separated coloured zones due to threonine (reddish pink) in the lower region and leucine (pink) in the middle region.

VALIDITY

The chromatogram obtained with solution (1) shows a pink spot (which may be separated into two spots) corresponding to leucine. A reddish pink spot occurs in the lower region corresponding to threonine. Between the spots corresponding to threonine and leucine there is an orange pink spot and a pink spot present, in order of increasing Rf value. Several spots are present below the spot corresponding to threonine.

TESTS

Ethanol

25% to 35% w/w (30% to 42% v/v), Appendix VIII F.

Dry residue

Not less than 1.0% w/w, Appendix XI P.

Relative density

0.956 to 0.968, Appendix V G.

Anti-T Lymphocyte Immunoglobulin for Human Use, Animal

General Notices

(Ph Eur monograph 1928)

Ph Eur

DEFINITION

Animal anti-T lymphocyte immunoglobulin for human use is a liquid or freeze-dried preparation containing immunoglobulins, obtained from serum or plasma of animals, mainly rabbits or horses, immunised with human lymphocytic antigens.

The immunoglobulin has the property of diminishing the number and function of immunocompetent cells, in particular T-lymphocytes. The preparation contains principally immunoglobulin G. It may contain antibodies against other lymphocyte subpopulations and against other cells. The preparation is intended for intravenous administration, after dilution with a suitable diluent where applicable.

Applicable provisions of the monograph on *Immunosera for human use, animal (0084)* are stated below.

PRODUCTION

GENERAL PROVISIONS

The production method has been shown to yield consistently immunoglobulins of acceptable safety, potency in man and stability.

Any reagent of biological origin used in production shall be free of contamination with bacteria, fungi and viruses. The method of preparation includes a step or steps that have been shown to remove or inactivate known agents of infection.

During development studies, it shall be demonstrated that the production method yields a product that:

— does not transmit infectious agents,

— is characterised by a defined pattern of immunological activity, notably: antigen binding, complement-dependent and independent cytotoxicity, cytokine release, induction of T-cell activation, cell death,

— does not contain antibodies that cross-react with human tissues to a degree that would impair clinical safety,

— has a defined maximum content of anti-thrombocyte antibody activity,

— has a defined maximum content of haemoglobin.

The product has been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated.

Reference preparation A batch shown to be suitable for checking the validity of the assay and whose efficacy has been demonstrated in clinical trials, or a batch representative thereof.

ANIMALS

The animals used are of a species approved by the competent authority, are healthy and exclusively reserved for production of anti-T lymphocyte immunoglobulin. They are tested and shown to be free from a defined list of infectious agents. The introduction of animals into a closed herd follows specified procedures, including definition of quarantine measures. Where appropriate, tests for additional specific agents are considered depending on the geographical localisation of the establishment used for the breeding and production of the animals. The feed originates from a controlled source and no animal proteins are added. The suppliers of animals are certified by the competent authority.

If the animals are treated with antibiotics, a suitable withdrawal period is allowed before collection of blood or plasma. The animals are not treated with penicillin antibiotics. If a live vaccine is administered, a suitable waiting period is imposed between vaccination and collection of serum or plasma for immunoglobulin production.

The species, origin and identification number of the animals are specified.

IMMUNISATION

The antigens used are identified and characterised, where appropriate. They are identified by their names and a batch number; information on the source and preparation are recorded.

The selected animals are isolated for at least 1 week before being immunised according to a defined schedule with booster injections at suitable intervals. Adjuvants may be used.

Animals are kept under general health surveillance and specific antibody production is controlled at each cycle of immunisation.

Animals are thoroughly examined before collection of blood or plasma. If an animal shows any pathological lesion not related to the immunisation process, it is not used, nor are any other of the animals in the group concerned, unless it is evident that their use will not impair the safety of the product.

Human antigens such as continuously growing T-lymphocyte cell lines or thymocytes are used to immunise the animals. Cells may be subjected to a sorting procedure. The immunising antigens are shown to be free from infectious agents by validated methods for relevant blood-borne pathogens, notably hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) and other relevant adventitious agents originating from the preparation of the antigen. The cells used comply with defined requirements for purity of the cell population and freedom from adventitious agents.

COLLECTION OF BLOOD OR PLASMA

Collection of blood is made by venepuncture or plasmapheresis. The puncture area is shaved, cleaned and disinfected. The animals may be anaesthetised under conditions that do not influence the quality of the product.

No antimicrobial preservative is added to the plasma and serum samples. The blood or plasma is collected in such a manner as to maintain sterility of the product. The blood or plasma collection is conducted at a site separate from the area where the animals are kept or bred and the area where the immunoglobulin is purified. If the serum or plasma is stored before further processing, precautions are taken to avoid microbial contamination.

Several single plasma or serum samples may be pooled before purification. The single or pooled samples are tested before purification for the following tests.

Tests for contaminating viruses

Each pool is tested for contaminating viruses by suitable in vitro tests including inoculation to cell cultures capable of detecting a wide range of viruses relevant for the particular product. Where applicable, in vitro tests for contaminating viruses are carried out on the adsorbed pool, after the last production stage that may introduce viral contaminants.

PURIFICATION AND VIRAL INACTIVATION

The immunoglobulins are concentrated and purified by fractional precipitation, chromatography, immuno-adsorption or by other suitable chemical or physical methods. The methods are selected and validated to avoid contamination at all steps of processing and to avoid formation of protein aggregates that effect immunobiological characteristics of the product.

Unless otherwise justified and authorised, validated procedures are applied for removal and/ or inactivation of viruses.

After purification and treatment for removal and/or inactivation of viruses, a stabiliser may be added to the intermediate product, which may be stored for a period defined in the light of stability data.

Only an intermediate product that complies with the following requirements may be used in the preparation of the final bulk.

If the method of preparation includes a step for adsorption of cross-reacting anti-human antibodies using material from human tissues and/or red blood cells, the human materials are submitted to a validated procedure for inactivation of infectious agents, unless otherwise justified and authorised. If erythrocytes are used for adsorption, the donors for such materials comply with the requirements for donors of blood and plasma of the monograph on *Human plasma for fractionation (0853).* If other human material is used, it is shown by validated methods to be free from relevant blood-borne pathogens, notably HBV, HCV and HIV. If substances are used for inactivation or removal of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the anti-T lymphocyte immunoglobulin.

FINAL BULK

The final bulk is prepared from a single intermediate product or from a pool of intermediate products obtained from animals of the same species. A stabiliser may be added. No antimicrobial preservative is added either during the manufacturing procedure or for preparation of the final bulk solution. During manufacturing, the solution is passed through a bacteria-retentive filter.

FINAL LOT

The final bulk of anti-T-lymphocyte immunoglobulin is distributed aseptically into sterile, tamper-proof containers. The containers are closed as to prevent contamination.

Only a final lot that complies with the requirements prescribed below under Identification, Tests and Assay may be released for use.

CHARACTERS

The liquid preparation is clear or slightly opalescent and colourless or pale yellow. The freeze-dried preparation is a white or slightly yellow powder or solid friable mass, which after reconstitution gives a liquid preparation corresponding to the description above.

IDENTIFICATION

A. Using a suitable range of species-specific antisera, carry out precipitation tests on the preparation to be examined. It is recommended that the test be carried out using antisera specific to the plasma proteins of each species of domestic animal commonly used in the preparation of materials of biological origin in the country concerned and antisera specific to human plasma proteins. The preparation is shown to contain proteins originating from the animal used for the anti-T lymphocyte immunoglobulin production.

B. Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal serum of the animal used for production, compare this serum and the preparation to be examined, both diluted to a concentration that will allow a clear gammaglobulin precipitation arc to be obtained on the gel. The main component of the preparation to be examined corresponds to the IgG component of normal serum of the animal used for production.

C. The preparation complies with the assay.

TESTS

Solubility

To a container of the preparation to be examined, add the volume of the liquid for reconstitution stated on the label. The preparation dissolves completely within the time stated on the label.

Extractable volume *(2.9.17)*

It complies with the requirement for extractable volume.

pH *(2.2.3)*

The pH is within the limits approved for the particular product.

Osmolality *(2.2.35)*

Minimum 240 mosmol/kg after dilution, where applicable.

Proteins *(2.5.33)*

90 per cent to 110 per cent of the amount stated on the label.

Stabiliser

Determine the amount of stabiliser by a suitable physico-chemical method. The preparation contains not less than 80 per cent and not more than 120 per cent of the quantity stated on the label.

Distribution of molecular size

Size exclusion chromatography *(2.2.30)*.

Test solution Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to a concentration suitable for the chromatographic system used. A concentration in the range 2-20 g/l is usually suitable.

Reference solution Dilute *human immunoglobulin (molecular size) BRP* with a 9 g/l solution of *sodium chloride R* to the same protein concentration as the test solution.

Column:

— *size:* *l* = 0.6 m, Ø = 7.5 mm,

— *stationary phase*: *silica gel for size-exclusion chromatography R*, a grade suitable for fractionation of globular proteins in the molecular mass range of 20 000 to 200 000.

Mobile phase Dissolve 4.873 g of *disodium hydrogen phosphate dihydrate R,* 1.741 g of *sodium dihydrogen phosphate monohydrate R* and 11.688 g of *sodium chloride R* in 1 litre of *water R.*

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 50-600 µg of protein.

Retention time Identify the peaks in the chromatogram obtained with the test solution by comparison with the chromatogram obtained with the reference solution; any peak with a retention time shorter than that of dimer corresponds to polymers and aggregates.

System suitability:

— *reference solution*: the principal peak corresponds to IgG monomer and there is a peak corresponding to dimer with a retention time relative to monomer of 0.85 ± 0.05,

— *test solution*: the relative retentions of monomer and dimer are 1 ± 0.05 with reference to the corresponding peaks in the chromatogram obtained with the reference solution.

Limits:

— *total monomer and dimer*: at least 95 per cent of the total area of the peaks,

— *total polymers and aggregates*: maximum 5 per cent of the total area of the peaks.

Purity

Polyacrylamide gel electrophoresis *(2.2.31)*, under non-reducing and reducing conditions.

Resolving gel Non-reducing conditions: 8 per cent acrylamide; reducing conditions: 12 per cent acrylamide.

Test solution Dilute the preparation to be examined to a protein concentration of 0.5-2 mg/ml.

Reference solution Dilute the reference preparation to the same protein concentration as the test solution.

Application 10 µl.

Detection Coomassie staining.

Results Compared with the electropherogram of the reference solution, no additional bands are found in the electropherogram of the test solution.

Anti-A and anti-B haemagglutinins *(2.6.20)*

The 1 to 64 dilution does not show agglutination.

Where applicable, dilute the preparation to be examined as prescribed for use before preparing the dilutions for the test.

Haemolysins

Prepare a 1 to 64 dilution of the preparation to be examined, diluted if necessary as stated on the label. Take 6 aliquots of the 1 to 64 dilution. To 1 volume of 3 of the aliquots, add 1 volume of a 10 per cent *V/V* suspension of group A1, group B and group O erythrocytes in a 9 g/l solution of *sodium chloride R*, respectively. To 1 volume of the remaining 3 aliquots, add 1 volume of a 10 per cent *V/V* suspension of group A1, group B and group O erythrocytes in a 9 g/l solution of *sodium chloride R*, respectively, and to each aliquot 1 volume of fresh group AB serum (as a source of complement). Mix and incubate at 37 °C for 1 h. Examine the supernatant liquids for haemolysis. No signs of haemolysis are present.

Thrombocyte antibodies

Examined by a suitable method, the level of thrombocyte antibodies is shown to be below that approved for the specific product.

Water *(2.5.12)*

Maximum 3 per cent.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

Unless otherwise justified and authorised, it complies with the test for pyrogens. Unless otherwise prescribed, inject 1 ml per kilogram of the rabbit's body mass.

ASSAY

The biological activity is determined by measuring the complement-dependent cytotoxicity on target cells. Flow cytometry is performed with read-out of dead cells stained using propidium iodide. The activity is expressed as the concentration of anti-T lymphocyte immunoglobulin in milligrams per millilitre which mediates 50 per cent cytotoxicity.

Lymphocyte separation medium Commercial separation media with low viscosity and a density of 1.077 g/ml.

Complement Commercial complement is suitable.

Buffered salt solution pH 7.2 Dissolve 8.0 g of *sodium chloride R*, 0.2 g of *potassium chloride R*, 3.18 g of *disodium hydrogen phosphate R* and 0.2 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 ml with the same solvent.

Buffer solution for flow cytometry Add 40 ml of 0.1 per cent *V/V sodium azide R* and 10 ml of foetal calf serum to 440 ml of buffered salt solution pH 7.2. The foetal calf serum is inactivated at 56 °C for 30 min prior to use. Store at 4 °C.

Propidium iodide solution Dissolve *propidium iodide R* in buffered salt solution pH 7.2, to a concentration of 1 mg/ml. Store this stock solution at 2-8 °C and use within 1 month. For the assay, dilute this solution with buffer solution for flow cytometry, to obtain a concentration of 5 µg/ml. Store at 2-8 °C and use within 3 h.

Microtitre plates Plates used to prepare immunoglobulin dilutions are U- or V-bottomed polystyrene or poly(vinyl chloride) plates without surface treatment.

Micronic tubes Suitable for flow cytometry measurement.

Cell suspension Collect blood in anticoagulant from at least one healthy donor. Immediately isolate the peripheral blood mononuclear cells (PBMC) by gradient centrifugation in lymphocyte separation medium so that the PBMC form a visible clean interface between the plasma and the separation medium. Collect the layer containing the cells and dispense into centrifuge tubes containing buffered salt solution pH 7.2. Centrifuge at 400 g at 2-8 °C for 10 min. Discard the supernatant. Suspend the cell pellet in buffer solution for flow cytometry. Repeat the centrifugation and resuspension procedure of the cells twice. After the third centrifugation, resuspend the cell pellet in 1 ml of buffer solution for flow cytometry. Determine the number and vitality of the cells using a haemocytometer. Cell viability of at least 90 per cent is required. Adjust the cell number to 7 × 10^6/ml by adding buffer solution for flow cytometry. Store the cell suspension at 4 °C and use within 12 h.

If necessary, the first PBMC pellet may be resuspended in buffered salt solution pH 7.2 containing 20 per cent fœtal calf serum and stored overnight at 2 °C. Centrifuge at 400 g at 2-8 °C for 10 min. Discard the supernatant. Suspend the cell pellet in buffer solution for flow cytometry. Determine the number and vitality of the cells using a haemocytometer. Cell viability of at least 90 per cent is required. Adjust the cell number to 7 × 10^6/ml by adding buffer solution for flow cytometry.

It is also possible for cells to be immediately frozen and stored in nitrogen using the following method.

Buffer solution for freezing To 20 ml of cell culture medium, add 25 ml of fœtal calf serum and 5 ml of dimethyl sulphoxide (DMSO). Store this solution at 2-8 °C and use within 3 h.

20 × 10^6 cells per ampoule are frozen. These ampoules are stored in liquid nitrogen.

Buffer solution for thawing To 450 ml of cell culture medium, add 50 ml of foetal calf serum. Store this solution at 2-8 °C and use within 3 h.

Each ampoule is thawed in a water-bath at 37 °C with shaking. Cell suspension is repeated in a buffer solution for thawing. Centrifuge at 200 g at 2-8 °C for 10 min. Discard the supernatant. Suspend the cell pellet in buffer solution for flow cytometry. Repeat the procedure for centrifugation and resuspension of cells once. After the second centrifugation, resuspend the cells pellet in 1 ml of buffer solution for flow cytometry. Determine the number and vitality of the cells using a haemocytometer. Cell viability of at least 90 per cent is required. Adjust the cell number to 7 × 10^6/ml by adding buffer solution for flow cytometry. Store the cell suspension at 4 °C and use within 3 h.

Test solutions For freeze-dried preparations, reconstitute as stated on the label. Prepare 3 independent series of not fewer than 7 dilutions using buffer solution for flow cytometry as diluent.

Reference solutions For freeze-dried preparations, reconstitute according to the instructions for use. Prepare 3 independent dilution series of not fewer than 7 dilutions using buffer solution for flow cytometry as diluent.

Distribute 75 µl of each of the dilutions of the test solution or reference solution to each of a series of wells of a microtitre plate. Add 25 µl of the cell suspension of PBMC into each well. Add 25 µl of rabbit complement to each of the wells. Incubate at 37 °C for 30 min.

Centrifuge the plates at 200 g at 4 °C for 8 min, discard the supernatant and keep the plate on ice. Preparation for flow cytometry measurement is done step-wise by using a certain number of wells in order to allow labelling with *propidium iodide R* solution and measurement within a defined time period. Resuspend carefully the cell pellet of a certain number of wells with 200 µl of propidium iodide solution. Transfer the suspension into tubes. Incubate at 25 °C for 10 min then place immediately on ice.

Proceed with fluorescence measurement in a flow cytometer. Define a region including all propidium iodide-positive cells on the basis of Forward-Scattered, light (FSC) and flourescence (FL2 or FL3 for propidium iodide). Measure the percentage of propidium iodide-positive cells, without gating but excluding debris. Analyse at least 3000 cells for each of the test and reference solutions.

Use the percentages of dead cells to estimate the potency as the concentration in milligrams per millilitre of the preparation to be examined necessary to induce 50 per cent of cytotoxicity by fitting a sigmoidal dose response curve to the data obtained with the test and the reference preparations and by using a 4-parameter logistic model (see, for example, chapter *5.3*) and suitable software. The test is not valid unless the percentage of propidium iodide-positive cells at the lower asymptote of the curve is less then 15 per cent and the percentage of propidium iodide-positive cells at the upper asymptote of the curve is at least 80 per cent.

The estimated activity is 70 per cent to 130 per cent of the activity approved for the particular product.

The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

STORAGE

Protected from light at the temperature stated on the label.

Expiry date The expiry date is calculated from the beginning of the assay.

LABELLING

The label states:

— for liquid preparations, the volume of the preparation in the container and the protein content,

— for freeze-dried preparations:
  — the name and the volume of the reconstitution liquid to be added,
  — the quantity of protein in the container,
  — that the immunoserum is to be used immediately after reconstitution,
  — the time required for complete dissolution,

— the animal species of origin,

— the name and amount of stabiliser, where applicable,

— the dilution to be made before use of the product.

Ph Eur

Botulinum Antitoxin

General Notices

(Ph Eur monograph 0085)

The label may state 'Bot/Ser' followed by a letter or letters indicating the type or types present.

When Mixed Botulinum Antitoxin or Botulinum Antitoxin is prescribed or demanded and the types to be present are not stated, Botulinum Antitoxin prepared from types A, B and E shall be dispensed or supplied.

Ph Eur

DEFINITION

Botulinum antitoxin is a preparation containing antitoxic globulins that have the power of specifically neutralising the toxins formed by *Clostridium botulinum* type A, type B or type E, or any mixture of these types.

PRODUCTION

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunised against *Cl. botulinum* type A, type B and type E toxins.

IDENTIFICATION

It specifically neutralises the types of *Cl. botulinum* toxins stated on the label, rendering them harmless to susceptible animals.

POTENCY

Not less than 500 IU of antitoxin per millilitre for each of types A and B and not less than 50 IU of antitoxin per millilitre for type E.

The potency of botulinum antitoxin is determined by comparing the dose necessary to protect mice against the lethal effects of a fixed dose of botulinum toxin with the quantity of the standard preparation of botulinum antitoxin necessary to give the same protection. For this comparison a reference preparation of each type of botulinum antitoxin, calibrated in International Units, and suitable preparations of botulinum toxins, for use as test toxins, are required. The potency of each test toxin is determined in relation to the specific reference preparation; the potency of the botulinum antitoxin to be examined is determined in relation to the potency of the test toxins by the same method.

International Units of the antitoxin are the specific neutralising activity for botulinum toxin type A, type B and type E contained in stated amounts of the International Standards which consist of dried immune horse sera of types A, B and E. The equivalence in International Units of the International Standard is stated from time to time by the World Health Organisation.

Selection of animals Use mice having body masses such that the difference between the lightest and the heaviest does not exceed 5 g.

Preparation of test toxins CAUTION: Botulinum toxin is extremely toxic: exceptional care must be taken in any procedure in which it is employed. Prepare type A, B and E toxins from sterile filtrates of approximately 7-day cultures in liquid medium of *Cl. botulinum* types A, B and E. To the filtrates, add 2 volumes of glycerol, concentrate, if necessary, by dialysis against glycerol and store at or slightly below 0 °C.

Selection of test toxins Select toxins of each type for use as test toxins by determining for mice the L+/10 dose and the LD_{50}, the observation period being 96 h. The test toxins contain at least 1000 LD_{50} in an L+/10 dose.

Determination of test doses of the toxins (L+/10 dose) Prepare solutions of the reference preparations in a suitable liquid such that each contains 0.25 IU of antitoxin per millilitre. Using each solution in turn, determine the test dose of the corresponding test toxin.

Prepare mixtures of the solution of the reference preparation and the test toxin such that each contains 2.0 ml of the solution of the reference preparation, one of a graded series of volumes of the test toxin and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using four mice for each mixture, inject a dose of 1.0 ml intraperitoneally into each mouse. Observe the mice for 96 h.

The test dose of toxin is the quantity in 1.0 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, the death of all four mice injected with the mixture within the observation period.

Determination of potency of the antitoxin Prepare solutions of each reference preparation in a suitable liquid such that each contains 0.25 IU of antitoxin per millilitre.

Prepare solutions of each test toxin in a suitable liquid such that each contains 2.5 test doses per millilitre.

Using each toxin solution and the corresponding reference preparation in turn, determine the potency of the antitoxin. Prepare mixtures of the solution of the test toxin and the antitoxin to be examined such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the antitoxin to be examined, and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (2.0 ml) that contains 0.5 IU, and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using four mice for each mixture, inject a dose of 1.0 ml intraperitoneally into each mouse. Observe the mice for 96 h.

The mixture that contains the largest volume of antitoxin that fails to protect the mice from death contains 0.5 IU. This quantity is used to calculate the potency of the antitoxin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the reference preparation die and all those injected with mixtures containing more survive.

LABELLING

The label states the types of *Cl. botulinum* toxin neutralised by the preparation.

Ph Eur

Diphtheria Antitoxin

General Notices

(Ph Eur monograph 0086)

The label may state 'Dip/Ser'.

Ph Eur

DEFINITION

Diphtheria antitoxin is a preparation containing antitoxic globulins that have the power of specifically neutralising the toxin formed by *Corynebacterium diphtheriae.*

PRODUCTION

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunised against diphtheria toxin.

IDENTIFICATION

It specifically neutralises the toxin formed by *C. diphtheriae*, rendering it harmless to susceptible animals.

ASSAY

Not less than 1000 IU of antitoxin per millilitre for antitoxin obtained from horse serum. Not less than 500 IU of antitoxin per millilitre for antitoxin obtained from the serum of other mammals.

The potency of diphtheria antitoxin is determined by comparing the dose necessary to protect guinea-pigs or rabbits against the erythrogenic effects of a fixed dose of diphtheria toxin with the quantity of the standard preparation of diphtheria antitoxin necessary to give the same protection. For this comparison a reference preparation of diphtheria antitoxin, calibrated in International Units, and a suitable preparation of diphtheria toxin, for use as a test toxin, are required. The potency of the test toxin is determined in relation to the reference preparation; the potency of the diphtheria antitoxin to be examined is determined in relation to the potency of the test toxin by the same method.

The International Unit of antitoxin is the specific neutralising activity for diphtheria toxin contained in a stated amount of the International Standard, which consists of a quantity of dried immune horse serum. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Preparation of test toxin Prepare diphtheria toxin from cultures of *C. diphtheriae* in a liquid medium. Filter the culture to obtain a sterile toxic filtrate and store at 4 °C.

Selection of test toxin Select a toxin for use as a test toxin by determining for guinea-pigs or rabbits the lr/100 dose and the minimal reacting dose, the observation period being 48 h. The test toxin has at least 200 minimal reacting doses in the lr/100 dose.

Minimal reacting dose This is the smallest quantity of toxin which, when injected intracutaneously into guinea-pigs or rabbits, causes a small, characteristic reaction at the site of injection within 48 h.

The test toxin is allowed to stand for some months before being used for the assay of antitoxin. During this time its toxicity declines and the lr/100 dose may be increased. Determine the minimal reacting dose and the lr/100 dose at frequent intervals. When experiment shows that the lr/100 dose is constant, the test toxin is ready for use and may be used for a long period. Store the test toxin in the dark at 0 °C to 5 °C. Maintain its sterility by the addition of toluene or other antimicrobial preservative that does not cause a rapid decline in specific toxicity.

Determination of test dose of toxin (lr/100 dose) Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.1 IU of antitoxin per millilitre.

Prepare mixtures of the solution of the reference preparation and of the test toxin such that each contains 1.0 ml of the solution of the reference preparation, one of a graded series of volumes of the test toxin and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 15 min to 60 min. Using two animals for each mixture, inject a dose of 0.2 ml intracutaneously into the shaven or depilated flanks of each animal. Observe the animals for 48 h.

The test dose of toxin is the quantity in 0.2 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, a small but characteristic erythematous lesion at the site of injection.

Determination of potency of the antitoxin Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.125 IU of antitoxin per millilitre.

Prepare a solution of the test toxin in a suitable liquid such that it contains 12.5 test doses per millilitre.

Prepare mixtures of the solution of the test toxin and of the antitoxin to be examined such that each contains 0.8 ml of the solution of the test toxin, one of a graded series of volumes of the antitoxin to be examined and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 0.8 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (0.8 ml) that contains 0.1 IU and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 15 min to 60 min. Using two animals for each mixture, inject a dose of 0.2 ml intracutaneously into the shaven or depilated flanks of each animal. Observe the animals for 48 h.

The mixture that contains the largest volume of antitoxin that fails to protect the guinea-pigs from the erythematous effects of the toxin contains 0.1 IU. This quantity is used to calculate the potency of the antitoxin in International Units per millilitre.

The test is not valid unless all the sites injected with mixtures containing 0.8 ml or less of the solution of the reference preparation show erythematous lesions and at all those injected with mixtures containing more there are no lesions.

Ph Eur

European Viper Venom Antiserum

General Notices

(Ph Eur monograph 0145)

The only poisonous snake native to the British Isles is the adder or common viper, *Vipera berus*. In a geographical region where other species of snake (including elapids) are found, antisera able to neutralise the venoms of the species of snake indigenous to the region should be used. When the preparation is intended to neutralise the venom or venoms of one or more snakes other than vipers, the title Snake Venom Antiserum is used.

Ph Eur

DEFINITION

European viper venom antiserum is a preparation containing antitoxic globulins that have the power of neutralising the venom of one or more species of viper. The globulins are obtained by fractionation of the serum of animals that have been immunised against the venom or venoms.

IDENTIFICATION

It neutralises the venom of *Vipera ammodytes*, or *Vipera aspis,* or *Vipera berus*, or *Vipera ursinii* or the mixture of these venoms stated on the label, rendering them harmless to susceptible animals.

ASSAY

Each millilitre of the preparation to be examined contains sufficient antitoxic globulins to neutralise not less than 100 mouse LD_{50} of *Vipera ammodytes* venom or *Vipera aspis* venom and not less than 50 mouse LD_{50} of the venoms of other species of viper. The potency of European viper venom antiserum is determined by estimating the dose necessary to protect mice against the lethal effects of a fixed dose of venom of the relevant species of viper.

Selection of test venoms Use venoms which have the normal physicochemical, toxicological and immunological characteristics of venoms from the particular species of vipers. They are preferably freeze-dried and stored in the dark at 5 ± 3 °C.

Select a venom for use as a test venom by determining the LD_{50} for mice, the observation period being 48 h.

Determination of the test dose of venom Prepare graded dilutions of the reconstituted venom in a 9 g/l solution of *sodium chloride R* or other isotonic diluent in such a manner that the middle dilution contains in 0.25 ml the dose expected to be the LD_{50}. Dilute with an equal volume of the same diluent. Using at least four mice, each weighing 18 g to 20 g, for each dilution, inject 0.5 ml intravenously into each mouse. Observe the mice for 48 h and record the number of deaths. Calculate the LD_{50} using the usual statistical methods.

Determination of the potency of the antiserum to be examined Dilute the reconstituted test venom so that 0.25 ml contains the test dose of 5 LD_{50} (test venom solution).

Prepare serial dilutions of the antiserum to be examined in a 9 g/l solution of *sodium chloride R* or other isotonic diluent, the dilution factor being 1.5 to 2.5. Use a sufficient number and range of dilutions to enable a mortality curve between 20 per cent and 80 per cent mortality to be established and to permit an estimation of the statistical variation.

Prepare mixtures such that 5 ml of each mixture contains 2.5 ml of one of the dilutions of the antiserum to be examined and 2.5 ml of the test venom solution. Allow the mixtures to stand in a water-bath at 37 °C for 30 min. Using not fewer than six mice, each weighing 18 g to 20 g, for each mixture, inject 0.5 ml intravenously into each mouse. Observe the mice for 48 h and record the number of deaths. Calculate the PD_{50}, using the usual statistical methods. At the same time verify the number of LD_{50} in the test dose of venom, using the method described above. Calculate the potency of the antiserum from the expression:

$$\frac{(T_v - 1)}{\mathrm{PD}_{50}}$$

T_v = number of LD_{50} in the test dose of venom.

In each mouse dose of the venom-antiserum mixture at the end point there is one LD_{50} of venom remaining unneutralised by the antiserum and it is this unneutralised venom that is responsible for the deaths of 50 per cent of the mice inoculated with the mixture. The amount of venom neutralised by the antiserum is thus one LD_{50} less than the total amount contained in each mouse dose. Therefore, as the potency of the antiserum is defined in terms of the number of LD_{50} of venom that are neutralised, rather than the number of LD_{50} in each mouse dose, the expression required in the calculation of potency is T_v - 1 rather than T_v.

Alternatively, the quantity of test venom in milligrams that is neutralised by 1 ml or some other defined volume of the antiserum to be examined may be calculated.

LABELLING

The label states the venom or venoms against which the antiserum is effective.

Warning: Because of the allergenic properties of viper venoms, inhalation of venom dust should be avoided by suitable precautions.

Ph Eur

Gas-gangrene Antitoxin (Novyi)

General Notices

Gas-gangrene Antitoxin (Oedematiens)
(Ph Eur monograph 0087)

The label may state 'Nov/Ser'.

Preparation

Mixed Gas-gangrene Antitoxin

Ph Eur

DEFINITION

Gas-gangrene antitoxin (novyi) is a preparation containing antitoxic globulins that have the power of neutralising the alpha toxin formed by *Clostridium novyi* (Former nomenclature: *Clostridium oedematiens*). It is obtained by fractionation from the serum of horses, or other mammals, that have been immunised against *Cl. novyi* alpha toxin.

IDENTIFICATION

It specifically neutralises the alpha toxin formed by *Cl. novyi,* rendering it harmless to susceptible animals.

ASSAY

Not less than 3750 IU of antitoxin per millilitre.

The potency of gas-gangrene antitoxin (novyi) is determined by comparing the dose necessary to protect mice or other suitable animals against the lethal effects of a fixed dose of *Cl. novyi* toxin with the quantity of the standard preparation of gas-gangrene antitoxin (novyi) necessary to give the same protection. For this comparison a reference preparation of gas-gangrene antitoxin (novyi), calibrated in International Units, and a suitable preparation of *Cl. novyi* toxin for use as a test toxin are required. The potency of the test toxin is determined in relation to the reference preparation; the potency of the gas-gangrene antitoxin (novyi) to be examined is determined in relation to the potency of the test toxin by the same method.

The International Unit of antitoxin is the specific neutralising activity for *Cl. novyi* toxin contained in a stated amount of the International Standard, which consists of a quantity of dried immune horse serum. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Selection of animals Use mice having body masses such that the difference between the lightest and the heaviest does not exceed 5 g.

Preparation of test toxin Prepare the test toxin from a sterile filtrate of an approximately 5-day culture in liquid medium of *Cl. novyi.* Treat the filtrate with *ammonium sulphate R*, collect the precipitate, which contains the toxin, dry *in vacuo* over *diphosphorus pentoxide R,* powder and store dry.

Selection of test toxin Select a toxin for use as a test toxin by determining for mice the L+ dose and the LD_{50}, the observation period being 72 h. The test toxin has an L+ dose of 0.5 mg or less and contains not less than 25 LD_{50} in each L+ dose.

Determination of test dose of toxin (L+ dose) Prepare a solution of the reference preparation in a suitable liquid such that it contains 12.5 IU of antitoxin per millilitre.

Prepare a solution of the test toxin in a suitable liquid such that 1 ml contains a precisely known amount such as 10 mg.

Prepare mixtures of the solution of the reference preparation and the solution of the test toxin such that each contains 0.8 ml of the solution of the reference preparation, one of a graded series of volumes of the solution of the test toxin and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.2 ml intramuscularly into each mouse. Observe the mice for 72 h.

The test dose of toxin is the quantity in 0.2 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, the death of all six mice injected with the mixture within the observation period.

Determination of potency of the antitoxin Prepare a solution of the reference preparation in a suitable liquid such that it contains 12.5 IU of antitoxin per millilitre.

Prepare a solution of the test toxin in a suitable liquid such that it contains 12.5 test doses per millilitre.

Prepare mixtures of the solution of the test toxin and the antitoxin to be examined such that each contains 0.8 ml of the solution of the test toxin, one of a graded series of volumes of the antitoxin to be examined and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 0.8 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (0.8 ml) that contains 10 IU and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.2 ml intramuscularly into each mouse. Observe the mice for 72 h.

The mixture that contains the largest volume of antitoxin that fails to protect the mice from death contains 10 IU. This quantity is used to calculate the potency of the antitoxin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 0.8 ml or less of the solution of the reference preparation die and all those injected with mixtures containing a larger volume survive.

Ph Eur

Gas-gangrene Antitoxin (Perfringens)

General Notices

(Ph Eur monograph 0088)

The label may state 'Perf/Ser'.

Preparation

Mixed Gas-gangrene Antitoxin

Ph Eur

DEFINITION

Gas-gangrene antitoxin (perfringens) is a preparation containing antitoxic globulins that have the power of specifically neutralising the alpha toxin formed by *Clostridium perfringens*. It is obtained by fractionation from the serum of horses, or other mammals, that have been immunised against *Cl. perfringens* alpha toxin.

IDENTIFICATION

It specifically neutralises the alpha toxin formed by *Cl. perfringens*, rendering it harmless to susceptible animals.

ASSAY

Not less than 1500 IU of antitoxin per millilitre.

The potency of gas-gangrene antitoxin (perfringens) is determined by comparing the dose necessary to protect mice or other suitable animals against the lethal effects of a fixed dose of *Cl. perfringens* toxin with the quantity of the standard preparation of gas-gangrene antitoxin (perfringens) necessary to give the same protection. For this comparison a reference preparation of gas-gangrene antitoxin (perfringens), calibrated in International Units, and a suitable preparation of *Cl. perfringens* toxin for use as a test toxin are required. The potency of the test toxin is determined in relation to the reference preparation; the potency of the gas-gangrene antitoxin (perfringens) to be examined is determined in relation to the potency of the test toxin by the same method.

The International Unit of antitoxin is the specific neutralising activity for *Cl. perfringens* toxin contained in a stated amount of the International Standard, which consists of a quantity of dried immune horse serum. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Selection of animals Use mice having body masses such that the difference between the lightest and the heaviest does not exceed 5 g.

Preparation of test toxin Prepare the test toxin from a sterile filtrate of an approximately 5-day culture in liquid medium of *Cl. perfringens*. Treat the filtrate with *ammonium sulphate R,* collect the precipitate, which contains the toxin, dry *in vacuo* over *diphosphorus pentoxide R*, powder and store dry.

Selection of test toxin Select a toxin for use as a test toxin by determining for mice the L+ dose and the LD_{50}, the observation period being 48 h. The test toxin has an L+ dose of 4 mg or less and contains not less than 20 LD_{50} in each L+ dose.

Determination of test dose of toxin (L+ dose) Prepare a solution of the reference preparation in a suitable liquid such that it contains 5 IU of antitoxin per millilitre.

Prepare a solution of the test toxin in a suitable liquid such that 1 ml contains a precisely known amount such as 10 mg.

Prepare mixtures of the solution of the reference preparation and the solution of the test toxin such that each contains 2.0 ml of the solution of the reference preparation, one of a graded series of volumes of the solution of the test toxin and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.5 ml intravenously into each mouse. Observe the mice for 48 h.

The test dose of toxin is the quantity in 0.5 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, the death of all six mice injected with the mixture within the observation period.

Determination of potency of the antitoxin Prepare a solution of the reference preparation in a suitable liquid such that it contains 5 IU of antitoxin per millilitre.

Prepare a solution of the test toxin in a suitable liquid such that it contains five test doses per millilitre.

Prepare mixtures of the solution of the test toxin and the antitoxin to be examined such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the antitoxin to be examined and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (2.0 ml) that contains 10 IU and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.5 ml intravenously into each mouse. Observe the mice for 48 h.

The mixture that contains the largest volume of antitoxin that fails to protect the mice from death contains 10 IU. This quantity is used to calculate the potency of the antitoxin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the reference preparation die and all those injected with mixtures containing a larger volume survive.

Ph Eur

Gas-gangrene Antitoxin (Septicum)

General Notices

(Ph Eur monograph 0089)

The label may state 'Sep/Ser'.

Preparation

Mixed Gas-gangrene Antitoxin

Ph Eur

DEFINITION

Gas-gangrene antitoxin (septicum) is a preparation containing antitoxic globulins that have the power of specifically neutralising the alpha toxin formed by *Clostridium septicum*. It is obtained by fractionation from the serum of horses, or other mammals, that have been immunised against *Cl. septicum* alpha toxin.

IDENTIFICATION

It specifically neutralises the alpha toxin formed by *Cl. septicum*, rendering it harmless to susceptible animals.

ASSAY

Not less than 1500 IU of antitoxin per millilitre.

The potency of gas-gangrene antitoxin (septicum) is determined by comparing the dose necessary to protect mice or other suitable animals against the lethal effects of a fixed dose of *Cl. septicum* toxin with the quantity of the standard preparation of gas-gangrene antitoxin (septicum) necessary to give the same protection. For this comparison a reference preparation of gas-gangrene antitoxin (septicum), calibrated in International Units, and a suitable preparation of *Cl. septicum* toxin for use as a test toxin are required. The potency of the test toxin is determined in relation to the reference preparation; the potency of the gas-gangrene antitoxin (septicum) to be examined is determined in relation to the potency of the test toxin by the same method.

The International Unit of antitoxin is the specific neutralising activity for *Cl. septicum* toxin contained in a stated amount of the International Standard, which consists of a quantity of dried immune horse serum. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Selection of animals Use mice having body masses such that the difference between the lightest and the heaviest does not exceed 5 g.

Preparation of test toxin Prepare the test toxin from a sterile filtrate of an approximately 5-day culture in liquid medium of *Cl. septicum*. Treat the filtrate with *ammonium sulphate R*, collect the precipitate, which contains the toxin, dry *in vacuo* over *diphosphorus pentoxide R*, powder and store dry.

Selection of test toxin Select a toxin for use as a test toxin by determining for mice the L+ dose and the LD_{50}, the observation period being 72 h. The test toxin has an L+ dose of 0.5 mg or less and contains not less than 25 LD_{50} in each L+ dose.

Determination of test dose of toxin (L+ dose) Prepare a solution of the reference preparation in a suitable liquid such that it contains 5 IU of antitoxin per millilitre.

Prepare a solution of the test toxin in a suitable liquid such that 1 ml contains a precisely known amount such as 20 mg.

Prepare mixtures of the solution of the reference preparation and the solution of the test toxin such that each contains 2.0 ml of the solution of the reference preparation, one of a graded series of volumes of the solution of the test toxin and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.5 ml intravenously into each mouse. Observe the mice for 72 h.

The test dose of toxin is the quantity in 0.5 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, the death of all six mice injected with the mixture within the observation period.

Determination of potency of the antitoxin Prepare a solution of the reference preparation in a suitable liquid such that it contains 5 IU of antitoxin per millilitre.

Prepare a solution of the test toxin in a suitable liquid such that it contains five test doses per millilitre.

Prepare mixtures of the solution of the test toxin and the antitoxin to be examined such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the antitoxin to be examined and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (2.0 ml) that contains 10 IU and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.5 ml intravenously into each mouse. Observe the mice for 72 h.

The mixture that contains the largest volume of antitoxin that fails to protect the mice from death contains 10 IU. This quantity is used to calculate the potency of the antitoxin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the reference preparation die and all those injected with mixtures containing more survive.

Ph Eur

Immunosera

General Notices

Antisera

(Immunosera for Human Use, Animal, Ph Eur monograph 0084)

Immunosera comply with the requirements of the European Pharmacopoeia monograph for Immunosera for Human Use, Animal. These requirements are reproduced below.

Ph Eur

DEFINITION

Animal immunosera for human use are liquid or freeze-dried preparations containing purified immunoglobulins or immunoglobulin fragments obtained from serum or plasma of immunised animals of different species.

The immunoglobulins or immunoglobulin fragments have the power of specifically neutralising or binding to the antigen used for immunisation. The antigens include microbial or other toxins, human antigens, suspensions of bacterial and viral antigens and venoms of snakes, scorpions and spiders. The preparation is intended for intravenous or intramuscular administration, after dilution where applicable.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently immunosera of acceptable safety, potency in man and stability.

Any reagent of biological origin used in the production of immunosera shall be free of contamination with bacteria, fungi and viruses. The general requirements of chapter *5.1.7. Viral safety* apply to the manufacture of animal immunosera for human use, in conjunction with the more specific requirements relating to viral safety in this monograph. The method of preparation includes a step or steps that have been shown to remove or inactivate known agents of infection.

Methods used for production are validated, effective, reproducible and do not impair the biological activity of the product.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

Reference preparation A batch shown to be suitable in clinical trials, or a batch representative thereof, is used as the reference preparation for the tests for high molecular mass proteins and purity.

ANIMALS

The animals used are of a species approved by the competent authority, are healthy and are exclusively reserved for production of immunoserum. They are tested and shown to be free from a defined list of infectious agents. The introduction of animals into a closed herd follows specified procedures, including definition of quarantine measures. Where appropriate, additional specific agents are considered depending on the geographical localisation of the establishment used for the breeding and production of the animals. The feed originates from a controlled source and no animal proteins are added. The suppliers of animals are certified by the competent authority.

If the animals are treated with antibiotics, a suitable withdrawal period is allowed before collection of blood or plasma. The animals are not treated with penicillin antibiotics. If a live vaccine is administered, a suitable waiting period is imposed between vaccination and collection of serum or plasma for immunoserum production.

IMMUNISATION

The antigens used are identified and characterised, where appropriate; where relevant, they are shown to be free from extraneous infectious agents. They are identified by their names and a batch number; information on the source and preparation are recorded.

The selected animals are isolated for at least 1 week before being immunised according to a defined schedule, with booster injections at suitable intervals. Adjuvants may be used.

Animals are kept under general health surveillance and specific antibody production is controlled at each cycle of immunisation.

Animals are thoroughly examined before collection of blood or plasma. If an animal shows any pathological lesion not related to the immunisation process, it is not used, nor are any other of the animals in the group concerned, unless it is evident that their use will not impair the safety of the product.

COLLECTION OF BLOOD OR PLASMA

Collection of blood is made by venepuncture or plasmapheresis. The puncture area is shaved, cleaned and disinfected. The animals may be anaesthetised under conditions that do not influence the quality of the product. Unless otherwise prescribed, an antimicrobial preservative may be added. The blood or plasma is collected in such a manner as to maintain sterility of the product. The blood or plasma collection is conducted at a site separate from the area where the animals are kept or bred and the area where the immunoserum is purified. If the serum or plasma is stored before further processing, precautions are taken to avoid microbial contamination.

Several single plasma or serum samples may be pooled before purification. The single or pooled samples are tested before purification for the following tests.

Tests for contaminating viruses

If an antimicrobial preservative is added, it must be neutralised before carrying out the tests, or the tests are carried out on a sample taken before addition of the antimicrobial preservative. Each pool is tested for contaminating viruses by suitable *in vitro* tests.

Each pool is tested for viruses by inoculation to cell cultures capable of detecting a wide range of viruses relevant for the particular product.

Potency

Carry out a biological assay as indicated in the monograph and express the result in International Units per millilitre, where applicable. A validated in vitro method may also be used.

Protein content

Dilute the product to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion *(2.5.9)* and calculate the content of protein by multiplying by 6.25. The protein content is within approved limits.

PURIFICATION AND VIRAL INACTIVATION

The immunoglobulins are concentrated and purified by fractional precipitation, chromatography, immunoadsorption or by other chemical or physical methods. They may be processed further by enzyme treatment. The methods are selected and validated to avoid contamination at all steps of processing and to avoid formation of protein aggregates that affect the immunobiological characteristics of the product. For products intended to consist of immunoglobulin fragments, the methods are validated to guarantee total fragmentation. The methods of purification used are such that they do not generate additional components that compromise the quality and the safety of the product.

Unless otherwise justified and authorised, validated procedures are applied for removal and/ or inactivation of viruses. The procedures are selected to avoid the formation of polymers or aggregates and, unless the product is intended to consist of Fab′ fragments, to minimise the splitting of F(ab′)2 into Fab′ fragments.

After purification and treatment for removal and/or inactivation of viruses, a stabiliser may be added to the intermediate product, which may be stored for a period defined in light of stability data.

Only an intermediate product that complies with the following requirements may be used in the preparation of the final bulk.

Purity

Examine by non-reducing polyacrylamide gel electrophoresis *(2.2.31)*, by comparison with the reference preparation. The bands are compared in intensity and no additional bands are found.

FINAL BULK

The final bulk is prepared from a single intermediate product or from a pool of intermediate products obtained from animals of the same species. Intermediate products with different specificities may be pooled.

An antimicrobial preservative and a stabiliser may be added. If an antimicrobial preservative has been added to the blood or plasma, the same substance is used as the antimicrobial preservative in the final bulk.

Only a final bulk that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable physico-chemical method. It contains not less than 85 per cent and not more than 115 per cent of the amount stated on the label.

Sterility *(2.6.1)*

It complies with the test for sterility.

FINAL LOT

The final bulk of immunoserum is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that complies with the requirements prescribed below under Identification, Tests and Assay may be released for use. Provided that the tests for osmolality, protein content, molecular-size distribution, antimicrobial preservative, stabiliser, purity, foreign proteins and albumin and the assay have been carried out with satisfactory results on the final bulk, they may be omitted on the final lot.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

The identity is established by immunological tests and, where necessary, by determination of biological activity. The assay may also serve for identification.

CHARACTERS

Immunosera are clear to opalescent and colourless to very faintly yellow liquids. They are free from turbidity. Freeze-dried products are white or slightly yellow powders or solid friable masses. After reconstitution they show the same characteristics as liquid preparations.

TESTS

Solubility

To a container of the preparation to be examined, add the volume of the liquid for reconstitution stated on the label. The preparation dissolves completely within the time stated on the label.

Extractable volume *(2.9.17)*

It complies with the requirement for extractable volume.

pH *(2.2.3)*

The pH is within the limits approved for the particular product.

Osmolality *(2.2.35)*

Minimum 240 mosmol/kg after dilution, where applicable.

Protein content

90 per cent to 110 per cent of the amount stated on the label, and, unless otherwise justified and authorised, not more than 100 g/l.

Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R.* Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion *(2.5.9)* and calculate the content of protein by multiplying by 6.25.

Molecular-size distribution

Examine by liquid chromatography *(2.2.29 or 2.2.30)*. It complies with the specification approved for the particular product.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable physicochemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Phenol *(2.5.15)*

Maximum 2.5 g/l for preparations containing phenol.

Stabiliser

Determine the amount of stabiliser by a suitable physico-chemical method. The preparation contains not less than 80 per cent and not more than 120 per cent of the quantity stated on the label.

Purity

Examine by non-reducing polyacrylamide gel electrophoresis *(2.2.31)*, by comparison with the reference preparation. No additional bands are found for the preparation to be examined.

Foreign proteins

When examined by precipitation tests with specific antisera, only protein from the declared animal species is shown to be present, unless otherwise prescribed, for example where material of human origin is used during production.

Albumin

Unless otherwise prescribed in the monograph, when examined electrophoretically, the content of albumin is not greater than the limit approved for the particular product and, in any case, is not greater than 3 per cent.

Water *(2.5.12)*

Maximum 3 per cent.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

Unless otherwise justified and authorised, it complies with the test for pyrogens. Unless otherwise prescribed, inject 1 ml per kilogram of the rabbit's body mass.

ASSAY

Carry out a biological assay as indicated in the monograph and express the result in International Units per millilitre, where appropriate. A validated in vitro method may also be used.

STORAGE

Protected from light, at the temperature stated on the label. Do not allow liquid preparations to freeze.

Expiry date The expiry date is calculated from the beginning of the assay.

LABELLING

The label states:

— the number of International Units per millilitre, where applicable;

— the amount of protein per container;

— for freeze-dried preparations:

— the name and volume of the reconstituting liquid to be added;

— that the immunoserum is to be used immediately after reconstitution;

— the time required for complete dissolution;

— the route of administration;

— the storage conditions;

— the expiry date, except for containers of less than 1 ml which are individually packed; the expiry date may be omitted from the label on the container, provided it is shown on the package and the label on the package states that the container must be kept in the package until required for use;

— the animal species of origin;

— the name and amount of any antimicrobial preservative, any stabiliser and any other substance added to the immunoserum.

Ph Eur

Mixed Gas-gangrene Antitoxin

General Notices

(Ph Eur monograph 0090)

The label may state 'Gas/Ser'.

Ph Eur

DEFINITION

Mixed gas-gangrene antitoxin is prepared by mixing gas-gangrene antitoxin (novyi), gas-gangrene antitoxin (perfringens) and gas-gangrene antitoxin (septicum) in appropriate quantities.

IDENTIFICATION

It specifically neutralises the alpha toxins formed by *Clostridium novyi* (former nomenclature: *Clostridium oedematiens*), *Clostridium perfringens* and *Clostridium septicum*, rendering them harmless to susceptible animals.

ASSAY

Gas-gangrene antitoxin (novyi), not less than 1000 IU of antitoxin per millilitre; gas-gangrene antitoxin (perfringens), not less than 1000 IU of antitoxin per millilitre; gas-gangrene antitoxin (septicum) not less than 500 IU of antitoxin per millilitre.

Carry out the assay for each component, as prescribed in the monographs on *Gas-gangrene antitoxin (novyi) (0087)*, *Gas-gangrene antitoxin (perfringens) (0088)* and *Gas-gangrene antitoxin (septicum) (0089)*.

Ph Eur

Monoclonal Antibodies for Human Use

General Notices

(Ph Eur monograph 2031)

The requirements of the monograph for Immunosera do not necessarily apply to the monograph for Monoclonal Antibodies for Human Use.

Ph Eur

DEFINITION

Monoclonal antibodies for human use are preparations of an immunoglobulin or a fragment of an immunoglobulin, for example, F(ab′)2, with defined specificity, produced by a single clone of cells. They may be conjugated to other substances, including for radiolabelling.

They can be obtained from immortalised B lymphocytes that are cloned and expanded as continuous cell lines or from rDNA-engineered cell lines.

Currently available rDNA-engineered antibodies include the following antibodies.

Chimeric monoclonal antibodies The variable heavy and light chain domains of a human antibody are replaced by those of a non-human species, which possess the desired antigen specificity.

Humanised monoclonal antibodies The 3 short hypervariable sequences (the complementarity determining regions) of non-human variable domains for each chain are engineered into the variable domain framework of a human antibody; other sequence changes may be made to improve antigen binding.

Recombinant human monoclonal antibodies The variable heavy and light chain domain of a human antibody are combined with the constant region of a human antibody.

Monoclonal antibodies obtained from cell lines modified by recombinant DNA technology also comply with the requirements of the monograph *Products of recombinant DNA technology (0784).*

This monograph applies to monoclonal antibodies for therapeutic and prophylactic use and for use as *in vivo* diagnostics. It does not apply to monoclonal antibodies used as reagents in the manufacture of medicinal products. Nor does it apply to monoclonal antibodies produced in ascites, for which requirements are decided by the competent authority.

PRODUCTION

GENERAL PROVISIONS

Production is based on a seed-lot system using a master cell bank and, if applicable, a working cell bank derived from the cloned cells. The production method is validated during development studies in order to prevent transmission of infectious agents by the final product. All biological materials and cells used in the production are characterised and are in compliance with chapter *5.2.8. Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products*. Where monoclonal antibodies for human use are manufactured using materials of human or animal origin, the requirements of chapter *5.1.7. Viral safety* also apply. Where an immunogen is used it is characterised and the method of immunisation is documented.

Process validation

During development studies, the production method is validated for the following aspects:

— consistency of the production process including fermentation, purification and, where applicable, fragmentation method;

— removal or inactivation of infectious agents;

— adequate removal of product- and process-related impurities (for example, host-cell protein and DNA, protein A, antibiotics, cell-culture components);

— specificity and specific activity of the monoclonal antibody;

— absence of non-endotoxin pyrogens;

— reusability of purification components (for example, column material), limits or acceptance criteria being set as a function of the validation;

— methods used for conjugation, where applicable.

Product characterisation

The product is characterised to obtain adequate information including: structural integrity, isotype, amino-acid sequence, secondary structure, carbohydrate moiety, disulphide bridges, conformation, specificity, affinity, specific biological activity and heterogeneity (characterisation of isoforms).

A battery of suitable analytical techniques is used including chemical, physical, immunochemical and biological tests (for example, peptide mapping, N- and C-terminal amino-acid sequencing, mass spectrometry, chromatographic, electrophoretic and spectroscopic techniques). Additional tests are performed to obtain information on cross-reactivity with human tissues.

For those products that are modified by fragmentation or conjugation, the influence of the methods used on the antibody is characterised.

Process intermediates

Where process intermediates are stored, an expiry date or a storage period justified by stability data is established for each.

Biological assay

The biological assay is chosen in terms of its correlation with the intended mode of action of the monoclonal antibody.

Reference preparation

A batch shown to be stable and shown to be suitable in clinical trials, or a batch representative thereof, is used as a reference preparation for the identification, tests and assay. The reference preparation is appropriately characterised as defined under Product characterisation, except that it is not necessary to examine cross-reactivity for each batch of reference preparation.

Definition of a batch

Definition of a batch (including batch size) is required throughout the process.

SOURCE CELLS

Source cells include fusion partners, lymphocytes, myeloma cells, feeder cells and host cells for the expression of the recombinant monoclonal antibody.

The origin and characteristics of the parental cell are documented, including information on the health of the donors, and on the fusion partner used (for example, myeloma cell line, human lymphoblastoid B-cell line).

Wherever possible, source cells undergo suitable screening for extraneous agents and endogenous agents. The choice of viruses for the tests is dependent on the species and tissue of origin.

CELL LINE PRODUCING THE MONOCLONAL ANTIBODY

The suitability of the cell line producing the monoclonal antibody is demonstrated by:

— documentation on the history of the cell line including description of the cell fusion, immortalisation or transfection and cloning procedure;

— characterisation of the cell line (for example, phenotype, isoenzyme analysis, immunochemical markers and cytogenetic markers);

— characterisation of relevant features of the antibody;

— stability of antibody secretion with respect to the characteristics of the antibody and level of expression and glycosylation up to or beyond the population doubling level or generation number used for routine production;

— for recombinant DNA products, stability of the host/vector genetic and phenotypic characteristics up to or beyond the population doubling level or generation number used for routine production.

CELL BANKS

The master cell bank is a homogeneous suspension of the cell line producing the monoclonal antibody, distributed in equal volumes in a single operation into individual containers for storage.

A working cell bank is a homogeneous suspension of the cell material derived from the master cell bank at a finite passage level, distributed in equal volumes in a single operation into individual containers for storage.

Post-production cells are cells cultured up to or beyond the population doubling level or generation number used for routine production.

The following tests are performed on the master cell bank: viability, identity, sterility (bacteria, fungi, mycoplasmas), characterisation of the monoclonal antibody produced. Non-endogenous viral contamination is tested with a suitable range of *in vivo* and *in vitro* tests. Retrovirus and other endogenous viral contamination is tested using a suitable range of in vitro tests.

The following tests are performed on the working cell bank: viability, identity, sterility (bacteria, fungi, mycoplasmas). Adventitious viral contamination is tested with a suitable range of *in vivo* and *in vitro* tests. For the first working cell bank, these tests are performed on post-production cells, generated from that working cell bank; for working cell banks subsequent to the first working cell bank, a single *in vitro* and *in vivo* test can be done either directly on the working cell bank or on post-production cells.

For the master cell bank and working cell bank, tests for specific viruses are carried out when potentially contaminated biological material has been used during preparation of the cell banks, taking into account the species of origin of this material. This may not be necessary when this material is inactivated using validated procedures.

The following tests are performed on the post-production cells: sterility (bacteria, fungi, mycoplasmas). Virus tests are performed on cells or cell culture supernatants. For this, non-endogenous viral contamination is tested with a suitable range of *in vivo* and *in vitro* tests. Retrovirus and other endogenous viral contamination is tested using a suitable range of in vitro tests.

CULTURE AND HARVEST

Production at finite passage level (single harvest)

Cells are cultivated up to a defined maximum number of passages or population doublings (in accordance with the stability of the cell line). Product is harvested in a single operation.

Continuous-culture production (multiple harvest)

Cells are continuously cultivated for a defined period (in accordance with the stability of the system and production consistency). Monitoring is necessary throughout the life of the culture; the required frequency and type of monitoring will depend on the nature of the production system.

Each harvest is tested for antibody content, bioburden, endotoxin and mycoplasmas. Routine general or specific tests for adventitious viruses are carried out at a suitable stage depending on the nature of the manufacturing process and the materials used. For processes using production at finite passage level (single harvest), at least 3 harvests are tested for adventitious viruses using a suitable range of *in vitro* methods.

The acceptance criteria for harvests for further processing are clearly defined and linked to the schedule of monitoring applied. If any adventitious viruses are detected, the process is carefully investigated to determine the cause of the contamination and the harvest is not further processed. Harvests in which an endogenous virus has been detected are not used for purification unless an appropriate action plan has been defined to prevent transmission of infectious agents by the final product.

PURIFICATION

Harvests may be pooled before further processing. The purification process includes steps that remove and/or inactivate non-enveloped and enveloped viruses. A validated purification process, for which removal and/or inactivation of infectious agents and removal of product- and process-related impurities has been demonstrated is used. Defined steps of the process lead to a purified antibody of constant quality and biological activity.

The test programme on the purified monoclonal antibody depends on the validation of the process, on demonstration of consistency and on the expected level of product- and process-related impurities. The purified monoclonal antibody is tested for bioburden and bacterial endotoxins, purity, integrity and potency by suitable analytical methods, comparing with the reference preparation where necessary.

If storage of intermediates is intended, adequate stability of these preparations and its impact on quality or shelf-life of the finished product are evaluated.

FINAL BULK

The final bulk is prepared from one or more batches of purified monoclonal antibody. Suitable stabilisers and other excipients may be added during preparation of the final bulk.

Only a final bulk that complies with the following requirements may be used in the preparation of the final lot.

Sterility *(2.6.1)*

Carry out the test using 10 ml for each medium.

Bacterial endotoxins *(2.6.14)*

It complies with the limit approved for the particular product.

Process-related impurities

Suitable tests for host-cell-derived proteins, host-cell- and vector-derived DNA and other process-related impurities are carried out on a suitable number of final bulks or batches of purified monoclonal antibodies. The final bulk complies with the limits approved for the particular product. When consistency of the purification process has been demonstrated, the tests may subsequently be omitted.

FINAL LOT

The final bulk is distributed aseptically into sterile containers, which are then closed so as to prevent contamination.

CHARACTERS

Liquid preparations are clear or slightly opalescent, colourless or slightly yellow liquids, without visible particles. Freeze-dried products are white or slightly yellow powders or solid friable masses. After reconstitution they show the same characteristics as liquid preparations.

IDENTIFICATION

The identity is established by suitable validated methods comparing the product with the reference preparation. The assay also contributes to identification.

TESTS

Appearance

Liquid or reconstituted freeze-dried preparations are clear or slightly opalescent and colourless or slightly yellow, without visible particles.

Solubility

Freeze-dried preparations dissolve completely in the prescribed volume of reconstituting liquid, within a defined time, giving a clear or slightly opalescent solution without visible particles.

pH *(2.2.3)*

It complies with the limits approved for the particular product.

Osmolality *(2.2.35)*

Minimum 240 mosmol/kg, diluted for use where applicable.

Extractable volume *(2.9.17)*

It complies with the test for extractable volume.

Total protein *(2.5.33)*

It complies with the limits approved for the particular product.

Molecular-size distribution

Molecular-size distribution is determined by a suitable method, for example, size-exclusion chromatography *(2.2.30)*. It complies with the limits approved for the particular product.

Molecular identity and structural integrity

Depending on the nature of the monoclonal antibody, its microheterogeneity and isoforms, a number of different tests can be used to demonstrate molecular identity and structural integrity. These tests may include peptide mapping, isoelectric focusing, ion-exchange chromatography, hydrophobic interaction chromatography, oligosaccharide mapping, monosaccharide content and mass spectrometry.

Purity

Examine by a suitable validated method, such as SDS-polyacrylamide gel electrophoresis *(2.2.31)* under non-reducing and reducing conditions or capillary electrophoresis *(2.2.47)*. Suitable tests for process-related and product-related impurities are carried out.

Stabiliser

Where applicable, it complies with the limits approved for the particular product.

Water *(2.5.12)*

Freeze-dried products comply with the limits approved for the particular product.

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

It complies with the limits approved for the particular product.

Tests applied to modified antibodies

Suitable tests are carried out depending on the type of modification.

ASSAY

Carry out a suitable assay against the reference preparation. Design of the assay and calculation of the results are made according to the usual principles *(for example, 5.3)*.

STORAGE

As stated on the label.

Expiry date The expiry date is calculated from the date of sterile filtration, the date of filling (for liquid preparations) or the date of freeze-drying (where applicable).

LABEL

The label states:

— the number of International Units per millilitre, where applicable;

— the quantity of protein per container;

— the quantity of monoclonal antibody in the container;

— for liquid preparations, the volume of the preparation in the container;

— for freeze-dried preparations:
  — the name and the volume of the reconstitution liquid to be added;
  — the period of time within which the monoclonal antibody is to be used after reconstitution;

— the dilution to be made before use of the product, where applicable.

Ph Eur

Tetanus Antitoxin

General Notices

(Tetanus Antitoxin for Human Use, Ph Eur monograph 0091)

The label may state 'Tet/Ser'.

Ph Eur

DEFINITION

Tetanus antitoxin for veterinary use is a preparation containing principally the globulins that have the power of specifically neutralising the neurotoxin formed by *Clostridium tetani*.

PRODUCTION

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunised against tetaunus toxin.

IDENTIFICATION

It specifically neutralises the toxin formed by *Cl. Tetani*, rendering it harmless to susceptible animals.

POTENCY

Not less than 1000 IU of antitoxin per millilitre when intended for prophylactic use. Not less than 3000 IU if antitoxin per millilitre when intended for therapeutic use.

The potency of tetanus antitoxin is determined by comparing the dose necessary to protect guinea-pigs or mice against the paralytic effects of a fixed dose of tetanus toxin with the quantity of the standard preparation of tetanus antitoxin necessary to give the same protection. In countries where the paralysis method is not obligatory the lethal method may be used. For this method the number of animals and the procedure are identical with those described for the paralysis method but the end-point is the death of the animal rather than the onset of paralysis and the L+/10 dose is used instead of the Lp/10 dose. For this comparison a reference preparation of tetanus antitoxin, calibrated in International Units, and a suitable preparation of tetanus toxin, for use as a test toxin, are required. The potency of the test toxin is determined in relation to the reference preparation; the potency of the tetanus antitoxin to be examined is determined in relation to the potency of the test toxin by the same method.

The International Unit of antitoxin is the specific neutralising activity for tetanus toxin contained in a stated amount of the International Standard which consists of a quantity of dried immune horse serum. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Selection of animals If mice are used, the body masses should be such that the difference between the lightest and the heaviest dose does not exceed 5 g.

Preparation of test toxin Prepare the text toxin from a sterile filtrate of an approximately 9-day culture in liquid medium of *Cl. tetani*. To the filtrate add 1 to 2 volumes of glycerol and store slightly below 0 °C. Alternatively, treat the filtrate with *ammonium sulphate R*, collect the precipitate, which contains the toxin, dry *in vacuo* over *diphosphorus pentoxide R*, powder and store and dry, either in sealed ampoules or *in vacuo* over *diphosphorous pentoxide R*.

Determination of test dose of toxin (Lp/10 dose) Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.5 IU of antitoxin per millilitre.

If the test toxin is stored dry, reconstitute it using a suitable liquid.

Prepare the mixtures of the solution of the reference preparation and the test toxin such that each contains 2.0 ml of the solution of the reference preparation, one of a graded series of volumes of the test toxin and a sufficient of a suitable liquid to bring the volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.5 ml subcutaneously into each mouse. Observe the mice for 96 h. Mice that become paralysed may be euthanised.

The test dose of toxin is the quantity in 0.5 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, paralysis in all six mice injected with the mixture within the observation period.

Determination of potency of the antitoxin Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.5 IU of antitoxin per millilitre.

Prepare a solution of the test toxin in a suitable liquid such that it contains five test doses per millilitre.

Prepare the mixtures of the solution of the test toxin and the antitoxin to be examined such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the antitoxin to be examined and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (2.0 ml) that contains 1 IU and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using six mice for each mixture, inject into each mouse subcutaneously a dose of 0.5 ml. Observe the mice for 96 h. Mice that become paralysed may be euthanised.

The mixture that contains the largest volume of antitoxin that fails to protect the mice from paralysis contains 1 IU. This quantity is used to calculate the potency of the antitoxin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 2.0 or less of the solution of the reference preparation show paralysis and all those injected with mixtures containing more do not.

Ph Eur

Ammonia [^{13} N] Injection

General Notices

(Ph Eur monograph 1492)

Ph Eur

DEFINITION

Ammonia (^{13}N) injection is a sterile solution of [^{13}N]ammonia for diagnostic use. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared nitrogen-13 radioactivity at the date and time stated on the label. Not less than 99 per cent of the total radioactivity corresponds to nitrogen-13 in the form of [^{13}N]ammonia. Not less than 99.0 per cent of the total radioactivity corresponds to nitrogen-13.

PRODUCTION

RADIONUCLIDE PRODUCTION

Nitrogen-13 is a radioactive isotope of nitrogen which may be produced by various nuclear reactions, such as proton irradiation of carbon-13 or oxygen-16, or deuteron irradiation of carbon-12.

RADIOCHEMICAL SYNTHESIS

[^{13}N]Ammonia may be prepared by proton irradiation of water followed by the reduction of the resulting [^{13}N]nitrates/nitrites mixture with a reducing agent. The [^{13}N]ammonia formed is distilled from the reaction mixture and trapped in a slightly acidic solution.

Other methods may produce [^{13}N]ammonia "in-target" by proton irradiation of water containing a small amount of ethanol or acetic acid, or by proton irradiation of a slurry of [^{13}C]carbon powder in water. The resulting solution can be purified, to remove radionuclidic and radiochemical impurities, using anion and cation exchange columns.

CHARACTERS

A clear, colourless solution.

Nitrogen-13 has a half-life of 9.96 min and emits positrons with a maximum energy of 1.198 MeV, followed by annihilation gamma radiation of 0.511 MeV.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with test (a) for radionuclidic purity (see Tests).

C. Examine the chromatograms obtained in the test for radiochemical purity. The principal peak in the radiochromatogram obtained with the test solution has approximately the same retention time as the principal peak in the radiochromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

The pH of the injection is 5.5 to 8.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, *V* being the maximum recommended dose in millilitres. The injection may be released for use before completion of the test.

CHEMICAL PURITY

Aluminium In a test-tube about 12 mm in internal diameter, mix 1 ml of *acetate buffer solution pH 4.6 R* and 2 ml of a 1 in 20 dilution of the preparation to be examined in *water R*. Add 0.05 ml of a 10 g/l solution of *chromazurol S R*. After 3 min, the colour of the solution is not more intense than that of a standard prepared at the same time and in the same manner using 2 ml of a 1 in 20 dilution of *aluminium standard solution (2 ppm Al) R* (2 ppm).

The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

(a) Half-life The half-life is between 9 min and 11 min.

(b) Gamma emitting impurities Retain a sample of the preparation to be examined for 2 h. Examine the gamma-ray spectrum of the decayed material for the presence of radionuclidic impurities, which should, where possible, be identified and quantified. The total gamma radioactivity due to these impurities does not exceed 1.0 per cent of the total radioactivity.

The injection may be released for use before completion of tests (a) and (b).

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Test solution The preparation to be examined.

Reference solution Dilute 1.0 ml of *dilute ammonia R2* to 10.0 ml with *water R*.

The chromatographic procedure may be carried out using:

— a column 0.04 m long and 4.0 mm in internal diameter packed with *cation exchange resin R* (10 µm);

— as mobile phase at a flow rate of 2 ml/min *0.002 M nitric acid* ;

— a suitable radioactivity detector;

— a conductivity detector;

— a loop injector;

maintaining the column at a constant temperature between 20 °C and 30 °C.

Inject separately the test solution and the reference solution. The chromatogram obtained with the radioactivity detector and the test solution shows a principal peak with approximately the same retention time as the peak in the chromatogram obtained with the reference solution and the conductivity detector. Not less than 99 per cent of the total radioactivity corresponds to nitrogen-13 in the form of ammonia.

The injection may be released for use before completion of the test.

RADIOACTIVITY

Measure the radioactivity using suitable equipment by comparison with a standardised fluorine-18 solution or by using an instrument calibrated with the aid of such a solution. Standardised fluorine-18 solutions are available from laboratories recognised by the competent authority.

IMPURITIES

A. $[^{13}N]O_2$-;

B. $[^{13}N]O_3$-;

C. [18F-];

D. $H_2[^{15}O]$.

Ph Eur

Carbon Monoxide [^{15} O]

General Notices

(Ph Eur monograph 1607)

Ph Eur

DEFINITION

Mixture of carbon [^{15}O]monoxide in the gaseous phase and a suitable vehicle such as *Medicinal air (1238),* for diagnostic use.

Purity:

— minimum 99 per cent of the total radioactivity corresponds to oxygen-15;

— minimum 97 per cent of the total radioactivity corresponds to oxygen-15 in the form of carbon monoxide (CO).

PRODUCTION

RADIONUCLIDE PRODUCTION

Oxygen-15 is a radioactive isotope of oxygen which may be produced by various nuclear reactions such as proton irradiation of nitrogen-15 or deuteron irradiation of nitrogen-14.

RADIOCHEMICAL SYNTHESIS

In order to recover oxygen-15 as molecular oxygen from the nitrogen target gas, carrier oxygen is added at concentrations generally ranging from 0.2 per cent *V/V* to 1.0 per cent *V/V*. After irradiation, the target gas is usually reacted with activated charcoal at a temperature of about 950 °C. The activated charcoal is preconditioned before use by flushing an inert gas at the production flow rate at a temperature of about 950 °C for not less than 1 h. The carbon [^{15}O]monoxide obtained is purified by passage through a carbon dioxide scavenger, such as soda lime, before mixing with the vehicle.

CHARACTERS

Appearance

Colourless gas.

HALF-LIFE AND NATURE OF RADIATION OF OXYGEN-15

See general chapter 5.7. Table of physical characteristics of radionuclides.

IDENTIFICATION

A. Gamma spectrometry.

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with the test for radionuclidic purity (see Tests).

C. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peaks in the chromatogram obtained with the test gas using the radioactivity detector are similar in retention times to the principal peaks corresponding to carbon monoxide in the chromatogram obtained with reference gas (a) using the thermal conductivity detector.

TESTS

The following tests are performed on carbon [^{15}O]monoxide as described under radiochemical synthesis before mixing with the vehicle.

Carbon monoxide

Gas chromatography (*2.2.28*) as described in the test for radiochemical purity.

The concentration of carbon monoxide in the test sample is determined before administration and is used to calculate the amount of carbon monoxide to be administered to the patient.

Injection Test sample, reference gas (b).

Examine the chromatogram obtained with the thermal conductivity detector and calculate the content of carbon monoxide.

RADIONUCLIDE PURITY

Oxygen-15: minimum 99 per cent of the total radioactivity.

A. Gamma spectrometry.

Comparison Standardised fluorine-18 solution, or by using an instrument calibrated with the aid of such a solution. Standardised fluorine-18 solutions and/or standardisation services are available from the competent authority.

Results The spectrum obtained with the solution to be examined does not differ significantly from that obtained with a standardised fluorine-18 solution.

B. Half-life: 1.9 min to 2.2 min.

The preparation may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Carbon [^{15}O]monoxide

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test sample Carbon [^{15}O]monoxide as described under radiochemical synthesis.

Reference gas (a) *Nitrogen gas mixture R.*

Reference gas (b) *Nitrogen R*, containing 2.0 per cent *V/V* of *carbon monoxide R1*.

Column:
- *size:* *l* = 1.8 m, Ø1 = 6.3 mm and Ø2 = 3.2 mm;
- *stationary phase*: *GC concentrical column R.*

Carrier gas *helium for chromatography R.*

Flow rate 65 ml/min.

Temperature:
- *column*: 40 °C;
- *injection port*: 40 °C;
- *thermal conductivity detector*: 70 °C.

Detection Thermal conductivity detector and radioactivity detector connected in series.

Injection Loop injector.

Run time 10 min.

Retention times Oxygen, nitrogen and carbon monoxide eluting from the inner column = about 0.4 min; carbon dioxide eluting from the inner column = about 0.8 min; oxygen eluting from the outer column = about 2.1 min; nitrogen eluting from the outer column = about 3.1 min; carbon monoxide eluting from the outer column = about 6.2 min.

System suitability Reference gas (a):

- 5 clearly separated principal peaks are observed in the chromatogram obtained using the thermal conductivity detector,
- *resolution*: minimum of 1.5 between the peaks due to carbon dioxide eluting from the inner column and oxygen eluting from the outer column, in the chromatogram obtained using the thermal conductivity detector.

Limits Examine the chromatogram obtained with the radioactivity detector and calculate the percentage content of oxygen-15 substances from the peak areas.

- carbon [^{15}O]monoxide: minimum 97 per cent of the total radioactivity.
- disregard the first peak corresponding to components co-eluting from the inner column.

RADIOACTIVITY

The radioactive concentration is determined before administration.

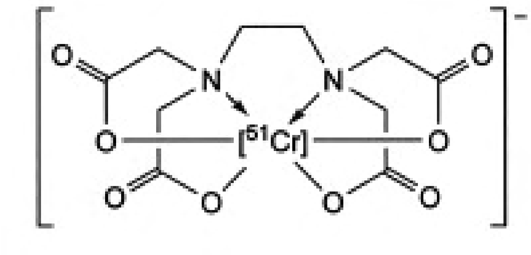
Measure the radioactivity using suitable equipment by comparison with a standardised fluorine-18 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Chromium [^{51} Cr] Edetate Injection

General Notices

(Ph Eur monograph 0266)



Ph Eur

DEFINITION

Sterile solution containing chromium-51 in the form of a complex of chromium(III) with (ethylenedinitrilo)tetraacetic acid, the latter being present in excess. It may be made isotonic by the addition of sodium chloride and may contain a suitable antimicrobial preservative such as benzyl alcohol.

Content:

— *chromium-51*: 90.0 per cent to 110.0 per cent of the declared chromium-51 radioactivity at the date and time stated on the label;

— *chromium*: maximum 1 mg/ml.

PRODUCTION

Chromium-51 is a radioactive isotope of chromium and may be prepared by neutron irradiation of chromium, either of natural isotopic composition or enriched in chromium-50.

CHARACTERS

Appearance

Clear, violet solution.

Half-life and nature of radiation of chromium-51

See general chapter *5.7 . Table of physical characteristics of radionuclides*.

IDENTIFICATION

A. Radionuclidic purity (see Tests).

B. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retardation factor to the principal peak in the chromatogram obtained with the reference solution.

TESTS

pH *(2.2.3)*

3.5 to 6.5.

Chromium

Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution The preparation to be examined.

Reference solution Dissolve 0.96 g of *chromic potassium sulphate R* and 2.87 g of *sodium edetate R* in 50 ml of *water R* , boil for 10 min, cool, adjust to pH 3.5-6.5 with *dilute sodium hydroxide solution R* and dilute to 100.0 ml with *water R* .

Measure the absorbance of the test solution and the reference solution at the absorption maximum at 560 nm. The absorbance of the test solution is not greater than that of the reference solution (1 mg/ml).

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Chromium-51

Minimum 99.9 per cent of the total radioactivity.

Gamma-ray spectrometry.

Results The only gamma photons have an energy of 0.320 MeV.

RADIOCHEMICAL PURITY

[^{51}Cr]Chromium edetate

Descending paper chromatography *(2.2.26)* .

Test solution The preparation to be examined.

Reference solution Use the reference solution from the test for chromium.

Chromate carrier solution Dissolve 0.1 g of *potassium chromate R* in 1 ml of *concentrated ammonia R1* and dilute to 100 ml with *water R* .

Paper *paper for chromatography R.*

Mobile phase *concentrated ammonia R1, ethanol (96 per cent) R, water R* (1:2:5 *V/V/V*).

Application Apply a band of a 50 g/l solution of *lead acetate R* to the paper at about 4 cm from the origin and dry in hot air. Apply 10 µl of the chromate carrier solution at the origin, followed by 10 µl of the test solution on the same spot. On a separate sheet, repeat the above procedure, applying 10 µl of the reference solution instead of the test solution.

Development Immediately, over a path of 14 cm.

Drying In air.

Detection Determine the distribution of radioactivity using a suitable radioactivity detector.

Retardation factors Impurity A = 0; impurity B = 0.2 to 0.4; [^{51}Cr]chromium edentate = 0.8 to 0.9.

System suitability The band of lead acetate turns yellow due to reaction with the chromate carrier solution. The retardation factor of the radioactive spot due to [^{51}Cr]chromium edetate in the radiochromatogram obtained with the test solution is similar to that of the violet spot in the chromatogram obtained with the reference solution.

Limit:

- *[^{51} Cr]chromium edetate*: minimum 97.0 per cent of the total radioactivity due to chromium-51.

RADIOACTIVITY

Measure the radioactivity using a calibrated instrument.

IMPURITIES

A. [^{51}Cr]chromium(III) ion,

B. [^{51}Cr]chromate ion.

Ph Eur

Cyanocobalamin [^{57} Co] Capsules

General Notices

(Ph Eur monograph 0710)

Ph Eur

DEFINITION

Cyanocobalamin (^{57}Co) capsules contain [^{57}Co]-α-(5,6-dimethylbenzimidazol-1-yl)cobamide cyanide and may contain suitable auxiliary substances. Cobalt-57 is a radioactive isotope of cobalt and may be produced by proton irradiation of nickel. Cyanocobalamin (^{57}Co) may be prepared by the growth of suitable micro-organisms on a medium containing (^{57}Co) cobaltous ion. Not less than 90 per cent of the cobalt-57 is in the form of cyanocobalamin. The capsules comply with the requirements for hard capsules in the monograph on *Capsules (0016),* unless otherwise justified and authorised.

CHARACTERS

Hard gelatin capsules.

Cobalt-57 has a half-life of 271 days and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised cobalt-57 solution. Standardised cobalt-57 and cobalt-58 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of cobalt-57 has an energy of 0.122 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The principal peak in the radio-chromatogram obtained with the test solution has a retention time similar to that of the peak in the chromatogram obtained with the reference solution.

TESTS

Disintegration

The capsules comply with the test for disintegration of tablets and capsules (*2.9.1*) except that one capsule is used in the test instead of six.

Uniformity of content

Determine by measurement in a suitable counting assembly and under identical geometrical conditions the radioactivity of each of not less than ten capsules. Calculate the average radioactivity per capsule. The radioactivity of no capsule differs by more than 10 per cent from the average. The relative standard deviation is less than 3.5 per cent.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument calibrated with the aid of standardised cobalt-57 and cobalt-58 solutions. The spectrum does not differ significantly from that of the stan-dardised cobalt-57 solution. Determine the relative amounts of cobalt-57, cobalt-56 and cobalt-58 present. Cobalt-56 has a half-life of 78 days and its presence is shown by gamma photons of energy 0.847 MeV. Cobalt-58 has a half-life of 70.8 days and its presence is shown by gamma photons of energy 0.811 MeV. Not more than 0.1 per cent of the total radioactivity is due to cobalt-56, cobalt-58 and other radionuclidic impurities.

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Test solution Dissolve the contents of a capsule in 1.0 ml of *water R* and allow to stand for 10 min. Centrifuge at 2000 r/min for 10 min. Use the supernatant.

Reference solution Dissolve 10 mg of *cyanocobalamin CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 2 ml of the solution to 100 ml with the mobile phase. Use within 1 h.

The chromatographic procedure may be carried out using:

- — a stainless steel column 0.25 m long and 4 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm),
- — as mobile phase at a flow rate of 1.0 ml/min a mixture prepared as follows: mix 26.5 volumes of *methanol R* and 73.5 volumes of a 10 g/l solution of *disodium hydrogen phosphate R* adjusted to pH 3.5 using *phosphoric acid R* and use within 2 days,
- — a radioactivity detector adjusted for cobalt-57,
- — as detector a spectrophotometer set at 361 nm,
- — a loop injector.

Inject 100 µl of the test solution and record the chromatogram for three times the retention time of cyanocobalamin. Determine the peak areas and calculate the percentage of cobalt-57 present as cyanocobalamin. Inject 100 µl of the reference solution and record the chromatogram for 30 min.

RADIOACTIVITY

The average radioactivity determined in the test for uniformity of content is not less than 90.0 per cent and not more than 110.0 per cent of the declared cobalt-57 radioactivity, at the date stated on the label.

STORAGE

Store in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Cyanocobalamin [^{57} Co] Solution

General Notices

(Ph Eur monograph 0269)

Ph Eur

DEFINITION

Cyanocobalamin (^{57}Co) solution is a solution of [^{57}Co]-α-(5,6-dimethylbenzimidazol-1-yl) cobamide cyanide and may contain a stabiliser and an antimicrobial preservative. Cobalt-57 is a radioactive isotope of cobalt and may be produced by the irradiation of nickel with protons of suitable energy. Cyanocobalamin (^{57}Co) may be prepared by the growth of suitable micro-organisms on a medium containing (^{57}Co) cobaltous ion. The solution contains not less than 90.0 per cent and not more than 110.0 per cent of the declared cobalt-57 radioactivity at the date stated on the label. Not less than 90 per cent of the cobalt-57 is in the form of cyanocobalamin.

CHARACTERS

A clear, colourless or slightly pink solution. Cobalt-57 has a half-life of 271 days and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised cobalt-57 solution. Standardised cobalt-57 and cobalt-58 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of cobalt-57 has an energy of 0.122 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The principal peak in the radiochromatogram obtained with the solution to be examined has a retention time similar to that of the peak in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

The pH of the solution is 4.0 to 6.0.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument calibrated with the aid of standardised cobalt-57 and cobalt-58 solutions. The spectrum does not differ significantly from that of the standardised cobalt-57 solution. Determine the relative amounts of cobalt-57, cobalt-56 and cobalt-58 present. Cobalt-56 has a half-life of 78 days and its presence is shown by gamma photons of energy 0.847 MeV. Cobalt-58 has a half-life of 70.8 days and its presence is shown by gamma photons of energy 0.811 MeV. Not more than 0.1 per cent of the total radioactivity is due to cobalt-56, cobalt-58 and other radionuclidic impurities.

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Reference solution Dissolve 10 mg of *cyanocobalamin CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 2 ml of the solution to 100 ml with the mobile phase. Use within 1 h.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4 mm in internal diameter packed with octylsilyl silica gel for chromatography R (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture prepared as follows: mix 26.5 volumes of *methanol R* and 73.5 volumes of a 10 g/l solution of *disodium hydrogen phosphate R* adjusted to pH 3.5 using *phosphoric acid R* and use within 2 days,

— a radioactivity detector adjusted for cobalt-57,

— as detector a spectrophotometer set at 361 nm,

— a loop injector.

Inject 100 µl of the solution to be examined and record the chromatogram for three times the retention time of cyanocobalamin. Determine the peak areas and calculate the percentage of cobalt-57 present as cyanocobalamin. Inject 100 µl of the reference solution and record the chromatogram for 30 min.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised cobalt-57 solution.

STORAGE

Store protected from light at a temperature of 2 °C to 8 °C.

Ph Eur

Cyanocobalamin [^{58} Co] Capsules

General Notices

(Ph Eur monograph 1505)

Ph Eur

DEFINITION

Cyanocobalamin (^{58}Co) capsules contain [^{58}Co]-α-(5,6-dimethylbenzimidazol-1-yl)cobamide cyanide and may contain suitable auxiliary substances. Cobalt-58 is a radioactive isotope of cobalt and may be produced by neutron irradiation of nickel. Cyanocobalamin (^{58}Co) may be prepared by the growth of suitable micro-organisms on a medium containing (^{58}Co) cobaltous ion. Not less than 84 per cent of the cobalt-58 is in the form of cyanocobalamin. The capsules comply with the requirements for hard capsules in the monograph on *Capsules (0016),* unless otherwise justified and authorised. The average radioactivity is not less than 90.0 per cent and not more than 110.0 per cent of the declared cobalt-58 radioactivity at the date stated on the label.

CHARACTERS

Hard gelatin capsules.

Cobalt-58 has a half-life of 70.9 days and emits beta (β^+) radiation and gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised cobalt-58 solution. Standardised cobalt-58 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photons of cobalt-58 have energies of 0.511 MeV (annihilation radiation) and 0.811 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The principal peak in the radiochromatogram obtained with the test solution has a retention time similar to that of the peak in the chromatogram obtained with the reference solution.

TESTS

Disintegration

The capsules comply with the test for disintegration of tablets and capsules (*2.9.1*) except that one capsule is used in the test instead of six.

Uniformity of content

Determine by measurement in a suitable counting assembly and under identical geometrical conditions the radioactivity of each of not less than ten capsules. Calculate the average radioactivity per capsule. The radioactivity of no capsule differs by more than 10 per cent from the average. The relative standard deviation is less than 3.5 per cent.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument calibrated with the aid of standardised cobalt-58, cobalt-57 and cobalt-60 solutions. The spectrum does not differ significantly from that of the standardised cobalt-58 solution. Standardised cobalt-58, cobalt-57 and cobalt-60 solutions are available from laboratories recognised by the competent authority. Determine the relative amounts of cobalt-58, cobalt-57 and cobalt-60 present. Cobalt-57 has a half-life of 272 days and its presence is shown by gamma photons of energy 0.122 MeV. Cobalt-60 has a half-life of 5.27 years and its presence is shown by gamma photons of energies 1.173 MeV and 1.333 MeV. Not more than 1 per cent of the total radioactivity is due to cobalt-60 and not more than 2 per cent of the total radioactivity is due to cobalt-57, cobalt-60 and other radionuclidic impurities.

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Test solution Dissolve the contents of a capsule in 1.0 ml of *water R* and allow to stand for 10 min. Centrifuge at 2000 r/min for 10 min. Use the supernatant.

Reference solution Dissolve 10 mg of *cyanocobalamin CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 2 ml of the solution to 100 ml with the mobile phase. Use within 1 h of preparation.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture prepared as follows: mix 26.5 volumes of *methanol R* and 73.5 volumes of a 10 g/l solution of *disodium hydrogen phosphate R* , adjusted to pH 3.5 with *phosphoric acid R* and use within 2 days,

— a radioactivity detector adjusted for cobalt-58,

— as detector a spectrophotometer set at 361 nm,

— a loop injector.

Inject 100 µl of the test solution and record the chromatogram for three times the retention time of cyanocobalamin. Determine the peak areas and calculate the percentage of cobalt-58 present as cyanocobalamin. Inject 100 µl of the reference solution and record the chromatogram for 30 min.

RADIOACTIVITY

The average radioactivity determined in the test for uniformity of content is not less than 90.0 per cent and not more than 110.0 per cent of the declared cobalt-58 radioactivity, at the date stated on the label.

STORAGE

Store in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

Ph Eur

Cyanocobalamin [^{58} Co] Solution

General Notices

(Ph Eur monograph 0270)

Ph Eur

DEFINITION

Cyanocobalamin (^{58}Co) solution is a solution of [^{58}Co]-α-(5,6-dimethylbenzimidazol-1-yl) cobamide cyanide and may contain a stabiliser and an antimicrobial preservative. Cobalt-58 is a radioactive isotope of cobalt and may be produced by neutron irradiation of nickel. Cyanocobalamin (^{58}Co) may be prepared by the growth of suitable micro-organisms on a medium containing (^{58}Co) cobaltous ion. The solution contains not less than 90.0 per cent and not more than 110.0 per cent of the declared cobalt-58 radioactivity at the date stated on the label. Not less than 90 per cent of the cobalt-58 is in the form of cyanocobalamin.

CHARACTERS

A clear, colourless or slightly pink solution.

Cobalt-58 has a half-life of 70.8 days and emits beta (β^+) radiation and gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised cobalt-58 solution. Standardised cobalt-58, cobalt-57 and cobalt-60 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photons of cobalt-58 have energies of 0.511 MeV (annihilation radiation) and 0.811 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The principal peak in the radiochromatogram obtained with the solution to be examined has a retention time similar to that of the peak in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

The pH of the solution is 4.0 to 6.0.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument having adequate resolution and calibrated with the aid of standardised cobalt-58, cobalt-57 and cobalt-60 solutions. The spectrum does not differ significantly from that of the standardised cobalt-58 solution. Determine the relative amounts of cobalt-58, cobalt-57 and cobalt-60 present. Cobalt-57 has a half-life of 271 days and its presence is shown by gamma photons of energy 0.122 MeV. Cobalt-60 has a half-life of 5.27 years and its presence is shown by gamma photons of energies 1.173 MeV and 1.332 MeV. Not more than 1 per cent of the total radioactivity is due to cobalt-60 and not more than 2 per cent of the total radioactivity is due to cobalt-57, cobalt-60 and other radionuclidic impurities.

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Reference solution Dissolve 10 mg of *cyanocobalamin CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 2 ml of the solution to 100 ml with the mobile phase. Use within 1 h.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture prepared as follows: mix 26.5 volumes of *methanol R* and 73.5 volumes of a 10 g/l solution of *disodium hydrogen phosphate R* adjusted to pH 3.5 using *phosphoric acid R* and use within 2 days,

— a radioactivity detector adjusted for cobalt-58,

— as detector a spectrophotometer set at 361 nm,

— a loop injector.

Inject 100 µl of the solution to be examined and record the chromatogram for three times the retention time of cyanocobalamin. Determine the peak areas and calculate the percentage of cobalt-58 present as cyanocobalamin. Inject 100 µl of the reference solution and record the chromatogram for 30 min.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised cobalt-58 solution or by measurement in an instrument calibrated with the aid of such a solution.

STORAGE

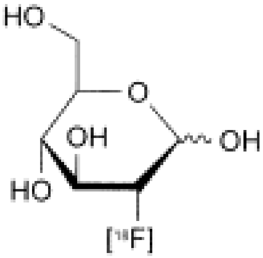
Store protected from light at a temperature of 2 °C to 8 °C.

Ph Eur

Fludeoxyglucose [^{18} F] Injection

General Notices

(Ph Eur monograph 1325)



Ph Eur

DEFINITION

Sterile solution containing 2-[^{18}F]fluoro-2-deoxy-D-glucopyranose (2-[^{18}F]fluoro-2-deoxy-D-glucose) prepared by nucleophilic substitution. It may also contain 2-[^{18}F]fluoro-2-deoxy-D-mannose.

Content:

— *fluorine-18*: 90 per cent to 110 per cent of the declared fluorine-18 radioactivity at the date and time stated on the label;

— *fluorine-18 in the form of 2-[^{18} F]fluoro-2-deoxy- D -glucose and 2-[^{18} F]fluoro-2-deoxy- D -mannose* : minimum 95 per cent of the total radioactivity;

— *2-[^{18} F]fluoro-2-deoxy- D -mannose* : maximum 10 per cent of the total radioactivity due to 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose;

— *2-fluoro-2-deoxy-d-glucose* : maximum 0.5 mg per maximum recommended dose, in millilitres.

PRODUCTION

RADIONUCLIDE PRODUCTION

The radionuclide fluorine-18 is most commonly produced by proton irradiation of water enriched in oxygen-18.

RADIOCHEMICAL SYNTHESIS

2-[^{18}F]Fluoro-2-deoxy-D-glucose is mostly prepared by phase transfer catalysed nucleophilic substitution of 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulphonyl-β-D-mannopyranose (tetraacetylmannose triflate) with [^{18}F]fluoride. Generally, [^{18}F]fluoride is adsorbed on an anion-exchange resin and eluted with a solution of potassium carbonate, which is then evaporated to dryness. Addition of a phase-transfer catalyst, such as an aminopolyether or a tetra-alkyl ammonium salt in dry acetonitrile, may be used to enhance the nucleophilicity of the [^{18}F]fluoride so that it reacts easily with the tetraacetylmannose triflate at elevated temperature. A variation of the method uses solid-phase-catalysed nucleophilic substitution on derivatised anion-exchange resin, for example, derivatised with 4-(4-methylpiperidin-1-yl) pyridine.

Hydrolysis under either alkaline or acidic conditions yields 2-[^{18}F]fluoro-2-deoxy-D-glucose. Depending on the conditions of hydrolysis, variable amounts of 2-chloro-2-deoxy-D-glucose and/or 2-[^{18}F]fluoro-2-deoxy-D-mannose may be formed as a by-product.

The preparation can be purified by serial chromatography on combinations of ion-retardation resin, ion-exchange resin, alumina and octadecylsilyl derivatised silica gel.

CHARACTERS

Appearance

Clear, colourless or slightly yellow solution.

Half-life and nature of radiation of fluorine-18: see general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Test A for radionuclidic purity (see Tests).

B. Determine the approximate half-life by no fewer than 3 measurements of the activity of a sample in the same geometrical conditions within a suitable period of time (for example, 30 min).

Result 105 to 115 min.

C. Examine the chromatograms obtained in test A for radiochemical purity (see Tests).

Result The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Particular tests for chemical impurities may be omitted if the substances mentioned are not used or cannot be formed in the production process.

pH *(2.2.3)*

4.5 to 8.5.

2-Fluoro-2-deoxy-D-glucose and impurity A

Liquid chromatography *(2.2.29)* .

Test solution The preparation to be examined.

Reference solution (a) Dissolve 1.0 mg of *2-fluoro-2-deoxy- D -glucose R* in *water R* and dilute to 2.0 ml with the same solvent. Dilute 1.0 ml of the solution to *V* with *water R* , *V* being the maximum recommended dose in millilitres.

Reference solution (b) Dissolve 1.0 mg of *2-chloro-2-deoxy- D -glucose R* (impurity A) in *water R* and dilute to 2.0 ml with the same solvent. Dilute 1.0 ml of the solution to *V* with *water R* , V being the maximum recommended dose in millilitres.

Reference solution (c) Dissolve 1.0 mg of *2-fluoro-2-deoxy- D -mannose R* in *water R* and dilute to 2.0 ml with the same solvent. Mix 0.5 ml of this solution with 0.5 ml of reference solution (a).

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *strongly basic anion exchange resin for chromatography R* (10 µm);

— *temperature*: constant, between 20 °C and 25 °C.

Mobile phase 4 g/l solution of *sodium hydroxide R* in *carbon dioxide-free water R* .

Flow rate 1 ml/min.

Detection Detector suitable for carbohydrates in the required concentration range, such as pulse amperometric detector and radioactivity detector connected in series.

Injection 20 µl.

Run time Twice the retention time of 2-fluoro-2-deoxy-D-glucose.

Relative retention With reference to 2-fluoro-2-deoxy-D-glucose (retention time = about 12 min): 2-fluoro-2-deoxy-D-mannose = about 0.9; impurity A = about 1.1.

System suitability Reference solution (c) using the carbohydrate detector:

— *resolution*: minimum 1.5 between the peaks due to 2-fluoro-2-deoxy-D-mannose and 2-fluoro-2-deoxy-D-glucose,

— *signal-to-noise ratio* : minimum 10 for the peak due to 2-fluoro-2-deoxy-D-glucose.

Limits In the chromatogram obtained with the carbohydrate detector:

— *2-fluoro-2-deoxy- D -glucose* : not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 mg/*V*);

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 mg/*V*).

Impurity B

Spot test.

Test solution The preparation to be examined.

Reference solution (a) *water R.*

Reference solution (b) Dissolve 11.0 mg of *aminopolyether R* (impurity B) in *water R* and dilute to 5.0 ml with the same solvent. Dilute 1 ml of the solution to *V* with *water R*, *V* being the maximum recommended dose in millilitres.

Plate *TLC silica gel plate for aminopolyether test R.*

Application 2.5 µl; in addition, apply 2.5 µl of the test solution and then 2.5 µl of reference solution (b) at the same place.

Detection Visually compare the spots 1 min after application.

System suitability:

- the spot due to the successive application of the test solution and reference solution (b) is similar in appearance to the spot due to reference solution (b), which is characterised by a number of concentric circles; the darker innermost circle (of intensity proportional to the concentration of impurity B) may be surrounded by a bluish-black ring, outside of which is a lighter circle surrounded by a peripheral dark edge;
- the spot due to reference solution (a) has a more diffuse inner circle, which is brownish-pink and without a distinct margin between it and the surrounding lighter zone;
- the spot due to reference solution (b) is clearly different from the spot due to reference solution (a).

Limit:

- the central portion of the spot due to the test solution is less intense than that of the spot due to reference solution (b) (2.2 mg/*V*).

Impurity C

Liquid chromatography *(2.2.29)*.

Test solution The preparation to be examined.

Reference solution (a) Dilute 2.1 ml of a 25.95 g/l solution of *tetrabutylammonium hydroxide R* (impurity C) to 20.0 ml with *water R*. Dilute 1.0 ml of this solution to *V* with *water R*, *V* being the maximum recommended dose in millilitres.

Reference solution (b) Dilute 1 ml of a 25.95 g/l solution of *tetrabutylammonium hydroxide R* to 10 ml with *water R*. Dilute 1 ml of this solution to 25 ml with *water R*.

Column:

- *size*: l = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: constant, between 20 °C and 25 °C.

Mobile phase 25 volumes of a 0.95 g/l solution of *toluenesulphonic acid R* and 75 volumes of *acetonitrile R*.

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl.

Run time Twice the retention time of tetrabutylammonium ions.

Retention time Tetrabutylammonium hydroxide = about 3.3 min.

System suitability Reference solution (b):

- *signal-to-noise ratio* : minimum 10 for the principal peak;
- *symmetry factor* : maximum 1.8 for the principal peak.

Limit:

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (2.75 mg/*V*).

Impurity D

Ultraviolet and visible absorption spectrophotometry *(2.2.25)* .

Test solution The preparation to be examined.

Reference solution Dissolve 20.0 mg of *4-(4-methylpiperidin-1-yl)pyridine R* (impurity D) in *water R* and dilute to 100.0 ml with the same solvent. Dilute 0.1 ml of the solution to *V* with *water R* , *V* being the maximum recommended dose in millilitres.

Measure the absorbance of the test solution and the reference solution at the absorption maximum of 263 nm.

Limit The absorbance of the test solution is not greater than that of the reference solution (0.02 mg/*V*).

Residual solvents

Limited according to the principles defined in general chapter *5.4*. The preparation may be released for use before completion of the test.

Sterility

It complies with the test for sterility prescribed in the monograph *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

Bacterial endotoxins *(2.6.14)*

Less than 175/*V* IU/ml, *V* being the maximum recommended dose in millilitres. The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

The preparation may be released for use before completion of test B.

A. Gamma-ray spectrometry.

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. Gamma-ray spectrometry.

Determine the amount of fluorine-18 and radionuclidic impurities with a half-life longer than 2 h. For the detection and quantification of impurities, retain the preparation to be examined for at least 24 h to allow the fluorine-18 to decay to a level that permits the detection of impurities.

Results The total radioactivity due to radionuclidic impurities is not more than 0.1 per cent.

RADIOCHEMICAL PURITY

A. Liquid chromatography *(2.2.29)* as described in the test for 2-fluoro-2-deoxy-D-glucose and impurity A. If necessary, dilute the test solution with *water R* to obtain a radioactivity concentration suitable for the radioactivity detector.

Injection Test solution and reference solutions (a) and (c).

Relative retention With reference to 2-[^{18}F]fluoro-2-deoxy-D-glucose (retention time = about 12 min): 2-[^{18}F]fluoro-2-deoxy-D-mannose = about 0.9. Partially and fully acetylated derivatives of both compounds hydrolyse under the chromatographic conditions and therefore elute as 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose.

Locate the peaks due to 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose using the chromatograms obtained with the carbohydrate detector and reference solutions (a) and (c).

Limits:

— *fluorine-18 in the form of 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose*: minimum 95 per cent of the total radioactivity;

— *2-[^{18}F]fluoro-2-deoxy-D-mannose*: maximum 10 per cent of the total radioactivity due to 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose.

B. Thin-layer chromatography *(2.2.27)*.

Test solution The preparation to be examined.

Reference solution Dissolve, with gentle heating, 30 mg of *1,2,3,4-tetra-O-acetyl-β-D-glucopyranose R* and 20 mg of *glucose R* in 1 ml of *water R*.

Plate *TLC silica gel plate R.*

Mobile phase *water R, acetonitrile R* (5:95 *V/V*).

Application About 2 µl.

Development Over a path of 8 cm.

Drying In air for 15 min.

Detection Determine the distribution of radioactivity using a suitable detector, then immerse the plate in a 75 g/l solution of *sulphuric acid R* in *methanol R* and dry with a heat gun or at 150 °C until the appearance of dark spots in the chromatogram obtained with the reference solution.

Retardation factors [^{18}F]fluoride = about 0; 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose = about 0.45; partially or fully acetylated derivatives of 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose = about 0.8 to 0.95.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated spots.

Limits:

— *fluorine-18 in the form of 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose*: minimum 95 per cent of the total radioactivity;

— *fluorine-18 in the form of fluoride and partially or fully acetylated derivatives of 2-[^{18} F] fluoro-2-deoxy- D -glucose and 2-[^{18} F]fluoro-2-deoxy- D -mannose* : maximum 5 per cent of the total radioactivity.

RADIOACTIVITY

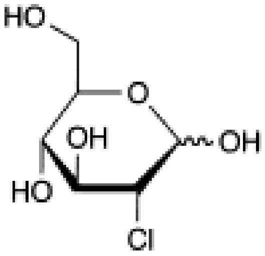
Measure the radioactivity using a calibrated instrument.

LABELLING

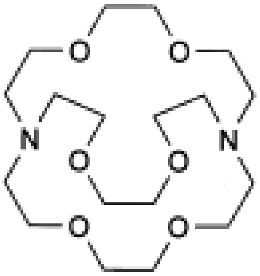
The label states the maximum recommended dose, in millilitres.

IMPURITIES

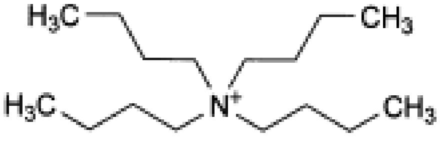
Specified impurities A, B, C, D, E.



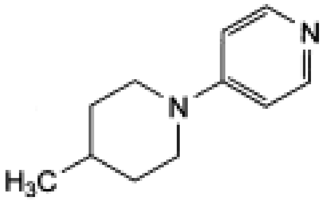
A. 2-chloro-2-deoxy-D-glucopyranose (2-chloro-2-deoxy-D-glucose),



B. 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (aminopolyether),



C. *N,N,N*-tributylbutan-1-aminium (tetrabutylammonium),



D. 4-(4-methylpiperidin-1-yl)pyridine,

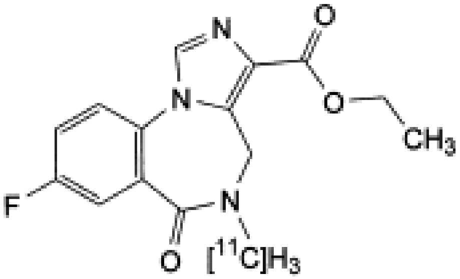
E. [^{18}F]fluoride.

Ph Eur

Flumazenil (*N*-[^{11} C]methyl) Injection

General Notices

(Ph Eur monograph 1917)



Ph Eur

DEFINITION

Sterile solution of ethyl 8-fluoro-5-[^{11}C]methyl-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4] benzodiazepine-3-carboxylate which may contain a stabiliser such as ascorbic acid.

Content

90 per cent to 110 per cent of the declared carbon-11 radioactivity at the date and time stated on the label.

Content of flumazenil

Maximum 50 µg in the maximum recommended dose in millilitres.

PRODUCTION

RADIONUCLIDE PRODUCTION

Carbon-11 is a radioactive isotope of carbon which is most commonly produced by proton irradiation of nitrogen. Depending on the addition of either trace amounts of oxygen or small amounts of hydrogen, the radioactivity is obtained as [^{11}C]carbon dioxide or [$_{11}$C]methane, respectively.

RADIOCHEMICAL SYNTHESIS

[5-Methyl-^{11}C]flumazenil may be prepared by *N*-alkylation of ethyl 8-fluoro-6-oxo-5,6-dihydro-

4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (demethylflumazenil) with iodo[^{11}C]methane or [^{11}C]methyl trifluoromethanesulphonate.

Synthesis of iodo[^{11}C]methane

Iodo[^{11}C]methane may be produced from [^{11}C]carbon dioxide or from [^{11}C]methane. The most frequently used method is reduction of [^{11}C]carbon dioxide with lithium aluminium hydride. The [^{11}C]methanolate formed is reacted with hydriodic acid. Alternatively [^{11}C]methane, either obtained directly in the target or by on-line processes from [^{11}C]carbon dioxide, is reacted with iodine.

Synthesis of [^{11}C]methyl trifluoromethanesulphonate

[^{11}C]methyl trifluoromethanesulphonate may be prepared from iodo[^{11}C]methane using a solid support such as graphitised carbon, impregnated with silver trifluoromethanesulphonate.

Synthesis of [5-methyl-^{11}C]flumazenil

The most widely used method to obtain [5-methyl-^{11}C]flumazenil is the N-alkylation of demethylflumazenil with iodo[^{11}C]methane in alkaline conditions in a solvent such as dimethylformamide or acetone. The resulting [5-methyl-^{11}C]flumazenil can be purified by semi-preparative liquid chromatography. For example, a column packed with octadecylsilyl silica gel for chromatography eluted with a mixture of ethanol and water is suitable.

PRECURSOR FOR SYNTHESIS

Demethylflumazenil

Melting point (2.2.14) 286 °C to 289 °C.

Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of demethylflumazenil.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of carbon-11

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with test B for radionuclidic purity (see Tests).

C. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

pH (*2.2.3*)

6.0 to 8.0.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres. The injection may be released for use before completion of the test.

Flumazenil and impurity A

Liquid chromatography (*2.2.29*).

Test solution The preparation to be examined.

Reference solution (a) Dissolve 2.5 mg of *flumazenil R* in 5 ml of *methanol R* .

Reference solution (b) Dissolve 2.5 mg of *demethylflumazenil R* in 50 ml of *methanol R.*

Reference solution (c) To 0.1 ml of reference solution (a) add 0.1 ml of reference solution (b) and dilute to *V* with a 0.9 g/l solution of *sodium chloride R* , *V* being the maximum recommended dose in millilitres.

Reference solution (d) Dilute 0.1 ml of reference solution (a) to 50 ml with *methanol R* . Dilute 1.0 ml of this solution to *V* with a 0.9 g/l solution of *sodium chloride R* , *V* being the maximum recommended dose in millilitres.

Column:

— *size: l* = 0.15 m, Ø = 3.9 mm,

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 440 m^2/g, a pore size of 100 nm and a carbon loading of 19 per cent,

— *temperature*: maintain at a constant temperature between 20-30 °C.

Mobile phase *methanol R* , *water R* (45:55 *V/V*).

Flow rate 1 ml/min.

Detection Spectrophotometer at 260 nm and radioactivity detector connected in series.

Injection 100 µl.

Run time 10 min.

Relative retention with reference to flumazenil: impurity A = about 0.74.

System suitability Reference solution (c):

— *resolution*: minimum 2.5 between the peaks due to flumazenil and impurity A.

Limits Examine the chromatogram obtained with the spectrophotometer:

— *flumazenil* : not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (50 µg/*V*),

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (5 µg/*V*),

— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1 µg/*V*).

Residual solvents

are limited according to the principles defined in the general chapter (*5.4*), using the general method (*2.4.24*). The preparation may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Carbon-11

Minimum 99 per cent of the total radioactivity.

The preparation may be released for use before completion of the test.

A. Gamma-ray spectrometry.

Results The spectrum obtained with the solution to be examined does not differ significantly from that obtained with a standardised fluorine-18 solution.

B. Half-life: 19.9 min to 20.9 min.

RADIOCHEMICAL PURITY

Liquid chromatography (*2.2.29*) as described in the test for flumazenil and impurity A, with the following modifications.

Injection Test solution and reference solution (a); if necessary, dilute the test solution to a radioactivity concentration suitable for the detector.

Limit Examine the chromatogram obtained with the radioactivity detector:

— *[5-methyl-^{11}C]flumazenil* : minimum 95 per cent of the total radioactivity.

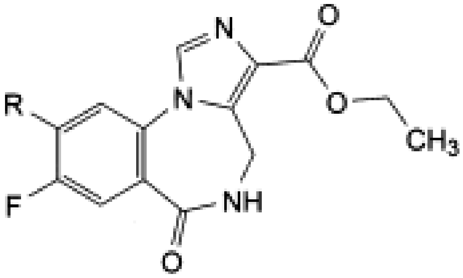
RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

LABELLING

The label states the maximum recommended dose in millilitres.

IMPURITIES



A. R = H: ethyl 8-fluoro-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (demethylflumazenil),

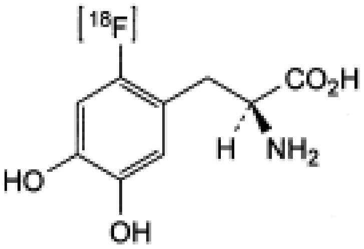
B. R = CH_2-CO-CH_3: ethyl 8-fluoro-6-oxo-9-(2-oxopropyl)-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4] benzodiazepine-3-carboxylate (acetone addition compound of demethylflumazenil).

Ph Eur

Fluorodopa [^{18} F] Injection

General Notices

(Fluorodopa (18F) (Prepared By Electrophilic Substitution) Injection, Ph Eur monograph 1918)



Ph Eur

DEFINITION

Sterile solution of (2*S*)-2-amino-3-(2-([^{18}F]fluoro)-4,5-dihydroxyphenyl)propanoic acid (6-[^{18}F] fluorolevodopa). It may contain stabilisers such as ascorbic acid and edetic acid.

This monograph applies to an injection containing 6-[^{18}F]fluorolevodopa produced by electrophilic substitution.

Content:

— *fluorine-18*: 90 per cent to 110 per cent of the declared fluorine-18 radioactivity at the date and time stated on the label;

— *dopa*: maximum 1 mg per maximum recommended dose in millilitres;

— *6-fluorolevodopa*: maximum 15 mg per maximum recommended dose in millilitres.

PRODUCTION

RADIONUCLIDE PRODUCTION

Fluorine-18 is a radioactive isotope of fluorine that may be produced by various nuclear reactions induced by proton irradiation of oxygen-18, deuteron irradiation of neon-20, or helium-3 or helium-4 irradiation of oxygen-16.

In order to obtain fluorine-18 in a chemical form suitable for electrophilic substitution reactions, such as fluorine gas or gaseous acetylhypofluorite, a small amount of non-radioactive fluorine gas (0.3-0.8 per cent of the target gas volume) must be added as a carrier at some step in the production process.

RADIOCHEMICAL SYNTHESIS

6-[^{18}F]Fluorolevodopa may be prepared by various radiochemical synthetic pathways, which lead to different products in terms of yield, specific radioactivity, by-products and possible impurities. Electrophilic pathways for production of 6-[^{18}F]fluorolevodopa may proceed by fluorodemetallation of a stannylated derivative of levodopa, with molecular [^{18}F]fluorine or [^{18}F]acetylhypofluorite, followed by hydrolysis of protecting groups and final purification by semipreparative liquid chromatography. Pathways using demercuration or dethallation must not be used.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of fluorine-18

See general chapter 5.7. *Table of physical characteristics of radionuclides*.

IDENTIFICATION

A. Test A for radionuclidic purity (see Tests).

B. Determine the approximate half-life by at least 3 measurements of the activity of a sample in the same geometrical conditions over a suitable period of time, for example 30 min.

Results 105 min to 115 min.

C. Examine the chromatograms obtained in the test for radiochemical purity (see Tests).

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the peak due to 6-fluorolevodopa in the chromatogram obtained with reference solution (a).

D. Examine the chromatograms obtained in the test for impurities C and D (see Tests).

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retardation factor to the peak due to 6-fluorolevodopa in the chromatogram obtained with reference solution (b).

TESTS

pH *(2.2.3)*

4.0 to 5.5.

Sterility

It complies with the test for sterility prescribed in the monograph *Radiopharmaceutical* *preparations (0125)* . The injection may be released for use before completion of the test.

Bacterial endotoxins *(2.6.14)*

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres. The injection may be released for use before completion of the test.

6-Fluorolevodopa, dopa, impurity A and impurity B

Liquid chromatography *(2.2.29)* . *Prepare the reference solutions immediately before use.*

Test solution The preparation to be examined.

Reference solution (a) Dissolve 18.0 mg of *6-fluorolevodopa hydrochloride R* in 5.0 ml of the mobile phase and dilute to V with the mobile phase, V being the maximum recommended dose in millilitres.

Reference solution (b). Dissolve 1.0 mg of *levodopa R* in 5 ml of the mobile phase and dilute to *V* with the mobile phase, *V* being the maximum recommended dose in millilitres.

Reference solution (c) Dissolve 1.0 mg of *trimethyltin chloride R* (impurity A) in 2.0 ml of the mobile phase. Dilute 1.0 ml of this solution to *V* with the mobile phase, V being the maximum recommended dose in millilitres.

Reference solution (d) Mix equal volumes of reference solutions (b) and (c).

Reference solution (e) Dissolve 2.0 mg of *6-hydroxydopa R* (impurity B) in 20.0 ml of the mobile phase. Dilute 0.25 ml of this solution to *V* with the mobile phase, *V* being the maximum recommended dose in millilitres.

Column:

— *size:* *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* ;

— *temperature*: maintain at a constant temperature between 20 °C and 30 °C.

Mobile phase 6.9 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 2.4 with a 4.8 g/l solution of *phosphoric acid R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 200 nm and radioactivity detector connected in series.

Injection 20 µl.

Run time 15 min.

Relative retention With reference to 6-fluorolevodopa (retention time = about 6 min): impurity A and impurity B = about 0.7; dopa = about 0.8.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to dopa and impurity A.

Limits Examine the chromatograms obtained with the spectrophotometer:

— *6-fluorolevodopa*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (15 mg/*V*);

— *dopa*: not more than the area of the peak due to levodopa in the chromatogram obtained with reference solution (b) (1.0 mg/*V*);

— *sum of impurities A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (corresponding to a limit of 0.5 mg/*V* of impurity A or a limit of 0.025 mg/*V* of impurity B, or to lower limits of each if both impurities are present).

Residual solvents

Limited according to the principles defined in general chapter 5.4. The preparation may be released for use before completion of the test.

RADIONUCLIDE PURITY

Fluorine-18

Minimum 99.9 per cent of the total radioactivity. The preparation may be released for use before completion of test B.

A. Gamma-ray spectrometry

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. Gamma-ray spectrometry

Determine the amount of fluorine-18 and radionuclidic impurities with a half-life longer than 2 h. For the detection and quantification of impurities, retain the preparation to be examined for a sufficient time to allow the fluorine-18 to decay to a level that permits the detection of impurities.

Results The spectrum obtained with the preparation to be examined does not differ significantly from a background spectrum.

RADIOCHEMICAL PURITY

Liquid chromatography *(2.2.29)* as described in the test for 6-fluorolevodopa, dopa, impurity A and impurity B.

Examine the chromatogram recorded using the radioactivity detector and locate the peak due to 6-[^{18}F]fluorolevodopa by comparison with the chromatogram obtained with reference solution (a) and the spectrophotometer.

Limit:

— *6-[18F]fluorolevodopa*: minimum 95 per cent of the total radioactivity due to fluorine-18.

Impurities C and D

Thin-layer chromatography *(2.2.27)* .

Test solution The preparation to be examined.

Reference solution (a) Dissolve 2 mg of *DL -6-fluorodopa hydrochloride R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 2 mg of *6-fluorolevodopa hydrochloride R* in *water R* and dilute to 10 ml with the same solvent.

Plate *octadecylsilyl TLC silica gel plate for chiral separations R*.

Mobile phase *methanol R* , *water R* (50:50 *V/V*).

Application 2 µl.

Development Over a path of 10 cm.

Drying In air for 5 min.

Detection Spray with a 2 g/l solution of *ninhydrin R* in *anhydrous ethanol R* and heat at 60 °C for 10 min; determine the distribution of radioactivity using a suitable detector.

Retardation factors impurity D = about 0; 6-[^{18}F]fluorolevodopa = about 0.3; impurity C = about 0.5.

System suitability Reference solution (a):

— the chromatogram shows 2 clearly separated spots.

Limits:

— *impurity C*: maximum 2 per cent of the total radioactivity due to fluorine-18;

— *impurity D*: maximum 4 per cent of the total radioactivity due to fluorine-18.

RADIOACTIVITY

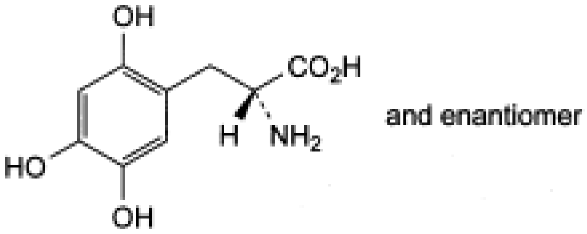
Measure the radioactivity using a calibrated instrument.

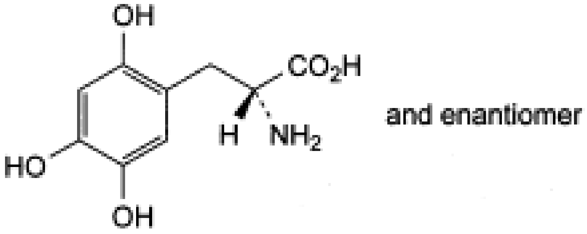
LABELLING

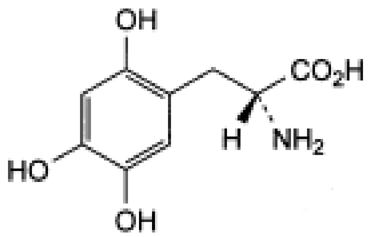
The label states the maximum recommended dose in millilitres.

IMPURITIES

A. Cl-Sn$(CH_3)_3$: chlorotrimethylstannane (trimethyltin chloride),

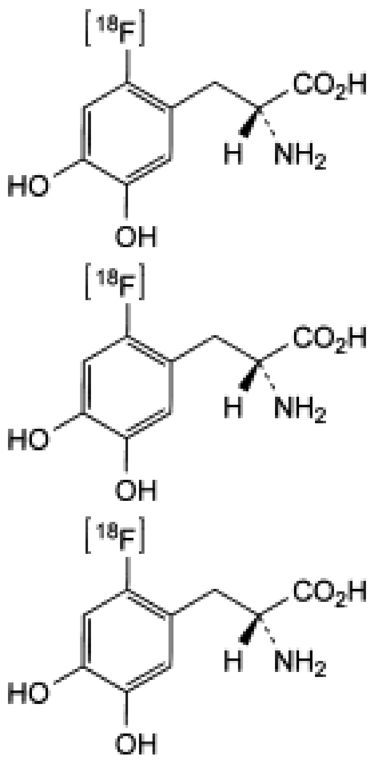






and enantiomer

B. (2*RS*)-2-amino-3-(2,4,5-trihydroxyphenyl)propanoic acid (6-hydroxydopa),



C. (2*R*)-2-amino-3-(2-[^{18}F]fluoro-4,5-dihydroxyphenyl)propanoic acid (6-[^{18}F]fluorodextrodopa),

D. [^{18}F]fluoride.

Ph Eur

Gallium [^{67} Ga] Citrate Injection

General Notices

(Ph Eur monograph 0555)

Ph Eur

DEFINITION

Gallium (^{67}Ga) citrate injection is a sterile solution of gallium-67 in the form of gallium citrate. It may be made isotonic by the addition of sodium chloride and sodium citrate and may contain a suitable antimicrobial preservative such as benzyl alcohol. Gallium-67 is a radioactive isotope of gallium and may be obtained by the irradiation, with protons of suitable energy, of zinc which may be enriched in zinc-68. Gallium-67 may be separated from zinc by solvent extraction or column chromatography. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared gallium-67 radioactivity at the date and hour stated on the label. Not more than 0.2 per cent of the total radioactivity is due to gallium-66.

CHARACTERS

A clear, colourless solution.

Gallium-67 has a half-life of 3.26 days and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised gallium-67 solution when measured either by direct comparison or by use of an instrument calibrated with the aid of such a solution. Standardised gallium-67 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photons have energies of 0.093 MeV, 0.185 MeV and 0.300 MeV.

B. To 0.2 ml of the injection to be examined add 0.2 ml of a solution containing 1 g/l of *ferric chloride R* and 0.1 per cent *V/V* of *hydrochloric acid R* and mix. Compare the colour with that of a solution containing 9 g/l of *benzyl alcohol R* and 7 g/l of *sodium chloride R* treated in the same manner. A yellow colour develops in the test solution only.

TESTS

pH (*2.2.3*)

The pH of the injection is 5.0 to 8.0.

Zinc

To 0.1 ml of the injection to be examined add 0.9 ml of *water R* , 5 ml of *acetate buffer solution pH 4.7 R* , 1 ml of a 250 g/l solution of *sodium thiosulphate R* and 5.0 ml of a dithizone solution prepared as follows: dissolve 10 mg of *dithizone R* in 100 ml of *methyl ethyl ketone R* allow to stand for 5 min, filter and immediately before use dilute the solution to ten times its volume with *methyl ethyl ketone R* . Shake vigorously for 2 min and separate the organic layer. Measure the absorbance (*2.2.25*) of the organic layer at 530 nm, using the organic layer of a blank solution as the compensation liquid. The absorbance is not greater than that of the organic layer obtained with 0.1 ml of *zinc standard solution (5 ppm Zn) R* treated in the same manner.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised gallium-67 solution, apart from any differences attributable to the presence of gallium-66. Gallium-66 has a half-life of 9.4 h and its most prominent gamma photon has an energy of 1.039 MeV. Not more than 0.2 per cent of the total radioactivity is due to gallium-66.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised gallium-67 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Indium [^{111} In] Chloride Solution

General Notices

(Ph Eur monograph 1227)

Ph Eur

DEFINITION

Indium (^{111}In) chloride solution is a sterile solution of indium-111 as the chloride in aqueous hydrochloric acid containing no additives. Indium-111 is a radioactive isotope of indium and may be produced by the irradiation of cadmium with protons of suitable energy. The solution contains not less than 90.0 per cent and not more than 110.0 per cent of the declared indium-111 radioactivity at the date and hour stated on the label. Not more than 0.25 per cent of the total radioactivity is due to radionuclides other than indium-111. Not less than 95 per cent of the radioactivity corresponds to indium-111 in the form of ionic indium(III). The method of preparation is such that no carrier is added and the specific radioactivity is not less than 1.85 GBq of indium-111 per microgram of indium.

CHARACTERS

A clear, colourless solution.

Indium-111 has a half-life of 2.8 days and emits gamma radiation and X-rays.

IDENTIFICATION

A. *Carry out the test after allowing sufficient time for short-lived impurities such as indium-110m to decay*. Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised indium-111 solution apart from any differences due to the presence of indium-114m, when measured either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised indium-111 and indium-114m solutions are available from laboratories recognised by the competent authority. The most prominent gamma photons of indium-111 have energies of 0.171 MeV and 0.245 MeV.

B. To 100 µl of *silver nitrate solution R2* add 50 µl of the solution. A white precipitate is formed.

C. It complies with the test for pH (see Tests).

D. Examine the chromatogram obtained in the test for radiochemical purity. The principal peak has an R_F value of 0.5 to 0.8.

TESTS

pH (*2.2.3*)

The pH of the solution is 1.0 to 2.0.

Cadmium

Not more than 0.40 µg/ml, determined by electrothermal atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 0.05 ml of the solution to be examined to a suitable volume with a suitable concentration of *hydrochloric acid R*.

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R,* diluted as necessary with the same concentration of *hydrochloric acid R* as in the solution to be examined.

Measure the absorbance at 228.8 nm using a cadmium hollow-cathode lamp as source of radiation.

Copper

Not more than 0.15 µg/ml, determined by electrothermal atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 0.1 ml the solution to be examined to a suitable volume with a suitable concentration of *hydrochloric acid R.*

Reference solutions Prepare the reference solutions using *copper standard solution (0.1 per cent) R* diluted as necessary with the same concentration of *hydrochloric acid R* as the solution to be examined.

Measure the absorbance at 324.8 nm using a copper hollow-cathode lamp as source of radiation.

Iron

Not more than 0.60 µg/ml, determined by electrothermal atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 0.1 ml of the solution to be examined to a suitable volume with a suitable concentration of *hydrochloric acid R.*

Reference solutions Prepare the reference solutions using *iron standard solution (0.1 per cent Fe) R* diluted as necessary with the same concentration of *hydrochloric acid R* as the solution to be examined.

Measure the absorbance at 248.3 nm using an iron hollow-cathode lamp as source of radiation.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The solution may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised solution of indium-111 apart from any differences due to the presence of indium-114m.

Indium-114m

Carry out the test after allowing sufficient time for short-lived impurities such as indium-110m to decay. Take a volume equivalent to 30 MBq and record the gamma-ray spectrum using a suitable detector with a shield of lead, 6 mm thick, placed between the sample and the detector. The response in the region corresponding to the 0.558 MeV photon and the 0.725 MeV photon of indium-114m does not exceed that obtained using 75 kBq of a standardised solution of indium-114m (0.25 per cent) measured under the same conditions, when all measurements are calculated with reference to the date and hour of administration. Standardised indium-111 and indium-114m solutions are available from laboratories recognised by the competent authority.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet.

Apply to the plate 5 µl of the solution to be examined. Develop immediately over a path of 15 cm using a 9.0 g/l solution of *sodium chloride R* adjusted to pH 2.3 ± 0.05 with *dilute hydrochloric acid R*. Allow the plate to dry in a current of cold air. Determine the distribution of radioactivity using a suitable detector. Indium-111 chloride migrates with an R_F value of 0.5 to 0.8. Not less than 95 per cent of the total radioactivity of the chromatogram corresponds to indium-111 chloride.

RADIOACTIVITY

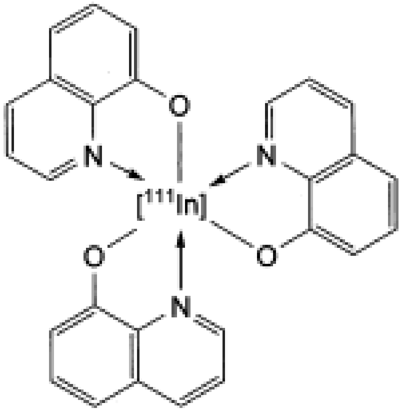
Measure the radioactivity using suitable counting equipment by comparison with a standardised indium-111 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Indium [^{111} In] Oxine Solution

General Notices

(Ph Eur monograph 1109)



$C_{27}H_{18}[^{111}In]N_3O_3$ 547.2

Ph Eur

DEFINITION

Indium (^{111}In) oxine solution is a sterile solution of indium-111 in the form of a complex with 8-hydroxyquinoline. It may contain suitable surface active agents and may be made iso-tonic by the addition of sodium chloride and a suitable buffer. Indium-111 is a radioactive isotope of indium and may be produced by the irradiation of cadmium with protons of suitable energy. The solution contains not less than 90.0 per cent and not more than 110.0 per cent of the declared indium-111 radioactivity at the date and hour stated on the label. Not more than 0.25 per cent of the total radioactivity is due to radionuclides other than indium-111. Not less than 90 per cent of the radioactivity corresponds to indium-111 complexed with oxine. The method of preparation is such that no carrier is added and the specific radioactivity is not less than 1.85 GBq of indium-111 per microgram of indium.

CHARACTERS

A clear, colourless solution.

Indium-111 has a half-life of 2.8 days and emits gamma radiation and X-rays.

IDENTIFICATION

A. *Carry out the test after allowing sufficient time for short-lived impurities such as indium-*

110m to decay. Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised indium-111 solution apart from any differences due to the presence of indium-114m, when measured either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised indium-111 and indium-114m solutions are available from laboratories recognised by the competent authority. The most prominent gamma photons of indium-111 have energies of 0.171 MeV and 0.245 MeV.

B. Place 5 mg to 10 mg of *magnesium oxide R* in a glass container of approximately 20 mm in internal diameter. Add 20 µl of the solution to be examined. Examine in ultraviolet light at 365 nm. Bright yellow fluorescence is produced.

C. The distribution of radioactivity between the organic and aqueous phases in the test for radiochemical purity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the solution is 6.0 to 7.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The solution may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised solution of indium-111, apart from any differences due to the presence of indium-114m.

Indium-114m

Carry out the test after allowing sufficient time for short-lived impurities such as indium-110m to decay. Take a volume equivalent to 30 MBq and record the gamma-ray spectrum using a suitable detector with a shield of lead, 6 mm thick, placed between the sample and the detector. The response in the region corresponding to the 0.558 MeV photon and the 0.725 MeV photon of indium-114m does not exceed that obtained using 75 kBq of a standardised solution of indium-114m (0.25 per cent) measured under the same conditions, when all measurements are calculated with reference to the date and hour of administration. (It should be noted that indium (^{111}In) oxine solution is a precursor used in the *in vitro* labelling of white blood cells or platelets prior to their re-injection into the patient. It is not intended for direct administration). Standardised indium-111 and indium-114m solutions are available from laboratories recognised by the competent authority.

RADIOCHEMICAL PURITY

To a silanised separating funnel containing 3 ml of a 9 g/l solution of *sodium chloride R* add 100 µl of the solution to be examined and mix. Add 6 ml of *octanol R* and shake vigorously. Allow the phases to separate and then run the lower layer into a suitable vial for counting. Allow the upper layer to drain completely into a similar vial. Add 1 ml of *octanol R* to the funnel, shake vigorously and drain into the vial containing the organic fraction. Add 5 ml of *dilute hydrochloric acid R* to the funnel, shake vigorously and drain these rinsings into a third vial. Seal each vial and, using a suitable instrument, measure the radioactivity in each. Calculate the radiochemical purity by expressing the radioactivity of the indium-111 oxine complex, found in the organic phase, as a percentage of the radioactivity measured in the three solutions. Not less than 90 per cent of the radioactivity corresponds to indium-111 complexed with oxine.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised indium-111 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Indium [^{111} In] Pentetate Injection

General Notices

(Ph Eur monograph 0670)

Ph Eur

DEFINITION

Indium (^{111}In) pentetate injection is a sterile and apyrogenic solution containing indium-111 in the form of indium diethylenetriaminepenta-acetate. It may contain calcium and may be made isotonic by the addition of sodium chloride and a suitable buffer. Indium-111 is a radioactive isotope of indium which may be obtained by proton irradiation, of appropriate energy, of cadmium which may be enriched with cadmium-111 or cadmium-112. The injection contains not less than 90 per cent and not more than 110 per cent of the declared indium-111 radioactivity at the date and hour stated on the label. The radioactivity due to indium-114m is not greater than 0.2 per cent of the total radioactivity at the date and hour of administration. Not less than 95 per cent of the radioactivity corresponds to indium-111 complexed with pentetate.

CHARACTERS

A clear, colourless solution.

Indium-111 has a half-life of 2.8 days and emits gamma radiation and X-rays.

IDENTIFICATION

A. Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised indium-111 solution apart from any differences due to the presence of indium-114m, when measured either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised indium-111 and indium-114m solutions are available from laboratories recognised by the competent authority. The most prominent gamma photons of indium-111 have energies of 0.171 MeV and 0.245 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the injection is 7.0 to 8.0.

Cadmium

Not more than 5.0 µg of Cd per millilitre, determined by atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Mix 0.1 ml of the injection to be examined with 0.9 ml of a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *water R* .

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R* and diluting with a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *water R* .

Measure the absorbance at 228.8 nm using a cadmium hollow-cathode lamp as source of radiation and an air-acetylene flame.

Uncomplexed diethylenetriaminepenta-acetic acid

In a micro test-tube, mix 100 µl of the injection to be examined with 100 µl of a freshly prepared 1 g/l solution of *hydroxynaphthol blue*, *sodium salt R* in *1 M sodium hydroxide* . Add 50 µl of a 0.15 g/l solution of *calcium chloride R* . The solution remains pinkish-violet or changes from blue to pinkish-violet (0.4 mg/ml).

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 14/V IU/ml, V being the maximum recommended dose in millilitres.

RADIONUCLIDIC PURITY

Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised solution of indium-111 apart from any differences due to the presence of indium-114m.

Indium-114m

Retain a sample of the injection to be examined for a sufficient time to allow the indium-111 radioactivity to decay to a sufficiently low level to permit the detection of radionuclidic impurities. Record the gamma-ray spectrum of the decayed material in a suitable instrument calibrated with the aid of a standardised indium-114m solution. Indium-114m has a half-life of 49.5 days and its most prominent gamma photon has an energy of 0.190 MeV. The radioactivity due to indium-114m is not greater than 0.2 per cent of the total radioactivity at the date and hour of administration.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for 10 min. Use a plate such that during development the mobile phase migrates over a distance of 10 cm to 15 cm in about 10 min.

Apply to the plate 5 µl to 10 µl of the injection to be examined and allow to dry. Develop over a path of 10 cm to 15 cm using a 9 g/l solution of *sodium chloride R* . Allow the plate to dry in air. Determine the distribution of radioactivity using a suitable detector. Indium pentetate complex migrates near to the solvent front. The radioactivity of the spot corresponding to indium pentetate complex represents not less than 95 per cent of the total radioactivity of the chromatogram.

RADIOACTIVITY

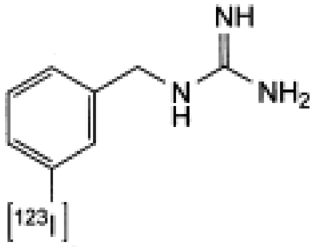
Measure the radioactivity using suitable counting equipment by comparison with a standardised indium-111 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Iobenguane [^{123} I] Injection

General Notices

(Ph Eur monograph 1113)



$C_8H_{10}[^{123}I]N_3$

Ph Eur

DEFINITION

Iobenguane (^{123}I) injection is a sterile, bacterial endotoxin-free solution of 1-(3-[^{123}I]iodobenzyl) guanidine or its salts. It may contain a suitable buffer, a suitable labelling catalyst such as ionic copper, a suitable labelling stabiliser such as ascorbic acid and antimicrobial preservatives. Iodine-123 is a radioactive isotope of iodine and may be obtained by proton irradiation of xenon enriched in xenon-124 (not less than 98 per cent) followed by the decay of caesium-123 formed via xenon-123. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared iodine-123 radioactivity at the date and hour stated on the label. Not less than 95 per cent of the radioactivity corresponds to iodine-123 in the form of iobenguane. The specific radioactivity is not less than 10 GBq of iodine-123 per gram of iobenguane base. Not more than 0.35 per cent of the total radioactivity is due to radionuclides other than iodine-123.

CHARACTERS

A clear, colourless or slightly yellow solution.

Iodine-123 has a half-life of 13.2 h and emits gamma radiation and X-rays.

IDENTIFICATION

A. Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-123 solution apart from any differences attributable to the presence of iodine-125, tellurium-121 and other radionuclidic impurities. The most prominent gamma photon of iodine-123 has an energy of 0.159 MeV.

Iodine-125 has a half-life of 59.4 days and emits an X-ray of 0.027 MeV and a photon of 0.035 MeV. Tellurium-121 has a half-life of 19.2 days and the most prominent photons have energies of 0.507 MeV and 0.573 MeV. Standardised iodine-123, iodine-125 and tellurium-121 solutions are available from laboratories recognised by the competent authority.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of the radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the solution is 3.5 to 8.0.

Specific radioactivity

The specific radioactivity is calculated from the results obtained in the test for radiochemical purity. Determine the content of iobenguane sulphate from the areas of the peaks corresponding to iobenguane in the chromatograms obtained with the test solution and reference solution (b). Calculate the concentration as iobenguane base by multiplying the result obtained in the assay by 0.85.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. Determine the relative amounts of iodine-125, tellurium-121 and other radionuclidic impurities present. No radionuclides with a longer half-life than iodine-125 are detected. For the determination of iodine-125, tellurium-121 and other radionuclidic impurities, retain the solution to be examined for a sufficient time to allow the radioactivity of iodine-123 to decrease to a level that permits the detection of radionuclidic impurities. Record the gamma-ray spectrum and the X-ray spectrum of the decayed material using a suitable instrument. Not more than 0.35 per cent of the total radioactivity is due to radionuclides other than iodine-123. The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Test solution The injection to be examined.

Reference solution (a) Dissolve 0.100 g of *sodium iodide R* in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *iobenguane sulphate CRS* in 25 ml of the mobile phase and dilute to 50.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.0 mm in internal diameter packed with *silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture of 1 volume of an 80 g/l solution of *ammonium nitrate R*, 2 volumes of *dilute ammonia R2* and 27 volumes of *methanol R*,

— a suitable radioactivity detector,

— a spectrophotometer set at 254 nm and provided with a flow-cell,

— a 10 µl loop injector.

Inject the test solution and the reference solutions. Not less than 95 per cent of the radioactivity of the chromatogram is found in the peak corresponding to iobenguane. Not more than 4 per cent of the radioactivity is found in the peak corresponding to iodide and not more than 1 per cent of the radioactivity is found in other peaks.

RADIOACTIVITY

Measure the radioactivity using a suitable counting apparatus by comparison with a standardised iodine-123 solution or by measurement in an instrument calibrated with the aid of such a solution.

STORAGE

Store protected from light.

LABELLING

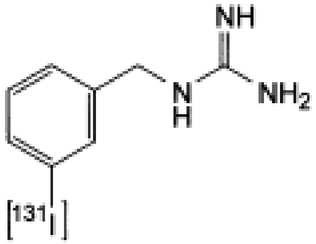
The label states the specific radioactivity expressed in GBq of iodine-123 per gram of iobenguane base.

Ph Eur

Iobenguane [^{131} I] Injection for Diagnostic Use

General Notices

(Ph Eur monograph 1111)



$C_8H_{10}[^{131}I]N_3$

Ph Eur

DEFINITION

Iobenguane (^{131}I) injection for diagnostic use is a sterile, bacterial endotoxin-free solution of 1-(3-[^{131}I]iodobenzyl)guanidine or its salts. It may contain a suitable buffer. It may also contain a suitable labelling catalyst such as ionic copper and a suitable labelling stabiliser such as ascorbic acid. It may contain antimicrobial preservatives. Iodine-131 is a radioactive isotope of iodine and may be obtained by neutron irradiation of tellurium or by extraction of uranium fission products. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared iodine-131 radioactivity at the date and hour stated on the label. Not less than 94 per cent of the radioactivity corresponds to iodine-131 in the form of iobenguane. The specific radioactivity is not less than 20 GBq of iodine-131 per gram of iobenguane base.

CHARACTERS

A clear, colourless or slightly yellow solution.

Iodine-131 has a half-life of 8.04 days and emits beta and gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-131 solution by direct comparison with such a solution. Standardised iodine-131 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of iodine-131 has an energy of 0.365 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of the radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the solution is 3.5 to 8.0.

Specific radioactivity

The specific radioactivity is calculated from the results obtained in the test for radiochemical purity. Determine the content of iobenguane sulphate from the areas of the peaks corresponding to iobenguane in the chromatograms obtained with the test solution and reference solution (b). Calculate the concentration as iobenguane base by multiplying the result obtained in the assay by 0.85.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-131 solution. Determine the relative amounts of iodine-131, iod-ine-133, iodine-135 and other radionuclidic impurities present. Iodine-133 has a half-life of 20.8 h and its most prominent gamma photons have energies of 0.530 MeV and 0.875 MeV. Iodine-135 has a half-life of 6.55 h and its most prominent gamma photons have energies of 0.527 MeV, 1.132 MeV and 1.260 MeV. Not less than 99.9 per cent of the total radioactivity is due to iodine-131.

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Test solution The injection to be examined.

Reference solution (a) Dissolve 0.100 g of *sodium iodide R* in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *iobenguane sulphate CRS* in 25 ml of the mobile phase and dilute to 50.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.0 mm in internal diameter packed with *silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture of 1 volume of an 80 g/l solution of *ammonium nitrate R*, 2 volumes of *dilute ammonia R2* and 27 volumes of *methanol R*,

— a suitable radioactivity detector,

— a spectrophotometer set at 254 nm and provided with a flow-cell,

— a 10 µl loop injector.

Inject the test solution and the reference solutions. Not less than 94 per cent of the radioactivity of the chromatogram is found in the peak corresponding to iobenguane. Not more than 5 per cent of the radioactivity is found in the peak corresponding to iodide and not more than 1 per cent of the radioactivity is found in other peaks.

RADIOACTIVITY

Measure the radioactivity using a suitable counting apparatus by comparison with a standardised iodine-131 solution or by measurement in an instrument calibrated with the aid of such a solution.

STORAGE

Store protected from light.

LABELLING

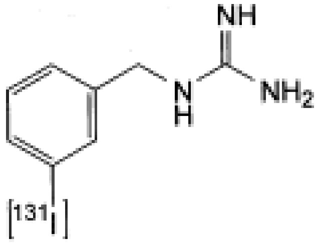
The label states the specific radioactivity expressed in gigabecquerels of iodine-131 per gram of iobenguane base.

Ph Eur

Iobenguane [^{131} I]) Injection for Therapeutic Use

General Notices

(Ph Eur monograph 1112)



$C_8H_{10}[^{131}I]N_3$

Ph Eur

DEFINITION

Iobenguane (^{131}I) injection for therapeutic use is a sterile, bacterial endotoxin-free solution of 1-(3-[^{131}I]iodobenzyl)guanidine or its salts. It may contain a suitable buffer, a suitable labelling catalyst such as ionic copper, a suitable labelling stabiliser such as ascorbic acid and antimicrobial preservatives. Iodine-131 is a radioactive isotope of iodine and may be obtained by neutron irradiation of tellurium or by extraction of uranium fission products. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared iodine-131 radioactivity at the date and hour stated on the label. Not less than 92 per cent of the radioactivity corresponds to iodine-131 in the form of iobenguane. The specific radioactivity is not less than 400 GBq of iodine-131 per gram of iobenguane base.

CHARACTERS

A clear, colourless or slightly yellow solution.

Iodine-131 has a half-life of 8.04 days and emits beta and gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-131 solution by direct comparison with such a solution. Standardised iodine-131 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of iodine-131 has an energy of 0.365 MeV.

B. Examine the chromatogram obtained in the test for radio-chemical purity. The distribution of the radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the solution is 3.5 to 8.0.

Specific radioactivity

The specific radioactivity is calculated from the results obtained in the test for radiochemical purity. Determine the content of iobenguane sulphate from the areas of the peaks corresponding to iobenguane in the chromatograms obtained with the test solution and reference solution (b). Calculate the concentration as iobenguane base by multiplying the result obtained in the assay by 0.85.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-131 solution. Determine the relative amounts of iodine-131, iodine-133, iodine-135 and other radionuclidic impurities present. Iodine-133 has a half-life of 20.8 h and its most prominent gamma photons have energies of 0.530 MeV and 0.875 MeV. Iodine-135 has a half-life of 6.55 h and its most prominent gamma photons have energies of 0.527 MeV, 1.132 MeV and 1.260 MeV. Not less than 99.9 per cent of the total radioactivity is due to iodine-131.

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Test solution The injection to be examined.

Reference solution (a) Dissolve 0.100 g of *sodium iodide R* in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *iobenguane sulphate CRS* in 25 ml of the mobile phase and dilute to 50.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.0 mm in internal diameter packed with *silica gel for chromatography R* (5 µm),

— as mobile phase at a flow rate of 1.0 ml/min a mixture of 1 volume of an 80 g/lsolution of *ammonium nitrate R*, 2 volumes of *dilute ammonia R2* and 27 volumes of *methanol R*,

— a suitable radioactivity detector,

— a spectrophotometer set at 254 nm and provided with a flow-cell,

— a 10 µl loop injector.

Inject the test solution and the reference solutions. Not less than 92 per cent of the radioactivity of the chromatogram is found in the peak corresponding to iobenguane. Not more than 7 per cent of the radioactivity is found in the peak corresponding to iodide and not more than 1 per cent of the radioactivity is found in other peaks.

RADIOACTIVITY

Measure the radioactivity using a suitable counting apparatus by comparison with a standardised iodine-131 solution or by measurement in an instrument calibrated with the aid of such a solution.

STORAGE

Store protected from light.

LABELLING

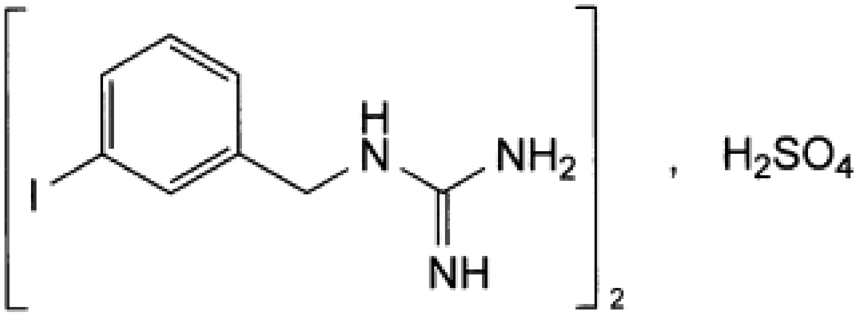
The label states the specific radioactivity expressed in gigabecquerels of iodine-131 per gram of iobenguane base.

Ph Eur

Iobenguane Sulphate for Radiopharmaceutical Preparations

General Notices

(Ph Eur monograph 2351)



$C_{16}H_{20}I_2N_6,H_2SO_4$ 648 *87862-25-7*

Ph Eur

DEFINITION

Bis[(3-iodobenzyl)guanidine] sulphate.

Content

98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white crystals.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of iobenguane sulphate.

B. Dissolve about 10 mg in 1 ml of *water R* with gentle heating. The solution gives reaction (a) of sulphates (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Test solution Dissolve 10.0 mg of the substance to be examined in 1 ml of *ethanol (96 per cent) R* with gentle heating and dilute to 5.0 ml with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *iobenguane sulphate CRS* in 1 ml of *ethanol (96 per cent) R* with gentle heating and dilute to 5.0 ml with the same solvent.

Reference solution (b) Dissolve 23.1 mg of *3-iodobenzylammonium chloride R* (salt of impurity A) in 1 ml of *ethanol (96 per cent) R* with gentle heating and dilute to 10.0 ml with the same solvent.

Reference solution (c) Mix 1 ml of reference solution (a) and 1 ml of reference solution (b).

Reference solution (d) Dilute 0.1 ml of reference solution (b) to 10.0 ml with *ethanol (96 per cent) R.*

Column:

- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *silica gel for chromatography R* (5 µm);
- *temperature*: maintain at a constant temperature between 20 °C and 30 °C.

Mobile phase Mix 40 ml of an 80 g/l solution of *ammonium nitrate R* , 80 ml of *dilute ammonia R2* and 1080 ml *of methanol R* .

Flow rate 1 ml/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µl of the test solution and reference solutions (c) and (d).

Run time 15 min.

Relative retention With reference to iobenguane (retention time = about 7 min): impurity A = about 0.2.

System suitability Reference solution (c):

- *resolution*: minimum 4.0 between the peaks due to iobenguane and impurity A.

Limit:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{16}H_{22}I_2N_6O_4S$ from the declared content of *iobenguane sulphate CRS.*

STORAGE

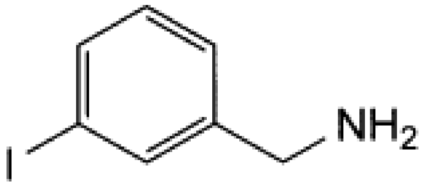
Protected from light, at a temperature below 25 °C.

LABELLING

The label recommends testing the substance in a production test before its use for the manufacture of radiopharmaceutical preparations. This ensures that, under specified production conditions, the substance yields the radiopharmaceutical preparation in the desired quantity and quality specified.

IMPURITIES

Specified impurities A.



A. 1-(3-iodophenyl)methanamine.

Ph Eur

Iodinated [^{125} I] Albumin Injection

General Notices

(Iodinated (^{125}I) Human Albumin Injection, Ph Eur monograph 1922)

Ph Eur

DEFINITION

Sterile, endotoxin-free solution of human albumin labelled with iodine-125. It may contain a suitable buffer and an antimicrobial preservative. The human albumin used complies with the requirements of the monograph on *Human albumin solution (0255).*

Content

90 per cent to 110 per cent of the declared iodine-125 radioactivity at the date stated on the label.

Purity:

— minimum of 99.0 per cent of the total radioactivity corresponds to iodine-125,

— minimum of 80 per cent of the total radioactivity is associated with the albumin fractions II to V,

— maximum of 5 per cent of the total radioactivity corresponds to unbound iodide.

Content of albumin

95 per cent to 105 per cent of the declared albumin content stated on the label.

CHARACTERS

Appearance

Clear, colourless to yellowish solution.

Half-life and nature of radiation of iodine-125

See general chapter *5.7 . Table of physical characteristics of radionuclides*.

IDENTIFICATION

A. Gamma-ray and X-ray spectrometry.

Comparison Standardised iodine-125 solution, or by using a calibrated instrument. Standardised iodine-125 solutions and/or standardisation services are available from the competent authority.

Results The spectrum obtained with the preparation to be examined does not differ significantly from that obtained with a standardised iodine-125 solution, apart from any differences attributable to the presence of iodine-126. The most prominent photon has an energy of 0.027 MeV, corresponding to the characteristic X-ray of tellurium, gamma photons of an energy of 0.035 MeV are also present. Iodine-126 has a half-life of 13.11 days and its most prominent gamma photons have energies of 0.388 MeV and 0.666 MeV.

B. Examine by a suitable immunoelectrophoresis technique (*2.7.1*). Using antiserum to normal human serum, compare normal human serum and the preparation to be examined, both diluted if necessary. The main component of the preparation to be examined corresponds to the main component of the normal human serum. The diluted solution may show the presence of small quantities of other plasma proteins.

TESTS

pH (*2.2.3*)

5.0 to 9.0.

Albumin

Reference solution Dilute *human albumin solution R* with a 9 g/l solution of *sodium chloride R* to a concentration of 5 mg of albumin per millilitre.

To 1.0 ml of the preparation to be examined and to 1.0 ml of the reference solution add 4.0 ml of *biuret reagent R* and mix. After exactly 30 min, measure the absorbance (*2.2.25*) of each solution at 540 nm, using as the compensation liquid a 9 g/l solution of *sodium chloride R* treated in the same manner. From the absorbances measured, calculate the content of albumin in the injection to be examined in milligrams per millilitre.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).*

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres.

RADIONUCLIDIC PURITY

Iodine-125

Minimum 99.0 per cent of the total radioactivity.

Gamma-ray and X-ray spectroscopy.

Comparison Standardised solution of iodine-125.

Determine the relative amounts of iodine-125 and iodine-126 present.

RADIOCHEMICAL PURITY

Iodine-125 in albumin fractions II to V, iodine-125 corresponding to unbound iodide

Size-exclusion chromatography (*2.2.30*).

Test solution Mix 0.25 ml of the preparation to be examined with 0.25 ml of the mobile phase. Use immediately after mixing.

Reference solution *Human albumin solution R* or another appropriate human albumin standard diluted with the mobile phase to a suitable albumin concentration.

Column:
- *size: l* = 0.6 m, Ø = 7.5 mm,
- *stationary phase*: *silica gel for size-exclusion chromatography R* ,
- *temperature*: 25 °C.

Mobile phase Dissolve 11.24 g of *potassium dihydrogen phosphate R* , 42.0 g of *disodium hydrogen phosphate R* , 11.70 g of *sodium chloride R* in 2000 ml of *water R* .

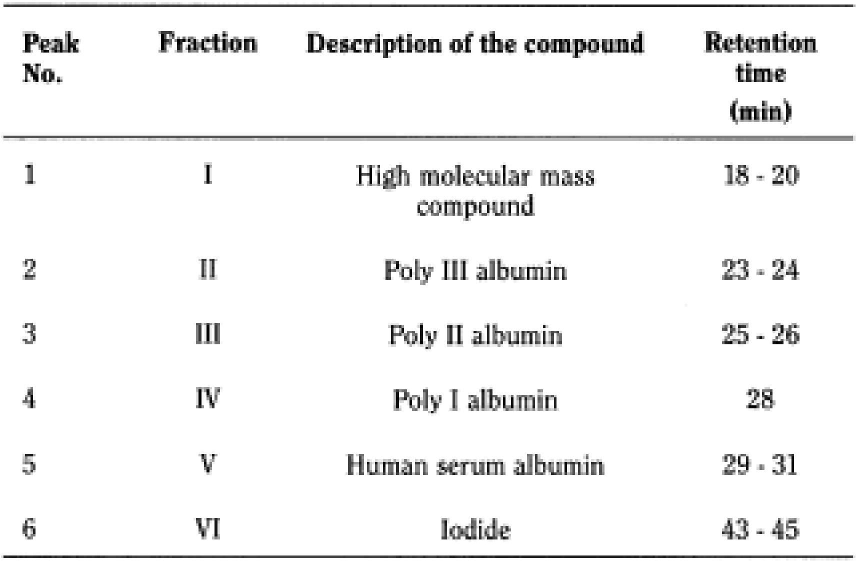
Flow rate 0.6 ml/min.

Detection Spectrophotometer at 280 nm and radioactivity detector set for iodine-125 connected in series.

Injection Loop injector.

Run time 85 min.

Retention times:



Peak No.	Fraction	Description of the compound	Retention time (min)
1	I	High molecular mass compound	18 - 20
2	II	Poly III albumin	23 - 24
3	III	Poly II albumin	25 - 26
4	IV	Poly I albumin	28
5	V	Human serum albumin	29 - 31
6	VI	Iodide	43 - 45

The main peak in the chromatogram obtained with the reference solution corresponds to fraction V.

Limits:
- *radioactivity in fractions II to V*: minimum 80 per cent of the total radioactivity applied to the column,
- *iodine-125 in fraction VI*: maximum 5 per cent of the total radioactivity.

RADIOACTIVITY

Measure the radioactivity using suitable equipment by comparison with a standardised iodine-125 solution or by measurement with a calibrated instrument.

LABELLING

The label states:

— the amount of albumin,

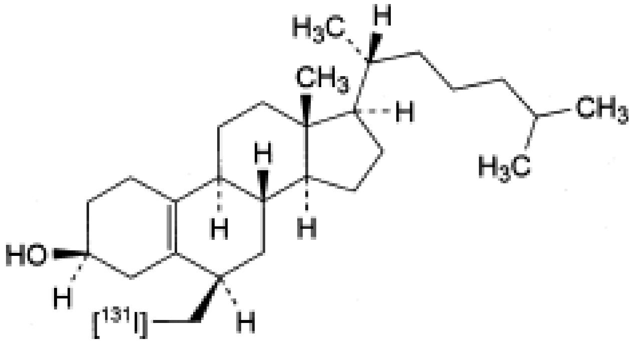
the maximum volume to be injected.

Ph Eur

Iodinated [^{131} I] Norcholesterol Injection

General Notices

(Ph Eur monograph 0939)



Ph Eur

DEFINITION

Iodinated (^{131}I) norcholesterol injection is a sterile, bacterial endotoxin-free solution of 6β-[^{131}I]iodomethyl-19-norcholest-5(10)-en-3β-ol. It may contain a suitable emulsifier such as polysorbate 80 and a suitable antimicrobial preservative such as benzyl alcohol. Iodine-131 is a radioactive isotope of iodine and may be obtained by neutron irradiation of tellurium or by extraction from uranium fission products. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared iodine-131 radioactivity at the date and hour stated on the label. Not less than 85 per cent of the radioactivity corresponds to iodine-131 in the form of 6β-[^{131}I]iodomethyl-19-norcholest-5(10)-en-3β-ol. Not more than 5 per cent of the radioactivity corresponds to iodine-131 in the form of iodide. The specific radioactivity is 3.7 GBq to 37 GBq per gram of 6β-iodomethylnorcholesterol.

CHARACTERS

A clear or slightly turbid, colourless or pale yellow solution.

Iodine-131 has a half-life of 8.04 days and emits beta and gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-131 solution by direct comparison with such a solution. The most prominent photon of iodine-131 has an energy of 0.365 MeV.

Standardised iodine-131 solutions are available from laboratories recognised by the competent authority.

B. Examine the chromatogram obtained in test (a) for radiochemical purity. The distribution of radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the solution is between 3.5 and 8.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-131 solution. Determine the relative amounts of iodine-131, iodine-133, iodine-135 and other radionuclidic impurities present. Iodine-133 has a half-life of 20.8 h and its most prominent gamma photons have energies of 0.530 MeV and 0.875 MeV. Iodine-135 has a half-life of 6.55 h and its most prominent gamma photons have energies of 0.527 MeV, 1.132 MeV and 1.260 MeV. Not less than 99.9 per cent of the total radioactivity is due to iodine-131.

RADIOCHEMICAL PURITY

a) Examine by thin-layer chromatography (*2.2.27*) using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution The injection to be examined.

Carrier solution Dissolve 10 mg of *potassium iodide R*, 20 mg of *potassium iodate R* and 0.1 g of *sodium hydrogen carbonate R* in *distilled water R* and dilute to 10 ml with the same solvent.

Apply to the plate up to 5 µl of the test solution and 10 µl of the carrier solution on the same spot. Develop over a path of 15 cm (about 60 min) using chloroform R. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Determine the distribution of radioactivity using a suitable detector. In the chromatogram obtained, not less than 85 per cent of the total radioactivity is found in the spot corresponding to 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol at an R_F value of about 0.5. Iodide ion remains near the starting-line.

b) Examine by thin-layer chromatography (*2.2.27*) using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution The injection to be examined.

Carrier solution Dissolve 10 mg *potassium iodide R*, 20 mg of *potassium iodate R* and 0.1 g of *sodium hydrogen carbonate R* in *distilled water R* and dilute to 10 ml with the same solvent.

Apply to the plate 10 µl of the carrier solution and then up to 5 µl of the test solution on the same spot. Develop over a path of 15 cm (about 90 min) using a mixture of equal volumes of *chloroform R* and *ethanol R*. Allow the plate to dry in air. Expose the plate to ultraviolet light at 254 nm for 5 min. A yellow spot corresponding to iodide develops at an R_F value of about 0.5. Determine the distribution of radio- activity using a suitable detector. The main peak of radio-activity is near to the solvent front. Other iodocholesterols migrate near the solvent front. In the chromatogram obtained, not more than 5 per cent of the total radioactivity is found in the spot corresponding to iodide.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised iodine-131 solution or by measurement in an instrument calibrated with the aid of such a solution.

STORAGE

Store protected from light at a temperature not exceeding - 18 °C.

Ph Eur

Krypton [^{81m} Kr] Inhalation Gas

General Notices

(Ph Eur monograph 1533)

Ph Eur

DEFINITION

Krypton (^{81m}Kr) inhalation gas is a mixture of krypton-81m and a suitable vehicle such as air.

Krypton-81m is formed by decay of its parent radionuclide rubidium-81. Rubidium-81 has a half-life of 4.58 h.

The krypton-81m formed is separated from the rubidium-81 with a flow of a suitable gas in a rubidium/krypton generator. Rubidium-81 is produced by proton irradiation of krypton isotopes or by helium-3 or helium-4 irradiation of bromine. After separation of rubidium-81 from the target, it is retained by a suitable support.

Krypton-81m is eluted at a suitable flow rate with a vehicle such as air. The level of moisture required in the eluent depends on the type of generator used. The transport tube for administration has a defined length and inner diameter. The radioactivity concentration is determined before administration.

The radioactivity due to radionuclides other than krypton-81m is not greater than 0.1 per cent, expressed as a percentage of the total radioactivity in the preparation and calculated with reference to the date and time of administration.

CHARACTERS

A clear, colourless gas.

Krypton-81m has a half-life of 13.1 s and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray and X-ray spectrum using a suitable instrument. The gamma photon of krypton-81m has an energy of 0.190 MeV.

B. The half-life is 11.8 s to 14.4 s.

TESTS

RADIONUCLIDIC PURITY

Elute the generator as prescribed. Pass a sufficient amount (2 litres to 10 litres) of eluate at a suitable flow rate through a suitable absorber such as water. Determine the amount of radioactivity eluted. Allow the krypton-81m to decay for 5 min and record the gamma and X-ray spectrum of the residual radioactivity on the absorber using a suitable instrument. Examine the gamma-ray and X-ray spectrum of the absorber for the presence of radioactive impurities, which must be identified and quantified. The absorbed radioactivity is not more than 0.1 per cent of the radioactivity passed through the absorber, calculated with reference to the date and time of administration.

RADIOACTIVITY

Determine the radioactive concentration of the preparation using suitable equipment such as an ionisation chamber or a gamma ray spectrometer. The measurement equipment may be calibrated by reference to a primary calibrated instrument at a laboratory recognised by the competent authority. The radioactivity is measured under defined operating conditions, such as gas flow rate and measurement geometry, that are identical to those used for the calibration of the instrument.

STORAGE

The storage conditions apply to the generator.

LABELLING

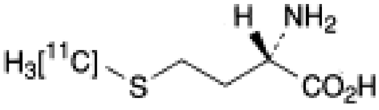
The labelling conditions apply to the generator.

Ph Eur

L-Methionine([^{11} C]-Methyl) Injection

General Notices

(Ph Eur monograph 1617)



Ph Eur

DEFINITION

Sterile solution of (2*S*)-2-amino-4-([^{11}C]methylsulphanyl)butanoic acid for diagnostic use.

Content

90 per cent to 110 per cent of the declared carbon-11 radioactivity at the date and time stated on the label.

Purity:

— minimum of 99 per cent of the total radioactivity corresponds to carbon-11,

— minimum of 95 per cent of the total radioactivity corresponds to carbon-11 in the form of L-[*methyl*-^{11}C]methionine and D-[*methyl*-^{11}C]methionine,

— maximum of 10 per cent of the total radioactivity corresponds to carbon-11 in the form of D-[*methyl*-^{11}C]methionine.

Content of methionine

Maximum of 2 mg per maximum recommended dose in millilitres.

PRODUCTION

RADIONUCLIDE PRODUCTION

Carbon-11 is a radioactive isotope of carbon which is most commonly produced by proton irradiation of nitrogen. Depending on the addition of either trace amounts of oxygen or small amounts of hydrogen, the radioactivity is obtained as [^{11}C]carbon dioxide or [^{11}C]methane.

RADIOCHEMICAL SYNTHESIS

L-[*Methyl*-^{11}C]methionine can be prepared by various chemical synthetic pathways. All methods rely on the alkylation of the sulphide anion of L-homocysteine with [^{11}C]methyl iodide or [^{11}C]methyl triflate. Variations in the procedures used to generate the sulphide anion of L-homocysteine and methods to obtain [^{11}C]methyl iodide lead to negligible differences with respect to quality in terms of specific radioactivity, enantiomeric purity and possible chemical and radiochemical impurities.

Synthesis of [^{11}C]methyl iodide

[^{11}C]Methyl iodide can be obtained either starting from [^{11}C]carbon dioxide or from [^{11}C]methane. The most frequently used method is the reduction of [^{11}C]carbon dioxide with lithium aluminium hydride. The formed [^{11}C]methanol is reacted with hydroiodic acid. Alternatively [^{11}C]methane, either obtained directly in the target or by on-line processes from [^{11}C]carbon dioxide, is reacted with iodine.

Synthesis of [^{11}C]methyl triflate

[^{11}C]methyl triflate can be prepared from [^{11}C]methyl iodide using a silver triflate-impregnated solid support such as graphitised carbon.

Synthesis of L-[*methyl*-^{11}C]methionine

The most widely used method to obtain L-[*methyl*-^{11}C]methionine is the alkylation of the sulphide anion, generated from L-homocysteine thiolactone, with [^{11}C]methyl iodide or [^{11}C]methyl triflate in alkaline conditions in a solvent such as acetone. The L-[*methyl*-^{11}C]methionine obtained can be purified by semi-preparative liquid chromatography. For example, a column packed with octadecylsilyl silica gel for chromatography eluted with a 9 g/l solution of sodium chloride is suitable.

L-Homocysteine thiolactone hydrochloride

Specific optical rotation *(2.2.7)* : + 20.5 to + 21.5, determined on a 10 g/l solution at 25 °C.

Infrared absorption spectrophotometry *(2.2.24)* .

Comparison Ph. Eur. reference spectrum of L -homocysteine thiolactone hydrochloride.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of carbon-11

See general chapter *5.7 . Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with the test for radionuclidic purity (see Tests).

C. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (b).

TESTS

pH *(2.2.3)*

4.5 to 8.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Bacterial endotoxins *(2.6.14)*

Less than 175/V IU/ml, *V* being the maximum recommended dose in millilitres. The injection may be released for use before completion of the test.

CHEMICAL PURITY

Impurity A, impurity B and methionine

Liquid chromatography (2.2.29).

Test solution The preparation to be examined.

Reference solution (a) Dissolve 0.6 mg of *L -homocysteine thiolactone hydrochloride R* , 2 mg of *DL -homocysteine R* and 2 mg of *DL -methionine R* in *water R* and dilute to *V*, *V* being the maximum recommended dose in millilitres.

Reference solution (b) Dissolve 2 mg of *L -methionine R* in the same solvent as used in the test solution and dilute to 10 ml with the same solvent.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface of 220 m2/g, a pore size of 8 nm and a carbon loading of 6.2 per cent,

— *temperature*: 25 °C.

Mobile phase 1.4 g/l solution of *potassium dihydrogen phosphate R.*

Flow rate 1 ml/min.

Detection Spectrophotometer at 225 nm and radioactivity detector connected in series.

Injection Loop injector.

Run time 10 min.

Relative retention With reference to methionine (retention time = about 2.6 min): impurity B = about 0.8, impurity A = about 2.7.

System suitability Reference solution (a):

— *resolution*: minimum of 2.5 between the peaks due to methionine and impurity B.

Limits Examine the chromatogram obtained with the spectrophotometer:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.6 mg/ *V*),

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (2 mg/ *V*),

— *methionine*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (2 mg/ *V*).

Residual solvents *(2.4.24)*

Maximum 50 mg/V for the concentration of acetone, V being the maximum recommended dose in millilitres. The preparation may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Carbon-11

Minimum 99 per cent of the total radioactivity.

A. Gamma-ray spectroscopy.

Comparison Standardised fluorine-18 solution, or by using an instrument calibrated with the aid of such a solution. Standardised fluorine-18 solutions and/or standardisation services are available from the competent authority.

Results The spectrum obtained with the solution to be examined does not differ significantly from that obtained with a standardised fluorine-18 solution.

B. Half-life: 19.9 min to 20.9 min.

The preparation may be released for use before completion of the test.

RADIOCHEMICAL PURITY

L-[Methyl-^{11}C]methionine and impurity E

Liquid chromatography *(2.2.29)* as described in the test for impurity A, impurity B and methionine.

Injection Test solution and reference solution (b).

Limits Examine the chromatogram obtained with the radioactivity detector:

— *total of* L *-[methyl-* ^{11} *C]methionine and impurity E*: minimum of 95 per cent of the total radioactivity,

— other peaks in the chromatogram may be due to impurity C, impurity D and impurity F.

ENANTIOMERIC PURITY

Impurity E

Thin-layer chromatography *(2.2.27)* .

Test solution The preparation to be examined.

Reference solution (a) Dissolve 2 mg of *L -methionine R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 4 mg of *DL -methionine R* in *water R* and dilute to 10 ml with the same solvent.

Plate *TLC octadecysilyl silica gel plate for chiral separations R.*

Mobile phase *methanol R* , *water R* (50:50 *V/V*).

Application 2-10 µl.

Development Over a path of 8 cm.

Drying In air for 5 min.

Detection Spray with a 2 g/l solution of *ninhydrin R* in *ethanol R* and heat at 60 °C for 10 min. Determine the distribution of radioactivity using a suitable detector.

Retardation factors L-[*methyl*-^{11}C]methionine = about 0.58; impurity E = about 0.51.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Limits:

— *total of L -[methyl- ^{11} C]methionine and impurity E*: minimum 95 per cent of the total radioactivity,

— *impurity E*: maximum 10 per cent of the total radioactivity.

The preparation may be released for use before completion of the test.

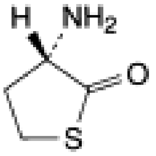
RADIOACTIVITY

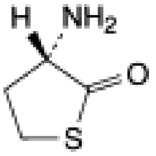
Measure the radioactivity using suitable equipment by comparison with a standardised fluorine-18 solution or by measurement in an instrument calibrated with the aid of such a solution.

LABELLING

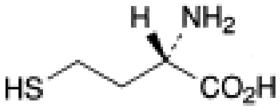
The accompanying information specifies the maximum recommended dose in millilitres.

IMPURITIES

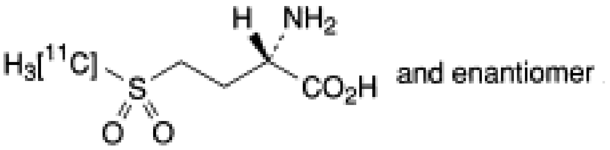


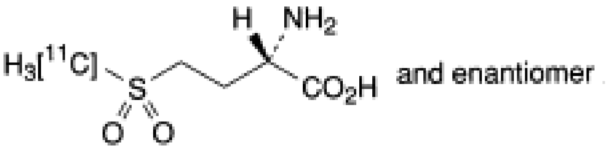


A. (3*S*)-3-aminodihydrothiophen-2(3*H*)-one (L-homocysteine thiolactone),

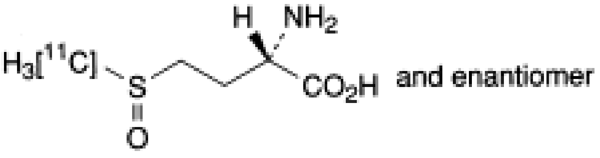


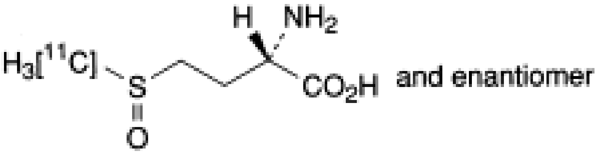
B. (2*S*)-2-amino-4-sulphanylbutanoic acid (L-homocysteine),


and enantiomer

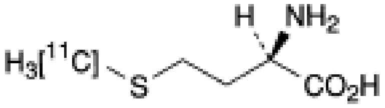

and enantiomer

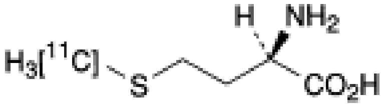
C. (2*RS*)-2-amino-4-([^{11}C]methylsulphonyl)butanoic acid (DL-[*methyl*-^{11}C]methionine *S*,*S*-dioxide),


and enantiomer


and enantiomer

D. (2*RS*)-2-amino-4-([^{11}C]methylsulphinyl)butanoic acid (DL-[*methyl*-^{11}C]methionine *S*-oxide)
,





E. (2*R*)-2-amino-4-([^{11}C]methylsulphanyl)butanoic acid (D-[*methyl*-^{11}C]methionine),

F. [^{11}C]methanol

Ph Eur

Oxygen [^{15} O]

General Notices

(Ph Eur monograph 1620)

Ph Eur

DEFINITION

Mixture of [^{15}O]oxygen in the gaseous phase and a suitable vehicle such as *Medicinal ai (1238),* for diagnostic use.

Purity:

— minimum 99 per cent of the total radioactivity corresponds to oxygen-15,

— minimum 97 per cent of the total radioactivity corresponds to oxygen-15 in the form of oxygen (O_2).

PRODUCTION

RADIONUCLIDIC PRODUCTION

Oxygen-15 is a radioactive isotope of oxygen which may be produced by various nuclear reactions such as proton irradiation of nitrogen-15 or deuteron irradiation of nitrogen-14.

RADIOCHEMICAL SYNTHESIS

In order to recover oxygen-15 as molecular oxygen from the nitrogen target gas, carrier oxygen is added at concentrations generally ranging from 0.2 per cent *V/V* to 1.0 per cent *V/V*. After irradiation, the target gas is usually passed through activated charcoal and a carbon dioxide scavenger, such as soda lime, before mixing with the vehicle.

CHARACTERS

Appearance

Colourless gas.

Half-life and nature of radiation of oxygen-15

See general chapter *5.7*. Table of physical characteristics of radionuclides.

IDENTIFICATION

A. Gamma spectrometry.

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with the test for radionuclidic purity (see Tests).

C. Examine the chromatograms obtained in the test for radiochemical purity.

Results The retention times of the principal peaks in the chromatogram obtained with the test gas using the radioactivity detector are similar to those of the principal peaks corresponding to oxygen in the chromatogram obtained with the reference gas using the thermal conductivity detector.

TESTS

The following tests are performed on [^{15}O]oxygen as described under radiochemical synthesis before mixing with the vehicle.

RADIONUCLIDIC PURITY

Oxygen-15

Minimum 99 per cent of the total radioactivity.

A. Gamma spectrometry.

Comparison Standardised fluorine-18 solution, or by using an instrument calibrated with the aid of such a solution. Standardised fluorine-18 solutions and/or standardisation services are available from the competent authority.

Results The spectrum obtained with the solution to be examined does not differ significantly from that obtained with a standardised fluorine-18 solution.

B. Half-life: 1.9 min to 2.2 min.

The preparation may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Oxygen-15 in the form of O_2

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test sample [^{15}O]oxygen as described under radiochemical synthesis.

Reference gas *nitrogen gas mixture R.*

Column:

— *size:* l = 1.8 m, Ø1 = 6.3 mm and Ø2 = 3.2 mm,

— *stationary phase*: *GC concentrical column R.*

Carrier gas *helium for chromatography R.*

Flow rate 65 ml/min.

Temperature:

- — *column*: 40 °C,
- — *injection port*: 40 °C,
- — *thermal conductivity detector*: 70 °C.

Detection Thermal conductivity detector and radioactivity detector connected in series.

Injection Loop injector.

Run time 10 min.

Retention times Oxygen, nitrogen and carbon monoxide eluting from the inner column = about 0.4 min; carbon dioxide eluting from the inner column = about 0.8 min; oxygen eluting from the outer column = about 2.1 min; nitrogen eluting from the outer column = about 3.1 min; carbon monoxide eluting from the outer column = about 6.2 min.

System suitability Reference gas:

- — 5 clearly separated principal peaks are observed in the chromatogram obtained using the thermal conductivity detector,
- — *resolution*: minimum of 1.5 between the peaks due to carbon dioxide eluting from the inner column and oxygen eluting from the outer column, in the chromatogram obtained using the thermal conductivity detector.

Limits Examine the chromatogram obtained with the radioactivity detector and calculate the percentage content of oxygen-15 substances from the peak areas.

- — *oxygen-15 gas in the form of* O_2: minimum 97 per cent of the total radioactivity,
- — disregard the first peak corresponding to components co-eluting from the inner column.

RADIOACTIVITY

The radioactive concentration is determined before administration.

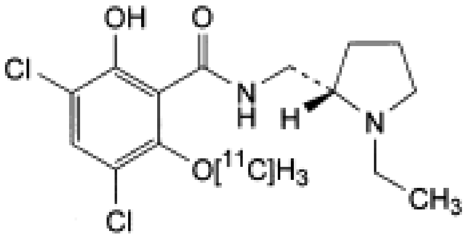
Measure the radioactivity using suitable equipment by comparison with a standardised fluorine-18 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Raclopride ([^{11} C]Methoxy) Injection

General Notices

(Ph Eur monograph 1924)



Ph Eur

DEFINITION

Sterile solution of 3,5-dichloro-*N*-[[(2*S*)-1-ethylpyrrolidin-2-yl]methyl]-2-hydroxy-6-([^{11}C] methoxy)benzamide.

Content

90 per cent to 110 per cent of the declared carbon-11 radioactivity at the date and time stated on the label.

Purity:

- — minimum of 99 per cent of the total radioactivity corresponds to carbon-11,
- — minimum of 95 per cent of the total radioactivity corresponds to carbon-11 in the form of [*methoxy*-^{11}C]raclopride.

Content of raclopride

Maximum of 10 µg per maximum recommended dose in millilitres.

PRODUCTION

RADIONUCLIDE PRODUCTION

Carbon-11 is a radioactive isotope of carbon most commonly produced by proton irradiation of nitrogen. Depending on the addition of either trace amounts of oxygen or small amounts of hydrogen, the radioactivity is obtained as [^{11}C]carbon dioxide or [^{11}C]methane, respectively.

RADIOCHEMICAL SYNTHESIS

[*Methoxy*-^{11}C]raclopride may be prepared by *O*-alkylation of the corresponding phenolate anion (*S*)-3,5-dichloro-2,6-dihydroxy-*N*-[(1-ethylpyrrolidin-2-yl)methyl]benzamide with iodo [^{11}C]methane or [^{11}C]methyl trifluoromethanesulphonate.

Synthesis of iodo[^{11}C]methane

Iodo[^{11}C]methane may be produced from [^{11}C]carbon dioxide or from [^{11}C]methane. The most frequently used method is reduction of [^{11}C]carbon dioxide with lithium aluminium hydride. The lithium aluminium [^{11}C]methanolate formed is reacted with hydroiodic acid to iodo[^{11}C] methane via [^{11}C]methanol. Alternatively [^{11}C]methane, either obtained directly in the target or by on-line processes from [^{11}C]carbon dioxide, is reacted with iodine.

Synthesis of [^{11}C]methyl trifluoromethanesulphonate

[^{11}C]Methyl trifluoromethanesulphonate may be prepared from iodo[^{11}C]methane using a solid support such as graphitised carbon impregnated with silver trifluoromethanesulphonate.

Synthesis of [*methoxy*-^{11}C]raclopride

Methylation with iodo[^{11}C]methane is performed under alkaline conditions in a solvent such as dimethyl sulphoxide. The methylation with [^{11}C]methyl trifluoromethanesulphonate is performed in a solvent such as dimethylformamide or acetone. The resulting [*methoxy*-^{11}C] raclopride may be purified by semi-preparative liquid chromatography using, for example, a column packed with octadecylsilyl silica gel for chromatography eluted with a mixture of 25 volumes of acetonitrile and 75 volumes of *0.01 M phosphoric acid*.

PRECURSOR FOR SYNTHESIS

(*S*)-3,5-Dichloro-2,6-dihydroxy-*N*-[(1-ethylpyrrolidin-2-yl)methyl]benzamide hydrobromide

Melting point (*2.2.14*): 211 °C to 213 °C.

Specific optical rotation (*2.2.7*): + 11.3 to + 11.5, determined on a 15.0 g/l solution in *ethanol R* at 22 °C.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of carbon-11

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with test B for radionuclidic purity (see Tests).

C. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (d).

TESTS

pH (*2.2.3*)

4.5 to 8.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres. The injection may be released for use before completion of the test.

CHEMICAL PURITY

Raclopride and impurity A

Liquid chromatography (*2.2.29*).

Test solution The preparation to be examined.

Reference solution (a) Dissolve 7.2 mg of *raclopride tartrate R* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b) Dissolve 1.2 mg of *(S)-3,5-dichloro-2,6-dihydroxy-N-[(1-ethylpyrrolidin-2-yl)methyl]benzamide hydrobromide R* in *methanol R* and dilute to 100 ml with the same solvent.

Reference solution (c) To 0.1 ml of reference solution (a) add 0.1 ml of reference solution (b) and dilute to *V* with *water R*, *V* being the maximum recommended dose in millilitres.

Reference solution (d) Dilute 1.0 ml of reference solution (a) to 10.0 ml with *water R*.

Column:

— *size:* *l* = 0.05 m, Ø = 4.6 mm,

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm) with a specific surface area of 175 m^2/g, a pore size of 12.5 nm, a pore volume of 0.7 cm^3/g and a carbon loading of 15 per cent,

— *temperature*: 30 °C.

Mobile phase Dissolve 2 g of *sodium heptanesulphonate R* in 700 ml of *water R,* adjust to pH 3.9 with *phosphoric acid R* and dilute to 1000 ml with *acetonitrile R*.

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm and radioactivity detector connected in series.

Injection Loop injector; inject the test solution and reference solutions (b) and (c).

Run time 10 min.

Relative retention With reference to raclopride: impurity A = about 0.46.

System suitability Reference solution (c):

— *resolution*: minimum of 5 between the peaks due to raclopride and to impurity A.

Limits Examine the chromatogram obtained with the spectrophotometer:

— *raclopride*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (10 µg/*V)*,

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1 µg/*V*).

Residual solvents

are limited according to the principles defined in the general chapter (*5.4*), using the general method (*2.4.24*). The preparation may be released for use before completion of the test.

RADIONUCLIDID PURITY

Carbon-11

Minimum 99 per cent of the total radioactivity.

The preparation may be released for use before completion of the test.

A. Gamma-ray spectrometry.

Comparison Standardised fluorine-18 solution, or by using a calibrated instrument. Standardised fluorine-18 solutions and/or standardisation services are available from the competent authority.

Results The spectrum obtained with the solution to be examined does not differ significantly from that obtained with a standardised fluorine-18 solution.

B. Half-life. 19.9 min to 20.9 min.

RADIOCHEMICAL PURITY

Liquid chromatography (*2.2.29*) as described in the test for raclopride and impurity A with the following modifications.

Injection Test solution and reference solution (d).

Limits Examine the chromatogram obtained with the radioactivity detector:

— *[Methoxy-^{11}C] raclopride*: minimum of 95 per cent of the total radioactivity.

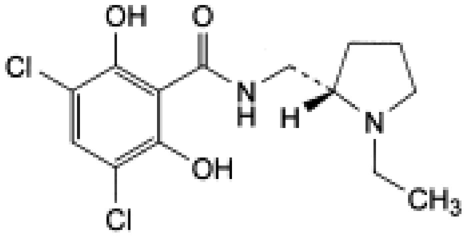
RADIOACTIVITY

Mesure the radioactivity using suitable equipment by comparison with a standardised fluorine-18 solution or by using a calibrated instrument.

LABELLING

The accompanying information specifies the maximum recommended dose in millilitres.

IMPURITIES



A. 3,5-dichloro-*N*-[[(2*S*)-1-ethylpyrrolidin-2-yl]methyl]-2,6-dihydroxybenzamide.

Ph Eur

Radiopharmaceutical Preparations

General Notices

(Ph Eur monograph 0125)

Radiopharmaceutical Preparations comply with the requirements of the European Pharmacopoeia. These requirements are reproduced below.

Ph Eur

DEFINITION

For the purposes of this general monograph, radiopharmaceutical preparations cover:

— radiopharmaceutical: any medicinal product which, when ready for use, contains one or more radionuclides (radioactive isotopes) included for a medicinal purpose,

— radionuclide generator: any system incorporating a fixed parent radionuclide from which is produced a daughter radionuclide which is to be removed by elution or by any other method and used in a radiopharmaceutical preparation,

— kit for radiopharmaceutical preparation: any preparation to be reconstituted and/or combined with radionuclides in the final radiopharmaceutical preparation, usually prior to its administration,

— radiopharmaceutical precursor: any other radionuclide produced for the radio-labelling of another substance prior to administration.

A nuclide is a species of atom characterised by the number of protons and neutrons in its nucleus (and hence by its atomic number Z, and mass number A) and also by its nuclear energy state. Isotopes of an element are nuclides with the same atomic number but different mass numbers. Nuclides containing an unstable arrangement of protons and neutrons will transform spontaneously to either a stable or another unstable combination of protons and neutrons with a constant statistical probability. Such nuclides are said to be radioactive and are called radionuclides. The initial unstable nuclide is referred to as the parent radionuclide and the resulting nuclide as the daughter nuclide.

The radioactive decay or transformation may involve the emission of charged particles, electron capture (EC) or isomeric transition (IT). The charged particles emitted from the nucleus may be alpha particles (helium nucleus of mass number 4) or beta particles (negatively charged, generally called electrons or positively charged, generally called positrons). The emission of charged particles from the nucleus may be accompanied by the emission of gamma rays. Gamma rays are also emitted in the process of isomeric transition. These emissions of gamma rays may be partly replaced by the ejection of electrons known as internal conversion electrons. This phenomenon, like the process of electron capture, causes a secondary emission of X-rays (due to the reorganisation of the electrons in the atom). This secondary emission may itself be partly replaced by the ejection of electrons known as Auger electrons. Radionuclides with a deficit of neutrons may decay by emitting positrons. These radionuclides are called positron emitters. Positrons are annihilated on contact with electrons, the process being accompanied by the emission of usually two gamma photons, each with an energy of 511 keV, generally emitted at 180° to each other, termed annihilation radiation.

The decay of a radionuclide is governed by the laws of probability with a characteristic decay constant and follows an exponential law. The time in which a given quantity of a radionuclide decays to half its initial value is termed the half-life ($T_{1/2}$).

The penetrating power of each radiation varies considerably according to its nature and its energy. Alpha particles are completely absorbed in a thickness of a few micrometers to some tens of micrometers of matter. Beta particles are completely absorbed in a thickness of several millimetres to several centimetres of matter. Gamma rays are not completely absorbed but only attenuated and a tenfold reduction may require, for example, several centimetres of lead. For most absorbents, the denser the absorbent, the shorter the range of alpha and beta particles and the greater the attenuation of gamma rays.

Each radionuclide is characterised by an invariable half-life, expressed in units of time and by the nature and energy of its radiation or radiations. The energy is expressed in electronvolts (eV), kilo-electronvolts (keV) or mega-electronvolts (MeV).

Generally the term "radioactivity" is used to describe the phenomenon of radioactive decay and to express the physical quantity (activity) of this phenomenon. The radioactivity of a preparation is the number of nuclear disintegrations or transformations per unit time.

In the International System (SI), radioactivity is expressed in becquerel (Bq) which is one nuclear transformation per second. Absolute radioactivity measurements require a specialised laboratory but identification and measurement of radiation can be carried out relatively by comparing with standardised preparations provided by laboratories recognised by the competent authority.

Radionuclidic purity

The ratio, expressed as a percentage, of the radioactivity of the radionuclide concerned to the total radioactivity of the radiopharmaceutical preparation. The relevant radionuclidic impurities are listed with their limits in the individual monographs.

Radiochemical purity

The ratio, expressed as a percentage, of the radioactivity of the radionuclide concerned which is present in the radiopharmaceutical preparation in the stated chemical form, to the total radioactivity of that radionuclide present in the radiopharmaceutical preparation. The relevant radiochemical impurities are listed with their limits in the individual monographs.

Chemical purity

In monographs on radiopharmaceutical preparations chemical purity is controlled by specifying limits on chemical impurities.

Isotopic carrier

A stable isotope of the element concerned either present or added to the radioactive preparation in the same chemical form as that in which the radionuclide is present.

Specific radioactivity

The radioactivity of a radionuclide per unit mass of the element or of the chemical form concerned.

Radioactive concentration

The radioactivity of a radionuclide per unit volume.

Total radioactivity

The radioactivity of the radionuclide, expressed per unit (vial, capsule, ampoule, generator, etc).

Starting materials

All the constituents which make up the radiopharmaceutical preparations.

Period of validity

The time during which specifications described in the monograph must be fulfilled. Expiry date and, if necessary, time must be clearly stated.

PRODUCTION

A radiopharmaceutical preparation monograph describes as precisely as possible the method of production of the radionuclide. A radiopharmaceutical preparation contains its radionuclide:

— as an element in atomic or molecular form, e.g. [^{133}Xe], [^{15}O]O_2,

— as an ion, e.g. [^{131}I]iodide, [^{99m}Tc]pertechnetate,

— included in or attached to organic molecules by chelation, e.g. [^{111}In]oxine or by covalent bonding, e.g. 2-[^{18}F]fluoro-2-deoxy-D-glucose.

The practical ways of producing radionuclides for use in, or as radiopharmaceutical preparations are:

— neutron bombardment of target materials (generally in nuclear reactors),

— charged particles bombardment of target materials (in accelerators such as cyclotrons),

— nuclear fission of heavy nuclides of target materials (generally after neutron or particle bombardment),

— from a radionuclide generator.

NEUTRON OR CHARGED PARTICLE BOMBARDMENT

The nuclear reaction and the probability of its occurrence in unit time are dependent on the nature and physical properties of the target material and the nature, energy and quantity of the incident particles.

The nuclear transformation occurring through particle bombardment may be written in the form:

target nucleus (bombarding particle, emitted particle or radiation) produced nucleus.

Examples: $^{58}\text{Fe}(n,\gamma)^{59}\text{Fe}$

$^{18}O(p,n)^{18}F$

In addition to the desired nuclear reaction adventitious transformations may occur. These will be influenced by the energy of the incident particle and the purity of the target material. Such adventitious transformations may give rise to radionuclidic impurities.

NUCLEAR FISSION

A small number of nuclides with a high atomic number are fissionable and the most frequently used reaction is the fission of uranium-235 by neutrons in a nuclear reactor. Iodine-131, molybdenum-99 and xenon-133 may be produced by nuclear fission of uranium-235. Their extraction from a mixture of more than 200 other radionuclides must be carefully controlled in order to minimise the radionuclidic impurities.

RADIONUCLIDE GENERATORS

Radionuclide generator systems use a relatively long-lived parent radionuclide which decays to a daughter radionuclide, usually with a shorter half-life.

By separating the daughter radionuclide from the parent radionuclide by a chemical or physical process, it is possible to use the daughter at a considerable distance from the production site of the generators despite its short half-life.

TARGET MATERIALS

The isotopic composition and purity of the target material will determine the relative percentages of the principal radionuclide and radionuclidic impurities. The use of isotopically enriched target material in which the abundance of the required target nuclide has been artificially increased, can improve the production yield and the purity of the desired radionuclide.

The chemical form, the purity, the physical state and the chemical additives, as well as the bombardment conditions and the direct physical and chemical environment will determine the chemical state and chemical purity of the radionuclides which are produced.

In the production of radionuclides and particularly of short-lived radionuclides it may not be possible to determine any of these quality criteria before further processing and manufacture of radiopharmaceutical preparations. Therefore each batch of target material must be tested in test production runs before its use in routine radionuclide production and manufacture of the radiopharmaceutical preparations, to ensure that under specified conditions, the target yields the radionuclide in the desired quantity and quality specified.

The target material is contained in a holder in gaseous, liquid or solid state, in order to be irradiated by a beam of particles. For neutron bombardment, the target material is commonly contained in quartz ampoules or high purity aluminium or titanium containers. It is necessary to ascertain that no interaction can occur between the container and its contents under the irradiation conditions (temperature, pressure, time).

For charged particle bombardment, the holder for target material is usually built of aluminium or another appropriate metal, with inlet and outlet ports, a surrounding cooling system and usually a thin metal foil target window. The nature and thickness of the target window have a particular influence on the yield of the nuclear reaction and may also affect the radionuclidic purity.

The production procedure clearly describes:

- — target material,
- — construction of the holder for target material,
- — loading of target material into the irradiation system,
- — method of irradiation (bombardment),
- — separation of the desired radionuclide,

and evaluates all effects on the efficiency of the production in terms of quality and quantity of the produced radionuclide.

The chemical state of the isolated radionuclide may play a major role in all further processing.

PRECURSORS FOR SYNTHESIS

Generally, these precursors are not produced on a large scale. Some precursors are synthesised by the radiopharmaceutical production laboratory, others are supplied by specialised producers or laboratories.

Tests for identity, for chemical purity and the assay must be performed by validated procedures.

When batches of precursors are accepted using data from the certificates of analysis, suitable evidence has to be established to demonstrate the consistent reliability of the supplier's analyses and at least one identity test must be conducted. It is recommended to test precursor materials in production runs before their use for the manufacture of radiopharmaceutical preparations, to ensure that under specified production conditions, the precursor yields the radiopharmaceutical preparation in the desired quantity and quality specified.

Performance of the production system

All operations, from the preparation of the target to the dispensing of the final radiopharmaceutical preparation, must be clearly documented including their impact on the purity of the final product and the efficiency of the procedure. Where possible, in-process controls are performed and the results recorded at each production step to identify at which level a possible discrepancy from the normal production pathway may have occurred.

a) The production of radiopharmaceutical preparations may make use of mechanical and automated processes that are used in the pharmaceutical industry, subject to adapting these to the specificity of the radioactive starting material and to the requirements of radioprotection.

b) For radiopharmaceutical preparations containing shortlived radionuclides, such as certain positron emitters, remotely controlled production and automated radiosynthesis are generally used. For radionuclides with a very short half-life (less than 20 min) the control of the performance of the production system is an important measure to assure the quality of the radiopharmaceutical preparation before its release.

c) Any production procedure must be validated in test production runs before its use in routine manufacture of radiopharmaceutical preparations, to ensure that under specified production conditions, the production system yields the radiopharmaceutical preparation in the desired quantity and specified quality.

d) The preparation of the dosage form of the final radiopharmaceutical preparation in the practice of nuclear medicine generally involves limited radioactivity starting from ready-to-use radiopharmaceutical preparations, generators, kits and radioactive precursors. All conditions which may affect the quality of the product (e.g. radiochemical purity and sterility) must be clearly defined and must include appropriate measures for radiation protection.

IDENTIFICATION

Radioactive decay

Radioactivity decays at an exponential rate with a decay constant characteristic of each radionuclide.

The curve of exponential decay (decay curve) is described by the equation:

$$A_t = A_o e^{-\lambda t}$$

A_t = the radioactivity at time t,

A_o = the radioactivity at time $t = 0$,

λ = the decay constant characteristic of each radionuclide,

e = the base of Napierian logarithms.

The half-life ($T_{1/2}$) is related to the decay constant (λ) by the equation:

$$T_{1/2} = \frac{\ln 2}{\lambda} \qquad (\ln 2 \approx 0.693)$$

The radionuclide is generally identified by its half-life or by the nature and energy of its radiation or radiations or by both, as prescribed in the monograph.

Measurement of half-life

The half-life is measured with a suitable detection apparatus such as an ionisation chamber, a Geiger-Müller counter, a scintillation counter (solid crystal, liquid) or a semiconductor detector. The preparation to be tested is used as such or diluted or dried in a capsule after appropriate dilution. The radioactivity chosen, having regard to experimental conditions, must be of a sufficiently high level to allow detection during several estimated half-lives, but not too high to minimise count rate losses, for example due to dead time.

The radioactive source is prepared in a manner that will avoid loss of material during handling. If it is a liquid (solution), it is contained in bottles or sealed tubes. If it is a solid (residue from drying in a capsule), it is protected by a cover consisting of a sheet of adhesive cellulose acetate or of some other material.

The same source is measured in the same geometrical conditions and at intervals usually corresponding to half of the estimated half-life throughout a time equal to about three half-lives. The correct functioning of the apparatus is checked using a source of long half-life and, if necessary, corrections for any changes of the count rate have to be applied (see Measurement of Radioactivity).

A graph can be drawn with time as the abscissa and the logarithm of the relative instrument reading (e.g. count rate) as the ordinate. The calculated half-life differs by not more than 5 per cent from the half-life stated in the Pharmacopoeia, unless otherwise stated.

Determination of the nature and energy of the radiation

The nature and energy of the radiation emitted may be determined by several procedures including the construction of an attenuation curve and the use of spectrometry. The attenuation curve can be used for analysis of electron radiation; spectrometry is mostly used for identification of gamma rays and detectable X-rays.

The *attenuation curve* is drawn for pure electron emitters when no spectrometer for beta rays is available or for beta/gamma emitters when no spectrometer for gamma rays is available. This method of estimating the maximum energy of beta radiation gives only an approximate value. The source, suitably mounted to give constant geometrical conditions, is placed in front of the thin window of a Geiger-Müller counter or a proportional counter. The source is protected as described above. The count rate of the source is then measured. Between the source and the counter are placed, in succession, at least six aluminium screens of increasing mass per unit area within such limits that with a pure beta emitter this count rate is not affected by the addition of further screens. The screens are inserted in such a manner that constant geometrical conditions are maintained. A graph is drawn showing, as the abscissa, the mass per unit area of the screen expressed in milligrams per square centimetre and, as the ordinate, the logarithm of the count rate for each screen examined. A graph is drawn in the same manner for a standardised preparation. The mass attenuation coefficients are calculated from the median parts of the curves, which are practically rectilinear.

The *mass attenuation coefficient* μ_m, expressed in square centimetres per milligram, depends on the energy spectrum of the beta radiation and on the nature and the physical properties of the screen. It therefore allows beta emitters to be identified. It is calculated using the equation:

$$\mu_m = \frac{\ln A_1 - \ln A_2}{m_2 - m_1}$$

m_1 = mass per unit area of the lightest screen,

m_2 = mass per unit area of the heaviest screen, m_1 and m_2 being within the rectilinear part of the curve,

A_1 = count rate for mass per unit area m_1,

A_2 = count rate for mass per unit area m_2.

The mass attenuation coefficient μ_m thus calculated does not differ by more than 10 per cent from the coefficient obtained under identical conditions using a standardised preparation of the same radionuclide.

The range of beta particles is a further parameter which can be used for the determination of the beta energy. It is obtained from the graph described above as the mass per unit area corresponding to the intersection of the extrapolations of the descending rectilinear part of the attenuation curve and the horizontal line of background radioactivity.

Liquid scintillation counting may be used to obtain spectra of α and β^- emitters (see measurement of radioactivity).

Gamma spectrometry is used to identify radionuclides by the energy and intensity of their gamma rays and X-rays.

The preferred detector for gamma and X-ray spectrometry is a germanium semiconductor detector. A thallium-activated sodium iodide scintillation detector is also used but this has a much lower energy resolution.

The gamma detector has to be calibrated using standard sources because the detection efficiency is a function of the energy of the gamma and X-rays as well as the form of the source and the source-to-detector distance. The detection efficiency may be measured using a calibrated source of the radionuclide to be measured or, for more general work, a graph of efficiency against gamma and X-ray energy may be constructed from a series of calibrated sources of various radionuclides.

The gamma and X-ray spectrum of a radionuclide which emits gamma and X-rays is unique to that nuclide and is characterised by the energies and the number of photons of particular energies emitted per transformation from one energy level to another energy level. This property contributes to the identification of radionuclides present in a source and to their quantification. It allows the estimation of the degree of radionuclidic impurity by detecting peaks other than those expected.

It is possible to establish the rate of the decay of radioactivity using gamma spectrometry since the peaks diminish in amplitude as a function of the half-life. If, in such a source, a radioactive impurity with a different half-life is present, it is possible to detect the latter by identification of the characteristic peak or peaks whose amplitudes decrease at a different rate from that expected for the particular radionuclide. A determination of the half-life of the additional peaks by repeated measurements of the sample will help to identify the impurity.

The *Table of physical characteristics of radionuclides mentioned in the European Pharmacopoeia* (*5.7*) summarises the most commonly accepted physical characteristics of radionuclides used in preparations which are the subject of monographs in the European Pharmacopoeia. In addition, the Table states the physical characteristics of the main potential impurities of the radionuclides mentioned in the monographs.

By "transition probability" is meant the probability of the transformation of a nucleus in a given energy state, via the transition concerned. Instead of "probability" the terms "intensity" and "abundance" are frequently used.

By "emission probability" is meant the probability of an atom of a radionuclide giving rise to the emission of the particles or radiation concerned.

Irrespective of whether the one or the other meaning is intended, probability is usually measured in terms of 100 disintegrations.

MEASUREMENT OF RADIOACTIVITY

The radioactivity of a preparation is stated at a given date and, if necessary, time.

The absolute measurement of the radioactivity of a given sample may be carried out if the decay scheme of the radionuclide is known, but in practice many corrections are required to obtain accurate results. For this reason it is common to carry out the measurement with the aid of a primary standard source. Primary standards may not be available for short-lived radionuclides e.g. positron emitters. Measuring instruments are calibrated using suitable standards for the particular radionuclides. Standards are available from the laboratories recognised by the competent authority. Ionisation chambers and Geiger-Müller counters may be used to measure beta and beta/gamma emitters; scintillation or semiconductor counters or ionisation chambers may be used for measuring gamma emitters; low-energy beta emitters require a liquid-scintillation counter. For the detection and measurement of alpha emitters, specialised equipment and techniques are required. For an accurate comparison of radioactive sources, it is essential for samples and standards to be measured under similar conditions.

Low-energy beta emitters may be measured by liquid-scintillation counting. The sample is dissolved in a solution containing one or more often two organic fluorescent substances (primary and secondary scintillators), which convert part of the energy of disintegration into photons of light, which are detected by a photomultiplier and converted into electrical impulses. When using a liquid-scintillation counter, comparative measurements are corrected for light-quenching effects. Direct measurements are made, wherever possible, under similar conditions, (e.g. volumes and type of solutions) for the source to be examined and the standard source.

All measurements of radioactivity must be corrected by subtracting the background due to radioactivity in the environment and to spurious signals generated in the equipment itself.

With some equipment, when measurements are made at high levels of radioactivity, it may be necessary to correct for loss by coincidence due to the finite resolving time of the detector and its associated electronic equipment. For a counting system with a fixed dead time τ following each count, the correction is:

$$N = \frac{N_{obs}}{1 - N_{obs}\tau}$$

N = the true count rate per second,

N_{obs} = the observed count rate per second,

τ = the dead time, in seconds.

With some equipment this correction is made automatically. Corrections for loss by coincidence must be made before the correction for background radiation.

If the time of an individual measurement, t_m is not negligible short compared with the half-life, $T_{1/2}$, the decay during this measurement time must be taken into account. After having corrected the instrument reading (count rate, ionisation current, etc.) for background and, if necessary, for losses due to electronic effects, the decay correction during measurement time is:

$$R_{corr} = \frac{R\dfrac{t_m \ln 2}{T_{1/2}}}{1 - \exp\left(-\dfrac{t_m \ln 2}{T_{1/2}}\right)}$$

R_{corr} = instrument reading corrected to the beginning of the individual measurement,

R = instrument reading before decay correction, but already corrected for background, etc.

The results of determinations of radioactivity show variations which derive mainly from the random nature of nuclear transformation. A sufficient number of counts must be registered in order to compensate for variations in the number of transformations per unit of time. The standard deviation is the square root of the counts, so at least 10 000 counts are necessary to obtain a relative standard deviation of not more than 1 per cent (confidence interval: 1 sigma).

All statements of radioactive content are accompanied by a statement of the date and, if necessary, the time at which the measurement was made. This statement of the radioactive content must be made with reference to a time zone (GMT, CET). The radioactivity at other times may be calculated from the exponential equation or from tables.

The radioactivity of a solution is expressed per unit volume to give the radioactive concentration.

RADIONUCLIDIC PURITY

In most of the cases, to state the radionuclidic purity of a radiopharmaceutical preparation, the identity of every radionuclide present and their radioactivity must be known. The most generally useful method for examination of radionuclidic purity is that of gamma spectrometry. It is not a completely reliable method because alpha- and beta-emitting impurities are not usually easily detectable and, when sodium iodide detectors are employed, the peaks due to gamma emitting impurities are often obscured by the spectrum of the principal radionuclide.

The individual monographs prescribe the radionuclidic purity required (for example, the gamma-ray spectrum does not significantly differ from that of a standardised preparation) and may set limits for specific radionuclidic impurities (for example, cobalt-60 in cobalt-57). While these requirements are necessary, they are not in themselves sufficient to ensure that the radionuclidic purity of a preparation is sufficient for human use. The manufacturer must examine the product in detail and especially must examine preparations of radionuclides of short half-life for impurities of long half-life after a suitable period of decay. In this way, information on the suitability of the manufacturing processes and the adequacy of the testing procedures may be obtained. In cases where two or more positron emitting radionuclides need to be identified and/or differentiated, as e.g. 18F-impurities in 13N-preparations, half-life determinations need to be carried out in addition to gamma spectrometry.

Due to differences in the half-lives of the different radionuclides present in a radiopharmaceutical preparation, the radionuclidic purity changes with time. The requirement of the radionuclidic purity must be fulfilled throughout the period of validity. It is sometimes difficult to carry out these tests before authorising the release for use of the batch when the half-life of the radionuclide in the preparation is short. The test then constitutes a control of the quality of production.

RADIOCHEMICAL PURITY

The determination of radiochemical purity requires separation of the different chemical substances containing the radionuclide and estimating the percentage of radioactivity associated with the declared chemical substance. Radiochemical impurities may originate from:

- radionuclide production,
- subsequent chemical procedures,
- incomplete preparative separation,
- chemical changes during storage.

The requirement of the radiochemical purity must be fulfilled throughout the period of validity.

In principle, any method of analytical separation may be used in the determination of radiochemical purity. For example, the monographs for radiopharmaceutical products may include paper chromatography (*2.2.26*), thin-layer chromatography (*2.2.27*), electrophoresis (*2.2.31*), size-exclusion chromatography (*2.2.30*), gas chromatography (*2.2.28*) and liquid chromatography (*2.2.29*). The technical description of these analytical methods is set out in the monographs. Moreover certain precautions special to radioactivity must also be taken for radiation protection.

In a hospital environment thin-layer and paper chromatography are mostly used. In paper and thin-layer chromatography, a volume equal to that described in the monograph is deposited on the starting-line as prescribed in the general methods for chromatography. It is preferable not to dilute the preparation to be examined but it is important to avoid depositing such a quantity of radioactivity that counting losses by coincidence occur during measurement of the radioactivity. On account of the very small quantities of the radioactive material applied, a carrier may be added when specified in a particular monograph. After development, the support is dried and the positions of the radioactive areas are detected by autoradiography or by measurement of radioactivity over the length of the chromatogram, using suitable collimated counters or by cutting the strips and counting each portion. The positions of the spots or areas permit chemical identification by comparison with solutions of the same chemical substances (non-radioactive) using a suitable detection method.

Radioactivity may be measured by integration using an automatic-plotting instrument or a digital counter. The ratios of the areas under the peaks give the ratios of the radioactive concentration of the chemical substances. When the strips are cut into portions, the ratios of the quantities of radioactivity measured give the ratio of concentrations of the radioactive chemical species.

SPECIFIC RADIOACTIVITY

Specific radioactivity is usually calculated taking into account the radioactive concentration (radioactivity per unit volume) and the concentration of the chemical substance being studied, after verification that the radioactivity is attributable only to the radionuclide (radionuclidic purity) and the chemical species (radiochemical purity) concerned.

Specific radioactivity changes with time. The statement of the specific radioactivity therefore includes reference to a date and, if necessary, time. The requirement of the specific radioactivity must be fulfilled throughout the period of validity.

CHEMICAL PURITY

The determination of chemical purity requires quantification of the individual chemical impurities specified in the monograph.

ENANTIOMERIC PURITY

Where appropriate, the stereoisomeric purity has to be verified.

PHYSIOLOGICAL DISTRIBUTION

A physiological distribution test is prescribed, if necessary, for certain radiopharmaceutical preparations. The distribution pattern of radioactivity observed in specified organs, tissues or other body compartments of an appropriate animal species (usually rats or mice) can be a reliable indication of the expected distribution in humans and thus of the suitability for the intended purpose.

The individual monograph prescribes the details concerning the performance of the test and the physiological distribution requirements which must be met for the radiopharmaceutical preparation. A physiological distribution conforming to the requirements will assure appropriate distribution of the radioactive compounds to the intended biological target in humans and limits its distribution to non-target areas.

In general, the test is performed as follows.

Each of three animals is injected intravenously with the preparation to be tested. If relevant, the species, sex, strain and weight and/or age of the animals is specified in the monograph. The test injection is the radiopharmaceutical preparation as it is intended for human use. Where applicable, products are reconstituted according to the manufacturer's instructions. In some cases, dilution immediately before injection may be necessary.

The administration will normally be made via the intravenous route for which purpose the caudal vein is used. Other veins such as the saphenous, femoral, jugular or penile veins may be used in special cases. Animals showing evidence of extravasation of the injection (observed at the time of injection or revealed by subsequent assay of tissue radioactivity) are rejected from the test.

Immediately after injection each animal is placed in a separate cage which will allow collection of excreta and prevent contamination of the body surface of the animal.

At the specified time after injection, the animals are euthanised by an appropriate method and dissected. Selected organs and tissues are assayed for their radioactivity using a suitable instrument as described elsewhere in this monograph. The physiological distribution is then calculated and expressed in terms of the percentage of the radioactivity which is found in each of the selected organs or tissues. For this purpose the radioactivity in an organ may be related to the injected radioactivity calculated from the radioactive content of the syringe measured before and after injection. For some radiopharmaceutical preparations it may be appropriate to determine the ratio of the radioactivity in weighed samples of selected tissues (radioactivity/mass).

For a preparation to meet the requirements of the test, the distribution of radioactivity in at least two of the three animals must comply with all the specified criteria.

STERILITY

Radiopharmaceutical preparations for parenteral administration must be prepared using precautions designed to exclude microbial contamination and to ensure sterility. The test for sterility is carried out as described in the general method for sterility (*2.6.1*). Special difficulties arise with radiopharmaceutical preparations because of the short half-life of some radionuclides small size of batches and the radiation hazards. It is not always possible to await the results of the test for sterility before authorisation of the release for use of the batch concerned. Parametric release (*5.1.1*) of the product manufactured by a fully validated process is the method of choice in such cases. When aseptic manufacturing is used, the test for sterility has to be executed as a control of the quality of production.

When the size of a batch of the radiopharmaceutical preparation is limited to one or a few samples (e.g. therapeutic or very short-lived radiopharmaceutical preparation), sampling the batch for sterility testing may not be applicable. If the radiopharmaceutical preparation is sterilised by filtration and/or aseptically processed (*5.1.1*) process validation is critical.

When the half-life of the radionuclide is very short (e.g. less than 20 min), the administration of the radiopharmaceutical preparation to the patient is generally on-line with a validated production system.

For safety reasons (high level of radioactivity) it is not possible to use the quantity of the radiopharmaceutical preparations as required in the test for sterility (*2.6.1*). The method by membrane filtration is to be preferred to limit irradiation of personnel.

Notwithstanding the requirements concerning the use of antimicrobial preservatives in *Parenteral preparations (0520)*, their addition to radiopharmaceutical preparations in multidose containers is not obligatory, unless prescribed in the monograph.

BACTERIAL ENDOTOXINS - PYROGENS

For certain radiopharmaceutical preparations a test for bacterial endotoxins is prescribed. The test is carried out as described in the general method (*2.6.14*), taking the necessary precautions to limit irradiation of the personnel carrying out the test.

The limit for bacterial endotoxins is indicated in the individual monograph.

When the nature of the radiopharmaceutical preparation results in an interference by inhibition or activation and it is not possible to eliminate the interfering factor(s), the test for pyrogens (*2.6.8*) may be specifically prescribed.

It is sometimes difficult to carry out these tests before releasing the batch for use when the half-life of the radionuclide in the preparation is short. The test then constitutes a control of the quality of production.

STORAGE

Store in an airtight container in a place that is sufficiently shielded to protect personnel from irradiation by primary or secondary emissions and that complies with national and international regulations concerning the storage of radioactive substances. During storage, containers may darken due to irradiation. Such darkening does not necessarily involve deterioration of the preparations.

Radiopharmaceutical preparations are intended for use within a short time and the end of the period of validity must be clearly stated.

LABELLING

The labelling of radiopharmaceutical preparations complies with the relevant national and European legislation.

The label on the direct container states:

— the name of the preparation and/or its reference,

— the name of the manufacturer,

— an identification number,

— for liquid and gaseous preparations: the total radioactivity in the container, or the radioactive concentration per millilitre at a stated date and, if necessary, time, and the volume of liquid in the container,

— for solid preparations (such as freeze-dried preparations): the total radioactivity at a stated date and, if necessary, time. After reconstitution with the appropriate solution, the preparation is considered as a liquid preparation,

— for capsules: the radioactivity per capsule at a stated date and, if necessary, time and the number of capsules in the container.

The labelling can be adapted in certain cases (e.g. radiopharmaceutical preparations containing short-lived radionuclides).

In addition, the label on the outer package states:

— the route of administration,

— the period of validity or the expiry date,

— the name and concentration of any added antimicrobial preservative,

— where applicable, any special storage conditions.

Ph Eur

Sodium Acetate ([1-^{11} C]) Injection

General Notices

(Ph Eur monograph 1920)

$CH_3{}^{11}COONa$

Ph Eur

DEFINITION

Sterile solution of sodium [1-^{11}C]acetate, in equilibrium with [1-^{11}C]acetic acid.

Content

90 per cent to 110 per cent of the declared carbon-11 radioactivity at the date and time stated on the label.

PRODUCTION

RADIONUCLIDE PRODUCTION

Carbon-11 is a radioactive isotope of carbon which is most commonly produced by proton irradiation of nitrogen. By the addition of trace amounts of oxygen, the radioactivity is obtained as [^{11}C]carbon dioxide.

RADIOCHEMICAL SYNTHESIS

[^{11}C]Carbon dioxide may be separated from the target gas mixture by cryogenic trapping or by trapping on a molecular sieve at room temperature. [^{11}C]Carbon dioxide is then released from the trap using an inert gas such as nitrogen at a temperature higher than the trapping temperature.

[1-^{11}C]Acetate is usually prepared by reaction of [^{11}C]carbon dioxide with methylmagnesium bromide in organic solvents such as ether or tetrahydrofuran.

Hydrolysis of the product yields [1-^{11}C]acetic acid. It is purified by chromatographic procedures. The eluate is diluted with sodium chloride solution.

PRECURSOR FOR SYNTHESIS

Methylmagnesium bromide

The reactivity of methylmagnesium bromide is tested by decomposition of a defined amount with water. The amount of methane released during this reaction is not less than 90 per cent of the theoretical value.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of carbon-11

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with test B for radionuclidic purity (see Tests).

C. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

4.5 to 8.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/*V* IU/ml, V being the maximum recommended dose in millilitres. The injection may be released for use before completion of the test.

CHEMICAL PURITY

Acetate

Liquid chromatography (*2.2.29*).

Test solution The preparation to be examined.

Reference solution Dissolve 28 mg of *sodium acetate R* in *water R* and dilute to *V*, *V* being the maximum recommended dose in millilitres.

Column:

- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *strongly basic anion exchange resin for chromatography R* (10 µm);
- *temperature*: 25 °C.

Mobile phase *0.1 M sodium hydroxide* protected from atmospheric carbon dioxide.

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm and radioactivity detector connected in series.

Injection Loop injector.

Run time 10 min.

System suitability Reference solution:

- *resolution*: minimum 4.0 between the peaks due to hold-up volume and acetate.

Limit Examine the chromatograms obtained with the spectrophotometer:

- *acetate*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (20 mg per *V*).

Residual solvents

are limited according to the principles defined in the general chapter (*5.4*), using the general method (*2.4.24*). The preparation may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Carbon-11

Minimum 99 per cent of the total radioactivity.

The preparation may be released for use before completion of the tests.

A. Gamma-ray spectrometry.

Comparison Standardised fluorine-18 solution, or by using a calibrated instrument. Standardised fluorine-18 solutions and/or standardisation services are available from laboratories recognised by the competent authority.

Results The spectrum obtained with the solution to be examined does not differ significantly from that obtained with a standardised fluorine-18 solution.

B. Half-life: 19.9 min to 20.9 min.

RADIOCHEMICAL PURITY

[1-^{11}C]Acetate

Liquid chromatography (*2.2.29*) as described in the test for acetate.

Limit Examine the chromatograms obtained with the spectrophotometer and the radioactivity detector:

- *total of [1-^{11}C]acetate*: minimum 95 per cent of the total radioactivity.

RADIOACTIVITY

Measure the radioactivity using suitable equipment by comparison with a standardised fluorine-18 solution or by measurement with a calibrated instrument.

LABELLING

The accompanying information specifies the maximum recommended dose in millilitres.

Ph Eur

Sodium Chromate [^{51} Cr] Sterile Solution

General Notices

(Ph Eur monograph 0279)

Ph Eur

DEFINITION

Sodium chromate (^{51}Cr) sterile solution is a sterile solution of sodium [^{51}Cr]chromate made isotonic by the addition of sodium chloride. Chromium-51 is a radioactive isotope of chromium and may be prepared by neutron irradiation of chromium, either of natural isotopic composition or enriched in chromium-50. The solution contains not less than 90.0 per cent and not more than 110.0 per cent of the declared chromium-51 radioactivity at the date and hour stated on the label. Not less than 90 per cent of the radioactivity corresponds to chromium-51 in the form of chromate. The specific radioactivity is not less than 370 MBq of chromium-51 per milligram of chromate ion.

CHARACTERS

A clear, colourless or slightly yellow solution.

Chromium-51 has a half-life of 27.7 days and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised chromium-51 solution. Standardised chromium-51 solution are available from laboratories recognised by the competent authority. The gamma photon has an energy of 0.320 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the solution is 6.0 to 8.5.

Total chromate

Not more than 2.7 µg of chromate ion (CrO_4^{2-}) per megabecquerel. Measure the absorbance of the solution (*2.2.25*) at the absorption maximum at 370 nm. Calculate the content of chromate using the absorbance of a standard consisting of a 1.7 mg/l solution of *potassium chromate R* . If necessary, adjust the solution to be examined and the standard to pH 8.0 by adding *sodium hydrogen carbonate solution R.*

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The solution may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised chromium-51 solution.

RADIOCHEMICAL PURITY

Examine by ascending paper chromatography (*2.2.26*).

Apply to the paper a quantity of the solution sufficient for the detection method. Begin the development immediately and develop for 2.5 h using a mixture of 25 volumes of *ammonia R*, 50 volumes of *alcohol R* and 125 volumes of *water R*. Chromic ions remain on the starting line. Determine the distribution of the radioactivity using a suitable detector. Not less than 90 per cent of the total radioactivity of the chromatogram is found in the spot with an R_F value of about 0.9, corresponding to sodium chromate.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised chromium-51 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Sodium Fluoride [^{18} F] Injection

General Notices

(Ph Eur monograph 2100)

Ph Eur

DEFINITION

Sterile solution containing fluorine-18 in the form of sodium fluoride. It may contain carrier fluoride and a suitable buffer.

Content

— *fluorine-18*: 90 per cent to 110 per cent of the declared fluorine-18 radioactivity at the date and hour stated on the label,

— *fluoride*: maximum 4.52 mg per maximum recommended dose in millilitres.

PRODUCTION

The radionuclide fluorine-18 is most commonly produced by proton irradiation of water enriched in oxygen-18. Fluorine-18 in the form of fluoride is recovered from the target water, generally by adsorption and desorption from anion-exchange resins or electrochemical deposition and redissolution.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of fluorine-18

See *Table of physical characteristics of radionuclides (5.7)*.

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with test B for radionuclidic purity (see Tests).

C. Examine the chromatograms obtained in the test for radiochemical purity (see Tests).

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution. In the chromatogram obtained with the reference solution, the peak due to fluoride is negative.

TESTS

pH *(2.2.3)*

5.0 to 8.5.

Fluoride

Liquid chromatography *(2.2.29)* .

Test solution The preparation to be examined.

Reference solution Dissolve 10 mg of *sodium fluoride R* in *water R* and dilute to *V* with the same solvent, *V* being the maximum recommended dose in millilitres.

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm,

— *stationary phase*: *strongly basic anion-exchange resin for chromatography R* (10 µm),

— *temperature*: constant, between 20 °C and 30 °C.

Mobile phase 4 g/l solution of *sodium hydroxide R*, protected from atmospheric carbon dioxide.

Flow rate 1 ml/min.

Detection Spectrophotometer at 220 nm and a radioactivity detector connected in series.

Injection 20 µl.

Run time 15 min.

System suitability Examine the chromatogram obtained with the reference solution using the spectrophotometer:

— *signal-to-noise ratio* : minimum 10 for the principal peak,

— *retention time of fluoride*: minimum 3 times the hold-up time.

Limit Examine the chromatogram obtained with the spectrophotometer:

— *fluoride*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (4.52 mg/*V*).

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

Bacterial endotoxins *(2.6.14)*

Less than 175/*V* IU/ml, *V* being the maximum recommended dose in millilitres. The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Fluorine-18

Minimum 99.9 per cent of the total radioactivity.

The preparation may be released for use before completion of the tests.

A. Gamma-ray spectrometry.

Determine the amount of fluorine-18 and radionuclidic impurities with a half-life longer than 2 h. For the detection and quantification of impurities, retain the preparation to be examined for a sufficient time to allow the fluorine-18 to decay to a level which permits the detection of impurities.

Results The spectrum obtained with the preparation to be examined does not differ significantly from that of a background spectrum.

B. Half-life: 105 min to 115 min.

RADIOCHEMICAL PURITY

[^{18}F]fluoride

Liquid chromatography *(2.2.29)* as described in the test for fluoride. If necessary, dilute the test solution with *water R* to obtain a radioactivity concentration suitable for the radioactivity detector.

Limit Examine the chromatogram obtained with the radioactivity detector:

— *[^{18}F]fluoride*: minimum 98.5 per cent of the total radioactivity.

RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

LABELLING

The label states the maximum recommended dose in millilitres.

Ph Eur

Sodium Iodide [^{123} I] Injection

General Notices

(Ph Eur monograph 0563)

Ph Eur

DEFINITION

Sterile solution containing iodine-123 in the form of sodium iodide; it may contain sodium thiosulphate or some other suitable reducing agent and a suitable buffer.

Content

90 per cent to 110 per cent of the declared iodine-123 radioactivity at the date and hour stated on the label.

PRODUCTION

Iodine-123 is obtained by proton irradiation of xenon enriched in xenon-124 (minimum 98 per cent) followed by the decay of xenon-123 which is formed directly and by the decay of caesium-123. No carrier iodide is added.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of iodine-123

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The spectrum obtained with the preparation to be examined does not differ significantly from that of a standardised iodine-123 solution. The most prominent gamma photon has an energy of 0.159 MeV and is accompanied by the principal X-ray of 0.027 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference

solution (a).

TESTS

pH (*2.2.3*)

7.0 to 10.0.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The preparation may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Iodine-123

Minimum 99.65 per cent of the total radioactivity.

Gamma-ray spectrometry.

Determine the relative amounts of iodine-123, iodine-125, tellurium-121 and other radionuclidic impurities present. For the detection of tellurium-121 and iodine-125, retain the preparation to be examined for a sufficient time to allow iodine-123 to decay to a level which permits the detection of radionuclidic impurities. No radionuclides with a half-life longer than that of iodine-125 are detected.

The preparation may be released for use before completion of the test.

RADIOCHEMICAL PURITY

[^{123}I]IODIDE

Liquid chromatography (2.2.29).

Test solution Dilute the preparation to be examined with a 2 g/l solution of *sodium hydroxide R* to a radioactive concentration suitable for the detector. Add an equal volume of a solution containing 1 g/l of *potassium iodide R* , 2 g/l of *potassium iodate R* and 10 g/l of *sodium hydrogen carbonate R* and mix.

Reference solution (a) Dilute 1 ml of a 26.2 mg/l solution of *potassium iodide R* to 10 ml with *water R* .

Reference solution (b) Dilute 1 ml of a 24.5 mg/l solution of *potassium iodate R* to 10 ml with *water R* . Mix equal volumes of this solution and reference solution (a).

Column:

— *size:* *l* = 0.25 m, Ø = 4.0 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: constant between 20 °C and 30 °C.

Mobile phase Dissolve 5.85 g of *sodium chloride R* in 1000 ml of *water R* , add 0.65 ml of *octylamine R* and adjust to pH 7.0 with *dilute phosphoric acid R* ; add 50 ml of *acetonitrile R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm and a radioactivity detector connected in series.

Injection 20 µl.

Run time 12 min.

Relative retention With reference to iodide (retention time = about 5 min): iodate = 0.2 to 0.3.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to iodide and iodate in the chromatogram recorded with the spectrophotometer.

Limit Examine the chromatogram obtained with the test solution using the radioactivity detector and locate the peak due to iodide by comparison with the chromatogram obtained with reference solution (a) using the spectrophotometer:

— *[^{123}I]iodide*: minimum 95 per cent of the total radioactivity.

RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

LABELLING

The label states any substance added.

IMPURITIES

A. [^{123}I]iodate ion.

Ph Eur

Sodium Iodide [^{123} I] Solution for Radiolabelling

General Notices

(Ph Eur monograph 2314)

Ph Eur

DEFINITION

Strongly alkaline solution containing iodine-123 in the form of sodium iodide.

Content

90 per cent to 110 per cent of the declared iodine-123 radioactivity at the date and hour stated on the label.

PRODUCTION

Iodine-123 is obtained by proton irradiation of xenon highly enriched in xenon-124 followed by the decay of directly formed xenon-123 and by the decay of caesium-123. No carrier iodide or reducing agents are added.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of iodine-123

See general chapter *5.7*. *Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The most prominent gamma photon of iodine-123 has an energy of 0.159 MeV and is accompanied by the principal X-ray of 0.027 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity (see Tests).

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Alkalinity (*2.2.4*)

The solution is strongly alkaline.

RADIONUCLIDIC PURITY

Iodine-123

Minimum 99.7 per cent of the total radioactivity.

Gamma-ray spectrometry.

Determine the relative amounts of iodine-123, iodine-125, tellurium-121 and other radionuclidic impurities present. For the detection of tellurium-121 and iodine-125, retain the solution to be examined for a sufficient time to allow iodine-123 to decay to a level which permits the detection of radionuclidic impurities. No radionuclides with a half-life longer than that of iodine-125 are detected.

The solution may be released for use before completion of the test.

RADIOCHEMICAL PURITY

$[^{123}I]$iodide

Liquid chromatography (*2.2.29*).

Test solution Dilute the solution to be examined with an equal volume of a solution containing 1 g/l of *potassium iodide R* , 2 g/l of *potassium iodate R* and 10 g/l of *sodium hydrogen carbonate R* and mix. If necessary, first dilute the solution to be examined with a 2 g/l solution of *sodium hydroxide R* to ensure that the final mixture has a radioactivity concentration suitable for the radioactivity detector.

Reference solution (a) Dissolve 10 mg of *potassium iodide R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *potassium iodate R* in *water R* and dilute to 10 ml with the same solvent. Mix equal volumes of this solution and reference solution (a).

Column:

- *size: l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: constant, between 20 °C and 30 °C.

Use stainless steel tubing.

Mobile phase Dissolve 5.85 g of *sodium chloride R* in 1000 ml of *water R* , add 0.65 ml of *octylamine R* and adjust to pH 7.0 with *dilute phosphoric acid R* ; add 50 ml of *acetonitrile R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm and a radioactivity detector connected in series.

Injection 20 µl.

Run time 12 min.

Relative retention With reference to iodide (retention time = about 5 min): iodate = 0.2 to 0.3.

System suitability Reference solution (b):

— *resolution*: minimum 2 between the peaks due to iodide and iodate in the chromatogram recorded with the spectrophotometer.

Examine the chromatogram obtained with the test solution using the radioactivity detector and locate the peak due to iodide by comparison with the chromatogram obtained with reference solution (a) using the spectrophotometer.

Limit:
— *[^{123}I]iodide*: minimum 95 per cent of the total radioactivity.

RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

LABELLING

The label states:

— the vehicle and any substance added;

— that the solution is not for direct administration to humans.

IMPURITIES

A. iodine-125,

B. tellurium-121,

C. [^{123}I]iodate ion.

Ph Eur

Sodium Iodide [^{131} I] Capsules for Diagnostic Use

General Notices

(Ph Eur monograph 0938)

Ph Eur

DEFINITION

Capsules for diagnostic use containing iodine-131 in the form of sodium iodide on a solid support; they may contain sodium thiosulphate or some other suitable reducing agents and a suitable buffering substance. A package contains 1 or more capsules.

Content:

— iodine-131: maximum 37 MBq per capsule; the average radioactivity determined in the test for uniformity of content is 90 per cent to 110 per cent of the declared iodine-131 radioactivity at the date and hour stated on the label;

— iodide: maximum 20 µg per capsule.

PRODUCTION

Iodine-131 is obtained by neutron irradiation of tellurium or by extraction from uranium fission products. No carrier iodide is added.

CHARACTERS

Half-life and nature of radiation of iodine-131

See general chapter *5.7*. Table of physical characteristics of radionuclides.

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The spectrum obtained with the preparation to be examined does not differ significantly from that of a standardised iodine-131 solution. The most prominent gamma photon has an energy of 0.365 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peak in the radiochromatogram obtained with test solution (b) is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Disintegration

The contents of the capsule dissolve completely within 15 min.

In a water-bath at 37 °C, warm in a small beaker about 20 ml of a 2.0 g/l solution of *potassium iodide R*. Add a capsule to be examined. Stir magnetically at 20 r/min.

Uniformity of content

Determine the radioactivity of each of not fewer than 10 capsules. Calculate the average radioactivity per capsule. The radioactivity of no capsule differs by more than 10 per cent from the average, the relative standard deviation is not greater than 3.5 per cent.

Iodide

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve a capsule to be examined in 10 ml of *water R*. Filter through a 0.2 µm filter.

Test solution (b) Dissolve a capsule to be examined in *water R*. Filter through a 0.2 µm filter and dilute the filtrate with a 2 g/l solution of *sodium hydroxide R* to a radioactive concentration suitable for the detector. Add an equal volume of a solution containing 1 g/l of *potassium iodide R*, 2 g/l of *potassium iodate R* and 10 g/l of *sodium hydrogen carbonate R* and mix.

Reference solution (a) Dilute 1 ml of a 26.2 mg/l solution of *potassium iodide R* to 10 ml with *water R.*

Reference solution (b) Dilute 1 ml of a 24.5 mg/l solution of *potassium iodate R* to 10 ml with *water R*. Mix equal volumes of this solution and reference solution (a).

Blank solution Prepare a solution containing 2 mg/ml of each constituent stated on the label, apart from iodide.

Column:
- *size:* *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: constant between 20 °C and 30 °C.

Mobile phase Dissolve 5.85 g of *sodium chloride R* in 1000 ml of *water R*, add 0.65 ml of *octylamine R* and adjust to pH 7.0 with *dilute phosphoric acid R*; add 50 ml of *acetonitrile R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm and radioactivity detector connected in series.

Injection 20 µl of test solution (a), reference solutions (a) and (b) and the blank solution.

Run time 12 min.

Relative retention With reference to iodide (retention time = about 5 min): iodate = 0.2 to 0.3.

System suitability:

— in the chromatogram obtained with the blank solution, none of the peaks has a retention time similar to that of the peak due to iodide,

— *resolution*: minimum 2 between the peaks due to iodide and iodate in the chromatogram obtained with reference solution (b) recorded with the spectrophotometer.

Limit Examine the chromatograms obtained with the spectrophotometer:

— *iodide*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (20 µg/capsule).

RADIONUCLIDIC PURITY

Iodine-131

Minimum 99.9 per cent of the total radioactivity.

Gamma-ray spectrometry.

Determine the relative amounts of iodine-131, iodine-133, iodine-135 and other radionuclidic impurities present.

RADIOCHEMICAL PURITY

[^{131}I]iodide

Liquid chromatography (*2.2.29*) as described in the test for iodide with the following modifications.

Injection 20 µl of test solution (b) and reference solution (a).

Limit Examine the chromatogram obtained with the test solution using the radioactivity detector and locate the peak due to iodide by comparison with the chromatogram obtained with reference solution (a) using the spectrophotometer:

— *[^{131}I]iodide*: minimum 95 per cent of the total radioactivity.

RADIOACTIVITY

Determine the radioactivity of the package using a calibrated instrument.

LABELLING

The label states any substance added and the number of capsules in the package.

IMPURITIES

A. [^{131}I]iodate ion.

Ph Eur

Sodium Iodide [^{131} I] Capsules for Therapeutic Use

General Notices

(Ph Eur monograph 2116)

Ph Eur

DEFINITION

Capsules for therapeutic use containing iodine-131 in the form of sodium iodide on a solid support; they contain sodium thiosulphate or some other suitable reducing agents and a suitable buffering substance.

Content:

— iodine-131: 90 per cent to 110 per cent of the declared radioactivity at the date and hour stated on the label,

— iodide: maximum 20 µg per capsule.

PRODUCTION

Iodine-131 is obtained by neutron irradiation of tellurium or by extraction from uranium fission products. No carrier iodide is added.

CHARACTERS

Half-life and nature of radiation of iodine-131

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The spectrum obtained with the preparation to be examined does not differ significantly from that of a standardised iodine-131 solution. The most prominent gamma photon of iodine-131 has an energy of 0.365 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity.

Results The principal peak in the radiochromatogram obtained with test solution (b) is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Disintegration

The contents of the capsule dissolve completely within 15 min.

In a water-bath at 37 °C, warm in a small beaker about 20 ml of a 2.0 g/l solution of *potassium iodide R* . Add a capsule to be examined. Stir magnetically at a rotation rate of 20 r/min.

Iodide

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve a capsule to be examined in 10 ml of *water R* . Filter through a 0.2 µm filter.

Test solution (b) Dissolve a capsule to be examined in *water R* . Filter through a 0.2 µm filter and dilute the filtrate with an equal volume of a solution containing 1 g/l of *potassium iodide R* , 2 g/l of *potassium iodate R* and 10 g/l of *sodium hydrogen carbonate R.* If necessary, first dilute the filtrate with a 2 g/l solution of *sodium hydroxide R* to ensure that the final mixture has a radioactivity concentration suitable for the radioactivity detector.

Reference solution (a) Dilute 1.0 ml of a 26.2 mg/l solution of *potassium iodide R* to 10.0 ml with *water R* .

Reference solution (b) Dilute 1 ml of a 24.5 mg/l solution of *potassium iodate R* to 10 ml with *water R* . Mix equal volumes of this solution with reference solution (a).

Blank solution Prepare a solution containing 2 mg/ml of each added substance stated on the label, apart from iodide.

Column:

- *size:* *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: constant, between 20 °C and 30 °C.

Use stainless steel tubing.

Mobile phase Dissolve 5.85 g of *sodium chloride R* in 1000 ml of *water R* , add 0.65 ml of *octylamine R* and adjust to pH 7.0 with *dilute phosphoric acid R* ; add 50 ml of *acetonitrile R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm and radioactivity detector connected in series.

Injection 20 µl of test solution (a), reference solutions (a) and (b) and the blank solution.

Run time 12 min.

Relative retention With reference to iodide (retention time = about 5 min): iodate = 0.2 to 0.3.

System suitability:

- in the chromatogram obtained with the blank solution, none of the peaks has a retention time similar to that of the peak due to iodide;
- *resolution*: minimum 2 between the peaks due to iodide and iodate in the chromatogram

obtained with reference solution (b) using the spectrophotometer.

Limits Examine the chromatograms obtained with the spectrophotometer; locate the peak due to iodide by comparison with the chromatogram obtained with reference solution (a):

— *iodide*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (20 µg/capsule).

RADIONUCLIDIC PURITY

Iodine-131

Minimum 99.9 per cent of the total radioactivity.

Gamma-ray spectrometry.

Determine the relative amounts of iodine-130, iodine-131, iodine-133, iodine-135 and other radionuclidic impurities present.

RADIOCHEMICAL PURITY

[^{131}I]iodide

Liquid chromatography (*2.2.29*) as described in the test for iodide with the following modifications.

Injection 20 µl of test solution (b) and reference solution (a).

Limits Examine the chromatogram obtained with test solution (b) using the radioactivity detector and locate the peak due to iodide by comparison with the chromatogram obtained with reference solution (a) using the spectrophotometer:

— *[^{131}I]iodide*: minimum 95 per cent of the total radioactivity.

RADIOACTIVITY

Determine the radioactivity of each capsule using a calibrated instrument.

LABELLING

The label states any substance added.

IMPURITIES

A. [^{131}I]iodate ion,

B. iodine-130,

C. iodine-133,

D. iodine-135.

Sodium Iodide [^{131} I] Solution For Radiolabelling

General Notices

(Ph Eur monograph 2121)

Ph Eur

DEFINITION

Strongly alkaline solution containing iodine-131 in the form of sodium iodide. It does not contain a reducing agent.

Content

90 per cent to 110 per cent of the declared iodine-131 radioactivity at the date and hour stated on the label.

PRODUCTION

Iodine-131 may be obtained by neutron irradiation of tellurium or by extraction from uranium fission products. No carrier iodide is added.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of iodine-131

See *Table of physical characteristics of radionuclides (5.7)*.

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The spectrum obtained with the preparation to be examined does not differ significantly from that of a standardised iodine-131 solution. The most prominent gamma photon of iodine-131 has an energy of 0.365 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity (see Tests).

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Alkalinity *(2.2.4)*

The preparation is strongly alkaline.

RADIONUCLIDIC PURITY

Iodine-131

Minimum 99.9 per cent of the total radioactivity.

Gamma-ray spectrometry.

Determine the relative amounts of iodine-130, iodine-131, iodine-133, iodine-135 and other radionuclidic impurities present.

RADIOCHEMICAL PURITY

[^{131}I]iodide

Liquid chromatography *(2.2.29)* .

Test solution Dilute the preparation to be examined with an equal volume of a solution containing 1 g/l of *potassium iodide R*, 2 g/l of *potassium iodate R* and 10 g/l of *sodium hydrogen carbonate R* and mix. If necessary, first dilute the preparation to be examined with a 2 g/l solution of *sodium hydroxide R* to ensure that the final mixture has a radioactivity concentration suitable for the radioactivity detector.

Reference solution (a) Dissolve 10 mg of *potassium iodide R* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b) Dissolve 20 mg of *potassium iodate R* in *water R* and dilute to 10 ml with the same solvent. Mix equal volumes of this solution and reference solution (a).

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: constant, between 20 °C and 30 °C.

Use stainless steel tubing.

Mobile phase Dissolve 5.85 g of *sodium chloride R* in 1000 ml of *water R*, add 0.65 ml of *octylamine R* and adjust to pH 7.0 with *dilute phosphoric acid R*; add 50 ml of *acetonitrile R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm and a radioactivity detector connected in series.

Injection 20 µl.

Run time 12 min.

Relative retention with reference to iodide (retention time = about 5 min): iodate = 0.2 to 0.3.

System suitability Reference solution (b):

- *resolution*: minimum 2 between the peaks due to iodide and iodate in the chromatogram recorded with the spectrophotometer.

Limit Examine the chromatogram obtained with the radioactivity detector:

- *[^{131}I]iodide*: minimum 95 per cent of the total radioactivity.

RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

LABELLING

The label states:

- the method of production of iodine-131,
- the vehicle and any substance added,
- that the preparation is not for direct human use.

IMPURITIES

A. [^{131}I]iodate ion.

Ph Eur

Sodium Iodide [^{131} I] Solution

General Notices

(Ph Eur monograph 0281)

Ph Eur

DEFINITION

Solution containing iodine-131 in the form of sodium iodide and also sodium thiosulphate or some other suitable reducing agent. It may contain a suitable buffer.

Content:

— *iodine-131*: 90 per cent to 110 per cent of the declared radioactivity at the date and hour stated on the label,

— *iodide*: maximum 20 µg in the maximum recommended dose in millilitres.

PRODUCTION

Iodine-131 is a radioactive isotope of iodine and may be obtained by neutron irradiation of tellurium or by extraction from uranium fission products. No carrier iodide is added.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of iodine-131

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The spectrum obtained with the preparation to be examined does not differ significantly from that of a standardised iodine-131 solution. The most prominent gamma photon has an energy of 0.365 MeV.

B. Examine the chromatograms obtained in the test for iodide.

Results The principal peak in the radiochromatogram obtained with test solution (a) is similar in retention time to the principal peak in the chromatogram obtained with reference

solution (a).

TESTS

pH (*2.2.3*)

7.0 to 10.0.

Sterility

If intended for parenteral use, it complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The solution may be released for use before completion of the test.

Iodide

Liquid chromatography (*2.2.29*).

Test solution (a) The preparation to be examined.

Test solution (b) Dilute the preparation to be examined with *0.05 M sodium hydroxide* until the radioactivity is equivalent to about 74 MBq/ml. Add an equal volume of a solution containing 1 g/l of *potassium iodide R*, 2 g/l of *potassium iodate R* and 10 g/l of *sodium hydrogen carbonate R* and mix.

Reference solution (a) Dilute 1 ml of a 26.2 mg/l solution of *potassium iodide R* to *V* with *water R*, *V* being the maximum recommended dose in millilitres.

Reference solution (b) Dilute 1 ml of a 24.5 mg/l solution of *potassium iodate R* to *V* with *water R*, *V* being the maximum recommended dose in millilitres. Mix equal volumes of this solution and of reference solution (a).

Blank solution Prepare a solution containing 2 mg/ml of each of the components stated on the label, apart from iodide.

Column:

- *size:* *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: maintain at a constant temperature between 20 °C and 30 °C.

Use stainless steel tubing.

Mobile phase Dissolve 5.844 g of *sodium chloride R* in 1000 ml of *water R*, add 650 µl of *octylamine R* and adjust to pH 7.0 with *phosphoric acid R*; add 50 ml of *acetonitrile R* and mix.

Flow rate 1.5 ml/min.

Detection Spectrophotometer at 220 nm and radioactivity detector connected in series.

Injection 25 µl; inject test solution (a), the blank solution and reference solutions (a) and (b).

Run time 12 min.

Relative retention With reference to iodide (retention time = about 5 min): iodate = 0.2 to 0.3.

System suitability:

- in the chromatogram obtained with the blank solution, none of the peaks shows a retention time similar to that of the peak due to iodide,
- *resolution*: minimum 2 between the peaks due to iodide and iodate in the chromatogram obtained with reference solution (b) recorded with the spectrophotometer.

Limit Examine the chromatogram obtained with the spectrophotometer; locate the peak due to iodide by comparison with the chromatogram obtained with reference solution (a):

- *iodide*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a).

RADIONUCLIDIC PURITY

Iodine-131

Minimum 99.9 per cent of the total radioactivity.

Gamma-ray spectrometry.

Determine the relative amounts of iodine-131, iodine-133, iodine-135 and other radionuclidic impurities present.

RADIOCHEMICAL PURITY

[^{131}I]Iodide

Liquid chromatography (*2.2.29*) as described in the test for iodide with the following modification.

Injection Test solution (b).

Limit Examine the chromatogram obtained with the radioactivity detector:

- *[^{131}I]iodide*: minimum 95 per cent of the total radioactivity.

RADIOACTIVITY

Measure the radioactivity using suitable equipment by comparison with a standardised iodine-131 solution or by using a calibrated instrument.

LABELLING

The label states:

- any substance added,
- the maximum recommended dose, in millilitres,
- where applicable, that the preparation is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES

A. [131I]iodate ion.

Ph Eur

Sodium Iodohippurate [^{123} I] Injection

General Notices

(Ph Eur monograph 0564)

Ph Eur

DEFINITION

Sodium iodohippurate (^{123}I) injection is a sterile solution of sodium (2-[^{123}I]iodobenzamido) acetate. It may contain a suitable buffer and a suitable antimicrobial preservative such as benzyl alcohol. Iodine-123 is a radioactive isotope of iodine and may be obtained by proton irradiation of xenon enriched in xenon-124 (not less than 98 per cent) followed by the decay of caesium-123 formed via xenon-123. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared iodine-123 radioactivity at the date and hour stated on the label. Not less than 96 per cent of the radioactivity corresponds to iodine-123 in the form of sodium 2-iodohippurate. The specific radioactivity is 0.74 GBq to 10.0 GBq of iodine-123 per gram of sodium 2-iodohippurate. Not more than 0.35 per cent of the total radioactivity is due to radionuclides other than iodine-123.

CHARACTERS

A clear, colourless liquid.

Iodine-123 has a half-life of 13.2 h and emits gamma radiation and X-rays.

IDENTIFICATION

A. Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-123 solution apart from any differences attributable to the presence of iodine-125, tellurium-121 and other radionuclidic impurities. Standardised iodine-123, iodine-125 and tellurium-121 solutions are available from laboratories recognised by the national authorities. The most prominent gamma photon of iodine-123 has an energy of 0.159 MeV and is accompanied by an X-ray of 0.027 MeV. Iodine-125 has a half-life of 59.4 days and emits an X-ray of 0.027 MeV and a photon of 0.035 MeV. Tellurium-121 has a half-life of 19.2 days and the most prominent photons have energies of 0.507 MeV and 0.573 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The spot corresponding to the main peak of radioactivity in the chromatogram obtained with the test solution is similar in position to the spot corresponding to 2-iodohippuric acid in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

The pH of the solution is 3.5 to 8.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. Determine the relative amounts of iodine-125, tellurium-121 and other radionuclidic impurities present. No radionuclides with longer half lives than iodine-125 are detected. For the determination of iodine-125, tellurium-121 and other radionuclidic impurities, retain the solution to be examined for a sufficient time to allow the radioactivity of iodine-123 to decrease to a level which permits the detection of radionuclidic impurities. Record the gamma-ray spectrum and X-ray spectrum of the decayed material using a suitable instrument. Not more than 0.35 per cent of the total radioactivity is due to radionuclides other than iodine-123. The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using *silica gel GF* $_{254}$ *R* as the coating substance.

Test solution Dissolve 1 g of *potassium iodide R* in 10 ml of *water R*, add 1 volume of this solution to 10 volumes of the injection to be examined and use within 10 min of mixing. If necessary, dilute with the reference solution (carrier) to give a radioactive concentration sufficient for the detection method, for example 3.7 MBq per millilitre.

Reference solution (carrier) Dissolve 40 mg of *2-iodohippuric acid R* and 40 mg of *2-iodobenzoic acid R* in 4 ml of *0.1 M sodium hydroxide*, add 10 mg of *potassium iodide R* and dilute to 10 ml with *water R.*

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm (about 75 min) using a mixture of 1 volume of *water R*, 4 volumes of *glacial acetic acid R*, 20 volumes of *butanol R* and 80 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The chromatogram obtained with the reference solution shows a spot corresponding to 2-iodohippuric acid and nearer to the solvent front a spot corresponding to 2-iodobenzoic acid. Iodide ion remains near the starting-line. Determine the distribution of radioactivity using a suitable detector. In the chromatogram obtained with the test solution, not less than 96 per cent of the total radioactivity is found in the spot corresponding to 2-iodohippuric acid and not more than 2 per cent of the total radioactivity is found in either of the spots corresponding to 2-iodobenzoic acid and to iodide ion.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised iodine-123 solution or by measurement in an instrument calibrated with the aid of such a solution.

STORAGE

Store protected from light.

LABELLING

The label states whether or not the preparation is suitable for renal plasma-flow studies.

Ph Eur

Sodium Iodohippurate [^{131} I] Injection

General Notices

(Ph Eur monograph 0282)

Ph Eur

DEFINITION

Sodium iodohippurate (^{131}I) injection is a sterile solution of sodium 2-(2-[^{131}I]iodobenzamido) acetate. It may contain a suitable buffer and a suitable antimicrobial preservative such as benzyl alcohol. Iodine-131 is a radioactive isotope of iodine and may be obtained by neutron irradiation of tellurium or by extraction from uranium fission products. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared iodine-131 radioactivity at the date and hour stated on the label. Not less than 96 per cent of the iodine-131 is in the form of sodium 2-iodohippurate. The specific radioactivity is 0.74 GBq to 7.4 GBq of iodine-131 per gram of sodium 2-iodohippurate.

CHARACTERS

A clear, colourless liquid.

Iodine-131 has a half-life of 8.04 days and emits beta and gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-131 solution. Standardised iodine-131 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of iodine-131 has an energy of 0.365 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The main peak of radioactivity in the chromatogram obtained with the test solution is similar in position to the spot corresponding to 2-iodohippuric acid in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

The pH of the injection is 6.0 to 8.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised iodine-131 solution. Determine the relative amounts of iodine-131, iodine-133, iodine-135 and other radionuclidic impurities present. Iodine-133 has a half-life of 20.8 h and its most prominent gamma photons have energies of 0.530 MeV and 0.875 MeV. Iodine-135 has a half-life of 6.55 h and its most prominent gamma photons have energies of 0.527 MeV, 1.132 MeV and 1.260 MeV. Not less than 99.9 per cent of the total radioactivity is due to iodine-131.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using *silica gel GF$_{254}$ R* as the coating substance.

Test solution Dissolve 1 g of *potassium iodide R* in 10 ml of *water R*, add 1 volume of this solution to 10 volumes of the injection to be examined and use within 10 min of mixing. If necessary dilute with the reference solution (carrier) to give a radioactive concentration sufficient for the detection method, for example 3.7 MBq per millilitre.

Reference solution (carrier) Dissolve 40 mg of *2-iodohippuric acid R* and 40 mg of *2-iodobenzoic acid R* in 4 ml of *0.1 M sodium hydroxide*, add 10 mg of *potassium iodide R* and dilute to 10 ml with *water R.*

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm (about 75 min) using a mixture of 1 volume of *water R,* 4 volumes of *glacial acetic acid R*, 20 volumes of *butanol R* and 80 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The chromatogram obtained with the reference solution shows a spot corresponding to 2-iodohippuric acid and nearer to the solvent front a spot corresponding to 2-iodobenzoic acid. Iodide ion remains near the starting-line. Determine the distribution of radioactivity using a suitable detector. In the chromatogram obtained with the test solution, not less than 96 per cent of the total radioactivity is found in the spot corresponding to 2-iodohippuric acid and not more than 2 per cent of the total radioactivity is found in either of the spots corresponding to 2-iodobenzoic acid and to iodide ion.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised iodine-131 solution or by measurement in an instrument calibrated with the aid of such a solution.

STORAGE

Store protected from light.

LABELLING

The label states that the preparation is not necessarily suitable for renal plasma-flow studies.

Ph Eur

Sodium Molybdate [^{99} Mo] Solution (Fission)

General Notices

(Ph Eur monograph 1923)

Ph Eur

DEFINITION

Alkaline solution of sodium [^{99}Mo]molybdate obtained by extraction of fission products of uranium-235. It may contain stabilisers.

Content

90 per cent to 110 per cent of the declared molybdenum-99 radioactivity at the date and time stated on the label.

PRODUCTION

Molybdenum-99 is usually produced by fission of uranium enriched in uranium-235, which is caused by the absorption of a thermal neutron, resulting in high-specific-activity molybdenum-99. By the fission of uranium after neutron capture, more than 200 different radionuclides are produced. In approximately 6 per cent of the fissions, molybdenum-99 is formed after decay of a number of short-lived parent radionuclides. After dissolution of the target, the molybdenum-99 is separated from the mixture of nuclides and purified by using chromatographic processes in order to obtain molybdenum-99 with a high level of radionuclidic purity.

CHARACTERS

Appearance

Clear, colourless or almost colourless solution.

Half-life and nature of radiation of molybdenum-99

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The most prominent gamma photon of molybdenum-99 has an energy of 0.740 MeV; a peak with an energy of 0.141 MeV, due to technetium-99-m is also visible.

B. Examine the chromatograms obtained in the test for radiochemical purity (see Tests).

Results The principal peak in the radiochromatogram obtained with the test solution has a similar retardation factor to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dilute the preparation to be examined to a radioactivity concentration of approximately 370 MBq/ml with a 2.42 g/l solution of *sodium molybdate R* .

Alkalinity

The preparation is alkaline (*2.2.4*).

RADIONUCLIDIC PURITY

Iodine-131, ruthenium-103 and tellurium-132:

— *iodine-131*: maximum 5×10^{-3} per cent of the total radioactivity;

— *ruthenium-103*: maximum 5×10^{-3} per cent of the total radioactivity;

— *tellurium-132*: maximum 5×10^{-3} per cent of the total radioactivity.

The following method has been found to be suitable; other validated methods, approved by the competent authority, may be used.

Gamma-ray spectrometry.

Condition a column with an internal volume of approximately 1.5 ml of *strongly basic anion exchange resin R* with a mixture of equal volumes of *glacial acetic acid R* and *water R* . All elutions of the column are made at a flow rate not exceeding 1 ml/min.

Test solution In a test-tube, successively add, with mixing, 1 ml of a 24.2 g/l solution of *sodium molybdate R* , 0.5 ml of *strong hydrogen peroxide solution R* , 2.5 ml of *glacial acetic acid R* , 1.0 ml of *iodine-123 and ruthenium-106 spiking solution R* and 1.0 ml of solution S. Allow to stand for 30 min at room temperature.

Reference solution Mix 1.0 ml of *iodine-123 and ruthenium-106 spiking solution R* and 4.0 ml of *water R.*

Apply the test solution to the column and elute. Just before the disappearance of the liquid from the top of the column, add 6 ml of a mixture of equal volumes of *glacial acetic acid R* and *water R* and elute. Transfer 5.0 ml of the combined eluates to a counting tube. Determine the radioactivity of iodine-123, iodine-131, ruthenium-103, ruthenium-106 and iodine-132 at the gamma-ray energies of 0.159 MeV for iodine-123, 0.365 MeV for iodine-131, 0.497 MeV for ruthenium-103, 0.512 MeV for ruthenium-106 and 0.668 MeV for iodine-132. Determine in the same way the radioactivity of iodine-123 and ruthenium-106 in the reference solution and calculate the recovery of iodine-123 and ruthenium-106 in the combined eluates.

Calculate the radioactivity of iodine-131, iodine-132 and ruthenium-103 in the combined eluates, taking into account the recovery, the fraction of eluate used, the counting efficiency and the radioactive decay. From the radioactivity of iodine-132 (daughter radionuclide of tellurium-132), calculate the radioactivity of tellurium-132, taking into account the time of the test and the time of separation of molybdenum-99.

Total radioactivity due to strontium-89 and strontium-90

Maximum 6 × 10^{-5} per cent of the total radioactivity.

The following method has been found to be suitable; other validated methods, approved by the competent authority, may be used.

Liquid scintillation spectrometry.

Connect 2 columns, each with an internal volume of approximately 1.5 ml of *strongly basic anion exchange resin R*, in series and condition the columns with 10 ml of a 4 g/l solution of *sodium hydroxide R*. All elutions of the columns are made at a flow rate not exceeding 1 ml/min.

Test solution In a test-tube, successively add, with mixing, 1.0 ml of solution S, 50 µl of *strontium-85 spiking solution R* and 0.05 ml of *strong sodium hypochlorite solution R*. Allow to stand for 10 min at room temperature.

Reference solution Mix 50 µl of *strontium-85 spiking solution R* with 5.0 ml of a 9.5 g/l solution of *nitric acid R* in a vial for liquid scintillation counting and add 10 ml of *liquid scintillation cocktail R*.

Apply the test solution to the upper of the 2 columns and elute. Just before the disappearance of the liquid from the top of the upper column, add 3 ml of a 4 g/l solution of *sodium hydroxide R* and elute until the columns are dry. Combine the eluates and add 4 ml of a 947 g/l solution of *nitric acid R* (molybdenum-poor eluate). Determine the radioactivity due to molybdenum-99 using gamma-ray spectrometry. If the radioactivity due to molybdenum-99 is higher than 6 × 10^{-7} per cent of the radioactivity due to molybdenum-99 in 1 ml of solution S, repeat the above procedure using 2 new columns.

Condition a column with an internal volume of approximately 2 ml of *strontium selective extraction resin R* with 5 ml of a 473 g/l solution of *nitric acid R* and dry the column. All elutions of the column are made at a flow rate not exceeding 1 ml/min. Apply to the column the molybdenum-poor eluate and elute. Just before the disappearance of the liquid from the top of the column, add 20 ml of a 473 g/l solution of *nitric acid R* and elute until the column is dry. Rinse the column with 2 ml of a 9.5 g/l solution of *nitric acid R*, dry the column and discard the eluate. Elute the column with 8.0 ml of a 9.5 g/l solution of *nitric acid R* until the column is dry. Transfer 5.0 ml of the eluate into a vial for liquid scintillation counting and add 10 ml of *liquid scintillation cocktail R*.

Determine the total radioactivity due to strontium-89 and strontium-90 in this solution by liquid scintillation spectrometry, and the radioactivity due to strontium-85 by gamma-ray spectrometry. Determine the radioactivity due to strontium-85 in the reference solution by gamma-ray spectrometry. Calculate the recovery of strontium-85 in the eluate. Calculate the measured total radioactivity of strontium-89 and strontium-90 in the eluate, taking into account the recovery of strontium and the fraction of eluate used.

Total radioactivity due to alpha-particle-emitting impurities

Maximum 1 × 10^{-7} per cent of the total radioactivity.

The following method has been found to be suitable; other validated methods, approved by the competent authority, may be used.

Alpha-ray spectrometry.

Test solution To 0.2 ml of the preparation to be examined add 1.0 ml of *plutonium-242 spiking solution R,* 1.0 ml of *americium-243 spiking solution R* and 9.0 ml of a 927 g/l solution of *hydrochloric acid R* . Evaporate the sample to dryness. Dissolve the residue in 2 ml of a 927 g/l solution of *hydrochloric acid R* . Evaporate again to dryness. Dissolve the residue in 2 ml of a 10.3 g/l solution of hydrochloric acid R.

Apply the test solution to a column containing 0.7 g of *anion exchange resin R1* . Collect the eluate and wash the column with 1 ml of a 10.3 g/l solution of *hydrochloric acid R* . Evaporate the combined eluates to dryness and dissolve the residue in 2 ml of a 10.3 g/l solution of *hydrochloric acid R* . Apply this solution to a 2nd column containing 0.7 g of *anion exchange resin R1* . Collect the eluate and wash the column with 1 ml of a 10.3 g/l solution of *hydrochloric acid R* . Evaporate the combined eluates to dryness and dissolve the residue in 1 ml of *nitric acid R.* Evaporate to dryness. Dissolve the residue again in 1 ml of *nitric acid R.*

Add 1 ml of a 42.6 g/l solution of *anhydrous sodium sulphate R* and evaporate to dryness. Add 0.3 ml of *sulphuric acid R* . Warm until the residue is dissolved. Add 4 ml of *distilled water R* and 0.01 ml of *thymol blue solution R* . Add *concentrated ammonia R* dropwise until the colour changes from red to yellow.

Prepare an electrodeposition cell as follows. An electropolished stainless steel planchet is fitted in the cap of a 20 ml polyethylene scintillation vial. The bottom of the vial has been cut off and a hole has been drilled through the centre of the cap for electrical connection to the planchet cathode. The planchet, 20 mm in diameter and 0.5 mm thick, is rinsed with *acetone R* and *water R* prior to use. The anode, a platinum spiral, is introduced through the bottom of the vial and fitted 5 mm from the cathode.

Pour the solution prepared as described above into the electrodeposition cell and rinse the container with a total of 5 ml of a 10 g/l solution of *sulphuric acid R* (the solution becomes slightly pink). Adjust to pH 2.1-2.4. with *concentrated ammonia R* or with a 200 g/l solution of *sulphuric acid R* . Electrolyse at 1.2 A for 75 min without stirring.

Add 1 ml of *concentrated ammonia R* about 1 min prior to switching off the current. Rinse the planchet with a 57 g/l solution of *ammonia R* . Rinse the planchet with *acetone R* and remove any residual solvent by patting the planchet with absorbent paper. Heat the planchet on a hot plate at 180 °C for 10 min.

Determine the radioactivity of alpha emitters by alpha-ray spectrometry, taking into account the recovery of the alpha-particle-emitting radionuclides (measured using the plutonium-242 and americium-243 spiking solutions).

Total of gamma-ray-emitting radionuclides other than molybdenum-99, technetium-99m, iodine-131, ruthenium-103 and tellurium-132

Maximum 1×10^{-2} per cent of the total radioactivity.

The following method has been found to be suitable; other validated methods, approved by the competent authority, may be used.

Gamma-ray spectrometry.

Allow the preparation to decay for 4-6 weeks. Examine the gamma-ray spectrum for the presence of other gamma-ray-emitting impurities. Identify and quantify other gamma-ray-emitting impurities. The preparation may be released for use before completion of the test.

RADIOCHEMICAL PURITY

[^{99}Mo]Molybdate

The following method has been found to be suitable; other validated methods, approved by the competent authority, may be used.

Thin-layer chromatography (*2.2.27*).

Test solution Dilute the preparation to be examined with a 4.0 g/l solution of *sodium hydroxide R* to a radioactivity concentration suitable for the detector.

Reference solution 50 g/l solution of *sodium molybdate R* in a 4.0 g/l solution of *sodium hydroxide R* .

Plate *TLC silica gel plate R.*

Mobile phase 10.6 g/l solution of *anhydrous sodium carbonate R* .

Application 5 µl of the test solution and 2 µl of the reference solution.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Determine the distribution of radioactivity using a suitable detector and spray with a 2 g/l solution of *phenylhydrazine R* in *glacial acetic acid R* ; heat at 100-105 °C for 5 min.

Retardation factor Molybdate and pertechnetate = about 0.9.

Limit:

— *sum of [^{99}Mo]molybdate and [^{99m}Tc]pertechnetate*: minimum 95 per cent of the total radioactivity.

RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

LABELLING

The label states that the preparation is only suitable for the preparation of technetium-99m generators.

IMPURITIES

A. iodine-131,

B. ruthenium-103,

C. tellurium-132,

D. strontium-89,

E. strontium-90.

Sodium Pertechnetate [^{99m} Tc] Injection (Fission)

General Notices

(Ph Eur monograph 0124)

Ph Eur

This monograph applies to sodium pertechnetate (^{99m} Tc) injection obtained from molybdenum-99 extracted from fission products of uranium. Sodium pertechnetate (^{99m} Tc) injection obtained from molybdenum-99 produced by the neutron irradiation of molybdenum is described in the monograph on Sodium pertechnetate (^{99m} Tc) injection (non-fission) (0283).

DEFINITION

Sodium pertechnetate (^{99m}Tc) injection (fission) is a sterile solution containing technetium-99m in the form of pertechnetate ion and made isotonic by the addition of sodium chloride. Technetium-99m is a radionuclide formed by the decay of molybdenum-99. Molybdenum-99 is a radioactive isotope of molybdenum extracted from uranium fission products. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 95 per cent of the radioactivity corresponds to technetium-99m in the form of pertechnetate ion.

The radioactivity due to radionuclides other than technetium-99m, apart from that due to technetium-99 resulting from the decay of technetium-99m, is not greater than that shown below, expressed as a percentage of the total radioactivity and calculated with reference to the date and hour of administration.

Molybdenum-99	0.1 per cent
Iodine-131	5×10^{-3} per cent
Ruthenium-103	5×10^{-3} per cent
Strontium-89	6×10^{-5} per cent
Strontium-90	6×10^{-6} per cent
Alpha-emitting impurities	1×10^{-7} per cent
Other gamma-emitting impurities	0.01 per cent

The injection may be prepared from a sterile preparation of molybdenum-99 under aseptic conditions.

CHARACTERS

A clear, colourless solution.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by measurement in an instrument calibrated with the aid of such a solution. Standardised technetium-99m, molybdenum-99, iodine-131, ruthenium-103, strontium-89 and strontium/yttrium-90 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.0 to 8.0.

Aluminium

In a test tube about 12 mm in internal diameter, mix 1 ml of *acetate buffer solution pH 4.6 R* and 2 ml of a 1 in 2.5 dilution of the injection in *water R* . Add 0.05 ml of a 10 g/l solution of *chromazurol S R* . After 3 min, the colour of the solution is not more intense than that of a standard prepared at the same time and in the same manner using 2 ml of *aluminium standard solution (2 ppm Al) R* (5 ppm).

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Preliminary test To obtain an approximate estimate before use of the injection, take a volume equivalent to 37 MBq and determine the gamma-ray spectrum using a sodium iodide detector with a shield of lead, of thickness 6 mm, interposed between the sample and the detector. The response in the region corresponding to the 0.740 MeV photon of molybdenum-99 does not exceed that obtained using 37 kBq of a standardised molybdenum-99 solution measured under the same conditions, when all measurements are expressed with reference to the date and hour of administration.

Definitive test Retain a sample of the injection for a sufficient time to allow the technetium-99m radioactivity to decay to a sufficiently low level to permit the detection of radionuclidic impurities. All measurements of radioactivity are expressed with reference to the date and hour of administration.

— *Molybdenum-99*. Record the gamma-ray spectrum of the decayed material in a suitable instrument calibrated with the aid of a standardised molybdenum-99 solution. The most prominent photons have energies of 0.181 MeV, 0.740 MeV and 0.778 MeV. Molybdenum-99 has a half-life of 66.0 h. Not more than 0.1 per cent of the total radioactivity is due to molybdenum-99.

— *Iodine-131*. Record the gamma-ray spectrum of the decayed material in a suitable instrument calibrated with the aid of a standardised iodine-131 solution. The most prominent photon has an energy of 0.365 MeV. Iodine-131 has a half-life of 8.04 days. Not more than 5×10^{-3} per cent of the total radioactivity is due to iodine-131.

— *Ruthenium-103*. Record the gamma-ray spectrum of the decayed material in a suitable instrument calibrated using a standardised ruthenium-103 solution. The most prominent photon has an energy of 0.497 MeV. Ruthenium-103 has a half-life of 39.3 days. Not more than 5×10^{-3} per cent of the total radioactivity is due to ruthenium-103.

— *Strontium-89*. Determine the presence of strontium-89 in the decayed material with an instrument suitable for the detection of beta rays, by comparison with a standardised strontium-89 solution. It is usually necessary first to carry out chemical separation of the strontium so that the standard and the sample may be compared in the same physical and chemical form. Strontium-89 decays with a beta emission of 1.492 MeV maximum energy and has a half-life of 50.5 days. Not more than 6×10^{-5} per cent of the total radioactivity is due to strontium-89.

— *Strontium-90*. Determine the presence of strontium-90 in the decayed material with an instrument suitable for the detection of beta rays. To distinguish strontium-90 from strontium-89, compare the radioactivity of yttrium-90, the daughter nuclide of strontium-90, with an yttrium-90 standard after the chemical separation of the yttrium. If prior chemical separation of the strontium is necessary, the conditions of radioactive equilibrium must be ensured. The yttrium-90 standard and the sample must be compared in the same physical and chemical form. Strontium-90 and yttrium-90 decay with respective beta emissions of 0.546 MeV and 2.284 MeV maximum energy and half-lives of 29.1 years and 64.0 h. Not more than 6×10^{-6} per cent of the total radioactivity is due to strontium-90.

— *Other gamma-emitting impurities*. Examine the gamma-ray spectrum of the decayed material for the presence of other radionuclidic impurities, which should, where possible, be identified and quantified. The total gamma radioactivity due to these impurities does not exceed 0.01 per cent of the total radioactivity.

— *Alpha-emitting impurities*. Measure the alpha radioactivity of the decayed material to detect any alpha-emitting radionuclidic impurities, which should, where possible, be identified and quantified. The total alpha radioactivity due to these impurities does not exceed 1×10^{-7} per cent of the total radioactivity.

RADIOCHEMICAL PURITY

Examine by descending paper chromatography (*2.2.26*).

Test solution Dilute the preparation to be examined with *water R* to a suitable radioactive concentration.

Apply 5 µl of the test solution. Develop for 2 h using a mixture of 20 volumes of *water R* and 80 volumes of *methanol R*. Allow the paper to dry. Determine the distribution of radioactivity using a suitable detector. Not less than 95 per cent of the total radioactivity is in the spot corresponding to pertechnetate ion, which has an R_F value of about 0.6.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Sodium Pertechnetate [^{99m} Tc] Injection (Non-fission)

General Notices

(Ph Eur monograph 0283)

Ph Eur

This monograph applies to sodium pertechnetate (^{99m} *Tc) injection obtained from molybdenum-99 produced by neutron irradiation of molybdenum. Sodium pertechnetate (* ^{99m} *Tc) injection obtained from molybdenum-99 extracted from fission products of uranium is described in the monograph on Sodium pertechnetate(* ^{99m} *Tc) injection (fission) (0124).*

DEFINITION

Sodium pertechnetate (^{99m}Tc) injection (non-fission) is a sterile solution containing technetium-99m in the form of pertechnetate ion and made isotonic by the addition of sodium chloride. Technetium-99m is a radionuclide formed by the decay of molybdenum-99. Molybdenum-99 is a radioactive isotope of molybdenum produced by neutron irradiation of molybdenum. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 95 per cent of the radioactivity corresponds to technetium-99m in the form of pertechnetate ion.

The radioactivity due to radionuclides other than technetium-99m, apart from that due to technetium-99 resulting from the decay of technetium-99m is not greater than that shown below, expressed as a percentage of the total radioactivity and calculated with reference to the date and hour of administration.

Molybdenum-99	0.1 per cent
Other radionuclidic impurities	0.01 per cent

The injection may be prepared from a sterile preparation of molybdenum-99 under aseptic conditions.

CHARACTERS

A clear, colourless solution.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.0 to 8.0.

Aluminium

In a test tube about 12 mm in internal diameter, mix 1 ml of *acetate buffer solution pH 4.6 R* and 2 ml of a 1 in 2.5 dilution of the injection in *water R.* Add 0.05 ml of a 10 g/l solution of *chromazurol S R* . After 3 min, the colour of the solution is not more intense than that of a standard prepared at the same time in the same manner using 2 ml of *aluminium standard solution (2 ppm Al) R* (5 ppm).

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125) .* The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Preliminary test To obtain an approximate estimate before use of the injection, take a volume equivalent to 37 MBq and record the gamma-ray spectrum using a sodium iodide detector with a shield of lead, of thickness 6 mm, interposed between the sample and the detector. The response in the region corresponding to the 0.740 MeV photon of molybdenum-99 does not exceed that obtained using 37 kBq of a standardised solution of molybdenum-99 measured under the same conditions, when all measurements are expressed with reference to the date and hour of administration.

Definitive test Retain a sample of the injection for a sufficient time to allow the technetium-99m radioactivity to decay to a sufficiently low level to permit the detection of radionuclidic impurities. All measurements of radioactivity are expressed with reference to the date and hour of administration.

— *Molybdenum-99*. Record the gamma-ray spectrum of the decayed material in a suitable instrument calibrated using a standardised molybdenum-99 solution. The most prominent gamma photons have energies of 0.181 MeV, 0.740 MeV and 0.778 MeV. Molybdenum-99 has a half-life of 66.0 h. Not more than 0.1 per cent of the total radioactivity is due to molybdenum-99.

— *Other gamma-emitting impurities*. Examine the gamma-ray spectrum of the decayed material for the presence of other radionuclidic impurities, which should, where possible, be identified and quantified. The total radioactivity due to other radionuclidic impurities does not exceed 0.01 per cent of the total radioactivity.

RADIOCHEMICAL PURITY

Examine by descending paper chromatography (*2.2.26*).

Test solution Dilute the injection with *water R* to a suitable radioactive concentration.

Apply 5 µl of the test solution. Develop for 2 h using a mixture of 20 volumes of *water R* and 80 volumes of *methanol R*. Allow the paper to dry in air. Determine the distribution of radioactivity using a suitable detector. Not less than 95 per cent of the total radioactivity is found in the spot corresponding to pertechnetate ion, which has an R_F value of about 0.6.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Sodium Phosphate [^{32} P] Injection

General Notices

(Ph Eur monograph 0284)

Ph Eur

DEFINITION

Sodium phosphate (^{32}P) injection is a sterile solution of disodium and monosodium (^{32}P) orthophosphates made isotonic by the addition of sodium chloride. Phosphorus-32 is a radioactive isotope of phosphorus and may be produced by neutron irradiation of sulphur. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared phosphorus-32 radioactivity at the date and hour stated on the label. Not less than 95 per cent of the radioactivity corresponds to phosphorus-32 in the form of orthophosphate ion. The specific radioactivity is not less than 11.1 MBq of phosphorus-32 per milligram of orthophosphate ion.

CHARACTERS

A clear, colourless solution.

Phosphorus-32 has a half-life of 14.3 days and emits beta radiation.

IDENTIFICATION

A. Record the beta-ray spectrum or the beta-ray absorption curve using a suitable method. The spectrum or curve does not differ significantly from that of a standardised phosphorus-32 solution obtained under the same conditions. Standardised phosphorus-32 solutions are available from laboratories recognised by the competent authority. The maximum energy of the beta radiation is 1.71 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the injection is 6.0 to 8.0.

Phosphates

Dilute the injection with *water R* to give a radioactive concentration of 370 kBq of phosphorus-32 per millilitre. Mix in a volumetric flask, with shaking, 1.0 ml of the solution with a mixture of 0.5 ml of a 2.5 g/l solution of *ammonium vanadate R,* 0.5 ml of *ammonium* *molybdate solution R* and 1 ml of *perchloric acid R* and dilute to 5.0 ml with *water R*. After 30 min, the solution is not more intensely coloured than a standard prepared at the same time in the same manner using 1.0 ml of a solution containing 33 mg of orthophosphate ion per litre.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Record the beta-ray spectrum or the beta-ray absorption curve using a suitable method. The spectrum or curve does not differ significantly from that of a standardised phosphorus-32 solution obtained under the same conditions.

RADIOCHEMICAL PURITY

Examine by ascending paper chromatography (*2.2.26*).

Test solution Dilute the injection with *water R* until the radioactivity is equivalent to 10 000 to 20 000 counts per minute per 10 µl

Reference solution Prepare a solution of *phosphoric acid R* containing 2 mg of phosphorus per millilitre.

Using a strip of paper 25 mm wide and about 300 mm long, apply 10 µl of the reference solution. Apply to the same starting-point 10 µl of the test solution. Develop for 16 h using a mixture of 0.3 ml of *ammonia R*, 5 g of *trichloroacetic acid R*, 25 ml of *water R* and 75 ml of 2- *propanol R.* Allow the paper to dry in air. Determine the position of the inactive phosphoric acid by spraying with a 50 g/l solution of *perchloric acid R* and then with a 10 g/l solution of *ammonium molybdate R*. Expose the paper to *hydrogen sulphide R*. A blue colour develops. Determine the position of the radioactive spot by autoradiography or by measuring the radioactivity over the whole length of the chromatogram. Not less than 95 per cent of the total radioactivity of the chromatogram is found in the spot corresponding to phosphoric acid.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised phosphorus-32 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Strontium [^{89} Sr] Chloride Injection

General Notices

(Ph Eur monograph 1475)

Ph Eur

DEFINITION

Strontium (^{89}Sr) chloride injection is a sterile solution of [^{89}Sr]strontium chloride. Strontium-89 is a radioactive isotope of strontium and is produced by neutron irradiation of strontium enriched in strontium-88. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared strontium-89 radioactivity at the date stated on the label. Not more than 0.6 per cent of the total radioactivity is due to radionuclides other than strontium-89. The specific radioactivity is not less than 1.8 MBq of strontium-89 per milligram of strontium. The injection contains 6.0 mg/ml to 12.5 mg/ml of strontium.

CHARACTERS

A clear, colourless solution.

Strontium-89 has a half-life of 50.5 days and emits beta radiation with a maximum energy of 1.492 MeV.

IDENTIFICATION

A. Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised strontium-89 solution, when measured either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised strontium-89 solutions are available from laboratories recognised by the competent authority. The gamma photon detected has an energy of 0.909 MeV and is due to the short-lived daughter product, yttrium-89m (formed in 0.01 per cent of the disintegrations), in equilibrium with the strontium-89.

B. To 0.1 ml of the injection to be examined, add 1 ml of a freshly prepared 1 g/l solution of *sodium rhodizonate R* . Mix and allow to stand for 1 min. A reddish-brown precipitate is formed.

C. To 0.1 ml of *silver nitrate solution R2* add 50 µl of the injection to be examined. A white precipitate is formed.

TESTS

pH (*2.2.3*)

The pH of the solution is 4.0 to 7.5.

Note: the following tests for aluminium, iron and lead may be carried out simultaneously with the test for strontium. If this is not the case, the reference solutions are prepared such that they contain strontium at approximately the same concentration as in the test solution.

Aluminium

Not more than 2.0 µg/ml, determined by atomic emission spectrometry (plasma or arc method) (*2.2.22, Method I*).

Test solution Dilute 0.2 ml of the injection to be examined to a suitable volume with *dilute nitric acid R* .

Reference solutions Prepare the reference solutions using *aluminium standard solution (10 ppm Al) R* diluted as necessary with *dilute nitric acid R.*

Iron

Not more than 5.0 µg/ml, determined by atomic emission spectrometry (plasma or arc method) (*2.2.22, Method I*).

Test solution Dilute 0.2 ml of the injection to be examined to a suitable volume with *dilute nitric acid R.*

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R* diluted as necessary with *dilute nitric acid R.*

Lead

Not more than 5.0 µg/ml, determined by atomic emission spectrometry (plasma or arc method) (*2.2.22, Method I*).

Test solution Dilute 0.2 ml of the injection to be examined to a suitable volume with *dilute nitric acid R.*

Reference solutions Prepare the reference solutions using *lead standard solution (10 ppm Pb) R* diluted as necessary with *dilute nitric acid R* .

Strontium

6.0 mg/ml to 12.5 mg/ml. Examine by atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dilute 0.2 ml of the injection to be examined to a suitable volume with *dilute nitric acid R* .

Reference solutions Prepare the reference solutions using *strontium standard solution (1.0 per cent Sr) R* diluted as necessary with *dilute nitric acid R* .

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).*

RADIONUCLIDIC PURITY

Gamma emitters

Record the gamma-ray and X-ray spectrum of the injection to be examined using a suitable instrument. Not more than 0.4 per cent of the total radioactivity in the preparation to be examined is due to gamma emitting radionuclides other than yttrium-89m.

Beta emitters

Evaporate to dryness 100 µl of the injection to be examined under a radiant heat source. Dissolve the residue in 2 ml of *47 per cent hydrobromic acid R*, evaporate to dryness under the radiant heat source and dissolve the residue in 2 ml of *dilute hydrobromic acid R1.* Transfer the solution to the top of a column, 5 mm to 6 mm in diameter, packed with approximately 2 ml of *cationic exchange resin R1* (100 µm to 250 µm), previously conditioned with *dilute hydrobromic acid R1* and elute the column with the same solvent until 10 ml of eluate has been collected into a container containing 50 µl of a 15 g/l solution of *anhydrous sodium sulphate R* in *1 M hydrochloric acid*.

To a liquid scintillation vial add an appropriate volume of scintillation liquid followed by 1 ml of *water R*, 0.1 ml of a 15 g/l solution of *anhydrous sodium sulphate R* in *1 M hydrochloric acid* and 100 µl of eluate. Shake to obtain a clear solution. Using suitable counting equipment determine the radioactivity due to sulphur-35 and phosphorus-32 in the sample.

Taking into account the recovery efficiency of the separation, counting efficiency and radioactive decay, determine the radioactive concentration of sulphur-35 and phosphorus-32 in the sample and hence the percentage of total beta emitting impurities in the injection to be examined. Not more than 0.2 per cent of the total radioactivity in the injection to be examined is due to the sum of sulphur-35 and phosphorus-32 radioactivities.

RADIOACTIVITY

Measure the radioactivity using suitable equipment by comparison with a standardised strontium-89 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Table of Physical Characteristics of Radionuclides Mentioned in the European Pharmacopoeia

General Notices

The following table is given to complete the general monograph *Radiopharmaceutical preparations (0125)*.

The values are obtained from the database of the National Nuclear Data Center (NNDC) at Brookhaven National Laboratory, Upton. N.Y., USA, directly accessible via Internet at the address: 'http://www.nndc.bnl.gov/nndc/nudat/radform.html'.

In case another source of information is preferred (more recent values), this source is explicitly mentioned.

Other data sources:

* DAMRI (Département des Applications et de la Métrologie des Rayonnements Ionisants, CEA Gif-sur-Yvette, France),

** PTB (Physikalisch-Technische Bundesanstalt, Braunschweig, Germany),

*** NPL (National Physical Laboratory, Teddington, Middlesex, UK).

The uncertainty of the half-lives are given in parentheses. In principle the digits in parentheses are the standard uncertainty of the corresponding last digits of the indicated numerical value ('Guide to the Expression of Uncertainty in Measurement', International Organisation for Standardisation (ISO), 1993, ISBN 92-67-10188-9).

The following abbreviations are used:

e_A = Auger electrons,

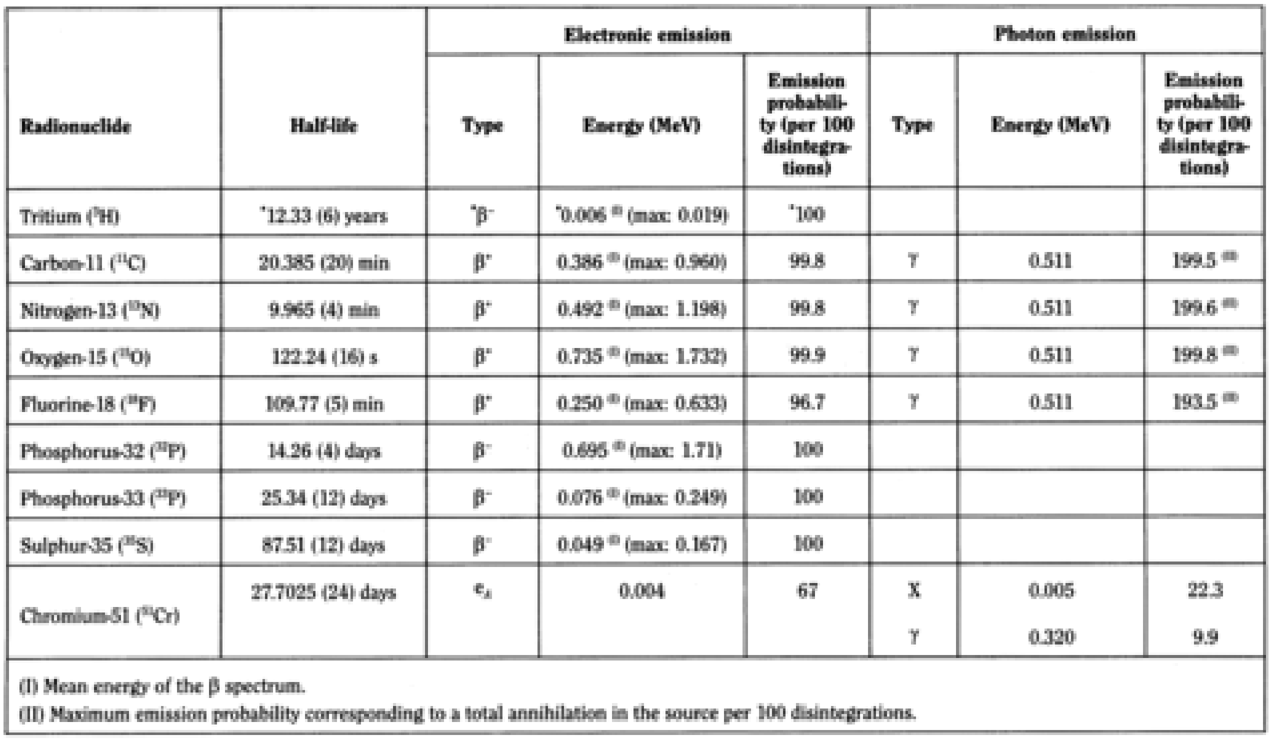
ce = conversion electrons,

β^- = electrons,

β^+ = positrons,

γ = gamma rays,

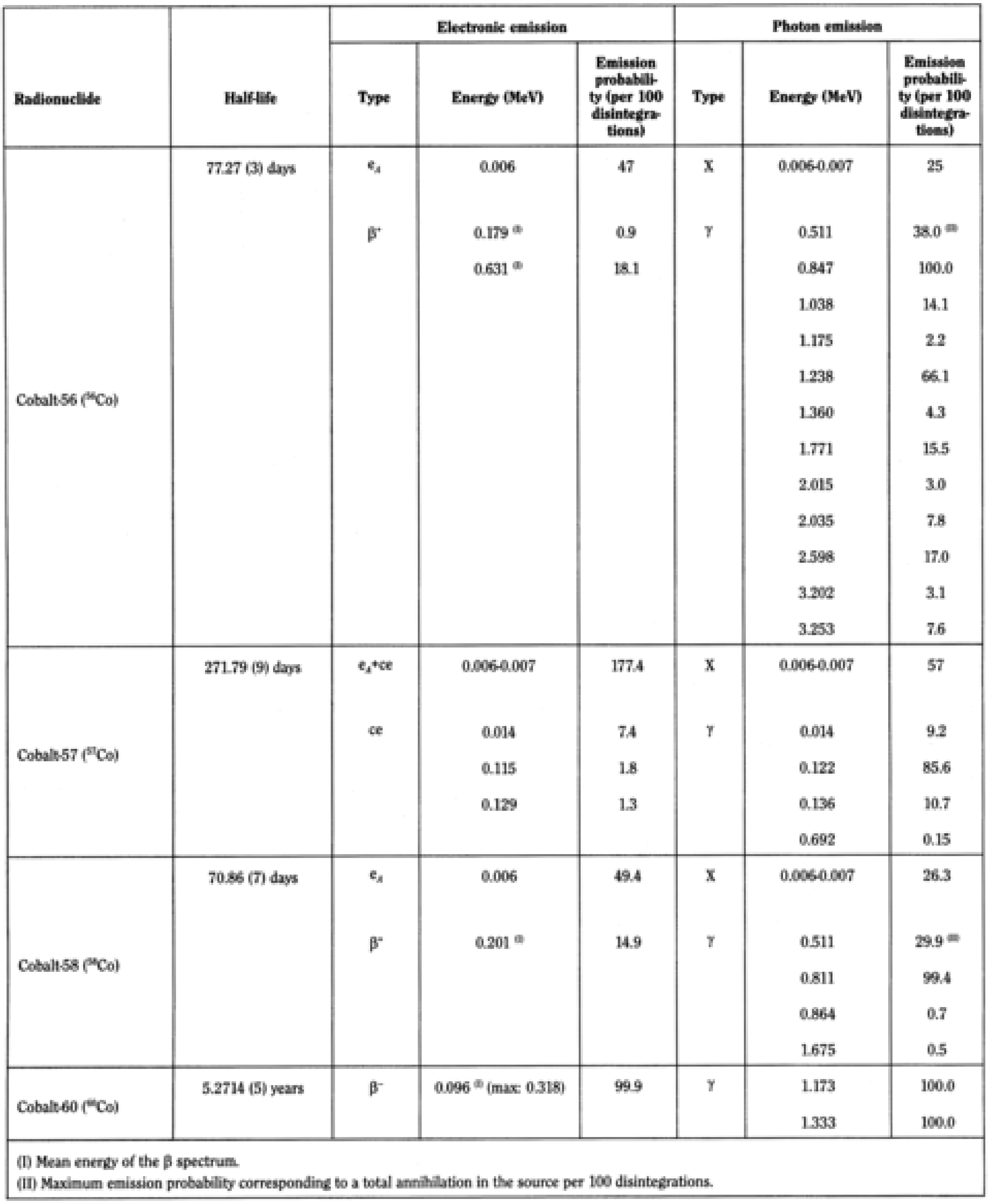
X = X-rays.



Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Tritium (^{3}H)	*12.33 (6) years	*β^-	*0.006 $^{(I)}$ (max: 0.019)	*100			
Carbon-11 (^{11}C)	20.385 (20) min	β^+	0.386 $^{(I)}$ (max: 0.960)	99.8	γ	0.511	199.5 $^{(II)}$
Nitrogen-13 (^{13}N)	9.965 (4) min	β^+	0.492 $^{(I)}$ (max: 1.198)	99.8	γ	0.511	199.6 $^{(II)}$
Oxygen-15 (^{15}O)	122.24 (16) s	β^+	0.735 $^{(I)}$ (max: 1.732)	99.9	γ	0.511	199.8 $^{(II)}$
Fluorine-18 (^{18}F)	109.77 (5) min	β^+	0.250 $^{(I)}$ (max: 0.633)	96.7	γ	0.511	193.5 $^{(II)}$
Phosphorus-32 (^{32}P)	14.26 (4) days	β^-	0.695 $^{(I)}$ (max: 1.71)	100			
Phosphorus-33 (^{33}P)	25.34 (12) days	β^-	0.076 $^{(I)}$ (max: 0.249)	100			
Sulphur-35 (^{35}S)	87.51 (12) days	β^-	0.049 $^{(I)}$ (max: 0.167)	100			
Chromium-51 (^{51}Cr)	27.7025 (24) days	e_A	0.004	67	X	0.005	22.3
					γ	0.320	9.9

(I) Mean energy of the β spectrum.

(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.



Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Cobalt-56 (^{56}Co)	77.27 (3) days	e_A	0.006	47	X	0.006-0.007	25
		β^+	0.179 (I)	0.9	γ	0.511	38.0 (II)
			0.631 (I)	18.1		0.847	100.0
						1.038	14.1
						1.175	2.2
						1.238	66.1
						1.360	4.3
						1.771	15.5
						2.015	3.0
						2.035	7.8
						2.598	17.0
						3.202	3.1
						3.253	7.6
Cobalt-57 (^{57}Co)	271.79 (9) days	e_A+ce	0.006-0.007	177.4	X	0.006-0.007	57
		ce	0.014	7.4	γ	0.014	9.2
			0.115	1.8		0.122	85.6
			0.129	1.3		0.136	10.7
						0.692	0.15
Cobalt-58 (^{58}Co)	70.86 (7) days	e_A	0.006	49.4	X	0.006-0.007	26.3
		β^+	0.201 (I)	14.9	γ	0.511	29.9 (II)
						0.811	99.4
						0.864	0.7
						1.675	0.5
Cobalt-60 (^{60}Co)	5.2714 (5) years	β^-	0.096 (I) (max: 0.318)	99.9	γ	1.173	100.0
						1.333	100.0

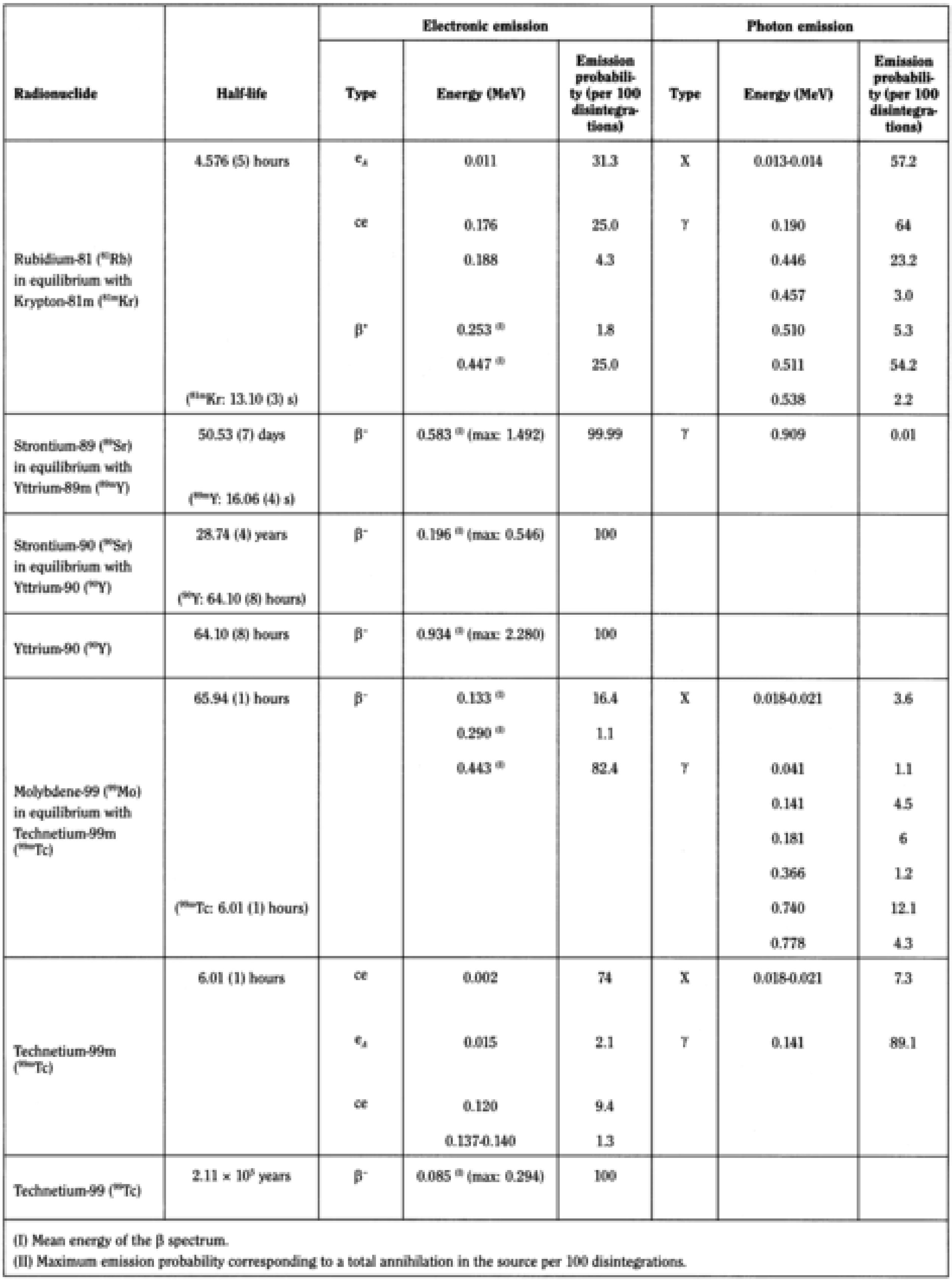
(I) Mean energy of the β spectrum.

(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.

Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Gallium-66 (^{66}Ga)	9.49 (7) hours	e_A	0.008	21	X	0.009-0.010	19.1
		β^+	0.157 (I)	1	γ	0.511	112 (II)
			0.331 (I)	0.7		0.834	5.9
			0.397 (I)	3.8		1.039	37
			0.782 (I)	0.3		1.333	1.2
			1.90 (I)	50		1.919	2.1
						2.190	5.6
						2.423	1.9
						2.752	23.4
						3.229	1.5
						3.381	1.5
						3.792	1.1
						4.086	1.3
						4.295	4.1
						4.807	1.8
Gallium-67 (^{67}Ga)	3.2612 (6) days	e_A	0.008	62	X	0.008-0.010	57
		ce	0.082-0.084	30.4	γ	0.091-0.093	42.4
			0.090-0.092	3.6		0.185	21.2
			0.175	0.3		0.209	2.4
						0.300	16.8
						0.394	4.7
						0.888	0.15
Germanium-68 (^{68}Ge) in equilibrium with Gallium-68 (^{68}Ga)	270.82 (27) days	e_A	0.008	42.4	X	0.009-0.010	44.1
	(^{68}Ga: 67.629 (24) min)	β^+	0.353 (I)	1.2	γ	0.511	178.3
			0.836 (I)	88.0		1.077	3.0
Gallium-68 (^{68}Ga)	67.629 (24) min	e_A	0.008	5.1	X	0.009-0.010	4.7
		β^+	0.353 (I)	1.2	γ	0.511	178.3
			0.836 (I)	88.0		1.077	3.0
Krypton-81m (^{81m}Kr)	13.10 (3) s	ce	0.176	26.4	X	0.012-0.014	17.0
			0.189	4.6			
					γ	0.190	67.6

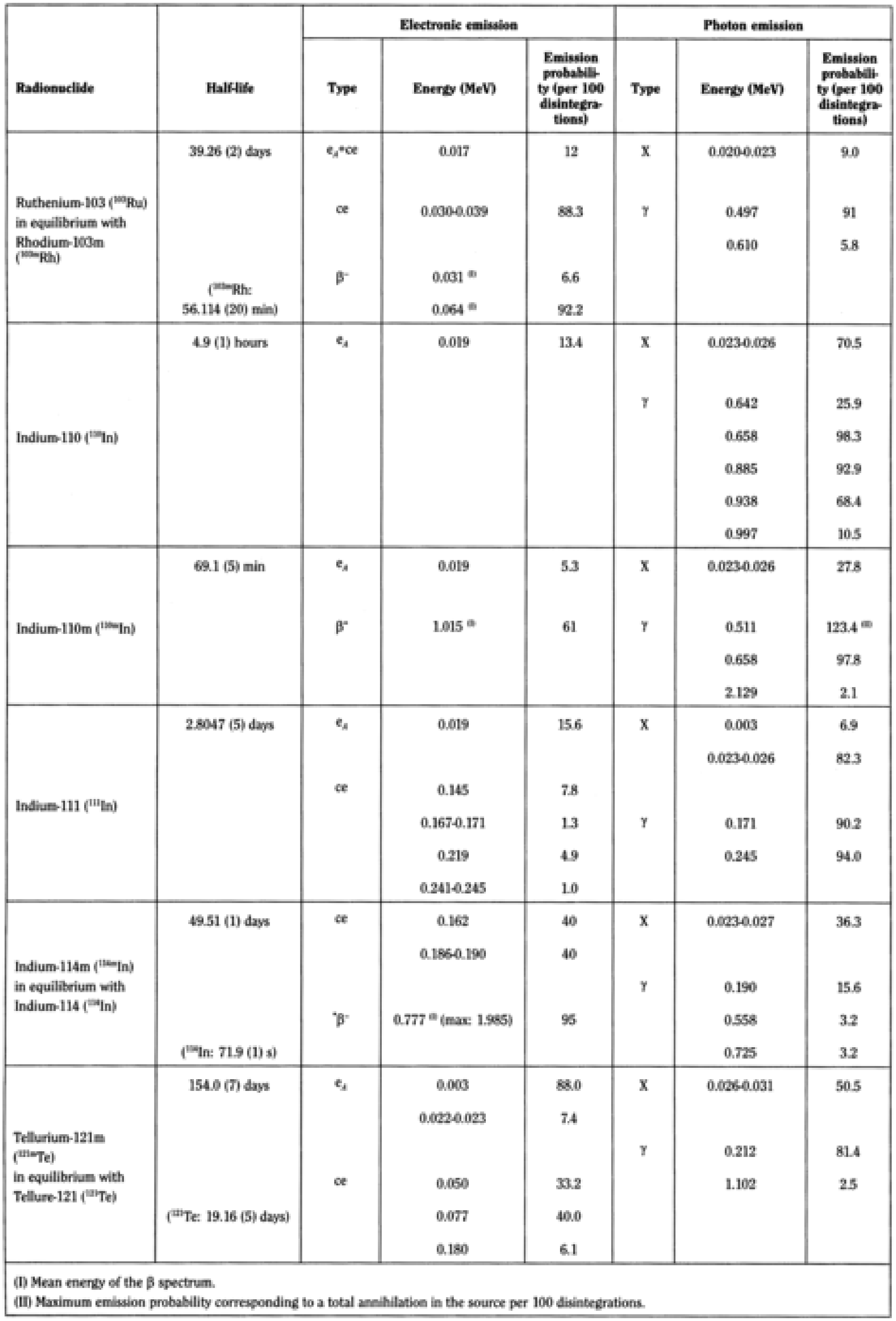
(I) Mean energy of the β spectrum.

(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.



Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Rubidium-81 (^{81}Rb) in equilibrium with Krypton-81m (^{81m}Kr)	4.576 (5) hours	e_A	0.011	31.3	X	0.013-0.014	57.2
		ce	0.176	25.0	γ	0.190	64
			0.188	4.3		0.446	23.2
						0.457	3.0
		β^+	0.253 $^{(I)}$	1.8		0.510	5.3
			0.447 $^{(I)}$	25.0		0.511	54.2
	(^{81m}Kr: 13.10 (3) s)					0.538	2.2
Strontium-89 (^{89}Sr) in equilibrium with Yttrium-89m (^{89m}Y)	50.53 (7) days (^{89m}Y: 16.06 (4) s)	β^-	0.583 $^{(I)}$ (max: 1.492)	99.99	γ	0.909	0.01
Strontium-90 (^{90}Sr) in equilibrium with Yttrium-90 (^{90}Y)	28.74 (4) years (^{90}Y: 64.10 (8) hours)	β^-	0.196 $^{(I)}$ (max: 0.546)	100			
Yttrium-90 (^{90}Y)	64.10 (8) hours	β^-	0.934 $^{(I)}$ (max: 2.280)	100			
Molybdene-99 (^{99}Mo) in equilibrium with Technetium-99m (^{99m}Tc)	65.94 (1) hours	β^-	0.133 $^{(I)}$	16.4	X	0.018-0.021	3.6
			0.290 $^{(I)}$	1.1			
			0.443 $^{(I)}$	82.4	γ	0.041	1.1
						0.141	4.5
						0.181	6
						0.366	1.2
	(^{99m}Tc: 6.01 (1) hours)					0.740	12.1
						0.778	4.3
Technetium-99m (^{99m}Tc)	6.01 (1) hours	ce	0.002	74	X	0.018-0.021	7.3
		e_A	0.015	2.1	γ	0.141	89.1
		ce	0.120	9.4			
			0.137-0.140	1.3			
Technetium-99 (^{99}Tc)	2.11 × 10^5 years	β^-	0.085 $^{(I)}$ (max: 0.294)	100			

(I) Mean energy of the β spectrum.
(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.



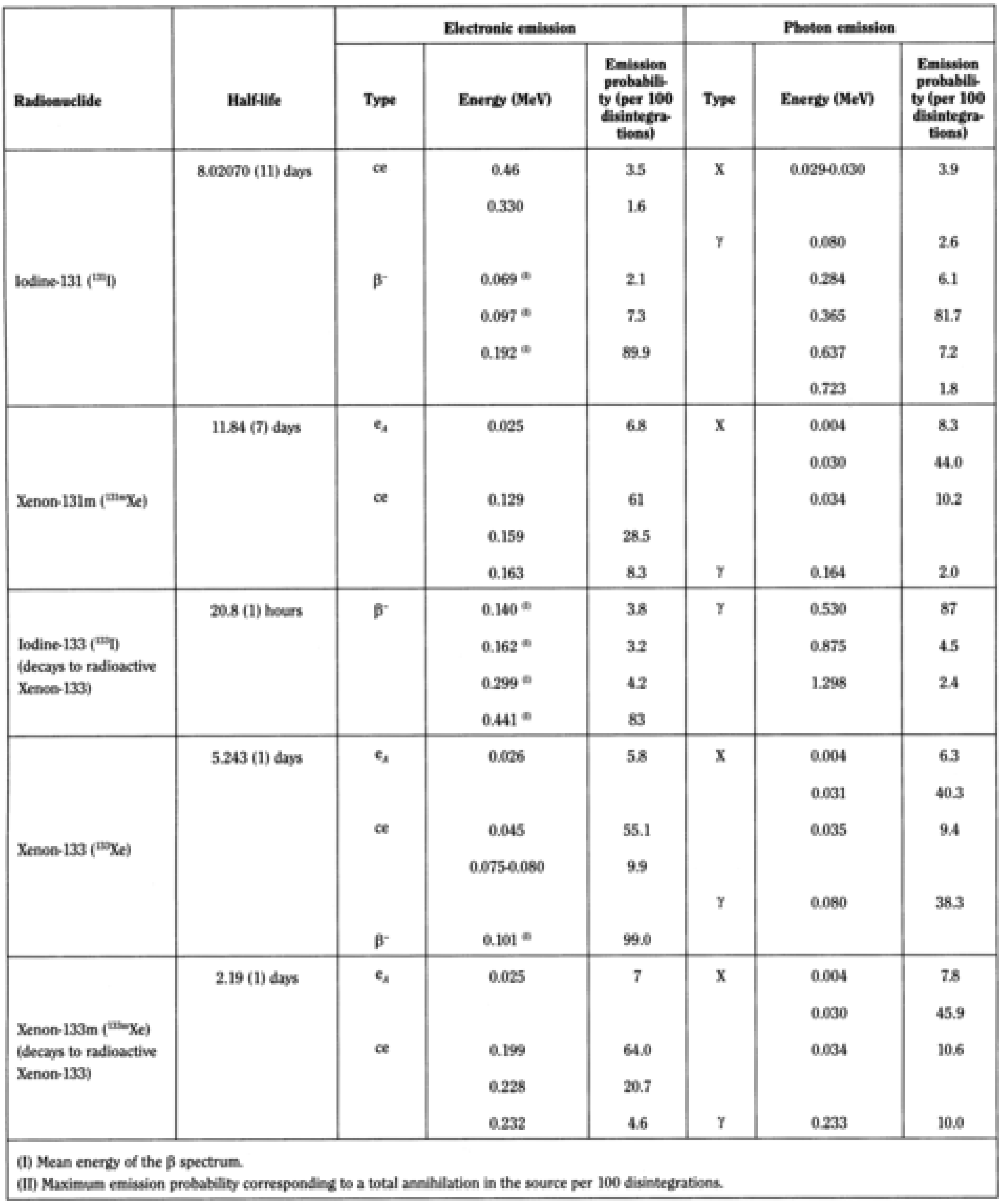
Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Ruthenium-103 (^{103}Ru) in equilibrium with Rhodium-103m (^{103m}Rh)	39.26 (2) days	e_A+ce	0.017	12	X	0.020-0.023	9.0
		ce	0.030-0.039	88.3	γ	0.497	91
						0.610	5.8
		β^-	0.031 (I)	6.6			
	(^{103m}Rh: 56.114 (20) min)		0.064 (I)	92.2			
Indium-110 (^{110}In)	4.9 (1) hours	e_A	0.019	13.4	X	0.023-0.026	70.5
					γ	0.642	25.9
						0.658	98.3
						0.885	92.9
						0.938	68.4
						0.997	10.5
Indium-110m (^{110m}In)	69.1 (5) min	e_A	0.019	5.3	X	0.023-0.026	27.8
		β^+	1.015 (I)	61	γ	0.511	123.4 (II)
						0.658	97.8
						2.129	2.1
Indium-111 (^{111}In)	2.8047 (5) days	e_A	0.019	15.6	X	0.003	6.9
						0.023-0.026	82.3
		ce	0.145	7.8			
			0.167-0.171	1.3	γ	0.171	90.2
			0.219	4.9		0.245	94.0
			0.241-0.245	1.0			
Indium-114m (^{114m}In) in equilibrium with Indium-114 (^{114}In)	49.51 (1) days	ce	0.162	40	X	0.023-0.027	36.3
			0.186-0.190	40			
					γ	0.190	15.6
		β^-	0.777 (I) (max: 1.985)	95		0.558	3.2
	(^{114}In: 71.9 (1) s)					0.725	3.2
Tellurium-121m (^{121m}Te) in equilibrium with Tellure-121 (^{121}Te)	154.0 (7) days	e_A	0.003	88.0	X	0.026-0.031	50.5
			0.022-0.023	7.4			
					γ	0.212	81.4
		ce	0.050	33.2		1.102	2.5
	(^{121}Te: 19.16 (5) days)		0.077	40.0			
			0.180	6.1			

(I) Mean energy of the β spectrum.

(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.

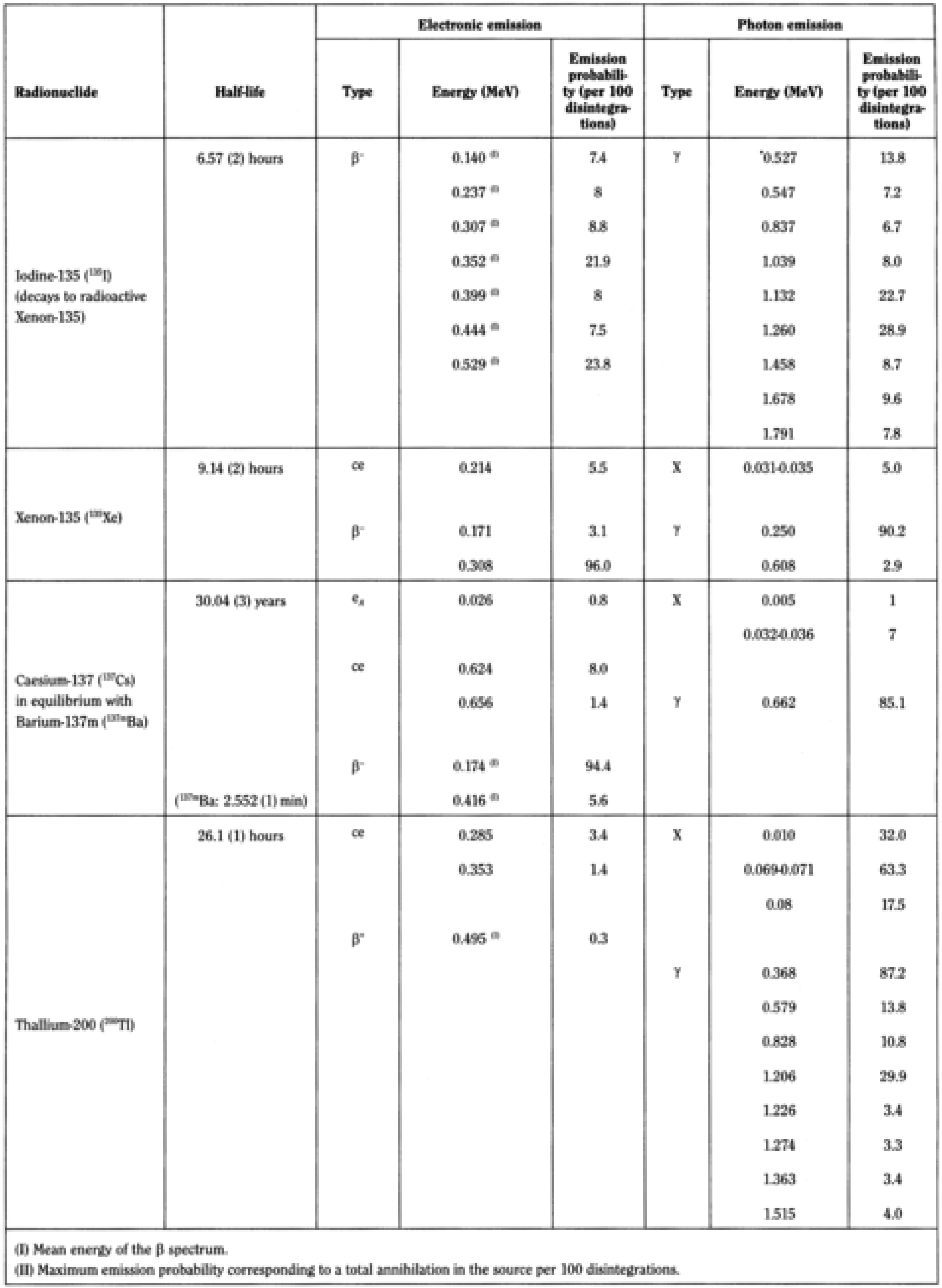
Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Tellurium-121 (^{121}Te)	$^{-}$19.16 (5) days	e_A	0.022	11.6	X	0.026-0.030	75.6
					γ	0.470	1.4
						0.508	17.7
						0.573	80.3
Iodine-123 (^{123}I)	13.27 (8) hours	e_A	0.023	12.3	X	0.004	9.3
						0.027-0.031	86.6
		ce	0.127	13.6			
			0.154	1.8	γ	0.159	83.3
			0.158	0.4		0.346	0.1
						0.440	0.4
						0.505	0.3
						0.529	1.4
						0.538	0.4
Iodine-125 (^{125}I)	59.402 (14) days	e_A+ce	0.004	80	X	0.004	15.5
			0.023-0.035	33		0.027	114
						0.031	26
					γ	0.035	6.7
Iodine-126 (^{126}I)	13.11 (5) days	e_A	0.023	6	X	0.027-0.031	42.2
		ce	0.354	0.5	γ	0.388	34
			0.634	0.1		0.491	2.9
						0.511	2.3 $^{(II)}$
		β^-	0.109 $^{(I)}$	3.6		0.666	33
			0.290 $^{(I)}$	32.1		0.754	4.2
			0.459 $^{(I)}$	8.0		0.880	0.8
						1.420	0.3
		β^+	0.530 $^{(I)}$	1			

(I) Mean energy of the β spectrum.
(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.



Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Iodine-131 (^{131}I)	8.02070 (11) days	ce	0.46	3.5	X	0.029-0.030	3.9
			0.330	1.6			
					γ	0.080	2.6
		β⁻	0.069 (I)	2.1		0.284	6.1
			0.097 (I)	7.3		0.365	81.7
			0.192 (I)	89.9		0.637	7.2
						0.723	1.8
Xenon-131m (^{131m}Xe)	11.84 (7) days	e_A	0.025	6.8	X	0.004	8.3
						0.030	44.0
		ce	0.129	61		0.034	10.2
			0.159	28.5			
			0.163	8.3	γ	0.164	2.0
Iodine-133 (^{133}I) (decays to radioactive Xenon-133)	20.8 (1) hours	β⁻	0.140 (I)	3.8	γ	0.530	87
			0.162 (I)	3.2		0.875	4.5
			0.299 (I)	4.2		1.298	2.4
			0.441 (I)	83			
Xenon-133 (^{133}Xe)	5.243 (1) days	e_A	0.026	5.8	X	0.004	6.3
						0.031	40.3
		ce	0.045	55.1		0.035	9.4
			0.075-0.080	9.9			
					γ	0.080	38.3
		β⁻	0.101 (I)	99.0			
Xenon-133m (^{133m}Xe) (decays to radioactive Xenon-133)	2.19 (1) days	e_A	0.025	7	X	0.004	7.8
						0.030	45.9
		ce	0.199	64.0		0.034	10.6
			0.228	20.7			
			0.232	4.6	γ	0.233	10.0

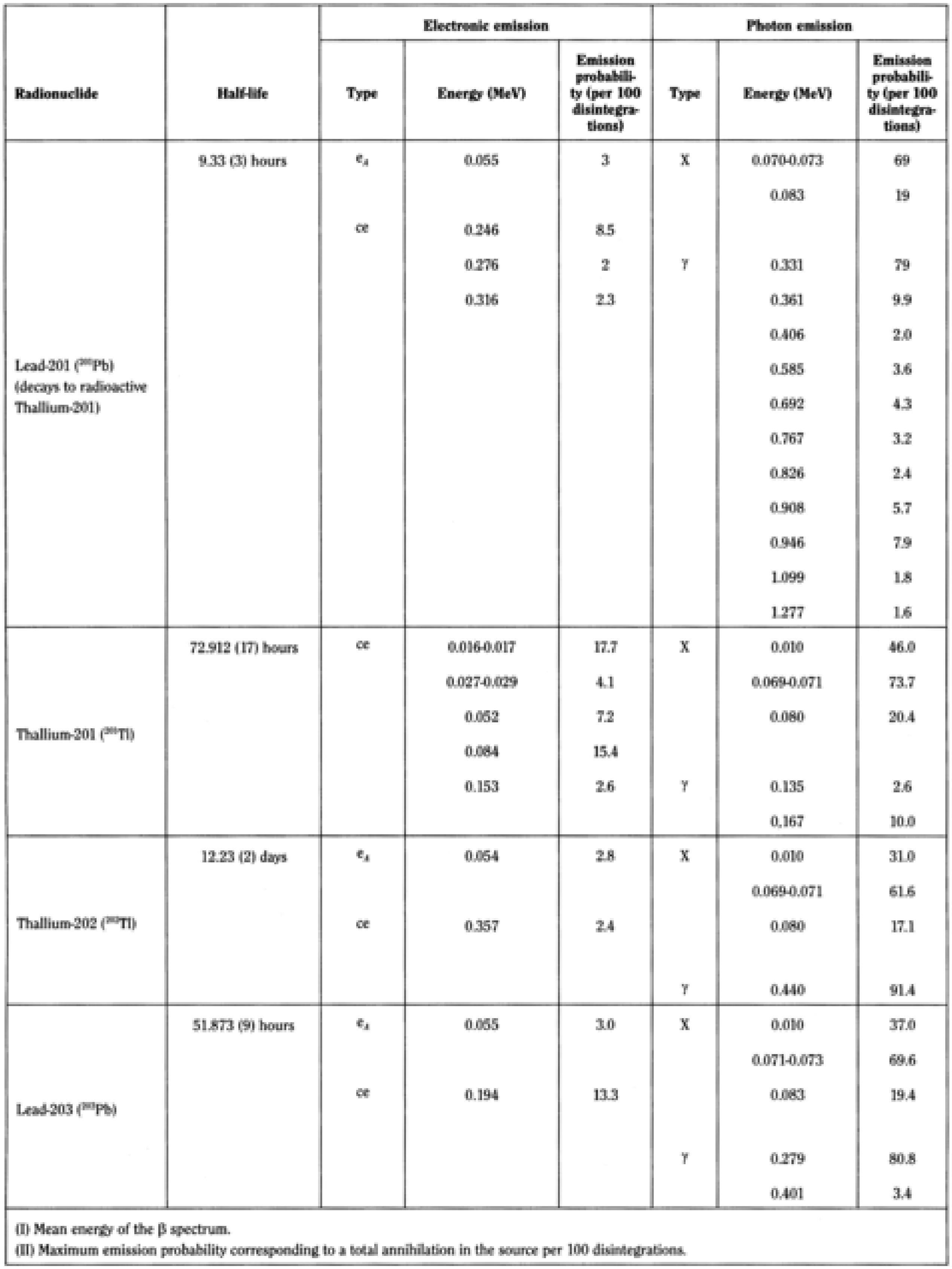
(I) Mean energy of the β spectrum.
(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.



Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Iodine-135 (^{135}I) (decays to radioactive Xenon-135)	6.57 (2) hours	β^-	0.140 (I)	7.4	γ	0.527	13.8
			0.237 (I)	8		0.547	7.2
			0.307 (I)	8.8		0.837	6.7
			0.352 (I)	21.9		1.039	8.0
			0.399 (I)	8		1.132	22.7
			0.444 (I)	7.5		1.260	28.9
			0.529 (I)	23.8		1.458	8.7
						1.678	9.6
						1.791	7.8
Xenon-135 (^{135}Xe)	9.14 (2) hours	ce	0.214	5.5	X	0.031-0.035	5.0
		β^-	0.171	3.1	γ	0.250	90.2
			0.308	96.0		0.608	2.9
Caesium-137 (^{137}Cs) in equilibrium with Barium-137m (^{137m}Ba)	30.04 (3) years	e_A	0.026	0.8	X	0.005	1
						0.032-0.036	7
		ce	0.624	8.0			
			0.656	1.4	γ	0.662	85.1
		β^-	0.174 (I)	94.4			
	(^{137m}Ba: 2.552 (1) min)		0.416 (I)	5.6			
Thallium-200 (^{200}Tl)	26.1 (1) hours	ce	0.285	3.4	X	0.010	32.0
			0.353	1.4		0.069-0.071	63.3
						0.08	17.5
		β^+	0.495 (I)	0.3			
					γ	0.368	87.2
						0.579	13.8
						0.828	10.8
						1.206	29.9
						1.226	3.4
						1.274	3.3
						1.363	3.4
						1.515	4.0

(I) Mean energy of the β spectrum.

(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.



Radionuclide	Half-life	Electronic emission			Photon emission		
		Type	Energy (MeV)	Emission probability (per 100 disintegrations)	Type	Energy (MeV)	Emission probability (per 100 disintegrations)
Lead-201 (^{201}Pb) (decays to radioactive Thallium-201)	9.33 (3) hours	e_A	0.055	3	X	0.070-0.073	69
						0.083	19
		ce	0.246	8.5			
			0.276	2	γ	0.331	79
			0.316	2.3		0.361	9.9
						0.406	2.0
						0.585	3.6
						0.692	4.3
						0.767	3.2
						0.826	2.4
						0.908	5.7
						0.946	7.9
						1.099	1.8
						1.277	1.6
Thallium-201 (^{201}Tl)	72.912 (17) hours	ce	0.016-0.017	17.7	X	0.010	46.0
			0.027-0.029	4.1		0.069-0.071	73.7
			0.052	7.2		0.080	20.4
			0.084	15.4			
			0.153	2.6	γ	0.135	2.6
						0,167	10.0
Thallium-202 (^{202}Tl)	12.23 (2) days	e_A	0.054	2.8	X	0.010	31.0
						0.069-0.071	61.6
		ce	0.357	2.4		0.080	17.1
					γ	0.440	91.4
Lead-203 (^{203}Pb)	51.873 (9) hours	e_A	0.055	3.0	X	0.010	37.0
						0.071-0.073	69.6
		ce	0.194	13.3		0.083	19.4
					γ	0.279	80.8
						0.401	3.4

(I) Mean energy of the β spectrum.
(II) Maximum emission probability corresponding to a total annihilation in the source per 100 disintegrations.

Technetium [^{99m} Tc] Albumin Injection

General Notices

(Technetium (^{99m}Tc) Human Albumin Injection, Ph Eur monograph 0640)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) human albumin injection is a sterile, apyrogenic solution of human albumin labelled with technetium-99m. It contains a reducing substance, such as a tin salt in an amount not exceeding 1 mg of Sn per millilitre; it may contain a suitable buffer and an antimicrobial preservative. Although, at present, no definite value for a maximum limit of tin can be fixed, available evidence tends to suggest the importance of keeping the ratio of tin to albumin as low as possible. The human albumin used complies with the requirements of the monograph on *Human albumin solution (0255).* The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the quantity of albumin stated on the label. Not less than 80 per cent of the radioactivity is associated with the albumin fractions II to V. Not more than 5.0 per cent of the radioactivity due to technetium-99m corresponds to free pertechnetate, as determined by the method described in the test for radiochemical purity.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile and apyrogenic ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A clear, colourless or pale-yellow solution.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Using a suitable range of species-specific antisera, carry out precipitation tests on the preparation to be examined. The test is to be carried out using antisera specific to the plasma proteins of each species of domestic animal currently used in the preparation of materials of biological origin in the country concerned. The injection is shown to contain proteins of human origin and gives negative results with antisera specific to plasma proteins of other species.

C. Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and the injection to be examined, both diluted if necessary. The main component of the injection to be examined corresponds to the main component of the normal human serum. The diluted solution may show the presence of small quantities of other plasma proteins.

TESTS

pH (*2.2.3*)

The pH of the injection is 2.0 to 6.5.

Albumin

Reference solution Dilute *human albumin solution R* with a 9 g/l solution of *sodium chloride R* to a concentration of 5 mg of albumin per millilitre.

To 1.0 ml of the injection to be examined and to 1.0 ml of the reference solution add 4.0 ml of *biuret reagent R* and mix. After exactly 30 min, measure the absorbance (*2.2.25*) of each solution at 540 nm, using as the compensation liquid a 9 g/l solution of *sodium chloride R* treated in the same manner. From the absorbances measured, calculate the content of albumin in the injection to be examined in milligrams per millilitre.

Tin

Test solution To 1.0 ml of the injection to be examined add 1.0 ml of *2 M hydrochloric acid R* . Heat in a water-bath at 100 °C for 30 min. Cool and centrifuge at 300 g for 10 min. Dilute 1.0 ml of the supernatant liquid to 10 ml with *1 M hydrochloric acid*.

Reference solution Dissolve 95 mg of *stannous chloride R* in *1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

To 1.0 ml of each solution add 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R* , 0.05 ml of *thioglycollic acid R* , 0.1 ml of *dithiol reagent R* and 3.0 ml of *0.2 M hydrochloric acid* . Mix. Measure the absorbance (*2.2.25*) of each solution at 540 nm, using *0.2 M hydrochloric acid* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution (1 mg of Sn per millilitre).

Physiological distribution

Inject a volume not greater than 0.5 ml and containing not more than 1.0 mg of albumin into a suitable vein such as a caudal vein or a saphenous vein of each of three male rats, each weighing 150 g to 250 g. Measure the radioactivity in the syringe before and after the injection. Euthanise the rats 30 min after the injection. Take one millilitre of blood by a suitable method and remove the liver and, if a caudal vein has been used for the injection, the tail. Using a suitable instrument determine the radioactivity in 1 ml of blood, in the liver and, if a caudal vein has been used for the injection, in the tail. Determine the percentage of radioactivity in the liver and in 1 ml of blood with respect to the total radioactivity calculated as the difference between the measurements made on the syringe minus the activity in the tail (if a caudal vein has been used for the injection). Correct the blood concentration by multiplying by a factor of *m*/200 where *m* is the body mass of the rat in grams. In not fewer than two of the three rats used, the radioactivity in the liver is not more than 15 per cent and that in blood, after correction, is not less than 3.5 per cent.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/V IU/ml, V being the maximum recommended dose in millilitres.

RADIOCHEMICAL PURITY

A. Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for 10 min. Use a plate such that during development the mobile phase migrates over a distance of 10 cm to 15 cm in about 10 min.

Apply to the plate 5 µl to 10 µl of the injection to be examined and allow to dry. Develop over a path of 10 cm to 15 cm using *methyl ethyl ketone R* . Allow the plate to dry. Determine the distribution of radioactivity using a suitable detector. The technetium-99m human albumin complex remains at the starting-point and pertechnetate ion migrates near to the solvent front. Not more than 5.0 per cent of the technetium-99m radioactivity corresponds to technetium in the form of pertechnetate ion.

B. Examine by size-exclusion chromatography (*2.2.30*).

Mobile phase (concentrated) Dissolve 1.124 g of *potassium dihydrogen phosphate R* , 4.210 g of *disodium hydrogen phosphate R* , 1.17 g of *sodium chloride R* and 0.10 g of *sodium azide R* in 100 ml of *water R.*

Test solution Mix 0.25 ml of the injection to be examined with 0.25 ml of the mobile phase (concentrated). Use immediately after dilution.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.6 m long and 7.5 mm in internal diameter, packed with *silica gel for size-exclusion chromatography R,*

— as the mobile phase at a flow rate of 0.6 ml/min a mixture of equal volumes of mobile phase (concentrated) and *water R,*

— a radioactivity detector set for technetium-99m,

— a loop injector.

Inject 200 µl of the test solution. Continue the chromatography for at least 10 min after background level is reached.

Peaks are eluted with the following retention times:

I	High molecular mass compound	19-20 min
II	Poly III-albumin	23-24 min
III	Poly II-albumin	25-27 min

IV	Poly V I-albumin	28-29 min
V	Human serum albumin	32-33 min
VI	Tin colloid	40-47 min
VII	Pertechnetate	48 min

At least 80 per cent of the radioactivity applied to the column is associated with the albumin fractions II to V.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

LABELLING

The label states:

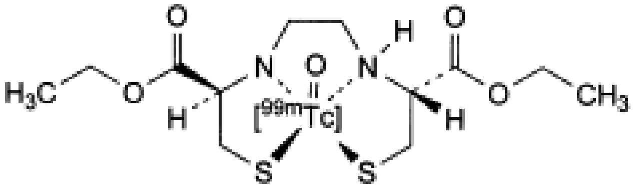
- — the amount of albumin,
- — the amount of tin, if any.

Ph Eur

Technetium [^{99m} Tc] Bicisate Injection

General Notices

(Ph Eur monograph 2123)



Ph Eur

DEFINITION

Sterile solution of a complex of technetium-99m with diethyl *N*,*N′*-ethylenedi-L-cysteinate. It may contain stabilisers and inert additives such as *Mannitol (0559)* and *Disodium edetate (0232).*

Content

90 per cent to 110 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label.

PRODUCTION

It is prepared from *N*,*N′*-(1,2-ethylenediyl)bis[(2*R*)-2-amino-3-sulphanylpropanoic acid] diethyl ester and *Sodium pertechnetate (* ^{99m} *Tc) injection (fission) (0124)* or *Sodium pertechnetate (* ^{99m} *Tc) injection (non-fission) (0283)* in the presence of reducing agents such as a stannous salt.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of technetium-99m

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The most prominent gamma photon of technetium-99m has an energy of 0.141 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity (see Tests).

Results The principal peak in the chromatogram obtained with the test solution is similar in retardation factor to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

pH (*2.2.3*)

6.5 to 7.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Impurities A, B, C, D, E, F

Thin-layer chromatography (*2.2.27*).

Test solution The preparation to be examined.

Reference solution (a) To vial B of *bicisate labelling kit CRS* in lead shielding add 2 ml of sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) containing 400-800 MBq. Dissolve the contents of vial A of *bicisate labelling kit CRS* in 3 ml of a 9 g/l solution of *sodium chloride R* . Immediately transfer 1.0 ml of the solution contained in vial A to vial B. Mix and allow to stand for 30 min at room temperature.

Reference solution (b) Sodium pertechnetate (^{99m}Tc) injection (fission or non fission).

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R.*

Application 5 µl, allow the spots to dry for 5-10 min.

Development Over 4/5 of the plate.

Drying In air.

Detection Determine the distribution of radioactivity using a suitable detector.

Retardation factors Technetium-99m bicisate = more than 0.4; impurities A, B, C, D, E and F = less than 0.2.

System suitability The retardation factor of the principal peak in the chromatogram obtained with reference solution (a) is clearly different from the retardation factor of the peak in the chromatogram obtained with reference solution (b).

Limit:

— *sum of impurities A, B, C, D, E and F*: not more than 6 per cent of the total radioactivity.

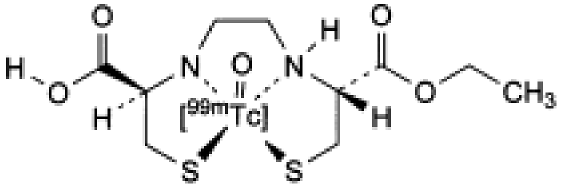
RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

IMPURITIES

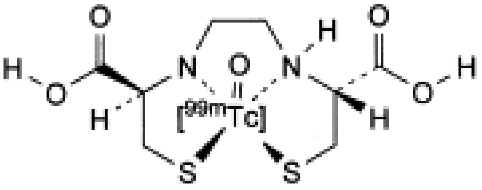
A. technetium-99m in colloidal form,

B. [^{99m}Tc]pertechnetate ion,



and isomers

C. complex of technetium-99m with ethyl hydrogen *N*,*N*′-ethylenedi-L-cysteinate,



D. complex of technetium-99m with *N*,*N*′-ethylenedi-L-cysteine,

E. complex of technetium-99m with mannitol,

F. complex of technetium-99m with disodium edetate.

Ph Eur

Technetium [^{99m} Tc] Colloidal Rhenium Sulphide Injection

General Notices

(Ph Eur monograph 0126)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) colloidal rhenium sulphide injection is a sterile, apyrogenic colloidal dispersion of rhenium sulphide the micelles of which are labelled with technetium-99m. It is stabilised with gelatin. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 92 per cent of the radioactivity corresponds to technetium-99m in colloidal form. The pH of the injection may be adjusted by the addition of a suitable buffer such as a citrate buffer solution. The injection contains a variable amount of colloidal rhenium sulphide, not exceeding 0.22 mg of rhenium (Re) per millilitre, according to the method of preparation.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile, apyrogenic ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A light-brown liquid.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the injection.

C. To 1 ml add 5 ml of *hydrochloric acid R* , 5 ml of a 50 g/l solution of *thiourea R* and 1 ml of a 200 g/l solution of *stannous chloride R* in *hydrochloric acid R* . A yellow colour is produced.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.0 to 7.0.

Rhenium

Test solution Use 1 ml of the injection to be examined.

Reference solutions Using a solution containing 100 µg of *potassium perrhenate R* (equivalent to 60 ppm of Re) and 240 µg of *sodium thiosulphate R* per millilitre, prepare a suitable range of solutions and dilute to the same final volume with *water R* .

To the test solution and to 1 ml of each of the reference solutions add 5 ml of *hydrochloric acid R* , 5 ml of a 50 g/l solution of *thiourea R* and 1 ml of a 200 g/l solution of *stannous chloride R* in *hydrochloric acid R* and dilute to 25.0 ml with *water R* . Allow to stand for 40 min and measure the absorbance (*2.2.25*) of each solution at 400 nm, using a reagent blank as the compensation liquid. Using the absorbances obtained with the reference solutions, draw a calibration curve and calculate the concentration of rhenium in the injection to be examined.

Physiological distribution

Inject a volume not greater than 0.2 ml into the caudal vein of each of three mice each weighing 20 g to 25 g. Euthanise the mice 20 min after the injection, remove the liver, spleen and lungs and measure the radioactivity in the organs using a suitable instrument. Measure the radioactivity in the rest of the body after having removed the tail. Determine the percentage of radioactivity in the liver, the spleen and the lungs from the expression:

$$\frac{A}{B} \times 100$$

A = radioactivity of the organ concerned,

B = total radioactivity in the liver, the spleen, the lungs and the rest of the body.

In each of the three mice at least 80 per cent of the radioactivity is found in the liver and spleen and not more than 5 per cent in the lungs. If the distribution of radioactivity in one of the three mice does not correspond to the prescribed proportions, repeat the test on a further three mice. The preparation complies with the test if the prescribed distribution of radioactivity is found in five of the six mice used. The injection may be released for use before completion of the test.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

Pyrogens

It complies with the test for pyrogens prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . Inject not less than 0.1 ml per kilogram of the rabbit's mass. The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by ascending paper chromatography (*2.2.26*).

Apply to the paper 10 µl of the injection. Develop immediately over a path of 10 cm to 15 cm using a 9 g/l solution of *sodium chloride R* . Allow the paper to dry. Determine the distribution of radioactivity using a suitable detector. Technetium-99m in colloidal form remains at the starting-point and pertechnetate ion migrates with an R_F of about 0.6. There may be other impurities with an R_F of 0.8 to 0.9. The radioactivity corresponding to technetium-99m in colloidal form represents not less than 92 per cent of the total radioactivity of the chromatogram.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

LABELLING

The label states, in particular, the quantity of rhenium per millilitre.

Ph Eur

Technetium [^{99m} Tc] Colloidal Sulphur Injection

General Notices

(Ph Eur monograph 0131)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) colloidal sulphur injection is a sterile, apyrogenic colloidal dispersion of sulphur, the micelles of which are labelled with technetium-99m. It may be stabilised with a colloid-protecting substance based on gelatin. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 92 per cent of the radioactivity corresponds to technetium-99m in colloidal form. The pH of the injection may be adjusted by the addition of a suitable buffer, such as an acetate, citrate or phosphate buffer solution. The injection contains a variable amount of colloidal sulphur, according to the method of preparation.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile, apyrogenic ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A clear to opalescent, colourless to yellowish liquid.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the injection.

C. In a test-tube 100 mm long and 16 mm in internal diameter, evaporate 0.2 ml of the injection to dryness. Dissolve the sulphur by shaking the residue with 0.2 ml of *pyridine R* and add about 20 mg of *benzoin R* . Cover the open end of the tube with a filter paper moistened with *lead acetate solution R* . Heat the test-tube in a bath containing glycerol at 150 °C. The paper slowly becomes brown.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.0 to 7.0.

Physiological distribution

Inject a volume not greater than 0.2 ml into the caudal vein of each of 3 mice, each weighing 20 g to 25 g. Euthanise the mice 20 min after the injection, remove the liver, spleen and lungs and measure the radioactivity in the organs using a suitable instrument. Measure the radioactivity in the rest of the body after having removed the tail. Determine the percentage of radioactivity in the liver, the spleen and the lungs from the expression:

$$\frac{A}{B} \times 100$$

A = radioactivity of the organ concerned,

B = total radioactivity in the liver, the spleen, the lungs and the rest of the body.

In each of the 3 mice at least 80 per cent of the radioactivity is found in the liver and spleen and not more than 5 per cent in the lungs. If the distribution of radioactivity in 1 of the 3 mice does not correspond to the prescribed proportions, repeat the test on a further 3 mice. The preparation complies with the test if the prescribed distribution of radioactivity is found in 5 of the 6 mice used. The injection may be released for use before completion of the test.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

Pyrogens

It complies with the test for pyrogens prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . Inject not less than 0.1 ml per kilogram of the rabbit's mass. The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by ascending paper chromatography (*2.2.26*).

Apply to the paper 10 µl of the injection. Develop immediately over a path of 10 cm to 15 cm with a 9 g/l solution of *sodium chloride R* . Allow the paper to dry. Determine the distribution of radioactivity using a suitable detector. Technetium-99m in colloidal form remains at the starting-point and pertechnetate ion migrates with an R_F of 0.6. There may be other impurities of R_F 0.8 to 0.9. The radioactivity corresponding to technetium-99m in colloidal form represents not less than 92 per cent of the total radioactivity of the chromatogram.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Technetium [^{99m} Tc] Colloidal Tin Injection

General Notices

(Ph Eur monograph 0689)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) colloidal tin injection is a sterile, colloidal dispersion of tin labelled with technetium-99m. The injection contains a variable quantity of tin not exceeding 1 mg of Sn per millilitre; it contains fluoride ions, it may be stabilised with a suitable, apyrogenic colloid-protecting substance and it may contain a suitable buffer. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 95 per cent of the radioactivity corresponds to technetium-99m in colloidal form.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration. Syringes for handling the eluate intended for labelling of the final product, or the final product, should not contain rubber parts.

CHARACTERS

A clear or opalescent, colourless liquid.

Technetium-99m has a half life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Mix 0.05 ml of *zirconyl nitrate solution R* with 0.05 ml of *alizarin S solution R.* Add 0.05 ml of the injection to be examined. A yellow colour is produced.

TESTS

pH (*2.2.3*)

The pH of the injection to be examined is 4.0 to 7.0.

Tin

Test solution Dilute 3.0 ml of the injection to be examined to 50.0 ml with *1 M hydrochloric acid*.

Reference solution Dissolve 0.115 g of *stannous chloride R* in *1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

To 1.0 ml of each solution add 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R*, 0.05 ml of *thioglycollic acid R*, 0.1 ml of *dithiol reagent R* and 3.0 ml of *0.2 M hydrochloric acid*. Mix. Measure the absorbance (*2.2.25*) of each solution at 540 nm, using *0.2 M hydrochloric acid* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution (1 mg of Sn per millilitre).

Physiological distribution

Inject not more than 0.2 ml into a caudal vein of each of three mice, each weighing 20 g to 25 g. Euthanise the mice 20 min after the injection and remove the liver, spleen and lungs. Measure the radioactivity in the organs using a suitable instrument. Measure the radioactivity in the rest of the body, after having removed the tail. Determine the percentage of radioactivity in the liver, the spleen and the lungs with respect to the total radioactivity of all organs and the rest of the body excluding the tail.

In each of the three mice at least 80 per cent of the radioactivity is found in the liver and spleen and not more than 5 per cent in the lungs. If the distribution of radioactivity in one of the three mice does not correspond to the prescribed proportions, repeat the test on a further three mice. The preparation complies with the test if the prescribed distribution of radioactivity is found in five of the six mice used.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for 10 min. Use a plate such that during development the mobile phase migrates over a distance of 10 cm to 15 cm in about 10 min.

Apply to the plate 5 µl to 10 µl of the injection to be examined. Develop immediately over a path of 10 cm to 15 cm using a 9 g/l solution of *sodium chloride R* purged with *nitrogen R*. Allow the plate to dry. Determine the distribution of radioactivity using a suitable detector. Technetium-99m in colloidal form remains at the starting point and pertechnetate ion migrates near to the solvent front. Not less than 95 per cent of the technetium-99m radioactivity corresponds to technetium in colloidal form.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Technetium [^{99m} Tc] Etifenin Injection

General Notices

(Ph Eur monograph 0585)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) etifenin injection is a sterile solution which may be prepared by mixing sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) with solutions of etifenin [[(2,6-diethylphenyl)carbamoylmethylimino]di-acetic acid; $C_{16}H_{22}N_2O_5$] and stannous chloride. The injection contains a variable quantity of tin (Sn) not exceeding 0.2 mg/ml. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 95.0 per cent of the radioactivity corresponds to technetium-99m complexed with etifenin.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable, sterile ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A clear, colourless solution.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine by liquid chromatography (*2.2.29*).

Test solution Dilute the injection to be examined with *methanol R* to obtain a solution containing about l mg of etifenin per millilitre.

Reference solution Dissolve 5.0 mg of *etifenin CRS* in *methanol R* and dilute to 5.0 ml with the same solvent.

The chromatographic procedure may be carried out using:

— a column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica* *gel for chromatography R* (5 µm to 10 µm),

— as mobile phase at a flow rate of 1 ml/min a mixture of 20 volumes of *methanol R* and 80 volumes of a 14 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 2.5 by the addition of *phosphoric acid R* ,

— a spectrophotometer set at 230 nm.

Inject 20 µl of each solution. The principal peak in the chromatogram obtained with the test solution has a similar retention time to the principal peak in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.0 to 6.0.

Physiological distribution

Inject 0.1 ml (equivalent to about 3.7 MBq) into a caudal vein of each of three mice, each weighing 20 g to 25 g. Euthanise the mice I h after the injection. Remove the liver, gall-bladder, small intestine, large intestine and kidneys, collecting excreted urine. Measure the radioactivity in the organs using a suitable instrument. Measure the radioactivity of the rest of the body, after having removed the tail. Determine the percentage of radioactivity in each organ from the expression:

$$\frac{A}{B} \times 100$$

A = radioactivity of the organ concerned,

B = radioactivity of all organs and the rest of the body, excluding the tail.

In not fewer than two mice the sum of the percentages of radioactivity in the gall-bladder and small and large intestine is not less than 80 per cent. Not more than 3 per cent of the radioactivity is present in the liver, and not more than 2 per cent in the kidneys

Tin

Test solution Dilute 1.0 ml of the injection to be examined to 5.0 ml with *1 M hydrochloric acid* .

Reference solution Prepare a reference solution containing 0.075 mg of *stannous chloride R* per millilitre in *1 M hydrochloric acid* .

To 1.0 ml of each solution add 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R* , 0.05 ml of *thioglycollic acid R* , 0.I ml of *dithiol reagent R* and 3.0 ml of *0.2 M hydrochloric acid* . Mix. Measure the absorbance (*2.2.25*) of each solution at 540 nm, using *0.2 M hydrochloric acid* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution (0.2 mg of Sn per millilitre).

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using silicic acid as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for l0 min. The plate used should be such that during development the mobile phase moves over a distance of 10 cm to 15 cm in about 15 min.

Apply to the plate 5 µl to 10 µl of the injection to be examined. Develop immediately over a path of 10 cm to 15 cm using a 9 g/l solution of *sodium chloride R*. Allow the plate to dry. Determine the distribution of radioactivity using a suitable detector. Technetium-99m complexed with etifenin migrates almost to the middle of the chromatogram and pertechnetate ion migrates with the solvent front. Impurities in colloidal form remain at the starting point. The radioactivity corresponding to technetium-99m complexed with etifenin represents not less than 95.0 per cent of the total radioactivity of the chromatogram.

RADIOACTIVITY

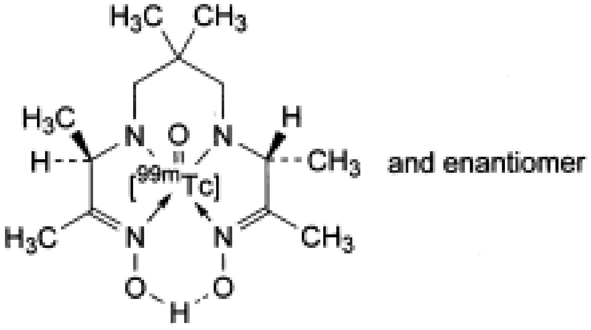
Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Technetium [^{99m} Tc] Exametazime Injection

General Notices

(Ph Eur monograph 1925)



Ph Eur

DEFINITION

Sterile solution of lipophilic technetium-99m exametazime which may be prepared by dissolving a racemic mixture of (3*RS*,9*RS*)-4,8-diaza-3,6,6,9-tetramethylundecane-2,10-dione bisoxime in the presence of a stannous salt in *Sodium pertechnetate (^{99m} Tc) injection (fission) (0124)* or *Sodium pertechnetate (^{99m} Tc) injection (non-fission) (0283)*. It may contain stabilisers and inert additives.

Content

90 per cent to 110 per cent of the declared technetium-99m radioactivity at the date and time stated on the label.

Purity

Minimum of 80 per cent of the total radioactivity corresponds to lipophilic technetium-99m exametazime and its meso isomer.

CHARACTERS

Appearance

Clear solution.

Half-life and nature of radiation of technetium-99m

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Comparison Standardised technetium-99m solution, or by using a calibrated instrument. Standardised technetium-99m solutions and/or standardisation services are available from the competent authority.

Results The spectrum obtained with the solution to be examined does not differ significantly from that obtained with a standardised technetium-99m solution. The most prominent gamma photon has an energy of 0.141 MeV.

B. Examine the chromatograms obtained in the test Impurity A under Radiochemical purity.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the peak due to lipophilic technetium-99m exametazime in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

5.0 to 10.0.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Impurity C

Thin-layer chromatography (*2.2.27*).

Test solution The preparation to be examined.

Plate *TLC silica gel plate R* ; use a glass-fibre plate.

Mobile phase 9 g/l solution of *sodium chloride R.*

Application About 5 µl.

Development Immediate, over 2/3 of the plate.

Drying In air.

Detection Determine the distribution of radioactivity using a suitable detector.

Retardation factors Impurity C = 0.8 to 1.0; lipophilic technetium-99m exametazime and impurities A, B, D and E do not migrate.

Limits:

— *impurity C*: maximum 10 per cent of the total radioactivity.

Total of lipophilic technetium-99m exametazime and impurity A

Thin-layer chromatography (*2.2.27*).

Test solution The preparation to be examined.

Plate *TLC silica gel plate R* ; use a glass-fibre plate.

Mobile phase *methyl ethyl ketone R* .

Application About 5 µl.

Development Immediate, over 2/3 of the plate.

Drying In air.

Detection Determine the distribution of radioactivity using a suitable detector.

Retardation factors Lipophilic technetium-99m exametazime = 0.8 to 1.0, impurity A = 0.8 to 1.0, impurity C = 0.8 to 1.0; impurities B, D and E do not migrate.

Limits Calculate the percentage of radioactivity due to impurities B, D and E from test B *(B)* and the percentage of the radioactivity due to impurity C from test A *(A)*. Calculate the total percentage of lipophilic technetium-99m exametazime and impurity A from the expression:

$$100 - A - B$$

— *total of lipophilic technetium-99m exametazime and impurity A*: minimum 80 per cent of the total radioactivity.

Impurity A

Liquid chromatography (*2.2.29*).

Test solution The preparation to be examined.

Reference solution Dissolve the contents of a vial of *meso-rich exametazime CRS* in 0.5 ml of a 9 g/l solution of *sodium chloride R* and transfer to a lead-shielded, nitrogen-filled vial. Add 6 µl of a freshly prepared 1 g/l solution of *stannous chloride R* in *0.05 M hydrochloric acid* and 2.5 ml of sodium pertechnetate (99mTc) injection (fission or non-fission) containing 370-740 MBq. Mix carefully and use within 30 min of preparation.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 13 nm and a carbon loading of 11 per cent.

Mobile phase Mix 33 volumes of *acetonitrile R* and 67 volumes of *0.1 M phosphate buffer solution pH 3.0 R* .

Flow rate 1.5 ml/min.

Detection Radioactivity detector.

Injection Loop injector.

Run time 20 min.

Relative retention With reference to lipophilic technetium-99m exametazime: impurity A = about 1.2.

System suitability Reference solution:

— chromatogram similar to the chromatogram provided with *meso-rich exametazime CRS,*

— *resolution*: minimum of 2 between the peaks due to lipophilic technetium-99m exametazime and to impurity A.

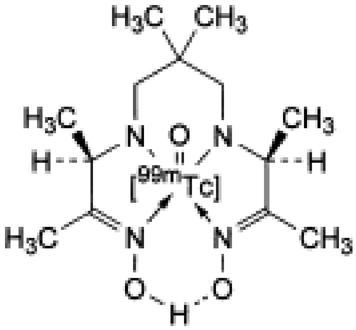
Limits:

— *impurity A*: maximum 5 per cent of the radioactivity due to lipophilic technetium-99m exametazime and impurity A.

RADIOACTIVITY

Measure the radioactivity using suitable equipment by comparison with a standardised technetium-99m solution or by using a calibrated instrument.

IMPURITIES



A. *meso* isomer of lipophilic technetium-99m exametazime,

B. technetium-99m in colloidal form,

C. [^{99m}Tc]pertechnetate ion,

D. non lipophilic technetium-99m exametazime complex,

E. *meso* isomer of non lipophilic technetium-99m exametazime complex.

Ph Eur

Technetium [^{99m} Tc] Gluconate Injection

General Notices

(Ph Eur monograph 1047)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) gluconate injection is a sterile solution, which may be prepared by mixing solutions of calcium gluconate and a stannous salt or other suitable reducing agent with sodium pertechnetate (^{99m}Tc) injection (fission or non-fission). The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 90 per cent of the radioactivity corresponds to technetium-99m gluconate complex.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A slightly opalescent solution.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. 5 µl of the solution complies with identification A prescribed in the monograph on *Calcium gluconate (0172).*

C. Examine the chromatograms obtained in the test for radiochemical purity. The distribution of the radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the solution is 6.0 to 8.5.

Physiological distribution

Inject a volume not greater than 0.2 ml into the caudal vein of each of three rats weighing 150 g to 250 g. Measure the radioactivity of the syringe before and after injection. Euthanise the rats 30 min after the injection. Remove at least 1 g of blood by a suitable method and remove the kidneys, the liver, the bladder plus voided urine and the tail. Weigh the sample of blood.

Determine the radioactivity in the organs, the blood sample and the tail using a suitable instrument. Calculate the percentage of radioactivity in each organ and in 1 g of blood with respect to the total radioactivity calculated as the difference between the two measurements made on the syringe minus the activity in the tail. Correct the blood concentration by multiplying by a factor of $m/200$ where m is the body mass of the rat in grams.

In not fewer than two of the three rats used, the radioactivity in the kidneys is not less than 15 per cent, that in the bladder plus voided urine is not less than 20 per cent and that in the liver is not more than 5 per cent. The radioactivity in the blood, after correction, is not more than 0.50 per cent.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for 10 min. Use a plate such that during development the mobile phase migrates over a distance of 10 cm to 15 cm in about 10 min.

a) Apply to the plate 5 µl to 10 µl of the solution to be examined. Develop immediately over a path of 10 cm to 15 cm using a 9 g/l solution of *sodium chloride R* . Allow the plate to dry. Determine the distribution of radioactivity using a suitable detector. Impurities in colloidal form remain at the starting point. Technetium gluconate complex and pertechnetate ion migrate near to the solvent front.

b) Apply to the plate 5 µl to 10 µl of the solution to be examined and allow to dry. Develop over a path of 10 cm to 15 cm using *methyl ethyl ketone R* . Dry in a current of warm air. Determine the distribution of radioactivity using a suitable detector. Pertechnetate ion impurity migrates near to the solvent front. Technetium gluconate complex and technetium in colloidal form remain at the starting point.

The sum of the percentages of radioactivity corresponding to impurities in the chromatograms obtained in test (a) and (b) does not exceed 10 per cent.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Technetium [^{99m} Tc] Macrosalb Injection

General Notices

(Ph Eur monograph 0296)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) macrosalb injection is a sterile, apyrogenic suspension of human albumin in the form of irregular insoluble aggregates obtained by denaturing human albumin in aqueous solution; the particles are labelled with technetium-99m. The injection contains reducing substances, such as tin salts in an amount not exceeding 3 mg of Sn per millilitre; it may contain a suitable buffer such as acetate, citrate or phosphate buffer and also non-denatured human albumin and an antimicrobial preservative such as benzyl alcohol. The human albumin employed complies with the requirements prescribed in the monograph on *Human albumin solution (0255)*. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 90 per cent of the technetium-99m is bound to the particles of the suspension as determined by the test for non-filterable radioactivity. The particles have a typical diameter between 10 µm and 100 µm. The specific radioactivity is not less than 37 MBq of technetium-99m per milligram of aggregated albumin at the date and hour of administration.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile and apyrogenic ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A white or almost white suspension which may separate on standing.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. The tests for non-filterable radioactivity and particle size contribute to the identification of the preparation.

C. Transfer 1 ml of the injection to a centrifuge tube and centrifuge at 2500 g for 5 min to 10 min. Decant the supernatant liquid. To the residue add 5 ml of *cupri-tartaric solution R2* , mix and allow to stand for 10 min. If necessary, heat to dissolve the particles and allow to cool. Add rapidly 0.5 ml of *dilute phosphomolybdotungstic reagent R* , mixing immediately. A blue colour develops.

TESTS

pH (*2.2.3*)

The pH of the injection is 3.8 to 7.5.

Non-filterable radioactivity

Use a polycarbonate membrane filter 13 mm to 25 mm in diameter, 10 µm thick and with circular pores 3 µm in diameter. Fit the membrane into a suitable holder. Place 0.2 ml of the injection on the membrane and filter, adding 20 ml of a 9 g/l solution of *sodium chloride R* during the filtration. The radioactivity remaining on the membrane represents not less than 90 per cent of the total radioactivity of the injection.

Particle size

Examine using a microscope. Dilute the injection if necessary so that the number of particles is just low enough for individual particles to be distinguished. Using a syringe fitted with a needle having a calibre not less than 0.35 mm, place a suitable volume in a suitable counting chamber such as a haemocytometer cell, taking care not to overfill the chamber. Allow the suspension to settle for 1 min and carefully add a cover slide without squeezing the sample. Scan an area corresponding to at least 5000 particles. Not more than 10 particles have a maximum dimension greater than 100 µm. No particle having a maximum dimension greater than 150 µm is present.

Aggregated albumin

Test solution Transfer a volume of the injection expected to contain about 1 mg of aggregated albumin to a centrifuge tube and centrifuge at about 2500 g for 5 min to 10 min. Decant the supernatant liquid. Resuspend the sediment in 2.0 ml of a 9 g/l solution of *sodium chloride R* . Centrifuge at 2500 g for 5 min to 10 min. Decant the supernatant liquid. Resuspend the sediment in 5.0 ml of *sodium carbonate solution R1* . Heat in a water-bath at 80 °C to 90 °C to dissolve the aggregated albumin. Allow to cool, transfer to a volumetric flask and dilute to 10.0 ml with *sodium carbonate solution R1.*

Reference solutions Prepare a range of reference solutions containing 0.05 mg to 0.2 mg of human albumin per millilitre in *sodium carbonate solution R1.*

Introduce 3.0 ml of each solution separately into 25 ml flasks. To each flask add 15.0 ml of *cupri-tartaric solution R2* , mix and allow to stand for 10 min. Add rapidly 1.5 ml of *dilute phosphomolybdotungstic reagent R* and mix immediately. Allow to stand for 30 min and measure the absorbance (*2.2.25*) at 750 nm using *sodium carbonate solution R1* as the compensation liquid. Using the absorbances obtained with the reference solutions, draw a calibration curve and calculate the content of aggregated albumin in the injection.

Tin

Test solution To 1.0 ml of the injection add 1.0 ml of *2 M hydrochloric acid R* . Heat in a water-bath for 30 min. Cool and centrifuge for 10 min at 300 g. Dilute 1.0 ml of the supernatant liquid to 25.0 ml with *1 M hydrochloric acid*.

Reference solution Dissolve 0.115 g of *stannous chloride R* in *1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

To 1.0 ml of each solution add 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R*, 0.05 ml of *thioglycollic acid R*, 0.1 ml of *dithiol reagent R* and 3.0 ml of *0.2 M hydrochloric acid*. Mix. Measure the absorbance (*2.2.25*) of each solution at 540 nm, using *0.2 M hydrochloric acid* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution (3 mg of Sn per millilitre).

Physiological distribution

Inject a volume not greater than 0.2 ml into the caudal vein of each of three rats weighing 150 g to 250 g. Euthanise the rats 15 min after the injection, remove the liver, the spleen and the lungs and measure the radioactivity in the organs using a suitable instrument. Measure the radioactivity in the rest of the body, including the blood, after having removed the tail. Determine the percentage of radioactivity in the lungs, the liver and the spleen from the expression:

$$\frac{A}{B} \times 100$$

A = radioactivity of the organ concerned,

B = total radioactivity in the liver, the spleen, the lungs and the rest of the body.

In not fewer than two of the three rats used, at least 80 per cent of the radioactivity is found in the lungs and not more than a total of 5 per cent in the liver and spleen. The injection may be released for use before completion of the test.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

Pyrogens

It complies with the test for pyrogens prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. Inject into the animals not less than 0.1 ml per kilogram of the rabbit's mass. The injection may be released for use before completion of the test.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

LABELLING

The label states:

- — that the preparation should be shaken before use,
- — the quantity of tin per millilitre, if any,
- — that the preparation is not to be used if after shaking, the suspension does not appear homogeneous.

Ph Eur

Technetium [^{99m} Tc] Medronate Injection

General Notices

(Ph Eur monograph 0641)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) medronate injection is a sterile solution which may be prepared by mixing solutions of sodium methylenediphosphonate and a stannous salt with sodium pertechnetate (^{99m}Tc) injection (fission or non-fission). The injection contains a variable quantity of tin (Sn) not exceeding 3 mg/ml; it may contain antimicrobial preservatives, antioxidants, stabilisers and buffers. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Radioactivity present as chemical forms other than technetium-99m medronate complex is not greater than 5.0 per cent of the total radioactivity.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A clear, colourless solution.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The distribution of the radioactivity contributes to the identification of the preparation.

C. Examine by thin-layer chromatography (*2.2.27*) using cellulose as the coating substance.

Test solution Dilute the injection to be examined with water R to obtain a solution containing about 0.1 mg to 0.5 mg of sodium medronate per millilitre.

Reference solution Dissolve a suitable quantity (1 mg to 5 mg) of *medronic acid CRS* in a mixture of a 9.0 g/l solution of *sodium chloride R* and *water R* and dilute to 10 ml with the same solvent so as to obtain a solution similar to the test solution with regard to medronate and sodium chloride concentrations.

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm to 14 cm (development time about 4 h) using a mixture of 20 volumes of *2-propanol R*, 30 volumes of *1 M hydrochloric acid* and 60 volumes of *methyl ethyl ketone R*. Allow the plate to dry in air and spray with *ammonium molybdate solution R4*. Expose the plate to ultraviolet light at 254 nm for about 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the spot in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

The pH of the solution is 3.5 to 7.5.

Tin

Test solution Dilute 1.0 ml of the solution to 50.0 ml with *1 M hydrochloric acid*.

Reference solution Dissolve 0.115 g of *stannous chloride R* in *1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

To 1.0 ml of each solution add 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R*, 0.05 ml of *thioglycollic acid R*, 0.1 ml of *dithiol reagent R* and 3.0 ml of *0.2 M hydrochloric acid*. Mix. Measure the absorbance (*2.2.25*) of each solution at 540 nm, using *0.2 M hydrochloric acid* as compensation liquid. The absorbance of the test solution is not greater than that of the reference solution (3 mg of Sn per millilitre).

Physiological distribution

Inject a volume not greater than 0.2 ml, equivalent to not more than 0.05 mg of sodium medronate into a suitable vein such as a caudal vein or the saphenous vein of each of three rats, each weighing 150 g to 250 g. Measure the radioactivity in the syringe before and after injection. Euthanise the rats 2 h after the injection. Remove one femur, the liver, and some blood. Weigh the blood. Remove the tail if a caudal vein has been used for the injection. Using a suitable instrument measure the radioactivity in the femur, liver, and blood, and in the tail if a caudal vein has been used for the injection. Determine the percentage of radioactivity in each sample from the expression:

$$\frac{A}{B} \times 100$$

A = radioactivity of the sample concerned,

B = total radioactivity, which is equal to the difference between the two measurements made on the syringe minus the radioactivity in the tail if a caudal vein has been used for the injection.

Calculate the radioactivity per unit mass in the blood. Correct the blood concentration by multiplying by a factor *m*/200 where *m* is the body mass of the rat in grams.

In not fewer than two of the three rats: not less than 1.5 per cent of the radioactivity is found in the femur; not more than 1.0 per cent is found in the liver and not more than 0.05 per cent per gram is found in the blood.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Use plates such that during development, the mobile phase migrates 10 cm to 15 cm in about 10 min. Determine hydrolysed technetium and technetium in colloidal form by test (a) and pertechnetate ion by test (b).

(a) Apply to the plate 5 µl to 10 µl of the injection. Develop immediately over a path of 10 cm to 15 cm using a 136 g/l solution of *sodium acetate R* . Allow the plate to dry in air. Determine the distribution of radioactivity using a suitable detector. Hydrolysed technetium and technetium in colloidal form remain at the starting point. Technetium medronate complex and pertechnetate ion migrate near to the solvent front.

(b) Apply to the plate 5 µl to 10 µl of the injection and dry quickly. Develop over a path of 10 cm to 15 cm using *methyl ethyl ketone R* . Allow the plate to dry. Determine the distribution of radioactivity using a suitable detector. Pertechnetate ion migrates near to the solvent front. Technetium medronate complex and technetium in colloidal form remain at the starting-point.

The percentage of radioactivity corresponding to pertechnetate ion in the chromatogram obtained in test (b) is not greater than 2.0 per cent and the sum of the percentages of radioactivity corresponding to impurities in the chromatograms obtained in test (a) and test (b) (including pertechnetate ion) is not greater than 5.0 per cent.

RADIOACTIVITY

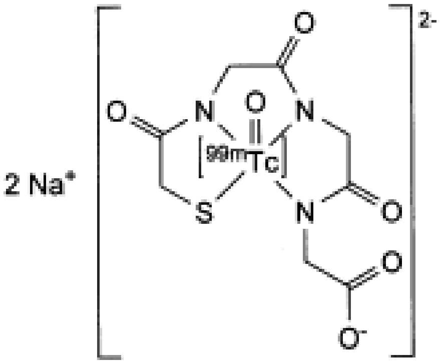
Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Technetium [^{99m} Tc] Mertiatide Injection

General Notices

(Ph Eur monograph 1372)



Ph Eur

DEFINITION

Technetium (^{99m}Tc) mertiatide injection is a sterile solution which may be prepared by either heating a mixture containing S-benzoylmercaptoacetyltriglycine (betiatide), a weak chelating agent such as tartrate, a stannous salt and sodium pertechnetate (^{99m}Tc) injection (fission or non-fission), or by mixing solutions of mercaptoacetyltriglycine (mertiatide), a stannous salt and sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) at alkaline pH. It may contain stabilisers and a buffer. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and time stated on the label. Not less than 94 per cent of the radioactivity corresponds to technetium-99m in the form of [^{99m}Tc]technetium mertiatide.

CHARACTERS

A clear, colourless solution.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine the chromatogram obtained in test (b) for radiochemical purity. The principal peak in the chromatogram obtained with the test solution has approximately the same retention time as the principal peak in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

The pH of the injection is 5.0 to 7.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

(a) Examine by ascending paper chromatography (*2.2.26*) using a suitable paper as the stationary phase.

Test solution The solution to be examined.

Apply 2 µl of the test solution to the paper. Develop over a path of 15 cm using a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R* . Allow the paper to dry and determine the distribution of the radioactivity using a suitable detector. Not more than 2.0 per cent of the total radioactivity is retained at the origin (R_F value 0.0 to 0.1).

(b) Examine by liquid chromatography (*2.2.29*).

Test solution The solution to be examined.

Reference solution Dissolve with heating on a water-bath 5 mg of *S-benzylmercaptoacetyltriglycine CRS* in 5 ml of water R. To 1 ml of this solution in a closed vial filled with *nitrogen R* , add 0.5 ml of a 40 g/l solution of *sodium potassium tartrate R* , 25 µl of a 4 g/l solution of *stannous chloride R* in *0.05 M hydrochloric acid R* and 370 MBq to 740 MBq of sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) in a volume not exceeding 3 ml. Heat the mixture on a water-bath for 10 min and allow to cool to room temperature.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.0 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R (5 µm),*

— as mobile phase at a flow rate of 1.0 ml/min:

Mobile phase A A mixture of 7 volumes of *ethanol R* with 93 volumes of a solution of a 1.36 g/l solution of *potassium dihydrogen phosphate R* , adjusted to pH 6.0 with *0.1 M sodium hydroxide* ,

Mobile phase B A mixture of 10 volumes of *water R* with 90 volumes of *methanol R* ,

— a suitable radioactivity detector,

— a 20 µl loop injector.

Equilibrate the column with mobile phase A for 20 min. Inject the test solution and the reference solution. Switch 10 min after each injection to mobile phase B and continue the chromatographic procedure for 15 min.

The test is not valid unless in the chromatogram obtained with the test solution the principal peak has approximately the same retention time as the principal peak in the chromatogram obtained with the reference solution. In the chromatogram obtained with the test solution the sum of the areas preceding the principal peak (corresponding to hydrophilic impurities, including [^{99m}Tc]pertechnetate) is not greater than 3.0 per cent of the sum of the areas of all peaks. The sum of the peaks following the principal peak (corresponding to lipophilic impurities) is not greater than 4.0 percent of the sum of the area of all peaks.

Not less than 94 per cent of the radioactivity corresponds to [^{99m}Tc]technetium mertiatide.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Technetium [^{99m} Tc] Microspheres Injection

General Notices

(Ph Eur monograph 0570)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) microspheres injection is a sterile, apyrogenic suspension of human albumin which has been denatured to form spherical insoluble particles; the particles are labelled with technetium-99m. The injection contains reducing substances, such as tin salts in an amount not exceeding 3 mg of Sn per millilitre; it may contain a suitable buffer such as acetate, citrate or phosphate and additives such as wetting agents. The human albumin used complies with the requirements of the monograph on *Human albumin solution (0255).* The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 95 per cent of the technetium-99m is bound to the particles of the suspension as determined by the test for non-filterable radioactivity. The particles have a typical diameter between 10 µm and 50 µm. The radioactivity is not less than 185 MBq of technetium-99m per million particles at the date and hour of administration.

Technetium (^{99m}Tc) microspheres injection is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile and apyrogenic ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A suspension of white, yellow or artificially coloured particles which may separate on standing.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. The tests for non-filterable radioactivity and particle size contribute to the identification of the preparation.

C. Transfer 1 ml of the injection to a centrifuge tube and centrifuge at 2500 g for 5 min to 10 min. Decant the supernatant liquid. To the residue add 5 ml of *cupri-tartaric solution R2*, mix and allow to stand for 10 min. If necessary, heat to dissolve the particles and allow to cool. Add rapidly 0.5 ml of *dilute phosphomolybdotungstic reagent R*, mix immediately and allow to stand. A blue colour develops.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.0 to 9.0.

Non-filterable radioactivity

Use a polycarbonate membrane filter 13 mm to 25 mm in diameter, 10 µm thick and with circular pores 3 µm in diameter. Fit the membrane into a suitable holder. Place 0.2 ml of the injection on the membrane and filter, adding 20 ml of a 9 g/l solution of *sodium chloride R* during the filtration. The radioactivity remaining on the membrane represents not less than 95 per cent of the total radioactivity of the injection.

Particle size

Examine using a microscope. Dilute the injection if necessary so that the number of particles is just low enough for individual particles to be distinguished. Using a syringe fitted with a needle having a calibre not less than 0.35 mm, place a suitable volume in a suitable counting chamber such as a haemocytometer cell, taking care not to overfill the chamber. Allow the suspension to settle for 1 min and carefully add a cover slide without squeezing the sample. Scan an area corresponding to at least 5000 particles. The particles have a uniform spherical appearance. Not more than 10 particles have a maximum dimension greater than 75 µm. No particle having a maximum dimension greater than 100 µm is present.

Number of particles

Examine using a microscope. Fill a suitable counting chamber such as a haemocytometer cell with a suitable dilution of the injection taking care that particles do not separate during the transfer. Count the number of particles in the chamber. Repeat this procedure twice and calculate the number of particles per millilitre of the injection.

Tin

Test solution To 1.0 ml of the injection add 0.5 ml of *sulphuric acid R* and 1.5 ml of *nitric acid R*. Heat and evaporate to approximately 1 ml. Add 2 ml of *water R* and evaporate again to approximately 1 ml. Repeat this procedure twice, cool and dilute to 25.0 ml with *1 M hydrochloric acid*.

Reference solution Dissolve 0.115 g of *stannous chloride R* in *1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

To 1.0 ml of each solution add 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R*, 0.05 ml of *thioglycollic acid R*, 0.1 ml of *dithiol reagent R* and 3.0 ml of *0.2 M hydrochloric acid*. Mix. Measure the absorbance (*2.2.25*) of each solution at 540 nm, using *0.2 M hydrochloric acid* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution (3 mg of Sn per millilitre).

Physiological distribution

Inject a volume not greater than 0.2 ml into a caudal vein of each of three rats weighing 150 g to 250 g. Euthanise the rats 15 min after the injection, remove the liver, the spleen and the lungs and measure the radioactivity in the organs using a suitable instrument. Measure the radioactivity in the rest of the body, including the blood and voided urine, after having discarded the tail. Determine the percentage of radioactivity in the liver, the spleen and the lungs from the expression:

$$\frac{A}{B} \times 100$$

A = radioactivity of the organ concerned,

B = total radioactivity in the liver, the spleen, the lungs and the rest of the body, including voided urine.

In not fewer than two of the three rats used, not less than 80 per cent of the radioactivity is found in the lungs and not more than a total of 5 per cent in the liver and spleen. The injection may be released for use before completion of the test.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Pyrogens

It complies with the test for pyrogens prescribed in the monograph on *Radiopharmaceutical preparations (0125)* . Inject not less than 0.1 ml per kilogram of the rabbit's mass. The injection may be released for use before completion of the test.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

LABELLING

The label states:

— the quantity of tin per millilitre, if any,

— that the preparation should be shaken before use.

Ph Eur

Technetium [^{99m} Tc] Pentetate Injection

General Notices

(Ph Eur monograph 0642)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) pentetate injection is a sterile solution which may be prepared by mixing solutions of sodium diethylenetriaminepenta-acetate or calcium trisodium diethylenetriaminepenta-acetate and a stannous salt with a solution of sodium pertechnetate (^{99m}Tc). It contains a variable quantity of tin (Sn) not exceeding 1 mg/ml; it may contain suitable antimicrobial preservatives, antioxidants, stabilisers and buffers. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 95.0 per cent of the radioactivity corresponds to technetium-99m complexed with sodium pentetate or calcium trisodium pentetate.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable, sterile ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A clear, colourless or slightly yellow solution.

Technetium-99m has a half life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the preparation.

C. Place in a clean, dry 10 ml glass tube a volume of the injection to be examined containing 2 mg of pentetate. Dilute, if necessary, to 1 ml with *water R*. Place in a second tube 1 ml of *water R* (blank). To each tube add 0.1 ml of a 1 g/l solution of *nickel sulphate R*, 0.5 ml of a 50 per cent *V/V* solution of *glacial acetic acid R* and 0.75 ml of a 50 g/l solution of *sodium hydroxide R*. Mix and verify that the pH is not above 5. To each tube add 0.1 ml of a 10 g/l solution of *dimethylglyoxime R* in *alcohol R*. Mix and allow to stand for 2 min. Adjust the pH in each tube to not less than 12 by adding a 100 g/l solution of *sodium hydroxide R*. Mix and check that the pH is not below 12. Allow to stand for 2 min. Heat the tubes gently on a water-bath for 2 min. The solution in the tube containing the injection to be examined remains clear and colourless throughout. The solution in the blank tube becomes red on addition of dimethylglyoxime solution and a red precipitate is formed when the tube is heated on a water-bath.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.0 to 7.5.

Tin

Test solution Dilute 1.5 ml of the injection to 25.0 ml with *1 M hydrochloric acid*.

Reference solution Dissolve 0.115 g of *stannous chloride R* in *1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

To 1.0 ml of each solution add 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R*, 0.05 ml of *thioglycollic acid R*, 0.1 ml of *dithiol reagent R* and 3.0 ml of *0.2 M hydrochloric acid*. Mix. Measure the absorbance (*2.2.25*) of each solution at 540 nm, using *0.2 M hydrochloric acid* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution (1 mg of Sn per millilitre).

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125)*. The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for 10 min. Use a plate such that during development the mobile phase migrates over a distance of 10 cm to 15 cm in about 10 min.

(a) Apply to the plate 5 µl to 10 µl of the injection to be examined. Develop immediately over a path of 10 cm to 15 cm using a 9 g/l solution of *sodium chloride R*. Allow the plate to dry in air. Determine the distribution of radioactivity using a suitable detector. Impurities in colloidal form remain at the starting point. Technetium pentetate complex and pertechnetate ion migrate near to the solvent front.

(b) Apply to the plate 5 µl to 10 µl of the injection to be examined and allow to dry. Develop over a path of 10 cm to 15 cm using *methyl ethyl ketone R*. Allow the plate to dry. Determine the distribution of radioactivity using a suitable detector. Pertechnetate ion migrates near to the solvent front. Technetium pentetate complex and impurities in colloidal form remain at the starting point.

The sum of the percentages of radioactivity corresponding to impurities in the chromatograms obtained in test (a) and (b) does not exceed 5.0 per cent.

RADIOACTIVITY

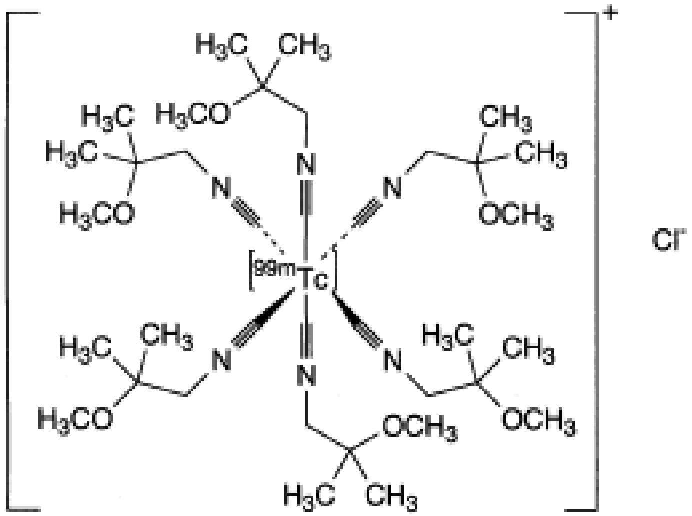
Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Technetium [^{99m} Tc] Sestamibi Injection

General Notices

(Ph Eur monograph 1926)



Ph Eur

DEFINITION

Sterile solution of (*OC*-6-11)-hexakis[1-(isocyano-κ*C*)-2-methoxy-2-methylpropane][^{99m}Tc] technetium(I) chloride, which may be prepared by heating a mixture containing [tetrakis(2-methoxy-2-methylpropyl-1-isocyanide)copper (1+)] tetrafluoroborate, a weak chelating agent, a stannous salt and *Sodium pertechnetate (^{99m} Tc) injection (fission) (0124)* or *Sodium pertechnetate (^{99m} Tc) injection (non-fission) (0283).*

Content

90 per cent to 110 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label.

CHARACTERS

Appearance

Clear, colourless solution.

Half-life and nature of radiation of technetium-99m

See general chapter *5.7. Table of physical characteristics of radionuclides.*

IDENTIFICATION

A. Gamma-ray spectrometry.

Results The spectrum obtained with the solution to be examined does not differ significantly from that of a standardised technetium-99m solution. The most prominent gamma photon has an energy of 0.141 MeV.

B. Examine the chromatograms obtained in the test for impurity C under Radiochemical purity.

Results The principal peak in the radiochromatogram obtained with the test solution is similar in retention time to the principal peak in the radiochromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

5.0 to 6.0.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Impurity A and other polar impurities

Thin-layer chromatography (*2.2.27*).

Test solution The preparation to be examined.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase Mix 10 volumes of *tetrahydrofuran R* , 20 volumes of a 38.5 g/l solution of *ammonium acetate R* , 30 volumes of *methanol R* and 40 volumes of *acetonitrile R* .

Application About 5 µl.

Development Immediately over a path of 6 cm.

Drying In air.

Detection Determine the distribution of radioactivity using a radioactivity detector.

Retardation factors Impurity B and apolar impurities = 0 to 0.1; impurity C and technetium-99m sestamibi = 0.3 to 0.6; impurity A and other polar impurities = 0.9 to 1.0.

Limit See test for impurity B.

Impurity B

Paper chromatography *(2.2.26) . If no activity is found at retardation factor 0 to 0.1 in the test for impurity A and other polar impurities, impurity B is absent and the test for impurity B may be omitted.*

Test solution The preparation to be examined.

Paper *paper for chromatography R.*

Mobile phase Mix equal volumes of *acetonitrile R* , *0.5 M acetic acid* and a 20 g/l solution of *sodium chloride R .*

Application About 5 µl.

Development Over a path of 10 cm.

Drying In air.

Detection Determine the distribution of radioactivity using a radioactivity detector.

Retardation factors Impurity B = 0 to 0.1; impurity A, impurity C and technetium-99m sestamibi = 0.8 to 1.0.

Limit:

— *sum of impurity A and other polar impurities, and impurity B*: maximum 5 per cent of the total radioactivity.

Impurity C

Liquid chromatography (*2.2.29*).

Test solution The preparation to be examined.

Reference solution To a vial of *sestamibi labelling kit CRS* add 3 ml of a 9 g/l solution of *sodium chloride R* containing 700 MBq to 900 MBq of sodium pertechnetate (^{99m}Tc) injection (fission or non-fission). Heat the mixture in a water-bath for 10 min and allow to cool to room temperature.

Column:

— *size:* *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *acetonitrile R,* 35 volumes of a 6.6 g/l solution of *ammonium sulphate R* and 45 volumes of *methanol R* .

Flow rate 1.5 ml/min.

Detection Radioactivity detector.

Injection 25 µl.

Run time 25 min.

Relative retention

With reference to technetium-99m sestamibi: impurity C = about 1.3.

System suitability Reference solution:

— the chromatogram is similar to the chromatogram provided with *sestamibi labelling kit*

CRS,

— *relative retention* with reference to technetium-99m sestamibi: impurity C = minimum 1.2.

Limits:

— *impurity C*: not more than 3 per cent of the total radioactivity,

— *technetium-99m sestamibi*: minimum 94 per cent of the total radioactivity.

Calculate the percentage of radioactivity due to technetium-99m sestamibi from the expression:

$$\frac{(100 - B) \times T}{100}$$

B = percentage of radioactivity due to impurity B determined in the test for impurity B under Radiochemical purity,

T = area of the peak due to technetium-99m sestamibi in the chromatogram obtained with the test solution.

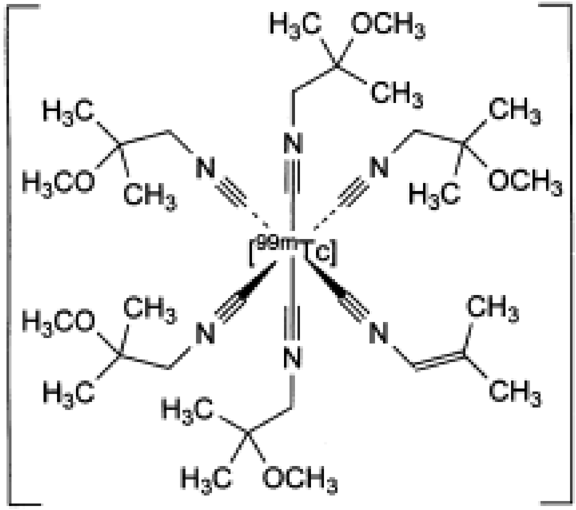
RADIOACTIVITY

Determine the radioactivity using a calibrated instrument.

IMPURITIES

A. $[^{99m}Tc]O_4^-$: (^{99m}Tc)pertechnetate ion,

B. technetium-99m in colloidal form,



C. (*OC*-6-22)-pentakis[1-(isocyano-κ*C*)-2-methoxy-2-methylpropane][1-(isocyano-κ*C*)-2-methylprop-1-ene][^{99m}Tc]technetium (1+).

Technetium [^{99m} Tc] Succimer Injection

General Notices

(Ph Eur monograph 0643)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) succimer injection is a sterile solution of *meso*-2,3-dimercaptosuccinic acid labelled with technetium-99m. It contains a reducing substance, such as a tin salt in an amount not exceeding 1 mg of Sn per millilitre, and may contain stabilisers, antioxidants such as ascorbic acid, and inert additives. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 95.0 per cent of the radioactivity corresponds to technetium-99m succimer complex.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration. Syringes for handling the eluate intended for labelling of the final product, or for handling the final product should not contain rubber parts.

CHARACTERS

A clear, colourless solution.

Technetium-99m has a half life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine the chromatogram obtained in the test for radiochemical purity. The distribution of the radioactivity contributes to the identification of the preparation.

C. Place 1 ml of the injection to be examined in a test-tube and add 1 ml of a 20 g/l solution of *sodium nitroprusside R* and 0.1 ml of *glacial acetic acid R* . Mix. Place carefully at the top of the solution a layer of *concentrated ammonia R* . A violet ring develops between the layers.

TESTS

pH (*2.2.3*)

The pH of the injection is 2.3 to 3.5.

Tin

Test solution Dilute 1.5 ml of the injection to be examined to 25.0 ml with *1 M hydrochloric acid* .

Reference solution Dissolve 0.115 g of *stannous chloride R* in *1 M hydrochloric acid* and dilute to 1000.0 ml with the same acid.

To 1.0 ml of each solution add 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R* , 0.05 ml of *thioglycollic acid R* , 0.1 ml of *dithiol reagent R* and 3.0 ml of *0.2 M hydrochloric acid* . Mix. Allow to stand for 60 min. Measure the absorbance (*2.2.25*) of each solution at 540 nm, using *0.2 M hydrochloric acid* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution (1 mg of Sn per millilitre).

Physiological distribution

Inject a volume not greater than 0.2 ml and containing not more than 0.1 mg of dimercaptosuccinic acid into a suitable vein, such as a caudal vein or a saphenous vein, of each of three rats each weighing 150 g to 250 g. Measure the radioactivity in the syringe before and after the injection. Euthanise the rats 1 h after the injection. Remove the kidneys, the liver, the stomach, the lungs and, if a caudal vein has been used for the injection, the tail. Using a suitable instrument determine the radioactivity in the organs and, if a caudal vein has been used for injection, in the tail. Determine the percentage of radioactivity in each organ with respect to the total radioactivity calculated as the difference between the two measurements made on the syringe minus the activity in the tail (if a caudal vein has been used for the injection).

In not fewer than two of the three rats used, the radioactivity in the kidneys is not less than 40 per cent, that in the liver is not more than 10.0 per cent, that in the stomach is not more than 2.0 per cent and that in the lungs is not more than 5.0 per cent.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for 10 min. Use a plate such that during development the mobile phase migrates over a distance of 10 cm to 15 cm in about 10 min.

Apply to the plate 5 µl to 10 µl of the injection to be examined. Develop immediately over a path of 10 cm to 15 cm using *methyl ethyl ketone R* . Allow the plate to dry. Determine the distribution of radioactivity using a suitable detector. Technetium succimer complex remains at the starting point. Pertechnetate ion migrates near to the solvent front. Not less than 95.0 per cent of the total radioactivity is found in the spot corresponding to technetium succimer complex. The radioactivity corresponding to pertechnetate ion represents not more than 2.0 per cent of the total radioactivity.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

STORAGE

Store protected from light.

Ph Eur

Technetium [^{99m} Tc] Tin Pyrophosphate Injection

General Notices

(Ph Eur monograph 0129)

Ph Eur

DEFINITION

Technetium (^{99m}Tc) tin pyrophosphate injection is a sterile, apyrogenic solution which may be prepared by mixing solutions of sodium pyrophosphate and stannous chloride with sodium pertechnetate (^{99m}Tc) injection (fission or non-fission). The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared technetium-99m radioactivity at the date and hour stated on the label. Not less than 90 per cent of the radioactivity corresponds to technetium-99m complexed with tin pyrophosphate. The injection contains a quantity of sodium pyrophosphate ($Na_4P_2O_7,10H_2O$) that may vary from 1 mg to 50 mg per millilitre and a variable quantity of tin (Sn) not exceeding 3.0 mg per millilitre.

It is prepared from sodium pertechnetate (^{99m}Tc) injection (fission or non-fission) using suitable sterile, apyrogenic ingredients and calculating the ratio of radionuclidic impurities with reference to the date and hour of administration.

CHARACTERS

A clear, colourless solution.

Technetium-99m has a half-life of 6.02 h and emits gamma radiation.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised technetium-99m solution either by direct comparison or by using an instrument calibrated with the aid of such a solution. Standardised technetium-99m and molybdenum-99 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photon of technetium-99m has an energy of 0.140 MeV.

B. Examine the chromatograms obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the injection.

C. To 1 ml add 1 ml of *acetic acid R*. Heat on a water-bath for 1 h. After cooling, add 10 ml of *nitro-vanadomolybdic reagent R* and allow to stand for 30 min. A yellow colour develops.

D. To 1 ml add 2 ml of a 30 per cent *V/V* solution of *sulphuric acid R*, 1 ml of *hydrochloric acid R*, 0.05 ml of *thioglycollic acid R*, 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R* and 0.1 ml of *dithiol reagent R* and allow to stand for 30 min. A pink colour develops.

TESTS

pH (*2.2.3*)

The pH of the injection is 6.0 to 7.0.

Sodium pyrophosphate

Test solution Use 1 ml of the injection to be examined or a suitable dilution of it.

Reference solutions Using a solution containing *sodium pyrophosphate R* and *stannous chloride R* in the same proportions as in the injection to be examined, prepare a range of solutions and dilute to the same final volume with *water R* .

To the test solution and to 1 ml of each of the reference solutions add successively 10 ml of a 1 g/l solution of *disodium hydrogen phosphate R* , 10 ml of *iron standard solution (8 ppm Fe) R* , 5 ml of *glacial acetic acid R* and 5 ml of a 1 g/l solution of *hydroxylamine hydrochloride R.* Dilute each solution to 40 ml with *water R* and heat in a water-bath at 40 °C for 1 h. To each solution add 4 ml of a 1 g/l solution of *phenanthroline hydrochloride R* and dilute to 50.0 ml with *water R* . Measure the absorbance (*2.2.25*) of each solution at 515 nm using as the compensation liquid a reagent blank containing hydrochloric acid (1.1 g/l HCl) instead of the *iron standard solution (8 ppm Fe) R* . Using the absorbances obtained with the reference solutions, draw a calibration curve and calculate the concentration of sodium pyrophosphate in the injection to be examined.

Tin

Test solution Use 1 ml of the injection to be examined or a suitable dilution of it.

Reference solutions Using a solution in hydrochloric acid (6.2 g/l HCl) containing *sodium pyrophosphate R* and *stannous chloride R* in the same proportions as in the injection to be examined, prepare a range of solutions and dilute to the same volume with hydrochloric acid (6.2 g/l HCl).

To the test solution and to 1 ml of each of the reference solutions add 2 ml of a 300 g/l solution of *sulphuric acid R* , 1 ml of *hydrochloric acid R* , 0.05 ml of *thioglycollic acid R* , 0.4 ml of a 20 g/l solution of *sodium laurilsulfate R* and 0.1 ml of *dithiol reagent R* and dilute to 15 ml with hydrochloric acid (6.2 g/l HCl). Allow the solutions to stand for 30 min and measure the absorbance (*2.2.25*) of each solution at 530 nm, using as the compensation liquid a reagent blank containing the same quantity of *sodium pyrophosphate R* as the injection to be examined. Using the absorbances obtained with the reference solutions, draw a calibration curve and calculate the concentration of tin in the injection to be examined.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Pyrogens

It complies with the test for pyrogens prescribed in the monograph on *Radiopharmaceutical preparations (0125).* Inject not less than 0.1 ml per kilogram of the rabbit's mass. The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

(a) Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for 10 min. The plate used should be such that during development the mobile phase migrates over a distance of 10 cm to 15 cm in about 10 min.

Apply to the plate 5 µl to 10 µl of the injection and dry in a stream of nitrogen. Develop over a path of 10 cm to 15 cm using *methyl ethyl ketone R* through which nitrogen has been bubbled in the chromatography tank for 10 min immediately before the chromatography. Allow the plate to dry. Determine the distribution of radioactivity using a suitable detector. The technetium-99m tin pyrophosphate complex remains at the starting-point and pertechnetate ion migrates with an R_F of 0.95 to 1.0.

(b) Examine by thin-layer chromatography (*2.2.27*) using silica gel as the coating substance on a glass-fibre sheet. Heat the plate at 110 °C for 10 min. The plate used should be such that during development the mobile phase migrates over a distance of 10 cm to 15 cm in about 10 min.

Apply to the plate 5 µl to 10 µl of the injection. Develop immediately over a path of 10 cm to 15 cm using a 136 g/l solution of *sodium acetate R*. Allow the plate to dry and measure the distribution of radioactivity using a suitable detector. Impurities in colloidal form remain at the starting-point and technetium-99m tin pyrophosphate complex and pertechnetate ion migrate with an R_F of 0.9 to 1.0.

Add together the percentages of radioactivity corresponding to impurities in the chromatograms obtained in test (a) and test (b). The sum does not exceed 10 per cent.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution.

LABELLING

The label states, in particular, the quantity of sodium pyrophosphate per millilitre and the quantity of tin per millilitre.

Ph Eur

Thallous [^{201} Tl] Chloride Injection

General Notices

(Ph Eur monograph 0571)

Ph Eur

DEFINITION

Thallous (^{201}Tl) chloride injection is a sterile solution of thallium-201 in the form of thallous chloride. It may be made isotonic by the addition of *Sodium chloride (0193)* and may contain a suitable antimicrobial preservative such as *Benzyl alcohol (0256).* Thallium-201 is a radioactive isotope of thallium formed by the decay of lead-201. Lead-201 is a radioactive isotope of lead and may be obtained by irradiation, with protons of suitable energy, of thallium which may be enriched in thallium-203. Thallium-201 may be separated from lead-201 by passing through a column of an ion-exchange resin. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared thallium-201 radioactivity at the date and hour stated on the label. Not more than 2.0 per cent of the total radioactivity is due to thallium-202 and not less than 97.0 per cent is due to thallium-201. Not less than 95.0 per cent of the radioactivity is due to thallium in the form of thallous ions. The specific radioactivity is not less than 3.7 GBq per milligram of thallium.

CHARACTERS

A clear, colourless solution.

Thallium-201 has a half-life of 3.05 days and emits gamma radiation and X-rays.

IDENTIFICATION

A. Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised thallium-201 solution when measured either by direct comparison or by use of an instrument calibrated with the aid of such a solution. Standardised thallium-201 and thallium-202 solutions are available from laboratories recognised by the competent authority. The most prominent gamma photons have energies of 0.135 MeV, 0.166 MeV and 0.167 MeV. The X-rays have energies of 0.069 MeV to 0.083 MeV.

B. Examine the electropherogram obtained in the test for radiochemical purity. The distribution of radioactivity contributes to the identification of the preparation.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.0 to 7.0.

Thallium

To 0.5 ml of the injection add 0.5 ml of hydrochloric acid (220 g/l HCl) and 0.05 ml of *bromine water R* and mix. Add 0.1 ml of a 30 g/l solution of *sulphosalicylic acid R*. After decolorisation add 1.0 ml of a 1 g/l solution of *rhodamine B R*. Add 4 ml of *toluene R* and shake for 60 s. Separate the toluene layer. The toluene layer is not more intensely coloured than the toluene layer of a standard prepared at the same time in the same manner using 0.5 ml of *thallium standard solution (10 ppm Tl) R*.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

Record the gamma-ray and X-ray spectrum using a suitable instrument calibrated with the aid of standardised thallium-201 and thallium-202 solutions. The spectrum does not differ significantly from that of the standardised thallium-201 solution. Determine the relative amounts of thallium-201 and thallium-202 and other radionuclidic impurities present. Thallium-202 has a half-life of 12.2 days and its most prominent gamma photon has an energy of 0.440 MeV. Thallium-200 has a half-life of 1.09 days and its most prominent gamma photons have energies of 0.368 MeV, 0.579 MeV, 0.828 MeV and 1.206 MeV. Lead-201 has a half-life of 9.4 h and its most prominent gamma photon has an energy of 0.331 MeV. Lead-203 has a half-life of 2.17 days and its most prominent gamma photon has an energy of 0.279 MeV. Not more than 2.0 per cent of the total radioactivity is due to thallium-202 and not less than 97.0 per cent is due to thallium-201.

RADIOCHEMICAL PURITY

Examine by zone electrophoresis (*2.2.31*), using a suitable strip of cellulose acetate, as the support and a 18.6 g/l solution of *sodium edetate R* as the electrolyte solution. Soak the strip in the electrolyte solution for 45-60 min. Remove the strip with forceps taking care to handle the outer edges only. Place the strip between 2 absorbent pads and blot to remove excess solution.

Test solution Mix equal volumes of the injection to be examined and the electrolyte solution.

Apply not less than 5 µl of the test solution to the centre of the strip and mark the point of application. Apply an electric field of 17 V/cm for at least 10 min. Allow the strip to dry in air. Determine the distribution of radioactivity using suitable equipment. Not less than 95.0 per cent of the radioactivity migrates towards the cathode.

RADIOACTIVITY

Measure the radioactivity using suitable counting equipment by comparison with a standardised thallium-201 solution or by measurement in an instrument calibrated with the aid of such a solution.

Ph Eur

Tritiated [^{3} H] Water Injection

General Notices

(Ph Eur monograph 0112)

Ph Eur

DEFINITION

Tritiated (^{3}H) water injection is water for injections in which some of the water molecules contain tritium atoms in place of protium atoms. It may be made isotonic by the addition of sodium chloride. Tritium (^{3}H) may be obtained by the neutron irradiation of lithium. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared tritium activity at the date stated on the label.

CHARACTERS

A clear, colourless liquid.

Tritium has a half-life of 12.3 years and emits beta radiation.

IDENTIFICATION

Record the beta-ray spectrum by the method prescribed in the test for radionuclidic purity. The spectrum does not differ significantly from that of a standardised tritiated (^{3}H) water. Standardised tritiated (^{3}H) water is available from laboratories recognised by the competent authority. The maximum energy of the beta radiation is 0.019 MeV.

TESTS

pH (*2.2.3*)

The pH of the injection is 4.5 to 7.0.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).*

RADIONUCLIDIC PURITY

(a) Mix 100 µl of a suitable dilution of the injection with 10 ml of a scintillation liquid consisting of 1000 ml of *dioxan R* , 100 g of *naphthalene R* , 7 g of *diphenyloxazole R* and 0.3 g of *methylphenyloxazolylbenzene R* , the reagents being of an analytical grade suitable for liquid scintillation. Measure the radioactivity of the mixture in a liquid scintillation counter fitted with a discriminator. The count should be about 5000 impulses per second at the lowest setting of the discriminator. Record the count at different discriminator settings. For each measurement count at least 10 000 impulses over a period of at least 1 min. Immediately determine in the same conditions the count for a standardised tritiated (^{3}H) water having approximately the same activity.

Plot the counts at each discriminator setting, correcting for background activity, on semi-logarithmic paper, the discriminator settings being in arbitrary units as the abscissae.The vertical distance between the two curves obtained is constant. They obey the mathematical relationship:

$$\frac{\frac{A_1}{B_1} - \frac{A_2}{B_2}}{\frac{A_1}{B_1}} \times 100 < 20$$

A_1 = radioactivity recorded for the standardised preparation at the lowest discriminator setting,

B_1 = radioactivity recorded for the preparation to be examined at the lowest discriminator setting,

A_2 = radioactivity recorded for the standard at the discriminator setting such that,

$$A_2 \approx A_1 \times 10^{-3}$$

B_2 = radioactivity recorded for the preparation to be examined at the latter discriminator setting.

(b) Record the gamma-ray spectrum. The instrument registers only background activity.

RADIOCHEMICAL PURITY

Place a quantity of the injection equivalent to about 2 µCi (74 kBq), diluted to 50 ml with *water R* , in an all-glass distillation apparatus of the type used for the determination of distillation range (*2.2.11*). Determine the radioactive concentration. Distil until about 25 ml of distillate has been collected. Precautions must be taken to avoid contamination of the air. If the test is carried out in a fume cupboard, the equipment must be protected from draughts. Determine the radioactive concentration of the distillate and of the liquid remaining in the distillation flask. Neither of the radioactive concentrations determined after distillation differs by more than 5 per cent from the value determined before distillation.

RADIOACTIVITY

Determine the radioactivity using a liquid scintillation counter.

Ph Eur

Water [^{15} O] Injection

General Notices

(Ph Eur monograph 1582)

Ph Eur

DEFINITION

Water (^{15}O) injection is a sterile solution of [^{15}O]water for diagnostic use. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared oxygen-15 radioactivity at the date and time stated on the label. Not less than 99 per cent of the total radioactivity corresponds to oxygen-15 in the form of water.

PRODUCTION

RADIONUCLIDE PRODUCTION

Oxygen-15 is a radioactive isotope of oxygen which may be produced by various nuclear reactions such as proton irradiation of nitrogen-15 or deuteron irradiation of nitrogen-14.

RADIOCHEMICAL SYNTHESIS

In order to recover oxygen-15 as molecular oxygen from the nitrogen target gas, carrier oxygen is added at concentrations generally ranging from 0.2 per cent *V/V* to 1.0 per cent *V/V*. [^{15}O]Water can be prepared from [^{15}O]oxygen by reaction with hydrogen using a suitable catalyst.

An alternative method is to produce [^{15}O]water "in-target" by adding hydrogen to the irradiated target gas at a concentration generally ranging from 2 per cent *V/V* to 5 per cent *V/*V.

The [^{15}O]water vapour contained in the gas-stream is either bubbled through a reservoir of a sterile 9 g/l solution of sodium chloride, or is exchanged by diffusion into such a solution through a membrane filter for dialysis.

Ammonia is a possible chemical impurity in [^{15}O]water. This may arise either from catalytic conversion of hydrogen and nitrogen on the catalyst or by radiolysis if "in-target" production is used. In addition, there is the possibility of contamination by oxygen-15-labelled oxides of nitrogen. Although these contaminants can be effectively removed from the gas phase by soda lime and charcoal adsorbers, they may break through and be present in the final preparation.

CHARACTERS

A clear, colourless solution.

Oxygen-15 has a half-life of 2.04 min and emits positrons with a maximum energy of 1.732 MeV, followed by annihilation gamma radiation of 0.511 MeV.

IDENTIFICATION

A. Record the gamma-ray spectrum using a suitable instrument. The only gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.

B. It complies with the test for radionuclidic purity (see Tests).

C. Examine the chromatogram obtained in the test for radiochemical purity. The retention time of the second peak is due to the radioactivity eluting in the void volume.

TESTS

pH (*2.2.3*)

The pH of the injection is 5.5 to 8.5.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical preparations (0125).* The injection may be released for use before completion of the test.

Bacterial endotoxins (*2.6.14*)

Less than 175/*V* IU/ml, *V* being the maximum administered volume in millilitres. The injection may be released for use before completion of the test.

CHEMICAL PURITY

(a) *Ammonium* (*2.4.1*). 1 ml complies with the limit test for ammonium (10 ppm).

(b) *Nitrates*. To 1 ml add 49 ml of *nitrate-free water R* . Place 5 ml of this solution in a test-tube immersed in iced water, add 0.4 ml of a 100 g/l solution of *potassium chloride R* , 0.1 ml of *diphenylamine solution R* and, dropwise with shaking, 5 ml of *sulphuric acid R* . Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 4.5 ml of *nitrate-free water R* and 0.5 ml of *nitrate standard solution (2 ppm* NO_3*) R* (10 ppm).

The injection may be released for use before completion of tests (a) and (b).

RADIONUCLIDIC PURITY

Record the gamma-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised fluorine-18 solution. Standardised fluorine-18 solutions are available from the laboratories recognised by the competent authority.

The half-life is between 1.9 min and 2.2 min. Not less than 99 per cent of total radioactivity corresponds to oxygen-15.

The injection may be released for use before completion of the test.

RADIOCHEMICAL PURITY

Examine by liquid chromatography (*2.2.29*).

Test solution The preparation to be examined.

The chromatographic procedure may be carried out using:

- — a column 0.25 m long and 4.0 mm in internal diameter packed with *aminopropylsilyl silica gel for chromatography R* (10 µm),
- — as mobile phase at a flow rate of 1 ml/min a 10 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3 with *phosphoric acid R* ,
- — a suitable radioactivity detector,
- — a loop injector,
- — an internal recovery detection system, consisting of a loop of the chromatographic tubing between the injector and the column through the radioactivity detector, which has been calibrated for count recovery,

maintaining the column at a constant temperature between 20 °C and 30 °C.

Inject the test solution. Continue the chromatography for 10 min. In the chromatogram obtained, the first peak corresponds to the injected radioactivity of the test solution, the second peak corresponds to the amount of radioactivity as [^{15}O]water. Calculate the percentage content of [^{15}O]water from the areas of the peaks in the chromatogram obtained with the test solution. Not less than 99 per cent of the total radioactivity injected corresponds to oxygen-15 in the form of water.

The injection may be released for use before completion of the test.

RADIOACTIVITY

Measure the radioactivity using suitable equipment by comparison with a standardised fluorine-18 solution or by using an instrument calibrated with the aid of such a solution.

Ph Eur

Xenon [^{133} Xe] Injection

General Notices

(Ph Eur monograph 0133)

Ph Eur

DEFINITION

Xenon (^{133}Xe) injection is a sterile solution of xenon-133 that may be made isotonic by the addition of sodium chloride. Xenon-133 is a radioactive isotope of xenon and is obtained by separation from the other products of uranium fission. The injection contains not less than 80 per cent and not more than 130 per cent of the declared xenon-133 radioactivity at the date and hour stated on the label.

The injection is presented in a container that allows the contents to be removed without introducing air bubbles. The container is filled as completely as possible and any gas bubble present does not occupy more than 1 per cent of the volume of the injection as judged by visual comparison with a suitable standard.

CHARACTERS

A clear, colourless solution.

Xenon-133 has a half-life of 5.29 days and emits beta and gamma radiation and X-rays.

IDENTIFICATION

Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised xenon-133 solution in a 9 g/l solution of *sodium chloride R* , apart from any differences attributable to the presence of xenon-131m and xenon-133m. If standardised xenon-133 solutions are not readily available, suitable standardised ionisation chambers are obtainable from laboratories recognised by the relevant competent authority. The most prominent gamma photon of xenon-133 has an energy of 0.081 MeV and there is an X-ray (resulting from internal conversion) of 0.030 MeV to 0.035 MeV. Xenon-131m has a half-life of 11.9 days and emits a gamma photon of 0.164 MeV. Xenon-133m has a half-life of 2.19 days and emits a gamma photon of 0.233 MeV.

TESTS

pH (*2.2.3*)

The pH of the injection is 5.0 to 8.0.

Sterility

It complies with the test for sterility prescribed in the monograph on *Radiopharmaceutical* *preparations (0125).* The injection may be released for use before completion of the test.

RADIONUCLIDIC PURITY

(a) Record the gamma-ray and X-ray spectrum using a suitable instrument. The spectrum does not differ significantly from that of a standardised xenon-133 solution in a 9 g/l solution of *sodium chloride R* , apart from any differences attributable to the presence of xenon-131m and xenon-133m.

(b) Transfer 2 ml of the injection to an open flask and pass a current of air through the solution for 30 min, taking suitable precautions concerning the dispersion of radioactivity. Measure the residual beta and gamma activity of the solution. The activity does not differ significantly from the background activity detected by the instrument.

RADIOACTIVITY

Weigh the container with its contents. Determine its total radioactivity using suitable counting equipment by comparison with a standardised xenon-133 solution or by measurement in an instrument calibrated with the aid of such a solution, operating in strictly identical conditions. If an ionisation chamber is used its inner wall should be such that the radiation is not seriously attenuated. Remove at least half the contents and re-weigh the container. Measure the radioactivity of the container and the remaining contents as described above. From the measurements, calculate the radioactive concentration of xenon-133 in the injection.

CAUTION

Significant amounts of xenon-133 may be present in the closures and on the walls of the container. This must be taken into account in applying the rules concerning the transport and storage of radioactive substances and in disposing of used containers.

Ph Eur

Absorbent Cotton

General Notices

(Ph Eur monograph 0036)

Ph Eur

DEFINITION

Absorbent cotton consists of new fibres or good quality combers obtained from the seed-coat of various species of the genus *Gossypium* L., cleaned, purified, bleached and carefully carded. It may not contain any compensatory colouring matter.

CHARACTERS

It is white or almost white and is composed of fibres of average length not less than 10 mm, determined by a suitable method, and contains not more than traces of leaf residue, pericarp, seed-coat or other impurities. It offers appreciable resistance when pulled. It does not shed any appreciable quantity of dust when gently shaken.

IDENTIFICATION

A. Examined under a microscope, each fibre is seen to consist of a single cell, up to about 4 cm long and up to 40 µm wide, in the form of a flattened tube with thick and rounded walls and often twisted.

B. When treated with *iodinated zinc chloride solution R*, the fibres become violet.

C. To 0.1 g add 10 ml of *zinc chloride-formic acid solution R.* Heat to 40 °C and allow to stand for 2 h 30 min, shaking occasionally. It does not dissolve.

TESTS

Solution S

Place 15.0 g in a suitable vessel, add 150 ml of *water R*, close the vessel and allow to macerate for 2 h. Decant the solution, squeeze the residual liquid carefully from the sample with a glass rod and mix. Reserve 10 ml of the solution for the test for surface-active substances and filter the remainder.

Acidity or alkalinity

To 25 ml of solution S add 0.1 ml of *phenolphthalein solution R* and to another 25 ml add 0.05 ml of *methyl orange solution R*. Neither solution is pink.

Foreign fibres

Examined under a microscope, it is seen to consist exclusively of typical cotton fibres, except that occasionally a few isolated foreign fibres may be present.

Fluorescence

Examine a layer about 5 mm in thickness under ultraviolet light at 365 nm. It displays only a slight brownish-violet fluorescence and a few yellow particles. It shows no intense blue fluorescence, apart from that which may be shown by a few isolated fibres.

Neps

Spread about 1 g evenly between 2 colourless transparent plates each 10 cm square. Examine for neps by transmitted light and compare with *Absorbent cotton RM*. The product to be examined is not more neppy than the standard.

Absorbency

Apparatus A dry cylindrical copper wire basket 8.0 cm high and 5.0 cm in diameter. The wire of which the basket is constructed is about 0.4 mm in diameter, the mesh is 1.5 cm to 2.0 cm wide and the mass of the basket is 2.7 ± 0.3 g.

Sinking time Not more than 10 s. Weigh the basket to the nearest centigram (m_1). Take a total of 5.00 g in approximately equal quantities from 5 different places in the product to be examined, place loosely in the basket and weigh the filled basket to the nearest centigram (m_2). Fill a beaker 11 cm to 12 cm in diameter to a depth of 10 cm with water at about 20 °C. Hold the basket horizontally and drop it from a height of about 10 mm into the water. Measure with a stopwatch the time taken for the basket to sink below the surface of the water. Calculate the result as the average of 3 tests.

Water-holding capacity Not less than 23.0 g of water per gram. After the sinking time has been measured, remove the basket from the water, allow it to drain for exactly 30 s suspended in a horizontal position over the beaker, transfer it to a tared beaker (m_3) and weigh to the nearest centigram (m_4). Calculate the water-holding capacity per gram of absorbent cotton using the following expression:

$$\frac{m_4 - (m_2 + m_3)}{m_2 - m_1}$$

Calculate the result as the average of 3 tests.

Ether-soluble substances

Not more than 0.50 per cent. In an extraction apparatus, extract 5.00 g with *ether R* for 4 h at a rate of at least 4 extractions per hour. Evaporate the ether extract and dry the residue to constant mass at 100 °C to 105 °C.

Extractable colouring matter

In a narrow percolator, slowly extract 10.0 g with *alcohol R* until 50 ml of extract is obtained. The liquid obtained is not more intensely coloured (*2.2.2, Method II*) than reference solution Y_5, GY_6 or a reference solution prepared as follows: to 3.0 ml of blue primary solution add 7.0 ml of hydrochloric acid (10 g/l HCl). Dilute 0.5 ml of this solution to 10.0 ml with hydrochloric acid (10 g/l HCl).

Surface-active substances

Introduce the 10 ml portion of solution S reserved before filtration into a 25 ml graduated ground-glass-stoppered cylinder with an external diameter of 20 mm and a wall thickness of not greater than 1.5 mm, previously rinsed 3 times with *sulphuric acid R* and then with *water R*. Shake vigorously 30 times in 10 s, allow to stand for 1 min and repeat the shaking. After 5 min, any foam present must not cover the entire surface of the liquid.

Water-soluble substances

Not more than 0.50 per cent. Boil 5.000 g in 500 ml of *water R* for 30 min, stirring frequently. Replace the water lost by evaporation. Decant the liquid, squeeze the residual liquid carefully from the sample with a glass rod and mix. Filter the liquid whilst hot. Evaporate 400 ml of the filtrate (corresponding to 4/5 of the mass of the sample taken) and dry the residue to constant mass at 100 °C to 105 °C.

Loss on drying *(2.2.32)*

Not more than 8.0 per cent, determined on 5.000 g by drying in an oven at 105 °C.

Sulphated ash *(2.4.14)*

Not more than 0.40 per cent. Introduce 5.00 g into a previously heated and cooled, tared crucible. Heat cautiously over a naked flame and then carefully to dull redness at 600 °C. Allow to cool, add a few drops of *dilute sulphuric acid R*, then heat and incinerate until all the black particles have disappeared. Allow to cool. Add a few drops of *ammonium carbonate solution R*. Evaporate and incinerate carefully, allow to cool and weigh again. Repeat the incineration for periods of 5 min to constant mass.

STORAGE

Store in a dust-proof package in a dry place.

Ph Eur

Absorbent Viscose Wadding

General Notices

(Ph Eur monograph 0034)

Ph Eur

DEFINITION

Absorbent viscose wadding consists of bleached, carefully carded, new fibres of regenerated cellulose obtained by the viscose process, with or without the addition of titanium dioxide, of linear density 1.0 dtex to 8.9 dtex (dtex = mass of 10 000 m of fibre, expressed in grams) and cut to a suitable staple length. It does not contain any compensatory colouring matter.

CHARACTERS

It is white or very slightly yellow, has a lustrous or matt appearance, and is soft to the touch.

IDENTIFICATION

A. Viscose rayon fibres may be solid or hollow; hollow fibres may have a continuous lumen or be compartmented. The fibres have an average length of 25 mm to 80 mm and when examined under a microscope in the dry state, or when mounted in *alcohol R* and *water R*, the following characters are observed. They are usually of a more or less uniform width, with many longitudinal parallel lines distributed unequally over the width. The ends are cut more or less straight. Matt fibres contain numerous granular particles of approximately 1 µm average diameter.

Solid fibres In longitudinal view, the surface of the fibres may be uneven or crenate. Fibres having an approximately circular or elliptical cross section have a diameter of about 10 µm to 20 µm and those that are flattened and twisted ribbons vary in width from 15 µm to 20 µm as the twisting of the filament reveals first the major axis and then the minor axis. They are about 4 µm in thickness. Other solid cross sections are Y-shaped and have protruding limbs with the major axis 5 µm to 25 µm in length and the minor axis 2 µm to 8 µm wide.

Hollow fibres Fibres with a continuous, hollow lumen have a diameter of up to about 30 µm; they are thin-walled, with a wall thickness of about 5 µm. When mounted in *alcohol R* and *water R*, the lumen is clearly indicated in many fibres by the presence of many entrapped air bubbles.

Compartmented fibres These fibres may have a diameter of up to 80 µm; they are hollow, having a central lumen which is divided up into several compartments. Individual compartments vary in size but typically may be up to about 60 µm in length and there may be more than one compartment across the width of each fibre. Some compartments show entrapped air bubbles when the fibres are mounted in *alcohol R* and *water R.*

B. When treated with *iodinated zinc chloride solution R*, the fibres become violet.

C. To 0.1 g add 10 ml of *zinc chloride-formic acid solution R*. Heat to 40 °C and allow to stand for 2 h 30 min, shaking occasionally. It dissolves completely except for the matt variety where titanium dioxide particles remain.

D. Dissolve the residue obtained in the test for sulphated ash by warming gently with 5 ml of *sulphuric acid R*. Allow to cool and add 0.2 ml of *dilute hydrogen peroxide solution R*. The solution obtained from the lustrous variety undergoes no change in colour; that from the matt variety shows an orange-yellow colour, the intensity of which depends on the quantity of titanium dioxide present.

TESTS

Solution S

Place 15.0 g in a suitable vessel, add 150 ml of *water R*, close the vessel and allow to macerate for 2 h. Decant the solution, squeeze the residual liquid carefully from the sample with a glass rod and mix. Reserve 10 ml of the solution for the test for surface-active substances and filter the remainder.

Acidity or alkalinity

To 25 ml of solution S add 0.1 ml of *phenolphthalein solution R* and to another 25 ml add 0.05 ml of *methyl orange solution R*. Neither solution is pink.

Foreign fibres

Examined under a microscope, it is seen to consist exclusively of viscose fibres, except that occasionally a few isolated foreign fibres may be present.

Fluorescence

Examine a layer about 5 mm in thickness under ultraviolet light at 365 nm. It displays only a slight brownish-violet fluorescence. It shows no intense blue fluorescence, apart from that which may be shown by a few isolated fibres.

Absorbency

Apparatus A dry cylindrical copper-wire basket 8.0 cm high and 5.0 cm in diameter. The wire of which the basket is constructed is about 0.4 mm in diameter, the mesh is 1.5 cm to 2.0 cm wide and the mass of the basket is 2.7 ± 0.3 g.

Sinking time Not more than 10 s. Weigh the basket to the nearest centigram (m_1). Take a total of 5.00 g in approximately equal quantities from 5 different places in the product to be examined, place loosely in the basket and weigh the filled basket to the nearest centigram (m_2). Fill a beaker 11 cm to 12 cm in diameter to a depth of 10 cm with water at about 20 °C. Hold the basket horizontally and drop it from a height of about 10 mm into the water. Measure with a stopwatch the time taken for the basket to sink below the surface of the water. Calculate the result as the average of 3 tests.

Water-holding capacity Not less than 18.0 g of water per gram. After the sinking time has been measured, remove the basket from the water, allow it to drain for exactly 30 s suspended in a horizontal position over the beaker, transfer it to a tared beaker (m_3) and weigh to the nearest centigram (m_4). Calculate the water-holding capacity per gram of absorbent viscose wadding using the following expression:

$$\frac{m_4 - (m_2 + m_3)}{m_2 - m_1}$$

Calculate the result as the average of 3 tests.

Ether-soluble substances

Not more than 0.30 per cent. In an extraction apparatus, extract 5.00 g with *ether R* for 4 h at a rate of at least 4 extractions per hour. Evaporate the ether extract and dry the residue to constant mass at 100 °C to 105 °C.

Extractable colouring matter

In a narrow percolator, slowly extract 10.0 g with *alcohol R* until 50 ml of extract is obtained. The liquid obtained is not more intensely coloured (*2.2.2, Method II*) than reference solution Y_5, GY_6 or a reference solution prepared as follows: to 3.0 ml of blue primary solution add 7.0 ml of hydrochloric acid (10 g/l HCl) and dilute 0.5 ml of this solution to 10.0 ml with hydrochloric acid (10 g/l HCl).

Surface-active substances

Introduce the 10 ml portion of solution S reserved before filtration into a 25 ml graduated ground-glass-stoppered cylinder with an external diameter of 20 mm and a wall thickness of not greater than 1.5 mm, previously rinsed 3 times with *sulphuric acid R* and then with *water R*. Shake vigorously 30 times in 10 s, allow to stand for 1 min and repeat the shaking. After 5 min, any foam present does not cover the entire surface of the liquid.

Water-soluble substances

Not more than 0.70 per cent. Boil 5.00 g in 500 ml of *water R* for 30 min, stirring frequently. Replace the water lost by evaporation. Decant the liquid, squeeze the residual liquid carefully from the sample with a glass rod and mix. Filter the liquid whilst hot. Evaporate 400 ml of the filtrate (corresponding to 4/5 of the mass of the sample taken) and dry the residue to constant mass at 100 °C to 105 °C.

Hydrogen sulphide

To 10 ml of solution S add 1.9 ml of *water R*, 0.15 ml of *dilute acetic acid R* and 1 ml of *lead acetate solution R*. After 2 min, the solution is not more intensely coloured than a reference solution prepared at the same time using 0.15 ml of *dilute acetic acid R*, 1.2 ml of *thioacetamide reagent R*, 1.7 ml of *lead standard solution (10 ppm Pb) R* and 10 ml of solution S.

Loss on drying (*2.2.32*)

Not more than 13.0 per cent, determined on 5.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*)

Not more than 0.45 per cent for the lustrous variety and not more than 1.7 per cent for the matt variety. Introduce 5.00 g into a previously heated and cooled, tared crucible. Heat cautiously over a naked flame and then carefully to dull redness at 600 °C. Allow to cool, add a few drops of *dilute sulphuric acid R*, then heat and incinerate until all the black particles have disappeared. Allow to cool. Add a few drops of *ammonium carbonate solution R*. Evaporate and incinerate carefully, allow to cool and weigh again. Repeat the incineration for periods of 5 min to constant mass.

STORAGE

Store in a dust-proof package in a dry place.

Ph Eur

Sterile Catgut

General Notices

(Ph Eur monograph 0317)

Ph Eur

DEFINITION

Sterile catgut consists of sutures prepared from collagen taken from the intestinal membranes of mammals. After cleaning, the membranes are split longitudinally into strips of varying width, which, when assembled in small numbers, according to the diameter required, are twisted under tension, dried, polished, selected and sterilised. The sutures may be treated with chemical substances such as chromium salts to prolong absorption and glycerol to make them supple, provided such substances do not reduce tissue acceptability.

Appropriate harmonised standards may be considered when assessing compliance with respect to origin and processing of raw materials and with respect to biocompatibility.

Sterile catgut is a surgical wound-closure device. Being an absorbable suture it serves to approximate tissue during the healing period and is subsequently metabolised by proteolytic activity.

PRODUCTION

Production complies with relevant regulations on the use of animal tissues in medical devices notably concerning the risk of transmission of animal spongiform encephalopathy agents.

Appropriate harmonised standards may apply with respect to appropriate validated methods of sterilisation, environmental control during manufacturing, labelling and packaging.

It is essential for the effectiveness and the performance characteristics during use and during the functional lifetime of catgut that the following physical properties are specified: consistent diameter, sufficient initial strength and firm needle attachment.

The requirements outlined below have been established, taking into account stresses which occur during normal conditions of use. These requirements can be used to demonstrate that individual production batches of sterile catgut are suitable for wound closure according to usual surgical techniques.

TESTS

If stored in a preserving liquid, remove the sutures from the sachet and measure promptly and in succession the length, diameter and breaking load. If stored in the dry state, immerse the sutures in alcohol R or a 90 per cent V/V solution of 2-propanol R for 24 h and proceed with the measurements as indicated below.

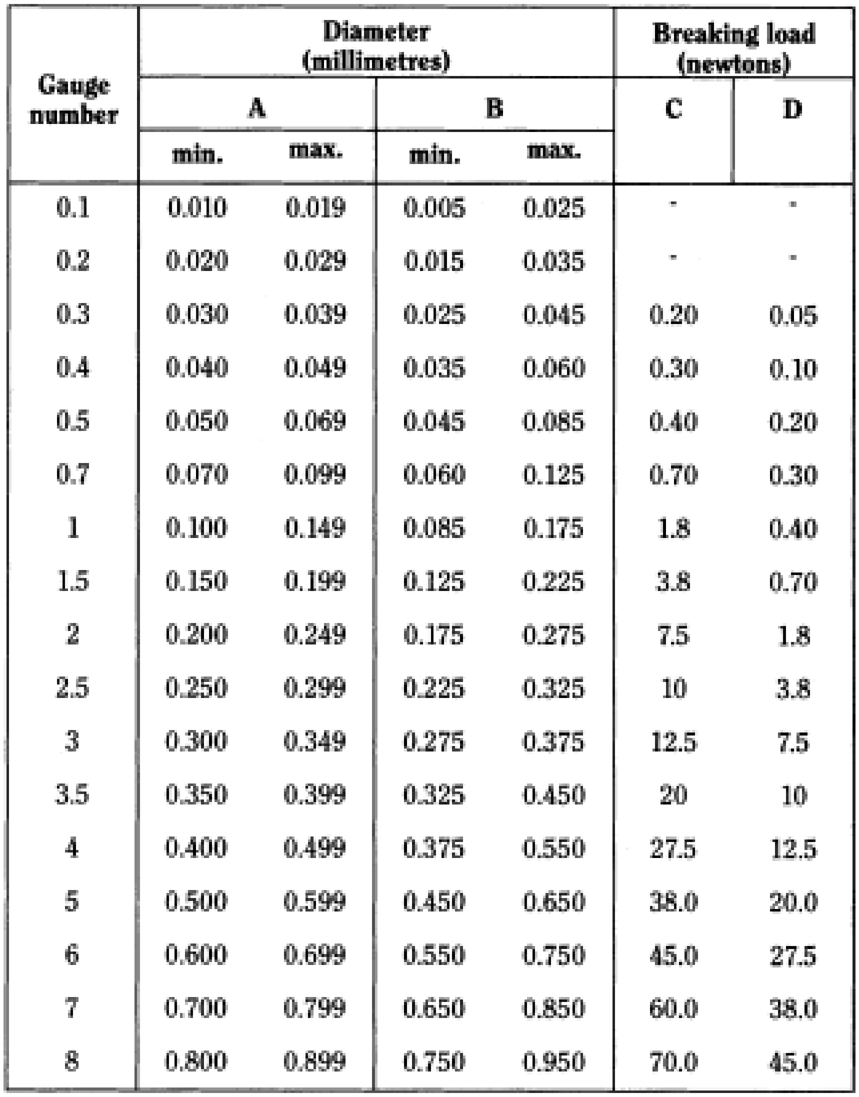
Length

Measure the length without applying to the suture more tension than is necessary to keep it straight. The length of each suture is not less than 90 per cent of the length stated on the label and does not exceed 350 cm.

Diameter

Carry out the test on 5 sutures. Use a suitable instrument capable of measuring with an accuracy of at least 0.002 mm and having a circular pressor foot 10 mm to 15 mm in diameter. The pressor foot and the moving parts attached to it are weighted so as to apply a total load of 100 ± 10 g to the suture being tested. When making the measurement, lower the pressor foot slowly to avoid crushing the suture. Measure the diameter at intervals of 30 cm over the whole length of the suture. For a suture less than 90 cm in length, measure at 3 points approximately evenly spaced along the suture. The suture is not subjected to more tension than is necessary to keep it straight during measurement. The average of the measurements carried out on the sutures being tested and not less than two-thirds of the measurements taken on each suture are within the limits given in the columns under A in Table 0317.-1 for the gauge number concerned. None of the measurements is outside the limits given in the columns under B in Table 0317.-1 for the gauge number concerned.

Table 0317.-1.– *Diameters and Breaking Loads*



Gauge number	Diameter (millimetres)				Breaking load (newtons)	
	A		B		C	D
	min.	max.	min.	max.		
0.1	0.010	0.019	0.005	0.025	-	-
0.2	0.020	0.029	0.015	0.035	-	-
0.3	0.030	0.039	0.025	0.045	0.20	0.05
0.4	0.040	0.049	0.035	0.060	0.30	0.10
0.5	0.050	0.069	0.045	0.085	0.40	0.20
0.7	0.070	0.099	0.060	0.125	0.70	0.30
1	0.100	0.149	0.085	0.175	1.8	0.40
1.5	0.150	0.199	0.125	0.225	3.8	0.70
2	0.200	0.249	0.175	0.275	7.5	1.8
2.5	0.250	0.299	0.225	0.325	10	3.8
3	0.300	0.349	0.275	0.375	12.5	7.5
3.5	0.350	0.399	0.325	0.450	20	10
4	0.400	0.499	0.375	0.550	27.5	12.5
5	0.500	0.599	0.450	0.650	38.0	20.0
6	0.600	0.699	0.550	0.750	45.0	27.5
7	0.700	0.799	0.650	0.850	60.0	38.0
8	0.800	0.899	0.750	0.950	70.0	45.0

Minimum breaking load

The minimum breaking load is determined over a simple knot formed by placing one end of a suture held in the right hand over the other end held in the left hand, passing one end over the suture and through the loop so formed (see Figure 0317.-1) and pulling the knot tight. Carry out the test on 5 sutures. Submit sutures of length greater than 75 cm to 2 measurements and shorter sutures to one measurement. Determine the breaking load using a suitable tensilometer. The apparatus has 2 clamps for holding the suture, one of which is mobile and is driven at a constant rate of 30 cm/min. The clamps are designed so that the suture being tested can be attached without any possibility of slipping. At the beginning of the test the length of suture between the clamps is 12.5 cm to 20 cm and the knot is midway between the clamps. Set the mobile clamp in motion and note the force required to break the suture. If the suture breaks in a clamp or within 1 cm of it, the result is discarded and the test repeated on another suture. The average of all the results, excluding those legitimately discarded, is equal to or greater than the value given in column C in Table 0317.-1 and no individual result is less than that given in column D for the gauge number concerned.

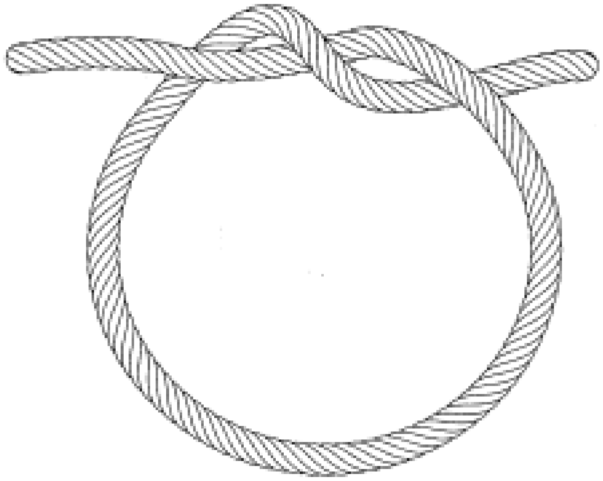


Figure 0317.-1. – *Simple knot*

Soluble chromium compounds

Place 0.25 g in a conical flask containing 1 ml of *water R* per 10 mg of catgut. Stopper the flask, allow to stand at 37 ± 0.5 °C for 24 h, cool and decant the liquid. Transfer 5 ml to a small test tube and add 2 ml of a 10 g/l solution of *diphenylcarbazide R* in *alcohol R* and 2 ml of *dilute sulphuric acid R*. The solution is not more intensely coloured than a standard prepared at the same time using 5 ml of a solution containing 2.83 µg of *potassium dichromate R* per millilitre, 2 ml of *dilute sulphuric acid R* and 2 ml of a 10 g/l solution of *diphenylcarbazide R* in *alcohol R* (1 ppm of Cr).

Needle attachment

If the catgut is supplied with an eyeless needle attached that is not stated to be detachable, it complies with the test for needle attachment. Carry out the test on 5 sutures. Use a suitable tensilometer, such as that described for the determination of the minimum breaking load. Fix the needle and suture (without knot) in the clamps of the apparatus in such a way that the swaged part of the needle is completely free of the clamp and in line with the direction of pull on the suture. Set the mobile clamp in motion and note the force required to break the suture or to detach it from the needle. The average of the 5 determinations and all individual values are not less than the respective values given in Table 0317.-2 for the gauge number concerned. If not more than one individual value fails to meet the individual requirement, repeat the test on an additional 10 sutures. The catgut complies with the test if none of these 10 values is less than the individual value in Table 0317.-2 for the gauge number concerned.

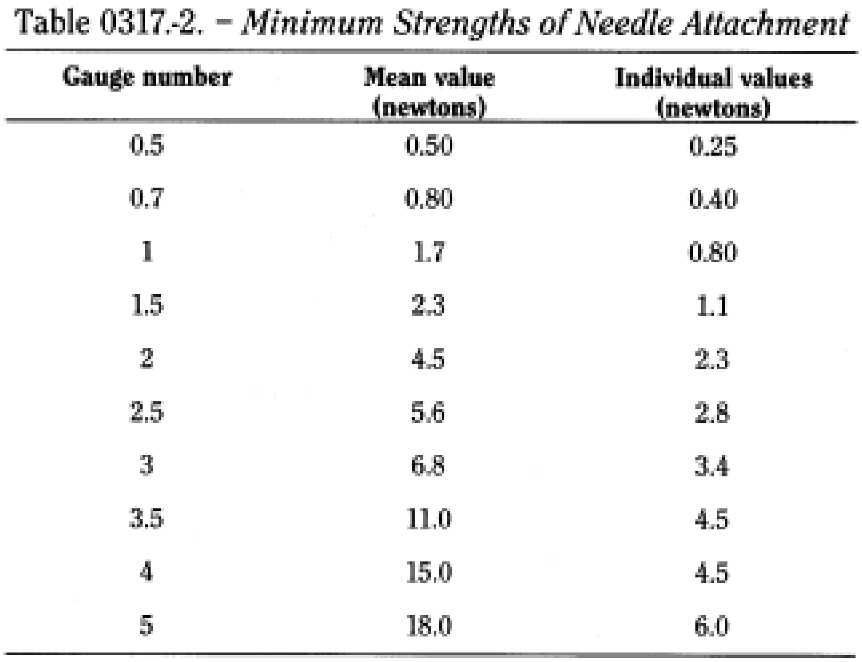


Table 0317.-2. – *Minimum Strengths of Needle Attachment*

Gauge number	Mean value (newtons)	Individual values (newtons)
0.5	0.50	0.25
0.7	0.80	0.40
1	1.7	0.80
1.5	2.3	1.1
2	4.5	2.3
2.5	5.6	2.8
3	6.8	3.4
3.5	11.0	4.5
4	15.0	4.5
5	18.0	6.0

STORAGE (PACKAGING)

Sterile catgut sutures are presented in individual sachets that maintain sterility and allow the withdrawal and use of the sutures in aseptic conditions. Sterile catgut may be stored dry or in a preserving liquid to which an antimicrobial agent but not an antibiotic may be added.

Sutures in their individual sachets (primary packaging) are kept in a protective cover (box) which maintains the physical and mechanical properties until the time of use.

The application of appropriate harmonised standards for packaging of medical devices shall be considered.

LABELLING

Reference may be made to the appropriate harmonised standards for labelling of medical devices.

The details strictly necessary for the user to identify the product properly are indicated on or in each sachet (primary packaging) and on the protective cover (box) and include at least:

- — gauge number;
- — length in centimetres or metres;
- — if appropriate, that the needle is detachable;
- — name of the product;
- — intended use (surgical suture, absorbable).

Ph Eur

Sterile Non-absorbable Sutures

General Notices

Sterile Non-absorbable Ligatures

(Ph Eur monograph 0324)

NOTE: *The name Nylon 6 as a synonym for Polyamide 6 and Nylon 6/6 as a synonym for Polyamide 6/6 may be used freely in many countries, including the United Kingdom, but exclusive proprietary rights in this name are claimed in certain other countries.*

Ph Eur

DEFINITION

Sterile non-absorbable sutures are sutures which, when introduced into a living organism, are not metabolised by that organism. Sterile non-absorbable sutures vary in origin, which may be animal, vegetable, metallic or synthetic. They occur as cylindrical monofilaments or as multifilament sutures consisting of elementary fibres which are assembled by twisting, cabling or braiding; they may be sheathed; they may be treated to render them non-capillary, and they may be coloured.

Appropriate harmonised standards may be considered when assessing compliance with respect to origin and processing of raw materials and with respect to biocompatibility.

Sterile non-absorbable surgical sutures serve to approximate tissue during the healing period and provide continuing wound support.

Commonly used materials include the following:

Silk (Filum bombycis)

Sterile braided silk suture is obtained by braiding a number of threads, according to the diameter required, of degummed silk obtained from the cocoons of the silkworm *Bombyx mori* L.

Linen (Filum lini)

Sterile linen thread consists of the pericyclic fibres of the stem of *Linum usitatissimum* L. The elementary fibres, 2.5 cm to 5 cm long, are assembled in bundles 30 cm to 80 cm long and spun into continuous lengths of suitable diameter.

Poly(ethylene terephthalate) (Filum ethyleni polyterephthalici)

Sterile poly(ethylene terephthalate) suture is obtained by drawing poly(ethylene terephthalate) through a suitable die. The suture is prepared by braiding very fine filaments in suitable numbers, depending on the gauge required.

Polyamide-6 (Filum polyamidicum-6)

Sterile polyamide-6 suture is obtained by drawing through a suitable die a synthetic plastic material formed by the polymerisation of ∊-caprolactam. It consists of smooth, cylindrical monofilaments or braided filaments, or lightly twisted sutures sheathed with the same material.

Polyamide-6/6 (Filum polyamidicum-6/6)

Sterile polyamide-6/6 suture is obtained by drawing through a suitable die a synthetic plastic material formed by the polycondensation of hexamethylenediamine and adipic acid. It consists of smooth, cylindrical monofilaments or braided filaments, or lightly twisted sutures sheathed with the same material.

Polypropylene (Filum polypropylenicum)

Polypropylene suture is obtained by drawing polypropylene through a suitable die. It consists of smooth cylindrical mono-filaments.

Monofilament and multifilament stainless steel (Filum aciei irrubiginibilis monofilamentum/multifilamentum)

Sterile stainless steel sutures have a chemical composition as specified in ISO 5832-1 - Metallic Materials for surgical implants - Part 1: Specification for wrought stainless steel and comply with ISO 10334 - Implants for surgery - Malleable wires for use as sutures and other surgical applications.

Stainless steel sutures consist of smooth, cylindrical monofilaments or twisted filaments or braided filaments.

Poly(vinylidene difluoride) (PVDF) (Filum poly(vinylideni difluoridum))

Sterile PVDF suture is obtained by drawing through a suitable die a synthetic plastic material which is formed by polymerisation of 1,1-difluorethylene. It consists of smooth cylindrical monofilaments.

IDENTIFICATION

Non-absorbable sutures may be identified by chemical tests. Materials from natural origin may also be identified by microscopic examination of the morphology of these fibres. For synthetic materials, identification by infrared spectrophotometry (*2.2.24*) or by differential scanning calorimetry may be applied.

Identification of silk

A. Dissect the end of a suture, using a needle or fine tweezers, to isolate a few individual fibres. The fibres are sometimes marked with very fine longitudinal striations parallel to the axis of the suture. Examined under a microscope, a cross-section is more or less triangular to semi-circular, with rounded edges and without a lumen.

B. Impregnate isolated fibres with *iodinated potassium iodide solution R*. The fibres are coloured pale yellow.

Identification of linen

A. Dissect the end of a suture, using a needle or fine tweezers, to isolate a few individual fibres. Examined under a microscope, the fibres are seen to be 12 µm to 31 µm wide and, along the greater part of their length, have thick walls, sometimes marked with fine longitudinal striations, and a narrow lumen. The fibres gradually narrow to a long, fine point. Sometimes there are unilateral swellings with transverse lines.

B. Impregnate isolated fibres with *iodinated zinc chloride solution R*. The fibres are coloured violet-blue.

Identification of poly(ethyleneterephthalate)

It is practically insoluble in most of the usual organic solvents, but is attacked by strong alkaline solutions. It is incompatible with phenols.

A. 50 mg dissolves with difficulty when heated in 50 ml of *dimethylformamide R.*

B. To about 50 mg add 10 ml of *hydrochloric acid R1*. The material remains intact even after immersion for 6 h.

Identification of polyamide-6

It is practically insoluble in the usual organic solvents; it is not attacked by dilute alkaline solutions (for example a 100 g/l solution of *sodium hydroxide R*) but is attacked by dilute mineral acids (for example a 20 g/l solution of *sulphuric acid R*), by hot *glacial acetic acid R* and by a 70 per cent *m/m* solution of *anhydrous formic acid R.*

A. Heat about 50 mg with 0.5 ml of *hydrochloric acid R1* in a sealed glass tube at 110 °C for 18 h and allow to stand for 6 h. No crystals appear.

B. 50 mg dissolves in 20 ml of a 70 per cent *m/m* solution of *anhydrous formic acid R*.

Identification of polyamide-6/6

It is practically insoluble in the usual organic solvents; it is not attacked by dilute alkaline solutions (for example a 100 g/l solution of *sodium hydroxide R*) but is attacked by dilute mineral acids (for example a 20 g/l solution of *sulphuric acid R*), by hot *glacial acetic acid R* and by an 80 per cent *m/m* solution of *anhydrous formic acid R*.

A. In contact with a flame it melts and burns, forming a hard globule of residue and gives off a characteristic odour resembling that of celery.

B. Place about 50 mg in an ignition tube held vertically and heat gently until thick fumes are evolved. When the fumes fill the tube, withdraw it from the flame and insert a strip of *nitrobenzaldehyde paper R*. A violet-brown colour slowly appears on the paper and fades slowly in air; it disappears almost immediately on washing with *dilute sulphuric acid R.*

C. To about 50 mg add 10 ml of *hydrochloride acid R1*. The material disintegrates in the cold and dissolves within a few minutes.

D. 50 mg does not dissolve in 20 ml of a 70 per cent *m/m* solution of *anhydrous formic acid R* but dissolves in 20 ml of an 80 per cent *m/m* solution of *anhydrous formic acid R*.

Identification of polypropylene

Polypropylene is soluble in decahydronaphthalene, 1-chloronaphthalene and trichloroethylene. It is not soluble in alcohol, in ether and in cyclohexanone.

A. It softens at temperatures between 160 °C and 170 °C. It burns with a blue flame giving off an odour of burning paraffin wax and of octyl alcohol.

B. To 0.25 g add 10 ml of *toluene R* and boil under a reflux condenser for about 15 min. Place a few drops of the solution on a disc of *sodium chloride R* slide and evaporate the solvent in an oven at 80 °C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *polypropylene CRS.*

C. To 2 g add 100 ml of *water R* and boil under a reflux condenser for 2 h. Allow to cool. The relative density (*2.2.5*) of the material is 0.89 g/ml to 0.91 g/ml, determined using a hydrostatic balance.

Identification of stainless steel

Stainless steel sutures are identified by confirming that the composition is in accordance with ISO 5832 Part 1.

Identification of poly(vinylidene difluoride)

It is soluble in warm dimethylformamide. It is insoluble in ethanol, hot and cold isopropyl alcohol, ethyl acetate, tetrachlorethylene.

A. The strand melts between 170 °C and 180 °C. It melts in a flame and does not burn after removal of the flame. Place a small piece of suture on an annealed copper wire or sheet. Heat in an oxidising flame. No green colour is produced.

B. Dissolve 0.25 g of the suture in 10 ml of *dimethylformamide R* and boil under a reflux condenser for about 15 min. Place a few drops of the solution on a *sodium chloride R* slide and evaporate the solvent in an oven at 80 °C (1 h). Examine by infrared absorption spectrophotometry (*2.2.24*). The spectrum shows absorption maxima at the following wave-numbers: 838.3 ± 0.5 cm^{-1}, 873.3 ± 1 cm^{-1}, 1070.0 ± 2 cm^{-1}, 1165.0 ± 10 cm^{-1}, 1275 ± 0.5 cm^{-1}, 1399 ± 5 cm^{-1}.

C. To 2 g of suture add 100 ml of *water R* and boil under a reflux condenser for 2 h. Allow to cool. The relative density (*2.2.5*) of the material is 1.71 to 1.78.

PRODUCTION

The appropriate harmonised standards may apply with respect to appropriate validated methods of sterilisation, environmental control during manufacturing, labelling and packaging.

It is essential for the effectiveness and the performance characteristics during use and during the functional lifetime of these sutures that the following physical properties are specified: consistent diameter, sufficient initial strength and firm needle attachment.

The requirements below have been established, taking into account stresses which occur during normal conditions of use. These requirements can be used to demonstrate that individual production batches of these sutures are suitable for wound closure in accordance with usual surgical techniques.

TESTS

Remove the sutures from the sachet and measure promptly and in succession the length, diameter and minimum load.

If linen is tested the sutures are conditioned as follows: if stored in the dry state, expose to an atmosphere with a relative humidity of 65 ± 5 per cent at 20 ± 2 °C for 4 h immediately before measuring the diameter and for the determination of minimum breaking load immerse in *water R* at room temperature for 30 min immediately before carrying out the test.

Length

Measure the length without applying more tension than is necessary to keep them straight. The length of the suture is not less than 95 per cent of the length stated on the label and does not exceed 400 cm.

Diameter

Unless otherwise prescribed, measure the diameter by the following method using 5 sutures. Use a suitable mechanical instrument capable of measuring with an accuracy of at least

0.002 mm and having a circular pressor foot 10-15 mm in diameter. The pressor foot and the moving parts attached to it are weighted so as to apply a total load of 100 ± 10 g to the suture being tested. When making the measurements, lower the pressor foot slowly to avoid crushing the suture. Measure the diameter at intervals of 30 cm over the whole length of the suture. For a suture less than 90 cm in length, measure at 3 points approximately evenly spaced along the suture. During the measurement submit monofilament sutures to a tension not greater than that required to keep them straight. Submit multifilament sutures to a tension not greater than one-fifth of the minimum breaking load shown in column C of Table 0324.-1 appropriate to the gauge number and type of material concerned or 10 N whichever is less. Stainless steel sutures do not require tension to be applied during the measurement of diameter. For multifilament sutures of gauge number above 1.5 make 2 measurements at each point, the second measurement being made after rotating the suture through 90°. The diameter of that point is the average of the 2 measurements. The average of the measurements carried out on the sutures being tested and not less than two-thirds of the measurements taken on each suture are within the limits given in the column under A in Table 0324.-1 for the gauge number concerned. None of the measurements are outside the limits given in the columns under B in Table 0324.-1 for the gauge number concerned.

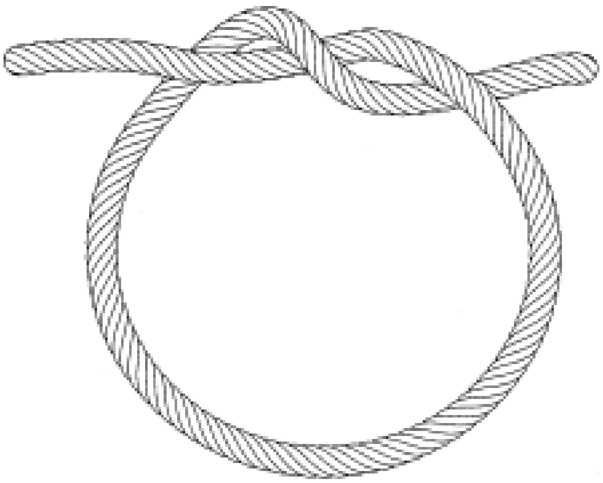


Figure 0324.-1. – *Simple knot*

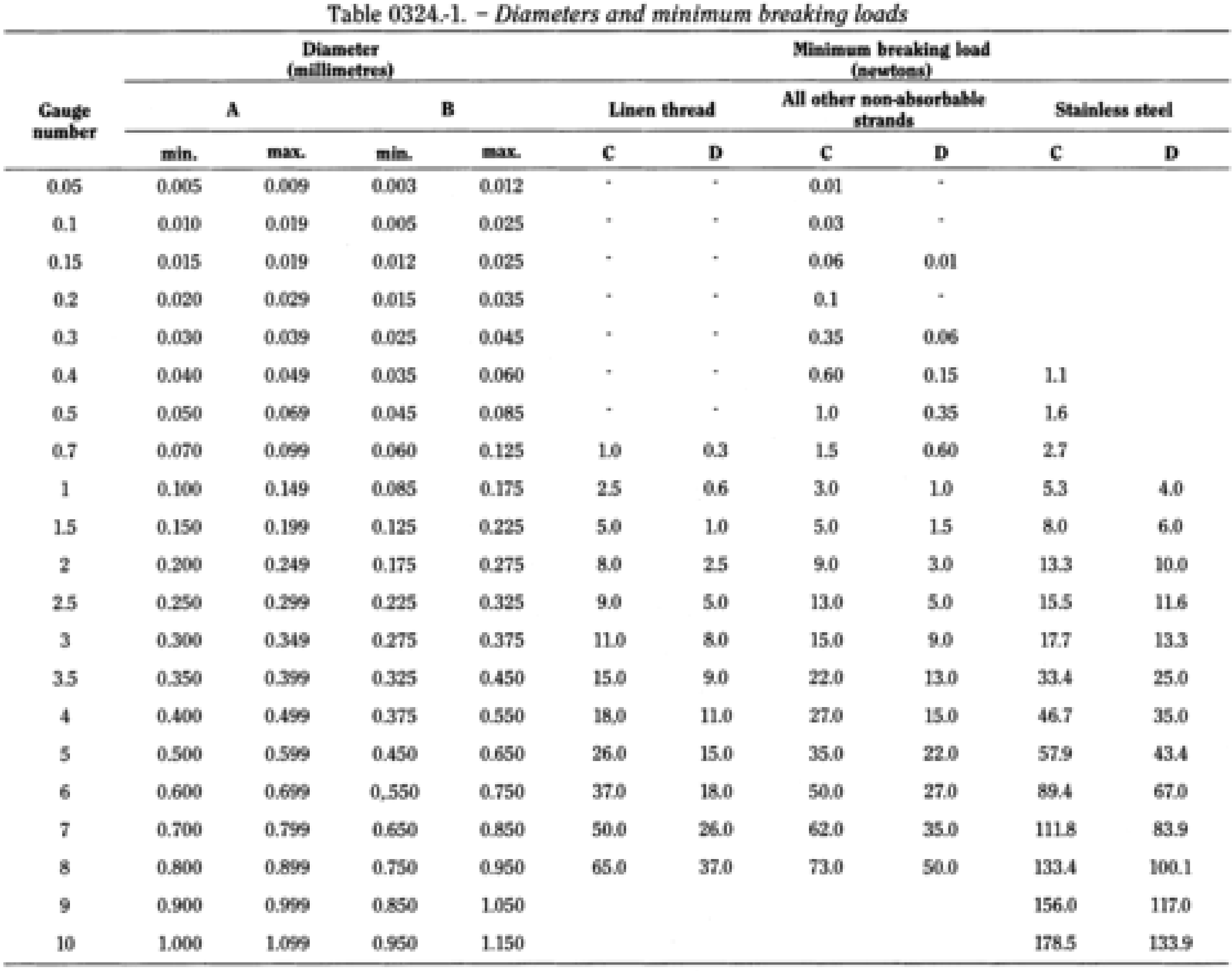


Table 0324.-1. – *Diameters and minimum breaking loads*

Gauge number	Diameter (millimetres)				Minimum breaking load (newtons)					
	A		B		Linen thread		All other non-absorbable strands		Stainless steel	
	min.	max.	min.	max.	C	D	C	D	C	D
0.05	0.005	0.009	0.003	0.012	-	-	0.01	-		
0.1	0.010	0.019	0.005	0.025	-	-	0.03	-		
0.15	0.015	0.019	0.012	0.025	-	-	0.06	0.01		
0.2	0.020	0.029	0.015	0.035	-	-	0.1	-		
0.3	0.030	0.039	0.025	0.045	-	-	0.35	0.06		
0.4	0.040	0.049	0.035	0.060	-	-	0.60	0.15	1.1	
0.5	0.050	0.069	0.045	0.085	-	-	1.0	0.35	1.6	
0.7	0.070	0.099	0.060	0.125	1.0	0.3	1.5	0.60	2.7	
1	0.100	0.149	0.085	0.175	2.5	0.6	3.0	1.0	5.3	4.0
1.5	0.150	0.199	0.125	0.225	5.0	1.0	5.0	1.5	8.0	6.0
2	0.200	0.249	0.175	0.275	8.0	2.5	9.0	3.0	13.3	10.0
2.5	0.250	0.299	0.225	0.325	9.0	5.0	13.0	5.0	15.5	11.6
3	0.300	0.349	0.275	0.375	11.0	8.0	15.0	9.0	17.7	13.3
3.5	0.350	0.399	0.325	0.450	15.0	9.0	22.0	13.0	33.4	25.0
4	0.400	0.499	0.375	0.550	18.0	11.0	27.0	15.0	46.7	35.0
5	0.500	0.599	0.450	0.650	26.0	15.0	35.0	22.0	57.9	43.4
6	0.600	0.699	0,550	0.750	37.0	18.0	50.0	27.0	89.4	67.0
7	0.700	0.799	0.650	0.850	50.0	26.0	62.0	35.0	111.8	83.9
8	0.800	0.899	0.750	0.950	65.0	37.0	73.0	50.0	133.4	100.1
9	0.900	0.999	0.850	1.050					156.0	117.0
10	1.000	1.099	0.950	1.150					178.5	133.9

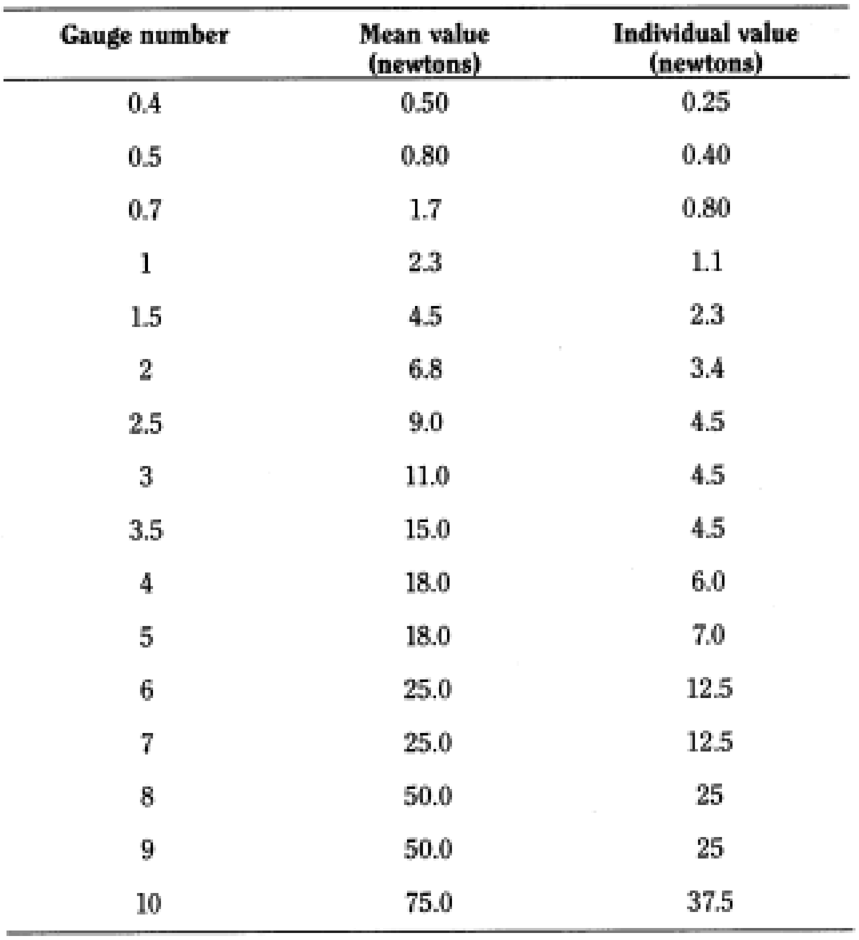
Minimum breaking load

Unless otherwise prescribed, determine the minimum breaking load by the following method using sutures in the condition in which they are presented. The minimum breaking load is determined over a simple knot formed by placing one end of a suture held in the right hand over the other end held in the left hand, passing one end over the suture and through the loop so formed (see Figure 0324.-1) and pulling the knot tight. For stainless steel sutures gauges 3.5 and above, the minimum breaking load is determined on a straight pull. Carry out the test on 5 sutures. Submit sutures of length greater than 75 cm to 2 measurements and shorter sutures to 1 measurement. Determine the breaking load using a suitable tensilometer. The apparatus has 2 clamps for holding the suture, 1 of which is mobile and is driven at a constant rate of 30 cm/min. The clamps are designed so that the suture being tested can be attached without any possibility of slipping. At the beginning of the test the length of suture between the clamps is 12.5 cm to 20 cm and the knot is midway between the clamps. Set the mobile clamp in motion and note the force required to break the suture. If the suture breaks in a clamp or within 1 cm of it, the result is discarded and the test repeated on another suture. The average of all the results, excluding those legitimately discarded, is equal to or greater than the value given in column C in Table 0324.-1 and no value is less than that given in column D for the gauge number and type of material concerned.

Needle attachment

If the sutures are supplied with an eyeless needle attached that is not stated to be detachable, they comply with the test for needle attachment. Carry out the test on 5 sutures. Use a suitable tensilometer, such as that described for the determination of the minimum breaking load. Fix the needle and suture (without knot) in the clamps of the apparatus in such a way that the swaged part of the needle is completely free of the clamp and in line with the direction of pull on the suture. Set the mobile clamp in motion and note the force required to break the suture or to detach it from the needle. The average of the 5 determinations and all individual values are not less than the respective values given in Table 0324.-2 for the gauge number concerned. If not more than 1 individual value fails to meet the individual requirement, repeat the test on an additional 10 sutures. The attachment complies with the test if none of these 10 values is less than the individual value in Table 0324.-2 for the gauge number concerned.

Table 0324.-2. – *Minimum strengths of needle attachment*



Gauge number	Mean value (newtons)	Individual value (newtons)
0.4	0.50	0.25
0.5	0.80	0.40
0.7	1.7	0.80
1	2.3	1.1
1.5	4.5	2.3
2	6.8	3.4
2.5	9.0	4.5
3	11.0	4.5
3.5	15.0	4.5
4	18.0	6.0
5	18.0	7.0
6	25.0	12.5
7	25.0	12.5
8	50.0	25
9	50.0	25
10	75.0	37.5

Extractable colour

Sutures that are dyed and intended to remain so during use comply with the test for extractable colour. Place 0.25 g of the suture to be examined in a conical flask, add 25.0 ml of *water R* and cover the mouth of the flask with a short-stemmed funnel. Boil for 15 min, cool and adjust to the original volume with *water R*. Depending on the colour of the suture, prepare the appropriate reference solution as described in Table 0324.-3 using the primary colour solutions (*2.2.2*).

The test solution is not more intensely coloured than the appropriate reference solution.

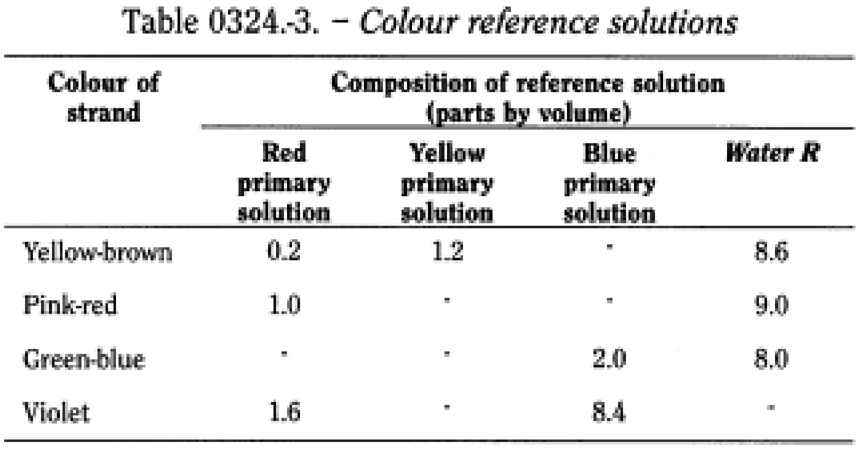


Table 0324.-3. – *Colour reference solutions*

Colour of strand	Composition of reference solution (parts by volume)			
	Red primary solution	Yellow primary solution	Blue primary solution	*Water R*
Yellow-brown	0.2	1.2	-	8.6
Pink-red	1.0	-	-	9.0
Green-blue	-	-	2.0	8.0
Violet	1.6	-	8.4	-

Monomer and oligomers

Polyamide-6 suture additionally complies with the following test for monomer and oligomers. In a continuous-extraction apparatus, treat 1.00 g with 30 ml of *methanol R* at a rate of at least 3 extractions per hour for 7 h. Evaporate the extract to dryness, dry the residue at 110 °C for 10 min, allow to cool in a desiccator and weigh. The residue weighs not more than 20 mg (2 per cent).

STORAGE (PACKAGING)

Sterile non-absorbable sutures are presented in a suitable sachet that maintains sterility and allows the withdrawal and use of a suture in aseptic conditions. They may be stored dry or in a preserving liquid to which an antimicrobial agent but no antibiotic may be added.

Sterile non-absorbable sutures are intended to be used only on the occasion when the sachet is first opened.

Sutures in their individual sachets (primary packaging) are kept in a protective cover (box) which maintains the physical and mechanical properties until the time of use.

The application of appropriate harmonised standards for packaging of medical devices shall be considered in addition.

LABELLING

Reference may be made to the appropriate harmonised standards for the labelling of medical devices.

The details strictly necessary for the user to identify the product properly are indicated on or in each sachet (primary packaging) and on the protective cover (box) and include at least:

— gauge number;

— length, in centimetres or metres;

— if appropriate, that the needle is detachable;

— name of the product;

— intended use (surgical suture, non-absorbable);

— if appropriate, that the suture is coloured;

— if appropriate, the structure (braided, monofilament, sheathed).

Ph Eur

Sterile Synthetic Absorbable Braided Sutures

General Notices

(Ph Eur monograph 0667)

Ph Eur

DEFINITION

Sterile synthetic absorbable braided sutures consist of sutures prepared from a synthetic polymer, polymers or copolymers which, when introduced into a living organism, are absorbed by that organism and cause no undue tissue irritation. They consist of completely polymerised material. They occur as multifilament sutures consisting of elementary fibres which are assembled by braiding. The sutures may be treated to facilitate handling and they may be coloured.

Appropriate harmonised standards may be considered when assessing compliance with respect to origin and processing of raw materials and with respect to biocompatibility.

Sterile synthetic absorbable braided sutures are wound- closure devices. Being absorbable they serve to approximate tissue during the healing period and subsequently lose tensile strength by hydrolysis.

PRODUCTION

Appropriate harmonised standards may apply with respect to appropriate validated methods of sterilisation, environmental control during manufacturing, labelling and packaging.

It is essential for the effectiveness and the performance characteristics during use and during the functional lifetime of these sutures that the following physical properties are specified: consistent diameter, sufficient initial strength and firm needle attachment.

The requirements below have been established, taking into account stresses which occur during normal conditions of use. These requirements can be used to demonstrate that individual production batches of these sutures are suitable for wound closure according to usual surgical techniques.

TESTS

Carry out the following tests on the sutures in the state in which they are removed from the sachet.

Length

Measure the length of the suture without applying more tension than is necessary to keep it straight. The length of each suture is not less than 95 per cent of the length stated on the label and does not exceed 400 cm.

Diameter

Unless otherwise prescribed, measure the diameter by the following method, using five sutures in the condition in which they are presented. Use a suitable instrument capable of measuring with an accuracy of at least 0.002 mm and having a circular pressor foot 10 mm to 15 mm in diameter. The pressor foot and the moving parts attached to it are weighted so as to apply a total load of 100 ± 10 g to the suture being tested. When making the measurements, lower the pressor foot slowly to avoid crushing the suture. Measure the diameter at intervals of 30 cm over the whole length of the suture. For a suture less than 90 cm in length, measure at three points approximately evenly spaced along the suture. During the measurement, submit the sutures to a tension not greater than one-fifth of the minimum breaking load shown in column C of Table 0667.-1 appropriate to the gauge number and type of material or 10 N whichever is less. For sutures of gauge number above 1.5 make two measurements at each point, the second measurement being made after rotating the suture through 90°. The diameter of that point is the average of the two measurements. The average of the measurements carried out on the sutures being tested and not less than two-thirds of the measurements taken on each suture are within the limits given in the columns under A in Table 0667.-1 for the gauge number concerned. None of the measurements is outside the limits given in the columns under B in Table 0667.-1 for the gauge number concerned.

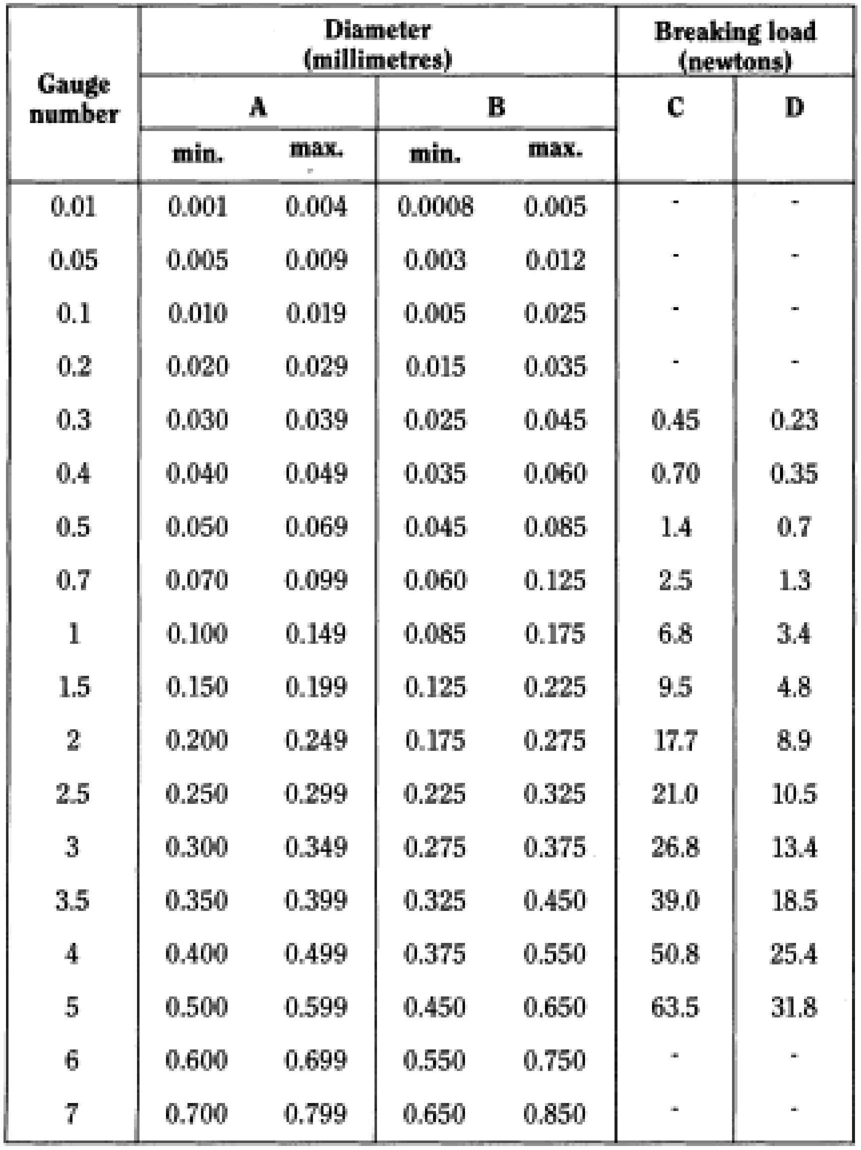


Table 0667.-1. – *Diameters and breaking loads*

Gauge number	Diameter (millimetres)				Breaking load (newtons)	
	A		B		C	D
	min.	max.	min.	max.		
0.01	0.001	0.004	0.0008	0.005	-	-
0.05	0.005	0.009	0.003	0.012	-	-
0.1	0.010	0.019	0.005	0.025	-	-
0.2	0.020	0.029	0.015	0.035	-	-
0.3	0.030	0.039	0.025	0.045	0.45	0.23
0.4	0.040	0.049	0.035	0.060	0.70	0.35
0.5	0.050	0.069	0.045	0.085	1.4	0.7
0.7	0.070	0.099	0.060	0.125	2.5	1.3
1	0.100	0.149	0.085	0.175	6.8	3.4
1.5	0.150	0.199	0.125	0.225	9.5	4.8
2	0.200	0.249	0.175	0.275	17.7	8.9
2.5	0.250	0.299	0.225	0.325	21.0	10.5
3	0.300	0.349	0.275	0.375	26.8	13.4
3.5	0.350	0.399	0.325	0.450	39.0	18.5
4	0.400	0.499	0.375	0.550	50.8	25.4
5	0.500	0.599	0.450	0.650	63.5	31.8
6	0.600	0.699	0.550	0.750	-	-
7	0.700	0.799	0.650	0.850	-	-

Minimum breaking load

The minimum breaking load is determined over a simple knot formed by placing one end of a suture held in the right hand over the other end held in the left hand, passing one end over the suture and through the loop so formed (see Figure 0667.-1) and pulling the knot tight.

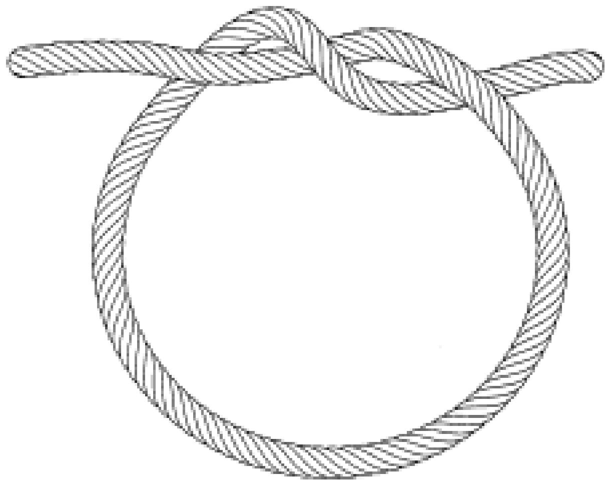


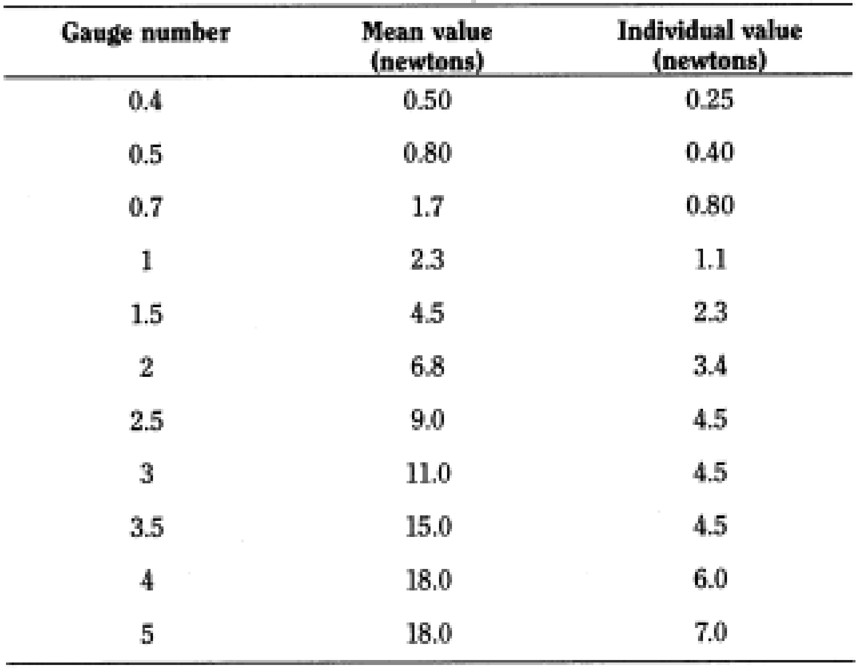
Figure 0667.-1. – *Simple knot*

Carry out the test on five sutures. Submit sutures of length greater than 75 cm to two measurements and shorter sutures to one measurement. Determine the breaking load using a suitable tensilometer. The apparatus has two clamps for holding the suture, one of which is mobile and is driven at a constant rate of 25 cm to 30 cm per minute. The clamps are designed so that the suture being tested can be attached without any possibility of slipping. At the beginning of the test the length of suture between the clamps is 12.5 cm to 20 cm and the knot is midway between the clamps. Set the mobile clamp in motion and note the force required to break the suture. If the suture breaks in a clamp or within 1 cm of it, the result is discarded and the test repeated on another suture. The average of all the results excluding those legitimately discarded is equal to or greater than the value given in column C in Table 0667.-1 and no individual result is less than that given in column D for the gauge number concerned.

Needle attachment

If the suture is supplied with an eyeless needle attached that is not stated to be detachable the attachment, it complies with the test for needle attachment. Carry out the test on five sutures. Use a suitable tensilometer, such as that described for the determination of the minimum breaking load. Fix the needle and suture (without knot) in the clamps of the apparatus in such a way that the swaged part of the needle is completely free of the clamp and in line with the direction of pull on the suture. Set the mobile clamp in motion and note the force required to break the suture or to detach it from the needle. The average of the five deter-minations and all individual values are not less than the respective values given in Table 0667.-2 for the gauge number concerned. If not more than one individual value fails to meet the individual requirement, repeat the test on an additional ten sutures. The attachment complies with the test if none of the ten values is less than the individual value in Table 0667.-2 for the gauge number concerned.

Table 0667.-2. – *Minimum strengths of needle attachment*



Gauge number	Mean value (newtons)	Individual value (newtons)
0.4	0.50	0.25
0.5	0.80	0.40
0.7	1.7	0.80
1	2.3	1.1
1.5	4.5	2.3
2	6.8	3.4
2.5	9.0	4.5
3	11.0	4.5
3.5	15.0	4.5
4	18.0	6.0
5	18.0	7.0

STORAGE (PACKAGING)

Sterile synthetic absorbable braided sutures are presented in a suitable sachet that maintains sterility and allows the withdrawal and use of the sutures in aseptic conditions. The sutures must be stored dry.

They are intended to be used only on the occasion when the sachet is first opened.

Sutures in their individual sachets (primary packaging) are kept in a protective cover (box) which maintains the physical and mechanical properties until the time of use.

The application of appropriate harmonised standards for packaging of medical devices may be considered in addition.

LABELLING

Reference may be made to the appropriate harmonised standards for the labelling of medical devices.

The details strictly necessary for the user to identify the product properly are indicated on or in each sachet (primary packaging) and on the protective cover (box) and include at least:

— gauge number;

— length in centimetres or metres;

— if appropriate, that the needle is detachable;

— name of the product;

— intended use (surgical absorbable suture);

— if appropriate, that the suture is coloured;

— the structure (braided).

Sterile Synthetic Absorbable Monofilament Sutures

General Notices

(Ph Eur monograph 0666)

Ph Eur

DEFINITION

Sterile synthetic absorbable monofilament sutures consist of sutures prepared from a synthetic polymer, polymers or copolymers which, when introduced into a living organism, are absorbed by that organism and cause no undue tissue irritation. They consist of completely polymerised material. They occur as monofilament sutures. The sutures may be treated to facilitate handling and they may be coloured.

Appropriate harmonised standards may be considered when assessing compliance with respect to origin and processing of raw materials and with respect to biocompatibility.

Sterile synthetic absorbable monofilament sutures are wound- closure devices. Being absorbable they serve to approximate tissue during the healing period and subsequently lose tensile strength by hydrolysis.

PRODUCTION

The appropriate harmonised standards may apply with respect to appropriate validated methods of sterilisation, environmental control during manufacturing, labelling and packaging.

It is essential for the effectiveness and the performance characteristics during use and during the functional lifetime of these sutures that the following physical properties are specified: consistent diameter, sufficient initial strength and firm needle attachment.

The requirements below have been established, taking into account stresses which occur during normal conditions of use. These requirements can be used to demonstrate that individual production batches of these sutures are suitable for wound closure according to usual surgical techniques.

TESTS

Carry out the following tests on the sutures in the state in which they are removed from the sachet.

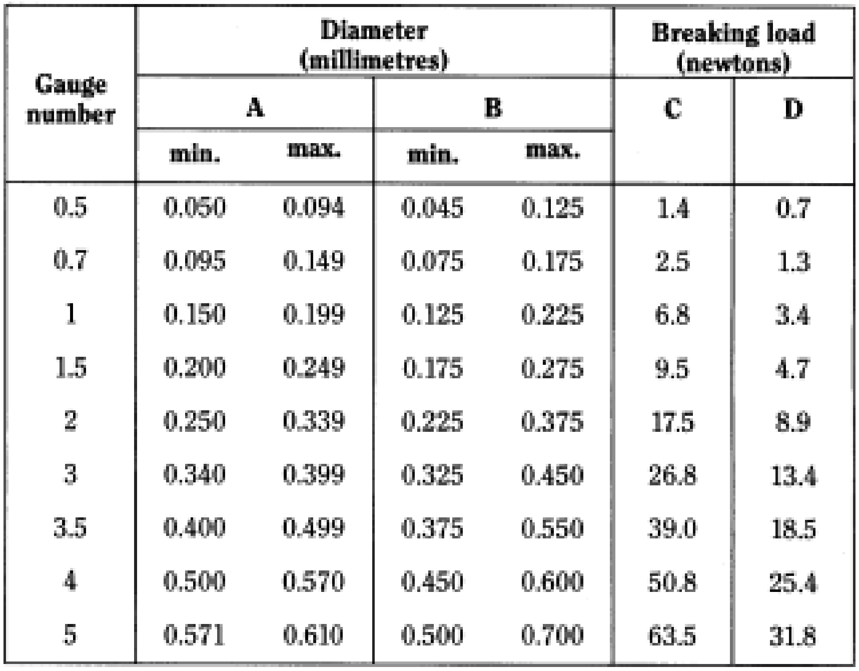
Length

Measure the length of the suture without applying more tension than is necessary to keep it straight. The length of each suture is not less than 95 per cent of the length stated on the label and does not exceed 400 cm.

Diameter

Unless otherwise prescribed, measure the diameter by the following method, using five sutures in the condition in which they are presented. Use a suitable instrument cap-able of measuring with an accuracy of at least 0.002 mm and having a circular pressor foot 10 mm to 15 mm in diameter. The pressor foot and the moving parts attached to it are weighted so as to apply a total load of 100 ± 10 g to the suture being tested. When making the measurements, lower the pressor foot slowly to avoid crushing the suture. Measure the diameter at intervals of 30 cm over the whole length of the suture. For a suture less than 90 cm in length, measure at three points approximately evenly spaced along the suture. During the measurement, submit the sutures to a tension not greater than that required to keep them straight. The average of the measurements carried out on the sutures being tested and not less than two-thirds of the measurements taken on each suture are within the limits given in the columns under A in Table 0666.-1 for the gauge number concerned. None of the measurements is outside the limits given in the columns under B in Table 0666.-1 for the gauge number concerned.

Table 0666.-1. – *Diameters and breaking loads*



Gauge number	Diameter (millimetres)				Breaking load (newtons)	
	A		B		C	D
	min.	max.	min.	max.		
0.5	0.050	0.094	0.045	0.125	1.4	0.7
0.7	0.095	0.149	0.075	0.175	2.5	1.3
1	0.150	0.199	0.125	0.225	6.8	3.4
1.5	0.200	0.249	0.175	0.275	9.5	4.7
2	0.250	0.339	0.225	0.375	17.5	8.9
3	0.340	0.399	0.325	0.450	26.8	13.4
3.5	0.400	0.499	0.375	0.550	39.0	18.5
4	0.500	0.570	0.450	0.600	50.8	25.4
5	0.571	0.610	0.500	0.700	63.5	31.8

Minimum breaking load

The minimum breaking load is determined over a simple knot formed by placing one end of a suture held in the right hand over the other end held in the left hand, passing one end over the suture and through the loop so formed (see Figure 0666.-1) and pulling the knot tight.

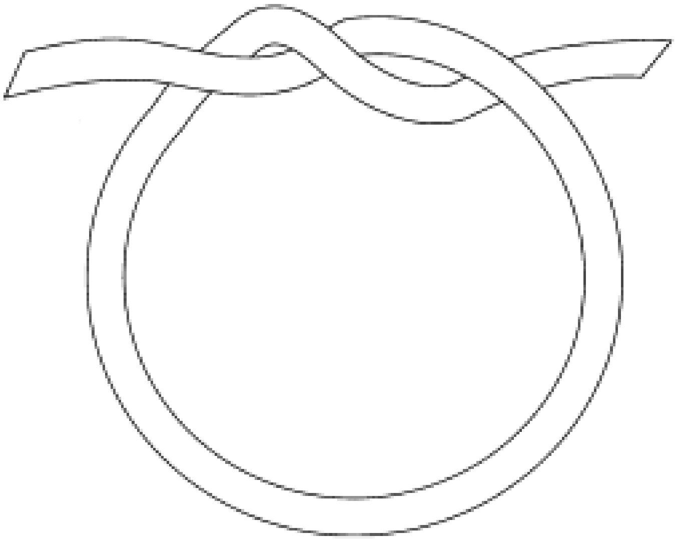


Figure 0666.-1. – *Simple knot*

Carry out the test on five sutures. Submit sutures of length greater than 75 cm to two measurements and shorter sutures to one measurement. Determine the breaking load using a suitable tensilometer. The apparatus has two clamps for holding the suture, one of which is mobile and is driven at a constant rate of 25 cm to 30 cm per minute. The clamps are designed so that the suture being tested can be attached without any possibility of slipping. At the beginning of the test the length of suture between the clamps is 12.5 cm to 20 cm and the knot is midway between the clamps. Set the mobile clamp in motion and note the force required to break the suture. If the suture breaks in a clamp or within 1 cm of it, the result is discarded and the test repeated on another suture. The average of all the results excluding those legitimately discarded is equal to or greater than the value given in column C in Table 0666.-1 and no individual result is less than that given in column D for the gauge number concerned.

Needle attachment

If the suture is supplied with an eyeless needle attached that is not stated to be detachable, the attachment complies with the test for needle attachment. Carry out the test on five sutures. Use a suitable tensilometer, such as that described for the determination of the minimum breaking load. Fix the needle and suture (without knot) in the clamps of the apparatus in such a way that the swaged part of the needle is completely free of the clamp and in line with the direction of pull on the suture. Set the mobile clamp in motion and note the force required to break the suture or to detach it from the needle. The average of the five determinations and all individual values are not less than the respective values given in Table 0666.-2 for the gauge number concerned. If not more than one individual value fails to meet the individual requirement, repeat the test on an additional ten sutures. The attachment complies with the test if none of the ten values is less than the individual value in Table 0666.-2 for the gauge number concerned.

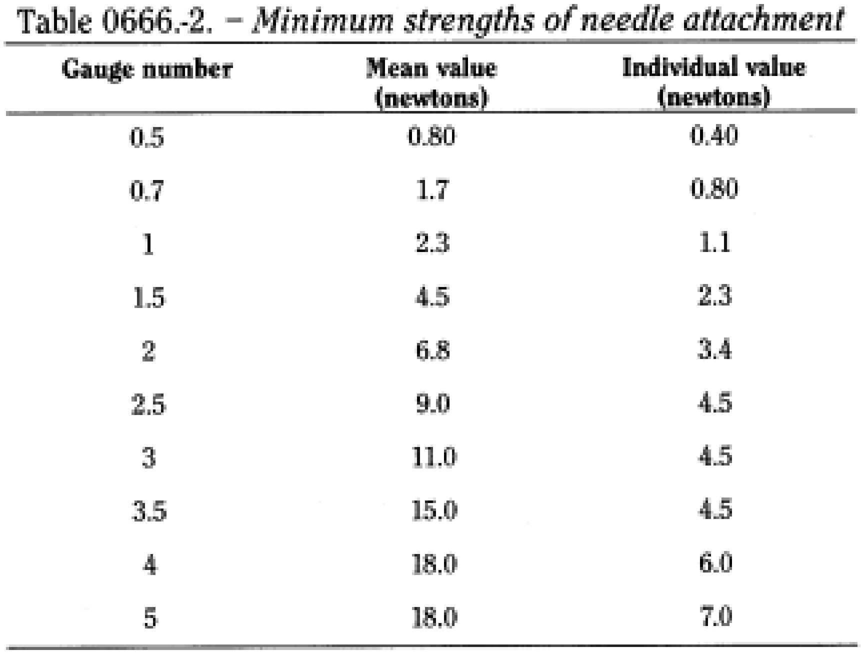


Table 0666.-2. – *Minimum strengths of needle attachment*

Gauge number	Mean value (newtons)	Individual value (newtons)
0.5	0.80	0.40
0.7	1.7	0.80
1	2.3	1.1
1.5	4.5	2.3
2	6.8	3.4
2.5	9.0	4.5
3	11.0	4.5
3.5	15.0	4.5
4	18.0	6.0
5	18.0	7.0

STORAGE (PACKAGING)

Sterile synthetic absorbable monofilament sutures are presented in a suitable sachet that maintains sterility and allows the withdrawal and use of the sutures in aseptic conditions. The sutures must be stored dry.

They are intended to be used only on the occasion when the sachet is first opened.

Sutures in their individual sachets (primary packaging) are kept in a protective cover (box) which maintains the physical and mechanical properties until the time of use.

The application of appropriate harmonised standards for packaging of medical devices may be considered in addition.

LABELLING

Reference may be made to appropriate harmonised standards for the labelling of medical devices.

The details strictly necessary for the user to identify the product properly are indicated on or in each sachet (primary packaging) and on the protective cover (box) and include at least:

— gauge number;

— length in centimetres or metres;

— if appropriate, that the needle is detachable;

— name of the product;

— intended use (surgical absorbable suture);

— if appropriate, that the suture is coloured;

— the structure (monofilament).

Ph Eur

SUTURES

General Notices

(Ph Eur monograph 90004)

Ph Eur

INTRODUCTION

The following monographs apply to sutures for human use: Catgut, sterile (0317), Sutures, sterile non-absorbable (0324), Sutures, sterile synthetic absorbable braided (0667) and Sutures, sterile synthetic absorbable monofilament (0666). They cover performance characteristics of sutures and may include methods of identification. Sutures are medical devices as defined in Directive 93/42/EEC.

These monographs can be applied to show compliance with essential requirements as defined in Article 3 of Directive 93/42/EEC covering the following:

Physical performance characteristics: diameter, breaking load, needle attachment, packaging, sterility, information supplied by the manufacturer (see Section 13 of Annex 1 of Directive 93/42/EEC), labelling.

To show compliance with other essential requirements, the application of appropriate harmonised standards as defined in Article 5 of Directive 93/42/EEC may be considered.

Ph Eur

Acebutolol Capsules

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Acebutolol Capsules contain Acebutolol Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of acebutolol, $C_{18}H_{28}N_2O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum* of a 0.7% w/w dispersion of the contents of the capsules in *potassium bromide* , Appendix II A, is concordant with the *reference spectrum* of acebutolol hydrochloride *(RS 380)*.

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *glacial acetic acid* , 20 volumes of *dimethylformamide* and 60 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add to a quantity of the contents of the capsules containing the equivalent of 0.4 g of acebutolol 20 ml of a mixture of equal volumes of *chloroform* and *methanol* , shake for 2 minutes, centrifuge and use the supernatant liquid. For solution (2), dilute 3 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *chloroform* and *methanol* and further dilute 1 volume of this solution to 10 volumes with the same mixture of solvents. For solution (3), dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *chloroform* and *methanol* and further dilute 1 volume of this solution to 10 volumes with the same mixture of solvents. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.1%). Disregard any spot remaining on the line of application.

ASSAY

To a quantity of the mixed contents of 20 capsules containing the equivalent of 0.15 g of acebutolol add 150 ml of *water*, shake for 10 minutes, add sufficient *water* to produce 250 ml, centrifuge and dilute 10 ml of the supernatant liquid to 100 ml with *water*. To 10 ml of this solution add 10 ml of 0.1M *hydrochloric acid* and sufficient *water* to produce 100 ml. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 233 nm and calculate the content of $C_{18}H_{28}N_2O_4$ in the capsules taking 643 as the value of A(1%, 1 cm) at the maximum at 233 nm.

STORAGE

Acebutolol Capsules should be protected from light.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of acebutolol.

Acebutolol Tablets

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Acebutolol Tablets contain Acebutolol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of acebutolol, $C_{18}H_{28}N_2O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum* of a 0.7% w/w dispersion of the powdered tablets in *potassium bromide*, Appendix II A, is concordant with the *reference spectrum* of acebutolol hydrochloride *(RS 380)*.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *glacial acetic acid*, 20 volumes of *dimethylformamide* and 60 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add to a quantity of the powdered tablets containing the equivalent of 0.4 g of acebutolol 20 ml of a mixture of equal volumes of *chloroform* and *methanol*, shake for 2 minutes, centrifuge and use the supernatant liquid. For solution (2), dilute 3 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *chloroform* and *methanol* and further dilute 1 volume of this solution to 10 volumes with the same mixture of solvents. For solution (3), dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *chloroform* and *methanol* and further dilute 1 volume of this solution to 10 volumes with the same mixture of solvents. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.1%). Disregard any spot remaining on the line of application.

ASSAY

Shake a number of whole tablets containing the equivalent of 4 g of acebutolol with 250 ml of *water* until completely disintegrated, add sufficient *water* to produce 1000 ml, filter and dilute 10 ml of the filtrate to 250 ml with *water*. To 10 ml of this solution add 20 ml of 0.1M *hydrochloric acid* and sufficient *water* to produce 200 ml. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 233 nm and calculate the content of $C_{18}H_{28}N_2O_4$ in the tablets taking 643 as the value of A (1%, 1 cm) at the maximum at 233 nm.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of acebutolol.

Acenocoumarol Tablets

General Notices

Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant.

DEFINITION

Acenocoumarol Tablets contain Acenocoumarol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of acenocoumarol, $C_{19}H_{15}NO_6$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat a quantity of the powdered tablets containing 50 mg of Acenocoumarol with 30 ml of *acetone* under a reflux condenser for 5 minutes, filter and wash the residue with two 10 ml quantities of *acetone*. Evaporate the combined filtrate and washings to 5 ml, add *water* drop wise until the solution becomes turbid, heat on a water bath until the solution is clear and allow to stand. Filter, wash the crystals with a mixture of equal volumes of *acetone* and *water* and dry at 100° at a pressure of 2 kPa for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of acenocoumarol *(RS 001)*.

B. The *light absorption*, Appendix II B, of the final solution obtained in the Assay exhibits maxima at 283 nm and 306 nm.

C. Heat 25 mg of the residue obtained in test A with 2.5 ml of *glacial acetic acid*, 0.5 ml of *hydrochloric acid* and 0.2 g of *zinc powder* on a water bath for 5 minutes, cool and filter. To the filtrate add 0.05 ml of *sodium nitrite solution* and add the mixture to 10 ml of a 1% w/v solution of *2-naphthol* containing 3 ml of 5M *sodium hydroxide*. A bright red precipitate is produced.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 20 volumes of *glacial acetic acid*, 50 volumes of *chloroform* and 50 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Acenocoumarol with 5 ml of *acetone*, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *acetone* . After removal of the plate, allow it to dry in air and immediately examine under *ultraviolet light (254 nm)* . Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Uniformity of content

Tablets containing 4 mg or less of Acenocoumarol comply with the requirements stated under Tablets using the following method of analysis. Finely crush one tablet, add 30 ml of *methanol* , stir the mixture for 30 minutes and filter through sintered glass, washing the residue with three 15 ml quantities of *methanol* . To the combined filtrate and washings add 10 ml of 1M *hydrochloric acid* and sufficient *methanol* to produce 100 ml. If necessary dilute further with a solvent prepared by diluting 1 volume of 1M *hydrochloric acid* to 10 volumes with *methanol* to produce a solution containing about 0.001% w/v of Acenocoumarol. Measure the *absorbance* of the resulting solution at the maximum at 306 nm, Appendix II B. Calculate the content of $C_{19}H_{15}NO_6$ taking 521 as the value of A (1%, 1 cm) at the maximum at 306 nm.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 1 mg of Acenocoumarol add 30 ml of *methanol* , stir the mixture for 30 minutes and filter through sintered glass, washing the residue with three 15 ml quantities of *methanol* . To the combined filtrate and washings add 10 ml of 1M *hydrochloric acid* and sufficient *methanol* to produce 100 ml and measure the *absorbance* of the resulting solution at the maximum at 306 nm, Appendix II B. Calculate the content of $C_{19}H_{15}NO_6$ taking 521 as the value of A (1%, 1 cm) at the maximum at 306 nm.

Acetazolamide Tablets

General Notices

Action and use

Carbonic anhydrase inhibitor; diuretic; treatment of glaucoma, ocular hypertension, mountain sickness.

DEFINITION

Acetazolamide Tablets contain Acetazolamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of acetazolamide, $C_4H_6N_4O_3S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Acetazolamide with 2 ml of 1M *sodium hydroxide* and filter. Neutralise the filtrate with *glacial acetic acid*, filter and dry the resulting precipitate at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of acetazolamide *(RS 002)*.

B. Triturate a quantity of the powdered tablets containing 0.5 g of Acetazolamide with a mixture of 5 ml of *water* and 1 ml of 1M *sodium hydroxide*, transfer to a test tube, add 0.2 g of *zinc powder* and 0.5 ml of *hydrochloric acid* and immediately place a piece of *lead acetate paper* over the mouth of the tube. The paper exhibits a brownish black colour.

C. To a quantity of the powdered tablets containing 25 mg of Acetazolamide add 5 ml of *water*, 0.15 ml of 1M *sodium hydroxide* and 0.1 ml of *weak copper sulphate solution*. A greenish blue colour or precipitate is produced.

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF$_{254}$* as the coating substance and a freshly prepared mixture of 50 volumes of *propan-2-ol*, 30 volumes of *ethyl acetate* and 20 volumes of 13.5M *ammonia* as the mobile phase. Use the tank without lining the walls and allow to saturate for 1 hour before development. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Acetazolamide for 20 minutes with 10 ml of a mixture of equal volumes of *ethanol (96%)* and *ethyl acetate* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the same solvent mixture. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary* *spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.4 g of Acetazolamide add 90 ml of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 22.22 mg of $C_4H_6N_4O_3S_2$.

Acetylcysteine Injection

General Notices

Action and use

Sulphydryl donor; antidote to paracetamol poisoning; mucolytic.

DEFINITION

Acetylcysteine Injection is a sterile solution in Water for Injections of acetylcysteine sodium, prepared by the interaction of Acetylcysteine with Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of acetylcysteine, $C_5H_9NO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume containing the equivalent of 0.8 g of Acetylcysteine add 3M *hydrochloric acid* until the pH of the solution is 2. Add, while stirring continuously, two 200-mg portions of finely powdered *sodium chloride* followed, if necessary, by further 25-mg portions of *sodium chloride* until a precipitate begins to appear. Allow to stand for 15 minutes, filter and dry the residue at 70^{c} at a pressure not exceeding 0.7 kPa for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of acetylcysteine *(RS 003)*. Examine as discs prepared using *potassium bromide*.

TESTS

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

Specific optical rotation

+21 to +27, Appendix V F. To a volume containing the equivalent of 1.25 g of Acetylcysteine add 1 ml of a 0.1% w/v solution of *disodium edetate* and sufficient *mixed phosphate buffer pH 7.0* to produce 25 ml.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. With the exception of solution (3), the solutions should be prepared immediately before use. For solution (1) dilute the injection with the mobile phase to produce a solution containing the equivalent of 0.2% w/v of Acetylcysteine. Solution (2) is a 0.2% w/v solution of N-*acetyl*-L-*cysteine* in the mobile phase. Prepare solution (3) in the same manner as solution

(2) but store at room temperature for at least 2 hours before use. For solution (4) dissolve 20 mg of L- *cysteine* and 20 mg of L- *cystine* in 10 ml of 1M *hydrochloric acid* , add 40 mg of N-*acetyl*- L-*cysteine* and immediately dilute to 100 ml with the mobile phase. Dilute 10 ml of the resulting solution to 200 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 10 volumes of *methanol* and 90 volumes of a 0.5% w/v solution of *ammonium sulphate* containing 0.02M *sodium pentanesulphonate* , the solution being adjusted to pH 2 using 2M *hydrochloric acid* , and (c) a detection wavelength of 205 nm.

Inject 20 µl of solution (4). The chromatogram obtained shows three peaks with retention times of about 3.6 minutes (cystine), about 4 minutes (cysteine) and about 6 minutes (acetylcysteine). Adjust the sensitivity so that the height of the peak due to cysteine is about 20% of full-scale deflection. The test is not valid unless, in the chromatogram obtained with solution (4), the height of the trough separating the peaks due to cysteine and cystine is less than one quarter of the height of the peak due to cysteine. Inject 20 µl of solutions (2) and (3) and allow the chromatography to continue for three times the retention time of acetylcysteine. In the chromatogram obtained with solution (3) a peak due to *N,N′*-diacetylcystine appears which has a retention time of about 13 minutes. The area of this peak is greater than the area of any corresponding peak in the chromatogram obtained with solution (2).

In the chromatogram obtained with solution (1) the area of any peak corresponding to *N,N′*-diacetylcystine is not greater than the area of the peak due to acetylcysteine in the chromatogram obtained with solution (4) (1%), the area of any peak due to cysteine or cystine is not greater than the corresponding peak in the chromatogram obtained with solution (4) (0.5%) and the sum of the areas of any other *secondary peaks* is not greater than the area of the peak due to acetylcysteine in the chromatogram obtained with solution (4) (1%).

Hydrogen sulphide

Place a quantity of the injection containing the equivalent of 0.4 g of Acetylcysteine in a round-bottomed, three-necked flask containing 40 ml of *water*. The flask is fitted with a gas inlet tube which reaches nearly to the bottom of the flask, a dropping funnel containing *hydrochloric acid* and an outlet tube leading to a 100 ml graduated flask containing a mixture of 1 ml of 5M *sodium hydroxide* and 50 ml of *water*. Pass through the flask a steady current of nitrogen and add 10 ml of *hydrochloric acid* from the dropping funnel. Maintain the current of nitrogen for 30 minutes and then disconnect the absorption flask. Add to the flask 10 ml of a solution prepared by dissolving 0.1 g of N,N-*dimethyl*-p-*phenylenediamine dihydrochloride* in a mixture of 45 ml of *hydrochloric acid* and 55 ml of *water* decolourised with *activated charcoal* before use, if necessary, and 5 ml of a 5% w/v solution of *iron(III) chloride hexahydrate* in 1M *hydrochloric acid* and allow to stand for 20 minutes protected from light. Add sufficient *water* to produce 100 ml and measure the *absorbance* of the solution, Appendix II B, at 665 nm using a 4-cm pathlength and using in the reference cell a solution prepared in the same manner but without the injection being examined.

Prepare a 0.4% w/v solution of *sodium sulphide* . Standardise this solution in the following manner. To 25 ml of 0.05M *iodine VS* add 8 ml of *hydrochloric acid* and 25 ml of the sodium sulphide solution. Titrate with 0.1M *sodium thiosulphate solution VS* using *starch solution* , added towards the end point, as indicator. Repeat the operation without the sodium sulphide solution. The concentration of the sodium sulphide solution expressed in parts per million of hydrogen sulphide is the difference between the titrations multiplied by 68.16. Prepare a solution containing the equivalent of 20 ppm of hydrogen sulphide by appropriate dilution of the sodium sulphide solution with *water.*

Repeat the procedure carried out on the injection using 2 ml of the 20-ppm hydrogen sulphide solution in place of the injection being examined. The *absorbance* of the solution obtained from the injection is not greater than the absorbance of the solution obtained from the standard (100 ppm with reference to the content of acetylcysteine).

Pyrogens

Complies with the *test for pyrogens* , Appendix XIV D. Use per kg of the rabbit's weight a volume containing the equivalent of 0.3 g of Acetylcysteine.

ASSAY

Add 20 ml of *glacial acetic acid* to a volume containing the equivalent of 0.4 g of Acetylcysteine and titrate with 0.05M *iodine VS* until a permanent pale yellow colour is obtained. Each ml of 0.05M *iodine VS* is equivalent to 16.23 mg of $C_5H_9NO_3S$.

STORAGE

Acetylcysteine Injection should be protected from light.

LABELLING

The strength is stated in terms of an equivalent percentage w/v of Acetylcysteine.

Aciclovir Cream

General Notices

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Cream contains Aciclovir in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption* , Appendix II B, in the range 230 to 350 nm of the solution prepared in the Assay exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with solution (2) is similar in position and intensity to that in the chromatogram obtained with solution (3).

TEST

Guanine

Carry out the method for *thin-layer chromatography* , Appendix III A, using *cellulose* F_{254} as the coating substance (Merck cellulose F plates are suitable). Apply separately to the plate 10 µl of each of the following solutions. For solution (1) transfer a quantity of the well-mixed cream containing 30 mg of Aciclovir into a 10 ml graduated, stoppered centrifuge tube, add 3 ml of 0.1M *sodium hydroxide* and shake to disperse the cream. Add 5 ml of a mixture of 1 volume of *chloroform* and 2 volumes of *propan-1-ol* , shake well, centrifuge and dilute the upper aqueous layer to 5 ml with 0.1M *sodium hydroxide* , mix, centrifuge and use the upper aqueous layer. For solution (2) dilute 1 volume of solution (1) to 10 volumes with 0.1M *sodium hydroxide* . For solution (3) dissolve 6.0 mg of *aciclovir BPCRS* in 10 ml of 0.1M *sodium hydroxide* . For solution (4) dissolve 6.0 mg of *guanine* in 100 ml of 0.1M *sodium hydroxide* . Use *ethyl acetate* for the first mobile phase but allow the solvent front to ascend to the top of the plate. After removal of the plate, dry it in a current of air and repeat the development in the same direction using a mixture of 10 volumes of *propan-1-ol* , 30 volumes of 13.5M *ammonia* and 60 volumes of a 5% w/v solution of *ammonium sulphate* as the mobile phase and allowing the solvent front to ascend 8 cm above the line of application. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . Any *secondary spot* corresponding to guanine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (1.0%). Disregard any spot that appears just below the solvent front.

ASSAY

Shake a quantity of the well-mixed cream containing about 7.5 mg of Aciclovir with 50 ml of 0.5M *sulphuric acid*. Shake well with 50 ml of *ethyl acetate*, allow to separate and collect the clear lower aqueous layer. Wash the organic layer with 20 ml of 0.5M *sulphuric acid* and dilute the combined washings and the aqueous layer to 100 ml with 0.5M *sulphuric acid*. Mix well and filter (Whatman GF/F is suitable). Discard the first few ml of filtrate and to 10 ml of the filtrate add sufficient *water* to produce 50 ml. Measure the *absorbance* of the resulting solution at the maximum at 255 nm, Appendix II B. Calculate the content of $C_8H_{11}N_5O_3$ taking 562 as the value of A (1%, 1 cm) at the maximum at 255 nm.

Aciclovir Eye Ointment

General Notices

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Eye Ointment is a sterile preparation containing Aciclovir in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption* , Appendix II B, in the range 230 to 350 nm of the solution prepared in the Assay exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with solution (2) is similar in position and intensity to that in the chromatogram obtained with solution (3).

TEST

Guanine

Complies with the test described under Aciclovir Intravenous Infusion but preparing solution (1) in the following manner. Disperse a quantity of the eye ointment containing 30 mg of Aciclovir in 100 ml of *hexane* , extract with 5 ml of 0.1M *sodium hydroxide* , allow to separate and retain the lower aqueous layer.

ASSAY

Disperse a quantity of the eye ointment containing 10 mg of Aciclovir in 60 ml of *hexane* . Extract with three 30 ml quantities of 0.1M of *sodium hydroxide* , add sufficient 0.1M *sodium hydroxide* to produce 100 ml and filter. To 15 ml of this solution add 5 ml of 2M *hydrochloric acid* and sufficient *water* to produce 100 ml. Measure the *absorbance* of the resulting solution at the maximum at 255 nm, Appendix II B. Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the value of A (1%, 1 cm) at the maximum at 255 nm.

Aciclovir Intravenous Infusion

General Notices

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Intravenous Infusion is a sterile solution of aciclovir sodium in Water for Injections. It is prepared by dissolving Aciclovir Sodium for Intravenous Infusion in the requisite amount of Water for Injections before use.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

TEST

Bacterial endotoxins

Carry out the test for *bacterial endotoxins* , Appendix XIV C. The endotoxin limit concentration of the intravenous infusion, diluted, if necessary, with *water BET* to give a solution containing the equivalent of 25 mg of Aciclovir per ml is 4.37 IU per ml. Carry out the test using the maximum valid dilution of the above defined solution calculated from the declared sensitivity of the lysate used in the test.

STORAGE

Aciclovir Intravenous Infusion should be used within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

LABELLING

The strength is stated as the equivalent amount of Aciclovir in a suitable dose-volume.

ACICLOVIR SODIUM FOR INTRAVENOUS INFUSION

DEFINITION

Aciclovir Sodium for Intravenous Infusion is a sterile material prepared from Aciclovir with the aid of a suitable alkali. It may contain excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution prepared in the Assay exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with solution (2) is similar in position and intensity to that in the chromatogram obtained with solution (3).

C. Yield reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

Dissolve the contents of a sealed container in sufficient *water for injections* to produce a solution containing the equivalent of 2.5% w/v of Aciclovir (solution A). The pH of solution A is 10.7 to 11.7, Appendix V L.

Clarity and colour of solution

Solution A is not more opalescent than *reference suspension II*, Appendix IV A, and not more intensely coloured than *reference solution* Y_6, Appendix IV B, Method II.

Guanine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *cellulose* F_{254} as the coating substance (Merck cellulose F plates are suitable) and a mixture of 10 volumes of *propan-1-ol*, 30 volumes of 13.5M *ammonia* and 60 volumes of a 5% w/v solution of *ammonium sulphate* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dissolve the contents of a sealed container in sufficient 0.1M *sodium hydroxide* to produce a solution containing the equivalent of 0.5% w/v of Aciclovir. For solution (2) dilute 1 volume of solution (1) to 10 volumes with 0.1M *sodium hydroxide*. For solution (3) dissolve 5 mg of *aciclovir BPCRS* in 10 ml of 0.1M *sodium hydroxide*. For solution (4) dissolve 5 mg of *guanine* in 100 ml of 0.1M *sodium hydroxide*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* corresponding to guanine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (1%). Disregard any spot that appears just below the solvent front.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 2 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 2 µl of each of the following freshly prepared solutions. For solution (1) dissolve the contents of a sealed container in sufficient *dimethyl sulphoxide* to produce a solution containing the equivalent of 2.5% w/v of Aciclovir. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *dimethyl sulphoxide*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any *secondary spot* with an Rf value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Dissolve the total contents of 10 containers in sufficient 0.1M *hydrochloric acid* to produce 500 ml. Dilute 3 ml of the resulting solution to 100 ml with 0.1M *hydrochloric acid* and dilute 5 ml of the resulting solution with the same solvent to produce a solution containing the equivalent of 0.0015% w/v of Aciclovir. Measure the *absorbance* at the maximum at 255 nm, Appendix II B. Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the value of A (1%, 1 cm) at the maximum at 255 nm.

LABELLING

The label of the sealed container states the quantity of aciclovir sodium in terms of the equivalent amount of Aciclovir.

Aciclovir Oral Suspension

General Notices

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Oral Suspension is a suspension of Aciclovir in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 250 nm of the solution prepared in the Assay before the final dilution exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with solution (2) is similar in position and intensity to that in the chromatogram obtained with solution (3). If the Rf values of the principal spots in the chromatograms obtained with solutions (2) and (3) are different, the oral suspension complies if the chromatogram obtained with solution (5) shows a single, compact spot.

TESTS

Acidity

pH, 4.0 to 7.0, Appendix V L.

Guanine

Carry out the test described under Aciclovir Intravenous Infusion but applying 5 µl of each of the following solutions. For solution (1) add 20 ml of 0.1M *sodium hydroxide* to a quantity of the oral suspension containing 0.6 g of Aciclovir, shake to disperse and add sufficient *absolute ethanol* to produce 100 ml. Mix well and allow any suspended material to settle before application to the plate. For solution (2) dilute 1 volume of solution (1) to 10 volumes with a mixture of 35 volumes of 0.1M *sodium hydroxide* and 65 volumes of *absolute ethanol*. For solution (3) dissolve 6 mg of *aciclovir BPCRS* in 10 ml of a mixture of 35 volumes of 0.1M *sodium hydroxide* and 65 volumes of *absolute ethanol*. For solution (4) dissolve 6 mg of *guanine* in 100 ml of a mixture of 35 volumes of 0.1M *sodium hydroxide* and 65 volumes of *absolute ethanol* (1%). Solution (5) contains equal volumes of solutions (2) and (3).

ASSAY

To a weighed quantity containing 0.4 g of Aciclovir add 400 ml of *water* and 25 ml of 1M *sulphuric acid*, shake well, disperse with the aid of ultrasound for 10 minutes and add sufficient *water* to produce 500 ml. Filter the resulting solution, discard the first few ml of filtrate and dilute 5 ml of the filtrate to 200 ml with 0.05M *sulphuric acid*. Add 10 ml of the resulting solution to 5 ml of a 0.01% w/v solution of *cetrimide* in 0.05M *sulphuric acid*, add sufficient 0.05M *sulphuric acid* to produce 100 ml and measure the *fluorescence,* Appendix II E, using an excitation wavelength of 308 nm and an emission wavelength of 415 nm. Set the instrument to zero using a 0.0005% w/v solution of *cetrimide* in 0.05M *sulphuric acid*. Calculate the content of $C_8H_{11}N_5O_3$ in the oral suspension from the *fluorescence* obtained by carrying out the operation at the same time using a mixture prepared by adding 10 ml of a 0.002% w/v solution of *aciclovir BPCRS* in 0.05M *sulphuric acid* and beginning at the words 'to 5 ml of a 0.01% w/v solution of *cetrimide* ...'. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_8H_{11}N_5O_3$, weight in volume, using the declared content of $C_8H_{11}N_5O_3$ in *aciclovir BPCRS*.

Aciclovir Tablets

General Notices

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Aciclovir Tablets contain Aciclovir.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution prepared in the Assay exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with solution (2) is similar in position and intensity to that in the chromatogram obtained with solution (3).

TESTS

Guanine

Comply with the test described under Aciclovir Intravenous Infusion but preparing solution (1) in the following manner. Shake a quantity of the powdered tablets containing 0.25 g of Aciclovir with 25 ml of 0.1M *sodium hydroxide* for 10 minutes. Add a sufficient quantity of 0.1M *sodium hydroxide* to produce 50 ml, allow to stand and allow any undissolved material to settle before application to the plate.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 25 ml of the medium and filter. Measure the *absorbance* of the filtered solution, diluted if necessary with 0.1M *hydrochloric acid*, at the maximum at 255 nm, Appendix II B. Calculate the total content of aciclovir, $C_8H_{11}N_5O_3$, in the medium taking 560 as the value of A (1%, 1 cm) at the maximum at 255 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 2 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 2 µl of each of the following freshly prepared solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Aciclovir with 10 ml of *dimethyl sulphoxide* for 15 minutes and filter. For solution (2) dilute 0.7 volumes of solution (1) to 100 volumes with *dimethyl sulphoxide*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any *secondary spot* with an Rf value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) (0.7%).

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing 0.1 g of Aciclovir add 60 ml of 0.1M *sodium hydroxide* and disperse with the aid of ultrasound for 15 minutes. Add a sufficient quantity of 0.1M *sodium hydroxide* to produce 100 ml, mix well and filter. To 15 ml of the filtrate add 50 ml of *water* and 5.8 ml of 2M *hydrochloric acid* and sufficient *water* to produce 100 ml. To 5 ml of the solution add sufficient 0.1M *hydrochloric acid* to produce 50 ml and mix well. Measure the *absorbance* of the resulting solution at the maximum at 255 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the value of A (1%, 1 cm) at the maximum at 255 nm.

Adrenaline and Cocaine Intranasal Solution / Epinephrine and Cocaine Intranasal Solution

General Notices

NOTE : There are currently no licensed formulations in the United Kingdom.

Action and use

Adrenoceptor agonist + local anaesthetic.

DEFINITION

Adrenaline and Cocaine Intranasal Solution contains Adrenaline Acid Tartrate and Cocaine Hydrochloride in a suitable vehicle.

The intranasal solution complies with the requirements stated under Nasal Preparations, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of adrenaline, $C_9H_{13}NO_3$

95.0 to 105.0% of the stated amount.

Content of cocaine hydrochloride, $C_{17}H_{21}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay for adrenaline, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

B. In the Assay for cocaine hydrochloride, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

C. To 10 ml of the intranasal solution add 2 ml of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour and remove excess iodine by adding 0.1M *sodium thiosulphate* drop wise. A red colour is produced.

D. Yields reaction A characteristic of *chlorides* , Appendix VI.

TESTS

Acidity

pH, 2.0 to 4.0, Appendix V L.

Related substances (for cocaine hydrochloride)

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the intranasal solution with sufficient mobile phase to produce a solution containing 0.04% w/v of Cocaine Hydrochloride.

(2) 0.0008% w/v of *benzoylecgonine hydrate* in the mobile phase.

(3) 0.0008% w/v of *benzoic acid* in the mobile phase.

(4) 0.0008% w/v of each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil 10 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

1 volume of 9M *perchloric acid*, 35 volumes of *methanol* and 64 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to benzoylecgonine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to benzoic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%).

The sum of the contents of the two impurities is not greater than 2%.

ASSAY

For adrenaline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a suitable volume of the intranasal solution with sufficient mobile phase to produce a solution containing the equivalent of 0.011% w/v of adrenaline.

(2) 0.02% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase.

(3) 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS.

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 205 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

Dissolve 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* in a mixture of 950 ml of *water* and 50 ml of *methanol* and adjust the pH to 3.5 with 1M *sodium hydroxide.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_9H_{13}NO_3$ in the intranasal solution from the chromatograms obtained and using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

For cocaine hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the intranasal solution containing 40 mg of Cocaine Hydrochloride with sufficient *water* to produce 100 ml.

(2) 0.04% w/v of *cocaine hydrochoride BPCRS* in *water*.

(3) 0.0008% w/v of each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil 10 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

1 volume of 9M *perchloric acid* , 35 volumes of *methanol* and 64 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{21}NO_4$,HCl in the intranasal solution from the chromatograms obtained and using the declared content of $C_{17}H_{21}NO_4$,HCl in *cocaine hydrochoride BPCRS*.

STORAGE

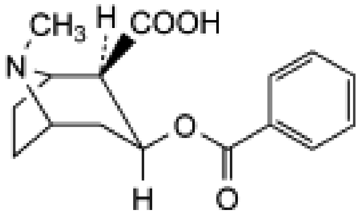
Adrenaline and Cocaine Intranasal Solution should be protected from light.

LABELLING

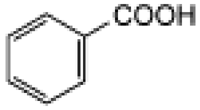
The quantity of adrenaline acid tartrate is stated in terms of the equivalent amount of adrenaline (epinephrine).

IMPURITIES

The impurities limited by the requirements of this monograph include:



A. Benzoylecgonine



B. Benzoic acid

Adrenaline Eye Drops / Epinephrine Eye Drops

General Notices

Neutral Adrenaline Eye Drops

Neutral Epinephrine Eye Drops

Action and use

Adrenoceptor agonist; treatment of glaucoma.

DEFINITION

Adrenaline Eye Drops are a sterile solution of Adrenaline in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of adrenaline, $C_9H_{13}NO_3$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (2) is the same as that of the principal peak in the chromatogram obtained with solution (1).

B. To 1 ml of a dilution of the eye drops containing 0.1% w/v of Adrenaline adjusted, if necessary, to a neutral or slightly acidic pH add, drop wise, a 0.25% w/v solution of *iron (II) chloride hexahydrate* until a green colour is produced. On the gradual addition of *sodium hydrogen carbonate solution* , the solution changes first to blue and then to red.

C. To 1 ml of a dilution of the eye drops containing 0.1% w/v of Adrenaline add 2 ml of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour. Remove excess iodine by adding 0.2M *sodium thiosulphate* drop wise. A red colour is produced.

TESTS

Acidity or alkalinity

pH, 5.5 to 7.6, Appendix V L.

Noradrenaline

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. Solution (1) contains 0.0018% w/v of *noradrenaline acid tartrate* in the mobile phase. For solution (2) dilute the eye drops with the mobile phase to produce a solution containing 0.10% w/v of Adrenaline. Solution (3) contains 0.0018% w/v of *noradrenaline acid tartrate* and 0.0018% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 4.0 g of *tetramethylammonium hydrogen sulphate* , 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* in a mixture of 950 ml of *water* and 50 ml of *methanol* , the pH of the mixture being adjusted to 3.5 with 1M *sodium hydroxide* , and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

In the chromatogram obtained with solution (2) the area of any peak corresponding to noradrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. Solution (1) contains 0.2% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase. For solution (2) dilute the eye drops with sufficient mobile phase to produce a solution containing 0.1% w/v of Adrenaline. Solution (3) contains 0.2% w/v of *adrenaline acid tartrate BPCRS* and 0.2% w/v of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute, a solution prepared by adding 4.0 g of *tetra-methylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* to a mixture of 950 ml of *water* and 50 ml of *methanol* , the pH of the mixture being adjusted to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

Calculate the content of $C_9H_{13}NO_3$ in the eye drops using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS* .

STORAGE

Adrenaline Eye Drops should be protected from light.

Adrenaline Injection / Epinephrine Injection

General Notices

Adrenaline Tartrate Injection

Epinephrine Tartrate Injection

Action and use

Adrenoceptor agonist.

DEFINITION

Adrenaline Injection is a sterile, isotonic solution containing 0.18% w/v of Adrenaline Acid Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of adrenaline, $C_9H_{13}NO_3$

0.09 to 0.11% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as that in the chromatogram obtained with solution (1).

B. To 1 ml add a 0.25% w/v solution of *iron (III) chloride hexahydrate* drop wise until a green colour is produced. On the gradual addition of *sodium hydrogen carbonate solution*, the solution changes first to blue and then to red.

C. To 10 ml add 2 ml of a 10% w/v solution of *Disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour and remove excess iodine by adding 0.1M *sodium thiosulphate* drop wise. A red colour is produced.

TESTS

Acidity

pH, 2.8 to 3.6, Appendix V L.

Noradrenaline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0018% w/v of *noradrenaline acid tartrate* in the mobile phase. For solution (2) use the injection. Solution (3) contains 0.0018% w/v of *adrenaline acid tartrate BPCRS* and 0.0018% w/v of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* in a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusting the pH to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

In the chromatogram obtained with solution (2) the area of any peak corresponding to noradrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase. For solution (2) dilute 1 volume of the injection to 10 volumes with the mobile phase. Solution (3) contains 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* to a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusting the pH of the mixture to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

Calculate the content of $C_9H_{13}NO_3$ in the injection using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

STORAGE

Adrenaline Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of adrenaline (epinephrine).

Adrenaline Injection contains the equivalent of adrenaline (epinephrine), 1 in 1000 (1 mg in 1 ml).

Adrenaline Solution / Epinephrine Solution

General Notices

Adrenaline Tartrate Solution

Epinephrine Tartrate Solution

Action and use

Adrenoceptor agonist.

DEFINITION

Adrenaline Solution is an isotonic *cutaneous solution* containing 0.18% w/v of Adrenaline Acid Tartrate with a suitable combination of an antioxidant and an antimicrobial preservative in Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of adrenaline, $C_9H_{13}NO_3$

0.09 to 0.11% w/v.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as that in the chromatogram obtained with solution (1).

B. To 1 ml add a 0.25% w/v solution of *iron (III) chloride hexahydrate* drop wise until a green colour is produced. On the gradual addition of *sodium hydrogen carbonate solution* , the solution changes first to blue and then to red.

C. To 10 ml add 2 ml of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour and remove excess iodine by adding 0.1M *sodium thiosulphate* drop wise. A red colour is produced.

TESTS

Acidity

pH, 2.7 to 3.6, Appendix V L.

Noradrenaline

Complies with the test described under Adrenaline Eye Drops but using the preparation being examined as solution (2).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase. For solution (2) dilute 1 volume of the preparation being examined to 10 volumes with the mobile phase. Solution (3) contains 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulphate* , 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* to a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusting the pH of the mixture to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

Calculate the content of $C_9H_{13}NO_3$ in the preparation being examined using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS* .

STORAGE

Adrenaline Solution should be kept in a well-filled glass container suitable for parenteral preparations, Appendix XIX B, and should be protected from light.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

The quantity of active ingredient is stated in terms of the equivalent amount of adrenaline (epinephrine).

The label indicates the pharmaceutical form as 'cutaneous solution'.

Adrenaline Solution contains the equivalent of adrenaline (epinephrine), 1 in 1000 (1 mg in 1 ml).

When a solution of adrenaline hydrochloride is prescribed or demanded, a solution complying with the requirements of this monograph may be dispensed or supplied.

Alginate Raft-forming Oral Suspension

General Notices

DEFINITION

Alginate Raft-forming Oral Suspension is a suspension containing Sodium Alginate, Sodium Bicarbonate and Calcium Carbonate in a suitable flavoured vehicle. It may be coloured. The suspension forms a raft.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of active constituents

90.0 to 110.0 % of the stated amount of sodium alginate.

PRODUCTION

The content of the active constituents in the oral suspension is measured using suitably validated methods.

IDENTIFICATION

A. Dilute a quantity of the oral suspension containing 0.25 g of Sodium Alginate, accurately weighed, in sufficient *water* to produce 200 ml. Filter and dilute a portion of this solution with sufficient *water* to produce a solution containing 0.0125% w/v of Sodium Alginate. To 1 ml of this solution, add 1 ml of a freshly prepared 4% w/v solution of *resorcinol* and 6 ml of *sulphuric acid* . An orange-pink colour is produced.

Evaporate to dryness a quantity of the oral suspension containing 1 g of Sodium Alginate on a water bath. The residue complies with the following tests.

B. Shake 0.2 g with 20 ml of *water*. To 5 ml of this solution add 1 ml of *calcium chloride solution* . A voluminous gelatinous mass is formed.

C. Yields the reactions characteristic of *sodium*, Appendix VI.

TESTS

Alkalinity

pH, 7.0 to 9.5, Appendix V L.

Relative density

1.03 to 1.07 g/ml, Appendix V G.

Raft strength

The mean raft strength is not less than 7.5 g, determined by the following method. Carry out the method for *texture analysis of semi-solids or gels*, Appendix XVII F. Introduce 150 ml of 0.1M *hydrochloric acid* into a 250 ml beaker having an internal diameter of 60 to 70 mm. Place in a water bath such that the volume of water in the bath is level with the top of the acid in the beaker and allow to equilibrate to 36.5° to 37.5°. Suspend an L-shaped probe comprising 1 mm diameter 316 gauge stainless steel having a 90 mm vertical arm with a hook at the top and a 20 mm horizontal arm such that the vertical arm of the probe hangs down the centre axis of the beaker and the horizontal arm is in the lower third of the acid.

Using a syringe (without needle), remove a quantity of suspension, previously shaken, equivalent to one dose. Where a dosage range is specified, use the maximum dose. Wipe the outside of the syringe and add the suspension evenly into the medium (the time taken to add the entire dose is approximately 5 seconds).

After 30 minutes, remove the beaker from the water bath, dry the outside of the beaker and transfer to a suitable texture analyser. Attach the probe to the arm of the texture analyser, and lift the arm so that the probe is lifted through the raft at a speed of 5 mm/sec. Record the peak raft strength in g.

LABELLING

The label states the sodium content of the suspension.

Alimemazine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Alimemazine Tablets contain Alimemazine Tartrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of alimemazine tartrate, $C_{36}H_{44}N_4S_2,C_4H_6O_6$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 40 mg of Alimemazine Tartrate add 10 ml of *water* and 2 ml of 1M *sodium hydroxide* , shake and extract with 15 ml of *ether* . Wash the ether layer with 5 ml of *water*, dry with *anhydrous sodium sulphate* and evaporate the ether to dryness. Dissolve the residue in 0.4 ml of *dichloromethane* . The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of alimemazine *(RS 005)* .

B. To a quantity of the powdered tablets containing 1 mg of Alimemazine Tartrate add 1 ml of a mixture of equal volumes of *formaldehyde solution* and *sulphuric acid* . A purple colour is produced.

TESTS

Related substances

Comply with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and applying separately to the plate 20 µl of each of the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Alimemazine Tartrate with 10 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture.

ASSAY

Carry out the following procedure protected from light. Add 150 ml of 0.1M *hydrochloric acid* to 10 tablets, shake for 10 minutes, mix with the aid of ultrasound for 1 minute, dilute with 0.1M *hydrochloric acid* to produce a solution containing 0.050% w/v of Alimemazine Tartrate and filter (solution A). Dilute 10 ml of solution A to 100 ml with *water* (solution B). To a further

10 ml of solution A add 2 ml of *peroxyacetic acid solution*, mix, allow to stand for 5 minutes and add sufficient *water* to produce 100 ml (solution C). Measure the *absorbance* of solution C at the maximum at 342 nm, Appendix II B, using solution B in the reference cell and measure the *absorbance* of solution B at the same wavelength using *water* in the reference cell. Repeat the procedure using a 0.05% w/v solution of *alimemazine tartrate BPCRS* in 0.1M *hydrochloric acid* in place of solution A, beginning at the words 'Dilute 10 ml of solution A ...' and calculate the content of $C_{36}H_{44}N_4S_2,C_4H_6O$ using the declared content of $C_{36}H_{44}N_4S_2,C_4H_6O$ in *alimemazine tartrate BPCRS*. The test is not valid if the absorbance of solution B is more than 0.10.

Allopurinol Tablets

General Notices

Action and use

Xanthine oxidase inhibitor; treatment of gout and hyperuricaemia.

DEFINITION

Allopurinol Tablets contain Allopurinol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of allopurinol, $C_5H_4N_4O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 250 nm.

B. Shake a quantity of the powdered tablets containing 0.1 g of Allopurinol with 5 ml of 1.25M *sodium hydroxide* and add 3 ml of *phosphomolybdotungstic reagent* and 5 ml of a 20% w/v solution of *sodium carbonate*. A greyish blue colour is produced.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Allopurinol with 10 ml of 0.1M *sodium hydroxide* for about 1 minute, immediately dilute to 200 ml with mobile phase A and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A and further dilute 1 volume to 10 volumes with mobile phase A.

(3) Dissolve 10 mg of *allopurinol impurity A EPCRS* and 5 mg each of *allopurinol impurity B EPCRS, allopurinol impurity C EPCRS, allopurinol impurity D EPCRS* and *allopurinol impurity E EPCRS* in mobile phase A. Add 20 ml of solution (1) and immediately dilute to 100 ml with mobile phase A. Dilute 1 ml of the resulting solution to 100 ml with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 5µ is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

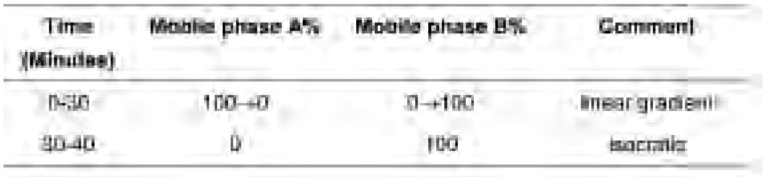
(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

Mobile phase A A mixture of 1 volume of *methanol* and 9 volumes of a 0.125% w/v solution of *potassium dihydrogen orthophosphate* .

Mobile phase B A mixture of 3 volumes of *methanol* with 7 volumes of a 0.125% w/v solution of *potassium dihydrogen orthophosphate* .



Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-30	100→0	0→100	linear gradient
30-40	0	100	isocratic

SYSTEM SUITABILITY

The test is not valid unless in solution (3) the *resolution factor* between the peaks corresponding to impurity A and allopurinol is at least 3.

Inject solution (3). When the chromatogram is recorded in the prescribed conditions, the retention times are: impurity A, about 4.2 minutes; impurities B and C, about 6.1 minutes; allopurinol, about 7.7 minutes; impurity D, about 26.1 minutes; impurity E, about 27.8 minutes. Inject solution (1) and solution (2). Continue the chromatography of solution (1) for 5 times the retention time of allopurinol.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.2%);

— the area of any unresolved double peak corresponding to impurities B and C is not greater than the area of the corresponding double peak in the chromatogram obtained with solution (3) (0.2%);

— the area of any peaks corresponding to impurity D or impurity E is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

— the area of any other *secondary peak* is not greater than the area of the peak due to allopurinol in the chromatogram obtained with solution (2) (0.1%);

— the sum of the areas of any other *secondary peaks* is not greater than 3 times the area of the peak due to allopurinol in the chromatogram obtained with solution (2) (0.3%).

Disregard any peak with an area less than 0.2 times that of the peak due to allopurinol in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Allopurinol with 20 ml of 0.05M *sodium hydroxide* for 20 minutes, add 80 ml of 0.1M *hydrochloric acid* , shake for 10 minutes, add sufficient 0.1M *hydrochloric acid* to produce 250 ml, filter and dilute 10 ml of the filtrate to 250 ml with 0.1M *hydrochloric acid* . Measure the *absorbance* of the resulting solution at the maximum at 250 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the content of $C_5H_4N_4O$ taking 563 as the value of A (1%, 1 cm) at the maximum at 250 nm.

Almond Oil Ear Drops

General Notices

DEFINITION

Almond Oil Ear Drops are Virgin Almond Oil in a suitable container.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

IDENTIFICATION

Carry out the test for the *identification of fixed oils by thin-layer chromatography* , Appendix X N. The chromatogram obtained from the oil being examined shows spots corresponding to those in the *typical chromatogram* for almond oil.

TESTS

Acid value

Not more than 2.0, Appendix X B. Use 5 g dissolved in 50 ml of the prescribed mixture of solvents.

Peroxide value

Not more than 10, Appendix X F.

Relative density

0.911 to 0.920, Appendix V G.

Unsaponifiable matter

Not more than 0.7% w/w, Appendix X H, Method II. Use 5 g.

Apricot-kernel oil and peach-kernel oil

Shake 2 ml for 5 minutes with a mixture of 1 ml of *fuming nitric acid* and 1 ml of *water* and allow to separate. No pink or brown colour develops in either layer.

Foreign fixed oils

Carry out the test for *foreign oils by gas chromatography* , Appendix X N. The fatty-acid fraction of the oil has the following composition.

Saturated fatty acids of chain length less than C_{16} Not more than 0.1%.

Palmitic acid 4.0 to 9.0%.

Palmitoleic acid (equivalent chain length on polyethylene glycol adipate, 16.3) Not more than 0.6%.

Margaric acid Not more than 0.2%.

Stearic acid Not more than 3.0%.

Oleic acid (equivalent chain length on polyethylene glycol adipate, 18.3) 62.0 to 86.0%.

Linoleic acid (equivalent chain length on polyethylene glycol adipate, 18.9) 20.0 to 30.0%.

Linolenic acid (equivalent chain length on polyethylene glycol adipate, 19.7) Not more than 0.4%.

Arachidic acid Not more than 0.1%.

Gadoleic acid (equivalent chain length on polyethylene glycol adipate, 20.3) Not more than 0.1%.

Behenic acid Not more than 0.1%.

Erucic acid (equivalent chain length on polyethylene glycol adipate, 22.3) Not more than 0.1%.

Sterols

Carry out the test for *sterols in fatty oils*, Appendix X N. The sterol fraction of the oil has the following composition.

Cholesterol Not more than 0.7%.

Campesterol Not more than 4.0%.

Stigmasterol Not more than 3.0%.

β-*Sitosterol* 73.0% to 87.0%.

Δ^5-*Avenasterol* At least 10.0%.

Δ^7-*Avenasterol* Not more than 3.0%.

Δ^7-*Stigmastenol* Not more than 3.0 per cent.

Fucosterol Not more than 2.0 per cent.

Brassicasterol Not more than 0.3%.

Sesame oil

Shake 10 ml with 5 ml of a mixture of 0.5 volume of a 0.35% v/v solution of *furfuraldehyde* in *acetic anhydride* and 4.5 volumes of *acetic anhydride* for 1 minute, filter through a filter paper impregnated with *acetic anhydride* and add 0.2 ml of *sulphuric acid*. No bluish green colour develops.

STORAGE

Almond Oil Ear Drops should be kept in a well-filled container and protected from light.

Aloxiprin Tablets

General Notices

Action and use

Salicylate; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Aloxiprin Tablets contain Aloxiprin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of total salicylates

92.5 to 107.5% of the stated amount, calculated as *O*-acetylsalicylic acid, $C_9H_8O_4$.

IDENTIFICATION

To 0.5 g of the powdered tablets add 5 ml of *hydrochloric acid* , boil, cool and filter. Wash the residue with 20 ml of *water* and combine the filtrate and washings. The resulting solution yields the reaction characteristic of *aluminium salts* and, after neutralisation with 1M *sodium hydroxide* , yields reaction A characteristic of *salicylates* , Appendix VI.

TESTS

Disintegration

Maximum time, 5 minutes, Appendix XII A1.

Free salicylates

To a quantity of the powdered tablets containing the equivalent of 0.6 g of total salicylates add 50 ml of dry *ether* and shake for 30 minutes. Filter rapidly through fluted filter paper and wash the paper with several portions of dry *ether* . To the combined filtrate and washings add 10 ml of 1M *sodium hydroxide* , swirl to mix and evaporate the ether on a water bath. Cool, adjust the pH to between 2.40 and 2.50 with 1M *hydrochloric acid* and dilute to 100 ml with *water*. To 20 ml of the resulting solution add 4 ml of *iron (III) chloride solution* and dilute to 50 ml with *acetate buffer pH 2.45* . Allow to stand for 30 minutes, filter, if necessary, through a pair of fluted filter papers and measure the *absorbance* of the solution at the maximum at 530 nm, Appendix II B, using in the reference cell a solution prepared by diluting 4 ml of *iron (III) chloride solution* to 50 ml with *acetate buffer pH 2.45* . The absorbance is not more than that obtained using 5 ml of a 0.036% w/v solution of *salicylic acid* diluted to 20 ml with *water* in place of the solution being examined and beginning at the words 'add 4 ml of *iron (III) chloride solution* ...' (1.5%, calculated with reference to the content of total salicylates).

Combined salicylate

Not more than 15.0%, calculated as salicylic acid, $C_7H_6O_3$, with reference to the content of total salicylates when determined by the following method. To a quantity of the finely powdered tablets containing the equivalent of 0.15 g of total salicylates add 40 ml of a 0.5% w/v solution of *sodium fluoride* in 0.1M *hydrochloric acid* and shake for 5 minutes. Allow the mixture to stand for 10 minutes, shaking at frequent intervals, extract with six 20-ml quantities of *chloroform*, filter the combined extracts through *anhydrous sodium sulphate*, wash with 30 ml of *chloroform* and dilute to 200 ml with *chloroform*. Dilute 20 ml of the solution to 100 ml with *chloroform* and measure the *absorbance* of the resulting solution at the maximum at 308 nm, Appendix II B. Calculate the content of $C_7H_6O_3$ taking 293 as the value of A (1%, 1 cm) at the maximum at 308 nm.

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powder containing the equivalent of 0.3 g of total salicylates add 50 ml of 1M *sodium hydroxide* and boil gently for 15 minutes with occasional swirling. Cool, adjust the pH of the mixture to between 2.40 and 2.50 with 1M *hydrochloric acid* and dilute to 500 ml with *water*. Filter a portion of the suspension; to 5 ml of the filtrate add 35 ml of *acetate buffer pH 2.45* and 4 ml of *iron (III) chloride solution* and dilute to 50 ml with *water*. Allow to stand for 30 minutes and measure the *absorbance* of the resulting solution at the maximum at 530 nm, Appendix II B, using in the reference cell a solution prepared by diluting 4 ml of *iron (III) chloride solution* to 50 ml with *acetate buffer pH 2.45*. Calculate the content of total salicylates as *O*-acetylsalicylic acid, $C_9H_8O_4$, from the *absorbance* obtained by repeating the procedure using 4 ml of a 0.05% w/v solution of *salicylic acid* in place of the solution being examined and beginning at the words 'add 35 ml of *acetate buffer pH 2.45* ...'. Each g of salicylic acid is equivalent to 1.305 g of $C_9H_8O_4$.

LABELLING

The quantity of active ingredient is stated both as the amount of Aloxiprin and in terms of the equivalent amount of total salicylates calculated as *O*-acetylsalicylic acid, $C_9H_8O_4$.

Aluminium Acetate Ear Drops

General Notices

DEFINITION

Aluminium Sulphate	225 g
Calcium Carbonate	100 g
Tartaric Acid	45 g
Acetic Acid (33 per cent)	250 ml
Purified Water	750 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Aluminium Sulphate in 600 ml of the Purified Water, add the Acetic Acid and then the Calcium Carbonate mixed with the remainder of the Purified Water and allow to stand for not less than 24 hours in a cool place, stirring occasionally. Filter, add the Tartaric Acid to the filtrate and mix.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of aluminium, Al

1.7 to 1.9% w/v.

CHARACTERISTICS

A clear liquid.

Weight per ml

1.06 to 1.08 g, Appendix V G.

ASSAY

Dilute 10 ml to 100 ml with *water*. To 10 ml of the resulting solution add 40 ml of 0.05M *disodium edetate VS* , 90 ml of *water* and 0.15 ml of *methyl red solution* . Neutralise by the drop wise addition of 1M *sodium hydroxide* and warm on a water bath for 30 minutes. Cool, add 1 ml of 2M *nitric acid* and 5 g of *hexamine* and titrate with 0.05M *lead nitrate VS* using 0.5 ml of *xylenol orange solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 1.349 mg of Al.

STORAGE

Aluminium Acetate Ear Drops should be kept in a well-filled container.

When aluminium acetate solution or Burow's Solution is prescribed or demanded a solution complying with the requirements of this monograph shall be dispensed or supplied.

Aluminium Chloride Solution

General Notices

DEFINITION

Aluminium Chloride Solution is a *cutaneous solution*. It contains Aluminium Chloride Hexahydrate in a suitable ethanolic vehicle.

PRODUCTION

In making Aluminium Chloride Solution, Industrial Methylated Spirits may be used provided that the law and the statutory regulations governing the use of Industrial Methylated Spirits are observed.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of aluminium chloride hexahydrate, $AlCl_3,6H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dilute the solution with *water* to produce a solution containing 2% w/v of Aluminium Chloride Hexahydrate. The solution yields reaction A characteristic of *aluminium salts* and reaction A characteristic of *chlorides* , Appendix VI.

TESTS

Iron

Dilute the solution being examined with *water* to produce a solution containing 10.0% w/v of Aluminium Chloride Hexahydrate. 10 ml of this solution complies with the *limit test for iron* , Appendix VII (10 ppm, determined with respect to the content of aluminium chloride hexahydrate).

Ethanol

Not less than 70.0% v/v, Appendix VIII F.

ASSAY

To a weighed quantity containing about 0.4 g of Aluminium Chloride Hexahydrate add 25 ml of *water* and carry out the *complexometric titration of aluminium* , Appendix VIII D. Each ml of 0.1M *disodium edetate VS* is equivalent to 24.14 mg of $AlCl_3,6H_2O$.

If the declared content is in terms of weight in volume, determine the *weight per ml* of the solution, Appendix V G, and hence calculate the content of $AlCl_3,6H_2O$, weight in volume.

STORAGE

Aluminium Chloride Solution should be stored upright.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored; (3) that the solution is flammable.

The label state indicates the pharmaceutical form as 'cutaneous solution'.

Aluminium Hydroxide Oral Suspension

General Notices

DEFINITION

Aluminium Hydroxide Oral Suspension is an aqueous suspension of hydrated aluminium oxide together with varying quantities of basic aluminium carbonate. It contains the equivalent of 4% w/w of aluminium oxide and has a peppermint flavour.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of aluminium oxide, Al_2O_3

The equivalent of 3.5 to 4.4% w/w.

CHARACTERISTICS

A white suspension from which small amounts of clear liquid may separate on standing. It may exhibit thixotropic properties.

IDENTIFICATION

A solution in 2M *hydrochloric acid* yields the reaction characteristic of *aluminium salts*, Appendix VI.

TESTS

Alkalinity

pH, when diluted with an equal volume of *carbon dioxide-free water*, not more than 7.5, Appendix V L.

Neutralising capacity

Disperse 5 g in 100 ml of *water*, heat to 37°, add 100 ml of 0.1M *hydrochloric acid VS* previously heated to 37° and stir continuously, maintaining the temperature at 37°. The pH of the solution, at 37°, after 10, 15 and 20 minutes, is not less than 1.8, 2.3 and 3.0 respectively and at no time during this period is it more than 4.0. Add 10 ml of 0.5M *hydrochloric acid VS* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate the solution with 0.1M *sodium hydroxide VS* to pH 3.5. Not more than 50 ml of 0.1M *sodium hydroxide VS* is required.

Ammonium salts

To 25 g in an ammonia-distillation apparatus add 25 ml of 5M *sodium hydroxide* and 250 ml of *water*, distil about 100 ml, collecting the distillate in 25 ml of 0.1M *hydrochloric acid VS*, and titrate the excess of acid with 0.1M *sodium hydroxide VS* using *methyl red solution* as indicator. Not less than 20.0 ml of 0.1M *sodium hydroxide VS* is required.

Arsenic

Dissolve 2.0 g in 18 ml of *brominated hydrochloric acid* and 32 ml of *water*. 25 ml of the resulting solution complies with the *limit test for arsenic*, Appendix VII (1 ppm).

Heavy metals

Dissolve 2.0 g in 20 ml of 1M *hydrochloric acid* and 10 ml of *water*, add 0.5 ml of *nitric acid* and boil for about 30 seconds. Cool, add 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with two 10 ml quantities of a mixture of equal volumes of *isoamyl alcohol* and *ether*. To the aqueous layer add 2 g of *citric acid* and dilute to 40 ml with *water*. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (20 ppm).

Chloride

Dissolve 0.30 g in 2 ml of 2M *nitric acid*, boil, cool, dilute to 250 ml with *water* and filter. 15 ml of the filtrate complies with the *limit test for chlorides*, Appendix VII (0.3%).

Sulphate

Dissolve 0.50 g in 5 ml of 2M *hydrochloric acid,* boil, cool, dilute to 200 ml with *water* and filter. 12.5 ml of the filtrate, diluted to 15 ml with 2M *hydrochloric acid*, complies with the *limit test for sulphates*, Appendix VII (0.5%).

Microbial contamination

Carry out a quantitative evaluation for Enterobacteria and certain other Gram-negative bacteria, Appendix XVI B1. 0.01 ml of the preparation gives a negative result, Table I (most probable number of bacteria per gram fewer than 10^2).

ASSAY

Dissolve 5 g in 3 ml of *hydrochloric acid* by warming on a water bath, cool to below 20° and dilute to 100 ml with *water*. To 20 ml of this solution add 40 ml of 0.05M *disodium edetate VS*, 80 ml of *water* and 0.15 ml of *methyl red solution* and neutralise by the drop wise addition of 1M *sodium hydroxide*. Heat on a water bath for 30 minutes, add 3 g of *hexamine* and titrate with 0.05M *lead nitrate VS* using 0.5 ml of *xylenol orange solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

STORAGE

Aluminium Hydroxide Oral Suspension should be kept at a temperature not exceeding 30°. It should not be allowed to freeze.

Aluminium Hydroxide Tablets

General Notices

DEFINITION

Aluminium Hydroxide Tablets contain, in each, 500 mg of Dried Aluminium Hydroxide in a suitable basis with a peppermint flavour.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aluminium oxide, Al_2O_3

Not less than the equivalent of 0.225 g.

IDENTIFICATION

The powdered tablets yield the reaction characteristic of *aluminium salts* , Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Aluminium Hydroxide Tablets.

Neutralising capacity

Pass a sufficient quantity of the powder prepared for use in the Assay through a sieve with a nominal mesh aperture of 150 µm. Mix a quantity of the powder containing 0.5 g of Dried Aluminium Hydroxide with a small quantity of *water* to give a smooth paste and slowly add further quantities of *water* to a total volume of 100 ml. Warm to 37°, add 100 ml of 0.1M *hydrochloric acid VS* previously heated to 37° and stir continuously, maintaining the temperature at 37°. The pH of the solution at 37° after 10, 15 and 20 minutes is not less than 1.6, 1.8 and 2.2 respectively and at no time during this period is it more than 4.0. Add 10 ml of 0.5M *hydrochloric acid VS* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate the solution with 0.1M *sodium hydroxide VS* to pH 3.5. Subtract the volume of 0.1M *sodium hydroxide VS* from 150 to obtain the number of ml of 0.1M *hydrochloric acid VS* required for the neutralisation. Calculate the number of ml of 0.1M *hydrochloric acid VS* required for the total weight of the tablets taken for the Assay and divide by the number of tablets. The result is not less than 115.

ASSAY

Weigh and powder 20 tablets, avoiding frictional heating. Dissolve a quantity of the powder containing 0.4 g of Dried Aluminium Hydroxide as completely as possible in a mixture of 3 ml of *hydrochloric acid* and 3 ml of *water* by warming on a water bath, cool to below 20° and dilute to 100 ml with *water*. To 20 ml of this solution add 40 ml of 0.05M *disodium edetate VS* , 80 ml of *water* and 0.15 ml of *methyl red solution* and neutralise by the drop wise addition of

1M *sodium hydroxide VS* . Heat on a water bath for 30 minutes, add 3 g of *hexamine* and titrate with 0.05M *lead nitrate VS* using 0.5 ml of *xylenol orange solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

LABELLING

The label states that the tablets should be chewed before swallowing.

Alverine Capsules

General Notices

Action and use

Smooth muscle relaxant; antispasmodic.

DEFINITION

Alverine Capsules contain Alverine Citrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of alverine citrate, $C_{20}H_{27}N,C_6H_8O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.12 g of Alverine Citrate with 5 ml of *methanol* for 5 minutes, filter through a 0.45 µ PTFE filter, evaporate the filtrate to dryness under a stream of nitrogen using a warm water bath and dry the residue for 1 hour at 50° under vacuum. The *infrared absorption spectrum* of the residue*,* Appendix II A, is concordant with the *reference spectrum* of alverine citrate (*RS 409*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter through a membrane filter with a pore size of 0.45 µm, discarding the first 10 ml of the filtrate. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. Solution (1) contains 0.006% w/v of *alverine citrate BPCRS* in 0.1M *hydrochloric acid*. Solution (2) is the filtrate from the dissolution vessel, diluted, if necessary with 0.1M *hydrochloric acid* to produce a solution expected to contain about 0.006% w/v of Alverine Citrate.

The chromatographic conditions described under Related substances may be used.

Calculate the total content of alverine citrate, $C_{20}H_{27}N,C_6H_8O_7$, in the medium from the chromatograms obtained and using the declared content of $C_{20}H_{27}N,C_6H_8O_7$ in *alverine citrate BPCRS.*

Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) add 80 ml of *methanol* to a quantity of the mixed contents of the capsules containing 0.6 g of Alverine Citrate. Mix with the aid of ultrasound for 1 hour, allow to cool to room temperature, add sufficient *methanol* to produce 100 ml, mix and filter (Whatman GF/C is suitable). Solution (2) is *alverine citrate impurity standard solution BPCRS.*

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute 0.01M *sodium dodecyl sulphate* in a mixture of 55 volumes of *acetonitrile* and 45 volumes of *water* adjusting the pH of the mixture to 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 220 nm.

Inject 20 µl of solution (2). The test is not valid unless the chromatogram obtained closely resembles the reference chromatogram supplied with *alverine citrate impurity standard solution BPCRS.*

In the chromatogram obtained with solution (1), the areas of any peaks corresponding to alverine citrate impurities A, C and D are not greater than the areas of the corresponding peaks in the chromatogram obtained with solution (2) (0.2%, 0.5% and 0.5% respectively).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. To a quantity of the mixed contents of 20 capsules containing the equivalent of 0.6 g Alverine Citrate add 100 ml of *methanol,* mix with the aid of ultrasound for 1 hour and add sufficient *methanol* to produce 500 ml. Stir vigorously for 10 minutes and filter (Cellulose nitrate filter 0.45 µ is suitable). Dilute 10 volumes of the filtrate to 20 volumes with *water*. For solution (2) dilute 1 volume of a 0.6% w/v solution of *alverine citrate BPCRS* in *methanol* to 10 volumes with *water.* Solution (3) is *alverine citrate impurity standard solution BPCRS.*

The chromatographic conditions described under Related substances may be used.

Inject 20 µl of solution (3). The test is not valid unless the chromatogram obtained closely resembles the reference chromatogram supplied with *alverine citrate impurity standard solution BPCRS.*

Calculate the content of $C_{20}H_{27}N,C_6H_8O_7$ in the capsules using the declared content of $C_{20}H_{27}N,C_6H_8O_7$ in *alverine citrate BPCRS.*

STORAGE

Alverine Capsules should be stored in a well-closed container and protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Alverine Citrate.

Amantadine Capsules

General Notices

Action and use

Viral replication inhibitor (influenza A); dopamine receptor agonist; treatment of influenza and Parkinson's disease.

DEFINITION

Amantadine Capsules contain Amantadine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of amantadine hydrochloride, $C_{10}H_{17}N,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 200 mg of Amantadine Hydrochloride in 10 ml of 0.1M *hydrochloric acid* on a water bath and filter. Add 1 ml of 5M *sodium hydroxide* to the filtrate, extract with 5 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate*, wash the sodium sulphate with 2 ml of *dichloromethane* and evaporate the solution to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of amantadine *(RS 006)*.

B. The contents of the capsules yield the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using 1 µl or other suitable volume of the following solution. Dissolve a quantity of the contents of the capsules containing 0.1 g of Amantadine Hydrochloride in 2 ml of *water*, add 2 ml of a 20% w/v solution of *sodium hydroxide* and 2 ml of *chloroform* and shake for 10 minutes. Separate the chloroform layer, dry over *anhydrous sodium sulphate* and filter.

The chromatographic procedure may be carried out using a glass column (1.8 m × 2 mm) containing a packing material prepared in the following manner. Mix 19.5 g of *silanised diatomaceous support* (Chromosorb G/AW/DMCS is suitable) with 60 ml of a 0.33% w/v solution of *potassium hydroxide* in *methanol* and evaporate the solvent under reduced pressure while slowly rotating the mixture. Dissolve 0.4 g of *low-vapour pressure hydrocarbons (type L)* (Apiezon L is suitable) in 60 ml of *toluene* (dissolution requires up to 5 hours), add this solution to the prepared silanised diatomaceous support and evaporate the solvent under reduced pressure while slowly rotating the mixture. Program the temperature of the column to increase from 100° to 200° at a constant rate of 6° per minute with the inlet port at 220° and the detector at 300°. Use a flow rate of 30 ml per minute for the carrier gas. Record the chromatogram for at least 2.5 times the retention time of the principal peak.

The area of any *secondary peak* is not greater than 0.3% and the sum of the areas of any secondary peaks is not greater than 1% by *normalisation*.

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 0.5 g of Amantadine Hydrochloride as completely as possible in 10 ml of *water* by heating on a water bath while shaking and cool. Add 10 ml of 5M *sodium hydroxide* and 50 ml of *hexane*, shake gently for 15 minutes and centrifuge. To 10 ml of the supernatant liquid add 30 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 18.77 mg of $C_{10}H_{17}N,HCl$.

STORAGE

Amantadine Capsules should be kept in an airtight container and protected from light.

Amantadine Oral Solution

General Notices

Action and use

Viral replication inhibitor (influenza A); dopamine receptor agonist; treatment of influenza and Parkinson's disease.

DEFINITION

Amantadine Oral Solution is a solution of Amantadine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of amantadine hydrochloride, $C_{10}H_{17}N,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Acidify a volume of the oral solution containing 0.1 g of Amantadine Hydrochloride with 1M *hydrochloric acid* and extract with three 15 ml quantities of *ether*. Discard the ether layer, add 2M *sodium hydroxide* to the aqueous layer until it is just alkaline, extract with two 10 ml quantities of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate*, evaporate the filtrate to dryness under reduced pressure and dry the residue at room temperature over *phosphorus pentoxide* at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of amantadine *(RS 006)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method described under Assay using 1 µl of the following solution. Mix, with swirling, a volume of the oral solution containing 0.1 g of Amantadine Hydrochloride with 4 ml of a 20% w/v solution of *sodium hydroxide*, add 10 ml of *toluene*, shake the mixture for 10 minutes, allow the layers to separate and use the upper layer.

The area of any *secondary peak* is not greater than 0.3% and the sum of the areas of any *secondary peaks* is not greater than 1% by *normalisation*.

ASSAY

Prepare a 0.6% w/v solution of *naphthalene* (internal standard) in *toluene* (solution A). Carry out the method for *gas chromatography*, Appendix III B, using 1 µl of each of the following solutions. For solution (1) mix, with swirling, a weighed quantity of the oral solution containing 0.1 g of Amantadine Hydrochloride with 4 ml of a 20% w/v solution of *sodium hydroxide* and add 10 ml of solution A. Shake the mixture for 10 minutes, allow the layers to separate and use the upper layer. Prepare solution (2) in the same manner but using 0.1 g of *amantadine hydrochloride BPCRS* in 10 ml of *water* in place of the preparation being examined.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *silanised diatomaceous support* (Chromosorb WHP is suitable) and coated with 10% polyethylene glycol compound (Carbowax 20M is suitable) previously treated with a 5% w/v solution of *potassium hydroxide* in *methanol*. Maintain at 150° with the inlet port at 180° and the detector at 250° and use a flow rate of 40 ml per minute for the carrier gas.

Calculate the content of $C_{10}H_{17}N,HCl$ using the ratios of the area of the peak corresponding to Amantadine Hydrochloride to the area of the peak due to the internal standard in the chromatograms obtained with solutions (1) and (2) and using the declared content of $C_{10}H_{17}N,HCl$ in *amantadine hydrochloride BPCRS.*

Amikacin Injection

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Amikacin Injection is a sterile solution of Amikacin Sulphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of amikacin, $C_{22}H_{43}N_5O_{13}$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the test for related substances, the principal peak in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (5).

Acidity

pH, 3.5 to 5.5, Appendix V L.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection, if necessary, with sufficient *water* to produce a solution containing the equivalent of 1.0% w/v of amikacin.

(2) Dilute 1 volume of solution (1) to 10 volumes with *water*.

(3) 0.015% w/v of *amikacin impurity A EPCRS* in *water*.

(4) 0.01% w/v of *amikacin sulphate EPCRS* and 0.01% w/v of *amikacin impurity A EPCRS* in *water*.

(5) 0.13% w/v of *amikacin sulphate EPCRS* in *water*.

(6) *Water* (blank solution).

Derivatise the solutions prior to analysis using the following method:

Transfer 0.2 ml of the solution under test to a ground glass stoppered vial. Add 2 ml of a 1% w/v solution of *2,4,6-trinitrobenzenesulphonic acid*. To this solution add 3 ml of *pyridine* and close the vial tightly. Shake vigorously for 30 seconds and heat on a water bath at 75° for 2 hours. Cool in cold water for 2 minutes and add 2 ml of *glacial acetic acid*. Shake vigorously for 30 seconds. Store the derivatised solutions at 10° prior to and during analysis.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 340 nm.

(f) Inject 20 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for 4 times the retention time of amikacin.

MOBILE PHASE

A mixture of 30 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate,* adjusted to pH 6.5 with a 2.2% w/v solution of *potassium hydroxide*, and 70 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to amikacin and impurity A is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.5%);

the sum of the areas of all such peaks is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (3) (3%).

Disregard any peaks corresponding to the peaks in the chromatogram obtained with solution (6), any peak eluting before the principal peak and any peak with an area of less than 0.067 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing the equivalent of 10 mg per ml of amikacin (solution A). The endotoxin limit concentration of solution A is 3.3 IU of endotoxin per ml.

Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (2) and (5) described under Related substances.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{43}N_5O_{13}$ in the injection using the declared content of $C_{22}H_{43}N_5O_{13},2H_2SO_4$ in *amikacin sulphate EPCRS*. Each mg of $C_{22}H_{43}N_5O_{13},2H_2SO_4$ is equivalent to 0.7488 mg of $C_{22}H_{43}N_5O_{13}$.

LABELLING

The strength is stated as the equivalent amount of amikacin in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Amikacin Sulphate.

Amiloride Tablets

General Notices

Action and use

Sodium channel blocker; potassium-sparing diuretic.

DEFINITION

Amiloride Tablets contain Amiloride Hydrochloride.

With the exception of the requirements for shape, the tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing the equivalent of 0.5 mg of anhydrous amiloride hydrochloride with 100 ml of 0.1M *hydrochloric acid* and filter. The *light absorption* of the filtrate, Appendix II B, in the range 230 to 380 nm exhibits two maxima, at 285 nm and at 361 nm.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (5).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following four solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 17.5 mg of anhydrous amiloride hydrochloride with 10 ml of a mixture of 1 volume of *acetonitrile* and 3 volumes of *water*, disperse with the aid of ultrasound for 5 minutes and centrifuge. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 1 volume of *acetonitrile* and 3 volumes of *water*. For solution (3) dilute 1 volume of solution (2) to 10 volumes with a mixture of 1 volume of *acetonitrile* and 3 volumes of *water*. Solution (4) contains 0.0010% w/v of *methyl 3,5-diamino-6-chloropyrazine-2-carboxylate EPCRS* in a mixture of 1 volume of *acetonitrile* and 3 volumes of *water*. Solution (5) contains 0.175% w/v of *amiloride hydrochloride BPCRS* in a mixture of 1 volume of *acetonitrile* and 3 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm)

(Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute, a mixture of 5 volumes of *tetramethylammonium hydroxide solution* , 250 volumes of *acetonitrile* and 745 volumes of *water*, the pH of the mixture being adjusted to 7.0 using a mixture of 1 volume of *orthophosphoric acid* and 9 volumes of *water*, and (c) a detection wavelength of 254 nm.

Inject 20 µl of solution (4) and adjust the concentration of acetonitrile so that the retention time of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate is 5 to 6 minutes (an increase in the concentration of acetonitrile reduces the retention time). Inject 20 µl of solution (2) and adjust the concentrations of tetramethylammonium hydroxide and orthophosphoric acid so that the retention time of amiloride is 9 to 12 minutes keeping the pH at 7.0 (an increase in the concentrations reduces the retention time).

Inject 20 µl of solution (3). The test is not valid unless the *signal-to-noise ratio* of the peak due to amiloride in the chromatogram obtained is at least 5.0.

Inject 20 µl of each of solutions (1) and (4) and record the chromatograms for 5 times the retention time of amiloride. In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than the area of the peak due to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with solution (4) (0.6%) . Disregard any peak with an area less than 10% of the area of the peak due to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with solution (4) (0.06%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 10 mg of anhydrous amiloride hydrochloride add 10 ml of *methanol* and shake for 10 minutes. Add 60 ml of 0.1M *sodium hydroxide* , shake for 30 minutes and dilute to 100 ml with 0.1M *sodium hydroxide* . Immediately transfer 4 ml of the resulting solution to a stoppered centrifuge tube and add 10 ml of 0.1M *sodium hydroxide* and 20 ml of *tributyl orthophosphate* that has been washed with *water* before use. Shake vigorously for 2 minutes and centrifuge for 5 minutes. Remove the upper layer and repeat the extraction with a further 20 ml of the water-washed tributyl orthophosphate. To the combined extracts add 2 ml of *methanol* and sufficient of the water-washed tributyl orthophosphate to produce 50 ml and centrifuge to remove traces of water. Measure the *absorbance* of the resulting solution at the maximum at 363 nm, Appendix II B, using in the reference cell a mixture of 48 volumes of the water-washed tributyl orthophosphate and 2 volumes of *methanol* . Calculate the content of $C_6H_8ClN_7O,HCl$ taking 692 as the value of A(1%, 1 cm) at the maximum at 363 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous amiloride hydrochloride.

Aminoglutethimide Tablets

General Notices

Action and use

Inhibitor of adrenal corticosteroid synthesis; used in chemical adrenalectomy.

DEFINITION

Aminoglutethimide Tablets contain Aminoglutethimide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aminoglutethimide, $C_{13}H_{16}N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.25 g of Aminoglutethimide with 10 ml of *methanol* for 10 minutes, filter through a 0.45-µm glass microfibre filter (Whatman GF/C is suitable) and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of aminoglutethimide *(RS 403).*

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of the medium and filter through a membrane filter with a nominal pore size not greater than 0.45 µm, discarding the first 15 ml of filtrate. Dilute the filtered solution with sufficient of a buffer solution prepared by dissolving 6.804 g of *potassium dihydrogen orthophosphate* in sufficient 0.04M *sodium hydroxide* to produce 1000 ml and adjusting the pH to 7.5 with 0.2M *sodium hydroxide* to produce a solution expected to contain about 0.001% w/v of Aminoglutethimide. Measure the *absorbance* of this solution, Appendix II B, at 237 nm using in the reference cell dissolution medium that has been diluted in the same manner as the test solution. Calculate the total content of aminoglutethimide, $C_{13}H_{16}N_2O_2$, in the medium from the *absorbance* obtained from a solution prepared by diluting a 0.025% w/v solution of *aminoglutethimide BPCRS* in dissolution medium to contain 0.001% w/v with the buffer solution and using the declared content of $C_{13}H_{16}N_2O_2$ in *aminoglutethimide BPCRS* .

3-Aminoglutethimide

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) add 75 ml of *methanol* to a quantity of the powdered tablets containing 0.5 g of Aminoglutethimide, shake for 30 minutes, dilute to 100 ml with *methanol*, centrifuge and dilute 10 ml of the clear supernatant liquid to 100 ml with *water*. For solution (2) dilute 1 volume of a 0.005% w/v solution of *aminoglutethimide impurity A EPCRS* in *methanol* to 5 volumes with *water*. For solution (3) dilute 1 volume of solution (1) to 10 volumes with *methanol*, mix 1 volume of this solution with 1 volume of a 0.005% w/v solution of *aminoglutethimide impurity A EPCRS* in *methanol* and dilute to 5 volumes with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 0.5 volume of *triethylamine*, 160 volumes of *acetonitrile* and 800 volumes of *water* and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to aminoglutethimide impurity A is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 2M *ammonia*, 4 volumes of *methanol* and 5 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) add 5 ml of *methanol* to a quantity of the powdered tablets containing 0.1 g of Aminoglutethimide, shake vigorously for 10 minutes, centrifuge and use the clear supernatant liquid. Solution (2) contains 2% w/v of *aminoglutethimide BPCRS* in *methanol*. *For* solution (3) dilute 1 volume of solution (1) to 200 volumes with *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* Expose the plate to nitrous fumes in a closed glass tank for 15 minutes (the nitrous fumes may be generated by adding 100 ml of 1M *hydrochloric acid* to 6.9 g of *sodium nitrite*). Place the plate in a current of warm air for 15 minutes and spray with a 0.5% w/v solution of N-*(1-naphthyl) ethylenediamine dihydrochloride* in *ethanol (50%)* containing 1% v/v of 1M *hydrochloric acid*. By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than that in the chromatogram obtained with solution (3) (0.5%).

Azo-glutethimide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 200 ml of *dimethylsulphoxide* to a quantity of the powdered tablets containing 0.25 g of Aminoglutethimide, shake for 15 minutes, protected from light, add sufficient *dimethylsulphoxide* to produce 250 ml and filter. Solution (2) contains 0.00003% w/v of *aminoglutethimide impurity D EPCRS* in *dimethylsulphoxide*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 350 volumes of a solution prepared by dissolving 0.285 g of *disodium edetate* in *water*, adding 7.5 ml of 2M *acetic acid*, 50 ml of 0.1M *potassium hydroxide* and sufficient *water* to produce 1000 ml, adjusting the pH to 5.0 with *glacial acetic acid* and 650 volumes of *methanol* and (c) a detection wavelength of 328 nm. Pass the mobile phase through the column until the retention times obtained with solution (2) are constant.

Inject *dimethylsulphoxide* as a blank solution.

In the chromatogram obtained with solution (1) the area of any peak corresponding to aminoglutethimide impurity D is not greater than the area of the peak in the chromatogram obtained with solution (2) (300 ppm).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 75 ml of *methanol* to a quantity of the powdered tablets containing 0.5 g of Aminoglutethimide, shake for 30 minutes, dilute to 100 ml with *methanol*, centrifuge and dilute 10 ml of the clear supernatant liquid to 100 ml with *water*. For solution (2) dilute 1 volume of a 0.5% w/v solution of *aminoglutethimide BPCRS* in *methanol* to 10 volumes with *water*. For solution (3) dilute 1 volume of a 0.5% w/v solution of *aminoglutethimide BPCRS* in *methanol* to 100 volumes with *methanol* and mix 1 volume of this solution and 1 volume of a 0.005% w/v solution of *aminoglutethimide impurity A EPCRS* in *methanol* to 10 volumes with *water*.

The chromatographic conditions described in the test for 3-Aminoglutethimide may be used.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.0.

Calculate the content of $C_{13}H_{16}N_2O_2$ in the tablets using the declared content of $C_{13}H_{16}N_2O_2$ in *aminoglutethimide BPCRS*.

STORAGE

Aminoglutethimide Tablets should be protected from light and moisture and stored at a temperature not exceeding 30°.

Aminophylline Injection

General Notices

Action and use

Non-selective phosphodiesterase inhibitor; treatment of reversible airways obstruction.

DEFINITION

Aminophylline Injection is a solution of Aminophylline or Aminophylline Hydrate in Water for Injections free from carbon dioxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of theophylline, $C_7H_8N_4O_2$

73.25 to 88.25% of the stated amount of aminophylline.

Content of ethylenediamine, $C_2H_8N_2$

Not more than 0.295 g for each g of anhydrous theophylline, $C_7H_8N_4O_2$, determined in the Assay for theophylline.

IDENTIFICATION

A. To a volume containing the equivalent of 0.1 g of aminophylline add 0.5 ml of 2M *hydrochloric acid* with constant stirring, allow to stand for a few minutes and filter. Wash the residue with small quantities of cold *water*, recrystallise from hot *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of theophylline *(RS 333)*.

B. To a volume containing the equivalent of 0.1 g of aminophylline add 2 ml of a 1% w/v solution of *copper(II) sulphate* and shake. A purplish blue colour is produced.

C. Evaporate a volume containing the equivalent of 60 mg of aminophylline to dryness in a porcelain dish. To the residue add 1 ml of *hydrochloric acid* and 0.1 g of *potassium chlorate* and evaporate to dryness. A reddish residue is produced, which becomes purple on exposure to the vapour of 5M *ammonia*.

TESTS

Alkalinity

pH, 8.8 to 10.0, Appendix V L.

ASSAY

For theophylline

To a volume containing the equivalent of 0.1 g of aminophylline add sufficient 0.01M *sodium hydroxide* to produce 250 ml, dilute 5 ml to 250 ml with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_7H_8N_4O_2$ taking 650 as the value of A (1%, 1 cm) at the maximum at 275 nm.

For ethylenediamine

To a volume containing the equivalent of 0.5 g of aminophylline add, if necessary, sufficient *water* to produce 20 ml and titrate with 0.05M *sulphuric acid VS* , using *bromocresol green solution* as indicator, until the colour changes from blue to green. Each ml of 0.05M *sulphuric acid VS* is equivalent to 3.005 mg of $C_2H_8N_2$. Calculate the weight of $C_2H_8N_2$ present for each g of $C_7H_8N_4O_2$ found.

LABELLING

When the injection is prepared from Aminophylline Hydrate, Theophylline or Theophylline Hydrate the strength is stated as the equivalent amount of aminophylline in a suitable dose-volume.

Aminophylline Tablets

General Notices

DEFINITION

Aminophylline Tablets contain Aminophylline or Aminophylline Hydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of theophylline, $C_7H_8N_4O_2$

81.4 to 90.0% of the stated amount of Aminophylline.

Content of ethylenediamine, $C_2H_8N_2$

Not less than 10.9% and not more than 12.1% of the stated amount of Aminophylline.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Aminophylline with 20 ml of *water*, filter, add to the filtrate with constant stirring 1 ml of 2M *hydrochloric acid*, allow to stand for a few minutes and again filter. Reserve the filtrate for test C. Wash the residue with small quantities of cold *water*, recrystallise from hot *water* and dry at 105°. The *melting point* of the residue is about 271°, Appendix V A.

B. The *infrared absorption spectrum* of the residue obtained in test A, Appendix II A, is concordant with the *reference spectrum* of theophylline *(RS 333)*.

C. To the filtrate reserved in test A add 0.2 ml of *benzoyl chloride*, make alkaline with 5M *sodium hydroxide* and shake vigorously. Filter, wash the residue with cold *water* and recrystallise from a mixture of 1 volume of *water* and 3 volumes of *ethanol (96%)*. The *melting point* of the crystals, after drying at 100°, is about 250°, Appendix V A.

D. Shake a quantity of the powdered tablets containing 0.25 g of Aminophylline with 5 ml of *water* and filter. To 2 ml of the filtrate add 2 ml of a 1% w/v solution of *copper (II) sulphate* and shake. A purplish blue colour is produced.

TESTS

Dissolution of theophylline

Comply with the *dissolution test for tablets and capsules*, Appendix XII D, using Apparatus II. Use as the medium 900 ml of *phosphate buffer pH 7.0* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is a 0.001% w/v solution of *theophylline BPCRS* in *phosphate buffer pH 7.0*. Use the filtered dissolution medium as solution (2), diluted with *phosphate buffer pH 7.0*, if necessary.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically-bonded phenyl groups (5 µm) (Apex Phenyl is suitable), (b) a mixture of 45 volumes of *methanol* and 55 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 273 nm.

Calculate the total content of theophylline, $C_7H_8N_4O_2$, in the medium taking 100% as the content of $C_7H_8N_4O_2$ in *theophylline BPCRS* .

ASSAY

For theophylline

Weigh and powder 20 tablets. Shake a quantity of the powder containing 80 mg of Aminophylline with a mixture of 20 ml of 0.1M *sodium hydroxide* and 60 ml of *water* for 10 minutes, add sufficient *water* to produce 200 ml, mix and filter. Dilute 5 ml of the filtrate to 250 ml with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_7H_8N_4O_2$ taking 650 as the value of A (1%, 1 cm) at the maximum at 275 nm.

For ethylenediamine

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.3 g of Aminophylline with 20 ml of *water*, heat to 50° for 30 minutes and titrate with 0.05M *sulphuric acid VS* , using *bromocresol green solution* as indicator, until the colour changes from blue to green. Each ml of 0.05M *sulphuric acid VS* is equivalent to 3.005 mg of $C_2H_8N_2$.

STORAGE

Aminophylline Tablets should be kept in an airtight container and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous Aminophylline.

Amiodarone Intravenous Infusion

General Notices

Action and use

Potassium channel blocker; class III antiarrhythmic.

DEFINITION

Amiodarone Intravenous Infusion is a sterile solution of Amiodarone Hydrochloride in Water for Injections. It is prepared immediately before use by diluting Amiodarone Sterile Concentrate with Glucose Intravenous Infusion in accordance with the manufacturer's instructions.

The intravenous infusion complies with the requirements stated under Parenteral Preparations.

AMIODARONE STERILE CONCENTRATE

DEFINITION

Amiodarone Sterile Concentrate is a sterile solution of Amiodarone Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of amiodarone hydrochloride, $C_{25}H_{29}I_2NO_3,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a volume of the concentrate containing 0.3 g of Amiodarone Hydrochloride with three 25 ml volumes of *dichloromethane* . Dry the combined extracts over *anhydrous sodium sulphate* , filter and evaporate to dryness. To the residue add 2 ml of 1M *sodium hydroxide* and extract with 25 ml of *ether* . Dry the extract over *anhydrous sodium sulphate* , filter and evaporate to dryness. Dry the residue obtained under reduced pressure over *phosphorus pentoxide* and dissolve in 2.5 ml of *dichloromethane* . The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of amiodarone *(RS 008)* .

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak in the chromatogram obtained with solution (2).

TESTS

Colour of solution

Not more intense than *reference solution BY* $_4$ or *GY* $_4$, Appendix IV B, Method II.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 5 volumes of *anhydrous formic acid,* 10 volumes of *methanol* and 85 volumes of *dichloromethane* as the mobile phase. Pre-wash the plate with the mobile phase and allow to dry in air before use. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a volume of the concentrate containing 50 mg of Amiodarone Hydrochloride to 20 ml with *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.004% w/v of *2-butyl-3-(4-hydroxy-3,5-di-iodobenzoyl) benzofuran BPCRS* in *methanol*. Solution (4) contains 0.1% w/v of *benzyl alcohol* in *methanol.* After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to 2-butyl-3-(4-hydroxy-3,5-di-iodobenzoyl) benzofuran is not more intense than the spot in the chromatogram obtained with solution (3) (1.6%) and any other *secondary spot,* other than any spot corresponding to benzyl alcohol, is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the concentrate containing 50 mg of Amiodarone Hydrochloride to 50 ml with the mobile phase and dilute 10 ml of this solution to 100 ml with the mobile phase. Solution (2) contains 0.01% w/v of *amiodarone hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (7.5 cm × 3.9 mm) packed with silica particles the surface of which have been modified by chemically-bonded nitrile groups (4 µm) (Nova-Pak CN HP is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 45 volumes of 0.01M *sodium perchlorate* and 55 volumes of *acetonitrile*, the pH of the mixture being adjusted to 3.0 with 2M *orthophosphoric acid* and (c) a detection wavelength of 244 nm.

Calculate the content of $C_{25}H_{29}I_2NO_3,HCl$ using the declared content of $C_{25}H_{29}I_2NO_3,HCl$ in *amiodarone hydrochloride BPCRS.*

STORAGE

Amiodarone Sterile Concentrate should be protected from light.

LABELLING

The label states (1) 'Amiodarone Sterile Concentrate'; (2) that the solution must be diluted with Glucose Intravenous Infusion.

Amiodarone Tablets

General Notices

Action and use

Potassium channel blocker; class III antiarrhythmic.

DEFINITION

Amiodarone Tablets contain Amiodarone Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amiodarone hydrochloride, $C_{25}H_{29}I_2NO_3,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.3 g of Amiodarone Hydrochloride with 25 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. To the residue add 2 ml of 1M *sodium hydroxide* and extract with 25 ml of *ether*. Dry the extract over *anhydrous sodium sulphate*, filter and evaporate to dryness. Dry the residue obtained under reduced pressure over *phosphorus pentoxide* and dissolve in 2.5 ml of *dichloromethane*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of amiodarone *(RS 008)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the peak in the chromatogram obtained with solution (1).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 50 mg of Amiodarone Hydrochloride with 50 ml of *methanol*, allow to cool and filter through a 0.45-µm PTFE filter. Dilute 1 volume of the solution to 2 volumes with a mixture of equal volumes of *acetonitrile* and *water*. For solution (2) dissolve 10 mg each of *amiodarone impurity D EPCRS*, *amiodarone impurity E EPCRS* and *amiodarone hydrochloride BPCRS* in *methanol* and dilute to 50 ml with the same solvent. Dilute 1 ml of the solution to 200 ml with a mixture of equal volumes of *acetonitrile* and *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS(2) is suitable) and maintained at 30^{c}, (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 30 volumes of *methanol*, 40 volumes of *acetonitrile* and 30 volumes of a mixture prepared in the following manner: to 800 ml of *water*, add 3 ml of *glacial acetic acid* , adjust to pH 4.9 with *dilute ammonia R1* and dilute to 1000 ml with *water* and (c) a detection wavelength of 240 nm. Inject 20 µl of each solution.

The relative retentions with reference to amiodarone (retention time = 24 minutes) are: impurity A = about 0.26; impurity D = about 0.29; impurity E = about 0.37; impurity B = about 0.49; impurity C = about 0.55; impurity G = about 0.62; impurity F = about 0.69. The test is not valid unless in the chromatogram obtained with solution (2) the *resolution factor* between the peaks due to impurity D and impurity E is at least 3.5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the peak due to amiodarone in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the peak due to Amiodarone in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.1 times the area of the peak due to amiodarone in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) mix with the aid of ultrasound for 15 minutes a quantity of the powdered tablets containing 0.05 g of Amiodarone Hydrochloride with 100 ml of *methanol* , allow to cool and filter through a 0.45 µm PTFE filter. Dilute 1 volume of the solution to 5 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

For solution (2) dilute 1 volume of a 0.05% w/v solution of *amiodarone hydrochloride BPCRS* in *methanol* to 5 volumes with a mixture of equal volumes of *acetonitrile* and *water*.

The chromatographic procedure described under the test for Related substances may be used.

Calculate the content of $C_{25}H_{29}I_2NO_3$,HCl using the declared content of $C_{25}H_{29}I_2NO_3$,HCl in *amiodarone hydrochloride BPCRS.*

STORAGE

Amiodarone Tablets should be protected from light.

Amitriptyline Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Amitriptyline Tablets contain Amitriptyline Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amitriptyline hydrochloride, $C_{20}H_{23}N,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 5 mg of Amitriptyline Hydrochloride with 20 ml of *methanol* and filter. To 1 ml of the filtrate add 1 ml of a 2.5% w/v solution of *sodium hydrogen carbonate* , 1 ml of a 2% w/v solution of *sodium periodate* and 1 ml of a 0.3% w/v solution of *potassium permanganate* , allow to stand for 15 minutes, acidify with 1M *sulphuric acid* and extract with 10 ml of *2,2,4-trimethylpentane* . The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 265 nm.

B. Triturate a quantity of the powdered tablets containing 0.1 g of Amitriptyline Hydrochloride with 10 ml of *chloroform* , filter and evaporate the filtrate to a low volume. Add *ether* until a turbidity is produced and allow to stand. Dissolve 50 mg of the precipitate in 3 ml of *water* and add 0.05 ml of a 2.5% w/v solution of *quinhydrone* in *methanol* . No red colour is produced within 15 minutes (distinction from nortriptyline).

C. The precipitate obtained in test B yields reaction A characteristic of *chlorides* , Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, protected from light, using *silica gel G* as the coating substance and a mixture of 3 volumes of *diethylamine* , 15 volumes of *ethyl acetate* and 85 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application in an unlined tank. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 20 mg of Amitriptyline Hydrochloride with 5 ml of a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *ethanol (96%)*, centrifuge and use the supernatant liquid. Solution (2) contains 0.0010% w/v of *dibenzosuberone EPCRS* in *chloroform*. Solution (3) contains 0.0040% w/v of *cyclobenzaprine hydrochloride EPCRS*. After removal of the plate, allow it to dry in air, spray with a freshly prepared mixture of 4 volumes of *formaldehyde* and 96 volumes of *sulphuric acid*, heat at 100° to 105° for 10 minutes and examine under *ultraviolet light (365 nm)*. Any spot corresponding to dibenzosuberone in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%). Examine the plate under *ultraviolet light (254 nm)*. Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) dissolve 50 mg of *amitriptyline hydrochloride BPCRS* in 10 ml of *methanol* and dilute to 200 ml with *methanol (50%)*. For film-coated tablets, prepare solution (2) in the following manner. Add 50 ml of 0.1M *hydrochloric acid* to 20 tablets, shake vigorously until the tablets are completely disintegrated, add 100 ml of *methanol* and shake for 30 minutes. Dilute the suspension to 200 ml with *methanol*, centrifuge and dilute a volume of the supernatant liquid containing 25 mg of Amitriptyline Hydrochloride to 100 ml with *methanol (50%)*. For sugar-coated tablets prepare solution (2) in the following manner. Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Amitriptyline Hydrochloride with 50 ml of 0.1M *hydrochloric acid* for 30 minutes, add 100 ml of *methanol* and shake for 30 minutes. Dilute the suspension to 200 ml with *water*, centrifuge and use the supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute 0.03M *sodium hexanesulphonate* in a mixture of equal volumes of *water* and *acetonitrile*, adjusted to pH 4.5 by the addition of *glacial acetic acid*, and (c) a detection wavelength of 239 nm.

Calculate the content of $C_{20}H_{23}N,HCl$ using the declared content of $C_{20}H_{23}N,HCl$ in *amitriptyline hydrochloride BPCRS*.

Ammonium Chloride Mixture

General Notices

Ammonium Chloride Oral Solution

DEFINITION

Ammonium Chloride Mixture is an *oral solution* containing 10% w/v of Ammonium Chloride in a suitable vehicle containing Aromatic Ammonia Solution and Liquorice Liquid Extract.

Extemporaneous preparation

It is recently prepared according to the following formula.

Ammonium Chloride	100 g
Aromatic Ammonia Solution	50 ml
Liquorice Liquid Extract	100 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ammonium chloride, NH_4Cl

9.50 to 10.66% w/v.

ASSAY

To 1 ml add 20 ml of *water* and titrate with 0.1M *silver nitrate VS* determining the end point potentiometrically. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.349 mg of NH_4Cl.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Amoxicillin Capsules

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Amoxicillin Capsules contain Amoxicillin Trihydrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$

92.5 to 110.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing the equivalent of 0.5 g of amoxicillin with 5 ml of *water* for 5 minutes, filter, wash the residue first with *absolute ethanol* and then with *ether* and dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of amoxicillin trihydrate *(RS 011)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dissolve a quantity of the residue in sufficient *sodium hydrogen carbonate solution* to produce a solution containing the equivalent of 0.25% w/v of amoxicillin. Solution (2) contains 0.25% w/v of *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*. Solution (3) contains 0.25% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*. After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, injecting 50 µl of each of the following solutions. For solution (1) add 80 ml of mobile phase A to a quantity of the mixed capsule contents containing the equivalent of 0.15 g of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 ml, mix and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Solution (3) contains 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 254 nm.

Mobile phase A Mix 1 volume of *acetonitrile* and 99 volumes of a pH 5.0 buffer solution prepared in the following manner. To 250 ml of 0.2M *potassium dihydrogen orthophosphate* add 2M *sodium hydroxide* until the pH reaches 5.0 and add sufficient *water* to produce 1000 ml.

Mobile phase B Mix 20 volumes of *acetonitrile* and 80 volumes of the pH 5.0 buffer solution.

Equilibrate the column with a mobile phase ratio A:B of 92:8. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase.

Inject solutions (1) and (2) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase ratio A:B of 1:100 over a period of 25 minutes. Continue the chromatography with mobile phase B for 15 minutes then equilibrate the column for 15 minutes with the mobile phase chosen originally. Inject mobile phase A and use the same elution gradient to obtain a blank.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, injecting 50 µl of each of the following solutions. For solution (1) add 80 ml of mobile phase A to a quantity of the mixed contents of 20 capsules containing the equivalent of 60 mg of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 ml, mix and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.070% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A. Solution (3) contains 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 8 volumes of mobile phase B and 92 volumes of mobile phase A as described below and (c) a detection wavelength of 254 nm.

Mobile phase A Mix 1 volume of *acetonitrile* and 99 volumes of a 25% v/v solution of 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide*.

Mobile phase B Mix 20 volumes of *acetonitrile* and 80 volumes of a 25% v/v solution of 0.2M *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide*.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ in the capsules from the chromatograms obtained and from the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of amoxicillin.

Amoxicillin Injection

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Amoxicillin Injection is a sterile solution of Amoxicillin Sodium in Water for Injections. It is prepared by dissolving Amoxicillin Sodium for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Amoxicillin Injection should be used immediately after preparation.

AMOXICILLIN SODIUM FOR INJECTION

DEFINITION

Amoxicillin Sodium for Injection is a sterile material consisting of Amoxicillin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of amoxicillin sodium *(RS 010)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dissolve a quantity of the contents of a sealed container in sufficient *sodium hydrogen carbonate solution* to produce a solution containing the equivalent of 0.25% w/v of amoxicillin. Solution (2) contains 0.25% w/v of *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution*. Solution (3) contains 0.25% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate*

BPCRS in *sodium hydrogen carbonate solution* . After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

C. Yields reaction B characteristic of *sodium salts* , Appendix VI.

TESTS

Alkalinity

pH of a solution containing the equivalent of 10% w/v of amoxicillin, 8.0 to 10.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, injecting 50 µl of each of the following solutions. For solution (1) add 80 ml of mobile phase A to a quantity of the contents of a sealed container containing the equivalent of 0.15 g of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 ml, mix and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with mobile phase A. For solution (3) add 1 ml of *water* to 0.2 g of *amoxicillin trihydrate BPCRS* , shake and add, dropwise, *dilute sodium hydroxide solution* to obtain a solution. (The pH of the solution is about 8.5.) Store the solution at room temperature for 4 hours and dilute 0.5 ml to 50 ml with mobile phase A. Solution (4) contains 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 254 nm.

Mobile phase A Mix 1 volume of *acetonitrile* and 99 volumes of a pH 5.0 buffer solution prepared in the following manner. To 250 ml of 0.2M *potassium dihydrogen orthophosphate* add 2M *sodium hydroxide* until the pH reaches 5.0 and add sufficient *water* to produce 1000 ml.

Mobile phase B Mix 20 volumes of *acetonitrile* and 80 volumes of the pH 5.0 buffer solution.

Equilibrate the column with a mobile phase ratio A:B of 92:8. The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase.

Inject solutions (1) and (2) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase ratio A:B of 1:100 over a period of 25 minutes. Continue the chromatography with mobile phase B for 15 minutes then equilibrate the column for 15 minutes with the mobile phase chosen originally. Inject mobile phase A and use the same elution gradient to obtain a blank.

Inject solution (3). The three main peaks eluted after the principal peak correspond to amoxicillin diketopiperazine, amoxicillin dimer and amoxicillin trimer. The retention times of these peaks relative to that of the principal peak are about 3.4, 4.1 and 4.5 respectively.

In the chromatogram obtained with solution (1) the area of any peak corresponding to amoxicillin dimer is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%), the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%) and the sum of the areas of all the *secondary peaks* is not greater than 9 times the area of the principal peak in the chromatogram obtained with solution (2) (9%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Water

Not more than 4.0% w/w, Appendix IX C. Use 0.3 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg per ml of amoxicillin (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, injecting 50 µl of each of the following solutions. For solution (1) add 80 ml of mobile phase A to a quantity of the mixed contents of the 10 containers containing the equivalent of 60 mg of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 ml, mix and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.070% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A. Solution (3) contains 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

The chromatographic conditions described under Amoxicillin Capsules may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ in a container of average content weight from the chromatograms obtained and from the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS*.

LABELLING

The label of the sealed container states the quantity of Amoxicillin Sodium contained in it in terms of the equivalent amount of amoxicillin.

Amoxicillin Oral Suspension

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Amoxicillin Oral Suspension is a suspension of Amoxicillin Trihydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$

When freshly constituted not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dilute a quantity of the oral suspension in sufficient *sodium hydrogen carbonate solution* to produce a solution containing the equivalent of 0.25% w/v of amoxicillin. Solution (2) contains 0.25% w/v of *amoxicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution* . Solution (3) contains 0.25% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *sodium hydrogen carbonate solution* . After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

TESTS

Acidity or alkalinity

pH, 4.0 to 7.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, injecting 50 µl of each of the following solutions. For solution (1) dilute a weighed quantity of the oral suspension containing the equivalent of 60 mg of amoxicillin with sufficient mobile phase A to produce 100 ml, mix and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.070% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A. Solution (3) contains 0.0004% w/v of *cefadroxil BPCRS* and 0.003% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A.

The chromatographic conditions described under Amoxicillin Capsules may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{19}N_3O_5S$, weight in volume, using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS*.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be kept at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of amoxicillin.

Amphotericin Lozenges

General Notices

Action and use

Antifungal.

DEFINITION

Amphotericin Lozenges are *compressed lozenges* containing Amphotericin.

The lozenges comply with the requirements stated under Oromucosal Preparations and with the following requirements.

IDENTIFICATION

To a quantity of the powdered lozenges containing the equivalent of 12.5 mg of amphotericin B add 2.5 ml of *dimethyl sulphoxide* and shake for 5 minutes. Add 15 ml of *methanol* , shake for a further 10 minutes, add sufficient *methanol* to produce 25 ml and filter. Dilute 1 ml of the filtrate to 100 ml with *methanol* . The *light absorption* of the resulting solution, Appendix II B, in the range 300 to 450 nm exhibits three maxima, at 362, 381 and 405 nm. The ratio of the *absorbance* at the maximum at 362 nm to that at the maximum at 381 nm is 0.5 to 0.6. The ratio of the *absorbance* at 381 nm to that at the maximum at 405 nm is about 0.9.

TESTS

Content of tetraenes

Not more than 13.3% w/w of the stated amount of amphotericin B when determined by the following method. For solution (1) add to a quantity of the powdered lozenges containing the equivalent of 37.5 mg of amphotericin B 5 ml of *dimethyl sulphoxide* and 25 ml of *methanol* and shake for 10 minutes, add sufficient *methanol* to produce 50 ml, mix thoroughly and filter. Dilute 4 ml of the filtrate to 50 ml with *methanol* . Prepare solution (2) by dissolving 50 mg of *amphotericin B EPCRS* in 5 ml of *dimethyl sulphoxide* , adding sufficient *methanol* to produce 50 ml and diluting 4 ml to 50 ml with *methanol* . For solution (3) dissolve 25 mg of *nystatin EPCRS* in 25 ml of *dimethyl sulphoxide* , dilute to 250 ml with *methanol* and dilute 4 ml of the resulting solution to 50 ml with *methanol* . Measure the *absorbances* of solutions (1) and (2) at the maximum at 282 nm and that of solution (3) at the same wavelength, Appendix II B, using a 0.8% v/v solution of *dimethyl sulphoxide* in *methanol* in the reference cell. Then measure the *absorbance* of solution (3) at the maximum at 304 nm and those of solutions (1) and (2) at the same wavelength. Calculate the A(1%, 1 cm) of the preparation being examined, at both wavelengths, each with reference to the content obtained in the assay. Calculate the A(1%, 1 cm) of *nystatin EPCRS* and of *amphotericin B EPCRS* , at both wavelengths, each with reference to the dried substance. Calculate the percentage content of tetraenes (*x*) from the expression:

$x = F + 100 (B_1 S_2 - B_2 S_1) / (N_2 B_1 - N_1 B_2)$

where S_1 and S_2 are the specific absorbances of the preparation being examined at 282 nm and 304 nm respectively, N_1 and N_2 are the specific absorbances of *nystatin EPCRS* at 282 nm and 304 nm respectively, B_1 and B_2 are the specific absorbances of *amphotericin B EPCRS* at 282 nm and 304 nm respectively and F is the declared content of tetraenes in *amphotericin B EPCRS* .

Dissolution

The requirement for dissolution does not apply to Amphotericin Lozenges.

ASSAY

Weigh and powder 20 lozenges. Triturate a quantity of the powder containing the equivalent of 10 mg of amphotericin B with 10 ml of *water*, add sufficient *dimethyl sulphoxide* to produce 100 ml, shake for 20 minutes and filter. Carry out the *microbiological assay of antibiotics* , Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of amphotericin B in the lozenges taking each 1000 IU found to be equivalent to 1 mg of amphotericin B. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

STORAGE

Amphotericin Lozenges should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of amphotericin B.

Amphotericin Oral Suspension

General Notices

Action and use

Antifungal.

DEFINITION

Amphotericin Oral Suspension is a suspension of Amphotericin in a suitable flavoured vehicle.

Amphotericin Oral Suspension should not be diluted.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

IDENTIFICATION

To a quantity containing the equivalent of 35 mg of amphotericin B add 10 ml of *dimethyl sulphoxide* , mix, add sufficient *methanol* to produce 250 ml and mix. If the solution is not clear, centrifuge a portion until clear. Dilute 2 ml of the clear supernatant liquid to 100 ml with *methanol* . The *light absorption* of the resulting solution, Appendix II B, in the range 300 to 450 nm exhibits three maxima, at 362, 381 and 405 nm. The ratio of the *absorbance* at the maximum at 362 nm to that at the maximum at 381 nm is 0.5 to 0.6. The ratio of the *absorbance* at the maximum at 381 nm to that at 405 nm is about 0.9.

TESTS

Content of tetraenes

Complies with the test described under Amphotericin Lozenges but preparing solution (1) in the following manner. To a quantity containing the equivalent of 37.5 mg of amphotericin B add 5 ml of *dimethyl sulphoxide* and 25 ml of *methanol* and shake for 10 minutes, add sufficient *methanol* to produce 50 ml, mix thoroughly and filter. Dilute 4 ml of the filtrate to 50 ml with *methanol* .

ASSAY

To a weighed quantity containing the equivalent of 0.5 g of amphotericin B add 60 ml of *dimethyl sulphoxide* and shake for 20 minutes. Add sufficient *dimethyl sulphoxide* to produce 100 ml, mix, filter (Whatman No. 1 filter paper is suitable) and dilute 10 ml of the filtrate to 50 ml with *dimethyl sulphoxide* . Carry out the *microbiological assay of antibiotics* , Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of amphotericin B in the oral suspension taking each 1000 IU found to be equivalent to 1 mg of amphotericin B. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated content.

STORAGE

Amphotericin Oral Suspension should be protected from light and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of amphotericin B. The label states the appropriate directions for using the preparation.

Aprotinin Injection

General Notices

Action and use

Antifibrinolytic.

DEFINITION

Aprotinin Injection is a sterile solution of Aprotinin or a sterile dilution of Aprotinin Concentrated Solution in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of aprotinin

90.0 to 110.0% of the stated number of Ph Eur Units.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a 10% w/v solution of *sodium acetate* in a mixture of 50 volumes of *glacial acetic acid* and 40 volumes of *water* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use the undiluted injection. For solution (2) dilute *aprotinin solution EPBRP* with sufficient *water* to give a solution containing the same number of Ph Eur Units per ml as solution (1). After removal of the plate, allow it to dry in air and spray with a solution containing 0.1 g of *ninhydrin* in a mixture of 70 ml of *absolute ethanol*, 21 ml of *glacial acetic acid* and 6 ml of a 1% w/v solution of *copper (II) chloride*. Dry the plate at 60°. The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. Dilute a volume of the injection containing 15 Ph Eur Units to 50 ml with *phosphate buffer pH 7.2*. Mix 1 ml of the resulting solution with 1 ml of a solution containing 0.01% w/v of *trypsin EPCRS* in 0.002M *hydrochloric acid* and allow to stand for 10 minutes. Add 1 ml of a 0.2% w/v solution of *casein* in *phosphate buffer pH 7.2* and incubate at 35° for 30 minutes. Cool in ice and add 0.5 ml of a mixture of 50 volumes of *absolute ethanol*, 49 volumes of *water* and 1 volume of *glacial acetic acid*. Shake and allow to stand at room temperature for 15 minutes; the solution becomes cloudy. Repeat the operation using *phosphate buffer pH 7.2* in place of the diluted injection; the solution remains clear.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.0, Appendix V L.

Proteins of higher molecular weight

Carry out the method for *size-exclusion chromatography*, Appendix III C, equilibrating the column with 3M *acetic acid*. Freeze dry a volume of the injection containing 300 Ph Eur Units at -30° at a pressure of 2.7 Pa; the procedure, including a period of drying at 15° to 25°, should take 6 to 12 hours. Dissolve the residue thus obtained in 1 ml of 3M *acetic acid* and apply to the top of the column.

The chromatographic procedure may be carried out using (a) a column (80 to 100 cm × 25 mm) packed with a cross-linked dextran suitable for fractionation of proteins in the range of molecular weights from 1,500 to 30,000 (Sephadex G-50-SF is suitable), (b) 3M *acetic acid* as the mobile phase and (c) a detection wavelength of 277 nm.

Collect the eluate in 2 ml fractions. No peak is eluted in advance of the principal peak.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary with *water BET* to give a solution containing 5.56 Ph Eur units of aprotinin per ml (solution A). The endotoxin limit concentration of solution A is 0. 778 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the Assay described under Aprotinin.

STORAGE

Aprotinin Injection should be protected from light.

LABELLING

The label states the number of Ph Eur Units per ml.

Aqueous Cream

General Notices

DEFINITION

Emulsifying Ointment	300 g
Phenoxyethanol	10 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

The suitability of the Cream for use as a diluent should be confirmed before use.

Extemporaneous preparation

The following directions apply.

Dissolve the Phenoxyethanol in sufficient Purified Water at about 60° to produce a total weight of about 700 g. Melt the Emulsifying Ointment, add the phenoxyethanol solution when both are at about 60° and mix. Stir gently until cool, add sufficient of the Purified Water to produce 1000 g and mix.

The cream complies with the requirements stated under Topical Semi-solid Preparations.

Arachis Oil Enema

General Notices

DEFINITION

Arachis Oil Enema is Arachis Oil in a suitable container.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

IDENTIFICATION

Carry out the test for *identification of fixed oils by thin-layer chromatography* , Appendix X N. The chromatogram obtained from the oil being examined shows spots corresponding to those in the *typical chromatogram* for arachis oil.

TESTS

Acid value

Not more than 0.6, Appendix X B.

Alkaline impurities

Complies with the test for *alkaline impurities*, Appendix X N.

Peroxide value

Not more than 5.0, Appendix X F.

Relative density

0.912 to 0.918, Appendix V G.

Unsaponifiable matter

Not more than 1.0% w/w, Appendix X H, Method II. Use 5 g.

Foreign fixed oils

Carry out the test for *foreign oils by gas chromatography* , Appendix X N. The fatty-acid fraction of the oil has the following composition.

Saturated fatty acids of chain length less than C_{16} Not more than 0.4%.

Palmitic acid 7.0 to 16.0%.

Stearic acid 1.3 to 6.5%.

Oleic acid 35.0 to 72.0%.

Linoleic acid (equivalent chain length on polyethylene glycol adipate 18.9) 13.0 to 43.0%.

Linolenic acid (equivalent chain length on polyethylene glycol adipate 19.7) Not more than 0.6%.

Arachidic acid 1.0 to 3.0%.

Gadoleic acid (equivalent chain length on polyethylene glycol adipate 20.3) 0.5 to 2.1%.

Behenic acid 1.0 to 5.0%.

Erucic acid (equivalent chain length on polyethylene glycol adipate 22.3) Not more than 0.5%.

Lignoceric acid 0.5 to 3.0%.

The ratio of linoleic acid to behenic acid is not more than 15.

Semi-drying oils

To 1.0 g of the oil being examined add 5 ml of a mixture of 3 volumes of 2M *ethanolic potassium hydroxide* and 1 volume of *ethanol (96%)*, boil under a reflux condenser for 5 minutes, add 1.5 ml of 5M *acetic acid* and 50 ml of *ethanol (70%)* and heat until the solution is clear. Allow to cool or cool very slowly with a thermometer in the liquid. The temperature at which the liquid begins to become cloudy is not lower than 36°.

Sesame oil

Shake 10 ml with 5 ml of a mixture of 0.5 volume of a 0.35% v/v solution of *furfuraldehyde* in *acetic anhydride* and 4.5 volumes of *acetic anhydride* for 1 minute, filter the solution through a filter paper impregnated with *acetic anhydride* and add 0.2 ml of *sulphuric acid*. No bluish green colour develops.

Arginine Hydrochloride Intravenous Infusion

General Notices

Action and use

Amino acid; nutrient.

DEFINITION

Arginine Hydrochloride Intravenous Infusion is a sterile solution of Arginine Hydrochloride in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of arginine hydrochloride, $C_6H_{14}N_4O_2$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Ninhydrin-positive substances the spot in the chromatogram obtained with solution (2) is similar in position, size and intensity to the spot in the chromatogram obtained with solution (4).

B. Dilute a suitable volume of the intravenous infusion with sufficient *water* to produce a solution containing 1.25% w/v of Arginine Hydrochloride. To 2 ml of the resulting solution add 1 ml of *1-naphthol solution* and 2 ml of a mixture of equal volumes of *sodium hypochlorite solution (3% Cl)* and *water*. A red colour develops.

C. Dilute a suitable volume of the intravenous infusion with sufficient *water* to produce a solution containing 0.5% w/v of Arginine Hydrochloride. The resulting solution yields reaction A characteristic of *chlorides* .

TESTS

Particulate contamination

When supplied in a container with a nominal volume of more than 100 ml, complies with the test for *sub-visible particles* , Appendix XIII A.

Acidity

pH, 5.0 to 6.5, Appendix V L.

Ninhydrin-positive substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel* *plate* and a mixture of 30 volumes of *concentrated ammonia* and 70 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute the intravenous infusion with *water* to contain 1.0% w/v of Arginine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 50 volumes with *water.* For solution (3) dilute 1 volume of solution (2) to 4 volumes with *water*. Solution (4) contains 0.02% w/v of *arginine hydrochoride BPCRS* in *water*. Solution (5) contains 0.04% w/v each of *arginine hydrochoride BPCRS* and *lysine hydrochloride EPCRS* in *water*. After removal of the plate, heat it at 100° to 105° until the ammonia is removed. Spray with *ninhydrin solution* and heat at 100° to 105° for 15 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%). The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated spots.

Bacterial endotoxins

The endotoxin limit concentration is 0.5 IU per ml, Appendix XIV C. Dilute infusions containing more than 5.0% w/v of Arginine Hydrochloride with *water BET* to contain 5% w/v.

ASSAY

Dissolve 28 g of *potassium hydroxide* and 2 g of *potassium sodium (+)-tartrate* in 100 ml of *water*, add to the cooled solution 0.1 g of *2,4-dichloro-1-naphthol*, 180 ml of *ethanol (96%)* and 20 ml of a solution prepared by diluting 1 volume of *sodium hypochlorite solution (3% Cl)* to 6 volumes with *water* and allow the mixture to stand for 1 hour (solution A). Dilute a suitable volume of the intravenous infusion with sufficient *water* to produce a solution containing 0.004% w/v of Arginine Hydrochloride. To 5 ml add 5 ml of a 0.3% w/v solution of *potassium iodide* , mix and allow to stand for 15 minutes. Add 15 ml of solution A, mix and allow to stand for 15 minutes. Add 5 ml of a solution prepared by diluting 1 volume of *sodium hypochlorite solution (3% Cl)* to 15 volumes with *water* and allow to stand for 15 minutes. Measure the *absorbance* of the resulting solution at the maximum at 520 nm, Appendix II B, using *water* in the reference cell. Repeat the procedure using 5 ml of a 0.004% w/v solution of *arginine hydrochloride BPCRS* in *water* beginning at the words 'add 5 ml of a 0.3% w/v solution of *potassium iodide* …'. Calculate the content of $C_6H_{14}N_4O_2$,HCl from the absorbances obtained using the declared content of $C_6H_{14}N_4O_2$,HCl in *arginine hydrochloride BPCRS* .

LABELLING

The strength is stated as the percentage w/v of Arginine Hydrochloride. The label states that solutions containing visible solid particles must not be used.

IMPURITIES

The impurities limited by the requirements of the monograph include ornithine.

Aromatic Ammonia Solution

General Notices

Sal Volatile Solution

DEFINITION

Ammonium Bicarbonate	25 g
Nutmeg Oil	0.3 ml
Lemon Oil	0.5 ml
Ethanol (90 per cent)	37.5 ml
Strong Ammonia Solution	67.5 ml
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Ammonium Bicarbonate in 800 ml of the Purified Water. Separately dissolve the Lemon Oil and the Nutmeg Oil in the Ethanol (90 per cent). Add the ethanolic solution to the aqueous solution and add the Strong Ammonia Solution and sufficient Purified Water to produce 1000 ml. Add 25 g of previously sterilised Purified Talc, shake, allow to stand for a few hours, shaking occasionally, and filter.

Content of free ammonia, NH_3

1.12 to 1.25% w/v.

Content of ammonium carbonate, $(NH_4)_2CO_3$

2.76 to 3.24% w/v.

TESTS

Ethanol content

2.6 to 3.5% v/v when determined by the following method.

Carry out the method for *gas chromatography* , Appendix III B, using the following solutions. Solution (1) contains 1.5% v/v of *absolute ethanol* and 1.5% v/v of *propan-1-ol* (internal standard). For solution (2) dilute a volume of the preparation being examined with *water* to contain between 1.0 and 2.0% v/v of ethanol. Prepare solution (3) in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 1.5% v/v.

The chromatographic procedure may be carried out using a column (1.5 m × 4 mm) packed with porous polymer beads (100 to 120 mesh) (Porapak Q and Chromosorb 101 are suitable) and maintained at 150° with both the inlet port and the detector at 170°.

Calculate the percentage content of ethanol from the areas of the peaks due to ethanol in the chromatograms obtained with solutions (1) and (3).

Weight per ml

0.980 to 1.005 g, Appendix V G.

ASSAY

For free ammonia

To 20 ml add 50 ml of 1M *hydrochloric acid VS*, boil, cool and titrate the excess of acid with 1M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 1M *hydrochloric acid VS*, after subtraction of the volume of 1M *sodium hydroxide VS* required in the Assay for ammonium carbonate, is equivalent to 17.03 mg of NH_3.

For ammonium carbonate

To 20 ml add 25 ml of 1M *sodium hydroxide VS* and 40 ml of *barium chloride solution*, heat on a water bath for 15 minutes, cool, add 10 ml of *formaldehyde solution* previously neutralised to *thymol blue solution* and titrate the excess of alkali with 1M *hydrochloric acid VS*, using *thymol blue solution* as indicator, to the grey colour indicative of pH 8.8. Each ml of 1M *sodium hydroxide VS* is equivalent to 48.04 mg of $(NH_4)_2CO_3$.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

Aromatic Ammonia Spirit

General Notices

Sal Volatile Spirit

DEFINITION

Nutmeg Oil	3 ml
Lemon Oil	5 ml
Ethanol (90 per cent)	750 ml
Ammonium Bicarbonate	25 g
Strong Ammonia Solution	67.5 ml
Purified Water	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Distil a mixture of the Lemon Oil, the Nutmeg Oil, the Ethanol (90 per cent) and 375 ml of Purified Water. Reserve the first 875 ml of distillate. Distil a further 55 ml and add the Ammonium Bicarbonate and the Strong Ammonia Solution to the distillate. Heat on a water bath to 60° in a sealed bottle of not less than 120 ml capacity, shaking occasionally, until solution is complete, cool, filter through absorbent cotton, mix the filtrate with the reserved distillate, add sufficient Purified Water to produce 1000 ml and mix.

The spirit complies with the requirements stated under Spirits and with the following requirements.

Content of free ammonia, NH_3

1.12 to 1.30% w/v.

Content of ammonium carbonate, $(NH_4)_2CO_3$

2.76 to 3.24% w/v.

TESTS

Ethanol content

64 to 70% v/v, Appendix VIII F.

Weight per ml

0.880 to 0.893 g, Appendix V G.

ASSAY

For free ammonia

To 20 ml add 50 ml of 1M *hydrochloric acid VS*, boil, cool and titrate the excess of acid with 1M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 1M *hydrochloric acid VS*, after subtraction of the volume of 1M *sodium hydroxide VS* required in the Assay for ammonium carbonate, is equivalent to 17.03 mg of NH_3.

For ammonium carbonate

To 20 ml add 25 ml of 1M *sodium hydroxide VS* and 40 ml of *barium chloride solution*, heat on a water bath for 15 minutes, cool, add 10 ml of *formaldehyde solution* previously neutralised to *thymol blue solution* and titrate the excess of alkali with 1M *hydrochloric acid VS*, using *thymol blue solution* as indicator, to the grey colour indicative of pH 8.8. Each ml of 1M *sodium hydroxide VS* is equivalent to 48.04 mg of $(NH_4)_2CO_3$.

Ascorbic Acid Injection

General Notices

Action and use

Vitamin C.

DEFINITION

Ascorbic Acid Injection is a sterile solution of Ascorbic Acid in Water for Injections containing Sodium Bicarbonate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ascorbic acid, $C_6H_8O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless liquid.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 120 volumes of *ethanol (96%)* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute the injection, if necessary, with *water* to contain 0.5% w/v of Ascorbic Acid. Solution (2) contains 0.5% w/v of *ascorbic acid EPCRS* in *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. To a volume containing 50 mg of Ascorbic Acid add 0.2 ml of 2M *nitric acid* and 0.2 ml of 0.1M *silver nitrate* . A grey precipitate is produced.

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

Oxalic acid

Dilute a volume containing 0.25 g to 5 ml with *water*, neutralise to *litmus paper* with 2M *sodium hydroxide* , add 1 ml of 2M *acetic acid* and 0.5 ml of *calcium chloride solution* and allow to stand for 1 hour. Any opalescence produced is not more intense than that in a solution prepared at the same time and in the following manner. Dissolve 70 mg of *oxalic acid* in 500 ml of *water* and to 5 ml of the resulting solution add 1 ml of 2M *acetic acid* and 0.5 ml of *calcium chloride solution* (0.3%).

ASSAY

To a volume containing 0.2 g add 5 ml of 2M *sulphuric acid* and titrate with 0.05M *iodine VS* using *starch mucilage* as indicator. Each ml of 0.05M *iodine VS* is equivalent to 8.806 mg of $C_6H_8O_6$.

STORAGE

Ascorbic Acid Injection should be protected from light and stored at a temperature of 2° to 8°.

When vitamin C injection is prescribed or demanded, Ascorbic Acid Injection shall be dispensed or supplied.

Ascorbic Acid Tablets

General Notices

Action and use

Vitamin C.

DEFINITION

Ascorbic Acid Tablets contain Ascorbic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ascorbic acid, $C_6H_8O_6$

95.0 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 120 volumes of *ethanol (96%)* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Ascorbic Acid with 10 ml of *water* for 15 minutes and filter. Solution (2) contains 0.5% w/v of *ascorbic acid EPCRS* in *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets with *water* and filter. The filtrate is acidic to *litmus solution* , decolourises *2,6-dichlorophenolindophenol solution* and reduces *silver nitrate solution* immediately at room temperature producing a black precipitate.

TESTS

Disintegration

The requirement for Disintegration does not apply to Ascorbic Acid Tablets containing 500 mg or more of Ascorbic Acid.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.15 g of Ascorbic Acid as completely as possible in a mixture of 30 ml of *water* and 20 ml of 1M *sulphuric acid* and titrate with 0.1M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 8.806 mg of $C_6H_8O_6$.

STORAGE

Ascorbic Acid Tablets should be kept free from contact with metal and protected from light and moisture.

LABELLING

For tablets containing 500 mg or more of Ascorbic Acid the label states that the tablets should be chewed before swallowing.

When vitamin C tablets are prescribed or demanded, Ascorbic Acid Tablets shall be dispensed or supplied.

Aspirin and Caffeine Tablets

General Notices

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Aspirin and Caffeine Tablets contain, in each, 350 mg of Aspirin and 30 mg of Caffeine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$

330 to 370 mg.

Content of caffeine, $C_8H_{10}N_4O_2$

27.5 to 32.5 mg.

IDENTIFICATION

A. Boil 1 g of the powdered tablets with 10 ml of 1M *sodium hydroxide* , cool and filter. Acidify the filtrate with 1M *sulphuric acid* ; a white precipitate is produced. To a solution of the precipitate add *iron(III) chloride solution R1* ; a deep violet colour is produced.

B. Shake 0.5 g of the powdered tablets with 10 ml of *water* for 5 minutes, filter and add 10 ml of 1M *sodium hydroxide* . Extract with three 30 ml quantities of *dichloromethane* , washing each extract with the same 10 ml of *water*. Filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Reserve a quantity of the residue for test C. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid* , add 0.1 g of *potassium chlorate* and evaporate to dryness in a porcelain dish. A reddish residue remains, which becomes purple on exposure to ammonia vapour.

C. The *light absorption* , Appendix II B, in the range 240 to 350 nm of a 0.001% w/v solution of the residue reserved in test B exhibits a maximum at 273 nm.

TESTS

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 ml of *chloroform* and 10 ml of *water*, shake well and allow to separate. Filter the chloroform layer through a dry filter paper and evaporate 10 ml of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 ml of *ethanol (96%)* , stir well, dilute to 100 ml with *water* at a temperature not exceeding 10°, filter immediately, rapidly transfer 50 ml to a *Nessler* *cylinder*, add 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1* to a mixture of 3 ml of a freshly prepared 0.050% w/v solution of *salicylic acid*, 2 ml of *ethanol (96%)* and sufficient *water* to produce 50 ml contained in a second *Nessler cylinder* (3.0%).

Dissolution

For aspirin

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of a pH 4.5 buffer prepared by mixing 29.9 g of *sodium acetate* and 16.6 ml of *glacial acetic acid* with sufficient *water* to produce 10 litres and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at 265 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *aspirin BPCRS* in the dissolution medium and calculate the total content of aspirin, $C_9H_8O_4$, in the medium using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

ASSAY

Weigh and powder 20 tablets.

For aspirin

To a quantity of the powder containing 0.7 g of Aspirin add 20 ml of *water* and 2 g of *sodium citrate* and boil under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 ml of warm *water* and titrate with 0.5M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 45.04 mg of $C_9H_8O_4$.

For caffeine

To a quantity of the powder containing 30 mg of Caffeine add 200 ml of *water* and shake for 30 minutes. Add sufficient *water* to produce 250 ml and filter. To 10 ml of the filtrate add 10 ml of 1M *sodium hydroxide* and extract immediately with five 30 ml quantities of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the combined chloroform extracts, if necessary, through absorbent cotton previously moistened with *chloroform*. Evaporate the solution to dryness and dissolve the residue as completely as possible in *water*, warming gently if necessary. Cool, add sufficient *water* to produce 100 ml, mix and filter if necessary. Measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_8H_{10}N_4O_2$ taking 504 as the value of A(1%, 1 cm) at the maximum at 273 nm.

LABELLING

The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets. This requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed.

Aspirin Tablets

General Notices

Acetylsalicylic Acid Tablets

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Aspirin Tablets contain Aspirin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Boil 0.5 g of the powdered tablets for 2 to 3 minutes with 10 ml of 5M *sodium hydroxide* , cool and add an excess of 1M *sulphuric acid* ; a crystalline precipitate is produced. To a solution of the precipitate in *water* add *iron(III) chloride solution R1* ; a deep violet colour is produced.

TESTS

Salicylic acid

Shake a quantity of the powdered tablets containing 0.20 g of Aspirin with 4 ml of *ethanol (96%)* and dilute to 100 ml with *water* at a temperature not exceeding 10°. Filter immediately, transfer 50 ml of the filtrate to a *Nessler cylinder*, add 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1* , mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1* to a mixture of 3 ml of a freshly prepared 0.10% w/v solution of *salicylic acid* , 2 ml of *ethanol (96%)* and sufficient *water* to produce 50 ml contained in a second *Nessler cylinder* (3.0%).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using as the medium 500 ml of a pH 4.5 buffer prepared by mixing 29.9 g of *sodium acetate* and 16.6 ml of *glacial acetic acid* with sufficient *water* to produce 10 litres and rotating the basket at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at 265 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *aspirin BPCRS* in the dissolution medium and calculate the total content of aspirin, $C_9H_8O_4$, in the medium using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.5 g of Aspirin add 30 ml of 0.5M *sodium hydroxide VS*, boil gently for 10 minutes and titrate the excess of alkali with 0.5M *hydrochloric acid VS* using *phenol red solution* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of sodium hydroxide required. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 45.04 mg of $C_9H_8O_4$.

LABELLING

The label states that the tablets contain Aspirin, unless this word appears in the name of the tablets. This requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed.

Atenolol Injection

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Atenolol Injection is a sterile solution of Atenolol in Water for Injections containing Citric Acid Monohydrate and Sodium Chloride.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of atenolol, $C_{14}H_{22}N_2O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a volume of the injection containing 5 mg of Atenolol add sufficient 1M *sodium hydroxide* to make it alkaline (about 0.5 ml) and extract with three 10 ml quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform* . Filter the combined extracts through *anhydrous sodium sulphate* and evaporate to dryness in a current of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of atenolol *(RS 015)* .

TESTS

Acidity

pH, 5.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) use the injection being examined. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. For solution (3) dissolve 10 mg of *atenolol impurity standard BPCRS* in 0.1 ml of *dimethyl sulphoxide* , with the aid of gentle heat, and dilute to 20 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 20 volumes of *tetrahydrofuran* , 180 volumes of *methanol* and 800 volumes of 0.025M *potassium dihydrogen orthophosphate* containing 1.0 g of *sodium octanesulphonate* and 0.4 g of *tetrabutylammonium hydrogen sulphate* per litre and adjusted to pH 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 226 nm.

The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram provided with *atenolol impurity standard BPCRS* in that the peak due to bis ether precedes, and is separated from, that due to tertiary amine, which is normally a doublet. If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase; increasing the concentration increases the retention time of the tertiary amine.

In the chromatogram obtained with solution (1) the area of any peak corresponding to blocker acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to either tertiary amine or bis ether is not greater than half of the area of the peak in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Dilute a volume of the injection containing 10 mg of Atenolol to 100 ml with *methanol* and measure the *absorbance* at the maximum at about 275 nm, Appendix II B. Calculate the content of $C_{14}H_{22}N_2O_3$ taking 53.7 as the value of A(1%, 1 cm) at the maximum at 275 nm.

STORAGE

Atenolol Injection should be protected from light.

Atenolol Oral Solution

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Atenolol Oral Solution is a solution of Atenolol in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of atenolol, $C_{14}H_{22}N_2O_3$

94.0 to 106.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 1 volume of 18M *ammonia* and 99 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1), make a volume of the oral solution containing 50 mg of Atenolol alkaline with 1M *sodium hydroxide* (about 0.5 ml), extract with three 10 ml quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform* , filter the combined extracts through *anhydrous sodium sulphate* , evaporate to dryness under reduced pressure with gentle heat and dissolve the residue in 0.5 ml of *methanol* . Solution (2) contains 1.0% w/v of *atenolol BPCRS* in *methanol* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2). Disregard any spots due to excipients at Rf values of 0.69 and 0.80.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dilute the oral solution with the mobile phase to produce a solution containing 0.2% w/v of Atenolol. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. For solution (3) dissolve 10 mg of *atenolol impurity standard BPCRS* in 0.1 ml of *dimethyl sulphoxide*, with the aid of gentle heat, and dilute to 20 ml with the mobile phase.

The chromatographic procedure described under Atenolol Injection may be used.

The test is not valid unless the chromatogram obtained with solution (3) resembles that of the reference chromatogram provided with *atenolol impurity standard BPCRS* in that the peak due to bis ether precedes, and is separated from, that due to tertiary amine, which is normally a doublet. If necessary adjust the concentration of sodium octanesulphonate in the mobile phase; increasing the concentration increases the retention time of the tertiary amine.

In the chromatogram obtained with solution (1) the area of any peak corresponding to blocker acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to either tertiary amine or bis ether is not greater than half of the area of the peak in the chromatogram obtained with solution (2) (0.25%). Allow the chromatography to proceed for twice the retention time of the blocker acid.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the oral solution with the mobile phase to produce a solution containing 0.025% w/v of Atenolol. Solution (2) contains 0.025% w/v of *atenolol BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 1 volume of *sulphuric acid (10%)*, 25 volumes of *acetonitrile* and 74 volumes of *water* containing 0.93 g per litre of *sodium octyl sulphate* adjusted to pH 3 with 2M *sodium hydroxide* and (c) a detection wavelength of 275 nm. For solution (1) allow the chromatography to proceed for at least 30 minutes.

Calculate the content of $C_{14}H_{22}N_2O_3$ in the oral solution using the declared content of $C_{14}H_{22}N_2O_3$ in *atenolol BPCRS*.

Atenolol Tablets

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Atenolol Tablets contain Atenolol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of atenolol, $C_{14}H_{22}N_2O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat a quantity of the powdered tablets containing 0.1 g of Atenolol with 15 ml of *methanol* to 50°, shake for 5 minutes, filter (Whatman No. 42 paper is suitable) and evaporate the filtrate to dryness on a water bath. Warm the residue with 10 ml of 0.1M *hydrochloric acid* , shake and filter. Add to the filtrate sufficient 1M *sodium hydroxide* to make it alkaline, extract with 10 ml of *chloroform* , dry by shaking with *anhydrous sodium sulphate* , filter, evaporate the filtrate to dryness on a water bath and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of atenolol *(RS 015)* .

B. The *light absorption* , Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits maxima at 275 nm and 282 nm.

TESTS

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Atenolol with 25 ml of the mobile phase, mix with the aid of ultrasound for 20 minutes, filter (Whatman GF/C filter paper is suitable) and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. For solution (3) dissolve 10 mg of *atenolol impurity standard BPCRS* in 0.1 ml of *dimethyl sulphoxide* , with the aid of gentle heat, add 10 ml of the mobile phase and mix.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared as described below and (c) a detection wavelength of 226 nm. For the mobile phase dissolve 0.8 g of *sodium octanesulphonate* and 0.4 g of *tetrabutylammonium hydrogen sulphate* in 1 litre of a mixture of 20 volumes of *tetrahydrofuran*, 180 volumes of *methanol* and 800 volumes of a 0.34% w/v solution of *potassium dihydrogen orthophosphate*; adjust the pH to 3.0 with *orthophosphoric acid*. Inject 20 µl of each solution.

The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram provided with *atenolol impurity standard BPCRS* in that the peak due to bis ether precedes, and is separated from, that due to tertiary amine, which is normally a doublet. If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase; increasing the concentration increases the retention time of the tertiary amine.

In the chromatogram obtained with solution (1) the area of any peak corresponding to blocker acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to either tertiary amine or bis ether is not greater than half of the area of the peak in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Powder 20 tablets. Transfer the powder to a 500 ml flask using 300 ml of *methanol*, heat the resulting suspension to 60° and shake for 15 minutes. Cool, dilute to 500 ml with *methanol*, filter through a fine glass micro-fibre filter paper (Whatman GF/C is suitable) and dilute a suitable volume of the filtrate with sufficient *methanol* to produce a solution containing 0.01% w/v of Atenolol. Measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{14}H_{22}N_2O_3$ taking 53.7 as the value of A(1%, 1 cm) at the maximum at 275 nm.

Atropine Eye Drops

General Notices

Action and use

Anticholinergic.

DEFINITION

Atropine Eye Drops are a sterile solution of Atropine Sulphate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Comply with test A for Identification described under Atropine Injection, using for the preparation of solution (1) a volume of the eye drops containing 5 mg of Atropine Sulphate.

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to atropine sulphate in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

For eye drops containing less than 0.1% w/v of Atropine Sulphate For solution (1) use the eye drops being examined. Solution (2) contains 0.05% w/v of *atropine sulphate BPCRS* and 0.05% w/v of *homatropine hydrobromide BPCRS* in the mobile phase. Inject 100 µl.

For eye drops containing 0.1% w/v of Atropine Sulphate or more For solution (1) dilute the eye drops to contain 0.1% w/v of Atropine Sulphate with *water*. Solution (2) contains 0.1% w/v of *atropine sulphate BPCRS* and 0.1% w/v of *homatropine hydrobromide BPCRS* in the mobile phase. Inject 20 µl.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid* and (c) a detection wavelength of 257 nm.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to atropine sulphate and homatropine hydrobromide is at least 2.5.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the eye drops using the declared content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in *atropine sulphate BPCRS* .

Atropine Eye Ointment

General Notices

Action and use

Anticholinergic.

DEFINITION

Atropine Eye Ointment is a sterile preparation containing Atropine Sulphate in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Atropine Injection but preparing solution (1) in the following manner. Dissolve a quantity of the ointment containing 10 mg of Atropine Sulphate as completely as possible in 10 ml of *petroleum spirit (boiling range, 40° to 60°)* and extract with two 10 ml quantities of 0.05M *sulphuric acid*, washing each acid solution with the same 5 ml of *petroleum spirit (boiling range, 40° to 60°)*. Mix the acid solutions, make alkaline with 5M *ammonia* and extract with two 15 ml quantities of *chloroform*. Evaporate the chloroform and dissolve the residue in 2 ml of *ethanol (96%)*.

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to atropine sulphate in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the eye ointment containing 10 mg of Atropine Sulphate in 10 ml of *ether* and extract with two 10 ml quantities of 0.01M *hydrochloric acid*. Use the combined extracts. Solution (2) contains 0.05% w/v of *atropine sulphate BPCRS* and 0.05% w/v of *homatropine hydrobromide BPCRS* in the mobile phase. Inject 100 µl.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid* and (c) a detection wavelength of 257 nm.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to atropine sulphate and homatropine hydrobromide is at least 2.5.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the eye ointment using the declared content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in *atropine sulphate BPCRS* .

Atropine Injection

General Notices

Action and use

Anticholinergic.

DEFINITION

Atropine Injection is a sterile solution of Atropine Sulphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and a mixture of 50 volumes of *chloroform* , 40 volumes of *acetone* and 10 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) evaporate a volume of the injection containing 5 mg of Atropine Sulphate to dryness on a water bath, triturate the residue with 1 ml of *ethanol (96%)* , allow to stand and use the supernatant liquid. Solution (2) contains 0.5% w/v of *atropine sulphate BPCRS* in *ethanol (96%)* . After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution* . The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to atropine sulphate in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.8 to 4.5, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

For injections containing less than 0.1% w/v of Atropine Sulphate For solution (1) use the injection being examined. Solution (2) contains *atropine sulphate BPCRS* and *homatropine hydrobromide BPCRS* in the mobile phase, both at the same concentration as the solution being examined. Inject 100 µl.

For injections containing 0.1% w/v or more of Atropine Sulphate For solution (1) dilute the injection, if necessary, to contain 0.1% w/v of Atropine Sulphate with *water*. Solution (2) contains 0.1% w/v of *atropine sulphate BPCRS* and 0.1% w/v of *homatropine hydrobromide BPCRS* in the mobile phase. Inject 20 µl.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid* and (c) a detection wavelength of 257 nm.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to atropine sulphate and homatropine hydrobromide is at least 2.5.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the injection using the declared content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in *atropine sulphate BPCRS* .

Atropine Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Atropine Tablets contain Atropine Sulphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Comply with test A for Identification described under Atropine Injection using as solution (1) the supernatant liquid obtained by shaking a quantity of the powdered tablets containing 10 mg of Atropine Sulphate with 2 ml of *ethanol (96%)* and centrifuging.

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the peak due to atropine sulphate in the chromatogram obtained with solution (2).

TESTS

Uniformity of content

Tablets containing 600 µg of Atropine Sulphate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) shake 1 tablet with 2 ml of the mobile phase with the aid of ultrasound until fully disintegrated and filter. Solution (2) contains 0.03% w/v of *atropine sulphate BPCRS* and 0.03% w/v of *homatropine hydrobromide BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid* and (c) a detection wavelength of 257 nm.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution*

factor between the peaks due to atropine sulphate and homatropine hydrobromide is at least 2.5.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in each tablet using the declared content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in *atropine sulphate BPCRS* .

ASSAY

Use the average of the 10 individual results determined in the test for Uniformity of content.

Azapropazone Capsules

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Azapropazone Capsules contain Azapropazone.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of azapropazone, $C_{16}H_{20}N_4O_2,2H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with the *reference spectrum* of azapropazone *(RS 016)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the following operations in subdued light using low-actinic glassware without delay. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the capsules as completely as possible in sufficient of a mixture of 1 volume of *phosphate buffer pH 4.0* and 3 volumes of *methanol* to produce a solution containing 0.10% w/v of Azapropazone and filter. Solutions (2), (3) and (4) are solutions in a mixture of 1 volume of *phosphate buffer pH 4.0* and 3 volumes of *methanol* containing (2) 0.00010% w/v of *azapropazone impurity A BPCRS*, (3) 0.00025% w/v of *azapropazone impurity B BPCRS* and (4) 0.00025% w/v of *azapropazone impurity C BPCRS*. For solution (5) dilute 1 volume of solution (1) to 100 volumes with a mixture of 1 volume of *phosphate buffer pH 4.0* and 3 volumes of *methanol* and further dilute 1 volume of this solution to 10 volumes with the same solvent mixture. For solution (6) dilute 1 volume of solution (5) to 2 volumes with a mixture of 1 volume of *phosphate buffer pH 4.0* and 3 volumes of *methanol*. Solution (7) contains 0.1% w/v of *azapropazone impurity standard BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm)

(µBondapak C18 is suitable), (b) a mixture of 1 volume of *glacial acetic acid*, 36 volumes of *methanol* and 63 volumes of a 0.068% w/v solution of *sodium butanesulphonate* in *water* as the mobile phase with a flow rate of 2.5 ml per minute and (c) a detection wavelength of 254 nm.

Inject solution (7) and continue the chromatography for 5 times the retention time of the principal peak. The test is not valid unless the chromatogram obtained with solution (7) closely resembles the reference chromatogram supplied with the azapropazone impurity standard. If necessary adjust the proportion of methanol in the mobile phase to give the required retention times.

In the chromatogram obtained with solution (1) the area of any peaks corresponding to azapropazone impurities A, B or C are not greater than the areas of the corresponding peaks in the chromatograms obtained with solutions (2), (3) and (4), respectively (0.1%, 0.25% and 0.25%). The area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (5) (0.1%). Calculate the content of impurities A, B and C using the respective reference solutions and the content of any unnamed impurities using solution (5). The total nominal content of impurities is not greater than 0.5%. Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (6) (0.05%).

ASSAY

Carry out the following operations in subdued light using low-actinic glassware without delay. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 20 mg of Azapropazone with 40 ml of a mixture of 1 volume of *phosphate buffer pH 4.0* and 3 volumes of *methanol* (solvent A) and add sufficient solvent A to produce 100 ml. Solution (2) contains 0.02% w/v of *azapropazone BPCRS* in solvent A.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 1 volume of *glacial acetic acid*, 36 volumes of *methanol* and 63 volumes of a 0.068% w/v solution of *sodium butanesulphonate* in *water* as the mobile phase at a flow rate of 2.5 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{16}H_{20}N_4O_2,2H_2O$ using the declared content of $C_{16}H_{20}N_4O_2,2H_2O$ in *azapropazone BPCRS*.

STORAGE

Azapropazone Capsules should be protected from light.

Azapropazone Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Azapropazone Tablets contain Azapropazone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of azapropazone, $C_{16}H_{20}N_4O_2,2H_2O$

95.0 to 105.0% of the stated content.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Azapropazone with 10 ml of *methanol,* filter (Whatman GF/C paper is suitable), evaporate the filtrate and dry the residue at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of anhydrous azapropazone *(RS 017).*

B. The *light absorption* , Appendix II B, in the range 210 to 350 nm of solution (1) obtained in the Assay is concordant with that of solution (2).

TEST

Related substances

Carry out the following operations in subdued light using low-actinic glassware without delay. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions in a mixture of 1 volume of *phosphate buffer pH 4.0* and 3 volumes of *methanol* (solvent A). For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Azapropazone with 70 ml of solvent A, dilute to 100 ml and filter. Solution (2) contains 0.00025% w/v of *azapropazone impurity A BPCRS.* Solution (3) contains 0.00075% w/v of *azapropazone impurity C BPCRS.* For solution (4) dilute 10 ml of solution (1) to 100 ml and dilute 1 ml of the resulting solution to 100 ml. Solution (5) contains 0.1% w/v of *azapropazone impurity standard BPCRS* . For solution (6) dilute 1 volume of solution (4) to 2 volumes.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 1 volume of *glacial acetic acid* , 36 volumes of *methanol* and 63 volumes of a 0.068% w/v solution of *sodium butanesulphonate* in *water* as the mobile phase with a flow rate of 2.5 ml per minute and (c) a detection wavelength of 254 nm.

Inject solution (5) and continue the chromatography for 5 times the retention time of the principal peak. The test is not valid unless the chromatogram obtained with solution (5) closely resembles the reference chromatogram. If necessary adjust the proportion of methanol in the mobile phase to give the required retention times.

In the chromatogram obtained with solution (1) the area of any peaks corresponding to azapropazone impurities A and C are not greater than the areas of the corresponding peaks in the chromatograms obtained with solutions (2) and (3) (0.25% and 0.75% respectively). The area of any other *secondary peak* other than any peak corresponding to azapropazone impurity B is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.1%). Calculate the content of impurities A and C using the respective reference solutions and the content of any unnamed impurities using solution (4). The total content of impurities is not greater than 1%. Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (6) (0.05%).

ASSAY

Carry out the following operations in subdued light using low-actinic glassware without delay. Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 0.6 g of Azapropazone add 20 ml of *water,* shake for 30 minutes, add 60 ml of *methanol,* shake for 10 minutes and dilute to 100 ml with *water*. Centrifuge a portion of the solution at 3000 revolutions per minute for 10 minutes and filter the supernatant liquid through a 0.45-µm membrane filter. Dilute 5 ml of the filtrate to 500 ml with *water* and further dilute 15 ml of the resulting solution to 200 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 253 nm, Appendix II B. Calculate the content of $C_{16}H_{20}N_4O_2,2H_2O$, taking 1033 as the value of A(1%, 1 cm) at the maximum at 253 nm.

STORAGE

Azapropazone Tablets should be protected from light.

Azathioprine Tablets

General Notices

Action and use

Immunosuppressant.

DEFINITION

Azathioprine Tablets contain Azathioprine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of azathioprine, $C_9H_7N_7O_2S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method described under the test for 5-Chloro-1-methyl-4-nitroimidazole and 6-mercaptopurine applying separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Azathioprine with 50 ml of 6M *ammonia* , filter through a glass micro fibre paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.4% w/v of *azathioprine EPCRS* in 6M *ammonia* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Heat a quantity of the powdered tablets containing 20 mg of Azathioprine with 100 ml of *water* and filter. To 5 ml of the filtrate add 1 ml of *hydrochloric acid* and 10 mg of *zinc powder* and allow to stand for 5 minutes; a yellow colour is produced. Filter, cool in ice, add 0.1 ml of a 10% w/v solution of *sodium nitrite* and 0.1 g of *sulphamic acid* and shake until the bubbles disappear. Add 1 ml of *2-naphthol solution* ; a pale pink precipitate is produced.

TEST

5-Chloro-1-methyl-4-nitroimidazole and 6-mercaptopurine

Carry out the method for *thin-layer chromatography* , Appendix III A, using *cellulose* F_{254} as the coating substance and *butan-1-ol* saturated with 6M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions prepared immediately before use. For solution (1) shake a quantity of the powdered tablets containing 0.20 g of Azathioprine with 10 ml of 6M *ammonia* and filter through a glass micro fibre filter paper (Whatman GF/C is suitable). Solution (2) contains 2.0% w/v of *azathioprine EPCRS* and 0.020% w/v of *6-mercapto-purine* in 6M *ammonia* . Solution (3) contains 0.020% w/v of *6-mercaptopurine* in 6M *ammonia* . Solution (4) contains 0.020% w/v of *chloromethylnitroimidazole EPCRS* in 6M *ammonia*. After removal of the plate, dry it at 50° and examine under *ultraviolet light (254 nm)*. Any spot in the chromatogram obtained with solution (1) corresponding to 6-mercaptopurine in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (3). Any spot corresponding to 5-chloro-1-methyl-4-nitroimidazole in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Azathioprine with 20 ml of *dimethyl sulphoxide* for 15 minutes and dilute to 500 ml with 0.1M *hydrochloric acid*. Avoid heating or the use of ultrasound. Filter, dilute 25 ml of the filtrate to 1000 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of $C_9H_7N_7O_2S$ taking 628 as the value of A(1%, 1 cm) at the maximum at 280 nm.

STORAGE

Azathioprine Tablets should be protected from light.

Compound Alginate Antacid Oral Suspension

General Notices

DEFINITION

Compound Alginate Antacid Oral Suspension is a suspension containing an alginate in a suitable flavoured vehicle. It may be coloured. The suspension has an acid neutralising capacity.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of aluminium, Al; calcium, Ca; magnesium, Mg; potassium, K; sodium, Na, as appropriate

84.0 to 116.0% of the requisite amount, this being calculated from the stated amounts of the relevant constituents.

IDENTIFICATION

Evaporate to dryness on a water bath a quantity of the oral suspension containing the equivalent of 1 g of the alginate component. The residue complies with the following tests.

A. Shake 0.2 g in 20 ml of *water*. To 5 ml of this solution add 1 ml of *calcium chloride solution* . A voluminous gelatinous mass is formed.

B. To 10 ml of the solution prepared in Identification test A add 1 ml of *dilute sulphuric acid* . A gelatinous mass is formed.

C. It complies with the appropriate assays (see Assay).

TESTS

Alkalinity

pH, 7.5 to 9.5, Appendix V L.

Relative density

1.04 to 1.14 g/ml, Appendix V G.

Raft-forming ability

Introduce 150 ml of 0.1M *hydrochloric acid* into a 250 ml beaker having an internal diameter of 60 to 70 mm. Place in a water bath such that the volume of water in the bath is level with the top of the acid in the beaker and allow to equilibrate to 36.5° to 37.5°. Using a syringe (without needle), remove a quantity of suspension, previously shaken, equivalent to one dose. Where a dosage range is specified, use the maximum dose. Wipe the outside of the syringe and add the suspension evenly into the centre of the beaker (the time taken to add the entire dose is approximately 5 seconds). After 30 minutes, remove the beaker from the water bath, dry the outside of the beaker and examine the contents. A mass is formed.

Neutralising capacity

Not less than 30 ml of 0.1M *hydrochloric acid VS* is required to neutralise 5 ml of the oral suspension when determined by the following method.

To 5 ml of the oral suspension, accurately weighed in a 500 ml conical flask, add 250 ml of 0.1M *hydrochloric acid VS* and mix well. Place the flask in a water bath maintained at 36.5° to 37.5° for 1 hour, swirling frequently. Allow to cool and filter. Collect the filtrate in a clean dry flask and titrate to pH 4.0 using 0.5M *sodium hydroxide VS*. Calculate the neutralising capacity using the following expression:

$$\left[\left(250 \times \frac{C_1}{0.100}\right) - \left(T \times \frac{C_2}{0.500} \times 5\right)\right] \times \frac{W_m \times 5}{W}$$

where

C_1 = the concentration of 0.1M *hydrochloric acid VS* in moles per litre,

T = the volume of 0.5M *sodium hydroxide VS* in ml,

C_2 = the concentration of 0.5M *sodium hydroxide VS* in moles per litre,

W_m = the weight per ml of the suspension in g/ml,

W = the weight of the suspension in g.

ASSAY

For aluminium

Dilute a quantity of the suspension containing the equivalent of 125 mg of Al, based on the labelled amount, in *water* to produce 500 ml and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 396 nm. Use *aluminium standard solution* (*200 ppm Al*), suitably diluted with the strontium chloride solution, for the standard solutions.

For calcium

Dilute a quantity of the suspension containing the equivalent of 120 mg of Ca, based on the labelled amount, in *water* to produce 500 ml and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 393 nm. Use *calcium standard solution* (*400 ppm Ca*), suitably diluted with the strontium chloride solution, for the standard solutions.

For magnesium

Dilute a quantity of the suspension containing the equivalent of 125 mg of Mg, based on the labelled amount, in *water* to produce 500 ml and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 285 nm. Use *magnesium standard solution* (*100 ppm Mg*), suitably diluted with the strontium chloride solution, for the standard solutions.

For potassium

Dilute a quantity of the suspension containing the equivalent of 120 mg of K, based on the labelled amount, in *water* to produce 250 ml and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm. Use *potassium standard solution* (*600 ppm K*), suitably diluted with the strontium chloride solution, for the standard solutions.

For sodium

Dilute a quantity of the suspension containing the equivalent of 125 mg of Na, based on the labelled amount, in *water* to produce 500 ml and filter. Dilute a suitable volume of the filtrate with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm. Use *sodium standard solution* (*200 ppm Na*), suitably diluted with the strontium chloride solution, for the standard solutions.

LABELLING

The label states (1) the sodium content of the suspension and (2) the potassium content of the suspension, as applicable.

Compound Aluminium Paste

General Notices

Baltimore Paste

DEFINITION

Compound Aluminium Paste contains 20% w/w of Aluminium Powder and 40% w/w of Zinc Oxide in a suitable hydrophobic liquid basis.

Extemporaneous preparation

The following formula and directions apply.

Aluminium Powder	200 g
Zinc Oxide	400 g
Liquid Paraffin	400 g

Mix the Aluminium Powder and the Zinc Oxide with the Liquid Paraffin until smooth.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of aluminium, Al

15.8 to 20.0% w/w.

Content of zinc oxide, ZnO

37.0 to 42.0% w/w.

IDENTIFICATION

A. To 5 ml of solution A prepared in the Assay for aluminium add 2 ml of *ammonia buffer pH 10.9*, centrifuge and reserve the supernatant liquid. Dissolve the residue in the minimum quantity of 2M *hydrochloric acid* and add 0.25 ml of *ammonium acetate solution* and 0.25 ml of a 0.1% w/v solution of *mordant blue 3*. An intense purple colour is produced.

B. Add 2 ml of *sodium sulphide solution* to the supernatant liquid reserved in test A, centrifuge, dissolve the sediment in the minimum volume of 1M *sulphuric acid* and add 0.05 ml of a 0.1% w/v solution of *copper(II) sulphate* and 2 ml of *ammonium mercurithiocyanate reagent*. A violet precipitate is produced.

ASSAY

For aluminium

Disperse 1 g in a mixture of 20 ml of *hydrochloric acid* and 20 ml of *water* with the aid of gentle heat, filter, wash the filter with *water*, cool the combined filtrate and washings and dilute to 100 ml with *water* (solution A). Neutralise 10 ml of solution A to *congo red paper* with 5M *sodium hydroxide*, add 50 ml of 0.05M *disodium edetate VS*, heat on a water bath for 30 minutes, cool, add 3 g of *hexamine* and titrate the excess of disodium edetate with 0.05M *lead nitrate VS*, using *xylenol orange solution* as indicator, to a purplish red colour. Add 1 g of *sodium fluoride* to the resulting solution, heat on a water bath for 15 minutes, cool and continue the titration to a purplish red colour. The volume of 0.05M *lead nitrate VS* used in the second titration represents the amount of disodium edetate liberated from the complex. Each ml of 0.05M *disodium edetate VS* is equivalent to 1.349 mg of Al.

For zinc oxide

The difference between the volume of 0.05M *disodium edetate VS* added in the Assay for aluminium and the total volume of 0.05M *lead nitrate VS* used represents the amount of zinc present. Each ml of 0.05M *disodium edetate VS* is equivalent to 4.068 mg of ZnO.

Concentrated Anise Water

General Notices

DEFINITION

Anise Oil or Star Anise Oil	20 ml
Ethanol (90 per cent)	700 ml
Water	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Anise Oil or Star Anise Oil in the Ethanol (90 per cent) and add gradually, with vigorous shaking after each addition, sufficient Water to produce 1000 ml. Add 50 g of previously sterilised Purified Talc, or other suitable filtering aid, allow to stand for a few hours, shaking occasionally, and filter.

The water complies with the requirements stated under Aromatic Waters and with the following requirements.

TESTS

Ethanol content

60 to 64% v/v, Appendix VIII F.

Weight per ml

0.898 to 0.908 g, Appendix V G.

Dilute Adrenaline Injection 1 in 10,000 / Dilute Epinephrine Injection 1 in 10,000

General Notices

Action and use

Adrenoceptor agonist.

DEFINITION

Dilute Adrenaline Injection 1 in 10,000 is a sterile, isotonic solution containing either 0.018% w/v of Adrenaline Acid Tartrate or 0.01% w/v of adrenaline hydrochloride, prepared by the interaction of Adrenaline and Hydrochloric Acid, in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of adrenaline, $C_9H_{13}NO_3$

0.009 to 0.011% w/v.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. To 10 ml add 2 ml of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a brown colour and remove excess iodine by adding 0.1M *sodium thiosulphate* drop wise. A red or pink colour is produced.

TESTS

Acidity

pH, 2.2 to 5.0, Appendix V L.

Noradrenaline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00018% w/v of *noradrenaline acid tartrate* in the mobile phase. For solution (2) use the injection. Solution (3) contains 0.00018% w/v of *adrenaline* *acid tartrate BPCRS* and 0.00018% w/v of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* in a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusting the pH to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

In the chromatogram obtained with solution (2) the area of any peak corresponding to noradrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (1%, calculated with respect to the content of adrenaline).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase. Solution (2) is the injection. Solution (3) contains 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* to a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusting the pH of the mixture to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the content of $C_9H_{13}NO_3$ in the injection using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

STORAGE

Dilute Adrenaline Injection 1 in 10,000 should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of adrenaline (epinephrine).

Dilute Adrenaline Injection contains the equivalent of adrenaline (epinephrine), 1 in 10,000 (100 µg in 1 ml).

Dilute Ammonia Solution

General Notices

DEFINITION

Dilute Ammonia Solution contains 10% w/w of ammonia. It is prepared by diluting Strong Ammonia Solution with freshly boiled and cooled Purified Water.

Content of ammonia, NH_3

9.5 to 10.5% w/w.

TESTS

Relative density

0.958 to 0.962, Appendix V G.

ASSAY

Weigh 6 g into 50 ml of 1M *hydrochloric acid VS* and titrate the excess of acid with 1M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 1M *hydrochloric acid VS* is equivalent to 17.03 mg of NH_3.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

When ammonia solution is prescribed or demanded, Dilute Ammonia Solution shall be dispensed or supplied.

Dispersible Aciclovir Tablets

General Notices

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Dispersible Aciclovir Tablets contain Aciclovir in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aciclovir, $C_8H_{11}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution prepared in the Assay exhibits a maximum at 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with solution (2) is similar in position and intensity to that in the chromatogram obtained with solution (3).

TESTS

Guanine

Comply with the test described under Aciclovir Intravenous Infusion but preparing solution (1) in the following manner. Shake a quantity of the powdered tablets containing 0.25 g of Aciclovir with 25 ml of 0.1M *sodium hydroxide* for 10 minutes. Add a sufficient quantity of 0.1M *sodium hydroxide* to produce 50 ml, allow to stand and allow any undissolved material to settle before application to the plate.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 2 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 2 µl of each of the following freshly prepared solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Aciclovir with 10 ml of *dimethyl sulphoxide* for 15 minutes and filter. For solution (2) dilute 0.7 volumes of solution (1) to 100 volumes with *dimethyl sulphoxide*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any *secondary spot* with an Rf value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) (0.7%).

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing 0.1 g of Aciclovir add 60 ml of 0.1M *sodium hydroxide* and disperse with the aid of ultrasound for 15 minutes. Add sufficient 0.1M *sodium hydroxide* to produce 100 ml, mix well and filter. To 15 ml of the filtrate add 50 ml of *water*, 5.8 ml of 2M *hydrochloric acid* and sufficient *water* to produce 100 ml. To 5 ml of the solution add sufficient 0.1M *hydrochloric acid* to produce 50 ml and mix well. Measure the *absorbance* of the resulting solution at the maximum at 255 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the value of A (1%, 1 cm) at the maximum at 255 nm.

LABELLING

The label states that the tablets should be dispersed in water immediately before use.

Dispersible Aspirin Tablets

General Notices

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Dispersible Aspirin Tablets contain Aspirin in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Disperse a quantity of the powdered tablets containing 50 mg of Aspirin in 10 ml of warm *water*, boil and add 0.5 ml of *iron(III) chloride solution R1*. A violet-red colour is produced.

TESTS

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 ml of *chloroform*, 2 ml of 2.5M *sulphuric acid* and shake vigorously for 2 minutes. Filter the chloroform extract through a dry filter paper containing 1 g of *anhydrous sodium sulphate* and evaporate 5 ml of the filtrate to dryness at room temperature using a rotary evaporator. Dissolve the residue in 2 ml of *ethanol (96%)*, transfer to a *Nessler cylinder* with a further 1 ml of *ethanol (96%)*, dilute to 50 ml with *water* at a temperature not exceeding 10°, add 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1* to a mixture of 3 ml of a freshly prepared 0.050% w/v solution of *salicylic acid*, 3 ml of *ethanol (96%)* and sufficient *water* to produce 50 ml contained in a second *Nessler cylinder* (3.0%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of Aspirin in 10 ml of 1M *sulphuric acid* and boil under a reflux condenser for 1 hour. Cool, transfer to a separating funnel, rinsing the flask and condenser with small quantities of *water* and extract the liberated salicylic acid with four 20 ml quantities of *ether*. Wash the combined ether extracts with two 5 ml quantities of *water*, evaporate the ether in a current of air at a temperature not exceeding 30°, dissolve the residue in 20 ml of 0.5M *sodium hydroxide VS* and dilute to 200 ml with *water*. Transfer 50 ml to a stoppered flask, add 50 ml of 0.05M *bromine VS* and 5 ml of *hydrochloric acid*, protect the solution from light, shake repeatedly for 15 minutes and allow to stand for 15 minutes. Add 20 ml of *dilute potassium iodide solution*, shake thoroughly and titrate with 0.1M *sodium thiosulphate VS*, using *starch mucilage*, added towards the end point, as indicator. Each ml of 0.05M *bromine VS* is equivalent to 3.003 mg of $C_9H_8O_4$.

LABELLING

The label states (1) that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed); (2) that the tablets should be dispersed in water immediately before use.

When Dispersible Aspirin Tablets are prescribed or demanded, no strength being stated, tablets containing 300 mg shall be dispensed or supplied.

When soluble aspirin tablets are prescribed, Dispersible Aspirin Tablets shall be dispensed.

Effervescent Soluble Aspirin Tablets

General Notices

Effervescent Aspirin Tablets

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Effervescent Soluble Aspirin Tablets contain Aspirin in a suitable soluble, effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve with vigorous effervescence on the addition of warm *water* to produce a clear solution.

B. Dissolve a quantity of the powdered tablets containing 50 mg of Aspirin in 10 ml of warm *water*, boil and add 0.5 ml of *iron(III) chloride solution R1*. A violet-red colour is produced.

TESTS

Disintegration

Comply with the requirement for Effervescent Tablets stated under Tablets.

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 ml of *chloroform*, 2 ml of 2.5M *sulphuric acid* and shake vigorously for 2 minutes. Filter the chloroform extract through a dry filter paper containing 1 g of *anhydrous sodium sulphate* and evaporate 5 ml of the filtrate to dryness at room temperature using a rotary evaporator. Dissolve the residue in 2 ml of *ethanol (96%)*, transfer to a *Nessler cylinder* with a further 1 ml of *ethanol (96%)*, dilute to 50 ml with *water* at a temperature not exceeding 10°, add 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1* to a mixture of 3 ml of a freshly prepared 0.050% w/v solution of *salicylic acid*, 3 ml of *ethanol (96%)* and sufficient *water* to produce 50 ml contained in a second *Nessler cylinder* (3.0%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of Aspirin in 10 ml of 1M *sulphuric acid* and boil under a reflux condenser for 1 hour. Cool, transfer to a separating funnel, rinsing the flask and condenser with small quantities of *water* and extract the liberated salicylic acid with four 20 ml quantities of *ether*. Wash the combined ether extracts with two 5 ml quantities of *water*, evaporate the ether in a current of air at a temperature not exceeding 30°, dissolve the residue in 20 ml of 0.5M *sodium hydroxide VS* and dilute to 200 ml with *water*. Transfer 50 ml to a stoppered flask, add 50 ml of 0.05M *bromine VS* and 5 ml of *hydrochloric acid*, protect the solution from light, shake repeatedly during 15 minutes and allow to stand for 15 minutes. Add 20 ml of *dilute potassium iodide solution* shake thoroughly and titrate with 0.1M *sodium thiosulphate VS*, using *starch mucilage*, added towards the end point, as indicator. Each ml of 0.05M *bromine VS* is equivalent to 3.003 mg of $C_9H_8O_4$.

LABELLING

The label states (1) that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed); (2) that the tablets should be dissolved in water immediately before use.

When Effervescent Soluble Aspirin Tablets are prescribed or demanded, no strength being stated, tablets containing 300 mg shall be dispensed or supplied.

When soluble aspirin tablets are prescribed, Dispersible Aspirin Tablets shall be dispensed.

Gastro-resistant Aspirin Tablets

General Notices

Enteric-coated Aspirin Tablets

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Gastro-resistant Aspirin Tablets contain Aspirin. They are covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Boil a quantity of the powdered tablets containing 0.3 g of Aspirin for 2 to 3 minutes with 10 ml of 5M *sodium hydroxide* , cool and add an excess of 1M *sulphuric acid* ; a crystalline precipitate is produced. To a solution of the precipitate in *water* add *iron(III) chloride solution R1 ;* a deep violet colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using as the medium 1000 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. After 2 hours, withdraw a sample of the medium, filter and measure the *absorbance* of the filtrate, Appendix II B, at 276 nm using 0.1M *hydrochloric acid* in the reference cell. Measure the *absorbance* of a suitable solution of *aspirin BPCRS* in 0.1M *hydrochloric acid* and calculate the total content of aspirin, $C_9H_8O_4$, in the medium using the declared content of $C_9H_8O_4$ in *aspirin BPCRS* . The amount of aspirin released is not more than 5% of the stated amount.

Replace the 0.1M *hydrochloric acid* in the vessel with 900 ml of *mixed phosphate buffer pH 6.8* , previously held at 36.5° to 37.5°. After 45 minutes, withdraw a sample of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium, if necessary, at 265 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *aspirin BPCRS* in the dissolution medium and calculate the total content of aspirin, $C_9H_8O_4$, in the medium using the declared content of $C_9H_8O_4$ in *aspirin BPCRS* .

Salicylic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 60 ml of *acetonitrile* and 1 ml of *formic acid* to a quantity of powdered tablets containing 0.3 g of Aspirin, shake for 15 minutes and add sufficient *acetonitrile* to produce 100 ml, mix and filter. Solution (2) contains 0.009% w/v of *salicylic acid* in a mixture of 99 volumes of *acetonitrile* and 1 volume of *formic acid*. Solution (3) contains 0.3% w/v of *aspirin BPCRS* and 0.009% w/v of *salicylic acid* in a mixture of 99 volumes of *acetonitrile* and 1 volume of *formic acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Luna C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 1 volume of *acetonitrile* and 3 volumes of 0.05M *sodium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* and (c) a detection wavelength of 280 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to salicylic acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 60 ml of *acetonitrile* and 1 ml of *formic acid* to a quantity of the powdered tablets containing 0.3 g of Aspirin, shake for 15 minutes and add sufficient *acetonitrile* to produce 100 ml, mix and filter. Dilute 1 volume to 4 volumes with a mixture of 99 volumes of *acetonitrile* and 1 volume of *formic acid*. Solution (2) contains 0.075% w/v of *aspirin BPCRS* in a mixture of 99 volumes of *acetonitrile* and 1 volume of *formic acid*. Solution (3) contains 0.075% w/v of *aspirin BPCRS* and 0.0015% w/v of *salicylic acid* in a mixture of 99 volumes of *acetonitrile* and 1 volume of *formic acid*.

The chromatographic conditions described under Salicylic acid may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

Calculate the content $C_9H_8O_4$ in the tablets using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

STORAGE

Gastro-resistant Aspirin Tablets should be protected from moisture.

LABELLING

The label states (1) that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed); (2) that the tablets should be swallowed whole and not chewed.

Paediatric Alimemazine Oral Solution

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Paediatric Alimemazine Oral Solution is a solution containing 0.15% w/v of Alimemazine Tartrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of alimemazine tartrate, $C_{36}H_{44}N_4S_2,C_4H_6O_6$

0.135 to 0.165% w/v.

IDENTIFICATION

Dilute 50 ml of the oral solution with 175 ml of *water* and add 7 ml of 1M *sodium hydroxide* . Extract with 100 ml of *ether* , wash the ether layer with 15 ml of *water* and dry over *anhydrous sodium sulphate* (solution A). Evaporate 30 ml of solution A to dryness and dissolve the residue in 0.2 ml of *dichloromethane* . The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of alimemazine *(RS 005)* .

TESTS

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) dilute 15 ml with an equal volume of *water*, add 2 ml of 1M *sodium hydroxide* and extract with two 15-ml quantities of *chloroform* . Dry the chloroform extracts with *anhydrous sodium sulphate* , filter and evaporate the filtrate to dryness. Dissolve the residue as completely as possible in 1 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* . For solution (2) dilute 1 volume of solution (1) to 50 volumes with the same solvent mixture.

ASSAY

Carry out the following procedure protected from light. To a weighed quantity containing 15 mg of Alimemazine Tartrate add 25 ml of *water* and 5 ml of a 5% w/v solution of *sodium hydroxide* . Extract the mixture with two 50 ml quantities of *chloroform* , shaking vigorously for 1 minute each time, evaporate the combined extracts to dryness at about 30° at a pressure of 2 kPa and dissolve the residue in sufficient 0.1M *hydrochloric acid* to produce 50 ml (solution B). Dilute 10 ml of solution B to 50 ml with *water* (solution C). To a further 10 ml of solution B add 5 ml of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50 ml (solution D). Measure the *absorbance* of solution D at the maximum at 342 nm, Appendix II B, using solution C in the reference cell and measure the *absorbance* of solution C at the same wavelength using *water* in the reference cell. Repeat the procedure using a 0.03% w/v solution of *alimemazine tartrate BPCRS* in 0.1M *hydrochloric acid* in place of solution B and beginning at the words 'Dilute 10 ml of ...'. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{36}H_{44}N_4S_2,C_4H_6O_6$, weight in volume, using the declared content of $C_{36}H_{44}N_4S_2,C_4H_6O_6$ in *alimemazine tartrate BPCRS*. The result is not valid if the absorbance of solution C is more than 0.10.

STORAGE

Paediatric Alimemazine Oral Solution should be protected from light.

Paediatric Alimemazine Oral Solution contains 7.5 mg of Alimemazine Tartrate in 5 ml.

Prolonged-release Aminophylline Tablets

General Notices

Prolonged-release Aminophylline Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Non-selective phosphodiesterase inhibitor; treatment of reversible airways obstruction.

DEFINITION

Prolonged-release Aminophylline Tablets contain Aminophylline or Aminophylline Hydrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Aminophylline. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of theophylline, $C_7H_8N_4O_2$

81.4 to 90.0% of the stated amount of aminophylline.

Content of ethylenediamine, $C_2H_8N_2$

Not less than 10.9% and not more than 12.1% of the stated amount of aminophylline.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Aminophylline with 20 ml of *water*, filter, add to the filtrate with constant stirring 1 ml of 2M *hydrochloric acid* , allow to stand for a few minutes and again filter. Reserve the filtrate for test C. Wash the residue with small quantities of cold *water*, recrystallise from hot *water* and dry at 105°. The *melting point* of the residue is about 271°, Appendix V A.

B. The *infrared absorption spectrum* of the residue obtained in test A, Appendix II A, is concordant with the *reference spectrum* of theophylline *(RS 333)* .

C. To the filtrate reserved in test A add 0.2 ml of *benzoyl chloride* , make alkaline with 5M *sodium hydroxide* and shake vigorously. Filter, wash the residue with cold *water* and recrystallise from a mixture of 1 volume of *water* and 3 volumes of *ethanol (96%)* . The *melting point* of the crystals, after drying at 100°, is about 250°, Appendix V A.

D. Shake a quantity of the powdered tablets containing 0.25 g of Aminophylline with 5 ml of *water* and filter. To 2 ml of the filtrate add 2 ml of a 1% w/v solution of *copper (II) sulphate* and shake. A purplish blue colour is produced.

ASSAY

For theophylline

Weigh and powder 20 tablets. Shake a quantity of the powder containing 80 mg of Aminophylline with a mixture of 20 ml of 0.1M *sodium hydroxide* and 60 ml of *water* for 10 minutes, add sufficient *water* to produce 200 ml, mix and filter. Dilute 5 ml of the filtrate to 250 ml with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_7H_8N_4O_2$ taking 650 as the value of A (1%, 1 cm) at the maximum at 275 nm.

For ethylenediamine

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.3 g of Aminophylline with 20 ml of *water*, heat to 50° for 30 minutes and titrate with 0.05M *sulphuric acid VS*, using *bromocresol green solution* as indicator, until the colour changes from blue to green. Each ml of 0.05M *sulphuric acid VS* is equivalent to 3.005 mg of $C_2H_8N_2$.

STORAGE

Prolonged-release Aminophylline Tablets should be kept in an airtight container and protected from light.

Strong Ammonium Acetate Solution

General Notices

DEFINITION

Ammonium Bicarbonate	470 g
Glacial Acetic Acid	453 g
Strong Ammonia Solution	A sufficient quantity
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Ammonium Bicarbonate by adding gradually to the Glacial Acetic Acid diluted with 350 ml of Purified Water. Add Strong Ammonia Solution until 0.05 ml of the resulting solution diluted with 0.5 ml of *water* gives a full blue colour with 0.05 ml of *bromothymol blue solution R3* and a full yellow colour with 0.05 ml of *thymol blue solution* ; about 100 ml of Strong Ammonia Solution is required. Add sufficient Purified Water to produce 1000 ml.

Content of ammonium acetate, $C_2H_7NO_2$

55.0 to 60.0% w/v.

TESTS

Acidity or alkalinity

pH of a 10% v/v solution, 7.0 to 8.0, Appendix V L.

Weight per ml

1.085 to 1.095 g, Appendix V G.

ASSAY

To 5 ml add 50 ml of *water* and 12 ml of *formaldehyde solution* previously neutralised to *phenolphthalein solution R1* and titrate with 1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 1M *sodium hydroxide VS* is equivalent to 77.08 mg of $C_2H_7NO_2$.

STORAGE

Strong Ammonium Acetate Solution should be kept in lead-free glass containers.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

When ammonium acetate solution or dilute ammonium acetate solution is prescribed or demanded, Strong Ammonium Acetate Solution diluted to eight times its volume with freshly boiled and cooled Purified Water, shall be dispensed or supplied.

Strong Paediatric Alimemazine Oral Solution

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Strong Paediatric Alimemazine Oral Solution is a solution containing 0.6% w/v of Alimemazine Tartrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of alimemazine tartrate, $C_{36}H_{44}N_4S_2,C_4H_6O_6$

0.54 to 0.66% w/v.

IDENTIFICATION

Dilute 15 ml of the oral solution with 175 ml of *water* and add 7 ml of 1M *sodium hydroxide* . Extract with 100 ml of *ether* , wash the ether layer with 15 ml of *water* and dry over *anhydrous sodium sulphate* (solution A). Evaporate 30 ml of solution A to dryness and dissolve the residue in 0.2 ml of *dichloromethane* . The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of alimemazine *(RS 005)* .

TESTS

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) dilute 5 ml with 15 ml of *water*, add 2 ml of 1M *sodium hydroxide* and extract with two 15 ml quantities of *chloroform* . Dry the chloroform extracts with *anhydrous sodium sulphate* , filter and evaporate the filtrate to dryness. Dissolve the residue as completely as possible in 1 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* . For solution (2) dilute 1 volume of solution (1) to 50 volumes with the same solvent mixture.

ASSAY

Carry out the following procedure protected from light. To a weighed quantity containing 30 mg of Alimemazine Tartrate add 25 ml of *water* and 5 ml of a 5% w/v solution of *sodium hydroxide* . Extract the mixture with two 50-ml quantities of *chloroform* , shaking vigorously for 1 minute each time, evaporate the combined extracts to dryness at about 30° at a pressure of 2 kPa and dissolve the residue in sufficient 0.1M *hydrochloric acid* to produce 100 ml (solution B). Dilute 10 ml of solution B to 50 ml with *water* (solution C). To a further 10 ml of solution B add 5 ml of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50 ml (solution D). Measure the *absorbance* of solution D at the maximum at 342 nm, Appendix II B, using solution C in the reference cell and measure the *absorbance* of solution C at the same wavelength using *water* in the reference cell. Repeat the procedure using a 0.03% w/v solution of *alimemazine tartrate BPCRS* in 0.1M *hydrochloric acid* in place of solution B and beginning at the words 'Dilute 10 ml of ...'. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{36}H_{44}N_4S_2,C_4H_6O_6$, weight in volume, using the declared content of $C_{36}H_{44}N_4S_2,C_4H_6O_6$ in *alimemazine tartrate BPCRS*. The result is not valid if the absorbance of solution C is more than 0.10.

STORAGE

Strong Paediatric Alimemazine Oral Solution should be protected from light.

Strong Paediatric Alimemazine Oral Solution contains 30 mg of Alimemazine Tartrate in 5 ml.

Baclofen Oral Solution

General Notices

Action and use

Skeletal muscle relaxant.

DEFINITION

Baclofen Oral Solution is a solution of Baclofen in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of baclofen, $C_{10}H_{12}ClNO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and a mixture of 20 volumes of *glacial acetic acid* , 20 volumes of *water* and 80 volumes of *butan-1-ol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a volume of the oral solution containing 5 mg of Baclofen to 100 ml with a mixture of 35 volumes of *acetonitrile* and 65 volumes of *water*. Solution (2) contains 0.005% w/v of *baclofen BPCRS* in the same solvent mixture. After removal of the plate, allow the solvent to evaporate. Place an evaporating dish containing a mixture of 4 ml of *water*, 1 ml of 7M *hydrochloric acid* and 0.5 g of *potassium permanganate* in a chromatography tank, close the tank and allow to stand for 2 minutes. Place the plate in the tank, close the tank and leave the plate in contact with the vapour for 1 minute. After removal of the plate, place it in a current of cold air until an area of coating below the line of application shows only a faint blue colour on the addition of 0.05 ml of *potassium iodide and starch solution* . Spray the plate with *potassium iodide and starch solution* and examine in daylight. The chromatogram obtained with solution (1) exhibits a spot that corresponds to the spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TEST

Lactam

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use solution (1) prepared for the Assay. Solution (2) contains 0.0002% w/v of *baclofen impurity A EPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of *baclofen BPCRS*, 0.0003% w/v of *propyl 4-hydroxybenzoate*, 0.0003% w/v of *methyl 4-*

hydroxybenzoate and 0.0002% w/v of *baclofen impurity A EPCRS* in the mobile phase.

The chromatographic procedure described under Assay may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to methyl-4-hydroxybenzoate and baclofen impurity A and between the peaks due to baclofen impurity A and propyl-4-hydroxybenzoate is at least 5.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to baclofen impurity A (lactam) is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a weighed quantity of the oral solution containing 5 mg of Baclofen to 50 ml with the mobile phase. Solution (2) contains 0.01% w/v of *baclofen BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of *baclofen BPCRS*, 0.0003% w/v of *propyl 4-hydroxybenzoate* and 0.0002% w/v of *baclofen impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 5 g of *sodium dodecyl sulphate* in a mixture of 5 ml of *orthophosphoric acid* and 650 ml of *water* and diluting to 1000 ml with *acetonitrile* and (c) a detection wavelength of 218 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to baclofen impurity A and propyl-4-hydroxybenzoate is at least 5.0.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{10}H_{12}ClNO_2$, weight in volume, using the declared content of $C_{10}H_{12}ClNO_2$ in *baclofen BPCRS*.

STORAGE

Baclofen Oral Solution should be stored below 25° and protected from light. It should not be refrigerated.

Baclofen Tablets

General Notices

Action and use

Skeletal muscle relaxant.

DEFINITION

Baclofen Tablets contain Baclofen.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of baclofen, $C_{10}H_{12}ClNO_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 80 volumes of *butan-1-ol*, 20 volumes of *glacial acetic acid* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Baclofen with 20 ml of a mixture of 4 volumes of *absolute ethanol* and 1 volume of *glacial acetic acid* for 30 minutes and filter. Solution (2) contains 0.1% w/v of *baclofen BPCRS* in a mixture of 4 volumes of *absolute ethanol* and 1 volume of *glacial acetic acid*. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 100° for 10 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Lactam

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing 0.10 g of Baclofen with 50 ml of the mobile phase for 30 minutes, shaking occasionally to disperse the sample, and filter through a glass-fibre filter (Whatman GF/C is suitable). Solution (2) contains 0.004% w/v of *baclofen impurity A EPCRS* in the mobile phase. Solution (3) contains 0.2% w/v of *baclofen BPCRS* and 0.004% w/v of *baclofen impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS

1 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 5 volumes of *glacial acetic acid*, 440 volumes of *methanol* and 560 volumes of *water,* the mixture containing 1.822 g per litre of *sodium hexanesulphonate* and (c) a detection wavelength of 266 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to baclofen and baclofen impurity A is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to baclofen impurity A (lactam) is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter through a membrane filter with a nominal pore size not greater than 0.45 µm, discarding the first 10 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.001% w/v of *baclofen BPCRS* in the mobile phase. Solution (2) is the filtrate from the dissolution vessel.

The chromatographic conditions described under Assay may be used.

Calculate the total content of baclofen, $C_{10}H_{12}ClNO_2$, in the medium from the declared content of $C_{10}H_{12}ClNO_2$ in *baclofen BPCRS*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add a quantity of whole tablets containing 0.1 g of Baclofen to 25 ml of a mixture of 100 volumes of *water* and 1 volume of *glacial acetic acid* and disperse with the aid of ultrasound. Dilute to 50 ml with *methanol*, filter and use the filtrate. Solution (2) contains 0.2% w/v of *baclofen BPCRS* in a mixture of 100 volumes of *methanol*, 100 volumes of *water* and 1 volume of *glacial acetic acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.01M *sodium hexanesulphonate* in a mixture of 100 volumes of *methanol*, 100 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 265 nm.

Calculate the content of $C_{10}H_{12}ClNO_2$ using the declared content of $C_{10}H_{12}ClNO_2$ in *baclofen BPCRS*.

Barium Sulphate Oral Suspension

General Notices

DEFINITION

Barium Sulphate Oral Suspension is a suspension of Barium Sulphate for Suspension in a suitable aqueous vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of barium sulphate, $BaSO_4$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A smooth, white or creamy white suspension.

IDENTIFICATION

Evaporate 1 ml to dryness and ignite to constant weight. The residue complies with the following tests.

A. To 0.2 g add 5 ml of a 50% w/v solution of *sodium carbonate* and boil for 5 minutes, add 10 ml of *water* and filter. Reserve the residue for test B. Acidify the filtrate with 2M *hydrochloric acid* . The resulting solution yields the reactions characteristic of *sulphates* , Appendix VI.

B. Wash the residue reserved in test A with *water*, add 5 ml of 2M *hydrochloric acid* , mix well and filter. Add 0.3 ml of 1M *sulphuric acid* to the filtrate. A white precipitate is produced which is insoluble in 2M *hydrochloric acid* .

Acidity or alkalinity

pH, 3.5 to 8.5, Appendix V L.

ASSAY

Evaporate to dryness a quantity containing 0.6 g of Barium Sulphate in a platinum dish on a water bath and carry out the Assay described under Barium Sulphate for Suspension, beginning at the words 'add 5 g of *sodium carbonate* …'. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the percentage of $BaSO_4$, weight in volume.

Beclometasone Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Cream contains Anhydrous Beclometasone Dipropionate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform* , 10 volumes of *acetone* and 5 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the preparation being examined containing 0.5 mg of Anhydrous Beclometasone Dipropionate in 20 ml of *methanol (80%)* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *chloroform* , shake vigorously, allow the layers to separate, evaporate the chloroform layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *chloroform* . Solution (2) contains 0.025% w/v of *beclometasone dipropionate BPCRS* in *chloroform* . Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and, while hot, spray with *alkaline tetrazolium blue solution* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to beclometasone dipropionate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

For creams containing 0.025% w/w of Anhydrous Beclometasone Dipropionate prepare solution (1) by mixing 10 ml of *methanol (80%)* containing 0.01% w/v of *beclometasone* *dipropionate BPCRS* and 0.0005% w/v of *beclometasone 17-propionate BPCRS* with 2 ml of a 0.05% w/v solution of *testosterone propionate* (internal standard) in *methanol (80%)* and diluting to 50 ml with the same solvent. For solution (2) disperse a quantity of the preparation being examined containing 1 mg of Anhydrous Beclometasone Dipropionate in 20 ml of hot *methanol (90%)*, add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower layer through a small plug of absorbent cotton previously washed with *methanol (80%)*. Repeat the extraction of the trimethylpentane solution with two further 10 ml quantities of *methanol (80%)*, filtering the extracts through the absorbent cotton. Combine the extracts and add sufficient *methanol (80%)* to produce 50 ml. If the resulting solution is more than slightly cloudy, filter. Prepare solution (3) in the same manner as solution (2) but add 2 ml of a 0.05% w/v solution of the internal standard in *methanol (80%)* before diluting to 50 ml.

For creams containing 0.5% w/w of Anhydrous Beclometasone Dipropionate prepare solution (1) by mixing 25 ml of *methanol (80%)* containing 0.04% w/v of *beclometasone dipropionate BPCRS* and 0.002% w/v of *beclometasone 17-propionate BPCRS* with 20 ml of a 0.05% w/v solution of *testosterone propionate* (internal standard) in *methanol (80%)* and dilute to 200 ml with the same solvent. For solution (2) add 100 ml of *methanol (80%)* to a quantity of the preparation being examined containing 10 mg of Anhydrous Beclometasone Dipropionate and heat on a water bath, with swirling, until the preparation has dispersed. Cool, dilute to 200 ml with *methanol (80%)* and filter. Prepare solution (3) in the same manner as solution (2) but add 20 ml of a 0.05% w/v solution of the internal standard in *methanol (80%)* before diluting to 200 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of *methanol* and *water* such that the *resolution factor* between the peaks due to beclometasone 17-propionate (retention time about 1.5 minutes) and beclometasone dipropionate (retention time about 2 minutes) is more than 2.0 (a mixture of 70 volumes of *methanol* and 30 volumes of *water* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{28}H_{37}ClO_7$ in the preparation being examined using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*.

STORAGE

Beclometasone Cream should be protected from light.

Beclometasone Nasal Spray

General Notices

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Nasal Spray is a suspension of Anhydrous Beclometasone Dipropionate in a suitable liquid in a suitable pressurised container fitted with a suitable nasal delivery system.

The intranasal solution complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

Complies with the test described under Beclometasone Pressurised Inhalation.

TESTS

Related substances

Complies with the test described under Beclometasone Pressurised Inhalation when determined on the residue collected from a sufficient number of discharges.

ASSAY

Carry out the Assay described under Beclometasone Pressurised Inhalation.

LABELLING

The label states the amount of active ingredient delivered by each actuation of the valve and the number of deliveries available from the container.

Beclometasone Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Ointment contains Anhydrous Beclometasone Dipropionate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Beclometasone Cream using the following as solutions (1) and (2). For solution (1) dissolve a quantity of the ointment containing 0.5 mg of Anhydrous Beclometasone Dipropionate in 2 ml of *chloroform* by heating on a water bath, add 10 ml of *methanol* and heat on the water bath until the mixture begins to boil. Shake vigorously, cool in ice for 30 minutes and filter. Evaporate the filtrate to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *chloroform* . Solution (2) contains 0.05% w/v of *beclometasone dipropionate BPCRS* in *chloroform* .

B. In the Assay the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to beclometasone dipropionate in the chromatogram obtained with solution (1).

ASSAY

Carry out the Assay described under Beclometasone Cream, preparing the solutions in the following manner. For solution (1) mix 10 ml of *methanol (80%)* containing 0.01% w/v of *beclometasone dipropionate BPCRS* and 0.0005% w/v of *beclometasone 17-propionate BPCRS* with 2 ml of a 0.05% w/v solution of *testosterone propionate* (internal standard) in *methanol (80%)* and dilute to 50 ml with the same solvent. For solution (2) disperse a quantity of the ointment containing 1 mg of Anhydrous Beclometasone Dipropionate in 25 ml of hot *2,2,4-trimethylpentane* , cool and extract the mixture with successive quantities of 20, 10 and 10 ml of *methanol (80%)*, filtering each extract in turn through a small plug of absorbent cotton previously washed with *methanol (80%)*. Combine the filtrates and dilute to 50 ml with *methanol (80%)*. Prepare solution (3) in the same manner as solution (2) but add 2 ml of a 0.05% w/v solution of the internal standard in *methanol (80%)* before diluting to 50 ml.

STORAGE

Beclometasone Ointment should be protected from light.

Beclometasone Powder for Inhalation

General Notices

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Powder for Inhalation consists of Anhydrous Beclometasone Dipropionate or Beclometasone Dipropionate Monohydrate in *microfine powder* either alone or admixed with Lactose. It may be presented either as pre-metered units for use in a designated powder inhaler or as a powder reservoir within a metered-dose powder inhaler.

The powder for inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRE-METERED UNITS

DEFINITION

Beclometasone pre-metered unit powder inhalers consist of Anhydrous Beclometasone Dipropionate or Beclometasone Dipropionate Monohydrate in *microfine powder* either alone or admixed with Lactose in a suitable container for use within a suitable device.

PRODUCTION

Complies with the requirements for Uniformity of delivered dose stated under Preparations for Inhalation using the method described in the European Pharmacopoeia or other methodology where justified and authorised.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

When supplied as disks, 90.0 to 110.0% of the stated amount per pre-metered unit. When supplied as capsules, 80.0 to 120.0% of the stated amount per pre-metered unit.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Disperse a quantity of powder containing the equivalent of 200 µg of beclometasone dipropionate in 5 ml of 1M *sodium hydroxide* in a 25 ml conical flask. Add three drops of *copper sulphate solution* and shake. A precipitate may form which dissolves to give a clear blue solution. Heat the solution to boiling; a red precipitate is produced.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the mixed contents of 20 units containing the equivalent of 1.2 mg of beclometasone dipropionate in the mobile phase and dilute to 5 ml with the mobile phase. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase. Solution (3) contains 0.0040% w/v each of *beclometasone 17-propionate BPCRS* and *beclometasone 21-propionate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 or Lichrospher RP18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 350 volumes of *water* and 600 volumes of *acetonitrile* diluted to 1000 volumes with *water* and (c) a detection wavelength of 254 nm.

When the chromatograms are recorded under the prescribed conditions, the retention time for beclometasone 17-propionate is about 6 minutes, for beclometasone 21-propionate, about 7 minutes and for beclometasone dipropionate, about 13 minutes.

Inject 100 µl of solution (3). The test is not valid unless the *resolution factor* between the peaks due to beclometasone 17-propionate and beclometasone 21-propionate is at least 1.4. If this resolution is not achieved, adjust the concentration of acetonitrile in the mobile phase.

Inject separately 100 µl of each of solutions (1) and (2) and record the chromatograms for twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1%); the sum of the areas of all the *secondary peaks* is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak the area of which is less than 0.025 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Uniformity of delivered dose

Complies with the requirements stated under Powders for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), collect single doses of the preparation being examined using the procedure described under Powders for Inhalation, Uniformity of delivered dose and dissolve the collected dose in sufficient of a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile* to produce a solution containing the equivalent of 0.00005% w/v of beclometasone dipropionate. For solution (2) dilute 10 ml of a solution containing 0.0005% w/v of *beclometasone dipropionate BPCRS* in *methanol* to 100 ml with a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm x 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable), (b) as the mobile phase with a flow rate of 1.3 ml per minute a mixture of 25 volumes of *water* and 75 volumes of *methanol* and (c) a detection wavelength of 240 nm.

Inject 100 µl of each solution.

Calculate the amount of beclometasone dipropionate, $C_{28}H_{37}ClO_7$, per delivered dose using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*. Repeat the procedure nine times as described for pre-metered systems under Powders for Inhalation, Uniformity of delivered dose.

Deposition of the emitted dose

Carry out the test for *aerodynamic assessment of fine particles*, Appendix XII C7, using Apparatus A but determining the content of active ingredient as described below. Use 7 ml of a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile* in the upper impingement chamber and 30 ml of the same solvent mixture in the lower impingement chamber. Use the solvent mixture to wash the coupling tube, E, and transfer the combined solution and washings in the lower impingement chamber to a 100-ml graduated flask and dilute the combined solution and washings to 100 ml with the solvent mixture. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) use the diluted solution from the lower impingement chamber diluted with a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile* , if necessary, to contain the equivalent of 0.00005% w/v of beclometasone dipropionate. For solution (2) dilute 10 ml of a solution containing 0.0005% w/v of *beclometasone dipropionate BPCRS* in *methanol* to 100 ml with a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile* .

The chromatographic procedure described under Uniformity of delivered dose may be used.

Inject 100 µl of each solution. Calculate the amount of beclometasone dipropionate, $C_{28}H_{37}ClO_7$, delivered to the lower impingement chamber per actuation of the valve using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*. Not less than 10% of the average amount of beclometasone dipropionate delivered per actuation of the valve, determined in the Assay, is deposited in the lower impingement chamber.

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the mixed contents of 20 units in sufficient of the mobile phase to produce a solution containing the equivalent of 0.001% w/v of beclometasone dipropionate. Solution (2) contains 0.001% w/v of *beclometasone dipropionate BPCRS* in the mobile phase. Solution (3) contains 0.001% w/v each of *beclometasone 17-propionate BPCRS* and *beclometasone 21-propionate BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

Inject 100 µl of solution (3). The test is not valid unless the *resolution factor* between the peaks due to beclometasone 17-propionate and beclometasone 21-propionate is at least 1.4. If this resolution is not achieved, adjust the concentration of acetonitrile in the mobile phase.

Inject separately 100 µl of each of solutions (1) and (2). Calculate the content of $C_{28}H_{37}ClO_7$ using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*.

Labelling

The quantity of active ingredient is stated in terms of the equivalent amount of beclometasone dipropionate per unit.

METERED DOSE POWDER INHALER

DEFINITION

Beclometasone metered dose powder inhalers consist of Anhydrous Beclometasone Dipropionate or Beclometasone Dipropionate Monohydrate in *microfine powder* either alone or admixed with Lactose in a suitable device.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

80.0 to 120.0% of the stated amount per delivered dose.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Disperse a quantity of the powder containing the equivalent of 0.25 g of beclometasone dipropionate in 5 ml of *water*. Add 5 ml of 6M *ammonia* and heat in a water-bath at 80° for 10 minutes. An orange-red colour is produced.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the powder for inhalation containing the equivalent of 1.2 mg of beclometasone dipropionate in the mobile phase and dilute to 5 ml with the mobile phase. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase. Solution (3) contains 0.0040% w/v each of *beclometasone 17-propionate BPCRS* and *beclometasone 21-propionate BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances for Pre-metered units may be used.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2.0%) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of all the *secondary peaks* is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak the area of which is less than 0.025 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Uniformity of delivered dose

Carry out the test for Uniformity of delivered dose as stated in the requirements for the Pre-metered unit.

Deposition of the emitted dose

Carry out the test for Deposition of emitted dose as stated in the requirements for the Pre-metered unit.

ASSAY

Use the average of the 10 individual results obtained in the test for Uniformity of delivered dose.

Labelling

The quantity of active ingredient is stated in terms of the equivalent amount of beclometasone dipropionate per actuation.

Beclometasone Pressurised Inhalation

General Notices

Beclometasone Pressurised Inhalation prepared using different propellant gases, whilst complying with the requirements of the monograph, may not necessarily be interchangeable.

Action and use

Glucocorticoid.

DEFINITION

Beclometasone Pressurised Inhalation is a solution or suspension of Anhydrous Beclometasone Dipropionate in a suitable liquid in a suitable pressurised container.

The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of beclometasone dipropionate, $C_{28}H_{37}ClO_7$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of beclometasone dipropionate (2) (*RS 379*). Examine the substance as a dispersion in *potassium bromide* prepared in the following manner. Discharge the container a sufficient number of times, under conditions of very low relative humidity (less than 5%), into a mortar to obtain 2 mg of Anhydrous Beclometasone Dipropionate. Heat at 110° for 2 hours at a pressure of 2 kPa, cool, grind the residue thoroughly with 0.1 g of *potassium bromide*, add a further 0.2 g of *potassium bromide* and mix thoroughly.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to beclometasone dipropionate in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *acetone*.

(1) Discharge the container into a small, dry flask a sufficient number of times to obtain 0.5 mg of Anhydrous Beclometasone Dipropionate and dissolve the residue in 2 ml of *acetone*. Evaporate the solution to a volume such that the whole solution can be applied to the plate.

(2) 0.1% w/v of *beclometasone dipropionate BPCRS*.

(3) Dilute 1 volume of solution (2) to 2 volumes.

(4) Dilute 1 volume of solution (2) to 4 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase described below.

(c) Apply separately to the plate the whole of solution (1) and 10 µl of each of solutions (2), (3) and (4).

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray with *alkaline tetrazolium blue solution* and heat at 50° for 5 minutes. Cool and spray again with *alkaline tetrazolium blue solution*.

MOBILE PHASE

3 volumes of *methanol* and 97 volumes of *1,2-dichloroethane.*

LIMITS

In the chromatogram obtained with solution (1):

— any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (2%);

— not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%);

— not more than two *such spots* are more intense than the spot in the chromatogram obtained with solution (4) (0.5%).

Disregard any spot with an Rf value of more than 0.85.

Deposition of the emitted dose

Carry out the test for *aerodynamic assessment of fine particles*, Appendix XII C7, using Apparatus A with 7 ml of *methanol* in the upper impingement chamber and 30 ml of *methanol* in the lower impingement chamber but determining the content of active ingredient as described below. Use *methanol* to wash the coupling tube, E, and transfer the combined solution and washings in the lower impingement chamber to a flask containing sufficient *testosterone propionate BPCRS* (internal standard) in *methanol* that, on dilution to volume with appropriate amounts of *water* and *methanol*, the final solution contains 0.00015% w/v each of testosterone propionate and beclometasone dipropionate in the methanol-water mixture in the proportions 70:30 by volume.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.00015% w/v each of *testosterone propionate BPCRS* and *beclometasone dipropionate BPCRS* in the mobile phase.

(2) The diluted solution from the lower impingement chamber.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 239 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

70 volumes of *methanol* and 30 volumes of *water*, adjusted if necessary so that the *resolution factor* between the peaks due to beclometasone dipropionate and testosterone propionate is at least 2.0

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the amount of beclometasone dipropionate, $C_{28}H_{37}ClO_7$, delivered to the lower impingement chamber per actuation of the valve using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS.* For pressurised inhalations containing 50 µg and 100 µg of Anhydrous Beclometasone Dipropionate, not less than 35% of the average amount of beclometasone delivered per actuation of the valve, calculated as the average of the three results determined in the Assay, is deposited in the lower impingement chamber. For pressurised inhalations containing 200 µg and 250 µg of Anhydrous Beclometasone Dipropionate, not less than 25% of the average amount of beclometasone dipropionate delivered per actuation of the valve, calculated as the average of the three results determined in the Assay, is deposited in the lower impingement chamber.

ASSAY

Determine the content of active ingredient delivered by the first 10 successive combined actuations of the valve after priming. Carry out the procedure for Content of active ingredient delivered by actuation of the valve described under Pressurised Inhalations, beginning at the words 'Remove the pressurised container from the actuator ...' and ending at the words '... to the volume specified in the monograph', using 35 ml of *methanol* in the vessel. Transfer the combined solution and washings obtained from the set of 10 combined actuations to a flask containing sufficient *testosterone propionate BPCRS* (internal standard) in *methanol* that, on dilution to volume with appropriate amounts of *water* and *methanol* , the final solution contains 0.00015% w/v each of testosterone propionate and beclometasone dipropionate in the methanol-water mixture in the proportions 70:30 by volume (solution A). Determine the content of active ingredient in the 10 combined actuations using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Solution A.

(2) 0.00015% w/v of *beclometasone dipropionate BPCRS* in the mobile phase.

(3) 0.00015% w/v each of *testosterone propionate BPCRS* and *beclometasone dipropionate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Deposition of the emitted dose may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the average content of $C_{28}H_{37}ClO_7$ delivered by a single actuation of the valve using the declared content of $C_{28}H_{37}ClO_7$ in *beclometasone dipropionate BPCRS*.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle 10 and on the last 10 successive combined actuations of the valve, as estimated from the number of deliveries available from the container as stated on the label. For each of the three determinations the average content of $C_{28}H_{37}ClO_7$ delivered by a single actuation of the valve is within the limits stated under Content of beclometasone dipropionate.

Bendroflumethiazide Tablets

General Notices

Action and use

Thiazide diuretic.

DEFINITION

Bendroflumethiazide Tablets contain Bendroflumethiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bendroflumethiazide, $C_{15}H_{14}F_3N_3O_4S_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Bendroflumethiazide with 10 ml of *acetone* for 10 minutes and filter. Solution (2) contains 0.1% w/v of *bendroflumethiazide EPCRS* in *acetone*. After removal of the plate, allow it to dry in air, examine under *ultraviolet light (254 nm)* and then reveal the spots by *Method I*. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Bendroflumethiazide with 25 ml of *acetone* for 10 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 2.5 ml of *acetone*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, allow it to dry in air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.0%).

ASSAY

Weigh and powder 20 tablets. Mix a quantity of the powder containing 15 mg of Bendroflumethiazide with 50 ml of 0.1M *sodium hydroxide* with the aid of ultrasound for 10 minutes and dilute to 100 ml with 0.1M *sodium hydroxide* . Mix, filter, dilute 10 ml of the filtrate to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{15}H_{14}F_3N_3O_4S_2$ taking 410 as the value of A(1%, 1 cm) at the maximum at 275 nm.

Benorilate Oral Suspension

General Notices

Action and use

Salicylate-paracetamol derivative; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Benorilate Oral Suspension is a suspension of Benorilate in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of benorilate, $C_{17}H_{15}NO_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the oral suspension containing 0.5 g of Benorilate add 10 ml of *water*, mix and extract with 50 ml of *dichloromethane*. Wash the extract with 10 ml of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of benorilate *(RS 023)*.

B. To 10 mg add 10 ml of 6M *hydrochloric acid* and boil until completely dissolved. To 5 ml of the resulting solution add 0.1 ml of *strong 1-naphthol solution*, mix and add sufficient 1M *sodium hydroxide* to make the solution just alkaline. A blue colour is produced which can be extracted into *butan-1-ol*.

C. *Melting point*, about 179°, Appendix V A.

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel HF_{254} precoated plate (Analtech plates are suitable) and a mixture of 5 volumes of *glacial acetic acid*, 15 volumes of *ether* and 80 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 40 µl of solution (1) and 10 µl of each of solutions (2), (3) and (4). For solution (1) add 20 ml of *methanol* to a quantity of the oral suspension containing 0.40 g of

Benorilate, mix well, add 20 ml of *dichloromethane* , shake and filter. For solutions (2) and (3) dilute 1 volume of solution (1) with *methanol* to 25 volumes and 125 volumes respectively. Solution (4) contains 0.0080% w/v of *paracetamol* in *methanol* . After removal of the plate, allow it to dry in air and develop in a second mobile phase consisting of a mixture of 10 volumes of *formic acid* , 45 volumes of *ether* and 45 volumes of *2,2,4-trimethylpentane* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . In the chromatogram obtained with solution (1) any spot corresponding to paracetamol is not more intense than the spot obtained with solution (4) (0.2%) and any *secondary spot* immediately above that of the principal spot [Rf value approximately 1.1 relative to benorilate] is not more intense than the principal spot in the chromatogram obtained with solution (2) (1%). Not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.2%). Disregard any spot with an Rf value higher than 1.5 relative to benorilate in the chromatogram obtained with solutions (1), (2) and (3).

Salicylic acid

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dilute the oral suspension with *methanol (80%)* to produce a solution containing 0.40% w/v of Benorilate. Solution (2) contains 0.00040% w/v of *salicylic acid* in *methanol (80%)*. Solution (3) contains 0.0010% w/v of *salicylic acid* and 0.020% w/v of *sorbic acid* in *methanol (80%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with particles of silica 5 µm in diameter the surface of which has been modified with chemically-bonded hexyl groups (Spherisorb hexyl is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 5 volumes of a 9.0% w/v solution of *disodium hydrogen orthophosphate* adjusted to pH 6.5 with *orthophosphoric acid* , 30 volumes of *acetonitrile* and 65 volumes of *water*, the mixture adjusted to pH 2.2 with *orthophosphoric acid* , and (c) a detection wavelength of 305 nm.

The test is not valid unless the *resolution factor* between the peaks due to salicylic acid and sorbic acid in the chromatogram obtained with solution (3) is at least 2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to salicylic acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

To a quantity of the oral suspension containing 0.25 g of Benorilate, add 10 ml of *water*, mix, extract with two 40-ml quantities of *dichloromethane* filtering each extract successively through a plug of absorbent cotton saturated with *dichloromethane* and wash the plug with 10 ml of *dichloromethane* . Combine the extracts and washings and dilute to 100 ml with *dichloromethane* . Dilute 5 ml to 50 ml with *absolute ethanol* and dilute 2 ml of the resulting solution to 50 ml with *absolute ethanol* . Measure the *absorbance* of the final solution at the maximum at 240 nm, Appendix II B. Calculate the content of $C_{17}H_{15}NO_5$ taking 740 as the value of A(1%, 1 cm) at the maximum at 240 nm. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the percentage of $C_{17}H_{15}NO_5$, weight in volume.

Benorilate Tablets

General Notices

Action and use

Salicylate-paracetamol derivative; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Benorilate Tablets contain Benorilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of benorilate, $C_{17}H_{15}NO_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 1 g of Benorilate with 30 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* for 10 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of benorilate *(RS 023)*.

B. To 10 mg add 10 ml of 6M *hydrochloric acid* and boil until completely dissolved. To 5 ml of the resulting solution add 0.1 ml of *strong 1-naphthol solution*, mix and add sufficient 1M *sodium hydroxide* to make the solution just alkaline. A blue colour is produced which can be extracted into *butan-1-ol*.

C. *Melting point*, about 179°, Appendix V A.

TESTS

4-Aminophenol

Shake a quantity of the powdered tablets containing 2.5 g of Benorilate with 100 ml of *water* for 15 minutes and filter. If the filtrate is opalescent, warm on a water bath until it becomes clear and allow to cool. To 20 ml of the filtrate add 0.2 ml of *sodium nitroprusside-carbonate solution*, mix and allow to stand for 30 minutes. The solution is not more intensely coloured than a solution prepared at the same time and in the same manner but using 2 ml of a solution of *4-aminophenol* containing 5 µg per ml and 18 ml of *water* in place of the filtrate (20 ppm).

Salicylic acid

Shake a quantity of the powdered tablets containing 0.50 g of Benorilate with 100 ml of *water* for 15 minutes and filter. If the filtrate is opalescent, warm on a water bath until it becomes clear and allow to cool. Transfer 10 ml of the filtrate to a *Nessler cylinder*, dilute to 50 ml with *water*, add 0.2 ml of a 0.5% w/v solution of *iron(III) chloride* and allow to stand for 1 minute. The colour obtained is not more intense than that of a solution prepared at the same time by adding 0.2 ml of a 0.5% w/v solution of *iron(III) chloride* to a mixture of 1 ml of a 0.025% w/v solution of *salicylic acid* in *ethanol (96%)* and sufficient *water* to produce 50 ml (0.5%).

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel HF_{254} precoated plate (Analtech plates are suitable) and a mixture of 5 volumes of *glacial acetic acid* , 15 volumes of *ether* and 80 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. Solution (1) contains 4.0% w/v of the residue obtained in the tests for Identification in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* . For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* . For solution (3) dilute 1 volume of solution (1) to 500 volumes with a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* . Solution (4) contains 0.0080% w/v of *paracetamol* in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* . After removal of the plate, allow it to dry in air and develop in a second mobile phase consisting of a mixture of 10 volumes of *formic acid* , 45 volumes of *ether* and 45 volumes of *2,2,4-trimethylpentane* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* .

Any spot corresponding to paracetamol in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.2%). Any *secondary spot* in the chromatogram obtained with solution (1) with an Rf value slightly higher than that of the principal spot is not more intense than the principal spot in the chromatogram obtained with solution (2) (1%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Benorilate with 100 ml of *absolute ethanol* for 30 minutes and filter. Dilute 5 ml of the filtrate to 250 ml with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 240 nm, Appendix II B. Calculate the content of $C_{17}H_{15}NO_5$ taking 740 as the value of A(1%, 1 cm) at the maximum at 240 nm.

Benzatropine Injection

General Notices

Action and use

Anticholinergic.

DEFINITION

Benzatropine Injection is a sterile solution of Benzatropine Mesilate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of benzatropine mesilate, $C_{21}H_{25}NO,CH_4O_3S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a suitable volume with sufficient 2M *hydrochloric acid* to produce a solution containing 0.08% w/v of Benzatropine Mesilate. The *light absorption* of the resulting solution, Appendix II B, exhibits maxima at 253 nm and 258 nm and inflections at 249, 264 and 268 nm.

B. To a volume containing 10 mg of Benzatropine Mesilate add 5 ml of *picric acid solution R1*, mix and allow to stand for 1 hour. The *melting point* of the precipitate, after drying at 105°, is about 185°, Appendix V A.

TESTS

Acidity or alkalinity

pH, 5.0 to 8.0, Appendix V L.

Tropine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 75 volumes of *ethanol (96%)* and 15 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) evaporate to dryness using a rotary evaporator a volume containing 10 mg of Benzatropine Mesilate, extract the residue with two 2 ml quantities of *acetone* and evaporate the combined extracts to dryness. Dissolve the residue in 0.5 ml of *acetone*. Solution (2) contains 0.010% w/v of *tropine* in *acetone*. After removal of the plate, allow it to dry in air and spray with *sodium iodobismuthate solution* and then with a 0.4% w/v solution of *sulphuric acid*. Any spot corresponding to tropine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution

(2).

ASSAY

To a volume containing 25 mg of Benzatropine Mesilate add 10 ml of 5M *sodium hydroxide* , 10 ml of *water* and an excess of *sodium chloride* . Extract with four 25 ml quantities of *ether* , combine the ether extracts and extract with four 10 ml quantities of 2M *hydrochloric acid* , remove any residual ether from the combined extracts in a current of air, dilute to 50 ml with 2M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{21}H_{25}NO,CH_4O_3S$ from the *absorbance* obtained by repeating the operation using 25 mg of *benzatropine mesilate BPCRS* beginning at the words 'add 10 ml of 5M *sodium hydroxide* ...' and from the declared content of $C_{21}H_{25}NO,CH_4O_3S$ in *benzatropine mesilate BPCRS* .

Benzatropine Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Benzatropine Tablets contain Benzatropine Mesilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of benzatropine mesilate, $C_{21}H_{25}NO,CH_4O_3S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a suitable quantity of the powdered tablets with 2M *hydrochloric acid* and filter. Dilute the filtrate with sufficient 2M *hydrochloric acid* to produce a solution containing 0.1% w/v of Benzatropine Mesilate. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 253 nm and 258 nm.

B. Extract a quantity of the powdered tablets containing 10 mg of Benzatropine Mesilate with 10 ml of *ethanol (96%)* and filter. Evaporate the filtrate to about 2 ml, pour into 5 ml of hot *picric acid solution R1* and allow to cool. The *melting point* of the precipitate, after drying at 105°, is about 185°, Appendix V A.

TESTS

Tropine

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and a mixture of 75 volumes of *ethanol (96%)* and 15 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Benzatropine Mesilate with 4 ml of *acetone* for 5 minutes, centrifuge, evaporate 2 ml of the supernatant liquid to dryness and dissolve the residue in 0.5 ml of *acetone* . Solution (2) contains 0.010% w/v of *tropine* in *acetone* . After removal of the plate, allow it to dry in air and spray with *sodium iodobismuthate solution* and then with a 0.4% w/v solution of *sulphuric acid* . Any spot corresponding to tropine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Uniformity of content

Tablets containing 2 mg or less of Benzatropine Mesilate comply with the requirement stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of *benzatropine mesilate BPCRS* in the mobile phase. For solution (2) shake one tablet with 8 ml of the mobile phase for 5 minutes, add sufficient of the mobile phase to produce 10 ml, centrifuge for 5 minutes and use the supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP8 or Spherisorb C8 is suitable), (b) a mixture of 65 volumes of *acetonitrile* and 35 volumes of *octylamine phosphate buffer pH 3.0* as the mobile phase with a flow rate of 1.3 ml per minute and (c) a detection wavelength of 259 nm.

Calculate the content of $C_{21}H_{25}NO,CH_4O_3S$ in each tablet using the declared content of $C_{21}H_{25}NO,CH_4O_3S$ in *benzatropine mesilate BPCRS*.

ASSAY

Weigh and powder 30 tablets. To a quantity of the powder containing 50 mg of Benzatropine Mesilate add 50 ml of *water* and shake for 15 minutes. Add 10 ml of a 50% w/v solution of *sodium hydroxide* and an excess of *sodium chloride* and extract with successive quantities of 50, 25, 25 and 25 ml of *ether*. Extract the combined ether layers with successive quantities of 25, 25, 25 and 15 ml of 2M *hydrochloric acid*, dilute the combined extracts to 100 ml with 2M *hydrochloric acid*, mix and filter if necessary. Measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{21}H_{25}NO,CH_4O_3S$ from the *absorbance* obtained by repeating the operation using 50 mg of *benzatropine mesilate BPCRS* in place of the powdered tablets and from the declared content of $C_{21}H_{25}NO,CH_4O_3S$ in *benzatropine mesilate BPCRS*.

Benzoic Acid Solution

General Notices

DEFINITION

Benzoic Acid	50 g
Propylene Glycol	750 ml
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Benzoic Acid in the Propylene Glycol and add sufficient Purified Water, in small quantities and with constant stirring, to produce 1000 ml.

Content of benzoic acid, $C_7H_6O_2$

4.75 to 5.25% w/v.

IDENTIFICATION

A. To 5 ml add 30 ml of 1M *sulphuric acid* and extract the precipitated acid with three 25 ml quantities of *petroleum spirit (boiling range, 40° to 60°)*. Wash the combined extracts with three 25 ml quantities of *water*, filter through absorbent cotton and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of benzoic acid *(RS 025)*.

B. *Melting point* of the residue obtained in test A, about 121°, Appendix V A.

TESTS

Weight per ml

1.045 to 1.055 g, Appendix V G.

ASSAY

To 10 ml add 20 ml of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 12.21 mg of $C_7H_6O_2$.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

Benzoin Inhalation

General Notices

DEFINITION

Benzoin Inhalation is an *inhalation vapour, solution*.

Sumatra Benzoin crushed	100 g
Prepared storax of commerce	50 g
Ethanol (96 per cent)	Sufficient to produce 1000 ml

In making Benzoin Inhalation, Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit[1].

Extemporaneous preparation

The following directions apply.

Macerate the crushed Sumatra Benzoin and the prepared storax with 750 ml of Ethanol (96 per cent) for 24 hours. Filter and pass sufficient Ethanol (96 per cent) through the filter to produce 1000 ml.

The inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of total balsamic acids

Not less than 3.0% w/v, calculated as cinnamic acid, $C_9H_8O_2$.

Total solids

9.0 to 12.0% w/v when determined by drying at 105° for 4 hours, Appendix XI A. Use 2 ml.

ASSAY

Boil 10 ml with 25 ml of *ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Evaporate the ethanol, disperse the residue in 50 ml of hot *water*, cool, add 80 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and allow to stand for 10 minutes. Filter, wash the residue on the filter with 20 ml of *water*, acidify the combined filtrate and washings with *hydrochloric acid* and extract with four 40 ml quantities of *ether*. Discard the aqueous solution, combine the ether extracts and extract with successive quantities of 20, 20, 10, 10 and 10 ml of *sodium hydrogen carbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, carefully acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each extract through *anhydrous sodium sulphate* supported on absorbent cotton. Distil the chloroform from the combined filtrates until 10 ml remains and remove the remainder in a current of air. Dissolve the residue, with the aid of gentle heat, in 10 ml of *ethanol (96%)*, previously neutralised to *phenol red solution*, cool and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 14.82 mg of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

LABELLING

The label indicates the pharmaceutical form as 'inhalation vapour'.

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[1] The statutory regulations governing the use of Industrial Methylated Spirit must be observed.

Benzoyl Peroxide Cream

General Notices

DEFINITION

Benzoyl Peroxide Cream contains Hydrous Benzoyl Peroxide in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 50 volumes of *toluene* , 2 volumes of *dichloromethane* and 1 volume of *glacial acetic acid* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the preparation being examined containing the equivalent of 50 mg of anhydrous benzoyl peroxide with 10 ml of *chloroform* and filter. Solution (2) contains 0.5% w/v of *benzoyl peroxide* in *chloroform* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dilute 2 volumes of solution (2) to 100 volumes with the mobile phase. For solution (2) disperse a quantity of the preparation being examined containing the equivalent of 0.10 g of anhydrous benzoyl peroxide in 25 ml of *acetonitrile* , add sufficient *water* to produce 50 ml, mix and filter. Solution (3) contains 0.020% w/v of *benzoic acid* in the mobile phase. Solution (4) contains 0.0020% w/v of *ethyl benzoate* in the mobile phase. Solution (5) contains 0.0020% w/v of *benzaldehyde* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 500 volumes of *acetonitrile* , 500 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 235 nm.

In the chromatogram obtained with solution (2) the areas of any peaks corresponding to benzoic acid, ethyl benzoate and benzaldehyde are not greater than the areas of the principal peaks in the chromatograms obtained with solutions (3), (4) and (5) respectively. The area of any other *secondary peak* is not greater than half of the area of the principal peak in the

chromatogram obtained with solution (1).

ASSAY

Mix a quantity of the preparation being examined containing the equivalent of 0.25 g of anhydrous benzoyl peroxide with 50 ml of *acetone* and add sufficient *acetone* to produce 100 ml. To 10 ml add 25 ml of a 20% w/v solution of *potassium iodide*, mix, stopper the flask and allow to stand for 15 minutes protected from light. Add 25 ml of *acetone* and titrate with 0.01M *sodium thiosulphate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.01M *sodium thiosulphate VS* is equivalent to 1.211 mg of $C_{14}H_{10}O_4$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Benzoyl Peroxide Gel

General Notices

DEFINITION

Benzoyl Peroxide Gel is a solution of Hydrous Benzoyl Peroxide in a suitable water-soluble basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION; RELATED SUBSTANCES

Complies with the tests described under Benzoyl Peroxide Cream.

ASSAY

Carry out the Assay described under Benzoyl Peroxide Cream.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Benzoyl Peroxide Lotion

General Notices

DEFINITION

Benzoyl Peroxide Lotion is a *cutaneous suspension*. It contains Hydrous Benzoyl Peroxide in a suitable non-greasy vehicle.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION; RELATED SUBSTANCES

Complies with the tests described under Benzoyl Peroxide Cream.

ASSAY

Carry out the Assay described under Benzoyl Peroxide Cream.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

The label indicates the pharmaceutical form as 'cutaneous suspension'.

Benzydamine Cream

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Benzydamine Cream contains Benzydamine Hydrochloride in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of benzydamine hydrochloride, $C_{19}H_{23}N_3O,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat a quantity of the cream containing 25 mg of Benzydamine Hydrochloride with 50 ml of *absolute ethanol* until the cream is completely dissolved and place in an ice-bath until a white precipitate forms. Allow to warm to 20°, dilute to 100 ml with *absolute ethanol* and filter. Dilute 10 ml of the filtrate to 100 ml with *absolute ethanol* . The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 308 nm.

B. In the test for 1-Benzyl-1*H*-indazol-3-ol, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

1-Benzyl-1*H*-indazol-3-ol

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 30 volumes of *triethylamine* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) extract a quantity of the cream containing 60 mg of Benzydamine Hydrochloride with 25 ml of hot *methanol* , cool the solution in ice and filter; repeat the extraction twice, filtering each extract and evaporate the combined extracts to dryness using a rotary evaporator; dissolve the residue in 5 ml of *methanol* . Solution (2) contains 1.2% w/v of *benzydamine hydrochloride BPCRS* in *methanol*. Solution (3) contains 0.0024% w/v of *1-benzyl-1*H*-indazol-3-ol BPCRS* in *methanol* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm* and *365 nm)*. By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

To a quantity of the cream containing 25 mg of Benzydamine Hydrochloride add 50 ml of *ethanol (96%)*, heat until the cream is completely dissolved and cool in an ice-bath until a white precipitate forms. Allow to warm to 20°, dilute to 100 ml with *ethanol (96%)* and filter. Dilute 10 ml of the filtrate to 100 ml with *ethanol (96%)* and measure the *absorbance* of the resulting solution at the maximum at about 308 nm, Appendix II B, using *ethanol (96%)* in the reference cell. Calculate the content of $C_{19}H_{23}N_3O,HCl$ from the *absorbance* obtained with a solution containing 0.0025% w/v of *benzydamine hydrochloride BPCRS* in *ethanol (96%)* and using the declared content of $C_{19}H_{23}N_3O,HCl$ in *benzydamine hydrochloride BPCRS*.

Benzydamine Mouthwash

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Benzydamine Mouthwash is a solution of Benzydamine Hydrochloride in a suitable flavoured and coloured vehicle.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of benzydamine hydrochloride, $C_{19}H_{23}N_3O,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 30 volumes of *triethylamine* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) dilute the mouthwash, if necessary, with *absolute ethanol* to contain 0.15% w/v of Benzydamine Hydrochloride. Solution (2) contains 0.15% w/v of *benzydamine hydrochloride BPCRS* in *absolute ethanol* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

pH

5.0 to 7.0, Appendix V L.

1-Benzyl-1*H*-indazol-3-ol

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 10 volumes of *glacial acetic acid,* 20 volumes of *chloroform* and 70 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) extract a quantity of the mouthwash containing 15 mg of Benzydamine Hydrochloride with seven 90 ml quantities of *chloroform* . Filter each extract through phase separating paper, evaporate the combined extracts to dryness and dissolve the residue in 10 ml of *methanol*. Solution (2) contains 0.0015% w/v of *1-benzyl-1*H*-indazol-3-ol BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Prepare a 0.075% w/v solution of *1-benzyl-3-(3-diethylamino-propoxy)-1*H*-indazole BPCRS* (internal standard) in *water* (solution A). For solution (1) add 10 ml of solution A, 5 ml of *water*, 5 ml of 1M *sodium hydroxide* and 20 ml of *chloroform* to 5 ml of a solution containing 0.15% w/v of *benzydamine hydrochloride BPCRS* in *water*, shake for 5 minutes, centrifuge and use the chloroform layer. Prepare solution (2) in the same manner as solution (1) but using a volume of the mouthwash containing 7.5 mg of Benzydamine Hydrochloride, diluted, if necessary, to 5 ml with *water* in place of the 5 ml of solution containing 0.15% w/v of *benzydamine hydrochloride BPCRS* in *water*.

The chromatographic procedure may be carried out using a glass column (2 m × 2 mm) packed with *acid-washed, diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 260° with a flame ionisation detector.

Calculate the content of $C_{19}H_{23}N_3O,HCl$ using the declared content of $C_{19}H_{23}N_3O,HCl$ in *benzydamine hydrochloride BPCRS*.

LABELLING

The label states, where appropriate, that the preparation is also suitable for use as a gargle.

Benzydamine Oromucosal Spray

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Benzydamine Oromucosal Spray is a solution of Benzydamine Hydrochloride in a suitable flavoured vehicle in a suitable metered-dose container.

The oromucosal spray complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of benzydamine hydrochloride, $C_{19}H_{23}N_3O,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 30 volumes of *triethylamine* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) dilute the oromucosal spray, if necessary, with *absolute ethanol* to contain 0.15% w/v of Benzydamine Hydrochloride. Solution (2) contains 0.15% w/v of *benzydamine hydrochloride BPCRS* in *absolute ethanol* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

pH

5.0 to 7.0, Appendix V L.

Uniformity of weight

Weigh one unit. Fire one shot and reweigh the unit. Repeat four times, then repeat the entire process with 3 more units (20 shots). Determine the average weight delivered per shot. Not more than two of the individual weights deviate from the average weight by more than 10% and none deviates by more than 20%.

1-Benzyl-1*H*-indazol-3-ol

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 10 volumes of *glacial acetic acid,* 20 volumes of *chloroform* and 70 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) extract a quantity of the oromucosal spray containing 15 mg of Benzydamine Hydrochloride with seven 90 ml quantities of *chloroform*. Filter each extract through phase separating paper, evaporate the combined extracts to dryness and dissolve the residue in 10 ml of *methanol*. Solution (2) contains 0.0015% w/v of *1-benzyl-1*H*-indazol-3-ol BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Prepare a 0.075% w/v solution of *1-benzyl-3-(3-diethylamino-propoxy)-1*H*-indazole BPCRS* (internal standard) in *water* (solution A). For solution (1) add 10 ml of solution A, 5 ml of *water*, 5 ml of 1M *sodium hydroxide* and 20 ml of *chloroform* to 5 ml of a solution containing 0.15% w/v of *benzydamine hydrochloride BPCRS* in *water*, shake for 5 minutes, centrifuge and use the chloroform layer. Prepare solution (2) in the same manner as solution (1) but using a quantity of the oromucosal spray containing 7.5 mg of Benzydamine Hydrochloride, diluted, if necessary, to 5 ml with *water* in place of the 5 ml of solution containing 0.15% w/v of *benzydamine hydrochloride BPCRS* in *water*.

The chromatographic procedure may be carried out using a glass column (2 m × 2 mm) packed with *acid-washed, diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 260°, with a flame ionisation detector.

Calculate the content of $C_{19}H_{23}N_3O,HCl$ from the chromatograms obtained using the declared content of $C_{19}H_{23}N_3O,HCl$ in *benzydamine hydrochloride BPCRS*.

LABELLING

The label states (1) the date after which the oromucosal spray is not intended to be used; (2) the conditions under which it should be stored.

Benzyl Benzoate Application

General Notices

DEFINITION

Benzyl Benzoate Application is a *cutaneous emulsion*. It contains 25% w/v of Benzyl Benzoate in a suitable oil-in-water emulsified basis.

Extemporaneous preparation

The following formula and directions apply.

Benzyl Benzoate	250 g
Emulsifying Wax	20 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Melt the Emulsifying Wax, add the Benzyl Benzoate and mix. Pour the mixture into sufficient warm Purified Water to produce 1000 ml and stir thoroughly until cold.

The application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of benzyl benzoate, $C_{14}H_{12}O_2$

23.1 to 26.9% w/v.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0050% w/v of *benzyl benzoate BPCRS* in the mobile phase. For solution (2) dissolve 1 g of the application in sufficient of the mobile phase to produce 100 ml and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 230 nm.

Determine the *weight per ml* of the application, Appendix V G, and calculate the content of $C_{14}H_{12}O_2$, weight in volume, using the declared content of $C_{14}H_{12}O_2$ in *benzyl benzoate BPCRS*.

LABELLING

The label states that the contents of the container should be shaken before use.

The label indicates the pharmaceutical form as 'cutaneous emulsion'.

Benzylpenicillin Injection

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Benzylpenicillin Injection is a sterile solution of Benzylpenicillin Potassium or Benzylpenicillin Sodium in Water for Injections. It is prepared by dissolving Benzylpenicillin for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Benzylpenicillin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

BENZYLPENICILLIN FOR INJECTION

DEFINITION

Benzylpenicillin for Injection is a sterile material consisting of Benzylpenicillin Potassium or Benzylpenicillin Sodium with or without excipients. It is supplied in a sterile container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of penicillins, calculated as $C_{16}H_{18}N_2O_4S$

95.0 to 105.0% of the content of benzylpenicillin stated on the label.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the spectrum of *benzylpenicillin potassium EPCRS* or *benzylpenicillin sodium EPCRS* as appropriate.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of three solutions in *water* containing (1) sufficient of the contents of the sealed container to give a solution containing the equivalent of 0.5% w/v of benzylpenicillin, (2) 0.5% w/v of *benzylpenicillin sodium EPCRS* or 0.5% w/v of *benzylpenicillin potassium EPCRS*, as appropriate, and (3) 0.5% w/v of *benzylpenicillin sodium EPCRS* or 0.5% w/v of *benzylpenicillin potassium EPCRS*, as appropriate, and 0.5% w/v of *phenoxymethylpenicillin potassium EPCRS*. After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

C. Yield reaction A characteristic of *potassium salts* or reaction A characteristic of *sodium salts*, Appendix VI, as appropriate.

TESTS

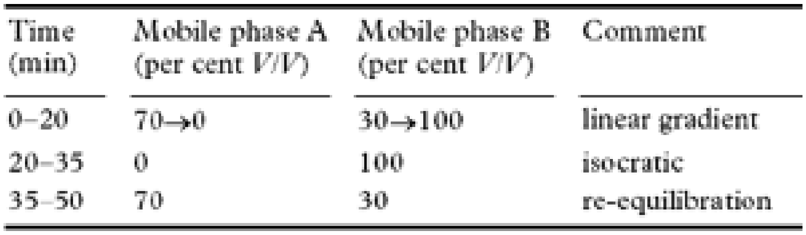
Acidity or alkalinity

pH of a solution containing the equivalent of 10% w/v of benzylpenicillin, 5.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use. For solution (1) dissolve a quantity of the contents of a sealed container containing the equivalent of 80 mg of benzylpenicillin in 20 ml of *water*, mix, filter and use the filtrate. Solution (2) contains 0.0040% w/v of *benzylpenicillin sodium EPCRS* in *water*.

The chromatographic conditions described under Assay may be used. Inject 20 µl of solution (2) and elute isocratically with a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B. Inject 20 µl of solution (1) and elute isocratically with a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B. Immediately after elution of the benzylpenicillin peak start the following linear gradient.



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)	Comment
0–20	70→0	30→100	linear gradient
20–35	0	100	isocratic
35–50	70	30	re-equilibration

Inject *water* and use the same elution pattern to obtain a blank. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried to constant weight at 105°, lose not more than 1.0% of their weight. Use 1 g.

Pyrogens

Comply with the *test for pyrogens*, Appendix XIV D. Use per kg of the rabbit's weight 1 ml of a solution in *water for injections* containing the equivalent of 1.5 mg of benzylpenicllin per ml.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use. For solution (1) dissolve a quantity of the mixed contents of the 10 containers containing the equivalent of 80 mg of benzylpenicillin in 20 ml of *water*, mix, filter and dilute 1 volume of the filtrate to 4 volumes with *water*. Solution (2) contains 0.10% w/v of *benzylpenicillin sodium EPCRS* in *water*. Solution (3) contains 0.020% w/v of *benzylpenicillin sodium EPCRS* and 0.020% w/v of *phenylacetic acid EPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as mobile phase at a flow rate of 1 ml per minute a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B prepared as described below and (c) a detection wavelength of 225 nm.

Mobile phase A Mix 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 3.5 with a 50% w/v solution of *dilute orthophosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*.

Mobile phase B Mix 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 3.5 with a 50% w/v solution of *dilute orthophosphoric acid*, 40 volumes of *water* and 50 volumes of *methanol*.

Equilibrate the column with the mobile phase.

Inject 20 µl of solution (3). The assay is not valid unless, in the chromatogram obtained, the *resolution factor* between the two principal peaks is at least 6.0 (if necessary adjust the ratio A:B of the mobile phase).

Inject alternately solution (1) and solution (2). Calculate the content of penicillins, as $C_{16}H_{18}N_2O_4S$, in a container of average content weight using the declared content of $C_{16}H_{17}N_2NaO_4S$ in *benzylpenicillin sodium EPCRS*. Each mg of $C_{16}H_{17}N_2NaO_4S$ is equivalent to 0.9383 mg of $C_{16}H_{18}N_2O_4S$.

STORAGE

The sealed container should be stored at a temperature not exceeding 30°.

LABELLING

The label of the sealed container states (1) whether the contents are Benzylpenicillin Potassium or Benzylpenicillin Sodium; (2) the quantity of Benzylpenicillin Potassium or Benzylpenicillin Sodium contained in it in terms of the equivalent amount of benzylpenicillin.

Betahistine Dihydrochloride Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Betahistine Dihydrochloride Tablets contain Betahistine Dihydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of betahistine dihydrochloride, $C_8H_{14}Cl_2N_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 24 mg of Betahistine Dihydrochloride with 50 ml of *water* and filter. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 256 nm and a less well-defined maximum at 265 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of the mobile phase to a quantity of the powdered tablets containing 32 mg of Betahistine Dihydrochloride, shake for 10 minutes, add sufficient mobile phase to produce 100 ml, mix, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 500 volumes with the mobile phase. Solution (3) contains 0.00064% w/v of N-*methyl-bis[β-(2-pyridyl)ethyl]amine trihydrochloride BPCRS* in the mobile phase. Solution (4) contains 0.000032% w/v of *2-vinylpyridine hydrochloride* in *acetonitrile*. Solution (5) contains 0.00064% w/v of N-*methyl-bis[β-(2-pyridyl)ethyl]amine trihydrochloride BPCRS* and 0.00064% w/v of *betahistine dihydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax XDB Eclipse is suitable) and maintained at 30°, (b) as mobile phase with a flow rate of 2 ml per minute a mixture prepared as follows: dissolve 0.4 g of *hexylamine* in 600 ml of a solution containing 0.46% w/v of *sodium dihydrogen orthophosphate monohydrate* and 0.27% w/v of *sodium dodecyl sulphate*, add 400 ml of *acetonitrile*, mix, adjust the pH to 3.5 using *orthophosphoric acid* and (c) a detection wavelength of 254 nm.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the two principal peaks is at least 3.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *N*-methyl-bis[β-(2-pyridyl)ethyl]amine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%), the area of any peak corresponding to 2-vinylpyridine is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any other *secondary peaks* is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2%). Disregard any peak with an area less than 0.05% of that of the principal peak in the chromatogram obtained with solution (1) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of the mobile phase to a quantity of the powdered tablets containing 32 mg of Betahistine Dihydrochloride, shake for 10 minutes, add sufficient mobile phase to produce 100 ml, mix, centrifuge and use the supernatant liquid. Solution (2) is a 0.032% w/v solution of *betahistine dihydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.00064% w/v of N-*methyl-bis[β-(2-pyridyl)ethyl]amine trihydrochloride BPCRS* and 0.00064% w/v of *betahistine dihydrochloride BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

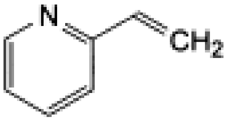
Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

Calculate the content of $C_8H_{14}Cl_2N_2$ in the tablets using the declared content of $C_8H_{14}Cl_2N_2$ in *betahistine dihydrochloride BPCRS*.

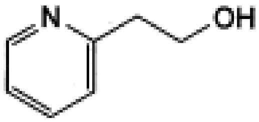
STORAGE

Betahistine Dihydrochloride Tablets should be protected from light and moisture.

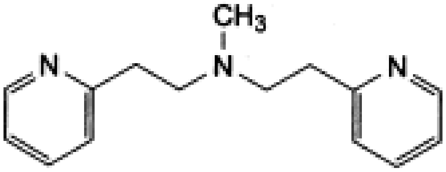
IMPURITIES



A. 2-ethenylpyridine (2-vinylpyridine),



B. 2-(pyridin-2-yl)ethanol,



C. *N*-methyl-2-(pyridin-2-yl)-*N*-[2-(pyridin-2-yl)ethyl]ethanamine.

Betamethasone and Clioquinol Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone and Clioquinol Cream contains Betamethasone Valerate and Clioquinol, the latter in *very fine powder*, in a suitable basis.
The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

Content of clioquinol, C_9H_5ClINO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the preparation being examined containing the equivalent of 0.5 mg of betamethasone in 20 ml of *methanol (80%)* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice and centrifuge. Transfer 10 ml of the supernatant liquid to a separating funnel, add 3 ml of *water* and 5 ml of *chloroform*, shake vigorously, allow the layers to separate and evaporate the chloroform layer to dryness in a current of *nitrogen* with gentle heating. Dissolve the residue in 1 ml of *chloroform*. Solution (2) contains 0.03% w/v of *betamethasone valerate BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay for betamethasone the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (1).

C. In the Assay for clioquinol the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clioquinol in the chromatogram obtained with solution (1).

ASSAY

For betamethasone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix 10 ml of a solution containing 0.024% w/v of *betamethasone valerate BPCRS* and 0.0012% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (80%)* with 5 ml of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (80%)* and dilute to 50 ml with the same solvent. For solution (2) shake a quantity of the cream containing the equivalent of 2 mg of betamethasone with 100 ml of hot *hexane* for 2 minutes, cool, extract the mixture with 20 ml of *ethanol (96%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (75%)*. Repeat the extraction of the hexane mixture with two 10 ml quantities of *ethanol (75%),* filtering each extract in turn through the absorbent cotton and dilute the combined filtrates to 50 ml with *ethanol (75%)*. Prepare solution (3) in the same manner as solution (2) but add 5 ml of a 0.072% w/v solution of the internal standard in *ethanol (80%)* before diluting to 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60 °, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of *absolute ethanol* and *water* adjusted so that the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21-valerate (retention time about 7 minutes) is more than 1.0 (a mixture of 42 volumes of *absolute ethanol* and 58 volumes of *water* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in the cream using the declared content of $C_{22}H_{29}FO_5$ in *betamethasone valerate BPCRS* and using peak areas.

For clioquinol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix 5 ml of a solution containing 0.024% w/v of *clioquinol BPCRS* in an 80% v/v solution of *2-methoxyethanol* and 1 ml of a solution containing 1% w/v of *nickel(II) chloride hexahydrate* in *water* and dilute to 50 ml with the mobile phase. For solution (2) add 80 ml of a hot 80% v/v solution of *2-methoxyethanol* to a quantity of the cream containing 30 mg of Clioquinol and heat on a water bath for 5 minutes, swirling vigorously. Cool to room temperature, dilute to 100 ml with the same solvent, mix and filter. To 5 ml of the filtrate add 1 ml of a solution containing 1% w/v of *nickel(II) chloride hexahydrate* and dilute to 50 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically bonded phenyl groups (5 µm) (Spherisorb Phenyl is suitable), (b) a solution containing 0.024% w/v of *nickel(II) chloride hexahydrate* in a mixture of 2 volumes of *methanol*, 3 volumes of *acetonitrile* and 5 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 273 nm.

Calculate the content of C_9H_5ClINO in the cream using the declared content of C_9H_5ClINO in *clioquinol BPCRS*.

STORAGE

Betamethasone and Clioquinol Cream should be protected from light.

LABELLING

The quantity of active ingredient with respect to Betamethasone Valerate is stated in terms of the equivalent amount of betamethasone.

Betamethasone and Clioquinol Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone and Clioquinol Ointment contains Betamethasone Valerate and Clioquinol, the latter in *very fine powder*, in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

Content of clioquinol, C_9H_5ClINO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Betamethasone and Clioquinol Cream but preparing the solutions in the following manner. For solution (1) disperse a quantity of the ointment containing the equivalent of 1 mg of betamethasone with 10 ml of *methanol* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice and filter. Evaporate the filtrate to dryness in a current of nitrogen and dissolve the residue in 0.5 ml of *chloroform*. Solution (2) contains 0.24% w/v of *betamethasone valerate BPCRS* in *chloroform*.

B. In the Assay for betamethasone the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (1).

C. In the Assay for clioquinol the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clioquinol in the chromatogram obtained with solution (1).

ASSAY

For betamethasone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix 10 ml of a solution containing 0.024% w/v of *betamethasone valerate BPCRS* and 0.0012% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (65%)* with 5 ml of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (65%)* and dilute to 50 ml with *ethanol (65%)*. For solution (2) disperse a quantity of the ointment containing the equivalent of 2 mg of betamethasone in 100 ml of hot *hexane*, cool, extract with 20 ml of *ethanol (65%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (65%)*; repeat the extraction of the hexane mixture with two 10 ml quantities of *ethanol (65%)*, filtering each extract in turn through the absorbent cotton and dilute the combined filtrates to 50 ml with *ethanol (65%)*. Prepare solution (3) in the same manner as solution (2) but add 5 ml of the 0.072% w/v solution of the internal standard in *ethanol (65%)* before diluting to 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60 °, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of *absolute ethanol* and *water* adjusted so that the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21-valerate (retention time about 7 minutes) is more than 1.0 (a mixture of 42 volumes of *absolute ethanol* and 58 volumes of *water* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in the ointment using the declared content of $C_{22}H_{29}FO_5$ in *betamethasone valerate BPCRS*.

For clioquinol

Carry out the Assay described under Betamethasone and Clioquinol Cream.

STORAGE

Betamethasone and Clioquinol Ointment should be protected from light.

LABELLING

The quantity of active ingredient with respect to Betamethasone Valerate is stated in terms of the equivalent amount of betamethasone.

Betamethasone Eye Drops

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Eye Drops are a sterile solution of Betamethasone Sodium Phosphate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of betamethasone sodium phosphate, $C_{22}H_{28}FNa_2O_8P$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 60 volumes of *butan-1-ol*, 20 volumes of *acetic anhydride* and 20 volumes of *water*, prepared immediately before use, as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use the eye drops, diluted if necessary with *water* to contain 0.1% w/v of Betamethasone Sodium Phosphate. Solution (2) contains 0.1% w/v of *betamethasone sodium phosphate BPCRS* in *water*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). Solution (4) is a mixture of equal volumes of solution (2) and a 0.1% w/v solution of *prednisolone sodium phosphate BPCRS* in *water*. After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and examine under *ultraviolet light (254 nm)*. The chromatograms obtained with solutions (1), (2) and (3) show single principal spots with similar Rf values. The chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone sodium phosphate in the chromatogram obtained with solution (1).

C. To a volume containing 0.2 mg of Betamethasone Sodium Phosphate, add slowly 1 ml of *sulphuric acid* and allow to stand for 2 minutes. A brownish yellow colour but no red colour or yellowish green fluorescence is produced.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following three solutions in the mobile phase. For solution (1) dilute 1 volume of solution (2) to 50 volumes with *water*. For solution (2) dilute the eye drops if necessary to give a solution containing 0.10% w/v of Betamethasone Sodium Phosphate. Solution (3) contains 0.0060% w/v each of *betamethasone sodium phosphate BPCRS* and *betamethasone*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) a mixture of 60 volumes of *citro-phosphate buffer pH 5.0* and 40 volumes of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 241 nm.

Inject 20 µl of solution (3). The test is not valid unless the *resolution factor* between the peaks due to betamethasone sodium phosphate and betamethasone is at least 3.5.

Inject separately 20 µl of each of solutions (1) and (2) and record the chromatogram for three times the retention time of the principal peak. In the chromatogram obtained with solution (2) the area of any peak corresponding to betamethasone is not greater than 1.3 times the area of the principal peak in the chromatogram obtained with solution (1), the area of any other *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (1) and the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (1). Disregard any peak the area of which is less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is a mixture of 5 ml of a 0.1% w/v solution of *betamethasone sodium phosphate BPCRS* in *water* (solution A) and 10 ml of a 0.06% w/v solution of *hydrocortisone* (internal standard) in *methanol* and sufficient *water* to produce 25 ml. For solution (2) mix a quantity of the eye drops containing 5 mg of Betamethasone Sodium Phosphate with 10 ml of *methanol* and dilute to 25 ml with *water*. Prepare solution (3) in the same manner as solution (2) but using 10 ml of the internal standard solution in place of the methanol.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) a mixture of 55 volumes of *citro-phosphate buffer pH 5.0* and 45 volumes of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 241 nm.

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in solution A by measuring the *absorbance*, Appendix II B, of an aliquot diluted with *water* to contain 0.002% w/v of Betamethasone Sodium Phosphate at the maximum at 241 nm and taking 297 as the value of A(1%, 1 cm) at the maximum at 241 nm. Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in the eye drops using peak areas.

STORAGE

Betamethasone Eye Drops should be protected from light.

Betamethasone Injection

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a volume containing the equivalent of 4 mg of betamethasone add 1 ml of *water* and sufficient *absolute ethanol* to produce 40 ml. Place 2 ml of the solution in a stoppered tube, add 10 ml of *phenylhydrazine-sulphuric acid solution*, mix, warm in a water bath at 60° for 20 minutes and cool immediately. The *absorbance* of the resulting solution at the maximum at 450 nm is not more than 0.1, Appendix II B.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 60 volumes of *butan-1-ol*, 20 volumes of *acetic anhydride* and 20 volumes of *water*, prepared immediately before use, as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) use the injection, if necessary diluted with *water* to contain the equivalent of 2 mg of betamethasone per ml. Solution (2) contains 0.25% w/v solution of *betamethasone sodium phosphate BPCRS* in *water*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). Solution (4) is a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *prednisolone sodium phosphate BPCRS* in *water*. After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and examine under *ultraviolet light (254 nm)*. The chromatograms obtained with solutions (1), (2) and (3) show single spots with similar Rf values. The chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values. *Secondary spots* due to excipients may also be observed in the chromatograms obtained with solutions (1), (3) and (4).

C. Evaporate a volume containing the equivalent of 2 mg of betamethasone to dryness on a water bath, dissolve the residue in 2 ml of *sulphuric acid* and allow to stand for 2 minutes. No red colour is produced.

TESTS

Alkalinity

pH, 8.0 to 9.0, Appendix V L.

Colour

The injection, diluted if necessary with *water* to contain the equivalent of 2 mg of betamethasone per ml, is not more intensely coloured than *reference solution* BY_4, Appendix IV B, Method I.

Related substances

Complies with test described under Betamethasone Eye Drops using the following three solutions. For solution (1) dilute 1 volume of solution (2) to 50 volumes with the mobile phase. For solution (2) dilute the injection with the mobile phase to give a solution containing the equivalent of 0.10% w/v of betamethasone. Solution (3) contains 0.0060% w/v each of *betamethasone sodium phosphate BPCRS* and *betamethasone*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. For solution (1) dilute a volume of the injection containing 8 mg of betamethasone to 50 ml with *methanol (50%)*. For solution (2) dilute 5 ml of a 0.045% w/v solution of *betamethasone sodium phosphate BPCRS* in *water* (solution A) to 10 ml with *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 241 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in the injection, determining the exact strength of $C_{22}H_{29}FO_5$ in solution (2) as follows. Dilute 3 ml of solution A to 50 ml with *water* and measure the *absorbance*, Appendix II B, of the resulting solution at the maximum at 241 nm, taking 391 as the value of A(1%, 1 cm) for betamethasone.

STORAGE

Betamethasone Injection should be stored at a temperature not exceeding 30° and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone in a suitable dose-volume.

Betamethasone Sodium Phosphate Tablets

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Sodium Phosphate Tablets contain Betamethasone Sodium Phosphate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 60 volumes of *butan-1-ol*, 20 volumes of *acetic anhydride* and 20 volumes of *water* prepared immediately before use as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dissolve a quantity of the powdered tablets containing the equivalent of 2 mg of betamethasone in 25 ml of *water*, add 2.5 g of *sodium chloride* and 1 ml of *hydrochloric acid*, extract with 25 ml of *chloroform* and discard the chloroform layer. Extract with 25 ml of *tributyl orthophosphate* and discard the aqueous layer. Prepare solution (2) in the same manner as solution (1) but using 2.5 mg of *betamethasone sodium phosphate BPCRS* in place of the powdered tablets. Solution (3) is a mixture of equal volumes of solutions (1) and (2). Solution (4) is a mixture of equal volumes of solution (1) and a solution prepared in the same manner as solution (1) but using 2.5 mg of *prednisolone sodium phosphate BPCRS* in place of the powdered tablets. After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes, spray the hot plate with *ethanolic sulphuric acid (20%)* and again heat at 110° for 10 minutes. The chromatograms obtained with solutions (1), (2) and (3) show single spots with similar Rf values. The chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

B. Mix a quantity of the powdered tablets containing the equivalent of 0.4 mg of betamethasone with 1 ml of *sulphuric acid* and allow to stand for 5 minutes. A pale yellow colour is produced (distinction from prednisolone sodium phosphate tablets).

TESTS

Disintegration

Maximum time, 5 minutes, Appendix XII A1.

Uniformity of content

Tablets containing less than the equivalent of 2 mg of betamethasone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. Solution (1) is a mixture of equal volumes of a solution containing 0.0065% w/v of *betamethasone sodium phosphate BPCRS* in *water* and a solution containing 0.006% w/v of *hydrocortisone* in *methanol* (internal standard solution). For solution (2) dissolve one tablet as completely as possible in 5 ml of *water* and add 5 ml of *methanol*. For solution (3) dissolve one tablet as completely as possible in 5 ml of *water* and add 5 ml of the internal standard solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) a mixture of 55 volumes of *citro-phosphate buffer pH 5.0* and 45 volumes of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 241 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in each tablet, determining the exact strength of the solution of *betamethasone sodium phosphate BPCRS* as described in the Assay.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. For solution (1) shake a quantity of the powdered tablets containing 2.5 mg of betamethasone for 20 minutes with 25 ml of *water*, dilute to 50 ml with *methanol*, mix and filter through a glass fibre filter (Whatman GF/C is suitable). For solution (2) dilute 5 ml of a 0.014% w/v solution of *betamethasone sodium phosphate BPCRS* in *water* (solution A) to 10 ml with *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 241 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets, determining the exact strength of $C_{22}H_{29}FO_5$ in solution (2) as follows. Dilute 5 ml of solution A to 25 ml with *water* and measure the *absorbance*, Appendix II B, of the resulting solution at the maximum at 241 nm, taking 391 as the value of A(1%, 1 cm) for betamethasone.

STORAGE

Betamethasone Sodium Phosphate Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

Betamethasone Tablets

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Tablets contain Betamethasone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 25 mg of Betamethasone with 150 ml of *dichloromethane* for 30 minutes, filter, wash the filtrate with 20 ml of *water*, dry over *anhydrous sodium sulphate*, evaporate the solution to dryness and dry the residue at 105° for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of betamethasone *(RS 029)*.

B. The residue obtained in test A complies with the test for the *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase A*. At a fourth point apply to the plate 2 µl of a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *dexamethasone BPCRS* in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*. The chromatogram obtained with this solution shows two principal spots with almost identical Rf values.

C. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone in the chromatogram obtained with solution (1).

TESTS

Uniformity of content

Tablets containing less than 2 mg of Betamethasone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, protected from light using the following solutions. Solution (1) contains 0.0025% w/v of *betamethasone BPCRS* and 0.002% w/v of *hydrocortisone* (internal standard) in *methanol (50%)*. For solution (2) finely crush one tablet, add 20 ml of a 0.002% w/v solution of *hydrocortisone* in *methanol (50%)*, shake for 10 minutes and filter through a glass-fibre filter paper (Whatman GF/C is suitable).

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 53 volumes of *water* and 47 volumes of *methanol* as the mobile phase with a flow rate of 1.4 ml per minute and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in each tablet taking 100.0% as the content of $C_{22}H_{29}FO_5$ in *betamethasone BPCRS*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light using the following solutions. Solution (1) contains 0.0125% w/v of *betamethasone BPCRS* and 0.010% w/v of *hydrocortisone* (internal standard) in *methanol (50%)*. For solution (2) weigh and powder 20 tablets; to a quantity of the powder containing 2.5 mg of Betamethasone add 20 ml of *methanol (50%)*, shake for 10 minutes and filter through a glass-fibre filter paper (Whatman GF/C is suitable). Prepare solution (3) in the same manner as solution (2) but use 20 ml of a 0.01% w/v solution of *hydrocortisone* in *methanol (50%)* in place of the 20 ml of methanol (50%).

The chromatographic conditions described under Uniformity of content may be used.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets from the declared content of $C_{22}H_{29}FO_5$ in *betamethasone BPCRS*.

STORAGE

Betamethasone Tablets should be protected from light.

Betamethasone Valerate Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Valerate Cream contains Betamethasone Valerate in a suitable basis.
The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Betamethasone Valerate Lotion using the following solutions. For solution (1) disperse a quantity of the cream containing the equivalent of 0.5 mg of betamethasone in 20 ml of *methanol (80%)* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *chloroform*, shake vigorously, allow the layers to separate and evaporate the chloroform layer to dryness in a current of nitrogen with gentle heating. Dissolve the residue in 1 ml of *chloroform*. Solution (2) contains 0.03% w/v of *betamethasone valerate BPCRS* in *chloroform*.

C. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix 10 ml of a solution containing 0.024% w/v of *betamethasone valerate BPCRS* and 0.0012% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (80%)* with 5 ml of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (80%)* and dilute to 50 ml with the same solvent. For solution (2) shake a quantity of the cream containing the equivalent of 2 mg of betamethasone with 100 ml of hot *hexane* for 2 minutes, cool, extract the mixture with 20 ml of *ethanol (96%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (75%)*. Repeat the extraction of the hexane mixture with two 10 ml quantities of *ethanol (75%)*, filtering each extract in turn through the absorbent cotton and dilute the combined filtrates to 50 ml with *ethanol (75%)*. Prepare solution (3) in the same manner as solution (2) but add 5 ml of a 0.072% w/v solution of the internal standard in *ethanol (80%)* before diluting to 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of *absolute ethanol* and *water* adjusted so that the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21-valerate (retention time about 7 minutes) is more than 1.0 (a mixture of 42 volumes of *absolute ethanol* and 58 volumes of *water* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in the cream using the declared content of $C_{22}H_{29}FO_5$ in *betamethasone valerate BPCRS* and using peak areas.

STORAGE

Betamethasone Valerate Cream should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

Betamethasone Valerate Lotion

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Valerate Lotion is a *cutaneous solution*. It contains Betamethasone Valerate in a suitable vehicle.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform*, 10 volumes of *acetone* and 5 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the preparation being examined containing the equivalent of 1 mg of betamethasone with 5 ml of *methanol (80%)* by heating on a water bath until the methanol begins to boil. Shake vigorously, cool in ice and filter. Transfer the filtrate to a separating funnel and add 0.5 ml of *water* and 1 ml of *chloroform*. Shake vigorously, allow the layers to separate and use the chloroform layer. Solution (2) contains 0.05% w/v of *betamethasone valerate BPCRS* in *chloroform*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix 20 ml of a solution containing 0.018% w/v of *betamethasone valerate BPCRS* and 0.0010% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (65%)* with 5 ml of a 0.11% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (65%)*. For solution (2) disperse a quantity of the preparation being examined containing the equivalent of 3 mg of betamethasone in a mixture of 10 ml of *ethanol (65%)* and 50 ml of *hexane*, shake for 2 minutes and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (65%)*. Repeat the extraction of the hexane mixture with two 5 ml quantities of *ethanol (65%)*, filtering the ethanol extracts through the absorbent cotton, add 5 ml of *ethanol (65%)* to the combined filtrates and mix. Prepare solution (3) in the same manner as solution (2) but adding 5 ml of a 0.11% w/v solution of the internal standard in *ethanol (65%)* in place of the 5 ml of ethanol (65%).

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60^{c}, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of *absolute ethanol* and *water* adjusted so that baseline separation is obtained between betamethasone valerate (retention time about 5 minutes) and betamethasone 21-valerate (retention time about 7 minutes) and between betamethasone 21-valerate and beclomethasone dipropionate (retention time about 8 minutes) (a mixture of 42 volumes of *absolute ethanol* and 58 volumes of *water* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in the preparation being examined using the declared content of $C_{22}H_{29}FO_5$ in *betamethasone valerate BPCRS*.

STORAGE

Betamethasone Valerate Lotion should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

The label indicates the pharmaceutical form as 'cutaneous solution'.

Betamethasone Valerate Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Valerate Ointment contains Betamethasone Valerate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Betamethasone Valerate Lotion preparing solution (1) in the following manner. Disperse a quantity of the ointment containing the equivalent of 1 mg of betamethasone in 10 ml of *methanol* by heating on a water bath until the methanol begins to boil, shake vigorously, cool in ice for 30 minutes and filter. Evaporate the filtrate to dryness in a current of nitrogen with gentle heating and dissolve the residue in 0.5 ml of *chloroform*. Solution (2) contains 0.24% w/v of *betamethasone valerate BPCRS* in *chloroform*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix 10 ml of a solution containing 0.024% w/v of *betamethasone valerate BPCRS* and 0.0012% w/v of *betamethasone 21-valerate BPCRS* in *ethanol (65%)* with 5 ml of a 0.072% w/v solution of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (65%)* and dilute to 50 ml with *ethanol (65%)*. For solution (2) disperse a quantity of the ointment containing the equivalent of 2 mg of betamethasone in 100 ml of hot *hexane*, cool, extract with 20 ml of *ethanol (65%)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (65%)*; repeat the extraction of the hexane mixture with two 10 ml quantities of *ethanol (65%)*, filtering each extract in turn through the absorbent cotton and dilute the combined filtrates to 50 ml with *ethanol (65%)*. Prepare solution (3) in the same manner as solution (2) but add 5 ml of the 0.072% w/v solution of the internal standard in *ethanol (65%)* before diluting to 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60^{c}, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of *absolute ethanol* and *water* adjusted so that the *resolution factor* between the peaks due to betamethasone valerate (retention time about 5 minutes) and betamethasone 21valerate (retention time about 7 minutes) is more than 1.0 (a mixture of 42 volumes of *absolute ethanol* and 58 volumes of *water* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in the ointment using the declared content of $C_{22}H_{29}FO_5$ in *betamethasone valerate BPCRS*.

STORAGE

Betamethasone Valerate Ointment should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

Betamethasone Valerate Scalp Application

General Notices

Action and use

Glucocorticoid.

DEFINITION

Betamethasone Valerate Scalp Application is a *cutaneous solution*. It contains Betamethasone Valerate in a suitable liquid basis.

The application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of betamethasone, $C_{22}H_{29}FO_5$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Betamethasone Valerate Lotion, preparing solution (1) by diluting a suitable volume of the application with *absolute ethanol* to give a solution containing the equivalent of 0.04% w/v of betamethasone.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to betamethasone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the Assay described under Betamethasone Valerate Lotion, preparing solutions (2) and (3) in the following manner. For solution (2) dilute a quantity of the application containing the equivalent of 3 mg of betamethasone to 25 ml with *ethanol (65%)*. For solution (3) add 5 ml of a 0.11% w/v solution of the internal standard to a quantity of the application containing the equivalent of 3 mg of betamethasone and dilute to 25 ml with *ethanol (65%)*.

STORAGE

Betamethasone Valerate Scalp Application should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

The label indicates the pharmaceutical form as 'cutaneous solution'.

Betaxolol Eye Drops, Solution

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Betaxolol Eye Drops, Solution are a sterile solution of Betaxolol Hydrochloride in Purified Water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of betaxolol, $C_{18}H_{29}O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 plates are suitable) and as the mobile phase a mixture of 30 volumes of a solution prepared by diluting 1 volume of 13.5M *ammonia* to 50 volumes with *propan-2-ol* immediately before use and 70 volumes of *chloroform*. Apply separately to the plate 5 µl of each of the following solutions in *water*. For solution (1) dilute the eye drops to produce a solution containing the equivalent of 0.1% w/v of betaxolol. Shake 1 ml of the solution with 4 ml of *water*, 0.1 ml of 13.5M *ammonia* and 2 ml of *chloroform*, centrifuge and use the chloroform layer. Prepare solution (2) in the same manner as solution (1) but using a 0.1% w/v solution of *betaxolol hydrochloride BPCRS* in place of the eye drops. Solution (3) is a mixture of equal volumes of solution (1) and solution (2). After removal of the plate, allow it to dry in air and spray with a solution prepared by dissolving 5 g of *iodine* and 10 g of *potassium iodide* in sufficient *water* to produce 100 ml and mixing 20 ml of the resulting solution with 30 ml of *water* and 50 ml of 2M *acetic acid*. Examine the plate immediately; spots due to betaxolol appear brown. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2) .

TESTS

Acidity or alkalinity

pH, 6.0 to 7.8, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For solution (1) dilute a suitable volume of the eye drops with sufficient of the mobile phase to produce a solution containing the equivalent of 0.02% w/v of betaxolol. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS-2 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 45 volumes of a buffer solution prepared as described below and 55 volumes of *acetonitrile* and (c) a detection wavelength of 220 nm. To prepare the buffer solution add 5 ml of *orthophosphoric acid* to 990 ml of *water*, adjust the pH to 3.0 with 2M *ammonia* and add sufficient *water* to produce 1000 ml. Dissolve 3 g of *sodium dodecyl sulphate* in 450 ml of the resulting solution.

The test is not valid unless the *column efficiency*, determined on the peak due to betaxolol in the chromatogram obtained with solution (2), is at least 8000 *theoretical plates* per metre and the *symmetry factor* of the principal peak is not more than 2.5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the area of not more than one such peak is greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops with sufficient of the mobile phase to produce a solution containing the equivalent of 0.01% w/v of betaxolol. Solution (2) contains 0.012% w/v of *betaxolol hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.012% w/v of *betaxolol hydrochloride BPCRS* and 0.006% w/v of *pilocarpine nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS-2 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water* containing 0.71% w/v of *anhydrous disodium hydrogen orthophosphate* and 0.91% w/v of *dimethylamine hydrochloride*, adjusted to pH 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 220 nm.

Inject 10 µl of each solution. The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to betaxolol and pilocarpine is at least 1.5.

Calculate the content of $C_{18}H_{29}O_3$ in the eye drops using the declared content of $C_{18}H_{29}O_3$ in *betaxolol hydrochloride BPCRS*.

STORAGE

Betaxolol Eye Drops, Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betaxolol.

Betaxolol Eye Drops, Suspension

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Betaxolol Eye Drops, Suspension are a sterile suspension of Betaxolol Hydrochloride in Purified Water containing suitable binding and suspending agents.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

The eye drops should be shaken vigorously before carrying out the following tests.

Content of betaxolol, $C_{18}H_{29}O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Comply with the tests described under Betaxolol Eye Drops, Solution.

TESTS

Particle size

Examine using an automated light obscuration instrument such as that described in Appendix XIII A. Not less than 99.5% of the particles are less than 25 µm, not less than 99.95% are less than 50 µm and none exceeds 75 µm.

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

Related substances

Comply with the test described under Betaxolol Eye Drops, Solution, but preparing solution (1) in the following manner. Dilute a suitable volume of the eye drops with sufficient of the mobile phase to produce a solution containing the equivalent of 0.02% w/v of betaxolol, centrifuge and use the supernatant liquid.

Bound betaxolol

45.0 to 65.0% of the content of betaxolol, when determined by the following method. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a weighed quantity of the eye drops with sufficient of the mobile phase to produce a suspension containing the equivalent of 0.01% w/v of betaxolol, mix with the aid of ultrasound for 15 minutes and allow to cool. Centrifuge at a speed of 2000 revolutions per minute for 10 minutes and use the supernatant liquid. For solution (2) centrifuge the eye drops at a speed of 25,000 revolutions per minute for 30 minutes and dilute a weighed quantity of the supernatant liquid with sufficient of the mobile phase to produce a solution expected to contain the equivalent of 0.01% w/v of betaxolol. Solution (3) contains 0.012% w/v of *betaxolol hydrochloride BPCRS* in the mobile phase.

The chromatographic conditions described under Assay may be used.

Determine the *weight per ml* of the eye drops, Appendix V G, and calculate the content of $C_{18}H_{29}O_3$, weight in volume, in solutions (1) and (2) using the declared content of $C_{18}H_{29}O_3$ in *betaxolol hydrochloride BPCRS*. Calculate the content of bound betaxolol from the difference between the contents of betaxolol found in solutions (1) and (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a weighed quantity of the eye drops with sufficient of the mobile phase to produce a suspension containing the equivalent of 0.01% w/v of betaxolol, mix thoroughly, centrifuge and use the supernatant liquid. Solution (2) contains 0.012% w/v of *betaxolol hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.012% w/v of *betaxolol hydrochloride BPCRS* and 0.006% w/v of *pilocarpine nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS-2 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water* containing 0.71% w/v of *anhydrous disodium hydrogen orthophosphate* and 0.91% w/v of *dimethylamine hydrochloride*, adjusted to pH 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 220 nm.

Inject 10 µl of each solution. The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to betaxolol and pilocarpine is at least 1.5.

Determine the *weight per ml* of the eye drops, Appendix V G, and calculate the content of $C_{18}H_{29}O_3$, weight in volume, using the declared content of $C_{18}H_{29}O_3$ in *betaxolol hydrochloride BPCRS*.

STORAGE

Betaxolol Eye Drops, Suspension should be protected from light and stored in accordance with the manufacturer's instructions.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of betaxolol.

Bezafibrate Tablets

General Notices

Action and use

Fibrate; lipid-regulating drug.

DEFINITION

Bezafibrate Tablets contain Bezafibrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bezafibrate, $C_{19}H_{20}ClNO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.2 g of Bezafibrate with two 10-ml quantities of *acetone* for 10 minutes, combine and filter the extracts (a Whatman GF/C is suitable) and evaporate the filtrate to dryness. The infrared absorption spectrum of the residue, Appendix II A, is concordant with the reference spectrum of bezafibrate *(RS 419)*.

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a pH 6.5 buffer solution prepared by dissolving 0.608 g of *sodium hydroxide* and 6.805 g of *potassium dihydrogen orthophosphate* in sufficient *water* to produce 1000 ml and adjusting the pH to 6.5 ± 0.05 using *sodium hydroxide solution* or *orthophosphoric acid*. Rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute 1 volume of the filtrate to 20 volumes with the dissolution medium. Measure the *absorbance* of the solution at the maximum at 229 nm, Appendix II B, using dissolution medium in the reference cell. Calculate the total content of bezafibrate, $C_{19}H_{20}ClNO_4$, in the medium from the *absorbance* of a 0.0011% w/v solution of *bezafibrate BPCRS* in the dissolution medium and using the declared content of $C_{19}H_{20}ClNO_4$ in *bezafibrate BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 100 mg of Bezafibrate with 15 ml of *methanol* for 2 minutes, shake for a further 10 minutes, cool, add sufficient of the mobile phase to produce 100 ml, mix and filter, discarding the first 20 ml of filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dissolve sufficient quantities of *bezafibrate BPCRS* and *chlorobenzoyltyramine BPCRS* in the minimum quantity of *methanol* and dilute with mobile phase to produce a solution containing 0.0002% w/v of *bezafibrate BPCRS* and 0.0002% w/v of *chlorobenzoyltyramine BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable)

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 239 nm.

(f) Injection volume of 20 µl for each solution.

The retention time of bezafibrate is about 5 minutes. Allow the chromatography to proceed for four times the retention time of the principal peak.

MOBILE PHASE

A mixture of 3.9 volumes of 40% w/v *tetrabutylammonium hydroxide*, 400 volumes of *acetonitrile* and 600 volumes of *water* and adjusting the final pH to 4.0 with 10% v/v *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to bezafibrate and chlorobenzoyltyramine is at least 7.0. If necessary, adjust the content of tetrabutylammonium hydroxide to obtain the required resolution.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total area of any such peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.05%).

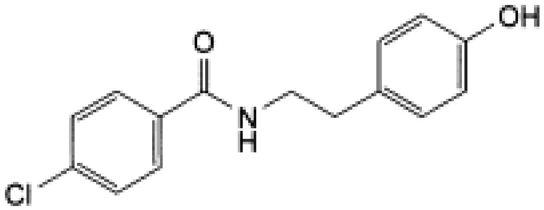
ASSAY

Weigh and powder 20 tablets. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing 100 mg of Bezafibrate with 70 ml of *methanol* for 2 minutes, shake for a further 10 minutes, cool, add sufficient *methanol* to produce 100 ml, mix and filter discarding the first 20 ml of filtrate; dilute 1 volume of the filtrate to 100 volumes with *methanol*. Solution (2) contains 0.001% w/v of *bezafibrate BPCRS* in *methanol*.

Measure the *absorbance* at the maximum at 229 nm, Appendix II B. Calculate the content of $C_{19}H_{20}ClNO_4$ in the tablets from the absorbances obtained and from the declared content of $C_{19}H_{20}ClNO_4$ in *bezafibrate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include,



4-chloro-*N*-[2-(4-hydroxyphenyl)ethyl]benzamide (chlorobenzoyltyramine).

Bisacodyl Suppositories

General Notices

Action and use

Stimulant laxative.

DEFINITION

Bisacodyl Suppositories contain Bisacodyl in a suitable suppository basis.
The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of bisacodyl, $C_{22}H_{19}NO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method described under Related substances applying to the plate 2 µl of each solution and using as solution (2) a 1% w/v solution of *bisacodyl EPCRS* in *acetone*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve a quantity of the suppositories containing 0.15 g of Bisacodyl as completely as possible in 150 ml of *petroleum spirit (boiling range, 40° to 60°)*, filter, wash the residue with *petroleum spirit (boiling range, 40° to 60°)* until free from fatty material and dry at about 100°. Wash with a very small quantity of warm *chloroform* and dissolve the residue in 10 ml of a 0.5% v/v solution of *sulphuric acid*. To 2 ml of the solution add 0.05 ml of *potassium tetraiodomercurate solution*. A white precipitate is produced.

C. To 2 ml of the solution obtained in test B add *sulphuric acid*. A reddish violet colour is produced.

D. Boil 2 ml of the solution obtained in test B with a little *nitric acid*; a yellow colour is produced. Cool and add 5M *sodium hydroxide*; the colour becomes yellowish brown.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of equal volumes of *butan-2-one* and *xylene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the suppositories containing 20 mg of Bisacodyl with 20 ml of *petroleum spirit (boiling range, 40° to 60°)*, filter, wash the residue with *petroleum spirit (boiling range, 40° to 60°)* until free from fat and dissolve in 2 ml of *acetone*. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

To a quantity of the suppositories containing 0.1 g of Bisacodyl add 80 ml of *anhydrous acetic acid* previously neutralised with 0.02M *perchloric acid VS* to *1-naphthol-benzein solution*, warm gently until solution is complete and immediately carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.02M *perchloric acid VS* and determining the end point potentiometrically. Each ml of 0.02M *perchloric acid VS* is equivalent to 7.228 mg of $C_{22}H_{19}NO_4$. Calculate the average content of bisacodyl, $C_{22}H_{19}NO_4$, in the suppositories.

Black Currant Syrup

General Notices

DEFINITION

Black Currant Syrup is prepared either from the clarified juice of Black Currant or from concentrated black currant juice of commerce. It contains a suitable antioxidant. Permitted food grade colours may be added.

PRODUCTION

It is prepared by dissolving 700 g of Sucrose either in 560 ml of clarified juice, previously diluted with Water to a weight per ml of 1.045 g, or in 560 ml of a solution of the same weight per ml prepared from the concentrated juice of commerce and Water, and adding to this solution sufficient Benzoic Acid to give a final concentration of not more than 800 ppm, or sufficient Sodium Metabisulphite or other suitable sulphite to give a final concentration of not more than 350 ppm of sulphur dioxide.

The syrup complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ascorbic acid[1], $C_6H_8O_6$

Not less than 0.055% w/w.

TESTS

Sulphur dioxide

Not more than 350 ppm, Appendix IX B.

Weight per ml

1.27 to 1.30 g, Appendix V G.

ASSAY

Mix 5 g with 25 ml of a freshly prepared 20% w/v solution of *metaphosphoric acid*, add 20 ml of *acetone* and dilute to 100 ml with *water*. To four 3 ml quantities of this solution add 0.4, 0.5, 0.6 and 0.7 ml, respectively, of *double-strength standard 2,6-dichlorophenolindophenol solution*, mix well by agitation with a fine stream of carbon dioxide, add 3 ml of *chloroform*, agitate for a further 15 seconds, examine the solutions against a white background and select the two that are on either side of the end point (that is, one colourless and one pink). Prepare a further six solutions as directed above, but adding to the first an amount of dye solution equal to that added to the selected colourless solution, successively increasing this volume by 0.02 ml increments in the second to the fifth solutions and adding to the sixth solution a volume equal to that added to the selected pink solution. Select the solution exhibiting the faintest pink colour. Each ml of *double-strength standard 2,6-dichlorophenolindophenol solution* added to this solution is equivalent to 0.200 mg of $C_6H_8O_6$.

STORAGE

Black Currant Syrup should be kept in a well-filled container and protected from light.

Black Currant Syrup contains, in 10 ml, about 7.5 mg of ascorbic acid.

[1] The requirement for Content of ascorbic acid does not apply when

Bleomycin Injection

General Notices

Action and use

Cytotoxic antibacterial.

DEFINITION

Bleomycin Injection is a sterile solution of Bleomycin Sulphate in a suitable liquid. It is prepared by dissolving Bleomycin Sulphate for Injection in the requisite amount of the liquid stated on the label before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Bleomycin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

BLEOMYCIN SULPHATE FOR INJECTION

DEFINITION

Bleomycin Sulphate for Injection is a sterile material consisting of Bleomycin Sulphate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of bleomycin sulphate *(RS 367)*.

B. In the test for Composition, the retention time and size of the two principal peaks in the chromatogram obtained with solution (1) are approximately the same as those of the two principal peaks in the chromatogram obtained with solution (2).

C. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

Dissolve a quantity in sufficient *carbon dioxide-free water* to produce a solution containing 10,000 IU of bleomycin per ml. The pH of the resulting solution is 4.5 to 6.0, Appendix V L.

Colour of solution

Dissolve a quantity in sufficient *water* to produce a solution containing 40,000 IU of bleomycin per ml. The *absorbance* of the solution at 430 nm is not greater than 0.15, Appendix II B.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, lose not more than 6.0% of their weight. Use the combined contents of two containers.

Composition

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of a sealed container in sufficient *water* to produce a solution containing 1000 IU of bleomycin per ml. Solution (2) contains 0.05% w/v of *bleomycin sulphate EPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (7 µm) (Nucleosil C18 is suitable), (b) as the mobile phases at a flow rate of 1.2 ml per minute the solutions described below and (c) a detection wavelength of 254 nm. Use as the initial mobile phase a mixture of 10 volumes of *methanol* and 90 volumes of a mixture prepared in the following manner: dissolve 0.960 g of *sodium pentanesulphonate* in 900 ml of 0.08M *acetic acid*, add 1.86 g of *disodium edetate*, dilute to 1000 ml with 0.08M *acetic acid* and adjust to pH 4.3 with 18M *ammonia*. Maintain the initial mobile phase for 15 minutes, increase the proportion of *methanol* to 40 volumes over 60 minutes and maintain the final mobile phase until demethylbleomycin A_2 is eluted (about 20 minutes; retention time 1.5 to 2.5, relative to bleomycin A_2).

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 5.

Calculate the percentage content by *normalisation*. The content of bleomycin A_2 (first principal peak) is between 55% and 70%; the content of bleomycin B_2 is between 25% and 32%; the sum of the contents of bleomycin A_2 and bleomycin B_2 is not less than 85%; the content of demethylbleomycin A_2 (retention time 1.5 to 2.5, relative to bleomycin A_2) is not greater than 8%; the total content of other related substances is not greater than 9.5%. Disregard any impurity present at less than 0.1%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to produce a solution containing 15,000 IU of bleomycin per ml (solution A). The endotoxin limit concentration of solution A is 50 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Mix the contents of the containers and carry out the *microbiological assay of antibiotics*, Appendix XIV A, Method A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

For a container of average content weight, the upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the total number of IU (Units) contained in it.

Bretylium Injection

General Notices

Action and use

Antiarahythmic.

DEFINITION

Bretylium Injection is a sterile solution of Bretylium Tosilate in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of bretylium tosilate, $C_{18}H_{24}BrNO_3S$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Dry a quantity of the injection containing 50 mg of Bretylium Tosilate over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 16 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of bretylium tosilate *(RS 030)*. If the spectra are not concordant, dissolve a sufficient quantity of the residue in the minimum volume of *acetone* by heating on a water bath at 50°, evaporate to dryness at room temperature under a current of nitrogen and prepare a new spectrum of the residue.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck plates are suitable) and a mixture of 15 volumes of *glacial acetic acid ,* 30 volumes of *water* and 75 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute the injection with *water*, if necessary, to contain 0.5% w/v of Bretylium Tosilate. Solution (2) contains 0.5% w/v of *bretylium tosilate BPCRS* in *water*. After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) the two principal spots correspond to those in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 5.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For solution (1) dilute the injection with sufficient of the mobile phase to produce a solution containing 0.2% w/v of Bretylium Tosilate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.05% w/v of *bretylium tosilate BPCRS* and 0.05% w/v of *2-bromobenzyldimethylamine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemically-bonded phenyl groups (5 µm) (Spherisorb Phenyl is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 0.5 volume of *triethylamine*, 2 volumes of *glacial acetic acid*, 19 volumes of *acetonitrile* and 81 volumes of 0. 01M *sodium octanesulphonate* and (c) a detection wavelength of 265 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 6.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%). Disregard any peak due to tosilate (retention time, about 2 minutes) and any peak with an area less than 0.05 times the area of the peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection with sufficient of the mobile phase to produce a solution containing 0.05% w/v of Bretylium Tosilate. Solution (2) contains 0.05% w/v of *bretylium tosilate BPCRS* in the mobile phase. Solution (3) contains 0.05% w/v of *bretylium tosilate BPCRS* and 0.05% w/v of *2-bromobenzyldimethylamine hydrochloride BPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 6.

Calculate the content of $C_{18}H_{24}BrNO_3S$ in the injection from the area of the peaks corresponding to bretylium and using the declared content of $C_{18}H_{24}BrNO_3S$ in *bretylium tosilate BPCRS*.

STORAGE

Bretylium Injection should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Bretylium Tosilate.

Bromocriptine Capsules

General Notices

Action and use

Dopamine receptor agonist.

DEFINITION

Bromocriptine Capsules contain Bromocriptine Mesilate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of bromocriptine, $C_{32}H_{40}BrN_5O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 10 mg of bromocriptine with 50 ml of *methanol* for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 20 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 380 nm exhibits a maximum at 305 nm and a minimum at 270 nm.

B. In the test for Related substances, the principal band in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (6).

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and as the mobile phase a mixture of 0.1 volume of 13.5M *ammonia*, 1.5 volumes of *water*, 3 volumes of *propan-2-ol*, 88 volumes of *dichloromethane* and 100 volumes of *ether*. Apply separately to the plate as 10-mm bands 50 µl of each of the following six solutions prepared immediately before use. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 20 mg of bromocriptine with 10 ml of *methanol* for 20 minutes and centrifuge. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. For solution (3) dilute 3 volumes of solution (1) to 100 volumes with *methanol*. For solution (4) dilute 1 volume of solution (1) to 100 volumes with *methanol*. For solution (5) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (6) contains 0.023% w/v of *bromocriptine mesilate BPCRS* in *methanol*. Apply solution (1) as the last solution and develop the chromatograms immediately in an unsaturated tank. After removal of the plate, dry it in a current of cold air for 2 minutes, spray with *ammonium molybdate solution R3* and heat at 100° until bands appear (about 10 minutes). In the chromatogram obtained with solution (1) any *secondary band* is not more intense than the band in the chromatogram obtained with solution (3) (3%). Not more than one such band is more intense than the band in the chromatogram obtained with solution (4) (1%) and not more than a further two such bands are more intense than the band in the chromatogram obtained with solution (5) (0.5%). Disregard any band within 20 mm of the line of application.

ASSAY

Prepare the solutions in subdued light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.011% w/v of *bromocriptine mesilate BPCRS* in *methanol (50%)*. For solution (2) mix a quantity of the mixed contents of 20 capsules containing the equivalent of 10 mg of bromocriptine with 70 ml of *methanol (50%)* with the aid of ultrasound for 5 minutes, filter and dilute to 100 ml with the same solvent. For solution (3) heat a 0.011% w/v solution of *bromocriptine mesilate BPCRS* in a mixture of 1 volume of 1M *acetic acid* and 9 volumes of *methanol* at 60° for 90 minutes and cool to room temperature.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 55 volumes of *acetonitrile* and 45 volumes of a 0.08% w/v solution of *ammonium carbonate* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 300 nm.

The assay is not valid unless the *resolution factor* between the two peaks obtained with solution (3) is not less than 3.0.

Calculate the content of $C_{32}H_{40}BrN_5O_5$ using the declared content of $C_{32}H_{40}BrN_5O_5$ in *bromocriptine mesilate BPCRS*.

STORAGE

Bromocriptine Capsules should be kept in an airtight container and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of bromocriptine.

Bromocriptine Tablets

General Notices

Action and use

Dopamine receptor agonist.

DEFINITION

Bromocriptine Tablets contain Bromocriptine Mesilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bromocriptine, $C_{32}H_{40}BrN_5O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of bromocriptine with 50 ml of *methanol* for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 20 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 380 nm exhibits a maximum at 305 nm and a minimum at 270 nm.

B. In the test for Related substances, the principal band in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (6).

C. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method described under Bromocriptine Capsules applying separately to the plate, as 10-mm bands, 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 10 mg of bromocriptine with 25 ml of a mixture of equal volumes of *chloroform* and *methanol* for 30 minutes, filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable) and wash the residue with two 5 ml quantities of the solvent mixture. Evaporate the filtrate and washings to dryness at 25° at a pressure of 2 kPa, dissolve the residue in 2 ml of the solvent mixture and centrifuge. For solution (2) dilute 1 volume of solution (1) to 10 volumes. For solution (3) dilute 3 volumes of solution (2) to 10 volumes. For solution (4) dilute 1 volume of solution (2) to 10 volumes. For solution (5) dilute 1 volume of solution (2) to 20 volumes, each with a mixture of equal volumes of *chloroform* and *methanol* as the diluent. Solution (6) contains 0.055% w/v of *bromocriptine mesilate BPCRS* in the same solvent mixture. In the chromatogram obtained with solution (1) any *secondary band* is not more intense than the band in the chromatogram obtained with solution (3) (3%). Not more than one such band is more intense than the band in the chromatogram obtained with solution (4) (1%) and not more than a further two such bands are more intense than the band in the chromatogram obtained with solution (5) (0.5%). Disregard any band within 20 mm of the line of application.

Uniformity of content

Tablets containing less than the equivalent of 2 mg of bromocriptine comply with the requirements stated under Tablets using the following method of analysis. Mix one tablet with 50 ml of *ethanol (50%)* with the aid of ultrasound until disintegrated, shake for 30 minutes and centrifuge. Measure the *absorbance* of the supernatant liquid at the maximum at 305 nm, Appendix II B. Calculate the content of $C_{32}H_{40}BrN_5O_5$ taking 144 as the value of A(1%, 1 cm) at the maximum at 305 nm.

ASSAY

Weigh and powder 20 tablets. Carry out the Assay described under Bromocriptine Capsules but preparing solution (2) in the following manner. Mix a quantity of the powdered tablets containing the equivalent of 10 mg of bromocriptine with 70 ml of *methanol (50%)* with the aid of ultrasound for 5 minutes, shake for 30 minutes, filter and dilute to 100 ml with the same solvent mixture.

STORAGE

Bromocriptine Tablets should be kept in an airtight container and protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of bromocriptine.

Brompheniramine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Brompheniramine Tablets contain Brompheniramine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of brompheniramine maleate, $C_{16}H_{19}BrN_2,C_4H_4O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the test for Related substances the retention time of the principal peak in the chromatogram obtained with solution (2) is similar to that of the principal peak in the chromatogram obtained with solution (3).

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Brompheniramine Maleate with 5 ml of *water* for 5 minutes, make the resulting suspension alkaline by adding 13.5M *ammonia* drop wise, add 2.5 ml of *toluene*, shake for a further 5 minutes, centrifuge and use the upper, toluene layer. For solution (2) dilute 1 volume of solution (1) to 50 volumes with *dichloromethane*. Solution (3) is a 0.016% w/v solution of *brompheniramine maleate EPCRS* in *dichloromethane*. Solution (4) contains 0.5% w/v of *brompheniramine maleate EPCRS* and 0.25% w/v of *chlorphenamine maleate EPCRS* in *dichloromethane*.

The chromatographic procedure may be carried out using a glass column (2.3 m × 2 mm) packed with *acid and base-washed*, *silanised diatomaceous support* (135 µm to 175 µm) (Chromosorb W AW-DMCS is suitable) impregnated with 3% w/w of *polymethylphenylsiloxane (50% phenyl)* (OV 17 is suitable) and maintaining the temperature of the column at 205° and that of the injection port and the detector at 250°.

Inject 1 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to brompheniramine and chlorpheniramine is at least 1.5. After injecting solution (1), continue the chromatography for at least 2.5 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than 1% of the area of the principal peak and no *secondary peak* has an area greater than 0.4% of the area of the principal peak. Disregard any peak with an area less than 0.1% of that of the principal peak in the chromatogram obtained with solution (1).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 4 mg of Brompheniramine Maleate with 50 ml of *water* for 10 minutes, adjust the pH to 11.0 with 0.1M *sodium hydroxide* and cool to room temperature. Extract the mixture with two 75 ml quantities of *petroleum spirit (boiling range, 40° to 60°)*. Extract the combined extracts with three 50 ml quantities of 1M *hydrochloric acid*, combine the acidic extracts and add sufficient 1M *hydrochloric acid* to produce 200 ml. Measure the *absorbance* of the resulting solution at the maximum at 265 nm, Appendix II B. Calculate the content of $C_{16}H_{19}BrN_2,C_4H_4O_4$ taking 199 as the value of A(1%, 1 cm) at the maximum at 265 nm.

STORAGE

Brompheniramine Tablets should be protected from light.

Buffered Cream

General Notices

DEFINITION

Emulsifying Ointment	300 g
Disodium Hydrogen Phosphate Dodecahydrate	25 g
Citric Acid Monohydrate	5 g
Chlorocresol	1 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

If another antimicrobial preservative replaces Chlorocresol in this formulation, the suitability of the Cream as a diluent should be confirmed before use.

Extemporaneous preparation

Melt the Emulsifying Ointment with the aid of gentle heat. In a vessel that can be closed, heat about 650 g of Purified Water to about 60°; add the Chlorocresol and, when it melts, vigorously shake the closed vessel to effect dissolution. Dissolve the Disodium Hydrogen Phosphate Dodecahydrate and the Citric Acid Monohydrate in the chlorocresol solution. Add the aqueous phase to the melted ointment when both are at about 60°. Stir gently until cool, add sufficient Purified Water to produce 1000 g and mix.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

TESTS

Acidity

pH, 5.7 to 6.3, determined directly on the cream, Appendix V L.

STORAGE

If Buffered Cream is kept in aluminium tubes, their inner surfaces should be coated with a suitable lacquer.

Bumetanide and Prolonged-release Potassium Tablets

General Notices

Bumetanide and Slow Potassium Tablets

Bumetanide and Prolonged-release Potassium Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Loop diuretic.

DEFINITION

Bumetanide and Prolonged-release Potassium Tablets contain Bumetanide and Potassium Chloride. They are formulated so that the Potassium Chloride is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Potassium Chloride. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bumetanide, $C_{17}H_{20}N_2O_5S$

92.5 to 107.5% of the stated amount.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances the spot in the chromatogram obtained with solution (1) is similar in position, size and intensity to the spot in the chromatogram obtained with solution (2).

B. Examine the filtrate obtained in the test for Uniformity of content by *fluorescence spectrophotometry*, Appendix II E, using an excitation wavelength of 350 nm. The solution emits light at 445 nm.

C. Dissolve a quantity of the powdered tablets containing 0.1 g of Potassium Chloride as completely as possible in 2 ml of *water* and filter. The filtrate yields reaction A characteristic of *potassium salts*, Appendix VI.

D. Dissolve a quantity of the powdered tablets containing 0.02 g of Potassium Chloride as completely as possible in 2 ml of *water* and filter. The filtrate yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

For bumetanide

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and measure the *fluorescence*, Appendix II E, using an excitation wavelength of 350 nm and an emission wavelength of 445 nm and *water* in the reference cell. Measure the *fluorescence* of a 0.04% w/v solution of *bumetanide BPCRS* in *ethanol (96%)* diluted to a suitable concentration with *water* under the same conditions and calculate the total content of $C_{17}H_{20}N_2O_5S$ in the medium from the fluorescences obtained and from the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a high-performance silica gel F_{254} plate (Merck 5629 plates are suitable) and a mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 8 cm above the line of application. Apply separately to the plate 10 µl of each of solutions (1) and (2) and 5 µl of solution (3). For solution (1) add 10 ml of a 0.1% w/v solution of *citric acid* to a number of whole tablets containing, in total, 1 mg of Bumetanide, shake for 10 minutes, remove and discard the cores, extract the solution with two 20 ml quantities of *ether*, evaporate the combined extracts to dryness under reduced pressure and dissolve the residue in 0.5 ml of *ethyl acetate*. For solution (2) add 2 ml of a 0.20% w/v solution of *bumetanide BPCRS* in *ethyl acetate* to 10 ml of a 0.1% w/v solution of *citric acid*, shake for 15 minutes, centrifuge and use the ethyl acetate layer. For solution (3) mix 1 ml of a 0.20% w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid BPCRS* in *ethyl acetate* and 1 ml of a 0.20% w/v solution of *bumetanide BPCRS* in *ethyl acetate*, add sufficient *ethyl acetate* to produce 100 ml, add 2 ml of this solution to 10 ml of a 0.1% w/v solution of *citric acid*, shake for 15 minutes and use the ethyl acetate layer. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) corresponding to 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not more intense than the corresponding spot in the chromatogram obtained with solution (3) (0.5%) and any other *secondary spot* is not more intense than the spot due to bumetanide in the chromatogram obtained with solution (3) (0.5%).

Uniformity of content

Tablets containing less than 2 mg of Bumetanide comply with the requirements stated below. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake one tablet in 0.5 ml of *methanol* for 3 minutes, add 9 ml of the mobile phase and shake for 30 minutes. Filter to remove the tablet core, add sufficient of the mobile phase to produce 10 ml, centrifuge for 15 minutes and use the supernatant liquid. If the supernatant liquid is cloudy, filter through a 0.45-µm membrane filter (Millipore Millex is suitable), discarding the first 2 ml of filtrate. For solution (2) dilute 5 ml of a 0.1% w/v solution of *bumetanide BPCRS* in *methanol* to 100 ml with the mobile phase.

The chromatographic procedure described under Assay may be used.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in each tablet using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*. The tablets comply with the test if not more than one of the individual values is outside the range 85% to 115% of the average value and none is outside the limits 75% to 125% of the average value. If two or three individual values are outside the limits 85% to 115% of the average value and none is outside the limits 75% to 125%, repeat the determination on a further 20 tablets taken at random. The tablets comply with the test if in the total number of tablets tested not more than three individual values are outside the limits 85% to 115% and none is outside the limits 75% to 125% of the average value.

ASSAY

For bumetanide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a number of whole tablets containing 5 mg of Bumetanide in 5 ml of *methanol* for 3 minutes, add 90 ml of the mobile phase and shake for 30 minutes. Filter to remove the tablet cores, add sufficient of the mobile phase to produce 100 ml, centrifuge for 15 minutes and use the supernatant liquid. If the supernatant liquid is cloudy, filter through a 0.45-µm membrane filter (Millipore Millex is suitable), discarding the first 2 ml of filtrate. For solution (2) dilute 5 ml of a 0.1% w/v solution of *bumetanide BPCRS* in *methanol* to 100 ml with the mobile phase. Solution (3) contains 0.005% w/v of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP-18 is suitable), (b) a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm. Inject separately 20 µl of each solution.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the tablets using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

For potassium chloride

Shake 20 whole tablets with 800 ml of *water* for 15 minutes, heating on a water bath if necessary, and allow to stand for 24 hours. Add sufficient *water* to produce 1000 ml, filter and dilute a portion of the filtrate to a suitable concentration. Carry out the method for *atomic emission spectrophotometry*, Appendix II D, measuring at 766.5 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions. Each mg of potassium is equivalent to 1.908 mg of KCl.

LABELLING

The label states that the tablets should be swallowed whole and not chewed.

Bumetanide Injection

General Notices

Action and use

Loop diuretic.

DEFINITION

Bumetanide Injection is a sterile solution of Bumetanide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of bumetanide, $C_{17}H_{20}N_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the injection containing 10 mg of Bumetanide with 20 ml of *ether*, filter the ether layer through *anhydrous sodium sulphate* and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of bumetanide *(RS 033)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.8, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform* as the mobile phase. Apply separately to the plate 25 µl of each of the following solutions. For solution (1) adjust the pH of a quantity of the injection containing 5 mg of Bumetanide to 12 using 0.1M *sodium hydroxide* and extract with two 20 ml quantities of *ether*. Discard the ether, adjust the pH to 4 using 1M *acetic acid*, extract with two further 20 ml quantities of *ether*, dry the ether by filtering through *anhydrous sodium sulphate*, wash the filter with 5 ml of *ether* and evaporate the combined filtrate and washings to dryness using a rotary evaporator. Dissolve the residue in 5 ml of *methanol* and centrifuge. Evaporate the supernatant liquid to dryness using a rotary evaporator and dissolve the residue in 0.5 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol* and further dilute 1 volume of this solution to 30 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) to 3 volumes with *methanol*. Solution (4) contains 0.005% w/v of *3-*

amino-4-phenoxy-5-sulphamoylbenzoic acid BPCRS in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) corresponding to 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%) and not more than two other such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the injection containing 2.5 mg of Bumetanide to 20 ml using a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*. For solution (2) dilute 10 ml of a 0.025% w/v solution of *bumetanide BPCRS* in a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* to 20 ml with *water*. Inject 20 µl of each solution. Solution (3) contains 0.0125% w/v of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak ODS is suitable), (b) a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

Calculate the content of $C_{17}H_{20}N_2O_5S$ using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

Bumetanide Oral Solution

General Notices

Action and use

Loop diuretic.

DEFINITION

Bumetanide Oral Solution is a solution of Bumetanide in a suitable flavoured vehicle.
The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of bumetanide, $C_{17}H_{20}N_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (5).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform* as the mobile phase. Apply separately to the plate 25 µl of each of the following solutions. For solution (1) mix a quantity of the oral solution containing 2 mg of Bumetanide with 10 ml of *water* and 0.6 ml of 1M *hydrochloric acid*, add 5 ml of *ethyl acetate*, shake for 15 minutes, centrifuge and decant the ethyl acetate. Add a further 5 ml of *ethyl acetate* to the residue, shake for 15 minutes, centrifuge and decant the ethyl acetate. Evaporate the combined ethyl acetate extracts to dryness using a rotary evaporator and dissolve the residue in 0.5 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) to 10 volumes with *methanol* and further dilute 1 volume of this solution to 3 volumes with *methanol*. For solution (4) dilute 1 volume of solution (2) to 100 volumes with *methanol*. Solution (5) contains 0.040% w/v of *bumetanide BPCRS* in *methanol*. Solution (6) contains 0.002% w/v of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) corresponding to 3-amino-4-phenoxy-5-sulphamoyl-benzoic acid is not more intense than the spot in the chromatogram obtained with solution (6) (0.5%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%) and not more than two other such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the oral solution containing 2.5 mg of Bumetanide with 12.5 ml of *water* and 0.8 ml of 1M *hydrochloric acid*, add 10 ml of *ethyl acetate*, shake for 15 minutes, centrifuge and decant the ethyl acetate. Repeat the extraction procedure twice using a further two 10 ml quantities of *ethyl acetate* and beginning at the words 'add 10 ml of ... '. Evaporate the combined ethyl acetate extracts to dryness using a rotary evaporator, dissolve the residue in 10 ml of a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* and dilute to 20 ml with *water*. For solution (2) dilute 5 ml of a 0.025% w/v solution of *bumetanide BPCRS* in a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* and dilute to 10 ml with *water*. Inject 20 µl of each solution. Solution (3) contains 0.0125% w/v of *3-amino-4-phenoxy-5-sulphamoyl-benzoic acid BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak ODS is suitable), (b) a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{17}H_{20}N_2O_5S$, weight in volume, using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

Bumetanide Tablets

General Notices

Action and use

Loop diuretic.

DEFINITION

Bumetanide Tablets contain Bumetanide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bumetanide, $C_{17}H_{20}N_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Bumetanide with 25 ml of *ether*, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of bumetanide *(RS 033)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake mechanically a quantity of the powdered tablets containing 12.5 mg of Bumetanide with 20 ml of a mixture of equal volumes of *acetonitrile* and *methanol* for 20 minutes, centrifuge for 10 minutes, decant and reserve the supernatant liquid. Extract the residue with 5 ml of a mixture of equal volumes of *acetonitrile* and *methanol,* shaking mechanically for 30 seconds, centrifuge for 10 minutes, decant and combine the extracts. Evaporate the combined extracts to dryness under reduced pressure, dissolve the residue in 0.5 ml of *methanol* and centrifuge for 10 minutes. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol* and further dilute 3 volumes of this solution to 10 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 10 volumes with *methanol* and further dilute 1 volume of this solution to 100 volumes with *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

Uniformity of content

Tablets containing less than 2 mg of Bumetanide comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve one tablet in 10 ml of a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*, shake with the aid of ultrasound for 5 minutes, dilute to 20 ml with *water*, filter and use the filtrate. For solution (2) dilute 10 ml of a 0.010% w/v solution of *bumetanide BPCRS* in a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* to 20 ml with *water*.

The chromatographic procedure described under Assay may be used.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in each tablet using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS.*

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the powdered tablets containing 2.5 mg of Bumetanide in 10 ml of a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*, shake with the aid of ultrasound for 5 minutes, dilute to 20 ml with *water*, filter and use the filtrate. For solution (2) dilute 10 ml of a 0.025% w/v solution of *bumetanide BPCRS* in a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol* to 20 ml with *water*. Inject 20 µl of each solution. Solution (3) contains 0.0125% w/v of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak ODS is suitable), (b) a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

Calculate the content of $C_{17}H_{20}N_2O_5S$ using the declared content of $C_{17}H_{20}N_2O_5S$ in *bumetanide BPCRS*.

Bupivacaine and Adrenaline Injection / Bupivacaine and Epinephrine Injection

General Notices

Action and use

Local anaesthetic.

DEFINITION

Bupivacaine and Adrenaline Injection is a sterile solution of Bupivacaine Hydrochloride and Adrenaline Acid Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl$

92.5 to 107.5% of the stated amount.

Content of adrenaline, $C_9H_{13}NO_3$

80.0 to 120.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel G60 plates are suitable) and a mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a quantity of the injection, if necessary, with *water* to produce a solution containing 0.2% w/v of Bupivacaine Hydrochloride. Solution (2) contains 0.2% w/v of *bupivacaine hydrochloride BPCRS* in *water*. Solution (3) contains 0.2% w/v of *bupivacaine hydrochloride BPCRS* and 0.2% w/v of *lidocaine hydrochloride BPCRS* in *water*. After removal of the plate, dry it in a current of cold air, heat at 110° for 1 hour, place the hot plate in a tank of chlorine gas prepared by the addition of *hydrochloric acid* to a 5% w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry the plate in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

B. In the Assay for adrenaline, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L.

2,6-Dimethylaniline; Related bases

Complies with the requirements stated under Bupivacaine Injection.

ASSAY

For anhydrous bupivacaine hydrochloride

Carry out the Assay described under Bupivacaine Injection.

For adrenaline

Dissolve 8.0 g of *tetramethylammonium hydrogen sulphate*, 2.2 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* in a mixture of 900 ml of *water* and 100 ml of *methanol*, adjust the pH to 3.5 with 1M *sodium hydroxide* and filter through glass micro fibre paper under reduced pressure (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 5 ml of a 0.001% w/v solution of *adrenaline acid tartrate BPCRS* to 10 ml with solution A. For solution (2) dilute the injection, if necessary, to produce a solution containing the equivalent of 0.0005% w/v of adrenaline and dilute 5 ml of the resulting solution to 10 ml with solution A. For solution (3) mix 5 ml of solution (1) with 5 ml of a 0.001% w/v solution of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* to a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusting the pH of the mixture to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

Calculate the content of $C_9H_{13}NO_3$ in the injection using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

STORAGE

Bupivacaine and Adrenaline Injection should be protected from light.

LABELLING

The quantities of the active ingredients are stated in terms of the equivalent amounts of anhydrous bupivacaine hydrochloride and adrenaline (epinephrine).

Bupivacaine Heavy Injection

General Notices

Bupivacaine and Dextrose Injection; Bupivacaine and Glucose Injection

Action and use

Local anaesthetic.

DEFINITION

Bupivacaine Heavy Injection is a sterile solution of Bupivacaine Hydrochloride and either Anhydrous Glucose or Glucose in Water for Injections. No preservative is added. The inclusion of glucose in the formulation assists the gravitational flow of the injection when administered.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl,H_2O$

95.0 to 105.0% of the stated amount.

Content of glucose monohydrate, $C_6H_{12}O_6,H_2O$

72.0 to 88.0 mg per ml.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. To a volume of the injection containing 50 mg of Bupivacaine Hydrochloride add sufficient 2M *sodium hydroxide* to obtain a pH of 11 and extract with 25 ml of n-*heptane*. Dry the heptane extract over *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness using a rotary evaporator and dry at 60° at a pressure of 2 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of bupivacaine *(RS 034)*.

B. Dip, for 1 second, a suitable stick with a reactive pad containing glucose-oxidase, peroxidase and a hydrogen-donating substance, such as *tetramethylbenzidine*, in the injection. Observe the colour of the reactive pad; within 60 seconds the colour changes from yellow to green or blue.

C. In the Assay for bupivacaine, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

D. When heated with *cupri-tartaric solution R1*, a copious precipitate of copper(I) oxide is produced.

TESTS

Acidity

pH, 4.0 to 6.0, Appendix V L.

2,6-Dimethylaniline

Buffer Solution

To 27.6g of *sodium dihydrogen phosphate monohydrate* add 7ml of a solution containing 8.9% of *disodium hydrogen orthophosphate dihydrate* and add sufficient water to produce 1000 ml, if necessary adjust the pH to 5.0 using 1M *orthophosphoric acid* or 1M *sodium hydroxide* (buffer solution).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the injection with mobile phase if necessary to contain 0.5% w/v Bupivacaine Hydrochloride.

(2) 0.0004% w/v *2,6-dimethylaniline*.

(3) 0.0002% w/v *2,6-dimethylaniline* and 0.0002% w/v *4-chloroaniline*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil Elite is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 ml per minute.

(d) Use ambient column temperature.

(e) Use an electrochemical detector (direct amperometry) with a glassy carbon working electrode, a silver-silver chloride reference electrode, held at + 0.9 V oxidation potential, and a detector sensitivity of 20 nA/V.

(f) Inject volume of 20 µl of each solution.

Under the prescribed conditions the retention time of 2,6-dimethylaniline is about 6.5 minutes.

MOBILE PHASE

A mixture of 4 volumes of *acetonitrile* and 6 volumes of buffer solution containing 0.006% w/v *disodium edetate* and 0.055% w/v *tetrabutylammonium hydrogen sulphate R1.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to 2,6-dimethylaniline and 4-chloroaniline is at least 1.5.

CHROMATOGRAPHY

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2,6-dimethylaniline is not greater than the area of any corresponding peak in the chromatogram obtained with solution (2) (800 ppm).

5-Hydroxymethylfurfural and light absorbing impurities

Dilute a volume of the injection containing 1.0 g of Glucose Monohydrate to 250 ml with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

Related substances

BUFFER SOLUTION

To 0.18g of *sodium dihydrogen phosphate monohydrate* and 2.9g of *disodium hydrogen orthophosphate dihydrate* add sufficient water to produce 1000 ml, if necessary adjust the pH to 8.0 using 1M *orthophosphoric acid* or 1M *sodium hydroxide* (buffer solution).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection if necessary to contain 0.5% w/v of Bupivacaine Hydrochloride.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength 240nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 4 volumes of buffer solution and 6 volumes of *acetonitrile.*

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total area of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%) and any peak corresponding to 2,6-dimethylaniline.

ASSAY

For bupivacaine hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute a volume of the injection if necessary to contain 0.05% w/v Bupivacaine Hydrochloride.

(2) 0.05% w/v of *bupivacaine hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{28}N_2O,HCl,H_2O$ in the injection using the declared content of $C_{18}H_{28}N_2O,HCl,H_2O$ in *bupivacaine hydrochloride BPCRS*.

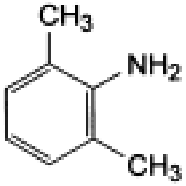
For glucose

To a quantity containing the equivalent of 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 ml of 5M *ammonia* and sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and measure the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the quantity of the injection taken for assay.

LABELLING

The label states the strength is stated in terms of the equivalent amount of bupivacaine hydrochloride and in terms of the equivalent amount of glucose, $C_6H_{12}O_6$ in a suitable dose-volume.

IMPURITIES



A. 2,6-Dimethylaniline,

B. 5-hydroxymethylfurfural.

Bupivacaine Injection

General Notices

Action and use

Local anaesthetic.

DEFINITION

Bupivacaine Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl$

92.5 to 107.5% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume of the injection containing the equivalent of 25 mg of anhydrous bupivacaine hydrochloride add 2 ml of 13.5M *ammonia*, shake and filter. Wash the precipitate with *water* and dry at 60° at a pressure of 2 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of bupivacaine *(RS 034)*.

B. To a volume of the injection containing the equivalent of 50 mg of anhydrous bupivacaine hydrochloride add 2 ml of a 10% w/v solution of *disodium hydrogen orthophosphate* and sufficient *iodinated potassium iodide solution* to produce a distinct brown colour. Remove the excess iodine by adding 0.1M *sodium thiosulphate*. No pink colour is produced.

TESTS

Acidity

pH, 4.0 to 6.5, Appendix V L.

2,6-Dimethylaniline

To a volume of the injection containing the equivalent of 25 mg of anhydrous bupivacaine hydrochloride add sufficient *water,* if necessary, to produce 10 ml, add 2M *sodium hydroxide* until the solution is just alkaline and extract with three 5 ml quantities of *dichloromethane*. Dry the combined dichloromethane extracts over *anhydrous sodium sulphate*, filter, wash with a further 5 ml of *dishloromethane* and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in 2 ml of *methanol*, add 1 ml of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand at room temperature for 10 minutes. The yellow colour produced is not more intense than the colour produced by repeating the operation using 10 ml of a solution in *water* containing 1 µg of *2,6-dimethylaniline* per ml in place of the injection (400 ppm).

Bupivacaine-related bases

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 0.1 volume of 13.5M *ammonia* and 100 volumes of *methanol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) evaporate a volume of the injection containing the equivalent of 0.1 g of anhydrous bupivacaine hydrochloride using a rotary evaporator, add sufficient *methanol* to the residue to produce 2 ml, mix, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing 0.0025% w/v of anhydrous bupivacaine hydrochloride. Solution (2) contains 0.0025% w/v of *bupivacaine hydrochloride BPCRS* in the mobile phase. For solution (3) prepare a 0.1% w/v solution of *2,6-dimethylaniline* in *acetonitrile*, dilute 10 volumes to 20 volumes with the mobile phase and then dilute 1 volume of the resulting solution to 100 volumes with solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 40 volumes of 0.02M *phosphate buffer pH 8.0* and 60 volumes of *acetonitrile* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 240 nm. Inject 20 µl of each solution.

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 8.

Calculate the content of $C_{18}H_{28}N_2O,HCl$ in the injection using the declared content of $C_{18}H_{28}N_2O,HCl$ in *bupivacaine hydrochloride BPCRS*.

LABELLING

The strength is stated in terms of the equivalent amount of anhydrous bupivacaine hydrochloride in a suitable dose-volume.

Busulfan Tablets

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Busulfan Tablets contain Busulfan. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of busulfan, $C_6H_{14}O_6S_2$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 10 mg of Busulfan with 10 ml of hot *acetone*, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of busulfan *(RS 035)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (3) is similar to that of the principal peak in the chromatogram obtained with solution (1).

TESTS

Disintegration

Maximum time, 15 minutes, Appendix XII A1.

Uniformity of content

Tablets containing less than 2 mg of Busulfan comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Prepare a 0.0001% w/v solution of *1,5-diiodopentane* (internal standard) in *acetone* (solution A). For solution (1) add 5 ml of a 30% w/v solution of *sodium iodide* in *acetone* to 5 ml of a 0.0001% w/v solution of *busulfan BPCRS* in *acetone*, stopper the flask lightly and heat in a water bath at 50° for 90 minutes. Cool, add 10 ml of solution A, mix, add 10 ml of *water* and 20 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer. Prepare solution (2) in the same manner as solution (3) but using 10 ml of *acetone* in place of solution A. For solution (3) add 1 ml of *water* to one tablet in a 50 ml graduated flask and mix with the aid of ultrasound until completely dispersed. Add 30 ml of *acetone*, shake for 15 minutes and dilute to 50 ml with *acetone*. Centrifuge and dilute a quantity of the supernatant liquid with *acetone* to produce a solution containing 0.0001% w/v of Busulfan. To 5 ml of the resulting solution add 5 ml of a 30% w/v solution of *sodium iodide* in *acetone*, stopper the flask lightly and heat in a water bath at 50° for 90 minutes. Cool, add 10 ml of solution A, mix, add 10 ml of *water* and 20 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 140° with an electron capture detector.

Calculate the content of $C_6H_{14}O_6S_2$ using the declared content of $C_6H_{14}O_6S_2$ in *busulfan BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method of analysis described under Uniformity of content but prepare solution (3) in the following manner. Add 5 ml of *water* to a quantity of powdered tablets containing 2.5 mg of Busulfan and mix with the aid of ultrasound until completely dispersed. Add 150 ml of *acetone*, shake for 15 minutes and dilute to 250 ml with *acetone*. Centrifuge and dilute 10 ml of the supernatant liquid to 100 ml with *acetone*. To 5 ml of the resulting solution add 5 ml of a 30% w/v solution of *sodium iodide* in *acetone*, stopper the flask lightly and heat in a water bath at 50° for 90 minutes. Cool, add 10 ml of solution A, mix, add 10 ml of *water* and 20 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

Calculate the content of $C_6H_{14}O_6S_2$ using the declared content of $C_6H_{14}O_6S_2$ in *busulfan BPCRS*.

Compound Benzoic Acid Ointment

General Notices

Whitfield's Ointment

DEFINITION

Compound Benzoic Acid Ointment contains 6.0% w/w of Benzoic Acid and 3.0% w/w of Salicylic Acid in a suitable emulsifying basis.

Extemporaneous preparation

The following formula and directions apply.

Benzoic Acid in *fine powder*	60 g
Salicylic Acid in *fine powder*	30 g
Emulsifying Ointment	910 g

Triturate the Benzoic Acid and the Salicylic Acid with a portion of the Emulsifying Ointment until smooth and gradually incorporate the remainder of the Emulsifying Ointment.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of benzoic acid, $C_7H_6O_2$

5.7 to 6.3% w/w.

Content of salicylic acid, $C_7H_6O_3$

2.7 to 3.3% w/w.

IDENTIFICATION

Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) warm 1 g of the ointment with 10 ml of *chloroform* , cool and filter. Solution (2) contains 0.6% w/v of *benzoic acid* and 0.3% w/v of *salicylic acid* in *chloroform* . After removal of the plate, allow the solvent to evaporate in a current of air and examine under *ultraviolet light (254 nm)* . The chromatogram obtained with solution (1) shows spots corresponding in colour and position to those in the chromatogram obtained with solution (2). Examine under *ultraviolet light (365 nm)* . The chromatogram obtained with solution (1) shows a blue fluorescent spot corresponding in colour and position to that in the chromatogram obtained with solution (2). Spray the plate with *iron(III) chloride solution R1*. The chromatogram obtained with solution (1) shows a purple spot corresponding in position to the blue fluorescent spot observed under ultraviolet light (365 nm) and corresponding in colour and position to the spot in the chromatogram obtained with solution (2).

ASSAY

For benzoic acid

To 2 g add 150 ml of *water*, warm until melted and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Reserve the solution for the Assay for salicylic acid. After the subtraction of 1 ml for each 13.81 mg of $C_7H_6O_3$ found in the Assay for salicylic acid, each ml of 0.1M *sodium hydroxide VS* is equivalent to 12.21 mg of $C_7H_6O_2$.

For salicylic acid

Cool the titrated solution obtained in the Assay for benzoic acid, dilute to 250 ml with *water* and filter. To 5 ml of the filtrate add sufficient *iron(III) nitrate solution* to produce 50 ml. Filter, if necessary, to remove haze and measure the *absorbance* of the resulting solution at the maximum at 530 nm, Appendix II B, using *iron(III) nitrate solution* in the reference cell. Calculate the content of $C_7H_6O_3$ from the absorbance obtained by repeating the operation using 5 ml of a 0.024% w/v solution of *salicylic acid* and beginning at the words 'add sufficient *iron(III) nitrate solution* ...'.

Compound Benzoin Tincture

General Notices

Friars' Balsam

DEFINITION

Barbados Aloes or Cape Aloes	20 g
Prepared storax of commerce	100 g
Sumatra Benzoin crushed	100 g
Ethanol (90 per cent)	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Macerate the Barbados Aloes or Cape Aloes, the prepared storax and the Sumatra Benzoin with 800 ml of Ethanol (90 per cent) in a closed vessel for not less than 2 days, shaking occasionally, filter and pass sufficient Ethanol (90 per cent) through the filter to produce 1000 ml.

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

Content of total balsamic acids

Not less than 4.5% w/v, calculated as cinnamic acid, $C_9H_8O_2$.

TESTS

Ethanol content

70 to 76% v/v, Appendix VIII F, Method III.

Dry residue

15 to 19% w/v.

Relative density

0.880 to 0.910, Appendix V G.

ASSAY

Carry out the Assay described under Benzoin Inhalation using 10 ml of the tincture. Each ml of 0.1M *sodium hydroxide* VS is equivalent to 14.82 mg of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

Gastro-resistant Bisacodyl Tablets

General Notices

Bisacodyl Tablets

Action and use

Stimulant laxative.

DEFINITION

Gastro-resistant Bisacodyl Tablets contain Bisacodyl. They are covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bisacodyl, $C_{22}H_{19}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Extract a quantity of the powdered tablets containing 50 mg of Bisacodyl with 20 ml of *dichloromethane*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of a 0.5% v/v solution of *sulphuric acid*. To 2 ml of the resulting solution add 0.05 ml of *potassium tetraiodomercurate solution*. A white precipitate is produced.

C. To 2 ml of the solution obtained in test B add *sulphuric acid*. A reddish violet colour is produced.

D. Boil 2 ml of the solution obtained in test B with a little *nitric acid*; a yellow colour is produced. Cool and add 5M *sodium hydroxide*; the colour becomes yellowish brown.

TESTS

Disintegration

Tablets covered with a gastro-resistant coating comply with the requirements stated under Tablets but using a 1.5% w/v solution of *sodium hydrogen carbonate* in place of the mixed phosphate buffer pH 6.8.

Related substances

SOLVENT A

A mixture of 4 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 66 volumes of *water*.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 25 mg of Bisacodyl with 40 ml of solvent A, dilute to 50 ml with solvent A and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A and further dilute 1 volume of the resulting solution to 10 volumes with solvent A.

(3) Dissolve the contents of a vial of *bisacodyl for system suitability EPCRS* in 1 ml of *acetonitrile* and mix with 1 ml of solvent A.

(4) Dissolve 5 mg of *bisacodyl for peak identification EPCRS* in 2.5 ml of *acetonitrile* and dilute to 5 ml with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 50 µl of each solution. Allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

MOBILE PHASE

A mixture of 45 volumes of *acetonitrile* and 55 volumes of 0.025M *ammonium formate* previously adjusted to pH 5.0 with *anhydrous formic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *bisacodyl for system suitability EPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

multiply any peak corresponding to impurity A by the correction factor of 0.7;

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%; taking into account the correction factor of 0.7);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak of the chromatogram obtained with solution (2) (0.1%);

the areas of any peaks corresponding to impurities C and E are not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any peak corresponding to impurity D is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any peak corresponding to impurity F is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other impurity is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of the impurities is not more than 1.0%.

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Bisacodyl with 40 ml of solvent A, dilute to 50 ml and filter. Dilute further 1 volume to 4 volumes with solvent A.

(2) 0.005% w/v of *bisacodyl BPCRS* in solvent A.

CHROMATOGRAPHIC PROCEDURE

The chromatographic procedure described under the test for Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *bisacodyl for system suitability EPCRS*.

DETERMINATION OF CONTENT

Calculate the total content of bisacodyl, $C_{22}H_{19}NO_{4.}$ in the tablets using the chromatogram obtained and the declared content of $C_{22}H_{19}NO_4$ in *bisacodyl BPCRS*.

Prolonged-release Bezafibrate Tablets

General Notices

Prolonged-release Bezafibrate Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Fibrate; lipid-regulating drug.

DEFINITION

Prolonged-release Bezafibrate Tablets contain Bezafibrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of bezafibrate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of bezafibrate, $C_{19}H_{20}ClNO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.2 g of Bezafibrate with two 10-ml quantities of *acetone* for 10 minutes, filter the combined extracts (Whatman GF/C is suitable) and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Bezafibrate *(RS 419)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 100 mg of Bezafibrate with 15 ml of *methanol* for 2 minutes, shake for a further 10 minutes, cool, add sufficient mobile phase to produce 100 ml, mix and filter, discarding the first 20 ml of filtrate.

(2) Dilute 1 volume of solution (1) to 200 volumes with the mobile phase.

(3) Dissolve sufficient quantities of *bezafibrate BPCRS* and *chlorobenzoyltyramine BPCRS* in the minimum quantity of *methanol* and dilute with mobile phase to produce a solution containing 0.0002% w/v of *bezafibrate BPCRS* and 0.0002% w/v of *chlorobenzoyltyramine BPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µl of each solution.

The retention time of bezafibrate is about 5 minutes. Allow the chromatography to proceed for four times the retention time of the principal peak.

MOBILE PHASE

A mixture of 3.9 volumes of a 40% w/v solution of *tetrabutylammonium hydroxide*, 400 volumes of *acetonitrile* and 600 volumes of *water*, adjusting the final pH to 4.0 with 10% v/v *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to bezafibrate and chlorobenzoyltyramine is at least 7.0. If necessary adjust the content of tetrabutylammonium hydroxide to obtain the required resolution.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the total area of any such peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.01%).

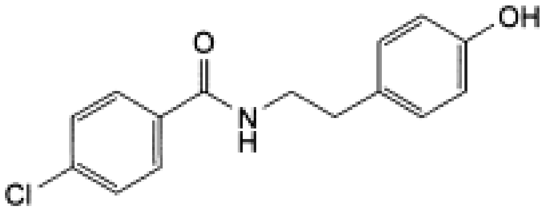
ASSAY

Weigh and powder 20 tablets. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Bezafibrate with 70 ml of *methanol* for 2 minutes, shake for a further 10 minutes, cool, add sufficient *methanol* to produce 100 ml, mix and filter discarding the first 20 ml of filtrate. Dilute 1 volume to 100 volumes with *methanol*. Solution (2) is a 0.001% w/v solution of *bezafibrate BPCRS* in *methanol*.

Measure the *absorbance* of the resulting solutions at the maximum at about 230 nm, Appendix II B. Calculate the content of $C_{19}H_{20}ClNO_4$ from the declared content of $C_{19}H_{20}ClNO_4$ in *bezafibrate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include:



4-chloro-*N*-[2-(4-hydroxyphenyl)ethyl]benzamide (chlorobenzoyltyramine).

Aqueous Calamine Cream

General Notices

DEFINITION

Aqueous Calamine Cream contains 4% w/w of Calamine and 3% w/w of Zinc Oxide in a suitable oil-in-water emulsified basis.

Extemporaneous preparation

The following formula and directions apply.

Calamine	40 g
Zinc Oxide	30 g
Liquid Paraffin	200 g
Self-emulsifying Glyceryl Monostearate	50 g
Cetomacrogol Emulsifying Wax	50 g
Phenoxyethanol	5 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

Melt the Cetomacrogol Emulsifying Wax with the Self-emulsifying Glyceryl Monostearate, add the Liquid Paraffin and heat to about 60°. Dissolve the Phenoxyethanol in about 620 g of Purified Water at about 60°, add the oily phase to the phenoxyethanol solution and mix. Stir until cool, add sufficient Purified Water to produce 930 g and mix. Triturate the Calamine and the Zinc Oxide and incorporate in the cream.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc, Zn

4.3 to 5.2% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Gently heat 4 g, taking precautions to avoid loss caused by spitting, until the basis is completely volatilised or charred, increase the temperature until the carbon is removed and ignite the residue to constant weight. Each g of residue is equivalent to 0.8034 g of Zn.

Aromatic Cardamom Tincture

General Notices

DEFINITION

Cardamom Oil	3 ml
Caraway Oil	10 ml
Cinnamon Oil	10 ml
Clove Oil	10 ml
Strong Ginger Tincture	60 ml
Ethanol (90 per cent)	Sufficient to produce 1000 ml

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

TESTS

Ethanol content

84 to 87% v/v, Appendix VIII F, Method III.

Relative density

0.825 to 0.845, Appendix V G.

Caffeine Citrate Injection

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Central nervous system stimulant.

DEFINITION

Caffeine Citrate Injection is a sterile solution of caffeine citrate, prepared by the interaction of Caffeine and Citric Acid Monohydrate, in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of caffeine citrate, $C_8H_{10}N_4O_2,C_6H_8O_7$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2) .

B. Yields the reaction characteristic of *citrates*, Appendix VI.

TESTS

Acidity

pH, 4.2 to 5.2, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a volume of the injection containing the equivalent of 50 mg of caffeine add 250 ml of *water* and filter through a 0.45-µm filter.

(2) 0.02% w/v of *caffeine BPCRS* in *water*.

(3) 0.02% w/v of *caffeine BPCRS* and 0.0004% w/v of *theophylline* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 10 µl of each solution.

MOBILE PHASE

4 volumes of *tetrahydrofuran*, 5 volumes of *acetonitrile* and 191 volumes of 0.01M *sodium acetate*, adjusted to pH 4.5 with *glacial acetic acid*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to caffeine and theophylline is at least 6.0.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2,C_6H_8O_7$ in the injection using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS*. Each mg of $C_8H_{10}N_4O_2$ is equivalent to 2 mg of $C_8H_{10}N_4O_2$, $C_6H_8O_7$.

LABELLING

The label states the quantity of active ingredient in terms of the amount of caffeine citrate and the equivalent amount of caffeine.

Caffeine Citrate Oral Solution

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Central nervous system stimulant.

DEFINITION

Caffeine Citrate Oral Solution is a solution of caffeine citrate, prepared by the interaction of Caffeine and Citric Acid Monohydrate, in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of caffeine citrate, $C_8H_{10}N_4O_2,C_6H_8O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2) .

B. Yields the reaction characteristic of *citrates*, Appendix VI.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing the equivalent of 50 mg of caffeine to 250 ml with *water* and filter through a 0.45-µm filter.

(2) 0.02% w/v of *caffeine BPCRS* in *water*.

(3) 0.02% w/v of *caffeine BPCRS* and 0.0004% w/v of *theophylline* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 275 nm.

(f) Inject 10 µl of each solution.

MOBILE PHASE

4 volumes of *tetrahydrofuran*, 5 volumes of *acetonitrile* and 191 volumes of 0.01M *sodium acetate*, adjusted to pH 4.5 with *glacial acetic acid*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to caffeine and theophylline is at least 6.0.

DETERMINATION OF CONTENT

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_8H_{10}N_4O_2,C_6H_8O_7$, weight in volume, using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS*. Each mg of $C_8H_{10}N_4O_2$ is equivalent to 2 mg of $C_8H_{10}N_4O_2,C_6H_8O_7$.

LABELLING

The quantity of active ingredient is stated in terms of the amount of caffeine citrate and the equivalent amount of caffeine.

Calamine and Coal Tar Ointment

General Notices

Compound Calamine Ointment

DEFINITION

Calamine and Coal Tar Ointment contains 12.5% w/w each of Calamine and Zinc Oxide and 2.5% w/w of Strong Coal Tar Solution in a suitable water-emulsifying basis.

Extemporaneous preparation

The following formula and directions apply.

Calamine, finely sifted	125 g
Zinc Oxide, finely sifted	125 g
Strong Coal Tar Solution	25 g
Hydrous Wool Fat	250 g
White Soft Paraffin	475 g

Melt together the Hydrous Wool Fat and the White Soft Paraffin. Triturate the Calamine and Zinc Oxide in the melted basis and stir gently, when cooled, to about 40°. Gradually incorporate the Strong Coal Tar Solution and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc compounds, calculated as ZnO

19.8 to 22.8% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Gently heat 1 g until the basis is completely volatilised or charred. Increase the heat until the carbon is removed and ignite the residue of ZnO until, after further ignition, two successive weighings do not differ by more than 0.2% of the weight of the residue. Each g of residue is equivalent to 0.8034 g of Zn.

STORAGE

Calamine and Coal Tar Ointment should be kept in a container that minimises evaporation losses.

Calamine Lotion

General Notices

DEFINITION

Calamine Lotion is a *cutaneous suspension*.

Calamine	150 g
Zinc Oxide	50 g
Bentonite	30 g
Sodium Citrate	5 g
Liquefied Phenol	5 ml
Glycerol	50 ml
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Triturate the Calamine, the Zinc Oxide and the Bentonite with a solution of the Sodium Citrate in about 700 ml of the Purified Water and add the Liquefied Phenol, the Glycerol and sufficient Purified Water to produce 1000 ml.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

IDENTIFICATION

A. To 2 ml add 2 ml of *periodic acid reagent*, shake, centrifuge and add 0.5 ml of the supernatant liquid to 2 ml of *ammoniacal silver nitrate solution* in a test tube. Heat on a water bath at 70º for 5 minutes. A silver mirror is produced on the side of the tube.

B. Mix 2 ml with 50 ml of *water*, centrifuge and decant the supernatant liquid. Suspend the residue in 20 ml of *water*, add 1 ml of *hydrochloric acid*, mix and filter. 5 ml of the filtrate, after neutralisation by drop wise addition of 2M *sodium hydroxide*, yields the reaction characteristic of *zinc salts*, Appendix VI.

Residue on ignition

14.5 to 18.0% w/w when determined by the following method. Evaporate 5 g to dryness and ignite until, after further ignition, two successive weighings do not differ by more than 0.2% of the weight of the residue.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous suspension'.

Calamine Ointment

General Notices

DEFINITION

Calamine Ointment contains 15% w/w of Calamine in a suitable hydrophobic basis.

Extemporaneous preparation

The following formula and directions apply.

Calamine, finely sifted	150 g
White Soft Paraffin	850 g

Triturate the Calamine with part of the White Soft Paraffin until smooth and gradually incorporate the remainder of the White Soft Paraffin.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc, Zn

7.8 to 9.4% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Gently heat 1 g until the basis is completely volatilised or charred, increase the heat until all the carbon is removed and ignite the residue until, after further ignition, two successive weighings do not differ by more than 0.2% of the weight of the residue. Each g of residue is equivalent to 0.8034 g of Zn.

Calcitonin (Salmon) Injection

General Notices

Action and use

Hormone.

DEFINITION

Calcitonin (Salmon) Injection is a sterile solution of Calcitonin (Salmon) in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of calcitonin (salmon), $C_{145}H_{240}N_{44}O_{48}S_2$

90.0 to 115.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.9 to 4.5, Appendix V L.

Calcitonin C

Carry out the method for *liquid chromatography*, Appendix III D, injecting 0.2 ml of the following solutions. For solution (1) dilute the injection, if necessary, with a 0.1M solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid* to give a final concentration of 10 µg of calcitonin (salmon) per ml. Solution (2) contains 10 µg of *calcitonin (salmon) BPCRS* per ml in the sodium dihydrogen orthophosphate solution. For solution (3) heat the injection at 75° for 15 hours and, if necessary, dilute as described for solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Vydac C18, 300 Å wide pore column for proteins and peptides is suitable) maintained at 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 100 volumes of a 0.363% w/v solution of *tetramethylammonium hydroxide pentahydrate* and 150 volumes of *acetonitrile* adjusted to pH 2.5 with *orthophosphoric acid* (mobile phase A) and a mixture of 450 volumes of a

0.402% w/v solution of *tetramethyl-ammonium hydroxide pentahydrate* and 50 volumes of *acetonitrile* adjusted to pH 2.5 with *orthophosphoric acid* (mobile phase B) and (c) a detection wavelength of 220 nm.

Equilibrate the column with a mixture of 35 volumes of mobile phase A and 65 volumes of mobile phase B. Operate by gradient elution increasing continuously and linearly the proportion of mobile phase A to 57% over a period of 21 minutes.

In the chromatogram obtained with solution (3) the peak due to calcitonin C is the largest peak to elute after the injection buffer salts and before the principal peak with a relative retention to that of calcitonin (salmon) of between 0.5 and 0.6.

The test is not valid unless the *resolution factor* between the peaks due to calcitonin C and calcitonin (salmon) in the chromatogram obtained with solution (3) is at least 3.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to calcitonin C is not greater than 7% by *normalisation.*

Related peptides

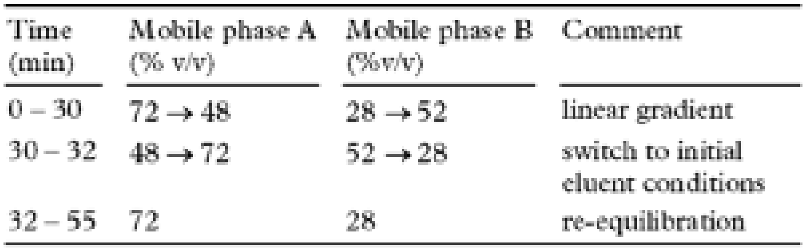
Carry out the method for *liquid chromatography*, Appendix III D, injecting 200 µl of each of the following solutions. For solution (1) dilute the injection, if necessary, with mobile phase A to give a final concentration of 10 µg of calcitonin (salmon) per ml. For solution (2) dissolve the contents of a vial of N*-acetyl-cys*^{1} *calcitonin EPCRS* in 0.4 ml of mobile phase A, dilute to 40 ml with mobile phase A and add 0.1 ml of solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) maintained at 65°, (b) as the mobile phase with a flow rate of 1.0 ml per minute the gradient elution programme described below and (c) a detection wavelength of 220 nm.

Mobile phase A Dissolve 3.26 g of *tetramethylammonium hydroxide pentahydrate* in 900 ml of *water*, adjust the pH to 2.5 with *orthophosphoric acid* and mix with 100 ml of *acetonitrile for chromatography*.

Mobile phase B Dissolve 1.45 g of *tetramethylammonium hydroxide pentahydrate* in 400 ml of *water*, adjust the pH to 2.5 with *orthophosphoric acid* and mix with 600 ml of *acetonitrile for chromatography*.

Equilibrate the column with a mixture of 72 volumes of mobile phase A and 28 volumes of mobile phase B. Carry out a gradient elution using the following gradient programme.



Time (min)	Mobile phase A (% v/v)	Mobile phase B (%v/v)	Comment
0 – 30	72 → 48	28 → 52	linear gradient
30 – 32	48 → 72	52 → 28	switch to initial eluent conditions
32 – 55	72	28	re-equilibration

When the chromatogram for solution (2) is recorded in the prescribed conditions, the relative retention time of *N*-acetyl-cys^1 calcitonin is about 1.15 relative to the principal peak. The test is not valid unless the *resolution factor* between the peaks corresponding to calcitonin and *N*-acetyl-cys^1 calcitonin is at least 5.0 and the *symmetry factor* for the *N*-acetyl-cys^1 calcitonin peak is not greater than 2.5. If necessary, adjust the initial ratio of A:B in the mobile phase.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than 3% of the total area of all the peaks and the sum of the areas of any such peaks is not greater than 5% of the total area of all the peaks by *normalisation*. Disregard any peak with an area less than 0.1 % of that of the principal peak.

ASSAY

Carry out the method described under the test for Calcitonin C.

Calculate the content of calcitonin (salmon) from the areas of the peak due to calcitonin (salmon) and that of any peak due to calcitonin C, using the declared content of $C_{145}H_{240}N_{44}O_{48}S_2$ in *calcitonin (salmon) BPCRS*.

STORAGE

Calcitonin (Salmon) Injection should be protected from light and stored at a temperature of 2° to 8°. Under these conditions it may be expected to retain its potency for not less than 2 years.

LABELLING

The label states the strength as the number of IU (Units) per ml. The label also states the equivalent number of micrograms of the peptide per ml.

Calcitriol Capsules

General Notices

Action and use

Vitamin D analogue.

DEFINITION

Calcitriol Capsules contain a solution of Calcitriol in a suitable fixed oil.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of calcitriol, $C_{27}H_{44}O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to calcitriol in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, in subdued light using the following solutions. For capsules containing 0.25 µg or less, use the mixed contents of 10 capsules as solution (1). For capsules containing more than 0.25 µg, prepare solution (1) by diluting a quantity of the mixed contents of 10 capsules containing 1.5 µg of Calcitriol to 1 ml with the mobile phase. Solution (2) contains 0.00015% w/v of *calcitriol EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si60 is suitable), (b) a mixture of 1 volume of *propan-1-ol*, 2 volumes of *methanol*, 40 volumes of *hexane* and 60 volumes of *ethyl acetate* as the mobile phase with a flow rate of 1.2 ml per minute and (c) a detection wavelength of 265 nm. If necessary, adjust the composition of the mobile phase so that the principal peak in the chromatogram obtained with solution (1) is clearly separated from the tail of the peak due to the excipient.

Calculate the content of calcitriol, $C_{27}H_{44}O_3$, using the declared content of $C_{27}H_{44}O_3$ in *calcitriol EPCRS*.

Calcium and Colecalciferol Tablets

General Notices

DEFINITION

Calcium and Colecalciferol Tablets contain Calcium Carbonate and Colecalciferol.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium

85.0 to 115.0% of the stated amount.

Content of colecalciferol, $C_{27}H_{44}O$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 µg of colecalciferol with 20 ml of the mobile phase with the aid of ultrasound for 15 minutes, cool to room temperature, centrifuge and use the supernatant liquid. Solution (2) contains 0.0001% w/v each of *colecalciferol BPCRS* and *ergocalciferol BPCRS* in the mobile phase. Solution (3) contains 0.0001% w/v of *colecalciferol BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 10 volumes of *water* and 90 volumes of *methanol* and (c) a detection wavelength of 265 nm.

Inject 50 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.4. If necessary, adjust the composition of the mobile phase to obtain the required resolution.

A peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to colecalciferol in the chromatogram obtained with solution (3).

B. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of calcium with 50 ml of *water* and filter. The solution yields reaction A characteristic of *calcium salts*, Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Calcium and Colecalciferol Tablets intended to be chewed. Tablets intended to be swallowed whole comply with the test for disintegration of uncoated tablets or of coated tablets, as appropriate.

Uniformity of content

Tablets containing less than 2 mg or 2% of Colecalciferol comply with the requirements stated under Tablets, with respect to the content of Colecalciferol, using the following method of analysis. Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 15 ml of *methanol (90%)*, mix with the aid of ultrasound until the tablet is dispersed and then for a further 5 minutes, dilute to 20 ml with *methanol (90%)*, mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable). Solution (2) contains 0.00005% w/v of *colecalciferol BPCRS* in *methanol (90%)*.

The chromatographic procedure described under Assay may be used.

Calculate the content of $C_{27}H_{44}O$ in each tablet using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS*.

ASSAY

For calcium

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 50 mg of calcium add 50 ml of *water* and 5 ml of *hydrochloric acid*. Heat the dispersion gently to boiling and continue to boil for about 2 minutes. Allow to cool and add 50 ml of 0.05M *disodium edetate VS*. Neutralise the solution using 2M *sodium hydroxide*, add 10 ml of *ammonia buffer pH 10.9* and 50 ml of *water*. Titrate the excess of disodium edetate with 0.05M *zinc chloride VS* using *mordant black II solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

For colecalciferol

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 mg of Colecalciferol with about 170 ml of *methanol (90%)* for 5 minutes and then mix with ultrasound for 5 minutes, dilute to 200 ml with *methanol (90%)*, mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable). Solution (2) contains 0.00005% w/v of *colecalciferol BPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 3 volumes of *water* and 97 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 264 nm. Inject 50 µl of each solution.

Calculate the content of $C_{27}H_{44}O$ in the tablets using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS*.

LABELLING

The label states (1) where appropriate, that the tablets should be chewed before swallowing; (2) the equivalent number of IU (Units) of antirachitic activity (vitamin D); (3) the equivalent amount of calcium.

Each microgram of Colecalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

Calcium and Ergocalciferol Tablets

General Notices

DEFINITION

Calcium and Ergocalciferol Tablets contain Calcium Lactate Pentahydrate, Calcium Phosphate and Ergocalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium

85.0 to 115.0% of the stated amount.

Content of ergocalciferol, $C_{28}H_{44}O$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 µg of ergocalciferol with 20 ml of the mobile phase with the aid of ultrasound for 15 minutes, cool to room temperature, centrifuge and use the supernatant liquid. Solution (2) contains 0.0001% w/v each of *colecalciferol BPCRS* and *ergocalciferol BPCRS* in the mobile phase. Solution (3) contains 0.0001% w/v of *ergocalciferol BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 10 volumes of *water* and 90 volumes of *methanol* and (c) a detection wavelength of 265 nm.

Inject 50 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.4. If necessary, adjust the composition of the mobile phase to obtain the required resolution.

A peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to ergocalciferol in the chromatogram obtained with solution (3).

B. Disperse a quantity of the powdered tablets containing the equivalent of 10 mg of calcium in 5 ml of *water* and filter. The filtrate yields reaction A characteristic of *lactates*, Appendix VI.

C. Warm a quantity of the powdered tablets containing the equivalent of 90 mg of calcium with 10 ml of 2M *nitric acid*, cool and filter. Add 10 ml of *ammonium molybdate solution* to the filtrate. A yellow precipitate is produced, characteristic of phosphates.

D. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of calcium with 50 ml of *water* and filter. The solution yields reaction A characteristic of *calcium salts*, Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Calcium and Ergocalciferol Tablets intended to be chewed. Tablets intended to be swallowed whole comply with the test for disintegration of uncoated tablets or of coated tablets, as appropriate.

Uniformity of content

Tablets containing less than 2 mg or 2% of Ergocalciferol comply with the requirements stated under Tablets, with respect to the content of Ergocalciferol, using the following method of analysis. Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 15 ml of *methanol (90%)*, mix with the aid of ultrasound until the tablet is dispersed and then for a further 5 minutes, dilute to 20 ml with *methanol (90%)*, mix, centrifuge and filter through a glass-fibre filter (Whatman GF/C is suitable). Solution (2) contains 0.00005% w/v of *ergocalciferol BPCRS* in *methanol (90%)*.

The chromatographic procedure described under Assay may be used.

Calculate the content of $C_{28}H_{44}O$ in each tablet using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS*.

ASSAY

For calcium

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 50 mg of calcium add 50 ml of *water* and 5 ml of *hydrochloric acid*. Heat the dispersion gently to boiling and continue for about 2 minutes. Allow to cool and add 50 ml of 0.05M *disodium edetate VS*. Neutralise the solution using 2M *sodium hydroxide*, add 10 ml of *ammonia buffer pH 10.9* and 50 ml of *water*. Titrate the excess of disodium edetate with 0.05M *zinc chloride VS* using *mordant black II solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

For ergocalciferol

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 mg of Ergocalciferol with about 170 ml of *methanol (90%)* for 5 minutes and then mix with the aid of ultrasound for 5 minutes, dilute to 200 ml with *methanol (90%)*, mix, centrifuge and filter through a glass fibre filter (Whatman GF/C is suitable). Solution (2) contains 0.00005% w/v of *ergocalciferol BPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) a mixture of 3 volumes of *water* and 97 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 264 nm. Inject 50 µl of each solution.

Calculate the content of $C_{28}H_{44}O$ in the tablets using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS.*

LABELLING

The label states (1) where appropriate, that the tablets should be chewed before swallowing; (2) the equivalent number of IU (Units) of antirachitic activity (vitamin D); (3) the equivalent amount of calcium.

Each microgram of Ergocalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

Calcium Chloride Injection

General Notices

DEFINITION

Calcium Chloride Injection is a sterile solution of Calcium Chloride Dihydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of calcium chloride dihydrate, $CaCl_2,2H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. 1 ml yields reaction C characteristic of *calcium salts*, Appendix VI.

B. Dilute 1 volume of the injection to 50 ml with *water*. The resulting solution yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity or alkalinity

pH 5.0 to 8.0, Appendix V L.

Colour of solution

The injection is not more intensely coloured than *reference solution* BY_6, Appendix IV B, Method II.

ASSAY

Carry out the *complexometric titration of calcium*, Appendix VIII D. Use a volume of the injection containing about 0.3 g of Calcium Chloride Dihydrate.

LABELLING

The label states (1) the percentage w/v of Calcium Chloride Dihydrate; (2) the concentration of calcium ion as millimoles in a suitable volume; (3) the concentration of chloride ion as millimoles in a suitable volume; (4) that the injection should be used in accordance with the manufacturer's instructions; (5) that solutions containing visible solid particles must not be used.

When calcium chloride intravenous infusion is prescribed or demanded, Calcium Chloride Injection shall be dispensed or supplied.

Calcium Folinate Injection

General Notices

Action and use

Vitamin B component.

DEFINITION

Calcium Folinate Injection is a sterile solution of Calcium Folinate. It is either supplied as a ready-to-use solution or it is prepared by dissolving Calcium Folinate for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations and, when supplied as a ready-to-use solution, with the following requirements.

Content of folinic acid, $C_{20}H_{23}N_7O_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume containing the equivalent of 20 mg of folinic acid add 40 ml of *acetone*, mix, allow to stand and then centrifuge, discarding the solvent. Suspend the residue in 40 ml of *acetone* and centrifuge. Dry the residue in a stream of nitrogen and then at a pressure of 0.7 kPa for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of calcium folinate *(RS 368)*.

B. To a volume of the injection containing 20 mg of Calcium Folinate add 5 ml of 5M *acetic acid* and carry out test B for *calcium salts*, Appendix VI, beginning at the words 'and add 0.05 ml of *potassium hexacyanoferrate(II) solution* ...'.

TESTS

Acidity or alkalinity

pH, 6.5 to 8.5, Appendix V L.

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute with *water*, if necessary, a volume of the injection to produce a solution containing the equivalent of 0.1% w/v of folinic acid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *water*. Solution (3) contains 0.001% w/v of *formylfolic acid EPCRS* in the mobile phase. For solution (4) dilute 1 volume of solution (2) to 10 volumes with *water*. For solution (5) mix 10 volumes of solution (2) with 5 volumes of solution (3).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 220 ml of *methanol* and 780 ml of a solution containing 2.0 ml of *tetrabutylammonium hydroxide solution* and 2.2 g of *disodium hydrogen orthophosphate* and previously adjusted to pH 7.5 with 10% v/v *orthophosphoric acid* and (c) a detection wavelength of 280 nm.

Inject solution (5). The test is not valid unless the *resolution factor* between the peaks corresponding to calcium folinate and formylfolic acid is at least 2.2. If necessary, adjust the methanol content in the mobile phase.

Inject solutions (1), (2), (3) and (4). In the chromatogram obtained with solution (1) the area of any peak corresponding to formylfolic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with *water* to produce a solution containing the equivalent of 0.01% w/v of folinic acid. Solution (2) contains 0.011% w/v of *calcium folinate EPCRS* in *water*. Solution (3) is a mixture of 5 volumes of a 0.001% w/v solution of *formylfolic acid EPCRS* in the mobile phase and 10 volumes of a 0.001% w/v solution of *calcium folinate EPCRS* in *water*.

The chromatographic conditions described under Related substances may be used.

Inject solution (3). The Assay is not valid unless the *resolution factor* between the peaks corresponding to calcium folinate and formylfolic acid is at least 2.2. If necessary, adjust the methanol content in the mobile phase.

Inject solutions (1) and (2). Calculate the content of folinic acid, $C_{20}H_{23}N_7O_7$, using the declared content of $C_{20}H_{21}CaN_7O_7$ in *calcium folinate EPCRS*. Each mg of $C_{20}H_{21}CaN_7O_7$ is equivalent to 0.926 mg of $C_{20}H_{23}N_7O_7$.

STORAGE

When supplied as a ready-to-use solution, Calcium Folinate Injection should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of folinic acid.

CALCIUM FOLINATE FOR INJECTION

DEFINITION

Calcium Folinate for Injection is a sterile material consisting of Calcium Folinate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of folinic acid, $C_{20}H_{23}N_7O_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of calcium folinate *(RS 368)*. If the spectra are not concordant prepare a solution containing 1% w/v of Calcium Folinate and carry out Identification Test A for the ready-to-use solution.

B. Yield reaction B characteristic of *calcium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 1.0% w/v of folinic acid, 6.5 to 8.5, Appendix V L.

Related substances

Carry out the test described for the ready-to-use solution but using the following solution as solution (1). Dissolve sufficient of the mixed contents of 10 containers in *water* to produce a solution containing the equivalent of 0.1% w/v of folinic acid.

ASSAY

Determine the weight of the contents of each of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the Assay described for the ready-to-use solution but using the following solution as solution (1). Dissolve sufficient of the mixed contents of the 10 containers in *water* to produce a solution containing the equivalent of 0.01% w/v of folinic acid.

Calculate the content of folinic acid, $C_{20}H_{23}N_7O_7$, in a container of average content weight using the declared content of $C_{20}H_{21}CaN_7O_7$ in *calcium folinate EPCRS*. Each mg of $C_{20}H_{21}CaN_7O_7$ is equivalent to 0.926 mg of $C_{20}H_{23}N_7O_7$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of folinic acid.

Calcium Folinate Tablets

General Notices

Action and use

Vitamin B component.

DEFINITION

Calcium Folinate Tablets contain Calcium Folinate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of folinic acid, $C_{20}H_{23}N_7O_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 15 mg of folinic acid with 150 ml of 0.1M *sodium hydroxide* and dilute to 200 ml with the same solvent. Filter and dilute 10 ml of the clear filtrate to 50 ml with 0.1M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 350 nm exhibits a maximum at about 282 nm.

B. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (4).

TESTS

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of *water* to a quantity of the powdered tablets containing the equivalent of 25 mg of folinic acid, shake for 15 minutes and dilute to 25 ml with *water*. Filter through a glass fibre filter (Whatman GF/C is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with *water*. Solution (3) contains 0.001% w/v of *formylfolic acid EPCRS* in the mobile phase. Solution (4) contains 0.001% w/v of *calcium folinate EPCRS* in *water*. Solution (5) contains 0.0001% w/v of *calcium folinate EPCRS* in *water*. For solution (6) mix 5 volumes of solution (3) with 10 volumes of solution (4).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 220 ml of *methanol* and 780 ml of a solution containing 2.0 ml of *tetrabutylammonium hydroxide solution* and 2.2 g of *disodium hydrogen orthophosphate* and previously adjusted to pH 7.5 with 10% v/v *orthophosphoric acid* and (c) a detection wavelength of 280 nm.

Inject solution (6). The test is not valid unless the *resolution factor* between the peaks corresponding to calcium folinate and formylfolic acid is at least 2.2. If necessary adjust the methanol content in the mobile phase.

Inject solutions (1), (2), (3), (4) and (5). In the chromatogram obtained with solution (1) the area of any peak corresponding to formylfolic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (5) (0.1%).

ASSAY

Protect the solutions from light. Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 200 ml of *water* to a quantity of the powdered tablets containing the equivalent of 25 mg of folinic acid, shake for 15 minutes and dilute to 250 ml with *water*. Filter through a glass fibre filter (Whatman GF/C is suitable). Solution (2) contains 0.011% w/v of *calcium folinate EPCRS*. Solution (3) is a mixture of 5 volumes of a 0.001% w/v solution of *formylfolic acid EPCRS* in the mobile phase and 10 volumes of a 0.001% w/v solution of *calcium folinate EPCRS* in *water*.

The chromatographic conditions described under Related substances may be used.

Inject solution (3). The assay is not valid unless the *resolution factor* between the peaks corresponding to calcium folinate and formylfolic acid is at least 2.2. If necessary adjust the methanol content in the mobile phase.

Inject solutions (1) and (2). Calculate the content of $C_{20}H_{23}N_7O_7$ using the declared content of $C_{20}H_{21}CaN_7O_7$ in *calcium folinate EPCRS*. Each mg of $C_{20}H_{21}CaN_7O_7$ is equivalent to 0.926 mg of $C_{20}H_{23}N_7O_7$.

STORAGE

Calcium Folinate Tablets should be stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of folinic acid.

Calcium Gluconate Injection

General Notices

DEFINITION

Calcium Gluconate Injection is a sterile solution of Calcium Gluconate for Injection in Water for Injections. Not more than 5.0% of the Calcium Gluconate may be replaced with calcium M-saccharate, or other suitable calcium salt, as a stabilising agent.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of calcium, Ca

8.5 to 9.4% of the content of Calcium Gluconate stated on the label.

IDENTIFICATION

A. To 1 ml add 0.05 ml of *iron(III) chloride solution R1*. An intense yellow colour is produced.

B. Warm a volume containing the equivalent of 0.5 g of Calcium Gluconate, add 0.65 ml of *glacial acetic acid* and 1 ml of *phenylhydrazine*. Heat on a water bath for 30 minutes, allow to cool and induce crystallisation. Filter, dissolve the residue in 10 ml of hot *water*, add a few mg of *activated charcoal*, shake, filter, allow the filtrate to cool and induce crystallisation. A white, crystalline precipitate is produced. The *melting point* of the crystals, after drying, is about 200°, with decomposition, Appendix V A.

C. Yields the reactions characteristic of *calcium salts*, Appendix VI.

TEST

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection in *water BET* if necessary to give a solution containing 100 mg of Calcium Gluconate per millilitre (solution A). The endotoxin limit concentration of solution A is 16.7 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

To a volume containing the equivalent of 0.5 g of Calcium Gluconate add 300 ml of *water* and carry out the *complexometric titration of calcium*, Appendix VIII D, beginning at the words 'add 6 ml of ...'.

LABELLING

The label states (1) the percentage w/v of Calcium Gluconate equivalent to the total amount of calcium present; (2) that solutions containing visible solid particles must not be used; (3) the name and the percentage of any added stabilising agent.

Calcium Gluconate Tablets

General Notices

DEFINITION

Calcium Gluconate Tablets contain Calcium Gluconate. Tablets that are intended to be chewed before swallowing are prepared using Chocolate Basis or other suitable basis with a chocolate flavour.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium gluconate, $C_{12}H_{22}CaO_{14},H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract five tablets, finely powdered, with two 25-ml quantities of *petroleum spirit (boiling range, 40° to 60°)*, discard the extracts and repeat the extraction with three 10 ml quantities of *water*, again discarding the extracts. Dissolve the residue as completely as possible in 30 ml of hot *water*, filter and to 0.5 ml of the filtrate add 0.05 ml of *iron(III) chloride solution R1*. An intense yellow colour is produced.

B. To a volume of the filtrate obtained in test A containing 0.5 g of Calcium Gluconate add 0.65 ml of *glacial acetic acid* and 1 ml of *phenylhydrazine*, heat on a water bath for 30 minutes, cool and induce crystallisation. Filter, dissolve the residue in 10 ml of hot *water*, add a few mg of *activated charcoal*, shake, filter, allow the filtrate to cool and induce crystallisation. A white, crystalline precipitate is produced. The *melting point* of the crystals, after drying, is about 201°, with decomposition, Appendix V A.

C. The powdered tablets yield the reactions characteristic of *calcium salts*, Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Calcium Gluconate Tablets.

Dissolution

Tablets intended to be swallowed whole comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, measuring at 422.7 nm using a calcium hollow-cathode lamp as the radiation source, an air-acetylene flame and the following solutions.

Test solution Use the filtered dissolution medium diluted, if necessary, with *water* to give a concentration suitable for the instrument used.

Standard solutions Use *calcium standard solution (100 ppm Ca)* suitably diluted with *water*.

Determine the total content of calcium in the dissolution medium and calculate the content of calcium gluconate taking each mg of calcium to be equivalent to 11.21 mg of $C_{12}H_{22}CaO_{14}$, H_2O.

ASSAY

Weigh and powder 20 tablets. Ignite a quantity of the powder containing 0.5 g of Calcium Gluconate, cool and dissolve the residue with gentle heat in 5 ml of 2M *hydrochloric acid*. Filter, wash the residue on the filter with *water* and dilute the combined filtrate and washings to 50 ml with *water*. Neutralise with 5M *ammonia*, using *methyl orange solution* as indicator, add 5 ml of 8M *sodium hydroxide* and titrate with 0.05M *disodium edetate VS* using *calconcarboxylic acid triturate* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 22.42 mg of $C_{12}H_{22}CaO_{14},H_2O$.

LABELLING

The label states, where appropriate, that the tablets should be chewed before swallowing.

When Calcium Gluconate Tablets are prescribed or demanded, no strength being stated, tablets containing 600 mg shall be dispensed or supplied.

Calcium Hydroxide Solution

General Notices

Lime Water

DEFINITION

Calcium Hydroxide	10 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Shake together thoroughly and repeatedly; allow to stand until clear. Siphon off the clear solution as required.

Content of calcium hydroxide, $Ca(OH)_2$

Not less than 0.15% w/v.

CHARACTERISTICS

A colourless liquid. It absorbs carbon dioxide from the air, a film of calcium carbonate being formed on the surface of the liquid. It becomes turbid when boiled and clear again on cooling.

IDENTIFICATION

Yields the reactions characteristic of *calcium salts*, Appendix VI.

ASSAY

Titrate 25 ml with 0.1M *hydrochloric acid VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 3.705 mg of $Ca(OH)_2$.

STORAGE

Calcium Hydroxide Solution should be kept in a well-filled container.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

Calcium Lactate Tablets

General Notices

DEFINITION

Calcium Lactate Tablets contain Calcium Lactate Pentahydrate or Calcium Lactate Trihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium lactate, calculated as $C_6H_{10}CaO_6,5H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Triturate a quantity of the powdered tablets containing 0.3 g of Calcium Lactate Pentahydrate or its equivalent with 5 ml of *methanol* and filter. To 0.2 ml of the filtrate add 2 ml of *sulphuric acid* , heat at 85° for 2 minutes, cool and add 4 mg of *4-hydroxybiphenyl* . A violet-red colour is produced.

B. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, impart a brick red colour to the flame.

Disintegration

Maximum time, 30 minutes, Appendix XII A1.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of Calcium Lactate Pentahydrate or its equivalent as completely as possible in 50 ml of *water* and titrate with 0.05M *disodium edetate VS* to within a few millilitres of the expected end point. Add 8 ml of 5M *sodium hydroxide* and 0.1 g of *solochrome dark blue mixture* and continue the titration until the colour changes from pink to full blue. Each ml of 0.05M *disodium edetate VS* is equivalent to 15.41 mg of $C_6H_{10}CaO_6,5H_2O$.

LABELLING

When the active ingredient is Calcium Lactate Trihydrate the quantity is stated in terms of the equivalent amount of Calcium Lactate Pentahydrate.

Captopril Oral Solution

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Angiotensin-converting enzyme inhibitor.

DEFINITION

Captopril Oral Solution is a solution containing Captopril in a suitable flavoured vehicle. It is supplied as a ready-to-use solution or it is prepared by dissolving Captopril Powder for Oral Solution in the requisite volume of the vehicle provided just before issue for use.

The oral solution complies with the requirements stated under Oral Liquids and with the requirements stated under Unlicensed Medicines.

STORAGE

The oral solution should be stored at the temperature and used within the period stated on the label.

When supplied as a ready-to-use solution, the oral solution complies with the following requirements.

Content of captopril, $C_9H_{15}NO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to captopril in the chromatogram obtained with solution (2).

TESTS

Captopril disulphide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the oral solution containing 25 mg of Captopril to 50 ml with *methanol* and mix.

(2) 0.0015% w/v of *captopril disulphide BPCRS* in *methanol*.

(3) Dilute 1 volume of solution (1) to 100 volumes with solution (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 0.5 volume of *orthophosphoric acid*, 450 volumes of *water* and 550 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to captopril and captopril disulphide is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to captopril disulphide is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing 25 mg of Captopril to 50 ml with mobile phase, mix and dilute 1 volume of the resulting solution to 5 volumes with the mobile phase.

(2) 0.01% w/v of *captopril BPCRS* and 0.0005% w/v of *captopril disulphide BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 0.5 volume of *orthophosphoric acid*, 450 volumes of *water* and 550 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to captopril and captopril disulphide is at least 2.0.

DETERMINATION OF CONTENT

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of

$C_9H_{15}NO_3S$, weight in volume, using the declared content of $C_9H_{15}NO_3S$ in *captopril BPCRS*.

CAPTOPRIL POWDER FOR ORAL SOLUTION

DEFINITION

Captopril Powder for Oral Solution is a dry powder consisting of Captopril with or without excipients. It is supplied in a sealed container.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

Content of captopril, $C_9H_{15}NO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to captopril in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a solution containing 2% w/v of Captopril, 2.0 to 2.6, Appendix V L.

Clarity of solution

A solution containing 2% w/v of Captopril in *carbon dioxide-free water* is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the contents of the sealed container containing 50 mg of Captopril in the mobile phase and add sufficient mobile phase to produce 100 ml.

(2) Dilute 2 volumes of solution (1) to 100 volumes with the mobile phase.

(3) Dissolve a quantity of the contents of the sealed container containing 10 mg of Captopril in mobile phase, add 0.25 ml of 0.05M *iodine* and sufficient mobile phase to produce 100 ml. Dilute 10 volumes of the resulting solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12.5 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for 3 times the retention time of Captopril.

MOBILE PHASE

A mixture of 0.5 volume of *orthophosphoric acid*, 500 volumes of *methanol* and 500 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three principal peaks and the *resolution factor* between the last two eluting principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and any peak with a retention time less than 1.4 minutes.

ASSAY

Mix the contents of 10 containers and dissolve a quantity of the mixed contents containing 0.15 g of Captopril in 30 ml of *water*. Titrate with 0.05M *iodine*, determining the end-point potentiometrically, Appendix VIII B, using a combined platinum electrode.

Each ml of 0.05M *iodine* is equivalent to 21.73 mg of $C_9H_{15}NO_3S$.

Captopril Tablets

General Notices

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Captopril Tablets contain Captopril.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of captopril, $C_9H_{15}NO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of Captopril in 25 ml of *methanol* with the aid of ultrasound and filter. Mix 1 ml of the filtrate with 0.5 g of *potassium bromide*, dry at room temperature at 2 kPa, grind to a uniform mixture and prepare a disc. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of captopril *(RS 038)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to captopril in the chromatogram obtained with solution (2).

TESTS

Captopril disulphide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) transfer a quantity of the powdered tablets containing 25 mg of Captopril to a centrifuge tube, add 25 ml of *methanol*, centrifuge for 15 minutes and use the supernatant liquid. Solution (2) contains 0.0030% w/v of *captopril disulphide BPCRS* in *methanol*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 0.5 volume of *orthophosphoric acid*, 450 volumes of *water* and 550 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 220 nm.

The test is not valid unless in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to captopril and captopril disulphide is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to captopril disulphide is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) transfer a quantity of the powdered tablets containing 25 mg of Captopril to a centrifuge tube, add 25 ml of the mobile phase, mix with the aid of ultrasound for 15 minutes and centrifuge. Dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase. Solution (2) contains 0.01% w/v of *captopril BPCRS* and 0.0005% w/v of *captopril disulphide BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 0.5 volume of *orthophosphoric acid*, 450 volumes of *water* and 550 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 220 nm.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to captopril and captopril disulphide is at least 2.0.

Calculate the content of $C_9H_{15}NO_3S$ using the declared content of $C_9H_{15}NO_3S$ in *captopril BPCRS*.

Carbamazepine Tablets

General Notices

Carbamazepine Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Action and use

Antiepileptic.

DEFINITION

Carbamazepine Tablets contain Carbamazepine.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Carbamazepine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of carbamazepine, $C_{15}H_{12}N_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 ml of *acetone*, filter the hot solution, wash the filtrate with two 5 ml quantities of hot *acetone*, cool in ice, evaporate the combined filtrates to dryness. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of carbamazepine (*RS 406*). Examine as a disc prepared using potassium bromide.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 ml of *methanol* for 15 minutes. Dilute to 200 ml with *water*, mix and filter. For solution (2) dissolve 7.5 mg each of *carbamazepine EPCRS*, *carbamazepine impurity A EPCRS* and *iminodibenzyl* (impurity E) in *methanol* and dilute to 100 ml with the same solvent. Dilute 1.0 ml of the resulting solution to 50 ml with a mixture of *methanol (50%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (10 µm) (Nucleosil 10 CN is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, adding 0.2 ml of *anhydrous formic acid* and 0.5 ml of *triethylamine* to 1000 ml of the solution and (c) a detection wavelength of 230 nm.

Inject 20 µl of solution (1) and continue the chromatography for 6 times the retention time of carbamazepine which is about 10 minutes. The test is not valid unless in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to carbamazepine and carbamazepine impurity A is at least 1.7.

The relative retention times with reference to carbamazepine are impurity B, about 0.7; impurity A, about 0.9; impurity C, about 1.6; impurity D, about 3.5; impurity E, about 5.1.

In the chromatogram obtained with solution (1), the areas of any peaks corresponding to carbamazepine impurities A and E are not greater than the areas of the corresponding peaks in the chromatogram obtained with solution (2) (0.1%). The area of any other *secondary peak* is not greater than the area of the peak due to carbamazepine (0.1%) and the sum of the areas of any such peaks is not greater than 5 times the area of the peak due to carbamazepine (0.5%). Disregard any peak with an area less than 0.5 times the area of the peak due to carbamazepine in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 ml of *methanol* for 15 minutes. Dilute to 200 ml with *water*, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50%)*. Solution (2) is a 0.03% w/v of *carbamazepine EPCRS* in *methanol (50%)*.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{15}H_{12}N_2O$ from the chromatograms obtained and using the declared content of $C_{15}H_{12}N_2O$ in *carbamazepine EPCRS*.

Carbaryl Lotion

General Notices

Action and use

Insecticide.

DEFINITION

Carbaryl Lotion is a *cutaneous solution*. It contains Carbaryl in a suitable vehicle.
The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of carbaryl, $C_{12}H_{11}NO_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 0.5 volume of *water*, 10 volumes of *methanol* and 90 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) disperse a quantity of the preparation being examined containing 4 mg of Carbaryl in a solution containing 10 ml of a saturated solution of *sodium chloride* and 50 ml of *ether*, shake, allow the layers to separate, wash the ether layer with two 10 ml quantities of *water*, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in 1 ml of *absolute ethanol*. Solution (2) contains 0.4% w/v of *carbaryl BPCRS* in *absolute ethanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

1-Naphthol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the preparation being examined with sufficient *acetonitrile* to produce a solution containing 0.1% w/v of Carbaryl. Solution (2) contains 0.003% w/v of *1-naphthol* in the mobile phase. Solution (3) contains 0.005% w/v of *carbaryl BPCRS* and 0.005% w/v of *1-naphthol* in the mobile phase.

The chromatographic conditions described under Assay may be used.

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 1-

naphthol is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the preparation being examined with sufficient *methanol* to produce a solution containing 0.005% w/v of Carbaryl. Solution (2) contains 0.005% w/v of *carbaryl BPCRS* in *methanol*. Solution (3) contains 0.005% w/v of *carbaryl BPCRS* and 0.005% w/v of *1-naphthol* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of 1 volume of *glacial acetic acid*, 25 volumes of *acetonitrile* and 75 volumes of *water* as the mobile phase with a flow rate of 2.5 ml per minute and (c) a detection wavelength of 280 nm.

Inject 20 µl of each solution. The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the content of $C_{12}H_{11}NO_2$ using the declared content of $C_{12}H_{11}NO_2$ in *carbaryl BPCRS*.

STORAGE

Carbaryl Lotion should be protected from light.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous solution'.

Carbimazole Tablets

General Notices

Action and use

Thionamide antithyroid drug.

DEFINITION

Carbimazole Tablets contain Carbimazole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of carbimazole, $C_7H_{10}N_2O_2S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 50 mg of Carbimazole with two 5 ml quantities of *dichloromethane*. Combine the dichloromethane extracts, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum*, Appendix II A, of the residue after drying at 60° at a pressure not exceeding 0.7 kPa for 30 minutes is concordant with the *reference spectrum* of carbimazole *(RS 042)*.

B. To a small quantity of the powdered tablets add 0.05 ml of *dilute potassium iodobismuthate solution*. A scarlet colour is produced.

Thiamazole and other related substances

Carry out the test protected from light and prepare the solutions immediately before use.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 20 mg of Carbimazole in 10 ml of *acetonitrile* with the aid of ultrasound for 5 minutes, filter through a nylon syringe filter and dilute 1 volume to 4 volumes with 5% v/v of *acetonitrile*.

(2) 0.00025% w/v of *carbimazole BPCRS* in 5% v/v of *acetonitrile*.

(3) 0.0005% w/v of *thiamazole* in 5% v/v of *acetonitrile.*

(4) 0.01% w/v of *carbimazole BPCRS* and 0.0005% w/v of *thiamazole* in 5% v/v of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use linear gradient elution using the mobile phases described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use ambient column temperature.

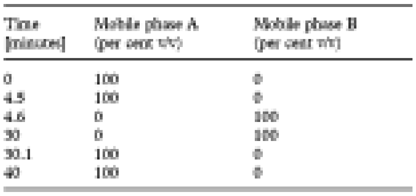
(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

Mobile phase A *acetonitrile* (5% v/v)

Mobile phase B *acetonitrile* (20% v/v)



Time [minutes]	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
4.5	100	0
4.6	0	100
30	0	100
30.1	100	0
40	100	0

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to carbimazole and thiamazole is at least 5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to thiamazole is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

— the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the test protected from light and prepare the solutions immediately before use.

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 20 mg of Carbimazole in 10 ml of *acetonitrile* with the aid of ultrasound for 5 minutes, filter through a nylon syringe filter and dilute 1 volume of this solution to 40 volumes with 5% v/v of *acetonitrile*.

(2) 0.005% w/v of *carbimazole BPCRS* in 5% v/v of *acetonitrile*.

(3) 0.01% w/v of *carbimazole BPCRS* and 0.0005% w/v of *thiamazole* in 5% v/v of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to carbimazole and thiamazole is at least 5.

DETERMINATION OF CONTENT

Calculate the content of $C_7H_{10}N_2O_2S$ in the tablets using the declared content of $C_7H_{10}N_2O_2S$ in *carbimazole BPCRS.*

Carbomer Eye Drops

General Notices

Action and use

Stabilizer in pharmaceutical products.

DEFINITION

Carbomer Eye Drops are a sterile viscous solution of Carbomer 940 or Carbomer 980 in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

A. Add 15 g of the eye drops to 85 ml of *water* and mix thoroughly. To 3 ml add 1 ml of *calcium chloride solution*, a fine white precipitate is produced.

B. Add 0.5 ml of *thymol blue solution* to 10 g of the eye drops. A yellow colour is produced. Add 0.5 ml of *cresol red solution* to 10 g of the eye drops. A yellow colour is produced.

TESTS

Acidity or alkalinity

pH, 6.6 to 7.1, Appendix V L.

Apparent viscosity

75 to 125% of the declared value when determined by the following method. Empty the contents of sufficient containers to obtain 200 g of the eye drops, taking care to avoid incorporation of air bubbles, and homogenise. Allow to stand at 25° for 60 minutes. Immerse the appropriate spindle of a rotational viscometer, switch on after 5 minutes and determine the *viscosity* at 25°, Appendix V H, Method III, using a shear speed of 5 per second.

Clarity of solution

The eye drops are not more opalescent than *reference suspension III*, Appendix IV A.

LABELLING

The label states the nominal viscosity in millipascal seconds.

Carboplatin Injection

General Notices

Action and use

Platinum-containing cytotoxic.

DEFINITION

Carboplatin Injection is a sterile solution of Carboplatin in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of carboplatin, $C_6H_{12}N_2O_4Pt$

90.0 to 105.0% of the stated amount.

Carry out the following procedures protected from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* and a mixture of 20 volumes of *water* and 80 volumes of *acetone* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions containing (1) the injection diluted, if necessary, with *water* to produce a solution containing 1.0% w/v of Carboplatin and (2) 1.0% w/v of *carboplatin BPCRS* in *water*. Use the solutions within 2 hours of preparation. After removal of the plate, allow it to dry in air for 2 hours. Spray the plate with a solution prepared immediately before use by dissolving 5.6 g of *tin(II)chloride* in 10 ml of *hydrochloric acid* (dissolution may not be complete, filter if necessary), adding 90 ml of *water* and 1 g of *potassium iodide* and stirring. Heat the plate at 100° for 10 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.0 to 7.0, Appendix V L.

Cyclobutane-1,1-dicarboxylic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), dilute the injection with *water* to produce a solution containing 0.1% w/v of Carboplatin; use within 2 hours of preparation. Solution (2) contains 0.001% w/v of *cyclobutane-1,1-dicarboxylic acid* in *water*. For solution (3) mix 1 volume of solution (1) with 1 volume of solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm

× 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Micro-bondapak C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 20 volumes of solution A prepared as described below, 100 volumes of *acetonitrile* and 880 volumes of *water* and (c) a detection wavelength of 220 nm. To prepare solution A dissolve 8.5 g of *tetrabutylammonium hydrogen sulphate* in 80 ml of *water*, add 3.4 ml of *orthophosphoric acid* and adjust the pH to 7.55 with 10M *sodium hydroxide*.

Inject 100 µl of each solution. For solution (1), allow the chromatography to proceed for 2.5 times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to carboplatin and that due cyclobutane-1,1-dicarboxylic acid is at least 2.5.

In the chromatogram obtained with solution (1), the area of any peak corresponding to cyclobutane-1,1-dicarboxylic acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection with *water BET*, if necessary, to contain 10 mg of Carboplatin per ml (solution A). The endotoxin limit concentration of this solution is 5.4 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions used within 2 hours of preparation. For solution (1) dilute the injection with *water* to produce a solution containing 0.1% w/v of Carboplatin. Solution (2) contains 0.1% w/v of *carboplatin BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *aminopropylsilyl silica gel for chromatography* (5 µm) (Micro-bondapak-NH_2 is suitable), (b) as the mobile phase a mixture of 130 volumes of *water* and 870 volumes of *acetonitrile* with a flow rate of 2 ml per minute and (c) a detection wavelength of 230 nm.

The test is not valid unless, in the chromatogram obtained with solution (1), the *capacity factor* is not less than 4.0, the number of *theoretical plates* is not less than 5000 and the *symmetry factor* is not more than 2.0.

Calculate the content of $C_6H_{12}N_2O_4Pt$ in the injection using the declared content of $C_6H_{12}N_2O_4Pt$ in *carboplatin BPCRS*.

STORAGE

Carboplatin Injection should be protected from light and free from contact with metals.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Carboplatin.

Carmellose Sodium Eye Drops

General Notices

Action and use

Used in the treatment of tear deficiency.

DEFINITION

Carmellose Sodium Eye Drops are a sterile colloidal solution of Carmellose Sodium in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of carmellose sodium

95.0 to 115.0% of the stated amount.

IDENTIFICATION

A. To 10 ml of the eye drops, add 1 ml of *copper sulphate solution*. A blue, cotton-like precipitate is formed.

B. In the Assay, the spectrum obtained with solution (1) shows maxima at about 295, 366, 519 and 635 nm. The spectrum obtained with solution (1) corresponds to the spectrum obtained with solution (2).

TESTS

Clarity and colour of solution

The eye drops are *clear*, Appendix IV A, and are not more intensely coloured than *reference solution* Y_6, Appendix IV B, Method II.

Acidity

pH, 6.0 to 7.0, Appendix V L.

Osmolality

The osmolality of the eye drops is 270 to 350 mosmol/kg, Appendix V N.

ASSAY

Dissolve 3.75 g of *diphenylamine* in 150 ml of *glacial acetic acid*, add 90 ml of *hydrochloric acid* and mix well (solution A).

For solution (1), use the eye drops, diluted if necessary, with *water* to contain 0.01% w/v of Carmellose Sodium. Pipette 2 ml of this solution into a glass stoppered test tube, add 5 ml of solution A, mix and immediately immerse the test tube in an oil bath maintained at 105° to 110° for 30 minutes, the bath temperature being kept uniform to within 0.1° during this time.

Remove the test tube, place in an ice bath until cooled and allow to equilibrate to room temperature for not more than 1 hour. For solution (2), prepare a solution containing 0.01% w/v of *carmellose sodium BPCRS* in *water* and treat at the same time and in the same manner as solution (1), starting at the words "pipette 2 ml of this solution ….". For solution (3), use *water* and treat at the same time and in the same manner as solution (1), starting at the words "pipette 2 ml of this solution….".

Measure the *absorbance* of solution (1) and solution (2) at the maximum at about 635 nm, Appendix II B, using solution (3) in the reference cell. Calculate the content of Carmellose Sodium in the eye drops from the values of the absorbances obtained and using the declared content of Carmellose Sodium in *carmellose sodium BPCRS*.

Carteolol Eye Drops

General Notices

DEFINITION

Carteolol Eye Drops are a sterile solution of Carteolol Hydrochloride in Purified Water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of carteolol hydrochloride, $C_{16}H_{24}N_2O_3$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 50 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute the eye drops with *water*, if necessary, to contain 0.5% w/v of Carteolol Hydrochloride. Solution (2) contains 0.5% w/v of *carteolol hydrochloride BPCRS* in *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 6.2 to 7.2, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops with the mobile phase to contain 0.20% w/v of Carteolol Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (YMC-Pack ODS-A is suitable), (b) as the mobile phase a mixture of 1 volume of *methanol*, 20 volumes of *acetonitrile* and 79 volumes of a 0.282% w/v solution of *sodium hexanesulphonate* at a flow rate such that the retention time of carteolol hydrochloride is about 14 minutes (1 ml per minute may be suitable) and (c) a detection wavelength of 252 nm.

The test is not valid unless the *column efficiency*, determined on the principal peak in the chromatogram obtained with solution (2), is at least 6000 *theoretical plates* per metre.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than twice the area of the peak in the chromatogram obtained with solution (3) (0.2%), the area of not more than one such peak is greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%) and the sum of the areas of all such peaks is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. For solution (1) dilute the eye drops to contain 0.002% w/v of Carteolol Hydrochloride. Solution (2) contains 0.002% w/v of *carteolol hydrochloride BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher C8 is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute a solution containing 1.0 g of *potassium dihydrogen orthophosphate* in a mixture of 80 ml of *acetonitrile* and 400 ml of *water* and (c) a detection wavelength of 252 nm.

The test is not valid unless the *column efficiency*, determined on the principal peak in the chromatogram obtained with solution (2), is at least 6000 *theoretical plates* per metre.

Calculate the content of $C_{16}H_{24}N_2O_3$,HCl in the eye drops from the chromatograms obtained and from the declared content of $C_{16}H_{24}N_2O_3$,HCl in *carteolol hydrochloride BPCRS*.

Cascara Tablets

General Notices

DEFINITION

Cascara Tablets contain Cascara Dry Extract. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of total hydroxyanthracene derivatives

17.0 to 23.0 mg, of which not less than 40% consists of cascarosides, both calculated as cascaroside A.

TESTS

Disintegration

Comply with the requirements stated under Tablets but for sugarcoated tablets the maximum time is 120 minutes.

Frangula

Comply with the requirements stated under Cascara Dry Extract, but preparing solution (1) with a quantity of the powdered tablets containing 32 mg of total hydroxyanthracene derivatives.

ASSAY

Weigh and powder 20 tablets. Carry out the Assay described under Cascara Dry Extract using a quantity of the powder containing 75 mg of total hydroxyanthracene derivatives.

Cefaclor Capsules

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefaclor Capsules contain Cefaclor.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of anhydrous cefaclor, $C_{15}H_{14}ClN_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 0.3 g of anhydrous cefaclor with 100 ml of *water*, filter and dilute 1 ml of the filtrate to 100 ml with *water*. The *light absorption*, Appendix II B, in the range 190 nm to 310 nm, of the final solution exhibits a maximum only at 264 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute the filtered solution, if necessary, with sufficient *water* to produce a solution expected to contain the equivalent of about 0.025% w/v of anhydrous cefaclor. Measure the *absorbance* of the solution at the maximum at 264 nm, Appendix II B, using *water* in the reference cell. Calculate the total content of anhydrous cefaclor, $C_{15}H_{14}ClN_3O_4S$, in the medium from the *absorbance* of a 0.025% w/v solution of *cefaclor EPCRS* in *water* and using the declared content of $C_{15}H_{14}ClN_3O_4S$ in *cefaclor EPCRS*.

Related substances

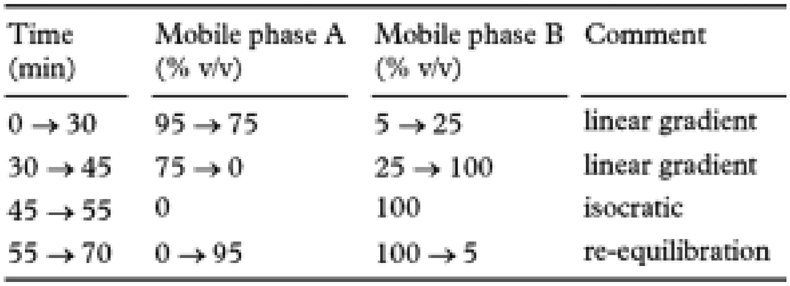
Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. Prepare a 0.27% w/v solution of *sodium dihydrogen orthophosphate* and adjust the pH to 2.5, if necessary, with *orthophosphoric acid* (solution A). For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 0.5 g of anhydrous cefaclor with 200 ml of solution A, add sufficient of solution A to produce 250 ml and filter. Solution (2) contains 0.002% w/v of *cefaclor EPCRS* in solution A. Solution (3) contains 0.0025% w/v of *cefaclor EPCRS* and 0.005% w/v of *delta-3-cefaclor EPCRS* in solution A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS-2 is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 220 nm.

Mobile phase A A 0.78% w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid*.

Mobile phase B Mix 450 volumes of *acetonitrile* with 550 volumes of mobile phase A.

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes. Inject the solutions and carry out the following gradient elution.



Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0 → 30	95 → 75	5 → 25	linear gradient
30 → 45	75 → 0	25 → 100	linear gradient
45 → 55	0	100	isocratic
55 → 70	0 → 95	100 → 5	re-equilibration

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%). Disregard any peak with an area less than 0.1 times that of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered, mixed contents of 20 capsules containing the equivalent of 75 mg of anhydrous cefaclor with the mobile phase, add sufficient mobile phase to produce 250 ml and filter. Solution (2) contains 0.03% w/v of *cefaclor EPCRS* in the mobile phase. Solution (3) contains 0.03% w/v of each of *cefaclor EPCRS* and *delta-3-cefaclor EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Beckman Ultrasphere ODS and Supelcosil LC-18-DB are suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 1 g of *sodium pentanesulphonate* in a mixture of 780 ml of *water* and 10 ml of *triethylamine*, adjusting the pH to 2.5 using *orthophosphoric acid*, adding 220 ml of *methanol* and mixing and (c) a detection wavelength of 265 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.5 and the *symmetry* *factor* of the peak due to cefaclor is at most 1.5.

Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the capsules from the chromatograms obtained and using the declared content of $C_{15}H_{14}ClN_3O_4S$ in *cefaclor EPCRS*.

STORAGE

Cefaclor Capsules should be stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

Cefaclor Oral Suspension

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefaclor Oral Suspension is a suspension of Cefaclor in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

STORAGE

The dry ingredients should be protected from light and stored at a temperature not exceeding 30°.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous cefaclor, $C_{15}H_{14}ClN_3O_4S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing the equivalent of 0.3 g of anhydrous cefaclor with 500 ml of *water*, filter and use the filtrate. The *light absorption*, Appendix II B, in the range 190 nm to 310 nm, of the final solution exhibits a maximum only at 264 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. Prepare a 0.27% w/v solution of *sodium dihydrogen orthophosphate* and adjust the pH to 2.5, if necessary, with *orthophosphoric acid* (solution A). For solution (1) shake a quantity of the oral suspension containing the equivalent of 0.25 g of anhydrous cefaclor with 200 ml of solution A, add sufficient of solution A to produce 250 ml and filter. Solution (2) contains 0.001% w/v of *cefaclor EPCRS* in solution A. Solution (3) contains 0.0025% w/v of *cefaclor EPCRS* and 0.005% w/v of *delta-3-cefaclor EPCRS* in solution A.

The chromatographic conditions described under Cefaclor Capsules may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%). Disregard any peak with an area less than 0.1 times that of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the oral suspension containing the equivalent of 75 mg of anhydrous cefaclor with 200 ml of the mobile phase, add sufficient of the mobile phase to produce 250 ml and filter. Solution (2) contains 0.03% w/v of *cefaclor EPCRS* in the mobile phase. Solution (3) contains 0.03% w/v of each of *cefaclor EPCRS* and *delta-3-cefaclor EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Beckman Ultrasphere ODS and Supelcosil LC-18-DB are suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 1 g of *sodium pentanesulphonate* in a mixture of 780 ml of *water* and 10 ml of *triethylamine*, adjusting the pH to 2.5 using *orthophosphoric acid*, adding 220 ml of *methanol* and mixing and (c) a detection wavelength of 265 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.5 and the *symmetry factor* of the peak due to cefaclor is at most 1.5.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{14}ClN_3O_4S$, weight in volume, using the declared content of $C_{15}H_{14}ClN_3O_4S$ in *cefaclor EPCRS*.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

Cefadroxil Capsules

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefadroxil Capsules contain Cefradroxil Monohydrate.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 plates are suitable) and a mixture of 3 volumes of a 6.7% w/v solution of *ninhydrin* in *acetone*, 80 volumes of a 0.1M solution of *disodium hydrogen orthophosphate* and 120 volumes of a 0.1M solution of *citric acid* as the mobile phase. Impregnate the plate by development with a 5% v/v solution of *n-tetradecane* in *hexane*. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) stir a quantity of the contents of the capsules containing the equivalent of 0.2 g of anhydrous cefadroxil with 100 ml of *water*, filter and use the filtrate. Solution (2) contains 0.2% w/v of *cefadroxil BPCRS* in *water*. After removal of the plate allow it to dry in air, spray with a 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat the plate at 110° for 10 minutes and allow to cool. The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of *water* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtered medium, diluted if necessary with *water*, at the maximum at 263 nm using *water* in the reference cell, Appendix II B. Calculate the total content of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$, in the medium from the *absorbance* obtained from a 0.003% w/v solution of *cefadroxil BPCRS* in *water* and using the declared content of $C_{16}H_{17}N_3O_5S$ in *cefadroxil BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) add 50 ml of the mobile phase to a quantity of the contents of the capsules containing the equivalent of 0.5 g of anhydrous cefadroxil, mix, stir magnetically for 10 minutes, filter and use the filtrate. Solution (2) contains 0.01% w/v of *cefadroxil BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of *D-α-(4-hydroxyphenyl)glycine EPCRS* (cefadroxil impurity A) in the mobile phase. Solution (4) contains 0.01% w/v of *7-aminodesacetoxycephalosporanic acid EPCRS* (cefadroxil impurity B) in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared as described below and (c) a detection wavelength of 254 nm. For the mobile phase add 200 ml of 1M *potassium hydroxide*, 40 ml of *0.4M tetrabutylammonium hydroxide* and 80 ml of *methanol* to 1600 ml of *water*, mix, add sufficient *water* to produce 2000 ml and adjust the pH to 7.0, if necessary, with *orthophosphoric acid*. For solution (1) allow the chromatography to proceed for 6 times the retention time of the principal peak.

When the chromatograms are recorded under the conditions described above the retention time of cefadroxil is 8 to 12 minutes. If necessary, adjust the composition of the mobile phase (increasing the methanol content decreases the retention time, decreasing the methanol content increases the retention time).

The test is not valid unless the *column efficiency*, determined on the peak due to cefadroxil in the chromatogram obtained with solution (2), is at least 1500 *theoretical plates* per metre and the *symmetry factor* of the principal peak is at most 1.6.

Inject solution (2) five times. The test is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

In the chromatogram obtained with solution (1) the area of any peak corresponding to cefadroxil impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%), the area of any peak corresponding to cefadroxil impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Water

The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 0.5 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing the equivalent of 0.2 g of anhydrous cefadroxil with 150 ml of a phosphate buffer prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate* in sufficient *water* to produce 2000 ml and adjusting the pH, if necessary, to 5.0 with 10M *potassium hydroxide* for 5 minutes. Add sufficient of the buffer solution to produce 200 ml and filter. Solution (2) contains 0.1% w/v of *cefadroxil BPCRS* in the buffer solution. Solution (3) contains 0.005% w/v of *cefadroxil BPCRS* and 0.05% w/v of *amoxicillin trihydrate BPCRS* in the buffer solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm

× 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm or 10 µm) (Hypersil ODS is suitable), (b) as the mobile phase at a flow rate of 1.0 ml per minute a mixture of 4 volumes of *acetonitrile* and 96 volumes of a 0.272% w/v solution of *potassium dihydrogen orthophosphate* and (c) a detection wavelength of 254 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefadroxil and amoxicillin is at least 5.0. If necessary, adjust the acetonitrile content in the mobile phase.

Calculate the content of $C_{16}H_{17}N_3O_5S$ in the capsules using the declared content of $C_{16}H_{17}N_3O_5S$ in *cefadroxil BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefadroxil.

Cefadroxil Oral Suspension

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefadroxil Oral Suspension is a suspension of Cefadroxil Monohydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

STORAGE

The dry ingredients should be stored at a temperature not exceeding 30°.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$

When freshly constituted not more than 110.0% of the stated amount. When stored at the temperature and for the period stated on the label, during which the Oral Suspension may be expected to be satisfactory for use, not less than 90.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 plates are suitable) and a mixture of 3 volumes of a 6.7% w/v solution of *ninhydrin* in *acetone*, 80 volumes of a 0.1M solution of *disodium hydrogen orthophosphate* and 120 volumes of a 0.1M solution of *citric acid* as the mobile phase. Impregnate the plate by development with a 5% v/v solution of *n-tetradecane* in *hexane*. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) dilute a volume of the oral suspension containing the equivalent of 0.2 g of anhydrous cefadroxil to 100 ml with *water*, filter and use the filtrate. Solution (2) contains 0.2% w/v of *cefadroxil BPCRS* in *water*. After removal of the plate, allow it to dry in air, spray with a 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat the plate at 110° for 10 minutes and allow to cool. The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the oral suspension with sufficient of mobile phase A to produce a solution containing the equivalent of 0.1% w/v of anhydrous cefadroxil, mix, stir magnetically for 10 minutes, filter through a 0.45-µm filter and use the filtrate. Solution (2) contains 0.001% w/v of *cefadroxil BPCRS* in mobile phase A. Solution (3) contains 0.001% w/v of *D-α-(4-hydroxyphenyl)glycine EPCRS* (cefadroxil impurity A) in the mobile phase. Solution (4) contains 0.001% w/v of *7-aminodesacetoxycephalosporanic acid EPCRS* (cefadroxil impurity B) in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP-18 is suitable), (b) as mobile phases A and B with a flow rate of 1 ml per minute the solutions described below and (c) a detection wavelength of 254 nm.

Mobile phase A Dissolve 5.44 g of *potassium dihydrogen orthophosphate* in 2000 ml of *water* and adjust the pH, if necessary, to 5.0 with a 1% w/v solution of *potassium hydroxide*.

Mobile phase B Add 400 ml of *acetonitrile* to 600 ml of mobile phase A and adjust the pH, if necessary to 5.0 with a 2% v/v solution of *orthophosphoric acid*.

Inject 20 µl of each solution and record the chromatograms under the following conditions. Elute initially with mobile phase A. After 5 minutes, use linear gradient elution increasing the concentration of mobile phase B to 32% after 35 minutes. Elute isocratically for 25 minutes with a mixture of 32% of mobile phase B and 68% of mobile phase A. Carry out a linear gradient elution for 1 minute to 100% of mobile phase A and elute for a further 9 minutes with mobile phase A.

When the chromatograms are recorded under the conditions described above the retention time of cefadroxil is 14 to 20 minutes. If necessary, adjust the proportion of mobile phase A to mobile phase B to achieve the stated retention time.

The test is not valid unless the *column efficiency*, determined on the peak due to cefadroxil in the chromatogram obtained with solution (2), is at least 2000 *theoretical plates* per metre and the *symmetry factor* of the principal peak is at most 1.5.

Inject solution (2) five times. The test is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

In the chromatogram obtained with solution (1) the area of any peak corresponding to cefadroxil impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%), the area of any peak corresponding to cefadroxil impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) shake a weighed quantity of the oral suspension containing the equivalent of 0.25 g of anhydrous cefadroxil with 250 ml of a phosphate buffer prepared by dissolving 13.6

g of *potassium dihydrogen orthophosphate* in sufficient *water* to produce 2000 ml and adjusting the pH, if necessary, to 5.0 with 10M *potassium hydroxide* for 5 minutes; filter and use the filtrate. Solution (2) contains 0.1% w/v of *cefadroxil BPCRS* in the buffer solution. Solution (3) contains 0.005% w/v of *cefadroxil BPCRS* and 0.05% w/v of *amoxicillin trihydrate BPCRS* in the buffer solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm or 10 µm) (Hypersil ODS is suitable), (b) as the mobile phase at a flow rate of 1.0 ml per minute a mixture of 4 volumes of *acetonitrile* and 96 volumes of a 0.272% w/v solution of *potassium dihydrogen orthophosphate* and (c) a detection wavelength of 254 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefadroxil and amoxicillin is at least 5.0. If necessary, adjust the acetonitrile content in the mobile phase.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{17}N_3O_5S$, weight in volume, using the declared content of $C_{16}H_{17}N_3O_5S$ in *cefadroxil BPCRS*.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

Cefadroxil Oral Suspension should be kept at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefadroxil.

Cefalexin Tablets

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefalexin Tablets contain Cefalexin Monohydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous cefalexin, $C_{16}H_{17}N_3O_4S$

92.5 to 110.0% of the stated amount.

IDENTIFICATION

Remove any coating. Shake a quantity of the powdered tablet cores containing the equivalent of 0.5 g of anhydrous cefalexin with 1 ml of *water* and 1.4 ml of 1M *hydrochloric acid*, add 0.1 g of *activated charcoal*, shake, filter and wash the filter with 1 ml of *water*. Add slowly to the filtrate a saturated solution of *sodium acetate* until precipitation occurs. Add 5 ml of *methanol*, filter and wash the precipitate with two 1 ml quantities of *methanol*. The residue, after drying at a pressure not exceeding 0.7 kPa, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cefalexin *(RS 049)*.

B. Complies with test B described under Cefalexin Capsules but preparing solution (1) in the following manner. Shake 0.2 g with 25 ml of a mixture of equal volumes of *methanol* and 0.067M *mixed phosphate buffer pH 7.0*, dilute to 50 ml with the same solvent mixture, filter and use the filtrate.

C. Mix 20 mg with 0.25 ml of a 1% v/v solution of *glacial acetic acid* and add 0.1 ml of a 1% w/v solution of *copper(II) sulphate* and 0.1 ml of 2M *sodium hydroxide*. An olive-green colour is produced.

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A1.

Related substances

Comply with the test described under Cefalexin Capsules but using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.25 g of anhydrous cefalexin with 10 ml of 2M *hydrochloric acid*, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with 2M *hydrochloric acid*. Solution (3) contains 0.025% w/v of *7-aminodesacetoxycephalosporanic acid EPCRS* in 2M *hydrochloric* *acid*. Solution (4) contains 0.025% w/v of DL-*phenylglycine* in 2M *hydrochloric acid*. Solution (5) contains 2.5% w/v of *cefalexin EPCRS* and 0.025% w/v of each of *7-aminodesacetoxycephalosporanic acid EPCRS* and DL-*phenylglycine* in 2M *hydrochloric acid*.

ASSAY

Weigh and powder 20 tablets. Carry out the Assay described under Cefalexin Capsules but using as solution (1) a solution prepared in the following manner. Shake a quantity of the powdered tablets containing the equivalent of 0.25 g of anhydrous cefalexin with 100 ml of *water* for 30 minutes, add sufficient *water* to produce 250 ml, filter and dilute 25 ml of the filtrate to 50 ml with *water*.

STORAGE

Cefalexin Tablets should be stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefalexin.

Cefazolin Injection

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefazolin Injection is a sterile solution of Cefazolin Sodium in Water for Injections. It is prepared by dissolving Cefazolin Sodium for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefazolin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFAZOLIN SODIUM FOR INJECTION

DEFINITION

Cefazolin Sodium for Injection is a sterile material consisting of Cefazolin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of cefazolin, $C_{14}H_{14}N_8O_4S_3$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient of a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0* to produce a solution containing the equivalent of 0.4% w/v of cefazolin.

(2) 0.4% w/v of *cefazolin EPCRS* in a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0*.

(3) 0.4% w/v each of *cefazolin EPCRS* and *cefoxitin sodium EPCRS* in a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silanised silica gel* HF_{254}.

(b) Mobile phase is a mixture of 15 volumes of *acetonitrile* and 85 volumes of a 15% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with 5M *acetic acid*.

(c) Develop to 15 cm.

(d) Application volume of 1 µl for each solution.

(e) After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm)*.

VALIDITY

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

B. Yield reaction A characteristic *of sodium salts*, Appendix VI.

TESTS

Acidity

pH of a solution containing the equivalent of 10.0% w/v of cefazolin, 4.0 to 6.0, Appendix V L.

Clarity of solution

A solution containing the equivalent of 10.0% w/v of cefazolin in *carbon dioxide-free water* is *clear,* Appendix IV A. The *absorbance* of the solution measured at 430 nm is not greater than 0.15*,* Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the contents of a sealed container in sufficient mobile phase A to produce a solution containing the equivalent of 0.25% w/v of cefazolin.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.

(3) Dissolve 20 mg of *cefazolin EPCRS* in 10 ml of a 0.2% w/v solution of *sodium hydroxide*, allow to stand for 15 to 30 minutes and dilute 1 volume to 20 volumes with mobile phase A.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Nucleosil 120-3 C18 is suitable).

(b) Gradient elution using the mobile phases described below.

(c) Flow rate of 1.2 ml per minute.

(d) Column temperature of 45°.

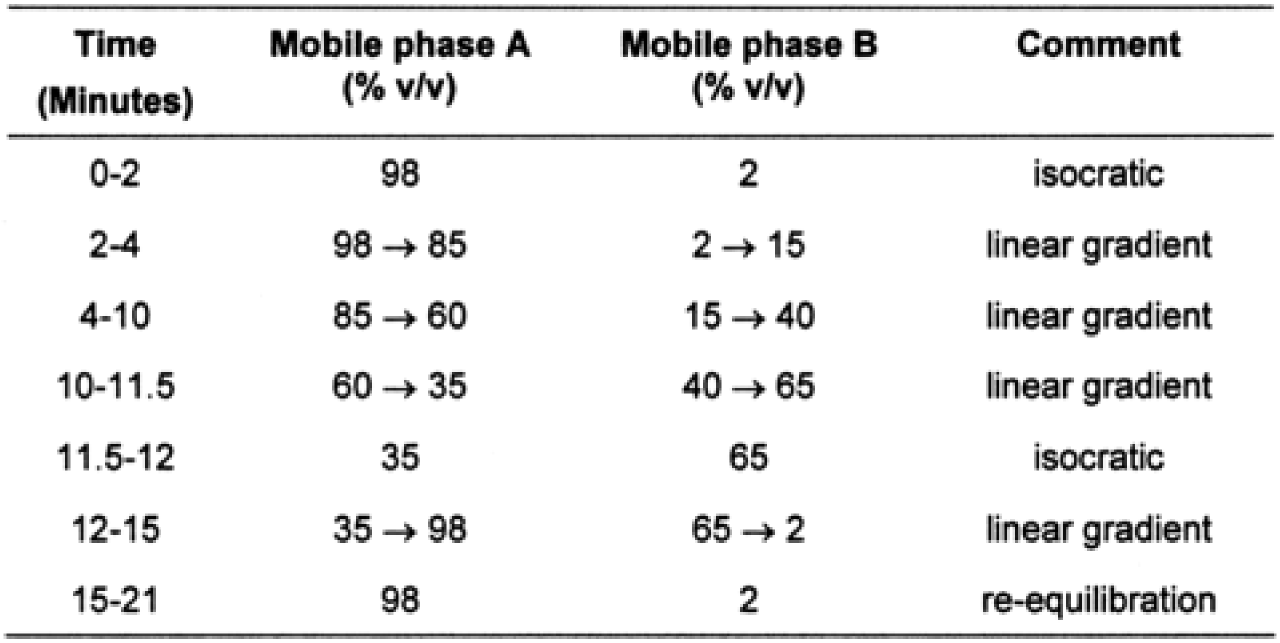
(e) Detection wavelength of 254 nm.

(f) Injection volume of 5 µl for each solution.

MOBILE PHASE

Mobile phase A A solution containing 1.454% w/v of *disodium hydrogen orthophosphate* and 0.353% w/v of *potassium dihydrogen orthophosphate*.

Mobile phase B Acetonitrile for chromatography.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	98	2	isocratic
2-4	98 → 85	2 → 15	linear gradient
4-10	85 → 60	15 → 40	linear gradient
10-11.5	60 → 35	40 → 65	linear gradient
11.5-12	35	65	isocratic
12-15	35 → 98	65 → 2	linear gradient
15-21	98	2	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefazolin and the peak with a retention time relative to cefazolin of about 1.1 (cefazolin impurity L) is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1);

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Water

Not more than 6.0% w/w, Appendix IX C, Method I. Use 0.3 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of cefazolin per ml (solution A). The endotoxin limit concentration of solution A is 1.5 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.1% w/v of cefazolin.

(2) 0.1% w/v of *cefazolin EPCRS*.

(3) 0.005% w/v of *cefuroxime sodium EPCRS* and 0.01% w/v of *cefazolin EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 270 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 10 volumes of *acetonitrile* and 90 volumes of a solution containing 0.277% w/v of *disodium hydrogen orthophosphate* and 0.186% w/v of *citric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefazolin and cefuroxime is at least 2.0. If necessary, adjust the concentration of acetonitrile in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{14}N_8O_4S_3$ in a container of average content weight from the declared content of $C_{14}H_{14}N_8O_4S_3$ in *cefazolin EPCRS*.

STORAGE

The sealed container should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The label on the sealed container states the quantity of Cefazolin Sodium contained in it in terms of the equivalent amount of cefazolin.

Cefotaxime Injection

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefotaxime Injection is a sterile solution of Cefotaxime Sodium in Water for Injections. It is prepared by dissolving Cefotaxime Sodium for Injection in the requisite amount of Water for Injections before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefotaxime Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFOTAXIME SODIUM FOR INJECTION

DEFINITION

Cefotaxime Sodium for Injection is a sterile material consisting of Cefotaxime Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of cefotaxime, $C_{16}H_{17}N_5O_7S_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cefotaxime sodium *(RS 044)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silanised silica gel* HF_{254} as the coating substance and as the mobile phase a mixture of 15 volumes of *acetone* and 85 volumes of a 15.4% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with *glacial acetic acid*. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dissolve a quantity of the contents of the sealed container in sufficient of a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0* to produce a solution containing the equivalent of 0.4% w/v of cefotaxime. Solution (2) contains 0.4% w/v of *cefotaxime sodium EPCRS* in a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0*. Solution (3) contains 0.4% w/v each of *cefotaxime sodium EPCRS* and *cefoxitin sodium EPCRS* in a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity

pH of a solution containing the equivalent of 10.0% w/v of cefotaxime, 4.5 to 6.5, Appendix V L.

Clarity of solution

A solution containing the equivalent of 10.0% w/v of cefotaxime in *carbon dioxide-free water* is *clear*, Appendix IV A. The *absorbance* of the solution at 430 nm is not greater than 0.60, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) dissolve a quantity of the contents of the sealed container in sufficient of the mobile phase to produce a solution containing the equivalent of 0.1% w/v of cefotaxime. Solution (2) contains 0.001% w/v of *cefotaxime acid EPCRS* in the mobile phase.

The chromatographic conditions described under Assay may be used.

Inject 10 µl of each solution. Allow the chromatography to proceed for at least 8 times the retention time (about 6 minutes) of cefotaxime. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of all the *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%).

Loss on drying

When dried in an oven at 100° to 105°, lose not more than 3.0% of their weight. Use 1 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of cefotaxime per ml (solution A). The endotoxin limit concentration of solution A is 0.5 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) dissolve a quantity of the mixed contents of the 10 containers in sufficient of the mobile phase to produce a solution containing the equivalent of 0.01% w/v of cefotaxime. Solution (2) contains 0.01% w/v of *cefotaxime acid EPCRS* in the mobile phase. For solution (3) add 1 ml of 2M *hydrochloric acid* to 4 ml of solution (1), heat the solution at 40° for 2 hours, add 5 ml of *phosphate buffer pH 6.6* and 1 ml of 2M *sodium* *hydroxide* and mix.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5µ ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared in the following manner: dissolve 3.5 g of *potassium dihydrogen orthophosphate* and 11.6 g of *disodium hydrogen orthophosphate* in 1000 ml of *water* at pH 7.0 and add 375 ml of *methanol*, and (c) a detection wavelength of 235 nm. Inject 10 µl of each solution.

Inject solution (2) and solution (3). Adjust the sensitivity of the detector so that the heights of the peaks in the chromatogram obtained with solution (3) are at least 50% of the full scale of the recorder. The test is not valid unless cefotaxime is eluted as the second of the principal peaks and the *resolution factor* between the two principal peaks is at least 3.5. The test is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 2.0.

When the chromatograms are recorded under the prescribed conditions the retention time of the principal peak is about 6 minutes. If necessary use another stationary phase or adjust the concentration of *methanol* in the mobile phase.

Calculate the content of $C_{16}H_{17}N_5O_7S_2$ in a container of average content weight from the declared content of $C_{16}H_{17}N_5O_7S_2$ in *cefotaxime acid EPCRS*.

STORAGE

The sealed container should be protected from light.

LABELLING

The label on the sealed container states the quantity of Cefotaxime Sodium contained in it in terms of the equivalent amount of cefotaxime.

Cefoxitin Injection

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefoxitin Injection is a sterile solution of Cefoxitin Sodium in Water for Injections. It is prepared by dissolving Cefoxitin Sodium for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefoxitin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFOXITIN SODIUM FOR INJECTION

DEFINITION

Cefoxitin Sodium for Injection is a sterile material consisting of Cefoxitin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of cefoxitin, $C_{16}H_{17}N_3O_7S_2$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of cefoxitin sodium *(RS 045)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silanised silica gel HF*$_{254}$ as the coating substance and as the mobile phase a mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* previously adjusted to pH 6.2 with 5M *acetic acid*. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dissolve a quantity of the contents of a sealed container in sufficient of a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0* to produce a solution containing the equivalent of 0.4% w/v of cefoxitin. Solution (2) contains 0.4% w/v of *cefoxitin sodium EPCRS* in a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0*. Solution (3) contains 0.4% w/v of each of *cefoxitin sodium EPCRS* and *cefazolin sodium EPCRS* in a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

C. Yield reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 1.0% w/v of cefoxitin, 4.2 to 7.0, Appendix V L.

Clarity and colour of solution

A solution containing the equivalent of 10.0% w/v of cefoxitin in *carbon dioxide-free water* is *clear*, Appendix IV A, and not more intensely coloured than intensity *5* of the range of reference solutions of the most appropriate colour, Appendix IV B, Method II.

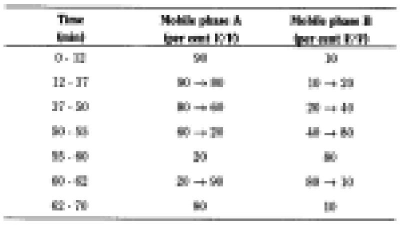
Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use. Dilute 20 ml of a 3.48% w/v solution of *dipotassium hydrogen orthophosphate*, adjusted to pH 6.8 with *orthophosphoric acid*, to 1000 ml with *water* (solution A). For solution (1) dissolve a quantity of the contents of a sealed container in sufficient of solution A to produce a solution containing the equivalent of 0.5% w/v of cefoxitin. For solution (2) dilute 1 volume of solution (1) to 100 volumes with solution A. For solution (3) add 7 ml of *water* and 2 ml of *methanol* to 1 ml of a 0.5% w/v solution of *cefoxitin sodium EPCRS* in solution A. Add 25 mg of *sodium carbonate*, stir for 10 minutes at room temperature, heat in a water-bath at 70° for 30 minutes and allow to cool. Add 3 drops of *glacial acetic acid* and 0.4 ml of a 0.5% w/v solution of *cefoxitin sodium EPCRS* in solution A and mix (generation of cefoxitin lactone).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *phenylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 300 m^2 / g and a pore size of 7 nm (Zorbax SB Phenyl is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 235 nm. Inject 20 µl of each solution.

Mobile phase A *water*, which has been adjusted to pH 2.7 with *anhydrous formic acid*.

Mobile phase B *acetonitrile.*



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 12	90	10
12 - 37	90 → 80	10 → 20
37 - 50	80 → 60	20 → 40
50 - 55	60 → 20	40 → 80
55 - 60	20	80
60 - 62	20 → 90	80 → 10
62 - 70	90	10

Inject solution (3). When the chromatogram is recorded using the prescribed conditions the retention time of cefoxitin is about 34 minutes. The test is not valid unless the *resolution factor* between the peak due to cefoxitin and the peak with a retention time relative to cefoxitin of about 1.31 (cefoxitin lactone) is at least 5.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%), the area of not more than three such peaks is greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all the *secondary peaks* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Water

Not more than 1.0% w/w, Appendix IX C, Method I. Use 0.5 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of cefoxitin per ml (solution A). The endotoxin limit concentration of solution A is 1.3 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. For solution (1) dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.1% w/v of cefoxitin. Solution (2) contains 0.1% w/v of *cefoxitin sodium EPCRS*. Solution (3) contains 0.08% w/v of *2-(2-thienyl)acetic acid*. For solution (4) mix 1 volume of solution (2) and 5 volumes of solution (3).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 1 volume of 5M *acetic acid*, 19 volumes of *acetonitrile* and 81 volumes of *water* and (c) a detection wavelength of 254 nm. Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.5.

Calculate the content of $C_{16}H_{17}N_3O_7S_2$ in a container of average content weight from the declared content of $C_{16}H_{16}N_3NaO_7S_2$ in *cefoxitin sodium EPCRS*. Each mg of $C_{16}H_{16}N_3NaO_7S_2$ is equivalent to 0.9510 mg of $C_{16}H_{17}N_3O_7S_2$.

STORAGE

The sealed container should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The label on the sealed container states the quantity of Cefoxitin Sodium contained in it in terms of the equivalent amount of cefoxitin.

Cefradine Capsules

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefradine Capsules contain Cefradine.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of cephalosporins, calculated as the sum of cefradine, ($C_{16}H_{19}N_3O_4S$) and cefalexin ($C_{16}H_{17}N_3O_4S$)

90.0 to 105.0% of the stated amount of Cefradine.

IDENTIFICATION

A. The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with the *reference spectrum* of cefradine *(RS 050)*. If the spectra are not concordant, dissolve a quantity of the contents containing 30 mg of Cefradine in 10 ml of *methanol*, evaporate to dryness at 40° at a pressure of 2 kPa and prepare a new spectrum.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel G precoated plate (Analtech plates are suitable) and the mobile phase and method of visualisation described under Related substances. Impregnate the plate as described under Related substances and apply separately 5 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Cefradine with 25 ml of 0.01M *ammonia* for 45 minutes and dilute 1 ml of the resulting solution to 10 ml with 0.01M *ammonia*. Solution (2) contains 0.040% w/v of *cefradine BPCRS* in 0.01M *ammonia*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.12M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtered solution, diluted if necessary with 0.12M *hydrochloric acid*, at the maximum at 255 nm, Appendix II B, using 0.12M *hydrochloric acid* in the reference cell. Calculate the total content of cephalosporins by determining the sum of the contents of $C_{16}H_{19}N_3O_4S$ and $C_{16}H_{17}N_3O_4S$ in the medium from the *absorbance* obtained from a 0.0025% w/v solution of *cefradine BPCRS* and using as the declared content the sum of the contents of $C_{16}H_{19}N_3O_4S$ (cefradine) and $C_{16}H_{17}N_3O_4S$ (cefalexin) in *cefradine BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel G precoated plate (Analtech plates are suitable) and using a mixture of 3 volume of *acetone*, 80 volumes of 0.2M *anhydrous disodium hydrogen orthophosphate* and 120 volumes of 0.1M *citric acid* as the mobile phase. Impregnate the plate by placing it in a tank containing a shallow layer of a 5% v/v solution of *n-tetradecane* in *n-hexane*, allowing the impregnating solvent to ascend to the top, removing the plate from the tank and allowing the solvent to evaporate; use with the flow of the mobile phase in the same direction that the impregnation was carried out. Apply separately to the plate, as bands about 3 cm wide, 40 µl of each of the following freshly prepared solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Cefradine with 25 ml of 0.01M *ammonia* for 45 minutes. For solution (2) dilute 1 ml of solution (1) to 100 ml with 0.01M *ammonia*. Solution (3) contains 0.0040% w/v of *cyclohexa-1,4-dienylglycine EPCRS* and 0.0040% w/v of *7-aminodesacetoxycephalosporanic acid EPCRS* in 0.01M *ammonia*. Solution (4) contains 0.040% w/v of *cefalexin EPCRS* in 0.01M *ammonia*.

After removal of the plate, heat at 90° for 2 to 3 minutes and spray the hot plate with a 0.1% w/v solution of *ninhydrin* in the mobile phase. Heat at 90° for 15 minutes in a circulating air oven with the plates parallel to the airflow, cool for 15 minutes protected from light and examine in daylight. In the chromatogram obtained with solution (1) any bands corresponding to cyclohexa-1,4-dienylglycine and 7-amino-desacetoxycephalosporanic acid are not more intense than the corresponding bands in the chromatogram obtained with solution (3) (1%) and any other *secondary band* is not more intense than the band in the chromatogram obtained with solution (2) (1%). Disregard any band that corresponds to the band in the chromatogram obtained with solution (4).

Cefalexin

Not more than 10.0%, calculated as the percentage of $C_{16}H_{17}N_3O_4S$ in the sum of $C_{16}H_{19}N_3O_4S$ and $C_{16}H_{17}N_3O_4S$ determined in the Assay.

Loss on drying

The contents of the capsules, when dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, lose not more than 7.0% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µl of each of the following solutions in the mobile phase. For solution (1) dissolve a quantity of the powdered mixed contents of 20 capsules to produce a solution containing 0.05% w/v of Cefradine. Solution (2) contains 0.05% w/v of *cefradine BPCRS.* Solution (3) contains 0.005% w/v each of *cefradine BPCRS* and *cefalexin EPCRS.*

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm or 10 µm) (Hypersil ODS is suitable), (b) as mobile phase with a flow rate of 1 ml per minute, a mixture of 1 volume of 5M *acetic acid ,* 17 volumes of a 3.62% w/v solution of *sodium acetate*, 200 volumes of *methanol* and 782 volumes of *water* and (c) a detection wavelength of 254 nm.

Inject solution (3). Adjust the sensitivity to obtain peaks with a height of at least 50% of the full-scale deflection on the recorder. The test is not valid unless the *resolution factor* between the peaks corresponding to cefalexin and cefradine is at least 5.0. If necessary, adjust the methanol content in the mobile phase. Inject separately solutions (1) and (2).

Calculate the content of cephalosporins by determining the sum of the contents of $C_{16}H_{19}N_3O_4S$ and $C_{16}H_{17}N_3O_4S$ using as the declared content the sum of the contents of

$C_{16}H_{19}N_3O_4S$ (cefradine) and $C_{16}H_{17}N_3O_4S$ (cefalexin) in *cefradine BPCRS*.

Cefradine Oral Suspension

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefradine Oral Suspension is a suspension of Cefradine in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for the Preparation of Oral Liquids stated under Oral Liquids.

For the following tests prepare the Oral Suspension as directed on the label. The suspension, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cephalosporins, calculated as the sum of cefradine ($C_{16}H_{19}N_3O_4S$) and cefalexin ($C_{16}H_{17}N_3O_4S$)

When freshly constituted, not more than 110.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the Oral Suspension may be expected to be satisfactory for use, not less than 90.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 25 mg of Cefradine with 100 ml of *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 261 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Analtech plates are suitable) and a mixture of 3 volumes of a 6.7% w/v solution of *ninhydrin* in *acetone*, 80 volumes of 0.1M *anhydrous disodium hydrogen orthophosphate* and 120 volumes of 0.1M *citric acid* as the mobile phase. Impregnate the plate by placing it in a tank containing a shallow layer of a 5% w/v solution of n-*tetradecane* in n-*hexane*, allowing the impregnating solvent to ascend to the top, removing the plate from the tank and allowing the solvent to evaporate; use with the flow of the mobile phase in the same direction as the impregnation was carried out. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the oral suspension with sufficient *water* to produce a solution containing 0.3% w/v of Cefradine, filter and use the filtrate. Solution (2) contains 0.3% w/v of *cefradine BPCRS* in *water*. After removal of the plate allow it to dry at 110° and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

TESTS

Acidity

pH, 3.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel G precoated plate (Analtech plates are suitable) and using a mixture of 3 volumes of *acetone*, 80 volumes of 0.2M *anhydrous disodium hydrogen orthophosphate* and 120 volumes of 0.1M *citric acid* as the mobile phase. Impregnate the plate by placing it in a tank containing a shallow layer of a 5% v/v solution of n-*tetradecane* in n-*hexane*, allowing the impregnating solvent to ascend to the top, removing the plate from the tank and allowing the solvent to evaporate; use with the flow of the mobile phase in the same direction that the impregnation was carried out. Apply separately to the plate, as bands about 3 cm wide, 40 µl of each of the following freshly prepared solutions. For solution (1) shake a quantity of the oral suspension containing 0.1 g of Cefradine with 25 ml of 0.01M *ammonia* for 45 minutes. For solution (2) dilute 1 volume of solution (1) to 100 volumes with 0.01M *ammonia*. Solution (3) contains 0.004% w/v of *cyclohexa-1,4-dienylglycine EPCRS* and 0.004% w/v of *7-aminodesacetoxycephalosporanic acid EPCRS* in 0.01M *ammonia*. Solution (4) contains 0.04% w/v of *cefalexin EPCRS* in 0.01M *ammonia*.

After removal of the plate, heat at 90^c for 2 to 3 minutes and spray the hot plate with a 0.1% w/v solution of *ninhydrin* in the mobile phase. Heat at 90^c for 15 minutes in a circulating air oven with the plates parallel to the air flow, cool for 15 minutes protected from light and examine in daylight. In the chromatogram obtained with solution (1) any bands corresponding to cyclohexa-1,4-dienylglycine and 7-aminodesacetoxycephalosporanic acid are not more intense than the corresponding bands in the chromatogram obtained with solution (3) (1%) and any other *secondary band* is not more intense than the band in the chromatogram obtained with solution (2) (1%). Disregard any band that corresponds to the band in the chromatogram obtained with solution (4).

Cefalexin

Not more than 10.0%, calculated as the percentage of $C_{16}H_{17}N_3O_4S$ in the sum of $C_{16}H_{19}N_3O_4S$ and $C_{16}H_{17}N_3O_4S$ determined in the Assay.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a weighed quantity of the oral suspension with sufficient of the mobile phase to produce a solution containing 0.05% w/v of Cefradine and filter through a 0.5-µm filter, discarding the first few ml of filtrate. Solution (2) contains 0.05% w/v of *cefradine BPCRS* in the mobile phase. Solution (3) contains 0.05% w/v of each of *cefradine BPCRS* and *cefalexin EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm or 10 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 3 volumes of 0.7M *glacial acetic acid*, 15 volumes of 0.5M *sodium acetate*, 200 volumes of *methanol* and 782 volumes of *water* and (c) a detection wavelength of 254 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to cefradine and cefalexin is at least 2.0.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of cephalosporins, weight in volume, by determining the sum of the contents of $C_{16}H_{19}N_3O_4S$ and $C_{16}H_{17}N_3O_4S$ using as the declared content the sum of the contents of $C_{16}H_{19}N_3O_4S$ (cefradine) and $C_{16}H_{17}N_3O_4S$ (cefalexin) in *cefradine BPCRS*.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Suspension should be stored at the temperature and used within the period stated on the label.

Ceftriaxone Injection

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Ceftriaxone Injection is a sterile solution of Ceftriaxone Sodium in Water for Injections. It is prepared by dissolving Ceftriaxone Sodium for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Ceftriaxone Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFTRIAXONE SODIUM FOR INJECTION

DEFINITION

Ceftriaxone Sodium for Injection is a sterile material prepared from Ceftriaxone Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of ceftriaxone, $C_{18}H_{18}N_8O_7S_3$

92.0 to 108.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of ceftriaxone sodium *(RS 046)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

C. Yield reaction A characteristic *of sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 12.0% w/v of ceftriaxone, 6.0 to 8.0, Appendix V L.

Clarity of solution

A solution containing the equivalent of 1.20% w/v of ceftriaxone in *carbon dioxide-free water* is *clear,* Appendix IV A, and not more intensely coloured than *reference solution* Y_5 or BY_5, Appendix IV B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1) and (4) as described under Assay.

The chromatographic conditions described under Assay may be used.

Inject 20 µl of each solution. Allow the chromatography to proceed for at least twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%) and the sum of the areas of all the *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (4) (5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Water

Not more than 11.0% w/w, Appendix IX C, Method I. Use 0.1 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of ceftriaxone per ml (solution A). The endotoxin limit concentration of solution A is 2.0 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. For solution (1) dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.030% w/v of ceftriaxone. Solution (2) contains 0.030% w/v of *ceftriaxone sodium EPCRS.* Solution (3) contains 0.0050% w/v each of *ceftriaxone sodium EPCRS* and *ceftriaxone sodium* E-*isomer EPCRS.* For solution (4) dilute 1 volume of solution (1) to 100 volumes.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute, a mixture prepared in the following manner: dissolve 2 g of *tetradecylammonium bromide* and 2 g of *tetraheptylammonium bromide* in a mixture of 440 ml of *water,* 55 ml of *0.067*M *mixed phosphate buffer pH 7.0*, 5 ml of a citrate buffer solution pH 5.0 prepared by dissolving 20.17 g of *citric acid* in 800 ml of *water*, adjusting to pH 5.0 with 10M *sodium hydroxide* and diluting to 1000 ml with *water*, and 500 ml of *acetonitrile*, and (c) a detection wavelength of 254 nm. Inject 20 µl of each solution.

Inject solution (3). Adjust the sensitivity of the detector so that the heights of the peaks are at least 50% of the full scale of the recorder. The test is not valid unless the *resolution factor* between the two principal peaks is at least 3.0. Inject alternately solution (1) and solution (2).

Calculate the content of $C_{18}H_{18}N_8O_7S_3$ in a container of average content weight from the declared content of $C_{18}H_{16}N_8Na_2O_7S_3,3½H_2O$ in *ceftriaxone sodium EPCRS.* Each mg of $C_{18}H_{16}N_8Na_2O_7S_3,3½H_2O$ is equivalent to 0.8383 mg of $C_{18}H_{18}N_8O_7S_3$.

STORAGE

The sealed container should be stored at a temperature not exceeding 30°.

LABELLING

The label on the sealed container states the quantity of Ceftriaxone Sodium contained in it in terms of the equivalent amount of ceftriaxone.

Cefuroxime Axetil Tablets

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefuroxime Axetil Tablets contain Cefuroxime Axetil. They may be coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cefuroxime, $C_{16}H_{16}N_4O_8S$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing the equivalent of 0.1 g of cefuroxime with 5 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cefuroxime axetil *(RS 047)*.

B. In the Assay, the retention times of the principal peaks in the chromatogram obtained with solution (1) correspond to those of the peaks due to diastereoisomers A and B of cefuroxime axetil in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the procedure described under Assay. In the chromatogram obtained with solution (1) the sum of the areas of the pair of peaks corresponding to the *E*-isomers in the chromatogram obtained with solution (3) is not greater than 1.5% by *normalisation*, the sum of the areas of any peaks corresponding to the Δ^3-isomers in the chromatogram obtained with solution (2) is not greater than 2.0% by *normalisation* and the area of any other *secondary peak* is not greater than 1.0% by *normalisation*.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtered sample, diluted if necessary with 0.1M *hydrochloric acid*, at the maximum at 278 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell, and compare with that of a standard solution in 0.1M *hydrochloric acid* having a known concentration of *cefuroxime axetil BPCRS* equivalent to about 10 to 20 µg of $C_{16}H_{16}N_4O_8S$ per ml. Calculate the total content of cefuroxime axetil, $C_{20}H_{22}N_4O_{10}S$, in the medium using the declared content of $C_{20}H_{22}N_4O_{10}S$ in *cefuroxime axetil BPCRS*. Each mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse 10 tablets in 0.2M *ammonium dihydrogen orthophosphate*, previously adjusted to pH 2.4 with *orthophosphoric acid*, using 10 ml per g of the stated content of cefuroxime. Immediately add sufficient *methanol* to produce a solution containing the equivalent of 0.5% w/v of cefuroxime and shake vigorously. Filter (Whatman GF/C is suitable) and dilute a quantity of the filtrate with sufficient of the mobile phase to produce a solution containing the equivalent of 0.025% w/v of cefuroxime. For solution (2) heat a quantity of solution (1) at 60° for 1 hour or until sufficient impurities (Δ^3-isomers) have been generated. For solution (3) expose a quantity of solution (1) to ultraviolet light (254 nm) for 24 hours or until sufficient impurities (*E*-isomers) have been generated. Solution (4) contains 0.03% w/v of *cefuroxime axetil BPCRS* in the mobile phase. All solutions containing cefuroxime axetil, if not to be used for immediate analysis, should be stored in the dark at a temperature between 2° and 8° before analysis.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica (5 µm) the surface of which has been modified by chemically-bonded trimethylsilyl groups (Hypersil SAS is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture of 38 volumes of *methanol* and 62 volumes of 0.2M *ammonium dihydrogen orthophosphate* adjusted, if necessary, so that the *resolution factors* between the peaks corresponding to the cefuroxime axetil diastereoisomers A and B in solution (4) and between the peaks corresponding to cefuroxime axetil diastereoisomer A and the cefuroxime axetil Δ^3-isomer in solution (2) are each not less than 1.5 and (c) a detection wavelength of 278 nm.

The retention times relative to cefuroxime axetil diastereoisomer A are approximately 0.9 for cefuroxime axetil diastereoisomer B, 1.2 for the cefuroxime axetil Δ^3-isomers and 1.7 and 2.1 for the *E*-isomers. The relative standard deviation for the response factor of cefuroxime axetil for replicate injections of solution (1) is not more than 2.0%.

Calculate the content of $C_{20}H_{22}N_4O_{10}S$ as the sum of the areas of the two peaks corresponding to diastereoisomers A and B of cefuroxime axetil and using the declared content of $C_{20}H_{22}N_4O_{10}S$ in *cefuroxime axetil BPCRS.* Each mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of cefuroxime.

Cefuroxime Injection

General Notices

Action and use

Cephalosporin antibacterial.

DEFINITION

Cefuroxime Injection is a sterile solution or suspension of Cefuroxime Sodium in Water for Injections. It is prepared by dissolving or suspending Cefuroxime Sodium for Injection in the requisite amount of Water for Injections before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cefuroxime Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CEFUROXIME SODIUM FOR INJECTION

DEFINITION

Cefuroxime Sodium for Injection is a sterile material consisting of Cefuroxime Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of cefuroxime, $C_{16}H_{16}N_4O_8S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cefuroxime sodium *(RS 048)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silanised silica gel* HF_{254} as the coating substance and as the mobile phase a mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of a 15.0% w/v solution of *ammonium acetate*, previously adjusted to pH 6.2 with *glacial acetic acid*. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dissolve a quantity of the contents of a sealed container in sufficient of a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0* to produce a solution containing the equivalent of 0.4% w/v of cefuroxime. Solution (2) contains 0.4% w/v of *cefuroxime sodium EPCRS* in a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0*. Solution (3) contains 0.4% w/v each of *cefuroxime sodium EPCRS* and *cefoxitin sodium EPCRS* in a mixture of equal volumes of *methanol* and *0.067*M *mixed phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

C. Yield reaction A characteristic *of sodium salts*, Appendix VI.

TESTS

Acidity

pH of a solution containing the equivalent of 10.0% w/v of cefuroxime, 5.5 to 8.5, Appendix V L.

Clarity of solution

A solution containing the equivalent of 10.0% w/v of cefuroxime in *carbon dioxide-free water* is not more opalescent than *reference suspension II*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1), (3) and (4) described under Assay.

The chromatographic conditions described under Assay may be used.

Allow the chromatography to proceed for at least 3 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to descarbamoyl-cefuroxime (located by comparison with the chromatogram obtained with solution (3)) is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%) and the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (4) (3%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (4) (0.1%) .

Water

Not more than 3.5% w/w, Appendix IX C. Use 0.4 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of cefuroxime per ml (solution A). The endotoxin limit concentration of solution A is 1.0 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. For solution (1) dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.1% w/v of cefuroxime. Solution (2) contains 0.1% w/v of *cefuroxime sodium EPCRS*. For solution (3) heat 20 ml of solution (2) in a water bath at 60° for 10 minutes, cool and inject immediately. For solution (4) dilute 1 volume of solution (2) to 100 volumes.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically-bonded hexylsilyl groups (5 µm) (Spherisorb S5 C6 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 1 volume of *acetonitrile* and 99 volumes of *acetate buffer pH 3.4* and (c) a detection wavelength of 273 nm.

Inject solution (3). The chromatogram obtained shows peaks corresponding to cefuroxime and descarbamoyl-cefuroxime. The assay is not valid unless the *resolution factor* between the two principal peaks is at least 2.0. If necessary adjust the concentration of *acetonitrile* in the mobile phase.

Inject solution (4). Adjust the sensitivity of the detector so that the height of the principal peak is not less than 25% of the full scale of the recorder. The assay is not valid unless the *symmetry factor* of the cefuroxime peak is 1.5 or less.

Calculate the content of $C_{16}H_{16}N_4O_8S$ in a container of average content weight from the declared content of $C_{16}H_{15}N_4NaO_8S$ in *cefuroxime sodium EPCRS*. Each mg of $C_{16}H_{15}N_4NaO_8S$ is equivalent to 0.9508 mg of $C_{16}H_{16}N_4O_8S$.

STORAGE

The sealed container should be protected from light.

LABELLING

The label on the sealed container states the quantity of Cefuroxime Sodium contained in it in terms of the equivalent amount of cefuroxime.

Celiprolol Tablets

General Notices

Action and use

Beta-adrenoceptor agonist.

DEFINITION

Celiprolol Tablets contain Celiprolol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of celiprolol hydrochloride, $C_{20}H_{33}N_3O_4$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix with the aid of ultrasound a quantity of the powdered tablets containing 200 mg of Celiprolol Hydrochloride with 100 ml of *dichloromethane* for 30 minutes, filter (Whatman filter paper No. 42 is suitable), remove the dichloromethane using a rotary evaporator and dry the residue over *phosphorus pentoxide* at 110^{c} at a pressure not exceeding 2 kPa for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of celiprolol hydrochloride (*RS 420*).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter; to 1 volume of the filtrate add sufficient *water* to produce 20 volumes and filter using a 0.45 µm membrane filter. Measure the *absorbance* of the filtrate, suitably diluted with *water* if necessary, at the maximum at 231 nm, Appendix II B, using *water* in the reference cell. Measure the *absorbance* of a 0.011% w/v solution of *celiprolol hydrochloride BPCRS* in the dissolution medium and calculate the total content of celiprolol hydrochloride, $C_{20}H_{33}N_3O_4$,HCl, in the medium using the declared content of $C_{20}H_{33}N_3O_4$,HCl in *celiprolol hydrochloride BPCRS*.

Related substances

Carry out method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Celiprolol Hydrochloride in 100 ml of the mobile phase for 15 minutes with occasional shaking, cool and filter using a 0.45-µm membrane filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase and further dilute 1 volume of this solution to 20 volumes with the mobile phase

(3) Prepare a solution containing 0.1% w/v of *celiprolol hydrochloride BPCRS* in *water,* add 5 drops of 5M *sodium hydroxide* and heat at 70° for 20 minutes (generation of impurity A).

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Waters µBondapak is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 233 nm.

(f) Injection volume of 20 µl for each solution.

For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

MOBILE PHASE

A mixture of 25 volumes of *acetonitrile* and 75 volumes of 0.025M *sodium dihydrogen phosphate monohydrate* adjusted to pH 3.0 with 3M *orthophosphoric acid*. If necessary adjust the composition of the mobile phase so that, in the chromatogram obtained with solution (3), the peak due to celiprolol impurity A is resolved from the solvent front.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the peak due to celiprolol impurity A is resolved from the solvent front.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to celiprolol impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

not more than one such peak has an area not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area of less than a fifth of the area of the peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Carry out method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Celiprolol Hydrochloride in 100 ml of the mobile phase for 15 minutes, cool and filter. Dilute 1 volume of the filtrate to 50 volumes with the mobile phase and filter using a 0.45 µm membrane filter.

(2) 0.002% w/v solution of *celiprolol hydrochloride BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

DETERMINATION OF CONTENT

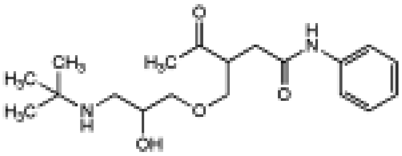
Calculate the content of $C_{20}H_{33}N_3O_4$,HCl in the tablets using the declared content of $C_{20}H_{33}N_3O_4$,HCl in *celiprolol hydrochloride BPCRS*.

STORAGE

Celiprolol Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Celiprolol Hydrochloride and the following.



1. 3-acetyl-4-{3-(1,1-dimethyl-ethylamino)-2-hydroxy}propoxybutyranilide

Cetomacrogol Emulsifying Ointment

General Notices

Action and use

Emulsifying agent.

DEFINITION

White Soft Paraffin	500 g
Cetomacrogol Emulsifying Wax	300 g
Liquid Paraffin	200 g

Extemporaneous preparation

The following directions apply.

Melt together and stir until cold.
The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Cetomacrogol Emulsifying Wax

General Notices

Non-ionic Emulsifying Wax

Action and use

Emulsifying agent.

DEFINITION

Cetostearyl Alcohol	800 g
Macrogol Cetostearyl Ether (22)	200 g

Extemporaneous preparation

The following directions apply.

Melt together and stir until cold.

CHARACTERISTICS

A white or almost white, waxy solid or flakes melting when heated to a clear almost colourless liquid.

Practically insoluble in *water*, producing an emulsion; moderately soluble in *ethanol (96%)*; partly soluble in *ether*.

IDENTIFICATION

A. Incinerate at a temperature not exceeding 450° until free from carbon and cool. The residue is negligible (distinction from Emulsifying Wax).

B. Complies with the test for Sulphated ash.

TESTS

Acid value

Not more than 0.5, Appendix X B.

Refractive index

At 60°, 1.435 to 1.439, Appendix V E.

Solidifying point

45° to 53°, Appendix V B, using the following modifications. Place in the inner test tube sufficient of the melted substance to fill the tube to a depth of about 50 mm. Stir the substance gently and steadily, without scraping the wall of the tube, while the tube and its contents are allowed to cool. The temperature at which the level of the mercury in the thermometer remains stationary for a short time is regarded as the solidifying point.

Alkalinity

Dissolve 10 g in 10 ml of *water* and 10 ml of *ethanol (96%)*. Not more than 0.5 ml of 0.1M *hydrochloric acid VS* is required for neutralisation using *phenolphthalein solution R1* as indicator.

Hydroxyl value

175 to 192, Appendix X D. Use 3.5 g.

Saponification value

Not more than 2.0, Appendix X G. Use 20 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

Cetrimide Cream

General Notices

Action and use

Antiseptic.

DEFINITION

Cetrimide Cream contains the stated percentage w/w of Cetrimide in a suitable basis.

Extemporaneous preparation

The following formula and directions apply.

Cetrimide	5 g, or a sufficient quantity
Cetostearyl Alcohol	50 g
Liquid Paraffin	500 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

Melt the Cetostearyl Alcohol and heat with the Liquid Paraffin to about 60°. Dissolve the Cetrimide in sufficient Purified Water to produce about 450 g. Add the aqueous solution to the oily phase when both are at about 60^{c} and mix. Stir gently until cool, add sufficient of the Purified Water to produce 1000 g and mix.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of cetrimide, $C_{17}H_{38}BrN$

88.0 to 106.0% of the stated amount.

IDENTIFICATION

Mix 1 g with 50 ml of *water*. The diluted cream complies with the following tests.

A. To 10 ml add 2 ml of *potassium hexacyanoferrate(III) solution*. A yellow precipitate is produced.

B. Shake 3 ml of *water* with 1 ml of 1M *sulphuric acid*, 2 ml of *chloroform* and 0.5 ml of *methyl orange solution*. Add 2 ml of the diluted cream, shake and allow to separate. A yellow colour develops in the chloroform layer.

ASSAY

To a quantity containing 5 mg of Cetrimide add 10 ml of hot *water* and shake gently until dispersed. Add 5 ml of 1M *sulphuric acid*, 20 ml of *chloroform* and 0.25 ml of *dimethyl yellow solution* and titrate with 0.001M *dioctyl sodium sulphosuccinate VS*. Each ml of 0.001M *dioctyl* *sodium sulphosuccinate VS* is equivalent to 0.3364 mg of $C_{17}H_{38}BrN$.

LABELLING

The strength is stated as the percentage w/w of Cetrimide.

When Cetrimide Cream is prescribed or demanded, no strength being stated, a cream containing 5% w/v shall be dispensed or supplied.

Cetrimide Emulsifying Ointment

General Notices

Action and use

Antiseptic.

DEFINITION

White Soft Paraffin	500 g
Cetostearyl Alcohol	270 g
Liquid Paraffin	200 g
Cetrimide	30 g

Extemporaneous preparation

The following directions apply.

Melt together the White Soft Paraffin, Cetostearyl Alcohol and Liquid Paraffin, add the Cetrimide and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of cetrimide, $C_{17}H_{38}BrN$

2.5 to 3.3% w/w.

ASSAY

To 0.3 g in a stoppered cylinder, add 10 ml of hot *water* and shake until the solid is dispersed. Add 5 ml of 1M *sulphuric acid* , 20 ml of *chloroform* and 1 ml of *dimethyl yellow and oracet blue B solution* and titrate with 0.001M *sodium dodecyl sulphate VS*. Each ml of 0.001M *sodium dodecyl sulphate VS* is equivalent to 0.3364 mg of $C_{17}H_{38}BrN$.

Cetrimide Solution

General Notices

Action and use

Antiseptic.

DEFINITION

Cetrimide Solution is a 1% w/v *cutaneous solution* of cetrimide prepared by appropriately diluting Strong Cetrimide Solution with Purified Water. Cetrimide Solution is freshly prepared.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of cetrimide, $C_{17}H_{38}BrN$

0.90 to 1.10% w/v.

IDENTIFICATION

A. To 10 ml add 2 ml of a 5% w/v solution of *potassium hexacyanoferrate(III)*. A yellow precipitate is produced.

B. Shake together 5 ml of *water*, 1 ml of 1M *sulphuric acid*, 2 ml of *chloroform* and 0.05 ml of *methyl orange solution*; the chloroform layer is colourless. Add 2 ml of the solution being examined and shake; a yellow colour is produced slowly in the chloroform layer.

C. Yields reaction A characteristic *of bromides*, Appendix VI.

ASSAY

To 100 ml of the solution add 25 ml of *chloroform*, 10 ml of 0.1M *sodium hydroxide* and 10 ml of a freshly prepared 8.0% w/v solution of *potassium iodide*. Shake well, allow to separate and discard the chloroform layer. Wash the aqueous layer with three 10-ml quantities of *chloroform* and discard the washings. Add 40 ml of *hydrochloric acid*, cool and titrate with 0.05M *potassium iodate* VS until the deep brown colour is almost discharged. Add 2 ml of *chloroform* and continue the titration, with shaking, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 10 ml of the freshly prepared 8.0% w/v potassium iodide solution, 20 ml of *water* and 40 ml of *hydrochloric acid*. The difference between the titrations represents the amount of potassium iodate required. Each ml of 0.05M *potassium iodate* VS is equivalent to 0.03364 g of $C_{17}H_{38}BrN$.

LABELLING

The label states that the solution should not be used later than 1 week after the container is first opened.

The label indicates the pharmaceutical form as 'cutaneous solution'.

STERILE CETRIMIDE SOLUTION

DEFINITION

Sterile Cetrimide Solution is Cetrimide Solution that has been sterilised by *heating in an autoclave*.

Content of cetrimide; Identification

Complies with the requirements stated under Cetrimide Solution.

ASSAY

Carry out the Assay described under Cetrimide Solution.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

LABELLING

The label states (1) 'Sterile Cetrimide Solution'; (2) that the solution is not intended for injection; (3) that the contents should not be used later than 1 week after the container is first opened.

The label indicates the pharmaceutical form as 'cutaneous solution'.

Chewable Calcium Carbonate Tablets

General Notices

DEFINITION

Chewable Calcium Carbonate Tablets contain Calcium Carbonate. They may be flavoured. *The tablets comply with the requirements stated under Tablets and with the following requirements.*

Content of calcium, Ca

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The powdered tablets yield reaction B characteristic of *calcium salts*, Appendix VI.

B. The powdered tablets yield the reactions characteristic of *carbonates*, Appendix VI.

Disintegration

The requirement for Disintegration does not apply to Chewable Calcium Carbonate Tablets.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 0.25 g of calcium add 50 ml of *water* and 5 ml of *hydrochloric acid*. Heat the dispersion gently to boiling and continue to boil for about 2 minutes. Allow to cool, add sufficient *water* to produce 250 ml and filter, if necessary. To 50 ml of this solution add 50 ml of 0.05M *disodium edetate VS*. Neutralise the solution using 2M *sodium hydroxide* and add 10 ml of *ammonia buffer pH 10.9* and 50 ml of *water*. Titrate the excess of disodium edetate with 0.05M *zinc chloride VS* using *mordant black* 11 *solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

LABELLING

The label states (1) the quantity of the active ingredient in terms of the equivalent amount of calcium; (2) that the tablets should be chewed before swallowing.

STORAGE

Chewable Calcium Carbonate Tablets should be protected from moisture.

Chlorambucil Tablets

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Chlorambucil Tablets contain Chlorambucil. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlorambucil, $C_{14}H_{19}Cl_2NO_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 10 mg of Chlorambucil with 10 ml of 2M *hydrochloric acid*, allow to stand for 30 minutes, shaking occasionally, and filter. To 5 ml of the filtrate add 0.5 ml of *potassium tetraiodomercurate solution*; a precipitate is produced. To the remainder of the filtrate add 0.15 ml of *dilute potassium permanganate solution*; the colour of the permanganate is discharged.

TESTS

Uniformity of content

Tablets containing 2 mg or less of Chlorambucil comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0020% w/v of *chlorambucil BPCRS* in a mixture of 9 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid*. For solution (2) dissolve one tablet as completely as possible in 10 ml of 0.1M *hydrochloric acid*, add 40 ml of *acetonitrile* and mix for 5 minutes with the aid of ultrasound. Add sufficient *acetonitrile* to produce a solution containing 0.002% w/v of Chlorambucil, filter through a glass microfibre filter paper (Whatman GF/C is suitable), discarding the first 20 ml of filtrate, and use the filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 and Spherisorb ODS 1 are suitable), (b) a mixture of 60 volumes of *acetonitrile* and 40 volumes of 0.02M *potassium dihydrogen orthophosphate* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{14}H_{19}Cl_2NO_2$ in the tablet using the declared content of $C_{14}H_{19}Cl_2NO_2$ in *chlorambucil BPCRS*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution (1) contains 0.0020% w/v of *chlorambucil BPCRS* in a mixture of 9 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid*. For solution (2) dissolve as completely as possible a quantity of the powdered tablets containing 10 mg of Chlorambucil in a mixture of 25 ml of 0.1M *hydrochloric acid* and 100 ml of *acetonitrile* by mixing for 10 minutes with the aid of ultrasound. Dilute to 250 ml with *acetonitrile*, filter through a glass microfibre filter paper (Whatman GF/C is suitable), discarding the first 20 ml of filtrate, and dilute 50 ml to 100 ml with a mixture of 9 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid*.

The chromatographic procedure described under Uniformity of content may be used.

Calculate the content of $C_{14}H_{19}Cl_2NO_2$ using the declared content of $C_{14}H_{19}Cl_2NO_2$ in *chlorambucil BPCRS*.

Chloramphenicol Capsules

General Notices

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Capsules contain Chloramphenicol.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Suspend a quantity of the contents of the capsules containing 0.1 g of Chloramphenicol in 60 ml of *water* and extract with two 20 ml quantities of *petroleum spirit (boiling range, 120° to 160°)*. Wash the combined extracts with two 15-ml quantities of *water*, add the washings to the aqueous layer, extract with four 50-ml quantities of *ether* and evaporate the combined ether extracts. The residue complies with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water* as the mobile phase. Apply separately to the plate 1 µl of each of two solutions in *ethanol (96%)* containing (1) 1% w/v of the residue and (2) 1% w/v of *chloramphenicol EPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve 10 mg in 2 ml of *ethanol (50%)*, add 4.5 ml of 1M *sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of 10M *sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 278 nm, Appendix II B. Calculate the total content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$, in the medium taking 297 as the value of A(1%, 1 cm) at the

maximum at 278 nm.

2-Amino-1-(4-nitrophenyl)propane-1,3-diol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0002% w/v of *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase. For solution (2) shake a quantity of the contents of the capsules containing 40 mg of Chloramphenicol with 100 ml of the mobile phase for 10 minutes, add sufficient mobile phase to produce 200 ml, mix and filter.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm), packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 85 volumes of a 0.21% w/v solution of *sodium pentanesulphonate*, 15 volumes of *acetonitrile* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 272 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 2-amino-1-(4-nitrophenyl)-propane-1,3-diol is not greater than the area of the peak in the chromatogram obtained with solution (1).

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 0.2 g of Chloramphenicol in 800 ml of *water*, warming if necessary to effect solution, and add sufficient *water* to produce 1000 ml. Dilute 10 ml to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Chloramphenicol Ear Drops

General Notices

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Ear Drops are a solution of Chloramphenicol in a suitable vehicle.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dilute a volume of the ear drops containing 0.1 g of Chloramphenicol to 10 ml with *ethanol (96%)*. Solution (2) contains 1% w/v of *chloramphenicol EPCRS* in *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

B. Dilute a volume of the ear drops containing 50 mg of Chloramphenicol to 10 ml with *ethanol (50%)*. To 2 ml add 4.5 ml of 1M *sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of 10M *sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

TESTS

2-Amino-1-(4-nitrophenyl)propane-1,3-diol

Comply with the test described under Chloramphenicol Eye Drops using the following solutions. For solution (1) dilute the ear drops with the mobile phase to contain 0.050% w/v of Chloramphenicol. Solution (2) contains 0.0025% w/v of *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

ASSAY

Dilute a quantity containing 25 mg of Chloramphenicol to 250 ml with *water*. Dilute 10 ml to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the value of A

(1%, 1 cm) at the maximum at 278 nm.

STORAGE

Chloramphenicol Ear Drops should be protected from light.

Chloramphenicol Eye Drops

General Notices

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Eye Drops are a sterile solution of Chloramphenicol in Purified Water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a volume containing 50 mg of Chloramphenicol add 15 ml of *water* and extract with four 25 ml quantities of *ether*. Combine the extracts and evaporate to dryness. The dried residue complies with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water* as the mobile phase. Apply separately to the plate 1 µl of each of two solutions in *ethanol (96%)* containing (1) 1% w/v of the residue and (2) 1% w/v of *chloramphenicol EPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve 10 mg in 2 ml of *ethanol (50%)*, add 4.5 ml of 1M *sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of 10M *sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

TESTS

Acidity or alkalinity

pH, 7.0 to 7.5, Appendix V L.

2-Amino-1-(4-nitrophenyl)propane-1,3-diol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops with the mobile phase to contain 0.050% w/v of Chloramphenicol. Solution (2) contains 0.0040% w/v of *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm), packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 85 volumes of a 0.21% w/v solution of *sodium pentanesulphonate*, 15 volumes of *acetonitrile* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 272 nm.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-amino-1-(4-nitrophenyl)propane-1,3-diol is not greater than the area of the peak in the chromatogram obtained with solution (2).

ASSAY

Dilute a volume containing 25 mg of Chloramphenicol to 250 ml with *water*. Dilute 10 ml to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the value of A (1%, 1 cm) at the maximum at 278 nm.

STORAGE

Chloramphenicol Eye Drops should be protected from light.

Chloramphenicol Eye Ointment

General Notices

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Eye Ointment is a sterile preparation containing Chloramphenicol in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity containing 30 mg of Chloramphenicol with 10 ml of *petroleum spirit (boiling range, 40° to 60°)*, centrifuge and discard the supernatant liquid. Repeat this procedure using three 10 ml quantities of the same solvent. The dried residue complies with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water* as the mobile phase. Apply separately to the plate 1 µl of each of two solutions in *ethanol (96%)* containing (1) 1% w/v of the residue and (2) 1% w/v of *chloramphenicol EPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve 10 mg of the residue in 2 ml of *ethanol (50%)*, add 4.5 ml of 1M *sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of 10M *sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

TESTS

2-Amino-1-(4-nitrophenyl)propane-1,3-diol

Complies with the test described under Chloramphenicol Eye Drops using the following solutions. For solution (1) add 25 ml of a 0.21% w/v solution of *sodium pentanesulphonate* to a quantity of the eye ointment containing 10 mg of Chloramphenicol and heat in a water bath until melted. Stir thoroughly with a glass rod, cool until the ointment base solidifies and filter through absorbent cotton. Repeat the extraction with a further 20 ml the sodium pentanesulphonate solution and dilute to 50 ml with the same solution. Solution (2) contains

0.00020% w/v of *2-amino-1-(4-nitrophenyl)propane-1,3-diol BPCRS* in the mobile phase.

ASSAY

Suspend a quantity containing 10 mg of Chloramphenicol in 50 ml of *petroleum spirit (boiling range, 40° to 60°)* and extract with successive quantities of 50, 50, 50 and 30 ml of warm *water.* Combine the extracts, dilute to 200 ml with *water*, mix well and filter, discarding the first 20 ml of the filtrate. Dilute 10 ml of the filtrate to 50 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Chloramphenicol Sodium Succinate Injection

General Notices

Action and use

Antibacterial.

DEFINITION

Chloramphenicol Sodium Succinate Injection is a sterile solution of Chloramphenicol Sodium Succinate in Water for Injections. It is prepared by dissolving Chloramphenicol Sodium Succinate for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Chloramphenicol Sodium Succinate Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

CHLORAMPHENICOL SODIUM SUCCINATE FOR INJECTION

DEFINITION

Chloramphenicol Sodium Succinate for Injection is a sterile material consisting of Chloramphenicol Sodium Succinate with or without excipients. It is supplied in a sealed container .

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of chloramphenicol sodium succinate, calculated as $C_{11}H_{12}Cl_2N_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of 2M *acetic acid* , 14 volumes of *methanol* and 85 volumes of *chloroform* as the mobile phase. Apply separately to the plate 2 µl of each of three solutions in *acetone* containing (1) sufficient of the contents of the sealed container to produce a solution containing the equivalent of 1% w/v of chloramphenicol, (2) 1% w/v of *chloramphenicol sodium succinate EPCRS* and (3) 1% w/v of *chloramphenicol EPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The two principal spots in the chromatogram obtained with solution (1) are similar in position and size to those in the chromatogram obtained with solution (2) and their positions are different from that of the principal spot in the chromatogram obtained with solution (3).

B. Dissolve 10 mg in 1 ml of *ethanol (50%)*, add 3 ml of a 1% w/v solution of *calcium chloride* and 50 mg of *zinc powder* and heat on a water bath for 10 minutes. Filter the hot solution, allow to cool, add 0.1 ml of *benzoyl chloride* and shake for 1 minute. Add 0.5 ml of *iron(III) chloride solution R1* and 2 ml of *chloroform* and shake. The aqueous layer is light violet-red to purple.

C. Yields reaction A characteristic *of sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 20% w/v of chloramphenicol, 6.0 to 7.0, Appendix V L.

Chloramphenicol and chloramphenicol disodium disuccinate

Carry out the method for *liquid chromatography*, Appendix III D, using four solutions in the mobile phase containing (1) 0.00050% w/v of *chloramphenicol disodium disuccinate EPCRS*, (2) 0.00050% w/v of *chloramphenicol EPCRS*, (3) sufficient of the contents of the sealed container to give a solution containing the equivalent of 0.018% w/v of chloramphenicol and (4) 0.00050% w/v each of *chloramphenicol disodium disuccinate EPCRS* and *chloramphenicol EPCRS* and sufficient of the contents of the sealed container to give a solution containing the equivalent of 0.025% w/v of chloramphenicol.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (LiChrosorb RP-18, Hypersil ODS and Polygosil C18 5 µm are suitable), (b) a mixture of 5 volumes of a 2% w/v solution of *orthophosphoric acid*, 40 volumes of *methanol* and 55 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 275 nm.

Inject 20 µl of each solution. The test is not valid unless the two peaks in the chromatogram obtained with solution (4) corresponding to those in the chromatograms obtained with solutions (1) and (2) are clearly separated from the peaks corresponding to the two principal peaks in the chromatogram obtained with solution (3). If necessary, adjust the methanol content of the mobile phase.

In the chromatogram obtained with solution (3) the areas of any peaks corresponding to chloramphenicol and chloramphenicol disodium disuccinate are not greater than those of the principal peaks in the chromatograms obtained with solutions (2) and (1) respectively (2% of each).

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

Pyrogens

Comply with the *test for pyrogens*, Appendix XIV D. Use per kg of the rabbit's weight 2 ml of a solution in *water for injections* containing the equivalent of 0.18% w/v of chloramphenicol.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Dissolve a quantity of the mixed contents of the 10 containers containing the equivalent of 0.2 g of chloramphenicol in *water* and add sufficient *water* to produce 500 ml. Dilute 5 ml of this solution to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 276 nm, Appendix II B. Calculate the content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$, in a container of average content weight taking 297 as the value of A(1%, 1 cm) at the maximum at 276 nm.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the quantity of Chloramphenicol Sodium Succinate contained in it in terms of the equivalent amount of chloramphenicol.

Chlordiazepoxide Capsules

General Notices

Action and use

Benzodiazepine.

DEFINITION

Chlordiazepoxide Capsules contain Chlordiazepoxide Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of chlordiazepoxide hydrochloride, $C_{16}H_{14}ClN_3O,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Dilute 1 volume of the final solution obtained in the Assay to 2 volumes with 0.1M *hydrochloric acid*. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 246 nm and 308 nm.

B. Shake a quantity of the contents of the capsules containing 25 mg of Chlordiazepoxide Hydrochloride with 2.5 ml of *water*, add 0.5 ml of 6M *ammonia*, mix, allow to stand for 5 minutes and filter. The filtrate, after making acidic with 2M *nitric acid*, yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of 13.5M *ammonia* as the mobile phase. Prepare the following solutions immediately before use in *acetone* containing 2% v/v of 13.5M *ammonia* and 8% v/v of *water*. For solution (1) shake a quantity of the contents of the capsules containing 0.10 g of Chlordiazepoxide Hydrochloride with 10 ml of the solvent mixture, allow to settle and use the clear supernatant liquid. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with the same solvent mixture. Solution (3) contains 0.01% w/v of *2-amino-5-chlorobenzophenone*. Apply separately to the plate 2-µl and 20-µl quantities of solution (1), 2 µl of solution (2) and 20 µl of solution (3). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with 2 µl of solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (3%). Spray the plate with a freshly prepared 1% w/v solution of *sodium nitrite* in 1M *hydrochloric acid*, dry it in a current of cold air and spray with a 0.4% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (96%)*. Any violet spot corresponding to 2-amino-5-chlorobenzophenone in the chromatogram obtained with 20 µl of solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted, if necessary, with 0.1M *hydrochloric acid* at the maximum at 308 nm, Appendix II B. Calculate the total content of chlordiazepoxide hydrochloride, $C_{16}H_{14}ClN_3O,HCl$, in the medium taking 292 as the value of A(1%, 1 cm) at the maximum at 308 nm.

ASSAY

Carry out the following procedure protected from light. Shake a quantity of the mixed contents of 20 capsules containing 20 mg of Chlordiazepoxide Hydrochloride with 150 ml of 0.1M *hydrochloric acid* for 20 minutes. Add sufficient 0.1M *hydrochloric acid* to produce 200 ml and filter. Dilute 10 ml of the filtrate to 50 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 308 nm, Appendix II B. Calculate the content of $C_{16}H_{14}ClN_3O,HCl$ taking 292 as the value of A(1%, 1 cm) at the maximum at 308 nm.

STORAGE

Chlordiazepoxide Capsules should be protected from light.

Chlordiazepoxide Hydrochloride Tablets

General Notices

Action and use

Benzodiazepine.

DEFINITION

Chlordiazepoxide Hydrochloride Tablets contain Chlordiazepoxide Hydrochloride.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlordiazepoxide, $C_{16}H_{14}ClN_3O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute 1 volume of the final solution obtained in the Assay to 2 volumes with 0.1M *hydrochloric acid*. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 246 nm and 308 nm.

B. To a quantity of the powdered tablets containing the equivalent of 0.2 g of chlordiazepoxide add 4 ml of hot 2M *hydrochloric acid*, heat at 100° for 10 minutes, cool and filter. 2 ml of the filtrate yields the reaction characteristic of *primary aromatic amines*, Appendix VI, producing a reddish precipitate.

C. Shake a quantity of the powdered tablets containing the equivalent of 25 mg of chlordiazepoxide with 2.5 ml of *water*, add 0.5 ml of 6M *ammonia*, mix, allow to stand for 5 minutes and filter. The filtrate, after making acidic with 2M *nitric acid*, yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of 13.5M *ammonia* as the mobile phase. Prepare the following solutions immediately before use in *acetone* containing 2% v/v of 13.5M *ammonia* and 8% v/v of *water*. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.10 g of chlordiazepoxide with 10 ml of the solvent mixture, allow to settle and use the supernatant liquid. For solution (2) dilute 5 volumes of solution (1) to 100 volumes with the same solvent mixture. For solution (3) dilute 1 volume of solution (1) to 100 volumes with the same solvent. Solution (4) contains 0.01% w/v of *2-amino-5-chlorobenzophenone*. Apply separately to the plate 2-µl and 20-µl quantities of solution (1), 2 µl of each of solutions (2) and (3) and 20 µl of solution (4). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with 2 µl of solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%). Spray the plate with a freshly prepared 1% w/v solution of *sodium nitrite* in 1M *hydrochloric acid*, dry it in a current of cold air and spray with a 0.4% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (96%)*. Any violet spot corresponding to 2-amino-5-chlorobenzophenone in the chromatogram obtained with 20 µl of solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (1%).

ASSAY

Shake 10 whole tablets with 150 ml of 0.1M *hydrochloric acid* for 20 minutes, add sufficient 0.1M *hydrochloric acid* to produce 250 ml and filter. Dilute 10 ml of the filtrate with sufficient 0.1M *hydrochloric acid* to produce a solution containing the equivalent of 0.0020% w/v of chlordiazepoxide and measure the *absorbance* of the resulting solution at the maximum at 308 nm, Appendix II B. Calculate the content of $C_{16}H_{14}ClN_3O$ taking 327 as the value of A (1%, 1 cm) at the maximum at 308 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of chlordiazepoxide.

Chlorhexidine Gluconate Gel

General Notices

Action and use

Antiseptic.

DEFINITION

Chlorhexidine Gluconate Gel is a solution of Chlorhexidine Gluconate in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of chlorhexidine gluconate, $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the gel containing 5 mg of Chlorhexidine Gluconate in 10 ml of *water*, mix and dilute to 500 ml with *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 200 to 320 nm exhibits two maxima, at 231 nm and 255 nm; and two minima, at 222 nm and 242 nm.

B. Place a quantity of the gel on a white tile and add a drop of *bromine water*. A reddish-yellow colour is produced.

C. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to chlorhexidine in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 5.0 to 7.0, Appendix V L.

4-Chloroaniline

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

SOLVENT A

Dissolve 80 mg of *2,6-dimethylaniline* (internal standard) in 1 ml of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100 ml and dilute 1 volume of this solution to 100 volumes with 0.01M *hydrochloric acid*.

(1) Shake a quantity of the gel containing 0.5 g of Chlorhexidine Gluconate with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution*, allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica treated filter paper

(Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

(2) Add 2 ml of solvent A to 50 ml of the gel, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution,* allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

REFERENCE SOLUTIONS

Prepare a series of reference solutions in the following manner. Dissolve 25 mg of *4-chloroaniline* in 1 ml of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 ml and dilute 1 volume to 10 volumes with the same solvent. To separate 0, 2, 4, 6 and 8 ml volumes of this solution (containing 0, 25, 50, 75 and 100 µg of 4-chloroaniline) add 2 ml of solvent A and sufficient *water* to produce 50 ml, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution* allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *silanised diatomaceous support* (100 to 120 mesh) coated with 15% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable).

(b) Use a flow rate of 50 ml per minute.

(c) A column temperature of 190°.

(d) Use an inlet port temperature of 200°.

(e) Use a detector temperature of 270°.

(f) Use *nitrogen* as the carrier gas.

(g) Use an electron capture detector.

(h) Inject 1 µl of each solution.

Inject the reference solutions and construct a calibration curve of the concentration of 4-chloroaniline against the ratio of the area of the peak corresponding to 4-chloroaniline to the area of the peak due to the internal standard.

LIMITS

In the chromatogram obtained with solution (2) determine the ratio of the area of any peak due to 4-chloroaniline to the area of the peak due to the internal standard and hence calculate the content of 4-chloroaniline in the gel. Not more than 20ppm is detected.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 ml of a 0.080% w/v solution of *diphenylamine* (internal standard) in the mobile

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phase to 5 ml of a 0.070% w/v solution of *chlorhexidine acetate BPCRS* in the mobile phase and dilute to 100 ml with the mobile phase.

(2) Mix a quantity of the gel containing 1 g of Chlorhexidine Gluconate with sufficient of the mobile phase to produce 100 ml.

(3) Prepare in the same manner as solution (2) but add 5 ml of the internal standard solution before diluting to 100 ml.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 100 µl of each solution.

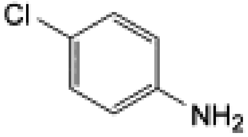
MOBILE PHASE

0.01 M *sodium octanesulphonate* in *methanol (73%)* adjusted to pH 3.0 with *glacial acetic acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ from the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS*. Each mg of $C_{22}H_{30}Cl_2N_{10}$ is equivalent to 1.775 mg of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

IMPURITIES



A. 4-chloroaniline.

Chlorhexidine Irrigation Solution

General Notices

Action and use

Antiseptic.

DEFINITION

Chlorhexidine Irrigation Solution is either a sterile solution of Chlorhexidine Acetate in Water for Irrigation or a sterile dilution of Chlorhexidine Gluconate Solution in Water for Irrigation. *The solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.*

Content of chlorhexidine acetate, $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$ or chlorhexidine gluconate, $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Chlorhexidine Irrigation Solution prepared using Chlorhexidine Acetate complies with tests A, B and C. Chlorhexidine Irrigation Solution prepared using Chlorhexidine Gluconate Solution complies with tests A and B only.

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (2) is the same as that of the peak due to chlorhexidine in the chromatogram obtained with solution (1).

B. To 10 ml of the irrigation solution add 5 ml of a warm 1% w/v solution of *cetrimide*, 1 ml of 5M *sodium hydroxide* and 1 ml of *bromine water*. A deep red colour is produced.

C. Evaporate or dilute a volume of the irrigation solution containing the equivalent of about 5 mg of chlorhexidine to about 5 ml. The resulting solution yields reaction B characteristic of *acetates*, Appendix VI.

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

4-Chloroaniline

Not more than 1.5 ppm when determined in the following manner. Dissolve 80 mg of *2,6-dimethylaniline* (internal standard) in 1 ml of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100 ml and dilute 1 volume of this solution to 100 volumes with 0.01M *hydrochloric acid* (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) shake 50 ml of the irrigation solution with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of

0.6M *sodium hydrogen carbonate solution*, allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer. For solution (2) add 2 ml of solution A to 50 ml of the irrigation solution, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate …'. Prepare a series of reference solutions in the following manner. Dissolve 25 mg of *4-chloroaniline* in 1 ml of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 ml and dilute 1 volume to 10 volumes with the same solvent. To separate 0, 2, 4, 6 and 8 ml volumes of this solution (containing 0, 25, 50, 75 and 100 µg of 4-chloroaniline) add 2 ml of solution A and sufficient *water* to produce 50 ml, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate …'.

The chromatographic procedure may be carried out using (a) a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 15% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable) at a temperature of 190° with the inlet port at 200° and the detector at 270°, (b) *nitrogen* as the carrier gas at a flow rate of 50 ml per minute and (c) an electron capture detector.

Inject the reference solutions and construct a calibration curve of the concentration of 4-chloroaniline against the ratio of the area of the peak corresponding to 4-chloroaniline to the area of the peak due to the internal standard. In the chromatogram obtained with solution (2) determine the ratio of the area of any peak due to 4-chloroaniline to the area of the peak due to the internal standard and hence calculate the content of 4-chloroaniline in the irrigation solution.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the irrigation solution. For solution (2) dilute 3 volumes of the irrigation solution to 100 volumes with the mobile phase. For solution (3) dilute 1 volume of solution (2) to 50 volumes with the mobile phase. Solution (4) contains 0.15% w/v of *chlorhexidine for performance test EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase at a flow rate of 1.0 ml per minute a solution containing 0.20% w/v of *sodium octanesulphonate* in a mixture of 120 volumes of *glacial acetic acid*, 270 volumes of *water* and 730 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Equilibrate the column with mobile phase for at least 1 hour. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 10 µl of solution (3) is at least 50% of the full scale of the recorder.

Inject 10 µl of solution (4). The test is not valid unless the resulting chromatogram is similar to the specimen chromatogram provided with *chlorhexidine for performance test EPCRS* in that the peaks due to impurity A and impurity B precede that due to chlorhexidine. If necessary, adjust the concentration of acetic acid in the mobile phase (increasing the concentration decreases the retention times).

Inject separately 10 µl of solutions (1), (2) and (3). Record the chromatograms of solutions (2) and (3) until the peak due to chlorhexidine has been eluted and record the chromatogram of solution (1) for six times the retention time of the peak due to chlorhexidine. In the chromatogram obtained with solution (1), the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3%). Disregard any peak with a relative retention time of 0.25 or less with respect to the principal peak and any peak the area of which is less than that of the principal peak in the chromatogram obtained with solution (3) (0.06%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.008% w/v of *chlorhexidine acetate BPCRS* in the mobile phase. For solution (2) dilute a quantity of the solution being examined containing 4 mg of Chlorhexidine Acetate or 5 mg of chlorhexidine gluconate with sufficient of the mobile phase to produce 50 ml.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$ or $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ from the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS*. Each mg of chlorhexidine, $C_{22}H_{30}Cl_2N_{10}$, is equivalent to 1.238 mg of $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$ or 1.775 mg of $C_{22}H_{30}Cl_2N_{10}$, $2C_6H_{12}O_7$.

STORAGE

Chlorhexidine Irrigation Solution should be protected from light.

LABELLING

The label on the container states whether the preparation contains Chlorhexidine Acetate or chlorhexidine gluconate.

Chlorhexidine Mouthwash

General Notices

Action and use

Antiseptic.

DEFINITION

Chlorhexidine Mouthwash contains Chlorhexidine Gluconate Solution in a suitable flavoured and coloured vehicle.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of chlorhexidine gluconate, $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}0_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak due to chlorhexidine in the chromatogram obtained with solution (2).

TESTS

4-Chloroaniline

Not more than 0.3% of the content of chlorhexidine gluconate when determined in the following manner. Dissolve 80 mg of *2,6-dimethylaniline* (internal standard) in 1 ml of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100 ml and dilute 1 volume of this solution to 100 volumes with 0.01M *hydrochloric acid* (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) dilute a volume of the mouthwash containing the equivalent of 25 mg of chlorhexidine gluconate to 50 ml with *water*, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution*, allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer. For solution (2) dilute a volume of the mouthwash containing the equivalent of 25 mg of chlorhexidine gluconate to 50 ml with *water*, add 2 ml of solution A, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate …'. Prepare a series of reference solutions in the following manner. Dissolve 25 mg of *4-chloroaniline* in 1 ml of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 ml and dilute 1 volume to 10 volumes with the same solvent. To separate 0, 2, 4, 6 and 8 ml volumes of this solution (containing 0, 25, 50, 75 and 100 µg of 4-chloroaniline) add 2 ml of solution A and sufficient *water* to produce 50 ml, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate …'.

The chromatographic procedure may be carried out using (a) a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 15% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable) at a temperature of 190° with the inlet port at 200° and the detector at 270°, (b) *nitrogen* as the carrier gas at a flow rate of 50 ml per minute and (c) an electron capture detector.

Inject the reference solutions and construct a calibration curve of the concentration of 4-chloroaniline against the ratio of the area of the peak corresponding to 4-chloroaniline to the area of the peak due to the internal standard. In the chromatogram obtained with solution (2) determine the ratio of the area of any peak due to 4-chloroaniline to the area of the peak due to the internal standard and hence calculate the content of 4-chloroaniline in the mouthwash with respect to the labelled content of chlorhexidine gluconate.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the mouthwash with sufficient of the mobile phase to produce a solution containing 0.01% w/v of chlorhexidine gluconate. Solution (2) contains 0.008% w/v of *chlorhexidine acetate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a solution containing 2 g of *sodium octanesulphonate* in a mixture of 120 ml of *glacial acetic acid*, 270 ml of *water* and 730 ml of *methanol* and (c) a detection wavelength of 254 nm.

Equilibrate the column with the mobile phase for at least 1 hour.

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}0_7$ from the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS*. Each mg of chlorhexidine, $C_{22}H_{30}Cl_2N_{10}$, is equivalent to 1.775 mg of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}0_7$.

STORAGE

Chlorhexidine Mouthwash should be protected from light.

Chlormethine Injection

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Chlormethine Injection is a sterile solution of Chlormethine Hydrochloride in Water for Injections or Sodium Chloride Intravenous Infusion. It is prepared by dissolving Chlormethine Hydrochloride for Injection in the requisite amount of Water for Injections or Sodium Chloride Intravenous Infusion immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Chlormethine Injection deteriorates rapidly on storage and should be used immediately after preparation.

CHLORMETHINE HYDROCHLORIDE FOR INJECTION

DEFINITION

Chlormethine Hydrochloride for Injection is a sterile material consisting of Chlormethine Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of chlormethine hydrochloride, $C_5H_{11}Cl_2N,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Dissolve the contents of a sealed container in 1 ml of *water* and add 0.02 ml of *potassium tetra-iodomercurate solution*. A cream precipitate is produced.

TESTS

Uniformity of content

The content of chlormethine hydrochloride, $C_5H_{11}Cl_2N,HCl$, in each of 10 individual containers as determined in the Assay is not less than 80.0% and not more than 120.0% of the average amount.

ASSAY

Dissolve the contents of a sealed container in 2 ml of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1*, add 0.1 ml of *phenolphthalein solution R1* and titrate with 0.01M *sodium hydroxide VS* (carbonate-free). Each ml of 0.01M *sodium hydroxide VS* is equivalent to 1.925 mg of $C_5H_{11}Cl_2N,HCl$. Calculate the content of $C_5H_{11}Cl_2N,HCl$ in the sealed container . Repeat the procedure with a further nine sealed containers. Calculate the average content of $C_5H_{11}Cl_2N,HCl$ per container from the 10 individual results thus obtained.

LABELLING

The label of the sealed container states (1) the amount of the active ingredient contained in it; (2) that the contents are strongly vesicant.

Chloroform and Morphine Tincture

General Notices

Chlorodyne

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Chloroform	125 ml
Morphine Hydrochloride	2.29 g
Peppermint Oil	1 ml
Ether	30 ml
Purified Water	50 ml
Ethanol (90 per cent)	125 ml
Liquorice Liquid Extract	125 ml
Treacle, of commerce	125 ml
Syrup sufficient to produce	1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Peppermint Oil in the Ethanol (90 per cent), add the Purified Water, dissolve the Morphine Hydrochloride in the mixture and add the Chloroform and the Ether. Separately, mix the Liquorice Liquid Extract and the treacle with 400 ml of Syrup. Mix the two solutions, add sufficient Syrup to produce 1000 ml and mix.

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

TESTS

Content of anhydrous morphine, $C_{17}H_{19}NO_3$

0.157 to 0.191% w/v.

Chloroform content

11.25 to 13.75% v/v when determined by the following method. Carry out the method for *gas* *chromatography*, Appendix III B, using the following solutions. Solution (1) contains 5% v/v of *chloroform* and 5% v/v of *propan-1-ol* (internal standard) in a mixture of 2 volumes of *methanol* and 1 volume of *water*. For solution (2) dilute 40 ml of the tincture to 100 ml with a mixture of 2 volumes of *methanol* and 1 volume of *water*. For solution (3) dilute 40 ml of the tincture and 5 ml of *propan-1-ol* to 100 ml with *methanol*.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with porous polymer beads (80 to 100 mesh) (Chromosorb 101 is suitable) and maintained at 120°.

Calculate the content of $CHCl_3$ from the areas of the peaks due to chloroform and propan-1-ol in the chromatograms obtained with solutions (1) and (3).

Ethanol content

12 to 15% v/v when determined by the method described under Chloroform content but using for solution (1) a solution containing 5% v/v of *absolute ethanol* and 5% v/v of *propan-1-ol* (internal standard) in a mixture of 2 volumes of *methanol* and 1 volume of *water*.

Calculate the content of C_2H_6O from the areas of the peaks due to ethanol and propan-1-ol in the chromatograms obtained with solutions (1) and (3).

Relative density

1.22 to 1.26, Appendix V G.

ASSAY

To 13 g add 1 ml of 5M *ammonia* and 4 ml of *water*, extract with 30 ml of a mixture of equal volumes of *ethanol (96%)* and *chloroform* and then with two 22.5-ml quantities of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)*. Wash each extract with the same 20 ml of a mixture of equal volumes of *ethanol (96%)* and *water* and evaporate the combined extracts to dryness. Dissolve the residue in 100 ml of 1M *hydrochloric acid*, dilute to 500 ml with *water* and filter. Dilute 10 ml of the filtrate with 10 ml of *water,* add 8 ml of a freshly prepared 1% w/v solution of *sodium nitrite*, allow to stand in the dark for 15 minutes, add 12 ml of 5M *ammonia* and dilute to 50 ml with *water*. Measure the *absorbance* of a 4-cm layer of the resulting solution at the maximum at 442 nm, Appendix II B, using in the reference cell a solution prepared in the same manner and at the same time but using 8 ml of *water* in place of the solution of sodium nitrite. Calculate the content of $C_{17}H_{19}NO_3$ from a calibration curve prepared from quantities of 2, 4, 6 and 8 ml of a 0.008% w/v solution of *anhydrous morphine* in 0.1M *hydrochloric acid*, each diluted to 20 ml with 0.1M *hydrochloric acid* and using the method described above beginning at the words 'add 8 ml ...'. Using the *weight per ml* of the tincture, calculate the content of $C_{17}H_{19}NO_3$, weight in volume.

STORAGE

Chloroform and Morphine Tincture should be kept in an airtight container.

LABELLING

The label states that the tincture must be shaken well before use.

Chloroform Spirit

General Notices

DEFINITION

Chloroform	50 ml
Ethanol (90 per cent)	Sufficient to produce 1000 ml

The spirit complies with the requirements stated under Spirits and with the following requirements.

TESTS

Ethanol content

83 to 87% v/v, Appendix VIII F.

Weight per ml

0.856 to 0.862 g, Appendix V G.

Chloroquine Phosphate Tablets

General Notices

Action and use

Antiprotozoal (malaria).

DEFINITION

Chloroquine Phosphate Tablets contain Chloroquine Phosphate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chloroquine phosphate, $C_{18}H_{26}ClN_3,2H_3PO_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of Chloroquine Phosphate in a mixture of 10 ml of *water* and 2 ml of 2M *sodium hydroxide* and extract with two 20 ml quantities of *chloroform*. Wash the chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chloroquine *(RS 054)*.

B. Extract a quantity of the powdered tablets containing 25 mg of Chloroquine Phosphate with 20 ml of *water*, filter and add 8 ml of *picric acid solution R1* to the filtrate. The *melting point* of the precipitate, after washing successively with *water*, *ethanol (96%)* and *ether*, is about 207°, Appendix V A.

C. Extract a quantity of the powdered tablets containing 0.5 g of Chloroquine Phosphate with 25 ml of *water* and filter. To the filtrate add 2.5 ml of 5M *sodium hydroxide* and extract with three 10 ml quantities of *ether*. The aqueous layer, after neutralisation with 2M *nitric acid*, yields the reactions characteristic *of phosphates*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 344 nm, Appendix II B. Calculate the total content of chloroquine phosphate, $C_{18}H_{26}ClN_3,2H_3PO_4$, in the medium taking 371 as the value of A(1%, 1 cm) at the maximum at 344 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 1 g of Chloroquine Phosphate with 20 ml of *water* for 30 minutes, centrifuge and use the supernatant liquid; if necessary filter through a glass fibre paper. For solution (2) dilute 1 ml of solution (1) to 100 ml with *water*. For solution (3) dilute 25 ml of solution (2) to 50 ml with *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Chloroquine Phosphate in 20 ml of 1M *sodium hydroxide* and extract with four 25-ml quantities of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 25.79 mg of $C_{18}H_{26}ClN_3,2H_3PO_4$.

250 mg of Chloroquine Phosphate is approximately equivalent to 155 mg of chloroquine.

Chloroquine Sulphate Injection

General Notices

Action and use

Antiprotozoal (malaria).

DEFINITION

Chloroquine Sulphate Injection is a sterile solution of Chloroquine Sulphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of chloroquine, $C_{18}H_{26}ClN_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume containing the equivalent of 60 mg of chloroquine add 2 ml of 2M *sodium hydroxide* and extract with two 20-ml quantities of *chloroform*. Wash the chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chloroquine *(RS 054)*.

B. Dilute a volume containing the equivalent of 15 mg of chloroquine to 20 ml with *water* and add 8 ml of *picric acid solution R1*. The *melting point* of the precipitate, after washing successively with *water*, *ethanol (96%)* and *ether*, is about 207°, Appendix V A.

C. Yields the reactions characteristic *of sulphates*, Appendix VI.

TESTS

Acidity

pH, 4.0 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *diethylamine,* 40 volumes of *cyclohexane* and 50 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 2 µl of each of the following three solutions. For solution (1) use the injection being examined. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *water*. For solution (3) dilute 1 volume of solution (2) to 2 volumes with *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

To a volume containing the equivalent of 0.4 g of chloroquine add 20 ml of 1M *sodium hydroxide* and extract with four 25 ml quantities of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 15.99 mg of $C_{18}H_{26}ClN_3$.

LABELLING

The strength is stated as the equivalent amount of chloroquine in a suitable dose-volume.

40 mg of chloroquine is approximately equivalent to 55 mg of Chloroquine Sulphate.

Chloroquine Sulphate Tablets

General Notices

Action and use

Antiprotozoal (malaria).

DEFINITION

Chloroquine Sulphate Tablets contain Chloroquine Sulphate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chloroquine sulphate, $C_{18}H_{26}ClN_3,H_2SO_4,H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of Chloroquine Sulphate in a mixture of 10 ml of *water* and 2 ml of 2M *sodium hydroxide* and extract with two 20-ml quantities of *chloroform*. Wash the chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chloroquine *(RS 054)*.

B. Shake a quantity of the powdered tablets containing 0.1 g of Chloroquine Sulphate with 10 ml of *water* and 1 ml of 2M *hydrochloric acid* and filter. To the filtrate add 1 ml of *barium chloride solution*. A white precipitate is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 344 nm, Appendix II B. Calculate the total content of chloroquine sulphate, $C_{18}H_{26}ClN_3,H_2SO_4,H_2O$, in the medium taking 450 as the value of A(1%, 1 cm) at the maximum at 344 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 2.0 g of Chloroquine Sulphate with 50 ml of *water* for 30 minutes, centrifuge and use the supernatant liquid; if necessary filter through a glass fibre paper. For solution (2) dilute 1 ml of solution (1) to 100 ml with *water*. For solution (3) dilute 25 ml of solution (2) to 50 ml with *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Chloroquine Sulphate in 20 ml of 1M *sodium hydroxide* and extract with four 25 ml quantities of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 20.90 mg of $C_{18}H_{26}ClN_3,H_2SO_4$.

200 mg of Chloroquine Sulphate is approximately equivalent to 146 mg of chloroquine.

Chloroxylenol Solution

General Notices

Action and use

Antiseptic.

DEFINITION

Chloroxylenol Solution is a *cutaneous solution*.

Chloroxylenol	50.0 g
Potassium Hydroxide	13.6 g
Oleic Acid	7.5 ml
Virgin Castor Oil	63.0 g
Terpineol	100 ml
Ethanol (96 per cent)	200 ml
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

In making Chloroxylenol Solution, the Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit[1].

Extemporaneous preparation

The following directions apply.

Dissolve the Potassium Hydroxide in 15 ml of Purified Water, add a solution of the Virgin Castor Oil in 63 ml of Ethanol (96 per cent), mix, allow to stand for 1 hour or until a small portion of the mixture remains clear when diluted with 19 times its volume of Purified Water and then add the Oleic Acid. Mix the Terpineol with a solution of the Chloroxylenol in the remainder of the Ethanol (96 per cent), pour into the soap solution and add sufficient Purified Water to produce 1000 ml.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of chloroxylenol, C_8H_9ClO

4.75 to 5.25% w/v.

TESTS

Ethanol content

16 to 21% v/v, Appendix VIII F.

ASSAY

Dissolve 0.4 g of *4-chloro-o-cresol* (internal standard) in sufficient *chloroform* to produce 50 ml (solution A). Carry out the method for *gas chromatography*, Appendix III B, using solutions prepared in the following manner. For solution (1) dissolve 0.10 g of *chloroxylenol BPCRS* in 10 ml of solution A and dilute to 20 ml with *chloroform*. For solution (2) place 4 ml of the solution being examined in a separating funnel, add 20 ml of *chloroform*, mix, add 4 ml of 2M *hydrochloric acid* and shake. Extract with two further 10 ml quantities of *chloroform*. Combine the chloroform extracts, dry by shaking with *anhydrous sodium sulphate* and filter. Prepare solution (3) in the same manner as solution (2) but adding 20 ml of solution A in place of the 20 ml of chloroform.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of polyethylene glycol (Carbowax 20M is suitable) and maintained at 160°.

Calculate the content of C_8H_9ClO using the declared content of C_8H_9ClO in *chloroxylenol BPCRS*.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

The label indicates the pharmaceutical form as 'cutaneous solution'.

[1] The law and statutory regulations governing the use of Industrial Methylated Spirit must be observed.

Chlorphenamine Injection

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Chlorphenamine Injection is a sterile solution of Chlorphenamine Maleate in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of chlorphenamine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 20 volumes of 1M *acetic acid*, 30 volumes of *methanol* and 50 volumes of *ethyl acetate* as the mobile phase. Heat the plate at 105° for 30 minutes before use. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) evaporate an appropriate volume to dryness in a current of nitrogen using the minimum amount of heat, dissolve the residue as completely as possible in sufficient *chloroform* to produce a solution containing 0.5% w/v of Chlorphenamine Maleate and centrifuge. Solution (2) contains 0.5% w/v of *chlorphenamine maleate BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The two principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2). Spray the plate with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.2, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *diethylamine*, 40 volumes of *chloroform* and 50 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) evaporate a suitable volume of the injection to dryness in a current of nitrogen using the minimum amount of heat, dissolve the residue as completely as possible in sufficient *chloroform* to produce a solution containing 5% w/v of Chlorphenamine Maleate and centrifuge. For solution (2) dilute 1 volume of solution (1) to 500 volumes with *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot remaining on the line of application.

ASSAY

Dilute a volume containing 10 mg of Chlorphenamine Maleate to 500 ml with 0.25M *sulphuric acid* and measure the *absorbance* of the resulting solution at the maximum at 265 nm, Appendix II B. Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ taking 212 as the value of A(1%, 1 cm) at the maximum at 265 nm.

STORAGE

Chlorphenamine Injection should be protected from light.

Chlorphenamine Oral Solution

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Chlorphenamine Oral Solution is a solution of Chlorphenamine Maleate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of chlorphenamine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the test for Related substances, the principal spot in the chromatogram obtained with solution (3) corresponds to the spot in the chromatogram obtained with solution (4).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 50 volumes of *ethyl acetate*, 30 volumes of *methanol* and 20 volumes of 1M *acetic acid* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a volume of the oral solution containing 10 mg of Chlorphenamine Maleate with an equal volume of *water*, add 20 ml of a 10% w/v solution of *sodium hydroxide* and extract with four 15 ml quantities of *chloroform*. Add 1 g of *anhydrous sodium sulphate* to the combined extracts, filter, evaporate at a temperature not exceeding 40° at a pressure of 2 kPa and dissolve the residue in 1 ml of *chloroform*. For solution (2) dilute 1 volume of solution (1) to 500 volumes with *chloroform*. For solution (3) dilute 1 volume of solution (1) to 10 volumes with *chloroform*. Solution (4) contains 0.1% w/v of *chlorphenamine maleate BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Solution (1) contains 0.15% w/v of *chlorphenamine maleate BPCRS* and 0.15% w/v of *N-phenylcarbazole* (internal standard) in *chloroform*. Prepare solution (2) in the same manner as solution (3), but omitting the internal standard. For solution (3) add 10 ml of a 10% w/v solution of *sodium hydroxide* in *methanol* and 5 ml of a 0.060% w/v solution of *N-phenylcarbazole* in *chloroform* to a quantity of the oral solution containing 3 mg of Chlorphenamine Maleate, extract with four 25 ml quantities of *chloroform* and wash each extract with the same 10 ml of *water*. Combine the chloroform extracts, shake with *anhydrous* *sodium sulphate*, filter, evaporate at a temperature not exceeding 40° at a pressure of 2 kPa and dissolve the residue in 2 ml of *chloroform*.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) (Gas Chrom Q or Diatomite CQ is suitable) coated with 3% w/w of dimethyl silicone fluid (OV-101 is suitable) and maintained at 220° with the inlet port at 250°.

Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ using the declared content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ in *chlorphenamine maleate BPCRS*.

STORAGE

Chlorphenamine Oral Solution should be protected from light.

Chlorphenamine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Chlorphenamine Tablets contain Chlorphenamine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlorphenamine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Comply with the test described under Chlorphenamine Injection using as solution (1) a solution prepared in the following manner. Extract a quantity of the powdered tablets containing 5 mg of Chlorphenamine Maleate with *chloroform*, filter, evaporate to dryness and dissolve the residue in 1 ml of *chloroform*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *diethylamine*, 40 volumes of *chloroform* and 50 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 50 mg of Chlorphenamine Maleate with *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*. For solution (2) dilute 1 volume of solution (1) to 500 volumes with *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 3 mg of Chlorphenamine Maleate with 20 ml of 0.05M *sulphuric acid* for 5 minutes, add 20 ml of *ether* , shake carefully and filter the acid layer into a second separating funnel. Extract the ether layer with two 10-ml quantities of 0.05M *sulphuric acid* , filter each acid layer into the second separating funnel and wash the filter with 0.05M *sulphuric acid* . Make the combined acid extracts and washings just alkaline to *litmus paper* with 1M *sodium hydroxide*, add 2 ml in excess and extract with two 50 ml quantities of *ether*. Wash each ether extract with the same 20 ml of *water* and extract with successive quantities of 20, 20 and 5 ml of 0.25M *sulphuric acid* . Dilute the combined acid extracts to 50 ml with 0.25M *sulphuric acid* , dilute 10 ml to 25 ml with 0.25M *sulphuric acid* and measure the *absorbance* of the resulting solution at the maximum at 265 nm, Appendix II B. Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ taking 212 as the value of A(1%, 1 cm) at the maximum at 265 nm.

Chlorpromazine Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Chlorpromazine Injection is a sterile solution of Chlorpromazine Hydrochloride in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume containing 0.1 g of Chlorpromazine Hydrochloride add 20 ml of *water* and 2 ml of 10M *sodium hydroxide*. Shake and extract with 25 ml of *ether*. Wash the ether layer with two 5 ml quantities of *water*, dry with *anhydrous sodium sulphate*, evaporate the ether and dissolve the residue in 1 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chlorpromazine *(RS 056)*.

B. Complies with the test for *identification of phenothiazines*, Appendix III A. For solution (1) dilute the injection with *water* to give a solution containing 0.2% w/v of Chlorpromazine Hydrochloride.

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

Related substances

Carry out the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and applying separately to the plate 20 µl of each of the following freshly prepared solutions. For solution (1) dilute a volume of the injection, if necessary, with sufficient of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* to produce a solution containing 0.5% w/v of Chlorpromazine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 20 volumes with the same solvent. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the same solvent. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Carry out the following procedure protected from light. Dilute a suitable volume with sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.0005% w/v of Chlorpromazine Hydrochloride and measure the *absorbance* at the maximum at 254 nm, Appendix II B. Calculate the content of $C_{17}H_{19}ClN_2S,HCl$ taking 915 as the value of A(1%, 1 cm) at the maximum at 254 nm.

STORAGE

Chlorpromazine Injection should be protected from light.

Chlorpromazine Oral Solution

General Notices

Chlorpromazine Elixir

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Chlorpromazine Oral Solution is a solution of Chlorpromazine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *identification of phenothiazines*, Appendix III A. For solution (1) dilute a suitable volume of the oral solution with *water* to give a solution containing 0.2% w/v of Chlorpromazine Hydrochloride.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light under an atmosphere of nitrogen using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *acetone*, 10 volumes of *diethylamine* and 80 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following freshly prepared solutions. For solution (1) add 40 ml of *water* and 5 ml of a 20% w/v solution of *sodium hydroxide* to a quantity of the oral solution containing 20 mg of Chlorpromazine Hydrochloride in a separating funnel and swirl to mix. Extract with two 25 ml quantities of *chloroform*, combine the chloroform extracts and filter through *anhydrous sodium sulphate*. Wash the sodium sulphate with a further 25 ml of *chloroform* and evaporate the combined filtrate and washings to dryness at about 30° in a gentle current of *nitrogen*. Dissolve the residue in 2 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent. Solution (3) is a 0.030% w/v solution of *chlorpromazine sulphoxide BPCRS* in the same solvent. After removal of the plate, allow it to dry in air, spray with a 20% w/w solution of *perchloric acid* and heat at 100° for 5 minutes. In the chromatogram obtained with solution (1) any spot corresponding to chlorpromazine sulphoxide is not more intense than the spot in the chromatogram obtained with solution (3) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2). Disregard any spot remaining on the line of application.

ASSAY

Carry out the following procedure protected from light. Dilute a quantity containing 0.1 g of Chlorpromazine Hydrochloride to 500 ml with 2M *hydrochloric acid*. To 10 ml of this solution add 20 ml of *water*, make distinctly alkaline to *litmus paper* with 13.5M *ammonia* and extract with six 25 ml quantities of *ether*. Extract the combined ether solutions with four 25 ml quantities of a mixture containing 1 volume of *hydrochloric acid* and 99 volumes of *water*, discard the ether, remove any dissolved ether from the combined extracts with a current of air and dilute to 250 ml with a mixture containing 1 volume of *hydrochloric acid* and 99 volumes of *water*. Measure the *absorbance* of the resulting solution at the maximum at 254 nm, Appendix II B. Calculate the content of $C_{17}H_{19}ClN_2S,HCl$ taking 914 as the value of A(1%, 1 cm) at the maximum at 254 nm.

STORAGE

Chlorpromazine Oral Solution should be protected from light.

Chlorpromazine Suppositories

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Chlorpromazine Suppositories contain Chlorpromazine in a suitable suppository basis.
The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of chlorpromazine, $C_{17}H_{19}ClN_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Comply with the test for *identification of phenothiazines*, Appendix III A. For solution (1) dissolve a quantity of the suppositories containing 0.1 g of Chlorpromazine in 50 ml of *chloroform*. Use *chlorpromazine hydrochloride EPCRS* to prepare solution (2).

TESTS

Related substances

Comply with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) dissolve a quantity of the suppositories containing 0.2 g of Chlorpromazine in sufficient *chloroform* to produce 20 ml. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *chloroform*.

ASSAY

Weigh five suppositories. Dissolve a quantity containing 0.5 g of Chlorpromazine in sufficient *chloroform* to produce 100 ml and dilute 20 ml to 100 ml with *ethanol (96%)*. Dilute 10 ml of this solution to 100 ml with *ethanol (96%)* and further dilute 5 ml of this solution to 100 ml with the same solvent. Measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B, using *ethanol (96%)* in the reference cell. Calculate the content of $C_{17}H_{19}ClN_2S$ taking 1150 as the value of A(1%, 1 cm) at the maximum at 258 nm.

STORAGE

Chlorpromazine Suppositories should be protected from light.

Chlorpromazine Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Chlorpromazine Tablets contain Chlorpromazine Hydrochloride. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 40 mg of Chlorpromazine Hydrochloride add 10 ml of *water* and 2 ml of 10M *sodium hydroxide*. Shake and extract with 15 ml of *ether*. Wash the ether layer with two 5 ml quantities of *water*, dry with *anhydrous sodium sulphate* and evaporate the ether. Dissolve the residue in 0.4 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of chlorpromazine *(RS 056)*.

B. Comply with the test for *identification of phenothiazines*, Appendix III A. For solution (1) shake a quantity of the powdered tablets with sufficient *chloroform* to produce a solution containing 0.20% w/v of Chlorpromazine Hydrochloride, centrifuge and use the supernatant liquid.

TESTS

Related substances

Comply with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Chlorpromazine Hydrochloride with 10 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2, protected from light. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute a suitable volume of the filtrate with 0.1M *hydrochloric acid* to produce a solution containing 0.0005% w/v of Chlorpromazine Hydrochloride. Measure the *absorbance* of this solution, Appendix II B, at 254 nm using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of

$C_{17}H_{19}ClN_2S,HCl$ in the medium taking 914 as the value of A(1%, 1 cm) at the maximum at 254 nm.

ASSAY

Carry out the following procedure protected from light. Powder 10 tablets without loss, triturate the powder with 10 ml of *absolute ethanol* , add about 300 ml of 0.1M *hydrochloric acid* and shake for 15 minutes. Add sufficient 0.1M *hydrochloric acid* to produce 500 ml, filter, dilute a volume of the filtrate containing 5 mg of Chlorpromazine Hydrochloride to 100 ml with 0.1M *hydrochloric acid* and further dilute 10 ml to 100 ml with the same solvent. Measure the *absorbance* of the resulting solution at the maximum at 254 nm, Appendix II B. Calculate the content of $C_{17}H_{19}ClN_2S,HCl$ taking 915 as the value of A(1%, 1 cm) at the maximum at 254 nm.

Chlorpropamide Tablets

General Notices

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Chlorpropamide Tablets contain Chlorpropamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlorpropamide, $C_{10}H_{13}ClN_2O_3S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 1 g of Chlorpropamide with five 4-ml quantities of *acetone*, filter and evaporate the filtrate carefully to dryness on a water-bath. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of chlorpropamide *(RS 057)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 6.8 by the addition of 1M *sodium hydroxide* and rotating the basket at 100 revolutions per minute. Withdraw a 10-ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 230 nm, Appendix II B. Calculate the total content of chlorpropamide, $C_{10}H_{13}ClN_2O_3S$, in the medium taking 469 as the value of A(1%, 1 cm) at the maximum at 230 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel 60* as the coating substance and a mixture of 11.5 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane*, 50 volumes of *methanol* and 100 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.5 g of Chlorpropamide with 10 ml of *acetone* for 10 minutes and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.015% w/v of *4-chlorobenzenesulphonamide* in *acetone*. Solution (3) contains 0.015% w/v of *1,3-dipropylurea* in *acetone*. For solution (4) dilute 0.3 volume of solution (1) to 100 volumes with *acetone*. For solution (5) dilute 1 volume of solution (4) to 3 volumes with *acetone*. For solution (6) dissolve 5 mg of *4-chlorobenzenesulphonamide* and 5 mg of *1,3-*

dipropylurea in 2 ml of solution (1) and add sufficient *acetone* to produce 10 ml. After removal of the plate, dry it in a current of cold air and heat at 110° for 10 minutes. Place the hot plate in a tank of chlorine gas prepared by the addition of *hydrochloric acid* to a 5% w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application gives at most a very faint blue colour with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. In the chromatogram obtained with solution (1) any spot corresponding to 4-chlorobenzenesulphonamide is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%), any spot corresponding to 1,3-dipropylurea is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4) (0.3%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (5) (0.1%). The test is not valid unless the chromatogram obtained with solution (6) shows three clearly separated spots with Rf values of approximately 0.4, 0.6 and 0.9 corresponding to chlorpropamide, 4-chlorobenzenesulphonamide and 1,3-dipropylurea, respectively.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.25 g of Chlorpropamide with 40 ml of *methanol* for 20 minutes, add sufficient *methanol* to produce 50 ml, mix, filter and dilute 5 ml of the filtrate to 100 ml with 0.1M *hydrochloric acid*. Dilute 10 ml of this solution to 250 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 232 nm, Appendix II B. Calculate the content of $C_{10}H_{13}ClN_2O_3S$ taking 598 as the value of A(1 cm, 1%) at the maximum at 232 nm.

Chlortalidone Tablets

General Notices

Action and use

Thiazide-like diuretic.

DEFINITION

Chlortalidone Tablets contain Chlortalidone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of chlortalidone, $C_{14}H_{11}ClN_2O_4S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Heat a quantity of the powdered tablets containing 0.2 g of Chlortalidone with 20 ml of *acetone* on a water bath for 10 minutes, cool and filter. Add 40 ml of *water* to the filtrate and heat on a water bath for 20 minutes using a gentle current of air to remove the acetone. Cool the solution to room temperature, allow to stand, filter and dry the crystals at 105° for 4 hours. The *infrared spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of chlortalidone *(RS 058)*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *toluene*, 10 volumes of *xylene*, 20 volumes of 18M *ammonia*, 30 volumes of *1,4-dioxan* and 30 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) add a quantity of the powdered tablets containing 0.1 g of Chlortalidone to 5 ml of *ethanol (96%)*, mix with the aid of ultrasound for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *ethanol (96%)*. Solution (3) contains 0.020% w/v of *2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid EPCRS* in *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to *2*-(4-chloro-3-sulphamoylbenzoyl)benzoic acid in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Boil a quantity of the powder containing 0.1 g of Chlortalidone under a reflux condenser with 30 ml of *methanol* for 5 minutes, shake vigorously for 15 minutes, cool and filter. Wash the residue and filter with *methanol* and dilute the combined filtrate and washings to 100 ml with *methanol*. To 5 ml add 2 ml of 1M *hydrochloric acid* and sufficient *methanol* to produce 50 ml and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{14}H_{11}ClN_2O_4S$ taking 57.4 as the value of A(1%, 1 cm) at the maximum at 275 nm.

Chlortetracycline Eye Ointment

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Chlortetracycline Eye Ointment is a sterile preparation containing Chlortetracycline Hydrochloride in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of chlortetracycline hydrochloride, $C_{22}H_{23}ClN_2O_8,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the eye ointment containing 10 mg of Chlortetracycline Hydrochloride in 10 ml of *dichloromethane*, extract with two 10 ml quantities of 0.01M *hydrochloric acid*, filter and dilute the filtrate to 100 ml with 0.01M *hydrochloric acid*. Dilute 20 ml of the resulting solution to 100 ml with 0.01M *hydrochloric acid*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 420 nm exhibits two maxima, at 266 nm and 368 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Adjust the pH of a 10% w/v solution of *disodium edetate* to 8.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 ml for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. At the time of use, dry the plate in an oven at 110° for 1 hour. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) disperse a quantity of the eye ointment containing 25 mg of Chlortetracycline Hydrochloride in 25 ml of *dichloromethane*, extract with two quantities of 0.01M *hydrochloric acid*, filter the aqueous layer and dilute to 50 ml with *water*. Solution (2) contains 0.05% w/v of *chlortetracycline hydrochloride BPCRS* in *water*. Solution (3) contains 0.05% w/v of each of *chlortetracycline hydrochloride BPCRS*, *tetracycline hydrochloride BPCRS* and *metacycline hydrochloride EPCRS* in *water*. Allow the plate to dry in a current of air and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

C. To a quantity of the eye ointment containing 0.5 mg of Chlortetracycline Hydrochloride add 2 ml of *sulphuric acid*; a deep blue colour is produced, which becomes bluish green. Add 1 ml of *water*; a brown colour is produced.

TESTS

Tetracycline hydrochloride and 4-epichlortetracycline hydrochloride

Not more than 8.0% and 6.0% respectively, determined as described under Assay. Inject separately solutions (1) and (4).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the eye ointment containing 25 mg of Chlortetracycline Hydrochloride in 20 ml of *chloroform* and 50 ml of 0.01M *hydrochloric acid* and shake for 15 minutes, dilute to 100 ml with 0.01M *hydrochloric acid*, mix thoroughly, allow to separate and filter the aqueous layer. For solution (2) dissolve 25 mg of *chlortetracycline hydrochloride BPCRS* in 20 ml of *chloroform* and 50 ml of 0.01M *hydrochloric acid* and treat in the same manner as solution (1) beginning at the words 'and shake for 15 minutes …'. Solution (3) contains 0.025% w/v of each of *chlortetracycline hydrochloride BPCRS* and *4-epichlortetracycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*. Solution (4) contains 0.002% w/v of *tetracycline hydrochloride BPCRS* and 0.0015% w/v of *4-epichlortetracycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 80 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 2.2 with *triethylamine* and 20 volumes of *dimethylformamide* and (c) a detection wavelength of 355 nm.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 1.5.

Calculate the content of $C_{22}H_{23}ClN_2O_8,HCl$ in the eye ointment using the declared content of $C_{22}H_{23}ClN_2O_8,HCl$ in *chlortetracycline hydrochloride BPCRS.*

STORAGE

Chlortetracycline Eye Ointment should be protected from light.

Chlortetracycline Ointment

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Chlortetracycline Ointment contains Chlortetracycline Hydrochloride in a suitable basis.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of chlortetracycline hydrochloride, $C_{22}H_{23}ClN_2O_8$,HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the ointment containing 10 mg of Chlortetracycline Hydrochloride in 10 ml of *dichloromethane*, extract with two 10 ml quantities of 0.01M *hydrochloric acid,* combine the aqueous extracts, filter and extract the filtrate with two 10-ml quantities of *ether* . Discard the ether extracts and dilute the aqueous layer to 100 ml with 0.01M *hydrochloric acid*. Dilute 20 ml of the resulting solution to 100 ml with 0.01M *hydrochloric acid*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 420 nm exhibits two maxima, at 266 nm and 368 nm.

B. Complies with test B for Identification described under Chlortetracycline Eye Ointment but using the following solutions. For solution (1) disperse a quantity of the ointment containing 25 mg of Chlortetracycline Hydrochloride in 25 ml of *dichloromethane,* extract with two quantities of 0. 01M *hydrochloric acid,* filter the aqueous layer and dilute to 50 ml with *water*. Solution (2) contains 0.05% w/v of *chlortetracycline hydrochloride BPCRS* in *water.* Solution (3) contains 0.05% w/v each of *chlortetracycline hydrochloride BPCRS*, *tetracycline hydrochloride BPCRS* and *metacycline hydrochloride EPCRS* in *water*.

C. To a quantity of the ointment containing 0.5 mg of Chlortetracycline Hydrochloride add 2 ml of *sulphuric acid*; a deep blue colour is produced, which becomes bluish green. Add 1 ml of *water*; a brown colour is produced.

TESTS

Tetracycline hydrochloride and 4-epichlortetracycline hydrochloride

Not more than 8.0% and 6.0% respectively, determined as described under Assay. Inject separately solutions (1) and (4).

ASSAY

Carry out the Assay described under Chlortetracycline Eye Ointment but using the following solutions. For solution (1) disperse a quantity of the ointment containing 25 mg of Chlortetracycline Hydrochloride in 20 ml of *chloroform* and 50 ml of 0.01M *hydrochloric acid* and shake for 15 minutes, dilute to 100 ml with 0.01M *hydrochloric acid*, mix thoroughly, allow to separate and filter the aqueous layer. For solution (2) dissolve 25 mg of *chlortetracycline hydrochloride BPCRS* in 20 ml of *chloroform* and 50 ml of 0.01M *hydrochloric acid* and treat in the same manner as solution (1) beginning at the words 'and shake for 15 minutes ...'. Solution (3) contains 0.025% w/v each of *chlortetracycline hydrochloride BPCRS* and *4-epichlortetracycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*. Solution (4) contains 0.002% w/v of *tetracycline hydrochloride BPCRS* and 0.0015% w/v of *4-epichlortetracycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 1.5.

Calculate the content of $C_{22}H_{23}ClN_2O_8$,HCl in the ointment using the declared content of $C_{22}H_{23}ClN_2O_8$,HCl in *chlortetracycline hydrochloride BPCRS*.

STORAGE

Chlortetracycline Ointment should be protected from light.

Choline Salicylate Ear Drops

General Notices

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Choline Salicylate Ear Drops are a solution of choline salicylate in Propylene Glycol. They are prepared by diluting Choline Salicylate Solution.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of choline salicylate, $C_{12}H_{19}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Heat 2 ml with *sodium hydroxide* until fumes are evolved. Triethylamine vapour is evolved, which turns moist *red litmus paper* blue.

B. Dilute a quantity containing 2 g of choline salicylate to 20 ml with *water*. The resulting solution yields the reactions characteristic *of salicylates*, Appendix VI.

TESTS

ASSAY

To a quantity containing 0.4 g of choline salicylate add 50 ml of *1,4-dioxan* and 5 ml of *acetic anhydride* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 ml of *methyl orange-xylene cyanol FF solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 24.13 mg of $C_{12}H_{19}NO_4$.

LABELLING

The strength is stated as the percentage w/v of choline salicylate.

Choline Salicylate Oromucosal Gel

General Notices

Choline Salicylate Dental Gel

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Choline Salicylate Oromucosal Gel is a solution of choline salicylate in a suitable water-miscible basis. It is prepared from Choline Salicylate Solution.

The oromucosal gel complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of choline salicylate, $C_{12}H_{19}NO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity containing 0.1 g of choline salicylate with 9 ml of *water* (solution A). To 1 ml of solution A add 1 ml of *ammonium reineckate solution*, filter and wash the precipitate produced. The precipitate is pink.

B. 1 ml of solution A yields reaction A characteristic *of salicylates*, Appendix VI.

C. Mix a quantity containing 0.2 g of choline salicylate with 15 ml of *water*, heat on a water bath for 15 minutes and filter. The filtrate yields reaction B characteristic *of salicylates*, Appendix VI.

TESTS

ASSAY

To a quantity containing 0.5 g of choline salicylate in a conical flask add 25 ml of *1,4-dioxan* and 5 ml of *acetic anhydride*. Shake well until the gel has completely dispersed and wash the inside wall of the flask with 25 ml of *1,4-dioxan*. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 ml of *methyl orange-xylene cyanol FF solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 24.13 mg of $C_{12}H_{19}NO_4$.

LABELLING

The quantity of active ingredient is stated as the amount of choline salicylate.

Choline Theophyllinate Tablets

General Notices

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

DEFINITION

Choline Theophyllinate Tablets contain Choline Theophyllinate. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of choline theophyllinate, $C_{12}H_{21}N_5O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Choline Theophyllinate with 20 ml of *absolute ethanol* for 10 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of choline theophyllinate *(RS 060)*.

B. The *light absorption* of the final solution obtained in the Assay, Appendix II B, exhibits a maximum at 275 nm.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* HF_{254} as the coating substance and a mixture of 95 volumes of *chloroform* and 5 volumes of *ethanol (96%)* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Choline Theophyllinate with 10 ml of *ethanol (96%)* for 10 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Choline Theophyllinate add 500 ml of *water* shake vigorously for 2 minutes, dilute to 1000 ml with *water*, mix and filter. To 10 ml of the filtrate add 10 ml of 0.1M *sodium hydroxide* and dilute to 100 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{12}H_{21}N_5O_3$ taking 415 as the value of A(1%, 1 cm) at the maximum at 275 nm.

STORAGE

Choline Theophyllinate Tablets should be protected from light.

Chorionic Gonadotrophin Injection

General Notices

Action and use

Gonadotrophic hormone.

DEFINITION

Chorionic Gonadotrophin Injection is a sterile solution of Chorionic Gonadotrophin in Water for Injections. It is prepared by dissolving Chorionic Gonadotrophin for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Chorionic Gonadotrophin Injection should be used immediately after preparation.

CHORIONIC GONADOTROPHIN FOR INJECTION

DEFINITION

Chorionic Gonadotrophin for Injection is a sterile material consisting of Chorionic Gonadotrophin with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

CHARACTERISTICS

A white or almost white, amorphous powder.

IDENTIFICATION

Cause an increase in the weight of the seminal vesicles or the prostate glands of immature male rats when administered as directed under the Assay.

TESTS

Acidity or alkalinity

pH of a 1% w/v solution, 6.0 to 8.0, Appendix V L.

Clarity and colour of solution

A 1.0% w/v solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 500 IU of chorionic gonadotrophin per ml (solution A). The endotoxin limit concentration of solution A is 15 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the Assay described under Chorionic Gonadotrophin. The estimated potency is not less than 80% and not more than 125% of the stated potency. The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE

The sealed container should be protected from light and stored at a temperature not exceeding 20°. Under these conditions, the contents may be expected to retain their potency for not less than 3 years.

LABELLING

The label of the sealed container states the number of IU (Units) contained in it.

Cimetidine Injection

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Cimetidine Injection is a sterile solution in Water for Injections of cimetidine hydrochloride, prepared by the interaction of Cimetidine and Hydrochloric Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of cimetidine, $C_{10}H_{16}N_6S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 310 nm of solution A used in the Assay exhibits a maximum at about 218 nm.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (8).

TESTS

Acidity or alkalinity

pH, 4.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and using the following solutions. For solution (1) dilute a quantity of the injection with sufficient *methanol* to give a solution containing the equivalent of 5.0% w/v of Cimetidine. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 20 volumes of the resulting solution to 100 volumes with *methanol*. For solution (4) dilute 5 volumes of solution (3) to 10 volumes with *methanol*. For solution (5) dilute 5 volumes of solution (4) to 10 volumes with *methanol*. For solution (6) dissolve 2.24 mg of *2-carbamoyl-1-methyl-3-[2-(5-methylimidazol-4-ylmethylthio)ethyl]guanidine dihydrochloride BPCRS* ('amide' impurity) in 5 ml of a 1.65% w/v solution of *sodium chloride* in *methanol (50%)*. For solution (7) dissolve 6.6 mg of *1-methyl-3-[2-(5-methylimidazol-4-yl-methylthio)ethyl]guanidine dihydrochloride BPCRS* ('guanidine' impurity) in 5 ml of a 1.65% w/v solution of *sodium chloride* in *methanol (50%)*. For solution (8) dissolve 5.0 mg of *cimetidine BPCRS* in 1 ml of *methanol*. Carry out the following tests.

A. Apply separately to the plate 4 µl of each solution. Allow the plate to stand for 15 minutes in the tank saturated with vapour from the mobile phase which consists of a mixture of 15 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 65 volumes of *ethyl acetate*. After development and removal of the plate, dry it in a current of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under *ultraviolet light (254 nm)*.

B. Apply separately to the plate 4 µl of each solution. Develop using a mixture of 8 volumes of 13.5M *ammonia*, 8 volumes of *methanol* and 84 volumes of *ethyl acetate*. After removal of the plate, dry it in a current of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under *ultraviolet light (254 nm)*.

The following limits apply to both methods. Any spot in the chromatogram obtained with solution (1) corresponding to the 'amide' impurity is not more intense than the principal spot in the chromatogram obtained with solution (6) (0.7%, calculated as the 'amide' base and with reference to cimetidine) and any spot corresponding to the 'guanidine' impurity is not more intense than the principal spot in the chromatogram obtained with solution (7) (2%, calculated as the 'guanidine' base and with reference to cimetidine). Any other *secondary spot* is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.2%) and not more than two such spots are more intense than the principal spot in the chromatogram obtained with solution (4) (0.1% each). The tests are not valid unless the chromatograms obtained with solution (5) show clearly visible spots.

ASSAY

Dilute a volume containing the equivalent of 0.5 g of Cimetidine with sufficient 0.05M *sulphuric acid* to produce a solution containing 0.001% w/v (solution A). Prepare a 0.001% w/v solution of *cimetidine BPCRS* in 0.05M *sulphuric acid* (solution B). Measure the *absorbance* of solutions A and B at the maximum at 218 nm and at 260 nm, Appendix II B. Calculate the content of $C_{10}H_{16}N_6S$ using the difference between the absorbances of solutions A and B at the two wavelengths and the declared content of $C_{10}H_{16}N_6S$ in *cimetidine BPCRS*.

LABELLING

The strength is stated in terms of the equivalent amount of Cimetidine in a suitable dose-volume.

Cimetidine Oral Solution

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Cimetidine Oral Solution is a solution containing Cimetidine in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cimetidine, $C_{10}H_{16}N_6S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 15 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 65 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a volume of the preparation with *methanol* to contain 0.04% w/v of Cimetidine. Solution (2) contains 0.04% w/v of *cimetidine BPCRS* in *methanol*. After removal of the plate, allow it to dry and expose it to iodine vapour until maximum contrast between the spots is obtained. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the preparation with the mobile phase to contain 0.050% w/v of Cimetidine. Solutions (2) to (6) are solutions in the mobile phase containing (2) 0.0020% w/v of *1-methyl-3-(2-(5-methylimidazol-4-yl-methylthio)ethyl)guanidine dihydrochloride BPCRS* ('guanidine' impurity), (3) 0.0019% w/v of *2-carbamoyl-1-methyl-3-(2-(5-methylimidazol-4-ylmethylthio)ethyl)guanidine dihydrochloride BPCRS* ('amide' impurity), (4) 0.0015% w/v of *2-cyano-1-methyl-3-(2-(5-methylimidazol-4-yl-methylsulphinyl)ethyl)guanidine BPCRS* ('sulphoxide' impurity) and (5) 0.00025% w/v each of 'guanidine', 'amide' and 'sulphoxide reference substances and 0.0002% w/v of *saccharin*. For solution (6) dilute 1 volume of solution (1) to 200 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with particles of silica (10 µm) *phenyl silica gel for chromatography* (µBondapak phenyl is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 4 volumes of *acetonitrile* and 96 volumes of a 0.25% v/v solution of *orthophosphoric acid*, the pH of the mixture being adjusted to 3.0 using 10M *potassium hydroxide* and (c) a detection wavelength of 228 nm. For solution (1) allow the chromatography to proceed for 3 times the retention time of the peak due to cimetidine.

The test is not valid unless the chromatogram obtained with solution (5) shows four clearly separated peaks.

The areas of any peaks in the chromatogram obtained with solution (1) corresponding to 'guanidine' and 'amide' are not greater than the areas of the principal peaks in the chromatograms obtained with solutions (2) and (3) respectively (3% each, calculated as the bases and with reference to cimetidine). The areas of any other *secondary peaks* in the chromatogram obtained with solution (1), except that of any peak corresponding to saccharin, are not greater than the area of the principal peak in the chromatogram obtained with solution (6) (0.5%). Calculate the content of the individual named impurities using the respective reference solutions and the content of unnamed impurities using solution (6). The total content of impurities is not greater than 4.0%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a weighed quantity of the preparation containing 0.2 g of Cimetidine with sufficient of the mobile phase to produce 200 ml. Dilute 5 ml of this solution to 100 ml with the mobile phase. Solution (2) contains 0.005% w/v of *cimetidine BPCRS* in the mobile phase. Solution (3) contains 0.004% w/v of *saccharin* in solution (2).

The chromatographic procedure described under Related substances may be used.

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal peaks.

Determine the *weight per ml* of the preparation, Appendix V G, and calculate the content of $C_{10}H_{16}N_6S$ using the declared content of $C_{10}H_{16}N_6S$ in *cimetidine BPCRS*.

STORAGE

Cimetidine Oral Solution should be protected from light.

Cimetidine Oral Suspension

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Cimetidine Oral Suspension is a suspension containing Cimetidine in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of cimetidine, $C_{10}H_{16}N_6S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

Complies with the tests described under Cimetidine Oral Solution. For test A prepare solution (1) in the following manner. Shake a volume of the oral suspension containing 40 mg of Cimetidine with 50 ml of *methanol*, dilute to 100 ml with *methanol*, mix and filter.

TESTS

Alkalinity

pH, 7.0 to 8.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the conditions and solutions (2) to (6) described under Cimetidine Oral Solution. For solution (1) use the filtrate obtained in the assay.

The area of any peak in the chromatogram obtained with solution (1) corresponding to the 'sulphoxide' impurity is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (3%). The areas of any other *secondary peaks* in the chromatogram obtained with solution (1), except that of the peak due to saccharin, are not greater than the area of the principal peak in the chromatogram obtained with solution (6) (0.5%). Calculate the content of the individual named impurities using the respective reference solutions and the content of unnamed impurities using solution (6). The total content of impurities is not greater than 4.0%.

ASSAY

Carry out the Assay described under Cimetidine Oral Solution preparing solution (1) in the following manner. To a weighed quantity of the oral suspension containing 0.1 g of Cimetidine add 100 ml of the mobile phase and shake for 10 minutes. Dilute to 200 ml with the mobile phase, mix and filter through a Whatman GF/F filter paper. Dilute 5 ml of the filtrate to 50 ml with the mobile phase.

STORAGE

Cimetidine Oral Suspension should be protected from light.

Cimetidine Tablets

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Cimetidine Tablets contain Cimetidine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cimetidine, $C_{10}H_{16}N_6S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.10 g of Cimetidine with 10 ml of *methanol* for 10 minutes, filter (Whatman GF/A is suitable) and evaporate to dryness on a rotary evaporator using gentle heat. Dissolve the residue in 5 ml of *chloroform* and evaporate to dryness with the aid of a current of air. Dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cimetidine *(RS 061)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (6).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and using the following solutions. For solution (1) add 20 ml of *methanol* to a quantity of the powdered tablets containing 1 g of Cimetidine, mix with the aid of ultrasound for 2 minutes, shake for 3 minutes and filter using a suitable 0.2-µm filter. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) to 20 volumes with *methanol*. For solution (4) dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 20 volumes of this solution to 100 volumes with *methanol*. For solution (5) dilute 5 volumes of solution (4) to 10 volumes with *methanol*. Solution (6) contains 0.50% w/v of *cimetidine BPCRS* in *methanol*. Carry out the following tests.

A. Apply separately to the plate 4 µl of each solution. Allow the plate to stand for 15 minutes in the tank saturated with vapour from the mobile phase which consists of a mixture of 15 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 65 volumes of *ethyl acetate*. After development and removal of the plate, dry it in a current of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under *ultraviolet light (254 nm)*.

B. Apply separately to the plate 4 µl of each solution. Develop using a mixture of 8 volumes of 13.5M *ammonia*, 8 volumes of *methanol* and 84 volumes of *ethyl acetate*. After removal of the plate, dry it in a current of cold air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under *ultraviolet light (254 nm)*.

The following limits apply to both methods. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.5%) and not more than two such spots are more intense than the principal spot in the chromatogram obtained with solution (4) (0.2% of each). The tests are not valid unless the chromatograms obtained with solution (5) show clearly visible spots.

ASSAY

Weigh and finely powder 20 tablets. Shake a quantity of the powdered tablets containing 0.1 g of Cimetidine with 300 ml of 0.05M *sulphuric acid* for 20 minutes, add sufficient 0.05M *sulphuric acid* to produce 500 ml and filter (Whatman GF/C is suitable). Dilute 5 ml of the filtrate to 100 ml with 0.05M *sulphuric acid* (solution A). Prepare a 0.001% w/v solution of *cimetidine BPCRS* in 0.05M *sulphuric acid* (solution B). Measure the *absorbance* of solutions A and B at the maximum at 218 nm and at 260 nm, Appendix II B. Calculate the content of $C_{10}H_{16}N_6S$ using the difference between the absorbances of solutions A and B at the two wavelengths and the declared content of $C_{10}H_{16}N_6S$ in *cimetidine BPCRS*.

Ciprofloxacin Intravenous Infusion

General Notices

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Ciprofloxacin Intravenous Infusion is a sterile solution, in Glucose Intravenous Infusion or in Sodium Chloride Intravenous Infusion, of ciprofloxacin lactate prepared by the interaction of Ciprofloxacin and Lactic Acid.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} high-performance precoated plate (Merck silica gel 60 F_{254} HPTLC plates are suitable) and a mixture of 10 volumes of *acetonitrile*, 20 volumes of 13.5M *ammonia*, 40 volumes of *dichloromethane* and 40 volumes of *methanol* as the mobile phase. Apply separately to the plate, as bands, 10 µl of each of the following solutions. For solution (1) dilute a quantity of the intravenous infusion with sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin. Solution (2) contains 0.058% w/v of *ciprofloxacin hydrochloride EPCRS* in *water*. For solution (3) mix 1 volume of solution (1) and 1 volume of solution (2). After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm* and *365 nm)*. The principal band in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) . The principal band in the chromatogram obtained with solution (3) appears as a single, compact band.

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the preparation being examined diluted, if necessary, with *water* to produce a solution containing the equivalent of 0.2% w/v of ciprofloxacin. Solution (2) contains 0.07% w/v of *lithium lactate BPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 7.8 mm) packed with a strong cation-exchange resin of sulphonated, cross-linked *styrene-divinylbenzene copolymer* in the hydrogen form (7 to 11 µm) (Aminex HPX-87 H is suitable), (b) as the mobile phase with a flow rate of about 0.6 ml per minute a degassed mixture of 15 volumes of *acetonitrile* and 85 volumes of 0.0025M *sulphuric acid* and (c) a detection wavelength of 208 nm. Maintain the temperature of the column at 40°. After each analysis the column should be rinsed with a mixture of 0.005M *sulphuric acid* and *acetonitrile* and then regenerated with 0.025M *sulphuric acid* .

The chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

For infusions prepared in Glucose Intravenous Infusion pH, 3.5 to 4.6, Appendix V L.

For infusions prepared in Sodium Chloride Intravenous Infusion pH, 3.9 to 4.5, Appendix V L.

Colour of solution

The intravenous infusion is not more intensely coloured than *reference solution* GY_6, Appendix IV B, Method II.

5-Hydroxymethylfurfural

Infusions prepared in Glucose Intravenous Infusion comply with the following test. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 1 volume of the intravenous infusion to 4 volumes with the mobile phase (contains 1.25% w/v of glucose). Solution (2) contains 0.000625% w/v of *5-hydroxymethylfurfural* in the mobile phase.

The chromatographic conditions described under Assay may be used.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 5-hydroxymethylfurfural is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.05%, calculated with reference to the glucose content).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the intravenous infusion with sufficient of the mobile phase to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin. Solution (2) contains 0.05% w/v of *ciprofloxacin hydrochloride for peak identification EPCRS* in the mobile phase. For solution (3) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 2 volumes with the mobile phase. For solution (4) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 5 volumes with the mobile phase. For solution (1) allow the chromatography to proceed for twice the retention time of ciprofloxacin.

The chromatographic conditions described under Assay may be used.

When the chromatograms are recorded under the prescribed conditions the retention time of ciprofloxacin is about 9 minutes. Retention times relative to ciprofloxacin are: impurity E, about 0.4; impurity F, about 0.5; impurity B, about 0.6; impurity C, about 0.7; impurity D, about 1.2.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to ciprofloxacin impurity B and ciprofloxacin impurity C is at least 1.3.

Identify any peaks in the chromatogram obtained with solution (1) corresponding to ciprofloxacin impurities B, C, D and E using solution (2) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4 and 6.7 respectively.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (ciprofloxacin impurity C) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%) and the total area of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

Bacterial endotoxins

The endotoxin limit concentration is 0.5 IU per ml, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the intravenous infusion with sufficient of the mobile phase to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin. Solution (2) contains 0.058% w/v of *ciprofloxacin hydrochloride EPCRS* in the mobile phase. Inject 5 µl of each solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 120-C18 and LiChrospher 100 RP 18 are suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 13 volumes of *acetonitrile* and 87 volumes of a 0.245% w/v solution of *orthophosphoric acid* the pH of which has been adjusted to 3.0 with *triethylamine* and (c) a detection wavelength of 278 nm. Maintain the temperature of the column at 40°.

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the intravenous infusion using the declared content of $C_{17}H_{18}FN_3O_3$,HCl in *ciprofloxacin hydrochloride EPCRS*. Each mg of $C_{17}H_{18}FN_3O_3$,HCl is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

STORAGE

Ciprofloxacin Intravenous Infusion should be protected from light. It should not be refrigerated.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ciprofloxacin.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity C listed under Ciprofloxacin.

Ciprofloxacin Tablets

General Notices

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Ciprofloxacin Tablets contain Ciprofloxacin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} high-performance precoated plate (Merck silica gel 60 F_{254} HPTLC plates are suitable) and a mixture of 10 volumes of *acetonitrile*, 20 volumes of 13.5M *ammonia*, 40 volumes of *dichloromethane* and 40 volumes of *methanol* as the mobile phase. Apply separately to the plate, as bands, 10 µl of each of the following solutions. For solution (1) use solution (1) described under Assay. Solution (2) contains 0.058% w/v of *ciprofloxacin hydrochloride EPCRS* in *water*. For solution (3) mix 1 volume of solution (1) and 1 volume of solution (2). After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm* and *365 nm)*. The principal band in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal band in the chromatogram obtained with solution (3) appears as a single, compact band.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. After 30 minutes withdraw a 10 ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with *water,* if necessary, at the maximum at 276 nm, Appendix II B, using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *ciprofloxacin hydrochloride EPCRS* in *water* at the maximum at 276 nm using dissolution medium in the reference cell. Calculate the total content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$, in the medium from the absorbances obtained and from the declared content of $C_{17}H_{18}FN_3O_3$,HCl in *ciprofloxacin hydrochloride EPCRS*. Each mg of $C_{17}H_{18}FN_3O_3$,HCl is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$. The amount of ciprofloxacin released is not less than 80% of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D. For solution (1) use solution (1) as described under Assay. Solution (2) contains 0.05% w/v of *ciprofloxacin hydrochloride for peak identification EPCRS* in the mobile phase. For solution (3) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 2 volumes. For solution (4) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 5 volumes. For solution (1) allow the chromatography to proceed for twice the retention time of ciprofloxacin.

The chromatographic conditions described under Assay may be used.

When the chromatograms are recorded under the prescribed conditions the retention time of ciprofloxacin is about 9 minutes. Retention times relative to ciprofloxacin are: impurity E, about 0.4; impurity F, about 0.5; impurity B, about 0.6; impurity C, about 0.7; impurity D, about 1.2.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to ciprofloxacin impurity B and ciprofloxacin impurity C is at least 1.3.

Identify any peaks in the chromatogram obtained with solution (1) corresponding to ciprofloxacin impurities B, C, D and E using solution (2) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4 and 6.7 respectively.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (ciprofloxacin impurity C) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%) and the total area of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Weigh and powder 20 tablets. For solution (1) add a quantity of the powdered tablets containing the equivalent of 2 g of ciprofloxacin to 750 ml of the mobile phase, mix with the aid of ultrasound for 20 minutes, add sufficient mobile phase to produce 1000 ml and mix. Filter a portion of the resulting suspension (Whatman GF/C filter paper is suitable) and dilute the filtrate with sufficient mobile phase to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin. Solution (2) contains 0.058% w/v of *ciprofloxacin hydrochloride EPCRS* in the mobile phase. Inject 5 µl of each solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 120-C18 and LiChrospher 100 RP 18 are suitable), (b) as the mobile phase at a flow rate of 1.5 ml per minute a mixture of 13 volumes of *acetonitrile* and 87 volumes of a 0.245% w/v solution of *orthophosphoric acid* the pH of which has been adjusted to 3.0 with *triethylamine* and (c) a detection wavelength of 278 nm. Maintain the temperature of the column at 40°.

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the tablets using the declared content of $C_{17}H_{18}FN_3O_3$,HCl in *ciprofloxacin hydrochloride EPCRS*. Each mg of $C_{17}H_{18}FN_3O_3$,HCl is equivalent to 0.9010 mg of $C_{17}H_{18}FN_3O_3$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ciprofloxacin.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity C listed under Ciprofloxacin Hydrochloride.

Cisplatin Injection

General Notices

Action and use

Platinum-containing cytotoxic.

DEFINITION

Cisplatin Injection is a sterile solution of Cisplatin. It is either supplied as a ready-to-use solution or it is prepared by dissolving Cisplatin for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use solution, the injection complies with the following requirements.

CHARACTERISTICS

A clear, colourless to pale yellow solution.

Content of cisplatin, $Cl_2H_6N_2Pt$

90.0 to 105.0% of the stated amount.

With the exception of identification test A, carry out the following procedures protected from light.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a solution diluted, if necessary, to contain 0.1% w/v of Cisplatin exhibits a maximum at 300 nm.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L.

Trichloroammineplatinate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light.

(1) Dissolve sufficient *potassium trichloroammineplatinate BPCRS* in a 0.9% w/v solution of *sodium chloride* to produce a solution containing 0.0015% w/v of trichloroammineplatinate.

(2) Use the injection diluted, if necessary, with a 0.9% w/v solution of *sodium chloride* to produce a solution containing 0.05% w/v of Cisplatin.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with silica gel with a chemically bonded, strongly basic quaternary ammonium anion-exchange coating (10 µm) (Spherisorb SAX is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 209 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.04% w/v solution of *ammonium sulphate* adjusted, if necessary, to a pH of 5.8 to 6.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the *resolution factor* between the peaks corresponding to sodium chloride and trichloroammineplatinate is at least 2.0.

LIMITS

In the chromatogram obtained with solution (2):

— the area of any peak corresponding to trichloroammineplatinate is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (3.0%).

Transplatin

Not more than 2.0% when determined by the method for *liquid chromatography*, Appendix III D, using the following solutions. Where specified, use as the solvent a 0.9% w/v solution of *sodium chloride* (saline solution).

(1) Add 10 ml of a 0.005% w/v solution of *transplatin BPCRS* in saline solution to 25 mg of *cisplatin EPCRS*, dilute to 25 ml with saline solution, shake for 30 minutes to effect dissolution and add sufficient saline solution to produce 50 ml (solution A). Mix 5 ml of a freshly prepared 0.5% w/v solution of *thiourea*, 5 ml of 1M *hydrochloric acid* and 10 ml of solution A, heat an aliquot in a reaction vial at 60° for 1 hour and cool.

(2) Prepare in the same manner as solution (1) but using 10 ml of the injection diluted, if necessary with saline solution to produce a solution containing 0.05% w/v of Cisplatin in place of the 10 ml of solution A.

(3) Prepare in the same manner as solution (1) but using a mixture of 10 ml of a solution containing 0.005% w/v of *cisplatin EPCRS* in saline solution and 10 ml of a 0.005% w/v solution of *transplatin BPCRS* in saline solution in place of 10 ml of solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with silica gel with a chemically bonded, strongly acidic cation-exchange coating (10 µm) (Maxsil SCX is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use a column temperature of 45°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µl of each solution.

(g) Condition the column by pumping the mobile phase at a flow rate of 2 ml per minute for 30 minutes, at 0.5 ml per minute for 30 minutes and then again at 2 ml per minute for 30 minutes.

(h) When the chromatogram obtained with solution (3) is recorded under the prescribed conditions, the retention time of the derivatised transplatin is 5 to 9 minutes; if necessary adjust the composition of the mobile phase and re-condition the column.

MOBILE PHASE

2.5% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.2 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless:

the *column efficiency*, determined on the peak due to transplatin in the chromatogram obtained with solution (1), is greater than 2,500 theoretical plates per metre and unless, in the chromatogram obtained with solution (3);

the *resolution factor* between the peaks corresponding to cisplatin and transplatin is at least 1.7.

LIMITS

In the chromatogram obtained with solution (2):

— the area of any peak corresponding to the derivative of transplatin is not greater than the area of the corresponding peak in the chromatogram obtained with solution (1) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.1% w/v of *cisplatin EPCRS* in a 0.9% w/v solution of *sodium chloride*.

(2) Use the injection being examined diluted, if necessary, with a 0.9% w/v solution of *sodium chloride* to produce a solution containing 0.1% w/v of Cisplatin.

(3) 0.05% w/v of *cisplatin EPCRS* and 0.005% w/v of *transplatin BPCRS* in a 0.9% w/v solution of *sodium chloride*; shake for 30 minutes to effect dissolution of the transplatin.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemically bonded amine groups (10 µm) (Lichrosorb NH_2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µl of each solution.

(g) Ignore any peak with a long retention time.

MOBILE PHASE

10 volumes of *water* and 90 volumes of *acetonitrile.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to transplatin and cisplatin is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $Cl_2H_6N_2Pt$ in the injection using the declared content of $Cl_2H_6N_2Pt$ in *cisplatin EPCRS*.

STORAGE

When supplied as a ready-to-use solution, Cisplatin Injection should be protected from light. It should not be refrigerated.

CISPLATIN FOR INJECTION

DEFINITION

Cisplatin for Injection is a sterile material consisting of Cisplatin with Mannitol and Sodium Chloride. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of cisplatin, $Cl_2H_6N_2Pt$

95.0 to 105.0% of the stated amount.

With the exception of identification test A, carry out the procedures protected from light.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a solution containing 0.1% w/v of Cisplatin in 0.1M *hydrochloric acid* exhibits a maximum only at 300 nm.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Acidity

pH of a solution containing 0.1% w/v of Cisplatin, 3.5 to 6.5, Appendix V L.

Trichloroammineplatinate

Complies with the test described for the ready-to-use solution above but preparing solution (2) in the following manner.

(2) Dissolve the contents of a sealed container in sufficient of a 0.9% w/v solution of *sodium chloride* to produce a solution containing 0.05% w/v of Cisplatin.

Transplatin

Complies with the test described for the ready-to-use solution above.

(2) Prepare in the same manner as solution (1) but using 10 ml of a solution prepared by shaking the contents of a sealed container with sufficient saline solution for 30 minutes to produce a solution containing 0.1% w/v of Cisplatin in place of 10 ml of the solution of *transplatin BPCRS* and *cisplatin EPCRS.*

ASSAY

Carry out the method described for the ready-to-use formulation above. For solution (2) dissolve the contents of a sealed container in sufficient of a 0.9% w/v solution of *sodium chloride* solution to produce a solution containing 0.1% w/v of Cisplatin.

Calculate the content of $Cl_2H_6N_2Pt$ in the sealed container using the declared content of $Cl_2H_6N_2Pt$ in *cisplatin EPCRS*. Repeat the procedure with a further nine sealed containers. Calculate the average content of $Cl_2H_6N_2Pt$ per container from the 10 individual results thus obtained.

STORAGE

The sealed container should be protected from light. It should not be refrigerated.

Clarithromycin Tablets

General Notices

Action and use

Macrolide antibacterial.

DEFINITION

Clarithromycin Tablets contain Clarithromycin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clarithromycin, $C_{38}H_{69}NO_{13}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Clarithromycin with 10 ml of *water* and extract with 20 ml of *dichloromethane*. Separate the lower dichloromethane layer and centrifuge. Filter the supernatant (Whatman GF/C is suitable) and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after drying under vacuum at room temperature for 2 hours, is concordant with the *reference spectrum* of clarithromycin *(RS 424)*.

TESTS

Dissolution

Carry out the *dissolution test for tablets and capsules*, Appendix XII BI.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 ml of a solution containing 1000 volumes of a 1.361% w/v solution of *sodium acetate* and 350 volumes of 0.1M *acetic acid*, adjusted to pH 5.0 with 0.1M *acetic acid*, at a temperature of 37º ± 0.5º, as the medium.

PROCEDURE

After 45 minutes, withdraw a sample of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the filtered dissolution medium, diluted with mobile phase if necessary, to produce a solution expected to contain 0.011% w/v of Clarithromycin.

(2) 0.011% w/v of *clarithromycin BPCRS* in the mobile phase.

(3) 0.011% w/v of each of *clarithromycin BPCRS* and *clarithromycin impurity E BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Superspher ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µl of each solution.

MOBILE PHASE

35 volumes of 0.067M *potassium dihydrogen orthophosphate* and 65 volumes of *methanol* adjusted to pH 4.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the approximate retention times for clarithromycin and clarithromycin impurity E are 4 and 6 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the total content of clarithromycin, $C_{38}H_{69}NO_{13}$, in the medium using the declared content of $C_{38}H_{69}NO_{13}$ in *clarithromycin BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of powdered tablets containing 75 mg of Clarithromycin in 40 ml of a mixture of equal volumes of *acetonitrile R1* and *water* (solution A), mix with the aid of ultrasound, add sufficient solution A to produce 50 ml and filter through a Whatman GF/C filter and then through a 0.45-µm PTFE filter.

(2) Dilute 5 volumes of solution (1) to 100 volumes with solution A.

(3) Dilute 10 volumes of solution (2) to 100 volumes with solution A.

(4) 0.15% w/v of *clarithromycin for peak identification EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Kingsorb C18 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1.1 ml per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

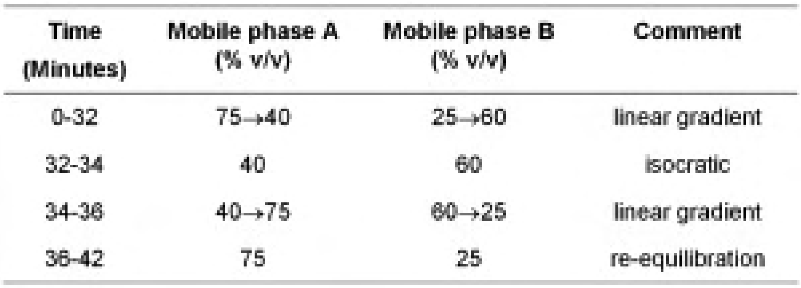
(f) Inject 10 µl of each solution.

MOBILE PHASE

Mobile phase A A 0.476% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 4.4 with either 2M *orthophosphoric acid* or a 4.5% w/v solution of *potassium hydroxide*, filtered through a C18 filtration kit (3M Empore is suitable).

Mobile phase B acetonitrile R1.

Use the following gradient.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-32	75→40	25→60	linear gradient
32-34	40	60	isocratic
34-36	40→75	60→25	linear gradient
36-42	75	25	re-equilibration

When the chromatograms are recorded using the prescribed conditions the retention times relative to clarithromycin (retention time = about 11 minutes) are: impurity I = about 0.38; impurity A = about 0.42; impurity J = about 0.63; impurity L = about 0.74; impurity B = about 0.79; impurity M = about 0.81; impurity C = about 0.89; impurity D = about 0.96; impurity N = about 1.15; impurity E = about 1.27; impurity F = about 1.33; impurity P = about 1.35; impurity O = about 1.41; impurity K = about 1.59; impurity G = about 1.72; impurity H = about 1.82.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2) the *symmetry factor* of the peak due to clarithromycin is less than 1.75;

in the chromatogram obtained with solution (4) the *peak to valley ratio* is at least 3.0 where H_p is the height above the baseline of the peak due to impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to clarithromycin;

the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *clarithromycin for peak identification EPCRS*.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities G and H using solution (4) and multiply the areas of these peaks by the corresponding correction factors; impurity G, 0.27; impurity H, 0.15.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (1%) and not more than four such peaks have an area greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of all the *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (3) (3.5%).

Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). Disregard any peaks eluting before impurity I and after impurity H.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely powder a quantity of tablets containing 2 g of Clarithromycin and quantitatively transfer the powder to a volumetric flask using about 350 ml of *methanol*. Mix with the aid of ultrasound for 15 minutes, shake vigorously for 15 minutes, allow to cool, add sufficient *methanol* to produce 500 ml and mix. Filter the solution (Whatman GF/C paper is suitable), dilute 1 volume of the filtrate to 40 volumes with mobile phase and filter through a 0.45-µm filter.

(2) 0.01% w/v of *clarithromycin BPCRS* in the mobile phase.

(3) 0.01% w/v of each of *clarithromycin BPCRS* and *clarithromycin impurity E BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Dissolution may be used.

When the chromatograms are recorded under the prescribed conditions the approximate retention times for clarithromycin and clarithromycin impurity E are 4 and 6 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{38}H_{69}NO_{13}$ in the tablets using the declared content of $C_{38}H_{69}NO_{13}$ in *clarithromycin BPCRS*.

Clemastine Oral Solution

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Clemastine Oral Solution contains Clemastine Fumarate in a suitable vehicle.
The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of clemastine, $C_{21}H_{26}ClNO$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

1-(4-Chlorophenyl)-1-phenylethanol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the oral solution containing the equivalent of 0.5 mg of clemastine to 25 ml with a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*. Solution (2) contains 0.00008% w/v of *1-(4-chlorophenyl)-1-phenylethanol EPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.* Solution (3) contains 0.000335% w/v of *clemastine fumarate BPCRS* and 0.00008% w/v of *1-(4-chlorophenyl)-1-phenylethanol EPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*.

The chromatographic procedure described under Assay may be used. Inject 100 µl of each solution.

The test is not valid unless the *resolution factor* between the peaks due to clemastine fumarate and 1-(4-chlorophenyl)-1-phenylethanol in the chromatogram obtained with solution (3) is at least 2.2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 1-(4-chlorophenyl)-1-phenylethanol is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%, calculated with reference to clemastine fumarate).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck plates are suitable) and a mixture of 1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *stabiliser-free tetrahydrofuran* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 20 ml of *water*, 20 ml of a saturated solution of *sodium chloride* and 2 ml of 13.5M *ammonia* to a quantity of the oral solution containing the equivalent of 8 mg of clemastine, extract with four 40 ml quantities of *dichloromethane*, washing each extract with the same 40 ml of *water*, filter the dichloromethane extracts and evaporate to dryness at a temperature of 30° to 40° under reduced pressure. Dissolve the residue in 50 ml of *methanol*, evaporate to dryness under the same conditions and dissolve the residue in 4 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. Solution (3) contains 0.027% w/v of *clemastine fumarate BPCRS* in *methanol*. Solution (4) contains 0.00135% w/v of *clemastine fumarate BPCRS* in *methanol*. Solution (5) contains 0.0135% w/v of each of *clemastine fumarate BPCRS* and *diphenhydramine hydrochloride EPCRS* in *methanol*. Solution (6) contains 0.0054% w/v of *2-(2-hydroxyethyl)-1-methylpyrrolidine BPCRS* in *methanol*. After removal of the plate, dry it in a current of cold air for 5 minutes, spray with a freshly prepared mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of 2M *acetic acid* and then with *hydrogen peroxide solution (10 vol)*. Cover the plate immediately with a glass plate of the same size and examine the chromatograms after 2 minutes. In the chromatogram obtained with solution (1) any spot corresponding to 2-(2-hydroxyethyl)-1-methylpyrrolidine is not more intense than the spot in the chromatogram obtained with solution (6) (2%, with reference to clemastine fumarate) and any orange-brown *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%, with reference to clemastine fumarate). Disregard any spot remaining on the line of application and any spot with an Rf value greater than that of the principal spot. The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated spots.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the oral solution containing the equivalent of 0.5 mg of clemastine to 20 ml with a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate.* Solution (2) contains 0.00335% w/v of *clemastine fumarate BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 0.1 volume of *orthophosphoric acid*, 45 volumes of *acetonitrile* and 55 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 220 nm. Inject 10 µl of each solution.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{21}H_{26}ClNO$, weight in volume, using the declared content of $C_{21}H_{26}ClNO$ in *clemastine fumarate BPCRS*.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of clemastine.

Clemastine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Clemastine Tablets contain Clemastine Fumarate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clemastine, $C_{21}H_{26}ClNO$

93.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

B. In the test for 1-(4-Chlorophenyl)-1-phenylethanol, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (3).

TESTS

1-(4-Chlorophenyl)-1-phenylethanol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add to a quantity of the powdered tablets containing the equivalent of 10 mg of clemastine 200 ml of a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*, shake vigorously for 45 minutes, centrifuge at a speed of at least 400 revolutions per minute for 10 minutes and use the supernatant liquid. Solution (2) contains 0.0000335% w/v of *1-(4-chlorophenyl)-1-phenylethanol EPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*. Solution (3) contains 0.0067% w/v of *clemastine fumarate BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*. Solution (4) contains 0.000335% w/v of *clemastine fumarate BPCRS* and 0.000064% w/v of *1-(4-chlorophenyl)-1-phenylethanol EPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*.

The chromatographic procedure described under Assay may be used. Inject 100 µl of each solution.

The test is not valid unless the *resolution factor* between the peaks due to clemastine fumarate and 1-(4-chlorophenyl)-1-phenylethanol in the chromatogram obtained with solution (4) is at least 2.2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 1-(4-chlorophenyl)-1-phenylethanol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%, calculated with reference to clemastine fumarate).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck plates are suitable) and a mixture of 1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *stabiliser-free tetrahydrofuran* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 8 mg of clemastine with 4 ml of *methanol* for 15 minutes, centrifuge at 4000 revolutions per minute for 10 minutes and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. Solution (3) contains 0.027% w/v of *clemastine fumarate BPCRS* in *methanol*. Solution (4) contains 0.00135% w/v of *clemastine fumarate BPCRS* in *methanol*. Solution (5) contains 0.0135% w/v of each of *clemastine fumarate BPCRS* and *diphenhydramine hydrochloride EPCRS* in *methanol*. Solution (6) contains 0.00135% w/v of *2-(2-hydroxyethyl)-1-methylpyrrolidine BPCRS* in *methanol*. After removal of the plate, dry it in a current of cold air for 5 minutes, spray with a freshly prepared mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of 2M *acetic acid* and then with *hydrogen peroxide solution (10 vol)*. Cover the plate immediately with a glass plate of the same size and examine the chromatograms after 2 minutes. In the chromatogram obtained with solution (1) any spot corresponding to 2-(2-hydroxyethyl)-1-methylpyrrolidine is not more intense than the spot in the chromatogram obtained with solution (6) (0.5%, with reference to clemastine fumarate) and any orange-brown *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%, with reference to clemastine fumarate). Disregard any spot remaining on the line of application and any spot with an Rf value greater than that of the principal spot. The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated spots.

Uniformity of content

Tablets containing the equivalent of less than 2 mg of clemastine comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) vigorously shake one tablet with 40 ml of a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate* for 45 minutes and centrifuge until a clear supernatant liquid is obtained. Solution (2) contains 0.00335% w/v of *clemastine fumarate BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 0.1 volume of *orthophosphoric acid*, 50 volumes of *acetonitrile* and 50 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 220 nm. Inject 10 µl of each solution.

Calculate the content of $C_{21}H_{26}ClNO$ in each tablet using the declared content of $C_{21}H_{26}ClNO$ in *clemastine fumarate BPCRS*.

ASSAY

For tablets containing the equivalent of 2 mg or more of clemastine

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III

D, using the following solutions. For solution (1) add to a quantity of the powdered tablets containing the equivalent of 10 mg of clemastine 200 ml of a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*, shake vigorously for 45 minutes, centrifuge at a speed of at least 400 revolutions per minute for 10 minutes and use the supernatant liquid. Solution (2) contains 0.0067% w/v of *clemastine fumarate BPCRS* in a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 0.1 volume of *orthophosphoric acid*, 45 volumes of *acetonitrile* and 55 volumes of a 1% w/v solution of *ammonium dihydrogen orthophosphate* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 220 nm. Inject 10 µl of each solution.

Calculate the content of $C_{21}H_{26}ClNO$ in the tablets using the declared content of $C_{21}H_{26}ClNO$ in *clemastine fumarate BPCRS*.

For tablets containing the equivalent of less than 2 mg of clemastine

Use the average of the individual results obtained in the test for Uniformity of content.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of clemastine.

Clindamycin Capsules

General Notices

Action and use

Lincosamide antibacterial.

DEFINITION

Clindamycin Capsules contain Clindamycin Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 30 mg of clindamycin with 15 ml of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of clindamycin hydrochloride *(RS 064)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the capsule contents containing the equivalent of 50 mg of clindamycin with 50 ml of the mobile phase for 15 minutes and filter (Whatman GF/C filter is suitable). For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase. Solution (3) contains 0.1% w/v of *clindamycin hydrochloride EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS 5 µm is suitable), (b) as the mobile phase a mixture of 45 volumes of *acetonitrile* and 55 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 7.5 with a 25% w/v solution of *potassium hydroxide* with a flow rate of 1 ml per minute and (c) a detection wavelength of 210 nm. For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak.

When the chromatograms are recorded using the prescribed conditions the retention time of the peak due to clindamycin is about 10 minutes. The chromatogram obtained with solution (3) shows peaks with retention times relative to clindamycin of about 0.4 [Ph Eur impurity A (lincomycin)], about 0.65 [Ph Eur impurity B (clindamycin B)] and about 0.8 [Ph Eur impurity C (7-epiclindamycin)].

In the chromatogram obtained with solution (1) the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%), the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (4%), the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (6%). Disregard any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Water

The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing the equivalent of 50 mg of clindamycin with 50 ml of the mobile phase for 15 minutes and filter (Whatman GF/C filter is suitable). Solution (2) contains 0.11% w/v of *clindamycin hydrochloride EPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the capsules using the declared content of $C_{18}H_{33}ClN_2O_5S$, HCl in *clindamycin hydrochloride EPCRS*. Each mg of $C_{18}H_{33}ClN_2O_5S$, HCl is equivalent to 0.9209 mg of $C_{18}H_{33}ClN_2O_5S$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of clindamycin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Clindamycin Hydrochloride.

Clindamycin Injection

General Notices

Action and use

Lincosamide antibacterial.

DEFINITION

Clindamycin Injection is a sterile solution of Clindamycin Phosphate in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of clindamycin, $C_{18}H_{33}ClN_2O_5S$

90.0 to 105.0% of the stated amount.

CHARACTERISTICS

An almost colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 1.5 volumes of 18M *ammonia*, 30 volumes of *toluene* and 70 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in *methanol* containing (1) a volume of the injection containing the equivalent of 50 mg of clindamycin diluted to 10 ml and (2) 0.5% w/v of *clindamycin phosphate EPCRS*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to clindamycin phosphate in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 5.5 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with the mobile phase to contain the equivalent of 0.3% w/v of clindamycin. Solution (2) contains 0.012% w/v of *lincomycin hydrochloride EPCRS*, 0.024% w/v of *clindamycin phosphate EPCRS* and 0.0015% v/v of *benzyl alcohol* in the mobile phase.

The chromatographic conditions described under Assay may be used. The order of the peaks in the chromatogram obtained with solution (2) is lincomycin hydrochloride, clindamycin phosphate and benzyl alcohol.

The test is not valid unless the *resolution factor* between the peaks due to lincomycin hydrochloride and clindamycin phosphate is at least 7.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than 8.0% by *normalisation*. Disregard any peak due to benzyl alcohol.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection in *water BET* to give a solution containing the equivalent of 10 mg of clindamycin per ml (solution A). The endotoxin limit concentration of solution A is 6 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with the mobile phase to contain the equivalent of 0.015% w/v of clindamycin. Solution (2) contains 0.018% w/v of *clindamycin phosphate EPCRS* in the mobile phase. Solution (3) contains 0.012% w/v of *lincomycin hydrochloride EPCRS*, 0.024% w/v of *clindamycin phosphate EPCRS* and 0.0015% v/v of *benzyl alcohol* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Zorbax C8 is suitable), (b) as the mobile phase a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 2.5 with *orthophosphoric acid* with a flow rate of 1 ml per minute and (c) a detection wavelength of 210 nm. Record the chromatograms for 3 times the retention time of the peak due to clindamycin.

The test is not valid unless the *resolution factor* between the peaks due to lincomycin hydrochloride and clindamycin phosphate is at least 7.7.

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the injection using the declared content of $C_{18}H_{34}ClN_2O_8PS$ in *clindamycin phosphate EPCRS*. Each mg of $C_{18}H_{34}ClN_2O_8PS$ is equivalent to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

STORAGE

Clindamycin Injection should be stored at a temperature of 8° to 30°.

LABELLING

The strength is stated as the equivalent amount of clindamycin in a suitable dose-volume.

Clobazam Capsules

General Notices

Action and use

Benzodiazepine.

DEFINITION

Clobazam Capsules contain Clobazam.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of clobazam, $C_{16}H_{13}ClN_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 20 mg of Clobazam with 10 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. Dissolve the residue in the minimum amount of *methanol*, evaporate to dryness and dry the residue at 105° for 10 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of clobazam *(RS 066)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (5).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of 0.1M *hydrochloric acid* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the filtered dissolution medium as solution (1). For solution (2) dissolve a sufficient quantity of *clobazam BPCRS* in 50 ml of *methanol* and dilute 2 ml of the resulting solution to 50 ml with 0.1M *hydrochloric acid*; the concentration of the final solution should be the same as that expected for solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed *octadecylsilyl silica gel for chromatography* (5 µm) (Superspher 100RP-18 is suitable), (b) a mixture of 470 volumes of *acetonitrile* and 530 volumes of *water* as the mobile phase with a flow rate of 0.7 ml per minute and (c) a detection wavelength of 230 nm.

Inject 50 µl of each solution. Calculate the total content of clobazam, $C_{16}H_{13}ClN_2O_2$, in the medium using the declared content of $C_{16}H_{13}ClN_2O_2$ in *clobazam BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF$_{254}$* as the coating substance and a mixture of 40 volumes of *acetone* and 60 volumes of *hexane* as the mobile phase. Use an unlined tank for the development of the plate. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 40 mg of Clobazam with three 10 ml quantities of *dichloromethane,* combine the filtered extracts and evaporate to dryness; dissolve the residue in 2 ml of *methanol* . For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) to 2.5 volumes with *methanol*. Solution (4) contains 0.010% w/v of *7-chloro-1,5-dihydro-5-phenyl-1,5-benzodiazepine-2,4 (3*H)-*dione BPCRS* in *methanol*. Solution (5) contains 0.010% w/v of *clobazam BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* Any spot corresponding to 7-chloro-1,5-dihydro-5-phenyl-1,5-benzodiazepine-2,4 (3*H*)-dione in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) weigh 20 capsules. Open the capsules without losing any part of the shell and transfer the contents as completely as possible to a 200 ml flask. Wash the shells with three 30 ml quantities of *methanol*, add the washings to the flask and dilute to volume. Allow the shells to dry at room temperature and weigh. The difference between the weights represents the weight of the total contents. Mix the contents of the flask with the aid of ultrasound for 10 minutes and stir magnetically for 20 minutes. Centrifuge a portion of the suspension and dilute a volume of the resulting supernatant liquid containing 5 mg of Clobazam to 100 ml with *methanol*. Solution (2) contains 0.005% w/v of *clobazam BPCRS* in *methanol*. Solution (3) contains 0.006% w/v of *7-chloro-1,5-dihydro-5-phenyl-1,5-benzodiazepine-2,4(3*H)-*dione BPCRS* (desmethylclobazam) and 0.0125% w/v of *clobazam BPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 470 volumes of *acetonitrile* and 530 volumes of *water* as the mobile phase with a flow rate of 0.7 ml per minute and (c) a detection wavelength of 230 nm. Adjust the composition of the mobile phase, if necessary, such that the *resolution factor* between the peaks corresponding to desmethylclobazam and clobazam in the chromatogram obtained with solution (3) is at least 3.0.

Calculate the content of $C_{16}H_{13}ClN_2O_2$ using the declared content of $C_{16}H_{13}ClN_2O_2$ in *clobazam BPCRS*.

Clobetasol Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Clobetasol Cream contains Clobetasol Propionate in a suitable basis.
The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clobetasol propionate, $C_{25}H_{32}ClFO_5$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. Prepare solution (1) in the following manner. Transfer a quantity of the cream containing 0.75 mg of Clobetasol Propionate to a 25-ml centrifuge tube, add 10 ml of *methanol* and heat in a water-bath at 60° for 4 minutes. Remove from the water-bath and shake vigorously. Repeat the heating and shaking, cool to room temperature, add 3.5 ml of *water* and mix. Centrifuge for 10 minutes. Transfer 10 ml of the clear supernatant liquid to a 100-ml separating funnel, add 1 g of *sodium chloride* and 10 ml of *water* and mix. Add 5 ml of *dichloromethane* and shake for 1 minute. Evaporate the dichloromethane layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 0.5 ml of *dichloromethane*. Solution (2) contains 0.05% w/v of *clobetasol propionate BPCRS* in *dichloromethane*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clobetasol propionate in the chromatogram obtained with solution (1).

ASSAY

CAUTION Carry out the preparation of solutions (2) and (3) with full facial protection and wearing heat-resistant gloves.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *clobetasol propionate BPCRS* and 0.01% w/v of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (50%)*. For solution (2) add 10 ml of *absolute ethanol* to a quantity of the cream containing 1 mg of Clobetasol Propionate, stopper firmly using a plastic stopper, heat on a water bath with intermittent shaking until the cream is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 10 ml with water. Prepare solution (3) in the same manner as solution (2) but add 5 ml of a 0.04% w/v solution of *beclometasone dipropionate BPCRS* in *absolute ethanol* and 5 ml of *absolute ethanol*. Solutions (2) and (3) may assume a gel-like appearance.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *stationary phase C* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 45 volumes of *absolute ethanol* and 55 volumes of *water* and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{25}H_{32}ClFO_5$ using peak areas and the declared content of $C_{25}H_{32}ClFO_5$ in *clobetasol propionate BPCRS*.

STORAGE

Clobetasol Cream should be stored at a temperature not exceeding 30°.

Clobetasol Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Clobetasol Ointment contains Clobetasol Propionate in a suitable basis.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clobetasol propionate, $C_{25}H_{32}ClFO_5$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. Prepare solution (1) in the following manner. Transfer a quantity of the ointment containing 0.5 mg of Clobetasol Propionate to a 25-ml centrifuge tube, add 10 ml of *methanol* and heat in a water bath at 70° for 4 minutes. Remove from the water bath and shake vigorously. Repeat the heating and shaking, cool in ice for 5 minutes and centrifuge for 10 minutes. Transfer 5 ml of the clear supernatant liquid to a suitable vial, evaporate to dryness in a current of nitrogen and dissolve the residue in 0.5 ml of *dichloromethane*. Solution (2) contains 0.05% w/v of *clobetasol propionate BPCRS* in *dichloromethane*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clobetasol propionate in the chromatogram obtained with solution (1).

ASSAY

CAUTION Carry out the preparation of solutions (2) and (3) with full facial protection and wearing heat-resistant gloves.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *clobetasol propionate BPCRS* and 0.01% w/v of *beclometasone dipropionate BPCRS* (internal standard) in *ethanol (50%)*. For solution (2) add 10 ml of *absolute ethanol* to a quantity of the ointment containing 1 mg of Clobetasol Propionate, stopper firmly using a plastic stopper, heat on a water-bath with intermittent shaking until the ointment is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 10 ml with *water*. Prepare solution (3) in the same manner as solution (2) but add 5 ml of a 0.04% w/v solution of *beclometasone dipropionate BPCRS* in *absolute ethanol* and 5 ml of *absolute ethanol*. Solutions (2) and (3) may assume a gel-like appearance.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 45 volumes of *absolute ethanol* and 55 volumes of *water* and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{25}H_{32}ClFO_5$ using peak areas and the declared content of $C_{25}H_{32}ClFO_5$ in *clobetasol propionate BPCRS*.

STORAGE

Clobetasol Ointment should be stored at a temperature not exceeding 30°.

Clobetasone Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Clobetasone Cream contains Clobetasone Butyrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clobetasone butyrate, $C_{26}H_{32}ClFO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using *silica gel 60 GF*$_{254}$ as the coating substance and a mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the cream containing 0.5 mg of Clobetasone Butyrate in a mixture of 5 volumes of *ethanol (80%)* and 10 volumes of n-*hexane*, taking 15 ml of the solvent mixture for each g of cream. Shake the mixture, allow to separate, filter the aqueous layer and add 1 ml of *water* for every 10 ml of n-*hexane* used. Cool the solution in ice for 30 minutes, centrifuge, filter the supernatant liquid and dilute with 10 ml of *water* for every 10 ml of n-*hexane* used. Add 1 g of *sodium chloride* for every 10 ml of *water* used and extract with 5 ml of *chloroform* for every 10 ml of *water* used. Evaporate the chloroform layer to dryness in a current of dry nitrogen with gentle heating and dissolve the residue in 0.5 ml of *chloroform*. Solution (2) contains 0.1% w/v of *clobetasone butyrate BPCRS* in *chloroform*. Solution (3) contains a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) . The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clobetasone butyrate in the chromatogram obtained with solution (1).

ASSAY

CAUTION Carry out the preparation of solutions (2) and (3) with full facial protection and wearing heat-resistant gloves.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *clobetasone butyrate BPCRS* and 0.0028% w/v of *clobetasol propionate BPCRS* (internal standard) in *ethanol (50%)*. For solution (2) add 10 ml of *absolute ethanol* to a quantity of the cream containing 1 mg of Clobetasone Butyrate.

Stopper firmly using a plastic stopper and heat on a water-bath with intermittent shaking until the cream is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 10 ml with *water*. Prepare solution (3) in the same manner as solution (2) but adding 5 ml of *absolute ethanol* and 5 ml of a 0.014% w/v solution of the internal standard in *absolute ethanol*. Solutions (2) and (3) may assume a gel-like appearance.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 40 volumes of *absolute ethanol* and 60 volumes of *water* and (c) a detection wavelength of 241 nm. The proportions of the mobile phase may be adjusted to give a retention time for clobetasone butyrate of about 5.5 minutes.

Calculate the content of $C_{26}H_{32}ClFO_5$ in the cream using peak areas and the declared content of $C_{26}H_{32}ClFO_5$ in *clobetasone butyrate* BPCRS.

STORAGE

Clobetasone Cream should be stored at a temperature not exceeding 30°.

Clobetasone Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Clobetasone Ointment contains Clobetasone Butyrate in a suitable basis.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clobetasone butyrate, $C_{26}H_{32}ClFO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel 60 GF*$_{254}$ as the coating substance and a mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the ointment containing 0.5 mg of Clobetasone Butyrate in a mixture of 5 volumes of *ethanol (80%)* and 10 volumes of n-*hexane*, taking 15 ml of the solvent mixture for each g of ointment. Shake the mixture, allow to separate, filter the aqueous layer and add 10 ml of *water* and 1 g of *sodium chloride* for every 15 ml of the solvent mixture used. Extract the resulting aqueous solution with 5 ml of *chloroform* for each g of *sodium chloride* used and evaporate the chloroform layer to dryness in a current of dry nitrogen with gentle heating. Dissolve the residue in 0.5 ml of *chloroform*. Solution (2) contains 0.1% w/v of *clobetasone butyrate BPCRS* in *chloroform*. Solution (3) contains a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to clobetasone butyrate in the chromatogram obtained with solution (1).

ASSAY

CAUTION Carry out the preparation of solutions (2) and (3) with full facial protection and wearing heat-resistant gloves.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *clobetasone butyrate BPCRS* and 0.0028% w/v of *clobetasol propionate BPCRS* (internal standard) in *ethanol (50%)*. For solution (2) add 10 ml of *absolute ethanol* to a quantity of the ointment containing 1 mg of Clobetasone Butyrate. Stopper firmly using a plastic stopper and heat on a water-bath with intermittent shaking until the ointment is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 10 ml with *water*. Prepare solution (3) in the same manner as solution (2) but adding 5 ml of *absolute ethanol* and 5 ml of a 0.014% w/v solution of the internal standard in *absolute ethanol*. Solutions (2) and (3) may assume a gel-like appearance.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 40 volumes of *absolute ethanol* and 60 volumes of *water* and (c) a detection wavelength of 241 nm. The proportions of the mobile phase may be adjusted to give a retention time for clobetasone butyrate of about 5.5 minutes.

Calculate the content of $C_{26}H_{32}ClFO_5$ in the ointment using peak areas and the declared content of $C_{26}H_{32}ClFO_5$ in *clobetasone butyrate BPCRS*.

STORAGE

Clobetasone Ointment should be stored at a temperature not exceeding 30°.

Clofazimine Capsules

General Notices

Action and use

Antileprosy drug.

DEFINITION

Clofazimine Capsules contain Clofazimine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of clofazimine, $C_{27}H_{22}Cl_2N_4$

95.0% to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 260 to 600 nm of the final solution obtained in the Assay, exhibits two maxima, at 289 nm and 491 nm.

B. Dissolve a quantity of the contents of the capsules containing 2 mg of Clofazimine in 3 ml of *acetone* and add 0.1 ml of *hydrochloric acid*; an intense violet colour is produced. Add 0.5 ml of 5M *sodium hydroxide*; the colour changes to orange-red.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the capsules containing 0.5 g of Clofazimine in 100 ml of the mobile phase, filter and dilute 1 volume of this solution to 100 volumes with the mobile phase. Solution (2) contains 0.0000125% w/v of *iminophenazine BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *clofazimine BPCRS* and 0.0005% w/v of *iminophenazine BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 35 volumes of a solution prepared by dissolving 2.25 g of *sodium dodecyl sulphate*, 0.85 g of *tetrabutylammonium hydrogen sulphate* and 0.885 g of *disodium hydrogen orthophosphate* in 500 ml of *water* and adjusting the pH to 3.0 with *orthophosphoric acid* and 65 volumes of *acetonitrile* and (c) a detection wavelength of 280 nm.

Inject 20 µl of solution (3). The test is not valid unless the *resolution factor* between the two principal peaks is at least 2, the retention time of iminophenazine relative to that of clofazimine is about 0.7 and the *column efficiency*, determined on the peak due to clofazimine is at least 3000 *theoretical plates*.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any such peaks is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 100 ml of the mobile phase to a quantity of the mixed contents of 20 capsules containing 0.5 g of Clofazimine, filter and dilute 1 volume of this solution to 100 volumes with the mobile phase. Solution (2) contains 0.005% w/v of *clofazimine BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *clofazimine BPCRS* and 0.0005% w/v of *iminophenazine BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

Inject 20 µl of solution (3). The test is not valid unless the *resolution factor* between the two principal peaks is at least 2, the retention time of iminophenazine relative to that of clofazimine is about 0.7 and the *column efficiency*, determined on the peak due to clofazimine is at least 3000 *theoretical plates*.

Calculate the percentage content of $C_{27}H_{22}Cl_2N_4$ in the capsules using the declared content of $C_{27}H_{22}Cl_2N_4$ in *clofazimine BPCRS*.

Clofibrate Capsules

General Notices

Action and use

Fibrate; lipid-regulating drug.

DEFINITION

Clofibrate Capsules contain Clofibrate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

IDENTIFICATION

The contents of the capsules comply with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the spectrum of *clofibrate EPCRS*.

B. The *light absorption*, Appendix II B, in the range 220 to 250 nm of a 0.001% w/v solution in *methanol* exhibits a maximum only at 226 nm. The A(1%, 1 cm) at the maximum is about 460.

C. The *light absorption*, Appendix II B, in the range 250 to 350 nm of a 0.01% w/v solution in *methanol* exhibits two maxima, at 280 nm and 288 nm. The A(1%, 1 cm) at the maxima are about 44 and about 31, respectively.

TESTS

Acidity

Add 10 ml of the contents of the capsules to 100 ml of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* with 0.1M *sodium hydroxide VS* and titrate with 0.1M *sodium hydroxide VS*. Not more than 2.5 ml is required to change the colour of the solution.

Refractive index

1.500 to 1.505, determined on the contents of the capsules, Appendix V E.

Relative density

1.138 to 1.147, determined on the contents of the capsules, Appendix V G.

4-Chlorophenol

Carry out the method for *gas chromatography*, Appendix III B, injecting 2 µl of each of the following solutions. Solution (1) contains 0.0025% w/v of *4-chlorophenol* in *chloroform*. For solution (2) extract a volume of the contents of the capsules containing 10.0 g of Clofibrate with 20 ml of 1M *sodium hydroxide*, wash the lower layer with 5 ml of *water*, add the washings to the aqueous layer and reserve the organic layer for the test for Volatile related substances.

Extract the combined aqueous layer and washings with two 5 ml quantities of *chloroform*, discard the chloroform and acidify the aqueous layer by the drop wise addition of *hydrochloric acid*. Extract with three 3 ml quantities of *chloroform*, combine the organic extracts and dilute to 10 ml with *chloroform*.

The chromatographic procedure may be carried out using a column (1.5 m × 4 mm) maintained at 185° and packed with either *acid-washed, silanised diatomaceous support* (Gas Chrom Q or Chromosorb W/AW/DMCS are suitable) (40 to 60 mesh) impregnated with 30% w/w of dimethyl silicone fluid (E-301 and SE-30 are suitable) or *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 10% w/w of dimethyl silicone fluid.

The area of any peak corresponding to 4-chlorophenol in the chromatogram obtained with solution (2) is not greater than the area of the peak in the chromatogram obtained with solution (1) (25 ppm).

Volatile related substances

Carry out the method for *gas chromatography*, Appendix III B, injecting 2 µl of each of the following solutions. For solution (1) dry the organic layer reserved in the test for 4-Chlorophenol with *anhydrous sodium sulphate*. For solution (2) use a solution of the contents of the capsules in *chloroform* containing 0.012% w/v of Clofibrate. Solution (3) is a 0.012% w/v solution of *methyl 2-(4-chlorophenoxy)-2-methylpropionate EPCRS* in the contents of the capsules.

The chromatographic procedure may be carried out as described in the test for 4-Chlorophenol.

In the chromatogram obtained with solution (3) measure from the baseline the height of the peak corresponding to methyl 2-(4-chlorophenoxy)-2-methylpropionate (a) and the height of the lowest part of the curve separating this peak from the peak corresponding to clofibrate (b). The test is not valid unless a is equal to at least 30% of full-scale deflection and a-b is greater than 75% of a.

The sum of the areas of any *secondary peaks* in the chromatogram obtained with solution (1) is not greater than 10 times the area of the peak due to clofibrate in the chromatogram obtained with solution (2) (0.1%).

Uniformity of weight

Carry out the test for Uniformity of weight for Soft Capsules described under Capsules. The average weight of the contents of the 20 capsules is 95.0 to 105.0% of the stated weight of Clofibrate.

Clofibrate, $C_{12}H_{15}ClO_3$

Not less than 97.0% w/w when determined by the following method. To a weighed quantity of the mixed contents of 10 capsules containing 1 g of Clofibrate add 25 ml of 0.5M *ethanolic potassium hydroxide* and heat under a reflux condenser fitted with a soda lime guard tube for 2 hours, rotating the flask frequently. Allow to cool, add 10 ml of *water* down the condenser and titrate the contents of the flask with 0.2M *hydrochloric acid VS* using 1 ml of *phenolphthalein solution R1* as indicator. Repeat the operation without the contents of the capsules. The difference between the titrations represents the amount of hydrochloric acid required. Each ml of 0.2M *hydrochloric acid VS* is equivalent to 48.54 mg of $C_{12}H_{15}ClO_3$.

Clomethiazole Capsules

General Notices

Action and use

Hypnotic.

DEFINITION

Clomethiazole Capsules contain a solution of Clomethiazole in a suitable fixed oil.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of clomethiazole, C_6H_8ClNS

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 20 mg of Clomethiazole with 70 ml of 0.1M *hydrochloric acid* for 15 minutes and dilute to 100 ml with the same solvent. Filter and dilute 10 ml of the filtrate to 50 ml with 0.1M *hydrochloric acid*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 257 nm.

B. To a quantity of the contents of the capsules containing 0.3 g of Clomethiazole add 10 ml of 0.1M *hydrochloric acid* and shake. To the aqueous layer add 5 ml of 1M *sodium hydroxide*, mix and extract with 15 ml of *chloroform*. Dry the chloroform layer with *anhydrous sodium sulphate*, filter and evaporate to remove the solvent. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of clomethiazole *(RS 051)*.

C. Mix a quantity of the contents of the capsules containing 0.1 g of Clomethiazole with 0.2 g of powdered *sodium hydroxide*, heat to fusion and continue heating for a further few seconds. Cool, add 0.5 ml of *water* and a slight excess of 2M *hydrochloric acid* and warm. Any fumes evolved do not turn moistened *starch iodate paper* blue (distinction from clomethiazole edisilate).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 100 ml of 1M *sulphuric acid* to whole capsules containing a total of 0.96 g of Clomethiazole, immerse in a water bath for 20 seconds, remove from the bath and shake vigorously. Repeat this treatment until the capsule shells have dissolved without exceeding a total heating time of 1 minute. Cool, centrifuge, filter the aqueous layer (Whatman GF/C paper is suitable) and dilute 5 ml of the filtrate to 50 ml with the mobile phase. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. For solution (3) dilute 1 volume of a 0.030% w/v solution of *4-methyl-5-vinylthiazole edisilate BPCRS* in *methanol* (solution A) to 50 volumes with the mobile phase. For solution (4) dilute 1 volume of a 0.020% w/v solution of *5-(2-chloroethyl)-4-methyl-3-[2-(4-methylthiazol-5-yl)ethyl]thiazolium chloride BPCRS* (quaternary dimer) in *methanol* (solution B) to 50 volumes with the mobile phase. For solution (5) dilute 1 volume of a 0.020% w/v solution of *4-methyl-5-(2-hydroxyethyl)thiazole BPCRS* in *methanol* (solution C) to 50 volumes with the mobile phase. For solution (6) add 1 ml each of solutions A, B and C to 5 ml of the filtrate obtained in the preparation of solution (2) and dilute to 50 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 70 volumes of a solution containing 0.13% w/v of *sodium hexanesulphonate* and 2.7% w/v of *tetramethylammonium hydrogen sulphate*, adjusted to pH 2.0 with 5M *sodium hydroxide*, and 30 volumes of *methanol* and (c) a detection wavelength of 257 nm.

The test is not valid unless in the chromatogram obtained with solution (6) baseline separation is achieved between the peaks due to the three specified impurities and also between each of these peaks and the principal peak.

Calculate the content of each of the specified impurities with reference to the stated content of Clomethiazole in the capsules expressing the content of 4-methyl-5-vinyl-thiazole as the base (1 mg of 4-methyl-5-vinylthiazole edisilate is equivalent to 0.568 mg of its base). Calculate the content of each of any other impurities from the areas of their peaks in the chromatogram obtained with solution (1) using the principal peak in the chromatogram obtained with solution (2) as reference and assuming the same response as clomethiazole. The content of each of the three specified impurities is not greater than 1.5% and the content of any other impurity is not greater than 0.5%. The total content of all the impurities is not greater than 3.0%.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using a quantity of the mixed contents of 20 capsules containing 0.4 g of Clomethiazole and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 16.16 mg of C_6H_8ClNS.

Clomethiazole Intravenous Infusion

General Notices

Action and use

Hypnotic.

DEFINITION

Clomethiazole Intravenous Infusion is a sterile solution of Clomethiazole Edisilate in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of clomethiazole edisilate, $(C_6H_8ClNS)_2,C_2H_6O_6S_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a volume of the intravenous infusion containing 40 mg of Clomethiazole Edisilate add 4 ml of 2M *sodium hydroxide*, mix and extract with 25 ml of *dichloromethane*. Dry the extract with *anhydrous sodium sulphate,* filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of clomethiazole *(RS 051)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel G precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 5 volumes of *water*, 10 volumes of 13.5M *ammonia*, 20 volumes of *butan-1-ol* and 65 volumes of *acetone* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 90 ml of *acetone* to a volume of the preparation containing about 50 mg of Clomethiazole Edisilate, mix and centrifuge. Wash the oily residue with three 10 ml quantities of *acetone* and then with three 10 ml quantities of *ether*. Dry the residue with a current of warm air and then dry under reduced pressure at 35° for 30 minutes. Dissolve the residue in 1 ml of 1M *acetic acid*, dilute to 5 ml with *methanol*, mix and filter through a 0.45-µm membrane filter. For solution (2) dissolve 0.1 g of *disodium ethanedisulphonate* in 5 ml of 1M *acetic acid* and dilute to 25 ml with *methanol*. After removal of the plate, dry it in a current of air until the ammonia is removed and spray with a 0.1% w/v solution of *bromocresol purple* in *ethanol (96%)* previously adjusted to a purple colour with 2M *ammonia* and examine immediately. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH of the infusion, 5.0 to 7.5, Appendix V L.

Related substances

Complies with the test described under Clomethiazole Capsules, calculating the content of each of the stated impurities with reference to the equivalent content of clomethiazole in the intravenous infusion (1 mg of clomethiazole edisilate is equivalent to 0.629 mg of base) and using the following solution as solution (1). Dilute a volume of the intravenous infusion with the mobile phase to produce a solution containing 0.04% w/v of Clomethiazole Edisilate.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) dilute a volume of the preparation with the mobile phase to produce a solution containing 0.01% w/v of Clomethiazole Edisilate. Solution (2) contains 0.01% w/v of *clomethiazole edisilate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 70 volumes of a solution containing 0.13% w/v of *sodium hexanesulphonate* and 2.7% w/v of *tetramethylammonium hydrogen sulphate* adjusted to pH 2.0 with 5M *sodium hydroxide* and 30 volumes of *methanol* and (c) a detection wavelength of 257 nm.

Calculate the content of $(C_6H_8ClNS)_2,C_2H_6O_6S_2$ from the declared content of $(C_6H_8ClNS)_2,C_2H_6O_6S_2$ in *clomethiazole edisilate BPCRS*.

STORAGE

Clomethiazole Intravenous Infusion should be stored at a temperature of 2° to 8°.

Clomethiazole Oral Solution

General Notices

Action and use

Hypnotic.

DEFINITION

Clomethiazole Oral Solution is a solution of Clomethiazole Edisilate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of clomethiazole edisilate, $(C_6H_8ClNS)_2,C_2H_6O_6S_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a volume of the oral solution containing 0.1 g of Clomethiazole Edisilate add 4 ml of 2M *sodium hydroxide*, mix and extract with 25 ml of *dichloromethane*. Dry the extract with *anhydrous sodium sulphate,* filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of clomethiazole *(RS 051)*.

B. Complies with test B for Identification described under Clomethiazole Intravenous Infusion.

TEST

Related substances

Complies with the test prescribed under Clomethiazole Capsules, calculating the content of each of the stated impurities with reference to the equivalent content of clomethiazole in the oral solution (1 mg of clomethiazole edisilate is equivalent to 0.629 mg of base) and using the following solution as solution (1). Dilute a volume of the oral solution with the mobile phase to produce a solution containing 0.05% w/v of Clomethiazole Edisilate.

ASSAY

Carry out the Assay described under Clomethiazole Intravenous Infusion.

STORAGE

Clomethiazole Oral Solution should be stored at a temperature of 2° to 8°.

Clomifene Tablets

General Notices

Action and use

Estrogen receptor modulator.

DEFINITION

Clomifene Tablets contain Clomifene Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clomifene citrate, $C_{26}H_{28}ClNO,C_6H_8O_7$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 350 nm of the solution obtained in the Assay exhibits two maxima, at 235 nm and 292 nm.

B. Dissolve a quantity of the powdered tablets containing 5 mg of Clomifene Citrate in 5 ml of a mixture of 1 volume of *acetic anhydride* and 5 volumes of *pyridine* and heat in a water bath. A dark red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of *water* and rotating the basket at 100 revolutions per minute. Withdraw a 10 ml sample of the medium and filter. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted, if necessary, with 0.1M *hydrochloric acid*, at the maximum at 232 nm, Appendix II B. Calculate the total content of clomifene citrate, $C_{26}H_{28}ClNO,C_6H_8O_7$, in the medium taking 317 as the value of A(1%, 1 cm) at the maximum at 232 nm.

Z-Isomer

30.0 to 50.0% of the content of clomifene citrate as determined in the Assay when determined in the following manner. Carry out the method for *liquid chromatography*, Appendix III D, using the following solution. Shake a quantity of the powdered tablets containing 50 mg of Clomifene Citrate with 50 ml of 0.1M *hydrochloric acid* for 10 minutes and filter. To 25 ml of the filtrate add 5 ml of 1M *sodium hydroxide* and extract with three 25-ml quantities of *ethanol-free chloroform*. Wash the combined extracts with 10 ml of *water*, dry over *anhydrous sodium sulphate* and add sufficient *ethanol-free chloroform* to produce 100 ml. To 20 ml of the solution add 0.1 ml of *triethylamine* and sufficient *hexane* to produce 100 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *silica gel for chromatography* (10 µm) (Porasil is suitable), (b) a mixture of *ethanol-free chloroform* and *hexane*, each containing 0.10% v/v of *triethylamine*, adjusted so that baseline separation is obtained between *E*- and *Z*-clomifene, as the mobile phase with a flow rate of 2 ml per minute (a mixture of 20 volumes of *ethanol-free chloroform* and 80 volumes of *hexane* is usually suitable) and (c) a detection wavelength of 302 nm. Pass the mobile phase through the system until stabilisation is achieved (about 250 ml).

In the chromatogram obtained with the solution being examined, a peak due to *E*-clomifene precedes the peak due to *Z*-clomifene. The test is not valid unless baseline separation is achieved between *E*- and *Z*-clomifene and the *column efficiency* is greater than 10,000 *theoretical plates* per metre determined using the peak due to the *E*-isomer.

Calculate the percentage of *Z*-isomer from the expression $100A_Z/(1.08A_D + A_Z)$ where A_Z and A_D are the areas of the peaks due to the *Z*- and *E*-isomers, respectively.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Clomifene Citrate for 30 minutes with 70 ml of 0.1M *hydrochloric acid* prepared using a 30% v/v solution of *propan-2-ol* as solvent, dilute to 100 ml with the propanolic hydrochloric acid and filter. Dilute 5 ml of the filtrate to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 292 nm, Appendix II B, using in the reference cell a solution prepared by diluting 5 ml of the propanolic hydrochloric acid to 100 ml with 0.1M *hydrochloric acid*. Calculate the content of $C_{26}H_{28}ClNO,C_6H_8O_7$ taking 175 as the value of A (1%, 1 cm) at the maximum at 292 nm.

STORAGE

Clomifene Tablets should be protected from light.

Clomipramine Capsules

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Clomipramine Capsules contain Clomipramine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of clomipramine hydrochloride, $C_{19}H_{23}ClN_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Triturate a quantity of the contents of the capsules containing 0.15 g of Clomipramine Hydrochloride with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after recrystallisation from hot *acetone* and drying at 110° for 30 minutes, is concordant with the *reference spectrum* of clomipramine hydrochloride *(RS 069)*.

TESTS

Related substances

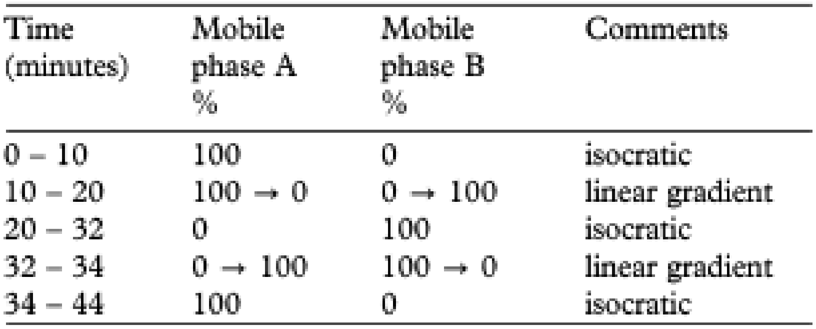
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the mixed contents of 20 capsules containing 40 mg of Clomipramine Hydrochloride with 15 ml of mobile phase A with the aid of ultrasound for 15 minutes, cool, dilute to 20 ml with the same solvent and filter through a 0.45 µm PTFE filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Solution (3) contains 0.002% w/v of *clomipramine impurity B EPCRS* and 0.0004% w/v of each *clomipramine impurity C EPCRS*, *clomipramine impurity D EPCRS* and *clomipramine impurity F EPCRS* in mobile phase A. Solution (4) contains 0.005% w/v of *clomipramine hydrochloride EPCRS* and 0.0015% w/v of *clomipramine impurity C EPCRS* in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25.0 cm x 4.6 mm) packed with *cyanopropylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS CN is suitable) and maintained at 30º, (b) as the mobile phase at a flow rate of 1.5 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 254nm

Mobile phase A

Dissolve 1.2 g of *sodium dihydrogen orthophosphate* in 950 ml of *water*, add 1.1 ml of *nonylamine*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 ml (solution A). Mix 75 volumes of solution A with 25 volumes of *acetonitrile*.

Mobile phase B Mix 65 volumes of solution A and 35 volumes of *acetonitrile*.



Time (minutes)	Mobile phase A %	Mobile phase B %	Comments
0 – 10	100	0	isocratic
10 – 20	100 → 0	0 → 100	linear gradient
20 – 32	0	100	isocratic
32 – 34	0 → 100	100 → 0	linear gradient
34 – 44	100	0	isocratic

The retention times relative to clomipramine are about 0.5, 0.7, 0.9, 1.7, 2.5, 3.4 and 4.3 for impurities A, B, C, D, E, F and G, respectively.

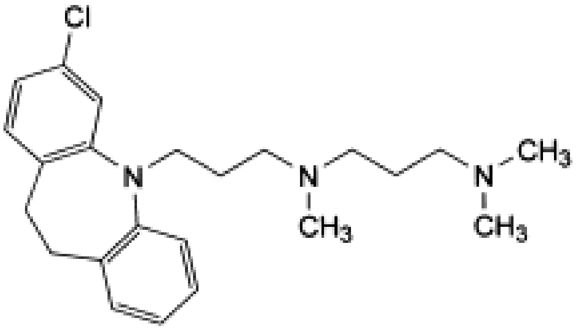
The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to clomipramine and clomipramine impurity C is at least 3.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to clomipramine impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (1%), the area of any peak corresponding to impurity C or impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.2%), the area of any peak corresponding to impurity F is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.2%), the area of any *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any such peaks is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%). The sum of the impurities is not greater than 1.0%. Disregard any peak with an area not greater than 0.01 times the area of the principal peak in the chromatogram obtained with solution (2) (0.01%).

ASSAY

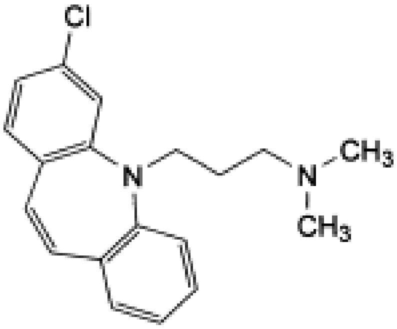
Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Clomipramine Hydrochloride with 200 ml of 0.1M *hydrochloric acid* for 1 hour, dilute to 250 ml with 0.1M *hydrochloric acid* and filter. Dilute 15 ml of the filtrate to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 252 nm, Appendix II B. Calculate the content of $C_{19}H_{23}ClN_2$,HCl taking 226 as the value of A(1%, 1 cm) at the maximum at 252 nm.

IMPURITIES

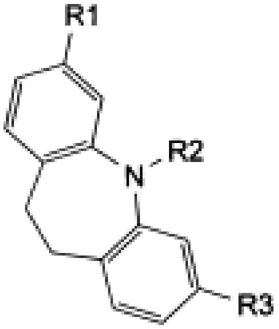


A. *N*-[3-(3-chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)propyl]-*N,N′,N′*-trimethylpropane-1,3-diamine,

B. imipramine,



C. 3-(3-chloro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine,



D. R1 = R3 = Cl, R2 = CH_2-CH_2-CH_2-$N(CH_3)_2$: 3-(3,7-dichloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine,

E. R1 = R2 = R3 = H: 10,11-dihydro-5*H*-dibenzo[*b,f*]azepine (iminodibenzyl),

F. R1 = Cl, R2 = R3 = H: 3-chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine,

G. R1 = Cl, R2 = CH_2-CH=CH_2, R3 = H: 3-chloro-5-(prop-2-enyl)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine.

Clonazepam Injection

General Notices

Action and use

Benzodiazepine.

DEFINITION

Clonazepam Injection is a sterile solution of Clonazepam. It is prepared immediately before use by diluting Sterile Clonazepam Concentrate with Water for Injections in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations.

STERILE CLONAZEPAM CONCENTRATE

DEFINITION

Sterile Clonazepam Concentrate is a sterile solution of Clonazepam in a suitable solvent.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of clonazepam, $C_{15}H_{10}ClN_3O_3$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless or slightly greenish yellow solution.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel GF_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia*, 15 volumes of n-*heptane*, 30 volumes of *nitromethane* and 60 volumes of *ether* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute 3 ml of a solution containing 3 mg of Clonazepam in a stoppered tube with an equal volume of *water*, shake with 1 ml of *chloroform*, allow to separate and use the chloroform layer. For solution (2) dissolve 3 mg of *clonazepam EPCRS* in 1 ml of *chloroform*. After removal of the plate, dry it in a current of cold air, spray with 2M *sodium hydroxide* and heat at 120° for 15 minutes. The yellow spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). Spots due to excipients may also be observed.

TESTS

Acidity

pH, 3.4 to 4.3, Appendix V L.

Related substances

Carry out the test protected from light. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance. For the first development use a mixture of 20 volumes of *chloroform* and 80 volumes of *ether* as the mobile phase. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) dilute, if necessary, a volume of the solution containing 10 mg of Clonazepam to 20 ml with *water* and extract with three 3 ml quantities of *chloroform*. Wash each chloroform extract separately with the same 10 ml volume of *water*, combine the extracts and add sufficient *chloroform* to produce 10 ml. Solutions (2) and (3) contain 0.00050% w/v and 0.00020% w/v respectively of *2-amino-2′-chloro-5-nitrobenzophenone EPCRS* ('nitrobenzophenone') in *chloroform*. Solution (4) contains 0.00020% w/v of *3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2-one EPCRS* ('carbostyril') in *chloroform*. After removal of the plate, allow it to dry in a current of cold air. For the second development use a mixture of 10 volumes of *ether* and 90 volumes of *nitromethane*. After removal of the plate, heat at a pressure of 2kPa at 120° for 3 hours, allow to cool and spray with a 10% w/v solution of *zinc chloride* in 0.1M *hydrochloric acid*. Allow the plate to dry in air and visualise by *Method I*, Appendix III A, beginning at the words 'expose to nitrous fumes ...'. Any spots in the chromatogram obtained with solution (1) corresponding to the nitrobenzophenone and carbostyril impurities are not more intense than the spots in the chromatograms obtained with solutions (3) and (4) respectively (0.2%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Protect the solutions from light throughout the assay. To a volume of the solution containing 20 mg of Clonazepam add sufficient *propan-2-ol* to produce 100 ml and dilute 10 ml to 100 ml with *propan-2-ol*. Measure the *absorbance* of the resulting solution at the maximum at 310 nm, Appendix II B. Calculate the content of $C_{15}H_{10}ClN_3O_3$ taking 364 as the value of A(1%, 1 cm) at the maximum at 310 nm.

STORAGE

Clonazepam Injection should be protected from light.

LABELLING

The label states (1) 'Sterile Clonazepam Concentrate'; (2) that the diluted injection is to be given by intravenous injection.

Clonidine Injection

General Notices

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

DEFINITION

Clonidine Injection is a sterile solution of Clonidine Hydrochloride in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of clonidine hydrochloride, $C_9H_9Cl_2N_3$,HCl

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Dilute a volume containing 0.3 mg of Clonidine Hydrochloride to 5 ml with 0.01M *hydrochloric acid*. The *light absorption* of the resulting solution, Appendix II B, in the range 245 to 350 nm exhibits maxima at 272 nm and 279 nm and an inflection at 265 nm.

B. To a volume containing 0.15 mg of Clonidine Hydrochloride add 1 ml of a 10% w/v solution of *ammonium reineckate* and allow to stand for 5 minutes. A pink precipitate is produced.

TESTS

Acidity or alkalinity

pH, 4.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and as the mobile phase the filtered upper layer obtained by shaking together 10 volumes of *glacial acetic acid*, 40 volumes of *butan-1-ol* and 50 volumes of *water* and allowing the layers to separate. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) add 10 ml of *methanol* to a volume containing 0.75 mg of Clonidine Hydrochloride, evaporate to dryness and dissolve the residue in 0.5 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution R2*. Allow to dry in air for 1 hour, spray again with the same reagent and immediately spray with a 5% w/v solution of *sodium nitrite*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

To a volume containing 0.15 mg of Clonidine Hydrochloride add 25 ml of *citro-phosphate buffer pH 7.6*. Add 5 ml of *water* and 1 ml of a solution containing 0.15% w/v of *bromothymol blue* and 0.15% w/v of *anhydrous sodium carbonate*. Add 30 ml of *chloroform*, shake for 1 minute and centrifuge. To 15 ml of the chloroform layer add 10 ml of *boric acid solution* and measure the *absorbance* of the resulting solution at the maximum at 420 nm, Appendix II B, using in the reference cell a solution prepared by diluting 10 ml of *boric acid solution* to 25 ml with *chloroform*. Repeat the operation using 5 ml of a 0.003% w/v solution of *clonidine hydrochloride BPCRS*, adding 20 ml of *citro-phosphate buffer pH 7.6* and completing the procedure described above, beginning at the words 'Add 5 ml of *water* ...'. Calculate the content of $C_9H_9Cl_2N_3$,HCl in the injection using the declared content of $C_9H_9Cl_2N_3$,HCl in *clonidine hydrochloride BPCRS*.

Clonidine Tablets

General Notices

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

DEFINITION

Clonidine Tablets contain Clonidine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clonidine hydrochloride, $C_9H_9Cl_2N_3$,HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 0.5 mg of Clonidine Hydrochloride add 30 ml of *water* and 5 ml of 1M *sodium hydroxide*. Swirl gently and extract with 20 ml of *chloroform*. Centrifuge the chloroform layer, dry with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 8 ml of 0.01M *hydrochloric acid*. The resulting solution complies with the following tests.

A. The *light absorption*, Appendix II B, in the range 245 to 350 nm exhibits maxima at 272 nm and 279 nm and an inflection at 265 nm.

B. Add 1 ml of a 10% w/v solution of *ammonium reineckate* and allow to stand for 5 minutes. A pink precipitate is produced.

TEST

Uniformity of content

Tablets containing less than 2 mg of Clonidine Hydrochloride comply with the requirements stated under Tablets using the following method of analysis.

For tablets containing 0.3 mg or more of Clonidine Hydrochloride, add a suitable volume of *citro-phosphate buffer pH 7.6* to one tablet, shake until disintegrated and dilute with *citro-phosphate buffer pH 7.6,* if necessary, to give a solution containing about 0.0015% w/v of Clonidine Hydrochloride. To 5 ml of the supernatant liquid add 1 ml of a solution containing 0.15% w/v of *bromothymol blue* and 0.15% w/v of *anhydrous sodium carbonate*, add 10 ml of *chloroform*, shake and allow to separate. Filter the chloroform layer through absorbent cotton and dilute 5 ml of the filtrate to 10 ml with *boric acid solution*. Measure the *absorbance* of a 2-cm layer of the resulting solution at the maximum at 420 nm, Appendix II B, using 5 ml of *boric acid solution* diluted to 10 ml with *chloroform* in the reference cell. Calculate the content of $C_9H_9Cl_2N_3$,HCl from the *absorbance* obtained by repeating the procedure using a solution prepared by diluting 5 ml of a 0.030% w/v solution of *clonidine hydrochloride BPCRS* to 100 ml with *citro-phosphate buffer pH 7.6*, transferring 5 ml to a separating funnel and completing the procedure described above, beginning at the words 'add 1 ml of a solution …' and using the declared content of $C_9H_9Cl_2N_3$,HCl in *clonidine hydrochloride BPCRS*.

For tablets containing less than 0.3 mg of Clonidine Hydrochloride, use the same procedure but with a concentration of 0.001% w/v or 0.0005% w/v of Clonidine Hydrochloride as appropriate and with correspondingly smaller concentrations of *clonidine hydrochloride BPCRS*.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 mg of Clonidine Hydrochloride add 25 ml of *citro-phosphate buffer pH 7.6* and shake for 15 minutes. Add 5 ml of *water* and 1 ml of a solution containing 0.15% w/v of *bromothymol blue* and 0.15% w/v of *anhydrous sodium carbonate* and shake to disperse. Add 30 ml of *chloroform*, shake continuously for 1 minute and centrifuge. To 15 ml of the chloroform layer add 10 ml of *boric acid solution* and measure the *absorbance* of the resulting solution at the maximum at 420 nm, Appendix II B, using in the reference cell a solution prepared by diluting 10 ml of *boric acid solution* to 25 ml with *chloroform*. Repeat the operation using 5 ml of a 0.003% w/v solution of *clonidine hydrochloride BPCRS*, adding 20 ml of *citro-phosphate buffer pH 7.6* and completing the procedure described above, beginning at the words 'Add 5 ml of *water* …'. Calculate the content of $C_9H_9Cl_2N_3$,HCl in the tablets using the declared content of $C_9H_9Cl_2N_3$, HCl in *clonidine hydrochloride BPCRS*.

Clotrimazole Cream

General Notices

Action and use

Antifungal.

DEFINITION

Clotrimazole Cream contains Clotrimazole in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of clotrimazole, $C_{22}H_{17}ClN_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity containing 40 mg of Clotrimazole with 20 ml of a mixture of 1 volume of 1M *sulphuric acid* and 4 volumes of *methanol* and shake with two 50-ml quantities of *dichloromethane*, discarding the organic layers. Make the aqueous phase alkaline with 2M *ammonia*, add a further 5 ml of 2M *ammonia* and extract with two 40-ml quantities of *dichloromethane*. Combine the *dichloromethane* extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and add sufficient *dichloromethane* to the filtrate to produce 100 ml. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 262 nm and 265 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and *di-isopropyl ether* as the mobile phase in a chromatography tank containing 25 ml of 13.5M *ammonia* in a beaker. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the cream containing 20 mg of Clotrimazole with 4 ml of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.5% w/v of *clotrimazole BPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in a current of air and spray with *dilute potassium iodobismuthate solution*. The spot in the chromatogram obtained with solution (1) is reddish brown and corresponds to the spot in the chromatogram obtained with solution (2).

TESTS

2-Chlorotritanol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) extract a quantity of the cream containing 20 mg of Clotrimazole by warming with 20 ml of *methanol* in a *water* bath at 50° for 5 minutes, shaking occasionally. Remove from the water-bath, shake the mixture vigorously while cooling to room temperature, cool in ice for 15 minutes, centrifuge for 5 minutes and decant the supernatant liquid. Repeat the extraction with two further 20-ml quantities of *methanol*. To the combined methanol extracts add 10 ml of *methanol* and dilute to 100 ml with *water.* Cool in ice and filter through a glass microfibre filter paper (Whatman GF/C is suitable). Solution (2) contains 0.0002% w/v of *2-chlorotritanol BPCRS* in a mixture of 7 volumes of *methanol* and 3 volumes of *water*. For solution (3) dilute 1 volume of solution (1) to 50 volumes with a mixture of 30 volumes of water and 70 volumes of *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 or Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 30 volumes of 0.02M *orthophosphoric acid* and 70 volumes of *methanol*, the pH of the mixture being adjusted to 7.5 with a 10% v/v solution of *triethylamine* in *methanol* and (c) a detection wavelength of 215 nm.

The *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (3), should be at least 9000 theoretical plates per metre.

Record the chromatogram for solution (1) for 1.5 times the retention time of the principal peak. The area of any peak corresponding to 2-chlorotritanol in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) treat a quantity of the cream containing 20 mg of Clotrimazole as described in the test for 2-Chlorotritanol and dilute 1 volume of the filtrate to 5 volumes with a mixture of 7 volumes of *methanol* and 3 volumes of *water.* For solution (2) dissolve 20 mg of *clotrimazole BPCRS* in 70 ml of *methanol*, add sufficient *water* to produce 100 ml and dilute 1 volume of the resulting solution to 5 volumes with a mixture of 30 volumes of *water* and 70 volumes of *methanol*.

The chromatographic procedure may be carried out using the conditions described in the test for 2-Chlorotritanol.

The *column efficiency*, determined using the peak in the chromatogram obtained with solution (2), should be at least 9000 theoretical plates per metre.

Calculate the content of $C_{22}H_{17}ClN_2$ in the cream using the declared content of $C_{22}H_{17}ClN_2$ in *clotrimazole BPCRS*.

Clotrimazole Pessaries

General Notices

Action and use

Antifungal.

DEFINITION

Clotrimazole Pessaries are *vaginal tablets* containing Clotrimazole.

The pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of clotrimazole, $C_{22}H_{17}ClN_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the powdered pessaries containing 40 mg of Clotrimazole with 20 ml of a mixture of 1 volume of 1M *sulphuric acid* and 4 volumes of *methanol* and shake with two 50-ml quantities of *dichloromethane*, discarding the organic layers. Make the aqueous phase alkaline with 2M *ammonia* and extract with two 40-ml quantities of *dichloromethane*. Combine the *dichloromethane* extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and add sufficient *dichloromethane* to the filtrate to produce 100 ml. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 262 nm and 265 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and *di-isopropyl ether* as the mobile phase in a tank containing 25 ml of 13.5M *ammonia* in a beaker. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered pessaries containing 20 mg of Clotrimazole with 4 ml of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.5% w/v of *clotrimazole BPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in a current of air and spray with *dilute potassium iodobismuthate solution*. The spot in the chromatogram obtained with solution (1) is reddish brown and corresponds to the spot in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of *methanol* to a quantity of the powdered pessaries containing 0.1 g of Clotrimazole and shake for 20 minutes. Dilute to 100 ml with *methanol* and filter. To 20 ml of the filtrate add 50 ml of *methanol* and sufficient *water* to produce 100 ml. Solution (2) contains 0.0002% w/v of *2-chlorotritanol BPCRS* in a mixture of 30 volumes of *water* and 70 volumes of *methanol*. For solution (3) dilute 1 volume of solution (1) to 50 volumes with a mixture of 30 volumes of *water* and 70 volumes of *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 or Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 30 volumes of 0.02M *orthophosphoric acid* and 70 volumes of *methanol*, the pH of the mixture being adjusted to 7.5 with a 10% v/v solution of *triethylamine* in *methanol* and (c) a detection wavelength of 215 nm.

The *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (3), should be at least 9000 theoretical plates per metre.

Record the chromatograms for solution (1) for 1.5 times the retention time of the principal peak. The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) weigh and powder 20 pessaries. To a quantity of the powder containing 0.1 g of Clotrimazole add 50 ml of *methanol* and shake for 20 minutes. Dilute to 250 ml with *methanol*, filter and to 10 ml of the filtrate add 60 ml of *methanol* and sufficient *water* to produce 100 ml. For solution (2) dissolve 20 mg of *clotrimazole BPCRS* in 70 ml of *methanol*, add sufficient *water* to produce 100 ml and dilute 1 volume of the resulting solution to 5 volumes with a mixture of 30 volumes of *water* and 70 volumes of *methanol*.

The chromatographic procedure may be carried out using the conditions described in the test for Related substances.

The *column efficiency*, determined using the peak in the chromatogram obtained with solution (2), should be at least 9000 theoretical plates per metre.

Calculate the content of $C_{22}H_{17}ClN_2$ using the declared content of $C_{22}H_{17}ClN_2$ in *clotrimazole BPCRS*.

Co-amilofruse Tablets

General Notices

Amiloride and Furosemide Tablets

Action and use

Potassium sparing diuretic + loop diuretic.

DEFINITION

Co-amilofruse Tablets contain Amiloride Hydrochloride and Furosemide in the proportions one part of anhydrous amiloride hydrochloride to eight parts of Furosemide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$

95.0 to 105.0% of the stated amount.

Content of furosemide, $C_{12}H_{11}ClN_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spots in the chromatogram obtained with solution (2) correspond to those in the chromatograms obtained with solutions (4) and (5) respectively.

B. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) are the same as those in the chromatogram obtained with solution (4).

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 5 volumes of *glacial acetic acid,* 5 volumes of *methanol* and 90 volumes of *chloroform* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing 80 mg of Furosemide with 16 ml of *methanol* for 5 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) to 20 volumes with *methanol*. Solution (4) contains 0.05% w/v of *furosemide BPCRS* in *methanol*. Solution (5) contains 0.00625% w/v of *amiloride hydrochloride BPCRS* in *methanol*. Solution (6) contains 0.0025% w/v of *4-chloro-5-sulphamoylanthranilic acid BPCRS* in *methanol*. Solution (7) contains 0.00031% w/v of *methyl 3,5-diamino-6-chloropyrazine-2-carboxylate EPCRS* in *methanol*. After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm).* In the chromatogram obtained with solution (1) any *secondary spot* other than any spot remaining on the line of application or any spots corresponding to either of the named impurities is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%, with reference to the content of furosemide). Examine under *ultraviolet light (365 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate is not more intense than the spot in the chromatogram obtained with solution (7) (0.5%, with reference to the content of anhydrous amiloride hydrochloride). Reveal the spots by *Method I*. In the chromatogram obtained with solution (1) any spot corresponding to 4-chloro-5-sulphamoylanthranilic acid is not more intense than the spot in the chromatogram obtained with solution (6) (0.5%, with reference to the content of furosemide).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 40 mg of Furosemide in 70 ml of a mixture of 4 volumes of *water* and 6 volumes of *methanol* adjusted to pH 2.0 with *orthophosphoric acid*, mix with the aid of ultrasound for 20 minutes, dilute to 100 ml with the same solvent mixture, mix, filter and dilute 20 ml of the filtrate to 50 ml with the same solvent mixture. Solutions (2) and (3) contain 0.002% w/v of *amiloride hydrochloride BPCRS* and 0.016% w/v of *furosemide BPCRS* respectively in a mixture of 4 volumes of *water* and 6 volumes of *methanol* adjusted to pH 2.0 with *orthophosphoric acid*. Solution (4) contains 0.002% w/v of *amiloride hydrochloride BPCRS* and 0.016% w/v of *furosemide BPCRS* in a mixture of 4 volumes of *water* and 6 volumes of *methanol* adjusted to pH 2.0 with *orthophosphoric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS1 is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a 0.02M solution of *sodium hexanesulphonate* in a mixture of 4 volumes of *water* and 6 volumes of *methanol*, the pH of the solution adjusted to 4.0 with 1M *acetic acid* and (c) a detection wavelength of 361 nm.

The assay is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to furosemide and amiloride is at least 2.5.

Calculate the content of $C_6H_8ClN_7O,HCl$ and $C_{12}H_{11}ClN_2O_5S$ using the declared content of $C_6H_8ClN_7O,HCl$ and $C_{12}H_{11}ClN_2O_5S$ in *amiloride hydrochloride BPCRS* and *furosemide BPCRS*, respectively.

STORAGE

Co-amilofruse Tablets should be protected from light.

LABELLING

The label states the quantities of Amiloride Hydrochloride, in terms of the equivalent amount of anhydrous amiloride hydrochloride, and of Furosemide in each tablet.

Co-amilozide Oral Solution

General Notices

Amiloride and Hydrochlorothiazide Oral Solution

Action and use

Potassium-sparing diuretic + thiazide diuretic.

DEFINITION

Co-amilozide Oral Solution is a solution containing Amiloride Hydrochloride and Hydrochlorothiazide in the proportions one part of anhydrous amiloride hydrochloride to ten parts of Hydrochlorothiazide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$

95.0 to 105.0% of the stated amount.

Content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 88 volumes of *tetrahydrofuran* and 12 volumes of 3M *ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a volume of the oral solution containing 0.1 g of Hydrochlorothiazide in *methanol*, dilute to 50 ml with *methanol* and mix. Solution (2) contains 0.2% w/v of *hydrochlorothiazide BPCRS* in *methanol*. Solution (3) contains 0.02% w/v of *amiloride hydrochloride BPCRS* in *methanol*. Solution (4) contains equal volumes of solutions (1), (2) and (3). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with solutions (2) and (3). If the Rf values of the spots obtained with solution (1) do not correspond exactly with those of the principal spots in the chromatograms obtained with solutions (2) and (3), the chromatogram obtained with solution (4) exhibits only one compact spot at each of these Rf values.

B. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (3) correspond to those of the peaks in the chromatograms obtained with solutions (1) and (2).

TESTS

Acidity

pH, 2.8 to 3.2, Appendix V L.

4-Amino-6-chlorobenzene-1,3-disulphonamide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00050% w/v of *4-amino-6-chlorobenzene-1,3-disulphonamide BPCRS* in the mobile phase. For solution (2) disperse a volume of the oral solution containing 50 mg of Hydrochlorothiazide in the mobile phase, dilute to 100 ml with the mobile phase and mix. Prepare solution (3) in the same manner as solution (2) but using solution (1) in place of the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 76 volumes of *water*, 20 volumes of *methanol* and 4 volumes of *phosphate buffer pH 3.0* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 265 nm.

The test is not valid unless a peak due to 4-amino-6-chlorobenzene-1,3-disulphonamide appears immediately before the principal peak in the chromatogram obtained with solution (3) and the height of the trough separating the two peaks is less than 5% of the height of the peak due to 4-amino-6-chlorobenzene-1,3-disulphonamide. The resolution between these two peaks may be improved by decreasing the methanol content in the mobile phase.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 4-amino-6-chlorobenzene-1,3-disulphonamide is not greater than the area of the peak in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 50 mg of *hydrochlorothiazide BPCRS* in a mixture of 20 ml of *methanol* and 4 ml of *phosphate buffer pH 3.0* and dilute to 100 ml with *water*. For solution (2) dissolve 50 mg of *amiloride hydrochloride BPCRS* in 200 ml of *methanol* and to 20 ml of the resulting solution add 4 ml of *phosphate buffer pH 3.0* and dilute to 100 ml with *methanol*. For solution (3) disperse a volume of the oral solution containing 50 mg of Hydrochlorothiazide in the mobile phase, dilute to 100 ml with the mobile phase and mix.

The chromatographic procedure may be carried out as described in the test for 4-Amino-6-chlorobenzene-1,3-disulphonamide but using a detection wavelength of 286 nm.

The assay is not valid unless the validity criteria of the test for 4-Amino-6-chlorobenzene-1,3-disulphonamide are met.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_6H_8ClN_7O,HCl$ and $C_7H_8ClN_3O_4S_2$, weight in volume, using the declared content of $C_6H_8ClN_7O,HCl$ and $C_7H_8ClN_3O_4S_2$ in *amiloride hydrochloride BPCRS* and *hydrochlorothiazide BPCRS* respectively.

LABELLING

The label states the quantities of Amiloride Hydrochloride, in terms of anhydrous amiloride hydrochloride, and of Hydrochlorothiazide in a suitable dose-volume.

Co-amilozide Tablets

General Notices

Amiloride and Hydrochlorothiazide Tablets

Action and use

Potassium-sparing diuretic + thiazide diuretic.

DEFINITION

Co-amilozide Tablets contain Amiloride Hydrochloride and Hydrochlorothiazide in the proportions one part of anhydrous amiloride hydrochloride to ten parts of Hydrochlorothiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$

95.0 to 105.0% of the stated amount.

Content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Hydrochlorothiazide with 50 ml of *acetone*, filter, evaporate the filtrate to dryness and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of hydrochlorothiazide *(RS 178)*.

B. In the test for Methyl 3,5-diamino-6-chloropyrazine-2-carboxylate, the principal spot in the chromatogram obtained with solution (3) corresponds to that in the chromatogram obtained with solution (4).

C. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (4) correspond to those in the chromatograms obtained with solutions (2) and (3).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 85 volumes of *ethyl acetate* and 15 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake vigorously a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide with 10 ml of *acetone* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Disregard any spot remaining on the line of application.

Methyl 3,5-diamino-6-chloropyrazine-2-carboxylate

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a freshly prepared mixture of 90 volumes of *1,4-dioxan* and 12 volumes of 3M *ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 17.5 mg of anhydrous amiloride hydrochloride with 10 ml of *methanol* and centrifuge. Solution (2) contains 0.0010% w/v of *methyl 3,5-diamino-6-chloropyrazine-2-carboxylate EPCRS* in *methanol*. For solution (3) dilute 1 volume of solution (1) to 20 volumes with *methanol*. Solution (4) contains 0.010% w/v of *amiloride hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any spot corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0010% w/v of *4-amino-6-chlorobenzene-1,3-disulphonamide BPCRS* in solution (3). For solution (2) dissolve 50 mg of *amiloride hydrochloride BPCRS* in sufficient *methanol* to produce 200 ml; to 20 ml of the resulting solution add 4 ml of 0.1M *hydrochloric acid* and dilute to 100 ml with *water*. For solution (3) dissolve 50 mg of *hydrochlorothiazide BPCRS* in a mixture of 20 ml of *methanol* and 4 ml of 0.1M *hydrochloric acid* and dilute to 100 ml with *water*. For solution (4) add a mixture of 20 ml of *methanol* and 4 ml of 0.1M *hydrochloric acid* to a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide, mix with the aid of ultrasound for 15 minutes, dilute to 100 ml with *water*, mix and filter.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 76 volumes of *water*, 20 volumes of *methanol* and 4 volumes of *phosphate buffer pH 3.0* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 286 nm.

The assay is not valid unless a peak due to 4-amino-6-chlorobenzene-1,3-disulphonamide appears immediately before the principal peak in the chromatogram obtained with solution (1). Increase the sensitivity, if necessary, to obtain at least 10% of full-scale deflection on the chart paper for this peak. The assay is also not valid unless the height of the trough separating the two peaks is less than 10% of the height of the peak due to 4-amino-6-chlorobenzene-1,3-disulphonamide. The resolution between the two peaks may be improved by decreasing the methanol content of the mobile phase.

Calculate the content of $C_6H_8ClN_7O,HCl$ and $C_7H_8ClN_3O_4S_2$ using the declared content of $C_6H_8ClN_7O,HCl$ and $C_7H_8ClN_3O_4S_2$ in *amiloride hydrochloride BPCRS* and *hydrochlorothiazide BPCRS* respectively.

STORAGE

Co-amilozide Tablets should be protected from light.

LABELLING

The label states the quantities of Amiloride Hydrochloride, in terms of the equivalent amount of anhydrous amiloride hydrochloride, and of Hydrochlorothiazide in each tablet.

Co-amoxiclav Injection

General Notices

Amoxicillin and Potassium Clavulanate Injection

Action and use

Penicillin antibacterial + beta-lactamase inhibitor.

DEFINITION

Co-amoxiclav Injection is a sterile solution of Amoxicillin Sodium and Potassium Clavulanate in Water for Injections. It is prepared by dissolving Co-amoxiclav for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Co-amoxiclav Injection should be used immediately after preparation.

CO-AMOXICLAV FOR INJECTION

DEFINITION

Co-amoxiclav for Injection is a sterile material consisting of Amoxicillin Sodium and Potassium Clavulanate with or without excipients. It is supplied in a sealed container.

PRODUCTION

The methods of production, extraction and purification of Potassium Clavulanate used in the formulation of Co-amoxiclav Injection are such that potassium clavam-2-carboxylate is eliminated or present at a level not exceeding 0.01%.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}N_3O_5S$

90.0 to 107.5% of the stated amount.

Content of clavulanic acid, $C_8H_9NO_5$

90.0 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use. Use as the mobile phase a mixture of 1 volume of *butan-1-ol*, 2 volumes of a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate*. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the sealed container containing the equivalent of 0.4 g of clavulanic acid in 100 ml of a mixture of 4 volumes of *methanol* and 6 volumes of 0.1M *mixed phosphate buffer pH 7.0* and filter. Solution (2) contains 0.4% w/v of *lithium clavulanate EPCRS* and 0.8% w/v of *amoxicillin trihydrate BPCRS* in a mixture of 4 volumes of *methanol* and 6 volumes of 0.1M *mixed phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* The principal spots in the chromatogram obtained with solution (1) are similar in position and colour to those in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH of a solution containing the equivalent of 10% w/v of amoxicillin, 8.0 to 10.0, Appendix V L.

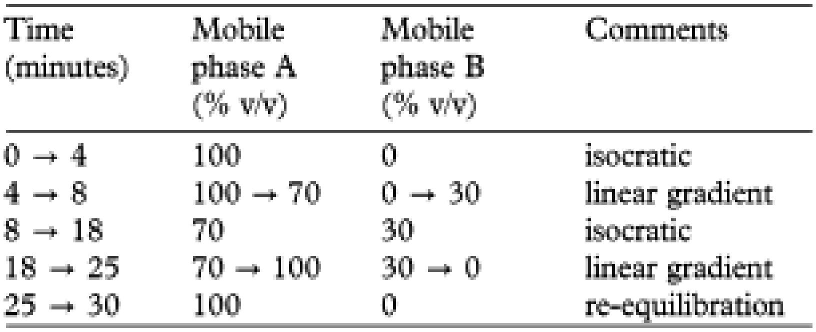
Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) dilute a quantity of the contents of a sealed container containing the equivalent of 0.1 g of amoxicillin in sufficient mobile phase A to produce 200 ml. Solution (2) contains 0.005% w/v of *amoxicillin trihydrate BPCRS* in mobile phase A. Solution (3) contains 0.05% w/v of *amoxicillin impurity standard BPCRS* in mobile phase A. Inject 20 µl of each solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb S3 ODS2 is suitable) and maintained at 20°, (b) as the mobile phase with a flow rate of 1.5 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 230 nm.

Mobile phase A Dissolve 15.6 g of *sodium dihydrogen orthophosphate* in 1000 ml of *water* and adjust the pH to 4.2 with *orthophosphoric acid*.

Mobile phase B Mix 10 volumes of mobile phase A with 90 volumes of *methanol*.



Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0 → 4	100	0	isocratic
4 → 8	100 → 70	0 → 30	linear gradient
8 → 18	70	30	isocratic
18 → 25	70 → 100	30 → 0	linear gradient
25 → 30	100	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention time of amoxicillin is about 4.3 minutes.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to amoxicillin and the peak with a retention time relative to amoxicillin of about 0.77 (α-penicilloic acid) is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak due to α-penicilloic acid is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (5%), the area of any peak with a retention time relative to amoxicillin of about 2.42 (amoxicillin dimer; the peak may appear as a doublet) is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (5%), the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%) and the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (15%).

Bacterial endotoxins

Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg per ml of amoxicillin (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

Water

Not more than 3.5% w/w, Appendix IX C. Use 0.5 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve, with shaking, a quantity of the mixed contents of the 10 containers containing the equivalent of 0.1 g of amoxicillin in sufficient *water* to produce 100 ml, mix and filter. Solution (2) contains 0.1% w/v of *amoxicillin trihydrate BPCRS* and 0.02% w/v of *lithium clavulanate EPCRS* in *water.*

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 80 volumes of *methanol* and 920 volumes of a 1.56% w/v solution of *sodium dihydrogen orthophosphate* in *water*, the pH being adjusted to 4.0 with *orthophosphoric acid*, and (c) a detection wavelength of 230 nm. Inject separately 10 µl of each solution.

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to amoxicillin and lithium clavulanate is at least 8.0.

Calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ in a container of average content weight using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

LABELLING

The label of the sealed container states the quantity of Amoxicillin Sodium contained in it, in terms of the equivalent amount of amoxicillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

The label of the sealed container states that the preparation contains penicillin.

Co-amoxiclav Tablets

General Notices

Amoxicillin and Potassium Clavulanate Tablets

Action and use

Penicillin antibacterial + beta-lactamase inhibitor.

DEFINITION

Co-amoxiclav Tablets contain Amoxicillin Trihydrate and either Potassium Clavulanate or Diluted Potassium Clavulanate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of amoxicillin, $C_{16}H_{19}O_5N_3S$

90.0 to 105.0% of the stated amount.

Content of clavulanic acid, $C_8H_9NO_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 F_{254} plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use. Use as the mobile phase a mixture of 1 volume of *butan-1-ol*, 2 volumes of a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate.* Apply separately to the plate 1 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.4 g of clavulanic acid in 100 ml of a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0* and filter. Solution (2) contains 0.4% w/v of *lithium clavulanate EPCRS* and 0.8% w/v of *amoxicillin trihydrate BPCRS* in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0.* After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spots in the chromatogram obtained with solution (1) are similar in position and colour to those in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 20 ml of the medium, filter through a 0.45-µm membrane filter and discard the first 10 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For solution (1) use the filtered dissolution medium diluted, if necessary, to produce a solution containing the equivalent of 0.025% w/v of amoxicillin. Solution (2) contains 0.025% w/v of *amoxicillin trihydrate BPCRS* and 0.01% w/v of *lithium clavulanate EPCRS* in *water*.

The chromatographic procedure described under Assay may be used.

Calculate the total content of amoxicillin, $C_{16}H_{19}N_3O_5S$, and of clavulanic acid, $C_8H_9NO_5$, in the medium using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve, with shaking, a quantity of the powdered tablets containing the equivalent of 0.25 g of amoxicillin in 400 ml of *water*, add sufficient *water* to produce 500 ml, mix and filter. Solution (2) contains 0.05% w/v of *amoxicillin trihydrate BPCRS* and 0.02% w/v of *lithium clavulanate EPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 5 volumes of *methanol* and 95 volumes of a 0.78% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 4.4 with *orthophosphoric acid*, and (c) a detection wavelength of 220 nm. Inject separately 20 µl of each solution.

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to amoxicillin and lithium clavulanate is at least 3.5 and the *symmetry factor* of the peak due to lithium clavulanate is at most 1.5.

Calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ in the tablets using the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxicillin trihydrate BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

STORAGE

Co-amoxiclav Tablets should be protected from light and stored in an airtight container.

LABELLING

The label states the quantity of Amoxicillin Trihydrate, in terms of the equivalent amount of amoxicillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid, in each tablet.

The label states that the preparation contains penicillin.

Co-beneldopa Capsules

General Notices

Benserazide Hydrochloride and Levodopa Capsules

Action and use

Dopa decarboxylase inhibitor + dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Co-beneldopa Capsules contain Benserazide Hydrochloride and Levodopa in the proportions, by weight, 1 part benserazide to 4 parts levodopa.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of benserazide, $C_{10}H_{15}N_3O_5$

95.0 to 105.0% of the stated amount.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a precoated cellulose plate (Merck plates are suitable) and a mixture of 10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol* as the mobile phase, but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the capsule contents containing 0.2 g of Levodopa with 40 ml of 0.1M *hydrochloric acid* for 10 minutes, filter and use the filtrate. Solution (2) contains 0.5% w/v of *levodopa BPCRS* in 0.1M *hydrochloric acid*. Solution (3) contains 0.142% w/v of *benserazide hydrochloride BPCRS* in 0.1M *hydrochloric acid*. After removal of the plate, allow it to dry in a current of warm air for 5 minutes, spray with *dilute phosphomolybdotungstic reagent*, dry in a current of warm air for 30 seconds and spray with a 10% w/v solution of *sodium hydroxide*. The chromatogram obtained with solution (1) shows two clearly separated spots, the spot with the higher Rf value corresponding to the spot in the chromatogram obtained with solution (2) and the spot with the lower Rf value corresponding to the spot in the chromatogram obtained with solution (3).

B. In the Assay, the chromatogram obtained with solution (1) exhibits two peaks with the same retention times as those due to benserazide and levodopa in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution* *test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), use the filtered dissolution medium diluted with the mobile phase to produce a solution expected to contain 0.04 mg of Levodopa per ml. For solution (2) dilute 1 volume of a 0.1% w/v solution of *levodopa BPCRS* in 0.1M *orthophosphoric acid* to 25 volumes with the mobile phase.

The chromatographic procedure described under Assay may be used. Disregard the peak due to benserazide.

Calculate the total content of levodopa, $C_9H_{11}NO_4$, in the medium using the declared content of $C_9H_{11}NO_4$ in *levodopa BPCRS*.

Related substances

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be prepared in mobile phase that has been cooled to 4° and injected immediately. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 0.1 g of benserazide with 100 ml of the mobile phase, mix with the aid of ultrasound for 3 minutes, shaking occasionally, and filter through a 0.45-µm filter, discarding the first 5 ml of filtrate. Solution (2) contains 0.0005% w/v of *benserazide impurity A EPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of each of *benserazide hydrochloride BPCRS* and *benserazide impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP8 is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture prepared by dissolving 4.76 g of *potassium dihydrogen orthophosphate* in 800 ml of *water*, adding 200 ml of *acetonitrile* and 1.22 g of *sodium decanesulphonate* and adjusting the pH to 3.5 with *orthophosphoric acid* and (c) a detection wavelength of 220 nm.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 2.0.

For solution (1) allow the chromatography to proceed for nine times the retention time of benserazide. In the chromatogram obtained with solution (1) the area of any peak corresponding to benserazide impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%), the area of any other *secondary peak* is not greater than the area of the peak due to benserazide in the chromatogram obtained with solution (3) (0.5%) and the sum of the areas of any such peaks is not greater than twice the area of the peak due to benserazide in the chromatogram obtained with solution (3) (1%). Disregard any peak with an area less than 0.1 times the area of the peak due to benserazide in the chromatogram obtained with solution (3) (0.05%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated cellulose plate (Merck plates are suitable) and a mixture of 10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate, as bands 20 mm long, 10 µl of each of solutions (1) and (2) and 20 µl of solution (3) and dry in a current of air. Solution (1) should be prepared immediately before use. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of *anhydrous formic acid* and *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) is a mixture of equal volumes of solution (1) and a solution prepared by dissolving 30 mg of L-*tyrosine* in 1 ml of *anhydrous formic acid* and diluting to 100 ml with *methanol*. After removal of the plate, allow it to dry in a current of warm air, spray with a freshly prepared mixture containing equal volumes of a 10% w/v solution of *iron(III) chloride hexahydrate* and a 5% w/v solution of *potassium hexacyanoferrate(III)* and examine the plate immediately. Any *secondary band* in the chromatogram obtained with solution (1) is not more intense than the band in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (3) shows a distinct band, at a higher Rf value than the principal band, which is more intense than the band in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 0.1 g of Levodopa with 80 ml of 0.1M *orthophosphoric acid* for 5 minutes, mix with the aid of ultrasound for 30 minutes, cool, add sufficient 0.1M *orthophosphoric acid* to produce 100 ml and mix. Filter the resulting solution through a 0.45-µm filter (Whatman GF/C is suitable), discarding the first 5 ml of filtrate, and dilute 10 volumes of the filtrate to 100 volumes with the mobile phase. For solution (2) dissolve 28.7 mg of *benserazide hydrochloride BPCRS* and 0.1 g of *levodopa BPCRS* in sufficient 0.1M *orthophosphoric acid* to produce 100 ml, mix and dilute 10 volumes of the resulting solution to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP8 is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture prepared by dissolving 4.76 g of *potassium dihydrogen orthophosphate* in 800 ml of *water*, adding 200 ml of *acetonitrile* and 1.22 g of *sodium decanesulphonate* and adjusting the pH to 3.5 with *orthophosphoric acid* and (c) a detection wavelength of 220 nm. Inject 20 µl of each solution.

The chromatogram obtained with solution (2) shows two principal peaks; the retention time of the peak due to benserazide is about three times that of the peak due to levodopa.

Calculate the content of $C_{10}H_{15}N_3O_5$ and of $C_9H_{11}NO_4$ in each capsule using the declared contents of $C_{10}H_{15}N_3O_5$ in *benserazide hydrochloride BPCRS* and of $C_9H_{11}NO_4$ in *levodopa BPCRS*.

STORAGE

Co-beneldopa Capsules should protected from moisture.

LABELLING

The label states the quantity of Benserazide Hydrochloride, in terms of the equivalent amount of benserazide, and the amount of Levodopa in each capsule.

Cocaine Eye Drops

General Notices

Action and use

Local anaesthetic.

DEFINITION

Cocaine Eye Drops are a sterile solution of Cocaine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of cocaine hydrochloride, $C_{17}H_{21}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Add 5 ml of 0.2M *ammonia* to a volume of the eye drops containing 40 mg of Cocaine Hydrochloride and extract with two 5-ml quantities of *dichloromethane*, filter the extracts through a phase separating paper (Whatman IPS is suitable) and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cocaine (RS 071).

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 2.5 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the eye drops with sufficient mobile phase to produce a solution containing 0.04% w/v of Cocaine Hydrochloride. Solution (2) contains 0.0008% w/v of *benzoylecgonine hydrate* in the mobile phase. Solution (3) contains 0.0008% w/v of *benzoic acid* in the mobile phase. Solution (4) contains 0.0008% w/v each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil 10 ODS is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1 volume of 9M *perchloric acid*, 35 volumes of *methanol* and 64 volumes of *water* and (c) a detection wavelength of 240 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to benzoylecgonine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%), the area of any peak corresponding to benzoic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%) and the sum of the two impurities is not greater than 2%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the eye drops containing 40 mg of Cocaine Hydrochloride with sufficient *water* to produce 100 ml. Solution (2) contains 0.04% w/v of *cocaine hydrochoride BPCRS*. Solution (3) contains 0.0008% w/v each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1)

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

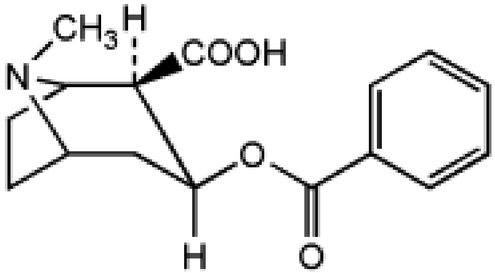
Calculate the content of $C_{17}H_{21}NO_4$,HCl in the eye drops from the chromatograms obtained and using the declared content of $C_{17}H_{21}NO_4$,HCl in *cocaine hydrochoride BPCRS*.

STORAGE

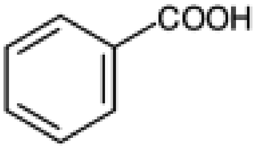
Cocaine Eye Drops should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include:



A. Benzoylecgonine



B. Benzoic acid

Cocaine Paste

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Local anaesthetic.

DEFINITION

Cocaine Paste contains Cocaine Hydrochloride in a suitable basis. It is intended for intranasal administration.

The paste complies with the requirements stated under Topical Semi-solid Preparations, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of cocaine hydrochloride, $C_{17}H_{21}NO_4,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Accurately weigh a quantity of the preparation being examined containing 0.4 g of Cocaine Hydrochloride, add 10 ml of *water,* stir and shake vigorously to fully suspend the preparation; centrifuge and collect the aqueous layer. Repeat the extraction with a further three 10-ml quantities of *water*, shaking and re-suspending the solution each time. Combine the aqueous extracts and dilute to 100 ml with *water*. Filter a portion of the solution and dilute 10 ml to 100 ml with *water*.

(2) 0.0008% w/v of *benzoylecgonine hydrate* in the mobile phase.

(3) 0.0008% w/v of *benzoic acid* in the mobile phase.

(4) 0.0008% w/v of each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil 10 ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

1 volume of 9M *perchloric acid*, 35 volumes of *methanol* and 64 volumes of *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to benzoylecgonine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any peak corresponding to benzoic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%).

The sum of contents of the two impurities is not greater than 2%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Accurately weigh a quantity of the preparation containing 0.4 g of Cocaine Hydrochloride, add 10 ml of *water*, stir and shake vigorously to fully suspend the preparation; centrifuge and collect the aqueous layer. Repeat the extraction with a further three 10-ml quantities of *water*, re-suspending and shaking the solution each time. Combine the aqueous extracts and dilute to 100 ml with *water*. Filter a portion of the solution and dilute 10 ml to 100 ml with *water*.

(2) 0.04% w/v of *cocaine hydrochoride BPCRS* in *water*.

(3) 0.0008% w/v of each of *benzoylecgonine hydrate* and *benzoic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS.

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

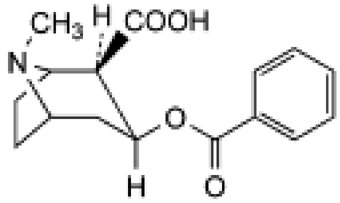
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to benzoylecgonine and benzoic acid is at least 2.0.

DETERMINATION OF CONTENT

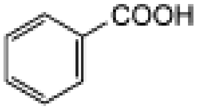
Calculate the content of $C_{17}H_{21}NO_4$,HCl in the paste from the chromatograms obtained and using the declared content of $C_{17}H_{21}NO_4$,HCl in *cocaine hydrochoride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:



A. Benzoylecgonine



B. Benzoic acid

Co-careldopa Tablets

General Notices

Levodopa and Carbidopa Tablets

Action and use

Dopa decarboxylase inhibitor + dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Co-careldopa Tablets contain Carbidopa and Levodopa.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous carbidopa, $C_{10}H_{14}N_2O_4$

90.0 to 110.0% of the stated amount.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (3) exhibits two peaks with the same retention times as those due to carbidopa and levodopa in the chromatogram obtained with solution (1).

B. To a quantity of the powdered tablets containing the equivalent of 1 mg of anhydrous carbidopa add 5 ml of 0.05M *sulphuric acid*, shake for 2 minutes and filter. Add 5 ml of *dimethylaminobenzaldehyde reagent* to the filtrate. A yellow colour is produced.

C. To a quantity of the powdered tablets containing 50 mg of Levodopa add 4 ml of *ethanol (96%)* and 1 ml of 1M *sulphuric acid* and shake for 2 minutes. Add 2 ml of *cinnamaldehyde*, allow to stand for 20 minutes, add 50 ml of 0.1M *hydrochloric acid*, shake for 2 minutes and allow to stand. Filter the aqueous layer obtained and to 5 ml add 0.1 ml of *iron(III) chloride solution R1*. To half of the solution add an excess of 5M *ammonia*; a purple colour is produced. To the remainder add an excess of 2M *sodium hydroxide*; a deep red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 750 ml of 0.1M *hydrochloric acid* and rotating the basket at 50 revolutions per minute. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0050% w/v of *levodopa BPCRS* and 0.00054% w/v of *carbidopa BPCRS* in 0.1M *hydrochloric acid*. Use the filtered dissolution medium as solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP8 is suitable), (b) 0.1M *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with 1M *orthophosphoric acid* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 282 nm.

Calculate the content of $C_{10}H_{14}N_2O_4$ and of $C_9H_{11}NO_4$ in the medium using the declared contents of $C_{10}H_{14}N_2O_4$ in *carbidopa BPCRS* and of $C_9H_{11}NO_4$ in *levodopa BPCRS*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.050% w/v of *levodopa BPCRS*, 0.0054% w/v of *carbidopa BPCRS* and 0.0050% w/v of *methyldopa BPCRS* (internal standard) in 0.1M *hydrochloric acid*. For solution (2) weigh and powder 20 tablets, shake a quantity of the powder containing 0.25 g of Levodopa with 60 ml of 0.1M *hydrochloric acid* for 15 minutes, add sufficient 0.1M *hydrochloric acid* to produce 100 ml and filter. Dilute 10 ml of the clear filtrate to 50 ml with 0.1M *hydrochloric acid*. For solution (3) add 5 ml of a 0.050% w/v solution of the internal standard in 0.1M *hydrochloric acid* to 10 ml of the clear filtrate obtained in the preparation of solution (2) and add sufficient 0.1M *hydrochloric acid* to produce 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP8 is suitable), (b) 0.1M *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with 1M *orthophosphoric acid* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 282 nm.

Calculate the content of $C_{10}H_{14}N_2O_4$ and of $C_9H_{11}NO_4$ in each tablet using the declared contents of $C_{10}H_{14}N_2O_4$ in *carbidopa BPCRS* and of $C_9H_{11}NO_4$ in *levodopa BPCRS*.

LABELLING

The label states the quantity of Carbidopa, in terms of the equivalent amount of anhydrous carbidopa, and the quantity of Levodopa in each tablet.

Co-codamol Capsules

General Notices

Codeine Phosphate and Paracetamol Capsules

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-codamol Capsules contain Codeine Phosphate and Paracetamol.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$

95.0 to 105.0% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 20 ml of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 24 mg of Codeine Phosphate with 30 ml of *water* for 1 minute and centrifuge. Decant, add 10 ml of 1M *sodium hydroxide* and 30 ml of *dichloromethane* to the supernatant liquid, shake for 1 minute and filter the dichloromethane layer through glass-fibre paper (Whatman GF/C is suitable). Solution (2) contains 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%)*. Solution (3) contains 0.08% w/v of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%)*. After removal of the plate, allow it to dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* with respect to the content of Paracetamol, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 5.8* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Dilute the filtrate with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00075% w/v of Paracetamol. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 257 nm using 0.1M *sodium hydroxide* in the reference cell. Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.001% w/v of *4-aminophenol* in the mobile phase. For solution (2) shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 50 ml of the mobile phase for 10 minutes and filter.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.01M *sodium butanesulphonate* in a mixture of 0.4 volume of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 272 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 4-aminophenol is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (0.1%). In the chromatogram obtained with solution (2) peaks with a long retention time may occur due to excipients.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 50 mg of Codeine Phosphate with 50 ml of 0.1M *hydrochloric acid* for 10 minutes and filter. Make the filtrate alkaline with 5M *sodium hydroxide* and extract with two 40 ml quantities of *dichloromethane*. Wash the combined extracts with 10 ml of *water*, filter through a layer of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with *dichloromethane*, evaporate the filtrate to dryness at a temperature not exceeding 45° using a rotary evaporator and dissolve the residue in 2 ml of *dichloromethane*. For solution (2) dilute 1.5 volumes of solution (1) to 100 volumes with *dichloromethane*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *dichloromethane*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *toluene,* 25 volumes of *acetone* and 65 volumes of *dichloromethane* as the mobile phase. Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank and allow the solvent front to ascend 14 cm above the line of application. Apply separately to the plate

200 µl of solution (1) and 40 µl of each of solutions (2), (3) and (4). For solution (1) transfer a quantity of the contents of the capsules containing 1.0 g of Paracetamol to a ground-glass-stoppered 15 ml centrifuge tube, add 5 ml of *peroxide-free ether*, shake mechanically for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or until a clear supernatant liquid is obtained and use the supernatant liquid. For solution (2) dilute 1 ml of solution (1) to 10 ml with *ethanol* (*96%*). Solution (3) contains 0.0050% w/v of *4′-chloroacetanilide* in *ethanol* (*96%*). For solution (4) dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol* (*96%*) to produce 100 ml. After removal of the plate, dry it in a current of warm air and examine under *ultraviolet light* (*254 nm*). Any spot corresponding to 4′-chloroacetanilide in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.005%). Any *secondary spot* in the chromatogram obtained with solution (2) with an Rf value lower than that of 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.25%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

Uniformity of content

Capsules containing less than 2% of Codeine Phosphate comply with the requirements stated under Capsules, with respect to the content of Codeine Phosphate, using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase. For solution (2) add 100 ml of the mobile phase to the contents of the capsules and mix with the aid of ultrasound until completely dispersed. Shake for 10 minutes, dilute to 200 ml with the mobile phase, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute 0.01M *sodium pentanesulphonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, the pH of the solution being adjusted to 2.8 using 2M *hydrochloric acid*, and (c) a detection wavelength of 220 nm.

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in each capsule using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

ASSAY

For codeine phosphate

Weigh the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase. For solution (2) shake a quantity of the contents of the capsules containing 8 mg of Codeine Phosphate with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

The chromatographic conditions described under Uniformity of content may be used.

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

For paracetamol

Weigh the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *paracetamol BPCRS* in the mobile phase. For solution (2) shake a quantity of the contents of the capsules containing 500 mg of Paracetamol with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 ml of the filtrate to 250 ml with the mobile phase.

The chromatographic conditions described under Uniformity of content may be used but with a detection wavelength of 243 nm.

Calculate the content of $C_8H_9NO_2$ using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

LABELLING

The label states the quantities of Codeine Phosphate and of Paracetamol in each capsule.

When Co-codamol Capsules are prescribed or demanded no strength being stated, capsules containing 8 mg of Codeine Phosphate and 500 mg of Paracetamol shall be dispensed or supplied.

Co-codamol Tablets

General Notices

Codeine Phosphate and Paracetamol Tablets

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-codamol Tablets contain Codeine Phosphate and Paracetamol.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$

95.0 to 105.0% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 ml of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 24 mg of Codeine Phosphate with 30 ml of *water* for 1 minute and centrifuge. Decant, add 10 ml of 1M *sodium hydroxide* and 30 ml of *chloroform* to the supernatant liquid, shake for 1 minute and filter the chloroform layer through glass-fibre paper (Whatman GF/C is suitable). Solution (2) contains 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%)*. Solution (3) contains 0.08% w/v each of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%)*. After removal of the plate, allow it to dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2) . The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* with respect to the content of Paracetamol, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 5.8* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Dilute the filtrate with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00075% w/v of Paracetamol. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 257 nm using 0.1M *sodium hydroxide* in the reference cell. Calculate the total content of paracetamol, $C_8H_9NO_2$ in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.001% w/v of *4-aminophenol* in the mobile phase. For solution (2) shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 ml of the mobile phase for 10 minutes and filter.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.01M *sodium butanesulphonate* in a mixture of 0.4 volume of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 272 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (1) (0.1%). In the chromatogram obtained with solution (2) peaks with a long retention time may occur due to excipients.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Codeine Phosphate with 50 ml of 0.1M *hydrochloric acid* for 10 minutes and filter. Make the filtrate alkaline with 5M *sodium hydroxide* and extract with two 40 ml quantities of *chloroform*. Wash the combined extracts with 10 ml of *water*, filter through a layer of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with *chloroform*, evaporate the filtrate to dryness at a temperature not exceeding 45° using a rotary evaporator and dissolve the residue in 2 ml of *chloroform*. For solution (2) dilute 1.5 volumes of solution (1) to 100 volumes with *chloroform*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *chloroform*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *toluene,* 25 volumes of *acetone* and 65 volumes of *chloroform* as the mobile phase. Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank and allow the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 200 µl of solution (1) and 40 µl of each of solutions (2), (3) and (4). For solution (1) transfer a quantity of the finely-powdered tablets containing 1.0 g of Paracetamol to a ground-glass-stoppered 15 ml centrifuge tube, add 5 ml of *peroxide-free ether*, shake mechanically for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or until a clear supernatant liquid is obtained and use the supernatant liquid. For solution (2) dilute 1 ml of solution (1) to 10 ml with *ethanol* (*96%*). Solution (3) contains 0.0050% w/v of *4′-chloroacetanilide* in *ethanol* (*96%*). For solution (4) dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol* (*96%*) to produce 100 ml. After removal of the plate, dry it in a current of warm air and examine under *ultraviolet light* (*254 nm*). Any spot corresponding to 4′-chloroacetanilide in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.005%). Any *secondary spot* in the chromatogram obtained with solution (2) with an Rf value lower than that of 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.25%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

Uniformity of content

Tablets containing less than 2 mg or 2% of Codeine Phosphate comply with the requirements stated under Tablets, with respect to the content of Codeine Phosphate, using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase. For solution (2) add 100 ml of the mobile phase to one tablet and mix with the aid of ultrasound until completely dispersed. Shake for 10 minutes, dilute to 200 ml with the mobile phase, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute 0.01M *sodium pentanesulphonate* in a mixture of 78 volumes of *water* and 22 volumes of *methanol*, the pH of the solution being adjusted to 2.8 using 2M *hydrochloric acid*, and (c) a detection wavelength of 220 nm.

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in each tablet using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

ASSAY

For codeine phosphate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase. For solution (2) shake a quantity of the powdered tablets containing 8 mg of Codeine Phosphate with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

The chromatographic conditions described under Uniformity of content may be used.

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

For paracetamol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *paracetamol BPCRS* in the mobile phase. For solution (2) shake a quantity of the powdered tablets containing 500 mg of Paracetamol with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 ml of the filtrate to 250 ml with the mobile phase.

The chromatographic conditions described under Uniformity of content may be used but with a detection wavelength of 243 nm.

Calculate the content of $C_8H_9NO_2$ using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

LABELLING

The label states the quantities of Codeine Phosphate and of Paracetamol in each tablet.

When Co-codamol Tablets are prescribed or demanded no strength being stated, tablets containing 8 mg of Codeine Phosphate and 500 mg of Paracetamol shall be dispensed or supplied.

Co-codaprin Tablets

General Notices

Aspirin and Codeine Tablets

Action and use

Opioid analgesic + antipyretic; analgesic; anti-inflammatory.

DEFINITION

Co-codaprin Tablets contain Codeine Phosphate and Aspirin in the proportions, by weight, 1 part to 50 parts.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4, \frac{1}{2}H_2O$

90.0 to 110.0% of the stated amount.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Boil 1 g of the powdered tablets with 10 ml of 1M *sodium hydroxide*, cool and filter. Acidify the filtrate with 1M *sulphuric acid*; a white precipitate is produced. To a solution of the precipitate add *iron(III) chloride solution R1*; a deep violet colour is produced.

B. Shake 1 g of the powdered tablets with a mixture of 20 ml of *water* and 1 ml of 1M *sulphuric acid* for 5 minutes and filter. Reserve the residue for test C. The filtrate yields the reactions characteristic of *phosphates*, Appendix VI. Make the remainder of the filtrate alkaline with 5M *ammonia*, extract with *dichloromethane*, separate the dichloromethane layer and evaporate the dichloromethane. Place a small quantity of the residue on the surface of a drop of *nitric acid*; a yellow, but no red, colour is produced.

C. Dissolve 0.1 g of the residue obtained in test B in 1 ml of *sulphuric acid*, add 0.05 ml of *iron(III) chloride solution R1* or 0.05 ml of *ammonium molybdate-sulphuric acid solution* and warm gently. A bluish violet colour is produced which changes to red on the addition of 0.05 ml of 2M *nitric acid*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* with respect to the content of Aspirin, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of a pH 4.5 buffer prepared by mixing 29.9 g of *sodium acetate* and 16.6 ml of *glacial acetic acid* with sufficient *water* to produce 10 litres and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at 265 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *aspirin BPCRS* in the dissolution medium and calculate the total content of aspirin, $C_9H_8O_4$, in the medium using the declared content of $C_9H_8O_4$ in *aspirin BPCRS*.

Foreign alkaloids

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Codeine Phosphate with 50 ml of 0.1M *hydrochloric acid*, filter and make the filtrate alkaline with 5M *sodium hydroxide*. Extract with two 40 ml quantities of *chloroform* and wash the combined extracts with 10 ml of *water*. Filter through a layer of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with *chloroform*. Evaporate the filtrate to dryness and dissolve the residue in 2 ml of *chloroform*. For solution (2) dilute 1.5 volumes of solution (1) to 100 volumes with *chloroform*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *chloroform*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 ml of *chloroform* and 10 ml of *water*, shake well and allow to separate. Filter the chloroform layer through a dry filter paper and evaporate 10 ml of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 ml of *ethanol (96%)*, stir well, dilute to 100 ml with *water* at a temperature not exceeding 10°, filter immediately and rapidly transfer 50 ml to a *Nessler cylinder*. Add 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1* to a mixture of 3 ml of a freshly prepared 0.050% w/v solution of *salicylic acid*, 2 ml of *ethanol (96%)* and sufficient *water* to produce 50 ml contained in a second *Nessler cylinder* (3.0%).

ASSAY

Weigh and powder 20 tablets.

For codeine phosphate

To a quantity of the powder containing 24 mg of Codeine Phosphate add 5 ml of 5M *sodium hydroxide* and 15 ml of *water*, shake for 2 minutes and extract with three 50 ml quantities of *chloroform*. Wash each extract with the same 10 ml of *water*, filter through absorbent cotton previously moistened with *chloroform* and evaporate the combined extracts to about 60 ml on a water bath in a current of air. Cool, add 25 ml of *water*, 5 ml of *acetate buffer pH 2.8* and 5 ml of *dimethyl yellow and oracet blue B solution* and titrate with 0.01M *dioctyl sodium sulphosuccinate VS* with vigorous swirling until near the end point, then add the titrant drop wise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end point is indicated by the appearance of a permanent pinkish grey colour in the chloroform layer. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of dioctyl sodium sulphosuccinate required.

Dissolve 40 mg of *codeine phosphate BPCRS* in 25 ml of water and 5 ml of *acetate buffer pH 2.8*, add 60 ml of *chloroform* and 5 ml of *dimethyl yellow and oracet blue B solution*, shake well to dissolve the codeine phosphate and carry out the method described above beginning at the words 'and titrate …'. Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

For aspirin

To a quantity of the powder containing 0.8 g of Aspirin add 20 ml of *water* and 2 g of *sodium citrate* and boil under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 ml of warm *water* and titrate with 0.5M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 45.04 mg of $C_9H_8O_4$.

STORAGE

Co-codaprin Tablets should be protected from light.

LABELLING

The label states (1) that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed); (2) the quantities of Codeine Phosphate and of Aspirin in each tablet.

Co-danthrusate Capsules

General Notices

Dantron and Docusate Sodium Capsules

Action and use

Stimulant laxative.

DEFINITION

Co-danthrusate Capsules contain Dantron and Docusate Sodium in the proportions, by weight, 5 parts to 6 parts.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of dantron, $C_{14}H_8O_4$

90.0 to 110.0% of the stated amount.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 50 mg of Dantron with 10 ml of *dichloromethane*, filter and dilute 2 ml of the filtrate to 20 ml with *dichloromethane*.

(2) 0.06% w/v of *docusate sodium BPCRS* in *dichloromethane*.

(3) 0.05% w/v of *dantron BPCRS* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution.

(d) After removal of the plate, allow it to dry in air, spray with *ethanolic sulphuric acid (10%)* and heat at 120° for 15 minutes.

MOBILE PHASE

85 volumes of *ethyl acetate* and 15 volumes of *methanol*.

VALIDITY

The two principal spots in the chromatogram obtained with solution (1) correspond to the principal spots in the chromatograms obtained with solutions (2) and (3).

B. Shake a quantity of the contents of the capsules containing 60 mg of Docusate Sodium with 50 ml of *water*. To 5 ml of this mixture add 1 ml of 2M *sulphuric acid*, 10 ml of *dichloromethane* and 0.2 ml of *dimethyl yellow solution* and mix; a red colour is produced in the dichloromethane layer. Add 50 mg of *cetrimide* and mix; the dichloromethane layer is yellow.

C. To a quantity of the contents of the capsules containing 50 mg of Dantron add 10 ml of *dichloromethane* and 5 ml of 1M *ammonia* and mix. A red colour is produced in the aqueous layer.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Dantron with 20 ml of *tetrahydrofuran* for 5 minutes and dilute to 100 ml with the mobile phase.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase.

(3) Dissolve 50 mg of *dantron impurity standard BPCRS* in 20 ml of *tetrahydrofuran* and dilute to 100 ml with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µl of each solution.

(g) For solution (2) allow the chromatography to proceed for 1.5 times the retention time of the principal peak.

MOBILE PHASE

2.5 volumes of *glacial acetic acid*, 40 volumes of *tetrahydrofuran* and 60 volumes of *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

a peak due to 1-hydroxyanthraquinone appears immediately before the principal peak, as indicated in the reference chromatogram supplied with *dantron impurity standard BPCRS;*

the height of the trough separating the two peaks is not greater than one third of the height of the peak due to 1-hydroxyanthraquinone.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to 1-hydroxyanthraquinone is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.3%

taking into account the correction factor for the impurity);

— the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

— disregard any peak with a retention time less than one third of that of the principal peak.

ASSAY

For dantron

To a quantity of the mixed contents of 20 capsules containing 35 mg of Dantron add 50 ml of *absolute ethanol*, heat on a water bath for 30 minutes and cool. Add sufficient *absolute ethanol* to produce 100 ml and filter through glass-fibre paper (Whatman GF/C is suitable). Dilute 5 ml of the filtrate to 100 ml with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 430 nm, Appendix II B. Calculate the content of $C_{14}H_8O_4$ taking 458 as the value of A(1%, 1 cm) at the maximum at 430 nm.

For docusate sodium

To a quantity of the mixed contents of 20 capsules containing 0.3 g of Docusate Sodium add 25 ml of *absolute ethanol*, heat to 60° for 5 minutes and cool. Add sufficient *water* to produce 200 ml and filter through glass-fibre paper (Whatman GF/C is suitable). To 25 ml of the filtrate add 10 ml of *water* and 15 ml of *chloroform* and titrate with 0.004M *benzethonium chloride VS*, using *dimidium bromide-sulphan blue mixed solution* as indicator, to the first appearance of a green colour in the chloroform layer and shaking vigorously towards the end point. Each ml of 0.004M *benzethonium chloride VS* is equivalent to 1.778 mg of $C_{20}H_{37}NaO_7S$.

LABELLING

The label states the quantities of Dantron and of Docusate Sodium in each capsule.

Codeine Linctus

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Codeine Linctus is an *oral solution* containing 0.3% w/v of Codeine Phosphate or an equivalent concentration of Codeine Phosphate Sesquihydrate in a suitable flavoured vehicle.

The title 'Diabetic Codeine Linctus' may be used for a preparation that complies with the requirements of this monograph and that is formulated with a vehicle appropriate for administration to diabetics.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of codeine phosphate $C_{18}H_{21}NO_3,H_3PO_4$, ½H_2O

0.285 to 0.315% w/v.

IDENTIFICATION

To 20 ml add 20 ml of *water* and 30 ml of *chloroform*, shake and discard the chloroform layer. To the aqueous layer add 10 ml of 1M *sodium hydroxide* and extract with 30 ml of *chloroform*. Wash the chloroform layer with two 10 ml quantities of 0.1M *sodium hydroxide* followed by 10 ml of *water*. Dry the chloroform layer with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dry the residue at 60°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of codeine *(RS 072)*.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 72 volumes of *ethanol (96%)*, 30 volumes of *cyclohexane* and 6 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 20 ml of *water* and 2 ml of 13.5M *ammonia* to 20 ml of the preparation being examined and extract with two 20 ml quantities of *chloroform*. Dry the combined extracts with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*. For solution (2) dilute 1.5 volumes of solution (1) to 100 volumes with *chloroform*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *chloroform*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.06% w/v of *codeine phosphate BPCRS* in *water*. For solution (2) mix a quantity of the preparation being examined containing 15 mg of codeine phosphate with sufficient *water* to produce 25 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute 0.01M *sodium octanesulphonate* in a mixture of 55 volumes of *water*, 45 volumes of *methanol* and 1 volume of *glacial acetic acid* and (c) a detection wavelength of 285 nm.

A large peak due to sodium benzoate may be present. If this peak interferes with the peak due to codeine phosphate adjust the chromatographic conditions to achieve a satisfactory separation. An increase in the content of water in the mobile phase or an increase in the concentration of sodium octanesulphonate will increase the retention time of the peak due to codeine phosphate relative to that of the peak due to sodium benzoate.

Determine the *weight per ml* of the linctus, Appendix V G, and calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$, weight in volume, using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

STORAGE

Codeine Linctus should be protected from light.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

When Diabetic Codeine Linctus is prescribed or demanded, Codeine Linctus formulated with a vehicle appropriate for administration to diabetics, whether or not labelled 'Diabetic Codeine Linctus', shall be dispensed or supplied.

Codeine Phosphate Injection

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Codeine Phosphate Injection is a sterile solution of Codeine Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing 60 mg of Codeine Phosphate add 0.05 ml of *hydrochloric acid* and sufficient *water* to produce 7.5 ml. Shake with three 10-ml quantities of *chloroform* and discard the chloroform. Add sufficient 6M *ammonia* to make the aqueous solution alkaline to litmus paper, extract with three 10-ml quantities of *chloroform*, evaporate the combined extracts to dryness and dry the residue at 80° for 4 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of codeine (RS 072).

B. Yields reaction B characteristic of *phosphates*, Appendix VI.

TESTS

Acidity

pH, 3.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute, if necessary, the injection with *methanol (60%)* to produce a solution containing 0.6% w/v of Codeine Phosphate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (60%).*

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture prepared by adding 600 ml of *methanol* containing 2.22 g of *dioctyl sodium sulphosuccinate* to 100 ml of *water* containing 1.36 g of *sodium acetate*, diluting to 1 litre with *water* and adjusting the pH to 5.5 with *glacial acetic acid* and (c) a detection wavelength of 285 nm.

For solution (1) allow the chromatography to proceed for 2.5 times the retention time of the principal peak.

The sum of the areas of any *secondary peaks* in the chromatogram obtained with solution (1) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.06% w/v of *codeine phosphate BPCRS* in *methanol (60%).* For solution (2) dilute the injection, if necessary, with *methanol (60%)* to produce a solution containing 0.06% w/v of Codeine Phosphate.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in the injection using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS.*

STORAGE

Codeine Phosphate Injection should be protected from light.

Codeine Phosphate Oral Solution

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Codeine Phosphate Oral Solution is a solution containing 0.5% w/v of Codeine Phosphate or an equivalent concentration of Codeine Phosphate Sesquihydrate in a suitable flavoured vehicle.

Extemporaneous preparation

The following formula and directions apply.

Codeine Phosphate	5 g
Water	15 ml
Chloroform Spirit	25 ml
Syrup	Sufficient to produce 1000 ml

Dissolve the Codeine Phosphate in the Water, add 750 ml of the Syrup and mix. Add the Chloroform Spirit and sufficient of the Syrup to produce 1000 ml and mix. It should be recently prepared.
The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of codeine phosphate $C_{18}H_{21}NO_3,H_3PO_4, ½H_2O$

0.475 to 0.525% w/v.

IDENTIFICATION

Related substances

Complies with the requirements stated under Codeine Linctus.

ASSAY

Carry out the Assay described under Codeine Linctus.

STORAGE

Codeine Phosphate Oral Solution should be protected from light.

Codeine Phosphate Tablets

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Codeine Phosphate Tablets contain Codeine Phosphate or Codeine Phosphate Sesquihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Macerate a quantity of the powdered tablets containing 50 mg of codeine phosphate or its equivalent with 5 ml of 1M *sulphuric acid* and 15 ml of *water*. Filter, make alkaline with 5M *ammonia*, extract with successive quantities of *chloroform* and evaporate the chloroform extracts on a water bath. Place a few mg of the residue, in powder, on the surface of a drop of *nitric acid*. A yellow but no red colour is produced (distinction from morphine).

B. Heat 10 mg of the residue obtained in test A on a water bath with 1 ml of *sulphuric acid* and 0.05 ml of *iron(III) chloride solution R1*. A blue colour is produced which changes to red on the addition of 0.05 ml of *nitric acid*.

C. Extract the powdered tablets with *water* and filter. The filtrate yields reaction B characteristic *of phosphates*, Appendix VI.

TESTS

Foreign alkaloids

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of codeine phosphate or its equivalent with 10 ml of a mixture of 4 volumes of 0.01M *hydrochloric acid* and 1 volume of *absolute ethanol* for 15 minutes and filter. For solution (2) dilute 1.5 volumes of solution (1) to 100 volumes with a mixture of 4 volumes of 0.01M *hydrochloric acid* and 1 volume of *absolute ethanol*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with a mixture of 4 volumes of 0.01M *hydrochloric acid* and 1 volume of *absolute ethanol*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of codeine phosphate or its equivalent as completely as possible in 20 ml of 0.25M *sulphuric acid*, filter and wash the residue on the filter with 0.25M *sulphuric acid* until *complete extraction* of the alkaloid is effected, Appendix XI G. Make alkaline with 5M *ammonia* and extract with successive quantities of *chloroform* until *complete extraction* of the alkaloid is effected. Wash each chloroform solution with the same 10 ml of *water* and evaporate the chloroform. To the residue add 5 ml of *ethanol (96%)* previously neutralised to *methyl red solution* and remove the ethanol by evaporation. Dissolve the residue in 1 ml of neutralised *ethanol (96%)*, add 10 ml of 0.1M *hydrochloric acid VS* and 10 ml of *water* and titrate with 0.1M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 40.64 mg of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$.

STORAGE

Codeine Phosphate Tablets should be protected from light.

LABELLING

When the active ingredient is codeine phosphate sesquihydrate, the quantity is stated in terms of the equivalent amount of codeine phosphate.

Codergocrine Tablets

General Notices

Action and use

Vasodilator.

DEFINITION

Codergocrine Tablets contain Codergocrine Mesilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codergocrine mesilate

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (2) shows four major peaks with retention times corresponding to the peaks due to codergocrine mesilate in the chromatogram obtained with solution (1).

B. To a quantity of the powdered tablets containing 10 mg of Codergocrine Mesilate add 1 ml of *methanol* and 5 ml of a 1% w/v solution of *(+)-tartaric acid*. Stir for 15 minutes and filter through sintered glass (ISO 4793, porosity grade 4, is suitable). To 1 ml of the filtrate add slowly 2 ml of *dimethylaminobenzaldehyde solution R6* and mix. A deep blue colour is produced.

TESTS

Related substances

Carry out in subdued light the method for *thin-layer chromatography,* Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable), scored at a distance of 15 cm from the line of application, and a freshly prepared mixture of 1 volume of 13.5M *ammonia*, 3 volumes of *methanol*, 50 volumes of *dichloromethane* and 50 volumes of *ethyl acetate* as the mobile phase. Use an unlined tank closed with an ungreased lid and develop the chromatogram immediately after introducing the mobile phase into the tank. Apply separately to the plate 20 µl of each of the following solutions prepared immediately before use in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*. Solutions (1) and (2) contain 0.0040% w/v and 0.0010% w/v respectively of *dihydroergocristine mesilate BPCRS*. For solution (3) shake a sufficient quantity of the powdered tablets with the solvent mixture to produce a solution containing 0.20% w/v of Codergrocrine Mesilate, filter and use the filtrate. Allow the solvent front to ascend for exactly 90 minutes, remove the plate and dry it in a current of cold air for not longer than 1 minute. Using a freshly prepared mobile phase, repeat the development for a further 90 minutes, remove the plate and dry it in a current of cold air for not longer than 1 minute. Spray with a 1% w/v solution of *dimethylaminobenzaldehyde* in a mixture of equal volumes of *hydrochloric acid* and *ethanol (96%)*, dry in a current of cold air for not longer than 2 minutes and heat at 40° for 15 minutes. In the chromatogram obtained with solution (3), any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (1) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, protecting all solutions from light. Use as the medium 500 ml of 0.1M *hydrochloric acid* and rotate the basket at 120 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter, discarding the first 10 ml of the filtrate. Measure the *fluorescence* of the solution, Appendix II E, using an excitation wavelength of 285 nm and an emission wavelength of 350 nm using 0.1M *hydrochloric acid* to set the instrument to zero. Measure the *fluorescence* of a solution of *codergrocrine mesilate BPCRS* in 0.1M *hydrochloric acid* containing the equivalent amount of Codergrocrine Mesilate as that expected in the test solution. Carry out the measurements as quickly as possible.

Calculate the total content of Codergrocrine Mesilate in the medium from the absorbances obtained and using the declared content of codergrocrine mesilate in *codergrocrine mesilate BPCRS*.

Uniformity of content

Tablets containing less than 2 mg of Codergrocrine Mesilate comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. Shake one tablet for 30 minutes with sufficient of a 1.0% w/v solution of *(+)-tartaric acid* to produce a solution containing 0.006% w/v of Codergrocrine Mesilate and filter through sintered glass (ISO 4793, porosity grade 4, is suitable). To 3 ml of the filtrate add 6 ml of *dimethylaminobenzaldehyde solution R6*, mix, cool to room temperature and allow to stand for exactly 30 minutes. Measure the *absorbance* of the resulting solution at the maximum at 580 nm, Appendix II B, using in the reference cell a solution prepared by treating 3 ml of a 1.0% w/v solution of *(+)-tartaric acid* and 6 ml of *dimethylaminobenzaldehyde solution R6* in the same manner. Prepare a 0.006% w/v solution of *codergrocrine mesilate BPCRS* in a 1.0% w/v solution of *(+)-tartaric acid*; to 3 ml of this solution add 6 ml of *dimethylaminobenzaldehyde solution R6* and complete the operation described above, beginning at the words 'mix, cool …'. From the absorbances so obtained, and using the declared content of codergrocrine mesilate in *codergrocrine mesilate BPCRS*, calculate the content of codergrocrine mesilate in the tablets.

Composition

Using the chromatograms obtained in the Assay calculate the percentage contents of the methanesulphonates of dihydroergocornine, of dihydroergocryptine (α- and β-forms) and of dihydroergocristine with respect to the sum of these components in solution (1), using the products of the molecular weights and peak area figures, and determine the ratio of the peak area of dihydro-α-ergocryptine to that of dihydro-β-ergocryptine. The content of each of dihydroergocornine, dihydroergocryptine and dihydroergocristine is not less than 30.0% and not more than 36.5% of the sum of the three components and the ratio of the peak area of dihydro-α-ergocryptine to that of dihydro-β-ergocryptine is between 1.5 and 2.5.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Prepare solution (1) in the following manner. Use a sufficient number of whole tablets to produce a final solution containing 0.060% w/v of Codergrocrine Mesilate. Stir the tablets for 15 minutes with 50 ml of a mixture of 2 volumes of a 1.0% w/v solution of *(+)-tartaric acid* and 1 volume of *absolute ethanol*, filter through a sintered glass filter (ISO 4793, porosity grade 4, is suitable), wash the filter with three 10 ml quantities of the same solvent mixture and dilute the combined filtrate and washing to 100 ml with the same solvent mixture. Solution (2) contains 0.060% w/v of *codergrocrine mesilate BPCRS* in a mixture of 2 volumes of a 1% w/v solution of *(+)-tartaric acid* and 1 volume of *absolute ethanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 5 volumes of *triethylamine*, 50 volumes of *acetonitrile* and 150 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 280 nm. The components are eluted in the following order: dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine.

The *resolution factor* between the peaks due to dihydro-α-ergocryptine and dihydroergocristine should be not less than 1.2; this value may be obtained by appropriate adjustment of the acetonitrile content of the mobile phase.

Calculate the content of codergrocrine mesilate in the tablets using the expression:

$$\frac{[A_1M_1+A_2M_2+A_3M_3+A_4M_2]_{\mathrm{S}}\,w_{\mathrm{R}}\,p_{\mathrm{R}}}{[A_1M_1+A_2M_2+A_3M_3+A_4M_2]_{\mathrm{R}}\,w_{\mathrm{S}}}$$

where A_1, A_2, A_3, A_4 = the peak areas of the components in order of elution;

M_1, M_2, M_3 = the molecular weights of the methanesulphonates of dihydroergocornine, dihydroergocryptine (α-and β-forms) and dihydroergocristine;

w_{R} = the percentage w/v of *codergrocrine mesilate BPCRS* in solution (2) ('R');

w_{S} = the declared content of codergrocrine mesilate in each tablet multiplied by the number of tablets used('S');

p_{R} = the declared content of codergrocrine mesilate in *codergrocrine mesilate BPCRS.*

STORAGE

Codergrocrine Tablets should be protected from light.

Co-dydramol Tablets

General Notices

Dihydrocodeine and Paracetamol tablets

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-dydramol Tablets contain Dihydrocodeine Tartrate and Paracetamol in the proportions, by weight, 1 part to 50 parts.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 ml of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 30 mg of Dihydrocodeine Tartrate with 10 ml of *water* for 1 minute and centrifuge. Decant, add 10 ml of 1M *sodium hydroxide* and 30 ml of *chloroform* to the supernatant liquid, shake for 1 minute and filter the chloroform layer through glass-fibre paper (Whatman GF/C is suitable). Solution (2) contains 0.1% w/v of *dihydro-codeine tartrate BPCRS* in *methanol (50%)*. Solution (3) contains 0.1% w/v of *codeine phosphate BPCRS* in *methanol (50%)*. Solution (4) is a mixture of equal volumes of solutions (2) and (3). After removal of the plate, allow it to dry in air, spray with *ethanolic iron (III) chloride solution* and heat at 105° for 10 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots of different colours.

C. In the Assay for dihydrocodeine tartrate, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* with respect to the content of Paracetamol, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 5.8* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Dilute the filtrate with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00075% w/v of Paracetamol. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 257 nm using 0.1M *sodium hydroxide* in the reference cell. Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.001% w/v of *4-aminophenol* in the mobile phase. For solution (2) shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 ml of the mobile phase for 10 minutes and filter.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.01M *sodium butanesulphonate* in a mixture of 0.4 volume of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 272 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (1) (0.1%). In the chromatogram obtained with solution (2) peaks with a long retention time may occur due to excipients.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Dihydrocodeine Tartrate with 0.5M *hydrochloric acid*, filter, make the filtrate alkaline with 5M *sodium hydroxide* and extract with four 25 ml quantities of *chloroform*. Wash each chloroform extract with 10 ml of each of 0.01M *sodium hydroxide* and *water*, filter the combined chloroform extracts, evaporate the filtrate to dryness at a temperature not exceeding 45° and dissolve the residue in 2 ml of *chloroform*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *chloroform*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *chloroform*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *toluene,* 25 volumes of *acetone* and 65 volumes of *chloroform* as the mobile phase. Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank and allow the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 200 µl of solution (1) and 40 µl of each of solutions (2), (3) and (4). For solution (1) transfer a quantity of the finely-powdered tablets containing 1.0 g of Paracetamol to a ground-glass-stoppered

15 ml centrifuge tube, add 5 ml of *peroxide-free ether*, shake mechanically for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or until a clear supernatant liquid is obtained and use the supernatant liquid. For solution (2) dilute 1 ml of solution (1) to 10 ml with *ethanol* (*96%*). Solution (3) contains 0.0050% w/v of *4′-chloroacetanilide* in *ethanol* (*96%*). For solution (4) dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol* (*96%*) to produce 100 ml. After removal of the plate, dry it in a current of warm air and examine under *ultraviolet light* (*254 nm*). Any spot corresponding to 4′-chloroacetanilide in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.005%). Any *secondary spot* in the chromatogram obtained with solution (2) with an Rf value lower than that of 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.25%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

Uniformity of content

Tablets containing 10 mg or less of Dihydrocodeine Tartrate comply with the requirements stated under Tablets, with respect to the content of dihydrocodeine tartrate, using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *dihydrocodeine tartrate BPCRS* in *methanol (25%)*. For solution (2) add 50 ml of *methanol* to one tablet and mix with the aid of ultrasound until completely dispersed. Add 100 ml of *water*, shake for 10 minutes, dilute to 200 ml with *water*, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute 0.01M *sodium pentanesulphonate* in a mixture of 78 volumes of *water* and 22 volumes of *methanol*, the pH of the solution being adjusted to 2.8 using 2M *hydrochloric acid* and (c) a detection wavelength of 225 nm.

Calculate the content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in each tablet using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS*.

ASSAY

For dihydrocodeine tartrate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *dihydrocodeine tartrate BPCRS* in *methanol (25%)*. For solution (2) shake a quantity of the powdered tablets containing 10 mg of Dihydrocodeine Tartrate with 50 ml of *methanol* for 1 minute, add 100 ml of *water* and shake for a further 10 minutes. Dilute to 200 ml with *water*, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate.

The chromatographic conditions described under Uniformity of content may be used.

Calculate the content of $C_{18}H_{23}NO_3,C_4H_6O_6$ using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS*.

For paracetamol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution (1) contains 0.005% w/v of *paracetamol BPCRS* in *water*. For solution (2) dilute 1 volume of solution (2) obtained in the Assay for dihydrocodeine tartrate to 50 volumes with *water*.

The chromatographic conditions described under Uniformity of content may be used but with a detection wavelength of 243 nm.

Calculate the content of $C_8H_9NO_2$ using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

LABELLING

The label states the quantities of Dihydrocodeine Tartrate and of Paracetamol in each tablet.

Co-fluampicil Capsules

General Notices

Flucloxacillin and Ampicillin Capsules

Action and use

Penicillin antibacterial.

DEFINITION

Co-fluampicil Capsules contain Flucloxacillin Sodium and Ampicillin Trihydrate, in the proportions, by weight, 1 part flucloxacillin to 1 part ampicillin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ampicillin, $C_{16}H_{19}N_3O_4S$

92.5 to 107.5% of the stated amount.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture 10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 0.125 g of each of ampicillin and flucloxacillin with 50 ml of *phosphate buffer pH 7.0* and filter through glass-fibre paper (Whatman GF/C is suitable). Solution (2) contains 0.28% w/v of *ampicillin trihydrate BPCRS* in *phosphate buffer pH 7.0*. Solution (3) contains 0.26% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. Solution (4) contains 0.28% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solutions (1) to (3) use the solutions described under test A. Solution (4) contains 0.26% w/v of each of *cloxacillin sodium BPCRS*, *dicloxacillin sodium EPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer* *pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows three clearly separated principal spots.

C. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those of the principal peaks in the chromatograms obtained with solutions (2) and (3).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) add a quantity of the mixed contents of 20 capsules containing the equivalent of 0.25 g of each of ampicillin and flucloxacillin to 350 ml of *water*, mix with the aid of ultrasound for 15 minutes, cool and dilute to 500 ml with *water*, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.05% w/v of *anhydrous ampicillin BPCRS*. Solution (3) contains 0.056% w/v of *flucloxacillin sodium BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 50 volumes of *methanol* and 50 volumes of a buffer solution containing 0.01M *diammonium hydrogen orthophosphate* and 0.005M *tetrabutylammonium hydroxide*, the pH of the solution being adjusted to 7.0 with *orthophosphoric acid* and (c) a detection wavelength of 262 nm.

Calculate the content of $C_{16}H_{19}N_3O_4S$ and of $C_{19}H_{17}ClFN_3O_5S$ using the declared content of $C_{16}H_{19}N_3O_4S$ in *anhydrous ampicillin BPCRS* and the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

LABELLING

The label states the quantity of Ampicillin Trihydrate in terms of the equivalent amount of ampicillin and the quantity of Flucloxacillin Sodium in terms of the equivalent amount of flucloxacillin.

The label states that the preparation contains penicillin.

Co-fluampicil Oral Suspension

General Notices

Flucloxacillin and Ampicillin Oral Suspension

Action and use

Penicillin antibacterial.

DEFINITION

Co-fluampicil Oral Suspension is a suspension containing equal amounts of Flucloxacillin Magnesium Octahydrate and Ampicillin Trihydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral suspension as directed on the label. The suspension examined immediately after preparation, unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ampicillin, $C_{16}H_{19}N_3O_4S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dilute a quantity of the oral suspension containing the equivalent of 0.125 g of each of ampicillin and flucloxacillin to 50 ml with *phosphate buffer pH 7.0*. Solution (2) contains 0.28% w/v of *ampicillin trihydrate BPCRS* in *phosphate buffer pH 7.0*. Solution (3) contains 0.26% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. Solution (4) contains 0.28% w/v of each of *amoxicillin trihydrate BPCRS* and *ampicillin trihydrate BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate (Merck silanised silica gel 60 F_{254s} (RP-18) plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solutions (1) to (3) use the solutions described under test A. Solution (4) contains 0.26% w/v of each of *cloxacillin sodium BPCRS*, *dicloxacillin sodium EPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. In the chromatogram obtained with solution (1) the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (4) shows three clearly separated principal spots.

TESTS

Acidity

pH, 4.8 to 5.6, Appendix V L.

ASSAY

To a weighed quantity of the oral suspension containing the equivalent of 0.1 g of each of ampicillin and flucloxacillin add sufficient *water* to produce 100 ml (solution A).

For ampicillin

Dilute 2 ml of solution A to 50 ml with *buffered copper sulphate solution pH 5.2*, transfer 10 ml to a stoppered test tube and heat in a water bath at 75° for 30 minutes. Rapidly cool to room temperature, dilute to 20 ml with *buffered copper sulphate solution pH 5.2* and measure the *absorbance* of the resulting solution at the maximum at 320 nm, Appendix II B, using in the reference cell a solution prepared by diluting 2 ml of solution A to 100 ml with *buffered copper sulphate solution pH 5.2*. Calculate the content of $C_{16}H_{19}N_3O_4S$ from the *absorbance* obtained by carrying out the operation at the same time using 2 ml of a solution prepared by dissolving 0.1 g of *anhydrous ampicillin BPCRS* in 100 ml of *water*, diluting to 50 ml with *buffered copper sulphate solution pH 5.2* and beginning at the words 'transfer 10 ml ...' and from the declared content of $C_{16}H_{19}N_3O_4S$ in *anhydrous ampicillin BPCRS*. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{16}H_{19}N_3O_4S$, weight in volume.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

For flucloxacillin

Dilute 2 ml of solution A to 100 ml with 1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 352 nm, Appendix II B, after exactly 11 minutes using 1M *hydrochloric acid* in the reference cell. Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ from the *absorbance* obtained by carrying out the operation at the same time using 2 ml of a solution prepared by dissolving 0.11 g of *flucloxacillin sodium BPCRS* in 100 ml of *water* and from the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS.* Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{19}H_{17}ClFN_3O_5S$, weight in volume.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The oral suspension should be stored at the temperature and used within the period stated on the label.

LABELLING

The label states the quantity of Ampicillin Trihydrate in terms of the equivalent amount of ampicillin and the quantity of Flucloxacillin Magnesium Octahydrate in terms of the equivalent amount of flucloxacillin.

The label states that the preparation contains penicillin.

Colchicine Tablets

General Notices

DEFINITION

Colchicine tablets contain Colchicine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of colchicine, $C_{22}H_{25}NO_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the powdered tablets containing 1 mg of Colchicine with 0.2 ml of *sulphuric acid* in a white dish; a lemon colour is produced. Add 0.05 ml of *nitric acid*; the colour changes to greenish blue and then rapidly becomes reddish and finally yellow or almost colourless. Add an excess of 5M *sodium hydroxide*; the colour changes to red.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using *silica gel* HF_{254} as the coating substance and a mixture of 1 volume of 13.5M *ammonia*, 25 volumes of *1,2-dichloroethane* and 50 volumes of *acetone* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 5 mg of Colchicine with 5 ml of *chloroform*, filter and evaporate the filtrate to dryness in a current of air. Dissolve the residue as completely as possible in 0.1 ml of *ethanol (96%)*, allow to settle and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 50 volumes with *ethanol (96%)*. For solution (3) dilute 1 volume of solution (2) to 2 volumes with *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

Uniformity of content

Tablets containing less than 2 mg of Colchicine comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure in subdued light. Place one tablet in a centrifuge tube and add 10 ml of *absolute ethanol*. Crush the tablet to a fine powder, shake for 30 minutes, centrifuge and wash the residue with *absolute ethanol*. Combine the extract and washings and add sufficient *absolute ethanol* to produce a 0.001% w/v solution. Measure the *absorbance* of the resulting solution at the maximum at 350 nm, Appendix II B. Calculate the content of $C_{22}H_{25}NO_6$ taking 440 as the value of *A* (1%, 1 cm) at the maximum at 350 nm.

ASSAY

Weigh and powder 20 tablets. Carry out the following procedure in subdued light. To a quantity of the powder containing 0.5 mg of Colchicine add 10 ml of *absolute ethanol* and shake for 30 minutes. Centrifuge, wash the residue with *absolute ethanol*, dilute the combined extract and washings to 50 ml with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 350 nm, Appendix II B. Calculate the content of $C_{22}H_{25}NO_6$ taking 440 as the value of *A* (1%, 1 cm) at the maximum at 350 nm.

STORAGE

Colchicine Tablets should be protected from light.

Colecalciferol Injection

General Notices

Action and use

Vitamin D analogue (Vitamin D_3).

DEFINITION

Colecalciferol Injection is a sterile solution containing 0.75% w/v of Colecalciferol in Ethyl Oleate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of colecalciferol, $C_{27}H_{44}O$

0.67 to 0.83% w/v.

CHARACTERISTICS

A pale yellow, oily liquid.

IDENTIFICATION

To 1 ml of a 0.2% v/v solution of the injection in *ethanol-free chloroform* add 9 ml of *antimony trichloride solution*. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 500 nm.

ASSAY

Carry out the following procedure in subdued light. Dilute 0.1 g of the injection to 50 ml with dry *1,2-dichloroethane* that has been purified by passing it through a column of *silica gel*. To 1 ml of this solution add rapidly 9 ml of *antimony trichloride in dichloroethane solution* and measure the *absorbance* of the solution at 500 and 550 nm, Appendix II B, 90 to 120 seconds after adding the reagent. Repeat the operation using 1 ml of a 0.002% w/v solution of *colecalciferol BPCRS* in the dry, purified 1,2-dichloroethane, beginning at the words 'add rapidly 9 ml of …'. Calculate the result of the assay from the difference between the absorbances at 500 and 550 nm and using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS*. Calculate the percentage w/v of colecalciferol taking 0.87 g as the value of the weight per ml of the injection.

STORAGE

Colecalciferol injection should be protected from light.

LABELLING

The label states (1) that the preparation is for intramuscular use only; (2) the equivalent number of IU (units) of antirachitic activity (vitamin D) in 1 ml.

Each µg of Colecalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

When calciferol injection is prescribed or demanded, Colecalciferol Injection or Ergocalciferol Injection shall be dispensed or supplied.

Colecalciferol Tablets

General Notices

Action and use

Vitamin D analogue (Vitamin D_3).

DEFINITION

Colecalciferol Tablets contain Colecalciferol.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of colecalciferol, $C_{27}H_{44}O$

90.0 to 125.0% of the stated amount.

IDENTIFICATION

A. In the test for Uniformity of content, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

B. Extract one tablet, in powder, with 5 ml of *ethanol-free chloroform*, filter and to 1 ml of the filtrate add 9 ml of *antimony trichloride solution*. A brownish red colour is produced.

TESTS

Uniformity of content

Tablets containing less than 2 mg of colecalciferol comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure in subdued light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.001% w/v of *colecalciferol BPCRS* in *hexane*. For tablets containing more than 0.25 mg prepare solution (2) in the following manner. Add 4 ml of *water* to one tablet in an amber flask and disperse with the aid of ultrasound. Add 12 ml of *dimethyl sulphoxide*, mix, extract with 100 ml of *hexane* by shaking for 30 minutes, centrifuge the hexane layer and use the clear supernatant liquid. For tablets containing 0.25 mg or less carry out the same procedure but using 2 ml of *water*, 6 ml of *dimethyl sulphoxide* and 25 ml of *hexane*. For solution (3) dissolve 0.5 g of *colecalciferol for performance test EPCRS* in 2 ml of *toluene* and dilute to 10 ml with the mobile phase; heat under a reflux condenser in a water bath at 90° for 45 minutes and cool.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Partisil is suitable), (b) a mixture of 8 volumes of *pentan-1-ol* and 992 volumes of *hexane* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Inject a suitable volume of solution (3) and record the chromatogram using a sensitivity such that the height of the peak due to colecalciferol is more than 50% of full-scale deflection. When the chromatogram is recorded under the prescribed conditions, approximate retention times relative to colecalciferol are 0.4 for precolecalciferol and 0.5 for *trans*-colecalciferol. The test is not valid unless the *resolution factor* between the peaks corresponding to precolecalciferol and *trans*-colecalciferol is at least 1.0. If necessary, adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution.

Inject a suitable volume of solution (1) and record the chromatogram using a sensitivity such that the height of the peak due to colecalciferol is more than 50% of full-scale deflection. Repeat the operation using solution (2) and injecting the same volume.

Calculate the content of colecalciferol, $C_{27}H_{44}O$, in each tablet using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS* and using peak heights.

ASSAY

Carry out the following procedure in subdued light. Weigh and powder 25 tablets, or more if necessary. To a quantity of the powder containing 6 mg of colecalciferol add 50 ml of *ethanol (96%)*, 14 ml of *glycerol* and 20 ml of a 50% w/v solution of *potassium hydroxide*. Boil under a reflux condenser for 30 minutes, swirling occasionally, add 110 ml of *water* and allow to stand for 10 minutes with occasional stirring. Cool and add sufficient *ethanol (96%)* to produce 250 ml. Shake 5 ml with 25 ml of *petroleum spirit (boiling range, 40° to 60°)* for 3 minutes and evaporate duplicate 5 ml portions of the extract to dryness in a current of *oxygen-free nitrogen*. Dissolve each residue in 1 ml of *ethanol-free chloroform*, add rapidly 9 ml of *antimony trichloride solution* and measure the *absorbance* of each solution at 500 nm and at 550 nm, 90 to 120 seconds after adding the reagent, Appendix II B. Repeat the operation using duplicate 1 ml portions of a solution containing a known amount of *colecalciferol BPCRS* in *ethanol-free chloroform* and beginning at the words 'add rapidly 9 ml of *antimony trichloride solution* …'. Subtract the absorbance at 550 nm from that at 500 nm and calculate the content of colecalciferol in mg from the average value so obtained and from the amount of colecalciferol in the reference solution using the declared content of $C_{27}H_{44}O$ in *colecalciferol BPCRS*.

LABELLING

The label states the equivalent number of IU (units) of antirachitic activity (vitamin D) per tablet.

Each µg of Colecalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

When calciferol tablets are prescribed or demanded, Colecalciferol Tablets or Ergocalciferol Tablets shall be dispensed or supplied.

Colestipol Granules

General Notices

Action and use

Lipid-regulating drug.

DEFINITION

Colestipol Granules contain Colestipol Hydrochloride with or without suitable excipients.

The granules comply with the requirements stated under Granules and with the following requirements.

IDENTIFICATION

Carry out the method for *gas chromatography*, Appendix III B, using a suitable gas chromatograph fitted with a pyrolysis unit.

To prepare the sample, mix 1 part of n*-eicosane* and 4 parts of the granules and grind the mixture in a mortar with *chloroform* until the preparation being examined is uniformly coated with the *n*-eicosane. Prepare the standard in the same manner but adding 4 parts of *colestipol hydrochloride BPCRS* in place of the preparation being examined. Load the sample and the standard separately into the pyrolysis unit.

The chromatographic procedure may be carried out using a glass column (1.8 m × 3 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) (Chromosorb W is suitable) coated with 0.25% w/w of *potassium hydroxide* and 5% w/w of *polyethylene glycol 20,000* (Carbowax 20M is suitable) maintained at about 85° with the detector at about 270°. The pyrolysis unit is capable of attaining a temperature of about 1000° when fitted with a platinum ribbon probe and using *helium* as the carrier gas with a flow rate of 60 ml per minute. Operate the unit in accordance with the manufacturer's instructions to obtain a pyrogram for *colestipol hydrochloride BPCRS* that is similar to that supplied with the reference material.

The pyrogram obtained with the substance being examined is concordant with that obtained with *colestipol hydrochloride BPCRS*.

TESTS

Acidity or alkalinity

Shake a suspension containing 10% w/w of colestipol hydrochloride in a stoppered vial at approximately 10-minute intervals for 1 hour, and centrifuge. Transfer a portion of the clear supernatant liquid to a suitable container and record the pH as soon as the reading has stabilised. The pH is 5.3 to 7.5, Appendix V L.

Uniformity of weight

Comply with the test for Uniformity of weight for effervescent granules described under Granules.

Water absorption capacity

A quantity of the granules containing 1 g of colestipol hydrochloride absorbs not less than 3.3 g and not more than 5.3 g of *water* when determined in the following manner. Transfer a quantity of the granules containing 5 g of colestipol hydrochloride to a dry, plastic container and add 80 g of *water*. Cover the container and allow the resulting suspension to equilibrate for 72 hours. Filter the resulting slurry through a medium-porosity fritted-glass funnel (KIMAX 60 ml-40M is suitable) at a pressure of 2 kPa; collect the filtrate in a tared, plastic container, disconnecting the vacuum 2 minutes after collection of the last portion of the filtrate. Immediately weigh the container and the filtrate and determine the weight, in g, of the filtrate. Calculate the weight of water absorbed from the difference between the weight of the filtrate and the original weight of *water* used in the test.

Cholate binding capacity

A quantity of the granules containing 1 g of colestipol hydrochloride binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate, determined by the following method. Prepare a solution containing 1.0% w/v of *sodium cholate* and 0.9% w/v of *sodium chloride* in *water* containing 1.8% v/v of 1M *sodium hydroxide* (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 1 volume of solution A with 1 volume of the mobile phase. For solution (2) transfer a quantity of the granules containing 1 g of colestipol hydrochloride to a ground-glass-stoppered flask, add 100 ml of solution A and shake vigorously for 90 minutes with the flask positioned horizontally. Remove the flask from the shaker, allow the contents to settle for 5 minutes and filter through a 0.45-µm filter, discarding the first 5 ml of filtrate. Dilute 1 volume of the filtrate with 1 volume of the mobile phase. Solution (3) contains 0.45% w/v of *cholic acid BPCRS* in a solution prepared by mixing equal volumes of *acetonitrile* containing 2 ml per litre of *orthophosphoric acid* and *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 mm) (Zorbax C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of a solution containing 2.76 g of *anhydrous sodium dihydrogen orthophosphate* in 450 ml of *water* adjusted to pH 3.0 with *orthophosphoric acid*, 100 ml of *methanol* and 450 ml of *acetonitrile* and (c) a detection wavelength of 214 nm.

Inject 20 µl of each solution. The retention time of the peak due to cholic acid is about 6 minutes. From the chromato-grams obtained determine the exact concentration of sodium cholate in solutions (1) and (2) in mg per ml using the declared content of cholic acid in *cholic acid BPCRS* and taking each g of cholic acid to be equivalent to 1.054 g of sodium cholate. The cholate binding capacity in mEq is determined by the expression:

(concentration of sodium cholate in solution (1) – concentration of sodium cholate in solution (2)) × 0.2325.

Colestyramine Oral Powder

General Notices

Action and use

Lipid-regulating drug.

DEFINITION

Colestyramine Oral Powder contains Colestyramine with suitable excipients.

The oral powder complies with the requirements stated under Oral Powders and with the following requirements.

Content of anhydrous colestyramine

85.0 to 115.0% of the stated amount.

IDENTIFICATION

To a quantity of the powder containing the equivalent of 0.5 g of anhydrous colestyramine add 100 ml of 0.1M *hydrochloric acid*, stir and heat on a water bath for 10 minutes. Filter, wash the residue with three 50 ml quantities of *water* and dry at 70° at a pressure not exceeding 7 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of colestyramine *(RS 369)*.

TESTS

Styrene

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powder containing the equivalent of 2 g of anhydrous colestyramine with 10 ml of *acetone* for 30 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.00002% w/v of *styrene* in *acetone*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of equal volumes of *acetonitrile* and *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Inject separately 20 µl of each solution. In the chromatogram obtained with solution (1) the area of any peak corresponding to styrene is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1 ppm).

ASSAY

Prepare a solution of *sodium glycocholate* by dissolving 1.5 g in 40 ml of hot *water*, cooling and diluting to 50 ml with *water* (solution A). To a quantity of the powder containing the equivalent of about 0.1 g of anhydrous colestyramine add 25 ml of *water* and shake for 15 minutes. Centrifuge and carefully decant and discard the liquid layer. Repeat the procedure with a further 25 ml of *water*. Dry the washed residue at 100° for 2 hours. To the residue add

10 ml of solution A, shake mechanically for 2 hours and then centrifuge (solution B). Prepare solution C in the same manner using 0.11 g of *colestyramine BPCRS*, beginning at the words 'add 10.0 ml of solution A…'. Dilute, separately, 2 ml of solution A, 2 ml of solution B and 2 ml of solution C to 200 ml with *water*. To 1 ml of each solution in separate 10 ml graduated flasks add 4 ml of *sulphuric acid (80%)*. Loosely stopper the flasks and heat at 60° for 15 minutes, cool and dilute to volume with *sulphuric acid (80%)*. Allow the solutions to stand for 1 hour. Measure the *absorbance* of each solution at the maximum at about 318 nm, Appendix II B, using *water* in the reference cell. Calculate the quantity of colestyramine in the powder using the following expression:

$$\frac{M(A_1 - A_2)W_1W_3}{2(A_1 - A_3)W_2}$$

where M = stated value, in g, of sodium glycocholate absorbed per g of colestyramine BPCRS,

A_1 = absorbance of solution A,

A_2 = absorbance of solution B,

A_3 = absorbance of solution C,

W_1 = weight of *colestyramine BPCRS*,

W_2 = weight of powder being examined,

W_3 = average weight of content of sachet.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of anhydrous cholestyramine.

Colistimethate Injection

General Notices

Action and use

Antibacterial.

DEFINITION

Colistimethate Injection is a sterile solution of Colistimethate Sodium in Sodium Chloride Intravenous Infusion. It is prepared by dissolving Colistimethate Sodium for Injection in the requisite amount of Sodium Chloride Intravenous Infusion.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Colistimethate Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

COLISTIMETHATE SODIUM FOR INJECTION

Colistimethate Sodium for Injection is a sterile material consisting of Colistimethate Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using a *TLC silica gel G plate* and a mixture of 25 parts of *water* and 75 parts of *phenol* as the mobile phase. Apply separately to the plate, as 10-mm bands, 5 µl of each of the following solutions. For solution (1) dissolve a quantity containing 62,500 IU in 1 ml of a mixture of equal volumes of *hydrochloric acid* and *water*, heat at 135° in a sealed tube for 5 hours, evaporate to dryness on a waterbath, continue the heating until any residual hydrochloric acid has evaporated and dissolve the residue in 0.5 ml of *water*. Solutions (2) to (5) contain (2) 0.2% w/v of *leucine*, (3) 0.2% w/v of *threonine*, (4) 0.2% w/v of *phenylalanine* and (5) 0.2% w/v of *serine* in *water*, respectively. Place the plate in the chromatographic tank so that it is not in contact with the mobile phase. Allow the plate to become impregnated with the vapour of the solvent for at least 12 hours and develop over a path of 12 cm using the same mobile phase. Dry the plate at 100° to 105°, spray with *ninhydrin solution R1*, and heat at 110° for 5 minutes. The chromatogram obtained with solution (1) shows zones corresponding to those in the chromatograms obtained with solutions (2) and (3) but shows no zones corresponding to those in the chromatograms obtained with solutions (4) and (5). The chromatogram obtained with solution (1) also shows a zone with a very low Rf value (2,4-diaminobutyric acid).

B. Dissolve a quantity containing 125,000 IU in 5 ml of *water*. Heat 0.5 ml of the solution with 0.5 ml of *chromotropic acid–sulphuric acid solution* at 100° for 30 minutes. A purple colour is produced (distinction from colistin sulphate).

C. Dissolve a quantity containing 625,000 IU in 1 ml of 1M *hydrochloric acid* and add 0.5 ml of 0.01M *iodine*. The colour is discharged (distinction from colistin sulphate) and the resulting solution yields reaction A characteristic of *sulphates*, Appendix VI.

D. Yield reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

Dissolve a quantity in sufficient *carbon dioxide-free water* to produce a solution containing 125,000 IU per ml. The pH of the resulting solution, measured 30 minutes after preparation, is 6.5 to 8.5, Appendix V L.

Free colistin

Dissolve a quantity containing 1,000,000 IU in 3 ml of *water*, add 0.1 ml of a 10% w/v solution of *silicotungstic acid* and allow to stand for 10 to 20 seconds. The resulting solution is not more opalescent than *reference suspension II*, Appendix IV A.

Loss on drying

When dried over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa for 3 hours, lose not more than 5.0% of their weight. Use 1 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C, using method D. Dissolve the contents of the sealed container in *water BET* to give a solution containing 250,000 IU per ml (solution A). The endotoxin limit concentration of solution A is 43.75 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of each of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Mix the contents of the 10 containers and carry out the *microbiological assay of antibiotics,* Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. For a container of average content weight, the upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the total number of IU (units) contained in it.

Colistin Tablets

General Notices

Action and use

Antibacterial.

DEFINITION

Colistin Tablets contain Colistin Sulphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

To a quantity of the powdered tablets containing 200,000 IU add 10 ml of *water*, shake and filter. Use the filtrate for the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using *silica gel G* as the coating substance and a mixture of 75 parts of *phenol* and 25 parts of *water* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions as 10-mm bands. For solution (1) add 0.5 ml of *hydrochloric acid* to 0.5 ml of the filtrate, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water bath, continue to heat until any residual hydrogen chloride has been removed, dissolve the residue in 0.5 ml of *water* and centrifuge, if necessary. Solutions (2) to (5) are solutions in *water* containing 0.2% w/v each of (2) L-*leucine*, (3) L-*threonine*, (4) L-*phenylalanine* and (5) L-*serine*. Place the plate in the tank so that it is not in contact with the mobile phase and expose it to the vapour of the mobile phase for at least 12 hours. Develop the chromatograms, remove the plate, heat it at 100° to 105°, spray with *ninhydrin solution R1* and heat the plate at 110° for 5 minutes. The bands in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with solutions (2) and (3) and do not correspond to those in the chromatograms obtained with solutions (4) and (5). The chromatogram obtained with solution (1) also shows a band with a very low Rf value (2,4-diaminobutanoic acid).

B. Heat 0.5 ml of the filtrate with 0.5 ml of *chromotropic acid–sulphuric acid solution* at 100° for 30 minutes. No purple colour is produced (distinction from colistin sulphomethate).

C. The filtrate yields reaction A characteristic of *sulphates*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Dissolve a suitable quantity of the powder in *phosphate buffer pH 6.0* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated number of IU.

STORAGE

Colistin Tablets should be protected from light.

LABELLING

The strength is stated as the number of IU (units) in each tablet.

Co-magaldrox Oral Suspension

General Notices

Magnesium Hydroxide and Aluminium Hydroxide Oral Suspension

Action and use

Antacid.

DEFINITION

Co-magaldrox Oral Suspension is a suspension containing Magnesium Hydroxide and Dried Aluminium Hydroxide in a suitable flavoured vehicle. The amount of dried aluminium hydroxide is adjusted to give the required content of Al_2O_3.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium hydroxide, $Mg(OH)_2$

90.0 to 110.0% of the stated amount.

Content of Al_2O_3

45.4 to 58.8% of the stated amount of dried aluminium hydroxide.

IDENTIFICATION

A. Mix 5 ml with 10 ml of 2M *hydrochloric acid*, add 5 drops of *methyl red solution* and heat to boiling. Add 6M *ammonia* until the solution becomes yellow, continue boiling for 2 minutes and filter. To 1 ml of the filtrate add 1 ml of 6M *ammonia* and 1 ml of 2M *ammonium chloride*; no precipitate is produced. Add 0.25M *disodium hydrogen orthophosphate*; a white, crystalline precipitate is produced which is insoluble in 6M *ammonia*.

B. To 5 ml add 10 ml of 2M *hydrochloric acid*. The solution yields the reaction characteristic *of aluminium salts*, Appendix VI.

TESTS

Heavy metals

Not more than 10 ppm when determined by the following method. Dissolve 8.0 g in 20 ml of 3M *hydrochloric acid* with the aid of heat, filter if necessary and add sufficient *water* to produce 50 ml (solution A). Transfer 25 ml of solution A to a 50 ml *Nessler cylinder*, adjust the pH to between 3.0 and 4.0 using 6M *ammonia*, add sufficient *water* to produce 40 ml and mix. Add 10 ml of freshly prepared *hydrogen sulphide solution*, mix and allow to stand for 5 minutes. When viewed down the vertical axis of the tube against a white background the colour of the solution is not more intense than that of a solution prepared at the same time and in the same manner using a solution of 2 ml of *lead standard solution (20 ppm Pb)* diluted to 25 ml with *water* in place of solution A adjusted to a pH between 3.0 and 4.0 using either

1M *acetic acid* or 6M *ammonia* and beginning at the words 'add sufficient *water* to produce 40 ml …' (standard solution). The colour of the standard solution is not as intense as that of a solution prepared at the same time and in the same manner but using a mixture of 25 ml of solution A and 2 ml of *lead standard solution (20 ppm Pb)* adjusted to a pH between 3.0 and 4.0 using either 1M *acetic acid* or 6M *ammonia* and beginning at the words 'add sufficient *water* to produce 40 ml …'.

Microbial contamination

Carry out a quantitative evaluation for enterobacteria and certain other Gram-negative bacteria, Appendix XVI B1. 0.01 ml of the preparation gives a negative result, Table I (most probable number of bacteria per gram fewer than 10^2).

ASSAY

For Al_2O_3

To a weighed quantity containing 1.5 g of dried aluminium hydroxide add 20 ml of *water*, stir and slowly add 10 ml of *hydrochloric acid*. Heat gently, if necessary, to aid solution, cool, filter, wash the filter well with *water*, dilute the combined filtrate and washings to 200 ml with *water* and mix. Reserve a portion of the solution for the Assay for magnesium hydroxide. To 10 ml add 20 ml of *water* and, with continuous stirring, 25 ml of 0.05M *disodium edetate VS* followed by 20 ml of a mixture of equal volumes of 2M *ammonium acetate* and 2M *acetic acid*. Heat near the boiling point for 5 minutes, cool, add 50 ml of *absolute ethanol* and 3 ml of a freshly prepared 0.025% w/v solution of *dithizone* in *absolute ethanol*. Titrate the excess of disodium edetate with 0.05M *zinc sulphate VS* until the colour of the solution changes from greenish blue to reddish violet. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3. Determine the *weight per ml* of the suspension, Appendix V G, and calculate the percentage content of Al_2O_3, weight in volume.

For magnesium hydroxide

To a volume of the solution reserved in the Assay for Al_2O_3 containing about 40 mg of magnesium hydroxide add 200 ml of *water* and 20 ml of *triethanolamine* and stir. Add 10 ml of *ammonia buffer pH 10.9* and cool the solution to between 3° and 4° by immersion in iced water. Titrate the cooled solution with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.916 mg of $Mg(OH)_2$. Using the weight per ml of the suspension, calculate the percentage content of $Mg(OH)_2$, weight in volume.

STORAGE

Co-magaldrox Oral Suspension should not be allowed to freeze.

Co-magaldrox Tablets

General Notices

Magnesium Hydroxide and Aluminium Hydroxide Tablets

Action and use

Antacid.

DEFINITION

Co-magaldrox Tablets contain Magnesium Hydroxide and Dried Aluminium Hydroxide. The amount of Dried Aluminium Hydroxide is adjusted to give the required content of Al_2O_3.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of magnesium hydroxide, $Mg(OH)_2$

90.0 to 110.0% of the stated amount.

Content of Al_2O_3

45.0 to 55.0% of the stated amount of dried aluminium hydroxide.

IDENTIFICATION

A. To a quantity of the powdered tablets containing about 0.2 g of magnesium hydroxide in 10 ml of 2M *hydrochloric acid* add 0.25 ml of *methyl red solution* and heat to boiling. Add 6M *ammonia* until the solution becomes yellow, continue boiling for 2 minutes and filter. To 1 ml of the filtrate add 1 ml of 6M *ammonia* and 1 ml of 2M *ammonium chloride*; no precipitate is produced. Add 0.25M *disodium hydrogen orthophosphate*; a white crystalline precipitate is produced which is insoluble in 6M *ammonia*.

B. Shake a quantity of the powdered tablets containing 0.25 g of dried aluminium hydroxide with 25 ml of 2M *hydrochloric acid* and filter. The filtrate yields the reaction characteristic of *aluminium salts*, Appendix VI.

ASSAY

For Al_2O_3

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 1.5 g of dried aluminium hydroxide add 20 ml of *water*, stir and slowly add 30 ml of 3M *hydrochloric acid*. Heat gently, if necessary, to aid solution, cool, filter, wash the filter well with *water*, dilute the combined filtrate and washings to 200 ml with *water* and mix. Reserve a portion of the solution for the Assay for magnesium hydroxide. To 10 ml add 20 ml of *water* and, with continuous stirring, 25 ml of 0.05M *disodium edetate VS* followed by 20 ml of a mixture of equal volumes of 2M *ammonium acetate* and 2M *acetic acid*. Heat near the boiling point for 5 minutes, cool, add 50 ml of *absolute ethanol* and 3 ml of a freshly prepared 0.025% w/v solution of *dithizone* in *absolute ethanol*. Titrate the excess of disodium edetate with 0.05M *zinc sulphate VS* until the colour of the solution changes from greenish blue to reddish violet.

Each ml of 0.05M *disodium edetate VS* is equivalent to 2.549 mg of Al_2O_3.

For magnesium hydroxide

To a volume of the solution reserved in the Assay for Al_2O_3 containing about 40 mg of magnesium hydroxide add 200 ml of *water* and 20 ml of *triethanolamine* and stir. Add 10 ml of *ammonia buffer pH 10.9* and cool the solution to between 3° and 4° by immersion in iced water. Titrate the cooled solution with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.916 mg of $Mg(OH)_2$.

Compound Cardamom Tincture

General Notices

DEFINITION

Cardamom Oil	0.450 ml
Caraway Oil	0.400 ml
Cinnamon Oil	0.225 ml
Cochineal, in *moderately coarse powder*	7 g
Glycerol	50 ml
Ethanol (60 per cent)	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Moisten the Cochineal with a sufficient quantity of Ethanol (60 per cent) and prepare 900 ml of tincture by *percolation*, Appendix XI F. Add the Cardamom Oil, the Caraway Oil, the Cinnamon Oil and the Glycerol and sufficient Ethanol (60 per cent) to produce 1000 ml; mix. Filter, if necessary.

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

TESTS

Ethanol content

52 to 57% v/v, Appendix VIII F, Method III.

Glycerol

4.5 to 5.5% v/v when determined by the following method. Dilute 20 ml to 100 ml with *water*. To 20 ml of this solution add 100 ml of *water* and 1 g of *activated charcoal* and boil under a reflux condenser for 15 minutes. Filter and wash the filter and charcoal with sufficient *water* to produce 150 ml. Add 0.25 ml of *bromocresol purple solution* and neutralise with 0.1M *sodium hydroxide* or 0.05M *sulphuric acid* to the blue colour of the indicator. Add 1.4 g of *sodium periodate* and allow to stand for 15 minutes. Add 3 ml of *propane-1,2-diol*, shake and allow to stand for 5 minutes. Add 0.25 ml of *bromocresol purple solution* and titrate with 0.1M *sodium hydroxide VS* to the same blue colour. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 9.210 mg of glycerol. Calculate the percentage v/v of glycerol, taking its weight per ml to be 1.260 g.

Relative density

0.925 to 0.937, Appendix V G.

Concentrated Camphor Water

General Notices

DEFINITION

Racemic Camphor	40 g
Ethanol (90 per cent)	600 ml
Water	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Racemic Camphor in the Ethanol (90 per cent) and add, gradually, with vigorous shaking after each addition, sufficient Water to produce 1000 ml.

The water complies with the requirements stated under Aromatic Waters and with the following requirement.

Ethanol content

51 to 55% v/v, Appendix VIII F.

Concentrated Cinnamon Water

General Notices

DEFINITION

Cinnamon Oil	20 ml
Ethanol (90 per cent)	600 ml
Water	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Cinnamon Oil in the Ethanol (90 per cent) and add gradually, with vigorous shaking after each addition, sufficient Water to produce 1000 ml. Add 50 g of previously sterilised Purified Talc, or other suitable filtering aid, allow to stand for a few hours, shaking occasionally, and filter.

The water complies with the requirements stated under Aromatic Waters and with the following requirements.

TESTS

Ethanol content

52 to 56% v/v, Appendix VIII F.

Weight per ml

0.914 to 0.922 g, Appendix V G.

Co-proxamol Tablets

General Notices

Dextropropoxyphene Hydrochloride and Paracetamol Tablets

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Co-proxamol Tablets contain Dextropropoxyphene Hydrochloride and Paracetamol in the proportions, by weight, 1 part to 10 parts.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dextropropoxyphene hydrochloride, $C_{22}H_{29}NO_2,HCl$

95.0 to 105.0% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Dextropropoxyphene Hydrochloride with 20 ml of 0.1M *hydrochloric acid* for 5 minutes and filter. To the filtrate add 5 ml of 2M *sodium hydroxide*, extract with two 25 ml quantities of *dichloromethane*, wash the combined extracts with 10 ml of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2 ml of *dichloromethane* and add 50 µl, drop wise, onto the surface of a disc prepared from about 0.3 g of *potassium bromide*, allowing the solvent to evaporate between applications; dry the disc at 50° for 2 minutes. The *infrared absorption spectrum* of the resulting thin film, Appendix II A, is concordant with the *reference spectrum* of dextropropoxyphene *(RS 091)*.

B. Shake a quantity of the powdered tablets containing 0.325 g of Paracetamol with 10 ml of *acetone* for 5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 ml of *phosphate buffer pH 5.8*, at a temperature of 37°, as the medium.

PROCEDURE

After 45 minutes withdraw a 20 ml sample of the medium and filter. Dilute the filtrate with 0.1M *sodium hydroxide*, if necessary, to give a solution expected to contain about 0.00075% w/v of paracetamol. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 257 nm using 0.1M *sodium hydroxide* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of *A*(1%, 1 cm) at the maximum at 257 nm.

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions .

(1) 0.001% w/v of *4-aminophenol* in the mobile phase.

(2) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 ml of the mobile phase for 10 minutes and filter.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column ((25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.01M *sodium butanesulphonate* in a mixture of 0.4 volume of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water.*

LIMIT

In the chromatogram obtained with solution (2):

— the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (1) (0.1%);

— peaks with a long retention time may occur due to excipients.

Related substances

A Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.0005% w/v of *4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride BPCRS* and 0.0005% w/v of *(1S,2R)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate*

BPCRS in a mixture of 1 volume of *acetonitrile* and 4 volumes of *water*.

(2) Shake a quantity of the powdered tablets containing 25 mg of Dextropropoxyphene Hydrochloride with 5 ml of *acetonitrile* for 2 minutes, add 5 ml of *water*, shake for a further 5 minutes, dilute to 25 ml with *water*, mix and filter (Whatman GF/F filter paper is suitable).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µl of each solution.

(g) The peaks, in order of emergence, are 4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride and (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of 0.2M *sodium perchlorate*, previously adjusted to pH 2.0 using 7M *hydrochloric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the resolution factor between the two peaks is greater than 1.5.

LIMITS

In the chromatogram obtained with solution (2):

— the areas of any peaks corresponding to 4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride and (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate are not greater than the areas of the respective peaks in the chromatogram obtained with solution (1) (0.5%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Transfer a quantity of the finely-powdered tablets containing 1.0 g of Paracetamol to a ground-glass-stoppered 15 ml centrifuge tube, add 5 ml of *peroxide-free ether*, shake mechanically for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or until a clear supernatant liquid is obtained and use the supernatant liquid.

(2) Dilute 1 ml of solution (1) to 10 ml with *ethanol* (*96%*).

(3) 0.0050% w/v of *4′-chloroacetanilide* in *ethanol* (*96%*).

(4) Dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol* (*96%*) to produce 100 ml.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* $F_{254.}$

(b) Use the mobile phase described below.

(c) Apply 200 µl of solution (1) and 40 µl of each of solutions (2), (3) and (4).

(d) Develop the plate to 14 cm.

(e) After removal of the plate, dry in air, and examine under *ultraviolet light* (*254 nm*).

MOBILE PHASE

10 volumes of *toluene,* 25 volumes of *acetone* and 65 volumes of *chloroform.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

LIMITS

In the chromatogram obtained with solution (1):

— any spot corresponding to 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.005%).

In the chromatogram obtained with solution (2):

— any *secondary spot* with an Rf value lower than that of 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.25%).

ASSAY

Weigh and powder 20 tablets.

For dextropropoxyphene hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Contains 0.0065% w/v of *dextropropoxyphene hydrochloride BPCRS* in a mixture of 40 volumes of *acetonitrile* and 60 volumes of 0.02M *hydrochloric acid*.

(2) Disperse a quantity of the powdered tablets containing 32.5 mg of Dextropropoxyphene Hydrochloride in 100 ml of 0.02M *hydrochloric acid*, mix with the aid of ultrasound for 15 minutes, allow to cool, dilute to 500 ml with a mixture of equal volumes of *acetonitrile* and 0.02M *hydrochloric acid* and filter (Whatman GF/C filter paper is suitable)

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 215 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

40 volumes of *acetonitrile* and 60 volumes of 0.2M *sodium perchlorate*, previously adjusted to pH 2.0 using 7M *hydrochloric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the resolution factor between the two peaks is greater than 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}NO_2$,HCl in the tablets using the declared content of $C_{22}H_{29}NO_2$,HCl in *dextropropoxyphene hydrochloride BPCRS*.

For paracetamol

Disperse a quantity of the powdered tablets containing 0.325 g of Paracetamol in 5 ml of *water*, add 100 ml of *methanol* and shake. Add 300 ml of *water*, shake for 5 minutes, allow to cool, dilute to 500 ml with *water*, mix and filter. Dilute 5 ml of the filtrate to 250 ml with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ in the tablets taking 715 as the value of *A*(1%, 1 cm) at the maximum at 257 nm.

STORAGE

Co-proxamol Tablets should be protected from light.

LABELLING

The label states the quantities of Dextropropoxyphene Hydrochloride and of Paracetamol in each tablet.

Cortisone Tablets

General Notices

Action and use

Corticosteroid.

DEFINITION

Cortisone Tablets contain Cortisone Acetate in *fine powder*.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cortisone acetate, $C_{23}H_{30}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.1 g of cortisone acetate with 5 ml of *chloroform*, filter and evaporate the chloroform. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with a spectrum prepared from the residue obtained by evaporating 5 ml of a 2% w/v solution of *cortisone acetate BPCRS* in *chloroform*. Use the same method of evaporation of the chloroform as that for the tablet extract. Prepare *potassium bromide* discs.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase B.*

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use. For solution (1) mix a quantity of the powdered tablets containing 25 mg of cortisone acetate with 10 ml of the mobile phase, place in an ultrasonic bath for 10 minutes and filter (Whatman GF/C filter is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.002% w/v each *of cortisone acetate BPCRS* and *hydrocortisone acetate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared as follows: mix 400 ml *of acetonitrile* with 550 ml of *water*, allow to equilibrate and adjust the volume to 1000 ml with *water* and (c) a detection wavelength of 254 nm.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 30 minutes.

Inject 20 µl of solution (2). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50% of the full scale of the recorder.

Inject 20 µl of solution (3). When the chromatograms are recorded in the prescribed conditions the retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the *resolution factor* between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject 20 µl of solution (1) and 20 µl of solution (2). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%); the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a 0.3% w/v solution of *sodium dodecyl sulphate* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtrate at the maximum at 242 nm, Appendix II B, using the dissolution medium in the reference cell. Calculate the total content of $C_{23}H_{30}O_6$ in the medium from the *absorbance* obtained from a 0.0028% w/v solution of *cortisone acetate BPCRS* in the medium and from the declared content of $C_{23}H_{30}O_6$ in *cortisone acetate BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 50 ml of a solution in *methanol* containing 0.02% w/v each of *cortisone acetate BPCRS* and *prednisolone* to 100 ml with *water*. For solution (2) add 50 ml of *methanol* to a quantity of the powdered tablets containing 10 mg of cortisone acetate, shake, mix with the aid of ultrasound for 2 minutes, dilute to 100 ml with *water*, shake, centrifuge and use the supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) *methanol (60%)* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{23}H_{30}O_6$ using the declared content of $C_{23}H_{30}O_6$ in *cortisone acetate BPCRS*. The assay is not valid unless the *resolution factor* between the peaks due to cortisone acetate and prednisolone in the chromatogram obtained with solution (1) is at least 5.0.

STORAGE

Cortisone Tablets should be protected from light.

Co-tenidone Tablets

General Notices

Atenolol and Chlortalidone Tablets

Action and use

Beta-adrenoreceptor antagonist + thiazide like diuretic.

DEFINITION

Co-tenidone Tablets contain Atenolol and Chlortalidone in the proportions, by weight, 4 parts to 1 part.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of atenolol, $C_{14}H_{22}N_2O_3$

95.0 to 105.0% of the stated amount.

Content of chlortalidone, $C_{14}H_{11}ClN_2O_4S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF$_{254}$* as the coating substance and a mixture of 30 volumes of 18M *ammonia* and 150 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) remove any film coating from the tablets, powder and shake a quantity of the powdered tablets containing 0.1 g of atenolol with 10 ml of *methanol* for 15 minutes and filter. Solution (2) contains 1.0% w/v of *atenolol BPCRS* in *methanol*. Solution (3) contains 0.25% w/v of *chlortalidone BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) the two principal spots correspond in position, size and intensity to those of the principal spots in the chromatograms obtained with solutions (2) and (3).

B. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those of the principal peaks in the chromatograms obtained with solutions (2) and (3).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) remove any film coating from the tablets, powder and shake a quantity of the powder containing 0.1 g of atenolol (and 0.025 g of chlortalidone) with 25 ml of the mobile phase for 30 minutes with the aid of ultrasound. Filter through a suitable filter (Whatman No I is suitable) and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. For solution (3) dissolve 50 mg of *atenolol impurity standard BPCRS* in 0.1 ml of *dimethyl sulphoxide*, with the aid of gentle heat, and dilute to 100 ml with the mobile phase. Solution (4) contains 0.002% w/v of *2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid*

EPCRS in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 20 volumes of *tetrahydrofuran*, 180 volumes of *methanol* and 800 volumes of 0.025M *potassium dihydrogen orthophosphate* containing 1.0 g of *sodium octanesulphonate* and 0.4 g of *tetrabutylammonium hydrogen sulphate* per litre and adjusted to pH 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 226 nm.

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *atenolol impurity standard BPCRS* and the peaks corresponding to tertiary amine, which is usually a doublet, and bis ether are clearly separated. If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase; increasing the concentration increases the retention time of the tertiary amine.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(4-chloro-3-sulphamoyl-benzoyl)benzoic acid is not greater than the area of the peak in the chromatogram obtained with solution (4) (2%, with reference to the content of chlortalidone), the area of any peak corresponding to blocker acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%, with reference to the content of atenolol), the area of any peak corresponding to either tertiary amine or bis ether is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.25%, with reference to the content of atenolol).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), weigh 20 tablets, remove the film coating, reweigh and powder. Extract a quantity of the powder containing 0.1 g of atenolol with 70 ml of the mobile phase by shaking with the aid of ultrasound for 30 minutes, allow to cool, add sufficient of the mobile phase to produce 100 ml, filter and use the filtrate. Solution (2) contains 0.1% w/v of *atenolol BPCRS* in the mobile phase. Solution (3) contains 0.025% w/v of *chlortalidone BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 10 volumes of *sulphuric acid (10%)*, 50 volumes of *propan-2-ol*, 200 volumes of *acetonitrile* and 740 volumes of *water* containing 0.5 g per litre of *sodium octanesulphonate* and adjusted to pH 3.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 275 nm.

Calculate the content of $C_{14}H_{22}N_2O_3$ and of $C_{14}H_{11}ClN_2O_4S$ using the declared contents of $C_{14}H_{22}N_2O_3$ and of $C_{14}H_{11}ClN_2O_4S$ in *atenolol BPCRS* and in *chlortalidone BPCRS,* respectively.

When atenolol and chlorthalidone tablets are prescribed or demanded, Co-tenidone Tablets shall be dispensed or supplied.

Co-triamterzide Tablets

General Notices

Triamterene and Hydrochlorothiazide Tablets

Action and use

Potassium-sparing diuretic + thiazide diuretic.

DEFINITION

Co-triamterzide Tablets contain Triamterene and Hydrochlorothiazide in the proportions, by weight, 2 parts to 1 part.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of triamterene, $C_{12}H_{11}N_7$

95.0 to 105.0% of the stated amount.

Content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of hydrochlorothiazide with 25 ml of *acetone*, filter, evaporate the filtrate to dryness and dry the residue at 100° for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of hydrochlorothiazide *(RS 178)*.

B. In test A for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (3).

C. In the Assay, the two principal peaks in the chromatogram obtained with solution (3) have the same retention times as the peak due to triamterene in the chromatogram obtained with solution (1) and as the peak due to hydrochlorothiazide in the chromatogram obtained with solution (2).

TESTS

5-Nitroso-2,4,6-triaminopyrimidine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* HF_{254} as the coating substance. Apply separately to the plate, as 1.5-cm bands, two 10-µl applications of each of the following four freshly prepared solutions. For solution (1) shake a quantity of the powdered tablets containing 0.10 g of triamterene with 10 ml of *anhydrous formic acid* for 5 minutes, centrifuge and use the clear supernatant liquid. For solution (2) dissolve 5 mg of *5-nitroso-2,4,6-triaminopyrimidine EPCRS* in 50 ml of *anhydrous formic acid* and dilute 1 volume of the solution to 10 volumes with the same solvent. Prepare solution (3) in the same manner as solution (1) but shaking with 10 ml of solution (2) in place of the formic acid. For solution (4) dissolve 5 mg of *hydrochlorothiazide BPCRS* in 1 ml of *acetone*.

Develop over a path of 5 cm using *ether* as the mobile phase, remove the plate, allow it to dry in air and develop over a path of 10 cm using a 0.05% w/v solution of *fluorescein sodium* in a mixture of 10 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 80 volumes of *ethyl acetate* as the mobile phase. After removal of the plate, dry it in a current of air, expose to ammonia vapour for a few seconds and examine under *ultraviolet light (254* and *365 nm)*. Any band corresponding to 5-nitroso-2,4,6-triaminopyrimidine in the chromatogram obtained with solution (1) is not more intense than the band in the chromatogram obtained with solution (2) (0.1%). The test is not valid unless, in the chromatogram obtained with solution (3), a band corresponding to the band due to hydrochlorothiazide (obtained with solution (4)), appears above, and is clearly separated from, the band due to 5-nitroso-2,4,6-triaminopyrimidine.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of 18M *ammonia,* 10 volumes of *methanol* and 90 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.10 g of triamterene with 20 ml of *dimethyl sulphoxide*, centrifuge and dilute 2 volumes of the supernatant liquid to 50 volumes with *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. For solution (3) dissolve 20 mg of *triamterene BPCRS* in 4 ml of *dimethyl sulphoxide* and add sufficient *methanol* to produce 100 ml. After removal of the plate, allow it to dry in air until the solvent has evaporated and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%, with reference to the content of triamterene) .

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 15 volumes of *propan-2-ol* and 85 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) vigorously shake a quantity of the powdered tablets containing 50 mg of hydrochlorothiazide with 50 ml of *acetone*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of *acetone*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%, with reference to the content of hydrochlorothiazide). Disregard any spot close to the line of application.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 25 ml of *acetonitrile* and 4 ml of *glacial acetic acid* to a quantity of the powdered tablets containing 25 mg of hydrochlorothiazide, mix, immediately add 20 ml of *water*, shake for 15 minutes, dilute to 100 ml with *water* and filter through glass-fibre paper (Whatman GF/A is suitable). Dilute 10 ml of the filtrate to 100 ml with *water*. For solution (2) dissolve 50 mg of *triamterene BPCRS* in 25 ml of *acetonitrile*, add 4 ml of *glacial acetic acid* and immediately add sufficient *water* to produce 100 ml. Dilute 10 ml to 100 ml with *water*. For solution (3) dissolve 25 mg of *hydrochlorothiazide BPCRS* in 25 ml of *acetonitrile*, add 4 ml of *glacial acetic acid* and immediately add sufficient *water* to produce 100 ml. Dilute 10 ml to 100 ml with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm)

(µBondapak C18 is suitable), (b) a mixture of 2 volumes of *methanol*, 18 volumes of *acetonitrile*, 40 volumes of a 0.5% w/v solution of *ammonium chloride* and 40 volumes of 0.01M *sodium perchlorate* as the mobile phase with a flow rate of 2.0 ml per minute and (c) a detection wavelength of 273 nm.

Calculate the content of $C_{12}H_{11}N_7$ and $C_7H_8ClN_3O_4S_2$ using the declared contents of $C_{12}H_{11}N_7$ and $C_7H_8ClN_3O_4S_2$ in *triamterene BPCRS* and *hydrochlorothiazide BPCRS* respectively.

STORAGE

Co-triamterzide Tablets should be protected from moisture.

Co-trimoxazole Intravenous Infusion

General Notices

Trimethoprim and Sulfamethoxazole Intravenous Infusion

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Co-trimoxazole Intravenous Infusion is a sterile solution containing Trimethoprim and the sodium derivative of Sulfamethoxazole. It is prepared immediately before use by diluting Sterile Co-trimoxazole Concentrate with between 25 and 35 times its volume of Glucose Intravenous Infusion or Sodium Chloride Intravenous Infusion.

The intravenous infusion complies with the requirements stated under Parenteral Preparations.

STERILE CO-TRIMOXAZOLE CONCENTRATE

DEFINITION

Sterile Co-trimoxazole Concentrate is a sterile solution of Trimethoprim and sulfamethoxazole sodium, prepared by the interaction of Sulfamethoxazole and Sodium Hydroxide, in the proportion 1 part to 5 parts, in Water for Injections containing 40 to 45% v/v of Propylene Glycol.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

92.5 to 107.5% of the stated amount of trimethoprim.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

92.5 to 107.5% of the stated amount of sulfamethoxazole.

CHARACTERISTICS

A colourless or slightly yellow solution.

IDENTIFICATION

A. Add drop wise to 75 ml of 0.1M *hydrochloric acid* a volume of the solution containing 0.8 g of sulfamethoxazole, stirring continuously. Allow the suspension to stand for 5 minutes and filter through a sintered-glass filter. Wash the residue with 10 ml of *water*, recrystallise from *ethanol (96%)* and dry at 105°. Reserve part of the residue for test D and dissolve the remainder in the minimum volume of a 5% w/v solution of *sodium carbonate*, add 1M *hydrochloric acid* drop wise until precipitation is complete, filter, wash the residue sparingly with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulfamethoxazole *(RS 327)*.

B. To a volume containing 80 mg of trimethoprim add 30 ml of 0.1M *sodium hydroxide* and extract with two 50 ml quantities of *chloroform*. Wash the combined extracts with two 10 ml quantities of 0.1M *sodium hydroxide* and then with 10 ml of *water*, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim *(RS 354)*.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform*, 10 volumes of *methanol* and 5 volumes of *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) evaporate to dryness a volume of the solution containing 0.16 g of sulfamethoxazole, shake the residue with 8 ml of *methanol* and filter. Solution (2) contains 2.0% w/v of *sulfamethoxazole BPCRS* in *methanol*. Solution (3) contains 0.4% w/v of *trimethoprim BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. One of the principal spots in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the other corresponds to that in the chromatogram obtained with solution (3).

TESTS

Alkalinity

pH, 9.5 to 11.0, Appendix V L.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the sterile concentrate with *water BET* to give a solution containing 1 mg of trimethoprim and 5 mg of sulphamethoxazole [6 mg of co-trimoxazole] per ml (solution A). The endotoxin limit concentration of solution A is 0.5 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

For trimethoprim

To a volume containing 48 mg of trimethoprim add 30 ml of 0.1M *sodium hydroxide* and extract with four 50 ml quantities of *chloroform*, washing each extract with the same two 10 ml quantities of 0.1M *sodium hydroxide*. Combine the chloroform extracts, extract with four 50 ml quantities of 1M *acetic acid*, wash the combined extracts with 5 ml of *chloroform* and dilute the aqueous extracts to 250 ml with 1M *acetic acid*. To 10 ml of the resulting solution add 10 ml of 1M *acetic acid* and sufficient *water* to produce 100 ml and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm.

For sulfamethoxazole

To a volume of the solution containing 0.4 g of sulfamethoxazole add 60 ml of *water* and 10 ml of *hydrochloric acid*. Add 3 g of *potassium bromide*, cool in ice and titrate slowly with 0.1M *sodium nitrite VS*, stirring constantly and determining the end point electrometrically. Each ml of 0.1M *sodium nitrite VS* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

STORAGE

Sterile Co-trimoxazole Concentrate should be protected from light.

LABELLING

The label states (1) 'Sterile Co-trimoxazole Concentrate'; (2) the contents of Trimethoprim and of Sulfamethoxazole in a suitable volume; (3) that the solution should be protected from light; (4) that the solution must be diluted with between 25 and 35 times its volume of Glucose Intravenous Infusion or Sodium Chloride Intravenous Infusion immediately before use; (5) that the Co-trimoxazole Intravenous Infusion so prepared is to be administered intravenously; (6) that the intravenous infusion should be discarded if any visible particles appear.

Co-trimoxazole Oral Suspension

General Notices

Trimethoprim and Sulfamethoxazole Oral Suspension

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Co-trimoxazole Oral Suspension is a suspension containing 1.6% w/v of Trimethoprim and 8.0% w/v of Sulfamethoxazole in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

1.44 to 1.76% w/v.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

7.40 to 8.60% w/v.

IDENTIFICATION

A. To a quantity containing 50 mg of trimethoprim add 30 ml of 0.1M *sodium hydroxide* and extract with four 50 ml quantities of *chloroform*. Wash the combined extracts with two 10 ml quantities of 0.1M *sodium hydroxide* and extract with two 50 ml quantities of *chloroform*. Wash the combined chloroform extracts with two 10 ml quantities of 0.1M *sodium hydroxide* and then with 10 ml of *water*. Shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim *(RS 354)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform*, 10 volumes of *methanol* and 5 volumes of *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) add 20 ml of *methanol* to 5 ml of the oral suspension, mix, shake with 10 g of *anhydrous sodium sulphate*, centrifuge and use the supernatant liquid. Solution (2) contains 2.0% w/v of *sulfamethoxazole BPCRS* in *methanol*. Solution (3) contains 0.4% w/v of *trimethoprim BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. One of the principal spots in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the other corresponds to the spot in the chromatogram obtained with solution (3).

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

ASSAY

For trimethoprim

Extract the chloroform solution reserved in the Assay for sulfamethoxazole with four 50 ml quantities of 1M *acetic acid*. Wash the combined extracts with 5 ml of *chloroform* and dilute the aqueous extracts to 250 ml with 1M *acetic acid*. To 10 ml of this solution add 10 ml of 1M *acetic acid* and sufficient *water* to produce 100 ml and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm. Using the weight per ml of the oral suspension, calculate the content of $C_{14}H_{18}N_4O_3$, weight in volume.

For sulfamethoxazole

To 4 g of the oral suspension add 30 ml of 0.1M *sodium hydroxide*, shake and extract with four 50 ml quantities of *chloroform*, washing each extract with the same two 10 ml quantities of 0.1M *sodium hydroxide*. Reserve the combined chloroform extracts for the Assay for trimethoprim. Dilute the combined aqueous solution and washings to 250 ml with *water*, filter and dilute 5 ml of the filtrate to 200 ml with *water* (solution A). Carry out the following procedure protected from light using 2 ml of solution A. Add 0.5 ml of 4M *hydrochloric acid* and 1 ml of a 0.1% w/v solution of *sodium nitrite* and allow to stand for 2 minutes. Add 1 ml of a 0.5% w/v solution of *ammonium sulphamate* and allow to stand for 3 minutes. Add 1 ml of a 0.1% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride* and allow to stand for 10 minutes. Dilute the resulting solution to 25 ml with *water* and measure the *absorbance* at 538 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but using 2 ml of *water* in place of solution A. Dissolve 0.25 g of *sulfamethoxazole BPCRS* in 50 ml of 0.1M *sodium hydroxide* and dilute to 250 ml with *water*. Dilute 5 ml of the resulting solution to 200 ml with *water* (solution B). Repeat the procedure using 2 ml of solution B and beginning at the words 'Add 0.5 ml of …'. Calculate the content of $C_{10}H_{11}N_3O_3S$ from the values of the absorbances obtained using the declared content of $C_{10}H_{11}N_3O_3S$ in *sulfamethoxazole BPCRS*. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{10}H_{11}N_3O_3S$, weight in volume.

STORAGE

Co-trimoxazole Oral Suspension should be protected from light and stored at a temperature not exceeding 30°.

Co-trimoxazole Oral Suspension contains, in 5 ml, 80 mg of Trimethoprim and 400 mg of Sulfamethoxazole.

Co-trimoxazole Tablets

General Notices

Trimethoprim and Sulfamethoxazole Tablets

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Co-trimoxazole Tablets contain Trimethoprim and Sulfamethoxazole in the proportions, by weight, 1 part to 5 parts.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

92.5 to 107.5% of the stated amount of trimethoprim.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

92.5 to 107.5% of the stated amount of sulfamethoxazole.

IDENTIFICATION

A. Filter the aqueous layer reserved in the Assay for trimethoprim. Add, drop wise, sufficient 2M *hydrochloric acid* to the filtrate to make it just acidic and extract with 50 ml of *ether*. Wash the ether layer with 10 ml of *water*, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in the minimum volume of a 5% w/v solution of *sodium carbonate*, add 1M *hydrochloric acid* drop wise until precipitation is complete and filter. Wash the residue sparingly with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulfamethoxazole *(RS 327)*.

B. To a quantity of the powdered tablets containing 50 mg of trimethoprim add 30 ml of 0.1M *sodium hydroxide* and extract with two 50 ml quantities of *chloroform*. Wash the combined chloroform extracts with two 10 ml quantities of 0.1M *sodium hydroxide* and then with 10 ml of *water*. Shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim *(RS 354)*.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform*, 10 volumes of *methanol* and 5 volumes of *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.4 g of sulfamethoxazole with 20 ml of *methanol* and filter. Solution (2) contains 2.0% w/v of *sulfamethoxazole BPCRS* in *methanol*. Solution (3) contains 0.4% w/v of *trimethoprim BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. One of the principal spots in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the other corresponds to the spot in the chromatogram obtained with solution (3).

TESTS

ASSAY

For trimethoprim

To a quantity of the powder containing 50 mg of trimethoprim add 30 ml of 0.1M *sodium hydroxide* and extract with four 50 ml quantities of *chloroform*, washing each extract with the same two 10 ml quantities of 0.1M *sodium hydroxide*. Reserve the aqueous layer for test A for Identification. Combine the chloroform extracts and extract with four 50 ml quantities of 1M *acetic acid*. Wash the combined extracts with 5 ml of *chloroform* and dilute the aqueous extracts to 250 ml with 1M *acetic acid*. To 10 ml of the solution add 10 ml of 1M *acetic acid* and sufficient *water* to produce 100 ml and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm.

For sulfamethoxazole

Weigh and powder 20 tablets. Dissolve, as completely as possible, a quantity of the powder containing 0.5 g of sulfamethoxazole in 60 ml of *water* and 10 ml of *hydrochloric acid*. Add 3 g of *potassium bromide*, cool in ice and titrate slowly with 0.1M *sodium nitrite VS*, stirring constantly and determining the end point electrometrically. Each ml of 0.1M *sodium nitrite VS* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

LABELLING

The label states the quantities of Trimethoprim and of Sulfamethoxazole in each tablet.

Crotamiton Cream

General Notices

Action and use

Acaricide.

DEFINITION

Crotamiton Cream contains Crotamiton in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of crotamiton, $C_{13}H_{17}NO$

93.0 to 107.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the cream containing 0.5 g of crotamiton with 50 ml of *water* and then slowly add 50 ml of 1M *sodium hydroxide* while stirring vigorously. Filter the mixture, adjust the filtrate to pH 7 with 5M *hydrochloric acid* and extract with 50 ml of *ether*. Wash the ether layer with 10 ml of a saturated solution of *sodium chloride*, dry the organic layer over *anhydrous sodium sulphate*, filter and evaporate to an oily residue. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a 0.003% w/v solution of the residue in *cyclohexane* exhibits a maximum only at 242 nm. The A(1%, 1 cm) at the maximum is about 315.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mobile phase prepared in the following manner. Shake 98 volumes of *chloroform* with 2 volumes of 18M *ammonia*, dry over *anhydrous sodium sulphate*, filter and mix 97 volumes of the filtrate with 3 volumes of *propan-2-ol*. Apply separately to the plate 5 µl of each of the following solutions in *absolute ethanol*. Solution (1) contains 0.25% w/v of the residue obtained in test A. Solution (2) contains 0.25% w/v of *crotamiton BPCRS*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2), but if not, the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

C. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of solutions (1), (4), (5), (6) and (7) as described under Assay. For solution (1) allow the chromatography to proceed for 2.5 times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the peaks corresponding to the *E*-isomer and to crotamiton impurity A is at least 4.5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to crotamiton impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (4) (3%), the sum of the areas of any *secondary peaks* apart from any peaks corresponding to the *Z*-isomer and to crotamiton impurity A is not greater than the sum of the areas of the peaks corresponding to the *E*- and *Z*-isomers in the chromatogram obtained with solution (5) (1%). Disregard any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with solution (5) (0.02%) and any peak with the same retention time as the principal peak in the chromatogram obtained with solution (7).

***Z*-Isomer**

Not more than 15% of the total content of *E*- and *Z*-isomers determined in the Assay.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 2 ml of *water* and 100 ml of *cyclohexane* to a quantity of the preparation being examined containing 0.1 g of crotamiton, shake for 10 minutes and separate the lower, aqueous layer. Repeat the extraction using two 10 ml quantities of *cyclohexane*, filter the combined extracts and add sufficient *cyclohexane* to produce 200 ml. For solution (2) dilute 1 volume of solution (1) to 20 volumes with *cyclohexane*. Solution (3) contains 0.0025% w/v of *crotamiton BPCRS* in *cyclohexane*. Solution (4) contains 0.0015% w/v of *crotamiton impurity A EPCRS* in *cyclohexane*. For solution (5) dilute 1 volume of solution (1) to 100 volumes with *cyclohexane*. For solution (6) dilute 1 volume of a 0.015% w/v solution of *crotamiton impurity A EPCRS* to 10 volumes with solution (1). Solution (7) contains 0.001% w/v of *methyl hydroxybenzoate* in *cyclohexane*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si60 is suitable), (b) a mixture of 8 volumes of *tetrahydrofuran* and 92 volumes of *cyclohexane* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 242 nm.

Inject 20 µl of each of solutions (4) and (6). When the chromatograms are recorded under the prescribed conditions, the retention times relative to the principal peak (*E*-crotamiton) are: *Z*-isomer, about 0.5; *N*-ethyl-*N*-(2-methylphenyl)but-3-enamide (crotamiton impurity A), about 0.8. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with solution (4) is at least 70% of the full scale of the recorder. The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the peaks corresponding to the *E*-isomer and to crotamiton impurity A is at least 4.5.

Inject 20 µl of solutions (2) and (3). Calculate the contents of the *E*- and *Z*-isomers in the preparation being examined using the declared contents of *E*- and *Z*-crotamiton in *crotamiton BPCRS* and hence calculate the content of $C_{13}H_{17}NO$ in the preparation being examined.

Crotamiton Lotion

General Notices

DEFINITION

Crotamiton Lotion is a *cutaneous suspension*. It contains Crotamiton in a suitable vehicle.
The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of crotamiton, $C_{13}H_{17}NO$

93.0 to 107.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the lotion containing 0.5 g of crotamiton add 30 ml of 1M *hydrochloric acid* and shake to produce a uniform suspension. Extract with 100 ml of *petroleum spirit (boiling range, 40° to 60°)*, wash the petroleum spirit layer with 10 ml of 1M *hydrochloric acid*, with two 10 ml quantities of 1M *sodium hydroxide* and then with 10 ml of a saturated solution of *sodium chloride*. Dry the organic layer over *anhydrous sodium sulphate*, filter and evaporate to an oily residue. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a 0.003% w/v solution of the residue in *cyclohexane* exhibits a maximum only at 242 nm. The A(1%, 1 cm) at the maximum is about 315.

B. Carry out Identification test B described under Crotamiton Cream using the residue from test A above in the preparation of solution (1).

In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

TESTS

Related substances

Complies with the test described under Crotamiton Cream.

Z-Isomer

Not more than 15% of the total content of *E*- and *Z*-isomers determined in the Assay.

ASSAY

Carry out the Assay described under Crotamiton Cream using a quantity of the lotion containing 0.1 g of crotamiton.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous suspension'.

Cyanocobalamin Tablets

General Notices

Action and use

Vitamin B_{12} analogue.

DEFINITION

Cyanocobalamin Tablets contain Cyanocobalamin. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyanocobalamin, $C_{63}H_{88}CoN_{14}O_{14}P$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. In the test for Uniformity of content, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Gently shake 10 tablets with 20 ml of *chloroform* to remove the coating and dry the tablet cores in a current of air. Transfer to a clean flask and powder with the aid of a glass rod. To a quantity of the powdered tablet cores containing 0.2 mg of cyanocobalamin, add 10 ml of a mixture of 1 volume of *2-ethoxyethanol* and 3 volumes of *water*, shake vigorously for 5 minutes, centrifuge at 3000 revolutions per minute for 15 minutes and filter using a filter with a pore size of 0.45 µm. The *light absorption* of the filtrate, Appendix II B, in the range 345 to 560 nm exhibits maxima at about 361 nm and 550 nm. No maximum is exhibited at 351 nm (distinction from hydroxocobalamin).

TESTS

Uniformity of content

Tablets containing less than 2 mg of cyanocobalamin comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using 100 µl of the following solutions. For solution (1) shake one finely-crushed tablet with 6 ml of *water* for 5 minutes, dilute to 10 ml, centrifuge for 15 minutes and use the supernatant solution. Solution (2) contains 0.0005% w/v, or other appropriate concentration, of *cyanocobalamin BPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemically-bonded hexylsilyl groups (5 µm) (Spherisorb Hexyl is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1 volume of *methanol* and 3 volumes of a pH 3 buffer solution prepared by mixing 1000 ml of a 2.1% w/v solution of *citric acid* with 250 ml of a 2.84% w/v solution of *anhydrous disodium hydrogen orthophosphate* and (c) a detection wavelength of 361 nm.

Calculate the content of cyanocobalamin, $C_{63}H_{88}CoN_{14}O_{14}P$, in each tablet using the declared content of $C_{63}H_{88}CoN_{14}O_{14}P$ in *cyanocobalamin BPCRS* .

ASSAY

Use the average of the 10 individual results obtained in the test for Uniformity of content.

STORAGE

Cyanocobalamin Tablets should be protected from light.

When vitamin B_{12} tablets are prescribed or demanded, Cyanocobalamin Tablets shall be dispensed or supplied.

Cyclizine Injection

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Cyclizine Injection is a sterile solution in Water for Injections containing 5% w/v of cyclizine lactate prepared by the interaction of Cyclizine and Lactic Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of cyclizine lactate, $C_{18}H_{22}N_2,C_3H_6O_3$

4.65 to 5.25% w/v.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. To 0.5 ml add 10 ml of *water* followed by 5M *sodium hydroxide* until strongly alkaline to *litmus paper* . Extract with two 10 ml quantities of *chloroform* and reserve the aqueous layer for test B. Wash each chloroform extract with 5 ml of *water*, dry the combined extracts with *anhydrous sodium sulphate* , filter and evaporate the filtrate to dryness. Dissolve the residue in *ethanol (96%)* and evaporate to dryness. The *infrared absorption spectrum* of the final residue, Appendix II A, is concordant with the *reference spectrum* of cyclizine *(RS 075)* .

B. The aqueous solution reserved in test A, after acidification with 1M *sulphuric acid* , yields the reaction characteristic of *lactates* , Appendix VI.

TESTS

Acidity

pH, 3.3 to 3.7, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and as the mobile phase the lower layer obtained after shaking together a mixture of 2 volumes of 13.5M *ammonia* , 8 volumes of *methanol* and 90 volumes of *dichloromethane* and allowing the layers to separate. Apply separately to the plate 20 µl of each of the following freshly prepared solutions. For solution (1) dilute 1 volume of the injection to 5 volumes with *methanol* . For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol* . Solution (3) contains 0.020% w/v of N-*methylpiperazine* in *methanol* . For solution (4) dilute 1 volume of solution (1) to 10 volumes with a 0.10% w/v solution of *hydroxyzine hydrochloride EPCRS* in *methanol* . After removal of the plate, allow it to dry in air and expose to iodine vapour for 10 minutes. Any spot corresponding to N-*methylpiperazine* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (2%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

ASSAY

Dilute 5 ml to 100 ml with 1M *sulphuric acid* . To 20 ml of this solution add 2 g of *sodium chloride* , shake with two 50 ml quantities of *ether* , allow to separate and wash the ether with the same two 10 ml quantities of *water*. To the combined aqueous solution and washings add 20 ml of 5M *sodium hydroxide* and extract with three 50 ml quantities of *ether* . Combine the ether extracts and wash with two 10 ml quantities of a saturated solution of *sodium chloride* . Extract the ether layer with two 25 ml quantities of 0.05M *sulphuric acid* and then with two 10 ml quantities of *water*. Combine the acidic and aqueous extracts and dilute to 100 ml with *water*. Dilute 5 ml of this solution to 200 ml with 0.05M *sulphuric acid* and measure the *absorbance* of the resulting solution at the maximum at 225 nm, Appendix II B. Calculate the content of $C_{18}H_{22}N_2,C_3H_6O_3$ taking 331 as the value of A(1%, 1 cm) at the maximum at 225 nm.

LABELLING

The strength is stated in terms of the equivalent amount of cyclizine lactate in a suitable dose-volume.

Cyclizine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Cyclizine Tablets contain Cyclizine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyclizine hydrochloride, $C_{18}H_{22}N_2,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.1 g of Cyclizine Hydrochloride with 10 ml of *ethanol (96%)*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Cyclizine Hydrochloride *(RS 076)*.

B. Extract a quantity of the powdered tablets containing 0.5 g of Cyclizine Hydrochloride with 20 ml of *water* and filter. The filtrate yields reaction A characteristic of *chlorides*, Appendix VI.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and as the mobile phase the lower layer obtained after shaking together a mixture of 2 volumes of 13.5M *ammonia*, 8 volumes of *methanol* and 90 volumes of *dichloromethane* and allowing the layers to separate. Apply separately to the plate 20 µl of each of the following freshly prepared solutions. For solution (1) triturate a quantity of the powdered tablets containing 0.10 g of Cyclizine Hydrochloride with 10 ml of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.0050% w/v of N*-methylpiperazine* in *methanol*. For solution (4) dilute 1 volume of solution (1) to 10 volumes with a 0.10% w/v solution of *hydroxyzine hydrochloride EPCRS* in *methanol*. After removal of the plate, allow it to dry in air and expose to iodine vapour for 10 minutes. Any spot corresponding to N*-methylpiperazine* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.125 g of Cyclizine Hydrochloride with 400 ml of 0.05M *sulphuric acid* for 15 minutes. Add sufficient 0.05M *sulphuric acid* to produce 500 ml, filter, dilute 5 ml of the filtrate to 100 ml with 0.05M *sulphuric acid* and measure the *absorbance* of the resulting solution at the maximum at 225 nm, Appendix II B. Calculate the content of $C_{18}H_{22}N_2$,HCl taking 390 as the value of A(1%, 1 cm) at the maximum at 225 nm.

Cyclopenthiazide Tablets

General Notices

Action and use

Thiazide-like diuretic.

DEFINITION

Cyclopenthiazide Tablets contain Cyclopenthiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyclopenthiazide, $C_{13}H_{18}ClN_3O_4S_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 5 mg of Cyclopenthiazide with 5 ml of *acetone* and filter. Solution (2) contains 0.1% w/v of *cyclopenthiazide BPCRS* in *acetone*. After removal of the plate, dry it in a current of air, examine under *ultraviolet light (254 nm)* and then reveal the spots by *Method I*. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Cyclopenthiazide with 50 ml of *acetone*, filter, evaporate the filtrate to dryness and dissolve the residue in 2 ml of *acetone*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Uniformity of content

Tablets containing less than 2 mg of Cyclopenthiazide comply with the requirements stated under Tablets using the following method of analysis. To one tablet add 50 ml of *methanol*, shake for 20 minutes, filter and measure the *absorbance* of the filtrate at the maximum at

273 nm, Appendix II B. Calculate the content of $C_{13}H_{18}ClN_3O_4S_2$ taking 585 as the value of A (1%, 1 cm) at the maximum at 273 nm.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 7.5 mg of Cyclopenthiazide with 50 ml of *methanol* for 10 minutes and dilute to 100 ml with *methanol* . Mix, filter, dilute 20 ml of the filtrate to 100 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_{13}H_{18}ClN_3O_4S_2$ taking 585 as the value of A(1%, 1 cm) at the maximum at 273 nm.

Cyclopentolate Eye Drops

General Notices

Action and use

Anticholinergic.

DEFINITION

Cyclopentolate Eye Drops are a sterile solution of Cyclopentolate Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of cyclopentolate hydrochloride, $C_{17}H_{25}NO_3,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Add 2M *ammonia* to a volume of the eye drops containing 25 mg of cyclopentolate hydrochloride until alkaline and extract immediately with 50 ml of *ether*. Wash the extract with 5 ml of *water*, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of cyclopentolate *(RS 078)*.

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 50 volumes of *propan-2-ol*, 30 volumes of *butyl acetate*, 15 volumes of *water* and 5 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) use the eye drops diluted, if necessary, to contain 0.5% w/v of cyclopentolate hydrochloride. For solution (2) dilute 1 volume of solution (1) to 50 volumes with *water*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *water*. After removal of the plate, dry it at 120° for 5 minutes, spray with *ethanolic sulphuric acid (10%)*, heat at 120° for 30 minutes and examine under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a solution containing 0.25% w/v of *4-chlorophenol* (internal standard) in *methanol* (solution A). For solution (1) add 4 ml of solution A to 4 ml of a 0.5% w/v solution of *cyclopentolate hydrochloride BPCRS* in *water* and dilute to 10 ml with the mobile phase. For solution (2) dilute a volume of the eye drops containing 20 mg of cyclopentolate hydrochloride to 10 ml with the mobile phase. For solution (3) add 4 ml of solution A to a volume of the eye drops containing 20 mg of cyclopentolate hydrochloride and dilute to 10 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 55 volumes of *methanol* and 45 volumes of 0.2M *sodium dihydrogen orthophosphate*, the mixture adjusted to pH 3.0 with *orthophosphoric acid*, as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

The method is not valid unless the *resolution factor* between the peaks due to cyclopentolate hydrochloride and the internal standard is greater than 4.

Calculate the content of $C_{17}H_{25}NO_3$,HCl using the declared content of $C_{17}H_{25}NO_3$,HCl in *cyclopentolate hydrochloride BPCRS*.

Cyclophosphamide Injection

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Cyclophosphamide Injection is a sterile, isotonic solution of Cyclophosphamide in Water for Injections. It is prepared by dissolving Cyclophosphamide for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Cyclophosphamide Injection deteriorates on storage and should be used immediately after preparation.

CYCLOPHOSPHAMIDE FOR INJECTION

DEFINITION

Cyclophosphamide for Injection is a sterile material consisting of Cyclophosphamide with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P$

92.5 to 107.5% of the stated amount of anhydrous cyclophosphamide.

IDENTIFICATION

A. Shake a quantity containing the equivalent of 0.2 g of anhydrous cyclophosphamide with 2 ml of *chloroform* and filter. The *infrared absorption spectrum* of the filtrate, Appendix II A, is concordant with the *reference spectrum* of cyclophosphamide *(RS 079)*.

B. Extract a quantity containing the equivalent of 0.1 g of anhydrous cyclophosphamide with *ether* and evaporate the extract to dryness. Dissolve the residue in 10 ml of *water* and add 5 ml of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *nitric acid* but soluble in 5M *ammonia* from which it is reprecipitated on the addition of *nitric acid*.

TESTS

Acidity

pH of a freshly prepared 2% w/v solution, 4.0 to 6.0, Appendix V L.

Uniformity of content

Sealed containers containing the equivalent of 1 g or less of anhydrous cyclophosphamide comply with the requirements stated under Parenteral Preparations, Powders for Injections. Use the individual results obtained in the Assay.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 2 volumes of *anhydrous formic acid*, 4 volumes of *acetone*, 12 volumes of *water* and 80 volumes of *butan-2-one* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dissolve a quantity of the contents of the sealed container containing the equivalent of 0.2 g of anhydrous cyclophosphamide in sufficient *ethanol (96%)* to produce 10 ml. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *ethanol (96%)*. After removal of the plate, dry it in a current of warm air and heat at 100° for 10 minutes. Place the plate while hot in a chromatography tank in which is placed an evaporating dish containing equal volumes of a 5% w/v solution of *potassium permanganate* and *hydrochloric acid*, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a very faint blue colour with *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and allow to stand for 5 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%). Disregard any spot remaining on the line of application.

ASSAY

Dissolve the contents of a sealed container containing the equivalent of 0.1 g of anhydrous cyclophosphamide in 30 ml of *chloroform*, shake vigorously for 15 minutes, filter (Whatman GF/F is suitable) and wash the filter with 15 ml of *chloroform*. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 50 ml of a 0.1% w/v solution of *sodium hydroxide* in *ethane-1,2-diol*. Boil the solution under a reflux condenser for 30 minutes and allow to cool. Rinse the condenser with 25 ml of *water*, add 75 ml of *propan-2-ol*, 15 ml of 2M *nitric acid*, 10 ml of 0.1M *silver nitrate VS* and 2 ml of *ammonium iron(III) sulphate solution R2* and titrate with 0.1M *ammonium thiocyanate VS*. Each ml of 0.1M *silver nitrate VS* is equivalent to 13.05 mg of $C_7H_{15}Cl_2N_2O_2P$. Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in the sealed container.

Repeat the procedure with a further nine sealed containers. Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ per container from the average of the 10 individual results thus obtained.

LABELLING

The label of the sealed container states (1) the weight of Cyclophosphamide contained in it; (2) the equivalent amount of anhydrous cyclophosphamide.

Cyclophosphamide Tablets

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Cyclophosphamide Tablets contain Cyclophosphamide. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P,H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.2 g of cyclophosphamide with 2 ml of *chloroform* and filter. The *infrared absorption spectrum* of the filtrate, Appendix II A, is concordant with the *reference spectrum* of cyclophosphamide *(RS 079)*.

B. Extract a quantity of the powdered tablets containing 0.1 g of cyclophosphamide with *ether* and evaporate the extract to dryness. Dissolve the residue in 10 ml of *water* and add 5 ml of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *nitric acid* but dissolves in 5M *ammonia* from which it is reprecipitated on the addition of *nitric acid*.

TESTS

Acidity

Shake a quantity of the powdered tablets containing 0.25 g of cyclophosphamide with 20 ml of *carbon dioxide-free water*, filter and titrate the filtrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 0.2 ml of 0.1M *sodium hydroxide VS* is required to change the colour of the solution.

Related substances

Comply with the test described under Cyclophosphamide Injection but preparing solution (1) in the following manner. Shake vigorously a quantity of the powdered tablets containing 0.2 g of cyclophosphamide with 50 ml of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of *ethanol (96%)*.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets containing 0.1 g of cyclophosphamide in 30 ml of *chloroform*, shake vigorously for 15 minutes, filter (Whatman GF/F is suitable) and wash the filter with 15 ml of *chloroform*. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 50 ml of a 0.1% w/v solution of *sodium hydroxide* in *ethane-1,2-diol*. Boil under a reflux condenser for 30 minutes and allow to cool. Rinse the condenser with 25 ml of *water*, add 75 ml of *propan-2-ol*, 15 ml of 2M *nitric acid*, 10 ml of 0.1M *silver nitrate VS* and 2 ml of *ammonium iron(III) sulphate solution R2* and titrate with 0.1M *ammonium thiocyanate VS*. Each ml of 0.1M *silver nitrate VS* is equivalent to 13.94 mg of $C_7H_{15}Cl_2N_2O_2P,H_2O$.

Cyproheptadine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Cyproheptadine Tablets contain Cyproheptadine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous cyproheptadine hydrochloride, $C_{21}H_{21}N,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing the equivalent of 20 mg of anhydrous cyproheptadine hydrochloride add 10 ml of *water* and 2.5 ml of 0.1M *sodium hydroxide* , extract with 10 ml of *dichloromethane* , filter through *anhydrous sodium sulphate* moistened with *dichloromethane* on absorbent cotton and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cyproheptadine *(RS 080)* .

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (3) corresponds to that in the chromatogram obtained with solution (5).

C. Extract a quantity of the powdered tablets containing the equivalent of 20 mg of anhydrous cyproheptadine hydrochloride with 7 ml of *water*, filter, add 0.3 ml of 5M *ammonia* to the filtrate and filter again. The filtrate yields reaction A characteristic of *chlorides* , Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase a mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane* . Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous cyproheptadine hydrochloride to 5 ml of the mobile phase, shake mechanically for 10 minutes and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.002% w/v of *dibenzocycloheptene EPCRS* in the mobile phase. For solution (3) dilute 1 volume of solution (1) to 10 volumes with the mobile phase.

For solution (4) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume to 10 volumes with the mobile phase. Solution (5) contains 0.1% w/v of *cyproheptadine hydrochloride EPCRS* in the mobile phase. Allow the plate to dry in air and spray with *ethanolic sulphuric acid (20%).* Heat at 110° for 30 minutes and examine under *ultraviolet light (365 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to dibenzocycloheptene is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 1.5 mg of anhydrous cyproheptadine hydrochloride add sufficient *ethanol (96%)* to produce 100 ml and filter if necessary. Measure the *absorbance* of the resulting solution at the maximum at 286 nm, Appendix II B. Calculate the content of $C_{21}H_{21}N,HCl$ taking 355 as the value of A (1%, 1 cm) at the maximum at 286 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cyproheptadine hydrochloride.

Cyproterone Tablets

General Notices

Action and use

Anti-estrogen.

DEFINITION

Cyproterone Tablets contain Cyproterone Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of cyproterone acetate, $C_{24}H_{29}ClO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of cyproterone acetate with 20 ml of *dichloromethane* , filter through a glass-fibre filter paper (Whatman GF/C is suitable) and evaporate the filtrate to dryness using a rotary evaporator and a water bath at 40°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of cyproterone acetate *(RS 395)* .

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2. For tablets containing less than 100 mg of Cyproterone Acetate use as the medium 900 ml of a 0.07% w/v solution of *sodium dodecyl sulphate* in 0.1M *hydrochloric acid* . For tablets containing 100 mg or more of Cyproterone Acetate use as the medium 900 ml of a 0.10% w/v solution of *sodium dodecyl sulphate* in 0.1M *hydrochloric acid* . Rotate the paddle at 100 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at the maximum at 285 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *cyproterone acetate BPCRS* in the dissolution medium and calculate the total content of cyproterone acetate, $C_{24}H_{29}ClO_4$, in the medium using the declared content of $C_{24}H_{29}ClO_4$ in *cyproterone acetate BPCRS* .

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of cyproterone acetate with 50 ml of a 4:1 mixture of *acetonitrile* and *water* for 5 minutes, dilute to 100 ml with *water* and filter through a glass-fibre filter paper (Whatman GF/C is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) dilute 1 volume of a solution containing 0.01% w/v each of *cyproterone acetate BPCRS* and *medroxyprogesterone acetate BPCRS* in a 4:1 mixture of *acetonitrile* and *water* to 10 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water* and (c) a detection wavelength of 254 nm.

Inject 20 µl of each solution. For solution (1) continue the chromatography for twice the retention time of cyproterone acetate. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

ASSAY

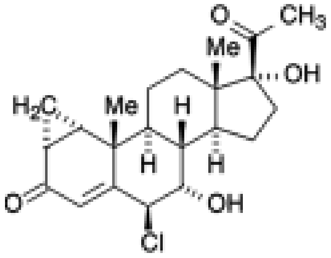
Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of cyproterone acetate with 50 ml of a 4:1 mixture of *acetonitrile* and *water* for 5 minutes, dilute to 100 ml with the same solvent mixture and filter through a glass-fibre filter paper (Whatman GF/C is suitable); dilute 1 volume of the filtrate to 10 volumes with the mobile phase. For solution (2) dilute 1 volume of a 0.05% w/v solution of *cyproterone acetate BPCRS* in a 4:1 mixture of *acetonitrile* and *water* to 10 volumes with the mobile phase. For solution (3) dilute 1 volume of a solution containing 0.01% w/v each of *cyproterone acetate BPCRS* and *medroxyprogesterone acetate BPCRS* in a 4:1 mixture of *acetonitrile* and *water* to 10 volumes with the mobile phase.

The chromatographic procedure described under Related substances may be used.

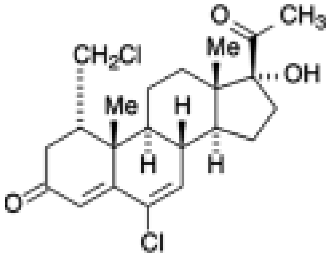
Calculate the content of $C_{24}H_{29}ClO_4$ in the tablets, using the declared content of $C_{24}H_{29}ClO_4$ in *cyproterone acetate BPCRS*.

IMPURITIES

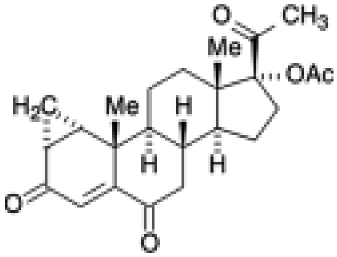
The impurities limited by the requirements of this monograph include those listed in the monograph for Cyproterone Acetate and the following:



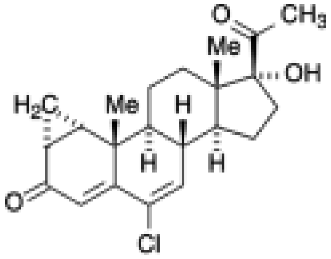
A. 6β-Chloro-7α ,17α-dihydroxy-1α ,2α-methylenepregna-4-ene-3,20-dione (*chlorohydrin derivative*)



B. 17α-Hydroxy-6-chloro-1α-chloromethylpregna-4,6-diene-3,20-dione (*1-chloromethyl derivative*)



C. 1α ,2α-Methylene-3,6,20-trioxopregna-4-en-17α-yl acetate



D. 17α-Hydroxy-6-chloro-1α,2α-methylenepregna-4,6-diene-3,20-dione (*cyproterone*)

Cytarabine Injection

General Notices

Action and use

Pyrimidine analogue; cytotoxic.

DEFINITION

Cytarabine Injection is a sterile solution of Cytarabine in Water for Injections. It is supplied as a ready-to-use injection or it is prepared immediately before use by dissolving Cytarabine for Injection in the requisite amount of the liquid stated on the label.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use injection, the injection complies with the following requirements.

Content of cytarabine, $C_9H_{13}N_3O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Evaporate a volume of the injection containing 0.1 g of Cytarabine to dryness at 60° at a pressure of 0.7 kPa, mix the residue with a minimum amount of hot *ethanol (96%)*, filter, allow the filtrate to cool and induce crystallisation if necessary. Filter, wash the crystals with 2 ml of *ethanol (96%)* and dry at 60° at a pressure of 0.7 kPa. The *infrared absorption spectrum* of the dried crystals, Appendix II A, is concordant with the *reference spectrum* of cytarabine *(RS 081)*.

TESTS

Acidity or alkalinity

pH, 7.0 to 9.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck plates are suitable) and a mixture of 15 volumes of *water*, 20 volumes of *acetone* and 65 volumes of *butan-2-one* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions in *water*. For solution (1) dilute, if necessary, a volume of the injection to produce a solution containing 2% w/v of Cytarabine. For solution (2) dilute 1 volume of solution (1) to 200 volumes. Solution (3) contains 0.040% w/v of *uracil arabinoside EPCRS*. Solution (4) contains 0.020% w/v each of *uridine* and *uracil arabinoside EPCRS.* After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* In the chromatogram obtained with solution (1) any spot corresponding to uracil arabinoside is not more intense than the spot in the chromatogram obtained with solution (3) (2%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 20 mg of *cytarabine BPCRS* in *water* and dilute to 100 ml with the same solvent. Dilute 10 ml to 100 ml with *water*. For solution (2) dilute a volume of the injection to produce a solution containing 0.002% w/v of Cytarabine.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable) and maintained at 30°, (b) a mixture of 7.5 volumes of *methanol* and 100 volumes of 0.005M *sodium pentanesulphonate* adjusted to pH 2.8 using *glacial acetic acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 280 nm.

Calculate the content of $C_9H_{13}N_3O_5$ using the declared content of $C_9H_{13}N_3O_5$ in *cytarabine BPCRS*.

CYTARABINE FOR INJECTION

DEFINITION

Cytarabine for Injection is a sterile material consisting of Cytarabine with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of cytarabine, $C_9H_{13}N_3O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the contents of the sealed container containing 0.1 g of Cytarabine with 10 ml of hot *ethanol (96%)*, filter, allow the filtrate to cool and induce crystallisation if necessary. Filter, wash the crystals with 2 ml of *ethanol (96%)* and dry at 60° at a pressure of 0.7 kPa. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of cytarabine.

Acidity

pH of a solution containing 2% w/v of cytarabine in the solvent stated on the label, 4.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 15 volumes of *water*, 20 volumes of *acetone* and

65 volumes of *butan-2-one* as the mobile phase. Apply separately to the plate 10 µl of each of the following four solutions in *water*. For solution (1) dissolve the contents of the sealed container in a sufficient volume to produce a solution containing 2% w/v of Cytarabine. For solution (2) dilute 1 volume of solution (1) to 200 volumes. Solution (3) contains 0.020% w/v of *uracil arabinoside EPCRS*. Solution (4) contains 0.020% w/v each of *uridine* and *uracil arabinoside EPCRS.* After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to uracil arabinoside is not more intense than the spot in the chromatogram obtained with solution (3) (1%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

Water

Not more than 3.0% w/w, Appendix IX C. Use 0.8 g.

ASSAY

Determine the weight of the contents of each of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections. Carry out the Assay described for the ready-to-use injection but using the following solution as solution (2). Dissolve sufficient of the mixed contents of the 10 containers in *water* to produce a solution containing 0.002% w/v of Cytarabine.

Calculate the content of $C_9H_{13}N_3O_5$ in a container of average content weight using the declared content of $C_9H_{13}N_3O_5$ in *cytarabine BPCRS.*

Dispersible Co-beneldopa Tablets

General Notices

Dispersible Benserazide Hydrochloride and Levodopa Tablets

Action and use

Dopa decarboxylase inhibitor + dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Dispersible Co-beneldopa Tablets contain Benserazide Hydrochloride and Levodopa in the proportions, by weight, 1 part benserazide to 4 parts levodopa in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of benserazide, $C_{10}H_{15}N_3O_5$

93.0 to 105.0% of the stated amount.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a precoated cellulose plate (Merck plates are suitable) and a mixture of 10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol* as the mobile phase, but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Levodopa with 40 ml of 0.1M *hydrochloric acid* for 10 minutes, filter and use the filtrate. Solution (2) contains 0.5% w/v of *levodopa BPCRS* in 0.1M *hydrochloric acid*. Solution (3) contains 0.142% w/v of *benserazide hydrochloride BPCRS* in 0.1M *hydrochloric acid*. After removal of the plate, allow it to dry in a current of warm air for 5 minutes, spray with *dilute phosphomolybdotungstic reagent*, dry in a current of warm air for 30 seconds and spray with a 10% w/v solution of *sodium hydroxide*. The chromatogram obtained with solution (1) shows two clearly separated spots, the spot with the higher Rf value corresponding to the spot in the chromatogram obtained with solution (2) and the spot with the lower Rf value corresponding to the spot in the chromatogram obtained with solution (3).

B. In the Assay, the chromatogram obtained with solution (1) exhibits two peaks with the same retention times as those due to benserazide and levodopa in the chromatogram obtained with solution (2).

TESTS

Disintegration

Comply with the requirements for Dispersible Tablets.

Related substances

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be prepared in mobile phase that has been cooled to 4° and injected immediately. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.1 g of benserazide with 100 ml of the mobile phase, mix with the aid of ultrasound for 3 minutes, shaking occasionally, and filter through a 0.45-µm filter, discarding the first 5 ml of filtrate. Solution (2) contains 0.0005% w/v of *benserazide impurity A EPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of each of *benserazide hydrochloride BPCRS* and *benserazide impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP8 is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture prepared by dissolving 4.76 g of *potassium dihydrogen orthophosphate* in 800 ml of *water*, adding 200 ml of *acetonitrile* and 1.22 g of *sodium decanesulphonate* and adjusting the pH to 3.5 with *orthophosphoric acid* and (c) a detection wavelength of 220 nm.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

For solution (1) allow the chromatography to proceed for nine times the retention time of benserazide. In the chromatogram obtained with solution (1) the area of any peak corresponding to benserazide impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%), the area of any other *secondary peak* is not greater than the area of the peak due to benserazide in the chromatogram obtained with solution (3) (0.5%) and the sum of the areas of any such peaks is not greater than twice the area of the peak due to benserazide in the chromatogram obtained with solution (3) (1%). Disregard any peak with an area less than 0.1 times the area of the peak due to benserazide in the chromatogram obtained with solution (3) (0.05%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated cellulose plate (Merck plates are suitable) and a mixture of 10 volumes of a 10% v/v solution of *hydrochloric acid*, 20 volumes of *water* and 70 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate, as bands 20 mm long, 10 µl of each of solutions (1) and (2) and 20 µl of solution (3) and dry in a current of air. Solution (1) should be prepared immediately before use. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of *anhydrous formic acid* and *methanol*, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) is a mixture of equal volumes of solution (1) and a solution prepared by dissolving 30 mg of L-*tyrosine* in 1 ml of *anhydrous formic acid* and diluting to 100 ml with *methanol*. After removal of the plate, allow it to dry in a current of warm air, spray with a freshly prepared mixture containing equal volumes of a 10% w/v solution of *iron(III) chloride hexahydrate* and a 5% w/v solution of *potassium hexacyanoferrate(III)* and examine the plate immediately. Any *secondary band* in the chromatogram obtained with solution (1) is not more intense than the band in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (3) shows a distinct band, at a higher Rf value than the principal band, which is more intense than the band in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 80 ml of 0.1M *orthophosphoric acid* for 5 minutes, mix with the aid of ultrasound for 30 minutes, cool, add sufficient 0.1M *orthophosphoric acid* to produce 100 ml and mix. Filter the resulting solution through a 0.45-µm filter (Whatman GF/C is suitable), discarding the first 5 ml of filtrate, and dilute 10 volumes of the filtrate to 100 volumes with the mobile phase. For solution (2) dissolve 28.7 mg of *benserazide hydrochloride BPCRS* and 0.1 g of *levodopa BPCRS* in sufficient 0.1M *orthophosphoric acid* to produce 100 ml, mix and dilute 10 volumes of the resulting solution to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP8 is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture prepared by dissolving 4.76 g of *potassium dihydrogen orthophosphate* in 800 ml of *water*, adding 200 ml of *acetonitrile* and 1.22 g of *sodium decanesulphonate* and adjusting the pH to 3.5 with *orthophosphoric acid* and (c) a detection wavelength of 220 nm. Inject 20 µl of each solution.

The chromatogram obtained with solution (2) shows two principal peaks; the retention time of the peak due to benserazide is about three times that of the peak due to levodopa.

Calculate the content of $C_{10}H_{15}N_3O_5$ and of $C_9H_{11}NO_4$ in each tablet using the declared contents of $C_{10}H_{15}N_3O_5$ in *benserazide hydrochloride BPCRS* and of $C_9H_{11}NO_4$ in *levodopa BPCRS*.

STORAGE

Dispersible Co-beneldopa Tablets should be protected from moisture.

LABELLING

The label states the quantity of Benserazide Hydrochloride, in terms of the equivalent amount of benserazide, and the amount of Levodopa in each tablet.

Dispersible Co-codaprin Tablets

General Notices

Dispersible Aspirin and Codeine Tablets

Action and use

Opioid analgesic + antipyretic; analgesic; anti-inflammatory.

DEFINITION

Dispersible Co-codaprin Tablets contain Codeine Phosphate and Aspirin in the proportions, by weight, 1 part to 50 parts in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4, ½H_2O$

90.0 to 110.0% of the stated amount.

Content of aspirin, $C_9H_8O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Effervesce on the addition of *water*.

B. Boil 1 g of the powdered tablets with 10 ml of 1M *sodium hydroxide,* cool and filter. Acidify the filtrate with 1M *sulphuric acid ;* a white precipitate is produced. To a solution of the precipitate add *iron(*III*) chloride solution R1*; a deep violet colour is produced.

C. Shake 1 g of the powdered tablets with a mixture of 20 ml of *water* and 1 ml of 1M *sulphuric acid* for 5 minutes and filter. Reserve the residue for test D. The filtrate yields the reactions characteristic *of phosphates,* Appendix VI. Make the remainder of the filtrate alkaline with 5M *ammonia,* extract with *chloroform,* separate the chloroform layer and evaporate the chloroform. Place a small quantity of the residue on the surface of a drop of *nitric acid ;* a yellow but no red colour is produced.

D. Dissolve 0.1 g of the residue obtained in test C in 1 ml of *sulphuric acid ,* add 0.05 ml of *iron(*III*) chloride solution R1* or 0.05 ml of *ammonium molybdate-sulphuric acid solution* and warm gently. A bluish violet colour is produced which changes to red on the addition of 0.05 ml of 2M *nitric acid .*

TEST

Salicylic acid

To a quantity of the powdered tablets containing 0.50 g of Aspirin add 50 ml of *chloroform* and a mixture of 2 ml of 1M *hydrochloric acid* and 8 ml of *water* shake well and allow to separate. Filter the chloroform layer through a dry filter paper and evaporate 10 ml of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 ml of *ethanol (96%)*, stir well, dilute to 100 ml with *water* at a temperature not exceeding 10°, filter immediately and rapidly transfer 50 ml to a *Nessler cylinder*. Add 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1*, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 ml of freshly prepared *ammonium iron(III) sulphate solution R1* to a mixture of 3 ml of a freshly prepared 0.050% w/v solution of *salicylic acid*, 2 ml of *ethanol (96%)* and sufficient *water* to produce 50 ml contained in a second *Nessler cylinder* (3.0%).

ASSAY

Weigh and powder 20 tablets.

For codeine phosphate

To a quantity of the powder containing 16 mg of Codeine Phosphate add 20 ml of *water* and 1 g of *disodium edetate*, swirl gently until effervescence ceases, shake to dissolve, add 5 ml of 5M sodium *hydroxide* and 15 ml of *water*, shake for 2 minutes and extract with three 50 ml quantities of *chloroform.* Wash each extract with the same 10 ml of *water*, filter through absorbent cotton previously moistened with *chloroform* and evaporate the combined extracts to about 60 ml on a water bath in a current of air. Cool, add 25 ml of *water*, 5 ml of *acetate buffer pH 2.8* and 5 ml of *dimethyl yellow and oracet blue B solution* and titrate with 0.01M *dioctyl sodium sulphosuccinate VS* with vigorous swirling until near the end point, then add the titrant drop wise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end point is indicated by the appearance of a permanent pinkish grey colour in the chloroform layer. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of dioctyl sodium sulphosuccinate required.

Dissolve 40 mg of *codeine phosphate BPCRS* in 25 ml of *water* and 5 ml of *acetate buffer pH 2.8*, add 60 ml of *chloroform* and 5 ml of *dimethyl yellow and oracet blue B solution*, shake well to dissolve the codeine phosphate and complete the method described above beginning at the words 'and titrate …' Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

For aspirin

To a quantity of the powder containing 0.8 g of Aspirin add 15 ml of *water* and swirl until effervescence ceases. Add 5 ml of 0.5M *sulphuric acid* and extract with 50 ml of *ether* followed by three 30 ml quantities of *ether*. Wash the combined extracts with 10 ml of *water*, filter through absorbent cotton previously moistened with *ether*, washing the separating funnel and filter with *ether*. Evaporate the ether in a water bath at 30° in a current of air. Dissolve the residue in 5 ml of *acetone* and evaporate in a water bath at 30°; again dissolve the residue in 5 ml of *acetone* and evaporate in a water bath at 30°. Dissolve the residue in 25 ml of *ethanol (96%)* previously neutralised to *phenol red solution* and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 18.02 mg of $C_9H_8O_4$.

STORAGE

Dispersible Co-codaprin Tablets should be protected from light.

LABELLING

The label states (1) that the tablets contain Aspirin, unless this word appears in the name of the tablets (this requirement does not apply in countries where exclusive proprietary rights in the name Aspirin are claimed); (2) the quantities of Codeine Phosphate and of Aspirin in each tablet; (3) that the tablets should be dispersed in water immediately before use.

Dispersible Co-trimoxazole Tablets

General Notices

Dispersible Trimethoprim and Sulfamethoxazole Tablets

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Dispersible Co-trimoxazole Tablets contain Trimethoprim and Sulfamethoxazole and in the proportions, by weight, 1 part to 5 parts in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

92.5 to 107.5% of the stated amount of trimethoprim.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

92.5 to 107.5% of the stated amount of sulfamethoxazole.

IDENTIFICATION

A. Filter the aqueous layer reserved in the Assay for trimethoprim. Add, drop wise, sufficient 2M *hydrochloric acid* to the filtrate to make it just acidic and extract with 50 ml of *ether*. Wash the ether layer with 10 ml of *water*, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in the minimum volume of a 5% w/v solution of *sodium carbonate*, add 1M *hydrochloric acid* drop wise until precipitation is complete and filter. Wash the residue sparingly with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulfamethoxazole *(RS 327)*.

B. To a quantity of the powdered tablets containing 50 mg of trimethoprim add 30 ml of 0.1M *sodium hydroxide* and extract with two 50 ml quantities of *chloroform*. Wash the combined chloroform extracts with two 10 ml quantities of 0.1M *sodium hydroxide* and then with 10 ml of *water*. Shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim *(RS 354)*.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform*, 10 volumes of *methanol* and 5 volumes of *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.4 g of sulfamethoxazole with 20 ml of *methanol* and filter. Solution (2) contains 2.0% w/v of *sulfamethoxazole BPCRS* in *methanol*. Solution (3) contains 0.4% w/v of *trimethoprim BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. One of the principal spots in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the other corresponds to the spot in the chromatogram obtained with solution (3).

Disintegration

The tablets disintegrate within 2 minutes when examined by the *disintegration test for tablets and capsules*, Appendix XII A1, but using *water* at 19° to 21°.

ASSAY

For trimethoprim

To a quantity of the powder containing 50 mg of trimethoprim add 30 ml of 0.1M *sodium hydroxide* and extract with four 50 ml quantities of *chloroform*, washing each extract with the same two 10 ml quantities of 0.1M *sodium hydroxide*. Reserve the aqueous layer for test A for Identification. Combine the chloroform extracts and extract with four 50 ml quantities of 1M *acetic acid*. Wash the combined extracts with four 5 ml quantities of *chloroform* and dilute the aqueous extracts to 250 ml with 1M *acetic acid*. To 10 ml of the solution add 10 ml of 1M *acetic acid* and sufficient *water* to produce 100 ml and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm.

For sulfamethoxazole

Weigh and powder 20 tablets. Dissolve, as completely as possible, a quantity of the powder containing 0.5 g of sulfamethoxazole in 60 ml of *water* and 10 ml of *hydrochloric acid*. Add 3 g of *potassium bromide*, cool in ice and titrate slowly with 0.1M *sodium nitrite VS*, stirring constantly and determining the end point electrometrically. Each ml of 0.1M *sodium nitrite VS* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

LABELLING

The label states (1) the quantities of Trimethoprim and of Sulfamethoxazole in each tablet; (2) that the tablets should be dispersed in water immediately before use.

Double-strength Chloroform Water

General Notices

DEFINITION

Chloroform	5 ml
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Chloroform in the Purified Water by shaking.
The water complies with the requirements stated under Aromatic Waters.

Effervescent Calcium Gluconate Tablets

General Notices

DEFINITION

Effervescent Calcium Gluconate Tablets contain Calcium Gluconate in a suitable effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of calcium gluconate, $C_{12}H_{22}CaO_{14},H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 1 g of Calcium Gluconate in 20 ml of hot *water*, cool and filter. To 0.5 ml of the filtrate add 0.05 ml of *iron(III) chloride solution R1*. An intense yellow colour is produced.

B. To 5 ml of the filtrate obtained in test A add 0.65 ml of *glacial acetic acid* and 1 ml of *phenylhydrazine*, heat on a water bath for 30 minutes, allow to cool and induce crystallisation. Filter, dissolve the residue in 10 ml of hot *water*, add a suitable quantity of *activated charcoal*, shake, filter, allow the filtrate to cool and induce crystallisation. A white, crystalline precipitate is produced. The *melting point* of the crystals, after drying, is about 201°, with decomposition, Appendix V A.

C. The powdered tablets yield reaction B characteristic of *calcium salts*, Appendix VI.

D. The tablets effervesce on the addition of *water*.

ASSAY

Weigh and powder 20 tablets. Ignite a quantity of the powder containing 0.5 g of Calcium Gluconate, cool and dissolve the residue with gentle heat in 5 ml of 2M *hydrochloric acid*. Filter, wash the residue on the filter with *water* and dilute the combined filtrate and washings to 50 ml with *water*. Neutralise with 5M *ammonia*, using *methyl orange solution* as indicator, add 5 ml of 8M *sodium hydroxide* and titrate with 0.05M *disodium edetate VS* using *calconcarboxylic acid triturate* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 22.42 mg of $C_{12}H_{22}CaO_{14},H_2O$.

LABELLING

The label states that the tablets should be dissolved in water immediately before use.

Effervescent Co-codamol Tablets

General Notices

Codeine Phosphate and Paracetamol Effervescent Tablets

Action and use

Opioid analgesic + analgesic; antipyretic.

DEFINITION

Effervescent Co-codamol Tablets contain Codeine Phosphate and Paracetamol in a suitable soluble, effervescent basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4, ½H_2O$

92.5 to 107.5% of the stated amount.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* F_{254} *plate* (Merck plates are suitable) and a mixture of 1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) mix a quantity of the powdered tablets containing 24 mg of Codeine Phosphate in 30 ml of *water* until effervescence ceases, add 10 ml of 1M *sodium hydroxide* and 30 ml of *dichloromethane* and shake for 1 minute. Filter the dichloromethane layer through a glass-fibre filter (Whatman GF/C is suitable). Solution (2) contains 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%)*. Solution (3) contains 0.08% w/v each of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%)*. After removal of the plate, allow it to dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

B. In the Assay for codeine phosphate, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution (1) contains 0.001% w/v of *4-aminophenol* in the mobile phase. For solution (2) stir a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 ml of the mobile phase for 10 minutes and filter.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.01M *sodium butanesulphonate* in a mixture of 0.4 volume of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 272 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (1) (0.1%). In the chromatogram obtained with solution (2) peaks with a long retention time may occur due to excipients.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel G plate* (Analtech plates are suitable) and a mixture of 6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) carefully mix a quantity of the powdered tablets containing 50 mg of Codeine Phosphate with 50 ml of 0.1M *hydrochloric acid* for 10 minutes and filter. Make the filtrate alkaline with 5M *sodium hydroxide* and extract with two 40-ml quantities of *dichloromethane*. Wash the combined extracts with 10 ml of *water*, filter through a layer of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with *dichloromethane*, evaporate the filtrate to dryness at a temperature not exceeding 45° using a rotary evaporator and dissolve the residue in 2 ml of *dichloromethane*. For solution (2) dilute 1.5 volumes of solution (1) to 100 volumes with *dichloromethane*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *dichloromethane*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

Uniformity of content

Tablets containing less than 2mg or 2% of Codeine Phosphate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase. For solution (2) add mobile phase to one tablet and mix with the aid of ultrasound until completely dispersed. Shake for 10 minutes, dilute with sufficient mobile phase to produce a solution containing 0.004% w/v of Codeine Phosphate, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute 0.01M *sodium pentanesulphonate* in a mixture of 78 volumes of *water* and 22 volumes of *methanol*, the pH of the solution being adjusted to 2.8 using 2M *hydrochloric acid*, and (c) a detection wavelength of 220 nm.

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4$, ½H_2O in each tablet using the declared content of $C_{18}H_{21}NO_3,H_3PO_4$, ½H_2O in *codeine phosphate BPCRS*.

ASSAY

For codeine phosphate

Use the average of the 10 individual results determined in the test for Uniformity of content.

For paracetamol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *paracetamol BPCRS* in the mobile phase. For solution (2) mix a quantity of the powdered tablets containing 0.5 g of Paracetamol with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 ml of the filtrate to 250 ml with the mobile phase.

The chromatographic conditions described under Uniformity of content may be used but with a detection wavelength of 243 nm.

Calculate the content of $C_8H_9NO_2$ using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

STORAGE

Effervescent Co-codamol Tablets should be protected from moisture.

LABELLING

The label states the quantities of Codeine Phosphate and of Paracetamol in each tablet.

When Co-codamol Effervescent Tablets are prescribed or demanded no strength being stated, tablets containing 8 mg of Codeine Phosphate and 500 mg of Paracetamol shall be dispensed or supplied.

Flexible Collodion

General Notices

Action and use

Skin protective.

DEFINITION

Flexible Collodion is a solution of Colophony in a mixture of Virgin Castor Oil and Collodion.

Extemporaneous preparation

The following formula and directions apply.

Colophony	25 g
Virgin Castor Oil	25 g
Collodion	Sufficient to produce 1000 ml

Mix the ingredients and stir until the colophony has dissolved; allow any deposit to settle and decant the clear liquid.

Flexible collodion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

IDENTIFICATION

A. Expose a thin layer to the air. A thin, tenacious film is left which, when ignited, burns rapidly with a yellow flame.

B. Mix with an equal volume of *water*. A white, viscid, stringy mass is obtained.

Ethanol content

20 to 23% v/v when determined by the following method. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions in *ether*. Solution (1) contains 5% v/v of *absolute ethanol* and 5% v/v of *acetonitrile* (internal standard). Solution (2) contains 20% v/v of the substance being examined. Solution (3) contains 20% v/v of the substance being examined and 5% v/v of the internal standard.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with porous polymer beads (100 to 120 mesh) (Porapak Q is suitable) and maintained at 120° with a flow rate of 40 ml per minute for the carrier gas.

Calculate the content of C_2H_6O from the areas of the peaks due to ethanol and acetonitrile in the chromatograms obtained with solution (1) and solution (3).

For preparations in which industrial methylated spirit has been used, determine the content of ethanol as described above.

Determine the concentration of methanol in the following manner. Carry out the chromatographic procedure described above but using the following solutions. Solution (1) contains 0.25% v/v of *methanol* and 0.25% v/v of *acetonitrile* (internal standard). For solution (2) dilute a volume of the preparation being examined with *ether* to contain between 0.2% and 0.3% v/v of *methanol*. Prepare solution (3) in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 0.25% v/v.

The sum of the contents of ethanol and methanol is 20 to 23% v/v and the ratio of the content of methanol to that of ethanol is commensurate with industrial methylated spirit having been used.

COLLODION FOR THE PREPARATION OF FLEXIBLE COLLODION

DEFINITION

Collodion is a solution of Pyroxylin in a mixture of Ether and Ethanol (90 per cent).

PRODUCTION

It may be prepared by adding 100 g of pyroxylin to 900 ml of a mixture of 3 volumes of solvent ether and 1 volume of ethanol (90 per cent) and agitating continuously until dissolved. The viscosity of the resulting solution is determined and the solution is diluted with the solvent mixture until it complies with the requirement for kinematic viscosity.

In making Collodion the ethanol (90 per cent) may be replaced by industrial methylated spirit diluted so as to be of equivalent alcoholic strength, provided that the law and the statutory regulations governing the use of industrial methylated spirit are observed.

CHARACTERISTICS

A clear, viscid, colourless or pale straw-coloured liquid.

TESTS

Weight per ml

0.785 to 0.795 g, Appendix V G.

Kinematic viscosity

405 to 700 $mm^2 s^{-1}$, when determined using a falling sphere viscometer complying with British Standard 188: 1977 (Methods for the determination of viscosity of liquids). Fill the fall tube with the collodion to about 10 mm above the 220 mm mark, place vertically in the bath and allow to stand for air bubbles to clear and for temperature equilibrium to be attained. Clean the sphere, immerse it in a portion of the liquid being examined maintained at a temperature of 19.9° to 20.1° and when it is at this temperature introduce it, without wiping, into the delivery tube. Observe the time for the lowest part of the sphere to pass through the planes of the tops of the 175-mm mark and the 25-mm mark, using a telescope or other suitable device to avoid errors due to parallax. The average of three readings concordant to within 0.5% is taken as the time of fall. Calculate the kinematic viscosity (ν) in square millimetres per second ($mm^2 s^{-1}$) from the expression:

$$\nu = \frac{d^2 g(\delta - \rho)}{0.18 v \rho} \times 0.867$$

where d = the diameter of the sphere in cm,

δ = density of the sphere in g cm^{-3},

ρ = density of the collodion being examined in g cm^{-3},

v = velocity of the fall in cm s^{-1},

g = local acceleration due to gravity in cm s^{-2}.

When Collodion is prescribed or demanded, Flexible Collodion shall be dispensed or supplied.

Paediatric Codeine Linctus

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Paediatric Codeine Linctus is an *oral solution* containing 0.06% w/v of Codeine Phosphate or an equivalent concentration of Codeine Phosphate Sesquihydrate in a suitable flavoured vehicle.

Extemporaneous preparation

It may be prepared by diluting Codeine Linctus with a suitable vehicle in accordance with the manufacturer's instructions.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of codeine phosphate $C_{18}H_{21}NO_3,H_3PO_4, \frac{1}{2}H_2O$

0.057 to 0.063% w/v.

IDENTIFICATION

To 50 ml add 20 ml of *water* and 30 ml of *chloroform*, shake and discard the chloroform layer. To the aqueous layer add 10 ml of 1M *sodium hydroxide* and extract with 30 ml of *chloroform*. Wash the chloroform layer with two 10 ml quantities of 0.1M *sodium hydroxide* followed by 10 ml of *water*. Dry the chloroform layer with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dry the residue at 60°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of codeine *(RS 072)*.

TESTS

Related substances

Complies with the test described under Codeine Linctus, applying to the plate 20 µl of each of the following solutions. For solution (1) add 20 ml of *water* and 2 ml of 13.5M *ammonia* to 40 ml of the linctus and extract with two 20 ml quantities of *chloroform*. Dry the combined extracts with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*. For solution (2) dilute 1.5 volumes of solution (1) to 100 volumes with *chloroform*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *chloroform*.

ASSAY

Carry out the Assay described under Codeine Linctus using the following solutions. Solution (1) contains 0.012% w/v of *codeine phosphate BPCRS* in *water*. For solution (2) mix a quantity of the linctus containing 3 mg of codeine phosphate with sufficient *water* to produce

25 ml.

STORAGE

Paediatric Codeine Linctus should be protected from light.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Paediatric Co-trimoxazole Oral Suspension

General Notices

Paediatric Trimethoprim and Sulfamethoxazole Oral Suspension

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Paediatric Co-trimoxazole Oral Suspension is a suspension containing 0.8% w/v of Trimethoprim and 4% w/v of Sulfamethoxazole in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

0.72 to 0.88% w/v.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

3.60 to 4.40% w/v.

IDENTIFICATION

Complies with the tests described under Co-trimoxazole Oral Suspension but in test B for solution (2) use a 1.0% w/v solution of *sulfamethoxazole BPCRS* in *methanol* and for solution (3) use a 0.20% w/v solution of *trimethoprim BPCRS* in *methanol*. Apply to the plate 10 µl of each of the three solutions.

Acidity

pH, 5.0 to 6.5, Appendix V L.

ASSAY

Carry out the Assays for trimethoprim and sulfamethoxazole described under Co-trimoxazole Oral Suspension but using 8 g of the oral suspension.

STORAGE

Paediatric Co-trimoxazole Oral Suspension should be protected from light and stored at a temperature not exceeding 30°.

Paediatric Co-trimoxazole Oral Suspension contains, in 5 ml, 40 mg of Trimethoprim and 200 mg of Sulfamethoxazole.

Paediatric Co-trimoxazole Tablets

General Notices

Paediatric Trimethoprim and Sulfamethoxazole Tablets

Action and use

Dihydrofolate reductase inhibitor + sulfonamide antibacterial.

DEFINITION

Paediatric Co-trimoxazole Tablets contain, in each, 20 mg of Trimethoprim and 100 mg of Sulfamethoxazole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

0.0185 to 0.0215 g.

Content of sulfamethoxazole, $C_{10}H_{11}N_3O_3S$

0.0925 to 0.1075 g.

IDENTIFICATION

Comply with the tests described under Co-trimoxazole Tablets.

ASSAY

Carry out the Assays for trimethoprim and for sulfamethoxazole described under Co-trimoxazole Tablets.

LABELLING

The label states the quantities of Trimethoprim and of Sulfamethoxazole in each tablet.

Prolonged-release Cefaclor Tablets

General Notices

Slow Cefaclor Tablets

Prolonged-release Cefaclor Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Cephalosporin antibacterial.

DEFINITION

Prolonged-release Cefaclor Tablets contain Cefaclor. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Cefaclor. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous cefaclor, $C_{15}H_{14}ClN_3O_4S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 0.3 g of anhydrous cefaclor with 100 ml of *water*, filter and dilute 1 ml of the filtrate to 100 ml with *water*. The *light absorption*, Appendix II B, in the range 190 nm to 310 nm, of the final solution exhibits a maximum only at 264 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. Prepare a 0.27% w/v solution of *sodium dihydrogen orthophosphate* and adjust the pH to 2.5, if necessary, with *orthophosphoric acid* (solution A). For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.75 g of anhydrous cefaclor with 200 ml of solution A, add sufficient of solution A to produce 250 ml and filter. Solution (2) contains 0.003% w/v of *cefaclor EPCRS* in solution A. Solution (3) contains 0.0025% w/v of *cefaclor EPCRS* and 0.005% w/v of *delta-3-cefaclor EPCRS* in solution A.

The chromatographic conditions described under Cefaclor Capsules may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obained with solution (2) (0.6%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%). Disregard any peak with an area less than 0.1 times that of the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 75 mg of anhydrous cefaclor with 200 ml of the mobile phase, add sufficient of the mobile phase to produce 250 ml and filter. Solution (2) contains 0.03% w/v of *cefaclor EPCRS* in the mobile phase. Solution (3) contains 0.03% w/v of each of *cefaclor EPCRS* and *delta-3-cefaclor EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Beckman Ultrasphere ODS and Supelcosil LC-18-DB are suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 1 g of *sodium pentanesulphonate* in a mixture of 780 ml of *water* and 10 ml of *triethylamine*, adjusting the pH to 2.5 using *orthophosphoric acid*, adding 220 ml of *methanol* and mixing and (c) a detection wavelength of 265 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cefaclor and delta-3-cefaclor is at least 2.5 and the *symmetry factor* of the peak due to cefaclor is at most 1.5.

Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the tablets from the chromatograms obtained and using the declared content of $C_{15}H_{14}ClN_3O_4S$ in *cefaclor EPCRS*.

STORAGE

Prolonged-release Cefaclor Tablets should be stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

Prolonged-release Clarithromycin Tablets

General Notices

Action and use

Macrolide antibacterial.

Prolonged-release Clarithromycin Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Prolonged-release Clarithromycin Tablets contain Clarithromycin. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Clarithromycin. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of clarithromycin, $C_{38}H_{69}NO_{13}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Clarithromycin with 10 ml of *water* and extract with 20 ml of *dichloromethane*. Separate the lower dichloromethane layer and centrifuge. Filter the supernatant (Whatman GF/C is suitable) and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after drying under vacuum at room temperature for 2 hours, is concordant with the *reference spectrum* of clarithromycin *(RS 424)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of powdered tablets containing 75 mg of Clarithromycin in 40 ml of a mixture of equal volumes of *acetonitrile R1* and *water* (solution A), mix with the aid of ultrasound, add sufficient solution A to produce 50 ml and filter through a Whatman GF/C filter and then through a 0.45-µm PTFE filter.

(2) Dilute 5 volumes of solution (1) to 100 volumes with solution A.

(3) Dilute 10 volumes of solution (2) to 100 volumes with solution A.

(4) 0.15% w/v of *clarithromycin for peak identification EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Kingsorb C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.1 ml per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 205 nm.

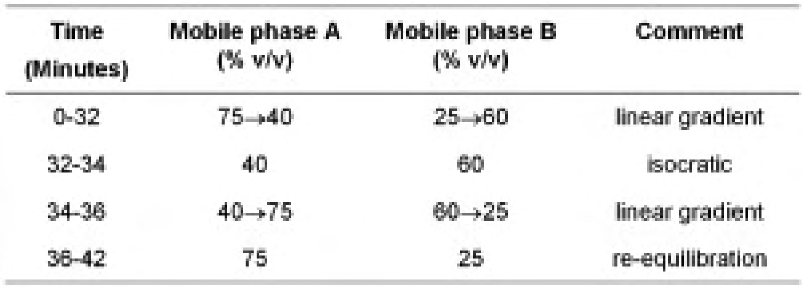
(f) Inject 10 µl of each solution.

MOBILE PHASE

Mobile phase A A 0.476% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 4.4 with either 2M *orthophosphoric acid* or a 4.5% w/v solution of *potassium hydroxide*, filtered through a C18 filtration kit (3M Empore is suitable).

Mobile phase B *acetonitrile R1.*

Use the following gradient.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-32	75→40	25→60	linear gradient
32-34	40	60	isocratic
34-36	40→75	60→25	linear gradient
36-42	75	25	re-equilibration

When the chromatograms are recorded using the prescribed conditions the retention times relative to clarithromycin (retention time = about 11 minutes) are: impurity I = about 0.38; impurity A = about 0.42; impurity J = about 0.63; impurity L = about 0.74; impurity B = about 0.79; impurity M = about 0.81; impurity C = about 0.89; impurity D = about 0.96; impurity N = about 1.15; impurity E = about 1.27; impurity F = about 1.33; impurity P = about 1.35; impurity O = about 1.41; impurity K = about 1.59; impurity G = about 1.72; impurity H = about 1.82.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2) the *symmetry factor* of the peak due to clarithromycin is less than 1.75;

in the chromatogram obtained with solution (4) the *peak to valley ratio* is at least 3.0 where H_p is the height above the baseline of the peak due to impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to clarithromycin;

the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *clarithromycin for peak identification EPCRS*.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities G and H using solution (4) and multiply the areas of these peaks by the corresponding correction factors; impurity G, 0.27; impurity H, 0.15.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (1%) and not more than four such peaks have an area greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of all the *secondary peaks* is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (3) (3.5%).

Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). Disregard any peaks eluting before impurity I and after impurity H.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely powder a quantity of tablets containing 2 g of Clarithromycin and quantitatively transfer the powder to a volumetric flask using about 350 ml of *methanol*. Mix with the aid of ultrasound for 15 minutes, shake vigorously for 15 minutes, allow to cool, add sufficient *methanol* to produce 500 ml and mix. Filter the suspension (Whatman GF/C paper is suitable), dilute 1 volume of the filtrate to 40 volumes with mobile phase and filter through a 0.45-µm filter.

(2) 0.01% w/v of *clarithromycin BPCRS* in the mobile phase.

(3) 0.01% w/v of each of *clarithromycin BPCRS* and *clarithromycin impurity E BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Superspher ODS2 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 210 nm.

(f) Inject 50 µl of each solution.

MOBILE PHASE

35 volumes of 0.067M *potassium dihydrogen orthophosphate* and 65 volumes of *methanol* adjusted to pH 4.0 with *orthophosphoric acid*.

When the chromatograms are recorded under the prescribed conditions the approximate retention times for clarithromycin and clarithromycin impurity E are 4 and 6 minutes respectively.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution*

factor between the two principal peaks is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{38}H_{69}NO_{13}$ in the tablets using the declared content of $C_{38}H_{69}NO_{13}$ in *clarithromycin BPCRS*.

Compound Docusate Enema

General Notices

Action and use

Stimulant laxative; faecal softener.

DEFINITION

Compound Docusate Enema is a *rectal solution* containing Docusate Sodium and Glycerol in a suitable water-miscible vehicle.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia*, 20 volumes of *ethanol (96%)*, 40 volumes of *water* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) dissolve, by heating on a water bath, a quantity of the enema containing 50 mg of Docusate Sodium in 5 ml of *water*. Pass 1 ml of this solution with the aid of vacuum through a solid-phase extraction column of 1 ml capacity and containing 0.1 g of an octadecyl-bonded silica sorbent (a Sep-pak C18 column is suitable) previously washed with 2 ml of *methanol*, followed by 5 ml of *water*. Wash the column with 2 ml of *water*, discarding the aqueous eluate and then elute the Docusate Sodium with 4 ml of *methanol*, retaining the methanol solution. Solution (2) contains 0.25% w/v of *docusate sodium BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and expose to iodine vapour. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Mix 1 g with 0.5 ml of *nitric acid* and superimpose 0.5 ml of *potassium dichromate solution*. A blue ring develops at the interface of the liquids, which does not diffuse into the lower layer within 10 minutes.

TESTS

Acidity or alkalinity

pH, 5.0 to 8.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of *ether* to a quantity of the enema containing 50 mg of Docusate Sodium, shake thoroughly and allow to stand for 16 hours. Filter the ether extract through *anhydrous sodium sulphate* using a glass-fibre filter (Whatman GF/C is suitable). Extract the aqueous layer with a further 50 ml quantity of *ether* and filter through *anhydrous sodium sulphate*. Evaporate the combined ether extracts to dryness and dissolve the residue obtained in 100 ml of a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*. Solution (2) contains 0.05% w/v of *docusate sodium BPCRS* in a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with silica gel with a chemically bonded, strongly basic quaternary ammonium anion-exchange coating (10 µm) (Spherisorb SAX is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1.6 volumes of *orthophosphoric acid*, 350 volumes of *water* and 650 volumes of *acetonitrile* and (c) a detection wavelength of 214 nm.

Calculate the content of $C_{20}H_{37}NaO_7S$ in the enema using the declared content of $C_{20}H_{37}NaO_7S$ in *docusate sodium BPCRS*.

Dacarbazine Injection

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Dacarbazine Injection is a sterile solution of Dacarbazine in Water for Injections. It is prepared by dissolving Dacarbazine for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Dacarbazine Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

DACARBAZINE FOR INJECTION

DEFINITION

Dacarbazine for Injection is a sterile material consisting of Dacarbazine with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of dacarbazine, $C_6H_{10}N_6O$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A white or very pale yellow powder.

IDENTIFICATION

A. Dissolve a quantity of the contents of the sealed container containing 0.1 g of Dacarbazine in 200 ml of 0.1M *mixed phosphate buffer pH 7.0* , dilute with the buffer solution to 250 ml and dilute 3 ml to 200 ml with the same buffer solution. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 237 nm and 330 nm.

B. In the test for 5-aminoimidazole-4-carboxamide, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

TESTS

5-Aminoimidazole-4-carboxamide hydrochloride

Carry out the following procedure protected from light and inject samples within 1 hour of preparation. Use low-actinic glassware. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the sealed container containing 0.20 g of Dacarbazine in 40 ml of 0.1M *acetic acid* and add sufficient 0.1M *acetic acid* to produce 50 ml. For solution (2) dilute 1 volume of solution (1) to 100 volumes with 0.1M *acetic acid*. Solution (3) contains 0.004% w/v of *dacarbazine BPCRS* in 0.1M *acetic acid*. Solution (4) contains 0.0024% w/v of *5-aminoimidazole-4-carboxamide hydrochloride* in 0.1M *acetic acid*.

The chromatographic procedure described under Related substances may be used but using 0.005M *dioctyl sodium sulphosuccinate* in a mixture of 3 volumes of *glacial acetic acid*, 87 volumes of *water* and 110 volumes of *methanol* as the mobile phase.

The area of any peak corresponding to 5-aminoimidazole-4-carboxamide hydrochloride in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.6%).

Related substances

Carry out the following procedure protected from light and inject samples within 1 hour of preparation. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0040% w/v of *2-azahypoxanthine BPCRS* in 0.25M *acetic acid*. For solution (2) dissolve a quantity of the contents of the sealed container containing 0.20 g of Dacarbazine in 40 ml of 0.25M *acetic acid* and add sufficient 0.25M *acetic acid* to produce 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.005M *dioctyl sodium sulphosuccinate* in a mixture of 1.5 volumes of *glacial acetic acid* and 98.5 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm. After use the column should be thoroughly flushed with *methanol* to remove dacarbazine which does not elute with the mobile phase.

In the chromatogram obtained with solution (2) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (1%), not more than one such peak has an area greater than half the area of the peak in the chromatogram obtained with solution (1) (0.5%) and the sum of the areas of all such peaks is not greater than three times the area of the peak in the chromatogram obtained with solution (1) (3%).

Uniformity of content

Sealed containers containing 200 mg or less of Dacarbazine comply with the requirements stated under Parenteral Preparations, Powders for Injections. Use the individual results obtained in the Assay.

ASSAY

Carry out the following procedure protected from light. Dissolve the contents of one container in 0.1M *hydrochloric acid* and dilute with sufficient 0.1M *hydrochloric acid* to produce a final solution containing 0.0008% w/v of Dacarbazine. Measure the *absorbance* of the resulting solution at the maximum at 323 nm, Appendix II B. Calculate the content of $C_6H_{10}N_6O$ in the sealed container taking 1090 as the value of A(1%, 1 cm) at the maximum at 323 nm.

Repeat the procedure with a further nine sealed containers and calculate the average content of $C_6H_{10}N_6O$ per container from the 10 individual results thus obtained.

STORAGE

The sealed container should be protected from light and stored at a temperature of 2° to 8°.

Dantrolene Oral Suspension

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Skeletal muscle relaxant.

DEFINITION

Dantrolene Oral Suspension is a suspension of Dantrolene Sodium in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of dantrolene sodium, $C_{14}H_9N_4NaO_5,3\frac{1}{2}H_2O$

95.0 to 105.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 0.1 g of Dantrolene Sodium with sufficient 0.01M *sodium hydroxide* to produce 100 ml, dilute 1 ml to 100 ml with 0.01M *sodium hydroxide* , filter and use the filtrate. The *light absorption* , Appendix II B, in the range 230 nm to 350 nm, of the final solution, exhibits a maximum at 314 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions, using 900 ml of 0.1M *hydrochloric acid* as the dissolution medium and rotating the paddle at 50 revolutions per minute. Use a volume of the oral suspension containing one dose.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the oral suspension containing 50 mg of Dantrolene Sodium in 20 ml of *tetrahydrofuran* and 2 ml of *glacial acetic acid* and dilute with sufficient *absolute ethanol* to produce 100 ml.

(2) Dilute 1 ml of solution (1) to 100 ml with *absolute ethanol*.

(3) Dissolve 5 mg of *dantrolene sodium BPCRS* and 0.1 g of *theophylline BPCRS* in 20 ml of *tetrahydrofuran* and 2 ml of *glacial acetic acid* and dilute with sufficient *absolute ethanol* to produce 100 ml. Further dilute 10 ml of this solution to 100 ml with *absolute ethanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Zorbax Sil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Adjust the flow rate of the mobile phase so that the retention time of the peak corresponding to Dantrolene Sodium is about 8 minutes.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 300 nm.

(f) Inject 10 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak.

MOBILE PHASE

9 volumes of *absolute ethanol*, 10 volumes of *glacial acetic acid* and 90 volumes of *hexane*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to theophylline and dantrolene is at least 6.

LIMITS

In the chromatogram obtained with solution (1):

the total area of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 50 ml of *dimethylformamide* to a weighed quantity of the oral suspension containing 60 mg of Dantrolene Sodium and dilute 1 volume of this solution to 100 volumes with the mobile phase.

(2) Dilute 1 volume of a 0.12% w/v solution of *dantrolene sodium BPCRS* in *dimethylformamide* to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with spherical particles of silica, 5 µm in diameter, the surface of which has been modified with chemically-bonded nitrile groups (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 262 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

15 volumes of *acetonitrile* and 85 volumes of a phosphate buffer pH 6.8 prepared by dissolving 11.88 g of *disodium hydrogen orthophosphate* and 9.08 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*.

DETERMINATION OF CONTENT

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{14}H_9N_4NaO_5,3½H_2O$, weight in volume, using the declared content of $C_{14}H_9N_4NaO_5$ in *dantrolene sodium BPCRS* . Each mg of $C_{14}H_9N_4NaO_5$ is equivalent to 1.1873 mg of $C_{14}H_9N_4NaO_5,3½H_2O$.

STORAGE

Dantrolene Oral Suspension should be protected from light.

Dapsone Tablets

General Notices

Action and use

Folic acid synthesis inhibitor; treatment of leprosy.

DEFINITION

Dapsone Tablets contain Dapsone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dapsone, $C_{12}H_{12}N_2O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of dapsone with 20 ml of *acetone* , filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dapsone *(RS 084)* .

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) is similar in position, colour and size to that in the chromatogram obtained with solution (5).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using 900 ml of 0.1M *hydrochloric acid* as the medium and rotating the basket at 100 revolutions per minute. Withdraw a sample of 20 ml of the medium. To a volume of the filtered sample expected to contain 0.1 mg of dapsone add 1 ml of a freshly prepared 0.5% w/v solution of *sodium nitrite* , allow to stand for 3 minutes, add 1.5 ml of a 1% w/v solution of *sulphamic acid* , allow to stand for 3 minutes, add 2.5 ml of a freshly prepared 0.2% w/v solution of N-*(1-naphthyl) -ethylenediamine dihydrochloride* in 0.1M *hydrochloric acid* and dilute to 20 ml with 1M *hydrochloric acid* (solution A). At the same time carry out the procedure using a volume of 0.1M *hydrochloric acid* equal to that of the filtered sample and beginning at the words 'add 1 ml of a freshly prepared 0.5% w/v solution of *sodium nitrite*...' (solution B). Allow solutions A and B to stand for at least 2 minutes and measure the *absorbance* of solution A at the maximum at 538 nm, Appendix II B, using solution B in the reference cell. Repeat the operation using a solution containing 0.1 mg of *dapsone BPCRS* in a volume of 0.1M *hydrochloric acid* equal to that of the filtered sample and beginning at the words 'add 1 ml of a freshly prepared 0.5% w/v solution of *sodium nitrite* ...'. Calculate the total content of dapsone, $C_{12}H_{12}N_2O_2S$, in the medium from the absorbances obtained using the declared content of $C_{12}H_{12}N_2O_2S$ in *dapsone BPCRS* .

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance, a mixture of 1 volume of 13.5M *ammonia* , 6 volumes of *methanol* , 20 volumes of *ethyl acetate* and 20 volumes of n-*heptane* , as the mobile phase and an unsaturated tank. Apply separately to the plate 10 µl of each of solutions (1), (3) and (4) and 1 µl of each of solutions (2) and (5). For solution (1) shake a quantity of the powdered tablets containing 0.1 g of dapsone with 10 ml of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol* . For solution (3) dilute 1 volume of solution (2) to 10 volumes with *methanol* . For solution (4) dilute 1 volume of solution (3) to 5 volumes with *methanol* . Solution (5) contains 0.1% w/v of *dapsone BPCRS* . After removal of the plate, allow it to dry in air, spray with a 0.1% w/v solution of *4-dimethylaminocinnamaldehyde* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *ethanol (96%)* and examine in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense that the spot in the chromatogram obtained with solution (3) (1%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.25 g of dapsone in a mixture of 15 ml of *water* and 15 ml of 2M *hydrochloric acid* , add 3 g of *potassium bromide* , cool in ice and titrate slowly with 0.1M *sodium nitrite VS* , stirring constantly and determining the end point electrometrically. Each ml of 0.1M *sodium nitrite VS* is equivalent to 12.42 mg of $C_{12}H_{12}N_2O_2S$.

Demeclocycline Capsules

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Demeclocycline Capsules contain Demeclocycline Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of demeclocycline hydrochloride, $C_{21}H_{21}ClN_2O_8,HCl$

90.0 to 107.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 ml for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. At the time of use, dry the plate in an oven at 110° for 1 hour. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) extract a quantity of the contents of the capsules containing 10 mg of demeclocycline hydrochloride with 20 ml of *methanol* and centrifuge. Solution (2) contains 0.05% w/v of *demeclocycline hydrochloride EPCRS* in *methanol*. Solution (3) contains 0.05% w/v each of *demeclocycline hydrochloride EPCRS*, *oxytetracycline hydrochloride EPCRS* and *metacycline hydrochloride EPCRS* in *methanol*. Allow the plate to dry in a stream of air and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

B. Add 20 ml of warm *methanol* to a quantity of the contents of the capsules containing 10 mg of demeclocycline hydrochloride, allow to stand for 20 minutes, filter and evaporate the filtrate to dryness on a water bath. To 0.5 mg of the residue add 2 ml of *sulphuric acid*; a purple colour is produced. Add 1 ml of *water*; the colour changes to yellow.

C. To 1 mg of the residue obtained in test B add 7 ml of *water* and 7 ml of 2M *hydrochloric acid* and heat gently for 30 seconds. No colour is produced immediately.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the filtered solution, if necessary, with 0.1M *hydrochloric acid* to give a solution expected to contain about 0.015% w/v of demeclocycline hydrochloride. Solution (2) contains 0.015% w/v of *demeclocycline hydrochloride EPCRS* in 0.1M *hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 20 volumes of *dimethylformamide* and 80 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 2.2 with *triethylamine* and (c) a detection wavelength of 355 nm.

Calculate the total content of demeclocycline hydrochloride, $C_{21}H_{21}ClN_2O_8$,HCl, in the medium using the declared content of $C_{21}H_{21}ClN_2O_8$,HCl, in *demeclocycline hydrochloride EPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1) and (3) described under Assay.

The chromatographic conditions described under Dissolution may be used.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (10%) and the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (3) (15%).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the contents of the capsules lose not more than 5.0% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the mixed contents of 20 capsules containing 0.5 g of demeclocycline hydrochloride with 50 ml of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 100 ml, filter and dilute 1 volume of the filtrate to 5 volumes with 0.01M *hydrochloric acid*. Solution (2) contains 0.10% w/v of *demeclocycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*. Solution (3) contains 0.010% w/v of *demeclocycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*. Solution (4) contains 0.015% w/v each of *demeclocycline hydrochloride EPCRS* and *4-epidemeclocycline hydrochloride EPCRS* in 0.01M *hydrochloric acid*.

The chromatographic conditions described under Dissolution may be used.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (4) is at least 3.0.

Calculate the content of $C_{21}H_{21}ClN_2O_8$,HCl in the capsules using the declared content of

$C_{21}H_{21}ClN_2O_8$,HCl in *demeclocycline hydrochloride EPCRS* .

Desferrioxamine Injection

General Notices

Action and use

Chelating agent (iron).

DEFINITION

Desferrioxamine Injection is a sterile solution of Desferrioxamine Mesilate in Water for Injections. It is prepared by dissolving Desferrioxamine Mesilate for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Desferrioxamine Injection deteriorates on storage and should be used immediately after preparation. Cloudy solutions should be discarded.

DESFERRIOXAMINE MESILATE FOR INJECTION

DEFINITION

Desferrioxamine Mesilate for Injection is a sterile material consisting of Desferrioxamine Mesilate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of desferrioxamine mesilate, $C_{25}H_{48}N_6O_8,CH_4SO_3$

94.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dissolve 40 mg of the contents of the sealed container in 2 ml of *absolute ethanol* by heating on a water bath at 60°, cool in ice until the substance begins to crystallise and evaporate to dryness at room temperature under a gentle current of *nitrogen*. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of desferrioxamine mesilate *(RS 086)*.

B. In the test for Related substances the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (3).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light. For solution (1) dissolve a quantity of the contents of the sealed containers containing 75 mg of desferrioxamine mesilate in sufficient of the mobile phase to produce 50 ml. For solution (2) dilute 1 volume of solution (1) to 25 volumes with the mobile phase. Solution (3) contains 0.15% w/v of *desferrioxamine mesilate EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 55 volumes of *tetrahydrofuran* and 950 volumes of a solution containing 0.039% w/v of *disodium edetate* and 0.139% w/v of *ammonium phosphate* adjusted to pH 2.8 with *orthophosphoric acid* in *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 220 nm.

Inject 20 µl of solution (3). Adjust the sensitivity of the detector so that the height of the peak with a relative retention time of about 0.8 is not less than 15% of the full scale of the recorder. The test is not valid unless the *resolution factor* between the peak with the relative retention time of about 0.8 and the principal peak is at least 1.0.

Inject 20 µl of solutions (1) and (2). Continue the chromatography for three times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (4%) and the sum of the areas of any such peaks is not greater than 1.75 times the area of the principal peak in the chromatogram obtained with solution (2) (7%). Disregard any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with solution (2) (0.08%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Dissolve a quantity of the mixed contents of the 10 containers containing 0.3 g of desferrioxamine mesilate in 15 ml of *water* and add 2 ml of 0.05M *sulphuric acid*. Titrate slowly with 0.1M *ammonium iron(III) sulphate VS* determining the end point potentiometrically and using a platinum electrode and a calomel reference electrode. Each ml of 0.1M *ammonium iron(III) sulphate VS* is equivalent to 65.68 mg of $C_{25}H_{48}N_6O_8,CH_4SO_3$. Calculate the content of $C_{25}H_{48}N_6O_8,CH_4SO_3$ in a container of average content weight.

Desipramine Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Desipramine Tablets contain Desipramine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of desipramine hydrochloride, $C_{18}H_{22}N_2,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Triturate a quantity of the powdered tablets containing 0.25 g of Desipramine Hydrochloride with 10 ml of *chloroform*, filter and evaporate the filtrate to 3 ml. Add *ether* until a slight turbidity is produced and allow to stand; filter, wash the crystals with a mixture of 1 volume of *chloroform* and 5 volumes of *ether* and dry at 100° at a pressure of 2 kPa for 90 minutes. The *infrared absorption spectrum* of the dried crystals, Appendix II A, is concordant with the *reference spectrum* of desipramine hydrochloride *(RS 087)*.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using *silica gel G* as the coating substance and a mixture of 50 volumes of *glacial acetic acid*, 50 volumes of *toluene* and 5 volumes of *water* as the mobile phase but allowing the solvent front to ascend 7 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Desipramine Hydrochloride with three 10 ml quantities of *chloroform* and filter; evaporate the combined filtrates to dryness and dissolve the residue in 10 ml of a mixture of equal volumes of *chloroform* and *absolute ethanol*. For solution (2) dilute 1 volume of solution (1) to 500 volumes with the same chloroform–ethanol mixture. After removal of the plate, dry it for 10 minutes in a current of air, spray with a 0.5% w/v solution of *potassium dichromate* in *sulphuric acid (20%)* and examine immediately. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 75 mg of Desipramine Hydrochloride with 100 ml of 0.1M *hydrochloric acid* for 30 minutes, add sufficient 0.1M *hydrochloric acid* to produce 250 ml and filter. Dilute 5 ml to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 250 nm, Appendix II B. Calculate the content of $C_{18}H_{22}N_2$,HCl taking 270 as the value of A (1%, 1 cm) at the maximum at 250 nm.

Desmopressin Injection

General Notices

Action and use

Vasopressin analogue; treatment of diabetes insipidus; nocturnal enuresis; haemophillia; von Willebrand's disease.

DEFINITION

Desmopressin Injection is a sterile solution of Desmopressin in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure.*

(1) Dilute the injection, if necessary, with *water* to give a final concentration of 0.0004% w/v of the peptide.

(2) Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture EPCRS* in 25ml of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for* *chromatography* (5 µm).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

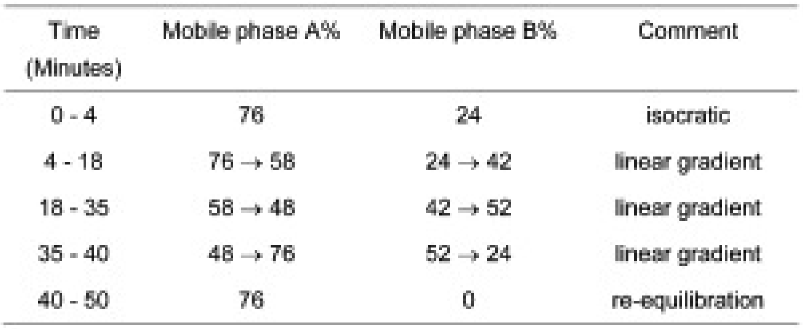
(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µl of each solution.

MOBILE PHASE

Mobile phase A *0.067 M mixed phosphate buffer solution pH 7.0*.

Mobile phase B A mixture of *acetonitrile for chromatography* and mobile phase A in equal volumes.



Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0 - 4	76	24	isocratic
4 - 18	76 → 58	24 → 42	linear gradient
18 - 35	58 → 48	42 → 52	linear gradient
35 - 40	48 → 76	52 → 24	linear gradient
40 - 50	76	0	re-equilibration

The retention time of desmopressin is about 16 minutes and the retention time for oxytocin is about 17 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any *secondary peak* is not more than 4.0%;

— the total area of any such peaks is not more than 5.0%.

Disregard any peak due to the solvent or with an area less than 2.0%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 10 IU per µg peptide.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection in *water* to give a final concentration of 0.0004 % w/v of the peptide.

(2) 0.0004% w/v of *desmopressin EPCRS* in *water*.

(3) Dissolve the contents of a vial of o*xytocin/desmopressin validation mixture EPCRS* in 25 ml of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µl of each solution.

MOBILE PHASE

20 volumes of *acetonitrile for chromatography* and 80 volumes of *0.067* M *mixed phosphate buffer solution, pH 7.0*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks at least 1.5.

The retention time of desmopressin is about 5 minutes; if necessary adjust the concentration of acetonitrile in the mobile phase to obtain a retention time of about 5 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in the injection from the chromatograms obtained and from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin EPCRS*.

STORAGE

Desmopressin Injection should be protected from light and stored at a temperature of 2° to 8°.

Desmopressin Intranasal Solution

General Notices

Action and use

Vasopressin analogue; treatment of diabetes insipidus; haemophillia; von Willebrand's disease.

DEFINITION

Desmopressin Intranasal Solution is a solution of Desmopressin containing suitable buffering agents and preservatives.

The intranasal solution complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 5.2, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure.*

(1) Dilute a volume of the intranasal solution in *water* to give a final concentration of 0.0025% w/v of the peptide.

(2) Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture EPCRS* in 10ml of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

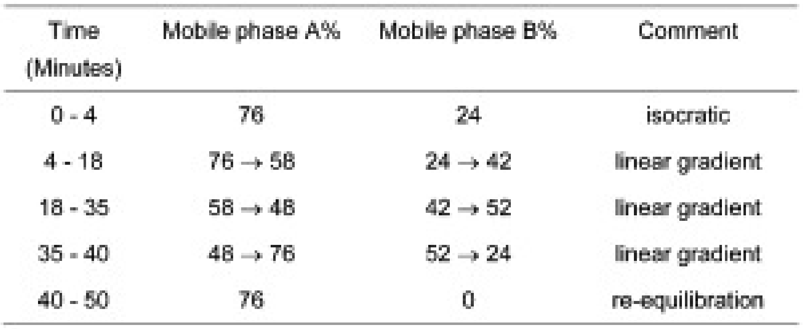
(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µl of each solution.

MOBILE PHASE

Mobile phase A *0.067M mixed phosphate buffer solution, pH 7.0*.

Mobile phase B 10 volumes of a*cetonitrile for chromatography* and 10 volumes of mobile phase A.



Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0 - 4	76	24	isocratic
4 - 18	76 → 58	24 → 42	linear gradient
18 - 35	58 → 48	42 → 52	linear gradient
35 - 40	48 → 76	52 → 24	linear gradient
40 - 50	76	0	re-equilibration

The retention time of desmopressin is about 16 minutes and the retention time of oxytocin is about 17 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.5;

The peak due to desmopressin is clearly separated from the peak due to the antimicrobial preservative stated on the label.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than 4.0%.

the total area of any such peaks is not more than 5.0%.

Disregard any peak due to the solvent, any antimicrobial preservative stated on the label and any peak with an area less than 0.3%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions:

(1) Dilute a volume of the intranasal solution in *water* to give a final concentration of 0.0025% w/v of the peptide.

(2) 0.0025% w/v of *desmopressin EPCRS* in *water*.

(3) Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture EPCRS* in 1ml of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 200 µl of each solution.

MOBILE PHASE

A mixture of 2 volumes of *acetonitrile for chromatography* and 8 volumes of *0.067* M *mixed phosphate buffer solution, pH 7.0*.

SYSTEM SUITABILITY

The test is not valid unless:

— in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is equal to or greater than 1.5.

— the peak due to desmopressin is clearly separated from the peak due to the antimicrobial preservative stated on the label.

The retention time of desmopressin is about 5 minutes. If necessary adjust the concentration of acetonitrile in the mobile phase to obtain the correct retention time and *resolution factor*.

DETERMINATION OF CONTENT

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in the intranasal solution from the chromatograms obtained and from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin EPCRS*.

STORAGE

Desmopressin Intranasal Solution should be protected from light and stored at a temperature of 2° to 8°.

Desmopressin Tablets

General Notices

Action and use

Vasopressin analogue; treatment of diabetes insipidus; nocturnal enuresis; haemophillia; von Willebrand's disease.

DEFINITION

Desmopressin Tablets contain Desmopressin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

IDENTIFICATION

In the test for Uniformity of content, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules* , Appendix XII B 1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.

(b) Use 500 ml of *water* as the medium.

PROCEDURE

(c) After 45 minutes, withdraw a 20 ml sample of the medium and filter.

(d) Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

(1) Use the filtered dissolution medium.

(2) Prepare a solution of *desmopressin EPCRS* in *water* with a final concentration equal to that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the total content of desmopressin $C_{46}H_{64}N_{14}O_{12}S_2$, in the medium using the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin EPCRS* .

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions and the *normalisation procedure* :

(1) Shake a quantity of powdered tablets containing 0.5 mg with 1 ml *water* to give a final concentration of 0.05% w/v of the peptide.

(2) Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture EPCRS* in 0.5 ml of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use linear gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

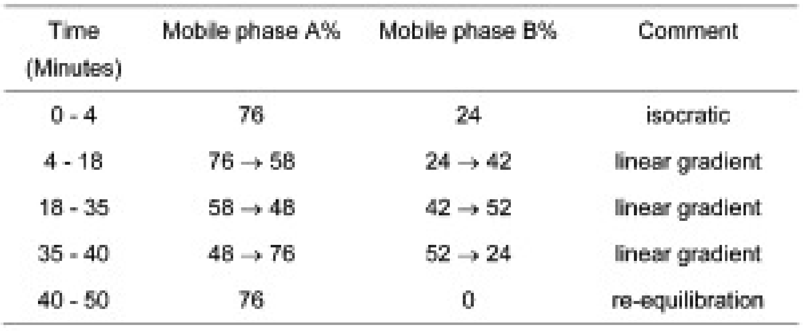
(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 50 µl of each solution.

Mobile phase A *0.067 M mixed phosphate buffer solution pH 7.0.*

Mobile phase B 10 volumes of a*cetonitrile for chromatography* and 10 volumes of mobile phase A.



Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0 - 4	76	24	isocratic
4 - 18	76 → 58	24 → 42	linear gradient
18 - 35	58 → 48	42 → 52	linear gradient
35 - 40	48 → 76	52 → 24	linear gradient
40 - 50	76	0	re-equilibration

The retention time of desmopressin is about 16 minutes and the retention time for oxytocin is about 17 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not more than 2.0%.

the total area of any such peaks is not more than 4.0%.

Disregard any peak due to the solvent or with a relative amount less than 0.05%.

Uniformity of content

Tablets containing less than 2 mg of Desmopressin comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions:

(1) Add sufficient *water* to one tablet to produce a solution containing 0.01% w/v of Desmopressin.

(2) 0.01% w/v of *desmopressin EPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm x 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 50 µl of each solution.

MOBILE PHASE

4 volumes of *acetonitrile for chromatography* and 6 volumes of *0.067 M mixed phosphate buffer solution pH 7.0*.

The retention time of desmopressin is about 5 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in each tablet from the chromatograms obtained and from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin EPCRS*.

ASSAY

Use the average of the 10 individual results obtained in the test for Uniformity of content.

STORAGE

Desmopressin Tablets should be protected from moisture.

Desogestrel Tablets

General Notices

Action and use

Progestogen.

DEFINITION

Desogestrel Tablets contain Desogestrel.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of desogestrel, $C_{22}H_{30}O$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography* , Appendix III A, using the following solutions in *dichloromethane* .

(1) Disperse a quantity of powdered tablets containing 0.75 mg of Desogestrel in 8 ml of *dichloromethane* , mix with the aid of ultrasound and dilute to 10 ml with *dichloromethane* and filter.

(2) 0.0075% w/v of *desogestrel BPCRS.*

(3) 0.0075% w/v each of *desogestrel BPCRS* and *lynestrenol BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel 60 precoated plate for high performance thin-layer chromatography (Merck silica gel 60 HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 2 µl of each solution.

(d) After removal of the plate, dry in air, spray it with *ethanolic sulphuric acid* (2%), heat at 110° for 10 minutes and examine under *ultraviolet light (365 nm)* .

MOBILE PHASE

A mixture of 20 volumes of *ethyl acetate* and 80 volumes of *toluene.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in colour, position and size to the principal spot in the chromatogram obtained with solution (2).

TESTS

Solution A

80 volumes of *acetonitrile* and 20 volumes of *water.*

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 ml of a 0.3% w/v solution of *sodium lauryl sulphate*, at a temperature of 37°, as the medium.

PROCEDURE

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

(1) After 45 minutes withdraw a sample of the medium and filter. Use the filtered medium, diluted with a 0.3% w/v solution of *sodium lauryl sulphate* if necessary, expected to contain 0.000015% w/v of Desogestrel.

(2) 0.000015% w/v of *desogestrel BPCRS* in a mixture of 1 volume of *2-propanol* and 99 volumes of a 0.3% w/v solution of *sodium lauryl sulphate.*

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Zorbax ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use a column temperature of 40°C.

(e) Use a detection wavelength of 205 nm.

(f) Inject 200 µl of each solution.

MOBILE PHASE

5 volumes of *water* and 95 volumes of *acetonitrile.*

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}O$ in the medium from the chromatograms obtained and using the declared content of $C_{22}H_{30}O$ in *desogestrel BPCRS* .

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions in 80 volumes of *acetonitrile* and 20 volumes of *water* (Solution A).

(1) Disperse a quantity of powdered tablets containing 0.75 mg of Desogestrel in 15 ml of solution A, mix with the aid of ultrasound and dilute with sufficient solution A to produce 20 ml, mix and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 2 ml of this solution to 10 ml with solution A.

(3) 0.00375% w/v of *desogestrel BPCRS* , 0.000075% w/v of *desogestrel impurity D BPCRS* and 0.0000375% w/v of *desogestrel impurity E BPCRS* .

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Zorbax ODS is suitable).

(b) Use a gradient elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use a column temperature of 40°C.

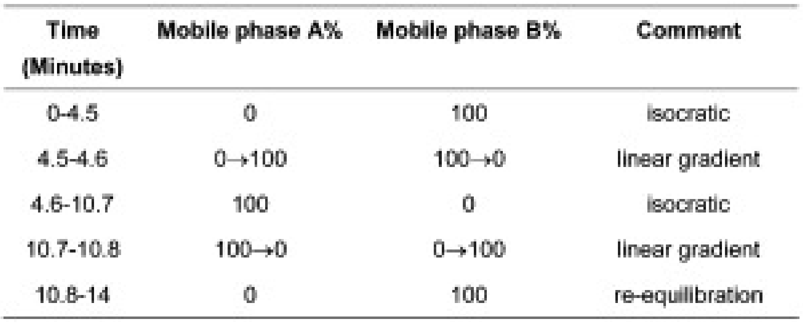
(e) Use detection wavelengths of 230 nm and 210 nm.

(f) Inject 25 µl of each solution.

MOBILE PHASE

Mobile Phase A *acetonitrile.*

Mobile phase B 50 volumes of *acetonitrile* and 50 volumes of *water.*



Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-4.5	0	100	isocratic
4.5-4.6	0→100	100→0	linear gradient
4.6-10.7	100	0	isocratic
10.7-10.8	100→0	0→100	linear gradient
10.8-14	0	100	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to desogestrel impurity D and desogestrel is at least 1.5 and the retention time of desogestrel impurity D is not greater than 6 minutes.

LIMITS

In the chromatogram obtained with solution (1) at 210 nm:

the area of any peak due to desogestrel impurity E is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (1%);

the area of any other peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

In the chromatogram obtained with solution (1) at 230 nm:

the area of any peak due to desogestrel impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (2%).

Uniformity of content

Tablets containing less than 2 mg of Desogestrel comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 80 volumes of *acetonitrile* and 20 volumes of *water* (Solution A).

(1) To one tablet add 5 ml of solution A, mix with the aid of ultrasound and add sufficient solution A to produce a solution expected to contain 0.00075% w/v of Desogestrel.

(2) 0.00075% w/v of *desogestrel BPCRS*.

(3) 0.00075% w/v of *desogestrel BPCRS*, 0.000015% w/v of *desogestrel impurity D BPCRS* and 0.0000075% w/v of *desogestrel impurity E BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with the following amendments:

(a) Use a detection wavelength of 210 nm.

(b) Inject 125 µl of each solution.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to desogestrel impurity D and desogestrel is at least 1.5 and the retention time of desogestrel impurity D is not greater than 6 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}O$ in the tablets using the declared content of $C_{22}H_{30}O$ in *desogestrel BPCRS* .

ASSAY

For tablets containing 2 mg or more of desogestrel

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions in 80 volumes of *acetonitrile* and 20 volumes of *water* (Solution A).

(1) To a quantity of powdered tablets containing 0.75 mg of Desogestrel add 10 ml of solution A, mix with the aid of ultrasound and add sufficient solution A to produce 20 ml.

(2) 0.00375% w/v of *desogestrel BPCRS* .

(3) 0.00375% w/v of *desogestrel BPCRS* , 0.000075% w/v of *desogestrel impurity D BPCRS* and 0.0000375% w/v of *desogestrel impurity E BPCRS* .

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used with a detection wavelength of 210 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to desogestrel impurity D and desogestrel is at least 1.5 and the retention time of desogestrel impurity D is not greater than 6 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{30}O$ in the tablets using the declared content of $C_{22}H_{30}O$ in *desogestrel BPCRS* .

For tablets containing less than 2 mg of desogestrel

Use the average of the ten individual results obtained in the test for Uniformity of content.

IMPURITIES

D. 13-ethyl-17-hydroxy-11-methylidene-18,19-dinor-17α-pregn-4-en-20-yn-3-one (European Pharmacopoeia impurity D),

E. 13-ethyl-11-methylidene-18,19-dinor-17α-pregn-4-en-20-yne-3α,17-diol (European Pharmacopoeia impurity E).

Dexamethasone and Neomycin Ear Spray

General Notices

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone and Neomycin Ear Spray is an emulsion containing Dexamethasone in *microfine powder* and Neomycin Sulphate in a suitable vehicle in a suitable metered-dose container. It may contain acetic acid.

The ear spray complies with the requirements stated under Ear Preparations and with the following requirements.

Content of dexamethasone, $C_{22}H_{29}FO_5$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

Shake the ear spray vigorously before carrying out the following tests.

IDENTIFICATION

A. In the Assay for dexamethasone, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

B. Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel F $_{254}$ plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *butan-2-ol* saturated with *water*, 10 volumes of *toluene* and 85 volumes of *ether* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) discharge the container a sufficient number of times to obtain a suitable quantity and dilute with *methanol* , if necessary, to produce a solution containing 0.1% w/v of Dexamethasone. Solution (2) contains 0.1% w/v of *dexamethasone BPCRS* in *methanol* . Solution (3) contains 0.1% w/v each of *dexamethasone BPCRS* and *betamethasone BPCRS* in *methanol* . Allow the plate to dry in air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) is similar in position and size to the principal spot in the chromatogram obtained with solution (2). Spray with *alcoholic solution of sulphuric acid* . Heat at 120° for 10 minutes or until the spots appear. Allow to cool. Examine the chromatograms in daylight and under *ultraviolet light (365 nm).* The principal spot in the chromatogram obtained with solution (1) is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two spots which may, however, not be completely separated.

C. In the test for Neomycin C the principal spot in the chromatogram obtained with solution

(1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (4).

Dexamethasone and Neomycin Ear Spray containing acetic acid complies with the following additional test.

D. Discharge the container a sufficient number of times to produce 0.2 g. The solution yields reaction A characteristic of *acetates*, Appendix VI.

TESTS

Acidity

pH, 2.0 to 3.0, Appendix V L.

Neamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* and a mixture of 10 volumes of *dichloromethane*, 20 volumes of 13.5M *ammonia* and 30 volumes of *methanol* as the mobile phase. Apply separately to the plate, as 5-mm bands, 5 µl of each of the following solutions. For solution (1) discharge the container a sufficient number of times to obtain a suitable quantity (contains 0.5% w/w of neomycin sulphate). Solution (2) contains 0.01% w/v of *neamine EPCRS* in *water*. For solution (3) mix 1 volume of solution (1) and 1 volume of solution (2). After removal of the plate dry it at 100° to 105° for 10 minutes, spray with *ninhydrin and stannous chloride reagent*, heat at 110° for 15 minutes, spray with the same reagent and heat at 110° for 15 minutes. In the chromatogram obtained with solution (1) any spot corresponding to neamine is not more intense than the spot in the chromatogram obtained with solution (2) (2%). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

Neomycin C

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* and a mixture of 20 volumes of *methanol* and 80 volumes of a 20% w/v solution of *sodium chloride* as the mobile phase. Apply separately to the plate, as 5-mm bands, 5 µl of each of the following solutions. For solution (1) discharge the container a sufficient number of times to obtain a suitable quantity (contains 0.5% w/w of neomycin sulphate). Solution (2) contains 0.075% w/v of *framycetin sulphate EPCRS* in *water*. For solution (3) dilute 1 volume of solution (2) to 5 volumes with *water*. Solution (4) contains 0.5% w/v of *neomycin sulphate EPCRS* in *water.* After removal of the plate, dry it at 100° to 105° for 10 minutes, spray with *ninhydrin solution R1* and heat at 100° to 105° for 10 minutes. In the chromatogram obtained with solution (1) the spot with an Rf value slightly less than that of the principal spot (neomycin C) is not more intense than the spot in the chromatogram obtained with solution (2) (15%) but is more intense than the spot in the chromatogram obtained with solution (3) (3%). The test is not valid unless, in the chromatogram obtained with solution (4), a spot appears with an Rf value slightly less than that of the principal spot.

Related substances

Dexamethasone

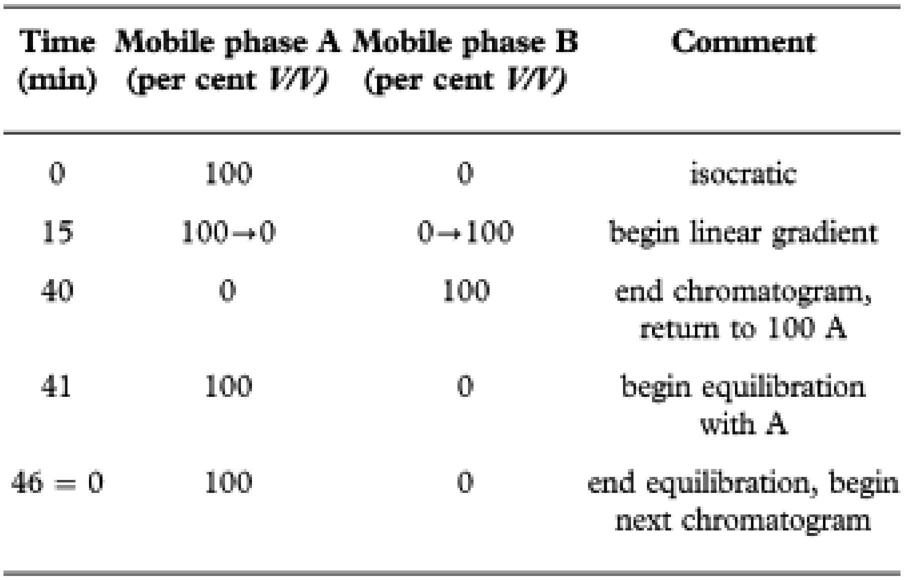
Carry out the method for *liquid chromatography*, Appendix III D. For solution (1) after priming the pump, discharge the container a sufficient number of times to obtain 2.5 mg of Dexamethasone, add 1.5 ml of *acetonitrile R* and 5 ml of mobile phase A. Mix with the aid of ultrasound, add sufficient mobile phase A to produce 10 ml and filter through a 0.45-µm filter.

For solution (2) dilute 1 ml of solution (1) to 100 ml with mobile phase A. Solution (3) contains 0.002% w/v of each of *methylprednisolone BPCRS* and *dexamethasone BPCRS* in mobile phase A. For solution (4) dilute 1 ml of solution (2) to 20 ml with mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) maintained at 45 °C, (b) as the mobile phase with a flow rate of 2.5 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 254 nm.

Mobile phase A 25% v/v *acetonitrile.*

Mobile phase B *acetonitrile.*



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0	100	0	isocratic
15	100→0	0→100	begin linear gradient
40	0	100	end chromatogram, return to 100 A
41	100	0	begin equilibration with A
46 = 0	100	0	end equilibration, begin next chromatogram

Inject 20 µl of solutions (3) and (4). When the chromatograms are recorded in the prescribed conditions, the retention times are: methylprednisolone about 12 minutes and dexamethasone about 14 minutes. The test is not valid unless: (a) in the chromatogram obtained with solution (3) the *resolution factor* between the peaks corresponding to methylprednisolone and dexamethasone is at least 1.5 (if necessary, adjust the concentration of acetonitrile in mobile phase A) and (b) in the chromatogram obtained with solution (4) the *signal to noise ratio* of the peak due to dexamethasone is at least 10.

Inject 20 µl of mobile phase A, solution (1) and solution (2). In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak due to mobile phase A and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (2) (0.05%).

ASSAY

For dexamethasone

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions protected from light. For solution (1) after priming the pump, discharge the container a sufficient number of times to obtain 1 mg of Dexamethasone, add 10 ml of *methanol*, place in an ultrasonic bath for 10 minutes, cool, dilute to 25 ml with *water*, mix and filter through a 0.45-µm PTFE filter. For solution (2) dilute 10 volumes of a 0.010% w/v solution of *dexamethasone BPCRS* in *methanol* to 25 volumes with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a filtered (Whatman GF/F is suitable) mixture of 10 volumes of *glacial acetic acid*, 350 volumes of *acetonitrile* and 640 volumes of *water* and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{22}H_{29}FO_5$ using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS*.

For neomycin sulphate

Prime the pump and discharge the container a sufficient number of times to obtain a quantity of the emulsion containing 3250 IU; dilute to 50 ml with *sterile phosphate buffer pH 8.0*. Dilute 10 ml of the resulting solution to 100 ml with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per ml.

STORAGE

Dexamethasone and Neomycin Ear Spray should not be allowed to freeze.

LABELLING

The strength with respect to Neomycin Sulphate is stated as the number of IU (Units) per ml.

Dexamethasone Eye Drops, Suspension

General Notices

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone Eye Drops, Suspension are a sterile suspension of Dexamethasone in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of dexamethasone, $C_{22}H_{29}FO_5$

95.0 to 105.0% of the stated amount.

The eye drops should be shaken vigorously before carrying out the following tests.

IDENTIFICATION

Mix a quantity of the Eye drops containing 20 mg of Dexamethasone with 5 ml of 0.1M *sodium hydroxide* , add 50 ml of *dichloromethane* and mix with the aid of ultrasound for 20 minutes, filter the dichloromethane layer and evaporate to dryness using a rotary evaporator. Dry the residue at 105^{c} for 2 hours. The *infrared absorption* spectrum of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dexamethasone *(RS 089)* .

TESTS

Particle size

The eye drops are a suspension and comply with the following test:

Examine using an automated light obscuration instrument such as that described in Appendix XIII A. Not more than 20 particles greater than 25 µm, not more than 2 particles greater than 50 µm and no particles greater than 90 µm.

Acidity

pH, 5.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of Dexamethasone in 70 ml of mobile phase, mix with the aid of ultrasound for 10 minutes, dilute with sufficient mobile phase to produce 100 ml and filter.

(2) Dilute 3 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.02% w/v of *dexamethasone impurity standard BPCRS* .

(4) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, dilute 1 ml of this solution to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for six times the retention time of dexamethasone.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of a 0.3% w/v solution of *orthophosphoric acid* that has been previously adjusted to pH 3.0 with *dilute sodium hydroxide* .

SYSTEM SUITABILITY

The test is not valid unless, the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity 3 and dexamethasone is at least 1.5;

closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS* .

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any peaks, apart from the principal peak, is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of Dexamethasone in 70 ml of mobile phase, mix with the aid of ultrasound for 10 minutes, dilute with sufficient mobile phase to produce 100 ml and filter.

(2) 0.02% w/v of *dexamethasone BPCRS*.

(3) 0.01% w/v of *dexamethasone impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity 3 and dexamethasone is at least 1.5;

closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS*.

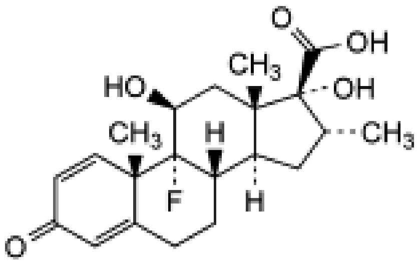
DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{29}FO_5$ in the eye drops using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS*.

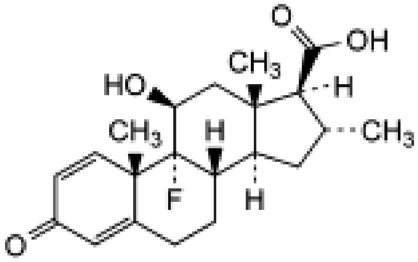
STORAGE

Dexamethasone Eye Drops, Suspension should be stored in accordance with the manufacturer's instructions.

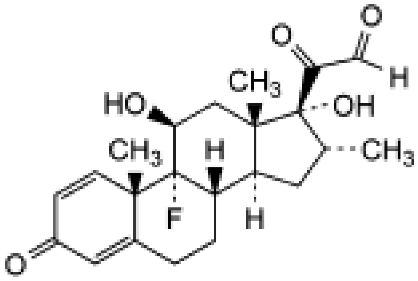
IMPURITIES



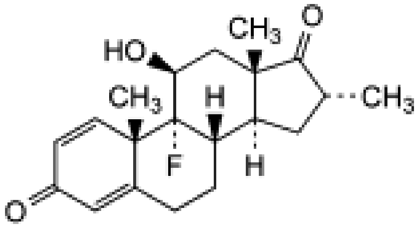
1. Dexamethasone-17β-carboxylic acid.



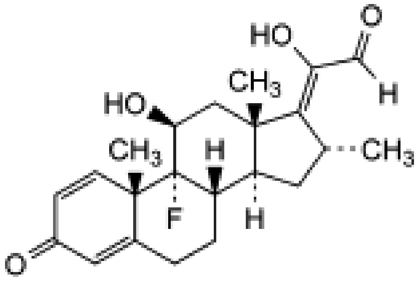
2. Dexamethasone-17α-dehydroxy-17β-carboxylic acid.



3. Dexamethasone-21-aldehyde.



4. Dexamethasone-17-ketone.



5. Dexamethasone-17(20)-enol-21-aldehyde.

Dexamethasone Sodium Phosphate Eye Drops, Solution

General Notices

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone Sodium Phosphate Eye Drops, Solution are a sterile Solution of Dexamethasone Sodium Phosphate in a suitable vehicle.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of dexamethasone sodium phosphate, $C_{22}H_{28}FNa_2O_8P$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the eye drops containing 20 mg of Dexamethasone Sodium Phosphate with 5 ml of 0.1M *sodium hydroxide* , add 50 ml of *dichloromethane* and mix with the aid of ultrasound for 20 minutes, filter the dichloromethane layer and evaporate to dryness using a rotary evaporator. Dry the residue at 105° for 2 hours. The *infrared absorption* spectrum of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dexamethasone *(RS 089)* .

TESTS

Acidity

pH, 7.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of Dexamethasone Sodium Phosphate in 70 ml of the mobile phase, mix with the aid of ultrasound for 10 minutes, dilute with sufficient mobile phase to produce 100 ml and filter.

(2) Dilute 3 volumes of solution (1) to 100 volumes with the mobile phase.

(3) 0.02% w/v of *dexamethasone impurity standard BPCRS* .

(4) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 ml of this solution to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for six times the retention time of dexamethasone sodium phosphate.

MOBILE PHASE

27 volumes of *acetonitrile* and 73 volumes of a 0.3% w/v solution of *orthophosphoric acid* that has been previously adjusted to pH 3.0 with *dilute sodium hydroxide* .

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity 3 and dexamethasone is at least 1.5;

closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS* .

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any peaks, apart from the principal peak, is not greater than the area of the peak in the chromatogram obtained with solution (2) (3%).

Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the eye drops containing 20 mg of Dexamethasone Sodium Phosphate in 70 ml of the mobile phase, mix with the aid of ultrasound for 10 minutes, dilute with sufficient of the mobile phase to produce 100 ml and filter.

(2) 0.015% w/v of *dexamethasone BPCRS* .

(3) 0.02% w/v of *dexamethasone impurity standard BPCRS* .

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity 3 and dexamethasone is at least 1.5;

closely resembles the chromatogram supplied with *dexamethasone impurity standard BPCRS* .

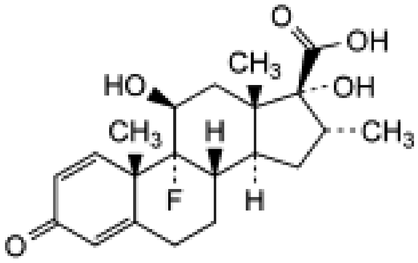
DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in the eye drops using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS* , each mg of $C_{22}H_{28}FNa_2O_8P$ is equivalent to 0.760 mg of $C_{22}H_{29}FO_5$.

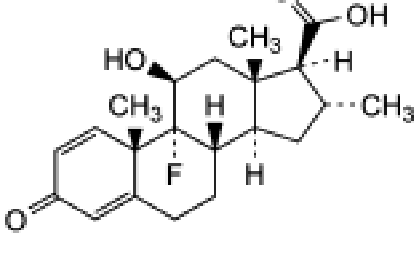
STORAGE

Dexamethasone Sodium Phosphate Eye Drops should be stored in accordance with the manufacturer's instructions.

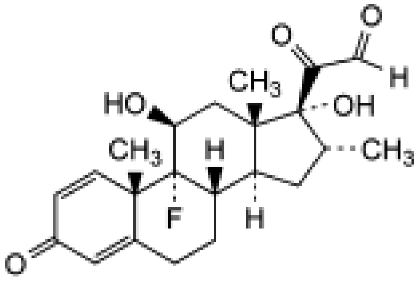
IMPURITIES



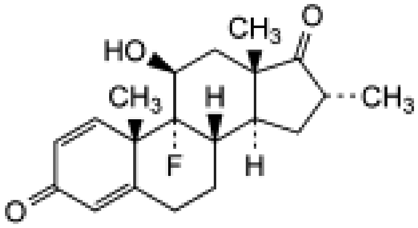
1. Dexamethasone-17β-carboxylic acid.



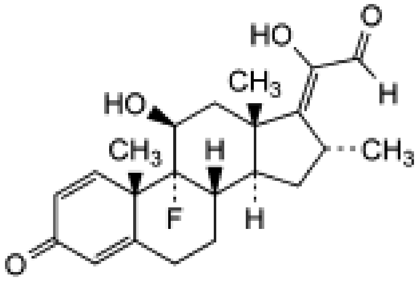
2. Dexamethasone-17α-dehydroxy-17β-carboxylic acid.



3. Dexamethasone-21-aldehyde.



4. Dexamethasone-17-ketone.



5. Dexamethasone-17(20)-enol-21-aldehyde.

Dexamethasone Sodium Phosphate Injection

General Notices

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone Sodium Phosphate Injection is a sterile solution of Dexamethasone Sodium Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dexamethasone phosphate, $C_{22}H_{30}FO_8P$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel F $_{254}$ plate* (Merck plates are suitable) and, as the mobile phase, a mixture of 20 volumes of *water*, 20 volumes of *acetic acid* and 60 volumes of *butan-1-ol* . Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute, if necessary, a volume of the injection to contain the equivalent of 0.1% w/v of dexamethasone phosphate in *methanol* . Solution (2) contains 0.1% w/v of *dexamethasone phosphate BPCRS* in *methanol* . Solution (3) contains 0.1% w/v each of *dexamethasone phosphate BPCRS* and *prednisolone sodium phosphate BPCRS* . After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes, spray the hot plate with *ethanolic sulphuric acid (20%)* and heat at 120° for 10 minutes; cool and examine in daylight and under *ultraviolet light (365 nm)* . The principal spot in the chromatogram obtained with solution (1) is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two spots which may, however, not be completely separated.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 7.0 to 8.5, Appendix V L.

Free dexamethasone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with the mobile phase to produce a solution containing the equivalent of 0.25% w/v of dexamethasone phosphate. Solution (2) contains 0.00125% w/v of *dexamethasone BPCRS* in the mobile phase. Solution (3) contains 0.25% w/v of *dexamethasone phosphate BPCRS*, 0.01% w/v of *propyl hydroxybenzoate* and 0.001% w/v of *dexamethasone BPCRS* in the mobile phase. Solution (4) contains 0.01% w/v of *propyl hydroxybenzoate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Hypersil ODS is suitable), (b) as mobile phase with a flow rate of 1 ml per minute a mixture prepared as follows: to 1.360 g of *potassium dihydrogen phosphate* add 0.600 g of *hexylamine*, mix, allow to stand for 10 minutes, dissolve in 182.5 ml of *water*, add 67.5 ml of *acetonitrile*, mix and filter (0.45 µm) and (c) a detection wavelength of 254 nm.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 45 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 20 µl of solution (2) is at least 50% of the full scale of the recorder.

Inject 20 µl of solution (3). The test is not valid unless the peak corresponding to dexamethasone is completely separated from the peaks due to dexamethasone phosphate and propyl hydroxybenzoate. If necessary, adjust slightly the concentration of acetonitrile or increase the concentration of water in the mobile phase.

Inject separately 20 µl each of solution (1) and solution (2). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of the peak corresponding to dexamethasone is not greater than that of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with the mobile phase to produce a solution containing the equivalent of 0.008% w/v of dexamethasone phosphate. Solution (2) contains 0.008% w/v of *dexamethasone phosphate BPCRS* in the mobile phase. Solution (3) contains 0.002% w/v each of *dexamethasone phosphate BPCRS* and *betamethasone sodium phosphate BPCRS* in the mobile phase.

The chromatographic conditions described under Free dexamethasone may be used.

Inject 20 µl of solution (3). The test is not valid unless the *resolution factor* between the peaks corresponding to betamethasone sodium phosphate and dexamethasone phosphate is at least 2.2. If necessary, adjust slightly the concentration of acetonitrile or increase the concentration of water in the mobile phase.

Inject separately 20 µl each of solution (1) and solution (2).

Calculate the content of $C_{22}H_{30}FO_8P$ in the injection from the peak areas obtained and from the declared content of $C_{22}H_{30}FO_8P$ in *dexamethasone phosphate BPCRS* .

STORAGE

Dexamethasone Sodium Phosphate Injection should be protected from light. It should not be allowed to freeze.

LABELLING

The content of active ingredient is stated as the amount of dexamethasone phosphate in a suitable dose-volume.

Dexamethasone Tablets

General Notices

Action and use

Glucocorticoid.

DEFINITION

Dexamethasone Tablets contain Dexamethasone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dexamethasone, $C_{22}H_{29}FO_5$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Mix a quantity of the powdered tablets containing 20 mg of Dexamethasone with 5 ml of 0.1M *sodium hydroxide* , add 50 ml of *dichloromethane* and mix with the aid of ultrasound for 20 minutes, filter and evaporate to dryness using a rotary evaporator. Dry the residue at 105° for 2 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of dexamethasone *(RS 089)* .

TESTS

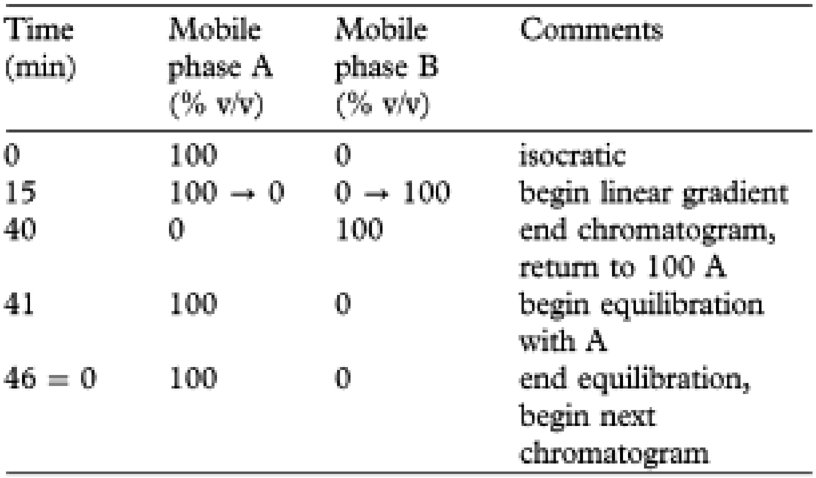
Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) to a quantity of the powdered tablets containing 2.5 mg of Dexamethasone add 10 ml of *acetonitrile* , mix with the aid of ultrasound and filter through a 0.45-µm filter. Dilute 4 ml of the filtrate to 10 ml with *water.* For solution (2) dilute 1 ml of solution (1) to 100 ml with mobile phase A. For solution (3) dissolve 2 mg of each of *dexamethasone BPCRS* and *methylprednisolone BPCRS* in mobile phase A and dilute to 100 ml with the same solvent.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) maintained at 45 °C, (b) Carry out a linear gradient elution with a flow rate of 2.5 ml per minute using the following conditions:

Mobile phase A 15% v/v *acetonitrile.*

Mobile phase B *acetonitrile.*



Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0	100	0	isocratic
15	100 → 0	0 → 100	begin linear gradient
40	0	100	end chromatogram, return to 100 A
41	100	0	begin equilibration with A
46 = 0	100	0	end equilibration, begin next chromatogram

and (c) a detection wavelength of 254 nm.

Inject 20 µl of solution (3). When the chromatograms are recorded in the prescribed conditions, the retention times are: methylprednisolone about 13 min and dexamethasone about 16 min. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and dexamethasone is at least 2.8; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject 20 µl of mobile phase A, solution (1) and solution (2). In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%); the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). Disregard any peak due to mobile phase A and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (2) (0.05%).

Uniformity of content

Tablets containing less than 2 mg of Dexamethasone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. Solution (1) contains 0.0025% w/v of *dexamethasone BPCRS* in *methanol (50%)*. For solution (2) to one tablet, add sufficient *methanol (50%)* to produce a solution containing 0.0025% w/v of Dexamethasone, shake for 10 minutes and filter through glass-fibre filter paper (Whatman GF/C is suitable).

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) *methanol (47%)* as the mobile phase with a flow rate of 1.4 ml per minute and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{22}H_{29}FO_5$ in each tablet using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS* .

ASSAY

For tablets containing 2 mg or more of dexamethasone

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to a quantity of the powdered tablets containing 2.5 mg of Dexamethasone add 20 ml of *methanol (50%)*, shake for 20 minutes and filter through glass-fibre filter paper (Whatman GF/C is suitable). Solution (2) contains 0.0125% w/v of *dexamethasone BPCRS* in *methanol (50%)*.

The chromatographic conditions described under Uniformity of content may be used.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets using the declared content of $C_{22}H_{29}FO_5$ in *dexamethasone BPCRS*.

For tablets containing less than 2 mg of dexamethasone

Use the average of the ten individual results obtained in the test for Uniformity of content.

STORAGE

Dexamethasone Tablets should be protected from light.

Dexamfetamine Tablets

General Notices

Action and use

Amfetamine.

DEFINITION

Dexamfetamine Tablets contain Dexamfetamine Sulphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dexamfetamine sulphate, $(C_9H_{13}N)_2,H_2SO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of dexamfetamine sulphate as completely as possible in 20 ml of *water*, filter, add 2 ml of 5M *sodium hydroxide* and extract with three 25 ml quantities of *ether*, washing the combined extracts with 5 ml of *water*. To the ether solution add 10 ml of 0.05M *sulphuric acid* and shake well. The acid layer, after warming to dispel residual ether and cooling to 20°, is dextrorotatory.

B. Extract a quantity of the powdered tablets containing 50 mg of dexamfetamine sulphate with 10 ml of *water*, filter, cool to about 15°, add 3 ml of 1M *sodium hydroxide* and shake for 2 minutes with 1 ml of a mixture of 1 volume of *benzoyl chloride* and 2 volumes of *ether*. Filter, wash the residue with 15 ml of *water* and recrystallise twice from *ethanol (50%)*. The *melting point* of the crystals, after drying at 105° for 1 hour, is about 156°, Appendix V A.

ASSAY

Weigh and powder 20 tablets, or more if necessary. Dissolve a quantity of the powder containing 0.1 g of dexamfetamine sulphate as completely as possible in 20 ml of *water*, add 8 g of *sodium chloride* and 2 ml of 5M *sodium hydroxide* and extract with successive quantities of 50, 20, 20 and 20 ml of *ether*. Extract the combined ether extracts with four 10 ml quantities of 0.1M *hydrochloric acid* and make the combined acid extracts alkaline with 5M *sodium hydroxide*. Dilute to 120 ml with *water* and distil into 20 ml of 0.05M *hydrochloric acid VS* until only 5 ml of liquid remains in the distillation flask. Boil, cool and titrate the excess of acid with 0.05M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 0.05M *hydrochloric acid VS* is equivalent to 9.212 mg of $(C_9H_{13}N)_2,H_2SO_4$.

Dextran 40 Intravenous Infusion

General Notices

Dextran 40 Injection

Action and use

Plasma substitute.

DEFINITION

Dextran 40 Intravenous Infusion is a sterile solution, in Glucose Intravenous Infusion or in Sodium Chloride Intravenous Infusion, of Dextran 40 for Injection.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dextrans

9.0 to 11.0% w/v.

CHARACTERISTICS

An almost colourless, slightly viscous solution.

TESTS

Acidity

Titrate 25 ml with 0.01M *sodium hydroxide VS* using *phenol red solution* as indicator. Not more than 2.0 ml of 0.01M *sodium hydroxide VS* is required to neutralise the solution.

Molecular size

For solutions in Glucose Intravenous Infusion, before proceeding with tests A, B and C add 4 volumes of *ethanol (96%)*, centrifuge and dissolve the residue in sufficient Sodium Chloride Intravenous Infusion to restore the original volume.

A. Determine the viscosities, Appendix V H, Method I, at 37°, using a U-tube viscometer (size C) of solutions in *saline solution* containing about 3.5, 2.5, 1.5 and 0.75% w/v of Dextrans, accurately determined. Calculate the viscosity ratio by dividing the time taken for the meniscus to fall from E to F using the liquid being examined by the time taken using *saline solution*. For each solution plot (viscosity ratio – 1.00)/concentration against concentration. The intercept on the viscosity ratio axis of a straight line through the points represents the intrinsic viscosity. The intrinsic viscosity is 0.16 to 0.20.

B. Dilute the solution being examined with *saline solution* to contain 6% w/v of Dextrans.

Place 100 ml in each of five stoppered flasks and adjust the temperature to 24.9° to 25.1°. Maintaining this temperature, add slowly with continuous stirring sufficient *absolute ethanol* to produce a faint cloudiness (about 45 ml). To the separate flasks add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of *absolute ethanol* , stopper the flasks and immerse in a water bath at about 35°, shaking occasionally, until clear solutions are obtained. Transfer the flasks to a water bath maintained at 24.9° to 25.1° and allow to stand overnight or until two clear liquid phases are formed. Discard the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 ml, remove the ethanol by evaporation at a pressure of 2 kPa, dilute to 25.0 ml with *water* and determine the *optical rotation* , Appendix V F. From the optical rotations calculate the amounts of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10% of the dextrans present in the infusion and determine its intrinsic viscosity by the method described under test A using a U-tube viscometer (size A). The intrinsic viscosity is not more than 0.27.

C. Place in each of four stoppered flasks 100 ml of the diluted intravenous infusion in *saline solution* containing 6% w/v of Dextrans and add slowly, with continuous stirring, 80, 90, 100 and 110 ml respectively of *absolute ethanol* . Stopper the flasks, transfer to a water bath maintained at 24.9° to 25.1° and allow to stand overnight or until two clear liquid phases are formed. Separate the supernatant solutions from the syrupy residues. Remove the ethanol from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against *water* to remove sodium chloride, adjust the volume to 25.0 ml with *water*, add sufficient *sodium chloride* to produce solutions containing 0.9% w/v and determine the *optical rotation* , Appendix V F. From the optical rotations calculate the amounts of dextrans present as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10% of the dextrans present in the infusion and determine the intrinsic viscosity by the method in test A above. The intrinsic viscosity is not less than 0.08.

Content of glucose

For solutions in Glucose Intravenous Infusion, 4.5 to 5.5% w/v, when determined by the following method. Dilute 15 ml to 50 ml with *water*. To 5 ml in a stoppered flask add 25 ml of a buffer solution containing 14.3% w/v of *sodium carbonate* and 4.0% w/v of *potassium iodide* and 25 ml of 0.05M *iodine VS* . Stopper the flask, allow to stand for exactly 30 minutes at 20°, add 35 ml of 2M *hydrochloric acid* and titrate immediately with 0.1M *sodium thiosulphate VS* . Repeat the operation using 5 ml of *water* beginning at the words 'add 25 ml...'. The difference between the titrations represents the amount of iodine required to oxidise the glucose. Each ml of 0.05M *iodine VS* is equivalent to 9.01 mg of glucose.

Bacterial endotoxins

The endotoxin limit concentration is 1.25 IU per ml, Appendix XIV C.

ASSAY

For solutions in Glucose Intravenous Infusion

Add 0.05 ml of 5M *ammonia* to the required volume and measure the *optical rotation* , Appendix V F. Calculate the content of dextrans from the expression

$$0.5076(\alpha - 0.528D)$$

where α is the observed angular rotation and D is the content of glucose as a percentage w/v determined in the test for Content of glucose.

For solutions in Sodium Chloride Intravenous Infusion

Measure the *optical rotation*, Appendix V F, and multiply the value obtained by 0.5076.

STORAGE

Dextran 40 Intravenous Infusion should not be exposed to undue fluctuations of temperature.

LABELLING

The strength is stated as the percentage w/v of dextrans. The label states (1) the name of the solvent; (2) that the infusion should not be used if it is cloudy or if a deposit is present.

Dextran 70 Intravenous Infusion

General Notices

Dextran 70 Injection

Action and use

Plasma substitute.

DEFINITION

Dextran 70 Intravenous Infusion is a sterile solution, in Glucose Intravenous Infusion or in Sodium Chloride Intravenous Infusion, of Dextran 70 for Injection.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dextrans

5.5 to 6.5% w/v.

CHARACTERISTICS

An almost colourless, slightly viscous solution.

TESTS

Acidity

Titrate 25 ml with 0.01M *sodium hydroxide VS* using *phenol red solution* as indicator. Not more than 1.25 ml of 0.01M *sodium hydroxide VS* is required to neutralise the solution.

Molecular size

For solutions in Glucose Intravenous Infusion, before proceeding with tests A, B and C add 4 volumes of *ethanol (96%)*, centrifuge and dissolve the residue in sufficient Sodium Chloride Intravenous Infusion to restore the original volume.

A. Determine the viscosities, Appendix V H, Method I, at 37°, using a U-tube viscometer (size C) of solutions in *saline solution* containing about 3.5, 2.5, 1.5 and 0.75% w/v of Dextrans, accurately determined. Calculate the viscosity ratio by dividing the time taken for the meniscus to fall from E to F using the liquid being examined by the time taken using *saline solution*. For each solution plot (viscosity ratio – 1.00)/concentration against concentration. The intercept on the viscosity ratio axis of a straight line through the points represents the intrinsic viscosity. The intrinsic viscosity is 0.22 to 0.27.

B. Place 100 ml in each of five stoppered flasks and adjust the temperature to 24.9° to

25.1 °. Maintaining this temperature, add slowly with continuous stirring sufficient *absolute ethanol* to produce a faint cloudiness (about 45 ml). To the separate flasks add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of *absolute ethanol*, stopper the flasks and immerse in a water bath at about 35°, shaking occasionally, until clear solutions are obtained. Transfer the flasks to a water bath maintained at 24.9° to 25.1° and allow to stand overnight or until two clear liquid phases are formed. Discard the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 ml, remove the ethanol by evaporation at a pressure of 2 kPa, dilute to 25.0 ml with *water* and determine the *optical rotation*, Appendix V F. From the optical rotations calculate the amounts of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10% of the dextrans present in the infusion and determine its intrinsic viscosity by the method described under test A using a U-tube viscometer (size A). The intrinsic viscosity is not more than 0.36.

C. Dilute the solution being examined with *saline solution* to contain 6% w/v of Dextrans. Place 100 ml in each of four stoppered flasks and add slowly, with continuous stirring, 80, 90, 100 and 110 ml respectively of *absolute ethanol*. Stopper the flasks, transfer to a water bath maintained at 24.9° to 25.1° and allow to stand overnight or until two clear liquid phases are formed. Separate the supernatant solutions from the syrupy residues. Remove the ethanol from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against *water* to remove sodium chloride, adjust the volume to 25.0 ml with *water*, add sufficient *sodium chloride* to produce solutions containing 0.9% w/v and determine the *optical rotation*, Appendix V F. From the optical rotations calculate the amounts of dextrans present as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10% of the dextrans present in the infusion and determine the intrinsic viscosity by the method in test A. The intrinsic viscosity is not less than 0.13.

Content of glucose

For solutions in Glucose Intravenous Infusion, 4.5 to 5.5% w/v, when determined by the following method. Dilute 15 ml to 50 ml with *water*. To 5 ml in a stoppered flask add 25 ml of a buffer solution containing 14.3% w/v of *sodium carbonate* and 4.0% w/v of *potassium iodide* and 25 ml of 0.05M *iodine VS*. Stopper the flask, allow to stand for exactly 30 minutes at 20°, add 35 ml of 2M *hydrochloric acid* and titrate immediately with 0.1M *sodium thiosulphate VS*. Repeat the operation using 5 ml of *water* and beginning at the words 'add 25 ml...'. The difference between the titrations represents the amount of iodine required to oxidise the glucose. Each ml of 0.05M *iodine VS* is equivalent to 9.01 mg of glucose.

Bacterial endotoxins

The endotoxin limit concentration is 1.21 IU per ml, Appendix XIV C.

ASSAY

For solutions in Glucose Intravenous Infusion

Add 0.05 ml of 5M *ammonia* to the required volume and measure the *optical rotation*, Appendix V F. Calculate the content of dextrans from the expression

$$0.5076(\alpha - 0.528D)$$

where α is the observed angular rotation and D is the content of glucose as a percentage w/v determined in the test for Content of glucose.

For solutions in Sodium Chloride Intravenous Infusion

Measure the *optical rotation*, Appendix V F, and multiply the value obtained by 0.5076.

STORAGE

Dextran 70 Intravenous Infusion should not be exposed to undue fluctuations in temperature.

LABELLING

The strength is stated as the percentage w/v of dextrans. The label states (1) the name of the solvent; (2) that the infusion should not be used if it is cloudy or if a deposit is present.

Dextromoramide Tablets

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Dextromoramide Tablets contain Dextromoramide Tartrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dextromoramide, $C_{25}H_{32}N_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 30 mg of dextromoramide with 10 ml of *water* and filter. Make the filtrate alkaline with 5M *ammonia* and extract with two 5 ml quantities of *ether* . Dry the combined ether extracts over *anhydrous sodium sulphate* , filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dextromoramide *(RS 090)* .

B. Shake a quantity of the powdered tablets containing the equivalent of 25 mg of dextromoramide with 25 ml of 1M *hydrochloric acid* and filter. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits maxima at 254, 259 and 264 nm.

TEST

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 20 mg of dextromoramide with 1 ml of *methanol* for 5 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol* . After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution* . Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 0.1 g of dextromoramide with 25 ml of 1M *hydrochloric acid* for 5 minutes. Add 25 ml of *water*, make alkaline with 5M *sodium hydroxide* and extract with four 25 ml quantities of *chloroform*. Wash the combined chloroform extracts with two 10 ml quantities of *water* and extract the combined washings with 10 ml of *chloroform*. Filter the combined chloroform extracts through absorbent cotton moistened with *chloroform* and wash the filter with a small quantity of *chloroform*. Heat on a water bath until the volume is reduced to about 25 ml, cool, add 50 ml of *anhydrous acetic acid*, previously neutralised to *crystal violet solution*, and titrate with 0.02M *perchloric acid VS* using *crystal violet solution* as indicator. Each ml of 0.02M *perchloric acid VS* is equivalent to 7.851 mg of $C_{25}H_{32}N_2O_2$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of dextromoramide.

Dextropropoxyphene Capsules

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Dextropropoxyphene Capsules contain Dextropropoxyphene Napsilate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of dextropropoxyphene, $C_{22}H_{29}NO_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing the equivalent of 0.15 g of dextropropoxyphene with 5 ml of *chloroform* and filter. The filtrate complies with the following tests.

A. Evaporate 3 ml to dryness and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dextropropoxyphene napsilate *(RS 092)*.

B. Evaporate 0.05 ml in a porcelain dish and streak the spot with *sulphuric acid* containing 0.05 ml of *formaldehyde solution* per ml. A purple colour is produced.

C. Evaporate 0.4 ml on a piece of filter paper and burn the residue by the method for *oxygen-flask combustion*, Appendix VIII C, using 5 ml of 1.25M *sodium hydroxide* as the absorbing liquid. When the process is complete, dilute the liquid to 25 ml with *water*. To 5 ml of the solution so obtained add 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of 1M *hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*. The solution becomes turbid.

ASSAY

Stir a quantity of the mixed contents of 20 capsules containing the equivalent of 0.5 g of dextropropoxyphene with 25 ml of *chloroform* and filter through absorbent cotton, washing the flask and filter with small quantities of *chloroform*. Add to the combined filtrates a mixture of 50 ml of *water* and 5 ml of 5M *sodium hydroxide*, shake, allow the layers to separate and wash the chloroform extract with 25 ml of *water*. Extract the aqueous layer with five 25 ml quantities of *chloroform*, washing each extract with the 25 ml of *water* and adding it to the original extract. Dry the combined extracts with *anhydrous sodium sulphate*, evaporate to about 3 ml on a water bath in a current of air, remove from the water bath and allow to evaporate to dryness at room temperature. Carry out Method I for *non-aqueous titration*, Appendix VIII A, on the residue using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 33.95 mg of $C_{22}H_{29}NO_2$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of dextropropoxyphene.

Diamorphine Injection

General Notices

Action and use

Opioid receptor agonist analgesic.

DEFINITION

Diamorphine Injection is a sterile solution of Diamorphine Hydrochloride in Water for Injections. It is prepared by dissolving Diamorphine Hydrochloride for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Diamorphine Injection deteriorates on storage and should be used immediately after preparation.

DIAMORPHINE HYDROCHLORIDE FOR INJECTION

DEFINITION

Diamorphine Hydrochloride for Injection is a sterile material prepared from Diamorphine Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of diamorphine hydrochloride, $C_{21}H_{23}NO_5,HCl,H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a sufficient quantity of the contents of the sealed container in the minimum volume of *dichloromethane* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of diamorphine hydrochloride *(RS 093)*.

TESTS

6-*O*-Acetylmorphine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the sealed container containing the equivalent of 0.2 g of Diamorphine Hydrochloride in 10 ml of *water*. For solution (2) dilute 1 volume of solution (1) to 20 volumes with *water*. For solution (3) dissolve a sufficient quantity of the contents of the sealed container to give a solution containing the equivalent of 0.1% w/v of Diamorphine Hydrochloride in 0.01M *sodium hydroxide* ; the solution should be freshly prepared.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-select B is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a solution containing 0.11% w/v of *sodium octanesulphonate* in a mixture of 10 volumes of *glacial acetic acid* , 10 volumes of *methanol* , 115 volumes of *acetonitrile* and 365 volumes of *water* and (c) a detection wavelength of 283 nm.

The chromatogram obtained with solution (3) exhibits two *secondary peaks* with retention times relative to the principal peak of about 0.23 (morphine) and 0.43 (6-*O*-acetyl-morphine). The test is not valid unless the *resolution factor* between the peaks due to morphine and 6-*O*-acetyl-morphine is at least 2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 6-*O*-acetylmorphine is not greater than the area of the peak in the chromatogram obtained with solution (2) (5%).

Uniformity of content

The content of $C_{21}H_{23}NO_5,HCl,H_2O$ in each of 10 individual containers as determined in the Assay is not less than 90.0% and not more than 110.0% of the average except that in one container the content may be not less than 80.0% and not more than 120.0% of the average.

ASSAY

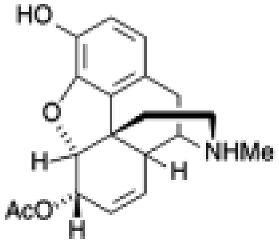
Dissolve the contents of a sealed container in a suitable volume of *water* and dilute with sufficient *water* to produce a solution containing the equivalent of 0.025% w/v of Diamorphine Hydrochloride. To 5 ml add 5 ml of 1M *hydrochloric acid* and boil vigorously under a reflux condenser for 15 minutes. Cool, add 5 ml of a freshly prepared 0.5% w/v solution of *sodium nitrite* , mix and add 2.5 ml of 5M *ammonia* and sufficient *water* to produce 25 ml. Repeat the operation with a further nine sealed containers. Measure the *absorbance* of each of the resulting solutions at the maximum at 435 nm, Appendix II B, and calculate the content of $C_{21}H_{23}NO_5,HCl,H_2O$ in each container by comparing the absorbances with that obtained by treating a mixture of 5 ml of a 0.015% w/v solution of *anhydrous morphine* in 1M *hydrochloric acid* and 5 ml of *water* in the same manner, beginning at the words 'add 5 ml of a freshly prepared...'. Each g of *anhydrous morphine* is equivalent to 1.486 g of $C_{21}H_{23}NO_5,HCl,H_2O$. Calculate the average content of $C_{21}H_{23}NO_5,HCl,H_2O$ per container from the 10 individual results thus obtained.

STORAGE

The sealed container should be protected from light.

IMPURITIES

The impurity limited by the requirements of this monograph is:


HO
O
H
NHMe
H
AcO
H

A. 6-*C*-Acetylmorphine.

Diazepam Injection

General Notices

Action and use

Benzodiazepine.

DEFINITION

Diazepam Injection is a sterile solution of Diazepam in Water for Injections or other suitable solvent.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of diazepam, $C_{16}H_{13}ClN_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption* , Appendix II B, of the solution obtained in the Assay exhibits a maximum at 368 nm.

B. Complies with test B for Identification described under Diazepam Tablets applying separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a suitable volume of the injection with sufficient *methanol* to produce a solution containing 0.10% w/v of Diazepam. Solution (2) contains 0.10% w/v of *diazepam BPCRS* in *methanol* .

TESTS

Acidity or alkalinity

pH, 6.2 to 7.0, Appendix V L.

ASSAY

To a volume containing 10 mg of Diazepam add 20 ml of *mixed phosphate buffer pH 7.0* and extract with four 20 ml quantities of *chloroform* , passing each extract through the same 5 g of *anhydrous sodium sulphate* . Combine the chloroform extracts, dilute to 100 ml with *chloroform* and mix. Evaporate 10 ml to dryness in a current of nitrogen, dissolve the residue in 25 ml of 0.05M *methanolic sulphuric acid* , mix and measure the *absorbance* of the resulting solution at the maximum at 368 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 151 as the value of A(1%, 1 cm) at the maximum at 368 nm.

STORAGE

Diazepam Injection should be protected from light.

Diazepam Oral Solution

General Notices

Action and use

Benzodiazepine.

DEFINITION

Diazepam Oral Solution is a solution of Diazepam in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of diazepam, $C_{16}H_{13}ClN_2O$

95.0 to 115.0% of the stated amount.

IDENTIFICATION

A. The *light absorption* , Appendix II B, in the range 320 to 400 nm of the final solution obtained in the Assay exhibits a maximum at 368 nm. The *light absorption* in the range 230 to 330 nm of a solution prepared by diluting 1 volume of the final solution obtained in the Assay to 5 volumes with 0.1M *methanolic sulphuric acid* exhibits two maxima, at 243 nm and 286 nm.

B. Carry out the method described under Related substances applying separately to the plate 10 µl of each of solution (1) and solution (2) and using as solution (2) a 0.4% w/v solution of *diazepam BPCRS* in *ethanol (96%)* . After removal of the plate, allow the solvent to evaporate and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.6, Appendix V L.

Related substances

Carry out in subdued light the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of equal volumes of *ethyl acetate* and *hexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 25 µl of each of the following solutions. For solution (1) add 40 ml of *water* to a volume of the oral solution containing 8 mg of Diazepam and extract with three 50-ml quantities of *ether* . Wash the combined ether extracts with 30 ml of 1M *sodium hydroxide* followed by two 40 ml quantities of *water*. Shake the extract with *anhydrous sodium sulphate* , filter, evaporate to dryness and dissolve the residue in 2 ml of *ethanol (96%)* . Solution (2) contains 0.0080% w/v of *5-chloro-2-methylaminobenzophenone BPCRS* in *ethanol (96%)* . Solution (3) contains 0.0040% w/v of *3-amino-6-chloro-1-methyl-4-phenylquinolin-2-ol BPCRS* in *ethanol (96%)* . For solution (4) dilute 2 ml of solution (1) to 100 ml with *ethanol (96%)* and dilute 1 ml of the resulting solution to 10 ml with the same solvent. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . In the chromatogram obtained with solution (1) any spot corresponding to 5-chloro-2-methylaminobenzophenone is not more intense than the spot in the chromatogram obtained with solution (2) and any spot corresponding to 3-amino-6-chloro-1-methyl-4-phenylquinolin-2-ol is not more intense than the spot in the chromatogram obtained with solution (3). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4).

ASSAY

To a weighed quantity containing 1 mg of Diazepam add 25 ml of a mixture of equal volumes of 1M *sodium hydroxide* and *methanol* and shake for 2 minutes. Extract with five 25 ml quantities of *chloroform* , shaking for 2 minutes each time. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate* and filter. Evaporate to dryness, dissolve the residue in 25 ml of 0.1M *methanolic sulphuric acid* and filter. Measure the *absorbance* of the filtrate, Appendix II B, at the maximum at 368 nm. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 151 as the value of A(1%, 1 cm) at the maximum at 368 nm. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{16}H_{13}ClN_2O$, weight in volume.

STORAGE

Diazepam Oral Solution should be protected from light.

Diazepam Rectal Solution

General Notices

Action and use

Benzodiazepine.

DEFINITION

Diazepam Rectal Solution is a solution of Diazepam in a suitable vehicle.

The rectal solution complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of diazepam, $C_{16}H_{13}ClN_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the solution obtained in the Assay exhibits a maximum at 368 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *methanol* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a suitable volume of the rectal solution with sufficient *methanol* to produce a solution containing 0.10% w/v of Diazepam. Solution (2) contains 0.10% w/v of *diazepam BPCRS* in *methanol*. After removal of the plate, spray it with a 10% v/v solution of *sulphuric acid* in *absolute ethanol*, heat at 105° for 10 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.2 to 7.2, Appendix V L.

ASSAY

To a volume containing 10 mg of Diazepam add 20 ml of *mixed phosphate buffer pH 7.0* and extract with four 20 ml quantities of *chloroform*, passing each extract through the same 5 g of *anhydrous sodium sulphate*. Combine the chloroform extracts, dilute to 100 ml with *chloroform* and mix. Evaporate 10 ml to dryness in a current of nitrogen, dissolve the residue in 25 ml of 0.05M *methanolic sulphuric acid*, mix and measure the *absorbance* of the resulting solution at the maximum at 368 nm, Appendix II B. Calculate the content of

$C_{16}H_{13}ClN_2O$ taking 151 as the value of A(1%, 1 cm) at the maximum at 368 nm.

STORAGE

Diazepam Rectal Solution should be protected from light.

Diazepam Tablets

General Notices

Action and use

Benzodiazepine.

DEFINITION

Diazepam Tablets contain Diazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diazepam, $C_{16}H_{13}ClN_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits two maxima, at 242 nm and 284 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform* and 10 volumes of *methanol* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 0.50% w/v of Diazepam, allow to settle and decant the supernatant liquid. Solution (2) contains 0.5% w/v of *diazepam BPCRS* in *methanol*. After removal of the plate, spray it with a 10% v/v solution of *sulphuric acid* in *absolute ethanol*, heat at 105° for 10 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances and decomposition products

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of equal volumes of *ethyl acetate* and *hexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate (1) 20 µl of a solution freshly prepared by shaking a quantity of the powdered tablets containing 50 mg of Diazepam with 5 ml of *ethanol (96%)* and filtering and (2) 5 µl of a solution freshly prepared by diluting 1 volume of solution (1) to 50 volumes with *ethanol (96%)*. After removal of the plate, allow the solvent to evaporate and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the

chromatogram obtained with solution (2).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, with 0.1M *hydrochloric acid*, at the maximum at 286 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of diazepam, $C_{16}H_{13}ClN_2O$, in the medium taking 488 as the value of A(1%, 1 cm) at the maximum at 286 nm.

Uniformity of content

Tablets containing 2 mg or less of Diazepam comply with the requirement stated under Tablets using the following method of analysis. To one tablet add 1 ml of *water*, allow the tablet to disintegrate and stand for 15 minutes. Add 80 ml of a 0.5% w/v solution of *sulphuric acid* in *methanol*, shake for 15 minutes, add sufficient of the methanolic sulphuric acid to produce 100 ml and filter. Measure the *absorbance* of the filtrate at the maximum at 284 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the value of A(1%, 1 cm) at the maximum at 284 nm.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 10 mg of Diazepam add 5 ml of *water*, mix and allow to stand for 15 minutes. Add 70 ml of a 0.5% w/v solution of *sulphuric acid* in *methanol*, shake for 15 minutes, add sufficient of the methanolic sulphuric acid to produce 100 ml and filter. Dilute 10 ml of the filtrate to 50 ml with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 284 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the value of A(1%, 1 cm) at the maximum at 284 nm.

STORAGE

Diazepam Tablets should be protected from light.

Diazoxide Injection

General Notices

Action and use

Vasodilator; treatment of hypertension.

DEFINITION

Diazoxide Injection is a sterile solution of Diazoxide in Water for Injections, prepared with the aid of Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of diazoxide, $C_8H_7ClN_2O_2S$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

To a volume containing 0.3 g of Diazoxide add 2 ml of 2M *hydrochloric acid* , stir, filter the precipitate and wash the filter thoroughly with *water* until the filtrate is free from acid. The precipitate, after drying at 105°, complies with the following tests.

A. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of diazoxide *(RS 094)* .

B. The *light absorption* , Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in 0.1M *sodium hydroxide* exhibits a maximum only at 280 nm.

C. Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 50 volumes of *toluene* , 30 volumes of *ether* and 20 volumes of *acetone* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) use the injection, diluted if necessary, with *methanol* to contain 0.02% w/v of Diazoxide. Solution (2) contains 0.02% w/v of *diazoxide EPCRS* in *methanol* . After removal of the plate, allow it to dry in air until the solvent has evaporated, examine under *ultraviolet light (254 nm)* and then treat the plate by *Method I* and examine again. The principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 11.2 to 11.9, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF $_{254}$* as the coating substance and a mixture of 7 volumes of 18M *ammonia*, 25 volumes of *methanol* and 68 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) use the injection diluted, if necessary, with 0.1M *sodium hydroxide* to contain 1.5% w/v of Diazoxide. For solution (2) dilute 1 volume of solution (1) to 200 volumes with 0.1M *sodium hydroxide*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

To a volume containing 75 mg of Diazoxide add sufficient 0.1M *sodium hydroxide* to produce 500 ml. Dilute 5 ml to 100 ml with 0.1M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of $C_8H_7ClN_2O_2S$ taking 585 as the value of A(1%, 1 cm) at the maximum at 280 nm.

STORAGE

Diazoxide Injection should be protected from light.

Diazoxide Tablets

General Notices

Action and use

Vasodilator; treatment of hypertension.

DEFINITION

Diazoxide Tablets contain Diazoxide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diazoxide, $C_8H_7ClN_2O_2S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.2 g of Diazoxide with 50 ml of *absolute ethanol*, filter and evaporate the filtrate to dryness at a pressure of 2 kPa. The residue complies with the following tests.

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in 0.1M *sodium hydroxide* exhibits a maximum only at 280 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 50 volumes of *toluene*, 30 volumes of *ether* and 20 volumes of *acetone* as the mobile phase. Apply separately to the plate 20 µl of each of two solutions in *methanol* containing (1) 0.02% w/v of the residue and (2) 0.02% w/v of *diazoxide EPCRS*. After removal of the plate, allow it to dry in air until the solvent has evaporated, examine under *ultraviolet light (254 nm)* and then treat the plate by *Method I* and examine again. The principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 7 volumes of 18M *ammonia*, 25 volumes of *methanol* and 68 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.75 g of Diazoxide with 40 ml of 0.1M *sodium hydroxide* for 30 minutes, filter and dilute the filtrate to 50 ml with 0.1M *sodium hydroxide*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with 0.1M *sodium hydroxide* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 50 mg of Diazoxide add 70 ml of *methanol* , shake for 1 hour, add sufficient *methanol* to produce 100 ml, mix and filter. Dilute 5 ml of the filtrate to 250 ml with 0.1M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of $C_8H_7ClN_2O_2S$ taking 585 as the value of A(1%, 1 cm) at the maximum at 280 nm.

Dichlorophen Tablets

General Notices

Action and use

Antihelminthic.

DEFINITION

Dichlorophen tablets contain Dichlorophen.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dichlorophen, $C_{13}H_{10}Cl_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of dichlorophen with 50 ml of 0.1M *sodium hydroxide* for 15 minutes, add sufficient 0.1M *sodium hydroxide* to produce 100 ml, centrifuge and dilute a suitable volume of the supernatant liquid with 0.1M *sodium hydroxide* to produce a solution containing 0.002% w/v of dichlorophen. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 350 nm exhibits two maxima, at 245 nm and 304 nm. The *absorbances* at the maxima are about 1.3 and about 0.54, respectively.

B. Shake a quantity of the powdered tablets containing 0.2 g of dichlorophen with a mixture of 5 ml of *water* and 5 ml of 5M *sodium hydroxide* , filter, cool in ice and add a solution prepared by mixing 1 ml of *sodium nitrite solution* with a cold solution containing 0.15 ml of *aniline* in a mixture of 4 ml of *water* and 1 ml of *hydrochloric acid* . A reddish brown precipitate is produced.

C. Fuse a quantity of the powdered tablets containing 0.5 g of dichlorophen with 2 g of *anhydrous sodium carbonate* , cool, extract the residue with *water* and filter. The filtrate yields reaction A characteristic *of chlorides* , Appendix VI.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.50 g of dichlorophen with 20 ml of *methanol* for 10 minutes, filter, add 7 ml of *water* and dilute to 50 ml with the mobile phase. Solution (2) contains 1.0% w/v of *dichlorophen impurity standard BPCRS* in the mobile phase. Solution (3) contains 0.0010% w/v of *4-chlorophenol* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm

× 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 1 volume of *glacial acetic acid* and 25 volumes of *water* and sufficient *methanol* to produce a chromatogram with solution (2) closely resembling the reference chromatogram supplied with the impurity standard (75 volumes of *methanol* is usually suitable) and (c) a detection wavelength of 280 nm. Record the chromatograms until all of the peaks named on the reference chromatogram have emerged.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-chlorophenol is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%). Calculate the content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-α,α′-diyl)diphenol and the sum of the nominal contents of any other impurities, excluding 4-chlorophenol, with reference to dichlorophen using the declared content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-α,α′-diyl)diphenol in *dichlorophen impurity standard BPCRS*. The content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-α,α′-diyl) diphenol does not exceed 8.0% w/w and the sum of the nominal contents of any other impurities does not exceed 2% w/w.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of dichlorophen with 50 ml of 0.1M *sodium hydroxide* for 15 minutes and add sufficient 0.1M *sodium hydroxide* to produce 100 ml. Centrifuge, dilute 10 ml of the clear supernatant liquid to 100 ml with 0.1M *sodium hydroxide*, dilute 20 ml of this solution to 100 ml with 0.1M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 304 nm, Appendix II B. Calculate the content of $C_{13}H_{10}Cl_2O_2$ taking 275 as the value of A(1%, 1 cm) at the maximum at 304 nm.

Diclofenac Gel

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Diclofenac Gel contains Diclofenac Diethylamine in a suitable basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diclofenac diethylamine, $C_{18}H_{22}Cl_2N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel F_{254} plates are suitable) and a mixture of 1 volume of *hydrochloric acid*, 1 volume of *water*, 6 volumes of *glacial acetic acid* and 11 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) add to a quantity of the gel containing 50 mg of Diclofenac Diethylamine 12.5 ml of 0.5M *lithium chloride* and shake until a homogeneous suspension is obtained. Add 12.5 ml of *chloroform*, shake briefly and mix with the aid of ultrasound for 5 minutes. Allow to separate, filter the chloroform layer through a glass fibre filter (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.2% w/v of *diclofenac diethylamine BPCRS* in a mixture of equal volumes of 0.5M *lithium chloride* and *dichloromethane*. After removal of the plate, dry it in a stream of warm air for 10 minutes. Spray with *ninhydrin solution* and heat at 110° for 15 minutes. The two principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to the corresponding spots in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the gel containing 50 mg of Diclofenac Diethylamine with 50 ml of *acetone* for 10 minutes, filter and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 10 ml of a mixture of 40 volumes of *water* and 60 volumes of *methanol*, dilute 1 volume of this solution to 5 volumes with the mobile phase and filter through a glass fibre filter (Whatman GF/C is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. Solution (3) contains 0.01% w/v of *diclofenac sodium BPCRS* and 0.01% w/v of *diclofenac impurity A EPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (end-capped Zorbax C8 is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute a mixture of 34 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 2.5 and 66 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Inject 20 µl of solution (3). When the chromatograms are recorded in the prescribed conditions, the retention times are about 25 minutes for diclofenac and about 12 minutes for diclofenac impurity A. Adjust the sensitivity of the system so that the height of the peaks in the chromatogram obtained with solution (3) is not less than 50% of the full scale of the recorder. Continue the chromatography for 1.5 times the retention time of diclofenac. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

Inject 20 µl of solution (1) and 20 µl of solution (2). In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the gel containing 50 mg of Diclofenac Diethylamine with 50 ml of *acetone* for 10 minutes, filter and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 100 ml of a mixture of 40 volumes of *water* and 60 volumes of *methanol*, dilute 1 volume of this solution to 10 volumes with the mobile phase and filter through a glass fibre filter (Whatman GF/C is suitable). Solution (2) contains 0.005% w/v of *diclofenac sodium BPCRS* in *methanol*. Solution (3) contains 0.01% w/v of *diclofenac sodium BPCRS* and 0.01% w/v of *diclofenac impurity A EPCRS* in *methanol*.

The chromatographic conditions described under Related substances may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

Calculate the percentage content of $C_{18}H_{22}Cl_2N_2O_2$ in the gel using the declared content of $C_{14}H_{10}Cl_2NNaO_2$ in *diclofenac sodium BPCRS* and taking each mg of $C_{14}H_{10}Cl_2NNaO_2$ to be equivalent to 1.1609 mg of $C_{18}H_{22}Cl_2N_2O_2$.

LABELLING

The label states the quantity of the active ingredient both in terms of Diclofenac Diethylamine and the equivalent of Diclofenac Sodium.

Dicycloverine Oral Solution

General Notices

Action and use

Anticholinergic.

DEFINITION

Dicycloverine Oral Solution is a solution of Dicycloverine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of dicycloverine hydrochloride, $C_{19}H_{35}NO_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume containing 0.1 g of dicycloverine hydrochloride add 10 ml of *water* and 1 ml of *hydrochloric acid* , shake with 30 ml of *ether* and allow to separate. Extract the aqueous layer with 30 ml of *chloroform* , wash the extract with two 10 ml quantities of *water* and filter the chloroform solution through *anhydrous sodium sulphate* . Evaporate the filtrate to dryness, recrystallise the residue from hot *acetone* and dry the precipitate at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dicycloverine hydrochloride (*RS 098*).

B. Acidify the oral solution with 2M *nitric acid* and add *silver nitrate solution* . A white precipitate is produced.

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel G plate* and a mixture of 5 volumes of 13.5M *ammonia* , 10 volumes of *ethyl acetate* , 10 volumes of *water* and 75 volumes of *propan-1-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 10 ml of *water* and 1 ml of *hydrochloric acid* to a volume containing 0.1 g of dicycloverine hydrochloride, shake with 30 ml of *ether* and allow to separate. Extract the aqueous layer with 30 ml of *dichloromethane* , wash the extract with two 10 ml quantities of *water*, shake with *anhydrous sodium sulphate* , filter (Whatman 1PS paper is suitable), evaporate the filtrate to dryness and dissolve the residue in 4 ml of *dichloromethane* . For solution (2) dilute 1 volume of solution (1) to 500 volumes with *dichloromethane* . Solution (3) contains 0.1% w/v of each of *dicycloverine hydrochloride EPCRS* and *tropicamide EPCRS* in *methanol* . After removal of the plate, dry it in a current of warm air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot remaining on the line of application. The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

ASSAY

To a weighed quantity containing 5 mg of dicycloverine hydrochloride add 5 ml of *sulphuric acid (10%)* and 2 ml of 0.02M *potassium permanganate*, mix, allow to stand, add 20 ml of *water* and 20 ml of *chloroform* to the decolourised solution and titrate with 0.001M *sodium dodecyl sulphate VS* using 1 ml of *dimethyl yellow solution* as indicator. Each ml of 0.001M *sodium dodecyl sulphate VS* is equivalent to 0.3460 mg of $C_{19}H_{35}NO_2,HCl$. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{19}H_{35}NO_2$, HCl, weight in volume.

STORAGE

Dicycloverine Oral Solution should be protected from light.

Dicycloverine Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Dicycloverine Tablets contain Dicycloverine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dicycloverine hydrochloride, $C_{19}H_{35}NO_2,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.2 g of dicycloverine hydrochloride with 20 ml of *chloroform* , filter, evaporate the filtrate to dryness, recrystallise the residue from *acetone* and dry at 105° for 4 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dicycloverine hydrochloride *(RS 098)* .

B. Shake a quantity of the powdered tablets containing 10 mg of dicycloverine hydrochloride with 5 ml of *water* and 0.2 ml of 2M *nitric acid* , filter and add 0.5 ml of *silver nitrate solution* to the filtrate. A white precipitate is produced.

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel G plate* and a mixture of 5 volumes of 13.5M *ammonia* , 10 volumes of *ethyl acetate* , 10 volumes of *water* and 75 volumes of *propan-1-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of dicycloverine hydrochloride with 8 ml of *water* and 2 ml of 13.5M *ammonia* , extract with two 20 ml quantities of *dichloromethane* , shake with *anhydrous sodium sulphate* , filter (Whatman 1PS paper is suitable), evaporate the filtrate to dryness and dissolve the residue in 4 ml of *dichloromethane* . For solution (2) dilute 1 volume of solution (1) to 500 volumes with *dichloromethane* . Solution (3) contains 0.1% w/v of each of *dicycloverine hydrochloride EPCRS* and *tropicamide EPCRS* in *methanol* . After removal of the plate, dry it in a current of warm air and spray with *dilute potassium iodobismuthate solution* . Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 30 mg of dicycloverine hydrochloride add 20 ml of *water* and shake. Add 10 ml of 1M *sulphuric acid* , 1 ml of *dimethyl yellow solution* and 40 ml of *chloroform* , shake and titrate with 0.004M *sodium dodecyl sulphate VS* , shaking vigorously and allowing the layers to separate after each addition, until a permanent orange–pink colour is produced in the chloroform layer. Each ml of 0.004M *sodium dodecyl sulphate VS* is equivalent to 1.384 mg of $C_{19}H_{35}NO_2$,HCl.

Diethylamine Salicylate Cream

General Notices

DEFINITION

Diethylamine Salicylate Cream is a dispersion of Diethylamine Salicylate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diethylamine salicylate, $C_{11}H_{17}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To 5 g add 50 ml of 2M *sodium hydroxide* , shake well and filter. Acidify 30 ml of the filtrate with 2M *hydrochloric acid* , cool and allow to stand. The *infrared absorption spectrum* of the resulting precipitate, after recrystallisation from *water* and drying at 105°, Appendix II A, is concordant with the *reference spectrum* of salicylic acid *(RS 316)* .

B. To 5 ml of the filtrate obtained in test A, add 10 ml of *water* and 0.05 ml of *iron(III) chloride solution R1* . A deep violet colour is produced.

C. Mix 3 g with 25 ml of *chloroform* and shake with 20 ml of 0.1M *hydrochloric acid* . To the acidic layer add 10 ml of 2M *sodium hydroxide* and boil. Diethylamine vapour is evolved, which turns moist *red litmus paper* blue.

ASSAY

To a quantity containing 50 mg of diethylamine salicylate add 30 ml of 0.5M *sodium hydroxide* and 15 ml of *ethanol (96%)* , mix, heat under a reflux condenser for 30 minutes and cool. To the resulting mixture add 20 ml of 1M *hydrochloric acid* , mix, dilute to 250 ml with *water* and filter. Shake 15 ml of the filtrate with 25 ml of *chloroform* for 2 minutes. To 5 ml of the chloroform layer add 10 ml of *iron(III) nitrate solution* and shake for 5 minutes. Centrifuge the aqueous layer and measure the *absorbance* at the maximum at 530 nm, Appendix II B, using *iron(III) nitrate solution* in the reference cell. Calculate the content of salicylate as salicylic acid, $C_7H_6O_3$, from the *absorbance* obtained by repeating the operation using 15 ml of a 0.013% w/v solution of *salicylic acid* in 0.02M *hydrochloric acid* , beginning at the words 'Shake 15 ml...'. Each g of salicylic acid is equivalent to 1.530 g of $C_{11}H_{17}NO_3$.

Diethylcarbamazine Tablets

General Notices

Action and use

Antihelminthic.

DEFINITION

Diethylcarbamazine Tablets contain Diethylcarbamazine Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diethylcarbamazine citrate, $C_{10}H_{21}N_3O, C_6H_8O_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 0.15 g of Diethylcarbamazine Citrate add 15 ml of *ethanol (96%),* shake for 5 minutes, filter and evaporate the filtrate to dryness. To the residue add 10 ml of 2M *sodium hydroxide,* filter if necessary, and extract with three 10-ml quantities of *dichloromethane.* Dry the combined extracts over *anhydrous sodium sulphate,* filter and evaporate. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of diethylcarbamazine (*RS 411) .*

B. In the test for related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to citric acid in the chromatogram obtained with solution (3).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. Carry out the method for *liquid chromatography* , Appendix III D. Dissolve 31.24 g of *potassium dihydrogen orthophosphate* in *water*, add sufficient *water* to produce 1000 ml and mix (solution A). Solution (1) contains 0.0025% w/v of *diethylcarbamazine citrate BPCRS* in solution A. For solution (2) dilute 10 ml of the filtered dissolution medium with an equal volume of a 6.248% w/v solution of *potassium dihydrogen orthophosphate* , filter and dilute with solution A to produce a solution expected to contain about 0.0025% w/v of Diethylcarbamazine Citrate.

The chromatographic procedure described under Assay may be used.

Calculate the total content of diethylcarbamazine citrate, $C_{10}H_{21}N_3O,C_6H_8O_7$, in the medium using the declared content of $C_{10}H_{21}N_3O,C_6H_8O_7$ in *diethylcarbamazine citrate BPCRS.*

Dimethylpiperazine and methylpiperazine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 5 volumes of 13.5M *ammonia,* 30 volumes of *butan-2-one* and 65 volumes of *methanol* as the mobile phase. Allow the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.50 g of Diethylcarbamazine Citrate with 20 ml of *methanol* and filter. For solution (2) dissolve 0.05 g of *diethylcarbamazine citrate BPCRS* in *methanol* and dilute to 2.0 ml with the same solvent. For solution (3) dissolve 10 mg of *methylpiperazine* in *methanol* and dilute to 200 ml with the same solvent. For solution (4) dissolve 10 mg of *dimethylpiperazine* in *methanol* and dilute to 200 ml with the same solvent. Dry the plate at 105° and expose to iodine vapour for 30 minutes. Any spots corresponding to methylpiperazine and dimethylpiperazine in the chromatogram obtained with solution (1) are not more intense than the spots in the chromatogram obtained with solutions (3) and (4) respectively (0.2% of each).

Related substances

Weigh and finely powder 20 tablets. Dissolve 31.24 g of *potassium dihydrogen orthophosphate* in *water*, add sufficient *water* to produce 1000 ml and mix (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.3 g of Diethylcarbamazine Citrate in 100 ml of solution A, centrifuge and use the clear supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume of the resulting solution to 10 volumes with solution A to produce a solution containing 0.0003% w/v Diethylcarbamazine Citrate. Solution (3) contains 0.2% w/v of *citric acid* in solution A.

The chromatographic procedure described under Assay may be used.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%). Disregard any peak with the same retention time as citric acid in the chromatogram obtained with solution (1).

ASSAY

Weigh and finely powder 20 tablets. Dissolve 31.24 g of *potassium dihydrogen orthophosphate* in *water*, add sufficient *water* to produce 1000 ml and mix (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the powdered tablets containing 25 mg of Diethylcarbamazine Citrate in 100 ml of solution A, shake and filter. Dilute 10 volumes to 50 volumes with solution A. Solution (2) contains 0.005% w/v *diethylcarbamazine citrate BPCRS* in solution A.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm X 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry is suitable), (b) as the mobile phase with a flow rate of 0.8 ml per minute a mixture of 100 ml of *methanol* and 900 ml of a solution containing 10 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and (c) a detection wavelength of 220 nm.

Calculate the content of $C_{10}H_{21}N_3O,C_6H_8O_7$ in the tablets using the declared content of

$C_{10}H_{21}N_3O,C_6H_8O_7$ in *diethylcarbamazine citrate BPCRS.*

Diethylstilbestrol Pessaries

General Notices

Action and use

Estrogen.

DEFINITION

Diethylstilbestrol Pessaries are moulded pessaries containing Diethylstilbestrol and Propylene Glycol in a suitable basis.

The pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of diethylstilbestrol, $C_{18}H_{20}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 450 nm of the irradiated solution prepared as directed in the Assay exhibits maxima at 292 nm and 418 nm.

B. Carry out the method of extraction described under the Assay and dissolve the residue obtained in 0.2 ml of *glacial acetic acid*. Add 1 ml of *orthophosphoric acid* and heat in a water bath for 3 minutes. A deep yellow colour is produced, which almost disappears on dilution with 3 ml of *glacial acetic acid*.

ASSAY

Weigh five pessaries, melt together by warming and allow to cool, stirring continuously. Dissolve a quantity of the mass containing 2.5 mg of Diethylstilbestrol in 25 ml of 2M *hydrochloric acid*, cool and dilute to 250 ml with *water*. Extract 50 ml with three 50 ml quantities of *chloroform*. Discard the aqueous layer and extract the combined chloroform extracts with three 15 ml quantities of 1M *sodium hydroxide*. Discard the chloroform layer and wash the combined aqueous extracts with two 10 ml quantities of *chloroform*. Adjust the pH of the aqueous layer to 9.5 with a 16% v/v solution of *orthophosphoric acid* and extract with four 20 ml quantities of *chloroform*. Filter the combined chloroform extracts through a layer of *anhydrous sodium sulphate*, wash the filter with *chloroform* and evaporate the combined chloroform extracts to dryness. Dissolve the residue in 25 ml of *absolute ethanol*, add 25 ml of a solution prepared by dissolving 1 g of *dipotassium hydrogen orthophosphate* in 55 ml of *water*, transfer a portion of the mixture to a 1-cm closed quartz cell, place the cell 10 cm from a 15-watt, short-wave ultraviolet lamp and irradiate for 10 minutes. Measure the *absorbance* of the irradiated solution at the maximum at 418 nm, Appendix II B, and calculate the content of $C_{18}H_{20}O_2$ from the *absorbance* obtained by repeating the operation using 25 ml of a 0.002% w/v solution of *diethylstilbestrol EPCRS* in *absolute ethanol* and beginning at the words 'add 25 ml of a solution...'.

Diethylstilbestrol Tablets

General Notices

Action and use

Estrogen.

DEFINITION

Diethylstilbestrol Tablets contain Diethylstilbestrol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diethylstilbestrol, $C_{18}H_{20}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 3 mg of Diethylstilbestrol with *ether* , filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *light absorption* , Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in *absolute ethanol* exhibits a maximum only at 241 nm.

B. The *light absorption* , Appendix II B, in the range 230 to 450 nm of the irradiated solution prepared as directed in the Assay exhibits two maxima, at 292 nm and 418 nm.

C. Dissolve 0.5 mg in 0.2 ml of *glacial acetic acid* , add 1 ml of *orthophosphoric acid* and heat in a water bath for 3 minutes. A deep yellow colour is produced which almost disappears on dilution with 3 ml of *glacial acetic acid* (distinction from dienoestrol).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 5 mg of Diethylstilbestrol add 50 ml of *absolute ethanol* , shake for 15 minutes, add sufficient *absolute ethanol* to produce 100 ml and centrifuge. Dilute 20 ml of the clear supernatant liquid to 50 ml with *absolute ethanol* and to 25 ml of the resulting solution add 25 ml of a solution prepared by dissolving 1 g of *dipotassium hydrogen orthophosphate* in 55 ml of *water*. Transfer a portion of the mixture to a 1 cm, closed quartz cell, place the cell 10 cm from a 15-watt, short-wave, ultraviolet lamp and irradiate for 10 minutes. Measure the *absorbance* of the irradiated solution at the maximum at 418 nm, Appendix II B, and calculate the content of $C_{18}H_{20}O_2$ from the *absorbance* obtained by repeating the operation using *diethylstilbestrol EPCRS* in place of the powdered tablets.

STORAGE

Diethylstilbestrol Tablets should be protected from light.

Diflucortolone Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Diflucortolone Cream contains Diflucortolone Valerate in a suitable non-oily basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diflucortolone valerate, $C_{27}H_{36}F_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of *diethylamine*, 50 volumes of *cyclohexane* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the cream containing 1 mg of Diflucortolone Valerate in 5 ml of *dichloromethane* by heating on a water bath, shake vigorously for 15 seconds, cool in ice, filter the dispersion and use the filtrate. Solution (2) contains 0.02% w/v of *diflucortolone valerate BPCRS* in *dichloromethane*. Solution (3) contains a mixture of equal volumes of solutions (1) and (2). Develop the chromatogram twice drying the plate in air for 15 minutes between developments. After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to diflucortolone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µl of each of the following solutions. For solution (1) dissolve 20 mg of *diflucortolone valerate BPCRS* in a 0.02% w/v solution of *clocortolone hexanoate BPCRS* (internal standard) in *methanol* and dilute to 200 ml with the internal standard solution. Add 30 ml of *methanol* to 10 ml of the resulting solution and dilute to 50 ml with *water*. For solution (2) add 40 ml of *methanol* to a quantity of the cream containing 1 mg of Diflucortolone Valerate, disperse by heating on a water bath at 60° for 5 minutes and shake for 30 seconds. Add 10 ml of *water,* cool in ice for

10 minutes and filter. Prepare solution (3) in the same manner as solution (2) but adding 10 ml of a 0.02% w/v solution of *clocortolone hexanoate BPCRS* in *methanol* and 30 ml of *methanol* .

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 250 volumes of *water* and 750 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{27}H_{36}F_2O_5$ in the cream using the declared content of $C_{27}H_{36}F_2O_5$ in *diflucortolone valerate BPCRS* .

Diflucortolone Oily Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Diflucortolone Oily Cream contains Diflucortolone Valerate in a suitable oily basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diflucortolone valerate, $C_{27}H_{36}F_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of *diethylamine* , 50 volumes of *cyclohexane* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) disperse a quantity of the cream containing 1 mg of Diflucortolone Valerate in 10 ml of *dichloromethane* by heating on a water bath, shake vigorously for 1 minute, cool in ice, filter the dispersion and use the filtrate. Solution (2) contains 0.01% w/v of *diflucortolone valerate BPCRS* in *dichloromethane* . Solution (3) contains a mixture of equal volumes of solutions (1) and (2). Develop the chromatogram twice drying the plate in air for 15 minutes between developments. After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to diflucortolone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, injecting 20 µl of each of the following solutions. For solution (1) dissolve 20 mg of *diflucortolone valerate BPCRS* in a 0.02% w/v solution of *clocortolone hexanoate BPCRS* (internal standard) in *methanol* and dilute to 200 ml with the internal standard solution. Add 30 ml of *methanol* to 10 ml of the resulting solution and dilute to 50 ml with *water*. For solution (2) add 40 ml of *methanol* to a quantity of the cream containing 1 mg of Diflucortolone Valerate, disperse by heating on a water bath at 60° for 5 minutes and shake for 30 seconds. Add 10 ml of *water,* cool in ice for

10 minutes and filter. Prepare solution (3) in the same manner as solution (2) but adding 10 ml of a 0.02% w/v solution of *clocortolone hexanoate BPCRS* in *methanol* and 30 ml of *methanol* .

The chromatographic procedure described under Diflucortolone Cream may be used.

Calculate the content of $C_{27}H_{36}F_2O_5$ in the cream using the declared content of $C_{27}H_{36}F_2O_5$ in *diflucortolone valerate BPCRS* .

Diflucortolone Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Diflucortolone Ointment contains Diflucortolone Valerate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of diflucortolone valerate, $C_{27}H_{36}F_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of *diethylamine* , 50 volumes of *cyclohexane* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the ointment containing 1 mg of Diflucortolone Valerate in 5 ml of *dichloromethane* by heating on a water bath, shake vigorously for 15 seconds, cool in ice, filter the dispersion and use the filtrate. Solution (2) contains 0.02% w/v of *diflucortolone valerate BPCRS* in *dichloromethane* . Solution (3) contains a mixture of equal volumes of solutions (1) and (2). Develop the chromatogram twice, drying the plate in air for 15 minutes between developments. After removal of the plate, allow it to dry in air for 15 minutes and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to diflucortolone valerate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, injecting 20 µl of each of the following solutions. For solution (1) dissolve 20 mg of *diflucortolone valerate BPCRS* in a 0.02% w/v solution of *clocortolone hexanoate BPCRS* (internal standard) in *methanol* and dilute to 200 ml with the internal standard solution. Add 30 ml of *methanol* to 10 ml of the resulting solution and dilute to 50 ml with *water*. For solution (2) shake a quantity of the ointment containing 1 mg of Diflucortolone Valerate with 10 ml of a 0.02% w/v solution of *clocortolone hexanoate BPCRS* in *methanol* and add 30 ml of *methanol* . Disperse by heating on a water bath at 60° for 5 minutes and shake for 30 seconds. Add 10 ml of *water*, cool in ice for 10 minutes and filter.

The chromatographic procedure described under Diflucortolone Cream may be used.

Calculate the content of $C_{27}H_{36}F_2O_5$ in the ointment using the declared content of $C_{27}H_{36}F_2O_5$ in *diflucortolone valerate BPCRS* .

Diflunisal Tablets

General Notices

Action and use

Salicylate; non-selective cyclo-oxygenase inhibitor; antipyretic; analgesic; anti-inflammatory.

DEFINITION

Diflunisal Tablets contain Diflunisal. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diflunisal, $C_{13}H_8F_2O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Diflunisal with 20 ml of *ether* for 15 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of diflunisal (form B) *(RS 101)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.1 M *methanolic hydrochloric acid* exhibits two maxima, at 251 nm and 315 nm.

C. Dissolve 2 mg in 10 ml of *ethanol (96%)* and add 0.1 ml of *iron(III) chloride solution*. A deep purple colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a pH 7.2 buffer prepared as described below and rotate the paddle at 50 revolutions per minute. For the dissolution medium, dissolve 121 g of *tris(hydroxymethyl) methylamine* in 9 litres of *water*, adjust the pH to 7.45 at 25° with a 7.0% w/v solution of *anhydrous citric acid*, add sufficient *water* to produce 10 litres, allow to equilibrate at 37° and adjust the pH to 7.2 if necessary. Withdraw a sample of 20 ml of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at the maximum at 306 nm using dissolution medium in the reference cell. Calculate the total content of diflunisal, $C_{13}H_8F_2O_3$, in the medium taking 118 as the value of *A* (1%, 1 cm) at the maximum at 306 nm.

Related substances

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *glacial acetic acid* , 20 volumes of *acetone* and 70 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. Solution (1) contains 2.0% w/v of the residue obtained in the tests for Identification in *methanol* . Solution (2) contains 0.0030% w/v of *4-hydroxybiphenyl* in *methanol* . After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light (254 nm)* . Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.15%).

B. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions in a mixture of 4 volumes of *acetonitrile* and 1 volume of *water*. Solution (1) contains 0.00055% w/v of *fluoranthene* (internal standard). Solution (2) contains 0.50% w/v of the residue obtained in the tests for Identification and 0.00055% w/v of the internal standard.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 2 volumes of *glacial acetic acid* , 25 volumes of *methanol* , 55 volumes of *water* and 70 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

In the chromatogram obtained with solution (2) the sum of the areas of any peaks with retention times greater than that of the internal standard is not greater than the area of the peak due to the internal standard.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 0.25 g of Diflunisal with 100 ml of 0.1M *methanolic hydrochloric acid* for 15 minutes, dilute to 250 ml with 0.1M *methanolic hydrochloric acid* and filter through a glass-fibre filter (Whatman GF4 is suitable). Dilute 5 ml of the filtrate to 100 ml with 0.1M *methanolic hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 315 nm, Appendix II B. Calculate the content of $C_{13}H_8F_2O_3$ taking 130 as the value of A(1%, 1 cm) at the maximum at 315 nm.

Digoxin Injection

General Notices

Action and use

Na /K-ATPase inhibitor; cardiac glycoside.

DEFINITION

Digoxin	25 mg
Ethanol (80 per cent)	12.5 ml
Propylene Glycol	40 ml
Citric Acid Monohydrate	75 mg
Disodium Hydrogen Phosphate Dodecahydrate	0.45 g
Water for Injections	Sufficient to produce 100 ml

Extemporaneous preparation

The following directions apply.

Dissolve the digoxin in the ethanol (80 per cent) and add the propylene glycol, a solution of the citric acid monohydrate and the disodium hydrogen phosphate dodecahydrate in Water for Injections and sufficient Water for Injections to produce 100 ml. Sterilise by *heating in an autoclave* .

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of digoxin, $C_{41}H_{64}O_{14}$

0.0225 to 0.0275% w/v.

IDENTIFICATION

Evaporate 2 ml to dryness, dissolve the residue in 1 ml of *glacial acetic acid* containing 0.01% w/v of *iron(III) chloride* and cautiously add 1 ml of *sulphuric acid* without mixing. A brown ring, free from red colour, is produced at the interface and, after a short time, an indigo colour is produced in the upper layer.

TEST

Acidity or alkalinity

pH, 6.7 to 7.3, Appendix V L.

ASSAY

Transfer 20 ml to a separating funnel containing 10 ml of *water*. Make alkaline with 5M *ammonia* and extract with four 25 ml quantities of *chloroform*. Wash each extract with the same 10 ml of *water*. Evaporate the combined chloroform extracts to dryness on a water bath, dry the residue at 105° for 15 minutes, cool, dissolve the residue in 5 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and add 20 ml of *glacial acetic acid* (solution A). To 5 ml of a 0.2% w/v solution of *digoxin EPCRS* in *glacial acetic acid* add 10 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and sufficient *glacial acetic acid* to produce 50 ml (solution B). Dilute 5 ml of solution A to 25 ml with *digoxin reagent*, mix, allow to stand for 1 hour and measure the *absorbance* of the resulting solution at 590 nm, Appendix II B, using *water* in the reference cell. Calculate the content of $C_{41}H_{64}O_{14}$ from the *absorbance* obtained by treating 5 ml of solution B at the same time and in the same manner.

STORAGE

Digoxin Injection should be protected from light.

Digoxin Tablets

General Notices

Action and use

Na /K-ATPase inhibitor; cardiac glycoside.

DEFINITION

Digoxin tablets contain digoxin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of digoxin, $C_{41}H_{64}O_{14}$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 0.25 mg of Digoxin, add 1 ml of *glacial acetic acid* containing 0.01% w/v of *iron(III) chloride hexahydrate*, shake for a few minutes, filter through sintered glass and cautiously add 1 ml of *sulphuric acid* without mixing. A brown ring, free from red colour, is produced at the interface and, after a short time, an indigo colour is produced in the upper layer.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, placing six tablets in the basket, using as the medium 600 ml of *water* freshly prepared by distillation and rotating the basket at 120 revolutions per minute for 60 minutes. Withdraw a sample of 5 ml of the medium. Filter the sample through a membrane filter disc with a nominal pore size not greater than 0.8 µm, discarding the first 1 ml of the filtrate, and transfer 1 ml to a 10 ml graduated flask. Add 3 ml of a 0.1% w/v solution of L-*ascorbic acid* in *methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide, prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with 0.02M *potassium permanganate VS*, mix and dilute to volume with *hydrochloric acid*. After exactly 2 hours measure the *fluorescence* of the solution, Appendix II E, using an excitation wavelength of 360 nm and an emission wavelength of 490 nm and setting the instrument to zero with *water* and to 100 with a solution prepared at the same time as the test solution in the following manner. Dilute 2.5 ml of a 0.100% w/v solution of *digoxin EPCRS* in *ethanol (80%)* to 100 ml with *water*, dilute the resulting solution further with *water* to produce a solution containing in 1 ml an amount of Digoxin equal to one-hundredth of the strength of the tablets being examined, transfer 1 ml of the solution to a 10 ml graduated flask and carry out the operation described above, beginning at the words 'Add 3 ml ... '. The amount of digoxin, $C_{41}H_{64}O_{14}$, per tablet in solution is not less than 75% of the stated amount.

Uniformity of content

Tablets containing less than 2 mg of Digoxin comply with the requirements stated under Tablets using the following method of analysis. For tablets containing 0.125 mg of digoxin, place one tablet in 5 ml of *water* at 37°, agitate to disintegrate, add 28 ml of *ethanol (96%)*, shake for 1 hour and add sufficient *ethanol (80%)* to produce 50 ml. For tablets containing more or less than 0.125 mg of Digoxin carry out the same procedure but using correspondingly greater or smaller quantities of *water*, *ethanol (96%)* and *ethanol (80%)*. Filter the resulting solution through a suitable membrane filter disc with a nominal pore size not greater than 0.8 µm, discarding the first few ml of the filtrate, and transfer 1.0 ml to a 10 ml graduated flask. Add 3 ml of a 0.1% w/v solution of L-*ascorbic acid* in *methanol*, 0.2 ml of a 0.009M solution of hydrogen peroxide prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with 0.02M *potassium permanganate VS*, mix and dilute to volume with *hydrochloric acid*. After exactly 2 hours measure the *fluorescence* of the solution, Appendix II E, using an excitation wavelength of 360 nm and an emission wavelength of 490 nm and setting the instrument to zero with *water*. Calculate the content of digoxin, $C_{41}H_{64}O_{14}$, from the *fluorescence* obtained by carrying out the operation at the same time using a solution containing 2.5 µg per ml of *digoxin EPCRS* in *ethanol (80%)* and beginning at the words 'transfer 1.0 ml ...'.

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powder containing 1.25 mg of Digoxin add 3 ml of *water*, swirl to disperse the powder and allow to stand for 10 minutes swirling occasionally. Add 25 ml of *glacial acetic acid*, shake for 1 hour and filter (Whatman No. 1 paper is suitable), discarding the first few ml. To 4 ml of the filtrate add 1 ml of *dimethyl sulphoxide*, dilute to 25 ml with *xanthydrol reagent*, mix well and allow to stand in the dark for 4 hours (solution A). At the same time prepare two further solutions in the same manner but using for solution B 4 ml of *digoxin standard solution* and for solution C 4 ml of a mixture of 25 volumes of *glacial acetic acid* and 3 volumes of *water* and beginning at the words 'add 1 ml of *dimethyl sulphoxide* ...'. Measure the *absorbances* of solutions A and B at the maximum at 545 nm, Appendix II B, using solution C in the reference cell.

Dihydrocodeine Injection

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Dihydrocodeine Injection is a sterile solution of Dihydrocodeine Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3,C_4H_6O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Add 0.05 ml to a mixture of 5 ml of *sulphuric acid* and 0.05 ml of *formaldehyde solution* . A purple colour is produced (distinction from pholcodine).

B. To a volume containing 10 mg of Dihydrocodeine Tartrate add 0.05 ml of *nitric acid* . A yellow but no red colour is produced (distinction from morphine).

C. Evaporate a volume containing 0.1 g of Dihydrocodeine Tartrate to dryness on a water bath. Dissolve the residue in 1 ml of *sulphuric acid* , add 0.05 ml of *iron(III) chloride solution R1* and warm gently. A brownish yellow colour is produced which does not become red on the addition of 0.05 ml of 2M *nitric acid* (distinction from codeine and morphine).

D. Yields reaction B characteristic *of tartrates* , Appendix VI.

TESTS

Acidity

pH, 3.0 to 4.5, Appendix V L.

ASSAY

Dilute a volume containing 50 mg of Dihydrocodeine Tartrate to 500 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 284 nm, Appendix II B. Calculate the content of $C_{18}H_{23}NO_3,C_4H_6O_6$ taking 35.7 as the value of A(1%, 1 cm) at the maximum at 284 nm.

STORAGE

Dihydrocodeine Injection should be protected from light.

Dihydrocodeine Oral Solution

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Dihydrocodeine Oral Solution is a solution of Dihydrocodeine Tartrate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of dihydrocodeine tartrate, $C_{18}H_{23}NO_3,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume of the oral solution containing 20 mg of Dihydrocodeine Tartrate add 10 ml of *water* and extract with two 25 ml quantities of *chloroform* . Discard the chloroform extracts, add 3 ml of 5M *sodium hydroxide* to the aqueous layer, mix and extract with 25 ml of *chloroform* . Wash the chloroform layer with 10 ml of *water*, shake with *anhydrous sodium sulphate* and filter. Wash the filter with *chloroform* and evaporate the combined filtrate and washings to dryness. Dissolve the residue in 1 ml of *dichloromethane* and apply 0.2 ml drop wise on to the surface of a disc prepared using 0.3 g of *potassium bromide* , allowing the solvent to evaporate between applications, and dry the disc at 50° for 2 minutes. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of dihydrocodeine *(RS 102)* .

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 90 volumes of *dichloromethane* , 10 volumes of *methanol* and 1 volume of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 20 µl of each of solutions (1), (2), (3) and (4) and 40 µl of solution (5). For solution (1) add 10 ml of *water* and 3 ml of 5M *sodium hydroxide* to a quantity of the oral solution containing 20 mg of Dihydrocodeine Tartrate, mix, extract with two 30 ml quantities of *chloroform* and wash each extract successively with the same 10 ml of *water*. Filter each extract in turn through *anhydrous sodium sulphate* on a plug of absorbent cotton moistened with *chloroform* , wash the filter with *chloroform* , evaporate the combined filtrate and washings to dryness and dissolve the residue in 2 ml of *methanol* . For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol* . For solution (3) dilute 1 volume of solution (2) to 2 volumes with *methanol* . Solution (4) contains 0.0050% w/v of *codeine* *phosphate BPCRS* in *methanol*. Solution (5) is a mixture of equal volumes of solution (1) and solution (4). After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any spot in the chromatogram obtained with solution (1) corresponding to the spot due to codeine in the chromatogram obtained with solution (5) is not more intense than the spot in the chromatogram obtained with solution (4). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3). The method is not valid unless, in the chromatogram obtained with solution (5), the spot due to codeine is clearly separated from the principal spot.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.04% w/v of *dihydrocodeine tartrate BPCRS* in *water*. For solution (2) disperse a weighed quantity of the oral solution containing 10 mg of Dihydrocodeine Tartrate in *water*, dilute to 25 ml with *water* and mix.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute, 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in a mixture of 60 volumes of *methanol* and 40 volumes of *water*, the pH of the mixture being adjusted to 5.5 with *glacial acetic acid* and (c) a detection wavelength of 284 nm.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{18}H_{23}NO_3,C_4H_6O_6$, weight in volume, using the declared content of $C_{18}H_{23}NO_3,C_4H_6O_6$ in *dihydrocodeine tartrate BPCRS*.

STORAGE

Dihydrocodeine Oral Solution should be protected from light.

Dimenhydrinate Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Dimenhydrinate Tablets contain Dimenhydrinate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dimenhydrinate, $C_{17}H_{21}NO,C_7H_7ClN_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.1 g of Dimenhydrinate with 10 ml of a mixture of equal volumes of *chloroform* and *ether* for 5 minutes, filter and evaporate the filtrate to an oily residue on a water bath. Add 3 ml of *ether* to the residue and scratch the interface between the residue and the ether gently with a glass rod until crystals appear in the liquid. Transfer the suspension of crystalline material in ether to a watch glass, allow the solvent to evaporate in a current of air and dry the residue at 60°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dimenhydrinate *(RS 104)*.

TEST

Theophylline and substances related to diphenhydramine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 1 volume of 13.5M *ammonia*, 9 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Dimenhydrinate with three 10 ml quantities of *chloroform*, filter, evaporate the combined filtrates almost to dryness and dissolve the residue in 5 ml of *chloroform*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *chloroform*. Solution (3) contains 0.010% w/v of *theophylline* in *chloroform*. After removal of the plate, dry it in a current of cold air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to theophylline in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Spray the plate with *potassium iodobismuthate solution*, allow it to dry in air and spray with *hydrogen peroxide solution (10 vol)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%). Disregard any spot extending from the line of application to an Rf value of about 0.1.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.1 g of Dimenhydrinate as completely as possible in 20 ml of *water*, add 10 ml of 5M *ammonia* , mix, extract with successive quantities of 15, 15, 15, 10 and 10 ml of *ether* and wash the combined extracts with 10 ml of *water*. Evaporate the ether, warm the residue with 10 ml of *ethanol (96%)* until dissolved, cool, add 50 ml of 0.01M *hydrochloric acid VS* and titrate the excess of acid with 0.01M *sodium hydroxide VS* using *methyl red mixed solution* as indicator. Each ml of 0.01M *hydrochloric acid VS* is equivalent to 4.700 mg of $C_{17}H_{21}NO$, $C_7H_7ClN_4O_2$.

Dimercaprol Injection

General Notices

Action and use

Chelating agent for use in heavy metal poisoning.

DEFINITION

Dimercaprol	5 g
Benzyl Benzoate	9.6 ml
Arachis Oil	Sufficient to produce 100 ml

Extemporaneous preparation

The following directions apply.

Dissolve the dimercaprol in the benzyl benzoate and add sufficient arachis oil to produce 100 ml. Add sufficient of a 35% v/v solution of strong ammonia solution in ethanol (96 per cent) until the acidity, when determined by the method described below, corresponds to a pH of 6.8 to 7.0. Add about 0.2 g of activated charcoal, stir, allow to stand for not less than 1 hour and filter. Distribute the solution in ampoules, the air in which is replaced by nitrogen or other suitable gas and seal immediately. Sterilise by *dry heat* at a minimum of 150° for not less than 1 hour.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dimercaprol, $C_3H_8OS_2$

4.75 to 5.25% w/v.

CHARACTERISTICS

A bright, pale yellow solution.

TESTS

Acidity

Shake with an equal volume of *water* for 2 minutes and allow to separate. The pH of the aqueous layer, after filtration through neutral filter paper, is 4.5 to 6.5, Appendix V L.

Refractive index

1.482 to 1.486, Appendix V E.

Weight per ml

0.940 to 0.955 g, Appendix V G.

ASSAY

To 1 g add 20 ml of 0.1M *hydrochloric acid* and titrate with 0.05M *iodine VS*. Each ml of 0.05M *iodine VS* is equivalent to 6.21 mg of $C_3H_8OS_2$. Use the *weight per ml* of the injection to calculate the percentage w/v of $C_3H_8OS_2$.

STORAGE

Dimercaprol Injection should be protected from light.

LABELLING

The label states (1) the nature of the solvent; (2) that the preparation is intended for intramuscular injection only.

Diphenhydramine Oral Solution

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Diphenhydramine Oral Solution is a solution of Diphenhydramine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of diphenhydramine hydrochloride, $C_{17}H_{21}NO,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and a mixture of 50 volumes of *ethanol (96%)* , 30 volumes of *glacial acetic acid* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following freshly prepared solutions. For solution (1) acidify a quantity of the oral solution containing 50 mg of diphenhydramine hydrochloride with 2M *hydrochloric acid* , shake with three 20 ml quantities of *ether* , discard the ether, extract with two 20 ml quantities of *chloroform* , dry the combined extracts over *anhydrous sodium sulphate* , filter, evaporate the chloroform and dissolve the cooled residue in 5 ml of *chloroform* . Solution (2) contains 1% w/v of *diphenhydramine hydrochloride EPCRS* in *chloroform* . After removal of the plate, allow it to dry in air and spray with a solution containing 0.25% w/v of *chloroplatinic(IV) acid* and 5% w/v of *potassium iodide* . The principal spot in the chromatogram obtained with solution (1) corresponds in colour and position to that in the chromatogram obtained with solution (2).

B. Evaporate to dryness 1 ml of solution (1) obtained in test A, dissolve the residue in 0.15 ml of *water* and add 2 ml of *sulphuric acid* ; a yellow colour is produced which, on the addition of 0.5 ml of *nitric acid* , changes to red. Add 15 ml of *water*, cool, add 5 ml of *chloroform* , shake and allow to separate; the chloroform layer is violet.

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel H* as the coating substance and a mixture of 80 volumes of *chloroform* and 20 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) use solution (1) described under test A for Identification. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *chloroform* . After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Acidify a quantity containing 0.1 g of diphenhydramine hydrochloride with 2M *hydrochloric acid*, shake with three 20 ml quantities of *ether*, discard the ether, make the aqueous solution alkaline with 5M *sodium hydroxide* and extract with successive 15 ml quantities of *ether* until extraction is complete. Wash the combined ether extracts with two 5 ml quantities of *water*, extract the combined washings with 15 ml of *ether* and evaporate the combined ether extracts to dryness. Dissolve the residue in 15 ml of 0.05M *sulphuric acid VS* and titrate the excess of acid with 0.1M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 0.05M *sulphuric acid VS* is equivalent to 29.18 mg of $C_{17}H_{21}NO,HCl$.

STORAGE

Diphenhydramine Oral Solution should be protected from light.

Dipipanone and Cyclizine Tablets

General Notices

Action and use

Opioid receptor agonist.

DEFINITION

Dipipanone and Cyclizine Tablets contain, in each, 10 mg of Dipipanone Hydrochloride and 30 mg of Cyclizine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dipipanone hydrochloride, $C_{24}H_{31}NO,HCl,H_2O$

9.0 to 11.0 mg.

Content of cyclizine hydrochloride, $C_{18}H_{22}N_2,HCl$

27.75 to 32.25 mg.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (3) exhibits two peaks having the same retention times as those due to dipipanone and cyclizine in the chromatogram obtained with solution (1).

B. Shake a quantity of the powdered tablets containing 60 mg of Cyclizine Hydrochloride with 10 ml of *acetone* and filter, reserving the filtrate for test C. Add 10 ml of *chloroform* to the residue, mix with the aid of ultrasound for 2 to 3 minutes, filter and evaporate to dryness. The residue yields reaction A characteristic of *chlorides* , Appendix VI.

C. Evaporate the filtrate reserved in test B to dryness. The residue yields reaction A characteristic of *chlorides* , Appendix VI.

TEST

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and as the mobile phase the lower layer obtained after shaking together a mixture of 2 volumes of 13.5M *ammonia* , 8 volumes of *methanol* and 90 volumes of *dichloromethane* and allowing the layers to separate. Apply separately to the plate 20 µl of each of the following freshly prepared solutions. For solution (1) shake a quantity of the powdered tablets containing 30 mg of Cyclizine Hydrochloride with 50 ml of a mixture of equal volumes of *methanol* and *dichloromethane*, filter, evaporate the filtrate to dryness and dissolve the residue in 3 ml of the same solvent mixture. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.0050% w/v of N-*methylpiperazine* in *methanol*. Solution (4) contains 0.10% w/v of each of *cyclizine hydrochloride BPCRS* and *hydroxyzine hydrochloride EPCRS* in *methanol*. After removal of the plate, allow it to dry in air and expose to iodine vapour for 10 minutes. Any spot corresponding to N-*methylpiperazine* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

ASSAY

Weigh and finely powder 20 tablets. Dissolve 0.5 g of *chlorcyclizine hydrochloride BPCRS* (internal standard) in sufficient *dichloromethane* to produce 100 ml (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 25 ml of solution A to 0.15 g of *cyclizine hydrochloride BPCRS* and 50 mg of *dipipanone hydrochloride BPCRS* and dilute to 50 ml with *dichloromethane*. For solution (2) add 40 ml of *dichloromethane* to a quantity of the powdered tablets containing 50 mg of Dipipanone Hydrochloride and mix with the aid of ultrasound for 20 minutes; dilute to 50 ml with *dichloromethane* and filter. Prepare solution (3) in the same manner as solution (2) but add 25 ml of solution A and 15 ml of *dichloromethane* in place of the 40 ml of dichloromethane.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of silicone grease (Apiezon L is suitable) and 5% w/w of *potassium hydroxide* and maintained at 250°. In the chromatogram obtained with solution (2) the peaks, in order of emergence, are due to cyclizine, chlorcyclizine and dipipanone.

Calculate the content of $C_{24}H_{31}NO,HCl,H_2O$ using the declared content of $C_{24}H_{31}NO,HCl,H_2O$ in *dipipanone hydrochloride BPCRS* and the content of $C_{18}H_{22}N_2,HCl$ using the declared content of $C_{18}H_{22}N_2,HCl$ in *cyclizine hydrochloride BPCRS*.

Dipivefrine Eye Drops

General Notices

Action and use

Adrenaline prodrug; treatment of glaucoma.

DEFINITION

Dipivefrine Eye Drops are a sterile solution of Dipivefrine Hydrochloride in purified water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of dipivefrine hydrochloride, $C_{19}H_{29}NO_5,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck plates are suitable) and a mixture of 1 volume of *formic acid* , 10 volumes of *methanol* and 30 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use the eye drops diluted with *water*, if necessary, to produce a solution containing 0.1% w/v of dipivefrine hydrochloride. Solution (2) is a 0.1% w/v solution of *dipivefrine hydrochloride BPCRS* in *water*. After removal of the plate, allow it to dry in air and spray with a mixture of 5 volumes of *ethylenediamine* , 45 volumes of *ethanol (96%)* and 50 volumes of a 0.1% w/v solution of *potassium hexacyanoferrate(III)* until saturated, allow to dry, heat at 100° for 10 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Clarity of solution

The eye drops are *clear*, Appendix IV A.

Acidity

pH, 2.5 to 3.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) use the eye drops diluted, if necessary, to contain 0.1% w/v of dipivefrine hydrochloride. For solution (2) dilute 4 volumes of solution (1) to 10 volumes with *water* and further dilute 1 volume of this solution to 100 volumes with *water*. For solution (3) dissolve the contents of one vial of *dipivefrine impurity standard BPCRS* (the impurity standard contains 99 mg of dipivefrine hydrochloride and 1 mg of dipivalyladrenalone hydrochloride) in 0.0015M *hydrochloric acid*, wash the container with the same solvent and dilute the solution and washings to 100 ml with the same solvent.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 1 volume of *glacial acetic acid*, 15 volumes of 0.014M *sodium dodecyl sulphate* and 24 volumes of *acetonitrile*, (c) a detection wavelength of 254 nm and (d) an electronic integrator.

The test is not valid unless the chromatogram obtained with solution (3) resembles the appropriate chromatogram provided with the impurity standard.

In the chromatogram obtained with solution (1) the area of any peak corresponding to dipivalyladrenalone hydrochloride is not greater than the area of the peak corresponding to dipivalyladrenalone hydrochloride in the chromatogram obtained with solution (3) (1%) and the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.4%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the eye drops diluted, if necessary, with *water* to contain 0.1% w/v of dipivefrine hydrochloride. Solution (2) contains 0.1% w/v of *dipivefrine hydrochloride BPCRS* in *water*. For solution (3) use solution (3) prepared for the Related substances.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless the chromatogram obtained with solution (3) resembles the appropriate chromatogram provided with the impurity standard.

Calculate the content of $C_{19}H_{29}NO_5,HCl$ in the eye drops from the chromatograms obtained and using the declared content of $C_{19}H_{29}NO_5,HCl$ in *dipivefrine hydrochloride BPCRS*.

Dipotassium Hydrogen Phosphate Injection

General Notices

Dipotassium Phosphate Injection

DEFINITION

Dipotassium Hydrogen Phosphate Injection is a sterile solution of Dipotassium Hydrogen Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of dipotassium hydrogen phosphate, K_2HPO_4

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *potassium salts* and reaction B characteristic of *phosphates* , Appendix VI.

TESTS

Alkalinity

pH, 9.0 to 10.0, Appendix V L.

ASSAY

Dilute, if necessary, a volume of the injection with sufficient *carbon dioxide-free water* to produce a solution containing 8.71% w/v (1 millimole of potassium ion per ml) of dipotassium hydrogen phosphate. Titrate 20 ml with 0.5M *hydrochloric acid VS* using a mixture of 4 volumes of *bromocresol green solution* and 1 volume of *methyl red solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 87.1 mg of K_2HPO_4.

LABELLING

The label states (1) the percentage w/v of Dipotassium Phosphate; (2) the concentration of the potassium ion in millimoles; (3) that the injection should be used in accordance with the manufacturer's instructions; (4) that solutions containing visible solid particles must not be used.

Dipyridamole Tablets

General Notices

Action and use

Adenosine reuptake inhibitor; inhibitor of platelet aggregation.

DEFINITION

Dipyridamole Tablets contain Dipyridamole. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dipyridamole, $C_{24}H_{40}N_8O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Dipyridamole with 20 ml of *chloroform* , filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dipyridamole *(RS 108)* .

B. To a quantity of the powdered tablets containing 10 mg of Dipyridamole add 50 ml of *methanol* , warm slightly, shake for 15 minutes and allow to cool. Add sufficient *methanol* to produce 100 ml, filter and to 10 ml of the filtrate add 1 ml of 1M *hydrochloric acid* and sufficient *methanol* to produce 100 ml. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 450 nm exhibits three maxima, at 230, 285 and 405 nm.

TEST

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Dipyridamole with 100 ml of the mobile phase for 15 minutes, filter (Whatman GF/C filter is suitable) and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. For solution (3) dilute 1 volume of a 0.1% w/v solution of *diltiazem hydrochloride EPCRS* to 20 ml with solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP8 5µ is suitable) maintained at 30°, (b) as the mobile phase with a flow rate of 1.3 ml per minute a mixture prepared as described below and (c) a detection wavelength of 290 nm. For the mobile phase dissolve 0.504 g of *potassium dihydrogen orthophosphate* in 370 ml of *water* and adjust the pH to 3.0 with *orthophosphoric acid* , add 80 ml of *acetonitrile* and 550 ml of *methanol*. Inject 20 µl of each solution. Continue the chromatography of solution (1) for nine times the retention time of dipyridamole.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to diltiazem and dipyridamole is at least 2.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.1 times that of the peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

To 10 whole tablets add 300 ml of 1M *hydrochloric acid*, heat at 40° for 20 minutes with shaking, allow to cool and add sufficient 1M *hydrochloric acid* to produce 500 ml. Filter and dilute, if necessary, with 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of Dipyridamole. Dilute 1 volume to 50 volumes with *water* and measure the *absorbance* of the resulting solution at the maximum at 283 nm, Appendix II B. Dilute 1 volume of a 0.05% w/v solution of *dipyridamole BPCRS* in 1M *hydrochloric acid* to 50 volumes with *water*. Calculate the content of $C_{24}H_{40}N_8O_4$ in the tablets from the absorbances obtained using the declared content of $C_{24}H_{40}N_8O_4$ in *dipyridamole BPCRS*.

Disopyramide Capsules

General Notices

Action and use

Class I antiarrhythmic.

DEFINITION

Disopyramide Capsules contain Disopyramide.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of disopyramide, $C_{21}H_{29}N_3O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.2 g of Disopyramide with 50 ml of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 ml of *chloroform*. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of disopyramide *(RS 110)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 269 nm.

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 1 volume of 18M *ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µl of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.20 g of Disopyramide with 20 ml of *methanol* for 30 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 400 volumes with *methanol*. After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

ASSAY

To a quantity of the mixed contents of 20 capsules containing 40 mg of Disopyramide, add 40 ml of 0.05M *methanolic sulphuric acid*, shake for 15 minutes, dilute to 100 ml with the same solvent and filter. Dilute 5 ml of the filtrate to 100 ml with 0.05M *methanolic sulphuric acid* and measure the *absorbance* of the resulting solution at the maximum at 269 nm, Appendix II B. Calculate the content of $C_{21}H_{29}N_3O$ taking 198.5 as the value of A(1%, 1 cm) at the maximum at 269 nm.

Disopyramide Phosphate Capsules

General Notices

Action and use

Class I antiarrhythmic.

DEFINITION

Disopyramide Phosphate Capsules contain Disopyramide Phosphate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of disopyramide, $C_{21}H_{29}N_3O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Suspend a quantity of the contents of the capsules containing the equivalent of 0.2 g of disopyramide in 50 ml of *chloroform* , add 2 ml of 13.5M *ammonia* , shake and filter through *anhydrous sodium sulphate* . Evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 ml of *chloroform* . The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of disopyramide *(RS 110)* .

B. The *light absorption* , Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 269 nm and a shoulder at 263 nm.

C. Shake a quantity of the contents of the capsules containing the equivalent of 0.4 g of disopyramide with 20 ml of *water* and filter. The filtrate yields the reactions characteristic of *phosphates* , Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 262 nm, Appendix II B. Calculate the total content of disopyramide, $C_{21}H_{29}N_3O$, in the medium taking 87 as the value of A(1%, 1 cm) at the maximum at 262 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 1 volume of 18M *ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 0.20 g of disopyramide with 20 ml of *methanol* for 30 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

To a quantity of the mixed contents of 20 capsules containing the equivalent of 40 mg of disopyramide, add 40 ml of 0.05M *methanolic sulphuric acid*, shake for 15 minutes, dilute to 100 ml with the same solvent and filter. Dilute 5 ml of the filtrate to 100 ml with 0.05M *methanolic sulphuric acid* and measure the *absorbance* of the resulting solution at the maximum at 269 nm, Appendix II B. Calculate the content of $C_{21}H_{29}N_3O$ taking 198.5 as the value of A(1%, 1 cm) at the maximum at 269 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of disopyramide.

Dispersible Doxycycline Tablets

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Dispersible Doxycycline Tablets contain Doxycycline Monohydrate in a suitable dispersible basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous doxycycline, $C_{22}H_{24}N_2O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 plates are suitable) and a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Spray the plate evenly with a 10% w/v solution of *disodium edetate* the pH of which has been adjusted to 9.0 with 10M *sodium hydroxide* (about 10 ml for a 100 mm × 200 mm plate). Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous doxycycline with 100 ml of *methanol* for 1 to 2 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.05% w/v of *doxycycline hyclate BPCRS* in *methanol* . Solution (3) contains 0.05% w/v of each of *doxycycline hyclate BPCRS* and *tetracycline hydrochloride BPCRS* in *methanol* . After removal of the plate, dry it in a current of air and examine under *ultraviolet light (365 nm)* . The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Disintegration

Comply with the requirements for Dispersible Tablets.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a solution prepared by dissolving 2 g of *sodium chloride* in 7 ml of *hydrochloric acid* and sufficient *water* to produce 1000 ml and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 15 ml of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at 276 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *doxycycline hyclate BPCRS* in the dissolution medium and calculate the total content of anhydrous doxycycline, $C_{22}H_{24}N_2O_8$, in the medium using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS*.

Light-absorbing impurities

Dissolve a quantity of the powdered tablets as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0% w/v of anhydrous doxycycline and filter. The *absorbance* of the filtrate at 490 nm is not more than 0.20, calculated with reference to the dried powdered tablets, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use. For solution (1) add a quantity of the powdered tablets containing the equivalent of 80 mg of anhydrous doxycycline to 80 ml of 0.01M *hydrochloric acid*, mix with the aid of ultrasound and add sufficient 0.01M *hydrochloric acid* to produce 100 ml. Centrifuge and use the supernatant liquid. Solutions (2) to (5) are solutions in 0.01M *hydrochloric acid* containing (2) 0.080% w/v of *doxycycline hyclate BPCRS,* (3) 0.080% w/v of *6-epidoxycycline hydrochloride EPCRS*, (4) 0.080% w/v of *metacycline hydrochloride EPCRS* and (5) 0.0016% w/v each of *6-epidoxycycline hydrochloride EPCRS* and *metacycline hydrochloride EPCRS.* For solution (6) dilute a mixture of 4 volumes of solution (2), 1.5 volumes of solution (3) and 1 volume of solution (4) to 25 volumes with 0.01M *hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene co-polymer* (8 µm) with a pore size of 10 nm (PLRP-S from Polymer Laboratories is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared as described below and (c) a detection wavelength of 254 nm. For the mobile phase add 60 g of *2-methylpropan-2-ol* to a graduated flask with the aid of 200 ml of *water*, add 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* previously adjusted to pH 8.0 with 2M *sodium hydroxide* and 10 ml of a 4% w/v solution of *disodium edetate* previously adjusted to pH 8.0 with 2M *sodium hydroxide* and dilute to 1000 ml with *water*. Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the first peak (metacycline) and the second peak (6-epidoxycycline) is at least 1.25 and the *resolution factor* between the second peak and the third peak (doxycycline) is at least 2.0. If necessary, adjust the content of 2-methylpropan-2-ol in the mobile phase.

In the chromatogram obtained with solution (1) the area of any peak corresponding to metacycline or 6-epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with solution (5) (2%) and the area of any other *secondary peak* is not greater than 0.25 times the area of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with solution (5) (0.5%).

Loss on drying

When dried at 60° at a pressure of 2 kPa for 2 hours, the powdered tablets lose not more than 6.0% of their weight. Use 1 g.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add a quantity of the powdered tablets containing the equivalent of 0.1 g of anhydrous doxycycline to 20 ml of 0.1M *hydrochloric acid*, mix with the aid of ultrasound until fully dispersed (about 10 minutes), add sufficient *water* to produce 200 ml, mix, centrifuge and use the supernatant liquid. For solution (2) dissolve 0.115 g of *doxycycline hyclate BPCRS* in 20 ml of 0.1M *hydrochloric acid* and add sufficient *water* to produce 200 ml.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{22}H_{24}N_2O_8$ in the tablets using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous doxycycline.

Disulfiram Tablets

General Notices

Action and use

Aldehyde dehyrogenase inhibitor; treatment of alcoholism.

DEFINITION

Disulfiram Tablets contain Disulfiram.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of disulfiram, $C_{10}H_{20}N_2S_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.2 g of Disulfiram by shaking with 5 ml of *dichloromethane* and filter. Evaporate the filtrate to dryness and dry the residue at 40° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of disulfiram *(RS 111)* .

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

C. Extract a quantity of the powdered tablets containing 0.3 g of disulfiram with *ethanol (96%)* , filter and evaporate the filtrate to dryness. Dissolve 50 mg of the residue in 5 ml of *ethanol (96%)* and add 1 ml of *potassium cyanide solution* . A yellow colour is produced which becomes green and then darkens to bluish green.

TESTS

Diethyldithiocarbamate

Shake a quantity of the powdered tablets containing 0.1 g of Disulfiram with 10 ml of *chloroform* and filter. Add 10 ml of 0.1M *sodium hydroxide* to the filtrate, shake, discard the chloroform layer and wash the aqueous layer with three 10 ml quantities of *chloroform* . To the aqueous layer add 0.25 ml of a 0.4% w/v solution of *copper(II) sulphate* and 2 ml of *dichloromethane* , shake and allow to separate. The lower layer is not more intensely coloured than a standard prepared at the same time and in the same manner by adding 10 ml of 0.1M *sodium hydroxide* to 0.2 ml of a freshly prepared 0.0075% w/v solution of *sodium diethyldithiocarbamate* and beginning at the words 'add 0.25 ml of a 0.4% w/v solution of *copper(II) sulphate* ...' (0.01%, calculated as the acid).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 30 volumes of *butyl acetate* and 70 volumes of n-*hexane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 0.50 g of Disulfiram with 20 ml of *ethyl acetate* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *ethyl acetate*. Solution (3) contains 0.025% w/v of *disulfiram BPCRS* in *ethyl acetate*. Solution (4) contains 0.050% w/v of *monosulfiram BPCRS* in *ethyl acetate*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to monosulfiram is not more intense than the spot in the chromatogram obtained with solution (4) (2%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.4 g of Disulfiram add 75 ml of *methanol* and shake for 30 minutes. Add sufficient *methanol* to produce 100 ml, filter and dilute 5 ml of the filtrate to 100 ml with *methanol*. To 5 ml of this solution add sufficient of a 0.1% w/v solution of *copper(II) chloride* in *methanol* to produce 25 ml, mix and allow to stand for 1 hour. Measure the *absorbance* of the resulting solution at the maximum at 400 nm, Appendix II B, using in the reference cell a solution prepared by diluting 5 ml of *methanol* to 25 ml with the copper(II) chloride solution. Repeat the operation using 5 ml of a 0.020% w/v solution of *disulfiram BPCRS* in *methanol,* beginning at the words 'add sufficient of a 0.1% w/v solution of *copper(II) chloride* ...'. Calculate the content of $C_{10}H_{20}N_2S_4$ from the *absorbances* obtained and using the declared content of $C_{10}H_{20}N_2S_4$ in *disulfiram BPCRS*.

STORAGE

Disulfiram Tablets should be protected from light.

Dithranol Cream

General Notices

Action and use

Coal tar extract; treatment of psoriasis.

DEFINITION

Dithranol Cream contains Dithranol in a suitable oil-in-water emulsified basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of dithranol, $C_{14}H_{10}O_3$

85.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay the principal peak in the chromatogram obtained with solution (2) has the same retention time as that in the chromatogram obtained with solution (1).

B. Suspend a quantity containing 0.5 mg of dithranol in 5 ml of 1M *sodium hydroxide* and heat on a water bath for 1 minute while stirring. A pink colour is produced.

TESTS

Dihydroxyanthraquinone and dithranol dimer

Not more than 10.0% of the stated amount of dithranol, calculated as the sum of the amounts of dihydroxyanthraquinone and dithranol dimer when determined by the method for *liquid chromatography* , Appendix III D, using the following solutions. For creams containing more than 0.5% w/v of Dithranol, use as solution (1) solution A obtained in the Assay. For solution (2) add 4 ml of *glacial acetic acid* to 20 ml of a solution containing 0.005% w/v of *1,8-dihydro-xyanthraquinone* and 0.005% w/v of *dithranol impurity C EPCRS (dithranol dimer)* in *dichloromethane* and add sufficient n-*hexane* to produce 100 ml. For creams containing 0.5% w/v or less of Dithranol, use as solution (1) solution B obtained in the Assay. For solution (2) add 20 ml of *glacial acetic acid* to 10 ml of a solution containing 0.005% w/v of *1,8-dihydroxyanthraquinone* and 0.005% w/v of *dithranol impurity C EPCRS (dithranol dimer)* in *dichloromethane* and add sufficient *dichloromethane* to produce 100 ml.

The chromatographic procedure described under Assay may be used but using a detection wavelength of 380 nm.

In the chromatogram obtained with solution (2) the peaks, other than the solvent peak, are, in order of emergence, due to dihydroxyanthraquinone and dithranol dimer. Calculate the amounts of dihydroxyanthraquinone and dithranol dimer in the cream with reference to the corresponding peaks in the chromatogram obtained with solution (2).

1-Hydroxy-9-anthrone

Carry out the method for *liquid chromatography*, Appendix III D, in subdued light using the following freshly prepared solutions. For solution (1) disperse a quantity of the cream containing 10 mg of Dithranol in 30 ml of *water* at about 50°, add 30 ml of a warm, saturated solution of *sodium chloride*, cool and extract with three 40 ml quantities of *chloroform*. Filter the extract successively through *anhydrous sodium sulphate* on a suitable filter (Whatman GF/C is suitable). Evaporate the filtrate under reduced pressure to a volume of about 2 ml, add 25 ml of warm *methanol*, shake, cool in ice for 15 minutes and filter (Whatman GF/C paper is suitable). Evaporate the filtrate to dryness under reduced pressure and dissolve the residue in 10 ml of a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *acetonitrile*; filter if necessary. For solution (2) dissolve 25 mg of *dithranol impurity D EPCRS (1-hydroxy-9-anthrone)* in 0.5 ml of *glacial acetic acid* and add sufficient *acetonitrile* to produce 50 ml (solution A); dilute 1 ml of solution A to 20 ml with *acetonitrile*. For solution (3) dilute 1 volume of solution (1) to 40 ml with a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *acetonitrile*. For solution (4) dissolve 50 mg of *dithranol BPCRS* in 0.5 ml of *glacial acetic acid*, add 2 ml of solution A and sufficient *acetonitrile* to produce 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 and Spherisorb ODS 2 are suitable), (b) a mixture of 2.5 volumes of *glacial acetic acid*, 40 volumes of *tetrahydrofuran* and 60 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *dithranol BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak corresponding to the principal peak in the chromatogram obtained with solution (2) (1-hydroxy-9-anthrone) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For creams containing more than 0.5% w/w of Dithranol prepare solution (1) in the following manner, protected from light. Disperse by shaking a quantity of the cream containing 50 mg of Dithranol in 10 ml of *glacial acetic acid* and 20 ml of *dichloromethane*, filter through a glass microfibre filter paper (Whatman GF/C is suitable) into a 100 ml graduated flask, wash the filter with *dichloromethane* and add sufficient *dichloromethane* to produce 100 ml. Shake the flask vigorously and allow to stand until two layers are obtained, add *dichloromethane* until the dichloromethane and water interface is level with the calibration mark, shake vigorously and again allow to stand until two layers are obtained. Dilute 20 volumes of the lower layer to 50 volumes with n-*hexane* (solution A). Dilute a suitable volume of solution A with n-*hexane* to contain 0.002% w/v of Dithranol. For creams containing 0.5% w/w or less of Dithranol prepare solution (1) in the following manner, protected from light. Disperse, by shaking, a quantity of the cream containing 5 mg of Dithranol in 10 ml of *glacial acetic acid* and 20 ml of *dichloromethane*, filter through a glass microfibre filter paper (Whatman GF/C is suitable) into a 50 ml graduated flask, wash the filter with *dichloromethane* and add sufficient *dichloromethane* to produce 50 ml. Shake the flask vigorously and allow to stand until two layers are obtained, add *dichloromethane* until the dichloromethane and water interface is level with the calibration mark, shake vigorously and again allow to stand until two layers are obtained (solution B). Dilute a suitable volume of solution B with n-*hexane* to contain 0.002% w/v of Dithranol. For solution (2) add 1 ml of *glacial acetic acid* to 10 ml of a 0.02% w/v solution of *dithranol BPCRS* in *dichloromethane* and add sufficient of the mobile phase to produce 100 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si 60 is suitable), (b) a mixture of 1 volume of *glacial acetic acid* , 5 volumes of *dichloromethane* and 82 volumes of n-*hexane* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 354 nm.

Calculate the content of $C_{14}H_{10}O_3$ in the cream using the declared content of $C_{14}H_{10}O_3$ in *dithranol BPCRS* .

STORAGE

Dithranol Cream should be protected from light.

Dithranol Ointment

General Notices

Action and use

Coal tar extract; treatment of psoriasis.

DEFINITION

Dithranol Ointment contains Dithranol, in *fine powder*, in a suitable hydrophobic basis.

Extemporaneous preparation

The following directions apply.

Triturate dithranol, in *fine powder*, with Yellow Soft Paraffin, add sufficient Yellow Soft Paraffin to produce an ointment of the required strength and mix thoroughly. Part of the Yellow Soft Paraffin may be replaced by Hard Paraffin to produce an ointment of stiffer consistency.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of dithranol, $C_{14}H_{10}O_3$

85.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (2) corresponds to the principal peak in the chromatogram obtained with solution (1).

B. Heat a quantity containing 0.5 mg of dithranol with 5 ml of 1M *sodium hydroxide* on a water bath with constant stirring. A pink colour is produced in the aqueous layer.

TESTS

Dihydroxyanthraquinone and dithranol dimer

Not more than 10.0% of the stated amount of dithranol, calculated as the sum of the amounts of dihydroxyanthraquinone and dithranol dimer, when determined by the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 ml of *glacial acetic acid* to 20 ml of a solution containing 0.0050% w/v of *1,8-dihydroxyanthraquinone* and 0.0050% w/v of *dithranol impurity C EPCRS (dithranol dimer)* in *dichloromethane* and add sufficient *hexane* to produce 100 ml. For solution (2) disperse a quantity of the ointment containing 20 mg of Dithranol in 20 ml of *dichloromethane*, add 1 ml of *glacial acetic acid*, dilute to 100 ml with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C is suitable).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si 60 is suitable), (b) a mixture of 82 volumes of *hexane*, 5 volumes of *dichloromethane* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 380 nm.

In the chromatogram obtained with solution (1) the peaks, other than the solvent peak, in order of emergence are due to dihydroxyanthraquinone and dithranol dimer. Calculate the amounts of dihydroxyanthraquinone and dithranol dimer in the ointment with reference to the corresponding peaks in the chromatogram obtained with solution (1).

1-Hydroxy-9-anthrone

Carry out the method for *liquid chromatography*, Appendix III D, in subdued light using the following freshly prepared solutions. For solution (1) disperse a quantity of the ointment containing 10 mg of Dithranol in 25 ml of *chloroform*, evaporate under reduced pressure to a volume of about 2 ml, add 25 ml of warm *methanol*, shake, cool in ice for 15 minutes and filter (Whatman GF/C paper is suitable). Evaporate the filtrate to dryness under reduced pressure and dissolve the residue in 10 ml of a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *acetonitrile*; filter if necessary. For solution (2) dissolve 25 mg of *dithranol impurity D EPCRS (1-hydroxy-9-anthrone)* in 0.5 ml of *glacial acetic acid* and add sufficient *acetonitrile* to produce 50 ml (solution A); dilute 1 ml of solution A to 20 ml with *acetonitrile*. For solution (3) dilute 1 volume of solution (1) to 40 ml with a mixture of 5 volumes of *glacial acetic acid* and 95 volumes of *acetonitrile*. For solution (4) dissolve 50 mg of *dithranol BPCRS* in 0.5 ml of *glacial acetic acid*, add 2 ml of solution A and sufficient *acetonitrile* to produce 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 and Spherisorb ODS 2 are suitable), (b) a mixture of 2.5 volumes of *glacial acetic acid*, 40 volumes of *tetrahydrofuran* and 60 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the chromatogram supplied with *dithranol BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak corresponding to the principal peak in the chromatogram obtained with solution (2) (1-hydroxy-9-anthrone) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 ml of *glacial acetic acid* to 20 ml of a 0.025% w/v solution of *dithranol BPCRS* in *dichloromethane* and add sufficient *hexane* to produce 100 ml. For solution (2) disperse a quantity of the ointment containing 5 mg of Dithranol in 20 ml of *dichloromethane*, add 1 ml of *glacial acetic acid*, dilute to 100 ml with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C is suitable).

The chromatographic procedure described under Dihydroxyanthraquinone and dithranol dimer may be used but using a detection wavelength of 354 nm.

Calculate the content of $C_{14}H_{10}O_3$ in the ointment using the declared content of $C_{14}H_{10}O_3$ in *dithranol BPCRS* .

STORAGE

Dithranol Ointment should be protected from light.

Dithranol Paste

General Notices

Action and use

Coal tar extract; treatment of psoriasis.

DEFINITION

Dithranol Paste contains Dithranol in a suitable hydrophobic basis containing 24% w/w each of Zinc Oxide and Starch and 2% w/w of Salicylic Acid.

Extemporaneous preparation

The following formula and directions apply.

Dithranol	1 g, or a sufficient quantity
Zinc and Salicylic Acid Paste	Sufficient to produce 1000 g

Mix the Dithranol with a portion of the Zinc and Salicylic Acid Paste until a smooth, even dispersion is obtained and gradually incorporate the remainder of the Zinc and Salicylic Acid Paste.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of dithranol, $C_{14}H_{10}O_3$

85.0 to 110.0% of the stated amount.

Content of salicylic acid, $C_7H_6O_3$

1.9 to 2.1% w/w.

Content of zinc oxide, ZnO

22.5 to 25.5% w/w.

IDENTIFICATION

A. In the Assay for dithranol, the principal peak in the chromatogram obtained with solution (2) corresponds to the principal peak in the chromatogram obtained with solution (1).

B. Suspend a quantity containing 0.5 mg of Dithranol in 5 ml of 1M *sodium hydroxide* and heat on a water bath for 1 minute while stirring. A pink colour is produced.

C. Disperse 0.5 g in 10 ml of *chloroform*, filter, extract the filtrate with 10 ml of a 1% w/v solution of *sodium hydrogen carbonate* and wash the extract with 5 ml of *chloroform*. To 5 ml add 3 ml of *iron(III) chloride solution R1*. A violet colour is produced which persists after the addition of 0.5 ml of 5M *acetic acid*.

TEST

Dihydroxyanthraquinone and dithranol dimer

Not more than 10.0% of the stated amount of dithranol, calculated as the sum of the amounts of dihydroxyanthraquinone and dithranol dimer, when determined by the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 ml of *glacial acetic acid* to 20 ml of a solution containing 0.0050% w/v of *1,8-dihydroxyanthraquinone* and 0.0050% w/v of *dithranol impurity C EPCRS (dithranol dimer)* in *dichloromethane* and add sufficient *hexane* to produce 100 ml. For solution (2) disperse a quantity of the paste containing 20 mg of Dithranol in 20 ml of *dichloromethane* with warming and stirring, centrifuge and decant the supernatant layer into a 100 ml flask. Disperse the residue in 20 ml of *hexane*, centrifuge, decant the supernatant layer into the 100 ml flask and repeat the procedure with a further two 20 ml quantities of *hexane*. Add 1 ml of *glacial acetic acid* to the combined extracts, dilute to 100 ml with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C paper is suitable).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si 60 is suitable), (b) a mixture of 82 volumes of *hexane*, 5 volumes of *dichloromethane* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 380 nm.

In the chromatogram obtained with solution (1) the peaks, other than the solvent peak, in order of emergence are due to dihydroxyanthraquinone and dithranol dimer. Calculate the amounts of dihydroxyanthraquinone and dithranol dimer in the paste with reference to the corresponding peaks in the chromatogram obtained with solution (1).

ASSAY

For dithranol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 ml of *glacial acetic acid* to 20 ml of a 0.025% w/v solution of *dithranol BPCRS* in *dichloromethane* and add sufficient *hexane* to produce 100 ml. For solution (2) disperse a quantity of the paste containing 5 mg of Dithranol in 20 ml of *dichloromethane* with warming and stirring, centrifuge and decant the supernatant layer into a 100 ml flask. Disperse the residue in 20 ml of *hexane*, centrifuge, decant the supernatant layer into the 100 ml flask and repeat the procedure with a further two 20 ml quantities of *hexane*. Add 1 ml of *glacial acetic acid* to the combined extracts, dilute to 100 ml with *hexane* and filter through a fine glass microfibre filter paper (Whatman GF/C paper is suitable).

The chromatographic procedure described under Dihydroxyanthraquinone and dithranol dimer may be used but using a detection wavelength of 354 nm.

Calculate the content of $C_{14}H_{10}O_3$ in the paste using the declared content of $C_{14}H_{10}O_3$ in *dithranol BPCRS*.

For salicylic acid

Shake 0.5 g with 10 ml of 1M *hydrochloric acid* and 10 ml of *ether* until fully dispersed. Decant and reserve the aqueous layer. Extract the ether layer with two further 10 ml quantities of 1M *hydrochloric acid*, combine the aqueous extracts with the reserved aqueous layer, wash with 10 ml of *ether* and reserve for the Assay for zinc oxide. Combine the ether extracts and washings, add 15 ml of *petroleum spirit (boiling range, 40° to 60°)* and extract with successive quantities of 20 ml, 10 ml and 10 ml of *borate buffer pH 9.0*. Dilute the combined extracts to 100 ml with 2M *hydrochloric acid*, dilute 15 ml of the resulting solution to 50 ml with the same solvent and measure the *absorbance* of the final solution at the maximum at 302 nm. Calculate the content of $C_7H_6O_3$ taking 260 as the value of *A* (1%, 1 cm) at the maximum at 302 nm.

For zinc oxide

To the combined aqueous extracts obtained in the Assay for salicylic acid add 20 ml of 1M *sodium hydroxide* and 50 mg of *xylenol orange triturate*. To the resulting solution add sufficient *hexamine* to change the colour of the solution to red, and then a further 3 g of *hexamine,* and titrate with 0.1M *disodium edetate VS*. Each ml of 0.1M *disodium edetate VS* is equivalent to 8.137 mg of ZnO.

STORAGE

Dithranol Paste should be protected from light.

Dobutamine Intravenous Infusion

General Notices

Action and use

Beta$_1$-adrenoceptor agonist.

DEFINITION

Dobutamine Intravenous Infusion is a sterile solution of Dobutamine Hydrochloride. It is supplied as a ready-to-use solution or it is prepared in accordance with the manufacturer's instructions either from Sterile Dobutamine Concentrate or from Dobutamine Hydrochloride for Injection.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C, Method D. Dilute the intravenous infusion, if necessary, with *water BET* to give a solution containing the equivalent of 10 mg of dobutamine per ml (solution A). The endotoxin limit concentration of solution A is 10 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

STORAGE

Dobutamine Intravenous Infusion prepared from Sterile Dobutamine Concentrate or from Dobutamine Hydrochloride for Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

When supplied as a ready-to-use solution, the intravenous infusion complies with the following requirements.

Content of dobutamine, $C_{18}H_{25}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate and a mixture of 2 volumes of *water*, 6 volumes of *glacial acetic acid*, 12 volumes of *ether* and 18 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use the intravenous infusion being examined diluted, if necessary, in a mixture of 1 volume of *water* and 4 volumes of *methanol* to produce a solution containing the equivalent of 0.05% w/v of dobutamine. Solution (2) contains 0.05% w/v of *dobutamine hydrochloride BPCRS* in a mixture of 1 volume of *water* and 4 volumes of *methanol* . After removal of the plate, allow it to dry in air and spray with a 0.5% w/v solution of *potassium permanganate* in 1M *sodium hydroxide* . The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

Related substances

Complies with the test described in the requirements for Dobutamine Sterile Concentrate but using the following solutions. For solution (1) use the intravenous infusion diluted, if necessary, with a mixture of equal volumes of mobile phase A and mobile phase B to produce a solution containing the equivalent of 0.05% w/v of dobutamine. For solution (2) dilute 4 volumes of solution (1) to 100 volumes with a 0.0005% w/v solution of *anisaldehyde* in a mixture of equal volumes of mobile phase A and mobile phase B and dilute 1 volume of the solution to 10 volumes with the same mixture of mobile phases. For solution (3) dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

ASSAY

Carry out the Assay described under the requirements for Sterile Dobutamine Concentrate but for solution (1) use the intravenous infusion being examined diluted, if necessary, with the mobile phase to contain the equivalent of 0.05% w/v of dobutamine.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of dobutamine.

STERILE DOBUTAMINE CONCENTRATE

DEFINITION

Sterile Dobutamine Concentrate is a sterile solution of Dobutamine Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of dobutamine, $C_{18}H_{25}NO_3$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or pale yellow solution.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel 60

F_{254} precoated plate and a mixture of 5 volumes of *glacial acetic acid*, 15 volumes of *water*, 40 volumes of *propan-1-ol* and 100 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use the concentrate being examined diluted, if necessary, with *water* to produce a solution containing the equivalent of 1.25% w/v of dobutamine. Solution (2) contains 1.25% w/v of *dobutamine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* Spray the plate with a 6.0% w/v solution of *iron(III) chloride hexahydrate* and then with a 2% w/v solution of *potassium hexacyanoferrate(III)*. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of the concentrate diluted, if necessary, to contain the equivalent of 1.25% w/v of dobutamine, 2.5 to 4.0, Appendix V L.

Clarity of solution

Dilute the concentrate, if necessary, to contain the equivalent of 1.25% w/v of dobutamine. The solution is not more opalescent than *reference suspension I*, Appendix IV A.

Light absorption

The *light absorption*, Appendix II B, at 400 nm of the concentrate diluted, if necessary, to contain the equivalent of 1.25% w/v of dobutamine is not more than 0.20.

Related substances

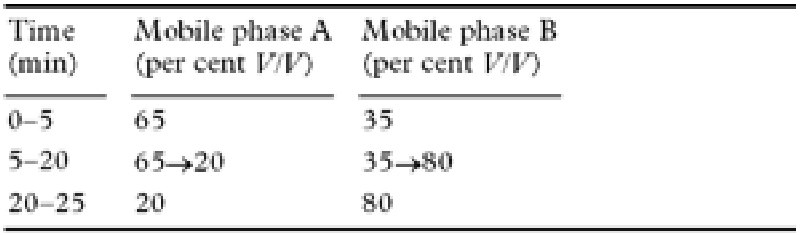
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the concentrate with a mixture of equal volumes of mobile phase A and mobile phase B to produce a solution containing the equivalent of 0.5% w/v of dobutamine. For solution (2) dilute 4 volumes of solution (1) to 100 volumes with a 0.005% w/v solution of *anisaldehyde* in a mixture of equal volumes of mobile phase A and mobile phase B and dilute 1 volume of the solution to 10 volumes with the same mixture of mobile phases. For solution (3) dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute the solvent mixture and gradient programme described below and (c) a detection wavelength of 280 nm.

Inject 20 µl of each solution.

Mobile phase A Dissolve 2.60 g of *sodium octanesulphonate* in 1000 ml of *water* and add 3 ml of *triethylamine*; adjust to pH 2.5 with *orthophosphoric acid*,

Mobile phase B A mixture of 18 volumes of *acetonitrile* and 82 volumes of *methanol*.



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0–5	65	35
5–20	65→20	35→80
20–25	20	80

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks corresponding to dobutamine hydrochloride and anisaldehyde is at least 4.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%) and the sum of the areas of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%). Disregard any peak due to the solvent and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the concentrate with the mobile phase to produce a solution containing the equivalent of 0.05% w/v of dobutamine. Solution (2) contains 0.06% w/v of *dobutamine hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.06% w/v of *dobutamine hydrochloride BPCRS* and 0.03% w/v of *4-(4-hydroxyphenyl)butan-2-one* in the mobile phase.

The chromatographic procedure may be carried out using (a) stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Supelcosil LC-18-DB is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute a mixture of 14 volumes of *methanol*, 28 volumes of *acetonitrile* and 58 volumes of a solution prepared by dissolving 3.38 g of *sodium octanesulphonate* in 1000 ml of *water*, adding 3 ml of *triethylamine* and adjusting the pH to 2.5 with *orthophosphoric acid* and (c) a detection wavelength of 280 nm.

Inject 20 µl of each solution. The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to dobutamine hydrochloride and 4-(4-hydroxyphenyl)butan-2-one is at least 1.5.

Calculate the content of $C_{18}H_{25}NO_3$ in the concentrate from the chromatograms obtained using the declared content of $C_{18}H_{25}NO_3$ in *dobutamine hydrochloride BPCRS*.

LABELLING

The label states (1) the quantity of active ingredient in terms of the equivalent amount of dobutamine; (2) that the solution must not be diluted with alkaline solutions.

DOBUTAMINE HYDROCHLORIDE FOR INJECTION

DEFINITION

Dobutamine Hydrochloride for Injection is a sterile material consisting of Dobutamine

Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of dobutamine, $C_{18}H_{25}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate and a mixture of 2 volumes of *water*, 6 volumes of *glacial acetic acid*, 12 volumes of *ether* and 18 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dissolve the contents of the sealed container in sufficient of a mixture of 1 volume of *water* and 4 volumes of *methanol* to produce a solution containing the equivalent of 1.0% w/v of dobutamine. Solution (2) contains 1% w/v of *dobutamine hydrochloride BPCRS* in a mixture of 1 volume of *water* and 4 volumes of *methanol*. After removal of the plate, allow it to dry in air and spray with a 0.5% w/v solution of *potassium permanganate* in 1M *sodium hydroxide*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a solution containing the equivalent of 2.5% w/v of dobutamine, 2.5 to 4.5, Appendix V L.

Clarity of solution

A solution containing the equivalent of 2.5% w/v of dobutamine is not more opalescent than *reference suspension II*, Appendix IV A.

Light absorption

The *absorbance*, Appendix II B, at 480 nm of a solution containing the equivalent of 2.5% w/v of dobutamine is not more than 0.065.

Related substances

Comply with the test described in the requirements for Sterile Dobutamine Concentrate but preparing solution (1) in the following manner. Dissolve the contents of the sealed container in a mixture of equal volumes of mobile phase A and mobile phase B to produce a solution containing the equivalent of 0.5% w/v of dobutamine.

Water

Not more than 2.0%, Appendix IX C. Use the contents of a single container and add 7 g of *salicylic acid* and 20 ml of *formamide* before the determination.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of one container in the mobile phase to produce a solution containing the equivalent of 0.05% w/v of dobutamine. Solution (2) contains 0.06% w/v of *dobutamine hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.06% w/v of *dobutamine hydrochloride BPCRS* and 0.03% w/v of *4-(4-hydroxyphenyl)butan-2-one* in the mobile phase.

The chromatographic procedure described under Assay in the requirements for Sterile Dobutamine Concentrate may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to dobutamine hydrochloride and 4-(4-hydroxyphenyl)butan-2-one is at least 1.5.

Calculate the content of $C_{18}H_{25}NO_3$ in the container using the declared content of $C_{18}H_{25}NO_3$ in *dobutamine hydrochloride BPCRS*.

Repeat the procedure on the contents of a further two containers. Determine the content of $C_{18}H_{25}NO_3$ using the average of the three individual results obtained.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of dobutamine.

Docusate Capsules

General Notices

Action and use

Stimulant laxative; faecal softener.

DEFINITION

Docusate Capsules contain a solution of Docusate Sodium in a suitable water miscible vehicle.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia* , 20 volumes of *ethanol (96%)*, 40 volumes of *water* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) dissolve, by heating on a water bath, a capsule in sufficient *water* to produce a solution containing 1% w/v of docusate sodium. Pass 1 ml of this solution with the aid of vacuum through a solid-phase extraction column of 1 ml capacity and containing 0.1 g of an octadecyl-bonded silica sorbent (a Sep-pak C18 column is suitable) previously washed with 2 ml of *methanol* , followed by 5 ml of *water*. Wash the column with 2 ml of *water*, discarding the aqueous eluate and then elute the docusate sodium with 4 ml of *methanol* , retaining the methanol solution. Solution (2) contains 0.25% w/v of *docusate sodium BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and expose to iodine vapour. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) add 200 ml of *water* to a number of whole capsules containing 1 g of docusate sodium and warm on a water bath until the capsules have dissolved. Add 200 ml of *acetonitrile,* shake the mixture thoroughly and cool. Add sufficient of a mixture of equal volumes of *acetonitrile* and *water* to produce 1000 ml, mix well and filter to obtain a clear solution (Whatman No. 1 paper followed by a 0.4-µm filter is suitable). Solution (2) contains

0.1% w/v of *docusate sodium BPCRS* in a mixture of equal volumes of *acetonitrile* and *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Ultracarb ODS is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 30 volumes of 0.005M *tetrabutylammonium dihydrogen orthophosphate* and 70 volumes of *acetonitrile* and (c) a detection wavelength of 214 nm.

Calculate the content of $C_{20}H_{37}NaO_7S$ in the capsules using the declared content of $C_{20}H_{37}NaO_7S$ in *docusate sodium BPCRS* .

STORAGE

Docusate Capsules should be kept in an airtight container .

Docusate Oral Solution

General Notices

Action and use

Stimulant laxative; faecal softener.

DEFINITION

Docusate Oral Solution contains 1.0% w/v of Docusate Sodium in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$

0.95 to 1.05% w/v.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia*, 20 volumes of *ethanol (96%)*, 40 volumes of *water* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) pass a volume of the oral solution containing 10 mg of docusate sodium, with the aid of vacuum, through a solid phase extraction column of 1 ml capacity and containing 0.1 g of an octadecyl-bonded silica sorbent (a Sep-pak C18 column is suitable) which has been previously washed with 2 ml of *methanol*, followed by 5 ml of *water*. Wash the column with 2 ml of *water*, discarding the aqueous eluate and then elute the docusate sodium with 4 ml of *methanol*, retaining the methanol solution. Solution (2) contains 0.25% w/v of *docusate sodium BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and expose to iodine vapour. The chromatogram obtained with solution (1) exhibits a spot with the same colour and Rf value as the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) pass a weighed quantity of the oral solution containing 10 mg of docusate sodium, with the aid of vacuum, through a solid-phase extraction column of 1 ml capacity and containing 0.1 g of an octadecyl-bonded silica sorbent (a Sep-pak C18 column is suitable) previously washed with 2 ml of *methanol*, followed by 5 ml of *water.* Wash the column with 2 ml of *water*, discarding the aqueous eluate. Elute the docusate sodium with 4 ml of *methanol* and dilute to 20 ml with a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water.* Solution (2) contains 0.05% w/v of *docusate sodium BPCRS* in a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with silica gel with a chemically bonded, strongly basic quaternary ammonium anion-exchange coating (10 µm) (Spherisorb SAX is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1.6 volumes of *orthophosphoric acid* , 350 volumes of *water* and 650 volumes of *acetonitrile* and (c) a detection wavelength of 214 nm.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{20}H_{37}NaO_7S$, weight in volume, using the declared content of $C_{20}H_{37}NaO_7S$ in *docusate sodium BPCRS* .

Domperidone Tablets

General Notices

Action and use

Peripheral dopamine receptor antagonist; antiemetic.

DEFINITION

Domperidone Tablets contain Domperidone Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of domperidone, $C_{22}H_{24}ClN_5O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of a solution prepared by dissolving 1.36 g of *sodium acetate* in 50 ml of *water*, adjusting the pH to 4.7 with *dilute acetic acid* and adding sufficient *water* to produce 100 ml, 18 volumes of *methanol*, 23 volumes of *dichloromethane* and 54 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 10 mg of domperidone with 10 ml of a mixture of equal volumes of *dichloromethane* and *methanol* and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) contains 0.127% w/v of *domperidone maleate BPCRS* in a mixture of equal volumes of *dichloromethane* and *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* Spray the plate with *potassium iodobismuthate solution* and examine again. Using each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at 286 nm using dissolution medium in the reference cell. Measure the *absorbance* of a 0.001% w/v solution of *domperidone maleate BPCRS* in the dissolution medium and calculate the total content of domperidone, $C_{22}H_{24}ClN_5O_2$, in the medium using the declared content of $C_{22}H_{24}ClN_5O_2$ in *domperidone maleate BPCRS.*

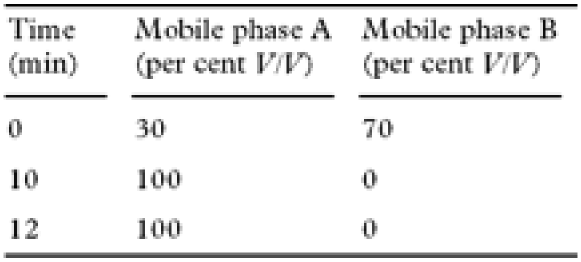
Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use. For solution (1) to a quantity of the powdered tablets containing the equivalent of 50 mg of domperidone add 10 ml of a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol,* mix with the aid of ultrasound for 20 minutes and filter through a glass microfibre filter (Whatman GF/C is suitable). For solution (2) dilute 1 volume of solution (1) to 200 volumes with a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol*. Dilute 1 volume of the resulting solution to 2 volumes with a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol*. Solution (3) contains 0.01% w/v of *domperidone maleate BPCRS* and 0.015% w/v of *droperidol EPCRS* in a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol.*

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable). Carry out a linear gradient elution with a flow rate of 1.5 ml per minute using the following conditions.

Mobile phase A Methanol.

Mobile phase B A 0.5% w/v solution of *ammonium acetate*.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0	30	70
10	100	0
12	100	0

Use a detection wavelength of 280 nm. Equilibrate the column for at least 30 minutes with *methanol* and equilibrate with the initial mobile phase for at least 5 minutes. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 10 µl of solution (2) is at least 50% of the full scale of the recorder.

Inject separately 10 µl of a mixture of equal volumes of 0.01M *hydrochloric acid* and *methanol* as a blank and 10 µl of each of solutions (1), (2) and (3). The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2. If necessary adjust the concentration of *methanol* in the mobile phase or adjust the time programme for the linear gradient.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak in the chromatogram obtained with the blank solution and any peak with an area less than 0.2 times the area of the peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) add sufficient *methanol* to 10 whole tablets to produce a solution containing 0.02% w/v of domperidone, mix with the aid of ultrasound for 20 minutes and filter through a glass microfibre filter (Whatman GF/C is suitable). To 50 ml of the filtrate add 1 ml of 0.1M *hydrochloric acid* and sufficient *water* to produce 100 ml. Solution (2) contains 0.0127% w/v of *domperidone maleate BPCRS* in a mixture of equal volumes of 0.002M *hydrochloric acid* and *methanol* .

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{22}H_{24}ClN_5O_2$ in the tablets using the declared content of $C_{22}H_{24}ClN_5O_2$ in *domperidone maleate BPCRS.*

STORAGE

Domperidone Tablets should be stored in an airtight container.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of domperidone.

Dopamine Intravenous Infusion

General Notices

Action and use

Dopamine receptor antagonist; beta_1-adrenoceptor agonist; alpha-adrenoceptor agonist.

DEFINITION

Dopamine Intravenous Infusion is a sterile solution of Dopamine Hydrochloride. It is supplied as a ready-to-use solution in Glucose Intravenous Infusion (50 g per litre) or it is prepared immediately before use in accordance with the manufacturer's instructions either from Sterile Dopamine Concentrate or from Dopamine Hydrochloride for Injection.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

When supplied as a ready-to-use solution, the intravenous infusion complies with the following requirements.

Content of dopamine hydrochloride, $C_8H_{11}NO_2,HCl$

95.0% to 105.0% of the stated amount.

Content of glucose, $C_6H_{12}O_6$

4.75 to 5.25% w/v.

CHARACTERISTICS

A colourless liquid.

IDENTIFICATION

A. Saturate a volume containing 0.1 g of dopamine hydrochloride with *sodium chloride* and extract with three 20 ml quantities of *butan-1-ol* . Filter the combined extracts through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dopamine hydrochloride *(RS 113)* .

B. To a volume containing 10 mg of dopamine hydrochloride add 0.1 ml of *iron(III) chloride solution R1* . An intense green colour is produced.

TESTS

Acidity

pH, 3.0 to 4.5, Appendix V L.

5-Hydroxymethylfurfural

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the intravenous infusion. Solution (2) contains 0.0025% w/v of *5-hydroxymethylfurfural* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) 0.05M *disodium hydrogen orthophosphate* adjusted to pH 7.0 with *orthophosphoric acid* as the mobile phase with a flow rate of 2.0 ml per minute and (c) a detection wavelength of 284 nm.

The area of any peak corresponding to 5-hydroxymethylfurfural in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.05%, determined with reference to the content of glucose).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the infusion with the mobile phase to produce a solution containing 0.032% w/v of dopamine hydrochloride. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase. For solution (3) dilute 1 volume of solution (1) and 1 volume of a solution containing 0.030% w/v each of *4-ethylcatechol* and *3,4-dimethoxyphenethylamine* to 50 volumes with the mobile phase.

The chromatographic conditions described under the Assay for dopamine hydrochloride may be used.

In the prescribed conditions, the retention time of dopamine is about 5 minutes, of 4-ethylcatechol about 3 minutes and of 3,4-dimethoxyphenethylamine, about 12 minutes. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to 4-ethylcatechol and dopamine is at least 3.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%) and not more than one such peak has an area greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the intravenous infusion, if necessary, with *water BET* to give a solution containing 1.6 mg per ml (solution A). The endotoxin limit concentration of solution A is 26.67 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

For dopamine hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0032% w/v of *dopamine hydrochloride BPCRS* in the mobile phase. For solution (2) dilute a suitable volume of the infusion with the mobile phase to produce a solution containing 0.0032% w/v of dopamine hydrochloride.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 700 volumes of 0.005M *sodium dodecyl sulphate*, 300 volumes of *acetonitrile*, 10 volumes of *glacial acetic acid* and 2 volumes of 0.1M *disodium edetate* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 280 nm.

Calculate the content of $C_8H_{11}NO_2$,HCl using the declared content of $C_8H_{11}NO_2$,HCl in *dopamine hydrochloride BPCRS*.

For glucose

To 50 ml add 0.2 ml of 5M *ammonia* and sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and determine the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the volume taken for assay.

STERILE DOPAMINE CONCENTRATE

DEFINITION

Sterile Dopamine Concentrate is a sterile solution of Dopamine Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of dopamine hydrochloride, $C_8H_{11}NO_2$,HCl

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or pale yellow solution.

IDENTIFICATION

A. Extract a volume containing 0.1 g of dopamine hydrochloride with 10 ml of *butan-1-ol*. Filter the extract through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dopamine hydrochloride *(RS 113)*.

B. To a volume containing 10 mg of dopamine hydrochloride add 0.05 ml of *iron(III) chloride solution R1*. An intense green colour is produced.

TESTS

Acidity

pH, 2.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 2 volumes of *anhydrous formic acid*, 7 volumes of *water,* 36 volumes of *methanol* and 52 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a volume of the concentrate containing 0.15 g of dopamine hydrochloride to 5 ml with *methanol*. Solution (2) contains 0.0075% w/v of *4-*O*-methyldopamine hydrochloride* in *methanol*. Solution (3) contains 0.0075% w/v each of *3-*O*-methyldopamine hydrochloride* and *4-*O*-methyldopamine hydrochloride* in *methanol*. After removal of the plate, allow it to dry in air for 15 minutes and spray evenly and abundantly with a mixture, prepared immediately before use, of equal volumes of *iron(III) chloride solution R1* and *potassium hexacyanoferrate(III) solution*. In the chromatogram obtained with solution (1) any spot with an Rf value higher than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the sterile concentrate with *water BET* to give a solution containing 1.6 mg per ml (solution A). The endotoxin limit concentration of solution A is 26.67 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for Assay of dopamine hydrochloride described in the requirements for the ready-to-use infusion.

STORAGE

Sterile Dopamine Concentrate should be protected from light.

LABELLING

The label states that the solution must not be diluted with alkaline solutions.

DOPAMINE HYDROCHLORIDE FOR INJECTION

DEFINITION

Dopamine Hydrochloride for Injection is a sterile material consisting of Dopamine Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of dopamine hydrochloride, $C_8H_{11}NO_2,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A white or almost white, crystalline powder.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of dopamine hydrochloride *(RS 113)*.

B. Dissolve 10 mg in 2 ml of *water* and add 0.05 ml of *iron(III) chloride solution R1*. An intense green colour is produced.

TESTS

Acidity

pH of a 4% w/v solution in a 1.0% w/v solution of *sodium metabisulphite*, 2.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 2 volumes of *anhydrous formic acid*, 7 volumes of *water*, 36 volumes of *methanol* and 52 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of three solutions in *methanol* containing (1) 3.0% w/v of the substance being examined, (2) 0.0075% w/v of *4*-O-*methyldopamine hydrochloride* and (3) 0.0075% w/v each of *3*-O-*methyldopamine hydrochloride* and *4*-O-*methyldopamine hydrochloride*. After removal of the plate, allow it to dry in air for 15 minutes and spray evenly and abundantly with a mixture, prepared immediately before use, of equal volumes of *iron(III) chloride solution R1* and *potassium hexacyanoferrate(III) solution*. In the chromatogram obtained with solution (1) any spot with an Rf value higher than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 1.6 mg per ml (solution A). The endotoxin limit concentration of solution A is 26.67 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0032% w/v of *dopamine hydrochloride BPCRS* in the mobile phase. For solution (2) dissolve sufficient of the mixed contents of the 10 containers in the mobile phase to produce a solution containing 0.0032% w/v of dopamine hydrochloride.

The chromatographic procedure described under Assay for dopamine hydrochloride in the requirements for the ready-to-use infusion may be used.

Calculate the content of $C_8H_{11}NO_2,HCl$ in a container of average content weight using the declared content of $C_8H_{11}NO_2,HCl$ in *dopamine hydrochloride BPCRS*.

Dosulepin Capsules

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Dosulepin Capsules contain Dosulepin Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of dosulepin hydrochloride, $C_{19}H_{21}NS,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the contents of the capsules containing 0.1 g of Dosulepin Hydrochloride with 20 ml of *absolute ethanol* , filter and remove the ethanol from the filtrate by evaporation. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dosulepin hydrochloride *(RS 114)* .

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 13.5M *ammonia* , 10 volumes of *propan-2-ol* and 90 volumes of *1,2-dichloroethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) extract a quantity of the contents of the capsules containing 0.25 g of Dosulepin Hydrochloride by shaking for 2 minutes with 5 ml of *chloroform* , centrifuge and use the supernatant liquid. For solution (2) dilute 2 ml of solution (1) to 5 ml with *chloroform* . Solution (3) contains 0.010% w/v each of *3-(dibenzo*[b,e]*thiepin-11(6H)-ylidene)*-N,N-*dimethylaminopropan-1-amine* S-*oxide hydrochloride BPCRS* and *dibenzo*[b,e]*thiepin-11(6H)-one BPCRS* in *chloroform* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . In the chromatogram obtained with solution (3) the spot with the lower Rf value is more intense than any corresponding spot in the chromatogram obtained with solution (2) (0.5%). In the chromatogram obtained with solution (1), any *secondary spot* other than any spot corresponding to the spot with the lower Rf value in the chromatogram obtained with solution (3) is not more intense than the proximate spot in the chromatogram obtained with solution (3) (0.2%).

Z-Isomer

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Solution (1) contains 0.50% w/v of *dosulepin hydrochloride BPCRS* in *methanol*. For solution (2) extract a quantity of the mixed contents of 20 capsules containing 25 mg of Dosulepin Hydrochloride with 5 ml of *methanol*, centrifuge and use the supernatant liquid.

The chromatographic procedure may be carried out using a glass column (1.8 m × 3 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225 is suitable) and maintained at 200°.

In the chromatogram obtained with solution (1) a peak due to *Z*-dosulepin is present with a retention time of approximately 0.83 relative to the retention time of the principal peak which is due to *E*-dosulepin. In the chromatogram obtained with solution (2) the area of any peak corresponding to *Z*-dosulepin is not greater than 7.5% of the sum of the areas of the peaks due to *Z*-dosulepin and *E*-dosulepin.

ASSAY

Extract a quantity of the mixed contents of 20 capsules containing 0.5 g of Dosulepin Hydrochloride with 20 ml followed by four 10 ml quantities of *chloroform*, filtering each extract through the same filter. Evaporate the combined extracts to dryness, dissolve the residue in 100 ml of *acetone*, add 10 ml of *mercury(II) acetate solution* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 33.19 mg of $C_{19}H_{21}NS,HCl$.

Dosulepin Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Dosulepin Tablets contain Dosulepin Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dosulepin hydrochloride, $C_{19}H_{21}NS, HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove the coating. Shake a quantity of the powdered tablet cores containing 0.2 g of Dosulepin Hydrochloride with 20 ml of *dichloromethane* , centrifuge, filter the supernatant liquid and evaporate the filtrate to dryness. Dissolve the residue in the minimum quantity of *ethanol (70%)* and add an excess of *ether* . Filter the precipitate, wash with *ether* and dry at 60° at a pressure of 2 kPa for 5 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of dosulepin hydrochloride *(RS 114)* . Examine the substance as a dispersion in *liquid paraffin* .

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 13.5M *ammonia* , 10 volumes of *propan-2-ol* and 90 volumes of *1,2-dichloroethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Dosulepin Hydrochloride for 2 minutes with 5 ml of *dichloromethane* , centrifuge and use the supernatant liquid. For solution (2) dilute 2 ml of solution (1) to 5 ml with *dichloromethane* . Solution (3) contains 0.010% w/v each of *3-(dibenzo*[b,e]*thiepin-11(6H)-ylidene)*-N,N-*dimethylaminopropan-1-amine* S-*oxide hydrochloride BPCRS* and *dibenzo*[b,e]*thiepin-11(6H)-one BPCRS* in *chloroform* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . In the chromatogram obtained with solution (3) the spot with the lower Rf value is more intense than any corresponding spot in the chromatogram obtained with solution (2) (0.5%). In the chromatogram obtained with solution (1) any *secondary spot* other than any spot corresponding to the spot with the lower Rf value in the chromatogram obtained with solution (3) is not more intense than the proximate spot in the chromatogram obtained with solution (3) (0.2%).

Z-Isomer

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Solution (1) contains 0.50% w/v of *dosulepin hydrochloride BPCRS* in *methanol*. For solution (2) shake a quantity of the powdered tablets containing 25 mg of Dosulepin Hydrochloride with 5 ml of *methanol* for 15 minutes, centrifuge and use the supernatant liquid.

The chromatographic procedure may be carried out using a glass column (1.8 m × 3 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225 is suitable) and maintained at 200°.

In the chromatogram obtained with solution (1) a peak due to *Z*-dosulepin is present with a retention time of approximately 0.83 relative to the retention time of the principal peak which is due to *E*-dosulepin. In the chromatogram obtained with solution (2) the area of any peak corresponding to *Z*-dosulepin is not greater than 7.5% of the sum of the areas of the peaks due to *Z*-dosulepin and *E*-dosulepin.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Dosulepin Hydrochloride with 60 ml of 0.1M *hydrochloric acid* for 30 minutes, dilute to 100 ml with 0.1M *hydrochloric acid* and filter. Discard the first 25 ml of the filtrate. Dilute 25 ml of the remaining filtrate to 100 ml with 0.1M *hydrochloric acid*. Solution (2) contains 0.0125% w/v of *dosulepin hydrochloride BPCRS* in 0.1M *hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 10 volumes of *tetrahydrofuran*, 40 volumes of *acetonitrile* and 50 volumes of a 0.5% w/v solution of *potassium dihydrogen orthophosphate*, the pH of the mixture being adjusted to 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 231 nm.

Calculate the content of $C_{19}H_{21}NS,HCl$ using the declared content of $C_{19}H_{21}NS,HCl$ in *dosulepin hydrochloride BPCRS*.

Doxapram Injection

General Notices

Action and use

Respiratory stimulant.

DEFINITION

Doxapram Injection is a sterile solution of Doxapram Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of doxapram hydrochloride, $C_{24}H_{30}N_2O_2,HCl,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume containing 50 mg of Doxapram Hydrochloride add 10 ml of *water* and 2 ml of 1M *sodium hydroxide* and extract with two 10 ml quantities of *ether*. Wash the combined extracts with 5 ml of *water*, dry over *anhydrous sodium sulphate*, filter and evaporate to dryness. Recrystallise the residue from 10 ml of 0.01M *methanolic hydrochloric acid*. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of doxapram hydrochloride *(RS 116)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits maxima at 253, 258 and 265 nm.

C. Yields the reactions characteristic *of chlorides*, Appendix VI.

TEST

Acidity

pH of a solution containing 1% w/v of doxapram hydrochloride, 3.5 to 5.0, Appendix V L.

ASSAY

Dilute a volume containing 0.2 g of Doxapram Hydrochloride to 250 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{24}H_{30}N_2O_2,HCl,H_2O$ in the injection from the *absorbance* of a 0.08% w/v solution of *doxapram hydrochloride BPCRS* using the declared content of $C_{24}H_{30}N_2O_2,HCl,H_2O$ in *doxapram hydrochloride BPCRS.*

STORAGE

Doxapram Injection should not be allowed to freeze.

Doxorubicin Injection

General Notices

Action and use

Anthracycline antibacterial; cytotoxic.

DEFINITION

Doxorubicin Injection is a sterile solution of Doxorubicin Hydrochloride in Water for Injections or Sodium Chloride Injection. It is either supplied as a ready-to-use solution or it is prepared by dissolving Doxorubicin Hydrochloride for Injection in the requisite amount of Water for Injections or Sodium Chloride Injection.

The injection complies with the requirements stated under Parenteral Preparations and, when supplied as a ready-to-use solution, with the following requirements.

Content of doxorubicin hydrochloride, $C_{27}H_{29}NO_{11},HCl$

95.0 to 110.0% of the stated amount.

CHARACTERISTICS

A red solution.

IDENTIFICATION

A. Dilute the injection with sufficient *ethanol (96%)* to produce a solution containing 0.001% w/v of Doxorubicin Hydrochloride. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 550 nm exhibits two maxima, at 234 and 252 nm, and four less clearly defined maxima at 288, 475, 495 and 530 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.5 to 3.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. The solutions should be prepared immediately before use. For solution (1) dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing

0.1% w/v of Doxorubicin Hydrochloride. Solution (2) contains 0.002% w/v of each of *doxorubicin hydrochloride EPCRS* and *epirubicin hydrochloride EPCRS* in the mobile phase. For solution (3) dissolve 10 mg of *doxorubicin hydrochloride EPCRS* in a mixture of 5 ml of *water* and 5 ml of *orthophosphoric acid* and allow to stand for 30 minutes. Adjust the pH to 2.6 with an 8% w/v solution of *sodium hydroxide*, add 15 ml of *acetonitrile* and 10 ml of *methanol* and mix (generation of doxorubicin aglycone [doxorubicinone]). For solution (4) dilute 5 volumes of solution (2) to 20 volumes with the mobile phase. Solution (5) contains 0.001% w/v of *methyl 4-hydroxybenzoate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 5 µm) is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.288% w/v of *sodium dodecyl sulphate* and 0.225% w/v of *orthophosphoric acid* and (c) a detection wavelength of 254 nm. Inject 10 µl of each solution.

When the chromatograms are recorded under the prescribed conditions the retention time of doxorubicin is about 8 minutes. For solution (1) allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to doxorubicin and epirubicin is at least 2.0.

Use solution (3) to identify the peak due to doxorubicin aglycone (second most abundant peak). In the chromatogram obtained with solution (1) the area of any peak corresponding to doxorubicin aglycone is not greater than six times the area of the peak due to doxorubicin in the chromatogram obtained with solution (4) (3%) and the area of any other *secondary peak* is not greater than the area of the peak due to doxorubicin in the chromatogram obtained with solution (4) (0.5%). Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (5).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 2 mg of Doxorubicin Hydrochloride per ml (solution A). The endotoxin limit concentration of solution A is 4.4 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be prepared immediately before use. For solution (1) dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing 0.01% w/v of Doxorubicin Hydrochloride. Solution (2) contains 0.01% w/v of *doxorubicin hydrochloride EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 5 µm is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.288% w/v of *sodium dodecyl sulphate* and 0.225% w/v of *orthophosphoric acid* and (c) a detection wavelength of 254 nm. Inject 10 µl of each solution.

Calculate the content of $C_{27}H_{29}NO_{11},HCl$ in the injection using the declared content of

$C_{27}H_{29}NO_{11}$,HCl in *doxorubicin hydrochloride EPCRS*.

STORAGE

When supplied as a ready-to-use solution, Doxorubicin Injection should be stored at a temperature of 2° to 8°.

Doxorubicin Injection prepared by dissolving the contents of a sealed container in the liquid stated on the label should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

DOXORUBICIN HYDROCHLORIDE FOR INJECTION

DEFINITION

Doxorubicin Hydrochloride for Injection is a sterile material consisting of Doxorubicin Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of doxorubicin hydrochloride, $C_{27}H_{29}NO_{11}$,HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powder containing 10 mg of Doxorubicin Hydrochloride with 5 ml of 0.01M *hydrochloric acid*, extract 2 ml of the resulting solution with two 5 ml quantities of *ether* and discard the ether layer. Dilute the aqueous solution with *ethanol (96%)* to contain 0.001% w/v of doxorubicin hydrochloride. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 550 nm exhibits two maxima, at 234 and 252 nm, and four less clearly defined maxima at 288, 475, 495 and 530 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a solution containing 0.2% w/v of Doxorubicin Hydrochloride in *Water for Injections*, 4.5 to 6.5, Appendix V L.

Related substances

Complies with the test described for the ready-to-use solution but preparing solution (1) in the following manner. Dissolve a quantity of the powder in sufficient of the mobile phase to produce a solution containing 0.1% w/v of Doxorubicin Hydrochloride.

In the chromatogram obtained with solution (1) the area of any peak corresponding to doxorubicin aglycone is not greater than twice the area of the peak due to doxorubicin in the chromatogram obtained with solution (4) (1%) and the area of any other *secondary peak* is not greater than the area of the peak due to doxorubicin in the chromatogram obtained with solution (4) (0.5%). Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (5).

Water

Not more than 4.0% w/w, Appendix IX C. Use 0.1 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 10 mg of Doxorubicin Hydrochloride per ml (solution A). The endotoxin limit concentration of solution A is 22 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be prepared immediately before use. For solution (1) dissolve a quantity of the mixed contents of the 10 containers in sufficient of the mobile phase to produce a solution containing 0.01% w/v of Doxorubicin Hydrochloride. Solution (2) contains 0.01% w/v of *doxorubicin hydrochloride EPCRS* in the mobile phase.

The chromatographic procedure described under the Assay for the ready-to-use solution may be used.

Calculate the content of $C_{27}H_{29}NO_{11}$,HCl in a container of average content weight using the declared content of $C_{27}H_{29}NO_{11}$,HCl in *doxorubicin hydrochloride EPCRS*.

Doxycycline Capsules

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Doxycycline Capsules contain Doxycycline Hyclate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of anhydrous doxycycline, $C_{22}H_{24}N_2O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 6 volumes of *water,* 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Spray the plate evenly with a 10% w/v solution of *disodium edetate* the pH of which has been adjusted to 9.0 with 10M *sodium hydroxide* (about 10 ml for a 100 mm × 200 mm plate). Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 50 mg of anhydrous doxycycline with 100 ml of *methanol* for 1 to 2 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.05% w/v of *doxycycline hyclate BPCRS* in *methanol*. Solution (3) contains 0.05% w/v each of *doxycycline hyclate BPCRS* and *tetracycline hydrochloride BPCRS* in *methanol*. After removal of the plate, dry it in a current of air and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. To 0.5 mg of the contents of the capsules add 2 ml of *sulphuric acid*. A yellow colour is produced.

C. The contents of the capsules yield the reactions characteristic *of chlorides*, Appendix VI.

TESTS

Light-absorbing impurities

Dissolve the contents of five capsules as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0% w/v of anhydrous doxycycline and filter. The *absorbance* of the filtrate at 490 nm is not more than 0.20, calculated with reference to the dried capsule contents, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use. For solution (1) dissolve a quantity of the contents of the capsules containing the equivalent of 7.0 mg of anhydrous doxycycline in 10 ml of 0.01M *hydrochloric acid*, filter and use the filtrate. Solutions (2) to (5) are solutions in 0.01M *hydrochloric acid* containing (2) 0.080% w/v of *doxycycline hyclate BPCRS*, (3) 0.080% w/v of *6-epidoxycycline hydrochloride EPCRS*, (4) 0.080% w/v of *metacycline hydrochloride EPCRS* and (5) 0.0016% w/v each of *6-epidoxycycline hydrochloride EPCRS* and *metacycline hydrochloride EPCRS*. For solution (6) dilute a mixture of 4 volumes of solution (2), 1.5 volumes of solution (3) and 1 volume of solution (4) to 25 volumes with 0.01M *hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene co-polymer* (8 µm) with a pore size of 10 nm (PLRP-S from Polymer Laboratories is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared as described below and (c) a detection wavelength of 254 nm. For the mobile phase add 60 g of *2-methylpropan-2-ol* to a graduated flask with the aid of 200 ml of *water*, add 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* previously adjusted to pH 8.0 with 2M *sodium hydroxide* and 10 ml of a 4% w/v solution of *disodium edetate* previously adjusted to pH 8.0 with 2M *sodium hydroxide* and dilute to 1000 ml with *water*. Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the first peak (metacycline) and the second peak (6-epidoxycycline) is at least 1.25 and the *resolution factor* between the second peak and the third peak (doxycycline) is at least 2.0. If necessary, adjust the content of 2-methylpropan-2-ol in the mobile phase.

In the chromatogram obtained with solution (1) the area of any peak corresponding to metacycline or 6-epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with solution (5) (2%, with reference to doxycycline hyclate) and the area of any other *secondary peak* is not greater than 0.25 times the area of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with solution (5) (0.5%, with reference to doxycycline hyclate).

Loss on drying

When dried at 105° for 2 hours, the contents of the capsules lose not more than 8.5% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the mixed contents of 20 capsules containing the equivalent of 17.5 mg of anhydrous doxycycline in sufficient 0.01M *hydrochloric acid* to produce 25 ml and dilute 4 ml of the solution to 25 ml with the same solvent. Solution (2) contains 0.0128% w/v of *doxycycline hyclate BPCRS* in 0.01M *hydrochloric acid*.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{22}H_{24}N_2O_8$ using the declared content of $C_{22}H_{24}N_2O_8$ in *doxycycline hyclate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous doxycycline.

Droperidol Injection

General Notices

Action and use

Dopamine receptor antagonist; beta$_1$-adrenoceptor agonist; alpha-adrenoceptor agonist neuroleptic.

DEFINITION

Droperidol Injection is a sterile solution of Droperidol in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of droperidol, $C_{22}H_{22}FN_3O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to droperidol in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a volume of the injection containing 10 mg of Droperidol with two successive 10 ml quantities of *dichloromethane* and use the combined extracts. Solution (2) contains 0.05% w/v of *droperidol BPCRS* in *dichloromethane.* After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* . The principal spot in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.5 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, with a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* to give a solution containing 0.5% w/v of Droperidol. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* and dilute 5 volumes of this solution to 20 volumes with the same solvent mixture. Solution (3) contains 0.0025% w/v each of *droperidol BPCRS* and *benperidol EPCRS* in a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulphate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil C18 BDS 3µ is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* changing by linear gradient to a mixture of 60 volumes of a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* and 40 volumes of *acetonitrile* over 15 minutes, followed by the final mixture for 5 minutes and (c) a detection wavelength of 275 nm. Equilibrate the column with *acetonitrile* for at least 30 minutes before use and then equilibrate at the initial mobile phase composition for at least 30 minutes.

Inject 20 µl of solution (3). When the chromatogram is recorded under the prescribed conditions, the retention times for benperidol and droperidol are about 6.5 minutes and about 7 minutes respectively. The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the peaks due to droperidol and benperidol is at least 2.0. If necessary, adjust the final concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient.

When solution (1) is prepared by diluting Droperidol Injection inject 20 µl of a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* as a blank solution. When solution (1) is the undiluted injection, inject 20 µl of *water* as a blank solution. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak obtained with the blank solution and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, with *methanol (50%)* to give a solution containing 0.010% w/v of Droperidol. Solution (2) contains 0.010% w/v of *droperidol BPCRS* in *methanol (50%)*. Solution (3) contains 0.0025% w/v of each of *droperidol BPCRS* and *benperidol EPCRS* in *methanol (50%)*.

The chromatographic procedure described under Related substances may be used.

Inject 20 µl of solution (3). When the chromatogram is recorded under the prescribed conditions, the retention times for benperidol and droperidol are about 6.5 minutes and about 7 minutes respectively. The assay is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the peaks due to droperidol and benperidol is at least 2.0. If necessary, adjust the final concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient.

Calculate the content of $C_{22}H_{22}FN_3O_2$ in the injection using the declared content of $C_{22}H_{22}FN_3O_2$ in *droperidol BPCRS*.

Droperidol Tablets

General Notices

Action and use

Dopamine receptor antagonist; $beta_1$-adrenoceptor agonist; alpha-adrenoceptor agonist; neuroleptic.

DEFINITION

Droperidol Tablets contain Droperidol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of droperidol, $C_{22}H_{22}FN_3O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Droperidol with 10 ml of *dichloromethane* for 10 minutes, filter, evaporate the filtrate to dryness and dry the residue for 30 minutes at 90°. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of droperidol (*RS 384*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to droperidol in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter through a 15-µm membrane filter and immediately measure the *absorbance* of the filtered solution, diluted if necessary with the dissolution medium, at the maximum at 247 nm, Appendix II B, using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *droperidol BPCRS* in dissolution medium and calculate the total content of droperidol, $C_{22}H_{22}FN_3O_2$, in the medium using the declared content of $C_{22}H_{22}FN_3O_2$ in *droperidol BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Droperidol with 20 ml of *dimethylformamide* for 20 minutes, filter and dilute 5 volumes of the filtrate to 10 volumes with a 1% w/v solution of *tetrabutylammonium hydrogen sulphate*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture containing equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* and further dilute 5 volumes of this solution to 20 volumes with the same solvent mixture. Solution (3) contains 0.0025% w/v each of *droperidol BPCRS* and *benperidol EPCRS* in a mixture containing equal volumes of *dimethylformam*ide and a 1% w/v solution of *tetrabutylammonium hydrogen sulphate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil C18 BDS 3µ is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* changing by linear gradient to a mixture of 60 volumes of a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* and 40 volumes of *acetonitrile* over 15 minutes, followed by the final mixture for 5 minutes and (c) a detection wavelength of 275 nm. Equilibrate the column with *acetonitrile* for at least 30 minutes before use and then equilibrate at the initial mobile phase composition for at least 30 minutes.

Inject 20 µl of solution (3). When the chromatogram is recorded under the prescribed conditions, the retention times for benperidol and droperidol are about 6.5 minutes and about 7 minutes respectively. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to droperidol and benperidol is at least 2.0. If necessary, adjust the final concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient.

Inject 20 µl of a mixture of equal volumes of *dimethylformamide* and a 1% w/v solution of *tetrabutylammonium hydrogen sulphate* as a blank solution. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak obtained with the blank solution and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Droperidol with 75 ml of *methanol* for 30 minutes, dilute to 100 ml with *methanol*, filter and dilute 10 ml of the resulting solution to 20 ml with *water*. Solution (2) contains 0.010% w/v of *droperidol BPCRS* in *methanol (50%)*. Solution (3) contains 0.0025% w/v each of *droperidol BPCRS* and *benperidol EPCRS* in *methanol (50%)*.

The chromatographic procedure described under Related substances may be used.

Inject 20 µl of solution (3). When the chromatogram is recorded under the prescribed conditions, the retention times for benperidol and droperidol are about 6.5 minutes and about 7 minutes respectively. The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to droperidol and benperidol is at least 2.0. If necessary, adjust the final concentration of acetonitrile in the mobile phase or adjust the time programme for the linear gradient.

Calculate the content of $C_{22}H_{22}FN_3O_2$ in the tablets using the declared content of $C_{22}H_{22}FN_3O_2$ in *droperidol BPCRS*.

Dydrogesterone Tablets

General Notices

Action and use

Progestogen.

DEFINITION

Dydrogesterone Tablets contain Dydrogesterone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of dydrogesterone, $C_{21}H_{28}O_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 60 mg of Dydrogesterone with 20 ml of *methanol* , filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of dydrogesterone *(RS 118)* .

B. Complies with the test for *identification of steroids* , Appendix III A, using *impregnating solvent II* and *mobile phase E*.

TESTS

Related substances

Comply with the test described under Dydrogesterone using the following solutions. For solution (1) extract a quantity of the powdered tablets containing 20 mg of Dydrogesterone with 50 ml of a 0.020% w/v solution of *1,2,3,4-tetraphenylcyclopenta-1,3-diene* (internal standard) in *chloroform* and filter. For solution (2) extract a quantity of the powdered tablets containing 20 mg of Dydrogesterone with 20 ml of *chloroform* , filter, evaporate to dryness using a rotary evaporator and dissolve the residue in 1 ml of *chloroform* . Prepare solution (3) in the same manner as solution (2) but extracting the powdered tablets with 20 ml of a 0.0010% w/v solution of the internal standard in *chloroform* .

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 10 mg of Dydrogesterone for 15 minutes with 70 ml of *methanol* , dilute to 200 ml with *methanol* and filter. Dilute 5 ml of the filtrate to 100 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 286 nm, Appendix II B. Calculate the content of $C_{21}H_{28}O_2$ taking 845 as the value of *A* (1%, 1 cm) at the maximum at 286 nm.

STORAGE

Dydrogesterone Tablets should be protected from light.

Gastro-resistant Diclofenac Tablets

General Notices

Diclofenac Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Gastro-resistant Diclofenac Tablets contain Diclofenac Sodium. They are made gastro-resistant by enteric-coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove the coating from 10 tablets and powder the cores. Add 0.5 ml of *glacial acetic acid* and 15 ml of *methanol* to a quantity of the powdered tablet cores containing 0.15 g of Diclofenac Sodium and mix with the aid of ultrasound. Shake gently for 1 minute, filter and collect the filtrate in 15 ml of *water*. Filter the precipitate under reduced pressure (Whatman GF/C filter paper is suitable), wash with four 5 ml quantities of *water* and dry at 105° for 2 to 3 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of diclofenac *(RS 096)*.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Diclofenac Sodium with 70 ml of the mobile phase for 30 minutes, add sufficient of the mobile phase to produce 100 ml, mix, centrifuge an aliquot and filter the supernatant liquid through a 0.45-µm filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 5 volumes with the mobile phase. Solution (3) contains 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (end-capped Zorbax C8 is suitable), (b) as mobile phase with a flow rate of 1 ml per minute a mixture of 34 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 66 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Inject solution (3). When the chromatograms are recorded in the prescribed conditions, the retention times are about 25 minutes for diclofenac and about 12 minutes for diclofenac impurity A. Continue the chromatography for 1.5 times the retention time of diclofenac. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

Inject solution (1) and solution (2). In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peaks with retention times relative to the principal peak of 0.67 and 0.1.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 10 tablets with 700 ml of *methanol (50%)* for 30 minutes with the aid of ultrasound, add sufficient mobile phase to produce 1000 ml, mix, centrifuge an aliquot and filter the supernatant liquid through a 0.45-µm filter. Dilute the filtrate with the mobile phase to produce a solution containing 0.005% w/v of Diclofenac Sodium. Solution (2) contains 0.005% w/v of *diclofenac sodium BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A EPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the tablets from the chromatograms obtained and from the declared content of $C_{14}H_{10}Cl_2NNaO_2$ in *diclofenac sodium BPCRS*.

STORAGE

Gastro-resistant Diclofenac Tablets should be protected from moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for diclofenac sodium.

Paediatric Digoxin Injection

General Notices

Action and use

Na /K-ATPase inhibitor; cardiac glycoside.

DEFINITION

Digoxin	10 mg
Ethanol (80 per cent)	12.5 ml
Propylene Glycol	40 ml
Citric Acid Monohydrate	75 mg
Disodium Hydrogen Phosphate Dodecahydrate	0.45 g
Water for Injections	Sufficient to produce 100 ml

Extemporaneous preparation

The following directions apply.

Dissolve the digoxin in the ethanol (80 per cent) and add the propylene glycol, a solution of the citric acid monohydrate and the disodium hydrogen phosphate dodecahydrate in Water for Injections and sufficient Water for Injections to produce 100 ml. Sterilise by *heating in an autoclave.*

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of digoxin, $C_{41}H_{64}O_{14}$

0.0090 to 0.0110% w/v.

IDENTIFICATION

Evaporate 5 ml to dryness, dissolve the residue in 1 ml of *glacial acetic acid* containing 0.01% w/v of *iron(III) chloride* and cautiously add 1 ml of *sulphuric acid* without mixing. A brown ring, free from red colour, is produced at the interface and, after a short time, an indigo colour is produced in the upper layer.

Acidity or alkalinity

pH, 6.7 to 7.3, Appendix V L.

ASSAY

Carry out the Assay described under Digoxin Injection, using 50 ml.

STORAGE

Paediatric Digoxin Injection should be protected from light.

Paediatric Digoxin Oral Solution

General Notices

Action and use

Na /K-ATPase inhibitor; cardiac glycoside.

DEFINITION

Paediatric Digoxin Oral Solution is a solution containing 0.005% w/v of Digoxin in a suitable flavoured vehicle.

Paediatric Digoxin Oral Solution should not be diluted.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of digoxin, $C_{41}H_{64}O_{14}$

0.0045 to 0.0055% w/v.

IDENTIFICATION

Extract 5 ml with four 20 ml quantities of *chloroform* , washing each extract with the same 10 ml of *water*, evaporate the combined extracts to dryness and dissolve the residue in 1 ml of *glacial acetic acid* containing 0.01% w/v of *iron (III) chloride hexahydrate.* Cautiously add 1 ml of *sulphuric acid* without mixing. A brown ring, free from red colour, is produced at the interface and, after a short time, an indigo colour is produced in the upper layer.

Acidity or alkalinity

pH, 6.8 to 7.2, Appendix V L.

ASSAY

Extract 100 ml with four 25 ml quantities of *chloroform* , washing each extract with the same 5 ml of *water*, and evaporate the combined extracts to dryness. To the residue add 3 ml of *absolute ethanol* and carefully evaporate to dryness on a water bath with the aid of a gentle current of air. Repeat the evaporation using a further 3 ml of *absolute ethanol* and cool. Dissolve the residue in 5 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* , add 20 ml of *glacial acetic acid* and filter if necessary. Dilute 5 ml of the filtrate to 25 ml with *digoxin reagent* , allow to stand for 2 hours and measure the *absorbance* of the resulting solution at the maximum at 590 nm, Appendix II B. Calculate the content of $C_{41}H_{64}O_{14}$ from the *absorbance* obtained by carrying out the operation at the same time but using a solution prepared by mixing 5 ml of a 0.2% w/v solution of *digoxin EPCRS* in *glacial acetic acid* with 10 ml of a mixture of 65 volumes *chloroform* and 35 volumes of *methanol* and adding sufficient *glacial acetic acid* to produce 50 ml, beginning at the words 'Dilute 5 ml of the filtrate …' and using *water* in the reference cell.

STORAGE

Paediatric Digoxin Oral Solution should be protected from light.

Paediatric Docusate Oral Solution

General Notices

Action and use

Stimulant laxative; faecal softener.

DEFINITION

Paediatric Docusate Oral Solution contains 0.25% w/v of Docusate Sodium in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of docusate sodium, $C_{20}H_{37}NaO_7S$

0.238 to 0.263% w/v.

IDENTIFICATION

Complies with the tests described under Docusate Oral Solution.

ASSAY

Carry out the Assay described under Docusate Oral Solution but preparing solution (1) in the following manner. For solution (1) pass a weighed quantity of the oral solution containing 5 mg of docusate sodium, with the aid of vacuum, through a solid-phase extraction column of 1 ml capacity and containing 0.1 g of an *octadecyl-bonded silica sorbent* (a Sep-pak C18 column is suitable) previously washed with 2 ml of *methanol* , followed by 5 ml of *water*. Wash the column with 2 ml of *water*, discarding the aqueous eluate. Elute the docusate sodium with 4 ml of *methanol* and dilute to 10 ml with a mixture of equal volumes of *acetonitrile* and *water*.

Prolonged-release Diclofenac Capsules

General Notices

Prolonged-release Diclofenac Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Prolonged-release Diclofenac Capsules contain Diclofenac Sodium. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Diclofenac Sodium. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Add 0.5 ml of *glacial acetic acid* and 15 ml of *methanol* to a quantity of the powdered capsule contents containing 0.15 g of Diclofenac Sodium and mix with the aid of ultrasound for 40 minutes. Shake gently for 1 minute, filter and collect the filtrate in 15 ml of *water*. Filter the precipitate (Whatman GF/C is suitable) under reduced pressure, wash with four 5-ml quantities of *water* and dry at 105° for 2 to 3 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of diclofenac *(RS 096)* .

TESTS

RELATED SUBSTANCES

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

To 30 volumes of *acetonitrile for chromatography* add 70 volumes of *water* (solvent A).

(1) Add 30 ml of solvent A to a quantity of the powdered contents of the capsules containing 0.1 g of Diclofenac Sodium and mix with the aid of ultrasound for 10 minutes with occasional shaking. Cool, add sufficient solvent A to produce 50 ml and filter (Whatman GF/C is suitable).

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A and dilute 1 volume of this solution to 5 volumes with solvent A.

(3) 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A EPCRS* in solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP Select B or equivalent is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 230 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 5 volumes of *tetrahydrofuran*, 30 volumes of *acetonitrile* and 65 volumes of 0.05M *ammonium dihydrogen orthophosphate,* previously adjusted to pH 5.0 using 18M *ammonia.*

When the chromatograms are recorded under the prescribed conditions, the retention times are about 18 minutes for diclofenac and about 26 minutes for diclofenac impurity A. Continue the chromatography for 5 times the retention time of diclofenac.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

— the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and adjust the pH to 6.8 with 1M *sodium hydroxide* (solution A). To a quantity of the mixed contents of 20 capsules containing 100 mg of Diclofenac Sodium add 10 ml of *ethanol (96%)* and mix with the aid of ultrasound for 20 minutes or until completely dispersed. Add 150 ml of solution A and mix with the aid of ultrasound for a further 20 minutes or until completely dispersed. Cool to room temperature, dilute to 250 ml with solution A and shake thoroughly. Filter the resulting solution and dilute 5 ml to 100 ml with solution A. Prepare a reference standard in the following manner. Dissolve 50 mg of *diclofenac sodium BPCRS* in 10 ml of *ethanol (96%)* with the aid of ultrasound for 5 minutes. Add 150 ml of solution A and mix with the aid of ultrasound for a further 5 minutes. Cool to room temperature, dilute to 250 ml with solution A and shake thoroughly. Dilute 5 ml of the resulting solution to 50 ml with solution A. Measure the *absorbance* , Appendix II B, of the solutions at 275 nm using in the reference cell a 0.4% v/v solution of *ethanol (96%)* in solution A.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the capsules using the *absorbances* at the maximum at 275 nm and the declared content of $C_{14}H_{10}Cl_2NNaO_2$ in *diclofenac sodium BPCRS* .

STORAGE

Prolonged-release Diclofenac Capsules should be protected from moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Diclofenac Sodium.

Prolonged-release Diclofenac Tablets

General Notices

Slow Diclofenac Tablets

Prolonged-release Diclofenac Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Prolonged-release Diclofenac Tablets contain Diclofenac Sodium. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of diclofenac sodium. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove the coating from 10 tablets and powder the cores. Add 0.5 ml of *glacial acetic acid* and 15 ml of *methanol* to a quantity of the powdered tablet cores containing 0.15 g of Diclofenac Sodium and mix with the aid of ultrasound. Shake gently for 1 minute, filter and collect the filtrate in 15 ml of *water*. Filter the precipitate under reduced pressure (Whatman GF/C filter paper is suitable), wash with four 5-ml quantities of *water* and dry at 105° for 2 to 3 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of diclofenac *(RS 096)*.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Diclofenac Sodium with 70 ml of the mobile phase for 30 minutes, add sufficient of the mobile phase to produce 100 ml, mix, centrifuge an aliquot and filter the supernatant liquid through a 0.45-µm filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 5 volumes with the mobile phase. Solution (3) contains 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (end-capped Zorbax C8 is suitable), (b) as mobile phase with a flow rate of 1 ml per minute a mixture of 34 volumes of a mixture of equal volumes of a 0.1% w/v solution of *orthophosphoric acid* and a 0.16% w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 66 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Inject solution (3). When the chromatograms are recorded in the prescribed conditions, the retention times are about 25 minutes for diclofenac and about 12 minutes for diclofenac impurity A. Continue the chromatography for 1.5 times the retention time of diclofenac. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

Inject solution (1) and solution (2). In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 10 tablets with 800 ml of *methanol (50%)* for 30 minutes, add sufficient of the mobile phase to produce 1000 ml, mix, centrifuge an aliquot and filter the supernatant liquid through a 0.45-µm filter. Dilute the resulting solution with the mobile phase to produce a solution containing 0.005% w/v of Diclofenac Sodium. Solution (2) contains 0.005% w/v of *diclofenac sodium BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *diclofenac sodium BPCRS* and 0.0005% w/v of *diclofenac impurity A EPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to diclofenac and diclofenac impurity A is at least 6.5.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the tablets from the chromatograms obtained and from the declared content of $C_{14}H_{10}Cl_2NNaO_2$ in *diclofenac sodium BPCRS*.

STORAGE

Prolonged-release Diclofenac Tablets should be protected from moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Diclofenac Sodium.

Econazole Cream

General Notices

Action and use

Antifungal.

DEFINITION

Econazole Cream contains Econazole Nitrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of econazole nitrate, $C_{18}H_{15}Cl_3N_2O,HNO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the cream containing 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of 1M *sulphuric acid* and 4 volumes of *methanol* and shake with two 50-ml quantities of *carbon tetrachloride* discarding the organic layers. Make the aqueous phase alkaline with 2M *ammonia* and extract with two 40-ml quantities of *chloroform* . Combine the *chloroform* extracts, shake with 5 g of *anhydrous sodium sulphate* , filter and dilute the filtrate to 100 ml with *chloroform.* Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *propan-2-ol* . The *light absorption* of the resulting solution, Appendix II B, in the range 240 to 350 nm exhibits maxima at 265, 271 and 280 nm. The ratio of the *absorbance* at the maximum at 271 nm to that at the maximum at 280 nm is 1.55 to 1.77. The test is not valid unless the ratio of the *absorbance* in the test for *resolution* is at least 2.

B. In the Assay, the principal peak in the chromatogram obtained with solution (3) has the same retention time as the peak due to econazole in the chromatogram obtained with solution (1).

ASSAY

Prepare a phosphate buffer solution by dissolving 2.5 g of *potassium dihydrogen orthophosphate* and 2.5 g of *dipotassium hydrogen orthophosphate* in 1000 ml of *water*. Prepare a 0.05% w/v solution of miconazole nitrate (internal standard) in *methanol* (solution A). Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. Solution (1) is a mixture of 10 ml of a 0.1% w/v solution of *econazole nitrate BPCRS* in *methanol,* 20 ml of solution A, 45 ml of *methanol* and 25 ml of phosphate buffer solution. For solution (2) mix a quantity of the cream containing 10 mg of Econazole Nitrate with 20 ml of solution (A) and 55 ml of *methanol* , warm on a water-bath for 30 seconds, shake for 1 minute, repeat the process twice and add 25 ml of phosphate buffer solution. Cool in an ice bath for 15 minutes, centrifuge for 10 minutes and use the supernatant liquid, filtered if necessary. Prepare solution (3) in the same manner as solution (2) but using 20 ml of *methanol* in place of solution A.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 2.0 ml per minute a mixture of 1 volume of phosphate buffer solution and 3 volumes of *methanol* and (c) a detection wavelength of 232 nm.

Calculate the content of $C_{18}H_{15}Cl_3N_2O,HNO_3$ in the cream using the declared content of $C_{18}H_{15}Cl_3N_2O,HNO_3$ in *econazole nitrate BPCRS*.

STORAGE

If Econazole Cream is kept in aluminium tubes, their inner surfaces should be coated with a suitable lacquer.

Econazole Pessaries

General Notices

Action and use

Antifungal.

DEFINITION

Econazole Pessaries are either moulded pessaries or *vaginal tablets* containing Econazole Nitrate in a suitable basis.

The pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of econazole nitrate, $C_{18}H_{15}Cl_3N_2O,HNO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the crushed pessaries containing 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of 1M *sulphuric acid* and 4 volumes of *methanol* and shake with two 50-ml quantities of *hexane* , discarding the organic layers. Make the aqueous phase alkaline with 2M *ammonia* and extract with two 40-ml quantities of *chloroform* . Combine the *chloroform* extracts, shake with 5 g of *anhydrous sodium sulphate* , filter and dilute the filtrate to 100 ml with *chloroform* . Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *propanol-2-ol*. The *light absorption* of the resulting solution, Appendix II B, in the range 240 to 350 nm exhibits maxima at 265, 271 and 280 nm. The ratio of the *absorbance* at the maximum at 271 nm to that at the maximum at 280 nm is 1.55 to 1.77. The test is not valid unless the ratio of the *absorbance* in the test for *resolution* is at least 2.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (3).

TEST

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 70 volumes of *dichloromethane* , 20 volumes of *methanol* and 10 volumes of an 85% w/v solution of *formic acid* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) mix a quantity of the crushed pessaries containing 40 mg of Econazole Nitrate with 40 ml of *methanol* and heat under a reflux condenser for 15 minutes. Allow to cool, filter (Whatman No. 1 paper is suitable), wash the filter paper with *methanol* and evaporate the filtrate and washings to a volume of about 5 ml. Filter (Whatman No. 50

paper is suitable), wash the paper with *methanol*, evaporate the filtrate and washings to dryness and dissolve the residue in 2 ml of *methanol*. For solution (2) dilute 0.5 ml of solution (1) to 100 ml with *methanol*. Solution (3) contains 2% w/v of *econazole nitrate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and expose to iodine vapour for 1 hour. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Disregard any spot with an Rf value higher than 0.9.

ASSAY

Dissolve five pessaries in 250 ml of *anhydrous acetic acid* with the aid of gentle heat, allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using a quantity of the solution containing 0.3 g of Econazole Nitrate and determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 44.47 mg of $C_{18}H_{15}Cl_3N_2O,HNO_3$.

Edrophonium Injection

General Notices

Action and use

Cholinesterase inhibitor.

DEFINITION

Edrophonium Injection is a sterile solution of Edrophonium Chloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of edrophonium chloride, $C_{10}H_{16}ClNO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a sufficient volume of the injection with 0.1M *sodium hydroxide* to give a solution containing 0.001% w/v of Edrophonium Chloride. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 350 nm exhibits two maxima, at 240 nm and 294 nm. The *absorbances* at the maxima are about 1.1 and about 0.34 respectively.

B. To a volume containing 20 mg of Edrophonium Chloride add 0.05 ml of *iron(III) chloride solution R1*. A reddish violet colour is produced.

C. Yields reaction A characteristic *of chlorides*, Appendix VI.

TESTS

Acidity

pH, 5.0 to 6.0, Appendix V L.

Dimethylaminophenol

To a volume containing 50 mg of Edrophonium Chloride add 10 ml of *phosphate buffer pH 8.0* and extract with two 20 ml quantities of *chloroform*. Wash the extracts successively with two 10 ml quantities of *water*, extract with 10 ml of 0.1M *sodium hydroxide* and discard the chloroform. The *absorbance* of the resulting solution at 293 nm is not more than 0.125, Appendix II B.

ASSAY

To a volume containing 50 mg of Edrophonium Chloride add sufficient *water* to produce 100 ml. Dilute 10 ml of this solution to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_{10}H_{16}ClNO$ taking 110 as the value of *A* (1%, 1 cm) at the maximum at 273 nm.

STORAGE

Edrophonium Injection should be protected from light.

Emulsifying Ointment

General Notices

DEFINITION

Emulsifying Wax	300 g
White Soft Paraffin	500 g
Liquid Paraffin	200 g

Extemporaneous preparation

The following directions apply.

Melt together and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Emulsifying Wax

General Notices

Anionic Emulsifying Wax

DEFINITION

Emulsifying Wax contains Cetostearyl Alcohol and either Sodium Lauryl Sulphate or sodium salts of similar sulphated higher primary aliphatic alcohols.

Extemporaneous preparation

The following formula and directions apply.

Cetostearyl Alcohol	90 g
Sodium Lauryl Sulphate	10 g
Purified Water	4 ml

Melt the Cetostearyl Alcohol and heat to about 95°. Add the Sodium Lauryl Sulphate, mix, add the Purified Water, heat to 115° and maintain at this temperature, stirring vigorously, until frothing ceases and the product is translucent. Cool quickly.

CHARACTERISTICS

An almost white or pale yellow, waxy solid or flakes, becoming plastic when warmed.

Practically insoluble in *water*, forming an emulsion; partly soluble in *ethanol (96%)* .

IDENTIFICATION

A. *Melting point* of the residue obtained in the test for Unsaponifiable matter, about 52°, Appendix V A.

B. Complies with the test for sulphated ash.

TESTS

Acidity

To 20 g add a mixture of 40 ml of *ether* and 75 ml of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and warm gently until solution is effected. Titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator until a pink colour which persists for at least 15 seconds is obtained. Not more than 1.0 ml of 0.1M *sodium hydroxide VS* is required.

Alkalinity

Disperse 5.0 g in 25 ml of warm *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and cool. No colour is produced on the addition of 0.5 ml of *phenolphthalein solution R1* .

Alcohols

To 3.5 g of the residue obtained in the test for Unsaponifiable matter add 12 g of *stearic anhydride* and 10 ml of *xylene* and heat gently under a reflux condenser for 30 minutes. Cool, add a mixture of 40 ml of *pyridine* and 4 ml of *water*, heat under a reflux condenser for a further 30 minutes and titrate the hot solution with 1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Repeat the operation without the residue. The difference between the titrations is 12.8 to 14.2 ml.

Iodine value

Not more than 3.0 *(iodine monochloride method)*, Appendix X E.

Saponification value

Not more than 2.0, Appendix X G. Use 20 g.

Sodium alkyl sulphates

Not less than 8.7%, calculated as $C_{12}H_{25}O_4SNa$ with reference to the anhydrous substance, when determined by the following method. Dissolve 0.25 g as completely as possible in 15 ml of *chloroform* , add 30 ml of *water*, 10 ml of 1M *sulphuric acid* and 1 ml of *dimethyl yellow and oracet blue B solution* and titrate with 0.004M *benzethonium chloride VS*, shaking vigorously and allowing the layers to separate after each addition, until the chloroform layer acquires a permanent clear green colour. Each ml of 0.004M *benzethonium chloride VS* is equivalent to 1.154 mg of $C_{12}H_{25}O_4SNa$.

Sulphated ash

1.8 to 3.3%, Appendix IX A.

Unsaponifiable matter

Not less than 86.0%, calculated with reference to the anhydrous substance, Appendix X H. Use 5 g and omit the titration of the residue.

Water

Not more than 4.0% w/w, Appendix IX C. Use 0.6 g.

Enalapril Tablets

General Notices

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Enalapril Tablets contain Enalapril Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of enalapril maleate, $C_{20}H_{28}N_2O_5,C_4H_4O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 HPTLC plates are suitable) and a mixture of 15 volumes of *glacial acetic acid*, 25 volumes of *water* and 60 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Enalapril Maleate with 10 ml of *ethanol (90%)* for 10 minutes, centrifuge and use the clear supernatant liquid (if necessary, filter through a 0.45 µm membrane filter). Solution (2) contains 0.2% w/v of *enalapril maleate BPCRS* in *ethanol (90%).* After removal of the plate, allow it to dry in air, spray with a solution prepared by mixing equal volumes of a 40% w/v solution of *potassium iodide* in *water* and a solution prepared by dissolving 0.85 g of *bismuth oxynitrate* in a mixture of 10 ml of *glacial acetic acid* and 40 ml of *water* and diluting 10 volumes of the mixture with 20 volumes of *glacial acetic acid* and 70 volumes of *water* immediately before use, then spray with *dilute hydrogen peroxide solution*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter, discarding the first 10 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the filtered dissolution medium diluted, if necessary, to produce a solution expected to contain about 0.00028% w/v of Enalapril Maleate. Solution (2) contains 0.00028% w/v of *enalapril maleate BPCRS* in *water*.

The chromatographic procedure described under Related substances may be used.

Calculate the total content of enalapril maleate, $C_{20}H_{28}N_2O_5,C_4H_4O_4$, in the medium using the declared content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in *enalapril maleate BPCRS.*

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use. Dissolve 0.136 g of *potassium dihydrogen orthophosphate* in 800 ml of *water*, adjust the pH of the solution to 2.0 with *orthophosphoric acid*, add sufficient *water* to produce 1000 ml and filter through a 0.45 µm filter (solution A). For solution (1) shake a quantity of the powdered tablets containing 50 mg of Enalapril Maleate with 50 ml of Solution A for 15 minutes, centrifuge and use the clear supernatant liquid (if necessary, filter through a 0.45 µm membrane filter). For solution (2) dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume to 5 volumes with solution A. For solution (3) dilute 3 volumes of a 0.01% w/v solution of *enalaprilat BPCRS* in solution A (solution B) to 20 volumes with Solution A. For solution (4) dilute 1 volume of a 0.01% w/v solution of *enalapril diketopiperazine BPCRS* in Solution A (solution C) to 20 volumes with solution A. For solution (5) mix 1 volume of solution B and 1 volume of solution C and dilute to 20 volumes with Solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) and maintained at 70°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 40 volumes of *acetonitrile* and 60 volumes of solution A and (c) a detection wavelength of 215 nm.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to enalaprilat and enalapril diketopiperazine is at least 3.0 and the *resolution factor* between the peaks due to enalapril diketopiperazine and enalapril is at least 2.0. If necessary, adjust the ratio of the components of the mobile phase.

In the chromatogram obtained with solution (1) the area of any peak corresponding to enalaprilat is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.5%), the area of any peak corresponding to enalapril diketopiperazine is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%), the area of any other *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%) and the sum of the areas of any *secondary peaks* other than any peak corresponding to enalaprilat or enalapril diketopiperazine is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Dissolve 0.136 g of *potassium dihydrogen orthophosphate* in 800 ml of *water*, adjust the pH of the solution to 2.0 with *orthophosphoric acid*, add sufficient *water* to produce 1000 ml and filter through a 0.45 µm filter (solution A). For solution (1) shake a quantity of the powdered tablets containing 10 mg of Enalapril Maleate with 50 ml of solution A for 15 minutes, centrifuge and use the clear supernatant (if necessary, filter through a 0.45 µm membrane filter). Solution (2) contains 0.02% w/v of *enalapril maleate BPCRS* in solution A. Solution (3) contains 0.01% w/v of *enalaprilat BPCRS* in solution A. Solution (4) contains 0.01% w/v of *enalapril diketopiperazine BPCRS* in solution A. For solution (5) mix 1 volume of solution (3) and 1 volume of solution (4) and dilute to 20 volumes with solution (1).

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to enalaprilat and enalapril diketopiperazine is at least 3.0 and the *resolution factor* between the peaks due to enalapril diketopiperazine and enalapril is at least 2.0. If necessary, adjust the ratio of the components of the mobile phase.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in the tablets using the declared content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in *enalapril maleate BPCRS.*

STORAGE

Enalapril Tablets should be protected from light and moisture.

Ephedrine Elixir

General Notices

Ephedrine Oral Solution

Action and use

Adrenoceptor agonist.

DEFINITION

Ephedrine Elixir is an *oral solution* containing 0.3% w/v of Ephedrine Hydrochloride in a suitable flavoured vehicle containing a sufficient volume of Ethanol (96%) or of an appropriate Dilute Ethanol to give a final concentration of 12% v/v of ethanol.

The elixir complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

0.27 to 0.33% w/v.

IDENTIFICATION

A. To 30 ml of the elixir add 2 ml of 2M *hydrochloric acid* , extract with two 20 ml quantities of *ether* and discard the ether. Add sufficient 5M *ammonia* to the aqueous phase to make it alkaline, extract with two 30 ml quantities of *ether* , wash the combined ether extracts with three 15 ml quantities of *water*, dry with *anhydrous sodium sulphate* , filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ephedrine *(RS 121)* .

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Ethanol content

11 to 13% v/v, Appendix VIII F.

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 80 volumes of *propan-2-ol* , 15 volumes of 13.5M *ammonia* and 5 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add sufficient 5M *ammonia* to 50 ml of the elixir to make it alkaline, extract with two 100 ml quantities of *ether*, wash the combined extracts with 10 ml of *water*, dry with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the oily residue in sufficient *methanol* to produce 5 ml. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (4) contains 0.30% w/v of *ephedrine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 100° for 5 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Disregard any spot of lighter colour than the background.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a weighed quantity of the elixir containing 60 mg of ephedrine hydrochloride to 50 ml with *methanol (65%)*. Solution (2) contains 0.12% w/v of *ephedrine hydrochloride BPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.005M *dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 263 nm.

Determine the *weight per ml* of the elixir, Appendix V G, and calculate the content of $C_{10}H_{15}NO,HCl$, weight in volume, using the declared content of $C_{10}H_{15}NO,HCl$ in *ephedrine hydrochloride BPCRS*.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Ephedrine Hydrochloride Tablets

General Notices

Action and use

Adrenoceptor agonist.

DEFINITION

Ephedrine Hydrochloride Tablets contain Ephedrine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the tablets containing 0.1 g of Ephedrine Hydrochloride with 20 ml of 0.1M *hydrochloric acid* , filter, wash the filtrate with two 20 ml quantities of *chloroform* and discard the chloroform. Make the aqueous layer alkaline with 5M *ammonia* and extract with two 30 ml quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol (96%)* . Dry the combined extracts over *anhydrous sodium sulphate* , filter and evaporate to a low volume at a pressure of 2 kPa. Prepare a disc using 0.3 g of *potassium bromide* , apply the chloroform solution to the surface of the disc and heat at 50° for 2 minutes. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of ephedrine *(RS 121)* .

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

C. Triturate a quantity of the powdered tablets containing 0.4 g of Ephedrine Hydrochloride with two 10 ml quantities of *chloroform* and discard the chloroform. Macerate the residue with 30 ml of warm *ethanol (96%)* for 20 minutes, filter, evaporate the filtrate to dryness on a water bath and dry the residue at 80°. Dissolve 10 mg of the residue in 1 ml of *water* and add 0.1 ml of *weak copper sulphate solution* followed by 1 ml of 5M *sodium hydroxide* ; a violet colour is produced. Add 1 ml of *ether* and shake; the ether layer is purple and the aqueous layer is blue.

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and a mixture of 80 volumes of *propan-2-ol* , 15 volumes of 13.5M *ammonia* and 5 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 0.10 g of Ephedrine Hydrochloride with 5 ml of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol*. Solution (3) contains 0.20% w/v of *ephedrine hydrochoride BPCRS* in *methanol*. For solution (4) dilute 1 volume of solution (1) to 200 volumes with *methanol*. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4). Disregard any spot of lighter colour than the background.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Ephedrine Hydrochloride with 30 ml of *methanol* for 10 minutes, add sufficient *water* to produce 50 ml, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.1% w/v of *ephedrine hydrochloride BPCRS* in *methanol (60%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.005M *dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 263 nm.

Calculate the content of $C_{10}H_{15}NO,HCl$ using the declared content of $C_{10}H_{15}NO,HCl$ in *ephedrine hydrochloride BPCRS*.

Ephedrine Nasal Drops

General Notices

Action and use

Adrenoceptor agonist.

DEFINITION

Ephedrine Nasal Drops are a solution of Ephedrine Hydrochloride in a suitable aqueous vehicle.

The nasal drops comply with the requirements stated under Nasal Preparations and with the following requirements.

Content of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the nasal drops containing 0.1 g of Ephedrine Hydrochloride add 2 ml of 2M *hydrochloric acid* , shake with two 20 ml quantities of *chloroform* and discard the chloroform. Add 5M *ammonia* until the aqueous layer is alkaline, extract with two 30 ml quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol* , dry the combined extracts over *anhydrous sodium sulphate* , filter and evaporate to dryness at a pressure of 2 kPa, heating gently to remove the last traces of solvent. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ephedrine *(RS 121)* .

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Acidity or alkalinity

pH, 4.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 80 volumes of *propan-2-ol* , 15 volumes of 13.5M *ammonia* and 5 volumes of *chloroform* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) use the nasal drops diluted, if necessary, with *water* to contain 0.5% w/v of Ephedrine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 5 volumes with *methanol* .

For solution (3) dilute 1 volume of solution (1) to 200 volumes with *water*. Solution (4) contains 0.1% w/v of *ephedrine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 100° for 5 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Disregard any spot of lighter colour than the background and any spot remaining on the line of application.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is a 0.1% w/v solution of *ephedrine hydrochloride BPCRS* in *methanol (65%)*. For solution (2) dilute the nasal drops with *methanol (80%)* to contain 0.1% w/v of Ephedrine Hydrochloride.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.005M *dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 263 nm.

Calculate the content of $C_{10}H_{15}NO,HCl$ in the nasal drops using the declared content of $C_{10}H_{15}NO,HCl$ in *ephedrine hydrochloride BPCRS*.

When ephedrine nasal drops are prescribed or demanded no strength being stated, nasal drops containing 0.5% w/v of ephedrine hydrochloride shall be dispensed or supplied.

Ergocalciferol Injection

General Notices

Action and use

Vitamin D analogue (Vitamin D_2).

DEFINITION

Ergocalciferol Injection is a sterile solution containing 0.75% w/v of Ergocalciferol in Ethyl Oleate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ergocalciferol, $C_{28}H_{44}O$

0.67 to 0.83% w/v.

CHARACTERISTICS

A pale yellow, oily liquid.

IDENTIFICATION

To 1 ml of a 0.2% v/v solution of the injection in *ethanol-free chloroform* add 9 ml of *antimony trichloride solution*. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 500 nm.

ASSAY

Carry out the following procedure in subdued light. Dilute 0.1 g of the injection to 50 ml with dry *1,2-dichloroethane* that has been purified by passing it through a column of *silica gel*. To 1 ml of this solution add rapidly 9 ml of *antimony trichloride in dichloroethane solution* and measure the *absorbance* of the solution at 500 and 550 nm, Appendix II B, 90 to 120 seconds after adding the reagent. Repeat the operation using 1 ml of a 0.002% w/v solution of *ergocalciferol BPCRS* in the dry, purified 1,2-dichloroethane, beginning at the words 'add rapidly 9 ml of *antimony trichloride solution* …'. Calculate the result of the assay from the difference between the absorbances at 500 and 550 nm and using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS*. Calculate the percentage w/v of ergocalciferol taking 0.87 g as the value of the weight per ml of the injection.

STORAGE

Ergocalciferol Injection should be protected from light.

LABELLING

The label states (1) that the preparation is for intramuscular use only; (2) the equivalent number of IU (units) of antirachitic activity (vitamin D) in 1 ml.

Each µg of ergocalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

When calciferol injection is prescribed or demanded, Colecalciferol Injection or Ergocalciferol Injection shall be dispensed or supplied.

Ergocalciferol Tablets

General Notices

Action and use

Vitamin D analogue (Vitamin D_2).

DEFINITION

Ergocalciferol Tablets contain Ergocalciferol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ergocalciferol, $C_{28}H_{44}O$

90.0 to 125.0% of the stated amount.

IDENTIFICATION

A. In the test for Uniformity of content, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

B. Extract one tablet, in powder, with 5 ml of *dichloromethane*, filter and to 1 ml of the filtrate add 9 ml of *antimony trichloride solution*. A brownish red colour is produced.

TESTS

Uniformity of content

Tablets containing less than 2 mg of Ergocalciferol comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.001% w/v of *ergocalciferol BPCRS* in *hexane*. For tablets containing more than 0.25 mg of ergocalciferol prepare solution (2) in the following manner. Add 4 ml of *water* to one tablet in an amber flask and disperse with the aid of ultrasound. Add 12 ml of *dimethyl sulphoxide*, mix, extract with 100 ml of *hexane* by shaking for 30 minutes, centrifuge the hexane layer and use the clear supernatant liquid. For tablets containing 0.25 mg or less carry out the same procedure but using 2 ml of *water*, 6 ml of *dimethyl sulphoxide* and 25 ml of *hexane*. For solution (3) dissolve 0.5 g of *cholecalciferol for performance test EPCRS* in 2 ml of *toluene* and dilute to 10 ml with the mobile phase; heat under a reflux condenser in a water bath at 90° for 45 minutes and cool.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Partisil is suitable), (b) a mixture of 8 volumes of *pentan-1-ol* and 992 volumes of *hexane* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Inject a suitable volume of solution (3) and record the chromatogram using a sensitivity such that the height of the peak due to colecalciferol is more than 50% of full-scale deflection. When the chromatogram is recorded under the prescribed conditions, approximate retention times relative to colecalciferol are 0.4 for precolecalciferol and 0.5 for *trans*-colecalciferol. The test is not valid unless the *resolution factor* between the peaks corresponding to precolecalciferol and *trans*-colecalciferol is at least 1.0. If necessary, adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution.

Inject a suitable volume of solution (1) and record the chromatogram using a sensitivity such that the height of the peak due to ergocalciferol is more than 50% of full-scale deflection. Repeat the operation using solution (2) and injecting the same volume.

Calculate the content of ergocalciferol, $C_{28}H_{44}O$, in each tablet using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS* and using peak heights.

ASSAY

For tablets containing 2 mg or more of ergocalciferol

Carry out the following procedure in subdued light. Weigh and powder 25 tablets, or more if necessary. To a quantity of the powdered tablets containing 6 mg of Ergocalciferol add 50 ml of *ethanol (96%)*, 14 ml of *glycerol* and 20 ml of a 50% w/v solution of *potassium hydroxide*. Boil under a reflux condenser for 30 minutes, swirling occasionally, add 110 ml of *water* and allow to stand for 10 minutes with occasional stirring. Cool and add sufficient *ethanol (96%)* to produce 250 ml. Shake 5 ml with 25 ml of *petroleum spirit (boiling range, 40° to 60°)* for 3 minutes and evaporate duplicate 5 ml portions of the extract to dryness in a current of *oxygen-free nitrogen*. Dissolve each residue in 1 ml of *ethanol-free chloroform*, add rapidly 9 ml of *antimony trichloride solution* and measure the *absorbance* of each solution at 500 nm and at 550 nm, 90 to 120 seconds after adding the reagent, Appendix II B. Repeat the operations using duplicate 1 ml portions of a solution containing a known amount of *ergocalciferol BPCRS* in *ethanol-free chloroform* and beginning at the words 'add rapidly 9 ml of *antimony trichloride solution…*'. Subtract the absorbance at 550 nm from that at 500 nm and calculate the content of ergocalciferol in mg from the average value so obtained and from the amount of ergocalciferol in the reference solution using the declared content of $C_{28}H_{44}O$ in *ergocalciferol BPCRS*.

For tablets containing less than 2 mg of ergocalciferol

Use the average of the 10 individual results obtained in the test for Uniformity of content.

LABELLING

The label states the equivalent number of IU (units) of antirachitic activity (vitamin D) per tablet.

Each µg of ergocalciferol is equivalent to 40 IU of antirachitic activity (vitamin D).

When calciferol tablets are prescribed or demanded, Colecalciferol Tablets or Ergocalciferol Tablets shall be dispensed or supplied.

Ergometrine and Oxytocin Injection

General Notices

Action and use

Oxytocic.

DEFINITION

Ergometrine and Oxytocin Injection is a sterile solution containing Ergometrine Maleate and either Oxytocin or Oxytocin Bulk Solution in Water for Injections. The acidity of the solution is adjusted to pH 3.3 by the addition of maleic acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$

90.0 to 110.0% of the stated amount.

Content of oxytocin, $C_{43}H_{66}N_{12}O_{12}S_2$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. In the test for Substances related to ergometrine, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

B. To a volume containing 0.1 mg of Ergometrine Maleate, add 0.5 ml of *water* and 2 ml of *dimethylaminobenzaldehyde solution R6*. After about 5 minutes a deep blue colour is produced.

C. In the Assay for oxytocin a peak in the chromatogram obtained with solution (2) corresponds to the peak due to oxytocin in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 2.9 to 3.5, Appendix V L.

Substances related to ergometrine

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 75 volumes of *chloroform*, 25 volumes of *methanol* and 3 volumes of *water* as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) add 10 ml of *absolute ethanol* to a volume of the injection containing 0.5 mg of Ergometrine Maleate and evaporate to dryness at a temperature not exceeding 30° at a pressure of 2 kPa; to the residue add 0.2 ml of a mixture of 1 volume of 13.5M *ammonia* and 9 volumes of *ethanol (80%)*, mix, centrifuge and use the supernatant liquid. Solutions (2), (3), (4), (5) and (6) contain 0.250% w/v, 0.0250% w/v, 0.0125% w/v, 0.0050% w/v and 0.00250% w/v of *ergometrine maleate BPCRS* respectively in a mixture of 1 volume of 3.5M *ammonia* and 9 volumes of *ethanol (80%)*. After removal of the plate, dry it in a current of cold air, spray with *dimethylaminobenzaldehyde solution R7* and dry the plate at 105° for 2 minutes. Assess the intensities of any *secondary spots* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with solutions (3) to (6), making allowance for area in assessing the intensities of spots of different Rf values. The sum of the intensities so assessed does not exceed 10% of the intensity of the principal spot.

ASSAY

For ergometrine maleate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.05% w/v of *ergometrine maleate BPCRS*. Solution (2) is the injection diluted, if necessary, to give a solution containing 0.05% w/v of Ergometrine Maleate.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable) and maintained at 40°, (b) a mixture of 92 volumes of a 0.2% v/v solution of *orthophosphoric acid* and 8 volumes of *acetonitrile* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 320 nm.

Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ from the declared content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in *ergometrine maleate BPCRS*.

For oxytocin

Carry out the method for *liquid chromatography*, Appendix III D, injecting 0.2 ml of each of the following solutions. Solution (1) contains 8 µg of *oxytocin EPCRS* per ml. Solution (2) is the injection diluted, if necessary, to give a solution containing about 8 µg of Oxytocin per ml.

The chromatographic procedure may be carried out using a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable) and maintained at 40°, (b) a mixture of 85 volumes of a 0.2% v/v solution of *orthophosphoric acid* and 15 volumes of *acetonitrile* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 220 nm. If necessary adjust the content of *acetonitrile* in the mobile phase so that the retention time of oxytocin is about 14 minutes. Record the chromatogram for sufficient time to ensure elution of any preservatives.

The *column efficiency*, determined using the peak due to oxytocin in the chromatogram obtained with solution (1), should be not less than 50,000 theoretical plates per metre.

Calculate the content of $C_{43}H_{66}N_{12}O_{12}S_2$ from the declared content of peptide in *oxytocin EPCRS.*

STORAGE

Ergometrine and Oxytocin Injection should be protected from light and stored at a temperature of 2° to 8°. Under these conditions it may be expected to retain its potency for not less than 3 years.

LABELLING

The strength with respect to oxytocin is stated as the number of IU (units) per ml. The label also states the equivalent number of µg of oxytocin per ml.

Ergometrine Injection

General Notices

Action and use

Oxytocic.

DEFINITION

Ergometrine Injection is a sterile solution of Ergometrine Maleate in Water for Injections. The acidity of the solution is adjusted to pH 3 by the addition of maleic acid.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless or faintly yellow solution.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (5).

B. Exhibits a blue fluorescence.

C. To a volume containing 0.1 mg of Ergometrine Maleate, add 0.5 ml of *water* and 2 ml of *dimethylaminobenzaldehyde solution R6*. A deep blue colour is produced after about 5 minutes.

TESTS

Acidity

pH, 2.7 to 3.5, Appendix V L.

Related substances

Carry out the following procedure in subdued light and protect from light any solutions not used immediately. Carry out the method for *thin-layer chromatography*, Appendix III A, using a suspension of *silica gel G* in 0.1M *sodium hydroxide* to prepare the plate and a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) evaporate a volume of the injection containing 1 mg of Ergometrine Maleate to dryness at 20° at a pressure of 2 kPa and dissolve the residue in 0.25 ml of *methanol*. Solutions (2), (3), (4) and (5) are solutions of *ergometrine maleate BPCRS* in *methanol* containing 0.010% w/v, 0.020% w/v, 0.040% w/v and 0.40% w/v respectively. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Assess the intensities of any *secondary spots* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with solutions (2), (3) and (4). The total of the intensities so assessed does not exceed 10% of the intensity of the principal spot.

ASSAY

Carry out the following procedure protected from light. Dilute a suitable volume with sufficient *water* to produce a solution containing 0.004% w/v of Ergometrine Maleate. To 3 ml add 6 ml of *dimethylaminobenzaldehyde solution R6*, mix, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time prepare solution B in the same manner but using 3 ml of a 0.004% w/v solution of *ergometrine maleate BPCRS* and beginning at the words 'add 6 ml ...'. Measure the *absorbance* of solution B at the maximum at 545 nm, Appendix II B, using in the reference cell a solution prepared by mixing 6 ml of *dimethylaminobenzaldehyde solution R6* and 3 ml of *water*. Without delay replace solution B with solution A, using the same cell, and measure the *absorbance* of solution A at the same wavelength. Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ using the declared content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in *ergometrine maleate BPCRS*.

STORAGE

Ergometrine Injection should be protected from light and stored at a temperature of 2° to 8°.

Ergometrine Tablets

General Notices

Action and use

Oxytocic.

DEFINITION

Ergometrine Tablets contain Ergometrine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (5).

B. Extract a quantity of the powdered tablets containing 2 mg of Ergometrine Maleate with 20 ml of *water*, filter and wash the residue with sufficient *water* to produce 20 ml. The solution exhibits a blue fluorescence.

C. To 2 ml of the solution obtained in test B add 4 ml of *dimethylaminobenzaldehyde solution R6*. A deep blue colour is produced after a few minutes.

TESTS

Related substances

Carry out the method for *thin-layer chromatography* protected from light, Appendix III A, using a suspension of *silica gel G* in 0.1M *sodium hydroxide* to prepare the plate and a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) triturate a quantity of the powdered tablets containing 1 mg of Ergometrine Maleate with 0.2 ml of a 1% w/v solution of *domiphen bromide*, add 2 ml of *methanol*, centrifuge and remove the supernatant liquid. Extract the residue with two 1 ml quantities of *methanol*, evaporate the combined extracts to dryness at 20° at a pressure of 2 kPa and dissolve the residue in 0.25 ml of *methanol*; centrifuge if necessary. Solutions (2), (3), (4) and (5) are solutions of *ergometrine maleate BPCRS* in *methanol* containing 0.010% w/v, 0.020% w/v, 0.040% w/v and 0.40% w/v respectively. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Assess the intensities of any *secondary spots* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with solutions (2), (3) and (4). The sum of the intensities so assessed does not exceed 10% of the intensity of the principal spot. In addition, no single *secondary spot* in the chromatogram obtained with solution (1) is more intense than the spot in the chromatogram obtained with solution (2).

Uniformity of content

Tablets containing less than 2 mg of Ergometrine Maleate comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. To one tablet add 10 ml of a 1% w/v solution of *(+)-tartaric acid*, shake for 30 minutes and centrifuge. Carry out the Assay described under Ergometrine Injection, beginning at the words 'Dilute a suitable volume…' and calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 2 mg of Ergometrine Maleate with 50 ml of a 1% w/v solution of *(+)-tartaric acid* for 30 minutes, centrifuge and use the supernatant liquid. Carry out the Assay described under Ergometrine Injection, beginning at the words 'To 3 ml...'.

STORAGE

Ergometrine Tablets should be protected from light.

Ergotamine Sublingual Tablets

General Notices

Action and use

Oxytocic.

DEFINITION

Ergotamine Sublingual Tablets contain Ergotamine Tartrate. They may be flavoured.
The tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of ergotamine tartrate, $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (5).

B. Triturate a quantity of the powdered tablets containing 2 mg of Ergotamine Tartrate with 5 ml of *petroleum spirit (boiling range, 40° to 60°)*, discard the petroleum spirit and triturate the residue with 10 ml of *chloroform* saturated with 13.5M *ammonia*. Filter and evaporate the chloroform. Dissolve 1 mg of the residue in a mixture of 5 ml of *glacial acetic acid* and 5 ml of *ethyl acetate*. To 1 ml of this solution add 1 ml of *sulphuric acid*, with continuous shaking and cooling; a blue colour with a red tinge is produced. Add 0.1 ml of *iron(III) chloride solution R1* previously diluted with an equal volume of *water*; the red tinge becomes less apparent and the blue colour more pronounced.

TESTS

Related substances

Carry out as rapidly as possible the method for *thin-layer chromatography* protected from light, Appendix III A, using a silica gel F_{254} precoated plate and a mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 1 mg of Ergotamine Tartrate with 2 ml of a mixture of equal volumes of *dichloromethane* and *methanol* and centrifuge. Remove the supernatant liquid, extract the residue with two 1 ml quantities of the solvent mixture, evaporate the combined extracts to dryness at 20° at a pressure of 2 kPa and dissolve the residue in 0.25 ml of a mixture of equal volumes of *dichloromethane* and *methanol*. Centrifuge if necessary. Solutions (2), (3), (4) and (5) contain 0.010% w/v, 0.020% w/v, 0.040% w/v and 0.40% w/v respectively of *ergotamine tartrate BPCRS* in a mixture of equal volumes of *dichloromethane* and *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Assess the intensities of any *secondary spots* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with solutions (2), (3) and (4). The sum of the intensities so assessed does not exceed 10% of the intensity of the principal spot in the chromatogram obtained with solution (1). In addition, any single *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2.5%).

Uniformity of content

Tablets containing less than 2% w/w of ergotamine tartrate comply with the requirements stated under Oromucosal Preparations using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.008% w/v of *ergotamine tartrate BPCRS* in *methanol (50%)* containing 1% v/v of 1M *hydrochloric acid*. For solution (2) shake one tablet with *methanol (50%)* containing 1% v/v of 1M *hydrochloric acid* for 15 minutes and add a sufficient volume of the same solvent to produce a solution containing 0.008% w/v of Ergotamine Tartrate; filter if necessary.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with particles of silica 5 µm in diameter the surface of which has been modified with chemically bonded hexyl groups (Spherisorb C6 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 5 volumes of a 9.0% w/v solution of *disodium hydrogen orthophosphate* adjusted to pH 5.0 with *orthophosphoric acid*, 45 volumes of *water* and 50 volumes of *methanol*, the mixture adjusted to pH 3.5 with *orthophosphoric acid*, and (c) a detection wavelength of 210 nm.

Calculate the content of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ in each tablet using the declared content of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ in *ergotamine tartrate BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.008% w/v of *ergotamine tartrate BPCRS* in *methanol (50%)* containing 1% v/v of 1M *hydrochloric acid*. For solution (2) shake a quantity of powdered tablets containing 8 mg of Ergotamine Tartrate with 50 ml of *methanol (50%)* containing 1% v/v of 1M *hydrochloric acid* for 15 minutes and add sufficient of the same solvent to produce 100 ml; filter if necessary.

The chromatographic procedure described under Uniformity of content may be used.

Calculate the content of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ using the declared content of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ in *ergotamine tartrate BPCRS*.

LABELLING

The label states that the tablets should be placed under the tongue and allowed to dissolve slowly.

Erythromycin and Zinc Acetate Lotion

General Notices

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin and Zinc Acetate Lotion is a *cutaneous solution*. It contains 4% w/v of Erythromycin and 1.2% w/v of Zinc Acetate in a suitable ethanolic vehicle. It is prepared by dissolving the dry ingredients in the requisite volume of the vehicle provided before use.

The lotion complies with the requirements stated under Liquids for Cutaneous Application.

The dry ingredients comply with the requirements for Powders for Lotions stated under Liquids for Cutaneous Application and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$)

95.0 to 105.0% of the stated amount of Erythromycin.

Content of zinc acetate, $C_4H_6O_4Zn,2H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay for erythromycin, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Mix a quantity of the powder with sufficient *water* to produce a 5% w/v solution and filter. Add 0.5 ml of a 0.25M solution of *potassium hexacyanoferrate(II)* to 5 ml of the resulting solution and mix; a white precipitate is produced. Add 5 ml of 4M *hydrochloric acid*; the precipitate does not dissolve.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1), (2), (3), (4) and (6) described under Assay for erythromycin.

The chromatographic conditions described under the Assay for erythromycin may be used.

Inject 100 µl of each solution. For solution (1) allow the chromatography to proceed for 6 times the retention time of the peak corresponding to erythromycin A.

When the chromatograms are recorded using the prescribed conditions the retention time of erythromycin A is about 15 minutes. The retention times relative to erythromycin A are: impurity A, about 0.3; impurity B, about 0.45; erythromycin C, about 0.5; impurity C, about 0.9;

impurity D, about 1.4; impurity F, about 1.5; erythromycin B, about 1.8; impurity E, about 4.3.

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities E and F using solution (6) and multiply the areas of these peaks by the corresponding correction factors: impurity E, 0.09; impurity F, 0.15. In the chromatogram obtained with solution (1) the area of any peak other than those peaks corresponding to erythromycin A, erythromycin B and erythromycin C, identified from the peaks in the chromatograms obtained with solutions (2) and (3), is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (3%) and the sum of the areas of any such peaks is not greater than 2.3 times the area of the principal peak in the chromatogram obtained with solution (4) (7%). Disregard any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with solution (4) (0.06%).

The content of each of erythromycin B and erythromycin C, as determined under Assay, is not more than 5%.

ASSAY

For erythromycin

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of a mixture of 1 volume of *methanol* and 3 volumes of *citro-phosphate buffer pH 7.0* (solvent A) to a quantity of the powder containing 80 mg of Erythromycin, mix with the aid of ultrasound for 5 minutes and allow to stand until phase separation has been completed. Centrifuge the lower layer and use the resulting clear solution. Solution (2) contains 0.4% w/v of *erythromycin A BPCRS* in solvent A. Solution (3) contains 0.02% w/v of each of *erythromycin B BPCRS* and *erythromycin C BPCRS* in solvent A. Solution (4) contains 0.012% w/v of *erythromycin A BPCRS* in solvent A. For solution (5) dissolve 5 mg of N-*demethylerythromycin A EPCRS* in solution (3), add 1 ml of solution (2) and sufficient of solution (3) to produce 25 ml. For solution (6) transfer 40 mg of *erythromycin A BPCRS* to a glass vial and spread evenly such that it forms a layer not more than about 1 mm thick. Heat at 130° for 4 hours, allow to cool and dissolve in sufficient of solvent A to produce 10 ml (generation of impurities E and F). The solutions can be used within one day if stored at 5°.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene copolymer* (8 µm) with a pore size of 100 nm (PLRP-S is suitable), (b) as the mobile phase with a flow rate of 2.0 ml per minute a solution prepared in the following manner: to 50 ml of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 9.0 with 1M *orthophosphoric acid* add 400 ml of *water*, 165 ml of *2-methylpropan-2-ol* and 30 ml of *acetonitrile* and dilute to 1000 ml with *water* and (c) a detection wavelength of 215 nm. Maintain the temperature of the column at 70° using a water bath for the column and at least one third of the tubing preceding the column.

Inject 100 µl of solution (5). Adjust the sensitivity of the detector so that the height of the peaks is at least 25% of the full scale of the recorder. The substances are eluted in the following order: *N*-demethylerythromycin A (impurity B), erythromycin C, erythromycin A and erythromycin B. The test is not valid unless the *resolution factor* between the peaks corresponding to *N*-demethylerythromycin A and erythromycin C is at least 0.8 and the *resolution factor* between the peaks corresponding to *N*-demethylerythromycin A and erythromycin A is at least 5.5. If necessary, adjust the concentration of 2-methylpropan-2-ol in the mobile phase (180 ml has been found to be suitable) or reduce the flow rate to 1.5 or 1.0 ml per minute.

Inject alternately 100 µl of solutions (1), (2) and (3). Calculate the content of erythromycin A using the chromatograms obtained with solutions (1) and (2) and from the declared content of $C_{37}H_{67}NO_{13}$ in *erythromycin A BPCRS*. Calculate the contents of erythromycin B and erythromycin C using the chromatograms obtained with solutions (1) and (3) and from the declared contents of $C_{37}H_{67}NO_{12}$ and $C_{36}H_{65}NO_{13}$ in *erythromycin B BPCRS* and *erythromycin C BPCRS* respectively.

For zinc acetate

To a quantity of the powder containing 0.2 g of Zinc Acetate add 5 ml of *dilute acetic acid*. Carry out the *complexometric titration of zinc*, Appendix VIII D. Each ml of 0.1M *disodium edetate VS* is equivalent to 21.95 mg of $C_4H_6O_4Zn,2H_2O$.

For the following tests prepare the lotion as directed on the label. The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$)

90.0 to 110.0% of the stated amount of Erythromycin.

Content of zinc acetate, $C_4H_6O_4Zn,2H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay for erythromycin, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Evaporate a suitable volume of the lotion to dryness on a water bath, add 20 ml of water to the residue, mix, with shaking, and filter. Add 1 ml of a 0.25M solution of *potassium hexacyanoferrate(II)* to the resulting filtrate and mix; a white precipitate is produced. Add 10 ml of 4M *hydrochloric acid*; the precipitate does not dissolve.

TEST

Clarity and colour of solution

The solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

ASSAY

For erythromycin

Carry out the Assay for erythromycin described in the requirements for the dry ingredients. For solution (1) use a volume of the lotion containing 80 mg of Erythromycin in place of the powder.

For zinc acetate

To a volume of the lotion containing 0.2 g of Zinc Acetate add 5 ml of *dilute acetic acid*. Carry out the *complexometric titration of zinc*, Appendix VIII D. Each ml of 0.1M *disodium edetate VS* is equivalent to 21.95 mg of $C_4H_6O_4Zn,2H_2O$.

STORAGE

Erythromycin and Zinc Acetate Lotion should be stored at the temperature and used within the period stated on the label.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous solution'.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Erythromycin.

Erythromycin Estolate Capsules

General Notices

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Estolate Capsules contain Erythromycin Estolate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

IDENTIFICATION

A. The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with the *reference spectrum* of erythromycin estolate *(RS 124)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) is similar in position and colour to that in the chromatogram obtained with solution (3).

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A1. Use a 0.6% v/v solution of *hydrochloric acid* in place of *water*.

Water

The contents of the capsules contain not more than 5.0% w/w of water, Appendix IX C. Use 0.5 g.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase a mixture of 1 volume of a 15% w/v solution of *ammonium acetate* previously adjusted to pH 7.0, 15 volumes of *ethanol (96%)* and 85 volumes of *chloroform*. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 0.4 g of erythromycin with 100 ml of *acetone*, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 4 volumes with *acetone*. Solution (3) contains 0.13% w/v of *erythromycin estolate BPCRS* in *acetone*. Solution (4) contains 0.1% w/v each of *erythromycin estolate BPCRS* and *erythromycin ethylsuccinate BPCRS* in *acetone*. Solution (5) contains 0.01% w/v of *erythromycin BPCRS* in *acetone*. After removal of the plate, allow it to dry in air, spray with *anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (5) (2.5%, with respect to erythromycin). The test is not valid unless the chromatogram obtained with solution

(4) shows two clearly separated spots.

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing the equivalent of 0.25 g of erythromycin in 400 ml of *methanol* and add 200 ml of sterile *phosphate buffer pH 7.0* and sufficient *Water for Injections* to produce 1000 ml. Maintain the solution at 60° for 3 hours, cool, filter and carry out the *microbiological assay of antibiotics* for erythromycin, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of erythromycin in a capsule of average content weight, taking each 1000 IU found to be equivalent to 1 mg of erythromycin. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of erythromycin.

Erythromycin Ethyl Succinate Oral Suspension

General Notices

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Ethyl Succinate Oral Suspension is a suspension of Erythromycin Ethyl Succinate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral suspension as directed on the label. The suspension examined immediately after preparation, unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

IDENTIFICATION

Dilute a quantity of the oral suspension containing the equivalent of 0.1 g of erythromycin to 25 ml with *water* and extract with two 10 ml quantities of *dichloromethane*. Wash the combined extracts with five 10 ml quantities of *water*, filter using silicone-treated filter paper (Phase Separator paper is suitable) and evaporate to dryness. The residue, after drying at 105° for 15 minutes complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of erythromycin ethyl succinate *(RS 125)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 1 volume of a 15% w/v solution of *ammonium acetate* previously adjusted to pH 7.0, 15 volumes of *ethanol (96%)* and 85 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dissolve a quantity of the residue in *acetone* to produce a solution containing the equivalent of 0.1% w/v of erythromycin. Solution (2) contains 0.1% w/v of *erythromycin ethylsuccinate BPCRS* in *acetone*. Solution (3) contains 0.1% w/v of *erythromycin ethylsuccinate BPCRS* and 0.1% w/v of *erythromycin estolate BPCRS* in *acetone.* After removal of the plate, allow it to dry in air, spray with *anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. The principal spot in the chromatogram obtained with solution (1) is similar in position and colour to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

TESTS

Acidity or alkalinity

pH, 6.5 to 9.5, Appendix V L.

ASSAY

To a weighed quantity of the oral suspension containing the equivalent of 0.25 g of erythromycin add 10 ml of *water* and swirl to mix. Add 200 ml of *methanol*, shake for 1 hour and dilute to 500 ml with *methanol*. Dilute 10 ml to 100 ml with *phosphate buffer pH 8.0*, stand at room temperature for 16 hours and carry out the *microbiological assay of antibiotics* for erythromycin, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

Calculate the content of erythromycin in the oral suspension taking each 1000 IU found to be equivalent to 1 mg of erythromycin. When freshly constituted the lower fiducial limit of error is not more than 120.0% of the stated amount and when stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, the upper fiducial limit of error is not less than 90.0% of the stated content.

STORAGE

The oral suspension should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of erythromycin.

Erythromycin Ethyl Succinate Tablets

General Notices

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Ethyl Succinate Tablets contain Erythromycin Ethyl Succinate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of erythromycin with 20 ml of a mixture of equal volumes of *chloroform* and *methanol* for 15 minutes. Centrifuge and evaporate the upper layer to dryness. Dissolve the residue in a minimum volume of *dichloromethane,* evaporate to dryness and dry at 105° for 15 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of erythromycin ethyl succinate *(RS 125)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) is similar in position and colour to that in the chromatogram obtained with solution (3). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 1 volume of a 15% w/v solution of *ammonium acetate* previously adjusted to pH 7.0, 15 volumes of *ethanol (96%)* and 85 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.1 g of erythromycin with 25 ml of a mixture of equal volumes of *chloroform* and *methanol* for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 4 volumes with the same solvent. Solution (3) contains 0.1% w/v of *erythromycin ethylsuccinate BPCRS* in *acetone.* Solution (4) contains 0.1% w/v of *erythromycin ethylsuccinate BPCRS* and 0.1% w/v of *erythromycin estolate BPCRS* in *acetone.* Solution (5) contains 0.020% w/v of *erythromycin BPCRS* in *acetone*. After removal of the plate, allow it to dry in air, spray with *anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (5) (5%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing the equivalent of 0.25 g of erythromycin add 200 ml of *methanol*, shake for 1 hour and dilute to 500 ml with *methanol*. Dilute 10 ml to 100 ml with *phosphate buffer pH 8.0*, stand at room temperature for 16 hours and carry out the *microbiological assay of antibiotics* for erythromycin, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of erythromycin in the tablets taking each 1000 IU found to be equivalent to 1 mg of erythromycin. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

STORAGE

Erythromycin Ethyl Succinate Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of erythromycin.

Erythromycin Lactobionate Intravenous Infusion

General Notices

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Lactobionate Intravenous Infusion is a sterile solution of Erythromycin Lactobionate in Sodium Chloride Intravenous Infusion. It is prepared before use by dissolving Erythromycin Lactobionate for Intravenous Infusion in the requisite amount of Water for Injections and diluting the resulting solution with the requisite amount of Sodium Chloride Intravenous Infusion.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

STORAGE

Erythromycin Lactobionate Intravenous Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

ERYTHROMYCIN LACTOBIONATE FOR INTRAVENOUS INFUSION

DEFINITION

Erythromycin Lactobionate for Intravenous Infusion is a sterile material consisting of Erythromycin Lactobionate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$)

90.0 to 110.0% of the stated amount of erythromycin.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of erythromycin lactobionate *(RS 126)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the contents of a sealed container in sufficient *methanol* to produce a solution containing the equivalent of 0.2% w/v of erythromycin.

(2) 0.2% w/v of *erythromycin A BPCRS* in *methanol*.

(3) 0.1% w/v of *lactobionic acid* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel H* as the coating substance.

(b) Use the mobile phase described below.

(c) Apply 5 µl of each solution.

(d) Develop to 15 cm.

(e) Remove the plate, allow it to dry in air and spray with a 0.5% w/v solution of *potassium permanganate* in 1M *sodium hydroxide* and heat at 110° for 5 minutes.

MOBILE PHASE

A mixture of 3 volumes of *glacial acetic acid*, 10 volumes of *water* and 90 volumes of *methanol*.

CONFIRMATION

The chromatogram obtained with solution (1) shows two spots, one of which corresponds in position, colour and size to the principal spot in the chromatogram obtained with solution (2) and the other to the principal spot in the chromatogram obtained with solution (3).

C. To a quantity of the contents of a sealed container containing the equivalent of 5 mg of erythromycin add 5 ml of a 0.02% w/v solution of *xanthydrol* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of 5M *acetic acid*. A red colour develops.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 1.34% w/v of erythromycin, 6.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1), (3) and (6) described under Assay.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used. For solution (1) allow the chromatography to proceed for 5 times the retention time of the peak corresponding to erythromycin A.

When the chromatograms are recorded using the prescribed conditions the retention time of erythromycin A is about 15 minutes. The retention times relative to erythromycin A are: impurity A, about 0.3; impurity B, about 0.45; erythromycin C, about 0.5; impurity C, about 0.9; impurity D, about 1.4; impurity F, about 1.5; erythromycin B, about 1.8; impurity E, about 4.3.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities E and F using solution (6) and multiply the areas of these peaks by the corresponding correction factors: impurity E, 0.09; impurity F, 0.15.

In the chromatogram obtained with solution (1):

the area of any peak other than those peaks corresponding to erythromycin A, erythromycin B and erythromycin C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3%);

the sum of the areas of any such peaks is not greater than 2.3 times the area of the principal peak in the chromatogram obtained with solution (3) (7%).

Disregard any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with solution (3) (0.06%).

The content of each of erythromycin B and erythromycin C, as determined under Assay, is not more than 5%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 50 mg of erythromycin per ml; further dilute this solution with *water BET* to give a solution containing the equivalent of 0.2 mg of erythromycin per ml (solution A). The endotoxin limit concentration of solution A is 0.07 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of each of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions can be used within one day if stored at 5°.

(1) Dissolve a quantity of the mixed contents of the 10 containers containing the equivalent of 40 mg of erythromycin in a mixture of 1 volume of *methanol* and 3 volumes of *citro-phosphate buffer pH 7.0* and dilute to 10 ml with the same mixture of solvents.

(2) 0.4% w/v of *erythromycin A BPCRS* in a mixture of 1 volume of *methanol* and 3 volumes of *citro-phosphate buffer pH 7.0.*

(3) 0.012% w/v of *erythromycin A BPCRS* in a mixture of 1 volume of *methanol* and 3 volumes of *citro-phosphate buffer pH 7.0*.

(4) 0.02% w/v of each of *erythromycin B BPCRS* and *erythromycin C BPCRS* in a mixture of 1 volume of *methanol* and 3 volumes of *citro-phosphate buffer pH 7.0*.

(5) Dissolve 5 mg of N-*demethylerythromycin A EPCRS* in solution (4), add 1 ml of solution (2) and sufficient of solution (4) to produce 25 ml.

(6) Transfer 40 mg of *erythromycin A BPCRS* to a glass vial and spread evenly such that it forms a layer not more than about 1 mm thick. Heat at 130° for 4 hours, allow to cool and dissolve in sufficient of solvent A to produce 10 ml (generation of impurities E and F).

CHROMATOGRAPHIC CONDITIONS

(a) Use a column (25 cm × 4.6 mm) packed with styrene-divinylbenzene copolymer (8 µm) with a pore size of 100 nm (PLRP-S is suitable),

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Maintain the temperature of the column at 70° using a water bath for the column and at least one third of the tubing preceding the column.

(e) Use a detection wavelength of 215 nm.

(f) Inject 100 µl of each solution.

MOBILE PHASE

To 50 ml of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate*, adjusted to pH 9.0 with 1M *orthophosphoric acid*, add 400 ml of *water*, 165 ml of *2-methylpropan-2-ol* and 30 ml of *acetonitrile R1* and dilute to 1000 ml with *water*.

SYSTEM SUITABILITY

Inject solution (5). Adjust the sensitivity of the system so that the height of the peaks is at least 25% of the full-scale of the recorder. The substances are eluted in the following order: *N*-demethylerythromycin A, erythromycin C, erythromycin A and erythromycin B. The test is not valid unless the *resolution factor* between the peaks corresponding to *N*-demethylerythromycin A and erythromycin C is at least 0.8 and the *resolution factor* between the peaks corresponding to *N*-demethylerythromycin A and erythromycin A is at least 5.5. If necessary, adjust the concentration of 2-methylpropan-2-ol in the mobile phase or reduce the flow rate to 1.5 or 1.0 ml per minute.

DETERMINATION OF CONTENT

Calculate the percentage content of erythromycin A using the chromatograms obtained with solutions (1) and (2) and from the declared content of $C_{37}H_{67}NO_{13}$ in *erythromycin A BPCRS*. Calculate the percentage contents of erythromycin B and erythromycin C using the chromatograms obtained with solutions (1) and (4) and from the declared contents of $C_{37}H_{67}NO_{12}$ and $C_{36}H_{65}NO_{13}$ in *erythromycin B BPCRS* and *erythromycin C BPCRS* respectively.

LABELLING

The label of the sealed container states the quantity of active ingredient contained in it in terms of the equivalent amount of erythromycin.

Erythromycin Stearate Tablets

General Notices

Action and use

Macrolide antibacterial.

DEFINITION

Erythromycin Stearate Tablets contain Erythromycin Stearate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

A. To a quantity of the powdered tablets containing the equivalent of 0.1 g of erythromycin add 10 ml of *water* and shake well. Decant the supernatant liquid and discard. Extract the residue by shaking with 10 ml of *methanol*, filter the extract and evaporate to dryness. The *infrared absorption spectrum*, Appendix II A, of the residue after drying at a pressure not exceeding 0.7 kPa is concordant with the *reference spectrum* of erythromycin stearate *(RS 127)*.

B. Dissolve a quantity of the powdered tablets containing the equivalent of 3 mg of erythromycin in 2 ml of *acetone* and add 2 ml of *hydrochloric acid*; an orange colour is produced which changes to red and then to deep violet–red. Add 2 ml of *chloroform* and shake; the chloroform layer becomes violet.

C. Extract a quantity of the powdered tablets containing the equivalent of 50 mg of erythromycin with 10 ml of *chloroform*, filter and evaporate to dryness. Heat 0.1 g of the residue gently with 5 ml of 2M *hydrochloric acid* and 10 ml of *water* until the solution boils; oily globules rise to the surface. Cool, remove the fatty layer, heat it with 3 ml of 0.1M *sodium hydroxide* and allow to cool; the solution sets to a gel. Add 10 ml of hot *water* and shake; the solution froths. To 1 ml add a 10% w/v solution of *calcium chloride*; a granular precipitate is produced which is insoluble in *hydrochloric acid*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a 2.722% w/v solution of *sodium acetate*, the pH of which has been adjusted to 5.0 with *glacial acetic acid* and rotate the paddle at 50 revolutions per minute. Transfer 5 ml of a filtered sample to a graduated flask, add 40 ml of *glacial acetic acid* and 10 ml of a 0.5% w/v solution of *4-dimethylaminobenzaldehyde* in *glacial acetic acid* and dilute to 100 ml with a mixture of 35 volumes of *glacial acetic acid* and 70 volumes of *hydrochloric acid*. Allow to stand for 15 minutes and measure the *absorbance* of the resulting solution at the maximum at 485 nm, Appendix II B, using in the reference cell dissolution medium that has been subjected to the conditions of the test. Prepare a suitable solution of *erythromycin stearate BPCRS* in the dissolution medium and filter. Transfer 5 ml of the filtered solution to a graduated flask, add 40 ml of *glacial acetic acid* and 10 ml of a 0.5% w/v solution of *4-dimethylaminobenzaldehyde* in *glacial acetic acid* and dilute to 100 ml with a mixture of 35 volumes of *glacial acetic acid* and 70 volumes of *hydrochloric acid*. Allow to stand for 15 minutes and measure the *absorbance* of the resulting solution at the maximum at 485 nm, Appendix II B, using in the reference cell dissolution medium that has been subjected to the conditions of the test. Calculate the total content of $C_{37}H_{67}NO_{13}$ in the medium from the *absorbances* obtained using the declared content of $C_{37}H_{67}NO_{13}$ in *erythromycin stearate BPCRS*.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing the equivalent of 25 mg of erythromycin as completely as possible in sufficient *methanol* to produce 100 ml and carry out the *microbiological assay of antibiotics* for erythromycin, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. Calculate the content of erythromycin in the tablets, taking each 1000 IU found to be equivalent to 1 mg of erythromycin. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

STORAGE

Erythromycin Stearate Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of erythromycin.

Estradiol and Norethisterone Acetate Tablets

General Notices

Action and use

Estrogen.

DEFINITION

Estradiol and Norethisterone Acetate Tablets contain Estradiol Hemihydrate and Norethisterone Acetate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of estradiol, $C_{18}H_{24}O_2$

95.0 to 105.0% of the stated amount.

Content of norethisterone acetate, $C_{22}H_{28}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of *dichloromethane* as the mobile phase, allowing the solvent front to ascend 8 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) add 0.2 ml of *water* to two tablets and shake to disperse. Add sufficient *ethanol (96%)* to produce a solution containing 0.035% w/v of Norethisterone Acetate, centrifuge and use the clear supernatant liquid. Solution (2) contains a suitable concentration of *estradiol hemihydrate BPCRS* and *norethisterone acetate BPCRS* in *ethanol (96%)*. After removal of the plate, allow it to dry in air and spray with *ethanolic sulphuric acid (5%)*. Heat the plate at 105° for 15 minutes and examine under *ultraviolet light (365 nm)*. The chromatogram obtained with solution (1) shows two clearly separated spots with R_f values corresponding to those observed in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows two peaks having the same retention times as the peaks due to estradiol and norethisterone acetate in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of a 0.3% w/v solution of *sodium lauryl sulphate* in *water* and rotate the paddle at 50 revolutions per minute. After 45 minutes, withdraw a sample of the medium and filter, discarding the first 5 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the filtered dissolution medium. For solution (2) dissolve a sufficient quantity of *estradiol hemihydrate BPCRS* and *norethisterone acetate BPCRS* in *methanol (80%)* and dilute an aliquot with a 0.3% w/v solution of *sodium lauryl sulphate* in *water*; the concentration of the final solution should be the same as that expected for solution (1). Solution (3) contains 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone EPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of 450 volumes of *water* and 550 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 235 nm.

Inject 200 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

Calculate the total content of estradiol, $C_{18}H_{24}O_2$, and norethisterone acetate, $C_{22}H_{28}O_3$, in the medium using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{22}H_{28}O_3$ in *norethisterone acetate BPCRS*.

Estrone and norethisterone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) powder 20 tablets. Add 20 ml of the mobile phase to a quantity of the powdered tablets containing the equivalent of 5 mg of estradiol, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 ml. Centrifuge and use the clear supernatant liquid. For solution (2) add 20 ml of the mobile phase to a quantity of the powdered tablets containing 2.5 mg of Norethisterone Acetate, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 ml. Centrifuge and use the clear supernatant liquid. Solution (3) contains 0.0001% w/v of *estrone EPCRS* in the mobile phase. Solution (4) contains 0.00005% w/v of *norethisterone BPCRS* in the mobile phase. Solution (5) contains 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone EPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

The chromatographic conditions described under Dissolution may be used.

Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to estrone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). In the chromatogram obtained with solution (2) the area of any peak corresponding to norethisterone is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%).

Uniformity of content

Comply with the requirements stated under Tablets using the following method of analysis. Tablets containing the equivalent of 2 mg or 2% w/w of estradiol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid* *chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of the mobile phase to one tablet, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 ml and centrifuge. Dilute the supernatant liquid if necessary, with the mobile phase to produce a solution containing the equivalent of 0.002% w/v of estradiol. For solution (2) dissolve sufficient quantities of *estradiol hemihydrate BPCRS* and *norethisterone acetate BPCRS* in the mobile phase and dilute an aliquot with the mobile phase; the concentrations in the final solution are the same as those expected for solution (1). Solution (3) contains 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone EPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

The chromatographic conditions described under Dissolution may be used.

Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

Calculate the contents of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{22}H_{28}O_3$ in *norethisterone acetate BPCRS*.

ASSAY

For estradiol

For tablets containing the equivalent of less than 2 mg or 2% w/w of estradiol Use the average of the 10 individual results obtained in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more or 2% w/w or more of estradiol Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of the mobile phase to a quantity of powdered tablets containing the equivalent of 10 mg of estradiol, mix with the aid of ultrasound and centrifuge. Dilute 10 ml of the clear supernatant liquid to 100 ml with the mobile phase. Solution (2) contains 0.002% w/v of *estradiol hemihydrate BPCRS* in the mobile phase. Solution (3) contains 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone EPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

The chromatographic conditions described under Dissolution may be used.

Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

Calculate the content of $C_{18}H_{24}O_2$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS*.

For norethisterone acetate

Use the average of the 10 individual results obtained in the test for Uniformity of content.

STORAGE

Estradiol and Norethisterone Acetate Tablets should be protected from light.

LABELLING

The label states the quantity of estradiol hemihydrate in terms of the equivalent amount of estradiol.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A. Estrone,

B. Norethisterone.

Estradiol and Norethisterone Tablets

General Notices

Action and use

Estrogen.

DEFINITION

Estradiol and Norethisterone Tablets contain Estradiol Hemihydrate and Norethisterone. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of estradiol, $C_{18}H_{24}O_2$

95.0 to 105.0% of the stated amount.

Content of norethisterone, $C_{20}H_{26}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *acetone* and 90 volumes of *dichloromethane* as the mobile phase, allowing the solvent front to ascend 8 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) add 0.2 ml of *water* to two tablets and shake to disperse. Add sufficient *ethanol (96%)* to produce a solution containing 0.035% w/v of Norethisterone, centrifuge and use the clear supernatant liquid. Solution (2) contains a suitable concentration of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in *ethanol (96%)*. After removal of the plate, allow it to dry in air and spray with *ethanolic sulphuric acid (5%)*. Heat the plate at 105° for 15 minutes and examine under *ultraviolet light (365 nm)*. The chromatogram obtained with solution (1) shows two clearly separated spots with R_f values corresponding to those observed in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows two peaks having the same retention times as those due to estradiol and norethisterone in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of a 0.3% w/v solution of *sodium lauryl sulphate* in *water* and rotate the paddle at 50 revolutions per minute. After 45 minutes, withdraw a sample of the medium and filter, discarding the first 5 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the filtered dissolution medium. For solution (2) dissolve a sufficient quantity of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in *methanol (80%)* and dilute an aliquot with a 0.3% w/v solution of *sodium lauryl sulphate* in *water*; the concentration of the final solution should be the same as that expected for solution (1). Solution (3) contains 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone EPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of 450 volumes of *water* and 550 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 235 nm.

Inject 200 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

Calculate the total content of estradiol, $C_{18}H_{24}O_2$, and norethisterone, $C_{20}H_{26}O_2$, in the medium using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and $C_{20}H_{26}O_2$ in *norethisterone BPCRS.*

Estrone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) powder 20 tablets. Add 20 ml of the mobile phase to a quantity of the powdered tablets containing the equivalent of 5 mg of estradiol, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 ml. Centrifuge and use the clear supernatant liquid. Solution (2) contains 0.0001% w/v of *estrone EPCRS* in the mobile phase. Solution (3) contains 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone EPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

The chromatographic conditions described under Dissolution may be used.

Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to estrone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Uniformity of content

Comply with the requirements stated under Tablets using the following method of analysis. Tablets containing the equivalent of 2 mg or 2% w/w of estradiol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of the mobile phase to one tablet, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 ml and centrifuge. Dilute the supernatant liquid, if necessary, with the mobile phase to produce a solution containing the equivalent of 0.002% w/v of estradiol. For solution (2) dissolve sufficient quantities of *estradiol hemihydrate BPCRS* and *norethisterone BPCRS* in the mobile phase and dilute an aliquot with the mobile phase; the concentrations in the final solution are the same as those expected for solution (1). Solution (3) contains 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone EPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

The chromatographic conditions described under Dissolution may be used.

Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

Calculate the content of $C_{18}H_{24}O_2$ and $C_{20}H_{26}O_2$ in each tablet using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS* and the declared content of $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

ASSAY

For estradiol

For tablets containing the equivalent of less than 2 mg or 2% w/w of estradiol Use the average of the 10 individual results obtained in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more or 2% w/w or more of estradiol Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of the mobile phase to a quantity of powdered tablets containing the equivalent of 10 mg of estradiol, mix with the aid of ultrasound and centrifuge. Dilute 1 volume of the clear supernatant liquid to 10 volumes with mobile phase. Solution (2) contains 0.002% w/v of *estradiol hemihydrate BPCRS* in the mobile phase. Solution (3) contains 0.0017% w/v of *estradiol hemihydrate BPCRS*, 0.00084% w/v of *norethisterone acetate BPCRS*, 0.00066% w/v of *estrone EPCRS* and 0.00034% w/v of *norethisterone BPCRS* in the mobile phase.

The chromatographic conditions described under Dissolution may be used.

Inject 20 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between each pair of peaks (estradiol and norethisterone, and estrone and norethisterone acetate) is at least 1.0.

Calculate the total content of estradiol, $C_{18}H_{24}O_2$, using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS*.

For norethisterone

Use the average of the 10 individual results obtained in the test for Uniformity of content.

STORAGE

Estradiol and Norethisterone Tablets should be protected from light.

LABELLING

The label states the quantity of estradiol hemihydrate in terms of the equivalent amount of estradiol.

IMPURITIES

The impurities limited by the requirements of this monograph include:

A. Estrone.

Estradiol Injection

General Notices

Action and use

Estrogen.

DEFINITION

Estradiol Injection is a sterile solution of Estradiol Benzoate in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these. It may contain suitable alcohols.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of estradiol benzoate, $C_{25}H_{28}O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 20 volumes of *ethyl acetate* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) add 10 ml of *2,2,4-trimethylpentane* to a volume of the injection containing 2 mg of Estradiol Benzoate and extract with three 10 ml quantities of *ethanol (70%)*. Wash the combined extracts with 15 ml of *2,2,4-trimethylpentane*, evaporate the ethanolic extract to dryness using a rotary evaporator and dissolve the residue in 2 ml of *chloroform*. Solution (2) contains 0.1% w/v of *estradiol benzoate BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air, spray with *ethanolic sulphuric acid (20%)*, heat at 105° for 10 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D.

For injections containing 0.2% w/v or more of estradiol benzoate

Dissolve 15 mg of *4-hydroxybenzaldehyde* (internal standard) in 5 ml of *1,4-dioxan,* add sufficient *cyclohexane* to produce 10 ml (solution A) and use the following solutions. For solution (1) add 1.0 ml of solution A to 10 mg of *estradiol benzoate BPCRS* and add sufficient of a mixture of 9 volumes of *cyclohexane* and 1 volume of *1,4-dioxan* to produce 10 ml. Prepare solution (2) in the same manner as solution (1) using a quantity of the injection containing 10 mg of Estradiol Benzoate but omitting the addition of solution A. Prepare solution (3) in the same manner as solution (1) using a quantity of the injection containing 10 mg of Estradiol Benzoate.

For injections containing less than 0.2% w/v of estradiol benzoate

Dissolve 15 mg of the internal standard in 10 ml of *1,4-dioxan*, add sufficient *cyclohexane* to produce 100 ml (solution B) and use the following solutions. For solution (1) add 10 ml of solution B to 10 mg of *estradiol benzoate BPCRS* and add sufficient of a mixture of 9 volumes of *cyclohexane* and 1 volume of *1,4-dioxan* to produce 100 ml. For solution (2) dilute a quantity of the injection containing 1 mg of Estradiol Benzoate to 10 ml with a mixture of 9 volumes of *cyclohexane* and 1 volume of *1,4-dioxan*. For solution (3) add 1 ml of solution B to a quantity of the injection containing 1 mg of Estradiol Benzoate and dilute to 10 ml with the same solvent mixture.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable), (b) a mixture of 1 volume of *1,4-dioxan* and 9 volumes of *cyclohexane* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

The assay is not valid unless the *resolution factor* between the peaks due to benzyl alcohol (if present) and estradiol benzoate and between the peaks due to estradiol benzoate and the internal standard is more than 1.5.

Calculate the content of $C_{25}H_{28}O_3$ using the declared content of $C_{25}H_{28}O_3$ in *estradiol benzoate BPCRS*.

STORAGE

Estradiol Injection should be protected from light. If solid matter separates on standing it should be redissolved by warming before use.

LABELLING

The label states (1) that the preparation is for intramuscular injection only; (2) that any solid matter that has separated on standing should be redissolved by warming before use.

Estradiol Transdermal Patches

General Notices

Action and use

Estrogen.

DEFINITION

Estradiol Transdermal Patches contain Estradiol Hemihydrate in a suitable matrix or reservoir presentation.

PRODUCTION

A suitable test is carried out to demonstrate the appropriate release of estradiol.

The transdermal patches comply with the requirements stated under Transdermal Patches and with the following requirements.

Content of estradiol, $C_{18}H_{24}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (100 mm × 100 mm) (Merck plates are suitable) and a mixture of 2 volumes of *acetone* and 8 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions and allow the solvent front to ascend 7 cm above the line of application. For solution (1) remove the release liner from a patch, score the exposed surface and cover the sticky surface with a small piece of glass wool. Place the patch in a centrifuge tube and add sufficient *methanol* to produce a solution containing the equivalent of 2 mg of estradiol in 5 ml of solvent, mix with the aid of ultrasound, shake for 15 minutes and, if necessary, filter through a glass-fibre filter paper (Whatman GF/C is suitable) followed by a 0.45-µm membrane filter. Solution (2) contains 0.04% w/v of *estradiol hemihydrate BPCRS* in *absolute ethanol*, dissolved with the aid of ultrasound. After removal of the plate, allow it to dry in a stream of warm air for 5 minutes, spray with *methanolic sulphuric acid (50%)* and heat at 110° for 10 minutes. Allow to cool and examine under *ultraviolet light (366 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds in colour, position and size to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For solution (1) remove the release liners from 10 patches, score the exposed surfaces and cover the sticky surfaces with small pieces of glass wool. Place the patches in a flask containing sufficient mobile phase to produce a solution containing the equivalent of 0.01% w/v of Estradiol, mix with the aid of ultrasound for 10 minutes, shake mechanically for 1 hour and filter through a glass-fibre filter paper (Whatman GF/C is suitable). If the filtrate is not clear, filter it through a membrane filter with a pore size of 0.45 µm. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecysilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2.0 ml per minute a mixture of 35 volumes of *acetonitrile* and 65 volumes of *water* and (c) a detection wavelength of 280 nm.

Inject 50 µl of each solution and allow the chromatography to proceed for twice the retention time of the principal peak. The test is not valid unless in the chromatogram obtained with solution (2) the *signal to-noise ratio* for the peak due to Estradiol not less than 10.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%). Disregard any peak due to diethyltoluamide.

Uniformity of content

Comply with the requirements stated under *uniformity of content*, Appendix XII C3, Test C, with respect to the individual content of each dosage unit and using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) remove the release liner from a patch, score the exposed surface and cover the sticky surface with a small piece of glass wool. Place the patch in a flask containing sufficient mobile phase to produce a solution containing the equivalent of 0.01% w/v of estradiol, mix with the aid of ultrasound for 10 minutes, shake mechanically for 1 hour and, if necessary, filter through a glass-fibre filter paper (Whatman GF/C is suitable). Using a disposable syringe, collect part of the supernatant liquid and filter through a membrane filter with a pore size of 0.45 µm. For solution (2) dilute 1 volume of a 0.1% w/v solution of *estradiol hemihydrate BPCRS* in *acetonitrile* to 10 volumes with the mobile phase.

Inject 20 µl of each solution.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{18}H_{24}O_2$ in the transdermal patch using the declared content of $C_{18}H_{24}O_2$ in *estradiol hemihydrate BPCRS*.

ASSAY

Use the average of the 10 results obtained in the test for Uniformity of content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of estradiol per patch.

Estramustine Phosphate Capsules

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Estramustine Phosphate Capsules contain Estramustine Sodium Phosphate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of estramustine phosphate, $C_{23}H_{32}Cl_2NO_6P$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 0.2 g of Estramustine Phosphate with 10 ml of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of estramustine sodium phosphate *(RS 128)*. In preparing the potassium bromide disc precautions should be taken to exclude moisture and avoid excessive grinding; if necessary heat the prepared disc at 90° for 2 minutes.

B. A 1% w/v solution of the residue obtained in test A yields the reactions characteristic *of sodium salts*, Appendix VI.

TESTS

Related substances

Comply with the test described under Estramustine Sodium Phosphate using for solutions (1) and (2) the residue obtained in test A for Identification in place of the substance being examined.

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing the equivalent of 0.28 g of Estramustine Phosphate with 20 ml of *water*, dilute to 50 ml with *water* and filter. Dilute 5 ml of the filtrate to 100 ml with *ethanol (50%)* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{23}H_{32}Cl_2NO_6P$ taking 15.4 as the value of A (1%, 1 cm) at the maximum at 275 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of estramustine phosphate.

Estriol Cream

General Notices

Action and use

Estrogen.

DEFINITION

Estriol Cream contains Estriol in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of estriol, $C_{18}H_{24}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 high performance precoated plate (Merck HPTLC silica gel 60 is suitable) and a mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) to a quantity of cream containing 2 mg of Estriol add 50 ml of *methanol*, heat on a water bath and mix until the cream has completely dispersed, cool the contents in ice, centrifuge and use the supernatant liquid. Solution (2) contains 0.004% w/v of *estriol BPCRS* in *methanol*. Solution (3) contains 0.004% w/v of each of *estriol BPCRS* and *prednisolone BPCRS* in *methanol*. Dry the plate in a horizontal position at 100 to 105°. Dilute 2 ml of *sulphuric acid* to 100 ml with *ethanol (96%)* and spray the solution evenly onto the plate. Examine the plate in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 4.5, Appendix V L.

Disperse 10 g of the cream in 10 ml of *water*, with continuous stirring throughout the pH determination.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to a quantity of cream containing 0.5 mg of Estriol add 20 ml of *methanol*, heat on a water bath and mix until the cream has completely dissolved, allow to cool to room temperature and add sufficient *methanol* to produce 25 ml. Cool the contents in ice for 15 minutes and centrifuge, use the supernatant liquid. For solution (2) dilute 5 volumes of solution (1) to 100 volumes with *methanol*, further dilute 10 volumes of this solution to 100 volumes with *methanol*. Solution (3) contains 0.01% w/v of *estriol impurity standard BPCRS* in *methanol*. For solution (4) dilute 10 volumes of solution (2) to 100 volumes with *methanol.*

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable) and maintained at 35°, (b) as the mobile phase, with a flow rate of 1 ml per minute, a mixture of 5 volumes of *methanol*, 38 volumes of *acetonitrile* and 57 volumes of *water* and (c) a detection wavelength of 280 nm.

Inject 10 µl of solutions (3) and (4). When the chromatograms are recorded under the prescribed conditions, the retention times for estriol and 16-epi-estriol are about 5 minutes and about 7 minutes respectively. The test is not valid unless, (a) in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to estriol and 16-epi-estriol is at least 2.5 and (b) in the chromatogram obtained with solution (4) the *signal-to-noise ratio* of the principal peak is at least 10.

Inject 10 µl of solution (1) and solution (2). In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). The sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to a quantity of cream containing 0.5 mg of Estriol add 20 ml of *methanol*, heat on a water bath and mix until the cream has completely dispersed, allow to cool to room temperature and add sufficient *methanol* to produce 25 ml, transfer to a centrifuge tube and cool the contents in ice for 15 minutes, centrifuge and dilute 5 volumes of the supernatant liquid to 100 volumes with *methanol*. Solution (2) contains 0.0001% w/v of *estriol BPCRS* in *methanol*. Solution (3) contains 0.01% w/v of *estriol impurity standard BPCRS* in *methanol*.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to estriol and 16-epi-estriol is at least 2.5.

Calculate the content of $C_{18}H_{24}O_3$ in the cream using the declared content of $C_{18}H_{24}O_3$ in *estriol BPCRS.*

Estropipate Tablets

General Notices

Action and use

Estrogen.

DEFINITION

Estropipate Tablets contain Estropipate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of estropipate, $C_{18}H_{22}O_5S,C_4H_{10}N_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Free estrone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 5 mg of Estropipate with 20 ml of *methanol (50%)* for 30 minutes, centrifuge and filter the supernatant liquid. Solution (2) contains 0.0005% w/v of *estrone EPCRS* in *methanol (50%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 35 volumes of *acetonitrile* and 65 volumes of 0.025M *potassium dihydrogen orthophosphate* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 213 nm. The peak due to estrone has a retention time, relative to the peak due to estropipate, of about 5.

In the chromatogram obtained with solution (1), the area of any peak corresponding to estrone is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

Tablets containing less than 2 mg of estropipate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of *water* to one tablet and shake for 30 minutes, add 20 ml of *methanol* to the mixture and shake for a further 30 minutes, dilute to 50 ml with *methanol (50%)* and filter. Dilute 10 ml of the filtrate to 20 ml with *methanol (50%)*. Solution (2) contains 0.0015% w/v of *estropipate BPCRS* in *methanol (50%)*.

The chromatographic procedure described under Free estrone may be used.

Calculate the content of $C_{18}H_{22}O_5S,C_4H_{10}N_2$ in each tablet using the declared content of $C_{18}H_{22}O_5S,C_4H_{10}N_2$ in *estropipate BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 3 mg of Estropipate with 40 ml of *methanol (50%)* for 30 minutes, dilute to 100 ml with the same solvent and filter. Solution (2) contains 0.003% w/v of *estropipate BPCRS* in *methanol (50%)*.

The chromatographic procedure described under Free estrone may be used.

Calculate the content of $C_{18}H_{22}O_5S,C_4H_{10}N_2$ in the tablets using the declared content of $C_{18}H_{22}O_5S,C_4H_{10}N_2$ in *estropipate BPCRS*.

Ethambutol Tablets

General Notices

Action and use

Antituberculosis drug.

DEFINITION

Ethambutol Tablets contain Ethambutol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ethambutol hydrochloride, $C_{10}H_{24}N_2O_2$,2HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 50 mg of ethambutol hydrochloride with 5 ml of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ethambutol hydrochloride *(RS 134)*.

B. Shake a quantity of the powdered tablets containing 0.1 g of ethambutol hydrochloride with 10 ml of *water*, filter and add to the filtrate 2 ml of a 1% w/v solution of *copper(II) sulphate* followed by 1 ml of 1M *sodium hydroxide*. A blue colour is produced.

2-Aminobutanol

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of 13.5M *ammonia*, 15 volumes of *water* and 75 volumes of *methanol* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.50 g of ethambutol hydrochloride for 5 minutes with sufficient *methanol* to produce 10 ml. Solution (2) contains 0.050% w/v of *2-aminobutan-1-ol* in *methanol*. After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes, cool, spray with *ninhydrin solution R1* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Add 20 ml of 2M *sodium hydroxide* to a quantity of the powdered tablets containing 0.2 g of ethambutol hydrochloride, mix with the aid of ultrasound for 5 minutes and extract with three successive 25 ml quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *propan-2-ol*. Filter each extract successively through the same filter consisting of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with a mixture of 3 volumes of *chloroform* and 1 volume of *propan-2-ol* and wash the plug with 10 ml of the same solvent mixture. Add 100 ml of *anhydrous acetic acid* to the combined extracts and washings and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 13.86 mg of $C_{10}H_{24}N_2O_2$,2HCl.

Ethanolamine Oleate Injection

General Notices

DEFINITION

Ethanolamine Oleate Injection is a sterile solution containing 5% w/v of Ethanolamine Oleate, prepared by the interaction of Ethanolamine and Oleic Acid, in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of oleic acid, $C_{18}H_{34}O_2$

3.9 to 4.5% w/v.

Content of ethanolamine, C_2H_7NO

0.85 to 0.95% w/v.

TESTS

Alkalinity

pH, 8.0 to 9.0, Appendix V L.

ASSAY

For oleic acid

To 10 ml add 20 ml of 0.05M *sulphuric acid VS* and extract with three 25 ml volumes of *chloroform*, washing each extract with the same 10 ml of *water*. Reserve the aqueous solution and washings for the Assay for ethanolamine. Evaporate the combined extracts to dryness, dissolve the residue in *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 28.25 mg of $C_{18}H_{34}O_2$.

For ethanolamine

Titrate the excess of acid in the combined aqueous solution and washings reserved in the Assay for oleic acid with 0.1M *sodium hydroxide VS* using *methyl orange solution* as indicator. Each ml of 0.05M *sulphuric acid VS* is equivalent to 6.108 mg of C_2H_7NO.

STORAGE

Ethanolamine Oleate Injection should be protected from light.

LABELLING

The label states (1) that the preparation contains 5% of ethanolamine oleate; (2) that the preparation is intended for intravenous injection.

Ethinylestradiol Tablets

General Notices

Action and use

Estrogen.

DEFINITION

Ethinylestradiol Tablets contain Ethinylestradiol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ethinylestradiol, $C_{20}H_{24}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (96%)* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 mg of Ethinylestradiol with four 20 ml quantities of *acetone*, filter each extract in turn, evaporate the combined filtrates to dryness on a water bath in a current of *nitrogen* and dissolve the residue in 0.25 ml of *acetone*. Solution (2) contains 0.1% w/v of *ethinylestradiol BPCRS* in *acetone*. After removal of the plate, allow it to dry in air, spray with *ethanolic sulphuric acid (20%)*, heat at 110° for 10 minutes and examine under *ultraviolet light (365 nm)* and in daylight. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Triturate a quantity of the powdered tablets containing 0.1 mg of Ethinylestradiol with 0.5 ml of 0.1M *sodium hydroxide* and 5 ml of *water*, allow to stand for 5 minutes, filter, acidify the filtrate with 0.15 ml of *sulphuric acid*, add 3 ml of *ether*, shake and allow to separate. Evaporate the ether layer to dryness and heat the residue on a water bath for 5 minutes with 0.2 ml of *glacial acetic acid* and 2 ml of *orthophosphoric acid*. A pink colour with an intense orange fluorescence is produced.

TESTS

Uniformity of content

Tablets containing less than 2 mg of Ethinylestradiol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0025% w/v of *ethinylestradiol BPCRS* in the mobile phase. For tablets containing less than 50 µg of Ethinylestradiol, solution (1) contains 0.0005% w/v of *ethinylestradiol BPCRS* in the mobile phase. For solution (2) add 2 ml of the mobile phase to one tablet, allow to stand for 5 minutes, mix with the aid of ultrasound for 5 minutes, centrifuge and use the clear supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax ODS is suitable), (b) a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 280 nm.

Calculate the content of $C_{20}H_{24}O_2$ using the declared content of $C_{20}H_{24}O_2$ in *ethinylestradiol BPCRS*.

ASSAY

Use the average of the 10 individual results obtained in the test for Uniformity of content.

STORAGE

Ethinylestradiol tablets should be protected from light.

Ethosuximide Capsules

General Notices

Action and use

Antiepileptic.

DEFINITION

Ethosuximide Capsules contain Ethosuximide.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ethosuximide, $C_7H_{11}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Heat a quantity of the contents of the capsules containing 0.1 g of Ethosuximide with 0.2 g of *resorcinol* and 0.1 ml of *sulphuric acid* at 140° for 5 minutes, add 5 ml of *water*, make alkaline with 5M *sodium hydroxide* and add 0.2 ml to a large volume of *water*. A bright green fluorescence is produced.

B. To a quantity of the contents of the capsules containing 0.5 g of Ethosuximide add 15 ml of a 40% w/v solution of *sodium hydroxide*. Boil under a reflux condenser for 30 minutes, cool, acidify with *hydrochloric acid* and extract with three 30 ml quantities of *ether*. Wash the combined extracts with 5 ml of *water* and evaporate to dryness. The *melting point* of the residue, after recrystallisation from *toluene* and *petroleum spirit (boiling range, 40° to 60°)*, is about 102°, Appendix V A.

ASSAY

Weigh 20 capsules. Open the capsules carefully without loss of shell material, express as much of the contents as possible and reserve the expressed material. Wash the shells with *ether*, discard the washings, allow the shells to stand at room temperature until the ether has evaporated and weigh. The difference between the weights represents the weight of the total contents. Dissolve a quantity of the contents containing 0.2 g of Ethosuximide in 30 ml of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using *magneson solution* as indicator and 0.1M *tetrabutylammonium hydroxide VS* as titrant. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 14.12 mg of $C_7H_{11}NO_2$.

Ethosuximide Oral Solution

General Notices

Action and use

Antiepileptic.

DEFINITION

Ethosuximide Oral Solution is a solution of Ethosuximide in a suitable flavoured vehicle.
The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ethosuximide, $C_7H_{11}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity containing 0.5 g of Ethosuximide with two 30 ml quantities of *chloroform*, filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Heat 0.1 g of the residue with 0.2 g of *resorcinol* and 0.1 ml of *sulphuric acid* at 140° for 5 minutes, cool, add 5 ml of *water*, make alkaline with 5M *sodium hydroxide* and add 0.2 ml to a large volume of *water*. A bright green fluorescence is produced.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as that due to *ethosuximide BPCRS* in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 2 ml of a 3.0% w/v solution of *dimethyl phthalate* (internal standard) in *chloroform* to 25 ml of a 0.2% w/v solution of *ethosuximide BPCRS* in *chloroform*. Prepare solution (2) in the same manner as solution (3) but omit the internal standard. For solution (3) add 10 ml of *water* and 2 g of *sodium hydrogen carbonate* to a weighed quantity of the oral solution containing 0.25 g of Ethosuximide and extract with five 25 ml quantities of *chloroform*, washing each extract with the same 10 ml of *water*. To the combined extracts add 10 ml of the internal standard solution, shake with 10 g of *anhydrous sodium sulphate* and filter.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225 is suitable) and maintained at 165°.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_7H_{11}NO_2$, weight in volume, using the declared content of $C_7H_{11}NO_2$ in *ethosuximide BPCRS*.

Etodolac Capsules

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Etodolac Capsules contain Etodolac.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of etodolac $C_{17}H_{21}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the contents of the capsules containing about 0.1 g of Etodolac add 4 ml of 0.01M *hydrochloric acid* and mix with the aid of ultrasound for 5 minutes, shaking occasionally, centrifuge for 10 minutes, discard the supernatant liquid and wash the residue with 4 ml of *water*. Shake to disperse, centrifuge for 10 minutes and discard the supernatant liquid. Add 4 ml of 0.01M *sodium hydroxide* to the residue and mix with the aid of ultrasound for 5 minutes, shaking occasionally and centrifuge for 10 minutes. Transfer the supernatant liquid to a second centrifuge tube, add about 1 ml of 0.1M *hydrochloric acid* (the pH of the supernatant liquid should be 2 or less). Centrifuge for 10 minutes, discard the supernatant liquid and wash the residue with 4 ml of *water*, shake to disperse and centrifuge for 10 minutes. Discard the supernatant liquid and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of etodolac *(RS 139)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to etodolac in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of *phosphate buffer pH 7.5* and rotating the basket at 100 revolutions per minute. Withdraw a 10 ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 278 nm, Appendix II B. Measure the *absorbance* of a suitable solution of *etodolac BPCRS* prepared by dissolving 30 mg in 100 ml of the dissolution medium and diluting to a suitable volume with the dissolution medium and using dissolution medium in the reference cell. Calculate the total content of etodolac, $C_{17}H_{21}NO_3$ in the medium from the absorbances obtained and from the declared content of $C_{17}H_{21}NO_3$ in *etodolac BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable), previously activated by heating at 105° for 1 hour, and a mixture of 0.5 volumes of *glacial acetic acid*, 30 volumes of *absolute ethanol* and 70 volumes of *toluene* as the mobile phase. Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes. Apply separately to the plate 10 µl of each of the following solutions in *acetone*. For solution (1) shake a quantity of the contents of the capsules containing 0.2 g of Etodolac with 20 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *acetone* and for solution (3) dilute 1 volume of solution (2) to 2 volumes with *acetone.* After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.25%).

Etodolac acid dimer

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable), previously activated by heating at 105° for 1 hour, and a mixture of 3 volumes of *glacial acetic acid*, 17 volumes of *1,4-dioxan* and 60 volumes of *toluene* as the mobile phase. Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.6 g of Etodolac with 20 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter. Solution (2) contains 0.003% w/v of *etodolac acid dimer BPCRS* in *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* Any *secondary spot* in the chromatogram obtained with solution (1) corresponding to the acid dimer is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%).

Total methyl analogue impurities

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Etodolac with 40 ml of *methanol*, mix with the aid of ultrasound for 5 minutes, filter and dilute 10 ml of the filtrate to 25 ml with *water*. For solution (2) dilute 1 volume of a solution containing 0.025% w/v each of *etodolac 1-methyl analogue BPCRS* and *etodolac 8-methyl analogue BPCRS* in *methanol* to 50 volumes with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 13 volumes of *acetonitrile*, 19 volumes of *methanol* and 68 volumes of a 1.74% w/v solution of *dipotassium hydrogen orthophosphate* as the mobile phase with a flow rate of 1.0 ml per minute and (c) a detection wavelength of 225 nm. Adjust the mobile phase, if necessary, to give a retention time of 14 to 20 minutes for etodolac. The order of elution of peaks in the chromatogram obtained with solution (2) is etodolac 8-methyl analogue and etodolac 1-methyl analogue. For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two methyl analogue impurities is at least 0.75.

Calculate the percentage content of etodolac 1-methyl analogue and etodolac 8-methyl analogue in etodolac by reference to the corresponding peaks in the chromatogram obtained with solution (2). The total content is not greater than 1.0%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 50 mg of Etodolac with about 70 ml of 0.1M *sodium hydroxide* for 30 minutes, dilute to 100 ml with 0.1M *sodium hydroxide*, mix and filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 2 ml of the filtrate to 100 ml with the mobile phase. For solution (2) dilute 2 ml of a 0.05% w/v solution of *etodolac BPCRS* in 0.1M *sodium hydroxide* to 100 ml with the mobile phase. For solution (3) add 2 ml of a 0.05% w/v solution of *etodolac 1-methyl analogue BPCRS* in 0.1M *sodium hydroxide* to 2 ml of a 0.05% w/v solution of *etodolac BPCRS* in 0.1M *sodium hydroxide* and dilute to 100 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 45 volumes of *acetonitrile* and 55 volumes of *phosphate buffer pH 4.75* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 225 nm.

The test is not valid unless the *resolution factor* between etodolac and etodolac 1-methyl analogue in the chromatogram obtained with solution (3) is at least 1.5.

Calculate the content of $C_{17}H_{21}NO_3$ using the declared content of $C_{17}H_{21}NO_3$ in *etodolac BPCRS*.

Etodolac Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Etodolac Tablets contain Etodolac.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of etodolac $C_{17}H_{21}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of etodolac with 30 ml of *hexane* for 5 minutes, centrifuge, discard the clear hexane layer and add about 40 ml of *ether* to the residue; shake for 5 minutes, centrifuge for 5 minutes, decant the ether layer and filter if necessary. Evaporate the solution to dryness under nitrogen and add about 5 ml of 0.1M *hydrochloric acid* to the residue. Warm on a water bath until the residue begins to crystallise and triturate with a glass rod to promote crystallisation. Cool the mixture in an ice bath, filter through a glass-fibre filter (Whatman GF/C is suitable) and dry the crystals at a pressure of 2 kPa at 60° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of etodolac *(RS 139)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to etodolac in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of *phosphate buffer pH 7.5* and rotating the basket at 100 revolutions per minute. Withdraw a 10 ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 278 nm, Appendix II B. Measure the *absorbance* of a suitable solution of *etodolac BPCRS* prepared by dissolving 20 mg in 100 ml of the dissolution medium and diluting to a suitable volume with the dissolution medium and using dissolution medium in the reference cell. Calculate the total content of etodolac, $C_{17}H_{21}NO_3$ in the medium from the absorbances obtained and from the declared content of $C_{17}H_{21}NO_3$ in *etodolac BPCRS*.

Related substances

Comply with the test described under Etodolac Capsules using a quantity of the powdered tablets containing 0.2 g of etodolac in place of the contents of the capsules.

Etodolac acid dimer

Comply with the test described under Etodolac Capsules using a quantity of the powdered tablets containing 0.6 g of etodolac in place of the contents of the capsules.

Total methyl analogue impurities

Comply with the test described under Etodolac Capsules but preparing solution (1) in the following manner. Shake a quantity of the powdered tablets containing 0.1 g of etodolac with 40 ml of *methanol*, mix with the aid of ultrasound for 5 minutes, filter and dilute 10 ml of the filtrate to 25 ml with *water*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of etodolac with about 70 ml of 0.1M *sodium hydroxide* for 30 minutes, dilute to 100 ml with 0.1M *sodium hydroxide*, mix, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 2 ml of the resulting solution to 100 ml with the mobile phase. For solution (2) dilute 2 ml of a 0.05% w/v solution of *etodolac BPCRS* in 0.1M *sodium hydroxide* to 100 ml with the mobile phase. For solution (3) add 2 ml of a 0.05% w/v solution of *etodolac 1-methyl analogue BPCRS* in 0.1M *sodium hydroxide* to 2 ml of a 0.05% w/v solution of *etodolac BPCRS* in 0.1M *sodium hydroxide* and dilute to 100 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 45 volumes of *acetonitrile* and 55 volumes of *phosphate buffer pH 4.75* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 225 nm.

The test is not valid unless the *resolution factor* between etodolac and etodolac 1-methyl analogue in the chromatogram obtained with solution (3) is at least 1.5.

Calculate the content of $C_{17}H_{21}NO_3$ using the declared content of $C_{17}H_{21}NO_3$ in *etodolac BPCRS*.

Etoposide Capsules

General Notices

Action and use

Inhibitor of DNA topoisomerase type II; cytotoxic.

DEFINITION

Etoposide Capsules contain a solution of Etoposide in a suitable water-miscible vehicle.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of etoposide, $C_{29}H_{32}O_{13}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Add a quantity of the contents of the capsules containing 0.1 g of Etoposide to a separating funnel containing 100 ml of *water*, extract with two 20 ml quantities of *dichloromethane*, dry the combined organic extracts over *anhydrous sodium sulphate* and filter. Extract the filtrate with 30 ml of *water*, filter the dichloromethane layer through *anhydrous sodium sulphate* and evaporate to dryness at 25° to 35° under reduced pressure. Dissolve the oily residue in 5 ml of *water*, shake gently and allow to stand for 30 minutes. Filter through a sintered-glass funnel, wash the precipitate in the funnel with three 20 ml quantities of *water* and dry the precipitate in the funnel at 40° at a pressure of 2 kPa for 90 minutes. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of etoposide (*RS 396*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a pH 4.5 buffer prepared by dissolving 2.99 g of *sodium acetate* and 14 ml of 2M *acetic acid* in sufficient *water* to produce 1000 ml and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the filtrate diluted, if necessary, with dissolution medium to produce a solution expected to contain about 0.005% w/v of Etoposide. Solution (2) contains 0.005% w/v of *etoposide EPCRS* in the dissolution medium. Solution (3) contains 0.005% w/v of *etoposide EPCRS* and 0.00025% w/v of *ethyl parahydroxybenzoate* in the dissolution medium.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *phenyl silica gel for chromatography* (10 µm) (µBondapak phenyl is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 26 volumes of *acetonitrile* and 74 volumes of a 0.272% w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid* and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the total content of etoposide, $C_{29}H_{32}O_{13}$, in the medium using the declared content of $C_{29}H_{32}O_{13}$ in *etoposide EPCRS*.

***cis*-Etoposide**

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 80 ml of the mobile phase to 10 whole capsules and stir mechanically for 15 minutes with occasional shaking. Add sufficient mobile phase to produce 100 ml and stir for a further 5 minutes. Dilute, if necessary, a volume of the solution with the mobile phase to give a solution containing 0.5% w/v of Etoposide; use immediately. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase. For solution (3) prepare a 0.5% w/v solution of *etoposide EPCRS* in a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

The chromatographic procedure described under Dissolution may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the principal peak and the peak immediately following the principal peak (*cis*-etoposide) is at least 1.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *cis*-etoposide is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 400 ml of the mobile phase to 10 whole capsules and stir mechanically for 15 minutes with occasional shaking. Add sufficient mobile phase to produce 500 ml and stir for a further 5 minutes. Dilute a volume of the solution with the mobile phase to give a solution containing 0.04% w/v of Etoposide. Solution (2) contains 0.04% w/v of *etoposide EPCRS* in the mobile phase. Solution (3) contains 0.005% w/v of *etoposide EPCRS* and 0.00025% w/v of *ethyl parahydroxybenzoate* in the mobile phase.

The chromatographic procedure described under Dissolution may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the content of $C_{29}H_{32}O_{13}$ in the capsules from the chromatograms obtained and using the declared content of $C_{29}H_{32}O_{13}$ in *etoposide EPCRS*.

STORAGE

Etoposide capsules should not be refrigerated.

Etoposide Intravenous Infusion

General Notices

Action and use

Inhibitor of DNA topoisomerase type II; cytotoxic.

DEFINITION

Etoposide Intravenous Infusion is a sterile solution of Etoposide. It is prepared immediately before use in accordance with the manufacturer's instructions from Sterile Etoposide Concentrate.

The intravenous infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Etoposide Intravenous Infusion should be used immediately after preparation.

STERILE ETOPOSIDE CONCENTRATE

DEFINITION

Sterile Etoposide Concentrate is a sterile solution of Etoposide in a suitable ethanolic vehicle.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of etoposide, $C_{29}H_{32}O_{13}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck plates are suitable) and a mixture of 0.5 volume of *water*, 2.5 volumes of *ethanol (96%)*, 25 volumes of *acetone* and 80 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 17 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a volume of the concentrate containing 20 mg of Etoposide to 25 ml with a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol*. Solution (2) contains 0.08% w/v of *etoposide EPCRS* in a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

Dilute a volume of the concentrate containing 0.1g of Etoposide to 50 ml with *carbon dioxide-free water*. The pH of the solution is 3.0 to 4.0, Appendix V L.

cis-Etoposide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the concentrate with the mobile phase to produce a solution containing 0.5% w/v of Etoposide. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase. For solution (3) prepare a 0.5% w/v solution of *etoposide EPCRS* in a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *phenyl silica gel for chromatography* (10 µm) (µBondapak phenyl is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 26 volumes of *acetonitrile* and 74 volumes of a 0.272% w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid* and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the principal peak and the peak immediately following the principal peak (*cis*-etoposide) is at least 1.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *cis*-etoposide is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the concentrate with the mobile phase to give a solution containing 0.04% w/v of Etoposide. Solution (2) contains 0.04% w/v of *etoposide EPCRS* in the mobile phase.

The chromatographic procedure described under *cis*-Etoposide may be used.

Calculate the content of $C_{29}H_{32}O_{13}$ in the concentrate from the chromatograms obtained and using the declared content of $C_{29}H_{32}O_{13}$ in *etoposide EPCRS*.

STORAGE

Sterile Etoposide Concentrate should be protected from light.

LABELLING

The label states (1) 'Sterile Etoposide Concentrate'; (2) that the diluted injection is to be given by intravenous injection; (3) that the concentrate should be protected from light.

Gastro-resistant Erythromycin Tablets

General Notices

Erythromycin Tablets

Action and use

Macrolide antibacterial.

DEFINITION

Gastro-resistant Erythromycin Tablets contain Erythromycin. They are made gastro-resistant by enteric-coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of erythromycins, calculated as the sum of erythromycin A ($C_{37}H_{67}NO_{13}$), erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$)

90.0 to 110.0% of the stated amount of Erythromycin.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Erythromycin with 5 ml of *dichloromethane*, decolourise, if necessary, with *activated charcoal*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, after drying at a pressure not exceeding 0.7 kPa, is concordant with the *reference spectrum* of erythromycin *(RS 123)*.

B. Dissolve a quantity of the powdered tablets containing 3 mg of Erythromycin as completely as possible in 2 ml of *acetone* and add 2 ml of *hydrochloric acid*; an orange colour is produced which changes to red and then to deep purplish red. Add 2 ml of *chloroform* and shake; the chloroform layer becomes purple.

TESTS

Disintegration

Tablets covered with a gastro-resistant coating comply with the requirements stated under Tablets but operating the apparatus for 1 hour in 0.1M *hydrochloric acid*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1), (2), (3), (4) and (6) described under Assay.

The chromatographic conditions described under Assay may be used.

Inject 0.1 ml of each solution. For solution (1) allow the chromatography to proceed for 5 times the retention time of the peak corresponding to erythromycin A.

When the chromatograms are recorded using the prescribed conditions the retention time of erythromycin A is about 15 minutes. The retention times relative to erythromycin A are: impurity A, about 0.3; impurity B, about 0.45; erythromycin C, about 0.5; impurity C, about 0.9; impurity D, about 1.4; impurity F, about 1.5; erythromycin B, about 1.8; impurity E, about 4.3.

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities E and F using solution (6) and multiply the areas of these peaks by the corresponding correction factors: impurity E, 0.09; impurity F, 0.15. In the chromatogram obtained with solution (1) the area of any peak other than those peaks corresponding to erythromycin A, erythromycin B and erythromycin C, identified from the peaks in the chromatograms obtained with solutions (2) and (3), is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (3%) and the sum of the areas of any such peaks is not greater than 2.3 times the area of the principal peak in the chromatogram obtained with solution (4) (7%). Disregard any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with solution (4) (0.06%).

The content of each of erythromycin B and erythromycin C, as determined under Assay, is not more than 5%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Weigh and powder 20 tablets (if necessary, remove the coating from 20 tablets using a sharp blade, taking care not to damage the cores, weigh the cores and powder). For solution (1) dissolve a quantity of the powdered tablets containing 40 mg of Erythromycin in 10 ml of a mixture of 1 volume of *methanol* and 3 volumes of *citro-phosphate buffer pH 7.0*, filter and use the filtrate. Solution (2) contains 0.4% w/v of *erythromycin A BPCRS* in a mixture of 1 volume of *methanol* and 3 volumes of *citro-phosphate buffer pH 7.0* (solvent A). Solution (3) contains 0.02% w/v of each of *erythromycin B BPCRS* and *erythromycin C BPCRS* in solvent A. Solution (4) contains 0.012% w/v of *erythromycin A BPCRS* in solvent A. For solution (5) dissolve 5 mg of N*-demethylerythromycin A EPCRS* in solution (3), add 1 ml of solution (2) and sufficient of solution (3) to produce 25 ml. For solution (6) transfer 40 mg of *erythromycin A BPCRS* to a glass vial and spread evenly such that it forms a layer not more than about 1 mm thick. Heat at 130° for 4 hours, allow to cool and dissolve in sufficient of solvent A to produce 10 ml (generation of impurities E and F). The solutions can be used within one day if stored at 5°.

The chromatographic procedure may be carried out using (a) a column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene copolymer* (8 µm) with a pore size of 100 nm (PLRP-S is suitable), (b) as mobile phase at a flow rate of 2.0 ml per minute a solution prepared in the following manner: to 50 ml of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 9.0 with 1M *orthophosphoric acid* add 400 ml of *water*, 165 ml of *2-methylpropan-2-ol* and 30 ml of *acetonitrile* and dilute to 1000 ml with *water* and (c) a detection wavelength of 215 nm. Maintain the temperature of the column at 70° using a water bath for the column and at least one third of the tubing preceding the column.

Inject 0.1 ml of solution (5). Adjust the sensitivity of the detector so that the height of the peaks is at least 25% of the full scale of the recorder. The substances are eluted in the following order: *N*-demethylerythromycin A, erythromycin C, erythromycin A and erythromycin B. The test is not valid unless the *resolution factor* between the peaks corresponding to *N*-demethylerythromycin A and erythromycin C is at least 0.8 and the *resolution factor* between the peaks corresponding to *N*-demethylerythromycin A and erythromycin A is at least 5.5. If necessary, adjust the concentration of 2-methylpropan-2-ol in the mobile phase (180 ml has been found to be suitable) or reduce the flow rate to 1.5 or 1.0 ml per minute.

Inject alternately 0.1 ml of solutions (1), (2) and (3). Calculate the content of erythromycin A using the chromatograms obtained with solutions (1) and (2) and from the declared content of $C_{37}H_{67}NO_{13}$ in *erythromycin A BPCRS*. Calculate the contents of erythromycin B and erythromycin C using the chromatograms obtained with solutions (1) and (3) and from the declared contents of $C_{37}H_{67}NO_{12}$ and $C_{36}H_{65}NO_{13}$ in *erythromycin B BPCRS* and *erythromycin C BPCRS* respectively.

STORAGE

Gastro-resistant Erythromycin Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Erythromycin.

Famotidine Tablets

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

DEFINITION

Famotidine Tablets contain Famotidine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of famotidine, $C_8H_{15}N_7O_2S_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Fischer Silica Gel GF plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia*, 20 volumes of *toluene*, 25 volumes of *methanol* and 40 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 40 mg of Famotidine with 4 ml of *glacial acetic acid* with the aid of ultrasound, dilute to 10 ml with the same solvent, centrifuge and use the clear supernatant liquid. Solution (2) contains 0.4% w/v of *famotidine BPCRS* in *glacial acetic acid*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Rotate the paddle at 50 revolutions per minute and use as the medium 900 ml of phosphate buffer pH 4.5 prepared in the following manner. Dissolve 13.61 g of *potassium dihydrogen orthophosphate* in *water*, adjust the pH of the solution with *orthophosphoric acid* or 1M *potassium hydroxide* as necessary and add sufficient *water* to produce 1000 ml. Withdraw a sample of 10 ml of the medium, filter and centrifuge at 2000 revolutions per minute for 20 minutes. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.001% w/v of *famotidine BPCRS* in the dissolution medium. For solution (2) use the filtered and centrifuged dissolution medium, diluted if necessary, to give a solution expected to contain about 0.001% w/v of Famotidine.

The chromatographic procedure described under Related substances may be used. Inject 50 µl of each solution.

Calculate the total content of famotidine, $C_8H_{15}N_7O_2S_3$, in the medium using the declared content of $C_8H_{15}N_7O_2S_3$ in *famotidine BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 200 ml of 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 6.0 with 1M *potassium hydroxide* (solution A) to a quantity of whole tablets containing 0.2 g of Famotidine, mix with the aid of ultrasound for 5 minutes, add about 200 ml of *methanol*, shake for 60 minutes and add sufficient solution A to produce 500 ml. For solution (2) dilute 1 volume of solution (1) to 100 volumes with solution A. For solution (3) dilute 1 volume of solution (1) to 10 volumes with solution A and dilute 1 volume of the resulting solution to 50 volumes with solution A. For solution (4) add 40 ml of *acetonitrile* to a mixture of 2 mg of each of *famotidine impurity C BPCRS*, *famotidine degradation impurity 1 BPCRS* and *famotidine degradation impurity 2 BPCRS,* mix with the aid of ultrasound for 2 minutes, cool, add 40 ml of *methanol* and sufficient solution A to produce 200 ml. Dilute 1 volume of the resulting solution to 5 volumes with solution A. For solution (5) dissolve 8 mg of *famotidine BPCRS* in 15 ml of solution A with the aid of ultrasound, cool and add sufficient solution A to produce 20 ml (solution B); to 1 ml of this solution add 0.05 ml of *hydrogen peroxide solution (10 vol)* *(*generates famotidine degradation impurity 3). For solution (6) dilute 1 volume of solution B with 100 volumes of solution A and further dilute a suitable volume with an equal volume of solution (4).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Inertsil ODS-2 is suitable) maintaining the column temperature at 40°, (b) a mixture of 7 volumes of *acetonitrile* and a mixture of 93 volumes of 0.1M *sodium acetate* containing 0.1% v/v of *triethylamine* the pH adjusted to 6.0 with *glacial acetic acid* and as the mobile phase with a flow rate of 1.4 ml per minute and (c) a detection wavelength of 275 nm. Condition the column for approximately 30 minutes before the first injection.

Inject separately 50 µl of each solution. The substances are eluted in the following order: famotidine degradation impurity 3, famotidine degradation impurity 1, famotidine impurity C, famotidine and famotidine degradation impurity 2. The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the peaks due to famotidine and famotidine impurity C is at least 1.4 and that between the peaks due to famotidine and famotidine degradation impurity 2 is at least 1.4. If this resolution is not achieved adjust the content of acetonitrile or decrease the concentration of sodium acetate in the mobile phase.

In the chromatogram obtained with solution (1) the areas of any peaks corresponding to famotidine impurity C or famotidine degradation impurities 1 and 2 are not greater than the areas of the corresponding peaks in the chromatogram obtained with solution (4) (0.5% of each), the area of any peak with a relative retention time of about 0.4 with reference to famotidine in the chromatogram obtained with solution (5) (famotidine degradation impurity 3) is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%). Calculate the contents of each of impurity C and degradation impurities 1 and 2 using the chromatogram obtained with solution (4) and the nominal content of any other impurities using the chromatograms obtained with solution (2) and (3). The sum of the contents of all the impurities so calculated is not greater than 2.5%. Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

ASSAY

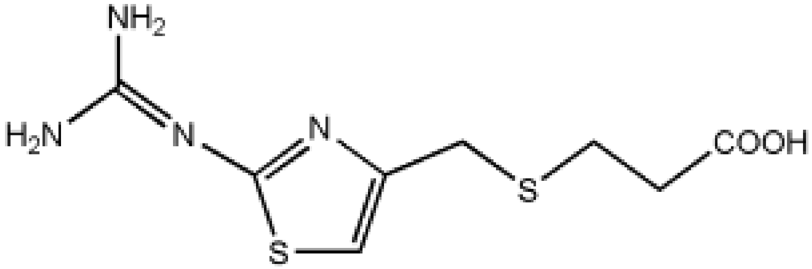
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 200 ml of 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 6.0 with 1M *potassium hydroxide* (solution A) to a quantity of whole tablets containing 0.2 g of Famotidine, mix with the aid of ultrasound for 5 minutes, add about 200 ml of *methanol*, shake for 60 minutes and add sufficient solution A to produce 500 ml. Dilute 1 volume of this solution to 5 volumes with solution A. For solution (2) dissolve 8 mg of *famotidine BPCRS* in 4 ml of *methanol*, mix with the aid of ultrasound for 5 minutes and add sufficient solution A to produce 20 ml. Dilute 1 volume of this solution to 5 volumes with solution A.

The chromatographic conditions described under Related substances may be used. Inject 50 µl of each solution.

Calculate the content of $C_8H_{15}N_7O_2S_3$ in the tablets using the declared content of $C_8H_{15}N_7O_2S_3$ in *famotidine BPCRS.*

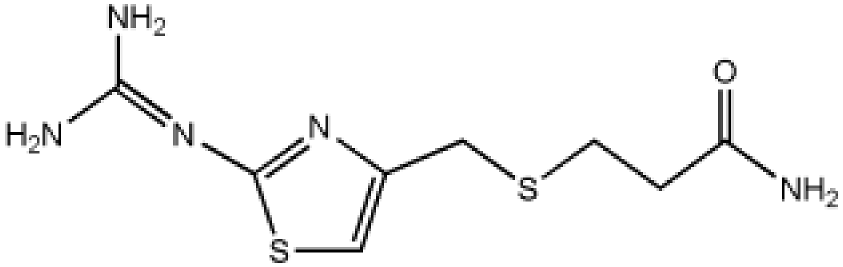
IMPURITIES

The impurities limited by the requirements of this monograph include impurity C listed under famotidine and the following degradation impurities.



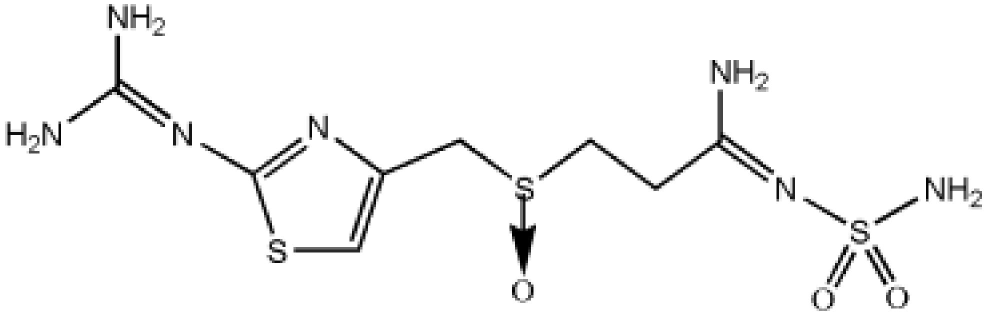
1.3-[2-(Diaminomethyleneamino)-1,3-thiazol-4-ylmethylthio]propanoic acid,

Corresponds to impurity F listed under Famotidine



2.3-[2-(Diaminomethyleneamino)-1,3-thiazol-4-ylmethylthio]propanamide,

Corresponds to impurity D listed under Famotidine



3.3-[2-(Diaminomethyleneamino)-1,3-thiazol-4-ylmethylsulphinyl]-*N*-sulphamoylpropanamidine.

Felbinac Cutaneous Foam

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Felbinac Cutaneous Foam is a fluid foam containing Felbinac in solution in a suitable pressurised container.

The cutaneous foam complies with the requirements stated under Medicated Foams and with the following requirements.

Content of felbinac, $C_{14}H_{12}O_2$

95.0 to 105.0% of the stated amount.

In each of the following tests, discharge a quantity of the foam into a suitable vessel with the can upright. Quantitatively transfer the foam using methanol. Determine the sample weight by difference.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of *glacial acetic acid*, 25 volumes of *acetone* and 50 volumes of *hexane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dissolve a weighed quantity of the foam containing 60 mg of felbinac in *methanol*, warming slightly if necessary to break the foam, and add sufficient *methanol* to produce 100 ml. Solution (2) is a 0.06% w/v solution of *felbinac BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). Spray the plate with a mixture of equal volumes of *formaldehyde solution* and *sulphuric acid* and heat at 110° for 10 minutes. The principal spots in the chromatograms obtained with solutions (1) and (2) are an intense purple colour.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH of the foam, 7.0 to 8.6, Appendix V L. Ensure good contact between the foam and the electrode.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a weighed quantity of the foam containing 30 mg of felbinac in *methanol*, warming slightly if necessary to break the foam, and add sufficient *methanol* to produce 50 ml. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 10 volumes with the same solvent. Solution (3) contains 0.00006% w/v of *4-acetylbiphenyl* and 0.00006% w/v of *biphenyl* in *methanol*. Solution (4) contains 0.001% w/v of *felbinac BPCRS* and 0.001% w/v of *o-phenylbenzoic acid* in the mobile phase. Inject 50 µl of each solution.

The chromatographic procedure described under Assay may be used.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.

For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peaks corresponding to 4-acetylbiphenyl or biphenyl is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1% of each) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a weighed quantity of the foam containing 30 mg of felbinac in 60 ml of *methanol*, warming slightly, if necessary, to break the foam, add slowly, and with mixing, sufficient *water* to produce 100 ml and dilute 1 volume of the resulting solution to 20 volumes with the mobile phase. Solution (2) contains 0.0015% w/v of *felbinac BPCRS* in the mobile phase. Solution (3) contains 0.0015% w/v of *felbinac BPCRS* and 0.0015% w/v of *o-phenylbenzoic acid* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS3 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 45 volumes of a 0.1% v/v solution of *glacial acetic acid* and 55 volumes of *methanol* and (c) a detection wavelength of 254 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.

Calculate the content of $C_{14}H_{12}O_2$ in the foam from the chromatograms obtained and using the declared content of $C_{14}H_{12}O_2$ in *felbinac BPCRS*.

STORAGE

Felbinac Cutaneous Foam should be kept protected from light. It should not be refrigerated.

Felbinac Gel

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; antinflammatory.

DEFINITION

Felbinac Gel is a solution of Felbinac in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of felbinac, $C_{14}H_{12}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of *glacial acetic acid*, 25 volumes of *acetone* and 50 volumes of *hexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a quantity of the gel containing 30 mg of felbinac with sufficient *acetone* to produce 5 ml and mix. Solution (2) is a 0.6% w/v solution of *felbinac BPCRS* in *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). Spray the plate with a mixture of equal volumes of *formaldehyde solution* and *sulphuric acid* and heat at 110° for 10 minutes. The principal spots in the chromatograms obtained with solutions (1) and (2) are an intense purple colour.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 7.0 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a weighed quantity of the gel containing 30 mg of felbinac in *methanol* and add sufficient *methanol* to produce 50 ml. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 10 volumes with the same solvent. Solution (3) contains 0.00006% w/v of *4-acetylbiphenyl* and 0.00006% w/v of *biphenyl* in *methanol*. Solution (4) contains 0.001% w/v of *felbinac BPCRS* and 0.001% w/v of *o-phenylbenzoic acid* in the mobile phase. Inject 50 µl of each solution.

The chromatographic procedure described under Assay may be used.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.

For solution (1) allow the chromatography to proceed for at least twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peaks corresponding to 4-acetylbiphenyl or biphenyl is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1% of each) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the gel containing 30 mg of felbinac in 70 ml of the mobile phase, add sufficient of the mobile phase to produce 100 ml, mix and dilute 1 volume of the resulting solution to 20 volumes with the mobile phase. Solution (2) contains 0.0015% w/v of *felbinac BPCRS* in the mobile phase. Solution (3) contains 0.0015% w/v of *felbinac BPCRS* and 0.0015% w/v of o*-phenylbenzoic acid* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS3 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 45 volumes of a 0.1% v/v solution of *glacial acetic acid* and 55 volumes of *methanol* and (c) a detection wavelength of 254 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.

Calculate the content of $C_{14}H_{12}O_2$ in the gel from the chromatograms obtained and using the declared content of $C_{14}H_{12}O_2$ in *felbinac BPCRS*.

Fenbufen Capsules

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Fenbufen Capsules contain Fenbufen.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of fenbufen, $C_{16}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the contents of the capsules containing 0.9 g of fenbufen add 10 ml of *acetone*, triturate using a glass pestle, filter through filter paper wetted with *acetone* into 100 ml of *petroleum spirit (boiling point, 40° to 60°)*, stir rapidly with a glass rod to induce crystallisation and allow to stand for 15 minutes. Filter through a fine porosity sintered-glass funnel, rinse the crystals with 25 ml of *petroleum spirit (boiling point, 40° to 60°)*, remove the solvent under reduced pressure and dry the crystals at 105° for 15 minutes. The *infrared absorption spectrum* of the dried crystals, Appendix II A, is concordant with the *reference spectrum* of fenbufen *(RS 140)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a phosphate buffer prepared by dissolving 6.69 g of *potassium dihydrogen orthophosphate* and 1.63 g of *sodium hydroxide* in sufficient *water* to produce 1000 ml, adjusting the pH to 7.5 with 5M *sodium hydroxide* if necessary, and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter (Whatman 541 paper is suitable) and dilute 1 ml of the filtrate to 50 ml with the dissolution medium. Measure the *absorbance* of this solution, Appendix II B, at 285 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *fenbufen BPCRS* prepared by dissolving 50 mg in 50 ml of *methanol* and diluting to a suitable volume with the dissolution medium and using dissolution medium in the reference cell. Calculate the total content of fenbufen, $C_{16}H_{14}O_3$ in the medium from the absorbances obtained and from the declared content of $C_{16}H_{14}O_3$ in *fenbufen BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add to a quantity of the contents of the capsules containing 0.25 g of fenbufen 20 ml of *dimethylformamide*, mix with the aid of ultrasound for 20 minutes, dilute to 50 ml with the initial mobile phase and filter (Whatman GF/F paper is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with the initial mobile phase and further dilute 1 volume of this solution to 10 volumes with the initial mobile phase. Solution (3) contains 0.0025% w/v of *fenbufen BPCRS* and 0.0006% w/v of *3-(4-chlorobenzoyl)propionic acid*.

The chromatographic procedure may be carried out using a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable). Use as the initial mobile phase a mixture of 32 volumes of *acetonitrile* and 68 volumes of a solution consisting of 1 volume of *glacial acetic acid* and 55 volumes of *water* and as the final mobile phase a mixture of 45 volumes of *acetonitrile* and 55 volumes of a solution consisting of 1 volume of *glacial acetic acid* and 55 volumes of *water*. Maintain the initial mobile phase for 15 minutes, carry out a linear gradient elution with a flow rate of 2 ml per minute for 5 minutes and maintain the final mobile phase for 15 minutes with the same flow rate. Use a detection wavelength of 254 nm.

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 10.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add to a quantity of the mixed contents of 20 capsules containing 0.1 g of fenbufen 30 ml of *methanol*, mix with the aid of ultrasound for 15 minutes, add sufficient of the mobile phase to produce 100 ml and dilute 1 volume of this solution to 10 volumes with the mobile phase. For solution (2) dissolve *fenbufen BPCRS* in *methanol*, add sufficient of the mobile phase to produce a 0.1% w/v solution and dilute 1 volume of this solution to 10 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), maintained about 40°, (b) a mixture of 1 volume of *glacial acetic acid*, 44 volumes of *acetonitrile* and 55 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 280 nm.

Calculate the content of $C_{16}H_{14}O_3$ in the capsules using the declared content of $C_{16}H_{14}O_3$ in *fenbufen BPCRS*.

Fenbufen Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Fenbufen Tablets contain Fenbufen.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fenbufen, $C_{16}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 0.9 g of fenbufen add 10 ml of *acetone*, triturate using a glass pestle, filter through filter paper wetted with *acetone* into 100 ml of *petroleum spirit (boiling point, 40° to 60°)*, stir rapidly with a glass rod to induce crystallisation and allow to stand for 15 minutes. Filter through a fine porosity sintered-glass funnel, rinse the crystals with 25 ml of *petroleum spirit (boiling point, 40° to 60°)*, remove the solvent under reduced pressure and dry the crystals at 105° for 15 minutes. The *infrared absorption spectrum* of the dried crystals, Appendix II A, is concordant with the *reference spectrum* of fenbufen *(RS 140)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a phosphate buffer prepared by dissolving 6.69 g of *potassium dihydrogen orthophosphate* and 1.63 g of *sodium hydroxide* in sufficient *water* to produce 1000 ml, adjusting the pH to 7.5 with 5M *sodium hydroxide* if necessary, and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter (Whatman 541 paper is suitable) and dilute 1 ml of the filtrate to 50 ml with the dissolution medium. Measure the *absorbance* of this solution, Appendix II B, at 285 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *fenbufen BPCRS* prepared by dissolving 50 mg in 50 ml of *methanol* and diluting to volume with the dissolution medium and using dissolution medium in the reference cell. Calculate the total content of fenbufen, $C_{16}H_{14}O_3$ in the medium from the absorbances obtained and from the declared content of $C_{16}H_{14}O_3$ in *fenbufen BPCRS*.

Related substances

Comply with the test described under Fenbufen Capsules but using as solution (1) a solution prepared in the following manner. To a quantity of the powdered tablets containing 0.25 g of fenbufen add 20 ml of *dimethylformamide*, mix with the aid of ultrasound for 20 minutes, add sufficient of the initial mobile phase to produce 50 ml and filter (Whatman GF/F paper is suitable).

ASSAY

Weigh and powder 20 tablets. Carry out the Assay described under Fenbufen Capsules but using as solution (1) a solution prepared in the following manner. To a quantity of the powdered tablets containing 0.1 g of fenbufen add 30 ml of *methanol*, mix with the aid of ultrasound for 15 minutes, add sufficient of the mobile phase to produce 100 ml, filter through a 0.45-µm filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase.

Fenoprofen Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Fenoprofen Tablets contain Fenoprofen Calcium. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fenoprofen, $C_{15}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits two maxima, at 272 nm and 278 nm, and a shoulder at 266 nm.

B. Suspend a quantity of the powdered tablets containing the equivalent of 0.3 g of fenoprofen in 10 ml of 0.1M *hydrochloric acid*. Extract with 20 ml of *chloroform*, filter the extract through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of fenoprofen *(RS 141)*.

C. Ignite a quantity of the powdered tablets. The residue yields the reactions characteristic *of calcium salts*, Appendix VI.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 80 ml of the mobile phase to a quantity of the powdered tablets containing the equivalent of 0.5 g of fenoprofen, mix with the aid of ultrasound, allow to cool, add sufficient mobile phase to produce 100 ml and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.04% w/v of *fenoprofen calcium* and 0.0015% w/v of *4,4′-dimethoxybenzophenone*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (7 to 8 µm) (Zorbax ODS is suitable), (b) a mixture of 61 volumes of *water*, 30 volumes of *acetonitrile*, 7 volumes of *tetrahydrofuran* and 2 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 270 nm. For solution (1) allow the chromatography to proceed for 3 times the retention time of the peak due to fenoprofen.

The test is not valid unless the *resolution factor* between the peaks corresponding to fenoprofen calcium and 4′,4′-dimethoxybenzophenone in the chromatogram obtained with solution (3) is at least 3.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than twice the area of the peak in the chromatogram obtained with solution (2), not more than one such peak has an area greater than the area of the peak in the chromatogram obtained with solution (2) and the sum of the areas of all such peaks is not greater than four times the area of the peak in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 0.2 g of fenoprofen add 5 ml of *glacial acetic acid* and shake for 1 minute. Add 100 ml of *methanol*, shake for 5 minutes, dilute to 200 ml with *methanol* and filter. Dilute 10 ml of the filtrate to 200 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 272 nm, Appendix II B. Calculate the content of $C_{15}H_{14}O_3$ taking 80.7 as the value of A (1%, 1 cm) at the maximum at 272 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of fenoprofen.

Fenoterol Pressurised Inhalation

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Fenoterol Pressurised Inhalation is a suspension of Fenoterol Hydrobromide in a suitable liquid in a suitable pressurised container.

The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of fenoterol hydrobromide, $C_{17}H_{21}NO_4,HBr$

85.0 to 115.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated HPTLC plate (Merck plates are suitable) and a mixture of 5 volumes of *anhydrous formic acid*, 15 volumes of *water*, 90 volumes of *dichloromethane* and 90 volumes of *absolute ethanol* as the mobile phase, but allowing the solvent front to ascend about 4 cm above the line of application. Apply separately to the plate under a gentle current of nitrogen 1 µl of each of the following solutions. For solution (1) make a small hole in the ferrule of one pressurised container, previously cooled, and allow the propellant to evaporate for about 1 minute, transfer the contents of the container to a flask and stir mechanically for about 10 minutes. Add 45 ml of 0.01M *hydrochloric acid*, continue stirring until the propellant has evaporated completely (about 1 hour), filter the resulting aqueous solution and dilute the filtrate with 0.01M *hydrochloric acid* to produce a solution containing 0.01% w/v of fenoterol hydrobromide. Solution (2) contains 0.010% w/v of *fenoterol hydrobromide BPCRS* in 0.01M *hydrochloric acid*. After removal of the plate, dry it at 60° for about 15 minutes and examine under *ultraviolet light (254 nm)*. Spray the plate with a freshly prepared 0.1% w/v solution of *fast red B salt* in a 5% w/v solution of *sodium carbonate*. By each method of visualisation, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. Make a small hole in the ferrule of one pressurised container, previously cooled, and allow the propellant to evaporate. Empty the contents of the container into a separating funnel, add 10 ml of *water* and 15 ml of *ether*, shake, allow to separate and filter the aqueous phase. Add 0.1 ml of *nitric acid* to the clear filtrate followed by 0.2 ml of a 4% w/v solution of *silver nitrate*. A pale, yellow flocculent precipitate is produced.

TESTS

Related substances

Examine the chromatograms obtained in the Assay. In the chromatogram obtained with solution (1) the area of any peak corresponding to fenoterol degradation impurity A is not greater than the area of the peak due to fenoterol degradation impurity A in the chromatogram obtained with solution (3) (1%).

Deposition of the emitted dose

Carry out the test for *aerodynamic assessment of fine particles*, Appendix XII C7, using apparatus A but determining the content of active ingredient as described below. Use 7 ml of 0.001M *hydrochloric acid* in the upper impingement chamber and 40 ml of a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol* in the lower impingement chamber. Use a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol* to wash the coupling tube, E, and transfer the combined solution and washings in the lower impingement chamber to a 100 ml graduated flask and dilute the combined solution and washings to 100 ml with a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol*.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the diluted solution from the lower impingement chamber. Solution (2) is a 0.0005% w/v solution of *fenoterol hydrobromide BPCRS* in a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol*. Solution (3) contains 0.00005% w/v of *fenoterol degradation impurity A BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 5 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 270 volumes of *acetonitrile* and 1000 volumes of a solution prepared by dissolving 2.5 g of *sodium heptanesulphonate* in 1000 ml of *water* and adjusting the pH to 3.2 with 0.05M *orthophosphoric acid*, and (c) a detection wavelength of 210 nm.

Inject 40 µl of each solution. For solution (1) allow the chromatography to proceed for four times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.5.

Calculate the amount of fenoterol hydrobromide, $C_{17}H_{21}NO_4,HBr$, delivered to the lower impingement chamber per actuation of the valve using the declared content of $C_{17}H_{21}NO_4,HBr$ in *fenoterol hydrobromide BPCRS*. Not less than 25% of the average amount of fenoterol hydrobromide delivered per actuation of the valve, calculated as the average of the three results determined in the Assay, is deposited in the lower impingement chamber.

Water

Not more than 0.02% w/w when determined by the following method. Weigh one pressurised container, cool the container, make a small hole in the ferrule and allow the propellant to evaporate (about 2 hours). Open the container and pour the contents into a vessel suitable for the determination of water, rinsing the pressurised container with *anhydrous methanol*. Reweigh the dried container together with its component parts. Carry out the method for the determination of water, Appendix IX C, on the combined contents and washings and hence calculate the percentage content of water in the pressurised container.

ASSAY

Determine the content of active ingredient delivered by the first 10 successive combined actuations of the valve after priming. Carry out the procedure for Content of active ingredient delivered by actuation of the valve described under Pressurised Inhalations, beginning at the words 'Remove the pressurised container from the actuator …' and ending at the words '… to the volume specified in the monograph', using 20 ml of a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol* in the vessel. Sparge the resulting solution with *helium* for about 30 minutes.

Determine the amount of active ingredient in the 10 combined actuations using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the solution prepared above diluted, if necessary, with a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol* to produce a solution containing 0.005% w/v of fenoterol hydrobromide. Solution (2) contains 0.005% w/v of *fenoterol hydrobromide BPCRS* in a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol*. Solution (3) contains 0.00005% w/v of *fenoterol degradation impurity A BPCRS* in solution (2).

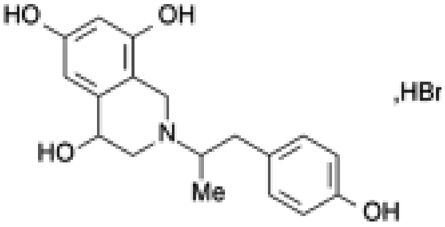
The chromatographic conditions described under Deposition of the emitted dose may be used. For solution (1) allow the chromatography to proceed for four times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.5.

Calculate the average content of $C_{17}H_{21}NO_4$,HBr delivered by a single actuation of the valve using the declared content of $C_{17}H_{21}NO_4$,HBr in *fenoterol hydrobromide BPCRS*.

Determine the content of active ingredient a second and a third time by repeating the procedure on the middle 10 and on the last 10 successive combined actuations of the valve, as estimated from the number of deliveries available from the container as stated on the label. For each of the three determinations the average content of $C_{17}H_{21}NO_4$,HBr delivered by a single actuation of the valve is within the limits stated under Content of fenoterol hydrobromide.

IMPURITIES



A. 2-(1-*p*-Hydroxyphenyl)-1-methylethyl-4,6,8-trihydroxy-1,2,3,4 tetrahydroisoquinoline hydrobromide (*fenoterol degradation impurity A*).

Fentanyl Injection

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Fentanyl Injection is a sterile solution of Fentanyl Citrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fentanyl, $C_{22}H_{28}N_2O$

95.0% to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the injection diluted, if necessary, to contain the equivalent of 0.005% w/v of fentanyl exhibits two maxima at 251 and 257 nm and a shoulder at 262 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yields reaction B characteristic *of citrates*, Appendix VI.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the injection being examined, diluted with the mobile phase, if necessary, to contain the equivalent of 0.005% w/v of fentanyl. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 5 volumes of the resulting solution to 20 volumes with the mobile phase. Solution (3) contains 0.00005% w/v of *fentanyl impurity A BPCRS* in the mobile phase. For solution (4) dissolve 10 mg of *fentanyl citrate BPCRS* in 10 ml of 2M *hydrochloric acid*, heat on a waterbath under a reflux condenser for 4 hours and neutralise with 10 ml of 2M *sodium hydroxide*. Evaporate to dryness on a waterbath, cool, dissolve the residue in 10 ml of *methanol* and filter. Dilute 1 volume of the filtrate to 10 volumes with the mobile phase (generation of impurity D).

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Bondclone C18 10µ is suitable), (b) as the mobile phase with a flow rate of 1.25 ml per minute a solution containing 0.3% w/v of *potassium dihydrogen orthophosphate* in a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 56 volumes of *water,* the solution adjusted to pH 3.2 with *orthophosphoric acid* and (c) a detection wavelength of 215 nm.

Inject 100 µl of *methanol* as a blank solution and 100 µl of each of solutions (1), (2), (3) and

(4). For solution (1) and solution (2) allow the chromatography to proceed for twice the retention time of the principal peak. The chromatogram obtained with solution (4) exhibits two peaks, due to fentanyl impurity D and fentanyl. The retention time of fentanyl impurity D is about 0.8 relative to fentanyl. In the chromatogram obtained with solution (1) the area of any peak corresponding to fentanyl impurity A is not greater than half the area of the peak in the chromatogram obtained with solution (3) (0.5%); the area of any peak corresponding to fentanyl impurity D is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (0.5%); the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any *secondary peaks* apart from any peaks corresponding to fentanyl impurity A and fentanyl impurity D is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%). Disregard any peak obtained with the blank solution and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is the injection, diluted if necessary with the mobile phase, to contain the equivalent of 0.005% w/v of fentanyl. Solution (2) is a 0.008% w/v solution of *fentanyl citrate BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{22}H_{28}N_2O$ in the injection using the declared content of $C_{22}H_{28}N_2O$ in *fentanyl citrate BPCRS*.

STORAGE

Fentanyl Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of fentanyl.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A and impurity D listed under Fentanyl Citrate.

Ferrous Fumarate and Folic Acid Tablets

General Notices

Action and use

Vitamin B component.

DEFINITION

Ferrous Fumarate and Folic Acid Tablets contain Ferrous Fumarate and Folic Acid. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Carry out the tests avoiding exposure to actinic light.

Content of ferrous fumarate, $C_4H_2FeO_4$

90.0 to 105.0% of the stated amount.

Content of folic acid, $C_{19}H_{19}N_7O_6$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. In the Assay for folic acid, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1) .

B. Heat a quantity of the powdered tablets containing 0.77 g of Ferrous Fumarate with 25 ml of a mixture of equal volumes of *hydrochloric acid* and *water* on a water bath for 15 minutes, cool and filter. Retain the residue for test C. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

C. Wash the residue reserved in test B with a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 ml of *sodium carbonate solution* and add *potassium permanganate solution* dropwise. The permanganate is decolourised and a brownish solution is produced.

TESTS

Ferric iron

Dissolve a quantity of the powder prepared for the Assay for ferrous fumarate containing 1.5 g of Ferrous Fumarate in a mixture of 100 ml of *water* and 10 ml of *hydrochloric acid* by heating rapidly to the boiling point. Boil for 15 seconds, cool rapidly, add 3 g of *potassium iodide*, stopper, allow to stand in the dark for 15 minutes and titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Repeat the operation without the substance being examined. The difference between the titrations is not more than 13.4 ml (5% ferric iron in Ferrous Fumarate).

Uniformity of Content

For folic acid

Tablets containing less than 2 mg of Folic Acid comply with the requirement stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0007% w/v of *folic acid EPCRS* in a mixture containing 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* and 135 volumes of *methanol*. For solution (2) place one tablet in 40 ml of a mixture containing 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* and 135 volumes of *methanol*, shake for 5 minutes with the aid of ultrasound, shake for a further 15 minutes, dilute to 50 ml a mixture containing 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* and 135 volumes of *methanol* and filter (0.45 µm nylon filter is suitable).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 135 volumes of *methanol* and 800 volumes of a solution containing 0.938% w/v of *sodium perchlorate* and 0.075% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 7.2 with 0.1M *potassium hydroxide* and diluted to 1000 volumes with *water* and (c) a detection wavelength of 277 nm.

Calculate the content of $C_{19}H_{19}N_7O_6$ in each tablet using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid EPCRS*.

ASSAY

Weigh and powder 20 tablets.

For ferrous fumarate

Disperse a quantity of the powder containing 0.3 g of Ferrous Fumarate in 7.5 ml of 1M *sulphuric acid* with gentle heating. Cool, add 25 ml of *water* and titrate immediately with 0.1M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 16.99 mg of $C_4H_2FeO_4$.

For folic acid

For tablets containing 2 mg or more of folic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0007% w/v of *folic acid EPCRS* in a mixture containing 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* and 135 volumes of *methanol*. For solution (2) mix a quantity of the powdered tablets containing 0.35 mg of Folic Acid in 40 ml of a mixture containing 800 volumes of a 0.57% w/v solution of *dipotassium hydrogen orthophosphate* and 135 volumes of *methanol*, shake for 5 minutes with the aid of ultrasound, shake for a further 15 minutes and dilute to 50 ml with the same solvent and filter (0.45 µm nylon filter is suitable).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silca gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 135 volumes of *methanol* and 800 volumes of a solution containing 0.938% w/v of *sodium perchlorate* and 0.075% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 7.2 with 0.1M *potassium hydroxide* and diluted to 1000 volumes with *water* and (c) a detection wavelength of 277 nm.

Calculate the content of $C_{19}H_{19}N_7O_6$ using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid EPCRS*.

For tablets containing less than 2 mg of folic acid

Use the average of the ten individual results obtained in the test for Uniformity of content.

STORAGE

Ferrous Fumarate and Folic Acid Tablets should be protected from light.

304 mg of ferrous fumarate is equivalent to 100 mg of ferrous iron.

Ferrous Fumarate Capsules

General Notices

DEFINITION

Ferrous Fumarate Capsules contain Ferrous Fumarate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ferrous iron, Fe(II)

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Heat 1 g of the contents of the capsules with 25 ml of a mixture of equal volumes of *hydrochloric acid* and *water* on a water bath for 15 minutes, cool and filter. Retain the residue for test B. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

B. Wash the residue reserved in test A with a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 ml of *dilute sodium carbonate solution* and add *dilute potassium permanganate solution* drop wise. The permanganate is decolourised and a brownish solution is produced.

C. Mix a quantity of the contents of the capsules containing 0.5 g of ferrous fumarate with 1 g of *resorcinol*. To 0.5 g of the mixture in a crucible add 0.15 ml of *sulphuric acid* and heat gently; a deep red, semi-solid mass is produced. Add the mass to a large volume of *water*; an orange–yellow solution is produced which exhibits no fluorescence.

TESTS

Ferric iron

In a flask with a ground-glass stopper dissolve a quantity of the mixed contents of 20 capsules prepared for the Assay containing 1.5 g of ferrous fumarate in a mixture of 10 ml of *hydrochloric acid* and 100 ml of *water* by heating rapidly to boiling. Boil for 15 seconds. Cool rapidly, add 3 g of *potassium iodide*, stopper the flask and allow to stand protected from light for 15 minutes. Add 2 ml of *starch solution* as indicator. Titrate the liberated iodine with 0.05M *sodium thiosulphate VS*. Carry out a blank test. The difference between the volumes used in the two titrations corresponds to the amount of iodine liberated by ferric ion. The difference between the titrations is not more than 5.4 ml (1%).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 100 ml of the medium and filter. Titrate the filtrate with 0.01M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Calculate the total content of Fe(II) in the medium taking each ml of 0.01M *ammonium cerium(IV) sulphate VS* to be equivalent to

0.5585 mg of Fe(II).

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 0.3 g of ferrous fumarate in 7.5 ml of 1M *sulphuric acid* with gentle heating. Cool, add 25 ml of *water* and titrate immediately with 0.1M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 5.585 mg of Fe(II).

LABELLING

The quantity of the active ingredient is stated both as the amount of ferrous fumarate and in terms of the equivalent amount of ferrous iron.

Ferrous Fumarate Oral Suspension

General Notices

DEFINITION

Ferrous Fumarate Oral Suspension is a suspension of ferrous fumarate in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ferrous iron, Fe(II)

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Mix a volume of the oral suspension containing about 0.6 g of ferrous fumarate with 20 ml of *water*, centrifuge and discard the supernatant liquid. Repeat with a further 20 ml of *water*. Add 15 ml of 3M *hydrochloric acid* to the residue, dissolve with minimum warming, cool and extract with two 50 ml quantities of *ether*. Wash the combined ether extracts with two 20 ml quantities of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fumaric acid *(RS 163)*.

B. Mix a volume of the oral suspension containing about 0.3 g of ferrous fumarate in 10 ml of *water*, centrifuge and discard the supernatant liquid. To 0.2 g of the residue add 10 ml of a mixture of equal volumes of *hydrochloric acid* and *water*, dissolve with minimum warming, cool and filter. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

TESTS

Ferric iron

Disperse a quantity of the oral suspension containing 0.28 g of ferrous fumarate in 20 ml of *water*, centrifuge and decant the supernatant liquid into a 500 ml graduated flask. Dissolve the residue in 20 ml of 1M *sulphuric acid* with minimum warming, cool and transfer the resulting solution to the same graduated flask, dilute to 500 ml with *water* and filter. To 5 ml of the filtrate in a *Nessler cylinder* add 40 ml of *water* and 5 ml of a 10% w/v solution of *ammonium thiocyanate* and mix. Any red colour produced is not more intense than that obtained by treating in the same manner a mixture of 42 ml of *water*, 0.2 ml of 1M *sulphuric acid* and 3 ml of a 0.0173% w/v solution of *ammonium iron(III) sulphate* in 0.01M *hydrochloric acid* [6.0%, with respect to the content of Fe(II)].

ASSAY

Mix a quantity of the oral suspension containing 0.28 g of ferrous fumarate with 10 ml of *water* and 25 ml of *nitric acid*, carefully warm until the vigorous reaction subsides, add a further 25 ml of *nitric acid*, evaporate to about 10 ml and allow to cool. Carefully add 10 ml of *sulphuric acid* to the cooled solution, evaporate until white fumes are evolved, cool, add 10 ml of *nitric acid* and again evaporate until white fumes are evolved. Repeat the evaporation with 10 ml quantities of *nitric acid* until all of the organic matter has been destroyed. To the resulting solution add 10 ml of *water* and 0.5 g of *ammonium oxalate*, evaporate until white fumes are evolved, add 50 ml of *water*, boil until a clear solution is obtained, cool, dilute to 100 ml with *water* and add *dilute potassium permanganate solution*, drop wise, until a faint pink colour persists for 10 seconds. Add 10 ml of *hydrochloric acid* and 3 g of *potassium iodide*, allow to stand for 15 minutes protected from light, add 10 ml of *chloroform* and titrate the liberated iodine with 0.1M *sodium thiosulphate VS*, with vigorous shaking, until the chloroform layer becomes colourless. Repeat the procedure without the preparation being examined. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 5.585 mg of Fe(II).

STORAGE

Ferrous Fumarate Oral Suspension should be protected from light.

LABELLING

The quantity of the active ingredient is stated both as the amount of ferrous fumarate and in terms of the equivalent amount of ferrous iron.

Ferrous Fumarate Tablets

General Notices

DEFINITION

Ferrous Fumarate Tablets contain Ferrous Fumarate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ferrous iron, Fe(II)

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Heat 1 g of the powdered tablets with 25 ml of a mixture of equal volumes of *hydrochloric acid* and *water* on a water bath for 15 minutes, cool and filter. Retain the residue for test B. The filtrate yields reaction A characteristic of *iron salts*, Appendix VI.

B. Wash the residue reserved in Test A with a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 ml of *dilute sodium carbonate solution* and add *dilute potassium permanganate solution* drop wise. The permanganate is decolourised and a brownish solution is produced.

C. Mix a quantity of the powdered tablets containing 0.5 g of ferrous fumarate with 1 g of *resorcinol*. To 0.5 g of the mixture in a crucible add 0.15 ml of *sulphuric acid* and heat gently; a deep red, semi-solid mass is produced. Add the mass to a large volume of *water*; an orange–yellow solution is produced which exhibits no fluorescence.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 100 ml of the medium and filter. Titrate the filtrate with 0.01M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Calculate the total content of Fe(II) in the medium taking each ml of 0.01M *ammonium cerium(IV) sulphate VS* to be equivalent to 0.5585 mg of Fe(II).

Ferric iron

In a flask with a ground-glass stopper dissolve a quantity of the powder prepared for the Assay containing 1.5 g of ferrous fumarate in a mixture of 10 ml of *hydrochloric acid* and 100 ml of *water* by heating rapidly to boiling. Boil for 15 seconds. Cool rapidly, add 3 g of *potassium iodide*, stopper the flask and allow to stand protected from light for 15 minutes. Add 2 ml of *starch solution* as indicator. Titrate the liberated iodine with 0.1M *sodium thiosulphate VS*. Carry out a blank test. The difference between the volumes used in the two titrations corresponds to the amount of iodine liberated by ferric ion. The difference between the titrations is not more than 13.4 ml (5%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of ferrous fumarate in 7.5 ml of 1M *sulphuric acid* with gentle heating. Cool, add 25 ml of *water* and titrate immediately with 0.1M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 5.585 mg of Fe (II).

LABELLING

The quantity of the active ingredient is stated both as the amount of ferrous fumarate and in terms of the equivalent amount of ferrous iron.

Ferrous Gluconate Tablets

General Notices

DEFINITION

Ferrous Gluconate Tablets contain Ferrous Gluconate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ferrous iron, Fe(II)

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of ferrous gluconate with 10 ml of 2M *hydrochloric acid*, filter and add to the filtrate 1 ml of *barium chloride solution*. An opalescence may be produced but no precipitate is produced.

B. The powdered tablets yield reaction A characteristic of *iron salts*, Appendix VI.

TESTS

Ferric iron

Dissolve a quantity of the powder prepared for the Assay containing 5 g of ferrous gluconate as completely as possible without heating in a mixture of 100 ml of freshly boiled and cooled *water* and 10 ml of *hydrochloric acid* and add 3 g of *potassium iodide*. Close the vessel, allow to stand in the dark for 5 minutes and titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Repeat the operation without the powder. The difference between the titrations represents the amount of iodine liberated by the ferric iron. Not more than 11.2 ml of 0.1M *sodium thiosulphate VS* is required.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 1 g of ferrous gluconate as completely as possible in a mixture of 30 ml of *water* and 20 ml of 1M *sulphuric acid* and titrate with 0.1M *ammonium cerium(IV)sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 5.585 mg of Fe (II).

LABELLING

The quantity of active ingredient is stated both as the amount of ferrous gluconate and in terms of the equivalent amount of ferrous iron.

Ferrous Sulphate Tablets

General Notices

DEFINITION

Ferrous Sulphate Tablets contain Dried Ferrous Sulphate. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ferrous iron, Fe(II)

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The powdered tablets yield reaction A characteristic of *iron salts*, Appendix VI.

B. Extract the powdered tablets with 2M *hydrochloric acid* and filter. The filtrate yields reaction A characteristic of *sulphates*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of dried ferrous sulphate as completely as possible in a mixture of 30 ml of *water* and 20 ml of 1M *sulphuric acid* and titrate with 0.1M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 5.585 mg of Fe(II).

LABELLING

The quantity of active ingredient is stated both as the amount of dried ferrous sulphate and in terms of the equivalent amount of ferrous iron.

Finasteride Tablets

General Notices

Action and use

5-Alpha reductase inhibitor; treatment of benign prostatic hyperplasia.

DEFINITION

Finasteride Tablets contain Finasteride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of finasteride, $C_{23}H_{36}N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Dissolution, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to finasteride in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to finasteride in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus II. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a 10 ml sample of the medium after 45 minutes and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use filtered dissolution medium. For solution (2) prepare a suitable solution of *finasteride BPCRS* in a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile*.

The chromatographic procedure may be carried out using (a) a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable) and maintained at 45°, (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 21 volumes of *water* and 29 volumes of *acetonitrile* and (c) a detection wavelength of 220 nm.

Inject 200 µl of solution (1) and solution (2). The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak corresponding to finasteride is less than 2.0.

Calculate the content of $C_{23}H_{36}N_2O_2$ in the medium from the chromatograms obtained using the declared content of $C_{23}H_{36}N_2O_2$ in *finasteride BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 7 ml of a mixture of equal volumes of *acetonitrile* and *water* to a quantity of powdered tablets containing 100 mg of Finasteride, dilute to 10 ml with the same mixture of solvents, centrifuge and filter the supernatant liquid. For solution (2) dilute 2 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *acetonitrile* and *water*, dilute 1 volume of this solution to 10 volumes with a mixture of equal volumes of *acetonitrile* and *water*. Solution (3) contains 1.0% w/v of *finasteride for system suitability EPCRS* in a mixture of equal volumes of *acetonitrile* and *water.* For solution (4) dilute 25 volumes of solution (2) to 100 volumes with equal volumes of *acetonitrile* and *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable) and maintained at 60°, (b) a mixture of 10 volumes of *acetonitrile,* 10 volumes of *tetrahydrofuran* and 80 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 210 nm.

Inject 20 µl of each solution.

When the chromatograms are recorded using the prescribed conditions the retention time of finasteride is about 28 minutes. The retention times relative to finasteride are: impurity A, about 0.9; impurity B, about 1.2; impurity C, about 1.4.

For solution (1) allow the chromatography to run for twice the retention time of the finasteride peak. The test is not valid unless, (a) in the chromatogram obtained with solution (3), the *peak to valley ratio* between finasteride impurity A and finasteride is at least 2.5 and, (b) in the chromatogram obtained with solution (4), the peak due to finasteride has a *signal to noise ratio* of at least 10.

In the chromatogram obtained with solution (1), (a) any peak corresponding to finasteride impurity A is not more than 0.3%, calculated using the area of the peak due to finasteride impurity A in solution (3) and the declared content of finasteride impurity A in *finasteride for system suitability EPCRS*; (b) the area of any peak corresponding to finasteride impurity B is not greater than 1.5 times the area of the principal peak in solution (2) (0.3%); (c) the area of any peak corresponding to finasteride impurity C is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%); (d) the area of any other peak is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the total content of impurities in solution (1) is not greater than (0.6%). Disregard any peak with an area less than 0.25 times the area of the peak due to finasteride in the chromatogram obtained with solution (2) (0.05%).

Uniformity of content

Tablets containing less than 2 mg of Finasteride, or less than 2% w/w of Finasteride comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), add 2.5 ml of *water* to one tablet, shake with the aid of ultrasound until the tablet is completely dispersed. Add 35 ml of a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile* (solvent A), mix with the aid of ultrasound for a further 30 minutes. Cool to room temperature, dilute to 50 ml with solvent A, centrifuge and use the clear supernatant liquid. For solution (2) prepare a suitable solution of *finasteride BPCRS* in solvent A.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) and maintained at 45°, (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by mixing equal volumes of *acetonitrile* and 0.0025M of *orthophosphoric acid* and (c) a detection wavelength of 240 nm.

Inject 20 µl of solution (1) and solution (2). The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to finasteride is less than 2.0.

Calculate the content of $C_{23}H_{36}N_2O_2$ in each tablet using the declared content of $C_{23}H_{36}N_2O_2$ in *finasteride BPCRS*.

ASSAY

For tablets containing 2 mg or more, and 2% w/w or more, of Finasteride Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), add 25 ml of *water* to a quantity of the powdered tablets containing 50 mg of Finasteride, add 350 ml of a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile* (solvent A), mix with the aid of ultrasound for 30 minutes. Allow to attain room temperature and dilute to 500 ml with solvent A, centrifuge and use the clear supernatant liquid. Solution (2) contains 0.010% w/v of *finasteride BPCRS* in solvent A.

The chromatographic conditions described under Uniformity of content may be used.

The assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to finasteride is less than 2.0.

Calculate the content of $C_{23}H_{36}N_2O_2$ in the tablets from the chromatograms obtained and using the declared content of $C_{23}H_{36}N_2O_2$ in *finasteride BPCRS*.

For tablets containing less than 2 mg or less than 2% w/w of Finasteride Use the average of the individual results obtained in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Finasteride.

Flavoxate Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Flavoxate Tablets contain Flavoxate Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of flavoxate hydrochloride, $C_{24}H_{25}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of flavoxate hydrochloride *(RS 143)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (5) corresponds to that in the chromatogram obtained with solution (6).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 1 volume of 18M *ammonia*, 80 volumes of *propan-2-ol* and 200 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate (1) 50 µl of a solution prepared by shaking a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 ml of *chloroform* and filtering, (2) 10 µl of a 0.030% w/v solution of *3-methylflavone-8-carboxylic acid BPCRS* in *chloroform*, (3) 10 µl of a 0.015% w/v solution of *3-methylflavone-8-carboxylic acid ethyl ester BPCRS* in *chloroform*, (4) 25 µl of a solution obtained by diluting one volume of solution (1) to 500 volumes with *chloroform*, (5) 10 µl of a solution obtained by diluting 1 volume of solution (1) to 20 volumes with *chloroform* and (6) 10 µl of a 0.1% w/v solution of *flavoxate hydrochloride BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* Any spot corresponding to 3-methylflavone-8-carboxylic acid ethyl ester in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.15%) and any other *secondary spot* in the chromatogram obtained with solution (1), other than the spot corresponding to 3-methyflavone-8-carboxylic acid, is not more intense than the spot in the chromatogram obtained with solution (4) (0.1%).

3-Methylflavone-8-carboxylic acid

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel GF$_{254}$ plate* (Merck silica gel 60 F$_{254}$ plates are suitable) and a mixture of 4 volumes of *glacial acetic acid*, 25 volumes of *ethyl acetate* and 70 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 ml of *chloroform* and filter. Solution (2) is a 0.010% w/v solution of *3-methylflavone-8-carboxylic acid BPCRS*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any spot corresponding to 3-methylflavone-8-carboxylic acid in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing 1 g of Flavoxate Hydrochloride add 600 ml of 0.1M *hydrochloric acid* and disperse with the aid of ultrasound for 10 minutes. Place in a water bath at 70° for 90 minutes, cool, add sufficient 0.1 M *hydrochloric acid* to produce 1 litre and filter. Dilute 5 ml to 250 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at 293 nm, Appendix II B. Calculate the content of $C_{24}H_{25}NO_4$,HCl from the absorbance obtained by repeating the measurement using a 0.002% w/v solution of *flavoxate hydrochloride BPCRS* in 0.1M *hydrochloric acid and* from the declared content of $C_{24}H_{25}NO_4$,HCl in *flavoxate hydrochloride BPCRS*.

STORAGE

Flavoxate tablets should be protected from light.

Flecainide Injection

General Notices

Action and use

Class I antiarrhythmic.

DEFINITION

Flecainide Injection is a sterile solution of Flecainide Acetate in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of flecainide acetate, $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless or almost colourless solution.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the solution obtained in the Assay in the range 230 nm to 350 nm exhibits a maximum at 296 nm.

B. In the test for (Piperidin-2-yl)methanamine, examine the chromatograms under *ultraviolet light (254 nm)* before spraying. The principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

(Piperidin-2-yl)methanamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck plates are suitable) and a mixture of 2 volumes of *methanol*, 5 volumes of 18M *ammonia*, 100 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 1 µl of solution (1) and 5 µl of each of solutions (2) and (3). Solution (1) contains 0.025% w/v of *flecainide acetate impurity B EPCRS* [(piperidin-2-yl) methanamine] in *methanol*. For solution (2) dilute the injection, if necessary, with *methanol* to contain 1% w/v of flecainide acetate. Solution (3) contains 1% w/v of *flecainide acetate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)* (for Identification test B). Spray the plate with a freshly prepared 0.2% w/v solution of *ninhydrin* in *methanol*, heat the plate at 105° for approximately 5 minutes and examine immediately. In the chromatogram obtained with solution (2) any bluish-purple spot corresponding to (piperidin-2-yl)methanamine is not more intense than the spot in the chromatogram obtained with solution (1) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is the injection diluted, if necessary, with a mixture of 29 volumes of *acetonitrile* and 71 volumes of *water* to contain 1.0% w/v of flecainide acetate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 29 volumes of *acetonitrile* and 71 volumes of *water* and further dilute 1 volume of this solution to 5 volumes with the same solvent mixture. Solution (3) contains 0.01% w/v of each of *flecainide acetate BPCRS* and *flecainide impurity A EPCRS* in a mixture of 29 volumes of *acetonitrile* and 71 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax C8 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 5 volumes of 1M *tetrabutylammonium hydroxide*, 10 volumes of *glacial acetic acid*, 290 volumes of *acetonitrile* and 710 volumes of *water*, the mixture adjusted to pH 5.8 using 18M *ammonia,* and (c) a detection wavelength of 254 nm.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to flecainide and flecainide impurity A is at least 3.5.

For solution (1) allow the chromatography to proceed for nine times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any such peaks is not greater than two and a half times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.05 times the area of the peak in the chromatogram obtained with solution (2) (0.01%).

ASSAY

Dilute the injection with a 0.2% v/v solution of *lactic acid* to produce a solution containing 0.01% w/v of flecainide acetate and measure the *absorbance* of the resulting solution at the maximum at about 296 nm, Appendix II B, using a 0.2% v/v solution of *lactic acid* in the reference cell. Calculate the content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in the injection from the *absorbance* obtained with a solution containing 0.01% w/v of *flecainide acetate BPCRS* in a 0.2% v/v solution of *lactic acid* and using the declared content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in *flecainide acetate BPCRS*.

STORAGE

Flecainide Injection should not be allowed to freeze.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and D stated under Flecainide Acetate.

Flecainide Tablets

General Notices

Action and use

Class I antiarrhythmic.

DEFINITION

Flecainide Tablets contain Flecainide Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of flecainide acetate, $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the solution obtained in the Assay in the range 230 nm to 350 nm exhibits a maximum at 296 nm.

B. Shake a quantity of the powdered tablets containing 0.1 g of flecainide acetate with 10 ml of *methanol* for 10 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of flecainide acetate *(RS 397)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 5 ml of the medium, filter and dilute the filtered solution, if necessary, with sufficient 0.1M *hydrochloric acid* to produce a solution expected to contain about 0.005% w/v of flecainide acetate. Measure the *absorbance* of the solution at the maximum at 298 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of flecainide acetate, $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$, in the medium from the absorbance of a 0.005% w/v solution of *flecainide acetate BPCRS* in 0.1M *hydrochloric acid* using the declared content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in *flecainide acetate BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* F_{254} *plate* (Merck plates are suitable) and a mixture of 2 volumes of *methanol*, 5 volumes of 18M *ammonia*, 100 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) add 2 ml of *methanol* to a quantity of the powdered tablets containing 0.2 g of flecainide acetate, shake, centrifuge and use the supernatant liquid. Solution (2) contains 0.05% w/v of *flecainide acetate BPCRS* in *methanol*. Solution (3) contains 0.05% w/v of *flecainide impurity A EPCRS* in *methanol*. Solution (4) contains 0.02% w/v of *flecainide impurity B EPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to 3-[2,5-bis(2,2,2-trifluoroethoxy)phenyl]-1,5,6,7,8,8ahexahydroimidazo[1,5-*a*]pyridine (impurity A) is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.5%). Spray the plate with a freshly prepared 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat the plate at 105° for approximately 5 minutes and examine immediately. In the chromatogram obtained with solution (1) any bluish-purple spot corresponding to (piperidin-2-yl) methanamine (impurity B) is not more intense than the principal spot in the chromatogram obtained with solution (4) (0.2%) and any other *secondary spot* is not more intense than the principal spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Shake 20 tablets with 100 ml of a 2% v/v solution of *lactic acid* until the tablets have disintegrated, add 650 ml of *water*, shake with the aid of ultrasound for 30 minutes, add sufficient *water* to produce 1000 ml, mix and filter (Whatman GF/F paper is suitable), discarding the first 100 ml of filtrate. Dilute the filtrate with a 0.2% v/v solution of *lactic acid* to produce a solution containing 0.01% w/v of flecainide acetate and measure the *absorbance* of the resulting solution at the maximum at about 296 nm, Appendix II B, using a 0.2% v/v solution of *lactic acid* in the reference cell. Calculate the content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in the tablets from the *absorbance* obtained with a solution containing 0.01% w/v of *flecainide acetate BPCRS* in a 0.2% v/v solution of *lactic acid* and using the declared content of $C_{17}H_{20}F_6N_2O_3,C_2H_4O_2$ in *flecainide acetate BPCRS*.

STORAGE

Flecainide Tablets should be stored at a temperature not exceeding 30°.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and D stated under Flecainide Acetate.

Flucloxacillin Capsules

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Flucloxacillin Capsules contain Flucloxacillin Sodium.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

92.5 to 110.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with the *reference spectrum* of flucloxacillin sodium *(RS 145)*.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the contents of the capsules containing the equivalent of 0.1 g of flucloxacillin with 80 ml of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 100 ml and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to cloxacillin and flucloxacillin is at least 2.5.

For solution (1) allow the chromatography to proceed for six times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing the equivalent of 50 mg of flucloxacillin with 40 ml of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 50 ml, filter and dilute 5 volumes of the resulting solution to 50 volumes with the mobile phase. Solution (2) contains 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (cloxacillin) and the second peak (flucloxacillin) is at least 2.5.

Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ in the capsules using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of flucloxacillin.

Flucloxacillin Injection

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Flucloxacillin Injection is a sterile solution of Flucloxacillin Sodium in Water for Injections. It is prepared by dissolving Flucloxacillin Sodium for Injection in the requisite amount of Water for Injections before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Flucloxacillin Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

FLUCLOXACILLIN SODIUM FOR INJECTION

DEFINITION

Flucloxacillin Sodium for Injection is a sterile material prepared from Flucloxacillin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of flucloxacillin sodium *(RS 145)*.

B. Yield reaction B characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 10% w/v of flucloxacillin, 5.0 to 7.0, Appendix V L.

Related substances

Comply with the test described under Flucloxacillin Capsules but using a solution prepared in the following manner as solution (1). Shake a quantity of the contents of the sealed container containing the equivalent of 0.1 g of flucloxacillin with 80 ml of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 100 ml and filter.

Water

Not more than 4.5% w/w, Appendix IX C. Use 0.3 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 9 mg of flucloxacillin per ml (solution A). The endotoxin limit concentration of solution A is 3.5 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the mixed contents of 10 containers containing the equivalent of 50 mg of flucloxacillin in sufficient of the mobile phase to produce 50 ml and dilute 5 volumes of the resulting solution to 50 volumes with the mobile phase. Solution (2) contains 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (cloxacillin) and the second peak (flucloxacillin) is at least 2.5.

Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ in a container of average content weight using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

LABELLING

The label of the sealed container states the quantity of Flucloxacillin Sodium contained in it in terms of the equivalent amount of flucloxacillin.

Flucloxacillin Oral Solution

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Flucloxacillin Oral Solution is a solution of Flucloxacillin Sodium in a suitable flavoured vehicle. It is prepared by dissolving the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral solution as directed on the label. The solution, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral solution may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dilute a quantity of the oral solution containing the equivalent of 50 mg of flucloxacillin to 20 ml with *phosphate buffer pH 7.0*. Solution (2) contains 0.25% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. Solution (3) contains 0.25% w/v of each of *cloxacillin sodium BPCRS*, *dicloxacillin sodium EPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

TESTS

Acidity or alkalinity

pH, 4.0 to 7.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For solution (1) dilute a weighed quantity of the oral solution containing the equivalent of 50 mg of flucloxacillin to 50 ml with the mobile phase and dilute 5 volumes of this solution to 50 volumes with the mobile phase. Solution (2) contains 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (cloxacillin) and the second peak (flucloxacillin) is at least 2.5.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{19}H_{17}ClFN_3O_5S$, weight in volume, using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

Repeat the procedure using a portion of the oral solution that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The oral solution should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of flucloxacillin.

Flucloxacillin Oral Suspension

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Flucloxacillin Oral Suspension is a suspension of Flucloxacillin Magnesium Octahydrate in a suitable flavoured vehicle. It is prepared by dispersing the dry ingredients in the specified volume of water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids.

For the following tests prepare the oral suspension as directed on the label. The suspension examined immediately after preparation, unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable) and a mixture of 30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dilute a quantity of the oral suspension containing the equivalent of 50 mg of flucloxacillin to 20 ml with *phosphate buffer pH 7.0*. Solution (2) contains 0.25% w/v of *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. Solution (3) contains 0.25% w/v of each of *cloxacillin sodium BPCRS*, *dicloxacillin sodium EPCRS* and *flucloxacillin sodium BPCRS* in *phosphate buffer pH 7.0*. After removal of the plate, allow it to dry in air, expose to iodine vapour until the spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

B. Dilute a quantity of the oral suspension containing the equivalent of 50 mg of flucloxacillin to 20 ml with *water*, shake to dissolve and filter if necessary. The filtrate yields reaction B characteristic of *magnesium salts*, Appendix VI.

TESTS

Acidity

pH, 4.8 to 5.8, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a weighed quantity of the oral suspension containing the equivalent of 50 mg of flucloxacillin to 50 ml with the mobile phase and dilute 5 volumes of the solution to 50 volumes with the mobile phase. Solution (2) contains 0.011% w/v of *flucloxacillin sodium BPCRS* in the mobile phase. Solution (3) contains 0.01% w/v of each of *flucloxacillin sodium BPCRS* and *cloxacillin sodium BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 5.0 with 2M *sodium hydroxide* and (c) a detection wavelength of 225 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (cloxacillin) and the second peak (flucloxacillin) is at least 2.5.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{19}H_{17}ClFN_3O_5S$, weight in volume, using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The oral suspension should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of flucloxacillin.

Flucytosine Tablets

General Notices

Action and use

Antifungal.

DEFINITION

Flucytosine Tablets contain Flucytosine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of flucytosine, $C_4H_4FN_3O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Flucytosine with 100 ml of *methanol* for 30 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of flucytosine *(RS 146)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution used in the Assay exhibits a maximum only at 286 nm.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a suitable silica gel as the coating substance and a mixture of 1 volume of *anhydrous formic acid*, 15 volumes of *water*, 25 volumes of *methanol* and 60 volumes of *ethyl acetate* as the mobile phase in an unsaturated tank but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.10 g of Flucytosine with 10 ml of a mixture of equal volumes of 13.5M *ammonia* and *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *methanol (60%)* and dilute 1 volume of the resulting solution to 100 volumes with *methanol (60%)*. For solution (3) dilute 1 volume of solution (1) to 10 volumes and dissolve 5 mg of *fluorouracil EPCRS* in 5 ml of the resulting solution. After removal of the plate, allow the solvent to evaporate, stand the plate in a tank of chlorine vapour prepared by the addition of *hydrochloric acid* to a 5% w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 15 minutes. Remove the plate and place it in a current of cold air until the excess of chlorine is removed and an area of the coating substance below the line of application does not give a blue colour with a drop of *potassium iodide and starch solution*. Spray the plate with *potassium iodide and starch solution* and examine the plate in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Flucytosine add 80 ml of 0.1M *hydrochloric acid* and shake for 15 minutes. Dilute to 100 ml with 0.1M *hydrochloric acid* and filter. Dilute 10 ml of the filtrate to 100 ml with 0.1M *hydrochloric acid* and further dilute 10 ml of the solution to 100 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 286 nm, Appendix II B. Calculate the content of $C_4H_4FN_3O$ taking 709 as the value of A(1%, 1 cm) at the maximum at 286 nm.

STORAGE

Flucytosine tablets should be protected from light.

Fludrocortisone Tablets

General Notices

Action and use

Mineralocorticoid.

DEFINITION

Fludrocortisone Tablets contain Fludrocortisone Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fludrocortisone acetate, $C_{23}H_{31}FO_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase B*. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 1 mg of fludrocortisone acetate with 20 ml of *chloroform* for 5 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 4 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*. Solution (2) contains 0.025% w/v of *fludrocortisone acetate BPCRS* in a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to fludrocortisone acetate in the chromatogram obtained with solution (1).

TESTS

Uniformity of content

Tablets containing less than 2 mg of fludrocortisone acetate comply with the requirement stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. Solution A contains 0.002% w/v of *norethisterone BPCRS* in *acetonitrile*. Solution (1) is a mixture of 4 volumes of a 0.0025% w/v solution of *fludrocortisone acetate BPCRS* in solution A and 1 volume of *water*. For solution (2) place a single tablet in a centrifuge tube, add 1 ml of *water*, shake on a vortex-type mixer for 1 minute, add 4.0 ml of solution A and shake again for 1 minute. Shake for a further 40 minutes on a mechanical shaker, centrifuge and use the clear supernatant solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 240 nm.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution A contains 0.01% w/v of *norethisterone BPCRS* (internal standard) in *acetonitrile*. Solution (1) is a mixture of 20 ml of solution A, 25 ml of a 0.01% w/v solution of *fludrocortisone acetate BPCRS* in *acetonitrile* and 10 ml of *water* diluted to 100 ml with *acetonitrile*. Prepare solution (2) in the same manner as solution (3) but using 8 ml of *acetonitrile* in place of 4 ml of solution A and 4 ml of acetonitrile. For solution (3) shake a quantity of the powdered tablets containing 0.5 mg of fludrocortisone acetate with 2 ml of *water* for 1 minute, add 4 ml of solution A and 4 ml of *acetonitrile* and shake on a mechanical shaker for 40 minutes. Dilute the mixture to 20 ml with *acetonitrile*, centrifuge and use the supernatant liquid.

The chromatographic conditions prescribed under the test for Uniformity of content may be used.

Calculate the content of $C_{23}H_{31}FO_6$ using the declared content of $C_{23}H_{31}FO_6$ in *fludrocortisone acetate BPCRS*.

Fluocinolone Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluocinolone Cream contains Fluocinolone Acetonide or Fluocinolone Acetonide Dihydrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocinolone acetonide, $C_{24}H_{30}F_2O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Fluocinolone Ointment.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to fluocinolone acetonide in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For creams containing 0.025% to 0.2% w/w of fluocinolone acetonide, solution (1) contains 0.025% w/v of *fluocinolone acetonide BPCRS* and 0.005% w/v of *phenacetin* (internal standard) in *chloroform*. Prepare solution (2) in the following manner. To a quantity of the cream containing 2.5 mg of fluocinolone acetonide add 60 ml of a solution prepared by adding 80 ml of *methanol* to 20 ml of a 25% w/v solution of *lithium chloride* and disperse by shaking vigorously. Add 100 ml of *cyclohexane*, shake gently for 2 minutes and separate the lower, aqueous methanolic layer, taking care to exclude any solid matter that separates at the interface. Repeat the extraction using a further 25 ml of the lithium chloride solution. To the combined extracts add a solution of 11 g of *aluminium potassium sulphate* in 214 ml of *water* followed by 50 ml of *chloroform*, shake vigorously for about 3 minutes, allow the layers to separate and filter the chloroform extract through filter paper (Whatman No. 1 is suitable), previously moistened with *chloroform*, again excluding any solid matter at the interface. Repeat the extraction with 50- and 10-ml quantities of *chloroform*, filtering the extracts as before. Evaporate the combined extracts to dryness on a water bath in a current of nitrogen, dissolve the residue in 5 ml of *chloroform*, transfer to a 10-ml graduated flask with the aid of *chloroform* and add sufficient *chloroform* to produce 10 ml. Prepare solution (3) in the same manner as solution (2) but adding 1.0 ml of a 0.050% w/v solution of *phenacetin* to the chloroform solution before dilution to 10 ml.

For creams containing 0.01% w/w of fluocinolone acetonide, solution (1) contains 0.01% w/v of *fluocinolone acetonide BPCRS* and 0.002% w/v of *phenacetin* (internal standard) in *chloroform*. Prepare solution (2) as described above but using a quantity of the cream containing 1 mg of fluocinolone acetonide. Prepare solution (3) in the same manner as solution (2) but adding 1 ml of a 0.02% w/v solution of *phenacetin* to the chloroform solution before diluting to 10 ml.

For creams containing 0.00625% w/w of fluocinolone acetonide, solution (1) contains 0.00625% w/v of *fluocinolone acetonide BPCRS* and 0.00125% w/v of *phenacetin* (internal standard) in *chloroform*. Prepare solution (2) as described above but using a quantity of the cream containing 0.62 mg of fluocinolone acetonide. Prepare solution (3) in the same manner as solution (2) but adding 1 ml of a 0.0125% w/v solution of *phenacetin* to the chloroform solution before diluting to 10 ml.

For creams containing 0.0025% w/w of fluocinolone acetonide, solution (1) contains 0.0025% w/v of *fluocinolone acetonide BPCRS* and 0.0005% w/v of *phenacetin* (internal standard) in *chloroform*. Prepare solution (2) as described above but using a quantity of the cream containing 0.25 mg of fluocinolone acetonide. Prepare solution (3) in the same manner as solution (2) but adding 1 ml of a 0.005% w/v solution of *phenacetin* to the chloroform solution before diluting to 10 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 58 volumes of *hexane*, 40 volumes of *chloroform*, 2 volumes of *methanol* and 0.1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 1.8 ml per minute and (c) a detection wavelength of 243 nm.

The assay is not valid unless the *resolution factor* (R_s) between the peaks due to fluocinolone acetonide and phenacetin is more than 2, and the *capacity factors* (k') of fluocinolone acetonide and phenacetin are about 3 and 2, respectively. If these conditions are not achieved, adjust the concentration of *methanol* in the mobile phase, increasing its concentration to reduce the value of k' and reducing its concentration to increase the value of k'. If the correct values cannot be obtained by this means, the column is faulty and must be repacked. If, when the correct k' values have been obtained, the value of R_s is less than 2, reduce the concentration of *chloroform* in the mobile phase by 5% to obtain an increased retention time for both fluocinolone acetonide and phenacetin and re-adjust the k' values to the specified values by increasing the concentration of *methanol*. Repeat the adjustment of chloroform and methanol concentrations until correct values for both R_s and k' have been obtained.

Calculate the content of $C_{24}H_{30}F_2O_6$ in the cream using the declared content of $C_{24}H_{30}F_2O_6$ in *fluocinolone acetonide BPCRS*.

LABELLING

When the active ingredient is Fluocinolone Acetonide Dihydrate, the quantity is stated in terms of the equivalent amount of fluocinolone acetonide.

Fluocinolone Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluocinolone Ointment contains Fluocinolone Acetonide or Fluocinolone Acetonide Dihydrate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocinolone acetonide, $C_{24}H_{30}F_2O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 60 volumes of n-*hexane*, 40 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *triethylamine* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) disperse, by shaking, a quantity of the preparation being examined containing 0.25 mg of fluocinolone acetonide in 2 ml of *chloroform*, add 10 ml of *methanol*, shake vigorously, cool in ice for 15 minutes, centrifuge at 3000 revolutions per minute for 15 minutes, decant the clear, supernatant liquid, evaporate to dryness on a water bath in a current of nitrogen and dissolve the residue in 1 ml of *chloroform*. Solution (2) contains 0.025% w/v solution of *fluocinolone acetonide BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and spray whilst hot with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to fluocinolone acetonide in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0050% w/v of *fluocinolone acetonide BPCRS* and 0.40% v/v of *toluene* (internal standard) in *methanol (80%)*.

For ointments containing 0.01% w/w or less of fluocinolone acetonide prepare solution (2) in the following manner. To a quantity of the ointment containing 0.5 mg of fluocinolone acetonide add 50 ml of *2,2,4-trimethylpentane* and 8 ml of *methanol (80%)*, warm gently on a water bath until the preparation is dispersed and shake vigorously for 2 minutes. Separate the lower, aqueous methanolic layer and wash the upper layer with 1 ml of *methanol (80%)*, adding the washing to the previous extract. To the combined extracts add sufficient *methanol*

(80%) to produce 10 ml. Prepare solution (3) in the same manner as solution (2) but adding 1 ml of a 4% v/v solution of the internal standard in *methanol* to the combined extracts before dilution to 10 ml.

For ointments containing 0.025% w/w of fluocinolone acetonide, prepare solution (2) in the following manner. To a quantity of the ointment containing 1.25 mg of fluocinolone acetonide add 50 ml of *2,2,4-trimethylpentane* and 20 ml of *methanol (80%)*, warm gently on a water bath until the preparation is dispersed and shake vigorously for 2 minutes. Separate the lower, aqueous methanolic layer and wash the upper layer with 2 ml of *methanol (80%)*, adding the washing to the previous extract. To the combined extracts add sufficient *methanol (80%)* to produce 25 ml. Prepare solution (3) in the same manner as solution (2) but adding 1 ml of a 10% v/v solution of the internal standard in *methanol* to the combined extracts before dilution to 25 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 55 volumes of *water*, 45 volumes of *acetonitrile* and 0.1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{24}H_{30}F_2O_6$ in the ointment using the declared content of $C_{24}H_{30}F_2O_6$ in *fluocinolone acetonide BPCRS*.

LABELLING

When the active ingredient is Fluocinolone Acetonide Dihydrate, the quantity is stated in terms of the equivalent amount of fluocinolone acetonide.

Fluocinonide Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluocinonide Cream contains Fluocinonide in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocinonide, $C_{26}H_{32}F_2O_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* F_{254} as the coating substance and a mixture of 12 volumes of *water*, 80 volumes of *methanol*, 150 volumes of *ether* and 770 volumes of *dichloromethane* as the mobile phase. Mix the water and the methanol before adding to the remaining solvents of the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (1) disperse a quantity of the cream containing 2.5 mg of fluocinonide in 25 ml of *methanol (80%)*, add 50 ml of *ether* and shake vigorously to produce a clear, homogeneous solution. Add 15 ml of *water*, shake and transfer the lower layer to a second separating funnel. Add 100 ml of *water*, extract with 10 ml of *chloroform*, filter the chloroform layer through filter paper and evaporate to a volume of about 1 ml in a current of air on a water bath. Solution (2) contains 0.1% w/v of *fluocinonide BPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the cream containing 1.5 mg of fluocinonide in 20 ml of *methanol (80%)*, warming gently over a water bath if necessary. Add 50 ml of *2,2,4-trimethylpentane*, shake for 2 minutes and transfer the lower aqueous methanol layer to a 50 ml flask. Repeat the extraction with a further 20 ml of *methanol (80%)*. Dilute the combined extracts to volume with the same solvent. Solution (2) contains 0.003% w/v of *fluocinonide BPCRS* in *methanol (80%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 0.1 volume of *glacial acetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{26}H_{32}F_2O_7$ in the cream using the declared content of $C_{26}H_{32}F_2O_7$ in *fluocinonide BPCRS*.

Fluocinonide Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluocinonide Ointment contains Fluocinonide in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocinonide, $C_{26}H_{32}F_2O_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A*,* using *silica gel* F_{254} as the coating substance and a mixture of 12 volumes of *water*, 80 volumes of *methanol*, 150 volumes of *ether* and 770 volumes of *dichloromethane* as the mobile phase. Mix the water and the methanol before adding to the remaining components of the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the ointment containing 2 mg of fluocinonide in 20 ml of *methanol (80%)* in a 100-ml separating funnel containing 50 ml of *2,2,4-trimethylpentane.* Warm the contents over a water bath and shake gently. Allow the layers to separate and transfer the lower layer to a 250-ml separating funnel containing 100 ml of *water*. Add 10 ml of *chloroform* and shake for 3 minutes. Allow the layers to separate, evaporate the lower layer to dryness on a water bath in a current of air and dissolve the residue in 1 ml of *chloroform*. Solution (2) contains 0.2% w/v of *fluocinonide BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air until the solvent has evaporated. Heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the ointment containing 1.5 mg of fluocinonide in 20 ml of *methanol (80%)* in a 100-ml separating funnel containing 50 ml of *2,2,4-tri-methylpentane*. Warm the contents over a water bath and shake gently for 2 minutes. Allow the layers to separate and transfer the lower layer to a 50 ml flask. Repeat the extraction with a further 20 ml of *methanol (80%)*. Dilute the combined extracts to 50ml with the same solvent. Solution (2) contains 0.003% w/v of *fluocinonide BPCRS* in *methanol (80%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 0.1 volume of *glacial acetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{26}H_{32}F_2O_7$ in the ointment using the declared content of $C_{26}H_{32}F_2O_7$ in *fluocinonide BPCRS.*

Fluocortolone Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluocortolone Cream contains Fluocortolone Pivalate and Fluocortolone Hexanoate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluocortolone pivalate, $C_{27}H_{37}FO_5$

90.0 to 110.0% of the stated amount.

Content of fluocortolone hexanoate, $C_{28}H_{39}FO_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silanised silica gel precoated plate the surface of which has been modified by chemically-bonded octadecylsilyl groups (Whatman KC18F plates are suitable) and a mixture of 35 volumes of *water* and 65 volumes of *acetonitrile* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) transfer a quantity of the preparation being examined containing 1 mg of fluocortolone pivalate to a separating funnel with the aid of 10 ml of *methanol*, add 50 ml of *2,2,4-trimethylpentane* and shake vigorously for 1 minute. Allow to stand, separate the lower methanol layer, add 100 ml of *water* and 50 ml of *dichloromethane*, shake vigorously for 1 minute and centrifuge at 1500 revolutions per minute for 5 minutes. Evaporate 25 ml of the dichloromethane layer to dryness on a water bath in a current of nitrogen and dissolve the residue in 1 ml of a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*. For solution (2) mix 0.5 ml of a 0.1% w/v solution of *fluocortolone pivalate BPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane* with 0.5 ml of a 0.1% w/v solution of *fluocortolone hexanoate BPCRS* in the same solvent mixture. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air for 10 minutes and examine under *ultraviolet light (254 nm)*. Two principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two compact spots.

B. In the Assay, the chromatogram obtained with solution (3) shows two peaks with the same retention times as the peaks due to fluocortolone pivalate and fluocortolone hexanoate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.004% w/v each of *fluocortolone pivalate BPCRS*, *fluocortolone hexanoate BPCRS* and *benzyl benzoate* (internal standard) in a mixture of 4 volumes of *acetonitrile* and 1 volume of *water*. For solution (2) add 20 ml of a mixture of 4 volumes of *acetonitrile* and 1 volume of *water* followed by 50 ml of *2,2,4-trimethylpentane* to a quantity of the preparation being examined containing 2 mg of fluocortolone pivalate and heat on a water bath, shaking intermittently, until the preparation is completely dispersed. Remove from the water bath, shake vigorously for 3 minutes, allow to separate and transfer the lower, aqueous acetonitrile layer to a flask. Repeat the extraction of the 2,2,4-trimethylpentane solution with a further 20 ml of the acetonitrile–water mixture and again transfer the lower, aqueous acetonitrile layer to the flask. Dilute the combined extracts to 50 ml with a mixture of 4 volumes of *acetonitrile* and 1 volume of *water* and filter a portion using a pressure filter (a Swinnex filter holder prepared with a double thickness of Whatman No. 42 filter paper is suitable). Prepare solution (3) in the same manner as solution (2) but adding 2 ml of a 0.100% v/v solution of *benzyl benzoate* in a mixture of 4 volumes of *acetonitrile* and 1 volume of *water* to the combined extracts before dilution to 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute, a mixture of *acetonitrile* and *water* each containing 1% v/v of *glacial acetic acid* adjusted so that the retention time of fluocortolone pivalate is about 9 minutes and baseline separation is obtained from benzyl benzoate and fluocortolone hexanoate (retention time, about 11 minutes) (a mixture of 53 volumes of *acetonitrile*, 47 volumes of *water* and 1 volume of *glacial acetic acid* is usually suitable) and (c) a detection wavelength of 238 nm.

Calculate the contents of $C_{27}H_{37}FO_5$ and $C_{28}H_{39}FO_5$ in the preparation being examined using the declared contents of $C_{27}H_{37}FO_5$ in *fluocortolone pivalate BPCRS* and $C_{28}H_{39}FO_5$ in *fluocortolone hexanoate BPCRS* respectively.

STORAGE

Fluocortolone Cream should be stored at a temperature not exceeding 30°.

LABELLING

The strength is stated as the percentages w/w of Fluocortolone Pivalate and Fluocortolone Hexanoate.

Fluorescein Eye Drops

General Notices

Action and use

Detection of corneal lesions, retinal angiography and pancreatic function testing.

DEFINITION

Fluorescein Eye Drops are a sterile solution of Fluorescein Sodium in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of fluorescein sodium, $C_{20}H_{10}Na_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Evaporate a volume containing 20 mg of fluorescein sodium and dry at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorescein sodium *(RS 151)*.

B. The eye drops are strongly fluorescent, even in extreme dilution; the fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

C. Dilute with *water* to produce a solution containing 0.05% w/v of fluorescein sodium. One drop of the solution, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to bromine vapour for 1 minute and then to ammonia vapour the yellow colour becomes deep pink.

TESTS

Acidity or alkalinity

pH, 7.0 to 9.0, Appendix V L.

Chloroform-soluble matter

To a volume containing 0.1 g of fluorescein sodium add 1 ml of 2M *sodium hydroxide*, extract with 10 ml of *chloroform* and dry the chloroform layer with *anhydrous sodium sulphate*. The *absorbance* of the resulting solution at 480 nm is not more than 0.05, Appendix II B, using *chloroform* in the reference cell.

Dimethylformamide

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Solution (1) contains 0.0020% v/v of *dimethylformamide* and 0.0020% v/v of *dimethylacetamide* (internal standard). For solution (2) dilute the eye drops with *water*, if necessary, to produce a solution containing 1.0% w/v of fluorescein sodium. To 5 ml of this solution add, with stirring, 0.3 ml of 1M *hydrochloric acid*, allow to stand for 15 minutes and centrifuge; dissolve 10 mg of *trisodium orthophosphate* in 2 ml of the supernatant liquid. Prepare solution (3) in the same manner as solution (2) but adding 1 ml of a 0.010% v/v solution of *dimethylacetamide* before the hydrochloric acid.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 10% w/w of *polyethylene glycol 1000* and maintained at 120°.

In the chromatogram obtained with solution (3) the ratio of the area of any peak corresponding to dimethylformamide to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with solution (1) (0.2%).

Related substances and resorcinol

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of solution (1) and 5 µl of each of solutions (2) to (6). For solution (1) dilute the eye drops with 0.1M *methanolic hydrochloric acid* to contain 0.5% w/v of fluorescein sodium. For solution (2) add to a volume of the eye drops containing 25 mg of fluorescein sodium 2 ml of *phosphate buffer pH 8.0*, 3 ml of *water* and 2.5 g of *sodium chloride*, shake to dissolve the sodium chloride and extract with two 25-ml quantities of *peroxide-free ether*. Dry the combined extracts over *anhydrous sodium sulphate*, evaporate to dryness under reduced pressure and dissolve the residue in 1 ml of 0.1M *methanolic hydrochloric acid*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with 0.1M *methanolic hydrochloric acid*. For solution (4) dilute 2 volumes of solution (3) to 5 volumes with 0.1M *methanolic hydrochloric acid*. Solution (5) contains 0.0125% w/v of *resorcinol* in 0.1M *methanolic hydrochloric acid*. For solution (6) mix 5 volumes of a 0.025% w/v solution of *resorcinol* in 0.1M *methanolic hydrochloric acid* with 2 volumes of solution (1) and add sufficient *methanol* to produce 10 ml. After removal of the plate, allow it to dry in air, expose the plate to iodine vapour for 30 minutes and examine in daylight and under *ultraviolet light (254 nm)*. On examination under ultraviolet light (254 nm), any *secondary spot* in the chromatogram obtained with solution (1) other than any spot corresponding to resorcinol is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (0.2%). On examination in daylight, any spot corresponding to resorcinol in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (5) (0.5%). The test is not valid unless the chromatogram obtained with solution (6) shows two clearly separated spots in daylight.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops with the mobile phase to produce a solution containing 0.005% w/v of fluorescein sodium. For solution (2) dissolve 55 mg of *diacetylfluorescein BPCRS* in a mixture of 5 ml of *ethanol (96%)* and 1 ml of 2.5M *sodium hydroxide*, heat on a water bath for 20 minutes, mixing frequently, cool and add sufficient *water* to produce 50 ml. Dilute 5 volumes of this solution to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 5 volumes of *triethylamine*, 400 volumes of *acetonitrile* and 595 volumes of *water*, the pH of the mixture adjusted to 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{20}H_{10}Na_2O_5$ in the eye drops from the chromatograms obtained and using the declared content of anhydrous diacetylfluorescein in *diacetylfluorescein BPCRS*. Each mg of anhydrous diacetylfluorescein is equivalent to 0.9037 mg of $C_{20}H_{10}Na_2O_5$.

STORAGE

Fluorescein Eye Drops should be protected from light.

LABELLING

The label states that the eye drops should be discarded after use on a single occasion.

Fluorescein Injection

General Notices

Action and use

Detection of corneal lesions, retinal angiography and pancreatic function testing.

DEFINITION

Fluorescein Injection is a sterile solution of Fluorescein Sodium in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fluorescein sodium, $C_{20}H_{10}Na_2O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Evaporate 1 ml to dryness on a water bath and dry the residue at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorescein sodium *(RS 151)*.

B. The injection is strongly fluorescent, even in extreme dilution; the fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

C. Dilute with *water* to produce a solution containing 0.05% w/v of fluorescein sodium. One drop of the solution, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to bromine vapour for 1 minute and then to ammonia vapour the yellow colour becomes deep pink.

TESTS

Alkalinity

pH, 8.0 to 9.8, Appendix V L.

Chloroform-soluble matter

Dilute the injection with *water*, if necessary, to produce a solution containing 5% w/v of fluorescein sodium. To 4 ml add 1 ml of 1M *sodium hydroxide*, dilute to 10 ml with *water*, extract with 10 ml of *chloroform* and dry the chloroform layer over *anhydrous sodium sulphate*. The *absorbance* of the resulting solution at 480 nm is not more than 0.10, Appendix II B, using *chloroform* in the reference cell.

Dimethylformamide

Complies with the test described under Fluorescein Eye Drops using the following solutions in place of solutions (2) and (3). For solution (2) dilute the injection with *water*, if necessary, to produce a solution containing 5.0% w/v of fluorescein sodium. To 10 ml of this solution add, with stirring, 1 ml of 3M *hydrochloric acid*, allow to stand for 15 minutes and centrifuge;

dissolve 0.1 g of *trisodium orthophosphate* in 5 ml of the supernatant liquid. Prepare solution (3) in the same manner as solution (2) but adding 1.0 ml of a 0.10% v/v solution of *dimethylacetamide* (internal standard) before the hydrochloric acid.

Related substances and resorcinol

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each solution. For solution (1) dilute the injection with 0.1M *methanolic hydrochloric acid* to produce a solution containing 1.0% w/v of fluorescein sodium. For solution (2) dilute the injection with *water* to produce a solution containing 5% w/v of fluorescein sodium. To 5 ml of this solution add 2 ml of *phosphate buffer pH 8.0*, 3 ml of *water* and 2.5 g of *sodium chloride*, shake to dissolve the sodium chloride and extract with two 25-ml quantities of *peroxide-free ether*. Dry the combined extracts over *anhydrous sodium sulphate*, evaporate to dryness under reduced pressure and dissolve the residue in 10 ml of 0.1M *methanolic hydrochloric acid*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with 0.1M *methanolic hydrochloric acid*. For solution (4) dilute 2 volumes of solution (3) to 5 volumes with 0.1M *methanolic hydrochloric acid*. Solution (5) contains 0.0125% w/v of *resorcinol* in 0.1M *methanolic hydrochloric acid*. For solution (6) mix 9 volumes of solution (5) with 1 volume of solution (1). After removal of the plate, allow it to dry in air, expose the plate to iodine vapour for 30 minutes and examine in daylight and under *ultraviolet light (254 nm)*. On examination under ultraviolet light (254 nm), any *secondary spot* in the chromatogram obtained with solution (1) other than any spot corresponding to resorcinol is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (0.2%). On examination in daylight, any spot corresponding to resorcinol in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (5) (0.5%). The test is not valid unless the chromatogram obtained with solution (6) shows two clearly separated spots in daylight.

ASSAY

Carry out the Assay described under Fluorescein Eye Drops using a solution prepared in the following manner as solution (1). Dilute the injection with *water* to produce a solution containing 1.0% w/v of fluorescein sodium and dilute 1 volume of this solution to 200 volumes with the mobile phase.

STORAGE

Fluorescein Injection should be protected from light.

Fluorometholone Eye Drops

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluorometholone Eye Drops are a sterile suspension of Fluorometholone in purified water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of fluorometholone, $C_{22}H_{29}FO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the eye drops containing 5 mg of fluorometholone with 20 ml of *acetone*, filter and evaporate the filtrate to dryness. Dissolve the residue in 10 ml of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorometholone *(RS 152)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the eye drops containing 1 mg of fluorometholone to 10 ml with a mixture of equal volumes of *methanol* and *water*. Solution (2) contains 0.00005% w/v of the substance being examined in *methanol* and solution (3) contains 0.00005% w/v each of *deltamedrane BPCRS* and *fluorometholone BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silca gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 40 volumes of *water* and 60 volumes of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to deltamedrane and fluorometholone is at least 1.5.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix the eye drops thoroughly and dilute a quantity containing 1 mg of fluorometholone to 25 ml with a mixture of equal volumes of *methanol* and *water*. For solution (2) prepare a 0.05% w/v solution of *fluorometholone BPCRS* in *methanol* and dilute 4 ml of the solution to 50 ml with a mixture of equal volumes of *methanol* and *water*. Solution (3) contains 0.00005% w/v each of *deltamedrane BPCRS* and *fluorometholone BPCRS*.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to deltamedrane and fluorometholone is at least 1.5.

Calculate the content of $C_{22}H_{29}FO_4$ in the eye drops using the declared content of $C_{22}H_{29}FO_4$ in *fluorometholone BPCRS*.

Fluorouracil Cream

General Notices

Action and use

Pyrimidine analogue; cytotoxic.

DEFINITION

Fluorouracil Cream contains Fluorouracil in a suitable basis.
The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluorouracil, $C_4H_3FN_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dry a quantity containing 50 mg of Fluorouracil at a pressure not exceeding 0.7 kPa, mix with 100 ml of *ether*, decant, wash the white, crystalline residue with 50 ml of *ether* and dry in air. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorouracil *(RS 153)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 266 nm.

TESTS

5-Hydroxyuracil

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 70 volumes of *ethyl acetate*, 40 volumes of *acetone* and 10 volumes of *water* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the cream containing 0.10 g of Fluorouracil with 10 ml of *water* for 5 minutes, add 10 ml of *ethanol (96%)*, mix and filter through glass-fibre paper. Solution (2) contains 0.00125% w/v of *5-hydroxyuracil* in *methanol (50%)*. After removal of the plate, allow it to dry in air, spray with a freshly prepared 0.5% w/v solution of *fast blue B salt* and then with 0.1M *sodium hydroxide*. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Urea

Carry out the method described under 5-Hydroxyuracil applying separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the cream containing 0.10 g of Fluorouracil with 10 ml of *water* for 5 minutes, add 10 ml of *ethanol (96%)*, mix and filter through glass-fibre paper. Solution (2) contains 0.0050% w/v of *urea* in *water*. After removal of the plate, allow it to dry in air, spray with a mixture of 10 volumes of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (96%)* and 1 volume of *hydrochloric acid* and heat at 100° until maximum intensity of the spots is obtained. Any spot corresponding to urea in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

To a quantity containing 50 mg of Fluorouracil add 50 ml of 0.1M *hydrochloric acid* and shake for 5 minutes. Extract with three 25-ml quantities of *chloroform*, discard the chloroform, dilute the aqueous solution to 250 ml with 0.1M *hydrochloric acid* and filter (Whatman No. 42 paper is suitable). Dilute 5 ml to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 266 nm, Appendix II B. Calculate the content of $C_4H_3FN_2O_2$ taking 552 as the value of A(1%, 1 cm) at the maximum at 266 nm.

Fluorouracil Injection

General Notices

Action and use

Pyrimidine analogue; cytotoxic.

DEFINITION

Fluorouracil Injection is a sterile solution in Water for Injections of fluorouracil sodium, prepared by the interaction of Fluorouracil and Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fluorouracil, $C_4H_3FN_2O_2$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Carefully acidify a volume containing the equivalent of 0.1 g of fluorouracil with *glacial acetic acid*, stir, cool and filter. Wash the precipitate with 1 ml of *water* and dry over *phosphorus pentoxide* at 80° at a pressure of 2 kPa for 4 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorouracil *(RS 153)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 266 nm.

TESTS

Alkalinity

pH, 8.5 to 9.1, Appendix V L.

Urea

Carry out the method described under Related substances applying separately to the plate 20 µl of each of the following solutions. For solution (1) dilute a suitable quantity of the injection with *water* to produce a solution containing the equivalent of 0.50% w/v of fluorouracil. Solution (2) contains 0.020% w/v of *urea* in *water*. After removal of the plate, allow it to dry in air, spray with a mixture of 10 volumes of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (96%)* and 1 volume of *hydrochloric acid* and heat at 100° until maximum intensity of the spots is obtained. Any spot corresponding to urea in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 70 volumes of *ethyl acetate*, 15 volumes of *methanol* and 15 volumes of *water* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a suitable quantity of the injection with *water* to produce a solution containing the equivalent of 2.0% w/v of fluorouracil. For solution (2) dilute 1 volume of solution (1) to 400 volumes with *methanol (50%)*. Solution (3) contains 0.0050% w/v of *5-hydroxyuracil* in *methanol (50%)*. After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Spray with a freshly prepared 0.5% w/v solution of *fast blue B salt* and then with 0.1M *sodium hydroxide*. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Disregard any spots with Rf values lower than 0.2.

ASSAY

To a volume containing the equivalent of 75 mg of fluorouracil add 20 ml of 1M *hydrochloric acid* and sufficient *water* to produce 200 ml. Dilute 3 ml to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 266 nm, Appendix II B. Calculate the content of $C_4H_3FN_2O_2$ taking 552 as the value of A(1%,1 cm) at the maximum at 266 nm.

STORAGE

Fluorouracil Injection should be protected from light. It should not be refrigerated.

LABELLING

The strength is stated in terms of the equivalent amount of Fluorouracil in a suitable dose-volume.

Fluoxetine Capsules

General Notices

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Fluoxetine Capsules contain Fluoxetine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of fluoxetine, $C_{17}H_{18}F_3NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the contents of the capsules containing the equivalent of 20 mg of fluoxetine with 10 ml of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluoxetine hydrochloride *(RS 385)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter, discarding the first 5 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a mixture of 250 volumes of *acetonitrile* and 1 volume of *diethylamine*, mix well and adjust the apparent pH to 3.5 with *orthophosphoric acid* (solution A); a white precipitate may form, keep the mixture well stirred. For solution (1) add to 5 ml of filtered medium 2 ml of solution A and mix well. For solution (2) add to 5 ml of a 0.0022% w/v solution of *fluoxetine hydrochloride BPCRS* 2 ml of solution A and mix well.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm) (Zorbax CN is suitable), (b) as the mobile phase at a flow rate of 2 ml per minute a mixture of 4 volumes of *diethylamine*, 400 volumes of *acetonitrile* and 600 volumes of *water*, the mixture adjusted to pH 3.5 with *orthophosphoric acid* and (c) a detection wavelength of 226 nm.

Inject 50 µl of each solution. Calculate the content of $C_{17}H_{18}F_3NO$ in the medium from the chromatograms obtained using the declared content of $C_{17}H_{18}F_3NO,HCl$ in *fluoxetine* *hydrochloride BPCRS* and taking each mg of $C_{17}H_{18}F_3NO,HCl$ to be equivalent to 0.8944 mg of $C_{17}H_{18}F_3NO$.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the capsules containing the equivalent of 20 mg of fluoxetine in sufficient of the mobile phase to produce 10 ml, filter and use the filtrate, discarding the first 2 ml. Solution (2) contains 0.001% w/v of *fluoxetine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (DuPont Zorbax SB-CN is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 35 volumes of *acetonitrile* and 65 volumes of a solution containing 1 volume of *triethylamine* and 99 volumes of *water*, and adjusting the pH to 6.0 with *orthophosphoric acid* and (c) a detection wavelength of 215 nm. If necessary, adjust the composition of the mobile phase so that, in the chromatogram obtained with solution (2), the retention time of the peak due to fluoxetine is between 10 and 20 minutes.

Inject 10 µl of each solution and for solution (1) allow the chromatography to proceed for twice the retention time of the peak due to fluoxetine hydrochloride.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than half the area of the peak in the chromatogram obtained with solution (2) (0.25%), the area of not more than two such peaks is greater than 0.2 times the area of the peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of all the *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the mixed contents of 20 capsules containing the equivalent of 20 mg of fluoxetine in sufficient of the mobile phase to produce 200 ml, filter and use the filtrate, discarding the first 2 ml. Solution (2) contains 0.011% w/v of *fluoxetine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (7.5 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (3.5 µm) (DuPont Zorbax RX-C8 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 33 volumes of a solution containing 0.3% w/v of *glacial acetic acid* and 0.64% w/v of *sodium pentanesulphonate*, adjusted to pH 5.0 with 5M *sodium hydroxide*, and 67 volumes of *methanol* and (c) a detection wavelength of 227 nm.

Inject solution (2) six times. The assay is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

Inject solutions (1) and (2). Calculate the content of $C_{17}H_{18}F_3NO$ in the capsules from the chromatograms obtained using the declared content of $C_{17}H_{18}F_3NO,HCl$ in *fluoxetine hydrochloride BPCRS* and taking each mg of $C_{17}H_{18}F_3NO,HCl$ to be equivalent to 0.8944 mg of $C_{17}H_{18}F_3NO$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of fluoxetine.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Fluoxetine Hydrochloride.

Fluoxetine Oral Solution

General Notices

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Fluoxetine Oral Solution is a solution of Fluoxetine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of fluoxetine, $C_{17}H_{18}F_3NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the oral solution containing the equivalent of 0.1 g of fluoxetine add 5 ml of 4M *sodium hydroxide*, extract with 10 ml of *dichloromethane*, filter the dichloromethane layer (Whatman 1PS is suitable) and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluoxetine *(RS 398)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 2.5 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Add 4.33 g of *sodium octanesulphonate* and 13.8 g of *sodium dihydrogen orthophosphate monohydrate* to 1000 ml of *water*, adjust the pH, if necessary, to 3.0 with *orthophosphoric acid* and filter (solution A). For solution (1) mix a quantity of the oral solution containing the equivalent of 20 mg of fluoxetine in 10 ml of a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A and use immediately. For solution (2) dilute 1 volume of solution (1) to 25 volumes with a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A and further dilute 1 volume to 10 volumes with the same solvent mixture. For solution (3) heat a 0.2% w/v solution of *fluoxetine hydrochloride BPCRS* in 0.5M *sulphuric acid* at 85° for 1 hour (generation of impurity A and chloro-impurity). For solution (4) dilute 1 volume of solution (3) to 100 volumes with a solution containing 0.1% w/v of *fluoxetine hydrochloride BPCRS* in a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A. Solution (5) contains 0.05% w/v of *benzoic acid* in a mixture of 1 volume of *acetonitrile*, 3 volumes of *methanol* and 6 volumes of solution A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelco Supelcosil LC-18-DB is suitable), (b) as mobile phases A and B with a flow rate of 1 ml per minute the solutions described below and (c) a detection wavelength of 215 nm.

Mobile phase A Add 21 volumes of *acetonitrile* and 26 volumes of *methanol* to 53 volumes of solution A and mix.

Mobile phase B Add 35 volumes of *acetonitrile* and 22 volumes of *methanol* to 43 volumes of solution A and mix.

Inject 20 µl of each solution and record the chromatograms under the following conditions. Equilibrate the column with mobile phase A for at least 10 minutes. Elute initially with mobile phase A. After 13 minutes, use linear gradient elution increasing the concentration of mobile phase B to 100% after 2 minutes. Elute isocratically with mobile phase B for 14 minutes. Carry out a linear gradient elution for 1 minute decreasing the concentration of mobile phase B to 0% and elute for at least a further 10 minutes with mobile phase A.

When the chromatograms are recorded under the conditions described above the retention time of fluoxetine is 20 to 30 minutes.

The test is not valid unless, in the chromatogram obtained with solution (4), the peaks which elute before fluoxetine elute before the start of the gradient programme and are well separated from any peaks due to excipients. If necessary, adjust the proportion of acetonitrile in mobile phase A.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.4%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.8%). Disregard any peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (5).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the oral solution with sufficient of the mobile phase to produce a solution containing the equivalent of 0.004% w/v of fluoxetine. Solution (2) contains 0.0045% w/v of *fluoxetine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica, the surface of which has been modified with chemically bonded cyano groups, (5 µm) (Dupont Zorbax CN Special is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of equal volumes of a 1% v/v solution of *triethylamine*, adjusted to pH 6.0 with *orthophosphoric acid*, and *acetonitrile* and (c) a detection wavelength of 215 nm. For solution (1) allow the chromatography to proceed for at least 10 minutes.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{17}H_{18}F_3NO$, weight in volume, using the declared content of $C_{17}H_{18}F_3NO,HCl$ in *fluoxetine hydrochloride BPCRS* and taking each mg of $C_{17}H_{18}F_3NO,HCl$ to be equivalent to 0.8944 mg of $C_{17}H_{18}F_3NO$.

STORAGE

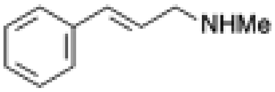
Fluoxetine oral solution should be protected from light.

LABELLING

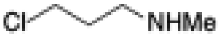
The quantity of active ingredient is stated in terms of the equivalent amount of fluoxetine.

IMPURITIES

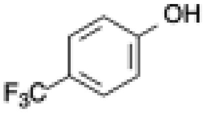
In addition to impurities A and B listed under Fluoxetine Hydrochloride, the impurities limited by the requirements of this monograph include:



1. 3-Methylamino-1-phenylprop-1-ene,



2. 3-Methylamino-1-chloropropane,



3. 4-Trifluoromethylphenol.

Flupentixol Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Flupentixol Injection is a sterile solution of Flupentixol Decanoate in a suitable vegetable oil. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of flupentixol decanoate, $C_{33}H_{43}F_3N_2O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute 1 volume of solution A obtained in the Assay to 2 volumes with *ethanol (96%)*. The *light absorption*, Appendix II B, in the range 205 to 300 nm of the resulting solution exhibits two maxima at 230 nm and 264 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 3 volumes of *diethylamine* and 90 volumes of *cyclohexane* as the mobile phase and an unsaturated tank. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute the injection with *ethanol (96%)* to contain 0.4% w/v of flupentixol decanoate. Solution (2) contains 0.4% w/v of *flupentixol decanoate dihydrochloride BPCRS* in *ethanol (96%).* After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *sodium molybdate* in *sulphuric acid*, heat at 110° for 20 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in colour, position and size to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*, protected from light. For solution (1) dilute the injection to contain 0.20% w/v of Flupentixol Decanoate. Solution (2) contains 0.0060% w/v of cis-*flupentixol BPCRS*. Solution (3) contains 0.001% w/v of *2-trifluoromethylthioxanthone BPCRS*. Solution (4) contains 0.002% w/v of trans-*flupentixol decanoate BPCRS*. Solution (5) contains 0.006% w/v of cis-*flupentixol BPCRS* and 0.001% w/v each of *2-trifluoromethylthioxanthone BPCRS* and trans-*flupentixol decanoate BPCRS* in solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable) maintained at a temperature of 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 0.1 volume of *orthophosphoric acid*, 25 volumes of a 20 millimole solution of *dioctyl sodium sulphosuccinate* (prepared by dissolving 8.89 g of *dioctyl sodium sulphosuccinate* in 500 ml of *water*, stirring for 6 to 8 hours and diluting to 1000 ml with *water*) and 75 volumes of *ethanol (96%)* and (c) a detection wavelength of 270 nm.

Inject solution (1) and allow the chromatography to proceed for 1.5 times the retention time of the principal peak. Inject 20 µl of each solution. The substances are eluted in the following order: 2-trifluoromethylthioxanthone, *cis*-flupentixol (free alcohol), flupentixol decanoate and *trans*-flupentixol decanoate. The test is not valid unless the chromatogram obtained with solution (5) shows clearly separated peaks.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *cis*-flupentixol is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3%), the area of any peak corresponding to 2-trifluoromethylthioxanthone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%) and the area of any peak corresponding to *trans*-flupentixol decanoate is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*, protected from light. For solution (1) dilute a volume of the injection to contain 0.20% w/v of Flupentixol Decanoate. Solution (2) contains 0.22% w/v of *flupentixol decanoate dihydrochloride BPCRS*.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{33}H_{43}F_3N_2O_2S$ using the declared content of $C_{33}H_{43}F_3N_2O_2S$ in *flupentixol decanoate dihydrochloride BPCRS*. Each mg of flupentixol decanoate dihydrochloride is equivalent to 0.8897 mg of flupentixol decanoate.

STORAGE

Flupentixol Injection should be protected from light.

Fluphenazine Decanoate Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Fluphenazine Decanoate Injection is a sterile solution of Fluphenazine Decanoate in refined sesame oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fluphenazine decanoate, $C_{32}H_{44}F_3N_3O_2S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a plate 200 mm × 200 mm in size and *silica gel GF*$_{254}$ as the coating substance. For the first development use *chloroform* as the mobile phase. Apply to the bottom right-hand corner of the plate a volume of the injection containing 25 µg of Fluphenazine Decanoate and develop over a path of 12 cm. After removal of the plate, allow it to dry in air, turn the plate through 90° in a clockwise direction, impregnate the coating with a 5% v/v solution of n*-tetradecane* in n*-hexane* and allow it to dry in air. Apply to the bottom right-hand corner of the plate, to the right of the solvent front of the first development, 1 µl of a 2.0% w/v solution of *fluphenazine decanoate EPCRS* in *ethanol (96%)*. For the second development use *methanol (90%)* as the mobile phase. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with the injection corresponds to that in the chromatogram obtained with the reference substance.

B. Shake a volume containing 5 mg of Fluphenazine Decanoate with 1 ml of a 1% w/v solution of *sucrose* in *hydrochloric acid* and allow to stand for 5 minutes. A red colour is produced in the acid layer.

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with sufficient *chloroform* to produce a solution containing 0.50% w/v of Fluphenazine Decanoate, dilute 1 volume of this solution to 25 volumes with *acetonitrile* and mix. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*. For solution (3) add 0.05 ml of 10M *sodium hydroxide* to 2 ml of solution (1) and allow to stand for 48 hours before use (generation of fluphenazine impurity). For solution (4) add 0.05 ml of *hydrogen peroxide solution (200 vol)* to 5 mg of *fluphenazine decanoate EPCRS*, allow to stand for 10 minutes, add sufficient *chloroform* to produce 1 ml, mix and dilute to 100 ml with *acetonitrile* (generation of mono- and di-*N*-oxide impurities).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) fitted with a guard column (2.5 cm × 4.6 mm) packed with the same material, (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared by adding 450 volumes of *acetonitrile* to a mixture of 75 volumes of a 1% w/v solution of *ammonium carbonate* and 450 volumes of *methanol* adjusted to pH 7.5 with 1M *acetic acid* and (c) a detection wavelength of 260 nm.

Inject separately 20 µl of each solution. After completion of the test, flush the column with a mixture of equal volumes of *methanol* and *water*.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to the two fluphenazine mono-*N*-oxides is at least 2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to the peak due to fluphenazine impurity in the chromatogram obtained with solution (3) is not greater than 4 times the area of the peak in the chromatogram obtained with solution (2) (4%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* other than any peak corresponding to fluphenazine is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (2%). Disregard any peak with a retention time relative to fluphenazine decanoate of 0.2 or less.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using a volume containing 0.15 g of Fluphenazine Decanoate diluted with 75 ml of *anhydrous acetic acid* and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 29.59 mg of $C_{32}H_{44}F_3N_3O_2S$.

STORAGE

Fluphenazine Decanoate Injection should be protected from light.

LABELLING

The label states that the injection is for intramuscular injection only.

Fluphenazine Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Fluphenazine Tablets contain Fluphenazine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fluphenazine hydrochloride, $C_{22}H_{26}F_3N_3OS,2HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *kieselguhr G* as the coating substance. Impregnate the dry plate by placing it in a tank containing a shallow layer of a mixture of 5 volumes of *2-phenoxyethanol*, 15 volumes of *formamide* and 180 volumes of *acetone*, allowing the impregnating solvent to ascend to the top, removing the plate from the tank and using it immediately. Use a mixture of 2 volumes of *diethylamine* and 100 volumes of *petroleum spirit (boiling range, 40° to 60°)* saturated with *2-phenoxyethanol* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 0.2% w/v of fluphenazine hydrochloride, centrifuge and use the supernatant liquid. Solution (2) contains 0.2% w/v of *fluphenazine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air, examine under *ultraviolet light (365 nm)* and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with *ethanolic sulphuric acid (20%)* and observe the colour produced. The principal spot in the chromatogram obtained with solution (1) corresponds in position, fluorescence and colour to that in the chromatogram obtained with solution (2).

B. Extract a quantity of the powdered tablets containing 5 mg of fluphenazine hydrochloride with 5 ml of *acetone*, filter and evaporate the filtrate to dryness. Add 2 ml of *sulphuric acid* to the residue and allow to stand for 5 minutes. An orange colour is produced.

C. Extract a quantity of the powdered tablets containing 10 mg of fluphenazine hydrochloride with 10 ml of *absolute ethanol* containing 0.2% v/v of 13.5M *ammonia* and evaporate the extract to dryness. Heat 0.5 ml of *chromic–sulphuric acid mixture* in a small test tube in a water bath for 5 minutes; the solution wets the side of the tube readily and there is no greasiness. Add 2 or 3 mg of the residue and again heat in a water bath for 5 minutes; the solution does not wet the side of the tube and does not pour easily from the tube.

TESTS

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 5 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 80 volumes of *acetone* as the mobile phase. Apply separately to the plate 50 µl of solution (1) and 25 µl of each of solutions (2) and (3). For solution (1) remove the coating from a suitable number of tablets; shake a quantity of the powdered tablet cores containing 20 mg of fluphenazine hydrochloride with 10 ml of 0.1M *methanolic sodium hydroxide* for 5 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 50 volumes with 0.1M *methanolic sodium hydroxide*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with 0.1M *methanolic sodium hydroxide*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Disregard any spot remaining on the line of application.

Uniformity of content

Tablets containing less than 2 mg of fluphenazine hydrochloride comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. Record *second-derivative ultraviolet absorption spectra* of the following solutions in the range 230 to 300 nm, Appendix II B. For solution (1) add 2 ml of 0.1M *hydrochloric acid* to one tablet and mix with the aid of ultrasound until the tablet is dispersed. Add 60 ml of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *ethanol (80%)*, shake for 20 minutes, dilute to 100 ml with the same solvent mixture, mix and filter. Solution (2) contains 0.001% w/v of *fluphenazine hydrochloride BPCRS*.

For each solution measure the amplitude from the peak at about 266 nm to the trough at about 258 nm. Calculate the content of $C_{22}H_{26}F_3N_3OS,2HCl$ in each tablet using the declared content of $C_{22}H_{26}F_3N_3OS,2HCl$ in *fluphenazine hydrochloride BPCRS*.

ASSAY

For tablets containing 2 mg or more of fluphenazine hydrochloride

Carry out the following procedure protected from light. Record *second-derivative ultraviolet absorption spectra* of the following solutions in the range 230 to 300 nm, Appendix II B. For solution (1) add 20 ml of 0.1M *hydrochloric acid* to 10 tablets, mix with the aid of ultrasound until the tablets are dispersed, add 600 ml of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *ethanol (80%)*, shake for 20 minutes, dilute to 1000 ml with the same solvent mixture, mix and filter. Solution (2) contains 0.001% w/v of *fluphenazine hydrochloride BPCRS*.

For each solution measure the amplitude from the peak at about 266 nm to the trough at about 258 nm. Calculate the content of $C_{22}H_{26}F_3N_3OS,2HCl$ in the tablets using the declared content of $C_{22}H_{26}F_3N_3OS,2HCl$ in *fluphenazine hydrochloride BPCRS*.

For tablets containing less than 2 mg of fluphenazine hydrochloride

Use the average of the 10 individual results obtained in the test for Uniformity of content.

Flurazepam Capsules

General Notices

Action and use

Benzodiazepine.

DEFINITION

Flurazepam Capsules contain Flurazepam Monohydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of flurazepam, $C_{21}H_{23}ClFN_3O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 0.15 g of flurazepam with 3 ml of *chloroform IR* and filter. The *infrared absorption spectrum* of the filtrate, Appendix II A, is concordant with the *reference spectrum* of flurazepam monohydrochloride *(RS 155)*.

B. The *light absorption*, Appendix II B, in the range 220 to 350 nm of the final solution obtained in the Assay exhibits two maxima, at 240 nm and 284 nm.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 2.5 volumes of *diethylamine* and 97.5 volumes of *ether* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake vigorously a quantity of the contents of the capsules containing the equivalent of 0.1 g of flurazepam with 2 ml of a mixture of 2 volumes of 13.5M *ammonia* and 98 volumes of *methanol* and centrifuge. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture. After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the following procedure protected from light. Transfer a quantity of the capsule contents containing the equivalent of 0.1 g of flurazepam into about 150 ml of 1M *methanolic sulphuric acid* , washing the inner surfaces of the capsule shells, shake for 10 minutes and add sufficient 1M *methanolic sulphuric acid* to produce 250 ml. Further dilute this solution with 1M *methanolic sulphuric acid* to produce a solution containing the equivalent of about 0.002% w/v of flurazepam and measure the *absorbance* of the resulting solution at the maximum at

284 nm, Appendix II B. Calculate the content of $C_{21}H_{23}ClFN_3O$ taking 319 as the value of A (1%, 1 cm) at the maximum at 284 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of flurazepam.

Flurbiprofen Eye Drops

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Flurbiprofen Eye Drops are a sterile solution of Flurbiprofen Sodium in purified water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of flurbiprofen sodium dihydrate, $C_{15}H_{12}FNaO_2,2H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 5 volumes of *propan-2-ol* and 95 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute the eye drops, if necessary, with a mixture of 25 volumes of *water* and 50 volumes of *methanol* to produce a solution containing 0.01% w/v of flurbiprofen sodium. Solution (2) contains 0.01% w/v of *flurbiprofen sodium BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.0, Appendix V L.

2-(Biphenyl-4-yl)propionic acid

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops, if necessary, with a mixture of 25 volumes of *water* and 50 volumes of *methanol* to produce a solution containing 0.030% w/v of flurbiprofen sodium. Solution (2) contains 0.00015% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*. Solution (3) contains 0.00050% w/v of *flurbiprofen sodium BPCRS* and 0.00050% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Resolve 5µ is suitable), (b) a mixture of 5 volumes of *glacial acetic acid*, 35 volumes of *acetonitrile* and 60 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm. Adjust the sensitivity so that the heights of the principal peaks in the chromatogram obtained with solution (3) are about 40% of full-scale deflection on the chart paper.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 1.5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(biphenyl-4-yl)propionic acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops, if necessary, with a mixture of 25 volumes of *water* and 50 volumes of *methanol* to produce a solution containing 0.015% w/v of flurbiprofen sodium. Solution (2) contains 0.015% w/v of *flurbiprofen sodium BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*. Solution (3) contains 0.0005% w/v of *flurbiprofen sodium BPCRS* and 0.0005% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS* in a mixture of 25 volumes of *water* and 50 volumes of *methanol*.

The chromatographic procedure described under the test for 2-(Biphenyl-4-yl)propionic acid may be used.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 1.5.

Calculate the content of $C_{15}H_{12}FNaO_2,2H_2O$ in the eye drops using the declared content of $C_{15}H_{12}FNaO_2,2H_2O$ in *flurbiprofen sodium BPCRS*.

Flurbiprofen Suppositories

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Flurbiprofen Suppositories contain Flurbiprofen in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of flurbiprofen, $C_{15}H_{13}FO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *glacial acetic acid*, 10 volumes of *methyl acetate* and 80 volumes of *trichloroethylene* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) cut a suitable number of the suppositories into small pieces, add 50 ml of *methanol* to a quantity containing 0.1 g of flurbiprofen, heat gently until melted, shake mechanically for 10 minutes, allow to cool at 2° to 8°, filter (Whatman No. 1 paper is suitable) and evaporate to dryness. Dissolve the residue in 5 ml of a mixture of equal volumes of *methanol* and *water*. Solution (2) contains 0.2% w/v of *flurbiprofen sodium BPCRS* in a mixture of equal volumes of *methanol* and *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) cut a suitable number of the suppositories into small pieces, add 50 ml of *methanol* to a quantity containing 0.1 g of flurbiprofen, heat gently until melted, shake mechanically for 10 minutes and filter (Whatman No. 1 paper is suitable). Dilute the filtrate with sufficient of a mixture containing 35 volumes of *acetonitrile* and 65 volumes of *water* to produce 100 ml and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water* and further dilute 1 volume to 5 volumes with the same solvent mixture. Solution (3) contains 0.0005% w/v of *2-(biphenyl-4-yl) propionic acid BPCRS* in a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*. Solution (4) contains 0.0005% w/v of *4-acetyl-2-fluorobiphenyl BPCRS* in a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*. For solution (5) dilute 1 volume of solution (1) to 200 volumes with solution (3).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Resolve 5µ is suitable) fitted with a stainless steel guard column (2 cm × 4.6 mm) packed with the same material, (b) a mixture of 5 volumes of *glacial acetic acid*, 35 volumes of *acetonitrile* and 60 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Adjust the sensitivity so that the heights of the principal peaks in the chromatogram obtained with solution (5) are about 40% of full-scale deflection on the chart paper.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the two principal peaks is at least 1.5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(biphenyl-4-yl)propionic acid is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%), the area of any peak corresponding to 4-acetyl-2-fluorobiphenyl is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.5%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) cut a suitable number of the suppositories into small pieces, add 50 ml of *methanol* to a quantity containing 0.1 g of flurbiprofen, heat gently until melted, shake mechanically for 10 minutes and filter (Whatman No. 1 paper is suitable). Dilute the filtrate with sufficient of a mixture containing 35 volumes of *acetonitrile* and 65 volumes of *water* to produce 100 ml, filter and dilute 1 volume of the filtrate to 25 volumes with the same solvent mixture. Solution (2) contains 0.005% w/v of *flurbiprofen sodium BPCRS* in a mixture of 35 volumes of *acetonitrile* and 65 volumes of *water*. Solution (3) contains 0.0005% w/v each of *flurbiprofen sodium BPCRS* and *2-(biphenyl-4-yl)propionic acid BPCRS* in a mixture of 35 volumes of *acetonitrile* and 65 volumes of *water*.

The chromatographic procedure described under Related substances may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.5.

Calculate the content of $C_{15}H_{13}FO_2$ in the suppositories using the declared content of $C_{15}H_{13}FO_2$ in *flurbiprofen sodium BPCRS*.

Flurbiprofen Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Flurbiprofen Tablets contain Flurbiprofen. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of flurbiprofen, $C_{15}H_{13}FO_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.5 g of flurbiprofen with 25 ml of *acetone*, filter, evaporate the filtrate to dryness with the aid of a current of air without heating and dry at 60° at a pressure of 2 kPa. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of flurbiprofen *(RS 156)*.

B. Heat 0.5 ml of *chromic–sulphuric acid mixture* in a small test tube in a water bath for 5 minutes; the solution wets the side of the tube readily and there is no greasiness. Add 2 or 3 mg of the residue and heat in a water bath for 5 minutes; the solution does not wet the side of the tube and does not pour easily from the tube.

C. *Melting point*, about 114°, Appendix V A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 0.5 g of flurbiprofen in 50 ml of *water*, add 200 ml of *acetonitrile*, mix, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water* and further dilute 1 volume to 5 volumes with the same solvent mixture. Solution (3) contains 0.0010% w/v of *2-(biphenyl-4-yl)propionic acid BPCRS* in a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*. For solution (4) dilute 1 volume of solution (1) to 200 volumes with solution (3).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Resolve 5µ is suitable), (b) a mixture of 5 volumes of *glacial acetic acid*, 35 volumes of *acetonitrile* and 60 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Adjust the sensitivity so that the heights of the principal peaks in the chromatogram obtained with solution (4) are about 40% of full-scale deflection on the chart paper.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (4) is at least 1.5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(biphenyl-4-yl)propionic acid is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any *secondary peaks* is not greater than five times the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of flurbiprofen with 60 ml of 0.1M *sodium hydroxide* for 5 minutes, dilute to 100 ml with 0.1M *sodium hydroxide*, filter if necessary and dilute 10 ml of the filtrate to 100 ml with the same solvent. Further dilute 10 ml to 100 ml with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 247 nm, Appendix II B. Calculate the content of $C_{15}H_{13}FO_2$ taking 802 as the value of A(1%, 1 cm) at the maximum at 247 nm.

Fluticasone Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluticasone Cream contains Fluticasone Propionate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) transfer a quantity of the cream containing 1 mg of fluticasone propionate to a separating funnel, add 25 ml of *acetonitrile* and 25 ml of n-*hexane*, shake for 3 minutes and allow to separate. Filter the lower layer through an absorbent cotton plug, previously washed with *acetonitrile*, into a 50 ml graduated flask, repeat the extraction with one 5 ml and one 2 ml quantities of *acetonitrile*, filter and add the extracts to the filtered layer; wash the absorbent cotton plug with 1 to 2 ml of *acetonitrile*, add the washings to the filtered layer and dilute the combined extracts to 50 ml with *acetonitrile*. Evaporate 4 ml of the resulting solution to dryness using a rotary evaporator at a temperature of 40° and dissolve the residue in 0.2 ml of *acetonitrile*. Solution (2) contains 0.04% w/v of *fluticasone propionate BPCRS* in *acetonitrile*. After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as that in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light. For solution (1) transfer a quantity of the cream containing 1 mg of fluticasone propionate to a separating funnel, add 25 ml of *ethanol (65%)*, stopper and shake until the cream is completely dispersed. Add 25 ml of n-*hexane*, shake for 3 minutes and allow to separate, filter the lower aqueous layer through an absorbent cotton plug, previously washed with *ethanol (65%)*, into a graduated flask and repeat the extraction with one 5 ml and then one 2 ml quantity of *ethanol (65%)*, filtering the aqueous ethanol extracts into the same graduated flask. Wash the absorbent cotton plug with *ethanol (65%)*, collecting the washings in the flask and dilute the combined extracts to 50 ml with *ethanol (65%)*. For solution (2) dilute 2 ml of a 0.05% w/v solution of *fluticasone propionate BPCRS* in *methanol (80%)* to 50 ml with *ethanol (65%)*. Solution (3) contains 0.002% w/v of *fluticasone propionate impurity standard BPCRS* in mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at a temperature of 50 ^{c}, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol* and (c) a detection wavelength of 240 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.6. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the cream using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*.

Fluticasone Nasal Drops

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluticasone Nasal Drops is a suspension of Fluticasone Propionate in a suitable liquid in a container fitted with an appropriate nasal delivery system.

The nasal drops comply with the requirements stated under Nasal Preparations and with the following requirements.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the nasal drops containing 2 mg of Fluticasone Propionate add sufficient *acetonitrile* to produce 5 ml, shake for 3 minutes and filter.

(2) 0.04% w/v of *fluticasone propionate BPCRS* in *acetonitrile.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 40 µl of each solution.

(d) Develop the plate to 12 cm

(e) After removal of the plate, allow it to dry in a current of air and examine *under ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the following operations in subdued light using low-actinic glassware. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the nasal drops containing 1 mg of Fluticasone Propionate to 5 ml with a mixture of equal volumes of mobile phase A and mobile phase B and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

(3) 0.02% w/v of *fluticasone propionate BPCRS* and 0.00004% w/v of *fluticasone impurity D EPCRS* in a mixture of equal volumes of mobile phase A and mobile phase B.

(4) Dilute 1 volume of solution (3) to 100 volumes with a mixture of equal volumes of mobile phase A and mobile phase B, dilute 5 volumes of this solution to 100 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 50 µl of each solution.

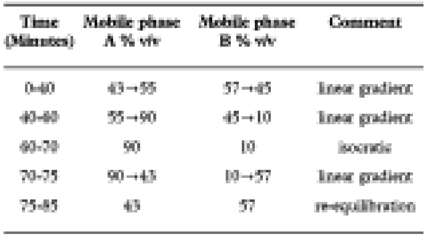
MOBILE PHASE

Mobile phase A A solution containing 0.05% v/v of *orthophosphoric acid* and 3.0% v/v of *methanol* in *acetonitrile.*

Mobile phase B A solution containing 0.05% v/v of *orthophosphoric acid* and 3.0% v/v of *methanol* in *water*.

Equilibrate the column with a mixture of 43 volumes of mobile phase A and 57 volumes of mobile phase B.

Use the following gradient.



Time (Minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comment
0-40	43→55	57→45	linear gradient
40-60	55→90	45→10	linear gradient
60-70	90	10	isocratic
70-75	90→43	10→57	linear gradient
75-85	43	57	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless (a) in the chromatogram obtained with solution (3), the *resolution* *factor* between fluticasone impurity D and fluticasone propionate is at least 1.5 and (b) in the chromatogram obtained with solution (4) the peak due to fluticasone propionate has a *signal to noise* ratio of at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (0.5%),

the sum of the areas of any such peaks is not greater than 4 times the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the following operations in subdued light using low-actinic glassware. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix the contents of 20 ampoules. Add 25 ml of *ethanol (65%)* to a quantity of the nasal drops containing 1 mg of Fluticasone Propionate, shake with the aid of ultrasound for 10 minutes. Add sufficient *ethanol (65%)* to produce 50 ml and filter.

(2) Dilute 2 ml of a 0.05% w/v solution of *fluticasone propionate BPCRS* in *methanol (80%)* to 50 ml with *ethanol (65%)*.

(3) 0.004% w/v of *fluticasone S-methyl impurity* BPCRS and 0.002% w/v of *fluticasone propionate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.5. If necessary, adjust the proportion of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the nasal drops using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*.

Fluticasone Nasal Spray

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluticasone Nasal Spray is a suspension of Fluticasone Propionate in a suitable liquid in a container fitted with an appropriate nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *silica gel F_{254} precoated plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) transfer a quantity of the nasal spray containing 1 mg of Fluticasone Propionate to a separating funnel, add 25 ml of *acetonitrile* and 25 ml of n-*hexane*, shake for 3 minutes and allow to separate. Retain the lower layer, repeat the extraction with two 5 ml quantities of *acetonitrile*. Filter the combined extracts and add sufficient *acetonitrile* to produce 50 ml. Evaporate 20 ml of the resulting solution to dryness using a rotary evaporator at a temperature not exceeding 40° and dissolve the residue in 1 ml of *acetonitrile*. Solution (2) contains 0.04% w/v of *fluticasone propionate BPCRS* in acetonitrile. After removal of the plate, allow it to dry in a current of air and examine *under ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the following operations in subdued light using low-actinic glassware. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the nasal spray containing 1 mg of Fluticasone Propionate to 5 ml with a mixture of equal volumes of mobile phase A and mobile phase B and filter.

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of equal volumes of mobile

phase A and mobile phase B.

(3) 0.02% w/v of fluticasone propionate BPCRS and 0.00004% w/v of fluticasone impurity D EPCRS in a mixture of equal volumes of mobile phase A and mobile phase B.

(4) Dilute 1 volume of solution (3) to 100 volumes with a mixture of equal volumes of mobile phase A and mobile phase B, further dilute 5 volumes of this solution to 100 volumes with a mixture of equal volumes of mobile phase A and mobile phase B.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Spherisorb ODS 1 is suitable).

(b) Gradient elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Column temperature of 40°.

(e) Detection wavelength of 240 nm.

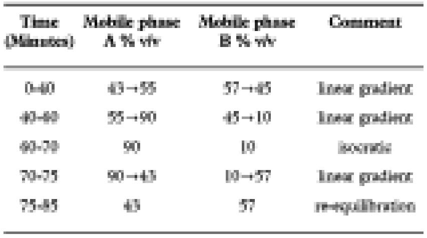
(f) Injection volume of 50 µl

MOBILE PHASE

Mobile phase A A solution containing 0.05% v/v of *orthophosphoric acid* and 3.0% v/v of *methanol* in *acetonitrile.*

Mobile phase B A solution containing 0.05% v/v of *orthophosphoric acid* and 3.0% v/v of *methanol* in *water*.

Equilibrate the column with a mixture of 43 volumes of mobile phase A and 57 volumes of mobile phase B. Carry out a gradient elution using the following gradient programme.



Time (Minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comment
0-40	43→55	57→45	linear gradient
40-60	55→90	45→10	linear gradient
60-70	90	10	isocratic
70-75	90→43	10→57	linear gradient
75-85	43	57	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless (a) in the chromatogram obtained with solution (3), the *resolution factor* between fluticasone impurity D and fluticasone propionate is at least 1.5 and (b) in the chromatogram obtained with solution (4) the peak due to fluticasone propionate has a *signal to noise* ratio of at least 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than 4 times the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.1 times the area of the peak due to fluticasone propionate in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the following operations in subdued light using low-actinic glassware. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) After priming the pump, discharge the container a sufficient number of times to obtain 1 mg of Fluticasone Propionate, add 25 ml of ethanol (65%) and shake with the aid of ultrasound for 10 minutes. Add sufficient ethanol (65%) to produce 50 ml, filter and use the filtrate.

(2) Dilute 2 ml of a 0.05% w/v solution of fluticasone propionate BPCRS in methanol (80%) to 50 ml with ethanol (65%).

(3) 0.0004% w/v of fluticasone S-methyl impurity *BPCRS* and 0.002% w/v of *fluticasone propionate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Spherisorb ODS 1 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Column temperature of 40°.

(e) Detection wavelength of 240 nm.

(f) Injection volume of 20 µl.

MOBILE PHASE

15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.5. If necessary, adjust the proportion of acetonitrile in the mobile phase if necessary.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the nasal spray using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*.

LABELLING

The label states the amount of active ingredient delivered by each actuation of the valve and the number of deliveries available from the container.

Fluticasone Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Fluticasone Ointment contains Fluticasone Propionate in a suitable basis.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of fluticasone propionate, $C_{25}H_{31}F_3O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) transfer a quantity of the ointment containing 0.1 mg of fluticasone propionate to a separating funnel, add 10 ml of *acetonitrile* and 50 ml of n-*hexane*, shake for 5 minutes and allow to separate. Filter the lower layer through an absorbent cotton plug previously washed with *acetonitrile*, extract the hexane layer with two 5 ml quantities of *acetonitrile*, filter each extract through the absorbent cotton plug and wash the absorbent cotton plug with 2 ml of *acetonitrile*. Add the washings to the combined filtrates, evaporate the resulting solution to dryness using a rotary evaporator at a temperature of 40° and dissolve the residue in 0.5 ml of *acetonitrile*. Solution (2) contains 0.020% w/v of *fluticasone propionate BPCRS* in *acetonitrile*. After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light. For solution (1) transfer a quantity of the ointment containing 0.1 mg of fluticasone propionate to a separating funnel, add 45 ml of n-*hexane*, previously heated to 60° in a water bath, stopper the funnel and shake until the ointment is dispersed, venting frequently. Wash the stopper and neck of the funnel with 5 ml of n-*hexane* collecting the washings in the funnel, allow the funnel to cool to room temperature, add 10 ml of *methanol (80%)*, stopper, shake for 1 minute and allow to separate. Filter the lower aqueous layer through an absorbent cotton plug, previously washed with *methanol (80%)*, into a graduated flask; repeat the extraction with two 5 ml quantities of *methanol (80%)*, filtering the aqueous methanol extracts into the same graduated flask. Wash the absorbent cotton plug with 2 ml of *methanol (80%)*, collecting the washings in the flask and dilute the extract to 25 ml with *methanol (80%)*. For solution (2) dilute 2 ml of a 0.005% w/v solution of *fluticasone propionate BPCRS* in *methanol (80%)* to 25 ml with the same solvent. Solution (3) contains 0.0004% w/v of *fluticasone* S-*methyl impurity BPCRS* and 0.002% w/v of *fluticasone propionate BPCRS* in *methanol (80%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at a temperature of 50^{c}, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 15 volumes of *acetonitrile*, 35 volumes of 0.01M *ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol* and (c) a detection wavelength of 240 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is at least 1.6. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the ointment using the declared content of $C_{25}H_{31}F_3O_5S$ in *fluticasone propionate BPCRS*.

Fluvoxamine Tablets

General Notices

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Fluvoxamine Tablets contain Fluvoxamine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of fluvoxamine maleate, $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Fluvoxamine Maleate with 10 ml of *acetonitrile* for 10 minutes, centrifuge and evaporate the supernatant liquid to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluvoxamine maleate *(RS 159)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel HF precoated plate (Analtech Uniplates are suitable) and a mixture of 5 volumes of 13.5M *ammonia* and 95 volumes of *ethanol (96%)* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Fluvoxamine Maleate with 5 ml of *methanol* for 10 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 1.0% w/v of *fluvoxamine maleate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* The two principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. After 20 minutes, withdraw a sample of 10 ml of the medium and centrifuge. Measure the *absorbance* of the clear supernatant liquid, Appendix II B, suitably diluted with *water* if necessary, at 244 nm using *water* in the reference cell. Calculate the total content of fluvoxamine maleate, $C_{15}H_{21}F_3N_2O_2$, $C_4H_4O_4$, in the medium taking 270 as the value of A (1%, 1 cm) at the maximum at 244 nm. The amount is not less than 70% of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Fluvoxamine Maleate with 125 ml of the mobile phase for 10 minutes, add sufficient mobile phase to produce 250 ml, mix, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) heat a 0.32% w/v solution of *fluvoxamine maleate impurity standard BPCRS* in 0.1M *hydrochloric acid* on a water bath for 10 minutes.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable) and maintained at 35°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 40 volumes of a solution containing 1.25% w/v of *diammonium hydrogen orthophosphate* and 0.275% w/v of *sodium heptanesulphonate monohydrate* and 60 volumes of *methanol*, adjusting the pH of the mixture to 3.5 with *orthophosphoric acid* and (c) a detection wavelength of 254 nm.

When the chromatograms are recorded under the conditions described above, the retention time of fluvoxamine maleate is 7 to 9 minutes. If necessary, adjust the composition of the mobile phase (increasing the methanol content decreases the retention time, increasing the water content increases the retention time).

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *fluvoxamine maleate impurity standard BPCRS* and the *resolution factor* between the peaks corresponding to fluvoxamine and the *Z*-isomer is at least 1.0.

In the chromatogram obtained with solution (1) identify the impurities from the chromatogram obtained with solution (3) and from the reference chromatogram supplied with the reference material. The area of any peak corresponding to the 'addition product' is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%) and the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard the peak corresponding to maleic acid (which elutes immediately after the solvent front) and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Fluvoxamine Maleate with 125 ml of the mobile phase for 10 minutes, add sufficient of the mobile phase to produce 250 ml, mix, centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with the mobile phase. Solution (2) contains 0.010% w/v of *fluvoxamine maleate BPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$ in the tablets using the declared content of $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$ in *fluvoxamine maleate BPCRS*.

STORAGE

Fluvoxamine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Fluvoxamine Maleate.

Folic Acid Tablets

General Notices

Action and use

Vitamin B component.

DEFINITION

Folic Acid Tablets contain Folic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of folic acid, $C_{19}H_{19}N_7O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 20 volumes of 13.5M *ammonia*, 20 volumes of *propan-1-ol* and 60 volumes of *ethanol (96%)* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 0.5 mg of folic acid with 1 ml of a mixture of 1 volume of 13.5M *ammonia* and 9 volumes of *methanol*, centrifuge and use the supernatant liquid. Solution (2) contains 0.05% w/v of *folic acid EPCRS* in a mixture of 2 volumes of 13.5M *ammonia* and 9 volumes of *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, fluorescence and size to that in the chromatogram obtained with solution (2).

TESTS

Hydrolysis products

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and protecting solutions (1) and (2) from light. Solution (1) is a solution in the mobile phase containing 0.5 µg of *4-aminobenzoic acid* and 2.0 µg of N-*(4-aminobenzoyl)*-L-*glutamic acid* per ml. For solution (2) shake a quantity of the powdered tablets containing 5.0 mg of folic acid with 50 ml of the mobile phase, centrifuge and use the supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) 0.05M *potassium dihydrogen orthophosphate*, adjusted to pH 5.5 with 5M *sodium hydroxide*, as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 269 nm.

In the chromatogram obtained with solution (1) two peaks, due to *N*-(4-aminobenzoyl)-L-glutamic acid and 4-aminobenzoic acid, are obtained in order of their emergence. The test is not valid unless the *resolution factor* between the two peaks is at least 3.

The areas of the peaks due to 4-aminobenzoic acid and *N*-(4-aminobenzoyl)-L-glutamic acid in the chromatogram obtained with solution (1) are greater than the areas of any corresponding peaks in the chromatogram obtained with solution (2).

Uniformity of content

Tablets containing less than 2 mg of folic acid comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 ml of 0.5M *hydrochloric acid* to 5 ml of a 0.0020% w/v solution of *folic acid EPCRS* in 0.1M *sodium hydroxide* and dilute to 10 ml with the mobile phase. For solution (2) shake one tablet with 5 ml of 0.1M *sodium hydroxide*, add sufficient mobile phase to produce a solution containing 0.001% w/v of folic acid, centrifuge and use the supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 93 volumes of 0.05M *potassium dihydrogen orthophosphate* and 7 volumes of *acetonitrile* adjusted to pH 6.0 with 5M *sodium hydroxide* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 283 nm.

Calculate the content of $C_{19}H_{19}N_7O_6$ in each tablet using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid EPCRS*.

ASSAY

For tablets containing 2 mg or more of folic acid

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions, protected from light. For solution (1) dilute 5 ml of a 0.020% w/v solution of *folic acid EPCRS* in 0.1M *sodium hydroxide* to 100 ml with the mobile phase. For solution (2) shake a quantity of the powdered tablets containing 20 mg of folic acid with 50 ml of 0.1M *sodium hydroxide*, dilute to 100 ml with the same solvent, centrifuge and dilute 5 ml of the supernatant liquid to 100 ml with the mobile phase.

The chromatographic procedure described under Uniformity of content may be used.

Calculate the content of $C_{19}H_{19}N_7O_6$ in the tablets using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid EPCRS*.

For tablets containing less than 2 mg of folic acid

Use the average of the 10 individual results obtained in the test for Uniformity of content.

STORAGE

Folic Acid Tablets should be protected from light.

Foscarnet Intravenous Infusion

General Notices

Action and use

Antiviral (cytomegalovirus).

DEFINITION

Foscarnet Intravenous Infusion is a sterile solution of Foscarnet Sodium in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of foscarnet sodium, $CNa_3O_5P,6H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Dry a quantity containing 48 mg of foscarnet sodium over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 16 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of foscarnet sodium *(RS 160)*. Disregard any peak occurring at 902 cm^{-1} or any shoulder at 1179 cm^{-1}.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 7.2 to 7.6, Appendix V L.

Phosphate and phosphite

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a solution containing 0.04% w/v of *sodium dihydrogen orthophosphate monohydrate* and 0.06% w/v of *sodium phosphite* (solution A). For solution (1) add 6 ml of solution A to 60 mg of *foscarnet sodium BPCRS* and dilute to 25 ml with *water*. For solution (2) dilute a volume of the intravenous infusion with sufficient *water* to produce a solution containing 0.24% w/v of foscarnet sodium.

The chromatographic procedure may be carried out using (a) a stainless steel column (5 cm × 4.6 mm) packed with an anion exchange resin (Waters IC PAK is suitable) at a temperature of 25°, (b) as the mobile phase with a flow rate of 1.4 ml per minute a solution prepared by dissolving 0.204 g of *potassium hydrogen phthalate* in *water*, adding 5 ml of 1M *nitric acid VS* and sufficient *water* to produce 2000 ml and (c) a detection wavelength of 290 nm. Inject 20 µl of each solution.

Allow the column to stabilise by passing mobile phase through it for at least 2 hours before starting the chromatography. The test is not valid unless, in the chromatogram obtained with solution (1), the peak due to phosphate (which elutes first) shows baseline separation from the peak due to phosphite.

In the chromatogram obtained with solution (2) the area of any peak corresponding to phosphate is not greater than 0.7 times the area of the corresponding peak in the chromatogram obtained with solution (1) and the area of any peak corresponding to phosphite is not greater than half the area of the corresponding peak in the chromatogram obtained with solution (1) (2.0% of phosphate and 1.0% of phosphite).

Related esters

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. Solutions (1) and (2) are solutions of the intravenous infusion diluted to contain (1) 1.0% w/v and (2) 0.0020% w/v respectively of foscarnet sodium. Solution (3) contains 0.02% w/v of *foscarnet sodium BPCRS* and 0.02% w/v of *foscarnet impurity B EPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Microsphere C18 is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute a solution prepared as described below and (c) a detection wavelength of 230 nm. Prepare two solutions containing (A) 100 ml of 0.5M *acetic acid*, 6 ml of 0.1M *sodium pyrophosphate* and 3.22 g of *sodium sulphate* in sufficient *water* to produce 1000 ml and (B) 6.8 g of *sodium acetate*, 6 ml of 0.1M *sodium pyrophosphate* and 3.22 g of *sodium sulphate* in sufficient *water* to produce 1000 ml. Mix solution A and solution B to produce a solution of pH 4.4 and to 1000 ml of this solution add 0.25 g of *tetrahexylammonium hydrogen sulphate* and 100 ml of *methanol*. Inject 5 µl of each solution.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to foscarnet sodium and foscarnet impurity B (disodium (ethoxyoxydophosphanyl)formate) is at least 7.

For solution (1) allow the chromatography to proceed for at least 2.5 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Bacterial endotoxins

The endotoxin limit concentration is 2 IU per ml, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase containing (1) a solution of the intravenous infusion diluted to contain 0.10% w/v of foscarnet sodium and (2) 0.10% w/v of *foscarnet sodium BPCRS*.

The chromatographic procedure described under Related esters may be used injecting 5 µl of each solution.

Calculate the content of $CNa_3O_5P,6H_2O$ in the intravenous infusion using the declared content of $CNa_3O_5P,6H_2O$ in *foscarnet sodium BPCRS*.

STORAGE

Foscarnet Intravenous Infusion should be stored at a temperature not exceeding 30°. It should not be refrigerated.

LABELLING

The label states that Foscarnet Intravenous Infusion may be administered as supplied via a central venous line or via a peripheral vein after dilution with Glucose Intravenous Infusion (50 g per litre) or Sodium Chloride Intravenous Infusion (0.9% w/v).

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Foscarnet Sodium with the exception of disodium ethoxycarbonyl phosphonate and sodium ethyl phosphonate.

Fosfestrol Injection

General Notices

Action and use

Estrogen.

DEFINITION

Fosfestrol Injection is a sterile solution of Fosfestrol Sodium in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous fosfestrol sodium, $C_{18}H_{18}Na_4O_8P_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (4).

B. The *light absorption*, Appendix II B, in the range 200 to 350 nm of the final solution obtained in the Assay exhibits a maximum at 240 nm.

TESTS

Alkalinity

pH, 7.7 to 8.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 15 volumes of each of *pentan-1-ol*, *triethylamine* and *water* and 55 volumes of *propan-1-ol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a volume of the injection containing the equivalent of 12 mg of anhydrous fosfestrol sodium to 5 ml with *methanol (50%)*. For solution (2) dilute 3 volumes of solution (1) to 200 volumes with *methanol (50%)*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *methanol (50%)*. Solution (4) contains 0.24% w/v of *fosfestrol sodium BPCRS* in *methanol (50%)*. Solution (5) contains 0.007% w/v of *diethylstilbestrol monophosphate BPCRS* (equivalent to 0.0079% w/v of disodium diethylstilbestrol monophosphate) in *methanol (50%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot in the chromatogram obtained with solution (1) corresponding to diethylstilbestrol monophosphate is not more intense than the spot in the chromatogram obtained with solution (5) (3.3%); any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Free diethylstilbestrol

Dilute a volume of the injection containing the equivalent of 0.6 g of anhydrous fosfestrol sodium with 20 ml of *water*, adjust the pH if necessary to between 7.9 and 8.2 with 0.1M *sodium hydroxide* and extract with two 30 ml quantities of *dichloromethane* and retain the aqueous phase. Wash the combined dichloromethane extracts with 30 ml of *water* and allow to separate. Extract the aqueous wash with 10 ml of *dichloromethane*, filter the dichloromethane extract, add to the original extract and add the aqueous extract to the original aqueous phase. Reserve the aqueous phase for the test for diethylstilbestrol disodium monophosphate. Evaporate the combined filtrates to dryness on a water bath and dissolve the residue in 5 ml of *absolute ethanol*. To the resulting solution add 5 ml of 0.01M *dipotassium hydrogen orthophosphate*. Transfer a portion of the mixture to a 1-cm closed silica cell, place the cell 10 cm from an 8-watt, short-wave ultraviolet lamp and irradiate for 60 minutes protected from daylight. The *absorbance* of the irradiated solution, measured within 1 hour after preparation of the solutions, at the maximum at 418 nm, Appendix II B, using *water* in the reference cell is not greater than that obtained by repeating the operation using a reference solution prepared in the following manner. To 20 ml of a 0.04% w/v solution of *diethylstilbestrol EPCRS* in *absolute ethanol* add 20 ml of 0.01M *dipotassium hydrogen orthophosphate* and mix (0.33%).

Diethylstilbestrol disodium monophosphate

Dilute the combined aqueous phase reserved in the test for Free diethylstilbestrol to 100 ml with *water* (solution A). Prepare a solution containing 0.0135% w/v of *diethylstilbestrol EPCRS* in *ethanol (96%)* (solution B). To 5 ml of each solution add 3 ml of *phosphomolybdotungstic reagent* and 2 ml of *ethanol (96%)*, dilute to 50 ml with a 5% w/v solution of *anhydrous sodium carbonate*, mix thoroughly and allow to stand for 30 minutes; filter the solutions through a polytetrafluoroethylene filter (0.45 µm) and measure the *absorbance*, Appendix II B, at 660 nm using *water* in the reference cell. The *absorbance* of solution A, multiplied by 1.46, is not greater than that obtained for solution B (3.3%).

ASSAY

Dilute a volume of the injection containing the equivalent of 60 mg of anhydrous fosfestrol sodium to 250 ml with a pH 7.9 buffer solution containing 1.134% w/v of *disodium hydrogen orthophosphate* and 0.04083% w/v of *potassium dihydrogen orthophosphate* and dilute 5 ml of the resulting solution to 100 ml with the same buffer solution. Measure the *absorbance* of the resulting solution at 240 nm, Appendix II B, and calculate the content of $C_{18}H_{18}Na_4O_8P_2$ taking 310 as the value of *A* (1%, 1 cm) at the maximum at 240 nm.

STORAGE

Fosfestrol Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous fosfestrol sodium.

IMPURITIES

The impurities limited by the requirements of the monograph include:

A. diethylstilbestrol disodium monophosphate

B. diethylstilbestrol.

Fosfestrol Tablets

General Notices

Action and use

Estrogen.

DEFINITION

Fosfestrol Tablets contain Fosfestrol Sodium. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous fosfestrol sodium, $C_{18}H_{18}Na_4O_8P_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds in position to that in the chromatogram obtained with solution (4).

B. The *light absorption* of the final solution obtained in the Assay, Appendix II B, in the range 200 to 350 nm, exhibits a maximum at 240 nm.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of *water* and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain the equivalent of 0.0024% w/v of anhydrous fosfestrol sodium. Measure the *absorbance* of this solution, Appendix II B, at 240 nm using dissolution medium in the reference cell. Calculate the total content of anhydrous fosfestrol sodium, $C_{18}H_{18}Na_4O_8P_2$, in the medium taking 313 as the value of *A* (1%, 1 cm) at 240 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 15 volumes each of *pentan-1-ol*, *triethylamine* and *water* and 55 volumes of *propan-1-ol* as the mobile phase, but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a quantity of the powdered tablets containing the equivalent of 12 mg of anhydrous fosfestrol sodium to 5 ml with *methanol (50%)*. For solution (2) dilute 3 volumes of solution (1) to 200 volumes with *methanol (50%)*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *methanol (50%)*. Solution (4) contains 0.24% w/v of *fosfestrol sodium BPCRS* in *methanol (50%)*. Solution (5) contains 0.007% w/v of *diethylstilbestrol monophosphate BPCRS* (equivalent to

0.0079% w/v of disodium diethylstilbestrol monophosphate) in *methanol (50%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot in the chromatogram obtained with solution (1) corresponding to diethylstilbestrol monophosphate is not more intense than the spot in the chromatogram obtained with solution (5) (3.3%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Free diethylstilbestrol

Suspend a quantity of the powdered tablets containing the equivalent of 0.24 g of anhydrous fosfestrol sodium in 30 ml of *water*, add 10 ml of *ethanol (96%)*, dilute to 50 ml with *water*, stir for 45 minutes and centrifuge at 10,000 revolutions per minute. Extract 25 ml of the supernatant liquid with two 20 ml quantities of *dichloromethane* and reserve the aqueous phase for the test for Diethylstilbestrol disodium monophosphate. Wash the combined dichloromethane extracts with 30 ml of *water* and drain into a 100 ml round-bottomed flask. Extract the aqueous wash with 10 ml of *dichloromethane*, filter the dichloromethane extract and add to the original extract. Evaporate the combined filtrates to dryness using a rotary evaporator and dissolve the residue in 5 ml of *absolute ethanol*. To the resulting solution add 5 ml of 0.01M *dipotassium hydrogen orthophosphate*. Transfer a portion of the mixture to a 1-cm closed silica cell, place the cell 10 cm from an 8-watt, short-wave ultraviolet lamp and irradiate for 60 minutes protected from daylight. The *absorbance* of the irradiated solution, measured within 1 hour after preparation of the solutions, at the maximum at 418 nm, Appendix II B, using *water* in the reference cell is not greater than that obtained by repeating the operation using a reference solution prepared in the following manner. To 20 ml of a 0.008% w/v solution of *diethylstilbestrol EPCRS* in *absolute ethanol* add 20 ml of 0.01M *dipotassium hydrogen orthophosphate* and mix (0.33%).

Diethylstilbestrol disodium monophosphate

Dilute the aqueous phase reserved in the test for Free diethylstilbestrol to 50 ml with *water* (solution A). Prepare a solution containing 0.005% w/v of *diethylstilbestrol EPCRS* in *ethanol (96%)* (solution B). To 5 ml of each solution add 3 ml of *phosphomolybdotungstic reagent* and 2 ml of *ethanol (96%)*, dilute to 50 ml with a 5% w/v solution of *anhydrous sodium carbonate*, mix thoroughly and allow to stand for 30 minutes; filter the solutions through a polytetrafluoroethylene filter (0.45 µm) and measure the *absorbance*, Appendix II B, at 660 nm using *water* in the reference cell. The *absorbance* of solution A, multiplied by 1.46, is not greater than that obtained for solution B (3.3%).

ASSAY

Weigh and powder 20 tablets. Dilute a quantity of the powdered tablets containing the equivalent of 0.1 g of anhydrous fosfestrol sodium to 250 ml with *water*, stir the suspension for 1 hour, filter, discard the first 50 ml of filtrate and dilute 5 ml of the resulting solution to 100 ml with a pH 7.9 buffer solution containing 1.134% w/v of *disodium hydrogen orthophosphate* and 0.04083% w/v of *potassium dihydrogen orthophosphate*. Measure the *absorbance* of the resulting solution at 240 nm, Appendix II B, and calculate the content of $C_{18}H_{18}Na_4O_8P_2$ taking 310 as the value of A(1%, 1 cm) at the maximum at 240 nm.

STORAGE

Fosfestrol Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous fosfestrol sodium.

IMPURITIES

The impurities limited by the requirements of the monograph include:

A. diethylstilbestrol disodium monophosphate

B. diethylstilbestrol.

Fructose Intravenous Infusion

General Notices

DEFINITION

Fructose Intravenous Infusion is a sterile solution of Fructose in Water for Injections.
The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of fructose, $C_6H_{12}O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or pale yellow solution.

IDENTIFICATION

A. Heat with *cupri-tartaric solution R1*. A copious red precipitate is produced.

B. The solution prepared in the Assay is laevorotatory.

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L, when determined on a solution diluted if necessary with *water for injections* to contain 5% w/v of Fructose and to which 0.30 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

5-Hydroxymethylfurfural and related substances

Dilute a volume containing 1.0 g of Fructose to 500 ml with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.50, Appendix II B.

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D. Use per kg of the rabbit's weight a volume containing 0.5 g of Fructose.

ASSAY

To a volume containing 5 g of Fructose add 0.2 ml of 5M *ammonia* and sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and determine the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.5427 represents the weight in g of fructose, $C_6H_{12}O_6$, in the volume taken for assay.

LABELLING

The strength is stated as the number of grams of Fructose per litre.

Furosemide Injection

General Notices

Action and use

Loop diuretic.

DEFINITION

Furosemide Injection is a sterile solution of furosemide sodium, prepared by the interaction of Furosemide with Sodium Hydroxide, in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of furosemide, $C_{12}H_{11}ClN_2O_5S$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 320 nm of the final solution obtained in the Assay exhibits two maxima, at 228 nm and 271 nm.

B. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (3).

TESTS

Alkalinity

pH, 8.0 to 9.3, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light. For solution (1) dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing 0.1% w/v of Furosemide. For solution (2) dilute 1 volume of solution (1) to 400 volumes with the mobile phase. Solution (3) contains 0.00025% w/v of *furosemide BPCRS* in the mobile phase. Solution (4) contains 0.00025% w/v of each of *furosemide BPCRS* and *furosemide impurity A EPCRS* in the mobile phase. Solution (5) contains 0.001% w/v of *4-chloro-5-sulphamoylanthranilic acid BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (ChromSpher C8 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared in the following manner: dissolve 0.2 g of *potassium dihydrogen orthophosphate* and 0.25 g of *cetrimide* in 70 ml of *water*, adjust the pH to 7.0 with 6M *ammonia* and add 30 ml of *propan-1-ol*, and (c) a detection wavelength of 238 nm.

Inject 100 µl of solution (4). Adjust the sensitivity of the system so that the heights of the two peaks in the chromatogram obtained are not less than 20% of the full-scale of the recorder. The test is not valid unless the *resolution factor* between the first peak (furosemide impurity A) and the second peak (furosemide) is at least 4.

Inject 100 µl of solution (1) and allow the chromatography to proceed for three times the retention time of the principal peak. The area of any peak corresponding to 4-chloro-5-sulphamoylanthranilic acid is not greater than the area of the peak in the chromatogram obtained with solution (5) (1%) and the sum of the areas of any other *secondary peaks* is not greater than twice the area of the first peak in the chromatogram obtained with solution (4) (0.5%). Disregard any peak with an area less than 0.1 times the area of the first peak in the chromatogram obtained with solution (4) (0.025%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection with *water BET*, if necessary, to contain the equivalent of 10 mg of Furosemide per ml (solution A). The endotoxin limit concentration of this solution is 35 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

To a volume containing the equivalent of 20 mg of Furosemide add sufficient *water* to produce 100 ml. Dilute 5 ml to 100 ml with 0.1M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{12}H_{11}ClN_2O_5S$ taking 580 as the value of A(1%, 1 cm) at the maximum at 271 nm.

STORAGE

Furosemide Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of Furosemide in a suitable dose-volume.

Furosemide Tablets

General Notices

Action and use

Loop diuretic.

DEFINITION

Furosemide Tablets contain Furosemide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of furosemide, $C_{12}H_{11}ClN_2O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 320 nm of the final solution obtained in the Assay exhibits two maxima, at 228 nm and 271 nm.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (3).

TESTS

Dissolution

Tablets containing less than 100 mg of Furosemide comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 5.8* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain about 0.001% w/v of Furosemide. Measure the *absorbance* of this solution, Appendix II B, at 277 nm using dissolution medium in the reference cell. Calculate the total content of furosemide, $C_{12}H_{11}ClN_2O_5S$, in the medium from the *absorbance* obtained from a 0.001% w/v solution of *furosemide BPCRS* in the dissolution medium and using the declared content of $C_{12}H_{11}ClN_2O_5S$ in *furosemide BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light. For solution (1) dissolve a quantity of the powdered tablets containing 20 mg of Furosemide in sufficient of the mobile phase to produce 50 ml and mix with the aid of ultrasound for 15 minutes. For solution (2) dilute 1 volume of solution (1) to 500 volumes with the mobile phase. Solution (3) contains 0.00008% w/v of *furosemide BPCRS* in the mobile phase. Solution (4) contains 0.00008% w/v of each of *furosemide BPCRS* and *furosemide impurity A EPCRS* in the mobile phase.

Solution (5) contains 0.00032% w/v of *4-chloro-5-sulphamoylanthranilic acid BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (ChromSpher C8 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared in the following manner: dissolve 0.2 g of *potassium dihydrogen orthophosphate* and 0.25 g of *cetrimide* in 70 ml of *water*, adjust the pH to 7.0 with 6M *ammonia* and add 30 ml of *propan-1-ol* and (c) a detection wavelength of 238 nm.

Inject 100 µl of solution (4). Adjust the sensitivity of the system so that the heights of the two peaks in the chromatogram obtained are not less than 20% of the full-scale of the recorder. The test is not valid unless the *resolution factor* between the first peak (furosemide impurity A) and the second peak (furosemide) is at least 4.

Inject 100 µl of solution (1) and allow the chromatography to proceed for three times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-chloro-5-sulphamoylanthranilic acid is not greater than the area of the peak in the chromatogram obtained with solution (5) (0.8%) and the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the area of the first peak in the chromatogram obtained with solution (4) (0.5%). Disregard any peak with an area less than 0.1 times the area of the first peak in the chromatogram obtained with solution (4) (0.02%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.2 g of Furosemide with 300 ml of 0.1M *sodium hydroxide* for 10 minutes, add sufficient 0.1M *sodium hydroxide* to produce 500 ml and filter. Dilute 5 ml to 250 ml with 0.1M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{12}H_{11}ClN_2O_5S$ taking 580 as the value of A(1%, 1 cm) at the maximum at 271 nm.

Fusidic Acid Cream

General Notices

Action and use

Antibacterial.

DEFINITION

Fusidic Acid Cream contains Fusidic Acid in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous fusidic acid, $C_{31}H_{48}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 12.5 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse, with shaking, a quantity of the cream containing the equivalent of 40 mg of anhydrous fusidic acid in 10 ml of *ethanol (96%)*, filter and use the filtrate. Solution (2) contains 0.5% w/v of *diethanolamine fusidate BPCRS* in *ethanol (96%).* Solution (3) contains 0.05% w/v of *potassium sorbate* in *water*. After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2) and is well separated from any spot corresponding to the spot in the chromatogram obtained with solution (3).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to fusidic acid in the chromatogram obtained with solution (2) .

TESTS

Acidity

pH, 4.5 to 6.0, determined directly on the cream, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the cream containing the equivalent of 15 mg of anhydrous fusidic acid in 25 ml of the mobile phase, heat the mixture until the cream has melted and shake vigorously for 15 minutes. Cool the mixture to below 10°, filter through a glass microfibre filter paper (Whatman GF/A is suitable), discarding the first few ml of filtrate, and allow to warm to room temperature. For solution (2) mix 1 volume of a 0.06% w/v solution of *3-ketofusidic acid EPCRS* in the mobile phase with 1 volume of solution (1) and dilute to 100 volumes with the mobile phase. For solution (3) dilute 30 µl of solution (1) to 100 ml with the mobile phase. Solution (4) contains 0.004% w/v of *potassium sorbate* in the mobile phase.

The chromatographic conditions described under Assay may be used. For solution (1) allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

The test is not valid unless (a) the *resolution factor* between the peaks corresponding to 3-ketofusidic acid and fusidic acid in the chromatogram obtained with solution (2) is at least 3.5 and (b) the principal peak in the chromatogram obtained with solution (3) has a *signal-to-noise ratio* of at least 3. If necessary, adjust the composition of the mobile phase.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than five times the area of the peak corresponding to fusidic acid in the chromatogram obtained with solution (2) (5%). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.03%) and any peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (4).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the cream containing the equivalent of 15 mg of anhydrous fusidic acid in 50 ml of the mobile phase, heat until the cream has melted and shake vigorously for 15 minutes. Cool the mixture to below 10°, filter through a glass microfibre filter paper (Whatman GF/A is suitable), discarding the first few ml of filtrate and allow to warm to room temperature. Solution (2) contains 0.0375% w/v of *diethanolamine fusidate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP-18 is suitable), (b) a mixture of 10 volumes of *methanol*, 40 volumes of 0.05M *orthophosphoric acid* and 50 volumes of *acetonitrile* as the mobile phase with a flow rate of 2.5 ml per minute and (c) a detection wavelength of 235 nm.

The assay is not valid unless the *column efficiency*, determined on the peak due to fusidic acid in the chromatogram obtained with solution (2), is at least 2000 *theoretical plates* per metre and the *symmetry factor* of the principal peak is 2.0 or less.

Calculate the content of $C_{31}H_{48}O_6$ in the cream using the declared content of $C_{31}H_{48}O_6$ in *diethanolamine fusidate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous fusidic acid.

Fusidic Acid Eye Drops

General Notices

Action and use

Antibacterial.

DEFINITION

Fusidic Acid Eye Drops are a sterile solution of Fusidic Acid in Purified Water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of anhydrous fusidic acid, $C_{31}H_{48}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 12.5 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse, with shaking, a quantity of the eye drops containing the equivalent of 20 mg of anhydrous fusidic acid in 10 ml of *ethanol (70%)*, filter and use the filtrate. Solution (2) contains 0.25% w/v of *diethanolamine fusidate BPCRS* in *ethanol (70%).* After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the peak due to fusidic acid in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 5.2 to 6.2, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops with a mixture of 10 volumes of *methanol*, 30 volumes of 1% w/v *glacial acetic acid* and 60 volumes of *acetonitrile* to contain the equivalent of 0.075% w/v of anhydrous fusidic acid. Shake vigorously for 15 minutes, add 0.5 g of *potassium nitrate* and shake for a further minute. Filter through a glass microfibre filter paper (Whatman GF/A is suitable), discarding the first few ml of filtrate, and dilute 10 volumes of the filtrate to 12.5 volumes with 0.05M *orthophosphoric acid*. For solution (2) mix 1 volume of a 0.06% w/v solution of *3-ketofusidic acid EPCRS* in the mobile phase with 1 volume of solution (1) and dilute to 100 volumes with the mobile phase. For solution (3) dilute 30 µl of solution (1) to 100 ml with the mobile phase.

The chromatographic conditions described under Assay may be used. For solution (1) allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

The test is not valid unless (a) the *resolution factor* between the peaks corresponding to 3-ketofusidic acid and fusidic acid in the chromatogram obtained with solution (2) is at least 3.5 and (b) the principal peak in the chromatogram obtained with solution (3) has a *signal-to-noise ratio* of at least 3. If necessary, adjust the composition of the mobile phase.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than four times the area of the peak corresponding to fusidic acid in the chromatogram obtained with solution (2) (4%). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.03%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the eye drops with a mixture of 10 volumes of *methanol*, 30 volumes of 1% w/v *glacial acetic acid* and 60 volumes of *acetonitrile* (solution A) to contain the equivalent of 0.075% w/v of anhydrous fusidic acid. Shake vigorously for 15 minutes, add 0.5 g of *potassium nitrate* and shake for a further minute. Filter through a glass microfibre filter paper (Whatman GF/A is suitable), discarding the first few ml of filtrate, and dilute 10 volumes of the filtrate to 12.5 volumes with 0.05M *orthophosphoric acid*. Solution (2) contains 0.075% w/v of *diethanolamine fusidate BPCRS* in a mixture of 40 volumes of solution A and 10 volumes of 0.05M *orthophosphoric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP-18 is suitable), (b) a mixture of 10 volumes of *methanol*, 40 volumes of 0.05M *orthophosphoric acid* and 50 volumes of *acetonitrile* as the mobile phase with a flow rate of 2.5 ml per minute and (c) a detection wavelength of 235 nm.

The assay is not valid unless the *column efficiency*, determined on the peak due to fusidic acid in the chromatogram obtained with solution (2), is at least 2000 *theoretical plates* per metre and the *symmetry factor* of the principal peak is 2.0 or less.

Calculate the content of $C_{31}H_{48}O_6$ in the eye drops using the declared content of $C_{31}H_{48}O_6$ in *diethanolamine fusidate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous fusidic acid.

Fusidic Acid Oral Suspension

General Notices

Action and use

Antibacterial.

DEFINITION

Fusidic Acid Oral Suspension is a suspension of Fusidic Acid in powder of suitable fineness in a suitable flavoured vehicle.

Fusidic Acid Oral Suspension should not be diluted.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous fusidic acid, $C_{31}H_{48}O_6$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity containing the equivalent of 0.1 g of anhydrous fusidic acid add 5 ml of *water* and extract with three 10 ml quantities of *chloroform*. Wash the combined extracts with two 10 ml quantities of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 1 ml of *chloroform IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of fusidic acid *(RS 166)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the peak due to fusidic acid in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 4.8 to 5.2, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following three solutions. For solution (1) extract a quantity of the oral suspension containing the equivalent of 0.10 g of anhydrous fusidic acid with two 20 ml quantities of *chloroform,* evaporate the combined extracts to dryness and dissolve the residue in 20 ml of the mobile phase. For solution (2) mix 1 ml of a 0.10% w/v solution of *3-ketofusidic acid EPCRS* in the mobile phase with 0.2 ml of solution (1) and dilute to 20 ml with the mobile phase. For solution (3) dilute 20 µl of solution (1) to 100 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 to 15 cm × 4 to 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP18 is suitable), (b) a mixture of 10 volumes of *methanol*, 20 volumes of a 1% w/v solution of *orthophosphoric acid*, 20 volumes of *water* and 50 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 235 nm.

Inject separately 20 µl of each solution. Continue the chromatography for at least 3.5 times the retention time of the principal peak. The test is not valid unless the *resolution factor* between the peaks corresponding to 3-ketofusidic acid and fusidic acid in the chromatogram obtained with solution (2) is at least 2.5 and unless the principal peak in the chromatogram obtained with solution (3) has a *signal-to-noise ratio* of at least 3.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than three times the area of the peak corresponding to fusidic acid in the chromatogram obtained with solution (2) (3%). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.02%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.07% w/v of *diethanolamine fusidate BPCRS* in the mobile phase. For solution (2) shake thoroughly a weighed quantity of the oral suspension containing the equivalent of 30 mg of anhydrous fusidic acid with 25 ml of the mobile phase, dilute to 50 ml with the mobile phase, filter and use the clear filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (LiChrosorb RP-18 is suitable), (b) a mixture of 60 volumes of *acetonitrile*, 30 volumes of a 1% v/v solution of *glacial acetic acid* and 10 volumes of *methanol* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 235 nm.

The *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (1), should be at least 14,000 theoretical plates per metre.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{31}H_{48}O_6$, weight in volume, using the equivalent declared content of $C_{31}H_{48}O_6$ in *diethanolamine fusidate BPCRS*.

STORAGE

Fusidic Acid Oral Suspension should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous fusidic acid.

When Fusidic Acid Oral Suspension is prescribed or demanded, no strength being stated, an Oral Suspension containing the equivalent of 250 mg of anhydrous fusidic acid in 5 ml shall be dispensed or supplied.

Paediatric Ferrous Sulphate Oral Solution

General Notices

DEFINITION

Paediatric Ferrous Sulphate Oral Solution is a solution containing 1.2% w/v of Ferrous Sulphate Heptahydrate and a suitable antioxidant in a suitable vehicle with an orange flavour. It is intended to be diluted well with water before use.

Extemporaneous preparation

The following formula and directions apply.

Ferrous Sulphate Heptahydrate	12 g
Ascorbic Acid	2 g
Orange Syrup	100 ml
Double-strength Chloroform Water	500 ml
Water	Sufficient to produce 1000 ml

Dissolve the Ascorbic Acid in the Double-strength Chloroform Water and use this solution to dissolve the Ferrous Sulphate Heptahydrate. Add the Orange Syrup and sufficient Water to produce 1000 ml and mix. The oral solution should be recently prepared.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ferrous sulphate heptahydrate, $FeSO_4,7H_2O$

1.10 to 1.30% w/v.

ASSAY

To 20 ml add 45 ml of *water*, 0.2 ml of *hydrochloric acid* and *dilute potassium permanganate solution* drop wise until a permanent pink colour is produced. Add 10 ml of *hydrochloric acid* and 3 ml of a 10% w/v solution of *ammonium thiocyanate* and titrate with 0.1M *titanium(III) chloride VS* maintaining a steady flow of *carbon dioxide* through the flask. Each ml of 0.1M *titanium(III) chloride VS* is equivalent to 27.80 mg of $FeSO_4,7H_2O$.

LABELLING

The label states that the oral solution should be diluted well with water before use.

Paediatric Ferrous Sulphate Oral Solution contains in 5 ml about 12 mg of ferrous iron.

Prolonged-release Felodipine Tablets

General Notices

Prolonged-release Felodipine Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Calcium channel blocker.

DEFINITION

Prolonged-release Felodipine Tablets contain Felodipine. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Felodipine. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of felodipine, $C_{18}H_{19}Cl_2NO_4$

92.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 15 mg of Felodipine with 100 ml of *ethanol (96%),* filter, and dilute 5 ml of the filtrate to 50 ml with *ethanol (96%).* The *light absorption,* Appendix II B, in the range 250 to 450 nm exhibits a maximum at 362 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 10 mg of Felodipine with 10 ml of *methanol* and filter through a 0.45-µm filter.

(2) 0.1% w/v of *felodipine BPCRS* in *methanol*.

(3) 0.1% w/v each of *felodipine BPCRS* and *nifedipine BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution.

(d) Develop to 15 cm.

(e) Dry the plate in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2) .

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

SOLVENT A

A mixture of 0.08% w/v of *orthophosphoric acid R* and 0.8% w/v of *sodium dihydrogen orthophosphate.*

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 10 mg of Felodipine with 10 ml of *methanol* and 20 ml of *acetonitrile* for 5 minutes, add 15 ml of solvent A and continue to disperse with the aid of ultrasound for a further 30 minutes. Cool, dilute to 50 ml with solvent A and filter through a 0.45-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

(4) 0.006% w/v of *felodipine impurity standard BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Nova-Pak is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.0 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

20 volumes of *methanol*, 40 volumes of *acetonitrile* and 40 volumes of solvent A.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least than 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to Impurity A is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%);

the sum of the areas of the peaks corresponding to impurity B (relative retention about 0.7 with respect to felodipine and impurity C (relative retention about 1.4 with respect to felodipine) is not greater than the area of the principal peak in the chromatogram obtained with solution (2);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

SOLVENT A

A mixture of 0.08% w/v of *orthophosphoric acid R* and 0.8% w/v of *sodium dihydrogen orthophosphate.*

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 10 mg of Felodipine with 10 ml of methanol and 20 ml of acetonitrile for 5 minutes, add 15 ml of solvent A and mix with the aid of ultrasound for a further 30 minutes. Cool, add sufficient solvent A to produce 50 ml and filter through a 0.45-µm PTFE filter. Dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) 0.002% w/v of felodipine BPCRS in mobile phase.

(3) 0.006% w/v of felodipine impurity standard BPCRS in mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{19}Cl_2NO_4$ in the tablets using the declared content of $C_{18}H_{19}Cl_2NO_4$ in *felodipine BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those impurities listed under Felodipine.

Prolonged-release Ferrous Sulphate Tablets

General Notices

Prolonged-release Ferrous Sulphate Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Prolonged-release Ferrous Sulphate Tablets contain Dried Ferrous Sulphate. They are formulated so that the medicament is released over a period of several hours. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of ferrous sulphate. The dissolution profile should reflect the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ferrous iron, Fe(II)

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The powdered tablets yield reaction A characteristic of *iron salts*, Appendix VI.

B. Extract a quantity of the powdered tablets containing 0.15 g of Dried Ferrous Sulphate with 10 ml of 2M *hydrochloric acid* and filter. The filtrate yields reaction A characteristic of *sulphates*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Dried Ferrous Sulphate as completely as possible in a mixture of 30 ml of *water* and 20 ml of 1M *sulphuric acid* and titrate with 0.1M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 5.585 mg of Fe(II).

LABELLING

The quantity of active ingredient is stated both as the amount of dried ferrous sulphate and in terms of the equivalent amount of Fe(II).

Acid Gentian Mixture

General Notices

Acid Gentian Oral Solution

DEFINITION

Acid Gentian Mixture is an *oral solution* containing 10% v/v of Concentrated Compound Gentian Infusion and 5% v/v of Dilute Hydrochloric Acid in a suitable vehicle.

Extemporaneous preparation

It is recently prepared according to the following formula.

Concentrated Compound Gentian Infusion	100 ml
Dilute Hydrochloric Acid	50 ml
Double-strength Chloroform Water	500 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of hydrochloric acid, HCl

0.48 to 0.56% w/v.

ASSAY

To 10 ml add 10 ml of *water*, adjust the pH to between 5.0 and 6.0 with 2M *sodium hydroxide* and dilute to 25 ml with *water*. Add 75 ml of *acetate buffer pH 5.0* and titrate with 0.1M *silver nitrate VS* determining the end point potentiometrically using a silver indicator electrode and a glass reference electrode and stirring throughout the titration. Each ml of 0.1M *silver nitrate VS* is equivalent to 3.646 mg of HCl.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Alkaline Gentian Mixture

General Notices

Alkaline Gentian Oral Solution

DEFINITION

Alkaline Gentian Mixture is an *oral solution* containing 10% v/v of Concentrated Compound Gentian Infusion and 5% w/v of Sodium Bicarbonate in a suitable vehicle.

Extemporaneous preparation

It is recently prepared according to the following formula.

Concentrated Compound Gentian Infusion	100 ml
Sodium Bicarbonate	50 g
Double-strength Chloroform Water	500 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

4.75 to 5.25% w/v.

ASSAY

To 10 ml of the mixture add 100 ml of *water* and 25 ml of 0.5M *hydrochloric acid VS*, boil for 10 minutes and titrate the excess of hydrochloric acid with 0.5M *sodium hydroxide VS* using 0.5 ml of *methyl red solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution

General Notices

St Marks Solution

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Prevention and treatment of dehydration.

DEFINITION

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution contains Anhydrous Glucose, Sodium Chloride and Sodium Citrate. It is prepared by dissolving the dry ingredients in the specified volume of Water just before issue for use.

Extemporaneous preparation

The following formula and directions apply.

Anhydrous Glucose	20 g
Sodium Chloride	3.5 g
Sodium Citrate	2.5 g
Water	Sufficient to produce 1000 ml

Dissolve the dry ingredients in Water and add sufficient Water to produce 1000 ml.

The oral solution complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of glucose, $C_6H_{12}O_6$

1.9 to 2.1% w/v.

Content of sodium chloride, NaCl

0.33 to 0.37% w/v.

Content of sodium citrate, $C_6H_5Na_3O_7,2H_2O$

0.24 to 0.26% w/v.

IDENTIFICATION

A. Heat 10 ml with *cupri-tartaric solution R1.* A red precipitate is produced.

B. Yields reaction A characteristic of *sodium* salts, reaction A characteristic of *chlorides* and the reactions characteristic of *citrates*, Appendix VI.

C. The solution prepared as directed in the Assay for glucose is dextrorotatory.

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

ASSAY

For glucose

To 100 ml of the solution add 0.2 ml of 5M *ammonia* and mix well. Allow to stand for 30 minutes and determine the *optical rotation* in a 2-dm tube, Appendix V F. Multiply the observed rotation in degrees by 0.9477, then by 1.002 and then by 10 to calculate the weight in grams of glucose, $C_6H_{12}O_6$, in the original powder.

For sodium chloride

Titrate 20 ml of the solution with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

For sodium citrate

Evaporate 25 ml of the solution almost to dryness on a rotary evaporator, add 10 ml of *acetone* and evaporate to dryness. Add 10 ml of *acetone*, 10 ml of *acetic anhydride* and 20 ml of *anhydrous acetic acid* to the residue and warm to about 60° until dissolution is complete. Allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 ml of *crystal violet solution* as indicator, titrating to a blue end point. Each ml of 0.1M *perchloric acid VS* is equivalent to 9.803 mg of $C_6H_5Na_3O_7,2H_2O$.

STORAGE

Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution should be stored at a temperature of 2° to 8°.

LABELLING

The label states that any portion of the oral solution that remains unused 24 hours after preparation should be discarded.

Gallamine Injection

General Notices

Action and use

Non-depolarizing neuromuscular blocker.

DEFINITION

Gallamine Injection is a sterile solution of Gallamine Triethiodide in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of gallamine triethiodide, $C_{30}H_{60}I_3N_3O_3$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 225 nm.

B. To 1 ml add 1 ml of *iodinated potassium iodide solution*. A brown precipitate is produced.

C. To 1 ml add 1 ml of *potassium tetraiodomercurate solution*. A yellow precipitate is produced.

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with sufficient of the mobile phase to produce a solution containing 0.060% w/v of Gallamine Triethiodide. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.3% w/v of *gallamine impurity standard BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with a mixture of chemically-bonded octylsilyl and octadecylsilyl groups and treated to reduce the level of residual silanol groups (5 µm) (HiChrom RPB is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared as described below, (c) a detection wavelength of 205 nm and (d) a column temperature of 40°. For the mobile phase dissolve 14 g of *sodium perchlorate* in 850 ml of a solution prepared by diluting 0.7 ml of *orthophosphoric acid* to 900 ml with *water*, adjusting to pH 3.0 with 10M *sodium hydroxide* and diluting to 1000 ml with *water*, and add 130 ml of *methanol*.

Inject separately 20 µl of each solution. For solution (1) allow the chromatography to proceed for 1.5 times the retention time of gallamine as perchlorate.

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *gallamine impurity standard BPCRS*.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%). Disregard the peak due to iodide (non-retained peak).

ASSAY

Dilute a volume containing 40 mg of Gallamine Triethiodide with sufficient 0.01M *hydrochloric acid* to produce 200 ml. Dilute 5 ml of this solution to 100 ml with 0.01M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 225 nm, Appendix II B. Calculate the content of $C_{30}H_{60}I_3N_3O_3$ taking 525 as the value of A(1%, 1 cm) at the maximum at 225 nm.

STORAGE

Gallamine Injection should be protected from light.

IMPURITIES

The impurities limited by this monograph include those listed in the monograph for Gallamine Triethiodide.

Gemfibrozil Capsules

General Notices

Action and use

Fibrate lipid-regulating drug.

DEFINITION

Gemfibrozil Capsules contain Gemfibrozil.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of gemfibrozil, $C_{15}H_{22}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing about 0.5 g of Gemfibrozil with 10 ml of n-*hexane* for 10 minutes, filter through a filter paper previously washed with 20 ml of n-*hexane*, evaporate the filtrate to dryness on a water bath and then dry over *silica gel* at a pressure of 2 kPa for 2 hours or until a waxy solid is obtained. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of gemfibrozil *(RS 167)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.2M *phosphate buffer pH 7.5* and rotate the paddle at 50 revolutions per minute. Withdraw a 10 ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 276 nm, Appendix II B. At the same time measure the *absorbance* at the maximum at 276 nm of a suitable solution of *gemfibrozil BPCRS* in the dissolution medium, adding the minimum volume of 0.1M *sodium hydroxide*, if necessary, to complete dissolution. Calculate the total content of gemfibrozil, $C_{15}H_{22}O_3$, in the medium from the absorbances obtained and from the declared content of $C_{15}H_{22}O_3$ in *gemfibrozil BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.4 g of Gemfibrozil with 100 ml of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 5 volumes with the mobile phase. Solution (3) contains 0.0004% w/v of *gemfibrozil impurity A BPCRS* in the mobile phase. Solution (4) contains 0.001% w/v of *gemfibrozil methyl ester BPCRS* and 0.0004% w/v of *gemfibrozil impurity A BPCRS* in solution (2).

The chromatographic procedure described under Assay may be used. For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to gemfibrozil and gemfibrozil methyl ester is at least 4.0 and the *resolution factor* between the peaks due to gemfibrozil methyl ester and gemfibrozil impurity A is at least 1.2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to gemfibrozil impurity A is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.1%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any *secondary peaks* other than any peak corresponding to gemfibrozil impurity A is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of *methanol* to a quantity of the mixed contents of 20 capsules containing 0.15 g of Gemfibrozil, shake on a mechanical shaker for 10 minutes, add 20 ml of *water*, 1 ml of *glacial acetic acid* and sufficient *methanol* to produce 100 ml, mix and filter (Whatman GF/C paper is suitable), discarding the first 20 ml of filtrate. Dilute 1 volume of the filtrate to 5 volumes with the mobile phase. For solution (2) dissolve 30 mg of *gemfibrozil BPCRS* in 80 ml of *methanol*, add 1 ml of *glacial acetic acid* and dilute to 100 ml with *water*. Solution (3) contains 0.01% w/v of *gemfibrozil methyl ester BPCRS* in a solution prepared by diluting 1 volume of solution (1) to 3 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 or Regis C18 are suitable), (b) a mixture of 1 volume of *glacial acetic acid*, 19 volumes of *water* and 80 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 276 nm.

Inject 20 µl of each solution. The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the peaks due to gemfibrozil and gemfibrozil methyl ester is at least 4.0.

Calculate the content of $C_{15}H_{22}O_3$ in the capsules from the declared content of $C_{15}H_{22}O_3$ in *gemfibrozil BPCRS.*

Gemfibrozil Tablets

General Notices

Action and use

Fibrate lipid-regulating drug.

DEFINITION

Gemfibrozil Tablets contain Gemfibrozil.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of gemfibrozil, $C_{15}H_{22}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the powdered tablets containing 0.3 g of Gemfibrozil with 10 ml of 0.1M *sodium hydroxide*, filter (Whatman 541 paper is suitable), acidify with a few drops of 2M *sulphuric acid*, shake and centrifuge. Wash the precipitate with *water*, allow to dry in air and dry over *anhydrous silica gel* at a pressure of 2 kPa for 4 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of gemfibrozil *(RS 167)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.2M *phosphate buffer pH 7.5* and rotate the paddle at 50 revolutions per minute. Withdraw a 10 ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 276 nm, Appendix II B. At the same time measure the *absorbance* at the maximum at 276 nm of a suitable solution of *gemfibrozil BPCRS* prepared by dissolving the substance in the minimum volume of *methanol* and diluting with 0.2M *phosphate buffer pH 7.5*. Calculate the total content of gemfibrozil, $C_{15}H_{22}O_3$, in the medium from the absorbances obtained and from the declared content of $C_{15}H_{22}O_3$ in *gemfibrozil BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.6 g of Gemfibrozil with 70 ml of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 5 volumes with the mobile phase. Solution (3) contains 0.0006% w/v of *gemfibrozil impurity A BPCRS* in the mobile phase. Solution (4) contains 0.001% w/v of *gemfibrozil methyl ester BPCRS* and 0.0004% w/v of *gemfibrozil impurity A*

BPCRS in solution (2).

The chromatographic procedure described under Assay may be used. For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to gemfibrozil and gemfibrozil methyl ester is at least 4.0 and the *resolution factor* between the peaks due to gemfibrozil methyl ester and gemfibrozil impurity A is at least 1.2.

In the chromatogram obtained with solution (1) the area of any peak corresponding to gemfibrozil impurity A is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.1%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any *secondary peaks* other than the peak corresponding to gemfibrozil impurity A is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 84 mg of Gemfibrozil with 80 ml of *methanol (80%)* on a mechanical shaker for 15 minutes, add sufficient *methanol (80%)* to produce 100 ml, mix and filter (Whatman 542 paper is suitable), discarding the first 20 ml of filtrate. Solution (2) contains 0.084% w/v of *gemfibrozil BPCRS* prepared by dissolving the substance in the minimum volume of *methanol* and diluting with *methanol (80%)*. Solution (3) contains 0.01% w/v of *gemfibrozil methyl ester BPCRS* in a solution prepared by diluting 1 volume of solution (1) to 10 volumes with a mixture of 2 volumes of *methanol* and 3 volumes of the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 or Regis C18 are suitable), (b) a mixture of 1 volume of *glacial acetic acid*, 19 volumes of *water* and 80 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 276 nm.

Inject 20 µl of each solution. The test is not valid unless the *resolution factor* between the peaks due to gemfibrozil and gemfibrozil methyl ester in the chromatogram obtained with solution (3) is at least 4.0.

Calculate the content of $C_{15}H_{22}O_3$ in the tablets from the declared content of $C_{15}H_{22}O_3$ in *gemfibrozil BPCRS.*

STORAGE

Gemfibrozil Tablets should be protected from light.

Gentamicin and Hydrocortisone Acetate Ear Drops

General Notices

Action and use

Aminoglycoside antibacterial (Gentamicin Sulphate); corticosteroid (Hydrocortisone Acetate).

DEFINITION

Gentamicin and Hydrocortisone Acetate Ear Drops contain Gentamicin Sulphate and Hydrocortisone Acetate suspended in a suitable vehicle.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions.

(1) Filter a volume of the ear drops diluted in *water*, if necessary, to produce a solution containing the equivalent of 0.3% w/v of gentamicin and use the filtrate.

(2) 0.5% w/v of *gentamicin sulphate EPCRS* in *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use as the mobile phase the lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *dichloromethane* and *methanol* and allowing to separate.

(c) Develop to 15 cm.

(d) Application volume of 10 µl for each solution.

(e) After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes.

CONFIRMATION

The three principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatogram obtained with solution (2).

B. Comply with the test for Composition of gentamicin sulphate.

C. Filter 20 ml of the well-mixed ear drops (Whatman No. 1 filter paper is suitable), wash the residue with the minimum amount of *water*, dry at 105° and dissolve 50 mg of the residue obtained in 50 ml of *ethanol (96%)*. Add 2 ml of *sulphuric acid* to 2 ml of the resulting solution; an intense yellow colour is produced which exhibits a green fluorescence which is particularly intense when viewed under *ultraviolet light (365 nm)*. Add the solution to 10 ml of *water* and mix; the fluorescence under ultraviolet light (365 nm) does not disappear.

D. In the Assay for hydrocortisone acetate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.0, Appendix V L.

Composition of gentamicin sulphate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 5 ml of *methanol* to 10 ml of a 0.065% w/v solution of *gentamicin sulphate EPCRS*, swirl and add 4 ml of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 ml, heat in a water bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

(2) Prepare solution (2) in the same manner as solution (1) but using 10 ml of a solution prepared by diluting a suitable volume of the ear drops with *water* to contain the equivalent of 0.045% w/v of gentamicin.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4.6 mm) packed with end-capped *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 330 nm.

(f) Injection volume of 5 µl for each solution.

MOBILE PHASE

0.025M *sodium heptanesulphonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to 20 minutes. The retention times relative to component C_2 are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (1), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (2) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the ear drops by *normalisation*. The proportions are within the following limits:

C_1, 25.0 to 50.0%;

C_{1a}, 10.0 to 35.0%;

C_2 plus C_{2a}, 25.0 to 55.0%.

ASSAY

For gentamicin

Dilute a volume of the ear drops containing the equivalent of 15 mg of gentamicin to 50 ml with sterile *phosphate buffer pH 8.0* and dilute 10 ml of the resulting solution to 50 ml with the same solvent. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the ear drops, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the ear drops containing 0.1 g of Hydrocortisone Acetate with 25 ml of *methanol*, add sufficient *methanol* to produce 100 ml and dilute 10 ml of this solution to 50 ml with mobile phase.

(2) Dilute 10 ml of a 0.1% w/v solution of *hydrocortisone acetate BPCRS* in *methanol* to 50 ml with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 256 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 400 volumes of *water* and 600 volumes of *methanol*.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}O_6$ in the ear drops using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

STORAGE

Gentamicin and Hydrocortisone Acetate Ear Drops should not be allowed to freeze.

LABELLING

The quantity of Gentamicin Sulphate is stated in terms of the equivalent amount of gentamicin.

Gentamicin Cream

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Cream is a viscous oil-in-water emulsion containing Gentamicin Sulphate dissolved in the aqueous phase.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase the lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) disperse a quantity of the cream containing the equivalent of 7.5 mg of gentamicin in 20 ml of *chloroform*, extract with 10 ml of *water* and use the aqueous layer. Solution (2) is a solution of *gentamicin sulphate EPCRS* in *water* containing the equivalent of 0.075% w/v of gentamicin. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes. The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulphate, the retention times of the four principal peaks in the chromatogram obtained with solution (2) correspond to those of the four principal peaks in the chromatogram obtained with solution (1)

TESTS

Composition of gentamicin sulphate

Complies with the test described under Gentamicin Injection but preparing solution (2) in the following manner. Disperse a quantity of the cream containing the equivalent of 15 mg of gentamicin in 10 ml of *chloroform*, add 10 ml of a 0.25% w/v solution of *sodium tetraborate*, shake vigorously, centrifuge and separate the aqueous layer. Repeat the extraction with two 5 ml quantities of the sodium tetraborate solution, dilute the combined aqueous extracts to 25 ml with the same solution and filter. To 10 ml of the clear filtrate add 5 ml of *methanol*, swirl, add 4 ml of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 ml, heat in a water bath at 60° for 15 minutes and cool.

ASSAY

Dissolve as completely as possible a quantity containing the equivalent of 3 mg of gentamicin in 20 ml of *chloroform* and shake vigorously with 75 ml of *phosphate buffer pH 8.0*. Dilute 10 ml of the aqueous extract to 50 ml with *phosphate buffer pH 8.0*. Carry out the *microbiological* *assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the cream, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of gentamicin.

Gentamicin Ear Drops

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Ear Drops are a solution of Gentamicin Sulphate in Purified Water.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase the lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate. Apply separately to the plate (1) a volume of the ear drops containing the equivalent of 30 µg of gentamicin and (2) 0.1 mg of *gentamicin sulphate EPCRS* dissolved in a volume of *water* equal to the volume of the ear drops used. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes. The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulphate, the retention times of the four principal peaks in the chromatogram obtained with solution (2) correspond to those of the four principal peaks in the chromatogram obtained with solution (1).

TESTS

Composition of gentamicin sulphate

Comply with the test described under Gentamicin Injection, using for solution (2) a solution prepared in the same manner as solution (1) but using 10 ml of a solution prepared by diluting a suitable volume of the ear drops with *water* to contain the equivalent of 0.045% w/v of gentamicin.

ASSAY

Dilute a volume of the ear drops containing the equivalent of 15 mg of gentamicin to 50 ml with sterile *phosphate buffer pH 8.0* and dilute 10 ml of the resulting solution to 50 ml with the same solvent. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the ear drops, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of gentamicin.

Gentamicin Eye Drops

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Eye Drops are a sterile solution of Gentamicin Sulphate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase the lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate. Apply separately to the plate (1) a volume of the eye drops containing the equivalent of 30 µg of gentamicin and (2) 0.1 mg of *gentamicin sulphate EPCRS* dissolved in a volume of *water* equal to the volume of the eye drops used. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes. The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulphate, the retention times of the four principal peaks in the chromatogram obtained with solution (2) correspond to those of the four principal peaks in the chromatogram obtained with solution (1).

TESTS

Composition of gentamicin sulphate

Comply with the test described under Gentamicin Injection, using for solution (2) a solution prepared in the same manner as solution (1) but using 10 ml of a solution prepared by diluting a suitable volume of the eye drops with *water* to contain the equivalent of 0.045% w/v of gentamicin.

ASSAY

Dilute a volume of the eye drops containing the equivalent of 15 mg of gentamicin to 50 ml with sterile *phosphate buffer pH 8.0* and dilute 10 ml of the resulting solution to 50 ml with the same solvent. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the eye drops, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of gentamicin.

Gentamicin Injection

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Injection is a sterile solution of Gentamicin Sulphate in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase the lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate. Apply separately to the plate (1) a volume of the injection containing the equivalent of 30 µg of gentamicin and (2) 0.1 mg of *gentamicin sulphate EPCRS* dissolved in a volume of *water* equal to the volume of the injection used. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes. The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulphate, the retention times of the four principal peaks in the chromatogram obtained with solution (2) correspond to those of the four principal peaks in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L.

Composition of gentamicin sulphate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 ml of *methanol* to 10 ml of a 0.065% w/v solution of *gentamicin sulphate EPCRS,* swirl and add 4 ml of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 ml, heat in a water bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours. Prepare solution (2) in the same manner as solution (1) but using 10 ml of a solution prepared by diluting a suitable volume of the injection with *water* to contain the equivalent of 0.045% of gentamicin.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 to 12.5 cm × 4.6 to 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) 0.025M *sodium heptanesulphonate monohydrate* in a mixture of 5 volumes of *glacial acetic acid*, 25 volumes of *water* and 70 volumes of *methanol* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 330 nm.

When the chromatograms are recorded under the prescribed conditions the retention time of component C_2 is 10 to 20 minutes. The retention times relative to component C_2 are: about 0.13 (reagent); about 0.27 (component C_1); about 0.65 (component C_{1a}); about 0.85 (component C_{2a}).

The test is not valid unless, in the chromatogram obtained with solution (1), the *resolution factor* between the peaks due to components C_{2a} and C_2 is at least 1.3.

Using the chromatogram obtained with solution (2) calculate the percentage content of components C_1, C_{1a}, C_2 and C_{2a} in the injection by *normalisation*. The proportions are within the following limits: C_1, 25.0 to 50.0%; C_{1a}, 10.0 to 35.0%; C_2 plus C_{2a}, 25.0 to 55.0%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing the equivalent of 10 mg of gentamicin per ml (solution A). The endotoxin limit concentration of solution A is 16.7 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the injection, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

LABELLING

The strength is stated in terms of the equivalent amount of gentamicin in a suitable dose-volume.

Gentamicin Ointment

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Gentamicin Ointment is a dispersion of Gentamicin Sulphate in *microfine powder* in White Soft Paraffin or other suitable anhydrous greasy basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase the lower layer obtained by shaking together equal volumes of 13.5M *ammonia*, *chloroform* and *methanol* and allowing to separate. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) disperse a quantity of the ointment containing the equivalent of 7.5 mg of gentamicin in 20 ml of *chloroform*, extract with 10 ml of *water* and use the aqueous layer. Solution (2) is a solution of *gentamicin sulphate EPCRS* in *water* containing the equivalent of 0.075% w/v of gentamicin. After removal of the plate, allow it to dry in air, spray with *ninhydrin solution R1* and heat at 105° for 2 minutes. The three principal spots in the chromatogram obtained with solution (1) correspond to the three principal spots in the chromatogram obtained with solution (2).

B. In the test for Composition of gentamicin sulphate, the retention times of the four principal peaks in the chromatogram obtained with solution (2) correspond to those of the four principal peaks in the chromatogram obtained with solution (1).

TESTS

Composition of gentamicin sulphate

Complies with the test described under Gentamicin Injection but preparing solution (2) in the following manner. Disperse a quantity of the ointment containing the equivalent of 20 mg of gentamicin in 10 ml of *chloroform*, add 20 ml of a 0.25% w/v solution of *sodium tetraborate*, shake vigorously, centrifuge and separate the aqueous layer. Filter and dilute to 50 ml with *water*. To 10 ml add 5 ml of *methanol*, swirl, add 4 ml of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 ml, heat in a water bath at 60° for 15 minutes and cool.

ASSAY

Dissolve as completely as possible a quantity containing the equivalent of 4 mg of gentamicin in 50 ml of *chloroform*, extract with three 20 ml quantities of *phosphate buffer pH 8.0* and dilute the combined extracts to 100 ml with *phosphate buffer pH 8.0*. Dilute 10 ml of the resulting solution to 50 ml with *phosphate buffer pH 8.0*. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of gentamicin in the ointment, taking each 1000 IU found to be equivalent to 1 mg of gentamicin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of gentamicin.

Glibenclamide Tablets

General Notices

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Glibenclamide Tablets contain Glibenclamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of glibenclamide, $C_{23}H_{28}ClN_3O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Dissolution

Prepare a mixture containing 0.8134% w/v of *anhydrous disodium hydrogen phosphate* and 0.1350% w/v of *potassium dihydrogen orthophosphate* (solution A).

Comply with the *dissolution test for tablets and capsules,* Appendix XII B1, using Apparatus 2. Use as the medium 1000 ml of solution A and rotate the paddle at 100 revolutions per minute. Withdraw 10 ml of the medium at 20 minutes and 90 minutes, filter (Minisart GS 25mm filters are suitable) discarding the first 5 ml of the filtrate. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dissolve *glibenclamide BPCRS* in the minimum quantity of *methanol* and dilute to an appropriate concentration with solution A.

(2) The filtrate from the dissolution vessel.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable).

(b) Isocratic system using the mobile phase below.

(c) Flow rate of 1.0 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 225 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 40 volumes of 0.1M *potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile.*

DETERMINATION OF CONTENT

Calculate the total content of glibenclamide, $C_{23}H_{28}ClN_3O_5S$, in the medium from the chromatograms obtained and using the declared content of $C_{23}H_{28}ClN_3O_5S$ in *glibenclamide BPCRS.*

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing 20 mg of Glibenclamide with four 5-ml quantities of a mixture of 20 volumes of *dichloromethane* and 10 volumes of *acetone*, evaporate the combined extracts to dryness at a temperature not exceeding 40° at a pressure of 2 kPa and dissolve the residue in 4 ml of a mixture of equal volumes of *chloroform* and *methanol*.

(2) 0.012% w/v of *4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulphonamide BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*.

(3) 0.0020% w/v of *methyl* N-*4-[2-(5-chloro-2-methoxybenzamido)ethyl] benzenesulphonylcarbamate BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*.

(4) 0.5% w/v of *glibenclamide BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel* GF_{254}.

(b) The mobile phase is a mixture of 5 volumes of *ethanol (96%)*, 5 volumes of *glacial acetic acid*, 45 volumes of *chloroform* and 45 volumes of *cyclohexane*.

(c) Application volume of 10 µl of each solution.

(d) Develop to 15 cm.

(e) Remove the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

LIMITS

In the chromatogram obtained with solution (1), any spots corresponding to 4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulphonamide and methyl *N*-4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulphonylcarbamate are not more intense than the spots in the chromatograms obtained with solutions (2)(2.4%) and (3)(0.4%) respectively.

Uniformity of content

Tablets containing 5 mg or less of Glibenclamide comply with the requirements stated under

Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Powder one tablet, add a mixture of 2 ml of *water* and 20 ml of *methanol*, mix with the aid of ultrasound until fully dispersed and filter through a 0.2-µm membrane filter (Anatop LC is suitable).

(2) Add 2 ml of *water* to 20 ml of a 0.025% w/v solution of *glibenclamide BPCRS* in *methanol*, mix with the aid of ultrasound until fully dispersed and filter (0.2-µm Anatop LC is suitable).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in each tablet using the declared content of $C_{23}H_{28}ClN_3O_5S$ in *glibenclamide BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix, with the aid of ultrasound, a quantity of the powdered tablets containing 5 mg of Glibenclamide with a mixture of 2 ml of *water* and 20 ml of *methanol* until fully dispersed and filter through a 0.2-µm membrane filter (Anatop LC is suitable).

(2) Dissolve 50 mg of *glibenclamide BPCRS* in 10 ml of *methanol* with the aid of ultrasound for 20 minutes, add sufficient *methanol* to produce 50 ml and dilute 1 volume of this solution to 4 volumes with *methanol*. To 20 ml of this solution add 2 ml of *water* and mix.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable).

(b) Isocratic system using the mobile phase below.

(c) Flow rate of 1.5 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 300 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 47 volumes of *acetonitrile* and 53 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ using the declared content of $C_{23}H_{28}ClN_3O_5S$ in *glibenclamide BPCRS*.

Gliclazide Tablets

General Notices

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Gliclazide Tablets contain Gliclazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of gliclazide, $C_{15}H_{21}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.16 g of Gliclazide with 20 ml of *dichloromethane*, centrifuge and evaporate the supernatant liquid to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of gliclazide *(RS 168)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the dissolution medium 900 ml of *phosphate buffer pH 7.4* and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter through a 0.5-µm filter. Measure the *absorbance* of the filtrate diluted, if necessary, with the dissolution medium to an expected concentration of 12.5 µg per ml of Gliclazide, Appendix II B, at 226 nm and 290 nm using the dissolution medium in the reference cell. Correct the absorbance obtained at 226 nm by subtracting the absorbance obtained at 290 nm. Calculate the total content of gliclazide, $C_{15}H_{21}N_3O_3S$, in the medium using the *absorbance* of a solution prepared by dissolving 62.0 mg of *gliclazide BPCRS* in 20 ml of *methanol*, adding sufficient dissolution medium to produce 1000 ml and diluting 1 volume of this solution to 5 volumes with the dissolution medium and using the declared content of $C_{15}H_{21}N_3O_3S$ in *gliclazide BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions without delay. For solution (1) shake a quantity of the powdered tablets containing 0.8 g of Gliclazide for 1 hour with 200 ml of *acetonitrile*, filter and dilute 10 ml of the filtrate to 50 ml with a mixture of 1 volume of *acetonitrile* and 2 volumes of *water*. For solution (2) dilute 1 volume of solution (1) to 500 volumes with a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*. For solution (3) dissolve 5 mg of *gliclazide BPCRS* and 15 mg of *1-(3-*

*azabicyclo[3.3.0]oct-3-yl)-3-*o*-tolylsulphonylurea BPCRS* in 25 ml of *acetonitrile*, dilute to 50 ml with *water* and dilute 1 volume of the resulting solution to 20 volumes with a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*. For solution (4) dissolve 8 mg of *1-(3-azabicyclo[3.3.0]oct-3-yl)-3-*o*-tolylsulphonylurea BPCRS* in 25 ml of *acetonitrile*, dilute to 50 ml with *water* and dilute 1 volume of the resulting solution to 100 volumes with a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (4 µm) (Superspher 60RP8 is suitable), (b) a mixture of 0.1 volume of *triethylamine*, 0.1 volume of *trifluoroacetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water* as the mobile phase with a flow rate of 0.9 ml per minute and (c) a detection wavelength of 235 nm. Inject separately 20 µl of each solution. For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak.

The test is not valid unless the *resolution factor* between the peaks in the chromatogram obtained with solution (3) is at least 1.8.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-*o*-tolylsulphonylurea is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any other *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%). Disregard any peak with an area less than one quarter of the area of the peak corresponding to gliclazide in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.8 g of Gliclazide for 1 hour with 200 ml of *acetonitrile*, filter and dilute 10 ml of the filtrate to 200 ml with a mixture of 2 volumes of *acetonitrile* and 3 volumes of *water*. For solution (2) dissolve 40 mg of *gliclazide BPCRS* in 10 ml of *acetonitrile* and dilute to 200 ml with a mixture of 2 volumes of *acetonitrile* and 3 volumes of *water*. For solution (3) dissolve 5 mg of *gliclazide BPCRS* and 15 mg of *1-(3-azabicyclo[3.3.0]oct-3-yl)-3-*o*-tolylsulphonylurea BPCRS* in 25 ml of *acetonitrile*, dilute to 50 ml with *water* and dilute 1 volume of the resulting solution to 20 volumes with a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless the *resolution factor* between the peaks in the chromatogram obtained with solution (3) is at least 1.8.

Calculate the content of $C_{15}H_{21}N_3O_3S$ in the tablets using the declared content of $C_{15}H_{21}N_3O_3S$ in *gliclazide BPCRS*.

Glipizide Tablets

General Notices

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Glipizide Tablets contain Glipizide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of glipizide $C_{21}H_{27}N_5O_4S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 320 nm of the final solution obtained in the Assay exhibits maxima at 226 nm and 274 nm.

B. Shake a quantity of the powdered tablets containing 25 mg of Glipizide with 10 ml of *dichloromethane* for 5 minutes, filter, dry the filtrate with *anhydrous sodium sulphate*, filter again and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of glipizide *(RS 169)*.

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel GF_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *ethyl acetate*, 20 volumes of *anhydrous formic acid* and 40 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Glipizide with four 10 ml quantities of *acetone*, evaporate the combined extracts to dryness under reduced pressure at a temperature not exceeding 30° and dissolve the residue in sufficient of a mixture of equal volumes of *dichloromethane* and *methanol* to produce 5 ml. For solution (2) dilute 1 volume of solution (1) to 200 volumes with a mixture of equal volumes of *dichloromethane* and *methanol* . For solution (3) dilute 1 volume of solution (1) to 500 volumes with the same solvent mixture. Solution (4) contains 0.010% w/v of *glipizide impurity A EPCRS* (4-[2-(5-methylpyrazine-2-carboxamido)ethyl]benzenesulphonamide) in a mixture of equal volumes of *dichloromethane* and *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to glipizide impurity A in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%). Any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 15 mg of Glipizide add 30 ml of *methanol*, heat gently on a water bath whilst shaking, cool and add sufficient *methanol* to produce 50 ml. Filter and dilute 5 ml of the filtrate to 50 ml with *methanol*. Measure the *absorbance* of the resulting solution at the maximum at 274 nm, Appendix II B, using *methanol* in the reference cell. Calculate the content of $C_{21}H_{27}N_5O_4S$ taking 237 as the value of A(1%, 1 cm) at the maximum at 274 nm.

Gliquidone Tablets

General Notices

Action and use

Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

DEFINITION

Gliquidone Tablets contain Gliquidone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of gliquidone, $C_{27}H_{33}N_3O_6S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 30 mg of Gliquidone in 10 ml of *methanol* with the aid of ultrasound, filter, evaporate the filtrate to dryness using a rotary evaporator and dry the residue at a temperature of 50° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of gliquidone *(RS 170)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a citro-phosphate buffer prepared by dissolving 35.6 g of *disodium hydrogen orthophosphate dihydrate* in sufficient *water* to produce 1000 ml, adjusting the pH to 8.5 with *citric acid*, and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 50 ml of the medium and filter (Whatman No. 1 filter paper is suitable), discarding the first 15 ml of filtrate. Measure the *absorbance* of the filtered solution, Appendix II B, at the maximum at 314 nm using a filtered solution of the dissolution medium in the reference cell. Measure the *absorbance* of a solution of *gliquidone BPCRS* prepared in the following manner. Dissolve about 30 mg of *gliquidone BPCRS* in 10 ml of *dimethylformamide*, add sufficient dissolution medium to produce 100 ml and mix. Dilute 10 ml of this solution to 100 ml with dissolution medium, mix and filter (Whatman No. 1 filter paper is suitable), discarding the first 15 ml of filtrate. Calculate the total content of gliquidone, $C_{27}H_{33}N_3O_6S$, in the medium from the absorbances obtained and from the declared content of $C_{27}H_{33}N_3O_6S$ in *gliquidone BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 5 volumes of *glacial acetic acid*, 5 volumes of *ethanol (96%)*, 45 volumes of *chloroform* and 45 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following five solutions. For solution (1) mix a quantity of the powdered tablets containing 0.1 g of Gliquidone with 8 ml of a mixture of 50 volumes of *dichloromethane* and 50 volumes of *methanol*, mix with the aid of ultrasound for 10 minutes, add sufficient of the same solvent mixture to produce 10 ml, filter (Whatman GF/C filter paper is suitable) and use the filtrate. Solution (2) contains 1.0% w/v of *gliquidone BPCRS* in a mixture of 50 volumes of *dichloromethane* and 50 volumes of *methanol*. Solution (3) contains 0.0030% w/v of *gliquidone BPCRS* in a mixture of 50 volumes of *dichloromethane* and 50 volumes of *methanol*. Solution (4) contains 0.010% w/v of *gliquidone sulphonamide BPCRS* in a mixture of 50 volumes of *dichloromethane* and 50 volumes of *methanol*. For solution (5) mix 1 volume of solution (3) and 1 volume of solution (4). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to gliquidone sulphonamide is not more intense than the spot in the chromatogram obtained with solution (4) (1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%). The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated principal spots.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 0.1 g of Gliquidone add 50 ml of *methanol*, mix with the aid of ultrasound for 10 minutes, allow to cool, add sufficient *methanol* to produce 100 ml and filter. Dilute 15 ml of the filtrate to 100 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 310 nm, Appendix II B. Calculate the content of $C_{27}H_{33}N_3O_6S$ in the tablets from the *absorbance* of a 0.015% w/v solution of *gliquidone BPCRS* in *methanol* and using the declared content of $C_{27}H_{33}N_3O_6S$ in *gliquidone BPCRS*.

Glucose Intravenous Infusion

General Notices

Glucose Injection

DEFINITION

Glucose Intravenous Infusion is a sterile solution of Anhydrous Glucose or Glucose in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of glucose, $C_6H_{12}O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution. Solutions containing the equivalent of 200 g per litre or more of glucose, $C_6H_{12}O_6$, may be not more than faintly yellow in colour.

IDENTIFICATION

A. Heat with *cupri-tartaric solution R1.* A red precipitate is produced.

B. The solution prepared as directed in the Assay is dextrorotatory.

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L, when determined on a solution diluted, if necessary, with *water for injections*, to contain not more than the equivalent of 5% w/v of glucose, $C_6H_{12}O_6$, and to which 0.30 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

5-Hydroxymethylfurfural and related substances

Dilute a volume containing the equivalent of 1.0 g of glucose, $C_6H_{12}O_6$, to 250 ml with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per ml, Appendix XIV C. Dilute infusions containing more than 5% w/v of Glucose with *water BET* to contain 5% w/v.

ASSAY

To a volume containing the equivalent of 2 g to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 ml of 5M *ammonia* and sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and determine the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the volume taken for assay.

LABELLING

The strength is stated as the equivalent number of grams of glucose, $C_6H_{12}O_6$, per litre.

When Glucose Intravenous Infusion is required as a diluent for Injections or Intravenous Infusions of the Pharmacopoeia, Glucose Intravenous Infusion (50 g per litre) shall be used.

Glucose Irrigation Solution

General Notices

DEFINITION

Glucose Irrigation Solution is a sterile solution of Anhydrous Glucose or Glucose in Water for Irrigation.

The irrigation solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.

Content of glucose, $C_6H_{12}O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution. Solutions containing the equivalent of 200 g per litre or more of glucose, $C_6H_{12}O_6$, may be not more than faintly straw coloured.

IDENTIFICATION

A. Heat with *cupri-tartaric solution R1*. A red precipitate is produced.

B. The solution prepared in the Assay is dextrorotatory.

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L, when determined in a solution diluted, if necessary, with *water* to contain not more than the equivalent of 5% w/v of glucose, $C_6H_{12}O_6$, and to which 0.30 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

5-Hydroxymethylfurfural and related substances

Dilute a volume containing the equivalent of 1.0 g of glucose, $C_6H_{12}O_6$, to 250 ml with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

ASSAY

To a volume containing the equivalent of 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 ml of 5M *ammonia* and sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and determine the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of glucose, $C_6H_{12}O_6$, in the volume taken for assay.

LABELLING

The strength is stated in terms of the equivalent amount of glucose, $C_6H_{12}O_6$.

Glutaraldehyde Solution

General Notices

DEFINITION

Glutaraldehyde Solution is a dilution of Strong Glutaraldehyde Solution in a mixture of Purified Water and Ethanol (96 per cent).

In making Glutaraldehyde Solution, Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit[1].

Content of glutaraldehyde, $C_5H_8O_2$

9.2 to 10.5% w/v.

IDENTIFICATION

A. Heat 5 ml with 10 ml of a solution containing 1 g of *hydroxylamine hydrochloride* and 2 g of *sodium acetate* on a water bath for 10 minutes, allow to cool and filter. The *melting point* of the residue, after washing with *water* and drying at 105°, is about 178°, Appendix V A.

To 1 ml add 2 ml of *ammoniacal silver nitrate solution* and mix gently for a few minutes. Silver is deposited.

TESTS

Ethanol content

50.0 to 60.0% v/v, Appendix VIII F.

ASSAY

Mix 10 ml with 100 ml of a 7% w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with 1M *sodium hydroxide VS* and allow to stand for 30 minutes. Add 20 ml of *petroleum spirit (boiling range, 40° to 60°)* and titrate with 1M *sodium hydroxide VS* until the colour of the aqueous phase matches that of a 7% w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with 1M *sodium hydroxide VS*. Each ml of 1M *sodium hydroxide VS* is equivalent to 50.05 mg of $C_5H_8O_2$.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

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[1] The law and the statutory regulations governing the use of Industrial Methylated Spirit must be observed.

Glycerol Eye Drops

General Notices

DEFINITION

Glycerol Eye Drops are a sterile solution of Glycerol in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of glycerol, $C_3H_8O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix 1 ml of the eye drops with 0.5 ml of *nitric acid* and superimpose 0.5 ml of *potassium dichromate solution*. A blue ring develops at the interface of the liquids, which does not diffuse into the lower layer within 10 minutes.

TESTS

Acidity or alkalinity

pH, 4.5 to 7.5, Appendix V L.

ASSAY

Dilute a suitable volume of the eye drops with sufficient *water* to produce a solution containing 3% w/v of Glycerol. To 5 ml add 150 ml of *water* and neutralise with 0.1M *sodium hydroxide VS*, using *bromocresol purple solution* as indicator. Add 1.6 g of *sodium periodate* and allow to stand protected from light for 15 minutes. Add 3 ml of *propan-1,2-diol*, shake, allow to stand protected from light for 5 minutes and titrate with 0.1M *sodium hydroxide VS*. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 9.21 mg of $C_3H_8O_3$.

STORAGE

Glycerol Eye Drops should be protected from light.

Strengths available

Eye Drops containing 10, 20, 30 and 50% w/v of glycerol are available.

Glycerol Suppositories

General Notices

Glycerin Suppositories

DEFINITION

Gelatin	14 g
Glycerol	70 g
Purified Water	A sufficient quantity

In tropical and subtropical countries the quantity of Gelatin may be increased to an amount not exceeding 18 g.

Extemporaneous preparation

The following directions apply.

Add the Gelatin to about 30 ml of Purified Water heated nearly to boiling, then add the Glycerol previously heated to 100° and heat the mixture on a water bath for 15 minutes or until solution is complete. Adjust the weight of the product to 100 g by the addition of hot Purified Water or by evaporation on a water bath and pour into suitable moulds.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of glycerol, $C_3H_8O_3$

66.5 to 73.5% w/w.

Disintegration

Maximum time, 1 hour, Appendix XII A2.

ASSAY

Dissolve a number of suppositories containing 8 g of Glycerol in 50 ml of *water* and add sufficient *water* to produce 250 ml. To 5 ml add 150 ml of *water* and 0.25 ml of *bromocresol purple solution* and neutralise with 0.1M *sodium hydroxide VS* to the blue colour of the indicator. Add 1.6 g of *sodium periodate* and allow to stand for 15 minutes. Add 3 ml of *propan-1,2-diol*, shake, allow to stand for 5 minutes and titrate with 0.1M *sodium hydroxide VS* to the same blue colour. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 9.210 mg of $C_3H_8O_3$.

Glyceryl Trinitrate Sublingual Spray

General Notices

DEFINITION

Glyceryl Trinitrate Sublingual Spray contains Glyceryl Trinitrate Solution in a suitable vehicle either in a suitable pressurised container or in a container fitted with a spray device.

PRODUCTION

A suitable test is carried out to demonstrate the uniformity of dose.

The oromucosal spray complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of glyceryl trinitrate, $C_3H_5N_3O_9$

80.0 to 120.0% of the amount stated to be delivered per metered dose.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel plates are suitable) and a mixture of 20 volumes of *ethyl acetate* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) expel a number of metered doses containing the equivalent of about 4 mg of glyceryl trinitrate into a beaker, add 5 ml of *acetone*, transfer to a graduated flask and add sufficient *acetone* to produce 10 ml. For solution (2) dilute a quantity of *glyceryl trinitrate solution BPCRS* with methanol (50%) to produce a solution containing 0.04% w/v of glyceryl trinitrate. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and spray with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) expel a number of metered doses containing the equivalent of about 5 mg of glyceryl trinitrate into a beaker, dilute to 5 ml with *methanol*, mix well and add sufficient *water* to produce 10 ml. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (50%).* For solution (3) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of glyceryl trinitrate and heat in a reaction vial at 100° for 30 minutes.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* and (c) a detection wavelength of 210 nm.

The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS* in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities with retention times relative to glyceryl trinitrate of approximately 0.5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of areas of any *secondary peaks* is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) collect 10 metered doses in a vessel avoiding loss of material, dissolve the expelled material in *methanol* and dilute with sufficient *methanol* to produce a solution containing the equivalent of 0.04% w/v of glyceryl trinitrate. For solution (2) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient *methanol (50%)* to produce a solution containing 0.04% w/v of glyceryl trinitrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.3 ml per minute a mixture of 50 volumes of *methanol* and 50 volumes of *water* and (c) a detection wavelength of 225 nm.

Calculate the content of $C_3H_5N_3O_9$ per metered dose using the declared content of $C_3H_5N_3O_9$ in *glyceryl trinitrate solution BPCRS*.

LABELLING

The label states (1) the instructions for using the spray; (2) the amount of glyceryl trinitrate delivered per metered dose; (3) the number of deliveries available from the container.

Glyceryl Trinitrate Tablets

General Notices

Glyceryl Trinitrate Sublingual Tablets; Trinitrin Tablets; Nitroglycerin Tablets

DEFINITION

Glyceryl Trinitrate Tablets are *sublingual tablets* made with Mannitol as the basis and may be prepared by adding Glyceryl Trinitrate Solution of an appropriate concentration to dried granules of Mannitol, mixing intimately, drying at a temperature not exceeding 50° or without heating for not more than 4 hours and compressing.

CAUTION Undiluted glyceryl trinitrate can be exploded by percussion or excessive heat. Appropriate precautions should be exercised and only exceedingly small amounts should be isolated.

The tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of glyceryl trinitrate, $C_3H_5N_3O_9$

85.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *toluene* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 0.5 mg of glyceryl trinitrate with 1 ml of *acetone* and centrifuge. For solution (2) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient *water* to produce a solution containing 0.05% w/v of glyceryl trinitrate. After removal of the plate, dry it in a stream of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (365 nm)*. Examine the plate in daylight. The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Extract a quantity of the powdered tablets containing 3 mg of glyceryl trinitrate with 5 ml of *ether* and filter. Evaporate the ether and dissolve the residue in 0.2 ml of *sulphuric acid* containing a trace of *diphenylamine*. An intense blue colour is produced.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the powdered tablets containing 2.5 mg of glyceryl trinitrate with 10 ml of *acetonitrile* with the aid of ultrasound, filter through a 4-µm filter and dilute one volume of the filtrate with an equal volume of *water*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of glyceryl trinitrate and heat in a reaction vial at 100° for 30 minutes.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water* and (c) a detection wavelength of 210 nm.

Inject 50 µl of each solution and, for solution (1), allow the chromatography to proceed for three times the retention time of the principal peak. The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS* in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities with retention times relative to glyceryl trinitrate of approximately 0.5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Tablets containing less than 2 mg of glyceryl trinitrate comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 2 ml of *acetonitrile* to one tablet, mix with the aid of ultrasound for 5 minutes, filter through a 4-µm filter and dilute 1 volume of the filtrate with an equal volume of *water*. For solution (2) dilute *glyceryl trinitrate solution BPCRS* with the mobile phase to produce a solution containing the equivalent amount of glyceryl trinitrate as that expected for solution (1). For solution (3) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of glyceryl trinitrate and heat in a reaction vial at 100° for 30 minutes.

The chromatographic conditions described under Related substances may be used.

Inject 50 µl of each solution. The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS* in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities with retention times relative to glyceryl trinitrate of approximately 0.5.

Calculate the content of $C_3H_5N_3O_9$ in each tablet from the chromatograms obtained and using the declared content of $C_3H_5N_3O_9$ in *glyceryl trinitrate solution BPCRS*.

ASSAY

Use the average of the 10 individual results obtained from the test for Uniformity of content.

STORAGE

Glyceryl Trinitrate Tablets should be protected from light and stored in a glass container closed by means of a screw closure lined with aluminium or tin foil; additional packing that absorbs glyceryl trinitrate should be avoided. Glyceryl Trinitrate Tablets should be issued for patients in containers of not more than 100 tablets.

LABELLING

The label states the amount of glyceryl trinitrate per tablet.

Glyceryl Trinitrate Transdermal Patches

General Notices

DEFINITION

Glyceryl Trinitrate Transdermal Patches contain glyceryl trinitrate in a suitable matrix or reservoir presentation. They are prepared from Glyceryl Trinitrate Solution.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of glyceryl trinitrate.

The transdermal patches comply with the requirements stated under Transdermal Patches and with the following requirements.

Content of glyceryl trinitrate, $C_3H_5N_3O_9$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel plates are suitable) and a mixture of 20 volumes of *ethyl acetate* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) clean the outer surface of a patch using a lint-free cloth moistened with *methanol*. Remove the release liner and score the exposed surface. Place the patch in a flask containing sufficient *methanol* to produce a solution containing 0.1% w/v of glyceryl trinitrate, mix with the aid of ultrasound in a water bath at 40° for 15 minutes and then shake mechanically at room temperature for 3 hours. Dilute the methanolic extract with sufficient *water* to produce a final concentration of 0.05% w/v of glyceryl trinitrate. For solution (2) dilute a quantity of *glyceryl trinitrate solution BPCRS* with *methanol (50%)* to produce a solution containing 0.05% w/v of glyceryl trinitrate. After removal of the plate, allow it to dry in air and spray with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds in colour, position and size to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) clean the outer surface of five patches using a lint-free cloth moistened with *methanol*. Remove the release liners and score the exposed surfaces. Place the patches in a flask containing sufficient *methanol* to produce a solution containing 0.1% w/v of glyceryl trinitrate, mix with the aid of ultrasound in a water bath at 40° for 15 minutes and then shake mechanically at room temperature for 3 hours. Dilute the methanolic extract with sufficient *water* to produce a final concentration of 0.05% w/v of glyceryl trinitrate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (50%).* For solution (3) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient 1M *hydrochloric acid* to produce a solution containing 0.05% w/v of glyceryl trinitrate and heat in a reaction vial at 100° for 30 minutes.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water* and (c) a detection wavelength of 210 nm.

The test is not valid unless the chromatogram obtained with solution (3) resembles the reference chromatogram supplied with *glyceryl trinitrate solution BPCRS* in that it shows a principal peak due to glyceryl trinitrate and two clearly separated peaks due to the dinitrate impurities with retention times relative to glyceryl trinitrate of approximately 0.5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of areas of any *secondary peaks* is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Uniformity of content

Comply with the requirements stated under *uniformity of content*, Appendix XII C3, Test C, with respect to the individual content of each dosage unit and using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) clean the outer surface of a patch using a lint-free cloth moistened with *methanol*. Remove the release liner and score the exposed surface. Place the patch in a flask containing sufficient *methanol* to produce a solution containing 0.1% w/v of glyceryl trinitrate, mix with the aid of ultrasound in a water bath at 40° for 15 minutes, shake mechanically at room temperature and dilute a volume of the solution with an equal volume of *water*. For solution (2) dilute a quantity of *glyceryl trinitrate solution BPCRS* with sufficient *methanol (50%)* to produce a solution containing 0.05% w/v of glyceryl trinitrate.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_3H_5N_3O_9$ in the transdermal patch using the declared content of $C_3H_5N_3O_9$ in *glyceryl trinitrate solution BPCRS*.

ASSAY

Use the average of the 10 results obtained in the test for Uniformity of content.

LABELLING

The quantity of active ingredient is stated in terms of the amount of glyceryl trinitrate per patch.

IMPURITIES

A. Glyceryl-1,2-dinitrate,

B. Glyceryl-1,3-dinitrate.

Glycine Irrigation Solution

General Notices

DEFINITION

Glycine Irrigation Solution is a sterile solution of Glycine in Water for Irrigation.
The irrigation solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.

Content of glycine, $C_2H_5NO_2$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Evaporate 5 ml to dryness on a water bath and dry at 105° for 1 hour. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of glycine *(RS 171)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 30 volumes of 13.5M *ammonia* and 70 volumes of *propan-1-ol* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute the irrigation solution with *water* to contain 0.25% w/v of Glycine. Solution (2) contains 0.25% w/v of *glycine* in *water*. After removal of the plate, dry it at 100° to 105° for 10 minutes, spray with *ninhydrin solution* and heat at 100° to 105° for 2 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 6.5, Appendix V L.

Ammonium compounds

Dilute a volume containing 0.2 g of Glycine to 70 ml with *water* in an ammonia-distillation apparatus, add 25 ml of a solution prepared by boiling 25 ml of 5M *sodium hydroxide* with 50 ml of *water* and 50 mg of *aluminium* until the volume is reduced to 25 ml and distil into 2 ml of a saturated solution of *boric acid* until 50 ml is obtained. Add 2 ml of a solution prepared by boiling 25 ml of 5M *sodium hydroxide* with 50 ml of *water* until the volume is reduced to 25 ml and 2 ml of *alkaline potassium tetraiodomercurate solution*. Any colour produced is not more intense than that obtained by treating 70 ml of *water* containing 4 ml of *ammonia standard solution (10 ppm NH_4)* in the same manner, beginning at the words 'add 25 ml of…' (200 ppm, calculated with reference to the content of glycine).

ASSAY

Dilute a volume containing 0.15 g of Glycine to 25 ml with *water* and add 10 ml of *formaldehyde solution* previously adjusted to pH 9.0 and 0.25 ml of a mixed indicator solution prepared by dissolving 75 mg of *phenolphthalein* and 25 mg of *thymol blue* in 100 ml of *ethanol (50%)*. Titrate with 0.1M *sodium hydroxide VS* until the yellow colour disappears and a faint violet colour is produced. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 7.507 mg of $C_2H_5NO_2$.

Griseofulvin Tablets

General Notices

Action and use

Antibacterial.

DEFINITION

Griseofulvin Tablets contain Griseofulvin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of griseofulvin, $C_{17}H_{17}ClO_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.125 g of Griseofulvin with 20 ml of *chloroform*, add 1 g of *anhydrous sodium sulphate*, shake and filter. Evaporate the filtrate to dryness and dry at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of griseofulvin *(RS 172)*.

B. Shake a quantity of the powdered tablets containing 80 mg of Griseofulvin with 150 ml of *ethanol (96%)* for 20 minutes. Dilute to 200 ml with *ethanol (96%)* and filter. Dilute 2 ml of the filtrate to 100 ml with *ethanol (96%)*. The *light absorption* of the resulting solution, Appendix II B, in the range 240 to 400 nm exhibits two maxima, at 291 nm and 325 nm, and a shoulder at 250 nm.

C. Dissolve 5 mg of the powdered tablets in 1 ml of *sulphuric acid* and add 5 mg of powdered *potassium dichromate*. A red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 1000 ml of a 1.5% w/v solution of *sodium dodecyl sulphate* and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtrate, suitably diluted if necessary with *methanol (80%)*, at the maximum at 291 nm, Appendix II B. Calculate the total content of $C_{17}H_{17}ClO_6$ in the medium taking 725 as the value of A(1%, 1 cm) at the maximum at 291 nm.

Related substances

Dissolve 50 mg of *9,10-diphenylanthracene* (internal standard) in sufficient *chloroform* to produce 50 ml (solution A). Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) dissolve 5 mg of *griseofulvin BPCRS* in *chloroform* and add 2 ml of solution A and sufficient *chloroform* to produce 200 ml. Evaporate 20 ml of the solution to about 1 ml. For solution (2) add 60 ml of *chloroform* to a quantity of the powdered tablets containing 50 mg of Griseofulvin, heat at 60° with shaking for 20 minutes, cool and dilute to 100 ml with *chloroform*. Centrifuge and evaporate 20 ml of the clear supernatant liquid to about 1 ml. Prepare solution (3) in the same manner as solution (2) but adding 1 ml of solution A before diluting to 100 ml with *chloroform*.

The chromatographic procedure may be carried out using a glass column (1.0 m × 4 mm) packed with acid-washed, *silanised diatomaceous support* (100 to 120 mesh) coated with 1% w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225 is suitable) and maintained at 250°.

In the chromatogram obtained with solution (3) the ratios of the areas of any peak corresponding to dechlorogriseofulvin (retention time about 0.6 times that of griseofulvin) and of any peak corresponding to dehydrogriseofulvin (retention time about 1.4 times that of griseofulvin) to the area of the peak due to the internal standard are, respectively, not greater than 0.6 times and not greater than 0.15 times the ratio of the area of the peak due to griseofulvin to that of the internal standard in the chromatogram obtained with solution (1).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 35 mg of Griseofulvin add 60 ml of *ethyl acetate*, mix and heat to 60° with shaking for 20 minutes. Allow to cool and dilute to 100 ml with *ethyl acetate*. Centrifuge and transfer two 5 ml aliquots of the clear supernatant liquid into separate 100 ml graduated flasks. Add 5 ml of 2M *methanolic methanesulphonic acid* to the first flask, allow to stand at 20° for 30 minutes and dilute to 100 ml with *methanol* (solution A). Dilute the contents of the second flask to 100 ml with *methanol* (solution B). To a third flask add 5 ml of 2M *methanolic methanesulphonic acid* and dilute to 100 ml with *methanol* (solution C). Measure the *absorbance* of each solution at 266 nm, Appendix II B. Calculate the content of $C_{17}H_{17}ClO_6$ from the difference between the absorbance obtained with solution A and the sum of the absorbances obtained with solutions B and C and from the difference obtained by repeating the operation using 35 mg of *griseofulvin BPCRS* in place of the powdered tablets and from the declared content of $C_{17}H_{17}ClO_6$ in *griseofulvin BPCRS*.

Guanethidine Tablets

General Notices

Action and use

Adrenergic neuron blocker.

DEFINITION

Guanethidine Tablets contain Guanethidine Monosulphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of guanethidine monosulphate, $C_{10}H_{22}N_4,H_2SO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 50 mg of Guanethidine Monosulphate with a mixture of 5 ml of *hydrochloric acid* and 45 ml of *water*, filter, neutralise 25 ml of the filtrate with 5M *sodium hydroxide* and add 20 ml of *picric acid solution R1*. The *melting point* of the precipitate, after washing with *water*, is about 153°, Appendix V A.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 30 mg of Guanethidine Monosulphate for 15 minutes with 50 ml of *water*, dilute to 100 ml with *water* and filter. To 5 ml of the filtrate add 10 ml of a solution prepared by dissolving 1 g of *sodium nitroprusside* and 1 g of *potassium hexacyanoferrate(II)* in 50 ml of a 0.5% w/v solution of *sodium hydroxide*, adding 5 ml of *hydrogen peroxide solution (100 vol)*, swirling gently and diluting to 100 ml with the solution of sodium hydroxide. Mix, allow to stand for 20 minutes, add sufficient *water* to produce 25 ml and measure the *absorbance* of the resulting solution at 520 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 ml of *water* in the same manner, beginning at the words 'add 10 ml of a solution…'. Repeat the operation using 5 ml of a 0.03% w/v solution of *guanethidine monosulphate BPCRS* beginning at the words 'add 10 ml of a solution …'. Calculate the content of $C_{10}H_{22}N_4,H_2SO_4$ in the tablets from the absorbances obtained using the declared content of $C_{10}H_{22}N_4,H_2SO_4$ in *guanethidine monosulphate BPCRS*.

STORAGE

Guanethidine Tablets should be protected from light.

Haemodialysis Solutions

General Notices

(Solutions for Haemodialysis, Ph Eur monograph 0128)

Ph Eur

DEFINITION

Solutions for haemodialysis are solutions of electrolytes with a concentration close to the electrolytic composition of plasma. Glucose may be included in the formulation.

Because of the large volumes used, haemodialysis solutions are usually prepared by diluting a concentrated solution with water of suitable quality (see the monograph on *Haemodialysis solutions concentrated, water for diluting (1167)*), using for example an automatic dosing device.

CONCENTRATED SOLUTIONS FOR HAEMODIALYSIS

Concentrated haemodialysis solutions are prepared and stored using materials and methods designed to produce solutions having as low a degree of microbial contamination as possible. In certain circumstances, it may be necessary to use sterile solutions.

During dilution and use, precautions are taken to avoid microbial contamination. Diluted solutions are to be used immediately after preparation.

Concentrated solutions for haemodialysis are supplied in:

- — rigid, semi-rigid or flexible plastic containers,
- — glass containers.

Three types of concentrated solutions are used:

1. Concentrated solutions with acetate or lactate

Several formulations of concentrated solutions are used. The concentrations of the components in the solutions are such that after dilution to the stated volume the concentrations of the components per litre are usually in the following ranges:

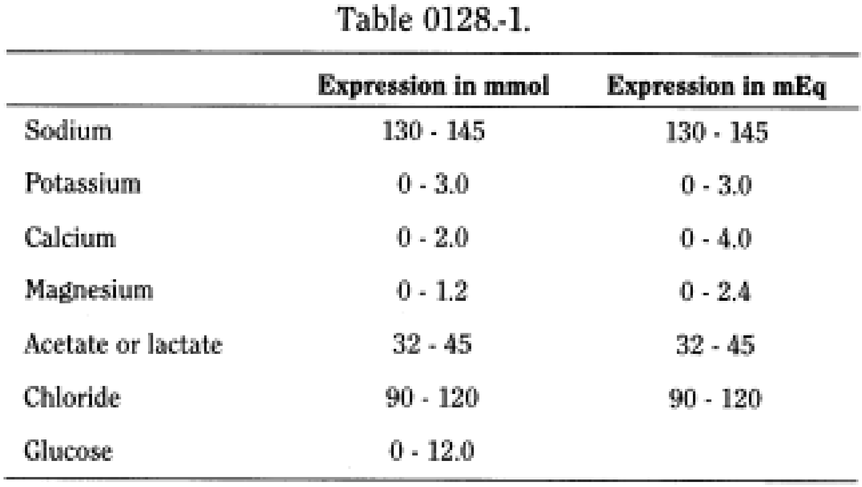


Table 0128.-1.

	Expression in mmol	Expression in mEq
Sodium	130 - 145	130 - 145
Potassium	0 - 3.0	0 - 3.0
Calcium	0 - 2.0	0 - 4.0
Magnesium	0 - 1.2	0 - 2.4
Acetate or lactate	32 - 45	32 - 45
Chloride	90 - 120	90 - 120
Glucose	0 - 12.0	

Concentrated solutions with acetate or lactate are diluted before use.

2. Concentrated acidic solutions

Several formulations of concentrated solutions are used. The concentrations of the components in the solutions are such that after dilution to the stated volume and before neutralisation with sodium hydrogen carbonate the concentrations of the components per litre are usually in the following ranges:

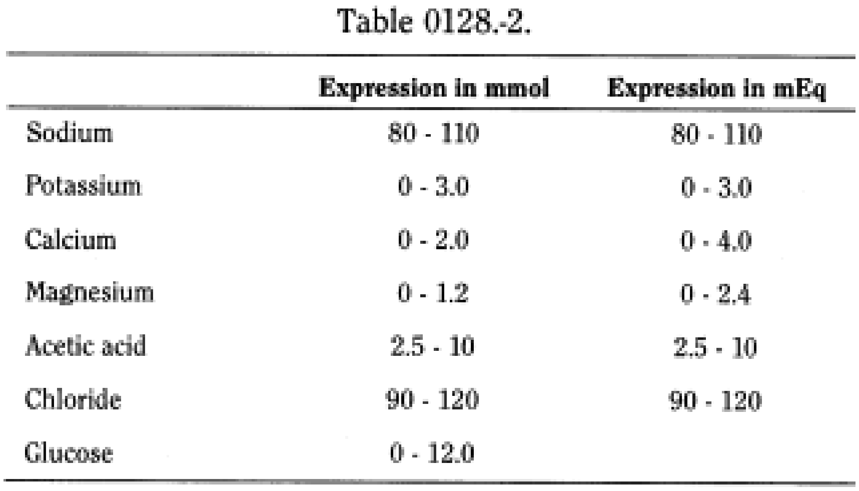


Table 0128.-2.

	Expression in mmol	Expression in mEq
Sodium	80 - 110	80 - 110
Potassium	0 - 3.0	0 - 3.0
Calcium	0 - 2.0	0 - 4.0
Magnesium	0 - 1.2	0 - 2.4
Acetic acid	2.5 - 10	2.5 - 10
Chloride	90 - 120	90 - 120
Glucose	0 - 12.0	

Sodium hydrogen carbonate must be added immediately before use to a final concentration of not more than 45 mmol/l. The concentrated solution of sodium hydrogen carbonate is supplied in a separate container. The concentrated acidic solutions and the concentrated solutions of sodium hydrogen carbonate are diluted and mixed immediately before use using a suitable device. Alternatively, solid sodium hydrogen carbonate may be used to prepare the solution.

3. Concentrated solutions without buffer

Several formulations of concentrated solutions without buffer are used. The concentrations of the components in the solutions are such that after dilution to the stated volume, the concentrations of the components per litre are usually in the following ranges:

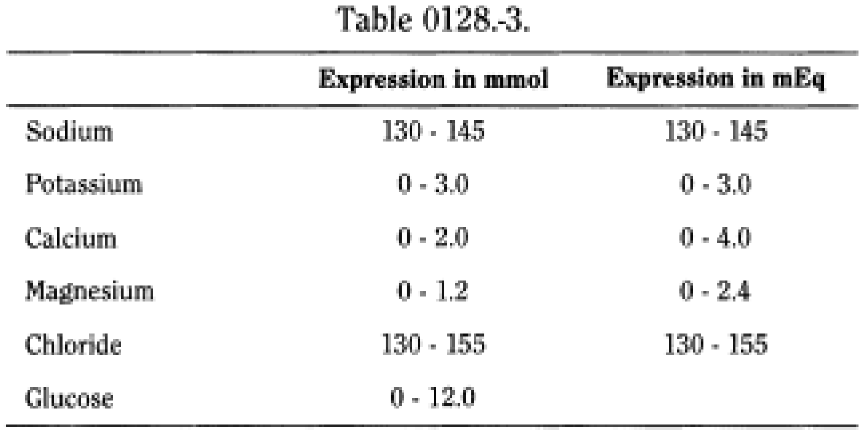


Table 0128.-3.

	Expression in mmol	Expression in mEq
Sodium	130 - 145	130 - 145
Potassium	0 - 3.0	0 - 3.0
Calcium	0 - 2.0	0 - 4.0
Magnesium	0 - 1.2	0 - 2.4
Chloride	130 - 155	130 - 155
Glucose	0 - 12.0	

Concentrated solutions without buffer are used together with parenteral administration of suitable hydrogen carbonate solutions.

IDENTIFICATION

According to the stated composition, the solution to be examined gives the following identification reactions (*2.3.1*):

— potassium: reaction (b);

— calcium: reaction (a);

— sodium: reaction (b);

— chlorides: reaction (a);

— lactates;

— carbonates and hydrogen carbonates;

— acetates:
 — if the solution is free from glucose, use reaction (b),
 — if the solution contains glucose, use the following method: to 5 ml of the solution to be examined add 1 ml of *hydrochloric acid R* in a test tube fitted with a stopper and a bent tube, heat and collect a few millilitres of distillate; carry out reaction (b) of acetates on the distillate;

— magnesium: to 0.1 ml of *titan yellow solution R* add 10 ml of water R, 2 ml of the solution to be examined and 1 ml of a 4.2 g/l solution of *sodium hydroxide R*; a pink colour is produced;

— glucose: to 5 ml of the solution to be examined, add 2 ml of *dilute sodium hydroxide solution R* and 0.05 ml of *copper sulphate solution R*; the solution is blue and clear; heat to boiling; an abundant red precipitate is formed.

TESTS

Appearance of solution

The solution to be examined is clear *(2.2.1)*. If it does not contain glucose, it is colourless *(2.2.2, Method I)*. If it contains glucose, it is not more intensely coloured than reference solution Y_7 *(2.2.2, Method I)*.

Aluminium *(2.4.17)*

Maximum 0.1 mg/l.

Prescribed solution Take 20 ml of the solution to be examined, adjust to pH 6.0 and add 10 ml of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 1 ml of aluminium *standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 9 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 10 ml of *water R*.

Extractable Volume *(2.9.17)*

The volume measured is not less than the nominal volume stated on the label.

Sterility *(2.6.1)*

If the label states that the concentrated haemodialysis solution is sterile, it complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 0.5 IU/ml in the solution diluted for use.

Pyrogens *(2.6.8)*

Solutions for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Dilute the solution to be examined with *water for injections R* to the concentration prescribed for use. Inject 10 ml of the solution per kilogram of the rabbit's mass.

ASSAY

Determine the density (2.2.5) of the concentrated solution and calculate the content in grams per litre and in millimoles per litre.

Sodium

97.5 per cent to 102.5 per cent of the content of sodium (Na) stated on the label.

Atomic emission spectrometry *(2.2.22, Method I)*.

Test solution Dilute 5.0 ml of the solution to be examined to 100.0 ml with *water R*. Dilute 2.0 ml of this solution to 50.0 ml with *water R*. To 1.0 ml of this solution add 10 ml of *lanthanum chloride solution R* and dilute to 100.0 ml with *water R*.

Reference solutions Into 4 identical volumetric flasks each containing 10 ml of *lanthanum chloride solution R*, introduce respectively 1.0 ml, 2.0 ml, 4.0 ml and 5.0 ml of *sodium standard solution (10 ppm Na) R* and dilute to 100.0 ml with *water R*.

Wavelength 589.0 nm.

Potassium

95.0 per cent to 105.0 per cent of the content of potassium (K) stated on the label.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dilute with *water R* an accurately weighed quantity of the solution to be examined to a concentration suitable for the instrument to be used. To 100 ml of this solution add 10 ml of a 22 g/l solution of *sodium chloride R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*. To 100 ml of each reference solution add 10 ml of a 22 g/l solution of *sodium chloride R*.

Source Potassium hollow-cathode lamp.

Wavelength 766.5 nm.

Atomisation device Air-acetylene flame.

Calcium

95.0 per cent to 105.0 per cent of the content of calcium (Ca) stated on the label.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dilute 5.0 ml of the solution to be examined to 100.0 ml with *water R*. To 3.0 ml of this solution add 5 ml of *lanthanum chloride solution R* and dilute to 50.0 ml with *water R*.

Reference solutions Into 4 keep volumetric flasks each containing 5 ml of *lanthanum chloride solution R*, introduce respectively 2.5 ml, 5.0 ml, 7.0 ml and 10.0 ml of *calcium standard solution (10 ppm Ca) R* and dilute to 50.0 ml with *water R*.

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Air-acetylene flame.

Magnesium

95.0 per cent to 105.0 per cent of the content of magnesium (Mg) stated on the label.

Atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Dilute 5.0 ml of the solution to be examined to 100.0 ml with *water R*. To 2.0 ml of this solution add 5 ml of *lanthanum chloride solution R* and dilute to 50.0 ml with *water R*.

Reference solutions Into 4 identical volumetric flasks each containing 5 ml of *lanthanum chloride solution R*, introduce respectively 1.0 ml, 2.0 ml, 3.0 ml and 4.0 ml of *magnesium standard solution (10 ppm Mg) R* and dilute to 50.0 ml with *water R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Air-acetylene flame.

Total chloride

95.0 per cent to 105.0 per cent of the content of chloride (Cl) stated on the label.

Dilute to 50 ml with *water R* an accurately measured volume of the solution to be examined containing the equivalent of about 60 mg of chloride. Add 5 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R*. Shake. Using 2 ml of *ferric ammonium sulphate solution R2* as indicator, titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained.

1 ml of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Acetate

95.0 per cent to 105.0 per cent of the content of acetate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of acetate, add 10.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of acetate.

Lactate

95.0 per cent to 105.0 per cent of the content of lactate stated on the label.

To a volume of the solution to be examined, corresponding to about 0.7 mmol of lactate, add 10.0 ml of *0.1 M hydrochloric acid*. Then add 50 ml of *acetonitrile R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of lactate.

Sodium hydrogen carbonate

95.0 per cent to 105.0 per cent of the content of sodium hydrogen carbonate stated on the label.

Titrate with *0.1 M hydrochloric acid* a volume of the solution to be examined corresponding to about 0.1 g of sodium hydrogen carbonate, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M hydrochloric acid* is equivalent to 8.40 mg of $NaHCO_3$.

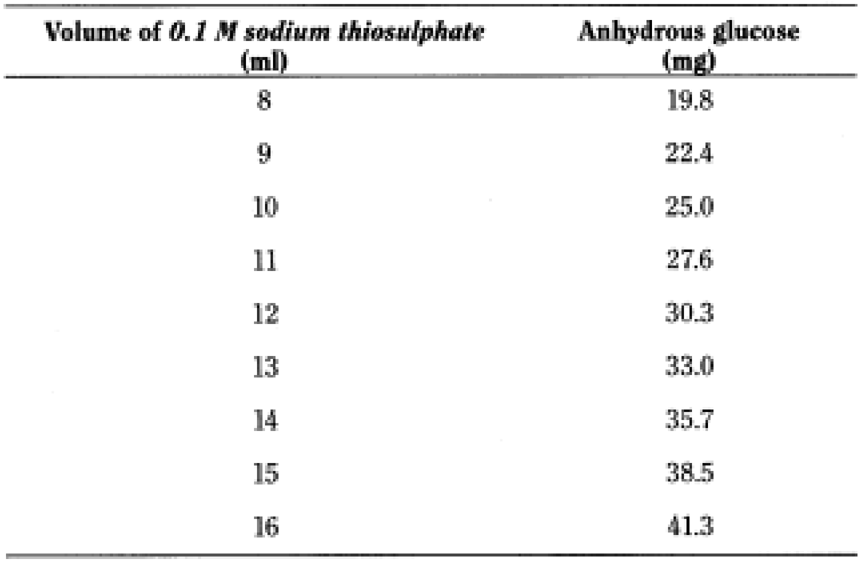
Reducing sugars (expressed as anhydrous glucose)

95.0 per cent to 105.0 per cent of the content of glucose stated on the label.

Transfer a volume of the solution to be examined containing the equivalent of 25 mg of glucose to a 250 ml conical flask with a ground-glass neck and add 25.0 ml of *cupri-citric solution R*. Add a few grains of pumice, fit a reflux condenser, heat so that boiling occurs within 2 min and maintain boiling for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 ml of *water R*. Carefully add, in small amounts, 25 ml of a 25 per cent *m/m* solution of *sulphuric acid R*. Titrate with *0.1 M sodium thiosulphate* using *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration using 25.0 ml of *water R*.

Calculate the content of reducing sugars, expressed as anhydrous glucose ($C_6H_{12}O_6$), using Table 0128.-4:

Table 0128.-4.



Volume of *0.1 M sodium thiosulphate* (ml)	Anhydrous glucose (mg)
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3

STORAGE

Store at a temperature not below 4 °C.

LABELLING

The label states:

— the formula of the concentrated solution for haemodialysis expressed in grams per litre and in millimoles per litre,

— the nominal volume of the solution in the container,

— where applicable, that the concentrated solution is sterile,

— the storage conditions,

— that the concentrated solution is to be diluted immediately before use,

— the dilution to be made,

— that the volume taken for use is to be measured accurately,

— the ionic formula for the diluted solution ready for use in millimoles per litre,

— that any unused portion of solution is to be discarded,

— where applicable, that sodium hydrogen carbonate is to be added before use.

Ph Eur

Haemofiltration and Haemodiafiltration Solutions

General Notices

(Solutions for Haemofiltration and for Haemodiafiltration, Ph Eur monograph 0861)

When the solution is intended for haemofiltration only, the title Haemofiltration Solution may be used.

Ph Eur

DEFINITION

Solutions for haemofiltration and for haemodiafiltration are preparations for parenteral use containing electrolytes with a concentration close to the electrolytic composition of plasma. Glucose may be included in the formulation.

Solutions for haemofiltration and for haemodiafiltration are supplied in:

— rigid or semi-rigid plastic containers,

— flexible plastic containers inside closed protective envelopes,

— glass containers.

The containers and closures comply with the requirements for containers for preparations for parenteral use (*3.2*. *Containers*).

In haemofiltration and haemodiafiltration, the following formulations are used. The concentrations of the components per litre of solution are usually in the following range:

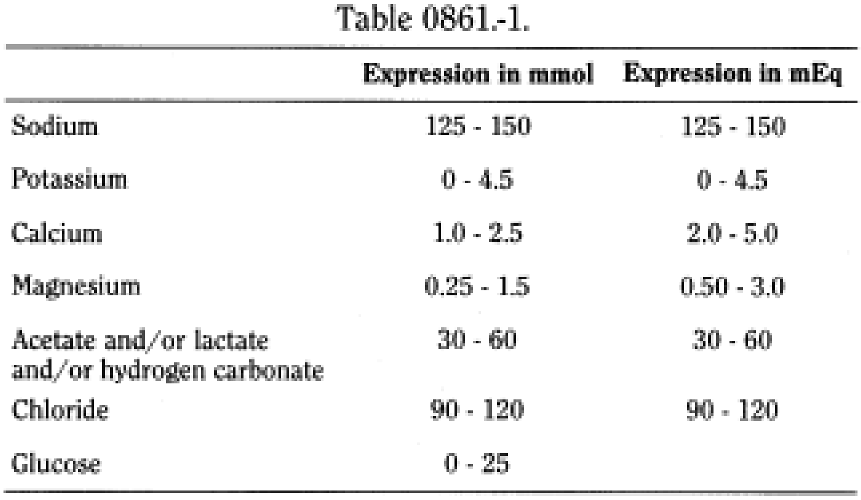


Table 0861.-1.

	Expression in mmol	Expression in mEq
Sodium	125 - 150	125 - 150
Potassium	0 - 4.5	0 - 4.5
Calcium	1.0 - 2.5	2.0 - 5.0
Magnesium	0.25 - 1.5	0.50 - 3.0
Acetate and/or lactate and/or hydrogen carbonate	30 - 60	30 - 60
Chloride	90 - 120	90 - 120
Glucose	0 - 25	

When hydrogen carbonate is present, the solution of sodium hydrogen carbonate is supplied in a container or a separate compartment and is added to the electrolyte solution immediately before use.

In haemofiltration and in haemodiafiltration, the following formulations may also be used:

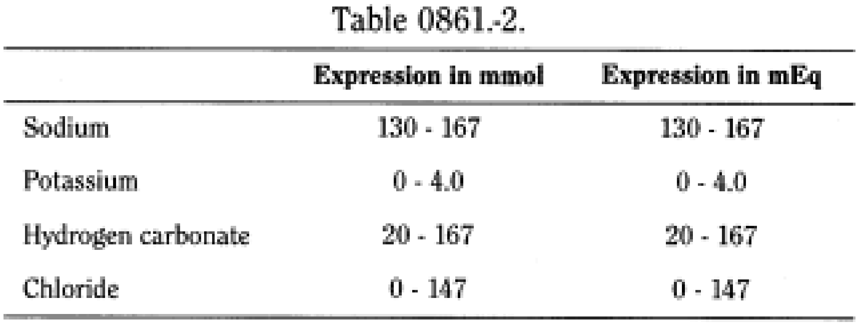


Table 0861.-2.

	Expression in mmol	Expression in mEq
Sodium	130 - 167	130 - 167
Potassium	0 - 4.0	0 - 4.0
Hydrogen carbonate	20 - 167	20 - 167
Chloride	0 - 147	0 - 147

Antioxidants such as metabisulphite salts are not added to the solutions.

IDENTIFICATION

According to the stated composition, the solution to be examined gives the following identification reactions (*2.3.1*):

— potassium: reaction (b);

— calcium: reaction (a);

— sodium: reaction (b);

— chlorides: reaction (a);

— acetates:
  — if the solution is free from glucose, use reaction (b),
  — if the solution contains glucose, use the following method: to 5 ml of the solution to be examined add 1 ml of *hydrochloric acid R* in a test-tube fitted with a stopper and a bent tube, heat and collect a few millilitres of distillate; carry out reaction (b) of acetates on the distillate;

— lactates;

— carbonates and hydrogen carbonates;

— magnesium: to 0.1 ml of titan yellow solution R add 10 ml of water R, 2 ml of the solution to be examined and 1 ml of 1 M sodium hydroxide; a pink colour is produced;

— glucose: to 5 ml of the solution to be examined, add 2 ml of dilute sodium hydroxide solution R and 0.05 ml of copper sulphate solution R; the solution is blue and clear; heat to boiling; an abundant red precipitate is formed.

TESTS

Appearance of solution

The solution is clear *(2.2.1)*. If it does not contain glucose, it is colourless (*2.2.2, Method I*). If it contains glucose, it is not more intensely coloured than reference solution Y_7 (*2.2.2, Method I*).

pH *(2.2.3)*

The pH of the solution is 5.0 to 7.5. If the solution contains glucose, the pH is 4.5 to 6.5. If the solution contains hydrogen carbonate, the pH is 7.0 to 8.5.

Hydroxymethylfurfural

To a volume of the solution containing the equivalent of 25 mg of glucose, add 5.0 ml of a 100 g/l solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R* and 1.0 ml of a 5 g/l solution of *barbituric acid R*. The absorbance *(2.2.25)*, determined at 550 nm after allowing the mixture to stand for 2 min to 3 min, is not greater than that of a standard prepared at the same time in the same manner using a solution containing 10 µg of *hydroxymethylfurfural R* in the same volume as the solution to be examined. If the solution contains hydrogen carbonate, use as the standard a solution containing 20 µg of *hydroxymethylfurfural R*.

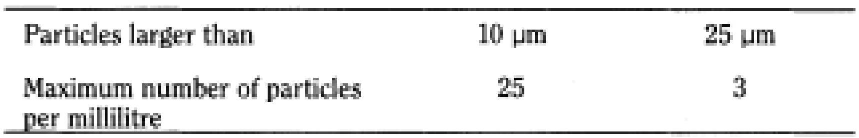
Aluminium *(2.4.17)*

Take 200 ml, adjust to pH 6.0 and add 10 ml of *acetate buffer solution pH 6.0 R*. The solution complies with the limit test for aluminium (10 µg/l). Use as the reference solution a mixture of 1 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate buffer solution pH 6.0 R* and 9 ml of *water R*. To prepare the blank use a mixture of 10 ml of *acetate buffer solution pH 6.0 R* and 10 ml of *water R*.

Particulate contamination

Carry out the test for sub-visible particles (*2.9.19*) using 50 ml of solution.

Table 0861.-3.



Particles larger than	10 µm	25 µm
Maximum number of particles per millilitre	25	3

Extractable Volume *(2.9.17)*

The solution complies with the test prescribed for parenteral infusions.

Sterility *(2.6.1)*

The solution complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/ml.

Pyrogens *(2.6.8)*

Solutions for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 ml of the solution.

ASSAY

Sodium

97.5 per cent to 102.5 per cent of the content of sodium (Na) stated on the label, determined by atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*.

Measure the absorbance at 589.0 nm using a sodium hollow-cathode lamp as source of radiation and an air-propane or an air-acetylene flame.

Potassium

95.0 per cent to 105.0 per cent of the content of potassium (K) stated on the label, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used. To 100 ml of the solution add 10 ml of a 22 g/l solution of *sodium chloride R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*. To 100 ml of each reference solution add 10 ml of a 22 g/l solution of *sodium chloride R*.

Measure the absorbance at 766.5 nm using a potassium hollow-cathode lamp as source of radiation and an air-propane or an air-acetylene flame.

Calcium

95.0 per cent to 105.0 per cent of the content of calcium (Ca) stated on the label, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *calcium standard solution (400 ppm Ca) R*.

Measure the absorbance at 422.7 nm using a calcium hollow-cathode lamp as source of radiation and an air-propane or an air-acetylene flame.

Magnesium

95.0 per cent to 105.0 per cent of the content of magnesium (Mg) stated on the label, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *magnesium standard solution (100 ppm Mg) R*.

Measure the absorbance at 285.2 nm using a magnesium hollow-cathode lamp as source of radiation and an air-propane or an air-acetylene flame.

Total chloride

95.0 per cent to 105.0 per cent of the content of chloride (Cl) stated on the label. Dilute to 50 ml with *water R* an accurately measured volume of the solution to be examined containing the equivalent of about 60 mg of chloride. Add 5 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R*. Shake. Using 2 ml of *ferric ammonium sulphate solution R2* as indicator, titrate with *0.1 M ammonium thiocyanate* until a reddish--yellow colour is obtained.

1 ml of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Acetate

95.0 per cent to 105.0 per cent of the content of acetate stated on the label. To a volume of the solution to be examined, corresponding to about 0.7 mmol of acetate, add 10.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of acetate.

Lactate

95.0 per cent to 105.0 per cent of the content of lactate stated on the label. To a volume of the solution to be examined, corresponding to about 0.7 mmol of lactate, add 10.0 ml of *0.1 M hydrochloric acid*. Add 50 ml of *acetonitrile R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of lactate.

Sodium hydrogen carbonate

95.0 per cent to 105.0 per cent of the content of sodium hydrogen carbonate stated on the label. Titrate with *0.1 M hydrochloric acid*, a volume of the solution to be examined corresponding to about 0.1 g of sodium hydrogen carbonate, determining the end-point potentiometrically *(2.2.20)*.

1 ml of *0.1 M hydrochloric acid* is equivalent to 8.40 mg of $NaHCO_3$.

Lactate and hydrogen carbonate

95.0 per cent to 105.0 per cent of the content of lactates and/or hydrogen carbonates stated on the label. Examine by liquid chromatography *(2.2.29)*.

Test solution Solution to be examined.

Reference solution Dissolve in 100 ml of *water for chromatography R* quantities of lactates and bicarbonates, accurately weighed, in order to obtain solutions having concentrations representing about 90 per cent, 100 per cent and 110 per cent of the concentrations indicated on the label.

The chromatographic procedure may be carried out using:

— a column 0.30 m long and 7.8 mm in internal diameter packed with cation exchange resin R (9 µm),

— as mobile phase at a flow rate of 0.6 ml/min 0.005 M sulphuric acid previously degassed with helium R,

— a differential refractometer detector,

maintaining the temperature of the column at 85 °C.

Inject in duplicate 20 µl of test solution and 20 µl of each reference solution. When the chromatograms are recorded in the prescribed conditions, the peaks elute in the following order: lactates then hydrogen carbonates.

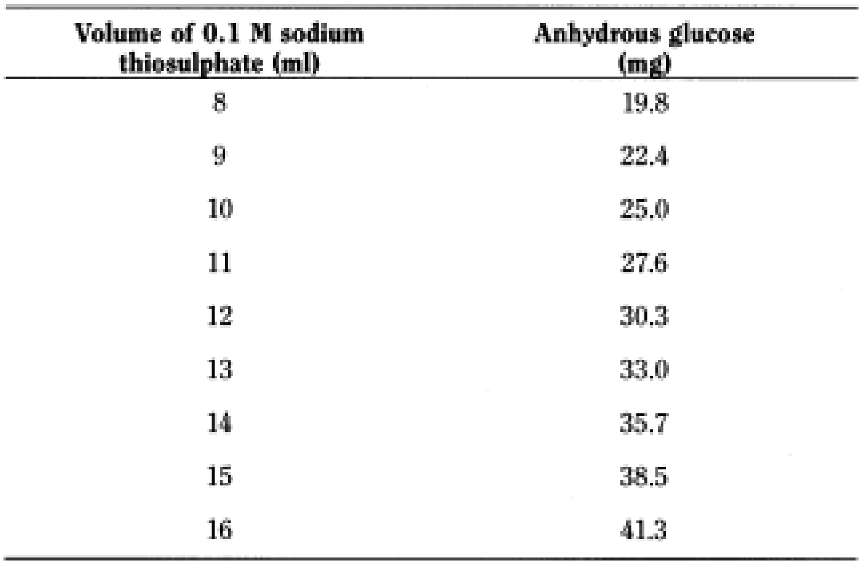
Determine the concentration of lactates and hydrogen carbonates in the test solution by interpolating the peak area for lactate and the peak height for hydrogen carbonate from the linear regression curve obtained with the reference solutions.

Reducing sugars (expressed as anhydrous glucose)

95.0 per cent to 105.0 per cent of the content of glucose stated on the label. Transfer a volume of the solution to be examined containing the equivalent of 25 mg of glucose to a 250 ml conical flask with a ground-glass neck and add 25.0 ml of *cupri-citric solution R*. Add a few grains of pumice, fit a reflux condenser, heat so that boiling occurs within 2 min and boil for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 ml of *water R*. Carefully add, in small amounts, 25 ml of a 25 per cent *m/m* solution of *sulphuric acid R*. Titrate with *0.1 M sodium thiosulphate* using *starch solution R*, added towards the end of titration, as indicator. Carry out a blank titration using 25.0 ml of *water R*.

Calculate the content of reducing sugars expressed as anhydrous glucose ($C_6H_{12}O_6$), using Table 0861.-4:

Table 0861.-4.



Volume of 0.1 M sodium thiosulphate (ml)	Anhydrous glucose (mg)
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3

STORAGE

Store at a temperature not lower than 4 °C.

LABELLING

The label states:

— the formula of the solution for haemofiltration or haemodiafiltration, expressed in grams per litre and in millimoles per litre,

— the calculated osmolarity, expressed in milliosmoles per litre,

— the nominal volume of the solution for haemofiltration or haemodiafiltration in the container,

— that the solution is free from bacterial endotoxins, or where applicable, that it is apyrogenic,

— the storage conditions,

— that any unused portion of solution is to be discarded.

Halibut-liver Oil Capsules

General Notices

DEFINITION

Halibut-liver Oil Capsules are soft capsules containing Halibut-liver Oil diluted, if necessary, with a suitable fixed oil to a volume of 0.12 to 0.18 ml in each capsule.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of vitamin A

3500 to 4700 IU.

ASSAY

Weigh 10 capsules. Open the capsules carefully without loss of shell material, express as much of the contents as possible and reserve the expressed material. Wash the shells with *ether*, discard the washings, allow the shells to stand at room temperature to constant weight. The difference between the weights represents the weight of the total contents. Determine the content of vitamin A in the reserved portion of the contents by the method described below and calculate the content in a capsule of average content weight.

Carry out the procedures as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper and iron) and acids.

Carry out Method A (ultraviolet absorption spectrophotometry) but if method A is found not to be valid, carry out method B (liquid chromatography).

Method A

To 1 g of the oil being examined in a round-bottomed flask, add 3 ml of a freshly prepared 50% w/w solution of *potassium hydroxide* and 30 ml of *ethanol*, boil under a reflux condenser in a current of *nitrogen* for 30 minutes and cool rapidly. Add 30 ml of *water* and extract with four 50 ml quantities of *ether* and discard the aqueous layer after complete separation of the final extract. Wash the combined ethereal layers with four 50 ml quantities of *water* and evaporate to dryness under a gentle current of *nitrogen* at a temperature not exceeding 30° or in a rotary evaporator at a temperature not exceeding 30° under reduced pressure. Dissolve the residue in sufficient *propan-2-ol R1* to give an expected concentration of vitamin A equivalent to 10 to 15 IU per ml. Measure the *absorbances* of the solution at 300 nm, 310 nm, 325 nm and 334 nm and at the wavelength of maximum absorption, Appendix II B, in a suitable spectrophotometer in 1-cm specially matched cells, using *propan-2-ol R1* in the reference cell.

Calculate the content of vitamin A, as *all-trans*-retinol, in IU per gram from the expression:

$$A_{325} \times \frac{1830}{100m} \times V$$

where A_{325} = absorbance at 325 nm,

m = weight of the oil being examined in grams,

V = total volume of solution containing 10 to 15 IU of vitamin A per ml,

1830 = conversion factor for the specific absorbance of *all-trans*-retinol in IU.

The above expression can be used only if A_{325} has a value of not greater than $A_{325.corr}/0.970$ where $A_{325,corr}$ is the corrected absorbance at 325 nm and is given by the equation:

$$A_{325,\ corr} = 6.815A_{325} - 2.555A_{310} - 4.260A_{324}$$

A designates the absorbance at the wavelength indicated by the subscript.

If A_{325} has a value greater than $A_{325,corr}/0.970$, calculate the content of vitamin A from the expression:

$$A_{325,corr} \times \frac{1830}{100m} \times V$$

The assay is not valid unless the wavelength of maximum absorption lies between 323 nm and 327 nm and the *absorbance* at 300 nm relative to that at 325 nm is at most 0.73.

Method B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare solution (1) in the following manner. To 2 g of the oil being examined in a round-bottomed flask add 5 ml of a freshly prepared 10% w/v solution of *ascorbic acid* and 10 ml of a freshly prepared 80% w/v solution of *potassium hydroxide* and 100 ml of *ethanol*, boil under a reflux condenser on a water bath for 15 minutes. Add 100 ml of a 1% w/v solution of *sodium chloride* and cool. Transfer the solution to a 500 ml separating funnel rinsing the round-bottomed flask with about 75 ml of a 1% w/v solution of *sodium chloride* and then with 150 ml of a mixture of equal volumes of *light petroleum R3* and *ether R*. Shake for 1 minute and when the layers have separated completely, discard the lower layer and wash the upper layer with 50 ml of a 3% w/v solution of *potassium hydroxide* in a 10% v/v solution of *ethanol* and then with three 50-ml quantities of a 1% w/v solution of *sodium chloride*. Filter the upper layer through 5 g of *anhydrous sodium sulphate* on a fast filter paper into a 250 ml flask suitable for a rotary evaporator. Wash the funnel with 10 ml of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30° under reduced pressure (water ejector) and fill with *nitrogen* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen* at a temperature not exceeding 30°. Dissolve the residue in *propan-2-ol*, transfer to a 25-ml graduated flask and dilute to 25 ml with *propan-2-ol*. Gentle heating with the aid of ultrasound may be required. (A large fraction of the white residue is cholesterol.) Solution (2) is a solution of *retinyl acetate EPCRS* in 2-*propan-2-ol R1* containing about 1000 IU per ml of *all-trans*-retinol. The exact concentration of solution (2) is assessed by *ultraviolet absorption spectrophotometry*, Appendix II B. Dilute the solution with *propan-2-ol R1* to a presumed concentration of 10 to 15 IU per ml and measure the *absorbance* at 326 nm in matched 1-cm cells using *propan-2-ol R1* in the reference cell. Calculate the content of vitamin A in IU per ml of solution (2) from the following expression, taking into account the assigned content of *retinyl acetate EPCRS*:

$$A_{326} \times \frac{1900 \times V_2}{100 \times V_1}$$

where A_{326} = absorbance at 326 nm,

V_2 = volume of the diluted solution,

V_1 = volume of reference solution (a) used,

1900 = conversion factor for the specific absorbance of *retinyl acetate EPCRS* in IU.

Prepare solution (3) in the same manner as solution (1) but using 2 ml of solution (2) in place of the oil being examined. The exact concentration of solution (3) is assessed by *ultraviolet absorption spectrophotometry*, Appendix II B. Dilute solution (3) with *propan-2-ol R1* to a presumed concentration of 10 to 15 IU per ml of *all-trans*-retinol and measure the *absorbance* at 325 nm in matched 1-cm cells using *propan-2-ol R1* in the reference cell. Calculate the content of *all-trans*-retinol in IU per ml of solution (3) from the expression:

$$A_{325} \times \frac{1830 \times V_4}{100 \times V_3}$$

where A_{325} = absorbance at 325 nm,

V_3 = volume of the diluted solution,

V_4 = volume of reference solution (b) used,

1830 = conversion factor for the specific absorbance of *all-trans*-retinol in IU.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm), (b) as mobile phase at a flow rate of 1 ml per minute a mixture of 3 volumes of *water* and 97 volumes of *methanol* and (c) as detector a spectrophotometer set at 325 nm.

Inject in triplicate 10 µl of solutions (1) and (3). The retention time of *all-trans*-retinol is 4 to 6 minutes.

The assay is not valid unless (a) the chromatogram obtained with solution (1) shows a peak corresponding to that of *all-trans*-retinol in the chromatogram obtained with solution (3), (b) when using the method of standard additions to solution (1) there is greater than 95% recovery of the added *retinyl acetate EPCRS* and (c) the recovery of *all-trans*-retinol in solution (3) as assessed by direct absorption spectrophotometry is greater than 95%.

Calculate the content of vitamin A using the following expression:

$$A_1 \times \frac{C \times V}{A_2} \times \frac{1}{m}$$

where A_1 = area of the peak corresponding to *all-trans*-retinol in the chromatogram obtained with solution (1),

A_2 = area of the peak corresponding to *all-trans*-retinol in the chromatogram obtained with solution (3),

C = concentration of *retinyl acetate EPCRS* in solution (2) as assessed before the saponification in International Units per millilitre (1000 IU per ml),

V = volume of solution (2) treated (2 ml),

m = weight of the oil being examined in solution (1) (2 g).

STORAGE

Halibut-liver Oil Capsules should be protected from light and stored at a temperature not exceeding 20°. When stored under these conditions, they may be expected to retain their potency for at least 3 years after the date of preparation.

LABELLING

The label states that each capsule contains 4000 IU (Units) of vitamin A activity.

Haloperidol Capsules

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Haloperidol Capsules contain Haloperidol.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the contents of the capsules containing 10 mg of Haloperidol add 10 ml of *water* and 1 ml of 1M *sodium hydroxide* and extract with 10 ml of *ether*. Filter the ether extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using as the coating substance a suitable silanised silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (Merck silanised silica gel 60 F_{254} plates are suitable) and a mixture of 0.5 volume of 13.5M *ammonia*, 20 volumes of *ammonium acetate solution*, 20 volumes of *water* and 60 volumes of *1,4-dioxan* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 10 mg of Haloperidol with 30 ml of *chloroform*, disperse with the aid of ultrasound for 15 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *chloroform*. After removal of the plate, dry it in a current of warm air for 15 minutes and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (2) shows a distinct and clearly visible spot.

Uniformity of content

Capsules containing less than 2 mg of Haloperidol comply with the requirements stated under

Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add sufficient of the mobile phase to the contents of one capsule to produce a solution containing 0.005% w/v of Haloperidol, disperse with the aid of ultrasound for 2 minutes, centrifuge and use the supernatant solution. Solution (2) contains 0.005% w/v of *haloperidol BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 45 volumes of *acetonitrile* and 55 volumes of a 1% w/v solution of *ammonium acetate* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 247 nm.

Calculate the content of $C_{21}H_{23}ClFNO_2$ in each capsule using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS*.

ASSAY

Use the average of the 10 individual results obtained in the test for Uniformity of content.

Haloperidol Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Haloperidol Injection is a sterile solution of Haloperidol in Lactic Acid diluted with Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume containing 20 mg of Haloperidol add 5 ml of *water* and 1 ml of 1M *sodium hydroxide* and extract with 10 ml of *chloroform*. Filter the chloroform extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 245 nm.

TESTS

Acidity

pH, 2.8 to 3.6, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 92 volumes of *chloroform*, 8 volumes of *methanol* and 1 volume of 13.5M *ammonia* as the mobile phase. Apply separately to the plate (1) a volume of the injection containing 0.1 mg of Haloperidol, (2) the same volume of a solution prepared by diluting 1 volume of the injection to 100 volumes with *methanol* and (3) the same volume of a solution prepared by diluting 1 volume of the injection to 200 volumes with *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

To a quantity containing 10 mg of Haloperidol add 8 ml of *water* and 10 ml of 1M *hydrochloric acid*. Extract with successive quantities of 25, 25, 10 and 10 ml of *ether*. Wash the combined ether extracts with 10 ml of *water*, combine the aqueous layers and remove the ether using a rotary evaporator. Add sufficient *water* to produce 100 ml and dilute 10 ml to 100 ml with *methanol*. Measure the *absorbance* of the resulting solution at the maximum at 245 nm, Appendix II B. Calculate the content of $C_{21}H_{23}ClFNO_2$ taking 346 as the value of A(1%, 1 cm) at the maximum at 245 nm.

STORAGE

Haloperidol Injection should be protected from light.

Haloperidol Oral Solution

General Notices

Haloperidol Oral Drops

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Haloperidol Oral Solution is an aqueous solution containing not more than 0.2% w/v of Haloperidol.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. To a volume containing 20 mg of Haloperidol add 1 ml of 1M *sodium hydroxide*, extract with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 245 nm.

TESTS

Acidity

pH, 2.5 to 3.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 92 volumes of *dichloromethane*, 8 volumes of *methanol* and 1 volume of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) dilute the oral solution if necessary with *methanol* to contain 0.1% w/v of Haloperidol. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *methanol*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

To a volume containing 10 mg of Haloperidol add 8 ml of *water* and 10 ml of 1M *hydrochloric acid* and extract with two 10ml quantities of *ether*. Wash the combined ether extracts with 10 ml of *water*, combine the aqueous solutions and remove the ether using a rotary evaporator. Add sufficient *water* to produce 100 ml and dilute 10 ml to 100 ml with *methanol*. Measure the *absorbance* of the resulting solution at the maximum at 245 nm, Appendix II B. Calculate the content of $C_{21}H_{23}ClFNO_2$ taking 346 as the value of A(1%, 1 cm) at the maximum at 245 nm.

STORAGE

Haloperidol Oral Solution should be protected from light. It should not be refrigerated.

When Haloperidol Oral Solution or Haloperidol Oral Drops is prescribed or demanded and no strength is stated, an Oral Solution containing 0.2% w/v of Haloperidol shall be dispensed or supplied.

Haloperidol Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Haloperidol Tablets contain Haloperidol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 10 mg of Haloperidol add 10 ml of *water* and 1 ml of 1M *sodium hydroxide* and extract with 10 ml of *ether*. Filter the ether extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

TESTS

Related substances

Carry out the method described under Haloperidol Capsules applying to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Haloperidol with 10 ml of *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *chloroform*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (2) shows a distinct and clearly visible spot.

Uniformity of content

Tablets containing less than 2 mg of Haloperidol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *haloperidol BPCRS* in the mobile phase. For solution (2) place one tablet in 10 ml of the mobile phase, disperse with the aid of ultrasound for 2 minutes and centrifuge. If necessary, dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005% w/v of Haloperidol.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil

ODS is suitable), (b) a mixture of 55 volumes of a 1% w/v solution of *ammonium acetate* and 45 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 247 nm.

Calculate the content of $C_{21}H_{23}ClFNO_2$ using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS*.

ASSAY

For tablets containing 2 mg or more of haloperidol

Weigh and powder 20 tablets and carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.020% w/v of *haloperidol BPCRS* in the mobile phase. For solution (2) add 60 ml of the mobile phase to a quantity of the powdered tablets containing 20 mg of Haloperidol, disperse with the aid of ultrasound for 2 minutes, add sufficient mobile phase to produce 100 ml, mix and filter.

The chromatographic procedure described under Uniformity of content may be used.

Calculate the content of $C_{21}H_{23}ClFNO_2$ using the declared content of $C_{21}H_{23}ClFNO_2$ in *haloperidol BPCRS*.

For tablets containing less than 2 mg of haloperidol

Use the average of the 10 individual results obtained in the test for Uniformity of content.

Heparin Injection

General Notices

Action and use

Anticoagulant.

DEFINITION

Heparin Injection is a sterile solution of Heparin Calcium or Heparin Sodium in Water for Injections. The pH of the solution may be adjusted by the addition of a suitable alkali.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

CHARACTERISTICS

A colourless or straw-coloured liquid, free from turbidity and from matter that deposits on standing.

IDENTIFICATION

A. Under the conditions of the assay, the clotting time is not less than 1.5 times the blank recalcification time.

B. Carry out the method for *zone electrophoresis*, Appendix III F, using *agarose for electrophoresis* as the supporting medium. To equilibrate the agarose and as electrolyte solution use a mixture of 50 ml of *glacial acetic acid* and 800 ml of *water* adjusted to pH 3 by the addition of *lithium hydroxide* and diluted to 1000 ml with *water*. Apply separately to the strip 2 µl to 3 µl of each of the following solutions. For solution (1) dilute a volume of the injection with *water* to give a solution containing 375 IU per ml. For solution (2), dilute *heparin sodium EPBRF* with an equal volume of *water*. Pass a current of 1 mA to 2 mA per centimetre of strip width at a potential difference of 300 volts for about 10 minutes. Stain the strips using a 0.1% w/v solution of *toluidine blue* and remove the excess by washing. The ratio of the distance migrated by the principal band or bands in the gel obtained with solution (1) to the distance migrated by the principal band in the gel obtained with solution (2) is 0.9 to 1.1.

C. When the injection contains Heparin Calcium, it yields reactions A and B characteristic of *calcium salts*, Appendix VI. When the injection contains Heparin Sodium, it yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 5.5 to 8.0, Appendix V L.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C.

For a preparation supplied in a container with a nominal content of less than 100 ml, dilute the injection if necessary with *water BET* to give a solution containing 1000 IU of heparin activity per ml (solution A). The endotoxin limit concentration of solution A is 10 IU of endotoxin per ml. Carry out the test using a lysate with a declared sensitivity not less sensitive than 0.0625 IU of endotoxin per ml and using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

For a preparation supplied in a container with a nominal content of 100 ml or more, the endotoxin limit concentration is 0.25 IU of endotoxin per ml.

For Heparin Injection prepared from Heparin Calcium it may be necessary to add divalent cations in order to achieve the validation criteria.

ASSAY

Carry out the *assay of heparin*, Appendix XIV J. The estimated potency is not less than 90% and not more than 111% of the stated potency. The fiducial limits of error are not less than 80% and not more than 125% of the stated potency.

STORAGE

Heparin Injection should preferably be kept in a container sealed by fusion of the glass.

LABELLING

The strength is stated as the number of IU (Units) in a suitable dose-volume except that for multi-dose containers the strength is stated as the number of IU (Units) per ml.

The label states (1) whether the material is the calcium or sodium salt; (2) when no antimicrobial preservative is present that the preparation contains no antimicrobial preservative and that any portion of the contents not used at once should be discarded.

Hexachlorophene Dusting Powder

General Notices

Zinc and Hexachlorophene Dusting Powder

Action and use

Antiseptic.

DEFINITION

Hexachlorophene Dusting Powder is a *cutaneous powder*. It is a mixture of Hexachlorophene and Zinc Oxide with a suitable inert diluent.

The dusting powder complies with the requirements stated under Topical Powders and with the following requirements.

Content of hexachlorophene, $C_{13}H_6Cl_6O_2$

90.0 to 110.0% of the stated amount.

Content of zinc oxide, ZnO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powder containing 30 mg of Hexachlorophene with 25 ml of *ether* for 15 minutes, filter, wash the filtrate with 10 ml of *water*, dry the ether layer over *anhydrous sodium sulphate*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hexachlorophene *(RS 174)*.

B. Shake a quantity of the powder containing 30 mg of Zinc Oxide with 10 ml of 2M *hydrochloric acid* for 15 minutes and filter. 5 ml of the filtrate yields the reaction characteristic *zinc salts*, Appendix VI.

ASSAY

For hexachlorophene

Shake a quantity of the powder containing 15 mg of Hexachlorophene with 50 ml of *methanolic tris(hydroxymethyl)methylamine solution* for 10 minutes, filter through a sintered-glass crucible (ISO 4793, porosity grade 3, is suitable), wash the filter with two 10 ml quantities of the tris(hydroxymethyl)methylamine solution and dilute the combined filtrate and washings to 100 ml with the same solution. Dilute 10 ml of the resulting solution to 50 ml with *methanolic tris(hydroxymethyl)methylamine solution* and measure the *absorbance* at the maximum at 312 nm, Appendix II B, using in the reference cell a solution prepared by diluting a further 10 ml of the solution to 50 ml with *acidified methanol*. An absorbance of 0.432 is equivalent to 15 mg of $C_{13}H_6Cl_6O_2$ in the weight of powder taken.

For zinc oxide

To a quantity of the powder containing 90 mg of Zinc Oxide add 10 ml of 2M *nitric acid* and 20 ml of *water*, boil for 2 minutes, cool, filter and wash the filter with two 25 ml quantities of *water*. To the combined filtrate and washings add 3 g of *hexamine* and titrate with 0.05M *disodium edetate VS* using 2 ml of *xylenol orange solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 4.068 mg of ZnO.

LABELLING

The label states that the preparation should not be applied to infants or to large areas of skin except in accordance with medical advice.

The label indicates the pharmaceutical form as 'cutaneous powder'.

Homatropine Eye Drops

General Notices

Action and use

Anticholinergic.

DEFINITION

Homatropine Eye Drops are a sterile solution of Homatropine Hydrobromide in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of homatropine hydrobromide, $C_{16}H_{21}NO_3,HBr$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume containing 60 mg of Homatropine Hydrobromide add 3 ml of 5M *ammonia*, extract with 15 ml of *chloroform*, dry the chloroform over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of homatropine *(RS 175)*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak derived from homatropine hydrobromide in the chromatogram obtained with solution (1).

C. To 1 ml of the eye drops, diluted with *water* if necessary to give a solution containing 1% w/v of Homatropine Hydrobromide, add 1 ml of 5M *ammonia*, shake with *chloroform* and evaporate the chloroform solution to dryness on a water bath. To the residue add 1.5 ml of a 2% w/v solution of *mercury(II) chloride* in *ethanol (60%)*. A yellow colour is produced which becomes red on gentle warming (distinction from most other alkaloids except atropine and hyoscyamine).

TESTS

Tropine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 33 volumes of *anhydrous formic acid*, 33 volumes of *water* and 134 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) use the eye drops diluted, if necessary, with *water* to contain 1% w/v of Homatropine Hydrobromide. Solution (2) contains 0.0050% w/v of *tropine*. After removal of the plate, dry it at 100° to 105° until the solvent has evaporated, allow to cool and spray with *dilute potassium iodobismuthate solution* until spots appear. Any spot corresponding to tropine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 1 ml of a 2% w/v solution of *atropine sulphate BPCRS* (internal standard) in *methanol* (solution A) and 1 ml of 5M *ammonia* to 5 ml of a 0.4% w/v solution of *homatropine hydrobromide BPCRS*. Extract with two 5 ml quantities of *chloroform*, shake the combined extracts with 1 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 10 ml of *dichloromethane*. To 1 ml of this solution add 0.2 ml of a mixture of 4 volumes of N,O-*bis(trimethylsilyl)acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes. Prepare solution (2) in the same manner as solution (3) but omitting the addition of solution A. For solution (3) add 1 ml of solution A and 1 ml of 5M *ammonia* to a volume of the eye drops containing 20 mg of Homatropine Hydrobromide, diluted if necessary to 5 ml with *water*, and complete the procedure described under solution (1), beginning at the words 'Extract with two 5-ml quantities of *chloroform*…'.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 220°.

Calculate the content of $C_{16}H_{21}NO_3,HBr$ using the declared content of $C_{16}H_{21}NO_3,HBr$ in *homatropine hydrobromide BPCRS*.

Hyaluronidase Injection

General Notices

Action and use

Used to promote absorption of fluid into tissues.

DEFINITION

Hyaluronidase Injection is a sterile solution of Hyaluronidase in Water for Injections. It is prepared by dissolving Hyaluronidase for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Hyaluronidase Injection should be used immediately after preparation.

HYALURONIDASE FOR INJECTION

DEFINITION

Hyaluronidase for Injection is a sterile material consisting of Hyaluronidase with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A solution containing 100 IU in 1 ml of a 0.9% w/v solution of *sodium chloride* depolymerises an equal volume of a 1% w/v solution of *sodium hyaluronate EPBRP* at 20° in 1 minute, as shown by a pronounced decrease in viscosity. This action is destroyed by heating the initial solution at 100° for 30 minutes.

TESTS

Acidity or alkalinity

pH of a 0.3% w/v solution in *carbon dioxide-free water*, 4.5 to 7.5, Appendix V L.

Clarity and colour of solution

A 1.0% w/v solution in *carbon dioxide-free water* is *clear*, Appendix IV A, and not more than faintly yellow.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 150 IU of Hyaluronidase per ml (solution A). The endotoxin limit concentration of solution A is 30 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the Assay described under Hyaluronidase. The estimated potency is not less than 90% and not more than 115% of the stated potency.

LABELLING

The label of the sealed container states (1) the total number of IU (Units) contained in it; (2) that the preparation is not intended for intravenous injection.

Hydralazine Injection

General Notices

Action and use

Vasodilator; treatment of hypertension.

DEFINITION

Hydralazine Injection is a sterile solution of Hydralazine Hydrochloride in Water for Injections. It is prepared by dissolving Hydralazine Hydrochloride for Injection in the requisite amount of Water for Injections immediately before use. For intravenous infusion, the Hydralazine Hydrochloride for Injection should be dissolved in, and then diluted with, an appropriate volume of a suitable diluent.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Hydralazine Injection deteriorates on storage and should be used immediately after preparation.

HYDRALAZINE HYDROCHLORIDE FOR INJECTION

DEFINITION

Hydralazine Hydrochloride for Injection is a sterile material consisting of Hydralazine Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of hydralazine hydrochloride, $C_8H_8N_4,HCl$

98.0 to 114.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydralazine hydrochloride *(RS 177)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution exhibits four maxima, at 240, 260, 305 and 315 nm.

C. Yield the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 2% w/v solution, 3.5 to 4.2, Appendix V L.

Clarity of solution

A 2.0% w/v solution is not more opalescent than *reference suspension II*, Appendix IV A.

Colour of solution

A 2.0% w/v solution in 0.01M *hydrochloric acid* is not more intensely coloured than *reference solution GY_6*, Appendix IV B, Method II.

Hydrazine

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase the upper layer of the mixture obtained by shaking together 80 volumes of *hexane*, 20 volumes of 13.5M *ammonia* and 20 volumes of *ethyl acetate* and allowing to stand. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) dissolve the contents of a sealed container in sufficient 0.1M *methanolic hydrochloric acid* to produce a solution containing 0.5% w/v of Hydralazine Hydrochloride. To 2 ml add 1 ml of a 2% w/v solution of *salicylaldehyde* in *methanol* and 0.1 ml of *hydrochloric acid*, centrifuge and decant the supernatant liquid. Prepare solution (2) in the same manner, but use 2 ml of a 0.00025% w/v solution of *hydrazine sulphate* in 0.1M *methanolic hydrochloric acid* in place of the solution of the material being examined. After removal of the plate, allow it to dry in air and spray with *dimethylaminobenzaldehyde solution R1*. Any spot corresponding to hydrazine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Uniformity of content

Sealed containers each containing 20 mg or less of Hydralazine Hydrochloride comply with the requirements stated under Parenteral Preparations, Powders for Injections using the following method of analysis. Dissolve the contents of a sealed container in sufficient *water* to produce 100 ml and dilute a volume containing 1 mg of Hydralazine Hydrochloride to 100 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_8H_8N_4$,HCl taking 535 as the value of A(1%, 1 cm) at the maximum at 260 nm.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Dissolve 0.1 g of the mixed contents of the 10 containers in a mixture of 25 ml of *water* and 35 ml of *hydrochloric acid* and titrate with 0.025M *potassium iodate VS* determining the end point potentiometrically using a platinum indicator electrode and a calomel reference electrode. Each ml of 0.025M *potassium iodate VS* is equivalent to 4.916 mg of $C_8H_8N_4$,HCl. Calculate the content of $C_8H_8N_4$,HCl in a container of average content weight.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states that solutions containing glucose should not be used in the preparation of the intravenous infusion.

Hydralazine Tablets

General Notices

Action and use

Vasodilator; treatment of hypertension.

DEFINITION

Hydralazine Tablets contain Hydralazine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydralazine hydrochloride, $C_8H_8N_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of Hydralazine Hydrochloride in 10 ml of *water*, make alkaline with 2 ml of 5M *ammonia* and extract with two 10-ml quantities of *chloroform*. Combine the chloroform extracts and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hydralazine *(RS 176)*.

B. Shake a quantity of the powdered tablets containing 25 mg of Hydralazine Hydrochloride with 30 ml of *methanol* for 15 minutes, add sufficient *methanol* to produce 50 ml and filter. Dilute 1 volume of the filtrate to 50 volumes with *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits four maxima, at 240, 260, 305 and 315 nm.

C. Extract a quantity of the powdered tablets containing 0.1 g of Hydralazine Hydrochloride with two 25-ml quantities of *methanol*, filter the combined extracts and evaporate to dryness. To 5 ml of a 1% w/v solution of the residue in *methanol* add 5 ml of a 2% w/v solution of *2-nitrobenzaldehyde* in *ethanol (96%)*. An orange precipitate is produced.

TEST

Hydrazine

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and as the mobile phase the upper layer obtained by shaking together 20 volumes of 13.5M *ammonia*, 20 volumes of *ethyl acetate* and 80 volumes of *hexane* and allowing to separate. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 50 mg of Hydralazine Hydrochloride with 10 ml of 0.1M *methanolic hydrochloric acid*; to 2 ml add 1 ml of a 2% w/v solution of *salicylaldehyde* in *methanol* and 0.1 ml of *hydrochloric acid*, centrifuge and use the supernatant liquid. Prepare solution (2) in the same manner, but using 2 ml of a 0.00025% w/v solution of *hydrazine sulphate* in 0.1M *methanolic hydrochloric acid* in place of the solution of the substance being examined. After removal of the plate, allow it to dry in air and spray with *dimethylaminobenzaldehyde solution R1*. Any spot corresponding to hydrazine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 50 mg of Hydralazine Hydrochloride add 50 ml of *water* and shake for 20 minutes. Add 60 ml of *hydrochloric acid* and titrate with 0.025M *potassium iodate VS* determining the end point potentiometrically using platinum and calomel electrodes. Each ml of 0.025M *potassium iodate VS* is equivalent to 4.916 mg of $C_8H_8N_4$,HCl.

STORAGE

Hydralazine Tablets should be protected from light.

Hydrochlorothiazide Tablets

General Notices

Action and use

Thiazide diuretic.

DEFINITION

Hydrochlorothiazide Tablets contain Hydrochlorothiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) triturate a quantity of the powdered tablets containing 10 mg of Hydrochlorothiazide with 10 ml of *acetone* and filter. Solution (2) contains 0.1% w/v of *hydrochlorothiazide EPCRS* in *acetone*. After removal of the plate, dry it in a current of air, examine under *ultraviolet light (254 nm)* and then treat the plate by *Method I* and examine again. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Related substances

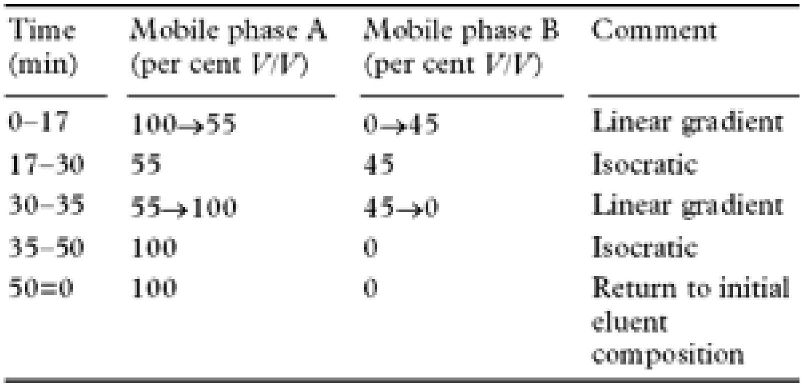
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide with 25 ml of a mixture of equal volumes of *acetonitrile* and *methanol* and dilute to 100 ml with *phosphate buffer solution pH 3.2 R1*. Filter through a glass-fibre filter (Whatman 0.45µ GD/X is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture containing 1 volume of *methanol*, 1 volume of *acetonitrile* and 2 volumes of *phosphate buffer solution pH 3.2 R1.* For solution (3) dissolve, with the aid of ultrasound, 15 mg each of *hydrochlorothiazide EPCRS* and *chlorothiazide EPCRS* in 25 ml of a mixture of equal volumes of *acetonitrile* and *methanol* and dilute to 100 ml with *phosphate buffer solution pH 3.2 R1*. Dilute 5 volumes of this solution to 100 volumes with the same solvent mixture.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Phenosphere ODS 3µ or Microsorb ODS 3µ is suitable), (b) as the mobile phase with a flow rate of 0.8 ml per minute the changing proportions of mobile phase A and mobile phase B described below and (c) a detection wavelength of 224 nm.

Mobile phase A A mixture of 10 volumes of *tetrahydrofuran*, 60 volumes of *methanol* and 940 volumes of *phosphate buffer solution pH 3.2 R1*.

Mobile phase B A mixture of 50 volumes of *tetrahydrofuran*, 500 volumes of *methanol* and 500 volumes of *phosphate buffer solution pH 3.2 R1*.

Equilibrate the column for at least 20 minutes with mobile phase A. Inject 20 µl. Carry out a linear gradient elution using the following gradient programme.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0–17	100→55	0→45	Linear gradient
17–30	55	45	Isocratic
30–35	55→100	45→0	Linear gradient
35–50	100	0	Isocratic
50=0	100	0	Return to initial eluent composition

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to chlorothiazide and hydrochlorothiazide is at least 2.5. If necessary, adjust the composition of the mobile phase or the time programme of the linear gradient.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 30 mg of Hydrochlorothiazide add 50 ml of 0.1M *sodium hydroxide*, shake for 20 minutes and dilute to 100 ml with 0.1M *sodium hydroxide*. Mix, filter, dilute 5 ml of the filtrate to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_7H_8ClN_3O_4S_2$ taking 520 as the value of A(1%, 1 cm) at the maximum at 273 nm.

Hydrocortisone Acetate and Neomycin Ear Drops

General Notices

Hydrocortisone and Neomycin Ear Drops

Action and use

Corticosteroid + Aminoglycoside antibacterial.

DEFINITION

Hydrocortisone Acetate and Neomycin Ear Drops are a suspension of Hydrocortisone Acetate in a solution of Neomycin Sulphate in Purified Water.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

90.0 to 110.0% w/v of the stated amount.

IDENTIFICATION

A. Comply with test A for Identification described under Hydrocortisone and Neomycin Cream using the following solutions. For solution (1) add 10 ml of *chloroform* to a quantity of the ear drops containing 5 mg of Hydrocortisone Acetate in a separating funnel, shake for 2 to 3 minutes and allow the layers to separate. Filter if necessary and use the chloroform layer. Solution (2) contains 0.05% w/v of *hydrocortisone acetate BPCRS* in *chloroform*.

B. In the Assay for hydrocortisone acetate the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

C. Comply with test C for Identification described under Hydrocortisone and Neomycin Cream. For solution (1) dilute a volume containing 3500 IU of Neomycin Sulphate with *water* to 2.5 ml, shake with 3 ml of *chloroform*, centrifuge and use the clear, upper layer.

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

Neamine

Comply with the test described under Hydrocortisone and Neomycin Cream. For solution (1) dilute a volume containing 3500 IU of Neomycin Sulphate with 2.5 ml of *water*, shake gently with 3 ml of *chloroform*, centrifuge and use the aqueous layer.

Neomycin C

Comply with the test described under Hydrocortisone and Neomycin Cream but using as solution (2) a solution prepared in the following manner. Dilute the ear drops with 0.02M *sodium tetraborate* to contain 700 IU per ml. To 0.5 ml of the resulting solution add 1.5 ml of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol*, heat in a water bath at 60° for 1 hour, cool and dilute the solution to 25 ml with the mobile phase; allow to stand and use the clear, lower layer.

ASSAY

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.020% w/v of *hydrocortisone acetate BPCRS* and 0.016% w/v of *fluoxymesterone BPCRS* (internal standard) in *chloroform*. For solution (2) shake a quantity of the ear drops containing 10 mg of Hydrocortisone Acetate with 25 ml of a 0.032% w/v solution of *fluoxymesterone BPCRS* in *chloroform*, add 25 ml of *chloroform*, mix and filter through *anhydrous sodium sulphate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable), (b) a mixture of 425 volumes of *butyl chloride*, 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the ear drops using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

For neomycin sulphate

Dilute a volume containing 3500 IU to 50 ml with sterile *phosphate buffer pH 8.0*, dilute 10 ml of the resulting solution to 100 ml with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per ml.

LABELLING

The strength with respect to Neomycin Sulphate is stated as the number of IU (Units) per ml.

Hydrocortisone Acetate and Neomycin Eye Drops

General Notices

Hydrocortisone and Neomycin Eye Drops

Action and use

Corticosteroid + Aminoglycoside antibacterial.

DEFINITION

Hydrocortisone Acetate and Neomycin Eye Drops are a sterile suspension of Hydrocortisone Acetate in a solution of Neomycin Sulphate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

90.0 to 110.0% w/v of the stated amount.

IDENTIFICATION

A. Comply with test A for Identification described under Hydrocortisone and Neomycin Cream using the following solutions. For solution (1) add 10 ml of *chloroform* to a quantity of the eye drops containing 5 mg of Hydrocortisone Acetate in a separating funnel, shake for 2 to 3 minutes and allow the layers to separate. Filter if necessary and use the chloroform layer. Solution (2) contains 0.05% w/v of *hydrocortisone acetate BPCRS* in *chloroform*.

B. In the Assay for hydrocortisone acetate the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

C. Comply with test C for Identification described under Hydrocortisone and Neomycin Cream. For solution (1) dilute a volume containing 3500 IU of Neomycin Sulphate with *water* to 2.5 ml, shake with 3 ml of *chloroform*, centrifuge and use the clear, upper layer.

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

Neamine

Comply with the test described under Hydrocortisone and Neomycin Cream. For solution (1) dilute a volume containing 3500 IU of Neomycin Sulphate with 2.5 ml of *water*, shake gently with 3 ml of *chloroform*, centrifuge and use the aqueous layer.

Neomycin C

Comply with the test described under Hydrocortisone and Neomycin Cream but using as solution (2) a solution prepared in the following manner. Dilute the eye drops with 0.02M *sodium tetraborate* to contain 700 IU per ml. To 0.5 ml of the resulting solution add 1.5 ml of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol*, heat in a water bath at 60° for 1 hour, cool and dilute the solution to 25 ml with the mobile phase; allow to stand and use the clear, lower layer.

ASSAY

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.020% w/v of *hydrocortisone acetate BPCRS* and 0.016% w/v of *fluoxymesterone BPCRS* (internal standard) in *chloroform*. For solution (2) shake a quantity of the eye drops containing 10 mg of Hydrocortisone Acetate with 25 ml of a 0.032% w/v solution of *fluoxymesterone BPCRS* in *chloroform*, add 25 ml of *chloroform*, mix and filter through *anhydrous sodium sulphate*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable), (b) a mixture of 425 volumes of *butyl chloride*, 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the eye drops using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

For neomycin sulphate

Dilute a volume containing 3500 IU to 50 ml with sterile *phosphate buffer pH 8.0*, dilute 10 ml of the resulting solution to 100 ml with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per ml.

LABELLING

The strength with respect to Neomycin Sulphate is stated as the number of IU (Units) per ml.

Hydrocortisone Acetate and Neomycin Eye Ointment

General Notices

Action and use

Corticosteroid + Aminoglycoside antibacterial.

DEFINITION

Hydrocortisone Acetate and Neomycin Eye Ointment is a sterile preparation containing Hydrocortisone Acetate and Neomycin Sulphate in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Hydrocortisone and Neomycin Cream using the following solutions. For solution (1) add 10 ml of *hexane* saturated with *acetonitrile* to a quantity of the ointment containing 5 mg of Hydrocortisone Acetate and shake for 2 to 3 minutes. Add 10 ml of *acetonitrile* saturated with *hexane*, shake for 10 minutes and allow the layers to separate. Centrifuge, filter the acetonitrile layer if necessary, evaporate 5 ml to dryness and dissolve the residue in 5 ml of a mixture of equal volumes of *chloroform* and *ethanol (96%)*. Solution (2) contains 0.05% w/v of *hydrocortisone acetate BPCRS* in a mixture of equal volumes of *chloroform* and *ethanol (96%)*.

B. In the Assay for hydrocortisone acetate the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

C. Complies with test C for Identification described under Hydrocortisone and Neomycin Cream. For solution (1) shake a quantity containing 7000 IU of Neomycin Sulphate with 10 ml of *chloroform*, add 5 ml of *water*, shake, centrifuge and use the clear, upper layer.

TESTS

Neamine

Complies with the test described under Hydrocortisone and Neomycin Cream. For solution (1) disperse a quantity containing 7000 IU of Neomycin Sulphate in 10 ml of *chloroform*, shake gently with 5 ml of *water*, centrifuge and use the aqueous layer.

Neomycin C

Complies with the test described under Hydrocortisone and Neomycin Cream.

ASSAY

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.025% w/v of *hydrocortisone acetate BPCRS* and 0.050% w/v of *fluoxymesterone BPCRS* (internal standard) in *chloroform*. For solution (2) shake a quantity of the ointment containing 25 mg of Hydrocortisone Acetate with 20 ml of a 0.25% w/v solution of *fluoxymesterone BPCRS* in *chloroform* and several glass beads for 30 minutes. Centrifuge; to 10 ml of the clear, supernatant layer add sufficient *chloroform* to produce 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable), (b) a mixture of 425 volumes of *butyl chloride*, 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the ointment using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

For neomycin sulphate

Dissolve a quantity containing 3500 IU in 50 ml of *ether*, extract the solution with three 30-ml quantities of sterile *phosphate buffer pH 8.0* and discard the ether phase. Pass *nitrogen* through the combined aqueous extracts to remove dissolved ether, dilute to 100 ml with sterile *phosphate buffer pH 8.0* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per g.

LABELLING

The strength with respect to Neomycin Sulphate is stated as the number of IU (Units) per g.

Hydrocortisone Acetate Cream

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Acetate Cream contains Hydrocortisone Acetate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions.

For creams containing more than 0.5% w/w of Hydrocortisone Acetate, prepare two solutions in the following manner. For solution (1) mix a quantity of the cream containing 25 mg of Hydrocortisone Acetate with 10 ml of *methanol (90%)*, add 50 ml of hot *hexane* and shake. Discard the upper layer, add 5 g of *anhydrous sodium sulphate* to the lower layer, mix and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) is a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *hydrocortisone acetate BPCRS* in *methanol*.

For creams containing 0.5% w/w or less of Hydrocortisone Acetate, prepare solution (1) in the same manner as solution (1) described above but using a quantity of the cream containing 5 mg of Hydrocortisone Acetate. Solution (2) is a mixture of equal volumes of solution (1) and a 0.05% w/v solution of *hydrocortisone acetate BPCRS* in *methanol*.

After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2), which appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For creams containing more than 0.5% w/w of Hydrocortisone Acetate, prepare solutions (1) and (2) in the following manner. For solution (1) dissolve 25 mg of *hydrocortisone acetate BPCRS* in 45 ml of *methanol*, add 5 ml of a 0.5% w/v solution of *betamethasone* (internal standard) in *methanol* and add sufficient *water* to produce 100 ml. For solution (2) disperse, by shaking, a quantity containing 25 mg of Hydrocortisone Acetate in 40 ml of a solution prepared by mixing 75 ml of *methanol* with 25 ml of a 15% w/v solution of *sodium chloride*. Add 50 ml of hot *hexane*, shake and separate the lower layer. Repeat the extraction using two 10 ml quantities of the methanolic sodium chloride solution. Add 5 ml of *methanol* to the combined extracts and sufficient *water* to produce 100 ml, mix and filter through a glass microfibre filter paper (Whatman GF/C is suitable).

For creams containing 0.5% w/w or less of Hydrocortisone Acetate, prepare solutions (1) and (2) in the following manner. For solution (1) dissolve 5 mg of *hydrocortisone acetate BPCRS* in 45 ml of *methanol* and add 5 ml of a 0.110% w/v solution of *betamethasone* (internal standard) in *methanol* and sufficient *water* to produce 100 ml. Prepare solution (2) in the same manner as solution (2) above but use a quantity containing 5 mg of Hydrocortisone Acetate.

For all creams prepare solution (3) in the same manner as solution (2) but adding 5 ml of the appropriate internal standard solution in place of the 5 ml of methanol before diluting to volume.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) *methanol (50%)* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the preparation being examined using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

Hydrocortisone Acetate Injection

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Acetate Injection is a sterile suspension of Hydrocortisone Acetate in Water for Injections. It is prepared using aseptic technique.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A white suspension that settles on standing but readily disperses on shaking. On examination under a microscope, the particles are seen to be crystalline and rarely exceed 30 µm in diameter.

IDENTIFICATION

Filter a volume containing 50 mg of Hydrocortisone Acetate through a sintered-glass filter, wash the residue with four 5-ml quantities of *water*, dissolve in 20 ml of *chloroform*, wash the chloroform solution with four 10-ml quantities of *water*, discard the washings, filter the chloroform solution through absorbent cotton and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydrocortisone acetate *(RS 179)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase B*.

C. Yields the reaction characteristic of *acetyl groups*, Appendix VI.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 25 mg of *hydrocortisone acetate BPCRS* in 50 ml of *methanol* and add sufficient *water* to produce 100 ml. For solution (2) add 70 ml of *methanol* to a volume of the injection containing 50 mg of Hydrocortisone Acetate, shake to produce a clear solution and dilute to 100 ml with *methanol*. Dilute 10 ml of the resulting solution to 20 ml with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) *methanol (50%)* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the preparation being examined using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

STORAGE

Hydrocortisone Acetate Injection should be protected from light.

LABELLING

The label states (1) that it is intended for local action; (2) that the container should be gently shaken before a dose is withdrawn.

Hydrocortisone Acetate Ointment

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Acetate Ointment contains Hydrocortisone Acetate in a suitable basis.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions.

For ointments containing more than 0.5% w/w of Hydrocortisone Acetate, prepare two solutions in the following manner. For solution (1) disperse a quantity containing 25 mg of Hydrocortisone Acetate in 5 ml of hot *hexane*, cool, extract with 10 ml of *methanol (90%)* and filter. Solution (2) is a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *hydrocortisone acetate BPCRS* in *methanol*.

For ointments containing 0.5% w/w or less of Hydrocortisone Acetate, prepare solution (1) in the same manner as solution (1) above but use a quantity containing 5 mg of Hydrocortisone Acetate. Solution (2) is a mixture of equal volumes of solution (1) and a 0.05% w/v solution of *hydrocortisone acetate BPCRS* in *methanol*.

After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) which appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For ointments containing more than 0.5% w/w of Hydrocortisone Acetate, prepare solutions (1) and (2) in the following manner. For solution (1) dissolve 25 mg of *hydrocortisone acetate BPCRS* in 45 ml of *methanol*, add 5 ml of a 0.5% w/v solution of *betamethasone* (internal standard) in *methanol* and sufficient *water* to produce 100 ml. For solution (2) disperse a quantity of the ointment containing 25 mg of Hydrocortisone Acetate in 100 ml of hot *hexane*, cool and extract with 20 ml of a solution prepared by mixing 3 volumes of *methanol* and 1 volume of a 15% w/v solution of *sodium chloride*. Repeat the extraction using two 10 ml quantities of the methanolic sodium chloride solution. Add 5 ml of *methanol* to the combined extracts and sufficient *water* to produce 100 ml, mix and filter through a glass microfibre filter (Whatman GF/C is suitable).

For ointments containing 0.5% w/w or less of Hydrocortisone Acetate, prepare solutions (1) and (2) in the following manner. For solution (1) dissolve 5 mg of *hydrocortisone acetate BPCRS* in 45 ml of *methanol*, add 5 ml of a 0.110% w/v solution of *betamethasone* (internal standard) in *methanol* and add sufficient *water* to produce 100 ml. Prepare solution (2) in the same manner as solution (2) above but use a quantity containing 5 mg of Hydrocortisone Acetate.

In each case prepare solution (3) in the same manner as solution (2) but add 5 ml of the internal standard solution in place of the 5 ml of methanol before diluting to volume.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) *methanol (50%)* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{23}H_{32}O_6$ in the ointment using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS*.

STORAGE

Hydrocortisone Acetate Ointment should be protected from light.

Hydrocortisone and Clioquinol Cream

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone and Clioquinol Cream contains Hydrocortisone and Clioquinol, the latter in *very fine powder*, in a suitable basis.
The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

92.5 to 107.5% of the stated amount.

Content of clioquinol, C_9H_5ClINO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) disperse, by warming and shaking, a quantity of the cream containing 2.5 g of Hydrocortisone in 10 ml of *ethanol (96%)*, cool, allow to stand at 0° for 30 minutes, filter and use the filtrate. Solution (2) contains 0.25% w/v of *hydrocortisone BPCRS* in *ethanol (96%)*. For solution (3) dissolve 12.5 mg of *hydrocortisone BPCRS* in 5 ml of solution (1). After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution.* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay for hydrocortisone, the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (1).

C. Fuse a quantity of the cream containing 0.1 g of Clioquinol with *anhydrous sodium carbonate*, dissolve the fused mass in *water* and acidify with 2M *nitric acid*. Add *silver nitrate solution*; a pale yellow precipitate is produced which is insoluble in 5M *ammonia*. Add 5M *ammonia* until the solution becomes alkaline, boil gently, filter and acidify the filtrate with 2M *nitric acid*; a white precipitate is produced which darkens on exposure to light.

ASSAY

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 5 mg of *hydrocortisone BPCRS* in 5 ml of a 4% v/v solution of *bromobenzene* (internal standard) in *methanol* and dilute to 50 ml with *methanol (80%)*. For solution (2) add 30 ml of *2,2,4-trimethylpentane* to a quantity of the cream containing 10 mg of Hydrocortisone and warm on a water bath until the preparation has melted. Extract the warm mixture with successive quantities of 30, 20 and 20 ml of *methanol (80%)*, combine the aqueous methanolic layers, cool to about 20° and dilute to 100 ml with the same solvent. Prepare solution (3) in the same manner as solution (2) but adding 10 ml of a 4% v/v solution of *bromobenzene* in *methanol* to the cooled methanolic extract.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) *methanol (65%)* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 242 nm.

Calculate the content of $C_{21}H_{30}O_5$ in the cream using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

For clioquinol

To a quantity of the cream containing 25 mg of Clioquinol add 80 ml of a hot mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* and heat on a water bath for 5 minutes. Cool in ice for 10 minutes, allow to warm to room temperature, dilute to 100 ml with the aqueous methoxyethanol, mix and filter. To 10 ml of the filtrate add 10 ml of *2-methoxyethanol* and 2 ml of a solution prepared by dissolving 0.5 g of *iron(III) chloride hexahydrate* in 80 ml of *2-methoxyethanol* and adding 0.1 ml of *hydrochloric acid* and sufficient *2-methoxyethanol* to produce 100 ml. Dilute the solution to 25 ml with *2-methoxyethanol* and measure the *absorbance* of the resulting solution at the maximum at 650 nm, Appendix II B, using in the reference cell a solution prepared by treating 10 ml of the aqueous methoxyethanol in the same manner beginning at the words 'add 10 ml of *2-methoxyethanol* …'.

Repeat the operation beginning at the words 'add 10 ml of *2-methoxyethanol* …' using 10 ml of a solution prepared in the following manner. Dissolve 0.125 g of *clioquinol BPCRS* in sufficient *2-methoxyethanol* to produce 50 ml, warming to effect solution; add 1 ml of *water* to 5 ml of the solution and add sufficient of a mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* to produce 50 ml. Calculate the content of C_9H_5ClINO from the *absorbances* obtained using the declared content of C_9H_5ClINO in *clioquinol BPCRS*.

STORAGE

Hydrocortisone and Clioquinol Cream should be protected from light.

Hydrocortisone and Clioquinol Ointment

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone and Clioquinol Ointment contains Hydrocortisone and Clioquinol, the latter in *very fine powder*, in a suitable basis.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

92.5 to 107.5% of the stated amount.

Content of clioquinol, C_9H_5ClINO

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) disperse, by warming and shaking, a quantity of the ointment containing 2.5 g of Hydrocortisone in 10 ml of *ethanol (96%)*, cool, allow to stand at 0 ^{c} for 30 minutes, filter and use the filtrate. Solution (2) contains 0.25% w/v of *hydrocortisone BPCRS* in *ethanol (96%)*. For solution (3) dissolve 12.5 mg of *hydrocortisone BPCRS* in 5 ml of solution (1). After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution.* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay for hydrocortisone the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (1).

C. Fuse a quantity of the ointment containing 0.1 g of Clioquinol with *anhydrous sodium carbonate*, dissolve the fused mass in *water* and acidify with 2M *nitric acid*. Add *silver nitrate solution*; a pale yellow precipitate is produced which is insoluble in 5M *ammonia*. Add 5M *ammonia* until the solution becomes alkaline, boil gently, filter and acidify the filtrate with 2M *nitric acid*; a white precipitate is produced which darkens on exposure to light.

ASSAY

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 5 mg of *hydrocortisone BPCRS* in 5 ml of a 4% v/v solution of *bromobenzene* (internal standard) in *methanol* and dilute to 50 ml with *methanol (80%)*. For solution (2) add 30 ml of *2,2,4-trimethylpentane* to a quantity of the ointment containing 10 mg of Hydrocortisone and warm on a water bath until the preparation has melted. Extract the warm mixture with successive quantities of 30, 20 and 20 ml of *methanol (80%)*, combine the aqueous methanolic layers, cool to about 20° and dilute to 100 ml with the same solvent. Prepare solution (3) in the same manner as solution (2) but adding 10 ml of a 4% v/v solution of *bromobenzene* in *methanol* to the cooled methanolic extract.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (Spherisorb ODS 1 is suitable), (b) *methanol (65%)* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 242 nm.

Calculate the content of $C_{21}H_{30}O_5$ in the ointment using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

For clioquinol

To a quantity containing 25 mg of Clioquinol add 80 ml of a hot mixture of 24 volumes of *2-methoxy-ethanol* and 6 volumes of *water* and heat on a water bath for 5 minutes. Cool in ice for 10 minutes, allow to warm to room temperature, dilute to 100 ml with the aqueous methoxyethanol, mix and filter. To 10 ml of the filtrate add 10 ml of *2-methoxyethanol* and 2 ml of a solution prepared by dissolving 0.5 g of *iron(III) chloride hexahydrate* in 80 ml of *2-methoxyethanol* and adding 0.1 ml of *hydrochloric acid* and sufficient *2-methoxyethanol* to produce 100 ml. Dilute the solution to 25 ml with *2-methoxyethanol* and measure the *absorbance* of the resulting solution at the maximum at 650 nm, Appendix II B, using in the reference cell a solution prepared by treating 10 ml of the aqueous methoxyethanol in the same manner beginning at the words 'add 10 ml of *2-methoxyethanol* …'.

Repeat the operation beginning at the words 'add 10 ml of *2-methoxyethanol* …' using 10 ml of a solution prepared in the following manner. Dissolve 0.125 g of *clioquinol BPCRS* in sufficient *2-methoxyethanol* to produce 50 ml, warming to effect solution; add 1 ml of *water* to 5 ml of the solution and add sufficient of a mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* to produce 50 ml. Calculate the content of C_9H_5ClINO from the *absorbances* obtained using the declared content of C_9H_5ClINO in *clioquinol BPCRS*.

STORAGE

Hydrocortisone and Clioquinol Ointment should be protected from light.

Hydrocortisone and Neomycin Cream

General Notices

Action and use

Corticosteroid + Aminoglycoside antibacterial.

DEFINITION

Hydrocortisone and Neomycin Cream contains Hydrocortisone and Neomycin Sulphate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 90 volumes of *dichloromethane* and 8 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 10 ml of *hexane* saturated with *acetonitrile* to a quantity of the preparation being examined containing 5 mg of Hydrocortisone and shake for 2 to 3 minutes. Add 10 ml of *acetonitrile* saturated with *hexane*, shake for 10 minutes and allow the layers to separate. Centrifuge, filter the acetonitrile layer if necessary, evaporate 5 ml to dryness and dissolve the residue in 5 ml of a mixture of equal volumes of *chloroform* and *ethanol (96%)*. Solution (2) contains 0.05% w/v of *hydrocortisone BPCRS* in a mixture of equal volumes of *chloroform* and *ethanol (96%)*. After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

B. In the Assay for hydrocortisone the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (1).

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 60 volumes of *methanol*, 40 volumes of 13.5M *ammonia* and 20 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) disperse a quantity containing 7000 IU of Neomycin Sulphate with 10 ml of *chloroform*, add 5 ml of *water*, shake, centrifuge and use the clear, upper layer. Solution (2) contains 0.2% w/v of *neomycin sulphate EPCRS* in *water*. After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 2 minutes. The principal red spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Neamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a freshly prepared 3.85% w/v solution of *ammonium acetate* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dissolve a quantity containing 7000 IU of Neomycin Sulphate in 10 ml of *chloroform*, shake gently with 5 ml of *water*, centrifuge and use the aqueous layer. Solution (2) contains 0.004% w/v of *neamine EPCRS* in *water*. After removal of the plate, dry it in a current of warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution* with *water* to contain 0.5% of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a very faint blue colour with a drop of a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to the cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. Any spot corresponding to neamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Neomycin C

Carry out the method for *liquid chromatography*, Appendix III D, injecting 10 µl of each of the following solutions. For solution (1) add 1.5 ml of a freshly prepared 2% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol* to 0.5 ml of a 0.10% w/v solution of *neomycin sulphate EPCRS* in 0.02M *sodium tetraborate*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 ml with the mobile phase, allow to stand and use the clear lower layer. For solution (2) shake a quantity containing 3500 IU of Neomycin Sulphate with 10 ml of *chloroform*, add 5 ml of 0.02M *sodium tetraborate*, mix, allow to separate and centrifuge the upper layer. Proceed as for solution (1) but using 0.5 ml of the clear supernatant liquid in place of 0.5 ml of the neomycin sulphate solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Nucleosil 100-5 is suitable), (b) as the mobile phase with a flow rate of 1.6 ml per minute a solution prepared by mixing 97 ml of *tetrahydrofuran*, 1.0 ml of *water* and 0.5 ml of *glacial acetic acid* with sufficient of a 2.0% v/v solution of *absolute ethanol* in *ethanol-free chloroform* to produce 250 ml and (c) a detection wavelength of 350 nm. Pass the mobile phase through the column for several hours before starting the analysis. Record the chromatogram for 1.4 times the retention time of the peak due to neomycin B.

The chromatogram obtained with solution (1) shows a principal peak due to neomycin B and a major *secondary peak* due to neomycin C with a retention time relative to neomycin B of about 0.6.

The *column efficiency*, determined using the peak due to neomycin B in the chromatogram obtained with solution (1), should be at least 13,000 *theoretical plates* per metre.

In the chromatogram obtained with solution (2) the area of the peak corresponding to neomycin C is 3 to 15% of the sum of the areas of the peaks corresponding to neomycin B and neomycin C.

ASSAY

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.010% w/v of *hydrocortisone BPCRS* and 0.018% w/v of *fluoxymesterone BPCRS* (internal standard) in *chloroform*. For solution (2) shake together a quantity of the preparation being examined containing 25 mg of Hydrocortisone, several small glass beads and 25 ml of a 0.036% w/v solution of *fluoxymesterone BPCRS* in *chloroform* for 15 minutes, add sufficient *chloroform* to produce 50 ml, mix and centrifuge. Remove any excipient material present at the interface and use the clear supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *silica gel for chromatography* (10 µm) (µPorasil is suitable), (b) a mixture of 425 volumes of *butyl chloride*, 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{21}H_{30}O_5$ in the cream using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

For neomycin sulphate

Stir a quantity containing 4200 IU with 15 ml of *chloroform* until the emulsion is completely broken. Transfer to a separating funnel with 25 ml of *phosphate buffer pH 8.0* and 5 ml of *chloroform*, shake vigorously, allow to separate and reserve the aqueous phase. Extract the chloroform layer with two 25-ml quantities of *phosphate buffer pH 8.0* and discard the chloroform layer. Pass *nitrogen* through the combined aqueous solutions to remove dissolved chloroform and dilute to 100 ml with sterile *phosphate buffer pH 8.0*. Dilute 10 ml of the resulting solution to 50 ml with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated amount.

LABELLING

The strength with respect to Neomycin Sulphate is stated as the number of IU (Units) per g.

Hydrocortisone Cream

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Cream contains Hydrocortisone in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions.

For creams containing more than 0.5% w/w of Hydrocortisone, prepare three solutions in the following manner. For solution (1) mix a quantity containing 25 mg of Hydrocortisone with 10 ml of *methanol (90%)*, add 50 ml of hot *hexane* and shake. Separate the lower layer, add 5 g of *anhydrous sodium sulphate*, mix and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) contains 0.25% w/v of *hydrocortisone BPCRS* in *methanol*. Solution (3) is a mixture of equal volumes of solutions (1) and (2).

For creams containing 0.5% w/w or less of Hydrocortisone, prepare solution (1) in the same manner as solution (1) above but use a quantity containing 5 mg of Hydrocortisone. Solution (2) contains 0.05% w/v of *hydrocortisone BPCRS* in *methanol*. Solution (3) is a mixture of equal volumes of solutions (1) and (2).

After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2); if it does not, the principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For creams containing more than 0.5% w/w of Hydrocortisone, prepare solutions (1) and (2) in the following manner. For solution (1) dissolve 25 mg of *hydrocortisone BPCRS* in 45 ml of *methanol*, add 5 ml of a 0.5% w/v solution of *betamethasone* (internal standard) in *methanol* and add sufficient *water* to produce 100 ml. For solution (2) disperse, by shaking, a quantity of the cream containing 25 mg of Hydrocortisone in 40 ml of a mixture of 3 volumes of *methanol* and 1 volume of a 15% w/v solution of *sodium chloride*. Add 50 ml of hot *hexane*, shake and separate the lower layer. Repeat the extraction using a further two 10-ml quantities of the methanolic sodium chloride solution. To the combined extracts add 5 ml of *methanol* and sufficient *water* to produce 100 ml, mix and filter through a glass microfibre paper (Whatman GF/C is suitable).

For creams containing 0.5% or less of Hydrocortisone, prepare solutions (1) and (2) in the following manner. For solution (1) dissolve 5 mg of *hydrocortisone BPCRS* in 45 ml of *methanol* and add 5 ml of a 0.110% w/v solution of *betamethasone* (internal standard) in *methanol* and sufficient *water* to produce 100 ml. Prepare solution (2) in the same manner as solution (2) above but use a quantity of the cream containing 5 mg of Hydrocortisone.

For all preparations prepare solution (3) in the same manner as solution (2) but add 5 ml of the appropriate internal standard solution in place of the 5 ml of methanol before diluting to volume.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) *methanol (50%)* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{21}H_{30}O_5$ in the preparation being examined using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS*.

Hydrocortisone Ointment

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Ointment contains Hydrocortisone in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Hydrocortisone Cream but preparing solution (1) in the following manner. For ointments containing more than 0.5% w/w of Hydrocortisone, disperse a quantity containing 25 mg of Hydrocortisone in 5 ml of hot *hexane*, cool, extract with 10 ml of *methanol (90%)* and filter. For ointments containing 0.5% w/w or less of Hydrocortisone, disperse a quantity containing 5 mg of Hydrocortisone in 50 ml of hot *hexane*, cool, extract with 10 ml of *methanol (90%)* and filter.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (1).

ASSAY

Carry out the Assay described under Hydrocortisone Cream but prepare solution (2) in the following manner. For ointments containing more than 0.5% w/w of Hydrocortisone, disperse a quantity containing 25 mg of Hydrocortisone in 100 ml of hot *hexane*, cool and extract with 20 ml of a solution prepared by mixing 3 volumes of *methanol* with 1 volume of a 15% w/v solution of *sodium chloride*. Repeat the extraction using a further two 10 ml quantities of the methanolic sodium chloride solution. To the combined extracts add 5 ml of *methanol* and sufficient *water* to produce 100 ml, mix and filter through a glass microfibre filter (Whatman GF/C is suitable). For ointments containing 0.5% w/w or less of Hydrocortisone, prepare solution (2) in the same manner as solution (2) above but use a quantity containing 5 mg of Hydrocortisone.

STORAGE

Hydrocortisone Ointment should be protected from light.

Hydrocortisone Oromucosal Tablets

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Oromucosal Tablets are mucoadhesive buccal tablets containing hydrocortisone sodium succinate prepared by buffering Hydrocortisone Hydrogen Succinate.

The tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *acetic anhydride*, 20 volumes of *water* and 60 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the tablets containing the equivalent of 25 mg of hydrocortisone with 10 ml of *methanol*, filter and use the filtrate. For solution (2) dissolve 50 mg of *hydrocortisone acetate BPCRS* in sufficient *methanol* to produce 20 ml. Solution (3) contains equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air, spray with *ethanolic sulphuric acid (20%)*, heat at 120° for 10 minutes, allow to cool and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows a single compact spot.

TESTS

Disintegration

The tablets disintegrate in not less than 10 minutes when examined by the *disintegration test for tablets and capsules*, Appendix XII A1, but using *water* at 24° to 26°.

Free hydrocortisone

Not more than 15% of the stated amount when determined by the following method. Weigh and powder 20 tablets. For solution (1) disperse a quantity of the powdered tablets containing the equivalent of 7.5 mg of hydrocortisone in 15 ml of a phosphate buffer prepared by dissolving 5.8 g of *anhydrous disodium hydrogen orthophosphate* and 1.6 g of *sodium dihydrogen orthophosphate* in 1000 ml of *water* and adjusting the pH to 7.4, if necessary, by adding 10 mg quantities of either *anhydrous disodium hydrogen orthophosphate* or *sodium dihydrogen orthophosphate*. Shake and extract with three 25-ml quantities of *chloroform*, combine the extracts in a second separating funnel, wash the combined extracts with 2 ml of *water*, filter the chloroform layer through a plug of absorbent cotton, previously moistened with *chloroform*, into a 250-ml round-bottomed flask, wash the funnel and absorbent cotton plug with two 10-ml quantities of *chloroform* and add the washings to the flask; evaporate to dryness using a rotary evaporator, allow to cool and dissolve the residue in 50 ml of *aldehyde-free ethanol (96%)*. Solution (2) contains 0.00225% w/v of *hydrocortisone BPCRS* in *aldehyde-free ethanol (96%)*.

Protect all the solutions from light. Transfer 10 ml of solution (1) into a stoppered test-tube and repeat for solution (2). Place in a water bath at 35° for 10 minutes, add 1 ml of *triphenyltetrazolium chloride solution* and 1 ml of *dilute tetramethylammonium hydroxide solution* to each tube, stopper and mix well; allow the tubes to stand in the water bath at 35° for a further 25 minutes, remove from the water bath and allow to stand in water for 5 minutes at room temperature. Measure the *absorbance* of the resulting solutions in a stoppered cell at the maximum at 485 nm, Appendix II B, using in the reference cell a solution prepared at the same time and in the same manner using 10 ml of *aldehyde-free ethanol (96%)*.

Calculate the content of free hydrocortisone.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing the equivalent of 25 mg of hydrocortisone with 50 ml of *water* for 10 minutes, dilute to 100 ml with *water*, filter and discard the first 20 ml of the filtrate; dilute 4 ml of the remaining filtrate to 100 ml with *water* and measure the *absorbance*, Appendix II B, at the maximum at 248 nm using *water* in the reference cell. Calculate the content of hydrocortisone, $C_{21}H_{30}O_5$, in the tablets using 449 as the value of A(1%, 1 cm) at the maximum at 248 nm.

LABELLING

The label states the quantity of hydrocortisone sodium succinate in terms of the equivalent amount of hydrocortisone.

Hydrocortisone Sodium Phosphate Injection

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Sodium Phosphate Injection is a sterile solution of Hydrocortisone Sodium Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

92.5 to 107.5% of the stated amount.

CHARACTERISTICS

A colourless or very pale yellow solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a freshly prepared mixture of 60 volumes of *butan-1-ol*, 20 volumes of *acetic anhydride* and 20 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a volume of the injection containing the equivalent of 0.25 g of hydrocortisone to 100 ml with *water*. Solution (2) contains 0.34% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). Solution (4) is a mixture of equal volumes of solution (1) and a 0.25% w/v solution of *betamethasone sodium phosphate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air until the solvent has evaporated, spray with *ethanolic sulphuric acid (20%)*, heat at 120° for 10 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot and the chromatogram obtained with solution (4) exhibits two principal spots with almost identical Rf values.

B. Evaporate 0.1 ml to dryness on a water bath and dissolve the residue in 2 ml of *sulphuric acid*. A yellowish green fluorescence is produced immediately (distinction from betamethasone sodium phosphate, dexamethasone sodium phosphate and prednisolone sodium phosphate).

TESTS

Alkalinity

pH, 7.5 to 8.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute a volume of the injection with *water* to contain the equivalent of 0.75% w/v of hydrocortisone. Solutions (2) and (3) contain 1.0% w/v of *hydrocortisone sodium phosphate BPCRS* and 0.020% w/v of *hydrocortisone BPCRS* in *methanol*, respectively. After removal of the plate, allow it to dry in air for 5 minutes and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Dilute a volume containing the equivalent of 0.2 g of hydrocortisone to 1000 ml with *water*. To 5 ml add 15 ml of *water*, 2.5 g of *sodium chloride* and 0.5 ml of *hydrochloric acid* and extract with three 25 ml quantities of *chloroform*. Wash each chloroform layer with the same 1 ml quantity of 0.1M *hydrochloric acid*, add the washings to the aqueous solution and discard the chloroform. Extract the aqueous solution with two 10 ml quantities of *tributyl orthophosphate*, discard the aqueous phase and extract the combined tributyl phosphate solutions with two 25 ml quantities of a solution containing 10% w/v of *sodium chloride* and 1% w/v of *anhydrous disodium hydrogen orthophosphate*. Filter the extracts successively through absorbent cotton and wash the filter with 10 ml of the chloride–phosphate solution. Dilute the combined filtrates to 100 ml with the chloride–phosphate solution and measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but using 20 ml of a solution containing 2.5 g of *sodium chloride* and 0.5 ml of *hydrochloric acid* and beginning at the words 'Extract the aqueous solution…'. Calculate the content of hydrocortisone sodium phosphate as $C_{21}H_{30}O_5$ taking 447 as the value of A(1%, 1 cm) at the maximum at 248 nm.

STORAGE

Hydrocortisone Sodium Phosphate Injection should be protected from light. The injection should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of hydrocortisone in a suitable dose-volume.

Hydrocortisone Sodium Phosphate Oral Solution

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Sodium Phosphate Oral Solution is a solution containing Hydrocortisone Sodium Phosphate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the oral solution containing the equivalent of 0.25 g of hydrocortisone to 100 ml with *water*.

(2) 0.34% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol* .

(3) A mixture of equal volumes of solutions (1) and (2).

(4) A mixture of equal volumes of solution (1) and a 0.25% w/v solution of *betamethasone sodium phosphate BPCRS* in *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase described below.

(c) Apply 5 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air until the solvent has evaporated, spray with *ethanolic sulphuric acid (20%)*, heat at 120° for 10 minutes and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

A freshly prepared mixture of 20 volumes of *acetic anhydride*, 20 volumes of *water* and 60 volumes of *butan-1-ol*.

SYSTEM SUITABILITY

The test is not valid unless the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot and the chromatogram obtained with solution (4) exhibits two principal spots with almost identical Rf values.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Evaporate 0.1 ml to dryness on a water bath and dissolve the residue in 2 ml of *sulphuric acid*. A yellowish green fluorescence is produced immediately (distinction from betamethasone sodium phosphate, dexamethasone sodium phosphate and prednisolone sodium phosphate).

TESTS

Alkalinity

pH, 7.5 to 8.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a suitable volume of the oral solution with *water* to contain the equivalent of 0.75% w/v of hydrocortisone.

(2) 1.0% w/v of *hydrocortisone sodium phosphate BPCRS* in *methanol*.

(3) A mixture of equal volumes of solutions (1) and (2).

(4) 0.020% w/v of *hydrocortisone BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase described below.

(c) Apply 2 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air for 5 minutes and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

A mixture of 1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (3%).

ASSAY

Dilute a volume of the oral solution containing the equivalent of 20 mg of hydrocortisone to 100 ml with *water*. To 5 ml add 15 ml of *water*, 2.5 g of *sodium chloride* and 0.5 ml of *hydrochloric acid* and extract with three 25 ml quantities of *chloroform*. Wash each chloroform layer with the same 1 ml quantity of 0.1M *hydrochloric acid*, add the washings to the aqueous solution and discard the chloroform. Extract the aqueous solution with two 10 ml quantities of *tributyl orthophosphate*, discard the aqueous phase and extract the combined tributyl phosphate solutions with two 25 ml quantities of a solution containing 10% w/v of *sodium chloride* and 1% w/v of *anhydrous disodium hydrogen orthophosphate*. Filter the extracts successively through absorbent cotton and wash the filter with 10 ml of the chloride–phosphate solution. Dilute the combined filtrates to 100 ml with the chloride–phosphate solution and measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but using 20 ml of a solution containing 2.5 g of *sodium chloride* and 0.5 ml of *hydrochloric acid* and beginning at the words 'Extract the aqueous solution …'. Calculate the content of hydrocortisone sodium phosphate as $C_{21}H_{30}O_5$ taking 447 as the value of A (1%, 1 cm) at the maximum at 248 nm.

STORAGE

Hydrocortisone Sodium Phosphate Oral Solution should be protected from light. The oral solution should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of hydrocortisone in a suitable dose-volume.

Hydrocortisone Sodium Succinate Injection

General Notices

Action and use

Corticosteroid.

DEFINITION

Hydrocortisone Sodium Succinate Injection is a sterile solution of hydrocortisone sodium succinate in Water for Injections. It is prepared by dissolving Hydrocortisone Sodium Succinate for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Hydrocortisone Sodium Succinate Injection deteriorates on storage and should be used immediately after preparation.

HYDROCORTISONE SODIUM SUCCINATE FOR INJECTION

DEFINITION

Hydrocortisone Sodium Succinate for Injection is a sterile material prepared from Hydrocortisone Hydrogen Succinate with the aid of a suitable alkali. It may contain excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of hydrocortisone, $C_{21}H_{30}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of hydrocortisone sodium succinate *(RS 180)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of *anhydrous formic acid*, 10 volumes of *absolute ethanol* and 150 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*. Solution (1) contains 0.1% w/v of the contents of the sealed container. Solution (2) contains 0.1% w/v of *hydrocortisone hydrogen succinate EPCRS*. Solution (3) contains 0.1% w/v each of *hydrocortisone hydrogen succinate EPCRS* and *methylprednisolone hydrogen succinate EPCRS*. After removal of the plate, allow it to dry in air and examine in daylight and under *ultraviolet light (365 nm)*. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) is similar in position to the spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two spots which may not be completely separated.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 5% w/v of hydrocortisone, 6.5 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a sufficient quantity of the contents of the sealed container in a mixture of equal volumes of *acetonitrile* and *water* to produce a solution containing the equivalent of 0.25% w/v of hydrocortisone. For solution (2) dilute 2 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *acetonitrile* and *water*. For solution (3) dilute a 0.035% w/v solution of *hydrocortisone BPCRS* in *acetonitrile* with an equal volume of *water*. For solution (4) dilute a solution containing 0.04% w/v each of *hydrocortisone hydrogen succinate EPCRS* and *dexamethasone BPCRS* in *acetonitrile* with an equal volume of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 330 volumes of *acetonitrile*, 600 volumes of *water* and 1 volume of *orthophosphoric acid* which is allowed to equilibrate and then diluted to 1000 volumes with *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

The test is not valid unless in the chromatogram obtained with solution (4) the *resolution factor* between the peaks corresponding to dexamethasone and hydrocortisone hydrogen succinate is at least 5.0.

For solution (1) allow the chromatography to proceed for twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to hydrocortisone is not greater than the area of the peak in the chromatogram obtained with solution (3) (7%) and the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

The content of hydrocortisone in each of 10 individual containers as determined in the Assay is not less than 92.5% and not more than 107.5% of the content of hydrocortisone stated on the label, except that in one container the weight may be not less than 85.0% and not more than 115.0% of the stated content.

ASSAY

Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.001% w/v of hydrocortisone. Measure the *absorbance* of the resulting solution at the maximum at 248 nm, Appendix II B, and calculate the content of $C_{21}H_{30}O_5$ taking 449 as the value of A(1%, 1 cm) at the maximum at 248 nm. Repeat the procedure with a further nine sealed containers and calculate the average content of $C_{21}H_{30}O_5$ per container from the ten individual results thus obtained.

LABELLING

The label of the sealed container states the quantity of hydrocortisone sodium succinate contained in it in terms of the equivalent amount of hydrocortisone.

Hydroflumethiazide Tablets

General Notices

Action and use

Thiazide diuretic.

DEFINITION

Hydroflumethiazide Tablets contain Hydroflumethiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydroflumethiazide, $C_8H_8F_3N_3O_4S_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Hydroflumethiazide with 10 ml of *acetone* for 10 minutes and filter. Solution (2) contains 0.1% w/v of *hydroflumethiazide BPCRS* in *acetone*. After removal of the plate, dry it in a current of air, examine under *ultraviolet light (254 nm)* and then treat the plate by *Method I* and examine again. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Hydroflumethiazide with 25 ml of *acetone* for 10 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 2.5 ml of *acetone*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, dry it in a current of air and reveal the spots by *Method I*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 15 mg of Hydroflumethiazide with 50 ml of *methanol* for 10 minutes and dilute to 100 ml with *methanol*. Mix, filter, dilute 10 ml of the filtrate to 100 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_8H_8F_3N_3O_4S_2$ taking 595 as the value of A(1%, 1 cm) at the maximum at 273 nm.

Hydrogen Peroxide Mouthwash

General Notices

DEFINITION

Hydrogen Peroxide Mouthwash is Hydrogen Peroxide Solution (6 per cent).

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of hydrogen peroxide, H_2O_2

5.0 to 7.0% w/v.

IDENTIFICATION

Acidity; Non-volatile matter; Organic stabilisers Complies with the requirements stated under Hydrogen Peroxide Solution (6 per cent).

ASSAY

Dilute 10 ml to 100 ml with *water*. To 10 ml of the resulting solution add 20 ml of 1M *sulphuric acid* and titrate with 0.02M *potassium permanganate VS*. Each ml of 0.02M *potassium permanganate VS* is equivalent to 1.701 mg of H_2O_2.

STORAGE

Hydrogen Peroxide Mouthwash should be protected from light.

LABELLING

The label states, where applicable, that the Mouthwash contains a stabilising agent.

Hydrotalcite Tablets

General Notices

Action and use

Antacid.

DEFINITION

Hydrotalcite Tablets contain Hydrotalcite.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydrotalcite, $Al_2Mg_6(OH)_{16}CO_3,4H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Add 20 ml of 2M *hydrochloric acid* to a quantity of the powdered tablets containing 1.0 g of Hydrotalcite, shake and filter. Add 30 ml of *water* to the filtrate and boil. Add 2M *ammonia* until just alkaline to *methyl red*, continue boiling for 2 minutes and filter. Wash the precipitate with 50 ml of a hot 2% w/v solution of *ammonium chloride* and dissolve in 15 ml of 2M *hydrochloric acid*. The resulting solution yields the reaction characteristic of *aluminium salts*, Appendix VI.

Neutralising capacity

Weigh and powder 20 tablets, pass the powder as completely as possible through a sieve of nominal mesh aperture about 225 µm and remix the sifted powder. Mix a quantity equivalent to one tablet with a small quantity of *water*, added slowly with stirring, to give a smooth paste and add gradually sufficient further quantities of *water* to produce 100 ml. Warm to 37°, add 100 ml of 0.1M *hydrochloric acid VS* previously heated to 37° and stir continuously using a paddle stirrer at a rate of about 200 revolutions per minute, maintaining the temperature at 37°. The pH of the solution at 37°, after 10 minutes and after 20 minutes, is 3.0 to 4.2, Appendix V L. Add 12 ml of 0.5M *hydrochloric acid VS* at 37°, stir continuously for 1 hour, maintaining the temperature at 37°, and titrate with 0.1M *sodium hydroxide VS* to pH 3.5. Subtract the number of ml of 0.1M *sodium hydroxide VS* from 160 ml to obtain the number of ml of 0.1M *hydrochloric acid VS* required for neutralisation. Not less than 130 ml of 0.1M *hydrochloric acid VS* is required to neutralise one tablet.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.3 g of Hydrotalcite add 2 ml of 7M *hydrochloric acid* and heat on a water bath for 15 minutes. Allow to cool, add 250 ml of *water* and 50 ml of 0.05M *disodium edetate VS* and neutralise with 1M *sodium hydroxide* using *methyl red solution* as indicator. Heat the solution on a water bath for 30 minutes and allow to cool. Add 3 g of *hexamine* and titrate the excess of disodium edetate with 0.05M *lead nitrate VS* using *xylenol orange solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 15.09 mg of $Al_2Mg_6(OH)_{16}CO_3,4H_2O$.

Hydrous Ointment

General Notices

Oily Cream

DEFINITION

Wool Alcohols Ointment	500 g
Phenoxyethanol	10 g
Dried Magnesium Sulphate	5 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 g

In preparing Hydrous Ointment the proportions of Hard Paraffin, Soft Paraffin and Liquid Paraffin used to make the Wool Alcohols Ointment may be varied to produce Hydrous Ointment having suitable properties.

When Hydrous Ointment is used in a white ointment, it should be prepared from Wool Alcohols Ointment made with White Soft Paraffin; when used in a coloured ointment, it should be prepared from Wool Alcohols Ointment made with Yellow Soft Paraffin.

Extemporaneous preparation

The following directions apply.

Dissolve the Phenoxyethanol and the Dried Magnesium Sulphate in sufficient warm Purified Water to produce about 500 g. Melt the Wool Alcohols Ointment and heat to about 60°; gradually add the aqueous solution at about 60° with vigorous stirring until a smooth cream is obtained. Stir until cool, add sufficient Purified Water to produce 1000 g and mix.

The ointment complies with the requirements stated under Topical Semi-solid Preparations.

STORAGE

Hydrous Ointment should be kept in a container made from non-absorbent material. If, on storage, some aqueous liquid separates, it is readily reincorporated by stirring.

Hydroxocobalamin Injection

General Notices

Action and use

Vitamin B_{12} analogue.

DEFINITION

Hydroxocobalamin Injection is a sterile solution of Hydroxocobalamin Acetate, Hydroxocobalamin Chloride or Hydroxocobalamin Sulphate in Water for Injections containing sufficient Acetic Acid, Hydrochloric Acid or Sulphuric Acid respectively to adjust the pH to about 4.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hydroxocobalamin, $C_{62}H_{89}CoN_{13}O_{15}P$

95.0 to 110.0% of the stated amount of anhydrous hydroxocobalamin.

IDENTIFICATION

Measure the *absorbance* at 351 nm and at 361 nm, Appendix II B. The ratio of the absorbance at 361 nm to that at 351 nm is about 0.65.

TESTS

Acidity

pH, 3.8 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µl of each of the following solutions. Solutions (1) to (3) are freshly prepared solutions of the injection, diluted if necessary with the mobile phase, containing the equivalent of (1) 0.10% w/v, (2) 0.005% w/v and (3) 0.00010% w/v of hydroxocobalamin. For solution (4) add 0.2 ml of a freshly prepared 2% w/v solution of *chloramine T* and 0.1 ml of 0.05M *hydrochloric acid* to a volume containing the equivalent of 5 mg of hydroxocobalamin, dilute to 10 ml with *water,* shake, allow to stand for 5 minutes and inject immediately. Protect the solutions from bright light.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrosorb 100 CH8/11 is suitable), (b) a mixture of 19.5 volumes of *methanol* and 80.5 volumes of a solution containing 1.5% w/v of *citric acid* and 0.81% w/v of *disodium hydrogen orthophosphate* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 351 nm.

The test is not valid unless the chromatogram obtained with solution (4) shows three principal peaks, the *resolution factor* between each pair of adjacent peaks is not less than 3.0 and the chromatogram obtained with solution (3) shows one principal peak with a *signal-to-noise ratio* of not less than 5.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (10%). Disregard any peak the area of which is less than that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the following procedure protected from light. Dilute a quantity containing the equivalent of 2.5 mg of anhydrous hydroxocobalamin to 100 ml with a solution containing 0.8% v/v of *glacial acetic acid* and 1.09% w/v of *sodium acetate* and measure the *absorbance* of the resulting solution at the maximum at 351 nm, Appendix II B. Calculate the content of $C_{62}H_{89}CoN_{13}O_{15}P$ taking 195 as the value of A(1%, 1 cm) at the maximum at 351 nm.

STORAGE

Hydroxocobalamin Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of anhydrous hydroxocobalamin in a suitable dose-volume.

When vitamin B_{12} injection is prescribed or demanded, Hydroxocobalamin Injection shall be dispensed or supplied.

Hydroxycarbamide Capsules

General Notices

Action and use

Cytotoxic alkylating drug.

DEFINITION

Hydroxycarbamide Capsules contain Hydroxycarbamide.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of hydroxycarbamide, $CH_4N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 30 mg of Hydroxycarbamide with 10 ml of *methanol* and filter. Evaporate the filtrate to dryness and dry the residue at 60° at a pressure of 2 kPa for 3 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hydroxycarbamide *(RS 184)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TEST

Urea

Carry out the method for *thin-layer chromatography*, Appendix III A, using a cellulose F precoated plate (Merck cellulose F plates are suitable) and a mixture of 1 volume of *glacial acetic acid*, 1 volume of *water* and 4 volumes of *butan-1-ol* as the mobile phase and developing the chromatograms for 18 hours. Apply separately to the plate 50 µl of each of the following solutions in several small volumes. For solution (1) shake a quantity of the contents of the capsules containing 0.5 g of Hydroxycarbamide with 10 ml of *water* for 15 minutes, dilute to 25 ml with *water* and filter. Solution (2) contains 0.01% w/v of *urea*. After removal of the plate, dry it in a current of air, spray with a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (96%)* containing 2% v/v of *hydrochloric acid*. In the chromatogram obtained with solution (1), any spot corresponding to urea is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed, finely powdered contents of 20 capsules containing 1 g of Hydroxycarbamide with 450 ml of *water* for 5 minutes, stir for 30 minutes with the aid of ultrasound, add sufficient *water* to produce 500 ml and mix well. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume of the filtrate with 1 volume of *water*. Solution (2) contains 0.10% w/v of *hydroxycarbamide BPCRS*. Solution (3) contains 0.10% w/v of *hydroxycarbamide BPCRS* and 0.4% w/v of *hydroxylamine hydrochloride*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) *water* as the mobile phase with a flow rate of 0.5 ml per minute and (c) a detection wavelength of 214 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between hydroxycarbamide and hydroxylamine hydrochloride is at least 1.0.

Calculate the content of $CH_4N_2O_2$ in the capsules using the declared content of $CH_4N_2O_2$ in *hydroxycarbamide BPCRS*.

STORAGE

Hydroxycarbamide Capsules should be protected from moisture.

Hydroxychloroquine Tablets

General Notices

Action and use

Antiprotozoal (malaria).

DEFINITION

Hydroxychloroquine Tablets contain Hydroxychloroquine Sulphate. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hydroxychloroquine sulphate, $C_{18}H_{26}ClN_3O,H_2SO_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powdered tablets containing 0.1 g of Hydroxychloroquine Sulphate in a mixture of 10 ml of *water* and 2 ml of 2M *sodium hydroxide* and extract with two 20 ml quantities of *chloroform*. Wash the chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of hydroxychloroquine *(RS 182)*.

B. Extract a quantity of the powdered tablets containing 0.2 g of Hydroxychloroquine Sulphate with 10 ml of *water* and filter. To the filtrate add 30 ml of hot *picric acid solution R1* and allow to cool. The *melting point* of the precipitate, after washing with 10 ml of *water*, is about 189°, Appendix V A.

C. Shake a quantity of the powdered tablets containing 0.1 g of Hydroxychloroquine Sulphate with 10 ml of *water* and filter. To the filtrate add 1 ml of 2M *hydrochloric acid* and 1 ml of *barium chloride solution*. A white precipitate is produced.

TEST

Disintegration

Maximum time, 45 minutes, Appendix XII A1.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Hydroxychloroquine Sulphate in 20 ml of 1M *sodium hydroxide* and extract with four 25 ml quantities of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 21.70 mg of $C_{18}H_{26}ClN_3O,H_2SO_4$.

200 mg of Hydroxychloroquine Sulphate is approximately equivalent to 156 mg of

hydroxychloroquine.

Hyoscine Butylbromide Injection

General Notices

Action and use

Anticholinergic.

DEFINITION

Hyoscine Butylbromide Injection is a sterile solution of Hyoscine Butylbromide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hyoscine butylbromide, $C_{21}H_{30}BrNO_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Evaporate to dryness a volume containing 0.1 g of Hyoscine Butylbromide, shake the residue with *chloroform*, filter, evaporate the filtrate to dryness and triturate the residue with 5 ml of *acetonitrile*. Evaporate to dryness and dry the residue at 50° at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hyoscine butylbromide *(RS 185)*. Retain the residue for use in test C.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits maxima at 252, 257 and 264 nm and a less well-defined maximum at 247 nm.

C. To 1 mg of the residue obtained in test A add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3% w/v solution of *potassium hydroxide* in *methanol*. A violet colour is produced.

TESTS

Acidity

pH, 3.7 to 5.5, Appendix V L.

Hyoscine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the injection diluted, if necessary, with 0.001M *hydrochloric acid* to contain 1.0% w/v of Hyoscine Butylbromide. Solution (2) contains 0.0010% w/v of *hyoscine hydrobromide BPCRS* in 0.001M *hydrochloric acid*. For solution (3) add 10 µl of solution (1) to 10 ml of solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb 10µ C8 is suitable), (b) a solution of 2.0 g of *sodium dodecyl sulphate* in a mixture of 370 ml of 0.001M *hydrochloric acid* and 680 ml of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 210 nm.

Inject 20 µl of solution (3). The test is not valid unless the *resolution factor* between the peaks corresponding to hyoscine and butylhyoscine is at least 5.

Inject 20 µl of each of solutions (1) and (2). The area of any peak corresponding to hyoscine in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} high-performance precoated plate (Merck silica gel 60 F_{254} HPTLC plates are suitable) and a mixture of 0.5 volume of *anhydrous formic acid* , 1.5 volumes of *water*, 9 volumes of *absolute ethanol* and 9 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 4 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute the injection, if necessary, with 0.01M *hydrochloric acid* to contain 2% w/v of Hyoscine Butylbromide. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with 0.01M *hydrochloric acid*. For solution (3) dilute 1 volume of solution (1) to 50 volumes with 0.01M *hydrochloric acid*. For solution (4) dilute 1 volume of solution (1) to 400 volumes with 0.01M *hydrochloric acid*. After removal of the plate, dry it at 60° for 15 minutes, spray with a solution prepared by mixing equal volumes of a 40% w/v solution of *potassium iodide* in *water* and a solution prepared by dissolving 0.85 g of *bismuth oxynitrate* in a mixture of 10 ml of *glacial acetic acid* and 40 ml of *water* and diluting 1 volume of the mixture with 2 volumes of *glacial acetic acid* and 10 volumes of *water* immediately before use. Allow the plate to dry in air, spray well with a 5% w/v solution of *sodium nitrite* and examine immediately. In the chromatogram obtained with solution (1) the principal spot has an Rf value of about 0.45. In the chromatogram obtained with solution (1) any *secondary spot* with an Rf value less than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) (3%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.25%); any *secondary spot* with an Rf value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (3) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (0.25%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection to contain 0.04% w/v of Hyoscine Butylbromide with 0.001M *hydrochloric acid*. Solution (2) contains 0.04% w/v of *hyoscine butylbromide BPCRS* in 0.001M *hydrochloric acid*.

The chromatographic procedure described under the test for Hyoscine may be used.

Calculate the content of $C_{21}H_{30}BrNO_4$ using the declared content of $C_{21}H_{30}BrNO_4$ in *hyoscine butylbromide BPCRS*.

STORAGE

Hyoscine Butylbromide Injection should be protected from light.

Hyoscine Butylbromide Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Hyoscine Butylbromide Tablets contain Hyoscine Butylbromide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hyoscine butylbromide, $C_{21}H_{30}BrNO_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Hyoscine Butylbromide with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and triturate the residue with 5 ml of *acetonitrile*. Evaporate to dryness and dry the residue at 50° at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of hyoscine butylbromide *(RS 185)*.

B. To 1 mg of the residue obtained in test A add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3% w/v solution of *potassium hydroxide* in *methanol*. A violet colour is produced.

C. Shake a quantity of the powdered tablets containing 50 mg of Hyoscine Butylbromide with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness, shake the residue with 50 ml of *water* and filter. The *light absorption* of the filtrate, Appendix II B, in the range 230 to 350 nm exhibits maxima at 252, 257 and 264 nm and a less well-defined maximum at 247 nm.

TESTS

Hyoscine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Hyoscine Butylbromide with 10 ml of 0.001M *hydrochloric acid* with the aid of ultrasound for 15 minutes, centrifuge and filter. Solution (2) contains 0.0010% w/v of *hyoscine hydrobromide BPCRS* in 0.001M *hydrochloric acid*. For solution (3) add 10 µl of solution (1) to 10 ml of solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb 10µ C8 is suitable), (b) a solution of 2.0 g of *sodium dodecyl sulphate* in a mixture of 370 ml of 0.001M *hydrochloric acid* and 680 ml of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 210 nm.

Inject 20 µl of solution (3). The test is not valid unless the *resolution factor* between the peaks corresponding to hyoscine and butylhyoscine is at least 5.

Inject 20 µl of each of solutions (1) and (2). The area of any peak corresponding to hyoscine in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} high-performance precoated plate (Merck silica gel 60 F_{254} HPTLC plates are suitable) and a mixture of 0.5 volume of *anhydrous formic acid*, 1.5 volumes of *water*, 9 volumes of *absolute ethanol* and 9 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 4 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Hyoscine Butylbromide with 5 ml of 0.01M *hydrochloric acid* and centrifuge. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with 0.01M *hydrochloric acid*. For solution (3) dilute 1 volume of solution (1) to 50 volumes with 0.01M *hydrochloric acid*. For solution (4) dilute 1 volume of solution (1) to 400 volumes with 0.01M *hydrochloric acid*. After removal of the plate, dry it at 60° for 15 minutes, spray with a solution prepared by mixing equal volumes of a 40% w/v solution of *potassium iodide* in *water* and a solution prepared by dissolving 0.85 g of *bismuth oxynitrate* in a mixture of 10 ml of *glacial acetic acid* and 40 ml of *water* and diluting 1 volume of the mixture with 2 volumes of *glacial acetic acid* and 10 volumes of *water* immediately before use. Allow the plate to dry in air, spray well with a 5% w/v solution of *sodium nitrite* and examine immediately. In the chromatogram obtained with solution (1) the principal spot has an Rf value of about 0.45. In the chromatogram obtained with solution (1) any *secondary spot* with an Rf value less than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) (3%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.25%); any *secondary spot* with an Rf value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (3) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (0.25%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 40 mg of Hyoscine Butylbromide with 60 ml of 0.001M *hydrochloric acid* with the aid of ultrasound for 15 minutes, dilute to 100 ml with the same solvent, centrifuge and filter to obtain a clear filtrate. Solution (2) contains 0.04% w/v of *hyoscine butylbromide BPCRS* in 0.001M *hydrochloric acid*.

The chromatographic procedure described under the test for Hyoscine may be used.

Calculate the content of $C_{21}H_{30}BrNO_4$ in the tablets using the declared content of $C_{21}H_{30}BrNO_4$ in *hyoscine butylbromide BPCRS*.

Hyoscine Eye Drops

General Notices

Action and use

Anticholinergic.

DEFINITION

Hyoscine Eye Drops are a sterile solution of Hyoscine Hydrobromide in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of hyoscine hydrobromide, $C_{17}H_{23}NO_4,HBr,3H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) evaporate a volume of the eye drops containing 5 mg of Hyoscine Hydrobromide to dryness on a water bath, triturate the residue with 1 ml of *ethanol (96%)*, allow to stand and use the supernatant liquid. Solution (2) contains 0.5% w/v of *hyoscine hydrobromide BPCRS* in *ethanol (96%).* After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *potassium iodobismuthate solution R1*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

B. In the Assay, the chromatogram obtained with solution (1) shows a peak of similar size and with the same retention time as the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. For solution (1) dilute the eye drops, if necessary, to produce a solution containing 0.125% w/v of Hyoscine Hydrobromide. Solution (2) contains 0.125% w/v of *hyoscine hydrobromide BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) and a stainless steel pre-column (1.0 cm × 4.6 mm) both packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1 volume of a 60% w/v solution of *perchloric acid*, 31 volumes of *methanol* and 68 volumes of *water*, adjusted to pH 2.5 with 13.5M *ammonia* and (c) a detection wavelength of 240 nm. When chlorhexidine is used as a preservative allow the chromatography for solution (1) to proceed until the chlorhexidine has eluted (this may be up to 20 times the retention time of the peak due to hyoscine hydrobromide).

Calculate the content of $C_{17}H_{21}NO_4$,HBr,$3H_2O$ using the declared content of $C_{17}H_{21}NO_4$,HBr in *hyoscine hydrobromide BPCRS*. Each mg of $C_{17}H_{21}NO_4$,HBr is equivalent to 1.141 mg of $C_{17}H_{21}NO_4$,HBr,$3H_2O$.

Hyoscine Injection

General Notices

Action and use

Anticholinergic.

DEFINITION

Hyoscine Injection is a sterile solution of Hyoscine Hydrobromide in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of hyoscine hydrobromide, $C_{17}H_{21}NO_4$,HBr,3H_2O

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) evaporate a volume of the injection containing 5 mg of Hyoscine Hydrobromide to dryness on a water bath, triturate the residue with 1 ml of *ethanol (96%),* allow to stand and use the supernatant liquid. Solution (2) contains 0.5% w/v of *hyoscine hydrobromide BPCRS* in *ethanol (96%).* After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *potassium iodobismuthate solution R1*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) .

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention as the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, to produce a solution containing 0.04% w/v of Hyoscine Hydrobromide. Solution (2) contains 0.04% w/v of *hyoscine hydrobromide BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) and a stainless steel pre-column (1.0 cm × 4.6 mm) both packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1 volume of a 60% w/v solution of *perchloric acid*, 31 volumes of *methanol* and 68 volumes of *water*, adjusted to pH 2.5 with 13.5M *ammonia* and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{17}H_{21}NO_4$,HBr,3H_2O using the declared content of $C_{17}H_{21}NO_4$,HBr in *hyoscine hydrobromide BPCRS*. Each mg of $C_{17}H_{21}NO_4$,HBr is equivalent to 1.141 mg of $C_{17}H_{21}NO_4$,HBr,3H_2O.

STORAGE

Hyoscine Injection should be protected from light.

Hyoscine Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Hyoscine Tablets contain Hyoscine Hydrobromide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of hyoscine hydrobromide, $C_{17}H_{21}NO_4,HBr,3H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *diethylamine*, 40 volumes of *acetone* and 50 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of powdered tablets containing 0.5 mg of Hyoscine Hydrobromide with 5 ml of 0.1M *hydrochloric acid*. Extract, with gentle swirling, with three 5 ml quantities of *dichloromethane* and discard the extracts. Add 1 ml of 5M *ammonia* to the aqueous phase and extract with two 5 ml quantities of *dichloromethane*. Dry the combined dichloromethane extracts over *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 0.5 ml of *ethanol (96%)*. Solution (2) contains 0.1% w/v of *hyoscine hydrobromide BPCRS* in *ethanol (96%)*. After removal of the plate, heat it at 105° for 20 minutes, allow to cool and spray with *potassium iodobismuthate solution R1*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Uniformity of content

Tablets containing less than 2 mg of Hyoscine Hydrobromide comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared in a 0.02% w/v solution of *atropine sulphate BPCRS* (internal standard) in *acetonitrile (25%)* (solution A). For solution (1) add 1 ml of solution A to one tablet and disintegrate with the aid of ultrasound. Shake the mixture for 2 minutes, centrifuge and filter the supernatant liquid using a suitable 0.45-µm filter. Solution (2) contains 0.015% w/v of *hyoscine hydrobromide BPCRS* in solution A.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 is suitable), (b) a solution containing 0.05M *sodium octanesulphonate* in a mixture of 1 volume of a 60% w/v solution of *perchloric acid*, 3 volumes of *methanol*, 21 volumes of *acetonitrile* and 75 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{17}H_{21}NO_4$,HBr,$3H_2O$ in each tablet using the declared content of $C_{17}H_{21}NO_4$,HBr in *hyoscine hydrobromide BPCRS*. Each mg of $C_{17}H_{21}NO_4$,HBr is equivalent to 1.141 mg of $C_{17}H_{21}NO_4$,HBr,$3H_2O$.

ASSAY

Prepare a 0.02% w/v solution of *atropine sulphate BPCRS* (internal standard) in *acetonitrile (25%)* (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.015% w/v of *hyoscine hydrobromide BPCRS* in solution A. For solution (2) add to 10 whole tablets 7 ml of *acetonitrile (25%)*, disintegrate with the aid of ultrasound and shake the mixture for 2 minutes. Add sufficient *acetonitrile (25%)* to produce a final solution containing 0.015% w/v of Hyoscine Hydrobromide, centrifuge and filter the supernatant liquid using a suitable 0.45-µm filter. Prepare solution (3) in the same manner as solution (2) but using solution A in place of acetonitrile (25%).

The chromatographic conditions described under Uniformity of content may be used.

Calculate the content of $C_{17}H_{21}NO_4$,HBr,$3H_2O$ using the declared content of $C_{17}H_{21}NO_4$,HBr in *hyoscine hydrobromide BPCRS.* Each mg of $C_{17}H_{21}NO_4$,HBr is equivalent to 1.141 mg of $C_{17}H_{21}NO_4$,HBr,$3H_2O$.

Hypromellose Eye Drops

General Notices

Alkaline Eye Drops; Artificial Tears

Action and use

Artificial tears.

Content

85.0 to 115.0% of stated amount.

DEFINITION

Hypromellose Eye Drops are a sterile solution of Hypromellose in Purified Water. They are isotonic with tear secretions.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

A. Heat 5 ml in a water bath, with stirring. Above 50° the solution becomes cloudy or a flocculent precipitate is produced. The solution becomes clear on cooling.

B. To 5 ml add 0.15 ml of 2M *acetic acid* and 1.2 ml of a 10% w/v solution of *tannic acid*. A yellowish white, flocculent precipitate is produced which dissolves in 6M *ammonia*.

TESTS

Clarity of solution

The eye drops are not more opalescent than *reference suspension IV,* Appendix IV A.

Alkalinity

pH, 8.2 to 8.6, Appendix V L.

Viscosity

70 to 130% of the declared value when determined by Appendix V H, Method I, at 20° and using a viscometer of a size appropriate to the nominal viscosity.

ASSAY

For solution A, dissolve 3.75 g of *diphenylamine* in 150 ml of *glacial acetic acid* then add 90 ml of *hydrochloric acid* and mix well.

For solution (1), use the eye drops, diluted if necessary, with water to contain 0.01% w/v of Hypromellose. Pipette 2 ml of this solution into a glass stoppered test tube, add 5 ml of solution A, mix and immediately immerse the test tube in an oil bath maintained at 105° to 110° for 30 minutes. Remove the test tube, cool in an ice bath for 10 minutes and allow to equilibrate to room temperature. For solution (2), prepare a solution containing 0.01% w/v of *hypromellose BPCRS* in *water*, and treat at the same time and in the same manner as solution (1), starting at the words "pipette 2 ml of this solution ….". For solution (3), use *water*, and treat at the same time and in the same manner as solution (1), starting at the words "pipette 2 ml of this solution….".

Measure the *absorbances* of solution (1) and solution (2) at the maximum at about 635 nm, Appendix II B, using solution (3) in the reference cell. Calculate the content of Hypromellose in the eye drops from the values of the absorbances obtained and using the declared content of Hypromellose in *hypromellose BPCRS*.

LABELLING

The label states the nominal viscosity in millipascal seconds.

When methylcellulose eye drops are prescribed or demanded, Hypromellose Eye Drops shall be dispensed or supplied.

Strong Haloperidol Oral Solution

General Notices

Strong Haloperidol Oral Drops

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Strong Haloperidol Oral Solution is an aqueous solution containing 1% w/v of Haloperidol. It is intended to be diluted before use.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of haloperidol, $C_{21}H_{23}ClFNO_2$

0.95 to 1.05% w/v.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. To 2 ml add 10 ml of *water* and 1 ml of 1M *sodium hydroxide*, extract with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of haloperidol *(RS 173)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 245 nm.

TESTS

Acidity

pH, 3.5 to 4.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 92 volumes of *dichloromethane*, 8 volumes of *methanol* and 1 volume of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. Solution (1) is the preparation being examined. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *water*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *water*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

To 1 ml add 10 ml of *water* and 10 ml of 1M *hydrochloric acid* and extract with two 10 ml quantities of *ether*. Wash the combined ether extracts with 10 ml of *water*, combine the aqueous solutions and remove the ether using a rotary evaporator. Add sufficient *water* to produce 100 ml and dilute 10 ml to 100 ml with *methanol*. Measure the *absorbance* of the resulting solution at the maximum at 245 nm, Appendix II B. Calculate the content of $C_{21}H_{23}ClFNO_2$ taking 346 as the value of A(1%, 1 cm) at the maximum at 245 nm.

STORAGE

Strong Haloperidol Oral Solution should be protected from light. It should not be refrigerated.

LABELLING

The label states that the preparation is to be diluted before use.

Water for Diluting Concentrated Haemodialysis Solutions

General Notices

(Haemodialysis Solutions, Concentrated, Water for Diluting, Ph Eur monograph 1167)

Ph Eur

The following monograph is given for information.

The analytical methods described and the limits proposed are intended to be used for validating the procedure for obtaining the water.

DEFINITION

Water for diluting concentrated haemodialysis solutions is obtained from potable water by distillation, by reverse osmosis, by ion exchange or by any other suitable method. The conditions of preparation, transfer and storage are designed to minimise the risk of chemical and microbial contamination.

When water obtained by one of the methods described above is not available, potable water may be used for home dialysis. Because the chemical composition of potable water varies considerably from one locality to another, consideration must be given to its chemical composition to enable adjustments to be made to the content of ions so that the concentrations in the diluted solution correspond to the intended use.

Attention has also to be paid to the possible presence of residues from water treatment (for example, chloramines) and volatile halogenated hydrocarbons.

For the surveillance of the quality of water for diluting concentrated haemodialysis solutions, the following methods may be used to determine the chemical composition and/or to detect the presence of possible contaminants together with suggested limits to be obtained.

CHARACTERS

Clear, colourless, tasteless liquid.

TESTS

Acidity or alkalinity

To 10 ml of the water to be examined, freshly boiled and cooled in a borosilicate glass flask, add 0.05 ml of *methyl red solution R*. The solution is not red. To 10 ml of the water to be examined add 0.1 ml of *bromothymol blue solution R1*. The solution is not blue.

Oxidisable substances

To 100 ml of the water to be examined add 10 ml of *dilute sulphuric acid R* and 0.1 ml of *0.02 M potassium permanganate* and boil for 5 min. The solution remains faintly pink.

Total available chlorine

Maximum 0.1 ppm.

In a 125 ml test-tube (A), place successively 5 ml of *buffer solution pH 6.5 R,* 5 ml of *diethylphenylenediamine sulphate solution R* and 1 g of *potassium iodide R*. In a second 125 ml test-tube (B), place successively 5 ml of *buffer solution pH 6.5 R* and 5 ml of *diethylphenylenediamine sulphate solution R*. Add as simultaneously as possible to tube A 100 ml of the water to be examined and to tube B a reference solution prepared as follows: to 1 ml of a 10 mg/l solution of *potassium iodate R*, add 1 g of *potassium iodide R* and 1 ml of *dilute sulphuric acid R*; allow to stand for 1 min, add 1 ml of *dilute sodium hydroxide solution R* and dilute to 100 ml with *water R*. Any colour in the mixture obtained with the water to be examined is not more intense than that in the mixture obtained with the reference solution.

Chlorides (2.4.4)

Maximum 50 ppm.

Dilute 1 ml of the water to be examined to 15 ml with *water R*. The solution complies with the limit test for chlorides.

Fluorides

Maximum 0.2 ppm.

Potentiometry (*2.2.36, Method I*): use as indicator electrode a fluoride-selective solid-membrane electrode and as reference electrode a silver-silver chloride electrode.

Test solution The water to be examined.

Reference solutions Dilute 2.0 ml, 4.0 ml and 10.0 ml of *fluoride standard solution (1 ppm F) R* respectively to 20.0 ml with *total-ionic-strength-adjustment buffer R1*.

Carry out the measurement of each solution.

Nitrates

Maximum 2 ppm.

Dilute 2 ml of the water to be examined to 100 ml with *nitrate-free water R*. Place 5 ml of the dilution in a test-tube immersed in iced water, add 0.4 ml of a 100 g/l solution of *potassium chloride R* and 0.1 ml of *diphenylamine solution R* and then, dropwise and with shaking, 5 ml of *sulphuric acid R*. Transfer the tube to a water-bath at 50 °C. Allow to stand for 15 min. Any blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 0.1 ml of *nitrate standard solution (2 ppm* NO_3*) R* and 4.9 ml of *nitrate-free water R*.

Sulphates (*2.4.13*)

Maximum 50 ppm.

Dilute 3 ml of the water to be examined to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates.

Aluminium (*2.4.17*)

Maximum 10 µg/l.

Prescribed solution To 400 ml of the water to be examined add 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R*.

Reference solution Mix 2 ml of *aluminium standard solution (2 ppm Al) R*, 10 ml of *acetate* *buffer solution pH 6.0 R* and 98 ml of *water R*.

Blank solution Mix 10 ml of *acetate buffer solution pH 6.0 R* and 100 ml of *water R.*

Ammonium

Maximum 0.2 ppm.

To 20 ml of the water to be examined in a flat-bottomed and transparent tube, add 1 ml of *alkaline potassium tetraiodomercurate solution R*. Allow to stand for 5 min. The solution is not more intensely coloured than a standard prepared at the same time and in the same manner using a mixture of 4 ml of *ammonium standard solution (1 ppm* NH_4*) R* and 16 ml of *ammonium-free water R*. Examine the solutions along the vertical axis of the tube.

Calcium

Maximum 2.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method* I).

Test solution The water to be examined.

Reference solutions Prepare reference solutions (1 ppm to 5 ppm) using *calcium standard solution (400 ppm Ca) R.*

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Oxidising air-acetylene flame.

Magnesium

Maximum 2.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 10 ml of the water to be examined to 100 ml with *distilled water R*.

Reference solutions Prepare reference solutions (0.1 ppm to 0.5 ppm) using *magnesium standard solution (100 ppm Mg) R.*

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Oxidising air-acetylene flame.

Mercury

Maximum 0.001 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Add 5 ml of *nitric acid R* per litre of the water to be examined. In a 50 ml borosilicate glass flask with a ground-glass-stopper, place 20 ml of the water to be examined and add 1 ml of *dilute nitric acid R* and shake. Add 0.3 ml of *bromine water R1*. Stopper the flask, shake and heat the stoppered flask at 45 °C for 4 h. Allow to cool. If the solution does not become yellow, add 0.3 ml of *bromine water R1* and re-heat at 45 °C for 4 h. Add 0.5 ml of a freshly prepared 10 g/l solution of *hydroxylamine hydrochloride R*. Shake. Allow to stand for 20 min.

Reference solutions Use freshly prepared reference solutions (0.0005 ppm to 0.002 ppm) obtained by diluting *mercury standard solution (1000 ppm Hg) R* with a 5 per cent *V/V* solution of *dilute nitric acid R* and treat as described for the test solution.

To a volume of solution suitable for the instrument to be used, add *stannous chloride solution R2* equal to 1/5 of this volume. Fit immediately the device for the entrainment of the mercury vapour. Wait 20 s and pass through the device a stream of *nitrogen R* as the carrier gas.

Source Mercury hollow-cathode tube or a discharge lamp.

Wavelength 253.7 nm.

Atomisation device Flameless system whereby the mercury can be entrained in the form of cold vapour.

Potassium

Maximum 2.0 ppm.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution (a) Dilute 50.0 ml of the water to be examined to 100 ml with *distilled water R*. Carry out a determination using this solution. If the potassium content is more than 0.75 mg/l, further dilute the water to be examined with *distilled water R*.

Test solution (b) Take 50.0 ml of the water to be examined or, if necessary, the water to be examined diluted as described in the preparation of test solution (a). Add 1.25 ml of *potassium standard solution (20 ppm K) R* and dilute to 100.0 ml with *distilled water R*.

Reference solutions Prepare reference solutions (0 ppm; 0.25 ppm; 0.50 ppm; 0.75 ppm; 1 ppm) using *potassium standard solution (20 ppm K) R.*

Wavelength 766.5 nm.

Calculate the potassium content of the water to be examined in parts per million from the expression:

$$\frac{p \times n_1 \times 0.5}{n_2 - n_1}$$

p = dilution factor used for the preparation of test solution (a),

n_1 = measured value of test solution (a),

n_2 = measured value of test solution (b).

Sodium

Maximum 50.0 ppm.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution The water to be examined. If the sodium content is more than 10 mg/l, dilute with *distilled water R* to obtain a concentration suitable for the apparatus used.

Reference solutions Prepare reference solutions (0 ppm; 2.5 ppm; 5.0 ppm; 7.5 ppm; 10

ppm) using *sodium standard solution (200 ppm Na) R.*

Wavelength 589 nm.

Zinc

Maximum 0.1 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*): use sampling and analytical equipment free from zinc or not liable to yield zinc under the conditions of use.

Test solution The water to be examined.

Reference solutions Prepare reference solutions (0.05 ppm to 0.15 ppm) using *zinc standard solution (100 ppm Zn) R.*

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Oxidising air-acetylene flame.

Heavy metals (*2.4.8*)

Maximum 0.1 ppm.

Heat 200 ml of the water to be examined in a glass evaporating dish on a water-bath until the volume is reduced to 20 ml. 12 ml of the solution complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Microbial contamination

Total viable aerobic count (*2.6.12*) not more than 10^2 micro-organisms per millilitre, determined by plate count.

Bacterial endotoxins (*2.6.14*)

Less than 0.25 IU/ml.

Ph Eur

Alcoholic Iodine Solution

General Notices

DEFINITION

Alcoholic Iodine Solution is a *cutaneous solution*.

Iodine	25 g
Potassium Iodide	25 g
Purified Water	25 ml
Ethanol (90 per cent)	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Potassium Iodide and the Iodine in the Purified Water and add sufficient Ethanol (90 per cent) to produce 1000 ml.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of iodine, I

2.4 to 2.7% w/v.

Content of potassium iodide, KI

2.4 to 2.7% w/v.

Ethanol content

83 to 88% v/v, Appendix VIII F.

ASSAY

For iodine

Dilute 10 ml with 20 ml of *water* and titrate with 0.1M *sodium thiosulphate VS*. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 12.69 mg of I.

For potassium iodide

To 10 ml add 30 ml of *water* and 50 ml of *hydrochloric acid* and titrate with 0.05M *potassium iodate VS* until the dark brown solution which is produced becomes pale brown. Add 1 ml of *amaranth solution* and continue the titration until the red colour just changes to pale yellow. From the number of ml of 0.05M *potassium iodate VS* required subtract half the number of ml of 0.1M *sodium thiosulphate VS* required in the Assay for iodine. Each ml of the remainder is equivalent to 16.60 mg of KI.

STORAGE

Alcoholic Iodine Solution should be kept in a well-closed container, the materials of which are resistant to iodine.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

The label indicates the pharmaceutical form as 'cutaneous solution'.

When iodine tinture is prescribed or demanded Alcoholic Iodine Solution shall be dispensed or supplied.

Aqueous Iodine Oral Solution

General Notices

DEFINITION

Iodine	50 g
Potassium Iodide	100 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Potassium Iodide and the Iodine in 100 ml of the Purified Water and add sufficient of the Purified Water to produce 1000 ml.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of iodine, I

4.75 to 5.25% w/v.

Content of potassium iodide, KI

9.5 to 10.5% w/v.

ASSAY

Dilute 25 ml to 100 ml with *water*.

For iodine

To 20 ml of the diluted solution add 10 ml of *water* and titrate with 0.1M *sodium thiosulphate VS*. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 12.69 mg of I.

For potassium iodide

To 10 ml of the diluted solution add 20 ml of *water* and 40 ml of *hydrochloric acid* and titrate with 0.05M *potassium iodate VS* until the dark brown solution which is produced becomes pale brown. Add 1 ml of *amaranth solution* and continue the titration until the red colour just changes to pale yellow. From the number of ml of 0.05M *potassium iodate VS* required subtract one quarter of the number of ml of 0.1M *sodium thiosulphate VS* required in the Assay for iodine. Each ml of the remainder is equivalent to 16.60 mg of KI.

STORAGE

Aqueous Iodine Oral Solution should be kept in a well-closed container, the materials of which are resistant to iodine.

LABELLING

The label states that the solution should be well diluted before use.

Aqueous Iodine Oral Solution contains in 1 ml 50 mg of Iodine and about 130 mg of total iodine, free and combined.

Aqueous Iodine Oral Solution is intended to be diluted before use.

When aqueous iodine solution is prescribed or demanded, Aqueous Iodine Oral Solution shall be dispensed or supplied.

Biphasic Insulin Injection

General Notices

Biphasic Insulin

(Ph Eur monograph 0831)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Biphasic insulin injection complies with the monograph on Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION

Biphasic insulin injection is a sterile suspension of crystals containing bovine insulin in a solution of porcine insulin.

CHARACTERS

A white or almost white suspension. When examined under a microscope, the majority of the particles are seen to be rhombohedral crystals, with a maximum dimension measured from corner to corner through the crystal greater than 10 µm but rarely exceeding 40 µm.

IDENTIFICATION

Examine the chromatograms obtained in the assay. The position of the peaks due to the two insulins in the chromatogram obtained with the test solution correspond to those of the principal peaks in the chromatogram obtained with the appropriate reference solution.

TESTS

pH *(2.2.3)*

The pH of the suspension to be examined is 6.6 to 7.2.

Insulin in the supernatant

22.0 per cent to 28.0 per cent of insulin in solution. Determine by the method described in the test for insulin in the supernatant in the monograph on *Insulin preparations, injectable (0854).*

Total zinc

26.0 µg to 37.5 µg per 100 IU of insulin. Determine by the method described in the monograph on *Insulin preparations, injectable (0854).*

Ph Eur

Biphasic Isophane Insulin Injection

General Notices

Biphasic Isophane Insulin
(Ph Eur monograph 0832)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Biphasic isophane insulin injection complies with the monograph on Insulin preparations, injectable (0854) with the exception of the test for Insulin in the supernatant and with the amendments prescribed below for the other tests.

DEFINITION

Biphasic isophane insulin injection is a sterile buffered suspension of either porcine or human insulin, complexed with protamine sulphate or another suitable protamine, in a solution of insulin of the same species.

PRODUCTION

Biphasic isophane insulin injection is prepared by carrying out the procedures described in the monograph on *Insulin preparations, injectable (0854)*.

Biphasic isophane insulin injection is produced by mixing, in defined ratios, soluble insulin injection and isophane insulin injection. The defined ratios shall be demonstrated by a test method which has been approved by the competent authority to comply with the label claim.

CHARACTERS

A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

IDENTIFICATION

Examine the chromatograms obtained in the Assay. The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak obtained with the appropriate reference solution.

TESTS

Total zinc

Not more than 40.0 µg per 100 IU of insulin, determined as described in the monograph on

Insulin preparations, injectable (0854).

LABELLING

The label states in addition to the indications mentioned in the monograph on *Insulin preparations, injectable (0854)* the ratio of soluble insulin injection to isophane insulin injection used in the manufacturing process of biphasic isophane insulin injection.

Ph Eur

Ibuprofen Cream

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Cream contains Ibuprofen in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 5 volumes of *anhydrous acetic acid*, 25 volumes of *ethyl acetate* and 75 volumes of n-*hexane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake vigorously a quantity of the cream containing 50 mg of Ibuprofen with 10 ml of *dichloromethane* for 5 minutes and filter (Whatman GF/C paper is suitable). Solution (2) contains 0.5% w/v of *ibuprofen BPCRS* in *dichloromethane*. After removal of the plate, dry it at 120° for 30 minutes, lightly spray the plate with a 1% w/v solution of *potassium permanganate* in 1M *sulphuric acid*, heat at 120° for 20 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of each of the following solutions. For solution (1) shake vigorously a quantity of the cream containing 0.1 g of Ibuprofen with 25 ml of *methanol* for 10 minutes, decant the solution into a 50 ml graduated flask, rinse the original flask with two 10 ml quantities of *methanol*, dilute the combined solution and rinsings to 50 ml with *methanol* and filter (Whatman GF/C paper is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. For solution (3) dissolve 50 mg of *ibuprofen BPCRS* in 2.5 ml of a 0.006% w/v solution of *2-(4-butylphenyl)propionic acid EPCRS* in *methanol* (prepared by diluting 1 volume of *2-(4-butylphenyl)propionic acid EPCRS* to 10 volumes with *methanol*) and add sufficient *methanol* to produce 25 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile* and 600 volumes of *water* diluted to 1000 volumes with *water* after equilibration and (c) a detection wavelength of 214 nm. Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with solution (2) is 70 to 90% of full-scale deflection on the chart paper. Record the chromatogram for 1.5 times the retention time of the principal peak.

Equilibrate the column with the mobile phase for about 45 minutes before starting the chromatography.

When the chromatograms are recorded under the conditions described above, the retention time of ibuprofen is about 20 minutes. In the chromatogram obtained with solution (3) measure the height (*a*) of the peak due to 2-(4-butylphenyl)-propionic acid and the height (*b*) of the lowest point of the curve separating this peak from that due to ibuprofen. The test is not valid unless *a* is greater than 1.5*b*. If necessary, adjust the concentration of acetonitrile in the mobile phase to obtain the required resolution.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(4-butylphenyl)-propionic acid is not greater than that of the peak due to 2-(4-butylphenyl) propionic acid in the chromatogram obtained with solution (3) (0.3%), the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%) and the sum of the areas of any such peaks is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%). Disregard any peak the area of which is less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake vigorously a quantity of the cream containing 50 mg of Ibuprofen with 25 ml of the mobile phase for 10 minutes, decant the solution into a 50 ml graduated flask, rinse the original flask with two 10 ml quantities of the mobile phase, dilute the combined solution and rinsings to 50 ml with the mobile phase and filter (Whatman GF/C paper is suitable). Solution (2) contains 0.1% w/v of *ibuprofen BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 264 nm.

Calculate the content of $C_{13}H_{18}O_2$ using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

Ibuprofen Gel

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Gel is a solution of Ibuprofen in a suitable water-miscible basis.
The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 5 volumes of *anhydrous acetic acid*, 25 volumes of *ethyl acetate* and 75 volumes of n-*hexane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake vigorously a quantity of the gel containing 0.125 g of Ibuprofen with 25 ml of *dichloromethane* for 5 minutes and use the upper layer. Solution (2) contains 0.5% w/v of *ibuprofen BPCRS* in *dichloromethane*. After removal of the plate, dry it at 120° for 30 minutes, lightly spray the plate with a 1% w/v solution of *potassium permanganate* in 1M *sulphuric acid*, heat at 120° for 20 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of each of the following solutions. For solution (1) disperse a quantity of the gel containing 0.1 g of Ibuprofen in 25 ml of warm *methanol*, cool and dilute to 50 ml with *methanol*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. For solution (3) dissolve 50 mg of *ibuprofen BPCRS* in 2.5 ml of a 0.006% w/v solution of *2-(4-butylphenyl)propionic acid EPCRS* in *methanol* (prepared by diluting 1 volume of *2-(4-butylphenyl)propionic acid EPCRS* to 10 volumes with *methanol*) and add sufficient *methanol* to produce 25 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile* and 600 volumes of *water* diluted to 1000 volumes with *water* after equilibration and (c) a detection wavelength of 214 nm. Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with solution (2) is 70 to 90% of full-scale deflection on the chart paper. Record the chromatogram for 1.5 times the retention time of the principal peak.

Equilibrate the column with the mobile phase for about 45 minutes before starting the chromatography.

When the chromatograms are recorded under the conditions described above, the retention time of ibuprofen is about 20 minutes. In the chromatogram obtained with solution (3) measure the height (*a*) of the peak due to 2-(4-butylphenyl)-propionic acid and the height (*b*) of the lowest point of the curve separating this peak from that due to ibuprofen. The test is not valid unless *a* is greater than 1.5*b*. If necessary, adjust the concentration of acetonitrile in the mobile phase to obtain the required resolution.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(4-butylphenyl)propionic acid is not greater than that of the peak due to 2-(4-butylphenyl)-propionic acid in the chromatogram obtained with solution (3) (0.3%), the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%) and the sum of the areas of any such peaks is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%). Disregard any peak the area of which is less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the gel containing 50 mg of Ibuprofen with 50 ml of warm *methanol* for 10 minutes, cool and add sufficient *methanol* to produce 100 ml. Dilute 10 volumes of this solution to 20 volumes with the mobile phase. For solution (2) dilute 10 volumes of a solution containing 0.05% w/v of *ibuprofen BPCRS* in *methanol* to 20 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 264 nm.

Calculate the content of $C_{13}H_{18}O_2$ using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

Ibuprofen Oral Suspension

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Oral Suspension is a suspension of Ibuprofen in a suitable flavoured vehicle.
The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 0.5 g of Ibuprofen with 20 ml of *chloroform*, allow to stand until the layers have separated, filter and evaporate the filtrate to dryness in a current of air without heating. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen *(RS 186)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 1 volume of *anhydrous acetic acid*, 5 volumes of *ethyl acetate* and 15 volumes of n*-hexane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a quantity of the oral suspension containing 0.1 g of Ibuprofen to 100 ml with *absolute ethanol*, shake vigorously for 5 minutes, filter (Whatman GF/C paper is suitable) and use the filtrate. Solution (2) contains 0.1% w/v of *ibuprofen BPCRS* in *absolute ethanol*. After removal of the plate dry it at 120° for 30 minutes, lightly spray the plate with a 1% w/v solution of *potassium permanganate* in 1M *sulphuric acid*, heat at 120° for 20 minutes and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). Disregard any spot remaining on the line of application.

TEST

4′-Isobutylacetophenone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions immediately after preparation. For solution (1) mix a quantity of the oral suspension containing 0.2 g of Ibuprofen with 30 ml of *acetonitrile*, add a further 10 ml of acetonitrile and 10 ml of 0.01M *orthophosphoric acid*, shake vigorously, dilute to 100 ml with 0.01M *orthophosphoric acid* and filter (Whatman GF/C paper is suitable). Solution (2) contains 0.0006% w/v of *4′-isobutylacetophenone BPCRS* in *acetonitrile*. Solution (3) contains 0.0006% w/v of *4′-isobutylacetophenone BPCRS* and 0.2% w/v of *ibuprofen BPCRS* in the

mobile phase.

The chromatographic procedure described under Assay may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to ibuprofen and 4′-isobutylacetophenone is at least 1.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 4′-isobutylacetophenone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions immediately after preparation. For solution (1) mix a quantity of the oral suspension containing 0.1 g of Ibuprofen with 30 ml of *acetonitrile*, add a further 10 ml of *acetonitrile* and 10 ml of 0.01M *orthophosphoric acid*, shake vigorously, dilute to 100 ml with 0.01M *orthophosphoric acid* and filter (Whatman GF/C paper is suitable). For solution (2) prepare a 0.1% w/v of *ibuprofen BPCRS* by dissolving a suitable quantity in 40 volumes of *acetonitrile* and adding 60 volumes of 0.01M *orthophosphoric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 400 volumes of *acetonitrile* and 600 volumes of 0.01M *orthophosphoric acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 220 nm. Inject 10 µl of each solution.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{13}H_{18}O_2$, weight in volume, from the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

STORAGE

Ibuprofen Oral Suspension should be protected from light.

Ibuprofen Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ibuprofen Tablets contain Ibuprofen. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ibuprofen, $C_{13}H_{18}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.5 g of Ibuprofen with 20 ml of *acetone*, filter and evaporate the filtrate to dryness in a current of air without heating. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ibuprofen *(RS 186)*.

B. *Melting point* of the residue obtained in test A, after recrystallisation from *petroleum spirit (boiling range, 40° to 60°)*, about 75°, Appendix V A.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of each of the following solutions. For solution (1) add 30 ml of *methanol* to a quantity of the powdered tablets containing 0.2 g of Ibuprofen, shake for 30 minutes, add 30 ml of *methanol* and sufficient *water* to produce 100 ml, mix and filter through a glass microfibre filter paper (Whatman GF/C is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) dissolve 50 mg of *ibuprofen BPCRS* in 2.5 ml of a 0.006% w/v solution of *2-(4-butylphenyl)propionic acid EPCRS* in *methanol* (prepared by diluting 1 volume of *2-(4-butylphenyl)propionic acid EPCRS* to 10 volumes with *methanol*) and add sufficient *methanol* to produce 25 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile* and 600 volumes of *water* diluted to 1000 volumes with *water* after equilibration and (c) a detection wavelength of 214 nm. Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with solution (2) is 70 to 90% of full-scale deflection on the chart paper. Record the chromatogram for 1.5 times the retention time of the principal peak.

Equilibrate the column with the mobile phase for about 45 minutes before starting the chromatography.

When the chromatograms are recorded under the conditions described above, the retention time of ibuprofen is about 20 minutes. In the chromatogram obtained with solution (3) measure the height (*a*) of the peak due to 2-(4-butylphenyl)-propionic acid and the height (*b*) of the lowest point of the curve separating this peak from that due to ibuprofen. The test is not valid unless *a* is greater than 1.5*b*. If necessary, adjust the concentration of acetonitrile in the mobile phase to obtain the required resolution.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(4-butylphenyl)propionic acid is not greater than that of the peak due to 2-(4-butylphenyl) propionic acid in the chromatogram obtained with solution (3) (0.3%), the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%) and the sum of the areas of any such peaks is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%). Disregard any peak the area of which is less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Ibuprofen with 30 ml of the mobile phase for 30 minutes, add sufficient of the mobile phase to produce 100 ml and mix thoroughly. Centrifuge 25 ml of the solution at 2500 *g* for 5 minutes and use the supernatant liquid. Solution (2) contains 0.2% w/v of *ibuprofen BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 264 nm.

Calculate the content of $C_{13}H_{18}O_2$ using the declared content of $C_{13}H_{18}O_2$ in *ibuprofen BPCRS*.

Idoxuridine Eye Drops

General Notices

Action and use

Pyrimidine nucleoside analogue; antiviral (herpesviruses).

DEFINITION

Idoxuridine Eye Drops are a sterile solution of Idoxuridine in Purified Water.
Except that they may be supplied in containers not exceeding 15 ml capacity, the eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of idoxuridine, $C_9H_{11}IN_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a suitable volume of the eye drops with 0.01M *sodium hydroxide* to produce a solution containing 0.004% w/v of Idoxuridine. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 279 nm.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak having the same retention time as the peak due to idoxuridine in the chromatogram obtained with solution (1).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00040% w/v of *2′-deoxyuridine*, 0.00080% w/v of *5-iodouracil*, 0.00040% w/v of *5-bromo-2′-deoxyuridine* and 0.00010% w/v of *sulphanilamide* (internal standard). For solution (2) dilute a suitable volume of the eye drops with *water* to give a final concentration of 0.080% w/v of Idoxuridine. For solution (3) dilute a suitable volume of the eye drops with sufficient of a suitable solution of *sulphanilamide* to give a final concentration of 0.080% w/v of Idoxuridine and 0.00010% w/v of the internal standard.

The chromatographic conditions described under the Assay may be used but using as the mobile phase a mixture of 4 volumes of *methanol* and 96 volumes of *water*. The order of elution of the peaks following the internal standard is deoxyuridine, iodouracil, bromodeoxyuridine and idoxuridine. Several peaks due to excipients may appear in the chromatogram obtained with solution (3) before the peak due to the internal standard.

In the chromatogram obtained with solution (3) the ratios of the areas of any peaks due to 2′-deoxyuridine, 5-iodouracil and 5-bromo-2′-deoxyuridine to the area of the peak due to sulphanilamide are not greater than the ratios of the areas of the corresponding peaks in the chromatogram obtained with solution (1).

ASSAY

Prepare a solution containing 0.12 g of *sulphathiazole* (internal standard) in 10 ml of *ethanol (96%)*, warming if necessary, and dilute to 100 ml with *water* (solution A). Prepare a solution by diluting a suitable volume of the eye drops with *water* if necessary to give a final concentration of 0.10% w/v of Idoxuridine (solution B). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 0.1 g of *idoxuridine BPCRS* with 50 ml of *water* until dissolved and then dilute to 100 ml with *water*; to 15 ml of this solution add 2 ml of solution A and dilute to 20 ml with *water*. For solution (2) add 2 ml of a 10% v/v solution of *ethanol (96%)* to 15 ml of solution B and dilute to 20 ml with *water*. For solution (3) add 2 ml of solution A to 15 ml of solution B and dilute to 20 ml with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 13 volumes of *methanol* and 87 volumes of *water* as the mobile phase with a flow rate of 1.7 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_9H_{11}IN_2O_5$ using the declared content of $C_9H_{11}IN_2O_5$ in *idoxuridine BPCRS*.

STORAGE

Idoxuridine Eye Drops should be stored at a temperature not exceeding 8° but should not be allowed to freeze.

LABELLING

The label states that the Eye Drops should not be used for continuous periods of treatment exceeding 21 days.

Ifosfamide Injection

General Notices

Action and use.

Cytotoxic alkylating agent.

DEFINITION

Ifosfamide Injection is a sterile solution of Ifosfamide in Water for Injections or a suitable liquid. It is prepared by dissolving Ifosfamide for Injection in the requisite amount of a suitable liquid immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Ifosfamide Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

IFOSFAMIDE FOR INJECTION

Ifosfamide for Injection is a sterile material consisting of Ifosfamide with or without excipients. It is supplied in a sealed container.

CAUTION Ifosfamide is Cytotoxic. Carry out the procedures described below exercising appropriate precautions.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of ifosfamide, $C_7H_{15}Cl_2N_2O_2P$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of ifosfamide (*RS 428*).

TESTS

Solution A

Dissolve 5.0 g in *carbon dioxide-free water* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Solution A is clear, Appendix IV A, and not more intensely coloured than *reference solution* Y_7, Appendix IV B.

Acidity or alkalinity

pH of an 8% w/v solution, 4.0 to 7.0, Appendix V L.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* and a mixture of 10 volumes of *water*, 15 volumes of *methanol*, 25 volumes of *acetic acid* and 50 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 15 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dissolve a sufficient quantity of the contents of the sealed container in a mixture of equal volumes of *methanol* and *water* to produce a solution containing 10.0% w/v of Ifosfamide. Solution (2) contains 0.025% w/v of *ifosfamide impurity A EPCRS* and 0.025% w/v of *chloroethylamine hydrochloride* (impurity C) in a mixture of equal volumes of *methanol* and *water.* Solution (3) contains 0.015% w/v of *ifosfamide impurity B EPCRS* in a mixture of equal volumes of *methanol* and *water*. Solution (4) contains 0.005% w/v of *ethanolamine,* 0.02% w/v of *ifosfamide impurity A EPCRS* and 0.08% w/v of *chloroethylamine hydrochloride* (impurity C) in a mixture of equal volumes of *methanol* and *water*. After removal of the plate, dry at 115° for 45 minutes. At the bottom of a chromatography tank, place an evaporating dish containing a 0.32% w/v solution of *potassium permanganate* and add an equal volume of *dilute hydrochloric acid*, close the tank and allow to stand for 10 minutes. Place the plate whilst still hot in the tank, avoiding contact of the stationary phase with the solution, and close the tank. Leave the plate in contact with the chlorine vapour for 20 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed (about 20 minutes) and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution*. Avoid prolonged exposure to cold air. Immerse the plate in a 0.1% w/v solution of *tetramethylbenzidine* in *ethanol (96%)* for 5 seconds. Allow the plate to dry and examine. In the chromatogram obtained with solution (1) any spot corresponding to impurity A or impurity C is not more intense than the corresponding spot in the chromatogram obtained with solution (2) (0.25%); any spot corresponding to impurity B is not more intense than the corresponding spot in the chromatogram obtained with solution (3) (0.15 %); any other spot is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.15%). The test is not valid unless the chromatogram obtained with solution (4) shows three clearly separated spots.

B. Carry out the method for *thin-layer chromatography*, Appendix II A, using a *TLC silica gel plate* and a mixture of 1 volume of *dichloromethane* and 10 volumes of *acetone* as the mobile phase but allowing the solvent front to ascend 15 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dissolve a sufficient quantity of the contents of the sealed container in a mixture of equal volumes of *methanol* and *water* to produce a solution containing 2.0% w/v of Ifosfamide. Solution (2) contains 0.005% w/v of *ifosfamide impurity E EPCRS* and 0.005% w/v of *ifosfamide impurity F EPCRS* in a mixture of equal volumes of *methanol* and *water.* Solution (3) contains 0.01% w/v of *ifosfamide impurity E EPCRS* and 0.01% w/v of *ifosfamide impurity F EPCRS* in a mixture of equal volumes of *methanol* and *water.* After removal of the plate dry at 115° for 45 minutes. At the bottom of a chromatography tank, place an evaporating dish containing a 0.32% w/v solution of *potassium permanganate* and add an equal volume of *dilute hydrochloric acid*, close the tank and allow to stand for 10 minutes. Place the plate whilst still hot in the tank, avoiding contact of the stationary phase with the solution, and close the tank. Leave the plate in contact with the chlorine vapour for 20 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed (about 20 minutes) and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution*. Avoid prolonged exposure to cold air. Immerse the plate in a 0.1% w/v solution of *tetramethylbenzidine* in *ethanol (96%)* for 5 seconds. Allow the plate to dry and examine. In the chromatogram obtained with solution (1) any spot corresponding to impurity E or impurity F is not more intense than the corresponding spot in the chromatogram obtained with solution (2) (0.25%). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

Water

Not more than 0.5% w/w, Appendix IX C. Use 1g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve a sufficient quantity of the contents of the sealed container to produce a solution containing 0.060% w/v of Ifosfamide.

(2) 0.060% w/v of *ifosfamide BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 195 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 30 volumes of *acetonitrile R1* and 70 volumes of *carbon dioxide-free water.*

DETERMINATION OF CONTENT

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in a container of average content weight using the declared content of $C_7H_{15}Cl_2N_2O_2P$ in *ifosfamide BPCRS*.

STORAGE

The sealed container should be kept protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ifosfamide. Related substances test A is intended to control degradation impurities and Related substances test B is intended to control synthetic impurities.

Imipramine Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Imipramine Tablets contain Imipramine Hydrochloride. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of imipramine hydrochloride, $C_{19}H_{24}N_2,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Triturate a quantity of the powdered tablets containing 0.25 g of Imipramine Hydrochloride with 10 ml of *chloroform*, filter, evaporate the filtrate to low volume, add *ether* until a turbidity is produced and allow to stand. The precipitate complies with the following tests.

A. *Melting point*, after recrystallisation from *acetone*, about 172°, Appendix V A.

B. Dissolve 5 mg in 2 ml of *nitric acid*. An intense blue colour is produced.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 5 volumes of *hydrochloric acid*, 5 volumes of *water*, 35 volumes of *glacial acetic acid* and 55 volumes of *ethyl acetate* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions prepared immediately before use. For solution (1) shake a quantity of the powdered tablets containing 0.20 g of Imipramine Hydrochloride with three 10 ml quantities of *chloroform*, filter the combined chloroform extracts, evaporate to dryness and dissolve the residue in 10 ml of *methanol*. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with *methanol* and dilute 1 volume of the resulting solution to 10 volumes with *methanol*. Solution (3) contains 0.0060% w/v of *iminodibenzyl* in *methanol*. After removal of the plate, allow it to dry for 5 minutes, spray with a 0.5% w/v solution of *potassium dichromate* in *sulphuric acid (20%)* and examine immediately. In the chromatogram obtained with solution (1) any spot corresponding to iminodibenzyl is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%).

ASSAY

Powder 10 tablets and transfer quantitatively without loss into 300 ml of 0.1M *hydrochloric acid*. Shake for 30 minutes, add sufficient 0.1M *hydrochloric acid* to produce 500 ml and filter through a fine glass microfibre filter (GF/C paper is suitable). Dilute a suitable volume of the filtrate with 0.1M *hydrochloric acid* to produce a solution containing 0.0025% w/v of Imipramine Hydrochloride and measure the *absorbance* of the resulting solution at the maximum at 250 nm, Appendix II B. Calculate the content of $C_{19}H_{24}N_2$,HCl taking 264 as the value of *A* (1%, 1 cm) at the maximum at 250 nm.

Indapamide Tablets

General Notices

Action and use

Thiazide-like diuretic.

DEFINITION

Indapamide Tablets contain Indapamide. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of indapamide hemihydrate, $C_{16}H_{16}ClN_3O_3S,½H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* F_{254} *plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *acetone* and 80 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) grind a quantity of the powdered tablets containing 50 mg of Indapamide with 10 ml of *acetone*, mix for 15 minutes, filter through a fine filter paper (Whatman 42 is suitable) and use the filtrate. Solution (2) contains 0.5% w/v of *indapamide EPCRS* in *acetone*. After removal of the plate, allow it to dry until the solvent has evaporated and examine under *ultraviolet light (254 nm)*. Spray the plate with a solution prepared by mixing 10 volumes of *potassium iodobismuthate solution* and 20 volumes of *glacial acetic acid* and diluting to 100 volumes with *water* and examine again. Finally, spray the plate with a 5% w/v solution of *sodium nitrite* in a mixture of equal volumes of *water* and *ethanol (96%)* and examine again. By each method of visualisation, the principal spot in the chromatogram obtained with solution (1) is similar in position, colour and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of 0.1M *hydrochloric acid* and rotate the paddle at 100 revolutions per minute. After 60 minutes, withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, suitably diluted with 0.1M *hydrochloric acid* if necessary, at 240 nm and at 275 nm using 0.1M *hydrochloric acid* in the reference cell. Prepare a reference solution by diluting 1 volume of a 0.10% w/v solution of *indapamide EPCRS* in *methanol* to 200 volumes with 0.1M *hydrochloric acid* and measure the *absorbance* of this solution at 240 nm and at

275 nm using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of indapamide hemihydrate, $C_{16}H_{16}ClN_3O_3S,½H_2O$, in the medium using the differences in absorbance at 240 nm and at 275 nm and using the declared content of $C_{16}H_{16}ClN_3O_3S$ in *indapamide EPCRS*. Each mg of $C_{16}H_{16}ClN_3O_3S$ is equivalent to 1.0246 mg of $C_{16}H_{16}ClN_3O_3S,½H_2O$. The amount of indapamide hemihydrate released is not less than 75% of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions. For solution (1) mix 10 whole tablets with 70 ml of *ethanol (96%)*, mix mechanically until the tablets have disintegrated and continue mixing for 2 hours. Add sufficient *ethanol (96%)* to produce 100 ml, mix and centrifuge. Dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005% w/v of Indapamide. For solution (2) dilute 1 volume of solution (1) to 10 volumes with the mobile phase and further dilute 1 volume of this solution to 100 volumes with the mobile phase. For solution (3) dilute 1 volume of a 0.00025% w/v solution of *indapamide impurity B EPCRS* in *ethanol (96%)* to 10 volumes with the mobile phase. For solution (4) dilute 1 volume of a 0.00025% w/v solution of *4-chloro-3-sulfamoylbenzoic acid* in *ethanol (96%)* to 10 volumes with the mobile phase. For solution (5) mix one volume of solution (1), one volume of a 0.00025% w/v solution of *indapamide impurity B EPCRS* in *ethanol (96%)* and 1 volume of a 0.00025% w/v solution of *4-chloro-3-sulfamoylbenzoic acid* in *ethanol (96%)* and add 7 volumes of the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.6 ml per minute a mixture of 6 volumes of a solution containing 5% w/v of *sodium dodecyl sulphate* and 3% v/v of *glacial acetic acid*, 10 volumes of *triethylamine*, 20 volumes of *butan-2-ol*, 310 volumes of *acetonitrile* and 690 volumes of *water*, the mixture being adjusted to pH 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 240 nm.

The test is not valid unless, in the chromatogram obtained with solution (5), the retention time of impurity B relative to indapamide is about 1.7 and the retention time of 4-chloro-3-sulfamoylbenzoic acid relative to indapamide is about 0.3.

In the chromatogram obtained with solution (1), the area of any peak corresponding to indapamide impurity B is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%), the area of any peak corresponding to 4-chloro-3-sulfamoylbenzoic acid is not greater than 0.4 times the area of the peak in the chromatogram obtained with solution (4) (0.2%) and the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%). The sum of the content of impurities, excluding indapamide impurity B, is not greater than 0.3%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions. For solution (1) mix 10 whole tablets with 70 ml of *ethanol (96%)*, mix mechanically until the tablets have disintegrated and continue mixing for 2 hours. Add sufficient *ethanol (96%)* to produce 100 ml, mix and centrifuge. Dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005% w/v of Indapamide. For solution (2) dilute 1 volume of a 0.025% w/v solution of *indapamide EPCRS* in *ethanol (96%)* to 5 volumes with the mobile phase.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{16}H_{16}ClN_3O_3S,½H_2O$ in the tablets from the chromatogram obtained and using the declared content of $C_{16}H_{16}ClN_3O_3S$ in *indapamide EPCRS*. Each mg of

$C_{16}H_{16}ClN_3O_3S$ is equivalent to 1.0246 mg of $C_{16}H_{16}ClN_3O_3S,\frac{1}{2}H_2O$.

STORAGE

Indapamide Tablets should be protected from light.

Indometacin Capsules

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Indometacin Capsules contain Indometacin.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of indometacin, $C_{19}H_{16}ClNO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.1 g of Indometacin with 5 ml of *chloroform*, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of indometacin *(RS 187)*.

B. The *light absorption*, Appendix II B, in the range 300 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 320 nm.

C. Mix a quantity of the contents of the capsules containing 25 mg of Indometacin with 2 ml of *water* and add 2 ml of 2M *sodium hydroxide*. A bright yellow colour is produced which fades rapidly.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 7.2* and rotate the paddle at 50 revolutions per minute. Withdraw a 10 ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 320 nm, Appendix II B, using *phosphate buffer pH 7.2* in the reference cell. Calculate the total content of indometacin, $C_{19}H_{16}ClNO_4$, in the medium taking 196 as the value of A(1%, 1 cm) at the maximum at 320 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a suspension of *silica gel* HF_{254} in a 4.68% w/v solution of *sodium dihydrogen orthophosphate* to coat the plate and a mixture of 70 volumes of *ether* and 30 volumes of *petroleum spirit (boiling range, 60° to 80°)* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.10 g of Indometacin with 5 ml of *chloroform*, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

To a quantity of the mixed contents of 20 capsules containing 50 mg of Indometacin add 10 ml of *water* and allow to stand for 10 minutes, swirling occasionally. Add 75 ml of *methanol*, shake well, add sufficient *methanol* to produce 100 ml and filter if necessary. To 5 ml of the filtrate add sufficient of a mixture of equal volumes of *methanol* and *phosphate buffer pH 7.2* to produce 100 ml. Measure the *absorbance* of the resulting solution at the maximum at 320 nm, Appendix II B. Calculate the content of $C_{19}H_{16}ClNO_4$ taking 193 as the value of A(1%, 1 cm) at the maximum at 320 nm.

Indometacin Suppositories

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Indometacin Suppositories contain Indometacin in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of indometacin, $C_{19}H_{16}ClNO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the suppositories containing 0.1 g of Indometacin as completely as possible in 50 ml of hot *water*, filter, wash the residue with hot *water* and allow to dry in air. Dissolve the residue in 5 ml of *chloroform* and evaporate to dryness. The *infrared absorption spectrum* of the final residue, Appendix II A, is concordant with the *reference spectrum* of indometacin *(RS 187)*.

B. Shake a quantity of the suppositories containing 25 mg of Indometacin with 5 ml of *water* until the basis dissolves; a white suspension is produced. Add 2 ml of 2M *sodium hydroxide*; a bright yellow colour is produced which fades rapidly.

TESTS

Disintegration

Carry out the *disintegration test for suppositories and pessaries*, Appendix XII A2, using a weighed suppository and *phosphate buffer pH 6.8* in place of *water* and operating the apparatus for 90 minutes. At the end of this period remove the suppository, dry with filter paper and weigh. Repeat the operation with two further weighed suppositories. Not less than 75% of each suppository is dissolved.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) dilute 3 volumes of solution (2) to 100 volumes with the mobile phase. For solution (2) powder or cut into small pieces a suitable number of the suppositories, dissolve a quantity containing 0.1 g of Indometacin in sufficient *methanol* to produce 50 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) a mixture of 40 volumes of 0.2% v/v solution of *orthophosphoric acid* and 60 volumes of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a

detection wavelength of 320 nm.

The *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (1), should be not less than 7500 theoretical plates per metre.

The sum of the areas of any *secondary peaks* that elute before the principal peak in the chromatogram obtained with solution (2) is not greater than the area of the peak in the chromatogram obtained with solution (1).

Repeat the procedure but using the following freshly prepared solutions and a detection wavelength of 240 nm. For solution (3) dilute 10 volumes of solution (2) to 20 volumes with the mobile phase. Solution (4) contains 0.001% w/v of *4-chlorobenzoic acid* in the mobile phase. In the chromatogram obtained with solution (3) the sum of the areas of any *secondary peaks* that elute before the principal peak, other than those determined in solution (2), is not greater than the area of the peak in the chromatogram obtained with solution (4).

ASSAY

Weigh 10 suppositories and powder or cut into small pieces. To a quantity containing 0.1 g of Indometacin add 50 ml of *methanol*, shake until dispersion is complete and, if necessary, filter. To 2 ml add sufficient of a mixture of equal volumes of *methanol* and *phosphate buffer pH 7.2* to produce 100 ml. Measure the *absorbance* of the resulting solution at the maximum at 318 nm, Appendix II B. Calculate the content of $C_{19}H_{16}ClNO_4$ taking 193 as the value of A (1%, 1 cm) at the maximum at 318 nm.

Indoramin Tablets

General Notices

Action and use

Alpha$_1$-adrenoceptor antagonist.

DEFINITION

Indoramin Tablets contain Indoramin Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of indoramin, $C_{22}H_{25}N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of indoramin with 25 ml of *water* for 5 minutes, filter (Whatman GF/C paper is suitable), make the filtrate alkaline with 2M *sodium hydroxide* and extract with 20 ml of *dichloromethane.* Wash the extracts with two 10 ml quantities of *water*, dry by shaking with *anhydrous sodium sulphate* and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of indoramin *(RS 188).*

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 18M *ammonia,* 20 volumes of *absolute ethanol* and 79 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 10 ml of *ethanol (96%)* to a quantity of the powdered tablets containing the equivalent of 0.1 g of indoramin, shake for 30 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *ethanol (96%).* For solution (3) dilute 1 volume of solution (2) to 5 volumes with *ethanol (96%).* After removal of the plate, allow it to dry in a current of warm air and examine it under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.1%). Disregard any spot remaining on the line of application.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use 900 ml of 0.1M *hydrochloric acid* as the medium and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 280 nm, Appendix II B. Calculate the total content of indoramin, $C_{22}H_{25}N_3O$, in the medium taking 186 as the value of A(1%, 1 cm) at the maximum at 280 nm.

ASSAY

Shake 10 whole tablets with 50 ml of 0.1M *hydrochloric acid* until the tablets have disintegrated, add 300 ml of *ethanol (96%)* and mix with the aid of ultrasound for 10 minutes, shaking occasionally. Add sufficient *ethanol (96%)* to produce 500 ml, filter (Whatman GF/C paper is suitable), dilute the filtrate with *ethanol (96%)* to contain 0.005% w/v of indoramin and measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of $C_{22}H_{25}N_3O$ taking 186 as the value of A(1%, 1 cm) at the maximum at 280 nm.

STORAGE

Indoramin Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of indoramin.

Injectable Insulin Preparations

General Notices

Insulin Preparations

(Ph Eur monograph 0854)

For preparations of Human Insulin, the label states the approved code in lower case letters indicative of the method of production.

The provisions of this monograph apply to sterile preparations of Human Insulin and its analogues, Bovine Insulin and Porcine Insulin.

The provisions of this monograph apply to the Injectable Insulin Preparations listed below.

Preparations

Biphasic Insulin Injection

Biphasic Isophane Insulin Injection

Insulin Injection

Insulin Aspart Injection

Insulin Lispro Injection

Insulin Zinc Suspension

Insulin Zinc Suspension (Amorphous)

Insulin Zinc Suspension (Crystalline)

Isophane Insulin Injection

Protamine Zinc Insulin Injection

Ph Eur

Injectable insulin preparations comply with the requirements for Injections prescribed in the monograph on Parenteral preparations (0520).

DEFINITION

Injectable insulin preparations are sterile preparations of *Insulin, human (0838)*, *Insulin, bovine (1637)* or *Insulin, porcine (1638)*. They contain not less than 90.0 per cent and not more than the equivalent of 110.0 per cent of the amount of insulin stated on the label. They are either solutions or suspensions or they are prepared by combining solutions and suspensions.

PRODUCTION

The methods of preparation are designed to confer suitable properties with respect to the onset and duration of therapeutic action.

The following procedures are carried out in a suitable sequence, depending on the method of preparation:

- addition of suitable antimicrobial preservatives;
- addition of a suitable substance or substances to render the preparation isotonic with blood;
- addition of a suitable substance or substances to adjust the pH to the appropriate value;
- determination of the strength of the insulin-containing component or components followed, where necessary, by adjustment so that the final preparation contains the requisite number of International Units per millilitre;
- sterilisation by filtration of the insulin-containing component or components; once this procedure has been carried out all subsequent procedures are carried out aseptically using materials that have been sterilised by a suitable method.

In addition, where appropriate, suitable substances are added and suitable procedures carried out to confer the appropriate physical form on the insulin-containing component or components. The final preparation is distributed aseptically into sterile containers which are closed so as to exclude microbial contamination.

TESTS

pH *(2.2.3)*

The pH of the solution or suspension is 6.9 to 7.8, unless otherwise prescribed in the specific monograph.

Insulin in the supernatant

For injectable insulin preparations that are suspensions, not more than 2.5 per cent of the total insulin content, unless otherwise stated. Centrifuge 10 ml of the suspension at 1500 g for 10 min and carefully separate the supernatant liquid and the residue. Determine the insulin content of the supernatant liquid (*S*) by a suitable method, for example using the chromatographic conditions described under Assay. Calculate the percentage of the insulin in solution from the expression:

$$\frac{100S}{T}$$

where T is the total insulin content determined as described under the Assay.

Impurities with molecular masses greater than that of insulin

Examine by size-exclusion chromatography *(2.2.30)*.

Test solution Add 4 µl of *6 M hydrochloric acid R* per millilitre of the preparation to be examined, whether a suspension or a solution, to obtain a clear acid insulin solution. When sampling a suspension, agitate the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 min of the initial addition of hydrochloric acid, add small aliquots of acid (less than 4 µl per millilitre) until a solution is obtained. Preparations with concentrations higher than 100 IU/ml need to be diluted with *0.01 M hydrochloric acid* to avoid overloading the column with insulin monomer.

Resolution solution Use a solution of insulin (approximately 4 mg/ml), containing more than

0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid R*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about ten days.

Maintain the solutions at 2 °C to 10 °C and use within 30 h (soluble insulin injection) or 7 days (other insulin preparations). If an automatic injector is used, maintain the temperature at 2 °C to 10 °C.

The chromatographic procedure may be carried out using:

— a column 0.3 m long and at least 7.5 mm in internal diameter packed with hydrophilic silica gel for chromatography R (5 µm to 10 µm), of a grade suitable for the separation of insulin monomer from dimers and polymers;

— as mobile phase at a flow rate of 0.5 ml/min a mixture consisting of 15 volumes of glacial acetic acid R, 20 volumes of acetonitrile R and 65 volumes of a 1.0 g/l solution of arginine R; filter and degas;

— as detector a spectrophotometer set at 276 nm.

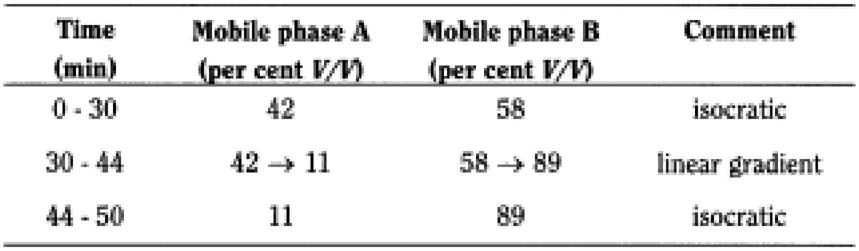
Equilibration of the column Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least three injections of the resolution solution. The column is equilibrated when repeatable results are obtained from two subsequent injections. If protamine-containing samples are to be analysed, the equilibration of the column is performed using a solution containing protamine.

Inject 100 µl of the resolution solution. When the chromatograms are recorded under the prescribed conditions, the retention times are: polymeric insulin complexes or covalent insulin-protamine complex: about 13 min to 17 min, covalent insulin dimer: about 17.5 min, insulin monomer: about 20 min, salts: about 22 min. If the sample solution contains preservatives, for example methyl paraben, m-cresol or phenol, these compounds elute later. The test is not valid unless the resolution, defined by the ratio of the height of the dimer peak to the height above the baseline of the valley separating the monomer and dimer peaks, is at least 2.0.

Inject 100 µl of the test solution. Record the chromatogram for approximately 35 min. In the chromatogram obtained, the sum of the areas of any peak with a retention time less than that of the insulin peak is not greater than 3.0 per cent (protamine containing preparations) or 2.0 per cent (non-protamine containing preparations) of the total area of the peaks. Disregard any peak with a retention time greater than that of the insulin peak.

Related proteins

Examine by liquid chromatography (*2.2.29*) as described under Assay, following the elution conditions as described in the table below:



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 30	42	58	isocratic
30 - 44	42 → 11	58 → 89	linear gradient
44 - 50	11	89	isocratic

Maintain the solutions at 2 °C to 10 °C and use within 24 h. Perform a system suitability check (resolution, linearity) as described under Assay. If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µl of the test solution and 20 µl of either reference solution (a), for insulin preparations containing 100 IU/ml, or reference solution (b), for insulin preparations containing 40 IU/ml. If necessary, adjust the injection volume to a volume between 10 µl and 20 µl in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for approximately 50 min. If necessary, make further adjustments to the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show a shorter retention time. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. In the chromatogram obtained with either reference solution (a), or reference solution (b), as appropriate, A21 desamido insulin appears as a small peak after the principal peak and has a retention time of about 1.3 relative to the principal peak, due to insulin. In the chromatogram obtained with the test solution the area of the peak due to A21 desamido insulin is not greater than 5.0 per cent of the total area of the peaks; the sum of the areas of any other peaks, apart from those due to insulin and A21 desamido insulin is not greater than 6.0 per cent of the total area of the peaks. Disregard the peaks due to the preservatives and protamine (early eluting peaks).

Total zinc

Not more than the amount stated in the individual monograph, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Use the following method, unless otherwise prescribed in the specific monograph.

Test solution Shake the preparation gently and dilute a volume containing 200 IU of insulin to 25.0 ml with *0.01 M hydrochloric acid*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/ml Zn) R* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Zinc in solution

Where applicable, not more than the amount stated in the individual monograph, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution Centrifuge the preparation to be examined and dilute 1 ml of the clear supernatant liquid obtained to 25.0 ml with *water R*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per millilitre) with *water R*.

Reference solutions Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/ml Zn) R* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins *(2.6.14)*

Less than 80 IU per 100 IU of insulin.

ASSAY

Examine by liquid chromatography *(2.2.29)*.

Test solution Add 4 µl of *6 M hydrochloric acid R* per millilitre of the preparation to be examined, whether a suspension or a solution, to obtain a clear solution. When sampling a suspension, shake the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 min of the initial addition of acid, add small aliquots of acid (less than 4 µl per millilitre) until a solution is obtained. For a preparation containing more than 100 IU ml, an additional dilution with *0.01 M hydrochloric acid* is necessary to avoid overloading the column.

Reference solution (a) For a preparation containing a single species of insulin, dissolve in *0.01 M hydrochloric acid*, as appropriate, the contents of a vial of *human insulin CRS*, *porcine insulin CRS* or *bovine insulin CRS* to obtain a concentration of 4.0 mg/ml. For a preparation containing both bovine and porcine insulins, mix 1.0 ml of a solution containing 4.0 mg of *bovine insulin CRS* per millilitre of *0.01 M hydrochloric acid* and 1.0 ml of a solution containing 4.0 mg of *porcine insulin CRS* per millilitre of *0.01 M hydrochloric acid*. *Reference solution (a) is used for the assay of insulin preparations containing 100 IU/ml*.

Reference solution (b) Dilute 4.0 ml of reference solution (a) to 10.0 ml with *0.01 M hydrochloric acid*. *Reference solution (b) is used for the assay of insulin preparations containing 40 IU/ml*.

Reference solution (c) Dissolve the contents of a vial of *human insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (d) Dissolve the contents of a vial of *porcine insulin CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg/ml.

Reference solution (e) Dilute 1.0 ml of reference solution (a) to 10.0 ml with *0.01 M hydrochloric acid*.

Reference solution (f) Dilute 1.0 ml of reference solution (b) to 10.0 ml with *0.01 M hydrochloric acid*.

Resolution solution Mix 1.0 ml of reference solution (c) and 1.0 ml of reference solution (d).

Maintain the solutions at 2 °C to 10 °C and use within 48 h. If an automatic injector is used, maintain the temperature at 2 °C to 10 °C.

The chromatographic procedure may be carried out using:

— a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with octadecylsilyl silica gel for chromatography R (5 µm);

— as mobile phase at a flow rate of 1 ml/min the following solutions prepared and maintained at a temperature not lower than 20 °C:

Mobile phase A Dissolve 28.4 g of *anhydrous sodium sulphate R* in *water R* and dilute to 1000 ml with the same solvent; add 2.7 ml of *phosphoric acid R*; adjust the pH to 2.3, if necessary, with *ethanolamine R*; filter and degas;

Mobile phase B Mix 550 ml of mobile phase A with 450 ml of *acetonitrile R*. Warm the solution to a temperature not lower than 20 °C in order to avoid precipitation (mixing of mobile phase A with acetonitrile is endothermic); filter and degas;

— as detector a spectrophotometer set at 214 nm;

maintaining the temperature of the column at 40 °C.

Elute with a mixture of 42 volumes of mobile phase A and 58 volumes of mobile phase B, adjusted if necessary.

Inject 20 µl of the resolution solution and 20 µl of reference solution (d). Record the chromatogram of the resolution solution until the peak corresponding to the principal peak in the chromatogram obtained with reference solution (d) is clearly visible. In the chromatogram obtained with the resolution solution, identify the peaks due to porcine insulin and human insulin. The test is not valid unless the resolution between the peaks due to human insulin and porcine insulin is at least 1.2. If necessary, adjust the concentration of acetonitrile in the mobile phase until this resolution is achieved.

Inject 20 µl of the test solution and 20 µl of either reference solutions (a) and (e), for insulin preparations containing 100 IU/ml, or 20 µl of reference solutions (b) and (f), for insulin preparations containing 40 IU/ml. If necessary, make further adjustments of the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution wash the column with a mixture of equal volumes of *acetonitrile R* and *water R* for a sufficient time to ensure elution of any interfering substances before injecting the next solution. The test is not valid unless the area of the principal peak in the chromatogram obtained with reference solution (a) or (b) is 10 ± 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) or (f). If this test fails, adjust the injection volume between 10 µl and 20 µl, in order to be in the linearity range of the detector.

Calculate the content of insulin plus A21 desamido insulin from the area of the peak due to the bovine, porcine or human insulin and that of any peak due to the A21 desamido insulin, using the declared content of insulin plus A21 desamido insulin in *bovine insulin CRS*, *porcine insulin CRS* or *human insulin CRS* , as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of the peaks due to the A21 desamido insulin(1) derivatives.

STORAGE

Unless otherwise prescribed, store in a sterile, airtight, tamper-proof container, protected from light, at a temperature of 2 °C to 8 °C. Insulin preparations are not to be frozen.

LABELLING

The label states:

— the potency in International Units per millilitre;

— the concentration in terms of the number of milligrams of insulin per millilitre (for preparations containing both bovine insulin and porcine insulin the concentration is stated as the combined amount of both insulins);

— where applicable, that the substance is produced by enzymatic modification of porcine insulin;

— where applicable, that the substance is produced by recombinant DNA technology;

— where applicable, the animal species of origin;

— that the preparation must not be frozen;

— where applicable, that the preparation must be resuspended before use.

Ph Eur

[1] 100 IU are equivalent to 3.47 mg of human insulin, to 3.45 mg of porcine insulin, and to 3.42 of bovine insulin.

Inositol Nicotinate Tablets

General Notices

Action and use

Vasodilator.

DEFINITION

Inositol Nicotinate Tablets contain Inositol Nicotinate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of inositol nicotinate, $C_{42}H_{30}N_6O_{12}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 1 g of Inositol Nicotinate with 30 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of inositol nicotinate *(RS 190)*. Retain the remainder of the residue for use in the Related substances test.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with 1M *hydrochloric acid* if necessary, at the maximum at 262 nm using 1M *hydrochloric acid* in the reference cell. Calculate the total content of inositol nicotinate, $C_{42}H_{30}N_6O_{12}$, in the medium taking 398 as the value of A(1%, 1 cm) at the maximum at 262 nm.

Free nicotinic acid

To a quantity of the powdered tablets containing 1 g of Inositol Nicotinate add 75 ml of water, shake for 15 minutes and titrate with 0.02M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 1.0 ml of 0.02M *sodium hydroxide VS* is required to produce the first pink colour.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a plate 200 mm × 200 mm in size and *silica gel* GF_{254} as the coating substance. For the first development use a mixture of 10 volumes of *methanol* and 90 volumes of *chloroform* as the mobile phase. Apply to the bottom right-hand corner of the plate 5 µl of solution (1) containing 5.0% w/v of the residue obtained in test A for Identification in a mixture of 1 volume of *methanol* and 9 volumes of *chloroform* and develop over a path of 12 cm. After removal of the plate, allow it to dry in air and turn the plate through 90° in a clockwise direction. Apply separately to the bottom right-hand corner of the plate, and to the right of the solvent front, 5 µl of each of two solutions of the residue obtained in test A for Identification in a mixture of 1 volume of *methanol* and 9 volumes of *chloroform* containing (2) 0.075% w/v and (3) 0.050% w/v, respectively. For the second development use a mixture of 5 volumes each of *glacial acetic acid*, *ethanol (96%)* and *water* and 50 volumes of *ethyl acetate* as the mobile phase. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 0.25 g of Inositol Nicotinate add 50 ml of *anhydrous acetic acid*, heat to boiling and allow to cool. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 13.51 mg of $C_{42}H_{30}N_6O_{12}$.

Insulin Aspart Injection

General Notices

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Insulin Aspart Injection is a sterile, neutral, aqueous solution of Insulin Aspart.
The injection complies with the requirements stated under Insulin Preparations with the modifications described below.

Content of insulin aspart, $C_{256}H_{381}N_{65}O_{79}S_6$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless liquid, free from turbidity and foreign matter; during storage traces of a very fine sediment may be deposited.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related proteins

Carry out the method for *liquid chromatography,* Appendix III D, as described under Assay using the *normalisation procedure*.

LIMITS

In the chromatogram obtained with solution (1), the area of the peak corresponding to B28isoAsp insulin aspart is not more than 2.5%, the total area of the peaks corresponding to A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart is not more than 5%, and the total area of other peaks corresponding to impurities is not more than 3.5%.

Total zinc

Not more than 40 µg per 100 units of insulin aspart, determined by *atomic absorption spectrometry*, Appendix II D Method I.

Test solution Shake the preparation gently and dilute a volume containing 100 units of insulin aspart to 25.0 ml with 0.01M *hydrochloric acid*. Dilute, if necessary, to a suitable concentration of zinc (for example 0.1 µg to 1.0 µg of Zn per millilitre) with 0.01M *hydrochloric acid*.

Reference solutions Use solutions containing a suitable range of concentrations, for example 0.20 µg, 0.40 µg, 0.60 µg, 0.80 µg and 1.00 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/ml Zn)* with 0.01M *hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 80 IU of endotoxin per 100 units of insulin aspart.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to produce a solution containing 0.4% w/v insulin aspart. Add 4µl of 6M *hydrochloric acid* per ml to this solution to obtain a clear acid solution. Maintain the solution at 2° to 8° and use within 48 hours.

(2) Dissolve the contents of a vial of *insulin aspart EPCRS* in 0.01M *hydrochloric acid* to produce a solution containing 0.4% w/v insulin aspart. Maintain the solution at 2° to 8° and use within 48 hours.

(3) Use an appropriate solution with a content of B3Asp insulin aspart and A21Asp insulin aspart of not less than 1%. This may be achieved by storing solution (2) at room temperature for 1 to 3 days. Maintain the solution at 2° to 8° and use within 72 hours.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).

(b) Gradient elution using the mobile phases described below.

(c) Flow rate of 1 ml per minute.

(d) Column temperature of 35°.

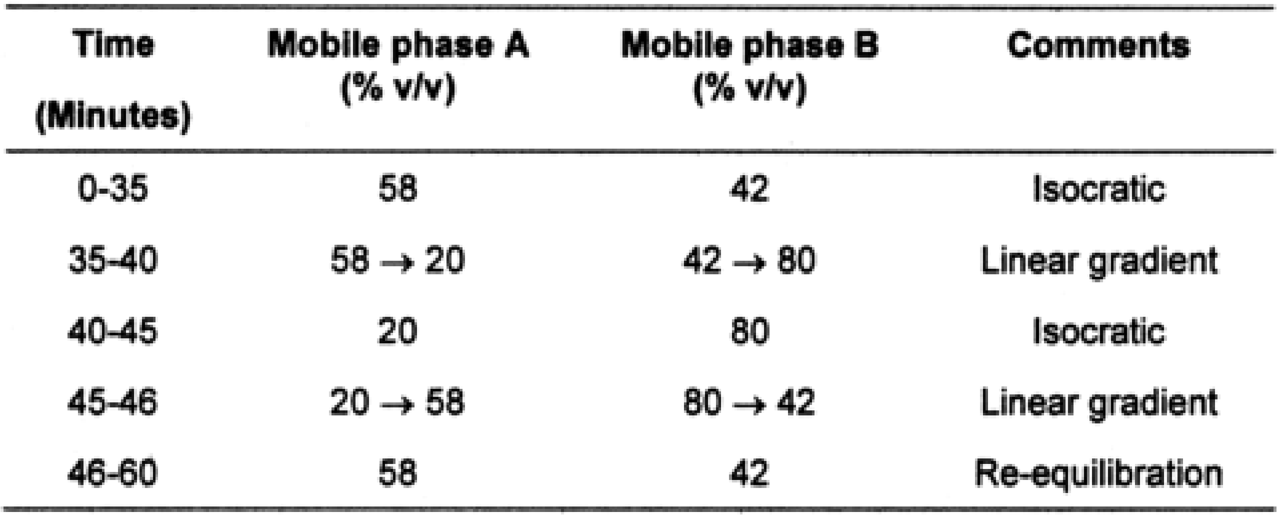
(e) Detection wavelength of 214 nm.

(f) An injection volume of 10 µl for each solution.

MOBILE PHASE

Mobile phase A Dissolve 70 g of *anhydrous sodium sulphate* in approximately 4500 ml of *water*; add 6.5 ml of *orthophosphoric acid* and adjust to pH 3.4, if necessary, with *dilute sodium hydroxide solution.* Dilute to 5000 ml with *water*; filter and degas. Mix 9 volumes of the solution with 1 volume of *acetonitrile for chromatography*.

Mobile phase B Mix equal volumes of *water* and *acetonitrile for chromatography*.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-35	58	42	Isocratic
35-40	58 → 20	42 → 80	Linear gradient
40-45	20	80	Isocratic
45-46	20 → 58	80 → 42	Linear gradient
46-60	58	42	Re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retention times with reference to insulin aspart (retention time, 20 to 26 minutes) are: B28isoAsp insulin aspart, approximately 0.9; B3Asp insulin aspart plus A21Asp insulin aspart (generally co eluted), approximately 1.3; B3isoAsp insulin aspart, approximately 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to insulin aspart and the peak due to A21Asp insulin aspart plus B3Asp insulin aspart is greater than 1.6.

The test is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 1.8.

DETERMINATION OF CONTENT

Calculate the content of insulin aspart $C_{256}H_{381}N_{65}O_{79}S_6$, together with the content of B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart using the areas of the corresponding peaks in the chromatograms obtained with solution (1) and solution (2) and the declared content of insulin aspart together with the content of B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart(1) in *insulin aspart EPCRS*.

LABELLING

The label states the potency in units per ml.

[1] 100 units are equivalent to 3.50 mg of insulin aspart.

Insulin Injection

General Notices

Neutral Insulin; Neutral Insulin Injection; Soluble Insulin
(Soluble Insulin Injection, Ph Eur monograph 0834)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Soluble insulin injection complies with the monograph on Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION

Soluble insulin injection is a neutral, sterile solution of bovine, porcine or human insulin.

CHARACTERS

A colourless liquid, free from turbidity and foreign matter; during storage, traces of a very fine sediment may be deposited.

IDENTIFICATION

Examine the chromatograms obtained in the assay. The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak obtained with the appropriate reference solution.

TESTS

Total zinc

Not more than 40.0 µg per 100 IU of insulin.

Determine by the method described in the monograph on *Insulin preparations, injectable (0854).*

Use the following test solution.

Test solution Dilute a volume of the gently shaken preparation containing 200 IU to 25.0 ml with *water R*. Dilute if necessary to a suitable concentration (for example, 0.4 µg to 1.6 µg of Zn per millilitre) with *water R*.

Ph Eur

Insulin Lispro Injection

General Notices

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Insulin Lispro Injection is a sterile, aqueous solution of Insulin Lispro.

The injection complies with the requirements stated under Insulin Preparations, with the exception of the test for Insulin in the supernatant, and with the modifications described below.

Content of insulin lispro, $C_{257}H_{383}N_{65}O_{77}S_6$

90.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear and colourless solution, free from turbidity and foreign matter.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Impurities with molecular masses greater than that of insulin lispro

The sum of the areas of the peaks in the chromatogram obtained with the test solution with a retention time less than that of the principal peak is not more than 1.5% of the total area of the peaks. Disregard any peak with a retention time greater than that of the peak due to insulin lispro monomer.

Related proteins

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to produce a solution containing 0.35% w/v of Insulin Lispro. Add 4 µl of 6M *hydrochloric acid* per ml of this solution. Maintain the solution at 2° to 8° and use within 56 hours.

(2) Prepare a 0.35% w/v solution of Insulin Lispro in 0.01M *hydrochloric acid* and add 4 µl of 6M *hydrochloric acid* per ml. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido insulin lispro.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for* *chromatography* (5 µm) with a pore size of 30 nm (Vydac Protein and Peptide C18 is suitable).

(b) Gradient system using the mobile phases described below.

(c) Flow rate of 1 ml per minute.

(d) Column temperature of 40°.

(e) Detection wavelength of 214 nm.

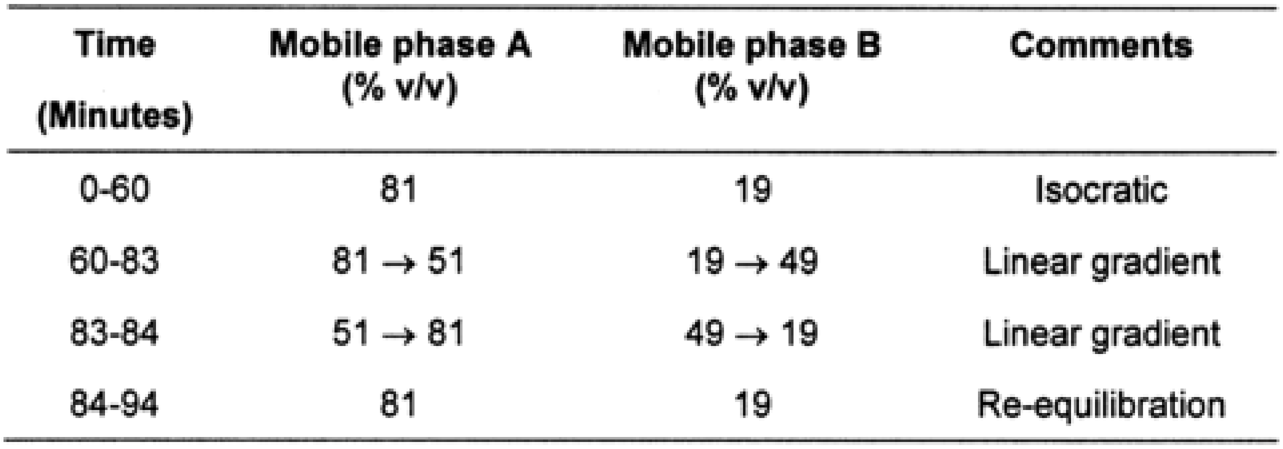
(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

Mobile phase A Mix 82 volumes of a 2.84% w/v solution of *anhydrous sodium sulphate* adjusted to pH 2.3 with *orthophosphoric acid* and 18 volumes of *acetonitrile for chromatography*.

Mobile phase B Mix equal volumes of a 2.84% w/v solution of *anhydrous sodium sulphate,* adjusted to pH 2.3 with *orthophosphoric acid ,* and *acetonitrile for chromatography*.

Use the following gradient.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-60	81	19	Isocratic
60-83	81 → 51	19 → 49	Linear gradient
83-84	51 → 81	49 → 19	Linear gradient
84-94	81	19	Re-equilibration

SYSTEM SUITABILITY

Adjust the mobile phase composition to obtain a retention time of about 41 minutes for insulin lispro; A21 desamido insulin lispro elutes near the start of the gradient elution. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the first peak (insulin lispro) and the second peak (A21 desamido insulin lispro) is at least 1.5 and the *symmetry factor* for the peak due to insulin lispro is less than 2.0.

LIMITS

In the chromatogram obtained with solution (1), the amount of A21 desamido insulin lispro is not more than 1.5%, and that of any other impurity is not greater than 0.5% by *normalisation.* The total amount of related proteins (excluding A21) is not greater than 4.0%.

Total zinc

14 to 35 µg per 100 units of insulin lispro, determined by *atomic absorption spectrometry*, Appendix II D, Method I.

Test solution Shake the preparation gently and dilute a volume containing 200 units of insulin lispro to 25.0 ml with 0.01M *hydrochloric acid*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per ml) with 0.01M *hydrochloric acid*.

Reference solutions Use solutions containing a suitable range of concentrations, for example 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per ml, freshly prepared by diluting *zinc standard solution (5 mg per ml Zn)* with 0.01M *hydrochloric acid.*

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 80 IU of endotoxin per 100 units of insulin lispro.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to obtain a 0.08% w/v solution. Add 4 µl of 6M *hydrochloric acid* per ml of this solution. Maintain the solution at 2 to 8° and use within 48 hours.

(2) Dissolve the contents of a vial of *insulin lispro EPCRS* in 0.01M *hydrochloric acid* to obtain a 0.08% w/v solution. Maintain the solution at 2° to 8° and use within 48 hours.

(3) Prepare a 0.1% w/v solution of the preparation being examined in 0.01M *hydrochloric acid* and add 4 µl of 6M *hydrochloric acid* per ml. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido insulin lispro. Maintain the solution at 2° to 8° and use within 14 days.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (10 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 0.8 ml per minute.

(d) Column temperature of 40°.

(e) Detection wavelength of 214 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

Mix 255 volumes of *acetonitrile for chromatography* and 745 volumes of a 2.84% w/v solution of *anhydrous sodium sulphate* adjusted to pH 2.3 with *orthophosphoric acid*.

SYSTEM SUITABILITY

When the chromatograms are recorded in the prescribed conditions, the retention time of insulin lispro is approximately 24 minutes. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the first peak (insulin lispro) and the second peak (A21 desamido insulin lispro), is greater than 1.8, and the maximum relative standard deviation for replicate injections is 1.1% after 3 injections.

DETERMINATION OF CONTENT

Calculate the content of insulin lispro $C_{257}H_{383}N_{65}O_{77}S_6$ from the chromatograms obtained and the declared content of $C_{257}H_{383}N_{65}O_{77}S_6$(1) in *insulin lispro EPCRS*.

LABELLING

The label states the potency in units per ml.

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[1] 100 IU are equivalent to 3.47 mg of insulin lispro.

Insulin Zinc Suspension (Amorphous)

General Notices

(Insulin Zinc Injectable Suspension (Amorphous), Ph Eur monograph 0835)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Insulin zinc injectable suspension (amorphous) complies with the monograph on Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION

Insulin zinc injectable suspension (amorphous) is a sterile neutral suspension of bovine, porcine or human insulin complexed with a suitable zinc salt; the insulin is in a form which is practically insoluble in water.

CHARACTERS

A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to have no uniform shape and a maximum dimension rarely exceeding 2 µm.

IDENTIFICATION

Examine the chromatograms obtained in the Assay. The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

TESTS

Total zinc

0.12 mg to 0.25 mg per 100 IU of insulin, determined as described in the monograph on *Insulin preparations, injectable (0854).*

Zinc in solution

20 per cent to 65 per cent of the total zinc is in the form of zinc in solution. Determine by the method described in the monograph on *Insulin preparations, injectable (0854).*

Ph Eur

Insulin Zinc Suspension (Crystalline)

General Notices

(Insulin Zinc Injectable Suspension (Crystalline), Ph Eur monograph 0836)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Insulin zinc injectable suspension (crystalline) complies with the monograph on Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION

Insulin zinc injectable suspension (crystalline) is a sterile neutral suspension of bovine, porcine or human insulin, complexed with a suitable zinc salt; the insulin is in a form which is practically insoluble in water.

CHARACTERS

A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rhombohedral crystals, the majority having a maximum dimension when measured from corner to corner through the crystal greater than 10 µm but rarely exceeding 40 µm.

IDENTIFICATION

Examine the chromatograms obtained in the Assay. The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

TESTS

Insulin not extractable with buffered acetone solution

Not less than 90 per cent of the total insulin content. Centrifuge a volume of the substance to be examined containing 200 IU of insulin and discard the supernatant liquid. Suspend the residue in 1.65 ml of *water R*, add 3.3 ml of *buffered acetone solution R*, stir for 3 min, again centrifuge, discard the supernatant liquid and repeat all the operations with the residue. Dissolve the residue using a suitable procedure, for example dissolve in *0.1 M hydrochloric acid* to give a final volume of 2.0 ml. Determine the insulin content of the residue (*R*) and determine the total insulin content (*T*) of an equal volume of the suspension by a suitable method. Calculate the percentage of insulin not extractable with buffered acetone solution from the expression:

$$\frac{100R}{T}$$

Total zinc

0.12 mg to 0.25 mg per 100 IU of insulin, determined as described in the monograph on *Insulin preparations, injectable (0854).*

Zinc in solution

20 per cent to 65 per cent of the total zinc is in the form of zinc in solution. Determine by the method described in the monograph on *Insulin preparations, injectable (0854).*

Ph Eur

Insulin Zinc Suspension

General Notices

Insulin Zinc Suspension, Mixed

(Insulin Zinc Injectable Suspension, Ph Eur monograph 0837)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Insulin zinc injectable suspension complies with the monograph on Insulin preparations, injectable (0854) with the amendments prescribed below.

DEFINITION

Insulin zinc injectable suspension is a sterile neutral suspension of bovine insulin and/or porcine insulin or of human insulin with a suitable zinc salt; the insulin is in a form which is practically insoluble in water.

PRODUCTION

Insulin zinc injectable suspension is prepared by carrying out the procedures described in the monograph on *Insulin preparations, injectable (0854).*

Insulin zinc injectable suspension is produced by mixing insulin zinc injectable suspension (crystalline) and insulin zinc injectable suspension (amorphous) in a ratio of 7 to 3.

CHARACTERS

A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the majority of the particles are seen to be rhombohedral crystals with a maximum dimension when measured from corner to corner through the crystal greater than 10 µm but rarely exceeding 40 µm; a considerable proportion of the particles are seen to have no uniform shape and a maximum dimension rarely exceeding 2 µm.

IDENTIFICATION

Examine the chromatograms obtained in the Assay.

For preparations made from a single species of insulin (bovine, porcine or human), the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution. For preparations made from a mixture of bovine and porcine insulin, the positions of the peaks due to the two insulins in the chromatogram obtained with the test solution correspond to those of the principal peaks in the chromatogram obtained with the appropriate reference solution.

TESTS

Insulin not extractable with buffered acetone solution

63 per cent to 77 per cent of the total insulin content. Centrifuge a volume of the substance to be examined containing 200 IU of insulin and discard the supernatant liquid. Suspend the residue in 1.65 ml of *water R*, add 3.3 ml of *buffered acetone solution R*, stir for 3 min, again centrifuge, discard the supernatant liquid and repeat all the operations with the residue. Dissolve the residue using a suitable procedure, for example dissolve in *0.1 M hydrochloric acid* to give a final volume of 2.0 ml. Determine the insulin content of the residue (*R*) and determine the total insulin content (*T*) of an equal volume of the suspension by a suitable method. Calculate the percentage of insulin not extractable with buffered acetone solution from the expression:

$$\frac{100R}{T}$$

Total zinc

0.12 mg to 0.25 mg per 100 IU of insulin, determined as described in the monograph on *Insulin preparations, injectable (0854).*

Zinc in solution

20 per cent to 65 per cent of the total zinc is in the form of zinc in solution. Determine by the method described in the monograph on *Insulin preparations, injectable (0854).*

Ph Eur

Inulin Injection

General Notices

Action and use

Diagnostic agent used in the determination of renal function (glomerular filtration rate).

DEFINITION

Inulin Injection is a sterile solution containing 10% w/v of Inulin and 0.8% w/v of Sodium Chloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of inulin

9.5 to 10.5% w/v.

Content of sodium chloride, NaCl

0.75 to 0.85% w/v.

Before carrying out the following tests dissolve any solid matter by heating in a water bath.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a suspension of *silica gel G* in a 0.3% w/v solution of *sodium acetate* to coat the plate, but spreading a layer 0.5 mm thick. Use a mixture of 10 volumes of *water*, 60 volumes of *chloroform* and 70 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) mix 1 volume of the injection with 3 volumes of *water*. For solution (2) mix 1 volume of the injection with 3 volumes of a 12.5% w/v solution of *oxalic acid*, boil the mixture for 10 minutes and cool before application. Solution (3) contains 2.5% w/v of D-*fructose* and 0.1% w/v of D-*glucose monohydrate* in *water*. Develop the chromatograms in a continuous elution tank for about 4 hours. After removal of the plate, evaporate the solvent in a current of warm air and spray with a solution in *acetone* containing 1% v/v of *aniline*, 1% v/v of *diphenylamine* and 1% v/v of *orthophosphoric acid* and heat for 10 minutes at 130°. The two principal spots in the chromatogram obtained with solution (2) correspond to those in the chromatogram obtained with solution (3). The spot in the chromatogram obtained with solution (1) remains on the line of application.

B. Dilute 1 ml to 20 ml with *water*. To 2 ml of the diluted solution add 3 ml of a 0.15% w/v solution of *resorcinol* in *ethanol (96%)* followed by 3 ml of *hydrochloric acid*, mix and heat at 80°. A red colour is produced.

C. Yields the *reactions* characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.0, Appendix V L.

Reducing sugars

Heat 5.0 ml of *cupri-tartaric solution R1* and titrate with the injection adding a few ml at a time and boiling for 10 to 15 seconds between each addition. When the solution becomes greenish-yellow, add 0.25 ml of a 1% w/v solution of *methylene blue* and continue the titration until the solution becomes orange. Repeat the operation using a 0.50% w/v solution of D-*fructose* in place of the injection. The volume of the fructose solution used is not greater than that of the injection.

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D. Use 10 ml per kg of the rabbit's weight.

ASSAY

For inulin

Dilute 50 ml with sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and measure the *optical rotation* using a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 1.299 represents the weight in g of inulin in the volume of the injection taken for assay.

For NaCl

Titrate 10 ml with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.845 mg of NaCl.

STORAGE

Inulin Injection deposits solid matter on storage. Before use, solid matter should be completely redissolved by heating for not more than 15 minutes; the solution should be cooled to a suitable temperature before administration and should not be reheated.

Iodised Oil Fluid Injection

General Notices

Action and use

Radio-opaque injection.

DEFINITION

Iodised Oil Fluid Injection is a sterile iodine addition product of the ethyl esters of the fatty acids obtained from poppy-seed oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of combined iodine

37.0 to 39.0% w/w.

CHARACTERISTICS

A straw-coloured or yellow, oily liquid.

Practically insoluble in *water*; soluble in *chloroform*, in *ether* and in *petroleum spirit (boiling range, 40° to 60°)*.

IDENTIFICATION

Boil 0.05 ml with 2 ml of *glacial acetic acid* and 0.1 g of *zinc powder* for 2 minutes, add 5 ml of *water*, shake, decant from any undissolved zinc and add 1 ml of *hydrogen peroxide solution (20 vol)*. Iodine vapour is evolved.

TESTS

Acidity

To 5 ml of *chloroform* add 5 ml of *ethanol (96%)* previously neutralised to *phenolphthalein solution R1* with 0.1M *ethanolic sodium hydroxide VS*, add 5 g of the injection, shake and titrate with 0.1M *ethanolic sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 1.0 ml is required to change the colour of the solution.

Weight per ml

1.28 to 1.30 g, Appendix V G.

Free iodine

Shake 1 g with 1 ml of *starch mucilage* and 1 ml of *cadmium iodide solution*. Any blue colour disappears on the addition of 0.4 ml of 0.01M *sodium thiosulphate*.

ASSAY

Boil 1 g with 10 ml of *glacial acetic acid* and 1 g of *zinc powder* under a reflux condenser for 1 hour. Add through the condenser 30 ml of hot *water*, filter through absorbent cotton, wash the flask with two 20 ml quantities of hot *water* and pass the washings through the filter. Cool the filtrate, add 25 ml of *hydrochloric acid* and 8 ml of *potassium cyanide solution* and titrate with 0.05M *potassium iodate VS* until the dark brown solution which is produced becomes light brown; add 5 ml of *starch mucilage* and continue the titration until the blue colour disappears. Each ml of 0.05M *potassium iodate VS* is equivalent to 12.69 mg of combined iodine.

STORAGE

Iodised Oil Fluid Injection should be kept in an atmosphere of carbon dioxide or nitrogen and protected from light.

Iofendylate Injection

General Notices

Action and use

Iodinated contrast medium.

DEFINITION

Iofendylate Injection is a sterile mixture of stereoisomers of ethyl 10-(4-iodophenyl) undecanoate ($C_{19}H_{29}IO_2$).
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of iofendylate, $C_{19}H_{29}IO_2$

98.0 to 101.0% w/w.

CHARACTERISTICS

Almost colourless to pale yellow, viscous liquid.

Very slightly soluble in *water*; freely soluble in *ethanol (96%)*; miscible with *chloroform* and with *ether*.

IDENTIFICATION

A. When heated with *sulphuric acid*, violet vapours of iodine are evolved.

B. To 1 ml add 15 ml of *water* and 7 g of *potassium dichromate*, cool and carefully add 10 ml of *sulphuric acid*. Allow to stand until the reaction ceases, heat under a reflux condenser for 2 hours, cool, pour into 25 ml of *water* and filter. Wash the residue with a little *water*, recrystallise from 10 ml of aqueous ethanol and sublime the crystals. The *melting point* of the sublimate is about 270°, Appendix V A.

TESTS

Acid value

Not more than 1.5, Appendix X B.

Refractive index

1.525 to 1.527, Appendix V E.

Saponification value

132 to 142, Appendix X G. Use 1 g.

Weight per ml

1.245 to 1.260 g, Appendix V G.

Aliphatic iodine

Mix 0.5 g with 10 ml of 1M *ethanolic potassium hydroxide* and boil vigorously under a reflux condenser in a water bath for 1 hour. Add 40 ml of *water* and 15 ml of *hydrochloric acid*, cool, filter and wash the residue with 10 ml of *water*. To the combined filtrate and washings add 10 ml of *potassium cyanide solution*, 5 ml of *starch mucilage* and 0.2 ml of 0.05M *potassium iodate*. A blue colour is not produced.

Free iodine

Absorbance of a 4-cm layer at 485 nm, not more than 0.20, Appendix II B.

ASSAY

Carry out the method for *oxygen-flask combustion for iodine*, Appendix VIII C, using 20 mg absorbed on filter paper surrounded by greaseproof paper. Each ml of 0.02M *sodium thiosulphate VS* is equivalent to 1.388 mg of $C_{19}H_{29}IO_2$.

STORAGE

Iophendylate Injection should be protected from light.

Action and use

Radio-opaque injection.

Iopanoic Acid Tablets

General Notices

Action and use

Iodinated contrast medium.

DEFINITION

Iopanoic Acid Tablets contain Iopanoic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of iopanoic acid, $C_{11}H_{12}I_3NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 30 mg of Iopanoic Acid with 10 ml of *ethanol (96%)*, filter, evaporate the filtrate to dryness and dry the residue at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of iopanoic acid *(RS 192)*.

B. Spray the chromatograms obtained in the test for Related substances with a 0.1% w/v solution of *dimethylamino-cinnamaldehyde* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *ethanol (96%)*. The principal spot in the chromatogram obtained with solution (2) is similar in position, colour and size to that in the chromatogram obtained with solution (4).

TESTS

Halide

To a quantity of the powdered tablets containing 0.8 g of Iopanoic Acid add just sufficient 0.2M *sodium hydroxide* to dissolve the iopanoic acid, dilute to 10 ml with *water*, add sufficient 2M *nitric acid* dropwise to ensure complete precipitation of the iodinated acid and add 3 ml in excess. Filter (Whatman GF/C filter paper is suitable), wash the precipitate with 5 ml of *water* and combine the solutions. 15 ml of the resulting solution complies with the *limit test for chlorides*, Appendix VII (180 ppm, calculated as chloride).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of 13.5M *ammonia*, 20 volumes of *methanol*, 20 volumes of *toluene* and 50 volumes of *1,4-dioxan* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 1.0 g of Iopanoic Acid with five 10 ml quantities of *ethanol (96%)*, filter, evaporate the combined filtrates to dryness using a rotary evaporator and dissolve the residue in 10 ml of a mixture of 3 volumes of 10M *ammonia* and 97 volumes of *methanol*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with a mixture of 3 volumes of 10M *ammonia* and 97 volumes of *methanol*. For solution (3) dilute 1 volume of solution (1) to 500 volumes with a mixture of 3 volumes of 10M *ammonia* and 97 volumes of *methanol*. Solution (4) contains 1.0% w/v of *iopanoic acid EPCRS* in a mixture of 3 volumes of 10M *ammonia* and 97 volumes of *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense that the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

Weigh and powder 20 tablets. Triturate a quantity of the powder containing 0.4 g of Iopanoic Acid with five 10-ml quantities of *ethanol (96%)*, decanting and filtering each extract through the same filter. Evaporate the combined filtrates almost to dryness on a water bath, cool, add 20 ml of *water*, 12 ml of 5M *sodium hydroxide* and 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 ml of *water*, filter through absorbent cotton and wash the flask and filter with two 20 ml quantities of *water*. To the combined filtrate and washings add 80 ml of *hydrochloric acid*, cool and titrate with 0.05M *potassium iodate VS* until the dark brown solution becomes light brown. Add 5 ml of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless. Each ml of 0.05M *potassium iodate VS* is equivalent to 19.03 mg of $C_{11}H_{12}I_3NO_2$.

STORAGE

Iopanoic Acid Tablets should be protected from light.

Ipratropium Nebuliser Solution

General Notices

DEFINITION

Ipratropium Nebuliser Solution is a solution of Ipratropium Bromide in Water for Injections and may contain sodium chloride.

The nebuliser solution complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of ipratropium bromide, $C_{20}H_{30}NO_3Br,H_2O$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Evaporate a volume of the nebuliser solution containing 1.5 mg of Ipratropium Bromide to dryness on a water bath. Shake the residue with 5 ml of methanol and filter (Whatman GF/C is suitable). Evaporate the filtrate to dryness on a water bath and dry the residue at room temperature at a pressure of 1 kPa for 15 minutes. The infrared absorption spectrum of the dried residue, Appendix II A, is concordant with a spectrum prepared from a mixture of 5 mg of ipratropium bromide BPCRS and 45 mg of sodium chloride dissolved in the minimum quantity of water, evaporated to dryness on a water bath and treated in a similar manner to the substance being examined beginning at the words "Shake the residue with 5 ml of methanol,……".

B. To a quantity of the nebuliser solution containing 0.5 mg of Ipratropium Bromide add 75 mg of lead dioxide and 0.25 ml of acetic acid, shake gently and allow to stand for 5 minutes. Add one drop to a strip of filter paper previously impregnated with decolorised fuchsin solution, a characteristic violet colour develops.

TESTS

Acidity

pH, 3.0 to 4.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the nebuliser solution, if necessary, with sufficient 0.001M *hydrochloric acid* to produce a solution containing 0.02% w/v of Ipratropium Bromide.

(2) Dilute 1 volume of solution (1) to 200 volumes with 0.001M *hydrochloric acid*.

(3) 0.005% w/v of *8*s*-isopropyl-3*b*-hydroxytropanium bromide BPCRS* and 0.005% w/v of *ipratropium bromide BPCRS* in 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm)(Columbus C8 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 0.5 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 210 nm.

(f) Injection volume of 20 µl for each solution.

For solution (1) allow the chromatography to proceed for 6 times the retention time of ipratropium (retention time of ipratropium, about 11 minutes).

MOBILE PHASE

A mixture of 4 volumes of freshly distilled *triethylamine,* 50 volumes of *propan-2-ol R1,* 100 volumes of *acetonitrile R1* and 850 volumes of a 0.1% w/v solution of *sodium methanesulphonate* previously adjusted to pH 3.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Osmolality

The osmolality of the solution is 245 mosmol/kg to 299 mosmol/kg, Appendix V N.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the nebuliser solution, if necessary, with sufficient 0.001M *hydrochloric acid* to produce a solution containing 0.02% w/v of Ipratropium Bromide.

(2) 0.02% w/v of *ipratropium bromide BPCRS* in 0.001M *hydrochloric acid*.

(3) 0.005% w/v of *8*s*-isopropyl-3*b*-hydroxytropanium bromide BPCRS* and 0.005% w/v of *ipratropium bromide BPCRS* in 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution*

factor between the two principal peaks is at least 1.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{30}NO_3Br,H_2O$ in the solution using the declared content of $C_{20}H_{30}NO_3Br,H_2O$ in *ipratropium bromide BPCRS.*

STORAGE

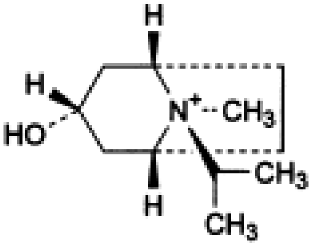
Ipratropium Nebuliser Solution should be stored protected from light in a sealed container.

LABELLING

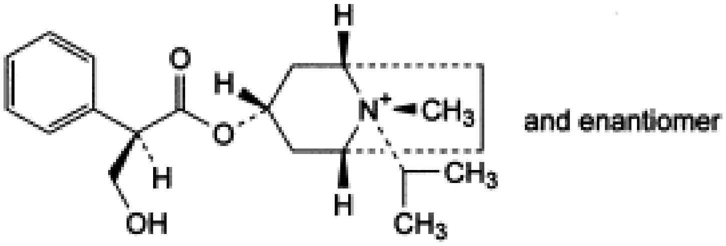
The label states the quantity of Ipratropium Bromide.

IMPURITIES

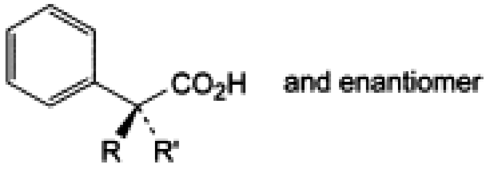
The impurities limited by the requirements of this monograph include.



A. (1*R*,3*r*,5*S*,8*r*)-3-hydroxy-8-methyl-8-(1-methylethyl)-8-azoniabicyclo [3.2.1]octane,



B. (1*R*,3*r*,5*S*,8*s*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane,



C. R = CH_2-OH, R′ = H: (2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid),

D. R + R′ = CH_2: 2-phenylpropenoic acid (atropic acid).

Ipratropium Powder for Inhalation

General Notices

DEFINITION

Ipratropium Powder for Inhalation consists of hard gelatin capsules containing Ipratropium Bromide admixed with Glucose. The contents of the capsules are in powder of a suitable fineness.

The powder for inhalation complies with the requirements stated under Preparations for Inhalation and the contents of the capsules comply with the following requirements.

Content of ipratropium bromide, $C_{20}H_{30}NO_3Br,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Vigorously shake for 2 minutes a quantity of the contents of the capsules containing 0.1 g of Ipratropium Bromide with 1 ml of *methanol*, centrifuge and use the supernatant.

(2) 0.1% w/v of *ipratropium bromide BPCRS* in a saturated solution of *glucose*.

(3) 0.0005% w/v of *tropic acid* in a saturated solution of *glucose*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254} (Merck HPTLC plates are suitable).

(b) The mobile phase is a mixture of 1 volume of *formic acid*, 3 volumes of *water*, 18 volumes of *ethanol* and 18 volumes of *dichloromethane*.

(c) Develop to 5 cm.

(d) Application volume of 10 µl for each solution.

(e) Remove the plate, dry for 15 minutes at 60^{c}, spray with *potassium iodobismuthate solution,* dry briefly in a current of air and spray with a 5% w/v solution of *sodium nitrite*.

VALIDITY

The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2).

B. Disperse a quantity of the contents of the capsules containing the equivalent of 0.3 mg of bromide ion in 0.5 ml of *water* add 75 mg of *lead dioxide* and 0.25 ml of *acetic acid*, shake gently and allow to stand for 5 minutes. Add one drop to a strip of filter paper previously impregnated with *decolorised fuchsin solution*, a characteristic violet colour develops.

C. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Vigorously shake for 2 minutes a quantity of the contents of the capsules containing 0.1 g of Ipratropium Bromide with 1 ml of *methanol*, centrifuge and use the supernatant.

(2) Dilute 1 volume of solution (1) to 200 volumes with a saturated solution of *glucose*.

(3) 0.0005% w/v of *tropic acid* in a saturated solution of *glucose*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel* GF_{254} (Merck HPTLC plates are suitable).

(b) The mobile phase is a mixture of 1 volume of *formic acid*, 3 volumes of *water*, 18 volumes of *ethanol* and 18 volumes of *dichloromethane*.

(c) Develop to 5 cm.

(d) Application volume of 10 µl for each solution.

(e) Remove the plate, dry for 15 minutes at 60°, spray with *potassium iodobismuthate solution,* dry briefly in a current of air and spray with a 5% w/v solution of *sodium nitrite*.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to tropic acid is not more intense than the spot in the chromatogram obtained with solution (2)(0.5%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2)(0.5%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules solution with sufficient mobile phase to produce a solution containing 0.02% w/v of Ipratropium Bromide.

(2) Dilute 1 volume of solution (1) to 200 volumes with mobile phase.

(3) 0.005% w/v of *8*s*-isopropyl-3*b*-hydroxytropanium bromide BPCRS* and 0.005% w/v of *ipratropium bromide BPCRS* in 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm), (Lichrospher 60 RP-select B is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 ml per minute.

(e) Detection wavelength of 210 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 3 volumes of *acetonitrile for chromatography and* 25 volumes of a 0.1% w/v solution of *sodium methanesulphonate* in 0.05M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules with sufficient 0.01M *hydrochloric acid* to produce a solution containing 0.0040% w/v of Ipratropium Bromide, filter and use the filtrate.

(2) 0.004% w/v of *ipratropium bromide BPCRS* in 0.01M *hydrochloric acid*.

(3) 0.005% w/v of *8s-isopropyl-3b-hydroxytropanium bromide BPCRS* and 0.005% w/v of *ipratropium bromide BPCRS* in 0.001M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm), (Lichrospher 60 RP-select B is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 ml per minute.

(d) Detection wavelength of 210 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 27 volumes of *acetonitrile for chromatography* and 100 volumes of a 0.25% w/v solution of *sodium heptanesulphonate monohydrate* in *water* adjusted to pH 3.2 with 0.05M *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.0.

DETERMINATION OF CONTENT

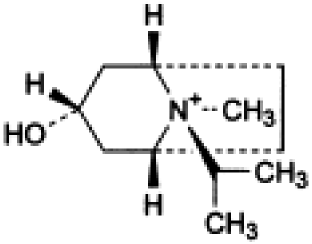
Calculate the content of $C_{20}H_{30}NO_3Br,H_2O$ in the powder for inhalation using the declared content of $C_{20}H_{30}BrNO_3,H_2O$ in *ipratropium bromide BPCRS.*

LABELLING

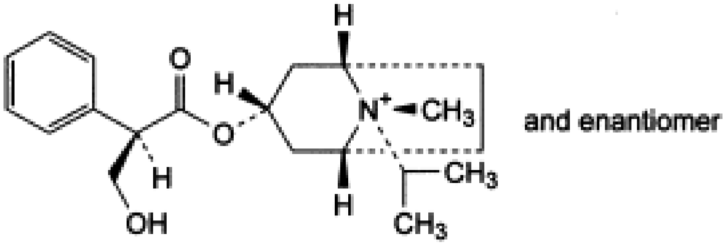
The label states the quantity of Ipratropium Bromide contained in each capsule.

IMPURITIES

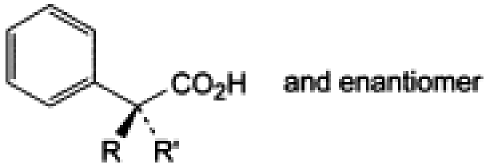
The impurities limited by the requirements of this monograph include.


H
H
HO
N+
CH3
CH3
H
CH3

A. (1*R*,3*r*,5*S*,8*r*)-3-hydroxy-8-methyl-8-(1-methylethyl)-8-azoniabicyclo [3.2.1]octane,


O
H
O
H
OH
H
N+
CH3
H
CH3
CH3
and enantiomer

B. (1*R*,3*r*,5*S*,8*s*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane,


CO2H
and enantiomer
R
R'

C. R = CH_2-OH, R′ = H: (2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid),

D. R + R′ = CH_2: 2-phenylpropenoic acid (atropic acid).

Ipratropium Pressurised Inhalation

General Notices

Action and use

Anticholinergic (antimuscarinic) bronchodilator.

DEFINITION

Ipratropium Pressurised Inhalation is a solution or suspension of Ipratropium Bromide in a suitable liquid in a suitable pressurised container.

The pressurised inhalation complies with the requirements stated under Preparations for Inhalations and with the following requirements.

Content of ipratropium bromide, $C_{20}H_{30}NO_3Br,H_2O$

85.0 to 115.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (6).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to ipratropium bromide in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a high performance silica gel precoated plate (Merck silica gel 60 HPTLC plates are suitable) and a mixture of 5 volumes of *water*, 8 volumes of *anhydrous formic acid*, 28 volumes of *methanol* and 70 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 6 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) punch a small hole in the ferrule of each of three cooled containers, allow the propellant to evaporate for about 1 minute and transfer the contents of the containers, through the punched holes, to a beaker. Stir, using a magnetic stirrer, for about 10 minutes, add 3.5 ml of 0.01M *hydrochloric acid* and continue stirring for about 1 hour, until the propellant has completely evaporated, filter, add 10 ml of *chloroform* to the filtrate and shake vigorously for 1 minute. Allow the phases to separate and use the upper layer. For solution (2) dilute 1 volume of solution (1) to 50 volumes with 0.01M *hydrochloric acid*. For solution (3) dilute 1 volume of solution (2) to 4 volumes with 0.01M *hydrochloric acid*. For solution (4) dilute 2 volumes of solution (3) to 5 volumes with 0.01M *hydrochloric acid*. Solution (5) contains 0.008% w/v of *8*s*-isopropyl-3*β*-hydroxytropanium bromide BPCRS* in 0.01M *hydrochloric acid*. Solution (6) contains 0.008% w/v of *ipratropium bromide BPCRS* in 0.01M *hydrochloric acid*. After removal of the plate, dry it in a current of warm air for about 30 minutes. Spray the plate with a mixture of 1 volume of *potassium iodobismuthate solution*, 2 volumes of *glacial acetic acid* and 10 volumes of *water*, allow to dry briefly, spray with a 5% w/v solution of *sodium nitrite* and immediately examine the plate. In the chromatogram obtained with solution (1) any spot corresponding to 8*s*-isopropyl-3β-hydroxytropanium bromide is not more intense than the spot in the chromatogram obtained with solution (2) (2%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%).

Deposition of the emitted dose

Carry out the test for *aerodynamic assessment of fine particles*, Appendix XII C7, but determining the content of active ingredient as described below. Use Apparatus A with 7 ml of 0.001M *hydrochloric acid* in the upper impingement chamber and 40 ml of a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol* in the lower impingement chamber. Use the same solvent mixture to wash the coupling tube, E, and transfer the combined solution and washings in the lower impingement chamber to a 100 ml graduated flask, rinsing the chamber with the solvent mixture and dilute the combined solution to 100 ml with the solvent mixture.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00007% w/v of *ipratropium bromide BPCRS* in a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol*. For solution (2) use the diluted solution from the lower impingement chamber.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (Lichrospher RP8 select B is suitable), (b) a mixture of 345 volumes of *acetonitrile* and 750 volumes of 0.012M *sodium heptanesulphonate* previously adjusted to pH 3.2 with 0.05M *orthophosphoric acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 210 nm.

The test is not valid unless in the chromatogram obtained with solution (1) the *symmetry factor* of the peak due to ipratropium bromide is less than 3.0 and unless the *signal-to-noise ratio* of the peak is at least 3.0.

Calculate the amount of ipratropium bromide, $C_{20}H_{30}BrNO_3,H_2O$, delivered to the lower impingement chamber per actuation of the valve using the declared content of $C_{20}H_{30}BrNO_3$, H_2O in *ipratropium bromide BPCRS*. Not less than 25% of the average amount of ipratropium bromide delivered per actuation of the valve, calculated as the average of the three results determined in the Assay, is deposited in the lower impingement chamber.

ASSAY

Determine the content of active ingredient delivered by the first 10 successive combined actuations of the valve after priming. Carry out the procedure for content of active ingredient delivered by actuation of the valve described under Pressurised Inhalations, Preparations for Inhalation, beginning at the words 'Remove the pressurised container from the actuator…' and ending at the words '… to the volume specified in the monograph' using 20 ml of a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol* in the vessel. Use the same solvent mixture to wash the pressurised container and to dilute the combined solution and washings obtained from the set of 10 combined actuations to 50 ml (solution A). Determine the amount of active ingredient in the 10 combined actuations using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00040% w/v of *ipratropium bromide BPCRS* in a mixture of equal volumes of 0.001M *hydrochloric acid* and *methanol*. For solution (2) use solution A.

The chromatographic conditions described under Deposition of the emitted dose may be used. In the chromatogram obtained with solution (1) long-running peaks due to excipients may appear.

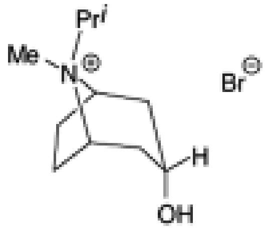
The test is not valid unless in the chromatogram obtained with solution (1) the *symmetry factor* for the peak due to ipratropium bromide is less than 3.0.

Calculate the average content of $C_{20}H_{30}BrNO_3,H_2O$ delivered by a single actuation of the valve using the declared content of $C_{20}H_{30}BrNO_3,H_2O$ in *ipratropium bromide BPCRS*.

Determine the content of active ingredient a second and a third time by repeating the procedure on the middle 10 and the last 10 successive combined actuations of the valve, as estimated from the number of deliveries available from the container as stated on the label. For each of the three determinations the average content of $C_{20}H_{30}BrNO_3,H_2O$ delivered by a single actuation of the valve is within the limits stated under Content of ipratropium bromide.

IMPURITIES

In addition to the impurities shown in the monograph for Ipratropium Bromide the degradation impurities limited by the requirements of this monograph include:



A. 8*s*-isopropyl-3β-hydroxytropanium bromide.

Iron Dextran Injection

General Notices

Action and use

Used in prevention and treatment of anaemia.

DEFINITION

Iron Dextran Injection is a sterile colloidal solution containing a complex of iron(III) hydroxide with dextrans of weight average molecular weight between 5000 and 7500.

PRODUCTION

Iron Dextran Injection is produced by a method of manufacture designed to provide an iron-dextran complex with appropriate iron absorption characteristics. This may be confirmed for routine control purposes by the use of an appropriate combination of physico-chemical tests, subject to the agreement of the competent authority.

The method of manufacture is validated to demonstrate that, if tested, the injection would comply with the following test.

Undue toxicity

Inject 0.10 ml into a tail vein of each of 10 mice; not more than three mice die within 5 days of injection. If more than three mice die within 5 days, repeat the test on another group of 20 mice. Not more than 10 of the 30 mice used in the combined tests die within 5 days of injection.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of iron, Fe

4.75 to 5.25% w/v.

Content of dextrans

17.0 to 23.0% w/v.

CHARACTERISTICS

A dark brown solution.

IDENTIFICATION

A. Add 5M *ammonia* to 0.2 ml of the injection previously diluted to 5 ml with *water*. No precipitate is produced.

B. Mix 1 ml with 100 ml of *water*. To 10 ml of this solution add 0.2 ml of *hydrochloric acid*, boil for 30 seconds, cool rapidly, add 4 ml of 13.5M *ammonia* and 10 ml of *hydrogen sulphide solution*, boil for at least 4 minutes to remove hydrogen sulphide, cool and filter.

Boil 5 ml of the filtrate with 5 ml of *cupri-tartaric solution R1*; the solution remains greenish in colour and no precipitate is produced. Boil a further 5 ml of the filtrate with 0.5 ml of *hydrochloric acid* for 5 minutes, cool, add 2.5 ml of 5M *sodium hydroxide* and 5 ml of *cupri-tartaric solution R1* and boil again; a reddish precipitate is produced.

C. To 1 ml add 20 ml of *water* and 5 ml of *hydrochloric acid* and boil for 5 minutes. Cool, add an excess of 13.5M *ammonia* and filter. Wash the precipitate with *water*, dissolve in the minimum volume of 2M *hydrochloric acid* and add sufficient *water* to produce 20 ml. The resulting solution yields reaction B characteristic of *iron salts*, Appendix VI.

TESTS

Acidity

pH, 5.2 to 6.5, Appendix V L.

Arsenic

To 5.0 ml in a Kjeldahl flask add 10 ml of *water* and 10 ml of *nitric acid* and heat until the vigorous evolution of brown fumes ceases. Cool, add 10 ml of *sulphuric acid* and heat again until fumes are evolved, adding *nitric acid* dropwise at intervals until oxidation is complete. Cool, add 30 ml of *water*, bring to the boil and continue boiling until the volume of liquid is reduced to about 20 ml. Cool and dilute to 50 ml with *water*. Reserve a portion of the solution for the test for Lead. To 5 ml of the solution add 10 ml of *water*, 15 ml of *stannated hydrochloric acid* and 3 ml of *tin*(II) *chloride solution AsT*. Connect to a condenser and distil 15 ml into 10 ml of *water*. To the distillate add 0.2 ml of *bromine water* and remove the excess of bromine with *tin*(II) *chloride solution AsT*. The solution complies with the *limit test for arsenic*, Appendix VII (2 µg per ml).

Copper

To 5.0 ml add 5 ml of *nitric acid* and heat until the vigorous evolution of brown fumes ceases. Cool, add 2 ml of *sulphuric acid* and heat again until fumes are evolved, adding *nitric acid* dropwise at intervals until oxidation is complete. Cool, add 25 ml of *hydrochloric acid*, warm to dissolve, cool and extract with four 25 ml quantities of *isobutyl acetate*, discarding the extracts. Evaporate the acid solution to dryness, adding *nitric acid* dropwise if charring occurs. Dissolve the residue in 10 ml of 1M *hydrochloric acid*, reserving a portion of the solution for the test for Zinc. To 1 ml add 25 ml of *water* and 1 g of *citric acid*, make alkaline to *litmus paper* with 5M *ammonia*, dilute to 50 ml with *water*, add 1 ml of *sodium diethyldithiocarbamate solution* and allow to stand for 5 minutes. Any colour produced is not more intense than that produced by treating in the same manner a mixture of 3 ml of *copper standard solution (10 ppm Cu)* and 1 ml of 1M *hydrochloric acid* beginning at the words 'add 25 ml of *water*…' (60 µg per ml).

Lead

To 16.0 ml of the solution reserved in the test for Arsenic add 50 ml of *hydrochloric acid* and extract with four 20 ml quantities of *isobutyl acetate*, discarding the extracts. Evaporate the acid solution to dryness and dissolve the residue in 20 ml of *water*. 12 ml of the solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard (25 µg per ml).

Zinc

To 5.0 ml of the solution reserved in the test for Copper add 15 ml of 1M *sodium hydroxide*, boil, filter, wash the residue with *water* and dilute the combined filtrate and washings to 25 ml with *water*. To 5 ml add 5 ml of 1M *hydrochloric acid* and 2 g of *ammonium chloride*, dilute to 50 ml with *water*, add 1 ml of freshly prepared *dilute potassium hexacyanoferrate*(II) *solution* and allow to stand for 20 minutes. Any opalescence produced is not more than that produced when 1 ml of freshly prepared *dilute potassium hexacyanoferrate*(II) *solution* is added to a solution prepared from 3 ml of *zinc standard solution (25 ppm Zn)*, 3 ml of 1M *sodium hydroxide*, 6 ml of 1M *hydrochloric acid* and 2 g of *ammonium chloride* diluted to 50 ml with *water* and allowed to stand for 20 minutes (150 µg per ml).

Chloride

To 5 ml add 75 ml of *water* and 0.05 ml of *nitric acid* and titrate immediately with 0.1M *silver nitrate VS* determining the end point potentiometrically. 6.8 to 9.6 ml of 0.1M *silver nitrate VS* is required.

Iron absorption

Using at least two rabbits, each weighing between 1.5 and 2.5 kg, clip the right hind leg free from hair over the injection site and swab the area with a bactericidal solution. Inject into each rabbit 0.4 ml of the injection per kg of the rabbit's weight using a 2 ml syringe fitted with a No. 12 hypodermic needle [22 gauge × 1.25 inch (0.7 × 32 mm approx.)]. Insert the needle at the distal end of the semitendinosus muscle, passing through the sartorius and entering the vastus medialis; the angle of the needle must be such that the full length is used. After 7 days, kill the rabbits and remove the legs into which the injections were made. Carefully cut open the muscles and examine the site of injection; it should be only very lightly stained and should show no dark brown deposits and no evidence of leakage along fascial planes. Skin the leg, dissect the flesh from the bone and cut into small pieces. Transfer the pieces to a 1000 ml beaker, add 75 ml of 2M *sodium hydroxide* and sufficient *water* to cover the flesh, cover the beaker with a watch glass and boil until most of the solid matter has disintegrated. Cool, cautiously add 50 ml of *sulphuric acid*, heat the mixture almost to boiling and add carefully ten 1 ml quantities of *fuming nitric acid* until no charring occurs when the excess of nitric acid has been boiled off. Cool, add 170 ml of *water,* boil until solution is complete, cool and dilute to 250 ml with *water.* To 5 ml of this solution add 3 ml of *sulphuric acid*, heat to fuming and complete the oxidation by the addition of small quantities of *nitric acid* until the solution is colourless. Cool, add 20 ml of *water,* boil for 3 minutes and add 10 ml of *ammonium citrate solution,* 10 ml of *ammonium mercaptoacetate solution,* 5M *ammonia* dropwise until the iron colour is fully developed, 1 ml of 5M *ammonia* in excess and sufficient *water* to produce 100 ml. Measure the *absorbance* of the resulting solution at 530 nm, Appendix II B.

Prepare a reference solution by adding 20 ml of *water* the same quantities of *ammonium citrate solution, ammonium mercaptoacetate solution* and 5M *ammonia* as used above, dilute to 100 ml with *water* and measure the *absorbance* at 530 nm. From the difference between the absorbances, calculate the amount of Fe present in the legs from a reference curve prepared by treating suitable aliquots of a solution of *ammonium iron*(III) *sulphate* containing 0.01% w/v of Fe by the procedure described above beginning at the words 'add 10 ml of *ammonium citrate solution*…'. Repeat the determination of Fe on the corresponding legs into which no injection was made, beginning at the words 'Carefully cut open the muscles…'. From the difference between the two amounts of iron, calculate the proportion of injected iron, as Fe, remaining in the leg tissues. Not more than 20% of the injected iron remains.

Bacterial endotoxins

The endotoxin limit concentration is 0.50 IU per mg of iron, Appendix XIV C.

ASSAY

For iron

To 2 g add 10 ml of *water* and 5 ml of *sulphuric acid* and stir for several minutes. Allow to stand for 5 minutes, cool and dilute to 50 ml with *water*. Prepare a suitable zinc amalgam by covering 300 g of *zinc shot* with a 2% w/v solution of *mercury*(II) *chloride* and stir for 10 minutes. Decant the solution, wash the residue three times with *water* and transfer it to a column (30 cm × 18 mm) fitted with a sintered-glass disc (ISO 4793, porosity grade 0, is suitable). Activate the zinc amalgam by passing through the column 200 ml of *sulphuric acid (5%)*. Pass the prepared solution slowly through the column and wash successively with 50 ml of *water*, four 25 ml quantities of *sulphuric acid (5%)* and 50 ml of *water*. Titrate the combined eluates with 0.1M *ammonium cerium*(IV) *sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium*(IV) *sulphate VS* is equivalent to 5.585 mg of Fe. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage w/v of Fe.

For dextrans

Dilute 1 g to 500 ml with *water*, dilute 10 ml of the solution to 100 ml with *water*, transfer 3 ml of the resulting solution to a test tube and cool to 0°. Add, to form a lower layer, 6 ml of a solution prepared and maintained at 0° containing 0.2% w/v of *anthrone* in a mixture of 19 volumes of *sulphuric acid* and 1 volume of *water,* mix and immediately heat on a water bath for 5 minutes. Cool and measure the *absorbance* of the resulting solution at the maximum at 625 nm, Appendix II B. Repeat the operation using 3 ml of *water* in place of the dilution of the injection. From the difference between the absorbances calculate the content of glucose present using a calibration curve prepared by treating suitable amounts of D-*glucose* in the same manner. Each g of D-*glucose* is equivalent to 0.94 g of dextrans. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage w/v of dextrans.

LABELLING

The strength is stated as the equivalent amount of iron, Fe, in a suitable dose-volume.

Iron Sucrose Injection

General Notices

Action and use

Used in prevention and treatment of anaemia.

DEFINITION

Iron Sucrose Injection is a sterile colloidal solution containing a complex of iron(III) hydroxide with sucrose of average molecular weight between 34000 and 60000.

PRODUCTION

Iron Sucrose Injection is produced by a method of manufacture designed to provide an iron-sucrose complex with appropriate iron absorption characteristics. This may be confirmed for routine control purposes by the use of an appropriate combination of physico-chemical tests, subject to the agreement of the competent authority.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of iron, Fe

1.90 to 2.10% w/v.

Content of sucrose

27.0 to 33.0% w/v.

IDENTIFICATION

A. To 1 ml of the injection add 20 ml of *water* and 5 ml of *hydrochloric acid* and boil for 5 minutes. Cool, add an excess of 13.5M *ammonia* and filter. Wash the precipitate with *water*, dissolve in the minimum volume of 2M *hydrochloric acid* and add sufficient *water* to produce 20 ml. The resulting solution yields reaction B characteristic of *iron salts*, Appendix VI.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH, 10.5 to 11.0, Appendix V L.

Osmolality

The osmolality, Appendix V N, of the injection is 1150 mosmol/l to 1350 mosmol/l.

Arsenic

To 10.0 ml of injection in a long-necked combustion flask add 20 ml of *water* and 20 ml of *nitric acid* and heat until the vigorous evolution of brown fumes ceases. Cool, add 20 ml of *sulphuric acid* and heat again until fumes are evolved, adding *nitric acid* dropwise until oxidation is complete. Cool, add 60 ml of *water,* bring to the boil and continue boiling until the volume of liquid is reduced to about 40 ml. Cool and dilute to 100 ml with *water*. Reserve a portion of the solution for the test for Lead. To 12.5 ml of the solution add 10 ml of *water*, 15 ml of *stannated hydrochloric acid* and 3 ml of *tin(II) chloride solution AsT*. Connect to a condenser and distil 15 ml into 10 ml of *water*. To the distillate add 0.2 ml of *bromine water* and remove the excess of bromine with *tin(II) chloride solution AsT*. The solution complies with the *limit test for arsenic*, Appendix VII (1 µg per ml).

Copper

To 5.0 ml of the injection add 5 ml of *nitric acid* and heat until the vigorous evolution of brown fumes ceases. Cool, add 2 ml of *sulphuric acid* and heat again until fumes are evolved, adding *nitric acid* dropwise until oxidation is complete. Cool, add 25 ml of *hydrochloric acid*, warm to dissolve, cool and wash with four 25-ml quantities of *isobutyl acetate*, discarding the washings. Evaporate the acid solution to dryness, adding *nitric acid* dropwise if charring occurs. Dissolve the residue in 10 ml of 1M *hydrochloric acid*, reserving a portion of the solution for the test for Zinc. To 1 ml add 25 ml of *water* and 1 g of *citric acid*, make alkaline to *litmus paper* with 5M *ammonia*, dilute to 300 ml with *water*, to 50 ml add 1 ml of *sodium diethyldithiocarbamate solution* and allow to stand for 5 minutes. Any colour produced is not more intense than that produced by treating in the same manner a mixture of 0.5 ml of *copper standard solution (10 ppm Cu)* and 1 ml of 1M *hydrochloric acid* beginning at the words 'add 25 ml of *water*…' (10 µg per ml).

Lead

To 40.0 ml of the solution reserved in the test for Arsenic add 50 ml of *hydrochloric acid* and wash with four 20-ml quantities of *isobutyl acetate*, discarding the washings. Evaporate the acid solution to dryness and dissolve the residue in 20 ml of *water*. 12 ml of the solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (2 ppm Pb)* to prepare the standard (10 µg per ml).

Chloride

To 10 ml of the injection add 75 ml of *water* and 0.05 ml of *nitric acid* and titrate immediately with 0.01M *silver nitrate VS* determining the end point potentiometrically. 4.0 to 8.0 ml of 0.01M *silver nitrate VS* is required.

Bacterial endotoxins

The endotoxin limit concentration is 0.50 IU per mg of iron, Appendix XIV C.

ASSAY

For iron

To 2 g add 10 ml of *water* and 5 ml of *sulphuric acid* and stir for several minutes. Allow to stand for 5 minutes, cool and dilute to 50 ml with *water*. Prepare a suitable zinc amalgam by covering 300 g of *zinc shot* with a 2% w/v solution of *mercury(II) chloride* and stir for 10 minutes. Decant the solution, wash the residue three times with *water* and transfer it to a column (30 cm × 18 mm) fitted with a sintered-glass disc (ISO 4793, porosity grade 0, is suitable). Activate the zinc amalgam by passing through the column 200 ml of *sulphuric acid*

(5%). Pass the prepared solution slowly through the column and wash successively with 50 ml of *water*, four 25-ml quantities of *sulphuric acid (5%)* and 50 ml of *water*. Titrate the combined eluates with 0.1M *ammonium cerium(IV) sulphate VS* using *ferroin solution* as indicator. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 5.585 mg of Fe. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage w/v of Fe.

For sucrose

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a volume of the injection containing 40mg of Iron Sucrose add 2.5 ml of a 3.0% w/v solution of *sodium dihydrogen orthophosphate monohydrate*, mix and allow to stand for 10 minutes, add sufficient *water* to produce 25 ml, centrifuge and filter discarding the first 2ml of filtrate.

(2) 1.30% w/v of *sucrose BPCRS* in mobile phase.

(3) 1.60% w/v of *sucrose BPCRS* in mobile phase.

(4) 1.80% w/v of *sucrose BPCRS* in mobile phase.

(5) 2.10% w/v of *sucrose BPCRS* in mobile phase.

(6) 2.30% w/v of *sucrose BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4 mm) packed with *aminopropylsilyl silica gel for chromatography* (3-10 µm).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a refractive index detector.

(f) Inject 20 µl of each solution.

MOBILE PHASE

21 volumes of *water* and 79 volumes of *acetonitrile.*

DETERMINATION OF CONTENT

Determine the peak areas of the principal peak in solutions (2), (3), (4), (5) and (6), plot the peak area for each solution as a function of concentration of sucrose and draw a straight line best fitting the five plotted points. From the graph obtained calculate the content of sucrose in the injection using the declared content of sucrose in *sucrose BPCRS*.

LABELLING

The strength is stated as the equivalent amount of iron, Fe, in a suitable dose-volume.

Isoconazole Pessaries

General Notices

Isoconazole Vaginal Tablets

Action and use

Antifungal.

DEFINITION

Isoconazole Pessaries are *vaginal tablets* containing Isoconazole Nitrate in a suitable basis.

The pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of isoconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 10 volumes of *methanol*, 30 volumes of *dichloromethane* and 60 volumes of *hexane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of powdered pessaries containing 10 mg of Isoconazole Nitrate with 10 ml of *dichloromethane* for 5 minutes and filter. Solution (2) contains 0.10% w/v of *isoconazole nitrate BPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and spray with *potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of powdered pessaries containing 0.10 g of Isoconazole Nitrate with 10 ml of the mobile phase for 30 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 400 volumes with the mobile phase. Solution (3) contains 0.0025% w/v each of *isoconazole nitrate BPCRS* and of *econazole nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS 3 µm is suitable), (b) a solution of 6.0 g of *ammonium acetate* in a mixture of 300 ml of *acetonitrile*, 320 ml of *methanol* and 380 ml of *water* as the mobile phase at a flow rate of 2 ml per minute and (c) a detection wavelength of 235 nm. Equilibrate the column with the mobile phase at a flow rate of 2 ml per minute for 30 minutes. In the chromatogram obtained with solution (1) allow the chromatography to proceed for 1.5 times the retention time of the principal peak.

In the chromatogram obtained with solution (3) the approximate retention times of econazole and isoconazole are 10 minutes and 14 minutes respectively. The test is not valid unless the *resolution factor* between the peaks due to econazole and isoconazole is at least 5.0. If necessary adjust the composition of the mobile phase.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of all such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peaks due to the solvent and to nitrate ion and any peak with an area less than 0.4 times that of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered pessaries containing 0.10 g of Isoconazole Nitrate with 100 ml of the mobile phase, mix and filter. Solution (2) contains 0.10% w/v of *isoconazole nitrate BPCRS* in the mobile phase. Solution (3) contains 0.10% w/v each of *isoconazole nitrate BPCRS* and of *econazole nitrate BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

In the chromatogram obtained with solution (3) the approximate retention times of econazole and isoconazole are 10 minutes and 14 minutes respectively. The assay is not valid unless the *resolution factor* between the peaks due to econazole and isoconazole is at least 5.0. If necessary adjust the composition of the mobile phase.

Calculate the content of $C_{18}H_{14}Cl_4N_2O,HNO_3$ in the pessaries using the declared content of $C_{18}H_{14}Cl_4N_2O,HNO_3$ in *isoconazole nitrate BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Isoconazole Nitrate.

Isoniazid Injection

General Notices

Action and use

Antituberculosis drug.

DEFINITION

Isoniazid Injection is a sterile solution of Isoniazid in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of isoniazid, $C_6H_7N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Using a rotary evaporator, evaporate a volume of the injection containing 50 mg of Isoniazid to dryness, extract the residue with two 10 ml quantities of *ethanol (96%)*, filter and evaporate the combined ethanol extracts to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of isoniazid *(RS 196)*.

B. To a volume containing 25 mg of Isoniazid add 5 ml of *ethanol (96%)*, 0.1 g of *sodium tetraborate* and 5 ml of a 5.0% w/v solution of *1-chloro-2,4-dinitrobenzene* in *ethanol (96%)*, evaporate to dryness on a water bath, heat for a further 10 minutes and dissolve the residue in 10 ml of *methanol*. A reddish purple colour is produced.

TEST

Acidity

pH, 5.6 to 6.0, Appendix V L.

ASSAY

Dilute a quantity containing 0.4 g of Isoniazid to 250 ml with *water*. To 25 ml of the solution in a glass-stoppered flask add 25 ml of 0.05M *bromine VS* and 5 ml of *hydrochloric acid*, shake for 1 minute, allow to stand for 15 minutes, add 1 g of *potassium iodide* and titrate with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Repeat the operation without the injection. The difference between the titrations represents the amount of bromine required. Each ml of 0.05M *bromine VS* is equivalent to 3.429 mg of $C_6H_7N_3O$.

STORAGE

Isoniazid Injection should be protected from light.

Isoniazid Tablets

General Notices

Action and use

Antituberculosis drug.

DEFINITION

Isoniazid Tablets contain Isoniazid.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isoniazid, $C_6H_7N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Isoniazid with 10 ml of *ethanol (96%)* for 15 minutes, centrifuge and decant the supernatant liquid. Extract the residue with two further 10-ml quantities of *ethanol (96%)* and evaporate the combined extracts to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of isoniazid *(RS 196)*.

B. Shake a quantity of the powdered tablets containing 1 mg of Isoniazid with 50 ml of *ethanol (96%)* and filter. To 5 ml of the filtrate add 0.1 g of *sodium tetraborate* and 5 ml of a 5% w/v solution of *1-chloro-2,4-dinitrobenzene* in *ethanol (96%)*, evaporate to dryness on a water bath and continue heating for a further 10 minutes. To the residue add 10 ml of *methanol* and mix. A reddish purple colour is produced.

TEST

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of *water* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 263 nm, Appendix II B. Calculate the total content of isoniazid, $C_6H_7N_3O$, in the medium taking 307 as the value of *A* (1%, 1 cm) at the maximum at 263 nm.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.4 g of Isoniazid as completely as possible in *water*, filter and wash the residue with sufficient *water* to produce 250 ml. To 50 ml of the resulting solution add 50 ml of *water*, 20 ml of *hydrochloric acid* and 0.2 g of *potassium bromide* and titrate with 0.0167M *potassium bromate VS* determining the end point electrometrically. Each ml of 0.0167M *potassium bromate VS* is equivalent to 3.429 mg of $C_6H_7N_3O$.

STORAGE

Isoniazid Tablets should be protected from light.

Isophane Insulin Injection

General Notices

Isophane Insulin; Isophane Insulin (NPH)
(Ph Eur monograph 0833)

Action and use

Hormone; treatment of diabetes mellitus.

Ph Eur

Isophane insulin injection complies with the monograph on Insulin preparations, injectable (0854) with the modifications prescribed below.

DEFINITION

Isophane insulin injection is a sterile suspension of bovine, porcine or human insulin, complexed with protamine sulphate or another suitable protamine.

PRODUCTION

Isophane insulin injection is prepared by carrying out the procedures described in the monograph on *Insulin preparations, injectable (0854)*.

The amount of protamine is based on the known isophane ratio and is not less than the equivalent of 0.3 mg and not more than the equivalent of 0.6 mg of protamine sulphate for each 100 IU of insulin in the insulin-protamine complex.

CHARACTERS

A white or almost white suspension which on standing deposits a white or almost white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

IDENTIFICATION

Examine the chromatograms obtained in the Assay. The position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

TESTS

Total zinc

Not more than 40.0 µg per 100 IU of insulin, determined as described in the monograph on *Insulin preparations, injectable (0854).*

Isoprenaline Injection

General Notices

Action and use

Adrenoceptor agonist.

DEFINITION

Isoprenaline Injection is a sterile solution of Isoprenaline Hydrochloride in Water for Injections. It is supplied either as a ready-to-use solution or prepared immediately before use in accordance with the manufacturer's instructions from Sterile Isoprenaline Concentrate.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use solution, the injection complies with the following requirements.

Content of isoprenaline hydrochloride, $C_{11}H_{17}NO_3,HCl$

90.0 to 115.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Record *second-derivative ultraviolet absorption spectra* of the following solutions, Appendix II B, in the range 250 to 330 nm. For solution (1) dilute the injection, if necessary, with *methanol* to contain 0.002% w/v of Isoprenaline Hydrochloride. Solution (2) is a 0.002% w/v solution of *isoprenaline hydrochloride BPCRS* in *methanol*. The spectrum obtained with solution (1) exhibits a maximum at 294 nm and a trough at 289 nm and is similar to the spectrum obtained with solution (2) in the range 280 to 298 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a reversed-phase high performance plate (Merck HPTLC RP18 F_{254} plates are suitable) and a mixture of 0.1 volume of *glacial acetic acid*, 40 volumes of *water* and 60 volumes of *methanol* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) evaporate a suitable volume of the solution just to dryness and dissolve the residue in sufficient *methanol* to produce a solution containing 0.01% w/v of Isoprenaline Hydrochloride. Solution (2) contains 0.01% w/v of *isoprenaline hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air, heat at 105° for 15 minutes and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. To 2 ml add 0.1 ml of *iron(III) chloride solution R1*. An emerald green colour is produced which, on the gradual addition of *sodium hydrogen carbonate solution*, changes first to blue and then to red.

TEST

Acidity

pH, 2.5 to 3.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the solution diluted, if necessary, with sufficient of a 0.1% w/v solution of *sodium metabisulphite* to produce a solution containing 0.002% w/v of Isoprenaline Hydrochloride. Solution (2) contains 0.002% w/v of *isoprenaline hydrochloride BPCRS* in a 0.1% w/v solution of *sodium metabisulphite*. Solution (3) is a mixture of 1 volume of a 0.02% w/v solution of *adrenaline acid tartrate* in the mobile phase containing 1.0% w/v of *sodium metabisulphite* and 18 volumes of solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 to 10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution prepared by dissolving 1.76 g of *sodium heptanesulphonate* in 800 ml of *water*, adding 200 ml of *methanol* and adjusting the pH to 3.0 with 1M *orthophosphoric acid* and (c) a detection wavelength of 280 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.5.

Calculate the content of $C_{11}H_{17}NO_3$,HCl in the injection using the declared content of $C_{11}H_{17}NO_3$,HCl in *isoprenaline hydrochloride BPCRS*.

STERILE ISOPRENALINE CONCENTRATE

DEFINITION

Sterile Isoprenaline Concentrate is a sterile solution of Isoprenaline Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of isoprenaline hydrochloride, $C_{11}H_{17}NO_3$,HCl

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or very pale yellow solution.

IDENTIFICATION

A. Record *second-derivative ultraviolet absorption spectra* of the following solutions, Appendix II B, in the range 250 to 330 nm. For solution (1) dilute the concentrate with *methanol* to contain 0.002% w/v of Isoprenaline Hydrochloride. Solution (2) is a 0.002% w/v solution of *isoprenaline hydrochloride BPCRS* in *methanol*. The spectrum obtained with solution (1) exhibits a maximum at 294 nm and a trough at 289 nm and is similar to the spectrum obtained with solution (2) in the range 280 to 298 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a reversed-phase high performance plate (Merck HPTLC RP18 F_{254} plates are suitable) and a mixture of 0.1 volume of *glacial acetic acid*, 40 volumes of *water* and 60 volumes of *methanol* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) dilute the concentrate with *methanol* to produce a solution containing 0.01% w/v of Isoprenaline Hydrochloride. Solution (2) contains 0.01% w/v of *isoprenaline hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air, heat at 105° for 15 minutes and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. To 2 ml add 0.1 ml of *iron(III) chloride solution R1*. An emerald green colour is produced which, on the gradual addition of *sodium hydrogen carbonate solution*, changes first to blue and then to red.

TEST

Acidity

pH, 2.5 to 3.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the concentrate diluted with sufficient of a 0.1% w/v solution of *sodium metabisulphite* to produce a solution containing 0.002% w/v of Isoprenaline Hydrochloride. Solution (2) contains 0.002% w/v of *isoprenaline hydrochloride BPCRS* in a 0.1% w/v solution of *sodium metabisulphite*. Solution (3) is a mixture of 1 volume of a 0.02% w/v solution of *adrenaline acid tartrate* in the mobile phase containing 1.0% w/v of *sodium metabisulphite* and 18 volumes of solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 to 10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution prepared by dissolving 1.76 g of *sodium heptanesulphonate* in 800 ml of *water*, adding 200 ml of *methanol* and adjusting the pH to 3.0 with 1M *orthophosphoric acid* and (c) a detection wavelength of 280 nm.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.5.

Calculate the content of $C_{11}H_{17}NO_3,HCl$ in the concentrate using the declared content of $C_{11}H_{17}NO_3,HCl$ in *isoprenaline hydrochloride BPCRS*.

STORAGE

Sterile Isoprenaline Concentrate should be protected from light.

Isosorbide Dinitrate Injection

General Notices

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Isosorbide Dinitrate Injection is a sterile solution of isosorbide dinitrate made isotonic with Sodium Chloride in Water for Injections. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of isosorbide dinitrate, $C_6H_8N_2O_8$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Evaporate a suitable volume of the injection under reduced pressure and below 40° to dryness. Shake a quantity of the residue containing 25 mg of isosorbide dinitrate with 10 ml of *dichloromethane* for 5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of isosorbide dinitrate (*RS 412*).

B. Yields the reactions characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

Acidity or alkalinity

pH, 3.5 to 7.0, Appendix V L.

Nitrite

To 40 ml of the injection add 2 ml of *sulphanilic acid solution*, 2 ml of *aminonaphthalenesulphonic acid solution* and sufficient *water* to produce 50 ml. Allow to stand at room temperature for 1 hour. Measure the *absorbance* of the resulting solution at the maximum at 524 nm, Appendix II B, using in the reference cell a solution prepared by treating 40 ml of *water* in the same manner, beginning at the words "add 2 ml of *sulphanilic acid solution* ...". The *absorbance* is not greater than that obtained by repeating the procedure using 2 ml of *nitrite standard solution (20 ppm)* diluted to 40 ml with *water* and treated at the same time and in the same manner, beginning at the words "add 2 ml of *sulphanilic acid solution* ..." (1 ppm, calculated with reference to the injection).

Isosorbide 5-nitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) evaporate a volume of the injection containing 50 mg of isosorbide dinitrate, under reduced pressure and below 40°, to dryness, triturate the residue with 50 ml of mobile phase*,* shake, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

Solution (2) contains 0.0005% w/v of *isosorbide 2-nitrate BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *isosorbide mononitrate BPCRS* in the mobile phase. Solution (4) contains 0.005% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 30 volumes of *methanol* and 70 volumes of *water* and (c) a detection wavelength of 220 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

In the chromatogram obtained with solution (1) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

Bacterial endotoxins

The endotoxin limit concentration is 5.0 IU per ml, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the injection, diluted, if necessary, with sufficient of a mixture of 25 volumes of *methanol* and 75 volumes of *water* to produce a solution containing 0.002% w/v of isosorbide dinitrate. For solution (2) prepare a 0.01% w/v solution of *isosorbide dinitrate BPCRS* in methanol. Filter and dilute 1 volume to 50 volumes with a mixture of 25 volumes of *methanol* and 75 volumes of *water.*

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *trimethylsilyl silica gel for chromatography* (3 µm) (Hypersil SAS is suitable), (b) as the mobile phase with a flow rate of 1.3 ml per minute a mixture containing 0.039% w/v of *ammonium acetate* in 25 volumes of *methanol R2* and 75 volumes of *water* and (c) a detection wavelength of 214 nm.

Calculate the content of $C_6H_8N_2O_8$ in the injection using the declared content of $C_6H_8N_2O_8$ in *isosorbide dinitrate BPCRS.*

Isosorbide Dinitrate Sublingual Tablets

General Notices

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Isosorbide Dinitrate Sublingual Tablets contain Diluted Isosorbide Dinitrate.

CAUTION Undiluted isosorbide dinitrate can be exploded by percussion or excessive heat. Appropriate precautions should be exercised and only exceedingly small amounts should be isolated.

The sublingual tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of isosorbide dinitrate, $C_6H_8N_2O_8$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *toluene* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 2 mg of isosorbide dinitrate with 1 ml of *ether* and centrifuge. Prepare solution (2) in the same manner but use *isosorbide dinitrate BPCRS* in place of the powdered tablets. After removal of the plate, dry it in a current of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing 50 mg of isosorbide dinitrate with warm *sulphuric acid (50%)* containing a trace of *diphenylamine.* An intense blue colour is produced.

TESTS

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel H plate* (Merck plates are suitable) and a mixture of 15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of isosorbide dinitrate with 5 ml of *ethanol (96%)* and filter. For solution (2) dissolve 10 mg of *potassium nitrate* in 1 ml of *water* and dilute to 100 ml with *ethanol (96%).* After removal of the plate thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes. Examine in daylight. In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Isosorbide 5-nitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of the mobile phase to a quantity of the powdered tablets containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.0005% w/v *of isosorbide 2-nitrate BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *isosorbide mononitrate BPCRS* in the mobile phase. Solution (4) contains 0.005% w/v each of *isosorbide dinitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *aminopropylsilyl silica gel for chromatography* (10 µm) (Lichrosorb NH_2 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 15 volumes of *absolute ethanol* and 85 volumes of *2,2,4-trimethylpentane* and (c) a detection wavelength of 215 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of the mobile phase to a quantity of the powdered tablets containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase. For solution (2) add 20 ml of the mobile phase to a quantity *of isosorbide dinitrate BPCRS* containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the mobile phase. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase. Solution (3) contains 0.005% w/v each *of isosorbide dinitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure described under Isosorbide 5-nitrate and isosorbide 2-nitrate may be used but using a detection wavelength of 230 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

Calculate the content of $C_6H_8N_2O_8$ in the tablets from the chromatograms obtained and using the declared content of $C_6H_8N_2O_8$ in *isosorbide dinitrate BPCRS*.

LABELLING

The label states that the tablets should be placed under the tongue and allowed to dissolve slowly.

When Isosorbide Dinitrate Tablets are prescribed or demanded, either Isosorbide Dinitrate Tablets or Isosorbide Dinitrate Sublingual Tablets may be dispensed or supplied, as appropriate.

Isosorbide Dinitrate Tablets

General Notices

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Isosorbide Dinitrate Tablets contain Diluted Isosorbide Dinitrate.

CAUTION Undiluted isosorbide dinitrate can be exploded by percussion or excessive heat. Appropriate precautions should be exercised and only exceedingly small amounts should be isolated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isosorbide dinitrate, $C_6H_8N_2O_8$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *toluene* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 2 mg of isosorbide dinitrate with 1 ml of *ether* and centrifuge. Prepare solution (2) in the same manner but use *isosorbide dinitrate BPCRS* in place of the powdered tablets. After removal of the plate, dry it in a current of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing 50 mg of isosorbide dinitrate with warm *sulphuric acid (50%)* containing a trace of *diphenylamine*. An intense blue colour is produced.

TESTS

Dissolution

Tablets intended to be swallowed whole comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter through a membrane filter with a nominal pore size not greater than 0.45 µm. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is a solution of *isosorbide dinitrate BPCRS* in the dissolution medium containing the same concentration of isosorbide dinitrate as that expected in the dissolution vessel. Solution (2) is the filtrate from the dissolution vessel.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute, a mixture of equal volumes of *methanol* and *water* and (c) a detection wavelength of 222 nm. Inject 100 µl of each solution.

Calculate the total content of isosorbide dinitrate, $C_6H_8N_2O_8$, in the medium using the declared content of $C_6H_8N_2O_8$ in *isosorbide dinitrate BPCRS*.

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel H plate* (Merck plates are suitable) and a mixture of 15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of isosorbide dinitrate with 5 ml of *ethanol (96%)* and filter. For solution (2) dissolve 10 mg of *potassium nitrate* in 1 ml of *water* and dilute to 100 ml with *ethanol (96%)*. After removal of the plate thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes. Examine in daylight. In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Isosorbide 5-nitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of the mobile phase to a quantity of the powdered tablets containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.0005% w/v of *isosorbide 2-nitrate BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *isosorbide mononitrate BPCRS* in the mobile phase. Solution (4) contains 0.005% w/v each of *isosorbide dinitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *aminopropylsilyl silica gel for chromatography* (10 µm) (Lichrosorb NH_2 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 15 volumes of *absolute ethanol* and 85 volumes of *2,2,4-trimethylpentane* and (c) a detection wavelength of 215 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

Disintegration

The requirement for Disintegration does not apply to Isosorbide Dinitrate Tablets.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III

D, using the following solutions. For solution (1) add 20 ml of the mobile phase to a quantity of the powdered tablets containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase. For solution (2) add 20 ml of the mobile phase to a quantity of *isosorbide dinitrate BPCRS* containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the mobile phase. Filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 1 volume to 10 volumes with the mobile phase. Solution (3) contains 0.005% w/v each of *isosorbide dinitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure described under Isosorbide 5-nitrate and isosorbide 2-nitrate may be used but using a detection wavelength of 230 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

Calculate the content of $C_6H_8N_2O_8$ in the tablets from the chromatograms obtained and using the declared content of $C_6H_8N_2O_8$ in *isosorbide dinitrate BPCRS*.

LABELLING

The label states whether the tablets are to be swallowed whole or chewed before being swallowed.

When Isosorbide Dinitrate Tablets are prescribed or demanded, either Isosorbide Dinitrate Tablets or Isosorbide Dinitrate Sublingual Tablets may be dispensed or supplied, as appropriate.

Isosorbide Mononitrate Tablets

General Notices

Action and use

Nitric oxide analogue; angina pectoris.

DEFINITION

Isosorbide Mononitrate Tablets contain Diluted Isosorbide Mononitrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isosorbide mononitrate, $C_6H_9NO_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 plates are suitable) and a mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 10 mg of isosorbide mononitrate with 10 ml of *ethanol (96%)* and filter. Prepare solution (2) in the same manner but using *isosorbide mononitrate BPCRS* in place of the powdered tablets. After removal of the plate, dry it in a current of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Analtech silica gel H plates are suitable) and a mixture of 15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of isosorbide mononitrate with 5 ml of *ethanol (96%)* and filter. For solution (2) dissolve 10 mg of *potassium nitrate* in 1 ml of *water* and dilute to 100 ml with *ethanol (96%).* After removal of the plate thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight. In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Isosorbide dinitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 15 ml of mobile phase to a quantity of the powdered tablets containing 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a membrane filter with a nominal pore size not greater than 0.45 µm and use the filtrate. Solution (2) contains 0.001% w/v of *isosorbide 2-nitrate BPCRS* in the mobile phase. Solution (3) contains 0.001% w/v of *isosorbide dinitrate BPCRS* in the mobile phase. Solution (4) contains 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octyldecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 30 volumes of *methanol* and 70 volumes of *water* and (c) a detection wavelength of 220 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

In the chromatogram obtained with solution (1) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 70 ml of mobile phase to a quantity of the powdered tablets containing 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes, dilute to 100 ml with the same solvent and centrifuge. Dilute 1 volume of the supernatant liquid to 5 volumes with mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm. For solution (2) add 20 ml of mobile phase to a quantity of *isosorbide mononitrate BPCRS* containing 25 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes, dilute to 25 ml with the same solvent and centrifuge. Dilute 1 volume of the supernatant liquid to 10 volumes with mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm. Solution (3) contains 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure described under Isosorbide dinitrate and isosorbide 2-nitrate may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

Calculate the content of $C_6H_9NO_6$ in the tablets from the chromatograms obtained and using the declared content of $C_6H_9NO_6$ in *isosorbide mononitrate BPCRS*.

Isotretinoin Capsules

General Notices

Action and use

Vitamin A analogue (retinoid); treatment of acne.

DEFINITION

Isotretinoin Capsules contain Isotretinoin.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of isotretinoin, $C_{20}H_{28}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (4).

B. The *light absorption* of the final solution obtained in the Assay, Appendix II B, in the range 300 to 400 nm, exhibits a maximum at 356 nm.

TESTS

Dissolution

Not less than 85% of the stated amount per capsule after 60 minutes. Protect the apparatus from light throughout the determination and flush the apparatus with nitrogen before use. Carry out the test using the apparatus for the *disintegration test for tablets and capsules*, Appendix XII A1, and 900 ml of 0.1M *sodium hydroxide* as the dissolution medium. Withdraw a 20-ml sample of the medium, filter immediately through a 0.2-µm filter, discarding the first 5 ml of filtrate, and dilute the filtrate with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00025% w/v of Isotretinoin. Measure the *absorbance* of this solution at 343 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell. Calculate the total content of isotretinoin, $C_{20}H_{28}O_2$, in the medium taking 1490 as the value of A(1%, 1 cm) at 343 nm and divide the result by 6 to obtain the amount per capsule.

Related substances

Protect the solutions from light throughout the procedure. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 10 mg of Isotretinoin with 10 ml of a mixture of 20 volumes of 0.1M *sodium hydroxide* and 80 volumes of *absolute ethanol* for 10 minutes, treat with ultrasound for 15 minutes and centrifuge for 5 minutes. Mix 5 ml of the supernatant liquid with 7.5 ml of 0.1M *hydrochloric acid* and extract with two 20-ml quantities of *dichloromethane*. Filter the combined extracts through phase separating paper (Whatman

1PS is suitable) and wash the filter with 5 ml of *dichloromethane*. Evaporate the combined filtrate and washing to dryness at 30° under reduced pressure and dissolve the residue in 5 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. Solution (3) contains 0.002% w/v of *tretinoin EPCRS* in *methanol*. Solution (4) contains 0.001% w/v of *isotretinoin EPCRS* in *methanol*. Solution (5) contains 0.001% w/v of each of *tretinoin EPCRS* and *isotretinoin EPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.4 ml per minute a 0.5% w/v solution of *glacial acetic acid* in a mixture of *methanol* and *water*, the proportions adjusted to give a retention time for tretinoin of about 15 minutes (a mixture of 23 volumes of *water* and 77 volumes of *methanol* is usually suitable) and (c) a detection wavelength of 353 nm.

Inject 10 µl of each solution and adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with solution (3) is at least 70% of the full scale of the recorder.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the peaks due to tretinoin and isotretinoin is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to tretinoin is not greater than twice the area of the peak in the chromatogram obtained with solution (3) (4%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than 1.5 times the area of the peak in the chromatogram obtained with solution (2) (1.5%).

ASSAY

Carry out the following procedure in subdued light. Slit 10 capsules with a scalpel avoiding loss of the capsule contents, place the capsules in a 500 ml graduated flask washing any material adhering to the scalpel into the flask with 25 ml of *dichloromethane* per capsule and mix with the aid of ultrasound for 15 minutes. Add sufficient *dichloromethane* to produce 500-ml, shake for 2 minutes and dilute with a solution prepared by diluting 5 ml of 0.1M *hydrochloric acid* to 250 ml with *ethanol (96%)* to produce a solution containing 0.0008% w/v of Isotretinoin. Measure the *absorbance* of this solution at the maximum at 356 nm, Appendix II B, using the ethanolic hydrochloric acid solution in the reference cell. Calculate the content of $C_{20}H_{28}O_2$ in each capsule taking 1350 as the value of A(1%, 1 cm) at the maximum at 356 nm.

STORAGE

Isotretinoin Capsules should be protected from light.

Isotretinoin Gel

General Notices

Action and use

Vitamin A analogue (retinoid); treatment of acne.

DEFINITION

Isotretinoin Gel is a solution of Isotretinoin in a suitable alcoholic basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of isotretinoin, $C_{20}H_{28}O_2$

90.0 to 110.0% of the stated amount.

Carry out all the operations as rapidly as possible protected from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2 volumes of *glacial acetic acid*, 4 volumes of *acetone*, 40 volumes of *peroxide-free ether* and 54 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1), shake to disperse a quantity of the gel containing 1 mg of Isotretinoin as completely as possible in 5 ml of *dichloromethane reagent* and 10 ml of *dichloromethane*, add 80 ml of n-*hexane* and shake the mixture vigorously; filter, evaporate the filtrate to dryness using a rotary evaporator at a temperature not exceeding 60° and dissolve the residue in 1 ml of *dichloromethane*. Solution (2) contains 0.1% w/v of *isotretinoin EPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), shake to disperse a quantity of the gel containing 2.5 mg of Isotretinoin as completely as possible in a mixture of 5 ml of *dichloromethane reagent* and 10 ml of *dichloromethane*, dilute to 50 ml with n-*hexane*, shake and filter. Solution (2) contains 0.0002% w/v of *tretinoin EPCRS* in n-*hexane*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with n-*hexane*. Solution (4) contains 0.0002% w/v of each of *isotretinoin EPCRS* and *tretinoin EPCRS* in n-*hexane*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb Si60 is suitable), (b) a mixture of 1 volume of *glacial acetic acid*, 30 volumes of *ethyl acetate* and 970 volumes of n-*hexane* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 365 nm.

Inject 50 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

In the chromatogram obtained with solution (1) the area of any peak due to tretinoin is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (4%) and the sum of the areas of any other *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (3) (1%).

ASSAY

Carry out the following procedure in subdued light. To a quantity of the gel containing 0.5 mg of isotretinoin, add 10 ml of *dichloromethane*, shake until all the gel has dispersed and dilute the solution to 100 ml with a solution prepared by diluting 5 ml of 0.1M *hydrochloric acid* to 250 ml with *ethanol (96%)*. Measure the *absorbance* of this solution at the maximum at 356 nm, Appendix II B, using the ethanolic hydrochloric acid solution in the reference cell. Calculate the content of $C_{20}H_{28}O_2$ in the gel taking 1350 as the value of A(1%, 1 cm) at the maximum at 356 nm.

Ispaghula Husk Effervescent Granules

General Notices

DEFINITION

Ispaghula Husk Effervescent Granules contain Ispaghula Husk in a suitable, effervescent basis.

The granules comply with the requirements stated under Granules and with the following requirements.

TESTS

Disintegration

Carry out the test stated under Effervescent Granules with the following modifications. Stir the contents of the beaker occasionally to disperse the mucilage formed; evolution of gas is complete after 5 minutes.

Swelling index

Not less than 40, Appendix XI C. Use a quantity of the powdered granules containing 1.0 g of Ispaghula Husk and a 100 ml ground-glass-stoppered cylinder graduated in 1 ml divisions.

Ispaghula Husk Granules

General Notices

DEFINITION

Ispaghula Husk Granules contain Ispaghula Husk with or without suitable excipients.

The granules comply with the requirements stated under Granules and with the following requirements.

IDENTIFICATION

A. Powder the granules and examine under a microscope using *lactic reagent*. Fragments of the episperm with polygonal cells filled with mucilage and fragments of the inner layers of the testa with brownish thin-walled cells often associated with the outer layers of the endosperm are seen.

B. When mounted in *ruthenium red solution*, the particles of the powder are stained red.

TESTS

Swelling index

Not less than 40, Appendix XI C. Use a quantity of the granules containing 1.0 g of Ispaghula Husk and a 100-ml ground-glass-stoppered cylinder graduated in 1 ml divisions.

Loss on drying

When dried at 100° to 105°, loses not more than 12.0% of its weight. Use 1 g.

Ash

Not more than 5.0%, Appendix XI J, Method II.

STORAGE

Ispaghula Husk Granules should be protected from moisture.

Ispaghula Husk Oral Powder

General Notices

DEFINITION

Ispaghula Husk Oral Powder contains Ispaghula Husk with or without suitable excipients.

The powder complies with the requirements stated under Oral Powders and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a suitable silica gel as the coating substance and a mixture of 15 volumes of *water* and 85 volumes of *acetonitrile* as the mobile phase. Apply separately to the plate, as bands, 10 µl of each of the following solutions. For solution (1) add 2 ml of a 23% w/v solution of *trifluoroacetic acid* to a quantity of the powder containing 10 mg of Ispaghula Husk in a thick-walled centrifuge tube, shake vigorously, close the tube and heat at 120° for 1 hour. Centrifuge the hydrolysate, transfer the clear supernatant liquid into a 50 ml flask, add 10 ml of *water* and evaporate the solution to dryness under reduced pressure. Take up the residue in 10 ml of *water*, again evaporate to dryness under reduced pressure and take up the residue in 2 ml of *methanol*. For solutions (2), (3) and (4) dissolve, separately, 10 mg of *arabinose*, 10 mg of *xylose* and 10 mg of *galactose* in small quantities of *water* and dilute each solution to 10 ml with *methanol*. After removal of the plate, allow it to dry in air, spray with *aminohippuric acid reagent*, heat at 120° for 5 minutes and examine in daylight. The chromatogram obtained with solution (1) shows two orange-pink zones (arabinose and xylose) and a yellow zone (galactose) similar in position and colour to the zones in the chromatograms obtained with solutions (2), (3) and (4).

B. When mounted in *ruthenium red solution*, the particles of the powder are stained red.

TESTS

Swelling index

Not less than 40, Appendix XI C. Use a quantity of the oral powder containing 1.0 g of Ispaghula Husk and a 100 ml ground-glass-stoppered cylinder graduated in 1 ml divisions.

Ash

Not more than 4.0%, Appendix XI J, Method II. Use a quantity of the powder containing 1 g of Ispaghula Husk.

STORAGE

Ispaghula Husk Oral Powder should be protected from moisture.

Isradipine Tablets

General Notices

Action and use

Calcium channel blocker.

DEFINITION

Isradipine Tablets contain Isradipine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isradipine, $C_{19}H_{21}N_3O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing 3 mg of Isradipine with 70 ml of *methanol (80%)* for 10 minutes, dilute to 100 ml with the same solvent and filter. The *light absorption* of the filtrate, Appendix II B, in the range 250 to 450 nm exhibits a maximum at 326 nm.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of a 0.1% w/v solution of N,N-*dimethyldodecylamine* N-*oxide* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 5 volumes of a 0.01% w/v solution *of isradipine BPCRS* in *methanol (80%)* to 100 volumes with a 0.1% w/v solution of N,N-*dimethyldodecylamine* N-*oxide.* Use the filtered dissolution medium as solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Brownlee Spheri ODS 5µ is suitable), (b) *methanol (80%)* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 326 nm. Inject 50 µl of each solution.

Calculate the content of $C_{19}H_{21}N_3O_5$ in the medium using the declared content of $C_{19}H_{21}N_3O_5$ in *isradipine BPCRS*.

Related substances

Carry out the test protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of *methanol (80%)* to a number of whole tablets containing 50 mg of Isradipine, shake for 1 hour, centrifuge the resulting mixture and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 400 volumes with *methanol (80%).* Solution (3) contains 0.0009% w/v *of isradipine impurity B BPCRS* in the mobile phase. Solution (4) contains 0.0005% w/v of *isradipine impurity D* in the mobile phase. Solution (5) contains 0.010% w/v each of *isradipine impurity D BPCRS* and *isradipine BPCRS* in *methanol (80%).* For solution (1) allow the chromatography to proceed for 5 times the retention time of the principal peak.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Brownlee Spheri ODS 5µ is suitable), (b) as the mobile phase a mixture of 125 volumes of *acetonitrile*, 270 volumes of *tetrahydrofuran* and 625 volumes of *water* with a flow rate of 1.2 ml per minute and (c) a detection wavelength of 230 nm.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the two principal peaks is at least 1.5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.25%), the area of any peak corresponding to isradipine impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.9%), and the area of any peak corresponding to isradipine impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (4) (0.5%). Calculate the percentage content of isradipine impurity B and isradipine impurity D using the respective reference solutions and the content of any unnamed impurities using solution (2). The total nominal content of impurities is not greater than 2%.

ASSAY

Carry out the assay protected from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of *methanol (80%)* to a number of whole tablets containing 50 mg of Isradipine, shake for 1 hour, centrifuge the resulting mixture and dilute a volume of the clear supernatant liquid with *methanol (80%)* to produce a solution containing 0.010% w/v of Isradipine. Solution (2) is a 0.010% w/v solution of *isradipine BPCRS* in *methanol (80%).* Solution (3) is a 0.010% w/v solution of *isradipine impurity D BPCRS* in solution (2).

The chromatographic procedure described under Related substances may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.5.

Calculate the content of $C_{19}H_{21}N_3O_5$ in the tablets using the declared content of $C_{19}H_{21}N_3O_5$ in *isradipine BPCRS*.

STORAGE

Isradipine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Isradipine.

Paediatric Ipecacuanha Emetic Mixture

General Notices

Paediatric Ipecacuanha Emetic; Paediatric Ipecacuanha Emetic Oral Solution

DEFINITION

Paediatric Ipecacuanha Emetic Mixture is an *oral solution*.

Ipecacuanha Liquid Extract	70 ml
Hydrochloric Acid	2.5 ml
Glycerol	100 ml
Syrup	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of total alkaloids

0.12 to 0.16% w/v, calculated as emetine, $C_{29}H_{40}N_2O_4$.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 90 volumes of *chloroform* and 10 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) mix 5 ml with 10 ml of 1M *sulphuric acid*, shake with two 10 ml quantities of *chloroform* and discard the chloroform. Add sufficient 5M *ammonia* to make the aqueous solution distinctly alkaline to *litmus paper*, extract with four 10 ml quantities of *chloroform*, evaporate the combined extracts to dryness, cool the residue and dissolve it in 0.5 ml of *ethanol (96%)*. Solution (2) contains 0.1% w/v of *cephaeline hydrochloride EPCRS* in *ethanol (96%)*. Solution (3) contains 0.1% w/v of *emetine hydrochloride EPCRS* in *ethanol (96%)*. After removal of the plate, dry it at 105° to 110° for 30 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution*. The principal spots in the chromatogram obtained with solution (1) correspond in colour and position to the spots in the chromatograms obtained with solutions (2) and (3). Disregard any *secondary spots*.

ASSAY

To 25 ml in a separating funnel add 20 ml of *water* and 5 ml of 1M *sulphuric acid*, shake with three 10 ml quantities of *chloroform* and wash each chloroform extract with a mixture of 20 ml of 0.05M *sulphuric acid* and 4 ml of *ethanol (96%)* contained in a second separating funnel. Transfer the acid–ethanol mixture from the second separating funnel to the first, make the combined liquids distinctly alkaline to *litmus paper* with 5M *ammonia* and extract with successive quantities of *chloroform* until *complete extraction* of the alkaloids is effected, Appendix XI G. Wash each chloroform extract with the same 10 ml of *water*, combine the chloroform extracts, evaporate the chloroform, add 2 ml of *ethanol (96%)* to the residue, evaporate to dryness and dry the residue at 80° in a current of air for 5 minutes. Dissolve the residue in 2 ml of *ethanol (96%)* previously neutralised to *methyl red solution*, add 10 ml of 0.01M *sulphuric acid VS* and titrate the excess of acid with 0.02M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 0.01M *sulphuric acid VS* is equivalent to 4.806 mg of $C_{29}H_{40}N_2O_4$.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Prolonged-release Isosorbide Mononitrate Tablets

General Notices

Prolonged-release Isosorbide Mononitrate Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Nitric oxide analogue; treatment of angina pectoris.

DEFINITION

Prolonged-release Isosorbide Mononitrate Tablets contain Diluted Isosorbide Mononitrate. They are formulated so that the medicament is released over a period of several hours. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of isosorbide mononitrate. The dissolution profile reflects the *in vivo* performance, which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of isosorbide mononitrate, $C_6H_9NO_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel 60 plates are suitable) and a mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing the equivalent of 10 mg of isosorbide mononitrate with 10 ml of *ethanol (96%)* and filter. Prepare solution (2) in the same manner but using *isosorbide mononitrate BPCRS* in place of the preparation being examined. After removal of the plate, dry it in a current of air, spray with *diphenylamine solution R1* and irradiate for 15 minutes with *ultraviolet light (254* and *365 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Inorganic nitrates

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Analtech silica gel H plates are suitable) and a mixture of 15 volumes of *glacial acetic* *acid*, 30 volumes of *acetone* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.1 g of isosorbide mononitrate with 5 ml of *ethanol (96%)* and filter. For solution (2) dissolve 10 mg of *potassium nitrate* in 1 ml of *water* and dilute to 100 ml with *ethanol (96%)*. After removal of the plate thoroughly dry it in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to *ultraviolet light (254 nm)* for 15 minutes and examine in daylight. In the chromatogram obtained with solution (1) any spot corresponding to potassium nitrate is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

Isosorbide dinitrate and isosorbide 2-nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 15 ml of mobile phase to a quantity of the powdered tablets containing the equivalent of 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a membrane filter with a nominal pore size not greater than 0.45 µm and use the filtrate. Solution (2) contains 0.001% w/v of *isosorbide 2-nitrate BPCRS* in the mobile phase. Solution (3) contains 0.001% w/v of *isosorbide dinitrate BPCRS* in the mobile phase. Solution (4) contains 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS2 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 30 volumes of *methanol* and 70 volumes of *water* and (c) a detection wavelength of 220 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

In the chromatogram obtained with solution (1) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 70 ml of mobile phase to a quantity of the powdered tablets containing the equivalent of 50 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes and dilute to 100 ml with the same solvent and centrifuge. Dilute 1 volume of the supernatant liquid to 5 volumes with the mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm. Solution (2) contains 0.01% w/v of *isosorbide mononitrate BPCRS* in the mobile phase. Solution (3) contains 0.001% w/v each of *isosorbide mononitrate BPCRS* and *isosorbide 2-nitrate BPCRS* in the mobile phase.

The chromatographic procedure described under Isosorbide dinitrate and isosorbide 2-nitrate may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

Calculate the content of $C_6H_9NO_6$ in the tablets from the chromatograms obtained and using the declared content of $C_6H_9NO_6$ in *isosorbide mononitrate BPCRS*.

Protamine Zinc Insulin Injection

General Notices

Protamine Zinc Insulin

Action and use

Antidote to heparin.

DEFINITION

Protamine Zinc Insulin Injection is a sterile buffered suspension of bovine, porcine or human insulin complexed with Protamine Sulphate or another suitable protamine and Zinc Chloride or another suitable zinc salt.

The injection complies with the requirements stated under Insulin Preparations and with the following requirements.

PRODUCTION

Protamine Zinc Insulin Injection is prepared by carrying out the procedures described under Insulin Preparations. A suitable protamine is added in the proportion of 1.0 to 1.7 mg of protamine sulphate per 100 IU of insulin.

DESCRIPTION

A white suspension which on standing deposits a white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by shaking. When examined under a microscope, approximately 50% of the particles are seen to have no uniform shape, with a maximum diameter rarely exceeding 2 µm. The remaining particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 10 µm but rarely exceeding 100 µm.

IDENTIFICATION

In the Assay the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the appropriate reference solution.

Total zinc

20 to 25 mg per 100 IU of insulin, determined as described under Insulin Preparations.

Kaolin and Morphine Mixture

General Notices

Kaolin and Morphine Oral Suspension

DEFINITION

Kaolin and Morphine Mixture is an *oral suspension* containing 20% w/v of Light Kaolin or Light Kaolin (Natural), 5% w/v of Sodium Bicarbonate and 4% v/v of Chloroform and Morphine Tincture in a suitable vehicle.

It should be recently prepared unless the kaolin has been sterilised.

Extemporaneous preparation

The following formula applies.

Light Kaolin or Light Kaolin (Natural)	200 g
Sodium Bicarbonate	50 g
Chloroform and Morphine Tincture	40 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

4.65 to 5.35% w/v.

Content of anhydrous morphine, $C_{17}H_{19}NO_3$

0.0055 to 0.0080% w/v.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 70 volumes of *toluene*, 20 volumes of *ethyl acetate* and 10 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) centrifuge 10 ml of the preparation, reserving the supernatant liquid. Extract the residue with four 10 ml quantities of a mixture of 38 ml of *acetone* and 2 ml of 5M *ammonia*, centrifuging between each extraction, and evaporate the combined extracts on a water bath to about 5 ml. Mix with the reserved supernatant liquid, add 10 ml of 1M *sulphuric acid*, shake with two 10 ml quantities of *chloroform* and discard the chloroform. Add sufficient 5M *ammonia* to make the aqueous solution alkaline to *litmus paper*, extract with three 10 ml quantities of a mixture of equal volumes of *chloroform* and *ethanol (96%)*, evaporate to dryness and dissolve the residue in 0.5 ml of *ethanol (96%)*. Solution (2) contains 0.10% w/v of *morphine hydrochloride*. After removal of the plate, heat it at 105° to 110° for 30 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution*.

The principal spot in the chromatogram obtained with solution (1) corresponds in colour and position to that in the chromatogram obtained with solution (2).

TESTS

Acid-insoluble matter

13.8 to 18.4% w/w when determined by the following method. To 3 g add 15 ml of *water*, cautiously add sufficient 2M *hydrochloric acid* to make the suspension acidic to *litmus paper*, boil for 5 minutes, replacing the water lost by evaporation, cool and filter the supernatant liquid. Boil the residue with 20 ml of *water* and 10 ml of 2M *hydrochloric acid*, cool, filter through the same filter and wash the residue with *water* until the washings are free from chloride. Dry and ignite the residue to constant weight at red heat.

ASSAY

For sodium bicarbonate

Boil 20 ml with 40 ml of *water* for 5 minutes, replacing the water lost by evaporation, add 50 ml of *ethanol (96%)* previously neutralised to *methyl red solution* and allow to stand for 1 hour. Filter and wash the residue with 100 ml of a mixture of equal volumes of the neutralised ethanol and *water*. Add 50 ml of 0.5M *hydrochloric acid VS* to the combined filtrate and washings, boil, cool and titrate the excess of hydrochloric acid with 0.5M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

For anhydrous morphine

Centrifuge 25 ml, reserving the supernatant liquid, and extract the residue with three 25 ml quantities of *ethanol (96%)*, centrifuging between each extraction and reserving each supernatant liquid. Add 40 ml of *water* and 7 ml of 5M *ammonia* to the combined supernatant liquids and extract the mixture with three 30 ml quantities of *chloroform*. Gently shake each extract with the same 15 ml of a mixture of 2 volumes of *water* and 1 volume of *ethanol (96%)* and discard the aqueous ethanol. Evaporate the combined chloroform solutions just to dryness, warm the residue with 10 ml of 1M *hydrochloric acid*, cool the solution, which may be slightly cloudy, add sufficient *water* to produce 50 ml and filter (Whatman No. 42 paper is suitable). To 20 ml of the filtrate add 8 ml of a freshly prepared 1% w/v solution of *sodium nitrite*, allow to stand in the dark for 15 minutes, add 12 ml of 5M *ammonia* and sufficient *water* to produce 50 ml and measure the *absorbance* of a 4-cm layer at 442 nm without delay, Appendix II B. Use in the reference cell a solution prepared in the same manner but using *water* in place of the sodium nitrite solution. Calculate the content of anhydrous morphine taking 124 as the value of A(1%, 1 cm) at the maximum at 442 nm.

STORAGE

Kaolin and Morphine Mixture should be kept in well-filled glass containers.

LABELLING

The label indicates the pharmaceutical form as 'oral suspension'.

Kaolin Mixture

General Notices

Kaolin Oral Suspension

DEFINITION

Kaolin Mixture is an *oral suspension* containing 20% w/v of Light Kaolin or Light Kaolin (Natural) and 5% w/v each of Light Magnesium Carbonate and Sodium Bicarbonate in a suitable vehicle with a peppermint flavour.

It should be recently prepared unless the kaolin has been sterilised.

Extemporaneous preparation

The following formula applies.

Light Kaolin or Light Kaolin (Natural)	200 g
Light Magnesium Carbonate	50 g
Sodium Bicarbonate	50 g
Concentrated Peppermint Emulsion	25 ml
Double-strength Chloroform Water	500 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium, Mg

1.04 to 1.25% w/w.

Content of sodium bicarbonate, $NaHCO_3$

4.05 to 4.65% w/w.

TESTS

Acid-insoluble matter

13.8 to 18.4% w/w when determined by the following method. Dry and ignite the residue reserved in the Assay for magnesium to constant weight at red heat.

ASSAY

For magnesium

To 3 g add 15 ml of *water*, make acidic to *litmus paper* by the cautious addition of 2M *hydrochloric acid*, boil for 5 minutes, replacing the water lost by evaporation, cool and filter the supernatant liquid. Boil the residue with 20 ml of *water* and 10 ml of 2M *hydrochloric acid*, cool, filter through the same filter and wash the residue with *water* until the washings are free from chloride. Reserve the residue for the test for Acid-insoluble matter. Dilute the combined filtrate and washings to 100 ml with *water*. To 20 ml add 0.1 g of L-*ascorbic acid*, make slightly alkaline to *litmus paper* with 5M *ammonia* and add 10 ml of *triethanolamine*, 10 ml of *ammonia buffer pH 10.9* and 1 ml of a 10% w/v solution of *potassium cyanide*. Titrate with 0.05M *disodium edetate VS*, using *mordant black 11 solution* as indicator, to a full blue colour. Each ml of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg.

For sodium bicarbonate

Boil 10 g with 100 ml of *water* for 5 minutes, filter, boil the residue with 100 ml of *water* for 5 minutes and filter. Combine the filtrates, cool and titrate with 0.5M *hydrochloric acid VS* using *methyl orange-xylene cyanol FF solution* as indicator. Add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. After subtracting one-fifth of the volume of 0.05M *disodium edetate VS*, each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

LABELLING

The label indicates the pharmaceutical form as 'oral suspension'.

Kaolin Poultice

General Notices

DEFINITION

Heavy Kaolin[1], finely sifted	527 g
Boric Acid, finely sifted	45 g
Methyl Salicylate	2 ml
Thymol	500 mg
Peppermint Oil	0.5 ml
Glycerol	425 g

Extemporaneous preparation

The following directions apply.

Mix the Heavy Kaolin, previously dried at 100°, and the Boric Acid with the Glycerol, heat at 120° for 1 hour, stirring occasionally, and allow to cool. Separately, dissolve the Thymol in the Methyl Salicylate, add to the cooled mixture, add the Peppermint Oil and mix thoroughly.

The heating step may be omitted if some other satisfactory means of mixing the solid ingredients with the Glycerol is used but in this case Heavy Kaolin that has been sterilised is used.

The poultice complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of boric acid, H_3BO_3

4.25 to 4.75% w/w.

ASSAY

Mix 20 g with a solution containing 10 g of D-*mannitol* in 40 ml of *water*, warm and stir until completely dispersed and titrate with 1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 1M *sodium hydroxide VS* is equivalent to 61.83 mg of H_3BO_3.

STORAGE

Kaolin Poultice should be kept in suitable containers that minimise absorption, diffusion or evaporation of the ingredients.

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[1] If an appropriate heating step, such as that described under Extemporaneous preparation, is not included in the manufacturing process, Heavy Kaolin that has been sterilised is used.

Ketamine Injection

General Notices

Action and use

Intravenous general anaesthetic.

DEFINITION

Ketamine Injection is a sterile solution of Ketamine Hydrochloride in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ketamine hydrochloride, $C_{13}H_{16}ClNO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume of the injection with a mixture of 1 volume of 1M *sodium hydroxide* and 49 volumes of *methanol* to produce a solution containing 0.08% w/v of ketamine hydrochloride. The *light absorption*, Appendix II B, in the range 230 to 350 nm of this solution exhibits a maximum at 301 nm and shoulders at 274, 268 and 261 nm.

B. Dilute a volume of the injection with 0.1M *hydrochloric acid* to produce a solution containing 0.029% w/v of ketamine hydrochloride. The *light absorption*, Appendix II B, in the range 230 to 350 nm of this solution exhibits two maxima at 276 nm and at 269 nm and a shoulder at 260 nm.

TESTS

Acidity

pH, 3.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, with the mobile phase to produce a solution containing 0.12% w/v of ketamine hydrochloride. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. For solution (3) dilute 1 volume of solution (2) to 2 volumes with the mobile phase. For solution (4) add 2 volumes of a 0.05% w/v solution of *ketamine impurity A EPCRS* in the mobile phase to 1 volume of solution (1) and dilute to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.0 mm) and a stainless steel pre-column (4 mm × 4.0 mm), both packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 5µ is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture prepared by dissolving 0.95 g of *sodium hexanesulphonate* in 1 litre of a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water* and adding 4 ml of 6M *acetic acid* and (c) a detection wavelength of 215 nm.

Inject solution (4). When the chromatograms are recorded in the prescribed conditions, the retention time is about 3 to 4.5 minutes for ketamine. The test is not valid unless the *resolution factor* between the peaks due to ketamine and ketamine impurity A is at least 1.5. If necessary, adjust the concentrations of water and acetonitrile in the mobile phase.

Inject solutions (1), (2) and (3). Continue the chromatography for 10 times the retention time of ketamine. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the area of not more than one such peak is greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.25%). Disregard any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Dilute a volume of the injection containing 25 mg of Ketamine Hydrochloride to 100 ml with 0.05M *hydrochloric acid* and mix. Measure the *absorbance* of the resulting solution at the maximum at 269 nm, Appendix II B. Measure the *absorbance* of a 0.025% w/v solution of *ketamine hydrochloride BPCRS* in 0.05M *hydrochloric acid* at the same wavelength.Calculate the content of $C_{13}H_{16}ClNO,HCl$ using the declared content of $C_{13}H_{16}ClNO,HCl$ in *ketamine hydrochloride BPCRS*.

STORAGE

Ketamine Injection should be protected from light and stored at a temperature not exceeding 30°.

Ketoprofen Capsules

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ketoprofen Capsules contain Ketoprofen.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ketoprofen, $C_{16}H_{14}O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.5 g of Ketoprofen with 50 ml of *chloroform* for 5 minutes, filter, evaporate to dryness using a rotary evaporator and induce crystallisation by prolonged scratching of the side of the container with a glass rod. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of ketoprofen *(RS 198)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a phosphate buffer prepared by dissolving 1.46 g of *potassium dihydrogen orthophosphate* and 20.06 g of *disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 ml, adjusting the pH to 7.5 if necessary with *orthophosphoric acid*, and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain about 0.001% w/v of Ketoprofen. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 260 nm using dissolution medium in the reference cell. Calculate the total content of ketoprofen, $C_{16}H_{14}O_3$, in the medium taking 662 as the value of A(1%, 1 cm) at the maximum at 260 nm.

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Ketoprofen with 100 ml of a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water* (solvent A), filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 50 volumes with solvent A and further dilute 1 volume to 10 volumes with solvent A. Solution (3) contains 0.0002% w/v of *impurity C EPCRS* (2-(3-carboxyphenyl)propionic acid) in solvent A. Solution (4) contains 0.0003% w/v of *impurity A EPCRS* (3-acetyl-benzophenone) in solvent A. For solution (5) dilute 1 volume of solution (1) to 100 volumes with solvent A; to 1 ml add 1 ml of solution (4).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 2 volumes of freshly prepared *phosphate buffer pH 3.5*, 43 volumes of *acetonitrile* and 55 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 233 nm.

Inject 20 µl of solution (5). The test is not valid unless the *resolution factor* between the peaks due to ketoprofen and 3-acetylbenzophenone is at least 7.

Inject separately 20 µl of solutions (1) to (4). Allow the chromatography to proceed for seven times the retention time of ketoprofen. In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-(3-carboxyphenyl)propionic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%), the area of any peak corresponding to 3-acetylbenzophenone is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.3%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of all the *secondary peaks* other than those due to the two named impurities is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Ketoprofen for 10 minutes with 300 ml of *methanol (75%)*, mix and dilute to 500 ml with *methanol (75%)*. Allow to stand, dilute 5 ml of the supernatant liquid to 100 ml with *methanol (75%)* and measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{16}H_{14}O_3$ taking 662 as the value of A(1%, 1 cm) at the maximum at 258 nm.

Ketoprofen Gel

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Ketoprofen Gel is a solution of Ketoprofen in a suitable water-miscible basis.
The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of ketoprofen, $C_{16}H_{14}O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (3).

TESTS

Acidity or alkalinity

pH of a 1% w/v dispersion of the gel in *carbon dioxide-free water*, 5.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of *formic acid*, 25 volumes of *toluene* and 75 volumes of *di-isopropyl ether* as the mobile phase. Apply separately to the plate 25 µl of each of the following solutions. For solution (1) shake a quantity of the gel containing 40 mg of Ketoprofen with 5 ml of *methanol* for 15 minutes, centrifuge the mixture for 5 minutes and use the supernatant liquid. Solution (2) contains 0.032% w/v of *ketoprofen ethyl ester BPCRS* in *methanol*. Solution (3) contains 0.8 % w/v of *ketoprofen BPCRS* in *methanol*. Solution (4) contains 0.004% w/v of *ketoprofen BPCRS* in *methanol*. Solution (5) contains 0.0016% w/v of *ketoprofen BPCRS* in *methanol*. After removal of the plate, dry it at 105° for 1 hour and examine under *ultraviolet light (254 nm)*. Spray the plate with a 0.4% w/v solution of *2,4-dinitrophenylhydrazine* in *methanol* containing 5% v/v of *hydrochloric acid* and dry the plate at 105° for 30 minutes. Spray the plate with a mixture of 10 volumes of *tetraethylammonium hydroxide solution* and 10 volumes of *methanol*, dry the plate at 105° for 5 minutes and examine in daylight. By each method of visualisation any spot corresponding to ketoprofen ethyl ester in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (2) (4%), any other *secondary spot* is not more intense than the principal spot in the chromatogram obtained with solution (4) (0.5%) and not more than three such spots are more intense than the principal spot in the chromatogram obtained with solution (5) (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the gel containing 10 mg of Ketoprofen with 50 ml of *methanol* for 15 minutes, centrifuge the mixture for 5 minutes, dilute 25 ml of the supernatant liquid with sufficient of a mixture of 270 ml of *acetonitrile* and 550 ml of a 0.5% w/v solution of *ammonium acetate* to produce 100 ml and mix. For solution (2) add 19 ml of *methanol* to 5 ml of a 0.1% w/v solution of *ketoprofen BPCRS* in *methanol*, add sufficient of a mixture of 270 ml of *acetonitrile* and 550 ml of a 0.5% w/v solution of *ammonium acetate* to produce 100 ml and mix.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 450 volumes of a solution containing 40% v/v of *methanol* and 60% v/v of *acetonitrile* and 550 volumes of a 0.5% w/v solution of *ammonium acetate*, adjusted to pH 5.9 by the addition of 10% w/w *nitric acid* and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{16}H_{14}O_3$ in the gel using the declared content of $C_{16}H_{14}O_3$ in *ketoprofen BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include ketoprofen ethyl ester and impurities A, B, E and F listed under Ketoprofen.

Labetalol Injection

General Notices

Action and use

Alpha- and beta-adrenoceptor antagonist.

DEFINITION

Labetalol Injection is a sterile solution of Labetalol Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of labetalol hydrochloride, $C_{19}H_{24}N_2O_3$,HCl

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless or very pale yellow solution.

IDENTIFICATION

A. Mix a volume containing 50 mg of Labetalol Hydrochloride with 50 ml of 0.1M *hydrochloric acid* and heat on a water bath for 30 minutes. Cool, filter, add 10 ml of *ammonia buffer pH 10.0* and extract with three 15 ml quantities of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of labetalol *(RS 199)*.

B. The *light absorption*, Appendix II B, in the range 250 to 400 nm of a 0.004% w/v solution of the residue obtained in test A in 0.1M *sodium hydroxide* exhibits a maximum only at 333 nm.

TESTS

Acidity

pH, 3.5 to 4.5, Appendix V L.

Diastereoisomer ratio

Examine by *gas chromatography*, Appendix III B, using a solution prepared in the following manner. Dilute a volume of the injection containing 20 mg of Labetalol Hydrochloride with *ethanol (96%)* to about 10 ml and evaporate to dryness using a rotary evaporator. Dissolve 2.0 mg of the residue in 1 ml of a 1.2% w/v solution of *1-butaneboronic acid* in *anhydrous pyridine* and allow to stand for 20 minutes.

The chromatographic procedure may be carried out using (a) a glass column (1.5 m × 4 mm) packed with *silanised diatomaceous support* (125 µm to 150 µm) impregnated with 3% w/w of *polymethylphenylsiloxane*, (b) *nitrogen for chromatography* as the carrier gas, (c) a flame-ionisation detector, maintaining the temperature of the column, the injection port and the detector at 300°. Inject 2 µl of the solution.

Two peaks each corresponding to a pair of diastereoisomers appear in the chromatogram. Adjust the sensitivity of the detector so that in the chromatogram obtained, the height of the taller of the diastereoisomer peaks is about 80% of the full scale of the recorder. The test is not valid unless the height of the trough separating these peaks is less than 5% of the full scale of the recorder. The area of each peak is not less than 45% and not more than 55% of the total area of both peaks.

Free carboxylic acid and other related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 5 volumes of 13.5M *ammonia*, 25 volumes of *methanol* and 75 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) dilute a volume of the injection containing 80 mg of Labetalol Hydrochloride to about 50 ml with *ethanol (96%)*, evaporate to dryness using a rotary evaporator and dissolve the residue in 1 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.160% w/v of *5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)-ethyl]salicylic acid hydrochloride BPCRS* in *methanol*. After removal of the plate, dry it in a current of warm air, heat at 105° for 30 minutes, cool and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino) ethyl]salicylic acid is not more intense that the spot in the chromatogram obtained with solution (3) (2%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Dilute a volume containing 50 mg of Labetalol Hydrochloride to 100 ml with *water*. To 10 ml of the solution add 10 ml of 0.05M *sulphuric acid* and dilute to 100 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_{19}H_{24}N_2O_3$,HCl taking 86 as the value of A(1%, 1 cm) at the maximum at 302 nm.

STORAGE

Labetalol Injection should be protected from light.

Labetalol Tablets

General Notices

Action and use

Alpha- and beta-adrenoceptor antagonist.

DEFINITION

Labetalol Tablets contain Labetalol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of labetalol hydrochloride, $C_{19}H_{24}N_2O_3,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 50 mg of Labetalol Hydrochloride add 50 ml of 0.1M *hydrochloric acid* and heat on a water bath for 30 minutes. Cool, filter, add 10 ml of *ammonia buffer pH 10.0* and extract with three 15 ml quantities of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of labetalol *(RS 199)*.

B. The *light absorption*, Appendix II B, in the range 250 to 400 nm of a 0.004% w/v solution in 0.1M *sodium hydroxide* of the residue obtained in test A exhibits a maximum only at 333 nm.

TESTS

Diastereoisomer ratio

Comply with the test described under Labetalol Injection using 2 mg of the residue prepared in the following manner. Shake a quantity of the powdered tablets containing 0.5 g of Labetalol Hydrochloride with 10 ml of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 5 volumes of 13.5M *ammonia*, 25 volumes of *methanol* and 75 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.5 g of Labetalol Hydrochloride with 10 ml of *methanol*, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) to 2 volumes with *methanol*. After removal of the plate, dry it in a current of warm air, heat at 105° for 30 minutes, cool and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 1 g of Labetalol Hydrochloride with 250 ml of 0.05M *sulphuric acid* for 30 minutes. Dilute the mixture to 500 ml with 0.05M *sulphuric acid*, mix, filter and dilute 10 ml of the filtrate to 250 ml with 0.05M *sulphuric acid*. Measure the *absorbance* of the resulting solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_{19}H_{24}N_2O_3$,HCl taking 86 as the value of A (1%, 1 cm) at the maximum at 302 nm.

Lacidipine Tablets

General Notices

Action and use

Calcium channel blocker.

DEFINITION

Lacidipine Tablets contain Lacidipine. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lacidipine, $C_{26}H_{33}NO_6$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light and prepare solutions immediately before use.

IDENTIFICATION

A. Shake a quantity of whole tablets containing 4 mg of Lacidipine with 50 ml of *absolute ethanol* with the aid of ultrasound for 30 minutes. Dilute to 100 ml with *absolute ethanol* and filter through a 0.45-µm membrane filter (Millipore Millex is suitable). The *light absorption* of the filtrate, Appendix II B, in the range 250 to 400 nm exhibits maxima only at 284 nm and 368 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use as the medium 500 ml of a solution prepared by mixing 100 ml of *water* with 10 ml of *polysorbate 20*, shaking gently and diluting to 1000 ml with *water* at a temperature of 37°.

PROCEDURE

(1) After 45 minutes, withdraw a sample of 10 ml of the medium and filter immediately through a 0.45-µm membrane filter (Millipore Millex is suitable), having first activated the filter with 3 ml of *methanol* followed by 5 ml of a 1% w/v solution of *polysorbate 20*. Measure the *absorbance* of the filtrate in a 2-cm cell, suitably diluted with the dissolution medium if necessary, at the maximum at 284 nm, Appendix II B, using the dissolution medium in the

reference cell.

(2) Measure the *absorbance,* in a 2 cm cell, of a solution of *lacidipine BPCRS* prepared by diluting a 0.02% w/v solution in *absolute ethanol* to a suitable volume with the dissolution medium at the maximum at 284 nm, Appendix II B, using the dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of lacidipine, $C_{26}H_{33}NO_6$, in the medium using the declared content of $C_{26}H_{33}NO_6$ in *lacidipine BPCRS*.

Related substances

Carry out method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 20 mg of Lacidipine with 50 ml of *absolute ethanol* with the aid of ultrasound for 15 minutes. Cool, dilute to 100 ml with *absolute ethanol*, filter through a 0.45-µm membrane filter (Millipore Millex is suitable) and dilute 5 volumes of the filtrate to 20 volumes with *hexane*.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *lacidipine impurity standard BPCRS* in *absolute ethanol* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µl of each solution.

(g) If necessary adjust the composition of the mobile phase so that, in the chromatogram obtained with solution (3), the retention time of the peak due to lacidipine is about 10 minutes.

(h) For solution (1) allow the chromatography to proceed for 2 times the retention time of the principal peak.

MOBILE PHASE

3 volumes of *absolute ethanol* and 97 volumes of n-*hexane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lacidipine impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak due to lacidipine impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%, taking into account the correction factor of 2);

— the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

— the total nominal content of impurities is not greater than 2.5%;

— disregard any peak with a relative retention time of 1.5 with respect to the peak due to lacidipine impurity B.

Uniformity of content

Tablets containing less than 2 mg or 2% of Lacidipine comply with the requirements stated under Tablets using the following method of analysis. Place one tablet in 50 ml of *absolute ethanol*, disperse the tablet completely with the aid of ultrasound for 30 minutes crushing with a glass rod if it does not disintegrate, and mix. Cool and filter through a 0.45-µm membrane filter (Millipore Millex is suitable). If necessary further dilute with *absolute ethanol* to produce a solution containing 0.004% w/v of Lacidipine. Measure the *absorbance* of the resulting solution at the maximum at 368 nm, Appendix II B, using *absolute ethanol* in the reference cell. Calculate the content of $C_{26}H_{33}NO_6$ in each tablet from the *absorbance* obtained from a 0.004% w/v solution of *lacidipine BPCRS* in *absolute ethanol* and using the declared content of $C_{26}H_{33}NO_6$ in *lacidipine BPCRS.*

ASSAY

Carry out method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse with the aid of ultrasound 10 whole tablets in 50 ml of *absolute ethanol* for 10 minutes, cool and filter through a 0.45-µm membrane filter (Millipore Millex is suitable). Dilute a quantity of the filtrate with sufficient mobile phase to produce a solution containing 0.01% w/v of Lacidipine.

(2) Dilute 5 volumes of a 0.04% w/v solution of *lacidipine BPCRS* in *absolute ethanol* to 20 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *lacidipine impurity standard BPCRS* in *absolute ethanol* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lacidipine impurity standard BPCRS*.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{33}NO_6$ in the tablets using the declared content of $C_{26}H_{33}NO_6$ in *lacidipine BPCRS*.

STORAGE

Lacidipine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Lacidipine.

Lactic Acid Pessaries

General Notices

DEFINITION

Lactic Acid Pessaries are moulded pessaries containing Lactic Acid in a suitable basis.
The pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

Content of lactic acid, $C_3H_6O_3$

95.0 to 105.0% of the stated amount.

ASSAY

Weigh five pessaries, melt together by warming and allow to cool, stirring continuously. Dissolve a quantity of the mass containing 1 g of Lactic Acid in 50 ml of *water*, add 50 ml of 0.5M *sodium hydroxide VS*, boil gently for 5 minutes, cool and titrate the excess of alkali with 0.5M *hydrochloric acid VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 45.04 mg of $C_3H_6O_3$.

Lactulose Oral Powder

General Notices

Action and use

Osmotic laxative.

DEFINITION

Lactulose Oral Powder consists of Lactulose with or without lesser amounts of other sugars including lactose and galactose.
The oral powder complies with the requirements stated under Oral Powders and with the following requirements.

Content of lactulose, $C_{12}H_{22}O_{11}$

95.0 to 102.0%.

Dissolve 3 g in carbon dioxide-free water and dilute to 50 ml with the same solvent (solution S).

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *glacial acetic acid*, 15 volumes of a 5% w/v solution of *boric acid*, 20 volumes of *methanol* and 55 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dissolve 50 mg of the oral powder in *water* and dilute to 10 ml with the same solvent. For solution (2) dissolve 50 mg of *lactulose EPCRS* in *water* and dilute to 10 ml with the same solvent. After removal of the plate, dry it at 100° to 105° for 5 minutes and allow to cool. Spray the plate with a 0.1% w/v solution of *1,3-dihydroxynaphthalene* in a mixture of 10 volumes of *sulphuric acid* and 90 volumes of *methanol*. Heat the plate at 110° for 5 minutes. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) is similar in position and size to the principal peak in the chromatogram obtained with solution (3).

C. Dissolve 0.05 g in 10 ml of *water*. Add 3 ml of *cupri-tartaric solution* and heat. A red precipitate is formed.

D. Dissolve 0.125 g in 5 ml of *water*. Add 5 ml of *ammonia*. Heat on a water bath at 80° for 10 minutes. A red colour develops.

E. Complies with the test for Specific optical rotation.

TESTS

Clarity and colour of solution

Solution S is *clear*, Appendix IV A, and not more intensely coloured than *reference solution*

BY_5, Appendix IV B, Method II.

pH

To 10 ml of solution S add 0.1 ml of a saturated solution of *potassium chloride*. The pH of the solution is 3.0 to 7.0, Appendix V L.

Specific optical rotation

Dissolve 1.25 g in *water*, add 0.2 ml of 13. 5M *ammonia* and dilute to 25 ml with *water*. The *specific optical rotation* is –46.0° to –50.0°, Appendix V G.

Related substances

In the Assay, in the chromatogram obtained with solution (1), the sum of the areas of any peaks corresponding to the principal peaks in the chromatograms obtained with solutions 5, 6, 7, 8 and 9 respectively (galactose, lactose, epilactose, tagatose and fructose) is not greater than the area of the peak corresponding to lactulose in the chromatogram obtained with solution (2) (3%).

Methanol

Carry out the method for *head-space gas chromatography*, Appendix III B, Method II. Mix 0.5 ml of *propan-1-ol* with 100 ml of *water*. Dilute 1 ml to 100 ml with *water*. Dilute 5 ml to 50 ml with *water* (internal standard solution). For solution (1) add 1 ml of the internal standard solution and 5 µl of a 0.1% v/v solution of *methanol* to 79 of the oral powder in a 20 ml vial. For solution (2) add 5 µl of a 0.1% v/v solution of *methanol* to 1 ml of the internal standard solution in a 20 ml vial.

The chromatographic procedure may be carried out using (a) a column (2 m × 2 mm) packed with *ethylvinylbenzene–divinylbenzene co-polymer* (180 µm), (b) *helium for chromatography* as the carrier gas at a flow rate of 30 ml per minute and (c) a flame-ionisation detector, maintaining the temperature of the column at 140°, that of the injection port at 200° and that of the detector at 220°. Maintain each solution at 60° for 1 hour, pressurise for 1 minute and transfer onto the column 1 ml of the gaseous phase.

In the chromatogram obtained with solution (1), the ratio of the area of the methanol peak to that of the internal standard peak is not greater than twice the corresponding ratio for the chromatogram obtained with solution (2) (50 ppm, calculated assuming the density of methanol to be 0.79 g/ml at 20°).

Boron

Avoid where possible the use of glassware. Prepare a reference solution as follows. Dissolve 50 mg of *boric acid* in *water* and dilute to 100 ml with the same solvent. Dilute 5 ml of this solution to 100 ml with *water*. Keep in a well-closed polyethylene container. In four polyethylene 25 ml flasks, place 0.50 g of the oral powder dissolved in 2 ml of *water* (solution A), 0.50 g of the oral powder dissolved in 1 ml of the reference solution and 1 ml of *water* (solution B), 1 ml of the reference solution and 1 ml of *water* (solution C) and 2 ml of *water* (solution D). To each flask, add 4 ml of *acetate-edetate buffer solution pH 5.5*. Mix and add 4 ml of freshly prepared *azomethine H solution*. Mix and allow to stand for 1 hour. Measure the *absorbance*, Appendix II B, of solutions A, B and C at 420 nm, using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is at least 0.25. The absorbance of solution B is not less than twice that of solution A (9 ppm of boron).

Lead

Complies with the *limit test for lead in sugars*, Appendix VII (0.5 ppm).

Water

Not more than 2.5%, Appendix IX C, Method I. Use 0.5 g.

Sulphated ash

Not more than 0.1%, Appendix IX A, Method II. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 1 g of the oral powder in 10 ml of *water*, add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25 ml with *water*. For solution (2) add 47.5 ml of *acetonitrile* to 3 ml of solution (1) with gentle heating and dilute to 100 ml with *water*. For solution (3) dissolve 1 g of *lactulose EPCRS* in 10 ml of *water*, add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25 ml with *water*. For solution (4) dissolve 20 mg of *lactulose EPCRS* and 20 mg of *epilactose EPCRS* in 2 ml of *water,* add 2.5 ml of *acetonitrile* with gentle heating and dilute to 5 ml with *water*. For solution (5) dissolve 0.2 g of *galactose* in 20 ml of *water*, add 25 ml of *acetonitrile* with gentle heating and dilute to 50 ml with *water*. For solution (6) dissolve 0.2 g of *lactose* in 20 ml of *water*, add 25 ml of *acetonitrile* with gentle heating and dilute to 50 ml with *water*. For solution (7) dissolve 20 mg of *epilactose EPCRS* in 2 ml of *water*, add 2.5 ml of *acetonitrile* with gentle heating and dilute to 5 ml with *water*. For solution (8) dissolve 0.2 g of *tagatose* in 20 ml of *water*, add 25 ml of *acetonitrile* with gentle heating and dilute to 50 ml with *water*. For solution (9) dissolve 0.2 g of *fructose* in 20 ml of *water*, add 25 ml of *acetonitrile* with gentle heating and dilute to 50 ml with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (5 cm × 4.6 mm) followed by a stainless steel column (15 cm × 4.6 mm), both packed with *aminopropylsilyl silica gel for chromatography* (3 µm) and maintained at 37° to 39°, (b) as mobile phase with a flow rate of 1.0 ml per minute a mixture prepared as follows: dissolve 0.253 g of *sodium dihydrogen orthophosphate* in 220 ml of *water* and add 780 ml of *acetonitrile*, and (c) as detector a refractometer maintained at a constant temperature.

When the chromatograms are recorded in the prescribed conditions, the retention time of lactulose is about 18.3 minutes and retention times relative to lactulose are about 0.38 for tagatose, 0.42 for fructose, 0.57 for galactose, 0.90 for epilactose and 1.17 for lactose.

Inject 20 µl of solution (4). The test is not valid unless the *resolution factor* between the peaks corresponding to lactulose and epilactose is at least 1.3. If necessary, adjust the concentration of *acetonitrile* in the mobile phase to between 75.0% and 82.0% v/v to achieve the prescribed resolution.

Inject separately 20 µl of solution (1) and 20 µl of solution (3) and continue the chromatography for 2.5 times the retention time of lactulose. Calculate the percentage content of $C_{12}H_{22}O_{11}$ (lactulose) from the areas of the peaks and from the declared content of $C_{12}H_{22}O_{11}$ in *lactulose EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Lactulose.

Lemon Spirit

General Notices

DEFINITION

Terpeneless Lemon Oil	100 ml
Ethanol (96 per cent)	Sufficient to produce 1000 ml

The spirit complies with the requirements stated under Spirits and with the following requirements.

Content of aldehydes

3.45 to 4.60% w/v, calculated as citral, $C_{10}H_{16}O$.

TESTS

Ethanol content

84 to 88% v/v, Appendix VIII F.

Weight per ml

0.814 to 0.823 g, Appendix V G.

ASSAY

Carry out the method for the *determination of aldehydes*, Appendix X K, using 10 ml, omitting the *toluene* and using a volume, not less than 7 ml, of *alcoholic hydroxylamine solution* that exceeds by 1 to 2 ml the volume of 0.5M *potassium hydroxide in ethanol (60%) VS* required. Each ml of 0.5M *potassium hydroxide in ethanol (60%) VS* is equivalent to 76.73 mg of $C_{10}H_{16}O$.

Lemon Syrup

General Notices

DEFINITION

Lemon Spirit	5 ml
Citric Acid Monohydrate	25 g
Invert Syrup	100 ml
Syrup	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Citric Acid Monohydrate in some of the Syrup, add the Invert Syrup, the Lemon Spirit and sufficient Syrup to produce 1000 ml and mix.

CHARACTERISTICS

Lemon Syrup has a weight per ml of about 1.33 g.

The syrup complies with the requirements stated under Oral Liquids and with the following requirements.

Content of citric acid monohydrate, $C_6H_8O_7,H_2O$

2.2 to 2.6% w/v.

ASSAY

Mix 8 g with 100 ml of *water* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 7.005 mg of $C_6H_8O_7,H_2O$. Determine the *weight per ml,* Appendix V G, and calculate the content of $C_6H_8O_7,H_2O$, weight in volume.

Levobunolol Eye Drops

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Levobunolol Eye Drops are a sterile solution of Levobunolol Hydrochloride in Purified Water. *The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.*

Content of levobunolol hydrochloride, $C_{17}H_{25}NO_3,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 350 nm of a solution prepared by diluting the eye drops with *ethanol (96%)* to contain 0.001% w/v of Levobunolol Hydrochloride exhibits two maxima, at 223 nm and at 255 nm and a broad peak at 315 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Related substances

The nominal total amount of related substances determined by tests A and B below is not more than 1.5% of the stated content of Levobunolol Hydrochloride.

A. Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of the following solutions. For solution (1) dilute a suitable volume of the eye drops with the mobile phase to produce a solution containing 0.10% w/v of Levobunolol Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.0010% w/v of *disodium edetate* in the mobile phase.

The chromatographic procedure described under Assay may be used.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Determine the sum of the areas of any *secondary peaks*. Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (3).

B. Carry out test A as described above but using a detection wavelength of 400 nm and injecting solution (1). The area of any peak with a retention time corresponding to that of the principal peak in the chromatogram obtained with solution (2) in test A is not greater than one-fifth of the area of that peak (0.5%, assuming a response factor of 5). Calculate the nominal percentage content of this impurity from the area of the peak in the chromatogram obtained with solution (1) taking one-fifth of the area of the peak in the chromatogram obtained with solution (2) in test A to be equivalent to 0.5%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of the following solutions. For solution (1) dilute a suitable volume of the eye drops with the mobile phase to produce a solution containing 0.005% w/v of Levobunolol Hydrochloride. Solution (2) contains 0.005% w/v of *levobunolol hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase a mixture of 5 volumes of *glacial acetic acid*, 450 volumes of 0.005M *sodium heptanesulphonate* and 550 volumes of *methanol* with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

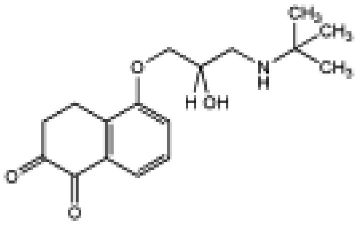
Calculate the content of $C_{17}H_{25}NO_3$,HCl in the eye drops from the declared content of $C_{17}H_{25}NO_3$,HCl in *levobunolol hydrochloride BPCRS*.

STORAGE

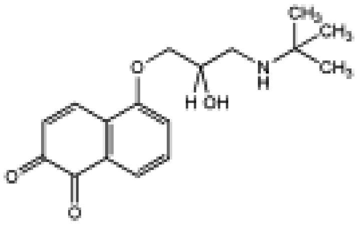
Levobunolol Eye Drops should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include:



A. 5-(3-*tert*-butylamino-2-hydroxypropoxy)-3,4-dihydro-1,2-naphthalene dione,



B. 5-(3-*tert*-butylamino-2-hydroxypropoxy)-1,2-naphthoquinone.

Levodopa Capsules

General Notices

Action and use

Dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Levodopa Capsules contain Levodopa.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve as completely as possible a quantity of the contents of the capsules containing 0.5 g of Levodopa in 25 ml of 1M *hydrochloric acid* and filter. Adjust to pH 3 with 5M *ammonia*, added dropwise with stirring, and allow to stand for several hours, protected from light. Filter, wash the precipitate and dry it at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of levodopa *(RS 201)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *microcrystalline cellulose* as the coating substance and a mixture of 25 volumes of *glacial acetic acid*, 25 volumes of *water* and 50 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of 1M *hydrochloric acid* and filter. Solution (2) contains 1% w/v of *levodopa BPCRS* in 1M *hydrochloric acid*. After removal of the plate, dry it in a current of warm air and spray with a freshly prepared mixture of equal volumes of a 10% w/v solution of *iron(III) chloride hexahydrate* and a 5% w/v solution of *potassium hexacyanoferrate(III)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. Shake a quantity of the contents of the capsules containing 20 mg of Levodopa with 5 ml of 0.1M *hydrochloric acid*. Add 0.1 ml of *iron(III) chloride solution R1*; a green colour is produced. To half of the solution add an excess of 5M *ammonia*; a purple colour is produced. To the remainder of the solution add an excess of 5M *sodium hydroxide*; a red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium. For capsules containing 0.25 g of Levodopa or less, filter and dilute 2 ml of the filtrate to 10 ml with 0.1M *hydrochloric acid*. For capsules containing more than 0.25 g of Levodopa, filter and dilute 1 ml of the filtrate to 10 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the solution, Appendix II B, at 280 nm using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of $C_9H_{11}NO_4$ in the medium taking 142 as the value of A(1%, 1 cm) at the maximum at 280 nm.

Specific optical rotation

–38.5 to –41.5, Appendix V F, when determined in the following manner. Shake a quantity of the contents of the capsules containing 1.25 g of Levodopa with 25 ml of 0.5M *hydrochloric acid* for 30 minutes, centrifuge and filter the supernatant liquid (Whatman No. 42 filter paper is suitable). To 10 ml of the filtrate add 10 ml of a 21.5% w/v solution of *aluminium sulphate*, 20 ml of a 21.8% w/v solution of *sodium acetate* and sufficient *water* to produce 50 ml and measure the *optical rotation*. Separately dilute 5 ml of the filtrate to 200 ml with 0.1M *hydrochloric acid*, mix well and dilute 10 ml to 200 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the content of levodopa, $C_9H_{11}NO_4$, in the filtrate taking 142 as the value of A(1%, 1 cm) at the maximum at 280 nm and hence calculate the *specific optical rotation*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated cellulose plate (Merck plates are suitable) and a mixture of 25 volumes of *glacial acetic acid*, 25 volumes of *water* and 50 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate, as bands 20 mm long, 10 µl of each of solutions (1) and (2) and 20 µl of solution (3) and dry in a current of air. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of *anhydrous formic acid* and *methanol*; prepare immediately before use. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) is a mixture of equal volumes of solution (1) and a solution prepared by dissolving 30 mg of L-*tyrosine* in 1 ml of *anhydrous formic acid* and diluting to 100 ml with *methanol*. After removal of the plate, dry it in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10% w/v solution of *iron(III) chloride hexahydrate* and a 5% w/v solution of *potassium hexacyanoferrate(III)* and examine the plate immediately. Any *secondary band* in the chromatogram obtained with solution (1) is not more intense than the band in the chromatogram obtained with solution (2) (0.5%). The test is not valid unless the chromatogram obtained with solution (3) shows a distinct band, at a higher Rf value than the principal band, which is more intense than the band in the chromatogram obtained with solution (2).

ASSAY

Dissolve as completely as possible a quantity of the mixed contents of 20 capsules containing 0.6 g of Levodopa in 10 ml of *anhydrous formic acid*, add 80 ml of *glacial acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *oracet blue B solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 19.72 mg of $C_9H_{11}NO_4$.

When L-dopa capsules are prescribed or demanded, Levodopa Capsules shall be dispensed or supplied.

Levodopa Tablets

General Notices

Action and use

Dopamine precursor; treatment of Parkinson's disease.

DEFINITION

Levodopa Tablets contain Levodopa.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of levodopa, $C_9H_{11}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve as completely as possible a quantity of the powdered tablets containing 0.5 g of Levodopa in 25 ml of 1M *hydrochloric acid* and filter. Adjust to pH 3 with 5M *ammonia*, added dropwise with stirring, and allow to stand for several hours, protected from light. Filter, wash the precipitate with *water* and dry it at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of levodopa *(RS 201)*.

B. Comply with test B for Identification described under Levodopa Capsules, but using as solution (1) a solution obtained by shaking a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of 1M *hydrochloric acid* and filtering.

C. Shake a quantity of the powdered tablets containing 20 mg of Levodopa with 5 ml of 0.1M *hydrochloric acid*. Add 0.1 ml of *iron*(III) *chloride solution R1*; a green colour is produced. To half of the solution add an excess of 5M *ammonia*; a purple colour is produced. To the remainder of the solution add an excess of 5M *sodium hydroxide*; a red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. For tablets containing 0.25 g of Levodopa or less, filter and dilute 2 ml of the filtrate to 10 ml with 0.1M *hydrochloric acid*. For tablets containing more than 0.25 g of Levodopa, filter and dilute 1 ml of the filtrate to 10 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the solution, Appendix II B, at 280 nm using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of $C_9H_{11}NO_4$ in the medium taking 142 as the value of A(1%, 1 cm) at the maximum at 280 nm.

Specific optical rotation

–38.5 to –41.5, Appendix V F, when determined in the following manner. Shake a quantity of the finely powdered tablets containing 1.25 g of Levodopa with 25 ml of 0.5M *hydrochloric acid* for 30 minutes, centrifuge and filter the supernatant liquid (Whatman No. 42 filter paper is suitable). To 10 ml of the filtrate add 10 ml of a 21.5% w/v solution of *aluminium sulphate*, 20 ml of a 21.8% w/v solution of *sodium acetate* and sufficient *water* to produce 50 ml and measure the *optical rotation*. Further dilute 5 ml of the filtrate to 200 ml with 0.1M *hydrochloric acid*, mix well and dilute 10 ml to 200 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 280 nm, Appendix II B. Calculate the concentration of levodopa, $C_9H_{11}NO_4$, in the filtrate taking 142 as the value of A(1%, 1 cm) at the maximum at 280 nm and hence calculate the *specific optical rotation*.

Related substances

Comply with the test described under Levodopa Capsules using as solution (1) a solution prepared immediately before use by shaking a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of *anhydrous formic acid* and *methanol*.

ASSAY

Weigh and powder 20 tablets. Dissolve as completely as possible a quantity of the powder containing 0.6 g of Levodopa in 10 ml of *anhydrous formic acid*, add 80 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *oracet blue B solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 19.72 mg of $C_9H_{11}NO_4$.

When L-dopa tablets are prescribed or demanded, Levodopa Tablets shall be dispensed or supplied.

Levomenthol Cream

General Notices

Menthol in Aqueous Cream

NOTE: There are currently no licensed formulations in the United Kingdom.

DEFINITION

Levomenthol Cream contains Levomenthol in Aqueous Cream.

Extemporaneous preparation

Levomenthol Cream may be prepared using the following directions.

Triturate the Levomenthol to a *fine powder.* If necessary, a small amount of glycerol may be added to minimise the creation of airborne menthol dust. Add the Aqueous Cream gradually and mix until smooth.

The cream complies with the requirements stated under Topical Semi-solid Preparations, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of levomenthol, $C_{10}H_{20}O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to levomenthol in the chromatogram obtained with solution (3).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following freshly prepared solutions.

(1) 0.1% w/v of *camphor* (internal standard) in *industrial methylated spirit (95%)*.

(2) Disperse a quantity of the well-mixed cream containing 50 mg of Levomenthol in 20 ml of *industrial methylated spirit (95%)* and mix with the aid of ultrasound for 15 minutes. Transfer the liquid contents to a 50 ml graduated flask, rinse the original flask with two 10-ml quantities of *industrial methylated spirit (95%)*, transfer the washings to the graduated flask and add sufficient *industrial methylated spirit (95%)* to produce 50 ml; mix well and filter (Whatman No. 1 paper is suitable) discarding the first 5 ml of filtrate. Add 10 ml of solution (1) to 10 ml of the filtrate and add sufficient *industrial methylated spirit (95%)* to produce 25 ml; mix well.

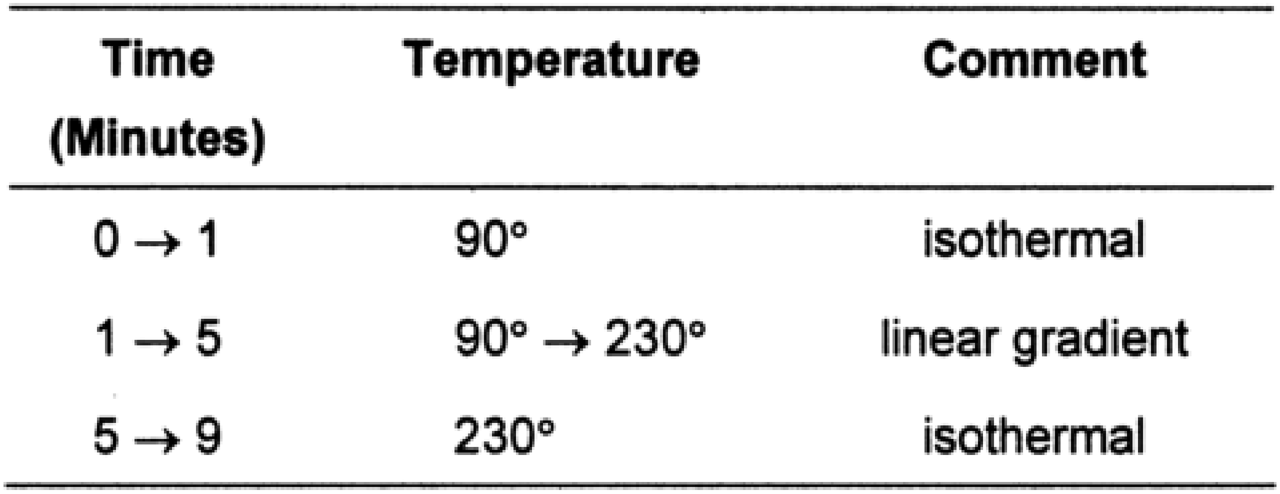
(3) Mix 5 ml of a 0.2% w/v solution of *levomenthol BPCRS* in *industrial methylated spirit (95%)* with 10 ml of solution (1) and dilute to 25 ml with *industrial methylated spirit (95%).*

CHROMATOGRAPHIC CONDITIONS

(a) Fused silica column (10 m × 0.53 mm) bonded with a 0.25-µm layer of dimethylpolysiloxane.

(b) Carrier gas Helium.

(c) Oven temperature:



Time (Minutes)	Temperature	Comment
0 → 1	90°	isothermal
1 → 5	90° → 230°	linear gradient
5 → 9	230°	isothermal

(d) Injector at 250°.

(e) Flame ionisation detector at 250°.

(f) Inject 1 µl of each solution.

When the chromatograms are recorded under the prescribed conditions the retention times are: levomenthol, about 2.1 minutes; camphor, about 2 minutes.

DETERMINATION OF CONTENT

Calculate the content of $C_{10}H_{20}O$ in the cream using the declared content of $C_{10}H_{20}O$ in *levomenthol BPCRS*.

Levomepromazine Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Levomepromazine Injection is a solution of Levomepromazine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of levomepromazine hydrochloride, $C_{19}H_{24}N_2OS,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume of the injection containing 50 mg of Levomepromazine Hydrochloride add 2 ml of 1M *sodium hydroxide*, shake, extract with 15 ml of *ether* and allow to separate. Wash the ethereal layer with 5 ml of *water,* filter through phase-separating paper (Whatman 1 PS is suitable) containing *anhydrous sodium sulphate*, evaporate the ether to dryness and dry the residue at 100^{c} for 3 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of levomepromazine (*RS 404)*.

TESTS

Acidity

pH, 4.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using a *TLC silica gel GF*$_{254}$ *plate* and a mixture of 5 volumes of *diethylamine*, 10 volumes of *acetone* and 85 volumes of *toluene* as the mobile phase, maintained at 35°. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a volume of the injection, if necessary, with sufficient of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* to produce a solution containing 0.5% w/v of Levomepromazine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and further dilute 1 volume to 2 volumes with the same solvent mixture. Solution (3) contains 0.005% w/v of *levomepromazine sulphoxide BPCRS* in a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to levomepromazine sulphoxide is not more intense than the spot in the chromatogram obtained with solution (3) (1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Carry out the following procedure protected from light. Dilute a volume of the injection containing 0.1 g of Levomepromazine Hydrochloride to 100 ml with *water* and dilute 1 volume of this solution to 25 volumes with *water*. Immediately measure the *absorbance* of the solution at the maximum at 302 nm, Appendix II B, using *water* in the reference cell. Measure the absorbance of a 0.004% w/v solution of *levomepromazine maleate BPCRS* in *water* and calculate the content of $C_{19}H_{24}N_2OS,HCl$ from the absorbances obtained using the declared content of $C_{19}H_{24}N_2OS,C_4H_4O_4$ in *levomepromazine maleate BPCRS*. Each mg of $C_{19}H_{24}N_2OS,C_4H_4O_4$ is equivalent to 0.8207 mg of $C_{19}H_{24}N_2OS,HCl$.

STORAGE

Levomepromazine Injection should be protected from light.

LABELLING

The label states that coloured solutions must not be used.

Levomepromazine Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Levomepromazine Tablets contain Levomepromazine Maleate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of levomepromazine maleate, $C_{19}H_{24}N_2OS,C_4H_4O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 50 mg of Levomepromazine Maleate add 10 ml of *water* and 2 ml of 1M *sodium hydroxide*, shake, extract with 15 ml of *ether* and allow to separate. Wash the ethereal layer with 5 ml of *water*, filter through phase-separating paper (Whatman 1 PS is suitable) containing *anhydrous sodium sulphate*, evaporate the ether to dryness and dry the residue at 100° for 3 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of levomepromazine (*RS 404)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Macherey-Nagel plates are suitable) and a mixture of 3 volumes of *water*, 7 volumes of *anhydrous formic acid* and 90 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) mix a quantity of the powdered tablets containing 0.2 g of Levomepromazine Maleate with 10 ml of a mixture of 1 volume of *water* and 9 volumes of *acetone*, shake with the aid of ultrasound for 5 minutes, allow to stand and use the clear supernatant liquid. Solution (2) contains 0.6% w/v of *maleic acid* in *anhydrous formic acid*. After removal of the plate, allow it to dry at 120° for 10 minutes and examine under *ultraviolet light (254 nm)*. The chromatogram obtained with solution (1) shows a spot remaining on the line of application and a spot which is similar in position to the principal spot in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. After 30 minutes, withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, suitably diluted with 0.1M *hydrochloric acid*, if necessary, at 311 nm using 0.1M *hydrochloric acid* in the reference cell. Measure the *absorbance* of a 0.005% w/v solution of *levomepromazine maleate BPCRS* using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of levomepromazine maleate, $C_{19}H_{24}N_2OS,C_4H_4O_4$, in the medium from the absorbances obtained and using the declared content of $C_{19}H_{24}N_2OS,C_4H_4O_4$ in *levomepromazine maleate BPCRS*. The amount is not less than 60% of the stated amount.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using a *TLC silica gel GF_{254} plate* and a mixture of 5 volumes of *diethylamine*, 10 volumes of *acetone* and 85 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) mix a quantity of the powdered tablets containing 0.1 g of Levomepromazine Maleate with 10 ml of a mixture of 1 volume of 18M *ammonia* and 99 volumes of *methanol*, shake with the aid of ultrasound for 5 minutes, mix, filter (Whatman No. 40 paper is suitable), discarding the first portion of filtrate, and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 50 mg of Levomepromazine Maleate add 15 ml of 0.2M *methanolic ammonia*, stir for 2 minutes, filter and collect the filtrate. Repeat the extraction with a further 3 successive 15-ml quantities of 0.2M *methanolic ammonia*, grinding the residue with a glass rod before extraction. Dilute the combined filtrates to 100 ml with 0.2M *methanolic ammonia*, mix and dilute 10 volumes of the solution to 100 volumes with *methanol* and further dilute 10 volumes of this solution to 100 volumes with the same solvent. Measure the *absorbance* of this solution at the maximum at 254 nm, Appendix II B, using *methanol* in the reference cell. Measure the *absorbance* of a 0.0005% w/v solution of *levomepromazine maleate BPCRS* in 0.002M *methanolic ammonia* at the same wavelength using *methanol* in the reference cell and calculate the content of $C_{19}H_{24}N_2OS,C_4H_4O_4$ in the tablets using the declared content of $C_{19}H_{24}N_2OS,C_4H_4O_4$ in *levomepromazine maleate BPCRS*.

STORAGE

Levomepromazine Tablets should be protected from light.

Levonorgestrel and Ethinylestradiol Tablets

General Notices

Action and use

Progestogen.

DEFINITION

Levonorgestrel and Ethinylestradiol Tablets contain Levonorgestrel and Ethinylestradiol.
The tablets comply with the requirements stated under Tablets and with the following requirements. For Levonorgestrel and Ethinylestradiol Tablets presented in 21-day or 28-day calendar packs, apply the requirements separately to tablets of each combination of different proportions, by weight, of the active ingredients. Where applicable, disregard any tablets that contain no active ingredient (placebo tablets).

Content of levonorgestrel, $C_{21}H_{28}O_2$

90.0 to 110.0% of the stated amount.

Content of ethinylestradiol, $C_{20}H_{24}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

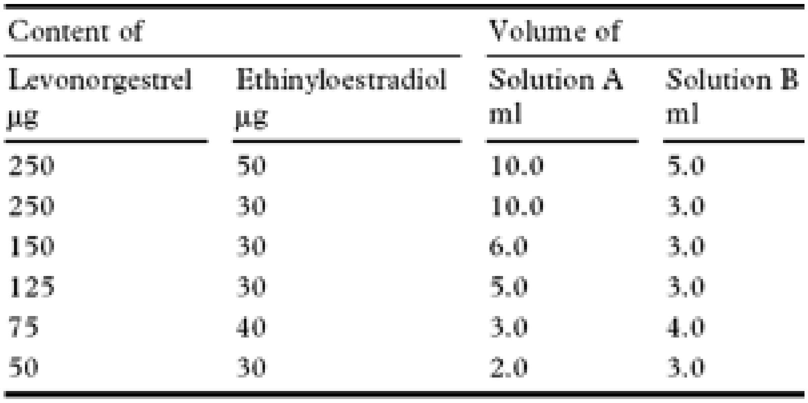
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of *methanol* and 99 volumes of *chloroform* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) extract 15 powdered tablets with 30 ml of *acetone*, filter, evaporate to dryness and dissolve the residue in 1 ml of *chloroform*. Solution (2) contains 0.075% w/v of *levonorgestrel BPCRS* in *chloroform*. Solution (3) contains 0.045% w/v of *ethinylestradiol BPCRS* in *chloroform*. Develop the chromatograms twice, drying the plates between developments. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. One of the principal spots in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the other principal spot corresponds to that in the chromatogram obtained with solution (3). Spray the plate with a 2% w/v solution of *toluene-p-sulphonic acid* in *water* and heat at 105° for 10 minutes and examine under *ultraviolet light (365 nm)*. The spots corresponding to levonorgestrel and ethinylestradiol appear as blue spots.

Uniformity of content

Comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Prepare the following stock solutions using a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*. Solution A contains 0.0625% w/v of *levonorgestrel BPCRS.* Solution B contains 0.025% of *ethinylestradiol BPCRS*. Solution C contains 0.020% w/v of *2-hydroxybiphenyl* (internal standard). For solution D dilute 1 ml of solution C to 20 ml with the solvent mixture.

For solution (1) mix the volumes of solutions A and B specified in the table with 5 ml of solution C and dilute to 100 ml with a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*. For solution (2) add 4 ml of solution D to one tablet, and heat at 60° in an ultrasonic bath for 25 minutes, shake and repeat the ultrasound treatment. Cool, centrifuge and use the clear supernatant liquid.



Content of		Volume of	
Levonorgestrel µg	Ethinyloestradiol µg	Solution A ml	Solution B ml
250	50	10.0	5.0
250	30	10.0	3.0
150	30	6.0	3.0
125	30	5.0	3.0
75	40	3.0	4.0
50	30	2.0	3.0

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 49 volumes of *acetonitrile* and 51 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 215 nm.

Calculate the content of $C_{21}H_{28}O_2$ and of $C_{20}H_{24}O_2$ in each tablet using the declared content of $C_{21}H_{28}O_2$ in *levonorgestrel BPCRS* and the declared content of $C_{20}H_{24}O_2$ in *ethinylestradiol BPCRS*.

ASSAY

For both levonorgestrel and ethinylestradiol use the average of the 10 individual results obtained in the test for Uniformity of content.

Levonorgestrel Tablets

General Notices

Action and use

Progestogen.

DEFINITION

Levonorgestrel Tablets contain Levonorgestrel.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of levonorgestrel.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of levonorgestrel, $C_{21}H_{28}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to a quantity of the tablets containing 0.15 mg of Levonorgestrel add 25 ml of *methanol (80%)*, heat in a water bath at 60° for 10 minutes, shaking occasionally, allow to cool and filter through a membrane with a pore size of 0.45 µm. Solution (2) contains 0.0006% w/v of *levonorgestrel BPCRS* in *methanol (80%)*. Solution (3) contains 0.0012% w/v of *norgestrel BPCRS* in *methanol (80%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution containing equal volumes of *methanol* and 1% w/v of *gamma-cyclodextrin* in *water* and (c) a detection wavelength of 242 nm.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to levonorgestrel and dextronorgestrel is at least 1.0.

A peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to levonorgestrel in the chromatogram obtained with solution (2).

B. In the test for uniformity of content, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 ml of a mixture of equal volumes of *methanol* and *water* to a quantity of the powdered tablets containing 0.18 mg of Levonorgestrel, mix with the aid of ultrasound for 30 minutes, stir vigorously for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *methanol* and *water*. Solution (3) contains 0.004% w/v each of *ethinylestradiol BPCRS* and *levonorgestrel BPCRS* in a mixture of equal volumes of *methanol* and *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable) and maintained at a temperature of 30^{c}, (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture of 100 volumes of *methanol*, 240 volumes of *acetonitrile* and 500 volumes of *water* and (c) a detection wavelength of 220 nm.

Inject separately 200 µl of each solution. For each solution allow the chromatography to proceed for twice the retention time of levonorgestrel.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to ethinylestradiol and levonorgestrel is at least 12.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

Tablets containing less than 2 mg of Levonorgestrel comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to 1 tablet add 5 ml of the mobile phase, disperse with the aid of ultrasound for 45 minutes, shaking every 15 minutes, centrifuge and use the clear supernatant liquid. Solution (2) contains 0.0006% w/v of *levonorgestrel BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1.3 ml per minute a mixture of equal volumes of *acetonitrile* and *water* and (c) a detection wavelength of 220 nm.

Inject 25 µl of each solution.

Calculate the content of $C_{21}H_{28}O_2$ in each tablet using the declared content of $C_{21}H_{28}O_2$ in *levonorgestrel BPCRS*.

ASSAY

Use the average of the 10 individual results obtained in the test for uniformity of content.

Levothyroxine Tablets

General Notices

Action and use

Thyroid hormone replacement.

DEFINITION

Levothyroxine Tablets contain Levothyroxine Sodium.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous levothyroxine sodium, $C_{15}H_{10}I_4NNaO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

B. To a quantity of the powdered tablets containing the equivalent of 0.5 mg of anhydrous levothyroxine sodium add a mixture of 3 ml of *ethanol (50%)* and 0.2 ml of *hydrochloric acid*, boil gently for 30 seconds, cool, filter, add 0.1 ml of a 10% w/v solution of *sodium nitrite* and boil; a yellow colour is produced. Cool and make alkaline with 5M *ammonia* ; the solution becomes orange.

Uniformity of content

Comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add sufficient 0.05M *sodium hydroxide* to one tablet to produce a solution containing the equivalent of about 6 µg per ml of anhydrous levothyroxine sodium, mix with the aid of ultrasound until the tablet is fully dispersed, cool and shake for 2 minutes. Add sufficient 0.05M *sodium hydroxide* to produce a solution containing the equivalent of 0.0004% w/v of anhydrous levothyroxine sodium, filter through glass microfibre filter paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.0004% w/v of *levothyroxine sodium EPCRS* in 0.05M *sodium hydroxide*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Nucleosil 5 CN is suitable), (b) a mixture of 5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile* and 700 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 225 nm. Inject 20 µl of each solution.

Calculate the content of $C_{15}H_{10}I_4NNaO_4$ in each tablet using the declared content of $C_{15}H_{10}I_4NNaO_4$ in *levothyroxine sodium EPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D.

For tablets containing the equivalent of less than 50 µg of anhydrous levothyroxine sodium, use the following solutions. For solution (1) disperse with the aid of ultrasound for 10 minutes a quantity of the powdered tablets containing the equivalent of 50 µg of anhydrous levothyroxine sodium with 8 ml of a mixture of equal volumes of *methanol* and 0.1M *sodium hydroxide* (solvent A). Shake for 2 minutes, cool, add sufficient solvent A to produce 10 ml, mix, filter through glass microfibre filter paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.0005% w/v of *levothyroxine sodium EPCRS* in solvent A. Solution (3) contains 0.0005% w/v of *liothyronine sodium EPCRS* and 0.0005% w/v of *levothyroxine sodium EPCRS* in solvent A.

For tablets containing the equivalent of 50 µg of anhydrous levothyroxine sodium or more, use the following solutions. For solution (1) disperse with the aid of ultrasound for 10 minutes a quantity of the powdered tablets containing the equivalent of 0.1 mg of anhydrous levothyroxine sodium with 8 ml of a mixture of equal volumes of *methanol* and 0.1M *sodium hydroxide* (solvent A). Shake for 2 minutes, cool, add sufficient solvent A to produce 10 ml, mix, filter through glass microfibre filter paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.001% w/v of *levothyroxine sodium EPCRS* in solvent A. Solution (3) contains 0.0005% w/v of *liothyronine sodium EPCRS* and 0.0005% w/v of *levothyroxine sodium EPCRS* in solvent A.

The chromatographic procedure may be carried out using the conditions described under the test for Uniformity of content.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 4.

Calculate the content of $C_{15}H_{10}I_4NNaO_4$ in the tablets using peak areas and the declared content of $C_{15}H_{10}I_4NNaO_4$ in *levothyroxine sodium EPCRS*.

STORAGE

Levothyroxine Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous levothyroxine sodium.

Lidocaine and Adrenaline Injection / Lidocaine and Epinephrine Injection

General Notices

Action and use

Local anaesthetic + class I antiarrhythmic; adrenoceptor agonist.

DEFINITION

Lidocaine and Adrenaline Injection is a sterile solution of Lidocaine Hydrochloride and Adrenaline Acid Tartrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of lidocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$

95.0 to 105.0% of the stated amount.

Content of adrenaline, $C_9H_{13}NO_3$

87.5 to 112.5% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel G60 plates are suitable) and a mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a quantity of the injection, if necessary, with *water* to produce a solution containing 0.2% w/v of Lidocaine Hydrochloride. Solution (2) contains 0.2% w/v of *lidocaine hydrochloride BPCRS* in *water*. Solution (3) contains 0.2% w/v of *lidocaine hydrochloride BPCRS* and 0.2% w/v of *bupivacaine hydrochloride BPCRS* in *water*. After removal of the plate, dry it in a current of cold air, heat at 110^{c} for 1 hour, place the hot plate in a tank of chlorine gas prepared by the addition of *hydrochloric acid* to a 5% w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry the plate in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

B. In the Assay for adrenaline, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

Acidity

pH, 3.0 to 4.5, Appendix V L.

ASSAY

For lidocaine hydrochloride

Carry out the assay described under Lidocaine Injection.

For adrenaline

Prepare a solution by adding 8.0 g of *tetramethylammonium hydrogen sulphate*, 2.2 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* to a mixture of 900 ml of *water* and 100 ml of *methanol*, adjust the pH to 3.5 using 1M *sodium hydroxide* and filter through glass microfibre paper under reduced pressure (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 5 ml of a 0.001% w/v solution of *adrenaline acid tartrate BPCRS* to 10 ml with solution A. For solution (2) dilute the injection, if necessary, to contain 0.0005% w/v of adrenaline and dilute 5 ml of the resulting solution to 10 ml with solution A. For solution (3) mix 5 ml of solution (1) with 5 ml of a 0.001% w/v solution of *noradrenaline acid tartrate* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* to a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusting the pH to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

Calculate the content of $C_9H_{13}NO_3$ in the injection using the declared content of $C_9H_{13}NO_3$ in *adrenaline acid tartrate BPCRS*.

STORAGE

Lidocaine and Adrenaline Injection should be protected from light.

LABELLING

The quantity of Adrenaline Acid Tartrate is stated in terms of the equivalent amount of adrenaline (epinephrine).

Lidocaine and Chlorhexidine Gel

General Notices

Action and use

Local anaesthetic; Class I antiarrhythmic and Antiseptic.

DEFINITION

Lidocaine and Chlorhexidine Gel is a sterile solution of Lidocaine Hydrochloride containing 0.25% v/v of Chlorhexidine Gluconate Solution in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous lidocaine hydrochloride, $C_{14}H_{22}N_2O,HCl$

95.0 to 105.0% of the stated amount.

Content of chlorhexidine gluconate solution

0.225 to 0.275% v/v.

IDENTIFICATION

To a quantity of the gel containing the equivalent of 80 mg of anhydrous lidocaine hydrochloride add 4 ml of *hydrochloric acid* and heat on a water bath for 10 minutes. Allow to cool, transfer to a separating funnel with the aid of 20 ml of *water*, add 5M *sodium hydroxide* until precipitation is complete and extract with two 20 ml quantities of *chloroform*. Filter the chloroform extracts through *anhydrous sodium sulphate* and evaporate the filtrate to dryness on a water bath using a current of nitrogen. The residue complies with tests A, B and C.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lidocaine *(RS 202)*.

B. Dissolve 20 mg in 1 ml of *ethanol (96%)*, add 0.5 ml of a 10% w/v solution of *cobalt(II) chloride* and 0.5 ml of 5M *sodium hydroxide* and shake for 2 minutes. A bluish-green precipitate is produced.

C. Dissolve 40 mg in 5 ml of a 1% w/v solution of *cetrimide* and add 1 ml of 5M *sodium hydroxide* and 1 ml of *bromine water*. An orange colour is produced.

D. In the Assay for chlorhexidine gluconate solution, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to chlorhexidine acetate in the chromatogram obtained with solution (1).

TESTS

Aromatic amines

Not more than 8 ppm of 2,6-dimethyl-aniline and not more than 1.5 ppm of 4-chloro-aniline when determined in the following manner. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) shake 0.2 g of the gel with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of a buffer solution prepared by adjusting the pH of 0.1M *sodium citrate* to 5.0 with 0.2M *sodium hydroxide*, allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica-treated filter paper (Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of 0.6M *sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer. For solution (2) dissolve 80 mg of *2,6-dimethylaniline* in 1 ml of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100 ml, dilute 1 volume of this solution to 50 volumes with 0.01M *hydrochloric acid* and further dilute 1 volume of this solution to 20 volumes with the same solvent. Shake 2 ml of this solution with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate …'. For solution (3) dissolve 30 mg of *4-chloroaniline* in 1 ml of 1M *hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 ml and dilute 1 volume to 50 volumes with the same solvent and further dilute 1 volume to 20 volumes with the same solvent. Shake 2 ml of this solution with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of 0.6M *sodium hydrogen carbonate solution* and continue in the same manner as for solution (1) beginning at the words 'allow to separate …'.

The chromatographic procedure may be carried out using (a) a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 15% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable) at a temperature of 190° with the inlet port at 200° and the detector at 270°, (b) *nitrogen* as the carrier gas at a flow rate of 50 ml per minute and (c) an electron capture detector.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2,6-dimethylaniline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) and the area of any peak corresponding to 4-chloroaniline is not greater than the area of the principal peak in the chromatogram obtained with solution (3).

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

For lidocaine hydrochloride

Carry out the Assay described under Lidocaine Gel .

For chlorhexidine gluconate solution

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 ml of a 0.080% w/v solution of *diphenylamine* (internal standard) in the mobile phase to 5 ml of a 0.070% w/v solution of *chlorhexidine acetate BPCRS* in the mobile phase and dilute to 100 ml with the mobile phase. For solution (2) mix 10 g of the gel with sufficient of the mobile phase to produce 100 ml. Prepare solution (3) in the same manner as solution (2) but adding 5 ml of the internal standard solution before diluting to 100 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute

0.01 M *sodium octanesulphonate* in *methanol (73%)* adjusted to pH 3.0 with *glacial acetic acid* and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ from the declared content of $C_{22}H_{30}Cl_2N_{10}$ in *chlorhexidine acetate BPCRS*. Each mg of $C_{22}H_{30}Cl_2N_{10}$ is equivalent to 1.775 mg of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$. Determine the *weight per ml* of the gel, Appendix V G, and express the result as the percentage volume in volume of Chlorhexidine Gluconate Solution, which contains 20% w/v of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

STORAGE

Lidocaine and Chlorhexidine Gel should be stored in accordance with the manufacturer's instructions.

LABELLING

The label states (1) the strength with respect to Lidocaine Hydrochloride in terms of the equivalent amount of anhydrous lidocaine hydrochloride; (2) that any of the gel not used in a single application should be discarded.

Lidocaine Gel

General Notices

Action and use

Local anaesthetic; Class I antiarrhythmic.

DEFINITION

Lidocaine Gel is a sterile solution of Lidocaine Hydrochloride in a suitable water-miscible basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of anhydrous lidocaine hydrochloride, $C_{14}H_{22}N_2O,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the gel containing the equivalent of 80 mg of anhydrous lidocaine hydrochloride add 4 ml of *hydrochloric acid* and heat on a water bath for 10 minutes. Allow to cool, transfer to a separating funnel with the aid of 20 ml of *water*, add 5M *sodium hydroxide* until precipitation is complete and extract with two 20 ml quantities of *chloroform*. Filter the chloroform extracts through *anhydrous sodium sulphate* and evaporate the filtrate to dryness on a water bath using a current of nitrogen. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lidocaine *(RS 202)*.

B. Dissolve 20 mg in 1 ml of *ethanol (96%)*, add 0.5 ml of a 10% w/v solution of *cobalt(II) chloride* and 0.5 ml of 5M *sodium hydroxide* and shake for 2 minutes. A bluish-green precipitate is produced.

C. Dissolve 40 mg in 5 ml of a 1% w/v solution of *cetrimide* and add 1 ml of 5M *sodium hydroxide* and 1 ml of *bromine water*. A yellow colour is produced.

TESTS

2,6-Dimethylaniline

Mix a quantity of the gel containing the equivalent of 15 mg of anhydrous lidocaine hydrochloride with sufficient *water* to produce 3 ml using a rotary vortex mixer. To 2 ml of the resulting solution add 1 ml of a freshly prepared 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol*, mix thoroughly using a rotary vortex mixer, add 2 ml of *glacial acetic acid* and allow to stand for 10 minutes. The yellow colour produced is not more intense than that obtained using a mixture of 2 ml of a solution of *2,6-dimethylaniline* in *methanol* containing 2 µg per ml in place of the solution of the gel.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

Disperse a quantity containing the equivalent of 10 mg of anhydrous lidocaine hydrochloride in 20 ml of *water*. Add 5 ml of *acetate buffer pH 2.8*, 120 ml of *chloroform* and 5 ml of *dimethyl yellow and oracet blue B solution* and titrate with 0.005M *dioctyl sodium sulphosuccinate VS*, swirling vigorously. Near the end point add the titrant dropwise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end point is indicated when the colour of the chloroform layer changes from green to pinkish-grey. Repeat the operation without the preparation being examined. The difference between the titrations represents the amount of dioctyl sodium sulphosuccinate required. Each ml of 0.005M *dioctyl sodium sulphosuccinate VS* is equivalent to 1.354 mg of $C_{14}H_{22}N_2O,HCl$. Determine the *weight per ml* of the gel, Appendix V G, and calculate the percentage of $C_{14}H_{22}N_2O,HCl$, weight in volume.

STORAGE

Lidocaine Gel should be stored in accordance with the manufacturer's instructions.

LABELLING

The label states (1) the strength in terms of the equivalent amount of anhydrous lidocaine hydrochloride; (2) that any of the gel not used in a single application should be discarded.

Lidocaine Injection

General Notices

Action and use

Local anaesthetic; Class I antiarrhythmic.

DEFINITION

Lidocaine Injection is a sterile solution of Lidocaine Hydrochloride in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of lidocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Make a volume containing 0.1 g of Lidocaine Hydrochloride alkaline with 5M *sodium hydroxide*, filter, wash the residue with *water*, dissolve it in 1 ml of *ethanol (96%)*, add 0.5 ml of a 10% w/v solution of *cobalt*(II) *chloride* and shake for 2 minutes. A bluish-green precipitate is produced.

B. To a volume containing 0.1 g of Lidocaine Hydrochloride add 10 ml of *picric acid solution R1*. The *melting point* of the precipitate, after washing with *water* and drying at 105°, is about 229°, Appendix V A.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

2,6-Dimethylaniline

To a volume of the injection containing 25 mg of Lidocaine Hydrochloride add *water* if necessary to produce 10 ml, add 2M *sodium hydroxide* until the solution is just alkaline and extract with three 5 ml quantities of *chloroform*. Dry the combined chloroform extracts over *anhydrous sodium sulphate*, filter, wash with a further 5 ml of *chloroform* and evaporate the filtrate to dryness at a pressure of 2 kPa. Dissolve the residue in 2 ml of *methanol*, add 1 ml of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand at room temperature for 10 minutes. Any yellow colour produced is not more intense than the colour obtained by repeating the operation using 10 ml of a solution of *2,6-dimethylaniline* in *water* containing 1 µg per ml in place of the injection (400 ppm).

ASSAY

Make a quantity containing 0.1 g of Lidocaine Hydrochloride alkaline with 2M *sodium hydroxide* and extract with three 20 ml quantities of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the washed extracts through a filter paper moistened with *chloroform* and wash the filter with 10 ml of *chloroform*. Add the washings to the filtrate and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.02M *perchloric acid VS* as titrant and *crystal violet solution* as indicator. Each ml of 0.02M *perchloric acid VS* is equivalent to 5.776 mg of $C_{14}H_{22}N_2O,HCl,H_2O$.

Lidocaine Ointment

General Notices

Action and use

Local anaesthetic; Class I antiarrhythmic.

DEFINITION

Lidocaine Ointment contains Lidocaine in a suitable hydrophilic basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of lidocaine, $C_{14}H_{22}N_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Warm a quantity of the ointment containing about 25 mg of Lidocaine until the basis has melted, add 1 ml of saturated *sodium chloride solution* and 0.2 ml of 1M *sodium hydroxide* and cool. Add 5 ml of *ether*, shake vigorously for 1 minute and allow the layers to separate. Filter the ether layer through *anhydrous sodium sulphate* and evaporate the *ether* to dryness. Dissolve the residue in the minimum volume of *chloroform IR*, apply the solution directly to a sodium chloride disc and allow the solvent to evaporate. The *infrared absorption spectrum* of the resulting thin film, Appendix II A, is concordant with the *reference spectrum* of lidocaine *(RS 405)*.

2,6-Dimethylaniline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a weighed quantity of the ointment containing 50 mg of Lidocaine in the mobile phase, dilute to 50 ml with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase. For solution (2) dilute a 0.1% w/v solution of *2,6-dimethylaniline* in *methanol* with the mobile phase to produce a solution containing 0.04 µg per ml of 2,6-dimethylaniline. For solution (3) mix equal volumes of a 0.01% w/v solution of *lidocaine BPCRS* in the mobile phase with a 0.005% w/v solution of *2,6-dimethylaniline* in the mobile phase.

The chromatographic procedure described under Assay may be used.

The chromatogram obtained with solution (3) shows a peak due to lidocaine and a peak due to 2,6-dimethylaniline with a retention time relative to lidocaine of about 0.5.

In the chromatogram obtained with solution (1), the area of any peak corresponding to 2,6-dimethylaniline is not greater than the area of the peak in the chromatogram obtained with solution (2) (400 ppm with reference to the content of Lidocaine).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For solution (1) dissolve a weighed quantity of the ointment containing 50 mg of Lidocaine in the mobile phase, dilute to 50 ml with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase. Solution (2) contains 0.010% w/v of *lidocaine BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Apex ODS 2 is suitable), (b) as the mobile phase with a flow rate of 0.8 ml per minute a mixture of 35 volumes of a phosphate buffer pH 8.0 prepared as described below and 65 volumes of *methanol* and (c) a detection wavelength of 230 nm. Prepare the phosphate buffer by adding a 0.408% w/v solution of *potassium dihydrogen orthophosphate* to a suitable volume of a 0.685% w/v solution of *dipotassium hydrogen orthophosphate* until pH 8.0 is attained.

Calculate the content of $C_{14}H_{22}N_2O$ in the ointment from the chromatograms obtained using the declared content of $C_{14}H_{22}N_2O$ in *lidocaine BPCRS*.

Lincomycin Capsules

General Notices

Action and use

Lincosamide antibacterial.

DEFINITION

Lincomycin Capsules contain Lincomycin Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of lincomycin, $C_{18}H_{34}N_2O_6S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the capsules contents containing 0.2 g of Lincomycin Hydrochloride with 5 ml of a mixture of 4 volumes of *chloroform* and 1 volume of *methanol*, filter and evaporate the filtrate. Dissolve the oily residue in 1 ml of *water*, add *acetone* until precipitation begins and add a further 20 ml of *acetone*. Filter the precipitate, wash with two 10-ml quantities of *acetone*, dissolve the residue in a little of the chloroform–methanol mixture, evaporate to dryness and dry at 60° at a pressure not exceeding 2 kPa for 4 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of lincomycin hydrochloride *(RS 203)*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to the trimethylsilyl derivative of lincomycin in the chromatogram obtained with solution (1).

TESTS

Lincomycin B

Examine solution (3) as described under the Assay but increasing the sensitivity by 8 to 10 times while recording the peak derived from lincomycin B, which is eluted immediately before that derived from lincomycin. The area of the peak derived from lincomycin B, when corrected for the sensitivity factor, is not more than 5% of the area of the peak derived from lincomycin.

Water

The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 0.3 g.

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 10 ml of a 0.8% w/w solution of *dotriacontane* (internal standard) in *chloroform* to 0.1 g of *lincomycin hydrochloride EPCRS*, dilute to 100 ml with a 2% w/v solution of *imidazole* in *chloroform* and shake to dissolve. Place 4 ml of the resulting solution in a 15-ml ground-glass-stoppered centrifuge tube, add 1 ml of a mixture of 99 volumes of N,O-*bis(trimethylsilyl)acetamide* and 1 volume of *trimethylchlorosilane* and swirl gently. Loosen the glass stopper and heat at 65° for 30 minutes. Prepare solution (2) in the same manner as solution (1) but omitting the internal standard and using a quantity of the mixed contents of 20 capsules containing the equivalent of 90 mg of lincomycin in place of the *lincomycin hydrochloride EPCRS*. Prepare solution (3) in the same manner as solution (1) but using a quantity of the mixed contents of 20 capsules containing the equivalent of 90 mg of lincomycin in place of the *lincomycin hydrochloride EPCRS*.

The chromatographic procedure may be carried out using a glass column (1.5 m × 3 mm) packed with *acid-washed silanised diatomaceous support* impregnated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 260° with both the inlet port and the detector at 260° to 290° and using *helium* as the carrier gas with a flow rate of about 45 ml per minute.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of lincomycin.

Lincomycin Injection

General Notices

Action and use

Lincosamide antibacterial.

DEFINITION

Lincomycin Injection is a sterile solution of Lincomycin Hydrochloride in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of lincomycin, $C_{18}H_{34}N_2O_6S$

92.5 to 107.5% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Add *acetone* to a volume of the injection containing 0.2 g of Lincomycin Hydrochloride until precipitation begins and add a further 20 ml of *acetone*. Filter the precipitate, wash with two 10-ml quantities of *acetone*, dissolve the residue in the minimum of a mixture of 4 volumes of *chloroform* and 1 volume of *methanol*, evaporate to dryness and dry at 60° at a pressure not exceeding 2 kPa for 4 hours. The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the *reference spectrum* of lincomycin hydrochloride *(RS 203)*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to the trimethylsilyl derivative of lincomycin in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 3.0 to 5.5, Appendix V L.

Lincomycin B

Examine solution (3) as described under the Assay but increase the sensitivity by 8 to 10 times while recording the peak derived from lincomycin B, which is eluted immediately before that derived from lincomycin. The area of the peak derived from lincomycin B, when corrected for the sensitivity factor, is not more than 5% of the area of the peak derived from lincomycin.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection if necessary with *water BET* to give a solution containing the equivalent of 10 mg of lincomycin per ml (solution A). The endotoxin limit concentration of solution A is 5.0 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 10 ml of a 0.8% w/w solution of *dotriacontane* (internal standard) in *chloroform* to 0.1 g of *lincomycin hydrochloride EPCRS*, dilute to 100 ml with a 2% w/v solution of *imidazole* in *chloroform* and shake to dissolve. Place 4 ml of the resulting solution in a 15-ml ground-glass-stoppered centrifuge tube, add 1 ml of a mixture of 99 volumes of N*,O-bis(trimethylsilyl)acetamide* and 1 volume of *trimethylchlorosilane* and swirl gently. Loosen the glass stopper and heat at 65° for 30 minutes. Prepare solution (2) in the same manner as solution (1) but omitting the internal standard and using the residue obtained by evaporating to dryness 1 ml of a solution prepared by diluting a volume of the injection containing the equivalent of 0.9 g of lincomycin to 10 ml with *methanol* in place of the *lincomycin hydrochloride EPCRS*. Prepare solution (3) in the same manner as solution (1) but using the residue obtained by evaporating to dryness 1 ml of a solution prepared by diluting a volume of the injection containing the equivalent of 0.9 g of lincomycin to 10 ml with *methanol* in place of the *lincomycin hydrochloride EPCRS*.

The chromatographic procedure may be carried out using a glass column (1.5 m × 3 mm) packed with *acid-washed silanised diatomaceous support* impregnated with 3% w/w of phenyl methyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 260° with both the inlet port and the detector at 260° to 290° and using *helium* as the carrier gas with a flow rate of about 45 ml per minute.

STORAGE

Lincomycin Injection should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The strength is stated as the equivalent amount of lincomycin in a suitable dose-volume.

Liothyronine Tablets

General Notices

Action and use

Thyroid hormone replacement.

DEFINITION

Liothyronine Tablets contain Liothyronine Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of liothyronine sodium, $C_{15}H_{11}I_3NNaO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

TEST

Uniformity of content

Comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add sufficient 0.05M *sodium hydroxide* to one tablet to produce a solution containing about 6 µg per ml of Liothyronine Sodium, mix with the aid of ultrasound until the tablet is fully dispersed, cool and shake for 2 minutes. Add sufficient 0.05M *sodium hydroxide* to produce a solution containing 0.0004% w/v of Liothryonine Sodium, filter through glass microfibre filter paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.0004% w/v of *liothyronine sodium EPCRS* in 0.05M *sodium hydroxide*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5 µm) (Nucleosil 5 CN is suitable), (b) a mixture of 5 volumes of *orthophosphoric acid*, 300 volumes of *acetonitrile* and 700 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 225 nm. Inject 20 µl of each solution.

Calculate the content of $C_{15}H_{11}I_3NNaO_4$ in each tablet using the declared content of $C_{15}H_{11}I_3NNaO_4$ in *liothyronine sodium EPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse with the aid of ultrasound for 10 minutes a quantity of the powdered tablets containing 0.1 mg of Liothyronine Sodium with 8 ml of a mixture of equal volumes of *methanol* and 0.1M *sodium hydroxide* (solvent A). Shake for 2 minutes, cool, add sufficient solvent A to produce 10 ml, mix, filter through glass microfibre filter paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.001% w/v of *liothyronine sodium EPCRS* in solvent A. Solution (3) contains 0.0005% w/v of *liothyronine sodium EPCRS* and 0.0005% w/v of *levothyroxine sodium EPCRS* in solvent A.

The chromatographic procedure may be carried out using the conditions described under the test for Uniformity of content.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 4.

Calculate the content of $C_{15}H_{11}I_3NNaO_4$ in the tablets using peak areas and the declared content of $C_{15}H_{11}I_3NNaO_4$ in *liothyronine sodium EPCRS*.

STORAGE

Liothyronine Tablets should be protected from light.

Lisinopril Tablets

General Notices

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Lisinopril Tablets contain Lisinopril Dihydrate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous lisinopril, $C_{21}H_{31}N_3O_5$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* GF_{254} *plate* and as the mobile phase a mixture of equal volumes of *glacial acetic acid*, *butan-1-ol*, *ethyl acetate* and *water*. Pre-wash the plate with *methanol* and allow to dry in air before use. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 10 mg of anhydrous lisinopril with 2 ml of *water* and add 8 ml of *methanol*. Solution (2) contains 0.11% w/v of *lisinopril dihydrate BPCRS* in a mixture of 20 volumes of *water* and 80 volumes of *methanol*. After removal of the plate, allow it to dry in air, spray with a 0.2% w/v solution of *ninhydrin* in *absolute ethanol*, heat at 105° for 10 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to lisinopril in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium after 30 minutes and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the filtered dissolution medium as solution (1). For solution (2) dissolve a quantity of *lisinopril dihydrate BPCRS* in sufficient of the dissolution medium to produce a solution of the same concentration as that expected for solution (1).

The chromatographic conditions described under Related substances may be used.

Calculate the total content of $C_{21}H_{31}N_3O_5$ in the medium from the chromatograms obtained and using the declared content of $C_{21}H_{31}N_3O_5$ in *lisinopril dihydrate BPCRS*. The amount of anhydrous lisinopril released is not less than 85% of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 20 mg of anhydrous lisinopril with a mixture of 1 volume of *methanol* and 4 volumes of *water* (solvent A) with the aid of ultrasound for 5 minutes, dilute to 100 ml with solvent A, mix with the aid of ultrasound again for about 10 minutes ensuring that the temperature of the solution does not rise above room temperature, and filter through a 0.45-µm filter. Solution (2) contains 0.0003% w/v of *lisinopril diketopiperazine BPCRS* in solvent A. For solution (3) dilute 1 volume of solution (1) to 100 volumes with solvent A. Solution (4) contains 0.022% w/v of *lisinopril dihydrate BPCRS* and 0.0002% w/v of *lisinopril diketopiperazine BPCRS* in solvent A.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP-8 is suitable) maintained at 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared by adding 200 volumes of *acetonitrile* to 800 volumes of a 0.408% w/v solution of *anhydrous potassium dihydrogen orthophosphate* adjusted to pH 2.0 with *orthophosphoric acid* containing 0.125% w/v of *sodium hexanesulphonate* and (c) a detection wavelength of 215 nm. Pass the mobile phase through the column for at least 30 minutes before starting the chromatography.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to lisinopril and lisinopril diketopiperazine is at least 5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to lisinopril diketopiperazine is not greater than the area of the peak in the chromatogram obtained with solution (2) (1.5%), the area of any other *secondary peak* is not greater than 0.3 times the area of the peak in the chromatogram obtained with solution (3) (0.3%) and the sum of the areas of any such peaks is not greater than 0.6 times the area of the peak in the chromatogram obtained with solution (3) (0.6%). The sum of the content of lisinopril diketopiperazine and the nominal total content of any other impurities is not more than 2%.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 10 whole tablets with a mixture of 1 volume of *methanol* and 4 volumes of *water* (solvent A) with the aid of ultrasound for 5 minutes, dilute to a suitable volume with solvent A, mix with the aid of ultrasound again for about 10 minutes ensuring that the temperature of the solution does not rise above room temperature, and filter through a 0.45-µm filter. Dilute a volume of the filtrate with solvent A to produce a solution containing the equivalent of 0.02% w/v of anhydrous lisinopril. Solution (2) contains 0.022% w/v of *lisinopril dihydrate BPCRS* in solvent A. Solution (3) contains 0.022% w/v of *lisinopril dihydrate BPCRS* and 0.0002% w/v of *lisinopril diketopiperazine BPCRS* in solvent A.

The chromatographic conditions described under Related substances may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to lisinopril and lisinopril diketopiperazine is at least 5.

Calculate the content of $C_{21}H_{31}N_3O_5$ in the tablets from the chromatograms obtained and using the declared content of $C_{21}H_{31}N_3O_5$ in *lisinopril dihydrate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous lisinopril.

IMPURITIES

The impurities controlled by this monograph include lisinopril diketopiperazine.

Lithium Carbonate Tablets

General Notices

DEFINITION

Lithium Carbonate Tablets contain Lithium Carbonate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lithium carbonate, Li_2CO_3

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A small quantity of the powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a carmine red colour to the flame.

ASSAY

Weigh and powder 20 tablets. Add a quantity of the powder containing 1 g of Lithium Carbonate to 100 ml of *water*, add 50 ml of 1M *hydrochloric acid VS* and boil for 1 minute to remove the carbon dioxide. Cool and titrate the excess of acid with 1M *sodium hydroxide VS* using *methyl orange solution* as indicator. Each ml of 1M *hydrochloric acid VS* is equivalent to 36.95 mg of Li_2CO_3.

Lithium Citrate Oral Solution

General Notices

DEFINITION

Lithium Citrate Oral Solution is a solution of Lithium Citrate in a suitable flavoured vehicle. *The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.*

Content of lithium citrate, $C_6H_5Li_3O_7,4H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The oral solution, when diluted with an equal volume of 3M *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a carmine red colour to the flame.

B. Boil 5 ml of the oral solution with 5 ml of *mercury(II) sulphate solution* and add a few drops of *dilute potassium permanganate solution*. The solution is decolorised and an off-white precipitate is produced.

TESTS

Acidity

pH, 4.0 to 5.5, Appendix V L.

ASSAY

Dilute a weighed quantity of the oral solution containing 0.4 g of Lithium Citrate to 1000 ml with *water* and add 0.05 ml of *polysorbate 80*. Determine the content of lithium by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 671 nm and using *lithium standard solution (100 ppm Li),* suitably diluted with *water* and 0.05 ml of *polysorbate 80*, to prepare the standard solutions. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of lithium citrate, weight in volume, taking each mg of Li to be equivalent to 13.54 mg of $C_6H_5Li_3O_7,4H_2O$.

STORAGE

Lithium Citrate Oral Solution should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The quantity of the active ingredient in a suitable dose-volume is stated (1) as the amount of Lithium Citrate; (2) as the concentration of lithium ions in millimoles; (3) in terms of the equivalent amount of Lithium Carbonate.

Lofepramine Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

LofepramineTablets contain Lofepramine Hydrochloride. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lofepramine, $C_{26}H_{27}ClN_2O$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Remove the majority of the coating. Wash the tablet with *dichloromethane* to remove the remaining coating. Place the tablet core in an ultrasound bath for 30 minutes with 10 ml of *dichloromethane*, filter the resulting suspension through a 0.45-µm PTFE filter; evaporate the filtrate to dryness using a rotary evaporator and a water bath at 30° and dry the residue at 50° at a pressure not exceeding 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of lofepramine hydrochloride (form B) *(RS 399B)*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of tablets containing the equivalent of 0.35 g of lofepramine for 1 hour with 50 ml of a mixture of 250 volumes of *acetonitrile*, 250 volumes of *water* and 1 volume of *orthophosphoric acid (85%)*, dilute the resulting solution to 200 ml with the same solvent mixture, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the same solvent mixture. Solution (3) contains 0.0002% w/v each of *desimpramine hydrochloride EPCRS* and *imipramine hydrochloride EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable) and maintained at 50°, (b) as the mobile phase with a flow rate of 1.5 ml per minute a 0.09% w/v solution of *sodium dodecyl sulphate* in a mixture of 550 volumes of *acetonitrile*, 325 volumes of *water* and 125 volumes of a buffer solution of pH 1.0 containing 0.015% w/v of *glycine*, 0.018% w/v of *sodium chloride* and 0.44% w/v of *hydrochloric acid* and (c) a detection wavelength of 254 nm.

Inject 20 µl of each solution. For solution (1) allow the chromatography to continue for 4 times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 0.9.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (2%). Disregard any peak with a retention time of less than 3 minutes.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 10 tablets for 1 hour with 300 ml of a mixture of 250 volumes of *acetonitrile*, 250 volumes of *water* and 1 volume of *orthophosphoric acid (85%)* and dilute to 500 ml with the same solvent mixture, mix and centrifuge; dilute the supernatant liquid with the same solvent mixture to produce a solution containing the equivalent of 0.028% w/v of lofepramine. Solution (2) contains 0.030% w/v of *lofepramine hydrochloride BPCRS* in the solvent mixture. Solution (3) contains 0.0002% w/v each of *desimpramine hydrochloride EPCRS* and *imipramine hydrochloride EPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 0.9.

Calculate the content of $C_{26}H_{27}ClN_2O$ in the tablets using the declared content of $C_{26}H_{27}ClN_2O$ in *lofepramine hydrochloride BPCRS*.

STORAGE

Lofepramine Tablets should be protected from light and moisture.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of lofepramine.

Lomustine Capsules

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Lomustine Capsules contain Lomustine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of lomustine, $C_9H_{16}ClN_3O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the following procedure in subdued light. Shake a quantity of the contents of the capsules containing 0.2 g of Lomustine with 10 ml of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator on a water bath maintained at not more than 60°. The residue, after drying at 60° at a pressure not exceeding 0.7 kPa for 30 minutes, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lomustine *(RS 204)*.

B. *Melting point*, about 88°, Appendix V A.

TEST

Related substances

A. Examine by *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 20 volumes of *glacial acetic acid* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.20 g of Lomustine with 10 ml of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 25 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) with 1 volume of *methanol*. For solution (4) dilute 1 volume of solution (2) to 10 volumes with *methanol*. For solution (5) dilute 1 volume of solution (2) to 20 volumes with *methanol*. For solution (6) dissolve 10 mg of *lomustine EPCRS* and 10 mg of *1,3-dicyclohexylurea* in *methanol* and dilute to 10 ml with the same solvent. After removal of the plate, dry it at 110° for 1 hour. At the bottom of a chromatography tank, place an evaporating dish containing a mixture of 1 volume of 7M *hydrochloric acid*, 1 volume of *water* and 2 volumes of a 1.5% w/v solution of *potassium permanganate*, close the tank and allow to stand for 15 minutes. Place the dried plate in the tank and close the tank. Leave the plate in contact with the chlorine vapour for 5 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the line of application does not give a blue colour with a drop of *potassium iodide and starch solution*. Spray with *potassium iodide and starch solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.4%) and at most one such spot is more intense than the spot in the chromatogram obtained with solution (5) (0.2%). The test is not valid unless the chromatogram obtained with solution (6) shows two clearly separated principal spots.

B. Examine by *liquid chromatography*, Appendix III D. For solution (1) shake a quantity of the contents of the capsules containing 0.20 g of Lomustine with 10 ml of *methanol* and filter. For solution (2) dilute 2 volumes of solution (2) to 100 volumes with *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 to 10 µm) (Nucleosil C18 is suitable), (b) as mobile phase with a flow rate of 2 ml per minute a mixture of 50 volumes of *methanol* and 50 volumes of *water*, (c) a detection wavelength of 230 nm and (d) a loop injector.

Inject 20 µl of solution (1). When the chromatograms are recorded in the prescribed conditions, the retention time of lomustine is about 25 minutes. When using a recorder, adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with solution (1) is not less than 50% of the full scale of the recorder.

Inject separately 20 µl of each solution. In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%). Disregard any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the following procedure in subdued light. Shake a quantity of the mixed contents of 20 capsules containing 40 mg of Lomustine with 70 ml of *ethanol (96%)* for 20 minutes, dilute to 100 ml with the same solvent and filter. Dilute 5 ml of the filtrate to 100 ml with *ethanol (96%)* and measure the *absorbance* of the resulting solution at the maximum at 230 nm, Appendix II B. Calculate the content of $C_9H_{16}ClN_3O_2$ taking 260 as the value of A(1%, 1 cm) at the maximum at 230 nm.

Loperamide Capsules

General Notices

Action and use

Opioid receptor agonist; antidiarrhoeal.

DEFINITION

Loperamide Capsules contain Loperamide Hydrochloride.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2.5 volumes of *acetate buffer pH 4.7*, 17.5 volumes of *methanol*, 27 volumes of *ethyl acetate* and 53 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 10 mg of Loperamide Hydrochloride with 10 ml of *methanol* for 5 minutes and filter. Solution (2) contains 0.10% w/v of *loperamide hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Expose the plate to iodine vapour and examine again. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of a buffer solution prepared by mixing 20 ml of 1M *acetic acid* with 60 ml of *water*, adjusting the pH to 4.7 with 1M *sodium hydroxide* and diluting to 100 ml with *water*, and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the filtered dissolution medium as solution (1). Solution (2) contains 0.00040% w/v of *loperamide hydrochloride BPCRS* in the dissolution medium.

The chromatographic procedure described under Assay may be used.

Calculate the total content of loperamide hydrochloride, $C_{29}H_{33}ClN_2O_2,HCl$, in the medium using the declared content of $C_{29}H_{33}ClN_2O_2,HCl$ in *loperamide hydrochloride BPCRS*.

Loperamide *N*-oxide

Carry out the method for *thin-layer chromatography*, Appendix III A, using a plate precoated with silica gel the surface of which has been modified with chemically-bonded octadecylsilyl groups (Whatman KC 18 plates are suitable) and a mixture of 30 volumes of *water*, adjusted to pH 3.0 by the addition of *orthophosphoric acid*, and 70 volumes of *methanol* as the mobile phase. Apply separately to the plate 25 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 20 mg of Loperamide Hydrochloride with 40 ml of *methanol* for 15 minutes, filter and evaporate the filtrate to dryness using a rotary evaporator and a water bath at 30°; to the residue add 2 ml of *methanol*, mix with the aid of ultrasound, centrifuge and use the supernatant liquid. Solution (2) contains 0.010% w/v of *loperamide* N*-oxide BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and expose to iodine vapour for 16 hours. In the chromatogram obtained with solution (1) any spot corresponding to loperamide *N*-oxide is not more intense than the principal spot in the chromatogram obtained with solution (2) (1%).

Uniformity of content

Capsules containing 2 mg of Loperamide Hydrochloride or less comply with the requirements stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 150 ml of *water* to one whole capsule and mix with the aid of ultrasound for 30 minutes, shake mechanically for 30 minutes and dilute to 200 ml with *acetonitrile*, filter and use the filtrate. For solution (2) dissolve 10 mg of *loperamide hydrochloride BPCRS* in 45 ml of *water* and dilute to 100 ml with *acetonitrile*. Dilute 1 volume of the resulting solution to 10 volumes with the same solvent mixture.

The chromatographic procedure described under Assay may be used.

Calculate the content of $C_{29}H_{33}ClN_2O_2$,HCl in each capsule using the declared content of $C_{29}H_{33}ClN_2O_2$,HCl in *loperamide hydrochloride BPCRS*.

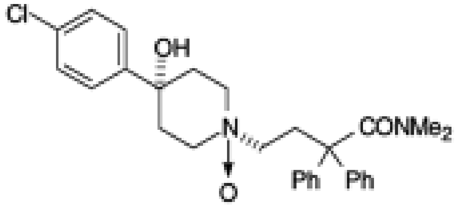
ASSAY

Prepare 0.005M *sodium octanesulphonate* containing, per 1000 ml, 1 ml of 13.5M *ammonia* and 0.5 ml of *triethylamine* and adjust the pH to 3.2 with *orthophosphoric acid* (solvent A). Carry out the method for *liquid chromatography* Appendix III D, using the following solutions. For solution (1) mix a quantity of the mixed contents of 20 capsules containing 20 mg of Loperamide Hydrochloride with 90 ml of *water* with the aid of ultrasound for 10 minutes, shake for 30 minutes, dilute to 200 ml with *acetonitrile* and filter. Solution (2) contains 0.010% w/v of *loperamide hydrochloride BPCRS* in a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile.*

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 45 volumes of solvent A and 55 volumes of *acetonitrile* and (c) a detection wavelength of 226 nm.

Calculate the content of $C_{29}H_{33}ClN_2O_2$,HCl in the capsules using the declared content of $C_{29}H_{33}ClN_2O_2$,HCl in *loperamide hydrochloride BPCRS*.

IMPURITIES



A. Loperamide *N*-oxide

Loprazolam Tablets

General Notices

Action and use

Benzodiazepine.

DEFINITION

Loprazolam Tablets contain Loprazolam Mesilate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of loprazolam, $C_{23}H_{21}ClN_6O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption,* Appendix II B, in the range 210 to 370 nm of the filtrate obtained in the Assay exhibits a maximum at 330 nm and a shoulder at 240 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 80 volumes of *chloroform* and 20 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 2 mg of loprazolam with 10 ml of a mixture of 46 volumes of *chloroform*, 46 volumes of *methanol* and 8 volumes of *water*, centrifuge and use the clear supernatant liquid. Solution (2) contains 0.02% w/v of *loprazolam mesilate BPCRS* in the same solvent mixture. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Complies with the test described under Loprazolam Mesilate, using 40 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 10 mg of loprazolam with 4 ml of a mixture of 46 volumes of *chloroform*, 46 volumes of *methanol* and 8 volumes of *water*, mix the suspension with the aid of ultrasound for 5 minutes, centrifuge and use the clear supernatant liquid. Solution (2) contains 0.00025% w/v of *loprazolam mesilate BPCRS* and solution (3) contains 0.00125% w/v of *6-(2-chlorophenyl)-2,4-dihydro-2-[(dimethylamino)methylene]-8-nitroimidazo-[1,2-a][1,4] benzodiazepin-1-one BPCRS* (dimethylamino analogue), both in the same solvent mixture. Any spot corresponding to the dimethylamino analogue in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2).

Uniformity of content

Tablets containing less than the equivalent of 2 mg of loprazolam comply with the requirements stated under Tablets using the following method of analysis. Shake one tablet with 8 ml of *water*, add 80 ml of *absolute ethanol*, mix with the aid of ultrasound for 10 minutes, cool and dilute to 200 ml with *absolute ethanol*. Mix, filter, discarding the first 20 ml of filtrate, and measure the *absorbance* of the resulting solution at the maximum at 330 nm, Appendix II B. Calculate the content of $C_{23}H_{21}ClN_6O_3$ taking 890 as the value of A(1%, 1 cm) at the maximum at 330 nm.

ASSAY

Use the average of the 10 individual results obtained in the test for Uniformity of content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of loprazolam.

Lorazepam Injection

General Notices

Action and use

Benzodiazepine.

DEFINITION

Lorazepam Injection is a sterile solution of Lorazepam in a suitable solvent.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of lorazepam, $C_{15}H_{10}Cl_2N_2O_2$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 5 ml of *hydrochloric acid* to a volume of the injection containing 10 mg of Lorazepam, heat in a water bath for 20 minutes and cool. Transfer the cooled solution to a separating funnel, add 8 ml of 10M *sodium hydroxide*, extract with two 25 ml quantities of *ether* and filter the combined extracts through absorbent cotton. Evaporate the filtrate to about 2 ml and dilute to 10 ml with *methanol*. For solution (2) add 5 ml of *hydrochloric acid* to 10 ml of a 0.1% w/v solution of *lorazepam BPCRS* in *absolute ethanol* and treat in the same manner as for solution (1) beginning at the words 'heat in a water bath…'. After removal of the plate, allow it to dry and spray with a freshly prepared 1.25% w/v solution of *sodium nitrite* in 0.5M *hydrochloric acid*. Heat the plate at 100° for 5 minutes, allow to cool and spray with a 0.1% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride* in *absolute ethanol*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 100 volumes of *chloroform* and 10 volumes of *methanol* as the mobile phase. Before use, stand the plate in *methanol* and allow the solvent front to ascend 17 cm. Allow the plate to dry in air, heat it at 100° to 105° for 1 hour and use with the flow of mobile phase in the same direction as that used for the pretreatment. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) add 10 ml of *water* to a volume of the injection containing 4 mg of Lorazepam, extract with two 10 ml quantities of *dichloromethane*, wash the combined extracts with 10 ml of *water* and evaporate the washed extract to dryness at 20° in a current of nitrogen. Dissolve the residue in 2 ml of *acetone*. Solution (2) contains 0.010% w/v of *6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde BPCRS* in *acetone*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *acetone*. For solution (4) dilute 1 volume of solution (1) to 200 volumes with *acetone*. Solution (5) contains 0.1% v/v of *benzyl alcohol* in *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to 6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4). Disregard any spot with an Rf value similar to that of the spot in the chromatogram obtained with solution (5).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.008% w/v of *lorazepam BPCRS* in the mobile phase. For solution (2) dilute a suitable volume of the injection with sufficient mobile phase to produce a solution containing 0.008% w/v of Lorazepam.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 70 volumes of *methanol* and 30 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ from the declared content of $C_{15}H_{10}Cl_2N_2O_2$ in *lorazepam BPCRS*.

STORAGE

Lorazepam Injection should be protected from light and stored at a temperature of 2° to 8°.

Lorazepam Tablets

General Notices

Action and use

Benzodiazepine.

DEFINITION

Lorazepam Tablets contain Lorazepam.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lorazepam, $C_{15}H_{10}Cl_2N_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 350 nm of the solution obtained in the Assay exhibits two maxima, at 230 nm and 316 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Lorazepam with 10 ml of *acetone* for 20 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 10 ml of *absolute ethanol*, add 5 ml of *hydrochloric acid*, heat on a water bath for 20 minutes and cool. Transfer the cooled solution to a separating funnel, add 8 ml of 10M *sodium hydroxide*, extract with two 25 ml quantities of *ether* and filter the combined extracts through absorbent cotton. Evaporate the filtrate to about 2 ml and dilute to 10 ml with *methanol*. For solution (2) treat 10 ml of a 0.1% w/v solution of *lorazepam BPCRS* in *absolute ethanol* in the same manner as described for solution (1) beginning at the words 'add 5 ml of *hydrochloric acid* …'. After removal of the plate, allow it to dry and spray with a freshly prepared 1.25% w/v solution of *sodium nitrite* in 0.5M *hydrochloric acid*. Heat the plate at 100° for 5 minutes, allow to cool and spray with a 0.1% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride* in *absolute ethanol*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII D, using Apparatus 1. Use as the medium 900 ml of 0.1M *hydrochloric acid*, and rotate the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute, if necessary, a quantity of filtrate with 0.1M *hydrochloric acid* to produce a solution expected to contain about

0.00011% w/v of Lorazepam. Using 2 cm cells, measure the *absorbance* of the solution at the maximum at 232 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the content of Lorazepam, $C_{15}H_{10}Cl_2N_2O_2$, in the medium taking 1115 as the value of A(1%, 1cm) at the maximum at 232 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *methanol* and 100 volumes of *chloroform* as the mobile phase. Before use, stand the plate in *methanol*, allowing the solvent front to ascend 17 cm, heat the plate at 100° to 105° for 1 hour. Use with the flow of mobile phase in the same direction as that used for the prewash. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) shake a quantity of powdered tablets containing 10 mg of Lorazepam with 4 ml of *acetone*, centrifuge and use the supernatant liquid. Solution (2) contains 0.0025% w/v of *6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde BPCRS* in *acetone*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *acetone*. For solution (4) dilute 1 volume of solution (1) to 200 volumes with *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to 6-chloro-4-(2-chlorophenyl)quinazoline-2-carboxaldehyde in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4).

Uniformity of content

Tablets containing less than 2 mg of Lorazepam comply with the requirements stated under Tablets using the following method of analysis. Crush one tablet to a fine powder, add 40 ml of *ethanol (96%)* and shake for 1 hour. Add sufficient *ethanol (96%)* to produce 50 ml, centrifuge and dilute the supernatant liquid with sufficient *ethanol (96%)* to produce a solution containing 0.0005% w/v of Lorazepam. Measure the *absorbance* of the resulting solution at the maximum at 230 nm, Appendix II B, and calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ taking 1150 as the value of A(1%, 1 cm) at the maximum at 230 nm.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 5 mg of Lorazepam add 40 ml of *ethanol (96%)* and shake for 1 hour. Add sufficient *ethanol (96%)* to produce 50 ml, centrifuge, dilute 5 ml to 100 ml with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 230 nm, Appendix II B. Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ taking 1150 as the value of A(1%, 1 cm) at the maximum at 230 nm.

STORAGE

Lorazepam Tablets should be protected from light.

Lormetazepam Tablets

General Notices

Action and use

Benzodiazepine.

DEFINITION

Lormetazepam Tablets contain Lormetazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lormetazepam, $C_{16}H_{12}Cl_2N_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 10 mg of Lormetazepam with 5 ml of *acetone*, filter, wash the residue with a further 5 ml of *acetone*, combine the acetone extracts and evaporate to dryness on a water bath using a current of *nitrogen*. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of lormetazepam *(RS 207)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *chloroform* and 1 volume of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Lormetazepam with 5 ml of *acetone* for 15 minutes and filter. Solution (2) contains 0.2% w/v of *lormetazepam BPCRS* in *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the powdered tablets containing 10 mg of Lormetazepam in 100 ml of *methanol (60%),* centrifuge and use the clear supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (60%)*. Solution (3) contains 0.0010% w/v each of *lormetazepam BPCRS* and *lorazepam BPCRS* in *methanol (60%)*.

The chromatographic procedure may be carried out using (a) a column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 48 volumes of *methanol* and 52 volumes of a phosphate buffer prepared by dissolving 4.91 g of *sodium dihydrogen orthophosphate* and 0.633 g of *disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 ml and (c) a detection wavelength of 230 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 4.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%). Disregard any peaks with a retention time of less than half that of lormetazepam.

Uniformity of content

Tablets containing less than 2 mg of Lormetazepam comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 3 ml of *water* to one tablet, allow to stand for 20 minutes with occasional shaking, mix with the aid of ultrasound for 2 minutes, add 35 ml of *methanol*, mix with the aid of ultrasound for 5 minutes, shake for a further 15 minutes and cool. For tablets containing 0.5 mg of Lormetazepam, dilute the resulting solution to 50 ml with *water* and for tablets containing 1 mg of Lormetazepam, dilute to 100 ml with *methanol (60%)*, centrifuge for 10 minutes and use the clear supernatant liquid. Solution (2) contains 0.001% w/v of *lormetazepam BPCRS* in *methanol (70%)*.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{16}H_{12}Cl_2N_2O_2$ in each tablet using the declared content of $C_{16}H_{12}Cl_2N_2O_2$ in *lormetazepam BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 10 mg of Lormetazepam in 500 ml of *methanol (60%)*, centrifuge for 15 minutes and use the clear supernatant liquid. Solution (2) contains 0.002% w/v of *lormetazepam BPCRS* in *methanol (60%)*.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{16}H_{12}Cl_2N_2O_2$ in the tablets from the declared content of $C_{16}H_{12}Cl_2N_2O_2$ in *lormetazepam BPCRS*.

Lymecycline Capsules

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Lymecycline Capsules contain Lymecycline.

The capsules comply with the requirements stated under Capsules and with the following requirements.

IDENTIFICATION

The contents of the capsules comply with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Adjust the pH of a 10% w/v solution of *disodium edetate* to 8.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 ml for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate in an oven at 110° for 1 hour. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 5 mg of Lymecycline with 10 ml of *methanol*, centrifuge and use the supernatant liquid. Solution (2) contains 0.05% w/v of *lymecycline BPCRS* in *methanol*. Solution (3) contains 0.05% w/v each of *tetracycline hydrochloride BPCRS*, *chlortetracycline hydrochloride BPCRS* and *doxycycline hyclate BPCRS* in *methanol*. After removal of the plate, allow it to dry in a current of air and examine under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

B. To 0.5 mg add 2 ml of *sulphuric acid*. A purplish red colour is produced.

C. Dissolve 50 mg in 5 ml of *water*, add 50 mg of *ninhydrin*, boil and add 15 ml of *water*. A bluish-violet colour is produced.

Dissolve 0.2 g in 5 ml of *water*, add 0.3 ml of *orthophosphoric acid* and distil. To 1 ml of the distillate add 10 ml of *chromotropic acid–sulphuric acid solution*. A violet colour is produced.

TESTS

Light-absorbing impurities

Dissolve the contents of five capsules as completely as possible in sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.15% w/v of Lymecycline and filter. The *absorbance* of the filtrate at 430 nm, when measured within 1 hour of preparing the solution, is not more than 0.60, calculated with reference to the anhydrous capsule contents, Appendix

II B.

Water

The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 0.3 g.

ASSAY

To a quantity of the mixed contents of 20 capsules containing 0.3 g of Lymecycline add 500 ml of 0.05M *hydrochloric acid*, mix and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of lymecycline in a capsule of average content weight taking each 1000 IU found to be equivalent to 1 mg of lymecycline. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

204 mg of Lymecycline is equivalent to approximately 150 mg of tetracycline.

Prolonged-release Lithium Carbonate Tablets

General Notices

Slow Lithium Carbonate Tablets

Prolonged-release Lithium Carbonate Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Prolonged-release Lithium Carbonate Tablets contain Lithium Carbonate and are formulated so as to release the medicament over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of lithium carbonate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of lithium carbonate, Li_2CO_3

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A small quantity of the powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a carmine red colour to the flame.

ASSAY

Weigh and powder 20 tablets. Add a quantity of the powder containing 1 g of Lithium Carbonate to 100 ml of *water*, add 50 ml of 1M *hydrochloric acid VS* and boil for 1 minute to remove the carbon dioxide. Cool and titrate the excess of acid with 1M *sodium hydroxide VS* using *methyl orange solution* as indicator. Each ml of 1M *hydrochloric acid VS* is equivalent to 36.95 mg of Li_2CO_3.

Sterile Lidocaine Solution

General Notices

Action and use

Local anaesthetic; Class I antiarrhythmic.

DEFINITION

Sterile Lidocaine Solution is a sterile *cutaneous solution*. It contains Lidocaine Hydrochloride in Purified Water.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of lidocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Make a volume containing 0.1 g of Lidocaine Hydrochloride alkaline with 5M *sodium hydroxide,* filter, wash the residue with *water*, dissolve it in 1 ml of *ethanol (96%)*, add 0.5 ml of a 10% w/v solution of *cobalt(II) chloride* and shake for 2 minutes. A bluish-green precipitate is produced.

B. To a volume containing 0.1 g of Lidocaine Hydrochloride add 10 ml of *picric acid solution R1*. The *melting point* of the precipitate, after washing with *water* and drying at 105°, is about 229°, Appendix V A.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

2,6-Dimethylaniline

Dilute the solution to contain 0.25% w/v of Lidocaine Hydrochloride. To 10 ml of this solution add 2M *sodium hydroxide* until the solution is just alkaline and extract with three 5 ml quantities of *chloroform*, dry the combined chloroform extracts over *anhydrous sodium sulphate*, filter, wash with a further 5 ml of *chloroform* and evaporate the filtrate to dryness at a pressure of 2 kPa. Dissolve the residue in 2 ml of *methanol,* add 1 ml of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand at room temperature for 10 minutes. Any yellow colour produced is not more intense than the colour obtained by repeating the operation using 10 ml of a solution of *2,6-dimethylaniline* in *water* containing 1 µg per ml in place of the solution being examined (400 ppm).

ASSAY

Make a quantity containing 0.1 g of Lidocaine Hydrochloride alkaline with 2M *sodium hydroxide* and extract with three 20 ml quantities of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the washed extracts through a filter paper moistened with *chloroform* and wash the filter with 10 ml of *chloroform*. Add the washings to the filtrate and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.02M *perchloric acid VS* as titrant and *crystal violet solution* as indicator. Each ml of 0.02M *perchloric acid VS* is equivalent to 5.776 mg of $C_{14}H_{22}N_2O,HCl,H_2O$.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

LABELLING

The label states (1) that the solution is not intended for injection; (2) that the solution should be used on one occasion only and that any remainder should be discarded; (3) the date after which the solution is not intended to be used; (4) the conditions under which it should be stored.

The label indicates the pharmaceutical form as 'cutaneous solution'.

White Liniment

General Notices

White Embrocation

DEFINITION

White Liniment is a *cutaneous emulsion*.

Oleic Acid	85 ml
Turpentine Oil	250 ml
Dilute Ammonia Solution	45 ml
Ammonium Chloride	12.5 g
Purified Water	625 ml

Extemporaneous preparation

The following directions apply.

Mix the Oleic Acid with the Turpentine Oil. Dilute the Dilute Ammonia Solution with 45 ml of the Purified Water, previously warmed, add to the oily solution and shake to form an emulsion. Separately dissolve the Ammonium Chloride in the remainder of the Purified Water, add to the emulsion and mix.

The liniment complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of volatile oil

24.5 to 27.5% v/w.

Refractive index

Of the oily liquid obtained in the Assay, 1.465 to 1.477, Appendix V E.

ASSAY

Acidify 100 g with 1M *sulphuric acid* using *methyl orange solution* as indicator, distil in steam and collect the distillate in a separating funnel; occasionally separate the aqueous portion of the distillate and return it to the flask. When all the volatile matter has been distilled, remove the aqueous portion of the distillate and measure the volume of the oily liquid.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous emulsion'.

Aromatic Magnesium Carbonate Mixture

General Notices

Aromatic Magnesium Carbonate Oral Suspension

DEFINITION

Aromatic Magnesium Carbonate Mixture is an *oral suspension* containing 3% w/v of Light Magnesium Carbonate and 5% w/v of Sodium Bicarbonate in a suitable vehicle containing Aromatic Cardamom Tincture.

Extemporaneous preparation

It is recently prepared according to the following formula.

Light Magnesium Carbonate	30 g
Sodium Bicarbonate	50 g
Aromatic Cardamom Tincture	30 ml
Double-strength Chloroform Water	500 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

4.7 to 5.3% w/v.

Content of magnesium, Mg

0.70 to 0.86% w/v.

ASSAY

For sodium bicarbonate

Boil 10 g with 100 ml of *water* for 5 minutes, filter, boil the residue with 100 ml of *water* for 5 minutes and filter. Combine the filtrates, cool and titrate with 0.5M *hydrochloric acid VS* using *methyl orange–xylene cyanol FF solution* as indicator. Add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. After subtracting one-fifth of the volume of 0.05M *disodium edetate VS*, each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$. Determine the *weight per ml* of the mixture, Appendix V G, and calculate the percentage of $NaHCO_3$, weight in volume.

For magnesium

Dissolve 30 g in the minimum quantity of 2M *hydrochloric acid* and dilute to 200 ml with *water*.

To 20 ml of this solution add 200 ml of *water* and 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg. Use the *weight per ml* of the mixture to calculate the percentage of Mg, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'oral suspension'.

Compound Magnesium Trisilicate Oral Powder

General Notices

DEFINITION

Magnesium Trisilicate	250 g
Chalk in powder	250 g
Sodium Bicarbonate	250 g
Heavy Magnesium Carbonate	250 g

The oral powder complies with the requirements stated under Oral Powders and with the following requirements.

Content of magnesium trisilicate, calculated as silica, SiO_2

11.0 to 15.2% w/w.

Content of sodium bicarbonate, $NaHCO_3$

23.7 to 26.9% w/w.

IDENTIFICATION

Shake 0.5 g with 5 ml of 2M *hydrochloric acid* and filter. The filtrate complies with the following tests.

A. To 0.05 ml add 0.2 ml of a 1% w/v solution of *glyoxal bis(2-hydroxyanil)* in *ethanol (96%)* and 0.05 ml of a 10% w/v solution of *sodium hydroxide*; a reddish brown precipitate is produced. Add *chloroform*; the precipitate dissolves to produce a red solution.

B. Add to the filtrate an excess of a 2.5% w/v solution of *ammonium oxalate*, filter and to the filtrate add 5M *sodium hydroxide* followed by *diphenylcarbazide solution*. A violet-red precipitate is produced.

ASSAY

For silica

Mix 1.5 g with 5 ml of *water* and 10 ml of 9M *perchloric acid* in a beaker, heat until dense white fumes are evolved, cover the beaker with a watch glass, continue heating for a further 3 hours and allow to cool. Add 30 ml of *water*, filter, wash the filter with 200 ml of hot *water*, discard the filtrate and washings, dry the filter paper and its contents, ignite at 1000°, cool and weigh. To the residue add 0.25 ml of *sulphuric acid* followed by 15 ml of *hydrofluoric acid*, heat cautiously on a sand bath until all the acid has evaporated, ignite at 1000°, cool and weigh. Subtract the weight of any residue so obtained from the weight of the residue obtained above and calculate the content of SiO_2 from the corrected weight of residue.

For sodium bicarbonate

Boil 2.5 g with 100 ml of *water* for 5 minutes, filter, boil the residue with 100 ml of *water* for 5 minutes and filter. Combine the filtrates, cool and titrate with 0.5M *hydrochloric acid VS* using *methyl orange–xylene cyanol FF solution* as indicator. Add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. After subtracting one-fifth of the volume of 0.05M *disodium edetate VS*, each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

Compound Magnesium Trisilicate Tablets

General Notices

Aluminium Hydroxide and Magnesium Trisilicate Tablets

DEFINITION

Compound Magnesium Trisilicate Tablets contain, in each, 250 mg of Magnesium Trisilicate and 120 mg of Dried Aluminium Hydroxide. They have a peppermint flavour.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of aluminium, Al

28 to 40 mg.

Content of magnesium, Mg

30 to 41 mg.

TESTS

Disintegration

The requirement for Disintegration does not apply to Compound Magnesium Trisilicate Tablets.

Neutralising capacity

Weigh and powder 20 tablets. Pass the powder as completely as possible, regrinding if necessary, through a sieve of nominal mesh aperture about 75 µm and remix the sifted powder. Mix a quantity equivalent to two tablets with a small quantity of *water*, added slowly with stirring, to give a smooth paste and slowly add further quantities of *water*, with stirring, to a total volume of 100 ml. Warm to 37°, add 100 ml of 0.1M *hydrochloric acid VS* previously heated to 37° and stir continuously, using a suitable paddle stirrer at a rate of about 200 revolutions per minute, maintaining the temperature at 37°. The pH of the suspension at 37°, after 10, 15 and 20 minutes, is not less than 2.0, 2.4 and 2.7 respectively and at no time during this period is it more than 4.0. Add 10 ml of 0.5M *hydrochloric acid VS* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate with 0.1M *sodium hydroxide VS* to pH 3.5. Subtract the volume of 0.1M *sodium hydroxide VS* from 150 ml to obtain the number of ml of 0.1M *hydrochloric acid VS* required for the neutralisation. Not less than 50 ml of 0.1M *hydrochloric acid VS* is required to neutralise one tablet.

ASSAY

For aluminium

Weigh and powder 20 tablets. To a quantity of the powder equivalent to two tablets add 7 ml of *hydrochloric acid* and 7 ml of *water*, stir and heat on a water bath for 15 minutes, stirring occasionally. Add 30 ml of hot *water*, mix and filter whilst hot, washing the filter well with hot *water*. Cool the combined filtrate and washings and dilute to 100 ml with *water*. Reserve 25 ml for the Assay for magnesium. To 30 ml add 70 ml of *water* and 40 ml of 0.05M *disodium edetate VS*. Add 0.2 ml of *methyl red solution*, neutralise to the orange colour of the indicator with 1M *sodium hydroxide* and heat on a water bath for 30 minutes. Cool, add 3 g of *hexamine* and titrate the excess of disodium edetate with 0.05M *lead nitrate VS* using *xylenol orange solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 1.349 mg of Al.

For magnesium

To 25 ml of the solution reserved in the Assay for aluminium add 1 g of *ammonium chloride* and 10 ml, or a quantity sufficient to redissolve the precipitate that is produced, of *triethanolamine*. Add 150 ml of *water* and 5 ml of *ammonia buffer pH 10.9* and titrate immediately with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg.

LABELLING

The label states that the tablets should be chewed before being swallowed.

Magaldrate Oral Suspension

General Notices

Action and use

Antacid.

DEFINITION

Magaldrate Oral Suspension contains Magaldrate in a suitable flavoured vehicle.
The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous magaldrate, $Al_5Mg_{10}(OH)_{31}(SO_4)_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the oral suspension containing the equivalent of 0.8 g of anhydrous magaldrate in 20 ml of 3M *hydrochloric acid*, add 30 ml of *water* and heat to boiling. Add 6M *ammonia* until a pH of 6.2 is obtained, continue boiling for a further 2 minutes, filter and retain the precipitate and the filtrate. To 2 ml of the filtrate add 2 ml of *ammonium chloride solution* and neutralise with a solution prepared by dissolving 2 g of *ammonium carbonate* and 2 ml of 6M *ammonia* in sufficient *water* to produce 20 ml; no precipitate is produced. Add *disodium hydrogen phosphate solution*; a white, crystalline precipitate is produced which is insoluble in 6M *ammonia*.

B. The precipitate retained in test A yields the reaction characteristic of *aluminium salts*, Appendix VI.

C. The filtrate obtained in test A yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Neutralising capacity

Disperse a quantity containing the equivalent of 0.8 g of anhydrous magaldrate in 70 ml of water, heat to 37° and mix for 1 minute. Maintain the temperature at 37° and, while stirring continuously, add from a pipette 30 ml of 1M *hydrochloric acid VS*. Stir for 15 minutes and, over a period not exceeding 5 minutes, titrate the excess acid with 1M *sodium hydroxide VS* to a pH of 3.5 which is stable for a period of 10 to 15 seconds. Not more than 12 ml of 1M *sodium hydroxide VS* is required.

Magnesium hydroxide

Not less than 49.2% and not more than 66.6% of the content of anhydrous magaldrate when determined by the following method. Mix a quantity of the oral suspension containing the equivalent of 1 g of magaldrate in 30 ml of 2M *hydrochloric acid* and add sufficient *water* to produce 100 ml (solution A). Dilute 10 ml of this solution to 200 ml with *water*. Add, while stirring, 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia buffer pH 10.9* and 0.4 ml of *mordant black 11 solution*. Titrate with 0.05M *disodium edetate VS* until a blue colour is obtained. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of disodium edetate required. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.916 g of $Mg(OH)_2$.

Aluminium hydroxide

Not less than 32.1% and not more than 45.9% of the stated content of anhydrous magaldrate when determined by the following method. Dilute 10 ml of solution A obtained in the test for Magnesium hydroxide with 20 ml of *water*. Add 25 ml of 0.05M *disodium edetate VS*, mix, allow to stand for 5 minutes and add 20 ml of *acetate buffer pH 4.4*, 60 ml of *ethanol (96%)* and 2 ml of a 0.026% w/v solution of *dithizone* in *ethanol (96%)*. Titrate with 0.05M *zinc sulphate VS* until a bright pink colour is obtained. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of disodium edetate required. Each ml of 0.05M *disodium edetate VS* is equivalent to 3.90 g of $Al(OH)_3$.

Microbial contamination

Carry out a quantitative evaluation for Enterobacteria and certain other Gram-negative bacteria, Appendix XVI B1. 0.01 ml of the preparation gives a negative result, Table 1 (most probable number of bacteria per gram fewer than 10^2).

ASSAY

To a quantity of the oral suspension containing the equivalent of 3 g of anhydrous magaldrate add 100 ml of 1M *hydrochloric acid VS* and mix using a magnetic stirrer to achieve dissolution. Titrate the excess acid with 1M *sodium hydroxide VS* to pH 3, determined potentiometrically. Repeat the procedure without the preparation being examined. The difference between the titrations represents the amount of hydrochloric acid required. Each ml of 1M *hydrochloric acid VS* is equivalent to 35.40 mg of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

LABELLING

The quantity of the active ingredient is stated in terms of the equivalent amount of anhydrous magaldrate.

Magnesium Chloride Injection

General Notices

DEFINITION

Magnesium Chloride Injection is a sterile solution of Magnesium Chloride Hexahydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of magnesium chloride hexahydrate, $MgCl_2,6H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

Yields the reactions characteristic of *magnesium salts* and of *chlorides*, Appendix VI.

Acidity or alkalinity

pH, 5.0 to 8.0, Appendix V L.

ASSAY

Dilute a volume containing 2.5 g of Magnesium Chloride Hexahydrate to 100 ml with *water*. To 20 ml of this solution add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.1M *disodium edetate VS* using 0.1 g of *mordant black 11 triturate* as indicator, until the pink tint is discharged from the blue colour. Each ml of 0.1M *disodium edetate VS* is equivalent to 20.33 mg of $MgCl_2,6H_2O$.

LABELLING

The strength is stated as the percentage w/v of Magnesium Chloride Hexahydrate and as the approximate concentration of magnesium ions (Mg^{2+}) in millimoles per ml.

For a preparation containing 10% w/v of Magnesium Chloride Hexahydrate, the concentration of magnesium ions is approximately 0.5 millimoles per millilitre (0.5 mmol Mg^{2+}/ml).

Magnesium Hydroxide Mixture

General Notices

Magnesium Hydroxide Oral Suspension

DEFINITION

Magnesium Hydroxide Mixture is an aqueous *oral suspension* of hydrated magnesium oxide. It may be prepared from a suitable grade of Light Magnesium Oxide.

Extemporaneous preparation

The following formula and directions apply.

Magnesium Sulphate	47.5 g
Sodium Hydroxide	15 g
Light Magnesium Oxide	52.5 g
Chloroform	2.5 ml
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Dissolve the Sodium Hydroxide in 150 ml of Purified Water, add the Light Magnesium Oxide, mix to form a smooth cream and then add sufficient Purified Water to produce 2500 ml. Pour this suspension in a thin stream into a solution of the Magnesium Sulphate in 2500 ml of Purified Water, stirring continuously during the mixing. Allow the precipitate to subside, remove the clear liquid, transfer the residue to a calico strainer, allow to drain and wash the precipitate with Purified Water until the washings give only a slight reaction for *sulphates*, Appendix VI. Mix the washed precipitate with Purified Water, dissolve the Chloroform in the mixture and add sufficient Purified Water to produce 1000 ml.

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of hydrated magnesium oxide, calculated as $Mg(OH)_2$

7.45 to 8.35% w/w.

CHARACTERISTICS

A white, uniform suspension which does not separate readily on standing.

TESTS

Heavy metals

Dissolve 12.5 g in 10 ml of *hydrochloric acid* and 20 ml of *water*, add 0.5 ml of *nitric acid*, boil to remove any carbon dioxide and filter. To the cooled filtrate add 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with two successive 10 ml quantities of a mixture of equal volumes of *isoamyl alcohol* and *ether*. To the aqueous layer add 2 g of *citric acid* and sufficient *water* to produce 50 ml. 12 ml of the resulting solution complies with *limit test A for heavy metals*, Appendix VII. Use *lead standard solution (1 ppm Pb)* to prepare the standard (4 ppm).

Sulphate

Dissolve 2.5 ml in 20 ml of *hydrochloric acid* and dilute to 500 ml with *water*. 15 ml of the solution, filtered if necessary, complies with the *limit test for sulphates*, Appendix VII (0.2%).

ASSAY

Mix 10 g with 50 ml of *water*, add 50 ml of 0.5M *sulphuric acid VS* and titrate the excess of acid with 1M *sodium hydroxide VS* using *methyl orange solution* as indicator. Each ml of 0.5M *sulphuric acid VS* is equivalent to 29.16 mg of hydrated magnesium oxide calculated as $Mg(OH)_2$.

STORAGE

Magnesium Hydroxide Mixture should not be kept in a cold place.

LABELLING

The label indicates the pharmaceutical form as 'oral suspension'.

Magnesium Sulphate Injection

General Notices

DEFINITION

Magnesium Sulphate Injection is a sterile solution of Magnesium Sulphate Heptahydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of magnesium sulphate heptahydrate, $MgSO_4,7H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

Yields the reactions characteristic of *magnesium salts* and of *sulphates*, Appendix VI.

TESTS

Acidity or alkalinity

pH of the injection diluted, if necessary, to contain 5% w/v of Magnesium Sulphate Heptahydrate, 5.5 to 7.0, Appendix V L.

ASSAY

Dilute a volume containing 1.25 g of Magnesium Sulphate Heptahydrate to 100 ml with *water*. To 20 ml of this solution add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using 0.1 g of *mordant black 11 triturate* as indicator, until the pink tint is discharged from the blue colour. Each ml of 0.05M *disodium edetate VS* is equivalent to 12.32 mg of $MgSO_4,7H_2O$.

LABELLING

The strength is stated as the percentage w/v of Magnesium Sulphate Heptahydrate and as the approximate concentration of magnesium ions (Mg^{2+}) in millimoles per ml.

For a preparation containing 50% w/v of Magnesium Sulphate Heptahydrate ($MgSO_4,7H_2O$) the concentration of magnesium ions is approximately 2 millimoles per millilitre (2 mmol Mg^{2+}/ml).

Magnesium Sulphate Mixture

General Notices

Magnesium Sulphate Oral Suspension

DEFINITION

Magnesium Sulphate Mixture is an *oral suspension* containing 40% w/v of Magnesium Sulphate Heptahydrate and 5% w/v of Light Magnesium Carbonate in a suitable vehicle with a peppermint flavour.

Extemporaneous preparation

It is recently prepared according to the following formula.

Magnesium Sulphate Heptahydrate	400 g
Light Magnesium Carbonate	50 g
Concentrated Peppermint Emulsion	25 ml
Double-strength Chloroform Water	300 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium sulphate heptahydrate, $MgSO_4,7H_2O$

36.0 to 44.0% w/v.

Content of magnesium carbonate, calculated as Mg

1.14 to 1.49% w/v.

IDENTIFICATION

A. Filter 10 ml of the mixture and reserve the residue. The filtrate yields reaction A characteristic of *magnesium salts*, Appendix VI. Wash the residue with 10 ml of *water*, dissolve 25 mg in 1 ml of 1M *hydrochloric acid* and adjust the pH to 6 to 7 with 1M *sodium hydroxide*. The resulting solution yields reaction A characteristic of *magnesium salts*, Appendix VI.

B. The residue obtained in test A yields reaction A characteristic of *carbonates*, Appendix VI.

C. The filtrate obtained in test A yields reaction A characteristic of *sulphates*, Appendix VI.

ASSAY

For magnesium sulphate heptahydrate

Boil 0.7 g with 50 ml of a mixture of equal volumes of *ethanol (96%)* and *water*, cool, filter and wash the residue with successive small quantities of the diluted ethanol until the washings are free from sulphate. Reserve the residue for the Assay for magnesium carbonate. To the combined filtrate and washings add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 12.32 mg of $MgSO_4,7H_2O$. Determine the *weight per ml* of the mixture, Appendix V G, and calculate the percentage of $MgSO_4,7H_2O$, weight in volume.

For magnesium carbonate

Dissolve the residue obtained in the Assay for magnesium sulphate in 10 ml of 1M *hydrochloric acid* and wash the filter with successive small quantities of *water* until the washings are free from chloride. To the combined filtrate and washings add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg. Using the *weight per ml*, calculate the percentage of Mg, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'oral suspension'.

Magnesium Sulphate Paste

General Notices

Morison's Paste

DEFINITION

Dried Magnesium Sulphate	A sufficient quantity
Phenol	0.5 g
Glycerol, previously heated at 120°	55 g
for 1 hour and cooled	

Extemporaneous preparation

The following directions apply.

Dry about 70 g of Dried Magnesium Sulphate at 150° for 1.5 hours or at 130° for 4 hours and allow to cool in a desiccator. Mix 45 g of the dried material in a warm mortar with the Phenol dissolved in the Glycerol.

In preparing larger quantities of the paste the period of heating the Dried Magnesium Sulphate should be increased, if necessary, to ensure that the dried material contains not less than 85% of magnesium sulphate, calculated as $MgSO_4$.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of magnesium sulphate, $MgSO_4$

36.0 to 41.0% w/w.

Content of phenol, C_6H_6O

0.45 to 0.55% w/w.

ASSAY

For magnesium sulphate

Dissolve 5 g in sufficient *water* to produce 100 ml. To 10 ml of the resulting solution add 150 ml of *water* and 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS*, using *mordant black 11 solution* as indicator, to a full blue colour. Each ml of 0.05M *disodium edetate VS* is equivalent to 6.018 mg of $MgSO_4$.

For phenol

Dissolve 5 g in 25 ml of *water*, add 25 ml of 0.05M *bromine VS* and 5 ml of *hydrochloric acid*, stopper the flask, allow to stand for 30 minutes with occasional swirling and then allow to stand for a further 15 minutes. Add 5 ml of a 20% w/v solution of *potassium iodide*, shake thoroughly, titrate with 0.1M *sodium thiosulphate VS* until only a faint yellow colour remains, add 0.25 ml of *starch mucilage* and 10 ml of *chloroform* and complete the titration with vigorous shaking. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of bromine required. Each ml of 0.05M *bromine VS* is equivalent to 1.569 mg of C_6H_6O.

LABELLING

The label states that the Paste should be stirred before use.

Magnesium Trisilicate Mixture

General Notices

Magnesium Trisilicate Oral Suspension; Compound Magnesium Trisilicate Mixture

DEFINITION

Magnesium Trisilicate Mixture is an *oral suspension* containing 5% w/v each of Magnesium Trisilicate, Light Magnesium Carbonate and Sodium Bicarbonate in a suitable vehicle with a peppermint flavour.

Extemporaneous preparation

It is recently prepared according to the following formula.

Magnesium Trisilicate	50 g
Light Magnesium Carbonate	50 g
Sodium Bicarbonate	50 g
Concentrated Peppermint Emulsion	25 ml
Double-strength Chloroform Water	500 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium, Mg

1.70 to 2.20% w/v.

Content of sodium bicarbonate, $NaHCO_3$

4.60 to 5.40% w/v.

IDENTIFICATION

A. Filter 50 ml of the mixture using a Buchner funnel, reserving the filtrate, wash the filter with 50 ml of *water* and dry the residue at 100° to 105° for 1 hour. The filtrate yields reaction B characteristic of *sodium salts* and reaction A characteristic of *bicarbonates*, Appendix VI.

B. Shake 0.5 g of the residue obtained in test A with 5 ml of 1M *hydrochloric acid* for 5 minutes, add 5 ml of 1M *sodium hydroxide* and centrifuge. The supernatant liquid yields reaction B characteristic of *magnesium salts*, Appendix VI.

C. The residue obtained in test A yields reaction A characteristic of *carbonates*, Appendix VI.

D. 30 mg of the residue obtained in test A yields the reaction characteristic of *silicates*, Appendix VI.

Neutralising capacity

To 5 g add 100 ml of *water* and heat to 37°c. Add 100 ml of 0.1M *hydrochloric acid VS* previously heated to 37°c and stir continuously using a suitable paddle stirrer rotating at a rate of about 200 revolutions per minute, maintaining the temperature at 37°c. The pH of the suspension at 37°c after 10 and 20 minutes is not less than 2.0 and not less than 2.4 respectively and at no time is it more than 4.0. Add 10 ml of 0.5M *hydrochloric acid VS* previously heated to 37°c, stir continuously for 1 hour, maintaining the temperature at 37°c, and titrate with 0.1M *sodium hydroxide VS* to pH 3.5. Subtract the volume of 0.1M *sodium hydroxide VS* from 150 to obtain the number of ml of 0.1M *hydrochloric acid VS* required for the neutralisation. Determine the *weight per ml* of the mixture, Appendix V G, and hence the volume of 0.1M *hydrochloric acid VS* required to neutralise 5 ml of the mixture. Not less than 100 ml of 0.1M *hydrochloric acid VS* is required.

ASSAY

For magnesium

To 5 g add 7 ml of *hydrochloric acid* and 7 ml of *water*, stir and heat on a water bath for 15 minutes. Add 30 ml of hot *water*, mix, filter while still hot and wash the filter with hot *water*. Cool the combined filtrate and washings and dilute to 100 ml with *water*. To 25 ml of the resulting solution add 150 ml of *water* and 5 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 1.215 mg of Mg. Using the weight per ml of the mixture calculate the percentage of Mg, weight in volume.

For sodium

bicarbonate Boil 20 g with 100 ml of *water* for 5 minutes, filter, boil the residue with 100 ml of *water* for 5 minutes and filter. Combine the filtrates, cool and titrate with 0.5M *hydrochloric acid VS* using *methyl orange–xylene cyanol FF solution* as indicator. Add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. After subtracting one-fifth of the volume of 0.05M *disodium edetate VS*, each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$. Using the weight per ml of the mixture calculate the percentage of $NaHCO_3$, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'oral suspension'.

Mannitol Intravenous Infusion

General Notices

DEFINITION

Mannitol Intravenous Infusion is a sterile solution of Mannitol in Water for Injections.
The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of mannitol, $C_6H_{12}O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Evaporate to dryness on a water bath a volume containing 0.2 g of Mannitol. The *melting point* of the residue, Appendix V A, is about 167°.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *water,* 70 volumes of *propan-1-ol* and 20 volumes of *ethyl acetate* as the mobile phase but allowing the solvent front to ascend 17 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute a volume of the intravenous infusion with *water* to contain 0.25% w/v of Mannitol. Solution (2) contains 0.25% w/v of *mannitol EPCRS* in *water*. After removal of the plate, allow it to dry in air and spray with a 0.2% w/v solution of *sodium periodate*. Allow the plate to dry in air for 15 minutes and spray with a 2% w/v solution of *4,4´-methylenebis-*N,N*-dimethylaniline* in a mixture of 1 volume of *glacial acetic acid* and 4 volumes of *acetone*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

C. Add 0.3 ml of a solution containing 10% w/v of Mannitol to 3 ml of a cooled mixture prepared by adding 6 ml of *sulphuric acid* to 3 ml of a freshly prepared 10% w/v solution of *catechol* while cooling in ice. Heat gently over a naked flame for about 30 seconds. A pink colour develops.

TESTS

Acidity or alkalinity

pH, 4.5 to 7.0, Appendix V L, when determined on solutions diluted, if necessary, with *water for injections* to contain 10% w/v of Mannitol and to which 0.30 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

Reducing sugars

Add 20 ml of *cupri-citric solution* and a few glass beads to a volume of the intravenous infusion containing 5 g of Mannitol. Heat so that boiling begins after 4 minutes and continue boiling for 3 minutes. Cool rapidly and add 100 ml of a 2.4% v/v solution of *glacial acetic acid* and 20 ml of 0.025M *iodine VS*. With continuous shaking add 25 ml of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water* and, when the precipitate has dissolved, titrate the excess of iodine with 0.05M *sodium thiosulphate VS* using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Not less than 12.8 ml of 0.05M *sodium thiosulphate VS* is required.

Bacterial endotoxins

The endotoxin limit concentration is 0.5 IU per ml, Appendix XIV C. Dilute infusions containing more than 10% w/v (100 g/l) of Mannitol with *water BET* to contain 10% w/v.

ASSAY

Dilute a quantity containing 0.4 g of Mannitol to 100 ml with *water*, transfer 10 ml to a stoppered flask, add 20 ml of 0.1M *sodium periodate* and 2 ml of 1M *sulphuric acid* and heat on a water bath for 15 minutes. Cool, add 3 g of *sodium hydrogen carbonate* and 25 ml of 0.1M *sodium arsenite VS*, mix, add 5 ml of a 20% w/v solution of *potassium iodide*, allow to stand for 15 minutes and titrate with 0.05M *iodine VS* until the first trace of yellow colour appears. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of iodine required. Each ml of 0.05M *iodine VS* is equivalent to 1.822 mg of $C_6H_{12}O_6$.

STORAGE

Mannitol Intravenous Infusion should be stored at a temperature of 20° to 30°. Exposure to lower temperatures may cause the deposition of crystals, which should be dissolved by warming before use.

LABELLING

The strength is stated as the percentage w/v of Mannitol.

Mebeverine Tablets

General Notices

Action and use

Smooth muscle relaxant; antispasmodic.

DEFINITION

Mebeverine Tablets contain Mebeverine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mebeverine hydrochloride, $C_{25}H_{35}NO_5$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Suspend a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride in 20 ml of *water*, add 5 ml of 5M *sodium hydroxide* and extract with two 25 ml quantities of *chloroform*. Dry the combined extracts over *anhydrous sodium sulphate* and evaporate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mebeverine *(RS 208)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum at 263 nm and a less well-defined maximum at 292 nm.

TEST

Related substances

Comply with the requirement stated under Mebeverine Hydrochloride using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride with 10 ml of *acetone* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *acetone*. Solution (3) contains 0.01% w/v of *veratric acid* in *acetone*.

ASSAY

Weigh and powder 20 tablets. Heat a quantity of the powdered tablets containing 0.5 g of Mebeverine Hydrochloride with 100 ml of 0.1M *hydrochloric acid* for 10 minutes on a water bath, shaking occasionally. Cool, add sufficient 0.1M *hydrochloric acid* to produce 250 ml and filter. To 10 ml of the filtrate add sufficient 0.1M *hydrochloric acid* to produce 100 ml and dilute 15 ml of the resulting solution to 100 ml with the same solvent. Measure the *absorbance* of the solution at the maximum at 263 nm, Appendix II B. Calculate the content of $C_{25}H_{35}NO_5$, HCl taking 263 as the value of A(1%, 1 cm) at the maximum at 263 nm.

STORAGE

Mebeverine Tablets should be kept in an airtight container, protected from light and stored at a temperature not exceeding 30°.

Medroxyprogesterone Injection

General Notices

Action and Use

Progestogen.

DEFINITION

Medroxyprogesterone Injection is a sterile suspension of Medroxyprogesterone Acetate in a suitable vehicle

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Dissolve a volume of the injection containing 50 mg of Medroxyprogesterone Acetate in 8 ml of *petroleum spirit* (*boiling range, 40° to 60°*) and extract with three 8-ml quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *petroleum spirit* (*boiling range, 40° to 60°*), dilute with *water* until the solution becomes turbid, allow to stand in ice for 2 hours and filter. Wash the precipitate with *water* and dry at 105°. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of medroxyprogesterone acetate (*RS 421*).

TESTS

Impurity F (6a-methyl-3,20-dioxo-5b-pregnan-17-yl acetate)

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) The injection, diluted with *dichloromethane* if necessary, to contain 2.0% w/v of Medroxyprogesterone Acetate.

(2) 2.0% w/v of *medroxyprogesterone acetate for performance test EPCRS* (containing 0.5% of impurity F) in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable)

(b) 10 volumes of *tetrahydrofuran*, 45 volumes of *1,1-dimethylethyl methyl ether* and 45 volumes of *hexane* as the mobile phase.

(c) Apply to the plate 10 µl of each of solutions (1) and (2).

(d) After removal of the plate, allow it to dry in air and carry out a second development in the same direction using a freshly prepared mobile phase.

(e) Dry the plate at 100° to 105° and allow to cool, spray with a 200 g/l solution of *toluenesulphonic acid* in *ethanol (96 per cent)*. Heat at 120 °C for 10 min, allow to cool and examine the plate in *ultraviolet light 365 nm*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1) any blue fluorescent spot with an Rf value higher than the principal spot is not more intense than the corresponding blue fluorescent spot due to impurity F in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a quantity of the injection containing 40 mg of Medroxyprogesterone Acetate with sufficient of the mobile phase to produce 100 ml.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.002% w/v of *medroxyprogesterone acetate BPCRS* and 0.005% w/v of *megestrol acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Prodigy ODS3 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 ml per minute.

(d) Maintain the temperature of the column at 40°.

(e) Detection wavelength of 241 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 100 volumes of *tetrahydrofuran,* 350 volumes of *acetonitrile,* 500 volumes of *water* and allowed to equilibrate and the volume adjusted to 1000 volumes with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the two principal peaks is at least 3.3;

the *symmetry factor* of the peak due to medroxyprogesterone acetate is not more than 1.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

disregard any peaks with an area not greater than 0.05 times the area of the principal peak in solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) The injection, diluted if necessary with the mobile phase, to contain 0.004% w/v of Medroxyprogesterone Acetate.

(2) 0.004% w/v of *medroxyprogesterone acetate BPCRS* in the mobile phase.

(3) 0.002% w/v of *medroxyprogesterone acetate BPCRS* and 0.005% w/v of *megestrol acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the two principal peaks is at least 3.3;

the *symmetry factor* of the peak due to medroxyprogesterone acetate is not more than 1.3.

DETERMINATION OF CONTENT

Calculate the content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$, in the injection using the declared content of $C_{24}H_{34}O_4$ in *medroxyprogesterone acetate BPCRS*.

STORAGE

Medroxyprogesterone Injection should be protected from light. On standing, solid matter may separate; it should be redissolved by heating before use.

IMPURITIES

A. 6-hydroxy-6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-hydroxymedroxyprogesterone acetate),

B. 17-hydroxy-6-methylpregn-4-ene-3,20-dione (medroxyprogesterone),

C. 6,17a-dimethyl-3,17-dioxo-*D*-homoandrost-4-en-17a-yl acetate,

D. 6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-epimedroxyprogesterone acetate),

E. 6-methylidene-3,20-dioxopregn-4-en-17-yl acetate (6-methylenehydroxyprogesterone acetate),

F. 6-methyl-3,20-dioxo-5-pregnan-17-yl acetate (4,5-dihydromedroxyprogesterone acetate),

G. 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate).

Medroxyprogesterone Tablets

General Notices

Action and Use

Progestogen.

DEFINITION

Medroxyprogesterone Tablets contain Medroxyprogesterone Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Disperse a quantity of the powdered tablets containing 50 mg of Medroxyprogesterone Acetate in 8 ml of *petroleum spirit* (*boiling range, 40° to 60°*) and extract with three 8-ml quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *petroleum spirit* (*boiling range, 40° to 60°*), dilute with *water* until the solution becomes turbid, allow to stand in ice for 2 hours and filter. Wash the precipitate with water and dry at 105°. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of medroxyprogesterone acetate *(RS 421)*.

TESTS

Dissolution

Comply with the dissolution test for tablets and capsules, Appendix XII D, using apparatus 2. Use as the medium 900 ml of 0.5% w/v *sodium lauryl sulphate* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of the medium and filter.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the filtrate add sufficient of a solution of 0.5% w/v *sodium lauryl sulphate* to produce a solution expected to contain 0.0028% w/v of Medroxyprogesterone Acetate.

(2) 0.0028% w/v of *medroxyprogesterone acetate BPCRS* in a solution of 0.5% w/v *sodium lauryl sulphate*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (8 cm × 4 mm) packed with *octylsilyl silica gel for chromatography R1* (10 µm) (Zorbax C8 is suitable)

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 254 nm.

(f) Injection volume of 20µl for each solution.

MOBILE PHASE

40 volumes of *water* and 60 volumes of *acetonitrile*.

DETERMINATION OF CONTENT

Calculate the content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$, in the medium using the declared content of $C_{24}H_{34}O_4$ in *medroxyprogesterone acetate BPCRS*.

Impurity F (6a-methyl-3,20-dioxo-5b-pregnan-17-yl acetate)

Carry out the method for *thin-layer chromatography*, Appendix III A using the following solutions.

(1) Shake a quantity of powdered tablets containing 0.2 g of Medroxyprogesterone Acetate with *dichloromethane*, add sufficient *dichloromethane* to produce 10 ml, centrifuge and use the supernatant liquid.

(2) 2.0% w/v of *medroxyprogesterone acetate for performance test EPCRS* (containing 0.5% of impurity F) in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as coating *silica gel* (Merck silica gel 60 plates are suitable)

(b) 10 volumes of *tetrahydrofuran*, 45 volumes of *1,1-dimethylethyl methyl ether* and 45 volumes of *hexane* as the mobile phase.

(c) Apply to the plate 10 µl of each of solutions (1) and (2).

(d) After removal of the plate, allow the plate to dry in air and carry out a second development in the same direction using a freshly prepared mobile phase.

(e) Dry the plate at 100° to 105° and allow to cool, spray with a 200 g/l solution of *toluenesulphonic acid* in *ethanol (96 per cent)*. Heat at 120 °C for 10 min, allow to cool and examine the plate in *ultraviolet light 365 nm*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1) any blue fluorescent spot with an Rf value higher than that of the principal spot is not more intense than the corresponding blue fluorescent spot due to impurity F in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 40 mg of Medroxyprogesterone Acetate with 50 ml of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 100 ml, mix, filter and use the filtrate.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.002% w/v of *medroxyprogesterone acetate BPCRS* and 0.005% w/v of *megestrol acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Prodigy ODS3 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 ml per minute.

(d) Maintain the temperature of the column at 40°.

(e) Detection wavelength of 241 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 100 volumes of *tetrahydrofuran,* 350 volumes of *acetonitrile,* 500 volumes of *water* and allowed to equilibrate and the volume adjusted to 1000 volumes with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the two principal peaks is at least 3.3;

the *symmetry factor* of the peak due to medroxyprogesterone acetate is not more than 1.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

disregard any peaks with an area not greater than 0.05 times the area of the principal peak in solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 40 mg of Medroxyprogesterone Acetate with 50 ml of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 100 ml, mix and filter. To 5ml of the filtrate add sufficient of the mobile phase to produce 50 ml.

(2) 0.004% w/v of *medroxyprogesterone acetate BPCRS* in the mobile phase.

(3) 0.002% w/v of *medroxyprogesterone acetate BPCRS* and 0.005% w/v of *megestrol acetate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the two principal peaks is at least 3.3;

the *symmetry factor* of the peak due to medroxyprogesterone acetate is not more than 1.3.

DETERMINATION OF CONTENT

Calculate the content of medroxyprogesterone acetate, $C_{24}H_{34}O_4$, in the tablets using the declared content of $C_{24}H_{34}O_4$ in *medroxyprogesterone acetate BPCRS*.

IMPURITIES

A. 6-hydroxy-6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-hydroxymedroxyprogesterone acetate),

B. 17-hydroxy-6-methylpregn-4-ene-3,20-dione (medroxyprogesterone),

C. 6,17a-dimethyl-3,17-dioxo-*D*-homoandrost-4-en-17a-yl acetate,

D. 6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-epimedroxyprogesterone acetate),

E. 6-methylidene-3,20-dioxopregn-4-en-17-yl acetate (6-methylenehydroxyprogesterone acetate),

F. 6-methyl-3,20-dioxo-5-pregnan-17-yl acetate (4,5-dihydromedroxyprogesterone acetate),

G. 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate).

Mefenamic Acid Capsules

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Mefenamic Acid Capsules contain Mefenamic Acid.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of mefenamic acid, $C_{15}H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with two 30-ml quantities of *ether*. Wash the combined extracts with *water*, evaporate to dryness on a water bath and dry the residue at 105°. Dissolve a sufficient quantity in the minimum volume of *absolute ethanol* and evaporate to dryness on a water bath. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mefenamic acid *(RS 210)*.

TESTS

Disintegration

Maximum time, 15 minutes, Appendix XII A1.

2,3-Dimethylaniline

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel G plate* and a mixture of 1 volume of 18M *ammonia*, 25 volumes of *1,4-dioxan* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with a mixture of 7.5 ml of *dichloromethane* and 2.5 ml of *methanol*, allow the insoluble matter to settle and use the supernatant liquid. Solution (2) contains 0.00025% w/v of *2,3-dimethylaniline* in a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol*. After removal of the plate, dry it in a current of warm air and visualise by *Method I*. Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (100 ppm).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel GF_{254} plate* and a mixture of 1 volume of *glacial acetic acid*, 25 volumes of *1,4-dioxan* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) use the supernatant liquid obtained in the test for 2,3-Dimethylaniline. For solution (2) dilute 1 volume of solution (1) to 500 volumes with a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol*. After removal of the plate, allow it to dry in air, expose to iodine vapour for 5 minutes and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 0.5 g of Mefenamic Acid in 100 ml of warm *absolute ethanol* previously neutralised to *phenol red solution* and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 24.13 mg of $C_{15}H_{15}NO_2$.

Mefenamic Acid Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Mefenamic Acid Tablets contain Mefenamic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mefenamic acid, $C_{15}H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.25 g of Mefenamic Acid with two 30-ml quantities of *ether*. Wash the combined extracts with *water*, evaporate to dryness on a water bath and dry the residue at 105°. Dissolve a sufficient quantity in the minimum volume of *absolute ethanol* and evaporate to dryness on a water bath. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mefenamic acid *(RS 210)*.

TESTS

2,3-Dimethylaniline

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel G plate* and a mixture of 1 volume of 18M *ammonia*, 25 volumes of *1,4-dioxan* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 40 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Mefenamic Acid with a mixture of 7.5 ml of *dichloromethane* and 2.5 ml of *methanol* for 10 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.00025% w/v of *2,3-dimethyl-aniline* in a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol*. After removal of the plate, dry it in a current of warm air and visualise by *Method I*. Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (100 ppm).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* GF_{254} *plate* and a mixture of 1 volume of *glacial acetic acid*, 25 volumes of *1,4-dioxan* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) use the supernatant liquid obtained in the test for 2,3-Dimethylaniline. For solution (2) dilute 1 volume of solution (1) to 500 volumes with a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol*. After removal of the plate, allow it to dry in air, expose to iodine vapour for 5 minutes and examine under *ultraviolet light (254* *nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot with an Rf value of 0.04 or less.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets containing 0.5 g of Mefenamic Acid in about 80 ml of warm *absolute ethanol* previously neutralised to *phenol red solution* alternating between heating and ultrasound to aid dissolution. Cool, add sufficient of the neutralised absolute ethanol to produce 100 ml, mix well and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 24.13 mg of $C_{15}H_{15}NO_2$.

Megestrol Tablets

General Notices

Action and use

Progestogen.

DEFINITION

Megestrol Tablets contain Megestrol Acetate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of megestrol acetate, $C_{24}H_{32}O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 40 mg of Megestrol Acetate with 250 ml of *chloroform* for 30 minutes, filter into a separating funnel, wash the filtrate with 20 ml of *water* and evaporate the chloroform. The residue, after drying at 60° at a pressure not exceeding 0.7 kPa for 2 hours, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of megestrol acetate *(RS 211)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase D*.

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A1.

Related foreign steroids

Carry out the method for *thin-layer chromatography,* Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 0.5 volume of *water,* 8 volumes of *methanol* and 92 volumes of *1,2-dichloroethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Megestrol Acetate with 10 ml of a mixture of 1 volume of *methanol* and 9 volumes of *chloroform* for 10 minutes, centrifuge and use the clear supernatant liquid. Solution (2) contains 0.005% w/v of *megestrol BPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*. After removal of the plate, allow it to dry in air, spray with *ethanolic sulphuric acid (20%),* heat at 110° for 10 minutes and examine under *ultraviolet light (365 nm).* Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense that the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Prepare a 0.016% w/v solution of *propyl 4-hydroxybenzoate* (internal standard) in *acetonitrile* (solution A) and carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix 4 ml of a 0.02% w/v solution of *megestrol acetate BPCRS* in *acetonitrile* with 10 ml of solution A and dilute to 50 ml with a 40% v/v solution of *acetonitrile*. For solution (2) shake a quantity of the powdered tablets containing 0.08 g of Megestrol Acetate with 10 ml of *water* for 10 minutes, add 75 ml of *acetonitrile*, shake for 30 minutes, dilute to 100 ml with *acetonitrile*, centrifuge and dilute 10 ml of the clear supernatant liquid to 50 ml with a 40% v/v solution of *acetonitrile*; to 5 ml of this solution add 10 ml of *acetonitrile* and dilute to 50 ml with a 40% v/v solution of *acetonitrile*. Prepare solution (3) in the same manner as solution (2) but adding 10 ml of solution A in place of the 10 ml of acetonitrile.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 32 volumes of *water* and 68 volumes of *acetonitrile* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 280 nm.

The assay is not valid unless in the chromatogram obtained with solution (1) the *resolution factor* between the peaks due to propyl 4-hydroxybenzoate and megestrol acetate is at least 8.

Calculate the content of $C_{24}H_{32}O_4$ using the declared content of $C_{24}H_{32}O_4$ in *megestrol acetate BPCRS*.

Meglumine Amidotrizoate Injection

General Notices

Action and use

Organic base used in the preparation of organic acids.

DEFINITION

Meglumine Amidotrizoate Injection is a sterile solution of the Meglumine salt of Amidotrizoic Acid Dihydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of meglumine amidotrizoate, $C_{11}H_9I_3N_2O_4,C_7H_{17}NO_5$

97.0 to 103.0% of the stated amount.

CHARACTERISTICS

A colourless to pale yellow, slightly viscous liquid.

IDENTIFICATION

A. Evaporate 2 ml of the injection to dryness and heat 50 mg of the residue. Violet vapours of iodine are evolved.

B. To 20 mg of the residue obtained by evaporating the injection to dryness add 5 ml of 1M *sodium hydroxide* and boil gently under a reflux condenser for 10 minutes. Cool, add 5 ml of 2M *hydrochloric acid* and cool in ice for 5 minutes. Add 4 ml of a 1% w/v solution of *sodium nitrite*, cool in ice for 5 minutes, add 0.3 g of *sulphamic acid*, swirl gently until effervescence ceases and add 2 ml of a 0.4% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride*. An orange-red colour is produced.

C. To a quantity containing 1 g of meglumine amidotrizoate add 50 ml of *water* and a slight excess of 2M *hydrochloric acid* and filter. Evaporate the filtrate to dryness on a water bath, dissolve the residue in a small quantity of boiling *ethanol (90%)*, filter and cool the filtrate in ice. The *melting point* of the dried precipitate is about 146°, Appendix V A.

TESTS

Acidity or alkalinity

pH, 6.0 to 7.0, Appendix V L.

Meglumine

22.9 to 25.3% of the stated content of meglumine amidotrizoate when determined by the following method. Measure the *optical rotation* of the injection, Appendix V F, using a 2-dm tube. Calculate the percentage content of meglumine using the expression 2.104*R* where *R* is the numerical value of the rotation.

Free amine

To a quantity containing 1 g of meglumine amidotrizoate in a 50 ml glass-stoppered graduated flask add sufficient *water* to produce 5 ml, 10 ml of 0.1M *sodium hydroxide* and 25 ml of *dimethyl sulphoxide*. Stopper the flask, mix the contents by gentle swirling and cool in ice, protected from light. After 5 minutes add slowly 2 ml of *hydrochloric acid*, mix and allow to stand for 5 minutes. Add 2 ml of a 2% w/v solution of *sodium nitrite*, mix and allow to stand for 5 minutes. Add 1 ml of an 8% w/v solution of *sulphamic acid*, mix and allow to stand for 5 minutes. Add 2 ml of a 0.1% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride* in a 70% v/v solution of *propane-1,2-diol* and mix. Remove the flask from the ice and allow to stand in water at 22° to 25° for 10 minutes, occasionally shaking gently. Add sufficient *dimethyl sulphoxide* to produce 50 ml and mix. Within 5 minutes of diluting to 50 ml measure the *absorbance* of the resulting solution at the maximum at 470 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 ml of *water* in the same manner. The absorbance is not more than 0.40.

Inorganic iodide

Dilute a quantity containing 0.80 g of meglumine amidotrizoate to 10 ml with *water*, add sufficient 2M *nitric acid* dropwise to ensure complete precipitation of the iodinated acid and add 3 ml in excess. Filter, wash the precipitate with 5 ml of *water*, add to the filtrate 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *chloroform* and shake. Any purple colour in the chloroform layer is not more intense than that obtained by adding 2 ml of *iodide standard solution (20 ppm I)* to a mixture of 3 ml of 2M *nitric acid* and sufficient *water* to equal the volume of the test solution, adding 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *chloroform* and shaking (50 ppm).

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D. Use per kg of the rabbit's weight a quantity containing 2.5 g of meglumine amidotrizoate.

ASSAY

Mix a quantity containing 0.5 g of meglumine amidotrizoate with 12 ml of 5M *sodium hydroxide* and 20 ml of *water*, add 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 ml of *water*, filter through absorbent cotton and wash the flask and filter with two 20 ml quantities of *water*. To the combined filtrate and washings add 80 ml of *hydrochloric acid*, cool and titrate with 0.05M *potassium iodate VS* until the dark brown solution becomes light brown. Add 5 ml of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless. Each ml of 0.05M *potassium iodate VS* is equivalent to 26.97 mg of $C_{11}H_9I_3N_2O_4,C_7H_{17}NO_5$. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage w/v of $C_{11}H_9I_3N_2O_4,C_7H_{17}NO_5$.

STORAGE

Meglumine Amidotrizoate Injection should be protected from light.

LABELLING

The strength is stated as the percentage w/v of meglumine amidotrizoate.

Meglumine Iodipamide Injection

General Notices

Action and use

Organic base used in the preparation of organic acids.

DEFINITION

Meglumine Iodipamide Injection is a sterile solution of the Meglumine salt of 3,3′-adipoyldiamino-bis(2,4,6-tri-iodobenzoic acid) (2:1) in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of meglumine iodipamide, $C_{20}H_{14}I_6N_2O_6,2C_7H_{17}NO_5$

97.0 to 103.0% of the stated amount.

CHARACTERISTICS

A colourless to pale yellow solution.

IDENTIFICATION

A. To a volume of the injection containing 0.6 g of meglumine iodipamide add 1 ml of 1M *hydrochloric acid*, stir until the precipitate becomes crystalline, add 5 ml of 1M *hydrochloric acid*, stir, filter and reserve the filtrate for test C. Wash the residue with 1M *hydrochloric acid* and then with *water* and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of iodipamide *(RS 191)*.

B. Heat 50 mg of the residue obtained in test A. Violet vapours of iodine are evolved.

C. Evaporate the filtrate reserved in test A to dryness on a water bath, dissolve the residue in a small quantity of boiling *ethanol (90%)*, filter and cool the filtrate in ice. The *melting point* of the dried precipitate is about 146°, Appendix V A.

TESTS

Acidity or alkalinity

pH, 6.0 to 7.1, Appendix V L.

Meglumine

11.8 to 13.7% of the stated content of meglumine iodipamide when determined by the following method. Measure the *optical rotation* of the injection, Appendix V G, using a 2-dm tube. Calculate the percentage content of meglumine using the expression 2.104*R* where *R* is the numerical value of the rotation.

Free amine

To a quantity containing 0.25 g of meglumine iodipamide add 25 ml of *water*, 8 ml of 1M *hydrochloric acid* and 4 ml of a 1.0% w/v solution of *sodium nitrite* and shake for 2 minutes. Add 6 ml of a 2% w/v solution of *ammonium sulphamate* and swirl gently until effervescence ceases. Add 0.4 ml of a 10% w/v solution of *1-naphthol* in *ethanol (96%)*, shake and add 3 ml of 5M *sodium hydroxide*. Dilute the solution to 50 ml with *water* and measure the *absorbance* at the maximum at 485 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but omitting the injection. The absorbance is not more than 0.17.

Inorganic iodide

Dilute a quantity containing 0.80 g of meglumine iodipamide to 10 ml with *water*, add sufficient 2M *nitric acid* dropwise to ensure complete precipitation of the iodinated acid and add 3 ml in excess. Filter, wash the precipitate with 5 ml of *water*, add to the filtrate 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *chloroform* and shake. Any purple colour in the chloroform layer is not more intense than that obtained by adding 2 ml of *iodide standard solution (20 ppm I)* to a mixture of 3 ml of 2M *nitric acid* and sufficient *water* to equal the volume of the test solution, adding 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *chloroform* and shaking (50 ppm).

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D. Use per kg of the rabbit's weight a quantity containing 1.25 g of meglumine iodipamide.

ASSAY

Mix 0.4 g with 12 ml of 5M *sodium hydroxide* and 20 ml of *water*, add 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 ml of *water*, filter through absorbent cotton and wash the flask and filter with two 20 ml quantities of *water*. To the combined filtrate and washings add 80 ml of *hydrochloric acid*, cool and titrate with 0.05M *potassium iodate VS* until the dark brown solution becomes light brown. Add 5 ml of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless. Each ml of 0.05M *potassium iodate VS* is equivalent to 25.50 mg of $C_{20}H_{14}I_6N_2O_6,2C_7H_{17}NO_5$. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage w/v of $C_{20}H_{14}I_6N_2O_6,2C_7H_{17}NO_5$.

LABELLING

The strength is stated as the percentage w/v of meglumine iodipamide.

Meloxicam Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Meloxicam Tablets contain Meloxicam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of meloxicam, $C_{14}H_{13}N_3O_4S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) To a quantity of the powdered tablets containing 50 mg of Meloxicam, add 5 ml of 0.1M *methanolic sodium hydroxide* and 20 ml of *methanol*, stir for 15 minutes and filter.

(2) Dissolve 50 mg of *meloxicam BPCRS* in 5 ml of 0.1M *methanolic sodium hydroxide* and add sufficient *methanol* to produce 25 ml of solution.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel* F_{254} *plate* (Merck HPTLC plates are suitable).

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

1 volume of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 ml of a buffer prepared by dissolving 13.61 g of *potassium dihydrogen orthophosphate* in 800 ml of *water*, adjusting the pH to 7.5 with 0.5M *sodium hydroxide*, adding sufficient *water* to produce 1000 ml, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and filter. Measure the absorbance of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at 362 nm using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a solution prepared by dissolving 30 mg of *meloxicam BPCRS* in 5 ml of *methanol*, adding 1 ml of 0.1M *sodium hydroxide* and adding sufficient dissolution medium to produce 100 ml. Dilute a volume of the resulting solution with sufficient dissolution medium to produce a solution containing 0.00075% w/v of Meloxicam.

DETERMINATION OF CONTENT

Calculate the total content of Meloxicam, $C_{14}H_{13}N_3O_4S_2$, in the medium using the declared content of $C_{14}H_{13}N_3O_4S_2$ in *meloxicam BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Moisten a quantity of powdered tablets containing 30 mg of Meloxicam with 10 ml of 1M *sodium hydroxide*, add 40 ml of *methanol* and mix with the aid of ultrasound for 5 minutes. Add a further 40 ml of *methanol*, mix for 3 hours using a magnetic stirrer and then with the aid of ultrasound for 5 minutes. Cool, add sufficient *methanol* to produce 100 ml and filter.

(2) Dilute 2 volumes of solution (1) to 100 volumes with *methanol*. Dilute 1 volume to 10 volumes with *methanol*.

(3) Dissolve 4.5 mg of *5-methylthiazole-2-ylamine* in 20 ml of 1M *sodium hydroxide* and 20 ml of *methanol*, cool and add sufficient *methanol* to produce 200 ml. Dilute 2 volumes to 100 volumes with *methanol*.

(4) Mix equal volumes of solution (1) and solution (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Kromasil 100 C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 0.8 ml per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

370 volumes of a mixture of 650 volumes of *methanol* and 100 volumes of *propan-2-ol* and 630 volumes of a 0.20% w/v solution of *diammonium hydrogen orthophosphate* adjusted to pH 7.0 with *dilute orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to 5-methylthiazole-2-ylamine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.15%);

— the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

— and the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Moisten a quantity of the powdered tablets containing 30 mg of Meloxicam with 10 ml of 1M *sodium hydroxide*, add 40 ml of *methanol* and mix with the aid of ultrasound for 5 minutes. Add a further 40 ml of *methanol*, mix for 3 hours using a magnetic stirrer and then with the aid of ultrasound for 5 minutes. Cool, add sufficient *methanol* to produce 100 ml and filter.

(2) Dissolve 30 mg of *meloxicam BPCRS* in 10 ml of 1M *sodium hydroxide* and 40 ml of *methanol*, cool and add sufficient *methanol* to produce 100 ml.

(3) Dissolve 4.5 mg of *2-amino-5-methylthiazole* in 20 ml of 1M *sodium hydroxide* and 20 ml of *methanol*, cool and add sufficient *methanol* to produce 200 ml. Dilute 2 volumes to 100 volumes with *methanol*.

(4) Mix equal volumes of solution (1) and solution (3).

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{13}N_3O_4S_2$ using the declared content of $C_{14}H_{13}N_3O_4S_2$ in *meloxicam BPCRS.*

Melphalan Injection

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Melphalan Injection is a sterile solution of melphalan hydrochloride. It is prepared immediately before use by dissolving Melphalan for Injection in a suitable solvent and then diluting with the requisite volume of a suitable diluent in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

TESTS

Acidity or alkalinity

6.0 to 7.0, Appendix V L.

Clarity of solution

Not more opalescent than *reference suspension II*, Appendix IV A.

STORAGE

Melphalan Injection deteriorates on storage and should be used immediately after preparation.

MELPHALAN FOR INJECTION

DEFINITION

Melphalan for Injection is a sterile material consisting of Melphalan with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of melphalan, $C_{13}H_{18}Cl_2N_2O_2$

90.0 to 110.0% of the stated amount of anhydrous melphalan.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in *methanol* exhibits a maximum at 260 nm and a less well-defined maximum at 310 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the principal peak in the chromatogram obtained with solution (1).

C. Dissolve a quantity containing the equivalent of 20 mg of anhydrous melphalan in 50 ml of *methanol* with the aid of gentle heat, add 1 ml of a 5% w/v solution of *4-(4-nitrobenzyl) pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 ml of hot *methanol* and add 0.1 ml of 13.5M *ammonia*. A red colour is produced.

D. Heat a quantity of the powder containing 0.1 g of anhydrous melphalan with 10 ml of 0.1M *sodium hydroxide* for 10 minutes on a water bath. The resulting solution, after acidification with 2M *nitric acid*, yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Ionisable chlorine

Dissolve a quantity containing the equivalent of 0.1 g of anhydrous melphalan in a mixture of 15 ml of *water* and 0.5 ml of *nitric acid*, allow to stand for 2 minutes and titrate with 0.02M *silver nitrate VS* determining the end point potentiometrically. Not more than 1.7 ml is required.

Uniformity of content

Sealed containers containing the equivalent of 100 mg or less of anhydrous melphalan comply with the requirements stated under Parenteral Preparations, Powders for Injections. Use the individual results obtained in the Assay.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.01% w/v of *melphalan BPCRS* in a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid*. For solution (2) dissolve the contents of one container in a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid* and dilute with sufficient of the same solvent mixture to produce a final solution containing the equivalent of 0.01% w/v of anhydrous melphalan.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 200 volumes of a 0.375% w/v solution of *ammonium carbonate*, 180 volumes of *methanol* and 2.7 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the amount of $C_{13}H_{18}Cl_2N_2O_2$ in the sealed container using the declared content of $C_{13}H_{18}Cl_2N_2O_2$ in *melphalan BPCRS*.

Repeat the procedure with a further nine sealed containers and calculate the average content of $C_{13}H_{18}Cl_2N_2O_2$ per container from the 10 individual results thus obtained.

LABELLING

The label of the sealed container states the equivalent amount of anhydrous melphalan contained in it.

Melphalan Tablets

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Melphalan Tablets contain Melphalan. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of melphalan, $C_{13}H_{18}Cl_2N_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 25 mg of Melphalan with 50 ml of hot *methanol*, filter and dilute 5 ml of the filtrate to 500 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 260 nm and a less well-defined maximum at 301 nm.

B. To the remainder of the filtrate obtained in test A add 1 ml of a 5% w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 ml of hot *methanol* and add 0.1 ml of 13.5M *ammonia*. A red colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a suitable volume of a 0.10% w/v solution of *melphalan BPCRS* in a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid* with sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.0002% w/v. For solution (2) dilute the filtered solution, if necessary, with 0.1M *hydrochloric acid* to give a solution expected to contain about 0.0002% w/v of Melphalan.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 2.7 volumes of *glacial acetic acid*, 180 volumes of *methanol* and 200 volumes of a 0.375% w/v solution of *ammonium carbonate* as the mobile phase with a flow rate of 2 ml per minute, (c) a detection wavelength of 254 nm and (d) a 100-µl loop injector.

Calculate the content of melphalan, $C_{13}H_{18}Cl_2N_2O_2$ in the medium using the declared content of $C_{13}H_{18}Cl_2N_2O_2$ in *melphalan BPCRS*.

Uniformity of content

Tablets containing 2 mg or less of Melphalan comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.01% w/v of *melphalan BPCRS* in a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid*. For solution (2) add 20 ml of a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid* to one tablet, mix with the aid of ultrasound for 10 minutes or until the tablet has disintegrated, filter, discarding the first 5 ml of filtrate, and use the filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 200 volumes of a 0.375% w/v solution of *ammonium carbonate*, 180 volumes of *methanol* and 2.7 volumes of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ using the declared content of $C_{13}H_{18}Cl_2N_2O_2$ in *melphalan BPCRS*.

ASSAY

For tablets containing more than 2 mg of Melphalan

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.01% w/v of *melphalan BPCRS* in a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid*. For solution (2) add about 300 ml of a mixture of 4 volumes of *acetonitrile* and 1 volume of 0.1M *hydrochloric acid* to 10 tablets, shake until the tablets have disintegrated (about 30 minutes), mix with the aid of ultrasound for 5 minutes, dilute to 500 ml with the same solvent mixture, filter, discarding the first 20 ml of filtrate, and use the filtrate.

The chromatographic procedure described under Uniformity of content may be used.

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the tablets using the declared content of $C_{13}H_{18}Cl_2N_2O_2$ in *melphalan BPCRS*.

For tablets containing 2 mg or less of Melphalan

Use the average of the 10 individual results determined in the test for Uniformity of content.

Menadiol Phosphate Injection

General Notices

Action and use

Vitamin K analogue.

DEFINITION

Menadiol Phosphate Injection is a sterile solution of Menadiol Sodium Phosphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of menadiol phosphate, $C_{11}H_{12}O_8P_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume of the injection containing the equivalent of 0.2 g of menadiol phosphate to 20 ml with *water* and add 20 ml of 1M *sulphuric acid*, 20 ml of 0.1M *cerium*(IV) *sulphate* and 2 ml of *hydrogen peroxide solution (20 vol)*. Extract with two 20 ml quantities of *chloroform*, evaporate the combined chloroform extracts to dryness on a water bath and dry the residue at 40° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of menadione *(RS 214)*.

B. To 50 mg of the residue obtained in test A add 5 ml of *water* followed by 75 mg of *sodium metabisulphite* and heat in a water bath, shaking vigorously, until an almost colourless solution is obtained. Dilute to 50 ml with *water*. To 2 ml of the resulting solution add 2 ml of a mixture of equal volumes of *ethanol (96%)* and 13.5M *ammonia*, shake and add 0.15 ml of *ethyl cyanoacetate*. A deep purplish-blue colour is produced which changes to green on the addition of 1 ml of 10M *sodium hydroxide*.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.0, Appendix V L.

Related substances

A. Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 50 volumes of *propan-1-ol*, 50 volumes of a 2% w/v solution of *ammonium chloride*, 5 volumes of *butan-1-ol* and 1.5 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) evaporate a volume of the injection containing the equivalent of 0.5 g of menadiol phosphate to dryness and dissolve the residue in 10 ml of *methanol (50%)*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol (50%)*. Solutions (3) and (4) contain 0.0025% w/v of *methyl 4-hydroxybenzoate* and 0.0025% w/v *of propyl 4-hydroxybenzoate* respectively in *methanol (50%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Disregard any spots corresponding to those in the chromatograms obtained with solutions (3) and (4).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 80 volumes of *cyclohexane*, 20 volumes of *ether* and 1 volume of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. Solution (1) is the injection diluted, if necessary, with *methanol* to contain the equivalent of 1.0% w/v of menadiol phosphate. Solution (2) contains 0.0050% w/v of *2-methyl-1,4-naphthoquinone* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to 2-methyl-1,4-naphthoquinone (menadione) in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Dilute, if necessary, a volume of the injection containing the equivalent of 50 mg of menadiol phosphate to 50 ml with *water*. Dilute 5 ml of the solution to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{11}H_{12}O_8P_2$ taking the value of A(1%, 1 cm), for a solution of $C_{11}H_8Na_4O_8P_2$ in 0.1M *hydrochloric acid*, to be 138 at the maximum at 290 nm. Each mg of $C_{11}H_8Na_4O_8P_2$ (menadiol sodium phosphate) is equivalent to 0.792 mg of $C_{11}H_{12}O_8P_2$ (menadiol phosphate).

LABELLING

The strength is stated in terms of the equivalent amount of menadiol phosphate in a suitable dose-volume.

Menadiol Phosphate Tablets

General Notices

Action and use

Vitamin K analogue.

DEFINITION

Menadiol Phosphate Tablets contain Menadiol Sodium Phosphate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of menadiol phosphate, $C_{11}H_{12}O_8P_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 0.15 g of menadiol phosphate with 15 ml of *water*, centrifuge and filter the supernatant liquid. To 10 ml of the filtrate add 10 ml of 1M *sulphuric acid*, 10 ml of 0.1M *cerium(IV) sulphate* and 1 ml of *hydrogen peroxide solution (20 vol)* and extract with two 10 ml quantities of *chloroform*. Evaporate the combined chloroform extracts to dryness in a water bath and dry the residue at 40° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of menadione *(RS 214)*.

B. To 50 mg of the residue obtained in test A add 5 ml of *water* followed by 75 mg of *sodium metabisulphite*, heat in a water bath, shaking vigorously, until an almost colourless solution is obtained and dilute to 50 ml with *water*. To 2 ml of the resulting solution add 2 ml of a mixture of equal volumes of 13.5M *ammonia* and *ethanol (96%)*, shake and add 0.15 ml of *ethyl cyanoacetate*. A deep purplish-blue colour is produced which changes to green on the addition of 1 ml of 10M *sodium hydroxide*.

TESTS

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 50 volumes of *propan-1-ol*, 50 volumes of a 2% w/v solution of *ammonium chloride*, 5 volumes of *butan-1-ol* and 1.5 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.25 g of menadiol phosphate with 10 ml of *methanol (50%)* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol (50%)*. Solution (3) contains 0.0080% w/v of *2-methyl-1,4-naphthoquinone* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Examine the plate under *ultraviolet light (365 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 10 mg of menadiol phosphate with 100 ml of 0.1M *hydrochloric acid* for 30 minutes. Dilute to 250 ml with 0.1M *hydrochloric acid*, filter and measure the *absorbance* of the filtrate at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{11}H_{12}O_8P_2$ taking the value of A (1%, 1 cm), for a solution of $C_{11}H_8Na_4O_8P_2$ in 0.1M *hydrochloric acid*, to be 138 at the maximum at 290 nm. Each mg of $C_{11}H_8Na_4O_8P_2$ (menadiol sodium phosphate) is equivalent to 0.792 mg of $C_{11}H_{12}O_8P_2$ (menadiol phosphate).

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of menadiol phosphate.

Menotrophin Injection

General Notices

Action and use

1:1 ratio of follicle stimulating hormone and luteinizing hormone.

DEFINITION

Menotrophin Injection is a sterile solution of Menotrophin in Water for Injections. It is prepared by dissolving Menotrophin for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Menotrophin Injection should be used immediately after preparation.

MENOTROPHIN FOR INJECTION

DEFINITION

Menotrophin for Injection is a sterile material consisting of Menotrophin with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

Cause enlargement of the ovaries of immature female rats and increase the weight of the seminal vesicles and prostate gland of immature male rats when administered as directed in the Assay.

TESTS

Acidity or alkalinity

Dissolve the contents of the sealed container in 3 ml of *water* (solution A). The pH is 6.0 to 8.0, Appendix V L.

Clarity and colour of solution

Solution A is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Water

Not more than 5% w/w when determined by *gas chromatography*, Appendix III B, using throughout dry glassware which may be siliconised and the following solutions. For solution (1) dilute 15 µl of *anhydrous methanol* (internal standard) with sufficient *propan-2-ol R1* to produce 100 ml. For solution (2) dissolve 4 mg of the contents of the sealed container in 0.5

ml of *propan-2-ol R1*. For solution (3) dissolve 4 mg of the contents of the sealed container in 0.5 ml of solution (1). For solution (4) add 10 µl of *water* to 50 ml of solution (1).

The chromatographic procedure may be carried out using a stainless steel column (1 m × 2 mm) packed with porous polymer beads (60 to 80 mesh) (Chromosorb 102 is suitable), maintained at 120° with a thermal conductivity detector maintained at 150° and using *helium* as the carrier gas.

From the chromatograms obtained and taking into account any water detectable in solution (1), calculate the percentage content of water taking 0.9972 g as its weight per ml at 20°.

Pyrogens

Comply with the *test for pyrogens*, Appendix XIV D. Use per kg of the rabbit's weight 1 ml of a solution in *sodium chloride injection* containing 5 IU of follicle stimulating hormone activity per ml.

ASSAY

Carry out the *biological assay of menotrophin* described under Menotrophin. The estimated potency for each component is not less than 80% and not more than 125% of the stated potency. The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE

The sealed container should be protected from light. The contents may be expected to retain their potency for not less than 3 years.

LABELLING

The label of the sealed container states the number of IU (Units) of follicle-stimulating hormone activity, the number of IU (Units) of luteinising hormone activity and, where applicable, the number of IU (Units) of chorionic gonadotrophin activity contained in it.

Menthol and Benzoin Inhalation

General Notices

DEFINITION

Menthol and Benzoin Inhalation is an *inhalation vapour, solution.*

Racementhol or Levomenthol	20 g
Benzoin Inhalation	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Racementhol or the Levomenthol in a portion of the Benzoin Inhalation, add sufficient Benzoin Inhalation to produce 1000 ml and mix.
The inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of total balsamic acids

Not less than 2.8% w/v, calculated as cinnamic acid, $C_9H_8O_2$.

Total solids

9.0 to 12.0% w/v when determined by drying at 105° for 4 hours, Appendix XI A. Use 2 ml.

ASSAY

Boil 10 ml with 25 ml of *ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Evaporate the ethanol, disperse the residue in 50 ml of hot *water*, cool, add 80 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and allow to stand for 10 minutes. Filter, wash the residue on the filter with 20 ml of *water*, acidify the combined filtrate and washings with *hydrochloric acid* and extract with four 40 ml quantities of *ether*. Discard the aqueous solution, combine the ether extracts and extract with successive quantities of 20, 20, 10, 10 and 10 ml of *sodium hydrogen carbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, carefully acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each extract through *anhydrous sodium sulphate* supported on absorbent cotton. Distil the chloroform from the combined filtrates until 10 ml remains and remove the remainder in a current of air. Dissolve the residue, with the aid of gentle heat, in 10 ml of *ethanol (96%)*, previously neutralised to *phenol red solution*, cool and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 14.82 mg of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

LABELLING

The label indicates the pharmaceutical form as 'inhalation vapour'.

Meptazinol Injection

General Notices

Action and use

Opioid receptor partial agonist; analgesic.

DEFINITION

Meptazinol Injection is a sterile solution of Meptazinol Hydrochloride in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of meptazinol, $C_{15}H_{23}NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 330 nm of the solution obtained in the Assay exhibits a maximum at 273 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 18M *ammonia*, 20 volumes of *absolute ethanol* and 79 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (1) dilute the injection, if necessary, with *ethanol (96%)* to contain the equivalent of 0.05% w/v of meptazinol. Solution (2) contains 0.05% w/v of *meptazinol hydrochloride BPCRS* in *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds in intensity, position and size to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 6.0, Appendix V L.

Colour of solution

The injection is not more intensely coloured than *reference solution* Y_5, Appendix IV B, Method II.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of 18M *ammonia*, 30 volumes of *chloroform* and 70 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a volume of the injection with *water* to produce a solution containing the equivalent of 1.0% w/v of meptazinol. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *water*. For solution (3) dilute a volume of solution (2) with an equal volume of *water*. After removal of the plate, allow it to dry in air, examine under *ultraviolet light (254 nm)*, expose to iodine vapour for 2 hours and examine again. By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Dilute a suitable volume of the injection with sufficient *water* to produce a solution containing the equivalent of 0.01% w/v of meptazinol and measure the *absorbance* at the maximum at 273 nm, Appendix II B. Calculate the content of $C_{15}H_{23}NO$ from the *absorbance* obtained using a 0.01% w/v solution of *meptazinol hydrochloride BPCRS* and from the declared content of $C_{15}H_{23}NO$ in *meptazinol hydrochloride BPCRS*.

LABELLING

The label of the sealed container states the quantity of active ingredient contained in it in terms of the equivalent amount of meptazinol.

Meptazinol Tablets

General Notices

Action and use

Opioid receptor partial agonist; analgesic.

DEFINITION

Meptazinol Tablets contain Meptazinol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of meptazinol, $C_{15}H_{23}NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 330 nm of the solution obtained in the Assay exhibits a maximum at 273 nm.

B. Comply with the test described under Meptazinol Injection but preparing solution (1) and solution (2) in the following manner. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 50 mg of meptazinol with 5 ml of *ethanol (96%)*, centrifuge and dilute 1 volume of the supernatant liquid to 50 volumes with *ethanol (96%)*. Solution (2) contains 0.02% w/v of *meptazinol hydrochloride BPCRS* in *ethanol (96%)*.

TEST

Related substances

Comply with the test described under Meptazinol Injection but using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.2 g of meptazinol with 20 ml of *ethanol (96%)*, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *ethanol (96%)*. For solution (3) dilute a volume of solution (2) with an equal volume of *ethanol (96%)*.

ASSAY

To a quantity of the powdered tablets containing the equivalent of 0.2 g of meptazinol add 40 ml of 0.06M *hydrochloric acid*, shake, dilute to 50 ml with the same solvent, filter through a glass-fibre paper (Whatman GF/C paper is suitable); dilute 5 ml of the filtrate to 250 ml with 0.06M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_{15}H_{23}NO$ from the *absorbance* obtained using a 0.0080% w/v solution of *meptazinol hydrochloride BPCRS* in 0.06M *hydrochloric acid* and from the declared content of $C_{15}H_{23}NO$ in *meptazinol hydrochloride BPCRS*.

LABELLING

The label states the quantity of active ingredient in terms of the equivalent amount of meptazinol.

Mepyramine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Mepyramine Tablets contain Mepyramine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mepyramine maleate, $C_{17}H_{23}N_3O,C_4H_4O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Mepyramine Maleate with 10 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mepyramine maleate *(RS 216)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) is similar in position and size to that in the chromatogram obtained with solution (4).

C. Dissolve a quantity of the powdered tablets containing 0.2 g of Mepyramine Maleate, freed as far as possible from any sugar coating, in 3 ml of *water*, add 2 ml of 5M *sodium hydroxide* and shake with three 3 ml quantities of *ether*. Warm the aqueous layer in a water bath for 10 minutes with 2 ml of *bromine solution*, heat to boiling, cool and add 0.2 ml to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*. A bluish-black colour is produced on heating for 15 minutes in a water bath.

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 2 volumes of *diethylamine* and 100 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions prepared immediately before use. For solution (1) shake a quantity of the powdered tablets containing 0.4 g of Mepyramine Maleate with 10 ml of *chloroform* and filter. For solution (2) dilute 1 volume of solution (1) to 10 volumes with *chloroform*. Solutions (3), (4), (5) and (6) contain 4% w/v, 0.4% w/v, 0.008% w/v and 0.004% w/v of *mepyramine maleate EPCRS* in *chloroform* respectively. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (5) (0.2%). The test is not valid unless the Rf values of the principal spots in the chromatograms obtained with solutions (1) and (3) are at least 0.2 and unless the spot in the chromatogram obtained with solution (6) is clearly visible. Disregard the spot due to maleic acid on the line of application.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Mepyramine Maleate add 75 ml of *water* and 5 ml of 2M *hydrochloric acid*, shake vigorously for 15 minutes and dilute to 100 ml with *water*. Centrifuge and dilute 10 ml of the clear, supernatant liquid to 100 ml with *water*. To 10 ml add 10 ml of 0.1M *hydrochloric acid* and dilute to 100 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 316 nm, Appendix II B. Calculate the content of $C_{17}H_{23}N_3O,C_4H_4O_4$ taking 206 as the value of A(1%, 1 cm) at the maximum at 316 nm.

Mercaptopurine Oral Suspension

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Thiopurine cytotoxic.

DEFINITION

Mercaptopurine Oral Suspension is a suspension of Mercaptopurine in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of mercaptopurine, $C_5H_4N_4S,H_2O$

90.0 to 110.0% of the stated amount.

Shake the oral suspension vigorously before carrying out the following tests.

IDENTIFICATION

A. Shake a quantity of the oral suspension containing 50 mg of Mercaptopurine with a mixture of 20 ml of *water* and 0.5 ml of 5M *sodium hydroxide* for not more than 5 minutes, add sufficient *water* to produce 100 ml, mix and filter. Dilute a portion of the filtrate with sufficient 0.1M *hydrochloric acid* to give a final concentration of 0.0005% w/v of Mercaptopurine. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 325 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.0, Appendix V L.

Dissolution

Complies with the requirements stated under Unlicensed Medicines, Oral Suspensions, using 900 ml of 0.1M h*ydrochloric acid* as the dissolution medium and rotating the paddle at 50 revolutions per minute. Use a volume of the oral suspension containing one dose.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 10 ml of a mixture of equal volumes of *methanol* and *water* to a weighed quantity of the oral suspension containing 15 mg of Mercaptopurine, mix with the aid of ultrasound for

15 minutes, shake for 1 hour and add sufficient of the mixture of equal volumes of *methanol* and *water* to produce 100 ml.

(2) 0.015% w/v of *mercaptopurine BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *end-capped base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS 5 µm is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 328 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *water* containing 0.1% w/v of *sodium heptanesulphonate monohydrate* and 0.3% w/v of *sulphuric acid*.

DETERMINATION OF CONTENT

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_5H_4N_4S,H_2O$, weight in volume, using the declared content of $C_5H_4N_4S,H_2O$ in *mercaptopurine BPCRS*.

STORAGE

Mercaptopurine Oral Suspension should be protected from light.

Mercaptopurine Tablets

General Notices

Action and use

Thiopurine cytotoxic.

DEFINITION

Mercaptopurine Tablets contain Mercaptopurine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mercaptopurine, $C_5H_4N_4S,H_2O$

90.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Mercaptopurine with 20 ml of *absolute ethanol*, filter and evaporate to dryness under reduced pressure. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mercaptopurine *(RS 426)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using the following conditions.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 0.1M *hydrochloric acid* as the dissolution medium at a temperature of 37°.

Withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium, if necessary, at the maximum at 325 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *mercaptopurine BPCRS* in the dissolution medium using the declared content of $C_5H_4N_4S,H_2O$ in *mercaptopurine BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of the powdered tablets containing 0.25 g of Mercaptopurine to 80 ml of 0.02M *methanolic sodium hydroxide* in a 100 ml volumetric flask, mix with the aid of ultrasound for 10 minutes, dilute to 100 ml and filter through a GF/F filter (Whatman Autotop

is suitable). Dilute 1 volume of the resulting solution to 20 volumes with 0.1% v/v of *anhydrous formic acid* .

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1% w/v of *anhydrous formic acid* and further dilute 1 volume of the resulting solution to 10 volumes with 0.1% w/v of *anhydrous formic acid* .

(3) 0.025% w/v of *hypoxanthine* in 0.02M *methanolic sodium hydroxide* diluted to 0.00025% w/v with 0.1% v/v of *anhydrous formic acid* .

(4) 0.03% w/v of *bis-(1,7-dihydro-6*H*-purine)-6,6-disulphide BPCRS* in 0.02M *methanolic sodium hydroxide* and dilute to 0.0003% w/v with 0.1% v/v of *anhydrous formic acid* .

(5) Dissolve 25 mg of *mercaptopurine impurity standard BPCRS* in 10 ml of 0.02M *methanolic sodium hydroxide.* Dilute 1 volume to 50 volumes with 0.1% v/v of *anhydrous formic acid* .

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *polar endcapped ether linked phenyl groups* (4 µm) (Phenomenex Synergi Polar RP is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

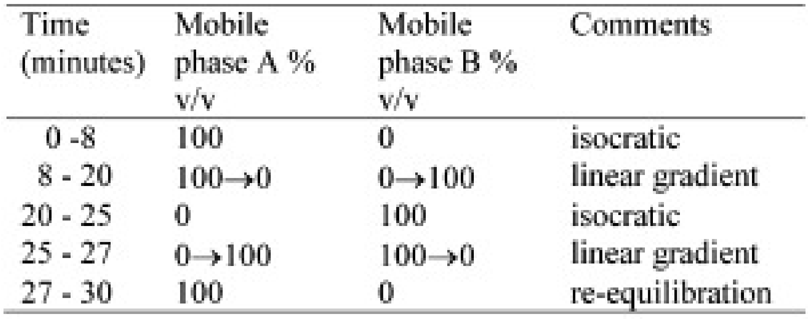
(e) Use a detection wavelength of 254 nm.

(f) Inject 50 µl of each solution and prepare the samples immediately before injection.

MOBILE PHASE

Mobile phase A 2 volumes of *methanol* and 98 volumes of 0.1% v/v of *anhydrous formic acid* .

Mobile phase B 50 volumes of *methanol* and 50 volumes of 0.1% v/v of *anhydrous formic acid* .



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0 -8	100	0	isocratic
8 - 20	100→0	0→100	linear gradient
20 - 25	0	100	isocratic
25 - 27	0→100	100→0	linear gradient
27 - 30	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless there is baseline resolution between the principal peaks corresponding to thioguanine, hypoxanthine and adenine in the chromatogram obtained with solution (5).

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to hypoxanthine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2.0%);

— the area of any peak corresponding to bis-(1,7-dihydro-6*H*-purine)-6,6-disulphide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2.5%);

— the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

— the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a quantity of the powdered tablets, containing 50 mg of Mercaptopurine in 100 ml of 0.02M *methanolic sodium hydroxide*, mix with the aid of ultrasound for 10 minutes and filter through a GF/F filter (Whatman Autotop is suitable). Dilute 1 volume to 20 volumes with 0.1% v/v *anhydrous formic acid*.

(2) 0.50% w/v of *mercaptopurine BPCRS* in 0.02M *methanolic sodium hydroxide* diluted to 0.0025% w/v with 0.1% v/v of *anhydrous formic acid*.

(3) Dissolve 25 mg of *mercaptopurine impurity standard BPCRS* in 10 ml of 0.02M *methanolic sodium hydroxide*. Dilute 1 volume to 50 volumes with 0.1% v/v of *anhydrous formic acid*. The chromatographic procedure described under Related substances may be used.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but with isocratic elution and the mobile phase described below.

MOBILE PHASE

2 volumes of *methanol* and 98 volumes of 0.1% v/v of *anhydrous formic acid*.

SYSTEM SUITABILITY

The test is not valid unless there is baseline resolution between the principal peaks corresponding to thioguanine, hypoxanthine and adenine in the chromatogram obtained with solution (5).

DETERMINATION OF CONTENT

Calculate the total content of mercaptopurine, $C_5H_4N_4S$,H2O, in the tablets using the chromatogram obtained and the declared content of $C_5H_4N_4S,H_2O$ in *mercaptopurine BPCRS*.

STORAGE

Mercaptopurine Tablets should be protected from light.

Metaraminol Injection

General Notices

Action and use

Adrenoceptor agonist.

DEFINITION

Metaraminol Injection is a sterile solution of Metaraminol Tartrate in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of metaraminol, $C_9H_{13}NO_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (5).

B. Dilute a volume containing the equivalent of 5 mg of metaraminol to 10 ml with *water* and extract with four 15 ml quantities of *chloroform*. To 0.5 ml of the aqueous layer add 0.5 ml of *phosphomolybdotungstic reagent* and 5 ml of *dilute sodium carbonate solution* and allow to stand for 5 minutes. An intense blue colour is produced.

C. To 4 ml of the aqueous layer obtained in test B add 5 ml of *borate buffer pH 9.6* and 1 ml of a freshly prepared 0.5% w/v solution of *sodium 1,2-naphthoquinone-4-sulphonate* and allow to stand for 1 minute. Add 0.2 ml of a 2% v/v solution of *benzalkonium chloride solution* and 5 ml of *toluene* and shake. A mauve colour is produced immediately in the toluene layer (distinction from phenylephrine).

TESTS

Acidity

pH, 3.2 to 4.5, Appendix V L.

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 10 volumes of 13.5M *ammonia*, 80 volumes of *chloroform* and 80 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) evaporate a volume of the injection containing the equivalent of 20 mg of metaraminol to dryness, dissolve the residue as completely as possible in 2 ml of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *methanol*. Solution (4) contains 0.1% w/v of *methyl 4-hydroxybenzoate* in *methanol*. Solution (5) contains 0.050% w/v of *metaraminol tartrate BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and spray with a solution prepared in the following manner. Mix 25 ml of a 0.45% w/v solution of *sulphanilic acid* in 1M *hydrochloric acid* with 1.5 ml of a 5% w/v solution of *sodium nitrite*, allow to stand for 5 minutes and mix cautiously with 25 ml of 2M *sodium carbonate*. Any *secondary spot* in the chromatogram obtained with solution (1), other than any spot corresponding to methyl 4-hydroxybenzoate, is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY

To a quantity containing the equivalent of 10 mg of metaraminol add sufficient *water* to produce 200 ml. Wash 25 ml with four 25 ml quantities of *chloroform*, discard the washings and filter the aqueous solution. Measure the *absorbance* of the filtrate at the maximum at 272 nm, Appendix II B. Calculate the content of $C_9H_{13}NO_2$ taking 111 as the value of A(1%, 1 cm) at the maximum at 272 nm.

STORAGE

Metaraminol Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of metaraminol in a suitable dose-volume.

Metformin Tablets

General Notices

Action and use

Biguanide; treatment of diabetes mellitus.

DEFINITION

Metformin Tablets contain Metformin Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of metformin hydrochloride, $C_4H_{11}N_5$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 20 mg of Metformin Hydrochloride with 20 ml of *absolute ethanol* , filter, evaporate the filtrate to dryness on a water bath and dry the residue at 105° for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metformin hydrochloride (*RS 217*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 6.8 by the addition of 1M *sodium hydroxide* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Filter, dilute 10 ml of the filtrate to 100 ml with *water* and dilute 10 ml of the resulting solution to 100 ml with *water*. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 233 nm, Appendix II B. Calculate the total content of metformin hydrochloride, $C_4H_{11}N_5$,HCl, in the medium taking 806 as the value of *A* (1%, 1 cm) at the maximum at 233 nm.

1-Cyanoguanidine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.50 g of Metformin Hydrochloride with 100 ml of the mobile phase, filter and use the filtrate. For solution (2) dissolve 20 mg of *1-cyanoguanidine* in *water*, dilute to 100 ml with *water* and dilute 1 ml of the resulting solution to 200 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with a regular, porous silica gel to which benzene sulphonic acid groups have been chemically bonded (5 µm) (Partisphere 5 µ SCX is suitable), (b) a 1.7% w/v solution of *ammonium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric* *acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 218 nm.

Inject 20 µl of solution (2). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is not less 50% of the full scale of the recorder.

Inject separately 20 µl of solutions (1) and (2). In the chromatogram obtained with solution (1) the area of any peak corresponding to 1-cyanoguanidine is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Metformin Hydrochloride with 70 ml of *water* for 15 minutes, dilute to 100 ml with *water* and filter, discarding the first 20 ml. Dilute 10 ml of the filtrate to 100 ml with *water* and dilute 10 ml of the resulting solution to 100 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 232 nm, Appendix II B. Calculate the content of the $C_4H_{11}N_5$,HCl taking 798 as the value of A(1%, 1 cm) at the maximum at 232 nm.

Methadone Linctus

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Methadone Linctus is an *oral solution* containing 0.04% w/v of Methadone Hydrochloride in a suitable vehicle with a tolu flavour.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$

0.036 to 0.044% w/v.

IDENTIFICATION

To 50 ml add 30 ml of *water* and add 1M *sulphuric acid* until the solution is acidic to *litmus paper*. Extract with two 20 ml quantities of *petroleum spirit (boiling range, 40° to 60°)*, discarding the extracts, and add 5M *sodium hydroxide* until the solution is alkaline to *litmus paper*. Add 4 g of *sodium chloride*, shake to dissolve, extract with two 25 ml quantities of *ether* and wash the combined ether extracts with five 20 ml quantities of *water*. Dry with *anhydrous sodium sulphate*, filter, evaporate to dryness and dry the residue over *phosphorus pentoxide* at a pressure of 2 kPa. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methadone *(RS 218)*.

B. To 5 mg add 0.05 ml of *dinitrobenzene solution* and 0.05 ml of a 50% w/v solution of *sodium hydroxide*. A purple colour is produced which changes slowly to dark brown.

ASSAY

To 30 g add 1 ml of *glacial acetic acid* and dilute to 100 ml with *water*. To 10 ml of the resulting solution add 10 ml of a 0.4% w/v solution of *picric acid* and 10 ml of *phosphate buffer pH 4.9*, extract with three 15 ml quantities of *chloroform* and dilute the combined chloroform extracts to 50 ml with *chloroform*. To 10 ml add sufficient *chloroform* to produce 20 ml and measure the *absorbance* of the resulting solution, Appendix II B, at the maximum at 350 nm using in the reference cell a solution prepared in the same manner but using 10 ml of a 1% v/v solution of *glacial acetic acid* and beginning at the words 'add 10 ml…'. Calculate the content of $C_{21}H_{27}NO,HCl$ taking 448 as the value of *A* (1%, 1 cm) at the maximum at 350 nm. Determine the *weight per ml*, Appendix V G, and calculate the content of $C_{21}H_{27}NO,HCl$, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Methadone Oral Solution (1 mg per ml)

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Methadone Oral Solution (1 mg per ml) contains 0.1% w/v of Methadone Hydrochloride in a suitable aqueous vehicle. It is supplied as a ready-to-use solution or it is prepared from Methadone Hydrochloride Oral Concentrate in accordance with the manufacturer's instructions.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$

0.090 to 0.110 % w/v.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel plates are suitable) and a mixture of 1.5 volumes of *concentrated ammonia R1* and 100 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add to 50 ml of the oral solution 30 ml of *water* and sufficient 1M *sulphuric acid* to make the solution acid to *litmus paper*. Extract with two 20 ml quantities of *petroleum spirit* (*boiling range, 40° to 60°),* discarding the extracts. Make the solution alkaline to *litmus paper* with 5M *sodium hydroxide*, add 4 g of *sodium chloride*, shake to dissolve, extract with two 25 ml quantities of *ether* and wash the combined extracts with five 20 ml quantities of *water*. Shake the ether extract with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dry the residue at a pressure of 2 kPa. Prepare a solution containing 0.5% w/v of the residue in *ethanol (96%)*. Solution (2) contains 0.55% w/v of *methadone hydrochloride BPCRS* in *ethanol (96%).* After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 1 volume of the oral solution to 10 volumes with the mobile phase. Solution (2) contains 0.01% w/v of *methadone hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm

× 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 10µ is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 50 volumes of *acetonitrile* and 50 volumes of 0.02M *potassium dihydrogen orthophosphate*, the mixture adjusted to pH 5.5 with 2M *orthophosphoric acid* or 2M *sodium hydroxide* and (c) a detection wavelength of 220 nm.

Calculate the content of $C_{21}H_{27}NO,HCl$ in the oral solution from the chromatograms obtained and using the declared content of $C_{21}H_{27}NO,HCl$ in *methadone hydrochloride BPCRS*.

METHADONE HYDROCHLORIDE ORAL CONCENTRATE

DEFINITION

Methadone Hydrochloride Oral Concentrate is a solution of Methadone Hydrochloride in a suitable aqueous vehicle.

The concentrate complies with the requirements stated under Oral Liquids and with the following requirements.

Content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck silica gel plates are suitable) and a mixture of 1.5 volumes of *concentrated ammonia R1* and 100 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add to 5 ml of the oral concentrate 30 ml of *water* and sufficient 1M *sulphuric acid* to make the solution acid to *litmus paper*. Extract with two 20 ml quantities of *petroleum spirit* (*boiling range, 40° to 60°),* discarding the extracts. Make the solution alkaline to *litmus paper* with 5M *sodium hydroxide*, add 4 g of *sodium chloride*, shake to dissolve, extract with two 25 ml quantities of *ether* and wash the combined extracts with five 20 ml quantities of *water*. Shake the ether extract with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dry the residue at a pressure of 2 kPa. Prepare a solution containing 0.5% w/v of the residue in *ethanol (96%)*. Solution (2) contains 0.55% w/v of *methadone hydrochloride BPCRS* in *ethanol (96%).* After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the concentrate with the mobile phase to produce a solution containing 0.01% w/v of Methadone Hydrochloride. Solution (2) contains 0.01% w/v of *methadone hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP18 10µ is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 50 volumes of *acetonitrile* and 50 volumes of 0.02M *potassium dihydrogen orthophosphate*, the mixture adjusted to pH 5.5 with 2M *orthophosphoric acid* or 2M *sodium hydroxide* and (c) a detection wavelength of 220 nm.

Calculate the content of $C_{21}H_{27}NO,HCl$ in the concentrate from the chromatograms obtained and using the declared content of $C_{21}H_{27}NO,HCl$ in *methadone hydrochloride BPCRS*.

LABELLING

The label states the directions for use of the concentrate.

At the specific request of the prescriber, the concentrate may be diluted to a concentration other than 0.1% w/v in accordance with the manufacturer's instructions.

Methadone Tablets

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Methadone Tablets contain Methadone Hydrochloride.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methadone hydrochloride, $C_{21}H_{27}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Methadone Hydrochloride with 20 ml of *water* and centrifuge. Make the supernatant liquid alkaline with 5M *sodium hydroxide*, stir with a glass rod until the precipitate solidifies, filter, wash with *water* and dry over *phosphorus pentoxide* at room temperature at a pressure of 2 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of methadone *(RS 218)*.

B. Extract a quantity of the powdered tablets containing 0.1 g of Methadone Hydrochloride with 10 ml of *water*, filter and wash the residue with sufficient *water* to bring the volume of the filtrate to 10 ml. Add to the filtrate 0.125 g of *picrolonic acid* dissolved in 50 ml of boiling *water*, stir and allow to stand for 2 hours. The *melting point* of the residue, after recrystallisation from *ethanol (20%)*, washing with *ethanol (20%)* and drying at 105°, is about 160° or about 180°, Appendix V A.

C. Extract a quantity of the powdered tablets containing 5 mg of Methadone Hydrochloride with 5 ml of *ethanol (96%)*, filter and evaporate the filtrate to dryness. To the residue add 0.05 ml of *dinitrobenzene solution* and 0.05 ml of a 50% w/v solution of *sodium hydroxide*. A purple colour is produced which changes slowly to dark brown.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 50 mg of Methadone Hydrochloride add 60 ml of *water* and 5 ml of *glacial acetic acid*, heat on a water bath for 5 minutes, mix with the aid of ultrasound for 10 minutes and dilute to 100 ml with water. Filter through a glass fibre paper, discarding the first 10 ml of filtrate, and dilute 20 ml to 100 ml with *water*. To 10 ml of the resulting solution add 10 ml of *phosphate buffer pH 4.9* and 5 ml of a 0.4% w/v solution of *picric acid*. Extract with three 15 ml quantities of *chloroform* and dilute the combined chloroform extracts to 50 ml with *chloroform*. To 10 ml add sufficient *chloroform* to produce 20 ml and measure the *absorbance* of the resulting solution, Appendix II B, at the maximum at 350 nm using in the reference cell a solution prepared in the same manner but using a 5% v/v solution of *glacial acetic acid* and beginning at the words 'Filter through a glass fibre paper…'. Calculate the content of $C_{21}H_{27}NO,HCl$ taking 448 as the value of *A* (1%, 1 cm) at the maximum at 350 nm.

Methotrexate Injection

General Notices

Action and use

Dihydrofolate reductase inhibitor; cytostatic.

DEFINITION

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injections containing Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methotrexate, $C_{20}H_{22}N_8O_5$

95.0 to 110.0% of the stated amount.

CHARACTERISTICS

A yellowish solution.

IDENTIFICATION

A. Dilute a suitable volume of the injection with 0.1M *sodium hydroxide* to produce a solution containing 0.001% w/v of Methotrexate. The *light absorption* of the resulting solution, Appendix II B, exhibits three maxima, at 258 nm, 303 nm and 371 nm.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Alkalinity

pH, 7.0 to 9.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection, if necessary, to produce a solution containing 0.01% w/v of Methotrexate.

(2) 0.00002% w/v of *methotrexate EPCRS.*

(3) 0.0003% w/v of *methotrexate impurity C EPCRS*.

(4) 0.0003% w/v each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µl of each solution.

(g) Continue the chromatography for three times the retention time of methotrexate.

MOBILE PHASE

7 volumes of *acetonitrile* and 93 volumes of a citro-phosphate buffer solution prepared by dissolving 7.8 g of *citric acid* and 17.9 g of *disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 ml.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to methotrexate and methotrexate impurity D is at least 2.0.

LIMITS

The approximate retention times relative to methotrexate are: impurity A, 0.2; impurity B, 0.3; impurity C, 0.4; impurity D, 0.8; impurity E, 2.3.

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity C is not greater that the area of the principal peak in the chromatogram obtained with solution (3) (3%);

— the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

— the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection, if necessary, to produce a solution containing 0.01% w/v of Methotrexate.

(2) 0.01% w/v of *methotrexate EPCRS.*

(3) 0.01% w/v each of the substance being examined and *methotrexate impurity D EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but using 302 nm as the detection wavelength.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to methotrexate and methotrexate impurity D is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{22}N_8O_5$ in the injection using the declared content of $C_{20}H_{22}N_8O_5$ in *methotrexate EPCRS*.

STORAGE

Methotrexate Injection should be protected from light.

LABELLING

When an antimicrobial preservative is present the label states that the contents are not intended for intrathecal injection.

Methotrexate Tablets

General Notices

Action and use

Dihydrofolate reductase inhibitor; cytostatic.

DEFINITION

Methotrexate Tablets contain Methotrexate or methotrexate sodium prepared by the interaction of Methotrexate with Sodium Hydroxide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methotrexate, $C_{20}H_{22}N_8O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 10 mg of Methotrexate with sufficient 0.1M *sodium hydroxide* to produce 100 ml, filter and dilute 10 ml of the filtrate to 100 ml with 0.1M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B in the range 230 to 400 nm, exhibits three maxima, at 258, 303 and 371 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A1.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 ml of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a 10 ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 303 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of *methotrexate EPCRS* in the dissolution

medium using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of methotrexate, $C_{20}H_{22}N_8O_5$, in the medium from the *absorbances* obtained and from the declared content of $C_{20}H_{22}N_8O_5$ in *methotrexate EPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the powdered tablets containing 10 mg of Methotrexate in 70 ml with the aid of ultrasound for 5 minutes and dilute to 100 ml. Mix and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) 0.00002% w/v of *methotrexate EPCRS.*

(3) 0.0003% w/v of *methotrexate impurity C EPCRS*.

(4) 0.0003% w/v each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 265 nm.

(f) Inject 20 µl of each solution.

(g) Continue the chromatography for three times the retention time of methotrexate.

MOBILE PHASE

7 volumes of *acetonitrile* and 93 volumes of a citro-phosphate buffer solution prepared by dissolving 7.8 g of *citric acid* and 17.9 g of *disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 ml.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to methotrexate and methotrexate impurity D is at least 2.0.

LIMITS

The approximate retention times relative to methotrexate are: impurity A, 0.2; impurity B, 0.3; impurity C, 0.4; impurity D, 0.8; impurity E, 2.3.

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity C is not greater that the area of the principal peak in the chromatogram obtained with solution (3) (3%);

— the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

— the sum of the areas of any other *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Disperse a quantity of the powdered tablets containing 10 mg of Methotrexate in 70 ml with the aid of ultrasound for 5 minutes and dilute to 100 ml. Mix and filter through a glass fibre filter (Whatman GF/C is suitable).

(2) 0.01% w/v of *methotrexate EPCRS.*

(3) 0.01% w/v each of *methotrexate EPCRS* and *methotrexate impurity D EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but using 302 nm as the detection wavelength.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to methotrexate and methotrexate impurity D is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{22}N_8O_5$ in the tablets using the declared content of $C_{20}H_{22}N_8O_5$ in *methotrexate EPCRS*.

STORAGE

Methotrexate Tablets should be protected from light.

LABELLING

When the active ingredient is methotrexate sodium, the quantity is expressed in terms of the equivalent amount of Methotrexate.

Methoxamine Injection

General Notices

Action and use

Adrenoceptor agonist.

DEFINITION

Methoxamine Injection is a sterile solution containing 2% w/v of Methoxamine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methoxamine hydrochloride, $C_{11}H_{17}NO_3$,HCl

1.90 to 2.10% w/v.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. The *light absorption* of the solution obtained in the Assay, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 290 nm.

B. Dilute 1 ml with 1 ml of *water*, add 5 ml of *diazotised nitroaniline solution* and 1 ml of *dilute sodium carbonate solution*, allow to stand for 2 minutes and add 1 ml of 1M *sodium hydroxide*. A deep red colour is produced which is extractable with *butan-1-ol*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 86 volumes of *chloroform*, 12 volumes of *methanol* and 2 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) use the injection being examined. Solution (2) contains 0.010% w/v of *2,5-dimethoxybenzaldehyde* in *methanol*. For solution (3) dilute 1 volume of the injection to 100 volumes with *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Any spot corresponding to 2,5-dimethoxybenzaldehyde in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Spray the plate with a 0.3% w/v solution of *ninhydrin* in *butan-1-ol* containing 3% v/v of *glacial acetic acid* and heat at 105° for 5 minutes. Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Dilute 1 ml to 100 ml with *water*, further dilute 20 ml of this solution to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{11}H_{17}NO_3$,HCl taking 137 as the value of A(1%, 1 cm) at the

maximum at 290 nm.

Methyl Salicylate Liniment

General Notices

DEFINITION

Methyl Salicylate is a *cutaneous emulsion*. It contains 25% v/v of Methyl Salicylate in Arachis Oil or other suitable fixed oil.

The liniment complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of methyl salicylate, $C_8H_8O_3$

23.0 to 26.5% v/v.

CHARACTERISTICS

The liniment has a characteristic odour.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as that due to methyl salicylate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using three solutions in *petroleum spirit (boiling range, 80° to 100°)* containing (1) 1% w/v of *benzyl alcohol* (internal standard) and 1% w/v of *methyl salicylate*, (2) 4% w/v of the liniment and (3) 4% w/v of the liniment and 1% w/v of the internal standard.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *diatomaceous support* (60 to 80 mesh) coated with 10% w/w of polyethylene glycol 1540 and maintained at 110°.

Determine the *weight per ml* of the liniment, Appendix V G, and calculate the content of $C_8H_8O_3$, volume in volume, taking the content of $C_8H_8O_3$ in *methyl salicylate* to be 100.0% and its *weight per ml* to be 1.18 g.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous emulsion'.

Methyl Salicylate Ointment

General Notices

Strong Methyl Salicylate Ointment

DEFINITION

Methyl Salicylate Ointment contains 50% w/w of Methyl Salicylate in a suitable water-emulsifying basis.

Extemporaneous preparation

The following formula and directions apply.

Methyl Salicylate	500 g
White Beeswax	250 g
Hydrous Wool Fat	250 g

Melt together the White Beeswax and the Hydrous Wool Fat, cool, add the Methyl Salicylate and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of methyl salicylate, $C_8H_8O_3$

45.0 to 52.5% w/w.

CHARACTERISTICS

The ointment has a characteristic odour.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as that due to methyl salicylate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using three solutions in *petroleum spirit (boiling range, 80° to 100°)* containing (1) 1% w/v of *benzyl alcohol* (internal standard) and 1% w/v of *methyl salicylate*, (2) 2% w/v of the ointment and (3) 2% w/v of the ointment and 1% w/v of the internal standard.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *diatomaceous support* (60 to 80 mesh) coated with 10% w/w of polyethylene glycol 1540 and maintained at 110°.

Calculate the content of $C_8H_8O_3$ taking 100.0% as the content of $C_8H_8O_3$ in *methyl salicylate*.

STORAGE

Certain plastic containers, such as those made from polystyrene, are unsuitable for Methyl Salicylate Ointment.

Methylcellulose Granules

General Notices

DEFINITION

Methylcellulose	64 g
Amaranth, food grade of commerce	20 mg
Saccharin Sodium	100 mg
Vanillin	200 mg
Acacia	4 g
Lactose	Sufficient to produce 100 g

Extemporaneous preparation

The following directions apply.

Mix the ingredients, in powder, add sufficient Water to form a coherent mass suitable for granulation, pass the mass through a sieve with a nominal mesh aperture of 2.8 mm, place the granules on a sieve with a nominal mesh aperture of 710 µm, discard the material passing through the sieve and dry the granules at a temperature not exceeding 60°.

The granules comply with the requirements stated under Granules and with the following requirements.

TESTS

Kinematic viscosity of aqueous solution

Not less than 2000 $mm^2 s^{-1}$ when determined by the following method. To 3.25 g in a wide-necked bottle add 100 ml of *water* previously heated to 85° to 90°, close the bottle with a device fitted with a stirrer, stir for 10 minutes, place the bottle in ice, continue stirring until the solution is of uniform consistency, remove the bottle from the ice and allow the solution to attain ambient temperature. Determine the *kinematic viscosity* of the solution, Appendix V H, Method II, using a suspended-level viscometer (size 4).

STORAGE

Methylcellulose Granules should be kept in a wide-mouthed, airtight container.

Methylcellulose Tablets

General Notices

DEFINITION

Methylcellulose Tablets contain Methylcellulose 450 in a suitable flavoured basis.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

A. Whilst stirring, introduce a quantity of the powdered tablets containing 1.0 g of Methylcellulose 450 into 50 ml of *carbon dioxide-free water* heated at 90°. Allow to cool, dilute to 100 ml with the same solvent and continue stirring until solution is complete (solution A). Heat 10 ml of solution A in a water bath, with stirring. At temperatures above 50° either the solution becomes cloudy or a flocculent precipitate is produced. The solution becomes clear on cooling.

B. Mix thoroughly a quantity of the powdered tablets containing 1 g of Methylcellulose 450 with 2 g of finely powdered *manganese(II) sulphate* in a test-tube about 16 cm long. Impregnate a strip of filter paper with a freshly prepared mixture of 1 volume of a 20% v/v solution of *diethanolamine* and 11 volumes of a 5% w/v solution of *sodium nitroprusside* adjusted to pH 9.8 with 1M *hydrochloric acid*. Insert the strip to a depth of 2 cm into the upper part of the tube, immerse the tube to a depth of 8 cm in a silicone oil bath and heat at 190° to 200°. The filter paper does not become blue within 10 minutes. Carry out a blank test.

TESTS

Kinematic viscosity

For tablets containing 500 mg of Methylcellulose 450, at 20°, 350 to 550 $mm^2 s^{-1}$, when determined by the following method. Place 2.6 g of the powdered tablets in a wide-mouthed bottle, add 100 ml of *water* heated to 85° to 90°, close the bottle with a stopper fitted with a stirrer and stir for 10 minutes. Cool in ice and continue stirring until the solution is of uniform consistence. Remove the ice and allow the solution to attain room temperature. Determine the *kinematic viscosity* of the solution, Appendix V H, Method II, using a suspended-level viscometer (size 3).

Disintegration

The requirement for Disintegration does not apply to Methylcellulose Tablets.

LABELLING

The label states that the tablets should be broken in the mouth before swallowing.

Methyldopa Tablets

General Notices

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

DEFINITION

Methyldopa Tablets contain Methyldopa. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous methyldopa, $C_{10}H_{13}NO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove the coating from a suitable number of tablets by washing with *chloroform*. To a quantity of the powdered tablet cores containing the equivalent of 5 g of anhydrous methyldopa add 35 ml of a mixture of equal volumes of *chloroform* and *methanol* and shake for 3 minutes. Centrifuge and discard the supernatant liquid. Repeat the operation with a further 35 ml of a mixture of equal volumes of *chloroform* and *methanol*. Dry the residue in a current of nitrogen, add 20 ml of *methanol* and 15 ml of 2M *hydrochloric acid*, shake for 2 minutes and filter. Adjust the pH of the filtrate to 4.9 with 5M *ammonia*, allow to stand for several hours at 2° to 8° and filter. Wash the precipitate with 15 ml of *water* and dry it at 50° at a pressure not exceeding 0.7 kPa for 3 hours. Reserve a portion of the residue for the test for Optical rotation. The remainder of the residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methyldopa *(RS 223)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *microcrystalline cellulose* as the coating substance and a mixture of 50 volumes of *butan-1-ol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in 1M *hydrochloric acid* containing (1) 1% w/v of the residue and (2) 1% w/v of *methyldopa BPCRS*. After removal of the plate, dry it in a current of warm air and spray with a freshly prepared mixture of equal volumes of a 10% w/v solution of *iron(III) chloride hexahydrate* and a 5% w/v solution of *potassium hexacyanoferrate(III)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. Add 0.1 ml of *iron(III) chloride solution R1* to 5 ml of a 0.4% w/v solution in 0.1M *hydrochloric acid*; a green colour is produced. To half of the solution add an excess of 5M *ammonia*; a purple colour is produced. To the remainder of the solution add an excess of 5M *sodium hydroxide*; a red colour is produced.

Optical rotation

Determine the content of $C_{10}H_{13}NO_4$ in the residue reserved in the test for Identification by carrying out Method I for *non-aqueous titration*, Appendix VIII A, using 0.2 g of the residue and *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 21.12 mg of $C_{10}H_{13}NO_4$. Dissolve a quantity of the residue containing 0.39 g of $C_{10}H_{13}NO_4$ in sufficient *aluminium chloride solution* to produce 10 ml. The *optical rotation* of the resulting solution at 25° is –0.98° to –1.09°, Appendix V F.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing the equivalent of 0.1 g of anhydrous methyldopa as completely as possible in sufficient 0.05M *sulphuric acid* to produce 100 ml and filter. To 5 ml of the filtrate add 2 ml of *iron(II) sulphate–citrate solution*, 8 ml of *glycine buffer solution* and sufficient *water* to produce 100 ml. Measure the *absorbance* of the resulting solution at the maximum at 545 nm, Appendix II B. Repeat the procedure using 5 ml of a 0.10% w/v solution of *methyldopa BPCRS* in place of 5 ml of the filtrate, beginning at the words 'add 2 ml of…'. Calculate the content of $C_{10}H_{13}NO_4$ using the declared content of $C_{10}H_{13}NO_4$ in *methyldopa BPCRS*.

STORAGE

Methyldopa Tablets should be protected from light.

LABELLING

The quantity of active ingredient is expressed in terms of the equivalent amount of anhydrous methyldopa.

Methyldopate Injection

General Notices

Action and use

Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

DEFINITION

Methyldopate Injection is a sterile solution prepared by dissolving Methyldopate Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methyldopate hydrochloride and methyldopa calculated as $C_{12}H_{17}NO_4$,HCl

The equivalent of 92.5 to 107.5% of the stated amount of Methyldopate Hydrochloride.

IDENTIFICATION

A. Dilute with 0.1M *hydrochloric acid* to produce a solution containing 0.004% w/v of Methyldopate Hydrochloride. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum only at 280 nm.

B. Dilute with 0.1M *hydrochloric acid* to produce a solution containing 2% w/v of Methyldopate Hydrochloride. The *optical rotation* of the resulting solution, measured at 25° in a 2-dm tube, is –0.28° to –0.35°, Appendix V F.

C. Yields reaction A characteristic *of chlorides*, Appendix VI.

TESTS

Acidity

pH, 3.0 to 4.2, Appendix V L.

Methyldopa

Not more than 10% of the declared content of Methyldopate Hydrochloride when determined as described in the Assay and calculated as $C_{12}H_{17}NO_4$,HCl.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.1% w/v of *methyldopate hydrochloride BPCRS* and 0.01% w/v of *methyldopa BPCRS* in the mobile phase. For solution (2) dilute a volume of the injection being examined with the mobile phase to contain 0.1% w/v of Methyldopate Hydrochloride.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a 0.3% w/v solution of *sodium dihydrogen orthophosphate* in a mixture of 155 volumes of *water* and 45 volumes of *methanol*, the mixture containing 0.1% v/v of *orthophosphoric acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 280 nm.

Determine the content of methyldopa, expressed as methyldopate hydrochloride, $C_{12}H_{17}NO_4$,HCl, using the declared content of $C_{10}H_{13}NO_4$ in *methyldopa BPCRS* and taking each mg of $C_{10}H_{13}NO_4$ as equivalent to 1.305 mg of $C_{12}H_{17}NO_4$,HCl. Determine the content of $C_{12}H_{17}NO_4$,HCl from the declared content of $C_{12}H_{17}NO_4$,HCl in *methyldopate hydrochloride BPCRS*. Calculate the total content of methyldopate hydrochloride and methyldopa as $C_{12}H_{17}NO_4$,HCl as the sum of the two determinations.

STORAGE

Methyldopate Injection should be protected from light.

Methylphenobarbital Tablets

General Notices

Action and use

Barbiturate.

DEFINITION

Methylphenobarbital Tablets contain Methylphenobarbital.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methylphenobarbital, $C_{13}H_{14}N_2O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Transfer a quantity of the finely powdered tablets containing 0.20 g of Methylphenobarbital to a long-stemmed, sintered glass filter (ISO 4793, porosity grade 3, is suitable), wash with 15 ml of *petroleum ether (boiling range 40° to 60°)*, stir for 2 to 3 minutes and filter; repeat the extraction with two 15 ml quantities of *petroleum ether (boiling range 40° to 60°)*. Discard the filtrates, extract the residues with two 15 ml quantities of *dichloromethane*, combine the extracts, evaporate to dryness on a water bath and dry the residue at 105° for 30 minutes to 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of methylphenobarbital *(RS 388)*.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2 and rotating the paddle at 75 revolutions per minute. Use as the medium 900 ml of a borate buffer of pH 10.0 prepared by dissolving 3.093 g of *boric acid*, 3.730 g of *potassium chloride* and 1.75 g of *sodium hydroxide* in 200 ml of *water* and diluting the solution to 1000 ml with *water*. Withdraw a sample of 20 ml of the medium and filter. Dilute the filtrate, if necessary, with the dissolution medium to give a solution expected to contain about 0.002% w/v of Methylphenobarbital. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 242 nm using dissolution medium in the reference cell. Calculate the total content of methylphenobarbital, $C_{13}H_{14}N_2O_3$, in the medium from the absorbance obtained from a 0.0020% w/v solution of *methylphenobarbital BPCRS* in dissolution medium and using the declared content of $C_{13}H_{14}N_2O_3$ in *methylphenobarbital BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 120 ml of *methanol* to a quantity of the powdered tablets containing 30 mg of Methylphenobarbital, mix with the aid of ultrasound for 10 minutes, add 50 ml of *water*, cool and dilute to 200 ml with *methanol*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (75%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with particles of silica 5 µm in diameter the surface of which has been modified with chemically bonded hexyl groups (Spherisorb Hexyl is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 55 volumes of 0.045M *disodium hydrogen orthophosphate* adjusted to pH 6.5 with *orthophosphoric acid* and 45 volumes of *methanol*, adjusted to pH 6.0 with *orthophosphoric acid* and (c) a detection wavelength of 235 nm.

Inject 20 µl of each solution. In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 120 ml of *methanol* to a quantity of the powdered tablets containing 30 mg of Methylphenobarbital, mix with the aid of ultrasound for 10 minutes, add 50 ml of *water*, cool and dilute to 200 ml with *methanol*. Prepare solution (2) in the same manner as solution (1) but using 30 mg of *methylphenobarbital BPCRS* in place of the powdered tablets.

The chromatographic procedure described under the test for Related substances may be used.

Inject 10 µl of each solution. Calculate the content of $C_{13}H_{14}N_2O_3$ using the declared content of $C_{13}H_{14}N_2O_3$ in *methylphenobarbital BPCRS*.

Methylprednisolone Acetate Injection

General Notices

Action and use

Glucocorticoid.

DEFINITION

Methylprednisolone Acetate Injection is a sterile suspension of Methylprednisolone Acetate in Water for Injections. It is prepared using aseptic technique.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methylprednisolone acetate, $C_{24}H_{32}O_6$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A white suspension which settles on standing but readily disperses on shaking. On examination under a microscope, the particles are seen to be crystalline and rarely exceed 20 µm in diameter.

IDENTIFICATION

Dilute a volume containing 0.1 g of Methylprednisolone Acetate to 5 ml with *water*, centrifuge and discard the supernatant liquid. Wash the residue with five 5 ml quantities of *water*, resuspending the residue in the *water* each time, centrifuging and discarding the washings. The residue, after drying at 105° for 3 hours, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methylprednisolone acetate *(RS 226)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase A*.

TESTS

Acidity or alkalinity

pH, 3.5 to 7.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Mix 0.12 g of *prednisone BPCRS* (internal standard) with 0.6 ml of *glacial acetic acid*, slowly add *chloroform* with the aid of ultrasound, shake to dissolve and dilute with sufficient *chloroform* to produce 20 ml (solution A). For solution (1) add 10 ml of solution A to a quantity of the injection containing 40 mg of Methylprednisolone Acetate, add sufficient *chloroform* to produce 25 ml and shake for 15 minutes or until the aqueous layer is clear; to 4 ml of the chloroform layer, add 30 ml of *chloroform* and 0.4 g of *anhydrous sodium sulphate*, shake for 5 minutes and use the clear solution. For solution (2) dissolve 20 mg of *methylprednisolone acetate BPCRS* in 5 ml of solution A and add sufficient *chloroform* to produce 100 ml.

The chromatographic procedure may be carried out using (a) a column (25 cm × 4 mm) packed with *silica gel for chromatography* (5 to 10 µm), (b) a mixture of 30 volumes of *glacial acetic acid*, 35 volumes of *methanol*, 70 volumes of *tetrahydrofuran*, 475 volumes of water-saturated *1-chloro-butane* and 475 volumes of *1-chlorobutane* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

The assay is not valid unless the *resolution factor* between the peaks due to methylprednisolone acetate and the internal standard is at least 2.5.

Calculate the content of $C_{24}H_{32}O_6$ from the declared content of $C_{24}H_{32}O_6$ in *methylprednisolone acetate BPCRS*.

STORAGE

Methylprednisolone Acetate Injection should be protected from light and stored at a temperature not exceeding 30°. It should not be allowed to freeze.

LABELLING

The label states (1) that the preparation is not to be given by intravenous injection; (2) that the container should be shaken gently before a dose is withdrawn.

Methylprednisolone Tablets

General Notices

Action and use

Glucocorticoid.

DEFINITION

Methylprednisolone Tablets contain Methylprednisolone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methylprednisolone, $C_{22}H_{30}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 50 mg with 100 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of methylprednisolone *(RS 225)*.

B. Complies with test B described under Methylprednisolone.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of *water* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 5 ml of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample at the maximum at 256 nm, Appendix II B. Calculate the total content of methylprednisolone, $C_{22}H_{30}O_{5,}$ in the medium taking 400 as the value of *A* (1%, 1 cm) at the maximum at 256 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using as the coating substance a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm and a mixture of 1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Methylprednisolone for 15 minutes with 2 ml of a mixture of 1 volume of *methanol* and 9 volumes of *chloroform*, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of the supernatant liquid to 50 volumes with the same solvent mixture. For solution (3) dilute 1 volume of solution (2) to 4 volumes with the same solvent mixture. For solution (4) dissolve 1 mg of *hydrocortisone* in a mixture of 0.1 ml of solution (1) and 0.9 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%). The test is not valid unless the chromatogram obtained with solution (4) shows two principal spots that are close together but separated.

ASSAY

Weigh and powder 20 tablets. Suspend a quantity of the powder containing 10 mg of Methylprednisolone in 10 ml of *water* and extract with successive quantities of 100, 50, 50 and 40 ml of *chloroform*. Wash each extract with the same 10 ml quantity of *water*, filter and dilute the combined filtrates to 250 ml with *chloroform*. Evaporate 25 ml just to dryness, dissolve the residue in sufficient *aldehyde-free ethanol (96%)* to produce a solution containing between 390 and 410 µg of Methylprednisolone in 10 ml and complete the *tetrazolium assay of steroids*, Appendix VIII J, beginning at the words 'Transfer 10 ml…'.

Dissolve a quantity of *methylprednisolone BPCRS* in sufficient *aldehyde-free ethanol (96%)* to produce a solution containing between 390 and 410 µg in 10 ml and complete the tetrazolium assay beginning at the words 'Transfer 10 ml…'. From the *absorbances* so obtained, and using the declared content of $C_{22}H_{30}O_5$ in *methylprednisolone BPCRS,* calculate the content of $C_{22}H_{30}O_5$ in the tablets.

STORAGE

Methylprednisolone Tablets should be protected from light.

Methylthioninium Injection

General Notices

Action and use

Reducing agent; antidote to methaemoglobinaemia.

DEFINITION

Methylthioninium Injection is a sterile solution of Methylthioninium Chloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of methylthioninium chloride, $C_{16}H_{18}ClN_3S,3H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 240 to 800 nm of a solution diluted, if necessary, with *dilute hydrochloric acid* to contain 0.0005% w/v of Methylthioninium Chloride exhibits maxima at 255 to 260 nm, 285 to 290 nm, 675 to 685 nm and 740 to 750 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck plates are suitable) and a mixture of 20 volumes of *anhydrous formic acid* and 80 volumes of *propan-1-ol* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) use the injection diluted, if necessary, with *methanol* to produce a solution containing 0.01% w/v of Methylthioninium Chloride. Solution (2) contains 0.01% w/v of *methylthioninium chloride EPCRS* in *methanol*. After removal of the plate, allow it to dry in air protected from light and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). A *secondary spot* may appear above the principal spot in both chromatograms.

TESTS

Acidity

pH, 3.0 to 4.5, Appendix V L.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 1% w/v of Methylthioninium Chloride (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

To a weighed quantity of the injection containing 0.3 g of Methylthioninium Chloride add 50 ml of *potassium dichromate solution R1*, dilute to 100 ml with *water* and allow to stand for 10 minutes. Filter and discard the first 20 ml of the filtrate. To 50 ml of the filtrate add 50 ml of 1M *sulphuric acid* and 8 ml of *potassium iodide solution*. Allow to stand protected from light for 5 minutes. Add 80 ml of *water* and titrate with 0.1M *sodium thiosulphate VS* using 2 ml of starch solution as indicator. Repeat the procedure without the preparation being examined. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 12.45 mg of $C_{16}H_{18}ClN_3S,3H_2O$. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage of $C_{16}H_{18}ClN_3S,3H_2O$, weight in volume.

Methysergide Tablets

General Notices

Action and use

Non-selective 5HT receptor antagonist.

DEFINITION

Methysergide Tablets contain Methysergide Maleate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of methysergide, $C_{21}H_{27}N_3O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (8).

B. To 5 ml of solution A obtained in the Assay add 10 ml of *dimethylaminobenzaldehyde solution R6*. A violet-blue colour is produced.

TESTS

Dissolution

Carry out the following procedure protected from light. The tablets comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 500 ml of 0.1M *hydrochloric acid* and rotating the basket at 120 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter through a membrane filter having a nominal pore size not greater than 0.45 µm, discarding the first 10 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light. For solution (1) dissolve 15 mg of *methysergide maleate BPCRS* in 500 ml of 0.1M *hydrochloric acid* and dilute 10 ml to 100 ml with 0.1M *hydrochloric acid*. Solution (2) is the filtrate from the dissolution vessel.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Spherisorb C8 is suitable), (b) a mixture of 65 volumes of 0.01M *diammonium hydrogen orthophosphate* and 35 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a fluorimetric detector with an excitation wavelength of 325 nm and an emission wavelength of 430 nm.

Calculate the total content of methysergide, $C_{21}H_{27}N_3O_2$, in the medium using the declared content of $C_{21}H_{27}N_3O_2$ in *methysergide maleate BPCRS*.

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a suspension of *silica gel G* in 0.1M *sodium hydroxide* to prepare the plate and a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) place a quantity of the powdered tablets containing the equivalent of 5.0 mg of methysergide in a sintered-glass filter, add 10 ml of *petroleum spirit (boiling range, 40° to 60°)*, stir for 2 minutes and filter. Repeat the washing with two further 10 ml quantities of the petroleum spirit and discard the washings. Extract with four 20 ml quantities of a solution prepared by shaking 100 ml of *chloroform* vigorously with 0.15 ml of 13.5M *ammonia*. Evaporate the filtrate to dryness at room temperature at a pressure of 2 kPa and dissolve the residue in 1 ml of a mixture of 1 volume of 13.5M *ammonia* and 100 volumes of *methanol*. Solutions (2), (3), (4), (5), (6) and (7) contain the equivalent of 0.0025% w/v, 0.005% w/v, 0.01% w/v, 0.015% w/v, 0.02% w/v and 0.025% w/v respectively of methysergide and are prepared by diluting appropriate volumes of solution (8) with a mixture of 1 volume of 13.5M *ammonia* and 100 volumes of *methanol*. For solution (8) dissolve 14 mg of *methysergide maleate BPCRS* in a mixture of 1 ml of *ethanol (96%)* and 19 ml of a 1% w/v solution of *(+)-tartaric acid*. Make alkaline by the addition of 0.15 ml of 13.5M *ammonia* and extract with successive quantities of 20, 10, 10 and 10 ml of *chloroform*. Filter the combined extracts and evaporate to dryness at room temperature at a pressure of 2 kPa. Dissolve the residue in 2 ml of a mixture of 1 volume of 13.5M *ammonia* and 100 volumes of *methanol*.

After removal of the plate, allow it to dry in air and examine under *ultraviolet light (365 nm)*. Assess the intensities of any *secondary spots* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with solutions (2) to (7), making allowance for area in assessing the intensities of spots with different Rf values and disregarding any spots less intense than the spot in the chromatogram obtained with solution (2). The sum of the intensities so assessed does not exceed 5% of the intensity of the principal spot in the chromatogram obtained with solution (1).

Uniformity of content

Tablets containing less than the equivalent of 2 mg of methysergide comply with the requirements stated under Tablets using the following method of analysis protected from light. To one tablet add 50 ml of a 1% w/v solution of *(+)-tartaric acid*, shake for 30 minutes and centrifuge. Measure the *absorbance* of the supernatant liquid at the maximum at 322 nm, Appendix II B. Calculate the content of $C_{21}H_{27}N_3O_2$ taking 227 as the value of A(1%, 1 cm) at the maximum at 322 nm.

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 20 mg of methysergide add 50 ml of a 1% w/v solution of *(+)-tartaric acid* and 0.2 ml of a 20% w/v solution of *benzalkonium chloride* and shake for 30 minutes. Dilute with the tartaric acid solution to 100 ml, mix and filter, discarding the first few ml. To 10 ml of the filtrate add sufficient of the tartaric acid solution to produce 100 ml and measure the *absorbance* at the maximum at 322 nm, Appendix II B. Calculate the content of $C_{21}H_{27}N_3O_2$ taking 227 as the value of A(1%, 1 cm) at the maximum at 322 nm.

STORAGE

Methysergide Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of methysergide.

Metipranolol Eye Drops

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Metipranolol Eye Drops are a sterile solution in Purified Water of metipranolol hydrochloride prepared by the interaction of Metipranolol with Hydrochloric Acid.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of metipranolol, $C_{17}H_{27}NO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume of the eye drops containing 5 mg of Metipranolol with sufficient *water* to produce 50 ml. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a broad maximum between 273 nm and 282 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the eye drops, if necessary, with sufficient *methanol* to produce a solution containing 0.10% w/v of Metipranolol. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.002% w/v of *desacetylmetipranolol BPCRS* in *methanol*. Solution (4) contains 0.02% w/v of *metipranolol BPCRS* and 0.02% w/v of *desacetylmetipranolol BPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1 volume of *perchloric acid*, 45 volumes of *methanol* and 54 volumes of *water*, the pH of the mixture being adjusted to 3.0 with 13.5M *ammonia* and (c) a detection wavelength of 275 nm. For solution (1) allow the chromatography to proceed for at least 3 times the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution*

factor between the two principal peaks is at least 5.

In the chromatogram obtained with solution (1) the area of any peak corresponding to desacetylmetipranolol is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (2%) and the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the eye drops, if necessary, with sufficient *methanol* to produce a solution containing 0.10% w/v of Metipranolol. Solution (2) is a 0.10% w/v solution of *metipranolol BPCRS* in *methanol*. Solution (3) contains 0.02% w/v of *metipranolol BPCRS* and 0.02% w/v of *desacetylmetipranolol BPCRS* in *methanol*.

The chromatographic procedure described under Related substances may be used.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 5.

Calculate the content of $C_{17}H_{27}NO_4$ in the eye drops using the declared content of $C_{17}H_{27}NO_4$ in *metipranolol BPCRS*.

STORAGE

Metipranolol Eye Drops should be protected from light. The eye drops should not be allowed to freeze.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Metipranolol.

Metoclopramide Injection

General Notices

Action and use

Dopamine receptor antagonist; antiemetic.

Metoclopramide Hydrochloride formulations should reflect the Hydrochloride salt.

DEFINITION

Metoclopramide Injection is a sterile solution of Metoclopramide Hydrochloride in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2,HCl$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. In the test for Related substances the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (3).

B. To a volume containing the equivalent of 50 mg of anhydrous metoclopramide hydrochloride add 5 ml of *water* and 5 ml of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in 1M *hydrochloric acid*. A yellowish-orange colour is produced.

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with sufficient of the mobile phase to produce a solution containing the equivalent of 0.1% w/v of anhydrous metoclopramide hydrochloride. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.0005% w/v of *metoclopramide hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS

1 is suitable), (b) 0.01M *sodium hexanesulphonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*, as the mobile phase with a flow rate such that the retention time of the principal peak is about 8 minutes (1.8 ml per minute may be suitable) and (c) a detection wavelength of 265 nm.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peaks with a retention time relative to the principal peak of 0.5 or less.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with sufficient *water* to produce a solution containing the equivalent of 0.01% w/v of anhydrous metoclopramide hydrochloride. Solution (2) contains 0.01% w/v of *metoclopramide hydrochloride BPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 20 volumes of a solution prepared by dissolving 2.25 g of *sodium octanesulphonate* and 0.3 g of *sodium acetate* in sufficient *water* to produce 1000 ml and adjusting the pH, if necessary, to 3.8 with *glacial acetic acid* and 80 volumes of *methanol* and (c) a detection wavelength of 305 nm.

Calculate the content of $C_{14}H_{22}ClN_3O_2,HCl$ in the injection using the declared content of $C_{14}H_{22}ClN_3O_2,HCl$ in *metoclopramide hydrochloride BPCRS*.

STORAGE

Metoclopramide Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

Metoclopramide Oral Solution

General Notices

Action and use

Dopamine receptor antagonist; antiemetic.

Metoclopramide Hydrochloride formulations should reflect the Hydrochloride salt.

DEFINITION

Metoclopramide Oral Solution is a solution of Metoclopramide Hydrochloride in a suitable flavoured vehicle.
The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, of the final solution obtained in the Assay exhibits a maximum at 534 nm.

B. In the test for Related substances the retention time of the principal peak in the chromatogram obtained with solution (2) is similar to the retention time of the principal peak in the chromatogram obtained with solution (3).

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the oral solution containing the equivalent of 10 mg of anhydrous metoclopramide hydrochloride with sufficient of the mobile phase to produce 10 ml and filter (Whatman GF/C filter paper is suitable). For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.0005% w/v of *metoclopramide hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) 0.01M *sodium hexanesulphonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*, as the mobile phase with a flow rate such that the retention time of the principal peak is about 8 minutes (1.8 ml per minute may be suitable) and (c) a detection wavelength of 265 nm.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peaks with a retention time relative to the principal peak of 0.5 or less.

ASSAY

Protect the solutions from light. Dilute a quantity of the oral solution containing the equivalent of 10 mg of anhydrous metoclopramide hydrochloride to 100 ml with *water*. To 5 ml of this solution add 5 ml of 1M *hydrochloric acid*, mix, add 5 ml of a freshly prepared solution containing 1% w/v of *sodium nitrite*, mix and allow to stand for 10 minutes. Add 5 ml of a freshly prepared solution containing 5% w/v of *ammonium sulphamate*, mix and allow to stand for 25 minutes. Add 5 ml of a freshly prepared solution containing 0.5% w/v of N*-(1-naphthyl)ethylenediamine dihydrochloride* in 1M *hydrochloric acid*, mix, add sufficient *water* to produce 100 ml, mix and allow to stand for 5 minutes. Measure the *absorbance* of the resulting solution at the maximum at 534 nm, Appendix II B, using *water* in the reference cell. Calculate the content of $C_{14}H_{22}ClN_3O_2,HCl$ from the *absorbance* obtained by repeating the procedure using 5 ml of a 0.01% w/v solution of *metoclopramide hydrochloride BPCRS* in place of the solution being examined, beginning at the words 'add 5 ml of 1M *hydrochloric acid*… ', and using the declared content of $C_{14}H_{22}ClN_3O_2,HCl$ in *metoclopramide hydrochloride BPCRS*.

STORAGE

Metoclopramide Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

Metoclopramide Tablets

General Notices

Action and use

Dopamine receptor antagonist; antiemetic.

Metoclopramide Hydrochloride formulations should reflect the Hydrochloride salt.

DEFINITION

Metoclopramide Tablets contain Metoclopramide Hydrochloride.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (3).

B. Shake a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous metoclopramide hydrochloride with 5 ml of *water*, filter and add to the filtrate 5 ml of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in 1M *hydrochloric acid*. A yellowish-orange colour is produced.

Related substances

Protect the solutions from light. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.1 g of anhydrous metoclopramide hydrochloride with 20 ml of *methanol* for 5 minutes, filter (Whatman GF/C filter paper is suitable) and add sufficient of the mobile phase to produce 100 ml. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.0005% w/v of *metoclopramide hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) 0.01M *sodium hexanesulphonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*, as the mobile phase with a flow rate such that the retention time of the principal peak is about 8 minutes (1.8 ml per minute may be suitable) and (c) a detection wavelength of 265 nm.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 20 mg of anhydrous metoclopramide hydrochloride with 80 ml of *water* with the aid of ultrasound for 10 minutes and then continue shaking mechanically for 20 minutes. Add sufficient *water* to produce 200 ml, filter and use the filtrate. Solution (2) contains 0.01% w/v of *metoclopramide hydrochloride BPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Novapak C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 20 volumes of a solution prepared by dissolving 2.25 g of *sodium octanesulphonate* and 0.3 g of *sodium acetate* in sufficient *water* to produce 1000 ml and adjusting the pH, if necessary, to 3.8 with *glacial acetic acid* and 80 volumes of *methanol* and (c) a detection wavelength of 305 nm.

Calculate the content of $C_{14}H_{22}ClN_3O_2,HCl$ in the tablets using the declared content of $C_{14}H_{22}ClN_3O_2,HCl$ in *metoclopramide hydrochloride BPCRS*.

STORAGE

Metoclopramide Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

Metoprolol Injection

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Metoprolol Injection is a sterile solution of Metoprolol Tartrate in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing 20 mg of Metoprolol Tartrate add 2 ml of 5M *ammonia*, mix, extract with 30 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate* and evaporate the filtrate to dryness using a rotary evaporator with gentle heating if necessary. Cool the residue to –18° for 30 minutes and allow to warm to room temperature. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metoprolol *(RS 228)*.

B. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (4).

C. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the injection being examined diluted, if necessary, with sufficient of the mobile phase to produce a solution containing 0.1% w/v of Metoprolol Tartrate. Solution (2) contains 0.2% w/v of *potassium sodium (+)-tartrate* in the mobile phase. For solution (3) mix 1 volume of solution (2) and 2 volumes of a solution containing 0.005% w/v of *fumaric acid* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemically bonded amine groups (10 µm) (Lichrosorb NH_2 is suitable), (b) a solution containing 1% w/v of *sodium chloride* in a mixture of 25 volumes of *methanol* and 75 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 210 nm.

The chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated peaks.

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) evaporate a volume of the injection containing 10 mg of Metoprolol Tartrate to dryness at a temperature not exceeding 40° and dissolve the residue in 2 ml of the mobile phase. For solution (2) dilute 6 volumes of solution (1) to 100 volumes with the mobile phase and further dilute 10 volumes of this solution to 100 volumes with the mobile phase. For solution (3) dilute 10 volumes of solution (2) to 20 volumes with the mobile phase. Solution (4) contains 0.0030% w/v of *metoprolol tartrate EPCRS* in the mobile phase. Solution (5) contains 0.0025% w/v of *metoprolol tartrate EPCRS* and 0.0015% w/v of *metoprolol impurity D EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable) maintained at a temperature of 40°, (b) as the *mobile phase* with a flow rate of 1 ml per minute a mixture of 2 volumes of *triethylamine*, 3 volumes of *orthophosphoric acid*, 10 volumes of *glacial acetic acid*, 190 volumes of *acetonitrile* and 810 volumes of a 0.48% w/v solution of *ammonium acetate* and (c) a detection wavelength of 275 nm. Equilibrate the column with the mobile phase with a flow rate of 1 ml per minute until a stable baseline is obtained (at least 20 minutes).

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 10 µl of solution (3) is not less than 50% of the full scale of the recorder.

Inject 10 µl of solution (5). When the chromatograms are recorded under the prescribed conditions, the retention times are metoprolol, about 10 minutes and metoprolol impurity D, about 13.5 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to metoprolol impurity D and metoprolol is at least 4.0. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately 10 µl of solution (1) and 10 µl of solution (3). Continue the chromatography for three times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%) and the sum of the areas of any such peaks is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). If any of the above limits are exceeded and if a secondary peak occurs with a retention time of about 5 minutes (metoprolol impurity C), prepare solution (6) in a fume cupboard in the following manner. Place an evaporating dish 10 cm in diameter containing 10 ml of a 0.1% w/v solution of *metoprolol tartrate EPCRS* in 0.1M *hydrochloric acid* so that the surface of the solution is 5 cm from a lamp emitting ultraviolet light at 254 nm for 6 hours. Dilute 1 volume of this solution to 50 volumes with the mobile phase (solution (6)).

In the chromatogram obtained with solution (1): divide the area of the peak corresponding to the principal peak in the chromatogram obtained with solution (6) (metoprolol impurity C) by 10: this divided area is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%); the sum of this divided area and the areas of any other *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.06%).

ASSAY

Dilute a volume of the injection containing 3 mg of Metoprolol Tartrate to 20 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 274 nm, Appendix II B. Calculate the content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ from the *absorbance* obtained with a 0.015% w/v solution of *metoprolol tartrate BPCRS* and from the declared content of $(C_{15}H_{25}NO_3)_2, C_4H_6O_6$ in *metoprolol tartrate BPCRS*.

Metoprolol Tartrate Tablets

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Metoprolol Tartrate Tablets contain Metoprolol Tartrate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity of the powdered tablets containing 50 mg of Metoprolol Tartrate with 25 ml of *water*, add 2 ml of 5M *ammonia*, extract with 20 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate*, wash the filter with 10 ml of *dichloromethane* and evaporate the combined filtrate and washings to dryness using a rotary evaporator, using gentle heat if necessary. Cool the residue to –18° for 30 minutes and allow to warm to room temperature. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metoprolol *(RS 228).*

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel H precoated plate (Analtech Uniplate plates are suitable) and a mixture of 40 volumes of *anhydrous formic acid* and 140 volumes of *ether* as the mobile phase, but allowing the solvent front to ascend 5 cm above the line of application. Pre-wash the plate with the mobile phase and allow to dry in air for at least 1 hour before use. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.5 g of Metoprolol Tartrate with 15 ml of *methanol* for 1 hour, add sufficient *methanol* to produce 25 ml and mix. Filter through a glass microfibre filter (Whatman GF/F is suitable) and use the filtrate. Solution (2) contains 2.0% w/v of *metoprolol tartrate BPCRS* in *methanol*. Solution (3) contains 0.5% w/v of *(+)-tartaric acid* in *methanol*. For solution (4) mix 1 volume of solution (1) with 1 volume of solution (3). After removal of the plate, allow it to dry in air for about 10 minutes, dry in an oven at 100° for 20 minutes and spray the plate with a solution prepared in the following manner. Dissolve 75 mg of *bromocresol green* and 25 mg of *bromophenol blue* in 100 ml of *absolute ethanol* (solution A); dissolve 0.25 g of *potassium permanganate* and 0.5 g of *sodium carbonate* in 100 ml of *water* (solution B); mix 9 volumes of solution A and 1 volume of solution B and use immediately. The chromatogram obtained with solution (1) closely resembles the chromatogram obtained with solution (2). In the chromatogram obtained with solution (1) the spot with the higher Rf value corresponds to the spot in the chromatogram obtained with solution (3). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots; the spot of low Rf value is purple and the spot of high Rf value, which is a single, compact spot, is yellow.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Metoprolol Tartrate with 10 ml of *chloroform* for 10 minutes, centrifuge, remove and retain the supernatant layer. Extract the residue with a further 10 ml quantity of *chloroform*, centrifuge and remove the supernatant layer. Evaporate the combined chloroform extracts to dryness at a temperature not exceeding 40°, add sufficient mobile phase to produce 50 ml, mix and filter. Solution (2) contains 0.0025% w/v of *metoprolol tartrate EPCRS* and 0.0015% w/v of *metoprolol impurity D EPCRS* in the mobile phase. For solution (3) dilute 3 volumes of solution (1) to 100 volumes with the mobile phase and further dilute 10 volumes of this solution to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable) maintained at a temperature of 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 2 volumes of *triethylamine*, 3 volumes of *orthophosphoric acid*, 10 volumes of *glacial acetic acid*, 190 volumes of *acetonitrile* and 810 volumes of a 0.48% w/v solution of *ammonium acetate* and (c) a detection wavelength of 275 nm. Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute until a stable baseline is obtained (at least 20 minutes).

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 10 µl of solution (3) is not less than 50% of the full scale of the recorder.

Inject 10 µl of solution (2). When the chromatograms are recorded under the prescribed conditions, the retention times are metoprolol, about 10 minutes and metoprolol impurity D, about 13.5 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to metoprolol impurity D and metoprolol is at least 4.0. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately 10 µl of solution (1) and 10 µl of solution (3). Continue the chromatography for three times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%) and the sum of the areas of any such peaks is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). If any of the above limits are exceeded and if a secondary peak occurs with a retention time of about 5 minutes (metoprolol impurity C), prepare solution (4) in a fume cupboard in the following manner. Place an evaporating dish 10 cm in diameter containing 10 ml of a 0.1% w/v solution of *metoprolol tartrate EPCRS* in 0.1M *hydrochloric acid* so that the surface of the solution is 5 cm from a lamp emitting ultraviolet light at 254 nm for 6 hours. Dilute 1 volume of this solution to 50 volumes with the mobile phase (solution (4)).

In the chromatogram obtained with solution (1): divide the area of the peak corresponding to the principal peak in the chromatogram obtained with solution (4) (metoprolol impurity C) by 10: this divided area is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%); the sum of this divided area and the areas of any other *secondary peaks* is not greater than 1.7 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.06%).

ASSAY

Weigh and powder 20 tablets. Add to a quantity of the powdered tablets containing 75 mg of

Metoprolol Tartrate 150 ml of *absolute ethanol*, shake with the aid of ultrasound for 15 minutes, allow to cool, add sufficient *absolute ethanol* to produce 200 ml and filter (Whatman GF/C paper is suitable). To 20 ml of the filtrate add sufficient *absolute ethanol* to produce 50 ml and measure the *absorbance* of the resulting solution at the maximum at 274 nm, Appendix II B. Calculate the content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ from the *absorbance* obtained with a 0.015% w/v solution of *metoprolol tartrate BPCRS* in *absolute ethanol* and from the declared content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ in *metoprolol tartrate BPCRS*.

Metronidazole Gel

General Notices

Action and use

Imidazole antibacterial.

DEFINITION

Metronidazole Gel contains Metronidazole in a suitable water-soluble basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Nitrite

Disperse 1.0 g of the gel in sufficient *water* to produce 100 ml. To 40 ml of the solution add 2 ml of *sulphanilic acid solution*, 2 ml of *aminonaphthalenesulphonic acid solution* and sufficient *water* to produce 50 ml. Allow to stand at room temperature for 1 hour. Measure the *absorbance* of the resulting solution at the maximum at 524 nm, Appendix II B, using in the reference cell a solution prepared by treating 40 ml of *water* in the same manner, beginning at the words 'add 2 ml of *sulphanilic acid solution*...'. The *absorbance* is not greater than that obtained by repeating the procedure using 1 ml of *nitrite standard solution (20 ppm)* diluted to 40 ml with *water* and treated at the same time and in the same manner, beginning at the words 'add 2 ml of *sulphanilic acid solution*...' (50 ppm, calculated with reference to the gel).

2-Methyl-5-nitroimidazole

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and as the mobile phase a mixture of 1 volume of *water*, 10 volumes of *ethanol (96%)*, 10 volumes of *diethylamine* and 80 volumes of *dichloromethane*. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake for 1 hour a quantity of the gel containing 45 mg of Metronidazole with 150 ml of *methanol*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of a mixture of equal volumes of *dichloromethane* and *methanol*. Solution

(2) contains 0.0023% w/v of *2-methyl-5-nitroimidazole* in a mixture of equal volumes of *dichloromethane* and *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Spray with *titanium(III) chloride solution* and heat at 110° until the blue-grey colour disappears, cool and spray with a 1% w/v solution of *fast blue B salt* followed by a mixture of 5 volumes of *ethanol (96%)*, 3 volumes of *water* and 2 volumes of 18M *ammonia*. Any *secondary spot* corresponding to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the gel in 10 ml of *methanol (8%)* to produce a solution containing 0.030% w/v of Metronidazole. For solution (2) dissolve 37.5 mg of *metronidazole BPCRS* in 10 ml of *methanol (8%)* and dilute 2 ml of this solution to 25 ml with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (5 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2) is suitable, (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 3 volumes of *methanol* and 97 volumes of 0.005M *potassium dihydrogen orthophosphate*, the pH of the mixture being adjusted to 4.0 with *orthophosphoric acid*, and (c) a detection wavelength of 240 nm.

Calculate the content of $C_6H_9N_3O_3$ in the gel using the declared content of $C_6H_9N_3O_3$ in *metronidazole BPCRS*.

Metronidazole Intravenous Infusion

General Notices

Action and use

Imidazole antibacterial.

DEFINITION

Metronidazole Intravenous Infusion is a sterile solution of Metronidazole in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$

95.0 to 110.0% of the stated amount.

CHARACTERISTICS

An almost colourless to pale yellow solution.

IDENTIFICATION

Shake a volume of the intravenous infusion containing 0.1 g of Metronidazole with 9 g of *sodium chloride* for 5 minutes, add 20 ml of *acetone*, shake for a further 5 minutes and allow to separate. Evaporate the upper layer to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metronidazole *(RS 229)*.

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Related substances

Carry out the test described under Metronidazole Tablets but preparing solution (2) in the following manner. Dilute a suitable volume of the intravenous infusion with sufficient mobile phase to produce a solution containing 0.1% w/v of Metronidazole.

In the chromatogram obtained with solution (2) the area of any *secondary peak* is not greater than the area of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (1) (0.5%).

Nitrite

To a volume of the infusion containing 2.5 mg of Metronidazole add 40 ml of *water*, 2 ml of *sulphanilic acid solution*, 2 ml of *aminonaphthalenesulphonic acid solution* and sufficient *water* to produce 50 ml. Allow to stand at room temperature for 1 hour. Measure the *absorbance* of the resulting solution at the maximum at 524 nm, Appendix II B, using in the reference cell a solution prepared by treating 1 ml of *water* in the same manner, beginning at the words 'add 40 ml…'. The *absorbance* is not greater than that obtained by repeating the procedure using 1 ml of *nitrite standard solution (20 ppm)* treated at the same time and in the same manner, beginning at the words 'add 40 ml of *water* …' (0.8%, calculated with reference to the content of metronidazole).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the intravenous infusion, if necessary, with *water BET* to give a solution containing 5 mg per ml (solution A). The endotoxin limit concentration of solution A is 3.5 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Dilute a volume of the infusion containing 50 mg of Metronidazole to 100 ml with 0.1M *hydrochloric acid*. Dilute 10 ml of the resulting solution to 250 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 277 nm, Appendix II B. Calculate the content of $C_6H_9N_3O_3$ taking 375 as the value of A(1%, 1 cm) at the maximum at 277 nm.

STORAGE

Metronidazole Intravenous Infusion should be protected from light.

LABELLING

The label states that solutions containing visible solid particles must not be used.

Metronidazole Oral Suspension

General Notices

Action and use

Imidazole antibacterial.

DEFINITION

Metronidazole Oral Suspension is a suspension of Metronidazole Benzoate in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a volume of the oral suspension containing the equivalent of 200 mg of metronidazole with 20 ml of *water*, filter under partial vacuum and wash the residue with three 10-ml quantities of *water* (Whatman GF/C filter paper is suitable). Dissolve the residue as completely as possible in 10 ml of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue after drying at 60°, Appendix II A, is concordant with the *reference spectrum* of metronidazole benzoate (*RS 408*). Examine as discs prepared using potassium bromide.

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

Metronidazole

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the oral suspension containing the equivalent of 200 mg of metronidazole with 150 ml of *methanol*, add sufficient *water*, with mixing and cooling, to produce 250 ml, mix and centrifuge. For solution (2) dissolve 20 mg of *metronidazole BPCRS* in 150 ml of *methanol* and add sufficient *water*, with mixing and cooling, to produce 250 ml. Dilute 1 volume to 10 volumes with *methanol (60%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 40 volumes of a 1.25% w/v solution of *ammonium acetate*, adjusted to pH 7.0 with *dilute acetic acid* or *dilute ammonia R1*, as appropriate, and 60 volumes of *methanol* and (c) a detection wavelength of 310 nm.

In the chromatogram obtained with solution (1) the area of any peak corresponding to metronidazole is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the oral suspension containing the equivalent of 200 mg of metronidazole with 150 ml of *methanol*, add sufficient *water*, with mixing and cooling, to produce 250 ml, mix and centrifuge. Dilute 1 volume to 10 volumes with *methanol (60%)*. For solution (2) dissolve 62.5 mg of *metronidazole benzoate BPCRS* in 1 ml of *dimethylformamide* and 30 ml of *methanol* and add sufficient *water*, with mixing and cooling, to produce 50 ml. Dilute 1 volume to 10 volumes with *methanol (60%)*.

The chromatographic procedure described under the test for Metronidazole may be used.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_6H_9N_3O_3$, weight in volume, using the declared content of $C_6H_9N_3O_3$ in *metronidazole benzoate BPCRS*.

STORAGE

Metronidazole Oral Suspension should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of metronidazole.

Metronidazole Suppositories

General Notices

Action and use

Imidazole antibacterial.

DEFINITION

Metronidazole Suppositories contain Metronidazole in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Dissolve a quantity of the suppositories containing 0.5 g of Metronidazole as completely as possible by heating with 30 ml of *petroleum spirit (boiling range, 40° to 60°)* on a water bath. Filter, wash the residue with the same solvent and dry at 100°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metronidazole *(RS 229)*.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) melt a quantity of the suppositories containing 0.25 g of Metronidazole using gentle heat, allow to cool and dissolve as completely as possible in 20 ml of *petroleum spirit (boiling range, 40° to 60°)* by warming on a water bath. Filter, wash the residue with *petroleum spirit (boiling range, 40° to 60°)*, dry in a current of air and dissolve in 250 ml of the mobile phase. Solutions (2) and (3) contain 0.00050% w/v of *2-methyl-5-nitroimidazole BPCRS* in the mobile phase and in solution (1) respectively.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 30 volumes of *methanol* and 70 volumes of 0.01M *potassium dihydrogen orthophosphate* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 315 nm. Record the chromatogram for 3 times the retention time of the principal peak in the chromatogram obtained with solution (2).

Adjust the sensitivity so that the height of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (3) is about 50% of full-scale deflection on the chart paper. Measure the height *(a)* of the peak due to 2-methyl-5-nitroimidazole and the height (*b*) of the lowest part of the curve separating this peak from the principal peak. The test is not valid unless *a* is greater than 10*b*.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh five suppositories, melt together by warming and allow to cool, stirring continuously until the mass is set. To a quantity containing 0.5 g of Metronidazole add 60 ml of *anhydrous acetic acid*, previously neutralised to *1-naphtholbenzein solution* with 0.1M *perchloric acid VS*, warm at 30° for 30 minutes and shake for 5 minutes. Cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 17.12 mg of $C_6H_9N_3O_3$.

STORAGE

Metronidazole Suppositories should be protected from light.

Metronidazole Tablets

General Notices

Action and use

Imidazole antibacterial.

DEFINITION

Metronidazole Tablets contain Metronidazole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of metronidazole, $C_6H_9N_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Metronidazole with 40 ml of *chloroform* for 15 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of metronidazole *(RS 229)*.

B. Shake a quantity of the powdered tablets containing 0.2 g of Metronidazole with 4 ml of 0.5M *sulphuric acid* and filter. To the filtrate add 10 ml of *picric acid solution R1* and allow to stand. The *melting point* of the precipitate, after washing with *water* and drying at 105°, is about 150°, Appendix V A.

C. Heat a quantity of the powdered tablets containing 10 mg of Metronidazole on a water bath with 10 mg of *zinc powder*, 1 ml of *water* and 0.25 ml of *hydrochloric acid* for 5 minutes, cool in ice, add 0.5 ml of *sodium nitrite solution* and remove the excess of nitrite with *sulphamic acid*. Add 0.5 ml of *2-naphthol solution* and 2 ml of 5M *sodium hydroxide*. An orange-red colour is produced.

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00050% w/v of *2-methyl-5-nitroimidazole BPCRS* in the mobile phase. For solution (2) shake a quantity of the powdered tablets containing 0.2 g of Metronidazole with 100 ml of the mobile phase for 5 minutes, dilute to 200 ml with the mobile phase, filter and use the filtrate. Solution (3) contains 0.00050% w/v of *2-methyl-5-nitroimidazole BPCRS* in solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 30 volumes of *methanol* and 70 volumes of 0.01M *potassium dihydrogen orthophosphate* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 315 nm. Record the chromatograms for 3 times the retention time of the principal peak in the chromatogram obtained with solution (2).

Adjust the sensitivity so that the height of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (3) is about 50% of full-scale deflection. Measure the height (*a*) of the peak due to 2-methyl-5-nitroimidazole and the height (*b*) of the lowest part of the curve separating this peak from the principal peak. The test is not valid unless *a* is greater than 10*b*.

The area of any *secondary peak* in the chromatogram obtained with solution (2) is not greater than the area of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with solution (1).

ASSAY

Weigh and powder 20 tablets. Transfer a quantity of the powder containing 0.2 g of Metronidazole to a sintered-glass crucible and extract with six 10 ml quantities of hot *acetone*. Cool, add to the combined extracts 50 ml of *acetic anhydride* and 0.1 ml of a 1% w/v solution of *brilliant green* in *anhydrous acetic acid* and titrate with 0.1M *perchloric acid VS* to a yellowish-green end point. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of perchloric acid required. Each ml of 0.1M *perchloric acid VS* is equivalent to 17.12 mg of $C_6H_9N_3O_3$.

Metyrapone Capsules

General Notices

Action and use

11-Beta hydroxylase inhibitor; inhibition of the formation of corticosteroids.

DEFINITION

Metyrapone Capsules contain Metyrapone.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of metyrapone, $C_{14}H_{14}N_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum* of the contents of the capsules, Appendix II A, is concordant with *reference spectrum 2* of metyrapone *(RS 231)*.

B. To a quantity of the contents of the capsules containing 50 mg of Metyrapone add 5 ml of 0.5M *sulphuric acid* and 0.2 ml of *potassium tetraiodomercurate solution*. A cream precipitate is produced.

TEST

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 5 volumes of 13.5M *ammonia,* 5 volumes of *water* and 90 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dissolve a quantity of the contents of the capsules containing 0.5 g of Metyrapone in 10 ml of *chloroform*. For solution (2) dilute 1 volume of solution (1) to 500 volumes with *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Weigh 10 capsules. Open the capsules carefully without loss of shell material, express as much of the contents as possible and reserve the expressed material. Wash the shells with *ether*, discard the washings, allow the shells to stand at room temperature for about 5 minutes and weigh. The difference between the weights represents the weight of the total contents. Dissolve a quantity of the contents containing 0.1 g of Metyrapone in 40 ml of 0.1M *hydrochloric acid* and add sufficient 0.1M *hydrochloric acid* to produce 100 ml. Dilute 5 ml to 50 ml with 0.1M *hydrochloric acid* and dilute 5 ml of this solution to 50 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{14}H_{14}N_2O$ taking 500 as the value of A(1%, 1 cm) at the maximum at 260 nm.

STORAGE

Metyrapone Capsules should be protected from light.

Mexenone Cream

General Notices

Action and use

Sunscreen.

DEFINITION

Mexenone Cream is a dispersion of Mexenone in a suitable basis.
The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mexenone, $C_{15}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *butan-2-one* and 100 volumes of *toluene* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) disperse a quantity of the cream containing 0.1 g of Mexenone in 50 ml of *water*, add 5 g of *sodium chloride*, extract with two 25 ml quantities of *chloroform*, wash the combined extracts with 50 ml of *water* and filter the chloroform solution through *kieselguhr*. Solution (2) contains 0.2% w/v of *mexenone BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

Acidity

pH of a dispersion prepared by mixing 4 g with 20 ml of *carbon dioxide-free water*, 4.0 to 5.0, Appendix V L.

ASSAY

To a quantity containing 20 mg of Mexenone add 20 ml of *methanol*, warm to disperse, dilute to 100 ml with *methanol* and filter, discarding the first portion of the filtrate. Dilute 20 ml of the clear filtrate to 100 ml with *methanol*, further dilute 20 ml of this solution to 100 ml with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 287 nm, Appendix II B. Calculate the content of $C_{15}H_{14}O_3$ taking 640 as the value of A(1%, 1 cm) at the maximum at 287 nm.

Mexiletine Capsules

General Notices

Action and use

Class I antiarrhythmic.

DEFINITION

Mexiletine Capsules contain Mexiletine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of mexiletine hydrochloride, $C_{11}H_{17}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 50 mg of Mexiletine Hydrochloride with 10 ml of *methanol* for 15 minutes, filter (Whatman GF/C filter paper is suitable), evaporate to dryness on a rotary evaporator and dry the residue at 105° for 2 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of mexiletine hydrochloride *(RS 233)*.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) has the same retention time as that of the principal peak in the chromatogram obtained with solution (3).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix, with the aid of ultrasound for 10 minutes, a quantity of the contents of the capsules containing 0.2 g of Mexiletine Hydrochloride with 10 ml of the mobile phase, filter through a 0.4-µm glass microfibre filter (Whatman GF/C is suitable) and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 10 volumes with the mobile phase. For solution (3) dissolve 2 mg of *mexiletine impurity C EPCRS* and 16 mg of *2,6-dimethylphenol* in the mobile phase and add sufficient mobile phase to produce 20 ml. Mix 1 volume of this solution with 2 volumes of solution (2) and add sufficient mobile phase to produce 100 volumes.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil is suitable), (b) as mobile phase with a flow rate of 1 ml per minute a mixture of 35 volumes of solution A described below and 65 volumes of *methanol R2* and (c) a detection wavelength of 262 nm. For solution (A) dissolve 11.5 g of *anhydrous sodium acetate* in 500 ml of *water*, add 3.2 ml of *glacial acetic acid*, mix and allow to cool. Adjust the pH to 4.8 with *glacial acetic acid* and add sufficient *water* to produce 1000 ml. For solution (1) allow the chromatography to proceed for 5.5 times the retention time of mexiletine.

When the chromatograms are recorded in the prescribed conditions, the retention time of mexiletine is about 4 minutes and retention times relative to mexiletine are about 0.7 for mexiletine impurity C (1,1′-[(3,3′,5,5′-tetramethylbiphenyl-4,4′-diyl)bisoxy]dipropan-2-amine) and about 1.8 for mexiletine impurity A (2,6-dimethylphenol).

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to mexiletine and impurity C is at least 5.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to impurity A is not greater than 2.5 times the area of the peak due to *2,6-dimethylphenol* in the chromatogram obtained with solution (3) (0.1%), the area of any peak corresponding to impurity C is not greater than 20 times the area of the peak due to impurity C in the chromatogram obtained with solution (3) (0.1%), the area of any other *secondary peak* is not greater than half the area of the peak due to mexiletine in the chromatogram obtained with solution (3) (0.1%) and the sum of the areas of all the peaks is not greater than 2.5 times the area of the peak due to mexiletine in the chromatogram obtained with solution (3) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Mexiletine Hydrochloride with 50 ml of 0.01M *hydrochloric acid* for 30 minutes. Dilute to 100 ml with 0.01M *hydrochloric acid* and centrifuge. Measure the *absorbance* of the supernatant liquid at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{11}H_{17}NO,HCl$ taking 11.6 as the value of A(1%, 1 cm) at the maximum at 260 nm.

Mexiletine Injection

General Notices

Action and use

Class I antiarrhythmic.

DEFINITION

Mexiletine Injection is a sterile solution of Mexiletine Hydrochloride in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of mexiletine hydrochloride, $C_{11}H_{17}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) has the same retention time as that of the principal peak in the chromatogram obtained with solution (3).

B. A volume containing 2.5 mg of Mexiletine Hydrochloride diluted to 2 ml yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 5.0 to 6.0, Appendix V L.

Related substances

Complies with the test described under Mexiletine Capsules but using a solution prepared in the following manner as solution (1). Dilute a volume of the injection with sufficient of the mobile phase to produce a solution containing 2.0% w/v of Mexiletine Hydrochloride.

ASSAY

Dilute a volume containing 0.125 g of Mexiletine Hydrochloride to 250 ml with 0.01M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{11}H_{17}NO,HCl$ taking 11.6 as the value of A(1%, 1 cm) at the maximum at 260 nm.

Mianserin Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tetracyclic antidepressant.

DEFINITION

Mianserin Tablets contain Mianserin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mianserin hydrochloride, $C_{18}H_{20}N_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Mianserin Hydrochloride with 10 ml of *methanol*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of mianserin hydrochloride *(RS 234)*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as that due to mianserin in the chromatogram obtained with solution (1).

C. The residue obtained in test A yields the reactions characteristic of *chlorides*, Appendix VI.

TEST

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) triturate a quantity of the powdered tablets containing 40 mg of Mianserin Hydrochloride with 2 ml of a mixture of 4 volumes of *methanol* and 1 volume of 13.5M *ammonia* and centrifuge. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture. For solution (3) dilute 1 volume of solution (1) to 500 volumes with the same solvent mixture. After removal of the plate, allow it to dry in a current of cold air for 5 minutes and then expose to iodine vapour for 20 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.2%). Disregard any spot with an Rf value lower than 0.15.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 3 ml of 1M *sodium hydroxide* and 10 ml of *toluene* containing 0.2% w/v of *triphenylamine* (internal standard) to 10 ml of a solution containing 0.2% w/v of *mianserin hydrochloride BPCRS* in 0.2M *hydrochloric acid*, mix for 5 minutes with a vortex mixer, centrifuge and use the clear upper layer. For solution (2) shake a quantity of the powdered tablets containing 60 mg of Mianserin Hydrochloride with 30 ml of 0.2M *hydrochloric acid* for 1 hour and filter. To 10 ml of the filtrate add 3 ml of 1M *sodium hydroxide* and 10 ml of *toluene*, mix for 5 minutes with a vortex mixer, centrifuge and use the clear upper layer. For solution (3) treat a further 10 ml of the filtrate obtained under solution (2) in the same manner as for solution (1) beginning at the words 'add 3 ml of 1M *sodium hydroxide…*'.

The chromatographic procedure may be carried out using a glass column (1.0 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of phenylmethyl silicone fluid (50% phenyl) (OV-17 is suitable) and maintained at 255°.

Calculate the content of $C_{18}H_{20}N_2$,HCl using the declared content of $C_{18}H_{20}N_2$,HCl in *mianserin hydrochloride BPCRS*.

STORAGE

Mianserin Tablets should be protected from light.

Miconazole and Hydrocortisone Acetate Cream

General Notices

Action and use

Antifungal azole.

DEFINITION

Miconazole and Hydrocortisone Acetate Cream contains Miconazole Nitrate and Hydrocortisone Acetate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$

95.0 to 105.0% of the stated amount.

Content of hydrocortisone acetate, $C_{23}H_{32}O_6$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *ammonium acetate,* 40 volumes of *dioxan* and 40 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the cream containing 0.15 g of Miconazole Nitrate by shaking with 25 ml of *methanol* for about 30 minutes, dilute to 50 ml with *methanol,* filter and use the filtrate. Solution (2) contains 0.3% w/v of *miconazole nitrate BPCRS* in *methanol.* Solution (3) contains 0.15% w/v of *hydrocortisone acetate BPCRS* in *methanol.* Solution (4) contains 0.15% w/v of *hydrocortisone BPCRS* and 0.15% w/v of *hydrocortisone acetate BPCRS* in *methanol.* After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight. The principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatograms obtained with solutions (2) and (3). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

B. In the Assay for miconazole nitrate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to miconazole nitrate in the chromatogram obtained with solution (2).

C. In the Assay for hydrocortisone acetate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to hydrocortisone acetate in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a 10% w/v dispersion in *carbon dioxide-free water*, 3.5 to 4.5, Appendix V L.

Related substances

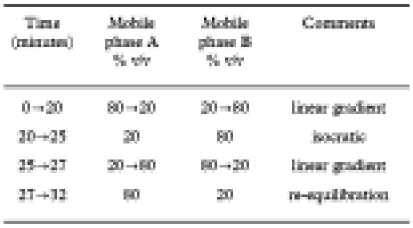
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 25 mg of Miconazole Nitrate with 30 ml of *methanol* for 30 minutes, add sufficient *methanol* to produce 50 ml and filter (Whatman GF/F is suitable). For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 235 nm.

Inject 20 µl of each solution.

Mobile phase A A solution containing 0.01M *ammonium dihydrogen orthophosphate* adjusted to pH 6.0 with *dilute ammonia R1*.

Mobile phase B A mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of *acetonitrile*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0→20	80→20	20→80	linear gradient
20→25	20	80	isocratic
25→27	20→80	80→20	linear gradient
27→32	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times are: hydrocortisone acetate, about 8 minutes; miconazole, about 18 minutes.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

In the chromatogram obtained with solution (1), the area of any peak other than those of Hydrocortisone acetate and Miconazole is not greater than the area of the miconazole peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak due to nitrate ion and any peak with an area less than 0.2 times the area of the peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For miconazole nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 50 ml of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter if necessary. Solution (2) contains 0.050% w/v of *miconazole nitrate BPCRS* in *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2,4-dichlorobenzenemethanol*.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the preparation being examined using the declared content of $C_{18}H_{14}Cl_4N_2O.HNO_3$ in *miconazole nitrate BPCRS.*

For hydrocortisone acetate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 25 mg of Hydrocortisone Acetate with 50 ml of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter if necessary. Solution (2) contains 0.025% w/v of *hydrocortisone acetate BPCRS* in *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2,4-dichlorobenzenemethanol*.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

Calculate the content of $C_{23}H_{32}O_6$ in the preparation being examined using the declared content of $C_{23}H_{32}O_6$ in *hydrocortisone acetate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Miconazole Nitrate and the following:

2,4-dichlorobenzenemethanol,

2,4-dichloro-β-[2,4-dichlorophenyl)-methoxy]benzene ethanamine,

N-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)-methoxy]ethyl]formamide.

Miconazole and Hydrocortisone Cream

General Notices

Action and use

Antifungal azole.

DEFINITION

Miconazole and Hydrocortisone Cream contains Miconazole Nitrate and Hydrocortisone in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$

95.0 to 105.0% of the stated amount.

Content of hydrocortisone, $C_{21}H_{30}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *ammonium acetate,* 40 volumes of *dioxan* and 40 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the cream containing 0.15 g of Miconazole Nitrate by shaking with 25 ml of *methanol* for about 30 minutes, dilute to 50 ml with *methanol,* filter and use the filtrate. Solution (2) contains 0.3% w/v of *miconazole nitrate BPCRS* in *methanol.* Solution (3) contains 0.15% w/v of *hydrocortisone BPCRS* in *methanol.* Solution (4) contains 0.15% w/v of *hydrocortisone BPCRS* and 0.15% w/v of *hydrocortisone acetate BPCRS* in *methanol.* After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight. The principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatograms obtained with solutions (2) and (3). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

B. In the Assay for miconazole nitrate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to miconazole nitrate in the chromatogram obtained with solution (2).

C. In the Assay for hydrocortisone, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a 10% w/v dispersion in *carbon dioxide-free water*, 2.6 to 3.6, Appendix V L.

Related substances

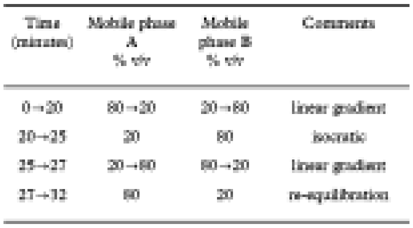
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 25 mg of Miconazole Nitrate with 30 ml of *methanol* for 30 minutes, add sufficient *methanol* to produce 50 ml and filter (Whatman GF/F is suitable). For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 235 nm.

Inject 20 µl of each solution.

Mobile phase A A solution containing 0.01M *ammonium dihydrogen orthophosphate* adjusted to pH 6.0 with *dilute ammonia R1*.

Mobile phase B A mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of *acetonitrile*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0→20	80→20	20→80	linear gradient
20→25	20	80	isocratic
25→27	20→80	80→20	linear gradient
27→32	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times are: hydrocortisone, about 8 minutes; miconazole, about 18 minutes.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

In the chromatogram obtained with solution (1), the area of any peak other than those due to Hydrocortisone and Miconazole is not greater than the area of the peak due to miconazole in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak due to nitrate ion and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For miconazole nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 50 ml of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter if necessary. Solution (2) contains 0.050% w/v of *miconazole nitrate BPCRS* in *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the preparation being examined using the declared content of $C_{18}H_{14}Cl_4N_2O.HNO_3$ in *miconazole nitrate BPCRS.*

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 25 mg of Hydrocortisone with 50 ml of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter if necessary. Solution (2) contains 0.025% w/v of *hydrocortisone BPCRS* in *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

Calculate the content of $C_{21}H_{30}O_5$ in the preparation being examined using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS.*

STORAGE

Miconazole and Hydrocortisone Cream should be stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Miconazole Nitrate and the following:

2,4-dichlorobenzenemethanol,

2,4-dichloro-β-[2,4-dichlorophenyl)-methoxy]benzene ethanamine,

N-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)-methoxy]ethyl]formamide.

Miconazole and Hydrocortisone Ointment

General Notices

Action and use

Antifungal azole.

DEFINITION

Miconazole and Hydrocortisone Ointment contains Miconazole Nitrate and Hydrocortisone in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$

95.0 to 105.0% of the stated amount.

Content of hydrocortisone, $C_{21}H_{30}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography,* Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *ammonium acetate,* 40 volumes of *dioxan* and 40 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the ointment containing 0.15 g of Miconazole Nitrate by shaking with 25 ml of *tetrahydrofuran* for about 30 minutes, dilute to 50 ml with *methanol,* filter and use the filtrate. Solution (2) contains 0.3% w/v of *miconazole nitrate BPCRS* in equal volumes of *methanol* and *tetrahydrofuran.* Solution (3) contains 0.15% w/v of *hydrocortisone BPCRS* in equal volumes of *methanol* and *tetrahydrofuran.* Solution (4) contains 0.15% w/v of *hydrocortisone BPCRS* and 0.15% w/v of *hydrocortisone acetate BPCRS* in equal volumes of *methanol* and *tetrahydrofuran.* After removal of the plate, allow it to dry in air and expose to iodine vapour until spots appear and examine in daylight. The principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatograms obtained with solutions (2) and (3). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

B. In the Assay for miconazole nitrate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to miconazole nitrate in the chromatogram obtained with solution (2).

C. In the Assay for hydrocortisone, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to hydrocortisone in the chromatogram obtained with solution (2).

TESTS

Related substances

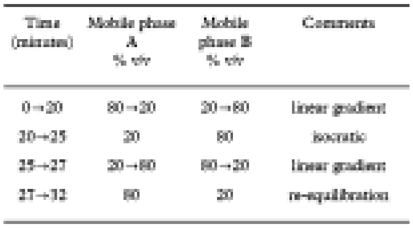
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the ointment containing 50 mg of Miconazole Nitrate with 50 ml of *tetrahydrofuran* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter (Whatman GF/F is suitable). For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2, 4-dichlorobenzenemethanol* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil BDS is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 235 nm.

Inject 20 µl of each solution.

Mobile phase A A solution containing 0.01M *ammonium dihydrogen orthophosphate* adjusted to pH 6.0 with *dilute ammonia R1*.

Mobile phase B A mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of *acetonitrile*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0→20	80→20	20→80	linear gradient
20→25	20	80	isocratic
25→27	20→80	80→20	linear gradient
27→32	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention times are: hydrocortisone, about 8 minutes; miconazole, about 18 minutes.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

In the chromatogram obtained with solution (1), the area of any peak other than those of Hydrocortisone and Miconazole is not greater than the area of the miconazole peak in the chromatogram obtained with solution (2) (0.5%) and the total area of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak due to nitrate ion and any peak with an area less than 0.2 times the area of the peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

For miconazole nitrate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the ointment containing 50 mg of Miconazole Nitrate with 50 ml of *tetrahydrofuran* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter if necessary. Solution (2) contains 0.050% w/v of *miconazole nitrate BPCRS* in *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the preparation being examined using the declared contents of $C_{18}H_{14}Cl_4N_2O.HNO_3$ in *miconazole nitrate BPCRS.*

For hydrocortisone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the ointment containing 25 mg of Hydrocortisone with 50 ml of *tetrahydrofuran* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter if necessary. Solution (2) contains 0.025% w/v of *hydrocortisone BPCRS* in *methanol*. Solution (3) contains 0.02% w/v each of *miconazole impurity A EPCRS* and *2,4-dichlorobenzenemethanol* in *methanol*.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 5.0.

Calculate the content of $C_{21}H_{30}O_5$ in the preparation being examined using the declared content of $C_{21}H_{30}O_5$ in *hydrocortisone BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include impurity A listed under Miconazole Nitrate and the following:

2,4-dichlorobenzenemethanol,

2,4-dichloro-β-[2,4-dichlorophenyl)-methoxy]benzene ethanamine,

N-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)-methoxy]ethyl]formamide.

Miconazole Cream

General Notices

Action and use

Antifungal azole.

DEFINITION

Miconazole Cream contains Miconazole Nitrate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Mix a quantity containing 40 mg of Miconazole Nitrate with 20 ml of a mixture of 1 volume of 1M *sulphuric acid* and 4 volumes of *methanol* and shake with two 50 ml quantities of *hexane*, discarding the organic layers. Make the aqueous phase alkaline with 2M *ammonia* and extract with two 40 ml quantities of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and dilute the filtrate to 100 ml with *chloroform*. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits maxima at 264, 272 and 280 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak due to miconazole in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 30 ml of a mixture of equal volumes of *methanol* and *tetrahydrofuran* for 30 minutes, add sufficient of the same solvent mixture to produce 50 ml and filter through a glass microfibre filter (Whatman GF/C is suitable). For solution (2) dilute 5 volumes of solution (1) to 100 volumes with a mixture of equal volumes of *methanol* and *tetrahydrofuran* and 5 volumes of the resulting solution to 100 volumes with the same solvent mixture. Solution (3) contains 0.0025% w/v of *miconazole nitrate BPCRS* and 0.0025% w/v of *econazole nitrate BPCRS* in a mixture of equal volumes of *methanol* and *tetrahydrofuran*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil 3 ODS is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a

0.6% w/v solution of *ammonium acetate* in a mixture of 300 volumes of *acetonitrile*, 320 volumes of *methanol* and 380 volumes of *water* and (c) a detection wavelength of 235 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to miconazole and econazole is at least 10. If necessary adjust the composition of the mobile phase.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 30 ml of a mixture of equal volumes of *methanol* and *tetrahydrofuran* for 30 minutes, add sufficient of the same solvent mixture to produce 50 ml and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) contains 0.1% w/v of *miconazole nitrate BPCRS* in a mixture of equal volumes of *methanol* and *tetrahydrofuran.* Solution (3) contains 0.0025% w/v of *miconazole nitrate BPCRS* and 0.0025% w/v of *econazole nitrate BPCRS* in a mixture of equal volumes of *methanol* and *tetrahydrofuran*.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to miconazole and econazole is at least 10. If necessary adjust the composition of the mobile phase.

Calculate the content of $C_{18}H_{14}Cl_4N_2O,HNO_3$ in the cream using the declared content of $C_{18}H_{14}Cl_4N_2O,HNO_3$ in *miconazole nitrate BPCRS*.

STORAGE

If Miconazole Cream is kept in aluminium tubes, their inner surfaces should be coated with a suitable lacquer.

Miconazole Oromucosal Gel

General Notices

Action and use

Antifungal azole.

DEFINITION

Miconazole Oromucosal Gel is a suspension of Miconazole, in *very fine powder*, in a suitable water-miscible basis. It may be flavoured.

The oromucosal gel complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of miconazole, $C_{18}H_{14}Cl_4N_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the gel containing 20 mg of Miconazole with sufficient *methanol* to produce a 0.05% w/v solution. The *light absorption* of this solution, Appendix II B, in the range 250 to 350 nm exhibits two maxima at 272 and 280 nm and a broad shoulder at about 263 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the gel containing 50 mg of Miconazole with 25 ml of *methanol* for 30 minutes, add sufficient *methanol* to produce 50 ml and filter through a glass microfibre filter (Whatman GF/C is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 5 volumes of the resulting solution to 20 volumes with *methanol*. Solution (3) contains 0.0025% w/v each of *miconazole nitrate BPCRS* and *econazole nitrate BPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a 0.6% w/v solution of *ammonium acetate* in a mixture of 300 volumes of *acetonitrile*, 320 volumes of *methanol* and 380 volumes of *water* and (c) a detection wavelength of 235 nm.

Inject 10 µl of solution (3). The test is not valid unless the *resolution factor* between the peaks due to miconazole and econazole is at least 10.

Inject separately 10 µl each of solution (1) and solution (2). For solution (1) continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the gel containing 50 mg of Miconazole with 25 ml of *methanol* for 30 minutes, add sufficient *methanol* to produce 50 ml and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) contains 0.12% w/v of *miconazole nitrate BPCRS* in *methanol*. Solution (3) contains 0.0025% w/v each of *miconazole nitrate BPCRS* and *econazole nitrate BPCRS* in *methanol*.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to miconazole and econazole is at least 10.

Calculate the content of $C_{18}H_{14}Cl_4N_2O$ in the gel using the declared content of $C_{18}H_{14}Cl_4N_2O$ in *miconazole nitrate BPCRS*.

STORAGE

Miconazole Oromucosal Gel should be protected from light.

Midazolam Injection

General Notices

Action and use

Benzodiazepine.

DEFINITION

Midazolam Injection is a sterile solution of Midazolam in Water for Injections containing Hydrochloric Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of midazolam, $C_{18}H_{13}ClFN_3$

95.0% to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing 20 mg of Midazolam add sufficient 5M *ammonia* to make the solution just alkaline, extract with two 10 ml quantities of *dichloromethane*, dry the combined extracts over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of midazolam *(RS 235)*.

B. In the test for Related substances, the chromatogram obtained with solution (1) shows a peak with the same retention time as that due to Midazolam in the chromatogram obtained with solution (3).

TESTS

Acidity

pH, 2.9 to 3.7, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Allow all the solutions to stand protected from light for at least 2 hours before testing. For solution (1) dilute a volume of the injection containing 10 mg of Midazolam to 20 ml with the mobile phase. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.001% w/v each of N-*desalkylflurazepam BPCRS* and *midazolam BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-18 non-endcapped is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 28 volumes of solution A prepared as described below and 72 volumes of *methanol* and (c) a detection wavelength of 220 nm. Maintain the temperature of the column at 40°. To prepare solution A mix equal volumes of 0.1M *orthophosphoric acid* and 0.03M *triethylamine* and adjust the pH to 3.5 by the addition of 0.1M *sodium hydroxide*.

For solution (1) allow the chromatography to continue for 3 times the retention time of the principal peak. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to midazolam and *N*-desalkylflurazepam is at least 3.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Allow all the solutions to stand protected from light for at least 2 hours before testing. For solution (1) dilute a volume of the injection containing 10 mg of Midazolam to 100 ml with the mobile phase. Solution (2) contains 0.01% w/v of *midazolam BPCRS* in the mobile phase. Solution (3) contains 0.001% w/v each of N-*desalkylflurazepam BPCRS* and *midazolam BPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to midazolam and *N*-desalkylflurazepam is at least 3.0.

Calculate the content of $C_{18}H_{13}ClFN_3$ in the injection using the declared content of $C_{18}H_{13}ClFN_3$ in *midazolam BPCRS*.

STORAGE

Midazolam Injection should be protected from light.

Minocycline Tablets

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Minocycline Tablets contain Minocycline Hydrochloride Dihydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of minocycline, $C_{23}H_{27}N_3O_7$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Extract a quantity of the powdered tablets containing the equivalent of 10 mg of minocycline with 20 ml of *methanol*, centrifuge and use the supernatant liquid.

(2) 0.05% w/v of *minocycline hydrochloride EPCRS* in *methanol*.

(3) 0.05% w/v of each of *minocycline hydrochloride EPCRS* and *doxycycline hyclate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel GF*$_{254}$ as the coating substance. Adjust the pH of a 10.0% w/v solution of *disodium edetate* to 9.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 ml for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Dry the plate at 110° for 1 hour immediately before use.

(b) The mobile phase is a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane.*

(c) Develop to 15 cm.

(d) Application volume of 2 µl for each solution.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (254 nm)*.

VALIDITY

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Shake a quantity of the powdered tablets containing the equivalent of 50 mg of minocycline with 5 ml of *water* and filter. The filtrate yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter, discarding the first few ml of filtrate. Dilute the filtered solution, if necessary, with 0.1M *hydrochloric acid* to give a solution expected to contain the equivalent of about 0.001% w/v of minocycline. Measure the *absorbance* of the solution at the maximum at 348 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of minocycline, $C_{23}H_{27}N_3O_7$, in the medium from the *absorbance* obtained from a 0.001% w/v solution of *minocycline hydrochloride EPCRS* in 0.1M *hydrochloric acid* and using the declared content of $C_{23}H_{27}N_3O_7$,HCl in *minocycline hydrochloride EPCRS*. Each mg of $C_{23}H_{27}N_3O_7$,HCl is equivalent to 0.9261 mg of $C_{23}H_{27}N_3O_7$.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Shake a quantity of the powdered tablets containing the equivalent of 25 mg of minocycline with 50 ml of *water*, dilute to 100 ml with the same solvent and filter.

(2) Dilute 1 volume of solution (1) to 50 volumes with *water*.

(3) Dilute 1.2 volumes of solution (1) to 100 volumes with *water*.

(4) Dissolve 10 mg of *minocycline hydrochloride EPCRS* in *water* and evaporate to dryness on a water bath. Dissolve the residue in 10 ml of mobile phase and add sufficient mobile phase to produce 25 ml.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (20 cm × 4.6 mm) packed with end-capped *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 280 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 25 volumes of a 0.4% w/v solution of *sodium edetate*, 27 volumes of *dimethylformamide* and 50 volumes of a 2.8% w/v solution of *ammonium oxalate*, adjusted to pH 7.0 using a 10.4% w/v solution of *tetrabutylammonium hydroxide*.

For solution (1) allow the chromatography to proceed for 1.5 times the retention time of minocycline.

When the chromatograms are recorded using the prescribed conditions the retention time of minocycline is about 15 minutes. The chromatogram obtained with solution (3) shows a peak with a retention time relative to minocycline of about 0.8 [4-epiminocycline (Ph.Eur. impurity A)].

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to minocycline and 4-epiminocycline is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak due to 4-epiminocycline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

— the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.2%);

— the sum of the areas of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Shake a quantity of the powdered tablets containing the equivalent of 25 mg of minocycline with 50 ml of water, dilute to 100 ml with the same solvent, filter and dilute 10 ml of the filtrate to 20 ml with water.

(2) 0.0125% w/v of *minocycline hydrochloride EPCRS* in *water*.

(3) Dissolve 10 mg of *minocycline hydrochloride EPCRS* in *water* and evaporate to dryness on a water bath. Dissolve the residue in 10 ml of mobile phase and add sufficient mobile phase to produce 25 ml.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

When the chromatograms are recorded using the prescribed conditions the retention time of minocycline is about 15 minutes. The chromatogram obtained with solution (3) shows a peak with a retention time relative to minocycline of about 0.8 [4-epiminocycline (Ph.Eur. impurity A)].

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{27}N_3O_7$ in the tablets from the chromatograms obtained and using the declared content of $C_{23}H_{27}N_3O_7$,HCl in *minocycline hydrochloride EPCRS*. Each mg of $C_{23}H_{27}N_3O_7$,HCl is equivalent to 0.9261 mg of $C_{23}H_{27}N_3O_7$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of minocycline.

Minoxidil Scalp Application

General Notices

Action and use

Vasodilator; treatment of hypertension; male pattern baldness.

DEFINITION

Minoxidil Scalp Application is a *cutaneous solution* of Minoxidil in a suitable ethanolic solvent. *The scalp application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.*

Content of minoxidil, $C_9H_{15}N_5O$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

Evaporate a volume of the scalp application containing about 50 mg of Minoxidil to dryness at a temperature of about 50^{c} under a stream of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of minoxidil *(RS 376)*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a suitable volume of the scalp application with the mobile phase to produce a solution containing 0.025% w/v of Minoxidil. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) add 1 ml of solution (1) to 10 ml of a 0.0025% w/v solution of *deoxyminoxidil EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 10 µm is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute, 3.0 g of *docusate sodium* in a mixture of 10 ml of *glacial acetic acid*, 300 ml of *water* and 700 ml of *methanol*, adjusting the pH of the resulting solution to 3.0 with *perchloric acid* and (c) a detection wavelength of 240 nm. Record the chromatogram obtained with solution (1) for twice the retention time of the principal peak.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to minoxidil and deoxyminoxidil is at least 2.0.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%). Disregard any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) prepare a solution containing 0.025% w/v of Minoxidil by serial dilution of the scalp application with the mobile phase. Solution (2) contains 0.025% w/v of *minoxidil BPCRS* in the mobile phase. Solution (3) contains 0.025% w/v of *deoxyminoxidil EPCRS* in solution (1).

The chromatographic procedure may be carried out as described under Related substances but using a detection wavelength of 254 nm.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to minoxidil and deoxyminoxidil is at least 2.0.

Calculate the content of $C_9H_{15}N_5O$ in the scalp application using the declared content of $C_9H_{15}N_5O$ in *minoxidil BPCRS*.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous solution'.

Mitobronitol Tablets

General Notices

Action and use

Cytotoxic.

DEFINITION

Mitobronitol Tablets contain Mitobronitol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of mitobronitol, $C_6H_{12}Br_2O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.5 g of Mitobronitol with 10 ml of hot *methanol*, filter and allow the filtrate to cool. The crystalline precipitate, after filtering and drying at 105° for 2 hours, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mitobronitol *(RS 236)*.

B. Dissolve 0.1 g in 10 ml of 1M *sodium hydroxide*, boil, cool, acidify with 2M *nitric acid* and add 1 ml of *silver nitrate solution*. A pale yellow, curdy precipitate is produced.

TESTS

Ionic halide

Shake a quantity of powdered tablets containing 1 g of Mitobronitol with 50 ml of *water* for 15 minutes, dilute to 100 ml with *water* and filter. To 50 ml of the filtrate add 10 ml of 2M *nitric acid* and 5 ml of 0.01M *silver nitrate VS* and mix. Titrate the excess of silver nitrate with 0.01M *ammonium thiocyanate VS* using *ammonium iron(III) sulphate solution R2* as indicator. Not more than 3.2 ml of 0.01M *silver nitrate VS* is required.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.2 g of Mitobronitol add 40 ml of 0.5M *sodium hydroxide*, heat to boiling, cool, add 25 ml of 0.1M *silver nitrate VS*, acidify with 5 ml of 5M *nitric acid* and titrate the excess of silver nitrate with 0.1M *ammonium thiocyanate VS* determining the end point potentiometrically. Each ml of 0.1M *silver nitrate VS* is equivalent to 15.40 mg of $C_6H_{12}Br_2O_4$.

STORAGE

Mitobronitol Tablets should be protected from light.

Mitoxantrone Intravenous Infusion

General Notices

Action and use

Cytotoxic.

DEFINITION

Mitoxantrone Intravenous Infusion is a sterile solution of Mitoxantrone Hydrochloride. It is prepared immediately before use by diluting Sterile Mitoxantrone Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the intravenous infusion, if necessary, with *water BET* to give a solution containing the equivalent of 2 mg of mitoxantrone per ml (solution A). The endotoxin limit concentration of solution A is 10 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

LABELLING

The strength is stated in terms of the equivalent amount of mitoxantrone in a suitable dose-volume.

STERILE MITOXANTRONE CONCENTRATE

DEFINITION

Sterile Mitoxantrone Concentrate is a sterile solution of Mitoxantrone Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of mitoxantrone, $C_{22}H_{28}N_4O_6$

90.0 to 105.0% of the stated amount.

CHARACTERISTICS

A dark blue solution.

IDENTIFICATION

A. Dilute a volume of the concentrate containing the equivalent of 2 mg of mitoxantrone to about 100 ml with *water* and add 20 ml of 1M *hydrochloric acid* and sufficient *water* to produce 200 ml. The *light absorption* of the solution, Appendix II B, in the range 220 nm to

350 nm shows a maximum at about 240 nm and a shoulder at 274 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH of a solution diluted, if necessary, with *water* to contain the equivalent of 0.2% w/v of mitoxantrone, 3.0 to 4.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the concentrate containing the equivalent of 20 mg of mitoxantrone to 50 ml with the mobile phase. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.2% w/v of *mitoxantrone impurity A EPCRS* and 0.04% w/v of *mitoxantrone hydrochloride EPCRS* in the mobile phase. For solution (4) dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with particles of silica the surface of which has been coated with chemicallybonded phenyl groups (10 µm) (µBondapak Phenyl is suitable), (b) as the mobile phase with a flow rate of 3 ml per minute a mixture of 750 volumes of *water*, 250 volumes of *acetonitrile* and 25 volumes of a solution prepared by dissolving 22 g of *sodium heptanesulphonate* in about 150 ml of *water*, filtering through a 0.45-µm filter, washing the filter with *water,* adding 32 ml of *glacial acetic acid* to the combined filtrate and washings and diluting to 250 ml with *water* and (c) a detection wavelength of 254 nm.

Inject separately 50 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

Record the chromatogram obtained with solution (1) for three times the retention time of the principal peak. In the chromatogram obtained, the area of any peak with a retention time of 1.69 relative to mitoxantrone (impurity D) is not greater than three times the area of the peak in the chromatogram obtained with solution (2) (3%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* other than any peak corresponding to mitoxantrone impurity D is not greater than five times the area of the peak in the chromatogram obtained with solution (2) (5%). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the concentrate containing the equivalent of 20 mg of mitoxantrone to 50 µl with the mobile phase. Solution (2) contains 0.04% w/v of *mitoxantrone hydrochloride EPCRS* in the mobile phase. Solution (3) contains 0.2% w/v of *mitoxantrone impurity A EPCRS* in solution (2).

The chromatographic procedure described under Related substances may be used.

Inject separately 50 µl of each solution. The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

Calculate the content of $C_{22}H_{28}N_4O_6$ from the peak areas in the chromatograms obtained using the declared content of $C_{22}H_{30}Cl_2N_4O_6$ in *mitoxantrone hydrochloride EPCRS* and taking each mg of $C_{22}H_{30}Cl_2N_4O_6$ to be equivalent to 0.8951 mg of $C_{22}H_{28}N_4O_6$.

LABELLING

The label states the concentration in terms of the equivalent amount of mitoxantrone in a suitable volume.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mitoxantrone Hydrochloride.

Mometasone Aqueous Nasal Spray

General Notices

Action and use

Glucocorticoid.

DEFINITION

Mometasone Aqueous Nasal Spray is an aqueous suspension of Mometasone Furoate in a suitable pressurised container fitted with an appropriate nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. In the test for Related substances the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (5).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to mometasone furoate in the chromatogram obtained with solution (5).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Discharge the container a sufficient number of times to obtain 1 mg of Mometasone Furoate, add 4 ml of *acetone*, mix with the aid of ultrasound and filter. Evaporate the filtrate to dryness and dissolve in 1 ml of *acetone*.

(2) 0.002% w/v of *mometasone furoate BPCRS* in *acetone*.

(3) Dilute 1 volume of solution (2) to 2 volumes with *acetone*.

(4) Dilute 1 volume of solution (2) to 4 volumes with *acetone*.

(5) 0.1% w/v of *mometasone furoate BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) 3 volumes of *methanol* and 97 volumes of *1,2-dichloroethane* as the mobile phase.

(c) Application volume of 50 µl for each solution.

(d) After removal of the plate, allow it to dry in air, spray with *alkaline tetrazolium blue solution* and heat at 50° for 5 minutes. Cool and spray again with *alkaline tetrazolium blue solution*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (2%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) discharge the container a sufficient number of times to obtain 1 mg of Mometasone Furoate, add 20 ml of hot *methanol (90%)* and add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80%)*. Repeat the extraction of the *2,2,4-trimethylpentane* layer with two further 10 ml quantities of *methanol (80%)*, filtering the extracts through the absorbent cotton. Combine the extracts and add sufficient *methanol (80%)* to produce 50 ml. Flter through a 0.45 µm nylon filter (Millipore Millex-HN is suitable).

(2) 0.002% w/v of *mometasone furoate BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC PROCEDURE

(a) Stainless steel column (10 cm × 5 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Column temperature of 60°.

(e) Detection wavelength of 238 nm.

(f) Injection volume of 20 µl.

MOBILE PHASE

A mixture of 45 volumes of *water* and 55 volumes of *methanol*.

SYSTEM SUITABILITY

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to mometasone furoate is about 9 minutes.

The test is not valid unless the *symmetry factor* of the peak due to mometasone furoate is not more than 1.2.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the preparation being examined using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS*.

LABELLING

The label states the amount of active ingredient delivered by each actuation of the valve and the number of deliveries available from the container.

Mometasone Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Mometasone Cream contains Mometasone Furoate in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the cream containing 0.5 mg of Mometasone Furoate in 20 ml of *methanol (80%)* by heating on a water bath until the solution begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *dichloromethane*, shake vigorously, allow the layers to separate, evaporate the *dichloromethane* layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *dichloromethane*.

(2) 0.025% w/v of *mometasone furoate BPCRS* in *dichloromethane*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase.

(c) Application volume of 10 µl for each solution.

(d) After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and, while hot, spray with *alcoholic sulphuric acid solution* and heat at 105° for a further 5 minutes.

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to mometasone furoate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the cream containing 1 mg of Mometasone Furoate in 20 ml of hot *methanol (90%)*, add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80%)*. Repeat the extraction of the *2,2,4-trimethylpentane* layer with two further 10 ml quantities of *methanol (80%)*, filtering the extracts through the absorbent cotton. Combine the extracts and add sufficient *methanol (80%)* to produce 50 ml. Flter through a 0.45 µm nylon filter (Millipore Millex-HN is suitable).

(2) 0.002% w/v of *mometasone furoate BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC PROCEDURE

(a) Stainless steel column (10 cm × 5 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Column temperature of 60°.

(e) Detection wavelength of 238 nm.

(f) Injection volume of 20 µl.

MOBILE PHASE

A mixture of 45 volumes of *water* and 55 volumes of *methanol*.

SYSTEM SUITABILITY

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to mometasone furoate is about 9 minutes.

The test is not valid unless the *symmetry factor* of the peak due to mometasone furoate is not more than 1.2.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the preparation being examined using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS*.

STORAGE

Mometasone Cream should be protected from light.

Mometasone Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Mometasone Ointment contains Mometasone Furoate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the ointment containing 0.5 mg of Mometasone Furoate in 20 ml of *methanol (80%)* by heating on a water bath until the solution begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *dichloromethane*, shake vigorously, allow the layers to separate, evaporate the *dichloromethane* layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *dichloromethane*.

(2) 0.025% w/v of *mometasone furoate BPCRS* in *dichloromethane*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase.

(c) Apply to the plate 10 µl of solutions (1), (2) and (3).

(d) After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and, while hot, spray with *alcoholic sulphuric acid solution* and heat at 105° for a further 5 minutes.

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to mometasone furoate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the ointment containing 1 mg of Mometasone Furoate in 20 ml of hot *methanol (90%)*, add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80%)*. Repeat the extraction of the *2,2,4-trimethylpentane* layer with two further 10 ml quantities of *methanol (80%)*, filtering the extracts through the absorbent cotton. Combine the extracts and add sufficient *methanol (80%)* to produce 50 ml. Flter through a 0.45 µm nylon filter (Millipore Millex-HN is suitable).

(2) 0.002% w/v of *mometasone furoate BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (10 cm × 5 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Column temperature of 60°.

(e) Detection wavelength of 238 nm

(f) Injection volume of 20 µl.

MOBILE PHASE

A mixture of 45 volumes of *water* and 55 volumes of *methanol*.

SYSTEM SUITABILITY

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to mometasone furoate is about 9 minutes.

The test is not valid unless the *symmetry factor* of the peak due to mometasone furoate is not more than 1.2.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the preparation being examined using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS*.

STORAGE

Mometasone Ointment should be protected from light.

Mometasone Scalp Application

General Notices

Action and use

Glucocorticoid.

DEFINITION

Mometasone Scalp Application is a cutaneous suspension of Mometasone Furoate in a suitable liquid basis.

The scalp application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of mometasone furoate, $C_{27}H_{30}Cl_2O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Disperse a quantity of the scalp application containing 0.5 mg of Mometasone Furoate in 20 ml of *methanol (80%)* by heating on a water bath until the solution begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *dichloromethane*, shake vigorously, allow the layers to separate, evaporate the *dichloromethane* layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *dichloromethane*.

(2) 0.025% w/v of *mometasone furoate BPCRS* in *dichloromethane*.

(3) A mixture of equal volumes of solutions (1) and (2).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane* as the mobile phase.

(c) Application volume of 10 µl for each solution.

(d) After removal of the plate, allow it to dry in air until the solvent has evaporated, heat at 105° for 5 minutes and, while hot, spray with *alcoholic sulphuric acid solution* and heat at 105° for a further 5 minutes.

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to mometasone furoate in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the scalp application containing 1 mg of Mometasone Furoate in 20 ml of hot *methanol (90%)*, add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80%)*. Repeat the extraction of the *2,2,4-trimethylpentane* layer with two further 10 ml quantities of *methanol (80%)*, filtering the extracts through the absorbent cotton. Combine the extracts and add sufficient *methanol (80%)* to produce 50 ml.

(2) 0.002% w/v of *mometasone furoate BPCRS* in *methanol (80%)*.

CHROMATOGRAPHIC PROCEDURE

(a) Stainless steel column (10 cm × 5 mm) packed with *base-deactivated, end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Column temperature of 60°.

(e) Detection wavelength of 238 nm.

(f) Injection volume of 20 µl.

MOBILE PHASE

A mixture of 45 volumes of *water* and 55 volumes of *methanol*.

When the chromatograms are recorded under the prescribed conditions the retention time of the peak due to mometasone furoate is about 9 minutes.

SYSTEM SUITABILITY

The test is not valid unless the *symmetry factor* of the peak due to mometasone furoate is not more than 1.2.

DETERMINATION OF CONTENT

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the preparation being examined using the declared content of $C_{27}H_{30}Cl_2O_6$ in *mometasone furoate BPCRS*.

STORAGE

Mometasone Scalp Application should be protected from light.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous suspension'.

Morphine and Atropine Injection

General Notices

Action and use

Opioid receptor agonist; analgesic; anticholinergic.

DEFINITION

Morphine and Atropine Injection is a sterile isotonic solution containing 1.0% w/v of Morphine Sulphate and 0.06% w/v of Atropine Sulphate in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$

0.054 to 0.066% w/v.

Content of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$

0.92 to 1.07% w/v.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 3 volumes of 13.5M *ammonia* and 200 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 1 ml of 5M *ammonia* to 1 ml of the injection, extract with two 5 ml quantities of *chloroform*, filter the combined extracts through *anhydrous sodium sulphate*, evaporate to dryness in a current of warm air and dissolve the residue in 0.5 ml of *chloroform*. For solution (2) treat 1 ml of a 0.06% w/v solution of *atropine sulphate BPCRS* in the same manner as for solution (1). For solution (3) treat 1 ml of a 1% w/v solution of *morphine sulphate BPCRS* in the same manner as for solution (1). After removal of the plate, allow it to dry in a current of air and spray with *dilute potassium iodobismuthate solution*. The spots in the chromatogram obtained with solution (1) correspond to the spots in the chromatograms obtained with solutions (2) and (3).

B. In the Assay for atropine sulphate, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

C. In the Assay for morphine sulphate, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel plates are suitable) and a freshly prepared mixture of 2.5

volumes of 18M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute the injection, if necessary, with a mixture of equal volumes of *ethanol (96%)* and *water* to produce a solution containing 1% w/v of Morphine Sulphate. For solution (2) dissolve 50 mg of *codeine phosphate* in 5 ml of solution (1) and dilute 1 volume of the resulting solution to 200 volumes with a mixture of equal volumes of *ethanol (96%)* and *water*. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with a mixture of equal volumes of *ethanol (96%)* and *water*. Solution (4) contains 0.06% w/v of *atropine sulphate* in a mixture of equal volumes of *ethanol (96%)* and *water*. After removal of the plate, dry it in a current of air, spray with *potassium iodobismuthate solution*, dry for 15 minutes in a current of air and spray with *hydrogen peroxide solution (10 vol)*. Examine the plates immediately. In the chromatograms obtained with solutions (1) and (2) the spots due to codeine are bluish-grey and the spots due to morphine are pinkish. In the chromatogram obtained with solution (1) any spot corresponding to codeine is not more intense than the spot due to codeine in the chromatogram obtained with solution (2) (0.5%), any other *secondary spot* is not more intense than the spot due to morphine in the chromatogram obtained with solution (2) (0.5%) and not more than two such spots are more intense than the spot due to morphine in the chromatogram obtained with solution (3) (0.2%). Disregard the spot in the chromatogram obtained with solution (1) which corresponds to the spot for atropine sulphate in the chromatogram obtained with solution (4). The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots.

ASSAY

For atropine sulphate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the injection being examined. Solution (2) contains 0.06% w/v of *atropine sulphate BPCRS* in *methanol (60%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid* and (c) a detection wavelength of 257 nm.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ using the declared content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in *atropine sulphate BPCRS*.

For morphine sulphate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 1 volume of the injection to 10 volumes with *methanol (60%)*. Solution (2) contains 0.1% w/v of *morphine sulphate BPCRS* in *methanol (60%)*.

The chromatographic procedure described under Assay for atropine sulphate may be used but with a detection wavelength of 285 nm.

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulphate BPCRS*.

Morphine Sulphate Injection

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Sulphate Injection is a sterile solution of Morphine Sulphate in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$

92.5 to 107.5% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

B. Evaporate a volume containing 5 mg of Morphine Sulphate to dryness on a water bath. Dissolve the residue in 5 ml of *water* and add 0.15 ml of *dilute potassium hexacyanoferrate (III) solution* and 0.05 ml of *iron(III) chloride solution R1*. A bluish-green colour is produced immediately, which changes rapidly to blue.

C. Yields reaction A characteristic of *sulphates*, Appendix VI.

TESTS

Related substances

Complies with the test described under Morphine Suppositories but using the following solutions. For solution (1) dilute the injection, if necessary, with a mixture of equal volumes of *ethanol (96%)* and *water* to produce a solution containing 1% w/v of Morphine Sulphate. For solution (2) dissolve 50 mg of *codeine phosphate* in 5 ml of solution (1) and dilute 1 volume of the resulting solution to 200 volumes with a mixture of equal volumes of *ethanol (96%)* and *water*. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with a mixture of equal volumes of *ethanol (96%)* and *water*.

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots. Disregard any spot with an Rf value of less than 0.1.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For solution (1) dilute the injection to contain 0.1% w/v of Morphine Sulphate with *methanol (60%)*. Solution (2) contains 0.1% w/v of *morphine sulphate BPCRS* in *methanol (60%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid* and (c) a detection wavelength of 285 nm.

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulphate BPCRS*.

STORAGE

Morphine Sulphate Injection should be protected from light.

Morphine Suppositories

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Suppositories contain Morphine Hydrochloride or Morphine Sulphate in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of morphine

As the hydrochloride, $C_{17}H_{19}NO_3,HCl,3H_2O$, or as the sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$, 95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a quantity of the suppositories containing 90 mg of Morphine Hydrochloride or Morphine Sulphate in 25 ml of *chloroform*, extract with 50 ml of 0.01M *hydrochloric acid* and wash the aqueous layer with two 20 ml quantities of *chloroform*. Make the aqueous layer alkaline with *ammonia buffer pH 10.0* and extract with two 40 ml quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *propan-2-ol* . Dry the combined extracts with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of morphine *(RS 237)*.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel plate* (Merck plates are suitable) and a mixture of 2.5 volumes of 13.5M *ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70%)* and 35 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the mixed suppositories prepared for the Assay containing 0.1 g of Morphine Hydrochloride or Morphine Sulphate in 10 ml of hot *water*, mix vigorously, allow to cool until the mass sets and filter the lower, aqueous layer. For solution (2) dissolve 50 mg of *codeine phosphate* in 5 ml of solution (1) and dilute 1 volume of the resulting solution to 200 volumes with *water*. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with *water*. After removal of the plate, dry it in a current of air, spray with *potassium iodobismuthate solution*, dry for 15 minutes in a current of air and spray with *hydrogen peroxide solution (10 vol)*. The spot due to codeine is bluish-grey and the spot due to morphine is pinkish. In the chromatogram obtained with solution (1) any spot corresponding to codeine is not more intense than the spot due to codeine in the chromatogram obtained with solution (2) (0.5%), any other *secondary spot* is not more intense than the spot due to morphine in the chromatogram obtained with solution (2) (0.5%) and not more than two such spots are more intense than the spot due to morphine in the chromatogram obtained with solution (3) (0.2%).

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 30 mg of *morphine sulphate BPCRS* in 60 ml of 0.01M *hydrochloric acid*, add 20 ml of 0.3M *dipotassium hydrogen orthophosphate* and dilute to 100 ml with *water*. For solution (2) weigh 10 suppositories, melt together by warming and allow to cool, stirring continuously until the mass has set. Dissolve a quantity containing 30 mg of Morphine Hydrochloride or Morphine Sulphate in 25 ml of *chloroform*, extract with two quantities of 40 ml and 20 ml of 0.01M *hydrochloric acid*, wash the combined aqueous layers with two 20 ml quantities of *chloroform*, add 20 ml of 0.3M *dipotassium hydrogen orthophosphate* and dilute to 100 ml with *water*. Solution (3) contains 0.03% w/v of *morphine sulphate BPCRS* and 0.04% w/v of *codeine phosphate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute, a solution containing 0.22% w/v of *dioctyl sodium sulphosuccinate* and 0.14% w/v of *sodium acetate* in a mixture of 60 volumes of *methanol* and 40 volumes of *water*, the pH of the solution being adjusted to 5.5 with *glacial acetic acid*, and (c) a detection wavelength of 285 nm.

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the peaks due to morphine and codeine is at least 2.0.

Calculate the content of $C_{17}H_{19}NO_3,HCl,3H_2O$ or $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$, as appropriate, using the declared content of $C_{17}H_{19}NO_3$ in *morphine sulphate BPCRS*. Each g of $C_{17}H_{19}NO_3$ is equivalent to 1.317 g of $C_{17}H_{19}NO_3,HCl,3H_2O$ or 1.330 g of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

LABELLING

The label states the quantity of Morphine Hydrochloride or of Morphine Sulphate in each suppository.

Morphine Tablets

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Morphine Tablets contain Morphine Sulphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 20 mg of Morphine Sulphate with 5 ml of *water*, filter and add to the filtrate 0.05 ml of *iron(III) chloride solution R1*. A blue colour is produced.

B. Extract a quantity of the powdered tablets containing 10 mg of Morphine Sulphate with 10 ml of *water*, filter and to 5 ml of the filtrate add 0.15 ml of *dilute potassium hexacyanoferrate (III) solution* and 0.05 ml of *iron(III) chloride solution R1*. A bluish-green colour is produced immediately which changes rapidly to blue.

C. The powdered tablets yield the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 6.5* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.001% w/v of *morphine sulphate BPCRS* in *phosphate buffer pH 6.5*. Solution (2) is the filtered dissolution medium, diluted with *phosphate buffer pH 6.5*, if necessary, to produce a solution containing about 0.001% w/v of Morphine Sulphate.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of 50 volumes of *methanol* and 50 volumes of 0.01M *sodium heptanesulphonate* in 0.1M *acetic acid* as the mobile phase with a flow rate of 2.0 ml per minute and (c) a detection wavelength of 211 nm.

Inject 50 µl of each solution. Calculate the total content of morphine sulphate, $(C_{17}H_{19}NO_3)_2$, $H_2SO_4,5H_2O$, in the medium using the declared content of $C_{17}H_{19}NO_3$ in *morphine sulphate*

BPCRS. Each g of $C_{17}H_{19}NO_3$ is equivalent to 1.330 g of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

Related substances

Complies with the test described under Morphine Suppositories but using 50 µl of each of the following solutions. For solution (1) remove any coating from the tablets and shake a quantity of the powdered tablet cores containing 10 mg of Morphine Sulphate with 10 ml of a mixture of *ethanol (96%)* and *water* for 10 minutes and filter. For solution (2) dissolve 5 mg of *codeine phosphate* in 5 ml of solution (1) and dilute 1 volume of the resulting solution to 200 volumes with a mixture of equal volumes of *ethanol (96%)* and *water*. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with a mixture of equal volumes of *ethanol (96%)* and *water*.

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots. Disregard any spot with an Rf value of less than 0.1.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Morphine Sulphate with 25 ml of *water* and 5 ml of 1M *sodium hydroxide*, add 1 g of *ammonium sulphate*, shake to dissolve, add 20 ml of *ethanol (96%)* and extract with successive quantities of 40, 20, 20 and 20 ml of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol (96%)*. Wash each extract with the same 5 ml of *water*, filter and evaporate the solvent. Dissolve the residue in 10 ml of 0.05M *hydrochloric acid VS*, boil, cool, add 15 ml of *water* and titrate the excess of acid with 0.05M *sodium hydroxide VS* using *methyl red solution* as indicator. Each ml of 0.05M *hydrochloric acid VS* is equivalent to 18.97 mg of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

STORAGE

Morphine Tablets should be protected from light.

Moxisylyte Tablets

General Notices

Action and use

Alpha-adrenoceptor antagonist.

DEFINITION

Moxisylyte Tablets contain Moxisylyte Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of moxisylyte, $C_{16}H_{25}NO_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 20 mg of moxisylyte with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of moxisylyte hydrochloride *(RS 238)*.

B. Dilute 20 ml of a 0.03% w/v solution to 100 ml with *water* (solution A). Dilute a further 20 ml of the same solution to 100 ml with 0.1M *sodium hydroxide* (solution B). The ratio of the *absorbance* of solution B at 302 nm, measured 30 minutes after preparation, to that of solution A at 275 nm is about 1.7, Appendix II B.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0040% w/v of *2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride BPCRS* and 0.00050% w/v of *2-thymoxyethyldimethylamine hydrochloride BPCRS* in the mobile phase. For solution (2) shake a quantity of the powdered tablets containing the equivalent of 0.18 g of moxisylyte with 100 ml of the mobile phase for 15 minutes, centrifuge and use the clear supernatant liquid. For solution (3) dilute 1 volume of solution (2) to 100 volumes with the mobile phase. For solution (4) dilute 1 volume of solution (3) to 10 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) 0.005M *sodium hexanesulphonate* in a mixture of 2 volumes of *glacial acetic acid*, 185 volumes of *water* and 315 volumes of *methanol* as the mobile phase with a flow rate of 1.0 ml per minute and (c) a detection wavelength of 276 nm.

The peaks in the chromatogram obtained with solution (1) are due to (a) 2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride and (b) 2-thymoxyethyldimethylamine hydrochloride in order of their elution. In the chromatogram obtained with solution (2) the areas of any peaks corresponding to (a) and (b) are not greater than the areas of the corresponding peaks in the chromatogram obtained with solution (1) (2% of 2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride and 0.25% of 2-thymoxyethyldimethylamine hydrochloride respectively), the area of any peak with a retention time relative to moxisylyte of 2.3 is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1% of 2-(6-chlorothymoxy)-ethyldimethylamine hydrochloride) and the area of any peak with a retention time relative to moxisylyte of 0.9 is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.1% of 2-(6-acetylthymoxy)ethyldimethylamine).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 13 mg of moxisylyte with 150 ml of 0.1M *hydrochloric acid*, add sufficient 0.1M *hydrochloric acid* to produce 250 ml and filter. Measure the *absorbance* of the filtrate at the maximum at 275 nm, Appendix II B. Calculate the content of $C_{16}H_{25}NO_3$ taking 79.7 as the value of *A* (1%, 1 cm) at the maximum at 275 nm.

STORAGE

Moxisylyte Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of moxisylyte.

40 mg of moxisylyte is equivalent to 45.2 mg of Moxisylyte Hydrochloride.

Mupirocin Cream

General Notices

Action and use

Antibacterial.

DEFINITION

Mupirocin Cream contains Mupirocin Calcium in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mupirocin, $C_{26}H_{44}O_9$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the cream containing the equivalent of 100 mg of mupirocin in a mixture of 20 ml of *dichloromethane* and 4 ml of 0.2M *sodium borate buffer pH 8.4,* with shaking, and centrifuge at 3000 revolutions per minute until separate layers are obtained. The upper layer (which may be cloudy) is separated from the lower layer by a band of creamy solid. Remove and retain the upper aqueous layer and re-extract the lower layer with a further 4 ml of 0.2M *sodium borate buffer pH 8.4.* Combine the aqueous extracts, add 10 ml of *dichloromethane* to the combined extracts and centrifuge at 3000 revolutions per minute. Remove the upper layer and adjust the pH to about 4 with 1M *hydrochloric acid* (a slight cloudiness may be observed), add 10 ml of *dichloromethane* and centrifuge. Separate and retain the lower layer; add a further 10 ml of *dichloromethane* to the upper layer and centrifuge. Combine the dichloromethane extracts and evaporate to dryness at room temperature under a current of nitrogen; a jelly-like residue is obtained. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of mupirocin *(RS 425)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the cream containing the equivalent of 50 mg of mupirocin in 5 ml of a mixture of 3 volumes of *tetrahydrofuran* and 1 volume of *water* and shake mechanically for 15 minutes. Add 5 ml of *acetate buffer pH 4.0* and mix thoroughly. Centrifuge for about 15 minutes; remove the lower layer and filter through a 0.5-µm filter. Dilute 1 ml of this solution to 50 ml with a solution containing equal volumes of *acetate buffer pH 4.0* and a mixture of 3 volumes of *tetrahydrofuran* and 1 volume of *water*, mix and filter through a

0.5-µm filter.

(2) 0.0002% w/v of *mupirocin lithium EPCRS* in 0.1M *phosphate buffer solution pH 6.3*.

(3) A solution containing equal volumes of *acetate buffer pH 4.0* and a mixture of 3 volumes of *tetrahydrofuran* and 1 volume of *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use a column temperature of 30°.

(e) Use a detection wavelength of 240 nm.

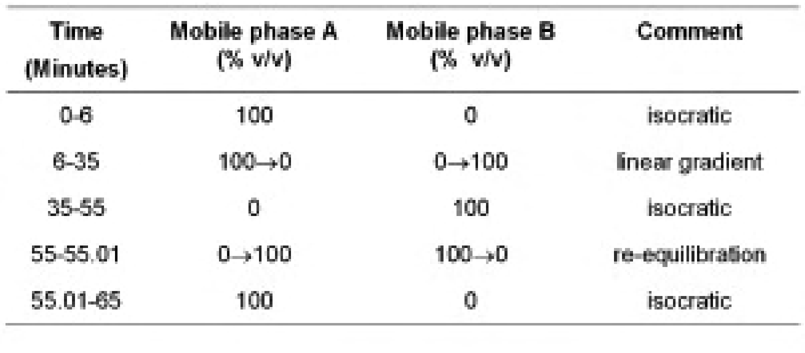
(f) Inject 20 µl of each solution.

MOBILE PHASE

Mobile phase A Dissolve 5.8 g of *glacial acetic acid* in 900 ml of *water*, adjust the pH to 5.7 with *ammonium hydroxide* and add sufficient *water* to produce 1000 ml (0.1M ammonium acetate solution). Mix 25 volumes of *tetrahydrofuran* and 75 volumes of the 0.1M ammonium acetate solution.

Mobile phase B 30 volumes of *tetrahydrofuran* and 70 volumes of 0.1M ammonium acetate solution.

Use the following gradient. Allow the system to equilibrate for 10 minutes at the start.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-6	100	0	isocratic
6-35	100→0	0→100	linear gradient
35-55	0	100	isocratic
55-55.01	0→100	100→0	re-equilibration
55.01-65	100	0	isocratic

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is between 19.4 and 23.7 minutes (about 21.5 minutes).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.7 (mupirocin impurity C) is at least 3.0;

the *column efficiency*, determined on the peak due to mupirocin, is at least 7000 *theoretical plates* per metre;

the *symmetry factor* of the principal peak is at most 1.75.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to mupirocin impurity C is not greater than 3.2 times the area of the principal peak in the chromatogram obtained with solution (2) (3.2%, with respect to the content of mupirocin);

the area of any peak with a retention time relative to mupirocin of about 0.6 (mupirocin impurity D) is not greater than 8.5 times the area of the principal peak in the chromatogram obtained with solution (2) (8.5%, with respect to the content of mupirocin);

the area of any peak with a retention time relative to mupirocin of about 0.63 (mupirocin impurity E) is not greater than 16 times the area of the principal peak in the chromatogram obtained with solution (2) (16%, with respect to the content of mupirocin);

the area of any other *secondary peak* is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%, with respect to the content of mupirocin);

the sum of the areas of all the *secondary peaks* is not greater than 30 times the area of the principal peak in the chromatogram obtained with solution (2) (30%, with respect to the content of mupirocin).

Disregard any peaks corresponding to the peaks in the chromatogram obtained with solution (3) and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%, with respect to the content of mupirocin).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the cream containing the equivalent of 10 mg of mupirocin in 25 ml of *tetrahydrofuran*, add 50 ml of 0.1M *phosphate buffer solution pH 6.3* and shake vigorously. Add sufficient 0.1M *phosphate buffer solution pH 6.3* to produce 100 ml and mix; filter the solution through a 0.5-µm filter and use the filtrate.

(2) 0.01% w/v of *mupirocin lithium EPCRS* in 0.1M *phosphate buffer solution pH 6.3.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

28 volumes of *acetonitrile* and 72 volumes of 0.1M *phosphate buffer solution pH 6.3*.

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 5 minutes.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.77 (mupirocin impurity C) is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{44}O_9$ in the cream using the declared content of $C_{26}H_{43}O_9Li$ in *mupirocin lithium EPCRS*. Each mg of $C_{26}H_{43}O_9Li$ is equivalent to 0.9882 mg of $C_{26}H_{44}O_9$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of mupirocin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mupirocin Calcium.

Mupirocin Nasal Ointment

General Notices

Action and use

Antibacterial.

DEFINITION

Mupirocin Nasal Ointment contains Mupirocin Calcium in a suitable basis.
The nasal ointment complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of mupirocin, $C_{26}H_{44}O_9$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the nasal ointment containing the equivalent of 100 mg of mupirocin in a mixture of 20 ml of *dichloromethane* and 4 ml of 0.2M *sodium borate buffer pH 8.4,* with shaking, and centrifuge at 3000 revolutions per minute until separate layers are obtained. Remove and retain the upper aqueous layer and re-extract the lower layer with a further 4 ml of 0.2M *sodium borate buffer pH 8.4.* Combine the aqueous extracts, add 10 ml of *dichloromethane* to the combined extracts and centrifuge at 3000 revolutions per minute. Remove the upper aqueous layer and adjust the pH to about 4 with 1M *hydrochloric acid* (a slight cloudiness may be observed), add 10 ml of *dichloromethane* and centrifuge. Separate and retain the lower layer; add a further 10 ml of *dichloromethane* to the upper layer and centrifuge. Combine the dichloromethane extracts and evaporate to dryness at room temperature under a current of nitrogen; a jelly-like residue is obtained. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of mupirocin *(RS 425)*.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the nasal ointment containing the equivalent of 50 mg of mupirocin in 5 ml of a solution containing 3 volumes of *tetrahydrofuran* and 1 volume of *water* and shake vigorously on a mechanical shaker for 1 hour. Add 5 ml of a solution prepared by dissolving 13.6 g of *sodium acetate* in 1000 ml of *water*, mixing and adjusting the pH to 4.0 with *glacial acetic acid*, shake for a further 15 minutes. Allow the layers to separate and filter the lower aqueous layer (Whatman GF/F filter paper is suitable).

(2) Dilute 1 volume of solution (1) to 50 volumes with a solution containing 3 volumes of

tetrahydrofuran and 1 volume of *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable) fitted with a suitable stainless steel guard column packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

25 volumes of *tetrahydrofuran* and 75 volumes of a solution prepared by dissolving 7.7 g of *ammonium acetate* in 900 ml of *water*, adjusting the pH to 5.7 with 0.1M *glacial acetic acid* and diluting to 1000 ml with *water.*

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 12 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.75 (mupirocin impurity C) is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity C is not greater than 4% by *normalisation*;

the area of any other *secondary peak* is not greater than 1% by *normalisation*;

the sum of the areas of all the *secondary peaks* is not greater than 10% by *normalisation*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the nasal ointment containing the equivalent of 10 mg of mupirocin in 50 ml of a solution containing 3 volumes of *tetrahydrofuran* and 1 volume of *water* and shake vigorously on a mechanical shaker for 1 hour. Add 50 ml of a solution prepared by dissolving 13.6 g of *sodium acetate* in 1000 ml of *water*, mixing and adjusting the pH to 4.0 with *glacial acetic acid*, shake for a further 15 minutes and filter (Whatman GF/F filter paper is suitable).

(2) 0.01% w/v of *mupirocin lithium EPCRS* in a solution containing 3 volumes of *tetrahydrofuran* and 1 volume of *water.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable) fitted with a suitable stainless steel guard column packed with the same material.

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

25 volumes of *tetrahydrofuran* and 75 volumes of a solution prepared by dissolving 7.7 g of *ammonium acetate* in 900 ml of *water*, adjusting the pH to 5.7 with 0.1M *glacial acetic acid* and diluting to 1000 ml with *water.*

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 12 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.75 (mupirocin impurity C) is at least 3.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{44}O_9$ in the nasal ointment using the declared content of $C_{26}H_{43}O_9Li$ in *mupirocin lithium EPCRS*. Each mg of $C_{26}H_{43}O_9Li$ is equivalent to 0.9882 mg of $C_{26}H_{44}O_9$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of mupirocin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mupirocin Calcium.

Mupirocin Ointment

General Notices

Action and use

Antibacterial.

DEFINITION

Mupirocin Ointment contains Mupirocin in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of mupirocin, $C_{26}H_{44}O_9$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the ointment containing 50 mg of Mupirocin in 50 ml of 0.1M *phosphate buffer solution pH 6.3*, swirl and allow to stand (with occasional swirling) until dissolution is almost complete. Shake vigorously for 10 minutes and add sufficient 0.1M *phosphate buffer solution pH 6.3* to produce 100 ml. Solution (2) contains 0.00054% w/v of *mupirocin lithium EPCRS* in 0.1M *phosphate buffer solution pH 6.3*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable) fitted with a stainless steel guard column packed with the same material (Novapak C8 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 28 volumes of *tetrahydrofuran* and 72 volumes of a solution prepared by dissolving 7.7 g of *ammonium acetate* in 900 ml of *water*, adjusting the pH to 5.7 with 0.1M *glacial acetic acid* and diluting to 1000 ml with *water* and (c) a detection wavelength of 240 nm.

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 13 minutes. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.65 (mupirocin impurity C) is at least 3.5.

In the chromatogram obtained with solution (1): the area of any peak corresponding to mupirocin impurity C is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (4%, with respect to the content of mupirocin); the area of any peak with a retention time relative to mupirocin of about 0.5 (mupirocin impurity D) is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%, with respect to the content of mupirocin); the area of any peak with a retention time relative to mupirocin of about 0.55 (mupirocin impurity E) is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (10%, with respect to the content of mupirocin); the area of any other *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%, with respect to the content of mupirocin) and the sum of the areas of all the *secondary peaks* is not greater than 20 times the area of the principal peak in the chromatogram obtained with solution (2) (20%, with respect to the content of mupirocin). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%, with respect to the content of mupirocin).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the ointment containing 50 mg of Mupirocin in 100 ml of 0.1M *phosphate buffer solution pH 6.3*, swirl and allow to stand (with occasional swirling) until dissolution is complete. Add 56 ml of *acetonitrile* and sufficient 0.1M *phosphate buffer solution pH 6.3* to produce 200 ml. Solution (2) contains 0.027% w/v of *mupirocin lithium EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 28 volumes of *acetonitrile* and 72 volumes of 0.1M *phosphate buffer solution pH 6.3* and (c) a detection wavelength of 230 nm.

When the chromatograms are recorded under the prescribed conditions the retention time of mupirocin is about 5 minutes. The assay is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peak due to mupirocin and the peak with a retention time relative to mupirocin of about 0.77 (mupirocin impurity C) is at least 1.5.

Calculate the content of $C_{26}H_{44}O_9$ in the ointment using the declared content of $C_{26}H_{43}O_9Li$ in *mupirocin lithium EPCRS*. Each mg of $C_{26}H_{43}O_9Li$ is equivalent to 0.9882 mg of $C_{26}H_{44}O_9$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Mupirocin.

Prolonged-release Minocycline Capsules

General Notices

Action and use

Tetracycline antibacterial.

Prolonged-release Minocycline Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Prolonged-release Minocycline Capsules contain Minocycline Hydrochloride Dihydrate. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Minocycline Hydrochloride. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of minocycline, $C_{23}H_{27}N_3O_7$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Add 150 ml of *methanol* to a quantity of the mixed capsule contents containing the equivalent of 0.1 g of minocycline, mix with the aid of ultrasound, add sufficient *methanol* to produce 200 ml and filter (Whatman No. 41 paper is suitable).

(2) 0.05% w/v of *minocycline hydrochloride EPCRS* in *methanol*.

(3) 0.05% w/v of each of *minocycline hydrochloride EPCRS* and *oxytetracycline hydrochloride EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC octadecylsilyl silica gel* F_{254} *plate* (Merck RP18 F_{254S} plates are suitable).

(b) The mobile phase is a mixture of 20 volumes of *acetonitrile*, 20 volumes of *methanol* and 60 volumes of a 6.3% w/v solution of *oxalic acid* previously adjusted to pH 2.0 with *ammonia*.

(c) Develop to 15 cm.

(d) Application volume of 5 µl for each solution.

(e) After removal of the plate allow it to dry in air and examine under *ultraviolet light (254 nm).*

VALIDITY

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Add 80 ml of mobile phase to a quantity of the mixed capsule contents containing the equivalent of 0.1 g of minocycline and mix with the aid of ultrasound. Add sufficient mobile phase to produce 100 ml, filter and dilute 25 ml of the filtrate to 100 ml with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) Dissolve 10 mg of *minocycline hydrochloride EPCRS* in *water* and evaporate to dryness on a water bath. Dissolve the residue in 10 ml of mobile phase and add sufficient mobile phase to produce 25 ml.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (20 cm × 4.6 mm) packed with end-capped *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 280 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 25 volumes of a 0.4% w/v solution of *sodium edetate*, 27 volumes of *dimethylformamide* and 50 volumes of a 2.8% w/v solution of *ammonium oxalate*, adjusted to pH 7.0 using a 10.4% w/v solution of *tetrabutylammonium hydroxide*.

For solution (1) allow the chromatography to proceed for 1.5 times the retention time of minocycline.

When the chromatograms are recorded using the prescribed conditions the retention time of minocycline is about 15 minutes. The chromatogram obtained with solution (3) shows a peak with a retention time relative to minocycline of about 0.8 [4-epiminocycline (Ph.Eur. impurity A)].

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to minocycline and 4-epiminocycline is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to 4-epiminocycline is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of any other *secondary peak* is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%);

the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light. Store the solutions at a temperature of 2° to 8° and use within 3 hours of preparation.

(1) Add 80 ml of mobile phase to a quantity of the mixed contents of 20 capsules containing the equivalent of 0.1 g of minocycline and mix with the aid of ultrasound. Add sufficient mobile phase to produce 100 ml, filter and dilute 5 ml of the filtrate to 100 ml with mobile phase.

(2) 0.005% w/v of *minocycline hydrochloride EPCRS* in the mobile phase.

(3) Dissolve 10 mg of *minocycline hydrochloride EPCRS* in *water* and evaporate to dryness on a water bath. Dissolve the residue in 10 ml of mobile phase and add sufficient mobile phase to produce 25 ml.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

When the chromatograms are recorded using the prescribed conditions the retention time of minocycline is about 15 minutes. The chromatogram obtained with solution (3) shows a peak with a retention time relative to minocycline of about 0.8 [4-epiminocycline (Ph.Eur. impurity A)].

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to minocycline and 4-epiminocycline is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{27}N_3O_7$ in the capsules from the chromatograms obtained and using the declared content of $C_{23}H_{27}N_3O_7$,HCl in *minocycline hydrochloride EPCRS*. Each mg of $C_{23}H_{27}N_3O_7$,HCl is equivalent to 0.9261 mg of $C_{23}H_{27}N_3O_7$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of minocycline.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Minocycline Hydrochloride Dihydrate.

Prolonged-release Morphine Tablets

General Notices

Prolonged-release Morphine Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Prolonged-release Morphine Tablets contain Morphine Sulphate. They are formulated so that the medicament is released over a period of several hours. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of morphine sulphate. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 20 mg of Morphine Sulphate with 5 ml of *water*, filter and add to the filtrate 0.05 ml of *iron(III) chloride solution R1*. A blue colour is produced.

B. Extract a quantity of the powdered tablets containing 10 mg of Morphine Sulphate with 10 ml of *water*, filter and to 5 ml of the filtrate add 0.15 ml of *dilute potassium hexacyanoferrate (III) solution* and 0.05 ml of *iron(III) chloride solution R1*. A bluish-green colour is produced immediately which changes rapidly to blue.

C. The powdered tablets yield the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Pseudomorphine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of powdered tablets containing 30 mg of Morphine Sulphate with 50 ml of the mobile phase, with the aid of ultrasound if necessary, dilute to 100 ml with the same solvent, mix and filter. Solution (2) contains 0.00033% w/v of *pseudomorphine trihydrate BPCRS* in the mobile phase. Solution (3) contains 0.03% w/v of *morphine sulphate BPCRS* and 0.0003 % w/v of *pseudomorphine BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 0.05 volume of *orthophosphoric acid*, 30 volumes of *acetonitrile* and 70 volumes of 0.01M *sodium octanesulphonate* and (c) a detection wavelength of 281 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to morphine and pseudomorphine is at least 4.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to pseudomorphine is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

Related substances

Comply with the test described under Morphine Suppositories but using 50 µl of each of the following solutions. For solution (1) remove any coating from the tablets and shake a quantity of the powdered tablet cores containing 10 mg of Morphine Sulphate with 10 ml of a mixture of *ethanol (96%)* and *water* for 10 minutes and filter. For solution (2) dissolve 5 mg of *codeine phosphate* in 5 ml of solution (1) and dilute 1 volume of the resulting solution to 200 volumes with a mixture of equal volumes of *ethanol (96%)* and *water*. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with a mixture of equal volumes of *ethanol (96%)* and *water*.

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots. Disregard any spot with an Rf value of less than 0.1.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of powdered tablets containing 30 mg of Morphine Sulphate with 50 ml of the mobile phase, with the aid of ultrasound if necessary, dilute to 100 ml with the same solvent, mix and filter. Solution (2) contains 0.03% w/v of *morphine sulphate BPCRS* and 0.00033% w/v of *pseudomorphine trihydrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 0.05 volume of *orthophosphoric acid*, 30 volumes of *acetonitrile* and 70 volumes of 0.01M *sodium octanesulphonate* and (c) a detection wavelength of 281 nm.

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to morphine and pseudomorphine is at least 4.0.

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ using the declared content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in *morphine sulphate BPCRS*.

STORAGE

Prolonged-release Morphine Tablets should be protected from light.

Gastro-resistant Naproxen Tablets

General Notices

Enteric-coated Naproxen Tablets

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Gastro-resistant Naproxen Tablets contain Naproxen. They are made gastro-resistant by enteric-coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of naproxen, $C_{14}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Remove the coating from a number of tablets and powder the uncoated cores. Extract a quantity of the powdered tablets containing 0.2 g of Naproxen with 20 ml of *methanol*, shake for 15 minutes, filter, evaporate the filtrate and dry the residue at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of naproxen *(RS 244)*.

TESTS

Dissolution

Prepare a phosphate buffer pH 7.4 by dissolving 2.62 g of *sodium dihydrogen orthophosphate monohydrate* and 11.5 g of *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 ml and adjusting the pH to 7.4, if necessary.

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. After 2 hours, withdraw about 10 ml of the medium from the centre of the vessel, filter through a 5-µm filter and measure the *absorbance* of the solution, Appendix II B, at 332 nm using 0.1M *hydrochloric acid* in the reference cell. Measure the *absorbance* of a suitable solution of *naproxen BPCRS* in the phosphate buffer pH 7.4 and calculate the total content of naproxen, $C_{14}H_{14}O_3$, in the medium using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS.* The amount of naproxen released is not more than 5% of the stated amount.

Replace the 0.1M hydrochloric acid in the vessel by 900 ml of the phosphate buffer pH 7.4 previously held at a temperature of 36.5° to 37.5°. After 45 minutes, withdraw a sample of 10 ml of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium, if necessary, at 332 nm using the phosphate buffer in the reference cell. Measure the *absorbance* of a suitable solution of *naproxen BPCRS* in the phosphate buffer and calculate the total content of naproxen, $C_{14}H_{14}O_3$, in the medium using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*. The amount of naproxen released is not less than 70% of the stated amount.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* GF_{254} *plate* and a mixture of 3 volumes of *glacial acetic acid*, 9 volumes of *tetrahydrofuran* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.5 g of Naproxen with 10 ml of *methanol* for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Naproxen with 70 ml of *methanol* for 10 minutes with the aid of ultrasound, add sufficient *methanol* to produce 100 ml and centrifuge at 3000 revolutions per minute for 10 minutes. Dilute 1 volume of the supernatant liquid to 10 volumes with a mixture of 32 volumes of *water* and 68 volumes of *methanol*. For solution (2) dilute 1 volume of a 0.1% w/v solution of *naproxen BPCRS* in *methanol* to 10 volumes with a mixture of 32 volumes of *water* and 68 volumes of *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 32 volumes of 0.01M *orthophosphoric acid* and 68 volumes of *methanol* and (c) a detection wavelength of 262 nm.

Calculate the content of $C_{14}H_{14}O_3$ in the tablets from the chromatograms obtained using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

STORAGE

Gastro-resistant Naproxen Tablets should be protected from light.

Nabumetone Oral Suspension

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Nabumetone Oral Suspension contains Nabumetone in a suitable flavoured vehicle.
The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nabumetone, $C_{15}H_{16}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel GF_{254} precoated plate (Merck 5715 plates are suitable) and *chloroform* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) add a volume of the well-shaken suspension containing 0.1 g of Nabumetone to 10 ml of *chloroform*, shake, allow to separate and use the lower layer. Solution (2) contains 1.0% w/v of *nabumetone BPCRS* in *chloroform*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position to the spot in the chromatogram obtained with solution (2) and the chromatogram obtained with solution (3) shows a single, compact spot at the same Rf value as the spot in the chromatogram obtained with solution (2).

B. In the Assay the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the peak in the chromatogram obtained with solution (2).

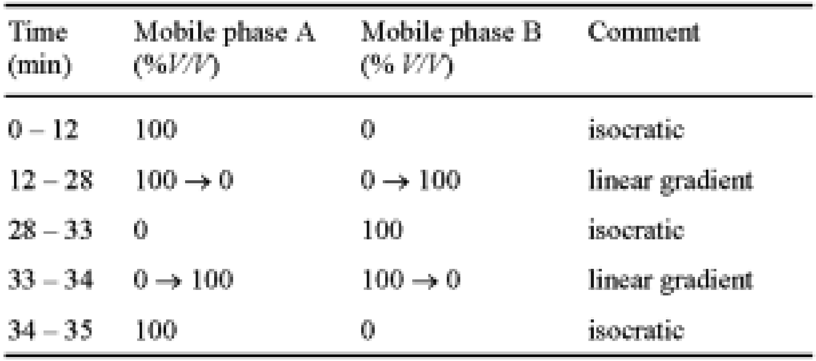
Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a volume of the suspension containing 0.5 g of Nabumetone with 10 ml of *methanol* until completely dispersed, add sufficient *acetonitrile* to produce 100 ml, shake for a further 5 minues and filter (Whatman No. 1 paper is suitable). Solution (2) contains 0.0025% w/v of *nabumetone BPCRS* in *acetonitrile*. Solution (3) contains 0.0015% w/v of *nabumetone impurity F BPCRS* in *acetonitrile*. Solution (4) contains 0.002% w/v of each of *nabumetone BPCRS* and *nabumetone impurity D EPCRS* in *acetonitrile*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (4 µm) (Genesis C18 4µ is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 254 nm. Maintain the temperature of the column at 40°.

Mobile phase A A mixture of 12 volumes of *tetrahydrofuran*, 28 volumes of *acetonitrile for* *chromatography* and 60 volumes of a 0.1% v/v solution of *glacial acetic acid* in *carbon dioxide-free water*.

Mobile phase B A mixture of 24 volumes of *tetrahydrofuran*, 56 volumes of *acetonitrile for chromatography* and 20 volumes of a 0.1% v/v solution of *glacial acetic acid* in *carbon dioxide-free water*.



Time (min)	Mobile phase A (%*V/V*)	Mobile phase B (% *V/V*)	Comment
0 – 12	100	0	isocratic
12 – 28	100 → 0	0 → 100	linear gradient
28 – 33	0	100	isocratic
33 – 34	0 → 100	100 → 0	linear gradient
34 – 35	100	0	isocratic

When the chromatograms are recorded under the prescribed conditions the retention time of nabumetone is about 11 minutes.

The test is not valid unless, in the chromatogram obtained with solution (4) the *resolution factor* between the two principal peaks is at least 1.5.

In the chromatogam obtained with solution (1) the area of any peak corresponding to nabumetone impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%) and the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add to a weighed quantity of the suspension containing 1 g of Nabumetone 70 ml of *ethanol (96%)*, mix with the aid of ultrasound for 45 minutes, add sufficient *ethanol (96%)* to produce 100 ml and filter (Whatman GF/C paper is suitable). Dilute 5 volumes of the filtrate to 100 volumes with the mobile phase and further dilute 5 volumes of the resulting solution to 50 volumes with the mobile phase. For solution (2) dilute 5 volumes of a 0.1% w/v solution of *nabumetone BPCRS* in *acetonitrile* to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (4 µm) (Genesis C18 4µ is suitable), (b) a mixture of 18 volumes of *tetrahydrofuran*, 40 volumes of a 0.1% v/v solution of *glacial acetic acid* in *carbon dioxide-free water* and 42 volumes of *acetonitrile for chromatography* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm. When the chromatograms are recorded under the prescribed conditions the retention time of nabumetone is about 4 minutes.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{16}O_2$, weight in volume, from the chromatograms obtained using the declared content of $C_{15}H_{16}O_2$ in *nabumetone BPCRS*.

Nabumetone Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Nabumetone Tablets contain Nabumetone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nabumetone, $C_{15}H_{16}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum* of the powdered tablets, Appendix II A, is concordant with the *reference spectrum* of nabumetone *(RS 239)*.

Related substances

Comply with the test described under Nabumetone Oral Suspension but using a solution prepared in the following manner as solution (1). Shake a quantity of the powdered tablets containing 0.25 g of Nabumetone with 50 ml of *acetonitrile* for 5 minutes, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) finely powder a quantity of whole tablets containing 5 g of Nabumetone, add 400 ml of *acetonitrile*, mix with the aid of ultrasound for 15 minutes, allow to cool, add sufficient *acetonitrile* to produce 500 ml, mix and filter through a glass-fibre filter (Whatman GF/C is suitable). Dilute 5 volumes of the filtrate to 100 volumes with the mobile phase and further dilute 5 volumes of the resulting solution to 50 volumes with the mobile phase. For solution (2) dilute 5 volumes of a 0.1% w/v solution of *nabumetone BPCRS* in *acetonitrile* to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (4 µm) (Genesis C18 4µ is suitable), (b) a mixture of 18 volumes of *tetrahydrofuran*, 40 volumes of a 0.1% v/v solution of *glacial acetic acid* in *carbon dioxide-free water* and 42 volumes of *acetonitrile for chromatography* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm. When the chromatograms are recorded under the prescribed conditions the retention time of nabumetone is about 4 minutes.

Calculate the content of $C_{15}H_{16}O_2$ in the tablets from the chromatograms obtained using the declared content of $C_{15}H_{16}O_2$ in *nabumetone BPCRS*.

Naftidrofuryl Capsules

General Notices

Action and use

Vasodilator.

DEFINITION

Naftidrofuryl Capsules contain Naftidrofuryl Oxalate.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of naftidrofuryl oxalate, $C_{26}H_{35}NO_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake, with the aid of ultrasound, a quantity of the powdered contents of the capsules containing 0.1 g of Naftidrofuryl Oxalate with 5 ml of *water* and filter (Whatman GF/C filter paper is suitable). Add 5 ml of 2M *sodium hydroxide* to the filtrate, extract with two 10 ml quantities of *chloroform*, wash the combined chloroform extracts with two 10 ml quantities of *water*, evaporate to dryness using a rotary evaporator and dry the oily residue over *phosphorus pentoxide* at a pressure of 2 kPa for 18 hours. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of naftidrofuryl *(RS 240)*.

B. Mix a quantity of the powdered contents of the capsules containing 0.25 g of Naftidrofuryl Oxalate with 5 ml of *water*, shake for 10 minutes and filter. Add *calcium chloride solution* to the filtrate; a white precipitate is produced. The residue dissolves in mineral acids but is practically insoluble in 2M *acetic acid* and in 6M *ammonia*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of the following solutions. For solution (1) mix with the aid of ultrasound a quantity of the powdered contents of the capsules with sufficient of the mobile phase to produce a solution containing 0.1% w/v of Naftidrofuryl Oxalate and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.001% w/v of *3-(1-naphthyl)-2-tetrahydrofurfurylpropionic acid BPCRS* in the mobile phase. Solution (3) contains 0.0002% w/v of *2-diethylaminoethyl-3-(1-naphthyl)-2-(1-naphthylmethyl)propionate oxalate BPCRS* in the mobile phase. Solution (4) contains 0.01% w/v of *naftidrofuryl oxalate BPCRS* and 0.005% w/v of *2-diethylaminoethyl-3-(1-naphthyl)-2-(1-naphthylmethyl)-propionate oxalate BPCRS* in the mobile phase.

The chromatographic conditions described under Assay may be used.

The test is not valid unless in the chromatogram obtained with solution (4) the *resolution factor* between the two principal peaks is at least 4.

Inject solution (1) and record the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 3-(1-naphthyl)-2-tetrahydrofurfurylpropionic acid is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%), the area of any peak corresponding to 2-diethylaminoethyl-3-(1-naphthyl)-2-(1-naphthylmethyl)-propionate oxalate is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.2%), the area of any other *secondary peak* is not greater than half the area of the peak in the chromatogram obtained with solution (3) (0.1%) and the sum of the areas of all the *secondary peaks* other than those corresponding to the named impurities is not greater than 1.5 times the area of the peak in the chromatogram obtained with solution (3) (0.3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of the following solutions. For solution (1) finely powder the contents of 10 capsules, mix with the aid of a spatula, dissolve with the aid of ultrasound in sufficient of the mobile phase to produce a solution containing 1% w/v of Naftidrofuryl Oxalate, filter (Whatman GF/C filter paper is suitable) and dilute 1 volume of the filtrate to 10 volumes with the mobile phase. Solution (2) contains 0.1% w/v of *naftidrofuryl oxalate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified by chemicallybonded phenyl groups (5 µm) (Spherisorb Phenyl is suitable), (b) a mixture of 40 volumes of 0.05M *sodium acetate*, adjusted to pH 4.0 with an 85% v/v solution of *orthophosphoric acid*, and 60 volumes of *acetonitrile* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 283 nm.

Calculate the content of $C_{26}H_{35}NO_7$ in the capsules using the declared content of $C_{26}H_{35}NO_7$ in *naftidrofuryl oxalate BPCRS*.

Nalidixic Acid Oral Suspension

General Notices

Action and use

Quinolone antibacterial.

DEFINITION

Nalidixic Acid Oral Suspension is a suspension of Nalidixic Acid in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nalidixic acid, $C_{12}H_{12}N_2O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

To 5 ml add 30 ml of *water* and 20 ml of *dilute sodium carbonate solution*, mix and shake with two 30 ml quantities of *chloroform*; discard the chloroform layers. Acidify the aqueous solution with 5M *hydrochloric acid*, shake with 40 ml of *chloroform*, wash the chloroform layer with 10 ml of *water* to which has been added 0.5 ml of 5M *hydrochloric acid*, filter the chloroform layer through absorbent cotton and evaporate the filtrate to dryness. Dissolve the residue in sufficient 0.1M *sodium hydroxide* to produce a solution containing 0.0008% w/v of Nalidixic Acid. The *light absorption* of the solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 258 nm and 334 nm.

ASSAY

Dilute a weighed quantity containing 0.12 g of Nalidixic Acid to 100 ml with 0.01M *sodium hydroxide*, dilute 2 ml to 250 ml with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 334 nm, Appendix II B. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{12}H_{12}N_2O_3$, weight in volume, taking 494 as the value of A(1%, 1 cm) at the maximum at 334 nm.

Nalidixic Acid Tablets

General Notices

Action and use

Quinolone antibacterial.

DEFINITION

Nalidixic Acid Tablets contain Nalidixic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nalidixic acid, $C_{12}H_{12}N_2O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 1 g of Nalidixic Acid add 50 ml of *chloroform*, shake for 15 minutes, filter and evaporate the filtrate to dryness. The residue, after drying at 105°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of nalidixic acid *(RS 241)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.0008% w/v solution in 0.1M *sodium hydroxide* exhibits maxima at 258 nm and 334 nm.

C. *Melting point*, about 228°, Appendix V A.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Rotate the paddle at 60 revolutions per minute and use as the medium 900 ml of a methanolic phosphate buffer prepared in the following manner: mix 2.3 volumes of 0.2M *sodium hydroxide* with 2.5 volumes of 0.2M *potassium dihydrogen orthophosphate* and 2.0 volumes of *methanol*, dilute to 10 volumes with *water* and, if necessary, adjust the pH to 8.6 using 1M *sodium hydroxide*. Withdraw a sample of 10 ml of the medium, filter and measure the *absorbance* of the solution, suitably diluted if necessary, at the maximum at 334 nm, Appendix II B. Calculate the total content of nalidixic acid, $C_{12}H_{12}N_2O_3$, in the medium taking 494 as the value of A(1%, 1 cm) at the maximum at 334 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of 5M *ammonia*, 20 volumes of *dichloromethane* and 70 volumes of *ethanol (96%)* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.10 g of Nalidixic Acid with 50 ml of *dichloromethane* for 15 minutes, filter, evaporate to dryness and dissolve the residue in 5 ml of *dichloromethane*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *dichloromethane* and further dilute 1 volume of the resulting solution to 2 volumes with *dichloromethane*. For solution (3) dilute 1 volume of solution (2) to 2.5 volumes with *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 0.1 g of Nalidixic Acid add 150 ml of 1M *sodium hydroxide*, shake for 3 minutes, dilute to 200 ml with 1M *sodium hydroxide*, mix and allow to stand for 15 minutes. Dilute 2 ml to 200 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 334 nm, Appendix II B, using 0.01M *sodium hydroxide* in the reference cell. Calculate the content of $C_{12}H_{12}N_2O_3$ taking 494 as the value of A(1%, 1 cm) at the maximum at 334 nm.

STORAGE

Nalidixic Acid Tablets should be protected from light.

Naloxone Injection

General Notices

Action and use

Opioid receptor antagonist.

DEFINITION

Naloxone Injection is a sterile solution of Naloxone Hydrochloride in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous naloxone hydrochloride, $C_{19}H_{21}NO_4,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance. Heat the plate at 105° for 15 minutes immediately before use. Use a mixture of 5 volumes of *methanol* and 95 volumes of the upper layer from a mixture of 60 ml of 2M *ammonia* and 100 ml of *butan-1-ol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) add 1 ml of *ammonia buffer pH 10.0* to a volume of the injection containing the equivalent of 2 mg of anhydrous naloxone hydrochloride, extract with three 20 ml quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform*, dry the combined extracts over *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *methanol*. Dilute 1 volume of this solution to 20 volumes with *methanol*. Solution (2) contains 0.010% w/v of *naloxone hydrochloride BPCRS* in *methanol*. Protect the plate from light during development. After removal of the plate, dry it in a current of air, spray with a freshly prepared 0.5% w/v solution of *potassium hexacyanoferrate(III)* in *iron(III) chloride solution R1* and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.0 to 4.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance. Heat the plate at 105° for 15 minutes immediately before use. Use a mixture of 5 volumes of *methanol* and 95 volumes of the upper layer from a mixture of 60 ml of 2M *ammonia* and 100 ml of *butan-1-ol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) add 1 ml of *ammonia buffer pH 10.0* to a volume of the injection containing the equivalent of 2 mg of anhydrous naloxone hydrochloride, extract with three 20 ml quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform*, dry the combined extracts over *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Protect the plate from light during development. After removal of the plate, dry it in a current of air, spray with a freshly prepared 0.5% w/v solution of *potassium hexacyanoferrate(III)* in *iron(III) chloride solution R1* and examine in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration of the injection, diluted if necessary with *water BET* to give a solution containing the equivalent of 0.04% w/v of anhydrous naloxone hydrochloride, is 70 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of the above defined solution calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection to contain the equivalent of 0.001% w/v of anhydrous naloxone hydrochloride with a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water*. Solution (2) contains 0.001% w/v of *naloxone hydrochloride BPCRS* in a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water*. Solution (3) contains 0.001% w/v of *naloxone hydrochloride BPCRS* and 0.0005% w/v of *noroxymorphone* in a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 to 10 µm) (Zorbax C18 7 to 8 µm is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a solution containing 0.068% w/v of *sodium octanesulphonate* and 0.1% w/v of *sodium chloride* in a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water* and (c) a detection wavelength of 229 nm.

The test is not valid unless the *resolution factor* between the peaks due to naloxone and noroxymorphone in the chromatogram obtained with solution (3) is at least 1.3.

Calculate the content of $C_{19}H_{21}NO_4$,HCl in the injection using the declared content of $C_{19}H_{21}NO_4$,HCl in *naloxone hydrochloride BPCRS*.

STORAGE

Naloxone Injection should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous naloxone hydrochloride.

When naloxone is prescribed for neonatal use, Neonatal Naloxone Injection (containing the equivalent of 20 micrograms per ml of anhydrous naloxone hydrochloride) shall be dispensed.

Nandrolone Decanoate Injection

General Notices

Action and use

Anabolic steroid; androgen.

DEFINITION

Nandrolone Decanoate Injection is a sterile solution of Nandrolone Decanoate in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of nandrolone decanoate, $C_{28}H_{44}O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate the surface of which has been modified by chemically-bonded octadecylsilyl groups (Whatman KC 18F plates are suitable) and a mixture of 20 volumes of *water*, 40 volumes of *acetonitrile* and 60 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following three solutions. For solution (1) dilute the injection with *chloroform* to give a solution containing 0.5% w/v of Nandrolone Decanoate. Solution (2) contains 0.5% w/v of *nandrolone decanoate BPCRS* in *chloroform*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air until the solvent has evaporated and heat at 100° for 10 minutes. Allow to cool and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

ASSAY

To a quantity containing 0.1 g of Nandrolone Decanoate add sufficient *chloroform* to produce 100 ml. Dilute 3 ml to 50 ml with *chloroform* and to 5 ml of this solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20 ml. Allow to stand for 45 minutes and measure the *absorbance* of the resulting solution at the maximum at 380 nm, Appendix II B, using in the reference cell 5 ml of *chloroform* treated in the same manner. Calculate the content of $C_{28}H_{44}O_3$ from the *absorbance* obtained by repeating the operation using a suitable quantity of *nandrolone BPCRS* and from the declared content of $C_{18}H_{26}O_2$ in *nandrolone BPCRS.* Each mg of $C_{18}H_{26}O_2$ is equivalent to 1.562 mg of $C_{28}H_{44}O_3$.

STORAGE

Nandrolone Decanoate Injection should be protected from light.

LABELLING

The label states that the preparation is for intramuscular injection only.

Nandrolone Phenylpropionate Injection

General Notices

Action and use

Anabolic steroid; androgen.

DEFINITION

Nandrolone Phenylpropionate Injection is a sterile solution of Nandrolone Phenylpropionate in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of nandrolone phenylpropionate, $C_{27}H_{34}O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Dissolve a volume containing 50 mg of Nandrolone Phenylpropionate in 8 ml of *petroleum spirit (boiling range, 40° to 60°)* and extract with three 8 ml quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *petroleum spirit (boiling range, 40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand for 2 hours in ice and filter. The precipitate, after washing with *water* and drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, complies with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate the surface of which has been modified by chemically-bonded octadecylsilyl groups (Whatman KC 18F plates are suitable) and a mixture of 20 volumes of *water*, 40 volumes of *acetonitrile* and 60 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following three solutions. Solution (1) contains 0.5% w/v of the dried precipitate in *chloroform*. Solution (2) contains 0.5% w/v of *nandrolone phenylpropionate BPCRS* in *chloroform*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air until the solvent has evaporated and heat at 100° for 10 minutes. Allow to cool and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. *Melting point*, about 97°, Appendix V A.

ASSAY

To a quantity containing 0.1 g of Nandrolone Phenylpropionate add sufficient *chloroform* to produce 100 ml. Dilute 3 ml to 50 ml with *chloroform* and to 5 ml of the solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20 ml. Allow to stand for 45 minutes and measure the *absorbance* of the resulting solution at the maximum at 380 nm, Appendix II B, using in the reference cell 5 ml of *chloroform* treated in the same manner. Calculate the content of $C_{27}H_{34}O_3$ from the *absorbance* obtained by repeating the operation using a 0.006% w/v solution of *nandrolone phenylpropionate BPCRS* in *chloroform* and beginning at the words 'to 5 ml…' and using the declared content of $C_{27}H_{34}O_3$ in *nandrolone phenylpropionate BPCRS*.

STORAGE

Nandrolone Phenylpropionate Injection should be protected from light.

LABELLING

The label states that the preparation is for intramuscular injection only.

Naproxen Oral Suspension

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Naproxen Oral Suspension is an aqueous suspension of Naproxen in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of naproxen, $C_{14}H_{14}O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Evaporate 50 ml of solution A obtained in the Assay to dryness using a rotary evaporator. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of naproxen *(RS 244)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.004% w/v solution in *methanol* exhibits four maxima, at 262, 271, 316 and 331 nm.

TESTS

Acidity

pH, 2.1 to 4.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 3 volumes of *glacial acetic acid*, 9 volumes of *tetrahydrofuran* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) evaporate solution A obtained in the Assay to dryness on a rotary evaporator and dissolve the residue in sufficient *methanol* to produce a solution containing 5.0% w/v of Naproxen. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

To a quantity of the oral suspension containing 0.5 g of Naproxen add 20 ml of 3.5M *hydrochloric acid*, mix, extract with three 50 ml quantities of *chloroform*, filter each extract through *anhydrous sodium sulphate*, combine the filtrates and add sufficient *chloroform* to produce 200 ml (solution A). To 5 ml of solution A add sufficient *methanol* to produce 250 ml and measure the *absorbance* of the resulting solution at the maximum at 331 nm, Appendix II B. Calculate the content of $C_{14}H_{14}O_3$ taking 81.0 as the value of A(1%, 1 cm) at the maximum at 331 nm.

Naproxen Suppositories

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Naproxen Suppositories contain Naproxen in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of naproxen $C_{14}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a quantity of the suppositories containing 0.5 g of Naproxen in 50 ml of *2,2,4-trimethylpentane* and extract with four 25 ml quantities of *methanol (80%)*. To the combined extracts add 100 ml of a 2% w/v solution of *sodium chloride*, extract with four 25 ml quantities of *chloroform*, dry the combined extracts over *anhydrous sodium sulphate*, filter and add sufficient *chloroform* to produce 200 ml (solution A). Evaporate 100 ml of solution A to dryness using a rotary evaporator. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of naproxen *(RS 244)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.004% w/v solution in *methanol* exhibits four maxima, at 262, 271, 316 and 331 nm.

Related substances

Comply with the test described under Naproxen Oral Suspension using the following solutions. For solution (1) weigh 20 suppositories and cut into small pieces. Dissolve a quantity of the suppositories containing 0.5 g of Naproxen in 50 ml of *2,2,4-trimethylpentane* and extract with four 25-ml quantities of *methanol (80%)*. Combine the extracts, add 100 ml of a 2% w/v solution of *sodium chloride*, extract with four 25-ml quantities of *chloroform* filtering each extract through a layer of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with *chloroform*. Evaporate the combined filtrates to dryness using a rotary evaporator with the aid of gentle heat. Shake the residue with 10 ml of *methanol*, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is a 0.01% w/v solution of *naproxen BPCRS* in the mobile phase. For solution (2) disperse ten suppositories in 500 ml of *methanol* on a water bath for 40 minutes with the aid of ultrasound and by swirling the flask. Cool at 5° for 1 hour, centrifuge and use the clear, supernatant liquid; if the solution is still cloudy filter through glass-fibre paper (Whatman GF/C is suitable). To 5 ml of the filtrate add sufficient of the mobile phase to produce a solution containing 0.01% w/v of naproxen. Solution (3) contains 0.01% w/v each of *naproxen BPCRS* and *2-naphthylacetic acid* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 1 volume of a 0.52% w/v solution of *sodium acetate*, adjusted to pH 5.8 using *glacial acetic acid*, and 1 volume of *methanol* and (c) a detection wavelength of 254 nm.

The assay is not valid unless the *resolution factor* between the peaks due to naproxen and 2-naphthylacetic acid in the chromatogram obtained with solution (3) is greater than 3.0.

Calculate the content of $C_{14}H_{14}O_3$ using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

STORAGE

Naproxen Suppositories should be protected from light.

Naproxen Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Naproxen Tablets contain Naproxen.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of naproxen, $C_{14}H_{14}O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 20 mg of Naproxen with sufficient *methanol* to produce 100 ml and filter. Reserve 10 ml of the filtrate for test B, evaporate the remainder and dry the residue at 105 ^{c}. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of naproxen *(RS 244)*.

B. Dilute the 10 ml portion of the filtrate reserved in test A to 100 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits maxima at 262, 271, 316 and 331 nm.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a phosphate buffer prepared by dissolving 2.62 g of *sodium dihydrogen orthophospate monohydrate* and 11.50 g of *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 ml, and adjusting the pH to 7.4 with either 0.1M *sodium hydroxide* or 0.1M *hydrochloric acid* if necessary, and rotating the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Immediately measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at 332 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *naproxen BPCRS* in the dissolution medium and calculate the total content of naproxen, $C_{14}H_{14}O_3$, in the medium using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

Related substances

Comply with the test described under Naproxen Oral Suspension using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.5 g of Naproxen with 10 ml of *methanol* for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Naproxen with 70 ml of *methanol* for 30 minutes, add sufficient *methanol* to produce 100 ml and filter. Dilute 10 ml of the filtrate to 50 ml with *methanol* and measure the *absorbance* at the maximum at 331 nm, Appendix II B. Calculate the content of $C_{14}H_{14}O_3$ from the *absorbance* obtained by repeating the operation using a 0.01% w/v solution of *naproxen BPCRS* in *methanol* and using the declared content of $C_{14}H_{14}O_3$ in *naproxen BPCRS*.

STORAGE

Naproxen Tablets should be protected from light.

Neomycin Eye Drops

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Neomycin Eye Drops are a sterile solution of Neomycin Sulphate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol* as the mobile phase. Apply separately to the plate (1) a volume of the eye drops containing 3.5 IU, (2) 1 µl of a 0.5% w/v solution of *neomycin sulphate EPCRS* in *water* and (3) 1 µl of a mixture of equal volumes of the eye drops and solution (2). After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 2 minutes. The principal red spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the principal red spot in the chromatogram obtained with solution (3) appears as a single spot.

B. Yield the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Neamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a freshly prepared 3.85% w/v solution of *ammonium acetate* as the mobile phase. Apply separately to the plate (1) a volume of the eye drops containing 3.5 IU and (2) the same volume of *water* containing 0.1 µg of *neamine EPCRS*. After removal of the plate, dry it in a current of warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution* with *water* to contain 0.5% w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives not more than a very faint blue colour with one drop of a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. Avoid prolonged exposure to the cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. Any spot corresponding to neamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Neomycin C

Carry out the method for *liquid chromatography*, Appendix III D, injecting 10 µl of each of the following solutions. For solution (1) add 1.5 ml of a freshly prepared 2% w/v solution of *1-*

fluoro-2,4-dinitrobenzene in *methanol* to 0.5 ml of a 0.10% w/v solution of *neomycin sulphate EPCRS* in 0.02M *sodium tetraborate*, heat in a water bath at 60° for 1 hour and cool; dilute the solution to 25 ml with the mobile phase, allow to stand and use the clear lower layer. For solution (2) dilute the eye drops with 0.02M *sodium tetraborate* to contain 700 IU per ml; proceed as for solution (1) but using 0.5 ml of the diluted solution in place of 0.5 ml of the neomycin sulphate solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Nucleosil 100-5 is suitable), (b) as the mobile phase with a flow rate of 1.6 ml per minute a solution prepared by mixing 97 ml of *tetrahydrofuran*, 1.0 ml of *water* and 0.5 ml of *glacial acetic acid* with sufficient of a 2.0% v/v solution of *absolute ethanol* in *ethanol-free chloroform* to produce 250 ml and (c) a detection wavelength of 350 nm.

Pass the mobile phase through the column for several hours before injecting the solutions. Record the chromatograms for 1.4 times the retention time of the peak due to neomycin B.

The chromatogram obtained with solution (1) shows a principal peak due to neomycin B and a major *secondary peak* due to neomycin C with a retention time relative to neomycin B of about 0.6.

The *column efficiency*, determined using the peak due to neomycin B in the chromatogram obtained with solution (1), should be not less than 13,000 theoretical plates per metre.

In the chromatogram obtained with solution (2) the area of the peak corresponding to neomycin C is not less than 3% and not more than 15% of the sum of the areas of the peaks corresponding to neomycin B and neomycin C.

ASSAY

Dilute a quantity containing 3500 IU to 50 ml with sterile *phosphate buffer pH 8.0*; dilute 10 ml of the resulting solution to 100 ml with the same solvent and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per ml.

STORAGE

Neomycin Eye Drops should be protected from light.

LABELLING

The strength is stated as the number of IU (Units) per ml.

Neomycin Eye Ointment

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Neomycin Eye Ointment is a sterile preparation containing Neomycin Sulphate in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) disperse a quantity of the eye ointment containing 14,000 IU in 100 ml of *chloroform*, extract with 5 ml of *water* and use the aqueous extract. Solution (2) contains 0.4% w/v of *neomycin sulphate EPCRS* in *water*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 2 minutes. The principal red spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the principal red spot in the chromatogram obtained with solution (3) appears as a single spot.

TESTS

Neamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a freshly prepared 3.85% w/v solution of *ammonium acetate* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) disperse a quantity containing 14,000 IU in 20 ml of *chloroform*, shake gently with 8 ml of *water*, allow the layers to separate and use the aqueous layer. Solution (2) contains 0.005% w/v of *neamine EPCRS*. After removal of the plate, dry it in a current of warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution* with *water* to contain 0.5% w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives not more than a very faint blue colour with one drop of a 0.5% w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage*. Any spot corresponding to neamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Neomycin C

Complies with the test described under Neomycin Eye Drops but preparing solution (2) in the following manner. Disperse a quantity of the eye ointment containing 3500 IU in 20 ml of *petroleum spirit (boiling range, 120° to 160°)*, add 5 ml of 0.02M *sodium tetraborate*, shake, separate the aqueous layer and centrifuge; proceed as for solution (1) but using 0.5 ml of the separated aqueous layer in place of 0.5 ml of the neomycin sulphate solution.

ASSAY

Dissolve a quantity containing 3500 IU in 25 ml of *chloroform*, extract with four 20 ml quantities of sterile *phosphate buffer pH 8.0*, combine the extracts and add sufficient of the buffer solution to produce 100 ml. Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU per g.

LABELLING

The strength is stated as the number of IU (Units) per g.

Neomycin Tablets

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Neomycin Tablets contain Neomycin Sulphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 70,000 IU with 25 ml of *water* and filter. Solution (2) contains 0.4% w/v of *neomycin sulphate EPCRS* in *water*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 2 minutes. The principal red spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the principal red spot in the chromatogram obtained with solution (3) appears as a single compact spot.

B. The powdered tablets yield the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Neamine

Comply with the test described under Neomycin Eye Drops but applying separately to the plate 2 µl of each of the following solutions. For solution (1) add 5 ml of *water* to a quantity of the powdered tablets containing 7000 IU, shake for 5 minutes and filter. Solution (2) contains 0.004% w/v of *neamine EPCRS*.

Neomycin C

Comply with the test described under Neomycin Eye Drops but preparing solution (2) in the following manner. Shake a quantity of the powdered tablets containing 17,500 IU with 20 ml of 0.02M *sodium tetraborate*, dilute to 25 ml with the same solvent, mix and centrifuge; proceed as for solution (1) but using 0.5 ml of the separated aqueous layer in place of the 0.5 ml of neomycin sulphate solution.

ASSAY

Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder containing 15,000 IU to a flask containing 150 ml of sterile *phosphate buffer pH 8.0* and add sufficient of the buffer solution to produce 250 ml. Allow to stand, dilute 10 ml of the clear supernatant liquid to 100 ml with the buffer solution and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated number of IU.

STORAGE

Neomycin Tablets should be protected from light and stored at a temperature not exceeding 30°.

LABELLING

The quantity of active ingredient is stated in terms of the number of IU (Units).

Neonatal Naloxone Injection

General Notices

Action and use

Opioid receptor antagonist.

DEFINITION

Neonatal Naloxone Injection is a sterile solution containing the equivalent of 20 micrograms per ml of anhydrous naloxone hydrochloride in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of anhydrous naloxone hydrochloride, $C_{19}H_{21}NO_4,HCl$

0.0185 to 0.0215% w/v.

IDENTIFICATION

A. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance. Heat the plate at 105° for 15 minutes immediately before use. Use a mixture of 5 volumes of *methanol* and 95 volumes of the upper layer from a mixture of 60 ml of 2M *ammonia* and 100 ml of *butan-1-ol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) add 1 ml of *ammonia buffer pH 10.0* to a volume of the injection containing the equivalent of 0.1 mg of anhydrous naloxone hydrochloride, extract with three 20 ml quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform*, dry the combined extracts over *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *methanol*. Solution (2) contains 0.010% w/v of *naloxone hydrochloride BPCRS* in *methanol*. Protect the plate from light during development. After removal of the plate, dry it in a current of air, spray with a freshly prepared 0.5% w/v solution of *potassium hexacyanoferrate(III)* in *iron(III) chloride solution R1* and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.0 to 4.5, Appendix V L.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is 3.5 IU per ml. Carry out the test using the maximum valid dilution of the injection calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection with a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water* to contain the equivalent of 0.001% w/v of anhydrous naloxone hydrochloride. Solution (2) contains 0.001% w/v of *naloxone hydrochloride BPCRS* in a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water*. Solution (3) contains 0.001% w/v of *naloxone hydrochloride BPCRS* and 0.0005% w/v of *noroxymorphone* in a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 to 10 µm) (Zorbax C18 7 to 8 µm is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a solution containing 0.068% w/v of *sodium octanesulphonate* and 0.1% w/v of *sodium chloride* in a mixture of 1 volume of *orthophosphoric acid*, 450 volumes of *methanol* and 550 volumes of *water* and (c) a detection wavelength of 229 nm.

The test is not valid unless the *resolution factor* between the peaks due to naloxone and noroxymorphone in the chromatogram obtained with solution (3) is at least 1.3.

Calculate the content of $C_{19}H_{21}NO_4$,HCl in the injection using the declared content of $C_{19}H_{21}NO_4$,HCl in *naloxone hydrochloride BPCRS*.

STORAGE

Neonatal Naloxone Injection should be protected from light.

LABELLING

The label states (1) the quantity of active ingredient in terms of the equivalent amount of anhydrous naloxone hydrochloride; (2) that the injection is for neonatal use.

Neostigmine Injection

General Notices

Action and use

Cholinesterase inhibitor.

DEFINITION

Neostigmine Injection is a sterile solution of Neostigmine Metilsulfate in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of neostigmine metilsulfate, $C_{13}H_{22}N_2O_6S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute, if necessary, a volume of the injection containing 2.5 mg of Neostigmine Metilsulfate to 5 ml with *water*, shake with three 10 ml quantities of *ether* and discard the ether extracts. The *light absorption* of the aqueous solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 260 nm and 266 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 5 volumes of *water*, 10 volumes of *formic acid*, 35 volumes of *methanol* and 50 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use the injection diluted, if necessary, with *water* to produce a solution containing 0.05% w/v of Neostigmine Metilsulfate. Solution (2) contains 0.05% w/v of *neostigmine metilsulfate EPCRS*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

C. To 1 ml add 0.5 ml of 5M *sodium hydroxide* and evaporate to dryness on a water bath. Heat rapidly on an oil bath to about 250° and maintain at this temperature for about 30 seconds. Cool, dissolve the residue in 1 ml of *water*, cool in ice and add 1 ml of *diazobenzenesulphonic acid solution*. An orange-red colour is produced.

TESTS

Acidity

pH, 4.5 to 6.5, Appendix V L.

(3-Hydroxy)trimethylanilinium methyl sulphate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is the injection diluted, if necessary, with *water* to contain 0.05% w/v of

Neostigmine Metilsulfate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *water*. For solution (3) add 0.05 ml of 5M *sodium hydroxide* to 1 ml of solution (1) and allow to stand for 5 minutes. Add 0.1 ml of 5M *hydrochloric acid* and use immediately.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable), (b) as the mobile phase at a flow rate of 1.1 ml per minute a 0.0015M solution of *sodium heptanesulphonate* in a mixture of 15 volumes of *acetonitrile* and 85 volumes of 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 215 nm.

In the chromatogram obtained with solution (3) the principal peak has a retention time of about 6.8 minutes (neostigmine metilsulfate) and there is a peak with a relative retention time of about 0.5 ((3-hydroxy)trimethylanilinium methylsulphate). In the chromatogram obtained with solution (1), the area of any *secondary peak* with a retention time corresponding to that of the peak due to (3-hydroxy)trimethylanilinium methylsulphate in the chromatogram obtained with solution (3) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Dilute a quantity containing 25 mg of Neostigmine Metilsulfate to 50 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{13}H_{22}N_2O_6S$ taking 14.35 as the value of A(1%, 1 cm) at the maximum at 260 nm.

STORAGE

Neostigmine Injection should be protected from light.

Neostigmine Tablets

General Notices

Action and use

Cholinesterase inhibitor.

DEFINITION

Neostigmine Tablets contain Neostigmine Bromide.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of neostigmine bromide, $C_{12}H_{19}BrN_2O_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Triturate a quantity of the powdered tablets containing 60 mg of Neostigmine Bromide with two 5 ml quantities of hot *chloroform* and filter. Evaporate the filtrate to dryness on a water bath, extract the residue with 5 ml of hot *water*, cool and filter. To 0.1 ml of the filtrate add 0.5 ml of 5M *sodium hydroxide* and evaporate to dryness on a water bath. Heat quickly in an oil bath to about 250^{c} and maintain at this temperature for about 30 seconds. Cool, dissolve the residue in 1 ml of *water*, cool in ice and add 1 ml of *diazobenzenesulphonic acid solution*. A cherry-red colour is produced.

B. The aqueous filtrate obtained in text A yields the reactions characteristic *of bromides*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Transfer a quantity of the powder containing 0.15 g of Neostigmine Bromide to a semi-micro ammonia-distillation apparatus, add 20 ml of a 50% w/v solution of *sodium hydroxide* and 0.5 ml of a 2% solution of *octan-2-ol* in *liquid paraffin*. Pass a current of steam through the mixture, collect the distillate in 50 ml of 0.01M *sulphuric acid VS* until the total volume is about 200 ml and titrate the excess of acid with 0.02M *sodium hydroxide VS* using *methyl red solution* as indicator. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of acid required to neutralise the dimethylamine produced. Each ml of 0.01M *sulphuric acid VS* is equivalent to 6.064 mg of $C_{12}H_{19}BrN_2O_2$.

STORAGE

Neostigmine Tablets should be protected from light.

Niclosamide Tablets

General Notices

Action and use

Antihelminthic.

DEFINITION

Niclosamide Tablets contain Anhydrous Niclosamide or Niclosamide Monohydrate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of anhydrous niclosamide, $C_{13}H_8Cl_2N_2O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Heat a quantity of the powdered tablets containing 0.5 g of anhydrous niclosamide with 25 ml of hot *ethanol (96%)*, filter while hot and evaporate the filtrate to dryness on a water bath. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of niclosamide *(RS 245)*. If the spectra are not concordant heat a suitable quantity of the residue at 120° for 1 hour and prepare a new spectrum.

B. Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 5 ml of 2M *sodium hydroxide* as the absorbing liquid. The resulting solution yields a white precipitate with *silver nitrate solution* which is insoluble in 2M *nitric acid* but soluble in 5M *ammonia*.

C. Heat 50 mg with 5 ml of 1M *hydrochloric acid* and 0.1 g of *zinc powder* in a water bath for 10 minutes, cool and filter. To the filtrate add 0.5 ml of a 1% w/v solution of *sodium nitrite* and allow to stand for 10 minutes. Add 2 ml of a 2% w/v solution of *ammonium sulphamate*, shake, allow to stand for 10 minutes and add 2 ml of a 0.5% w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride*. A deep red colour is produced.

TESTS

Disintegration

The requirement for Disintegration does not apply to Niclosamide Tablets.

2-Chloro-4-nitroaniline

Boil a quantity of the powdered tablets containing 0.10 g of anhydrous niclosamide with 20 ml of *methanol* for 2 minutes, cool, add sufficient 1M *hydrochloric acid* to produce 50 ml and filter. To 10 ml of the filtrate add 0.5 ml of a 0.5% w/v solution of *sodium nitrite* and allow to stand for 10 minutes. Add 1 ml of a 2% w/v solution of *ammonium sulphamate*, shake, allow to stand for 10 minutes and add 1 ml of a 0.5% w/v solution of N-*(1-naphthyl)ethylenediamine* *dihydrochloride*. Any colour produced is not more intense than that obtained by treating 20 ml of a solution in *methanol* containing 10 µg of *2-chloro-4-nitroaniline* in the same manner and at the same time, beginning at the words 'add sufficient 1M *hydrochloric acid* …'.

5-Chlorosalicylic acid

Boil a quantity of the powdered tablets containing 0.50 g of anhydrous niclosamide with 10 ml of *water* for 2 minutes, cool, filter and to the filtrate add 0.2 ml of *iron(III) chloride solution R1*. No red or violet colour is produced.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 1 volume of solution (2) to 100 volumes with *acetonitrile* and further dilute 1 volume of this solution to 20 volumes with *acetonitrile*. For solution (2) shake a quantity of the powdered tablets containing 0.1 g of anhydrous niclosamide with 80 ml of *methanol* for 15 minutes, add sufficient *methanol* to produce 100 ml and filter.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a mixture of 50 volumes of *acetonitrile* and 50 volumes of a solution containing 0.2% w/v of *potassium dihydrogen orthophosphate*, 0.2% w/v of *tetrabutylammonium hydrogen sulphate* and 0.1% w/v of *disodium hydrogen orthophosphate* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 230 nm. Adjust the sensitivity so that the height of the peak corresponding to niclosamide in the chromatogram obtained with solution (1) is not less than 20% of full-scale deflection. Record the chromatogram for twice the retention time of niclosamide.

The sum of the areas of any *secondary peaks* in the chromatogram obtained with solution (2) is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (1). Disregard any peak with an area less than 10% of the area of the area of the principal peak in the chromatogram obtained with solution (1).

ASSAY

Weigh and powder 20 tablets. Carry out Method II for *non-aqueous titration*, Appendix VIII A, using a quantity of the powdered tablets containing 0.3 g of anhydrous niclosamide dissolved in 60 ml of *dimethylformamide*, 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

STORAGE

Niclosamide Tablets should be protected from light.

LABELLING

The label states that the tablets should be chewed before swallowing.

When the active ingredient is Niclosamide Monohydrate the quantity is stated in terms of the equivalent amount of anhydrous niclosamide.

Nicotinamide Tablets

General Notices

DEFINITION

Nicotinamide Tablets contain Nicotinamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nicotinamide, $C_6H_6N_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Nicotinamide with 25 ml of *absolute ethanol* for 15 minutes, filter and evaporate the filtrate to dryness on a water bath. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of nicotinamide *(RS 246)*.

B. The *light absorption* of the solution obtained in the Assay, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 262 nm and two shoulders, at 258 nm and 269 nm.

C. Shake a quantity of the powdered tablets containing 50 mg of Nicotinamide with 50 ml of *water* and filter. To 2 ml of the filtrate add 2 ml of *cyanogen bromide solution* and 3 ml of a 2.5% v/v solution of *aniline* and shake. A yellow colour is produced.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *water*, 45 volumes of *ethanol (96%)* and 48 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Nicotinamide with 15 ml of *absolute ethanol* for 15 minutes, filter, evaporate to dryness on a water bath and dissolve the residue as completely as possible in 1 ml of *absolute ethanol*. For solution (2) dilute 1 volume of solution (1) to 400 volumes with *absolute ethanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Nicotinamide with 50 ml of *ethanol (96%)* for 15 minutes and dilute to 100 ml with *ethanol (96%)*. Mix, filter, dilute 5 ml of the filtrate to 100 ml with *ethanol (96%)* and measure the *absorbance* of the resulting solution at the maximum at 262 nm, Appendix II B. Calculate the content of $C_6H_6N_2O$ taking 241 as the value of A(1%, 1 cm) at the maximum at 262 nm.

STORAGE

Nicotinamide Tablets should be protected from light.

Nicotinic Acid Tablets

General Notices

DEFINITION

Nicotinic Acid Tablets contain Nicotinic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nicotinic acid, $C_6H_5NO_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 8 volumes of *water*, 45 volumes of *ethanol (96%)* and 48 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Nicotinic Acid with 50 ml of hot *ethanol (96%)*, filter and allow the filtrate to cool. Solution (2) contains 0.1% w/v of *nicotinic acid* in *ethanol (96%)*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing 0.1 g of Nicotinic Acid with *ethanol (96%)*, filter and evaporate the filtrate to dryness. To the residue add 10 mg of *citric acid* and 0.15 ml of *acetic anhydride* and heat on a water bath. A reddish-violet colour is produced.

C. Triturate a quantity of the powdered tablets containing 50 mg of Nicotinic Acid with 10 ml of *water* and filter. To 2 ml of the filtrate add 6 ml of *cyanogen bromide solution* and 1 ml of a 2.5% v/v solution of *aniline*. A golden yellow colour is produced.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.3 g of Nicotinic Acid add 40 ml of hot *ethanol (96%)*, previously neutralised to *phenolphthalein solution R1*, and shake. Allow to stand for 15 minutes, swirling occasionally, and then shake for 10 minutes. Filter through absorbent cotton and wash the filter with *ethanol (96%)*. Add 50 ml of *carbon dioxide-free water* and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 12.31 mg of $C_6H_5NO_2$.

Nicotinyl Alcohol Tablets

General Notices

Action and use

Vasodilator.

DEFINITION

Nicotinyl Alcohol Tablets contain Nicotinyl Alcohol Tartrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nicotinyl alcohol tartrate, $C_6H_7NO,C_4H_6O_6$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of 4 volumes of 13.5M *ammonia*, 16 volumes of *methanol*, 30 volumes of *1,4-dioxan* and 50 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Nicotinyl Alcohol Tartrate with 50 ml of *methanol*, filter and evaporate the filtrate to dryness at about 30° at a pressure of 2 kPa. Add 2 ml of *water* to the residue, shake, filter and add 0.1 ml of 13.5M *ammonia* to the filtrate. For solution (2) dilute 1 volume of solution (1) to 500 volumes with 0.1M *ammonia*. Solution (3) contains 0.025% w/v of *3-(aminomethyl)pyridine* in 0.1M *ammonia*. Solution (4) contains 0.025% w/v of *nicotinyl alcohol tartrate BPCRS* in 0.1M *ammonia*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Spray with a 2% w/v solution of *chloro-2,4,6-trinitrobenzene* in *absolute ethanol*, dry in a current of air and spray with a 5% w/v solution of *sodium carbonate*. In the chromatogram obtained with solution (1), any spot corresponding to 3-(aminomethyl)pyridine is not more intense than the spot in the chromatogram obtained with solution (3) (0.2%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Disregard any spot due to tartaric acid remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Nicotinyl Alcohol Tartrate with 150 ml of 0.1M *hydrochloric acid* for 10 minutes, dilute to 250 ml with the same solvent, mix and filter. Dilute 10 ml of the filtrate to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 261 nm, Appendix II B. Calculate the content of $C_6H_7NO,C_4H_6O_6$ taking 210 as the value of A(1%, 1 cm) at the maximum at 261 nm.

Nifedipine Capsules

General Notices

Action and use

Calcium channel blocker.

DEFINITION

Nifedipine Capsules contain Nifedipine.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of nifedipine, $C_{17}H_{18}N_2O_6$

95.0 to 105.0% of the stated amount.

Carry out all the following procedures in the dark or under long-wavelength light (greater than 420 nm). Prepare solutions immediately before use and protect them from light.

IDENTIFICATION

A. Add a quantity of the contents of the capsules containing 50 mg of Nifedipine to 10 ml of *water* and mix with the aid of ultrasound for 5 minutes, filter (Whatman GF/C paper is suitable), wash the residue with *water* and dry at 110°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of nifedipine *(RS 248)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane* as the mobile phase in an unsaturated tank. Apply separately to the plate 5 µl of each of the following solutions in a mixture of equal volumes of *dichloromethane* and *methanol*. For solution (1) shake a quantity of the contents of the capsules containing 20 mg of Nifedipine with 10 ml of the solvent mixture and filter. Solution (2) contains 0.2% w/v of *nifedipine BPCRS* in the solvent mixture. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, appearance under ultraviolet light and size to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium and measure the *absorbance* of a 2-cm layer of the solution, suitably diluted if necessary, at the maximum at 340 nm, Appendix II B. Measure the *absorbance* of a 2-cm layer of a solution prepared by diluting a 0.025% w/v solution of *nifedipine BPCRS* in *methanol* to a suitable volume with the dissolution medium and using the dissolution medium in the reference cell. Calculate the total content of nifedipine, $C_{17}H_{18}N_2O_6$, in the medium from the absorbances obtained and from the declared content of $C_{17}H_{18}N_2O_6$ in *nifedipine BPCRS*.

Nitro- and nitroso-phenylpyridine analogues

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the capsules containing 20 mg of Nifedipine in 8 ml of *methanol* and dilute to 20 ml with a mixture of 9 volumes of *acetonitrile,* 36 volumes of *methanol* and 55 volumes of *water*. Solution (2) contains 0.0010% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate EPCRS* (nitrophenylpyridine analogue) in a mixture of 5 volumes of *acetonitrile,* 33 volumes of *water* and 62 volumes of *methanol*. Solution (3) contains 0.00050% w/v of *dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate EPCRS* (nitrosophenylpyridine analogue) in a mixture of 5 volumes of *acetonitrile,* 33 volumes of *water* and 62 volumes of *methanol.* For solution (4) dilute 2 volumes of a solution in *methanol* containing 0.25% w/v of *nifedipine BPCRS*, 0.0025% w/v each of the nitrophenylpyridine analogue and nitrosophenylpyridine analogue and 0.0050% w/v of *propyl hydroxybenzoate* to 5 volumes with a mixture of 9 volumes of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *water.*

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable), (b) a mixture of 9 volumes of *acetonitrile*, 36 volumes of *methanol* and 55 volumes of *borate buffer pH 8.0* as the mobile phase with a flow rate of 2.0 ml per minute and (c) a detection wavelength of 220 nm.

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *nifedipine BPCRS*.

In the chromatogram obtained with solution (1) the areas of any peaks corresponding to the nitrophenylpyridine analogue and the nitrosophenylpyridine analogue are not greater than the areas of the corresponding peaks in the chromatograms obtained with solution (2) (1%) and solution (3) (0.5%), respectively.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) take ten capsules and break them beneath the surface of 30 ml of *acetonitrile*. Stir to dissolve the capsule contents. Transfer the resulting solution to a 250 ml graduated flask. Wash the capsule shells with several portions of the mobile phase, transferring the washings to the flask, add sufficient of the mobile phase to produce 250 ml and mix. If necessary, further dilute a portion of the solution with the mobile phase to give a solution containing 0.02% w/v of nifedipine. For solution (2) dissolve 50 mg of *nifedipine BPCRS* in 30 ml of *acetonitrile* and dilute to 250 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 is suitable), (b) a mixture of 40 volumes of *acetonitrile* and 60 volumes of a 0.03% v/v solution of *orthophosphoric acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 235 nm.

Calculate the content of $C_{17}H_{18}N_2O_6$ in the capsules using the declared content of $C_{17}H_{18}N_2O_6$ in *nifedipine BPCRS*.

Nikethamide Injection

General Notices

Action and use

Central nervous system stimulant.

DEFINITION

Nikethamide Injection is a sterile solution containing 25% w/v of Nikethamide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of nikethamide, $C_{10}H_{14}N_2O$

24.0 to 26.0% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Make 1 ml alkaline with 5M *sodium hydroxide*, extract with 5 ml of *dichloromethane* and evaporate the solvent. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of nikethamide *(RS 249)*.

TESTS

Acidity or alkalinity

pH, 6.0 to 8.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 25 volumes of *propan-1-ol* and 75 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute 1 ml of the injection to 5 ml with *methanol*. Solutions (2) and (3) contain 0.05% w/v and 0.005% w/v respectively of *ethylnicotinamide EPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to ethylnicotinamide in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Dilute 5 ml to 500 ml with *water*. To 5 ml of the solution add 5 ml of 1M *hydrochloric acid* and dilute to 500 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 263 nm, Appendix II B. Calculate the content of $C_{10}H_{14}N_2O$ taking 282 as the value of *A* (1%, 1 cm) at the maximum at 263 nm.

Nimodipine Intravenous Infusion

General Notices

Action and use

Calcium channel blocker.

DEFINITION

Nimodipine Intravenous Infusion is a sterile solution of Nimodipine in a suitable aqueous alcoholic solvent.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of nimodipine, $C_{21}H_{26}N_2O_7$

95.0 to 105.0% of the stated amount.

Carry out the following procedures protected from light or under long-wavelength light (greater than 420 nm). Prepare the solutions immediately before use and protect them from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions as bands. For solution (1) extract a volume of the intravenous infusion containing 4 mg of Nimodipine with 4 ml of *dichloromethane* and use the dichloromethane layer. Solution (2) is a 0.1% w/v solution of *nimodipine BPCRS* in *dichloromethane*. Solution (3) is a mixture of equal volumes of solution (1) and solution (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Spray the plate with a freshly prepared 0.1% w/v solution of *2,6-dichloroquinonechlorimide* in *ethanol* and heat at 110^{c} for about 3 minutes. The bands due to nimodipine are brownish-green to bluish-green. The principal band in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal band in the chromatogram obtained with solution (3) appears as a single compact band.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

Bacterial endotoxins

The endotoxin limit concentration is 5.0 IU per ml, Appendix XIV C.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the intravenous infusion, if necessary, with *absolute ethanol*, to contain 0.02% w/v of Nimodipine. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 2 volumes of this solution to 10 volumes with the mobile phase. Solution (3) contains 0.0001% w/v of *nimodipine impurity A EPCRS* in *absolute ethanol*. Solution (4) contains 0.0001% w/v each of *nimodipine BPCRS* and *nimodipine impurity A EPCRS* in *absolute ethanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher RP-18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 12 volumes of *acetonitrile,* 24 volumes of *tetrahydrofuran* and 64 volumes of *water* and (c) a detection wavelength of 235 nm. Maintain the column temperature at 30°.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to nimodipine and nimodipine impurity A is at least 1.5 and the *symmetry factor* of the peak due to nimodipine is not more than 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to nimodipine impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%). The sum of the areas of any *secondary peaks* other than any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the intravenous infusion, if necessary, with *absolute ethanol*, to produce a solution containing 0.02% w/v of Nimodipine. Solution (2) contains 0.02% w/v of *nimodipine BPCRS* in *absolute ethanol*. Solution (3) contains 0.002% w/v each of *nimodipine BPCRS* and *nimodipine impurity A EPCRS* in *absolute ethanol*.

The chromatographic conditions described under Related substances may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.3 and the *symmetry factor* of the peak due to nimodipine is not more than 2.0.

Calculate the content of $C_{21}H_{26}N_2O_7$ in the intravenous infusion using the declared content of $C_{21}H_{26}N_2O_7$ in *nimodipine BPCRS*.

STORAGE

Nimodipine Intravenous Infusion should be protected from light. It should not be allowed to come into contact with polyvinyl chloride (PVC).

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nimodipine.

Nimodipine Tablets

General Notices

Action and use

Calcium channel blocker.

DEFINITION

Nimodipine Tablets contain Nimodipine. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nimodipine,$C_{21}H_{26}N_2O_7$

95.0 to 105.0% of the stated amount.

Carry out the following procedures protected from light or under long-wavelength light (greater than 420 nm). Prepare solutions immediately before use and protect them from light.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck HPTLC silica gel 60 F_{254} plates are suitable) and a mixture of 40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 60 mg of Nimodipine with 10 ml of *ethyl acetate* for 15 minutes, centrifuge and use the supernatant liquid. Solution (2) is a 0.6% w/v solution of *nimodipine BPCRS* in *ethyl acetate*. Solution (3) is a mixture of equal volumes of solution (1) and solution (2). After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Spray the plate with a freshly prepared 0.1% w/v solution of *2, 6-dichloroquinonechlorimide* in *ethanol* and heat at 110° for about 3 minutes. The spot due to nimodipine is greyish-violet. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (3) appears as a single, compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of acetate buffer pH 4.5 prepared as described below containing 0.3% w/v of *sodium dodecyl sulphate* and rotate the paddle at 75 revolutions per minute. Prepare the buffer solution by dissolving 0.299 g of *sodium acetate* in 50 ml of *water*, add 0.174 g of *glacial acetic acid* and dilute to 100 ml with *water*. After 30 minutes withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtrate, Appendix II B, diluted with the dissolution medium if necessary, at the maximum at 340 nm using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *nimodipine BPCRS* in the dissolution medium and calculate the total content of nimodipine, $C_{21}H_{26}N_2O_7$, in the medium using the declared content of $C_{21}H_{26}N_2O_7$ in *nimodipine BPCRS*. The amount of nimodipine released is not less than 85% of the stated amount.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of *methanol* to a quantity of the powdered tablets containing 60 mg of Nimodipine, mix with the aid of ultrasound for 5 minutes, add sufficient *methanol* to produce 100 ml, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and dilute 2 volumes of this solution to 10 volumes with the mobile phase. Solution (3) contains 0.0003% w/v of *nimodipine impurity A EPCRS* in the mobile phase. Solution (4) contains 0.0002% w/v each of *nimodipine BPCRS* and *nimodipine impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (7 µm) (Lichrosorb RP-18 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 13 volumes of *acetonitrile*, 26 volumes of *tetrahydrofuran* and 60 volumes of *water* and (c) a detection wavelength of 235 nm. Maintain the column temperature at 40°.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to nimodipine and nimodipine impurity A is at least 1.5 and the *symmetry factor* of the peak due to nimodipine is not more than 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to nimodipine impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%) and the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%). The sum of the areas of any *secondary peaks* other than any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of *methanol* to a quantity of the powdered tablets containing 60 mg of Nimodipine, mix with the aid of ultrasound for 5 minutes, add sufficient *methanol* to produce 100 ml, centrifuge and use the supernatant liquid. Solution (2) contains 0.06% w/v of *nimodipine BPCRS* in *methanol*. Solution (3) contains 0.002% w/v of *nimodipine BPCRS* and 0.002% w/v of *nimodipine impurity A EPCRS* in *methanol*.

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to nimodipine and nimodipine impurity A is at least 1.5, and the *symmetry factor* of the peak due to nimodipine is not more than 2.

Calculate the content of $C_{21}H_{26}N_2O_7$ in the tablets using the declared content of $C_{21}H_{26}N_2O_7$ in *nimodipine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nimodipine.

Nitrazepam Oral Suspension

General Notices

Action and use

Benzodiazepine.

DEFINITION

Nitrazepam Oral Suspension is a suspension of Nitrazepam in a suitable flavoured vehicle, which may be coloured.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nitrazepam, $C_{15}H_{11}N_3O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 15 volumes of *ethyl acetate* and 85 volumes of *nitromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add a quantity of the oral suspension containing 20 mg of Nitrazepam to 40 ml of *water*, extract with successive quantities of 50, 20 and 20 ml of *chloroform*, filtering each extract through phase-separating paper, and add sufficient *chloroform* to the combined extracts to produce 100 ml. Solution (2) contains 0.02% w/v of *nitrazepam BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air and visualise the spots by *Method I*. A spot in the chromatogram obtained with solution (1) corresponds to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (2) has a similar retention time to the peak due to nitrazepam in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00625% w/v of *nitrazepam BPCRS* in *chloroform*. For solution (2) add a quantity of the oral suspension containing 12.5 mg of Nitrazepam to 25 ml of *water*, extract with three 50 ml quantities of *chloroform*, filtering each extract through phase-separating paper, and add sufficient *chloroform* to produce 200 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb or Partisil is suitable), (b) a mixture of 10 volumes of *absolute ethanol* and 90 volumes of n-*hexane* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{11}N_3O_3$, weight in volume, using the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS*.

STORAGE

Nitrazepam Oral Suspension should be protected from light.

Nitrazepam Tablets

General Notices

Action and use

Benzodiazepine.

DEFINITION

Nitrazepam Tablets contain Nitrazepam.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of Nitrazepam, $C_{15}H_{11}N_3O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 280 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *methanol* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 0.1% w/v of Nitrazepam, allow to settle and decant the supernatant liquid. Solution (2) contains 0.1% w/v of *nitrazepam BPCRS* in *methanol*. After removal of the plate, spray it with a 10% v/v solution of *sulphuric acid* in *absolute ethanol*, heat at 105° for 10 minutes and examine under *ultraviolet light* (*365 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. To a quantity of the powdered tablets containing 5 mg of Nitrazepam add 5 ml of *hydrochloric acid* and 10 ml of *water*, heat on a water bath for 15 minutes and filter. To the clear filtrate add 1 ml of a 0.1% w/v solution of *sodium nitrite*, allow to stand for 3 minutes and add 1 ml of a 0.5% w/v solution of *sulphamic acid*. Allow to stand for a further 3 minutes and add 1 ml of a 0.1% w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*. A red colour is produced.

TESTS

Carry out the following tests protected from light.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Measure the *absorbance,* Appendix II B, of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 280 nm, Appendix II B, using dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *nitrazepam BPCRS* in dissolution medium at the maximum at 280 nm, Appendix II B, using dissolution medium in the reference cell. Calculate the total content of nitrazepam, $C_{15}H_{11}N_3O_3$, in the medium using the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS.*

Decomposition products

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 15 volumes of *ethyl acetate* and 85 volumes of *nitromethane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following freshly prepared solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Nitrazepam with 4 ml of a mixture of equal volumes of *dichloromethane* and *methanol* for 5 minutes, centrifuge and use the clear supernatant liquid. For solution (2) dissolve 10 mg of *aminonitrobenzophenone* in *acetone* and dilute to 100 ml with the same solvent; dilute 25 ml of the solution to 50 ml with *acetone*. For solution (3) dissolve 10 mg of *nitrazepam impurity A EPCRS* (3-amino-6-nitro-4-phenylquinol-2(1*H*)-one) in *acetone* and dilute to 100 ml with the same solvent; dilute 25 ml to 50 ml with *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). In the chromatogram obtained with solution (1) any spot corresponding to aminonitrobenzophenone is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and any spot corresponding to 3-amino-6-nitro-4-phenylquinol-2(1*H*)-one is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

Uniformity of content

Tablets containing less than 2 mg or less than 2% w/w of Nitrazepam comply with the requirements stated under Tablets using the following method of analysis.

To a powdered tablet add 5 ml of *water*, mix and allow to stand for 15 minutes. Add 70 ml of a 0.5% v/v solution of *hydrochloric acid* in *methanol*, shake for 15 minutes, add sufficient of the 0.5% v/v solution of *hydrochloric acid* in *methanol* to produce 100 ml and filter. Dilute, if necessary, with sufficient of a 0.5% v/v solution of *hydrochloric acid* in *methanol* to produce a solution containing 0.001% w/v of nitrazepam. Measure the *absorbance* of the resulting solution immediately at the maximum at 280 nm, Appendix II B, using a 0.5% v/v solution of *hydrochloric acid* in *methanol* in the reference cell. Calculate the content of $C_{15}H_{11}N_3O_3$ in the solution from the absorbance obtained from a solution containing 0.001% w/v *nitrazepam BPCRS* in a 0.5% v/v solution of *hydrochloric acid* in *methanol* and from the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS.*

ASSAY

For tablets containing 2 mg or more or 2% w/w or more of Nitrazepam

Weigh and powder 20 tablets. To a quantity of the powder containing 5 mg of Nitrazepam add 5 ml of *water*, mix and allow to stand for 15 minutes. Add 70 ml of a 0.5% v/v solution of *hydrochloric acid* in *methanol*, shake for 15 minutes, add sufficient of the 0.5% v/v solution of *hydrochloric acid* in *methanol* to produce 100 ml and filter. Dilute 10 ml of the filtrate to 50 ml with the 0.5% v/v solution of *hydrochloric acid* in *methanol*. Measure the *absorbance* of the resulting solution immediately at the maximum at 280 nm using a 0.5% v/v solution of *hydrochloric acid* in *methanol* in the reference cell, Appendix II B. Calculate the content of $C_{15}H_{11}N_3O_3$ in the solution from the absorbance obtained from a solution containing 0.001% w/v of *nitrazepam BPCRS* in a 0.5% v/v solution of *hydrochloric acid* in *methanol* and from the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS.*

For tablets containing less than 2 mg or less than 2% w/w of Nitrazepam

Use the average of the 10 individual results obtained in the test for Uniformity of content.

STORAGE

Nitrazepam Tablets should be protected from light.

Nitrofurantoin Oral Suspension

General Notices

Action and use

Antibacterial.

DEFINITION

Nitrofurantoin Oral Suspension is a suspension of Nitrofurantoin in a suitable flavoured vehicle.
Nitrofurantoin Oral Suspension should not be diluted.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nitrofurantoin, $C_8H_6N_4O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 220 to 400 nm of the final solution obtained in the Assay exhibits two maxima, at 266 nm and 367 nm.

B. Dissolve 5 mg of the residue obtained by centrifuging a quantity of the oral suspension containing 50 mg of Nitrofurantoin in 5 ml of 0.1M *sodium hydroxide*. A deep yellow solution is produced, which changes to deep orange-red.

ASSAY

Carry out the following procedure in subdued light. To a weighed quantity containing 30 mg of Nitrofurantoin add, in successive small volumes, 50 ml of *dimethylformamide*, stirring well between each addition. Continue stirring until the sample is completely dissolved and dilute to 500 ml with an aqueous solution containing 1.8% w/v of *sodium acetate* and 0.14% v/v of *glacial acetic acid*. Dilute 10 ml of this solution to 100 ml with the sodium acetate-acetic acid solution and measure the *absorbance* of the resulting solution at the maximum at 367 nm, Appendix II B, using in the reference cell a 1% v/v solution of *dimethylformamide* in the sodium acetate-acetic acid solution. Calculate the content of $C_8H_6N_4O_5$ taking 765 as the value of A(1%, 1 cm) at the maximum at 367 nm. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_8H_6N_4O_5$, weight in volume.

STORAGE

Nitrofurantoin Oral Suspension should be protected from light.

Nitrofurantoin Tablets

General Notices

Action and use

Antibacterial.

DEFINITION

Nitrofurantoin Tablets contain Nitrofurantoin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nitrofurantoin, $C_8H_6N_4O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

The *light absorption*, Appendix II B, in the range 220 to 400 nm of the final solution obtained in the Assay exhibits two maxima, at 266 nm and 367 nm.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* HF_{254} as the coating substance and a mixture of 10 volumes of *methanol* and 90 volumes of *nitromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Nitrofurantoin with 10 ml of a mixture of 1 volume of *dimethylformamide* and 9 volumes of *acetone* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. After removal of the plate, allow it to dry in air, heat at 100° to 105° for 5 minutes and examine under *ultraviolet light* (*254 nm*). Spray the plate with *phenylhydrazine hydrochloride solution* and heat it at 100° to 105° for a further 10 minutes. By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the following procedure in subdued light. Weigh and powder 20 tablets. To a quantity of the powder containing 0.12 g of Nitrofurantoin add 50 ml of *dimethylformamide*, shake for 5 minutes, add sufficient *water* to produce 1000 ml and mix. Dilute 5 ml to 100 ml with a solution containing 1.8% w/v of *sodium acetate* and 0.14% v/v of *glacial acetic acid* and filter. Measure the *absorbance* of the filtrate at the maximum at 367 nm, Appendix II B, using the sodium acetate-acetic acid solution in the reference cell. Calculate the content of $C_8H_6N_4O_5$ taking 765 as the value of A(1%, 1 cm) at 367 nm.

STORAGE

Nitrofurantoin Tablets should be protected from light.

Nizatidine Intravenous Infusion

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer.

DEFINITION

Nizatidine Intravenous Infusion is a sterile solution of Nizatidine. It is prepared immediately before use from Nizatidine Sterile Concentrate in accordance with the manufacturer's instructions.

The intravenous infusion complies with the requirements stated under Parenteral Preparations.

NIZATIDINE STERILE CONCENTRATE

DEFINITION

Nizatidine Sterile Concentrate is a sterile solution of Nizatidine in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Infusions stated under Parenteral Preparations and with the following requirements.

Content of nizatidine, $C_{12}H_{21}N_5O_2S_2$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless to yellow solution that darkens on storage.

IDENTIFICATION

Shake a volume of the concentrate containing 50 mg of Nizatidine with 10 ml of *dichloromethane* for 5 minutes. Filter the *dichloromethane* layer through *anhydrous sodium sulphate* and evaporate the filtrate to dryness on a rotary evaporator at 40°. Cool the residue in a refrigerator for 15 hours and, if necessary, induce crystallisation by scratching the wall of the container with a glass rod. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of nizatidine (*RS 410*).

TESTS

Acidity or alkalinity

pH of the concentrate diluted with *water* to contain 1.0% w/v of Nizatidine, 6.0 to 8.0, Appendix V L.

Colour of solution

Dilute the concentrate with *water* to produce a solution containing 1.0% w/v of Nizatidine. The *absorbance* of the solution at 410 nm, Appendix II B, is not more than 0.40 determined using water in the reference cell.

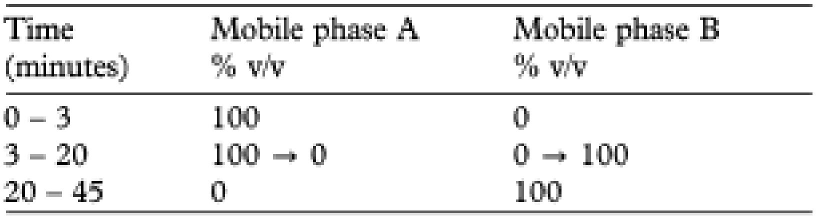
Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the concentrate with mobile phase A to produce a solution containing 0.5% w/v of Nizatidine. For solution (2) dilute 1 volume of solution (1) to 100 volumes with mobile phase A. Solution (3) contains 0.005% w/v of *nizatidine EPCRS* and 0.005% w/v of *nizatidine impurity F EPCRS* in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Supelco LC-18-DB and Kromasil C18 are suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute the gradient elution programme described below and (c) a detection wavelength of 254 nm.

Mobile phase A Dissolve 5.9 g of *ammonium acetate* in 760 ml of *water*, add 1 ml of *diethylamine* and adjust the pH to 7.5 with 6M *acetic acid* (solution A). Prepare a mixture of 24 volumes of *methanol* and 76 volumes of solution A.

Mobile phase B A mixture of 50 volumes of *methanol* and 50 volumes of solution A.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v
0 – 3	100	0
3 – 20	100 → 0	0 → 100
20 – 45	0	100

Inject 20 µl of each solution. The test is not valid unless the retention time of Nizatidine is between 10 and 20 minutes and the *symmetry factor* of the peak due to Nizatidine is not greater than 2.0.

Inject 20 µl of solution (3). The test is not valid unless the *resolution factor* between the peak due to Nizatidine (first peak) and Nizatidine impurity F (second peak) is at least 2.0.

In the chromatogram obtained with solution (1), the area of any peak with a relative retention time of about 0.7 (impurity E) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%), the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%), the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%) and the sum of the areas of all the *secondary peaks* is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the concentrate with mobile phase A to produce a solution containing 0.03% w/v of Nizatidine. Solution (2) is a 0.03% w/v solution of *nizatidine EPCRS* in mobile phase A.

The chromatographic procedure described under Related substances may be used.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to Nizatidine is not greater than 2.0.

Calculate the content $C_{12}H_{21}N_5O_2S_2$, in the concentrate from the chromatograms obtained using the declared content of $C_{12}H_{21}N_5O_2S_2$ in *nizatidine EPCRS.*

STORAGE

Nizatidine Sterile Concentrate should be protected from light.

Noradrenaline Injection / Norepinephrine Injection

General Notices

Action and use

Alpha-adrenoceptor agonist.

DEFINITION

Noradrenaline Injection is a sterile solution of Noradrenaline Acid Tartrate. It is either supplied as a ready-to-use solution or prepared immediately before use by diluting Sterile Noradrenaline Concentrate with Sodium Chloride and Glucose Intravenous Infusion or with Glucose Intravenous Infusion (5.0% w/v) in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use solution, the injection complies with the following requirements.

Content of noradrenaline acid tartrate, $C_8H_{11}NO_3,C_4H_6O_6,H_2O$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless liquid.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Mix a volume of the solution containing 1 mg of Noradrenaline Acid Tartrate with 10 ml of *phthalate buffer pH 3.6*, add 1 ml of 0.05M *iodine*, allow to stand for 5 minutes and add 2 ml of 0.1M *sodium thiosulphate*; not more than a very faint red colour is produced. Repeat the test using *phosphate buffer pH 6.6*; a strong reddish violet colour is produced.

TESTS

Acidity

pH, 3.0 to 4.6, Appendix V L.

Adrenaline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the injection diluted, if necessary, to contain 0.020% w/v of Noradrenaline Acid Tartrate. Solution (2) contains 0.0002% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase. Solution (3) contains 0.0002% w/v of *adrenaline acid tartrate BPCRS* and 0.0002% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm

× 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* in a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusting the pH to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to adrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection with the mobile phase, if necessary, to produce a solution containing 0.02% w/v of Noradrenaline Acid Tartrate. Solution (2) contains 0.02% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase. Solution (3) contains 0.02% w/v of *noradrenaline acid tartrate BPCRS* and 0.02% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase.

The chromatographic procedure described under the test for Adrenaline may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the content of $C_8H_{11}NO_3,C_4H_6O_6,H_2O$ in the injection using the declared content of $C_8H_{11}NO_3,C_4H_6O_6,H_2O$ in *adrenaline acid tartrate BPCRS*.

STORAGE

The ready-to-use solution should be protected from light. If the solution is brown, it should be discarded.

STERILE NORADRENALINE CONCENTRATE

STERILE NOREPINEPHINE CONCENTRATE

DEFINITION

Sterile Noradrenaline Concentrate is a sterile, isotonic solution containing Noradrenaline Acid Tartrate in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of noradrenaline acid tartrate, $C_8H_{11}NO_3,C_4H_6O_6,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Mix a volume containing 1 mg of Noradrenaline Acid Tartrate with 10 ml of *phthalate buffer pH 3.6*, add 1 ml of 0.05M *iodine*, allow to stand for 5 minutes and add 2 ml of 0.1M *sodium thiosulphate*; not more than a very faint red colour is produced. Repeat the test using *phosphate buffer pH 6.6*; a strong reddish violet colour is produced.

TESTS

Acidity

pH, 3.0 to 4.6, Appendix V L.

Noradrenalone

Dilute the concentrate with 0.01M *hydrochloric acid* to produce a solution containing 0.1% w/v of Noradrenaline Acid Tartrate. The *absorbance* of the solution measured at 310 nm, Appendix II B, is not greater than 0.17 (0.12%).

Adrenaline

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the concentrate being examined diluted, if necessary, to contain 0.2% w/v of Noradrenaline Acid Tartrate. Solution (2) contains 0.002% w/v of *adrenaline acid tartrate BPCRS* in the mobile phase. Solution (3) contains 0.002% w/v of *adrenaline acid tartrate BPCRS* and 0.002% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of 0.1M *disodium edetate* in a mixture of 50 ml of *methanol* and 950 ml of *water* and adjusting the pH to 3.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 205 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to adrenaline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute one volume of the concentrate being examined with the mobile phase to produce a solution containing 0.02% w/v of Noradrenaline Acid Tartrate. Solution (2) contains 0.02% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase. Solution (3) contains 0.02% w/v of *adrenaline acid tartrate BPCRS* and 0.02% w/v of *noradrenaline acid tartrate BPCRS* in the mobile phase.

The chromatographic procedure described under the test for Adrenaline may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

Calculate the content of $C_8H_{11}NO_3,C_4H_6O_6,H_2O$ from the chromatograms obtained and using the declared content of $C_8H_{11}NO_3,C_4H_6O_6,H_2O$ in *noradrenaline acid tartrate BPCRS*.

STORAGE

Sterile Noradrenaline Concentrate should be protected from light. If the solution is brown it should be discarded.

Norethisterone Tablets

General Notices

Action and use

Progestogen.

DEFINITION

Norethisterone Tablets contain Norethisterone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of norethisterone, $C_{20}H_{26}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 20 volumes of *ethyl acetate* and 80 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) crush one tablet in sufficient *ethanol* (*96%*) to produce a final solution containing approximately 0.035% w/v of Norethisterone, warm to 50° for 10 minutes, mix with the aid of ultrasound, allow to cool and centrifuge to obtain a clear supernatant liquid. Solution (2) contains 0.035% w/v of *norethisterone BPCRS* in *ethanol* (*96%*). After removal of the plate, allow it to dry in air and spray with a mixture of 30 volumes of *methanol* and 70 volumes of *sulphuric acid* and examine under *ultraviolet light* (*365 nm*). The Rf value of the principal spot in the chromatogram obtained with solution (1) is the same as that of the spot in the chromatogram obtained with solution (2).

TESTS

Uniformity of content

Tablets containing less than 2 mg of Norethisterone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.007% w/v of *norethisterone BPCRS* in *methanol* (*60%*). For solution (2) disperse one tablet in 2 ml of *water* with the aid of ultrasound for 15 minutes, add sufficient *methanol* to produce 5 ml, centrifuge for 10 minutes and use the clear supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 28 volumes of *water* and 72 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{20}H_{26}O_2$ in each tablet using the declared content of $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

ASSAY

For tablets containing less than 2 mg of norethisterone

Calculate the average of the 10 individual results obtained in the test for Uniformity of content.

For tablets containing 2 mg or more of norethisterone

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the conditions described in the test for Uniformity of content and using the following solutions. Solution (1) contains 0.02% w/v of *norethisterone BPCRS* in *methanol* (60%). For solution (2) mix a quantity of the powdered tablets containing 5 mg of Norethisterone in 10 ml of *water* with the aid of ultrasound for 15 minutes, add sufficient *methanol* to produce 25 ml, centrifuge for 10 minutes and use the supernatant liquid.

The chromatographic procedure described under Uniformity of content may be used.

Calculate the content of $C_{20}H_{26}O_2$ using the declared content of $C_{20}H_{26}O_2$ in *norethisterone BPCRS*.

STORAGE

Norethisterone Tablets should be protected from light.

Norfloxacin Eye Drops

General Notices

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Norfloxacin Eye Drops are a sterile solution of Norfloxacin in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of norfloxacin, $C_{16}H_{18}FN_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 210 to 400 nm of a solution prepared by diluting the eye drops with 0.1M *hydrochloric acid* to contain 0.006% w/v of Norfloxacin exhibits the same maxima and minima as that of a 0.006% w/v solution of *norfloxacin BPCRS* in 0.1M *hydrochloric acid*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 5.0 to 5.4, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions. For solution (1) dilute a suitable volume of the eye drops with 0.1% v/v *orthophosphoric acid* to produce a solution containing 0.006% w/v of Norfloxacin. Solution (2) contains 0.006% w/v of *norfloxacin BPCRS* in 0.1% v/v *orthophosphoric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Waters µBondapak C18 is suitable) and maintained at 50°, (b) a mixture of 150 volumes of *acetonitrile* and 850 volumes of 0.1% v/v *orthophosphoric acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 278 nm. Inject 10 µl of each solution.

Precondition the column using 0.01M *anhydrous sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with *orthophosphoric acid*, at a flow rate of 0.5 ml per minute for 8 hours. Equilibrate the column with the mobile phase for about 30 minutes before starting the chromatography. When the chromatograms are recorded under the conditions described above, the retention time of norfloxacin is about 4 minutes.

The assay is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 2.0.

Inject solution (2) six times. The assay is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

Calculate the content of $C_{16}H_{18}FN_3O_3$ in the eye drops using the declared content of $C_{16}H_{18}FN_3O_3$ in *norfloxacin BPCRS*.

STORAGE

Norfloxacin Eye Drops should be protected from light.

Norfloxacin Tablets

General Notices

Action and use

Fluoroquinolone antibacterial.

DEFINITION

Norfloxacin Tablets contain Norfloxacin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of norfloxacin, $C_{16}H_{18}FN_3O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a silica gel GF_{254} precoated plate which has been previously washed with *methanol* and allowed to dry in air (Analtech plates are suitable) and a mixture of 8 volumes of *water*, 14 volumes of *diethylamine*, 20 volumes of *toluene*, 40 volumes of *chloroform* and 40 volumes of *methanol* as the mobile phase. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) add 2 ml of *water* to a quantity of whole tablets containing 0.4 g of Norfloxacin and disperse with the aid of ultrasound. Add 100 ml of a solution containing 50 volumes of *dichloromethane* and 50 volumes of a solution prepared by adding 9 ml of *hydrochloric acid* to 1000 ml of *methanol*, mix with the aid of ultrasound until a uniform suspension is produced, add sufficient of the same solvent mixture to produce 200 ml and centrifuge 25 ml of the final solution. For solution (2) dissolve, with the aid of ultrasound, 50 mg of *norfloxacin BPCRS* in 15 ml of a solution containing 50 volumes of *dichloromethane* and 50 volumes of a solution prepared by adding 9 ml of *hydrochloric acid* to 1000 ml of *methanol* and add sufficient of the same solvent mixture to produce 25 ml. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm* and *365 nm*). By each method of visualisation, the principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2, protected from light. Use as the medium 750 ml of an acetate buffer solution prepared in the following manner and rotate the paddle at 50 revolutions per minute. Add 14.3 ml of *glacial acetic acid* to 4500 ml of *water*, mix, add slowly with stirring 2.5 ml of a 50% w/v solution of *sodium hydroxide* and add sufficient *water* to produce 5000 ml. If necessary, adjust the pH to 4.0 with *glacial acetic acid* or a 50% w/v solution of *sodium hydroxide*. After 30 minutes, withdraw a sample of 20 ml of the medium, filter and dilute the filtrate with sufficient of the dissolution medium to give a solution expected to contain about 0.0016% w/v of Norfloxacin. Measure the *absorbance* of this solution, Appendix II B, at 313 nm using dissolution medium in the reference cell. Calculate the total content of norfloxacin, $C_{16}H_{18}FN_3O_3$, in the medium from the *absorbance* obtained from a 0.0016% w/v solution of *norfloxacin BPCRS* in the dissolution medium and using the declared content of $C_{16}H_{18}FN_3O_3$ in *norfloxacin BPCRS*. The amount of norfloxacin released is not less than 85% of the stated amount.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions. For solution (1) add 80 ml of the mobile phase to a quantity of the powdered tablets containing 0.1 g of Norfloxacin, mix with the aid of ultrasound for at least 5 minutes, add sufficient of the mobile phase to produce 200 ml, mix and dilute 10 volumes of this solution to 25 volumes with the mobile phase. Solution (2) contains 0.02% w/v of *norfloxacin BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Waters µBondapak C18 is suitable) maintained at 40°, (b) a mixture of 150 volumes of *acetonitrile* and 850 volumes of 0.1% v/v solution of *orthophosphoric acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 275 nm.

Inject 10 µl of each solution. Precondition the column using 0.01M *anhydrous sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with *orthophosphoric acid*, at a flow rate of 0.5 ml per minute for 8 hours. Equilibrate the column with the mobile phase for about 30 minutes before starting the chromatography. When the chromatograms are recorded under the conditions described above, the retention time of norfloxacin is about 5 minutes.

The assay is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 2.0.

Inject solution (2) six times. The assay is not valid unless the relative standard deviation of the area of the principal peak is at most 2.0%.

Calculate the content of $C_{16}H_{18}FN_3O_3$ in the tablets using the declared content of $C_{16}H_{18}FN_3O_3$ in *norfloxacin BPCRS*.

STORAGE

Norfloxacin Tablets should be protected from light and moisture.

Norgestrel Tablets

General Notices

Action and use

Progestogen.

DEFINITION

Norgestrel Tablets contain Norgestrel. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of norgestrel.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of norgestrel, $C_{21}H_{28}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to a quantity of the tablets containing 0.15 mg of Norgestrel add 10 ml of *methanol (80%)*, heat on a water-bath at 60° for 10 minutes, shaking occasionally, allow to cool, dilute with *methanol (80%)* to produce a solution containing 0.00375% w/v of Norgestrel, mix and filter through a membrane with a pore size of 0.45 µm. Solution (2) contains 0.004% w/v of *norgestrel BPCRS* in *methanol (80%)*.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution containing equal volumes of *methanol* and 1% w/v of *gamma-cyclodextrin* in *water* and (c) a detection wavelength of 242 nm.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to levonorgestrel and dextronorgestrel is at least 1.0.

The two principal peaks in the chromatogram obtained with solution (1) have the same retention times as the peaks due to levonorgestrel and dextronorgestrel in the chromatogram obtained with solution (2).

B. In the test for Uniformity of content, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 ml of a mixture of equal volumes of *methanol* and *water* to a quantity of the powdered tablets containing 0.15 mg of Norgestrel, mix with the aid of ultrasound for 30 minutes, stir vigorously for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of equal volumes of *methanol* and *water*. Solution (3) contains 0.004% w/v each of *ethinylestradiol BPCRS* and *norgestrel BPCRS* in a mixture of equal volumes of *methanol* and *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable) maintained at 30°, (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture of 100 volumes of *methanol*, 240 volumes of *acetonitrile* and 500 volumes of *water* and (c) a detection wavelength of 220 nm.

Inject separately 200 µl of each solution. For each solution allow the chromatography to proceed for twice the retention time of norgestrel.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to ethinylestradiol and norgestrel is at least 12.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

Tablets containing less than 2 mg of Norgestrel comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to 1 tablet add 5 ml of the mobile phase, heat on a water bath at 60° for 15 minutes while shaking, disperse with the aid of ultrasound for 5 minutes, centrifuge and use the clear supernatant liquid. Dilute, if necessary, with the mobile phase to produce a solution containing 0.0015% w/v of Norgestrel. Solution (2) contains 0.0015% w/v of *norgestrel BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1.3 ml per minute a mixture of equal volumes of *acetonitrile* and *water* and (c) a detection wavelength of 220 nm.

Inject 25 µl of each solution.

Calculate the content of $C_{21}H_{28}O_2$ in each tablet using the declared content of $C_{21}H_{28}O_2$ in *norgestrel BPCRS*.

ASSAY

Use the average of the 10 individual results obtained in the test for Uniformity of content.

Nortriptyline Capsules

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Nortriptyline Capsules contain Nortriptyline Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of nortriptyline, $C_{19}H_{21}N$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing the equivalent of 5 mg of nortriptyline with 20 ml of *methanol* and filter. To 1 ml of the filtrate add 1 ml of a 2.5% w/v solution of *sodium hydrogen carbonate*, 1 ml of a 2% w/v solution of *sodium periodate* and 1 ml of a 0.3% w/v solution of *potassium permanganate*. Allow to stand for 15 minutes, acidify with 1M *sulphuric acid* and extract with 10 ml of *2,2,4-trimethylpentane*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 265 nm.

B. Triturate a quantity of the contents of the capsules containing the equivalent of 0.1 g of nortriptyline with 10 ml of *chloroform*, filter and evaporate the chloroform. Dissolve 50 mg of the residue in 3 ml of warm *water*, cool and add 0.05 ml of a 2.5% w/v solution of *quinhydrone* in *methanol*. A red colour develops (distinction from amitriptyline).

C. The residue obtained in test B yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Dibenzosuberone

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 3 volumes of *diethylamine*, 15 volumes of *ethyl acetate* and 85 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) extract a quantity of the contents of the capsules containing the equivalent of 20 mg of nortriptyline with 5 ml of a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *ethanol* (*96%*), centrifuge and use the supernatant liquid. Solution (2) contains 0.0010% w/v of *dibenzosuberone* in *ethanol* (*96%*). After removal of the plate, allow it to dry in air until the solvent has evaporated, spray with *sulphuric acid* containing 4% v/v of *formaldehyde solution* and examine immediately under *ultraviolet light* (*365 nm*). Any spot corresponding to dibenzosuberone in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using a quantity of the mixed contents of 20 capsules containing the equivalent of 0.1 g of nortriptyline, adding 3 ml of *mercury(II) acetate solution* and using 0.02M *perchloric acid VS* as titrant and *crystal violet solution* as indicator. Each ml of 0.02M *perchloric acid VS* is equivalent to 5.268 mg of $C_{19}H_{21}N$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of nortriptyline.

Nortriptyline Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Nortriptyline Tablets contain Nortriptyline Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of nortriptyline, $C_{19}H_{21}N$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 5 mg of nortriptyline with 20 ml of *methanol* and filter. To 1 ml of the filtrate add 1 ml of a 2.5% w/v solution of *sodium hydrogen carbonate*, 1 ml of a 2% w/v solution of *sodium periodate* and 1 ml of a 0.3% w/v solution of *potassium permanganate*. Allow to stand for 15 minutes, acidify with 1M *sulphuric acid* and extract with 10 ml of *2,2,4-trimethylpentane*. The *light absorption* of the resulting trimethylpentane solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 265 nm.

B. Triturate a quantity of the powdered tablets containing the equivalent of 0.1 g of nortriptyline with 10 ml of *chloroform*, filter and evaporate the filtrate to a low volume. Add *ether* until a turbidity is produced and allow to stand. Dissolve 50 mg of the precipitate in 3 ml of warm *water*, cool and add 0.05 ml of a 2.5% w/v solution of *quinhydrone* in *methanol*. A red colour develops (distinction from amitriptyline).

C. The precipitate obtained in test B yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Dibenzosuberone

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 3 volumes of *diethylamine*, 15 volumes of *ethyl acetate* and 85 volumes of *cyclohexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 5 µl each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing the equivalent of 20 mg of nortriptyline with 5 ml of a mixture of 1 volume of 2M *hydrochloric acid* and 9 volumes of *ethanol* (*96%*), centrifuge and use the supernatant liquid. Solution (2) contains 0.0010% w/v of *dibenzosuberone* in *ethanol* (*96%*). After removal of the plate, allow it to dry in air until the solvent has evaporated, spray with *sulphuric acid* containing 4% v/v of *formaldehyde solution* and examine immediately under *ultraviolet light* (*365 nm*). Any spot corresponding to dibenzosuberone in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using as solution (1) a 0.025% w/v solution of *nortriptyline hydrochloride BPCRS* in *methanol* (*50%*). For solution (2) add 50 ml of *water* to 20 tablets, shake vigorously until the tablets have completely disintegrated, add 100 ml of *methanol* and shake for 30 minutes. Add sufficient *water* to produce 200 ml, filter and dilute a volume of the filtrate containing the equivalent of 25 mg of nortriptyline to 100 ml with *methanol* (*50%*).

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a 0.56% w/v solution of *sodium hexanesulphonate* in a mixture of equal volumes of *water* and *acetonitrile* adjusted to pH 4.5 with *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 239 nm.

Calculate the content of $C_{19}H_{21}N$ using the declared content of $C_{19}H_{21}N$ in *nortriptyline hydrochloride BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of nortriptyline.

Nystatin Ointment

General Notices

Action and use

Antifungal.

DEFINITION

Nystatin Ointment is a dispersion of Nystatin in *microfine powder* in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

IDENTIFICATION

Disperse a quantity containing 25,000 IU in 10 ml of *chloroform*, add 40 ml of *methanol* and shake. Filter and dilute 1 ml of the filtrate to 25 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima, at 291, 305 and 319 nm. The ratios of the *absorbances* at the maxima at 291 nm and 319 nm to the *absorbance* at the maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use in the reference cell a solution prepared in exactly the same manner but omitting the preparation being examined.

ASSAY

Carry out the following procedure protected from light. Disperse a quantity containing 400,000 IU in 20 ml of *ether* in a stoppered flask, add 70 ml of *dimethylformamide* and shake for 15 minutes. Add 10 ml of *water*, shake vigorously for 2 minutes and filter. Dilute 10 ml of the filtrate to 100 ml with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

LABELLING

The strength is stated as the number of IU (Units) per g.

Nystatin Oral Suspension

General Notices

Nystatin Oral Drops

Action and use

Antifungal.

DEFINITION

Nystatin Oral Suspension is a suspension of Nystatin in a suitable flavoured vehicle.

Nystatin Oral Suspension should not be diluted.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

IDENTIFICATION

To a quantity containing 300,000 IU add a mixture of 5 ml of *glacial acetic acid* and 50 ml of *methanol* and shake. Add sufficient *methanol* to produce 100 ml, filter and dilute 1 ml of the filtrate to 100 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima, at 291, 305 and 319 nm. The ratios of the *absorbances* at the maxima at 291 nm and 319 nm to the *absorbance* at the maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use in the reference cell a solution prepared in exactly the same manner but omitting the preparation being examined.

ASSAY

Carry out the following procedure protected from light. Dissolve a quantity containing 200,000 IU in sufficient *dimethylformamide* to produce 50 ml, dilute 10 ml to 200 ml with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU.

STORAGE

Nystatin Oral Suspension should be protected from light. It should not be allowed to freeze.

LABELLING

The strength is stated as the number of IU (Units) per ml. The label also states the appropriate directions for using the preparation.

Nystatin Pastilles

General Notices

Action and use

Antifungal.

DEFINITION

Nystatin Pastilles contain Nystatin.

The pastilles comply with the requirements stated under Oromucosal Preparations and with the following requirements.

IDENTIFICATION

Add 100 ml of 0.1M *sodium hydroxide* to a quantity of the pastilles containing 100,000 IU and that have been cut into small pieces, warm on a water bath, shake until dissolved, cool, filter and dilute 1 volume of the filtrate to 50 volumes with 0.1M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima at 291, 305 and 319 nm.

TESTS

Acidity or alkalinity

pH of a solution prepared by dissolving a quantity of the pastilles containing 100,000 IU in *water* at 37° and allowing to cool, 6.0 to 7.5, Appendix V L.

Uniformity of weight

Weigh individually 20 pastilles taken at random and determine the average weight. The weight of not more than one pastille deviates from the average weight by more than 10% and none deviates by more than 15%.

ASSAY

Carry out the following procedure protected from light. Weigh 20 pastilles and cut them into small pieces. Cool a quantity of the pastilles containing 500,000 IU at 5° for 1 hour until hard, add 50 ml of *water* and homogenise. Transfer quantitatively to a 250 ml graduated flask using three 60 ml quantities of *dimethylformamide,* shake for 5 minutes and add sufficient *dimethylformamide* to produce 250 ml. Dilute 10 ml to 100 ml with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90% and the lower fiducial limit of error is not more than 110% of the stated number of IU.

LABELLING

The quantity of active ingredient contained in each pastille is stated as the number of IU

(Units).

Nystatin Pessaries

General Notices

Action and use

Antifungal.

DEFINITION

Nystatin Pessaries are *vaginal tablets* containing Nystatin.

The pessaries comply with the requirements stated under Vaginal Preparations and with the following requirements.

IDENTIFICATION

To a quantity of the powdered pessaries containing 300,000 IU add a mixture of 5 ml of *glacial acetic acid* and 50 ml of *methanol* and shake. Add sufficient *methanol* to produce 100 ml, filter and dilute 1 ml of the filtrate to 100 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima, at 291, 305 and 319 nm. The ratios of the *absorbances* at the maxima at 291 nm and 319 nm to the *absorbance* at the maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use in the reference cell a solution prepared in exactly the same manner but omitting the preparation being examined.

TESTS

Loss on drying

When dried to constant weight at 105°, the powdered pessaries lose not more than 5.0% of their weight. Use 1 g.

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 pessaries. Shake a quantity of the powder containing 200,000 IU with 50 ml of *dimethylformamide* for 1 hour. Centrifuge, dilute 10 ml of the clear supernatant liquid to 200 ml with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 125.0% of the stated number of IU.

LABELLING

The quantity of active ingredient is stated as the number of IU (Units).

Nystatin Tablets

General Notices

Action and use

Antifungal.

DEFINITION

Nystatin Tablets contain Nystatin. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 300,000 IU with a mixture of 5 ml of *glacial acetic acid* and 50 ml of *methanol*, add sufficient *methanol* to produce 100 ml and filter. Dilute 1 ml of the filtrate to 100 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits three maxima, at 291, 305 and 319 nm. The ratios of the *absorbances* at the maxima at 291 nm and 319 nm to the *absorbance* at the maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use in the reference cell a solution prepared in exactly the same manner but omitting the preparation being examined.

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A1, but using a 0.6% v/v solution of *hydrochloric acid* in place of *water*. If the tablets fail to disintegrate, wash them rapidly by immersion in *water* and continue the test using *phosphate buffer pH 6.8*; the tablets then disintegrate within a further 30 minutes.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the powdered tablets lose not more than 5.0% of their weight. Use 1 g.

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Shake a quantity of the powder containing 200,000 IU with 50 ml of *dimethylformamide* for 1 hour. Centrifuge, dilute 10 ml of the clear, supernatant liquid to 200 ml with a solution containing 9.56% w/v of *potassium dihydrogen orthophosphate* and 11.5% v/v of 1M *potassium hydroxide* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated number of IU.

LABELLING

The quantity of active ingredient is stated as the number of IU (Units).

Camphorated Opium Tincture

General Notices

DEFINITION

Opium Tincture	50 ml
Benzoic Acid	5 g
Racemic Camphor	3 g
Anise Oil or Star Anise Oil	3 ml
Ethanol (60 per cent)	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Benzoic Acid, the Racemic Camphor and the Anise Oil or Star Anise Oil in 900 ml of Ethanol (60 per cent), add the Opium Tincture and sufficient Ethanol (60 per cent) to produce 1000 ml and mix. Filter, if necessary.

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

Content of anhydrous morphine, $C_{17}H_{19}NO_3$

0.045 to 0.055% w/v.

TESTS

Ethanol content

56 to 60% v/v, Appendix VIII F, Method III.

Relative density

0.90 to 0.92, Appendix V G.

ASSAY

To 10 ml add 5 ml of *water* and 1 ml of 5M *ammonia* and extract with 30 ml of a mixture of equal volumes of *ethanol* (*96%*) and *chloroform* and then with two 22.5 ml quantities of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*), washing each extract with the same 20 ml of a mixture of equal volumes of *ethanol* (*96%*) and *water*. Evaporate the combined extracts almost to dryness, extract the residue with 10 ml of *calcium hydroxide solution*, filter and wash the filter with 10 ml of *calcium hydroxide solution*. To the combined filtrate and washings add 0.1 g of *ammonium sulphate*, extract with two 10 ml quantities of *ethanol-free chloroform*, wash the combined extracts with 10 ml of *water* and discard the chloroform solution. To the combined alkaline liquid and aqueous washings add 10 ml of 1M *hydrochloric acid*, heat on a water bath to remove any chloroform, cool and dilute to 100 ml with *water*. To 10 ml of this solution add 10 ml of 0.1M *hydrochloric acid* and 8 ml of a freshly prepared 1.0% w/v solution of *sodium nitrite*, allow to stand for 15 minutes, add 12 ml of 5M *ammonia*, dilute to 50 ml with *water* and measure the *absorbance* of a 4-cm layer of the resulting solution at the maximum at 442 nm, Appendix II B, using in the reference cell a solution prepared at the same time and in the same manner but using 8 ml of *water* in place of the solution of sodium nitrite. Calculate the content of $C_{17}H_{19}NO_3$ taking 124 as the value of A(1%, 1 cm) at the maximum at 442 nm.

Compound Orange Spirit

General Notices

DEFINITION

Terpeneless Orange Oil	2.5 ml
Terpeneless Lemon Oil	1.3 ml
Anise Oil or Star Anise Oil	4.25 ml
Coriander Oil	6.25 ml
Ethanol (90 per cent)	Sufficient to produce 1000 ml

The spirit complies with the requirements stated under Spirits and with the following requirements.

TESTS

Ethanol content

86 to 90% v/v, Appendix VIII F.

Weight per ml

0.828 to 0.841 g, Appendix V G.

Concentrated Camphorated Opium Tincture

General Notices

DEFINITION

Opium Tincture	400 ml
Benzoic Acid	40 g
Racemic Camphor	24 g
Anise Oil or Star Anise Oil	24 ml
Ethanol (96 per cent)	400 ml
Water	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Benzoic Acid, the Racemic Camphor and the Anise Oil or Star Anise Oil in the Ethanol (96 per cent), add the Opium Tincture and sufficient Water to produce 1000 ml, mix and filter if necessary.

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

Content of anhydrous morphine, $C_{17}H_{19}NO_3$

0.36 to 0.44% w/v.

TESTS

Ethanol content

54 to 59% v/v, Appendix VIII F, Method III.

Relative density

0.912 to 0.930, Appendix V G.

ASSAY

Dilute 10 ml to 100 ml with *ethanol* (*50%*) and carry out the Assay described under Camphorated Opium Tincture using 10 ml of the diluted solution.

Gastro-resistant Omeprazole Capsules

General Notices

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION

Gastro-resistant Omeprazole Capsules contain Omeprazole. They are covered with a gastro-resistant coating or prepared from granules or particles covered with a gastro-resistant coating.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of omeprazole, $C_{17}H_{19}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of finely powdered capsule contents containing 20 mg of Omeprazole with 50 ml of 0.1M *sodium hydroxide,* dilute to 100 ml, filter and further dilute 10 ml to 100 ml. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 276 nm and 305 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Carry out the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the media the solutions described sequentially below and rotate the paddles at 100 revolutions per minute.

Mix 11 volumes of 0.25M *trisodium orthophosphate* and 22 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 100 volumes with *water* and adjust the pH, if necessary, to 11.0 ± 0.05 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution A).

Mix 1 volume of 10M *sodium hydroxide* with 99 volumes of *0.05M phosphate buffer solution pH 4.5* (solution B).

Mix 5.2 volumes of 1M *anhydrous sodium dihydrogen orthophosphate* and 63.2 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 1000 volumes with *water* and adjust the pH, if necessary, to 7.6 ± 0.05 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution C).

First stage (pH 4.5)

Use as the medium 700 ml of *0.05M phosphate buffer solution pH 4.5.* After 45 minutes, withdraw 5 ml of the medium, filter the aliquot, dilute to 25 ml with solution A and retain the samples for analysis as described below. Proceed immediately to the final stage.

Final stage (pH 6.8)

Within 5 minutes, add 200 ml of solution B at 37±0.5° to the vessel. Maintain the rotation speed at 100 revolutions per minute and continue to operate the apparatus for 45 minutes. Withdraw 5 ml of the medium, filter the aliquot, dilute to 25 ml with solution A and retain the samples for analysis as described below.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the sample solutions taken above.

(2) Dissolve a sufficient quantity of *omeprazole BPCRS* in solution A and dilute with *water*; the concentration of the final solution should be the same as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (15 cm × 2 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Nucleosil C18 is suitable). Use a suitable guard column.

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 0.25 ml per minute.

(d) Column temperature of 30°.

(e) Detection wavelength of 302 nm.

(f) Injection volume of 10 µl.

Allow the chromatography to proceed for 8 times the retention time of omeprazole.

MOBILE PHASE

Mix 25 volumes of solution C, 35 volumes of *water* and 40 volumes of *acetonitrile*. Adjust the pH, if necessary, to 7.6 ± 0.05 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate.

SYSTEM SUITABILITY

Inject solution (2) six times. The test is not valid unless the relative standard deviation of the areas of the peak due to omeprazole is not more than 2.0% and the *symmetry factor* of the peak due to omeprazole is not more than 2.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_{17}H_{19}N_3O_3S$ in the medium using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

LIMITS

The amount of omeprazole released after the first stage is not more than 10% of the stated amount. The amount of omeprazole released after the final stage is not less than 65% (Q) of the stated amount.

Impurity C

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in solvent A.

Prepare a mixture of equal volumes of *methanol* and *dichloromethane* (solvent A).

(1) Weigh the contents of 20 capsules and grind to a fine powder. Disperse a quantity of the powder containing 50 mg of Omeprazole in 15 ml of solvent A, mix with the aid of ultrasound for 30 minutes, dilute with solvent A to produce 20 ml, mix and filter. Evaporate the filtrate to dryness and dissolve the residue in 1 ml of the same solvent.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A. Dilute 1 volume of this solution to 10 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel* F_{254} as the coating substance.

(b) The mobile phase is a mixture of 20 volumes of *propan-2-ol*, 40 volumes of *dichloromethane* that has been previously shaken with *concentrated ammonia* (shake 100 ml of *dichloromethane* with 30 ml of *concentrated ammonia* in a separating funnel; allow the layers to separate and use the lower layer) and 40 volumes of *dichloromethane*.

(c) Application volume of 10 µl for each solution.

(d) Develop to 15 cm.

(e) Remove the plate, dry in air and examine under *ultraviolet light (254 nm)*.

LIMITS

Any spot in the chromatogram obtained with solution (1) with a higher Rf value than that of the spot due to omeprazole is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Weigh the contents of 20 capsules and grind to a fine powder. Disperse a quantity of the powder containing 24 mg of Omeprazole in 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 ml, mix and filter.

(2) Dilute 5 volumes of solution (1) to 100 volumes with the mobile phase. Dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) Mix 10 mg each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS* in mobile phase and dilute to 100 ml with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil RP8 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 280 nm.

(f) Injection volume of 40 µl.

Allow the chromatography to proceed for 3 times the retention time of omeprazole.

MOBILE PHASE

A mixture of 27 volumes of *acetonitrile* and 73 volumes of a 0.14% w/v solution of *disodium hydrogen orthophosphate* that has been previously adjusted to pH 7.6 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and omeprazole is greater than 3.0 and the retention time of omeprazole is about 9 minutes.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak due to impurity D or any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). The sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Weigh the contents of 20 capsules and grind to a fine powder. Disperse a quantity of the powder containing 24 mg of Omeprazole in 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 ml, mix and filter and further dilute 10 volumes to 100 volumes with the mobile phase.

(2) 0.0012% w/v of *omeprazole BPCRS* in the mobile phase.

(3) Mix 10 mg each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS* in mobile phase and dilute to 100 ml with the same solvent.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under the test for Related substances may be used but with a detection wavelength of 305 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and omeprazole is greater than 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{19}N_3O_3S$ in the capsules from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

IMPURITIES

The impurities limited by this monograph include those listed under Omeprazole.

Gastro-resistant Omeprazole Tablets

General Notices

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

DEFINITION

Gastro-resistant Omeprazole Tablets contain Omeprazole. They are covered with a gastro-resistant coating or prepared from granules or particles covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of omeprazole, $C_{17}H_{19}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of powdered tablets containing 20 mg of Omeprazole with 50 ml of 0.1M *sodium hydroxide,* dilute to 100 ml, filter and further dilute 10 ml to 100 ml. The *light absorption*, Appendix II B, in the range 230 to 350 nm exhibits two maxima at 276 nm and 305 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Carry out the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the media the solutions described sequentially below and rotate the paddles at 100 revolutions per minute.

Mix 11 volumes of 0.25M *trisodium orthophosphate* and 22 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 100 volumes with *water* and adjust the pH, if necessary, to 11.0 ± 0.05 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution A).

Mix 1 volume of 10M *sodium hydroxide* with 99 volumes of *0.05M phosphate buffer solution pH 4.5* (solution B).

Mix 5.2 volumes of 1M *anhydrous sodium dihydrogen orthophosphate* and 63.2 volumes of 0.5M *anhydrous disodium hydrogen orthophosphate*, dilute to 1000 volumes with *water* and adjust the pH, if necessary, to 7.6 ± 0.05 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate (solution C).

First stage (pH 4.5)

Use as the medium 700 ml of *0.05M phosphate buffer solution pH 4.5.* After 45 minutes, withdraw 5 ml of the medium, filter the aliquot, dilute to 25 ml with solution A and retain the samples for analysis as described below. Proceed immediately to the final stage.

Final stage (pH 6.8)

Within 5 minutes, add 200 ml of solution B at 37±0.5° to the vessel. Maintain the rotation speed at 100 revolutions per minute and continue to operate the apparatus for 45 minutes. Withdraw 5 ml of the medium, filter the aliquot, dilute to 25 ml with solution A and retain the samples for analysis as described below.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the sample solutions taken above.

(2) Dissolve a sufficient quantity of *omeprazole BPCRS* in solution A and dilute with *water*; the concentration of the final solution should be the same as that expected for solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (15 cm × 2 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Nucleosil C18 is suitable). Use a suitable guard column.

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 0.25 ml per minute.

(d) Column temperature of 30°.

(e) Detection wavelength of 302 nm.

(f) Injection volume of 10 µl.

Allow the chromatography to proceed for 8 times the retention time of omeprazole.

MOBILE PHASE

Mix 25 volumes of solution C, 35 volumes of *water* and 40 volumes of *acetonitrile*. Adjust the pH, if necessary, to 7.6 ± 0.05 with *orthophosphoric acid* or 10M *sodium hydroxide*, as appropriate.

SYSTEM SUITABILITY

Inject solution (2) six times. The test is not valid unless the relative standard deviation of the areas of the peak due to omeprazole is not more than 2.0% and the *symmetry factor* of the peak due to omeprazole is not more than 2.0.

DETERMINATION OF CONTENT

Calculate the total content of $C_{17}H_{19}N_3O_3S$ in the medium using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

LIMITS

The amount of omeprazole released after the first stage is not more than 10% of the stated amount. The amount of omeprazole released after the final stage is not less than 65% (Q) of the stated amount.

Impurity C

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in solvent A.

Prepare a mixture of equal volumes of *methanol* and *dichloromethane* (solvent A).

(1) Disperse a quantity of the powdered tablets containing 50 mg of Omeprazole in 15 ml of solvent A, mix with the aid of ultrasound for 30 minutes, dilute with solvent A to produce 20 ml, mix and filter. Evaporate the filtrate to dryness and dissolve the residue in 1 ml of the same solvent.

(2) Dilute 1 volume of solution (1) to 100 volumes with solvent A. Dilute 1 volume of this solution to 10 volumes with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel* F_{254} as the coating substance.

(b) The mobile phase is a mixture of 20 volumes of *propan-2-ol*, 40 volumes of *dichloromethane* that has been previously shaken with *concentrated ammonia* (shake 100 ml of *dichloromethane* with 30 ml of *concentrated ammonia* in a separating funnel; allow the layers to separate and use the lower layer) and 40 volumes of *dichloromethane*.

(c) Application volume of 10 µl for each solution.

(d) Develop to 15 cm.

(e) Remove the plate, dry in air and examine under *ultraviolet light (254 nm)*.

LIMITS

Any spot in the chromatogram obtained with solution (1) with a higher Rf value than that of the spot due to omeprazole is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 24 mg of Omeprazole in 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 ml, mix and filter.

(2) Dilute 5 volumes of solution (1) to 100 volumes with the mobile phase. Dilute 1 volume of this solution to 10 volumes with mobile phase.

(3) Mix 10 mg each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS* in mobile phase and dilute to 100 ml with the same solvent.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography R* (5 µm) (Nucleosil RP8 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 280 nm.

(f) Injection volume of 40 µl.

Allow the chromatography to proceed for 3 times the retention time of omeprazole.

MOBILE PHASE

A mixture of 27 volumes of *acetonitrile* and 73 volumes of a 0.14% w/v solution of *disodium hydrogen orthophosphate* that has been previously adjusted to pH 7.6 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and omeprazole is greater than 3.0 and the retention time of omeprazole is about 9 minutes.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak due to impurity D or any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). The sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Disperse a quantity of the powdered tablets containing 24 mg of Omeprazole in 150 ml of mobile phase, mix with the aid of ultrasound for 30 minutes, dilute with sufficient mobile phase to produce 200 ml, mix, filter and further dilute 10 volumes to 100 volumes.

(2) 0.0012% w/v of *omeprazole BPCRS* in mobile phase.

(3) Mix 10 mg each of *omeprazole BPCRS* and *omeprazole impurity D EPCRS* in mobile phase and dilute to 100 ml with the same solvent.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under the test for Related substances may be used but with a detection wavelength of 305 nm.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity D and omeprazole is greater than 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{19}N_3O_3S$ in the tablets from the chromatograms obtained and using the declared content of $C_{17}H_{19}N_3O_3S$ in *omeprazole BPCRS*.

IMPURITIES

The impurities limited by this monograph include those listed under Omeprazole.

Olive Oil Ear Drops

General Notices

DEFINITION

Olive Oil Ear Drops are Virgin Olive Oil in a suitable container.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

IDENTIFICATION

Carry out the test for the *identification of fixed oils by thin-layer chromatography*, Appendix X N. The chromatogram obtained from the oil being examined shows spots corresponding to those in the *typical chromatogram* for olive oil. For certain types of olive oil the difference in the sizes of spots E and F is less pronounced than in the figure.

TESTS

Acid value

Not more than 2.0, Appendix X B. Use 5 g.

Light absorption

The *absorbance* of a 1% w/v solution in *cyclohexane* at the maximum at 270 nm is not more than 0.20, Appendix II B. The ratio of the *absorbance* at 232 nm to that at 270 nm is greater than 8.

Peroxide value

Not more than 20.0, Appendix X F.

Relative density

0.910 to 0.916, Appendix V G.

Unsaponifiable matter

Not more than 1.5% w/w when determined by the following method. To 5 g of the oil in a 150-ml flask add 50 ml of 2M *ethanolic potassium hydroxide* and heat under a reflux condenser on a water bath for 1 hour, shaking frequently. Add 50 ml of *water* through the condenser, shake, allow to cool and transfer the contents of the flask to a separating funnel, rinsing the flask with 50 ml of *petroleum spirit* (*boiling range, 40° to 60°*) in portions and adding the rinsings to the separating funnel. Shake vigorously for 1 minute, allow to separate and transfer the aqueous layer to a second separating funnel. If an emulsion forms add small quantities of *ethanol* (*96%*) or a concentrated solution of *potassium hydroxide*. Extract the aqueous layer with two 50-ml quantities of *petroleum spirit* (*boiling range, 40° to 60°*) and combine the organic layers in a third separating funnel. Wash with three 50-ml quantities of *ethanol* (*50%*), transfer the petroleum spirit layer to a weighed 250-ml flask, rinse the separating funnel with small quantities of *petroleum spirit* (*boiling range, 40° to 60°*) and add the rinsings to the flask.

Evaporate the solvent on a water bath, dry at 100° to 105° for 15 minutes with the flask in a horizontal position, allow to cool and weigh. Repeat the drying for successive 15-minute periods until the loss in weight between two successive weighings does not exceed 0.1%. Dissolve the residue in 20 ml of *ethanol* (*96%*) previously neutralised to 0.1 ml of *bromophenol blue solution* and, if necessary, titrate with 0.1M *hydrochloric acid VS*. Calculate the percentage content of unsaponifiable matter from the expression

$100(a-0.032b)/w$

where a is the weight, in g, of the residue, b is the volume, in ml, of 0.1M *hydrochloric acid VS* required and w is the weight, in g, of oil taken. The test is not valid if $0.032b$ is greater than 5% of a and must be repeated.

Foreign fixed oils

Carry out the test for *foreign oils by gas chromatography*, Appendix X N. The fatty-acid fraction of the oil has the following composition.

Saturated fatty acids of chain length less than C_{16}: Not more than 0.1%.

Palmitic acid: 7.5 to 20.0%.

Palmitoleic acid (equivalent chain length on polyethylene glycol adipate, 16.3): Not more than 3.5%.

Stearic acid: 0.5 to 5.0%.

Oleic acid (equivalent chain length on polyethylene glycol adipate, 18.3): 56.0 to 85.0%.

Linoleic acid (equivalent chain length on polyethylene glycol adipate, 18.9): 3.5 to 20.0%.

Linolenic acid (equivalent chain length on polyethylene glycol adipate, 19.7): Not more than 1.2%.

Arachidic acid: Not more than 0.7%.

Eicosenoic acid (equivalent chain length on polyethylene glycol adipate, 20.3): Not more than 0.4%.

Behenic acid: Not more than 0.2%.

Lignoceric acid: Not more than 0.2%.

Sterols

Carry out the test for the *determination of sterols*, Appendix X N. The sterol fraction of the oil has the following composition:

sum of contents of β-sitosterol, Δ5,23-stigmastadienol, clerosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol: not less than 93.0%.

cholesterol: not more than 0.5%.

Δ7-stigmasterol: not more than 0.5%.

campesterol: not more than 4.0%.

and the content of stigmasterol is not more than that of campesterol.

Sesame oil

Shake 10 ml for about 1 minute with a mixture of 0.5 ml of a 0.35% v/v solution of *furfuraldehyde* in *acetic anhydride* and 4.5 ml of *acetic anhydride* in a cylinder closed with a ground-glass stopper, filter through paper impregnated with *acetic anhydride* and add 0.2 ml of *sulphuric acid* to the filtrate. No bluish green colour develops.

STORAGE

Olive Oil Ear Drops should be kept in a well-filled container and protected from light.

Ondansetron Injection

General Notices

Action and use

Serotonin 5HT$_3$ antagonist; treatment of nausea and vomiting.

DEFINITION

Ondansetron Injection is a sterile solution of Ondansetron Hydrochloride Dihydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ondansetron, $C_{18}H_{19}N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume of the injection containing the equivalent of 40 mg of ondansetron add 3 ml of 10M *sodium hydroxide*, shake and filter. Wash the precipitate with *water* and dry at 60° at a pressure of 2 kPa. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of ondansetron *(RS 418)*.

TESTS

Acidity

pH, 3.3 to 4.0, Appendix V L.

Impurity B

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions in solvent A.

SOLVENT A

0.5 volumes of 13.5M *ammonia,* 100 volumes of *ethanol (96%)* and 100 volumes of *methanol*.

(1) Dilute the injection, if necessary, to give a solution containing the equivalent of 0.2% w/v of ondansetron.

(2) Dilute 1 volume of solution (1) to 50 volumes and dilute 4 volumes of the resulting solution to 20 volumes.

(3) 0.25% w/v solution of *ondansetron for TLC system suitability BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use *silica gel* F_{254} as the coating.

(b) Use the mobile phase described below.

(c) Apply 100 µl of each solution.

(d) Develop the plate to 15 cm.

(e) Remove the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 40 volumes of *methanol*, 50 volumes of *ethyl acetate* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows 3 clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* corresponding to impurity B is not more intense than the spot obtained with solution (2) (0.4%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection, if necessary, to give a solution containing the equivalent of 0.1% w/v of ondansetron.

(2) Dilute 1 volume of solution (1) to 50 volumes and further dilute 1 volume of the resulting solution to 10 volumes.

(3) 0.0001% w/v of *imidazole* (impurity E) and 0.0001% w/v of *2-methylimidazole* (impurity F) .

(4) 0.05% w/v of *ondansetron impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5µm) (Spherisorb CN is suitable).

(b) Use isocratic elution with the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 216 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

20 volumes of *acetonitrile* and 80 volumes of 0.02M *sodium dihydrogen orthophosphate dihydrate*, previously adjusted to pH 5.4 with 1M *sodium hydroxide.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peaks due to impurity E and impurity F is at least 1.3;

and in solution (4):

the *resolution factor* between the peaks due to impurity C and D is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D (the second eluting peak in solution (4)) is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the area of any peak corresponding to impurity C (the first eluting peak in solution (4)) is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.2%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the principal peak in solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection in *water BET* to give a solution containing 5 mg of ondansetron per ml (solution A). The endotoxin limit concentration of solution A is 21.88 IU endotoxin per ml.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dilute the injection to give a solution containing the equivalent of 0.01% w/v of ondansetron.

(2) 0.0125% w/v of *ondansetron hydrochloride dihydrate BPCRS*.

(3) 0.05% w/v of *ondansetron impurity standard BPCRS.*

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and impurity D is at least 2.5.

Determination of content

Calculate the content of $C_{18}H_{19}N_3O$ in the injection using the declared content of $C_{18}H_{19}N_3O$ in *ondansetron hydrochloride dihydrate BPCRS.* Each mg of $C_{18}H_{19}N_3O$ is equivalent to 1.25 mg $C_{18}H_{19}N_3O,HCl,2H_2O$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ondansetron Hydrochloride Dihydrate.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ondansetron.

Ondansetron Tablets

General Notices

Action and use

Serotonin $5HT_3$ antagonist; treatment of nausea and vomiting.

DEFINITION

Ondansetron Tablets contain Ondansetron Hydrochloride Dihydrate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ondansetron, $C_{18}H_{19}N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 30 mg of ondansetron with 20 ml of *acetonitrile,* filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ondansetron hydrochloride *(RS 419)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 ml of *water*, at a temperature of 37 º, as the medium.

PROCEDURE

(1) After 45 minutes, withdraw a 10 ml sample of the medium, and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 310 nm, Appendix II B, using *water* in the reference cell.

(2) Measure the *absorbance* of a 0.01% w/v solution of *ondansetron hydrochloride dihydrate BPCRS* using *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of ondansetron, $C_{18}H_{19}N_3O$, in the medium from the absorbances obtained and using the declared content of $C_{18}H_{19}N_3O$, in *ondansetron hydrochloride dihydrate BPCRS.* Each mg of $C_{18}H_{19}N_3O$ is equivalent to 1.25 mg $C_{18}H_{19}N_3O,HCl,2H_2O$.

Impurity B

Carry out the method for *thin-layer chromatography,* Appendix III A, using the following solutions in solvent A.

SOLVENT A

0.5 volumes of 13.5M *ammonia,* 100 volumes of *ethanol (96%)* and 100 volumes of *methanol.*

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of ondansetron with 25 ml *acetonitrile,* filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in 2 ml of solvent A.

(2) Dilute 1 volume of solution (1) to 50 volumes and dilute 4 volumes of the resulting solution to 20 volumes.

(3) 0.25% w/v solution of *ondansetron for TLC system suitability BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution.

(d) Develop the plate to 15 cm.

(e) Remove the plate, dry in air and examine under *ultraviolet light (254 nm).*

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 40 volumes of *methanol*, 50 volumes of *ethyl acetate* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows 3 clearly separated spots.

LIMIT

In the chromatogram obtained with solution (1):

— any *secondary spot* corresponding to impurity B is not more intense than the spot obtained in the chromatogram obtained with solution (2) (0.4%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of ondansetron with 25 ml of *acetonitrile,* filter and evaporate the filtrate to dryness. Dissolve the residue in 20 ml of mobile phase.

(2) Dilute 1 volume of solution (1) to 50 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(3) 0.0001% w/v of *imidazole* (impurity E) and 0.0001% w/v of *2-methylimidazole* (impurity F) in the mobile phase.

(4) 0.05% w/v of *ondansetron impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *nitrile silica gel for chromatography* (5µm) (Spherisorb CN is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 216 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

20 volumes of *acetonitrile* and 80 volumes of 0.02M *sodium dihydrogen orthophosphate dihydrate*, previously adjusted to pH 5.4 with 1M *sodium hydroxide.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity E and impurity F is at least 1.3 and in solution (4) the *resolution factor* between the peaks due to impurity C and impurity D is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity D (the second eluting peak in solution (4) is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

— the area of any peak corresponding to impurity C (the first eluting peak in solution (4)) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

— the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

— the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of ondansetron with 25 ml of *acetonitrile,* filter and evaporate the filtrate to dryness. Dissolve the residue in 200 ml of mobile phase and dilute 1 volume to 10 volumes with mobile phase.

(2) 0.0125% w/v of *ondansetron hydrochloride dihydrate BPCRS* in the mobile phase.

(3) 0.05% w/v of *ondansetron impurity standard BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and impurity D is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{19}N_3O$ in the tablets using the declared content of $C_{18}H_{19}N_3O$, in *ondansetron hydrochloride dihydrate BPCRS.*

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Ondansetron Hydrochloride Dihydrate.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ondansetron.

Oral Rehydration Salts

General Notices

Action and use

Prevention and treatment of dehydration.

DEFINITION

Oral Rehydration Salts are *oral powders* containing either Anhydrous Glucose or Glucose, Sodium Chloride, Potassium Chloride and Sodium Citrate or Sodium Bicarbonate. After being dissolved in the requisite volume of Water they are intended for the prevention and treatment of dehydration due to diarrhoea, including maintenance therapy. Oral Rehydration Salts may contain suitable flavouring agents. Where necessary they may also contain suitable flow agents in the minimum quantity required to achieve a satisfactory product.

The composition of one of the formulations in use is described below in terms of the amount in grams to be dissolved in sufficient Water to produce 1000 ml. It is that recommended by the Diarrhoeal Diseases Control Programme of the World Health Organization (WHO) and the United Nations Childrens Fund (UNICEF).

Sodium Chloride	2.6 g
Potassium Chloride	1.5 g
Sodium Citrate	2.9 g
Anhydrous Glucose	13.5 g

The oral powders comply with the requirements stated under Oral Powders and with the following requirements.

Content of potassium, K, sodium, Na, bicarbonate, HCO_3, chloride, Cl, and citrate, $C_6H_5O_7$, as appropriate,

90.0 to 110.0% of the requisite amount, the latter being calculated from the stated amounts of the relevant constituents.

Content of anhydrous glucose, $C_6H_{12}O_6$,

As appropriate 90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. When heated with *cupri-tartaric solution R1* a copious precipitate of copper(I) oxide is produced.

B. Yield reaction B characteristic of *potassium salts*, Appendix VI.

C. Yield reaction A characteristic of *sodium salts*, Appendix VI.

D. Yield reaction A characteristic of *chlorides*, Appendix VI.

E. For preparations containing Sodium Citrate a quantity of the oral powder containing the equivalent of 50 mg of citric acid yields reactions A and B characteristic of *citrates*, Appendix VI.

F. For preparations containing Sodium Bicarbonate add 2 ml of *hydrochloric acid*. A vigorous effervescence is produced.

ASSAY

Carry out the following assays on the well-mixed contents of an individual sachet or on a suitable sample from the well-mixed contents of a bulk container. Where the amount in an individual sachet is insufficient to carry out all the assays, thoroughly mix the contents of sufficient sachets to provide enough material for all of the assays.

For the Assays for potassium, for sodium, for chloride and for bicarbonate dissolve 8 g in sufficient *water* to produce 500 ml (solution A).

For potassium

Dilute a suitable volume of solution A with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm. Use *potassium standard solution* (*600 ppm K*), suitably diluted with the strontium chloride solution, for the standard solutions. Each mg of Potassium Chloride is equivalent to 0.5245 mg of K.

For sodium

Dilute a suitable volume of solution A with a solution of *strontium chloride* such that there is a 1500- to 2000-fold excess of strontium ions in the final solution and determine by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm. Use *sodium standard solution* (*200 ppm Na*), suitably diluted with the strontium chloride solution, for the standard solutions. Each mg of Sodium Chloride, of Sodium Bicarbonate and of Sodium Citrate is equivalent to 0.3934, 0.2737 and 0.2345 mg of Na respectively.

For chloride

Titrate a suitable volume (50 ml for the formula stated above) of solution A with 0.1M *silver nitrate VS* using a 5% w/v solution of *potassium chromate* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 3.545 mg of Cl. Each mg of Potassium Chloride and of Sodium Chloride is equivalent to 0.4756 mg and 0.6066 mg of Cl respectively.

For citrate

Disperse 2.8 g in 80 ml of *anhydrous acetic acid*, heat to about 50°, cool, dilute to 100 ml with *anhydrous acetic acid*, allow to stand for 10 minutes and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 20 ml of the supernatant liquid and *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 6.303 mg of $C_6H_5O_7$. Each mg of Sodium Citrate is equivalent to 0.6430 mg of $C_6H_5O_7$.

For bicarbonate

Titrate 200 ml of solution A with 0.1M *hydrochloric acid VS* using *methyl orange solution* as indicator. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 6.101 mg of HCO_3. Each mg of Sodium Bicarbonate is equivalent to 0.7263 mg of HCO_3.

For glucose

Dissolve 7.5 g in 40 ml of *water*, add 0.5 ml of 1M *ammonia*, dilute to 50 ml with *water*, mix well and allow to stand for not less than 5 hours. If necessary use a suitable method of filtration to obtain a clear solution. Determine the *optical rotation* of the solution, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of $C_6H_{12}O_6$ in the weight taken for the Assay.

STORAGE

Oral Rehydration Salts should be protected from moisture. They should be kept in sachets, preferably made of aluminium foil, containing sufficient for a single dose or for a single day's treatment. Powders for use in hospitals may be presented in bulk containers containing sufficient to produce a volume of solution appropriate to the daily requirements of the establishment concerned.

LABELLING

The label states (1) for sachets the total weights, in grams, of each of the constituents; (2) for bulk containers the weights, in grams, of each of the constituents in a stated quantity, in grams, of the oral powder; (3) the directions for use; (4) that any portion of the solution prepared using the oral powder that remains unused 24 hours after preparation should be discarded.

The label indicates the pharmaceutical form as 'oral powder'.

Orange Syrup

General Notices

DEFINITION

Orange Tincture	60 ml
Syrup	Sufficient to produce 1000 ml

The syrup complies with the requirements stated under Oral Liquids and with the following requirement.

TESTS

Weight per ml

1.29 to 1.31 g, Appendix V G.

Orciprenaline Oral Solution

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Orciprenaline Oral Solution is a solution of Orciprenaline Sulphate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of orciprenaline sulphate, $(C_{11}H_{17}NO_3)_2,H_2SO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds in position, colour and size to that in the chromatogram obtained with solution (5). The test is not valid unless the chromatogram obtained with solution (6) shows a single, compact spot.

B. To 2 ml add 2 ml of a 0.1% w/v solution of *2,6-dichloroquinone-4-chloroimide* in *ethanol* (*96%*) and 1 ml of *sodium carbonate solution*; a violet colour is produced immediately changing to greenish brown and then light brown over a period of 5 minutes. The final colour is not more intense than that of a blank prepared at the same time and in the same manner but using 2 ml of *ethanol* (*96%*) in place of the preparation being examined.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel G precoated plate (Merck plates are suitable) and a mixture of 4 volumes of 13.5M *ammonia*, 16 volumes of *water*, 30 volumes of *propan-2-ol* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) dilute the oral solution, if necessary, with a mixture of 5 volumes of *methanol* and 1 volume of *water* to produce a solution containing 0.20% w/v of Orciprenaline Sulphate. For solution (2) dilute 1 volume of solution (1) to 10 volumes with the same solvent. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the same solvent. Solution (4) contains 0.1% w/v of *methyl 4-hydroxybenzoate* in a mixture of 5 volumes of *methanol* and 1 volume of *water*. Solution (5) contains 0.020% w/v of *orciprenaline sulphate BPCRS* in a mixture of 5 volumes of *methanol* and 1 volume of *water*. For solution (6) mix 1 volume of solution (2) and 1 volume of solution (5). After removal of the plate, allow it to dry in air and expose to iodine vapour until spots appear. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Disregard any spot with an Rf value equal to or higher than that of the principal spot in the chromatogram obtained with solution (4). The test is not valid unless the spot in the chromatogram obtained with solution (3) is clearly visible.

ASSAY

To a weighed quantity of the oral solution containing 20 mg of Orciprenaline Sulphate add 5 ml of *water*, extract with 15 ml of *ether*, wash the ether layer with 20 ml of *water*, discard the ether and combine the aqueous layers. To the resulting solution add 10 ml of 0.0167M *potassium bromate VS*, 1 g of *potassium bromide* and 5 ml of *hydrochloric acid*, shake gently for 1 minute, add 0.5 g of *potassium iodide* and titrate with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Repeat the procedure without the oral solution. The difference between the titrations represents the amount of potassium bromate required. Each ml of 0.0167M *potassium bromate VS* is equivalent to 4.337 mg of $(C_{11}H_{17}NO_3)_2,H_2SO_4$. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $(C_{11}H_{17}NO_3)_2,H_2SO_4$, weight in volume.

STORAGE

Orciprenaline Oral Solution should be protected from light.

When Orciprenaline Oral Solution is prescribed or demanded, no strength being stated, an Oral Solution containing 10 mg in 5 ml shall be dispensed or supplied.

Orciprenaline Tablets

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Orciprenaline Tablets contain Orciprenaline Sulphate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of orciprenaline sulphate, $(C_{11}H_{17}NO_3)_2,H_2SO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.1 g of Orciprenaline Sulphate with *methanol*, filter and evaporate the filtrate to dryness. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.015% w/v solution of the residue in 0.01M *hydrochloric acid* exhibits a broad maximum at 278 nm. The *absorbance* over the range 276 to 280 nm is about 1.0.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 1.5 volumes of 13.5M *ammonia*, 10 volumes of *water* and 90 volumes of *aldehyde-free methanol* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Orciprenaline Sulphate with 10 ml of *ethanol* (*80%*) for 10 minutes with the aid of ultrasound and filter (Whatman GF/C filter paper is suitable). Solution (2) contains 0.1% w/v of *orciprenaline sulphate BPCRS* in *ethanol* (*80%*). Solution (3) contains 0.1% w/v of each of *orciprenaline sulphate BPCRS* and *salbutamol sulphate BPCRS* in *ethanol* (*80%*). Allow the plate to dry in air and spray with a 1% w/v solution of *potassium permanganate*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

C. The residue obtained in test A yields the reactions characteristic *of sulphates*, Appendix VI.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 4 volumes of 13.5M *ammonia*, 16 volumes of *water*, 30 volumes of *propan-2-ol* and 50 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Orciprenaline Sulphate with 10 ml of *ethanol* (*80%*) for 10 minutes with the aid of ultrasound and filter (Whatman GF/C filter paper is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with *ethanol* (*80%*). For solution (3) dilute 1 volume of solution (1) to 200 volumes with *ethanol* (*80%*). Allow the plate to dry in air and expose the plate to iodine vapour. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (2) (1%) and at most one such spot is more intense than the principal spot in the chromatogram obtained with solution (3) (0.5%). Disregard any spot with an Rf value lower than that of the principal spot in the chromatogram obtained with solution (1). The test is not valid unless the spot in the chromatogram obtained with solution (3) is clearly visible.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 80 mg of Orciprenaline Sulphate with 50 ml of 0.01M *hydrochloric acid*, filter and add sufficient 0.01M *hydrochloric acid* to the filtrate to produce 100 ml. Dilute 10 ml to 100 ml with 0.01M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 276 nm, Appendix II B. Calculate the content of $(C_{11}H_{17}NO_3)_2,H_2SO_4$ taking 72.3 as the value of A(1%, 1 cm) at the maximum at 276 nm.

STORAGE

Orciprenaline Tablets should be protected from light.

Organ Preservation Solutions

General Notices

(Solutions for Organ Preservation, Ph Eur monograph 1264)

Ph Eur

DEFINITION

Solutions for organ preservation are sterile, aqueous preparations, intended for storage, protection and/or perfusion of mammalian body organs that are in particular destined for transplantation.

They contain electrolytes that are typically at a concentration close to the intracellular electrolyte composition.

They may contain carbohydrates (such as glucose or mannitol), amino acids, calcium-complexing agents (such as citrate or phosphate), hydrocolloids (such as starch or gelatin derivatives) and other excipients, for example to make the preparation isotonic with blood, to adjust or buffer the pH, to prevent deterioration of the ingredients, but not to adversely affect the intended action of the preparation or, at the concentration used, to cause toxicity or undue local irritation. Solutions for organ preservation may also contain active substances or these may be added immediately before use.

Solutions for organ preservation, examined under suitable conditions of visibility, are clear and practically free from particles.

Solutions for organ preservation may also be presented as concentrated solutions. They are diluted to the prescribed volume with a prescribed liquid immediately before use. After dilution, they comply with the requirements for solutions for organ preservation.

Before use, the solutions for organ preservation are cooled below room temperature, typically to 2 °C to 6 °C, to reduce the temperature of the body organ and its metabolism.

Where applicable, the containers for solutions for organ preservation comply with the requirements for *Materials used for the manufacture of containers (3.1 and subsections)* and *Containers (3.2 and subsections)*. Solutions for organ preservation are supplied in glass containers *(3.2.1)* or in other containers such as plastic containers *(3.2.2 and 3.2.8)*. The tightness of the container is ensured by suitable means. Closures ensure a good seal, prevent the access of micro-organisms and other contaminants and usually permit the withdrawal of a part or the whole of the contents without removal of the closure. The plastic materials or elastomers of which the closure is composed are sufficiently firm and elastic to allow the passage of a needle with the least possible shedding of particles.

PRODUCTION

Solutions for organ preservation are prepared using materials and methods designed to ensure their sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in the text on *Methods of preparation of sterile products (5.1.1)*.

Unless otherwise justified and authorised, solutions for organ preservation are prepared from *water for injections R* and do not contain antimicrobial preservatives.

TESTS

pH *(2.2.3)*

Carry out the test at room temperature. The pH of the solution is 5.0 to 8.0.

Osmolality *(2.2.35)*

The osmolality of the solution is 250 mosmol/kg to 380 mosmol/kg.

Hydroxymethylfurfural

If the solution contains glucose, it complies with the following test: to a volume of the preparation to be examined containing the equivalent of 25 mg of glucose, add 5.0 ml of a 100 g/l solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R* and 1.0 ml of a 5 g/l solution of *barbituric acid R*. The absorbance *(2.2.25)*, determined at 550 nm after allowing the mixture to stand for 2 min to 3 min, is not greater than that of a standard prepared at the same time in the same manner using a solution containing 10 µg of *hydroxymethylfurfural R* in the same volume as the preparation to be examined.

Particulate contamination

Carry out the test for sub-visible particles *(2.9.19)* using 50 ml of preparation to be examined. The solution contains not more than 50 particles per millilitre larger than 10 µm and not more than 5 particles per millilitre larger than 25 µm.

Products for which the label states that the product is to be used with a final filter are exempted from these requirements.

Sterility *(2.6.1)*

The solution complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 0.5 IU/ml.

Pyrogens *(2.6.8)*

Solutions for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 ml of the solution, unless otherwise justified and authorised.

LABELLING

The label states:

- — that the solution is not to be used for injection,
- — the formula of the solution for organ preservation expressed in grams per litre and in millimoles per litre,
- — the nominal volume of the solution for organ preservation in the container,
- — the osmolality, expressed in milliosmoles per kilogram,

— that any unused portion of the ready-to-use solution, of the concentrated solution or of the diluted solution must be discarded,

— the storage conditions,

— if applicable, that the solution is to be used in conjunction with a final filter.

In addition, for concentrated solutions the label states that the solution must be diluted with a suitable liquid immediately before use.

Ph Eur

Orphenadrine Hydrochloride Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Orphenadrine Hydrochloride Tablets contain Orphenadrine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of orphenadrine hydrochloride, $C_{18}H_{23}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.15 g of Orphenadrine Hydrochloride with *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Dissolve 5 mg in 2 ml of *sulphuric acid*. An orange-red colour is produced.

B. Dissolve 50 mg in 10 ml of *ethanol* (*50%*), add 10 ml of *picric acid solution R1* and allow to stand. The *melting point* of the precipitate, after recrystallisation from *ethanol* (*96%*), is about 89° or about 107°, Appendix V A.

C. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 70 mg of Orphenadrine Hydrochloride as completely as possible in a mixture of 5 ml of *water* and 5 ml of 2M *hydrochloric acid*. Without delay extract with four 15 ml quantities of *chloroform*, filter the combined extracts and evaporate to about 20 ml. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.02M *perchloric acid VS*, adding 2 ml of *mercury(II) acetate solution* and determining the end point potentiometrically. Each ml of 0.02M *perchloric acid VS* is equivalent to 6.118 mg of $C_{18}H_{23}NO,HCl$.

Oxazepam Tablets

General Notices

Action and use

Benzodiazepine.

DEFINITION

Oxazepam Tablets contain Oxazepam.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxazepam, $C_{15}H_{11}ClN_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 20 mg of Oxazepam with 25 ml of *chloroform*, filter, evaporate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of oxazepam (*RS 253*).

B. The *light absorption*, Appendix II B, in the range 210 to 350 nm of the solution obtained in the Assay exhibits two maxima, at 230 nm and 316 nm.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, with 0.1M *hydrochloric acid*, at the maximum at 236 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of oxazepam, $C_{15}H_{11}ClN_2O_2$, in the medium taking 1080 as the value of A(1%, 1 cm) at the maximum at 236 nm.

Related substances

Carry out the test protected from light. Carry out the method for *thin-layer chromatography*, Appendix III A, using as the coating substance a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (Merck silica gel 60 F_{254} is suitable) and a mixture of 10 volumes of *methanol* and 100 volumes of *dichloromethane* as the mobile phase. Before use, wash the plate with *methanol* allowing the solvent front to ascend 17 cm above the line of application. Develop the plate in the same direction as the washing with methanol. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 30 mg of Oxazepam with 6 ml of *acetone* and centrifuge. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *acetone*. For solution (3) dilute 1 volume of solution (1) to 500 volumes with *acetone*. Solution (4) contains 0.10% w/v of each of *oxazepam EPCRS* and *bromazepam EPCRS* in *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.2%). The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 25 mg of Oxazepam add 150 ml of *ethanol* (*96%*) and shake for 30 minutes. Add sufficient *ethanol* (*96%*) to produce 250 ml, centrifuge, dilute 5 ml of the supernatant liquid to 100 ml with the same solvent and measure the *absorbance* of the resulting solution at the maximum at 230 nm, Appendix II B. Calculate the content of $C_{15}H_{11}ClN_2O_2$ taking 1250 as the value of A(1%, 1 cm) at the maximum at 230 nm.

STORAGE

Oxazepam Tablets should be protected from light.

Oxprenolol Tablets

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Oxprenolol Tablets contain Oxprenolol Hydrochloride. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxprenolol hydrochloride, $C_{15}H_{23}NO_3$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 50 mg of Oxprenolol Hydrochloride add 10 ml of *water* and 2 ml of 1.25M *sodium hydroxide*, extract with 10 ml of *chloroform* and reserve the aqueous layer for test B. Dry the chloroform extract over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of oxprenolol (*RS 255*).

B. The aqueous layer obtained in test A yields reaction A characteristic of *chlorides*, Appendix VI.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 2 volumes of 13.5M *ammonia*, 12 volumes of *methanol* and 88 volumes of *chloroform* as the mobile phase, but allowing the solvent front to ascend 13 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.25 g of Oxprenolol Hydrochloride with 5 ml of *water*, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *water*. Allow the spots to dry in air for 15 minutes before development. After removal of the plate, dry it in a current of warm air for 10 minutes, allow to cool, spray with *anisaldehyde solution*, heat at 100° to 105° for 5 to 10 minutes and examine in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Mix a quantity of the powder containing 20 mg of Oxprenolol Hydrochloride with 25 ml of 0.1M *hydrochloric acid* and add sufficient *water* to produce 250 ml. Mix with the aid of ultrasound for 5 minutes, shake for 15 minutes and filter. Measure the *absorbance* of the solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_{15}H_{23}NO_3$,HCl taking 74.5 as the value of A(1%, 1 cm) at the maximum at 273 nm.

Oxybuprocaine Eye Drops

General Notices

Action and use

Local anaesthetic.

DEFINITION

Oxybuprocaine Eye Drops are a sterile solution of Oxybuprocaine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of oxybuprocaine hydrochloride, $C_{17}H_{28}N_2O_3,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the eye drops containing 0.4% w/v of Oxybuprocaine Hydrochloride add 0.5 ml of 2M *hydrochloric acid* and 0.5 ml of a 10% w/v solution of *sodium nitrite* and mix. Add a solution prepared by dissolving 0.2 g of *2-naphthol* in the minimum volume of 18M *ammonia*; a red precipitate is produced.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 4.3, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the eye drops with sufficient of the mobile phase to produce a solution containing 0.016% w/v of Oxybuprocaine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) mix 1 volume of solution (1) with 1 volume of a 4% w/v solution of *sodium hydroxide* and allow to stand for 20 minutes. Add 1 volume of 2M *orthophosphoric acid*, dilute to 100 volumes with the mobile phase and dilute 1 volume of the final solution to 4 volumes with the mobile phase (generation of 4-amino-3-butoxybenzoic acid).

The chromatographic procedure described under Assay may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a suitable volume of the eye drops with sufficient of the mobile phase to produce a solution containing 0.016% w/v of Oxybuprocaine Hydrochloride. Solution (2) contains 0.016% w/v of *oxybuprocaine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 40 volumes of *acetonitrile* containing 0.1% v/v of *diethylamine* and 60 volumes of a 0.75% w/v solution of *ammonium acetate* which has been adjusted to pH 5.0 with *glacial acetic acid* and (c) a detection wavelength of 235 nm.

Calculate the content of $C_{17}H_{28}N_2O_3$,HCl in the eye drops using the declared content of $C_{17}H_{28}N_2O_3$,HCl in *oxybuprocaine hydrochloride BPCRS*.

STORAGE

Oxybuprocaine Eye Drops should be protected from light. They should not be allowed to freeze.

Oxybutynin Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Oxybutynin Tablets contain Oxybutynin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxybutynin hydrochloride, $C_{22}H_{31}NO_3,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for thin-layer chromatography, Appendix III A, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 50 mg of Oxybutynin Hydrochloride with 10 ml of methanol for 10 minutes with the aid of ultrasound, allow to stand for 30 minutes and use the supernatant liquid

(2) Contains 0.05% w/v of oxybutynin hydrochloride BPCRS in methanol.

CHROMATOGRAPHIC PROCEDURE

(a) Use as the coating TLC silica gel F254.

(b) Mobile phase is a mixture of 10 volumes of 18m ammonia, 10 volumes of water, 20 volumes of acetone and 70 volumes of propan-1-ol.

(c) Develop to 15 cm.

(d) Application volume of 10µl for each solution.

(e) Remove the plate, allow it to dry in air and examine under ultraviolet light (254 nm).

COMPARISON

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TEST

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of the powdered tablets containing 50 mg of Oxybutynin Hydrochloride with 40 ml of 0.01M *hydrochloric acid* with the aid of ultrasound for 15 minutes, add sufficient 0.01M *hydrochloric acid* to produce 50 ml, mix and filter (Whatman GF/A filters are suitable).

(2) Contains 0.0015% w/v of *oxybutynin impurity A EPCRS* in 0.01M *hydrochloric acid*.

(3) Contains 0.001% w/v of *phenylcyclohexylglycolic acid BPCRS* in 0.01M *hydrochloric acid*.

(4) Dilute 1 volume of solution (1) to 200 volumes with 0.01M *hydrochloric acid*.

(5) Contains 0.001% w/v of *oxybutynin impurity A EPCRS* in solution (4).

CHROMATOGRAPHIC PROCEDURE

(a) A stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography R1* (5 µm) (Symmetry C18 is suitable)

(b) Gradient elution using the mobile phase programme below.

(c) Flow rate of 1 ml per minute.

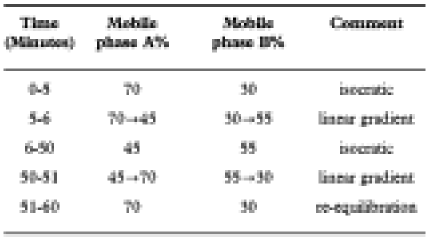
(d) Column temperature ambient.

(e) Detection wavelength of 210 nm.

(f) Injection volume of 50 µl for each solution.

Mobile phase A A solution containing 0.34% w/v of *potassium dihydrogen orthophosphate* and 0.436% of *dipotassium hydrogen orthophosphate*.

Mobile phase B *acetonitrile*.



Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-5	70	30	isocratic
5-6	70→45	30→55	linear gradient
6-50	45	55	isocratic
50-51	45→70	55→30	linear gradient
51-60	70	30	re-equilibration

VALIDITY

System suitability The test is not valid unless, in the chromatogram obtained with solution (5), the retention times are about 31 minutes for oxybutynin hydrochloride and about 47 minutes for oxybutynin impurity A and the *resolution factor* between the peaks due to oxybutynin hydrochloride and oxybutynin impurity A is at least 10.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to oxybutynin impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

In the chromatogram obtained with solution (1) the area of any peak corresponding to phenylcyclohexylglycolic acid is not greater than half the area of the peak in the chromatogram obtained with solution (3) (0.5%).

Other impurities The individual area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (4) (0.2%) and the sum of the areas of any such *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%).

Disregard limit Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix 10 whole tablets with 400 ml of 0.01M *hydrochloric acid* for 20 minutes with the aid of ultrasound, cool, add sufficient 0.01M *hydrochloric acid* to produce 500 ml, mix and filter (Whatman GF/A filters are suitable). Dilute a volume of this solution with sufficient 0.01M *hydrochloric acid* to produce a solution containing 0.005% w/v of Oxybutynin Hydrochloride.

(2) Contains 0.005% w/v of *oxybutynin hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC PROCEDURE

(a) A stainless steel column (15 cm × 4.6 mm) packed with *nitrile silica gel for chromatography R1* (5 µm) (Spherisorb nitrile is suitable).

(b) Isocratic elution using the mobile phase below.

(c) Flow rate of 2 ml per minute.

(d) Column temperature 40°.

(e) Detection wavelength of 200 nm.

(f) Injection volume of 20 µl for each solution.

Mobile phase A mixture of 300 volumes of *acetonitrile* and 700 volumes of a 0.48% w/v solution of *anhydrous potassium dihydrogen orthophosphate* previously adjusted to pH 3.0 to 3.5 with *orthophosphoric acid*

CALCULATION OF CONTENT

Calculate the content of $C_{22}H_{31}NO_3,HCl$ in the tablets from the chromatograms obtained using the declared content of $C_{22}H_{31}NO_3,HCl$ in *oxybutynin hydrochloride BPCRS*.

Oxymetholone Tablets

General Notices

Action and use

Anabolic steroid; androgen.

DEFINITION

Oxymetholone Tablets contain Oxymetholone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxymetholone, $C_{21}H_{32}O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 50 mg of Oxymetholone add 10 ml of *water*, extract with two 10 ml quantities of *chloroform* and evaporate the chloroform extract to dryness. The residue, after drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of oxymetholone (*RS 256*).

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase D*.

ASSAY

Weigh and powder 20 tablets. Extract a quantity of the powder containing 25 mg of Oxymetholone with 0.01M *ethanolic sodium hydroxide* and add sufficient 0.01M *ethanolic sodium hydroxide* to produce 250 ml. Filter if necessary, dilute 5 ml of the filtrate to 50 ml with 0.01M *ethanolic sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 315 nm, Appendix II B. Calculate the content of $C_{21}H_{32}O_3$ taking 547 as the value of A(1%, 1 cm) at the maximum at 315 nm.

STORAGE

Oxymetholone Tablets should be kept free from contact with ferrous metals and protected from light.

Oxytetracycline Capsules

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Oxytetracycline Capsules contain Oxytetracycline Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9$,HCl

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a suitable silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 ml for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate at 110° for 1 hour. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) extract a quantity of the contents of the capsules containing 10 mg of Oxytetracycline Hydrochloride with 20 ml of *methanol*, centrifuge and use the supernatant liquid. For solution (2) dissolve 5 mg of *oxytetracycline hydrochloride EPCRS* in sufficient *methanol* to produce 10 ml. For solution (3) dissolve 5 mg of *oxytetracycline hydrochloride EPCRS* and 5 mg of *demeclocycline hydrochloride EPCRS* in sufficient *methanol* to produce 10 ml. After removal of the plate, dry it in a current of air and examine under *ultraviolet light* (*365 nm*). The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. To 0.5 mg of the contents of the capsules add 2 ml of *sulphuric acid* ; a deep crimson colour is produced. Add 1 ml of *water*; the colour changes to yellow.

C. The contents of the capsules yield the reactions characteristic *of chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 353 nm, Appendix II B. Calculate the total content of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9$,HCl, in the medium taking 282 as the value of A (1%, 1 cm) at the maximum at 353 nm.

Light-absorbing impurities

Dissolve a portion of the mixed contents of five capsules as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce two solutions of Oxytetracycline Hydrochloride containing (1) 0.20% w/v and (2) 1.0% w/v and filter each solution. The *absorbance* of the filtrate obtained from solution (1) at 430 nm is not more than 0.75, Appendix II B, calculated with reference to the dried capsule contents. The *absorbance* of the filtrate obtained from solution (2) at 490 nm is not more than 0.40, calculated with reference to the dried capsule contents.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the contents of the capsules lose not more than 5.0% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake, with the aid of ultrasound, a quantity of the mixed contents of 20 capsules containing 0.1 g of Oxytetracycline Hydrochloride with 80 ml of 0.1M methanolic hydrochloric acid prepared by diluting 1 volume of 1M *hydrochloric acid* to 10 volumes with *methanol*. Add sufficient of the methanolic hydrochloric acid to produce 100 ml. Centrifuge and dilute 1 volume of the clear supernatant liquid to 20 volumes with the same solvent. Solution (2) contains 0.005% w/v of *oxytetracycline EPCRS* in the same solvent. Solution (3) contains 0.1% w/v of *4-epioxytetracycline EPCRS* in the same solvent. Solution (4) contains 0.1% w/v of *tetracycline hydrochloride BPCRS* in the same solvent. For solution (5) dilute a mixture containing 1.5 ml of a 0.1% w/v solution of *oxytetracycline EPCRS* in the 0.1M methanolic hydrochloric acid, 1 ml of solution (3) and 3 ml of solution (4) to 25 ml with the same solvent.

The chromatographic procedure may be carried out using (a) a column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene copolymer* (8 to 10 µm) (Polymer Laboratories, PLRP-S 100A, is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared as described below and (c) a detection wavelength of 254 nm. To 50.0 g of *2-methyl-propan-2-ol* add 200 ml of *water*, 60 ml of *0.33M phosphate buffer pH 7.5*, 50 ml of a 1.0% w/v solution of *tetrabutylammonium hydrogen sulphate* previously adjusted to pH 7.5 with 2M *sodium hydroxide* and 10 ml of a 0.04% w/v solution of *disodium edetate* previously adjusted to pH 7.5 with 2M *sodium hydroxide* and dilute to 1 litre with *water*.

Inject solution (5). The assay is not valid unless (a) the *resolution factor* between the first peak (4-epioxytetracycline) and the second peak (oxytetracycline) is at least 4.0, (b) the *resolution factor* between the second peak and the third peak (tetracycline) is at least 5.0 (if necessary reduce the content of 2-methylpropan-2-ol in the mobile phase to increase the resolution) and (c) the *symmetry factor* of the peak due to oxytetracycline is not more than 1.25.

Calculate the content of $C_{22}H_{24}N_2O_9$,HCl in the capsules using the declared content of $C_{22}H_{24}N_2O_9$ in *oxytetracycline EPCRS*. Each mg of $C_{22}H_{24}N_2O_9$ is equivalent to 1.079 mg of $C_{22}H_{24}N_2O_9$,HCl.

Oxytetracycline Tablets

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Oxytetracycline Tablets contain Oxytetracycline Dihydrate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of oxytetracycline dihydrate, $C_{22}H_{24}N_2O_9,2H_2O$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a suitable silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 ml for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate at 110° for 1 hour. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 10 mg of Oxytetracycline Dihydrate with 20 ml of *methanol*, centrifuge and use the supernatant liquid. For solution (2) dissolve 5 mg of *oxytetracycline hydrochloride EPCRS* in sufficient *methanol* to produce 10 ml. For solution (3) dissolve 5 mg of *oxytetracycline hydrochloride EPCRS* and 5 mg of *demeclocycline hydrochloride EPCRS* in sufficient *methanol* to produce 10 ml. After removal of the plate, dry it in a current of air and examine under *ultraviolet light* (*365 nm*). The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. To 2 mg of the powdered tablets add 2 ml of *sulphuric acid* ; a deep crimson colour is produced. Add 1 ml of *water*; the colour changes to yellow.

C. Shake 4 mg of the powdered tablets with 5 ml of a 1% w/v solution of *sodium carbonate* and add 2 ml of *diazobenzenesulphonic acid solution*. A light brown colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 353 nm, Appendix II B. Calculate the total content of oxytetracycline dihydrate, $C_{22}H_{24}N_2O_9,2H_2O$, in the medium taking 282 as the value of A(1%, 1 cm) at the maximum at 353 nm.

Light-absorbing impurities

Wash five tablets with *water* or *chloroform* until any coloured coating is removed, dry with filter paper and powder. Dissolve portions of the powder as completely as possible in sufficient of a mixture of 1 volume of 1M *hydrochloric acid* and 99 volumes of *methanol* to produce two solutions containing (1) 0.20% w/v of Oxytetracycline Dihydrate and (2) 1.0% w/v of Oxytetracycline Dihydrate and filter each solution. The *absorbance* of the filtrate obtained from solution (1) at 430 nm is not more than 0.50 and the *absorbance* of the filtrate obtained from solution (2) at 490 nm is not more than 0.40, Appendix II B.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake, with the aid of ultrasound, a quantity of the powdered tablets containing 50 mg of Oxytetracycline Dihydrate with 40 ml of 0.01M *hydrochloric acid*, add sufficient 0.01M *hydrochloric acid* to produce 50 ml, filter and dilute 1 volume of the clear filtrate to 20 volumes with the same solvent. Solution (2) contains 0.005% w/v of *oxytetracycline EPCRS* in the same solvent. Solution (3) contains 0.1% w/v of *4-epioxytetracycline EPCRS* in the same solvent. Solution (4) contains 0.1% w/v of *tetracycline hydrochloride BPCRS* in the same solvent. For solution (5) dilute a mixture containing 1.5 ml of a 0.1% w/v solution of *oxytetracycline EPCRS* in 0.01M *hydrochloric acid*, 1 ml of solution (3) and 3 ml of solution (4) to 25 ml with the same solvent.

The chromatographic procedure may be carried out using (a) a column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene copolymer* (8 to 10 µm) (Polymer Laboratories, PLRP-S 100A, is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared as described below and (c) a detection wavelength of 254 nm. To 50.0 g of *2-methyl-propan-2-ol* add 200 ml of *water*, 60 ml of *0.33*M *phosphate buffer pH 7.5*, 50 ml of a 1.0% w/v solution of *tetrabutylammonium hydrogen sulphate* previously adjusted to pH 7.5 with 2M *sodium hydroxide* and 10 ml of a 0.04% w/v solution of *disodium edetate* previously adjusted to pH 7.5 with 2M *sodium hydroxide* and dilute to 1 litre with *water*.

Inject solution (5). The assay is not valid unless (a) the *resolution factor* between the first peak (4-epioxytetracycline) and the second peak (oxytetracycline) is at least 4.0, (b) the *resolution factor* between the second peak and the third peak (tetracycline) is at least 5.0 (if necessary reduce the content of 2-methylpropan-2-ol in the mobile phase to increase the resolution) and (c) the *symmetry factor* of the peak due to oxytetracycline is not more than 1.25.

Calculate the content of $C_{22}H_{24}N_2O_9,2H_2O$ in the tablets using the declared content of $C_{22}H_{24}N_2O_9$ in *oxytetracycline EPCRS*. Each mg of $C_{22}H_{24}N_2O_9$ is equivalent to 1.078 mg of $C_{22}H_{24}N_2O_9,2H_2O$.

Oxytocin Injection

General Notices

Action and use

Oxytocic.

DEFINITION

Oxytocin Injection is a sterile solution of Oxytocin or a sterile dilution of Oxytocin Bulk Solution in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of oxytocin, $C_{43}H_{66}N_{12}O_{12}S_2$

90.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A clear, colourless liquid.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a high performance *silica gel G* precoated plate (Merck 5631 plates are suitable) and a mixture of 1 volume of *glacial acetic acid*, 6 volumes of *water*, 30 volumes of *methanol* and 70 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 8 cm above the line of application. Prepare a solution containing 17 µg per ml of *oxytocin EPCRS* by dissolving the contents of one vial in the requisite volume of a 1.56% w/v solution of *sodium dihydrogen orthophosphate* (solution A). For solution (1) evaporate 5 ml of the injection on a rotary evaporator at 30° and dissolve the residue in 0.5 ml of *water*.

For injections containing 10 IU per ml prepare solution (2) by evaporating 5 ml of solution A on a rotary evaporator at 30° and dissolving the residue in 0.5 ml of *water.* Apply separately to the plate 1 µl of solutions (1) and (2), dry in a stream of cold air and develop immediately.

For injections containing 5 IU per ml prepare solution (2) by evaporating 2.5 ml of solution A on a rotary evaporator at 30° and dissolving the residue in 0.5 ml of *water.* Apply separately to the plate 2 µl of solutions (1) and (2), dry in a stream of cold air and develop immediately.

For injections containing 1 IU per ml use solution A as solution (2). Apply separately to the plate 10 µl of solutions (1) and (2), dry in a stream of cold air and develop immediately.

After removal of the plate, allow it to dry in a stream of cold air for 10 minutes and develop again in a tank, previously equilibrated for 30 minutes with fresh mobile phase. After removal of the plate, allow it to dry in a stream of warm air for 10 minutes, cool and spray with *phosphomolybdotungstic reagent* until the plate appears translucent. Immediately place the plate in a tank, previously equilibrated for 30 minutes with 13.5M *ammonia,* for 5 minutes, remove and dry in a stream of warm air. The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

Acidity

pH, 3.5 to 4.5, Appendix V L.

ASSAY

Carry out the Assay for Oxytocin described under Ergometrine and Oxytocin Injection. For the test solution use the injection being examined. For the reference solution dissolve the contents of one vial of *oxytocin EPCRS* in a 1.65% w/v solution of *sodium dihydrogen orthophosphate* to produce a solution containing the same concentration in µg of oxytocin as that stated on the label of the injection. Inject 200 µl of each solution.

Calculate the content of $C_{43}H_{66}N_{12}O_{12}S_2$ from the declared content of peptide in *oxytocin EPCRS* and using peak areas.

LABELLING

The strength is stated as the number of IU (Units) per ml. The label also states the equivalent number of micrograms of oxytocin per ml.

Aqueous Phenol Injection

General Notices

DEFINITION

Aqueous Phenol Injection is a sterile solution of Phenol in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenol, C_6H_6O

95.0 to 105.0% w/v of the stated amount.

IDENTIFICATION

To 1 ml add 10 ml of *water* and 0.1 ml of *iron(III) chloride solution R1*. A violet colour is produced which disappears on addition of 5 ml of *propan-2-ol*.

TESTS

Acidity

pH, 3.3 to 6.5, Appendix V L.

ASSAY

Dilute a quantity containing 250 mg of Phenol with sufficient *water* to produce 250 ml, transfer 25 ml to a 500 ml glass-stoppered flask and add 50 ml of 0.05M *bromine VS* and 5 ml of *hydrochloric acid*, stopper, swirl occasionally during 30 minutes and allow to stand for a further 15 minutes. Add 5 ml of a 20% w/v solution of *potassium iodide*, taking care to avoid loss of bromine, shake thoroughly and titrate with 0.1M *sodium thiosulphate VS* until only a faint yellow colour remains. Add 0.1 ml of *starch mucilage* and 10 ml of *chloroform* and complete the titration with vigorous shaking. Repeat the operation without the injection. The difference between the titrations represents the amount of bromine required. Each ml of 0.05M *bromine VS* is equivalent to 1.569 mg of C_6H_6O. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage w/v of C_6H_6O.

STORAGE

Aqueous Phenol Injection should be protected from light.

LABELLING

The strength is stated as the percentage w/v of Phenol.

Compound Podophyllin Paint

General Notices

DEFINITION

Compound Podophyllin Paint is a *cutaneous solution*.

Podophyllum Resin	150 g
Compound Benzoin Tincture	Sufficient to produce 1000 ml

In making the Compound Benzoin Tincture used to prepare Compound Podophyllin Paint, the Ethanol (90 per cent) may be replaced by Industrial Methylated Spirit[1] diluted so as to be of equivalent ethanolic strength.

The paint complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* GF_{254} *plate* and a mixture of 1 volume of *methanol* and 25 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, as bands, 10 µl of each of the following solutions in *methanol* containing (1) 7% v/v of the paint, (2) 0.5% w/v of *podophyllotoxin* and (3) 0.1% w/v of *phenazone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*) to locate the quenching zone due to phenazone in solution (3). Spray the plate with *methanolic sulphuric acid* (*50%*) and heat at 130° for 10 minutes. The chromatogram obtained with solution (1) exhibits a purplish zone (podophyllotoxin) corresponding in position and colour to the principal zone in solution (2) and a purplish zone (4′-demethylpodophyllotoxin) corresponding in position to the quenching zone found in solution (3). Other coloured zones are present in the chromatogram obtained with solution (1).

TESTS

Weight per ml

0.925 to 0.975 g, Appendix V G.

Total solids

27.0 to 33.0% w/v when determined by evaporating 1 ml to dryness on a water bath and drying the residue at 105° for 4 hours.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous solution'.

[1] The law and the statutory regulations governing the use of Industrial Methylated Spirit must be observed.

Concentrated Peppermint Emulsion

General Notices

DEFINITION

Concentrated Peppermint Emulsion is a 2% v/v dispersion of Peppermint Oil in a suitable vehicle containing a non-ionic surface-active agent.

Extemporaneous preparation

The following formula and directions apply.

Peppermint Oil	20 ml
Polysorbate 20	1 ml
Double-strength Chloroform Water	500 ml
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

Shake the Peppermint Oil with the Polysorbate 20 and add gradually, shaking well after each addition, the Double-strength Chloroform Water and sufficient Purified Water to produce 1000 ml.

Dispersible Paracetamol Tablets

General Notices

Action and use

Analgesic; antipyretic.

DEFINITION

Dispersible Paracetamol Tablets contain Paracetamol in a suitable dispersible basis.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Disperse in *water* to form a uniform suspension.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 257 nm.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 40 µl of each of solutions (1), (2) and (3). Pour the mobile phase into the unlined tank, immediately place the prepared plate in the tank and close the tank. For solution (1) add 50 ml of *ethanol* (*96%*) to a quantity of the powdered tablets containing 0.10 g of Paracetamol, shake for 10 minutes, add sufficient *ethanol* (*96%*) to produce 100 ml and filter. Solution (2) contains 0.10% w/v of *paracetamol BPCRS* in *ethanol* (*96%*). For solution (3) dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol* (*96%*) to produce 100 ml. After removal of the plate, dry it in a current of warm air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

TESTS

Disintegration

Comply with the requirements for Dispersible Tablets.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light. For solution (1) disperse a quantity of powdered tablets containing to 0.2 g of Paracetamol in 8 ml of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 ml, mix well and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with mobile phase and dilute 1 volume of this solution to 20 volumes with mobile phase. Solution (3) contains 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase. Solution (4) contains 0.00002% w/v of *4′-chloroacetanilide* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute, with a temperature of 35°C, a mixture of 250 volumes of *methanol* containing 1.15 g of a 40% v/v solution of *tetrabutylammonium hydroxide* with 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate* and (c) a detection wavelength of 245 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

Inject solution (1) and allow the chromatography to proceed for 12 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in solution (3) (0.1%), the area of any peak corresponding to 4′-chloroacetanilide is not greater than the area of the principal peak in solution (4) (10 ppm) and no other impurity is greater than the area of the principal peak obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. Add 50 ml of 0.1M *sodium hydroxide* slowly to a quantity of the powder containing 0.2 g of Paracetamol, dilute with 100 ml of *water*, shake for 15 minutes and add sufficient *water* to produce 250 ml. Mix, filter and dilute 10 ml of the filtrate to 100 ml with *water*. Add 10 ml of the resulting solution to 10 ml of 0.1M *sodium hydroxide*, dilute to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

STORAGE

Dispersible Paracetamol Tablets should be protected from light.

LABELLING

The label states that the tablets should be dispersed in water immediately before use.

Effervescent Potassium Chloride Tablets

General Notices

DEFINITION

Effervescent Potassium Chloride Tablets contain potassium ions and chloride ions in an effervescent basis. The proportion of chloride ions to potassium ions may vary, but the stated molar proportion of chloride ions should be not less than 66% of that of potassium ions.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of potassium ions, K^+

95.0 to 105.0% of the stated amount.

Content of chloride ions, Cl^-

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The powdered tablets yield the reactions characteristic of *potassium salts* and of *chlorides*, Appendix VI.

B. Effervesce on the addition of *water*.

TESTS

Disintegration

The requirement for Disintegration does not apply to Effervescent Potassium Chloride Tablets.

Solution

One tablet dissolves in 100 ml of *water* at 20° with brisk effervescence in not more than 3 minutes. The resulting solution is not more than faintly opalescent.

ASSAY

Weigh and powder 20 tablets.

For potassium ions

Dissolve a quantity of the powder containing 35 mg of potassium ions in sufficient *water* to produce 500 ml. Dilute 5 ml of the resulting solution to 100 ml with a 0.6% w/v solution of *strontium chloride* and determine the content of potassium by Method I for *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution* (*600 ppm K*), suitably diluted with a 0.6% w/v solution of *strontium chloride*, to prepare the standard solutions.

For chloride ions

Dissolve a quantity of the powder containing 0.12 g of chloride ions in *water*, dilute to 50 ml with *water* and titrate with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 3.545 mg of Cl.

LABELLING

The label states (1) the content of potassium ions and the content of chloride ions in terms of the weight and the number of millimoles of ion present in each tablet; (2) that the tablets must be dissolved before administration; (3) directions for dissolving the tablets before administration.

When Effervescent Potassium Chloride Tablets are prescribed or demanded, the amount of potassium required in each tablet must be specified.

Gastro-resistant Pancreatin Tablets

General Notices

Pancreatin Tablets

DEFINITION

Gastro-resistant Pancreatin Tablets contain Pancreatin. They are made gastro-resistant by enteric-coating or by other means.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pancreatin

Not less than 90.0% of the stated minimum numbers of Units of free protease activity, of lipase activity and of amylase activity.

IDENTIFICATION

Triturate a quantity of powdered tablets containing 2.5 g of Pancreatin with 100 ml of *water* and filter. The filtrate complies with the tests described under Pancreatin.

ASSAY

Weigh and powder 20 tablets. Carry out the *assay of pancreatin* described under Pancreatin using in the determination of each activity a quantity of powder estimated to contain the number of Units specified in the method.

STORAGE

Gastro-resistant Pancreatin Tablets should be stored at a temperature of 2° to 8°.

LABELLING

The label states the minimum number of Units of free protease activity, of lipase activity and of amylase activity in each tablet.

Gastro-resistant Peppermint Oil Capsules

General Notices

DEFINITION

Gastro-resistant Peppermint Oil Capsules contain Peppermint Oil. They are enteric capsules. *The capsules comply with the requirements stated under Capsules and with the following requirements.*

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* GF_{254} plate and a mixture of 5 volumes of *ethyl acetate* and 95 volumes of *toluene* as the mobile phase. Apply separately to the plate as bands 20 µl of solution (1) and 10 µl of solution (2). For solution (1) dissolve a quantity of the contents of the capsules containing 0.1 g of Peppermint Oil in sufficient *toluene* to produce 10 ml. For solution (2) dissolve 10 mg of *thymol*, 10 µl of *menthyl acetate*, 20 µl of *cineole* and 50 mg of *menthol* in *toluene* and dilute to 10 ml with the same solvent. Allow the plate to dry in air until the solvent has evaporated and examine under *ultraviolet light* (*254 nm*). The chromatogram obtained with solution (1) may show quenching zones (carvone, pulegone) situated just below the level of the zone (thymol) in the chromatogram obtained with solution (2). Spray with *anisaldehyde solution* and examine in daylight for 5 to 10 minutes while heating at 100° to 105°. The chromatogram obtained with solution (2) shows, in order of increasing Rf value, an intense blue to violet zone (menthol) in the lower third; a violet-blue to brown zone (cineole); a pink zone (thymol); and a violet-blue zone (menthyl acetate). In the chromatogram obtained with solution (1) there is a zone due to menthol (the most intense) and a faint zone due to cineole; at Rf values between those of the cineole and thymol zones in the chromatogram obtained with solution (2), there may be light pink or greyish-blue or greenish-grey zones (carvone, pulegone, isomenthone); in the middle of the chromatogram, there is a violet-blue zone (menthyl acetate) and just below it a greenish-blue zone (menthone); an intense violet-red zone (hydrocarbons) appears near the solvent front and below it there may be a brownish-yellow zone (menthofuran); other less intensely coloured zones may also appear.

B. Examine the chromatograms obtained in the test for Chromatographic profile. The retention time of the principal peaks in the chromatogram obtained with solution (1) is similar to that of the principal peaks in the chromatogram obtained with solution (2). Carvone and pulegone may be absent from the chromatogram obtained with solution (1).

TESTS

Disintegration test

Complies with the requirements stated under Gastro-resistant Capsules.

Refractive index

1.457 to 1.467, determined on the contents of the capsules, Appendix V G.

Relative density

0.900 to 0.916, determined on the contents of the capsules, Appendix V E.

Composition of peppermint oil

Carry out the method for *gas chromatography,* Appendix III B, using the following solutions. For solution (1) use the contents of the capsules. For solution (2) dissolve 0.1 g of *limonene*, 0.2 g of *cineole,* 0.4 g of *menthone*, 0.1 g of *menthofuran*, 0.1 g of *isomenthone*, 0.4 g of *menthyl acetate*, 0.6 g of *menthol*, 0.2 g of *pulegone* and 0.1 g of *carvone* in 1 ml of *hexane*. Prepare immediately before use.

The chromatographic procedure may be carried out using (a) a fused-silica capillary column (60 m × 0.25 mm) coated with *macrogol 20,000* as the bonded phase, maintaining the temperature of the column at 60° for 10 minutes, then raising the temperature at a rate of 2° per minute to 180° and maintaining at 180° for 5 minutes, a split injection system with a split ratio of 100 to 1 and maintaining the temperature of the injection port and of the detector at 220° and (b) *helium* as the carrier gas at a flow rate of 1.5 ml per minute.

Inject about 0.2 µl of solution (2). When the chromatograms are recorded in the prescribed conditions, the components elute in the order indicated in the composition of solution (2); a type chromatogram is reproduced in the monograph for Peppermint Oil. Record the retention times of these substances.

The test is not valid unless the number of *theoretical plates* calculated from the limonene peak at 110° is at least 30,000 and the *resolution factor* between the peaks corresponding to limonene and cineole is at least 1.5.

Inject about 0.2 µl of solution (1). Using the retention times determined from the chromatogram obtained with solution (2), locate the components of solution (2) on the chromatogram obtained with solution (1) (disregard the peak due to hexane).

Determine the percentage content of the components by the normalisation procedure.

The percentages are within the following ranges:

Limonene	1.0 to 5.0%,
Cineole	3.5 to 14.0%,
Menthone	14.0 to 32.0%,
Menthofuran	1.0 to 9.0%,
Isomenthone	1.5 to 10.0%,
Menthyl acetate	2.8 to 10.0%,
Menthol	30.0 to 55.0%,
Pulegon	Not more than 4.0%,
Carvone	Not more than 1.0%.

The ratio of cineole content to limonene content is greater than 2.

STORAGE

Gastro-resistant Peppermint Oil Capsules should be protected from light.

Gastro-resistant Prednisolone Tablets

General Notices

Enteric-coated Prednisolone Tablets

Action and use

Glucocorticoid.

DEFINITION

Gastro-resistant Prednisolone Tablets contain Prednisolone. They are covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prednisolone, $C_{21}H_{28}O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets with *dichloromethane*, filter and evaporate the filtrate to dryness. The residue complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase A*.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak having the same retention time as the peak due to prednisolone in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1. Using the apparatus described under Test A for the *disintegration test for tablets and capsules*, Appendix XII A1, introduce one tablet into each of six tubes and operate the apparatus without the discs for 60 minutes in 0.1M *hydrochloric acid*. Remove the tablets and use these tablets to carry out the dissolution test using 900 ml of *mixed phosphate buffer pH 6.8* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter through a membrane filter having a nominal pore size not greater than 0.45 µm, discarding the first 10 ml of filtrate.

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µl of each of the following solutions. For solution (1) prepare a 0.05% w/v solution of *prednisolone BPCRS* in a mixture of 42 volumes of *water* and 58 volumes of *methanol* and dilute with the dissolution medium to contain 0.00025% w/v. For solution (2) use the filtered sample diluted, if necessary, with the dissolution medium to contain 0.00025% w/v of prednisolone.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography,* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 42 volumes of *water* and 58 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

The test is not valid unless the *column efficiency*, determined using the peak due to prednisolone in the chromatogram obtained with solution (1), is greater than 15,000 theoretical plates per metre.

Calculate the content of $C_{21}H_{28}O_5$ in the medium using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Prednisolone with 25 ml of *methanol* for 10 minutes and mix with the aid of ultrasound for 2 minutes. Filter the extract (Whatman GF/F is suitable), wash the filter with two 10-ml quantities of *methanol*, combine the filtrate and washings and evaporate to dryness using a rotary evaporator and a warm water bath; dissolve the residue in 10 ml of *tetrahydrofuran* and dilute to 20 ml with *water*. Solution (2) contains 0.002% w/v each of *prednisolone BPCRS* and *hydrocortisone BPCRS* in the mobile phase. For solution (3) dilute 1 volume of solution (1) to 100 volumes with a 50% v/v solution of *tetrahydrofuran*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Alltima C18 is suitable) and maintained at 45 °, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared as follows: mix 220 volumes of *tetrahydrofuran* with 700 volumes of *water*, allow to equilibrate, dilute to 1000 volumes with *water* and mix and (c) a detection wavelength of 254 nm.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 30 minutes. Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with solution (3) is at least 50% of the full scale of the recorder.

Inject 20 µl of solution (2). When the chromatograms are recorded in the prescribed conditions, the retention times are: prednisolone, about 14 minutes and hydrocortisone, about 15.5 minutes. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of tetrahydrofuran in the mobile phase.

Inject separately 20 µl of a 50% v/v solution of *tetrahydrofuran* as a blank, 20 µl of solution (1) and 20 µl of solution (3). Continue the chromatography for 4.5 times the retention time of the principal peak.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%) and the sum of the areas of any such peaks is not greater than four times the area of the principal peak in the chromatogram obtained with solution (3) (4%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%) and any peak with a retention time of 5 minutes or less.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *prednisolone BPCRS* and 0.0075% w/v of *dexamethasone* (internal standard) in a mixture of 42 volumes of *water* and 58 volumes of *methanol*. For solution (2) add 58 ml of *methanol* to a quantity of the powder containing 5 mg of Prednisolone, shake for 10 minutes, add sufficient *water* to produce 100 ml, mix and filter. Prepare solution (3) in the same manner as solution (2) but add 10 ml of a 0.075% w/v solution of *dexamethasone* in *methanol* and 48 ml of *methanol* in place of the 58 ml of methanol.

The chromatographic procedure described under Dissolution may be used.

The assay is not valid unless in the chromatogram obatained with solution (1), the *resolution factor* between the peaks due to prednisolone and dexamethasone is greater than 2.5 and the *column efficiency*, determined using the peak due to prednisolone is greater than 15,000 theoretical plates per metre.

Calculate the content of $C_{21}H_{28}O_5$ in the tablets using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

LABELLING

The label states that the tablets should be swallowed whole and not chewed.

Light Liquid Paraffin Eye Drops

General Notices

DEFINITION

Light Liquid Paraffin Eye Drops are Light Liquid Paraffin in a suitable container.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

TESTS

Acidity or alkalinity

To 10 ml add 20 ml of boiling *water* and shake vigorously for 1 minute. Separate the aqueous layer and filter. To 10 ml of the filtrate, add 0.1 ml of *phenolphthalein solution*. The solution is colourless. Not more than 0.1 ml of 0.1M *sodium hydroxide VS* is required to change the colour to pink.

Relative density

0.810 to 0.875, Appendix V G.

Viscosity

25 mPa.s to 80 mPa.s, Appendix V H, Method II.

Solid paraffins

Dry a suitable quantity of the eye drops by heating at 100° for 2 hours and cool in a desiccator over *sulphuric acid* . Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 hours, the liquid is sufficiently clear for a black line, 0.5 mm wide, to be easily seen against a white background held vertically behind the tube.

STORAGE

Light Liquid Paraffin Eye Drops should be protected from light.

Liquid Paraffin and Magnesium Hydroxide Oral Emulsion

General Notices

DEFINITION

Liquid Paraffin and Magnesium Hydroxide Oral Emulsion is a 25% v/v dispersion of Liquid Paraffin in an aqueous suspension containing 6% w/w of hydrated magnesium oxide prepared from a suitable grade of Light Magnesium Oxide.

Extemporaneous preparation

The following formula and directions apply.

Liquid Paraffin	250 ml
Chloroform Spirit	15 ml
Magnesium Hydroxide Mixture	Sufficient to produce 1000 ml

Mix the Chloroform Spirit with 650 ml of Magnesium Hydroxide Mixture, add to the Liquid Paraffin, add sufficient Magnesium Hydroxide Mixture to produce 1000 ml and pass through a homogeniser.

The oral emulsion complies with the requirements stated under Oral Liquids and with the following requirements.

Content of magnesium hydroxide, $Mg(OH)_2$

5.1 to 7.0% w/w.

Content of liquid paraffin

19.7 to 24.0% w/w.

ASSAY

For magnesium hydroxide

Dilute 4 g with 50 ml of *water*, shake with 25 ml of 0.5M *hydrochloric acid VS* and titrate with 0.5M *sodium hydroxide VS* using *methyl orange solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 14.58 mg of $Mg(OH)_2$.

For liquid paraffin

Extract the neutralised liquid obtained in the Assay for magnesium hydroxide with three 25 ml quantities of a mixture of equal volumes of *petroleum spirit* (*boiling range, 40° to 60°*) and *ether*, wash the combined extracts with two 10 ml quantities of *water*, discard the washings, evaporate the solvent, add 5 ml of *acetone* and again evaporate. Repeat the addition and evaporation of *acetone* until the residue is free from water, dry at 105° for 15 minutes and weigh.

Liquid Paraffin Oral Emulsion

General Notices

Liquid Paraffin Emulsion

DEFINITION

Liquid Paraffin	500 ml
Vanillin	500 mg
Chloroform	2.5 ml
Benzoic Acid Solution	20 ml
Methylcellulose 20	20 g
Saccharin Sodium	50 mg
Water	A sufficient quantity

Extemporaneous preparation

The following directions apply.

Heat about 120 ml of Water until it boils and add the Methylcellulose; when thoroughly hydrated add sufficient Water in the form of ice to produce 350 ml and stir until homogeneous. Mix the Chloroform and the Benzoic Acid Solution, dissolve the Vanillin in the mixture and add to the methylcellulose dispersion while stirring vigorously for 5 minutes. Add the Saccharin Sodium dissolved in Water and dilute to 500 ml with Water. Add the Liquid Paraffin, stirring constantly, and pass through a homogeniser.

The oral emulsion complies with the requirements stated under Oral Liquids and with the following requirements.

Content of liquid paraffin

44.0 to 49.0% w/w.

ASSAY

To 5 g add 10 ml of *water*, extract with two 40 ml quantities of a mixture of 2 volumes of *ethanol (96%)*, 3 volumes of *petroleum spirit* (*boiling range, 40° to 60°*) and 3 volumes of *ether* and then with 30 ml of a mixture of equal volumes of *petroleum spirit* (*boiling range, 40° to 60°*) and *ether*. Wash the combined extracts with 15 ml of 0.5M *sodium hydroxide* and then with 15 ml of *water*, evaporate the solvent, add 5 ml of *acetone* and evaporate again. Repeat the addition and evaporation of *acetone* until the residue is free from water, dry at 105° for 15 minutes and weigh.

Oily Phenol Injection

General Notices

DEFINITION

Oily Phenol Injection is a sterile solution containing 5% w/v of Phenol in a suitable fixed oil.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenol, C_6H_6O

4.75 to 5.25% w/v.

ASSAY

Dissolve 8 g in 50 ml of *ether* and extract with successive 10 ml quantities of 2M *sodium hydroxide* until extraction is complete, boil the combined extracts for 2 minutes, cool and dilute to 250 ml with *water*. To 20 ml of this solution in a glass-stoppered flask add 30 ml of 0.05M *bromine VS* and 6 ml of *hydrochloric acid*, stopper, shake repeatedly during 15 minutes and allow to stand for a further 15 minutes. Add 30 ml of *dilute potassium iodide solution*, taking care to avoid loss of bromine, and titrate the liberated iodine with 0.1M *sodium thiosulphate VS*. Repeat the operation without the injection. The difference between the titrations represents the amount of bromine required. Each ml of 0.05M *bromine VS* is equivalent to 1.569 mg of C_6H_6O. Determine the *weight per ml*, Appendix V G, and calculate the percentage w/v of C_6H_6O.

Paediatric Paracetamol Oral Solution

General Notices

Action and use

Analgesic; antipyretic.

DEFINITION

Paediatric Paracetamol Oral Solution is a solution containing 2.4% w/v of Paracetamol in a suitable flavoured vehicle.

Paediatric Paracetamol Oral Solution should not be diluted.
The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

2.28 to 2.52% w/v.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the preparation being examined with *methanol* to produce a solution containing 0.24% w/v Paracetamol and filter if necessary.

(2) 0.24% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution.

(d) Develop to 15 cm.

(e) Remove the plate, dry it in a current of warm air, examine under *ultraviolet light* (*254 nm*) and also reveal the spots using *Method 1.*

MOBILE PHASE

0.5 volume of *glacial acetic acid*, 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform.*

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to paracetamol in the chromatogram obtained with solution (1).

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 5 ml of the preparation being examined with 15 ml of the mobile phase, dilute to 25 ml with the mobile phase and filter if necessary.

(2) 0.0024% w/v of *4-aminophenol* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Use ambient column temperature.

(e) Detection wavelength of 272 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.01M *sodium butanesulphonate* in a mixture of 0.4 volumes of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water.*

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%);

— peaks with a long retention time may occur due preservatives in the preparation.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a weighed quantity of the preparation being examined containing 24 mg of Paracetamol with 100 ml of the mobile phase, dilute to 200 ml with the mobile phase and filter if necessary.

(2) 0.012% w/v of *paracetamol BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using the conditions described in the test for 4-Aminophenol but using a detection wavelength of 243 nm.

Determine the *weight per ml* of the preparation, Appendix V G, and calculate the percentage content of $C_8H_9NO_2$, weight in volume, using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

STORAGE

Paediatric Paracetamol Oral Solution should be protected from light. It should not be refrigerated.

Paediatric Phenobarbital Oral Solution

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Barbiturate.

DEFINITION

Paediatric Phenobarbital Oral Solution is an *oral solution* containing Phenobarbital Sodium in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of phenobarbital, $C_{12}H_{12}N_2O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, of the residue obtained in the Assay is concordant with the *reference spectrum* of phenobarbital (*RS 270*).

B. The oral solution yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

pH, 7.5 to 9.0, Appendix V L.

ASSAY

Acidify 50 ml of the preparation being examined to *litmus paper* with 2M *hydrochloric acid* and extract with four 25-ml quantities of *ether*. Wash the combined ether extracts with two 2-ml quantities of *water* and wash the combined aqueous washings with 10 ml of *ether*. Add the ether washings to the combined ether extracts, evaporate the ether and dry the residue of phenobarbital, $C_{12}H_{12}N_2O_{3.}$ to constant weight at 105°. Calculate the content of $C_{12}H_{12}N_2O_3$ in the oral solution as a percentage of the stated amount.

STORAGE

Paediatric Phenobarbital Oral Solution should be protected from light. It should not be refrigerated.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of phenobarbital.

Pamidronate Disodium Intravenous Infusion

General Notices

Action and use

Bisphosphonate; treatment of osteolytic lesions; Paget′s disease; hypercalcaemia of malignancy.

DEFINITION

Pamidronate Disodium Intravenous Infusion is a sterile solution of Pamidronate Disodium in Water for Injections. It is prepared by dissolving Pamidronate Disodium for Intravenous Infusion in Water for Injections and then diluting with the requisite volume of a suitable diluent in accordance with the manufacturer's instructions.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of anhydrous pamidronate disodium per ml (solution A). The endotoxin limit concentration of solution A is 30 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

STORAGE

Pamidronate Disodium Intravenous Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

PAMIDRONATE DISODIUM FOR INTRAVENOUS INFUSION

DEFINITION

Pamidronate Disodium for Intravenous Infusion is a sterile material prepared from Pamidronate Disodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of anhydrous pamidronate disodium, $C_3H_9NNa_2O_7P_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated cellulose plate (Avicel F1440 precoated 10 cm × 10 cm sheets are suitable) and a mixture of 1 g of *trichloroacetic acid*, 5 ml of 13.5M *ammonia*, 40 ml of *water* and 55 ml of *methanol* as the mobile phase. Before use, place the plate in an unlined tank and allow the mobile phase to ascend to the top of the sheet, dry it for 30 minutes in a current of warm air at about 50° and allow it to cool. Use with the flow of mobile phase in the same direction as that used for the prewash and use the same chamber and mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dissolve a quantity of the contents of the sealed container in sufficient *water* to produce a solution containing 0.3% w/v of anhydrous pamidronate disodium. Solution (2) contains 0.4% w/v of *disodium pamidronate BPCRS* in *water*. Allow the mobile phase to ascend 8 cm above the line of application. After removal of the plate, dry in a current of warm air for 30 minutes, spray with a solution prepared by dissolving 1 g of *ammonium molybdate* in 85 ml of *water* and adding 10 ml of *hydrochloric acid* and 5 ml of a 60% v/v solution of *perchloric acid*, heat at 80° for 10 minutes, allow to cool and expose to *ultraviolet light (365 nm)* for 2 minutes; examine the plate in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to pamidronate disodium in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

Dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.3% w/v of anhydrous pamidronate disodium. The pH of the solution is 6.0 to 7.0, Appendix V L.

β-Alanine

Carry out the method for *thin-layer chromatography* under subdued light, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 4 volumes of 13.5M *ammonia*, 8 volumes of *di-isopropyl ether* and 9 volumes of *methanol* as the mobile phase but allowing the solvent front to ascend 7 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.30% w/v of anhydrous pamidronate disodium. Solution (2) contains 0.0015% w/v of *3-aminopropionic acid*. After removal of the plate, allow it to dry in a current of warm air and spray with a 0.01% w/v solution of *fluorescamine* in *acetone*, immerse the plate for 2 seconds in a mixture of 1 volume of *liquid paraffin* and 2 volumes of n-*hexane*, allow to dry in air for 15 minutes and examine under *ultraviolet light (365 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to β-alanine (3-aminopropionic acid) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Determine the weight of the mixed contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the mixed contents of the 10 containers in *water* and dilute with the same solvent to produce a solution containing the equivalent of 0.20% w/v of anhydrous pamidronate disodium. Solution (2) contains 0.20% w/v of *disodium pamidronate BPCRS* in *water.*

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm

× 4.6 mm) packed with an anion-exchange stationary phase (5 µm) (IC Anion Super-sep is suitable) and maintained at 35^{c}, (b) as the mobile phase with a flow rate of 1.0 ml per minute a solution prepared by adding 0.47 ml of *formic acid* to 2500 ml of *water* and adjusting the solution to pH 3.5 with 2M *sodium hydroxide* and (c) a refractive index detector. Inject 100 µl of each solution.

Calculate the content of $C_3H_9NNa_2O_7P_2$ in a container of average content weight from the chromatograms obtained and using the declared content of $C_3H_9NNa_2O_7P_2$ in *disodium pamidronate BPCRS.*

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous pamidronate disodium.

IMPURITIES

The impurities limited by the requirements of this monograph include β-alanine.

Pancreatin Granules

General Notices

Action and use

Enzyme; treatment of pancreatic exocrine deficiency.

DEFINITION

Pancreatin Granules contain Pancreatin and may also contain suitable gastro-resistant substances.

The granules comply with the requirements stated under Granules and with the following requirements.

Content of pancreatin

Not less than 90.0% of the stated minimum number of Units of free protease activity, of lipase activity and of amylase activity.

IDENTIFICATION

Triturate a quantity of powdered granules containing 2.5 g of Pancreatin with 100 ml of *water*. The liquid complies with the tests described under Pancreatin.

ASSAY

Carry out the *assay of pancreatin* described under Pancreatin using in the determination of each activity a quantity of the powdered granules estimated to contain the number of Units specified in the method.

STORAGE

Pancreatin Granules should be stored at a temperature of 2° to 8°.

LABELLING

The label states (1) the minimum number of Units of free protease activity, of lipase activity and of amylase activity per g; (2) where applicable, that the granules contain gastro-resistant substances.

Pancuronium Injection

General Notices

Action and use

Non-depolarizing neuromuscular blocker.

DEFINITION

Pancuronium Injection is a sterile solution of Pancuronium Bromide in Sodium Chloride Intravenous Infusion.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of pancuronium bromide, $C_{35}H_{60}Br_2N_2O_4$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless liquid.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (2).

B. To a volume containing 5 mg of Pancuronium Bromide, diluted if necessary to 10 ml with *water*, add 10 ml of *1,2-dichloroethane* followed by 1 ml of *methyl orange solution*. Shake, centrifuge, allow the layers to separate and acidify the organic layer with 1M *sulphuric acid*. A red colour is produced.

C. Yields reaction A characteristic of *bromides*, Appendix VI.

TESTS

Acidity

pH, 3.8 to 4.2, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Evaporate a volume of the injection containing 20 mg of Pancuronium Bromide to dryness in a current of *nitrogen*, add 2 ml of *acetonitrile* to the residue and mix with the aid of ultrasound for 1 minute.

(2) 1.0% w/v of *pancuronium bromide BPCRS* in *acetonitrile*.

(3) Dilute 1 volume of solution (1) to 50 volumes with *acetonitrile*.

(4) Dilute 1 volume of solution (3) to 2 volumes with *acetonitrile*.

(5) 3% w/v of *pancuronium bromide for system suitability EPCRS* (containing 1.0 per cent of impurity D) in *methylene chloride*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 2 µl of each solution.

(d) Develop the plate to 15 cm in an unsaturated tank.

(e) After removal of the plate, dry it in a current of cold air and spray with a 1% w/v solution of *sodium nitrite* in *methanol*. Allow to stand for 2 minutes, spray the plate with *potassium iodobismuthate solution* and dry in a current of cold air.

MOBILE PHASE

5 volumes of a 20% w/v solution of *sodium iodide*, 10 volumes of *acetonitrile* and 85 volumes of *propan-2-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows two distinct spots and the Rf value of the spot due to impurity D relative to that due to pancuronium bromide is at least 1.2.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to dacuronium bromide (impurity A) or vecuronium bromide (impurity D) is not more intense than the spot in the chromatogram obtained with solution (5) (3%). Impurity A and impurity D have the same Rf value and if present in the same solution would produce a single spot;

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (2%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (1%).

ASSAY

To a volume containing 4 mg of Pancuronium Bromide add 1 ml of a 13.9% w/v solution of *hydroxylamine hydrochloride* followed by 1 ml of a 15% w/v solution of *sodium hydroxide*. Allow to stand for 10 minutes and then add 1 ml of a solution prepared by diluting *hydrochloric acid* with *water* to contain 12.76% w/v of HCl. Add 1 ml of a 10% w/v solution of *iron(III) chloride* in 0.1M *hydrochloric acid*, add sufficient *water* to produce 25 ml, centrifuge and measure the *absorbance* of the clear, supernatant liquid at the maximum at 510 nm, Appendix II B, using in the reference cell a solution prepared by carrying out the operation using an equal volume of *water* in place of the injection. Calculate the content of $C_{35}H_{60}Br_2N_2O_4$ from the *absorbance* obtained by repeating the operation using a suitable quantity of *pancuronium bromide BPCRS* dissolved in *water* and using the declared content of $C_{35}H_{60}Br_2N_2O_4$ in *pancuronium bromide BPCRS*.

STORAGE

Pancuronium Injection should be stored at a temperature of 2° to 8°.

Papaveretum Injection

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Papaveretum Injection is a sterile solution of Papaveretum in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of morphine, $C_{17}H_{19}NO_3$

59.7 to 69.3% of the stated amount of Papaveretum.

Content of papaverine, $C_{20}H_{21}NO_4$

6.2 to 7.7% of the stated amount of Papaveretum.

Content of codeine, $C_{18}H_{21}NO_3$

4.9 to 5.9% of the stated amount of Papaveretum.

CHARACTERISTICS

A clear, colourless to yellowish brown solution.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia,* 6 volumes of *ethanol (96%),* 40 volumes of *acetone* and 40 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. Solution (1) is the injection diluted, if necessary, to contain 1.6% w/v of Papaveretum. Solutions (2), (3) and (4) are solutions in *water* containing (2) 1.28% w/v of *morphine sulphate BPCRS*, (3) 0.115% w/v of *papaverine hydrochloride BPCRS* and (4) 0.10% w/v of *codeine hydrochloride BPCRS*. After removal of the plate, dry it at 100° to 105° for 15 minutes, allow to cool and spray with *potassium iodobismuthate solution* and then with a 0.4% v/v solution of *sulphuric acid*. The chromatogram obtained with solution (1) shows three principal spots corresponding to the principal spots in the chromatograms obtained with solutions (2), (3) and (4).

Acidity

pH, 2.5 to 4.0, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution (1) contains 0.128% w/v of *morphine sulphate BPCRS*, 0.0115% w/v of *papaverine hydrochloride BPCRS* and 0.010% w/v of *codeine hydrochloride BPCRS* in *methanol* (*60%*). Solution (2) contains 0.128% w/v of *morphine sulphate BPCRS* in *methanol* (*60%*). For solution (3) dilute a volume of the injection containing 80 mg of Papaveretum to 50 ml with *methanol* (*60%*).

The chromatographic procedure may be carried out using a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution containing 0.01M *sodium acetate* and 0.005M *dioctyl sodium sulphosuccinate* in *methanol* (*60%*) adjusted to pH 5.5 with *glacial acetic acid* and (c) a detection wavelength of 285 nm. If necessary, adjust the proportion of methanol in the mobile phase in the range 55% v/v to 65% v/v so that the retention time of morphine in solution (2) is 4 to 5 minutes. Adjust the pH of the mobile phase with either *glacial acetic acid* or 2M *sodium hydroxide* in order to obtain optimum separation of the three principal components in solution (1). The retention times of codeine and papaverine relative to that of morphine are about 1.3 and 1.7 respectively.

For morphine Calculate the content of $C_{17}H_{19}NO_3$ using the declared content of $C_{17}H_{19}NO_3$ in *morphine sulphate BPCRS*.

For papaverine Calculate the content of $C_{20}H_{21}NO_4$ using the declared content of $C_{20}H_{21}NO_4$ in *papaverine hydrochloride BPCRS*.

For codeine Calculate the content of $C_{18}H_{21}NO_3$ using the declared content of $C_{18}H_{21}NO_3$ in *codeine hydrochloride BPCRS*.

STORAGE

Papaveretum Injection should be protected from light.

LABELLING

The label states (1) the amount of Papaveretum in a suitable dose-volume; (2) the content of morphine in a suitable dose-volume.

Papaverine Injection

General Notices

Action and use

Phosphodiesterase inhibitor; smooth muscle relaxant.

DEFINITION

Papaverine Injection is a sterile solution of Papaverine Hydrochloride in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of papaverine hydrochloride, $C_{20}H_{21}NO_4$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing 60 mg of Papaverine Hydrochloride add 2 ml of *ethanol (96%)*, evaporate to dryness on a steam bath with the aid of a stream of *nitrogen* and dry the residue at 105° for 2 hours. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of papaverine hydrochloride *(RS 415).*

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 2.0 to 4.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection, if necessary, with *methanol (50%)* to produce a solution containing 0.06% w/v of Papaverine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol* (50%). Solution (3) contains 0.0005% w/v of *papaverine hydrochloride BPCRS* and 0.005% w/v of *noscapine* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *endcapped octadecylsilyl silica gel for chromatography* (5 µm) (Phenomenex Luna C(18)2 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared by adding 700 ml of *methanol* containing 2.22 g of *dioctyl sodium sulphosuccinate* to 100 ml of *water* containing 1.36 g of *sodium acetate*, diluting to 1 litre with *water* and adjusting the pH to 5.5 with *glacial acetic acid* and (c) a detection wavelength of 250 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to papaverine hydrochloride and noscapine is at least 3.0.

In the chromatogram obtained with solution (1), the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0045% w/v of *papaverine hydrochloride BPCRS* in *methanol (50%).* For solution (2) dilute a volume of the injection, if necessary, with *methanol* (50%) to produce a solution containing 0.0045% w/v of Papaverine Hydrochloride.

The chromatographic method described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to papaverine hydrochloride and noscapine is at least 3.0.

Calculate the content of $C_{20}H_{21}NO_4$,HCl in the injection using the declared content of $C_{20}H_{21}NO_4$,HCl in *papaverine hydrochloride BPCRS*.

STORAGE

Papaverine Injection should be protected from light.

Paracetamol Capsules

General Notices

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Capsules contain Paracetamol.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 20 ml of *acetone*, filter, evaporate the filtrate and dry at 105°.The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol (*RS 258*).

TESTS

Dissolution

Comply with the *dissolution test for tablets and capsules*, Appendix XII D, using Apparatus II. Use as the medium 900 ml of *phosphate buffer pH 5.8* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Dilute the filtrate with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00075% w/v of Paracetamol. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 257 nm using 0.1M *sodium hydroxide* in the reference cell. Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions immediately before use and protect from light.

(1) Disperse a quantity of the contents of the capsules containing 0.2 g of Paracetamol in 8 ml of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 ml, mix well and filter.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase and dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(3) 0.002% w/v of each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase.

(4) 0.00002% w/v of *4′-chloroacetanilide* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Column temperature 35°.

(e) Detection wavelength of 245 nm.

(f) Injection volume of 20 µl for each solution.

(g) Allow the chromatography to proceed for 12 times the retention time of the principal peak.

MOBILE PHASE

A mixture of 250 volumes of *methanol* containing 1.15 g of a 40% v/v solution of *tetrabutylammonium hydroxide* with 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to 4′-chloroacetanilide is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (10 ppm);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Weigh the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions immediately before use and protect from light.

(1) Disperse a quantity of the contents of the capsules containing 0.5 g of Paracetamol in 80 ml of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 100 ml, mix well, filter and dilute 1 volume of the resulting solution to 100 volumes with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

(3) 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Sainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Column temperature 35 ^{c}.

(e) Detection wavelength of 245 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 250 volumes of *methanol* containing 1.15 g of a 40% v/v solution of *tetrabutylammonium hydroxide*, 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate.*

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS.*

STORAGE

Paracetamol Capsules should be protected from light.

Paracetamol Oral Suspension

General Notices

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Oral Suspension is a suspension of Paracetamol in a suitable flavoured vehicle. *The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.*

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the preparation being examined with *methanol* to produce a solution containing 0.24% w/v Paracetamol and filter if necessary.

(2) 0.24% w/v of *paracetamol BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* $GF_{254.}$

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution.

(d) Develop the plate to 15 cm.

(e) Remove the plate, dry it in a current of warm air, examine under *ultraviolet light* (*254 nm*) and also reveal the spots using *Method 1.*

MOBILE PHASE

0.5 volume of glacial acetic acid, 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform.*

CONFIRMATION

By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to paracetamol in the chromatogram obtained with solution (1).

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 5 ml of the preparation being examined with 15 ml of the mobile phase, dilute with the mobile phase to contain 0.48% w/v of Paracetamol and filter if necessary.

(2) 0.0024% w/v of *4-aminophenol* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil ODS is suitable).

(b) Use an isocratic system using the mobile phase described below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.01 M *sodium butanesulphonate* in a mixture of 0.4 volumes of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water.*

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%);

— peaks with a long retention time may occur due preservatives in the preparation.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a weighed quantity of the preparation being examined containing 24 mg of Paracetamol with 100 ml of the mobile phase, dilute to 200 ml with the mobile phase and filter if necessary.

(2) 0.012% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure may be carried out using the conditions described in the test for 4-Aminophenol but using a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Determine the *weight per ml* of the preparation, Appendix V G, and calculate the percentage content of $C_8H_9NO_2$, weight in volume, using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

STORAGE

Paracetamol Oral Suspension should be protected from light.

When paediatric paracetamol oral suspension or paediatric paracetamol mixture is prescribed or demanded, Paracetamol Oral Suspension containing 2.4% w/v of Paracetamol shall be dispensed or supplied.

Paracetamol Suppositories

General Notices

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Suppositories contain Paracetamol in a suitable suppository basis.

The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* F_{254} *plate* and a mixture of 1 volume of *methanol* and 4 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add sufficient *methanol* to 1 suppository to produce a final solution containing 0.1% w/v of Paracetamol and warm on a water-bath until the suppository has melted. Allow the solution to cool, with occasional stirring, and filter. Solution (2) contains 0.1% w/v of *paracetamol BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. To a quantity of the suppositories containing 50 mg of Paracetamol cut into small pieces add 1 ml of *hydrochloric acid* and heat to boiling for 3 minutes, add 10 ml of *water* and cool. No precipitate is produced. Add 0.05 ml of *potassium dichromate solution R1*. A violet colour develops which does not change to red.

TESTS

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0005% w/v of *4-aminophenol* in the mobile phase. For solution (2) dissolve a minimum of 5 suppositories, cut into small pieces, in the minimum volume of *ethanol* (*96%*), warm if necessary, and dilute with sufficient *water* to obtain a solution containing 0.5% w/v of Paracetamol.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.01M *sodium butanesulphonate* in a mixture of 0.4 volume of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 272 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 4-

aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (1) (0.1%).

ASSAY

For suppositories containing more than 150 mg add to 1 suppository 30 ml of *water* and 100 ml of 1M *sulphuric acid*. Boil under a reflux condenser for 1 hour, cool and add 100 ml of *water*, 50 g of frozen *water*, 50 ml of *dilute hydrochloric acid* and 0.2 ml of *ferroin solution*. Titrate with 0.2M *ammonium cerium(IV) sulphate VS* until a yellow colour is obtained. Each ml of 0.2M *ammonium cerium(IV) sulphate VS* is equivalent to 15.12 mg of $C_8H_9NO_2$. Repeat the test using a further 4 suppositories and calculate the average content per suppository from the 5 individual results thus obtained.

For suppositories containing 150 mg or less add to 1 suppository 10 ml of *water* and 30 ml of *dilute sulphuric acid*. Boil under a reflux condenser for 1 hour, cool and add 40 ml of *water*, 40 g of frozen *water*, 15 ml of *dilute hydrochloric acid* and 0.1 ml of *ferroin solution*. Titrate with 0.1M *ammonium cerium(IV) sulphate VS* until a yellow colour is obtained. Each ml of 0.1M *ammonium cerium(IV) sulphate VS* is equivalent to 7.56 mg of $C_8H_9NO_2$. Repeat the test using a further 4 suppositories and calculate the average content per suppository from the 5 individual results thus obtained.

Paracetamol Tablets

General Notices

Action and use

Analgesic; antipyretic.

DEFINITION

Paracetamol Tablets contain Paracetamol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 ml of *acetone*, filter, evaporate the filtrate to dryness and dry at 105°. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of paracetamol (*RS 258*).

B. Boil 0.1 g with 1 ml of *hydrochloric acid* for 3 minutes, add 10 ml of *water* and cool; no precipitate is produced. Add 0.05 ml of 0.0167M *potassium dichromate*; a violet colour is produced slowly which does not turn red.

C. *Melting point*, about 169°, Appendix V A.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 5.8* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Dilute the filtrate with 0.1M *sodium hydroxide* to give a solution expected to contain about 0.00075% w/v of Paracetamol. Measure the *absorbance* of this solution, Appendix II B, at the maximum at 257 nm using 0.1M *sodium hydroxide* in the reference cell. Calculate the total content of paracetamol, $C_8H_9NO_2$, in the medium taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light. For solution (1) disperse a quantity of powdered tablets containing 0.2 g of Paracetamol in 8 ml of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 ml, mix well and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with mobile phase and dilute 1 volume of this solution to 20 volumes with mobile phase. Solution (3) contains 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase. Solution (4) contains 0.00002% w/v of *4′-chloroacetanilide* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute, at a temperature of 35°C, a mixture of 250 volumes of *methanol* containing 1.15 g of a 40% v/v solution of *tetrabutylammonium hydroxide* with 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate* and (c) a detection wavelength of 245 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

Inject solution (1) and allow the chromatography to proceed for 12 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in solution (3) (0.1%), the area of any peak corresponding to 4′-chloroacetanilide is not greater than the area of the principal peak in solution (4) (10 ppm) and no other impurity is greater than the area of the principal peak obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. Add a quantity of the powder containing 0.15 g of Paracetamol to 50 ml of 0.1M *sodium hydroxide*, dilute with 100 ml of *water*, shake for 15 minutes and add sufficient *water* to produce 200 ml. Mix, filter and dilute 10 ml of the filtrate to 100 ml with *water*. Add 10 ml of the resulting solution to 10 ml of 0.1M *sodium hydroxide*, dilute to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

STORAGE

Paracetamol Tablets should be protected from light.

Paracetamol, Codeine Phosphate and Caffeine Capsules

General Notices

Action and use

Analgesic; antipyretic; opioid receptor agonist.

DEFINITION

Paracetamol, Codeine Phosphate and Caffeine Capsules contain Paracetamol, Codeine Phosphate and Caffeine.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$

95.0 to 105.0% of the stated amount.

Content of caffeine, $C_8H_{10}N_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 20 ml of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 24 mg of Codeine Phosphate with 30 ml of *water* for 1 minute and centrifuge. Decant, add 10 ml of 1M *sodium hydroxide* and 30 ml of *dichloromethane* to the supernatant liquid, shake for 1 minute and filter the dichloromethane layer through glass-fibre paper (Whatman GF/C is suitable).

(2) 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%)*.

(3) 0.08% w/v each of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase as described below.

(c) Apply 10 µl of each solution.

(d) Develop to 15 cm.

(e) After removal of the plate, dry in air, spray with *ethanolic iron(III) chloride solution* and heat at 105° for 10 minutes and examine in daylight.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

D. Shake a quantity of the contents of the capsules containing 0.03 g of Caffeine with 10 ml of *water* for 5 minutes, filter and add 10 ml of 1M *sodium hydroxide*. Extract with three 30-ml quantities of *dichloromethane*, washing each extract with the same 10 ml of *water*. Filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness in a porcelain dish. A reddish colour is observed which changes to purple when exposed to ammonia vapour.

E. In the Assay for caffeine, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules,* Appendix XII B1, using the following conditions.

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use 900 ml *phosphate buffer pH 5.8* as the dissolution medium at a temperature of 37°. After 45 minutes withdraw a sample of 20 ml of the medium and filter.

PROCEDURE

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For paracetamol

(1) Use the filtered dissolution medium, diluted with *phosphate buffer pH 5.8,* if necessary, to contain 0.005%w/v of Paracetamol.

(2) 0.005% w/v solution of *paracetamol BPCRS* in *phosphate buffer pH 5.8*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used but with a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ in the medium using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

For codeine phosphate

(1) Use the filtered dissolution medium, diluted with *phosphate buffer pH 5.8* if necessary, to contain 0.001% w/v of Codeine Phosphate.

(2) 0.001% w/v solution of *codeine phosphate BPCRS* in *phosphate buffer pH 5.8*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in the medium using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

For caffeine

(1) Use the filtered dissolution medium, diluted with *phosphate buffer pH 5.8,* if necessary, to contain 0.003% w/v of Caffeine.

(2) 0.001% w/v solution of *caffeine BPCRS* in *phosphate buffer pH 5.8*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2$ in the medium using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS.*

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 0.5 g of Paracetamol with 50 ml of the mobile phase for 10 minutes and filter.

(2) 0.001% w/v of *4-aminophenol* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10µm) (Nucleosil C18 is suitable).

(b) Use an isocratic system and the mobile phase described below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.01M *sodium butanesulphonate* in a mixture of 0.4 volumes of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water.*

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%);

peaks with a long retention time may occur due to excipients.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 50 mg of Codeine Phosphate with 50 ml of 0.1M *hydrochloric acid* for 10 minutes and filter. Make the filtrate alkaline with 5M *sodium hydroxide* and extract with two 40 ml quantities of *dichloromethane*. Wash the combined extracts with 10 ml of *water*, filter through a layer of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with *dichloromethane*, evaporate the filtrate to dryness at a temperature not exceeding 45° using a rotary evaporator and dissolve the residue in 2 ml of *dichloromethane.*

(2) Dilute 1.5 volumes of solution (1) to 100 volumes with *dichloromethane*.

(3) Dilute 1 volume of solution (1) to 100 volumes with *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase described below.

(c) Apply 20 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow to dry in air and spray with *potassium iodobismuthate solution*.

MOBILE PHASE

6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%);

not more than one spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Transfer a quantity of the contents of the capsules containing 1.0 g of Paracetamol to a

ground-glass-stoppered 15 ml centrifuge tube, add 5 ml of *peroxide-free ether*, shake mechanically for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or until a clear supernatant liquid is obtained and use the supernatant liquid.

(2) Dilute 1 ml of solution (1) to 10 ml with *ethanol* (*96%*).

(3) 0.0050% w/v of *4′-chloroacetanilide* in *ethanol* (*96%*).

(4) Dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol BPCRS* in sufficient *ethanol* (*96%*) to produce 100 ml.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 200 µl of solution (1) and 40 µl of each of solutions (2), (3) and (4).

(d) Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank and allow the solvent front to ascend 14 cm above the line of application.

(e) After removal of the plate, dry in a current of warm air and examine under *ultraviolet light* (*254 nm*).

MOBILE PHASE

10 volumes of *toluene,* 25 volumes of *acetone* and 65 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

LIMITS

Any spot corresponding to 4′-chloroacetanilide in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.005%).

Any *secondary spot* in the chromatogram obtained with solution (2) with an Rf value lower than that of 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.25%).

Uniformity of content

Capsules containing less than 2% of Codeine Phosphate comply with the requirements stated under Capsules, with respect to the content of Codeine Phosphate, using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 ml of the mobile phase to the contents of one capsule and mix with the aid of ultrasound until completely dispersed. Shake for 10 minutes, dilute to 200 ml with the mobile phase, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with octadecylsilyl *silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use an isocratic system using the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.01M *sodium pentanesulphonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, the pH of the solution being adjusted to 2.8 using 2M *hydrochloric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in each capsule using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

ASSAY

For paracetamol

Mix the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 500 mg of Paracetamol with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 ml of the filtrate to 250 ml with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used but with a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

For codeine phosphate

Mix the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 8 mg of Codeine Phosphate with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in *codeine phosphate BPCRS*.

For caffeine

Mix the contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the contents of the capsules containing 30 mg of Caffeine with 100 ml of the mobile phase for 10 minutes and filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 ml of the filtrate to 50 ml with the mobile phase.

(2) 0.003%w/v of *caffeine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2$ in each capsule using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS.*

LABELLING

The label states the quantities of Paracetamol, Codeine Phosphate and Caffeine.

Paracetamol, Codeine Phosphate and Caffeine Tablets

General Notices

Action and use

Analgesic; antipyretic; opioid receptor agonist.

DEFINITION

Paracetamol, Codeine Phosphate and Caffeine Tablets contain Codeine Phosphate, Paracetamol and Caffeine.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

Content of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$

95.0 to 105.0% of the stated amount.

Content of caffeine, $C_8H_{10}N_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 ml of *acetone*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of paracetamol *(RS 258)*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 24 mg of Codeine Phosphate with 30 ml of *water* for 1 minute and centrifuge. Decant, add 10 ml of 1M *sodium hydroxide* and 30 ml of *dichloromethane* to the supernatant liquid, shake for 1 minute and filter the dichloromethane layer through glass-fibre paper (Whatman GF/C is suitable).

(2) 0.08% w/v of *codeine phosphate BPCRS* in *methanol (50%)*.

(3) 0.08% w/v each of *codeine phosphate BPCRS* and *dihydrocodeine tartrate BPCRS* in *methanol (50%)*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution.

(d) Remove the plate, dry in air, spray with *ethanolic iron(III) chloride solution* and heat at

105° for 10 minutes and examine in daylight.

MOBILE PHASE

1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots of different colours.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

C. In the Assay for codeine phosphate, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

D. Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 10 ml of *water* for 5 minutes, filter and add 10 ml of 1M *sodium hydroxide*. Extract with three 30-ml quantities of *dichloromethane*, washing each extract with the same 10 ml of *water*. Filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate*, evaporate to dryness in a porcelain dish and expose to ammonia vapour. A pink colour is observed.

E. In the Assay for caffeine, the chromatogram obtained with solution (1) shows a principal peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2 and rotate the paddle at 50 revolutions per minute.

(b) Use p*hosphate buffer pH 5.8* as the dissolution medium.

PROCEDURE

After 45 minutes, withdraw a sample of 20 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For paracetamol

(1) Use the filtered dissolution medium, diluted with *phosphate buffer pH 5.8*, if necessary, to contain 0.005% w/v of Paracetamol.

(2) 0.005% w/v solution of *paracetamol BPCRS* in *phosphate buffer pH 5.8*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used but with a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ in the medium using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

For codeine phosphate

(1) Use the filtered dissolution medium, diluted with *phosphate buffer pH 5.8*, if necessary, to contain 0.001% w/v of Codeine Phosphate.

(2) 0.001% w/v solution of *codeine phosphate BPCRS* in *phosphate buffer pH 5.8*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3$,H3PO4,½H2O in the medium using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

For caffeine

(1) Use the filtered dissolution medium, diluted with *phosphate buffer pH 5.8,* if necessary, to contain 0.003% w/v of Caffeine.

(2) 0.001% w/v solution of *caffeine BPCRS* in *phosphate buffer pH 5.8*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2$ in the medium using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS.*

4-Aminophenol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.5 g of Paracetamol with 50 ml of the mobile phase for 10 minutes and filter.

(2) 0.001% w/v of *4-aminophenol* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10µm) (Nucleosil ODS is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 272 nm.

(f) Injection 20 µl for each solution.

MOBILE PHASE

0.01M *sodium butanesulphonate* in a mixture of 0.4 volumes of *formic acid*, 15 volumes of *methanol* and 85 volumes of *water.*

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with solution (1) (0.1%),

peaks with a long retention time may occur due to excipients.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 50 mg of Codeine Phosphate with 50 ml of 0.1M *hydrochloric acid* for 10 minutes and filter. Make the filtrate alkaline with 5M *sodium hydroxide* and extract with two 40-ml quantities of *dichloromethane*. Wash the combined extracts with 10 ml of *water*, filter through a layer of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with *dichloromethane*, evaporate the filtrate to dryness at a temperature not exceeding 45° using a rotary evaporator and dissolve the residue in 2 ml of *dichloromethane.*

(2) Dilute 1.5 volumes of solution (1) to 100 volumes with *dichloromethane*.

(3) Dilute 1 volume of solution (1) to 100 volumes with *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G.*

(b) Use the mobile phase described below.

(c) Apply 20 µl of each solution.

(d) Develop the plate to 15 cm.

(e) Remove the plate, dry in air and spray with *potassium iodobismuthate solution*.

MOBILE PHASE

6 volumes of 13.5M *ammonia*, 30 volumes of *cyclohexane* and 72 volumes of *absolute ethanol*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1.5%),

not more than one spot with an Rf value higher than that of the principal spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Transfer a quantity of the finely powdered tablets containing 1.0 g of Paracetamol to a ground-glass-stoppered 15 ml centrifuge tube, add 5 ml of *peroxide-free ether*, shake mechanically for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or

until a clear supernatant liquid is obtained and use the supernatant liquid.

(2) Dilute 1 ml of solution (1) to 10 ml with *ethanol* (*96%*).

(3) 0.0050% w/v of *4′-chloroacetanilide* in *ethanol* (*96%*).

(4) Dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol* (*96%*) to produce 100 ml.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase described below.

(c) Apply 200 µl of solution (1) and 40 µl of each of solutions (2), (3) and (4).

(d) Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank.

(d) Develop the plate to 15 cm.

(d) Remove the plate, dry in a current of warm air and examine under *ultraviolet light* (*254 nm*).

MOBILE PHASE

10 volumes of *toluene,* 25 volumes of *acetone* and 65 volumes of *dichloromethane.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.005%).

In the chromatogram obtained with solution (2):

any *secondary spot* with an Rf value lower than that of 4′-chloroacetanilide is not more intense than the spot in the chromatogram obtained with solution (3) (0.25%).

Uniformity of content

Tablets containing less than 2 mg or 2% of Codeine Phosphate comply with the requirements stated under Tablets, with respect to the content of Codeine Phosphate, using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 ml of the mobile phase to one tablet and mix with the aid of ultrasound until completely dispersed. Shake for 10 minutes, dilute to 200 ml with the mobile phase, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

0.01M *sodium pentanesulphonate* in a mixture of 22 volumes of *methanol* and 78 volumes of *water*, the pH of the solution being adjusted to 2.8 using 2M *hydrochloric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in each tablet using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,\frac{1}{2}H_2O$ in *codeine phosphate BPCRS*.

ASSAY

For paracetamol

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 500 mg of Paracetamol with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 ml of the filtrate to 250 ml with the mobile phase.

(2) 0.005% w/v of *paracetamol BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used but with a detection wavelength of 243 nm.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_9NO_2$ using the declared content of $C_8H_9NO_2$ in *paracetamol BPCRS*.

For codeine phosphate

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 8 mg of Codeine Phosphate with 100 ml of the mobile phase for 10 minutes, dilute to 200 ml with the same solvent, filter through a glass-fibre filter (Whatman GF/C is suitable) and use the filtrate.

(2) 0.004% w/v of *codeine phosphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ using the declared content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$ in *codeine phosphate BPCRS*.

For caffeine

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 30 mg of Caffeine with 100 ml of the mobile phase for 10 minutes, filter through a glass-fibre filter (Whatman GF/C is suitable) and dilute 5 ml of the filtrate to 50 ml with the mobile phase.

(2) 0.003% w/v of *caffeine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_8H_{10}N_4O_2$ in each tablet using the declared content of $C_8H_{10}N_4O_2$ in *caffeine BPCRS.*

LABELLING

The label states the quantities of Paracetamol, Codeine Phosphate and Caffeine.

Paraffin Ointment

General Notices

DEFINITION

White Beeswax	20 g
Hard Paraffin	30 g
Cetostearyl Alcohol	50 g
White Soft Paraffin	900 g

Extemporaneous preparation

The following directions apply.

Mix the ingredients, heat gently with stirring until homogeneous and stir until cold.
The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Paraldehyde Injection

General Notices

DEFINITION

Paraldehyde Injection is Paraldehyde that has been sterilised. It does not contain any added antimicrobial preservatives.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. A 10.0% v/v solution in *carbon dioxide-free water* (solution A) is *clear*, Appendix IV A, but becomes turbid on warming.

B. Heat 5 ml with 0.1 ml of 1M *sulphuric acid*. Acetaldehyde is evolved.

C. To 5 ml of solution A in a test tube add 5 ml of *ammoniacal silver nitrate solution* and heat on a water bath. Silver is deposited as a mirror on the side of the tube.

TESTS

Acidity

Titrate 50 ml of solution A with 0.1M *sodium hydroxide VS* using 0.05 ml of *phenolphthalein solution* as indicator. Not more than 1.5 ml of 0.1M *sodium hydroxide VS* is required to change the colour of the solution.

Distillation range

Not more than 10% distils below 123° and not less than 95% distils below 126°, Appendix V C.

Freezing point

10° to 13°, Appendix V B.

Refractive index

1.403 to 1.406, Appendix V E.

Relative density

0.991 to 0.996, Appendix V G.

Acetaldehyde

Shake 5 ml with a mixture of 5 ml of *ethanol* (*60%*), 5 ml of *alcoholic hydroxylamine solution* and 0.2 ml of *methyl orange solution*. Not more than 0.8 ml of 0.5M *sodium hydroxide VS* is required to change the colour of the solution to pure yellow.

Peroxides

To 50 ml of solution A in a ground-glass-stoppered flask add 5 ml of 1M *sulphuric acid* and 10 ml of *potassium iodide solution*, close the flask and allow to stand in the dark for 15 minutes. Titrate with 0.1M *sodium thiosulphate VS* using 1 ml of *starch solution* as indicator; allow to stand for 5 minutes and, if necessary, complete the titration. Not more than 2.0 ml of 0.1M *sodium thiosulphate VS* is required.

STORAGE

Paraldehyde Injection should be supplied in single-dose glass ampoules sealed by fusion of the glass. It should be kept in complete darkness.

LABELLING

The label states that plastic syringes should not be used for the administration of the injection.

Paroxetine Tablets

General Notices

Action and use

Selective serotonin reuptake inhibitor; antidepressant.

DEFINITION

Paroxetine Tablets contain Paroxetine Hydrochloride Hemihydrate or Anhydrous Paroxetine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

PRODUCTION

The manufacturing process of Paroxetine Hydrochloride, used in the formulation of Paroxetine Tablets, is validated to show that the content of 4-(4-fluorophenyl)-1-methyl-1,2,3, 6-tetrahydropyridine is not more than 1 ppm.

Content of anhydrous paroxetine hydrochloride, $C_{19}H_{20}FNO_3$, HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption,* Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Dissolution test exhibits a maximum at 294 nm.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

DISSOLUTION

Comply with the *dissolution test for tablets and capsules,* Appendix XII D, using Apparatus II. Use as the medium 900 ml of 0.1Λ *hydrochloric acid,* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of the medium and filter through a plastic filter with a GF/A filter (Millipore Swinnex is suitable). Measure the a*bsorbance* of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at 294 nm, Appendix II B, using the dissolution medium in the reference cell. Measure the *absorbance* of a suitable solution of *paroxetine hydrochloride hemihydrate EPCRS* in 0.1M *hydrochloric acid* at the maximum at 294 nm using the dissolution medium in the reference cell. Calculate the total content of paroxetine hydrochloride, $C_{19}H_{20}FNO_3$,HCl, in the medium from the absorbances obtained and from the declared content of $C_{19}H_{20}FNO_3$,HCl in *paroxetine hydrochloride hemihydrate EPCRS.*

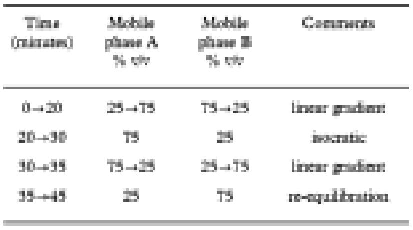
Related substances

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 50 mg of anhydrous paroxetine hydrochloride with 10 ml of *methanol* for 30 minutes. Filter through a 0.45-µm membrane filter (Gelman acrodisc GHP is suitable), dilute 1 volume of the solution with 1 volume of mobile phase B. For solution (2) dilute 1 volume of solution (1) to 100 volumes with mobile phase A and further dilute 1 volume of the resulting solution to 10 volumes with mobile phase A. Solution (3) is a 0.25% w/v solution of *paroxetine impurity standard BPCRS* in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable), (b) carry out a linear gradient elution using the following mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 265 nm.

Mobile phase A A mixture of 50% of *acetonitrile* and 50% of phosphate buffer pH 6.0 prepared by dissolving 4.9 g of *orthophosphoric acid* in about 800 ml of *water*, adjusting the pH to 6.0 with 1M *sodium hydroxide* and diluting to 1000 ml with *water*.

Mobile phase B Phosphate buffer pH 6.0 prepared by dissolving 4.9 g of *orthophosphoric acid* in about 800 ml, adjusting the pH to 6.0 with 1M *sodium hydroxide* and diluting to 1000 ml with *water*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v	Comments
0→20	25→75	75→25	linear gradient
20→30	75	25	isocratic
30→35	75→25	25→75	linear gradient
35→45	25	75	re-equilibration

Inject 20 µl of solution (3). The test is not valid unless the chromatogram obtained closely resembles the chromatogram supplied with *paroxetine impurity standard BPCRS.*

Inject 20 µl each of solution (1) and solution (2). In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-(4′-fluorphenyl)-3-hydroxymethyl piperidine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%, taking into account the correction factor of 0.5) the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) disperse with the aid of ultrasound a quantity of powdered tablets containing the equivalent of 25 mg of anhydrous paroxetine hydrochloride in 70 ml of *water* and 5 ml of 0.05M *hydrochloric acid*. Mix with the aid of ultrasound until all the granular material has dispersed. Add 150 ml of *propan-2-ol*, shake thoroughly and mix with the aid of ultrasound with occasional swirling for 15 minutes. Cool, add sufficient *propan-2-ol* to produce 250 ml, filter through a glass microfibre filter (Whatman GF/F is suitable) and use the filtrate. Solution (2) contains 0.01% w/v of *paroxetine hydrochloride hemihydrate EPCRS* in a solution containing 1 volume 0.01M *hydrochloric acid,* 14 volumes of *water* and 35 volumes of *propan-2-ol.* Solution (3) contains 0.25% w/v of *paroxetine impurity standard BPCRS* in mobile phase A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 50% of *acetonitrile* and 50% of phosphate buffer pH 6.0 prepared by dissolving 4.9 g of *orthophosphoric acid* in about 800 ml of *water*, adjusting the pH to 6.0 with 1M *sodium hydroxide* and diluting to 1000 ml with *water* and (c) a detection wavelength of 265 nm.

Inject 20 µl of solution (3). The test is not valid unless the chromatogram obtained closely resembles the chromatogram supplied with *paroxetine impurity standard BPCRS.*

Calculate the content of paroxetine hydrochloride, $C_{19}H_{20}FNO_3$,HCl, in the tablets using the declared content of $C_{19}H_{20}FNO_3$,HCl in *paroxetine hydrochloride hemihydrate EPCRS.*

LABELLING

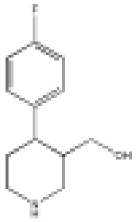
When the active ingredient is Paroxetine Hydrochloride Hemihydrate, the quantity is stated in terms of the equivalent amount of Anhydrous Paroxetine Hydrochloride.

STORAGE

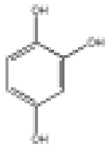
Paroxetine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirement of this monograph include:



4-(4′-fluorphenyl)-3-hydroxymethyl piperidine,



1,2,4-trihydroxybenzene.

Penicillamine Tablets

General Notices

Action and use

Disease-modifying antirheumatic drug; chelating agent; treatment of Wilson′s disease; heavy metal poisoning; cystinuria.

DEFINITION

Penicillamine Tablets contain Penicillamine. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of penicillamine, $C_5H_{11}NO_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Penicillamine with 4 ml of *water* and filter. Add to the filtrate 2 ml of *phosphotungstic acid solution* and allow to stand for a few minutes. A deep blue colour is produced.

B. Dissolve a quantity of the powdered tablets containing 10 mg of Penicillamine in 5 ml of *water* and add 0.3 ml of 5M *sodium hydroxide* and 20 mg of *ninhydrin*. An intense blue or violet-blue colour is produced immediately.

TESTS

Mercuric salts

Not more than 40 ppm, calculated with reference to the content of penicillamine, when determined by the following method. Disperse a quantity of the powdered tablets containing 1 g of Penicillamine in 10 ml of *water* in a stoppered flask, add 0.2 ml of 9M *perchloric acid* and swirl to dissolve. Add 1 ml of *ammonium pyrrolidinedithiocarbamate solution*, mix, add 2 ml of *4-methylpentan-2-one*, shake well for 1 minute and add sufficient *water* to produce 25 ml. Determine by *atomic absorption spectrophotometry*, Appendix II D, introducing the methylpentanone layer into the flame, measuring at 254 nm and using *4-methylpentan-2-one* in place of *water*. Use *mercury standard solution* (*100 ppm Hg*), suitably diluted with *water*, for the standard solutions, adjusted to contain the same concentrations of 9M perchloric acid, ammonium pyrrolidinedithiocarbamate solution and 4-methylpentan-2-one as the solution being examined.

Penicillamine disulphide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. The solutions should be prepared with de-gassed mobile phase and used immediately. For solution (1) shake a quantity of the powdered tablets containing 40 mg of Penicillamine with 10 ml of the mobile phase, filter and use the filtrate. Solution (2) contains 0.004% w/v of *penicillamine disulphide EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 5 C8 is suitable), (b) a solution containing 0.2% w/v of *methane sulphonic acid* and 0.01% w/v of *disodium edetate* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 220 nm.

In the chromatogram obtained with solution (1) the area of any peak corresponding to penicillamine disulphide is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.1 g of Penicillamine as completely as possible in 50 ml of *water* and filter. Add to the filtrate 5 ml of 1M *sodium hydroxide* and 0.2 ml of a 0.1% w/v solution of *dithizone in ethanol* (*96%*) and titrate with 0.02M *mercury*(*II*) *nitrate VS*. Each ml of 0.02M *mercury*(*II*) *nitrate VS* is equivalent to 5.968 mg of $C_5H_{11}NO_2S$.

Pentagastrin Injection

General Notices

Action and use

Gastrin analogue.

DEFINITION

Pentagastrin Injection is a sterile isotonic solution of Pentagastrin in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of pentagastrin, $C_{37}H_{49}N_7O_9S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance on each of three plates and the following solvent systems as the mobile phases: (A) a mixture of 25 volumes of a 3% v/v solution of 13.5M *ammonia* and 75 volumes of *butan-2-ol*, (B) the upper layer produced by shaking together 10 volumes of *glacial acetic acid*, 40 volumes of *butan-1-ol* and 50 volumes of *water* and allowing to separate and (C) a mixture of 25 volumes of *water,* 25 volumes of *ether* and 50 volumes of *glacial acetic acid*. Apply separately to each plate (1) a volume of the injection containing 5 µg of Pentagastrin and (2) 1 µl of a solution of *pentagastrin BPCRS* in 0.1M *ammonia* containing 0.5% w/v of pentagastrin. After removal of the plates, allow them to dry in air, heat at 100° for 2 minutes, spray with a 1.0% w/v solution of *4-dimethylaminobenzaldehyde* in a mixture of 3 volumes of *methanol* and 1 volume of *hydrochloric acid* and heat at 100° until purple spots are produced (about 2 minutes). Examine by transmitted light. On each plate, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *water*, 20 volumes of *glacial acetic acid* and 100 volumes of *ether* as the mobile phase. Apply separately to the plate (1) four successive 5-µl quantities of the injection diluted, if necessary, with *water* to contain 0.025% w/v of Pentagastrin and (2) 5 µl of the injection diluted, if necessary, with *water* to contain 0.005% w/v of Pentagastrin. Dry the plate in a current of nitrogen after each application. After removal of the plate, allow it to dry in air, heat at 100° for 2 minutes, spray with a 1.0% w/v solution of *4-dimethylaminobenzaldehyde* in a mixture of 3 volumes of *methanol* and 1 volume of *hydrochloric acid* and heat at 100° until purple spots are produced (about 2 minutes). Examine by transmitted light. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution

(2) (5%).

Acidity or alkalinity

pH, 7.0 to 8.5, Appendix V L.

ASSAY

Dilute a volume containing 0.75 mg of Pentagastrin to 10 ml with 0.01M *ammonia* and measure the *absorbance* at the maximum at about 280 nm, Appendix II B. Calculate the content of $C_{37}H_{49}N_7O_9S$ taking 70.0 as the value of A(1%, 1 cm) at the maximum at 280 nm.

STORAGE

Pentagastrin Injection should be protected from light and stored at a temperature of 2° to 8°. It should not be allowed to freeze.

Pentamidine Injection

General Notices

Action and use

Antiprotozoal.

DEFINITION

Pentamidine Injection is a sterile solution of Pentamidine Isetionate in Water for Injections. It is prepared by dissolving Pentamidine Isetionate for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Pentamidine Injection deteriorates on storage and should be used immediately after preparation.

PENTAMIDINE ISETIONATE FOR INJECTION

DEFINITION

Pentamidine Isetionate for Injection is a sterile material consisting of Pentamidine Isetionate without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of pentamidine isetionate, $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of pentamidine isetionate (*RS 259*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.01M *hydrochloric acid* exhibits a maximum only at 262 nm. The *absorbance* at 262 nm is about 0.95.

C. To 10 ml of a 0.05% w/v solution add 1 ml of a 0.1% w/v solution of *glyoxal sodium bisulphite* and 1 ml of a solution prepared by dissolving 4 g of *boric acid* in a mixture of 27 ml of 1M *sodium hydroxide* and sufficient *water* to produce 100 ml. Heat on a water bath for 10 minutes. A magenta colour is produced.

TESTS

Acidity

pH of a 5% w/v solution, 4.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.1% w/v of the contents of the sealed container in the mobile phase. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) add to 0.1 g of the contents of the sealed container 40 ml of *water* and some glass beads, adjust the pH to 10.5 with 2M *sodium hydroxide*, heat under a reflux condenser for 20 minutes, cool and dilute to 50 ml with *water*. Dilute 1 volume of this solution to 50 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 7 volumes of a 3% w/v solution of *ammonium acetate* and 13 volumes of *methanol*, the pH of the mixture being adjusted to 7.5 with *triethylamine*, and (c) a detection wavelength of 265 nm.

For solution (1) allow the chromatography to proceed for 3.5 times the retention time of the principal peak.

The test is not valid unless the chromatogram obtained with solution (3) shows two principal peaks and the *resolution factor* between these peaks is greater than 2.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than one fifth of the area of the peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of all such peaks is not greater than two fifths of the area of the peak in the chromatogram obtained with solution (2) (0.4%).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Dissolve 0.25 g of the mixed contents of the 10 containers in 50 ml of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 29.63 mg of $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$. Calculate the content of pentamidine isetionate, $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$, in a container of average content weight.

Pentazocine Capsules

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Pentazocine Capsules contain Pentazocine Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of pentazocine hydrochloride, $C_{19}H_{27}NO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 50 mg of Pentazocine Hydrochloride with 3 ml of 0.1M *sodium hydroxide* and 3 ml of *dichloromethane* and allow to separate. Filter the dichloromethane layer through a phase separation paper (Whatman 1PS is suitable) and evaporate the filtrate using a rotary evaporator at a temperature of 40°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pentazocine (form B) (*RS 261*).

B. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of *water* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtered solution, suitably diluted if necessary, at the maximum at 278 nm, Appendix II B. Calculate the total content of pentazocine hydrochloride, $C_{19}H_{27}NO,HCl$, in the medium taking 61.2 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Related substances

Comply with the test described under Pentazocine Tablets, but preparing solution (1) in the following manner. For solution (1) shake a quantity of the contents of the capsules containing 0.2 g of Pentazocine Hydrochloride with 10 ml of 0.1M *methanolic ammonia*, centrifuge and use the clear supernatant liquid.

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Pentazocine

Hydrochloride with 200 ml of *water* for 15 minutes, add 5 ml of 1M *hydrochloric acid* and sufficient *water* to produce 500 ml, mix and filter. Measure the *absorbance* of the filtrate at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{19}H_{27}NO,HCl$ taking 61.2 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Pentazocine Injection

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Pentazocine Injection is a sterile solution in Water for Injections of either Pentazocine Lactate or pentazocine lactate prepared by the interaction of Pentazocine and Lactic Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of pentazocine, $C_{19}H_{27}NO$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume containing the equivalent of 90 mg of pentazocine add 5 ml of 0.1M *sodium hydroxide* and shake the resulting solution with 5 ml of *chloroform*. Wash the chloroform extract with 2 ml of *water*, dry over *anhydrous sodium sulphate* and filter. Evaporate the chloroform using a rotary evaporator and dry the oily residue at a temperature not exceeding 25° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pentazocine (form B) (*RS 261*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of 18M *ammonia*, 10 volumes of *ethyl acetate*, 30 volumes of *water* and 50 volumes of *absolute ethanol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a quantity of the injection with sufficient *methanol* to produce a solution containing the equivalent of 0.75% w/v of pentazocine. Solution (2) contains 0.4% w/v of *calcium lactate* in *methanol*. After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *potassium permanganate* and examine in daylight. The principal spot in the chromatogram obtained with solution (1) is similar in colour, position and size to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 3 volumes of *methanol*, 3 volumes of *isopropylamine* and 94 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a quantity of the injection with sufficient *ethanol* (*96%*) to produce a solution containing the equivalent of 2.0% w/v of pentazocine. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *ethanol* (*96%*). For solution (3) dilute 1 volume of solution (1) to 200 volumes with *ethanol* (*96%*). For solution (4) dilute 1 volume of solution (1) to 400 volumes with *ethanol* (*96%*). After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Heat the plate at 105 ^{c} for 15 minutes, allow to cool, expose to iodine vapour and re-examine under *ultraviolet light* (*254 nm*). By each method of visualisation any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%), not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than four such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.25%). Disregard any spot remaining on the line of application.

ASSAY

To a quantity containing the equivalent of 0.15 g of pentazocine add sufficient *water* to produce 100 ml. To 5 ml add 1 ml of 1M *hydrochloric acid*, dilute to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 278.5 nm, Appendix II B. Calculate the content of $C_{19}H_{27}NO$ taking 69.0 as the value of A(1%, 1 cm) at the maximum at 278.5 nm.

STORAGE

Pentazocine Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of pentazocine in a suitable dose-volume.

Pentazocine Suppositories

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Pentazocine Suppositories contain Pentazocine Lactate in a suitable suppository basis.
The suppositories comply with the requirements stated under Rectal Preparations and with the following requirements.

Content of pentazocine, $C_{19}H_{27}NO$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the filtrate obtained in the Assay containing the equivalent of 0.1 g of pentazocine add sufficient 1M *sodium hydroxide* to make alkaline and extract with three 10 ml quantities of *dichloromethane*. Wash the combined extracts with 5 ml of *water*, dry over *anhydrous sodium sulphate* and filter. Evaporate the dichloromethane using a rotary evaporator and dry the oily residue at a temperature not exceeding 25° over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 16 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pentazocine (form B) (*RS 261*).

B. Disperse a suppository containing the equivalent of 50 mg of pentazocine in 5 ml of *petroleum spirit* (*boiling range, 40° to 60°*) and extract with 2 ml of 0.1M *sodium hydroxide*. Add 25 ml of *methanol* to the aqueous layer, evaporate to dryness using a rotary evaporator and dry the residue at 60° under reduced pressure for 16 hours. To the residue add 0.2 ml of *methanol* and 2 ml of *sulphuric acid* and heat the resulting solution at 85° for 2 minutes. Allow to cool and add a few mg of *4-hydroxybiphenyl*. A violet-red colour is produced within 5 minutes.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 3 volumes of *isopropylamine*, 3 volumes of *methanol* and 94 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) mix a quantity of the suppositories containing the equivalent of 0.2 g of pentazocine in 30 ml of *petroleum spirit* (*boiling range, 40° to 60°*) and extract with 10 ml of 0.01M *hydrochloric acid*. Add about 10 mg of *sodium carbonate* to the aqueous extract and shake to dissolve. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *chloroform*. For solution (3) dilute 5 volumes of solution (2) to 10 volumes with *chloroform*. For solution (4) dilute 5 volumes of solution (3) to 10 volumes with *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine under *ultraviolet light* (*254 nm*). In the chromatogram obtained with solution (1) any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%), not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than four such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.25% each).

ASSAY

Dissolve a quantity of the suppositories containing the equivalent of 0.5 g of pentazocine in 50 ml of *petroleum spirit* (*boiling range, 40° to 60°*) and extract with six 50 ml quantities of 0.01M *hydrochloric acid*. Wash the combined extracts with two 10 ml quantities of *chloroform* and dilute to 500 ml with 0.01M *hydrochloric acid*, mix and filter (Whatman No. 1 is suitable). Dilute 10 ml of the clear filtrate to 100 ml with 0.01M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B, using 0.01M *hydrochloric acid* in the reference cell. Calculate the content of $C_{19}H_{27}NO$ taking 69.0 as the value of A(1%, 1 cm) at the maximum at 278 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of pentazocine.

Pentazocine Tablets

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Pentazocine Tablets contain Pentazocine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pentazocine hydrochloride, $C_{19}H_{27}NO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.1 g of Pentazocine Hydrochloride with 10 ml of *water,* filter (Whatman GF/C is suitable), add 1 ml of 1M *sodium hydroxide* and shake the resulting solution with 20 ml of *chloroform*. Wash the chloroform extract with 5 ml of *water*, dry over *anhydrous sodium sulphate* and filter. Evaporate the chloroform using a rotary evaporator and dry the oily residue at 60° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the powdered residue, Appendix II A, is concordant with the *reference spectrum* of pentazocine (form B) (*RS 261*). If the spectra are not concordant, dissolve the residue in *chloroform*, evaporate to dryness and prepare a new spectrum of the residue.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 94 volumes of *chloroform*, 3 volumes of *methanol* and 3 volumes of *isopropylamine* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Pentazocine Hydrochloride with 10 ml of 0.1M *methanolic ammonia* for 10 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the same solvent. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the same solvent. For solution (4) dilute 1 volume of solution (1) to 400 volumes with the same solvent. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine under *ultraviolet light* (*254 nm*). By each method of visualisation any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2), not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) and not more than four such spots are more intense than the spot in the chromatogram obtained with solution (4). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 25 mg of Pentazocine Hydrochloride with 100 ml of *water* for 15 minutes, add 2.5 ml of 1M *hydrochloric acid* and sufficient *water* to produce 250 ml and filter. Measure the *absorbance* of the filtrate at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{19}H_{27}NO,HCl$ taking 61.2 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Pentobarbital Tablets

General Notices

Action and use

Barbiturate.

DEFINITION

Pentobarbital Tablets contain Pentobarbital Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pentobarbital sodium, $C_{11}H_{17}N_2NaO_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat 0.2 g of the residue obtained in the Assay on a water bath with the minimum quantity of *ethanol* (*25%*) until dissolved, filter while hot and allow the filtrate to cool. Filter, wash the crystals with a small quantity of *ethanol* (*25%*) and dry at 105°. The *infrared absorption spectrum* of the crystals, Appendix II A, is concordant with the *reference spectrum* of pentobarbital (*RS 264*).

B. The powdered tablets yield the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Isomer

Dissolve a quantity of the powdered tablets containing 0.3 g of Pentobarbital Sodium in 5 ml of a 5% w/v solution of *anhydrous sodium carbonate*, heating gently if necessary. Add 10 ml of a 3% w/v solution of *4-nitrobenzyl chloride* in *ethanol* (*96%*) and heat under a reflux condenser for 30 minutes. Cool to 25°, filter and wash the precipitate with five 5 ml quantities of *water*. Heat the precipitate with 25 ml of *ethanol* (*96%*) in a small flask under a reflux condenser until dissolved (about 10 minutes). Filter the hot solution, cool to 25° and, if necessary, scratch the side of the flask with a glass rod to induce crystallisation. Filter, wash the precipitate with two 5 ml quantities of *water* and dry at 100° to 105° for 30 minutes. The *melting point* of the dried precipitate is 136° to 148°, Appendix V A, Method I.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.3 g of Pentobarbital Sodium as completely as possible in 10 ml of a 2% w/v solution of *sodium hydroxide*, saturate with *sodium chloride*, acidify with *hydrochloric acid* and extract with successive 15 ml quantities of *ether* until complete extraction is effected. Wash the combined extracts with two 2 ml quantities of *water* and extract the combined washings with 10 ml of *ether*. Add the ether to the main ether layer, filter and wash the filter with *ether*. Evaporate the solvent and dry the residue to constant weight at 105°. Each g of residue is equivalent to

1.097 g of $C_{11}H_{17}N_2NaO_3$.

Peppermint Spirit

General Notices

Peppermint Essence

DEFINITION

Peppermint Oil	100 ml
Ethanol (90 per cent)	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Peppermint Oil in Ethanol (90 per cent) and add sufficient Ethanol (90 per cent) to produce 1000 ml. If the solution is not clear, shake with previously sterilised Purified Talc and filter.

The spirit complies with the requirements stated under Spirits and with the following requirements.

TESTS

Ethanol content

78 to 82% v/v, Appendix VIII F.

Weight per ml

0.830 to 0.840 g, Appendix V G.

Content of oil

9.0 to 11.0% v/v when determined by the following method. Add 25 ml of the spirit and 5 ml of *xylene* to 90 ml of a 10% w/v solution of *sodium chloride* containing 1.0% v/v of *hydrochloric acid* in a flask of about 150 ml capacity with a long neck graduated in 0.1 ml increments and of such a diameter that a 15-cm length has a capacity of 10 ml. Shake the mixture for about 30 minutes, allow to separate and raise the undissolved oily layer into the graduated part of the neck of the flask by the gradual addition of more of the acidified sodium chloride solution, allow to stand for 2 hours or until there is no further change in volume of the oily layer and measure the volume of the oily layer. The volume of the oily layer, after the subtraction of 5 ml, is taken to be the volume of oil.

Peritoneal Dialysis Solutions

General Notices

(Ph Eur monograph 0862)

Ph Eur

DEFINITION

Solutions for peritoneal dialysis are preparations for intraperitoneal use containing electrolytes with a concentration close to the electrolytic composition of plasma. They contain glucose in varying concentrations or other suitable osmotic agents.

Solutions for peritoneal dialysis are supplied in:

— rigid or semi-rigid plastic containers,

— flexible plastic containers fitted with a special connecting device; these are generally filled to a volume below their nominal capacity and presented in closed protective envelopes,

— glass containers.

The containers and closures comply with the requirements for containers for preparations for parenteral use *(3.2.1 and 3.2.2)*.

Several formulations are used. The concentrations of the components per litre of solution are usually in the following range:

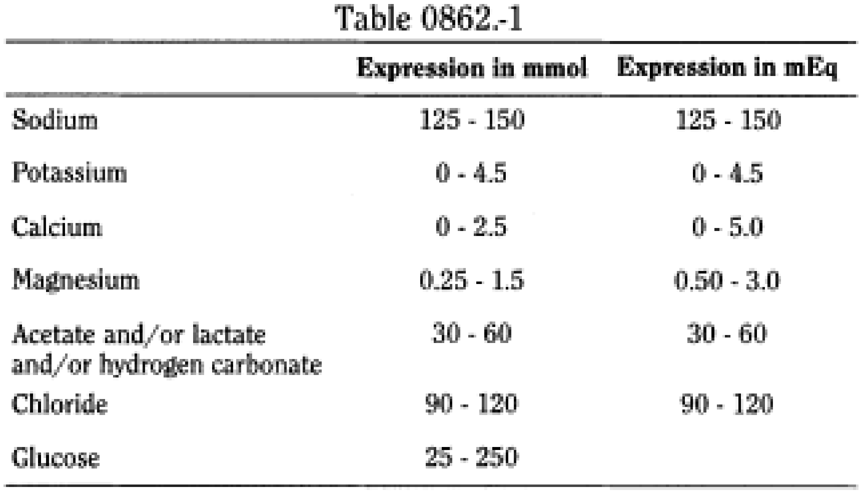


Table 0862.-1

	Expression in mmol	Expression in mEq
Sodium	125 - 150	125 - 150
Potassium	0 - 4.5	0 - 4.5
Calcium	0 - 2.5	0 - 5.0
Magnesium	0.25 - 1.5	0.50 - 3.0
Acetate and/or lactate and/or hydrogen carbonate	30 - 60	30 - 60
Chloride	90 - 120	90 - 120
Glucose	25 - 250	

When hydrogen carbonate is present, the solution of sodium hydrogen carbonate is supplied in a container or a separate compartment and is added to the electrolyte solution immediately before use.

Unless otherwise justified and authorised, antioxidants such as metabisulphite salts are not added to the solutions.

IDENTIFICATION

According to the stated composition, the solution to be examined gives the following identification reactions (*2.3.1*):

— potassium: reaction (b);

— calcium: reaction (a);

— sodium: reaction (b);

— chlorides: reaction (a);

— acetates: to 5 ml of the solution to be examined add 1 ml of *hydrochloric acid R* in a test-tube fitted with a stopper and a bent tube, heat and collect a few millilitres of distillate; carry out reaction (b) of acetates on the distillate;

— lactates, hydrogen carbonates; the identification is carried out together with the assay;

— magnesium: to 0.1 ml of *titan yellow solution R* add 10 ml of *water R*, 2 ml of the solution to be examined and 1 ml of *1 M sodium hydroxide*; a pink colour is produced;

— glucose: to 5 ml of the solution to be examined, add 2 ml of *dilute sodium hydroxide solution R* and 0.05 ml of *copper sulphate solution R*; the solution is blue and clear; heat to boiling; an abundant red precipitate is formed.

TESTS

Appearance of solution

The solution is clear *(2.2.1)* and not more intensely coloured than reference solution Y_4 *(2.2.2, Method I)*.

pH *(2.2.3)*

The pH of the solution is 5.0 to 6.5. If the solution contains hydrogen carbonate, the pH is 6.5 to 8.0.

Hydroxymethylfurfural

To a volume of the solution containing the equivalent of 25 mg of glucose, add 5.0 ml of a 100 g/l solution of *p-toluidine R* in *2-propanol R* containing 10 per cent *V/V* of *glacial acetic acid R* and 1.0 ml of a 5 g/l solution of *barbituric acid R*. The absorbance *(2.2.25)* determined at 550 nm after allowing the mixture to stand for 2 min to 3 min is not greater than that of a standard prepared at the same time in the same manner using a solution containing 10 µg of *hydroxymethylfurfural R* in the same volume as the solution to be examined. If the solution contains hydrogen carbonate, use as the standard a solution containing 20 µg of *hydroxymethylfurfural R*.

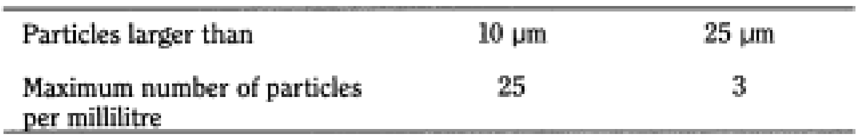
Aluminium *(2.4.17)*

Take 400 ml, adjust to pH 6.0 and add 10 ml of *acetate buffer solution pH 6.0 R*. The solution complies with the limit test for aluminium (15 µg/l). Use as the reference solution a mixture of 3 ml of *aluminium standard solution (2 ppm Al) R,* 10 ml of *acetate buffer solution pH 6.0 R* and 9 ml of *water R.* To prepare the blank use a mixture of 10 ml of *acetate buffer solution pH 6.0 R* and 10 ml of *water R*.

Particulate contamination

Carry out the test for sub-visible particles (*2.9.19*) using 50 ml of solution.

Table 0862.-2



Particles larger than	10 µm	25 µm
Maximum number of particles per millilitre	25	3

Extractable Volume *(2.9.17)*

The solution complies with the test prescribed for parenteral infusions.

Sterility *(2.6.1)*

The solution complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 0.25 IU/ml.

Pyrogens *(2.6.8)*

Solutions for which a validated test for bacterial endotoxins cannot be carried out comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 ml of the solution.

ASSAY

Sodium

97.5 per cent to 102.5 per cent of the content of sodium (Na) stated on the label, determined by atomic absorption spectrometry *(2.2.23, Method II)*.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*.

Measure the absorbance at 589.0 nm using a sodium hollow-cathode lamp as source of radiation and an air-propane or an air-acetylene flame.

Potassium

95.0 per cent to 105.0 per cent of the content of potassium (K) stated on the label, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used. To 100 ml of the solution add 10 ml of a 22 g/l solution of *sodium chloride R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*. To 100 ml of each reference solution add 10 ml of a 22 g/l solution of *sodium chloride R*.

Measure the absorbance at 766.5 nm, using a potassium hollow-cathode lamp as source of radiation and an air-propane or an air-acetylene flame.

Calcium

95.0 per cent to 105.0 per cent of the content of calcium (Ca) stated on the label, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *calcium standard solution (400 ppm Ca) R*.

Measure the absorbance at 422.7 nm using a calcium hollow-cathode lamp as source of radiation and an air-propane or an air-acetylene flame.

Magnesium

95.0 per cent to 105.0 per cent of the content of magnesium (Mg) stated on the label, determined by atomic absorption spectrometry *(2.2.23, Method I)*.

Test solution If necessary, dilute the solution to be examined with *water R* to a concentration suitable for the instrument to be used.

Reference solutions Prepare the reference solutions using *magnesium standard solution (100 ppm Mg) R*.

Measure the absorbance at 285.2 nm using a magnesium hollow-cathode lamp as source of radiation and an air-propane or an air-acetylene flame.

Total chloride

95.0 per cent to 105.0 per cent of the content of chloride (Cl) stated on the label. Dilute to 50 ml with *water R* an accurately measured volume of the solution to be examined containing the equivalent of about 60 mg of chloride. Add 5 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 2 ml of *dibutyl phthalate R*. Shake. Using 2 ml of *ferric ammonium sulphate solution R2* as indicator, titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained.

1 ml of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Acetate

95.0 per cent to 105.0 per cent of the content of acetate stated on the label. To a volume of the solution to be examined, corresponding to about 0.7 mmol of acetate, add 10.0 ml of *0.1 M hydrochloric acid*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.1 mmol of acetate.

Lactate

95.0 per cent to 105.0 per cent of the content of lactate stated on the label. To a volume of the solution to be examined, corresponding to about 0.7 mmol of lactate, add 10.0 ml of *0.1 M hydrochloric acid*. Then add 50 ml of *acetonitrile R*. Carry out a potentiometric titration *(2.2.20)*, using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 ml *of 0.1 M sodium hydroxide* is equivalent to 0.1 mmol of lactate.

Sodium hydrogen carbonate

95.0 per cent to 105.0 per cent of the content of sodium hydrogen carbonate stated on the label. Titrate with *0.1 M hydrochloric acid*, a volume of the solution to be examined corresponding to about 0.1 g of sodium hydrogen carbonate, determining the end-point potentiometrically *(2.2.20)*.

1 ml *of 0.1 M hydrochloric acid* is equivalent to 8.40 mg of $NaHCO_3$.

Lactate and hydrogen carbonate

95.0 per cent to 105.0 per cent of the content of lactates and/or hydrogen carbonates stated on the label. Examine by liquid chromatography *(2.2.29)*.

Test solution Solution to be examined.

Reference solution Dissolve in 100 ml of *water for chromatography R* quantities of lactates and hydrogen carbonates, accurately weighed, in order to obtain solutions having concentrations representing about 90 per cent, 100 per cent and 110 per cent of the concentrations indicated on the label.

The chromatographic procedure may be carried out using:

— a column 0.30 m long and 7.8 mm in internal diameter packed with *cation exchange resin R* (9 µm),

— as mobile phase at a flow rate of 0.6 ml/min *0.005 M sulphuric acid* previously degassed with *helium R*,

— a differential refractometer detector,

maintaining the temperature of the column at 85 °C.

Inject in duplicate 20 µl of test solution and 20 µl of each reference solution. When the chromatograms are recorded in the prescribed conditions, the peaks elute in the following order: lactates then hydrogen carbonates.

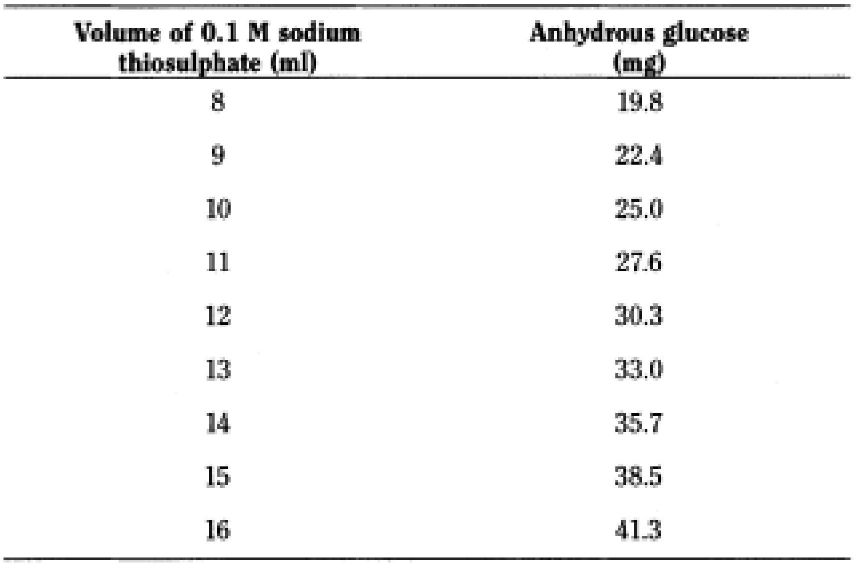
Determine the concentration of lactates and hydrogen carbonates in the test solution by interpolating the peak area for lactate and the peak height for hydrogen carbonate from the linear regression curve obtained with the reference solutions.

Reducing sugars (expressed as anhydrous glucose)

95.0 per cent to 105.0 per cent of the content of glucose stated on the label. Transfer a volume of solution to be examined containing the equivalent of 25 mg of glucose to a 250 ml conical flask with a ground-glass neck and add 25.0 ml of *cupri-citric solution R*. Add a few grains of pumice, fit a reflux condenser, heat so that boiling occurs within 2 min and boil for exactly 10 min. Cool and add 3 g of *potassium iodide R* dissolved in 3 ml of *water R.* Carefully add, in small amounts, 25 ml of a 25 per cent *m/m* solution of *sulphuric acid R*. Titrate with *0.1 M sodium thiosulphate* using *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration using 25.0 ml of *water R.*

Calculate the content of reducing sugars expressed as anhydrous glucose ($C_6H_{12}O_6$), by means of Table 0862.-3:

Table 0862.-3



Volume of 0.1 M sodium thiosulphate (ml)	Anhydrous glucose (mg)
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3

STORAGE

Store at a temperature not below 4 °C.

LABELLING

The label states:

- the formula of the solution for peritoneal dialysis, expressed in grams per litre and in millimoles per litre,
- the calculated osmolarity, expressed in milliosmoles per litre,
- the nominal volume of the solution for peritoneal dialysis in the container,
- that the solution is free from bacterial endotoxins, or where applicable, that it is apyrogenic,
- the storage conditions,
- that the solution is not to be used for intravenous infusion,
- that any unused portion of solution is to be discarded.

Ph Eur

Perphenazine Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Perphenazine Tablets contain Perphenazine. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of perphenazine, $C_{21}H_{26}ClN_3OS$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 40 mg of Perphenazine add 10 ml of *water* and 2 ml of 1M *sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the ether layer with 5 ml of *water*, dry with *anhydrous sodium sulphate* and evaporate the ether to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of perphenazine (*RS 265*).

B. Extract a quantity of the powdered tablets containing 20 mg of Perphenazine with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2 ml of *methanol*, pour into a solution of 0.4 g of *picric acid* in 10 ml of *methanol* at 50°, cool and allow to stand for 4 hours. The *melting point* of the precipitate, after recrystallisation from *methanol*, is about 248°, with decomposition, Appendix V A.

C. To a quantity of the powdered tablets containing 5 mg of Perphenazine add 5 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

TESTS

Related substances

Comply with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase C* and applying separately to the plate 50 µl of each of the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 20 mg of Perphenazine with 10 ml of *ethanol* (*96%*) and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *ethanol* (*96%*).

Uniformity of content

Tablets containing 2 mg or less of Perphenazine comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. Record *second-derivative ultraviolet absorption spectra* of the following solutions, Appendix II B, in the range 210 to 290 nm. For solution (1) add 5 ml of *water* to one tablet, mix with the aid of ultrasound for 15 minutes or until the tablet has completely disintegrated, heat on a water bath for 3 minutes, swirling continuously and cool. Add 50 ml of *ethanol* (*96%*), mix with the aid of ultrasound for 2 minutes, shake for 5 minutes, dilute to 100 ml with *ethanol* (*96%*) and filter through a glass microfibre filter paper. Dilute the filtrate, if necessary, with *ethanol* (*96%*) to produce a solution containing 0.001% w/v of Perphenazine. Solution (2) contains 0.001% w/v of *perphenazine BPCRS* in *ethanol* (*96%*).

For each solution measure the amplitude from the peak at 265 nm to the trough at 255 nm. Calculate the content of $C_{21}H_{26}N_3OS$ in each tablet using the declared content of $C_{21}H_{26}N_3OS$ in *perphenazine BPCRS*.

ASSAY

For tablets containing more than 2 mg of Perphenazine

Carry out the following procedure protected from light. Record *second-derivative ultraviolet absorption spectra* of the following solutions, Appendix II B, in the range 210 to 290 nm. For solution (1) add 50 ml of *water* to 10 tablets, mix with the aid of ultrasound for 15 minutes or until the tablets have completely disintegrated, heat on a water bath for 3 minutes, swirling continuously, cool, add 400 ml of *ethanol* (*96%*), mix with the aid of ultrasound for 2 minutes, shake for 5 minutes, dilute to 500 ml with *ethanol* (*96%*) and filter through a glass microfibre filter paper. Dilute the filtrate with *ethanol* (*96%*) to produce a solution containing 0.001% w/v of Perphenazine. Solution (2) contains 0.001% w/v of *perphenazine BPCRS* in *ethanol* (*96%*). Measure the amplitude from the peak at 265 nm to the trough at 255 nm. Calculate the content of $C_{21}H_{26}N_3OS$ using the declared content of $C_{21}H_{26}N_3OS$ in *perphenazine BPCRS*.

For tablets containing 2 mg or less of Perphenazine

Use the average of the 10 individual results obtained in the test for Uniformity of content.

Pethidine Injection

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Pethidine Injection is a sterile solution of Pethidine Hydrochloride in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

PRODUCTION

The manufacturing process of Pethidine Hydrochloride, used in the formulation of Pethidine Injection, is validated to show that the content of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is not more than 0.1 ppm.

Content of pethidine hydrochloride, $C_{15}H_{21}NO_2$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume containing 50 mg of Pethidine Hydrochloride add sufficient 1M *sodium hydroxide* to make strongly alkaline to *litmus paper* and extract with two 10 ml quantities of *chloroform*. Wash the combined extracts with 5 ml of *water*, dry over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Remove the last traces of chloroform by drying the residual oil at 60° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of pethidine (*RS 266*).

B. Yields the reactions characteristic of *chlorides*, Appendix VI.

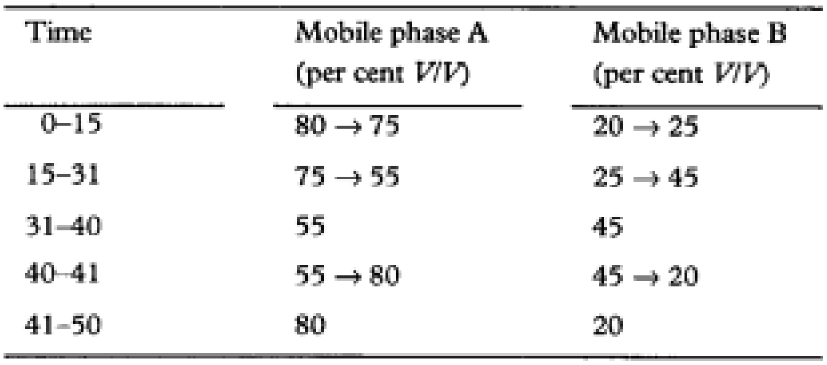
Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection containing 0.1 g of Pethidine Hydrochloride to 25 ml with a mixture of 20 volumes of *acetonitrile* and 80 volumes of *water*. For solution (2) dilute 0.5 volume of solution (1) to 100 volumes with a mixture of 20 volumes of *acetonitrile* and 80 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.0 mm) packed with *spherical end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 340 m^2/g, a pore size of 10 nm and a carbon loading of 19% (Kromasil C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute the gradient elution programme described below and (c) a detection wavelength of 210 nm.

Mobile phase A Mix equal volumes of a 4.2% w/v solution of *sodium perchlorate* and a 1.2% w/v solution of *orthophosphoric acid*. Adjust the pH to 2.0 with *triethylamine*.

Mobile phase B Acetonitrile.



Time	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0–15	80 → 75	20 → 25
15–31	75 → 55	25 → 45
31–40	55	45
40–41	55 → 80	45 → 20
41–50	80	20

Inject 20 µl of each of solutions (1) and (2).

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area 0.1 times the area of the peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.1% w/v of Pethidine Hydrochloride and further dilute 3 volumes of the resulting solution to 25 volumes with the mobile phase. For solution (2) dilute 3 volumes of a 0.10% w/v solution of *pethidine hydrochloride BPCRS* mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS1 is suitable) maintained at 40^{c}, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 11 volumes of *acetonitrile* and 9 volumes of a mixture prepared in the following manner: dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 10 ml *triethylamine*, mix well and adjust the solution to pH 7.0 with *orthophosphoric acid* and (c) a detection wavelength of 230 nm.

The *column efficiency*, determined on the peak due to pethidine in the chromatogram obtained with solution (2), should be at least 8000 *theoretical plates* per metre.

Calculate the content of $C_{15}H_{21}NO_2$,HCl in the injection using the declared content of $C_{15}H_{21}NO_2$,HCl in *pethidine hydrochloride BPCRS*.

Pethidine Tablets

General Notices

Action and use

Opioid receptor agonist; analgesic.

DEFINITION

Pethidine Tablets contain Pethidine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pethidine hydrochloride, $C_{15}H_{21}NO_2,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Pethidine Hydrochloride with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and dry the residue at a pressure of 2 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pethidine hydrochloride (*RS 267*).

B. Shake a quantity of the powdered tablets containing 0.2 g of Pethidine Hydrochloride with 20 ml of *water* and filter. To 5 ml of the filtrate add 10 ml of *picric acid solution R1*. The *melting point* of the crystals so obtained, after washing with *water*, is about 190°, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *kieselguhr G* as the coating substance but allowing the solvent front to ascend 12 cm above the line of application. Impregnate the dry plate by placing it in a closed tank containing a mixture of 10 volumes of *2-phenoxyethanol* and 90 volumes of *acetone* so that the plate dips about 5 mm beneath the surface of the liquid, allowing the impregnation solvent to ascend at least 15 cm, removing the plate from the tank and drying in a current of air. Use immediately, with the flow of the mobile phase in the same direction as the impregnation. Use as the mobile phase the upper layer obtained by shaking together 1 volume of *diethylamine*, 8 volumes of *2-phenoxyethanol* and 100 volumes of *petroleum spirit* (*boiling range, 50° to 70°*) and allowing it to settle.

Apply separately to the plate 5 µl of each of the following solutions. For solution (1) use the upper layer obtained by shaking a quantity of the powdered tablets containing 0.1 g of Pethidine Hydrochloride with 5 ml of *water*, filtering, shaking the filtrate with 0.5 ml of 5M *sodium hydroxide* and 2 ml of *ether* and allowing the layers to separate. For solution (2) dilute 0.5 ml of solution (1) to 50 ml with *ether*.

After removal of the plate, allow it to dry in air for 10 minutes, return the plate to the tank and repeat the development. Remove the plate, allow it to dry in air for 10 minutes and spray with a 0.2% w/v solution of *2,7-dichlorofluorescein* in *methanol*. Allow the plate to stand for 5 minutes and spray with *water* until the background is white to pale yellow. Examine in daylight. The chromatograms show red or orange spots. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%). Examine without delay under *ultraviolet light* (*365 nm*). The chromatograms show spots with intense yellow fluorescence. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.5 g of Pethidine Hydrochloride in 40 ml of *water*, add 2 ml of 5M *sodium hydroxide* and extract immediately with quantities of 25, 10 and 10 ml of *chloroform*. Wash each extract with the same 15 ml of *water* and filter into a dry flask. Titrate the combined extracts, which should be clear and free from droplets of *water*, with 0.05M *perchloric acid VS* using 0.15 ml of *1-naphtholbenzein solution* as indicator. Each ml of 0.05M *perchloric acid VS* is equivalent to 14.19 mg of $C_{15}H_{21}NO_2$,HCl.

Phenelzine Tablets

General Notices

Action and use

Monoamine oxidase inhibitor; antidepressant.

DEFINITION

Phenelzine Tablets contain Phenelzine Sulphate. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenelzine, $C_8H_{12}N_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 30 mg of phenelzine with 25 ml of *water* for 5 minutes and filter. Make the filtrate alkaline with 5M *sodium hydroxide* and extract with two 25 ml quantities of *chloroform*. Extract the combined chloroform layers with two 25 ml quantities of 0.05M *sulphuric acid* and dilute the combined extracts to 100 ml with 0.05M *sulphuric acid*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits three well-defined maxima, at 252, 258 and 263 nm.

B. Extract a quantity of the powdered tablets containing the equivalent of 30 mg of phenelzine with 10 ml of *water* and filter. Make 5 ml of the filtrate alkaline with 5M *sodium hydroxide* and add 1 ml of *cupri-tartaric solution R1*. A red precipitate is produced.

C. Extract a quantity of the powdered tablets containing the equivalent of 45 mg of phenelzine with 5 ml of *water* and filter. The filtrate yields the reactions characteristic of *sulphates*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 0.1 g of phenelzine add 20 ml of *water* and 2 ml of 2M *hydrochloric acid*, heat to boiling and boil for 30 seconds, stirring constantly. Add 25 ml of *water*, cool, add 2 g of *sodium hydrogen carbonate* and 50 ml of 0.05M *iodine VS*, stopper the flask and allow to stand for 90 minutes. Add 15 ml of 2M *hydrochloric acid* and titrate with 0.1M *sodium thiosulphate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the powder. The difference between the titrations represents the amount of iodine required. Each ml of 0.05M *iodine VS* is equivalent to 3.405 mg of $C_8H_{12}N_2$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of phenelzine.

Phenindione Tablets

General Notices

Action and use

Oral anticoagulant (indanedione).

DEFINITION

Phenindione Tablets contain Phenindione.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenindione, $C_{15}H_{10}O_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.2 g of Phenindione with 50 ml of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of phenindione (*RS 268*).

B. Recrystallise the residue obtained in test A from *ethanol* (*96%*), dissolve 20 mg in 10 ml of *ethanol* (*96%*) with the aid of heat, cool, dilute to 250 ml with 0.1M *sodium hydroxide* and dilute 5 ml to 100 ml with 0.1M *sodium hydroxide*. The *absorbance* of the resulting solution at the maximum at 278 nm is about 0.54 and at 330 nm is about 0.16, Appendix II B.

TESTS

Related substances

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and as the mobile phase a solution containing 0.02% w/v of *butylated hydroxytoluene* in a mixture of 4 volumes of *glacial acetic acid*, 20 volumes of *ethyl acetate* and 80 volumes of *toluene*. Allow the solvent front to ascend 4 cm, remove the plate and dry it in a current of cold air for 1 minute. Without delay apply separately to the plate 10 µl of each of the following solutions and develop immediately. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Phenindione with 15 ml of *dichloromethane*, filter, evaporate the filtrate to dryness and dissolve the residue in 5 ml of *dichloromethane*. For solution (2) dilute 1 volume of solution (1) to 50 volumes with *dichloromethane*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *dichloromethane*. After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Uniformity of content

Tablets containing 50 mg or less of Phenindione comply with the requirements stated under Tablets using the following method of analysis. Place one tablet in 50 ml of 0.1M *sodium hydroxide*, dissolve the tablet completely by shaking gently, add a further 100 ml of 0.1M *sodium hydroxide* and shake for 1 hour. Dilute to 250 ml with 0.1M *sodium hydroxide*, filter and dilute a portion of the filtrate with sufficient 0.1M *sodium hydroxide* to produce a solution containing 4 µg of Phenindione per ml. Measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{15}H_{10}O_2$ taking 1310 as the value of A(1%, 1 cm) at the maximum at 278 nm.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 50 mg of Phenindione with 150 ml of 0.1M *sodium hydroxide* for 1 hour, add sufficient 0.1M *sodium hydroxide* to produce 250 ml, filter and dilute 5 ml of the filtrate to 250 ml with 0.1M *sodium hydroxide*. Measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{15}H_{10}O_2$ taking 1310 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Phenobarbital Elixir

General Notices

Phenobarbital Oral Solution

Action and use

Barbiturate.

DEFINITION

Phenobarbital Elixir is an *oral solution* containing 0.3% w/v of Phenobarbital in a suitable flavoured vehicle containing a sufficient volume of Ethanol (96 per cent) or of an appropriate Dilute Ethanol to give a final concentration of 38% v/v of ethanol.

The elixir complies with the requirements stated under Oral Liquids and with the following requirements.

Content of phenobarbital, $C_{12}H_{12}N_2O_3$

0.27 to 0.33% w/v.

IDENTIFICATION

The residue obtained in the Assay complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of phenobarbital (*RS 270*).

B. *Melting point,* about 175°, Appendix V A.

TESTS

Ethanol content

36 to 40% v/v, Appendix VIII F.

ASSAY

Extract 50 ml with three 50 ml quantities of *ether*, wash the combined ether extracts with 20 ml of *water*, discard the washings, extract the ether solution with a mixture of 5 ml of 2M *sodium hydroxide* and 25 ml of *water* and then with two 5 ml quantities of *water*. Acidify the combined aqueous extracts to *litmus paper* with 2M *hydrochloric acid*, extract with four 25 ml quantities of *ether*, wash the combined ether extracts with two 2 ml quantities of *water*, wash the combined aqueous washings with 10 ml of *ether*, add the ether washings to the combined ether extracts, evaporate the ether and dry the residue of phenobarbital to constant weight at 105°. Calculate the content of $C_{12}H_{12}N_2O_3$ in the elixir as a % w/v.

STORAGE

Phenobarbital Elixir should be protected from light.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Phenobarbital Injection

General Notices

Action and use

Barbiturate.

DEFINITION

Phenobarbital Injection is a sterile solution containing 20% w/v of Phenobarbital Sodium in a mixture of nine volumes of Propylene Glycol and one volume of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenobarbital sodium, $C_{12}H_{11}N_2NaO_3$

19.0 to 21.0% w/v.

IDENTIFICATION

To 5 ml add 15 ml of *water*, make slightly acidic with 1M *sulphuric acid* and filter. The residue, after washing with *water* and drying at 105°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the reference spectrum of phenobarbital (*RS 270*).

B. *Melting point*, about 175°, Appendix V A.

C. Dissolve 50 mg in 2 ml of a 0.2% w/v solution of *cobalt*(*II*) *acetate* in *methanol*, warm, add 50 mg of powdered *sodium tetraborate* and heat to boiling. A bluish violet colour is produced.

TESTS

Alkalinity

pH, 10.0 to 11.0, Appendix V L.

Weight per ml

1.090 to 1.100 g, Appendix V G.

ASSAY

To 2 g add 30 ml of *water* and 3 g of *sodium carbonate*, stir to dissolve and titrate with 0.1M *silver nitrate VS* until a distinct turbidity is observed when viewed against a black background, the solution being stirred vigorously throughout the titration. Each ml of 0.1M *silver nitrate VS* is equivalent to 25.42 mg of $C_{12}H_{11}N_2NaO_3$. Use the *weight per ml* of the injection to calculate the percentage w/v of phenobarbital sodium, $C_{12}H_{11}N_2NaO_3$.

Phenobarbital Sodium Tablets

General Notices

Action and use

Barbiturate.

DEFINITION

Phenobarbital Sodium Tablets contain Phenobarbital Sodium.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenobarbital sodium, $C_{12}H_{11}N_2NaO_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Heat 0.2 g of the residue obtained in the Assay on a water bath with 15 ml of *ethanol* (*25%*) until dissolved, filter while hot and allow to cool. Filter through a sintered-glass crucible, wash with a small quantity of *ethanol* (*25%*) and dry at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of phenobarbital (*RS 270*).

B. The powdered tablets yield the reactions characteristic of *sodium salts*, Appendix VI.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets containing 0.3 g of Phenobarbital Sodium as completely as possible in 10 ml of a 2% w/v solution of *sodium hydroxide*, saturate with *sodium chloride*, acidify with *hydrochloric acid* and extract with successive 15 ml quantities of *ether* until complete extraction is effected. Wash the combined extracts with two 2 ml quantities of *water* and extract the combined washings with 10 ml of *ether*. Add the ether to the main ether layer, filter and wash the filter with *ether*. Evaporate the solvent and dry the residue of phenobarbital to constant weight at 105°. Each g of residue is equivalent to 1.095 g of $C_{12}H_{11}N_2NaO_3$. Calculate the quantity of $C_{12}H_{11}N_2NaO_3$ in the tablets as a percentage of the stated amount.

Phenobarbital Tablets

General Notices

Action and use

Barbiturate.

DEFINITION

Phenobarbital Tablets contain Phenobarbital.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenobarbital, $C_{12}H_{12}N_2O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Heat 0.2 g of the residue obtained in the Assay on a water bath with 15 ml of *ethanol (25%)* until dissolved, filter while hot and allow the filtrate to cool. Filter, wash the crystals with a small quantity of *ethanol (25%)* and dry at 105°. The residue complies with the following tests.

A. *Melting point*, about 175°, Appendix V A.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of phenobarbital (*RS 270*). If the spectra obtained are not concordant, heat the residue in a sealed tube at 105° for 1 hour and prepare a new spectrum of the residue.

C. Dissolve 50 mg in 2 ml of a 0.2% w/v solution of *cobalt(II) acetate* in *methanol*, warm, add 50 mg of powdered *sodium tetraborate* and heat to boiling. A bluish violet colour is produced.

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A1.

ASSAY

Weigh and powder 20 tablets. Extract a quantity of the powder containing 0.3 g of Phenobarbital in a continuous extraction apparatus with *ether* until complete extraction is effected. Remove the ether and dry the residue of phenobarbital to constant weight at 105°. Calculate the content of $C_{12}H_{12}N_2O_3$ in the tablets as a percentage of the stated amount.

Phenol and Glycerol Injection

General Notices

DEFINITION

Phenol and Glycerol Injection is a sterile solution containing 5% w/v of Phenol in Glycerol that has been previously dried at 120° for 1 hour.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenol, C_6H_6O

4.75 to 5.25% w/v.

CHARACTERISTICS

A pale straw-coloured, viscous solution.

IDENTIFICATION

A. Add *bromine water* to a 1% w/v solution. A white precipitate is produced, which, on the continued addition of *bromine water*, at first dissolves then reappears and becomes permanent.

B. To 0.5 ml add 5 ml of *water* and 0.05 ml of *sodium nitrite solution* and carefully pour on to the surface of *sulphuric acid*. A coloured zone, red above and green below, appears at the junction of the two layers.

C. When heated on a borax bead in a naked flame, it imparts a green colour to the flame.

ASSAY

Dissolve 2 g in sufficient *water* to produce 50 ml, transfer 25 ml to a 500 ml glass-stoppered flask and add 50 ml of 0.05M *bromine VS* and 5 ml of *hydrochloric acid*, stopper, swirl occasionally during 30 minutes and allow to stand for a further 15 minutes. Add 5 ml of a 20% w/v solution of *potassium iodide*, taking care to avoid loss of bromine, shake thoroughly and titrate with 0.1M *sodium thiosulphate VS* until only a faint yellow colour remains. Add 0.1 ml of *starch mucilage* and 10 ml of *chloroform* and complete the titration with vigorous shaking. Repeat the operation without the injection. The difference between the titrations represents the amount of bromine required. Each ml of 0.05M *bromine VS* is equivalent to 1.569 mg of C_6H_6O. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage w/v of C_6H_6O.

STORAGE

Phenol and Glycerol Injection should be protected from light.

LABELLING

The strength is stated as the percentage w/v of Phenol.

Phenoxybenzamine Capsules

General Notices

Action and use

Alpha-adrenoceptor antagonist.

DEFINITION

Phenoxybenzamine Capsules contain Phenoxybenzamine Hydrochloride.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of phenoxybenzamine hydrochloride, $C_{18}H_{22}ClNO,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the contents of the capsules containing 40 mg of Phenoxybenzamine Hydrochloride in 50 ml of *ethanol-free chloroform*, wash the solution with three 20 ml quantities of 0.01M *hydrochloric acid*, filter the chloroform solution and dilute 10 ml of the filtrate to 50 ml with *ethanol-free chloroform*. The *light absorption* of the resulting solution, Appendix II B, exhibits two maxima, at 272 nm and 279 nm.

B. The contents of the capsules yield reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Weigh 20 capsules. Open the capsules carefully without loss of shell material, remove the contents, wash the shells with three 10 ml quantities of *chloroform* and add the washings to the capsule contents. Allow the shells to dry at room temperature to constant weight. The difference between the weights represents the weight of the total contents. Evaporate the mixed capsule contents and washings to dryness, stirring continuously, and carry out Method I for *non-aqueous titration*, Appendix VIII A, adding 10 ml of *mercury(II) acetate solution* and using *oracet blue B solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 34.03 mg of $C_{18}H_{22}ClNO,HCl$.

Phenoxymethylpenicillin Oral Solution

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Phenoxymethylpenicillin Oral Solution is a solution of Phenoxymethylpenicillin Potassium in a suitable flavoured vehicle. It is prepared by dissolving the dry ingredients in the specified volume of Water just before issue for use.

The dry ingredients comply with the requirements for Powders and Granules for Oral Solutions and Suspensions stated under Oral Liquids.

For the following tests prepare the Oral Solution as directed on the label. The solution, examined immediately after preparation unless otherwise indicated, complies with the requirements stated under Oral Liquids and with the following requirements.

Content of phenoxymethylpenicillin, $C_{16}H_{18}N_20_5S$, calculated as the sum of the contents of phenoxymethylpenicillin and 4-hydroxyphenoxymethylpenicillin

When freshly constituted, not more than 120.0% of the stated amount of phenoxymethylpenicillin. When stored at the temperature and for the period stated on the label during which the Oral Solution may be expected to be satisfactory for use, not less than 80.0% of the stated amount of phenoxymethylpenicillin.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable) and a mixture of 20 volumes of *acetone* and 80 volumes of *mixed phosphate buffer pH 5.4* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) dilute a volume of the oral solution containing the equivalent of 0.1 g of phenoxymethylpenicillin to 100 ml with *mixed phosphate buffer pH 5.4*. Solution (2) contains 0.11% w/v of *phenoxymethylpenicillin potassium BPCRS* in *mixed phosphate buffer pH 5.4*. After removal of the plate, allow it to dry in air, spray with 0.1M *sodium hydroxide*, heat at 50° for 10 minutes, allow to cool and spray with a mixture of 100 volumes of *starch mucilage*, 6 volumes of *glacial acetic acid* and 2 volumes of a 1% w/v solution of *iodine* in a 4% w/v solution of *potassium iodide*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dilute a volume containing the equivalent of 25 mg of phenoxymethylpenicillin to 20 ml with *water*. To 10 ml add 0.5 ml of *neutral red solution* and sufficient 0.01M *sodium hydroxide* to produce a permanent orange colour and then add 1.0 ml of *penicillinase solution*. The colour changes rapidly to red.

ASSAY

To a weighed quantity of the oral solution containing the equivalent of 0.125 g of phenoxymethylpenicillin add sufficient *water* to produce 500 ml and dilute 25 ml to 100 ml with *water*. Place two 2 ml aliquots of the resulting solution into separate stoppered tubes. To one tube add 10 ml of *imidazole-mercury reagent*, mix, stopper the tube and immerse in a water bath at 60° for exactly 25 minutes, swirling occasionally. Remove from the water bath and cool rapidly to 20° (solution A). To the second tube add 10 ml of *water* and mix (solution B). Without delay measure the *absorbances* of solutions A and B at the maximum at 325 nm, Appendix II B, using in the reference cell a mixture of 2 ml of *water* and 10 ml of *imidazole-mercury reagent* for solution A and *water* for solution B. Calculate the content of $C_{16}H_{18}N_2O_5S$ from the difference between the absorbances of solutions A and B, from the difference obtained by repeating the operation using 0.14 g of *phenoxymethylpenicillin potassium BPCRS* in place of the oral solution and from the declared content of $C_{16}H_{18}N_2O_5S$ in *phenoxymethylpenicillin potassium BPCRS*. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{16}H_{18}N_2O_5S$, weight in volume.

Repeat the procedure using a portion of the oral solution that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

STORAGE

The Oral Solution should be stored at the temperature and used within the period stated on the label.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of phenoxymethylpenicillin.

Phenoxymethylpenicillin Tablets

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Phenoxymethylpenicillin Tablets contain Phenoxymethylpenicillin Potassium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenoxymethylpenicillin, $C_{16}H_{18}N_20_5S$, calculated as the sum of the contents of phenoxymethylpenicillin and 4-hydroxyphenoxymethylpenicillin

92.5 to 107.5% of the stated amount of phenoxymethylpenicillin.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 80 mg of phenoxymethylpenicillin with *water*, dilute to 250 ml with *water* and filter. The *light absorption* of the filtrate, Appendix II B, exhibits maxima at 268 nm and 274 nm and a minimum at 272 nm.

B. Shake a quantity of the powdered tablets containing the equivalent of 10 mg of phenoxymethylpenicillin with 10 ml of *water*, filter and add 0.5 ml of *neutral red solution*. Add sufficient 0.01M *sodium hydroxide* to produce a permanent orange colour and then add 1.0 ml of *penicillinase solution*. The colour changes rapidly to red.

C. Ignite 0.5 g of the powdered tablets, add 5 ml of 2M *hydrochloric acid*, boil, cool and filter. The filtrate yields reaction B characteristic of *potassium salts*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 6.8 by the addition of 1M *sodium hydroxide* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 268 nm, Appendix II B. At the same time measure the *absorbance* at the maximum at 268 nm of a suitable solution of *phenoxymethylpenicillin potassium BPCRS* in a solution containing 0.68% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 6.8 by the addition of 1M *sodium hydroxide*. Calculate the total content of phenoxymethylpenicillin, $C_{16}H_{18}N_2O_5S$, in the medium from the absorbances obtained and from the declared content of $C_{16}H_{18}N_2O_5S$ in *phenoxymethylpenicillin potassium BPCRS*.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 0.125 g of phenoxymethylpenicillin with 300 ml of *water* for 30 minutes, add sufficient *water* to produce 500 ml, dilute 25 ml to 100 ml with *water* and filter. Place two 2 ml aliquots of the filtrate in separate stoppered tubes. To one tube add 10 ml of *imidazole-mercury reagent*, mix, stopper the tube and immerse in a water bath at 60° for exactly 25 minutes, swirling occasionally. Remove from the water bath and cool rapidly to 20° (solution A). To the second tube add 10 ml of *water* and mix (solution B). Without delay measure the *absorbances* of solutions A and B at the maximum at 325 nm, Appendix II B, using in the reference cell a mixture of 2 ml of *water* and 10 ml of *imidazole-mercury reagent* for solution A and *water* for solution B. Calculate the content of $C_{16}H_{18}N_2O_5S$ from the difference between the absorbances of solutions A and B, from the difference obtained by repeating the operation using 0.14 g of *phenoxymethylpenicillin potassium BPCRS* in place of the preparation being examined and from the declared content of $C_{16}H_{18}N_2O_5S$ in *phenoxymethylpenicillin potassium BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of phenoxymethyl-penicillin.

Phentolamine Injection

General Notices

Action and use

Alpha-adrenoceptor antagonist.

DEFINITION

Phentolamine Injection is a sterile solution of Phentolamine Mesilate in Water for Injections containing Anhydrous Glucose.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phentolamine mesilate, $C_{17}H_{19}N_3O,CH_4SO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Acidity

pH, 3.5 to 5.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 5 volumes of 13.5M *ammonia*, 15 volumes of *acetone* and 85 volumes of *butan-2-one* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) dilute the injection, if necessary, with *ethanol* (*96%*) to give a solution containing 1% w/v of Phentolamine Mesilate. For solution (2) dilute 1 volume of solution (1) to 20 volumes with *ethanol* (*96%*). For solution (3) dilute 1 volume of solution (1) to 200 volumes with *ethanol* (*96%*). Solution (4) contains 0.05% w/v of *phentolamine mesilate BPCRS* in *ethanol* (*96%*). After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Dilute a volume of the injection with *methanol* to produce a solution containing of 0.004% w/v of Phentolamine Mesilate. Measure the *absorbance* at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{17}H_{19}N_3O,CH_4O_3S$ taking 204 as the value of A(1%, 1 cm) at the maximum at 278 nm.

STORAGE

Phentolamine Injection should be protected from light.

Phenylephrine Eye Drops

General Notices

Action and use

Alpha-adrenoceptor agonist.

DEFINITION

Phenylephrine Eye Drops are a sterile solution of Phenylephrine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of phenylephrine hydrochloride $C_9H_{13}NO_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

B. To a volume containing 10 mg of Phenylephrine Hydrochloride diluted with *water* if necessary to produce 1 ml add 0.05 ml of *copper sulphate solution* and 1 ml of 5M *sodium hydroxide*; a violet colour is produced. Add 1 ml of *ether* and shake; the ether layer remains colourless.

TESTS

Acidity or alkalinity

pH, 3.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 5 volumes of *chloroform,* 15 volumes of 10M *ammonia* and 80 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute the eye drops with sufficient *methanol* to give a solution containing 2% w/v of Phenylephrine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 40 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 100 volumes with *methanol*. Solution (4) contains 0.05% w/v of *phenylephrine hydrochloride BPCRS* in *methanol*. After removal of the plate, dry it in a current of cold air, spray with *ninhydrin solution*, heat at 100° to 105° for 5 to 10 minutes and examine in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. Solution (1) contains 0.1% w/v of *phenylephrine hydrochloride BPCRS* in *water*. For solution (2) dilute the eye drops with sufficient *water* to give a solution containing 0.1% w/v of Phenylephrine Hydrochloride.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil C18 is suitable) (b) a solution of 0.10% w/v *sodium heptanesulphonate* in a mixture of 2 volumes of *glacial acetic acid,* 400 volumes of *methanol* and 600 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 273 nm.

Calculate the content of $C_9H_{13}NO_2,HCl$ using the declared content of $C_9H_{13}NO_2,HCl$ in *phenylephrine hydrochloride BPCRS*.

STORAGE

Phenylephrine Eye Drops should be protected from light.

Phenylephrine Injection

General Notices

Action and use

Alpha-adrenoceptor agonist.

DEFINITION

Phenylephrine Injection is a sterile solution of Phenylephrine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenylephrine hydrochloride, $C_9H_{13}NO_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

B. To a volume containing 10 mg of Phenylephrine Hydrochloride add, if necessary, sufficient *water* to produce 1 ml and then add 0.05 ml of *weak copper sulphate solution* and 1 ml of 5M *sodium hydroxide*; a violet colour is produced. Add 1 ml of *ether* and shake; the ether layer remains colourless.

C. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 4.5 to 6.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of 5 volumes of *chloroform*, 15 volumes of 10M *ammonia* and 80 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) evaporate a volume of the injection containing 20 mg of Phenylephrine Hydrochloride to dryness and dissolve the residue in 1 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) to 2.5 volumes with *methanol*. Solution (4) contains 0.01% w/v of *phenylephrine hydrochloride BPCRS* in *methanol*. After removal of the plate, dry it in a current of cold air, spray with *ninhydrin solution*, heat at 100° to 105° for 10 minutes and examine in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

To a quantity containing 50 mg of Phenylephrine Hydrochloride add sufficient 0.5M *sulphuric acid* to produce 100 ml. Dilute 10 ml to 100 ml with 0.5M *sulphuric acid* and measure the *absorbance* of the resulting solution at the maximum at 273 nm, Appendix II B. Calculate the content of $C_9H_{13}NO_2,HCl$ taking 90 as the value of A(1%, 1 cm) at the maximum at 273 nm.

STORAGE

Phenylephrine Injection should be protected from light.

Phenytoin Capsules

General Notices

Action and use

Antiepileptic.

DEFINITION

Phenytoin Capsules contain Phenytoin Sodium.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Centrifuge the precipitated mixture obtained in test B, dissolve the residue in *methanol*, evaporate and dry at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of phenytoin (*RS 272*).

B. Shake a quantity of the contents of the capsules containing 0.5 g of Phenytoin Sodium with 10 ml of *water* and filter. The filtrate yields a white precipitate on the addition of 2M *hydrochloric acid*.

C. To 0.1 g of the residue obtained in test A add 0.5 ml of 1M *sodium hydroxide*, 10 ml of a 10% w/v solution of *pyridine* and 1 ml of *copper sulphate-pyridine reagent* and allow to stand for 10 minutes. A blue precipitate is produced.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using as the coating substance a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (Merck silica gel 60 F_{254} is suitable) and a mixture of 10 volumes of 13.5M *ammonia,* 45 volumes of *chloroform* and 45 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.20 g of Phenytoin Sodium with 5 ml of *methanol*, warm on a water bath with shaking and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.020% w/v of *benzophenone* in *methanol*. Allow the plate to dry in a current of cold air for 2 minutes before development of the chromatograms. After removal of the plate, dry it at 80° for 5 minutes and examine under *ultraviolet light* (*254 nm*). In the chromatogram obtained with solution (1) any spot corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing 0.25 g of Phenytoin Sodium with 40 ml of 0.01M *sodium hydroxide* for 5 minutes and dilute to 50 ml with 0.01M *sodium hydroxide*. Centrifuge, acidify 25 ml of the clear liquid with 10 ml of 0.1M *hydrochloric acid* and extract with successive quantities of 50, 40 and 25 ml of *ether*. Wash the combined extracts with 10 ml of *water*, evaporate to dryness and dry the residue at 105°. Dissolve in 50 ml of *anhydrous pyridine* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and a 0.3% w/v solution of *thymol blue* in *anhydrous pyridine* as indicator. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 27.43 mg of $C_{15}H_{11}N_2NaO_2$.

Phenytoin Injection

General Notices

Action and use

Antiepileptic.

DEFINITION

Phenytoin Injection is a sterile solution containing 5% w/v of Phenytoin Sodium in a mixture of 40% v/v of Propylene Glycol and 10% v/v of Ethanol in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$

4.75 to 5.25% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, of the residue obtained in the Assay for phenytoin sodium is concordant with the *reference spectrum* of phenytoin (*RS 272*).

B. Mix 0.2 g of the residue obtained in the Assay for phenytoin sodium with 1.4 g of *chlorotriphenylmethane* and 2 ml of *pyridine* and heat under a reflux condenser on a water bath for 1 hour. Cool, dissolve the mixture in 40 ml of warm *acetone*, add 50 mg of *activated charcoal*, mix well and filter. Evaporate the filtrate to about 20 ml and allow to stand overnight at about 4°. The *melting point* of the crystalline precipitate, after drying in a current of air, is about 176°, Appendix V A.

TESTS

Alkalinity

pH, 11.5 to 12.1, Appendix V L.

Weight per ml

1.025 to 1.035 g, Appendix V G.

Benzil and benzophenone

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 30 volumes of *1,4-dioxan* and 75 volumes of *hexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute 1 ml of the injection to 2.5 ml with *methanol*. Solution (2) contains 0.010% w/v of *benzophenone* in *ethanol* (*96%*). Solution (3) contains 0.010% w/v of *benzil* in *ethanol* (*96%*) . After removal of the plate, allow the solvent to evaporate and examine under *ultraviolet light* (*254 nm*). In the chromatogram obtained with solution (1) any spots corresponding to benzophenone and benzil are not more intense than the spots in the chromatograms obtained with solutions (2) and (3) respectively (0.5% of each).

Ethanol

9.0 to 11.0% v/v, when determined by the following method. Carry out the method for *gas chromatography*, Appendix III B, using three solutions containing (1) 2% v/v of *propan-1-ol* (internal standard) and 2% v/v of *absolute ethanol*, (2) the injection diluted with *water* to contain 2% v/v of Ethanol and (3) the injection diluted with a solution of the internal standard to contain 2% v/v of Ethanol and 2% v/v of propan-1-ol.

The chromatographic procedure may be carried out using a column (1.8 m × 2 mm) packed with porous polymer beads (Chromosorb 102 is suitable) (80 to 100 mesh) and maintained at 120°.

Propylene glycol

37.0 to 43.0% v/v, when determined by the following method. Carry out the method for *gas chromatography*, Appendix III B, using solutions containing (1) 4% w/v of *ethane-1,2-diol* (internal standard) and 4% w/v of *propane-1,2-diol*, (2) the injection diluted with *water* to contain 4% w/v of Propylene Glycol and (3) the injection diluted with a solution of the internal standard to contain 4% w/v of Propylene Glycol and 4% w/v of the ethane-1,2-diol.

The chromatographic procedure may be carried out using the conditions described under Ethanol but maintaining the column at 175°.

Calculate the content of $C_3H_8O_2$, volume in volume, taking 1.036 g as its weight per ml.

ASSAY

To 6 g add 25 ml of *water* and extract with successive quantities of 50, 30 and 30 ml of *ethyl acetate*, shaking each extract thoroughly for 1 minute, allowing to stand for 5 minutes and again shaking for 1 minute before separating the solvent. Wash each extract by shaking thoroughly for 1 minute with the same 15 ml of *water*, filter the extracts successively and wash the filter with 10 ml of *ethyl acetate*. Evaporate the solvent from the combined filtrates and washing and dry the residue to constant weight at 105°. Each g of residue is equivalent to 1.087 g of $C_{15}H_{11}N_2NaO_2$. Using the *weight per ml* of the injection, calculate the content of $C_{15}H_{11}N_2NaO_2$, weight in volume.

STORAGE

Phenytoin Injection should be protected from light. Solutions in which a haziness or precipitate develops should not be used.

Phenytoin Oral Suspension

General Notices

Action and use

Antiepileptic.

DEFINITION

Phenytoin Oral Suspension is a suspension of Phenytoin in a suitable flavoured vehicle.
The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of phenytoin, $C_{15}H_{12}N_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, of the final residue obtained in the Assay is concordant with the *reference spectrum* of phenytoin (*RS 272*).

TESTS

Acidity

pH, 4.5 to 5.5, Appendix V L.

Benzil and benzophenone

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 30 volumes of *1,4-dioxan* and 75 volumes of *hexane* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) add 5 ml of *water* and 2 ml of 2M *hydrochloric acid* to a quantity of the oral suspension containing 30 mg of Phenytoin, mix well and extract with five 20 ml quantities of *ether*. Combine the ether extracts, wash with three 10 ml quantities of *water*, evaporate to dryness and dissolve the residue in 1.5 ml of a mixture of 1 volume of *glacial acetic acid* and 9 volumes of *acetone*. Solution (2) contains 0.0040% w/v of *benzophenone* in *ethanol* (*96%*). Solution (3) contains 0.0040% w/v of *benzil* in *ethanol* (*96%*). After removal of the plate, allow the solvent to evaporate and examine under *ultraviolet light* (*254 nm*). Any spots corresponding to benzophenone and benzil in the chromatogram obtained with solution (1) are not more intense than the spots in the chromatograms obtained with solutions (2) and (3) respectively (0.2% of each).

ASSAY

To a weighed quantity containing 0.2 g of Phenytoin add 10 ml of 2M *hydrochloric acid* and 15 ml of *water*, extract with three 50 ml quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *propan-2-ol* and evaporate the combined extracts to dryness. Dry the residue at

105° for 1 hour, cool and dissolve in a mixture of 3 ml of 1M *sodium hydroxide* and 50 ml of *water* with the aid of gentle heat. Cool and pass a current of *carbon dioxide* through the solution until precipitation of phenytoin is complete. Filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable), wash the residue with two 10 ml quantities of *water* and dry at 105° for 2 hours. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{12}N_2O_2$, weight in volume.

Phenytoin Tablets

General Notices

Action and use

Antiepileptic.

DEFINITION

Phenytoin Tablets contain Phenytoin Sodium. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Phenytoin Sodium with 20 ml of *water*, filter, acidify with 2M *hydrochloric acid* and extract with *chloroform*. Wash the chloroform extract with *water*, dry with *anhydrous sodium sulphate* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of phenytoin (*RS 272*).

B. Triturate a quantity of the powdered tablets containing 0.5 g of Phenytoin Sodium with 10 ml of *water* and filter. Add 2M *hydrochloric acid*; a white precipitate is produced.

C. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, impart a yellow colour to the flame.

Related substances

Complies with the test described under Phenytoin Capsules but using a quantity of the powdered tablets containing 0.2 g of Phenytoin Sodium to prepare solution (1).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.25 g of Phenytoin Sodium with 40 ml of 0.01M *sodium hydroxide* for 5 minutes and dilute to 50 ml with 0.01M *sodium hydroxide*. Centrifuge, acidify 25 ml of the clear liquid with 10 ml of 0.1M *hydrochloric acid* and extract with successive quantities of 50, 40 and 25 ml of *ether*. Wash the combined extracts with 10 ml of *water*, evaporate to dryness and dry the residue at 105°. Dissolve the residue in 50 ml of *anhydrous pyridine* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and a 0.3% w/v solution of *thymol blue* in *pyridine* as indicator. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 27.43 mg of $C_{15}H_{11}N_2NaO_2$.

Pholcodine Linctus

General Notices

Action and use

Opioid receptor agonist; cough suppressant.

DEFINITION

Pholcodine Linctus is an *oral solution* containing 0.1% w/v of Pholcodine and 1% w/v of Citric Acid Monohydrate in a suitable flavoured vehicle.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of pholcodine, $C_{23}H_{30}N_2O_4,H_2O$

0.090 to 0.110% w/v.

IDENTIFICATION

To 20 ml add 20 ml of *water*, make alkaline to *litmus paper* with 5M *ammonia*, extract with two 20 ml quantities of *chloroform*, washing each extract with 5 ml of *water*, dry the combined extracts with *anhydrous sodium sulphate*, filter and evaporate to dryness. If necessary add 0.1 ml of *ether* and scratch the side of the vessel with a glass rod to induce crystallisation. The crystals, dried at a pressure not exceeding 2 kPa, comply with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of pholcodine (*RS 273*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.01% w/v solution in 0.01M *sodium hydroxide* exhibits a maximum only at 284 nm.

C. Dissolve the remainder of the crystals in 1 ml of *sulphuric acid* and add 0.05 ml of *ammonium molybdate-sulphuric acid solution*; a pale blue colour is produced. Warm gently; the colour changes to deep blue. Add 0.05 ml of 2M *nitric acid*; the colour changes to brownish red.

ASSAY

To 50 g add sufficient 5M *ammonia* to make the solution alkaline to *litmus paper* and extract with four 25-ml quantities of *chloroform*, washing each extract with the same 5 ml of *water*. Combine the extracts and evaporate until the volume is reduced to 15 ml. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.02M *perchloric acid VS* as titrant and *quinaldine red solution* as indicator. Each ml of 0.02M *perchloric acid VS* is equivalent to 4.165 mg of $C_{23}H_{30}N_2O_4,H_2O$. Determine the *weight per ml* of the linctus, Appendix V G, and calculate the content of $C_{23}H_{30}N_2O_4,H_2O$, weight in volume.

STORAGE

Pholcodine Linctus should be protected from light.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Phosphates Enema

General Notices

Sodium Phosphates Enema

DEFINITION

Phosphates Enema is a *rectal solution* containing suitable quantities of either Anhydrous Sodium Dihydrogen Phosphate, Sodium Dihydrogen Phosphate Monohydrate, Sodium Dihydrogen Phosphate Dihydrate or Phosphoric Acid and either Anhydrous Disodium Hydrogen Phosphate, Disodium Hydrogen Phosphate Dodecahydrate or Disodium Hydrogen Phosphate Dihydrate in freshly boiled and cooled Purified Water. A suitable antimicrobial preservative may be included.

The composition of two established formulations is described below.

Formula A

Sodium Dihydrogen Phosphate Dihydrate	160 g
Disodium Hydrogen Phosphate Dodecahydrate	60 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

A suitable antimicrobial preservative may be included.

Formula B

Sodium Dihydrogen Phosphate Dihydrate	100 g
Disodium Hydrogen Phosphate Dodecahydrate	80 g
Purified Water, freshly boiled and cooled	Sufficient to produce 1000 ml

A suitable antimicrobial preservative may be included.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of sodium dihydrogen phosphate dihydrate, $NaH_2PO_4,2H_2O$

Formula A: 15.2 to 16.8% w/v; *Formula B*: 9.5 to 10.5% w/v; *any other formulation*: 95.0 to 105.0% of the stated amount.

Content of disodium hydrogen phosphate dodecahydrate, $Na_2HPO_4,12H_2O$

Formula A: 5.6 to 6.4% w/v; *Formula B*: 7.5 to 8.5% w/v; *any other formulation*: 95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Yields the reactions characteristic of *sodium salts*, Appendix VI.

B. Yields the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Clarity and colour of solution

The enema being examined is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Acidity

pH, 5.0 to 6.2, Appendix V L.

ASSAY

For sodium dihydrogen phosphate dihydrate

To 20 ml add 80 ml of *water* and 25 g of *sodium chloride* and titrate with 0.5M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 78.00 mg of $NaH_2PO_4,2H_2O$.

For disodium hydrogen phosphate dodecahydrate

Titrate 50 ml with 0.5M *hydrochloric acid VS* using 2 ml of a mixture of 4 volumes of *bromocresol green solution* and 1 volume of *methyl red solution* as indicator and titrating to the colour indicative of pH 4.4. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 179.0 mg of $Na_2HPO_4,12H_2O$.

LABELLING

The label states (1) where appropriate, that the enema is Formula A or Formula B; (2) for any formulation other than Formula A or B, the content in terms of the amount or equivalent of Sodium Dihydrogen Phosphate Dihydrate and Disodium Hydrogen Phosphate Dodecahydrate; (3) the date after which the enema is not intended to be used; (4) the conditions under which it should be stored.

Phytomenadione Injection

General Notices

Action and use

Vitamin K analogue.

DEFINITION

Phytomenadione Injection is a sterile preparation of Phytomenadione in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of phytomenadione, $C_{31}H_{46}O_2$

90.0 to 115.0% of the stated amount.

CHARACTERISTICS

A clear to slightly opalescent liquid when maintained at a temperature below 25°.

IDENTIFICATION

A. Dilute with *absolute ethanol* to produce a solution containing 0.01% w/v of Phytomenadione. The *light absorption* of the solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 328 nm and a minimum at 292 nm.

B. Dilute a quantity of the solution used in test A with *absolute ethanol* to produce a solution containing 0.001% w/v of Phytomenadione. The *light absorption* of the solution, Appendix II B, in the range 230 to 350 nm exhibits maxima at 245, 249, 263 and 271 nm and minima at 256 nm and 266 nm.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.5, Appendix V L.

ASSAY

Carry out in subdued light the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.01% w/v of *phytomenadione EPCRS* in the mobile phase. For solution (2) dilute a volume of the injection containing 10 mg of Phytomenadione with sufficient of the mobile phase to produce 100 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 95 volumes of *ethanol* (*96%*) and 5 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{31}H_{46}O_2$ using the declared content of $C_{31}H_{46}O_2$ in *phytomenadione*

EPCRS.

STORAGE

Phytomenadione Injection deteriorates on exposure to light and should be stored in the dark. It should not be allowed to freeze.

LABELLING

The label states that the injection should not be used if separation has occurred or if oil droplets have appeared.

When vitamin K_1 injection is prescribed or demanded, Phytomenadione Injection shall be dispensed or supplied.

Phytomenadione Tablets

General Notices

Action and use

Vitamin K analogue.

DEFINITION

Phytomenadione Tablets contain Phytomenadione.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of phytomenadione, $C_{31}H_{46}O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Phytomenadione with 50 ml of *absolute ethanol* for 1 hour, allow to stand and dilute 5 ml of the clear supernatant liquid to 50 ml with *absolute ethanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 328 nm and a minimum at 292 nm. Dilute a suitable volume of the solution with sufficient *absolute ethanol* to produce a solution containing 0.001% w/v of Phytomenadione. The *light absorption* of this solution, in the range 230 to 350 nm, exhibits maxima at 245, 249, 263 and 271 nm and minima at 256 nm and 266 nm.

TESTS

Disintegration

The requirement for Disintegration does not apply to Phytomenadione Tablets.

Menadione

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 1 volume of *methanol*, 20 volumes of *ether* and 80 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 50 mg of Phytomenadione in 5 ml of *absolute ethanol* with the aid of ultrasound for 5 minutes, add 15 ml of *2,2,4-trimethylpentane*, shake for 1 minute, centrifuge and use the supernatant liquid. Solution (2) contains 0.0025% w/v of *menadione* in *2,4,4-trimethylpentane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* corresponding to menadione in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Carry out in subdued light the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.01% w/v of *phytomenadione EPCRS* in the mobile phase. For solution (2) add 5 ml of 0.5M *ammonia* to a quantity of the powdered tablets containing 10 mg of Phytomenadione and mix with the aid of ultrasound for 5 minutes. Add 90 ml of *ethanol* (*96%*) and mix with the aid of ultrasound for 10 minutes. Shake for 10 minutes, add sufficient *ethanol* (*96%*) to produce 100 ml, centrifuge and use the clear supernatant layer.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 5 volumes of *water* and 95 volumes of *ethanol* (*96%*) as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{31}H_{46}O_2$ using the declared content of $C_{31}H_{46}O_2$ in *phytomenadione EPCRS*.

LABELLING

The label states that the tablets should be chewed before swallowing or allowed to dissolve slowly in the mouth.

When vitamin K_1 tablets are prescribed or demanded, Phytomenadione Tablets shall be dispensed or supplied.

Pilocarpine Hydrochloride Eye Drops

General Notices

Action and use

Cholinoceptor agonist; treatment of glaucoma.

DEFINITION

Pilocarpine Hydrochloride Eye Drops are a sterile solution of Pilocarpine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of pilocarpine hydrochloride, $C_{11}H_{16}N_2O_2$,HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a volume containing 10 mg of Pilocarpine Hydrochloride add 0.1 ml of 1M *sulphuric acid* , 1 ml of *hydrogen peroxide solution* (*20 vol*), 1 ml of *toluene* and 0.05 ml of *potassium chromate solution*, shake well and allow to separate. The toluene layer is bluish violet and the aqueous layer remains yellow.

Pilocarpic acid

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of each of the following solutions. For solution (1) dilute a suitable volume with *water* to produce a solution containing 0.080% w/v of Pilocarpine Hydrochloride. For solution (2) dilute 2 volumes of solution (1) to 25 volumes with *water*. For solution (3) dissolve 8.0 mg of *pilocarpine nitrate BPCRS* in 10 ml of 0.0025M *sodium hydroxide*, heat in a boiling water bath for 30 minutes and cool.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase a mixture of 55 volumes of *methanol*, 60 volumes of *acetonitrile* and 885 volumes of 0.002M *tetrabutylammonium dihydrogen orthophosphate*, the pH of the mixture being adjusted to 7.75 using 6M *ammonia*, with a flow rate of 1.2 ml per minute and (c) a detection wavelength of 220 nm. Record the chromatogram of solution (1) for twice the retention time of the main peak.

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *pilocarpine nitrate BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak corresponding to pilocarpic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (8%).

ASSAY

Use Method A for eye drops that contain less than 1% w/v of Pilocarpine Hydrochloride and in which benzalkonium chloride is used as an antimicrobial preservative. For other eye drops use Method B.

A. To 2 ml in a stoppered flask add 5 ml of *mixed phosphate buffer pH 10*, 5 ml of *chloroform* and 0.1 ml of *bromophenol blue solution*. Titrate slowly with 0.01M *sodium tetraphenylborate VS*, shaking well between successive small additions, until the chloroform layer becomes colourless. Using a further 2 ml of the eye drops, carry out the procedure described under Method B beginning at the words 'add 5 ml of *acetate buffer pH 3.7* ...'. The difference between the titrations at pH 3.7 and pH 10 represents the amount of sodium tetraphenylborate required.

B. Dilute if necessary with *water* to produce a solution containing 1% w/v of Pilocarpine Hydrochloride. To 2 ml add 5 ml of *acetate buffer pH 3.7* and 15 ml of 0.01M *sodium tetraphenylborate VS*, mix and allow to stand for 10 minutes. Filter, wash the container and filter with 5 ml of *water*, combine the filtrate and washings and titrate with 0.005M *cetylpyridinium chloride VS* using 0.5 ml of *bromophenol blue solution* as indicator. Repeat the operation without the preparation being examined. The difference between the titrations represents the amount of sodium tetraphenylborate required.

Each ml of 0.01M *sodium tetraphenylborate VS* is equivalent to 2.447 mg of $C_{11}H_{16}N_2O_2$,HCl.

Pilocarpine Nitrate Eye Drops

General Notices

Action and use

Cholinoceptor agonist; treatment of glaucoma.

DEFINITION

Pilocarpine Nitrate Eye Drops are a sterile solution of Pilocarpine Nitrate in Purified Water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of pilocarpine nitrate, $C_{11}H_{16}N_2O_2,HNO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Evaporate to dryness a volume containing 20 mg of Pilocarpine Nitrate on a water bath and heat for 1 hour at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pilocarpine nitrate (*RS 274*).

TESTS

Acidity

pH 2.5 to 4.2, Appendix V L.

Pilocarpic acid

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of each of the following solutions. For solution (1) dilute a suitable volume with *water* to produce a solution containing 0.080% w/v of Pilocarpine Nitrate. For solution (2) dilute 1 volume of solution (1) to 25 volumes with *water*. For solution (3) dissolve 8.0 mg of *pilocarpine nitrate BPCRS* in 10 ml of 0.0025M *sodium hydroxide*, heat in a boiling water bath for 30 minutes and cool.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase a mixture of 55 volumes of *methanol*, 60 volumes of *acetonitrile* and 885 volumes of 0.002M *tetrabutylammonium dihydrogen orthophosphate*, the pH of the mixture being adjusted to 7.75 using 6M *ammonia*, with a flow rate of 1.2 ml per minute and (c) a detection wavelength of 220 nm. Record the chromatogram of solution (1) for twice the retention time of the main peak.

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *pilocarpine nitrate BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak corresponding to pilocarpic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (4.0%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using 20 µl of each of the following solutions. For solution (1) dilute a suitable volume of the eye drops with *water* to produce a solution containing 0.08% w/v of Pilocarpine Nitrate. Solution (2) contains 0.08% w/v of *pilocarpine nitrate BPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase a mixture of 55 volumes of *methanol*, 60 volumes of *acetonitrile* and 885 volumes of 0.002M *tetrabutylammonium dihydrogen orthophosphate*, the pH of the mixture being adjusted to 7.75 using 6M *ammonia*, with a flow rate of 1.2 ml per minute and (c) a detection wavelength of 220 nm. Two major peaks are obtained with both solutions. Disregard the peak that is due to nitric acid and has the shorter retention time. Record the chromato-gram of solution (1) for twice the retention time of the principal peak.

Calculate the content of $C_{11}H_{16}N_2O_2,HNO_3$ using the declared content of $C_{11}H_{16}N_2O_2,HNO_3$ in *pilocarpine nitrate BPCRS*.

Pimozide Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Pimozide Tablets contain Pimozide.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pimozide, $C_{28}H_{29}F_2N_3O$

95.0% to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Pimozide with 20 ml of *dichloromethane* for 5 minutes, filter through a glass microfibre filter (Whatman GF/C is suitable) and evaporate the filtrate to dryness under reduced pressure. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pimozide (*RS 389*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.01M *hydrochloric acid* and rotate the paddle at 100 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter through a 0.45-µm polytetrafluoroethylene filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the solution from the sample, if necessary, with the dissolution medium to give a solution containing 0.0002% w/v of Pimozide. Solution (2) contains 0.0002% w/v of *pimozide BPCRS*.

The chromatographic procedure described under Related substances may be used.

Calculate the total content of pimozide, $C_{28}H_{29}F_2N_3O$, in the medium using the declared content of $C_{28}H_{29}F_2N_3O$ in *pimozide BPCRS*.

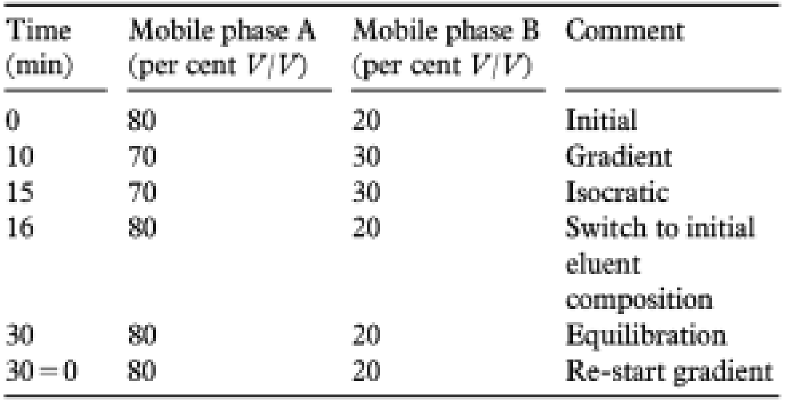
Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of *methanol* to a quantity of the powdered tablets containing 40 mg of Pimozide, shake for 30 minutes, mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid through a 0.45-µm polytetrafluoro-ethylene filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Solution (3) contains 0.005% w/v of *pimozide BPCRS* and 0.002% w/v of *mebendazole* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable). Carry out a linear gradient elution with a flow rate of 2 ml per minute using the following conditions.

Mobile phase A A solution containing 0.25% w/v of *ammonium acetate* and 0.85% w/v of *tetrabutylammonium hydrogen sulphate*.

Mobile phase B *Acetonitrile*.



Time (min)	Mobile phase A (per cent *V*/*V*)	Mobile phase B (per cent *V*/*V*)	Comment
0	80	20	Initial
10	70	30	Gradient
15	70	30	Isocratic
16	80	20	Switch to initial eluent composition
30	80	20	Equilibration
30 = 0	80	20	Re-start gradient

Use a detection wavelength of 280 nm. Equilibrate the column for at least 10 minutes with the initial mobile phase.

Inject 10 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 5.0. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%).

Uniformity of content

Tablets containing 2 mg or less of Pimozide comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) shake one tablet with 7 ml of *methanol* for 30 minutes, dilute with sufficient *methanol* to produce a solution containing 0.01% w/v of Pimozide, mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid through a 0.45-µm polytetrafluoroethylene filter. Solution (2) contains 0.010% w/v of *pimozide BPCRS* in *methanol*. Solution (3) contains 0.005% w/v of *pimozide BPCRS* and 0.002% w/v of *mebendazole* in *methanol*.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 5.0.

Calculate the content of $C_{28}H_{29}F_2N_3O$ in each tablet using the declared content of $C_{28}H_{29}F_2N_3O$ in *pimozide BPCRS.*

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Pimozide with 35 ml of *methanol* for 30 minutes, dilute to 50 ml with *methanol,* mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid through a 0.45-µm polytetrafluoroethylene filter. Solution (2) contains 0.04% w/v of *pimozide BPCRS* in *methanol*. Solution (3) contains 0.004% w/v of *pimozide BPCRS* and 0.002% w/v of *mebendazole EPCRS* in *methanol*.

The chromatographic procedure described under Related substances may be used.

Inject 10 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 5.0.

Calculate the content of $C_{28}H_{29}F_2N_3O$ in the tablets using the declared content of $C_{28}H_{29}F_2N_3O$ in *pimozide BPCRS*.

STORAGE

Pimozide Tablets should be kept protected from light.

Pindolol Tablets

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Pindolol Tablets contain Pindolol.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pindolol, $C_{14}H_{20}N_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Pindolol with 80 ml of *ether* for 30 minutes, filter and dry the extract with *anhydrous sodium sulphate*. Filter the dried extract, remove the ether using a rotary evaporator and dry the residue over *phosphorus pentoxide* at 110° at a pressure not exceeding 2 kPa for 1 hour. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of pindolol (*RS 275*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits two maxima, at 264 nm and 287 nm.

C. Shake a quantity of the powdered tablets containing 20 mg of Pindolol with 5 ml of a mixture of 1 volume of *glacial acetic acid* and 99 volumes of *methanol* for 45 minutes. Centrifuge and dilute 1 ml of the supernatant liquid to 50 ml with the acetic acid-methanol mixture. To 2 ml of this solution add 1 ml of *dimethylaminobenzaldehyde solution R7*. A violet-blue colour is produced.

Related substances

Carry out the following procedure as rapidly as possible protected from light. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a freshly prepared mixture of 4 volumes of 13.5M *ammonia*, 50 volumes of *ethyl acetate* and 50 volumes of *methanol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Pindolol with 5 ml of a mixture of 1 volume of *glacial acetic acid* and 99 volumes of *methanol* for 15 minutes, centrifuge and use the supernatant liquid; apply to the plate as the last solution. For solution (2) dilute 1 volume of solution (1) to 10 volumes with the acetic acid-methanol mixture and further dilute 7 volumes of this solution to 100 volumes with the same solvent mixture. For solution (3) dilute 1 volume of solution (1) to 10 volumes with the acetic acid-methanol mixture and further dilute 3 volumes of this solution to 100 volumes with the same solvent mixture. Develop the chromatograms without delay. After removal of the plate, spray immediately with *dimethylaminobenzaldehyde solution R7* and warm at 50° for 20 minutes. Any spot with an Rf value of about 0.1 in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.7%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.3%). Disregard any spot remaining on the line of application.

ASSAY

Shake a quantity of whole tablets containing 90 mg of Pindolol with 90 ml of *methanol* for 45 minutes. Centrifuge and dilute 15 ml of the supernatant liquid to 100 ml with *methanol*. Dilute 5 ml of this solution to 50 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 264 nm, Appendix II B. Calculate the content of $C_{14}H_{20}N_2O_2$ taking 338 as the value of A(1%, 1 cm) at the maximum at 264 nm. Make no allowance for the fact that fewer than 20 tablets may have been taken.

STORAGE

Pindolol Tablets should be protected from light.

Piperacillin Intravenous Infusion

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Piperacillin Intravenous Infusion is a sterile solution of piperacillin sodium in Water for Injections. It is prepared by dissolving Piperacillin for Intravenous Infusion in the requisite amount of Water for Injections before use.

The intravenous infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Piperacillin Intravenous Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

PIPERACILLIN FOR INTRAVENOUS INFUSION

DEFINITION

Piperacillin for Intravenous Infusion is a sterile material consisting of either Piperacillin Sodium or piperacillin sodium prepared by the interaction of Piperacillin with Sodium Bicarbonate, with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of anhydrous piperacillin, $C_{23}H_{27}N_5O_7S$

94.0 to 115.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the powder containing the equivalent of 0.25 g of anhydrous piperacillin in 20 ml of *water*, add 0.5 ml of *dilute hydrochloric acid*, mix well, allow to stand until a precipitate appears and filter through a membrane filter (Millipore GS or HA is suitable). The *infrared absorption spectrum* of the dried precipitate, Appendix II A, is concordant with the spectrum of *piperacillin EPCRS*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

Piperacillin Intravenous Infusion prepared from Piperacillin Sodium complies with the following additional test.

C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity

pH of a solution containing the equivalent of 20% w/v of anhydrous piperacillin, 4.8 to 6.8, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the mixed contents of the container in sufficient of the mobile phase to produce a solution containing the equivalent of 0.039% w/v of anhydrous piperacillin.

(2) 0.0008% w/v of *piperacillin EPCRS* in the mobile phase.

(3) 0.0004% w/v of *piperacillin EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Assay may be used.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak with a retention time relative to piperacillin of about 0.6 is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (3) (3.5%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Water

Not more than 2% w/w, Appendix IX C. Use 0.5 g.

If the contents of the sealed container have been freeze-dried *in situ*, not more than 0.9% w/w, Appendix IX C. Use 0.5 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of a sealed container in *water BET* to give a solution containing the equivalent of 50 mg of anhydrous piperacillin per ml (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions.

(1) Dissolve a quantity of the mixed contents of the 10 containers in sufficient of the mobile phase to produce a solution containing the equivalent of 0.039% w/v of anhydrous

piperacillin.

(2) Dissolve 40 mg of *piperacillin EPCRS* in *methanol* and dilute to 100 ml with the mobile phase.

(3) 0.01% w/v of *anhydrous ampicillin BPCRS* and 0.02% w/v of *piperacillin EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 to 10 µm) (Beckman Ultrasphere ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 10 µl of each solution.

MOBILE PHASE

3 volumes of 0.4M *tetrabutylammonium hydroxide*, 100 volumes of 0.2M *sodium dihydrogen orthophosphate monohydrate*, 447 volumes of *water* and 450 volumes of *methanol* which has been allowed to cool and the pH adjusted initially to 5.6 with 20% w/v *orthophosphoric acid* and finally to 5.5 with 2% w/v *orthophosphoric acid*.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 16.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{27}N_5O_7S$ in a container of average content weight from the declared content of $C_{23}H_{27}N_5O_7S$ in *piperacillin EPCRS*.

STORAGE

The sealed container should be protected from moisture.

LABELLING

The label of the sealed container states the quantity of active ingredient contained in it in terms of the equivalent amount of anhydrous piperacillin.

Piperazine Citrate Elixir

General Notices

Piperazine Citrate Oral Solution

Action and use

Antihelminthic.

DEFINITION

Piperazine Citrate Elixir is an *oral solution* containing 18.75% w/v of Piperazine Citrate in a suitable flavoured vehicle.

The elixir complies with the requirements stated under Oral Liquids and with the following requirements.

Content of piperazine citrate, $(C_4H_{10}N_2)_3,2C_6H_8O_7$

15.3 to 17.7% w/v.

IDENTIFICATION

A. To 1 ml add 5 ml of 2M *hydrochloric acid* and, with stirring, 1 ml of a freshly prepared 50% w/v solution of *sodium nitrite*, cool in ice for 15 minutes, induce crystallisation, wash the crystalline precipitate with *water* and dry at 105°. The *melting point* is about 159°, Appendix V A.

B. Warm 10 ml with *activated charcoal* and filter. Boil a portion of the filtrate with an excess of *mercury(II) sulphate solution*, filter, boil the filtrate and add 0.25 ml of *dilute potassium permanganate solution*. The potassium permanganate solution is decolourised and a white precipitate is produced.

C. Acidify a portion of the filtrate obtained in test B with 1M *sulphuric acid*, add 0.25 ml of *dilute potassium permanganate solution*, warm until the colour is discharged and add an excess of *bromine water*. A white precipitate is produced either immediately or on cooling.

TESTS

Weight per ml

1.20 to 1.30 g, Appendix V G.

ASSAY

To 1.5 g add 3.5 ml of 0.5M *sulphuric acid* and 10 ml of *water*, add 100 ml of *picric acid solution R1*, heat on a water bath for 15 minutes, allow to stand for 1 hour and filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable). Wash the residue with successive 10 ml quantities of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulphate, then wash the residue with five 10 ml quantities of *absolute ethanol* and dry to constant weight at 105°. Each g of residue is equivalent to 0.3935 g of $(C_4H_{10}N_2)_3,2C_6H_8O_7$. Use the weight per ml of the elixir to calculate the percentage weight in volume of $(C_4H_{10}N_2)_3,2C_6H_8O_7$.

STORAGE

Piperazine Citrate Elixir should be protected from light.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Piperazine Citrate Elixir contains, in 5 ml, the equivalent of about 750 mg of piperazine hydrate.

Piperazine Phosphate Tablets

General Notices

Action and use

Antihelminthic.

DEFINITION

Piperazine Phosphate Tablets contain Piperazine Phosphate. If the tablets are intended to be chewed before swallowing they may contain suitable flavouring agents.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of piperazine phosphate, $C_4H_{10}N_2,H_3PO_4,H_2O$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 1 g of Piperazine Phosphate with 20 ml of *water* and filter. The filtrate complies with the following tests.

A. Dilute 1 ml to 5 ml with *water*, add 0.5 g of *sodium hydrogen carbonate*, 0.5 ml of a freshly prepared 5% w/v solution of *potassium hexacyanoferrate*(*III*) and 0.1 ml of *mercury*, shake vigorously for 1 minute and allow to stand for 20 minutes. A reddish colour is produced slowly.

B. To 4 ml add 1 ml of *hydrochloric acid* and, with stirring, 1 ml of a 50% w/v solution of *sodium nitrite* and cool in ice for 15 minutes, stirring if necessary to induce crystallisation. The *melting point* of the crystals, after washing with 10 ml of cold *water* and drying at 105°, is about 159°, Appendix V A.

C. Yields the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Disintegration

The requirement for Disintegration does not apply to Piperazine Phosphate Tablets intended to be chewed before swallowing.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Piperazine Phosphate with 10 ml of *water* for 1 hour, filter and wash the residue with two 10 ml quantities of *water*. To the combined extract and washings add 5 ml of 1M *sulphuric acid* and 50 ml of *picric acid solution R1*, bring to the boil, allow to stand for several hours, filter through a sintered-glass crucible (ISO 4793, porosity grade 4, is suitable) and wash the residue with successive 10 ml quantities of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulphate. Wash the residue with five 10 ml quantities of *absolute ethanol* and dry to constant weight at 100° to 105°. Each g of residue is equivalent to 0.3714 g of $C_4H_{10}N_2,H_3PO_4,H_2O$.

LABELLING

The label states, where applicable, that the tablets are to be chewed before swallowing.

520 mg of Piperazine Phosphate is approximately equivalent to 500 mg of piperazine hydrate.

Piroxicam Capsules

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Piroxicam Capsules contain Piroxicam.
The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of piroxicam, $C_{15}H_{13}N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (3).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

2-Pyridylamine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of *diethylamine* and 8 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 80 mg of Piroxicam with 25 ml of *dichloromethane*, filter, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 ml of *dichloromethane*. Solution (2) contains 0.010% w/v of *2-pyridylamine* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any spot corresponding to 2-pyridylamine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *glacial acetic acid* and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 7.5 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 80 mg of Piroxicam with 25 ml of *dichloromethane*, filter, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 ml of *dichloromethane*. For solution (2) dilute 1 ml of solution (1) to 20 ml with *dichloromethane*. Solution (3) contains 0.20% w/v of *piroxicam*

BPCRS in *dichloromethane*. For solution (4) dilute 2 ml of solution (2) to 50 ml with *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.2%). Disregard any spot remaining on the line of application.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a 10 ml sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 242 nm, Appendix II B. Calculate the total content of piroxicam, $C_{15}H_{13}N_3O_4S$, in the medium taking 352 as the value of A(1%, 1 cm) at the maximum at 242 nm.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add a quantity of the mixed contents of 20 capsules containing 10 mg of Piroxicam to 150 ml of 0.01M *methanolic hydrochloric acid*, mix with the aid of ultrasound for 30 minutes, cool and dilute to 200 ml with the same solvent, filter through glass-fibre paper (Whatman GF/C is suitable) and use the filtrate. Solution (2) contains 0.005% w/v of *piroxicam BPCRS* in 0.01M *methanolic hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 60 volumes of *methanol* and 40 volumes of a buffer solution prepared by adding a solution containing 5.35 g of *disodium hydrogen orthophosphate* in 100 ml of *water* to a solution containing 7.72 g of *citric acid* in 400 ml of *water* and diluting to 1000 ml and (c) a detection wavelength of 242 nm.

Calculate the content $C_{15}H_{13}N_3O_4S$ using the declared content of $C_{15}H_{13}N_3O_4S$ in *piroxicam BPCRS*.

Piroxicam Gel

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Piroxicam Gel contains Piroxicam.
The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of piroxicam, $C_{15}H_{13}N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of *glacial acetic acid*, 10 volumes of *methanol* and 80 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) mix a quantity of the gel containing 10 mg of Piroxicam with 0.1 ml of a saturated solution of *sodium chloride* until the mixture becomes turbid. Dilute to 5 ml with 0.01M *methanolic hydrochloric acid*, shake well, centrifuge and use the clear supernatant solution. Filter the supernatant solution if necessary. Solution (2) contains 0.2% w/v of *piroxicam BPCRS* in 0.01M *methanolic hydrochloric acid*. After removal of the plate, dry it in a current of air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH of a 10% w/v solution of the gel, 7.2 to 8.2, Appendix V L.

2-Pyridylamine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 ml of 0. 01M *methanolic hydrochloric acid* to a quantity of the gel containing 5 mg of Piroxicam, shake gently for 30 minutes, add 50 ml of the mobile phase and shake vigorously for 30 minutes. Dilute to 100 ml with the mobile phase, mix and filter through a glass fibre membrane filter (1µm). For solution (2) dilute 5 volumes of a 0.00050% w/v solution of 2-*pyridylamine* in 0.01M *methanolic hydrochloric acid* to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm

× 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax stablebond C8 is suitable) and a stainless steel guard column (10 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm), both columns maintained at 40°, (b) a mixture of 15 volumes of *methanol*, 30 volumes of *acetonitrile* and 55 volumes of 0.05M *sodium dihydrogen orthophosphate* previously adjusted to pH 3.5 with 5M *orthophosphoric acid* as the mobile phase with a flow rate of 1.0 ml per minute and (c) a fluorimetric detector with an excitation wavelength of 313 nm and an emission wavelength of 380 nm.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 2-pyridylamine is not greater than that of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 ml of 0. 01M *methanolic hydrochloric acid* to a quantity of the gel containing 5 mg of Piroxicam, shake gently for 30 minutes, add 50 ml of the mobile phase and shake vigorously for 30 minutes. Dilute to 100 ml with the mobile phase, mix and filter through a glass fibre membrane filter (1 µm). For solution (2) prepare a 0.10% w/v solution of *piroxicam BPCRS* in 0.01M *methanolic hydrochloric acid*, with the aid of ultrasound if necessary, and dilute 5 volumes of this solution to 100 volumes with the mobile phase.

The chromatographic procedure described under 2-Pyridylamine may be used but use an ultraviolet spectrophotometer detector at a wavelength of 248 nm.

Calculate the content of $C_{15}H_{13}N_3O_4S$ in the gel using the declared content of $C_{15}H_{13}N_3O_4S$ in *piroxicam BPCRS*.

Pizotifen Tablets

General Notices

Action and use

Serotonin (5HT) receptor partial agonist; prophylaxis of migraine.

DEFINITION

Pizotifen Tablets contain Pizotifen Malate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pizotifen, $C_{19}H_{21}NS$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing the equivalent of 10 mg of pizotifen add 50 ml of *water*, mix with the aid of ultrasound for 15 minutes and centrifuge. Make the clear supernatant liquid alkaline with 5M *sodium hydroxide* and extract with three 25 ml quantities of *ether*, washing each extract with the same 20 ml of *water*. Combine the ether extracts, dry with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pizotifen (*RS 276*).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2).

C. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to pizotifen in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 1 volume of 13.5M *ammonia*, 15 volumes of *absolute ethanol* and 85 volumes of *toluene* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions prepared using a mixture of 200 volumes of *methanol*, 100 volumes of *chloroform* and 3 volumes of 13.5M *ammonia* (solvent A). For solution (1) mix a quantity of the powdered tablets containing the equivalent of 6 mg of pizotifen with 60 ml of solvent A for 20 minutes, centrifuge, evaporate 50 ml of the clear supernatant liquid to dryness at 40° at a pressure of 2 kPa and dissolve the residue in 2 ml of solvent A. Centrifuge and use the supernatant liquid. Solution (2) contains 0.36% w/v of *pizotifen malate BPCRS* in solvent A. For solution (3) dilute 1 volume of solution (1) to 100 volumes with solvent A. For solution (4) dilute 1 volume of solution (3) to 2 volumes with solvent A. After removal of the plate, dry it in a current of warm air for 5 minutes, spray with a mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of 2M *acetic acid* and then with *hydrogen peroxide solution* (*10 vol*), cover immediately with a glass plate and examine in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (1%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.5%). Disregard any spots with Rf values lower than 0.1.

Uniformity of content

Tablets containing less than the equivalent of 2 mg of pizotifen comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0073% w/v of *pizotifen malate BPCRS* in the mobile phase. For solution (2) add sufficient mobile phase to one tablet to produce a solution containing the equivalent of 0.0050% w/v of pizotifen, mix with the aid of ultrasound until the tablet has disintegrated, stir for 15 minutes and centrifuge.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable) and a pre-column (1 cm × 4.6 mm) packed with the same material placed between the pump and the injector, (b) a mixture of 0.3 volume of *triethylamine*, 45 volumes of *water* and 55 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 256 nm.

Calculate the content of pizotifen, $C_{19}H_{21}NS$, using the declared content of $C_{19}H_{21}NS$ in *pizotifen malate BPCRS*.

ASSAY

Calculate the content of $C_{19}H_{21}NS$ using the average of the 10 results obtained in the test for Uniformity of content.

STORAGE

Pizotifen Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of pizotifen.

Poldine Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Poldine Tablets contain Poldine Metilsulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of poldine metilsulfate, $C_{22}H_{29}NO_7S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 2 mg of Poldine Metilsulfate with 10 ml of *water* and allow to stand. Decant the supernatant liquid, add 20 ml of *ammonium cobaltothiocyanate solution* and 5 ml of *chloroform* and shake well. The chloroform layer becomes blue.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 5 volumes of *formic acid*, 5 volumes of *water,* 30 volumes of *methanol* and 60 volumes of *chloroform* as the mobile phase. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) add 4 ml of *methanol* to a quantity of the powdered tablets containing 20 mg of Poldine Metilsulfate, mix with the aid of ultrasound for 5 minutes, centrifuge and to 1 ml of the supernatant liquid add 0.2 ml of *formic acid*. For solution (2) dilute 1 volume of solution (1) to 100 volumes with a mixture of 5 volumes of *methanol* and 1 volume of *formic acid*. After removal of the plate, allow it to dry in air, spray with a 50% v/v solution of *sulphuric acid* and heat at 110° for 20 minutes. Any pink *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%). Disregard any brown spots due to excipients in the chromatogram obtained with solution (1).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 2 mg of Poldine Metilsulfate with 10 ml of *water* for 10 minutes and allow to stand. To 5 ml of the supernatant liquid add 20 ml of *ammonium cobaltothiocyanate solution*, shake vigorously for 1 minute and allow to stand for 5 minutes. Extract with four 5 ml quantities of *chloroform*, add to the combined extracts sufficient *chloroform* to produce 25 ml and centrifuge. Measure the *absorbance* of the clear, chloroform solution at the maximum at 322 nm, Appendix II B. Repeat the operation using 5 ml of a 0.02% w/v solution of *poldine metilsulfate BPCRS* beginning at the words 'add 20 ml …'. Calculate the content of $C_{22}H_{29}NO_7S$ in the tablets from the absorbances obtained using the declared content of $C_{22}H_{29}NO_7S$ in *poldine metilsulfate BPCRS.*

Polymyxin and Bacitracin Eye Ointment

General Notices

Action and use

Antibacterial and Polypeptide antibacterial.

DEFINITION

Polymyxin and Bacitracin Eye Ointment is a sterile preparation containing Polymyxin B Sulphate and Bacitracin Zinc in a suitable basis.

The eye ointment complies with the requirements stated under Eye Preparations and with the following requirements.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 60 volumes of *propan-2-ol*, 40 volumes of *water* and 10 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 20 ml of *chloroform* to a quantity of the eye ointment containing 2500 IU of Bacitracin Zinc, shake, add 5 ml of 0.1M *hydrochloric acid*, shake vigorously, centrifuge and use the aqueous layer. Solution (2) contains 0.13% w/v of *polymyxin B sulphate EPCRS* in 0.1M *hydrochloric acid*. Solution (3) contains 0.75% w/v of *bacitracin zinc EPCRS* in 0.1M *hydrochloric acid*. After removal of the plate, dry it at 105°, spray with a 0.5% w/v solution of *ninhydrin* in *butan-1-ol* and heat at 105° for 15 minutes. The two principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with solutions (2) and (3).

ASSAY

For polymyxin B sulphate

To a quantity containing 10,000 IU of Polymyxin B Sulphate add 15 ml of *chloroform* and 40 ml of sterile *phosphate buffer pH 6.0*, mix thoroughly, centrifuge and discard the chloroform layer. Using the aqueous layer, carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU of Polymyxin B Sulphate.

For bacitracin zinc

To a quantity containing 500 IU of Bacitracin Zinc add 15 ml of *chloroform* and 40 ml of 0.01M *hydrochloric acid*, mix thoroughly, centrifuge and discard the chloroform layer. Using the aqueous layer, carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 120.0% of the stated number of IU of Bacitracin Zinc.

LABELLING

The strength is stated as the number of IU (Units) of Polymyxin B Sulphate per g and the number of IU (Units) of Bacitracin Zinc per g.

Polythiazide Tablets

General Notices

Action and use

Thiazide diuretic.

DEFINITION

Polythiazide Tablets contain Polythiazide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of polythiazide, $C_{11}H_{13}ClF_3N_3O_4S_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF$_{254}$* as the coating substance and a mixture of 20 volumes of *acetone*, 30 volumes of *ether* and 50 volumes of *toluene* as the mobile phase. Apply separately to the plate 20 µl of each of following solutions. For solution (1) use the supernatant liquid obtained by extracting a quantity of the powdered tablets containing 4 mg of Polythiazide with 20 ml of *methanol* and centrifuging. Solution (2) contains 0.02% w/v of *polythiazide BPCRS* in *methanol*. After removal of the plate, allow it to dry in air until the solvent has evaporated, examine under *ultraviolet light* (*254 nm*) and then treat the plate by *Method I* and examine again. The principal spot in the chromatogram obtained with solution (1) corresponds in colour and intensity to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *methanol* and 90 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of solution (1) and quantities of 2, 4, 6, 8 and 10 µl of solution (2). For solution (1) use the supernatant liquid obtained by shaking a quantity of the powdered tablets containing 10 mg of Polythiazide with 2 ml of *acetone* and centrifuging. For solution (2) dilute 1 volume of solution (1) to 50 volumes with *acetone*. After removal of the plate, allow it to dry in air and reveal the spots using *Method I*. Assess the intensity of any *secondary spot* in the chromatogram obtained with solution (1) by reference to the spots in the chromatograms obtained with the applications of solution (2). The sum of the intensities so assessed does not exceed 3% of that of the spot in the chromatogram obtained with solution (1) and no such spot is more intense than the spot in the chromatogram obtained with 10 µl of solution (2) (2%). Disregard any brown spot.

Uniformity of content

Tablets containing less than 2 mg of Polythiazide comply with the requirements stated under

Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a 0.004% w/v solution of *polythiazide BPCRS* in *methanol* with an equal volume of 0.1M *potassium dihydrogen orthophosphate* adjusted to pH 3.5 with *orthophosphoric acid* (buffer solution). For solution (2) shake one tablet with 25 ml of the buffer solution for 10 minutes, add 25 ml of *methanol* and shake for a further 20 minutes. Centrifuge 5 ml of the resulting solution for 5 minutes at 2000 revolutions per minute and use the supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 55 volumes of 0.1M *potassium dihydrogen orthophosphate* adjusted to pH 3.5 with *orthophosphoric acid* and 45 volumes of *methanol* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 268 nm.

Calculate the content of $C_{11}H_{13}ClF_3N_3O_4S_3$ using the declared content of $C_{11}H_{13}ClF_3N_3O_4S_3$ in *polythiazide BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a 0.004% w/v solution of *polythiazide BPCRS* in *methanol* with an equal volume of 0.1M *potassium dihydrogen orthophosphate* adjusted to pH 3.5 with *orthophosphoric acid* (buffer solution). For solution (2) shake a quantity of the powdered tablets containing 4 mg of Polythiazide with 100 ml of *methanol* for 20 minutes, filter and mix equal volumes of the filtrate and the buffer solution.

The chromatographic procedure may be carried out using the conditions described under Uniformity of content.

Calculate the content of $C_{11}H_{13}ClF_3N_3O_4S_3$ using the declared content of $C_{11}H_{13}ClF_3N_3O_4S_3$ in *polythiazide BPCRS*.

STORAGE

Polythiazide Tablets should be protected from light.

Potassium Chloride and Glucose Intravenous Infusion

General Notices

Potassium Chloride and Dextrose Injection; Potassium Chloride and Glucose Injection; Potassium Chloride and Dextrose Intravenous Infusion

DEFINITION

Potassium Chloride and Glucose Intravenous Infusion is a sterile solution of Potassium Chloride and either Anhydrous Glucose or Glucose in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

Content of glucose, $C_6H_{12}O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or faintly straw-coloured solution.

IDENTIFICATION

A. When heated with *cupri-tartaric solution R1* a copious precipitate of copper(I) oxide is produced.

B. The residue on evaporation yields the reactions characteristic *of potassium salts* and of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L.

5-Hydroxymethylfurfural and related substances

Dilute a volume containing 1.0 g of glucose, $C_6H_{12}O_6$, to 500 ml with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

Sodium

The content of Na is not more than 1.0% of the content of K, calculated from the content of potassium chloride determined in the Assay. Determine the content of Na by Method II for *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution* (*200 ppm Na*), diluted if necessary with *water*, to prepare the standard solutions.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per ml, Appendix XIV C.

ASSAY

For potassium chloride

Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution* (*600 ppm K*), suitably diluted with *water*, to prepare the standard solutions.

For glucose

To a quantity containing 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 ml of 5M *ammonia* and sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and measure the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the quantity of the intravenous infusion taken for assay.

LABELLING

The strength is stated as the percentages w/v of Potassium Chloride and of glucose, $C_6H_{12}O_6$.

When the preparation is intended for intravenous infusion, the label also states (1) that rapid infusion may be harmful; (2) the approximate concentrations, in millimoles per litre, of the potassium ions and the chloride ions and the number of grams per litre of glucose, $C_6H_{12}O_6$.

For a preparation containing 0.3% w/v of Potassium Chloride, the concentration of each ion is stated as 40 millimoles per litre.

Potassium Chloride and Sodium Chloride Intravenous Infusion

General Notices

Potassium Chloride and Sodium Chloride Injection

DEFINITION

Potassium Chloride and Sodium Chloride Intravenous Infusion is a sterile solution of Potassium Chloride and Sodium Chloride in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

The residue on evaporation yields the reactions characteristic of *potassium salts*, the reactions characteristic of *sodium salts* and the reactions characteristic of *chlorides*, Appendix VI.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per ml, Appendix XIV C.

ASSAY

For potassium chloride

Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution* (*600 ppm K*), suitably diluted with *water*, to prepare the standard solutions.

For sodium chloride

Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution* (*200 ppm Na*), suitably diluted with *water*, to prepare the standard solutions.

LABELLING

The strength is stated as the percentages w/v of Potassium Chloride and of Sodium Chloride.

When the preparation is intended for intravenous infusion, the label also states (1) that rapid infusion may be harmful; (2) the approximate concentrations, in millimoles per litre, of the potassium ions, the sodium ions and the chloride ions.

For a preparation containing 0.3% w/v of Potassium Chloride and 0.9% w/v of Sodium Chloride, the concentrations of the ions are stated as 40 millimoles of potassium, 150 millimoles of sodium and 190 millimoles of chloride per litre.

Potassium Chloride Oral Solution

General Notices

Action and use

Used in the prevention and treatment of potassium deficiency and electrolyte imbalance.

DEFINITION

Potassium Chloride Oral Solution is a solution of Potassium Chloride in Purified Water.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *potassium salts* and the reactions characteristic of *chlorides*, Appendix VI.

Acidity or alkalinity

pH, 5.0 to 7.5, Appendix V L.

ASSAY

Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution* (*600 ppm K*), suitably diluted with *water*, to prepare the standard solutions.

When Potassium Chloride Oral Solution for paediatric or neonatal use is prescribed or demanded, a solution containing 1 mmol/ml or 2 mmol/ml shall be dispensed or supplied.

Potassium Chloride, Sodium Chloride and Glucose Intravenous Infusion

General Notices

Potassium Chloride, Sodium Chloride and Dextrose Injection; Potassium Chloride, Sodium Chloride and Glucose Injection; Potassium Chloride, Sodium Chloride and Dextrose Intravenous Infusion

DEFINITION

Potassium Chloride, Sodium Chloride and Glucose Intravenous Infusion is a sterile solution of Potassium Chloride, Sodium Chloride and either Anhydrous Glucose or Glucose in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

Content of sodium chloride, NaCl

0.17 to 0.19% w/v.

Content of glucose, $C_6H_{12}O_6$

3.8 to 4.2% w/v.

CHARACTERISTICS

A colourless or faintly straw-coloured solution.

IDENTIFICATION

A. When heated with *cupri-tartaric solution R1* a copious precipitate of copper(I) oxide is produced.

B. The residue on evaporation and ignition yields reaction B characteristic of *potassium salts*, reaction B characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L.

5-Hydroxymethylfurfural and related substances

Dilute 25 ml to 500 ml with *water*. The *absorbance* of the resulting solution at the maximum at 284 nm is not more than 0.25, Appendix II B.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per ml, Appendix XIV C.

ASSAY

For potassium chloride

Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 767 nm and using *potassium standard solution* (*600 ppm K*), suitably diluted with *water*, to prepare the standard solutions.

For sodium chloride

Dilute appropriately with *water* and determine by *atomic emission spectrophotometry*, Appendix II D, measuring at 589 nm and using *sodium standard solution* (*200 ppm Na*), suitably diluted with *water*, to prepare the standard solutions.

For glucose

To a quantity containing 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 ml of 5M *ammonia* and sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and measure the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the quantity of the intravenous infusion taken for assay.

STORAGE

During storage, the separation of small solid particles from a glass container may occur. A solution containing such particles must not be used.

LABELLING

The label states (1) the content of Potassium Chloride as a percentage w/v and that the preparation contains 0.18% w/v of Sodium Chloride and 4.0% w/v of glucose, $C_6H_{12}O_6$; (2) the concentration of Potassium Chloride in grams per litre and the approximate concentration of potassium ions in millimoles per litre; (3) that the preparation contains 1.8 grams per litre of Sodium Chloride, equivalent to approximately 30 millimoles of sodium ions per litre; (4) the approximate quantity of chloride ions present, in millimoles per litre; (5) that the preparation contains 40 grams per litre of glucose, $C_6H_{12}O_6$; (6) that rapid infusion may be harmful; (7) that solutions containing visible solid particles must not be used.

Potassium Citrate Mixture

General Notices

Potassium Citrate Oral Solution

DEFINITION

Potassium Citrate Mixture is an *oral solution* containing 30% w/v of Potassium Citrate and 5% w/v of Citric Acid Monohydrate in a suitable vehicle with a lemon flavour. It is intended to be diluted with water before use.

Extemporaneous preparation

It is recently prepared according to the following formula.

Potassium Citrate	300 g
Citric Acid Monohydrate	50 g
Lemon Spirit	5 ml
Quillaia Tincture	10 ml
Syrup	250 ml
Double-strength Chloroform Water	300 ml
Water	Sufficient to produce 1000 ml

The mixture complies with the requirements stated under Oral Liquids and with the following requirements.

Content of citric acid monohydrate, $C_6H_8O_7,H_2O$

4.75 to *5.25% w/v.*

Content of potassium citrate, $C_6H_5K_3O_7,H_2O$

28.5 to 31.5% w/v.

IDENTIFICATION

A. Yields reaction A characteristic of *potassium salts*, Appendix VI.

B. Yields reaction B characteristic of *citrates*, Appendix VI.

ASSAY

For citric acid monohydrate

To 5 ml add 100 ml of *water*, boil, cool and titrate with carbonate-free 0.1M *sodium hydroxide*

VS using *thymol blue solution* as indicator. Each ml of carbonate-free 0.1M *sodium hydroxide VS* is equivalent to 7.005 mg of $C_6H_8O_7,H_2O$.

For potassium citrate

To 1 g add 5 ml of *acetic anhydride* and 20 ml of *anhydrous acetic acid*, heat on a water bath for 20 minutes, allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 10.81 mg of $C_6H_5K_3O_7,H_2O$. Determine the *weight per ml* of the mixture, Appendix V G, and calculate the content of $C_6H_5K_3O_7,H_2O$, weight in volume.

LABELLING

The label states that the mixture should be well diluted with water before use.

The label indicates the pharmaceutical form as 'oral solution'.

Potassium Dihydrogen Phosphate Oral Solution

General Notices

Potassium Acid Phosphate Oral Solution

NOTE: There are currently no licensed formulations in the United Kingdom.

DEFINITION

Potassium Dihydrogen Phosphate Oral Solution is a 13.6% w/v solution of Potassium Dihydrogen Phosphate in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of potassium dihydrogen phosphate, KH_2PO_4

12.9 to 14.3% w/v.

IDENTIFICATION

The oral solution yields the reactions characteristic of *potassium salts* and the reactions characteristic of *phosphates,* Appendix VI.

TESTS

Acidity

pH, 3.5 to 5.0, Appendix V L.

ASSAY

Titrate 10 ml of the oral solution, saturated with *sodium chloride,* with 0.5M *sodium hydroxide VS* using *phenolphthalein solution* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 68.05 mg of KH_2PO_4.

LABELLING

The label states: (1) the strength as a percentage w/v of Potassium Dihydrogen Phosphate; (2) the approximate concentration of the potassium ions and the phosphate ions in millimoles in a suitable volume.

Potassium Hydroxide Solution

General Notices

Potash Solution

DEFINITION

Potassium Hydroxide Solution is a solution of Potassium Hydroxide in Purified Water.

Content of total alkali, calculated as KOH

4.9 to 5.1% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Strongly alkaline and when neutralised with 2M *hydrochloric acid* yields the reactions characteristic *of potassium salts*, Appendix VI.

Weight per ml

1.040 to 1.050 g, Appendix V G.

ASSAY

Titrate 20 ml with 1M *hydrochloric acid VS* using *methyl orange solution* as indicator. Each ml of 1M *hydrochloric acid VS* is equivalent to 56.11 mg of total alkali, calculated as KOH.

STORAGE

Potassium Hydroxide Solution should be kept in a well-closed container of lead-free glass or of a suitable plastic.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

Potassium Hydroxyquinoline Sulphate and Benzoyl Peroxide Cream

General Notices

DEFINITION

Potassium Hydroxyquinoline Sulphate and Benzoyl Peroxide Cream contains Potassium Hydroxyquinoline Sulphate and Hydrous Benzoyl Peroxide in a suitable astringent basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of potassium hydroxyquinoline sulphate, $(C_9H_7NO)_2,H_2SO_4:K_2SO_4$

90.0 to 110.0% of the stated amount.

Content of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF$_{254}$* as the coating substance and a mixture of 2 volumes of *glacial acetic acid*, 4 volumes of *dichloromethane* and 100 volumes of *toluene* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the cream containing the equivalent of 50 mg of anhydrous benzoyl peroxide with 10 ml of *chloroform* and filter. Solution (2) contains 0.5% w/v of *benzoyl peroxide* in *chloroform*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Add 20 ml of *methanol* to a quantity of the cream containing 10 mg of Potassium Hydroxyquinoline Sulphate, heat in a water bath, shaking occasionally, cool and filter through *anhydrous sodium sulphate*. To 10 ml of the filtrate add 5 ml of a 0.5% w/v solution of *iron*(*II*) *sulphate* in *methanol* (*70%*). A dark blue or green colour is produced immediately.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 2 volumes of solution (2) to 100 volumes with the mobile phase. For solution (2) disperse a quantity of the cream containing the equivalent of 0.10 g of anhydrous benzoyl peroxide with 25 ml of *acetonitrile* by shaking vigorously, add sufficient *water* to produce 50 ml, mix and filter. Solution (3) contains 0.020% w/v of *benzoic acid* in the mobile phase. Solution (4) contains 0.0020% w/v of *ethyl benzoate* in the mobile phase. Solution (5) contains 0.0020% w/v of *benzaldehyde* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 1 volume of *glacial acetic acid,* 500 volumes of *acetonitrile and* 500 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a

detection wavelength of 235 nm.

In the chromatogram obtained with solution (2) the areas of any peaks corresponding to benzoic acid, ethyl benzoate and benzaldehyde are not greater than the areas of the principal peaks in the chromatograms obtained with solutions (3) (10%), (4) (1%) and (5) (1%) respectively. The area of any other *secondary peak* is not greater than half of the area of the principal peak in the chromatogram obtained with solution (1) (0.5%).

ASSAY

For potassium hydroxyquinoline sulphate

Shake vigorously a quantity of the cream containing 35 mg of Potassium Hydroxyquinoline Sulphate with 50 ml of *chloroform* and 10 ml of 13.5M *ammonia* for 5 minutes. Separate and reserve the chloroform layer and extract the aqueous layer with a further 50 ml of *chloroform*. Extract the combined chloroform layers with two 50 ml quantities of 3.5M *hydrochloric acid*, combine the extracts, wash gently with three 50 ml quantities of *chloroform* and discard the chloroform layers. Add 50 ml of 0.01M *bromine VS*, stopper the flask, shake for 20 minutes and allow to stand for 20 minutes. Add 10 ml of *dilute potassium iodide solution* and titrate with 0.02M *sodium thiosulphate VS* using *starch solution*, added towards the end of the titration, as indicator. Repeat the titration using 100 ml of 3.5M *hydrochloric acid* and beginning at the words 'Add 50 ml...'. The difference between the titrations represents the amount of bromine required. Each ml of 0.01M *bromine VS* is equivalent to 1.407 mg of $(C_9H_7NO)_2,H_2SO_4{:}K_2SO_4$.

For benzoyl peroxide

Shake gently a quantity of the cream containing the equivalent of 0.4 g of anhydrous benzoyl peroxide with 50 ml of *chloroform* until the cream has dispersed and filter the lower layer excluding any solid matter that separates at the interface. Repeat the extraction with two further 50 ml quantities of *chloroform*, wash the filter with *chloroform*, combine the extracts and washings and dilute to 250 ml with *chloroform*. To 25 ml of the chloroform extract add 40 ml of a 0.0005% w/v solution of *iron(III) chloride hexahydrate* in *glacial acetic acid* and 10 ml of *dilute potassium iodide solution* and shake for 20 minutes protected from light. Add 60 ml of *water* and titrate with 0.0125M *sodium thiosulphate VS* using *starch solution*, added towards the end of the titration, as indicator. Repeat the titration using 25 ml of *chloroform* in place of the chloroform extract. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.0125M *sodium thiosulphate VS* is equivalent to 1.514 mg of $C_{14}H_{10}O_4$.

LABELLING

The content of Hydrous Benzoyl Peroxide is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Potassium Iodate Tablets

General Notices

DEFINITION

Potassium Iodate Tablets contain Potassium Iodate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of potassium iodate, KIO_3

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.4 g of Potassium Iodate with 10 ml of *water* and filter (solution A). 1 ml of solution A yields reaction B characteristic of *potassium salts*, Appendix VI.

B. Add 1 g of *potassium iodide* and 1 ml of *hydrochloric acid* to 5 ml of solution A and shake. A brown colour is produced.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of *water* and rotating the basket at 100 revolutions per minute. Filter the medium after first wetting the filter paper with *water* and note the volume of filtrate. Treat the filtrate as described under Assay beginning at the words 'To 50 ml of the filtrate ...'. Repeat the assay procedure without the dissolution medium. Calculate the total content of potassium iodate, KIO_3, in the dissolution medium from the difference between the titrations.

Iodide

Shake a quantity of the powdered tablets containing 2.5 g of Potassium Iodate with 50 ml *water* and filter (solution B). Add 1 ml of 1.8M *sulphuric acid* and 1 ml of *chloroform* to 25 ml of solution B and shake. Any violet colour produced is not more intense than that of a solution prepared at the same time and in the same manner but using 5 ml of solution B and 2 ml of *iodide standard solution (10 ppm I)* (20 ppm).

ASSAY

Weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing 0.3 g of Potassium Iodate to an iodine flask, add 100 ml of *water*, shake and filter. To 50 ml of the filtrate add 3 g of *potassium iodide* followed by 50 ml of 2M *hydrochloric acid*. Stopper the flask, mix and stand in the dark for 5 minutes. Titrate the resulting solution with 0.1M *sodium thiosulphate VS* to a light straw colour and then complete the titration to a colourless end point using *starch solution* as indicator. Repeat the titration without the powdered tablets. The difference between the titrations represents the amount of sodium thiosulphate required. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 3.567 mg of KIO_3.

Povidone-Iodine Mouthwash

General Notices

Action and use

Disinfectant.

DEFINITION

Povidone-Iodine Mouthwash is either an aqueous solution of Iodinated Povidone or it is prepared by the interaction between Iodine and Povidone.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of available iodine, I

0.085 to 0.120% w/v.

CHARACTERISTICS

A yellowish-brown solution.

IDENTIFICATION

A. Dilute 1 ml to 2 ml with *water* and add 1 ml of the resulting solution to a mixture of 1 ml of *starch mucilage* and 9 ml of *water*. A deep blue colour is produced.

B. Transfer 10 ml to a small flask and cover the mouth of the flask with filter paper moistened with 0.05 ml of *starch mucilage*. No blue colour is produced on the paper within 60 seconds.

C. To 10 ml add drop wise 0.1M *sodium thiosulphate* until the colour of the iodine is just discharged. To 5 ml of the solution add 10 ml of 1M *hydrochloric acid* and 5 ml of a 7.0% w/v solution of *potassium dichromate*. A red precipitate is produced.

D. To 5 ml add 2 ml of *ammonium cobaltothiocyanate solution* previously acidified with 5M *hydrochloric acid*. A blue precipitate is produced.

TESTS

Acidity

pH, 3.0 to 6.5, Appendix V L.

Iodide

Not more than 0.06% w/v when determined by the following method. To 50 ml of the mouthwash add *sodium metabisulphite* until the colour of iodine has disappeared. Add 25 ml of 0.1M *silver nitrate VS*, 10 ml of *nitric acid* and 5 ml of *ferric ammonium sulphate solution R2*. Titrate with 0.1M *ammonium thiocyanate VS*. Repeat the procedure without the solution being examined. Each ml of 0.1M *silver nitrate VS* is equivalent to 12.69 mg of total iodine.

Calculate the percentage content of total iodine and subtract the percentage content of available iodine determined in the Assay to obtain the percentage content of iodide.

ASSAY

To 100 ml add 10 ml of 0.1M *hydrochloric acid* and sufficient *water* to produce 150 ml and titrate with 0.1M *sodium thiosulphate VS* determining the end point potentiometrically. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 12.690 mg of I.

Povidone-Iodine Solution

General Notices

Action and use

Disinfectant.

DEFINITION

Povidone-Iodine Solution is either an aqueous solution of Iodinated Povidone or it is prepared by the interaction between Iodine and Povidone. It is a *cutaneous solution.*

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of available iodine, I

0.85 to 1.20% w/v.

CHARACTERISTICS

A deep brown liquid.

IDENTIFICATION

A. Dilute 1 ml to 20 ml with *water* and add 1 ml of the resulting solution to a mixture of 1 ml of *starch mucilage* and 9 ml of *water*. A deep blue colour is produced.

B. Transfer 10 ml to a small flask and cover the mouth of the flask with filter paper moistened with 0.05 ml of *starch mucilage*. No blue colour is produced on the paper within 60 seconds.

C. Dilute 20 ml to 100 ml with *water*. To 10 ml add drop wise 0.1M *sodium thiosulphate* until the colour of the iodine is just discharged. Reserve 5 ml of the resulting solution for test D. To 5 ml of the solution add 10 ml of 1M *hydrochloric acid* and 5 ml of a 7.0% w/v solution of *potassium dichromate*. A red precipitate is produced.

D. To 5 ml of the solution reserved in test C add 2 ml of *ammonium cobaltothiocyanate solution* previously acidified with 5M *hydrochloric acid*. A blue precipitate is produced.

TESTS

Acidity

pH, 3.0 to 6.5, Appendix V L.

Iodide

Not more than 0.6% w/v when determined by the following method. Dilute 5 ml of the solution being examined to 100 ml with *water* and add *sodium metabisulphite* until the colour of iodine has disappeared. Add 25 ml of 0.1M *silver nitrate VS*, 10 ml of *nitric acid* and 5 ml of *ferric ammonium sulphate solution R2*. Titrate with 0.1M *ammonium thiocyanate VS*. Repeat the procedure without the solution being examined. Each ml of 0.1M *silver nitrate VS* is equivalent to 12.69 mg of total iodine. Calculate the percentage content of total iodine and subtract the percentage content of available iodine determined in the Assay to obtain the percentage content of iodide.

ASSAY

To 10 ml add 10 ml of 0.1M *hydrochloric acid* and sufficient *water* to produce 150 ml and titrate with 0.02M *sodium thiosulphate VS* determining the end point potentiometrically. Each ml of 0.02M *sodium thiosulphate VS* is equivalent to 2.538 mg of I.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

The label states the pharmaceutical form as 'cutaneous solution'.

Pravastatin Tablets

General Notices

Action and use

HMG Co-A reductase inhibitor; lipid-regulating drug.

DEFINITION

Pravastatin Tablets contain Pravastatin Sodium.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pravastatin sodium, $C_{23}H_{35}NaO_7$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 10 mg of Pravastatin Sodium with 80 ml of *water* for 10 minutes, dilute to 100 ml with *water*, filter and dilute 1 ml to 10 ml with *water*. The *light absorption* of the resulting solution, Appendix II B, in the range 220 to 350 nm, exhibits a maximum only at 238 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Dissolve 4.10 g of *anhydrous sodium acetate* in 400 ml of *water*, adjust the pH to 5.6 with *glacial acetic acid* and add sufficient *water* to produce 500 ml (solution A). Mix 20 volumes of *methanol* with 80 volumes of solution A (solution B). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of solution A to a quantity of the powdered tablets containing 10 mg of Pravastatin Sodium, mix using a vortex mixer and then with the aid of ultrasound for 15 minutes. Centrifuge and dilute 1 volume of the clear supernatant liquid to 5 volumes with solution B. For solution (2) dilute 1 volume of solution (1) to 100 volumes with solution B. For solution (3) dilute 1 volume of solution (2) to 5 volumes with solution B. For solution (4) dilute 1 volume of solution (3) to 4 volumes with solution B. For solution (5) dissolve 2 mg of *pravastatin impurity A EPCRS* in the minimum volume of *methanol* and dilute to 20 ml with solution (1). Inject 20 µl of each solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5µm) (Ultrasphere ODS is suitable) maintained at 25°, (b) as the mobile phase with a flow rate of 1.3 ml per minute a mixture of 1 volume of *glacial acetic acid*, 1 volume of *triethylamine*, 450 volumes of *methanol* and 550 volumes of *water* and (c) a detection wavelength of 238 nm.

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution*

factor between the peaks corresponding to pravastatin impurity A and pravastatin is at least 7.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%), not more than one such peak has an area greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%), not more than a further one such peak has an area greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%) and the sum of the areas of all such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (3%). Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

ASSAY

Weigh and powder 20 tablets. Dissolve 4.10 g of *anhydrous sodium acetate* in 400 ml of *water*, adjust the pH to 5.6 with *glacial acetic acid* and add sufficient *water* to produce 500 ml (solution A). Mix 20 volumes of *methanol* with 80 volumes of solution A (solution B). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 20 ml of solution A to a quantity of the powdered tablets containing 10 mg of Pravastatin Sodium, mix using a vortex mixer and then with the aid of ultrasound for 15 minutes. Centrifuge and dilute 1 volume of the clear supernatant liquid to 5 volumes with solution B. For solution (2) dissolve 12.4 mg of *pravastatin 1,1,3,3,-tetramethylbutylamine EPCRS* in 20 ml of solution A and dilute 1 volume to 5 volumes with solution B. For solution (3) dissolve 2 mg of *pravastatin impurity A EPCRS* in the minimum volume of *methanol* and dilute to 20 ml with solution (1).

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to pravastatin impurity A and pravastatin is at least 7.0.

Calculate the content of $C_{23}H_{35}NaO_7$ in the tablets using the declared content of $C_{23}H_{36}O_7$ in *pravastatin 1,1,3,3,-tetramethylbutylamine EPCRS*. Each mg of $C_{23}H_{36}O_7$ is equivalent to 1.052 mg of $C_{23}H_{35}NaO_7$.

STORAGE

Pravastatin Tablets should be protected from light and moisture.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Pravastatin Sodium.

Prazosin Tablets

General Notices

Action and use

Alpha$_1$-adrenoceptor antagonist.

DEFINITION

Prazosin Tablets contain Prazosin Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prazosin, $C_{19}H_{21}N_5O_4$

90.0 to 110% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing the equivalent of 10 mg of prazosin with a mixture of 10 ml of *dichloromethane* and 10 ml of 0.05M *potassium hydroxide*, filter the organic layer through absorbent cotton, evaporate to dryness and dry the residue at 60° at a pressure not exceeding 2 kPa for 2 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of prazosin (*RS 281*).

TESTS

Uniformity of content

Tablets containing less than the equivalent of 2 mg of prazosin comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 96 volumes of *methanol*, 2 volumes of *glacial acetic acid* and 2 volumes of *water*. Solution (1) contains 0.00220% w/v of *prazosin hydrochloride BPCRS*. For solution (2) shake one tablet for 1 hour in a suitable volume of the solvent to produce a solution containing the equivalent of 0.002% w/v of prazosin, centrifuge and use the supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *silica gel for chromatography* (5 µm) (Zorbax Sil is suitable), (b) 0.01% w/v of *diethylamine* in a mixture of 96 volumes of *methanol*, 2 volumes of *glacial acetic acid* and 2 volumes of *water* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{19}H_{21}N_5O_4$ using the declared content of $C_{19}H_{21}N_5O_4$ in *prazosin hydrochloride BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel GF$_{254}$ precoated plate (Analtech Uniplates are suitable) and a mixture of 95 volumes of *ethyl acetate* and 5 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 5 mg of prazosin with 2 ml of a mixture of 95 volumes of *chloroform* and 5 volumes of *diethylamine*, centrifuge and pass the supernatant liquid through a 0.5 µm PTFE filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with the same solvent. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 96 volumes of *methanol*, 2 volumes of *glacial acetic acid* and 2 volumes of *water*. Solution (1) contains 0.00220% of *prazosin hydrochloride BPCRS*. For solution (2) shake a quantity of the powdered tablets containing the equivalent of 2 mg of prazosin with 100 ml of the solvent mixture for 30 minutes, centrifuge and use the supernatant liquid.

The chromatographic procedure described under Uniformity of content may be used.

Calculate the content of $C_{19}H_{21}N_5O_4$ using the declared content of $C_{19}H_{21}N_5O_4$ in *prazosin hydrochloride BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of prazosin.

Prednisolone Enema

General Notices

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Enema is a *rectal solution* containing Prednisolone Sodium Phophate in Purified Water. It contains suitable buffering and stabilising agents and a suitable antimicrobial preservative.

The enema complies with the requirements stated under Rectal Preparations and with the following requirements.

Content of prednisolone, $C_{21}H_{28}O_5$

90.0 to 115.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak having the same retention time as the peak due to prednisolone sodium phosphate in the chromatogram obtained with solution (2).

B. Evaporate 5 ml to dryness on a water bath, cool and dissolve the residue in 0.5 ml of *sulphuric acid* . A dark red colour is produced.

TESTS

Acidity or alkalinity

pH, 5.5 to 7.5, Appendix V L.

Free prednisolone

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF_{254}* as the coating substance and a mixture of equal volumes of *acetone* and *toluene* as the mobile phase. Apply separately to the plate 25 µl of each of the following solutions. For solution (1) dilute a volume of the enema containing the equivalent of 2.0 mg of prednisolone with sufficient *water* to produce 25 ml, add 2.5 g of *sodium chloride* and 1 ml of *hydrochloric acid*, extract with three 25-ml quantities of *chloroform* and wash each extract with the same 1 ml quantity of 0.1M *hydrochloric acid* in a second separating funnel. Evaporate the combined chloroform extracts to about 5 ml using a rotary evaporator, transfer to a 15 ml stoppered tube and rinse the rotary evaporator flask with a few millilitres of *chloroform*, adding the rinsings to the tube. Evaporate in a gentle current of nitrogen and dissolve the residue in 1 ml of *chloroform*. Solution (2) contains 0.0134% w/v of *prednisolone BPCRS* in *chloroform*. After removal of the plate, allow it to dry and examine under *ultraviolet light* (*254 nm*). Any spot in the chromatogram obtained with solution (1) corresponding to prednisolone is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute, if necessary, a quantity of the enema containing the equivalent of 2 mg of prednisolone to 10 ml with *water*, add 10 ml of *water*, extract with three 20-ml quantities of *chloroform* and discard the lower layer. For solution (2) dissolve 55 mg of *prednisolone sodium phosphate BPCRS* in *water* and dilute to 200 ml (solution A); to 10 ml of solution A add 10 ml of a 0.035% w/v solution of *betamethasone sodium phosphate* in *water* (internal standard), extract with three 20 ml quantities of *chloroform* and discard the lower layer. Prepare solution (3) in the same manner as solution (1) but add 10 ml of a 0.035% w/v solution of *betamethasone sodium phosphate* in *water* in place of the 10 ml of water.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 247 nm.

Calculate the content of $C_{21}H_{28}O_5$ in solution A by measuring the *absorbance*, Appendix II B, of a solution obtained by diluting 1 volume of solution A to 10 volumes with *water* at the maximum at 247 nm and taking 419 as the value of A(1%, 1 cm) at the maximum at 247 nm. Calculate the content of $C_{21}H_{28}O_5$ in the enema using peak areas.

STORAGE

Prednisolone Enema should be protected from light.

LABELLING

The content of Prednisolone Sodium Phosphate is stated in terms of the equivalent amount of prednisolone.

The label states (1) the date after which the enema is not intended to be used; (2) the conditions under which it should be stored.

Prednisolone Sodium Phosphate Ear Drops

General Notices

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Sodium Phosphate Ear Drops are a solution of Prednisolone Sodium Phosphate in Purified Water.

The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of prednisolone sodium phosphate, $C_{21}H_{27}Na_2O_8P$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

For ear drops containing less than 0.1% w/v of Prednisolone Sodium Phosphate carry out test B only.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Use the ear drops diluted, if necessary, with *water* to contain 0.1% w/v of Prednisolone Sodium Phosphate.

(2) 0.1% w/v of *prednisolone sodium phosphate BPCRS.*

(3) A mixture of equal volumes of solutions (1) and (2).

(4) A mixture of equal volumes of solution (2) and a 0.1% w/v solution of *betamethasone sodium phosphate BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase described below.

(c) Apply 10 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and examine under *ultraviolet light* (*254 nm*).

MOBILE PHASE

33 volumes of *water,* 47 volumes of *glacial acetic acid* and 120 volumes of *butan-1-ol*, prepared immediately before use.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

CONFIRMATION

The chromatograms obtained with solutions (1), (2) and (3) show single principal spots with similar Rf values.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to prednisolone sodium phosphate in the chromatogram obtained with solution (2).

C. To a volume containing 0.2 mg of Prednisolone Sodium Phosphate slowly add 1 ml of *sulphuric acid* and allow to stand for 2 minutes. A deep red colour is produced.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.5, Appendix V L.

Free Prednisolone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.00004% w/v of *prednisolone BPCRS* in the mobile phase.

(2) Dilute the ear drops, if necessary, with the mobile phase to produce a solution containing 0.010% w/v of Prednisolone Sodium Phosphate.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 0.2 ml of each solution.

MOBILE PHASE

45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0.*

LIMITS

In the chromatogram obtained with solution (2) the area of any peak corresponding to prednisolone is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (4%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*.

(1) Dilute a quantity of the ear drops with *water*, if necessary, to contain 0.001% w/v of

Prednisolone Sodium Phosphate.

(2) 0.0025% w/v of *prednisolone sodium phosphate BPCRS.*

(3) 0.001% w/v of *prednisolone sodium phosphate BPCRS.*

(4) 0.001% w/v of *each of prednisolone sodium phosphate BPCRS* and *betamethasone sodium phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 247 nm.

(f) Inject 50 µl of each solution.

MOBILE PHASE

45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0.*

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to betamethasone sodium phosphate and prednisolone sodium phosphate is at least 2.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{21}H_{27}Na_2O_8P$ in solution (2) by measuring the *absorbance*, Appendix II B, of solution (2) at the maximum at 247 nm and taking 312 as the value of A(1%, 1 cm) at the maximum at 247 nm. Calculate the content of $C_{21}H_{27}Na_2O_8P$ in the ear drops using the calculated content of $C_{21}H_{27}Na_2O_8P$ in *prednisolone sodium phosphate BPCRS*.

STORAGE

Prednisolone Sodium Phosphate Ear Drops should be stored protected from light.

Prednisolone Sodium Phosphate Eye Drops

General Notices

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Sodium Phosphate Eye Drops are a sterile solution of Prednisolone Sodium Phosphate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of prednisolone sodium phosphate, $C_{21}H_{27}Na_2O_8P$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

For eye drops containing less than 0.1% w/v of Prednisolone Sodium Phosphate carry out test B only.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 33 volumes of *water,* 47 volumes of *glacial acetic acid* and 120 volumes of *butan-1-ol*, prepared immediately before use, as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use the eye drops diluted, if necessary, with *water* to contain 0.1% w/v of Prednisolone Sodium Phosphate. Solution (2) contains 0.1% w/v of *prednisolone sodium phosphate BPCRS* in *water*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). Solution (4) is a mixture of equal volumes of solution (2) and a 0.1% w/v solution of *betamethasone sodium phosphate BPCRS* in *water*. After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and examine under *ultraviolet light* (*254 nm*). The chromatograms obtained with solutions (1), (2) and (3) show single principal spots with similar Rf values. The chromatogram obtained with solution (4) shows two principal spots with almost identical Rf values.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to prednisolone sodium phosphate in the chromatogram obtained with solution (2).

C. To a volume containing 0.2 mg of Prednisolone Sodium Phosphate slowly add 1 ml of *sulphuric acid* and allow to stand for 2 minutes. A deep red colour is produced.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.5, Appendix V L.

Free prednisolone

Carry out the method for *liquid chromatography*, Appendix III D. For eye drops containing 0.010% w/v or more of Prednisolone Sodium Phosphate use 50 µl of the following solutions. Solution (1) contains 0.00040% w/v of *prednisolone BPCRS* in the mobile phase. Prepare solution (2) in the following manner. Dilute the eye drops, if necessary, with the mobile phase to produce a solution containing 0.010% w/v of Prednisolone Sodium Phosphate.

For eye drops containing less than 0.010% w/v of Prednisolone Sodium Phosphate, use 0.2 ml of the following solutions. Solution (1) contains 0.00004% w/v of *prednisolone BPCRS* in the mobile phase. For solution (2) dilute the eye drops, if necessary, with the mobile phase to produce a solution containing 0.0010% w/v of Prednisolone Sodium Phosphate.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 247 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to prednisolone is not greater than the area of the principal peak in the chromatogram obtained with solution (1) (4%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using 50 µl of the following solutions. For solution (1) dilute a quantity of the eye drops, if necessary, to contain 0.001% w/v of Prednisolone Sodium Phosphate. For solution (2) dissolve 10 mg of *prednisolone sodium phosphate BPCRS* in sufficient *water* to produce 100 ml (solution A) and dilute 10 ml of the solution to 100 ml. For solution (3) add 10 ml of a 0.01% w/v solution of *betamethasone sodium phosphate BPCRS* in *water* to 10 ml of solution A and dilute to 100 ml with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 247 nm.

The assay is not valid unless the *resolution factor* between the peaks due to betamethasone sodium phosphate and prednisolone sodium phosphate in the chromatogram obtained with solution (3) is at least 2.5.

Calculate the content of $C_{21}H_{27}Na_2O_8P$ in solution A by measuring the *absorbance*, Appendix II B, of a solution obtained by diluting 1 volume of solution A to 4 volumes with *water* at the maximum at 247 nm and taking 312 as the value of A(1%, 1 cm) at the maximum at 247 nm. Calculate the content of $C_{21}H_{27}Na_2O_8P$ in the eye drops using peak areas.

STORAGE

Prednisolone Sodium Phosphate Eye Drops should be stored protected from light.

Prednisolone Tablets

General Notices

Action and use

Glucocorticoid.

DEFINITION

Prednisolone Tablets contain Prednisolone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prednisolone, $C_{21}H_{28}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets with *acetone*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of prednisolone (*RS 282*).

B. Complies with the test for the Identification of steroids, Appendix III A, using *impregnating solvent I* and *mobile phase A*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute.

Carry out the method for *liquid chromatography*, Appendix III D, injecting 0.2 ml of each of the following solutions. For solution (1) prepare a 0.1% w/v solution of *prednisolone BPCRS* in *methanol* and dilute with *water* to contain 0.0001% w/v. For solution (2) use the dissolution medium diluted, if necessary, with *water* to contain 0.0001% w/v of prednisolone.

The chromatographic procedure described under Uniformity of content may be used.

The test is not valid unless the *column efficiency*, determined using the peak due to prednisolone in the chromatogram obtained with solution (1), is greater than 15,000 theoretical plates per metre.

Calculate the total content of $C_{21}H_{28}O_5$ in the medium using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Prednisolone with 25 ml of *methanol* for 10 minutes and mix with the aid of ultrasound for 2 minutes; filter the extract (Whatman GF/F is suitable), wash the filter with two 10 ml quantities of *methanol*, combine the filtrate and washings and evaporate to dryness using a rotary evaporator and a warm water bath; dissolve the residue in 10 ml of *tetrahydrofuran* and dilute to 20 ml with *water*. Solution (2) contains 0.002% w/v each of *prednisolone BPCRS* and *hydrocortisone BPCRS* in the mobile phase. For solution (3) dilute 1 volume of solution (1) to 100 volumes with a 50% v/v solution of *tetrahydrofuran*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Alltima C18 is suitable) and maintained at 45°, (b) as mobile phase at a flow rate of 1 ml per minute a mixture prepared as follows: mix 220 volumes of *tetrahydrofuran* with 700 volumes of *water*, allow to equilibrate, dilute to 1000 volumes with *water* and mix and (c) a detection wavelength of 254 nm.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 30 minutes. Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with solution (3) is at least 50% of the full scale of the recorder.

Inject 20 µl of solution (2). When the chromatograms are recorded in the prescribed conditions, the retention times are: prednisolone about 14 minutes and hydrocortisone about 15.5 minutes. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of *tetrahydrofuran* in the mobile phase.

Inject separately 20 µl of the solvent mixture of solution (1) as a blank, 20 µl of solution (1) and of solution (3). Continue the chromatography for 4.5 times the retention time of the principal peak.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%) and the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (3) (3%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%) and any peak with a retention time of 3 minutes or less.

Uniformity of content

Tablets containing less than 2 mg of Prednisolone comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0050% w/v of *prednisolone BPCRS* and 0.0075% w/v of *dexamethasone* (internal standard) in the mobile phase. For solution (2) add 20 ml of the mobile phase to one tablet, mix with the aid of ultrasound for at least 10 minutes, mix thoroughly, centrifuge and use the supernatant liquid. Prepare solution (3) in the same manner as solution (2) but using a 0.0075% w/v solution of the internal standard in the mobile phase in place of the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 58 volumes of *methanol* and 42 volumes of *water* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

The test is not valid unless the *resolution factor* between the peaks due to prednisolone and dexamethasone is greater than 2.5 and the *column efficiency*, determined using the peak due to prednisolone in the chromatogram obtained with solution (1), is greater than 15,000 theoretical plates per metre.

Calculate the content of $C_{21}H_{28}O_5$ using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *prednisolone BPCRS* and 0.0075% w/v of *dexamethasone* (internal standard) in a mixture of 58 volumes of *methanol* and 42 volumes of *water*. For solution (2) add 58 ml of *methanol* to a quantity of the powder containing 5 mg of Prednisolone, shake for 10 minutes, add sufficient *water* to produce 100 ml, mix and filter. Prepare solution (3) in the same manner as solution (2) but add 10 ml of a 0.075% w/v solution of *dexamethasone* in *methanol* and 48 ml of *methanol* in place of the 58 ml of methanol.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 42 volumes of *water* and 58 volumes of *methanol* as the mobile phase with a flow rate of about 1 ml per minute and (c) a detection wavelength of 254 nm.

The assay is not valid unless the *resolution factor* between the peaks due to prednisolone and dexamethasone is greater than 2.5 and the *column efficiency*, determined using the peak due to prednisolone in the chromatogram obtained with solution (1), is greater than 15,000 *theoretical plates* per metre.

Calculate the content of $C_{21}H_{28}O_5$ in the tablets using the declared content of $C_{21}H_{28}O_5$ in *prednisolone BPCRS*.

STORAGE

Prednisolone Tablets should be protected from light.

Prilocaine Injection

General Notices

Action and use

Local anaesthetic.

DEFINITION

Prilocaine Injection is a sterile solution of Prilocaine Hydrochloride in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of prilocaine hydrochloride, $C_{13}H_{20}N_2O,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. To a volume containing 0.1 g of Prilocaine Hydrochloride add 2M *sodium hydroxide* until the solution is about pH 11. Add 20 ml of *ethyl acetate*, shake, allow the layers to separate, dry the organic layer over *anhydrous calcium chloride* at a pressure of 2 kPa, filter and evaporate the filtrate to dryness under reduced pressure. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of prilocaine (*RS 284*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (3) has the same retention time as the peak due to prilocaine in the chromatogram obtained with solution (1).

TESTS

Acidity

pH, 5.0 to 7.0, Appendix V L.

Aromatic amines

To 1 ml of the injection, diluted if necessary to contain 0.05% w/v of Prilocaine Hydrochloride, add 1 ml of a 1% w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid*. Shake and allow to stand for 10 minutes. Measure the *absorbance* of the solution at 430 nm, Appendix II B, using *water* in the reference cell. The absorbance is not more intense than that produced in a solution prepared at the same time and in the same manner but using 1 ml of a solution in *water* containing 5 µg per ml of o-*toluidine hydrochloride* (0.75%, calculated as *o*-toluidine).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.01% w/v of *prilocaine hydrochloride BPCRS* in the mobile phase. For solution (2) dilute the injection with the mobile phase to produce a solution containing 0.01% w/v of Prilocaine Hydrochloride.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of 30 volumes of 0.05M *mixed phosphate buffer pH 8* and 70 volumes of *methanol* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 240 nm.

Calculate the content of $C_{13}H_{20}N_2O,HCl$ in the injection using the declared content of $C_{13}H_{20}N_2O,HCl$ in *prilocaine hydrochloride BPCRS*.

Procainamide Tablets

General Notices

Action and use

Class I antiarrhythmic.

DEFINITION

Procainamide Tablets contain Procainamide Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of procainamide hydrochloride, $C_{13}H_{21}N_3O,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.25 g of Procainamide Hydrochloride with 25 ml of *water*, make alkaline with 5M *sodium hydroxide* and extract with two 5 ml quantities of *chloroform*. Filter the combined extracts through *anhydrous sodium sulphate*, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 5 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of procainamide (*RS 288*).

B. Triturate a quantity of the powdered tablets containing 2 g of Procainamide Hydrochloride with 20 ml of *water* and filter. Reserve 10 ml of the filtrate for test C. To the remainder add 10 ml of 5M *sodium hydroxide* and extract with 10 ml of *chloroform*. Add 10 ml of *toluene* to the extract, dry over *anhydrous sodium sulphate* and filter. Mix the filtrate with 5 ml of dry *pyridine*, add 1 ml of *benzoyl chloride* drop wise, heat on a water bath for 30 minutes and pour into 100 ml of 2.5M *sodium hydroxide*. Extract with 10 ml of *ether*, wash the extract with 20 ml of *water*, dilute with 30 ml of *ether* and allow to crystallise. The *melting point* of the crystals, after recrystallisation from *ethanol* (*45%*), is about 186°, Appendix V A.

C. 10 ml of the filtrate reserved in test B yields the reactions characteristic of *chlorides*, Appendix VI.

Related substances

Comply with the test described under Procainamide Injection using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.4 g of Procainamide Hydrochloride with 20 ml of *methanol* (*90%*) for 15 minutes and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.5 g of Procainamide Hydrochloride in a stoppered flask add 100 ml of 6M *hydrochloric acid*, shake for 15 minutes and titrate with 0.1M *sodium nitrite VS*, using *ferrocyphen solution* as indicator, until a violet colour is produced that is stable for not less than 3 minutes. Repeat the operation without the powdered tablets. The difference between the titrations represents the amount of sodium nitrite required. Each ml of 0.1M *sodium nitrite VS* is equivalent to 27.18 mg of $C_{13}H_{21}N_3O$, HCl.

Prochlorperazine Buccal Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Prochlorperazine Buccal Tablets contain Prochlorperazine Maleate in a suitable basis.

The tablets comply with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of prochlorperazine maleate, $C_{20}H_{24}ClN_3S,2C_4H_4O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 24 mg of Prochlorperazine Maleate add 10 ml of *water* and 1 ml of 1M *sodium hydroxide*, shake, extract with 25 ml of *dichloromethane* and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of prochlorperazine (*RS 390*).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *acetone*, 10 volumes of *diethylamine*, and 80 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 60 mg of Prochlorperazine Maleate with 5 ml of 0.18M *methanolic ammonia*, shake for 15 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.006% w/v of *prochlorperazine maleate BPCRS* in 0.18M *methanolic ammonia*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing 10 mg of Prochlorperazine Maleate add 50 ml of 1M *methanesulphonic acid*, shake for 5 minutes and disperse with the aid of ultrasound for 2 minutes. Add sufficient 1M *methanesulphonic acid* to produce 100 ml, mix, centrifuge the mixture and dilute 10 ml of the supernatant liquid to 100 ml with *water*. Measure the *absorbance* of the resulting solution at 254 nm, Appendix II B, using 0.1M *methanesulphonic acid* in the reference cell. Measure the *absorbance* of a solution prepared by diluting 1 volume of a 0.01% w/v solution of *prochlorperazine maleate BPCRS* in 1M *methanesulphonic acid* to 10 volumes with *water* using 0.1M *methanesulphonic acid* in the reference cell. Calculate the content of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$ from the absorbances obtained and from the declared content of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$ in *prochlorperazine maleate BPCRS*.

STORAGE

Prochlorperazine Buccal Tablets should be protected from light.

Prochlorperazine Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Prochlorperazine Injection is a sterile solution of Prochlorperazine Mesilate in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of prochlorperazine mesilate, $C_{20}H_{24}ClN_3S,2CH_4SO_3$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume containing 0.1 g of Prochlorperazine Mesilate add 20 ml of *water* and 2 ml of 10M *sodium hydroxide*. Shake and extract the mixture with 25 ml of *ether*. Wash the ether layer with two 5 ml quantities of *water*, dry with *anhydrous sodium sulphate* and evaporate to dryness. Dissolve the residue in 1 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of prochlorperazine (*RS 289*).

B. To a volume containing 5 mg of Prochlorperazine Mesilate add carefully 2 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. To a volume containing 0.2 g of Prochlorperazine Mesilate add 1.5 ml of 5M *sodium hydroxide* and extract with 10 ml of *ether*. Wash the ether layer with two 5 ml quantities of *water*, evaporate the ether layer to dryness, dissolve the residue in 10 ml of *methanol* and add a solution of 0.3 g of *picric acid* in a mixture of 5 ml of *methanol* and 5 ml of *water*. The *melting point* of the precipitate, after washing with 15 ml of *methanol*, is about 255°, with decomposition, Appendix V A.

TESTS

Acidity

pH, 5.5 to 6.5, Appendix V L.

Related substances

Carry out the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following solutions. For solution (1) use the injection being examined. For solution (2) dilute 1 volume of solution (1) to 40 volumes with *methanol* containing 0.5% v/v of 13.5M *ammonia* immediately before use. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the methanol–ammonia mixture immediately before use. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (2) (2.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the following procedure protected from light. To a quantity containing 12.5 mg of Prochlorperazine Mesilate add sufficient *absolute ethanol* containing 0.01% v/v of 13.5M *ammonia* to produce 100 ml. Dilute 5 ml of the solution to 100 ml with the ammoniacal ethanol and measure the *absorbance* of the resulting solution at the maximum at 258 nm, Appendix II B. Calculate the content of $C_{20}H_{24}ClN_3S,2CH_4SO_3$ taking 635 as the value of A(1%, 1 cm) at the maximum at 258 nm.

STORAGE

Prochlorperazine Injection should be protected from light.

Prochlorperazine Oral Solution

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Prochlorperazine Oral Solution contains Prochlorperazine Mesilate in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of prochlorperazine mesilate, $C_{20}H_{24}ClN_3S,2CH_4SO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume of the oral solution containing 50 mg of Prochlorperazine Mesilate add 50 ml of *water* and 2 ml of 10M *sodium hydroxide*, add *sodium chloride* and extract with two 50 ml quantities of *ether*. Wash the combined ether extracts with two 5 ml quantities of *water*, filter through *anhydrous sodium sulphate* and evaporate to dryness. Dissolve the residue in 10 ml of *methanol* and add a solution prepared by dissolving 0.1 g of *picric acid* in a mixture of 5 ml of *methanol* and 5 ml of *water*, wash the resulting precipitate with 15 ml of *methanol* and dry at 100° for 40 minutes. The *melting point* of the dried precipitate is about 255°, with decomposition, Appendix V A.

B. In the test for Prochlorperazine sulphoxide, the principal spot in the chromatogram obtained with solution (2) is similar in position, size and intensity to that in the chromatogram obtained with solution (3).

Prochlorperazine sulphoxide

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a precoated silica gel F_{254} plate activated by drying at 105° for 1 hour and a mixture of 5 volumes of *diethylamine*, 45 volumes of *acetone* and 50 volumes of *hexane* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) mix a quantity of the oral solution containing 10 mg of Prochlorperazine Mesilate with 35 ml of *water* and 5 ml of a 20% w/v solution of *sodium hydroxide*, extract with four 40 ml quantities of *chloroform*, shaking for 2 minutes each time, and filter each extract through *anhydrous sodium sulphate* washing the sodium sulphate with 5 ml of *chloroform*. Evaporate the combined chloroform extracts and washings to dryness at 30° under reduced pressure using a current of *nitrogen* to remove the last of the solvent and dissolve the residue in 1 ml of a mixture of equal volumes of *chloroform* and *methanol*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with a mixture of equal volumes of *chloroform* and *methanol*. Solution (3) contains 0.1% w/v of *prochlorperazine mesilate BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*. Solution (4) contains 0.03% w/v of *prochlorperazine sulphoxide BPCRS* in a mixture of equal volumes of *chloroform* and *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any spot corresponding to prochlorperazine sulphoxide in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (3%).

ASSAY

Carry out the following procedure protected from light. To a weighed quantity containing 10 mg of Prochlorperazine Mesilate add 25 ml of *water* and 5 ml of a 10% w/v solution of *sodium hydroxide*. Extract the mixture with three 50 ml quantities of *chloroform*, shaking vigorously for 1 minute, allowing to separate and shaking vigorously for two further 1-minute intervals each time. Evaporate the combined extracts to dryness at about 30° under reduced pressure and dissolve the residue in sufficient 0.1M *hydrochloric acid* to produce 25 ml (solution A). Dilute 10 ml of solution A to 50 ml with *water* (solution B). To a further 10 ml of solution A add 5 ml of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50 ml (solution C). Measure the *absorbance* of solution C at the maximum at 343 nm, Appendix II B, using solution B in the reference cell. Repeat the procedure using a 0.04% w/v solution of *prochlorperazine mesilate BPCRS* in 0.1M *hydrochloric acid* in place of solution A and beginning at the words 'Dilute 10 ml of...'. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{20}H_{24}ClN_3S,2CH_4SO_3$, weight in volume, using the declared content of $C_{20}H_{24}ClN_3S,2CH_4SO_3$ in *prochlorperazine mesilate BPCRS*.

STORAGE

Prochlorperazine Oral Solution should be protected from light.

Prochlorperazine Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Prochlorperazine Tablets contain Prochlorperazine Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of prochlorperazine maleate, $C_{20}H_{24}ClN_3S,2C_4H_4O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 50 mg of Prochlorperazine Maleate add 20 ml of *water* and 2 ml of 1M *sodium hydroxide*, shake and extract with 50 ml of *dichloromethane* and evaporate the dichloromethane layer to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of prochlorperazine (*RS 390*).

B. To a quantity of the powdered tablets containing 5 mg of Prochlorperazine Maleate add 5 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. To a quantity of the powdered tablets containing 0.2 g of Prochlorperazine Maleate add 2 ml of *water* and 1 ml of 5M *sodium hydroxide*, mix and extract with three 10 ml quantities of *ether*. Dry the combined extracts with *anhydrous sodium sulphate*, filter, evaporate to dryness, dissolve the residue in 10 ml of *methanol* and add a solution of 0.15 g of *picric acid* in 10 ml of *methanol*. The *melting point* of the precipitate, after washing with a small quantity of *methanol*, is about 255°, with decomposition, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using a silica gel GF_{254} precoated plate (Merck plates are suitable) and a mixture of 10 volumes of *acetone*, 10 volumes of *diethylamine* and 80 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 60 mg of Prochlorperazine Maleate with 5 ml of 0.18M *methanolic ammonia*, shake for 15 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.006% w/v of *prochlorperazine maleate BPCRS* in 0.18M *methanolic ammonia*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing 10 mg of Prochlorperazine Maleate add 50 ml of 1M *methanesulphonic acid*, shake for 5 minutes and disperse with the aid of ultrasound for 2 minutes. Add sufficient 1M *methanesulphonic acid* to produce 100 ml, mix, centrifuge the mixture and dilute 10 ml of the supernatant liquid to 100 ml with *water*. Measure the *absorbance* of the resulting solution at 254 nm, Appendix II B, using 0.1M *methanesulphonic acid* in the reference cell. Measure the *absorbance* of a solution prepared by diluting 1 volume of a 0.01% w/v solution of *prochlorperazine maleate BPCRS* in 1M *methanesulphonic acid* to 10 volumes with *water* using 0.1M *methanesulphonic acid* in the reference cell. Calculate the content of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$ from the absorbances obtained and from the declared content of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$ in *prochlorperazine maleate BPCRS*.

STORAGE

Prochlorperazine Tablets should be protected from light.

Procyclidine Injection

General Notices

Action and use

Anticholinergic.

DEFINITION

Procyclidine Injection is a sterile solution of Procyclidine Hydrochloride in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of procyclidine hydrochloride, $C_{19}H_{29}NO,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Extract a volume of the injection containing 30 mg of Procyclidine Hydrochloride with three 5 ml quantities of *dichloromethane*, dry the combined extracts with *anhydrous sodium sulphate*, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of procyclidine hydrochloride (*RS 292*).

B. Carry out the method described in the test for Related substances under Procyclidine Tablets but applying 2 µl of each of the following solutions. For solution (1) dilute the injection, if necessary, with *water* to give a solution containing 0.50% w/v of Procyclidine Hydrochloride. Solution (2) contains 0.50% w/v of *procyclidine hydrochloride BPCRS*. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 6.8, Appendix V L.

Related substances

Complies with the test for Related substances described under Procyclidine Tablets but applying 20 µl of each of the following solutions. For solution (1) dilute the injection, if necessary, with *water* to give a solution containing 0.50% w/v of Procyclidine Hydrochloride. Solution (2) contains 0.0010% w/v of *1-phenyl-3-pyrrolidinopropan-1-one hydrochloride BPCRS* in *chloroform*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *water*.

ASSAY

Record *second-derivative ultraviolet absorption spectra*, Appendix II B, of the following solutions in the range 220 to 280 nm. For solution (1) dilute a volume of the injection containing 25 mg of Procyclidine Hydrochloride to 100 ml with 0.1M *hydrochloric acid*. Solution (2) contains 0.025% w/v of *procyclidine hydrochloride BPCRS* in 0.1M *hydrochloric acid*.

For each solution measure the amplitude from the largest trough at about 257 nm to the largest peak at about 254 nm. Calculate the content of $C_{19}H_{29}NO,HCl$ using the declared content of $C_{19}H_{29}NO,HCl$ in *procyclidine hydrochloride BPCRS*.

Procyclidine Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Procyclidine Tablets contain Procyclidine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of procyclidine hydrochloride, $C_{19}H_{29}NO,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the powdered tablets containing 25 mg of Procyclidine Hydrochloride in 10 ml of *water*, shake with 20 ml of *ether* and discard the ether layer. Make the aqueous layer alkaline with 2M *sodium hydroxide* and extract with two 20 ml quantities of *ether*. Wash the combined ether extracts with two 10 ml quantities of *water*, dry by shaking with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. If necessary, induce crystallisation by scratching with a glass rod. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of procyclidine (*RS 291*).

B. The powdered tablets yield the reactions characteristic of *chlorides*, Appendix VI.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of 13.5M *ammonia* and 100 volumes of *ether* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Procyclidine Hydrochloride with 5 ml of *chloroform* and filter. Solution (2) contains 0.001% w/v of *1-phenyl-3-pyrrolidinopropan-1-one hydrochloride BPCRS* in *chloroform*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *chloroform*. After removal of the plate, dry it at 105° for 15 minutes and examine under *ultraviolet light* (*254 nm*). Any spot corresponding to 1-phenyl-3-pyrrolidinopropan-1-one in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%). Spray the plate with *dilute potassium iodobismuthate solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Disregard any spot due to excipient on the line of application.

ASSAY

Weigh and finely powder 20 tablets. Record *second-derivative ultraviolet absorption spectra* of the following solutions, Appendix II B, in the range 220 to 280 nm. For solution (1) add 80 ml of 0.1M *hydrochloric acid* to a quantity of the powdered tablets containing 25 mg of

Procyclidine Hydrochloride, mix with the aid of ultrasound for 15 minutes, cool, dilute to 100 ml with 0.1M *hydrochloric acid* and filter through a glass fibre paper having a maximum pore size of 0.7 µm (Whatman GF/F paper is suitable) discarding the first 10 ml of filtrate. Solution (2) contains 0.025% w/v of *procyclidine hydrochloride BPCRS* in 0.1M *hydrochloric acid*.

For each solution measure the amplitude from the largest trough at about 257 nm to the largest peak at about 254 nm. Calculate the content of $C_{19}H_{29}NO,HCl$ using the declared content of $C_{19}H_{29}NO,HCl$ in *procyclidine hydrochloride BPCRS*.

Progesterone Injection

General Notices

Action and use

Progestogen.

DEFINITION

Progesterone Injection is a sterile solution of Progesterone in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these. It may contain suitable alcohols.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of progesterone, $C_{21}H_{30}O_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Dissolve a volume containing 50 mg of Progesterone in 8 ml of *petroleum spirit* (*boiling range, 40° to 60°*) and extract with three 8 ml quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *petroleum spirit* (*boiling range, 40° to 60°*), dilute with *water* until the solution becomes turbid, allow to stand in ice for 2 hours and filter. The precipitate, after washing with *water* and drying at 105°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of progesterone (*RS 293*).

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase E*.

ASSAY

To a quantity containing 50 mg of Progesterone add sufficient *chloroform* to produce 100 ml. Dilute 3 ml to 50 ml with *chloroform* and to 5 ml of the solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20 ml. Allow to stand for 45 minutes and measure the *absorbance* of the resulting solution at the maximum at 380 nm, Appendix II B, using in the reference cell 5 ml of *chloroform* treated in the same manner. Calculate the content of $C_{21}H_{30}O_2$ from the *absorbance* obtained by repeating the operation using a 0.003% w/v solution of *progesterone BPCRS* in *chloroform* and beginning at the words 'to 5 ml ...'.

STORAGE

Progesterone Injection should be protected from light. On standing, solid matter may separate; it should be redissolved by heating before use.

LABELLING

The label states that the preparation is intended for intramuscular injection only.

Proguanil Tablets

General Notices

Action and use

Antiprotozoal (malaria).

DEFINITION

Proguanil Tablets contain Proguanil Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of proguanil hydrochloride, $C_{11}H_{16}ClN_5,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 0.2 g of Proguanil Hydrochloride add 25 ml of *methanol*, shake for 10 minutes, filter, evaporate the filtrate to dryness and dry the residue at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of proguanil hydrochloride (*RS 294*).

B. Boil a quantity of the powdered tablets containing 0.5 g of Proguanil Hydrochloride with 5 ml of 2M *hydrochloric acid*, cool and filter. To the filtrate add a slight excess of 5M *sodium hydroxide*, extract with 30 ml of *ether*, evaporate the ether and dry the residue at 105°. The *melting point* of the residue is about 131°, Appendix V A.

TESTS

4-Chloroaniline

To a quantity of the powdered tablets containing 0.1 g of Proguanil Hydrochloride add 5 ml of *ethanol* (*96%*) and shake for 10 minutes. Add 2.5 ml of 2M *hydrochloric acid* and 15 ml of *water*, mix and filter through a moistened filter paper, washing the filter with 5 ml of *water*. Cool the filtrate to 5°, add 1 ml of 0.05M *sodium nitrite*, allow to stand at 5° for 5 minutes, add 2 ml of a 5% w/v solution of *ammonium sulphamate* and allow to stand for 10 minutes. Add 2 ml of a 0.1% w/v solution of N-(*1-naphthyl*)*ethylenediamine dihydrochloride*, dilute to 50 ml with *water* and allow to stand for 30 minutes. Any magenta colour produced is not more intense than that obtained by treating in the same manner and at the same time 20 ml of a solution containing 1.25 µg per ml of *4-chloroaniline* (250 ppm).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 50 ml of *methanol* to a quantity of the powdered tablets containing 0.1 g of Proguanil Hydrochloride, shake for 10 minutes, dilute to 100 ml with *methanol*, mix, filter and dilute 5 ml of the filtrate to 50 ml with the mobile phase. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) mix 1 volume of a 0.05%

w/v solution of *1-(2,5-dichlorophenyl)-5-isopropylbiguanide hydrochloride BPCRS* in *methanol* with 9 volumes of a 0.010% w/v solution of *proguanil hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared by dissolving 1.89 g of *sodium hexanesulphonate* in a mixture of 500 ml of *methanol*, 500 ml of *water* and 5 ml of *glacial acetic acid* and (c) a detection wavelength of 254 nm.

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated peaks due to proguanil hydrochloride and 1-(2,5-dichlorophenyl)-5-isopropylbiguanide hydrochloride.

The sum of the areas of any *secondary peaks* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.2M *hydrochloric acid* containing 0.2% w/v of *sodium chloride* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute 2 ml of the filtrate to 10 ml with dissolution medium. Measure the *absorbance* of this solution, Appendix II B, at 242 nm using dissolution medium in the reference cell. Measure the *absorbance* of a 0.001% w/v solution of *proguanil hydrochloride BPCRS* in dissolution medium using dissolution medium in the reference cell. Calculate the total content of proguanil hydrochloride, $C_{11}H_{16}ClN_5,HCl$, in the medium from the absorbances obtained and from the declared content of $C_{11}H_{16}ClN_5,HCl$ in *proguanil hydrochloride BPCRS*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) weigh and powder 20 tablets, add 50 ml of *methanol* to a quantity of the powdered tablets containing 0.1 g of Proguanil Hydrochloride, shake for 10 minutes, dilute to 100 ml with *methanol*, mix and filter. To 5 ml of the filtrate add sufficient *methanol* to produce 50 ml and dilute 10 ml of the resulting solution to 50 ml with the mobile phase. Solution (2) contains 0.002% w/v of *proguanil hydrochloride BPCRS* in the mobile phase. For solution (3) mix 1 volume of a 0.05% w/v solution of *1-(2,5-dichlorophenyl)-5-isopropylbiguanide hydrochloride BPCRS* in *methanol* with 9 volumes of a 0.010% w/v solution of *proguanil hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

The assay is not valid unless the chromatogram obtained with solution (3) shows two clearly separated peaks due to proguanil hydrochloride and 1-(2,5-dichlorophenyl)-5-isopropylbiguanide hydrochloride.

Calculate the content of $C_{11}H_{16}ClN_5,HCl$ in the tablets using the declared content of $C_{11}H_{16}ClN_5,HCl$ in *proguanil hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Proguanil Hydrochloride.

Prolonged-release Potassium Chloride Tablets

General Notices

Slow Potassium Chloride Tablets

Prolonged-release Potassium Chloride Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

DEFINITION

Prolonged-release Potassium Chloride Tablets contain Potassium Chloride and are formulated so as to release the medicament over a period of several hours. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of potassium chloride. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Stir 3 tablets in successive 20 ml portions of *water* until the outer coating is removed and separate the cores from the water. The powdered cores comply with the following tests.

A. Yield reaction A characteristic of *chlorides*, Appendix VI.

B. Yield the reactions characteristic of *potassium salts*, Appendix VI.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *water* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium after 1, 2 and 6 hours and treat each sample in the following manner. Add 25 ml of *water*, 5 ml of a 25% v/v solution of *glacial acetic acid* and 0.1 ml of a saturated solution of *potassium sulphate* and titrate with 0.01M *silver nitrate VS* determining the end point potentiometrically. Each ml of 0.01M *silver nitrate VS* is equivalent to 0.7455 mg of KCl. The amount of potassium chloride released after 1 hour is not more than 50%, after 2 hours is not less than 25% and not more than 75%, and after 6 hours is not less than 75%, calculated with reference to the declared content of Potassium Chloride.

ASSAY

Shake 10 whole tablets with 400 ml of *water* for 30 minutes then heat on a water bath for 45 minutes. Cool, add sufficient *water* to produce 500 ml and allow to stand for 24 hours. Filter and dilute to give a suitable concentration. Carry out the method for *atomic emission spectrophotometry*, Appendix II D, measuring at 766.5 nm and using *potassium standard solution* (*600 ppm K*), suitably diluted with *water*, to prepare the standard solutions. Each mg of potassium is equivalent to 1.908 mg of KCl.

Prolonged-release Propranolol Capsules

General Notices

Prolonged-release Propranolol Capsules from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Beta-adrenoceptor agonist.

DEFINITION

Prolonged-release Propranolol Capsules contain Propranolol Hydrochloride. They are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Propranolol Hydrochloride. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of propranolol hydrochloride, $C_{16}H_{21}NO_2,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. The *light absorption* of the solution obtained in the Assay, Appendix II B, exhibits two maxima, at 290 nm and 319 nm, and a shoulder at 306 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* GF_{254} *plate* and a mixture of 1 volume of 18M *ammonia* and 99 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered capsule contents containing 20 mg of Propranolol Hydrochloride with 10 ml of *methanol* for 5 minutes and filter (Whatman No. 1 paper is suitable). Solution (2) contains 0.2% w/v of *propranolol hydrochloride* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, injecting 10 µl of each of the following solutions. Solution (1) contains 0.1% w/v of *propranolol hydrochloride for performance test EPCRS* in the mobile phase. For solution (2) add 100 ml of *methanol* to a quantity of the powdered capsule contents containing 0.1 g of Propranolol Hydrochloride, mix with the aid of ultrasound for 15 minutes, shaking occasionally, filter through a glass-microfibre filter (Whatman GF/C is suitable) and use the filtrate. For solution (3) dilute 1 volume of solution (1) to 500 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5µ is suitable), (b) as the mobile phase with a flow rate of 1.8 ml per minute a mixture of 1.15 g of *sodium dodecyl sulphate*, 10 ml of a mixture of 1 volume of *sulphuric acid* and 9 volumes of *water*, 20 ml of a 1.7% w/v solution of *tetrabutylammonium dihydrogen orthophosphate*, 370 ml of *water* and 600 ml of *acetonitrile*, the mixture adjusted to pH 3.3 with 2M *sodium hydroxide*, and (c) a detection wavelength of 292 nm.

Inject 20 µl of solution (1). When the chromatogram is recorded in the prescribed conditions, the appearance and acceptance criteria specified in the leaflet which accompanies *propranolol hydrochloride for performance test EPCRS* are met.

For solutions (2) and (3) record the chromatograms for five times the retention time of the principal peak. In the chromatogram obtained with solution (2) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%) and the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (3) (0.8%).

ASSAY

To a quantity of the powdered mixed contents of 20 capsules containing 80 mg of Propranolol Hydrochloride add about 150 ml of *methanol*, heat to boiling and boil for 2 minutes, remove from the heat, shake for 20 minutes, cool to 20° and add sufficient *methanol* to produce 200 ml. Filter (Whatman No. 1 paper is suitable), discarding the first 20 ml of filtrate, and dilute 10 volumes of the filtrate to 200 volumes with *methanol*. Measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B, using *methanol* in the reference cell. Calculate the content of $C_{16}H_{21}NO_2,HCl$ taking 206 as the value of A(1%, 1 cm) at the maximum at 290 nm.

Promazine Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Promazine Injection is a sterile solution of Promazine Hydrochloride in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of promazine hydrochloride, $C_{17}H_{20}N_2S,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume containing 0.1 g of Promazine Hydrochloride add 20 ml of *water* and 2 ml of 10M *sodium hydroxide*. Shake and extract the mixture with 25 ml of *ether*. Wash the ether with two 5 ml quantities of *water*, dry with *anhydrous sodium sulphate* and evaporate the ether to dryness. Dissolve the residue in 1 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promazine (*RS 295*).

B. To a volume containing 5 mg of Promazine Hydrochloride carefully add 2 ml of *sulphuric acid* and allow to stand for 5 minutes. An orange colour is produced.

C. To a volume containing 0.2 g of Promazine Hydrochloride add 1 ml of 1M *sodium hydroxide* and extract with four 10 ml quantities of *ether*. Wash the combined extracts with 10 ml of *water*, remove the ether and dissolve the residue in 4 ml of *methanol*. Heat on a water bath almost to boiling, immediately add 2 ml of a boiling 3.5% w/v solution of *picric acid* in *methanol* and boil for 2 minutes. Cool in ice, filter, wash the crystals with three quantities of *methanol*, dissolve in 10 ml of hot *methanol* and repeat the crystallisation and washing. The *melting point* of the rust-red crystals so obtained, after drying at 105° for 1 hour, is about 144°, Appendix V A.

TESTS

Acidity

pH, 4.4 to 5.2, Appendix V L.

Related substances

Carry out the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and applying separately to the plate 10 µl of each of the following freshly prepared solutions. For solution (1) dilute a volume of the injection with sufficient *methanol* to produce a solution containing 1.0% w/v of Promazine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 40 volumes with *methanol*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *methanol*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the following procedure protected from light. To a quantity containing 50 mg of Promazine Hydrochloride add 5 ml of 2M *hydrochloric acid* and sufficient *water* to produce 1000 ml. To 10 ml add 10 ml of 0.1M *hydrochloric acid*, dilute to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 251 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 935 as the value of A(1%, 1 cm) at the maximum at 251 nm.

STORAGE

Promazine Injection should be protected from light.

Promazine Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Promazine Tablets contain Promazine Hydrochloride. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of promazine hydrochloride, $C_{17}H_{20}N_2S,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 40 mg of Promazine Hydrochloride add 10 ml of *water* and 2 ml of 1M *sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the extract with 5 ml of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 0.4 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promazine (*RS 295*).

B. To a quantity of the powdered tablets containing 5 mg of Promazine Hydrochloride add 5 ml of *sulphuric acid* and allow to stand for 5 minutes. An orange colour is produced.

C. Triturate a quantity of the powdered tablets containing 0.2 g of Promazine Hydrochloride with 4 ml of *methanol*, centrifuge, filter the supernatant liquid, heat almost to boiling, immediately add 2 ml of a boiling 3.5% w/v solution of *picric acid* in *methanol* and boil for 2 minutes. Cool in ice, filter, wash the crystals with three quantities of *methanol*, dissolve in 10 ml of hot *methanol* and repeat the crystallisation and washing. The *melting point* of the rust red crystals so obtained, after drying at 105° for 1 hour, is about 144°, Appendix V A.

Related substances

Comply with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Promazine Hydrochloride with 10 ml of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*.

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Triturate a quantity of the powder containing 50 mg of Promazine Hydrochloride with 10 ml of 2M *hydrochloric acid* and then add 200 ml of *water*. Shake for 15 minutes, add sufficient *water* to produce 500 ml and centrifuge about 50 ml of the mixture. To 5 ml of the clear, supernatant liquid add 10 ml of 0.1M *hydrochloric acid* and sufficient *water* to produce 100 ml. Measure the *absorbance* of the resulting solution at the maximum at 251 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 935 as the value of A(1%, 1 cm) at the maximum at 251 nm.

Promethazine Hydrochloride Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Promethazine Hydrochloride Tablets contain Promethazine Hydrochloride. They are coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of promethazine hydrochloride, $C_{17}H_{20}N_2S,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 40 mg of Promethazine Hydrochloride add 10 ml of *water* and 2 ml of 1M *sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the ether with 5 ml of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 0.4 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promethazine (*RS 297*).

B. To a quantity of the powdered tablets containing 5 mg of Promethazine Hydrochloride add 5 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. Dissolve a quantity of the powdered tablets containing 0.2 g of Promethazine Hydrochloride in 2 ml of *water*, filter, saturate with *potassium carbonate sesquihydrate*, extract with two 10 ml quantities of *ether* and evaporate the combined extracts to dryness. Dissolve the residue in 2 ml of *methanol* and pour into a solution of 0.4 g of *picric acid* in 10 ml of *methanol* at about 50°. Cool, scratch the side of the tube to induce crystallisation, allow to stand for 3 to 4 hours and filter. The *melting point* of the crystals, after washing with *methanol* and drying, is about 160°, Appendix V A.

Related substances

Carry out the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase B* and applying separately to the plate 20 µl of each of the following freshly prepared solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Promethazine Hydrochloride with 10 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filter. Solution (2) contains 0.010% w/v of *isopromethazine hydrochloride BPCRS* in the same solvent. For solution (3) dilute 1 volume of solution (1) to 200 volumes with the same solvent. Any spot corresponding to isopromethazine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Triturate a quantity of the powder containing 50 mg of Promethazine Hydrochloride with 10 ml of 2M *hydrochloric acid* and add 200 ml of *water*. Shake for 15 minutes, add sufficient *water* to produce 500 ml and centrifuge about 50 ml of the mixture. To 5 ml of the clear, supernatant liquid add 10 ml of 0.1M *hydrochloric acid* and sufficient *water* to produce 100 ml. Measure the *absorbance* of the resulting solution at the maximum at 249 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 910 as the value of A(1%, 1 cm) at the maximum at 249 nm.

Promethazine Injection

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Promethazine Injection is a sterile solution of Promethazine Hydrochloride in Water for Injections free from dissolved air.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of promethazine hydrochloride, $C_{17}H_{20}N_2S,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. To a volume containing 0.1 g of Promethazine Hydrochloride add 20 ml of *water* and 2 ml of 10M *sodium hydroxide*. Shake and extract the mixture with 25 ml of *ether*. Wash the ether layer with two 5 ml quantities of *water*, dry with *anhydrous sodium sulphate* and evaporate the ether. Dissolve the residue in 1 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promethazine (*RS 297*).

B. To a volume containing 5 mg of Promethazine Hydrochloride carefully add 2 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. To a volume containing 0.2 g of Promethazine Hydrochloride add sufficient *potassium carbonate sesquihydrate* to saturate the solution, extract with two 10-ml quantities of *ether* and evaporate the combined extracts to dryness. Dissolve the residue in 2 ml of *methanol* and pour into a solution of 0.4 g of *picric acid* in 10 ml of *methanol*, previously warmed to about 50°. Cool, scratch the side of the tube to induce crystallisation, allow to stand for 3 to 4 hours and filter. The *melting point* of the residue, after washing with *methanol* and drying, is about 160°, Appendix V A.

TESTS

Acidity

pH, 5.0 to 6.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light under an atmosphere of nitrogen using *silica gel GF_{254}* as the coating substance (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 15 volumes of *diethylamine*, 45 volumes of *hexane* and 50 volumes of *acetone* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following freshly prepared solutions. For solution (1) use a volume of the injection diluted, if necessary, with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol* to contain 1.0% w/v of Promethazine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 200 volumes with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. Solution (3) contains 0.01% w/v of *isopromethazine hydrochloride BPCRS* in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. Solution (4) contains 0.025% w/v of *promethazine sulphoxide BPCRS* in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. After removal of the plate, allow it to dry in air, spray with a 20% w/v solution of *perchloric acid* and heat at 100° for 5 minutes. In the chromatogram obtained with solution (1) any spot corresponding to isopromethazine is not more intense than the principal spot in the chromatogram obtained with solution (3) (1%), any spot corresponding to promethazine sulphoxide is not more intense than the spot in the chromatogram obtained with solution (4) (2.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Carry out the following procedure protected from light. To a volume containing 25 mg of Promethazine Hydrochloride add sufficient 0.01M *hydrochloric acid* to produce 100 ml. Dilute 10 ml to 100 ml with 0.01M *hydrochloric acid*, dilute 10 ml of this solution to 50 ml with 0.01M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 249 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 910 as the value of A (1%, 1 cm) at the maximum at 249 nm.

STORAGE

Promethazine Injection should be protected from light.

Promethazine Oral Solution

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Promethazine Oral Solution is a solution containing Promethazine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of promethazine hydrochloride, $C_{17}H_{20}N_2S,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Carry out the method for *identification of phenothiazines*, Appendix III A, but applying as solution (1) 5 µl of a solution prepared by diluting a suitable volume of the oral solution with *water* to contain 0.08% w/v of Promethazine Hydrochloride.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light under an atmosphere of nitrogen using *silica gel GF_{254}* as the coating substance (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 15 volumes of *diethylamine*, 45 volumes of *hexane* and 50 volumes of *acetone* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following freshly prepared solutions. For solution (1) add 50 ml of *water* and 10 ml of a 5% w/v solution of *sodium hydroxide* to a quantity of the oral solution containing 20 mg of Promethazine Hydrochloride in a separating funnel and swirl to mix. Extract with two 100 ml quantities of *chloroform*, shaking vigorously for 1 minute each time, and filter successively through *anhydrous sodium sulphate*, washing the sodium sulphate with 50 ml of *chloroform*. Evaporate the combined extracts and washings to dryness at 30° using a rotary evaporator and dissolve the residue in 2 ml of a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent. Solution (3) contains 0.01% w/v of *isopromethazine hydrochloride BPCRS* in the same solvent. Solution (4) contains 0.025% w/v of *promethazine sulphoxide BPCRS* in the same solvent. After removal of the plate, allow it to dry in air, spray with a 20% w/v solution of *perchloric acid* and heat at 100° for 5 minutes. In the chromatogram obtained with solution (1) any spot corresponding to isopromethazine is not more intense than the principal spot in the chromatogram obtained with solution (3) (1%), any spot corresponding to promethazine sulphoxide is not more intense than the principal spot in the chromatogram obtained with solution (4) (2.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Carry out the following procedure protected from light. To a weighed quantity containing 10 mg of Promethazine Hydrochloride add 25 ml of *water* and 5 ml of a 5% w/v solution of *sodium hydroxide*. Extract the mixture with two 50 ml quantities of *chloroform*, shaking vigorously for 1 minute each time, evaporate the combined extracts to dryness at about 30° at a pressure of 2 kPa and dissolve the residue in sufficient 0.1M *hydrochloric acid* to produce 50 ml (solution A). Dilute 10 ml of solution A to 50 ml with *water* (solution B). To a further 10 ml of solution A add 5 ml of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50 ml (solution C). Measure the *absorbance* of solution C at the maximum at 336 nm, Appendix II B, using solution B in the reference cell and measure the *absorbance* of solution B at the same wavelength using *water* in the reference cell. Repeat the procedure using a 0.02% w/v solution of *promethazine hydrochloride BPCRS* in 0.1M *hydrochloric acid* in place of solution A and beginning at the words 'Dilute 10 ml of...'. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{17}H_{20}N_2S,HCl$, weight in volume, using the declared content of $C_{17}H_{20}N_2S,HCl$ in *promethazine hydrochloride BPCRS*. The result is not valid if the absorbance of solution B is more than 0.10.

STORAGE

Promethazine Oral Solution should be protected from light.

Promethazine Teoclate Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Promethazine Teoclate Tablets contain Promethazine Teoclate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of promethazine teoclate, $C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing 60 mg of Promethazine Teoclate add 10 ml of *water* and 2 ml of 1M *sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the extract with 5 ml of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 0.4 ml of *chloroform*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promethazine (*RS 297*).

B. To a quantity of the powdered tablets containing 5 mg of Promethazine Teoclate add 5 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. Extract a quantity of the powdered tablets containing 0.2 g of Promethazine Teoclate with *chloroform*, filter and evaporate the filtrate to dryness. Shake the residue with 10 ml of *water*, add 4 ml of 5M *ammonia* and extract with two 30 ml quantities of *ether*. Wash the combined extracts with 10 ml of *water* and reserve the aqueous layer and washings. To the combined aqueous layer and washings add 4 ml of *hydrochloric acid*; a white precipitate is produced. Filter, wash the residue with *water* and dry at 105°. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness. A reddish residue remains which becomes purple on exposure to the vapour of 5M *ammonia*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *diethylamine*, 10 volumes of *acetone* and 85 volumes of *cyclohexane* as the mobile phase. Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank and allow the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Promethazine Teoclate with 10 ml of a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol* for 5 minutes, filter (Whatman GF/C filter paper is suitable) and use the filtrate. Solution (2) contains 0.02% w/v of *isopromethazine hydrochloride BPCRS* in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. For solution (4) dilute 1 volume of solution (1) to 500 volumes with a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. Allow the plate to dry in air and examine under *ultraviolet light* (*254 nm*). In the chromatogram obtained with solution (1) any spot corresponding to isopromethazine hydrochloride is not more intense than the spot in the chromatogram obtained with solution (2) (1%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%). Disregard any spot remaining on the line of application.

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. To a quantity of the powder containing 50 mg of Promethazine Teoclate add 5 ml of *water* and 1 ml of 13.5M *ammonia* and allow to stand for 5 minutes. Add 50 ml of *absolute ethanol*, shake for 5 minutes and filter, washing the residue with five 5 ml quantities of *absolute ethanol*. Add sufficient *absolute ethanol* to the filtrate to produce 100 ml. Dilute 10 ml to 100 ml with *absolute ethanol* and dilute 10 ml of this dilution to 100 ml with *absolute ethanol*. Measure the *absorbance* of the resulting solution at the maximum at 255 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$ taking 755 as the value of A(1%, 1 cm) at the maximum at 255 nm.

STORAGE

Promethazine Teoclate Tablets should be protected from light.

Propantheline Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Propantheline Tablets contain Propantheline Bromide. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of propantheline bromide, $C_{23}H_{30}BrNO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Triturate a quantity of the powdered tablets containing 75 mg of Propantheline Bromide with 10 ml of *chloroform*, filter, evaporate the chloroform and stir the residue with 5 ml of *ether* until it solidifies. The solid complies with the following tests.

A. Dissolve 60 mg in 2 ml of *water*, add 2 ml of 5M *sodium hydroxide*, boil for 2 minutes, cool slightly, acidify with 2M *hydrochloric acid*, heat to boiling, add *ethanol* (*96%*) drop wise until the precipitate just dissolves, cool and filter. The *melting point* of the residue, after washing with *water*, recrystallising from *ethanol* (*50%*) and drying at 105° for 1 hour, is about 215°, Appendix V A. The crystals dissolve in *sulphuric acid* to produce a bright yellow solution which fluoresces strongly in ultraviolet light (365 nm).

B. Yields the reactions characteristic of *bromides*, Appendix VI.

TESTS

Xanthanoic acid

Shake the combined ether extracts reserved in the Assay with two 30 ml quantities of 0.1M *sodium hydroxide* containing 1.5% w/v of *sodium chloride*. Remove the ether from the combined aqueous extracts by heating on a water bath, add sufficient 0.1M *sodium hydroxide* to produce 100 ml and dilute 25 ml to 100 ml with 0.1M *sodium hydroxide*. The *absorbance* of the resulting solution at the maximum at 248 nm is not more than 0.31, Appendix II B.

ASSAY

Weigh and powder 20 tablets or more if necessary. Place a quantity of the powder containing 0.5 g of Propantheline Bromide on a sintered-glass filter, add 10 ml of *peroxide-free ether*, dried over sodium and distilled before use, stir and filter. Repeat the extraction with four 10 ml quantities of the ether and reserve the combined ether extracts for the test for Xanthanoic acid. Extract the residue on the filter with four 10 ml quantities of *chloroform*, evaporate the combined chloroform extracts to about 10 ml, add 10 ml of *mercury*(*II*) *acetate solution* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 44.84 mg of $C_{23}H_{30}BrNO_3$.

Propofol Injection

General Notices

Action and use

Intravenous general anaesthetic.

DEFINITION

Propofol Injection is a sterile emulsion of Propofol in a suitable basis.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of propofol, $C_{12}H_{18}O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a volume of the injection containing 0.1 g of Propofol with three 25 ml quantities of *hexane* and filter the combined extracts using a glass fibre filter (Whatman GF/C is suitable). Extract the filtrate with two 20 ml quantities of *methanol* (90%) and evaporate the combined extracts under reduced pressure. Dissolve the residue obtained in 2 ml of *absolute ethanol* and evaporate to dryness under reduced pressure. Dry the residue at 50° over *phosphorus pentoxide* for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of Propofol (*RS 416*).

In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 8.5, Appendix V L.

Propofol quinone and propofol dimer

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a suitable volume of the well shaken injection with *propan-2-ol* to produce a solution containing 0.08% w/v of Propofol. Solution (2) contains 0.0008% w/v of *propofol EPCRS*, 0.00008% w/v of *propofol impurity J EPCRS* and 0.0002% w/v of *propofol dimer BPCRS* in *propan-2-ol* containing 6.8% v/v of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 2.0 ml per minute a mixture of 40 volumes of *tetrahydrofuran* and 60 volumes of *water* and (c) a detection wavelength of 254 nm.

Inject 20 µl each of solutions (1) and (2). When the chromatograms are recorded using the prescribed conditions the retention time of the principal peak in the chromatogram obtained with solution (2) has a retention time of about 7 minutes. The retention times relative to the principal peak are: propofol impurity J, about 0.8, propofol dimer about 2.5.

Inject solution (1) and continue the chromatography for at least three times the retention time of the principal peak In the chromatogram obtained the area of any peaks corresponding to propofol quinone (propofol impurity J) and propofol dimer are not greater than the areas of the corresponding peaks in the chromatogram obtained with solution (2) (0.1% and 0.25% respectively).

Globule size

Not more than 5000 globules greater than or equal to 2 µm per ml of a 0.01% v/v dilution in a filtered 0.9% w/v solution of *sodium chloride*. By means of suitable equipment, pass the solution through a 50 µm aperture and count the number of globules by monitoring the change in electrical current between two electrodes immersed on either side of the aperture (a Coulter Counter is suitable). Repeat the procedure using the 0.9% w/v solution of *sodium chloride* only to determine the background count.

Free fatty acid

Not greater than 7 millimoles per litre, when determined by the following method. To 5 ml of a well shaken injection add 25 ml of a mixture of 40 volumes of *propan-2-ol*, 10 volumes of *n-heptane* and 1 volume of 0.5M *sulphuric acid* and shake for 1 minute. Allow to stand for 10 minutes, add 15 ml of *n-heptane* and 15 ml of *water*. Mix by inverting the container 10 times and allow to stand for 15 minutes. To 15 ml of the upper phase add 5 ml of *nile blue A solution* and pass a stream of *nitrogen*, previously passed through 0.1M *sodium hydroxide*, through the solution. Titrate with 0.02M *sodium hydroxide VS*, using a microburette. Calculate the content of free fatty acid from a calibration curve prepared from quantities of 1, 2, 4, 6 and 8 ml of a 1.282% w/v solution of *palmitic acid* in *n-heptane*, each diluted to 50 ml with *n-heptane*. Carry out the method described above, using 5 ml of each solution and beginning at the words 'add 25 ml of a mixture…'.

Lysolecithin

Not greater than 0.2% w/v when determined by the following method.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a suitable volume of well shaken injection with mobile phase to produce a solution containing 0.1% w/v of Propofol and filter through a 0.45-µm nylon filter. Solution (2) contains 0.01% w/v of *lysolecithin* in mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm X 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture of 7 volumes of *orthophosphoric acid*, 200 volumes of *water* and 300 volumes of *acetonitrile* and (c) a detection wavelength of 210 nm.

Inject 250 µl each of solution (1) and solution (2).

In the chromatogram obtained with solution (1) calculate the content of *lysolecithin* using the following expression.

$$\frac{A_1 \times C_2 \times f}{A_2}$$

where:

A_1 = peak area of lysolecithin in solution (1)

A_2 = peak area of lysolecithin in solution (2)

C_2 = concentration of lysolecithin in solution (2)

f = dilution factor used for solution (1)

Bacterial endotoxins

Carry out the test for *Bacterial endotoxins*, Appendix XIV C. Dilute the injection in *water BET* to give an emulsion containing 5 mg per ml (solution A). The endotoxin limit concentration of solution A is 1.65 IU endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a suitable volume of the well shaken injection with *propan-2-ol* to produce a solution containing 0.08% w/v of Propofol. Solution (2) contains 0.08% w/v of *propofol EPCRS* in *propan-2-ol* containing 6.8% v/v of *water*. Solution (3) contains 0.0008% w/v of *propofol EPCRS* and 0.00008% w/v of *propofol impurity J EPCRS* in *propan-2-ol* containing 6.8% v/v of *water*.

The chromatographic procedure described under Propofol quinone and propofol dimer may be carried out but using a detection wavelength of 275 nm. Inject 20 µl of each solution.

In the chromatogram obtained with solution (3) the principal peak has a retention time of approximately 7 minutes. The test is not valid unless the *resolution factor* between the peaks due to propofol and propofol impurity J is at least 2.5.

Calculate the content of $C_{12}H_{18}O$ in the injection from the chromatograms obtained and using the declared content of $C_{12}H_{18}O$ in *propofol EPCRS.*

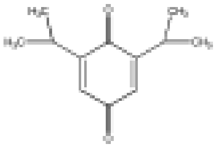
STORAGE

Propofol Injection should be stored at a temperature not exceeding 25°. It should not be allowed to freeze.

IMPURITIES

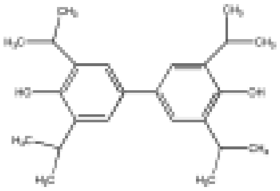
A. Propofol quinone (Ph Eur impurity J)

2,6-bis(1-methylethyl)-1,4-benzoquinone


H₃C
H₃C
O
CH₃
CH₃
O

B. Propofol dimer

3,3',5,5'-tetrakis(1-methylethyl)biphenyl-4,4'-diol


CH₃
H₃C
HO
H₃C
H₃C
CH₃
CH₃
OH
CH₃
H₃C

Propranolol Injection

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Propranolol Injection is a sterile solution of Propranolol Hydrochloride in Water for Injections containing Anhydrous Citric Acid or Citric Acid Monohydrate.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of propranolol hydrochloride, $C_{16}H_{21}NO_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Make alkaline with 1M *sodium hydroxide* a volume containing 10 mg of Propranolol Hydrochloride and extract with three 5 ml quantities of *ether*. Wash the combined extracts with *water* until the washings are free from alkali, dry with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dry the residue at 50° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of propranolol (*RS 298*).

B. The *light absorption* of the solution obtained in the Assay, Appendix II B, exhibits two maxima, at 290 nm and 319 nm, and a shoulder at 306 nm.

TESTS

Acidity

pH, 3.0 to 3.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, injecting 10 µl of each of the following solutions. Solution (1) contains 0.1% w/v of *propranolol hydrochloride for performance test EPCRS* in the mobile phase. For solution (2) dilute the injection being examined with *acetonitrile*, if necessary, to contain 0.10% w/v of Propranolol Hydrochloride. For solution (3) dilute 1 volume of solution (1) to 500 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5µ is suitable), (b) as the mobile phase with a flow rate of 1.8 ml per minute a mixture of 1.15 g of *sodium dodecyl sulphate*, 10 ml of a mixture of 1 volume of *sulphuric acid* and 9 volumes of *water*, 20 ml of a 1.7% w/v solution of *tetrabutylammonium dihydrogen orthophosphate*, 370 ml of *water* and 600 ml of *acetonitrile*, the mixture adjusted to pH 3.3 with 2M *sodium hydroxide*, and (c) a detection wavelength of 292 nm.

Inject 20 µl of solution (1). When the chromatogram is recorded in the prescribed conditions, the appearance and acceptance criteria specified in the leaflet which accompanies *propranolol hydrochloride for performance test EPCRS* are met.

For solutions (2) and (3) record the chromatograms for five times the retention time of the principal peak. In the chromatogram obtained with solution (2) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%) and the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (3) (0.8%).

ASSAY

To a volume containing 2 mg of Propranolol Hydrochloride add sufficient *methanol* to produce 100 ml and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{16}H_{21}NO_2,HCl$ taking 206 as the value of A(1%, 1 cm) at the maximum at 290 nm.

Propranolol Tablets

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Propranolol Tablets contain Propranolol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of propranolol hydrochloride, $C_{16}H_{21}NO_2,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Suspend a quantity of the powdered tablets containing 0.1 g of Propranolol Hydrochloride in 20 ml of *water*, filter, make the filtrate alkaline with 1M *sodium hydroxide* and extract with three 10 ml quantities of *ether*. Wash the combined extracts with *water* until the washings are free from alkali, dry with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dry the residue at 50° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of propranolol (*RS 298*).

B. The *light absorption* of the solution obtained in the Assay, Appendix II B, exhibits two maxima, at 290 nm and 319 nm, and a shoulder at 306 nm.

C. *Melting point* of the residue obtained in test A, about 94°, Appendix V A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, injecting 10 µl of each of the following solutions. Solution (1) contains 0.1% w/v of *propranolol hydrochloride for performance test EPCRS* in the mobile phase. For solution (2) add 100 ml of *methanol* to a quantity of the powdered tablets containing 0.1 g of Propranolol Hydrochloride, shake, filter through glass-microfibre paper (Whatman GF/C is suitable) and use the filtrate. For solution (3) dilute 1 volume of solution (1) to 500 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5µ is suitable), (b) as the mobile phase with a flow rate of 1.8 ml per minute a mixture of 1.15 g of *sodium dodecyl sulphate*, 10 ml of a mixture of 1 volume of *sulphuric acid* and 9 volumes of *water*, 20 ml of a 1.7% w/v solution of *tetrabutylammonium dihydrogen orthophosphate*, 370 ml of *water* and 600 ml of *acetonitrile*, the mixture adjusted to pH 3.3 with 2M *sodium hydroxide*, and (c) a detection wavelength of 292 nm.

Inject 20 µl of solution (1). When the chromatogram is recorded in the prescribed conditions, the appearance and acceptance criteria specified in the leaflet which accompanies *propranolol hydrochloride for performance test EPCRS* are met.

For solutions (2) and (3) record the chromatograms for five times the retention time of the principal peak. In the chromatogram obtained with solution (2) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.2%) and the sum of the areas of any *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (3) (0.8%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 20 mg of Propranolol Hydrochloride with 20 ml of *water* for 10 minutes. Add 50 ml of *methanol*, shake for a further 10 minutes, add sufficient *methanol* to produce 100 ml and filter. Dilute 10 ml of the filtrate to 50 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{16}H_{21}NO_2$,HCl taking 206 as the value of A(1%, 1 cm) at the maximum at 290 nm.

Propylthiouracil Tablets

General Notices

Action and use

Thiourea antithyroid drug.

DEFINITION

Propylthiouracil Tablets contain Propylthiouracil.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of propylthiouracil, $C_7H_{10}N_2OS$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 20 ml of *methanol* for 10 minutes, filter and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of propylthiouracil (*RS 300*).

B. Shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 60 ml of *methanol* for 20 minutes, dilute to 100 ml with *methanol* and filter. Dilute 5 ml of this solution to 250 ml with *methanol*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 274 nm.

C. Extract a quantity of the powdered tablets in a continuous extraction apparatus with *ether* and evaporate the solution to dryness. The *melting point* of the residue, after drying at 105°, is about 219°, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 0.2 volume of *glacial acetic acid*, 12 volumes of *propan-2-ol* and 100 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 5 ml of *methanol* for 15 minutes, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. Solution (3) contains 0.0010% w/v of *thiourea* in *methanol*. After removal of the plate, allow it to dry in air, examine under *ultraviolet light* (*254 nm*) and expose to iodine vapour for 10 minutes. Any spot corresponding to thiourea in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.1%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (1%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.15 g of

Propylthiouracil in a mixture of 20 ml of 0.1M *sodium hydroxide VS* and 75 ml of *water* with the aid of gentle heat. Cool, add 4 g of *sodium acetate*, just acidify the solution to *litmus paper* with 6M *acetic acid*, add 0.5 ml of a freshly prepared 0.5% w/v solution of *1,5-diphenylcarbazone* in *ethanol* (*96%*) and titrate with 0.02M *mercury nitrate VS* until a pinkish violet colour persists for 2 to 3 minutes. Each ml of 0.02M *mercury nitrate VS* is equivalent to 6.808 mg of $C_7H_{10}N_2OS$.

Protamine Sulphate Injection

General Notices

Action and use

Antidote to heparin.

DEFINITION

Protamine Sulphate Injection is a sterile solution of Protamine Sulphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of protamine sulphate

Not less than 80.0% of the stated amount.

IDENTIFICATION

A. Produces a precipitate under the conditions of the Assay.

B. Dilute the injection with *water* to give a solution containing 0.2% w/v of Protamine Sulphate. To 5 ml add 1 ml of a 10% w/v solution of *sodium hydroxide* and 1 ml of a 0.02% w/v solution of *1-naphthol* and mix. Cool to 5° and add 0.5 ml of *sodium hypobromite solution*. An intense pink colour is produced.

C. Heat 2 ml in a water bath at 60° and add 0.1 ml of *mercury*(*II*) *sulphate solution*; no precipitate is produced. Cool the mixture in ice; a white precipitate is produced.

D. Yields reaction A characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH, 2.5 to 3.5, Appendix V L.

Optical rotation

Dilute the injection with 0.5M *hydrochloric acid* to produce a solution containing 0.8% w/v of Protamine Sulphate. The *optical rotation* of the resulting solution is –0.52° to –0.68°, Appendix V F.

Light absorption

Dilute the injection, if necessary, with *water* to produce a solution containing 1% w/v of Protamine Sulphate. The *absorbance* at 260 to 280 nm is not more than 0.1, Appendix II B.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 10 mg of Protamine Sulphate per ml (solution A). The endotoxin limit concentration of solution A is 70 IU of endotoxin per ml. Carry out the test using the maximum allowable concentration of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the Assay described under Protamine Sulphate using solutions prepared by diluting the injection with *water* to contain (1) 0.015% w/v, (2) 0.010% w/v and (3) 0.005% w/v of Protamine Sulphate.

LABELLING

The label states that the dose is calculated from the results of determinations of the amount required to produce an acceptable blood clotting time in the patient.

Protriptyline Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Protriptyline Tablets contain Protriptyline Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of protriptyline hydrochloride, $C_{19}H_{21}N,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits a maximum only at 292 nm.

B. Shake a quantity of the powdered tablets containing 5 mg of Protriptyline Hydrochloride with 20 ml of *methanol* and filter. To 1 ml of the filtrate add 1 ml of a 2.5% w/v solution of *sodium hydrogen carbonate*, 1 ml of a 2% w/v solution of *sodium periodate* and 1 ml of a 0.3% w/v solution of *potassium permanganate*. Allow to stand for 15 minutes, acidify with 1M *sulphuric acid* and extract with 10 ml of *2,2,4-trimethylpentane*, washing the extract with 10 ml of 0.25M *sulphuric acid*. The *light absorption* of the resulting trimethylpentane solution, Appendix II B, does not exhibit a maximum at 265 nm (distinction from amitriptyline and nortriptyline).

C. Triturate a quantity of the powdered tablets containing 0.1 g of Protriptyline Hydrochloride with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. Dissolve part of the residue in 3 ml of *water* and add 0.05 ml of a 2.5% w/v solution of *quinhydrone* in *methanol*. A red colour develops.

ASSAY

Triturate 10 tablets for 15 minutes with 100 ml of a solution prepared by mixing 1 volume of 1M *hydrochloric acid* and 9 volumes of *methanol*, transfer to a graduated flask using sufficient of the solvent mixture to produce 250 ml, mix and filter through a fine filter paper. Dilute a volume of the filtrate containing 1 mg of Protriptyline Hydrochloride to 100 ml with the solvent mixture and measure the *absorbance* of the resulting solution at the maximum at 292 nm, Appendix II B. Calculate the content of $C_{19}H_{21}N,HCl$ taking 465 as the value of A(1%, 1 cm) at the maximum at 292 nm.

Proxymetacaine Eye Drops

General Notices

Action and use

Local anaesthetic.

DEFINITION

Proxymetacaine Eye Drops are a sterile solution of Proxymetacaine Hydrochloride in Purified Water. They may contain Glycerol.

With the exception that they may be supplied in containers not exceeding 15 ml in capacity, the eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of proxymetacaine hydrochloride, $C_{16}H_{26}N_2O_3$,HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 250 to 350 nm of the solution used in the Assay exhibits two maxima, at 268 nm and 310 nm.

B. To a volume containing 50 mg of Proxymetacaine Hydrochloride add 2 ml of 2M *hydrochloric acid* and extract with 25 ml of *chloroform*. Discard the chloroform layer, make alkaline with 5M *sodium hydroxide* and extract with two 25 ml quantities of *chloroform*. Filter the combined extracts through *anhydrous sodium sulphate* and evaporate the filtrate to dryness using a rotary evaporator. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of proxymetacaine (*RS 302*).

C. A volume containing 5 mg of Proxymetacaine Hydrochloride yields the reaction characteristic of *primary aromatic amines*, Appendix VI.

TESTS

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 5 volumes of *diethylamine*, 30 volumes of *ethyl acetate* and 75 volumes of *toluene* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use the eye drops, diluted if necessary to contain 0.5% w/v of Proxymetacaine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol*. For solution (3) dilute 1 volume of solution (2) with an equal volume of *methanol*. After removal of the plate, heat it at 105° for 10 minutes, allow to cool and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Disregard any spot remaining on the line of application.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 4 volumes of *glacial acetic acid*, 20 volumes of *cyclohexane* and 80 volumes of *1,4-dioxan* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) use solution (1) described in test A above. Solutions (2) and (3) are solutions of *3-amino-4-propoxybenzoic acid BPCRS* in *methanol* containing (2) 0.020% w/v and (3) 0.0050% w/v, respectively. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (4%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%). The principal spot remains on or near the line of application.

ASSAY

Dilute a volume containing 10 mg of Proxymetacaine Hydrochloride to 200 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 310 nm, Appendix II B. Calculate the content of $C_{16}H_{26}N_2O_3$,HCl taking 162 as the value of A(1%, 1 cm) at the maximum at 310 nm.

STORAGE

Proxymetacaine Eye Drops should be protected from light, stored at a temperature of 2° to 8° and should not be allowed to freeze.

Pseudoephedrine Oral Solution

General Notices

Action and use

Adrenoceptor agonist.

DEFINITION

Pseudoephedrine Oral Solution is a solution of Pseudoephedrine Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of pseudoephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the Oral Solution containing 0.3 g of Pseudoephedrine Hydrochloride, add 4 ml of 2M *hydrochloric acid*, extract with two 40-ml quantities of *ether* and discard the ether. Add sufficient 5M *ammonia* to the aqueous phase to make it alkaline, extract with two 60-ml quantities of *ether*, wash the combined ether extracts with three 30-ml quantities of *water*, dry with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, in the range 1550 to 1350 cm^{-1} and in the range 1250 to 400 cm^{-1} is concordant with the *reference spectrum* of pseudoephedrine *(RS 304)*.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add sufficient 5M *ammonia* to a volume of the oral solution containing 10 mg of Pseudoephedrine Hydrochloride to make it alkaline, extract with two 100-ml quantities of *ether*, wash the combined extracts with 10 ml of *water*, dry with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the oily residue in sufficient *methanol* to produce 5 ml.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.05% w/v each of *ephedrine hydrochloride BPCRS* and *pseudoephedrine hydrochloride BPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 5 volumes with the mobile phase.

(5) Dilute 1 volume of solution (2) to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with particles of *phenylsilyl silica gel for chromatography* (µBondapak Phenyl is suitable).

(b) Use isocratic elution and the mobile phase below.

(c) Use a flow rate of 1 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 257 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 6 volumes of *methanol* and 94 volumes of 0.15M *ammonium acetate*, adjusted to pH 4.0 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak due to ephedrine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.2%);

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing 60 mg of Pseudoephedrine Hydrochloride to 50 ml with *methanol (65%)*.

(2) 0.12% w/v of *pseudoephedrine hydrochloride BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 263 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

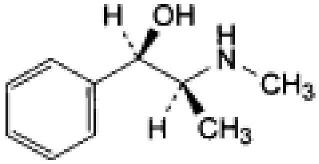
A solution containing 0.005M *dioctyl sodium sulphosuccinate* in a mixture of 1 volume of *glacial acetic acid*, 35 volumes of *water and* 65 volumes of *methanol*

DETERMINATION OF CONTENT

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{10}H_{15}NO,HCl$, weight in volume, using the declared content of $C_{10}H_{15}NO,HCl$ in *pseudoephedrine hydrochloride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:



Ephedrine.

Pseudoephedrine Tablets

General Notices

Action and use

Adrenoceptor agonist.

DEFINITION

Pseudoephedrine Tablets contain Pseudoephedrine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pseudoephedrine hydrochloride, $C_{10}H_{15}NO,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 60 mg of Pseudoephedrine Hydrochloride with 10 ml of *water* and filter. Shake the filtrate with 10 ml of *ether*, discard the ether, add 1 ml of 1M *sodium hydroxide* to the aqueous layer and extract with two 10 ml quantities of *ether*. Dry the combined ether extracts with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pseudoephedrine (*RS 304*).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 10 volumes of 5M *ammonia*, 10 volumes of *methanol*, 20 volumes of *acetone*, 20 volumes of *butan-1-ol* and 40 volumes of *butyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. Remove any film coating from the tablets before proceeding. For solution (1) add 25 ml of *methanol* to a quantity of the powdered tablets containing 0.5 g of Pseudoephedrine Hydrochloride, shake for 5 minutes, filter, wash the filter with *methanol* and evaporate the combined filtrate and washings to dryness. Dissolve the residue as completely as possible in 5 ml of *methanol*, centrifuge and dilute 1 volume of the supernatant liquid to 10 volumes with *methanol*. Solution (2) contains 1.0% w/v of *pseudephedrine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in a current of warm air, spray with a solution containing 0.3 g of *ninhydrin* in a mixture of 100 ml of *butan-1-ol* and 3 ml of *glacial acetic acid* and heat at 120° for 20 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Remove any film coating from the tablets before proceeding. For solution (1) add 25 ml of *methanol* to a quantity of the powdered tablets containing 0.5 g of Pseudoephedrine Hydrochloride, shake for 5 minutes, filter, wash the filter with *methanol* and evaporate the combined filtrate and washings to dryness. Dissolve the residue as completely as possible in 5 ml of *methanol*, centrifuge and dilute 1 ml of the supernatant liquid to 50 ml with the mobile phase. Solution (2) contains 0.002% w/v of *ephedrine hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.05% w/v of *ephedrine hydrochloride BPCRS* and 0.05% w/v of *pseudoephedrine hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with particles of silica the surface of which has been modified with chemically bonded phenyl groups (µBondapak Phenyl is suitable), (b) a mixture of 6 volumes of *methanol* and 94 volumes of 0.15M *ammonium acetate*, adjusted to pH 4.0 with *glacial acetic acid*, as the mobile phase with a flow rate of 1.0 ml per minute and (c) a detection wavelength of 257 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to ephedrine is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) is a 0.12% w/v solution of *pseudoephedrine hydrochloride BPCRS* in *methanol* (*50%*). For solution (2) add 50 ml of 0.1M *hydrochloric acid* to a quantity of the powdered tablets containing 0.12 g of Pseudoephedrine Hydrochloride, mix with the aid of ultrasound for 15 minutes, add sufficient *methanol* to produce 100 ml, filter and use the filtrate.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) 0.005M *dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 258 nm.

Calculate the content of $C_{10}H_{15}NO,HCl$ using the declared content of $C_{10}H_{15}NO,HCl$ in *pseudoephedrine hydrochloride BPCRS*.

Pyrazinamide Tablets

General Notices

Action and use

Antituberculosis drug.

DEFINITION

Pyrazinamide Tablets contain Pyrazinamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyrazinamide, $C_5H_5N_3O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.25 g of Pyrazinamide with 20 ml of *absolute ethanol*, filter, evaporate the filtrate to dryness and dry the residue at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pyrazinamide (*RS 306*).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 20 volumes of *glacial acetic acid*, 20 volumes of *water* and 60 volumes of *butan-1-ol* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.10 g of Pyrazinamide with 50 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*, filter, evaporate to dryness and dissolve the residue in sufficient of the same solvent mixture to produce 10 ml. For solution (2) dilute 1 volume of solution (1) to 500 volumes with a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*. After removal of the plate, allow it to dry in air and examine immediately under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Pyrazinamide add 200 ml of *water*, allow to stand for 10 minutes, swirling occasionally, mix with the aid of ultrasound for 10 minutes and dilute to 500 ml with *water*. Filter and discard the first 20 ml of filtrate. Dilute 5 ml of the filtrate to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 268 nm, Appendix II B. Calculate the content of $C_5H_5N_3O$ taking 650 as the value of A(1%, 1 cm) at the maximum at 268 nm.

Pyridostigmine Tablets

General Notices

Action and use

Cholinesterase inhibitor.

DEFINITION

Pyridostigmine Tablets contain Pyridostigmine Bromide.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyridostigmine bromide, $C_9H_{13}BrN_2O_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Triturate a quantity of the powdered tablets containing 0.1 g of Pyridostigmine Bromide with two 5 ml quantities of *water* and filter. Dilute the filtrate with *water* to contain 0.005% w/v of Pyridostigmine Bromide. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum at 270 nm. Reserve the filtrate for use in tests B and C.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) dilute 1 volume of the filtrate obtained in test A to 10 volumes with *methanol*.

(2) 0.1% w/v of *pyridostigmine bromide EPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 10 µl of each solution.

(d) Develop the plate to 20 cm.

(e) After removal of the plate, dry in air, spray with *dilute potassium iodobismuthate solution*.

MOBILE PHASE

A mixture of 5 volumes of *water*, 10 volumes of *formic acid*, 35 volumes of *methanol* and 50 volumes of *chloroform.*

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. The filtrate obtained in test A yields the reactions characteristic of *bromides*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing 0.1 g of Pyridostigmine Bromide with 100 ml of a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water* for 30 minutes and filter through a glass fibre filter (Whatman GF/C is suitable)

(2) Dilute 6 volumes of solution (1) to 100 volumes with a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*.

(3) Dilute 1 volume of solution (1) to 250 volumes with a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*.

(4) 0.0005% w/v each of *pyridostigmine impurity A EPCRS* and *pyridostigmine bromide EPCRS* in the mobile phase.

(5) Dilute 1 volume of solution (3) to 10 volumes with a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.1 ml per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 20 µl of each solution.

(g) Allow the chromatography to continue for at least twice the retention time of the peak due to Pyridostigmine.

MOBILE PHASE

30 volumes of *acetonitrile* and 70 volumes of a 0.43% w/v solution of *sodium dodecyl sulphate*, previously adjusted to pH 2.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to pyridostigmine impurity A and pyridostigmine bromide is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to pyridostigmine impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (6%);

the area of any peak corresponding to impurity pyridostigmine impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of any other *secondary peaks* is not greater than the principal peak in the chromatogram obtained with solution (3) (0.4%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.04%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 0.15 g of Pyridostigmine Bromide with 50 ml of *water* for 30 minutes, filter, wash the residue with *water* and add sufficient *water* to produce 250 ml. Dilute 5 ml to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 270 nm, Appendix II B. Calculate the content of $C_9H_{13}BrN_2O_2$ taking 186 as the value of A(1%, 1 cm) at the maximum at 270 nm.

STORAGE

Pyridostigmine Tablets should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Pyridostigmine Bromide.

Pyridoxine Tablets

General Notices

Action and use

Vitamin B_6.

DEFINITION

Pyridoxine Tablets contain Pyridoxine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyridoxine hydrochloride, $C_8H_{11}NO_3,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Pyridoxine Hydrochloride with 50 ml of *methanol* for 15 minutes, warming occasionally, filter and add sufficient 13.5M *ammonia* to make the filtrate alkaline. Evaporate the resulting solution to dryness, dissolve the residue as completely as possible in 15 ml of *chloroform* and filter. To the filtrate add 2 ml of a solution prepared by carefully adding 2 ml of *acetyl chloride* drop wise to 8 ml of cooled *methanol* and evaporate the resulting solution to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pyridoxine hydrochloride (*RS 308*).

B. Shake a quantity of the powdered tablets containing 20 mg of Pyridoxine Hydrochloride with 50 ml of *0.025M standard phosphate buffer* for 15 minutes and dilute to 100 ml with the same solvent. Mix, filter and dilute 5 ml of the filtrate to 100 ml with the same solvent. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 254 nm and 324 nm.

C. Triturate a quantity of the powdered tablets containing 20 mg of Pyridoxine Hydrochloride with 50 ml of *water* and allow to stand. To 1 ml of the supernatant liquid add 10 ml of a 5% w/v solution of *sodium acetate*, 1 ml of *water* and 1 ml of a 0.5% w/v solution of *2-6-dichloroquinone-4-chloroimide* in *ethanol* (*96%*) and shake; a blue colour is produced which fades rapidly and becomes brown. Repeat the operation but adding 1 ml of a 0.3% w/v solution of *boric acid* in place of the 1 ml of *water*; no blue colour is produced.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 9 volumes of 13.5M *ammonia*, 13 volumes of *tetrahydrofuran,* 13 volumes of *dichloromethane* and 65 volumes of *acetone* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 40 mg of Pyridoxine Hydrochloride with

10 ml of *water* for 15 minutes, filter and use the filtrate. For solution (2) dilute 1 ml of solution (1) to 200 ml with *water*. After removal of the plate, allow it to dry in air and spray with a 5% w/v solution of *sodium carbonate* in *ethanol* (*30%*). Allow the plate to dry in air, spray with a 0.1% w/v solution of *2,6-dichloroquinone-4-chloroimide* in *ethanol* (*96%*) and examine immediately. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 25 mg of Pyridoxine Hydrochloride add 50 ml of 0.1M *hydrochloric acid* and heat on a water bath for 15 minutes, swirling occasionally. Cool, dilute to 100 ml with 0.1M *hydrochloric acid* and filter, discarding the first 20 ml of filtrate. Dilute 5 ml of the filtrate to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_8H_{11}NO_3$,HCl taking 430 as the value of A(1%, 1 cm) at the maximum at 290 nm.

STORAGE

Pyridoxine Tablets should be protected from light and stored at a temperature not exceeding 30°.

When vitamin B_6 tablets are prescribed or demanded, Pyridoxine Tablets shall be dispensed or supplied.

Pyrimethamine Tablets

General Notices

Action and use

Dihydrofolate reductase inhibitor; antiprotozoal (malaria).

DEFINITION

Pyrimethamine Tablets contain Pyrimethamine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyrimethamine, $C_{12}H_{13}ClN_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 50 mg of Pyrimethamine with 50 ml of *ethanol (96%)* for 20 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pyrimethamine (*RS 309*).

B. Extract the powdered tablets with 1M *sulphuric acid*, filter and add *potassium tetraiodomercurate solution* to the filtrate. A creamy white precipitate is produced.

C. Extract a quantity of the powdered tablets containing 50 mg of Pyrimethamine with two 10 ml quantities of *chloroform* and evaporate the combined extracts to dryness. The *melting point* of the residue is about 240°, Appendix V A.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 4 volumes of *chloroform*, 8 volumes of *propan-1-ol*, 12 volumes of *glacial acetic acid* and 76 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 20 µl of each of the following freshly prepared solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Pyrimethamine with 5 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 400 volumes with the same solvent mixture. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powdered tablets containing 25 mg of Pyrimethamine add 50 ml of hot 0.1M *hydrochloric acid* and heat on a water bath for 10 minutes, swirling occasionally. Mix with the aid of ultrasound for 30 minutes, remove and cool to room temperature. Add sufficient 0.1M *hydrochloric acid* to produce 100 ml, filter and dilute

5 ml of the filtrate to 100 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 272 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the content of $C_{12}H_{13}ClN_4$ taking 316 as the value of A(1%, 1 cm) at the maximum at 272 nm.

Soluble Paracetamol Tablets

General Notices

Action and use

Analgesic; antipyretic.

DEFINITION

Soluble Paracetamol Tablets contain Paracetamol in either a suitable soluble basis or a suitable soluble, effervescent basis.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of paracetamol, $C_8H_9NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve, with or without vigorous effervescence as appropriate, on the addition of warm *water* to produce a slightly opalescent solution.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 257 nm.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform* as the mobile phase but allowing the solvent front to ascend 14 cm above the line of application. Apply separately to the plate 40 µl of each of solutions (1), (2) and (3). Pour the mobile phase into the unlined tank, immediately place the prepared plate in the tank and close the tank. For solution (1) add 50 ml of *ethanol* (*96%*) to a quantity of the powdered tablets containing 0.10 g of Paracetamol, shake for 10 minutes, add sufficient *ethanol* (*96%*) to produce 100 ml and filter. Solution (2) contains 0.10% w/v of *paracetamol BPCRS* in *ethanol* (*96%*). For solution (3) dissolve 0.25 g of *4′-chloroacetanilide* and 0.10 g of *paracetamol* in sufficient *ethanol* (*96%*) to produce 100 ml. After removal of the plate, dry it in a current of warm air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots, the spot corresponding to 4′-chloroacetanilide having the higher Rf value.

TESTS

Disintegration

Comply with the requirements for Soluble Tablets or with the requirement for Effervescent Tablets, as appropriate.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare the solutions immediately before use and protect from light. For solution (1) disperse a quantity of powdered tablets containing 0.2 g of Paracetamol in 8 ml of the mobile phase with the aid of ultrasound, add sufficient mobile phase to produce 10 ml, mix well and filter. For solution (2) dilute 1 volume of solution (1) to 20 volumes with mobile phase and dilute 1 volume of this solution to 20 volumes with mobile phase . Solution (3) contains 0.002% w/v each of *4-aminophenol* and *paracetamol BPCRS* in the mobile phase. Solution (4) contains 0.00002% w/v of *4′-chloroacetanilide* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax Rx C8 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute, with a temperature of 35°C, a mixture of 250 volumes of *methanol* containing 1.15 g of a 40% v/v solution of *tetrabutylammonium hydroxide* with 375 volumes of 0.05M *disodium hydrogen orthophosphate* and 375 volumes of 0.05M *sodium dihydrogen orthophosphate* and (c) a detection wavelength of 245 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 4.0.

Inject solution (1) and allow the chromatography to proceed for 12 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-aminophenol is not greater than the area of the corresponding peak in solution (3) (0.1%), the area of any peak corresponding to 4′-chloroacetanilide is not greater than the area of the principal peak in solution (4) (10 ppm) and no other impurity is greater than the area of the principal peak obtained with solution (2) (0.25%).

ASSAY

Weigh and powder 20 tablets. Add 50 ml of 0.1M *sodium hydroxide* slowly to a quantity of the powdered tablets containing 0.2 g of Paracetamol. If necessary, wait until effervescence has ceased, dilute with 100 ml of *water*, shake for 15 minutes and add sufficient *water* to produce 250 ml. Mix, filter and dilute 10 ml of the filtrate to 100 ml with *water*. Add 10 ml of the resulting solution to 10 ml of 0.1M *sodium hydroxide*, dilute to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 257 nm, Appendix II B. Calculate the content of $C_8H_9NO_2$ taking 715 as the value of A(1%, 1 cm) at the maximum at 257 nm.

STORAGE

Soluble Paracetamol Tablets should be protected from light.

LABELLING

The label states (1) that the tablets should be dissolved in water immediately before use; (2) where applicable, that the tablets are effervescent.

Sterile Potassium Chloride Concentrate

General Notices

DEFINITION

Sterile Potassium Chloride Concentrate is a sterile solution of Potassium Chloride in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of potassium chloride, KCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The residue on evaporation yields the reactions characteristic of *potassium salts* and the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity or alkalinity

Dilute, if necessary, the concentrate being examined with *carbon dioxide-free water* to contain 10.0% w/v of Potassium Chloride. To 50 ml add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of 0.01M *hydrochloric acid* or 0.01M *sodium hydroxide* is required to change the colour of the indicator.

Bacterial endotoxins

Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dilute the concentrate being examined with *water BET* to contain 0.5% w/v of Potassium Chloride and adjust the pH, if necessary, to 7 (solution A). The endotoxin limit concentration of solution A is 3.0 IU of endotoxin per ml. Carry out the test using a lysate with a declared sensitivity not less sensitive than 0.0625 IU of endotoxin per ml, and using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Dilute 5 ml to 100 ml with *water*. To a volume of the solution containing 0.15 g of Potassium Chloride add 30 ml of *water* and titrate with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 7.456 mg of KCl.

LABELLING

The label states (1) 'Sterile Potassium Chloride Concentrate'; (2) the strength as a percentage w/v of Potassium Chloride; (3) the approximate concentration of the potassium ions and the chloride ions in mmol in a suitable volume; (4) the volume of Sodium Chloride Intravenous Infusion (0.9% w/v) or other suitable diluent with which the concentrate must be diluted and mixed well before use.

Sterile Potassium Dihydrogen Phosphate Concentrate

General Notices

Potassium Acid Phosphate Injection

DEFINITION

Sterile Potassium Dihydrogen Phosphate Concentrate is a 13.6% w/v sterile solution of Potassium Dihydrogen Phosphate in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of potassium dihydrogen phosphate, KH_2PO_4

12.9 to 14.3% w/v.

IDENTIFICATION

The residue on evaporation yields the reactions characteristic of *potassium salts* and the reactions characteristic of *phosphates*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 5.0, Appendix V L.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

Titrate 10 ml, saturated with *sodium chloride*, with 0.5M *sodium hydroxide VS* using *phenolphthalein solution* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 68.05 mg of KH_2PO_4.

LABELLING

The label states (1) 'Sterile Potassium Dihydrogen Phosphate Concentrate'; (2) the strength as a percentage w/v of Potassium Dihydrogen Phosphate; (3) the approximate concentration of the potassium ions and the phosphate ions in millimoles in a suitable volume; (4) the volume of Sodium Chloride Intravenous Infusion (0.9% w/v) or other suitable diluent with which the concentrate must be diluted and mixed well before use.

Strong Pholcodine Linctus

General Notices

Action and use

Opioid receptor agonist; cough suppressant.

DEFINITION

Strong Pholcodine Linctus is an *oral solution* containing 0.2% w/v of Pholcodine and 2% w/v of Citric Acid Monohydrate in a suitable flavoured vehicle.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of pholcodine, $C_{23}H_{30}N_2O_4,H_2O$

0.180 to 0.220% w/v.

IDENTIFICATION

To 10 ml add 20 ml of *water*, make alkaline to *litmus paper* with 5M *ammonia*, extract with two 15 ml quantities of *chloroform*, washing each extract with 5 ml of *water*, dry the combined extracts with *anhydrous sodium sulphate*, filter and evaporate to dryness. If necessary add 0.1 ml of *ether* and scratch the side of the vessel with a glass rod to induce crystallisation. The crystals, dried at a pressure not exceeding 2 kPa, comply with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of pholcodine (*RS 273*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.01% w/v solution in 0.01M *sodium hydroxide* exhibits a maximum only at 284 nm.

C. Dissolve the remainder of the crystals in 1 ml of *sulphuric acid* and add 0.05 ml of *ammonium molybdate-sulphuric acid solution*; a pale blue colour is produced. Warm gently; the colour changes to deep blue. Add 0.05 ml of 2M *nitric acid*; the colour changes to brownish red.

ASSAY

To 25 g add sufficient 5M *ammonia* to make the solution alkaline to *litmus paper* and extract with four 25-ml quantities of *chloroform*, washing each extract with the same 5 ml of *water*. Combine the extracts and evaporate until the volume is reduced to 15 ml. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.02M *perchloric acid VS* as titrant and *quinaldine red solution* as indicator. Each ml of 0.02M *perchloric acid VS* is equivalent to 4.165 mg of $C_{23}H_{30}N_2O_4,H_2O$. Determine the *weight per ml* of the linctus, Appendix V G, and calculate the content of $C_{23}H_{30}N_2O_4,H_2O$, weight in volume.

STORAGE

Strong Pholcodine Linctus should be protected from light.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Quinidine Sulphate Tablets

General Notices

Action and use

Class I antiarrhythmic.

DEFINITION

Quinidine Sulphate Tablets contain Quinidine Sulphate.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quinidine sulphate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *diethylamine*, 20 volumes of *acetone* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 0.1 g of Quinidine Sulphate with 10 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*) and filter. Solution (2) contains 1.0% w/v of *quinidine sulphate EPCRS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*). Solution (3) contains 1.0% w/v each of *quinidine sulphate EPCRS* and *quinine sulphate EPCRS*. After removal of the plate, allow it to dry in air and spray with 0.05M *ethanolic sulphuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Shake a quantity of the powdered tablets containing 0.25 g of Quinidine Sulphate with 25 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*) and filter. Evaporate the filtrate to dryness and wash the residue with 10 ml of *ether*. Filter, wash the residue with 10 ml of *ether* and dry the residue at 60° at a pressure not exceeding 15 Pa for 2 hours. The pH of a 1% w/v solution of the residue is 6.0 to 6.8, Appendix V L.

C. Extract a quantity of the powdered tablets containing 0.1 g of Quinidine Sulphate with 20 ml of *water* and filter. The filtrate yields the reactions characteristic of *sulphates*, Appendix VI.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the powdered tablets containing 50 mg of Quinidine Sulphate with 20 ml of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 ml with the mobile phase and filter, discarding the first few ml of filtrate. For solution (2) dissolve 20 mg of *quinine sulphate EPCRS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase. Prepare solution (3) in the same manner as solution (2) but using *quinidine sulphate EPCRS* in place of *quinine sulphate EPCRS*. Solution (4) is a mixture of equal volumes of solutions (2) and (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject separately 10 µl of each of solutions (3) and (6). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µl of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µl of solution (1) and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinidine is not greater than 15%, the content of any related substance eluting before quinidine is not greater than 5% and the content of any other related substance is not greater than 2.5%.

ASSAY

Weigh and powder 20 tablets. Dissolve as completely as possible using heat a quantity of the powdered tablets containing 0.4 g of Quinidine Sulphate in 40 ml of *acetic anhydride* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 26.10 mg of $(C_{20}H_{24}N_2O_2)_2$, H_2SO_4,$2H_2O$.

STORAGE

Quinidine Sulphate Tablets should be protected from light.

Quinine Bisulphate Tablets

General Notices

Action and use

Antiprotozoal (malaria).

DEFINITION

Quinine Bisulphate Tablets contain Quinine Bisulphate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quinine bisulphate, $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *diethylamine*, 20 volumes of *acetone* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) remove any coating from the tablets and extract a quantity of the powdered tablet cores containing 0.1 g of Quinine Bisulphate with 10 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*) and filter. Solution (2) contains 1.0% w/v of *quinine sulphate EPCRS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*). Solution (3) contains 1.0% w/v each of *quinidine sulphate EPCRS* and *quinine sulphate EPCRS.* After removal of the plate, allow it to dry in air and spray with 0.05M *ethanolic sulphuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Shake a quantity of the powdered tablets containing 0.25 g of Quinine Bisulphate with 25 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*65%*) and filter. Evaporate the filtrate to dryness and wash the residue with 10 ml of *ether*. Filter, wash the residue with 10 ml of *ether* and dry at 60° at a pressure not exceeding 15 Pa for 2 hours. The pH of a 1% w/v solution is 2.8 to 3.4, Appendix V L.

C. Extract a quantity of the powdered tablets containing 0.5 g of Quinine Bisulphate with 10 ml of *water* and filter. The filtrate yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of

10 ml of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 348 nm, Appendix II B. Calculate the total content of quinine bisulphate, $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$, in the medium taking 99 as the value of A(1%, 1 cm) at the maximum at 348 nm.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) remove any coating from the tablets and mix a quantity of the powdered tablet cores containing 50 mg of Quinine Bisulphate with 20 ml of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 ml with the mobile phase and filter, discarding the first few ml of the filtrate. For solution (2) dissolve 20 mg of *quinine sulphate EPCRS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase. Prepare solution (3) in the same manner as solution (2) using *quinidine sulphate EPCRS* in place of *quinine sulphate EPCRS*. Solution (4) is a mixture of equal volumes of solutions (2) and (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject separately 10 µl of each of solutions (3) and (6). If necessary adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µl of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µl of solution (1) and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinine is not greater than 10%, the content of any related substance eluting before quinine is not greater than 5% and the content of any other related substance is not greater than 2.5%.

ASSAY

Weigh and powder 20 tablets. Dissolve as completely as possible a quantity of the powdered tablets containing 0.6 g of Quinine Bisulphate in 40 ml of *acetic anhydride* using heat. Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 54.86 mg of $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$.

STORAGE

Quinine Bisulphate Tablets that are not sugar-coated should be protected from light.

300 mg of Quinine Bisulphate is approximately equivalent to 177.5 mg of anhydrous quinine.

Quinine Dihydrochloride Intravenous Infusion

General Notices

Action and use

Antiprotozoal (malaria).

DEFINITION

Quinine Dihydrochloride Intravenous Infusion is a sterile solution of Quinine Dihydrochloride. It is prepared immediately before use in accordance with the manufacturer′s instructions from Quinine Dihydrochloride Sterile Concentrate.

The intravenous infusion complies with the requirements stated under Parenteral Preparations.

QUININE DIHYDROCHLORIDE STERILE CONCENTRATE

DEFINITION

Quinine Dihydrochloride Sterile Concentrate is a sterile solution of Quinine Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of quinine dihydrochloride, $C_{20}H_{24}N_2O_2,2HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

An almost colourless to light yellow solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel G plate* and a mixture of 10 volumes of *diethylamine*, 20 volumes of *acetone* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) extract a volume of the concentrate containing 0.1 g of Quinine Dihydrochloride with 10 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)* and filter. Solution (2) contains 1.0% w/v of *quinine sulphate EPCRS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)*. Solution (3) contains 1.0% w/v each of *quinidine sulphate EPCRS* and *quinine sulphate EPCRS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (96%)*. After removal of the plate, allow it to dry in air and spray with 0.05M *ethanolic sulphuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 1.5 to 3.0, Appendix V L.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the concentrate being examined diluted, if necessary, with the mobile phase to produce a solution containing 0.2% w/v of Quinine Dihydrochloride. For solution (2) dissolve 20 mg of *quinine sulphate EPCRS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase. Prepare solution (3) in the same manner as solution (2) but using *quinidine sulphate EPCRS* in place of *quinine sulphate EPCRS*. Solution (4) is a mixture of equal volumes of solution (2) and solution (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject 10 µl of each of solutions (3) and (6). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µl of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinine and a peak due to dihydroquinine, with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak, due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µl of solution (1) and record the chromatogram for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinine is not greater than 10%, the content of any related substance eluted before quinine is not greater than 5% and the content of any other related substance is not greater than 2.5%.

ASSAY

To a volume containing 0.6 g of Quinine Dihydrochloride, add 20 ml of *water* and 5 ml of 5M *sodium hydroxide*, extract with three 25-ml quantities of *chloroform* and wash the combined extracts with 20 ml of *water*. Dry the chloroform extracts with *anhydrous sodium sulphate*, evaporate to dryness at a pressure of 2 kPa, dissolve the residue in 50 ml of *anhydrous acetic acid* and add 20 ml of *acetic anhydride*. Carry out method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 19.87 mg of $C_{20}H_{24}N_2O_2$,2HCl.

STORAGE

Quinine Dihydrochloride Sterile Concentrate should be protected from light.

LABELLING

The label states (1) 'Quinine Dihydrochloride Sterile Concentrate', (2) that the concentrate must be diluted before administration, (3) that the concentrate should be protected from light.

Quinine Sulphate Tablets

General Notices

Action and use

Antiprotozoal (malaria).

DEFINITION

Quinine Sulphate Tablets contain Quinine Sulphate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of quinine sulphate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 10 volumes of *diethylamine*, 20 volumes of *acetone* and 80 volumes of *toluene* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) remove any coating from the tablets and extract a quantity of the powdered tablet cores containing 0.1 g of Quinine Sulphate with 10 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*) and filter. Solution (2) contains 1.0% w/v of *quinine sulphate EPCRS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*). Solution (3) contains 1.0% w/v each of *quinidine sulphate EPCRS* and *quinine sulphate EPCRS*. After removal of the plate, allow it to dry in air and spray with 0.05M *ethanolic sulphuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Extract a quantity of the powdered tablets containing 0.25 g of Quinine Sulphate with 25 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*) and filter. Evaporate the filtrate to dryness and wash the residue with 10 ml of *ether*. Filter and wash the residue with 10 ml of *ether*. Dry the residue at 60° at a pressure not exceeding 15 Pa for 2 hours. The pH of a 1% w/v suspension of the residue is 5.7 to 6.6, Appendix V L.

C. Extract a quantity of the powdered tablets containing 0.1 g of Quinine Sulphate with 20 ml of *water* and filter. The filtrate yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 348 nm, Appendix II B. Calculate the total content of quinine sulphate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$, in the medium taking 136 as the value of A(1%, 1 cm) at the maximum at 348 nm.

Other cinchona alkaloids

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) remove any coating from the tablets and mix a quantity of the powdered tablet cores containing 50 mg of Quinine Sulphate with 20 ml of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 ml with the mobile phase and filter, discarding the first few ml of the filtrate. For solution (2) dissolve 20 mg of *quinine sulphate EPCRS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase. Prepare solution (3) in the same manner as solution (2) using *quinidine sulphate EPCRS* in place of *quinine sulphate EPCRS*. Solution (4) is a mixture of equal volumes of solution (2) and solution (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject 10 µl of each of solutions (3) and (6). If necessary adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µl of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinine and a peak due to dihydroquinine, with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatogram obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µl of solution (1) and record the chromatogram for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinine is not greater than 10%, the content of any related substance eluted before quinine is not greater than 5% and the content of any other related substance is not greater than 2.5%.

ASSAY

Weigh and powder 20 tablets and dissolve as completely as possible, using heat, a quantity of the powdered tablets containing 0.4 g of Quinine Sulphate in 40 ml of *acetic anhydride*.

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 26.10 mg of $(C_{20}H_{24}N_2O_2)_2$, H_2SO_4,$2H_2O$.

STORAGE

Quinine Sulphate Tablets that are not sugar-coated should be protected from light.

300 mg of Quinine Sulphate is approximately equivalent to 248.6 mg of anhydrous quinine.

Compound Rhubarb Tincture

General Notices

DEFINITION

Rhubarb, in *moderately coarse powder*	100 g
Cardamom Oil	0.40 ml
Coriander Oil	0.03 ml
Glycerol	100 ml
Ethanol (60 per cent)	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Moisten the Rhubarb with a sufficient quantity of Ethanol (60 per cent) and prepare 850 ml of tincture by *percolation*, Appendix XI F. Add the Cardamom Oil, the Coriander Oil and the Glycerol and sufficient Ethanol (60 per cent) to produce 1000 ml. Mix and filter, if necessary.

The tincture complies with the requirements for Tinctures stated under Extracts and with the following requirements.

TESTS

Ethanol content

48 to 53% v/v, Appendix VIII F, Method III.

Glycerol

9.0 to 11.0% v/v when determined by the following method. Dilute 20 ml to 100 ml with *water*; to 10 ml of this solution add 100 ml of *water* and 1 g of *activated charcoal* and boil under a reflux condenser for 15 minutes. Filter, wash the filter and charcoal with sufficient *water* to produce 150 ml, add 0.25 ml of *bromocresol purple solution* and neutralise with 0.1M *sodium hydroxide* or 0.05M *sulphuric acid* to the blue colour of the indicator. Add 1.4 g of *sodium periodate*, allow to stand for 15 minutes, add 3 ml of *propane-1,2-diol*, shake and allow to stand for 5 minutes. Add 0.25 ml of *bromocresol purple solution* and titrate with 0.1M *sodium hydroxide VS* to the same blue colour. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 9.210 mg of glycerol. Calculate the percentage v/v of glycerol, taking 1.260 g as its weight per ml .

Relative density

0.958 to 0.977, Appendix V G.

Ramipril Capsules

General Notices

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Ramipril Capsules contain Ramipril.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ramipril, $C_{23}H_{32}N_2O_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the contents of the capsules containing 12.5 mg of Ramipril with 50 ml of *acetone,* centrifuge for 10 minutes, filter the supernatant liquid through a 0.45-µm filter, evaporate the filtrate to dryness on a *water bath* and dry the residue for 3 hours at 60°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of ramipril (*RS 417*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of 0.1M *hydrochloric acid* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of the medium and filter immediately through a 0.45-µm glass-fibre filter (Whatman GD/X is suitable), discarding the first 5 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the filtered dissolution medium diluted, if necessary, with 0.1M *hydrochloric acid* to produce a solution expected to contain 0.0025 mg of Ramipril per ml. Solution (2) contains 0.00025% w/v of *ramipril BPCRS* in 0.1M *hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-C18 is suitable) and maintained at 28°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture, adjusted to pH 2.1 with *orthophosphoric acid*, of 420 volumes of *acetonitrile R1* and 580 volumes of a solution containing 1.4% w/v of *sodium perchlorate* and 0.58% w/v of *orthophosphoric acid* adjusted to pH 2.5 with *triethylamine* and (c) a detection wavelength of 210 nm.

Inject 40 µl of each solution.

Calculate the total content of ramipril, $C_{23}H_{32}N_2O_5$, in the medium using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix, with the aid of ultrasound for 10 minutes and stirring, a quantity of the contents of the capsules containing 25 mg of Ramipril with 50 ml of the mobile phase and filter through a 0.45-µm filter.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase.

(3) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(4) 0.05% w/v of *ramipril BPCRS* and 0.0005% w/v of each of *ramipril impurity A EPCRS*, *ramipril impurity D EPCRS* and *ramipril impurity K BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-C18 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.0 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 210 nm.

(f) Injection volume of 15 µl for each solution.

When the chromatograms are recorded under the prescribed conditions, the retention time of ramipril is about 10 minutes and retention times relative to ramipril are about 0.6 for ramipril impurity K (diketopiperazine acid impurity), about 0.7 for ramipril impurity A (methyl ester impurity) and about 2.6 for ramipril impurity D (diketopiperazine impurity).

MOBILE PHASE

A mixture, adjusted to pH 2.6 with *orthophosphoric acid*, of 350 volumes of *acetonitrile R1* and 680 volumes of a solution adjusted to pH 3.9 with *triethylamine,* containing 1.4% w/v of sodium perchlorate and 0.58% w/v of orthophosphoric acid.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to ramipril impurity K and ramipril impurity A is at least 1.0.

LIMITS

For capsules containing more than 1.25 mg of Ramipril

In the chromatogram obtained with solution (1) the combined area of peaks corresponding to ramipril impurity D and ramipril impurity E is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (6.0%); the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). The sum of the areas of all the *secondary peaks* is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (6.0%).

For capsules containing 1.25 mg or less of Ramipril

In the chromatogram obtained with solution (1) the combined area of peaks corresponding to ramipril impurity D and ramipril impurity E is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%); the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). The sum of the areas of all the *secondary peaks* is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%).

Uniformity of content

Capsules containing less than 2 mg of Ramipril comply with the requirements stated under Capsules using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 80 ml of 0.1M *hydrochloric acid* to the contents and shell of one capsule, mix with the aid of ultrasound for 10 minutes and immediately stir magnetically for 15 minutes. Add sufficient 0.1M *hydrochloric acid* to produce 100 ml and filter through a 0.45-µm glass-fibre filter (Whatman GD/X is suitable). Dilute the resulting solution, if necessary, with sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.00025% w/v of Ramipril. Solution (2) contains 0.00025% w/v of *ramipril BPCRS* in 0.1M *hydrochloric acid*.

The chromatographic procedure described under Dissolution may be used.

Calculate the content of $C_{23}H_{32}N_2O_5$ in each capsule using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS*.

ASSAY

For capsules containing 2 mg or more of Ramipril

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 150 ml of 0.1M *hydrochloric acid* to a quantity of the mixed contents of 20 capsules containing 50 mg of Ramipril, mix with the aid of ultrasound for 10 minutes and immediately stir magnetically for 15 minutes. Add sufficient 0.1M *hydrochloric acid* to produce 200 ml and filter through a 0.45-µm glass-fibre filter (Whatman GD/X is suitable). Dilute the resulting solution with sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.00025% w/v of Ramipril.

(2) 0.00025% w/v of *ramipril BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}N_2O_5$ in the capsules using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS*.

For capsules containing less than 2 mg of Ramipril

Use the average of the 10 individual results obtained in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Ramipril.

Ramipril Tablets

General Notices

Action and use

Angiotensin converting enzyme inhibitor.

DEFINITION

Ramipril Tablets contain Ramipril.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ramipril, $C_{23}H_{32}N_2O_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 25 mg of Ramipril with 50 ml of *acetone,* centrifuge for 10 minutes, filter the supernatant liquid through a 0.45-µl filter, evaporate the filtrate to dryness on a *water bath* and dry the residue for 3 hours at 60°. The *infrared absorption spectrum* of the residue*,* Appendix II A, is concordant with the *reference spectrum* of ramipril (*RS 417*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 500 ml of 0.1M *hydrochloric acid* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of the medium and filter immediately through a 0.45-µm glass-fibre filter (Whatman GD/X is suitable), discarding the first 5 ml of filtrate. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the filtered dissolution medium diluted, if necessary, with 0.1M *hydrochloric acid* to produce a solution expected to contain 0.0025 mg of Ramipril per ml. Solution (2) contains 0.00025% w/v of *ramipril BPCRS* in 0.1M *hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-C18 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture, adjusted to pH 2.1 with *orthophosphoric acid*, of 420 volumes of *acetonitrile R1* and 580 volumes of a solution containing 1.4% w/v of *sodium perchlorate* and 0.58% w/v of *orthophosphoric acid* adjusted to pH 2.5 with *triethylamine* and (c) a detection wavelength of 210 nm.

Inject 40 µl of each solution.

Calculate the total content of ramipril, $C_{23}H_{32}N_2O_5$, in the medium using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound for 10 minutes, a quantity of the contents of the powdered tablets containing 25 mg of Ramipril with 50 ml of the mobile phase, centrifuge and use the clear supernatant liquid.

(2) Dilute 1 volume of solution (1) to 20 volumes with the mobile phase.

(3) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(4) 0.05% w/v of *ramipril BPCRS* and 0.0005% w/v of each of *ramipril impurity A EPCRS*, *ramipril impurity D EPCRS* and *ramipril impurity K BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 100-C18 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.0 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 210 nm.

(f) Injection volume of 15 µl for each solution.

When the chromatograms are recorded under the prescribed conditions, the retention time of ramipril is about 10 minutes and retention times relative to ramipril are about 0.6 for ramipril impurity K (diketopiperazine acid impurity), about 0.7 for ramipril impurity A (methyl ester impurity) and about 2.6 for ramipril impurity D (diketopiperazine impurity).

MOBILE PHASE

A mixture, adjusted to pH 2.6 with *orthophosphoric acid*, of 350 volumes of *acetonitrile R1* and 680 volumes of a solution adjusted to pH 3.9 with *triethylamine,* containing 1.4% w/v of sodium perchlorate and 0.58% w/v of orthophosphoric acid.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to ramipril impurity K and ramipril impurity A is at least 1.0.

LIMITS

For tablets containing more than 1.25 mg of Ramipril

In the chromatogram obtained with solution (1) the combined area of peaks corresponding to ramipril impurity D and ramipril impurity E is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (6.0%); the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). The sum of the areas of all the *secondary peaks* is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (6.0%).

For capsules containing 1.25 mg or less of Ramipril

In the chromatogram obtained with solution (1) the combined areas of peak corresponding to ramipril impurity D and ramipril impurity E is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%); the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%). The sum of the areas of all the *secondary peaks* is not greater than 1.6 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%).

Uniformity of content

Tablets containing less than 2 mg of Ramipril comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 ml of 0.1M *hydrochloric acid* to one tablet, mix with the aid of ultrasound for 10 minutes, dilute, if necessary, with sufficient 0.1M *hydrochloric acid* to produce a solution containing 0.025% w/v of Ramipril, centrifuge and use the supernatant liquid. Solution (2) contains 0.025% w/v of *ramipril BPCRS* in 0.1M *hydrochloric acid*.

The chromatographic procedure described under Dissolution may be used.

Calculate the content of $C_{23}H_{32}N_2O_5$ in each tablet using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS*.

ASSAY

For tablets containing 2 mg or more of Ramipril

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add 100 ml of 0.1M *hydrochloric acid* to a quantity of powdered tablets containing 25 mg of Ramipril, mix with the aid of ultrasound for 10 minutes and centrifuge, use the supernatant.

(2) 0.025% w/v of *ramipril BPCRS* in 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Dissolution may be used.

DETERMINATION OF CONTENT

Calculate the content of $C_{23}H_{32}N_2O_5$ in the tablets using the declared content of $C_{23}H_{32}N_2O_5$ in *ramipril BPCRS*.

For tablets containing less than 2 mg of Ramipril

Use the average of the 10 individual results obtained in the test for Uniformity of content.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Ramipril.

Ranitidine Injection

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

DEFINITION

Ranitidine Injection is a sterile solution of Ranitidine Hydrochloride in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of ranitidine, $C_{13}H_{22}N_4O_3S$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. To a volume of the injection containing the equivalent of 25 mg of ranitidine add 20 ml of *methanol*, mix and evaporate to dryness. Add 1 ml of *petroleum spirit* (*boiling range, 60° to 80°*) to the resulting residue, scratch the side of the vessel with a glass rod to induce crystallisation, evaporate to dryness and dry the residue at 60° for 10 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of ranitidine hydrochloride (*RS 311*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

Buffered formulations, pH 6.7 to 7.3; unbuffered formulations, pH 4.5 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute, if necessary, a volume of the injection with *water* to contain the equivalent of 0.1% w/v of ranitidine.

(2) Dilute 1 volume of solution (1) to 50 volumes with *water.*

(3) Dilute 1 volume of solution (1) to 100 volumes with *water*.

(4) Dilute 1 volume of solution (1) to 200 volumes with *water*.

(5) Dilute 1 volume of solution (3) to 5 volumes with *water*.

(6) Dissolve the contents of a vial of *ranitidine impurity J EPCRS* in 1 ml of solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters XTerra MS C18 is suitable).

(b) Gradient elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Column temperature of 35°.

(e) Detection wavelength of 230 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

Buffer solution

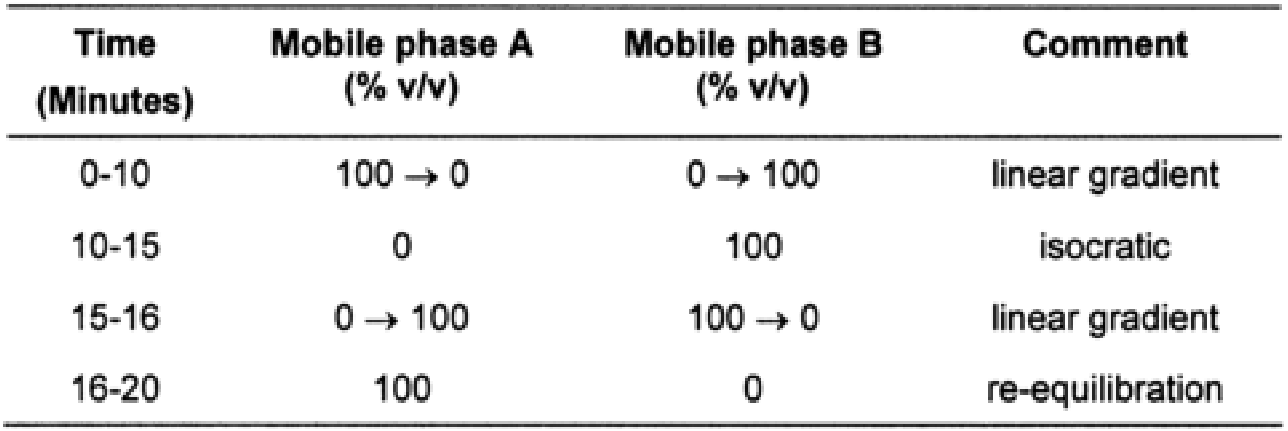
Adjust the pH of 950 ml of 0.05M *potassium dihydrogen orthophosphate to 7.1* by adding *strong sodium hydroxide solution* and dilute to 1 litre.

Mobile phase A

2 volumes of *acetonitrile* and 98 volumes of buffer solution.

Mobile phase B

22 volumes of *acetonitrile* and 78 volumes of buffer solution.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100 → 0	0 → 100	linear gradient
10-15	0	100	isocratic
15-16	0 → 100	100 → 0	linear gradient
16-20	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the two principal peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%);

the area of not more than one *secondary peak* is greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%);

the area of not more than two other *secondary peaks* is greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%);

the area of not more than two further *secondary peaks* is greater than the principal peak in solution (5) (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection with sufficient of the mobile phase to contain the equivalent of 0.01% w/v of ranitidine.

(2) 0.0112% w/v of *ranitidine hydrochloride BPCRS* in mobile phase.

(3) 0.0112% w/v of *ranitidine hydrochloride BPCRS* and 0.0002% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulphinylmethyl]furfuryl}amine BPCRS* in mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS 1 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 322 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

15 volumes of 0.1M *ammonium acetate* and 85 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless the peak due to ranitidine in the chromatogram obtained with solution (3) shows baseline separation from the peak due to dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulphinylmethyl]furfuryl}amine.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{22}N_4O_3S$ in the injection using the declared content of $C_{13}H_{22}N_4O_3S$ in *ranitidine hydrochloride BPCRS*.

STORAGE

Ranitidine Injection should be protected from light.

LABELLING

The label states (1) the quantity of active ingredient in terms of the equivalent amount of ranitidine; (2) where appropriate, that the injection is buffered.

Ranitidine Oral Solution

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

DEFINITION

Ranitidine Oral Solution is a solution of Ranitidine Hydrochloride in a suitable vehicle.
The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ranitidine, $C_{13}H_{22}N_4O_3S$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds in position to that in the chromatogram obtained with solution (3).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.7 to 7.5, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Prepare a solid phase extraction cartridge containing a C_{18} sorbent (Sep-Pak C_{18} cartridges are suitable) by passing 10 ml of *methanol* followed by 20 ml of 0.5M *ammonia* through the cartridge; attach the tip of a suitable syringe to the cartridge. Transfer a weighed quantity of the oral solution containing the equivalent of 10 mg of ranitidine to the barrel of the syringe, add 2 ml of 0.5M *ammonia* to the syringe, insert the plunger and slowly force the mixture through the cartridge, discarding the eluant; repeat with two 4 ml quantities of 0.5M *ammonia* discarding the eluant. Pass two 5-ml quantities of a mixture of 25 volumes of 0.1M *hydrochloric acid* and 75 volumes of *methanol* through the cartridge and collect the eluant; add 40 ml of *absolute ethanol* to the collected eluant, evaporate the resulting solution to dryness at a temperature not exceeding 30° under reduced pressure and dissolve the residue in 1 ml of *methanol*.

(2) Dilute 1 volume of solution (1) to 50 volumes with *methanol*.

(3) 0.0223% w/v of *ranitidine hydrochloride BPCRS* in *methanol*.

(4) 0.0112% w/v of *ranitidine hydrochloride BPCRS* in *methanol*.

(5) 0.0056% w/v of *ranitidine hydrochloride BPCRS* in *methanol*.

(6) 0.1% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulphinylmethyl]furfuryl}amine BPCRS* in solution (1).

(7) Prepare a solution in *water* containing 1.12% w/v of *ranitidine hydrochloride BPCRS* and 0.01% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulphinylmethyl]furfuryl}amine BPCRS* and repeat the sample preparation procedure applying 1 ml of the resulting solution to the barrel of the syringe.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Mobile phase is a mixture of 4 volumes of *water*, 8 volumes of 18M *ammonia*, 30 volumes of *propan-2-ol* and 50 volumes of *ethyl acetate.*

(c) Develop to 15 cm.

(d) Application volume of 20 µl for each solution.

(e) Remove the plate, allow it to dry thoroughly in air and expose to iodine vapour in a closed chamber until the spots are visible.

SYSTEM SUITABILITY

The test is not valid unless (a) the chromatogram obtained with solution (7) shows two clearly separated spots and the spot due to dimethyl{5-[2-(1-methylamino-2-nitrovinylamino) ethylsulphinylmethyl]furfuryl}amine is of equal intensity to that of the spot in the chromatogram obtained with solution (4) and (b) the chromatogram obtained with solution (6) shows two clearly separated spots.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (2%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (1%);

not more than two other such spots are more intense than the spot in the chromatogram obtained with solution (5) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute a weighed quantity of the oral solution containing the equivalent of 10 mg of ranitidine with sufficient of the mobile phase to produce 100 ml.

(2) 0.0112% w/v of *ranitidine hydrochloride BPCRS* in the mobile phase.

(3) 0.0112% w/v of *ranitidine hydrochloride BPCRS* and 0.0002% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulphinylmethyl]furfuryl}amine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS 1 is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 2 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 322 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

15 volumes of 0.1M *ammonium acetate* and 85 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless the peak due to ranitidine in the chromatogram obtained with solution (3) shows baseline separation from the peak due to dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulphinylmethyl]furfuryl}amine.

DETERMINATION OF CONTENT

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{13}H_{22}N_4O_3S$, weight in volume, using the declared content of $C_{13}H_{22}N_4O_3S$ in *ranitidine hydrochloride BPCRS.*

STORAGE

Ranitidine Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ranitidine.

Ranitidine Tablets

General Notices

Action and use

Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

DEFINITION

Ranitidine Tablets contain Ranitidine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ranitidine, $C_{13}H_{22}N_4O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 25 mg of ranitidine with 5 ml of *methanol* for 5 minutes, filter and evaporate the filtrate to dryness. Add 1 ml of *petroleum spirit* (*boiling range, 60° to 80°*) to the residue, scratch the side of the vessel to induce crystallisation, evaporate to dryness and dry the residue at 60° for 10 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of ranitidine hydrochloride (*RS 311*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of powdered tablets containing the equivalent of 0.13 g of ranitidine with 100 ml *water* and filter (Whatman No. 42. paper is suitable).

(2) Dilute 1 volume of solution (1) to 200 volumes with mobile phase A.

(3) Dilute 1 volume of solution (1) to 20 volumes with mobile phase A and dilute 3 volumes of the resulting solution to 50 volumes with mobile phase A.

(4) Dissolve the contents of a vial of *ranitidine impurity J EPCRS* in 1 ml of solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl amorphous organosilica polymer* (3.5 µm) (Waters Xterra MS C18 is suitable).

(b) Use gradient elution using the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

Buffer solution

Adjust the pH of 950 ml of 0.05M *potassium dihydrogen orthophosphate* to 7.1 by adding *strong sodium hydroxide solution* and dilute to 1 litre.

Mobile phase A

2 volumes of *acetonitrile* and 98 volumes of the buffer solution.

Mobile phase B

22 volumes of acetonitrile and 78 volumes of the buffer solution.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	100 → 0	0 → 100	linear gradient
10-15	0	100	isocratic
15-16	0 → 100	100 → 0	linear gradient
16-20	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

— the area of not more than one *secondary peak* is greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

— the area of not more than three other *secondary peaks* is greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake 10 tablets with 400 ml of the mobile phase until the tablets have completely disintegrated (about 15 minutes), dilute to 500 ml with the mobile phase, filter (Whatman GF/C paper is suitable) and dilute the filtrate with the mobile phase to produce a solution containing the equivalent of 0.01% w/v of ranitidine.

(2) 0.0112% w/v of *ranitidine hydrochloride BPCRS* in the mobile phase.

(3) 0.0112% w/v of *ranitidine hydrochloride BPCRS* and 0.0002% w/v of *dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulphinylmethyl]furfuryl}amine BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Partisil ODS 1 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 322 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

15 volumes of 0.1M *ammonium acetate* and 85 volumes of *methanol*.

SYSTEM SUITABILITY

The assay is not valid unless the peak due to ranitidine in the chromatogram obtained with solution (3) shows baseline separation from the peak due to dimethyl{5-[2-(1-methylamino-2-nitrovinylamino)ethylsulphinylmethyl]furfuryl}amine.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{22}N_4O_3S$ in the tablets using the declared content of $C_{13}H_{22}N_4O_3S$ in *ranitidine hydrochloride BPCRS*.

STORAGE

Ranitidine Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of ranitidine.

Ribavirin Nebuliser Solution

General Notices

Ribavirin Solution for Nebulisation

Action and use

Antiviral (Hepatitus C, respiratory syncytial virus).

DEFINITION

Ribavirin Nebuliser Solution is a sterile solution of Ribavirin in Water for Injections. It is prepared by dissolving Ribavirin for Nebulisation in the requisite amount of Water for Injections.

Sterility

Complies with the test *for sterility*, Appendix XVI A.

STORAGE

Ribavirin Nebuliser Solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

The contents of the sealed container when reconstituted comply with the requirements stated under Preparations for Inhalation.

RIBAVIRIN FOR NEBULISATION

DEFINITION

Ribavirin for Nebulisation is a sterile material consisting of Ribavirin with or without excipients. It is supplied in a sealed container.

Content of ribavirin, $C_8H_{12}N_4O_5$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 20 volumes of 0.1M *ammonium chloride* and 90 volumes of *acetonitrile* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in *water* containing (1) 1% w/v Ribavirin and (2) 1% w/v of *ribavirin BPCRS*. After removal of the plate, allow the spots to dry in air for 15 minutes and spray the plate with mixture of 0.5 ml of *anisaldehyde*, 0.5 ml of *sulphuric acid*, 0.1 ml of *glacial acetic acid* and 9 ml of *ethanol* (*96%*). Heat the plate at 105° for 40 minutes. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using two solutions of the contents of the container in the mobile phase containing (1) 0.050% w/v and (2) 0.000125% w/v of Ribavirin respectively. Solution (3) contains 0.050% w/v of *ribavirin impurity standard BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 7.8 mm) packed with a strong cation-exchange resin of sulphonated, cross-linked *styrene-divinylbenzene co-polymer* in the hydrogen form (7 to 11 µm) (Aminex HPAH is suitable) and maintained at 40°, (b) *water* adjusted to pH 2.5 with *sulphuric acid* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 207 nm. Inject separately 10 µl of each solution. For solution (1) allow the chromatography to proceed for ten times the retention time of the principal peak.

The test is not valid unless the *symmetry factor* for the principal peak in the chromatogram obtained with solution (1) is at least 0.7 and not more than 1.5 and unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *ribavirin impurity standard BPCRS*.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than that of the principal peak in the chromatogram obtained with solution (2) (0.25%) and the sum of the areas of any such peaks is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase. For solution (1) reconstitute the contents of one container as instructed on the label using an accurately measured volume of diluent, dilute a suitable volume of the solution to 200 volumes with the mobile phase to produce a solution containing 0.10% w/v of Ribavirin; mix and dilute 5 volumes of the resulting solution to 20 volumes with the mobile phase. Solution (2) contains 0.0025% w/v of *ribavirin BPCRS*.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_8H_{12}N_4O_5$ in the container using the declared content of $C_8H_{12}N_4O_5$ in *ribavirin BPCRS*.

LABELLING

The label of the sealed container states (1) the procedure for preparing the solution; (2) the delivery system to be used for the constituted solution; (3) the conditions under which it should be stored.

Rifampicin Capsules

General Notices

Action and use

Rifamycin antituberculosis drug.

DEFINITION

Rifampicin Capsules contain Rifampicin.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of rifampicin, $C_{43}H_{58}N_4O_{12}$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the contents of the capsules containing 0.15 g of Rifampicin with 5 ml of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of rifampicin (*RS 312*).

B. The *light absorption*, Appendix II B, in the range 220 to 500 nm of the final solution obtained in the Assay exhibits four maxima, at 237, 254, 334 and 475 nm.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium and filter. Measure the *absorbance* of the filtered sample, diluted if necessary with 0.1M *hydrochloric acid*, at the maximum at 336 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the total content of rifampicin, $C_{43}H_{58}N_4O_{12}$, in the medium taking 263 as the value of A(1%, 1 cm) at the maximum at 336 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions prepared in the solvent mixture described below. To 10 volumes of a 21.01% w/v solution of *citric acid* add 23 volumes of a 13.61% w/v solution of *potassium dihydrogen orthophosphate*, 77 volumes of a 17.42% w/v solution of *dipotassium hydrogen orthophosphate*, 250 volumes of *acetonitrile* and 640 volumes of *water* and mix. Prepare the solutions immediately before use. For solution (1) shake a quantity of the contents of the capsules containing 20 mg of Rifampicin with 10 ml of *acetonitrile*, centrifuge and dilute 5 ml of the clear supernatant liquid to 50 ml with the solvent mixture. For solution (2) dilute 1 volume of solution (1) to 100 volumes. Solution (3) contains 0.00080% w/v of *rifampicin quinone EPCRS*. Solution (4) contains 0.00030% w/v of *rifampicin* N-*oxide BPCRS*. Solution (5) contains 0.00010% w/v of *3-formylrifamycin SV BPCRS*. For solution (6) dilute 1 volume of solution (3) to 4 volumes and mix 1 volume of the resulting solution with 1 volume of solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Partisil C8 is suitable), (b) as mobile phase with a flow rate of 1.5 ml per minute a mixture of 35 volumes of *acetonitrile* and 65 volumes of a solution containing 0.1% v/v of *orthophosphoric acid*, 0.19% w/v of *sodium perchlorate*, 0.59% w/v of *citric acid* and 2.09% w/v of *potassium dihydrogen orthophosphate* and (c) a detection wavelength of 254 nm. Inject 20 µl of each solution.

Inject solution (6). Adjust the sensitivity of the detector so that the height of the two principal peaks is not less than half the full-scale of the recorder. The test is not valid unless the *resolution factor* between the two principal peaks is at least 4.0. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject solution (1) and allow the chromatography to proceed for at least 3 times the retention time of the peak due to rifampicin. The area of any peak corresponding to rifampicin quinone is not greater than the area of the peak in the chromatogram obtained with solution (3) (4%); the area of any peak corresponding to rifampicin *N*-oxide is not greater than the area of the peak in the chromatogram obtained with solution (4) (1.5%); the area of any peak corresponding to 3-formylrifamycin SV is not greater than the area of the peak in the chromatogram obtained with solution (5) (0.5%) and the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Shake a quantity of the mixed contents of 20 capsules containing 0.1 g of Rifampicin with 80 ml of *methanol*, add sufficient *methanol* to produce 100 ml and filter. Dilute 2 ml of the filtrate to 100 ml with *phosphate buffer pH 7.4* and measure the *absorbance* of the resulting solution at the maximum at 475 nm, Appendix II B. Calculate the content of $C_{43}H_{58}N_4O_{12}$ taking 187 as the value of A(1%, 1 cm) at 475 nm.

Rifampicin Oral Suspension

General Notices

Action and use

Rifamycin antituberculosis drug.

DEFINITION

Rifampicin Oral Suspension is a suspension of Rifampicin in powder of suitable fineness in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of rifampicin, $C_{43}H_{58}N_4O_{12}$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a quantity containing 0.1 g of Rifampicin add 30 ml of *water* and shake with two 50 ml quantities of *chloroform*. Dry the combined extracts with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness at a temperature not exceeding 70°. The residue, after washing with 1 ml of *ether* and drying at 70°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of rifampicin (*RS 312*).

B. Dissolve 10 mg of the residue in 10 ml of *methanol* and dilute 2 ml to 100 ml with *phosphate buffer pH 7.4*. The *light absorption* of the resulting solution, Appendix II B, in the range 240 to 500 nm exhibits three maxima, at 254, 334 and 475 nm.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions prepared in the solvent mixture described below. To 10 volumes of a 21.01% w/v solution of *citric acid* add 23 volumes of a 13.61% w/v solution of *potassium dihydrogen orthophosphate*, 77 volumes of a 17.42% w/v solution of *dipotassium hydrogen orthophosphate*, 250 volumes of *acetonitrile* and 640 volumes of *water* and mix. Prepare the solutions immediately before use. For solution (1) add 5 ml of *water* to a quantity of the oral suspension containing 20 mg of Rifampicin and extract with four 10 ml quantities of *dichloromethane*, filter the combined extracts and evaporate to dryness at a temperature not exceeding 40°. Dissolve the residue in 10 ml of *acetonitrile* and dilute 5 ml of the solution to 50 ml with the solvent mixture. For solution (2) dilute 1 volume of solution (1) to 100 volumes. Solution (3) contains 0.00030% w/v of *rifampicin quinone EPCRS*. Solution (4) contains 0.00020% w/v of *rifampicin* N*-oxide BPCRS*. Solution (5) contains 0.0010% w/v of *3-formylrifamycin SV BPCRS*. For solution (6) dilute 1 volume of solution (3) to 1.5 volumes and mix 1 volume of the resulting solution with 1 volume of solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Partisil C8 is suitable), (b) as mobile phase with a flow rate of 1.5 ml per minute a mixture of 35 volumes of *acetonitrile* and 65 volumes of a solution containing 0.1% v/v of *orthophosphoric acid*, 0.19% w/v of *sodium perchlorate*, 0.59% w/v of *citric acid* and 2.09% w/v of *potassium dihydrogen orthophosphate* and (c) a detection wavelength of 254 nm. Inject 20 µl of each solution.

Inject solution (6). Adjust the sensitivity of the detector so that the height of the two principal peaks is not less than half the full-scale of the recorder. The test is not valid unless the *resolution factor* between the two principal peaks is at least 4.0. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject solution (1) and allow the chromatography to proceed for at least 3 times the retention time of the peak due to rifampicin. The area of any peak corresponding to rifampicin quinone is not greater than the area of the peak in the chromatogram obtained with solution (3) (1.5%) ; the area of any peak corresponding to rifampicin *N*-oxide is not greater than the area of the peak in the chromatogram obtained with solution (4) (1%); the area of any peak corresponding to 3-formylrifamycin SV is not greater than the area of the peak in the chromatogram obtained with solution (5) (5%) and the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%). Disregard any peaks with retention times lower than that of the peak due to rifampicin quinone.

Acidity

pH, 4.2 to 4.8, Appendix V L.

ASSAY

Dilute a weighed quantity containing 0.4 g of Rifampicin to 500 ml with *methanol*, mix thoroughly, dilute 2 ml to 100 ml with *phosphate buffer pH 7.4* and measure the *absorbance* of the resulting solution at the maximum at 475 nm, Appendix II B. Calculate the content of $C_{43}H_{58}N_4O_{12}$ taking 187 as the value of A(1%, 1 cm) at 475 nm. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{43}H_{58}N_4O_{12}$, weight in volume.

Ritodrine Injection

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Ritodrine Injection is a sterile solution of Ritodrine Hydrochloride in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of ritodrine hydrochloride, $C_{17}H_{21}NO_3,HCl$

93.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute a suitable volume of the injection with sufficient *methanol* (*30%*) to produce a solution containing 0.010% w/v of Ritodrine Hydrochloride. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 275 nm and a shoulder at about 280 nm.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute, if necessary, a volume of the injection with sufficient of the mobile phase to produce a solution containing 0.10% w/v of Ritodrine Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase. For solution (3) heat a solution containing 0.10% w/v of *ritodrine hydrochloride BPCRS* in the mobile phase containing 5.6% v/v of *sulphuric acid* for 2 hours at 85°. Cool and mix 10 volumes of this solution with 8 volumes of a 10% w/v solution of *sodium hydroxide* (generation of *threo*-diastereoisomer).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of a solution containing 6.6 g of *diammonium hydrogen orthophosphate* and 1.1 g of *sodium heptanesulphonate* in 700 ml of *water* and 300 ml of *methanol* adjusted to pH 3.0 with an 85% v/v solution of *orthophosphoric acid* and (c) a detection wavelength of 275 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks (ritodrine and the *threo*-diastereoisomer) is at least 1.5.

In the chromatogram obtained with solution (1), the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (4%), not more than one such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%), not more than one such peak has an area greater than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all such peaks is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection with sufficient of the mobile phase to produce a solution containing 0.02% w/v of Ritodrine Hydrochloride. Solution (2) contains 0.02% w/v of *ritodrine hydrochloride BPCRS* in the mobile phase. For solution (3) heat a solution containing 0.1% w/v of *ritodrine hydrochloride BPCRS* in the mobile phase containing 5.6% v/v of *sulphuric acid* for 2 hours at 85°. Cool and mix 10 volumes with 8 volumes of a 10% w/v solution of *sodium hydroxide* (generation of *threo*-diastereoisomer).

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks (ritodrine and the *threo*-diastereoisomer) is at least 1.5.

Calculate the content of $C_{17}H_{21}NO_3,HCl$ in the injection from the chromatograms obtained and from the declared content of $C_{17}H_{21}NO_3,HCl$ in *ritodrine hydrochloride BPCRS*.

STORAGE

Ritodrine Injection should be protected from light.

Ritodrine Tablets

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Ritodrine Tablets contain Ritodrine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ritodrine hydrochloride, $C_{17}H_{21}NO_3,HCl$

93.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 10 mg of Ritodrine Hydrochloride with 30 ml of *methanol* for 20 minutes, filter (Whatman GF/C is suitable) and to 15 ml of the filtrate add sufficient *water* to produce 50 ml. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum at 275 nm and a shoulder at about 280 nm.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Ritodrine Hydrochloride in 50 ml of the mobile phase for 5 minutes, add sufficient mobile phase to produce 100 ml, mix and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) dissolve about 20 mg of *ritodrine hydrochloride BPCRS* in 50 ml of the mobile phase, add 5.6 ml of *sulphuric acid* and sufficient of the mobile phase to produce 100 ml, mix and heat at a temperature of 85° for 2 hours. Cool to room temperature, carefully mix 10 ml of the cooled solution with 8 ml of a 10% w/v solution of *sodium hydroxide* and allow to cool (generation of *threo*-diastereoisomer).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (7 µm) (Zorbax C8 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of a solution containing 6.6 g of *diammonium hydrogen orthophosphate* and 1.1 g of *sodium heptanesulphonate* in 700 ml of *water* and 300 ml of *methanol* adjusted to pH 3.0 with an 85% v/v solution of *orthophosphoric acid* and (c) a detection wavelength of 275 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks (ritodrine and the *threo*-diastereoisomer) is at least 1.5.

In the chromatogram obtained with solution (1), identify any peaks corresponding to tyramine (relative retention time to ritodrine, 0.3), *threo*-diastereoisomer (1.15) and 'aminoketone' (2.3). In the chromatogram obtained with solution (1) the area of any peaks corresponding to tyramine and the *threo*-diastereoisomer is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1% of each), the area of any peak corresponding to 'aminoketone' is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1% [relative response to ritodrine, 5]). The degradation products 4-hydroxybenzoic acid and 4-hydroxybenzaldehyde are formed in equimolar amounts with tyramine.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Ritodrine Hydrochloride in 50 ml of the mobile phase for 5 minutes, add sufficient of the mobile phase to produce 100 ml, mix and filter. Solution (2) contains 0.02% w/v of *ritodrine hydrochloride BPCRS* in the mobile phase. For solution (3) dissolve about 20 mg of *ritodrine hydrochloride BPCRS* in 50 ml of the mobile phase, add 5.6 ml of *sulphuric acid* and sufficient of the mobile phase to produce 100 ml, mix and heat at a temperature of 85° for 2 hours. Cool to room temperature, carefully mix 10 ml of the cooled solution with 8 ml of a 10% w/v solution of *sodium hydroxide* and allow to cool (generation of *threo*-diastereoisomer).

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks (ritodrine and the *threo*-diastereoisomer) is at least 1.5.

Calculate the content of $C_{17}H_{21}NO_3$,HCl in the tablets from the chromatograms obtained and from the declared content of $C_{17}H_{21}NO_3$,HCl in *ritodrine hydrochloride BPCRS*.

STORAGE

Ritodrine Tablets should be protected from light.

Compound Sodium Bicarbonate Tablets

General Notices

Soda Mint Tablets

DEFINITION

Compound Sodium Bicarbonate Tablets contain, in each, 300 mg of Sodium Bicarbonate. They have a peppermint flavour.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of total carbonate

275 to 325 mg, calculated as $NaHCO_3$.

IDENTIFICATION

A. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, impart a yellow colour to the flame.

B. The powdered tablets yield the reactions characteristic of *bicarbonates*, Appendix VI.

TESTS

Carbonate

Dissolve 2.5 g of the powder prepared for use in the Assay in 50 ml of *carbon dioxide-free water* . The pH of the resulting solution is not more than 8.6, Appendix V L.

Disintegration

The requirement for Disintegration does not apply to Compound Sodium Bicarbonate Tablets.

ASSAY

Weigh and powder 20 tablets. Dissolve 1 g of the powder in 20 ml of *water* and titrate with 0.5M *hydrochloric acid VS* using *methyl orange solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

LABELLING

The label states that the tablets should be allowed to dissolve slowly in the mouth.

Compound Sodium Chloride Mouthwash

General Notices

DEFINITION

Compound Sodium Chloride Mouthwash contains 1% w/v of Sodium Bicarbonate and 1.5% w/v of Sodium Chloride in a suitable vehicle with a flavour of peppermint.

Extemporaneous preparation

The following formula applies.

Sodium Bicarbonate	10 g
Sodium Chloride	15 g
Concentrated Peppermint Emulsion	25 ml
Double-strength Chloroform Water	500 ml
Water	Sufficient to produce 1000 ml

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

0.95 to 1.05% w/v.

Content of sodium chloride, NaCl

1.42 to 1.58% w/v.

ASSAY

For sodium bicarbonate

Titrate 20 ml with 0.1M *hydrochloric acid VS* using *methyl orange-xylene cyanol FF solution* as indicator. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 8.401 mg of $NaHCO_3$.

For sodium chloride

To 10 ml add 10 ml of *water* and carry out the method for *ion-selective potentiometry*, Appendix VIII E, using 0.1M *silver nitrate VS*. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

Compound Sodium Chloride Mouthwash should be diluted with an equal volume of warm water before use.

Compound Sodium Lactate Intravenous Infusion

General Notices

Compound Sodium Lactate Injection; Hartmann's Solution for Injection; Ringer-Lactate Solution for Injection

DEFINITION

Compound Sodium Lactate Intravenous Infusion is a sterile solution containing 0.32% w/v of sodium lactate with 0.6% w/v of Sodium Chloride, 0.04% w/v of Potassium Chloride and 0.027% w/v of Calcium Chloride Dihydrate in Water for Injections. It may be prepared from Sodium Lactate Solution or from Lactic Acid.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium, Na

0.27 to 0.32% w/v.

Content of potassium, K

0.019 to 0.022% w/v.

Content of total chloride, Cl

0.37 to 0.42% w/v.

Content of calcium chloride dihydrate, $CaCl_2,2H_2O$

0.025 to 0.029% w/v.

Content of lactate, calculated as $C_3H_6O_3$

0.23 to 0.28% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. When warmed with *potassium permanganate* , yields acetaldehyde.

B. The residue obtained by evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame. When viewed through a suitable blue glass, the flame is tinged with reddish purple.

C. Yields reaction C characteristic of *calcium salts* , Appendix VI.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.0, Appendix V L.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per ml, Appendix XIV C.

ASSAY

For Na

Prepare a suitable dilution in *water* and determine by *atomic emission spectrophotometry* , Appendix II D, measuring at 589 nm and using *sodium standard solution* (*200 ppm Na*), suitably diluted with *water*, for the standard solutions.

For K

Prepare a suitable dilution in *water* and determine by *atomic emission spectrophotometry* , Appendix II D, measuring at 767 nm and using *potassium standard solution* (*600 ppm K*), suitably diluted with *water*, for the standard solutions.

For total chloride

To 20 ml add 30 ml of *water*, 50 ml of 0.1M *silver nitrate VS* and 2 ml of *nitric acid* , filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with 0.1M *ammonium thiocyanate VS* using *ammonium iron* (*III*) *sulphate solution R2* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 3.545 mg of total Cl.

For calcium chloride dihydrate

To 50 ml add 5 ml of 0.01M *magnesium sulphate VS* and 5 ml of *ammonia buffer pH 10.9* and titrate with 0.01M *disodium edetate VS* using *mordant black 11 triturate* as indicator. From the volume of 0.01M *disodium edetate VS* required subtract the volume of 0.01M *magnesium sulphate VS* added. Each ml of the remainder is equivalent to 1.470 mg of $CaCl_2$, $2H_2O$.

For lactate, calculated as $C_3H_6O_3$

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. Solution (1) contains 0.28% w/v of *lithium lactate BPCRS* in the mobile phase. For solution (2) use the infusion.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 90 volumes of *water* and 10 volumes of a 2% v/v solution of *octylamine* in *acetonitrile* , the pH of the final mixture being adjusted to 7.0 with a 10% v/v solution of *orthophosphoric acid* , and (c) a detection wavelength of 210 nm.

Calculate the content of lactate as $C_3H_6O_3$ in the infusion using the declared equivalent content of $C_3H_6O_3$ in *lithium lactate BPCRS* .

LABELLING

The label states (1) that solutions containing visible solid particles must not be used; (2) that the Intravenous Infusion contains, in millimoles per litre, the following approximate amounts of the ions present: sodium, 131; potassium, 5; calcium, 2; bicarbonate (as lactate), 29 and chloride, 111.

Compound Sodium Picosulfate Powder for Oral Solution

General Notices

Sodium Picosulfate Oral Powder

DEFINITION

Compound Sodium Picosulfate Powder for Oral Solution contains Sodium Picosulfate, Light Magnesium Oxide and Anhydrous Citric Acid. On reconstitution the powder produces Sodium Picosulphate and Magnesium Citrate in solution; heat is evolved during the reconstitution.

The powder complies with the requirements stated under Powders and Granules for Oral Solutions and Suspensions under Oral Liquids and with the following requirements.

Content of sodium picosulfate, $C_{18}H_{13}NNa_2O_8S_2$

90.0 to 110.0% of the stated amount.

Content of light magnesium oxide, MgO

90.0 to 110.0% of the stated amount.

Content of anhydrous citric acid, $C_6H_8O_7$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. In the test for Uniformity of content, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak in the chromatogram obtained with solution (2).

B. Dissolve the contents of one sachet in *water*; heat is evolved.

C. Add 100 ml of *water* to the contents of one sachet and allow to cool. Centrifuge and dilute 10 ml of the supernatant liquid to 100 ml with *water*. 5 ml of the resulting solution yields reaction A characteristic of *citrates* , Appendix VI.

D. Place about 1 g of the oral powder in a silica crucible, moisten with *sulphuric acid* , ignite gently, again moisten with *sulphuric acid* and ignite at 800° until a carbon-free residue is obtained. Disperse about 0.1 g of the residue obtained in 10 ml of *water*, adjust the pH to about 3 with 2M *sulphuric acid* , shake and filter. The filtrate yields the reactions characteristic of *magnesium salts* , Appendix VI.

TESTS

Impurity A

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse the contents of one sachet in about 70 ml of *water*, allow to stand until effervescence has ceased, cool to room temperature, add sufficient *water* to produce a solution containing 0.01% w/v Sodium Picosulphate, mix and centrifuge; use the clear supernatant liquid. Solution (2) contains 0.0002% w/v of *sodium picosulfate BPCRS* in *water*. For solution (3) dissolve 10 mg of *sodium picosulfate BPCRS* in 2 ml of 0.1M *hydrochloric acid*, bring rapidly to the boil and heat for 1 minute. Cool in ice-water, add 2 ml of 0.1M *sodium hydroxide* and dilute to 10 ml with the mobile phase. Dilute 2 volumes to 50 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) a guard column (2.5 cm × 4.5 mm) packed with *octadecylsilyl silica gel for chromatography*, (c) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 15 volumes of *acetonitrile* and 85 volumes of *0.067* M *mixed phosphate buffer pH 7.0* and (d) a detection wavelength of 263 nm.

Inject 50 µl of each solution. The test is not valid unless the chromatogram obtained with solution (3) closely resembles the appropriate reference chromatogram supplied with *sodium picosulphate BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak due to impurity A (4-[(pyridin-2-yl)(4-hydroxyphenyl)methyl] phenyl sodium sulphate) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

Complies with the requirements stated under Powders with respect to the content of Sodium Picosulfate using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of one sachet in about 70 ml of *water*, allow to stand until effervescence has ceased, cool to room temperature, add sufficient *water* to produce 100 ml, mix and centrifuge; use the clear supernatant liquid. Solution (2) contains 0.01% w/v of *sodium picosulfate BPCRS* in *water*.

The chromatographic procedure described under Impurity A may be used.

Calculate the content of $C_{18}H_{13}NNa_2O_8S_2$ using the declared content of $C_{18}H_{13}NNa_2O_8S_2$ in *sodium picosulfate BPCRS.*

ASSAY

For sodium picosulfate

Use the average of the 10 individual results obtained in the test for Uniformity of content.

For light magnesium oxide

To a quantity of the powder containing 30 mg of Light Magnesium Oxide add 4 ml of 3M *hydrochloric acid*, dissolve by warming on a water bath, cool and add 50 ml of *water*. Carry out the *complexometric titration of magnesium*, Appendix VIII D, beginning at the words 'To the resulting solution... ', but using 0.05M *disodium edetate VS* as titrant. Each ml of 0.05M *disodium edetate VS* is equivalent to 2.015 mg of MgO.

For anhydrous citric acid

During the titration take care to exclude carbon dioxide. Dissolve a quantity of the powder containing 0.8 g of Anhydrous Citric Acid in 60 ml of *dimethylformamide* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant, determining the end point potentiometrically and taking the second inflection as the end point. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 96.05 mg of $C_6H_8O_7$.

LABELLING

The label states (1) the total weights, in grams, of the constituents in each sachet and (2) that heat is evolved when the contents of the sachet are added to water.

IMPURITIES

4-[(pyridin-2-yl)(4-hydroxyphenyl)methyl]phenyl sodium sulphate.

Dilute Sodium Hypochlorite Solution

General Notices

DEFINITION

Dilute Sodium Hypochlorite Solution is an aqueous *cutaneous solution* of sodium hypochlorite containing 1% of available chlorine. It may contain suitable stabilising agents and Sodium Chloride.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of available chlorine, Cl

0.90 to 1.10% w/w.

ASSAY

Add 10 ml of the solution to a mixture of 50 ml of *water*, 1 g of *potassium iodide* and 12.5 ml of 2M *acetic acid* . Titrate with 0.1M *sodium thiosulphate VS* using *starch solution* as indicator. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 3.545 mg of Cl. Determine the *weight per ml* of the solution, Appendix V G, and calculate the percentage w/w of available chlorine.

STORAGE

Dilute Sodium Hypochlorite Solution should be kept in a well-filled container and protected from light. It should be stored at a temperature not exceeding 20° and away from acids.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

The label indicates the pharmaceutical form as 'cutaneous solution'.

Gastro-resistant Sodium Valproate Tablets

General Notices

Enteric-coated Sodium Valproate Tablets

DEFINITION

Gastro-resistant Sodium Valproate Tablets contain Sodium Valproate. They are covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium valproate, $C_8H_{15}NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 10 ml of *water* and centrifuge. Acidify 5 ml of the supernatant liquid with 2M *sulphuric acid* , shake with 25 ml of *chloroform* and wash the chloroform layer with 5 ml of *water*. Dry by shaking with *anhydrous sodium sulphate* , filter and evaporate the chloroform to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of valproic acid (*RS 358*).

TEST

Related substances

Carry out the method for *gas chromatography* , Appendix III B, using the following solutions. Solution (1) contains 0.020% w/v of *octanoic acid* (internal standard) in *dichloromethane* . For solution (2) shake a quantity of the powdered tablets containing 0.50 g of Sodium Valproate with 10 ml of *water*, acidify with 2M *sulphuric acid* and shake with three 20 ml quantities of *dichloromethane* . Wash the combined dichloromethane extracts with 10 ml of *water*, shake with *anhydrous sodium sulphate* , filter and evaporate the filtrate to a volume of about 8 ml at a temperature not exceeding 30° using a rotary evaporator and dilute to 10 ml with dichloromehane. For solution (3) shake a quantity of the powdered tablets containing 0.50 g of Sodium Valproate with 10 ml of a 0.020% w/v solution of the internal standard in 0.1M *sodium hydroxide* and continue as described for solution (2), beginning at the words 'acidify with 2M *sulphuric acid* ...'.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 15% w/w of free fatty acid phase and 1% w/w of *orthophosphoric acid* and maintained at 170°.

In the chromatogram obtained with solution (3) the sum of the areas of any *secondary peaks* is not greater than the area of the peak due to the internal standard (0.4%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) shake 0.1 g of *sodium valproate BPCRS* with 10 ml of a 1% w/v solution of *octanoic acid* (internal standard) in 0.1M *sodium hydroxide*, acidify with 2M *sulphuric acid* and shake with three 20-ml quantities of *dichloromethane*. Wash the combined dichloromethane extracts with 10 ml of *water*, shake with *anhydrous sodium sulphate*, filter and wash the filter paper with 20 ml of *dichloromethane*. To 50 ml of the filtrate add sufficient *dichloromethane* to produce 100 ml. Prepare solution (2) in the same manner as solution (3) but using 10 ml of a solution of 0.1M *sodium hydroxide* in place of the internal standard solution. For solution (3) shake a quantity of the powdered tablets containing 0.1 g of Sodium Valproate with 10 ml of a 1% w/v solution of the internal standard in 0.1M *sodium hydroxide* and continue as described for solution (1) beginning at the words 'acidify with 2M *sulphuric acid* ... '.

The chromatographic conditions described under the test for Related substances may be carried out.

Calculate the content of $C_8H_{15}NaO_2$ in the tablets using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS*.

LABELLING

The label states that the tablets should be swallowed whole and not chewed.

Gastro-resistant Sulfasalazine Tablets

General Notices

Enteric-coated Sulfasalazine Tablets

Action and use

Aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Gastro-resistant Sulfasalazine Tablets contain Sulfasalazine. They are covered with a gastro-resistant coating.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulfasalazine, $C_{18}H_{14}N_4O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract for 30 minutes with the aid of ultrasound a quantity of the powdered tablets containing 0.2 g of Sulfasalazine with 100 ml of *methanol* , filter, evaporate the filtrate to dryness and dry at 35° at a pressure of 2 kPa for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulfasalazine *(RS 429)* .

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Sulfasalazine with 100 ml of 0.1M *ammonia* for 30 minutes, centrifuge and use the supernatant liquid.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *ammonia* .

(3) Contains 0.001% w/v of *sulfasalazine derivative for resolution EPCRS* in solution (2).

(4) Contains 0.001% w/v of *sulfasalazine EPCRS* in 0.1M *ammonia* .

(5) Dilute 1 volume of solution (2) to 20 volumes with 0.1M *ammonia* .

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phases described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 320 nm.

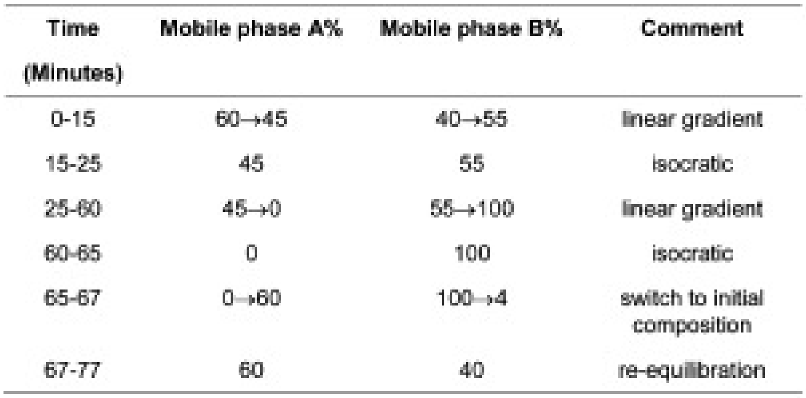
(f) Inject 20 µl of each solution.

MOBILE PHASE A

A solution containing 0.013% w/v of *sodium dihydrogen orthophosphate* and 0.25% w/v of *sodium acetate* adjusted to pH 4.8 with *glacial acetic acid* .

MOBILE PHASE B

Mix 1 volume of mobile phase A with 4 volumes of *methanol* .



Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-15	60→45	40→55	linear gradient
15-25	45	55	isocratic
25-60	45→0	55→100	linear gradient
60-65	0	100	isocratic
65-67	0→60	100→4	switch to initial composition
67-77	60	40	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the peaks corresponding to sulfasalazine and sulfasalazine derivative for resolution is at least 3.0.

When the chromatograms are recorded in the prescribed conditions, the approximate retention times relative to sulfasalazine are: impurity A, 2.00; impurity B, 1.85; impurity C, 0.80; impurity D, 1.90; impurity E, 1.63; impurity F, 0.85; impurity G, 1.39; impurity H, 0.16; impurity I, 0.28.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of any such peaks is not greater than 4 times the area of the peak in the chromatogram obtained with solution (2) (4%).

Disregard any peak with a retention time shorter than 6 minutes (corresponding to salicylic acid and sulfapyridine) and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.05%).

Salicylic acid and sulfapyridine

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 0.1 g of Sulfasalazine with 100 ml of 0.1M *ammonia* for 30 minutes, centrifuge and use the supernatant liquid.

(2) Prepare a solution containing 0.05% w/v of *salicylic acid* and 0.05% w/v of s*ulfapyridine EPCRS* in 0.1M *ammonia* and dilute 1 volume of this solution to 50 volumes with 0.1M *ammonia*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use Isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 300 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 70 volumes of mobile phase A described in the test for Related substances and 30 volumes of mobile phase B described in the test for Related substances.

SYSTEM SUITABILITY

The retention time of salicylic acid is about 6 minutes and that of sulfapyridine, about 7 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to salicylic acid and sulfapyridine is at least 2.

LIMITS

Allow the chromatography to proceed for 10 minutes. In the chromatogram obtained with solution (1) the areas of any peaks corresponding to salicylic acid and sulfapyridine are not greater than 0.5 times the areas of the corresponding peaks in the chromatogram obtained with solution (2) (0.5% of each).

Dissolution

Comply with the requirements for 'Monographs of the British Pharmacopoeia' in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2. Use as the, and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute a suitable volume of the filtrate with the dissolution medium to produce a solution containing 0.005% w/v of Sulfasalazine. Measure the *absorbance* of this solution, Appendix II B, at 406 nm using dissolution medium in the reference cell. Calculate the total content of $C_{18}H_{14}N_4O_5S$ in the medium from the *absorbance* of a 0.005% w/v solution of *sulfasalazine EPCRS* in dissolution medium and using the declared content of $C_{18}H_{14}N_4O_5S$ in *sulfasalazine EPCRS* .

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Sulfasalazine with about 50 ml of 0.1M *sodium hydroxide* , add sufficient of the same solvent to produce 100 ml, filter and discard the first 20 ml of filtrate. Dilute 5 ml of the filtrate with 750 ml of *water*, add 20 ml of 0.1M *acetic acid* , mix and add sufficient *water* to produce 1000 ml. Measure the *absorbance* of this solution at the maximum at 359 nm, Appendix II B. Calculate the content of $C_{18}H_{14}N_4O_5S$ in the tablets from the *absorbance* of a standard solution prepared in the same manner using 0.15 g of *sulfasalazine EPCRS* and using the declared content of $C_{18}H_{14}N_4O_5S$ in *sulfasalazine EPCRS* .

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Sulfasalazine.

Invert Syrup

General Notices

DEFINITION

Invert Syrup is a mixture of glucose and fructose prepared by hydrolysing a 66.7% w/w solution of Sucrose with a suitable mineral acid, such as hydrochloric acid, and neutralising the resulting solution using, for example, calcium carbonate or sodium carbonate. The degree of inversion is at least 95%.

The syrup complies with the requirements stated under Oral Liquids and with the following requirements.

Content of reducing sugars, expressed as invert sugar

Not less than 67.0% w/w.

CHARACTERISTICS

A clear, colourless to pale straw-coloured syrupy liquid.

Miscible with *water*, producing a clear solution; it dissolves in *ethanol (96%)* with the formation of an insoluble residue.

IDENTIFICATION

A. Heat 1 g with 10 ml of *water* and 5 ml of *cupri-tartaric solution*. A red precipitate is produced.

B. A solution in *water* is laevorotatory.

TESTS

Acidity

pH, 5.0 to 6.0, Appendix V L.

Arsenic

To 4.0 g add 50 ml of *water* and 10 ml of *brominated hydrochloric acid*, allow to stand for 5 minutes and remove the excess of bromine by adding *tin(II) chloride solution AsT* and dilute to 100 ml with *water*. 25 ml of the resulting solution complies with the *limit test for arsenic*, Appendix VII (1 ppm).

Lead

Prepare two solutions as follows. For solution (1) add 5 ml of 6M *acetic acid* to 12 g of the syrup. For solution (2) add 5 ml of 6M *acetic acid* and 2 ml of *lead standard solution (10 ppm Pb)* to 2.0 g of the syrup. Make solutions (1) and (2) alkaline with 5M *ammonia*, if necessary, and to each add 1 ml of *potassium cyanide solution PbT*. The solutions should not be more than faintly opalescent. If the colours of the solutions differ, equalise by the addition of a few drops of a highly diluted solution of burnt sugar or other non-reactive substance. Dilute each solution to 50 ml with *water*, add 0.1 ml of a 10% w/v solution of *sodium sulphide* to each and mix thoroughly. When viewed against a white background, the colour produced in solution (1) is not more intense than that produced in solution (2) (2 ppm).

Refractive index

1.4608 to 1.4630, Appendix V E.

Sulphur dioxide

Not more than 70 ppm, Appendix IX B.

Weight per ml

1.338 to 1.344 g, Appendix V G.

Sulphated ash

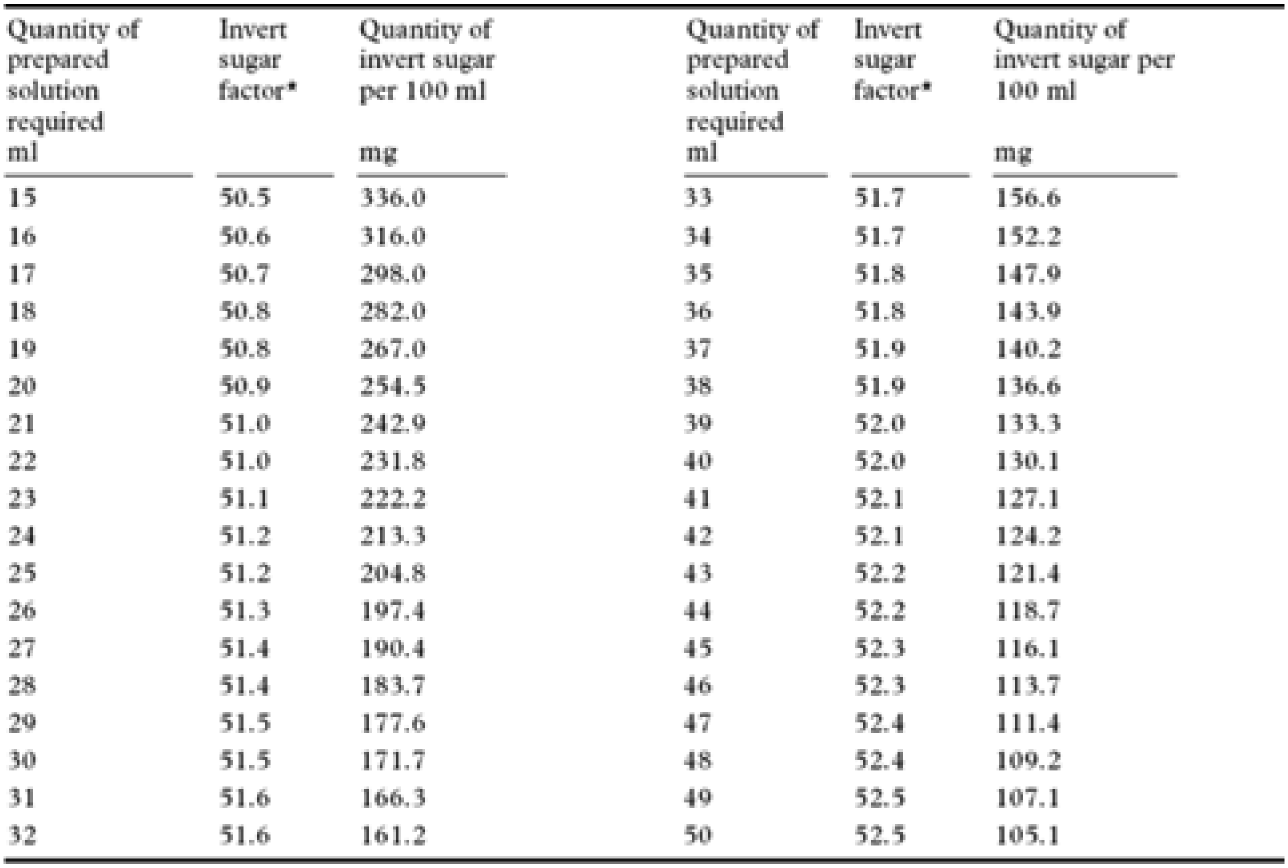
Not more than 0.1%, Appendix IX A.

ASSAY

Dilute the syrup so that the volume of the diluted solution required in the following method is between 15 and 50 ml. Add 10.00 ml of *cupri-tartaric solution R1* to a 300 ml conical flask, add from a burette 15 ml of the diluted solution, heat to boiling over wire gauze covered with insulating material and continue adding the diluted solution in quantities of about 5 ml at 15-second intervals until the colour of the mixture indicates that the reduction appears to be almost complete. Boil for 2 minutes, add 0.2 ml of a 1% w/v solution of *methylene blue* and continue the titration until the blue colour is discharged. Repeat the operation, but, before heating, add almost the full quantity of the diluted solution required to reduce all the copper and then boil moderately for 2 minutes. Without removing the flask from either the gauze or the flame during the remainder of the titration, add 0.2 ml of the methylene blue solution and continue the titration so that it is just complete in a total boiling time of exactly 3 minutes; the end point is indicated by the disappearance of the blue colour, the solution becoming orange. From the Table calculate the content of reducing sugars (expressed as invert sugar) in 100 ml of the diluted solution and hence the percentage weight in weight in the substance being examined.

STORAGE

Invert Syrup should be stored at a temperature of 35° to 45°.



Quantity of prepared solution required ml	Invert sugar factor*	Quantity of invert sugar per 100 ml mg	Quantity of prepared solution required ml	Invert sugar factor*	Quantity of invert sugar per 100 ml mg
15	50.5	336.0	33	51.7	156.6
16	50.6	316.0	34	51.7	152.2
17	50.7	298.0	35	51.8	147.9
18	50.8	282.0	36	51.8	143.9
19	50.8	267.0	37	51.9	140.2
20	50.9	254.5	38	51.9	136.6
21	51.0	242.9	39	52.0	133.3
22	51.0	231.8	40	52.0	130.1
23	51.1	222.2	41	52.1	127.1
24	51.2	213.3	42	52.1	124.2
25	51.2	204.8	43	52.2	121.4
26	51.3	197.4	44	52.2	118.7
27	51.4	190.4	45	52.3	116.1
28	51.4	183.7	46	52.3	113.7
29	51.5	177.6	47	52.4	111.4
30	51.5	171.7	48	52.4	109.2
31	51.6	166.3	49	52.5	107.1
32	51.6	161.2	50	52.5	105.1

* mg of invert sugar corresponding to 10.00 ml *of cupri-tartaric solution R1* .

Opiate Squill Linctus

General Notices

Compound Squill Linctus; Gee's Linctus

DEFINITION

Opiate Squill Linctus is an opalescent *oral solution* containing 33% v/v each of Squill Oxymel and Camphorated Opium Tincture in a suitable vehicle with a tolu flavour.

Extemporaneous preparation

The following formula applies.

Squill Oxymel	300 ml
Camphorated Opium Tincture	300 ml
Tolu Syrup	300 ml

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous morphine, $C_{17}H_{19}NO_3$

0.013 to 0.020% w/v.

TESTS

Ethanol content

18.0 to 22.0% v/v, Appendix VIII F.

ASSAY

To 12 g add 5 ml of *water* and 1 ml of 5M *ammonia* and extract with 30 ml of a mixture of equal volumes of *ethanol* (*96%*) and *chloroform* and then with two 22.5-ml quantities of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol* (*96%*), washing each extract with the same 20 ml of a mixture of equal volumes of *ethanol* (*96%*) and *water*. Evaporate the combined extracts, extract the residue with 10 ml of *calcium hydroxide solution* , filter and wash the filter with 10 ml of *calcium hydroxide solution* . To the combined filtrate and washings add 0.1 g of *ammonium sulphate* , extract with two 10 ml quantities of *ethanol-free chloroform* , wash the combined extracts with 10 ml of *water* and discard the chloroform solution. To the combined alkaline liquid and aqueous washings add 10 ml of 1M *hydrochloric acid* , heat on a water bath to remove any chloroform, cool and dilute to 100 ml with *water*. To 20 ml of this solution add 8 ml of a freshly prepared 1.0% w/v solution of *sodium nitrite* , allow to stand for 15 minutes, add 12 ml of 5M *ammonia* , dilute to 50 ml with *water* and measure the *absorbance* of a 4-cm layer of the resulting solution at the maximum at 442 nm, Appendix II B, using in the reference cell a solution prepared in the same manner and at the same time but using 8 ml of *water* in place of the solution of sodium nitrite. Calculate the content of $C_{17}H_{19}NO_3$ from a calibration curve prepared using quantities of 2, 4, 6 and 8 ml of a 0.008% w/v solution of *anhydrous morphine* in 0.1M *hydrochloric acid* , each diluted to 20 ml with 0.1M *hydrochloric acid* and using the method described above beginning at the words 'add 8 ml ...'. Determine the *weight per ml* of the linctus, Appendix V G, and calculate the content of $C_{17}H_{19}NO_3$, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Paediatric Opiate Squill Linctus

General Notices

Opiate Linctus for Infants

DEFINITION

Paediatric Opiate Squill Linctus is an *oral solution* containing 6% v/v each of Squill Oxymel and Camphorated Opium Tincture in a suitable vehicle with a tolu flavour.

Extemporaneous preparation

The following formula applies.

Squill Oxymel	60 ml
Camphorated Opium Tincture	60 ml
Tolu Syrup	60 ml
Glycerol	200 ml
Syrup	Sufficient to produce 1000 ml

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of anhydrous morphine, $C_{17}H_{19}NO_3$

0.0024 to 0.0036% w/v.

ASSAY

Carry out the Assay described under Opiate Squill Linctus with the following modifications. Use 32 g, add 5 ml of 1M *hydrochloric acid* to the combined alkaline liquid and aqueous washings and after heating and cooling dilute to 50 ml with *water*.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Paediatric Simple Linctus

General Notices

DEFINITION

Paediatric Simple Linctus is an *oral solution* containing 0.625% w/v of Citric Acid Monohydrate in a suitable vehicle with an anise flavour.

Extemporaneous preparation

It may be prepared extemporaneously by diluting Simple Linctus with a suitable vehicle in accordance with the manufacturer's instructions.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of free acid, calculated as citric acid monohydrate, $C_6H_8O_7,H_2O$

0.53 to 0.69% w/v.

IDENTIFICATION

Dilute 20 ml to 50 ml with *water*, add 2 g of *activated charcoal*, mix and filter. To 1 ml of the filtrate add 1 ml of *pyridine*, swirl to mix, add 5 ml of *acetic anhydride* and mix using, for example, a rotary vortex mixer. Immediately place in a water bath at about 30° for 5 minutes. A yellow colour is produced.

ASSAY

Dilute 15 g with 50 ml of *water* and titrate with 0.1M *sodium hydroxide VS* using 2 ml of *thymol blue solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 7.005 mg of $C_6H_8O_7,H_2O$. Determine the *weight per ml* of the linctus, Appendix V G, and calculate the content of $C_6H_8O_7,H_2O$, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Salbutamol Injection

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Injection is a sterile solution of Salbutamol Sulphate in Water for Injections.
The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of salbutamol, $C_{13}H_{21}NO_3$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless or very pale yellow solution.

IDENTIFICATION

A. Dilute a volume of the injection with sufficient 0.1M *hydrochloric acid* to produce a solution containing the equivalent of 0.004% w/v of salbutamol. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 276 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 50 volumes of *ethyl acetate*, 30 volumes of *propan-2-ol*, 16 volumes of *water* and 4 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) evaporate a suitable volume of the injection to dryness using a rotary evaporator, wash the residue with four 5-ml quantities of *absolute ethanol*, filter, evaporate the filtrate to dryness and dissolve the residue in sufficient *water* to produce a solution containing the equivalent of 0.1% w/v of salbutamol. Solution (2) contains 0.12% w/v of *salbutamol sulphate BPCRS*. After removal of the plate, allow it to dry in air until the solvent has evaporated, place it in an atmosphere saturated with *diethylamine* for a few minutes and spray with *diazotised nitroaniline solution*. The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. Dilute a volume containing the equivalent of 0.5 mg of salbutamol to 50 ml with *water*, add 1 ml of 5M *ammonia*, 1 ml of a 3% w/v solution of *4-aminophenazone*, 10 ml of a 2% w/v solution of *potassium hexacyanoferrate*(*III*) and 10 ml of *chloroform*, shake and allow to separate. An orange-red colour is produced in the chloroform layer.

D. A volume containing the equivalent of 1 mg of salbutamol yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH, 3.4 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection, if necessary, with the mobile phase to produce a solution containing the equivalent of 0.005% w/v of salbutamol. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.0004% w/v of *salbutamol impurity B EPCRS* and 0.0005% w/v of *salbutamol sulphate EPCRS* in the mobile phase. For solution (1) allow the chromatography to proceed for 25 times the retention time of salbutamol (retention time of salbutamol, about 1.9 minutes).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with spherical *end-capped octylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 335 m^2 per g, a pore size of 10 nm and a carbon loading of 11.7% (Waters Symmetry C8 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulphonate* and 0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.65 with 2M *orthophosphoric acid* and (c) a detection wavelength of 220 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0

In the chromatogram obtained with the solution (1) the area of any peak with a retention time relative to salbutamol of about 2.7 is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%), the area of any other *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%).

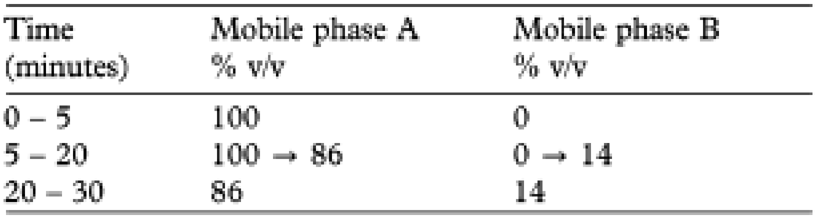
Salbutamol ketone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the injection solution, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.050% w/v of salbutamol. Solution (2) contains 0.00025% w/v of *salbutamol ketone impurity BPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C8 is suitable) maintained at 30°, (b) as the mobile phase with a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 276 nm.

Mobile phase A A mixture of 1.5 volumes of *propan-2-ol* and 98.5 volumes of 0.1M *ammonium acetate* adjusted to pH 4.5 with *glacial acetic acid*.

Mobile phase B *propan-2-ol*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v
0 – 5	100	0
5 – 20	100 → 86	0 → 14
20 – 30	86	14

In the chromatogram obtained with solution (1) the area of any peak corresponding to salbutamol ketone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection with the mobile phase to produce a solution containing the equivalent of 0.0025% w/v of salbutamol. Solution (2) contains 0.003% w/v of *salbutamol sulphate BPCRS* in the mobile phase. Solution (3) contains 0.003% w/v of *salbutamol sulphate BPCRS* and 0.003% w/v of *2-*tert-*butylamino-1-(4-hydroxy-3-methylphenyl)ethanol sulphate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with spherical particles of silica, 5 µm in diameter, the surface of which has been modified with chemically-bonded nitrile groups (Spherisorb CN is suitable), (b) as the mobile phase with a flow rate of 2.0 ml per minute a mixture of 15 volumes of *propan-2-ol*, 300 volumes of 0.05M *ammonium acetate* and 685 volumes of *water* the pH of the mixture being adjusted to 4.5 with *glacial acetic acid* and (c) a detection wavelength of 276 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 1.5.

Calculate the content of $C_{13}H_{21}NO_3$ using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of salbutamol.

Salbutamol Nebuliser Solution

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Nebuliser Solution is a solution of Salbutamol Sulphate in Water for Injections. *The nebuliser solution complies with the requirements stated under Preparations for Inhalation and with the following requirements.*

Content of salbutamol, $C_{13}H_{21}NO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dilute the solution being examined with sufficient 0.1M *hydrochloric acid* to produce a solution containing the equivalent of 0.008% w/v of salbutamol. The *light absorption* of the resulting solution, Appendix II B, in the range 230 nm to 350 nm exhibits a maximum only at 276 nm.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) corresponds to the salbutamol peak in the chromatogram obtained with solution (3).

C. Dilute the solution being examined with sufficient *water* to produce a solution containing the equivalent of 0.025% w/v of salbutamol. The resulting solution yields reaction A characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the nebuliser solution with sufficient of the mobile phase to produce a solution containing the equivalent of 0.05% w/v of salbutamol. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.0004% w/v of *salbutamol impurity B EPCRS* and 0.0005% w/v of *salbutamol sulphate EPCRS* in the mobile phase. For solution (1) allow the chromatography to proceed for 25 times the retention time of salbutamol (retention time of salbutamol, about 1.9 minutes).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with spherical *end-capped octylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 335 m^2 per g, a pore size of 10 nm and a carbon loading of

11.7% (Waters Symmetry C8 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulphonate* and 0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.65 with 2M *orthophosphoric acid* and (c) a detection wavelength of 220 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

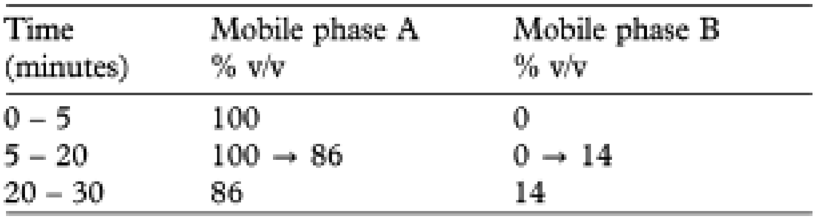
Salbutamol ketone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the nebuliser solution, if necessary, with sufficient *water* to produce a solution containing the equivalent of 0.050% w/v of salbutamol. Solution (2) contains 0.00025% w/v of *salbutamol ketone impurity BPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C8 is suitable) maintained at 30°, (b) as the mobile phase with a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 276 nm.

Mobile phase A A mixture of 1.5 volumes of *propan-2-ol* and 98.5 volumes of 0.1M *ammonium acetate* adjusted to pH 4.5 with *glacial acetic acid*.

Mobile phase B *propan-2-ol*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v
0 – 5	100	0
5 – 20	100 → 86	0 → 14
20 – 30	86	14

In the chromatogram obtained with solution (1) the area of any peak corresponding to salbutamol ketone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Dilute a volume of the nebuliser solution containing the equivalent of 4 mg of salbutamol to 50 ml with 0.1M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 276 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Calculate the content of $C_{13}H_{21}NO_3$ in the solution from the absorbance obtained from a solution prepared by diluting 1 volume of a 0.1% w/v solution of *salbutamol sulphate BPCRS* to 10 volumes with 0.1M *hydrochloric acid* and from the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*.

STORAGE

Salbutamol Nebuliser Solution should be protected from light.

LABELLING

The label states the quantity of active ingredient in terms of the equivalent amount of salbutamol.

Salbutamol Oral Solution

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Oral Solution is a solution of Salbutamol Sulphate in a suitable flavoured vehicle. *The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.*

Content of salbutamol, $C_{13}H_{21}NO_3$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

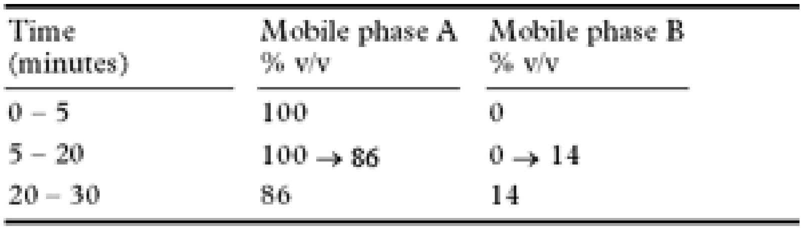
2-*tert*-Butylamino-1-(4-hydroxy-3-methylphenyl)ethanol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add to a volume of the oral solution containing the equivalent of 5 mg of salbutamol sufficient of the mobile phase to produce 50 ml, mix, filter through a glass microfibre filter (Whatman GF/C is suitable) and use the filtrate. Solution (2) is a 0.000050% w/v solution of *2-*tert*-butylamino-1-(4-hydroxy-3-methylphenyl)ethanol sulphate BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C8 is suitable) maintained at 30°, (b) a linear gradient elution with the conditions described below with a flow rate of 1 ml per minute and (c) a detection wavelength of 276 nm.

Mobile phase A A mixture of 98.5 volumes of 0.1M *ammonium acetate* adjusted to pH 4.5 with *glacial acetic acid* and 1.5 volumes of *propan-2-ol*.

Mobile phase B *propan-2-ol*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v
0 – 5	100	0
5 – 20	100 → 86	0 → 14
20 – 30	86	14

In the chromatogram obtained with solution (1) the area of any peak corresponding to

2-*tert*-butylamino-1-(4-hydroxy-3-methylphenyl)ethanol is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Carry out the method for *liquid chromatography* Appendix III D, using the following solutions. For solution (1) mix a quantity of the oral solution containing the equivalent of 2 mg of salbutamol with sufficient of the mobile phase to produce 50 ml and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) contains 0.004% w/v of *salbutamol BPCRS* in the mobile phase.

The chromatographic procedure described under 2-*tert*-Butylamino-1-(4-hydroxy-3-methylphenyl)ethanol sulphate may be used.

Calculate the content of $C_{13}H_{21}NO_3$ in the oral solution using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol BPCRS*.

STORAGE

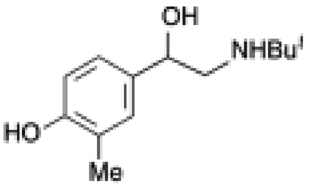
Salbutamol Oral Solution should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of salbutamol.

IMPURITIES

The impurity limited by this monograph is:



A. 2-*tert*-butylamino-1-(4-hydroxy-3-methylphenyl)ethanol.

Salbutamol Powder for Inhalation

General Notices

DEFINITION

Salbutamol Powder for Inhalation consists of Salbutamol Sulphate in *microfine powder* either alone or admixed with Lactose. It may be presented either as pre-metered units for use in a designated powder inhaler or as a powder reservoir within a metered-dose powder inhaler.

The powder for inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRE-METERED UNITS

DEFINITION

Salbutamol pre-metered unit powder inhalers consist of Salbutamol Sulphate in *microfine powder* either alone or admixed with Lactose in a suitable container for use within a suitable device.

PRODUCTION

Complies with the requirements for Uniformity of delivered dose stated under Preparations for Inhalation using the method described in the European Pharmacopoeia or other methodology where justified and authorised.

Content of salbutamol, $C_{13}H_{21}NO_3$

95.0 to 105.0% of the stated amount per pre-metered unit.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the powder for inhalation containing the equivalent of 1 mg of salbutamol in 10 ml of *water*.

(2) 0.010% w/v of *salbutamol sulphate BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating substance *silica gel G* (Merck plates are suitable).

(b) The mobile phase is a mixture of 4 volumes of 13.5M *ammonia*, 16 volumes of *water*, 30 volumes of *propan-2-ol* and 50 volumes of *ethyl acetate*.

(c) Application volume of 5 µl for each solution.

(d) Develop to 15 cm.

(e) Remove the plate, allow it to dry in air, spray with a 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol (90%)* and dry in air for 10 minutes. Spray the plate with a 2% w/v solution of *potassium hexacyanoferrate(III)* in a mixture of 1 volume of 18M *ammonia* and 3 volumes of *water* and spray again with the 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol (90%)*.

VALIDITY

The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

C. Dissolve 0.25 g in 5 ml of *water*. Add 5 ml of 6M *ammonia* and heat in a water bath at 80° for 10 minutes. An orange-red colour is produced.

D. Dissolve about 45 mg in 5 ml of *water*. The resulting solution yields reaction A characteristic of *sulphates*, Appendix VI.

TESTS

Related Substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powder for inhalation in sufficient mobile phase to produce a solution containing 0.020% w/v of Salbutamol Sulphate and filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, dilute 3 volumes to 10 volumes with the mobile phase.

(3) 0.00004% w/v of *salbutamol sulphate BPCRS* in the mobile phase.

(4) 0.00016% w/v of *salbutamol impurity F EPCRS* in the mobile phase.

(5) 0.00004% w/v of each of *salbutamol sulphate BPCRS* and *salbutamol impurity B EPCRS* in the mobile phase.

(6) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase, dilute 1 volumes to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (15 cm × 3.9 mm) packed with *spherical end-capped octylsilyl silica gel for chromatography* (5 µm) (Symmetry C8 is suitable),

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 220 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 22 volumes of *acetonitrile R1* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulphonate* and 0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.7 with 2M *orthosphosphoric acid.*

Allow the chromatography to proceed for 25 times the retention time of the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution factor* between the two principal peaks is at least 3.0. The retention time of the peak due to Salbutamol is about 2 minutes.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to salbutamol impurity F is not greater than the area of the peak in the chromatogram obtained with solution (4) (0.8%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (6) (0.05%).

The total related substances are not greater than 2.0%.

Uniformity of delivered dose

Complies with the requirements stated under Powders for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Powders for Inhalation, Uniformity of delivered dose and dissolve the collected dose in sufficient mobile phase to produce a solution containing the equivalent of 0.00009% w/v of salbutamol.

(2) 0.0001% w/v of *salbutamol sulphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with the exception of run time.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$, per delivered dose using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*. Repeat the procedure nine times as described for pre-metered systems under Powders for Inhalation, Uniformity of delivered dose.

Deposition of the emitted dose

Carry out the test for *aerodynamic assessment of fine particles*, Appendix XII F, using Apparatus A but determining the content of active ingredient as described below. Use 7 ml of *water* in the upper impingement chamber and 30 ml of *water* in the lower impingement chamber. Use *water* to wash the coupling tube, E, and transfer the combined solution and washings in the lower impingement chamber to a 100-ml graduated flask and dilute the combined solution and washings to 100 ml with *water*. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Use the diluted solution from the lower impingement chamber diluted, if necessary, with the mobile phase to produce a solution expected to contain the equivalent of 0.0003% w/v of salbutamol.

(2) 0.0003% w/v solution of *salbutamol sulphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used with the exception of run time.

DETERMINATION OF CONTENT

Calculate the amount of salbutamol, $C_{13}H_{21}NO_3$, delivered to the lower impingement chamber per actuation of the device using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*. Not less than 30% of the average amount of salbutamol delivered per actuation of the device, determined in the Assay, is deposited in the lower impingement chamber.

Water

4.5 to 5.5% w/w, Appendix IX C. Use 250 mg of the contents of the units and using a mixture of 1 volume of formamide and 2 volumes of methanol as the solvent.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve the mixed contents of 20 units [blister, capsule, etc] in sufficient *water* to produce a solution containing the equivalent of 0.0001% w/v of salbutamol.

(2) 0.0001% w/v of *salbutamol sulphate BPCRS* in *water*.

(3) 0.0001% w/v of *salbutamol sulphate BPCRS* and 0.0001% w/v of *salbutamol impurity B EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$, using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*.

LABELLING

The label states the equivalent amount of salbutamol per unit (blister, capsule etc).

When supplied as a powder reservoir within a metered-dose powder inhaler, the powder for inhalation complies with the following requirements.

METERED-DOSE POWDER INHALERS

DEFINITION

When supplied as a powder reservoir within a metered-dose powder inhaler, Salbutamol Powder for Inhalation consists of Salbutamol Sulphate in *microfine powder* either alone or admixed with Lactose in a suitable device.

Content of salbutamol, $C_{13}H_{21}NO_3$

80.0 to 120.0% of the stated amount per delivered dose.

IDENTIFICATION

For powders for inhalation containing salbutamol sulphate

Complies with Identification tests A, B and D stated above.

For powders for inhalation containing salbutamol sulphate admixed with lactose

Complies with Identification tests A, B, C and D stated above.

TESTS

Related Substances

Complies with the requirements stated above for Pre-metered units.

Uniformity of delivered dose

Complies with the requirements stated under Powders for Inhalation using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Collect single doses of the preparation being examined using the procedure described under Powders for Inhalation, Uniformity of delivered dose and dissolve the collected dose in sufficient *water* to produce a solution containing the equivalent of 0.0001% w/v of salbutamol.

(2) 0.0001% w/v of *salbutamol sulphate BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances for Pre-metered units may be used with the exception of run time.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$, per delivered dose using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*. Repeat the procedure nine times as described for reservoir systems under Powders for Inhalation, Uniformity of delivered dose.

Water

For powders for inhalation containing salbutamol sulphate admixed with lactose 4.5 to 5.5% w/w, Appendix IX C. Use 50 mg of the powder for inhalation and use a mixture of 1 volume of formamide and 2 volumes of methanol as the solvent.

Deposition of the emitted dose

Complies with the requirements stated under Deposition of the emitted dose for Pre-metered units.

ASSAY

Where the label states delivered dose, use the average of the 10 individual results obtained in the test for uniformity of delivered dose.

Where the label states metered dose, carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powder in sufficient *water* to produce a solution containing the equivalent of 0.0001% w/v of salbutamol.

(2) 0.0001% w/v of *salbutamol sulphate BPCRS* in *water*.

(3) 0.0001% w/v of *salbutamol sulphate BPCRS* and 0.0001% w/v of *salbutamol impurity B EPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances for Pre-metered units may be used with the exception of run time.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of salbutamol, $C_{13}H_{21}NO_3$, using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*.

LABELLING

The label states the dose in terms of the equivalent amount of salbutamol.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Salbutamol Sulphate.

Salbutamol Pressurised Inhalation

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Pressurised Inhalation is a suspension of either Salbutamol or Salbutamol Sulphate in a suitable liquid in a suitable pressurised container.

The pressurised inhalation complies with the requirements stated under Preparations for Inhalation and with the following requirements.

Content of salbutamol, $C_{13}H_{21}NO_3$

80.0 to 120.0% of the amount stated to be delivered by actuation of the valve.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, in the range 1650 to 400 cm^{-1} is concordant with the *reference spectrum* of either salbutamol (*RS 314*) or salbutamol sulphate (*RS 315*), as appropriate. Examine the substance as a dispersion in *potassium bromide* prepared in the following manner. Discharge the inhaler a sufficient number of times into a mortar to obtain 2 mg of Salbutamol, grind the residue thoroughly with 0.1 g of *potassium bromide*, add a further 0.2 g of *potassium bromide* and mix thoroughly.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) corresponds to the peak due to salbutamol in the chromatogram obtained with solution (3).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

For pressurised inhalations containing Salbutamol use the following solution as solution (1). Discharge the inhaler a sufficient number of times into a small, dry beaker to obtain 5 mg of Salbutamol and dissolve the residue in 10 ml of the mobile phase; mix with the aid of ultrasound until the solution is clear.

For pressurised inhalations containing Salbutamol Sulphate use the following solution as solution (1). Discharge the inhaler a sufficient number of times into a small, dry beaker to obtain the equivalent of 5 mg of salbutamol and dissolve the residue in 10 ml of the mobile phase; mix with the aid of ultrasound until the solution is clear.

For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.0004% w/v of *salbutamol impurity B EPCRS* and 0.0005% w/v of *salbutamol sulphate EPCRS* in the mobile phase. For solution (1) allow the chromatography to proceed for 25 times the retention time of salbutamol (retention time of salbutamol, about 1.9 minutes).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with spherical *end-capped octylsilyl silica gel for chromatography* (5 µm) with a specific surface area of 335 m^2 per g, a pore size of 10 nm and a carbon loading of 11.7% (Waters Symmetry C8 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.287% w/v of *sodium heptanesulphonate* and 0.25% w/v of *potassium dihydrogen orthophosphate* adjusted to pH 3.65 with 2M *orthophosphoric acid* and (c) a detection wavelength of 220 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

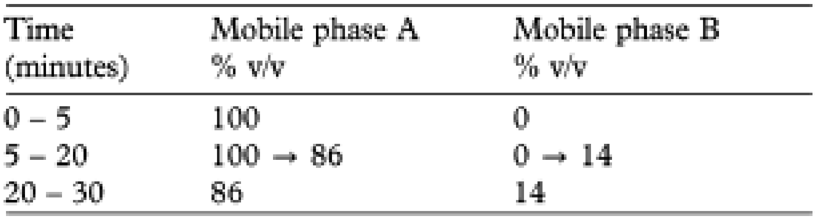
Salbutamol ketone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of solution (1) prepared above in the test for Related substances, with sufficient *water* to produce a solution containing the equivalent of 0.050% w/v of salbutamol. Solution (2) contains 0.00025% w/v of *salbutamol ketone impurity BPCRS* in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C8 is suitable) maintained at 30°, (b) as the mobile phase with a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 276 nm.

Mobile phase A A mixture of 1.5 volumes of *propan-2-ol* and 98.5 volumes of 0.1M *ammonium acetate* adjusted to pH 4.5 with *glacial acetic acid*.

Mobile phase B *propan-2-ol*.



Time (minutes)	Mobile phase A % v/v	Mobile phase B % v/v
0 – 5	100	0
5 – 20	100 → 86	0 → 14
20 – 30	86	14

In the chromatogram obtained with solution (1) the area of any peak corresponding to salbutamol ketone is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Deposition of the emitted dose

Carry out the test for *aerodynamic assessment of fine particles*, Appendix XII C7, using Apparatus A but determining the content of active ingredient as described below.

For pressurised inhalations containing Salbutamol use 7 ml of *methanol* in the upper impingement chamber and 30 ml of *methanol* in the lower impingement chamber. Use *methanol* to wash the coupling tube, E, and transfer the combined solution and washings in the lower impingement chamber to a 100 ml graduated flask containing 22.5 ml of a 0.1% w/v solution of *ammonium acetate*, rinsing the chamber with *methanol*, and dilute the combined solution and washings to 100 ml with *methanol*.

For pressurised inhalations containing Salbutamol Sulphate use 7 ml of *water* in the upper impingement chamber and 30 ml of *water* in the lower impingement chamber. Use a mixture of equal volumes of *methanol* and *water* to wash the coupling tube, E, and transfer the combined solution and washings in the lower impingement chamber to a 100 ml graduated flask containing 22.5 ml of a 0.1% w/v solution of *ammonium acetate*, rinsing the chamber with a mixture of equal volumes of *methanol* and *water*, and dilute the combined solution and washings to 100 ml with *water*.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0006% w/v of *2*-tert-*butylamino-1-*(*4-hydroxy-3-methylphenyl*)*ethanol sulphate BPCRS* and 0.0005% w/v of *salbutamol BPCRS* in the mobile phase. Solution (2) is a 0.0005% w/v solution of *salbutamol BPCRS* in the mobile phase. For solution (3) use the diluted solution from the lower impingement chamber.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 225 volumes of a 0.1% w/v solution of *ammonium acetate* and 800 volumes of *methanol* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 276 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (1) is at least 1.5.

Calculate the amount of salbutamol, $C_{13}H_{21}NO_3$, delivered to the lower impingement chamber per actuation of the valve using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol BPCRS*. Not less than 35% of the average amount of salbutamol delivered per actuation of the valve, calculated as the average of the three results determined in the Assay, is deposited in the lower impingement chamber.

ASSAY

Determine the content of active ingredient delivered by the first 10 successive combined actuations of the valve after priming.

For pressurised inhalations containing Salbutamol carry out the procedure for Content of active ingredient delivered by actuation of the valve described under Pressurised Inhalations, beginning at the words 'Remove the pressurised container from the actuator…' and ending at the words '… to the volume specified in the monograph', using 35 ml of *methanol* in the vessel. Add 22.5 ml of a 0.1% w/v solution of *ammonium acetate* to the combined solution and washings obtained from the set of 10 combined actuations and dilute to 100 ml with *methanol* (solution A).

For pressurised inhalations containing Salbutamol Sulphate carry out the procedure for Content of active ingredient delivered by actuation of the valve described under Pressurised Inhalations, beginning at the words 'Remove the pressurised container from the actuator…' and ending at the words '… to the volume specified in the monograph', using 35 ml of a mixture of equal volumes of *methanol* and *water* in the vessel. Add 22.5 ml of a 0.1% w/v solution of *ammonium acetate* to the combined solution and washings obtained from the set of 10 combined actuations and dilute to 100 ml with *water* (solution A).

Determine the amount of active ingredient in the 10 combined actuations using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.0012% w/v of *2-*tert*-butylamino-1-(4-hydroxy-3-methylphenyl)ethanol sulphate BPCRS* and 0.001% w/v of *salbutamol BPCRS* in the mobile phase. Solution (2) contains 0.001% w/v of *salbutamol BPCRS* in the mobile phase. For solution (3) use solution A.

The chromatographic conditions described under Deposition of the emitted dose may be used.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (1) is at least 1.5.

Calculate the average content of $C_{13}H_{21}NO_3$ delivered by a single actuation of the valve using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol BPCRS*.

Determine the content of active ingredient a second and a third time by repeating the procedure on the middle 10 and on the last 10 successive combined actuations of the valve, as estimated from the number of deliveries available from the container as stated on the label. For each of the three determinations the average content of $C_{13}H_{21}NO_3$ delivered by a single actuation of the valve is within the limits stated under Content of salbutamol.

LABELLING

The label on the container states (1) whether the preparation contains Salbutamol or Salbutamol Sulphate; (2) where applicable, that the preparation does not contain chlorofluorocarbon propellants (CFCs).

When the active ingredient is Salbutamol Sulphate, the quantity is stated in terms of the equivalent amount of salbutamol.

Salbutamol Tablets

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Salbutamol Tablets contain Salbutamol Sulphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of salbutamol, $C_{13}H_{21}NO_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method described under Related substances applying separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 10 mg of salbutamol with 10 ml of *methanol* (*80%*) and filter. Solution (2) contains 0.12% w/v of *salbutamol sulphate BPCRS*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Shake a quantity of the powdered tablets containing the equivalent of 2.5 mg of salbutamol with 50 ml of a 2% w/v solution of *sodium tetraborate*, add 1 ml of a 3% w/v solution of *4-aminophenazone*, 10 ml of a 2% w/v solution of *potassium hexacyanoferrate*(*III*) and 10 ml of *chloroform*, shake and allow to separate. An orange-red colour is produced in the chloroform layer.

C. Shake a quantity of the powdered tablets containing the equivalent of 4 mg of salbutamol with 10 ml of *water* and filter. The filtrate yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 3 volumes of 13.5M *ammonia*, 18 volumes of *water*, 35 volumes of *ethyl acetate*, 45 volumes of *propan-2-ol* and 50 volumes of *4-methylpentan-2-one* as the mobile phase but allowing the solvent front to ascend 18 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the tablets containing the equivalent of 48 mg of salbutamol with 60 ml of *ethanol* (*50%*) for 30 minutes, filter, evaporate to dryness using a rotary evaporator and gentle heat and dissolve the residue in 2 ml of *water*; if the residue is coloured, pass the final solution through a column (3 cm × 6 mm) packed with *activated acid aluminium oxide* (Brockmann Grade I is suitable). Solution (2) contains 0.058% w/v of *salbutamol sulphate*

BPCRS in *water*. Solution (3) contains 0.022% w/v of *salbutamol sulphate BPCRS* in *water*. After removal of the plate, allow it to dry in air until the solvent has evaporated and spray with a 0.1% w/v solution of *3-methylbenzothiazolin-2-one hydrazone hydrochloride* in *methanol* (*90%*) followed by a 2% w/v solution of *potassium hexacyanoferrate*(*III*) in a mixture of 1 volume of 18M *ammonia* and 3 volumes of *water* and spray again with the solution of methylbenzothiazolinone hydrazone hydrochloride. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.75%).

Uniformity of content

Tablets containing the equivalent of 2 mg or less of salbutamol comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III A, using the following solutions. Solution (1) contains 0.060% w/v of *2*-tert-*butylamino-1-*(*4-hydroxy-3-methylphenyl*)*ethanol sulphate BPCRS* and 0.060% w/v of *salbutamol sulphate BPCRS* in the mobile phase. Solution (2) contains 0.0024% w/v of *salbutamol sulphate BPCRS* in *water*. For solution (3) add 50 ml of *water* to one tablet, shake for 1 hour, add sufficient *water* to produce 100 ml, mix, centrifuge and use the supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 5 mm) packed with spherical particles of silica, 5 µm in diameter, the surface of which has been modified with chemically-bonded nitrile groups (Spherisorb CN is suitable), (b) as the mobile phase with a flow rate of 2.0 ml per minute a mixture of 65 volumes of *water*, 30 volumes of 0.05M *ammonium acetate* and 5 volumes of *propan-2-ol*, the pH of the mixture being adjusted to 4.5 with *glacial acetic acid*, and (c) a detection wavelength of 276 nm.

Calculate the content of $C_{13}H_{21}NO_3$ in each tablet using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*. The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (1) is at least 1.5.

ASSAY

For tablets containing the equivalent of more than 2 mg of salbutamol

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.048% w/v of *2*-tert-*butylamino-1-*(*4-hydroxy-3-methylphenyl*)*ethanol sulphate BPCRS* and 0.048% w/v of *salbutamol sulphate BPCRS* in *methanol* (*10%*). Solution (2) contains 0.048% w/v of *salbutamol sulphate BPCRS* in *water*. For solution (3) shake 10 tablets with 100 ml of *water* for 1 hour, add sufficient *water* to produce a solution containing the equivalent of 0.040% w/v of salbutamol, mix, centrifuge and use the supernatant liquid.

The chromatographic procedure described under Uniformity of content may be used.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (1) is at least 1.5.

Calculate the content of $C_{13}H_{21}NO_3$ using the declared content of $C_{13}H_{21}NO_3$ in *salbutamol sulphate BPCRS*.

For tablets containing the equivalent of 2 mg or less of salbutamol

Use the average of the 10 individual results obtained in the test for Uniformity of content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of salbutamol.

Salicylic Acid Collodion

General Notices

DEFINITION

Salicylic Acid	120 g
Flexible Collodion	Sufficient to produce 1000 ml

The collodion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of salicylic acid, $C_7H_6O_3$

13.0 to 15.8% w/w.

ASSAY

Dilute 2 g to 200 ml with *acetone* and to 2 ml of this solution add 35 ml of *acetate buffer pH 2.45* and 4 ml of a freshly prepared 0.5% w/v solution of *iron(III) chloride hexahydrate*. Dilute to 50 ml with *acetate buffer pH 2.45*, filter, discarding the first 10 ml of the filtrate, and measure the *absorbance* of the resulting solution at the maximum at 525 nm, Appendix II B. Calculate the content of $C_7H_6O_3$ from the *absorbance* obtained by repeating the operation using 2 ml of a 0.12% w/v solution of *salicylic acid* in *ethanol* (*96%*) and beginning at the words 'add 35 ml of *acetate buffer pH 2.45* ...'.

Salicylic Acid Ointment

General Notices

DEFINITION

Salicylic Acid Ointment contains 2% w/w of Salicylic Acid in a suitable water-emulsifying basis.

Extemporaneous preparation

The following formula and directions apply.

Salicylic Acid, finely sifted	20 g
Wool Alcohols Ointment	980 g

Melt the Wool Alcohols Ointment, gradually add the Salicylic Acid and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of salicylic acid, $C_7H_6O_3$

1.9 to 2.1% w/w.

IDENTIFICATION

Shake 1 g with 10 ml of *water*, filter and add to the filtrate *iron(III) chloride solution R1*; an intense reddish violet colour is produced which persists on the addition of 6M *acetic acid*. Add 2M *hydrochloric acid*; the colour disappears and a white, crystalline precipitate is produced.

ASSAY

Dissolve 10 g in a mixture of 20 ml of *ethanol (96%)*, previously neutralised to *phenol red solution*, and 20 ml of *ether* and titrate with 0.1M *sodium hydroxide VS* using *phenol red solution* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 13.81 mg of $C_7H_6O_3$.

Selegiline Oral Solution

General Notices

Action and use

Monoamine oxidase type B inhibitor; treatment of Parkinson's disease.

DEFINITION

Selegiline Oral Solution is a solution of Selegiline Hydrochloride in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of selegiline hydrochloride, $C_{13}H_{18}ClN$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

(*S*)-Selegiline

Carry out the method for *liquid chromatography*, Appendix III A, using the following solutions. For solution (1) add to a weighed quantity of the oral solution containing 20 mg of Selegiline Hydrochloride 1 ml of *propan-2-ol* and 10 µl of *butylamine*, dilute to 20 ml with the mobile phase, shake thoroughly, filter and use the filtrate. For solution (2) dissolve 8 mg of (RS)-*selegiline hydrochloride EPCRS* in a mixture of 10 µl of *butylamine* and 1 ml of *propan-2-ol* and dilute to 20 ml with the mobile phase. For solution (3) dilute 0.5 ml of solution (2) to 20 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel OD for chiral separation* (Chiralcel OD is suitable), (b) as the mobile phase at a flow rate of 0.5 ml per minute a mixture of 0.2 volume of *propan-2-ol* and 99.8 volumes of *cyclohexane* and (c) a detection wavelength of 220 nm.

Inject 20 µl of each solution. When the chromatograms are recorded in the prescribed conditions, the retention time of (*S*)-selegiline is about 10 minutes. Adjust the sensitivity of the system so that the height of the peaks in the chromatogram obtained with solution (3) is about 10% of the full scale of the recorder. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks corresponding to (*S*)-selegiline and (*R*)-selegiline is at least 1.5. If necessary, adjust the concentration of propan-2-ol in the mobile phase.

In the chromatogram obtained with solution (1), any peak corresponding to (*S*)-selegiline is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III D, using a *TLC silica gel F_{254} plate* and a mixture of 0.5 volume of 13.5M *ammonia*, 10 volumes of *1,4-dioxan*, 10 volumes of *propan-2-ol*, 10 volumes of *toluene* and 30 volumes of *xylene* as the mobile phase. Use an unlined tank and add the mobile phase immediately before placing the plate in the tank. Apply separately to the plate 50 µl of each of the following solutions. For solution (1) adjust the pH of a quantity of the oral solution containing 10 mg of Selegiline Hydrochloride to 12 with 1M *sodium hydroxide*, add 2 ml of *chloroform*, shake for 30 minutes, allow to separate and use the chloroform layer. Prepare solution (2) in the same manner as solution (1) but using 4 ml of a 0.25% w/v solution of *selegiline hydrochloride BPCRS* in 0.1M *hydrochloric acid* in place of the oral solution. For solution (3) adjust the pH of 1 ml of a 0.025% w/v solution of *selegiline hydrochloride BPCRS* in 0.1M *hydrochloric acid* to 12 with 1M *sodium hydroxide*, add 10 ml of *chloroform*, shake for 30 minutes, allow to separate and use the chloroform layer. For solution (4) dilute 2 volumes of solution (3) to 5 volumes with *chloroform*. For solution (5) adjust the pH of 2 ml of a 0.025% w/v solution of *methylamphetamine hydrochloride* in 0.1M *hydrochloric acid* to 12 with 1M *sodium hydroxide*, add 10 ml of *chloroform*, shake for 30 minutes, allow to separate and use the chloroform layer.

After removal of the plate, allow it to dry in air, spray with a solution prepared by mixing 2 volumes of a solution in *acetone* containing 10% w/v of *iron(III) chloride* and 4% w/v of *iodine* and 1 volume of a 40% w/v solution of *(+)-tartaric acid* in *water* and allowing to stand for 15 minutes before use. Examine the plate in daylight immediately after spraying. In the chromatogram obtained with solution (1), any spot corresponding to methylamphetamine is not more intense than the spot in the chromatogram obtained with solution (5) (1%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than two such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 10 ml of a mixture of equal volumes of *acetonitrile* and *methanol* to a weighed quantity of the oral solution containing 10 mg of Selegiline Hydrochloride, mix with the aid of ultrasound for 5 minutes, add 40 ml of a mixture of equal volumes of *acetonitrile* and *methanol* and shake mechanically for 15 minutes. Add sufficient *water* to produce 100 ml and dilute 5 volumes of the resulting solution to 10 volumes with the mobile phase. Solution (2) contains 0.005% w/v of *selegiline hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.005% w/v of *selegiline hydrochloride BPCRS* and 0.001% w/v of *nortriptyline hydrochloride EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared in the following manner: dilute 250 ml of *methanol* and 250 ml of *acetonitrile* to 1000 ml with a solution prepared by dissolving 4 ml of *butylamine* in 900 ml of *water*, adjusting the pH to 6.5 with *acetic acid*, and adding sufficient *water* to produce 1000 ml and (c) a detection wavelength of 215 nm.

Inject 20 µl of each solution.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{13}H_{18}ClN$, weight in volume, using the declared content of $C_{13}H_{18}ClN$ in *selegiline hydrochloride BPCRS*.

STORAGE

Selegiline Oral Solution should not be refrigerated.

Selegiline Tablets

General Notices

Action and use

Monoamine oxidase type B inhibitor; treatment of Parkinson's disease.

DEFINITION

Selegiline Tablets contain Selegiline Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of selegiline hydrochloride, $C_{13}H_{18}ClN$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Selegiline Hydrochloride with 2 ml of *water* and 1 ml of 1M *sodium hydroxide*. Extract with 10 ml of *dichloromethane*, filter the extract through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of selegiline (*RS 400*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

(*S*)-Selegiline

Carry out the method for *liquid chromatography*, Appendix III A, using the following solutions. For solution (1) add to a quantity of the powdered tablets containing 20 mg of Selegiline Hydrochloride 1 ml of *propan-2-ol* and 10 µl of *butylamine*, dilute to 10 ml with the mobile phase, shake thoroughly, filter and use the filtrate. For solution (2) dissolve 8 mg of (RS)-*selegiline hydrochloride EPCRS* in a mixture of 10 µl of *butylamine* and 1 ml of *propan-2-ol* and dilute to 10 ml with the mobile phase. For solution (3) dilute 0.5 ml of solution (2) to 20 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel OD for chiral separation* (Chiralcel OD is suitable), (b) as the mobile phase with a flow rate of 0.5 ml per minute a mixture of 0.2 volumes of *propan-2-ol* and 99.8 volumes of *cyclohexane* and (c) a detection wavelength of 220 nm.

Inject 20 µl of each solution. When the chromatograms are recorded in the prescribed conditions, the retention time of (*S*)-selegiline is about 10 minutes. Adjust the sensitivity of the system so that the height of the peaks in the chromatogram obtained with solution (3) is about 10% of the full scale of the recorder. The test is not valid unless the *resolution factor* between the peaks corresponding to (*S*)-selegiline and (*R*)-selegiline in the chromatogram obtained with solution (2) is at least 1.5. If necessary, adjust the concentration of propan-2-ol in the

mobile phase.

In the chromatogram obtained with solution (1), any peak corresponding to (*S*)-selegiline is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.5%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Selegiline Hydrochloride with 10 ml of a mixture of equal volumes of *methanol* and *acetonitrile*, filter and dilute 5 ml of the filtrate to 10 ml with *water*. For solution (2) dilute 1 volume of solution (1) to 10 volumes with the mobile phase and dilute 1 volume of this solution to 50 volumes with the mobile phase. Solution (3) contains 0.001% w/v of *methylamphetamine hydrochloride* in the mobile phase. Solution (4) contains 0.005% w/v of *selegiline hydrochloride BPCRS* and 0.001% w/v of *nortriptyline hydrochloride EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture prepared in the following manner: dilute 250 ml of *methanol* and 250 ml of *acetonitrile* to 1000 ml with a solution prepared by dissolving 4 ml of *butylamine* in 900 ml of *water*, adjusting the pH to 6.5 with *acetic acid*, and adding sufficient *water* to produce 1000 ml and (c) a detection wavelength of 215 nm.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

For solution (1) allow the chromatography to proceed for at least 2.5 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (0.5%), the area of not more than two such peaks is greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any such peaks is not greater than six times the area of the principal peak in the chromatogram obtained with solution (2) (1.2%).

If, in the chromatogram obtained with solution (1), the area of any peak corresponding to methylamphetamine is greater than half the area of the peak in the chromatogram obtained with solution (3), the following limits apply. The area of any peak corresponding to methylamphetamine is not greater than the area of the peak in the chromatogram obtained with solution (3) (1%), the area of any other *secondary peak* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (1%). In each case, disregard any peak with an area less than 0.1 times that of the area of the principal peak in the chromatogram obtained with solution (2) (0.02%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Selegiline Hydrochloride with 100 ml of a mixture of equal volumes of *methanol* and *acetonitrile*, filter and dilute 50 ml of the filtrate to 100 ml with *water*. Solution (2) contains 0.005% w/v of *selegiline hydrochloride BPCRS* in the mobile phase. Solution (3) contains 0.005% w/v of *selegiline hydrochloride BPCRS* and 0.001% w/v of *nortriptyline hydrochloride EPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

Calculate the content of $C_{13}H_{18}ClN$ in the tablets from the chromatograms obtained and using the declared content of $C_{13}H_{18}ClN$ in *selegiline hydrochloride BPCRS*.

STORAGE

Selegiline Tablets should be protected from light and moisture.

Selenium Sulphide Scalp Application

General Notices

Selenium Sulphide Application

DEFINITION

Selenium Sulphide Scalp Application is a *cutaneous suspension* of Selenium Sulphide in a suitable liquid basis.

The scalp application complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of selenium sulphide, SeS_2

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Gently boil a quantity containing 50 mg of Selenium Sulphide with 5 ml of *nitric acid* for 1 hour, dilute to 50 ml with *water* and filter. To 5 ml of the filtrate add 10 ml of *water* and 5 g of *urea*, boil, cool and add 2 ml of *dilute potassium iodide solution*. A yellow to orange colour is produced, which darkens rapidly on standing.

B. Allow the coloured solution obtained in test A to stand for 10 minutes and filter through *kieselguhr*. The filtrate yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH, 4.0 to 5.5, Appendix V L.

ASSAY

To a quantity containing 0.15 g of Selenium Sulphide add 25 ml of *fuming nitric acid*, heat on a water bath for 2 hours, cool and dilute to 250 ml with *water*. To 25 ml of the solution add 10 g of *urea* and 25 ml of *water*, boil, cool, add 10 ml of *dilute potassium iodide solution* and 10 ml of *chloroform* and titrate immediately with 0.05M *sodium thiosulphate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Shake vigorously as the end point is approached. Each ml of 0.05M *sodium thiosulphate VS* is equivalent to 1.789 mg of SeS_2.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous suspension'.

Senna Liquid Extract

General Notices

DEFINITION

Senna Fruit, Alexandrian or Tinnevelly, crushed	1000 g
Coriander Oil	6 ml
Ethanol (90 per cent)	250 ml
Purified Water, freshly boiled and cooled	A sufficient quantity

Extemporaneous preparation

The following directions apply.

Macerate the crushed Senna Fruit in 5 litres of Purified Water for 8 hours, decant the clear liquid and strain; repeat the process twice using 2 litres of Purified Water for each maceration. Lightly press the marc, strain the expressed liquid, mix the strained liquid with the previously decanted liquid and heat the combined liquids at 80° for 3 minutes in a covered vessel. Allow to stand for not less than 24 hours; filter.

Evaporate the filtrate to 750 ml under reduced pressure at a temperature not exceeding 60°. Separately, dissolve the Coriander Oil in the Ethanol (90 per cent), add the solution to the evaporated filtrate and add sufficient Purified Water to produce 1000 ml. Allow to stand for not less than 24 hours; filter.

The extract complies with the requirements stated under Extracts and with the following requirements.

TESTS

Ethanol content

21 to 24% v/v, Appendix VIII F, Method III.

Dry residue

17 to 25% w/v.

Relative density

1.02 to 1.09, Appendix V G.

Senna Tablets

General Notices

DEFINITION

Senna Tablets contain the powdered pericarp of Senna Fruit, Alexandrian or Tinnevelly.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of total sennosides, calculated as sennoside B

85.0 to 115.0% of the stated amount.

IDENTIFICATION

The powdered tablets exhibit diagnostic structures of senna pericarp. External epidermis of isodiametric cells with very thick outer walls. Occasional stomata. Trichomes few, unicellular, conical and warty. Parenchymatous cells from inner part of a two-to five-layered zone subjacent to the epidermis, each containing a single prism of calcium oxalate. Thick-walled fibres in two to four layers, the fibres of the outer and inner zones respectively with their long axes at right angles to each other. Sutural vascular strands sheathed by cells containing prisms of calcium oxalate; elements of seed tissue may also be present.

TESTS

Disintegration

Maximum time, 30 minutes, Appendix XII A.

ASSAY

Weigh and powder 20 tablets. Carry out the following procedure protected from light. To a quantity of the powder containing the equivalent of 7.5 mg of total sennosides add 30 ml of *water*, weigh, heat under a reflux condenser on a water bath for 15 minutes, allow to cool, weigh and restore the original weight with *water*. Centrifuge, transfer 20 ml of the supernatant liquid to a separating funnel, add 0.1 ml of 2M *hydrochloric acid*, shake with two 15 ml quantities of *chloroform*, allow to separate and discard the chloroform layers. Add 0.10 g of *sodium hydrogen carbonate* and shake for 3 minutes; centrifuge and transfer 10 ml of the liquid to a round-bottomed flask fitted with a ground-glass neck. Add a mixture of 8 ml of *iron(III) chloride solution* and 12 ml of *water* and mix. Heat under a reflux condenser on a water bath for 20 minutes, add 1 ml of *hydrochloric acid* and continue heating for a further 20 minutes, shaking frequently, until the precipitate is dissolved. Allow to cool, transfer the mixture to a separating funnel and extract with three 25 ml quantities of *ether* previously used to rinse the flask. Wash the combined ether extracts with two 15 ml quantities of *water* and add sufficient *ether* to produce 100 ml. Evaporate 10 ml just to dryness on a water-bath and dissolve the residue in 10 ml of 1M *potassium hydroxide*, filtering if necessary through a sintered-glass filter (ISO 4793, porosity grade 3, is suitable). Measure the *absorbance* of the resulting solution without delay at the maximum at 500 nm, Appendix II B. Calculate the content of total sennosides, as sennoside B, taking 200 as the value of A(1%, 1 cm) at the

maximum at 500 nm.

LABELLING

The quantity of active ingredient is stated in terms of total sennosides expressed as the equivalent content of sennoside B.

Simeticone Suspension for Infants

General Notices

Action and use

Silicon dioxide analogue; defoaming agent.

DEFINITION

Simeticone Suspension for Infants is a suspension of Simeticone for Oral Use in a suitable flavoured aqueous vehicle.

The suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of dimeticone

90.0 to 110.0% of the stated amount.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, of the sample solution prepared in the Assay is concordant with that obtained for the solution of *dimeticone EPCRS*.

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Defoaming activity

Dissolve 1.0 g of *octoxinol 9* in 100 ml of *water* (foaming solution). To a weight of the suspension containing 0.3 g of Dimeticone add sufficient *water* to produce 30 g and mix thoroughly (test solution). Into a suitable container introduce 100 ml of foaming solution and 0.5 ml of test solution. Close tightly and shake the mixture for 10 seconds at 300 oscillations per minute, at an angle of oscillation of about ±10° and an oscillation radius of about 13 cm. The time between the end of the shaking and the instant the first portion of foam-free liquid surface appears is not greater than 45 seconds.

ASSAY

Add 25 ml of *toluene*, pre-washed with 1M *sodium hydroxide*, and 50 ml of 1M *sodium hydroxide* to a quantity of the suspension containing 50 mg of dimeticone and shake mechanically for 5 minutes. Allow the mixture to stand for 5 minutes, centrifuge the upper layer and dry with *anhydrous sodium sulphate*. Record the *infrared absorption spectrum*, Appendix II A, of a 0.5 mm layer of the clear solution over the range 1375 to 1125 cm^{-1} (7.27 to 8.89 µm). Measure the absorbance of the CH_3Si stretching band at the maximum at 1261 cm^{-1} (7.93 µm). Repeat the operation using a 0.2% w/v solution of *dimeticone EPCRS* in *toluene*. Determine the *weight per ml* of the suspension, Appendix V G, and calculate the content of dimeticone weight in volume.

Simple Eye Ointment

General Notices

Eye Ointment Basis

DEFINITION

Wool Fat	100 g
Yellow Soft Paraffin	800 g
Liquid Paraffin	Sufficient to produce 1000 g

Extemporaneous preparation

The following directions apply.

Melt together the Wool Fat and the Yellow Soft Paraffin, add the Liquid Paraffin, filter the hot mixture through coarse filter paper placed in a heated funnel, sterilise the filtrate by *dry heat* at a minimum of 150 ° for not less than 1 hour and allow to cool.

The eye ointment complies with the requirements stated under Eye Preparations.

Simple Linctus

General Notices

DEFINITION

Simple Linctus is an *oral solution* containing 2.5% w/v of Citric Acid Monohydrate in a suitable vehicle with an anise flavour.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of free acid, calculated as citric acid monohydrate, $C_6H_8O_7,H_2O$

2.00 to 2.65% w/v.

IDENTIFICATION

Dilute 5 ml to 50 ml with *water*, add 2 g of *activated charcoal*, mix and filter. To 1 ml of the filtrate add 1 ml of *pyridine*, swirl to mix, add 5 ml of *acetic anhydride* and mix using, for example, a rotary vortex mixer. Immediately place in a water bath at about 30° for 5 minutes. A yellow colour is produced.

ASSAY

Dilute 15 g with 50 ml of *water* and titrate with 0.5M *sodium hydroxide VS* using 2 ml of *thymol blue solution* as indicator. Each ml of 0.5M *sodium hydroxide VS* is equivalent to 35.02 mg of $C_6H_8O_7,H_2O$. Determine the *weight per ml* of the linctus, Appendix V G, and calculate the content of $C_6H_8O_7,H_2O$, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Simple Ointment

General Notices

DEFINITION

Wool Fat	50 g
Hard Paraffin	50 g
Cetostearyl Alcohol	50 g
White Soft Paraffin or Yellow Soft Paraffin	850 g

Unless otherwise directed in the monograph, when Simple Ointment is used in a white ointment, it should be prepared with White Soft Paraffin; when used in a coloured ointment it should be prepared with Yellow Soft Paraffin.

Extemporaneous preparation

The following directions apply.

Mix the ingredients, heat gently with stirring until homogeneous and stir until cold.
The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Simvastatin Tablets

General Notices

Action and use

HMG Co-A reductase inhibitor; lipid-regulating drug.

DEFINITION

Simvastatin Tablets contain Simvastatin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of simvastatin, $C_{25}H_{38}O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Simvastatin with 20 ml of *dichloromethane*, filter through glass fibre filter paper (Whatman GF/C is suitable) and evaporate the filtrate to dryness using a rotary evaporator and a water bath at 40°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of simvastatin *(RS 423)*.

TESTS

Dissolution

Carry out the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.01M *sodium dihydrogen orthophosphate* containing 0.5% w/v of *sodium dodecyl sulphate* and adjusted to pH 7.0 with 1M *sodium hydroxide* and rotate the paddle at 50 revolutions per minute. After 30 minutes, withdraw a sample of 20 ml of the medium, filter and transfer 10 ml of the filtrate into a centrifuge tube containing 0.1 g of *pre-washed manganese(IV) oxide*. Shake the tube for 30 minutes, or until the manganese(IV) oxide is completely dispersed, centrifuge and measure the *absorbance* of the clear supernatant liquid, suitably diluted with dissolution medium if necessary, at the maximum at 247 nm and at the minimum at 257 nm, Appendix II B, using dissolution medium that has been similarly treated with *pre-washed manganese(IV) oxide* in the reference cell. At the same time measure the *absorbance* of a suitable solution of *simvastatin BPCRS,* prepared by dissolving *simvastatin BPCRS* in the dissolution medium and treating with *pre-washed manganese(IV) oxide* as described above, at 247 nm and at 257 nm.

Calculate the total content of simvastatin, $C_{25}H_{38}O_5$, in the medium using the differences in absorbance at 247 nm and at 257 nm and using the declared content of $C_{25}H_{38}O_5$ in *simvastatin BPCRS*.

Related substances

Mix 1 volume of a 0.3% v/v solution of *glacial acetic acid* adjusted to pH 4.0 with 1M *sodium* *hydroxide* with 4 volumes of *acetonitrile* (solution A).

Prepare a 0.51% w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.5 with a 25% v/v solution of *orthophosphoric acid* (pH 4.5 buffer solution).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 25 mg of Simvastatin with 20 ml of solution A for 10 minutes, shake for 15 minutes, cool and add sufficient solution A to produce 100 ml, filter through a 0.45 µm membrane filter.

(2) Dilute 1 volume of solution (1) to 500 volumes with solution A.

(3) 0.002% w/v *simvastatin BPCRS* and 0.002% w/v *lovastatin EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable).

(b) Gradient elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Column temperature of 30°.

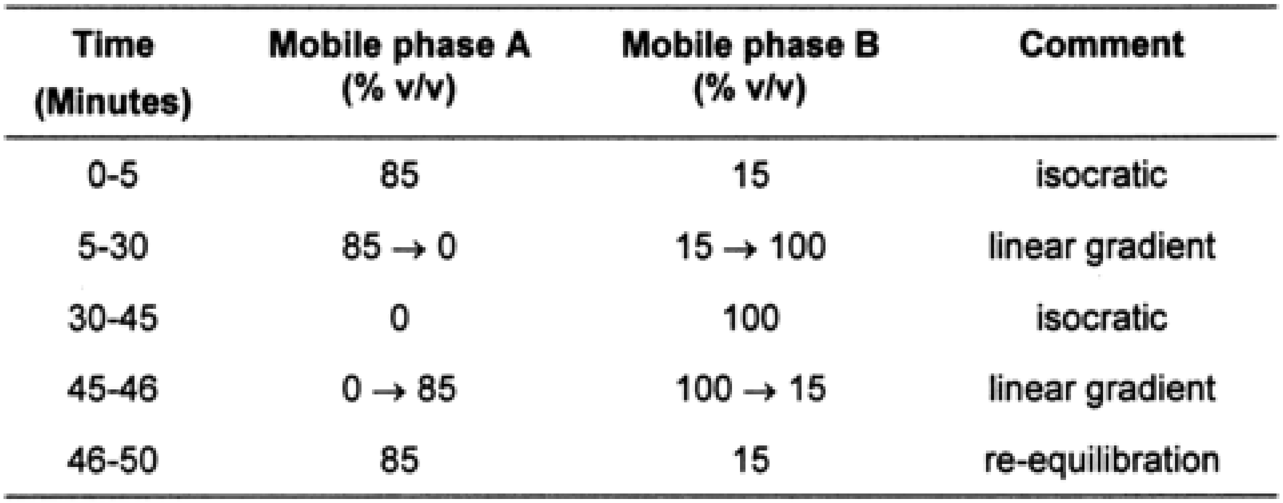
(e) Detection wavelength of 238 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

Mobile phase A Mix 40 volumes of *acetonitrile* with 60 volumes of pH 4.5 buffer solution.

Mobile phase B Mix 20 volumes of pH 4.5 buffer solution with 80 volumes of *acetonitrile*.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	85	15	isocratic
5-30	85 → 0	15 → 100	linear gradient
30-45	0	100	isocratic
45-46	0 → 85	100 → 15	linear gradient
46-50	85	15	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to simvastatin and lovastatin is at least 3.0

When the chromatograms are recorded under the prescribed conditions the retention times with respect to simvastatin are: simvastatin hydroxy acid about 0.52, lovastatin about 0.71, epilovastatin about 0.74, anhydrosimvastatin about 1.74 and simvastatin dimer about 2.42.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to lovastatin and epilovastatin is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any other *secondary peak* excluding any peaks corresponding to lovastatin and epilovastatin is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the sum of the areas of all the *secondary peaks* excluding any peaks corresponding to lovastatin and epilovastatin is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than a quarter of the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Add 3 ml of *glacial acetic acid* to 900 ml of *water*, adjust the pH to 4.0 with 1M *sodium hydroxide* and add sufficient *water* to produce 1000 ml. Mix 1 volume of this solution with 4 volumes of *acetonitrile* (solution A).

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix a quantity of whole tablets containing 0.16 g of Simvastatin in *water* and mix with the aid of ultrasound and shaking until completely dispersed. Add sufficient of solution A to produce about 75 ml, mix with the aid of ultrasound and shaking for 15 minutes, allow to cool to room temperature, add sufficient of solution A to produce 100 ml and centrifuge. Dilute a volume of the clear supernatant solution with sufficient of solution A to produce a solution containing 0.01% w/v of Simvastatin

(2) 0.01% w/v of *simvastatin BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 1.5 ml per minute.

(d) Column temperature of 45°.

(e) Detection wavelength of 238 nm.

(f) Injection volume of 20 µl for each solution.

MOBILE PHASE

A mixture of 7 volumes of a buffer solution prepared as described below and 13 volumes of *acetonitrile*. To prepare the buffer solution dissolve 5.1 g of *sodium dihydrogen orthophosphate* in 900 ml of *water*, adjust the pH to 4.5 with either *orthophosphoric acid* or 1M *sodium hydroxide* and add sufficient *water* to produce 1000 ml.

SYSTEM SUITABILITY

The test is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{25}H_{38}O_5$ in the tablets using the declared content of $C_{25}H_{38}O_5$ in *simvastatin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Simvastatin.

Soap Spirit

General Notices

DEFINITION

Soft Soap	650 g
Ethanol (90 per cent)	Sufficient to produce 1000 ml

In making Soap Spirit, the Ethanol (90 per cent) may be replaced by Industrial Methylated Spirit[1] diluted so as to be of equivalent ethanolic strength.

Extemporaneous preparation

The following directions apply.

Dissolve the Soft Soap in 300 ml of Ethanol (90 per cent), add sufficient Ethanol (90 per cent) to produce 1000 ml, mix, allow to stand and decant.

The spirit complies with the requirements stated under Spirits and with the following requirements.

TESTS

Acidity or alkalinity

10 ml requires for neutralisation not more than 1.0 ml of 0.1M *sodium hydroxide VS* or 0.2 ml of 0.1M *hydrochloric acid VS* using *phenolphthalein solution R1* as indicator.

Weight per ml

0.945 to 0.970 g, Appendix V G.

Ethanol content

28 to 32% v/v, Appendix VIII F.

Content of fatty acids

Not less than 27.0% w/v when determined by the following method. To 10 ml add 20 ml of *water* and 20 ml of 1M *hydrochloric acid*, extract with two 20 ml quantities of *petroleum spirit* (*boiling range, 40° to 60°*), wash the combined extracts with two 10 ml quantities of *water*, evaporate the petroleum spirit, add 5 ml of *acetone*, again evaporate and dry the residue at 80° for 2 hours.

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[1] The law and the statutory regulations governing the use of Industrial Methylated Spirit must be observed.

Sodium Amidotrizoate Injection

General Notices

DEFINITION

Sodium Amidotrizoate Injection is a sterile solution of Sodium Amidotrizoate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium amidotrizoate, $C_{11}H_8I_3N_2NaO_4$

97.0 to 103.0% of the stated amount.

CHARACTERISTICS

An almost colourless to pale yellow solution.

IDENTIFICATION

A. Dry 1 ml of the injection over *phosphorus pentoxide* at a pressure of 2 kPa for 16 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of sodium amidotrizoate (*RS 317*).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) is similar in position and size to the principal spot in the chromatogram obtained with solution (4).

TESTS

Acidity or alkalinity

pH, 6.6 to 7.6, Appendix V L. Take the first measurement 3 minutes after inserting the electrodes and repeat every 30 seconds until constant.

Free amine

To a volume of the injection containing 1.0 g of Sodium Amidotrizoate in a 50 ml glass-stoppered graduated flask add 5 ml of *water*, 10 ml of 0.1M *sodium hydroxide* and 25 ml of *dimethyl sulphoxide*. Stopper the flask, mix the contents by gently swirling and cool in ice, protected from light. After 5 minutes, slowly add 2 ml of *hydrochloric acid*, mix and allow to stand for 5 minutes. Add 2 ml of a 2% w/v solution of *sodium nitrite*, mix and allow to stand for 5 minutes. Add 1 ml of an 8% w/v solution of *sulphamic acid*, mix and allow to stand for 5 minutes. Add 2 ml of a 0.1% w/v solution of N-(*1-naphthyl*)*ethylenediamine dihydrochloride* in a 70% v/v solution of *propane-1,2-diol* and mix. Remove the flask from the ice and allow to stand in water at 22° to 25° for 10 minutes, with occasional gentle shaking. Add sufficient *dimethyl sulphoxide* to produce 50 ml and mix. Within 5 minutes of diluting to 50 ml measure the *absorbance* of the resulting solution at the maximum at 470 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 ml of *water* in the same manner. The *absorbance* is not more than 0.30.

Inorganic iodide

Dilute a quantity containing 0.80 g of Sodium Amidotrizoate to 10 ml with *water*, add sufficient 2M *nitric acid* drop wise to ensure complete precipitation of the iodinated acid and 3 ml in excess. Filter, wash the precipitate with 5 ml of *water*, add to the filtrate 1 ml of *hydrogen peroxide solution* (*100 vol*) and 1 ml of *chloroform* and shake. Any purple colour in the chloroform is not more intense than that obtained by adding 2 ml of *iodide standard solution* (*20 ppm I*) to a mixture of 3 ml of 2M *nitric acid* and sufficient *water* to equal the volume of the test solution, adding 1 ml of *hydrogen peroxide solution* (*100 vol*) and 1 ml of *chloroform* and shaking (50 ppm).

Related substances

Prepare the solutions in subdued light and develop the chromatograms protected from light. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 20 volumes of *anhydrous formic acid*, 25 volumes of *butan-2-one* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. Solutions (1), (2) and (3) are dilutions of the injection in a 3% v/v solution of 13.5M *ammonia in methanol* containing (1) 5.0% w/v, (2) 0.50% w/v and (3) 0.010% w/v of Sodium Amidotrizoate respectively. Solution (4) contains 0.50% w/v of *sodium amidotrizoate EPCRS* in a 3% v/v solution of 13.5M *ammonia* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.2%).

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D. Use per kg of the rabbit's weight a quantity containing 2.5 g of Sodium Amidotrizoate.

ASSAY

Mix a quantity containing 0.7 g of Sodium Amidotrizoate with 12 ml of 5M *sodium hydroxide* and 20 ml of *water*, add 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 ml of *water*, filter through absorbent cotton and wash the flask and the filter with two 20 ml quantities of *water*. To the combined filtrate and washings add 80 ml of *hydrochloric acid*, cool and titrate with 0.05M *potassium iodate VS* until the dark brown solution becomes light brown. Add 5 ml of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless. Each ml of 0.05M *potassium iodate VS* is equivalent to 21.20 mg of $C_{11}H_8I_3N_2NaO_4$. Determine the *weight per ml* of the injection, Appendix V G, and calculate the percentage w/v of sodium amidotrizoate, $C_{11}H_8I_3N_2NaO_4$.

STORAGE

Sodium Amidotrizoate Injection should be protected from light.

LABELLING

The strength is stated as the percentage w/v of Sodium Amidotrizoate.

Sodium Aurothiomalate Injection

General Notices

DEFINITION

Sodium Aurothiomalate Injection is a sterile solution of Sodium Aurothiomalate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of gold, Au

42.3 to 48.3% of the stated amount of Sodium Aurothiomalate.

CHARACTERISTICS

A pale yellow solution.

IDENTIFICATION

A. To a volume containing 20 mg of Sodium Aurothiomalate add 2 ml of *hydrogen peroxide solution* (*100 vol*) and 1 ml of 5M *sodium hydroxide* and boil for 30 seconds. A colloidal precipitate of gold is produced which appears bluish green by transmitted light.

B. To a volume containing 10 mg of Sodium Aurothiomalate add 0.1 ml of *potassium cyanide solution* and 0.1 ml of a 1.0% w/v solution of *sodium nitroprusside*. A deep magenta colour is produced.

Colour of solution

Dilute, if necessary, with *water* to give a solution containing 1.0% w/v of Sodium Aurothiomalate. The colour of the solution is not more intense than that of a 0.020% w/v solution of *potassium hexacyanoferrate*(*III*).

ASSAY

To a volume containing 0.1 g of Sodium Aurothiomalate, add 0.4 g of *potassium bromide* and 5 ml of *nitric acid*. Slowly evaporate the solution to dryness and continue heating until fumes cease to be evolved. Allow to cool, add 50 ml of *water*, warm, filter, wash the filter with hot *water*, dry and ignite the residue of gold, Au, for 3 hours at a temperature not lower than 600°.

STORAGE

Sodium Aurothiomalate Injection should be protected from light.

Sodium Bicarbonate Ear Drops

General Notices

DEFINITION

Sodium Bicarbonate	5 g
Glycerol	30 ml
Purified Water, freshly boiled and cooled	Sufficient to produce 100 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Sodium Bicarbonate in about 60 ml of Purified Water, add the Glycerol and sufficient Purified Water to produce 100 ml and mix.

Sodium Bicarbonate Ear Drops should be recently prepared.
The ear drops comply with the requirements stated under Ear Preparations and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

4.75 to 5.25% w/v.

Weight per ml

1.10 to 1.12 g, Appendix V G.

ASSAY

To 5 ml add 20 ml of *water* and titrate with 0.1M *hydrochloric acid VS* using *methyl orange-xylene cyanol FF solution* as indicator. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 8.401 mg of $NaHCO_3$.

Sodium Bicarbonate Eye Lotion

General Notices

DEFINITION

Sodium Bicarbonate Eye Lotion is a sterile aqueous solution of Sodium Bicarbonate.

Extemporaneous preparation

The following directions apply.

Dissolve the Sodium Bicarbonate in sufficient Purified Water, clarify by filtration, transfer the filtered solution into the final container, close the container so as to exclude micro-organisms and sterilise by *heating in an autoclave*.

The eye lotion complies with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *sodium salts* and the reactions characteristic of *bicarbonates*, Appendix VI.

ASSAY

Titrate a volume containing 1 g of Sodium Bicarbonate with 0.5M *hydrochloric acid VS* using *methyl orange solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

Containers

Sodium Bicarbonate Eye Lotion is supplied in suitable containers with a nominal volume of not more than 1 litre.

STORAGE

Containers that have previously been subjected to heating in an autoclave should not be re-used to keep Sodium Bicarbonate Eye Lotion.

LABELLING

The label states that any Eye Lotion not used within 24 hours of opening the container should be discarded.

When Sodium Bicarbonate Eye Lotion is prescribed or demanded no strength being stated, a lotion containing 2% w/v of Sodium Bicarbonate shall be dispensed or supplied.

Sodium Bicarbonate Intravenous Infusion

General Notices

Sodium Bicarbonate Injection

DEFINITION

Sodium Bicarbonate Intravenous Infusion is a sterile solution of Sodium Bicarbonate in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

94.0 to 106.0% of the stated amount.

IDENTIFICATION

A. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *sodium salts* and the reactions characteristic of *bicarbonates*, Appendix VI.

TESTS

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D. For intravenous infusions containing 2.5% w/v or less of Sodium Bicarbonate slowly inject 10 ml per kg of the rabbit's weight. For intravenous infusions containing more than 2.5% w/v of Sodium Bicarbonate dilute to contain 2.5% w/v of Sodium Bicarbonate and slowly inject 10 ml per kg of the rabbit's weight.

ASSAY

Titrate a volume containing 1 g of Sodium Bicarbonate with 0.5M *hydrochloric acid VS* using *methyl orange solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

STORAGE

Containers that have previously been subjected to heating in an autoclave should not be re-used to keep Sodium Bicarbonate Intravenous Infusion.

LABELLING

The strength is stated as the percentage w/v of Sodium Bicarbonate.

The label states (1) that containers containing visible particles must not be used; (2) the approximate concentrations, in millimoles per litre, of the sodium ions and the bicarbonate ions.

For a preparation containing 1.4% w/v of Sodium Bicarbonate the concentration of each ion is stated as 167 millimoles per litre.

Sodium Bicarbonate Oral Solution

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Antacid; used in the treatment of electrolyte deficiency.

DEFINITION

Sodium Bicarbonate Oral Solution is a solution of Sodium Bicarbonate in Purified Water.

The oral solution complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of sodium bicarbonate, $NaHCO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *sodium salts* and the reactions characteristic of *bicarbonates*, Appendix VI.

ASSAY

Titrate a volume containing 1 g of Sodium Bicarbonate with 0.5M *hydrochloric acid VS* using *methyl orange solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 42.00 mg of $NaHCO_3$.

When Sodium Bicarbonate Oral Solution for paediatric or neonatal use is prescribed or demanded, a solution containing 1 mmol/ml shall be dispensed or supplied.

Sodium Calcium Edetate Intravenous Infusion

General Notices

Sodium Calcium Edetate Injection

Action and use

Chelating agent.

DEFINITION

Sodium Calcium Edetate Intravenous Infusion is a sterile solution of Sodium Calcium Edetate. It is prepared immediately before use by diluting Sterile Sodium Calcium Edetate Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

LABELLING

The strength is stated as the equivalent amount of anhydrous sodium calcium edetate in a suitable dose-volume.

STERILE SODIUM CALCIUM EDETATE CONCENTRATE

DEFINITION

Sterile Sodium Calcium Edetate Concentrate is a sterile solution of Sodium Calcium Edetate in Water for Injections containing the equivalent of 20% w/v of anhydrous sodium calcium edetate.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of anhydrous sodium calcium edetate, $C_{10}H_{12}CaN_2Na_2O_8$

19.0 to 21.0% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Dilute 2.5 ml with 7.5 ml of *water*, make alkaline to *litmus paper* with 5M *ammonia* and add 5 ml of a 2.5% w/v solution of *ammonium oxalate*. Not more than a trace of precipitate is produced.

B. To 10 ml add 2 ml of a 10% w/v solution of *lead(II) nitrate*, shake and add 5 ml of *dilute potassium iodide solution*; no yellow precipitate is produced. Make alkaline to *litmus paper* with 5M *ammonia* and add 5 ml of a 2.5% w/v solution of *ammonium oxalate*; a white precipitate is produced.

C. Evaporate to dryness and ignite. The residue yields the reactions characteristic of *sodium salts* and *calcium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D. Use 2 ml per kg of the rabbit's weight.

ASSAY

To 2.5 ml add 90 ml of *water*, 7 g of *hexamine* and 5 ml of 2M *hydrochloric acid* and titrate with 0.05M *lead nitrate VS* using *xylenol orange solution* as indicator. Each ml of 0.05M *lead nitrate VS* is equivalent to 18.71 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

STORAGE

Sterile Sodium Calcium Edetate Concentrate should be kept in containers made from lead-free glass.

LABELLING

The label states (1) 'Sterile Sodium Calcium Edetate Concentrate'; (2) that the solution must be diluted with Sodium Chloride Intravenous Infusion or Glucose Intravenous Infusion before administration.

Sodium Chloride and Glucose Intravenous Infusion

General Notices

Sodium Chloride and Dextrose Injection; Sodium Chloride and Glucose Injection; Sodium Chloride and Dextrose Intravenous Infusion

DEFINITION

Sodium Chloride and Glucose Intravenous Infusion is a sterile solution of Sodium Chloride and either Anhydrous Glucose or Glucose in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

Content of glucose, $C_6H_{12}O_6$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or faintly straw-coloured solution.

IDENTIFICATION

A. When heated with *cupri-tartaric solution R1*, a copious precipitate of copper(I) oxide is produced.

B. Yields reaction B characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 6.5, Appendix V L.

5-Hydroxymethylfurfural and related substances

Dilute a volume containing 1.0 g of glucose, $C_6H_{12}O_6$, to 500 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 284 nm, Appendix II B. The *absorbance* is not more than 0.25.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per ml, Appendix XIV C. Dilute infusions containing more than 0.9% w/v of Sodium Chloride with *water BET* to contain 0.9% w/v.

ASSAY

For sodium chloride

Titrate a quantity containing 0.1 g of Sodium Chloride with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

For glucose

To a quantity containing 2 to 5 g of glucose, $C_6H_{12}O_6$, add 0.2 ml of 5M *ammonia* and sufficient *water* to produce 100 ml. Mix well, allow to stand for 30 minutes and measure the *optical rotation* in a 2-dm tube, Appendix V F. The observed rotation in degrees multiplied by 0.9477 represents the weight in g of glucose, $C_6H_{12}O_6$, in the volume of the injection taken for assay.

LABELLING

The strength is stated as the percentages w/v of Sodium Chloride and of glucose, $C_6H_{12}O_6$. The label states that solutions containing visible particles must not be used.

When the preparation is intended for intravenous infusion, the label states the approximate concentrations, in millimoles per litre, of the sodium ions and the chloride ions and the number of grams per litre of glucose, $C_6H_{12}O_6$. For a preparation containing 0.18% w/v of Sodium Chloride, the concentration of each ion is stated as 30 millimoles per litre.

Sodium Chloride Eye Drops

General Notices

DEFINITION

Sodium Chloride Eye Drops are a sterile solution of Sodium Chloride in Purified Water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium chloride, NaCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Yield the reactions characteristic of *sodium salts*, Appendix VI.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Titrate a volume containing 0.1 g of Sodium Chloride with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

When Sodium Chloride Eye Drops are prescribed or demanded no strength being stated, eye drops containing 0.9% w/v of Sodium Chloride shall be dispensed or supplied.

Sodium Chloride Eye Lotion

General Notices

DEFINITION

Sodium Chloride Eye Lotion is a sterile aqueous solution containing 0.9% w/v of Sodium Chloride.

Extemporaneous preparation

The following directions apply.

Dissolve the Sodium Chloride in sufficient Purified Water, clarify by filtration, transfer the filtered solution into the final container, close the container so as to exclude micro-organisms and sterilise by *heating in an autoclave*.

The eye lotion complies with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium chloride, NaCl

0.85 to 0.95% w/v.

IDENTIFICATION

Yields the reactions characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Sterility

Complies with the *test for sterility*, Appendix XVI A.

ASSAY

To 25 ml add 75 ml of *acetate buffer pH 5.0* and titrate with 0.1M *silver nitrate VS*, stirring constantly and determining the end point potentiometrically. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

Containers

Sodium Chloride Eye Lotion is supplied in suitable containers with a nominal volume of not more than 1 litre.

LABELLING

The label states that any Eye Lotion not used within 24 hours of opening the container should be discarded.

Sodium Chloride Intravenous Infusion

General Notices

Sodium Chloride Injection

DEFINITION

Sodium Chloride Intravenous Infusion is a sterile solution of Sodium Chloride in Water for Injections.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *sodium salts* and reaction A characteristic of *chlorides*, Appendix VI.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per ml, Appendix XIV C. Dilute infusions containing more than 0.9% w/v of Sodium Chloride with *water BET* to contain 0.9% w/v.

ASSAY

Titrate a volume containing 0.2 g of Sodium Chloride with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

LABELLING

The strength is stated as the percentage w/v of Sodium Chloride. The label states that solutions containing visible solid particles must not be used.

When the preparation is intended for intravenous infusion, the label states the approximate concentrations, in millimoles per litre, of the sodium ions and the chloride ions. For a preparation containing 0.9% w/v of Sodium Chloride the concentration of each ion is stated as 150 millimoles per litre.

For concentrated solutions supplied for the preparation of Sodium Chloride Intravenous Infusion by dilution, the label states 'Sterile Sodium Chloride Concentrate' as the title of the preparation.

When Sodium Chloride Intravenous Infusion or Sodium Chloride Injection is prescribed or demanded and no strength is stated, Sodium Chloride Intravenous Infusion (0.9% w/v) shall be dispensed or supplied.

When Sodium Chloride Injection is required as a diluent for Parenteral Preparations of the Pharmacopoeia, Sodium Chloride Intravenous Infusion (0.9% w/v) shall be used.

When normal saline solution for injection is prescribed or demanded, Sodium Chloride Intravenous Infusion (0.9% w/v) shall be dispensed or supplied.

Sodium Chloride Irrigation Solution

General Notices

DEFINITION

Sodium Chloride Irrigation Solution is a solution of Sodium Chloride in Water for Irrigation. *The irrigation solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.*

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Yields the reactions characteristic of *sodium salts*, Appendix VI.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Titrate a volume containing 0.2 g of Sodium Chloride with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

When normal saline is prescribed or demanded for use as an irrigation solution, Sodium Chloride Irrigation Solution containing 0.9% w/v of Sodium Chloride shall be dispensed or supplied.

Sodium Chloride Oral Solution

General Notices

NOTE: There are currently no licensed formulations in the United Kingdom.

Action and use

Used in the treatment of electrolyte deficiency.

DEFINITION

Sodium Chloride Oral Solution is a solution of Sodium Chloride in Purified Water.

The oral solution complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. When introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Titrate a volume containing 0.2 g of Sodium Chloride with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

When Sodium Chloride Oral Solution for paediatric or neonatal use is prescribed or demanded, a solution containing 1 mmol/ml or 5 mmol/ml shall be dispensed or supplied.

Sodium Chloride Solution

General Notices

DEFINITION

Sodium Chloride Solution is a 0.9% w/v *cutaneous solution* of Sodium Chloride in Purified Water. The solution may be clarified by filtration.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of sodium chloride, NaCl

0.85 to 0.95% w/v.

IDENTIFICATION

A. When introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

ASSAY

Titrate 20 ml with 0.1M *silver nitrate VS* using *potassium chromate solution* as indicator. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

LABELLING

The label states, where applicable, that the contents of the container are sterile.

The label indicates the pharmaceutical form as 'cutaneous solution'.

When normal saline is prescribed or demanded, Sodium Chloride Solution shall be dispensed or supplied.

If the label states that the contents of the container are sterile, the solution complies with the following additional requirements.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

LABELLING

The label states (1) 'Sterile Sodium Chloride Solution'; (2) that the solution is not intended for injection; (3) the date after which the solution is not intended to be used; (4) the conditions under which it should be stored.

Sodium Chloride Tablets

General Notices

DEFINITION

Sodium Chloride Tablets contain Sodium Chloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium chloride, NaCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The powdered tablets yield the reactions characteristic of *sodium salts* and the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Clarity of solution

Dissolve a quantity of the tablets containing 0.6 g of Sodium Chloride in 100 ml of *water*. The solution is *clear*, Appendix IV A.

Disintegration

The tablets dissolve completely within 15 minutes when examined by the *disintegration test for tablets and capsules*, Appendix XII A1, but using *water* at 19° to 21°.

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 0.2 g of Sodium Chloride in 35 ml of *water*, add 15 ml of 2M *nitric acid*, 5 ml of *dibutyl phthalate* and 50 ml of 0.1M *silver nitrate VS* and shake vigorously for about 1 minute. Add 5 ml of *ammonium iron(III) sulphate solution R2* and titrate with 0.1M *ammonium thiocyanate VS* until a reddish brown colour is obtained which, after shaking, does not fade within 5 minutes. Each ml of 0.1M *silver nitrate VS* is equivalent to 5.844 mg of NaCl.

LABELLING

The label states that the tablets should be dissolved in water before administration.

Sodium Citrate Eye Drops

General Notices

DEFINITION

Sodium Citrate Eye Drops are a sterile solution of Sodium Citrate in Purified Water.
The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium citrate, $C_6H_5Na_3O_7,2H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Yield reaction A characteristic of *sodium salts*, Appendix VI.

B. Yield reaction A characteristic of *citrates*, Appendix VI.

Acidity or alkalinity

pH, 7.0 to 7.5, Appendix V L.

ASSAY

Evaporate a volume containing 0.15 g of Sodium Citrate to dryness and dissolve the residue in *anhydrous acetic acid*, warming to about 50°. Allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 ml of *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 9.803 mg of $C_6H_5Na_3O_7,2H_2O$.

Sodium Citrate Irrigation Solution

General Notices

Sterile Sodium Citrate Solution for Bladder Irrigation

DEFINITION

Sodium Citrate Irrigation Solution is a sterile, solution of Sodium Citrate in Water for Irrigation containing Anhydrous Citric Acid or Citric Acid Monohydrate.

The irrigation solution complies with the requirements stated under Preparations for Irrigation and with the following requirements.

Content of sodium citrate, $C_6H_5Na_3O_7,2H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Yields reaction A characteristic of *sodium salts* and reaction A characteristic of *citrates*, Appendix VI.

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

ASSAY

Evaporate a volume containing 0.15 g of Sodium Citrate to dryness and dissolve the residue in *anhydrous acetic acid*, warming to about 50°. Allow to cool and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 ml of *1-naphtholbenzein solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 9.803 mg of $C_6H_5Na_3O_7,2H_2O$.

When Sodium Citrate Irrigation Solution is prescribed or demanded, no strength being stated, a solution containing 3.0% w/v of Sodium Citrate shall be dispensed or supplied.

Sodium Cromoglicate Eye Drops

General Notices

DEFINITION

Sodium Cromoglicate Eye Drops are a sterile solution of Sodium Cromoglicate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of sodium cromoglicate, $C_{23}H_{14}Na_2O_{11}$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 400 nm of a solution of the eye drops diluted to contain 0.002% w/v of Sodium Cromoglicate exhibits two maxima at 239 and 326 nm, and a shoulder at about 260 nm.

B. To 1 ml of the eye drops add 2.5 ml of 1M *sodium hydroxide* and boil for 1 minute; a yellow colour is produced. Add 0.5 ml of *diazobenzenesulphonic acid solution*; a deep red colour is produced.

TESTS

Acidity or alkalinity

pH, 4.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a precoated silica gel F_{254} plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *glacial acetic acid*, 50 volumes of *ethyl acetate* and 50 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) use the eye drops, diluted, if necessary with *water*, to contain 2% w/v of Sodium Cromoglicate. Solution (2) contains 0.01% w/v of *1,3-bis(2-acetyl-3-phenoxy)-2-propanol EPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Dilute the eye drops with *phosphate buffer pH 7.4* to give a solution containing 0.004% w/v of Sodium Cromoglicate and measure the *absorbance* of the resulting solution at the maximum at 326 nm, Appendix II B. Calculate the content of $C_{23}H_{14}Na_2O_{11}$ in the eye drops taking 164 as the value of A(1%, 1 cm) at the maximum at 326 nm.

Sodium Cromoglicate Powder for Inhalation

General Notices

DEFINITION

Sodium Cromoglicate Powder for Inhalation consists of hard gelatin capsules containing either Sodium Cromoglicate appropriately treated or Sodium Cromoglicate admixed with an approximately equal amount of Lactose. The contents of the capsules are in powder of a suitable fineness.

The powder for inhalation complies with the requirements stated under Preparations for Inhalation and the contents of the capsules comply with the following requirements.

Content of sodium cromoglicate, $C_{23}H_{14}Na_2O_{11}$

20.0 to 24.4 mg per capsule.

CHARACTERISTICS

A white powder; hygroscopic. When examined microscopically immediately after dispersion in *pentan-1-ol* by exposure for 20 seconds to low intensity ultrasonic waves, exhibit small, rounded particles of maximum dimension 10 µm, often present as loose agglomerates up to 30 µm. The contents of capsules containing Lactose also exhibit larger angular particles, usually axe-head in shape, of length 10 to 150 µm but mostly within the range 20 to 80 µm.

IDENTIFICATION

A. Dissolve a suitable quantity in sufficient *phosphate buffer pH 7.4* to produce a solution containing 0.001% w/v of Sodium Cromoglicate. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits two maxima, at 238 nm and 326 nm.

B. To a quantity containing 0.1 g of Sodium Cromoglicate add 2 ml of *water* and 2 ml of 1.25M *sodium hydroxide* and boil for 1 minute; a yellow colour is produced. Add 0.5 ml of *diazobenzenesulphonic acid solution* ; a blood-red colour is produced.

C. Yield reaction A characteristic of *sodium salts* , Appendix VI.

D. For capsules containing Lactose the contents when heated with *cupri-tartaric solution R1* produce a copious precipitate of copper(I) oxide.

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 5 volumes of *glacial acetic acid* , 50 volumes of *ethyl acetate* and 50 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dissolve a quantity containing 0.1 g of Sodium Cromoglicate in sufficient of a mixture of 1 volume of *acetone* , 4 volumes of *tetrahydrofuran* that has been freed from stabiliser by passage through a column of suitable alumina and 6 volumes of *water* to produce 5 ml and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The principal spot remains on the line of application.

Uniformity of weight

Weigh one capsule. Open without loss of shell material, remove the contents and weigh all parts of the shell; the difference between the weights represents the weight of the contents. Repeat the operation with a further 19 capsules and calculate the average weight of the contents of the 20 capsules.

For preparations containing no Lactose the weight of the contents of each capsule does not deviate from the average by more than 25%. For preparations containing Lactose the weight of the contents of each capsule does not deviate from the average weight by more than 15% except that, for two capsules, the weight of the contents may deviate by not more than 25%.

Loss on drying

When dried to constant weight at 100° at a pressure not exceeding 0.7 kPa, the contents of the capsules containing no Lactose lose 9.0 to 18.0% of their weight and the contents of capsules containing Lactose lose 5.5 to 10.0% of their weight.

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 0.1 g of Sodium Cromoglicate in sufficient *phosphate buffer pH 7.4* to produce 1000 ml, dilute 25 ml to 100 ml with *phosphate buffer pH 7.4* and measure the *absorbance* of the resulting solution at the maximum at 326 nm, Appendix II B. Calculate the content of $C_{23}H_{14}Na_2O_{11}$ in a capsule of average content weight taking 164 as the value of A(1%, 1 cm) at the maximum at 326 nm.

STORAGE

Sodium Cromoglicate Powder for Inhalation should be protected from moisture and stored at a temperature not exceeding 30°.

LABELLING

The label states (1) that each capsule contains 20 mg of Sodium Cromoglicate; (2) that the capsules are intended for use in an inhaler and are not to be swallowed; (3) where applicable, that the capsules contain Lactose.

Sodium Etacrynate Injection

General Notices

DEFINITION

Sodium Etacrynate Injection is a sterile solution of sodium etacrynate in Water for Injections. It is prepared by dissolving Sodium Etacrynate for Injection in the requisite amount of the liquid stated on the label.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Sodium Etacrynate Injection should be protected from light and used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

SODIUM ETACRYNATE FOR INJECTION

DEFINITION

Sodium Etacrynate for Injection is a sterile material consisting of the sodium salt of Etacrynic Acid with or without excipients.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of sodium etacrynate, calculated as etacrynic acid, $C_{13}H_{12}Cl_2O_4$

90.0 to 105.0% of the stated amount of Etacrynic Acid.

IDENTIFICATION

Suspend a quantity containing the equivalent of 50 mg of Etacrynic Acid in 25 ml of 0.1M *hydrochloric acid* and extract with two 40 ml quantities of *dichloromethane* . Dry the combined extracts with *anhydrous sodium sulphate* , filter and evaporate to dryness using a rotary evaporator. The residue complies with the following tests.

A. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of etacrynic acid (*RS 130*).

B. The *light absorption* , Appendix II B in the range 230 to 350 nm of a 0.005% w/v solution in 0.01M *methanolic hydrochloric acid* exhibits a maximum only at 270 nm and may exhibit a shoulder at 285 nm.

C. Dissolve a quantity containing the equivalent of 25 mg of Etacrynic Acid in 2 ml of 1M *sodium hydroxide* and heat on a water bath for 5 minutes. Cool, add 0.25 ml of a 50% v/v solution of *sulphuric acid* , 0.5 ml of a 10% w/v solution of *chromotropic acid sodium salt* and, carefully, 2 ml of *sulphuric acid*. An intense violet colour is produced.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 0.1% w/v of Etacrynic Acid, 6.3 to 7.7, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of a sealed container in sufficient of a mixture of 32 volumes of 0.05M *hydrochloric acid* and 70 volumes of *acetonitrile* to produce a solution containing the equivalent of 0.5% w/v of Etacrynic Acid. For solution (2) dilute 1 volume of solution (1) to 40 volumes with a mixture of 7 volumes of *acetonitrile* and 3 volumes of *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 57 volumes of *acetonitrile*, 43 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 270 nm.

In the chromatogram obtained with solution (1) the sum of the areas of all the peaks appearing after the principal peak is not more than twice the area of the principal peak in the chromatogram obtained with solution (2).

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections with the following modification. Instead of emptying the containers as completely as possible by gently tapping, dissolve the contents and use in the preparation of solutions (2) and (3) below.

Prepare a 0.15% w/v solution of *propyl 4-hydroxybenzoate* (internal standard) in *acetonitrile* (solution A). Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 50 mg of *etacrynic acid BPCRS* in 5 ml of solution A and dilute to 50 ml with a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*. For solutions (2) and (3) add 3.2 ml of 0.05M *hydrochloric acid* to each of 10 containers and shake to dissolve. Combine the resulting solutions and wash each container with a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water* and dilute the combined solutions and washings to 100 ml with the same solvent mixture (solution B). For solution (2) dilute 10 ml of solution B to 50 ml with the same solvent mixture. For solution (3) add 5 ml of solution A to 10 ml of solution B and dilute to 50 ml with the same solvent mixture.

The chromatographic procedure described under Related substances may be used but using as the mobile phase a mixture of 1 volume of *glacial acetic acid*, 40 volumes of *acetonitrile* and 60 volumes of *water*.

Calculate the content of $C_{13}H_{12}Cl_2O_4$ in a container of average content weight using the declared content of $C_{13}H_{12}Cl_2O_4$ in *etacrynic acid BPCRS*.

STORAGE

The sealed container should be protected from light.

LABELLING

The label of the sealed container states the quantity of sodium etacrynate contained in it in terms of the equivalent amount of Etacrynic Acid.

Sodium Feredetate Oral Solution

General Notices

Action and use

Source of iron.

DEFINITION

Sodium Feredetate Oral Solution contains Sodium Feredetate in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of ferric iron, Fe(III)

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Evaporate a quantity of the oral solution containing the equivalent of about 30 mg of ferric iron to dryness, ignite the residue and cool. Mix the cooled residue with 2 ml of *hydrochloric acid* , add sufficient *water* to produce 20 ml and filter. The filtrate yields reaction C characteristic of *iron salts* and a one in five dilution of the filtrate yields reaction B characteristic of *iron salts* , Appendix VI.

B. To a quantity of the oral solution containing the equivalent of 55 mg of ferric iron add sufficient 5M *ammonia* to make it alkaline to *litmus paper* followed by 5 ml of a 2.5% w/v solution of *ammonium oxalate* . Not more than a trace of a precipitate is produced.

C. To a quantity of the oral solution containing the equivalent of 55 mg of ferric iron add 2 ml of a 10% w/v solution of *lead(II) nitrate*, shake and add 5 ml of *dilute potassium iodide solution* ; no yellow precipitate is produced. Add sufficient 5M *ammonia* to make the solution alkaline to *litmus paper* and add 5 ml of a 2.5% w/v solution of *ammonium oxalate* ; a white precipitate is produced.

TESTS

Acidity

pH, 2.6 to 3.1, Appendix V L.

Free iron

Not more than 0.72% w/v of the content of ferric iron when determined in the following manner. Label three tubes A, B and C. Place a quantity of the oral solution containing the equivalent of 55 mg of ferric iron into each tube. Add 1 ml of a 1.0% w/v solution of *disodium* *catechol-3,5-disulphonate* into each of tubes A and B, add 4 ml of an iron standard solution prepared in the following manner to tube A and dilute each of solutions A, B and C to 25 ml with *water*. For the iron standard solution dilute 10 volumes of a 0.1726% w/v solution of *ammonium iron(III) sulphate* in 0.05M *sulphuric acid* to 20 volumes with *water* (100 ppm of Fe(III)). Measure the *absorbance* of solutions A and B at 670 nm, Appendix II B, using solution C in the reference cell using a 4-cm pathlength. The absorbance of solution A is not greater than twice that of solution B.

ASSAY

Mix a quantity of the oral solution containing the equivalent of 0.137 g of ferric iron with 20 ml of *water* and 20 ml of *hydrochloric acid*. Cool to room temperature and add 3 g of *potassium iodide*, stopper the flask and allow to stand for 5 minutes. Titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using 0.5 ml of *starch mucilage*, added just before the end point, as indicator. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of iodine liberated. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 5.585 mg of Fe.

LABELLING

The quantity of the active ingredient is stated both as the amount of Sodium Feredetate and in terms of the equivalent amount of ferric iron.

Sodium Fluoride Mouthwash

General Notices

DEFINITION

Sodium Fluoride Mouthwash is a solution of Sodium Fluoride in a suitable aqueous vehicle.

The mouthwash complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of sodium fluoride, NaF

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake 5 ml of the mouthwash with *activated charcoal* and filter. To 0.2 ml of the filtrate add 0.2 ml of a freshly prepared mixture of equal volumes of *alizarin S solution* and *zirconyl nitrate solution* . A yellow colour is produced.

B. The filtrate obtained in test A yields reaction A characteristic of *sodium salts* , Appendix VI.

ASSAY

Carry out Method I for *potentiometric determination of ionic concentration using ion-selective electrodes*, Appendix VIII E, using a fluoride selective electrode.

Prepare and store all solutions in plastic containers.

For solution (1) dilute a volume of the mouthwash containing 50 mg of Sodium Fluoride to 1000 ml. Solutions (2), (3), (4) and (5) contain 0.0110% w/v, 0.00550% w/v, 0.00220% w/v and 0.00110% w/v of *sodium fluoride* respectively. To 20 ml of each solution add 20 ml of a buffer solution prepared by mixing 57 ml of *glacial acetic acid* , 58 g of *sodium chloride* and 4 g of *cyclohexylenedinitrilotetra-acetic acid* in 500 ml of *water*, adjusting the pH to 5.2 with a 50% w/v solution of *sodium hydroxide* and diluting to 1000 ml with *water*.

In turn, stir each solution, immerse the electrodes and allow to stand for 5 minutes with constant stirring. Determine the potential difference between the electrodes for each of solutions (2), (3), (4) and (5) and plot on semi-logarithmic graph paper the potential difference obtained as a function of concentration of sodium fluoride. Using exactly the same conditions, determine the potential difference obtained with solution (1) and hence calculate the content of sodium fluoride in the mouthwash.

LABELLING

The label states the concentration of sodium fluoride and of fluoride ion in a suitable volume.

Sodium Fluoride Oral Drops

General Notices

DEFINITION

Sodium Fluoride Oral Drops are a solution of Sodium Fluoride in a suitable aqueous vehicle.

The oral drops comply with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium fluoride, NaF

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To 0.2 ml add 0.2 ml of a freshly prepared mixture of equal volumes of *alizarin S solution* and *zirconyl nitrate solution* . A yellow colour is produced.

B. Yield reaction A characteristic of *sodium salts* , Appendix VI.

ASSAY

Carry out Method I for *potentiometric determination of ionic concentration using ion-selective electrodes*, Appendix VIII E, using a fluoride selective electrode.

Prepare and store all solutions in plastic containers.

For solution (1) dilute a volume of the oral drops containing 11 mg of Sodium Fluoride to 200 ml. Solutions (2), (3), (4) and (5) contain 0.0110% w/v, 0.00550% w/v, 0.00220% w/v and 0.00110% w/v of *sodium fluoride* respectively. To 20 ml of each solution add 20 ml of a buffer solution prepared by mixing 57 ml of glacial acetic acid, 58 g of sodium chloride and 4 g of cyclohexylenedinitrilotetra-acetic acid in 500 ml of water, adjusting the pH to 5.2 with a 50% w/v solution of sodium hydroxide and diluting to 1000 ml with water.

In turn, stir each solution, immerse the electrodes and allow to stand for 5 minutes with constant stirring. Determine the potential difference between the electrodes for each of solutions (2), (3), (4) and (5) and plot on semi-logarithmic graph paper the potential difference obtained as a function of concentration of sodium fluoride. Using exactly the same conditions, determine the potential difference obtained with solution (1) and hence calculate the content of sodium fluoride in the oral drops.

LABELLING

The label states the concentration of sodium fluoride and of fluoride ion in a suitable dose-volume.

Sodium Fluoride Oral Solution

General Notices

NOTE : There are currently no licensed formulations in the United Kingdom.

Action and use

Used in the prevention of dental caries.

DEFINITION

Sodium Fluoride Oral Solution is a solution of Sodium Fluoride in a suitable aqueous vehicle.

The oral solution complies with the requirements stated under Oral Liquids, the requirements stated under Unlicensed Medicines and with the following requirements.

Content of sodium fluoride, NaF

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. To 0.2 ml add 0.2 ml of a freshly prepared mixture of equal volumes of *alizarin S solution* and *zirconyl nitrate solution* . A yellow colour is produced.

B. Yields reaction A characteristic of *sodium salts* , Appendix VI.

ASSAY

Carry out Method I for *potentiometric determination of ionic concentration using ion-selective electrodes*, Appendix VIII E, using a fluoride selective electrode.

Prepare and store all solutions in plastic containers.

For solution (1) dilute a volume of the oral solution containing 11 mg of Sodium Fluoride to 200 ml. Solutions (2), (3), (4) and (5) contain 0.0110% w/v, 0.00550% w/v, 0.00220% w/v and 0.00110% w/v of *sodium fluoride* respectively. To 20 ml of each solution add 20 ml of a buffer solution prepared by mixing 57 ml of *glacial acetic acid* , 58 g of *sodium chloride* and 4 g of *cyclohexylenedinitrilotetra-acetic acid* in 500 ml of *water*, adjusting the pH to 5.2 with a 50% w/v solution of *sodium hydroxide* and diluting to 1000 ml with *water*.

In turn, stir each solution, immerse the electrodes and allow to stand for 5 minutes with constant stirring. Determine the potential difference between the electrodes for each of solutions (2), (3), (4) and (5) and plot on semi-logarithmic graph paper the potential difference obtained as a function of concentration of sodium fluoride. Using exactly the same conditions, determine the potential difference obtained with solution (1) and hence calculate the content of sodium fluoride in the oral solution.

Sodium Fluoride Tablets

General Notices

DEFINITION

Sodium Fluoride Tablets contain Sodium Fluoride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium fluoride, NaF

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 5 mg of Sodium Fluoride with 5 ml of *water* for 5 minutes and filter (Solution A). To 2 ml of the filtrate add 0.2 ml of a freshly prepared mixture of equal volumes of *alizarin S solution* and *zirconyl nitrate solution* . A yellow colour is produced.

B. Solution A yields reaction A characteristic of *sodium salts* , Appendix VI.

Uniformity of content

Tablets containing less than 2.5 mg of Sodium Fluoride comply with the requirements stated under Tablets using the following method of analysis. Carry out Method I for *potentiometric determination of ionic concentration using ion-selective electrodes*, Appendix VIII E, using a fluoride selective electrode.

Prepare and store all solutions in plastic containers.

For solution (1) add a sufficient quantity of *water* to one tablet to produce a solution containing 0.0055% w/v of Sodium Fluoride, heat at 80° and mix with the aid of ultrasound until dissolved, cool to room temperature and filter. Solutions (2), (3), (4) and (5) contain 0.0110% w/v, 0.00550% w/v, 0.00220% w/v and 0.00110% w/v of *sodium fluoride* respectively. To 15 ml of each solution add 20 ml of a buffer solution prepared by mixing 57 ml of *glacial acetic acid* , 58 g of *sodium chloride* and 4 g of *cyclohexylenedinitrilotetra-acetic acid* in 500 ml of *water*, adjusting the pH to 5.2 with a 50% w/v solution of *sodium hydroxide* and diluting to 1000 ml with *water*.

In turn, stir each solution, immerse the electrodes and allow to stand for 5 minutes with constant stirring. Determine the potential difference between the electrodes for solutions (2), (3), (4) and (5) and plot on semi-logarithmic graph paper the potential difference obtained for each solution as a function of concentration of sodium fluoride. Using exactly the same conditions, determine the potential difference obtained with solution (1) and calculate the content of sodium fluoride in each tablet.

ASSAY

Use the average of the ten individual results obtained in the test for Uniformity of content.

LABELLING

The label stated the quantity of sodium fluoride and of fluoride ion per tablet.

Sodium Fusidate Ointment

General Notices

DEFINITION

Sodium Fusidate Ointment contains Sodium Fusidate in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of sodium fusidate, $C_{31}H_{47}NaO_6$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Disperse a quantity of the ointment containing 60 mg of Sodium Fusidate in 30 ml of n-*hexane* and shake with 6 ml of *water*. Allow to separate, remove the aqueous layer and add to it 5 ml of 0.2M *orthophosphoric acid* . Extract with two 10 ml quantities of *chloroform* , dry the combined chloroform extracts with *anhydrous sodium sulphate* , evaporate to dryness and dissolve the residue in 0.6 ml of *chloroform IR* . The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of fusidic acid (*RS 166*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (2) has the same retention time as the peak due to fusidic acid in the chromatogram obtained with solution (1).

TESTS

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following three solutions. For solution (1) add 25 ml of n-*hexane* to a quantity of the ointment containing 50 mg of Sodium Fusidate, stir, extract with 10 ml of *ethanol* (*70%*) and allow the layers to separate. Use the ethanol layer and filter if necessary. For solution (2) mix 1 ml of a 0.10% w/v solution of *3-ketofusidic acid EPCRS* in the mobile phase with 0.2 ml of solution (1) and dilute to 20 ml with the mobile phase. For solution (3) dilute 20 µl of solution (1) to 100 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 to 15 cm × 4 to 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher 100 RP18 is suitable) (b) a mixture of 10 volumes of *methanol* , 20 volumes of a 1% w/v solution of *orthophosphoric acid* , 20 volumes of *water* and 50 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 235 nm.

Inject separately 20 µl of each solution. Continue the chromatography for at least 3.5 times the retention time of the principal peak. The test is not valid unless the *resolution factor* between the peaks corresponding to 3-ketofusidic acid and sodium fusidate in the chromatogram obtained with solution (2) is at least 2.5 and unless the principal peak in the chromatogram obtained with solution (3) has a *signal-to-noise ratio* of at least 3.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than 4 times the area of the peak corresponding to sodium fusidate in the chromatogram obtained with solution (2) (4%). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.02%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.07% w/v of *diethanolamine fusidate BPCRS* in the mobile phase. For solution (2) add 10 ml of *n-hexane* and 25 ml of the mobile phase to a quantity of the ointment containing 30 mg of Sodium Fusidate, shake vigorously for 15 minutes and allow the layers to separate. Remove all of the lower layer, dilute to 50 ml with the mobile phase and filter through glass-fibre paper (Whatman GF/C is suitable).

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 is suitable), (b) a mixture of 60 volumes of *acetonitrile*, 30 volumes of a 1% v/v solution of *glacial acetic acid* and 10 volumes of *methanol* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 235 nm.

The *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (1), should be not less than 14,000 theoretical plates per metre.

Calculate the content of $C_{31}H_{47}NaO_6$ from the declared equivalent content of $C_{31}H_{47}NaO_6$ in *diethanolamine fusidate BPCRS*.

Sodium Iodide Injection

General Notices

DEFINITION

Sodium Iodide Injection is a sterile solution of Sodium Iodide in Water for Injections. It contains not more than 0.1% w/v of Sodium Thiosulphate or other suitable reducing agent.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium iodide, NaI

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Yields the reactions characteristic of *sodium salts* , Appendix VI.

B. Yields the reactions characteristic of *iodides* , Appendix VI.

ASSAY

Dilute a volume containing 0.3 g of Sodium Iodide to 10 ml with *water*, and add 10 ml of a mixture of 0.05 ml of a 1% w/v solution of *iodine* in *ethanol* (*96%*) and 100 ml of *iodide-free starch solution* . Titrate with 0.1M *silver nitrate VS* until only a pale yellow colour remains. Each ml of 0.1M *silver nitrate VS* is equivalent to 14.99 mg of NaI.

STORAGE

Sodium Iodide Injection should be kept free from contact with metals.

Sodium Lactate Intravenous Infusion

General Notices

Sodium Lactate Injection

DEFINITION

Sodium Lactate Intravenous Infusion is a sterile solution containing 1.85% w/v of sodium lactate in Water for Injections. It may be prepared from Sodium Lactate Solution or from Lactic Acid.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium lactate, $C_3H_5NaO_3$

1.75 to 1.95% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. When warmed with *potassium permanganate*, yields acetaldehyde.

B. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

C. Carry out reaction C characteristic of *calcium salts*, Appendix VI. No white precipitate is produced.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.0, Appendix V L.

Bacterial endotoxins

The endotoxin limit concentration is 0.25 IU per ml, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.30% w/v of *lithium lactate BPCRS* in the mobile phase. For solution (2) dilute 1 volume of the infusion to 5 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 90 volumes of *water* and 10 volumes of a 2% v/v solution of *octylamine* in *acetonitrile* , the pH of the final mixture being adjusted to 7.0 with a 10% v/v solution of *orthophosphoric acid* , and (c) a detection wavelength of 210 nm.

Calculate the content of $C_3H_5NaO_3$ in the infusion using the declared equivalent content of $C_3H_5NaO_3$ in *lithium lactate BPCRS* .

LABELLING

The label states (1) that solutions containing visible solid particles should not be used; (2) that the Intravenous Infusion is one-sixth molar and contains, in one litre, approximately 167 millimoles of sodium ions and of bicarbonate ions (as lactate).

Sodium Nitroprusside Intravenous Infusion

General Notices

DEFINITION

Sodium Nitroprusside Intravenous Infusion is a sterile solution of sodium nitroprusside in Glucose Intravenous Infusion containing 50 g of anhydrous glucose per litre. It is prepared immediately before use by dissolving Sodium Nitroprusside for Injection in the requisite amount of Glucose Intravenous Infusion (50 g per litre) and diluting the resulting solution with 250 to 500 times its volume of Glucose Intravenous Infusion (50 g per litre).

The intravenous infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Sodium Nitroprusside Intravenous Infusion should be used immediately after preparation.

SODIUM NITROPRUSSIDE FOR INJECTION

DEFINITION

Sodium Nitroprusside for Injection is a sterile material obtained from a solution of Sodium Nitroprusside by freeze drying. It is supplied in a sealed container and may contain excipients.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of sodium nitroprusside

The equivalent of 90.0 to 110.0% of the stated amount of sodium nitroprusside dihydrate, $Na_2Fe(CN)_5NO,2H_2O$.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 350 to 600 nm of a 1.6% w/v solution exhibits a maximum only at 395 nm. The *absorbance* at 395 nm is about 1.1.

B. Dissolve 20 mg in 2 ml of *water* and add 0.1 ml of *sodium sulphide solution*. A deep purple colour is produced.

C. Yields the reactions characteristic of *sodium salts*, Appendix VI.

Uniformity of content

Sealed containers containing the equivalent of 50 mg or less of sodium nitroprusside dihydrate comply with the requirements stated under Parenteral Preparations, Powders for Injections. Use the individual results obtained in the Assay.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions. Solution (1) contains 0.15% w/v of *sodium nitroprusside BPCRS* in *water*. For solution (2) dissolve the contents of a sealed container in 5 ml of *water* and dilute the resulting solution with *water* to produce a solution containing the equivalent of 0.15% w/v of sodium nitroprusside dihydrate. Solution (3) contains 0.015% w/v of *sodium nitroprusside BPCRS* and 0.015% w/v of *potassium hexacyanoferrate*(*III*) in *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with particles of silica 10 µm in diameter the surface of which has been modified with chemically-bonded phenyl groups (µBondapak phenyl is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture of 9 volumes of a solution containing 0.34% w/v of *tetrabutylammonium hydrogen sulphate* and 0.284% w/v of *anhydrous disodium hydrogen orthophosphate* adjusted to pH 6.0 with *orthophosphoric acid* and 6 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Calculate the content of $Na_2Fe(CN)_5NO,2H_2O$ in the sealed container using the declared content of $Na_2Fe(CN)_5NO,2H_2O$ in *sodium nitroprusside BPCRS*. The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to ferricyanide and nitroprusside is at least 3.0.

Repeat the procedure with a further nine sealed containers and calculate the average content of $Na_2Fe(CN)_5NO,2H_2O$ from the individual results thus obtained.

LABELLING

The label states (1) the content of sodium nitroprusside in terms of the equivalent of sodium nitroprusside dihydrate contained in it; (2) that the contents of the sealed container should be dissolved in, and immediately diluted with, the stated volume of Glucose Intravenous Infusion (50 g per litre); (3) that Sodium Nitroprusside Intravenous Infusion must be used immediately after preparation; (4) that highly coloured solutions must not be used; (5) that the diluted solution must be protected from light during infusion.

Sodium Picosulfate Oral Solution

General Notices

Sodium Picosulfate Elixir

Action and use

Stimulant laxative.

DEFINITION

Sodium Picosulfate Oral Solution is a solution of Sodium Picosulfate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of Sodium Picosulfate, $C_{18}H_{13}NNa_2O_8S_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to sodium picosulfate in the chromatogram obtained with solution (2).

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* F_{254} *plate* (Merck HPTLC plates are suitable) and a mixture of 2.5 volumes of *anhydrous formic acid*, 12.5 volumes of *water*, 25 volumes of *methanol* and 60 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute, if necessary, a volume of the oral solution with sufficient *methanol* to produce a solution containing 0.1% w/v of Sodium Picosulfate. Solution (2) contains 0.1% w/v of *sodium picosulfate BPCRS* in *methanol*. Dry the plate in a current of hot air for 15 minutes and examine under *ultraviolet light (254 nm).* Spray with a 20% w/v solution of *hydrochloric acid* in *methanol* and heat at 110° for 10 minutes. Spray the hot plate with a freshly prepared solution containing 5% w/v of *iron(III) chloride* and 0.1% w/v of *potassium hexacyanoferrate(III)* and examine the wet plate in daylight. By each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to the principal spot in the chromatogram obtained with solution (2).

TESTS

4-[(Pyridin-2-yl)(4-hydroxyphenyl)methyl]phenyl sodium sulphate

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute, if necessary, a volume of the oral solution with sufficient *water* to produce a solution containing 0.005% w/v of Sodium Picosulfate. Solution (2) contains 0.0001% w/v of *sodium picosulfate BPCRS*. For solution (3) dissolve 10 mg of *sodium picosulfate BPCRS* in 2 ml of 0.1M *hydrochloric acid* , bring rapidly to the boil and heat for 1 minute. Cool in ice-water, add 2 ml of 0.1M *sodium hydroxide* and dilute to 10 ml with the mobile phase. Dilute 2 volumes to 50 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (YMC Basic is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 620 volumes of a buffer solution containing 3.0 g of *disodium hydrogen orthophosphate* and 0.5 g of *cetyltrimethylammonium bromide* in 1000 ml of *water*, adjusting the pH to 5.0 with *orthophosphoric acid* , and 380 volumes of *acetonitrile* and (c) a detection wavelength of 263 nm.

Inject 50 µl of each solution. The test is not valid unless the chromatogram obtained with solution (3) closely resembles the appropriate reference chromatogram supplied with *sodium picosulfate BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak due to impurity A (4-[(pyridin-2-yl)(4-hydroxyphenyl)methyl]phenyl sodium sulphate) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

ASSAY

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dilute, if necessary, a volume of the oral solution with sufficient *water* to produce a solution containing 0.005% w/v of Sodium Picosulfate. Solution (2) contains 0.005% w/v of *sodium picosulfate BPCRS*.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{18}H_{13}NNa_2O_8S_2$ in the oral solution from the chromatograms obtained and from the declared content of $C_{18}H_{13}NNa_2O_8S_2$ in *sodium picosulfate BPCRS*.

STORAGE

Sodium Picosulfate Oral Solution should be protected from light.

When sodium picosulphate oral solution is prescribed or demanded, Sodium Picosulfate Oral Solution shall be dispensed or supplied.

IMPURITIES

The impurity limited by this monograph is:

A. 4-[(pyridin-2-yl)(4-hydroxyphenyl)methyl]phenyl sodium sulphate

Sodium Stibogluconate Injection

General Notices

Action and use

Trivalent ammonium compound; antihelminthic.

DEFINITION

Sodium Stibogluconate Injection is a sterile solution of Sodium Stibogluconate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of pentavalent antimony, Sb(V)

9.5 to 10.5% w/v.

CHARACTERISTICS

A colourless or faintly straw-coloured solution.

IDENTIFICATION

A. It is dextrorotatory.

B. Dilute 2 ml to 10 ml with *water* and pass *hydrogen sulphide* through the solution; no immediate precipitate is produced. Continue to pass *hydrogen sulphide* through the solution for several minutes; an orange precipitate is produced.

C. The residue after evaporation to dryness and incineration yields the reactions characteristic of *antimony compounds* and the reactions characteristic of *sodium salts* , Appendix VI.

Acidity

pH, 5.0 to 5.6, Appendix V L.

ASSAY

To 0.5 ml add 30 ml of *hydrochloric acid* , mix, add 70 ml of *orthophosphoric acid* and stir carefully until completely mixed. Titrate with 0.05M *ammonium iron (II) sulphate VS* prepared with *water* containing, in each litre, 20 ml of *sulphuric acid* (*50%*), determining the end point potentiometrically using a platinum electrode and a silver-silver chloride reference electrode. Each ml of 0.05M *ammonium iron (II) sulphate VS* is equivalent to 3.044 mg of Sb(V).

STORAGE

Sodium Stibogluconate Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of pentavalent antimony per ml.

Sodium Stibogluconate Injection contains, in 6 ml, the equivalent of 600 mg of pentavalent antimony or 2 g of Sodium Stibogluconate.

Sodium Tetradecyl Sulphate Injection

General Notices

DEFINITION

Sodium Tetradecyl Sulphate Injection is a sterile solution of Sodium Tetradecyl Sulphate Concentrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium tetradecyl sulphate, $C_{14}H_{29}NaO_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) boil a volume of the injection containing 0.1 g of sodium tetradecyl sulphate under a reflux condenser with 20 ml of 2M *hydrochloric acid* for 15 minutes, allow to cool, add 20 ml of *ethanol* (*96%*) and extract the mixture with two 10 ml quantities of n-*pentane*. Wash the combined pentane extracts with 20 ml of *water* and dry over *anhydrous sodium sulphate*. Solution (2) contains 0.35% w/v of *decan-1-ol* and 0.7% w/v of *dodecan-1-ol* in n-*pentane*.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of *polyethylene glycol* (Carbowax 20M is suitable) and maintained at 120°.

The retention time of the principal peak in the chromatogram obtained with solution (1) is less than the retention time of the peak due to dodecan-1-ol and more than that of the peak due to decan-1-ol in the chromatogram obtained with solution (2). A peak due to benzyl alcohol with a retention time less than that of the peak due to decan-1-ol may also be seen.

B. Mix 0.1 ml with 0.1 ml of a 0.1% w/v solution of *methylene blue* and 2 ml of 1M *sulphuric acid*, add 2 ml of *chloroform* and shake. The chloroform layer is intensely blue.

C. Dilute 0.5 ml with 10 ml of *ethanol* (*96%*) and heat to boiling on a water bath, shaking frequently. Filter immediately and evaporate the ethanol. Dissolve the residue in 8 ml of *water*, add 3 ml of 2M *hydrochloric acid*, evaporate the solution to half its volume and cool. Filter to remove the congealed fatty alcohols and add 1 ml of 0.25M *barium chloride* to the filtrate. A white crystalline precipitate is produced.

Alkalinity

pH, 7.5 to 7.9, Appendix V L.

ASSAY

Dilute a volume containing 0.12 g of sodium tetradecyl sulphate to 100 ml with *water*. To 20 ml add 15 ml of *chloroform* and 10 ml of *dimidium bromide-sulphan blue mixed solution* and titrate with *0.004* M *benzethonium bromide VS*, shaking vigorously and allowing the layers to separate after each addition, until the pink colour of the chloroform layer is completely discharged and a greyish blue colour is produced. Each ml of *0.004 M benzethonium chloride VS* is equivalent to 1.266 mg of $C_{14}H_{29}NaO_4S$.

STORAGE

Sodium Tetradecyl Sulphate Injection should be protected from light.

LABELLING

The label states (1) that the injection is for the treatment of varicose veins only; (2) the strength as the amount of sodium tetradecyl sulphate in a suitable dose-volume.

Sodium Thiosulphate Injection

General Notices

DEFINITION

Sodium Thiosulphate Injection is a sterile solution of Sodium Thiosulphate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sodium thiosulphate, $Na_2S_2O_3,5H_2O$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. Yields the reactions characteristic of *sodium salts* , Appendix VI.

B. To 1 ml add *iodinated potassium iodide solution* , the reagent is initially decolourised, until a very faint persistent yellow colour appears. The mixture does not yield reaction A characteristic of sulphates, Appendix VI.

C. To 5 ml add 1 ml of *hydrochloric acid* . A white precipitate which quickly changes to yellow is formed, and a gas is evolved which turns *starch iodate paper* blue.

TESTS

Acidity or alkalinity

pH, 7.0 to 9.0, Appendix V L.

ASSAY

Dilute a volume containing 0.5 g of Sodium Thiosulphate to about 20 ml with *water* and titrate with 0.05M *iodine VS* using 1 ml of *starch solution,* added towards the end of the titration, as indicator. Each ml of 0.05M *iodine VS* is equivalent to 24.82 mg of $Na_2S_2O_3,5H_2O$.

Sodium Valproate Oral Solution

General Notices

DEFINITION

Sodium Valproate Oral Solution is a solution of Sodium Valproate in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of sodium valproate, $C_8H_{15}NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the oral solution containing 0.25 g of Sodium Valproate with two 25 ml quantities of *chloroform* and discard the chloroform extracts. Add 10 ml of a saturated solution of *sodium chloride* and 10 ml of 2M *hydrochloric acid* , mix and shake with 25 ml of *chloroform* . Wash the chloroform layer with 5 ml of *water*, shake with *anhydrous sodium sulphate* , filter and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of valproic acid *(RS 358)* .

B. In the Assay, the chromatogram obtained with solution (2) exhibits a peak with the same retention time as the peak due to sodium valproate in the chromatogram obtained with solution (1).

Related substances

Carry out the method for *gas chromatography* , Appendix III B, using the following solutions. Solution (1) contains 0.020% w/v of *octanoic acid* (internal standard) in *dichloromethane* . For solution (2) shake a quantity of the oral solution containing 0.50 g of Sodium Valproate with 10 ml of *water*, acidify with 2M *sulphuric acid* and shake with three 20 ml quantities of *dichloromethane* . Wash the combined dichloromethane extracts with 10 ml of *water*, shake with *anhydrous sodium sulphate* , filter and evaporate the filtrate to a volume of about 8 ml, at a temperature not exceeding 30°, using a rotary evaporator and dilute to 10 ml with dichloromethane. For solution (3) shake a quantity of the oral solution containing 0.50 g of Sodium Valproate with 10 ml of a 0.020% w/v solution of the internal standard in 0.1M *sodium hydroxide* and continue as described for solution (2), beginning at the words 'acidify with 2M *sulphuric acid* ...'.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 15% w/w of free fatty acid phase and 1% w/w of *orthophosphoric acid* and maintained at 170°.

In the chromatogram obtained with solution (3) the sum of the areas of any *secondary peaks* is not greater than the area of the peak due to the internal standard (0.4%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) dissolve 0.16 g of *sodium valproate BPCRS* in 15 ml of the mobile phase, add 1 ml of a 10% v/v solution of *orthophosphoric acid*, mix and dilute to 50 ml with the mobile phase. For solution (2) add 1 ml of a 10% v/v solution of *orthophosphoric acid* to a weighed quantity of the oral solution containing 0.16 g of Sodium Valproate and dilute to 50 ml with the mobile phase. Solution (3) contains 0.002% w/v of *propyl parahydroxybenzoate* in solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 65 volumes of *methanol* and 35 volumes of 0.05M *potassium dihydrogen orthophosphate*, the pH of the final mixture being adjusted to 5.0 with a 10% v/v solution of *orthophosphoric acid*, and (c) a detection wavelength of 210 nm.

The method is not valid unless the *resolution factor* between the two peaks in the chromatogram obtained with solution (3) is at least 2.0.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_8H_{15}NaO_2$, weight in volume, from the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS*.

Sodium Valproate Tablets

General Notices

DEFINITION

Sodium Valproate Tablets contain Sodium Valproate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sodium valproate, $C_8H_{15}NaO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Sodium Valproate with 10 ml of *water* and centrifuge. Acidify 5 ml of the supernatant liquid with 2M *sulphuric acid*, shake with 25 ml of *chloroform* and wash the chloroform layer with 5 ml of *water*. Dry by shaking with *anhydrous sodium sulphate*, filter and evaporate the chloroform. The *infrared absorption spectrum* of a thin film of the residue, Appendix II A, is concordant with the *reference spectrum* of valproic acid (*RS 358*).

B. Shake a quantity of the powdered tablets containing 0.25 g of Sodium Valproate with 3 ml of *water* and centrifuge. To 2 ml of the supernatant liquid add 0.5 ml of a 10% w/v solution of *cobalt(II) nitrate*. A purple precipitate is produced which is soluble in *dichloromethane*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 6.8* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 20 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the filtrate from the dissolution vessel diluted, if necessary, to give a solution expected to contain 0.002% w/v of Sodium Valproate. Solution (2) contains 0.002% w/v of *sodium valproate BPCRS* in *phosphate buffer pH 6.8*.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (µBondapak C18 is suitable), (b) a mixture of 45 volumes of a 0.32% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid* and 55 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 220 nm.

Inject 50 µl of each solution. Calculate the content of sodium valproate, $C_8H_{15}NaO_2$, in the medium from the chromatogram obtained and using the declared content of $C_8H_{15}NaO_2$ in *sodium valproate BPCRS* .

Related substances

Carry out the method for *gas chromatography* , Appendix III B, using the following solutions. Solution (1) contains 0.020% w/v of *octanoic acid* (internal standard) in *dichloromethane* . For solution (2) shake a quantity of the powdered tablets containing 0.50 g of Sodium Valproate with 10 ml of *water*, acidify with 2M *sulphuric acid* and shake with three 20 ml quantities of *dichloromethane* . Wash the combined dichloromethane extracts with 10 ml of *water*, shake with *anhydrous sodium sulphate* , filter, evaporate the filtrate to a volume of about 8 ml, at a temperature not exceeding 30°, using a rotary evaporator and dilute to 10 ml with dichloromethane. For solution (3) shake a quantity of the powdered tablets containing 0.50 g of Sodium Valproate with 10 ml of a 0.020% w/v solution of the internal standard in 0.1M *sodium hydroxide* and continue as described for solution (2), beginning at the words 'acidify with 2M *sulphuric acid* ...'.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 15% w/w of free fatty acid phase and 1% w/w of *orthophosphoric acid* and maintained at 170°.

In the chromatogram obtained with solution (3) the sum of the areas of any *secondary peaks* is not greater than the area of the peak due to the internal standard (0.4%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.3 g of Sodium Valproate add 70 ml of *water*, shake for 10 minutes, dilute to 100 ml with *water* and filter. To 50 ml of the filtrate add 10 ml of a 30% w/v solution of *sodium chloride* and 10 ml of 2M *hydrochloric acid* . Extract with 40 ml of a mixture of 2 volumes of *ether* and 1 volume of *petroleum spirit* (*boiling range, 40° to 60°*) and allow to separate. Shake the aqueous layer with a further 40 ml of the ether-petroleum spirit mixture. Shake each of the ether extracts with the same three 10-ml quantities of a saturated solution of *sodium chloride* , combine the ether extracts and evaporate to a volume of about 1 ml at a temperature not exceeding 30°. Add 50 ml of *ethanol* (*96%*), previously neutralised with 0.1M *sodium hydroxide VS* , using *phenolphthalein solution R1* as indicator, and titrate with 0.1M *sodium hydroxide VS* . Each ml of 0.1M *sodium hydroxide VS* is equivalent to 16.62 mg of $C_8H_{15}NaO_2$.

Somatropin Injection

General Notices

SOMATROPIN INJECTION

Action and use

Growth hormone.

DEFINITION

Somatropin Injection is a sterile solution of somatropin in a suitable liquid. It is prepared by dissolving Somatropin for Injection in the liquid stated on the label.

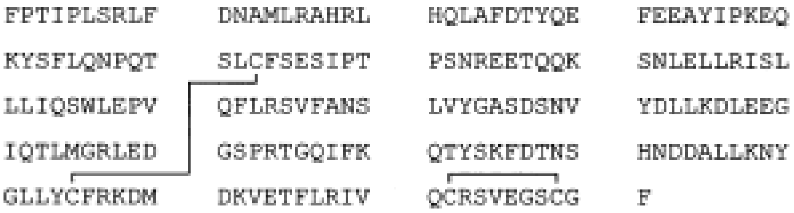
The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Somatropin Injection should be used within the period recommended by the manufacturer when prepared and stored strictly in accourdance with the manufacturer's instructions.

SOMATROPIN FOR INJECTION

(Ph Eur monograph 0952)



FPTIPLSRLF DNAMLRAHRL HQLAFDTYQE FEEAYIPKEQ
KYSFLQNPQT SLCFSESIPT PSNREETQQK SNLELLRISL
LLIQSWLEPV QFLRSVFANS LVYGASDSNV YDLLKDLEEG
IQTLMGRLED GSPRTGQIFK QTYSKFDTNS HNDDALLKNY
GLLYCFRKDM DKVETFLRIV QCRSVEGSCG F

$C_{990}H_{1528}N_{262}O_{300}S_{7}$ 22 125

Ph Eur

DEFINITION

Freeze-dried, sterile preparation of a protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary.

Content

89.0 per cent to 105.0 per cent of the amount of somatropin stated on the label.

By convention, for the purpose of labelling somatropin preparations, 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) is equivalent to 3.0 IU of biological activity.

Somatropin for injection complies with the requirements of the monograph *Parenteral preparations (0520).*

PRODUCTION

Somatropin for injection is prepared either from *Somatropin (0951*) or from *Somatropin concentrated solution (0950)*, or by a method based on recombinant DNA (rDNA) technology in which the injectable preparation is produced without the isolation of an intermediate solid or liquid bulk. In the latter case, during the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of not less than 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority. The purified preparation, to which buffers and stabilisers may be added, is filtered through a bacteria-retentive filter, aseptically distributed in sterile containers of glass type I (*3.2.1*) and freeze-dried. The containers are immediately sealed so as to exclude microbial contamination and moisture.

Somatropin for injection complies with the following additional requirements.

Host-cell-derived proteins

The limit is approved by the competent authority.

Host-cell- and vector-derived DNA

The limit is approved by the competent authority.

Where somatropin for injection is prepared from Somatropin (0951) or from Somatropin concentrated solution (0950) , compliance with the requirements for host-cell-derived proteins, host-cell- and vector-derived DNA, identification test A, identification test C and charged variants need not be reconfirmed by the manufacturer during subsequent production of somatropin for injection.

CHARACTERS

Appearance

White or almost white powder.

IDENTIFICATION

A. Capillary electrophoresis *(2.2.47)* as described in the test for charged variants with the following modifications.

Injection Test solution (b); under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

Results In the electropherogram obtained, only 1 principal peak, corresponding to somatropin, is detected: no doubling of this peak is observed.

B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

C. Peptide mapping *(2.2.55)* .

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a solution containing 2.0 mg/ml of somatropin and transfer about 1.0 ml to a tube made from a suitable material such as polypropylene. Prepare a 1 mg/ml solution of *trypsin for peptide mapping R* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* and add 30 µl to the solution of the substance to be examined. Cap the tube and place in a water-bath at 37 °C for 4 h. Remove from the water-bath and stop the reaction immediately, for example by freezing. If analysed immediately using an automatic injector, maintain at 2-8 °C.

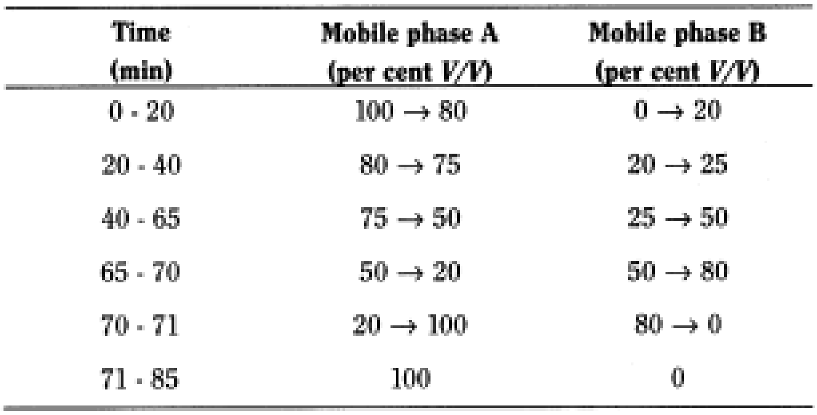
Reference solution Prepare at the same time and in the same manner as for the test solution, but using *somatropin CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION

Liquid chromatography *(2.2.29)* .

Column:
- size: l = 0.25 m, Ø = 4.6 mm;
- stationary phase: octylsilyl silica gel for chromatography R (5-10 µm) with a pore size of 30 nm;
- temperature: 30 °C.

Mobile phase:
- *mobile phase A*: dilute 1 ml of *trifluoroacetic acid R* to 1000 ml with water R;
- *mobile phase B*: to 100 ml of *water R* add 1 ml of *trifluoroacetic acid R* and dilute to 1000 ml with *acetonitrile for chromatography R* ;



Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 20	100 → 80	0 → 20
20 - 40	80 → 75	20 → 25
40 - 65	75 → 50	25 → 50
65 - 70	50 → 20	50 → 80
70 - 71	20 → 100	80 → 0
71 - 85	100	0

Flow rate 1 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µl.

System suitability The chromatograms obtained with the test solution and the reference solution are similar to the chromatogram of somatropin digest supplied with *somatropin CRS*.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h. If an automatic injector is used, maintain at 2-8 °C.*

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/ml of somatropin.

Reference solution Prepare a solution of *somatropin CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/ml of somatropin.

Resolution solution Dissolve the contents of a vial of *somatropin/desamidosomatropin resolution mixture CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a concentration of 2 mg/ml of somatropin.

Column:

— size: l = 0.25 m, Ø = 4.6 mm;

— stationary phase: a suitable singly end-capped butylsilyl silica gel, with a granulometry of 5 µm and a porosity of 30 nm; a silica saturation column is placed between the pump and the injector valve;

— temperature: 45 °C.

Mobile phase *propanol R* , *0.05 M tris-hydrochloride buffer solution pH 7.5 R* (29:71 *V/V*).

Flow rate 0.5 ml/min.

Detection Spectrophotometer at 220 nm.

Preconditioning of the column Rinse with 200-500 ml of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* in a 50 per cent *V/V* solution of *acetonitrile R* ; repeat as necessary, to improve column performance.

Injection 20 µl.

Relative retention With reference to somatropin (retention time = about 33 min; if necessary adjust the concentration of *propanol R* in the mobile phase): desamidosomatropin = about 0.85.

System suitability Resolution solution:

— resolution: minimum 1.0 between the peaks due to desamidosomatropin and somatropin;

— symmetry factor : 0.9 to 1.8 for the peak due to somatropin.

Limit:

— *total*: maximum 13.0 per cent.

Dimer and related substances of higher molecular mass

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Prepare a solution of the substance to be examined in *0.025 M phosphate buffer solution pH 7.0 R* , containing 1.0 mg/ml of somatropin.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/ml.

Resolution solution Place 1 vial of *somatropin CRS* in an oven at 50 °C for a period sufficient to generate 1-2 per cent of dimer (typically 12-24 h). Dissolve its contents in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/ml.

Column:

— size: l = 0.30 m, Ø = 7.8 mm;

— stationary phase: hydrophilic silica gel for chromatography R of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase *2-propanol R* , *0.063 M phosphate buffer solution pH 7.0 R* (3:97 *V/V*); filter and degas.

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µl.

Relative retention With reference to somatropin monomer (retention time = 12 min to 17 min): related substances of higher molecular mass = about 0.65; somatropin dimer = about 0.9.

System suitability Resolution solution:

— *peak-to-valley ratio* : minimum 2.5, where H_r = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limit:

— sum of the peaks with retention times less than that of the principal peak: maximum 6.0 per cent.

Charged variants

Capillary electrophoresis (*2.2.47*).

Test solution (a) Prepare a solution of the substance to be examined containing 1 mg/ml of somatropin.

Test solution (b) Mix equal volumes of test solution (a) and the reference solution.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *water R* and dilute with the same solvent to obtain a concentration of 1 mg/ml.

Capillary:
- material: uncoated fused silica;
- size: effective length = at least 70 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer 13.2 g/l solution of *ammonium phosphate R* adjusted to pH 6.0 with *phosphoric acid R* and filtered.

Detection Spectrophotometer at 200 nm.

Set the autosampler to store the samples at 4 °C during analysis.

Preconditioning of the capillary Rinse with *1 M sodium hydroxide* for 20 min, with *water R* for 10 min and with the CZE buffer for 20 min.

Between-run rinsing Rinse with *0.1 M sodium hydroxide* for 2 min and with the CZE buffer for 6 min.

Note Rinsing times may be adapted according to the length of the capillary and the equipment used.

Injection Test solution (a) and the reference solution; under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

The injection time and pressure may be adapted in order to meet the system suitability criteria.

Migration Apply a field strength of 217 V/cm (20 kV for capillaries of 92 cm total length) for 80 min, using CZE buffer as the electrolyte in both buffer reservoirs.

Relative migration With reference to somatropin: deamidated forms = 1.02 to 1.11.

System suitability Reference solution:

- the electropherogram obtained is similar to the electropherogram of somatropin supplied with *somatropin CRS*; 2 peaks (I_1, I_2) eluting prior to the principal peak and at least 2 peaks (I_3, I_4) eluting after the principal peak are clearly visible.

Note Peak I_2 corresponds to the cleaved form and peak I_4 corresponds to the deamidated forms, eluting as a doublet.

Limits:
- deamidated forms: maximum 6.5 per cent;
- any other impurity: for each impurity, maximum 2.0 per cent;
- total: maximum 11.5 per cent.

Water ***(2.5.32)***

Maximum 3.0 per cent, unless otherwise justified and authorised.

Bacterial endotoxins ***(2.6.14)***

Less than 5 IU/mg.

ASSAY

Size-exclusion chromatography *(2.2.30)* as described in the test for dimer and related substances of higher molecular mass.

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the declared content of $C_{990}H_{1528}N_{262}O_{300}S_7$ in *somatropin CRS*.

STORAGE

In a sterile, airtight, tamper-proof container, at a temperature of 2 °C to 8 °C.

LABELLING

The label states:

— the content of somatropin in the container, in milligrams;

— the composition and volume of the liquid to be added for reconstitution;

— the time within which the reconstituted solution shall be used and the storage conditions during this period;

— the name and quantity of any added substance;

— the storage temperature;

— that the preparation shall not be shaken during reconstitution.

Ph Eur

Sotalol Injection

General Notices

Action and use

Beta-adrenoceptor antagonist; class II and class III antiarrhythmic.

DEFINITION

Sotalol Injection is a sterile solution of Sotalol Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sotalol hydrochloride, $C_{12}H_{20}N_2O_3S,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless solution.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm, of the final solution obtained in the Assay exhibits a maximum only at 249 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 30 volumes of *methanol* and 70 volumes of *chloroform* as the mobile phase in a tank saturated with ammonia vapour. Allow the mobile phase to ascend 10 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute the injection with *water* to produce a solution containing 0.2% w/v of Sotalol Hydrochloride. Solution (2) contains 0.2% w/v of *sotalol hydrochloride BPCRS* in *water*. After removal of the plate, dry it in a current of air for at least 5 minutes and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 4.0 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection with the mobile phase to produce a solution containing 0.2% w/v of Sotalol Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of this solution to 3 volumes with the mobile phase. Solution (3) contains 0.0008% w/v of *4′-(2-isopropylaminoethyl)methanesulphonanilide hydrochloride BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 2 is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 210 volumes of *acetonitrile* and 790 volumes of *water* containing 2 g per litre of *sodium octanesulphonate* the pH of the mixture being adjusted to 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 228 nm.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 4′-(2-isopropylaminoethyl)methanesulphonanilide is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.4%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.3%) and the sum of the areas of any secondary peaks is not more than 1.65 times the area of the peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Dilute a volume of the injection containing 20 mg of Sotalol Hydrochloride to 100 ml with *water*. To 10 ml of this solution add 20 ml of 1M *sodium hydroxide* and sufficient *water* to produce 200 ml. Measure the *absorbance* of the resulting solution at the maximum at 249 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell. Calculate the content of $C_{12}H_{20}N_2O_3S,HCl$ taking 480 as the value of A(1%, 1 cm) at the maximum at 249 nm.

Sotalol Tablets

General Notices

Action and use

Beta-adrenoceptor antagonist; class II and class III antiarrhythmic.

DEFINITION

Sotalol Tablets contain Sotalol Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sotalol hydrochloride, $C_{12}H_{20}N_2O_3S,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm, of the final solution obtained in the Assay exhibits a maximum only at 249 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF$_{254}$* as the coating substance and a mixture of 30 volumes of *methanol* and 70 volumes of *chloroform* as the mobile phase in a tank saturated with ammonia vapour. Allow the mobile phase to ascend 10 cm above the line of application. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Sotalol Hydrochloride with 25 ml of *methanol* for 15 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.2% w/v of *sotalol hydrochloride BPCRS* in *water*. After removal of the plate, dry it in a current of air for at least 5 minutes and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds in position, size and intensity to that in the chromatogram obtained with solution (2).

Related substances

Comply with the test described under Sotalol Injection but preparing solution (1) in the following manner. Shake a quantity of the powdered tablets containing 50 mg of Sotalol Hydrochloride with 25 ml of the mobile phase for 15 minutes, centrifuge and use the supernatant liquid.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 50 mg of Sotalol Hydrochloride with 175 ml of *water* for 15 minutes, add sufficient *water* to produce 250 ml and centrifuge. To 10 ml of the supernatant liquid add 20 ml of 1M *sodium hydroxide* and sufficient *water* to produce 200 ml. Measure the *absorbance* of the resulting solution at the maximum at 249 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell. Calculate the content of $C_{12}H_{20}N_2O_3S,HCl$ taking 480 as the value of A(1%, 1 cm) at the maximum at 249 nm.

STORAGE

Sotalol Tablets should be protected from light.

Spironolactone Tablets

General Notices

Action and use

Aldosterone receptor antagonist; potassium-sparing diuretic.

DEFINITION

Spironolactone Tablets contain Spironolactone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of spironolactone, $C_{24}H_{32}O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.125 g of Spironolactone with two 10 ml quantities of *chloroform* , filter, evaporate the combined filtrates to dryness and dissolve the residue in 2.5 ml of *chloroform* . The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of spironolactone (*RS 321*).

B. Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 1 volume of *water*, 24 volumes of *cyclohexane* and 75 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 20 mg of Spironolactone with 10 ml of *dichloromethane* . Solution (2) contains 0.2% w/v of *spironolactone EPCRS* in *dichloromethane* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2. Use as the medium 1000 ml of 0.1M *hydrochloric acid* containing 0.1% w/v *sodium dodecyl sulphate* and rotate the paddle at 75 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and measure the *absorbance* of the filtrate, suitably diluted if necessary, at the maximum at 242 nm, Appendix II B. Calculate the total content of $C_{24}H_{32}O_4S$ in the medium taking 445 as the value of A(1%, 1 cm) at the maximum at 242 nm.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 62.5 mg of Spironolactone with 25 ml of *chloroform*, place in an ultrasonic bath for 5 minutes, shake for 10 to 15 minutes, centrifuge and filter the supernatant liquid through glass fibre paper (Whatman GF/C is suitable). Repeat the procedure on the residue with a further 25 ml of *chloroform*. Combine the chloroform extracts and evaporate to dryness using a rotary evaporator. Add 2.5 ml of *tetrahydrofuran* and 22.5 ml of the mobile phase to the residue. For solution (2) dilute 1 ml of solution (1) to 100 ml with the mobile phase. For solution (3) dissolve 25 mg of *canrenone EPCRS* in 1 ml of *tetrahydrofuran* and dilute to 10 ml with the mobile phase. For solution (4) dilute 1 ml of solution (3) to 100 ml with the mobile phase. For solution (5) mix 1 ml each of solutions (1) and (3) and dilute to 100 ml with the mobile phase. For solution (6) dilute 0.5 ml of solution (2) to 10 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Spherisorb C8 is suitable), (b) a mixture of 8 volumes of *acetonitrile*, 18 volumes of *tetrahydrofuran* and 74 volumes of *water* as the mobile phase with a flow rate of 1.8 ml per minute and (c) detection wavelengths of 254 nm and 283 nm as directed below.

Inject separately 20 µl of each of solutions (1), (2), (5) and (6) and record the chromatograms for twice the retention time of spironolactone using a detection wavelength of 254 nm. In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks*, other than any peak corresponding to canrenone, is not greater than the area of the peak due to spironolactone in the chromatogram obtained with solution (2) (1%). Disregard any peak the area of which is less than that of the principal peak in the chromatogram obtained with solution (6).

Inject 20 µl of each of solutions (1) and (4) and record the chromatograms using a detection wavelength of 283 nm. The test is not valid unless (a) the chromatogram obtained with solution (5) shows two peaks due to canrenone and spironolactone with a *resolution factor* greater than 1.4 and (b) the principal peak in the chromatogram obtained with solution (6) has a *signal-to-noise ratio* of at least 6.

In the chromatogram obtained with solution (1) the area of any peak corresponding to canrenone is not greater than that of the peak due to canrenone in the chromatogram obtained with solution (4) (1%). Calculate the percentage content of canrenone found when recording at 283 nm and the percentage content of the other related substances found when recording at 254 nm. The sum is not greater than 1.0%.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 25 mg of Spironolactone add 100 ml of *methanol* and heat just to boiling, with swirling. Cool, add sufficient *methanol* to produce 250 ml, dilute 10 ml to 100 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at 238 nm, Appendix II B. Calculate the content of $C_{24}H_{32}O_4S$ taking 470 as the value of A(1%, 1 cm) at the maximum at 238 nm.

STORAGE

Spironolactone Tablets should be protected from light.

Squill Oxymel

General Notices

DEFINITION

Squill, bruised or Indian Squill, bruised	50 g
Acetic Acid (33 per cent)	90 ml or a sufficient quantity
Purified Water, freshly boiled and cooled	250 ml
Purified Honey	A sufficient quantity

Extemporaneous preparation

The following directions apply.

Macerate the Squill or the Indian Squill with the Acetic Acid (33 per cent) and the Purified Water for 7 days with occasional agitation, strain, press out the liquid, heat the mixed liquids to boiling, filter whilst hot, cool, determine the content of acetic acid, add sufficient Acetic Acid (33 per cent) to the remainder of the filtrate to produce a solution containing about 8.5% w/v of acetic acid and mix. To every three volumes of the resulting solution add seven volumes of Purified Honey and mix thoroughly.

Content of acetic acid, $C_2H_4O_2$

2.2 to 2.7% w/v.

TESTS

Optical rotation

+0.6° to -3.0°, Appendix V F, when measured in a 25% w/v solution in *water* decolourised, if necessary, with *activated charcoal* .

Weight per ml

1.260 to 1.270 g, Appendix V G.

ASSAY

Dilute 20 ml with 20 ml of *carbon dioxide-free water* and titrate with 1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 1M *sodium hydroxide VS* is equivalent to 60.05 mg of $C_2H_4O_2$.

Standardised Senna Granules

General Notices

DEFINITION

Standardised Senna Granules contain Alexandrian Senna Fruit in powder form with suitable excipients. The granules contain 0.55% w/w of sennosides, calculated as sennoside B.

The granules comply with the requirements stated under Granules and with the following requirements.

Content of sennosides calculated as sennoside B

0.467 to 0.633% w/w.

IDENTIFICATION

A. To 25 mg, in *No. 180 powder*, add 50 ml of *water* and 2 ml of *hydrochloric acid*, heat in a water bath for 15 minutes, allow to cool and shake with 40 ml of *ether*. Dry the ether layer over *anhydrous sodium sulphate*, filter, evaporate 5 ml of the filtrate to dryness, cool and add 5 ml of 6M *ammonia* to the residue; a yellow or orange colour is produced. Heat on a water bath for 2 minutes; a reddish violet colour is produced.

B. In the Assay, the chromatogram obtained with solution (1) exhibits two peaks corresponding to the peaks due to sennoside A and sennoside B in the chromatogram obtained with solution (2).

Loss on drying

When dried at 105° for 5 hours, lose not more than 2.0% of their weight. Use 5 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 2 g of the granules with 50 ml of a 0.3% v/v solution of *acetic acid* adjusted to pH 5.9 with 1M *sodium hydroxide* for 30 minutes, centrifuge, filter through a glass fibre paper (Whatman GF/C is suitable) and use the filtrate. Prepare solution (2) in the same manner as solution (1) but using a quantity of *Alexandrian senna fruit powder BPCRS* 1 mg of sennoside B.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable), (b) a mixture of 83 volumes of a 1% v/v solution of *glacial acetic acid* and 17 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 350 nm.

Calculate the total content of sennosides A and B as sennoside B from the sum of the areas of the peaks corresponding to sennoside A and sennoside B and from the declared content of sennosides in *Alexandrian senna fruit powder BPCRS*.

STORAGE

Standardised Senna Granules should be kept in an airtight container.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of sennoside B.

Sterculia Granules

General Notices

DEFINITION

Sterculia Granules are Sterculia in granule form.

The granules comply with the requirements stated under Granules and with the following requirements.

CHARACTERISTICS

White or buff with a distinct odour of acetic acid; transparent, irregular shaped granules of about 1 to 4 mm which swell when treated with water.

IDENTIFICATION

A. Irregular or vermiform pieces, about 5 to 20 mm thick; greyish white with a brown or pink tinge; surface striated.

B. When powdered and mounted in *ethanol* (*96%*) it appears as small, transparent, angular particles of various sizes and shapes; the particles lose their sharp edges when *water* is added and each gradually swells until a large, indefinite, almost structureless mass results; when mounted in *ruthenium red solution* the particles are stained red; no blue coloured particles (starch) are visible when mounted in *iodine solution R1* .

C. Add 1 g to 80 ml of *water* and allow to stand for 24 hours, shaking occasionally. A tacky and viscous granular mucilage is produced. Retain the mucilage for use in test D.

D. Boil 4 ml of the mucilage obtained in test C with 0.5 ml of *hydrochloric acid* , add 1 ml of 5M *sodium hydroxide* , filter, add 3 ml of *cupri-tartaric solution R1* to the filtrate and heat. A red precipitate is produced.

TESTS

Acid-insoluble ash

Not more than 1.0%, Appendix XI K.

Ash

Not more than 7.0%, Appendix XI J.

Volatile acid

Not less than 13.0%, calculated as acetic acid, $C_2H_4O_2$, when determined by the following method. To 1 g contained in a 700 ml Kjeldahl flask add 100 ml of *water* and 5 ml of *orthophosphoric acid* , allow to stand for several hours, or until the granules are completely swollen, and boil gently under a reflux condenser for 2 hours. Steam distil until 800 ml of the distillate is obtained and the acid residue measures about 20 ml and titrate the distillate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of alkali required to neutralise the volatile acid. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 6.005 mg of volatile acid, calculated as $C_2H_4O_2$.

Loss on drying

The powdered granules, when dried to constant weight at 105°, lose not more than 20.0% of their weight. Use 1 g.

Microbial contamination

1.0 g is free from *Escherichia coli*, Appendix XVI B1.

STORAGE

Sterculia Granules should be stored in a dry place.

Sterile Silver Nitrate Solution

General Notices

DEFINITION

Sterile Silver Nitrate Solution is a sterile solution of Silver Nitrate in Purified Water.

When intended for ophthalmic use, the solution complies with the requirements stated under Eye Preparations and when intended for use as an irrigation solution, the solution complies with the requirements stated under Preparations for Irrigation. The solution also complies with the following requirements.

Content of silver nitrate, $AgNO_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Yields the reactions characteristic of *silver salts* and *of nitrates*, Appendix VI.

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Clarity and colour of solution

The solution is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

ASSAY

Dilute a volume containing 50 mg of Silver Nitrate with 20 ml of *water* and add 2 ml of *dilute nitric acid* and 2 ml of *ammonium iron(III) sulphate solution R2*. Titrate with 0.02M *ammonium thiocyanate VS* until a reddish-yellow colour is obtained. Each ml of 0.02M *ammonium thiocyanate VS* is equivalent to 3.397 mg of $AgNO_3$.

STORAGE

Sterile Silver Nitrate Solution should be protected from light.

LABELLING

The label states whether the solution is intended for ophthalmic use or for use as an irrigation solution.

Streptokinase Injection

General Notices

Action and use

Tissue-type plasminogen activator; fibrinolytic.

DEFINITION

Streptokinase Injection is a sterile solution of Streptokinase in Water for Injections. It is prepared by dissolving Streptokinase for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Streptokinase Injection should be used immediately after preparation.

STREPTOKINASE FOR INJECTION

DEFINITION

Streptokinase for Injection is a sterile material consisting of Streptokinase with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

CHARACTERISTICS

A white powder or a white friable solid; hygroscopic.

Freely soluble in *water*.

IDENTIFICATION

A. Place 0.5 ml of citrated human, canine or rabbit plasma in a haemolysis tube maintained in a water bath at 37°. Add 0.1 ml of a solution of the preparation being examined containing 10,000 IU of streptokinase activity per ml in *citro-phosphate buffer pH 7.2* and 0.1 ml of a solution of *thrombin* containing 20 IU per ml in *citro-phosphate buffer pH 7.2* and shake immediately. A clot forms and lyses within 30 minutes. Repeat the procedure using citrated bovine plasma. Lysis does not occur within 60 minutes.

B. Comply with test B for Identification described under Streptokinase.

TESTS

Acidity or alkalinity

pH of a solution in freshly boiled and cooled *water* containing 5,000 IU per ml, 6.8 to 7.5, Appendix V L.

Streptodornase

Comply with the test described under Streptokinase.

Streptolysin

Comply with the test described under Streptokinase.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 100,000 IU of Streptokinase per ml (solution A). The endotoxin limit concentration of solution A is 23.33 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the Assay described under Streptokinase. The estimated potency is not less than 90% and not more than 111% of the stated potency. The fiducial limits of error are not less than 80% and not more than 125% of the stated potency.

STORAGE

The sealed container should be protected from light and stored at a temperature of 2° to 8°. Under these conditions the contents may be expected to retain their potency for 2 years.

LABELLING

The label of the sealed container states (1) the total number of IU (Units) contained in it; (2) that the preparation is antigenic.

Streptomycin Injection

General Notices

Action and use

Aminoglycoside antibacterial; antituberculosis drug.

DEFINITION

Streptomycin Injection is a sterile solution of Streptomycin Sulphate in Water for Injections. It is either supplied as a ready-to-use solution or it is prepared by dissolving Streptomycin Sulphate for Injection in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

CHARACTERISTICS

A colourless to yellow solution.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing the equivalent of 10 mg of streptomycin per ml (solution A). The endotoxin limit concentration of solution A is 2.5 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

STORAGE

Streptomycin Injection prepared by dissolving the contents of a sealed container in Water for Injections should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

LABELLING

The strength is stated in terms of the equivalent amount of streptomycin in a suitable dose-volume.

When supplied as a ready-to-use solution, the injection also complies with the following requirements.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a 7% w/v solution of *potassium dihydrogen orthophosphate* as the mobile phase and a plate prepared in the following manner. Mix 0.3 g of *carbomer* (Carbopol 934 is suitable) with

240 ml of *water*, allow to stand with moderate shaking for 1 hour, adjust to pH 7 by the gradual addition, with constant shaking, of 2M *sodium hydroxide* and add 30 g of *silica gel H*. Spread a uniform layer of the resulting suspension 0.75 mm thick. Heat the plate at 110° for 1 hour, allow to cool and use immediately. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a suitable volume of the injection with sufficient *water* to produce a solution containing the equivalent of 0.08% w/v of streptomycin. Solution (2) contains 0.1% w/v of *streptomycin sulphate EPCRS* in *water*. Solution (3) contains 0.1% w/v of *streptomycin sulphate EPCRS*, 0.1% w/v of *neomycin sulphate EPCRS* and 0.1% w/v of *kanamycin monosulphate EPCRS* in *water*. Allow the solvent front to ascend 12 cm above the line of application. After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol* (*96%*) and a 46% w/v solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

B. Mix 0.05 ml with 4 ml of *water*, add 1 ml of 1M *sodium hydroxide* and heat in a water bath for 4 minutes. Add a slight excess of *hydrochloric acid* and 0.1 ml of *iron* (*III*) *chloride solution R1*. A violet colour is produced.

TESTS

Acidity

pH, 5.0 to 6.5, Appendix V L.

Streptomycin B

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 25 volumes of *glacial acetic acid*, 25 volumes of *methanol* and 50 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 13 to 15 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a volume of the injection containing the equivalent of 0.16 g of streptomycin in sufficient of a freshly prepared mixture of 3 volumes of *sulphuric acid* and 97 volumes of *methanol* to produce 5 ml, heat under a reflux condenser for 1 hour, cool, rinse the condenser with *methanol* and add sufficient *methanol* to produce 20 ml (1% w/v solution). For solution (2) dissolve 36 mg of D-*mannose* in sufficient of a freshly prepared mixture of 3 volumes of *sulphuric acid* and 97 volumes of *methanol* to produce 5 ml, heat under a reflux condenser for 1 hour, cool, rinse the condenser with *methanol* and add sufficient *methanol* to produce 50 ml. Dilute 5 ml of the resulting solution to 50 ml with *methanol*; this solution contains the equivalent of 0.03% w/v of streptomycin B (1 mg of D-mannose is equivalent to 4.13 mg of streptomycin B). After removal of the plate, allow it to dry in air and spray with a freshly prepared mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol* (*96%*) and a 20% v/v solution of *sulphuric acid* and heat at 110° for 5 minutes. Any spot corresponding to streptomycin B in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (3%).

ASSAY

Dilute a quantity containing the equivalent of 0.33 g of streptomycin to 100 ml with *water for injections* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of streptomycin in the injection, taking each 1000 IU found to be equivalent to 1 mg of streptomycin. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

STORAGE

Streptomycin Injection should be protected from light and stored at a temperature of 2° to 8°.

STREPTOMYCIN SULPHATE FOR INJECTION

DEFINITION

Streptomycin Sulphate for Injection is a sterile material consisting of Streptomycin Sulphate with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. Comply with test A for Identification described in the requirements for the ready-to-use solution but using as solution (1) a solution prepared by dissolving a quantity of the contents of the sealed container in sufficient *water* to produce a solution containing the equivalent of 0.08% w/v of streptomycin.

B. Dissolve 5 to 10 mg in 4 ml of *water* and add 1 ml of 1M *sodium hydroxide*. Heat in a water bath for 4 minutes. Add a slight excess of 2M *hydrochloric acid* and 0.1 ml of *iron(III) chloride solution R1*. A violet colour develops.

C. Yield the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 25% w/v of streptomycin, 4.5 to 7.0, Appendix V L.

Streptomycin B

Comply with the test described in the requirements for the ready-to-use solution but using as solution (1) a solution prepared in the following manner. Dissolve a quantity containing the equivalent of 0.16 g of streptomycin in sufficient of a freshly prepared mixture of 3 volumes of *sulphuric acid* and 97 volumes of *methanol* to produce 5 ml, heat under a reflux condenser for 1 hour, cool, rinse the condenser with *methanol* and add sufficient *methanol* to produce 20 ml (1% w/v solution).

Loss on drying

When dried over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 24 hours, lose not more than 7.0% of their weight. Use 1 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Dissolve a quantity of the mixed contents of the 10 containers containing the equivalent of 0.33 g of streptomycin in sufficient *water for injections* to produce 100 ml and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. Calculate the content of streptomycin in the injection, taking each 1000 IU found to be equivalent to 1 mg of streptomycin. For a container of average content weight, the upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated content.

LABELLING

The label on the sealed container states the quantity of streptomycin sulphate contained in it in terms of the equivalent amount of streptomycin.

Strong Sodium Hypochlorite Solution

General Notices

DEFINITION

Strong Sodium Hypochlorite Solution is an aqueous solution of sodium hypochlorite containing not less than 8% of available chlorine. It may contain suitable stabilising agents.

Content of available chlorine, Cl

Not less than 8.0% w/w.

Total alkalinity

Not more than 1.8% w/w, calculated as NaOH, when determined by the following method. To 25 ml add 50 ml of *water* and 35 ml of *hydrogen peroxide solution* (*20 vol*) and titrate with 0.5M *hydrochloric acid VS* using *methyl orange solution* as indicator. Each ml of 0.5M *hydrochloric acid VS* is equivalent to 20 mg of NaOH. Determine the *weight per ml* of the solution, Appendix V G, and calculate the total alkalinity as a percentage weight in weight.

ASSAY

Dilute 10 ml to 250 ml with *water*. Add 10 ml of the resulting solution to a mixture of 50 ml of *water*, 1 g of *potassium iodide* and 12.5 ml of 2M *acetic acid* . Titrate with 0.1M *sodium thiosulphate VS* using *starch solution* as indicator. Each ml of 0.1M *sodium thiosulphate VS* is equivalent to 3.545 mg of Cl. Determine the *weight per ml* of the solution, Appendix V G, and calculate the percentage w/w of available chlorine.

STORAGE

Strong Sodium Hypochlorite Solution should be kept in a well-filled container fitted with a glass stopper or a suitable plastic cap and protected from light. It should be stored at a temperature not exceeding 20° and away from acids.

LABELLING

The label states (1) that the solution must be diluted before use; (2) the date after which it is not intended to be used; (3) the conditions under which it should be stored.

Sulfadiazine Injection

General Notices

Action and use

Sulfonamide antibacterial.

DEFINITION

Sulfadiazine Injection is a sterile solution of the sodium derivative of Sulfadiazine in Water for Injections free from dissolved air.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sulfadiazine, $C_{10}H_{10}N_4O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Acidify a volume of the injection containing the equivalent of 0.5 g of Sulfadiazine with 6M *acetic acid* and filter. The *infrared absorption spectrum*, Appendix II A, of the residue, after washing with *water* and drying at 105°, is concordant with the *reference spectrum* of sulfadiazine (*RS 325*).

TESTS

Alkalinity

pH, 10.0 to 11.0, Appendix V L.

Colour of solution

An injection containing the equivalent of 1 g of Sulfadiazine in 4 ml of *water* is not more intensely coloured than *reference solution* BY_5, Appendix IV B, Method II.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a volume of the injection containing the equivalent of 0.5 g of Sulfadiazine with sufficient of a mixture of 1 volume of 13.5M *ammonia* and 9 volumes of *methanol* to produce 25 ml.

(2) Dilute 1 volume of solution (1) to 200 volumes with a mixture of 1 volume of 13.5M *ammonia* and 24 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Using as the coating *silica gel GF* $_{254}$.

(b) The mobile phase is a mixture of 3 volumes of 6M *ammonia*, 5 volumes of *water*, 40 volumes of *nitromethane* and 50 volumes of *1,4-dioxan.*

(c) Develop to 15 cm.

(d) Application volume of 5 µl for each solution.

(e) Remove the plate, dry it at 100° to 105° and examine under *ultraviolet light* (*254 nm*).

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

To a volume containing the equivalent of 0.5 g of Sulfadiazine add a mixture of 75 ml of *water* and 10 ml of *hydrochloric acid*, add 3 g of *potassium bromide*, cool in ice and titrate slowly with 0.1M *sodium nitrite VS*, stirring constantly and determining the end point electrometrically. Each ml of 0.1M *sodium nitrite VS* is equivalent to 25.03 mg of $C_{10}H_{10}N_4O_2S$.

STORAGE

Sulfadiazine Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of Sulfadiazine in a suitable dose-volume.

Sulfasalazine Tablets

General Notices

Action and use

Sulfonamide aminosalicylate; treatment of ulcerative colitis.

DEFINITION

Sulfasalazine Tablets contain Sulfasalazine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulfasalazine, $C_{18}H_{14}N_4O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 16 mg of Sulfasalazine with 100 ml of 0.1M *sodium hydroxide* until dispersed and filter. Dilute 5 ml of the filtrate to 100 ml with a mixture of 7 volumes of *water* and 2 volumes of 0.1M *acetic acid* . Record the *light absorbance* in the range 220 nm to 400 nm, Appendix II B. The absorbances at all wavelengths in the range are similar to those of a solution prepared from *sulfasalazine EPCRS* in the same manner.

B. In the test for Related substances, the principal peak in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

TESTS

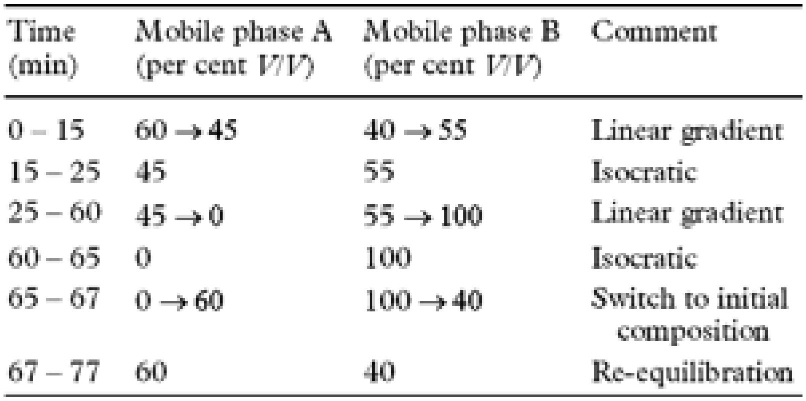
Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Sulfasalazine with 100 ml of 0.1M *ammonia* for 30 minutes, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with 0.1M *ammonia* . Solution (3) contains 0.001% w/v of *sulfasalazine derivative for resolution EPCRS* in solution (2). Solution (4) contains 0.001% w/v of *sulfasalazine EPCRS* in 0.1M *ammonia* .

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute the gradient elution programme described below and (c) a detection wavelength of 320 nm.

Mobile phase A A solution containing 0.013% w/v of *sodium dihydrogen orthophosphate* and 0.25% w/v of *sodium acetate* adjusted to pH 4.8 with *glacial acetic acid* .

Mobile phase B Mix 1 volume of mobile phase A with 4 volumes of *methanol* .



Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)	Comment
0 – 15	60 → 45	40 → 55	Linear gradient
15 – 25	45	55	Isocratic
25 – 60	45 → 0	55 → 100	Linear gradient
60 – 65	0	100	Isocratic
65 – 67	0 → 60	100 → 40	Switch to initial composition
67 – 77	60	40	Re-equilibration

Inject 20 µl of solution (2) and adjust the sensitivity of the detector so that the height of the peak in the chromatogram is at least 50% of the full scale of the recorder. Inject 20 µl of solution (3). The test is not valid unless the *resolution factor* between the peaks corresponding to sulfasalazine and sulfasalazine derivative for resolution is at least 3.0.

Inject 20 µl of each of solutions (1) and (2). When the chromatograms are recorded in the prescribed conditions, the approximate retention times relative to sulfasalazine are: impurity A, 2.00; impurity B, 1.85; impurity C, 0.80; impurity D, 1.90; impurity E, 1.63; impurity F, 0.85; impurity G, 1.39; impurity H, 0.16; impurity I, 0.28.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any such peaks is not greater than 4 times the area of the peak in the chromatogram obtained with solution (2) (4%). Disregard any peak with a retention time shorter than 6 minutes (corresponding to salicylic acid and sulfapyridine) and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Salicylic acid and sulfapyridine

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Sulfasalazine with 100 ml of 0.1M *ammonia* for 30 minutes, centrifuge and use the supernatant liquid. For solution (2) prepare a solution containing 0.05% w/v of *salicylic acid* and 0.05% w/v of s*ulfapyridine EPCRS* in 0.1M *ammonia* and dilute 1 volume of this solution to 50 volumes with 0.1M *ammonia* .

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 70 volumes of mobile phase A described in the test for Related substances and 30 volumes of mobile phase B described in the test for Related substances and (c) a detection wavelength of 300 nm.

Inject 20 µl of solution (2) and adjust the sensitivity so that the height of the principal peaks in the chromatogram obtained is at least 50% of the full scale of the recorder. When the chromatogram is recorded in the prescribed conditions the retention time of salicylic acid is about 6 minutes and that of sulfapyridine, about 7 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to salicylic acid and sulfapyridine is at least 2.

Inject 20 µl of each solution and allow the chromatography to proceed for 10 minutes. In the chromatogram obtained with solution (1) the areas of any peaks corresponding to salicylic acid and sulfapyridine are not greater than 0.5 times the areas of the corresponding peaks in the chromatogram obtained with solution (2) (0.5% of each).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of a phosphate buffer prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 1.66 g of *sodium hydroxide* in sufficient *water* to produce 1000 ml and adjusting the pH to 7.5 with 5M *sodium hydroxide* if necessary, and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute a suitable volume of the filtrate with the dissolution medium to produce a solution containing 0.005% w/v of Sulfasalazine. Measure the *absorbance* of this solution, Appendix II B, at 406 nm using dissolution medium in the reference cell. Calculate the total content of $C_{18}H_{14}N_4O_5S$ in the medium from the *absorbance* of a 0.005% w/v solution of *sulfasalazine EPCRS* in dissolution medium and using the declared content of $C_{18}H_{14}N_4O_5S$ in *sulfasalazine EPCRS*.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.15 g of Sulfasalazine with about 50 ml of 0.1M *sodium hydroxide*, add sufficient of the same solvent to produce 100 ml, filter and discard the first 20 ml of filtrate. Dilute 5 ml of the filtrate with 750 ml of *water*, add 20 ml of 0.1M *acetic acid*, mix and add sufficient *water* to produce 1000 ml. Measure the *absorbance* of this solution at the maximum at 359 nm, Appendix II B. Calculate the content of $C_{18}H_{14}N_4O_5S$ in the tablets from the *absorbance* of a standard solution prepared in the same manner using 0.15 g of *sulfasalazine EPCRS* and using the declared content of $C_{18}H_{14}N_4O_5S$ in *sulfasalazine EPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Sulfasalazine.

Sulfinpyrazone Tablets

General Notices

Action and use

Uricosuric.

DEFINITION

Sulfinpyrazone Tablets contain Sulfinpyrazone. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulfinpyrazone, $C_{23}H_{20}N_2O_3S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 0.2 g of Sulfinpyrazone with *chloroform*, filter and evaporate the filtrate to dryness on a water bath. The residue complies with the following tests.

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.01M *sodium hydroxide* exhibits a maximum only at 260 nm.

B. Dissolve 10 mg in 3 ml of *acetone* and add 0.05 ml of *iron(III) chloride solution R2* and 3 ml of *water*. A red colour is produced.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using as the coating substance a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *glacial acetic acid* and 80 volumes of *chloroform* as the mobile phase. Place the plate in a tank containing a beaker of *glacial acetic acid* and allow it to stand for 10 minutes. Heat the plate at 60° for 40 minutes, cool and pass a current of nitrogen over the plate for 10 minutes. Apply separately to the plate, under an atmosphere of nitrogen, 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Sulfinpyrazone with 10 ml of *chloroform*, filter and use the filtrate. For solution (2) dilute 2 volumes of solution (1) to 100 volumes with *acetone* and dilute 1 volume of the resulting solution to 10 volumes with *acetone*. For solution (3) dissolve 10 mg of *1,2-diphenyl-4-(2-phenylsulphonylethyl)pyrazolidine-3,5-dione EPCRS* (impurity A) in 10 ml of *acetone*. For solution (4) dilute 5 volumes of solution (3) to 10 volumes with *acetone*. For solution (5) dissolve 10 mg of *1,2-diphenyl-4-(2-phenylthioethyl)pyrazolidine-3,5 dione EPCRS* (impurity B) in 10 ml of *acetone*. For solution (6) dilute 5 volumes of solution (5) to 10 volumes with *acetone* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). In the chromatogram obtained with solution (1) any spots corresponding to impurity A and impurity B are not more intense than the spots in the chromatograms obtained with solutions (3) and (5) respectively (5% of each), not more than one such spot is more intense than the corresponding spot in the chromatogram obtained with solution (4) or (6) (2.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

ASSAY

Weigh and powder 20 tablets. Stir a quantity of the powder containing 0.5 g of Sulfinpyrazone with 10 ml of *petroleum spirit* (*boiling range, 40° to 60°*) for 10 minutes in a glass-stoppered flask. Decant through a sintered-glass filter, wash with two 7-ml quantities of *petroleum spirit* (*boiling range, 40° to 60°*) and discard the petroleum spirit. Warm to 80° to remove the solvent and extract the residue with six 20-ml quantities of hot *acetone* previously neutralised to *bromothymol blue solution R3* , decanting each solution and filtering through the sintered-glass filter. Titrate the combined extracts with 0.1M *sodium hydroxide VS* using *bromothymol blue solution R3* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 40.45 mg of $C_{23}H_{20}N_2O_3S$.

Sulindac Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Sulindac Tablets contain Sulindac.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulindac, $C_{20}H_{17}FO_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Sulindac with 10 ml of a mixture of equal volumes of *chloroform* and *methanol* for 10 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulindac (*RS 323*).

B. The *light absorption* , Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits two maxima, at 284 nm and 327 nm, and a less well-defined maximum at 258 nm.

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of *phosphate buffer pH 7.2* and rotate the paddle at 50 revolutions per minute. Withdraw a sample of 10 ml of the medium, filter and dilute with *phosphate buffer pH 7.2* to give a solution expected to contain 0.001% w/v of Sulindac. Measure the *absorbance* of this solution, Appendix II B, at 327 nm using *phosphate buffer pH 7.2* in the reference cell. Calculate the total content of $C_{20}H_{17}FO_3S$ in the medium taking 373 as the value of A(1%, 1 cm) at the maximum at 327 nm.

Related substances

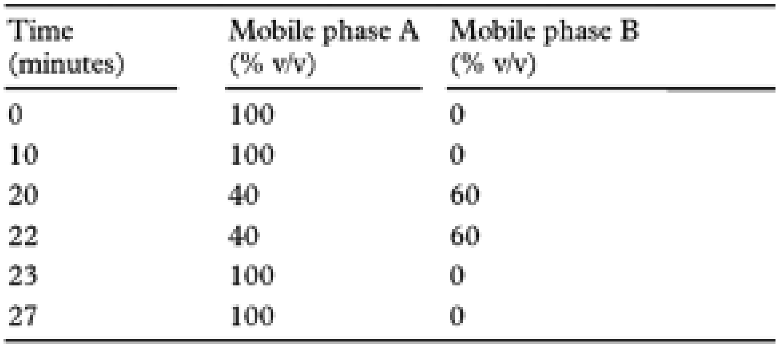
Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. Mix 160 volumes of *tetrahydrofuran* and 840 volumes of *methanol* (solution A). Mix equal volumes of solution A and a solution containing 0.358% w/v of *disodium hydrogen orthophosphate* and 0.34% w/v of *tetrabutylammonium hydrogen sulphate* (the mixture is solution B). For solution (1) shake a quantity of whole tablets containing 1 g of Sulindac with

175 ml of solution A mechanically for 15 minutes and then with the aid of ultrasound until the tablets have fully disintegrated (at least 15 minutes). Allow the resulting suspension to cool, add sufficient of solution B to produce 200 ml and mix. Dilute 10 ml of the resulting solution to 100 ml with solution B and filter through a 0.45-µm filter (Millipore Millex is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with solution B and further dilute 1 volume to 2 volumes with the same mixture of solvents. Solution (3) contains 0.050% w/v of *sulindac EPCRS* (which has an assigned content of the *E*-isomer) in solution B. For solution (4) add 0.05 ml of a 3% w/v solution of *potassium permanganate* to 5 ml of solution (1) (generation of sulphoxide impurity). For solution (5) add a few granules of *zinc*, in *fine powder* to 5 ml of solution (1), add 0.2 ml of *hydrochloric acid*, mix and filter through a 0.45-µm filter (generation of desoxy impurity). For solution (6) add 0.3 ml of *methanol* to 5 ml of solution (1), add 0.2 ml of *sulphuric acid* and mix (generation of methyl ester). For solution (7) add 0.3 ml of *absolute ethanol* to 5 ml of solution (1), add 0.2 ml of *sulphuric acid* and mix (generation of ethyl ester).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Beckman Ultrasphere C8 is suitable), (b) as mobile phases A and B with a flow rate of 2 ml per minute the solutions described below and (c) a detection wavelength of 330 nm. Carry out a linear gradient elution using the following conditions.

Mobile phase A Mix 160 volumes of *tetrahydrofuran* and 840 volumes of *methanol* (solution C). Prepare a solution containing 0.358% w/v of *disodium hydrogen orthophosphate* and 0.34% w/v of *tetrabutylammonium hydrogen sulphate* and mix 380 volumes of solution C with 620 volumes of this solution, adjust the pH of the mixture to 6.0 with *orthophosphoric acid* or a 50% w/v solution of *sodium hydroxide*, mix with the aid of ultrasound for 10 minutes and filter through a 0.45-µm filter.

Mobile phase B *Acetonitrile*.



Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	100	0
10	100	0
20	40	60
22	40	60
23	100	0
27	100	0

Inject 20 µl of solution (3) and continue the chromatography for twice the retention time of the principal peak. The chromatogram obtained shows a principal peak corresponding to sulindac and a peak corresponding to the *E*-isomer with a retention time relative to sulindac of about 1.25.

From the chromatograms obtained with solution (1) and solution (3) calculate the content of *E*-isomer in the preparation being examined using the declared content of the *E*-isomer in *sulindac EPCRS*; the *E*-isomer content is not more than 0.5%. In the chromatogram obtained with solution (1), the area of any peaks corresponding to the desoxy impurity, the sulphoxide impurity, the ethyl ester impurity or the methyl ester impurity (identified by reference to the chromatograms obtained with solutions (4) to (7)) are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 0.1 g of Sulindac with 80 ml of 0.1M *methanolic hydrochloric acid* for 10 minutes and dilute to 100 ml with the same solvent. Filter and dilute 3 ml of the filtrate to 200 ml with the same solvent. Measure the *absorbance* of the resulting solution at the maximum at 327 nm, Appendix II B, and calculate the content of $C_{20}H_{17}FO_3S$ taking 373 as the value of A(1%, 1 cm) at the maximum at 327 nm.

STORAGE

Sulindac Tablets should be protected from light.

Sulpiride Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Sulpiride Tablets contain Sulpiride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sulpiride, $C_{15}H_{23}N_3O_4S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 0.2 g of Sulpiride add 20 ml of *methanol* , shake for 5 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of sulpiride (*RS 391*).

Related substances

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel HF_{254} precoated plate (Analtech Uniplates are suitable) and a mixture of 2 volumes of 13.5M *ammonia* , 10 volumes of *1,4-dioxan* , 14 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 20 ml of *methanol* to a quantity of the powdered tablets containing 0.2 g of Sulpiride, shake for 5 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of *methanol* . Solution (2) contains 0.02% w/v of *sulpiride impurity A EPCRS* in *methanol* . For solution (3) dilute 1 volume of solution (1) to 100 volumes with *methanol* and dilute 1 volume of this solution to 10 volumes with *methanol* . After removal of the plate, allow it to dry in air, spray with *ninhydrin solution* and heat at 100° to 105° for 15 minutes. Any spot in the chromatogram obtained with solution (1) corresponding to the principal spot in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

B. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) add 20 ml of *methanol* to a quantity of the powdered tablets containing 0.2 g of Sulpiride, shake for 5 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in sufficient of the mobile phase to produce 200 ml. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with the mobile phase and dilute 1 volume of this solution to 10 volumes with the mobile phase. Solution (3) contains 0.01% w/v of *sulpiride BPCRS* and 0.01% w/v of *sulpiride impurity B EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Zorbax RX C8 is suitable), (b) as the mobile phase at a flow rate of 1.5 ml per minute a mixture of 10 volumes of *acetonitrile*, 10 volumes of *methanol* and 80 volumes of a solution containing 6.8% w/v of *potassium dihydrogen orthophosphate* and 0.1% w/v of *sodium octanesulphonate*, adjusted to pH 3.3 using *orthophosphoric acid*, and (c) a detection wavelength of 240 nm.

Adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with solution (2) is not less than 5% of the full scale of the recorder.

Inject 20 µl of solution (3). The test is not valid unless in the chromatogram obtained the *resolution factor* between the two principal peaks is at least 2.5.

Inject separately 20 µl of solution (1) and 20 µl of solution (2). Continue the chromatography for 2.5 times the retention time of sulpiride. In the chromatogram obtained with solution (1), the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

ASSAY

Weigh and powder 20 tablets. Mix a quantity of the powder containing 0.1 g of Sulpiride with about 50 ml of 0.1M *sodium hydroxide* for 5 minutes with the aid of ultrasound, add sufficient 0.1M *sodium hydroxide* to produce 100 ml and filter, discarding the first 10 ml of filtrate. Dilute 5 ml of the filtrate to 100 ml with 0.1M *sodium hydroxide* and measure the *absorbance* of the solution at the maximum at 291 nm, Appendix II B, using 0.1M *sodium hydroxide* in the reference cell. Calculate the content of $C_{15}H_{23}N_3O_4S$ in the tablets from the *absorbance* of a 0.005% w/v solution of *sulpiride BPCRS* in 0.1M *sodium hydroxide* using the declared content of $C_{15}H_{23}N_3O_4S$ in *sulpiride BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sulpiride.

Sumatriptan Injection

General Notices

Action and use

Serotonin $5HT_1$ receptor agonist; treatment of migraine.

DEFINITION

Sumatriptan Injection is a sterile isotonic solution of Sumatriptan Succinate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of sumatriptan succinate, $C_{14}H_{21}N_3O_2S,C_4H_6O_4$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear, colourless to pale yellow solution.

IDENTIFICATION

To a volume of the injection containing 40 mg of Sumatriptan Succinate add 1 ml of *saturated sodium chloride solution* and 1 ml of *saturated sodium carbonate solution* . Shake vigorously for 30 seconds, add two quantities of 2 ml of *propan-2-ol* , shake, allow to separate (this may take up to 24 hours) and discard the aqueous layer. Evaporate under a stream of nitrogen and dry at 100°. The *infrared absorption spectrum* is concordant with the *reference spectrum* of sumatriptan (*RS 414*).

TESTS

Acidity

pH, 4.2 to 5.3, Appendix V L.

Impurities A and H

Carry out the method for *liquid chromatography* , Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate* , the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile* (solution A).

(1) Dilute the injection, if necessary, with solution A, to contain 0.3% w/v of Sumatriptan Succinate.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A.

(3) Dilute the contents of a vial of *sumatriptan for system suitability EPCRS* (containing impurities A and H) to 1 ml with 1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *silica gel for chromatography* (Spherisorb silica S5W is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for 5 times the retention time of the principal peak.

MOBILE PHASE

A mixture of 10 volumes of 10M *ammonium acetate* and 90 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless in solution (3):

— the chromatogram resembles that supplied with *sumatriptan for system suitability EPCRS*;

— the *resolution factor* between impurity A and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%, taking into account the correction factor of 0.6 for impurity A);

— the area of any peak corresponding to impurity H is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5 and 1 volume *acetonitrile* (solution A).

(1) Dilute the injection, if necessary with solution A to contain 0.3% w/v of Sumatriptan Succinate.

(2) Dilute 3 volumes of solution (1) to 200 volumes with solution A.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* (contains impurities B, C, D and E) to 1 ml with 0.1M *hydrochloric acid*.

(4) 0.3% w/v of *sumatriptan impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use an isocratic system using the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.97 g of *dibutylamine,* 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 ml of *water,* adjusted to pH 7.5 with 10M *sodium hydroxide* and diluted to 1000 ml with *water.*

SYSTEM SUITABILITY

The test is not valid unless in solution (3):

— the chromatogram resembles that supplied with *sumatriptan impurity mixture EPCRS*;

— the *resolution factor* between impurity C and sumatriptan is at least 1.5.

LIMITS

In solution (4) identify impurities 1 and 2 from the reference chromatogram supplied with *sumatriptan impurity standard BPCRS.*

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity 1 is not greater than 4.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%, taking into account the correction factor of 4.5);

— the area of any peak corresponding to impurity 2 is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%, taking into account the correction factor of 3);

— the area of any other *secondary peak* is not greater than 0.66 times the principal peak in the chromatogram obtained with solution (2) (1.0%).

The total impurity content in the test for Impurities A and H and the test for Related substances is not greater than 4.0%.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 5 mg of Sumatriptan Succinate per ml (solution A). The endotoxin limit concentration is less than 350.0 IU of endotoxin per ml.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile*.

(1) Dilute the injection to contain 0.03% w/v of Sumatriptan Succinate.

(2) 0.03% w/v of *sumatriptan succinate EPCRS.*

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* (contains impurities B, C, D and E) to 1 ml with 0.1M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between impurity C and sumatriptan succinate is at least 1.5.

DETERMINATION OF CONTENT

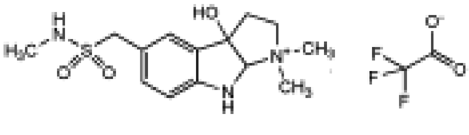
Calculate the content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in the injection using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate EPCRS*.

STORAGE

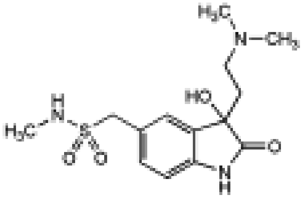
Sumatriptan Injection should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sumatriptan Succinate and also:



1. 3a-hydroxy-1,1-dimethyl-5-{[(methylamino)sulfonyl]methyl}-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-1-ium trifluoroacetate,



2. 1-{3-[2-(dimethylamino)ethyl]-3-hydroxy-2-oxo-2,3-dihydro-1*H*-indol-5-yl}-*N*-methylmethanesulfonamide.

Sumatriptan Nasal Spray

General Notices

Serotonin 5HT$_1$ receptor agonist; treatment of migraine.

DEFINITION

Sumatriptan Nasal Spray is a solution of Sumatriptan containing suitable buffering agents in a suitable container fitted with a suitable nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Content of sumatriptan, $C_{14}H_{21}N_3O_2S$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A clear, pale to dark yellow solution.

IDENTIFICATION

To a volume of nasal spray containing 40 mg of Sumatriptan add 1 ml of *saturated sodium chloride solution* and 1 ml of *saturated sodium carbonate solution*. Shake vigorously for 30 seconds, add two 2 ml quantities of *propan-2-ol*, shake, allow to separate and discard the aqueous layer. The separation may take up to 24 hours. Evaporate under a stream of nitrogen and dry at 100°.

The *infrared absorption spectrum* is concordant with the *reference spectrum* of sumatriptan *(RS 414)*.

TESTS

Acidity

pH, 5.0 to 6.0, Appendix V L.

Impurities A and H

Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5 and 1 volume *acetonitrile* (solution A).

(1) Dilute the nasal spray, if necessary, with sufficient of solution A to contain 0.1% w/v of Sumatriptan.

(2) Dilute 1 volume of solution (1) to 200 volumes with solution A.

(3) Dilute the contents of a vial of sumatriptan for system suitability EPCRS (containing impurities A and H) to 1 ml with 1M *hydrochloric acid* .

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb silica S5W is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µl of each solution.

(g) Allow the chromatography to proceed for 5 times the retention time of the principal peak.

MOBILE PHASE

A mixture of 10 volumes of 10M *ammonium acetate* and 90 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles that supplied with *sumatriptan for system suitability EPCRS* and the *resolution factor* between impurity A and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity A is not greater than 2.5 times the area of the principal peak obtained in the chromatogram obtained with solution (2) (1.5% taking into account the correction factor of 0.6 for impurity A);

— the area of any peak corresponding to impurity H is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Related substances

Carry out the method for *liquid chromatography* , Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate* , the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile* (solution A).

(1) Dilute the injection with solution A, if necessary, to contain 0.1% w/v of Sumatriptan.

(2) Dilute 3 volumes of solution (1) to 200 volumes with solution A.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* (containing

impurities B, C, D and E) with 0.1M *hydrochloric acid* and dilute to 1 ml with 0.1M *hydrochloric acid*.

(4) Prepare a 0.1% w/v of *sumatriptan impurity standard BPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution using the mobile phase below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 75 volumes of a solution containing 0.97 g of *dibutylamine,* 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 ml *water,* adjusted to pH to 6.5 with 10M *sodium hydroxide* and diluted to 1000 ml with *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and sumatriptan is at least 1.5.

Identify the peaks due to impurities 1 and 2, in the chromatogram obtained with solution (4), from the reference chromatogram supplied with *sumatriptan impurity standard BPCRS.*

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity 1 is not greater than 4.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5% taking into account the correction factor of 4.5);

— the area of any peak corresponding to impurity 2 is not greater than 3 times the area of the peak in the chromatogram obtained with solution (2) (1.5%, taking into account the correction factor of 3);

— the area of any other *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

The total impurity content in the test for Impurities A and H and the test for Related substances is not greater than 4.0%.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5, and 1 volume *acetonitrile* (solution A).

(1) Dilute the injection with solution A, if necessary, to contain 0.05% w/v of Sumatriptan.

(2) Prepare a 0.04% w/v of *sumatriptan succinate EPCRS* in solution A.

(3) Contains a mixture of 0.005% w/v of *sumatriptan impurity C EPCRS* and 0.005% w/v of *sumatriptan succinate EPCRS* in solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under the Related substances may be used.

SYSTEM SUITABILITY

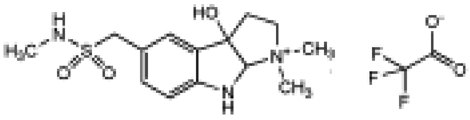
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 1.5.

DETERMINATION OF CONTENT

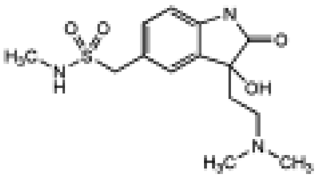
Calculate the content of $C_{14}H_{21}N_3O_2S$ using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate EPCRS*. 1 mg of $C_{14}H_{21}N_3O_2S$ is equivalent to 1.4 mg of $C_{14}H_{21}N_3O_2S, C_4H_6O_4$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sumatriptan Succinate and also:



3a-hydroxy-1,1-dimethyl-5-{[methylamino)sulfonyl]methyl}-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-1-ium trifluoroacetate]



1-{3-[2-(dimethylnitroyl)ethyl]-1H-indol-5-yl}-N-methylmethanesulfonamide

Sumatriptan Tablets

General Notices

Action and use

Serotonin 5HT$_1$ receptor agonist; treatment of migraine.

DEFINITION

Sumatriptan Tablets contain Sumatriptan Succinate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of sumatriptan succinate, $C_{14}H_{21}N_3O_2S,C_4H_6O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Add 10 ml of *methanol* to a quantity of powdered tablets containing 0.1 g of Sumatriptan Succinate , mix with the aid of ultrasound for 5 minutes, filter through a 0.4-µm PTFE filter, evaporate the filtrate and dry under reduced pressure for 1 hour. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the reference spectrum of sumatriptan succinate (*RS 413*).

TESTS

Impurities A and H

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

(1) Add 100 ml of 0.1M *hydrochloric acid* to a quantity of the powdered tablets containing 0.2 g of Sumatriptan Succinate, mix with the aid of ultrasound and filter through a 0.4-µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *hydrochloric acid* and dilute 3 volumes of the resulting solution to 4 volumes with the same solvent.

(3) Dilute the contents of a vial of *sumatriptan for system suitability EPCRS* (containing impurities A and H) to 1 ml with 0.1M *hydrochloric acid.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *silica gel for chromatography* (Spherisorb silica S5W is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2.0 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µl of each solution.

(g) For solution (1) allow the chromatography to proceed for 5 times the retention time of the principal peak.

MOBILE PHASE

A mixture of 10 volumes of 10M *ammonium acetate* and 90 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless in solution (3):

— the chromatogram resembles that supplied with *sumatriptan for system suitability EPCRS* ;

— the *resolution factor* between impurity A and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peak corresponding to impurity A is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75% taking into account the correction factor of 0.6);

— the area of any peak corresponding to impurity H is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

(1) Add 100 ml of 0.1M *hydrochloric acid* to a quantity of the powdered tablets containing 0.2 g of Sumatriptan Succinate, mix with the aid of ultrasound and filter through a 0.4-µm PTFE filter.

(2) Dilute 3 volumes of solution (1) to 100 volumes with 0.1M *hydrochloric acid* and dilute 1 volume of the resulting solution to 10 volumes with 0.1M *hydrochloric acid* .

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* (containing impurities B, C, D and E) to 1 ml with 0.1 M *hydrochloric acid* .

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µl of each solution.

MOBILE PHASE

A mixture of 25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.97 g of *dibutylamine,* 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 ml of *water,* adjusted to pH 7.5 with 10M *sodium hydroxide* and diluted to 1000 ml with *water.*

SYSTEM SUITABILITY

The test is not valid unless in solution (3):

— the chromatogram resembles that supplied with *sumatriptan impurity mixture EPCRS* ;

— the *resolution factor* between impurity C and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

— the area of any peaks corresponding to impurities B, C and D are not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%);

— the area of impurity E and any other *secondary peak* is not greater than area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

The total impurity content in the test for Impurities A and H and the test for Related substances is not greater than 2.0%.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions.

(1) Add 100 ml of 0.1M *hydrochloric acid* to a quantity of the powdered tablets containing 0.2 g of Sumatriptan Succinate, mix with the aid of ultrasound, filter through a 0.4-µm PTFE filter and dilute 1 volume to 10 volumes with 0.1M *hydrochloric acid* .

(2) 0.02% w/v of *sumatriptan succinate EPCRS* in 0.1M *hydrochloric acid*. For solution (3) dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* containing impurities B, C, D and E to 1 ml with 0.1 M *hydrochloric acid* .

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between sumatriptan and impurity C is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{21}N_3O_2S, C_4H_6O_4$ in the tablets using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate EPCRS* .

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Sumatriptan Succinate.

Surgical Spirit

General Notices

DEFINITION

Methyl Salicylate	5 ml
Diethyl Phthalate	20 ml
Castor Oil	25 ml
Industrial Methylated Spirit	Sufficient to produce 1000 ml

The spirit complies with the requirements stated under Spirits and with the following requirements.

Content of methyl salicylate, $C_8H_8O_3$

0.45 to 0.55% v/v.

Content of diethyl phthalate, $C_{12}H_{14}O_4$

1.80 to 2.20% v/v.

IDENTIFICATION

A. To 1 ml add 0.5 ml of 0.4M *iron (III) chloride hexahydrate*. A violet colour is produced.

B. To 1 ml add 50 mg of *resorcinol* and 1 ml of *sulphuric acid* , heat for 1 minute, cool, pour into *water* and make alkaline with 5M *sodium hydroxide* . A distinct green fluorescence is produced which disappears when the solution is made acidic and reappears when it is made alkaline.

TESTS

Weight per ml

0.817 to 0.827 g, Appendix V G.

ASSAY

For methyl salicylate

Dilute 5 ml to 100 ml with *absolute ethanol* , further dilute 10 ml of this solution to 100 ml with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 306 nm, Appendix II B. Calculate the content of $C_8H_8O_3$ taking 335 as the value of the *absorbance* of a 1% v/v solution of methyl salicylate at the maximum at 306 nm using a 1 cm cell.

For diethyl phthalate

Further dilute 10 ml of the final solution prepared in the Assay for methyl salicylate to 50 ml with *absolute ethanol* and measure the *absorbance* of the resulting solution at 227 nm, Appendix II B. Subtract from the observed absorbance the absorbance due to the methyl salicylate present, as determined above, taking 432 as the value of the *absorbance* of a 1% v/v solution of methyl salicylate using a 1 cm cell. For the purposes of calculation take 419 as the value of the *absorbance* of a 1% v/v solution of diethyl phthalate at 227 nm using a 1 cm cell.

LABELLING

The label states that the preparation is flammable and should be kept away from a naked flame.

Suxamethonium Chloride Injection

General Notices

Action and use

Depolarizing neuromuscular blocker.

DEFINITION

Suxamethonium Chloride Injection is a sterile solution of Suxamethonium Chloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of suxamethonium chloride, $C_{14}H_{30}Cl_2N_2O_4,2H_2O$

90.0 to 107.5% of the stated amount.

IDENTIFICATION

Dilute a volume containing 20 mg of Suxamethonium Chloride to 50 ml with *water*. To 0.5 ml add 2 ml of *chloroform*, 2 ml of a solution containing 0.16% w/v of *citric acid* and 6.6% w/v of *disodium hydrogen orthophosphate* and 0.1 ml of a solution containing 0.15% w/v of *bromothymol blue* and 0.15% w/v of *anhydrous sodium carbonate*. Shake for 2 minutes and allow to separate. The chloroform layer is yellow.

TESTS

Acidity

pH, 3.0 to 5.0, Appendix V L.

Hydrolysis products

The volume of 0.1M *sodium hydroxide VS* required for the preliminary neutralisation in the Assay is not more than one tenth of the total volume of 0.1M *sodium hydroxide VS* required for the preliminary neutralisation and the hydrolysis.

ASSAY

To a volume containing 0.2 g of Suxamethonium Chloride add 30 ml of *carbon dioxide-free water* and shake with five 20-ml quantities of *ether*. Wash the combined ether solutions with two 10-ml quantities of *water* and discard the ether. Shake the combined washings with two 10-ml quantities of *ether*, add the washings to the original aqueous solution and neutralise with 0.1M *sodium hydroxide VS* using *bromothymol blue solution R3* as indicator. Add 25 ml of 0.1M *sodium hydroxide VS*, heat under a reflux condenser for 40 minutes, allow to cool and titrate the excess of alkali with 0.1M *hydrochloric acid VS* using *bromothymol blue solution R3* as indicator. Repeat the operation using 40 ml of *carbon dioxide-free water* and beginning at the words 'Add 25 ml...'. The difference between the titrations represents the amount of sodium hydroxide required. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 19.87 mg of $C_{14}H_{30}Cl_2N_2O_4,2H_2O$.

STORAGE

Suxamethonium Chloride Injection should be stored at a temperature of 2° to 8°. It should not be allowed to freeze. Under these conditions it may be expected to continue to meet the requirements of the monograph for not less than 18 months after the date of preparation.

Syrup

General Notices

DEFINITION

Sucrose	667 g
Purified Water	Sufficient to produce 1000 g

One or more suitable antimicrobial preservatives may be added.

Extemporaneous preparation

The following directions apply.

Heat together until dissolved and add sufficient boiling Purified Water to produce 1000 g.

The syrup complies with the requirements stated under Oral Liquids and with the following requirements.

TESTS

Optical rotation

+56° to +60°, Appendix V F.

Weight per ml

1.315 to 1.333 g, Appendix V G.

STORAGE

Syrup should not be exposed to undue fluctuations in temperature.

LABELLING

The label states the names and proportions of any added antimicrobial preservatives.

When antimicrobial preservatives are added the suitability of the Syrup as a vehicle or diluent should be confirmed before use. The pH of Syrup may affect the solubility of basic or acidic materials.

Alpha Tocopheryl Succinate Tablets

General Notices

DEFINITION

Alpha Tocopheryl Succinate Tablets contain *RRR*-Alpha-Tocopheryl Hydrogen Succinate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of α-tocopherol, $C_{29}H_{50}O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel HF $_{254}$* as the coating substance and a mixture of 20 volumes of *ether* and 80 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.134 g of α-tocopherol with three 10 ml quantities of *ether* , filter, evaporate the combined filtrates to 2 ml and then evaporate the solution to dryness under a current of nitrogen. Prepare a 0.5% w/v solution from a portion of the resulting residue in *cyclohexane* . For solution (2) dissolve 10 mg of the residue obtained in the preparation of solution (1) in 2 ml of 5M *ethanolic sulphuric acid* , heat in a water bath for 1 minute, cool, add 2 ml each of *water* and *cyclohexane* and shake for 1 minute; use the upper layer. Solution (3) contains 0.5% w/v of RRR- α-*tocopheryl succinate EPCRS* in *cyclohexane* . Prepare solution (4) in the same manner as solution (2) but using 10 mg of RRR-α-*tocopheryl succinate EPCRS* in place of the substance being examined.

After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) is similar in position and size to that in the chromatogram obtained with solution (3). There are two spots in each of the chromato-grams obtained with solutions (2) and (4). Spray the plate with a mixture of 1 volume of *hydrochloric acid* , 4 volumes of a 0.25% w/v solution of *iron* (*III*) *chloride hexahydrate* in *ethanol* (*96%*) and 4 volumes of a 1% w/v solution of *1,10-phenanthroline hydrochloride* in *ethanol* (*96%*). In the chromatograms obtained with solutions (2) and (4) the spot of higher Rf value, due to α-tocopherol, is orange.

B. In the Assay the principal peak in the chromatogram obtained with solution (1) shows a peak with the same retention time as the peak due to the methylated alpha tocopheryl succinate in the chromatogram obtained with solution (2).

TESTS

Free tocopherol

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel HF* $_{254}$ as the coating substance and a mixture of 20 volumes of *ether* and 80 volumes of *cyclohexane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 134 mg of α-tocopherol with three 10 ml quantities of *ether*, filter and evaporate the combined filtrates to 2 ml, evaporate the final solution to dryness under a current of nitrogen and prepare a 0.5% w/v solution from the resulting residue in *cyclohexane*. Solution (2) contains 0.005% w/v of *α-tocopherol EPCRS* in *cyclohexane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. Dissolve 0.15 g of *dotriacontane* (internal standard) in sufficient *hexane* to produce 100 ml (solution A). For solution (1) mix a quantity of the powdered tablets containing the equivalent of 134 mg of α-tocopherol with 20 ml of *methanol*, mix with the aid of ultrasound for 5 minutes and centrifuge for 15 minutes. To 4 ml of the clear supernatant liquid add 2 ml of *2,2-dimethoxypropane* and 0.2 ml of *hydrochloric acid* and allow to stand in the dark at room temperature for 1 hour. Evaporate to dryness on a water bath with the aid of a current of nitrogen and dissolve the residue in 10 ml of solution A. For solution (2) dissolve 0.165 g of RRR- α *-tocopheryl succinate EPCRS* in 20 ml of *methanol* and continue in the same manner as solution (1) using 4 ml and beginning at the words 'add 2 ml of *2,2-dimethoxy-propane...*'.

The chromatographic procedure may be carried out using a borosilicate glass column (2 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) (Chromosorb W/AW is suitable) coated with 2 to 5% of polymethylsiloxane. Set the temperature of the column and the rate of flow of carrier gas at values such that the required resolution is achieved (a column temperature of 280° and a rate of flow of carrier gas of 40 ml per minute are suitable). Maintain the temperature of the injection port at 290° and the detector at 350°. The retention times of dotriacontane and methyl α-tocopheryl succinate (obtained in solution (1)) are about 8 minutes and 20 minutes, respectively.

Calculate the content of $C_{29}H_{50}O_2$ from the areas of the peaks due to dotriacontane and methyl α-tocopheryl succinate in the chromatograms obtained with solution (1) and solution (2) and from the declared content of $C_{29}H_{50}O_2$ in RRR- α *-tocopheryl succinate EPCRS*.

STORAGE

Alpha Tocopheryl Succinate Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of α-tocopherol.

Coal Tar and Salicylic Acid Ointment

General Notices

DEFINITION

Coal Tar and Salicylic Acid Ointment contains 2% w/v of each of Coal Tar and Salicylic Acid in a suitable emulsifying basis.

Extemporaneous preparation

The following formula and directions apply.

Coal Tar	20 g
Polysorbate 80	40 g
Salicylic Acid	20 g
Emulsifying Wax	114 g
White Soft Paraffin	190 g
Coconut Oil	540 g
Liquid Paraffin	76 g

Disperse the Coal Tar in the Polysorbate 80, incorporate the Salicylic Acid and mix with the previously melted Emulsifying Wax. Separately, melt the White Soft Paraffin and the Coconut Oil, incorporate the Liquid Paraffin warmed to the same temperature and add, with stirring, the resulting solution to the Coal Tar dispersion. Mix thoroughly and stir until cold.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of salicylic acid, $C_7H_6O_3$

1.90 to 2.10% w/w.

IDENTIFICATION

Disperse 2 g of the ointment in 20 ml of *water* with the aid of gentle heat, cool and filter. The filtrate yields reaction A characteristic of *salicylates* , Appendix VI.

ASSAY

To 2 g add 50 ml of *water*, warm until melted, cool and decant the supernatant liquid through moistened absorbent cotton. Repeat the operation with a further three 50 ml quantities of *water*. Dilute the combined aqueous extracts to 250 ml with *water*, filter and dilute 10 ml of the filtrate to 50 ml with *iron(III) nitrate solution*. Filter if necessary and measure the *absorbance* of the resulting solution at the maximum at 530 nm, Appendix II B, using in the reference cell a solution prepared by diluting 10 ml of the filtered extract to 50 ml with *water*. Calculate the content of $C_7H_6O_3$ from the *absorbance* of the solution obtained by diluting 10 ml of a 0.016% w/v solution of *salicylic acid* to 50 ml with *iron(III) nitrate solution*, filtering if necessary, and using *water* in the reference cell.

Coal Tar and Zinc Ointment

General Notices

DEFINITION

Coal Tar and Zinc Ointment contains 30% w/w of Zinc Oxide and 10% w/w of Strong Coal Tar Solution in a suitable hydrophobic basis.

Extemporaneous preparation

The following formula and directions apply.

Strong Coal Tar Solution	100 g
Zinc Oxide finely sifted	300 g
Yellow Soft Paraffin	600 g

Mix the Zinc Oxide with the Strong Coal Tar Solution, triturate with a portion of the Yellow Soft Paraffin until smooth, gradually incorporate the remainder of the Yellow Soft Paraffin and mix.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

28.5 to 31.5% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of 2M *acetic acid* and add sufficient *water* to produce 50 ml. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.1M *disodium edetate VS* until the solution becomes yellow. Each ml of 0.1M *disodium edetate VS* is equivalent to 8.138 mg of ZnO.

Coal Tar Paste

General Notices

DEFINITION

Strong Coal Tar Solution	75 g
Compound Zinc Paste	925 g

Extemporaneous preparation

The following directions apply.

Triturate the Strong Coal Tar Solution with a portion of the Compound Zinc Paste until smooth and gradually incorporate the remainder of the Compound Zinc Paste.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

21.0 to 25.0% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the paste gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of 2M *acetic acid* and add sufficient *water* to produce 50 ml. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.1M *disodium edetate VS* until the solution becomes yellow. Each ml of 0.1M *disodium edetate VS* is equivalent to 8.138 mg of ZnO.

Coal Tar Solution

General Notices

DEFINITION

Coal Tar	200 g
Polysorbate 80	50 g
Ethanol (96 per cent)	Sufficient to produce 1000 ml

In making Coal Tar Solution, the Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit[1].

Extemporaneous preparation

The following directions apply.

Mix the Coal Tar, warmed if necessary to render it fluid, with the Polysorbate 80, pour this mixture in a thin stream into 800 ml of Ethanol (96 per cent) in a closed vessel fitted with an agitator; continue agitation throughout the addition of the mixture and for 1 hour thereafter. Allow the mixture to stand for not less than 24 hours, decant and filter the supernatant liquid, wash the vessel and filter with Ethanol (96 per cent), combine the filtrate and washings and add sufficient Ethanol (96 per cent) to produce 1000 ml.

TESTS

Ethanol content

80 to 90% v/v, Appendix VIII F.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

[1] The law and the statutory regulations governing the use of Industrial Methylated Spirit must be observed.

Paediatric Compound Tolu Linctus

General Notices

DEFINITION

Paediatric Compound Tolu Linctus is an *oral solution* containing 0.6% w/v of Citric Acid Monohydrate in a suitable vehicle with a tolu flavour.

The linctus complies with the requirements stated under Oral Liquids and with the following requirements.

Content of total acid, calculated as citric acid monohydrate, $C_6H_8O_7,H_2O$

0.60 to 0.66% w/v.

ASSAY

To 15 g add 100 ml of *water* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 7.005 mg of $C_6H_8O_7,H_2O$. Determine the *weight per ml* of the linctus, Appendix V G, and calculate the content of $C_6H_8O_7,H_2O$, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'oral solution'.

Prolonged-release Theophylline Tablets

General Notices

Prolonged-release Theophylline Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

DEFINITION

Prolonged-release Theophylline Tablets contain Theophylline or Theophylline Hydrate. The are formulated so that the medicament is released over a period of several hours.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Theophylline. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of theophylline, $C_7H_8N_4O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

B. Extract a quantity of the powdered tablets containing the equivalent of 0.2 g of theophylline with 10 ml of a mixture of 60 volumes of *chloroform* and 40 volumes of *methanol* , filter and evaporate the filtrate to dryness. The residue yields the reaction characteristic of *xanthines* , Appendix VI.

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel F_{254} plate* and a mixture of 10 volumes of *concentrated ammonia* , 30 volumes of *acetone* , 30 volumes of *chloroform* and 40 volumes of *butan-1-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing the equivalent of 0.2 g of theophylline with 10 ml of a mixture of 60 volumes of *chloroform* and 40 volumes of *methanol* and centrifuge. For solution (2) dilute 1 volume of solution (1) to 200 volumes with a mixture of 60 volumes of *chloroform* and 40 volumes of *methanol* . After removal of the plate, allow it to dry in air and view under *ultraviolet light (254 nm).* Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the principal spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) add 1 ml of *methanol* to a quantity of the powdered tablets containing the equivalent of 0.1 g of theophylline, mix and add 50 ml of *water*. Dissolve with the aid of ultrasound, swirling occasionally, dilute to 100 ml with *water* and mix thoroughly. Dilute 5 ml to 50 ml with *water*. Solution (2) contains 0.01% w/v of *theophylline BPCRS* in water.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 1.8 ml per minute a mixture of 250 volumes of *methanol* and 750 volumes of *water* and (c) a detection wavelength of 270 nm.

Calculate the content of $C_7H_8N_4O_2$ in the tablets using the declared content of $C_7H_8N_4O_2$ in *theophylline BPCRS* .

LABELLING

When the active ingredient is Theophylline Hydrate the quantity is stated in terms of the equivalent amount of theophylline.

Strong Coal Tar Solution

General Notices

DEFINITION

Coal Tar	400 g
Polysorbate 80	50 g
Ethanol (96 per cent)	Sufficient to produce 1000 ml

In making Strong Coal Tar Solution the Ethanol (96 per cent) may be replaced by Industrial Methylated Spirit[1].

Extemporaneous preparation

The following directions apply.

Mix the Coal Tar, warmed if necessary to render it fluid, with the Polysorbate 80, pour this mixture in a thin stream into 700 ml of Ethanol (96 per cent) in a closed vessel fitted with an agitator; continue agitation throughout the addition of the mixture and for 1 hour thereafter. Allow the mixture to stand for not less than 24 hours, decant and filter the supernatant liquid, wash the vessel and filter with Ethanol (96 per cent), combine the filtrate and washings and add sufficient Ethanol (96 per cent) to produce 1000 ml.

TESTS

Ethanol content

70 to 80% v/v, Appendix VIII F.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

[1] The law and the statutory regulations governing the use of Industrial Methylated Spirit must be observed.

Talc Dusting Powder

General Notices

DEFINITION

Talc Dusting Powder is a sterile *cutaneous powder* of suitable fineness consisting of 10% of starch[1] and 90% of Purified Talc.

PRODUCTION

The starch is triturated with the Purified Talc and passed through a sieve of suitable mesh size (250 µm may be suitable). Either the Purified Talc is sterilised before use or the final product is subjected to a suitable sterilisation procedure.

The dusting powder complies with the requirements stated under Topical Powders and with the following requirement.

Acid-insoluble matter

86.0 to 91.0% when determined by the following method. Boil 0.5 g with 10 ml of 2M *hydrochloric acid* for 5 minutes, cool, filter through a tared sintered-glass crucible (ISO 4793, porosity grade 4, is suitable), wash the residue with *water* until the washings are free from acid and dry to constant weight at 105°.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous powder'.

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[1]

Tamoxifen Tablets

General Notices

Action and use

Selective estrogen receptor modulator.

DEFINITION

Tamoxifen Tablets contain Tamoxifen Citrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tamoxifen, $C_{26}H_{29}NO$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a quantity of the powdered tablets containing the equivalent of 0.1 g of tamoxifen add 20 ml of *water*, warm, add 2 ml of 5M *sodium hydroxide* and cool. Extract with two 10-ml quantities of *ether*, filtering each extract in turn. Combine the ether extracts and evaporate to dryness in a current of nitrogen at room temperature. Dry the residue at a pressure not exceeding 0.7 kPa for 30 minutes. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of tamoxifen (*RS 328*).

B. Shake a quantity of the powdered tablets containing the equivalent of 0.1 g of tamoxifen with 10 ml of *methanol*, filter, evaporate the filtrate to dryness on a water bath and dry the residue at 100° for 30 minutes. Dissolve 10 mg of the dried residue in 4 ml of *pyridine*, add 2 ml of *acetic anhydride* and heat on a water bath. A rose-pink to red colour is produced.

TESTS

E-Isomer and other related substances

Carry out the method for *liquid chromatography*, Appendix III D, protected from light and using the following solutions. For solution (1) to a quantity of the powdered tablets containing the equivalent of 50 mg of tamoxifen, add 35 ml of the mobile phase, mix with the aid of ultrasound for 5 minutes, dilute to 50 ml with the mobile phase, mix, centrifuge and filter the supernatant liquid through a membrane filter with a nominal pore size of 0.45 µm. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.15% w/v of *tamoxifen citrate for performance test EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Columbus C18 is suitable), (b) as the mobile phase with a flow rate of 1.2 ml per minute a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water* containing 0.09% w/v of *sodium dihydrogen orthophosphate* and 0.48% w/v of N,N-*dimethyloctylamine*, adjusted to pH 3.0 with *orthophosphoric acid* and (c) a detection wavelength of 240 nm.

Equilibrate the column with the mobile phase at a flow rate of 1.2 ml per minute for 30 minutes.

Adjust the sensitivity of the system so that the height of the peak due to the *E*-isomer in the chromatogram obtained with solution (3) is not less than 40% of the full scale of the recorder.

Inject 20 µl of each solution. Continue the chromatography for twice the retention time of the tamoxifen peak. The test is not valid unless the chromatogram obtained with solution (3) resembles that of the specimen chromatogram provided with the *tamoxifen citrate for performance test EPCRS* in that the *resolution factor* between the peaks due to the *E*-isomer and to tamoxifen impurity F is at least 3 and there is base-line separation of the peak due to tamoxifen impurity F from the following peak of the principal component.

In the chromatogram obtained with solution (1), the area of any peak due to the *E*-isomer is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%) and the sum of the areas of all the *secondary peaks* apart from any peak due to the *E*-isomer is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with a retention time of less than 2.5 minutes and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 1000 ml of 0.02M *hydrochloric acid* and rotating the basket at 150 revolutions per minute. Withdraw a sample of 5 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at 275 nm, Appendix II B. Calculate the total content of $C_{26}H_{29}NO$ in the medium taking 305 as the value of A(1%, 1 cm) at 275 nm.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 25 mg of tamoxifen add 100 ml of *methanol*, shake for 15 minutes and add sufficient *methanol* to produce 250 ml. Filter, dilute 10 ml of the filtrate to 100 ml with *methanol* and measure the *absorbance* of the resulting solution at 275 nm, Appendix II B. Calculate the content of $C_{26}H_{29}NO$ taking 325 as the value of A(1%, 1 cm) at 275 nm.

STORAGE

Tamoxifen Tablets should be protected from light.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of tamoxifen.

Temazepam Oral Solution

General Notices

Action and use

Benzodiazepine.

DEFINITION

Temazepam Oral Solution is a solution of Temazepam in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of temazepam, $C_{16}H_{13}ClN_2O_2$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a quantity of the oral solution with sufficient 0.1M *methanolic hydrochloric acid* to produce a solution containing 0.001% w/v of Temazepam. The *light absorption* of the resulting solution, Appendix II B, in the range 210 to 400 nm exhibits three maxima, at 238 nm, 283 nm and 358 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) add 5 ml of *water* to a quantity of the oral solution containing 10 mg of Temazepam and extract with two 10-ml quantities of *ether*. Evaporate the combined ether extracts almost to dryness and dissolve the residue in 2 ml of *acetone*. Solution (2) contains 0.5% w/v of *temazepam BPCRS* in *acetone*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. In the Assay, the chromatogram obtained with solution (2) exhibits a peak with the same retention time as that due to temazepam in the chromatogram obtained with solution (1).

TESTS

Alkalinity

pH, 7.3 to 8.3, Appendix V L.

6-Chloro-1,4-dihydro-1-methyl-4-phenylquinazolin-4-ol

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 92.5 volumes of *chloroform* and 7.5 volumes of *methanol* as the mobile phase but allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 5 ml of *water* to a quantity of the oral solution containing 10 mg of Temazepam and extract with two 10 ml quantities of *ether*. Evaporate the combined ether extracts almost to dryness and dissolve the residue in 1 ml of *acetone*. Solution (2) contains 0.010% w/v of *6-chloro-1,4-dihydro-1-methyl-4-phenylquinazolin-4-ol BPCRS*. After removal of the plate, allow it to dry in a current of warm air and examine under *ultraviolet light* (*365 nm*). In the chromatogram obtained with solution (1) any spot corresponding to 6-chloro-1,4-dihydro-1-methyl-4-phenylquinazolin-4-ol is not more intense than the spot in the chromatogram obtained with solution (2).

5-Chloro-2-methylaminobenzophenone

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.00050% w/v of *5-chloro-2-methylaminobenzophenone BPCRS* in *methanol* (*50%*). For solution (2) dilute a quantity of the oral solution with sufficient *methanol* (*50%*) to produce a solution containing 0.020% w/v of Temazepam.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 is suitable), (b) a mixture of 75 volumes of *methanol*, 25 volumes of *water* and 0.03 volume of *diethylamine* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

In the chromatogram obtained with solution (2) the area of any peak corresponding to 5-chloro-2-methylaminobenzophenone is not greater than the area of the peak in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of *temazepam BPCRS* in *methanol* (*50%*). For solution (2) add sufficient *methanol* (*50%*) to a weighed quantity of the oral solution containing 20 mg of Temazepam to produce 100 ml.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 60 volumes of *methanol*, 40 volumes of *water* and 0.03 volumes of *diethylamine* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_{16}H_{13}ClN_2O_2$, weight in volume, using the declared content of $C_{16}H_{13}ClN_2O_2$ in *temazepam BPCRS*.

STORAGE

Temazepam Oral Solution should be protected from light.

Temazepam Tablets

General Notices

Action and use

Benzodiazepine.

DEFINITION

Temazepam Tablets contain Temazepam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of temazepam, $C_{16}H_{13}ClN_2O_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption* , Appendix II B, in the range 210 to 400 nm of the final solution obtained in the Assay exhibits two maxima at 230 nm and at 315 nm and a shoulder at 250 nm.

B. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 10 volumes of *diethylamine* and 90 volumes of *ether* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Temazepam for 5 minutes with 20 ml of *methanol* and filter. Solution (2) contains 0.10% w/v of *temazepam BPCRS* in *methanol* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The chromatogram obtained with solution (1) exhibits a spot which corresponds to the spot in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2. Use as the medium 900 ml of 0.1M *hydrochloric acid* and rotate the paddle at 50 revolutions per minute. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, with 0.1M *hydrochloric acid* , at the maximum at 237 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell. Measure the *absorbance* of a suitable solution of *temazepam BPCRS* in the dissolution medium and calculate the total content of temazepam, $C_{16}H_{13}ClN_2O_2$, in the medium from the absorbances obtained and from the declared content of $C_{16}H_{13}ClN_2O_2$ in *temazepam BPCRS* .

Related substances

Carry out the test protected from light. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2 volumes of *methanol* and 98 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions, prepared immediately before use, in low-actinic glassware. For solution (1) shake a quantity of the powdered tablets containing 50 mg of Temazepam for 10 minutes with 5 ml of a mixture of equal volumes of *dichloromethane* and *methanol* and filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent mixture. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with the same solvent mixture. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 20 mg of Temazepam with 75 ml of *methanol* for 20 minutes, dilute to 100 ml with the same solvent and filter. Dilute 10 ml of the resulting filtrate to 250 ml with *methanol* and measure the *absorbance* of the resulting solution at the maximum at about 230 nm, Appendix II B. Calculate the content of $C_{16}H_{13}ClN_2O_2$ in the tablets from the *absorbance* obtained using a 0.0008% w/v solution of *temazepam BPCRS* in *methanol* and using the declared content of $C_{16}H_{13}ClN_2O_2$ in *temazepam BPCRS*.

STORAGE

Temazepam Tablets should be protected from light.

Tenoxicam Injection

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Tenoxicam Injection is a sterile solution of Tenoxicam in Water for Injections. It is prepared by dissolving Tenoxicam for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Tenoxicam Injection should be used immediately after preparation.

TENOXICAM FOR INJECTION

DEFINITION

Tenoxicam for Injection is a sterile material consisting of Tenoxicam with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of tenoxicam, $C_{13}H_{11}N_3O_4S_2$

104.5% to 115.5% of the stated amount.

IDENTIFICATION

A. In the test for Related substances, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak in the chromatogram obtained with solution (4).

B. The *light absorption*, Appendix II B, in the range 230 to 400 nm of the solution prepared in the Assay exhibits three maxima at 257 nm, 285 nm and 368 nm.

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F $_{254}$ plate* (Merck silica gel 60 F $_{254}$ plates are suitable) and a mixture of 2 volumes of *glacial acetic acid*, 45 volumes of *acetone* and 55 volumes of *toluene* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the sealed container containing 20 mg of Tenoxicam with 10 ml of a mixture containing equal volumes of *dichloromethane* and *methanol* for 5 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.2% w/v of *tenoxicam BPCRS* in a mixture of equal volumes of *dichloromethane* and *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Spray the plate with *acid potassium iodobismuthate solution* and examine again. Using each method of visualisation the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Alkalinity

pH of the injection when constituted in accordance with the manufacturer's instructions, 9.0 to 10.0, Appendix V L.

Clarity of solution

The injection, constituted in accordance with the manufacturer's instructions, is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the contents of the sealed container containing 20 mg of Tenoxicam in 20 ml of *acetonitrile* (*50%*) and dilute 1 volume of the resulting solution to 4 volumes with the mobile phase. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.0000625% w/v of *2-pyridylamine* in *acetonitrile* (*50%*). For solution (4) dilute 5 ml of a 0.1% w/v solution of *tenoxicam BPCRS* to 20 ml with the mobile phase. For solution (5) dilute 1 volume of a 0.1% w/v solution of *tenoxicam degradation impurity standard BPCRS* in *acetonitrile* (*50%*) to 4 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 is suitable) and a pre-column packed with *octylsilyl silica gel for chromatography* (10 µm) (Spheri-10 RP8, RP-GU pre-column is suitable), (b) as the mobile phase with a flow rate of 0.7 ml per minute a mixture prepared in the following manner: dissolve 0.12 g of *sodium dodecyl sulphate* in 700 ml of *methanol*, mix with 1000 ml of 0.05M *potassium dihydrogen orthophosphate* and adjust the pH to 2.8 with *orthophosphoric acid* and (c) 254 nm and 290 nm as the detection wavelengths. Condition the column with the mobile phase for 3 hours.

The test is not valid unless the chromatogram obtained with solution (5) closely resembles the reference chromatogram provided with *tenoxicam degradation impurity standard BPCRS.*

At a detection wavelength of 290 nm The area of any peak corresponding to 2-pyridylamine in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.25%).

At a detection wavelength of 254 nm In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Water

Not more than 4.0% w/w, Appendix IX C. Use 0.3 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Dissolve the contents of 10 containers in 0.1M *sodium hydroxide* and dilute to 1000 ml with 0.1M *sodium hydroxide*. Dilute the resulting solution with 0.1M *sodium hydroxide* to contain 0.0016% of Tenoxicam. Mix well, filter and measure the *absorbance* of the resulting solution at the maximum at 368 nm, Appendix II B.

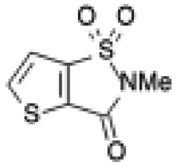
Calculate the total content of $C_{13}H_{11}N_3O_4S_2$ in a container of average content weight in the injection from the *absorbance* of a 0.0016% w/v solution of *tenoxicam BPCRS* in 0.1M *sodium hydroxide* using the declared content of $C_{13}H_{11}N_3O_4S_2$ in *tenoxicam BPCRS*.

STORAGE

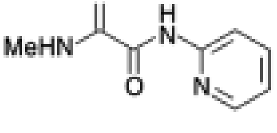
Where the sealed container is supplied with an ampoule of Water for Injections, this should not be allowed to freeze.

IMPURITIES

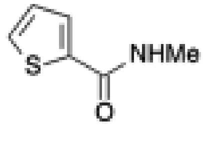
The impurities limited by the requirements of this monograph include those impurities listed under Tenoxicam and also the following.



A. 2-methylthieno[2,3-*d*]isothiazol-3(2*H*)-one-1,1-dioxide,



B. *N*-methyl-*N'*-(2-pyridyl)oxamide,



C. *N*-methylthiophene-2-carboxamide.

Tenoxicam Tablets

General Notices

Action and use

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

DEFINITION

Tenoxicam Tablets contain Tenoxicam.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tenoxicam, $C_{13}H_{11}N_3O_4S_2$

92.5 to 105.0% of the stated amount.

IDENTIFICATION

A. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the peak in the chromatogram obtained with solution (2).

B. Carry out method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel F $_{254}$ plate* (Merck silica gel 60 F $_{254}$ plates are suitable) and a mixture of 4 volumes of *anhydrous formic acid* , 30 volumes of *acetone* and 70 volumes of *dichloromethane* as the mobile phase but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) mix with the aid of ultrasound a quantity of the powdered tablets containing 20 mg of Tenoxicam with 20 ml of *dichloromethane* for 15 minutes, centrifuge and use the supernatant liquid. Solution (2) contains 0.1% w/v of *tenoxicam BPCRS* in *dichloromethane* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2 and rotating the paddle at 50 revolutions per minute. Use as the medium 900 ml of phosphate buffer pH 6.8 prepared in the following manner: dissolve 6.8 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, add 23 ml of 1M *sodium hydroxide* , dilute to 1000 ml and, if necessary, adjust the pH to 6.8 using either 1M *sodium hydroxide* or a 10% w/v solution of *orthophosphoric acid* . Withdraw a sample of 10 ml of the medium, filter and measure the *absorbance* of the solution, suitably diluted with the dissolution medium if necessary, at the maximum at 368 nm, Appendix II B. Measure the *absorbance* of a suitable solution of *tenoxicam BPCRS* in the dissolution medium and calculate the total content of tenoxicam, $C_{13}H_{11}N_3O_4S_2$, in the medium using the declared content of $C_{13}H_{11}N_3O_4S_2$ in *tenoxicam BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a number of whole tablets containing 0.1 g of Tenoxicam with 100 ml of *acetonitrile* (*50%*) for 70 minutes, mixing occasionally with the aid of ultrasound. Allow to stand for 10 minutes, dilute 5 volumes of the clear supernatant liquid to 20 volumes with the mobile phase and filter through a 0.45-µm membrane filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.0000625% w/v of *2-pyridylamine* in *acetonitrile* (*50%*). For solution (4) dilute 1 volume of a 0.1% w/v solution of *tenoxicam degradation impurity standard BPCRS* in *acetonitrile* (*50%*) to 4 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Nucleosil C8 5µ is suitable) and a pre-column packed with *octylsilyl silica gel for chromatography* (10 µm) (Spheri-10 RP8, RP-GU pre-column is suitable), (b) as the mobile phase with a flow rate of 0.7 ml per minute a mixture prepared in the following manner: dissolve 0.12 g of *sodium dodecyl sulphate* in 700 ml of *methanol*, mix with 1000 ml of 0.05M *potassium dihydrogen orthophosphate* and adjust the pH to 2.8 with *orthophosphoric acid* and (c) 254 nm and 290 nm as the detection wavelengths. Condition the column with the mobile phase for 3 hours.

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the reference chromatogram provided with *tenoxicam degradation impurity standard BPCRS*.

At a detection wavelength of 290 nm The area of any peak corresponding to 2-pyridylamine in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.25%).

At a detection wavelength of 254 nm In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any such peaks is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (2%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake 10 whole tablets with 200 ml of *acetonitrile* (*50%*) for 70 minutes, mixing occasionally with the aid of ultrasound. Allow to stand for 10 minutes, dilute a volume of the clear supernatant liquid with sufficient mobile phase to produce a solution containing 0.025% w/v of Tenoxicam and filter through a 0.45-µm membrane filter. For solution (2) dilute 5 ml of a 0.1% w/v solution of *tenoxicam BPCRS* in *acetonitrile* (*50%*) to 20 ml with the mobile phase. For solution (3) dilute 1 volume of a 0.1% w/v solution of *tenoxicam degradation impurity standard BPCRS* in *acetonitrile* (*50%*) to 4 volumes with the mobile phase.

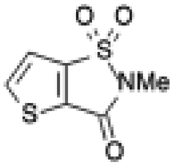
The chromatographic conditions described under Related substances may be used with a detection wavelength of 290 nm.

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram provided with *tenoxicam degradation impurity standard BPCRS*.

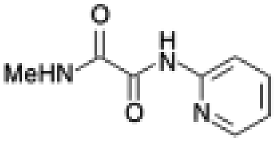
Calculate the content of $C_{13}H_{11}N_3O_4S_2$ in the tablets from the chromatogram obtained using the declared content of $C_{13}H_{11}N_3O_4S_2$ in *tenoxicam BPCRS*.

IMPURITIES

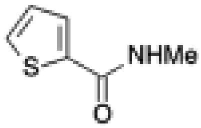
The impurities limited by the requirements of this monograph include those impurities listed under Tenoxicam and also the following.



A. 2-methylthieno[2,3-*d*]isothiazol-3(2*H*)-one-1,1-dioxide,



B. *N*-methyl-*N′*-(2-pyridyl)oxamide,



C. *N*-methylthiophene-2-carboxamide.

Terbutaline Tablets

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Terbutaline Tablets contain Terbutaline Sulphate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of terbutaline sulphate $(C_{12}H_{19}NO_3)_2,H_2SO_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 20 mg of Terbutaline Sulphate with 50 ml of 0.1M *sodium hydroxide* for 10 minutes, dilute to 100 ml with 0.1M *sodium hydroxide* and filter. Dilute 20 ml of the filtrate to 50 ml with 0.1M *sodium hydroxide* . The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 296 nm.

B. Carry out the method for *thin-layer chromatography* , Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 65 volumes of *propan-2-ol* , 25 volumes of *cyclohexane* and 5 volumes of *formic acid* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 10 mg of Terbutaline Sulphate with 4 ml of a mixture of equal volumes of *ethanol* (*96%*) and *water* for 10 minutes, centrifuge and use the clear solution. Solution (2) contains 0.25% w/v of *terbutaline sulphate BPCRS* in *water*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air, allow to stand for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised nitroaniline solution* . The spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2) and the principal spot in the chromatogram obtained with solution (3) appears as a single compact spot.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 75 mg of Terbutaline Sulphate with 35 ml of the mobile phase for 15 minutes, add sufficient of the mobile phase to produce 50 ml, mix and filter (Whatman GF/C paper is suitable). For solution (2) dilute 1 volume of solution (1) to 50 volumes with the mobile phase and further dilute 1 volume to 10 volumes with the mobile phase. Solution (3) contains 0.00045% w/v of *terbutaline sulphate BPCRS* and 0.00015% w/v of *terbutaline impurity C EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *base-deactivated octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil BDS C18 5µ is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute a solution prepared as described below and (c) a detection wavelength of 276 nm. For the mobile phase prepare a 0.05M solution of *ammonium formate* by dissolving 3.15 g of *ammonium formate* in 980 ml of *water*, adjust the pH to 3.0 by the addition of about 8 ml of *anhydrous formic acid* and add sufficient *water* to produce 1000 ml. Dissolve 4.23 g of *sodium hexanesulphonate* in 770 ml of the ammonium formate solution and add 230 ml of *methanol* .

Inject solution (3). When the chromatograms are recorded under the prescribed conditions the retention times are about 9 minutes for terbutaline impurity C and about 11 minutes for terbutaline sulphate. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to terbutaline sulphate and terbutaline impurity C is at least 2.

For solution (1) allow the chromatography to proceed for 6 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to terbutaline impurity C is not greater than twice the area of the peak due to terbutaline impurity C in the chromatogram obtained with solution (3) (0.2%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.4%). Disregard any peaks with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.02%) and any peaks with a retention time of less than 2.5 minutes.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 15 mg of Terbutaline Sulphate with 20 ml of 0.05M *sulphuric acid* for 15 minutes, add 1.6 ml of 1.5M *sodium acetate* and sufficient *water* to produce 25 ml and filter (Whatman GF/C paper is suitable). For solution (2) dissolve 30 mg of *terbutaline sulphate BPCRS* in 40 ml of 0.05M *sulphuric acid* , add 3.2 ml of 1.5M *sodium acetate* and add sufficient *water* to produce 50 ml.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $(C_{12}H_{19}NO_3)_2,H_2SO_4$ in the tablets from the chromatograms obtained and using the declared content of $(C_{12}H_{19}NO_3)_2,H_2SO_4$ in *terbutaline sulphate BPCRS* .

Terfenadine Oral Suspension

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Terfenadine Oral Suspension is a suspension of Terfenadine in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of terfenadine, $C_{32}H_{41}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a volume of the suspension containing 60 mg of Terfenadine with 10 ml of *water*, centrifuge and discard the liquid phase. Shake the residue sequentially with 10 ml of *water*, 10 ml of 0.2M *sodium hydroxide* , 10 ml of *water* and 10 ml of n-*hexane* , centrifuge and discard the liquid phase after each extraction. Dissolve the residue in 15 ml of *dichoromethane*, shake with 2 g of *anhydrous sodium sulphate* and filter. Add 0.5 ml of the filtrate to 0.3 g of *potassium bromide* in a mortar, mix with a pestle, warm to remove the solvent and prepare a disc from the resulting mixture. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of terfenadine (*RS 392*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

1-[4-(1,1-Dimethylethyl)phenyl]-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butan-1one

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dilute a quantity of the oral suspension containing 30 mg of Terfenadine to 70 ml with the mobile phase, shake for 1 hour, add sufficient mobile phase to produce 100 ml, mix and centrifuge. Solution (2) contains 0.00006% w/v of *terfenadine impurity A EPCRS* (1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butan-1-one) in the mobile phase. Solution (3) contains equal volumes of solution (1) and a solution containing 0.03% w/v of *terfenadine impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP8 is suitable), (b) 3 volumes of *acetonitrile* diluted to 5 volumes with *diethylammonium phosphate buffer solution pH 6.0* as the mobile phase with a flow rate of 1.0 ml per minute and (c) a detection wavelength of 254 nm.

Inject 20 µl of each solution and continue the chromatography for 5 times the retention time of terfenadine. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due terfenadine and terfenadine impurity A is at least 5.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to terfenadine impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%). In the chromatogram obtained with solution (1) peaks due to excipients with long retention times may be present.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add about 70 ml of the mobile phase to a weighed quantity of the oral suspension containing 30 mg of Terfenadine and shake for 1 hour. Dilute to 100 ml with the mobile phase, mix, centrifuge and, if necessary, filter the supernatant liquid. Solution (2) contains 0.030% w/v of *terfenadine BPCRS* in the mobile phase. Solution (3) contains 0.015% w/v of *terfenadine impurity A EPCRS* in a mixture of equal volumes of solution (2) and the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 6 volumes of *diethylamine,* 350 volumes of *water,* 500 volumes of *acetonitrile* and 100 volumes of phosphate buffer prepared by mixing 136.1 g of *potassium dihydrogen orthophosphate* with 3.2 ml of *orthophosphoric acid* and adding sufficient *water* to produce 1000 ml and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is greater than 1.4.

Calculate the content of $C_{32}H_{41}NO_2$ using the declared content of $C_{32}H_{41}NO_2$ in *terfenadine BPCRS*. Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{32}H_{41}NO_2$, weight in volume.

Terfenadine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Terfenadine Tablets contain Terfenadine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of terfenadine, $C_{32}H_{41}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.2 g of Terfenadine with 20 ml of *dichloromethane* , add 10 ml of 0.1M *sodium hydroxide* and shake again. Wash the dichloromethane layer with 10 ml of *water*, shake with 2 g of *anhydrous sodium sulphate* and filter. Add 0.2 ml of the filtrate to 0.3 g of *potassium bromide* in a mortar, mix with a pestle, warm to remove the solvent and prepare a disc from the resulting mixture. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of terfenadine (*RS 392*).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using Apparatus 2 and rotating the paddle at 50 revolutions per minute. Use as the medium 1000 ml of 0.1M *hydrochloric acid.* Withdraw a sample of 20 ml of the medium and filter. Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dilute the filtered sample, if necessary, with 0.1M *hydrochloric acid* to give a solution expected to contain about 0.006% w/v of Terfenadine. For solution (2) dilute 1 volume of a 0.06% w/v solution of *terfenadine BPCRS* in *methanol* to 10 volumes with 0.1M *hydrochloric acid.*

The chromatographic procedure described under Assay may be used but with a detection wavelength of 217 nm.

Calculate the total content of $C_{32}H_{41}NO_2$ in the medium using the declared content of

$C_{32}H_{41}NO_2$ in *terfenadine BPCRS.*

1-[4-(1,1-Dimethylethyl)phenyl]-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butan-1one

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 0.15 g of Terfenadine in 75 ml of the mobile phase with the aid of ultrasound for 15 minutes, cool to room temperature, dilute to 100 ml with the mobile phase, mix and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) contains 0.0003% w/v of *terfenadine impurity A EPCRS* (1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butan-1-one) in the mobile phase. Solution (3) contains a mixture of 1 volume of solution (1) and 9 volumes of a 0.015% w/v solution containing *terfenadine impurity A EPCRS* in the mobile phase.

The chromatographic procedure described under Assay may be used but using a detection wavelength of 217 nm.

Inject 20 µl of each solution and continue the chromatography for 5 times the retention time of terfenadine. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to terfenadine and that due to terfenadine impurity A is at least 5.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to terfenadine impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%). In the chromatogram obtained with solution (1) peaks due to excipients with long retention times may be present.

ASSAY

Weigh and finely powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) disperse a quantity of the powdered tablets containing 0.15 g of Terfenadine in 75 ml of the mobile phase with the aid of ultrasound for 15 minutes, cool to room temperature, dilute to 100 ml with mobile phase, mix and filter through a glass microfibre filter (Whatman GF/C is suitable). Solution (2) contains 0.15% w/v of *terfenadine BPCRS* in the mobile phase. Solution (3) contains 0.015% w/v each of *terfenadine BPCRS* and *terfenadine impurity A EPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP8 is suitable), (b) 3 volumes of *acetonitrile* diluted to 5 volumes with *diethylammonium phosphate buffer solution pH 6.0* as the mobile phase with a flow rate of 1.0 ml per minute and (c) a detection wavelength of 254 nm.

Inject 20 µl of each solution and continue the chromatography for 5 times the retention times of terfenadine. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peak due to terfenadine and that due to terfenadine impurity A is at least 5.0. In the chromatogram obtained with solution (1) peaks due to excipients with long retention times may be present.

Calculate the content of $C_{32}H_{41}NO_2$ in the tablets using the declared content of $C_{32}H_{41}NO_2$ in *terfenadine BPCRS.*

Testosterone Implants

General Notices

Action and use

Androgen.

DEFINITION

Testosterone Implants are sterile cylinders prepared by the fusion or heavy compression of Testosterone without the addition of any other substance.

The implants comply with the requirements stated under Parenteral Preparations and with the following requirements.

Content of testosterone, $C_{19}H_{28}O_2$

97 to 103%, calculated with reference to the dried substance, and 90 to 110% of the stated amount.

Diameter

Implants containing less than 50 mg, 2.0 to 2.5 mm; implants containing 50 mg or more, 4.25 to 4.75 mm.

Where appropriate, powder the implants before carrying out the following tests.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of testosterone (*RS 329*).

B. Comply with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase D*.

C. To 0.1 g in a stoppered tube add 3 ml of *anhydrous pyridine* and 0.6 ml of *acetic anhydride*. Heat on a waterbath for 3 hours, add *water* dropwise until crystals begin to form, then add slowly a further 15 ml of *water* and allow to stand until precipitation is complete. Filter the precipitate using a sintered-glass crucible and wash with *water* until the washings are neutral to *methyl red solution*. Recrystallise from *ethanol* (*96%*), adding a few drops of *water* if necessary to aid crystallisation, and dry at 105°. The *melting point* of the crystals is about 140°, Appendix V A.

TESTS

Melting point

152° to 156°, Appendix V A.

Specific optical rotation

In a 1% w/v solution in *absolute ethanol* , +106 to +112, Appendix V F, calculated with reference to the dried substance.

Carbonisation

A 1% w/v solution in *ethanol* (*96%*) is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Loss on drying

When dried to constant weight at 105°, lose not more than 1.0% of their weight. Use 1 g.

Sulphated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Weigh and powder a single implant. Dissolve 10 mg of the powdered implant in sufficient *absolute ethanol* to produce 100 ml, dilute 5 ml to 50 ml with *absolute ethanol* and measure the *absorbance* of the resulting solution at the maximum at 240 nm, Appendix II B. Calculate the content of $C_{19}H_{28}O_2$ taking 560 as the value of A(1%, 1 cm) at the maximum at 240 nm.

STORAGE

Testosterone Implants should be protected from light.

Testosterone Propionate Injection

General Notices

Action and use

Androgen.

DEFINITION

Testosterone Propionate Injection is a sterile solution of Testosterone Propionate in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of testosterone propionate, $C_{22}H_{32}O_3$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Dissolve a volume containing 50 mg of Testosterone Propionate in 8 ml of *petroleum spirit* (*boiling range, 40° to 60°*) and extract with three 8-ml quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *petroleum spirit* (*boiling range, 40° to 60°*), dilute with *water* until the solution becomes turbid, allow to stand for 2 hours in ice and filter. The precipitate, after washing with *water* and drying at 105°, complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of testosterone propionate (*RS 332*).

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent III* and *mobile phase F*.

ASSAY

To a volume containing 0.1 g of Testosterone Propionate add sufficient *chloroform* to produce 100 ml. Dilute 3 ml to 50 ml with *chloroform* and to 5 ml of the solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20 ml. Allow to stand for 45 minutes and measure the *absorbance* of the resulting solution at the maximum at 380 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 ml of *chloroform* in the same manner. Calculate the content of $C_{22}H_{32}O_3$ from the *absorbance* obtained by repeating the operation using a 0.006% w/v solution of *testosterone propionate BPCRS* in *chloroform*, beginning at the words 'to 5 ml...' and using the declared content of $C_{22}H_{32}O_3$ in *testosterone propionate BPCRS*.

STORAGE

Testosterone Propionate Injection should be protected from light.

LABELLING

The label states that the preparation is intended for intramuscular injection only.

Tetracaine Eye Drops

General Notices

Action and use

Local anaesthetic.

DEFINITION

Tetracaine Eye Drops are a sterile solution of Tetracaine Hydrochloride in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of tetracaine hydrochloride, $C_{15}H_{24}N_2O_2,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

To a volume containing 10 mg of Tetracaine Hydrochloride add 1 ml of a 5% w/v solution of *sodium acetate* and 1 ml of a 25% w/v solution of *ammonium thiocyanate* and mix. A white precipitate is produced which, after recrystallisation from *water* and drying at 80° for 2 hours, has a *melting point* of about 131°, Appendix V A.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF $_{254}$* as the coating substance and a mixture of 80 volumes of *dibutyl ether*, 16 volumes of *n-hexane* and 4 volumes of *glacial acetic acid* as the mobile phase. Place the plate in the tank, allow to stand until the solvent front has ascended about 12 cm, remove the plate and dry for a few minutes in a current of warm air. Allow to cool and apply separately to the plate 20 µl of solution (1) and 5 µl of solution (2). For solution (1) use the eye drops, diluted if necessary to contain 0.25% w/v of Tetracaine Hydrochloride. Solution (2) contains 0.0050% w/v of *4-aminobenzoic acid*. Allow the solvent front to ascend 10 cm above the line of application. After removal of the plate, dry it at 100° to 105° for 10 minutes and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). The principal spot in the chromatogram obtained with solution (1) remains on the line of application.

ASSAY

To a volume of the eye drops containing 20 mg of Tetracaine Hydrochloride add 5 ml of *acetate buffer pH 3.7* and 15 ml of 0.01M *sodium tetraphenylborate VS*, mix well, allow to stand for 10 minutes, filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable), wash the filter with 5 ml of *water* and titrate the excess of sodium tetraphenylborate with 0.005M *cetylpyridinium chloride VS* using 0.5 ml of *bromophenol blue solution* as indicator. Repeat the operation without the eye drops. The difference between the titrations represents the amount of sodium tetraphenylborate required. Each ml of 0.01M *sodium tetraphenylborate VS* is equivalent to 3.008 mg of $C_{15}H_{24}N_2O_2$,HCl.

STORAGE

Tetracaine Eye Drops should be protected from light.

Tetracosactide Injection

General Notices

Action and use

Corticotrophic peptide.

DEFINITION

Tetracosactide Injection is a sterile solution of Tetracosactide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of tetracosactide, $C_{136}H_{210}N_{40}O_{31}$

80.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

Mix 24 ml of *pyridine* and 16 ml of *glacial acetic acid* and add sufficient *water* to produce 100 ml (solution A). Carry out the method for *zone electrophoresis*, Appendix III F, using cellulose acetate foil as the support medium and an electrolyte solution containing 8% v/v of *glacial acetic acid* and 2% v/v of *pyridine*. Immerse the cellulose acetate foil in the electrolyte solution for 5 minutes and press dry between sheets of filter paper. Apply separately to the foil at points 1 cm from the anode edge and 2.5 cm apart 2 µl of each of the following solutions. For solution (1) evaporate to dryness a volume of the injection containing 0.5 mg of the peptide and dissolve the residue in 0.1 ml of solution A with the aid of gentle heat. For solution (2) dissolve 3.5 mg of *tetracosactide EPCRS* in 10 ml of *water* containing 10 µl of *glacial acetic acid*, 8.2 mg of *sodium acetate* and 81 mg of *sodium chloride*, evaporate 2 ml of the solution and dissolve the residue in 0.1 ml of solution A with the aid of gentle heat. Apply a voltage of 100 volts and allowing electrophoresis to proceed for 2 hours. Press the strips dry and immerse in a solution prepared by dissolving 1 g of *potassium hexacyanoferrate*(*III*) in 50 ml of *water* and adding 2 ml of a saturated solution of *iron* (*III*) *chloride hexahydrate*. Wash with a 5% v/v solution of *orthophosphoric acid* until the background is as pale as possible and finally wash with *water*. Examine the electrophoretograms while still moist. The principal spot in the electrophoretogram obtained with solution (1) corresponds to that in the electrophoretogram obtained with solution (2).

TESTS

Acidity

pH, 3.8 to 4.5, Appendix V L.

Tetracosactide sulphoxide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared with de-aerated solvents. For solution (1) dilute the injection, if necessary, with *water* to give a final concentration of 0.025% w/v of the peptide. For solution (2) add 10 µl of a solution prepared by diluting 1 volume of *hydrogen peroxide solution* (*20 vol*) to 200 volumes with *water* to 1 ml of solution (1) and allow to stand for 2 hours.

The chromatographic conditions described under Assay may be used. Take care to ensure that the syringe used to inject solution (1) is not contaminated with peroxide.

The chromatogram obtained with solution (2) exhibits a peak due to tetracosactide corresponding to the principal peak in the chromatogram obtained with solution (1) and a peak with a shorter retention time, due to tetracosactide sulphoxide, of significantly greater area than any corresponding peak in the chromatogram obtained with solution (1). In the chromatogram obtained with solution (1) the relative amount of tetracosactide sulphoxide is not more than 10% by *normalisation*.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute the injection, if necessary, with *water* to give a final concentration of 0.025% w/v of the peptide. For solution (2) dissolve an amount of *tetracosactide EPCRS* in sufficient *water* to produce a solution containing 0.025% w/v of the peptide.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) a mixture of 10 ml of *glacial acetic acid*, 365 ml of *acetonitrile* and 10 g of *ammonium sulphate* diluted to 2000 ml with *water* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 280 nm.

Calculate the content of the peptide $C_{136}H_{210}N_{140}O_{31}S$ from the chromatograms obtained and using the declared content of $C_{136}H_{210}N_{140}O_{31}S$ in *tetracosactide EPCRS*.

The result obtained is not valid unless the *resolution factor* between the peaks due to tetracosactide and tetracosactide sulphoxide in the chromatogram obtained with solution (1) in the test for Tetracosactide sulphoxide is at least 7.

STORAGE

Tetracosactide Injection should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The strength is stated in terms of the equivalent amount of the peptide in micrograms per ml.

The label also states that the preparation is for intravenous infusion or intramuscular injection only.

Tetracosactide Zinc Injection

General Notices

Action and use

Corticotrophic peptide.

DEFINITION

Tetracosactide Zinc Injection is a sterile aqueous suspension of Tetracosactide with zinc hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of tetracosactide, $C_{136}H_{210}N_{40}O_{31}$

80.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A white, flocculent suspension which settles slowly and is readily resuspended. On examination under a microscope the majority of the particles are seen as amorphous or microcrystalline particles or aggregates thereof. The maximum dimension of single particles rarely exceeds 50 µm. Under high power magnification a considerable proportion of the particles can be seen to have no uniform shape.

IDENTIFICATION

A. Carry out the test for Identification described under Tetracosactide Injection using the following solutions. For solution (1) freeze dry a quantity of the well-shaken suspension containing 2 mg of the peptide and dissolve the residue in a mixture of 0.1 ml of *formic acid* and 0.1 ml of *water* with the aid of gentle heat. For solution (2) dissolve 10.45 g of *zinc chloride* in 20 ml of *water*, add 95 ml of *formic acid* followed by 4.2 g of *disodium hydrogen orthophosphate* and 4.0 g of *sodium chloride* . Dissolve 6.04 g of *sodium hydroxide* in 40 ml of *water*. Mix the two solutions carefully with cooling and add sufficient *water* to produce 200 ml. Dissolve 2.8 mg of *tetracosactide EPCRS* in 0.2 ml of the resulting solution. The principal spot in the electrophoretogram obtained with solution (1) corresponds to that in the electrophoretogram obtained with solution (2).

B. Evaporate 1 ml of the well-shaken suspension to dryness in a crucible and heat strongly until combustion of the organic material is complete. The residue is yellow while hot and becomes white on cooling.

TESTS

Alkalinity

pH, 7.8 to 9.2, Appendix V L.

Light absorption

Centrifuge 7 ml of the well-shaken suspension for 10 minutes at about 2000 *g*. Shake the clear, supernatant liquid with five 5 ml quantities of *chloroform*, previously washed with *water*. Discard the chloroform and centrifuge the aqueous phase for 5 minutes at about 2000 g. The *absorbance* of the clear, supernatant liquid at 276 nm, Appendix II B, is not more than 0.38 for preparations containing 1 mg of the peptide per ml and not more than 0.19 for preparations containing 0.5 mg of the peptide per ml.

Sediment volume

Transfer 3.0 ml of the well-shaken suspension to a cuvette 10 mm × 10 mm in cross section. Allow to stand for 5 hours. The depth of the sediment is between 8 and 25 mm and the supernatant liquid is clear.

Zinc

To a volume of the well-shaken suspension containing 1 mg of the peptide add 5 ml of 0.1M *hydrochloric acid* and sufficient *water* to produce 1000 ml. Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, measuring at 214 nm and using *zinc standard solution* (*5 mg/ml Zn*), diluted if necessary with *water*, to prepare the standard solution. Preparations containing 1 mg of the peptide per ml contain 2.25 to 2.75 mg of zinc per ml; preparations containing 0.5 mg of the peptide per ml contain 1.35 to 1.65 mg of zinc per ml.

Tetracosactide sulphoxide

Carry out the test described under Tetracosactide Injection using the following solutions. For solution (1) mix a volume of the well-shaken suspension containing 1 mg of the peptide with 15 µl of *glacial acetic acid*. For solution (2) add 50 µl of a solution prepared by diluting 1 volume of *hydrogen peroxide solution* (*20 vol*) to 200 volumes to 1 ml of a 0.1% w/v solution of *tetracosactide EPCRS* in a 1% v/v solution of *glacial acetic acid* and allow to stand for 2 hours. The chromatogram obtained with solution (2) exhibits a peak due to tetracosactide and a significant peak with a shorter retention time, due to tetracosactide sulphoxide. In the chromatogram obtained with solution (1) the area of the peak due to tetracosactide sulphoxide is not more than 18% of the area of the peak due to tetracosactide. The peaks due to associated substances may be identified from the chromatogram obtained with solution (2) in the Assay.

ASSAY

Carry out the Assay described under Tetracosactide Injection using the following solutions. For solution (1) mix 1 ml of the well-shaken suspension with 15 µl of *glacial acetic acid*. For preparations containing 1 mg of the peptide per ml prepare solution (2) by dissolving quantities of *tetracosactide EPCRS* and *benzyl alcohol* in sufficient *water* to produce a solution containing the equivalent of 0.10% w/v of the peptide and 1.0% w/v of *benzyl alcohol* and adding 15 µl of *glacial acetic acid* per ml. For preparations containing 0.5 mg of the peptide per ml prepare solution (2) by dissolving quantities of *tetracosactide EPCRS* and *benzyl alcohol* in sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of the peptide and 1.0% w/v of *benzyl alcohol* and adding 15 µl of *glacial acetic acid* per ml. If necessary, adjust the ratio of the volumes of *acetonitrile* and *water* in the mobile phase so that in the chromatogram obtained with solution (2) the *resolution factor* between the peaks due to benzyl alcohol (eluted before tetracosactide) and tetracosactide is between 1.5

and 2.0.

STORAGE

Tetracosactide Zinc Injection should be protected from light and stored at a temperature of 2° to 8°. It should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of the peptide in mg per ml.

The label also states (1) that the preparation is for intramuscular injection only; (2) that the container should be gently shaken before a dose is withdrawn.

Tetracycline Capsules

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Tetracycline Capsules contain Tetracycline Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance. Adjust the pH of a 10% w/v solution of *disodium edetate* to 8.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 ml for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position at room temperature for at least 1 hour. Before use, dry the plate in an oven at 110° for 1 hour. Use a mixture of 6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 1 µl of each of the following solutions. For solution (1) extract a quantity of the contents of the capsules containing 10 mg of Tetracycline Hydrochloride with 20 ml of *methanol* and centrifuge. Solution (2) contains 0.05% w/v of *tetracycline hydrochloride BPCRS* in *methanol*. Solution (3) contains 0.05% w/v of each of *tetracycline hydrochloride BPCRS*, *chlortetracycline hydrochloride BPCRS* and *doxycycline hyclate BPCRS* in *methanol*. Dry the plate in a current of air and examine under *ultraviolet light* (*365 nm*). The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

B. To a quantity of the contents of the capsules containing 10 mg of Tetracycline Hydrochloride add 20 ml of warm *ethanol* (*96%*), allow to stand for 20 minutes, filter and evaporate the filtrate to dryness on a water bath. To 0.5 mg of the residue add 2 ml of *sulphuric acid*; a deep crimson colour is produced. Add 1 ml of *water*; the colour changes to deep yellow.

C. The residue obtained in test B yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 353 nm, Appendix II B. Calculate the total content of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8$,HCl, in the medium taking 310 as the value of A(1%, 1 cm) at the maximum at 353 nm.

4-Epitetracycline hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 25 mg of Tetracycline Hydrochloride in 80 ml of 0.01M *methanolic hydrochloric acid* for 10 minutes, dilute to 100 ml with the same solvent, mix and filter if necessary. Solution (2) contains 0.0020% w/v of *4-epitetracycline hydrochloride EPCRS* in 0.01M *methanolic hydrochloric acid*. Solution (3) contains 0.0015% w/v each of *4-epitetracycline hydrochloride EPCRS* and *tetracycline hydrochloride BPCRS* in 0.01M *methanolic hydrochloric acid*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 5 volumes of *dimethylformamide* and 95 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 3.9 with *triethylamine* and (c) a detection wavelength of 280 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to 4-epitetracycline is not greater than the area of the principal peak in the chromatogram obtained with solution (2).

Anhydrotetracycline hydrochloride and 4-epi-anhydrotetracycline hydrochloride

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 25 mg of Tetracycline Hydrochloride in 20 ml of 0.01M *methanolic hydrochloric acid* for 10 minutes, dilute to 25 ml with the same solvent, mix and filter if necessary. Solution (2) contains 0.0010% w/v each of *anhydrotetracycline hydrochloride EPCRS* and *4-epianhydrotetracycline hydrochloride EPCRS* in 0.01M *methanolic hydrochloric acid*. Solution (3) contains 0.0010% w/v each of *4-epianhydrotetracycline hydrochloride EPCRS* and *tetracycline hydrochloride BPCRS*.

The chromatographic conditions described under Epitetracycline hydrochloride may be used but use as the mobile phase a mixture of 10 volumes of *dimethylformamide*, 12 volumes of *acetonitrile* and 78 volumes of 0.1M *oxalic acid* the pH of which has been adjusted to 3.9 with *triethylamine*. The order of emergence of the peaks is tetracycline, 4-epianhydrotetracycline and anhydrotetracycline.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 6.0.

In the chromatogram obtained with solution (1) the area of any peaks corresponding to anhydrotetracycline hydrochloride and 4-epianhydrotetracycline hydrochloride is not greater than the area of the respective principal peak in the chromatogram obtained with solution (2).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the contents of the capsules lose not more than 3.0% of their weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the mixed contents of 20 capsules containing 25 mg of Tetracycline Hydrochloride in 80 ml of 0.01M *methanolic hydrochloric acid* for 10 minutes, dilute to 100 ml with the same solvent, mix and filter if necessary. Solution (2) contains 0.025% w/v of *tetracycline hydrochloride BPCRS* in 0.01M *methanolic hydrochloric acid*. Solution (3) contains 0.025% w/v each of 4-*epitetracycline hydrochloride EPCRS* and *tetracycline hydrochloride BPCRS* in 0.01M *methanolic hydrochloric acid*.

The chromatographic conditions described under 4-Epitetracycline hydrochloride may be used.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 2.0.

Calculate the content of $C_{22}H_{24}N_2O_8$,HCl in the capsules using the declared content of $C_{22}H_{24}N_2O_8$,HCl in *tetracycline hydrochloride BPCRS*.

Tetracycline Tablets

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Tetracycline Tablets contain Tetracycline Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Comply with test A described under Tetracycline Capsules using the following solution as solution (1). Extract a quantity of the powdered tablets containing 10 mg of Tetracycline Hydrochloride with 20 ml of *methanol* , centrifuge and use the supernatant liquid.

B. Extract a quantity of the powdered tablets containing 25 mg of Tetracycline Hydrochloride with 25 ml of *methanol* for 20 minutes, filter and evaporate the filtrate to dryness on a water bath. To 0.5 mg of the residue add 2 ml of *sulphuric acid* ; a purplish red colour is produced. Add 1 ml of *water*; the colour changes to deep yellow.

C. The residue obtained in test B yields the reactions characteristic of *chlorides* , Appendix VI.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using as the medium 900 ml of 0.1M *hydrochloric acid* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 353 nm, Appendix II B. Calculate the total content of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8$,HCl, in the medium taking 310 as the value of A(1%, 1 cm) at the maximum at 353 nm.

4-Epitetracycline hydrochloride

Complies with test described under Tetracycline Capsules. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Tetracycline Hydrochloride with 80 ml of 0.01M *methanolic hydrochloric acid* , dilute to 100 ml with the same solvent, mix and filter if necessary.

Anhydrotetracycline hydrochloride and 4-epianhydro-tetracycline hydrochloride

Complies with the test described under Tetracycline Capsules. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Tetracycline Hydrochloride with 20 ml of 0.01M *methanolic hydrochloric acid* , dilute to 25 ml with the same solvent, mix and filter if necessary.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, the powdered tablets lose not more than 3.0% of their weight. Use 1 g.

ASSAY

Weigh and powder 20 tablets and carry out the Assay described under Tetracycline Capsules. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Tetracycline Hydrochloride with 80 ml of 0.01M *methanolic hydrochloric acid* , dilute to 100 ml with the same solvent, mix and filter if necessary.

Calculate the content of $C_{22}H_{24}N_2O_8$,HCl in the tablets using the declared content of $C_{22}H_{24}N_2O_8$,HCl in *tetracycline hydrochloride BPCRS*.

Thiamine Injection

General Notices

Action and use

Vitamin B_1.

DEFINITION

Thiamine Injection is a sterile solution of Thiamine Hydrochloride in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS,HCl$

95.0 to 105.0% of the stated amount.

CHARACTERISTICS

A colourless or almost colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *cellulose* F_{254} as the coating substance and a mixture of 60 volumes of *butan-1-ol*, 25 volumes of *water* and 15 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) use the injection diluted if necessary with *water* to contain 0.1% w/v of Thiamine Hydrochloride. Solution (2) contains 0.1% w/v of *thiamine mononitrate BPCRS* in *water*. After removal of the plate, allow it to dry in air, heat at 105° for 30 minutes, spray with a mixture of equal volumes of a 0.3% w/v solution of *potassium hexacyanoferrate*(*III*) and a 10% w/v solution of *sodium hydroxide* and examine under *ultraviolet light* (*365 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. To a volume containing 20 mg of Thiamine Hydrochloride diluted, if necessary, to 10 ml with *water*, add 2 ml of 1M *acetic acid* and 1.6 ml of 1M *sodium hydroxide*, heat in a water bath for 30 minutes and cool. Add 5 ml of 5M *sodium hydroxide*, 10 ml of *dilute potassium hexacyano-ferrate*(*III*) *solution* and 10 ml of *butan-1-ol* and shake vigorously for 2 minutes. The upper layer shows an intense light blue fluorescence on exposure to ultraviolet light. Repeat the test but adding 0.9 ml of 1M *sodium hydroxide* and 0.2 g of *sodium sulphite* in place of the 1.6 ml of 1M *sodium hydroxide*. Not more than a slight fluorescence is produced.

C. To a mixture of 0.1 ml of *nitrobenzene* and 0.2 ml of *sulphuric acid* add a volume of the injection containing 5 mg of Thiamine Hydrochloride. Allow to stand for 10 minutes, cool in ice and add slowly with stirring 5 ml of *water* followed by 5 ml of 10M *sodium hydroxide*.

Add 5 ml of *acetone* and allow to stand. No violet colour is produced in the upper layer.

TESTS

Acidity

pH, 2.8 to 3.4, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.005% w/v of *thiamine mononitrate BPCRS* in 0.005M *hydrochloric acid*. For solution (2) dilute a volume of the injection containing 0.1 g of Thiamine Hydrochloride to 100 ml with 0.1M *hydrochloric acid* and further dilute 5 ml to 100 ml with *water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute a solution prepared by dissolving 1 g of *sodium heptane-sulphonate* in a mixture of 180 ml of *methanol* and 10 ml of *triethylamine*, diluting to 1000 ml with *water* and adjusting the pH to 3.2 with *orthophosphoric acid* and (c) a detection wavelength of 244 nm.

Calculate the content of $C_{12}H_{17}ClN_4OS,HCl$ using the declared content of $C_{12}H_{17}N_5O_4S$ in *thiamine mononitrate BPCRS*. Each mg of $C_{12}H_{17}N_5O_4S$ is equivalent to 1.030 mg of $C_{12}H_{17}ClN_4OS,HCl$.

STORAGE

Thiamine Injection should be protected from light.

When vitamin B_1 injection is prescribed or demanded, Thiamine Injection shall be dispensed or supplied.

Thiamine Tablets

General Notices

Action and use

Vitamin B_1.

DEFINITION

Thiamine Tablets contain Thiamine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS,HCl$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *cellulose* F_{254} as the coating substance and a mixture of 60 volumes of *butan-1-ol*, 25 volumes of *water* and 15 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 20 mg of Thiamine Hydrochloride with 20 ml of *water* and filter. Solution (2) contains 0.1% w/v of *thiamine mononitrate BPCRS*. After removal of the plate, allow it to dry in air, heat at 105° for 30 minutes, spray with a mixture of equal volumes of a 0.3% w/v solution of *potassium hexacyanoferrate*(*III*) and a 10% w/v solution of *sodium hydroxide* and examine under *ultraviolet light* (*365 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. Dissolve a quantity of the powdered tablets containing 20 mg of Thiamine Hydrochloride as completely as possible in 10 ml of *water* and 2 ml of 1M *acetic acid*, filter, add 1.6 ml of 1M *sodium hydroxide*, heat in a water bath for 30 minutes and cool. Add 5 ml of 5M *sodium hydroxide*, 10 ml of *dilute potassium hexacyanoferrate*(*III*) *solution* and 10 ml of *butan-1-ol* and shake vigorously for 2 minutes. The alcohol layer shows an intense light blue fluorescence on exposure to ultraviolet light. Repeat the test but adding 0.9 ml of 1M *sodium hydroxide* and 0.2 g of *sodium sulphite* in place of the 1.6 ml of 1M sodium hydroxide; not more than a slight fluorescence is produced.

ASSAY

Weigh and powder 20 tablets. Carry out the Assay described under Thiamine Injection using the following solutions. Solution (1) contains 0.006% w/v of *thiamine mononitrate BPCRS* in 0.005M *hydrochloric acid*. Prepare solution (2) in the following manner. For tablets containing less than 10 mg of thiamine hydrochloride, add 5 ml of 0.1M *hydrochloric acid* and 50 ml of *water* to a quantity of the powdered tablets containing 6 mg of Thiamine Hydrochloride, shake for 20 minutes, dilute to 100 ml with *water* and filter. For tablets containing 10 mg or more of Thiamine Hydrochloride, add 50 ml of 0.1M *hydrochloric acid* and 500 ml of *water* to a quantity of the powdered tablets containing 60 mg of Thiamine Hydrochloride, shake for 20 minutes, dilute to 1000 ml with *water* and filter.

STORAGE

Thiamine Tablets should be kept free from contact with metal and protected from light.

When vitamin B_1 tablets are prescribed or demanded, Thiamine Tablets shall be dispensed or supplied.

Thiopental Injection

General Notices

Action and use

Intravenous barbiturate; general anaesthetic.

DEFINITION

Thiopental Injection is a sterile solution of Thiopental Sodium in Water for Injections. It is prepared by dissolving Thiopental Sodium for Injection in the requisite amount of Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Thiopental Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

THIOPENTAL SODIUM FOR INJECTION

DEFINITION

Thiopental Sodium for Injection is a sterile material consisting of Thiopental Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of $C_{11}H_{18}N_2O_2S$

77.0 to 92.0% of the stated amount of Thiopental Sodium.

Content of Na

9.4 to 11.8% of the stated amount of Thiopental Sodium.

IDENTIFICATION

A. Dissolve 0.1 g in 5 ml of *water*, add 1 ml of 2M *hydrochloric acid* and extract with two 25 ml quantities of *chloroform*. Wash the extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dry the residue at 50° at a pressure of 2 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of thiopental (*RS 334*).

B. Yield the reaction characteristic of *non-nitrogen substituted barbiturates*, Appendix VI.

C. Yield reaction A characteristic of *sodium salts* , Appendix VI.

TESTS

Clarity and colour of solution

A 10.0% w/v solution in *carbon dioxide-free water* is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* GY_3 , Appendix IV B, Method II.

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel* GF_{254} as the coating substance and the lower layer of a mixture of 5 volumes of 13.5M *ammonia,* 15 volumes of *ethanol* (*96%*) and 80 volumes of *chloroform* as the mobile phase. Apply separately to the plate 20 µl of each of two solutions of the substance being examined in *water* containing (1) 1.0% w/v and (2) 0.005% w/v. Disregard any slight residue in solution (1). After removal of the plate, examine it immediately under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). Disregard any spot remaining on the line of application.

Loss on drying

When dried at 100° at a pressure not exceeding 2.7 kPa for 4 hours, lose not more than 2.5% of their weight. Use 0.5 g.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections. Carry out the following procedures using the mixed contents of the 10 containers.

For Na

Dissolve 0.6 g in 20 ml of *water* and titrate with 0.1M *hydrochloric acid VS* , using *methyl red solution* as indicator, until the yellow colour changes to pink; boil gently for 1 or 2 minutes, cool and if necessary continue the titration with 0.1M *hydrochloric acid VS* until the pink colour is restored. Each ml of 0.1M *hydrochloric acid VS* is equivalent to 2.299 mg of Na.

For $C_{11}H_{18}N_2O_2S$

To the liquid from the completed Assay for Na add a further 5 ml of 0.1M *hydrochloric acid* and extract with successive quantities of 25, 25, 20, 15, 15 and 10 ml of *chloroform* , washing each extract with the same 5 ml of *water*. Evaporate the chloroform from the mixed extracts and dry the residue of $C_{11}H_{18}N_2O_2S$ to constant weight at 105°. Calculate the content of $C_{11}H_{18}N_2O_2S$ in a container of average content weight.

LABELLING

The label of the sealed container states the weight of Thiopental Sodium contained in it.

Thiotepa Injection

General Notices

Action and use

Cytotoxic alkylating agent.

DEFINITION

Thiotepa Injection is a sterile solution of Thiotepa in Water for Injections. It is prepared by dissolving Thiotepa for Injection in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Thiotepa Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions. If solid matter separates, the solution should not be used.

THIOTEPA FOR INJECTION

DEFINITION

Thiotepa for Injection is a sterile material consisting of Thiotepa with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of thiotepa, $C_6H_{12}N_3PS$

95.0 to 110.0% of the stated amount.

CHARACTERISTICS

A white powder.

IDENTIFICATION

Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 5 ml of 1.25M *sodium hydroxide* as the absorbing liquid. When the process is complete, dilute to 25 ml with *water*. The resulting solution complies with the following tests.

A. To 5 ml add 0.1 ml of *hydrogen peroxide solution* (*100 vol*) and 1 ml of 1M *hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*. The solution becomes turbid.

B. To 2 ml add 40 ml of *water* and 4 ml of *ammonium molybdate-sulphuric acid solution*, mix, add 0.1 g of L-*ascorbic acid* and boil for 1 minute. A blue colour is produced.

TESTS

Acidity or alkalinity

Dissolve a quantity containing 20 mg of Thiotepa in 2 ml of *carbon dioxide-free water*. The pH of the resulting solution is 5.5 to 7.5, Appendix V L.

Clarity of solution

Dissolve a quantity containing 15 mg of Thiotepa in 4 ml of *water*. The solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. For solution (1) dissolve, with shaking, a quantity of the contents of the sealed container containing 15 mg of Thiotepa in 4 ml of *water*, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *water* and further dilute 1 volume to 10 volumes with *water*. For solution (3) dissolve 10 mg of *thiotepa BPCRS* in 2 ml of *methanol* in a ground-glass-stoppered tube, add 50 µl of a 0.1% v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (generation of methoxy-thiotepa). Allow the solution to cool and add 1 ml of *methanol*. For solution (4) dissolve 15 mg of *thiotepa BPCRS* in 10 ml of *water*, add 1 g of *sodium chloride*, boil in a water bath for 10 minutes and cool (generation of chloro-adduct).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) 15 volumes of *acetonitrile* and 85 volumes of 0.1M *mixed phosphate buffer pH 7.0* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 215 nm.

The chromatogram obtained with solution (3) shows a peak corresponding to methoxy-thiotepa with a retention time relative to thiotepa of about 1.3 and the chromatogram obtained with solution (4) shows a peak corresponding to the chloro-adduct with a retention time relative to thiotepa of about 3.75. The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 3.

For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to the 'chloro-adduct' (identified from the peak in the chromatogram obtained with solution (4)) is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%), the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%), the area of not more than two such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of all the *secondary peaks* is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of a sealed container in sufficient *water* to produce a solution containing 0.15% w/v of Thiotepa. Solution (2) contains 0.15% w/v of *thiotepa BPCRS*. For solution (3) dissolve 10 mg of *thiotepa BPCRS* in 2 ml of *methanol* in a ground-glass-stoppered tube, add 50 µl of a 0.1% v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (to produce a sufficient quantity of methoxy-thiotepa). Allow the solution to cool and add 1 ml of *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) 15 volumes of *acetonitrile* and 85 volumes of *0.1 M mixed phosphate buffer pH 7.0* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 215 nm.

The chromatogram obtained with solution (3) shows a peak corresponding to methoxy-thiotepa with a retention time relative to thiotepa of about 1.3. The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 3.

Calculate the amount of $C_6H_{12}N_3PS$ in the sealed container using the declared content of $C_6H_{12}N_3PS$ in *thiotepa BPCRS*.

Repeat the procedure with a further nine sealed containers. Calculate the average content of $C_6H_{12}N_3PS$ per container from the 10 individual results thus obtained.

STORAGE

The sealed container should be stored at a temperature of 2° to 8°.

LABELLING

The label of the sealed container states the amount of Thiotepa contained in it.

Tiabendazole Tablets

General Notices

Action and use

Benzimidazole antihelminthic.

DEFINITION

Tiabendazole Tablets contain Tiabendazole.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tiabendazole, $C_{10}H_7N_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dissolve a quantity of the powdered tablets containing 20 mg of Tiabendazole in 5 ml of 0.1M *hydrochloric acid* , add 3 mg of p-*phenylenediamine dihydrochloride* and shake until dissolved. Add 0.1 g of *zinc powder* , mix, allow to stand for 2 minutes and add 10 ml of *ammonium iron* (*III*) *sulphate solution R2*. A deep blue or bluish violet colour is produced.

TESTS

Disintegration

The requirement for Disintegration does not apply to Tiabendazole Tablets.

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel HF* $_{254}$ as the coating substance and a mixture of 2.5 volumes of *water*, 10 volumes of *acetone* , 25 volumes of *glacial acetic acid* and 62.5 volumes of *toluene* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.5 g of Tiabendazole with 50 ml of 0.1M *methanolic hydrochloric acid* , adjust the pH to 12 with 1M *methanolic sodium hydroxide* and filter (Whatman GF/C filter paper is suitable). For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol* . For solution (3) dilute 1 volume of solution (1) to 250 volumes with *methanol* . After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.4%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Tiabendazole add 75 ml of 0.1M *hydrochloric acid*, warm on a water bath for 15 minutes, shaking occasionally, cool, dilute to 100 ml with 0.1M *hydrochloric acid* and filter. Dilute 5 ml of the filtrate to 1000 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_{10}H_7N_3S$ taking 1230 as the value of A(1%, 1 cm) at the maximum at 302 nm.

LABELLING

The label states that the tablets should be chewed before swallowing.

Timolol Eye Drops

General Notices

Action and use

Beta-adrenoceptor antagonist; treatment of glaucoma.

DEFINITION

Timolol Eye Drops are a sterile solution of Timolol Maleate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of timolol, $C_{13}H_{24}N_4O_3S$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Add a volume of the eye drops containing the equivalent of 50 mg of timolol to an equal volume of *carbonate buffer pH 9.7* and extract with two 40 ml quantities of *dichloromethane* . Reserve the aqueous layer for test B, dry the extracts with *anhydrous sodium sulphate* , evaporate to dryness using a rotary evaporator and dry at 60 ° under reduced pressure for 15 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of timolol (*RS 339*).

B. Evaporate the aqueous solution reserved in test A to about 1 ml. Add 1 ml of *bromine solution* , heat in a water bath for 10 minutes, boil, cool and add 0.1 ml of the solution to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid* . A bluish black colour is produced on heating in a water bath for 15 minutes.

TESTS

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using 20 µl of each of the following solutions. For solution (1) dilute 1 volume of the eye drops to 250 volumes with the mobile phase. For solution (2) use the eye drops undiluted. For solution (3) dilute 1 volume of the eye drops to 500 volumes with the mobile phase. Solution (4) contains 0.30% w/v of *maleic acid* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm

× 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Nucleosil C18 is suitable), (b) as the mobile phase a mixture of 42.5 volumes of 0.02M *sodium octanesulphonate* and 57.5 volumes of *methanol*, adjusted to pH 3.0 using *glacial acetic acid*, with a flow rate of 2 ml per minute and (c) a detection wavelength of 295 nm. For solution (2) record the chromatogram for 4 times the retention time of the principal peak.

In the chromatogram obtained with solution (2) the area of any *secondary peak*, other than the peak corresponding to maleic acid, is not greater than the area of the peak obtained with solution (1) (0.4%) and not more than two such peaks have an area greater than that of the peak obtained with solution (3) (0.2%).

ASSAY

Dilute a volume containing the equivalent of 25 mg of timolol to 50 ml with *water*. To 5 ml add 15 ml of *carbonate buffer pH 9.7* and extract with three 20 ml quantities and one 10 ml quantity of *toluene*. Wash each extract successively with the same 10 ml volume of *carbonate buffer pH 9.7*, combine the toluene extracts and extract with four 20 ml quantities of 0.05M *sulphuric acid*. Combine the extracts, dilute to 100 ml, filter and measure the *absorbance* at the maximum at 295 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 ml of *water* in the same manner, beginning at the words 'add 15 ml...'. Calculate the content of $C_{13}H_{24}N_4O_3S$ taking 279 as the value of A(1%, 1 cm) at the maximum at 295 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of timolol.

Timolol Tablets

General Notices

Action and use

Beta-adrenoceptor antagonist.

DEFINITION

Timolol Tablets contain Timolol Maleate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of timolol maleate, $C_{13}H_{24}N_4O_3S,C_4H_4O_4$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel GF$_{254}$ plate* (Analtech Uniplates are suitable) and a mixture of 1 volume of 18M *ammonia*, 19 volumes of *methanol* and 80 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 30 mg of Timolol Maleate with 2 ml of 0.1M *hydrochloric acid* until a smooth paste is formed, add 5 ml of *methanol* and continue shaking for about 20 minutes. Add sufficient *methanol* to produce 50 ml, centrifuge until a clear solution is produced and use the supernatant liquid. For solution (2) dissolve, with shaking, 60 mg of *timolol maleate EPCRS* in 4 ml of 0.1M *hydrochloric acid* and add sufficient *methanol* to produce 100 ml. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). Disregard any spot remaining on the line of application (maleic acid).

B. To a quantity of the powdered tablets containing 70 mg of Timolol Maleate add 20 ml of *carbonate buffer pH 9.7* and extract with two 40-ml quantities of *dichloromethane*. Filter the aqueous layer and evaporate to about 1 ml. Add 1 ml of *bromine solution*, heat in a water bath for 10 minutes, boil, cool and add 0.1 ml of the solution to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*. A bluish black colour is produced on heating in a water bath for 15 minutes.

TESTS

Related substances

Comply with the test described under Timolol Eye Drops, but preparing solutions (1), (2) and (3) in the following manner. For solution (1) dilute 1 volume of solution (2) to 250 volumes with the mobile phase. For solution (2) shake a quantity of the powdered tablets containing 0.1 g of

Timolol Maleate with 10 ml of the mobile phase for 10 minutes and filter. For solution (3) dilute 1 volume of solution (2) to 500 volumes with the mobile phase.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 15 mg of Timolol Maleate add 25 ml of 0.05M *sulphuric acid*, shake for 20 minutes and centrifuge until clear. Add 5 ml of the resulting supernatant liquid to 15 ml of *carbonate buffer pH 9.7* and extract with three 20 ml quantities and one 10 ml quantity of *toluene*. Wash each extract successively with the same 10 ml volume of *carbonate buffer pH 9.7*, combine the toluene extracts and extract with four 20 ml quantities of 0.05M *sulphuric acid*. Combine the acid extracts, dilute to 100 ml, filter and measure the *absorbance* at the maximum at 295 nm, Appendix II B, using in the reference cell a solution prepared by treating a mixture of 5 ml of *water* and 15 ml of *carbonate buffer pH 9.7* in the same manner. Calculate the content of $C_{13}H_{24}N_4O_3S,C_4H_4O_4$ taking 204 as the value of A(1%, 1 cm) at the maximum at 295 nm.

Tioconazole Cream

General Notices

Action and use

Antifungal.

DEFINITION

Tioconazole Cream contains Tioconazole in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of tioconazole, $C_{16}H_{13}Cl_3N_2OS$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and, as the mobile phase, the upper layer obtained from a mixture of 40 volumes of *glacial acetic acid*, 40 volumes of *water* and 80 volumes of *4-methylpentan-2-one*. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add to a quantity of the cream containing 10 mg of Tioconazole 3 ml of n- *hexane* and 10 ml of *dichloromethane,* mix with the aid of ultrasound for 5 minutes and centrifuge. Shake 3 ml of the lower layer with 9 ml of 0. 5M *sulphuric acid* and centrifuge the aqueous layer. To 6 ml of the supernatant liquid add 1 ml of 8M *sodium hydroxide*, shake with 2 ml of *dichloromethane*, centrifuge and use the lower layer. Solution (2) contains 0.1% w/v of *tioconazole BPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

Mix 10 g with 10 ml of *carbon dioxide-free water*. The pH of the suspension is 4.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 40 ml of the mobile phase to a quantity of the cream containing 10 mg of Tioconazole, shake until fully dispersed, dilute to 50 ml with the mobile phase, mix and filter through a glass microfibre filter (0.2 µm). For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.20% w/v of *tioconazole impurity standard BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 1 volume of 0.005M *tetrabutylammonium dihydrogen orthophosphate* adjusted to pH 7.4 with 2M *ammonia* and 4 volumes of *methanol* as the mobile phase with a flow rate of 1.0 ml per minute and (c) a detection wavelength of 218 nm.

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the reference chromatogram supplied with *tioconazole impurity standard BPCRS*.

In the chromatogram obtained with solution (1) the areas of any peaks corresponding to tioconazole impurities A, B and C are not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare solution (1) in the same manner as that described for solution (1) under Related substances. Solution (2) contains 0.020% w/v of *tioconazole BPCRS* in the mobile phase.

The chromatographic conditions described under Related substances may be used.

Calculate the content of $C_{16}H_{13}Cl_3N_2OS$ in the cream using the declared content of $C_{16}H_{13}Cl_3N_2OS$ in *tioconazole BPCRS*.

Tioconazole Nail Solution

General Notices

Action and use

Antifungal.

DEFINITION

Tioconazole Nail Solution is a *cutaneous solution*. It contains Tioconazole in a suitable vehicle.

The nail solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of tioconazole, $C_{16}H_{13}Cl_3N_2OS$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel 60 F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and, as the mobile phase, the upper layer obtained from a mixture of 40 volumes of *glacial acetic acid*, 40 volumes of *water* and 80 volumes *4-methylpentan-2-one*. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) dilute a quantity of the nail solution containing 28 mg of Tioconazole to 10 ml with *methanol*. Solution (2) is a 0.28% w/v solution of *tioconazole BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2).

B. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (5).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute a quantity of the nail solution containing 20 mg of Tioconazole to 10 ml with the mobile phase. For solution (2) dilute 1 volume of solution (1) to 10 volumes with the mobile phase. For solution (3) dilute 1 volume of solution (2) to 20 volumes with the mobile phase. Solution (4) contains 0.20% w/v of *tioconazole impurity standard BPCRS* in the mobile phase. Solution (5) contains 0.02% w/v of *tioconazole BPCRS*.

The chromatographic procedure may be carried out using (a) stainless steel column (25 cm ×

4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 1 volume of 0.005M *tetrabutylammonium dihydrogen orthophosphate* adjusted to pH 7.4 with 2M *ammonia* and 4 volumes of *methanol* as the mobile phase with a flow rate of 1.0 ml per minute and (c) a detection wavelength of 218 nm.

The test is not valid unless the chromatogram obtained with solution (4) closely resembles the reference chromatogram supplied with *tioconazole impurity standard BPCRS* .

In the chromatogram obtained with solution (1) the areas of any peaks corresponding to tioconazole impurities A, B and C are not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5% of each).

ASSAY

Carry out Method I for *non-aqueous titration* , Appendix VIII A, using a quantity of the nail solution containing 0.28 g of Tioconazole. Each ml of 0.1M *perchloric acid VS* is equivalent to 38.77 mg of $C_{16}H_{13}Cl_3N_2OS$. Determine the *weight per ml* of the nail solution, Appendix V G, and calculate the content of $C_{16}H_{13}Cl_3N_2OS$, weight in volume.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous solution'.

Tioguanine Tablets

General Notices

Action and use

Purine analogue; cytostatic.

DEFINITION

Tioguanine Tablets contain Tioguanine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tioguanine, $C_5H_5N_5S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.5 g of Tioguanine with 10 ml of 1M *sodium hydroxide* and filter. Acidify the filtrate with *hydrochloric acid*, filter, dissolve the precipitate in 13.5M *ammonia*, evaporate to dryness and dry the residue at 105° at a pressure not exceeding 0.7 kPa for 5 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of tioguanine (*RS 340*).

B. Mix a quantity of the powdered tablets containing 5 mg of Tioguanine with 5 mg of *sodium formate* in a test tube and heat gently until melted. A gas is evolved which turns *lead acetate paper* black.

TESTS

Guanine

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF*$_{254}$ as the coating substance and a mixture of 90 volumes of *methanol* and 15 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) add sufficient 0.1M *sodium hydroxide* to a quantity of the powdered tablets containing 0.50 g of Tioguanine to produce 50 ml. Solution (2) contains 0.025% w/v of *guanine* in 0.1M *sodium hydroxide*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any spot corresponding to guanine in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 40 mg of Tioguanine add 20 ml of 0.1M *sodium hydroxide* and shake for 15 minutes. Add sufficient 0.1M *hydrochloric acid* to produce 200 ml, mix and filter through glass wool. Dilute 5 ml of the filtrate to 200 ml with 0.1M *hydrochloric acid*, mix and measure the *absorbance* of the resulting solution at the maximum at 348 nm, Appendix II B. Calculate the content of $C_5H_5N_5S$ taking 1240 as the value of A(1%, 1 cm) at the maximum at 348 nm.

Titanium Ointment

General Notices

DEFINITION

Titanium Ointment contains a large proportion of Titanium Dioxide with smaller quantities of titanium peroxide and titanium salicylate in a suitable basis containing 5.0% w/w of Dimeticone 350.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of total titanium, as titanium dioxide TiO_2

22.5 to 27.5% w/w.

Content of titanium salicylate

2.70 to 3.30% w/w.

CHARACTERISTICS

A yellow, greasy ointment.

IDENTIFICATION

A. Disperse 5 g in 20 ml of warm *dichloromethane* on a water bath and filter the resulting suspension (Whatman No 1 paper is suitable). Wash the residue with 50 ml of warm *dichloromethane* and transfer the residue to a 50 ml beaker with the aid of a few ml of *water*. Add carefully 10 ml of *sulphuric acid* and 5 g of *ammonium sulphate* and heat until a clear solution is obtained. Allow to cool, add sufficient *water* to produce about 30 ml and add 1 ml of *hydrogen peroxide solution* (*20 vol*). A bright orange colour is produced immediately.

B. Disperse 5 g in 20 ml of warm *dichloromethane* on a water bath and filter the resulting suspension (Whatman No 1 paper is suitable). Wash the residue with 50 ml of warm *dichloromethane* and transfer the residue to a 50 ml beaker with the aid of a few ml of *water*. Mix well, filter again and add 0.05 ml of a 5% w/v solution of *iron* (*III*) *chloride hexahydrate* to the filtrate. A purple colour is produced immediately.

TESTS

ASSAY

For TiO_2

Weigh 0.5 g into a tared silica crucible, heat gently over a small flame until the basis is completely volatilised or charred, increasing the rate of heating until almost all of the carbon has been removed. Heat at about 800^{c} for about 30 minutes, allow to cool in a desiccator and weigh. Repeat the heating at 800^{c} to constant weight. Calculate the content of total titanium as a percentage weight in weight using the formula:

$$\left(\frac{W_r}{W_s}\times 100\right)-1.55$$

where W_r = weight of the residue,

W_s = weight of the sample

1.55 = percentage correction for contribution from Dimeticone 350.

For titanium salicylate

To 1.5 g in an iodine flask add 50 ml of 2M *ammonia* and heat gently until the solution boils. Add 25 ml of 0.0167M *potassium bromate VS* and 10 ml of a 10% w/v solution of *potassium bromide*, add 20 ml of 7M *hydrochloric acid*, close the flask immediately and shake while cooling under running water. Allow the flask to stand for 30 minutes, protected from light. Add 10 ml of a 10% w/v solution of *potassium iodide*, shake and titrate the liberated iodine with 0.1M *sodium thiosulphate VS* using *starch solution*, added towards the end of the titration, as indicator. Each ml of 0.0167M *potassium bromate VS* is equivalent to 2.818 mg of titanium salicylate.

Tobramycin Injection

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Tobramycin Injection is a sterile solution of Tobramycin in Water for Injections containing Sulphuric Acid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable) and a mixture of 60 volumes of *methanol*, 40 volumes of 13.5M *ammonia* and 20 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a suitable volume of the injection with *water* to produce a solution containing 0.4% w/v of Tobramycin. Solution (2) contains 0.4% w/v of *tobramycin EPCRS* in *water*. Solution (3) contains 0.4% w/v each of *kanamycin sulphate EPCRS*, *neomycin sulphate EPCRS* and *tobramycin EPCRS*. After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol* (*96%*) and a 46% w/v solution of *sulphuric acid* and heat at 105° for 5 to 10 minutes. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

B. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH, 3.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel H* as the coating substance and a mixture of equal volumes of 13.5M *ammonia*, *butan-2-one* and *ethanol* (*96%*) as the mobile phase. Apply separately to the plate 4 µl of each of the following solutions. For solution (1) dilute a suitable volume of the injection with 0.01M *ammonia* to give a solution containing 40 mg of Tobramycin in 4 ml, shake with 10 ml of *ether* and use the aqueous layer. For solution (2) dilute 1 volume of solution (1) to 50 volumes with 0.01M *ammonia*. After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution* (*3% Cl*) with *water* to contain 0.5% w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a very faint blue colour with a drop of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dilute the injection, if necessary, with *water BET* to give a solution containing 10 mg of Tobramycin per ml (solution A). The endotoxin limit concentration of solution A is 20 IU per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of tobramycin in the injection, taking each 1000 IU found to be equivalent to 1 mg of tobramycin. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit is not more than 110.0% of the stated potency.

LABELLING

The strength is stated as the weight of Tobramycin in a suitable dose-volume.

Tolazamide Tablets

General Notices

Action and use

Inhibition of ATP-dependent potassium channels (sulphonylurea); treatment of diabetes mellitus.

DEFINITION

Tolazamide Tablets contain Tolazamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tolazamide, $C_{14}H_{21}N_3O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Triturate a quantity of the powdered tablets containing 0.25 g of Tolazamide with 50 ml of *acetone* and filter. Evaporate the filtrate to dryness and dry at 60° at a pressure of 2 kPa for 3 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of tolazamide (*RS 342*).

TESTS

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and a mixture of 23 volumes of 13.5M *ammonia,* 60 volumes of *cyclohexane* , 100 volumes of *methanol* and 200 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (1) shake a quantity of the powdered tablets containing 0.20 g of Tolazamide with 10 ml of *acetone* and filter. Solution (2) contains 0.010% w/v of *toluene*-p*-sulphonamide* in *acetone* . After removal of the plate, dry it in a current of cold air, heat at 110° for 10 minutes, place the hot plate in a tank of chlorine gas, prepared by the addition of *hydrochloric acid* to a 5% w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5% w/v solution of *potassium iodide* in *starch mucilage* ; avoid prolonged exposure to cold air. Spray the plate with a 0.5% w/v solution of *potassium iodide* in *starch mucilage* . Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense that the spot in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 0.5 g of Tolazamide with 50 ml of *chloroform*, filter, wash the residue with *chloroform* and evaporate the combined filtrate and washings to dryness. Dissolve the residue in 20 ml of *butan-2-one* with the aid of gentle heat. Allow to cool, add 30 ml of *ethanol* (*96%*) and titrate the resulting solution with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 31.14 mg of $C_{14}H_{21}N_3O_3S$.

Tolbutamide Tablets

General Notices

Action and use

Inhibition of ATP-dependent potassium channels (sulphonylurea); treatment of diabetes mellitus.

DEFINITION

Tolbutamide Tablets contain Tolbutamide.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tolbutamide, $C_{12}H_{18}N_2O_3S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Extract a quantity of the powdered tablets containing 1 g of Tolbutamide with 10 ml of *chloroform* , filter, evaporate the filtrate to dryness, scratching the side of the container, if necessary, to induce crystallisation, and dry the residue at 100° to 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of tolbutamide (*RS 343*).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules* , Appendix XII B1, using as the medium 900 ml of a solution containing 2.04% w/v of *disodium hydrogen orthophosphate* and 0.135% w/v of *potassium dihydrogen orthophosphate* and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of the filtered sample, suitably diluted if necessary, at the maximum at 228 nm, Appendix II B. Calculate the total content of tolbutamide, $C_{12}H_{18}N_2O_3S$, in the medium taking 417 as the value of A(1%, 1 cm) at the maximum at 228 nm.

Related substances

Carry out the method for *thin-layer chromatography* , Appendix III A, using *silica gel G* as the coating substance and a mixture of 2 volumes of *anhydrous formic acid* , 8 volumes of *methanol* and 90 volumes of *chloroform* as the mobile phase. Apply separately to the plate 5 µl of each of solutions (1) and (2) and 10 µl of solution (3). For solution (1) shake a quantity of the powdered tablets containing 0.50 g of Tolbutamide with 10 ml of *acetone* and filter.

Solution (2) contains 0.015% w/v of *toluene*-p-*sulphonamide* in *acetone*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, dry it in a current of warm air and heat at 110° for 10 minutes. While still hot, place the plate in a chromatography tank with an evaporating dish containing a 5% w/v solution of *potassium permanganate*, add an equal volume of *hydrochloric acid* and close the tank. Leave the plate in the tank for 2 minutes, then place it in a current of cold air until the excess of chlorine is removed and an area of coating below the line of application gives at most a very faint blue colour with *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and allow to stand for 5 minutes. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.3%). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.5 g of Tolbutamide add 50 ml of *ethanol* (*96%*) previously neutralised to *phenolphthalein solution R1*, warm to dissolve, add 25 ml of *water* and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 27.03 mg of $C_{12}H_{18}N_2O_3S$.

Tolu Syrup

General Notices

DEFINITION

Tolu-flavour Solution	100 ml
Syrup	Sufficient to produce 1000 ml

The syrup complies with the requirements stated under Oral Liquids and with the following requirement.

Weight per ml

1.29 to 1.32 g, Appendix V G.

Tolu-flavour Solution

General Notices

DEFINITION

Cinnamic Acid	5.0 g
Benzoic Acid	2.5 g
Ethyl Cinnamate	0.3 g
Vanillin	0.1 g
Cinnamon Oil	0.02 ml
Sucrose	500 g
Ethanol (96 per cent)	350 ml
Water	Sufficient to produce 1000 ml

Extemporaneous preparation

The following directions apply.

Dissolve the Sucrose in 320 ml of Water. Add 250 ml of the Ethanol (96 per cent), with mixing. Dissolve the Cinnamic Acid, Benzoic Acid, Ethyl Cinnamate, Vanillin and Cinnamon Oil in the remaining 100 ml of Ethanol (96 per cent), add this solution to the sucrose solution with mixing, dilute to 1000 ml with Water and mix. Allow to stand for a few hours before use.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF* $_{254}$ as the coating substance and a mixture of 75 volumes of n- *pentane*, 25 volumes of *hexane* and 15 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) use the solution being examined. Solution (2) contains 0.5% w/v of *cinnamic acid*, 0.25% w/v of *benzoic acid* and 0.03% v/v of *ethyl cinnamate* in *ethanol* (*90%*). After removal of the plate, allow it to dry in air for 15 minutes and repeat the development using the same mobile phase. Remove the plate, allow the solvent to evaporate and examine under *ultraviolet light* (*254 nm*). The spots in the chromatogram obtained with solution (1) are similar in size and correspond in position to those in the chromatogram obtained with solution (2).

TESTS

Ethanol content

31 to 36% v/v, Appendix VIII F.

Weight per ml

1.125 to 1.155 g, Appendix V G.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

Tramadol Capsules

General Notices

Action and use

Opioid receptor agonist; noradrenaline reuptake inhibitor; analgesic.

DEFINITION

Tramadol Capsules contain Tramadol Hydrochloride.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of tramadol hydrochloride, $C_{16}H_{25}NO_2,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography* , Appendix III A, using a *TLC silica gel F $_{254}$ plate* (Merck plates are suitable) and a mixture of 1 volume of *anhydrous formic acid* , 50 volumes of *acetone* and 50 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 25 ml of *methanol* to a quantity of the contents of the capsules containing 50 mg of Tramadol Hydrochloride, shake for 10 minutes and filter through a glass-fibre filter (Whatman GF/A is suitable). Solution (2) contains 0.2% w/v of *tramadol hydrochloride BPCRS* in *methanol* . After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1), mix with the aid of ultrasound, a quantity of the contents of the capsules containing 0.5 g of Tramadol Hydrochloride with 80 ml of the mobile phase, cool, add sufficient mobile phase to produce 100 ml and filter through a glass-fibre filter (Whatman GF/A is suitable). For solution (2) dilute 2 volumes of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume to 10 volumes with the mobile phase. Solution (3) contains 0.0015% w/v of *tramadol impurity A EPCRS* in the mobile phase. Solution (4) contains 0.0015% w/v each of *tramadol hydrochloride BPCRS* and *tramadol impurity A*

EPCRS in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 1 volume of *trifluoroacetic acid*, 30 volumes of *acetonitrile* and 69 volumes of *water* and (c) a detection wavelength of 271 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 3.0.

For solution (1) allow the chromatography to proceed for four times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to tramadol impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.3%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the sum of the areas of any *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), mix with the aid of ultrasound, a quantity of the mixed contents of 20 capsules containing 50 mg of Tramadol Hydrochloride with 150 ml of the mobile phase, cool, add sufficient mobile phase to produce 200 ml and filter through a glass-fibre filter (Whatman GF/A is suitable). Solution (2) contains 0.025% w/v of *tramadol hydrochloride BPCRS* in the mobile phase. Solution (3) 0.0015% w/v each of *tramadol hydrochloride BPCRS* and *tramadol impurity A EPCRS* in the mobile phase.

The chromatographic procedure described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 3.0.

Calculate the content of $C_{16}H_{25}NO_2$,HCl using the declared content of $C_{16}H_{25}NO_2$,HCl in *tramadol hydrochloride BPCRS.*

Tranexamic Acid Injection

General Notices

Action and use

Antifibrinolytic.

DEFINITION

Tranexamic Acid Injection is a sterile solution of Tranexamic Acid in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of tranexamic acid, $C_8H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume containing 0.4 g of Tranexamic Acid add 2 ml of *ether*, stir, add 5 ml of *methanol*, stir again and allow to crystallise. The crystals, after drying, comply with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of tranexamic acid (*RS 344*).

B. To 1 ml of a 1% w/v solution add 1 ml of a 0.2% w/v solution of *ninhydrin* in *ethanol* (*96%*) and heat on a water bath for 2 minutes. A dark bluish violet colour is produced.

C. Dissolve 0.2 g in 10 ml of 5M *sodium hydroxide*, add 0.2 ml of *benzoyl chloride* and shake vigorously for 10 minutes. Acidify to pH 4 with 2M *hydrochloric acid*, filter, wash the residue with 5 ml of *ether* and dry at 50° at a pressure of 2 kPa. The *melting point* of the residue is about 186°, Appendix V A.

TESTS

Acidity or alkalinity

pH, 6.5 to 8.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 1% w/v of Tranexamic Acid.

(2) 0.01% w/v of *tranexamic acid EPCRS* in *water*.

(3) 0.001% w/v of *tranexamic acid impurity C EPCRS* in *water*.

(4) 0.001% w/v of *4-aminomethylbenzoic acid* in *water*.

(5) 1% w/v of *tranexamic acid impurity standard BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Hypersil is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 0.9 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 220 nm.

(f) Injection volume of 20 µl.

MOBILE PHASE

Dissolve 11.0 g of *anhydrous sodium dihydrogen orthophosphate* in 500 ml of *water*, add 5 ml of *triethylamine* and 1.4 g of *sodium dodecyl sulphate*, adjust the pH to 2.5 with 2M *orthophosphoric acid* and add sufficient *water* to produce 600 ml. Add 400 ml of *methanol* and mix

Inject solution (1) and allow the chromatography to proceed for 3 times the retention time of tranexamic acid (about 13 minutes). Identify the impurities from the chromatogram obtained with solution (5) and from the reference chromatogram supplied with *tranexamic acid impurity standard BPCRS*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) closely resembles the reference chromatogram supplied with *tranexamic acid impurity standard BPCRS* and the *resolution factor* between the peaks corresponding to tranexamic acid and impurity C is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity B is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D, using 3 ml per kg of the rabbit's weight.

ASSAY

To a volume containing 0.1 g of Tranexamic Acid add 50 ml of *water* and adjust the solution to pH 7.0 with 0.1M *sodium hydroxide* or 0.1M *hydrochloric acid*. Add 25 ml of *formaldehyde solution*, previously adjusted to pH 7.0, and 20 ml of 0.1M *sodium hydroxide VS* and titrate with 0.1M *hydrochloric acid VS* determining the end point potentiometrically. Repeat the operation without the injection. The difference between the titrations represents the amount of sodium hydroxide required. Each ml of 0.1M *sodium hydroxide VS* is equivalent to 15.72 mg of $C_8H_{15}NO_2$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Tranexamic Acid.

Tranexamic Acid Tablets

General Notices

Action and use

Antifibrinolytic.

DEFINITION

Tranexamic Acid Tablets contain Tranexamic Acid.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tranexamic acid, $C_8H_{15}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 0.5 g of Tranexamic Acid with 5 ml of *water* for 15 minutes, filter and add 2 ml of *ether* to the filtrate. Stir, add 10 ml of *methanol* , stir again and allow to crystallise. The crystals, after drying, comply with the following tests.

A. The *infrared absorption spectrum* , Appendix II A, is concordant with the *reference spectrum* of tranexamic acid (*RS 344*).

B. To 1 ml of a 1% w/v solution add 1 ml of a 0.2% w/v solution of *ninhydrin* in *ethanol* (*96%*) and heat on a water bath for 2 minutes. A dark bluish violet colour is produced.

C. Dissolve 0.2 g in 10 ml of 5M *sodium hydroxide* , add 0.2 ml of *benzoyl chloride* and shake vigorously for 10 minutes. Acidify to pH 4 with 2M *hydrochloric acid* , filter, wash the residue with 5 ml of *ether* and dry at 50° at a pressure of 2 kPa. The *melting point* of the residue is about 186°, Appendix V A.

TESTS

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions.

(1) Shake a quantity of the powdered tablets containing 1 g of Tranexamic Acid with 100 ml of *water* for 10 minutes, filter and use the filtrate.

(2) 0.01% w/v of *tranexamic acid EPCRS* in *water*.

(3) 0.001% w/v of *tranexamic acid impurity C EPCRS* in *water*.

(4) 0.001% w/v of *4-aminomethylbenzoic acid* in *water*.

(5) 1% w/v of *tranexamic acid impurity standard BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography R* (5 µm) (Hypersil is suitable).

(b) Isocratic elution using the mobile phase described below.

(c) Flow rate of 0.9 ml per minute.

(d) Ambient column temperature.

(e) Detection wavelength of 220 nm.

(f) Injection volume of 20 µl.

MOBILE PHASE

Dissolve 11.0 g of *anhydrous sodium dihydrogen orthophosphate* in 500 ml of *water*, add 5 ml of *triethylamine* and 1.4 g of *sodium dodecyl sulphate*, adjust the pH to 2.5 with 2M *orthophosphoric acid* and add sufficient *water* to produce 600 ml. Add 400 ml of *methanol* and mix

Inject solution (1) and allow the chromatography to proceed for 3 times the retention time of tranexamic acid (about 13 minutes). Identify the impurities from the chromatogram obtained with solution (5) and from the reference chromatogram supplied with *tranexamic acid impurity standard BPCRS*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) closely resembles the reference chromatogram supplied with *tranexamic acid impurity standard BPCRS* and the *resolution factor* between the peaks corresponding to tranexamic acid and impurity C is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity B is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity C is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the area of any peak corresponding to impurity D is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets containing 0.25 g of Tranexamic Acid in 50 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 15.72 mg of $C_8H_{15}NO_2$.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Tranexamic Acid.

Tranylcypromine Tablets

General Notices

Action and use

Monoamine oxidase inhibitor; antidepressant.

DEFINITION

Tranylcypromine Tablets contain Tranylcypromine Sulphate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of tranylcypromine, $C_9H_{11}N$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *light absorption* , Appendix II B, in the range 230 to 350 nm of the solution obtained in the Assay exhibits three maxima, at 258, 264 and 271 nm. The *absorbances* at the maxima are about 1.2, about 1.3 and about 0.96, respectively.

B. Suspend a quantity of the powdered tablets containing the equivalent of 5 mg of tranylcypromine in 0.5 ml of *ethanol* (*96%*) and add 5 mg of *ninhydrin* . A purple colour is produced within 15 minutes.

C. The powdered tablets yield the reactions characteristic of *sulphates* , Appendix VI.

TESTS

Related substances

Dissolve 10 mg of *4-chloroaniline* (internal standard) in sufficient 0.1M *hydrochloric acid* to produce 20 ml (solution A). Carry out the method for *gas chromatography* , Appendix III B, using the following solutions. For solution (1) add 5 ml of 1M *sodium hydroxide* to 1 ml of solution A, extract with 10 ml of *dichloromethane* , add 1 ml of *trifluoroacetic anhydride* to the dichloromethane extract and allow to stand for 10 minutes. Evaporate the solution at a pressure of 2 kPa using a rotary evaporator and a water bath at 20° and dissolve the residue in 2 ml of *dichloromethane* . For solution (2) add 10 ml of 0.05M *sulphuric acid* to a quantity of the powdered tablets containing the equivalent of 75 mg of tranylcypromine, shake for 15 minutes, filter and wash the filter with two 5 ml quantities of 0.05M *sulphuric acid* . To the combined filtrate and washings add 1 ml of 5M *sodium hydroxide* and extract with 10 ml of *dichloromethane* . Dry the dichloromethane extract with *anhydrous sodium sulphate* , evaporate to about 5 ml without heating, add 1 ml of *trifluoroacetic anhydride* and allow to stand for 10 minutes. Evaporate the solvent at a pressure of 2 kPa using a rotary evaporator and a water bath at 20° and dissolve the residue in 2 ml of *dichloromethane*. For solution (3) add 10 ml of 0.05M *sulphuric acid* and 1 ml of solution A to a quantity of the powdered tablets containing the equivalent of 75 mg of tranylcypromine and complete the procedure described above, beginning at the words 'shake for 15 minutes…'.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable) and maintained at 170°.

In the chromatogram obtained with solution (3) the area of any *secondary peak* is not greater than that of the peak due to the trifluoroacetyl derivative of 4-chloroaniline (0.5%, calculated with reference to tranylcypromine sulphate).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 30 mg of tranylcypromine add 60 ml of 0.1M *sulphuric acid*, shake for 30 minutes, add sufficient 0.1M *sulphuric acid* to produce 100 ml and filter. Measure the *absorbance* of the resulting solution at the maximum at 271 nm and at 282 nm, Appendix II B. Calculate the content of $C_9H_{11}N$ from the difference between the absorbances taking 14.6 as the value of $\Delta A(1\%, 1\ \text{cm})$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of tranylcypromine.

Tretinoin Gel

General Notices

Action and use

Vitamin A analogue (retinoid); treatment of acne.

DEFINITION

Tretinoin Gel is a solution of Tretinoin in a suitable basis.

The gel complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of tretinoin, $C_{20}H_{28}O_2$

90.0 to 120.0% of the stated amount.

IDENTIFICATION

Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel GF $_{254}$* as the coating substance and a mixture of 70 volumes of *cyclohexane* and 30 volumes of *propan-2-ol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse a quantity of the gel containing 1.25 mg of Tretinoin as completely as possible in 25 ml of warm *methanol*, allow to cool to room temperature and extract with three 50 ml quantities of *hexane*. Wash the combined extracts with 20 ml of *water*, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness using a rotary evaporator at a temperature not exceeding 60°. Dissolve the residue in 5 ml of *methanol*. Solution (2) contains 0.025% w/v of *tretinoin EPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use solution (1) from the test for Identification. For solution (2) dilute 3 volumes of solution (1) to 100 volumes with *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.4 ml per minute a solution containing 0.5% v/v of *glacial acetic acid* in a mixture of *methanol* and *water* the proportions being adjusted to give a retention time for tretinoin of about 15 minutes (a mixture of 77 volumes of *methanol* and 23 volumes of *water* is usually suitable) and (c) a detection wavelength of 353 nm. Adjust the sensitivity of the instrument so that the height of the principal peak in the chromatogram obtained with solution (2) is about 70% of full-scale deflection.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (2).

ASSAY

Dissolve a quantity of the gel containing 0.5 mg of Tretinoin in sufficient *chloroform* to produce 100 ml and measure the *absorbance* of the resulting solution at the maximum at 365 nm, Appendix II B. Calculate the content of $C_{20}H_{28}O_2$ taking 1430 as the value of A(1%, 1 cm) at the maximum at 365 nm.

Tretinoin Solution

General Notices

Action and use

Vitamin A analogue (retinoid); treatment of acne.

DEFINITION

Tretinoin Solution is a *cutaneous solution* of Tretinoin in a suitable ethanolic solvent. It contains a suitable antioxidant.

The solution complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of tretinoin, $C_{20}H_{28}O_2$

90.0 to 120.0% of the stated amount.

CHARACTERISTICS

A clear, yellow liquid with the odour of ethanol.

IDENTIFICATION

Complies with the test described under Tretinoin Gel but preparing solution (1) in the following manner. Add 5 ml of *water* to a quantity containing 1.25 mg of Tretinoin and extract with two 20 ml quantities of *hexane* . Wash the combined extracts with two 10 ml quantities of *water*, filter through *anhydrous sodium sulphate* , evaporate the filtrate to dryness using a rotary evaporator at a temperature not exceeding 60° and dissolve the residue in 5 ml of *methanol* .

TESTS

Ethanol content

50 to 60% v/v, Appendix VIII F, Method II.

Related substances

Carry out the test described under Tretinoin Gel using as solution (1) the solution being examined and as solution (2) 3 volumes of the solution being examined diluted to 100 volumes with *methanol* . The sum of the areas of any *secondary peaks* in the chromatogram obtained with solution (1) is not greater than the area of the principal peak in the chromatogram obtained with solution (2).

ASSAY

To a quantity containing 0.5 mg of Tretinoin add 1 ml of 1M *hydrochloric acid* , dilute to 100 ml with *propan-2-ol* and measure the *absorbance* of the resulting solution at the maximum at 353 nm, Appendix II B. Determine the *weight per ml* , Appendix V G, and calculate the percentage w/w of $C_{20}H_{28}O_2$ taking 1520 as the value of A(1%, 1 cm) at the maximum at 353 nm.

STORAGE

Tretinoin Solution should be protected from light.

LABELLING

The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

The label indicates the pharmaceutical form as 'cutaneous solution'.

Triamcinolone Acetonide Injection

General Notices

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Acetonide Injection is a sterile suspension of Triamcinolone Acetonide, in very fine particles, in Water for Injections containing suitable dispersing agents.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A white suspension which settles on standing but readily disperses on shaking. On examination under a microscope, the particles are seen to be crystalline and rarely exceed 40 µm in diameter.

IDENTIFICATION

Extract a volume containing 50 mg of Triamcinolone Acetonide with two 10 ml quantities of *ether* and discard the ether extracts. Filter the aqueous layer through a sintered-glass filter, wash the residue with four 5 ml quantities of *water* and dry at 105° for 1 hour. The residue complies with the following tests.

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of triamcinolone acetonide (*RS 348*).

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent I* and *mobile phase B*.

TESTS

Acidity or alkalinity

pH, 5.0 to 7.5, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following two solutions in *methanol*. Solution (1) contains 0.01% w/v of *prednisolone BPCRS* (internal standard) and 0.02% w/v of *triamcinolone acetonide BPCRS*. Solution (2) is the clear supernatant liquid obtained by mixing a volume of the injection, diluted with *methanol*, with sufficient of a solution of *prednisolone BPCRS* in *methanol* to give a final concentration of 0.01% w/v of prednisolone and 0.02% w/v of triamcinolone acetonide and centrifuging. Prepare solution (3) in the same manner as solution (2) but omitting the internal standard.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 56 volumes of *methanol* and 44 volumes of *water* as the mobile with a flow rate of 1.4 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{24}H_{31}FO_6$ in the preparation being examined using peak heights and the declared content of $C_{24}H_{31}FO_6$ in *triamcinolone acetonide BPCRS*.

STORAGE

Triamcinolone Acetonide Injection should be protected from light. It should not be allowed to freeze.

Triamcinolone Cream

General Notices

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Cream contains Triamcinolone Acetonide in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 25 ml of *methanol* and 5 g of *anhydrous sodium sulphate* to a quantity of the preparation being examined containing 5 mg of Triamcinolone Acetonide, shake for 10 minutes and filter through a sintered-glass crucible (ISO 4793, porosity grade 4, is suitable) containing a 2 cm layer of *washed, flux-calcined diatomaceous filter-aid*, rinsing the flask and crucible with 10 ml of *methanol*; evaporate the combined filtrate and washings to dryness on a water bath and dissolve the residue in 10 ml of *chloroform*. Solution (2) contains 0.05% w/v of *triamcinolone acetonide BPCRS* in *chloroform*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). After removal of the plate, allow it to dry in air and spray with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the chromatogram obtained with solution (3) shows a single, compact principal spot.

B. In the Assay, the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to triamcinolone acetonide in the chromatogram obtained with solution (1).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 ml of a 0.1% w/v solution of *fluoxymesterone BPCRS* (internal standard) in *methanol* to 1 ml of a 0.1% w/v solution of *triamcinolone acetonide BPCRS* in *methanol* and add sufficient *methanol* to produce 10 ml. For solution (2) add 10 ml of *methanol* to a quantity of the preparation being examined containing 1 mg of Triamcinolone Acetonide, heat on a water bath at 60° to 70° until the preparation has melted, shake vigorously for 2 minutes, cool in ice and centrifuge; filter the supernatant liquid, if necessary. Prepare solution (3) in the same manner as solution (2) but use 1 ml of the internal standard solution and 9 ml of *methanol* in place of the 10 ml of methanol.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography,* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 65 volumes of *methanol* , 35 volumes of *water* and 0.025 volume of *triethylamine* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{24}H_{31}FO_6$ in the preparation being examined using the declared content of $C_{24}H_{31}FO_6$ in *triamcinolone acetonide BPCRS* .

Triamcinolone Hexacetonide Injection

General Notices

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Hexacetonide Injection is a suspension of Triamcinolone Hexacetonide, in *microfine powder* , in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of triamcinolone hexacetonide, $C_{30}H_{41}FO_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Dilute an appropriate volume of the injection to a concentration of 25 mg of Triamcinolone Hexacetonide per 7 ml with *water*, filter under reduced pressure, wash the residue with two 5 ml quantities of *water* and dry the filter and precipitate. Stir the dried filter and precipitate with 5 ml of *absolute ethanol* to dissolve the precipitate, decant the solution and evaporate to dryness with gentle heating and in a current of air. The *infrared absorption spectrum* of a dispersion of the residue in *potassium bromide* , Appendix II A, is concordant with the *reference spectrum* of triamcinolone hexacetonide (*RS 394*).

TESTS

Acidity or alkalinity

pH, 4.2 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) dilute a volume of the injection containing 20 mg of Triamcinolone Hexacetonide to 50 ml with *methanol* . For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase. Solution (3) contains 0.00040% w/v of *triamcinolone acetonide BPCRS* in the mobile phase. Solution (4) is a 0.0004% w/v solution of *triamcinolone hexacetonide BPCRS* in solution (3). Solution (5) contains 0.1% v/v of *benzyl alcohol* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) as the mobile phase with a flow rate of 1.4 ml per minute a mixture of 250 volumes of *water* and 750 volumes of *methanol* and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 10.

In the chromatogram obtained with solution (1) the area of any peak corresponding to triamcinolone acetonide is not greater than the area of the peak in the chromatogram obtained with solution (3) (1%) and the sum of the areas of any other *secondary peaks* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak corresponding to the peak in the chromatogram obtained with solution (5).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1), withdraw the nominal volume of a container and dilute with *methanol* to produce a solution containing 0.04% w/v of Triamcinolone Hexacetonide. Solution (2) contains 0.04% w/v of *triamcinolone hexacetonide BPCRS* in *methanol*. Solution (3) contains 0.04% w/v of *triamcinolone acetonide BPCRS* in solution (2).

The chromatographic conditions described under Related substances may be used.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 10.

Calculate the content of $C_{30}H_{41}FO_7$ from the chromatograms obtained using the declared content of $C_{30}H_{41}FO_7$ in *triamcinolone hexacetonide BPCRS*.

LABELLING

The label states the intended route of administration.

Triamcinolone Ointment

General Notices

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Ointment contains Triamcinolone Acetonide in a suitable basis.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Triamcinolone Cream but preparing solution (1) in the following manner. To a quantity of the ointment containing 5 mg of Triamcinolone Acetonide add 10 ml of *chloroform* and shake for 10 minutes. Add 25 ml of *methanol* and shake for a further 10 minutes. Filter through a sintered-glass crucible (ISO 4793, porosity grade 4, is suitable) containing a 2 cm layer of *washed, flux-calcined diatomaceous filter-aid* , rinsing the flask and crucible with 10 ml of *methanol* . Evaporate the combined filtrate and washings to dryness on a water bath and dissolve the residue in 10 ml of *chloroform* .

B. In the Assay, the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to triamcinolone acetonide in the chromatogram obtained with solution (1).

TESTS

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. For solution (1) add 4 ml of *dichloromethane* to a quantity of the preparation being examined containing 2 mg of Triamcinolone Acetonide, shake vigorously until it is completely dispersed, add 20 ml of *methanol* and shake vigorously for 1 minute. Cool in ice and centrifuge; evaporate the supernatant liquid or the liquid beneath the crust to dryness using a rotary evaporator, dissolve the residue as completely as possible in 1 ml of *methanol* , dilute to 2 ml with *water* and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol (50%)*. Solution (3) contains 0.002% w/v each of *triamcinolone acetonide BPCRS* and *triamcinolone BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm

× 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 475 volumes of *water* and 525 volumes of *methanol* and (c) a detection wavelength of 254 nm.

Inject 20 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 15.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 1 ml of a 0.1% w/v solution of *fluoxymesterone BPCRS* (internal standard) in *methanol*, 2 ml of *chloroform* and 8 ml of *methanol* to 1 ml of a 0.1% w/v solution of *triamcinolone acetonide BPCRS* in *methanol*. For solution (2) add 2 ml of *chloroform* to a quantity of the preparation being examined containing 1 mg of Triamcinolone Acetonide, shake vigorously until it is completely dispersed, add 10 ml of *methanol* and shake vigorously for 1 minute. Cool in ice and centrifuge; use the supernatant liquid or the liquid beneath the crust, filtered if necessary. Prepare solution (3) in the same manner as solution (2) but add 1 ml of the internal standard solution, 2 ml of *chloroform* and 9 ml of *methanol* in place of the 10 ml of methanol.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 0.025 volume of *triethylamine* as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 254 nm.

Calculate the content of $C_{24}H_{31}FO_6$ in the preparation being examined using the declared content of $C_{24}H_{31}FO_6$ in *triamcinolone acetonide BPCRS*.

Triamcinolone Oromucosal Paste

General Notices

Triamcinolone Dental Paste

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Oromucosal Paste contains Triamcinolone Acetonide in a suitable basis.

The oromucosal paste complies with the requirements stated under Oromucosal Preparations and with the following requirements.

Content of triamcinolone acetonide, $C_{24}H_{31}FO_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Complies with test A for Identification described under Triamcinolone Cream but preparing solution (1) in the following manner. To a quantity of the paste containing 5 mg of Triamcinolone Acetonide add 25 ml of *methanol* and shake for 10 minutes. Filter through a sintered-glass crucible (ISO 4793, porosity grade 4, is suitable) containing a 2 cm layer of *washed, flux-calcined diatomaceous filter-aid* , rinsing the flask and crucible with 10 ml of *methanol* . Evaporate the combined filtrate and washings to dryness on a water bath and dissolve the residue in 10 ml of *chloroform* .

B. In the Assay, the chromatogram obtained with solution (3) shows a peak with the same retention time as the peak due to triamcinolone acetonide in the chromatogram obtained with solution (1).

ASSAY

Carry out the Assay described under Triamcinolone Ointment.

Triamcinolone Tablets

General Notices

Action and use

Glucocorticoid.

DEFINITION

Triamcinolone Tablets contain Triamcinolone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of triamcinolone, $C_{21}H_{27}FO_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dissolve 1 mg of the residue obtained in the test for Related substances in 6 ml of *ethanol* (*96%*), add 5 ml of a 1% w/v solution of *butylated hydroxytoluene* in ethanol (*96%*) and 5 ml of 1M *sodium hydroxide* and heat on a water bath under a reflux condenser for 20 minutes. A pinkish lavender colour is produced.

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the peak due to triamcinolone in the chromatogram obtained with solution (1).

TESTS

Related substances

Carry out the method for *liquid chromatography* , Appendix III D, using the following solutions. Shake a quantity of the powdered tablets containing 15 mg of Triamcinolone with 15 ml of *absolute ethanol* for 15 minutes, filter under reduced pressure through fine filter paper (Whatman No. 42 is suitable) and evaporate to dryness using a rotary evaporator. Reserve 1 mg of the residue for test A for Identification. Dissolve the remainder of the residue in 15 ml of *methanol* (solution A). For solution (1) use solution (A). For solution (2) dilute 4 ml of solution A to 100 ml with *methanol* . For solution (3) dilute 5 ml of solution (A) to 50 ml with *methanol* and dilute 5 ml of the resulting solution to 50 ml with the same solvent.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase a mixture of *methanol* and *water*, adjusted so that the retention time of triamcinolone is about 5 minutes (a mixture of equal volumes of *methanol* and *water* is usually suitable) with a flow rate of 2 ml per minute and (c) a detection wavelength of 238 nm. For solution (3) record the chromatogram for 4 times the retention time of the triamcinolone peak.

The test is not valid unless the *column efficiency*, determined using the principal peak in the chromatogram obtained with solution (2), is at least 10,000 theoretical plates per metre.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (3) (2%) and not more than one such peak has an area greater than that of the principal peak in the chromatogram obtained with solution (3) (1%). The sum of the areas of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (4%).

ASSAY

Weigh and powder 20 tablets. Prepare a 0.06% w/v solution of *testosterone* (internal standard) in *methanol* (solution B) and carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 5 ml of solution B to 5 ml of a 0.08% w/v solution of *triamcinolone BPCRS* in *methanol* and add 15 ml of *methanol* (*50%*). For solution (2) shake a quantity of the powdered tablets containing 2.5 mg of Triamcinolone with 5 ml of *methanol* and 20 ml of a mixture of 5 volumes of *methanol* and 3 volumes of *water*. Shake for 15 minutes, mix with the aid of ultrasound for 10 minutes, centrifuge and use the supernatant liquid. Prepare solution (3) in the same manner as solution (2) but add 5 ml of solution B in place of the 5 ml of methanol.

The chromatographic procedure may be carried out using (a) a stainless steel column (20 cm × 4 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 70 volumes of *methanol*, 30 volumes of *water* and 0.1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 238 nm.

Calculate the content of $C_{21}H_{27}FO_6$ using the declared content of $C_{21}H_{27}FO_6$ in *triamcinolone BPCRS*.

Triamterene Capsules

General Notices

Action and use

Sodium channel blocker; potassium-sparing diuretic.

DEFINITION

Triamterene Capsules contain Triamterene.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of triamterene, $C_{12}H_{11}N_7$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

The final solution obtained in the Assay has a bluish fluorescence and the *light absorption*, Appendix II B, in the range 230 to 365 nm exhibits a maximum only at 360 nm.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 90 volumes of *ethyl acetate*, 10 volumes of *methanol* and 10 volumes of 18M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dissolve a quantity of the contents of the capsules containing 0.10 g of Triamterene in sufficient *dimethyl sulphoxide* to produce 20 ml and dilute 2 ml to 50 ml with *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. After removal of the plate, allow it to dry in air until the solvent has evaporated and examine under *ultraviolet light* (*365 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

Dissolve a quantity of the mixed contents of 20 capsules containing 0.1 g of Triamterene in 50 ml of a mixture of equal volumes of *glacial acetic acid* and *water* with the aid of gentle heat, cool and add sufficient *water* to produce 500 ml. Dilute 5 ml to 200 ml with 1M *acetic acid* and measure the *absorbance* of the resulting solution at the maximum at 360 nm, Appendix II B. Calculate the content of $C_{12}H_{11}N_7$ from a calibration curve prepared by treating suitable quantities of a solution of *triamterene BPCRS* in 1M *acetic acid* in the same manner and using the declared content of $C_{12}H_{11}N_7$ in *triamterene BPCRS*.

Triclofos Oral Solution

General Notices

Action and use

Hypnotic.

DEFINITION

Triclofos Oral Solution is a solution of Triclofos Sodium in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Content of triclofos sodium, $C_2H_3Cl_3NaO_4P$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Mix a quantity of the oral solution containing 0.25 g of Triclofos Sodium with 40 ml of *water*, add 5 ml of 1M *sulphuric acid* and extract with 25 ml of *isoamyl alcohol*. The extract complies with the following tests.

A. Evaporate 5 ml to dryness, heat the residue with 1 ml of a 50% v/v solution of *sulphuric acid* and 1 ml of a 5% w/v solution of *potassium permanganate* in a water bath for 5 minutes, add 7 ml of *water* and decolourise the solution with 1 ml of a 5% w/v solution of *oxalic acid*. To 1 ml add 1 ml of *pyridine* and 1 ml of 2M *sodium hydroxide*, heat in a water bath for 1 minute, stirring continuously. A pink colour is produced in the pyridine layer.

B. Evaporate 10 ml to dryness, heat the residue with 1 g of *anhydrous sodium carbonate* at a dull red heat for 10 minutes, cool, extract the residue with *water* and filter. The residue yields the reactions characteristic of *chlorides* and of *phosphates*, Appendix VI.

ASSAY

To a quantity containing 0.13 g of Triclofos Sodium add 15 ml of *water*, 1 ml of 1M *sodium hydroxide* and 15 ml of *ether*, shake for 1 minute, allow to separate, wash the ether layer with 1 ml of *water* and discard the ether solution. Add 2.5 ml of 1M *sulphuric acid* to the combined aqueous layer and washings, extract with four 10 ml quantities of *isoamyl alcohol*, allowing each extract to stand until good separation is achieved, combine the isoamyl alcohol extracts, remove any further water that separates and dilute to 50 ml with *isoamyl alcohol*. Transfer 10 ml of this solution to a 20 ml glass ampoule, add 10 ml of *alcoholic potassium hydroxide solution*, seal the ampoule, mix well and heat in an autoclave at 120° for 2 hours. Allow the ampoule to cool, transfer the contents to a flask with the aid of 20 ml of 2M *nitric acid* and add 25 ml of 0.02M *silver nitrate VS*. Titrate with 0.02M *ammonium thiocyanate VS* using *ammonium iron (III) sulphate solution R2* as indicator. Repeat the titration using 10 ml of *alcoholic potassium hydroxide solution* in place of the contents of the ampoule. The difference between the titrations represents the amount of silver nitrate required. Each ml of 0.02M *silver nitrate VS* is equivalent to 1.676 mg of $C_2H_3Cl_3NaO_4P$. Determine the *weight per ml* of the oral solution, Appendix V G, and calculate the content of $C_2H_3Cl_3NaO_4P$, weight in volume.

Trifluoperazine Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Trifluoperazine Tablets contain Trifluoperazine Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trifluoperazine, $C_{21}H_{24}F_3N_3S$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing the equivalent of 20 mg of trifluoperazine with 30 ml of 1M *hydrochloric acid* for 10 minutes, filter, make the filtrate alkaline to *litmus paper* with 5M *sodium hydroxide* and extract with two 20 ml quantities of *petroleum spirit* (*boiling range, 60° to 80°*). Combine the extracts, wash with 10 ml of *water*, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate carefully to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trifluoperazine (*RS 351*).

B. Extract a quantity of the powdered tablets containing the equivalent of 5 mg of trifluoperazine with 5 ml of *acetone*, filter and evaporate the filtrate to dryness. Add 2 ml of *sulphuric acid* to the residue and allow to stand for 5 minutes. An orange colour is produced.

C. Extract a quantity of the powdered tablets containing the equivalent of 7 mg of trifluoperazine with 5 ml of *acetone*, filter and evaporate the filtrate to dryness. Separately, heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test tube in a water bath for 5 minutes; the solution readily wets the sides of the tube and there is no greasiness. Add the residue to the acid mixture and heat again on a water bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

TESTS

Uniformity of content

Tablets containing the equivalent of less than 2 mg of trifluoperazine comply with the requirements stated under Tablets using the following method of analysis. Carry out the following procedure protected from light. Place one tablet in a 100 ml graduated flask, add 50 ml of a mixture of 5 volumes of *hydrochloric acid* and 95 volumes of *water*, shake until the tablet has completely disintegrated, dilute to volume with the same solvent, mix and filter, discarding the first 10 ml of filtrate. Measure the *absorbance* of the filtrate at the maximum at 256 nm, Appendix II B. Calculate the content of $C_{21}H_{24}F_3N_3S$ taking 743 as the value of A (1%, 1 cm) at the maximum at 256 nm.

ASSAY

Carry out the following procedure protected from light. Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 5 mg of trifluoperazine for 15 minutes with 400 ml of a mixture of 5 volumes of *hydrochloric acid* and 95 volumes of *water*, dilute to 500 ml with the same mixture, mix and filter. Measure the *absorbance* of the filtrate at the maximum at 256 nm, Appendix II B. Calculate the content of $C_{21}H_{24}F_3N_3S$ taking 743 as the value of A(1%, 1 cm) at the maximum at 256 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of trifluoperazine.

Trihexyphenidyl Tablets

General Notices

Action and use

Anticholinergic.

DEFINITION

Trihexyphenidyl Tablets contain Trihexyphenidyl Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trihexyphenidyl hydrochloride, $C_{20}H_{31}NO,HCl$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing Trihexyphenidyl Hydrochloride with 20 ml of *water* and filter. The filtrate yields a yellow precipitate with *picric acid solution R1* and a white precipitate with 5M *sodium hydroxide*.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets with sufficient *chloroform* to produce a solution containing 0.2% w/v of Trihexyphenidyl Hydrochloride and filter. Solution (2) contains 0.2% w/v of *trihexyphenidyl hydrochloride BPCRS* in *chloroform*. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) mix a quantity of the powdered tablets containing 50 mg of Trihexyphenidyl Hydrochloride with 10 ml of *water*, add 10 ml of *methanol*, disperse with the aid of ultrasound for 5 minutes, shake for 15 minutes, add sufficient *methanol* to produce 25 ml and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with *methanol* (*60%*). Solution (3) contains 0.01% w/v of *3-piperidylpropiophenone hydrochloride BPCRS* in solution (1).

The chromatographic procedure described under Uniformity of content may be used.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is greater than 4.0.

The area of any *secondary peak* in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the total area of any *secondary peaks* is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (2%).

Uniformity of content

Tablets containing 2 mg or less of Trihexyphenidyl Hydrochloride comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.008% w/v of *trihexyphenidyl hydrochloride BPCRS* and 0.004% w/v of *3-piperidylpropiophenone hydrochloride BPCRS* in the mobile phase. For solution (2) add 5 ml of *water* to one tablet and mix with the aid of ultrasound for 5 minutes or until the tablet is dispersed. Add 10 ml of *methanol*, shake for 15 minutes, dilute to 25 ml with *methanol*, mix and filter through a filter with a maximum pore size of 0.2 µm.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Resolve C18 is suitable), (b) as the mobile phase with a flow rate of 2 ml per minute, a mixture of 800 volumes of *acetonitrile*, 200 volumes of *water* and 0.2 volume of *triethylamine*, the pH of the mixture being adjusted to 4.0 with *orthophosphoric acid* and (c) a detection wavelength of 210 nm.

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (1) is greater than 4.0.

Calculate the content of $C_{20}H_{31}NO,HCl$ in each tablet using the declared content of $C_{20}H_{31}NO,HCl$ in *trihexyphenidyl hydrochloride BPCRS*.

ASSAY

For tablets containing more than 2 mg of trihexyphenidyl hydrochloride

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of *trihexyphenidyl hydrochloride BPCRS* and 0.01% w/v of *3-piperidylpropiophenone hydrochloride BPCRS* in the mobile phase. For solution (2) add 5 ml of *water* to a quantity of the powdered tablets containing 5 mg of Trihexyphenidyl Hydrochloride and mix with the aid of ultrasound for 5 minutes. Add 10 ml of *methanol*, shake for 15 minutes, dilute to 25 ml with *methanol* and filter through a filter with a maximum pore size of 0.2 µm.

The chromatographic procedure described under Uniformity of content may be used.

The assay is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (1) is greater than 4.0.

Calculate the content of $C_{20}H_{31}NO,HCl$ using the declared content of $C_{20}H_{31}NO,HCl$ in *trihexyphenidyl hydrochloride BPCRS*.

For tablets containing 2 mg or less of trihexyphenidyl hydrochloride

Use the average of the 10 individual results obtained in the test for Uniformity of content.

Trimethoprim Oral Suspension

General Notices

Action and use

Dihydrofolate reductase inhibitor; antibacterial.

DEFINITION

Trimethoprim Oral Suspension is a suspension of Trimethoprim in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* GF_{254} *plate* and a mixture of 2 volumes of 13.5M *ammonia*, 8 volumes of *methanol* and 90 volumes of *dichloromethane* as the mobile phase. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) add 15 ml of 0.1M *sodium hydroxide* to a quantity of the oral suspension containing 10 mg of Trimethoprim, extract with 50 ml of *dichloromethane* and wash the dichloromethane extract with two 10-ml quantities of 0.01M *sodium hydroxide*. Retain the sodium hydroxide solutions and repeat the extraction procedure with a further three 50-ml quantities of *dichloromethane*, washing the extracts each time with the same two 10-ml quantities of 0.01M *sodium hydroxide*. Evaporate the combined dichloromethane extracts until about 2 ml remains, transfer the residue to a 10-ml graduated flask with the aid of *dichloromethane*, add sufficient *dichloromethane* to produce 10 ml and mix well. Solution (2) contains 0.1% w/v of *trimethoprim BPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm).* The principal spot in the chromatogram obtained with solution (1) corresponds in position and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 10 ml of *methanol* and 10 ml of the mobile phase to a weighed quantity of the oral suspension containing 10 mg of Trimethoprim, mix with the aid of ultrasound for 5 minutes and add sufficient of the mobile phase to produce 50 ml, mix and filter. Solution (2) contains 0.02% w/v of *trimethoprim BPCRS* in *methanol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP 18 is suitable), (b) as the mobile phase at a flow rate of 1 ml per minute a mixture of 225 volumes of 0.003M *tetrabutylammonium bromide* in *water*, 225 volumes of a buffer solution prepared as described below and 550 volumes of *methanol* and (c) a detection wavelength of 260 nm. For the buffer solution dissolve 1.36 g of *sodium acetate* and 23.4 g of *sodium dihydrogen orthophosphate* in 450 ml of *water*, adjust the pH of the mixture, if necessary, to 5.0 with 0.1M *glacial acetic acid* or 0.1M *sodium hydroxide* and add sufficient *water* to produce 500 ml.

Determine the *weight per ml* of the oral suspension, Appendix V G, and calculate the content of $C_{14}H_{18}N_4O_3$, weight in volume, using the declared content of $C_{14}H_{18}N_4O_3$ in *trimethoprim BPCRS*.

Trimethoprim Tablets

General Notices

Action and use

Dihydrofolate reductase inhibitor; antibacterial.

DEFINITION

Trimethoprim Tablets contain Trimethoprim.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimethoprim, $C_{14}H_{18}N_4O_3$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Trimethoprim with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of trimethoprim (*RS 354*).

B. Shake a quantity of the powdered tablets containing 0.1 g of Trimethoprim with 60 ml of 0.1M *hydrochloric acid* for 20 minutes, add sufficient 0.1M *hydrochloric acid* to produce 100 ml, filter and dilute 5 ml of the filtrate to 250 ml with 0.1M *sodium hydroxide*. The *light absorption* of the resulting solution, Appendix II B, in the range 230 to 350 nm exhibits a maximum only at 287 nm.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.2 g of Trimethoprim with 50 ml of the mobile phase, filter and use the filtrate. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.3 ml per minute a 0.14% w/v solution of *sodium perchlorate* in *methanol* (*60%*) adjusted to pH 3.1 with 0.1M *hydrochloric acid* and (c) a detection wavelength of 280 nm.

The *column efficiency*, determined using the peak due to trimethoprim in the chromatogram obtained with solution (2), should be at least 8,000 theoretical plates per metre.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any such peaks is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 0.1 g of Trimethoprim add 100 ml of *glacial acetic acid*, shake for 20 minutes, dilute to 200 ml with *glacial acetic acid* and filter. To 5 ml of the filtrate add 15 ml of *glacial acetic acid* and dilute to 100 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 271 nm, Appendix II B. Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A(1%, 1 cm) at the maximum at 271 nm.

Trimipramine Tablets

General Notices

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION

Trimipramine Tablets contain Trimipramine Maleate. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of trimipramine, $C_{20}H_{26}N_2$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

Triturate a quantity of the powdered tablets containing the equivalent of 0.25 g of trimipramine with 10 ml of *chloroform*, filter and evaporate the filtrate to low volume. Add *ether* until a turbidity is produced and allow to stand. The *infrared absorption spectrum*, Appendix II A, of the precipitate, after recrystallisation from *acetone*, is concordant with the *reference spectrum* of trimipramine maleate (*RS 355*).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 0.7 volume of 13.5M *ammonia*, 10 volumes of *ethanol* (*96%*) and 90 volumes of *toluene* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions prepared immediately before use. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 0.18 g of trimipramine with three 10 ml quantities of *chloroform* and filter. Evaporate the combined filtrates to dryness and dissolve the residue in 10 ml of *methanol*. For solution (2) dilute 1 volume of solution (1) to 200 volumes with *methanol*. For solution (3) dilute 2 volumes of solution (2) to 5 volumes with *methanol*. Solution (4) contains 0.0125% w/v of *iminodibenzyl* in *methanol*. After removal of the plate, allow it to dry in air for 15 minutes and spray with a 0.5% w/v solution of *potassium dichromate* in *sulphuric acid* (*20% v/v*). Examine the plate immediately. Any spot corresponding to iminodibenzyl in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.7%, with reference to trimipramine). Any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (3) (0.2%). Disregard any spot remaining on the line of application.

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing the equivalent of 50 mg of trimipramine with 190 ml of a mixture of equal volumes of *acetonitrile* and *water* for 1 minute, mix with the aid of ultrasound for 5 minutes, shake for 1 minute and mix again with the aid of ultrasound for 5 minutes. Add sufficient of a mixture of equal volumes of *acetonitrile* and *water* to produce 200 ml, filter and dilute 10 ml of the filtrate to 100 ml with the same solvent. Measure the *absorbance* of the resulting solution at the maximum at 250 nm, Appendix II B. Calculate the content of $C_{20}H_{26}N_2$ taking 320 as the value of A(1%, 1 cm) at the maximum at 250 nm.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of trimipramine.

Triprolidine Tablets

General Notices

Action and use

Histamine H_1 receptor antagonist; antihistamine.

DEFINITION

Triprolidine Tablets contain Triprolidine Hydrochloride.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of triprolidine hydrochloride, $C_{19}H_{22}N_2,HCl,H_2O$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 10 mg of Triprolidine Hydrochloride with *ether*, filter, discard the ether and dry the residue. Extract the residue with *chloroform* and evaporate to dryness. Add 0.1 ml of *ether*, stir and allow to evaporate. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of triprolidine hydrochloride (*RS 356*).

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (4).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of equal volumes of *butan-2-one* and *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) extract a quantity of the powdered tablets containing 10 mg of Triprolidine Hydrochloride with *methanol*, filter, evaporate to dryness and dissolve the residue in 1 ml of *methanol*. Solution (2) contains 0.020% w/v of Z-*triprolidine hydrochloride BPCRS* in *methanol*. Solutions (3) and (4) contain 0.010% w/v and 1.0% w/v respectively of *triprolidine hydrochloride BPCRS* in *methanol*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). In the chromatogram obtained with solution (1) any spot corresponding to *Z*-triprolidine is not more intense than the spot in the chromatogram obtained with solution (2) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing 7.5 mg of Triprolidine Hydrochloride, add 15 ml of *water* and 1 g of *sodium chloride*, shake for 2 to 3 minutes and make alkaline with 5M *sodium hydroxide*. Extract with four 20 ml quantities of *ether* and wash the combined extracts with two 5 ml quantities of a mixture of equal volumes of a saturated solution of *sodium chloride* and *water*. Extract the ether solution with 20 ml of 0.1M *hydrochloric acid*, wash the ether with two 5 ml quantities of *water* and add the washings to the acid extract. Heat on a water bath for 30 minutes, cool and add sufficient *water* to produce 50 ml. Dilute 10 ml to 100 ml with 0.1M *hydrochloric acid* and measure the *absorbance* of the resulting solution at the maximum at 290 nm, Appendix II B. Calculate the content of $C_{19}H_{22}N_2,HCl,H_2O$ taking 290 as the value of A(1%, 1 cm) at the maximum at 290 nm.

Trisodium Edetate Intravenous Infusion

General Notices

Trisodium Edetate Injection

DEFINITION

Trisodium Edetate Intravenous Infusion is a sterile solution of trisodium edetate. It is prepared immediately before use by diluting Sterile Trisodium Edetate Concentrate with a suitable diluent in accordance with the manufacturer's instructions.

The intravenous infusion complies with the requirements stated under Parenteral Preparations and with the following requirements.

LABELLING

The strength is stated as the amount of trisodium edetate in a suitable dose-volume.

STERILE TRISODIUM EDETATE CONCENTRATE

DEFINITION

Sterile Trisodium Edetate Concentrate is a sterile solution in Water for Injections containing 20% w/v of trisodium edetate prepared by the interaction of Disodium Edetate and Sodium Hydroxide.

The concentrate complies with the requirements for Concentrated Solutions for Injections stated under Parenteral Preparations and with the following requirements.

Content of trisodium edetate, $C_{10}H_{13}N_2Na_3O_8$

19.0 to 21.0% w/v.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

A. Dilute 2.5 ml with 7.5 ml of *water* and add 0.5 ml of a 10% w/v solution of *calcium chloride*; make alkaline to *litmus paper* with 5M *ammonia* and add 5 ml of a 2.5% w/v solution of *ammonium oxalate*. No precipitate is produced.

B. Dilute 10 ml with 15 ml of *water*, add 2 ml of a 10% w/v solution of *lead(II) nitrate*, shake and add 5 ml of *dilute potassium iodide solution*; no yellow precipitate is produced. Make alkaline to *litmus paper* with 5M *ammonia* and add 5 ml of a 2.5% w/v solution of *ammonium oxalate*; no precipitate is produced.

C. Evaporate to dryness and ignite. The residue yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH, 7.0 to 8.0, Appendix V L.

Pyrogens

Complies with the *test for pyrogens*, Appendix XIV D, using per kg of the rabbit's weight 5 ml of a solution prepared as follows. To 1 volume of the solution being examined add 2.5 volumes of *calcium gluconate solution*. Dilute the resulting solution with sufficient *water for injections* to give a final concentration of 5.0% w/v of trisodium edetate.

ASSAY

Dilute 10 ml to 100 ml with *water* and use this solution to titrate a mixture of 25 ml of 0.05M *magnesium sulphate VS* and 10 ml of *ammonia buffer pH 10.9* using *mordant black 11 mixed triturate* as indicator. Each ml of 0.05M *magnesium sulphate VS* is equivalent to 17.91 mg of trisodium edetate, $C_{10}H_{13}N_2Na_3O_8$.

STORAGE

Sterile Trisodium Edetate Concentrate should be kept in containers made from lead-free glass.

LABELLING

The label states (1) 'Sterile Trisodium Edetate Concentrate'; (2) that the solution must be diluted with either Sodium Chloride Intravenous Infusion or Glucose Intravenous Infusion before administration.

Tropicamide Eye Drops

General Notices

Action and use

Anticholinergic.

DEFINITION

Tropicamide Eye Drops are a sterile solution of Tropicamide in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of tropicamide, $C_{17}H_{20}N_2O_2$

95.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Shake a volume of the eye drops containing 20 mg of Tropicamide with 10 ml of *chloroform*, dry the chloroform layer over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in the minimum quantity of *chloroform*, add drop wise to finely powdered *potassium bromide*, mix and dry at 60°. The *infrared absorption spectrum*, Appendix II A, of the dispersion examined as a disc is concordant with the *reference spectrum* of tropicamide (*RS 357*).

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the final solution obtained in the Assay exhibits a maximum only at 254 nm.

TESTS

Acidity

pH, 4.0 to 5.8, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 1 volume of 13.5M *ammonia*, 10 volumes of *methanol* and 190 volumes of *chloroform* as the mobile phase. Apply separately to the plate (1) a volume of the eye drops containing 0.2 mg of Tropicamide, (2) the same volume of a solution prepared by diluting 1 volume of the eye drops to 200 volumes and (3) the same volume of a solution prepared by diluting 1 volume of the eye drops to 500 volumes. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.2%).

ASSAY

To a quantity containing 30 mg of Tropicamide add sufficient *water* to produce 100 ml and mix. To 10 ml of the resulting solution add 2 ml of a 10% w/v solution of *anhydrous sodium carbonate* and extract with four 20 ml quantities of *chloroform*. Wash the combined chloroform extracts with 25 ml of a solution containing 0.0176% w/v of *disodium hydrogen orthophosphate* and 0.243% w/v of *sodium dihydrogen orthophosphate*. Wash the aqueous layer with 10 ml of *chloroform*, combine the chloroform extracts and shake with four 20 ml quantities of 0.5M *sulphuric acid*. Combine the acid extracts and add sufficient 0.5M *sulphuric acid* to produce 100 ml. Measure the *absorbance* of the resulting solution at the maximum at 254 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_2O_2$ in the eye drops taking 172 as the value of A(1%, 1 cm) at the maximum at 254 nm.

Urea Cream

General Notices

DEFINITION

Urea Cream contains Urea in a suitable basis.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of urea, CH_4N_2O

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable). Apply separately to the plate 10 µl of each of the following solutions. For solution (1) disperse with heating a quantity of the cream containing 50 mg of Urea in 1 ml of *water*, cool, add 4 ml of *acetone*, mix and filter. For solution (2) dissolve 50 mg of *urea* in 1 ml of *water* and add 4 ml of *acetone*. Solution (3) is a mixture of equal volumes of solutions (1) and (2). For the first development use *2,2,4-trimethylpentane* as the mobile phase. Remove the plate and allow it to dry in air. For the second development use as the mobile phase a mixture of 99 volumes of *absolute ethanol* and 1 volume of 13.5M *ammonia*. After removal of the plate, allow it to dry in air and spray with a solution containing 0.5% w/v of *4-dimethylaminobenzaldehyde* and 0.5% v/v of *sulphuric acid* in *absolute ethanol*. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows a single, compact principal spot.

B. To a quantity containing 0.1 g of Urea add 50 ml of *water* and heat until dispersed, cool in ice and filter through glass wool. Adjust the pH of the filtrate, which may not be clear, to between 6.0 and 7.0 using 0.1M *hydrochloric acid* or 0.1M *sodium hydroxide* as necessary. To 5 ml add 5 ml of a 0.1% w/v suspension of *urease-active meal* and allow to stand for 30 minutes in a stoppered flask at 37°. When the resulting solution is heated in a water bath, a vapour is produced that turns moist *red litmus paper* blue.

TESTS

Ammonia

Not more than 2.0% with respect to the content of urea, CH_4N_2O (determined in the Assay) when determined by the following method. Prepare a 0.035% w/v solution of *ammonium sulphate* in 1M *sulphuric acid* (solution A). Carry out the method for *ion-selective potentiometry*, Appendix VIII E, using an ammonia-selective electrode. Determine the response slope of the electrode using standard ammonia solutions and measure the emf in the following solutions. For solution (1) shake a quantity of the cream (w_1 g) containing 40 mg of Urea with 5 ml of 1M *sulphuric acid* to disperse, warming if necessary, add 10 ml of *2,2,4-trimethylpentane*, shake for 2 minutes and centrifuge. Dilute 3 ml of the aqueous layer to 50 ml with *water* and to 20 ml of the resulting solution add 4 ml of 1M *sodium hydroxide*. Prepare solution (2) in the same manner but shaking a quantity of the cream (w_2 g) containing 40 mg of Urea with 5 ml of solution A in place of 5 ml of 1M *sulphuric acid*.

Calculate C_1 and hence the concentration of ammonia in the cream from the expression:

$$\Delta E = a \log(w_2/w_1 + C_2/C_1)$$

where ΔE = the difference in the emf, in mV, obtained with the two solutions,

a = the response slope in mV per decade,

C_1 = the concentration of ammonia in solution (1) as % w/v,

C_2 = the concentration of standard added ammonia in solution (2) as % w/v (this is 0.0129 times the concentration of ammonium sulphate in solution A as % w/v), namely 0.000451% w/v.

ASSAY

Shake a quantity containing 42 mg of Urea with 150 ml of hot *water* for 20 minutes, allow to cool and dilute to 500 ml with *water*. Filter through a fine glass microfibre filter paper (Whatman GF/C is suitable), transfer 1 ml of the filtrate to a 100 ml graduated flask, add 2 ml of a 0.1% w/v suspension of *urease-active meal*, stopper the flask and allow to stand for 15 minutes at 37°. Immediately add 25 ml of a solution containing 12 g of *sodium salicylate* and 0.24 g of *sodium nitroprusside* in 200 ml and 25 ml of a solution prepared by diluting a volume of *sodium hypochlorite solution* containing the equivalent of 0.66 g of available chlorine with 0.2M *sodium hydroxide* to 1000 ml. Mix well, allow to stand at 37° for 5 minutes and dilute to 100 ml with *water*. Measure the *absorbance* of the resulting solution at the maximum at 665 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but using 1 ml of *water* in place of 1 ml of the filtrate. Repeat the operation using 42 mg of *urea BPCRS* in place of the cream being examined. Calculate the content of CH_4N_2O from the *absorbances* obtained using the declared content of CH_4N_2O in *urea BPCRS*.

STORAGE

Urea Cream should be stored in accordance with the manufacturer's instructions.

Urofollitropin Injection

General Notices

Action and use

Follicle-stimulating hormone.

DEFINITION

Urofollitropin Injection is a sterile solution of Urofollitropin in Sodium Chloride Intravenous Infusion. It is prepared by dissolving Urofollitropin for Injection in the requisite amount of Sodium Chloride Intravenous Infusion immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Urofollitropin Injection should be used immediately after preparation.

UROFOLLITROPIN FOR INJECTION

DEFINITION

Urofollitropin for Injection is a sterile material consisting of Urofollitropin with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

CHARACTERISTICS

An almost white or slightly yellow powder.

IDENTIFICATION

Causes enlargement of the ovaries of immature female rats when administered as directed in the Assay.

TESTS

Acidity or alkalinity

Dissolve the contents of the sealed container in 3 ml of *water* (solution A). The pH is 6.0 to 8.0, Appendix V L.

Clarity and colour of solution

Solution A is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Residual luteinising activity

Comply with the test described under Urofollitropin.

Water

Comply with the test described under Urofollitropin.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C, using Method C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 75 IU of urofollitropin per ml (solution A). The endotoxin limit concentration of solution A is 30 IU of endotoxin per ml. Carry out the test using a suitable dilution of solution A as described under Method C.

ASSAY

Carry out the Assay described under Urofollitropin. The estimated potency for each component is not less than 80% and not more than 125% of the stated potency. The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE

The sealed container should be protected from light. The contents may be expected to retain their potency for not less than 3 years.

LABELLING

The label of the sealed container states the number of IU (Units) of follicle-stimulating hormone activity contained in it.

Ursodeoxycholic Acid Capsules

General Notices

Action and use

Bile acid; treatment of gallstones.

DEFINITION

Ursodeoxycholic Acid Capsules contain Ursodeoxycholic Acid.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of ursodeoxycholic acid, $C_{24}H_{40}O_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the contents of the capsules containing 0.1 g of Ursodeoxycholic Acid with 10 ml of *ethanol* (*96%*), centrifuge and evaporate the supernatant liquid to dryness in a stream of nitrogen in a water bath at room temperature. Dry the residue at room temperature at a pressure of 0.7 kPa for 2 hours. The *infrared absorption spectrum*, Appendix II A, of the dried residue is concordant with the *reference spectrum* of ursodeoxycholic acid (*RS 402*).

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using Apparatus 2 and rotating the paddle at 75 revolutions per minute. Use as the medium 900 ml of a phosphate buffer of pH 7.5 prepared using 0.05M *potassium dihydrogen orthophosphate* and 0.05M *disodium hydrogen orthophosphate* in the ratio 1:1 and adjusting the pH to 7.5 using 1M *sodium hydroxide*. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the filtered dissolution medium as solution (1). For solution (2) add 0.7 ml of 0.1M *sodium hydroxide* to 28 mg of *ursodeoxycholic acid BPCRS* and add sufficient dissolution medium to produce 100 ml, mix with the aid of ultrasound for 20 minutes and leave to cool at room temperature.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Superspher 100 RP 18e is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 300 volumes of *acetonitrile* and 700 volumes of *water* previously adjusted to pH 1.7 with *orthophosphoric acid* and adjusting the pH of the mobile phase to 2.5 with 1M *sodium hydroxide* and (c) a detection wavelength of 210 nm.

Calculate the total content of ursodeoxycholic acid, $C_{24}H_{40}O_4$, in the medium using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable). Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the contents of the capsules containing 0.1 g of Ursodeoxycholic Acid with 10 ml of *ethanol* (*96%*) with the aid of ultrasound for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) prepare a solution containing 0.01% w/v of *lithocholic acid EPCRS*, 0.05% w/v of *cholic acid EPCRS* and 0.15% w/v of *chenodeoxycholic acid EPCRS* in *methanol* and dilute 1 volume of the resulting solution to 10 volumes with *acetone*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *ethanol* (*96%*). Use a mixture of 5 volumes of *acetic acid*, 5 volumes of *methanol* and 90 volumes of *chloroform* for the first mobile phase but allow the solvent front to ascend 10 cm above the line of application. After removal of the plate, dry it in a current of air and repeat the development in the same direction using a mixture of 1.25 volumes of *acetic acid*, 50 volumes of *ethyl acetate* and 50 volumes of *2,2,4-trimethylpentane* as the mobile phase but allowing the solvent front to ascend 8 cm above the line of application. After removal of the plate, allow it to dry in air and heat at 110° to 120° for 30 minutes. Spray immediately with a solution containing 5% w/v of *phosphomolybdic acid* in a mixture of 95 volumes of *glacial acetic acid* and 5 ml of *sulphuric acid*. Heat the plate for a further 5 to 10 minutes at 110° to 120° until dark blue spots appear on a white or pale blue background. In the chromatogram obtained with solution (1), any spot corresponding to lithocholic acid, cholic acid or chenodeoxycholic acid is not more intense than the corresponding spot in the chromatogram obtained with solution (2) (0.1%, 0.5% and 1.5% respectively); any other *secondary spot* is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh a quantity of the mixed contents of 20 capsules. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to a quantity of the mixed contents of the capsules containing 0.125 g of Ursodeoxycholic Acid add 5 ml of *methanol*, mix with the aid of ultrasound for 10 minutes and dilute to 50 ml with the mobile phase. For solution (2) dissolve 50 mg of *ursodeoxycholic acid BPCRS* in 2 ml of *methanol* with the aid of ultrasound for 10 minutes and dilute to 20 ml with the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (4 µm) (Superspher 100 RP 18e is suitable) maintained at 40°, (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 400 volumes of *acetonitrile* and 600 volumes of 0.001M *potassium dihydrogen orthophosphate* warmed to room temperature and adjusting the pH to 2.0 with *orthophosphoric acid* (*85%*) and filtering and (c) a detection wavelength of 210 nm.

Inject 30 µl of each solution.

Calculate the content of $C_{24}H_{40}O_4$ using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS*.

Ursodeoxycholic Acid Tablets

General Notices

Action and use

Bile acid; treatment of gallstones.

DEFINITION

Ursodeoxycholic Acid Tablets contain Ursodeoxycholic Acid. They may be coated.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of ursodeoxycholic acid, $C_{24}H_{40}O_4$

92.5 to 107.5% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing 0.1 g of Ursodeoxycholic Acid with 10 ml of *ethanol* (*96%*), centrifuge and evaporate the supernatant liquid to dryness in a stream of nitrogen in a water bath at room temperature. Dry the residue at room temperature at a pressure of 0.7 kPa for 2 hours. The *infrared absorption spectrum*, Appendix II A, of the dried residue is concordant with the *reference spectrum* of ursodeoxycholic acid (*RS 402*).

B. In the Assay, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (1).

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, rotating the basket at 100 revolutions per minute. Use as the medium a phosphate buffer of pH 8.0 prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and adjusting the pH to 8.0 using 2M *sodium hydroxide*. Withdraw a sample of 10 ml of the medium and filter. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the filtered dissolution medium as solution (1). For solution (2) dissolve 30 mg of *ursodeoxycholic acid BPCRS* in 1 ml of *ethanol* (*96%*) and dilute to 100 ml with the dissolution medium.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS is suitable), fitted with a stainless steel guard column packed with the same material (Lichrospher 100 RP 18e is suitable), both columns maintained at 40°, (b) as the mobile phase with a flow rate of 2 ml per minute a mixture of 280 volumes of *acetonitrile* and 720 volumes of a 0.05M phosphate buffer prepared by dissolving 6.81 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and adjusting the pH to 6.5 with 2M *sodium hydroxide* and (c) a detection wavelength of 210 nm. Inject 100 µl of each solution. Calculate the total content of ursodeoxycholic acid, $C_{24}H_{40}O_4$, in the medium using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS*.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel precoated plate (Merck silica gel 60 plates are suitable). Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.1 g of Ursodeoxycholic Acid with 10 ml of *ethanol* (*96%*) with the aid of ultrasound for 15 minutes, centrifuge and use the supernatant liquid. For solution (2) prepare a solution containing 0.01% w/v of *lithocholic acid EPCRS*, 0.05% w/v of *cholic acid EPCRS* and 0.15% w/v of *chenodeoxycholic acid EPCRS* in *methanol* and dilute 1 volume of the resulting solution to 10 volumes with *acetone*. For solution (3) dilute 1 volume of solution (1) to 200 volumes with *ethanol* (*96%*). Use a mixture of 5 volumes of *acetic acid*, 5 volumes of *methanol* and 90 volumes of *chloroform* for the first mobile phase but allow the solvent front to ascend 10 cm above the line of application. After removal of the plate, dry it in a current of air and repeat the development in the same direction using a mixture of 1.25 volumes of *acetic acid*, 50 volumes of *ethyl acetate* and 50 volumes of *2,2,4-trimethylpentane* as the mobile phase and allowing the solvent front to ascend 8 cm above the line of application. After removal of the plate, allow it to dry in air and heat at 110° to 120° for 30 minutes. Spray immediately with a solution containing 5% w/v of *phosphomolybdic acid* in a mixture of 95 volumes of *glacial acetic acid* and 5 ml of *sulphuric acid*. Heat the plate for a further 5 to 10 minutes at 110° to 120° until dark blue spots appear on a white or pale blue background. In the chromatogram obtained with solution (1), any spot corresponding to lithocholic acid, cholic acid or chenodeoxycholic acid is not more intense than the corresponding spot in the chromatogram obtained with solution (2) (0.1%, 0.5% and 1.5% respectively); any other *secondary spot* is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a solution containing 535 ml of *water*, 6.0 g of *sodium dihydrogen orthophosphate dihydrate*, 2.0 g of *disodium hydrogen orthophosphate dihydrate*, 65 ml of *tetrabutylammonium hydroxide solution* and 400 ml of *acetonitrile* (solvent A). For solution (1) mix a quantity of the powdered tablets containing 0.3 g of Ursodeoxycholic Acid with 20 ml of solvent A with the aid of ultrasound for 15 minutes, shake intermittently and filter, if necessary. Solution (2) contains 1.5% w/v of *ursodeoxycholic acid BPCRS* in solvent A.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) maintained at 50°, (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 500 volumes of *acetonitrile* and 500 volumes of a solution containing 1.2% w/v of *sodium dihydrogen orthophosphate dihydrate*, 0.4% w/v of *disodium hydrogen orthophosphate dihydrate* and 1.08% w/v of *tetrabutylammonium hydrogen sulphate* in *water* and (c) a detection wavelength of 210 nm. Inject 10 µl of each solution. Allow the chromatography to proceed for twice the retention time of ursodeoxycholic acid. Calculate the content of $C_{24}H_{40}O_4$ using the declared content of $C_{24}H_{40}O_4$ in *ursodeoxycholic acid BPCRS*.

Paediatric Vitamins A, C and D Oral Drops

General Notices

DEFINITION

Paediatric Vitamins A, C and D Oral Drops are a solution of vitamin A, Ascorbic Acid and Colecalciferol in a suitable, flavoured vehicle.

The oral drops comply with the requirements stated under Oral Liquids and with the following requirements.

Content of vitamin A

5200 to 6200 IU per ml.

Content of ascorbic acid

160 to 175 mg per ml.

Content of colecalciferol

2100 to 2500 IU per

IDENTIFICATION

A. In the Assay for vitamin A, the solution shows a maximum at 325 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel* F_{254} *plate* (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 20 volumes of *water* and 120 volumes of *ethanol* (*96%*) as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions. For solution (1) dilute the oral drops, if necessary, with *water* to contain the equivalent of 0.5% w/v of ascorbic acid. Solution (2) contains 0.5% w/v of *ascorbic acid EPCRS* in *water*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

C. In the Assay for colecalciferol, the chromatogram obtained with solution (1) shows a peak corresponding to the colecalciferol peak in the chromatogram obtained with solution (2).

ASSAY

FOR VITAMIN A

Carry out the test as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper and iron) and acids.

Examine by *ultraviolet absorption spectrophotometry*, Appendix II B (Method A). If method A is found not to be valid, examine by *liquid chromatography*, Appendix III D (Method B).

Method A

Test solution To a weighed quantity of the oral drops containing 50,000 IU in a round-bottomed flask, add 3 ml of a freshly prepared 50% w/w solution of *potassium hydroxide* and 30 ml of *absolute ethanol*. Boil under a reflux condenser in a current of *nitrogen* for 30 minutes. Cool rapidly and add 30 ml of *water*. Extract with four 50 ml quantities of *ether* discarding the lower layer after complete separation. Wash the combined upper layers with four 50 ml quantities of *water* and evaporate to dryness under a gentle current of *nitrogen* at a temperature not exceeding 30° or in a rotary evaporator at a temperature not exceeding 30° under reduced pressure (water ejector). Dissolve the residue in sufficient *propan-2-ol* to give an expected concentration of vitamin A equivalent to 10 to 15 IU per ml.

Measure the *absorbances*, Appendix II B, of the solution at 300 nm, 310 nm, 325 nm and 334 nm and at the wavelength of maximum absorption with a suitable spectrophotometer in 1 cm matched cells, using *propan-2-ol* as the compensation liquid.

Calculate the content of vitamin A, as *all-trans*-retinol, in IU per gram from the expression:

$$A_{325} \times \frac{1830}{100m} \times V$$

where A_{325} = absorbance at 325 nm

m = weight of the oral drops in grams

V = total volume of solution containing 10 IU to 15 IU of vitamin A per ml

1830 = conversion factor for the specific absorbance of *all-trans*-retinol in IU

The above expression can be used only if A_{325} has a value of not greater than $A_{325,\text{ corr}}$ / 0.970 where $A_{325,\text{ corr}}$ is the corrected absorbance at 325 nm and is given by the equation:

$$A_{325,\,\text{corr}} = 6.815A_{325} - 2.555A_{310} - 4.260A_{334}$$

where A designates the absorbance at the wavelength indicated by the subscript.

If A_{325} has a value greater than $A_{325,\text{ corr}}$ / 0.970, calculate the content of vitamin A from the expression:

$$A_{325,\,\text{corr}} \times \frac{1830}{100m} \times V$$

The assay is not valid unless:

(a) the wavelength of maximum absorption lies between 323 nm and 327 nm and

(b) the absorbance at 300 nm relative to that at 325 nm is at most 0.73.

Method B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Prepare solution (1) as follows. To a weighed quantity of the oral drops containing 50,000 IU in a round-bottomed flask, add 5 ml of a freshly prepared 10% w/v solution of *ascorbic acid* and 10 ml of a freshly prepared 80% w/v solution of *potassium hydroxide* and 100 ml of *ethanol* (*96%*). Boil under a reflux condenser on a water bath for 15 minutes. Add 100 ml of a 1% w/v solution of *sodium chloride* and cool. Transfer the solution to a 500 ml separating funnel rinsing the round-bottomed flask with about 75 ml of a 1% w/v solution of *sodium chloride* and then with 150 ml of a mixture of equal volumes of *petroleum spirit* (*boiling range, 40° to 60°*) and *ether*. Shake for 1 minute. When the layers have separated completely, discard the lower layer and wash the upper layer, first with 50 ml of a 3% w/v solution of *potassium hydroxide* in a 10% v/v solution of *ethanol* (*96%*) and then with three 50 ml quantities of a 1% w/v solution of *sodium chloride*. Filter the upper layer through 5 g of *anhydrous sodium sulphate* on a fast filter paper into a 250 ml flask suitable for a rotary evaporator. Wash the funnel with 10 ml of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30° under reduced pressure (water ejector) and fill with *nitrogen* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen* at a temperature not exceeding 30°. Dissolve the residue in *propan-2-ol*, transfer to a 25 ml volumetric flask and dilute to 25 ml with *propan-2-ol*. Gentle heating in an ultrasonic bath may be required. For solution (2) prepare a solution of *retinyl acetate EPCRS* in *propan-2-ol R1* so that 1 ml contains about 1000 IU of *all-trans*-retinol.

The exact concentration of solution (2) is assessed by ultraviolet absorption spectrophotometry, Appendix II B. Dilute solution (2) with *propan-2-ol R1* to a presumed concentration of 10 to 15 IU per ml and measure the *absorbance* at 326 nm in matched 1 cm cells using *propan-2-ol R1* as the compensation liquid.

Calculate the content of vitamin A in IU per ml of solution (2) from the following expression, taking into account the assigned content of *retinyl acetate EPCRS:*

$$A_{326} \times \frac{1900 \times V_2}{100 \times V_1}$$

where A_{326} = absorbance at 326 nm

V_2 = volume of the diluted solution

V_1 = volume of solution (2) used

1900 = conversion factor for the specific absorbance of *retinyl acetate EPCRS* in IU

For solution (3) proceed as described for solution (1) but use 2 ml of solution (2) in place of the oral drops.

The exact concentration of solution (3) is assessed by *ultraviolet absorption spectrophotometry*, Appendix II B. Dilute solution (3) with *propan-2-ol R1* to a presumed concentration of 10 to 15 IU per ml of *all-trans*-retinol and measure the absorbance at 325 nm in matched 1 cm cells using *propan-2-ol R1* as the compensation liquid.

Calculate the content of *all-trans*-retinol in IU per millilitre of solution (3) from the expression:

$$A_{325} \times \frac{1830 \times V_4}{100 \times V_3}$$

where A_{325} = absorbance at 325 nm

V_3 = volume of the diluted solution

V_4 = volume of solution (3) used

1830 = conversion factor for the specific absorbance of *all-trans*-retinol in IU

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm), (b) as mobile phase at a flow rate of 1 ml per minute a mixture of 3 volumes of *water* and 97 volumes of *methanol*, (c) a detection wavelength of 325 nm, (d) a 10 µl loop injector and (e) an electronic integrator.

Inject in triplicate solution (1) and solution (3). The retention time of *all-trans*-retinol is 5 ± 1 minutes.

The assay is not valid unless: (a) the chromatogram obtained with solution (1) shows a peak corresponding to that of *all-trans*-retinol in the chromatogram obtained with solution (3); (b) when using the method of standard additions to solution (1) there is greater than 95% recovery of the added *retinyl acetate EPCRS*; (c) the recovery of *all-trans*-retinol in solution (3), as assessed by direct absorption spectrophotometry, is greater than 95%.

Calculate the content of vitamin A using the following expression:

$$A_1 \times \frac{C \times V}{A_2} \times \frac{1}{m}$$

where A_1 = area of the peak corresponding to *all-trans*-retinol in the chromatogram obtained with solution (1)

A_2 = area of the peak corresponding to *all-trans*-retinol in the chromatogram obtained with solution (3)

C = concentration of *retinyl acetate EPCRS* in solution (2) as assessed prior to the saponification in International Units per ml (= 1000 IU per ml)

V = volume of solution (2) treated

m = weight of the oral drops in solution (1)

For ascorbic acid

To a volume of the oral drops containing the equivalent of 0.14 g of ascorbic acid, add 100 ml of 1% w/v *orthophosphoric acid* in *water* and 1 ml of *starch solution* and titrate with 0.05M *iodine VS* until a full blue colour is produced. Each ml of 0.05M *iodine VS* is equivalent to 0.0088 g of ascorbic acid.

For colecalciferol

Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) to a weighed quantity of the oral drops containing about 4000 IU of colecalciferol, add 10 ml of a 0.001% w/v solution of *ergocalciferol BPCRS* in *absolute ethanol*, 20 ml of *absolute ethanol* and 5 ml of a 60% w/v solution of *potassium hydroxide* in *water*. Pass a stream of nitrogen and boil under a reflux condenser for 20 minutes, cool and transfer the contents to a separating funnel using *ether*, shake and allow the layers to separate; discard the lower layer and wash the ether layer with four 50 ml quantities of *water*, swirling gently for each washing and discard the lower layer. Evaporate to dryness using a rotary evaporator and transfer the residue to a 4 ml vial using small quantities of *methanol* until 4 ml of solution is obtained. Prepare solution (2) in the same manner as solution (1) but using 10 ml each of 0.001% w/v each of *colecalciferol BPCRS* and *ergocalciferol BPCRS* respectively beginning at the words '20 ml of *absolute ethanol* …′.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with particles of silica the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm) (Spherisorb ODS 1 is suitable), (b) a mixture of 7 volumes of *water* and 93 volumes of *methanol* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

Allow the mobile phase to equilibrate for 30 minutes.

Inject 20 µl of each solution.

The assay is not valid unless the chromatogram obtained with solution (2) shows two distinct peaks. If complete resolution is not attained, add 3% v/v of *distilled water* to the mobile phase, allow to equilibrate and repeat the chromatography. If the resolution is still not achieved, use a new column and allow the mobile phase to equilibrate for 30 minutes.

Determine the *weight per ml* of the oral drops, Appendix V G, and calculate the content of colecalciferol, weight in volume, using the declared content of colecalciferol in *colecalciferol BPCRS.*

STORAGE

Paediatric Vitamins A, C and D Oral Drops should be kept in an airtight container and protected from light.

LABELLING

The label states the number of International Units of Vitamin A and Colecalciferol and the content of vitamin C in terms of the equivalent amount of ascorbic acid.

Prolonged-release Verapamil Tablets

General Notices

Prolonged-release Verapamil Tablets from different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable unless otherwise justified and authorised.

Action and use

Calcium channel blocker.

DEFINITION

Prolonged-release Verapamil Tablets contain Verapamil Hydrochloride. They are formulated so that the medicament is released over a period of several hours. They are coated.

PRODUCTION

A suitable dissolution test is carried out to demonstrate the appropriate release of Verapamil Hydrochloride. The dissolution profile reflects the *in vivo* performance which in turn is compatible with the dosage schedule recommended by the manufacturer.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of verapamil hydrochloride, $C_{27}H_{38}N_2O_4$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Verapamil Hydrochloride with 25 ml of 0.1M *hydrochloric acid* for 10 minutes, filter, extract the filtrate with 25 ml of *ether*, discard the extract and make the aqueous solution just alkaline with 2M *potassium carbonate sesquihydrate*. Extract with 25 ml of *ether*, filter the ether layer through *anhydrous sodium sulphate* and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the oily residue, Appendix II A, is concordant with the *reference spectrum* of verapamil (*RS 359*).

B. Shake a quantity of the powdered tablets containing 0.24 g of Verapamil Hydrochloride with 6 ml of *methanol* for a few minutes, allow to settle and dilute 0.7 ml of the supernatant liquid to 2 ml with *water*. The resulting solution yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) add 95 ml of a solution containing equal volumes of 0.01M *hydrochloric acid* and *methanol* (solvent A) to a quantity of the powdered tablets containing 0.24 g of Verapamil Hydrochloride, mix with the aid of ultrasound for 5 minutes, shaking occasionally, add sufficient solvent A to produce 100 ml and mix. Centrifuge 50 ml of the resulting solution; dilute the supernatant liquid with sufficient of solvent A to produce a solution containing 0.072% w/v of Verapamil Hydrochloride and filter through a 0.45-µm filter. For solution (2) dilute 1 volume of solution (1) to 200 volumes with solvent A. For solution (3) dilute 1 volume of solution (2) to 20 volumes with solvent A.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable) and maintained at 40°, (b) as the mobile phase with a flow rate of 0.8 ml per minute a solution prepared as described below and (c) a detection wavelength of 278 nm. For the mobile phase dissolve 0.82 g of *anhydrous sodium acetate* in 33 ml of *anhydrous acetic acid*, add sufficient *water* to produce 1000 ml and to 700 ml of this solution add 300 ml of *acetonitrile* and 3 ml of *2-heptylamine*.

Adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with solution (2) is at least 40% of the full scale of the recorder.

The test is not valid unless, in the chromatogram obtained with solution (3), a peak is observed which has the same retention time as the principal peak in the chromatogram obtained with solution (2).

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%) and the sum of the areas of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.24 g of Verapamil Hydrochloride with 150 ml of a solution containing 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *methanol* for several minutes, add sufficient of the same solvent mixture to produce 200 ml and filter. Dilute 4 ml of the filtrate to 100 ml with a solution containing 1 volume of 0.1M *hydrochloric acid* and 9 volumes of *methanol* and measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{27}H_{38}N_2O_4$,HCl in the tablets taking 122 as the value of A(1%, 1 cm) at the maximum at 278 nm.

STORAGE

Prolonged-release Verapamil Tablets should be protected from direct sunlight.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities D, E, F, G, I, J and K listed under Verapamil Hydrochloride.

Vancomycin Intravenous Infusion

General Notices

Vancomycin Injection

Action and use

Glycopeptide antibacterial.

DEFINITION

Vancomycin Intravenous Infusion is a sterile solution of Vancomycin Hydrochloride in Water for Injections. It is prepared by dissolving Vancomycin Hydrochloride for Intravenous Infusion in Water for Injections and then diluting with the requisite volume of a suitable diluent in accordance with the manufacturer's instructions.

The intravenous infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Vancomycin Intravenous Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

VANCOMYCIN HYDROCHLORIDE FOR INTRAVENOUS INFUSION

DEFINITION

Vancomycin Hydrochloride for Intravenous Infusion is a sterile material consisting of Vancomycin Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

IDENTIFICATION

A. In the test for Vancomycin B, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (4).

B. Yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a solution containing 5% w/v of Vancomycin Hydrochloride, 2.5 to 4.5, Appendix V L.

Clarity of solution

A solution containing 10.0% w/v of Vancomycin Hydrochloride is *clear*, Appendix IV A. The *absorbance* of the solution at 450 nm is not greater than 0.10, Appendix II B.

Vancomycin B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the solutions within 4 hours of preparation.

(1) Dissolve a quantity of the contents of the sealed container in sufficient mobile phase A to produce a solution containing 2,000 IU of vancomycin per ml.

(2) Dilute 1 volume of solution (1) to 25 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 40 volumes with mobile phase A.

(4) Heat a 0.050% w/v solution of *vancomycin hydrochloride EPCRS* in *water* at 65° for 24 hours and allow to cool.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 280 nm.

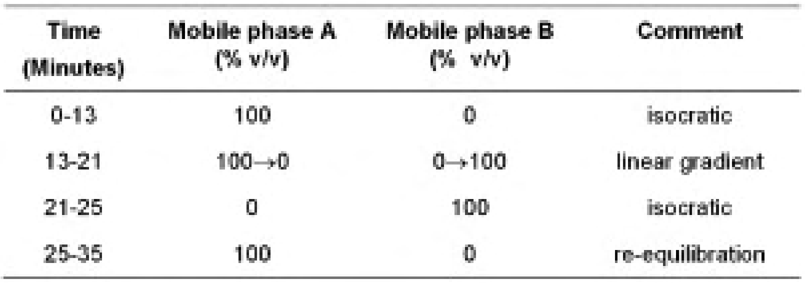
(f) Inject 20 µl of each solution.

MOBILE PHASE

Mobile phase A To 4 volumes of *triethylamine* add 1996 volumes of *water* and adjust to pH 3.2 with *orthophosphoric acid* (solution A). Add 10 volumes of *tetrahydrofuran* and 70 volumes of *acetonitrile* to 920 volumes of solution A.

Mobile phase B Add 10 volumes of *tetrahydrofuran* and 290 volumes of *acetonitrile* to 700 volumes of solution A.

Use the following gradient.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-21	100→0	0→100	linear gradient
21-25	0	100	isocratic
25-35	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the principal peak has a *signal-to-noise ratio* of at least 5;

in the chromatogram obtained with solution (2), the *symmetry factor* of the vancomycin peak is not greater than 1.6;

in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 5.0.

LIMITS

Not less than 88.0%, calculated using the following expression:

$$\frac{A_b \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

where,

A_b = area of the peak corresponding to vancomycin B in the chromatogram obtained with solution (2);

A_t = sum of the areas of the peaks corresponding to impurities in the chromatogram obtained with solution (1).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1), (2) and (3) described under Vancomycin B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Vancomycin B may be used.

LIMITS

In the chromatogram obtained with solution (1) calculate the percentage content of each impurity using the following expression:

$$\frac{\left(\frac{A_i}{25}\right) \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

where,

A_i = area of an impurity peak in the chromatogram obtained with solution (1),

A_b = area of the peak corresponding to vancomycin B in the chromatogram obtained with solution (2),

A_t = sum of the areas of the peaks corresponding to impurities in the chromatogram obtained with solution (1).

The content of any impurity is not greater than 4.0% and the sum of the contents of any such impurities is not greater than 12.0%.

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *tris-chloride buffer pH 7.4* prepared using *water BET* to give a solution containing 9000 IU of vancomycin per ml (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for Uniformity of weight under Parenteral Preparations, Powders for Injections.

Mix the contents of the 10 containers and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

For a container of average content weight, the upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

LABELLING

The label of the sealed container states (1) the total number of IU (Units) contained in it and (2) the number of IU (Units) per mg.

Vancomycin Oral Solution

General Notices

Action and use

Glycopeptide antibacterial.

DEFINITION

Vancomycin Oral Solution is a solution of Vancomycin Hydrochloride in a suitable flavoured vehicle. It is prepared by dissolving Vancomycin Hydrochloride for Oral Solution in the requisite amount of a suitable diluent.

The oral solution complies with the requirements stated under Oral Liquids.

STORAGE

Vancomycin Oral Solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

VANCOMYCIN HYDROCHLORIDE FOR ORAL SOLUTION

DEFINITION

Vancomycin Hydrochloride for Oral Solution is a dry powder consisting of Vancomycin Hydrochloride with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids and with the following requirements.

IDENTIFICATION

A. In the test for Vancomycin B, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (4).

B. Yield reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a solution containing 5% w/v of Vancomycin Hydrochloride, 2.5 to 4.5, Appendix V L.

Clarity of solution

A solution containing 10.0% w/v of Vancomycin Hydrochloride is *clear*, Appendix IV A. The *absorbance* of the solution at 450 nm is not greater than 0.10, Appendix II B.

Vancomycin B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Use the solutions within 4 hours of preparation.

(1) Dissolve a quantity of the contents of the sealed container in sufficient mobile phase A to produce a solution containing 2,000 IU of vancomycin per ml.

(2) Dilute 1 volume of solution (1) to 25 volumes with mobile phase A.

(3) Dilute 1 volume of solution (2) to 40 volumes with mobile phase A.

(4) Heat a 0.050% w/v solution of *vancomycin hydrochloride EPCRS* in *water* at 65° for 24 hours and allow to cool.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1 ml per minute.

(d) Use ambient column temperature.

(e) Use a detection wavelength of 280 nm.

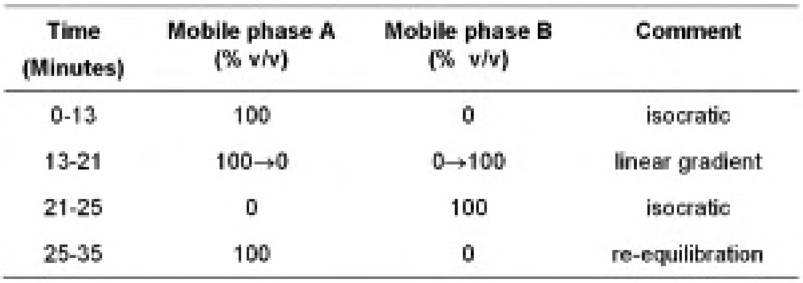
(f) Inject 20 µl of each solution.

MOBILE PHASE

Mobile phase A To 4 volumes of *triethylamine* add 1996 volumes of *water* and adjust to pH 3.2 with *orthophosphoric acid* (solution A). Add 10 volumes of *tetrahydrofuran* and 70 volumes of *acetonitrile* to 920 volumes of solution A.

Mobile phase B Add 10 volumes of *tetrahydrofuran* and 290 volumes of *acetonitrile* to 700 volumes of solution A.

Use the following gradient.



Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-13	100	0	isocratic
13-21	100→0	0→100	linear gradient
21-25	0	100	isocratic
25-35	100	0	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the principal peak has a *signal-to-noise ratio* of at least 5;

in the chromatogram obtained with solution (2), the *symmetry factor* of the vancomycin peak is not greater than 1.6;

in the chromatogram obtained with solution (4), the *resolution factor* between the two principal peaks is at least 5.0.

LIMITS

Not less than 88.0%, calculated using the following expression:

$$\frac{A_b \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

where,

A_b = area of the peak corresponding to vancomycin B in the chromatogram obtained with solution (2);

A_t = sum of the areas of the peaks corresponding to impurities in the chromatogram obtained with solution (1).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions (1), (2) and (3) described under Vancomycin B.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Vancomycin B may be used.

LIMITS

In the chromatogram obtained with solution (1) calculate the percentage content of each impurity using the following expression:

$$\frac{\left(\frac{A_i}{25}\right) \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

where,

A_i = area of an impurity peak in the chromatogram obtained with solution (1),

A_b = area of the peak corresponding to vancomycin B in the chromatogram obtained with solution (2),

A_t = sum of the areas of the peaks corresponding to impurities in the chromatogram obtained with solution (1).

The content of any impurity is not greater than 4.0% and the sum of the contents of any such impurities is not greater than 12.0%.

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

ASSAY

Mix the contents of 10 containers and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 115.0% of the stated number of IU.

LABELLING

The label of the sealed container states (1) the total number of IU (Units) contained in it and (2) the number of IU (Units) per mg.

Verapamil Injection

General Notices

Action and use

Calcium channel blocker.

DEFINITION

Verapamil Injection is a sterile solution of Verapamil Hydrochloride in Water for Injections. *The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of verapamil hydrochloride, $C_{27}H_{38}N_2O_4$,HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Dilute a volume containing 10 mg of Verapamil Hydrochloride to 5 ml with 0.1M *hydrochloric acid*, extract with 5 ml of *ether*, discard the extract and make the aqueous layer just alkaline with 2M *potassium carbonate sesquihydrate*. Extract with 5 ml of *ether*, filter the ether layer through *anhydrous sodium sulphate* and evaporate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of verapamil (*RS 359*).

B. To a volume containing 5 mg of Verapamil Hydrochloride add 0.2 ml of a 5% w/v solution of *mercury*(*II*) *chloride*. A white precipitate is produced.

C. To a volume containing 5 mg of Verapamil Hydrochloride add 0.5 ml of 3M *sulphuric acid* and 0.2 ml of *dilute potassium permanganate solution*. A violet precipitate is produced which quickly dissolves to produce a very pale yellow solution.

TESTS

Acidity

pH, 4.5 to 6.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) use the injection diluted, if necessary, with mobile phase to contain 0.125% w/v of Verapamil Hydrochloride. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 10 volumes with the same solvent. Solution (3) contains 0.005% w/v of *verapamil hydrochloride BPCRS* and 0.005% w/v of *verapamil impurity I EPCRS* in the mobile phase. Inject 10 µl of each solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 0.85 ml per minute a mixture of 1 volume of n-*heptylamine*, 4.7 volumes of *glacial acetic acid*, 58 volumes of *acetonitrile* and 137 volumes of 0.01M *sodium acetate* and (c) a detection wavelength of 278 nm.

Allow the chromatography to proceed for 4 times the retention time of verapamil. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to verapamil and verapamil impurity I is at least 2.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%). Disregard any peak with an area less than 0.5 times that of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Dilute a volume containing 5 mg of Verapamil Hydrochloride to 100 ml with 0.01M *hydrochloric acid*. Measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{27}H_{38}N_2O_4$,HCl taking 118 as the value of A(1%, 1 cm) at the maximum at 278 nm.

STORAGE

Verapamil Injection should be protected from light.

Verapamil Tablets

General Notices

Action and use

Calcium channel blocker.

DEFINITION

Verapamil Tablets contain Verapamil Hydrochloride. They are coated.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of verapamil hydrochloride, $C_{27}H_{38}N_2O_4$,HCl

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Shake a quantity of the powdered tablets containing 0.1 g of Verapamil Hydrochloride with 25 ml of 0.1M *hydrochloric acid*, filter, extract the filtrate with 25 ml of *ether*, discard the extract and make the aqueous solution just alkaline with 2M *potassium carbonate sesquihydrate*. Extract with 25 ml of *ether*, filter the ether layer through *anhydrous sodium sulphate* and evaporate to dryness. The *infrared absorption spectrum* of a thin film of the oily residue, Appendix II A, is concordant with the *reference spectrum* of verapamil (*RS 359*).

B. Shake a quantity of the powdered tablets containing 0.1 g of Verapamil Hydrochloride with 10 ml of *dichloromethane*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of *water*. To 2 ml of the resulting solution add 0.2 ml of a 5% w/v solution of *mercury(II) chloride*. A white precipitate is produced.

C. To 2 ml of the solution obtained in test B add 0.5 ml of 3M *sulphuric acid* and 0.2 ml of *dilute potassium permanganate solution*. A violet precipitate is produced which quickly dissolves to produce a very pale yellow solution.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing 0.24 g of Verapamil Hydrochloride with 90 ml of mobile phase, add sufficient of the mobile phase to produce 100 ml, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the mobile phase and further dilute 1 volume of the resulting solution to 10 volumes with the same solvent. Solution (3) contains 0.005% w/v of *verapamil hydrochloride BPCRS* and 0.005% w/v of *verapamil impurity I EPCRS* in the mobile phase. Inject 10 µl of each solution.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 0.85 ml per minute a mixture of 1 volume of n-*heptylamine*, 4.7 volumes of *glacial acetic acid*, 58 volumes of *acetonitrile* and 137 volumes of 0.01M *sodium acetate* and (c) a detection wavelength of 278 nm.

Allow the chromatography to proceed for 4 times the retention time of verapamil. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to verapamil and verapamil impurity I is at least 2.0.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%). Disregard any peak with an area less than 0.5 times that of the principal peak in the chromatogram obtained with solution (2) (0.05%).

ASSAY

Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Verapamil Hydrochloride with 150 ml of 0.1M *hydrochloric acid* for 10 minutes, add sufficient 0.1M *hydrochloric acid* to produce 200 ml and filter. Dilute 10 ml of the filtrate to 100 ml with *water* and measure the *absorbance* of the resulting solution at the maximum at 278 nm, Appendix II B. Calculate the content of $C_{27}H_{38}N_2O_4$,HCl taking 118 as the value of A(1%, 1 cm) at the maximum at 278 nm.

Vigabatrin Oral Powder

General Notices

Action and use

Antiepileptic.

DEFINITION

Vigabatrin Oral Powder contains Vigabatrin.

The oral powder complies with the requirements stated under Oral Powders and with the following requirements.

Content of vigabatrin, $C_6H_{11}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of vigabatrin (*RS 360*).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

5-Vinyl-2-pyrrolidone

In the Assay, the area of any peak corresponding to 5-vinyl-2-pyrrolidone in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve a quantity of the mixed contents of 20 sachets containing 0.2 g of Vigabatrin in sufficient of the mobile phase to produce 100 ml. Solution (2) contains 0.2% w/v of *vigabatrin BPCRS* in the mobile phase. Solution (3) contains 0.001% w/v of *5-vinyl-2-pyrrolidone BPCRS.* Solution (4) contains 0.002% w/v of *5-vinyl-2-pyrrolidone BPCRS*, 0.2% w/v of *povidone* and 0.2% w/v of *vigabatrin BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with cation exchange resin (10 µm) (Whatman Partisil SCX is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 1000 volumes of a 0.34% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 2.8 with *orthophosphoric acid* and (c) a detection wavelength of 210 nm.

Inject 20 µl of solution (4). When the chromatogram is recorded under the prescribed conditions, the retention times are povidone, about 4 minutes, 5-vinyl-2-pyrrolidone, about 5 minutes and vigabatrin, about 8 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to 5-vinyl-2-pyrrolidone and vigabatrin is at least 1.5 and the *resolution factor* between the peaks corresponding to povidone and 5-vinyl-2-pyrrolidone is at least 1.5.

Inject separately 20 µl of solutions (1) and (2). Calculate the content of $C_6H_{11}NO_2$ in one sachet from the areas of the peaks and using the declared content of $C_6H_{11}NO_2$ in *vigabatrin BPCRS*.

Vigabatrin Tablets

General Notices

Action and use

Antiepileptic.

DEFINITION

Vigabatrin Tablets contain Vigabatrin.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of vigabatrin, $C_6H_{11}NO_2$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a quantity of the powdered tablets containing 0.5 g of Vigabatrin add 10 ml of *water*, shake and filter through a 0.7 µm filter (Whatman GF/F is suitable). To 0.2 ml of the filtrate add 5 ml of *acetone* and allow the solvent to evaporate in a current of nitrogen. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of vigabatrin (*RS 360*).

TESTS

5-Vinyl-2-pyrrolidone

In the Assay, the area of any peak corresponding to 5-vinyl-2-pyrrolidone in the chromatogram obtained with solution (1) is not greater than the area of the peak in the chromatogram obtained with solution (3) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, injecting 20 µl of each of the following solutions. For solution (1) add 800 ml of the mobile phase to 10 whole tablets, stir vigorously until all of the tablets are uniformly dispersed into fine particles, add sufficient of the mobile phase to produce 1000 ml, mix well and filter through a 0.2 µm nylon-66 filter. Dilute 10 ml of the filtrate to 50 ml with the mobile phase. Solution (2) contains 0.10% w/v of *vigabatrin BPCRS* in the mobile phase. Solution (3) contains 0.0005% w/v of *5-vinyl-2-pyrrolidone BPCRS* in the mobile phase. Solution (4) contains 0.0005% w/v of *5-vinyl-2-pyrrolidone BPCRS*, 0.1% w/v of *povidone* and 0.1% w/v of *vigabatrin BPCRS* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with cation exchange resin (10 µm) (Whatman Partisil SCX is suitable), (b) as the mobile phase with a flow rate of 1.5 ml per minute, a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 1000 volumes of a 0.34% w/v solution of *potassium dihydrogen orthophosphate*, adjusting the pH to 2.8 with *orthophosphoric acid* and (c) a

detection wavelength of 210 nm.

In the chromatogram obtained with solution (4), the retention times are about 4 minutes for povidone, about 5 minutes for 5-vinyl-2-pyrrolidone and about 8 minutes for vigabatrin. The test is not valid unless the *resolution factor* between the peaks corresponding to 5-vinyl-2-pyrrolidone and vigabatrin in the chromatogram obtained with solution (4) is at least 1.5 and the *resolution factor* between the peaks corresponding to povidone and 5-vinyl-2-pyrrolidone is at least 1.5.

Calculate the percentage content of $C_6H_{11}NO_2$ in the tablets from the areas of the peaks for vigabatrin in the chromatograms obtained with solutions (1) and (2) and from the declared content of $C_6H_{11}NO_2$ in *vigabatrin BPCRS*.

Vinblastine Injection

General Notices

Action and use

Vinca alkaloid cytotoxic.

DEFINITION

Vinblastine Injection is a sterile solution of Vinblastine Sulphate in Water for Injections. It is prepared by dissolving Vinblastine Sulphate for Injection in the requisite amount of Sodium Chloride Intravenous Infusion containing 0.9% w/v of Sodium Chloride.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Vinblastine Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

VINBLASTINE SULPHATE FOR INJECTION

DEFINITION

Vinblastine Sulphate for Injection is a sterile material prepared from Vinblastine Sulphate. It may contain excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of vinblastine sulphate, $C_{46}H_{58}N_4O_9,H_2SO_4$

92.5 to 107.5% of the stated amount of anhydrous vinblastine sulphate.

IDENTIFICATION

A. In the test for Related substances the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

B. To 1 mg add 0.2 ml of a freshly prepared 1% w/v solution of *vanillin* in *hydrochloric acid*. A pink colour is produced in about 1 minute (distinction from vincristine sulphate).

C. Yield the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Acidity

pH of a solution containing the equivalent of 0.15% w/v of anhydrous vinblastine sulphate, 3.5 to 5.0, Appendix V L.

Clarity of solution

Dissolve the contents of a sealed container in 10 ml of *carbon dioxide-free water*. The resulting solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.10% w/v of anhydrous vinblastine sulphate. Solution (2) contains 0.10% w/v each of *vinblastine sulphate EPCRS* and *vincristine sulphate EPCRS* in *water*. Solutions (3), (4) and (5) contain 0.10% w/v, 0.0020% w/v and 0.00010% w/v, respectively, of *vinblastine sulphate EPCRS* in *water*. Keep the solutions in ice before use.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax C8 is suitable), (b) a guard column packed with a suitable silica gel placed between the pump and the injection device, (c) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 12 volumes of *acetonitrile*, 38 volumes of a 1.5% v/v solution of *diethylamine* adjusted to pH 7.5 with *orthophosphoric acid* and 50 volumes of *methanol* and (d) a detection wavelength of 262 nm. Inject 10 µl of each solution using a loop injector and record the chromatograms for 3 times the retention time of the peak due to vinblastine.

The test is not valid unless the *resolution factor* between the peaks due to vinblastine and vincristine in the chromato-gram obtained with solution (2) is at least 4 and unless the *signal-to-noise ratio* in the peak in the chromatogram obtained with solution (5) is at least 5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2%) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (4) (5%). Disregard any peak with an area less than that of the peak in the chromatogram obtained with solution (5) (0.1%).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 16 hours, lose not more than 17.0% of their weight.

Uniformity of content

The content of anhydrous vinblastine sulphate in each of 10 individual containers as determined in the Assay is not less than 90.0% and not more than 110.0% of the average except that in one container the content may be not less than 80.0% and not more than 120.0% of the average.

ASSAY

Dissolve the contents of a sealed container in a suitable volume of *methanol* and dilute with sufficient *methanol* to produce a solution containing 0.004% w/v of anhydrous vinblastine sulphate. Measure the *absorbance* of the resulting solution at the maximum at 267 nm, Appendix II B. Calculate the content of $C_{46}H_{58}N_4O_9,H_2SO_4$ in the sealed container taking 185 as the value of A(1%, 1 cm) at the maximum at 267 nm. Repeat the procedure with a further nine sealed containers. Calculate the average content of $C_{46}H_{58}N_4O_9,H_2SO_4$ per container from the 10 individual results thus obtained.

STORAGE

Vinblastine Sulphate for Injection should be stored at a temperature of 2° to 8°.

LABELLING

The label of the sealed container states the weight of anhydrous vinblastine sulphate contained in it.

Vincristine Injection

General Notices

Action and use

Vinca alkaloid cytotoxic.

DEFINITION

Vincristine Injection is a sterile solution prepared by dissolving Vincristine Sulphate for Injection in the requisite amount of Sodium Chloride Intravenous Infusion containing 0.9% w/v of Sodium Chloride.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Vincristine Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

VINCRISTINE SULPHATE FOR INJECTION

DEFINITION

Vincristine Sulphate for Injection is a sterile material consisting of a mixture of one part by weight of Vincristine Sulphate and ten parts by weight of Lactose with or without other excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of vincristine sulphate, $C_{46}H_{56}N_4O_{10},H_2SO_4$

92.5 to 107.5% of the stated amount of anhydrous vincristine sulphate.

IDENTIFICATION

A. In the test for Related substances the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (3).

B. Shake a quantity containing the equivalent of 1 mg of anhydrous vincristine sulphate with 3 ml of *chloroform*, filter and wash the filter with 2 ml of *chloroform*. Evaporate the combined chloroform solutions to dryness at 40°. Add 0.2 ml of a freshly prepared 1% w/v solution of *vanillin* in *hydrochloric acid* to the residue. An orange colour is produced in about 1 minute (distinction from vinblastine sulphate).

TESTS

Clarity of solution

Dissolve the contents of a sealed container in 10 ml of *carbon dioxide-free water*. The solution is *clear*, Appendix IV A.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.10% w/v of anhydrous vincristine sulphate. Solution (2) contains 0.10% w/v each of *vincristine sulphate EPCRS* and *vinblastine sulphate EPCRS* in *water*. Solutions (3), (4) and (5) contain 0.10% w/v, 0.0020% w/v and 0.00010% w/v, respectively, of *vincristine sulphate EPCRS* in *water*. Keep the solutions in ice before use.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Zorbax C8 is suitable), (b) a guard column packed with a suitable silica gel placed between the pump and the injection device, (c) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of 30 volumes of a 1.5% v/v solution of *diethylamine* adjusted to pH 7.5 with *orthophosphoric acid* and 70 volumes of *methanol* and (d) a detection wavelength of 297 nm. Inject 10 µl of each solution using a loop injector and record the chromatograms for 3 times the retention time of the peak due to vincristine.

The test is not valid unless the *resolution factor* between the peaks due to vincristine and vinblastine in the chromatogram obtained with solution (2) is at least 4 and unless the *signal-to-noise ratio* in the peak in the chromatogram obtained with solution (5) is at least 5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (2%) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (4) (5%). Disregard any peak with an area less than that of the peak in the chromatogram obtained with solution (5) (0.1%).

Uniformity of content

The content of anhydrous vincristine sulphate in each of 10 individual containers as determined in the Assay is not less than 90.0% and not more than 110.0% of the average except that in one container the content may be not less than 80.0% and not more than 120.0% of the average.

ASSAY

Dissolve the contents of a sealed container in a suitable volume of *methanol* and dilute with sufficient *methanol* to produce a solution containing the equivalent of 0.005% w/v of anhydrous vincristine sulphate. Measure the *absorbance* of the resulting solution at the maximum at 297 nm, Appendix II B. Calculate the content of $C_{46}H_{56}N_4O_{10},H_2SO_4$ in the sealed container taking 177 as the value of A(1%, 1 cm) at the maximum at 297 nm. Repeat the procedure with a further nine containers. Calculate the average content of $C_{46}H_{56}N_4O_{10},H_2SO_4$ per container from the 10 individual results thus obtained.

STORAGE

Vincristine Sulphate for Injection should be stored at a temperature of 2° to 8°.

LABELLING

The label of the sealed container states the weight of anhydrous vincristine sulphate contained in it.

Vindesine Injection

General Notices

Action and use

Vinca alkaloid cytotoxic.

DEFINITION

Vindesine Injection is a sterile solution of Vindesine Sulphate in a suitable liquid. It is prepared by dissolving Vindesine Sulphate for Injection in the requisite volume of a suitable diluent in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations.

STORAGE

Vindesine Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

VINDESINE SULPHATE FOR INJECTION

DEFINITION

Vindesine Sulphate for Injection is a sterile material consisting of a mixture of one part by weight of Vindesine Sulphate and five parts by weight of a suitable carrier with or without other excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Content of vindesine sulphate, $C_{43}H_{55}N_5O_7,H_2SO_4$

90.0 to 105.0% of the stated amount of anhydrous vindesine sulphate.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a *TLC silica gel F_{254} plate* and a mixture of 5 volumes of *diethylamine*, 5 volumes of *methanol* and 90 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl each of three solutions in *methanol* containing (1) sufficient of the contents of the sealed container to give a solution containing of the equivalent of 0.20% w/v of anhydrous vindesine sulphate, (2) 0.20% w/v of *vindesine sulphate EPCRS* and (3) 0.20% w/v each of *vinblastine sulphate EPCRS*, *vincristine sulphate EPCRS* and *vindesine sulphate EPCRS*. After removal of the plate, dry it in a current of air and examine under *ultraviolet light* (*254 nm*). The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

C. Yields the reactions characteristic of *sulphates*, Appendix VI.

TESTS

Clarity of solution

Dissolve the contents of a sealed container in 10 ml of *carbon dioxide-free water*. The solution is *clear*, Appendix IV A.

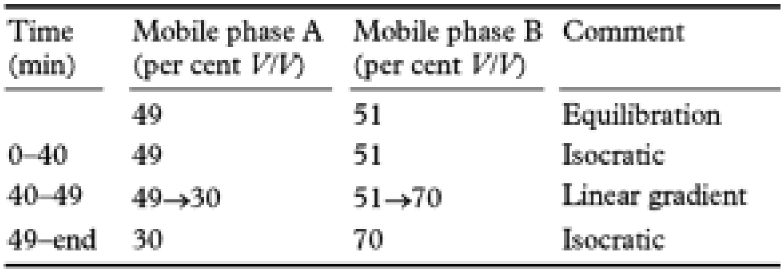
Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of a sealed container in sufficient *water* to produce a solution containing the equivalent of 0.10% w/v of anhydrous vindesine sulphate. For solution (2) dilute 1 volume of solution (1) to 50 volumes with *water*. For solution (3) dissolve 1 mg of *desacetylvinblastine EPCRS* in *water*, add 1 ml of solution (1) and dilute to 50 ml with *water*. For solution (4) dilute 1 volume of solution (2) to 20 volumes with *water*. Keep the solutions in ice before use.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Jones Apex ODS is suitable), (b) as the mobile phase at a flow rate of 2 ml per minute the changing proportions of mobile phase A and mobile phase B described below and (c) a detection wavelength of 270 nm.

Mobile phase A A 1.5% v/v solution of *diethylamine* adjusted to pH 7.4 with *orthophosphoric acid*

Mobile phase B Methanol.



Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
	49	51	Equilibration
0–40	49	51	Isocratic
40–49	49→30	51→70	Linear gradient
49–end	30	70	Isocratic

Inject separately 200 µl of each solution. Maintain the final concentration of the mobile phase until the total run time is twice the retention time of the principal peak in the chromatogram obtained with solution (1). The test is not valid unless, in the chromatogram obtained with solution (3), the retention time of vindesine is less than 40 minutes, the *symmetry factor* of the vindesine peak is not more than 2.0, and the *resolution factor* between the peaks due to vindesine and desacetylvinblastine is at least 2.0.

In the chromatogram obtained with the solution (1) the area of any *secondary peak* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3%) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (5%). Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

Uniformity of content

The content of anhydrous vindesine sulphate in each of 10 individual containers as determined in the Assay is not less than 90.0% and not more than 110.0% of the average except that in one container the content may be not less than 80.0% and not more than 120.0% of the average.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve the contents of a sealed container in *water* and add sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of anhydrous vindesine sulphate. For solution (2) dissolve and dilute the entire contents of a vial of *vindesine sulphate EPCRS* with *water* to yield a concentration of approximately 0.05% w/v. For solution (3) add 2 ml of solution (2) to 1 mg of *desacetylvinblastine EPCRS*. Keep the solutions in ice before use.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Jones Apex ODS is suitable), (b) as mobile phase at a flow rate of 1 ml per minute a mixture of 38 volumes of a 1.5% v/v solution of *diethylamine*, previously adjusted to pH 7.4 with *orthophosphoric acid*, and 62 volumes of *methanol* and (c) a detection wavelength of 270 nm.

Inject 20 µl of solution (3) five times. The assay is not valid unless the *resolution factor* between the peaks corresponding to vindesine sulphate and desacetylvinblastine sulphate is greater than 1.5, the *symmetry factor* of the vindesine peak is not more than 2.0, and the coefficient of variation of the area of the vindesine peak, calculated on the five injections is not greater than 2.0%.

Inject separately 20 µl of solution (1) and 20 µl of solution (2). Calculate the content of vindesine sulphate, $C_{43}H_{55}N_5O_7,H_2SO_4$, in the container using the declared content of $C_{43}H_{55}N_5O_7,H_2SO_4$ in *vindesine sulphate EPCRS*. Repeat the procedure with a further nine containers. Calculate the average content of $C_{43}H_{55}N_5O_7,H_2SO_4$ per container from the 10 individual results obtained.

STORAGE

Vindesine Sulphate for Injection should be stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed in the monograph for Vindesine Sulphate.

Vitamins B and C Injection

General Notices

DEFINITION

Vitamins B and C Injection is a sterile solution of Thiamine Hydrochloride, Pyridoxine Hydrochloride, Riboflavin Sodium Phosphate, Nicotinamide and Ascorbic Acid (as the sodium salt) in Water for Injections, containing Anhydrous Glucose for the intravenous injection or Benzyl Alcohol for the intramuscular injection.

Vitamins B and C Injection is prepared immediately before use by mixing the contents of two ampoules, (1) and (2). Ampoule (1) contains Thiamine Hydrochloride, Pyridoxine Hydrochloride, Riboflavin Sodium Phosphate and, where appropriate, Benzyl Alcohol. Ampoule (2) contains Nicotinamide and Ascorbic Acid and, where appropriate, Anhydrous Glucose. The air in ampoule (2) is replaced by nitrogen or other suitable inert gas.

The injection complies with the requirements stated under Parenteral Preparations and the contents of ampoules (1) and (2) comply with the following requirements, as appropriate.

Content of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS,HCl$

92.0 to 106.0% of the stated amount.

Content of pyridoxine hydrochloride, $C_8H_{11}NO_3,HCl$

90.0 to 110.0% of the stated amount.

Content of riboflavin, $C_{17}H_{20}N_4O_6$

90.0 to 110.0% of the stated amount.

Content of nicotinamide, $C_6H_6N_2O$

95.0 to 105.0% of the stated amount.

Content of ascorbic acid, $C_6H_8O_6$

95.0 to 105.0% of the stated amount.

Content of glucose, $C_6H_{12}O_6$

Where appropriate, 90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 60 volumes of *methanol* and 40 volumes of a solution containing 0.14% w/v of *potassium dihydrogen orthophosphate* and 0.5% w/v of *disodium edetate* as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions in *water*. For solution (1) use a volume of the contents of ampoule (1) diluted, if necessary, with *water* to contain 0.2% w/v of Pyridoxine Hydrochloride. For solution (2) use a volume of the injection in ampoule (2) diluted, if necessary, with *water* to contain 2% w/v of Ascorbic Acid. Solutions (3) and (4) contain 1% w/v and 0.4% w/v respectively of *thiamine mononitrate BPCRS*. Solution (5) contains 0.2% w/v of *pyridoxine hydrochloride BPCRS*. Solution (6) contains 0.022% w/v of *riboflavin sodium phosphate BPCRS*. Solution (7) contains 0.64% w/v of *nicotinamide BPCRS*. Solution (8) contains 2% w/v of *ascorbic acid EPCRS*. After removal of the plate, allow it to dry in air and examine under *ultraviolet light* (*254 nm and 365 nm*). The principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with either solutions (3), (5) and (6) or solutions (4), (5) and (6). The principal spots in the chromatogram obtained with solution (2) correspond to those in the chromatograms obtained with solutions (7) and (8).

B. For injections containing Anhydrous Glucose, heat 1 ml of the contents of ampoule (2) with *cupri-tartaric solution R1*. A copious precipitate of copper(I) oxide is produced.

ASSAY

For thiamine hydrochloride, pyridoxine hydrochloride and nicotinamide

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*. Solution (1) contains 0.01% w/v of *thiamine mononitrate BPCRS* and 0.002% w/v of *pyridoxine hydrochloride BPCRS*. Solution (2) contains 0.01% w/v of *thiamine mononitrate BPCRS* and 0.005% w/v of *pyridoxine hydrochloride BPCRS*. Solution (3) contains 0.016% w/v of *nicotinamide BPCRS*. For solution (4) dilute a suitable volume of the contents of ampoule (1) with *water* to contain 0.01% w/v of Thiamine Hydrochloride. For solution (5) dilute a suitable volume of the contents of ampoule (2) to contain 0.016% w/v of Nicotinamide.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute, 0.22% w/v of *sodium heptanesulphonate* in a mixture of 75 volumes of a 1.36% w/v solution of *potassium dihydrogen orthophosphate* and 25 volumes of *methanol*, the pH of the final mixture being adjusted to 3.0 with *orthophosphoric acid*, and (c) a detection wavelength of 280 nm.

Calculate the content of $C_{12}H_{17}ClN_4OS,HCl$ using the declared content of $C_{12}H_{17}N_5O_4S$ in *thiamine mononitrate BPCRS*. Each mg of $C_{12}H_{17}N_5O_4S$ is equivalent to 1.030 mg of $C_{12}H_{17}ClN_4OS,HCl$. Calculate the content of $C_8H_{11}NO_3,HCl$ using the declared content of $C_8H_{11}NO_3,HCl$ in *pyridoxine hydrochloride BPCRS*. Calculate the content of $C_6H_6N_2O$ using the declared content of $C_6H_6N_2O$ in *nicotinamide BPCRS*.

For riboflavin

Dilute a volume of the contents of ampoule (1) containing the equivalent of 4 mg of riboflavine with sufficient *phthalate buffer pH 4.0* to produce 200 ml and measure the *absorbance* of the resulting solution at the maximum at 446 nm, Appendix II B. Calculate the content of $C_{17}H_{20}N_4O_6$ taking 323 as the value of A(1%, 1 cm) at the maximum at 446 nm.

For ascorbic acid

Dilute a volume of the contents of ampoule (2) with *water* to produce a solution containing 1% w/v of Ascorbic Acid. To 20 ml of the resulting solution add 5 ml of 1M *sulphuric acid* and 50 ml of 0.05M *iodine VS* and titrate the excess iodine with 0.1M *sodium thiosulphate VS* using *starch mucilage* as indicator. Each ml of 0.05M *iodine VS* is equivalent to 8.806 mg of $C_6H_8O_6$.

For anhydrous glucose

For injections containing Anhydrous Glucose, dilute a volume of the contents of ampoule (2) containing 0.8 g of Anhydrous Glucose to 500 ml with *water* and dilute 2 ml of the resulting solution to 100 ml with *water*. Transfer 3 ml of this solution to a boiling tube previously cleaned with *chromic-sulphuric acid mixture* and rinsed with *water*. In two similar tubes place separately 3 ml of *glucose standard solution* and 3 ml of *water*. Add 6 ml of *anthrone reagent* in such a manner as to ensure rapid mixing, allow to stand for 10 minutes, cool quickly and measure the *absorbance*, Appendix II B, of the test solution and of the standard solution at the maximum at 625 nm using the solution prepared with *water* in the reference cell. Calculate the concentration of $C_6H_{12}O_6$ in the test solution.

STORAGE

Ampoules (1) and (2) for Vitamins B and C Injection should be protected from light.

LABELLING

For each ampoule the label states (1) the directions for the preparation of the injection; (2) whether the final injection is for intravenous or intramuscular use; (2) that the final injection is a high potency injection.

For ampoule (1) the label states the quantity of Riboflavin Sodium Phosphate in terms of the equivalent amount of riboflavin.

Warfarin Tablets

General Notices

Warfarin Tablets prepared from Warfarin Sodium are not necessarily interchangeable with Warfarin Tablets prepared from Warfarin Sodium Clathrate.

Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant (coumarin).

DEFINITION

Warfarin Tablets contain Warfarin Sodium or Warfarin Sodium Clathrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of warfarin sodium, $C_{19}H_{15}NaO_4$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Extract a quantity of the powdered tablets containing the equivalent of 0.1 g of warfarin sodium with 30 ml of *water*, add 0.1 ml of 2M *hydrochloric acid*, filter, wash the precipitate with *water* and dry. Warm the residue gently with 3 ml of *ethanol* (*96%*), filter and add the filtrate to 25 ml of *water* containing 0.1 ml of 2M *hydrochloric acid*. Filter, wash the precipitate with *water* and dry it at 105°. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of warfarin (*RS 361*).

B. The *melting point* of the residue obtained in test A is about 159°, Appendix V A.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1, using as the medium 900 ml of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 6.8 by the addition of 1M *sodium hydroxide* and rotating the basket at 100 revolutions per minute. For tablets containing 2 mg or less of warfarin sodium, place three tablets in the basket for each test; for tablets containing more than 2 mg of warfarin sodium, place a single tablet in the basket for each test. Withdraw a sample of 10 ml of the medium. Measure the *absorbance* of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maxima at 307 nm and 360 nm, Appendix II B, and calculate the difference between the two readings (ΔA). Calculate the total content of warfarin sodium, $C_{19}H_{15}NaO_4$, in the medium taking 428 as the value of $\Delta A(1\%, 1\ cm)$.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 50 volumes of *dichloromethane*, 50 volumes of *cyclohexane* and 20 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 40 mg of warfarin sodium with 30 ml of *water* for 15 minutes, add 0.1 ml of *hydrochloric acid* and extract with three 10 ml quantities of *chloroform*, drying each extract with *anhydrous sodium sulphate*; evaporate the combined extracts using a rotary evaporator at a temperature not exceeding 40° and dissolve the residue in 2 ml of *acetone*. Solution (2) contains 0.0020% w/v of (*E*)*-4-phenylbut-3-en-2-one* in *acetone*. Solution (3) contains 0.0020% w/v of *4-hydroxycoumarin* in *acetone*. For solution (4) dilute 1 volume of solution (1) to 200 volumes with *acetone*. After removal of the plate, allow it to dry in air, examine immediately in visible light noting the position of any coloured spots and then examine under *ultraviolet light* (*254 nm*), disregarding any spot that was noted in visible light. Any spots corresponding to (*E*)-4-phenylbut-3-en-2-one and 4-hydroxycoumarin in the chromatogram obtained with solution (1) are not more intense than the spots in the chromatograms obtained with solutions (2) and (3) respectively and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (4).

Uniformity of content

Tablets containing less than 2 mg of warfarin sodium comply with the requirements stated under Tablets but using in place of the content of active ingredient the individual peak areas obtained by the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solution. Shake one tablet with 10 ml of 0.01M *sodium hydroxide* for 15 minutes, add 10 ml of a 2% v/v solution of *glacial acetic acid* in *acetonitrile*, centrifuge for 10 minutes and use the clear supernatant liquid.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable), (b) a mixture of 55 volumes of *acetonitrile*, 45 volumes of *water* and 1 volume of *glacial acetic acid* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 283 nm.

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 5 mg of warfarin sodium with 50 ml of 0.01M *sodium hydroxide* for 15 minutes, add 50 ml of a 2% v/v solution of *glacial acetic acid* in *acetonitrile*, centrifuge for 10 minutes and use the clear supernatant liquid. For solution (2) dilute 5 ml of a 0.010% w/v solution of *warfarin BPCRS* in 0.01M *sodium hydroxide* to 10 ml with the mobile phase.

The chromatographic procedure may be carried out using the conditions described under Uniformity of content.

Calculate the content of $C_{19}H_{15}NaO_4$ in the tablets from the chromatogram obtained and using the declared content of $C_{19}H_{16}O_4$ in *warfarin BPCRS*. Each mg of $C_{19}H_{16}O_4$ is equivalent to 1.071 mg of $C_{19}H_{15}NaO_4$.

STORAGE

Warfarin Tablets should be protected from light.

LABELLING

When the active ingredient is Warfarin Sodium Clathrate the quantity is stated in terms of the equivalent amount of warfarin sodium.

Wool Alcohols Ointment

General Notices

DEFINITION

Wool Alcohols	60 g
Hard Paraffin	240 g
White Soft Paraffin or Yellow Soft Paraffin	100 g
Liquid Paraffin	600 g

In preparing Wool Alcohols Ointment, the proportions of Hard Paraffin, Soft Paraffin and Liquid Paraffin may be varied to produce Wool Alcohols Ointment having suitable properties.

When Wool Alcohols Ointment is used in a white ointment, it should be prepared with White Soft Paraffin; when used in a coloured ointment it should be prepared with Yellow Soft Paraffin.

Extemporaneous preparation

The following directions apply.

Melt together with the aid of gentle heat and stir until cold.
The ointment complies with the requirements stated under Topical Semi-solid Preparations.

Xylometazoline Nasal Drops

General Notices

Action and use

Alpha-adrenoceptor agonist.

DEFINITION

Xylometazoline Nasal Drops are a solution of Xylometazoline Hydrochloride in Purified Water. *The nasal drops comply with the requirements stated under Nasal Preparations and with the following requirements.*

Content of xylometazoline hydrochloride, $C_{16}H_{24}N_2$,HCl

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. To a volume of the nasal drops containing 50 mg of Xylometazoline Hydrochloride add 5 ml of 1M *sodium hydroxide*, extract with 10 ml of *dichloromethane IR*, evaporate to dryness and dissolve the residue in 0.5 ml of *dichloromethane IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of xylometazoline (*RS 362*).

B. To a volume containing 0.5 mg of Xylometazoline Hydrochloride add 0.2 ml of a 5% w/v solution of *sodium nitroprusside* and 0.1 ml of 5M *sodium hydroxide* and allow to stand for 10 minutes. Add 1 ml of a 10% w/v solution of *sodium hydrogen carbonate*. A violet colour is produced.

TESTS

Acidity

pH, 5.6 to 6.6, Appendix V L.

N-(2-Aminoethyl)-4-*tert*-butyl-2,6-xylylacetamide

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* HF_{254} as the coating substance and a mixture of 200 volumes of *methanol* and 3 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) add a volume of the nasal drops containing 10 mg of Xylometazoline Hydrochloride to 30 ml of *water*, add 5 ml of 5M *sodium hydroxide*, mix, extract with three 20 ml quantities of *dichloromethane*, evaporate the combined extracts to dryness and dissolve the residue in 1 ml of *dichloromethane*. Solution (2) contains 0.03% w/v of *xylometazoline impurity A EPCRS* in *dichloromethane*. After removal of the plate, allow it to dry in air and spray with a solution prepared by dissolving 0.3 g of *ninhydrin* in a mixture of 100 ml of *butan-1-ol* and 3 ml of *glacial acetic acid*. Heat at 100° for 10 minutes, allow to cool, and spray with *dilute potassium iodobismuthate solution*. Any spot corresponding to xylometazoline impurity A [*N*-(2-aminoethyl)-4-*tert*-butyl-2,6-xylylacetamide] in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

ASSAY

To a volume of the nasal drops containing 10 mg of Xylometazoline Hydrochloride add 5 ml of *water*, 10 ml of 2M *hydrochloric acid* and 10 ml of *dichloromethane* and shake for 1 minute. Discard the dichloromethane layer and repeat the extraction with two further 10 ml quantities of *dichloromethane*. Add to the aqueous extract 10 ml of 5M *sodium hydroxide* and 10 ml of *dichloromethane*, shake for 1 minute and allow to separate. Filter the dichloromethane extract through glass wool and repeat the extraction with four further 10 ml quantities of *dichloromethane*. Evaporate the combined dichloromethane extracts almost to dryness on a water bath, removing the final traces of solvent in a current of air, and dissolve the residue in 10 ml of 0.01M *hydrochloric acid*. To 2 ml of this solution add 3 ml of *water*, 2.5 ml of 1M *sodium hydroxide* and 2.5 ml of a 5% w/v solution of *sodium nitroprusside*, mix and allow to stand protected from light for 10 minutes. Add 10 ml of a freshly prepared 8.3% w/v solution of *sodium hydrogen carbonate*, dilute to 100 ml with *water*, allow to stand protected from light for 10 minutes and measure the *absorbance* of the resulting solution at the maximum at 560 nm, Appendix II B, using in the reference cell a solution prepared by treating 5 ml of *water* and 2.5 ml of 1M *sodium hydroxide* in the same manner, beginning at the words 'and 2.5 ml of a 5% w/v solution of *sodium nitroprusside* ...'. Repeat the operation using a 0.1% w/v solution of *xylometazoline hydrochloride BPCRS*, diluted if necessary with *water*, in place of the nasal drops. Calculate the content of $C_{16}H_{24}N_2$,HCl in the nasal drops from the absorbances obtained using the declared content of $C_{16}H_{24}N_2$,HCl in *xylometazoline hydrochloride BPCRS*.

STORAGE

Xylometazoline Nasal Drops should be protected from light.

Compound Zinc Paste

General Notices

DEFINITION

Compound Zinc Paste contains 25% w/w each of Zinc Oxide and Starch in a suitable hydrophobic basis.

Extemporaneous preparation

The following formula and directions apply.

Zinc Oxide, finely sifted	250 g
Starch, finely sifted	250 g
White Soft Paraffin	500 g

Melt the White Soft Paraffin, incorporate the Zinc Oxide and the Starch and stir until cold.
The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

23.5 to 26.5% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the paste gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of 2M *acetic acid* and add sufficient *water* to produce 50 ml. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.1M *disodium edetate VS* until the solution becomes yellow. Each ml of 0.1M *disodium edetate VS* is equivalent to 8.138 mg of ZnO.

Zinc and Castor Oil Ointment

General Notices

Zinc and Castor Oil Cream

DEFINITION

Zinc Oxide, finely sifted	75 g
Virgin Castor Oil	500 g
Cetostearyl Alcohol	20 g
White Beeswax	100 g
Arachis Oil	305 g

Extemporaneous preparation

The following directions apply.

Triturate the Zinc Oxide with a portion of the Virgin Castor Oil until smooth and add the mixture to the remainder of the ingredients previously melted together. Stir while cooling until the temperature is about 40°.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

7.0 to 8.0% w/w.

IDENTIFICATION

A. Heat 1.3 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. The residue obtained is yellow when hot and white when cold.

B. Dissolve the cooled residue obtained in test A in 5 ml of 1M *hydrochloric acid*. The resulting solution yields the reaction characteristic of *zinc salts*, Appendix VI.

ASSAY

Heat 1 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of 2M *acetic acid* and add sufficient *water* to produce 50 ml. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.05M *disodium edetate VS* until the solution becomes yellow. Each ml of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

Zinc and Coal Tar Paste

General Notices

White's Tar Paste

DEFINITION

Zinc and Coal Tar Paste contains 6% w/w each of Zinc Oxide and Coal Tar with 38% w/w of Starch in a suitable hydrophobic basis.

Extemporaneous preparation

The following formula and directions apply.

Emulsifying Wax	50 g
Coal Tar	60 g
Zinc Oxide, finely sifted	60 g
Starch	380 g
Yellow Soft Paraffin	450 g

Melt the Emulsifying Wax at 70°, add the Coal Tar and 225 g of the Yellow Soft Paraffin, stir at 70° until completely melted, add the remainder of the Yellow Soft Paraffin, cool to 30°, add the Zinc Oxide and the Starch, stirring constantly, and stir until cold.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

5.7 to 6.3% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 1.0 g of the paste gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of 2M *acetic acid* and add sufficient *water* to produce 50 ml. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.05M *disodium edetate VS* until the solution becomes yellow. Each ml of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

Zinc and Ichthammol Cream

General Notices

DEFINITION

Zinc and Ichthammol Cream contains 5% w/w of Ichthammol dispersed in a suitable basis of which about 82% w/w is Zinc Cream.

Extemporaneous preparation

The following formula and directions apply.

Ichthammol	50 g
Cetostearyl Alcohol	30 g
Wool Fat	100 g
Zinc Cream	Sufficient to produce 1000 g

Melt together the Wool Fat and the Cetostearyl Alcohol with the aid of gentle heat, triturate the mixture with 800 g of Zinc Cream until smooth, incorporate the Ichthammol, add sufficient Zinc Cream to produce 1000 g and mix.

The cream complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

23.4 to 29.3% w/w.

ASSAY

Gently heat 0.4 g, taking precautions to avoid loss caused by spitting, until the basis is completely volatilised or charred, cool, dissolve the residue in 10 ml of 2M *sulphuric acid* , add 40 ml of *water*, adjust the solution to about pH 10 with 5M *ammonia*, add 10 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

Zinc and Salicylic Acid Paste

General Notices

Lassar's Paste

DEFINITION

Zinc Oxide, finely sifted	240 g
Salicylic Acid, finely sifted	20 g
Starch, finely sifted	240 g
White Soft Paraffin	500 g

Extemporaneous preparation

The following directions apply.

Melt the White Soft Paraffin, incorporate the Zinc Oxide, the Salicylic Acid and the Starch and stir until cold.

The paste complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

22.5 to 25.5% w/w.

Content of salicylic acid, $C_7H_6O_3$

1.9 to 2.1% w/w.

IDENTIFICATION

A. Shake 1 g with 10 ml of *water*, filter and add *iron(III) chloride solution R1* to the filtrate. An intense reddish violet colour is produced which remains on the addition of 5M *acetic acid* but disappears on the addition of 2M *hydrochloric acid* with the separation of a white, crystalline precipitate.

B. Heat 0.5 g gently in a porcelain dish over a very small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. The residue is yellow when hot and white when cool.

TESTS

ASSAY

For salicylic acid

Shake 0.5 g with 10 ml of 1M *hydrochloric acid* and 10 ml of *ether* until fully dispersed.

Decant and reserve the aqueous layer. Extract the ether layer with two further 10 ml quantities of 1M *hydrochloric acid*, combine the aqueous extracts with the reserved aqueous layer, wash with 10 ml of *ether* and reserve for the Assay for zinc oxide. Combine the ether extracts, add 15 ml of *petroleum spirit* (*boiling range, 40° to 60°*) and extract with successive quantities of 20 ml, 10 ml and 10 ml of a mixture of equal volumes of *ethanol* (*90%*) and 1M *sodium hydroxide*. Dilute the combined extracts to 100 ml with 2M *hydrochloric acid*, further dilute 15 ml of the resulting solution to 50 ml with the same solvent and measure the *absorbance* of the final solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_7H_6O_3$ taking 260 as the value of A(1%, 1 cm) at the maximum at 302 nm.

For zinc oxide

To the combined aqueous extracts obtained in the Assay for salicylic acid add 20 ml of 1M *sodium hydroxide* and 50 mg of *xylenol orange triturate*. To the resulting solution add sufficient *hexamine* to change the colour of the solution to red and then a further 3 g of *hexamine* and titrate with 0.1M *disodium edetate VS*. Each ml of 0.1M *disodium edetate VS* is equivalent to 8.139 mg of ZnO.

Zinc Ointment

General Notices

DEFINITION

Zinc Ointment contains 15% w/w of Zinc Oxide in a suitable water-emulsifying basis.

Extemporaneous preparation

The following formula and directions apply.

Zinc Oxide, finely sifted	150 g
Simple Ointment	850 g

Triturate the Zinc Oxide with a portion of the Simple Ointment until smooth, gradually add the remainder of the Simple Ointment and mix thoroughly.

The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

14.0 to 16.0% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of 2M *acetic acid* and add sufficient *water* to produce 50 ml. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.05M *disodium edetate VS* until the solution becomes yellow. Each ml of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

Zinc Ointment

General Notices

DEFINITION

Zinc Ointment contains 15% w/w of Zinc Oxide in a suitable water-emulsifying basis.

Extemporaneous preparation

The following formula and directions apply.

Zinc Oxide, finely sifted	150 g
Simple Ointment	850 g

Triturate the Zinc Oxide with a portion of the Simple Ointment until smooth, gradually add the remainder of the Simple Ointment and mix thoroughly.
The ointment complies with the requirements stated under Topical Semi-solid Preparations and with the following requirements.

Content of zinc oxide, ZnO

14.0 to 16.0% w/w.

IDENTIFICATION

The residue obtained in the Assay is yellow when hot and white when cool.

ASSAY

Heat 0.5 g of the ointment gently in a porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of 2M *acetic acid* and add sufficient *water* to produce 50 ml. To the resulting solution add 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with 0.05M *disodium edetate VS* until the solution becomes yellow. Each ml of 0.05M *disodium edetate VS* is equivalent to 4.069 mg of ZnO.

Zinc Sulphate Eye Drops

General Notices

DEFINITION

Zinc Sulphate Eye Drops are a sterile solution containing 0.25% w/v of Zinc Sulphate Heptahydrate in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of zinc sulphate heptahydrate, $ZnSO_4,7H_2O$

0.22 to 0.28% w/v.

ASSAY

To 5 ml add 50 ml of *water* and 5 ml of *ammonia buffer pH 10.9* and titrate with 0.01M *disodium edetate VS* using *mordant black 11 solution* as indicator. Each ml of 0.01M *disodium edetate VS* is equivalent to 2.875 mg of $ZnSO_4,7H_2O$.

Zinc Sulphate Lotion

General Notices

DEFINITION

Zinc Sulphate Lotion is a *cutaneous solution*. It contains 1% w/v of Zinc Sulphate Heptahydrate in a suitable aqueous vehicle.

The lotion complies with the requirements stated under Liquids for Cutaneous Application and with the following requirements.

Content of zinc sulphate heptahydrate, $ZnSO_4,7H_2O$

0.95 to 1.05% w/v.

ASSAY

To 20 ml add 25 ml of 0.1M *hydrochloric acid* and 0.1 g of *activated charcoal*, shake, filter and wash the residue with 100 ml of 0.01M *hydrochloric acid*. To the combined filtrate and washings add 5 ml of *ammonia buffer pH 10.9* and titrate with 0.05M *disodium edetate VS* using *mordant black 11 solution* as indicator. Repeat the procedure using 20 ml of *water* in place of the preparation being examined. The difference between the titrations represents the amount of disodium edetate required. Each ml of 0.05M *disodium edetate VS* is equivalent to 14.38 mg of $ZnSO_4,7H_2O$.

LABELLING

The label indicates the pharmaceutical form as 'cutaneous solution'.

Zopiclone Tablets

General Notices

Action and use

Non-benzodiazepine hypnotic.

DEFINITION

Zopiclone Tablets contain Zopiclone.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of zopiclone, $C_{17}H_{17}ClN_6O_3$

95.0 to 105.0% of the stated amount.

Carry out all of the following procedures protected from light.

IDENTIFICATION

Shake a quantity of the powdered tablets containing 37.5 mg of Zopiclone with 30 ml of *acetone*, add sufficient *acetone* to produce 50 ml and filter. Evaporate the filtrate to dryness on a water bath and dry the residue in an oven at 60° for 2 hours. The *infrared absorption spectrum* of the dried residue, Appendix II A, is concordant with the *reference spectrum* of zopiclone *(RS 430)*.

TESTS

Dissolution

Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 500 ml of 0.1M *hydrochloric acid*, at a temperature of 37°, as the dissolution medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and measure the *absorbance* of the filtered sample, suitably diluted with the dissolution medium if necessary, at the maximum at 305 nm, Appendix II B using 0.1M *hydrochloric acid* in the reference cell.

(2) Measure the *absorbance* of a suitable solution of zopiclone *BPCRS* using 0.1M *hydrochloric acid* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of zopiclone, $C_{17}H_{17}ClN_6O_3$, in the medium using the declared content of $C_{17}H_{17}ClN_6O_3$ in *zopiclone BPCRS*.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Mix with the aid of ultrasound a quantity of the powdered tablets containing 37.5 mg of Zopiclone with 50 ml of the mobile phase for 30 minutes, shake for a further 20 minutes. Cool the solution and add sufficient of the mobile phase to produce 100 ml, mix and filter through a 0.45 µm PTFE filter.

(2) Dilute 1 volume of solution (1) to 100 volumes with the mobile phase.

(3) 0.0001% w/v of each of *zopiclone BPCRS, zopiclone oxide EPCRS* and *2-amino-5-chloropyridine BPCRS* in the mobile phase.

(4) Dilute 1 volume of solution (2) to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use a detection wavelength of 303 nm.

(e) Use a column temperature of 30°.

(f) Inject 20 µl of each solution.

(g) The retention times for 2-amino-5-chloropyridine, zopiclone oxide and zopiclone are about 7 minutes, about 20 minutes and about 26 minutes respectively.

MOBILE PHASE

375 volumes of *acetonitrile* and 625 volumes of a solution containing 0.5% w/v of *sodium dodecyl sulphate* and 0.01% w/v of *sodium dihydrogen orthophosphate* previously adjusted to pH 4.0 with *orthophosphoric acid*.

SYSTEM SUITABILITY

The test is not valid unless:

In the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to zopiclone and zopiclone-*N*-oxide is at least 3.0;

In the chromatogram obtained with solution (4), the *signal-to-noise ratio* of the principal peak is not less than 10.

LIMITS

In the chromatogram obtained with solution (1):

the area of the peak due to 2-amino-5-chloropyridine is not greater than twice the area of the peak due to 2-amino-5-chloropyridine in solution (3) (0.5%);

excluding 2-amino-5-chloropyridine, the area of any *secondary peak* is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the sum of the impurities is not greater than 1.5%.

Disregard any peak with an area less than 0.1 times the area of the principal peak in solution (2) (0.1%).

Uniformity of content

Tablets containing less than 2 mg or less than 2% w/w of Zopiclone comply with the requirements stated under Tablets using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Finely crush one tablet, add sufficient of the mobile phase to produce a solution with an expected concentration of 0.0075% w/v, shake and filter.

(2) 0.0075% w/v of *zopiclone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 ml per minute.

(d) Use a detection wavelength of 303 nm.

(e) Use a column temperature of 30°.

(f) Inject 10 µl of each solution.

MOBILE PHASE

1 volume of *acetonitrile* and 1 volume of a solution containing 0.5% w/v of *sodium dodecyl sulphate* and 0.1% w/v of *sodium dihydrogen orthophosphate*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to zopiclone is not more than 1.4.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{17}ClN_6O_3$ in the tablets using the declared content of $C_{17}H_{17}ClN_6O_3$ in *zopiclone BPCRS*.

ASSAY

For tablets containing 2 mg or more and 2% w/w or more of Zopiclone

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) shake a quantity of the powdered tablets containing 37.5 mg of Zopiclone with 60 ml of the mobile phase for 20 minutes, add sufficient of the mobile phase to produce 500 ml, mix and filter (Whatman GF/C is suitable), discarding the first 10 ml of filtrate.

(2) 0.0075% w/v solution of *zopiclone BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The Chromatographic conditions described under the test for Uniformity of content may be used.

SYSTEM SUITABILITY

The test is not valid unless in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to zopiclone is at least 1.4.

DETERMINATION OF CONTENT

Calculate the content of $C_{17}H_{17}ClN_6O_3$ in the tablets using the declared content of $C_{17}H_{17}ClN_6O_3$ in *zopiclone BPCRS*.

For tablets containing less than 2 mg or less than 2% w/w of Zopiclone

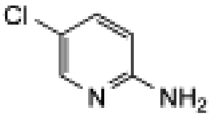
Use the average of the individual results obtained in the test for Uniformity of content

STORAGE

Zopiclone Tablets should be protected from light.

IMPURITIES

In addition to the impurities listed under Zopiclone, the impurities limited by the requirements of this monograph include



2-amino-5-chloropyridine.

Zuclopenthixol Acetate Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Zuclopenthixol Acetate Injection is a sterile solution of Zuclopenthixol Acetate in a suitable vegetable oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of zuclopenthixol acetate, $C_{24}H_{27}ClN_2O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 3 volumes of *diethylamine* and 90 volumes of *cyclohexane* as the mobile phase but using an unlined tank. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a volume of the injection with *ethanol* (*96%*) to contain 0.5% w/v of Zuclopenthixol Acetate. Solution (2) contains 0.5% w/v of *zuclopenthixol acetate dihydrochloride BPCRS* in *ethanol* (*96%*). Solution (3) contains 0.5% w/v each of *zuclopenthixol acetate dihydrochloride BPCRS* and *zuclopenthixol decanoate dihydrochloride BPCRS*. After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *sodium molybdate* in *sulphuric acid*, heat at 110° for 20 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable), a mixture of 10 volumes of *diethylamine*, 40 volumes of *dichloromethane* and 50 volumes of *cyclohexane* as the mobile phase and an unlined tank. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dissolve a quantity of the injection containing 0.10 g of Zuclopenthixol Acetate in sufficient *dichloromethane* to produce 50 ml. Solution (2) contains 0.0010% w/v of *2-chlorothioxanthone BPCRS* in *dichloromethane*. Solutions (3) to (5) contain (3) 0.0020% w/v, (4) 0.00040% w/v and (5) 0.00020% w/v respectively of *zuclopenthixol hydrochloride BPCRS* in *dichloromethane* containing a few drops of *diethylamine*. After removal of the plate, allow it to dry in air, spray with a mixture of equal volumes of *sulphuric acid* and *absolute ethanol*, heat at 110° for 5 minutes and examine under *ultraviolet light* (*365 nm*). In the chromatogram obtained with solution (1) any spot corresponding to 2-chlorothioxanthone is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and any spot corresponding to zuclopenthixol is not more intense than the spot in the chromatogram obtained with solution (3) (1%). Any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (4) (0.2%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (5) (0.1%).

***trans*-Isomer**

Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions. For solution (1) dissolve a quantity of the injection containing 20 mg of Zuclopenthixol Acetate in 25 ml of *dichloromethane* and dilute to 50 ml with *dichloromethane*. For solution (2) dissolve 23 mg of trans-*clopenthixol acetate dihydrochloride BPCRS* (equivalent to 20 mg of *trans*-clopenthixol acetate) in sufficient *dichloromethane* containing a few drops of *diethylamine* to produce 50 ml; dilute 1 ml of the resulting solution to 100 ml with *dichloromethane.* For solution (3) mix equal volumes of solution (1) and undiluted solution (2).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb S 5W is suitable), (b) a mixture of 0.08 volume of 13.5M *ammonia*, 45 volumes of n-*heptane*, 45 volumes of *dichloromethane* and 50 volumes of *acetonitrile* as the mobile phase with a flow rate of 2 ml per minute and (c) a detection wavelength of 254 nm.

Inject 50 µl of each solution. The test is not valid unless the *resolution factor* between the principal peaks in the chromatogram obtained with solution (3) is at least 2.6.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *trans*-clopenthixol acetate is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare a 0.2% w/v solution of cis-*flupenthixol propionate dihydrochloride BPCRS* (internal standard) in *dichloromethane*. For solution (1) dissolve a quantity of the injection containing 20 mg of Zuclopenthixol Acetate in 25 ml of *dichloromethane*, add 5 ml of the internal standard solution and dilute to 50 ml with *dichloromethane*. For solution (2) dissolve 20 mg of *zuclopenthixol acetate dihydrochloride BPCRS* in 25 ml of *dichloromethane* containing a few drops of *diethylamine*, add 5 ml of the internal standard solution and dilute to 50 ml with *dichloromethane*.

The chromatographic conditions described under *trans*-Isomer may be used. Inject 50 µl of each solution.

Determine the *weight per ml* of the injection, Appendix V G, and calculate the content of $C_{24}H_{27}ClN_2O_2S$ using the declared content of $C_{24}H_{27}ClN_2O_2S$ in *zuclopenthixol acetate dihydrochloride BPCRS*.

STORAGE

Zuclopenthixol Acetate Injection should be protected from light.

Zuclopenthixol Decanoate Injection

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Zuclopenthixol Decanoate Injection is a sterile solution of Zuclopenthixol Decanoate in a suitable vegetable oil.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of zuclopenthixol decanoate, $C_{32}H_{43}ClN_3O_2S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 3 volumes of *diethylamine* and 90 volumes of *cyclohexane* as the mobile phase but using an unlined tank. Before use, heat the plate at 110° for 30 minutes. Apply separately to the plate 5 µl of each of the following solutions. For solution (1) dilute a volume of the injection with *ethanol (96%)* to contain 0.5% w/v of Zuclopenthixol Decanoate. Solution (2) contains 0.5% w/v of *zuclopenthixol decanoate dihydrochloride BPCRS* in *ethanol (96%).* Solution (3) contains 0.5% w/v each of *zuclopenthixol decanoate dihydrochloride BPCRS* and *zuclopenthixol acetate dihydrochloride BPCRS*. After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *sodium molybdate* in *sulphuric acid*, heat at 110° for 20 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*, protected from light. For solution (1) dilute the injection to contain 0.50% w/v of Zuclopenthixol Decanoate. Solution (2) contains 0.015% w/v of *zuclopenthixol hydrochloride BPCRS*. Solution (3) contains 0.0025% w/v of *2-chlorothioxanthone BPCRS*. Solution (4) contains 0.005% w/v of trans-*clopenthixol decanoate BPCRS*. Solution (5) contains 0.015% w/v of *zuclopenthixol hydrochloride BPCRS*, 0.0025% w/v of *2-chlorothioxanthone BPCRS* and 0.005% w/v of trans-*clopenthixol decanoate BPCRS* in solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable) maintained at a temperature of 40°, (b) as the mobile phase with a flow rate of 1 ml per minute a mixture of 0.1 volume of *orthophosphoric acid*, 26 volumes of a 20 millimole solution of *dioctyl sodium sulphosuccinate* (prepared by dissolving 8.89 g of *dioctyl sodium sulphosuccinate* in 500 ml of *water*, stirring for 6 to 8 hours and diluting to 1000 ml with *water*) and 74 volumes of *ethanol (96%)* and (c) a detection wavelength of 270 nm.

Inject solution (1) and allow the chromatography to proceed for twice the retention time of the principal peak. Inject 20 µl of each solution. The test is not valid unless the chromatogram obtained with solution (5) shows clearly separated peaks.

In the chromatogram obtained with solution (1) the area of any peak corresponding to zuclopenthixol is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (3%), the area of any peak corresponding to 2-chlorothioxanthone is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%) and the area of any peak corresponding to *trans*-clopenthixol decanoate is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile* protected from light. For solution (1) dilute a volume of the injection to contain 0.50% w/v of Zuclopenthixol Decanoate. Solution (2) contains 0.57% w/v of *zuclopenthixol decanoate dihydrochloride BPCRS*.

The chromatographic procedure described under Related substances may be used.

Calculate the content of $C_{32}H_{43}ClN_3O_2S$ using the declared content of $C_{32}H_{43}ClN_3O_2S$ in *zuclopenthixol decanoate dihydrochloride BPCRS*. Each mg of zuclopenthixol decanoate dihydrochloride is equivalent to 0.8852 mg of zuclopenthixol decanoate.

STORAGE

Zuclopenthixol Decanoate Injection should be protected from light.

Zuclopenthixol Tablets

General Notices

Action and use

Dopamine receptor antagonist; neuroleptic.

DEFINITION

Zuclopenthixol Tablets contain Zuclopenthixol Hydrochloride.
The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of zuclopenthixol, $C_{22}H_{25}ClN_2OS$

95.0 to 105% of the stated amount.

IDENTIFICATION

A. The *light absorption,* Appendix II B, in the range 205 to 350 nm of solution A prepared in the Assay exhibits maxima at 230, 268 and 325 nm.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 2 volumes of 13.5M *ammonia*, 2 volumes of *propan-1-ol*, 40 volumes of *toluene* and 60 volumes of *acetone* as the mobile phase and an unsaturated tank. Apply separately to the plate 10 µl of each of the following solutions. For solution (1) shake a quantity of the tablets containing the equivalent of 10 mg of zuclopenthixol with 10 ml of *methanol*, centrifuge and use the supernatant liquid. Solution (2) contains 0.1% w/v of *zuclopenthixol hydrochloride BPCRS* in *methanol*. Solution (3) contains 0.1% w/v each of *zuclopenthixol hydrochloride BPCRS* and trans*-clopenthixol hydrochloride BPCRS.* After removal of the plate, allow it to dry in air, spray with a 1% w/v solution of *sodium molybdate* in *sulphuric acid*, heat at 110° for 20 minutes and examine in daylight. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of equal volumes of *methanol* and a phosphate buffer of pH 6.5 prepared by dissolving 2.56 g of *potassium dihydrogen orthophosphate* and 1.08 g of *dipotassium hydrogen orthophosphate* in 1000 ml of *water* and adjusting the pH if necessary to 6.5 ± 0.05 using *potassium hydroxide* or *orthophosphoric acid*. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 20 mg of zuclopenthixol with 35 ml of the phosphate buffer pH 6.5 until complete disintegration, add 65 ml of *methanol*, mix with the aid of ultrasound for 5 minutes, dilute to 200 ml with the solvent mixture, centrifuge and use the supernatant liquid. For solution (2) dilute 1 volume of solution (1) to 100 volumes with the same solvent mixture. Solution (3) contains 0.00010% w/v of *zuclopenthixol hydrochloride BPCRS* and 0.000010% w/v of *2-chlorothioxanthone BPCRS* in the solvent mixture.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C8 is suitable) maintained at a temperature of 45°, (b) as the mobile phase with a flow rate of 1.5 ml per minute a 0.077% w/v solution of *dodecyltrimethylammonium bromide* in a mixture of 18 volumes of *methanol*, 32 volumes of *acetonitrile* and 50 volumes of the phosphate buffer pH 6.5 and (c) a detection wavelength of 270 nm.

Inject 50 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to zuclopenthixol and 2-chlorothioxanthone is at least 2.5.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%) and the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (2.5%).

***trans*-Isomer**

Carry out the method for *liquid chromatography,* Appendix III D, protected from light, using the following solutions. For solution (1) shake a quantity of the powdered tablets containing the equivalent of 20 mg of zuclopenthixol in 10 ml of *water* for 10 minutes with the aid of ultrasound and filter (Whatman GF/C is suitable); to 5 ml of the filtrate add 25 ml of the mobile phase, shake thoroughly for 1 minute and dilute to 50 ml with the mobile phase; use the upper layer. For solution (2) prepare a 0.004% w/v solution of trans*-clopenthixol hydrochloride BPCRS* in *water*, shake 5 ml of this solution with 25 ml of the mobile phase for 1 minute and dilute to 50 ml with the mobile phase; use the upper layer. For solution (3) dissolve 10 mg of *zuclopenthixol hydrochloride BPCRS* in 5 ml of a 0.004% w/v solution of trans-*clopenthixol hydrochloride BPCRS*, add 25 ml of the mobile phase, shake thoroughly for 1 minute, dilute to 50 ml with the mobile phase and use the upper layer.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb S 5W is suitable), (b) a mixture of 0.2 volume of *water*, 0.3 volume of 13.5M *ammonia,*15 volumes of *propan-2-ol* and 85 volumes of n-*heptane* as the mobile phase with a flow rate of 1.5 ml per minute and (c) a detection wavelength of 254 nm.

The test is not valid unless the chromatogram obtained with solution (3) shows a similar resolution to that in the reference chromatogram supplied with trans-*clopenthixol hydrochloride BPCRS*.

In the chromatogram obtained with solution (1) the area of any peak corresponding to *trans*-clopenthixol hydrochloride is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution (2) (5%).

ASSAY

Weigh and powder 20 tablets. To a quantity of the powder containing the equivalent of 20 mg of zuclopenthixol add 40 ml of 0.1M *hydrochloric acid,* heat on a water bath for 30 minutes, shaking occasionally, cool, dilute to 200 ml with *water* and shake thoroughly. Centrifuge some of the resulting solution and dilute 20 ml of the supernatant liquid to 200 ml with *water* and mix (solution A). Prepare a reference standard in the following manner. Dissolve 25 mg of *zuclopenthixol hydrochloride BPCRS*, in 40 ml of 0.1M *hydrochloric acid*, dilute to 200 ml with *water* and shake thoroughly. Dilute 20 ml of the resulting solution to 200 ml with *water* and mix (solution B). Measure the *absorbance*, Appendix II B, of solution B and solution A at 230 nm using 0.002M *hydrochloric acid* in the reference cell. Calculate the content of

$C_{22}H_{25}ClN_2OS$ using the *absorbances* at the maximum at 230 nm and the declared content of $C_{22}H_{25}ClN_2OS$ in *zuclopenthixol hydrochloride BPCRS*.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of zuclopenthixol.

IMPURITIES

The impurities limited by the requirements of this monograph include those shown under Zuclopenthixol Hydrochoride.

Adsorbed Diphtheria and Tetanus Vaccine

General Notices

(Diphtheria and Tetanus Vaccine (Adsorbed), Ph Eur monograph 0444)

The label may state 'DT'.

Ph Eur

DEFINITION

Diphtheria and tetanus vaccine (adsorbed) is a preparation of diphtheria formol toxoid and tetanus formol toxoid with a mineral adsorbent. The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

PRODUCTION

GENERAL PROVISIONS

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

BULK PURIFIED DIPTHERIA AND TETANUS TOXOIDS

The bulk purified diphtheria and tetanus toxoids are prepared as described in the monographs on *Diphtheria vaccine (adsorbed) (0443*) and *Tetanus vaccine (adsorbed) (0452)* and comply with the requirements prescribed therein.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of suitable quantities of bulk purified diphtheria toxoid and tetanus toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

LABELLING

The label states:

— the minimum number of International Units of each component per single human dose,

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults,

— the name and the amount of the adsorbent,

— that the vaccine must be shaken before use,

— that the vaccine is not to be frozen.

Ph Eur

Adsorbed Diphtheria Vaccine

General Notices

(Diphtheria Vaccine (Adsorbed), Ph Eur monograph 0443)

Ph Eur

DEFINITION

Diphtheria vaccine (adsorbed) is a preparation of diphtheria formol toxoid with a mineral adsorbent. The formol toxoid is prepared from the toxin produced by the growth of *Corynebacterium diphtheriae.*

PRODUCTION

GENERAL PROVISIONS

Specific toxicity

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

BULK PURIFIED TOXOID

For the production of diphtheria toxin, from which toxoid is prepared, seed cultures are managed in a defined seed-lot system in which toxinogenicity is conserved and, where necessary, restored by deliberate reselection. A highly toxinogenic strain of *Corynebacterium diphtheriae* with known origin and history is grown in a suitable liquid medium. At the end of cultivation, the purity of each culture is tested and contaminated cultures are discarded. Toxin-containing culture medium is separated aseptically from the bacterial mass as soon as possible. The toxin content (Lf per millilitre) is checked (*2.7.27*) to monitor consistency of production. Single harvests may be pooled to prepare the bulk purified toxoid. The toxin is purified to remove components likely to cause adverse reactions in humans. The purified toxin is detoxified with formaldehyde by a method that avoids destruction of the immunogenic potency of the toxoid and reversion of the toxoid to toxin, particularly on exposure to heat. Alternatively, purification may be carried out after detoxification.

Only bulk purified toxoid that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

Absence of toxin and irreversibility of toxoid

Using the same buffer solution as for the final vaccine, without adsorbent, prepare a solution of bulk purified toxoid at 100 Lf/ml. Divide the solution into 2 equal parts. Maintain 1 part at 5 ± 3 °C and the other at 37 °C for 6 weeks. Carry out a test in Vero cells for active diphtheria toxin using 50 µl/well of both samples. The sample should not contain antimicrobial preservatives and detoxifying agents should be determined to be below the concentration toxic to Vero cells. Non-specific toxicity may be eliminated by dialysis.

Use freshly trypsinised Vero cells at a suitable concentration, for example 2.5×10^5 ml^{-1} and a reference diphtheria toxin diluted in 100 Lf/ml diphtheria toxoid. A suitable reference diphtheria toxin will contain either not less than 100 LD_{50}/ml or 67 to 133 lr/100 in 1 Lf and 25 000 to 50 000 minimal reacting doses for guinea-pig skin in 1 Lf (*diphtheria toxin BRP* is suitable for use as the reference toxin). Dilute the toxin in 100 Lf/ml diphtheria toxoid to a suitable concentration, for example 2×10^{-4} Lf/ml. Prepare serial twofold dilutions of the diluted diphtheria toxin and use undiluted test samples (50 µl/well). Distribute them in the wells of a sterile tissue culture plate containing a medium suitable for Vero cells. To ascertain that any cytotoxic effect noted is specific to diphtheria toxin, prepare in parallel dilutions where the toxin is neutralised by a suitable concentration of diphtheria antitoxin, for example 100 IU/ml. Include control wells without toxoid or toxin and with non-toxic toxoid at 100 Lf/ml on each plate to verify normal cell growth. Add cell suspension to each well, seal the plates and incubate at 37 °C for 5-6 days. Cytotoxic effect is judged to be present where there is complete metabolic inhibition of the Vero cells, indicated by the pH indicator of the medium. Confirm cytopathic effect by microscopic examination or suitable staining such as MTT dye. The test is invalid if 5×10^{-5} Lf/ml of reference diphtheria toxin in 100 Lf/ml toxoid has no cytotoxic effect on Vero cells or if the cytotoxic effect of this amount of toxin is not neutralised in the wells containing diphtheria antitoxin. The bulk purified toxoid complies with the test if no toxicity neutralisable by antitoxin is found in either sample.

Antigenic purity *(2.7.27)*

Not less than 1500 Lf per milligram of protein nitrogen.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of a suitable quantity of bulk purified toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

IDENTIFICATION

Diphtheria toxoid is identified by a suitable immunochemical method (*2.7.1*). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the absorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

LABELLING

The label states:

— the minimum number of International Units per single human dose,

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults,

— the name and the amount of the adsorbent,

— that the vaccine must be shaken before use,

— that the vaccine is not to be frozen.

Ph Eur

Adsorbed Diphtheria, Tetanus and Pertussis (Acellular Component) Vaccine

General Notices

(Diphtheria, Tetanus and Pertussis (Acellular, Component) Vaccine (Adsorbed), Ph Eur monograph 1931)

The label may state 'DTaP'.

Ph Eur

DEFINITION

Diphtheria, tetanus and pertussis (acellular, component) vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties, produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the latter harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins *(2.6.14)* in the bulk purified diphtheria toxoid, tetanus toxoid and pertussis components is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)* and *Pertussis vaccine (acellular, component, adsorbed) (1356)*.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid and pertussis components separately or together onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid, free formaldehyde and antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Osmolality *(2.2.35)*

The osmolality of the vaccine is within the limits approved for the particular preparation.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with specific antisera to the pertussis components of the vaccine.

TESTS

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent intraperitoneally into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than the minimum potency stated on the label.

Unless otherwise justified and authorised, the minimum potency stated on the label is 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

The vaccine complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the names and amounts of the pertussis components per single human dose;

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen;

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Ph Eur

Adsorbed Diphtheria, Tetanus and Pertussis Vaccine

General Notices

(Diphtheria, Tetanus and Pertussis Vaccine (Adsorbed), Ph Eur monograph 0445)

The label may state 'DTwP'.

Ph Eur

DEFINITION

Diphtheria, tetanus and pertussis vaccine (adsorbed) is a preparation of diphtheria formol toxoid and tetanus formol toxoid with a mineral adsorbent to which a suspension of inactivated *Bordetella pertussis* has been added. The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

PRODUCTION

GENERAL PROVISIONS

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

BULK PURIFIED DIPHTHERIA AND TETANUS TOXOIDS, BULK INACTIVATED B. PERTUSSIS SUSPENSION

The bulk purified diphtheria and tetanus toxoids and the inactivated *B. pertussis* suspension are prepared as described in the monographs on *Diphtheria vaccine (adsorbed) (0443), Tetanus vaccine (adsorbed) (0452)* and *Pertussis vaccine (adsorbed) (0161)*, respectively, and comply with the requirements prescribed therein.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of suitable quantities of bulk purified diphtheria toxoid and tetanus toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide and admixture of an appropriate quantity of a suspension of inactivated *B. pertussis*; the resulting mixture is approximately isotonic with blood. The *B. pertussis* concentration of the final bulk vaccine does not exceed that corresponding to an opacity of 20 IU per single human dose. If 2 or more strains of *B. pertussis* are used, the composition of consecutive lots of the final bulk vaccine shall be consistent with respect to the proportion of each strain as measured in opacity units. Suitable antimicrobial preservatives may be added to the bulk vaccine. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for specific toxicity of the pertussis component, antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method (*2.7.1*). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained; reserve the precipitate for identification test C. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (*2.7.1*). The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge to obtain a bacterial precipitate. Other suitable methods for separating the bacteria from the adsorbent may also be used. Identify pertussis vaccine by agglutination of the bacteria from the resuspended precipitate by antisera specific to *B. pertussis* or by the assay.

TESTS

Specific toxicity of the pertussis component

Use not fewer than 5 mice each weighing 14 g to 16 g for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 h before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with 0.5 ml, containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 9 g/l sterile solution of *sodium chloride R,* preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the groups of mice immediately before the injection and 72 h and 7 days after the injection. The vaccine complies with the test if: (a) at the end of 72 h the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than 60 per cent of that per control mouse; and (c) not more than 5 per cent of the vaccinated mice die during the test. The test may be repeated and the results of the tests combined.

Aluminium (*2.5.13*)

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (*2.4.18*)

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility (*2.6.1*)

The vaccine complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) (*2.7.6*).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) (*2.7.8*).

If the test is carried out in guinea-pigs, the lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose; if the test is carried out in mice, the lower confidence limit (P = 0.95) of the estimated potency is not less than 60 IU per single human dose.

Pertussis component

Carry out the assay of pertussis vaccine (*2.7.7*).

The estimated potency is not less than 4 IU per single human dose and the lower confidence limit (P = 0.95) of the estimated potency is not less than 2 IU per single human dose.

LABELLING

The label states:

— the minimum number of International Units of each component per single human dose,

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults,

— the name and the amount of the adsorbent,

— that the vaccine must be shaken before use,

— that the vaccine is not to be frozen.

Ph Eur

Adsorbed Diphtheria, Tetanus, Pertussis (Acellular Component) and Haemophilus Type b Conjugate Vaccine

General Notices

(Diphtheria, Tetanus, Pertussis (Acellular, Component) and Haemophilus Type b Conjugate Vaccine (Adsorbed), Ph Eur monograph 1932)

The label may state 'DTaP/Hib'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis (acellular, component) and haemophilus type b conjugate vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis;* polyribosylribitol phosphate (PRP) covalently bound to a carrier protein; a mineral absorbent such as aluminium hydroxide or hydrated aluminium phosphate. The product may be presented with the haemophilus type b component in a separate container, the contents of which are mixed with the other components immediately before use.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The acellular pertussis component may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate [$(C_{10}H_{19}O_{12}P)_n$], with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

If the vaccine is presented with the haemophilus component in a separate vial, as part of consistency studies the assays of the diphtheria, tetanus and pertussis components are carried out on a suitable number of batches of vaccine reconstituted as for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins *(2.6.14)* in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components and bulk PRP conjugate is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine; if the vaccine is presented with the haemophilus component in a separate container, the contents of the diphtheria, tetanus and pertussis antigens are in any case such that the final vial for these components contains less than 100 IU per single human dose.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

During development studies and wherever revalidation is necessary, it shall be demonstrated by tests in animals that the vaccine induces a T-cell-dependent B-cell immune response to PRP.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)*, *Pertussis vaccine (acellular, component, adsorbed) (1356)* and *Haemophilus type b conjugate vaccine (1219).*

FINAL BULK VACCINE

Different methods of preparation may be used: a final bulk vaccine may be prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid, acellular pertussis components and PRP conjugate onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate; or 2 final bulks may be prepared and filled separately, one containing the diphtheria, tetanus and pertussis components, the other the haemophilus component, which may be freeze-dried. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality shown below and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid and antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Osmolality *(2.2.35)*

The osmolality of the vaccine, reconstituted where applicable, is within the limits approved for the particular preparation.

pH *(2.2.3)*

The pH of the vaccine, reconstituted if necessary, is within the range approved for the particular product.

Free PRP

Unbound PRP is determined after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

IDENTIFICATION

If the vaccine is presented with the haemophilus component in a separate vial: identification tests A, B and C are carried out using the vial containing the diphtheria, tetanus and pertussis components; identification test D is carried out on the vial containing the haemophilus components.

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1*). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a specific antisera to the pertussis components of the vaccine.

D. The haemophilus component is identified by a suitable immunochemical method *(2.7.1)* for PRP.

TESTS

If the product is presented with the haemophilus component in a separate container: the tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with the diphtheria, tetanus and pertussis components; the tests for PRP content, water (where applicable), sterility and pyrogens are carried out on the container with the haemophilus component.

If the haemophilus component is freeze-dried, some tests may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component to be tested.

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent intraperitoneally into the third group of mice. After 5 days, inject intraperitoneally into each mouse 2 mg of histamine base in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

PRP

Minimum 80 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose *(2.5.31)* or phosphorus *(2.5.18)*, by an immunochemical method *(2.7.1)* or by anion-exchange liquid chromatography *(2.2.29)* with pulsed-amperometric detection.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Water *(2.5.12)*

Maximum 3.0 per cent for the freeze-dried haemophilus component.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a quantity of the vaccine equivalent to: 1 µg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 µg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 µg of PRP for a vaccine with OMP as carrier.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than the minimum potency stated on the label.

Unless otherwise justified and authorised, the minimum potency stated on the label is 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

The vaccine complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

LABELLING

The label states:

- — the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;
- — the names and amounts of the pertussis components per single human dose;

— the number of micrograms of PRP per single human dose;

— the type and nominal amount of carrier protein per single human dose;

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen;

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Ph Eur

Adsorbed Diphtheria, Tetanus, Pertussis (Acellular Component) and Hepatitis B (rDNA) Vaccine

General Notices

(Diphtheria, Tetanus, Pertussis (Acellular, Component) and Hepatitis B (rDNA) Vaccine (Adsorbed), Ph Eur monograph 1933)

The label may state 'DTaP/HepB'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis (acellular, component) and hepatitis B (rDNA) vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordella pertussis*; hepatitis B surface antigen; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties, produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the latter harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

Hepatitis B surface antigen is a component protein of hepatitis B virus; the antigen is obtained by recombinant DNA technology.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins *(2.6.14)* in the bulk purified diphtheria toxoid, tetanus toxoid and pertussis components is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452), Pertussis vaccine (acellular, component, adsorbed) (1356)* and *Hepatitis B vaccine (rDNA) (1056)*.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid, acellular pertussis components and hepatitis B surface antigen onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid and antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the content of free formaldehyde has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/

I, the test for free formaldehyde may be omitted on the final lot.

If an *in vivo* assay is used for the hepatitis B component, provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality *(2.2.35)*

The osmolality of the vaccine is within the limits approved for the particular preparation.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a specific antisera to the pertussis components of the vaccine.

D. The assay or, where applicable, the electrophoretic profile, serves also to identify the hepatitis B component of the vaccine.

TESTS

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

Pyrogens *(2.6.8)*

The vaccine complies with the test for pyrogens. Inject the equivalent of 1 human dose into each rabbit.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of *diphtheria vaccine (adsorbed) (2.7.6)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than the minimum potency stated on the label.

Unless otherwise justified and authorised, the minimum potency stated on the label is 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

The vaccine complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

Hepatitis B component

The vaccine complies with the assay of hepatitis B vaccine *(2.7.15)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the names and amounts of the pertussis components per single human dose;

— the amount of HBsAg per single human dose;

— the type of cells used for production of the hepatitis B component;

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen;

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Ph Eur

Adsorbed Diphtheria, Tetanus, Pertussis (Acellular Component) and Inactivated Poliomyelitis Vaccine

General Notices

(Diphtheria, Tetanus, Pertussis (Acellular, Component) and Poliomyelitis (Inactivated) Vaccine (Adsorbed), Ph Eur monograph 1934)

The label may state 'DTaP/IPV'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis (acellular, component) and poliomyelitis (inactivated) vaccine (adsorbed) is a combined vaccine containing: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; suitable strains of human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins (*2.6.14*) in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components and purified, inactivated monovalent poliovirus harvests is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)*, *Pertussis vaccine* (*acellular, component, adsorbed) (1356)* and *Poliomyelitis vaccine (inactivated) (0214).*

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid, acellular pertussis components and admixture of suitable quantities of purified monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable quantity of a trivalent pool of such purified monovalent harvests. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin

Determined on the poliomyelitis components by a suitable immunochemical method (*2.7.1*) after virus harvest and before addition of the adsorbent in the preparation of the final bulk vaccine, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the test for absence of residual pertussis toxin and irreversibility of pertussis toxoid, antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the determination of D-antigen content has been carried out with satisfactory results during preparation of the final bulk before addition of the adsorbent, it may be omitted on the final lot.

Provided that the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

The *in vivo* assay for the poliomyelitis component may be omitted once it has been demonstrated for a given product and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the in vivo assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the *in vivo* and *in vitro* assays must be evaluated, and the need for revalidation considered.

Osmolality ***(2.2.35)***

The osmolality of the vaccine is within the limits approved for the particular preparation.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a specific antisera to the pertussis components of the vaccine.

D. The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

TESTS

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent intraperitoneally into the third group of mice. After 5 days, inject intraperitoneally into each mouse 2 mg of histamine base in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than the minimum potency stated on the label.

Unless otherwise justified and authorised, the minimum potency stated on the label is 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

The vaccine complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

Poliomyelitis component

D-antigen content As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method (*2.7.1*) following desorption, using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test The vaccine complies with the *in vivo* assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the names and amounts of the pertussis components per single human dose;

— the types of poliovirus contained in the vaccine;

— the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the type of cells used for production of the poliomyelitis component;

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen;

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Ph Eur

Adsorbed Pertussis Vaccine (Acellular Component)

General Notices

(Pertussis Vaccine (Acellular, Component, Adsorbed), Ph Eur monograph 1356)

The label may state 'aP'.

Ph Eur

DEFINITION

Pertussis vaccine (acellular, component, adsorbed) is a preparation of individually prepared and purified antigenic components of *Bordetella pertussis* adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties, produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the latter harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Where a genetically modified form of *B. pertussis* is used for production consistency and genetic stability shall be established in conformity with the requirements of the monograph *Products of recombinant DNA technology (0784).*

Reference vaccine A batch of vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine is preferably stabilised by a method that has been shown to have no significant effect on the assay procedure when the stabilised and non-stabilised batches are compared.

CHARACTERISATION OF COMPONENTS

During development of the vaccine, the production process shall be validated to demonstrate that it yields consistently individual components that comply with the following requirements; after demonstration of consistency, the tests need not be applied routinely to each batch.

Adenylate cyclise

Not more than 500 ng in the equivalent of 1 dose of the final vaccine, determined by immunoblot analysis or another suitable method.

Tracheal cytotoxin

Not more than 2 pmol in the equivalent of 1 dose of the final vaccine, determined by a suitable method such as a biological assay or liquid chromatography *(2.2.29)*.

Absence of residual dermonecrotic toxin

Inject intradermally into each of 3 unweaned mice, in a volume of 0.1 ml, the amount of component or antigenic fraction equivalent to 1 dose of the final vaccine. Observe for 48 h. No dermonecrotic reaction is demonstrable.

Specific properties

The components of the vaccine are analysed by one or more of the methods shown below in order to determine their identity and specific properties (activity per unit amount of protein) in comparison with reference preparations.

Pertussis toxin Chinese hamster ovary (CHO) cell-clustering effect and haemagglutination as *in vitro* methods; lymphocytosis-promoting activity, histamine-sensitising activity and insulin secretory activity as *in vivo* methods. The toxin shows ADP-ribosyl transferase activity using transducin as the acceptor.

Filamentous haemagglutinin Haemagglutination and inhibition by specific antibody.

Pertactin, fimbrial-2 and fimbrial-3 antigens Reactivity with specific antibody.

Pertussis toxoid The toxoid induces in animals production of antibodies capable of inhibiting all the properties of pertussis toxin.

PURIFIED COMPONENTS

Production of each component is based on a seed-lot system. The seed cultures from which toxin is prepared are managed to conserve or where necessary restore toxinogenicity by deliberate selection.

None of the media used at any stage contains blood or blood products of human origin. Media used for the preparation of seed lots and inocula may contain blood or blood products of animal origin.

Pertussis toxin and, where applicable, filamentous haemagglutinin and pertactin are purified and, after appropriate characterisation, detoxified using suitable chemical reagents, by a method that avoids reversion of the toxoid to toxin, particularly on storage or exposure to heat. Other components such as fimbrial-2 and fimbrial-3 antigens are purified either separately or together, characterised and shown to be free from toxic substances. The purification procedure is validated to demonstrate appropriate clearance of substances used during culture or purification.

The content of bacterial endotoxins *(2.6.14)* is determined to monitor the purification procedure and to limit the amount in the final vaccine. The limits applied for the individual components are such that the final vaccine contains less than 100 IU per single human dose.

Before detoxification, the purity of the components is determined by a suitable method such as polyacrylamide gel electrophoresis (PAGE) or liquid chromatography. SDS-PAGE or immunoblot analysis with specific monoclonal or polyclonal antibodies may be used to characterise subunits. Requirements are established for each individual product.

Only purified components that comply with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility *(2.6.1)*

Carry out the test for sterility using for each medium a quantity of purified component equivalent to not less than 100 doses.

Absence of residual pertussis toxin

This test is not necessary for the product obtained by genetic modification. Use a group of not fewer than 5 histamine-sensitive mice each weighing 18-26 g. Inject into each mouse the equivalent of 1 human dose intravenously or twice the human dose intraperitoneally, diluted to not more than 0.5 ml with phosphate-buffered saline solution containing 2 g/l of gelatin. Inject diluent intraperitoneally into a second group of control mice. After 5 days, inject 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 h. If no animal dies, the preparation complies with the test.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

A validated test based on the clustering effect of the toxin for Chinese hamster ovary (CHO) cells may be used instead of the test in mice.

Residual detoxifying agents and other reagents

The content of residual detoxifying agents and other reagents is determined and shown to be below approved limits unless validation of the process has demonstrated acceptable clearance.

Antigen content

Determine the antigen content by a suitable immunochemical method *(2.7.1)* and protein nitrogen by sulphuric acid digestion *(2.5.9)* or another suitable method. The ratio of antigen content to protein nitrogen is within the limits established for the product.

FINAL BULK VACCINE

The vaccine is prepared by adsorption of suitable quantities of purified components, separately or together, onto aluminium hydroxide or hydrated aluminium phosphate. A suitable antimicrobial preservative may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for absence of residual pertussis toxin and irreversibility of pertussis toxoid, antimicrobial preservative, free formaldehyde and the assay have been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

IDENTIFICATION

Subject the vaccine to a suitable desorption procedure such as the following: dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 10 g/l solution; maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. Examined by a suitable immunochemical method *(2.7.1)*, the clear supernatant liquid reacts with specific antisera to the components stated on the label.

TESTS

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent intraperitoneally into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility ***(2.6.1)***

It complies with the test for sterility.

ASSAY

The capacity of the vaccine to induce the formation of specific antibodies is compared with the same capacity of a reference preparation examined in parallel; antibodies are determined using suitable immunochemical methods *(2.7.1)* such as enzyme-linked immunosorbent assay (ELISA). The test in mice shown below uses a three-point model but, after validation, for routine testing a single-dilution method may be used.

Requirement The capacity to induce antibodies is not significantly (P = 0.95) less than that of the reference vaccine.

The following test model is given as an example of a method that has been found to be satisfactory.

Selection and distribution of test animals Use in the test healthy mice (for example, CD1 strain) of the same stock 4 to 8 weeks old. Distribute the animals in 6 groups of a number appropriate to the requirements of the assay. Use 3 dilutions of the vaccine to be examined and 3 dilutions of a reference preparation and attribute each dilution to a group of mice. Inject intraperitoneally or subcutaneously into each mouse 0.5 ml of the dilution attributed to its group.

Collection of serum samples 4 to 5 weeks after vaccination, bleed the mice individually under anaesthesia. Store the sera at - 20 °C until tested for antibody content.

Antibody determination Assay the individual sera for content of specific antibodies to each component using a validated method such as the ELISA test shown below.

ELISA test Microtitre plates (poly(vinyl chloride) or polystyrene as appropriate for the specific antigen) are coated with the purified antigen at a concentration of 100 ng per well. After washing, unreacted sites are blocked by incubating with a solution of bovine serum albumin and then washed. Two-fold dilutions of sera from mice immunised with test or reference vaccines are made on the plates. After incubation at 22-25 °C for 1 h, the plates are washed. A suitable solution of anti-mouse IgG enzyme conjugate is added to each well and incubated at 22-25 °C for 1 h. After washing, a substrate is added from which the bound enzyme conjugate liberates a chromophore which can be quantified by measurement of absorbance *(2.2.25)*. The test conditions are designed to obtain a linear response for absorbance with respect to antibody content over the range of measurement used and absorbance values within the range 0.1 to 2.0.

A reference antiserum of assigned potency is used in the test and serves as the basis for calculation of the antibody levels in test sera. A standardised control serum is also included in the test.

The test is not valid if:

— the value found for the control serum differs by more than 2 standard deviations from the assigned value,

— the confidence limits (P = 0.95) are less than 50 per cent or more than 200 per cent of the estimated potency.

Calculation The antibody titres in the sera of mice immunised with reference and test vaccines are calculated and from the values obtained the potency of the test vaccine in relation to the reference vaccine is calculated by the usual statistical methods.

LABELLING

The label states:

— the names and amounts of the components present in the vaccine,

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification,

— the name and amount of the adsorbent,

— that the vaccine must be shaken before use,

— that the vaccine is not to be frozen.

Ph Eur

Adsorbed Pertussis Vaccine (Acellular, Co-purified)

General Notices

(Pertussis Vaccine (Acellular, Co-purified, Adsorbed), Ph Eur monograph 1595)

The label may state 'aP'.

Ph Eur

DEFINITION

Pertussis vaccine (acellular, co-purified, adsorbed) is a preparation of antigenic components of *Bordetella pertussis* adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate.

The vaccine contains an antigenic fraction purified without separation of the individual components. The antigenic fraction is treated by a method that transforms pertussis toxin to toxoid, rendering it harmless while maintaining adequate immunogenic properties of all the components and avoiding reversion to toxin. The antigenic fraction is composed of pertussis toxoid, filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. It may contain residual pertussis toxin up to a maximum level approved by the competent authority. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Reference vaccine A batch of vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine is preferably stabilised, by a method that has been shown to have no significant effect on the assay procedure when the stabilised and non-stabilised batches are compared.

CHARACTERISATION OF COMPONENTS

During development of the vaccine, the production process shall be validated to demonstrate that it yields consistently an antigenic fraction that complies with the following requirements; after demonstration of consistency, the tests need not be applied routinely to each batch.

Adenylate cyclise

Not more than 500 ng in the equivalent of 1 dose of the final vaccine, determined by immunoblot analysis or another suitable method.

Tracheal cytotoxin

Not more than 2 pmol in the equivalent of 1 dose of the final vaccine, determined by a suitable method such as a biological assay or liquid chromatography *(2.2.29)*.

Absence of residual dermonecrotic toxin

Inject intradermally into each of 3 unweaned mice, in a volume of 0.1 ml, the amount of antigenic fraction equivalent to 1 dose of the final vaccine. Observe for 48 h. No dermonecrotic reaction is demonstrable.

Specific properties

The antigenic fraction is analysed by one or more of the methods shown below in order to determine the identity and specific properties (activity per unit amount of protein) of its components in comparison with reference preparations.

Pertussis toxin Chinese hamster ovary (CHO) cell-clustering effect and haemagglutination as *in vitro* methods; lymphocytosis-promoting activity, histamine-sensitising activity and insulin secretory activity as *in vivo* methods. The toxin shows ADP-ribosyl transferase activity using transducin as the acceptor.

Filamentous haemagglutinin Haemagglutination and inhibition by specific antibody.

Pertactin, fimbrial-2 and fimbrial-3 antigens Reactivity with specific antibody.

Pertussis toxoid The toxoid induces in animals the production of antibodies capable of inhibiting all the properties of pertussis toxin.

PURIFIED ANTIGENIC FRACTION

Production of the antigenic fraction is based on a seed-lot system. The seed cultures are managed to conserve or, where necessary, restore toxinogenicity by deliberate selection.

None of the media used at any stage contains blood or blood products of human origin. Media used for the preparation of seed batches and inocula may contain blood or blood products of animal origin.

The antigenic fraction is purified and, after appropriate characterisation, detoxified using suitable reagents by a method that ensures minimal reversion of toxoid to toxin, particularly on storage or exposure to heat. The purification procedure is validated to demonstrate appropriate clearance of substances used during culture or purification.

The content of bacterial endotoxins *(2.6.14)* is determined to monitor the purification procedure and to limit the amount in the final vaccine. The limits applied are such that the final vaccine contains not more than 100 IU per single human dose.

Before detoxification, the purity of the antigenic fraction is determined by a suitable method such as polyacrylamide gel electrophoresis (PAGE) or liquid chromatography. SDS-PAGE or immunoblot analysis with specific monoclonal or polyclonal antibodies may be used to characterise subunits. Requirements are established for each individual product.

Only a purified antigenic fraction that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility *(2.6.1)*

Carry out the test for sterility using for each medium a quantity of purified antigenic fraction equivalent to not less than 100 doses of the final vaccine.

Test for residual pertussis toxin

Use 3 groups of not fewer than 5 histamine-sensitive mice each weighing 18 g to 26 g. Using phosphate-buffered saline containing 2 g/l of gelatin, prepare a series of dilutions of the purified antigenic fraction that have been shown to yield a graded response and attribute each dilution to a separate group of mice. Inject intraperitoneally into each mouse the dilution attributed to its group. Inject diluent intraperitoneally into a fourth group of control mice. After 5 days, inject intraperitoneally into each mouse 1 mg of histamine base in a volume not exceeding 0.5 ml. Record the number of animals that die within 24 h of histamine challenge. Calculate the weight or volume of a preparation that sensitises 50 per cent of the mice injected using a suitable statistical method such as probit analysis *(see 5.3)*. The residual activity of pertussis toxin does not exceed that of batches shown to be safe in clinical studies.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as described above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

A validated test based on the clustering effect of the toxin for Chinese hamster ovary (CHO) cells may be used instead of the test in mice.

Residual detoxifying agents and other reagents

The content of residual detoxifying agents and other reagents is determined and shown to be below approved limits unless validation of the process has demonstrated acceptable clearance.

Antigen content

Determine the complete quantitative antigen composition of the antigenic fraction by suitable immunochemical methods *(2.7.1)* and protein nitrogen by sulphuric acid digestion (*2.5.9*) or another suitable method. The ratio of total antigen content to protein nitrogen is within the limits established for the product.

FINAL BULK VACCINE

The vaccine is prepared by adsorption of a suitable quantity of the antigenic fraction onto aluminium hydroxide or hydrated aluminium phosphate. A suitable antimicrobial preservative may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for residual pertussis toxin, reversibility of toxoid, antimicrobial preservative, free formaldehyde and the assay have been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

IDENTIFICATION

Subject the vaccine to a suitable desorption procedure such as the following: dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 10 g/l solution; maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. Examined by a suitable immunochemical method *(2.7.1)*, the clear supernatant liquid reacts with specific antisera to the components in the vaccine.

TESTS

Test for residual pertussis toxin

Use 3 groups of not fewer than 5 histamine-sensitive mice (see under Production) each weighing 18 g to 26 g. Using phosphate-buffered saline containing 2 g/l of gelatin, prepare a series of dilutions of the vaccine to be examined that have been shown to yield a graded response and attribute each dilution to a separate group of mice. Inject intraperitoneally into each mouse the dilution attributed to its group. Inject diluent intraperitoneally into a fourth group of control mice. After 5 days, inject intraperitoneally into each mouse 1 mg of histamine base in a volume not exceeding 0.5 ml. Note the number of animals that die within 24 h of histamine challenge. Calculate the weight or volume of a preparation that sensitises 50 per cent of the mice injected using a suitable statistical method such as probit analysis *(see 5.3)*. The residual activity of pertussis toxin does not exceed that of batches shown to be safe in clinical studies.

Reversibility of toxoid

Carry out the test for residual pertussis toxin described above using the vaccine incubated at 37 °C for 4 weeks in parallel with a sample stored at 2 °C to 8 °C. The degree of reversibility does not exceed that of batches shown to be safe in clinical studies.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

The vaccine complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

LABELLING

The label states:

- — the names and amounts of the antigenic components present in the vaccine,
- — the maximum amount of residual pertussis toxin present in the vaccine,
- — the maximum degree of reversion of toxoid to toxin during the period of validity,
- — the name and amount of the adsorbent,
- — that the vaccine must be shaken before use,
- — that the vaccine is not to be frozen.

Ph Eur

Adsorbed Tetanus Vaccine

General Notices

(Tetanus Vaccine (Adsorbed), Ph Eur monograph 0452)

The label may state *Tet*'.

When Tetanus Vaccine is prescribed or demanded and the form is not stated, Adsorbed Tetanus Vaccine may be dispensed or supplied.

Ph Eur

DEFINITION

Tetanus vaccine (adsorbed) is a preparation of tetanus formol toxoid with a mineral adsorbent. The formol toxoid is prepared from the toxin produced by the growth of *Clostridium tetani.*

PRODUCTION

GENERAL PROVISIONS

Specific toxicity

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 21 days of the injection any of the animals shows signs of or dies from tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

BULK PURIFIED TOXOID

For the production of tetanus toxin, from which toxoid is prepared, seed cultures are managed in a defined seed-lot system in which toxinogenicity is conserved and, where necessary, restored by deliberate reselection. A highly toxinogenic strain of *Clostridium tetani* with known origin and history is grown in a suitable liquid medium. At the end of cultivation, the purity of each culture is tested and contaminated cultures are discarded. Toxin-containing culture medium is collected aseptically. The toxin content (Lf per millilitre) is checked *(2.7.27)* to monitor consistency of production. Single harvests may be pooled to prepare the bulk purified toxoid. The toxin is purified to remove components likely to cause adverse reactions in humans. The purified toxin is detoxified with formaldehyde by a method that avoids destruction of the immunogenic potency of the toxoid and reversion of toxoid to toxin, particularly on exposure to heat. Alternatively, purification may be carried out after detoxification.

Only bulk purified toxoid that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

Absence of toxin and irreversibility of toxoid

Using the same buffer solution as for the final vaccine, without adsorbent, prepare a solution of bulk purified toxoid at the same concentration as in the final vaccine. Divide the dilution into 2 equal parts. Keep one of them at 5 ± 3 °C and the other at 37 °C for 6 weeks. Test both dilutions as described below. Use 15 guinea-pigs, each weighing 250-350 g and that have not previously been treated with any material that will interfere with the test. Inject subcutaneously into each of 5 guinea-pigs 5 ml of the dilution incubated at 5 ± 3 °C. Inject subcutaneously into each of 5 other guinea-pigs 5 ml of the dilution incubated at 37 °C. Inject subcutaneously into each of 5 guinea-pigs at least 500 Lf of the non-incubated bulk purified toxoid in a volume of 1 ml. The bulk purified toxoid complies with the test if during the 21 days following the injection no animal shows signs of or dies from tetanus. If more than 1 animal dies from non-specific causes, repeat the test; if more than 1 animal dies in the second test, the toxoid does not comply with the test.

Antigenic purity *(2.7.27)*

Not less than 1000 Lf per milligram of protein nitrogen.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of a suitable quantity of bulk purified toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified toxoid or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

IDENTIFICATION

Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable tetanus antitoxin, giving a precipitate.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 40 IU per single human dose.

LABELLING

The label states:

— the minimum number of International Units per single human dose,

— the name and the amount of the adsorbent,

— that the vaccine must be shaken before use,

— that the vaccine is not to be frozen.

Ph Eur

Anthrax Vaccine for Human Use (Adsorbed, Prepared from Culture Filtrates)

General Notices

(Ph Eur monograph 2188)

The label may state 'Anthrax'.

Ph Eur

DEFINITION

Anthrax vaccine for human use (adsorbed, prepared from culture filtrates) is a preparation of *Bacillus anthracis* antigens precipitated by aluminium potassium sulphate. The antigens are prepared from a sterile culture filtrate produced by a non-encapsulated strain, either avirulent or attenuated, of *B. anthracis*.

The main virulence components of *B. anthracis* are the polyglutamic aicd capsule and 2 binary anthrax toxins, namely lethal toxin and œdema toxin, formed from the respective combination of protective antigen (PA) with either lethal factor (LF) or œdema factor (EF).

LF is a zinc-dependent endopeptidase and EF is a potent calmodulin and calcium-dependent adenylate cyclase. Cell-free cultures of *B. anthracis* contain PA and because expression of the 3 toxin-component genes is co-ordinately regulated, LF and EF are also present. In addition, the vaccine is likely to contain many other *B. anthracis* antigens, including membrane proteins, secreted proteins, cytoplasmic proteins, peptidoglycans, nucleic acids and carbohydrates.

PRODUCTION

GENERAL PROVISIONS

Cultures are managed in a seed-lot system. The vaccine strain is toxigenic but lacks the plasmid with the necessary genes for synthesis of the capsule, an important virulence factor.

The production method must be shown to yield a consistent and active product with a safety and efficacy profile that is adequate or equivalent to previous lots. The vaccine must show a level of protection against a virulent strain of *B. anthracis*, in a suitable animal infection model, that is equal to or greater than that of a reference vaccine. The vaccine must not show a level of toxicity that exceeds that of a reference vaccine.

The production method and stability of the final lot and relevant intermediates are evaluated using one or more indicator tests. Such tests include potency and specific toxicity, and may be supported by tests confirming the presence of relevant antigens and associated proteins. Release and shelf-life specifications are established based upon the results of stability testing so as to ensure satisfactory product performance during the approved period of validity.

SEED LOTS

The attenuated non-encapsulated strain of *B. anthracis* used is identified by historical records that include information on its origin and subsequent manipulation and the tests used to characterise the strain. These include morphological, cultural, biochemical and genetic properties of the strain. Only a master seed lot or, where applicable, working seed lots, that comply with the following requirements may be used.

Identification

Each seed lot is identified as containing *B. anthracis*.

Phenotypic parameters

Each seed lot must have a known biochemical and enzymatic profile and have a known history of absence of antibiotic resistance.

Microbial purity

Each seed lot complies with the requirements for absence of contaminating organisms. Purity of bacterial cultures is verified by methods of suitable sensitivity.

Virulence test

The absence of bacterial capsule is demonstrated for each seed lot by McFadyean stain and the specific toxicity (oedema) test.

REFERENCE PREPARATION

The potency and toxicity of the vaccine bulk are verified using reference standards derived from representative vaccine batches. These batches are extensively characterised for their intended purpose and are stored in suitably sized aliquots under conditions ensuring their stability.

PROPAGATION AND HARVEST

The attenuated strain is grown using suitable liquid media. At the end of cultivation, the purity of the culture is tested. The culture medium is separated from the bacterial mass by filtration. The pH of the filtrate is determined after dilution with a 0.9 g/l solution of *sodium chloride R* and is shown to be within limits suitable for stability. A suitable test for absence of live *B. anthracis*, including spores, is carried out. Aluminium potassium sulphate or an alternative adjuvant may be added at this stage. An antimicrobial preservative may be added to the suspension to form the purified harvest.

Only a purified harvest that complies with the following requirements may be used in the preparation of the final lot.

Immunological identity

Confirm the presence of *B. anthracis* protective antigen by a suitable immunochemical method *(2.7.1)*.

Antimicrobial preservative

Determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

FINAL BULK VACCINE

The purified harvest is diluted aseptically with sterile saline solution to make the final bulk vaccine.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof glass ampoules and heat-sealed to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the potency assay, the specific toxicity (oedema) test and the test for antimicrobial preservative have been carried out with satisfactory results on the purified harvest, they may be omitted on the final lot.

IDENTIFICATION

The presence of *B. anthracis* protective antigen is confirmed by a suitable immunochemical method *(2.7.1)*.

TESTS

Abnormal toxicity

Inject intraperitoneally up to 4 human doses of vaccine into each of at least 10 healthy mice, each weighing 17-22 g. Observe the mice daily for 7 days. The vaccine complies with the test if none of the animals shows signs of ill health.

Specific toxicity (oedema) test

Use not fewer than 2 rabbits per test. Prepare serial two-fold dilutions of vaccine with normal saline, corresponding to 4, 2, 1, 0.5 and 0.25 human doses. Inject intradermally 0.1 ml of each dilution of the test and of the reference vaccine into the shaved flanks of 2 rabbits. Each rabbit receives the 10 previously prepared injections (5 dilutions of the test vaccine and 5 dilutions of the reference vaccine). In one of the rabbits, the lower concentrations are injected at the anterior end and the higher concentrations at the posterior end. The reverse is used for the 2nd rabbit. The rabbits are monitored for 24 h for signs of oedema at the injection site. The vaccine complies with the test if the oedematous reaction is not greater than that observed with the reference vaccine.

Alternatively, specific *in vitro* assays for lethal factor and adenylate cyclase activity may be used, subject to validation.

Antimicrobial preservative

Determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the intended content.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

The potency of the anthrax vaccine is determined by comparing the dose required to protect guinea-pigs against intradermal challenge by a virulent strain of *B. anthracis* with the dose of a suitable reference preparation that gives the same protection. Use 9 groups of not fewer than 16 female guinea-pigs, each weighing 250-350 g. Prepare 4 dilutions of the vaccine and of the reference preparation containing 1.5, 0.5, 0.17 and 0.05 human doses in 0.5 ml. Allocate each dilution to a separate group. The remaining group receives 0.5 ml of saline and is used to verify the challenge dose. Inject subcutaneously into each guinea-pig 0.5 ml of the dilution allocated to its group on each of 2 occasions, 1 week apart. 7 days after the 2nd injection, inject intradermally into each guinea-pig 2000 spores of a virulent strain of *B. anthracis* (Vollum) in 0.1 ml. Observe the animals for 10 days and record the number of deaths per group. The test is not valid unless all the control animals die within 5 days of challenge. Using the proportions of animals that survive in each of the vaccinated groups, calculate the potency of the vaccine relative to the reference preparation using the usual statistical methods *(5.3)*. The vaccine complies with the test if:

— the relative potency estimate exceeds 1.0, or;

— the 95 per cent confidence interval for the relative potency includes 1.0, and the lower 95 per cent confidence limit is not less than 50 per cent of the relative potency estimate.

LABELLING

The label states that the vaccine is not to be frozen.

Ph Eur

Bacillus Calmette-Guérin Vaccine

General Notices

BCG Vaccine

(BCG Vaccine, Freeze-dried, Ph Eur monograph 0163)

The label may state 'BCG'.

Ph Eur

DEFINITION

Freeze-dried BCG vaccine is a preparation of live bacteria derived from a culture of the bacillus of Calmette and Guérin (*Mycobacterium bovis* BCG) whose capacity to protect against tuberculosis has been established.

PRODUCTION

GENERAL PROVISIONS

BCG vaccine shall be produced by a staff consisting of healthy persons who do not work with other infectious agents; in particular they shall not work with virulent strains of *Mycobacterium tuberculosis*, nor shall they be exposed to a known risk of tuberculosis infection. Staff are examined periodically for tuberculosis. BCG vaccine is susceptible to sunlight: the procedures for the preparation of the vaccine shall be designed so that all cultures and vaccines are protected from direct sunlight and from ultraviolet light at all stages of manufacture, testing and storage.

Production of the vaccine is based on a seed-lot system. The production method shall have been shown to yield consistently BCG vaccines that induce adequate sensitivity to tuberculin in man, that have acceptable protective potency in animals and are safe. The vaccine is prepared from cultures which are derived from the master seed lot by as few subcultures as possible and in any case not more than 8 subcultures. During the course of these subcultures the preparation is not freeze-dried more than once.

If a bioluminescence test or other biochemical method is used instead of viable count, the method is validated against the viable count for each stage of the process at which it is used.

BACTERIAL SEED LOTS

The strain used to establish the master seed lot is chosen for and maintained to preserve its characteristics, its capacity to sensitise man to tuberculin and to protect animals against tuberculosis, and its relative absence of pathogenicity for man and laboratory animals. The strain used shall be identified by historical records that include information on its origin and subsequent manipulation.

A suitable batch of vaccine is prepared from the first working seed lot and is reserved for use as the comparison vaccine. When a new working seed lot is established, a suitable test for delayed hypersensitivity in guinea-pigs is carried out on a batch of vaccine prepared from the new working seed lot; the vaccine is shown to be not significantly different in activity from the comparison vaccine. Antimicrobial agent sensitivity testing is also carried out.

Only a working seed lot that complies with the following requirements may be used for propagation.

Identification

The bacteria in the working seed lot are identified as *Mycobacterium bovis* BCG using microbiological techniques, which may be supplemented by molecular biology techniques (for example, nucleic acid amplification and restriction-fragment-length polymorphism).

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium. The working seed lot complies with the test for sterility except for the presence of mycobacteria.

Virulent mycobacteria

Examine the working seed lot as prescribed under Tests, using 10 guinea-pigs.

PROPAGATION AND HARVEST

The bacteria are grown in a suitable medium for not more than 21 days by surface or submerged culture. The culture medium does not contain substances known to cause toxic or allergic reactions in humans or to cause the bacteria to become virulent for guinea-pigs. The culture is harvested and suspended in a sterile liquid medium that protects the viability of the vaccine as determined by a suitable method of viable count.

FINAL BULK VACCINE

The final bulk vaccine is prepared from a single harvest or by pooling a number of single harvests. A stabiliser may be added; if the stabiliser interferes with the determination of bacterial concentration in the final bulk vaccine, the determination is carried out before addition of the stabiliser.

Only final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium. The final bulk vaccine complies with the test for sterility except for the presence of mycobacteria.

Count of viable units. Determine the number of viable units per millilitre by viable count on solid medium using a method suitable for the vaccine to be examined or by a suitable biochemical method. Carry out the test in parallel on a reference preparation of the same strain.

Count of viable units

Determine the number of viable units per millilitre by viable count on solid medium using a method suitable for the vaccine to be examined or by a suitable biochemical method. Carry out the test in parallel on a reference preparation of the same strain.

Bacterial concentration

Determine the total bacterial concentration by a suitable method, either directly by determining the mass of the micro-organisms, or indirectly by an opacity method that has been calibrated in relation to the mass of the organisms; if the bacterial concentration is determined before addition of a stabiliser, the concentration in the final bulk vaccine is established by calculation. The total bacterial concentration is within the limits approved for the particular product.

The ratio of the count of viable units to the total bacterial concentration is not less than that approved for the particular product.

FINAL LOT

The final bulk vaccine is distributed into sterile containers and freeze-dried to a moisture content favourable to the stability of the vaccine; the containers are closed either under vacuum or under an inert gas.

Except where the filled and closed containers are stored at a temperature of - 20 °C or lower, the expiry date is not later than 4 years from the date of harvest.

Only a final lot that complies with the following requirement for count of viable units and with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for virulent mycobacteria has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot. Provided the test for excessive dermal reactivity has been carried out with satisfactory results on the working seed lot and on 5 consecutive final lots produced from it, the test may be omitted on the final lot.

Count of viable units

Determine the number of viable units per millilitre of the reconstituted vaccine by viable count on solid medium using a method suitable for the vaccine to be examined or by a suitable biochemical method. The ratio of the count of viable units after freeze-drying to that before is not less than that approved for the particular product.

IDENTIFICATION

BCG vaccine is identified by microscopic examination of the bacilli in stained smears demonstrating their acid-fast property and by the characteristic appearance of colonies grown on solid medium. Alternatively, molecular biology techniques (for example nucleic acid amplification) may be used.

TESTS

Virulent mycobacteria

Inject subcutaneously or intramuscularly into each of 6 guinea-pigs, each weighing 250-400 g and having received no treatment likely to interfere with the test, a quantity of vaccine equivalent to at least 50 human doses. Observe the animals for at least 42 days. At the end of this period, euthanise the guinea-pigs and examine by autopsy for signs of infection with tuberculosis, ignoring any minor reactions at the site of injection. Animals that die during the observation period are also examined for signs of tuberculosis. The vaccine complies with the test if none of the guinea-pigs shows signs of tuberculosis and if not more than 1 animal dies during the observation period. If 2 animals die during this period and autopsy does not reveal signs of tuberculosis repeat the test on 6 other guinea-pigs. The vaccine complies with the test if not more than 1 animal dies during the 42 days following the injection and autopsy does not reveal any sign of tuberculosis.

Bacterial and fungal contamination

The reconstituted vaccine complies with the test for sterility *(2.6.1)* except for the presence of mycobacteria.

Excessive dermal reactivity

Use 6 healthy, white or pale-coloured guinea-pigs, each weighing not less than 250 g and having received no treatment likely to interfere with the test. Inject intradermally into each guinea-pig, according to a randomised plan, 0.1 ml of the reconstituted vaccine and of 2 tenfold serial dilutions of the vaccine and identical doses of the comparison vaccine. Observe the lesions formed at the site of the injection for 4 weeks. The vaccine complies with the test if the reaction it produces is not markedly different from that produced by the comparison vaccine.

Temperature stability

Maintain samples of the freeze-dried vaccine at 37 °C for 4 weeks. Determine the number of viable units in the heated vaccine and in unheated vaccine as described below. The number of viable units in the heated vaccine is not less than 20 per cent that in unheated vaccine.

Water

Not more than the limit approved for the particular product, determined by a suitable method.

ASSAY

Determine the number of viable units in the reconstituted vaccine by viable count on solid medium using a method suitable for the vaccine to be examined or by a suitable validated biochemical method. The number is within the range stated on the label. Determine the number of viable units in the comparison vaccine in parallel.

LABELLING

The label states:

— the minimum and maximum number of viable units per millilitre in the reconstituted vaccine,

— that the vaccine must be protected from direct sunlight.

Ph Eur

BCG for Immunotherapy

General Notices

(Ph Eur monograph 1929)

Ph Eur

DEFINITION

BCG for immunotherapy is a freeze-dried preparation of live bacteria derived from a culture of the bacillus of Calmette and Guérin (*Mycobacterium bovis* BCG) whose capacity for treatment has been established.

It complies with the monograph *Vaccines for human use (0153).*

PRODUCTION

GENERAL PROVISIONS

BCG for immunotherapy shall be produced by a staff consisting of healthy persons who do not work with other infectious agents; in particular they shall not work with virulent strains of *Mycobacterium tuberculosis*, nor shall they be exposed to a known risk of tuberculosis infection. Staff are examined periodically for tuberculosis. BCG for immunotherapy is susceptible to sunlight: the procedures for production shall be so designed that all products are protected from direct sunlight and from ultraviolet light at all stages of manufacture, testing and storage.

Production is based on a seed-lot system. The production method shall have been shown to yield consistently BCG products that can be used for treatment of superficial bladder cancer and are safe. The product is prepared from cultures which are separated from the master seed lot by as few subcultures as possible and in any case not more than 8 subcultures. During the course of these subcultures the preparation is not freeze-dried more than once.

If a bioluminescence test or other biochemical method is used instead of viable count, the method is validated against the viable count for each stage of the process at which it is used.

SEED LOTS

The strain used to establish the master seed lot is chosen for and maintained to preserve its characteristics, its capacity to treat and prevent superficial bladder cancer, and its relative absence of pathogenicity for man and laboratory animals. The strain used shall be identified by historical records that include information on its origin and subsequent manipulation.

Before establishment of a working seed lot a batch is prepared and reserved for use as the comparison product. When a new working seed lot is established, a suitable test for delayed hypersensitivity in guinea-pigs is carried out on a batch of product prepared from the new working seed lot; the product is shown to be not significantly different in activity from the comparison product. Antimicrobial agent sensitivity testing is also carried out.

Only a working seed lot that complies with the following requirements may be used for propagation.

Identification

The bacteria in the working seed lot are identified as *Mycobacterium bovis* BCG using microbiological techniques, which may be supplemented by molecular biology techniques (for example, nucleic acid amplification and restriction-fragment-length polymorphism).

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium. The working seed lot complies with the test for sterility, except for the presence of mycobacteria.

Virulent mycobacteria

Examine the working seed lot as prescribed under Tests, using 10 guinea-pigs.

PROPAGATION AND HARVEST

The bacteria are grown in a suitable medium for not more than 21 days by surface or submerged culture. The culture medium does not contain substances known to cause toxic or allergic reactions in human beings or to cause the bacteria to become virulent for guinea-pigs. The culture is harvested and suspended in a sterile liquid medium that protects the viability of the culture as determined by a suitable method of viable count.

FINAL BULK

The final bulk is prepared from a single harvest or by pooling a number of single harvests. A stabiliser may be added; if the stabiliser interferes with the determination of bacterial concentration on the final bulk, the determination is carried out before addition of the stabiliser.

Only final bulk that complies with the following requirements may be used in the preparation of the final lot.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml of final bulk for each medium. The final bulk complies with the test for sterility, except for the presence of mycobacteria.

Count of viable units

Determine the number of viable units per millilitre by viable count on solid medium using a method suitable for the product to be examined or by a suitable biochemical method. Carry out the test in parallel on a reference preparation of the same strain.

Bacterial concentration

Determine the total bacterial concentration by a suitable method, either directly by determining the mass of the micro-organisms, or indirectly by an opacity method that has been calibrated in relation to the mass of the micro-organisms; if the bacterial concentration is determined before addition of a stabiliser, the concentration in the final bulk is established by calculation. The total bacterial concentration is within the limits approved for the particular product.

The ratio of the count of viable units to the total bacterial concentration is not less than that approved for the particular product.

FINAL LOT

The final bulk is distributed into sterile containers and freeze-dried to a moisture content favourable to the stability of the product; the containers are closed either under vacuum or under an inert gas.

Except where the filled and closed containers are stored at a temperature of - 20 °C or lower, the expiry date is not later than 4 years from the date of harvest.

Only a final lot that complies with the following requirement for count of viable units and with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for virulent mycobacteria has been carried out with satisfactory results on the final bulk, it may be omitted on the final lot.

Count of viable units

Determine the number of viable units per millilitre of the reconstituted product by viable count on solid medium using a method suitable for the product to be examined, or by a suitable biochemical method. The ratio of the count of viable units after freeze-drying to that before is not less than that approved for the particular product.

IDENTIFICATION

BCG for immunotherapy is identified by microscopic examination of the bacilli in stained smears demonstrating their acid-fast property and by the characteristic appearance of colonies grown on solid medium. Alternatively, molecular biology techniques (for example, nucleic acid amplification) may be used.

TESTS

Virulent mycobacteria

Inject subcutaneously or intramuscularly into each of 6 guinea-pigs, each weighing 250-400 g and having received no treatment likely to interfere with the test, a quantity of the product to be examined equivalent to at least 1/25 of 1 human dose. Observe the animals for at least 42 days. At the end of this period, euthanise the guinea-pigs and examine by autopsy for signs of infection with tuberculosis, ignoring any minor reactions at the site of injection. Animals that die during the observation period are also examined for signs of tuberculosis. The product complies with the test if none of the guinea-pigs shows signs of tuberculosis and if not more than 1 animal dies during the observation period. If 2 animals die during this period and autopsy does not reveal signs of tuberculosis, repeat the test on 6 other guinea-pigs. The product complies with the test if not more than 1 animal dies during the 42 days following the injection and autopsy does not reveal any sign of tuberculosis.

Bacterial and fungal contamination

The reconstituted product complies with the test for sterility *(2.6.1)* except for the presence of mycobacteria.

Temperature stability

Maintain samples of the freeze-dried product at 37 °C for 4 weeks. Determine the number of viable units in the heated product and in unheated product as described below. The number of viable units in the heated product is within the limits approved for the particular product but in any case not less than 20 per cent of that in unheated product.

Water

Not more than the limit approved for the particular product, determined by a suitable method.

ASSAY

Determine the number of viable units in the reconstituted product by viable count on solid medium using a method suitable for the product to be examined or by a suitable validated biochemical method. The number is within the range stated on the label. Determine the number of viable units in the comparison control in parallel.

LABELLING

The label states:

— the minimum and the maximum number of viable units per dose in the reconstituted product,

— that the product must be protected from direct sunlight.

Ph Eur

Cholera Vaccine (Inactivated, Oral)

General Notices

(Ph Eur monograph 2327)

Ph Eur

DEFINITION

Cholera vaccine (inactivated, oral) is a homogeneous suspension of inactivated suitable strains of *Vibrio cholerae* serogroup O1, representing serotypes and biotypes of epidemic strains. The vaccine may contain the B subunit of cholera toxin (CTB). Just prior to ingestion, one dose of vaccine suspension is mixed with a suitable buffer as stated on the label.

PRODUCTION

GENERAL PROVISIONS

The production method must be validated to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The production process must be validated to show that no clinically significant quantities of active toxin are present in the product.

CHOICE OF VACCINE STRAIN

The vaccine consists of a mixture of epidemic *V. cholerae* strains inactivated by a suitable method such as heat or formalin inactivation. All strains express smooth lipopolysaccharide (LPS). The CTB is produced by recombinant DNA technology in a strain that lacks the gene for cholera toxin subunit A (*ctxA*$^-$). Selected *V. cholerae* strains are low cholera-toxin producers.

The World Health Organisation (WHO) can recommend new vaccine strains or antigens that may be used if necessary, in accordance with the regulations in force in the signatory states of the Convention on the Elaboration of a European Pharmacopoeia.

SEED LOTS

The strains of *V. cholerae* used shall be identified by historical records that include information on the origin of the strains and their subsequent manipulation. Characterisation and maintenance of the recombinant strains and plasmids used for production of the recombinant B subunit of cholera toxin (rCTB) and the origin of the gene for cholera toxin subunit B (*ctxB*) are documented. The stability of the rCTB plasmid in the recombinant strain during storage and beyond the passage level used in production is confirmed.

Characterisation of the rCTB is undertaken using a variety of analytical techniques including determination of molecular size, charge and amino acid composition. Techniques suitable for such purposes include sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and different liquid chromatographies. The identity of the product is confirmed by at least partial *N*-terminal and *C*-terminal amino acid sequencing.

Master seed lots are grown on agar plates, which may contain appropriate antibiotics. Colonies are used to produce working seed lots in liquid media that are free from antibiotics. Cultures derived from the working seed lot must have the same characteristics as the cultures of the strain from which the master seed lot was derived.

Only a seed lot that complies with the following requirements may be used in the preparation of the monovalent cell harvest.

IDENTIFICATION

Master seed lots are identified by colony morphology, and by biochemical characterisation, using suitable molecular assays or immunoassays. Working seed lots are identified by colony morphology and by molecular assays or immunoassays.

PURITY

Purity of master seed lots and working seed lots is verified by methods of suitable sensitivity.

PROPAGATION AND HARVEST

Each strain is grown separately from the working seed lot.

Cultures are checked at different stages of fermentation (subcultures and main culture) for purity, identity, cell opacity, pH and biochemical characteristics. Unsatisfactory cultures must be discarded.

Production cultures are shown to be consistent in respect of growth rate, pH and yield of cells or cell products.

MONOVALENT CELL HARVEST

Only a monovalent harvest that complies with established specifications for the following tests may be used.

pH *(2.2.3)*

Within the range approved for the particular product.

IDENTIFICATION

Relevant antigenic characteristics are verified by suitable immunological or biochemical assays.

Purity

Samples of culture are examined by microscopy of Gram-stained smears, by inoculation of appropriate culture media or by another suitable procedure.

Opacity

The absorbance at 600 nm *(2.2.25)* is within the range approved for the particular product.

INACTIVATED MONOVALENT CELL BULK

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. Bacteria are inactivated after washing, either by treatment with formaldehyde or by heating under conditions that ensure inactivation.

Only an inactivated monovalent cell bulk that complies with established specifications for the following tests may be used in the preparation of the final bulk.

pH ***(2.2.3)***

Within the range approved for the particular product.

Identification

Verified by slide agglutination.

Inactivation

Complete inactivation is verified by a suitable culture method.

Sterility ***(2.6.1)***

It complies with the test for sterility, carried out using 10 ml for each medium.

Opacity

The inactivation process may affect the accuracy of opacity measurements.

Purity

Samples of culture are examined by microscopy of Gram-stained smears, by inoculation of appropriate culture media or by another suitable procedure.

Smooth LPS content

Verified by a suitable immunoassay *(2.7.1)*.

Residual cholera toxin

The absence of residual cholera toxin is verified by a suitable immunoassay *(2.7.1)* or biochemical assay.

Free formaldehyde ***(2.4.18)***

Content to be determined where formaldehyde is used for inactivation.

PURIFIED RCTB

Production of the rCTB follows the guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant technology and is covered by the monograph *Products of recombinant DNA technology (0784)*. Prior to harvest, the cell culture is checked for purity and opacity. rCTB is harvested by suitable filtration, concentrated by diafiltration, purified by chromatography, filter-sterilised and stored under suitable conditions. The pH of the pooled eluate is adjusted prior to buffer exchange.

Only purified rCTB that complies with established specifications for the following tests may be used in the preparation of the final bulk.

pH ***(2.2.3)***

Within the range approved for the particular product.

Purity

Verified by SDS-PAGE *(2.2.31)* and an appropriate liquid chromatography method *(2.2.29)*.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium.

rCTB

The amount of rCTB is determined by a suitable immunoassay *(2.7.1)*.

FINAL BULK

The final bulk vaccine is prepared by aseptically mixing a suitable buffer with monovalent cell bulks. Where used, the rCTB bulk is added in appropriate amounts. Preservatives, if used, may be added at this stage.

Only a final bulk that complies with the following requirements may be used in the preparation of the final lot.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

FINAL LOT

The final bulk is mixed to homogeneity and filled aseptically into suitable containers.

Only a final lot that is within the limits approved for the particular product and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

IDENTIFICATION

Serotypes are detected by a suitable immunoassay *(2.7.1)* or molecular assay. rCTB is detected by a suitable immunoassay *(2.7.1)*. The antigen-content assays may also serve as an identity test.

TESTS

pH *(2.2.3)*

Within the range approved for the particular product.

Sterility *(2.6.1)*

It complies with the test for sterility.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l, where applicable.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

ASSAY

Antigen content

The amount of smooth LPS, and where applicable, the amount of rCTB, are within the limits approved for the particular product, determined by a suitable immunoassay *(2.7.1)*.

LABELLING

The label states:

— the method of inactivation;

— the serogroup, serotypes and biotypes of vaccine strains;

— the number of bacteria per human dose;

— the amount of rCTB.

Ph Eur

Cholera Vaccine, Freeze-dried

General Notices

(Ph Eur monograph 0155)

The label may state 'Dried/Cholera'.

Ph Eur

DEFINITION

Freeze-dried cholera vaccine is a preparation of a suitable strain or strains of *Vibrio cholerae*. The vaccine is reconstituted as stated on the label to give a uniform suspension containing not less than 8×10^9 bacteria in each human dose. The human dose does not exceed 1.0 ml of the reconstituted vaccine.

PRODUCTION

The vaccine is prepared using a seed-lot system. The vaccine consists of a mixture of equal parts of vaccines prepared from smooth strains of the 2 main serological types, Inaba and Ogawa. These may be of the classical biotype with or without the El-Tor biotype. A single strain or several strains of each type may be included. All strains must contain, in addition to their type O antigens, the heat-stable O antigen common to Inaba and Ogawa. If more than one strain each of Inaba and Ogawa are used, these may be selected so as to contain other O antigens in addition. The World Health Organisation recommends new strains which may be used if necessary in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia. In order to comply with the requirements for vaccination certificates required for international travel, the vaccine must contain not less than 8×10^9 organisms of the classical biotype. Each strain is grown separately. The bacteria are inactivated either by heating the suspensions (for example, at 56 °C for 1 h) or by treatment with formaldehyde or by a combination of the physical and chemical methods. Phenol is not used in the preparation. The vaccine is distributed into sterile containers and freeze-dried to a moisture content favourable to the stability of the vaccine. The containers are then closed so as to exclude contamination.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*) modified as follows: inject 0.5 ml of the vaccine into each mouse and 1.0 ml into each guinea pig.

IDENTIFICATION

The vaccine reconstituted as stated on the label is identified by specific agglutination tests.

TESTS

Phenol (*2.5.15*)

If phenol has been used in the preparation, the concentration is not more than 5 g/l.

Antibody production

Test the ability of the vaccine to induce antibodies (such as agglutinating, vibriocidal or haemagglutinating antibodies) in the guinea-pig, the rabbit or the mouse. Administer the reconstituted vaccine to a group of at least 6 animals. At the end of the interval of time necessary for maximum antibody formation, determined in preliminary tests, collect sera from the animals and titrate them individually for the appropriate antibody using a suitable method. The vaccine to be examined passes the test if each serotype has elicited a significant antibody response.

Sterility (*2.6.1*)

The reconstituted vaccine complies with the test for sterility.

LABELLING

The label states:

— the method used to inactivate the bacteria,

— the number of bacteria in each human dose.

Ph Eur

Cholera Vaccine

General Notices

(Ph Eur monograph 0154)

The label may state 'Cholera'.

Ph Eur

DEFINITION

Cholera vaccine is a homogeneous suspension of a suitable strain or strains of *Vibrio cholerae* containing not less than 8×10^9 bacteria in each human dose. The human dose does not exceed 1.0 ml.

PRODUCTION

The vaccine is prepared using a seed-lot system. The vaccine consists of a mixture of equal parts of vaccines prepared from smooth strains of the 2 main serological types, Inaba and Ogawa. These may be of the classical biotype with or without the El-Tor biotype. A single strain or several strains of each type may be included. All strains must contain, in addition to their type O antigens, the heat-stable O antigen common to Inaba and Ogawa. If more than one strain each of Inaba and Ogawa are used, these may be selected so as to contain other O antigens in addition. The World Health Organisation recommends new strains which may be used if necessary, in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia. In order to comply with the requirements for vaccination certificates required for international travel, the vaccine must contain not less than 8×10^9 organisms of the classical biotype. Each strain is grown separately. The bacteria are inactivated either by heating the suspensions (for example, at 56 °C for 1 h) or by treatment with formaldehyde or phenol or by a combination of the physical and chemical methods.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*) modified as follows: inject 0.5 ml of the vaccine into each mouse and 1.0 ml into each guinea pig.

IDENTIFICATION

It is identified by specific agglutination tests.

TESTS

Phenol *(2.5.15)*

If phenol has been used in the preparation, the concentration is not more than 5 g/l.

Antibody production

Test the ability of the vaccine to induce antibodies (such as agglutinating, vibriocidal or haemagglutinating antibodies) in the guinea-pig, the rabbit or the mouse. Administer the vaccine to a group of at least 6 animals. At the end of the interval of time necessary for maximum antibody formation, determined in preliminary tests, collect sera from the animals and titrate them individually for the appropriate antibody using a suitable method. The vaccine to be examined passes the test if each serotype has elicited a significant antibody response.

Sterility ***(2.6.1)***

It complies with the test for sterility.

LABELLING

The label states:

— the method used to inactivate the bacteria,

— the number of bacteria in each human dose.

Ph Eur

Diphtheria and Tetanus Vaccine (Adsorbed, Reduced Antigen(s) Content)

General Notices

Adsorbed Diphtheria and Tetanus Vaccine for Adults and Adolescents
(Ph Eur monograph 0647)

The label may state 'dT'.

For a vaccine for use in the United Kingdom, the amount of diphtheria toxoid used is adjusted so that the final vaccine contains not more than 2.0 flocculation equivalents (2.0 Lf) of diphtheria toxoid per dose.

Ph Eur

DEFINITION

Diphtheria and tetanus vaccine (adsorbed, reduced antigen(s) content) is a preparation of diphtheria formol toxoid and tetanus formol toxoid with a mineral adsorbent. The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively. It shall have been demonstrated to the competent authority that the quantity of diphtheria toxoid used does not produce adverse reactions in subjects from the age groups for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test once; if more than one animal dies in the second test, the vaccine does not comply with the test.

BULK PURIFIED DIPHTHERIA TOXOID AND TETANUS TOXOIDS

The bulk purified diphtheria and tetanus toxoids are prepared as described in the monographs on *Diphtheria vaccine (adsorbed) (0443)* and *Tetanus vaccine (adsorbed) (0452)* and comply with the requirements prescribed therein.

FINAL BULK VACCINE

The vaccine is prepared by adsorption of suitable quantities of bulk purified diphtheria toxoid and tetanus toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified toxoids or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method (*2.7.1*). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate. If a satisfactory result is not obtained with a vaccine adsorbed on aluminium hydroxide, carry out the test as follows. Centrifuge 15 ml of the vaccine to be examined and suspend the residue in 5 ml of a freshly prepared mixture of 1 volume of a 56 g/l solution of *sodium edetate R* and 49 volumes of a 90 g/l solution of *disodium hydrogen phosphate R*. Maintain at 37 °C for not less than 6 h and centrifuge. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 2 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 20 IU per single human dose.

LABELLING

The label states:

- the minimum number of International Units of each component per single human dose;
- the name and the amount of the adsorbent;
- that the vaccine must be shaken before use;
- that the vaccine is not to be frozen.

Ph Eur

Diphtheria Vaccine (Adsorbed, Reduced Antigen Content)

General Notices

Adsorbed Diphtheria Vaccine for Adults and Adolescents

(Ph Eur monograph 0646)

For a vaccine for use in the United Kingdom, the amount of toxoid used is adjusted so that the final vaccine contains not more than 2.0 flocculation equivalents (2.0 Lf) per dose.

Ph Eur

DEFINITION

Diphtheria vaccine (adsorbed, reduced antigen content) is a preparation of diphtheria formol toxoid with a mineral adsorbent. The formol toxoid is prepared from the toxin produced by the growth of *Corynebacterium diphtheriae*. It shall have been demonstrated to the competent authority that the quantity of diphtheria toxoid used does not produce adverse reactions in subjects from the age groups for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

Specific toxicity

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia, the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test once; if more than one animal dies in the second test, the vaccine does not comply with the test.

BULK PURIFIED TOXOID

The bulk purified toxoid is prepared as described in the monograph on *Diphtheria vaccine (adsorbed) (0443)* and complies with the requirements prescribed therein.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of a suitable quantity of bulk purified toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified toxoid or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

IDENTIFICATION

Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate. If a satisfactory result is not obtained with a vaccine adsorbed on aluminium hydroxide, carry out the test as follows. Centrifuge 15 ml of the vaccine to be examined and suspend the residue in 5 ml of a freshly prepared mixture of 1 volume of a 56 g/l solution of *sodium edetate R* and 49 volumes of a 90 g/l solution of *disodium hydrogen phosphate R*. Maintain at 37 °C for not less than 6 h and centrifuge. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 2 IU per single human dose.

LABELLING

The label states:

- the minimum number of International Units per single human dose;
- the name and the amount of the adsorbent;
- that the vaccine must be shaken before use;
- that the vaccine is not to be frozen.

Ph Eur

Diphtheria, Tetanus and Hepatitis B (rDNA) Vaccine (Adsorbed)

General Notices

(Ph Eur monograph 2062)

The label may state 'DT/HepB'.

Ph Eur

DEFINITION

Diphtheria, tetanus and hepatitis B (rDNA) vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; hepatitis B surface antigen (HBsAg); a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

HBsAg is a component protein of hepatitis B virus; the antigen is obtained by recombinant DNA technology.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The content of bacterial endotoxins *(2.6.14)* in the bulk purified diphtheria toxoid and tetanus toxoid is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and in any case the contents are such that the final vaccine contains less than 100 IU per single human dose.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443), Tetanus vaccine (adsorbed) (0452)* and *Hepatitis B vaccine (rDNA) (1056).*

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid and HBsAg onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the test for antimicrobial preservative and the assays for the diphtheria and tetanus components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the content of free formaldehyde has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

If an *in vivo* assay is used for the hepatitis B component, provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality *(2.2.35)*

The osmolality of the vaccine is within the limits approved for the particular preparation.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1).* The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37

°C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The assay or, where applicable, the electrophoretic profile, serves also to identify the hepatitis B component of the vaccine.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

It complies with the test for pyrogens. Inject the equivalent of 1 human dose into each rabbit.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 40 IU per single human dose.

Hepatitis B component

It complies with the assay of hepatitis B vaccine *(2.7.15)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose,

— the amount of HBsAg per single human dose,

— the type of cells used for production of the HBsAg component,

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults,

— the name and the amount of the adsorbent,

— that the vaccine must be shaken before use,

— that the vaccine is not to be frozen.

Ph Eur

Diphtheria, Tetanus and Poliomyelitis (Inactivated) Vaccine (Adsorbed, Reduced Antigen(s) Content)

General Notices

(Ph Eur monograph 2328)

The label may state 'Td/IPV'.

Ph Eur

DEFINITION

Diphtheria, tetanus and poliomyelitis (inactivated) vaccine (adsorbed, reduced antigen(s) content) is a combined vaccine containing: diphtheria formol toxoid; tetanus formol toxoid; suitable strains of human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The amount of diphtheria toxoid per single human dose is reduced compared to vaccines generally used for primary vaccination; the amount of tetanus toxoid may also be reduced.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between the monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test once; if more than one animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins (*2.6.14*) in bulk purified diphtheria toxoid, tetanus toxoid and inactivated monovalent poliovirus harvests is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)* and *Poliomyelitis vaccine (inactivated) (0214).*

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, separately or together, of suitable quantities of bulk purified diphtheria toxoid and tetanus toxoid, and an admixture of suitable quantities of purified monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable quantity of a trivalent pool of such purified monovalent harvests. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin

Determined on the poliomyelitis components by a suitable immunochemical method (*2.7.1*) after virus harvest and before addition of the adsorbent in the preparation of the final bulk vaccine, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the test for antimicrobial preservative and the assays for the diphtheria and tetanus components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided the determination of D-antigen content cannot be carried out on the final lot, it is carried out during preparation of the final bulk before addition of the adsorbent.

Provided the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

The *in vivo* assay for the poliomyelitis component may be omitted once it has been demonstrated for a given vaccine and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the *in vivo* assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the *in vivo* and *in vitro* assays must be evaluated, and the need for revalidation considered.

Osmolality *(2.2.35)*

The osmolality of the vaccine is within the limits approved for the particular preparation.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate. If a satisfactory result is not obtained with a vaccine adsorbed on aluminium hydroxide, carry out the test as follows. Centrifuge 15 ml of the vaccine to be examined and suspend the residue in 5 ml of a freshly prepared mixture of 1 volume of a 56 g/l solution of *sodium edetate R* and 49 volumes of a 90 g/l solution of *disodium hydrogen phosphate R*. Maintain at 37 °C for not less than 6 h and centrifuge. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility ***(2.6.1)***

It complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 2 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 20 IU per single human dose.

Poliomyelitis component

D-antigen content As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* following desorption, using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test The vaccine complies with the *in vivo* assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the types of poliovirus contained in the vaccine;

— the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the type of cells used for production of the poliomyelitis component;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen.

Diphtheria, Tetanus, Pertussis (Acellular, Component) and Poliomyelitis (Inactivated) Vaccine (Adsorbed, Reduced Antigen (s) Content)

General Notices

(Ph Eur monograph 2329)

The label may state 'dTaP/IPV'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis (acellular, component) and poliomyelitis (inactivated) vaccine (adsorbed, reduced antigen(s) content) is a combined vaccine containing: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; suitable strains of human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The amount of diphtheria toxoid per single human dose is reduced compared to vaccines generally used for primary vaccination; the amounts of tetanus toxoid and pertussis components may also be reduced.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between the monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test once; if more than one animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins *(2.6.14)* in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components and inactivated monovalent poliovirus harvests is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)*, *Pertussis vaccine (acellular, component, adsorbed) (1356)* and *Poliomyelitis vaccine (inactivated) (0214).*

FINAL BULK VACCINES

The final bulk vaccine is prepared by adsorption onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid and acellular pertussis components, and an admixture of suitable quantities of purified monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable quantity of a trivalent pool of such purified monovalent harvests. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin

Determined on the poliomyelitis components by a suitable immunochemical method *(2.7.1)* after virus harvest and before addition of the adsorbent in the preparation of the final bulk vaccine, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the test for absence of residual pertussis toxin and irreversibility of pertussis toxoid, the test for antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided the determination of D-antigen content cannot be carried out on the final lot, it is carried out during preparation of the final bulk before addition of the adsorbent.

Provided the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

The *in vivo* assay for the poliomyelitis component may be omitted once it has been demonstrated for a given vaccine and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the *in vivo* assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the *in vivo* and *in vitro* assays must be evaluated, and the need for revalidation considered.

Osmolality *(2.2.35)*

The osmolality of the vaccine is within the limits approved for the particular preparation.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate. If a satisfactory result is not obtained with a vaccine adsorbed on aluminium hydroxide, carry out the test as follows. Centrifuge 15 ml of the vaccine to be examined and suspend the residue in 5 ml of a freshly prepared mixture of 1 volume of a 56 g/l solution of *sodium edetate R* and 49 volumes of a 90 g/l solution of *disodium hydrogen phosphate R*. Maintain at 37 °C for not less than 6 h and centrifuge. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method *(2.7.1)*.

The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a specific antisera to the pertussis components of the vaccine.

D. The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

TESTS

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent intraperitoneally into the third group of mice. After 5 days, inject intraperitoneally into each mouse 2 mg of histamine base in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if one or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If one mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit (*P* = 0.95) of the estimated potency is not less than 2 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit (*P* = 0.95) of the estimated potency is not less than 20 IU per single human dose.

Pertussis component

The vaccine complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

Poliomyelitis component

D-antigen content As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* following desorption, using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test The vaccine complies with the *in vivo* assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the names and amounts of the pertussis components per single human dose;

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification;

— the types of poliovirus contained in the vaccine;

— the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the type of cells used for production of the poliomyelitis component;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen.

Ph Eur

Diphtheria, Tetanus, Pertussis (Acellular, Component) and Poliomyelitis (Inactivated) Vaccine (Adsorbed, Reduced Antigen (s) Content)

General Notices

(Ph Eur monograph 2329)

The label may state 'dTaP/IPV'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis (acellular, component) and poliomyelitis (inactivated) vaccine (adsorbed, reduced antigen(s) content) is a combined vaccine containing: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; suitable strains of human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The amount of diphtheria toxoid per single human dose is reduced compared to vaccines generally used for primary vaccination; the amounts of tetanus toxoid and pertussis components may also be reduced.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between the monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test once; if more than one animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins *(2.6.14)* in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components and inactivated monovalent poliovirus harvests is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)*, *Pertussis vaccine (acellular, component, adsorbed) (1356)* and *Poliomyelitis vaccine (inactivated) (0214).*

FINAL BULK VACCINES

The final bulk vaccine is prepared by adsorption onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid and acellular pertussis components, and an admixture of suitable quantities of purified monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable quantity of a trivalent pool of such purified monovalent harvests. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin

Determined on the poliomyelitis components by a suitable immunochemical method *(2.7.1)* after virus harvest and before addition of the adsorbent in the preparation of the final bulk vaccine, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the test for absence of residual pertussis toxin and irreversibility of pertussis toxoid, the test for antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided the determination of D-antigen content cannot be carried out on the final lot, it is carried out during preparation of the final bulk before addition of the adsorbent.

Provided the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

The *in vivo* assay for the poliomyelitis component may be omitted once it has been demonstrated for a given vaccine and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the *in vivo* assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the *in vivo* and *in vitro* assays must be evaluated, and the need for revalidation considered.

Osmolality *(2.2.35)*

The osmolality of the vaccine is within the limits approved for the particular preparation.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate. If a satisfactory result is not obtained with a vaccine adsorbed on aluminium hydroxide, carry out the test as follows. Centrifuge 15 ml of the vaccine to be examined and suspend the residue in 5 ml of a freshly prepared mixture of 1 volume of a 56 g/l solution of *sodium edetate R* and 49 volumes of a 90 g/l solution of *disodium hydrogen phosphate R*. Maintain at 37 °C for not less than 6 h and centrifuge. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method *(2.7.1)*.

The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained as described in identification test A reacts with a specific antisera to the pertussis components of the vaccine.

D. The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

TESTS

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent intraperitoneally into the third group of mice. After 5 days, inject intraperitoneally into each mouse 2 mg of histamine base in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if one or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If one mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit (*P* = 0.95) of the estimated potency is not less than 2 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit (*P* = 0.95) of the estimated potency is not less than 20 IU per single human dose.

Pertussis component

The vaccine complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

Poliomyelitis component

D-antigen content As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* following desorption, using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test The vaccine complies with the *in vivo* assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the names and amounts of the pertussis components per single human dose;

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification;

— the types of poliovirus contained in the vaccine;

— the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the type of cells used for production of the poliomyelitis component;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen.

Ph Eur

Diphtheria, Tetanus, Pertussis (Acellular, Component), Hepatitis B (rDNA), Poliomyelitis (Inactivated) and Haemophilus Type b Conjugate Vaccine (Adsorbed)

General Notices

(Ph Eur monograph 2067)

The label may state 'DTaP/HepB/IPV/Hib'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis (acellular, component), hepatitis B (rDNA), poliomyelitis (inactivated) and haemophilus type b conjugate vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; hepatitis B surface antigen (HBsAg); human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a suitable method; polyribosylribitol phosphate (PRP) covalently bound to a carrier protein. The antigens in the vaccine may be adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. The product may be presented with the haemophilus component in a separate container, the contents of which are mixed with the other components immediately before or during use.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The acellular pertussis component may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

Hepatitis B surface antigen is a component protein of hepatitis B virus; the antigen is obtained by recombinant DNA technology.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1 → 1)-ribitol-5-phosphate [$(C_{10}H_{19}O_{12}P)_n$], with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

If the vaccine is presented with the haemophilus component in a separate vial, as part of consistency studies the assays of the diphtheria, tetanus, pertussis, hepatitis B and poliomyelitis components are carried out on a suitable number of batches of vaccine reconstituted as for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria, toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins *(2.6.14)* in bulk purified diphtheria toxoid, bulk purified tetanus toxoid, bulk purified pertussis components, the hepatitis B surface antigen, the purified, inactivated monovalent poliovirus harvests and bulk PRP conjugate is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is not greater than the limit approved.

During development studies and wherever revalidation is necessary a test for pyrogens in rabbits *(2.6.8)* is carried out by injection of a suitable dose of the final lot. The vaccine is shown to be acceptable with respect to absence of pyrogenic activity.

During development studies and wherever revalidation is necessary, it shall be demonstrated by tests in animals that the vaccine induces a T-cell-dependent B-cell immune response to PRP.

The stability of the final lot and relevant intermediates is evaluated using one or more indicator tests. For the haemophilus component, such tests may include determination of molecular size, determination of free PRP in the conjugate and kinetics of depolymerisation. Taking account of the results of the stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on

Diphtheria vaccine (adsorbed) (0443), Tetanus vaccine (adsorbed) (0452), Pertussis vaccine (acellular, component, adsorbed) (1356), Hepatitis B vaccine (rDNA) (1056), Poliomyelitis vaccine (inactivated) (0214) and *Haemophilus type b conjugate vaccine (1219)*.

FINAL BULKS

Vaccine with all components in the same container The final bulk is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid, bulk purified acellular pertussis components and bulk purified hepatitis B surface antigen onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate and admixture of a suitable quantity of PRP conjugate and suitable quantities of purified and inactivated, monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable quantity of a trivalent pool of such monovalent harvests. Suitable antimicrobial preservatives may be added.

Vaccine with the haemophilus component in a separate container The final bulk of diphtheria, tetanus, pertussis, hepatitis B and poliovirus component is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid, bulk purified acellular pertussis components and bulk purified hepatitis B surface antigen onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate and admixture of suitable quantities of purified and inactivated, monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable pool of such monovalent harvests This final bulk is filled separately. Suitable antimicrobial preservatives may be added. The final bulk of the haemophilus component is prepared by dilution of the bulk conjugate to the final concentration with a suitable diluent. A stabiliser may be added.

Only final bulks that comply with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin

Determined on the poliomyelitis components by a suitable immunochemical method *(2.7.1)* after purification of the harvests and before preparation of the final bulk vaccine, before addition of the adsorbent, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

Where the haemophilus component is in a separate container, the final bulk of the haemophilus component is freeze-dried. Only a final lot that is satisfactory with respect to the test for osmolality shown below and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for osmolality, for absence of residual pertussis toxin and irreversibility of pertussis toxoid and for antimicrobial preservative and the diphtheria, tetanus and pertussis component assays have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens and the purified monovalent harvests or the trivalent pool of polioviruses or the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the test for bovine serum albumin has been carried out with satisfactory results on the trivalent pool of inactivated monovalent harvests of polioviruses or on the final bulk vaccine, it may be omitted on the final lot.

If an *in vivo* assay is used for the hepatitis B component, provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Provided the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

The *in vivo* assay for the poliomyelitis component may be omitted once it has been demonstrated for a given product and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the *in vivo* assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the *in vivo* and *in vitro* assays must be evaluated, and the need for revalidation considered.

Free PRP

For vaccines with all components in the same container, the free PRP content is determined on the non-absorbed fraction. Unbound PRP is determined on the haemophilus component after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

Bacterial endotoxins *(2.6.14)*

Less than the limit approved for the product concerned.

Osmolality *(2.2.35)*

The osmolality of the vaccine, reconstituted where applicable, is within the limits approved for the particular preparation.

IDENTIFICATION

If the vaccine is presented with the haemophilus component in a separate vial: identification tests A, B, C, D and E are carried out using the vial containing the diphtheria, tetanus, pertussis, hepatitis B and poliomyelitis components; identification test F is carried out on the vial containing the haemophilus components.

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The clear supernatant liquid obtained during identification test A reacts with a specific antisera to the pertussis components of the vaccine when examined by suitable immunochemical methods *(2.7.1)*.

D. The hepatitis B component is identified by a suitable immunochemical method *(2.7.1)*, for example the *in vitro* assay or by a suitable electrophoretic method *(2.2.31)*.

E. The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)*, such as determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

F. The PRP and its carrier protein are identified by a suitable immunochemical method *(2.7.1)*.

TESTS

If the product is presented with the haemophilus component in a separate container: the tests for absence of residual pertussis toxin and irreversibility of pertussis toxoid, free formaldehyde, aluminium, antimicrobial preservative and sterility are carried out on the container with the diphtheria, tetanus, pertussis, poliomyelitis and hepatitis B components; the tests for PRP, water, antimicrobial preservative (where applicable), aluminium (where applicable) and sterility are carried out on the container with the haemophilus component.

Some tests for the haemophilus component are carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component to be tested.

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent intraperitoneally into the third group of mice. After 5 days, inject intraperitoneally into each mouse 2 mg of histamine base in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

PRP

Minimum 80 per cent of the amount of PRP stated on the label, for a vaccine with the haemophilus component in a separate container.

For a vaccine with all components in the same container: the PRP content determined on the non-absorbed fraction is not less than that approved for the product.

PRP is determined either by assay of ribose *(2.5.31)* or phosphorus *(2.5. 18)*, by an immunochemical method *(2.7.1)* or by anion-exchange liquid chromatography *(2.2.29)* with pulsed-amperometric detection.

Aluminium *(2.5.13)*

Maximum 1.25 mg of aluminium (Al) per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l of free formaldehyde per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Water *(2.5.12)*

Maximum 3.0 per cent for the freeze-dried haemophilus component.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than the minimum potency stated on the label.

Unless otherwise justified and authorised, the minimum potency stated on the label is 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

The vaccine complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

Hepatitis B component

The vaccine complies with the assay of hepatitis B vaccine *(2.7.15)*.

Poliomyelitis component

D-antigen content As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* following desorption, using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test The vaccine complies with the in vivo assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the names and amounts of the pertussis components per single human dose;

— the amount of HBsAg per single human dose;

— the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the types of cells used for production of the poliomyelitis and the hepatitis B components;

— the number of micrograms of PRP per single human dose;

— the type and nominal amount of carrier protein per single human dose;

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen;

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Ph Eur

Diphtheria, Tetanus, Pertussis (Acellular, Component), Poliomyelitis (Inactivated) and Haemophilus Type b Conjugate Vaccine (Adsorbed)

General Notices

(Ph Eur monograph 2065)

The label may state 'DTaP/IPV/Hib'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis (acellular, component), poliomyelitis (inactivated) and haemophilus type b conjugate vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; suitable strains of human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a suitable method; polyribosylribitol phosphate (PRP) covalently bound to a carrier protein; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate. The product is presented with the haemophilus component in a separate container, the contents of which are mixed with the other components immediately before use.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The acellular pertussis component may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be co-purified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate $[(C_{10}H_{19}O_{12}P)_n]$, with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria, toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins *(2.6.14)* in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components, purified, inactivated monovalent poliovirus harvests and bulk PRP conjugate is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

During development studies and wherever revalidation is necessary, it shall be demonstrated by tests in animals that the vaccine induces a T-cell dependent B-cell immune response to PRP.

As part of consistency studies the assays of the diphtheria, tetanus, pertussis and poliomyelitis components are carried out on a suitable number of batches of vaccine reconstituted as for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)*, *Pertussis vaccine (acellular, component, adsorbed) (1356)*, *Poliomyelitis vaccine (inactivated) (0214)* and *Haemophilus type b conjugate vaccine (1219).*

FINAL BULKS

The final bulk of the diphtheria, tetanus, pertussis and poliomyelitis components is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid and bulk purified acellular pertussis components onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate and admixture of suitable quantities of purified, monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable quantity of a trivalent pool of such monovalent harvests. Suitable antimicrobial preservatives may be added.

The final bulk of the haemophilus component is prepared by dilution of the bulk conjugate to the final concentration with a suitable diluent. A stabiliser may be added.

Only final bulks that comply with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin

Determined on the poliomyelitis components by a suitable immunochemical method *(2.7.1)* during preparation of the final bulk vaccine, before addition of the adsorbent, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk of the haemophilus component is freeze-dried.

Only a final lot that is satisfactory with respect to the test for osmolality shown below and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the test for absence of residual pertussis toxin and irreversibility of pertussis toxoid, the test for antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided that the free formaldehyde content has been determined on the bulk purified antigens and the purified monovalent harvests or the trivalent pool of polioviruses or the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

The *in vivo* assay for the poliomyelitis component may be omitted once it has been demonstrated for a given product and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the in vivo assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the in vivo and in vitro assays must be evaluated, and the need for revalidation considered.

Osmolality *(2.2.35)*

The osmolality of the vaccine, reconstituted where applicable, is within the limits approved for the particular preparation.

Free PRP

Unbound PRP is determined on the haemophilus component after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

IDENTIFICATION

Identification tests A, B, C and D are carried out using the vial containing the diphtheria, tetanus, pertussis and poliomyelitis components; identification test E is carried out on the vial containing the haemophilus component.

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by suitable immunochemical methods *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with specific antisera to the pertussis components of the vaccine.

D. The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)*, such as determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

E. The haemophilus component is identified by a suitable immunochemical method *(2.7.1)* for PRP.

TESTS

The tests for absence of residual pertussis toxin and irreversibility of pertussis toxoid, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with the diphtheria, tetanus, pertussis and poliomyelitis components; the tests for PRP, water, sterility and pyrogens are carried out on the container with the haemophilus component.

Some tests for the haemophilus component may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component to be tested.

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2-8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent intraperitoneally into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Pertussis toxin BRP is suitable for use as a reference pertussis toxin.

PRP

Minimum 80 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose *(2.5.31)* or phosphorus *(2.5.18)*, by an immunochemical method *(2.7.1)* or by anion-exchange liquid chromatography *(2.2.29)* with pulsed-amperometric detection.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Water *(2.5.12)*

Maximum 3.0 per cent for the haemophilus component.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a quantity of the vaccine equivalent to: 1 µg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 µg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 µg of PRP for a vaccine with OMP as a carrier.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

Unless otherwise justified and authorised, the lower confidence limit ($P = 0.95$) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

It complies with the assay of pertussis vaccine (acellular) *(2.7.16)*.

Poliomyelitis component

D-antigen content As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* following desorption, using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test The vaccine complies with the *in vivo* assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the names and amounts of the pertussis components per single human dose;

— the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the type of cells used for production of the poliomyelitis component;

— the number of micrograms of PRP per single human dose;

— the type and nominal amount of carrier protein per single human dose;

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen;

— where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Ph Eur

Diphtheria, Tetanus, Pertussis and Poliomyelitis (Inactivated) Vaccine (Adsorbed)

General Notices

(Ph Eur monograph 2061)

The label may state 'DTwP/IPV'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis and poliomyelitis (inactivated) vaccine (adsorbed) is a combined vaccine containing: diphtheria formol toxoid; tetanus formol toxoid; an inactivated suspension of *Bordetella pertussis*; suitable strains of human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)*, *Pertussis vaccine (adsorbed) (0161)* and *Poliomyelitis vaccine (inactivated) (0214).*

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, separately or together, of suitable quantities of bulk purified diphtheria toxoid and bulk purified tetanus toxoid and admixture of suitable quantities of an inactivated suspension of *B. pertussis* and purified monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable quantity of a trivalent pool of such purified monovalent harvests. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin

Determined on the poliomyelitis components by a suitable immunochemical method *(2.7.1)* during preparation of the final bulk vaccine, before addition of the adsorbent, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for specific toxicity of the pertussis component and antimicrobial preservative, and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided that the free formaldehyde content has been determined on the bulk purified antigens, the inactivated *B. pertussis* suspension and the purified monovalent harvests or the trivalent pool of polioviruses or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

The *in vivo* assay for the poliomyelitis component may be omitted once it has been demonstrated for a given product and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the *in vivo* assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the *in vivo* and *in vitro* assays must be evaluated, and the need for revalidation considered.

Osmolality *(2.2.35)*

The osmolality of the vaccine is within the limits approved for the particular preparation.

IDENTIFICATION

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The centrifugation residue obtained in identification A may be used. Other suitable methods for separating the bacteria from the adsorbent may also be used. Identify pertussis vaccine by agglutination of the bacteria from the resuspended precipitate by antisera specific to *B. pertussis* or by the assay of the pertussis component prescribed under Assay.

D. The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

TESTS

Specific toxicity of the pertussis component

Use not fewer than 5 healthy mice each weighing 14-16 g for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 h before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with 0.5 ml, containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 9 g/l sterile solution of *sodium chloride R,* preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the groups of mice immediately before the injection and 72 h and 7 days after the injection. The vaccine complies with the test if: (a) at the end of 72 h the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than 60 per cent of that per control mouse; and (c) not more than 5 per cent of the vaccinated mice die during the test. The test may be repeated and the results of the tests combined.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

It complies with the test for sterility.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

If the test is carried out in guinea pigs, the lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose; if the test is carried out in mice, the lower confidence limit (P = 0.95) of the estimated potency is not less than 60 IU per single human dose.

Pertussis component

Carry out the assay of pertussis vaccine *(2.7.7)*.

The estimated potency is not less than 4 IU per single human dose and the lower confidence limit (P = 0.95) of the estimated potency is not less than 2 IU per single human dose.

Poliomyelitis component

D-antigen content As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* following desorption, using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test The vaccine complies with the *in vivo* assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the minimum number of International Units of pertussis vaccine per single human dose;

— the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the type of cells used for production of the poliomyelitis component;

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen.

Ph Eur

Diphtheria, Tetanus, Pertussis, Poliomyelitis (Inactivated) and Haemophilus Type b Conjugate Vaccine (Adsorbed)

General Notices

(Ph Eur monograph 2066)

The label may state 'DTwP/IPV/Hib'.

Ph Eur

DEFINITION

Diphtheria, tetanus, pertussis, poliomyelitis (inactivated) and haemophilus type b conjugate vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; an inactivated suspension of *Bordetella pertussis*; suitable strains of human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a suitable method; polyribosylribitol phosphate (PRP) covalently bound to a carrier protein; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate. The product is presented with the haemophilus component in a separate container, the contents of which are mixed with the other components immediately before use.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate [$(C_{10}H_{19}O_{12}P)_n$], with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

During development studies and wherever revalidation is necessary, it shall be demonstrated by tests in animals that the vaccine induces a T-cell-dependent B-cell immune response to PRP.

As part of consistency studies the assays of the diphtheria, tetanus, pertussis and poliomyelitis components are carried out on a suitable number of batches of vaccine reconstituted as for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

Reference vaccine(s) Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Specific toxicity of the diphtheria and tetanus components

The production method is validated to demonstrate that the product, if tested, would comply with the following test: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

PRODUCTION OF THE COMPONENTS

The production of the components complies with the requirements of the monographs on *Diphtheria vaccine (adsorbed) (0443)*, *Tetanus vaccine (adsorbed) (0452)*, *Pertussis vaccine (adsorbed) (0161)*, *Poliomyelitis vaccine (inactivated) (0214)* and *Haemophilus type b conjugate vaccine (1219).*

FINAL BULKS

The final bulk of the diphtheria, tetanus, pertussis and poliomyelitis components is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, and bulk purified tetanus toxoid onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate and admixture of suitable quantities of an inactivated suspension of *B. pertussis* and of purified, monovalent harvests of human poliovirus types 1, 2 and 3 or a suitable quantity of a trivalent pool of such monovalent harvests. Suitable antimicrobial preservatives may be added.

The final bulk of the haemophilus component is prepared by dilution of the bulk conjugate to the final concentration with a suitable diluent. A stabiliser may be added.

Only final bulks that comply with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin

Determined on the poliomyelitis components by a suitable immunochemical method *(2.7.1)* during preparation of the final bulk vaccine, before addition of the adsorbent, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk of the haemophilus component is freeze-dried.

Only a final lot that is satisfactory with respect to the test for osmolality shown below and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for specific toxicity of the pertussis component and antimicrobial preservative, and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided that the free formaldehyde content has been determined on the bulk purified antigens, the inactivated *B. pertussis* suspension and the purified monovalent harvests or the trivalent pool of polioviruses or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

The *in vivo* assay for the poliomyelitis component may be omitted once it has been demonstrated for a given product and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the *in vivo* assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the *in vivo* and *in vitro* assays must be evaluated, and the need for revalidation considered.

Osmolality *(2.2.35)*

The osmolality of the vaccine, reconstituted where applicable, is within the limits approved for the particular preparation.

Free PRP

Unbound PRP is determined on the haemophilus component after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

IDENTIFICATION

Identification tests A, B, C and D are carried out using the vial containing the diphtheria, tetanus, pertussis and poliomyelitis components; identification test E is carried out on the vial containing the haemophilus component.

A. Diphtheria toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method *(2.7.1)*. The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The centrifugation residue obtained in identification A may be used. Other suitable methods for separating the bacteria from the adsorbent may also be used. Identify pertussis vaccine by agglutination of the bacteria from the resuspended precipitate by antisera specific to *B. pertussis* or by the assay of the pertussis component prescribed under Assay.

D. The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)*, such as determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

E. The haemophilus component is identified by a suitable immunochemical method *(2.7.1)* for PRP.

TESTS

The tests for specific toxicity of the pertussis component, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with diphtheria, tetanus, pertussis and poliomyelitis components; the tests for PRP, water, sterility and pyrogens are carried out on the container with the haemophilus component.

Some tests for the haemophilus component may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component to be tested.

Specific toxicity of the pertussis component

Use not fewer than 5 healthy mice each weighing 14-16 g, for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 h before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with 0.5 ml, containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 9 g/l sterile solution of *sodium chloride R,* preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the groups of mice immediately before the injection and 72 h and 7 days after the injection. The vaccine complies with the test if: (a) at the end of 72 h the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than 60 per cent of that per control mouse; and (c) not more than 5 per cent of the vaccinated mice die during the test. The test may be repeated and the results of the tests combined.

PRP

Minimum 80 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose *(2.5.31)* or phosphorus *(2.5.18)*, by an immunochemical method *(2.7.1)* or by anion-exchange liquid chromatography *(2.2.29)* with pulsed-amperometric detection.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Water *(2.5.12)*

Maximum 3.0 per cent for the haemophilus component.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a quantity of the vaccine equivalent to: 1 µg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 µg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 µg of PRP for a vaccine with OMP as carrier.

ASSAY

Diphtheria component

Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) *(2.7.6)*.

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) *(2.7.8)*.

If the test is carried out in guinea-pigs, the lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose; if the test is carried out in mice, the lower confidence limit (P = 0.95) of the estimated potency is not less than 60 IU per single human dose.

Pertussis component

Carry out the assay of pertussis vaccine *(2.7.7)*.

The estimated potency is not less than 4 IU per single human dose and the lower confidence limit (P = 0.95) of the estimated potency is not less than 2 IU per single human dose.

Poliomyelitis component

D-antigen content As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* following desorption using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test The vaccine complies with the *in vivo* assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the minimum number of International Units of diphtheria and tetanus toxoid per single human dose;

— the minimum number of International Units of pertussis vaccine per single human dose;

— the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the type of cells used for production of the poliomyelitis component;

— the number of micrograms of PRP per single human dose;

— the type and nominal amount of carrier protein per single human dose;

— where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults;

— the name and the amount of the adsorbent;

— that the vaccine must be shaken before use;

— that the vaccine is not to be frozen.

Ph Eur

Haemophilus Type b and Meningococcal Group C Conjugate Vaccine

General Notices

The label may state 'Hib/MenC'.

DEFINITION

Haemophilus Type b and Meningococcal Group C Conjugate Vaccine is a combined vaccine. It is prepared from purified polysaccharides derived from a suitable strain of *Neisseria meningitidis* group C and from a suitable strain of *Haemophilus influenzae*, type b covalently bound to a carrier protein.

The product is presented as a combined lyophilisate of the haemophilus type b and meningococcal group C components together with the solvent in a separate container. The final product is prepared by mixing the contents of the two containers immediately before use.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the *test for abnormal toxicity for immunosera and vaccines*, Appendix XIV E. The stability of the final lot and relevant intermediates is evaluated using one or more indicator tests. Such tests may include determination of molecular size, determination of free saccharides in the conjugate or an immunogenicity test in animals. Taking account of the results of the stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

During development studies and wherever revalidation of the manufacturing process is necessary, it shall be demonstrated by tests in animals that the vaccine consistently induces a T-cell-dependent B-cell immune responses to PRP and to meningococcal group C polysaccharides.

PRODUCTION OF THE COMPONENTS

The production of the vaccine components complies with the requirements of the monographs for Haemophilus Type b Conjugate Vaccine and Meningococcal Group C Conjugate Vaccine.

FINAL BULK VACCINE

A final bulk may be prepared by mixing suitable quantities of the haemophilus and meningococcal C bulk conjugates and dilution to the final concentration with a suitable diluent. Different methods of preparation may be used. A suitable adjuvant, a suitable antimicrobial preservative and a stabiliser may be added to the components bulk conjugates.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than the minimum amount shown to be effective and not greater than 115% of the intended amount.

Sterility

Carry out the *test for sterility*, Appendix XVI A, using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification and Tests may be released for use. Provided the test for antimicrobial preservative has been carried out on the final bulk vaccine, it may be omitted on the final lot.

Acidity or alkalinity

pH of the reconstituted vaccine is within the range approved by the competent authority for the particular product, Appendix V L.

Free PRP

Unbound PRP is determined after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved by the competent authority for the particular product.

Free meningococcal C saccharide

Unbound saccharide is determined after removal of the conjugate, for example by anion-exchange liquid chromatography, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. The amount of free meningococcal C saccharide is not greater than that approved for the particular product.

The vaccine complies with the requirements stated under Vaccines and with the following requirements.

IDENTIFICATION

The haemophilus and menigococcal components are identified by a suitable immunochemical method, Appendix XIV B.

TESTS

PRP

Not less than 80% of the amount of PRP stated on the label. PRP is determined either by assay of ribose or phosphorus, Appendix XV G, by an immunochemical method, Appendix XIV B, or by anion-exchange liquid chromatography, Appendix III D, with pulsed-amperometric detection.

Meningococcal saccharide

Not less than 80% of the amount of meningococcal group C polysaccharide stated on the label. The saccharide content is determined by a suitable validated assay, for example sialic acid assay, Appendix XV G or anion-exchange liquid chromatography, Appendix III D, with pulsed amperometric detection.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than the minimum amount shown to be effective and not greater than 115% of the quantity stated on the label.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XVI C. Less than 25 IU per single human dose.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

LABELLING

The label states per human dose: (1) the number of micrograms of PRP; (2) the number of micrograms of meningococcal group C polysaccharide; (3) the type and nominal amount of carrier protein.

Haemophilus Type b Conjugate Vaccine

General Notices

(Ph Eur monograph 1219)

The label may state 'Hib'.

Ph Eur

DEFINITION

Haemophilus type b conjugate vaccine is a liquid or freeze-dried preparation of a polysaccharide, derived from a suitable strain of *Haemophilus influenzae* type b, covalently bound to a carrier protein. The polysaccharide, polyribosylribitol phosphate, referred to as PRP, is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate [$(C_{10}H_{19}O_{12}P)_n$], with a defined molecular size. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently haemophilus type b conjugate vaccines of adequate safety and immunogenicity in man. The production of PRP and of the carrier are based on seed-lot systems.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*).

During development studies and wherever revalidation of the manufacturing process is necessary, it shall be demonstrated by tests in animals that the vaccine consistently induces a T-cell-dependent B-cell immune response.

The stability of the final lot and relevant intermediates is evaluated using one or more indicator tests. Such tests may include determination of molecular size, determination of free PRP in the conjugate and the immunogenicity test in mice. Taking account of the results of the stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

BACTERIAL SEED LOTS

The seed lots of *H. influenzae* type b are shown to be free from contamination by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

No complex products of animal origin are included in the menstruum used for preservation of strain viability, either for freeze-drying or for frozen storage.

It is recommended that PRP produced by the seed lot be characterised using nuclear magnetic resonance spectrometry (*2.2.33*).

H. INFLUENZAE TYPE B POLYSACCHARIDE (PRP)

H. influenzae type b is grown in a liquid medium that does not contain high-molecular-mass polysaccharides; if any ingredient of the medium contains blood-group substances, the process shall be validated to demonstrate that after the purification step they are no longer detectable. The bacterial purity of the culture is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera. The culture may be inactivated. PRP is separated from the culture medium and purified by a suitable method. Volatile matter, including water, in the purified polysaccharide is determined by a suitable method such as thermogravimetry *(2.2.34)*; the result is used to calculate the results of certain tests with reference to the dried substance, as prescribed below.

Only PRP that complies with the following requirements may be used in the preparation of the conjugate.

Identification

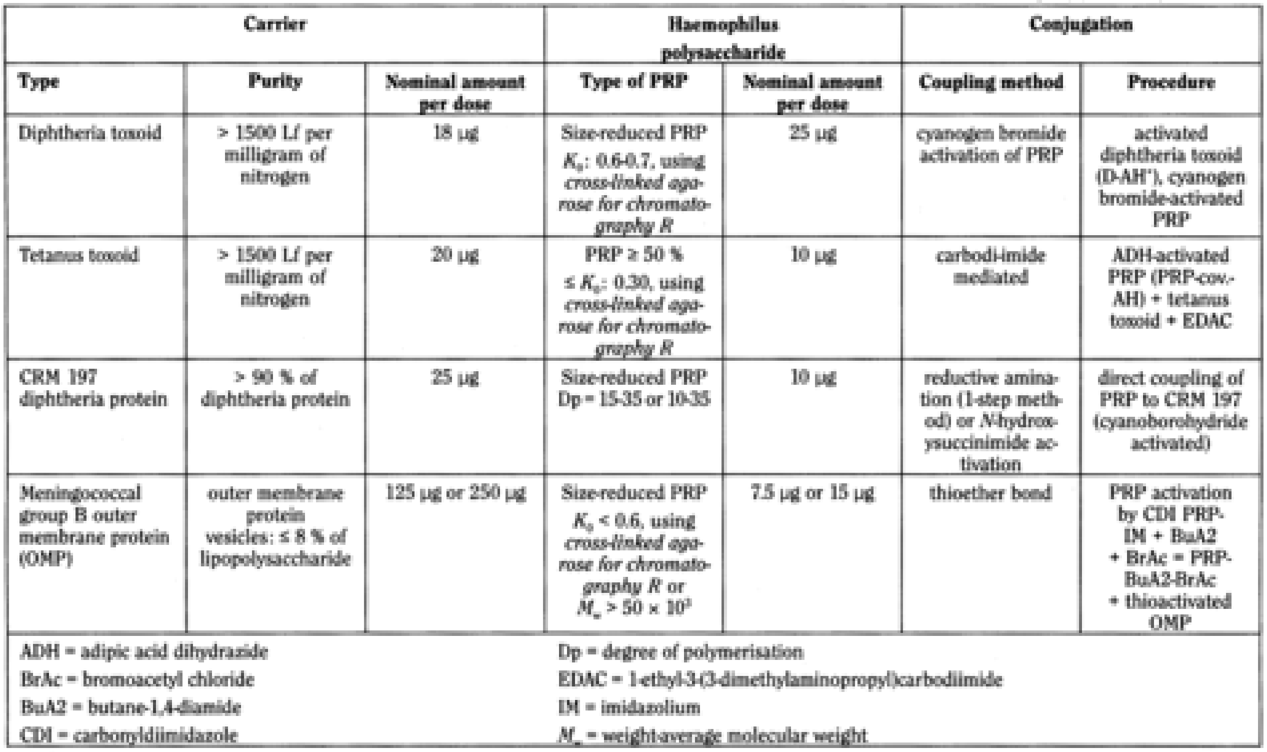
PRP is identified by an immunochemical method *(2.7.1)* or other suitable method, for example ^{1}H nuclear magnetic resonance spectrometry *(2.2.33)*.

Molecular-size distribution

The percentage of PRP eluted before a given K_0 value or within a range of K_0 values is determined by size-exclusion chromatography *(2.2.30)*; an acceptable value is established for the particular product and each batch of PRP must be shown to comply with this limit. Limits for currently approved products, using the indicated stationary phases, are shown for information in Table 1219.-1. Where applicable, the molecular-size distribution is also determined after chemical modification of the polysaccharide.

Liquid chromatography *(2.2.29)* with multiple-angle laser light-scattering detection may also be used for determination of molecular-size distribution.

Table 1219.-1. – *Product characteristics and specifications for PRP and carrier protein in currently approved products*



Carrier			**Haemophilus polysaccharide**		**Conjugation**	
Type	**Purity**	**Nominal amount per dose**	**Type of PRP**	**Nominal amount per dose**	**Coupling method**	**Procedure**
Diphtheria toxoid	> 1500 Lf per milligram of nitrogen	18 μg	Size-reduced PRP K_0: 0.6-0.7, using *cross-linked agarose for chromatography R*	25 μg	cyanogen bromide activation of PRP	activated diphtheria toxoid (D-AH⁺), cyanogen bromide-activated PRP
Tetanus toxoid	> 1500 Lf per milligram of nitrogen	20 μg	PRP ≥ 50 % ≤ K_0: 0.30, using *cross-linked agarose for chromatography R*	10 μg	carbodi-imide mediated	ADH-activated PRP (PRP-cov.-AH) + tetanus toxoid + EDAC
CRM 197 diphtheria protein	> 90 % of diphtheria protein	25 μg	Size-reduced PRP Dp = 15-35 or 10-35	10 μg	reductive amination (1-step method) or *N*-hydroxysuccinimide activation	direct coupling of PRP to CRM 197 (cyanoborohydride activated)
Meningococcal group B outer membrane protein (OMP)	outer membrane protein vesicles: ≤ 8 % of lipopolysaccharide	125 μg or 250 μg	Size-reduced PRP K_0 < 0.6, using *cross-linked agarose for chromatography R* or $M_w > 50 \times 10^3$	7.5 μg or 15 μg	thioether bond	PRP activation by CDI PRP-IM + BuA2 + BrAc = PRP-BuA2-BrAc + thioactivated OMP

ADH = adipic acid dihydrazide
BrAc = bromoacetyl chloride
BuA2 = butane-1,4-diamide
CDI = carbonyldiimidazole
Dp = degree of polymerisation
EDAC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
IM = imidazolium
M_w = weight-average molecular weight

A validated determination of the degree of polymerisation or of the weight-average molecular weight and the dispersion of molecular masses may be used instead of the determination of molecular size distribution.

Ribose *(2.5.31)*

Not less than 32 per cent, calculated with reference to the dried substance.

Phosphorus *(2.5.18)*

6.8 per cent to 9.0 per cent, calculated with reference to the dried substance.

Protein *(2.5.16)*

Not more than 1.0 per cent, calculated with reference to the dried substance. Use sufficient PRP to allow detection of proteins at concentrations of 1 per cent or greater.

Nucleic acid *(2.5.17)*

Not more than 1.0 per cent, calculated with reference to the dried substance.

Bacterial endotoxins *(2.6.14)*

Less than 25 IU per microgram of PRP.

Residual reagents

Where applicable, tests are carried out to determine residues of reagents used during inactivation and purification. An acceptable value for each reagent is established for the particular product and each batch of PRP must be shown to comply with this limit. Where validation studies have demonstrated removal of a residual reagent, the test on PRP may be omitted.

CARRIER PROTEIN

The carrier protein is chosen so that when the PRP is conjugated it is able to induce a T-cell-dependent B-cell immune response. Currently approved carrier proteins and coupling methods are listed for information in Table 1219.-1. The carrier proteins are produced by culture of suitable micro-organisms; the bacterial purity of the culture is verified; the culture may be inactivated; the carrier protein is purified by a suitable method.

Only a carrier protein that complies with the following requirements may be used in the preparation of the conjugate.

Identification

The carrier protein is identified by a suitable immunochemical method *(2.7.1)*.

Sterility *(2.6.1)*

Carry out the test using for each medium 10 ml or the equivalent of one-hundred doses, whichever is less.

Diphtheria toxoid

Diphtheria toxoid is produced as described in *Diphtheria vaccine (adsorbed) (0443)* and complies with the requirements prescribed therein for bulk purified toxoid.

Tetanus toxoid

Tetanus toxoid is produced as described in *Tetanus vaccine (adsorbed) (0452)* and complies with the requirements prescribed therein for bulk purified toxoid, except that the antigenic purity *(2.7.27)* is not less than 1500 Lf per milligram of protein nitrogen.

Diphtheria protein CRM 197

It contains not less than 90 per cent of diphtheria CRM 197 protein, determined by a suitable method. Suitable tests are carried out, for validation or routinely, to demonstrate that the product is nontoxic.

OMP (meningococcal group B Outer Membrane Protein complex)

OMP complies with the following requirements for lipopolysaccharide and pyrogens.

Lipopolysaccharide

Not more than 8 per cent of lipopolysaccharide, determined by a suitable method.

Pyrogens *(2.6.8)*

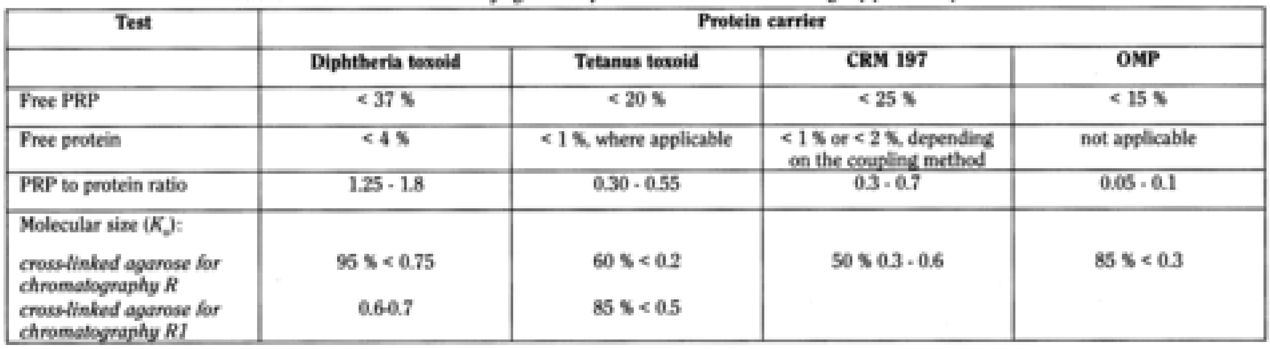
Inject into each rabbit 0.25 µg of OMP per kilogram of body mass.

BULK PRP CONJUGATE

PRP is chemically modified to enable conjugation; it is usually partly depolymerised either before or during this procedure. Reactive functional groups or spacers may be introduced into the carrier protein or PRP prior to conjugation. As a measure of consistency, the extent of derivatisation is monitored. The conjugate is obtained by the covalent binding of PRP and carrier protein. Where applicable, unreacted but potentially reactogenic functional groups are made unreactive by means of capping agents; the conjugate is purified to remove reagents.

Only a bulk conjugate that complies with the following requirements may be used in the preparation of the final bulk vaccine. For each test and for each particular product, limits of acceptance are established and each batch of conjugate must be shown to comply with these limits. Limits applied to currently approved products for some of these tests are listed for information in Table 1219.-2. For a freeze-dried vaccine, some of the tests may be carried out on the final lot rather than on the bulk conjugate where the freeze-drying process may affect the component being tested.

Table 1219.-2. – *Bulk conjugate requirements for currently approved products*



Test	Protein carrier			
	Diphtheria toxoid	**Tetanus toxoid**	**CRM 197**	**OMP**
Free PRP	< 37 %	< 20 %	< 25 %	< 15 %
Free protein	< 4 %	< 1 %, where applicable	< 1 % or < 2 %, depending on the coupling method	not applicable
PRP to protein ratio	1.25 - 1.8	0.30 - 0.55	0.3 - 0.7	0.05 - 0.1
Molecular size (K_d):				
cross-linked agarose for chromatography R	95 % < 0.75	60 % < 0.2	50 % 0.3 - 0.6	85 % < 0.3
cross-linked agarose for chromatography R1	0.6-0.7	85 % < 0.5		

PRP

The PRP content is determined by assay of phosphorus *(2.5.18)* or by assay of ribose *(2.5.31)* or by an immunochemical method *(2.7.1)*.

Protein

The protein content is determined by a suitable chemical method *(for example, 2.5.16)*.

PRP to protein ratio

Determine the ratio by calculation.

Molecular-size distribution

Molecular-size distribution is determined by size-exclusion chromatography *(2.2.30)*.

Free PRP

Unbound PRP is determined after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods.

Free carrier protein

Determine the content by a suitable method, either directly or by deriving the content by calculation from the results of other tests. The amount is within the limits approved for the particular product.

Unreacted functional groups

No unreacted functional groups are detectable in the bulk conjugate unless process validation has shown that unreacted functional groups detectable at this stage are removed during the subsequent manufacturing process (for example, owing to short half-life).

Residual reagents

Removal of residual reagents such as cyanide, EDAC (ethyldimethylaminopropylcarbodiimide) and phenol is confirmed by suitable tests or by validation of the process.

Sterility *(2.6.1)*

Carry out the test using for each medium 10 ml or the equivalent of 100 doses, whichever is less.

FINAL BULK VACCINE

An adjuvant, an antimicrobial preservative and a stabiliser may be added to the bulk conjugate before dilution to the final concentration with a suitable diluent.

Only a final bulk vaccine that complies with the following requirements may be used in preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification and Tests may be released for use. Provided the test for antimicrobial preservative has been carried out on the final bulk vaccine, it may be omitted on the final lot.

pH *(2.2.3)*

The pH of the vaccine, reconstituted if necessary, is within the range approved for the particular product.

Free PRP

Unbound PRP is determined after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

IDENTIFICATION

The vaccine is identified by a suitable immunochemical method *(2.7.1)* for PRP.

TESTS

PRP

Not less than 80 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose *(2.5.31)* or phosphorus *(2.5.18)*, by an immunochemical method *(2.7.1)* or by anion-exchange liquid chromatography with pulsed amperometric detection *(2.2.29)*.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than the minimum amount shown to be effective and not greater than 115 per cent of the quantity stated on the label.

Water *(2.5.12)*

Maximum 3.0 per cent for freeze-dried vaccines.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a quantity of the vaccine equivalent to: 1 µg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 µg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 µg of PRP for a vaccine with OMP as carrier.

LABELLING

The label states:

— the number of micrograms of PRP per human dose,

— the type and nominal amount of carrier protein per single human dose.

Ph Eur

Hepatitis A (Inactivated) and Hepatitis B (rDNA) Vaccine

General Notices

(Hepatitis A (Inactivated) and Hepatitis B (rDNA) Vaccine (Adsorbed), Ph Eur monograph 1526)

The label may state 'HepA/HepB'.

Ph Eur

DEFINITION

Hepatitis A (inactivated) and hepatitis B (rDNA) vaccine (adsorbed) is a suspension consisting of a suitable strain of hepatitis A virus, grown in cell cultures and inactivated by a validated method, and of hepatitis B surface antigen (HBsAg), a component protein of hepatitis B virus obtained by recombinant DNA technology; the antigens are adsorbed on a mineral carrier, such as aluminium hydroxide or hydrated aluminium phosphate.

PRODUCTION

GENERAL PROVISIONS

The two components are prepared as described in the monographs on *Hepatitis A vaccine (inactivated, adsorbed) (1107)* and *Hepatitis B vaccine (rDNA) (1056)* and comply with the requirements prescribed therein.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*).

Reference preparation The reference preparation is part of a representative batch shown to be at least as immunogenic in animals as a batch that, in clinical studies in young healthy adults, produced not less than 95 per cent seroconversion, corresponding to a level of neutralising antibody recognised to be protective, after a full-course primary immunisation. For hepatitis A, an antibody level not less than 20 mIU/ml determined by enzyme-linked immunosorbent assay is recognised as being protective. For hepatitis B, an antibody level not less than 10 mIU/ml against HBsAg is recognised as being protective.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated harvests of hepatitis A virus and one or more batches of purified antigen.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) have been carried out on the final bulk vaccine with satisfactory results, they may be omitted on the final lot. If the assay of the hepatitis A and/or the hepatitis B component is carried out in vivo, then provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

IDENTIFICATION

The vaccine is shown to contain hepatitis A virus antigen and hepatitis B surface antigen by suitable immunochemical methods *(2.7.1)*, using specific antibodies or by the mouse immunogenicity tests described under Assay.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 2 IU per human dose.

ASSAY

Hepatitis A component

The vaccine complies with the assay of hepatitis A vaccine *(2.7.14)*.

Hepatitis B component

The vaccine complies with the assay of hepatitis B vaccine (rDNA) *(2.7.15)*.

LABELLING

The label states:

- — the amount of hepatitis A virus antigen and hepatitis B surface antigen per container,
- — the type of cells used for production of the vaccine,
- — the name and amount of the adsorbent used,
- — that the vaccine must be shaken before use,
- — that the vaccine must not be frozen.

Ph Eur

Hepatitis A Vaccine (Inactivated, Virosome)

General Notices

(Ph Eur monograph 1935)

The label may state 'HepA'.

Ph Eur

DEFINITION

Hepatitis A vaccine (inactivated, virosome) is a suspension of a suitable strain of hepatitis A virus grown in cell cultures and inactivated by a validated method. Virosomes composed of influenza proteins of a strain approved for the particular product and phospholipids are used as adjuvants.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

Reference preparation A reference preparation of inactivated hepatitis A antigen is calibrated against a batch of hepatitis A vaccine (inactivated, virosome) that, in clinical studies in young healthy adults, produced not less than 95 per cent seroconversion, corresponding to a level of neutralising antibody accepted to be protective, after a full-course primary immunisation. An antibody level not less than 20 mIU/ml determined by enzyme-linked immunosorbent assay is recognised as being protective.

PREPARATION OF HEPATITIS A ANTIGEN

Production of the hepatitis A antigen is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability.

Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

SUBSTRATE FOR PROPAGATION OF HEPATITIS A VIRUS

The virus is propagated in a human diploid cell line *(5.2.3)*.

SEED LOTS OF HEPATITIS A VIRUS

The strain of hepatitis A virus used to prepare the master seed lot shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each master and working seed lot is identified as hepatitis A virus using specific antibodies.

Virus concentration

The virus concentration of each master and working seed lot is determined to monitor consistency of production.

Extraneous agents

The master and working seed lots comply with the requirements for seed lots for virus vaccines *(2.6.16)*.

PROPAGATION AND HARVEST OF HEPATITIS A VIRUS

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Animal serum (but not human serum) may be used in the cell culture media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator such as phenol red and antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells). Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Only a single harvest that complies with the following requirements may be used in the preparation of the vaccine. When the determination of the ratio of virus concentration to antigen content has been carried out on a suitable number of single harvests to demonstrate consistency, it may subsequently be omitted as a routine test.

Identification

The test for antigen content also serves to identify the single harvest.

Bacterial and fungal contamination *(2.6.1)*

The single harvest complies with the test for sterility, carried out using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

The single harvest complies with the test for mycoplasmas.

Control cells

The control cells of the production cell culture comply with a test for identity and the requirements for extraneous agents *(2.6.16)*.

Antigen content

Determine the hepatitis A antigen content by a suitable immunochemical method *(2.7.1)* to monitor production consistency; the content is within the limits approved for the particular product.

Ratio of virus concentration to antigen content

The consistency of the ratio of the concentration of infectious virus, as determined by a suitable cell culture method, to antigen content is established by validation on a suitable number of single harvests.

PURIFICATION AND PURIFIED HARVEST OF HEPATITIS A VIRUS

The harvest, which may be a pool of several single harvests, is purified by validated methods. If continuous cell lines are used for production, the purification process shall have been shown to reduce consistently the level of host-cell DNA.

Only a purified harvest that complies with the following requirements may be used in the preparation of the inactivated harvest.

Virus concentration

The concentration of infective virus in the purified harvest is determined by a suitable cell culture method to monitor production consistency and as a starting point for monitoring the inactivation curve.

Ratio of antigen to total protein

Determine the hepatitis A virus antigen content by a suitable immunochemical method *(2.7.1)*. Determine the total protein by a validated method. The ratio of hepatitis A virus antigen content to total protein content is within the limits approved for the particular product.

Bovine serum albumin

Maximum 50 ng per single human dose if foetal bovine serum is used, determined by a suitable immunochemical method *(2.7.1)*. Where appropriate in view of the manufacturing process, other suitable protein markers may be used to demonstrate effective purification.

Residual chemicals

If chemical substances are used during the purification process, tests for these substances are carried out on the purified harvest (or on the inactivated harvest), unless validation of the process has demonstrated total clearance. The concentration must not exceed the limits approved for the particular product.

INACTIVATION AND INACTIVATED HARVEST OF HEPATITIS A VIRUS

Several purified harvests may be pooled before inactivation. In order to avoid interference with the inactivation process, virus aggregation must be prevented or aggregates must be removed immediately before and/or during the inactivation process. The virus suspension is inactivated by a validated method; the method shall have been shown to be consistently capable of inactivating hepatitis A virus without destroying the antigenic and immunogenic activity; for each inactivation procedure, an inactivation curve is plotted representing residual live virus concentration measured on at least 3 occasions (for example, on days 0, 1 and 2 of the inactivation process). If formaldehyde is used for inactivation, the presence of excess free formaldehyde is verified at the end of the inactivation process.

Only an inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Inactivation

Carry out an amplification test for residual infectious hepatitis A virus by inoculating a quantity of the inactivated harvest equivalent to 5 per cent of the batch or, if the harvest contains the equivalent of 30 000 doses or more, not less than 1500 doses of vaccine into cell cultures of the same type as those used for production of the vaccine; incubate for a total of not less than 70 days making not fewer than 1 passage of cells within that period. At the end of the incubation period, carry out a test of suitable sensitivity for residual infectious virus. No evidence of hepatitis A virus multiplication is found in the samples taken at the end of the inactivation process. Use infective virus inocula concurrently as positive controls to demonstrate cellular susceptibility and absence of interference.

Sterility *(2.6.1)*

The inactivated viral harvest complies with the test for sterility, carried out using 10 ml for each medium.

Bacterial endotoxins *(2.6.14)*

Less than 2 IU of endotoxin in the equivalent of a single human dose.

Antigen content

Determine the hepatitis A virus antigen content by a suitable immunochemical method (*2.7.1*).

Residual chemicals

See under Purification and purified harvest.

PREPARATION OF INACTIVATED INFLUENZA VIRUS

The production of influenza viruses is based on a seed-lot system. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final production represents 1 passage from the working seed lot. The strain of influenza virus to be used is approved by the competent authority.

SUBSTRATE FOR PROPAGATION OF INFLUENZA VIRUS

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens *(5.2.2)* or in suitable cell cultures *(5.2.4)*, such as chick-embryo fibroblasts or chick kidney cells obtained from chicken flocks free from specified pathogens *(5.2.2)*. For production, the virus is grown in the allantoic cavity of fertilised hens' eggs from healthy flocks.

SEED LOTS OF INFLUENZA VIRUS

The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

Carry out the test for mycoplasmas, using 10 ml.

PROPAGATION AND HARVEST OF INFLUENZA VIRUS

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form the monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production is penicillin or streptomycin used.

POOLED HARVEST OF INFLUENZA VIRUS

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying antigenicity of haemagglutinin. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at a temperature of 5 ± 3 °C. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of CH_2O at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent *V/V* at any time during inactivation.

Only a pooled harvest that complies with the following requirements may be used in the preparation of the virosomes.

Haemagglutinin antigen

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it. Carry out the test at 20-25 °C.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Viral inactivation

Inoculate 0.2 ml of the harvest into the allantoic cavity of each of 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

Ovalbumin

Maximum 1 µg of ovalbumin in the equivalent of 1 human dose, determined by a suitable technique using a suitable reference preparation of ovalbumin.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Residual chemicals

Tests are carried out on the monovalent pooled harvest for the chemicals used for inactivation, the limits being approved by the competent authority.

PREPARATION OF VIROSOMES

Inactivated influenza virions are solubilised using a suitable detergent and are purified by high-speed centrifugation in order to obtain supernatants containing mainly influenza antigens. After the addition of suitable phospholipids, virosomes are formed by removal of the detergent either by adsorption chromatography or another suitable technique

Only virosomes that comply with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin content

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it.

Phospholipids

The content and identity of the phospholipids are determined by suitable immunochemical or physico-chemical methods.

Ratio of phospholipid to haemagglutinin

The ratio of phospholipid content to haemagglutinin content is within the limits approved for the particular product.

Residual chemicals

Tests are carried out for the chemicals used during the process. The concentration of each residual chemical is within the limits approved for the particular product.

FINAL BULK VACCINE

The bulk vaccine is prepared by adding virosomes to inactivated hepatitis A viruses to yield an approved hepatitis A antigen:haemagglutinin ratio. Several bulks may be pooled, and approved stabilisers and antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Protein content

The amount of protein is determined using a suitable technique, the limits being approved by the competent authorithy.

Phospholipids

The content and identity of the phospholipids are determined by suitable immunochemical or physico-chemical methods. The amount of phospholipids complies with the limits approved for the particular product.

Haemagglutinin content

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*. The amount of haemagglutinin must not exceed the limits approved for the particular product.

Hepatitis A antigen content

Determine the hepatitis A antigen content by a suitable immunochemical method. The amount of antigen must not exceed the limits approved for the particular product.

Ratio of hepatitis A antigen to haemagglutinin

The ratio of hepatitis A antigen content to haemagglutinin content is within the limits approved for the particular product.

Ovalbumin

Maximum 1 µg of ovalbumin per human dose, determined by a suitable technique using a suitable reference preparation of ovalbumin.

Virosome size

The size distribution of the virosome-hepatitis A virus mixture is within the limits approved for the particular product.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Residual chemicals

If chemical substances are used during the formulation process, tests for these substances are carried out, the limits being approved by the competent authorithy.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are then closed so as to avoid contamination.

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) have been carried out on the final bulk vaccine with satisfactory results, these tests may be omitted on the final lot. If the assay is carried out in vivo, provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

IDENTIFICATION

The vaccine is shown to contain hepatitis A virus antigen by a suitable immunochemical method *(2.7.1)* using specific antibodies.

TESTS

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 2 IU of endotoxin per human dose.

ASSAY

Determine the antigen content of the vaccine using a suitable immunochemical method *(2.7.1)* by comparison with the reference preparation. The acceptance criteria are approved for a given reference preparation by the competent authority.

LABELLING

The label states:

- — the biological origin of the cells used for the preparation of the vaccine,
- — that the carrier contains influenza proteins prepared in eggs,
- — that the vaccine is not to be frozen,
- — that the vaccine is to be shaken before use.

Ph Eur

Hepatitis B Vaccine (rDNA)

General Notices

(Ph Eur monograph 1056)

The label may state 'HepB'.

Ph Eur

DEFINITION

Hepatitis B vaccine (rDNA) is a preparation of hepatitis B surface antigen (HBsAg), a component protein of hepatitis B virus; the antigen may be adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. The antigen is obtained by recombinant DNA technology.

PRODUCTION

GENERAL PROVISIONS

The vaccine shall have been shown to induce specific, protective antibodies in man. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity and safety.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

Hepatitis B vaccine (rDNA) is produced by the expression of the viral gene coding for HBsAg in yeast (*Saccharomyces cerevisiae*) or mammalian cells (Chinese hamster ovary (CHO) cells or other suitable cell lines), purification of the resulting HBsAg and the rendering of this antigen into an immunogenic preparation. The suitability and safety of the cells are approved by the competent authority.

The vaccine may contain the product of the S gene (major protein), a combination of the S gene and pre-S2 gene products (middle protein) or a combination of the S gene, the pre-S2 gene and pre-S1 gene products (large protein).

Reference preparation The reference preparation is part of a representative batch shown to be at least as immunogenic in animals as a batch that, in clinical studies in young, healthy adults, produced not less than 95 per cent seroconversion, corresponding to a level of HBsAg neutralising antibody recognised to be protective, after a full-course primary immunisation. An antibody level not less than 10 mIU/ml is recognised as being protective.

CHARACTERISATION OF THE SUBSTANCE

Development studies are carried out to characterise the antigen. The complete protein, lipid and carbohydrate structure of the antigen is established. The morphological characteristics of the antigen particles are established by electron microscopy. The mean buoyant density of the antigen particles is determined by a physico-chemical method, such as gradient centrifugation. The antigenic epitopes are characterised. The protein fraction of the antigen is characterised in terms of the primary structure (for example, by determination of the amino-acid composition, by partial amino-acid sequence analysis and by peptide mapping).

CULTURE AND HARVEST

Identity, microbial purity, plasmid retention and consistency of yield are determined at suitable production stages. If mammalian cells are used, tests for extraneous agents and mycoplasmas are performed in accordance with *Tests for extraneous agents in viral vaccines for human use (2.6.16)*, but using 200 ml of harvest in the test in cell culture for other extraneous agents.

PURIFIED ANTIGEN

Only a purified antigen that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Total protein

The total protein is determined by a validated method. The content is within the limits approved for the specific product.

Antigen content and identification

The quantity and specificity of HBsAg is determined in comparison with the International Standard for HBsAg subtype *ad* or an in-house reference, by a suitable immunochemical method *(2.7.1)* such as radio-immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), immunoblot (preferably using a monoclonal antibody directed against a protective epitope) or single radial diffusion. The antigen/protein ratio is within the limits approved for the specific product.

The molecular weight of the major band revealed following sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) performed under reducing conditions corresponds to the value expected from the known nucleic acid and polypeptide sequences and possible glycosylation.

Antigenic purity

The purity of the antigen is determined by comparison with a reference preparation using liquid chromatography or other suitable methods such as SDS-PAGE with staining by acid blue 92 and silver. A suitable method is sensitive enough to detect a potential contaminant at a concentration of 1 per cent of total protein. Not less than 95 per cent of the total protein consists of hepatitis B surface antigen.

Composition

The content of proteins, lipids, nucleic acids and carbohydrates is determined.

Host-cell- and vector-derived DNA

If mammalian cells are used for production, not more than 10 pg of DNA in the quantity of purified antigen equivalent to a single human dose of vaccine.

Caesium

If a caesium salt is used during production, a test for residual caesium is carried out on the purified antigen. The content is within the limits approved for the specific product.

Sterility *(2.6.1)*

The purified antigen complies with the test for sterility, carried out using 10 ml for each medium.

Additional tests on the purified antigen may be required depending on the production method used: for example, a test for residual animal serum where mammalian cells are used for production or tests for residual chemicals used during extraction and purification.

FINAL BULK VACCINE

An antimicrobial preservative and an adjuvant may be included in the vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) have been carried out on the final bulk vaccine with satisfactory results, they may be omitted on the final lot. If the assay is carried out *in vivo*, then provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

IDENTIFICATION

The assay or, where applicable, the electrophoretic profile, serves also to identify the vaccine.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

Pyrogens *(2.6.8)*

The vaccine complies with the test for pyrogens. Inject the equivalent of one human dose into each rabbit.

ASSAY

The vaccine complies with the assay of hepatitis B vaccine (rDNA) *(2.7.15)*.

LABELLING

The label states:

— the amount of HBsAg per container,

— the type of cells used for production of the vaccine,

— the name and amount of the adsorbent used,

— that the vaccine must be shaken before use,

— that the vaccine must not be frozen.

Ph Eur

Inactivated Hepatitis A Vaccine

General Notices

(Hepatitis A Vaccine (Inactivated, Adsorbed), Ph Eur monograph 1107)

The label may state 'HepA'.

Ph Eur

DEFINITION

Hepatitis A vaccine (inactivated, adsorbed) is a suspension consisting of a suitable strain of hepatitis A virus grown in cell cultures, inactivated by a validated method and adsorbed on a mineral carrier.

PRODUCTION

Production of the vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

Reference preparation A part of a batch shown to be at least as immunogenic in animals as a batch that, in clinical studies in young healthy adults, produced not less than 95 per cent seroconversion, corresponding to a level of neutralising antibody accepted to be protective, after a full-course primary immunisation is used as a reference preparation. An antibody level of 20 mIU/ml determined by enzyme-linked immunosorbent assay is recognised as being protective.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in a human diploid cell line *(5.2.3)* or in a continuous cell line approved by the competent authority.

SEED LOTS

The strain of hepatitis A virus used to prepare the master seed lot shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each master and working seed lot is identified as hepatitis A virus using specific antibodies.

Virus concentration

The virus concentration of each master and working seed lot is determined to monitor consistency of production.

Extraneous agents

The master and working seed lots comply with the requirements for seed lots for virus vaccines *(2.6.16)*. In addition, if primary monkey cells have been used for isolation of the strain, measures are taken to ensure that the strain is not contaminated with simian viruses such as simian immunodeficiency virus and filoviruses.

VIRUS PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled. Animal serum (but not human serum) may be used in the cell culture media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator, such as phenol red, and antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells). Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Only a single harvest that complies with the following requirements may be used in the preparation of the vaccine. When the determination of the ratio of virus concentration to antigen content has been carried out on a suitable number of single harvests to demonstrate production consistency, it may subsequently be omitted as a routine test.

Identification

The test for antigen content also serves to identify the single harvest.

Bacterial and fungal contamination *(2.6.1)*

The single harvest complies with the test for sterility, carried out using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

The single harvest complies with the test for mycoplasmas, carried out using 1 ml for each medium.

Control cells

The control cells of the production cell culture comply with a test for identification and the requirements for extraneous agents *(2.6.16)*.

Antigen content

Determine the hepatitis A antigen content by a suitable immunochemical method (*2.7.1*) to monitor production consistency; the content is within the limits approved for the particular product.

Ratio of virus concentration to antigen content

The consistency of the ratio of the concentration of infectious virus, determined by a suitable cell culture method, to antigen content is established by validation on a suitable number of single harvests.

PURIFICATION AND PURIFIED HARVEST

The harvest, which may be a pool of several single harvests, is purified by validated methods. If continuous cell lines are used for production, the purification process shall have been shown to reduce consistently the level of host-cell DNA.

Only a purified harvest that complies with the following requirements may be used in the preparation of the inactivated harvest.

Virus concentration

The concentration of infectious virus in the purified harvest is determined by a suitable cell culture method to monitor production consistency and as a starting point for monitoring the inactivation curve.

Antigen:total protein ratio

Determine the hepatitis A virus antigen content by a suitable immunochemical method *(2.7.1)*. Determine the total protein by a validated method. The ratio of hepatitis A virus antigen content to total protein content is within the limits approved for the particular product.

Bovine serum albumin

Not more than 50 ng in the equivalent of a single human dose, determined by a suitable immunochemical method *(2.7.1)*. Where appropriate in view of the manufacturing process, other suitable protein markers may be used to demonstrate effective purification.

Residual host-cell DNA

If a continuous cell line is used for virus propagation, the content of residual host-cell DNA, determined using a suitable method, is not greater than 100 pg in the equivalent of a single human dose.

Residual chemicals

If chemical substances are used during the purification process, tests for these substances are carried out on the purified harvest (or on the inactivated harvest), unless validation of the process has demonstrated total clearance. The concentration must not exceed the limits approved for the particular product.

INACTIVATION AND INACTIVATED HARVEST

Several purified harvests may be pooled before inactivation. In order to avoid interference with the inactivation process, virus aggregation must be prevented or aggregates must be removed immediately before and/or during the inactivation process. The virus suspension is inactivated by a validated method; the method shall have been shown to be consistently capable of inactivating hepatitis A virus without destroying the antigenic and immunogenic activity; for each inactivation procedure, an inactivation curve is plotted representing residual live virus concentration measured at not fewer than three points in time (for example, on days 0, 1 and 2 of the inactivation process). If formaldehyde is used for inactivation, the presence of excess free formaldehyde is verified at the end of the inactivation process.

Only an inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Inactivation

Carry out an amplification test for residual infectious hepatitis A virus by inoculating a quantity of the inactivated harvest equivalent to 5 per cent of the batch or, if the harvest contains the equivalent of 30 000 doses or more, not less than 1500 doses of vaccine into cell cultures of the same type as those used for production of the vaccine; incubate for a total of not less than 70 days making not fewer than one passage of cells within that period. At the end of the incubation period, carry out a test of suitable sensitivity for residual infectious virus. No evidence of hepatitis A virus multiplication is found in the samples taken at the end of the inactivation process. Use infectious virus inocula concurrently as positive controls to demonstrate cellular susceptibility and absence of interference. Incubate for a total of not less than 70 days, making not fewer than one passage of cells within that period.

Sterility *(2.6.1)*

The inactivated viral harvest complies with the test for sterility, carried out using 10 ml for each medium.

Bacterial endotoxins *(2.6.14)*

Less than 2 IU in the equivalent of a single human dose.

Antigen content

Determine the hepatitis A virus antigen content by a suitable immunochemical method *(2.7.1)*.

Residual chemicals

See under Purification and purified harvest.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated harvests. Approved adjuvants, stabilisers and antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are then closed so as to avoid contamination.

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) have been carried out on the final bulk vaccine with satisfactory results, these tests may be omitted on the final lot. If the assay is carried out using mice or other animals, then provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

IDENTIFICATION

The vaccine is shown to contain hepatitis A virus antigen by a suitable immunochemical method *(2.7.1)* using specific antibodies or by the in vivo assay *(2.7.14)*.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

The vaccine complies with the assay of hepatitis A vaccine *(2.7.14)*.

LABELLING

The label states the biological origin of the cells used for the preparation of the vaccine.

Ph Eur

Inactivated Influenza Vaccine (Split Virion)

General Notices

(Influenza Vaccine (Split Virion, Inactivated), Ph Eur monograph 0158)

The label may state 'Flu'.

When Inactivated Influenza Vaccine or Influenza Vaccine is prescribed or demanded and the form is not stated, Inactivated Influenza Vaccine (Whole Virion), Inactivated Influenza Vaccine (Split Virion) or Inactivated Influenza Vaccine (Surface Antigen) may be dispensed or supplied.

Ph Eur

DEFINITION

Influenza vaccine (split virion, inactivated) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in fertilised hens' eggs, inactivated and treated so that the integrity of the virus particles has been disrupted without diminishing the antigenic properties of the haemagglutinin and neuraminidase antigens. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount.

The vaccine is a slightly opalescent liquid.

PRODUCTION

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

CHOICE OF VACCINE STRAIN

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends the strains that correspond to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

SUBSTRATE FOR VIRUS PROPAGATION

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) *(5.2.2)* or in suitable cell cultures *(5.2.4)*, such as chick-embryo fibroblasts or chick kidney cells obtained from SPF chicken flocks *(5.2.2)*. For production, the virus of each strain is grown in the allantoic cavity of fertilised hens' eggs from healthy flocks.

VIRUS SEED LOT

The production of vaccine is based on a seed-lot system. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents 1 passage from the working seed lot. The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

Carry out the test for mycoplasmas, using 10 ml.

VIRUS PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production is penicillin or streptomycin used.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process should cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at 5 ± 3 °C. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of CH_2O at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent *V/V* at any time during inactivation.

Before or after the inactivation procedure, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method and the virus particles are disrupted into component subunits by the use of approved procedures. For each new strain, a validation test is carried out to show that the monovalent bulk consists predominantly of disrupted virus particles.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it
[1]. Carry out the test at 20-25 °C.

For some vaccines, the physical form of the haemagglutinin particles prevents quantitative determination by immunodiffusion after inactivation of the virus. For these vaccines, a determination of haemagglutinin antigen is made on the monovalent pooled harvest before inactivation. The production process is validated to demonstrate suitable conservation of haemagglutinin antigen and a suitable tracer is used for formulation, for example, protein content.

Neuraminidase antigen

The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 monovalent pooled harvests from each working seed lot.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Residual infectious virus

Carry out the test described below under Tests.

Chemicals used for disruption

Tests are carried out on the monovalent pooled harvest for the chemicals used for disruption, the limits being approved by the competent authority.

FINAL BULK VACCINE

Appropriate quantities of the monovalent pooled harvests are blended to make the final bulk vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Tests and Assay may be released for use. Provided that the test for residual infectious virus has been performed with satisfactory results on each monovalent pooled harvest and that the tests for free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

IDENTIFICATION

The assay serves to confirm the antigenic specificity of the vaccine.

TESTS

Residual infectious virus

Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l, where applicable.

Ovalbumin

Not more than the quantity stated on the label and in any case not more than 1 µg per human dose, determined by a suitable immunochemical method *(2.7.1)* using a suitable reference preparation of ovalbumin.

Total protein

Not more than 6 times the total haemagglutinin content of the vaccine as determined in the assay, but in any case, not more than 100 µg of protein per virus strain per human dose and not more than a total of 300 µg of protein per human dose.

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 100 IU per human dose.

ASSAY

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it[1]. Carry out the test at 20-25 °C. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated haemagglutinin antigen content. The lower confidence limit (P = 0.95) is not less than 80 per cent of the amount stated on the label for each strain.

For some vaccines, quantitative determination of haemagglutinin antigen with respect to available reference preparations is not possible. An immunological identification of the haemagglutinin antigen and a semi-quantitative determination are carried out instead by suitable methods.

LABELLING

The label states:

- — that the vaccine has been prepared on eggs,
- — the strain or strains of influenza virus used to prepare the vaccine,
- — the method of inactivation,
- — the haemagglutinin content in micrograms per virus strain per dose,
- — the maximum amount of ovalbumin,
- — the season during which the vaccine is intended to protect.

Ph Eur

[1] *Reference haemagglutinin antigens are available from the National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, Great Britain.*

Inactivated Influenza Vaccine (Surface Antigen)

General Notices

(Influenza Vaccine (Surface Antigen, Inactivated), Ph Eur monograph 0869)

The label may state 'Flu' or 'Flu(adj)' as appropriate.

When Inactivated Influenza Vaccine or Influenza Vaccine is prescribed or demanded and the form is not stated, Inactivated Influenza Vaccine (Whole Virion), Inactivated Influenza Vaccine (Split Virion) or Inactivated Influenza Vaccine (Surface Antigen) may be dispensed or supplied.

Ph Eur

DEFINITION

Influenza vaccine (surface antigen, inactivated) is a sterile suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in fertilised hens' eggs, inactivated and treated so that the preparation consists predominantly of haemagglutinin and neuraminidase antigens, without diminishing the antigenic properties of these antigens. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount. The vaccine may contain an adjuvant.

PRODUCTION

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

CHOICE OF VACCINE STRAIN

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends the strains that correspond to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory states of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

SUBSTRATE FOR VIRUS PROPAGATION

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) *(5.2.2)* or in suitable cell cultures *(5.2.4)*, such as chick-embryo fibroblasts or chick kidney cells obtained from SPF chicken flocks *(5.2.2)*. For production, the virus of each strain is grown in the allantoic cavity of fertilised hens' eggs from healthy flocks.

VIRUS SEED LOT

The production of vaccine is based on a seed-lot system. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents one passage from the working seed lot. The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

Carry out the test for mycoplasmas, using 10 ml.

VIRUS PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production is penicillin or streptomycin used.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process should cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at 5 ± 3 °C. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of CH_2O at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent *V/V* at any time during inactivation.

Before or after the inactivation process, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method. Virus particles are disrupted into component subunits by approved procedures and further purified so that the monovalent bulk consists mainly of haemagglutinin and neuraminidase antigens.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it[1]. Carry out the test at 20-25 °C.

Neuraminidase antigen

The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 monovalent pooled harvests from each working seed lot.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Residual infectious virus

Carry out the test described below under Tests.

Purity

The purity of the monovalent pooled harvest is examined by polyacrylamide gel electrophoresis or by other approved techniques. Mainly haemagglutinin and neuraminidase antigens shall be present.

Chemicals used for disruption and purification

Tests are carried out on the monovalent pooled harvest for the chemicals used for disruption and purification, the limits being approved by the competent authority.

FINAL BULK VACCINE

Appropriate quantities of the monovalent pooled harvests are blended to make the final bulk vaccine. An adjuvant may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Tests and Assay may be released for use. Provided that the test for residual infectious virus has been performed with satisfactory results on each monovalent pooled harvest and that the tests for free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

If the ovalbumin and formaldehyde content cannot be determined on the final lot, owing to interference from the adjuvant, they are determined on the monovalent pooled harvest, the acceptance limits being set to ensure that the limits for the final product will not be exceeded.

If the vaccine contains an adjuvant, suitable tests for identity and other relevant quality criteria are carried out on the final lot. These tests may include chemical and physical analysis, determination of particle size and determination of the number of particles per unit volume.

IDENTIFICATION

The assay serves to confirm the antigenic specificity of the vaccine.

TESTS

Residual infectious virus

Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l, where applicable.

Ovalbumin

Not more than the quantity stated on the label and in any case not more than 1 µg per human dose, determined by a suitable immunochemical method *(2.7.1)* using a suitable reference preparation of ovalbumin.

Total protein

Not more than 40 µg of protein other than haemagglutinin per virus strain per human dose and not more than a total of 120 µg of protein other than haemagglutinin per human dose.

Sterility

It complies with the test for sterility *(2.6.1)*.

Bacterial endotoxins *(2.6.14)*

Less than 100 IU per human dose.

ASSAY

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it[1]. Carry out the test at 20-25 °C. The confidence limits (*P* = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated content. The lower confidence limit (*P* = 0.95) haemagglutinin antigen is not less than 80 per cent of the amount stated on the label for each strain.

LABELLING

The label states:

— that the vaccine has been prepared on eggs,

— the strain or strains of influenza virus used to prepare the vaccine,

— the method of inactivation,

— the haemagglutinin content in micrograms per virus strain per dose,

— the season during which the vaccine is intended to protect,

— the maximum amount of ovalbumin,

— where applicable, the name and the quantity of adjuvant used.

Ph Eur

[1] *Reference haemagglutinin antigens are available from the National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, Great Britain.*

Inactivated Influenza Vaccine (Whole Virion)

General Notices

(Influenza Vaccine (Whole Virion, Inactivated), Ph Eur monograph 0159)

The label may state 'Flu'.

When Inactivated Influenza Vaccine or Influenza Vaccine is prescribed or demanded and the form is not stated, Inactivated Influenza Vaccine (Whole Virion), Inactivated Influenza Vaccine (Split Virion) or Inactivated Influenza Vaccine (Surface Antigen) may be dispensed or supplied.

Ph Eur

DEFINITION

Influenza vaccine (whole virion, inactivated) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in fertilised hens' eggs and inactivated in such a manner that their antigenic properties are retained. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount.

The vaccine is a slightly opalescent liquid.

PRODUCTION

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

CHOICE OF VACCINE STRAIN

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends the strains that correspond to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

SUBSTRATE FOR VIRUS PROPAGATION

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) *(5.2.2)* or in suitable cell cultures *(5.2.4)*, such as chick-embryo fibroblasts or chick kidney cells obtained from SPF chicken flocks *(5.2.2)*. For production, the virus of each strain is grown in the allantoic cavity of fertilised hens' eggs from healthy flocks.

VIRUS SEED LOT

The production of vaccine is based on a seed-lot system. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents 1 passage from the working seed lot. The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

Carry out the test for mycoplasmas, using 10 ml.

VIRUS PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production is penicillin or streptomycin used.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process should cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at 5 ± 3 °C. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of CH_2O at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent *V/V* at any time during inactivation.

Before or after the inactivation process, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it
[1]. Carry out the test at 20-25 °C.

Neuraminidase antigen

The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 monovalent pooled harvests from each working seed lot.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Residual infectious virus

Carry out the test described below under Tests.

FINAL BULK VACCINE

Appropriate quantities of the monovalent pooled harvests are blended to make the final bulk vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Tests and Assay may be released for use. Provided that the test for residual infectious virus has been performed with satisfactory results on each monovalent pooled harvest and that the tests for free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

IDENTIFICATION

The assay serves to confirm the antigenic specificity of the vaccine.

TESTS

Residual infectious virus

Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l, where applicable.

Ovalbumin

Not more than the quantity stated on the label and in any case not more than 1 µg per human dose, determined by a suitable immunochemical method *(2.7.1)* using a suitable reference preparation of ovalbumin.

Total protein

Not more than 6 times the total haemagglutinin content of the vaccine as determined in the assay, but in any case, not more than 100 µg of protein per virus strain per human dose and not more than a total of 300 µg of protein per human dose.

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 100 IU per human dose.

ASSAY

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it[1]. Carry out the test at 20-25 °C. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated haemagglutinin antigen content. The lower confidence limit (P = 0.95) is not less than 80 per cent of the amount stated on the label for each strain.

LABELLING

The label states:

— that the vaccine has been prepared on eggs,

— the strain or strains of influenza virus used to prepare the vaccine,

— the method of inactivation,

— the haemagglutinin content in micrograms per virus strain per dose,

— the maximum amount of ovalbumin,

— the season during which the vaccine is intended to protect.

Ph Eur

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[1] *Reference haemagglutinin antigens are available from the National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, Great Britain.*

Inactivated Poliomyelitis Vaccine

General Notices

(Poliomyelitis Vaccine (Inactivated), Ph Eur monograph 0214)

The label may state 'IPV'.

Ph Eur

DEFINITION

Poliomyelitis vaccine (inactivated) is a liquid preparation of suitable strains of human poliovirus types 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method. It is a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The production method shall have been shown to yield consistently vaccines of acceptable safety and immunogenicity in man.

Production of the vaccine is based on a virus seed-lot system. Cell lines are used according to a cell-bank system. If primary, secondary or tertiary monkey kidney cells are used, production complies with the requirements indicated below.

Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more passages from the master seed lot than was used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in a human diploid cell line *(5.2.3)*, in a continuous cell line *(5.2.3)* or in primary, secondary or tertiary monkey kidney cells.

Primary, secondary or tertiary monkey kidney cells

The following special requirements for the substrate for virus propagation apply to primary, secondary or tertiary monkey kidney cells.

Monkeys used in the preparation of kidney cell cultures for production and control of the vaccine The animals used are of a species approved by the competent authority, in good health and, unless otherwise justified and authorised, have not been previously employed for experimental purposes. Kidney cells used for vaccine production and control are derived from monitored, closed colonies of monkeys bred in captivity, not from animals caught in the wild; a previously approved seed lot prepared using virus passaged in cells from wild monkeys may, subject to approval by the competent authority, be used for vaccine production if historical data on safety justify this.

Monitored, closed colonies of monkeys The monkeys are kept in groups in cages. Freedom from extraneous agents is achieved by the use of animals maintained in closed colonies that are subject to continuous and systematic veterinary and laboratory monitoring for the presence of infectious agents. The supplier of animals is certified by the competent authority. Each monkey is tested serologically at regular intervals during a quarantine period of not less than 6 weeks imposed before entering the colony, and then during its stay in the colony.

The monkeys used are shown to be tuberculin-negative and free from antibodies to simian virus 40 (SV40) and simian immunodeficiency virus. The blood sample used in testing for SV40 antibodies must be taken as close as possible to the time of removal of the kidneys. If *Macaca* sp. monkeys are used for production, the monkeys are also shown to be free from antibodies to herpesvirus B (cercopithecine herpesvirus 1) infection. Human herpesvirus 1 has been used as an indicator for freedom from herpesvirus B antibodies on account of the danger of handling herpesvirus B (cercopithecine herpesvirus 1).

Monkeys from which kidneys are to be removed are thoroughly examined, particularly for evidence of tuberculosis and herpesvirus B (cercopithecine herpesvirus 1) infection. If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of a seed lot or vaccine, it is not to be used nor are any of the remaining monkeys of the group concerned unless it is evident that their use will not impair the safety of the product.

All the operations described in this section are conducted outside the area where the vaccine is produced.

Monkey cell cultures for vaccine production Kidneys that show no pathological signs are used for preparing cell cultures. Each group of cell cultures derived from a single monkey forms a separate production cell culture giving rise to a separate single harvest.

The primary monkey kidney cell suspension complies with the test for mycobacteria *(2.6.2)*; disrupt the cells before carrying out the test.

If secondary or tertiary cells are used, it shall be demonstrated by suitable validation tests that cell cultures beyond the passage level used for production are free from tumorigenicity.

SEED LOTS

Each of the 3 strains of poliovirus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Only a working seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each working seed lot is identified as human poliovirus types 1, 2 or 3 by virus neutralisation in cell cultures using specific antibodies.

Virus concentration

The virus concentration of each working seed lot is determined to define the quantity of virus to be used for inoculation of production cell cultures.

Extraneous agents

The working seed lot complies with the requirements for seed lots for virus vaccines (*2.6.16*). In addition, if primary, secondary or tertiary monkey kidney cells have been used for isolation of the strain, measures are taken to ensure that the strain is not contaminated with simian viruses such as simian immunodeficiency virus, simian virus 40, filoviruses and herpesvirus B (cercopithecine herpesvirus 1). A working seed lot produced in primary, secondary or tertiary monkey kidney cells complies with the requirements given below under Virus propagation and harvest for single harvests produced in such cells.

PROPAGATION AND HARVEST

All processing of the cell bank and cell cultures is done under aseptic conditions in an area where no other cells or viruses are being handled. Approved animal serum (but not human serum) may be used in the cell culture media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells); where continuous cell lines in a fermenter are used for production, 200×10^6 cells are set aside to prepare control cells; where primary, secondary or tertiary monkey kidney cells are used for production, a cell sample equivalent to at least 500 ml of the cell suspension, at the concentration employed for vaccine production, is taken to prepare control cells.

Only a single harvest that complies with the following requirements may be used in the preparation of the vaccine. The tests for identification and bacterial and fungal contamination may be carried out instead on the purified, pooled monovalent harvest. After demonstration of consistency of production at the stage of the single harvest, the test for virus concentration may be carried out instead on the purified, pooled monovalent harvest.

Control cells

The control cells of the production cell culture comply with a test for identification (if a cell-bank system is used for production) and with the requirements for extraneous agents (*2.6.16*; where primary, secondary or tertiary monkey kidney cells are used, the tests in cell cultures are carried out as shown below under Test in rabbit kidney cell cultures and Test in cercopithecus kidney cell cultures).

Test in rabbit kidney cell cultures Test a sample of at least 10 ml of the pooled supernatant fluid from the control cultures for the absence of herpesvirus B (cercopithecine herpesvirus 1) and other viruses by inoculation onto rabbit kidney cell cultures. The dilution of supernatant in the nutrient medium is not greater than 1/4 and the area of the cell layer is at least 3 cm^2 per millilitre of inoculum. Set aside one or more containers of each batch of cells with the same medium as non-inoculated control cells. Incubate the cultures at 37 °C and observe for at least 2 weeks. The test is not valid if more than 20 per cent of the control cell cultures are discarded for non-specific, accidental reasons.

Test in cercopithecus kidney cell cultures Test a sample of at least 10 ml of the pooled supernatant fluid from the control cultures for the absence of SV40 virus and other extraneous agents by inoculation onto cell cultures prepared from the kidneys of cercopithecus monkeys, or other cells shown to be at least as sensitive for SV40, by the method described under Test in rabbit kidney cell cultures. The test is not valid if more than 20 per cent of the control cell cultures are discarded for non-specific, accidental reasons.

Identification

The single harvest is identified as containing human poliovirus types 1, 2 or 3 by virus neutralisation in cell cultures using specific antibodies.

Virus concentration

The virus concentration of each single harvest is determined by titration of infectious virus in cell cultures.

Bacterial and fungal contamination

The single harvest complies with the test for sterility *(2.6.1)*, carried out using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

The single harvest complies with the test for mycoplasmas, carried out using 10 ml.

Test in rabbit kidney cell cultures

Where primary, secondary or tertiary monkey kidney cells are used for production, test a sample of at least 10 ml of the single harvest for the absence of herpesvirus B (cercopithecine herpesvirus 1) and other viruses by inoculation onto rabbit kidney cell cultures as described above for the control cells.

Test in cercopithecus kidney cell cultures

Where primary, secondary or tertiary monkey kidney cells are used for production, test a sample of at least 10 ml of the single harvest for the absence of SV40 virus and other extraneous agents. Neutralise the sample by a high-titre antiserum against the specific type of poliovirus. Test the sample in primary cercopithecus kidney cell cultures or cells that have been demonstrated to be at least as susceptible for SV40. Incubate the cultures at 37 °C and observe for 14 days. At the end of this period, make at least one subculture of fluid in the same cell culture system and observe both primary cultures and subcultures for an additional 14 days.

PURIFICATION AND PURIFIED MONOVALENT HARVEST

Several single harvests of the same type may be pooled and may be concentrated. The monovalent harvest or pooled monovalent harvest is purified by validated methods. If continuous cell lines are used for production, the purification process shall have been shown to reduce consistently the content of substrate-cell DNA to not more than 100 pg per single human dose.

Only a purified monovalent harvest that complies with the following requirements may be used for the preparation of the inactivated monovalent harvest.

Identification

The virus is identified by virus neutralisation in cell cultures using specific antibodies or by determination of D-antigen.

Virus concentration

The virus concentration is determined by titration of infectious virus.

Specific activity

The ratio of the virus concentration or the D-antigen content, determined by a suitable immunochemical method *(2.7.1)*, to the total protein content (specific activity) of the purified monovalent harvest is within the limits approved for the particular product.

INACTIVATION AND INACTIVATED MONOVALENT HARVEST

Several purified monovalent harvests of the same type may be mixed before inactivation. To avoid failures in inactivation caused by the presence of virus aggregates, filtration is carried out before and during inactivation; inactivation is started within a suitable period, preferably not more than 24 h and in any case not more than 72 h, of the prior filtration. The virus suspension is inactivated by a validated method that has been shown to inactivate poliovirus without destruction of immunogenicity; during validation studies, an inactivation curve with at least 4 points (for example, time 0 h, 24 h, 48 h and 96 h) is established showing the decrease in concentration of live virus with time. If formaldehyde is used for inactivation, the presence of an excess of formaldehyde at the end of the inactivation period is verified.

Only an inactivated monovalent harvest that complies with the following requirements may be used in the preparation of a trivalent pool of inactivated monovalent harvests or a final bulk vaccine.

Test for effective inactivation

After neutralisation of the formaldehyde with sodium bisulphite (where applicable), verify the absence of residual live poliovirus by inoculation on suitable cell cultures of 2 samples of each inactivated monovalent harvest, corresponding to at least 1500 human doses. Take one sample not later than 3/4 of the way through the inactivation period and the other at the end. Inoculate the samples in cell cultures such that the dilution of vaccine in the nutrient medium is not greater than 1/4 and the area of the cell layer is at least 3 cm2 per millilitre of inoculum. Set aside one or more containers with the same medium as non-inoculated control cells. Observe the cell cultures for at least 3 weeks. Make not fewer than 2 passages from each container, one at the end of the observation period and the other 1 week before; for the passages, use cell culture supernatant and inoculate as for the initial sample. Observe the subcultures for at least 2 weeks. No sign of poliovirus multiplication is present in the cell cultures. At the end of the observation period, test the susceptibility of the cell culture used by inoculation of live poliovirus of the same type as that present in the inactivated monovalent harvest.

Sterility *(2.6.1)*

The inactivated monovalent harvest complies with the test for sterility, carried out using 10 ml for each medium.

D-antigen content

The content of D-antigen determined by a suitable immunochemical method *(2.7.1)* is within the limits approved for the particular preparation.

FINAL BULK VACCINE

The final bulk vaccine is prepared directly from the inactivated monovalent harvests of human poliovirus types 1, 2 and 3 or from a trivalent pool of inactivated monovalent harvests. If a trivalent pool of inactivated monovalent harvests is used, a test for effective inactivation is carried out on this pool instead of on the final bulk vaccine. A suitable stabiliser and a suitable antimicrobial preservative may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physicochemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Inactivation

Before addition of any antimicrobial preservative, a sample of at least 1500 ml or, for a purified and concentrated vaccine, the equivalent of 1500 doses is tested for residual live poliovirus in cell cultures, as described for the inactivated monovalent harvest. If the final bulk vaccine is prepared from a trivalent pool of inactivated monovalent harvests, the test for inactivation is carried out on that pool rather than on the final bulk vaccine.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde and antimicrobial preservative and the in vivo assay have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

The *in vivo* assay may be omitted once it has been demonstrated for a given product and for each poliovirus type that the acceptance criteria for the D-antigen determination are such that it yields the same result as the *in vivo* assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary, for example by heat treatment or other means of diminishing the immunogenic activity. Where there is a significant change in the manufacturing process of the antigens or their formulation, any impact on the *in vivo* and *in vitro* assays must be evaluated, and the need for revalidation considered.

Provided that the test for bovine serum albumin has been performed with satisfactory results on the trivalent pool of inactivated monovalent harvests or on the final bulk vaccine, it may be omitted on the final lot.

IDENTIFICATION

The vaccine is shown to contain human poliovirus types 1, 2 and 3 by a suitable immunochemical method *(2.7.1)* such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

TESTS

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physicochemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Protein nitrogen content *(Lowry method)*

Maximum 10 µg per single human dose.

Bovine serum albumin

Maximum 50 ng per single human dose, determined by a suitable immunochemical method *(2.7.1)*.

Sterility *(2.6.1)*

It complies with the test.

Bacterial endotoxins *(2.6.14)*

Less than 5 IU per single human dose.

ASSAY

D-antigen content

As a measure of consistency of production, determine the D-antigen content for human poliovirus types 1, 2 and 3 by a suitable immunochemical method (*2.7.1*) using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) BRP* is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In vivo test

The vaccine complies with the *in vivo* assay of poliomyelitis vaccine (inactivated) *(2.7.20)*.

LABELLING

The label states:

— the types of poliovirus contained in the vaccine;

— the nominal amount of virus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose;

— the cell substrate used to prepare the vaccine.

Ph Eur

Influenza Vaccine (Surface Antigen, Inactivated, Prepared in Cell Cultures)

General Notices

(Ph Eur monograph 2149)

The label may state 'Flu' or 'Flu(adj)' as appropriate.

Ph Eur

DEFINITION

Influenza vaccine (surface antigen, inactivated, prepared in cell cultures) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in cell cultures, inactivated and treated so that the preparation consists predominantly of haemagglutinin and neuraminidase antigens, preserving adequate antigenic properties of these antigens. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount. The vaccine is a clear or slightly opalescent liquid. The vaccine may contain an adjuvant. This monograph applies to vaccines produced in diploid or continuous cell lines of mammalian origin.

PRODUCTION

GENERAL PROVISIONS

Production of the vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

The production method is validated to demonstrate suitable reduction of residual host-cell protein. With the agreement of the competent authority and for each specific product, routine testing for residual host-cell proteins may be omitted based on the results of validation studies for the product. Guidance on the principles of such validation studies is given, for example, in the monograph on *Products of recombinant DNA technology (0784),* in particular in the chapters on "Validation of the production process - extraction and purification" and "Production consistency - Host-cell-derived proteins".

CHOICE OF VACCINE STRAIN

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends new strains corresponding to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory states of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

SUBSTRATE FOR VIRUS PROPAGATION

Influenza virus used in the preparation of seed lots is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) *(5.2.2)* or in suitable cell cultures *(5.2.3)*, such as chick-embryo fibroblasts, chick kidney cells obtained from SPF chicken flocks *(5.2.2)*, or a diploid or continuous cell line. The final passage for establishment of the working seed lot is prepared in the cell line used for routine production. For this production, the virus of each strain is propagated in a diploid or continuous cell line *(5.2.3)*.

VIRUS SEED LOT

The production of vaccine is based on a seed-lot system. Each of the strains of influenza virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents 1 passage from the working seed lot.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

The haemagglutinin and neuraminidase antigens of each master and working seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Virus concentration

The virus concentration of each working seed lot is determined. Where applicable, the virus concentration of each master seed lot is determined.

Extraneous agents *(2.6.16)*

The working seed lots comply with the requirements for seed lots. It is recognised that when a vaccine strain changes, there may be time constraints that make testing a virus seed for extraneous agents problematic, and the results of such testing may not be completely available before further processing. Rapid assays (e.g. multiplex PCR) are applied in parallel wherever possible.

If an agent is detected in a virus seed and the mammalian cells used for production are shown to be susceptible to this agent, the virus seed is not used for vaccine production.

If an agent is detected in a virus seed and the mammalian cells are not susceptible to the agent, validation of the production process to demonstrate removal or inactivation of the agent is carried out. If removal or inactivation cannot be demonstrated, the inactivated monovalent harvest is tested to demonstrate absence of any contaminant identified in the virus seed.

PROPAGATION AND SINGLE HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled at the same time. Animal serum (but no human serum) may be used in the cell culture media. Serum and trypsin used in the preparation of cell suspensions or media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator, such as phenol red, and antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells).

Only a single harvest that complies with the following requirements may be used in the preparation of the vaccine.

Identification

The test for antigen content also serves to identify the single harvest.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

Carry out the test for mycoplasmas, using 10 ml for each medium.

Control cells

The control cells of the production cell culture comply with a test for identification and the requirements for extraneous agents *(2.6.16)*.

Haemagglutinin antigen

Determine the haemagglutinin antigen content by a suitable immunochemical method *(2.7.1)*.

INACTIVATED AND PURIFIED MONOVALENT HARVEST

The harvest, which may be a pool of several single harvests of the same strain, is inactivated and purified by validated methods. Before or after the inactivation process, the monovalent harvest is concentrated and purified by high-speed centrifugation or another suitable method. The influenza virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process is designed so as to cause minimum alteration of the haemagglutinin and neuraminidase antigens.

Virus particles are disrupted into component subunits by approved procedures and further purified so that the monovalent bulk consists mainly of haemagglutinin and neuraminidase antigens.

If continuous cell lines are used for production, the purification process shall have been validated to reduce consistently host-cell DNA to a suitable level.

Only an inactivated, purified monovalent harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen

Determine the haemagglutinin antigen content by a suitable immunochemical method *(2.7.1).*

Antigen: total protein ratio

Determine the haemagglutinin antigen content by a suitable immunodiffusion test. Determine the total protein by a validated method. The ratio of haemagglutinin antigen content to total protein content is within the limits approved for the particular product.

Neuraminidase antigen

The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 monovalent harvests from each working seed lot.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Residual infectious virus

Carry out the test described below under Tests.

Purity

The purity of the monovalent harvest is examined by polyacrylamide gel electrophoresis or by other approved techniques. Mainly haemagglutinin and neuraminidase antigens are present.

Chemicals used for disruption and purification. Tests are carried out on the monovalent harvest for the chemicals used for disruption and purification, unless validation of the process has demonstrated total clearance. The concentration must not exceed the limits approved by the competent authority for the particular product.

FINAL BULK VACCINE

Appropriate quantities of the inactivated purified monovalent pooled harvests are blended to make the final bulk vaccine. An adjuvant may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Residual host-cell DNA

If a continuous cell line is used for virus propagation, the content of residual host-cell DNA, determined using a suitable method, is not greater than 10 ng in the equivalent of a single human dose.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Tests and Assay may be released for use. Provided that the test for residual infectious virus has been performed with satisfactory results on each inactivated and purified monovalent harvest and that the tests for free formaldehyde, bovine serum albumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

If the vaccine contains an adjuvant, suitable tests for identity and other relevant quality criteria are carried out on the final lot. These tests may include chemical and physical analysis, determination of particle size and determination of the number of particles per unit volume.

IDENTIFICATION

The assay serves to confirm the antigenic specificity of the vaccine.

TESTS

Residual infectious virus

Carry out an amplification test for residual infectious influenza virus by inoculating not less than 0.2 ml of the vaccine into cell cultures of the same type as used for production of the vaccine; incubate for not less than 4 days at 37 °C. Inoculate not less than 0.2 ml of the cell culture harvested medium into a new semiconfluent cell culture and incubate as before. At the end of the incubation period, examine for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage on cell cultures and test for haemagglutination; no haemagglutination occurs.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l, where applicable.

Bovine serum albumin

Maximum 50 ng per human dose, determined by a suitable immunochemical method *(2.7.1)*.

Total protein

Maximum 40 µg of protein other than haemagglutinin per virus strain per human dose.

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 25 IU per human dose.

ASSAY

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation [1] or with an antigen preparation calibrated against it. Carry out the test at 20-25 °C. The confidence limits (*P* = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated content. The lower confidence limit (*P* = 0.95) is not less than 80 per cent of the amount stated on the label for each strain.

LABELLING

The label states:

- — the biological origin of the cells used for the preparation of the vaccine;
- — the strain or strains of influenza virus used to prepare the vaccine;

— the method of inactivation;

— the haemagglutinin antigen content in micrograms per virus strain per dose;

— the season during which the vaccine is intended to protect;

— where applicable, the name and the quantity of adjuvant used.

Ph Eur

[1] *Reference haemagglutinin antigens are available from the National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, Great Britain.*

Influenza Vaccine (Surface Antigen, Inactivated, Virosome)

General Notices

(Ph Eur monograph 2053)

The label may state 'Flu'.

Ph Eur

DEFINITION

Influenza vaccine (surface antigen, inactivated, virosome) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in fertilised hens' eggs, inactivated and treated so that the preparation consists predominantly of haemagglutinin and neuraminidase antigens reconstituted to virosomes and without diminishing the antigenic properties of the antigens. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount.

The vaccine is a slightly opalescent liquid.

PRODUCTION

GENERAL PROVISIONS

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

CHOICE OF VACCINE STRAIN

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends the strains that correspond to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory states of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

SUBSTRATE FOR VIRUS PROPAGATION

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) *(5.2.2)* or in suitable cell cultures *(5.2.4)*, such as chick-embryo fibroblasts or chick kidney cells obtained from SPF chicken flocks *(5.2.2)*. For production, the virus of each strain is grown in the allantoic cavity of fertilised hens' eggs from healthy flocks.

VIRUS SEED LOT

The production of vaccine is based on a seed lot system. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents 1 passage from the working seed lot. The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

Carry out the test for mycoplasmas, using 10 ml.

VIRUS PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process is designed so as to cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at a temperature of 5 ± 3 °C. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of CH_2O at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent *V/V* at any time during inactivation.

Before or after the inactivation process, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or another suitable method.

Only a monovalent pooled harvest that complies with the following requirements may be used for the preparation of virosomes.

Provided the tests for haemagglutinin antigen, neuraminidase antigen and residual infectious virus have been carried out with satisfactory results on the monovalent virosomal preparation, they may be omitted on the monovalent pooled harvest when the manufacturing process is continuous between the monovalent pooled harvest and the monovalent virosomal preparation.

Haemagglutinin antigen

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation
[1] or with an antigen preparation calibrated against it. Carry out the test at 20-25 °C.

Neuraminidase antigen

The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 monovalent pooled harvests from each working seed lot.

Residual infectious virus

Carry out the test described under Tests.

PREPARATION OF MONOVALENT VIROSOMES

Virus particles are disrupted into component subunits by approved procedures and further purified so that the monovalent bulk consists mainly of haemagglutinin and neuraminidase antigens. Additional phospholipids may be added and virosomes may be formed by removal of the detergent either by adsorption chromatography or another suitable technique. Several monovalent virosomal preparations may be pooled.

Only a monovalent virosomal preparation that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation[1] or with an antigen preparation calibrated against it. Carry out the test at 20-25 °C.

Neuraminidase antigen

The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 virosomal preparations from each working seed lot.

Residual infectious virus

Carry out the test described under Tests. Provided this test has been carried out with satisfactory results on the monovalent pooled harvest, it may be omitted on the preparation of monovalent virosomes.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Purity

The purity of the monovalent virosomal preparation is examined by polyacrylamide gel electrophoresis *(2.2.31)* or by other approved techniques. Mainly haemagglutinin and neuraminidase antigens are present.

Chemicals used for disruption and purification

Tests for the chemicals used for disruption and purification are carried out on the monovalent virosomal preparation, the limits being approved by the competent authority.

Phospholipids

The content and identity of the phospholipids are determined by suitable immunochemical or physico-chemical methods.

Ratio of haemagglutinin to phospholipid

The ratio of haemagglutinin content to phospholipid content is within the limits approved for the particular product.

Virosome size

The average virosome diameter, determined by a suitable method such as photon-correlation spectroscopy, is not less than 100 nm and not greater than 300 nm. The polydispersity index is not greater than 0.4.

FINAL BULK VACCINE

Appropriate quantities of the monovalent virosomal preparations are blended to make the final bulk vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given under Tests and Assay may be released for use. Provided that the test for residual infectious virus has been performed with satisfactory results on each monovalent pooled harvest or, where appropriate, on the monovalent virosomal preparations, and that the tests for phospholipids, ratio of haemagglutinin to phospholipid, free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

IDENTIFICATION

The assay serves to confirm the antigenic specificity of the vaccine.

TESTS

Residual infectious virus

Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further 10 fertilised eggs and incubate at 33-37 °C for 3 days. The test is not valid unless at least 8 of the 10 embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

pH *(2.2.3)*

6.5 to 7.8

Phospholipids

The content and identity of the phospholipids is determined by a suitable immunochemical or physico-chemical method.

Ratio of haemagglutinin to phospholipid

The ratio of haemagglutinin content to phospholipid content is within the limits approved for the particular product.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l, where applicable.

Ovalbumin

Not more than the quantity stated on the label and in any case not more than 1 µg per human dose, determined by a suitable immunochemical method *(2.7.1)* using a suitable reference preparation of ovalbumin.

Total protein

Not more than 40 µg of protein other than haemagglutinin per virus strain per human dose, and not more than a total of 120 µg of protein other than hemagglutinin per human dose.

Sterility *(2.6.1)*

It complies with the test for sterility.

Virosome size

The average virosome diameter, determined by a suitable method such as photon-correlation spectroscopy, is not less than 100 nm and not greater than 300 nm. The polydispersity index is not greater than 0.4.

Bacterial endotoxins *(2.6.14)*

Less than 100 IU per human dose.

ASSAY

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation[1] or with an antigen preparation calibrated against it. Carry out the test at 20-25 °C. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated haemagglutinin antigen content. The lower confidence limit (P = 0.95) is not less than 80 per cent of the amount stated on the label for each strain.

LABELLING

The label states:

— that the vaccine has been prepared on eggs;

— the strain or strains of influenza virus used to prepare the vaccine;

— the method of inactivation;

— the haemagglutinin content, in micrograms per virus strain per dose;

— the maximum amount of ovalbumin;

— the season during which the vaccine is intended to protect.

Ph Eur

[1] *Reference haemagglutinin antigens are available from the National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, Great Britain.*

Influenza Vaccine (Whole Virion, Inactivated, Prepared in Cell Cultures)

General Notices

(Ph Eur monograph 2308)

The label may state 'Flu' or 'Flu(adj)' as appropriate.

Ph Eur

DEFINITION

Influenza vaccine (whole virion, inactivated, prepared in cell cultures) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in cell cultures and inactivated in such a manner that their antigenic properties are retained. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount. The vaccine is a slightly opalescent or opalescent liquid. The vaccine may contain an adjuvant. This monograph applies to vaccines produced in diploid or continuous cell lines of mammalian origin.

PRODUCTION

GENERAL PROVISIONS

Production of the vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

The production method is validated to demonstrate suitable reduction of residual host-cell protein. With the agreement of the competent authority and for each specific product, routine testing for residual host-cell proteins may be omitted based on the results of validation studies for the product. Guidance on the principles of such validation studies is given, for example, in the monograph on *Products of recombinant DNA technology (0784)*, in particular in the chapters on "Validation of the production process - extraction and purification" and "Production consistency - Host-cell-derived proteins".

CHOICE OF VACCINE STRAIN

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends new strains corresponding to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory states of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

SUBSTRATE FOR VIRUS PROPAGATION

Influenza virus used in the preparation of seed lots is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) *(5.2.2)* or in suitable cell cultures *(5.2.3)*, such as chick-embryo fibroblasts, chick kidney cells obtained from SPF chicken flocks *(5.2.2)*, or a diploid or continuous cell line. The final passage for establishment of the working seed lot is prepared in the cell line used for routine production. For this production, the virus of each strain is propagated in a diploid or continuous cell line *(5.2.3)*.

VIRUS SEED LOT

The production of vaccine is based on a seed-lot system. Each of the strains of influenza virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents 1 passage from the working seed lot.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

The haemagglutinin and neuraminidase antigens of each master and working seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Virus concentration

The virus concentration of each working seed lot is determined. Where applicable, the virus concentration of each master seed lot is determined.

Extraneous agents *(2.6.16)*

The working seed lots comply with the requirements for seed lots. It is recognised that when a vaccine strain changes, there may be time constraints that make testing a virus seed for extraneous agents problematic, and the results of such testing may not be completely available before further processing. Rapid assays (e.g. multiplex PCR) are applied in parallel wherever possible.

If an agent is detected in a virus seed and the mammalian cells used for production are shown to be susceptible to this agent, the virus seed is not used for vaccine production.

If an agent is detected in a virus seed and the mammalian cells are not susceptible to the agent, validation of the production process to demonstrate removal or inactivation of the agent is carried out. If removal or inactivation cannot be demonstrated, the inactivated monovalent harvest is tested to demonstrate absence of any contaminant identified in the virus seed.

PROPAGATION AND SINGLE HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled at the same time. Animal serum (but no human serum) may be used in the cell culture media. Serum and trypsin used in the preparation of cell suspensions or media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator, such as phenol red, and antibiotics at the lowest effective concentration. A sufficient quantity of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells).

Only a single harvest that complies with the following requirements may be used in the preparation of the vaccine.

Identification

The test for antigen content also serves to identify the single harvest.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)*, using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

Carry out the test for mycoplasmas, using 10 ml for each medium.

Control cells

The control cells of the production cell culture comply with a test for identification and the requirements for extraneous agents *(2.6.16)*.

Haemagglutinin antigen

Determine the haemagglutinin antigen content by a suitable immunochemical method *(2.7.1)*.

INACTIVATED AND PURIFIED MONOVALENT HARVEST

The harvest, which may be a pool of several single harvests of the same strain, is inactivated and purified by validated methods. Before or after the inactivation process, the monovalent harvest is concentrated and purified by high-speed centrifugation or another suitable method. The influenza virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process is designed so as to cause minimum alteration of the haemagglutinin and neuraminidase antigens.

If continuous cell lines are used for production, the purification process shall have been validated to reduce consistently host-cell DNA to a suitable level.

Only an inactivated, purified monovalent harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen

Determine the haemagglutinin antigen content by a suitable immunochemical method *(2.7.1)*.

Antigen: total protein ratio

Determine the haemagglutinin antigen content by a suitable immunodiffusion test. Determine the total protein by a validated method. The ratio of haemagglutinin antigen content to total protein content is within the limits approved for the particular product.

Neuraminidase antigen

The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 monovalent harvests from each working seed lot.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Residual infectious virus

Carry out the test described below under Tests.

FINAL BULK VACCINE

Appropriate quantities of the inactivated purified monovalent pooled harvests are blended to make the final bulk vaccine. An adjuvant may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility, using 10 ml for each medium.

Residual host-cell DNA

If a continuous cell line is used for virus propagation, the content of residual host-cell DNA, determined using a suitable method, is not greater than 10 ng in the equivalent of a single human dose.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Tests and Assay may be released for use. Provided that the test for residual infectious virus has been performed with satisfactory results on each inactivated and purified monovalent harvest and that the tests for free formaldehyde, bovine serum albumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

If the vaccine contains an adjuvant, suitable tests for identity and other relevant quality criteria are carried out on the final lot. These tests may include chemical and physical analysis, determination of particle size and determination of the number of particles per unit volume.

IDENTIFICATION

The assay serves to confirm the antigenic specificity of the vaccine.

TESTS

Residual infectious virus

Carry out an amplification test for residual infectious influenza virus by inoculating not less than 4 ml of the vaccine into cell cultures of the same type as used for production of the vaccine; incubate for not less than 7 days at 32 ± 2 °C. Inoculate not less than 10 ml of the cell culture harvested medium into a new cell culture and incubate as before. At the end of the incubation period, examine for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage on cell cultures and test for haemagglutination; no haemagglutination occurs.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l, where applicable.

Bovine serum albumin

Maximum 50 ng per human dose, determined by a suitable immunochemical method *(2.7.1)*.

Total protein

Not more than 6 times the total haemagglutinin content of the vaccine as determined in the assay, but in any case, not more than 100 µg of protein per virus strain per human dose.

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 25 IU per human dose.

ASSAY

Determine the content of haemagglutinin antigen by an immunodiffusion test *(2.7.1)*, by comparison with a haemagglutinin antigen reference preparation
[1] or with an antigen preparation calibrated against it. Carry out the test at 20-25 °C. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated content. The lower confidence limit (P = 0.95) is not less than 80 per cent of the amount stated on the label for each strain.

LABELLING

The label states:

— the biological origin of the cells used for the preparation of the vaccine;

— the strain or strains of influenza virus used to prepare the vaccine;

— the method of inactivation;

— the haemagglutinin antigen content in micrograms per virus strain per dose;

— the season during which the vaccine is intended to protect;

— where applicable, the name and the quantity of adjuvant used.

Ph Eur

[1] *Reference haemagglutinin antigens are available from the National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, Great Britain.*

Measles Vaccine, Live

General Notices

(Measles Vaccine (Live), Ph Eur monograph 0213)

The label may state 'Measles'.

Ph Eur

DEFINITION

Measles vaccine (live) is a freeze-dried preparation of a suitable attenuated strain of measles virus. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The production of vaccine is based on a virus seed-lot system and, if the virus is propagated in human diploid cells, a cell-bank system. The production method shall have been shown to yield consistently live measles vaccines of adequate immunogenicity and safety in man. Unless otherwise justified and authorised, the virus in the final vaccine shall have undergone no more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy; even with authorised exceptions, the number of passages beyond the level used for clinical studies shall not exceed 5.

The potential neurovirulence of the vaccine strain is considered during preclinical development, based on available epidemiological data on neurovirulence and neurotropism, primarily for the wild-type virus. In light of this, a risk analysis is carried out. Where necessary and if available, a test is carried out on the vaccine strain using an animal model that differentiates wild-type and attenuated virus; tests on strains of intermediate attenuation may also be needed.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*).

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in human diploid cells (*5.2.3*) or in cultures of chick-embryo cells derived from a chicken flock free from specified pathogens (*5.2.2*).

SEED LOT

The strain of measles virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are prepared in large quantities and stored at temperatures below - 20 °C if freeze-dried, or below - 60 °C if not freeze-dried.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

The master and working seed lots are identified as measles virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration

The virus concentration of the master and working seed lots is determined to monitor consistency of production.

Extraneous agents (*2.6.16*)

The working seed lot complies with the requirements for seed lots.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled during production. Suitable animal (but not human) serum may be used in the growth medium, but the final medium for maintaining cells during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 500 ml of the production cell cultures is set aside as uninfected cell cultures (control cells). The viral suspensions are harvested at a time appropriate to the strain of virus being used.

Only a single harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

The single harvest contains virus that is identified as measles virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration

The virus concentration in the single harvest is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents (*2.6.16*)

The single harvest complies with the tests for extraneous agents.

Control cells

If human diploid cells are used for production, the control cells comply with a test for identification. They comply with the tests for extraneous agents (*2.6.16*).

FINAL BULK VACCINE

Virus harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Bacterial and fungal contamination

The final bulk vaccine complies with the test for sterility (*2.6.1*), carried out using 10 ml for each medium.

FINAL LOT

A minimum virus concentration for release of the product is established such as to ensure, in light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the requirements for minimum virus concentration for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability

Maintain at least 3 vials of the final lot of freeze-dried vaccine in the dry state at 37 ± 1 °C for 7 days. Determine the virus concentration as described under Assay in parallel for the heated vaccine and for vaccine stored at the temperature recommended for storage. The virus concentration of the heated vaccine is not more than 1.0 log lower than that of the unheated vaccine.

IDENTIFICATION

When the vaccine reconstituted as stated on the label is mixed with specific measles antibodies, it is no longer able to infect susceptible cell cultures.

TESTS

Bacterial and fungal contamination

The reconstituted vaccine complies with the test for sterility (*2.6.1*).

Bovine serum albumin

Not more than 50 ng per single human dose, determined by a suitable immunochemical method (*2.7.1*).

Water (*2.5.12*)

Not more than 3.0 per cent, determined by the semi-micro determination of water.

ASSAY

Titrate the vaccine for infective virus, using at least 3 separate vials of vaccine and inoculating a suitable number of wells for each dilution step. Titrate 1 vial of an appropriate virus reference preparation in triplicate to validate each assay. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory. The relation with the appropriate European Pharmacopoeia Biological Reference Preparation is established and monitored at regular intervals if a manufacturer's reference preparation is used. Calculate the individual virus concentration for each vial of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations, using the usual statistical methods (for example, *5.3*). The combined estimate of the virus concentration for the 3 vials of vaccine is not less than that stated on the label; the minimum virus concentration stated on the label is not less than 3.0 log $CCID_{50}$ per single human dose.

The assay is not valid if:

- the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than ± 0.3 log $CCID_{50}$;
- the virus concentration of the reference preparation differs by more than 0.5 log $CCID_{50}$ from the established value.

The assay is repeated if the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 log $CCID_{50}$; data obtained from valid assays only are combined by the usual statistical methods (for example, *5.3*) to calculate the virus concentration of the sample. The confidence interval ($P = 0.95$) of the combined virus concentration is not greater than ± 0.3 log $CCID_{50}$.

Measles vaccine (live) BRP is suitable for use as a reference preparation.

Where justified and authorised, different assay designs may be used; this may imply the application of different validity and acceptance criteria. However, the vaccine must comply if tested as described above.

LABELLING

The label states:

- the strain of virus used for the preparation of the vaccine;
- the type and origin of the cells used for the preparation of the vaccine;
- the minimum virus concentration;
- that contact between the vaccine and disinfectants is to be avoided.

Ph Eur

Measles, Mumps and Rubella Vaccine, Live

General Notices

(Measles, Mumps and Rubella Vaccine (Live), Ph Eur monograph 1057)

The label may state 'MMR'.

Ph Eur

DEFINITION

Measles, mumps and rubella vaccine (live) is a freeze-dried preparation of suitable attenuated strains of measles virus, mumps virus and rubella virus.

The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The 3 components are prepared as described in the monographs *Measles vaccine (live) (0213)*, *Mumps vaccine (live) (0538)* and *Rubella vaccine (live) (0162)* and comply with the requirements prescribed therein.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*).

FINAL BULK VACCINE

Virus harvests for each component are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate. Suitable quantities of the pooled harvest for each component are mixed.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Bacterial and fungal contamination

Carry out the test for sterility (*2.6.1*), using 10 ml for each medium.

FINAL LOT

For each component, a minimum virus concentration for release of the product is established such as to ensure, in light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the requirements for minimum virus concentration of each component for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests may be released for use. Provided that the tests for bovine serum albumin and, where applicable, for ovalbumin have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Thermal stability

Maintain at least 3 vials of the final lot of freeze-dried vaccine in the dry state at 37 ± 1 °C for 7 days. Determine the virus concentration as described under Assay in parallel for the heated vaccine and for vaccine stored at the temperature recommended for storage. For each component, the virus concentration of the heated vaccine is not more than 1.0 log lower than that of the unheated vaccine.

IDENTIFICATION

When the vaccine reconstituted as stated on the label is mixed with antibodies specific for measles virus, mumps virus and rubella virus, it is no longer able to infect cell cultures susceptible to these viruses. When the vaccine reconstituted as stated on the label is mixed with quantities of specific antibodies sufficient to neutralise any 2 viral components, the 3rd viral component infects susceptible cell cultures.

TESTS

Bacterial and fungal contamination

The reconstituted vaccine complies with the test for sterility (*2.6.1*).

Bovine serum albumin

Not more than 50 ng per single human dose, determined by a suitable immunochemical method (*2.7.1*).

Ovalbumin

If the mumps component is produced in chick embryos, the vaccine contains not more than 1 µg of ovalbumin per single human dose, determined by a suitable immunochemical method (*2.7.1*).

Water (*2.5.12*)

Not more than 3.0 per cent, determined by the semi-micro determination of water.

ASSAY

The cell lines and/or neutralising antisera are chosen to ensure that each component is assayed without interference from the other 2 components.

Titrate the vaccine for infective measles, mumps and rubella virus, using at least 3 separate vials of vaccine and inoculating a suitable number of wells for each dilution step. Titrate 1 vial of the appropriate virus reference preparation in triplicate to validate each assay. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory. The relation with the appropriate European Pharmacopoeia Biological Reference Preparation is established and monitored at regular intervals if a manufacturer's reference preparation is used. Calculate the individual virus concentration for each vial of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations, using the usual statistical methods (for example, *5.3*).

The combined estimates of the measles, mumps and rubella virus concentrations for the 3 vials of vaccine are not less than that stated on the label; the minimum measles virus concentration stated on the label is not less than 3.0 log $CCID_{50}$ per single human dose; the minimum mumps virus concentration stated on the label is not less than 3.7 log $CCID_{50}$ per single human dose; the minimum rubella virus concentration stated on the label is not less than 3.0 log $CCID_{50}$ per single human dose.

The assay is not valid if:

- the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than ± 0.3 log $CCID_{50}$;
- the virus concentration of the reference preparation differs by more than 0.5 log $CCID_{50}$ from the established value.

The assay is repeated if the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 log $CCID_{50}$; data obtained from valid assays only are combined by the usual statistical methods (for example, *5.3*) to calculate the virus concentration of the sample. The confidence interval ($P = 0.95$) of the combined virus concentration is not greater than ± 0.3 log $CCID_{50}$.

Measles vaccine (live) BRP is suitable for use as a reference preparation.

Mumps vaccine (live) BRP is suitable for use as a reference preparation.

Rubella vaccine (live) BRP is suitable for use as a reference preparation.

Where justified and authorised, different assay designs may be used; this may imply the application of different validity and acceptance criteria. However, the vaccine must comply if tested as described above.

LABELLING

The label states:

- the strains of virus used in the preparation of the vaccine;
- where applicable, that chick embryos have been used for the preparation of the vaccine;
- the type and origin of the cells used for the preparation of the vaccine;
- the minimum virus concentration for each component of the vaccine;
- that contact between the vaccine and disinfectants is to be avoided;
- at the vaccine must not be given to a pregnant woman and that a woman must not become pregnant within 2 months after having the vaccine.

Ph Eur

Meningococcal Group C Conjugate Vaccine

General Notices

(Ph Eur monograph 2112)

The label may state 'MenC(conj)'.

Ph Eur

DEFINITION

Meningococcal group C conjugate vaccine is a liquid or freeze-dried preparation of purified capsular polysaccharide derived from a suitable strain of *Neisseria meningitidis* group C covalently linked to a carrier protein. Meningococcal group C polysaccharide consists of partly *O*-acetylated or *O*-deacetylated repeating units of sialic acids, linked with $2\alpha \rightarrow 9$ glycosidic bonds. The carrier protein, when conjugated to group C polysaccharide, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide. The vaccine may contain an adjuvant.

PRODUCTION

GENERAL PROVISIONS

The production method shall consistently have been shown to yield meningococcal group C conjugate vaccines of satisfactory immunogenicity and safety in man. The production of meningococcal group C polysaccharide and of the carrier protein are based on seed-lot systems.

During development studies and wherever revalidation is necessary, a test for pyrogens in rabbits *(2.6.8)* is carried out by injection of a suitable dose of the final lot. The vaccine is shown to be acceptable with respect to absence of pyrogenic activity.The production method is validated to demonstrate that the vaccine, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

During development studies and wherever revalidation of the manufacturing process is necessary, it shall be demonstrated by tests in animals that the vaccine consistently induces a T-cell-dependent B-cell immune response.

The stability of the final lot and relevant intermediates is evaluated using 1 or more indicator tests. Such tests may include determination of molecular size, determination of free saccharide in the conjugate or an immunogenicity test in animals.

BACTERIAL SEED LOTS

The bacterial strains used for master seed lots shall be identified by historical records that include information on their origin and the tests used to characterise the strain. Cultures from the working seed lot shall have the same characteristics as the strain that was used to prepare the master seed lot.

Purity of bacterial cultures is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

MENINGOCOCCAL GROUP C POLYSACCHARIDE

N. meningitidis is grown in a liquid medium that does not contain high-molecular-mass polysaccharides and is free from ingredients that will form a precipitate upon addition of cetyltrimethylammonium bromide (CTAB). The culture may be inactivated by heat and filtered before the polysaccharide is precipitated by addition of CTAB. The precipitate is further purified using suitable methods to remove nucleic acids, proteins and lipopolysaccharides and the final purification step consists of ethanol precipitation. An O-deacetylation step may also be included. Volatile matter, including water, in the purified polysaccharide is determined by a suitable method such as thermogravimetry *(2.2.34)*. The value is used to calculate the results of other tests with reference to the dried substance, as prescribed below.

Only meningococcal group C polysaccharide that complies with the following requirements may be used in the preparation of the conjugate.

Protein *(2.5.16)*

Maximum 1.0 per cent, calculated with reference to the dried substance.

Nucleic acid *(2.5.17)*

Maximum 1.0 per cent, calculated with reference to the dried substance.

O-acetyl groups

Examine by a suitable method *(for example 2.5.19)*. An acceptable value is established for the particular product and each batch of meningococcal group C polysaccharide must be shown to comply with this limit.

Sialic acid *(2.5.23)*

Minimum 0.800 g of sialic acid per gram of meningococcal group C polysaccharide using *N-acetylneuraminic acid R* to prepare the reference solution.

Residual reagents

Where applicable, tests are carried out to determine residues of reagents used during inactivation and purification. An acceptable value for each reagent is established for the particular product and each batch of meningococcal group C polysaccharide must be shown to comply with this limit. Where validation studies have demonstrated removal of a residual reagent, the test on purified meningococcal group C polysaccharide may be omitted.

Molecular-size distribution

Examine by size-exclusion chromatography *(2.2.30)*. An acceptable value is established for the particular product and each batch of meningococcal group C polysaccharide must be shown to comply with this limit. Where applicable, the molecular-size distribution is also determined after chemical modification of the meningococcal group C polysaccharide.

Identification and serological specificity

The identity and serological specificity are determined by a suitable immunochemical method *(2.7.1)* or other suitable method, for example ^{1}H nuclear magnetic resonance spectrometry

(2.2.33).

Bacterial endotoxins *(2.6.14)*

Less than 100 IU per microgram of meningococcal group C polysaccharide.

CARRIER PROTEIN

The carrier protein is chosen so that when meningococcal group C polysaccharide is conjugated it is able to induce a T-cell-dependent immune response. Tetanus toxoid and the non-toxic mutant of diphtheria toxin-like protein, CRM197 are suitable. The carrier protein is produced by culture of a suitable microorganism, the bacterial purity of which is verified.

Only a carrier protein that complies with the following requirements may be used in preparation of the conjugate.

Identification

The carrier protein is identified by a suitable immunochemical method *(2.7.1)*.

Diphtheria toxin-like protein

Where diphtheria toxin-like protein is used as the carrier, it contains not less than 90 per cent of CRM197, determined by a suitable method. Suitable tests are carried out, for validation or routinely, to demonstrate that the product is non-toxic.

Tetanus toxoid

Where tetanus toxoid is used as the carrier, it is produced as described in *Tetanus vaccine (adsorbed) (0452)* and complies with the requirements prescribed therein for bulk purified toxoid, except that the antigenic purity *(2.7.27)* is not less than 1500 Lf per milligram of protein nitrogen.

BULK CONJUGATE

Meningococcal group C polysaccharide is chemically modified to enable conjugation; it is usually partly depolymerised either before or during this procedure. The conjugate is obtained by the covalent bonding of activated meningococcal group C oligosaccharide and carrier protein. The conjugate purification procedures are designed to remove residual reagents used for conjugation. The removal of residual reagents and reaction by-products is confirmed by suitable tests or by validation of the purification process.

Only a bulk conjugate that complies with the following requirements may be used in the preparation of the final bulk vaccine. For each test and for each particular product, limits of acceptance are established and each batch of conjugate must be shown to comply with these limits.

Molecular-size distribution

Examine by size-exclusion chromatography *(2.2.30)*. An acceptable value is established for the particular product and each batch of bulk conjugate must be shown to comply with this limit.

Saccharide

The saccharide content is determined by a suitable validated assay, for example *(2.5.23)*. Anion-exchange liquid chromatography with pulsed amperometric detection *(2.2.29)* may also be used for determination of saccharide content. An acceptable value is established for the particular product and each batch of bulk conjugate must be shown to comply with this limit.

Protein

The protein content is determined by a suitable chemical method, for example *(2.5.16)*. An acceptable value is established for the particular product and each batch of bulk conjugate must be shown to comply with this limit.

Saccharide-to-protein ratio

Determine the ratio by calculation.

Free saccharide

Unbound saccharide is determined after removal of the conjugate, for example by anion-exchange liquid chromatography, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. An acceptable value is established for the particular product and each batch of bulk conjugate must be shown to comply with this limit.

Free carrier protein

Determine the content, either directly by a suitable method or by deriving the content by calculation from the results of other tests. An acceptable value is established for the particular product and each batch of bulk conjugate must be shown to comply with this limit.

Residual reagents

Removal of residual reagents such as cyanide is confirmed by suitable tests or by validation of the process.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium or the equivalent of 100 doses, whichever is less.

FINAL BULK VACCINE

An adjuvant and a stabiliser may be added to the bulk conjugate before dilution to the final concentration with a suitable diluent.

Only a final bulk vaccine that complies with the following requirement and is within the limits approved for the particular product may be used in preparation of the final lot.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that is within the limits approved for the particular product and is satisfactory with respect to each of the requirement given below under Identification, Tests and Assay may be released for use.

IDENTIFICATION

The vaccine is identified by a suitable immunochemical method *(2.7.1)*.

TESTS

pH *(2.2.3)*

The pH of the vaccine, reconstituted if necessary, is within ± 0.5 pH units of the limit approved for the particular product.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Water *(2.5.12)*

Maximum 3.0 per cent for freeze-dried vaccines.

Free saccharide

Unbound saccharide is determined after removal of the conjugate, for example by anion-exchange liquid chromatography, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. An acceptable value consistent with adequate immunogenicity, as shown in clinical trials, is established for the particular product and each final lot must be shown to comply with this limit.

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 25 IU per single human dose.

ASSAY

Saccharide

Minimum 80 per cent of the amount of meningococcal group C polysaccharide stated on the label. The saccharide content is determined by a suitable validated assay, for example sialic acid assay *(2.5.23)* or anion-exchange liquid chromatography with pulsed amperometric detection *(2.2.29)*.

LABELLING

The label states:

— the number of micrograms of meningococcal group C polysaccharide per human dose;

— the type and number of micrograms of carrier protein per human dose.

Ph Eur

Meningococcal Polysaccharide Vaccine

General Notices

(Ph Eur monograph 0250)

The label may state 'Men' plus relevant antigen. For example, 'MenAC'.

Ph Eur

DEFINITION

Meningococcal polysaccharide vaccine is a freeze-dried preparation of one or more purified capsular polysaccharides obtained from one or more suitable strains of *Neisseria meningitidis* group A, group C, group Y and group W135 that are capable of consistently producing polysaccharides.

N. meningitidis group A polysaccharide consists of partly *O*-acetylated repeating units of *N*-acetylmannosamine, linked with 1α→6 phosphodiester bonds.

N. meningitidis group C polysaccharide consists of partly *O*-acetylated repeating units of sialic acid, linked with 2α→9 glycosidic bonds.

N. meningitidis group Y polysaccharide consists of partly *O*-acetylated alternating units of sialic acid and D-glucose, linked with 2α→6 and 1α→4 glycosidic bonds.

N. meningitidis group W135 polysaccharide consists of partly *O*-acetylated alternating units of sialic acid and D-galactose, linked with 2α→6 and 1α→4 glycosidic bonds.

The polysaccharide component or components stated on the label together with calcium ions and residual moisture account for over 90 per cent of the mass of the preparation.

PRODUCTION

Production of the meningococcal polysaccharides is based on a seed-lot system. The production method shall have been shown to yield consistently meningococcal polysaccharide vaccines of satisfactory immunogenicity and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*).

SEED LOTS

The strains of *N. meningitidis* used for the master seed lots shall be identified by historical records that include information on their origin and by their biochemical and serological characteristics.

Cultures from each working seed lot shall have the same characteristics as the strain that was used to prepare the master seed lot. The strains have the following characteristics:

— colonies obtained from a culture are rounded, uniform in shape and smooth with a mucous, opalescent, greyish appearance;

— Gram staining reveals characteristic Gram-negative diplococci in "coffee-bean" arrangement;

— the oxidase test is positive;

— the culture utilises glucose and maltose;

— suspensions of the culture agglutinate with suitable specific antisera.

Purity of bacterial strains used for the seed lots is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

PROPAGATION AND HARVEST

The working seed lots are cultured on solid media that do not contain blood-group substances or ingredients of mammalian origin. The inoculum may undergo 1 or more subcultures in liquid medium before being used for inoculating the final medium. The liquid media used and the final medium are semisynthetic and free from substances precipitated by cetrimonium bromide (hexadecyltrimethylammonium bromide) and do not contain blood-group substances or high-molecular-mass polysaccharides.

The bacterial purity of the culture is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

The cultures are centrifuged and the polysaccharides precipitated from the supernatant by addition of cetrimonium bromide. The precipitate obtained is harvested and may be stored at - 20 °C awaiting further purification.

PURIFIED POLYSACCHARIDES

The polysaccharides are purified, after dissociation of the complex of polysaccharide and cetrimonium bromide, using suitable procedures to remove successively nucleic acids, proteins and lipopolysaccharides.

The final purification step consists of ethanol precipitation of the polysaccharides which are then dried and stored at - 20 °C. The loss on drying is determined by thermogravimetry *(2.2.34)* and the value is used to calculate the results of the other chemical tests with reference to the dried substance.

Only purified polysaccharides that comply with the following requirements may be used in the preparation of the final bulk vaccine.

Protein *(2.5.16)*

Not more than 10 mg of protein per gram of purified polysaccharide, calculated with reference to the dried substance.

Nucleic acids *(2.5.17)*

Not more than 10 mg of nucleic acids per gram of purified polysaccharide, calculated with reference to the dried substance.

O-Acetyl groups *(2.5.19)*

Not less than 2 mmol of *O*-acetyl groups per gram of purified polysaccharide for group A, not less than 1.5 mmol per gram of polysaccharide for group C, not less than 0.3 mmol per gram of polysaccharide for groups Y and W135, all calculated with reference to the dried substance.

Phosphorus *(2.5.18)*

Not less than 80 mg of phosphorus per gram of group A purified polysaccharide, calculated with reference to the dried substance.

Sialic acid *(2.5.23)*

Not less than 800 mg of sialic acid per gram of group C polysaccharide and not less than 560 mg of sialic acid per gram of purified polysaccharide for groups Y and W135, all calculated with reference to the dried substance. Use the following reference solutions.

Group C polysaccharide: a 150 mg/l solution of *N-acetylneuraminic acid R.*

Group Y polysaccharide: a solution containing 95 mg/l of *N-acetylneuraminic acid* R and 55 mg/l of *glucose R.*

Group W135 polysaccharide: a solution containing 95 mg/l of *N-acetylneuraminic acid R* and 55 mg/l of *galactose R.*

Calcium

If a calcium salt is used during purification, a determination of calcium is carried out on the purified polysaccharide; the content is within the limits approved for the particular product.

Distribution of molecular size

Examine by size-exclusion chromatography *(2.2.30)* using *agarose for chromatography R* or *cross-linked agarose for chromatography R*. Use a column about 0.9 m long and 16 mm in internal diameter equilibrated with a solvent having an ionic strength of 0.2 mol/kg and a pH of 7.0-7.5. Apply to the column about 2.5 mg of polysaccharide in a volume of about 1.5 ml and elute at about 20 ml/h. Collect fractions of about 2.5 ml and determine the content of polysaccharide by a suitable method. At least 65 per cent of group A polysaccharide, 75 per cent of group C polysaccharide, 80 per cent of group Y polysaccharide and 80 per cent of group W135 polysaccharide is eluted before a distribution coefficient (K_0) of 0.50 is reached. In addition, the percentages eluted before this distribution coefficient are within the limits approved for the particular product.

Identification and serological specificity

The identity and serological specificity are determined by a suitable immunochemical method (*2.7.1*). Identity and purity of each polysaccharide shall be confirmed; it shall be shown that there is not more than 1 per cent m/m of group-heterologous *N. meningitidis* polysaccharide.

Pyrogens *(2.6.8)*

The polysaccharide complies with the test for pyrogens. Inject into each rabbit per kilogram of body mass 1 ml of a solution containing 0.025 µg of purified polysaccharide per millilitre.

FINAL BULK VACCINE

One or more purified polysaccharides of 1 or more *N. meningitidis* groups are dissolved in a suitable solvent that may contain a stabiliser. When dissolution is complete, the solution is filtered through a bacteria-retentive filter.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are then closed so as to avoid contamination.

Only a final lot that is satisfactory with respect to each of the requirements prescribed below under Identification, Tests and Assay may be released for use.

CHARACTERS

A white or cream-coloured powder or pellet, freely soluble in water.

IDENTIFICATION

Carry out an identification test for each polysaccharide present in the vaccine by a suitable immunochemical method *(2.7.1)*.

TESTS

Distribution of molecular size

Examine by size-exclusion chromatography *(2.2.30)*. Use a column about 0.9 m long and 16 mm in internal diameter equilibrated with a solvent having an ionic strength of 0.2 mol/kg and a pH of 7.0-7.5. Apply to the column about 2.5 mg of each polysaccharide in a volume of about 1.5 ml and elute at about 20 ml/h. Collect fractions of about 2.5 ml and determine the content of polysaccharide by a suitable method.

For a divalent vaccine (group A + group C), use *cross-linked agarose for chromatography R*. The vaccine complies with the test if:

- — 65 per cent of group A polysaccharide is eluted before $K_0 = 0.50$;
- — 75 per cent of group C polysaccharide is eluted before $K_0 = 0.50$.

For a tetravalent vaccine (group A + group C + group Y + group W135), use *cross-linked agarose for chromatography R1* and apply a suitable immunochemical method *(2.7.1)* to establish the elution pattern of the different polysaccharides. The vaccine complies with the test if K_0 for the principal peak is:

- — not greater than 0.70 for group A and group C polysaccharide;
- — not greater than 0.57 for group Y polysaccharide;
- — not greater than 0.68 for group W135 polysaccharide.

Water *(2.5.12)*

Not more than 3.0 per cent, determined by the semi-micro determination of water.

Sterility *(2.6.1)*

It complies with the test for sterility.

Pyrogens *(2.6.8)*

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 ml of a solution containing:

- — 0.025 µg of polysaccharide for a monovalent vaccine;
- — 0.050 µg of polysaccharide for a divalent vaccine;
- — 0.10 µg of polysaccharide for a tetravalent vaccine.

ASSAY

Carry out an assay of each polysaccharide present in the vaccine.

For a divalent vaccine (group A + group C), use measurement of phosphorus *(2.5.18)* to determine the content of polysaccharide A and measurement of sialic acid *(2.5.23)* to determine the content of polysaccharide C. To determine sialic acid, use as reference solution a 150 mg/l solution of *N-acetylneuraminic acid R.*

For a tetravalent vaccine (group A + group C + group Y + group W135) a suitable immunochemical method *(2.7.1)* is used with a reference preparation of purified polysaccharide for each group.

The vaccine contains not less than 70 per cent and not more than 130 per cent of the quantity of each polysaccharide stated on the label.

LABELLING

The label states:

- — the group or groups of polysaccharides (A, C, Y or W135) present in the vaccine;
- — the number of micrograms of polysaccharide per human dose.

Ph Eur

Mumps Vaccine, Live

General Notices

(Mumps Vaccine (Live), Ph Eur monograph 0538)

The label may state 'Mumps'.

Ph Eur

DEFINITION

Mumps vaccine (live) is a freeze-dried preparation of a suitable attenuated strain of mumps virus. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The production of vaccine is based on a virus seed-lot system and, if the virus is propagated in human diploid cells, a cell-bank system. The production method shall have been shown to yield consistently live mumps vaccines of adequate immunogenicity and safety in man. Unless otherwise justified and authorised, the virus in the final vaccine shall have undergone no more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

The potential neurovirulence of the vaccine strain is considered during preclinical development, based on available epidemiological data on neurovirulence and neurotropism, primarily for the wild-type virus. In light of this, a risk analysis is carried out. Where necessary and if available, a test is carried out on the vaccine strain using an animal model that differentiates wild-type and attenuated virus; tests on strains of intermediate attenuation may also be needed.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in human diploid cells *(5.2.3)* or in chick-embryo cells or in the amniotic cavity of chick embryos derived from a chicken flock free from specified pathogens *(5.2.2)*.

SEED LOT

The strain of mumps virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are prepared in large quantities and stored at temperatures below - 20 °C if freeze-dried, or below - 60 °C if not freeze-dried.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

The master and working seed lots are identified as mumps virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration

The virus concentration of the master and working seed lots is determined to ensure consistency of production.

Extraneous agents *(2.6.16)*

The working seed lot complies with the requirements for seed lots.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled during the production. Suitable animal (but not human) serum may be used in the culture media. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 500 ml of the production cell cultures is set aside as uninfected cell cultures (control cells). If the virus is propagated in chick embryos, 2 per cent but not less than 20 eggs are set aside as uninfected control eggs. The viral suspensions are harvested at a time appropriate to the strain of virus being used.

Only a single harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

The single harvest contains virus that is identified as mumps virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration

The virus concentration in the single harvest is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents *(2.6.16)*

The single harvest complies with the tests for extraneous agents.

Control cells or eggs

If human diploid cells are used for production, the control cells comply with a test for identification; the control cells and the control eggs comply with the tests for extraneous agents *(2.6.16)*.

FINAL BULK VACCINE

Single harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Bacterial and fungal contamination

The final bulk vaccine complies with the test for sterility (2.6.1), carried out using 10 ml for each medium.

FINAL LOT

A minimum virus concentration for release of the product is established such as to ensure, in light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the requirements for minimum virus concentration for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests may be released for use. Provided that the tests for bovine serum albumin and, where applicable, for ovalbumin have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Thermal stability

Maintain at least 3 vials of the final lot of freeze-dried vaccine in the dry state at 37 ± 1 °C for 7 days. Determine the virus concentration as described under Assay in parallel for the heated vaccine and for vaccine stored at the temperature recommended for storage. The virus concentration of the heated vaccine is not more than 1.0 log lower than that of the unheated vaccine.

IDENTIFICATION

When the vaccine reconstituted as stated on the label is mixed with specific mumps antibodies, it is no longer able to infect susceptible cell cultures.

TESTS

Bacterial and fungal contamination

The reconstituted vaccine complies with the test for sterility *(2.6.1)*.

Bovine serum albumin

Not more than 50 ng per single human dose, determined by a suitable immunochemical method *(2.7.1)*.

Ovalbumin

If the vaccine is produced in chick embryos, it contains not more than 1 µg of ovalbumin per single human dose, determined by a suitable immunochemical method *(2.7.1)*.

Water *(2.5.12)*

Not more than 3.0 per cent, determined by the semi-micro determination of water.

ASSAY

Titrate the vaccine for infective virus, using at least 3 separate vials of vaccine and inoculating a suitable number of wells for each dilution step. Titrate 1 vial of an appropriate virus reference preparation in triplicate to validate each assay. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory. The relation with the appropriate European Pharmacopoeia Biological Reference Preparation is established and monitored at regular intervals if a manufacturer's reference preparation is used. Calculate the individual virus concentration for each vial of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations, using the usual statistical methods (for example, *5.3*). The combined estimate of the virus concentration for the 3 vials of vaccine is not less than that stated on the label; the minimum virus concentration stated on the label is not less than 3.7 log $CCID_{50}$ per single human dose.

The assay is not valid if:

— the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than ± 0.3 log $CCID_{50}$;

— the virus concentration of the reference preparation differs by more than 0.5 log $CCID_{50}$ from the established value.

The assay is repeated if the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 log $CCID_{50}$; data obtained from valid assays only are combined by the usual statistical methods (for example, *5.3*) to calculate the virus concentration of the sample. The confidence interval ($P = 0.95$) of the combined virus concentration is not greater than ± 0.3 log $CCID_{50}$.

Mumps vaccine (live) BRP is suitable for use as a reference preparation.

Where justified and authorised, different assay designs may be used; this may imply the application of different validity and acceptance criteria. However, the vaccine must comply if tested as described above.

LABELLING

The label states:

— the strain of virus used for the preparation of the vaccine;

— that the vaccine has been prepared in chick embryos or the type and origin of cells used for the preparation of the vaccine;

— the minimum virus concentration;

— that contact between the vaccine and disinfectants is to be avoided.

Ph Eur

Pertussis Vaccine (Adsorbed)

General Notices

(Ph Eur monograph 0161)

The label may state 'wP'.

Ph Eur

DEFINITION

Pertussis vaccine (adsorbed) is a sterile saline suspension of inactivated whole cells of one or more strains of *Bordetella pertussis* with a mineral adsorbent such as hydrated aluminium phosphate or aluminium hydroxide.

PRODUCTION

INACTIVATED *B. PERTUSSIS* SUSPENSION

Production is based on a seed-lot system. One or more strains of *B. pertussis* with known origin and history are used. Strains, culture medium and cultivation method are chosen in such a way that agglutinogens 1, 2 and 3 are present in the final vaccine. Each strain is grown for 24 h to 72 h in a liquid medium or on a solid medium; the medium used in the final cultivation stage does not contain blood or blood products. Human blood or blood products are not used in any culture media. The bacteria are harvested, washed to remove substances derived from the medium and suspended in a 9 g/l solution of sodium chloride or other suitable isotonic solution. The opacity of the suspension is determined not later than 2 weeks after harvest by comparison with the International Reference Preparation of Opacity and used as the basis of calculation for subsequent stages in vaccine preparation. The equivalence in International Units of the International Reference Preparation is stated by the World Health Organisation.

Single harvests are not used for the final bulk vaccine unless they have been shown to contain *B. pertussis* cells with the same characteristics, with regard to growth and agglutinogens, as the parent strain and to be free from contaminating bacteria and fungi. The bacteria are killed and detoxified in controlled conditions by means of a suitable chemical agent or by heating or by a combination of these methods. Freedom from live *B. pertussis* is tested using a suitable culture medium. The suspension is maintained at 5 ± 3 °C for a suitable period to diminish its toxicity.

FINAL BULK VACCINE

Suitable quantities of the inactivated single harvests are pooled to prepare the final bulk vaccine. A mineral adsorbent such as hydrated aluminium phosphate or aluminium hydroxide is added to the cell suspension. Suitable antimicrobial preservatives may be added. The bacterial concentration of the final bulk vaccine does not exceed that corresponding to an opacity of 20 IU per single human dose. If 2 or more strains of *B. pertussis* are used, the composition of consecutive lots of the final bulk vaccine shall be consistent with respect to the proportion of each strain as measured in opacity units.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility *(2.6.1)*

Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for specific toxicity, free formaldehyde and antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

IDENTIFICATION

Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge to obtain a bacterial precipitate. Other suitable methods for separating the bacteria from the adsorbent may also be used. Identify pertussis vaccine by agglutination of the bacteria from the resuspended precipitate by antisera specific to *B. pertussis* or by the assay.

TESTS

Specific toxicity

Use not fewer than 5 healthy mice each weighing 14 g to 16 g for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 h before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with 0.5 ml, containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 9 g/l sterile solution of *sodium chloride R*, preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the groups of mice immediately before the injection and 72 h and 7 days after the injection. The vaccine complies with the test if: (a) at the end of 72 h the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than 60 per cent of that per control mouse; and (c) not more than 5 per cent of the vaccinated mice die during the test. The test may be repeated and the results of the tests combined.

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

Carry out the assay of pertussis vaccine *(2.7.7)*.

The estimated potency is not less than 4 IU per single human dose and the lower confidence limit ($P = 0.95$) of the estimated potency is not less than 2 IU per single human dose.

LABELLING

The label states:

— the minimum number of International Units per single human dose,

— the name and the amount of the adsorbent,

— that the vaccine must be shaken before use,

— that the vaccine is not to be frozen.

Ph Eur

Pneumococcal Polysaccharide Conjugate Vaccine (Adsorbed)

General Notices

(Ph Eur monograph 2150)

The label may state 'Pneumo(conj)'.

Ph Eur

DEFINITION

Pneumococcal polysaccharide conjugate vaccine (adsorbed) is a sterile solution of capsular purified antigens (polysaccharides) obtained from *Streptococcus pneumoniae* serotypes individually conjugated to a carrier protein. The vaccine may be adsorbed on a suitable adjuvant or adsorbant.

Each serotype, produced from suitable pathogenic strains of *S. pneumoniae*, is grown in an appropriate medium.

The individual polysaccharides are purified through suitable purification methods (for example centrifugation, precipitation, ultrafiltration and column chromatography).

Each polysaccharide has a defined composition and a defined molecular size range.

The choice of polysaccharide depends on the frequency of the serotypes responsible for acute pathologies and their geographical distribution. It contains immunochemically different capsular polysaccharides.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently *S. pneumoniae* conjugate vaccines of adequate safety and immunogenicity in man. The production of polysaccharides and the carrier are based on a seed-lot system.

During development studies and wherever revalidation is necessary, a test for pyrogens in rabbits (*2.6.8*) is carried out by injection of a suitable dose of the final lot. The vaccine is shown to be acceptable with respect to absence of pyrogenic activity.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

During development studies and whenever revalidation of the manufacturing process is necessary, it shall be demonstrated by tests in animals that the vaccine consistently induces a T-cell-dependent B-cell immune response.

The stability of the final lot and pneumococcal saccharide is evaluated using suitable indicator tests. Such tests may include determination of molecular size, quantification of saccharide content and free polysaccharide content in the conjugate.

BACTERIAL SEED LOTS

The bacterial strains used for master seed lots shall be identified by historical records that include information on their origin and the tests used to characterise the strain.

Cultures obtained from the working seed lot shall have the same characteristics as the strain that was used to prepare the master seed lot.

Purity of bacterial cultures is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

PNEUMOCOCCAL POLYSACCHARIDES

Each strain of *S. pneumoniae* serotypes is individually grown in a liquid medium that does not contain high-molecular-mass polysaccharides; if any ingredient of the medium contains blood-group substances, the process is validated to demonstrate that after the purification step they are no longer detectable. The bacterial purity of the culture is verified by suitable methods. The culture is then inactivated. The polysaccharide is separated from the liquid culture and purified by suitable methods. Volatile matter, including water, in the purified polysaccharide is determined by a suitable method such as thermogravimetry *(2.2.34)*; the value is used to calculate the results of other chemical tests with reference to the dried substance, as prescribed below.

Only polysaccharides that comply with the following requirements may be used in the preparation of the conjugate.

Identification

Each polysaccharide is identified by an immunochemical method *(2.7.1)* or other suitable methods, for example ^{1}H nuclear magnetic resonance spectrometry *(2.2.33)* (assay for functional group).

Protein *(2.5.16)*

Depending on the serotype used, not more than the limit approved for the product, calculated with reference to the dried substance.

Nucleic acid *(2.5.17)*

Depending on the serotype used, not more than the limit approved for the product, calculated with reference to the dried substance.

Molecular size

The molecular size is evaluated by liquid chromatography *(2.2.29)* with multiple-angle laser light scattering detection or other suitable methods. The values are within the limit approved for each serotype. A validated determination of the degree of polymerisation or of the weight-average molecular weight and the dispersion of molecular masses may be used instead of the determination of molecular size distribution.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium or the equivalent of 100 doses for each medium, whichever is less.

Bacterial endotoxins *(2.6.14)*

Less than 0.5 IU of endotoxin per microgram of polysaccharide.

Residual reagents

Where applicable, suitable tests are carried out to determine residues of reagents used during inactivation and purification. An acceptable value for each reagent is established for the particular product and each batch of polysaccharide must be shown to comply with this limit. Where validation studies have demonstrated removal of residual reagents, the test on polysaccharides may be omitted.

ACTIVATED PNEUMOCOCCAL POLYSACCHARIDES

The polysaccharide is chemically activated prior to conjugation; it is usually partly depolymerised either before or during this procedure.

Only activated polysaccharides that comply with the following requirements may be used in the preparation of the conjugate.

Degree of oxidation

The degree of oxidation is represented by the ratio of the moles of saccharide repeat unit per mole of aldehyde and determined by a suitable method. The values are within the limits approved for each serotype.

Molecular size

The molecular size is evaluated by liquid chromatography *(2.2.29)* with multiple-angle laser light scattering detection or other suitable methods. The values are within the limits approved for each serotype.

CARRIER PROTEIN

The carrier protein is produced by culture of suitable micro-organisms; the bacterial purity of the culture is verified. The culture is inactivated. The carrier protein is purified by a suitable method.

Only a carrier protein that complies with the following requirements may be used in the preparation of the conjugate.

Identification

The carrier protein is identified by a suitable immunochemical method *(2.7.1)*.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium or the equivalent of 100 doses for each medium, whichever is less.

Endotoxin content *(2.6.14)*

Less than 1 IU per microgram of protein.

Carrier protein

Not less than 90 per cent of the total protein content, determined by a suitable method. Suitable tests are carried out for validation to demonstrate that the product is non-toxic.

MONOVALENT BULK CONJUGATE

The conjugate is obtained by the covalent binding of activated polysaccharides to the carrier protein.

The conjugate purification procedures are designed to remove residual reagents used for conjugation. The removal of residual reagents is confirmed by suitable tests or by validation of the purification process.

Only a bulk conjugate that complies with the following requirements may be used in the preparation of the final bulk vaccine. For each test, limits of acceptance are established and each batch of conjugate must be shown to comply with these limits.

Saccharide

The polysaccharide content is determined by a suitable physical or chemical method or by an immunochemical method *(2.7.1)*. The value complies with the requirement approved for each serotype.

Protein

The protein content is determined by a suitable physical or chemical method *(2.5.16, for example)*. The value complies with the requirement approved for each serotype.

Saccharide-to-protein ratio

Determine the saccharide-to-protein ratio by calculation. The value complies with the requirement approved for each serotype.

Free saccharide

Unbound polysaccharide is determined after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. A value consistent with adequate immunogenicity as shown in clinical trials is established for each serotype and each lot must be shown to comply with this limit.

Free carrier protein

Determine the content by a suitable method, either directly or by deriving the content by calculation from the results of other tests. The value complies with the requirement approved for each serotype.

Molecular size

The molecular size is evaluated by liquid chromatography *(2.2.29)* with multiple-angle laser light scattering detection or other suitable methods. The values are within the limits approved for each serotype.

Residual reagents

Where applicable, suitable tests are carried out to determine residues of reagents used during inactivation and purification. An acceptable value for each reagent is established for the particular product and each batch of conjugate must be shown to comply with this limit. Where validation studies have demonstrated removal of residual reagents, the test on conjugate polysaccharides may be omitted.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium or the equivalent of 100 doses for each medium, whichever is less.

Bacterial endotoxins *(2.6.14)*

Less than 0.75 IU of endotoxin per microgram of polysaccharide.

FINAL BULK VACCINE

The individual monovalent bulk conjugates are compounded to formulate the vaccine. An adjuvant may be added before the final dilution.

Only a final bulk vaccine that complies with the following requirements and is within the limits approved for the particular product may be used in the preparation of the final lot.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that is within the limits approved for the particular product and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

IDENTIFICATION

Each polysaccharide present in the vaccine is identified by a suitable immunochemical method *(2.7.1)*.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose.

Sterility *(2.6.1)*

It complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 12.5 IU per single human dose, unless otherwise justified and authorised.

ASSAY

Saccharide content

The polysaccharide content for each serotype is determined by a suitable immunochemical method (for example, nephelometry assay). The vaccine contains not less than 70 per cent and not more than 130 per cent of the quantity stated on the label for each polysaccharide. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated content.

LABELLING

The label states:

— the number of micrograms of each polysaccharide per single human dose;

— the number of micrograms of carrier protein per single human dose.

Ph Eur

Pneumococcal Polysaccharide Vaccine

General Notices

(Ph Eur monograph 0966)

The label may state 'Pneumo'.

Ph Eur

DEFINITION

Pneumococcal polysaccharide vaccine consists of a mixture of equal parts of purified capsular polysaccharide antigens prepared from suitable pathogenic strains of *Streptococcus pneumoniae* whose capsules have been shown to be made up of polysaccharides that are capable of inducing satisfactory levels of specific antibodies in man. It contains the 23 immunochemically different capsular polysaccharides listed in Table 0966-1.

The vaccine is a clear, colourless liquid.

PRODUCTION

Production of the vaccine is based on a seed-lot system for each type. The production method shall have been shown to yield consistently pneumococcal polysaccharide vaccines of adequate safety and immunogenicity in man.

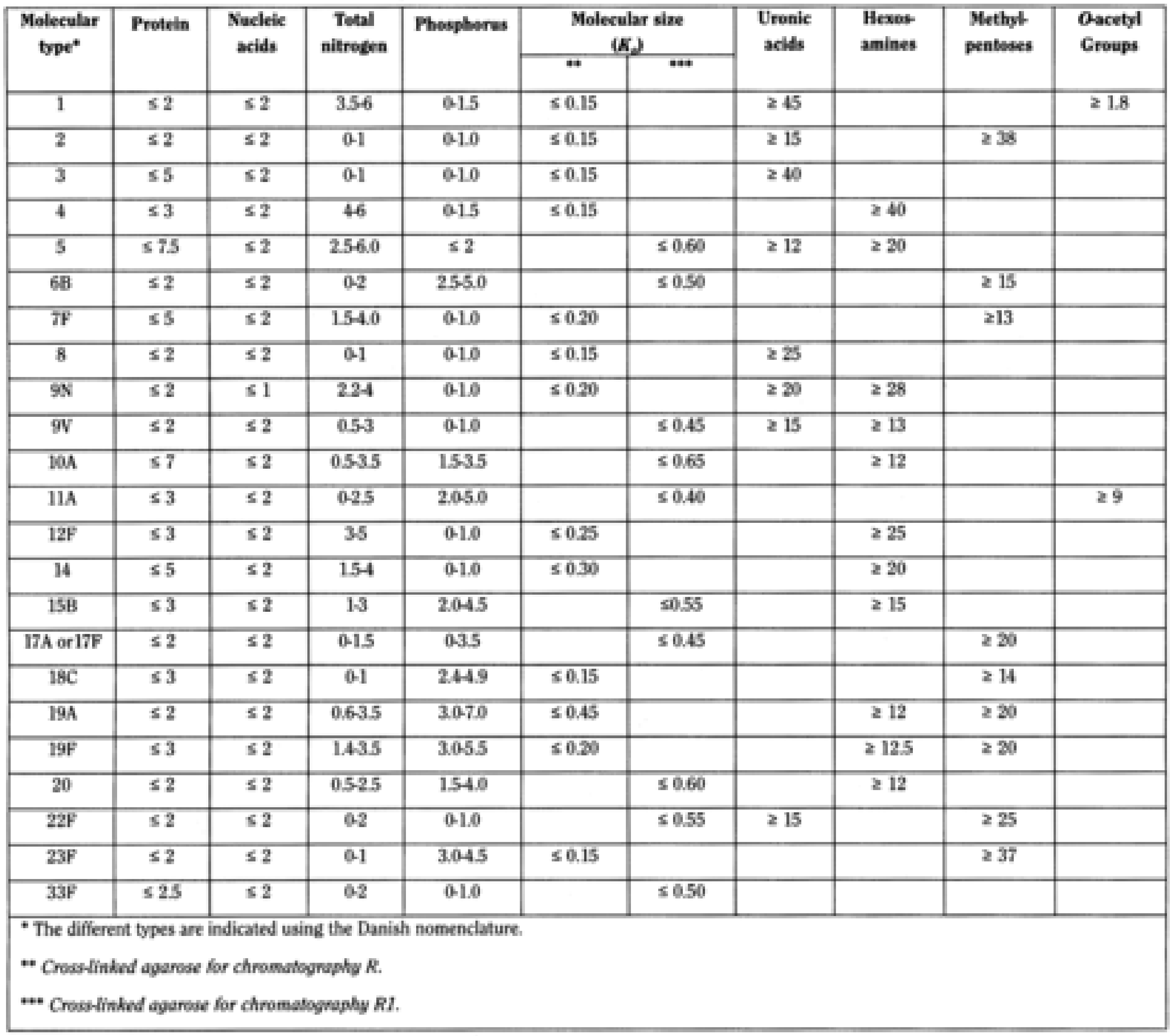
The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)* modified as follows for the test in guinea-pigs: inject 10 human doses into each guinea-pig and observe for 12 days.

MONOVALENT BULK POLYSACCHARIDES

The bacteria are grown in a suitable liquid medium that does not contain blood-group substances or high-molecular-mass polysaccharides. The bacterial purity of the culture is verified and the culture is inactivated with phenol. Impurities are removed by such techniques as fractional precipitation, enzymatic digestion and ultrafiltration. The polysaccharide is obtained by fractional precipitation, washed, and dried in a vacuum to a residual moisture content shown to be favourable to the stability of the polysaccharide. The residual moisture content is determined by drying under reduced pressure over diphosphorus pentoxide or by thermogravimetric analysis and the value obtained is used to calculate the results of the tests shown below with reference to the dried substance. The monovalent bulk polysaccharide is stored at a suitable temperature in conditions that avoid the uptake of moisture.

Only a monovalent bulk polysaccharide that complies with the following requirements may be used in the preparation of the final bulk vaccine. Percentage contents of components, determined by the methods prescribed below, are shown in Table 0966-1.

Table 0966.-1 – *Percentage contents of components of monovalent bulk polysaccharides*



Molecular type*	Protein	Nucleic acids	Total nitrogen	Phosphorus	Molecular size (K_d)		Uronic acids	Hexos-amines	Methyl-pentoses	O-acetyl Groups
					**	***				
1	≤ 2	≤ 2	3.5-6	0-1.5	≤ 0.15		≥ 45			≥ 1.8
2	≤ 2	≤ 2	0-1	0-1.0	≤ 0.15		≥ 15		≥ 38	
3	≤ 5	≤ 2	0-1	0-1.0	≤ 0.15		≥ 40			
4	≤ 3	≤ 2	4-6	0-1.5	≤ 0.15			≥ 40		
5	≤ 7.5	≤ 2	2.5-6.0	≤ 2		≤ 0.60	≥ 12	≥ 20		
6B	≤ 2	≤ 2	0-2	2.5-5.0		≤ 0.50			≥ 15	
7F	≤ 5	≤ 2	1.5-4.0	0-1.0	≤ 0.20				≥13	
8	≤ 2	≤ 2	0-1	0-1.0	≤ 0.15		≥ 25			
9N	≤ 2	≤ 1	2.2-4	0-1.0	≤ 0.20		≥ 20	≥ 28		
9V	≤ 2	≤ 2	0.5-3	0-1.0		≤ 0.45	≥ 15	≥ 13		
10A	≤ 7	≤ 2	0.5-3.5	1.5-3.5		≤ 0.65		≥ 12		
11A	≤ 3	≤ 2	0-2.5	2.0-5.0		≤ 0.40				≥ 9
12F	≤ 3	≤ 2	3-5	0-1.0	≤ 0.25			≥ 25		
14	≤ 5	≤ 2	1.5-4	0-1.0	≤ 0.30			≥ 20		
15B	≤ 3	≤ 2	1-3	2.0-4.5		≤0.55		≥ 15		
17A or17F	≤ 2	≤ 2	0-1.5	0-3.5		≤ 0.45			≥ 20	
18C	≤ 3	≤ 2	0-1	2.4-4.9	≤ 0.15				≥ 14	
19A	≤ 2	≤ 2	0.6-3.5	3.0-7.0	≤ 0.45			≥ 12	≥ 20	
19F	≤ 3	≤ 2	1.4-3.5	3.0-5.5	≤ 0.20			≥ 12.5	≥ 20	
20	≤ 2	≤ 2	0.5-2.5	1.5-4.0		≤ 0.60		≥ 12		
22F	≤ 2	≤ 2	0-2	0-1.0		≤ 0.55	≥ 15		≥ 25	
23F	≤ 2	≤ 2	0-1	3.0-4.5	≤ 0.15				≥ 37	
33F	≤ 2.5	≤ 2	0-2	0-1.0		≤ 0.50				

* The different types are indicated using the Danish nomenclature.

** *Cross-linked agarose for chromatography R.*

*** *Cross-linked agarose for chromatography R1.*

Protein ***(2.5.16)*.**

Nucleic acids ***(2.5.17)*.**

Total nitrogen ***(2.5.9)*.**

Phosphorus (*2.5.18*).

Molecular size

Determine by size-exclusion chromatography (2.2.30) using *cross-linked agarose for chromatography R* or *cross-linked agarose for chromatography R1.*

Uronic acids ***(2.5.22)*.**

Hexosamines ***(2.5.20)*.**

Methylpentoses ***(2.5.21)*.**

_O_-Acetyl groups _(2.5.19)_.

Identification _(2.7.1)_

Confirm the identity of the monovalent bulk polysaccharide by double immunodiffusion or electroimmunodiffusion (except for polysaccharides 7F, 14 and 33F), using specific antisera.

Specificity

No reaction occurs when the antigens are tested against all the antisera specific for the other polysaccharides of the vaccine, including factor sera for distinguishing types within groups. The polysaccharides are tested at a concentration of 50 µg/ml using a method capable of detecting 0.5 µg/ml.

FINAL BULK VACCINE

The final bulk vaccine is obtained by aseptically mixing the different polysaccharide powders. The uniform mixture is aseptically dissolved in a suitable isotonic solution so that one human dose of 0.50 ml contains 25 µg of each polysaccharide. An antimicrobial preservative may be added. The solution is sterilised by filtration through a bacteria-retentive filter.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

Sterility _(2.6.1)_

The final bulk vaccine complies with the test for sterility, using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers.

Only a final lot that is satisfactory with respect to each of the requirements given below under identification, tests and assay may be released for use. Provided that the tests for phenol and for antimicrobial preservative have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot. When consistency of production has been established on a suitable number of consecutive batches, the assay may be replaced by a qualitative test that identifies each polysaccharide, provided that an assay has been performed on each monovalent bulk polysaccharide used in the preparation of the final lot.

IDENTIFICATION

The assay serves also to identify the vaccine.

TESTS

pH _(2.2.3)_

The pH of the vaccine is 4.5 to 7.4.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Phenol ***(2.5.15)***

Not more than 2.5 g/l.

Sterility ***(2.6.1)***

It complies with the test for sterility.

Pyrogens ***(2.6.8)***

It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 ml of a dilution of the vaccine containing 2.5 µg/ml of each polysaccharide.

ASSAY

Determine the content of each polysaccharide by a suitable immunochemical method *(2.7.1)*, using antisera specific for each polysaccharide contained in the vaccine, including factor sera for types within groups, and purified polysaccharides of each type as standards.

The vaccine contains not less than 70 per cent and not more than 130 per cent of the quantity stated on the label for each polysaccharide. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated content.

LABELLING

The label states:

— the number of micrograms of each polysaccharide per human dose;

— the total amount of polysaccharide in the container.

Ph Eur

Poliomyelitis Vaccine, Live (Oral)

General Notices

(Poliomyelitis Vaccine (Oral), Ph Eur monograph 0215)

The label may state 'OPV'.

Ph Eur

DEFINITION

Oral poliomyelitis vaccine is a preparation of approved strains of live attenuated poliovirus type 1, 2 or 3 grown in *in vitro* cultures of approved cells, containing any one type or any combination of the 3 types of Sabin strains, presented in a form suitable for oral administration.

The vaccine is a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The vaccine strains and the production method shall have been shown to yield consistently vaccines that are both immunogenic and safe in man.

The production of vaccine is based on a virus seed-lot system. Cell lines are used according to a cell-bank system. If primary monkey kidney cell cultures are used, production complies with the requirements indicated below. Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more than 2 passages from the master seed lot.

REFERENCE STANDARDS

Poliomyelitis vaccine (oral) types 1, 2, 3 BRP is suitable for use as a virus reference preparation for the assay.

The International Standards for poliovirus type 2 (Sabin) for MAPREC (Mutant Analysis by PCR and Restriction Enzyme Cleavage) assays and poliovirus, type 3 (Sabin) synthetic DNA for MAPREC assays are suitable for use in the tests for genetic markers and the molecular tests for consistency of production.

Reference preparations of each poliovirus type at the Sabin Original + 2 passage level, namely WHO (SO + 2)/I for type 1 virus, WHO (SO + 2)/II for type 2 virus and WHO (SO + 2)/III for type 3 virus are available for comparison of the *in vivo* neurovirulence with that of homotypic vaccines. Requests for the WHO reference preparations for *in vivo* neurovirulence tests are to be directed to WHO, Biologicals, Geneva, Switzerland.

A suitable reference preparation is to be included in each test.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in human diploid cells (*5.2.3*), in continuous cell lines (*5.2.3*) or in primary monkey kidney cell cultures (including serially passaged cells from primary monkey kidney cells).

Primary monkey kidney cell cultures

The following special requirements for the substrate for virus propagation apply to primary monkey kidney cell cultures.

Monkeys used for preparation of primary monkey kidney cell cultures and for testing of virus If the vaccine is prepared in primary monkey kidney cell cultures, animals of a species approved by the competent authority, in good health, kept in closed or intensively monitored colonies and not previously employed for experimental purposes shall be used.

The monkeys shall be kept in well-constructed and adequately ventilated animal rooms in cages spaced as far apart as possible. Adequate precautions shall be taken to prevent cross-infection between cages. Not more than 2 monkeys shall be housed per cage and cage-mates shall not be interchanged. The monkeys shall be kept in the country of manufacture of the vaccine in quarantine groups for a period of not less than 6 weeks before use. A quarantine group is a colony of selected, healthy monkeys kept in one room, with separate feeding and cleaning facilities, and having no contact with other monkeys during the quarantine period. If at any time during the quarantine period the overall death rate of a shipment consisting of one or more groups reaches 5 per cent (excluding deaths from accidents or where the cause was specifically determined not to be an infectious disease), monkeys from that entire shipment shall continue in quarantine from that time for a minimum of 6 weeks. The groups shall be kept continuously in isolation, as in quarantine, even after completion of the quarantine period, until the monkeys are used. After the last monkey of a group has been taken, the room that housed the group shall be thoroughly cleaned and decontaminated before being used for a fresh group. If kidneys from near-term monkeys are used, the mother is quarantined for the term of pregnancy.

Monkeys from which kidneys are to be removed shall be anaesthetised and thoroughly examined, particularly for evidence of tuberculosis and cercopithecid herpesvirus 1 (B virus) infection.

If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of a seed lot or vaccine, it shall not be used, nor shall any of the remaining monkeys of the quarantine group concerned be used unless it is evident that their use will not impair the safety of the product.

All the operations described in this section shall be conducted outside the areas where the vaccine is produced.

The monkeys used shall be shown to be free from antibodies to simian virus 40 (SV40), simian immunodeficiency virus and spumaviruses. The blood sample used in testing for SV40 antibodies must be taken as close as possible to the time of removal of the kidneys. If *Macaca* spp. are used for production, the monkeys shall also be shown to be free from antibodies to cercopithecid herpesvirus 1 (B virus). Human herpesvirus has been used as an indicator for freedom from B virus antibodies on account of the danger of handling cercopithecid herpesvirus 1 (B virus). Monkeys used for the production of new seed lots are shown to be free from antibodies to simian cytomegalovirus (sCMV).

Primary monkey kidney cell cultures for vaccine production Kidneys that show no pathological signs are used for preparing cell cultures. If the monkeys are from a colony maintained for vaccine production, serially passaged monkey kidney cell cultures from primary monkey kidney cells may be used for virus propagation, otherwise the monkey kidney cells are not propagated in series. Virus for the preparation of vaccine is grown by aseptic methods in such cultures. If animal serum is used in the propagation of the cells, the maintenance medium after virus inoculation shall contain no added serum.

Each group of cell cultures derived from a single monkey or from foetuses from no more than 10 near-term monkeys is prepared and tested as an individual group.

VIRUS SEED LOTS

The strains of poliovirus used shall be identified by historical records that include information on the origin and subsequent manipulation of the strains.

Working seed lots are prepared by a single passage from a master seed lot and at an approved passage level from the original Sabin virus. Virus seed lots are prepared in large quantities and stored at a temperature below - 60 °C.

Only a virus seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each working seed lot is identified as poliovirus of the given type, using specific antibodies.

Virus concentration

Determined by the method described below, the virus concentration is the basis for the quantity of virus used in the neurovirulence test.

Extraneous agents *(2.6.16)*

If the working seed lot is produced in human diploid cells or in a continuous cell line, it complies with the requirements for seed lots for virus vaccines. If the working seed lot is produced in primary monkey kidney cell cultures, it complies with the requirements given below under Virus Propagation and Harvest and Monovalent Pooled Harvest and with the tests in adult mice, suckling mice and guinea-pigs given in chapter *2.6.16*.

In addition to the requirements in chapter *2.6.16*, for vaccines produced in cell lines and when the seed lot was produced in primary monkey kidney cell cultures, a validated test for sCMV is performed.

Working seed lots shall be free from detectable DNA sequences from simian virus 40 (SV40).

Neurovirulence

Each master and working seed lot complies with the test for neurovirulence of poliomyelitis vaccine (oral) in monkeys *(2.6.19)*. In addition, at least the first 4 consecutive batches of monovalent pooled harvest prepared from these seed lots shall be shown to comply with the test for neurovirulence of poliomyelitis vaccine (oral) in monkeys *(2.6.19)* before the seed lot is deemed suitable for use. Furthermore, the seed lot shall cease to be used in vaccine production if the frequency of failure of the monovalent pooled harvests produced from it is greater than predicted statistically. This statistical prediction is calculated after each test on the basis of all the monovalent pooled harvests tested; it is equal to the probability of false rejection on the occasion of a first test (i.e.1 per cent), the probability of false rejection on retest being negligible. If the test is carried out only by the manufacturer, the test slides are provided to the control authority for assessment.

Genetic markers

Each working seed lot is tested for its replicating properties at temperatures ranging from 36 °C to 40 °C as described under Monovalent pooled harvest. A profile (i.e. percentage of mutant) of the seed virus using the MAPREC assay is prepared. Type 3 virus seed lots comply with the MAPREC assay as described under Monovalent pooled harvest.

VIRUS PROPAGATION AND HARVEST

All processing of the cell banks and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled during the production. Suitable animal (but not human) serum may be used in the culture media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from live extraneous agents. The cell-culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. On the day of inoculation with the virus working seed lot, not less than 5 per cent or 1000 ml, whichever is the less, of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells). Special requirements, given below, apply to control cells when the vaccine is produced in primary monkey kidney cell cultures. The virus suspension is harvested not later than 4 days after virus inoculation. After inoculation of the production cell culture with the virus working seed lot, inoculated cells are maintained at a fixed temperature, shown to be suitable, within the range 33-35 °C; the temperature is maintained constant to ± 0.5 °C; control cell cultures are maintained at 33-35 °C for the relevant incubation periods.

Only a single virus harvest that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Virus concentration

The virus concentration of virus harvests is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Molecular tests for consistency of production

The MAPREC assay is performed on each virus harvest. The acceptance/rejection criteria for consistency of production are determined for each manufacturer and for each working seed by agreement with the competent authority. These criteria are periodically reviewed and updated to the satisfaction of the competent authority. An investigation of consistency occurs if a virus harvest gives results that are inconsistent with previous production history.

Control cells

The control cells of the production cell culture from which the virus harvest is derived comply with a test for identity and with the requirements for extraneous agents *(2.6.16)* or, where primary monkey kidney cell cultures are used, as shown below.

Primary monkey kidney cell cultures

The following special requirements apply to virus propagation and harvest in primary monkey kidney cell cultures.

Cell cultures On the day of inoculation with the virus working seed lot, each cell culture is examined for degeneration caused by an infective agent. If, in this examination, evidence is found of the presence in a cell culture of any extraneous agent, the entire group of cultures concerned shall be rejected.

On the day of inoculation with the virus working seed lot, a sample of at least 30 ml of the pooled fluid removed from the cell cultures of the kidneys of each single monkey or from foetuses from not more than 10 near-term monkeys is divided into 2 equal portions. 1 portion of the pooled fluid is tested in monkey kidney cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other portion of the pooled fluid is, where necessary, tested in monkey kidney cell cultures from another species so that tests on the pooled fluids are done in cell cultures from at least 1 species known to be sensitive to SV40. The pooled fluid is inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet is at least 3 cm^2/ml of pooled fluid. At least 1 bottle of each type of cell culture remains uninoculated to serve as a control. If the monkey species used for vaccine production is known to be sensitive to SV40, a test in a 2nd species is not required. Animal serum may be used in the propagation of the cells, provided that it does not contain SV40 antibody, but the maintenance medium after inoculation of test material contains no added serum except as described below.

The cultures are incubated at a temperature of 35-37 °C and are observed for a total period of at least 4 weeks. During this observation period and after not less than 2 weeks' incubation, at least 1 subculture of fluid is made from each of these cultures in the same cell culture system. The subcultures are also observed for at least 2 weeks.

Serum may be added to the original culture at the time of subculturing, provided that the serum does not contain SV40 antibody.

Fluorescent-antibody techniques may be useful for detecting SV40 virus and other viruses in the cells.

A further sample of at least 10 ml of the pooled fluid is tested for cercopithecid herpesvirus 1 (B virus) and other viruses in rabbit kidney cell cultures. Serum used in the nutrient medium of these cultures shall have been shown to be free from inhibitors of B virus. Human herpesvirus has been used as an indicator for freedom from B virus inhibitors on account of the danger of handling cercopithecid herpesvirus 1 (B virus). The sample is inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet is at least 3 cm^2/ml of pooled fluid. At least 1 bottle of the cell cultures remains uninoculated to serve as a control.

The cultures are incubated at a temperature of 35-37 °C and observed for at least 2 weeks.

A further sample of 10 ml of the pooled fluid removed from the cell cultures on the day of inoculation with the seed lot virus is tested for the presence of extraneous agents by inoculation into human cell cultures sensitive to measles virus.

The tests are not valid if more than 20 per cent of the culture vessels have been discarded for non-specific accidental reasons by the end of the respective test periods.

If, in these tests, evidence is found of the presence of an extraneous agent, the single harvest from the whole group of cell cultures concerned is rejected.

If the presence of cercopithecid herpesvirus 1 (B virus) is demonstrated, the manufacture of oral poliomyelitis vaccine shall be discontinued and the competent authority shall be informed. Manufacturing shall not be resumed until a thorough investigation has been completed and precautions have been taken against any reappearance of the infection, and then only with the approval of the competent authority.

If these tests are not done immediately, the samples of pooled cell-culture fluid shall be kept at a temperature of - 60 °C or below, with the exception of the sample for the test for B virus, which may be held at 4 °C, provided that the test is done not more than 7 days after it has been taken.

Control cell cultures On the day of inoculation with the virus working seed lot, 25 per cent (but not more than 2.5 litres) of the cell suspension obtained from the kidneys of each single monkey or from not more than 10 near-term monkeys is taken to prepare uninoculated control cell cultures. These control cell cultures are incubated in the same conditions as the inoculated cultures for at least 2 weeks and are examined during this period for evidence of cytopathic changes. The tests are not valid if more than 20 per cent of the control cell cultures have been discarded for non-specific, accidental reasons. At the end of the observation period, the control cell cultures are examined for degeneration caused by an infectious agent. If this examination or any of the tests required in this section shows evidence of the presence in a control culture of any extraneous agent, the poliovirus grown in the corresponding inoculated cultures from the same group shall be rejected.

Tests for haemadsorbing viruses At the time of harvest or within 4 days of inoculation of the production cultures with the virus working seed lot, a sample of 4 per cent of the control cell cultures is taken and tested for haemadsorbing viruses. At the end of the observation period, the remaining control cell cultures are similarly tested. The tests are carried out as described in chapter *2.6.16*.

Tests for other extraneous agents At the time of harvest, or within 7 days of the day of inoculation of the production cultures with the working seed lot, a sample of at least 20 ml of the pooled fluid from each group of control cultures is taken and tested in 2 kinds of monkey kidney cell culture, as described above.

At the end of the observation period for the original control cell cultures, similar samples of the pooled fluid are taken and the tests referred to in this section in the 2 kinds of monkey kidney cell culture and in the rabbit cell cultures are repeated, as described above under Cell cultures.

If the presence of cercopithecid herpesvirus 1 (B virus) is demonstrated, the production cell cultures shall not be used and the measures concerning vaccine production described above must be undertaken.

The fluids collected from the control cell cultures at the time of virus harvest and at the end of the observation period may be pooled before testing for extraneous agents. A sample of 2 per cent of the pooled fluid is tested in each of the cell culture systems specified.

Single harvests

Tests for neutralised single harvests in primary monkey kidney cell cultures A sample of at least 10 ml of each single harvest is neutralised by a type-specific poliomyelitis antiserum prepared in animals other than monkeys. In preparing antisera for this purpose, the immunising antigens used shall be prepared in non-simian cells.

Half of the neutralised suspension (corresponding to at least 5 ml of single harvest) is tested in monkey kidney cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other half of the neutralised suspension is tested, if necessary, in monkey kidney cell cultures from another species so that the tests on the neutralised suspension are done in cell cultures from at least 1 species known to be sensitive to SV40.

The neutralised suspensions are inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet is at least 3 cm^2/ml of neutralised suspension. At least 1 bottle of each type of cell culture remains uninoculated to serve as a control and is maintained by nutrient medium containing the same concentration of the specific antiserum used for neutralisation.

Animal serum may be used in the propagation of the cells, provided that it does not contain SV40 antibody, but the maintenance medium, after the inoculation of the test material, contains no added serum other than the poliovirus neutralising antiserum, except as described below.

The cultures are incubated at a temperature of 35-37 °C and observed for a total period of at least 4 weeks. During this observation period and after not less than 2 weeks' incubation, at least 1 subculture of fluid is made from each of these cultures in the same cell-culture system. The subcultures are also observed for at least 2 weeks.

Serum may be added to the original cultures at the time of subculturing, provided that the serum does not contain SV40 antibody.

Additional tests are made for extraneous agents on a further sample of the neutralised single harvests by inoculation of 10 ml into human cell cultures sensitive to measles virus. This test is also validated for the detection of sCMV.

Fluorescent-antibody techniques may be useful for detecting SV40 virus and other viruses in the cells.

The tests are not valid if more than 20 per cent of the culture vessels have been discarded for non-specific accidental reasons by the end of the respective test periods.

If any cytopathic changes occur in any of the cultures, the causes of these changes are investigated. If the cytopathic changes are shown to be due to unneutralised poliovirus, the test is repeated. If there is evidence of the presence of SV40 or other extraneous agents attributable to the single harvest, that single harvest is rejected.

MONOVALENT POOLED HARVEST

Monovalent pooled harvests are prepared by pooling a number of satisfactory single harvests of the same virus type. Monovalent pooled harvests from continuous cell lines may be purified. Each monovalent pooled harvest is filtered through a bacteria-retentive filter.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

Each monovalent pooled harvest is identified as poliovirus of the given type, using specific antibodies.

Virus concentration

The virus concentration is determined by the method described below and serves as the basis for calculating the dilutions for preparation of the final bulk, for the quantity of virus used in the neurovirulence test and to establish and monitor production consistency.

Genetic markers

For Sabin poliovirus type 3, a validated MAPREC assay is performed. In this analysis the amount of the mutation at position 472 of the genome (472-C) is estimated and expressed as a ratio relative to the International Standard for MAPREC analysis of poliovirus type 3 (Sabin). A poliovirus type 3 monovalent pooled harvest found to have significantly more 472-C than the International Standard for MAPREC analysis of poliovirus type 3 (Sabin) fails in the MAPREC assay.

The MAPREC analysis of poliovirus type 3 (Sabin) is carried out using a standard operating procedure approved by the competent authority. A suitable procedure (*Mutant analysis by PCR and restriction enzyme cleavage (MAPREC) for oral poliovirus (Sabin) vaccine*) is available from WHO, Quality and Safety of Biologicals (QSB), Geneva. A laboratory must demonstrate to the competent authority that it is competent to perform the assay. The manufacturer and the competent authority shall agree on the procedure and the criteria for deciding whether a monovalent pooled harvest contains significantly more 472-C than the International Standard.

Acceptance/rejection criteria for assessment of consistency of production are determined for each manufacturer and for each working seed lot by agreement with the competent authority. These criteria are updated as each new bulk is prepared and analysed. An investigation of consistency occurs if a monovalent pooled harvest gives results that are inconsistent with previous production history.

As the MAPREC assay for type 3 poliovirus (Sabin) is highly predictive of *in vivo* neurovirulence, if a filtered monovalent pooled harvest of type 3 poliovirus (Sabin) fails the MAPREC assay then this triggers an investigation of the consistency of the manufacturing process. This investigation also includes a consideration of the suitability of the working seed lot.

Monovalent pooled harvests passing the MAPREC assay are subsequently tested for in vivo neurovirulence.

For poliovirus type 3, results from the MAPREC assay and the monkey neurovirulence test (*2.6.19*) are used concomitantly to assess the impact of changes in the production process or when a new manufacturer starts production.

Pending validation of MAPREC assays for poliovirus types 1 and 2, for these viruses filtered bulk suspension is tested for the property of reproducing at temperatures of 36 °C and 40 °C. A ratio of the replication capacities of the virus in the monovalent pooled harvest is obtained over a temperature range between 36 °C and 40 °C in comparison with the seed lot or a reference preparation for the marker tests and with appropriate rct/40- and rct/40+ strains of poliovirus of the same type. The incubation temperatures used in this test are controlled to within ± 0.1 °C. The monovalent pooled harvest passes the test if, for both the virus in the harvest and the appropriate reference material, the titre determined at 36 °C is at least 5.0 log greater than that determined at 40 °C. If growth at 40 °C is so low that a valid comparison cannot be established, a temperature in the region of 39.0-39.5 °C is used, at which temperature the reduction in titre of the reference material must be in the range 3.0-5.0 log of its value at 36 °C; the acceptable minimum reduction is determined for each virus strain at a given temperature. If the titres obtained for 1 or more of the reference viruses are not concordant with the expected values, the test must be repeated.

Neurovirulence *(2.6.19)*

Each monovalent pooled harvest complies with the test for neurovirulence of poliomyelitis vaccine (oral). If the monkey neurovirulence test is carried out only by the manufacturer, the test slides are provided to the competent authority for assessment. The TgPVR21 transgenic mouse model provides a suitable alternative to the monkey neurovirulence test for neurovirulence testing of types 1, 2 or 3 vaccines once a laboratory qualifies as being competent to perform the test and the experience gained is to the satisfaction of the competent authority. The test is carried out using a standard operating procedure approved by the competent authority. A suitable procedure (*Neurovirulence test of type 1, 2 or 3 live poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus*) is available from WHO, Quality and Safety of Biologicals, Geneva.

Primary monkey kidney cell cultures

The following special requirements apply to monovalent pooled harvests derived from primary monkey kidney cell cultures.

Retroviruses The monovalent pooled harvest is examined using a reverse transcriptase assay. No indication of the presence of retroviruses is found.

Test in rabbits A sample of the monovalent pooled harvest is tested for cercopithecid herpesvirus 1 (B virus) and other viruses by injection of not less than 100 ml into not fewer than 10 healthy rabbits each weighing 1.5-2.5 kg. Each rabbit receives not less than 10 ml and not more than 20 ml, of which 1 ml is given intradermally at multiple sites since the maximum volume to be given intradermally at each site is 0.1 ml, and the remainder subcutaneously. The rabbits are observed for at least 3 weeks for death or signs of illness.

All rabbits that die after the first 24 h of the test and those showing signs of illness are examined by autopsy, and the brain and organs removed for detailed examination to establish the cause of death.

The test is not valid if more than 20 per cent of the inoculated rabbits show signs of intercurrent infection during the observation period. The monovalent pooled harvest passes the test if none of the rabbits shows evidence of infection with B virus or with other extraneous agents or lesions of any kind attributable to the bulk suspension.

If the presence of B virus is demonstrated, the measures concerning vaccine production described above under Cell cultures are taken.

Test in guinea-pigs. If the primary monkey kidney cell cultures are not derived from monkeys kept in a closed colony, the monovalent pooled harvest shall be shown to comply with the following test Administer to each of not fewer than 5 guinea-pigs, each weighing 350-450 g, 0.1 ml of the monovalent pooled harvest by intracerebral injection (0.05 ml in each cerebral hemisphere) and 0.5 ml by intraperitoneal injection. Measure the rectal temperature of each animal on each working day for 6 weeks. At the end of the observation period carry out autopsy on each animal.

In addition, administer to not fewer than 5 guinea-pigs 0.5 ml by intraperitoneal injection and observe as described above for 2-3 weeks. At the end of the observation period, carry out a passage from these animals to not fewer than 5 guinea-pigs using blood and a suspension of liver or spleen tissue. Measure the rectal temperature of the latter guinea-pigs for 2-3 weeks. Examine by autopsy all animals that, after the first day of the test, die or are euthanised because they show disease, or show on 3 consecutive days a body temperature higher than 40.1 °C; carry out histological examination to detect infection with filoviruses; in addition, inject a suspension of liver or spleen tissue or of blood intraperitoneally into not fewer than 3 guinea-pigs. If any signs of infection with filoviruses are noted, confirmatory serological tests are carried out on the blood of the affected animals. The monovalent pooled harvest complies with the test if not fewer than 80 per cent of the guinea-pigs survive to the end of the observation period and remain in good health, and no animal shows signs of infection with filoviruses.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more satisfactory monovalent pooled harvests and may contain more than one virus type. Suitable flavouring substances and stabilisers may be added.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Bacterial and fungal contamination

Carry out the test for sterility (*2.6.1*), using 10 ml for each medium.

FINAL LOT

Only a final lot that complies with the following requirement for thermal stability and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

IDENTIFICATION

The vaccine is shown to contain poliovirus of each type stated on the label, using specific antibodies.

TESTS

Bacterial and fungal contamination

The vaccine complies with the test for sterility (*2.6.1*).

Thermal stability

Maintain not fewer than 3 vials of the final lot at 37 ± 1 °C for 48 h. Determine the total virus concentration as described under Assay in parallel for the heated vaccine and for vaccine maintained at the temperature recommended for storage. The estimated difference between the total virus concentration of the unheated and heated vaccines is not greater than 0.5 log infectious virus units ($CCID_{50}$) per single human dose.

ASSAY

Titrate the vaccine for infectious virus, using not fewer than 3 separate vials of vaccine, following the method described below. Titrate 1 vial of an appropriate virus reference preparation in triplicate to validate each assay. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory. If the vaccine contains more than one poliovirus type, titrate each type separately, using an appropriate type-specific antiserum (or preferably a monoclonal antibody) to neutralise each of the other types present.

Calculate the individual virus concentration for each vial of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations, using the usual statistical methods (for example, *5.3*).

For a trivalent vaccine, the combined estimated virus titres per single human dose must be:

— not less than 6.0 log infectious virus units ($CCID_{50}$) for type 1;

— not less than 5.0 log infectious virus units ($CCID_{50}$) for type 2; and

— not less than 5.5 log infectious virus units ($CCID_{50}$) for type 3.

For a monovalent or divalent vaccine, the minimum virus titres are decided by the competent authority.

Method Inoculate a suitable number of wells in a microtitre plate with a suitable volume of each of the selected dilutions of virus followed by a suitable volume of a cell suspension of the Hep-2 (Cincinnati) line. Examine the cultures between days 7 and 9.

The assay is not valid if:

— the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than ± 0.3 log $CCID_{50}$;

— the virus concentration of the reference preparation differs by more than 0.5 log $CCID_{50}$ from the established value. The relation with the appropriate European Pharmacopoeia Biological Reference Preparation is established and monitored at regular intervals when a manufacturer's reference preparation is used.

The assay is repeated if the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 log $CCID_{50}$; data obtained from valid assays only are combined by the usual statistical methods (for example, 5.3) to calculate the virus concentration of the sample. The confidence interval ($P = 0.95$) of the combined virus concentration is not greater than ± 0.3 log $CCID_{50}$.

Poliomyelitis vaccine (oral) BRP is suitable for use as a reference preparation.

Where justified and authorised, different assay designs may be used; this may imply the application of different validity and acceptance criteria. However, the vaccine must comply if tested as described above.

LABELLING

The label states:

- the types of poliovirus contained in the vaccine;
- the minimum amount of virus of each type contained in a single human dose;
- the cell substrate used for the preparation of the vaccine;
- that the vaccine is not to be injected.

Ph Eur

Rabies Vaccine

General Notices

(Rabies Vaccine for Human Use Prepared in Cell Cultures, Ph Eur monograph 0216)

The label may state 'Rab'.

Ph Eur

DEFINITION

Rabies vaccine for human use prepared in cell cultures is a freeze-dried preparation of a suitable strain of fixed rabies virus grown in cell cultures and inactivated by a validated method.

The vaccine is reconstituted immediately before use as stated on the label to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

GENERAL PROVISIONS

The production of the vaccine is based on a virus seed-lot system and, if a cell line is used for virus propagation, a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability. Unless otherwise justified and authorised, the virus in the final vaccine must not have undergone more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy; even with authorised exceptions, the number of passages beyond the level used for clinical studies must not exceed 5.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (*2.6.9*).

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in a human diploid cell line (*5.2.3*), in a continuous cell line approved by the competent authority, or in cultures of chick-embryo cells derived from a flock free from specified pathogens (*5.2.2*).

SEED LOTS

The strain of rabies virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Working seed lots are prepared by not more than 5 passages from the master seed lot.

Only a working seed lot that complies with the following tests may be used for virus propagation.

Identification

Each working seed lot is identified as rabies virus using specific antibodies.

Virus concentration

The virus concentration of each working seed lot is determined by a cell culture method using immunofluorescence, to ensure consistency of production.

Extraneous agents (*2.6.16*)

The working seed lot complies with the requirements for virus seed lots. If the virus has been passaged in mouse brain, specific tests for murine viruses are carried out.

VIRUS PROPAGATION AND ACTIVATION

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Approved animal (but not human) serum may be used in the media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum; the media may contain human albumin. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells). The virus suspension is harvested on one or more occasions during incubation. Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Only a single harvest that complies with the following requirements may be used in the preparation of the inactivated viral harvest.

Identification

The single harvest contains virus that is identified as rabies virus using specific antibodies.

Virus concentration

Titrate for infective virus in cell cultures; the titre is used to monitor consistency of production.

Control cells

The control cells of the production cell culture from which the single harvest is derived comply with a test for identification and with the requirements for extraneous agents (*2.6.16*).

PURIFICATION AND INACTIVATION

The virus harvest may be concentrated and/or purified by suitable methods; the virus harvest is inactivated by a validated method at a fixed, well-defined stage of the process, which may be before, during or after any concentration or purification. The method shall have been shown to be capable of inactivating rabies virus without destruction of the immunogenic activity. If betapropiolactone is used, the concentration shall at no time exceed 1:3500.

Only an inactivated viral suspension that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Residual infectious virus

Carry out an amplification test for residual infectious rabies virus immediately after inactivation or using a sample frozen immediately after inactivation and stored at - 70 °C. Inoculate a quantity of inactivated viral suspension equivalent to not less than 25 human doses of vaccine into cell cultures of the same type as those used for production of the vaccine. A passage may be made after 7 days. Maintain the cultures for a total of 21 days and then examine the cell cultures for rabies virus using an immunofluorescence test. The inactivated virus harvest complies with the test if no rabies virus is detected.

Residual host-cell DNA

If a continuous cell line is used for virus propagation, the content of residual host-cell DNA, determined using a suitable method as described in *Products of recombinant DNA technology (0784),* is not greater than 10 ng per single human dose.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated viral suspensions. An approved stabiliser may be added to maintain the activity of the product during and after freeze-drying.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Glycoprotein content

Determine the glycoprotein content by a suitable immunochemical method (*2.7.1*), for example, single-radial immunodiffusion, enzyme-linked immunosorbent assay or an antibody-binding test. The content is within the limits approved for the particular product.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers and freeze-dried to a moisture content shown to be favourable to the stability of the vaccine. The containers are then closed so as to avoid contamination and the introduction of moisture.

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for residual infectious virus has been carried out with satisfactory results on the inactivated viral suspension and the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

IDENTIFICATION

The vaccine is shown to contain rabies virus antigen by a suitable immunochemical method (2.7.1) using specific antibodies, preferably monoclonal; alternatively, the assay serves also to identify the vaccine.

TESTS

Residual infectious virus

Inoculate a quantity equivalent to not less than 25 human doses of vaccine into cell cultures of the same type as those used for production of the vaccine. A passage may be made after 7 days. Maintain the cultures for a total of 21 days and then examine the cell cultures for rabies virus using an immunofluorescence test. The vaccine complies with the test if no rabies virus is detected.

Bovine serum albumin

Maximum 50 ng per single human dose, determined by a suitable immunochemical method *(2.7.1)*.

Sterility *(2.6.1)*

It complies with the test.

Bacterial endotoxins *(2.6.14)*

Less than 25 IU per single human dose.

Pyrogens *(2.6.8)*

It complies with the test. Unless otherwise justified and authorised, inject into each rabbit a single human dose of the vaccine diluted to 10 times its volume.

Water *(2.5.12)*

Maximum 3.0 per cent.

ASSAY

The potency of rabies vaccine is determined by comparing the dose necessary to protect mice against the effects of a lethal dose of rabies virus, administered intracerebrally, with the quantity of a reference preparation of rabies vaccine necessary to provide the same protection. For this comparison a reference preparation of rabies vaccine, calibrated in International Units, and a suitable preparation of rabies virus for use as the challenge preparation are necessary.

The International Unit is the activity contained in a stated quantity of the International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

The test described below uses a parallel-line model with at least 3 points for the vaccine to be examined and the reference preparation. Once the analyst has experience with the method for a given vaccine, it is possible to carry out a simplified test using a single dilution of the vaccine to be examined. Such a test enables the analyst to determine that the vaccine has a potency significantly higher than the required minimum, but does not give full information on the validity of each individual potency determination. The use of a single dilution allows a considerable reduction in the number of animals required for the test and must be considered by each laboratory in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Selection and distribution of the test animals Use healthy female mice, about 4 weeks old, each weighing 11-15 g, and from the same stock. Distribute the mice into 6 groups of a size suitable to meet the requirements for validity of the test and, for titration of the challenge suspension, 4 groups of 5.

Preparation of the challenge suspension Inoculate mice intracerebrally with the Challenge Virus Standard (CVS) strain of rabies virus and when the mice show signs of rabies, but before they die, euthanise them, then remove the brains and prepare a homogenate of the brain tissue in a suitable diluent. Separate gross particulate matter by centrifugation and use the supernatant liquid as the challenge suspension. Distribute the suspension in small volumes in ampoules, seal and store at a temperature below - 60 °C. Thaw one ampoule of the suspension and make serial dilutions in a suitable diluent. Allocate each dilution to a group of 5 mice and inject intracerebrally into each mouse 0.03 ml of the dilution allocated to its group. Observe the mice for 14 days. Calculate the LD_{50} of the undiluted suspension using the number in each group that, between the 5th and 14th days, die or develop signs of rabies.

Determination of potency of the vaccine Prepare 3 fivefold serial dilutions of the vaccine to be examined and 3 fivefold serial dilutions of the reference preparation. Prepare the dilutions such that the most concentrated suspensions may be expected to protect more than 50 per cent of the animals to which they are administered and the least concentrated suspensions may be expected to protect less than 50 per cent of the animals to which they are administered. Allocate the 6 dilutions, 1 to each of the 6 groups of mice, and inject by the intraperitoneal route into each mouse 0.5 ml of the dilution allocated to its group. After 7 days, prepare 3 identical dilutions of the vaccine to be examined and of the reference preparation and repeat the injections. 7 days after the second injection, prepare a suspension of the challenge virus such that, on the basis of the preliminary titration, 0.03 ml contains about 50 LD_{50}. Inject intracerebrally into each vaccinated mouse 0.03 ml of this suspension. Prepare 3 suitable serial dilutions of the challenge suspension. Allocate the challenge suspension and the 3 dilutions, 1 to each of the 4 groups of 5 control mice, and inject intracerebrally into each mouse 0.03 ml of the suspension or dilution allocated to its group. Observe the animals in each group for 14 days and record the number in each group that die or show signs of rabies in the period 5-14 days after challenge.

The test is not valid unless:

— for both the vaccine to be examined and the reference preparation the 50 per cent protective dose lies between the largest and smallest doses given to the mice;

— the titration of the challenge suspension shows that 0.03 ml of the suspension contained not less than 10 LD_{50};

— the statistical analysis shows a significant slope and no significant deviations from linearity or parallelism of the dose-response curves;

— the confidence limits ($P = 0.95$) are not less than 25 per cent and not more than 400 per cent of the estimated potency.

The vaccine complies with the test if the estimated potency is not less than 2.5 IU per human dose.

Application of alternative end-points Once a laboratory has established the above assay for routine use, the lethal end-point is replaced by an observation of clinical signs and application of an end-point earlier than death to reduce animal suffering. The following is given as an example.

The progress of rabies infection in mice following intracerebral injection can be represented by 5 stages defined by typical clinical signs:

Stage 1: ruffled fur, hunched back;

Stage 2: slow movements, loss of alertness (circular movements may also occur);

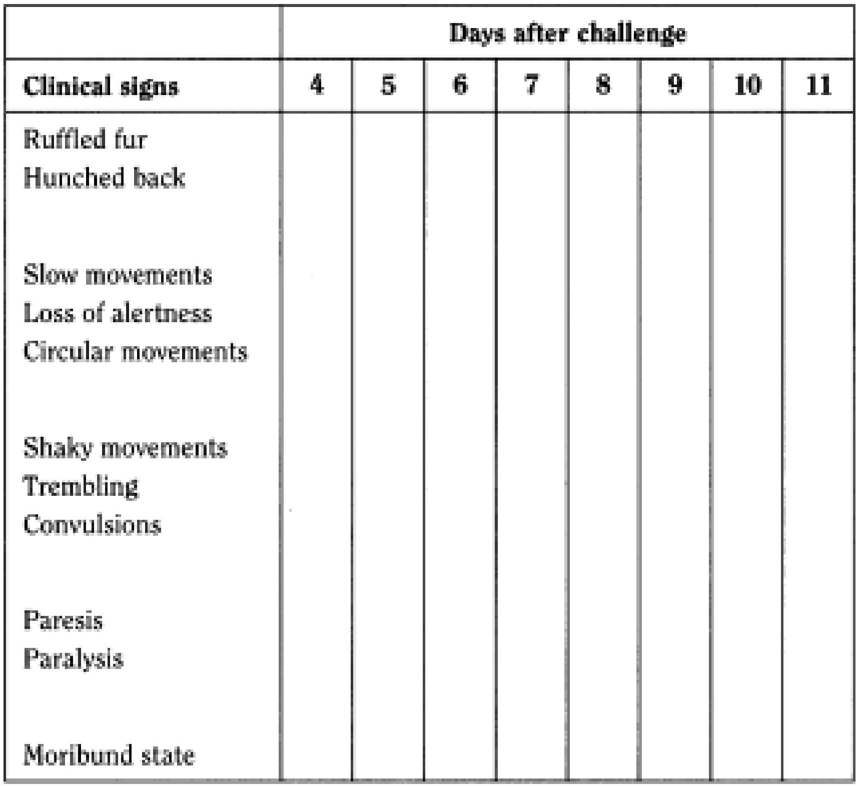
Stage 3: shaky movements, trembling, convulsions;

Stage 4: signs of paresis or paralysis;

Stage 5: moribund state.

Mice are observed at least twice daily from day 4 after challenge. Clinical signs are recorded using a chart such as that shown in Table 0216.-1. Experience has shown that using stage 3 as an end-point yields assay results equivalent to those found when a lethal end-point is used. This must be verified by each laboratory by scoring a suitable number of assays using both the clinical signs and the lethal end-point.

Table 0216.-1. – *Example of a chart used to record clinical signs in the rabies vaccine potency test*



	Days after challenge							
Clinical signs	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Ruffled fur								
Hunched back								
Slow movements								
Loss of alertness								
Circular movements								
Shaky movements								
Trembling								
Convulsions								
Paresis								
Paralysis								
Moribund state								

LABELLING

The label states the biological origin of the cells used for the preparation of the vaccine.

Ph Eur

Rubella Vaccine, Live

General Notices

(Ph Eur monograph 0162)

The label may state 'Rubella'.

Ph Eur

DEFINITION

Rubella vaccine (live) is a freeze-dried preparation of a suitable attenuated strain of rubella virus. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The production of vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently live rubella vaccines of adequate immunogenicity and safety in man. Unless otherwise justified and authorised, the virus in the final vaccine shall have undergone no more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

The potential neurovirulence of the vaccine strain is considered during preclinical development, based on available epidemiological data on neurovirulence and neurotropism, primarily for the wild-type virus. In light of this, a risk analysis is carried out. Where necessary and if available, a test is carried out on the vaccine strain using an animal model that differentiates wild-type and attenuated virus; tests on strains of intermediate attenuation may also be needed.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in human diploid cells *(5.2.3)*.

SEED LOT

The strain of rubella virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are prepared in large quantities and stored at temperatures below - 20 °C if freeze-dried, or below - 60 °C if not freeze-dried.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

The master and working seed lots are identified as rubella virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration

The virus concentration of the master and working seed lots is determined to ensure consistency of production.

Extraneous agents *(2.6.16)*

The working seed lot complies with the requirements for seed lots.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled during the production. Suitable animal (but not human) serum may be used in the growth medium, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 500 ml of the production cell cultures is set aside as uninfected cell cultures (control cells). The temperature of incubation is controlled during the growth of the virus. The virus suspension is harvested, on one or more occasions, within 28 days of inoculation. Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Only a single harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

The single harvest contains virus that is identified as rubella virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration

The virus concentration in the single harvest is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents *(2.6.16)*

The single harvest complies with the tests for extraneous agents.

Control cells

The control cells comply with a test for identification and with the tests for extraneous agents *(2.6.16)*.

FINAL BULK VACCINE

Single harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Bacterial and fungal contamination

The final bulk vaccine complies with the test for sterility *(2.6.1)*, carried out using 10 ml for each medium.

FINAL LOT

A minimum virus concentration for release of the product is established such as to ensure, in light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the requirements for minimum virus concentration for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability

Maintain at least 3 vials of the final lot of freeze-dried vaccine in the dry state at 37 ± 1 °C for 7 days. Determine the virus concentration as described under Assay in parallel for the heated vaccine and for vaccine stored at the temperature recommended for storage. The virus concentration of the heated vaccine is not more than 1.0 log lower than that of the unheated vaccine.

IDENTIFICATION

When the vaccine reconstituted as stated on the label is mixed with specific rubella antibodies, it is no longer able to infect susceptible cell cultures.

TESTS

Bacterial and fungal contamination

The reconstituted vaccine complies with the test for sterility *(2.6.1)*.

Bovine serum albumin

Not more than 50 ng per single human dose, determined by a suitable immunochemical method *(2.7.1)*.

Water *(2.5.12)*

Not more than 3.0 per cent, determined by the semi-micro determination of water.

ASSAY

Titrate the vaccine for infective virus, using at least 3 separate vials of vaccine and inoculating a suitable number of wells for each dilution step. Titrate 1 vial of an appropriate virus reference preparation in triplicate to validate each assay. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory. The relation with the appropriate European Pharmacopoeia Biological Reference Preparation is established and monitored at regular intervals if a manufacturer's reference preparation is used. Calculate the individual virus concentration for each vial of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations, using the usual statistical methods (for example, *5.3*). The combined estimate of the virus concentration for the 3 vials of vaccine is not less than that stated on the label; the minimum virus concentration stated on the label is not less than 3.0 log $CCID_{50}$ per single human dose.

The assay is not valid if:

- the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than ± 0.3 log $CCID_{50}$;
- the virus concentration of the reference preparation differs by more than 0.5 log $CCID_{50}$ from the established value.

The assay is repeated if the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 log $CCID_{50}$; data obtained from valid assays only are combined by the usual statistical methods (for example, 5.3) to calculate the virus concentration of the sample. The confidence interval ($P = 0.95$) of the combined virus concentration is not greater than ± 0.3 log $CCID_{50}$.

Rubella vaccine (live) BRP is suitable for use as a reference preparation.

Where justified and authorised, different assay designs may be used; this may imply the application of different validity and acceptance criteria. However, the vaccine must comply if tested as described above.

LABELLING

The label states:

- the strain of virus used for the preparation of the vaccine;
- the type and origin of the cells used for the preparation of the vaccine;
- the minimum virus concentration;
- that contact between the vaccine and disinfectants is to be avoided;
- that the vaccine must not be given to a pregnant woman and that a woman must not become pregnant within 2 months after having the vaccine.

Ph Eur

Smallpox Vaccine (Live)

General Notices

(Ph Eur monograph 0164)

The label may state 'SMV(live)'.

Ph Eur

DEFINITION

Smallpox vaccine (live) is a liquid or freeze-dried preparation of live vaccinia virus grown *in ovo* in the membranes of the chick embryo, in cell cultures or in the skin of living animals.

This monograph applies to vaccines produced using strains of confirmed efficacy in man, in particular those used during eradication of smallpox, for example the Lister strain (sometimes referred to as the Lister/Elstree strain) and the New York City Board of Health (NYCBOH) strain. It does not apply to non-replicative strains such as Modified Virus Ankara (MVA).

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently smallpox vaccines of adequate safety and immunogenicity in man. The strain used shall have been shown to produce typical vaccinia skin lesions in man. Production is based on a seed-lot system.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity of immunosera and vaccines for human use (*2.6.9*).

The International Reference Preparation for smallpox vaccine is suitable for use as a reference preparation in virus titration.

SUBSTRATE FOR VIRUS PROPAGATION

Animals used for production of skin-derived vaccines

If the vaccine is prepared in animals skins, the animals used are of a species approved by the competent authority, are in good health, are kept in closed or intensively monitored colonies, and have not previously been employed for experimental purposes. Only animals susceptible to infection by dermal inoculations with vaccinia virus may be used for vaccine production.

The animals are kept in well-constructed and adequately ventilated animals rooms with cages spaced as far apart as possible. Adequate precautions are taken to prevent cross-infection between cages. Not more than 1 large animal is housed per stall. Not more than 2 small animals are housed per cage and cage-mates must not be interchanged. The animals must be kept in the country of production of the vaccine in quarantine groups for a period of not less than 6 weeks before use.

If at any time during the quarantine period the overall death rate of the group reaches 5 per cent, no animals from that entire group may be used for vaccine production.

The groups are kept continuously in isolation, as in quarantine, even after completion of the quarantine period, until the animals are used. After the last animal of a group has been taken, the room that housed the group is thoroughly cleaned and decontaminated before receiving a new group.

Animals that are to be inoculated are anaesthetised and thoroughly examined. If an animal shows any pathological lesion, it is not used in the preparation of a seed lot or a vaccine, nor are any of the remaining animals of the quarantine group concerned unless it is evident that their use will not impair the safety of the product.

The prophylactic and diagnostic measures adopted to exclude the presence of infectious disease are approved by the competent authority. According to the species of animals used and the diseases to which that animal is liable in the country where the vaccine is being produced, these measures may vary. Consideration must also be given to the danger of spreading diseases to other countries to which the vaccine may be shipped. Special attention must always be given to foot-and-mouth disease, brucellosis, Q fever, tuberculosis and dermatomycosis, and it may also be necessary to consider diseases such as contagious pustular dermatitis (orf), anthrax, rinderpest, haemorrhagic septicaemia, Rift valley fever and others.

Embryonated eggs

Embryonated eggs used for production are obtained from a flock free from specified pathogens (SPF) (*5.2.2*).

Human diploid cells, continuous cell lines

Human diploid cells and continuous cell lines comply with the requirements for cell substrates (*5.2.3*).

Primary chick embryo cells

Primary chick embryo cells are derived from an SPF flock (*5.2.2*).

Primary rabbit kidney cells

Only healthy rabbits derived from a closed colony approved by the competent authority are used as a source. The animals, preferably 2-4 weeks old, are tested to ensure freedom from specified pathogens or their antibodies.

Where new animals are introduced into the colony, they are maintained in quarantine for a minimum of 2 months and shown to be free from specified pathogens. Animals to be used to provide kidneys shall not have been previously employed for experimental purposes, especially those involving infectious agents. The colony is monitored for zoonotic viruses and markers of contamination at regular intervals.

At the time the colony is established, all animals are tested to determine freedom from antibodies to possible viral contaminants for which there is evidence of capacity for infecting humans or evidence of capacity to replicate *in vitro* in cells of human origin. A test for retroviruses using a sensitive polymerase chain reaction (PCR)-based reverse transcriptase assay is also included. Nucleic acid amplification tests (*2.6.21*) for retroviruses may also be used.

After the colony is established, it is monitored by testing a representative group of at least 5 per cent of the animals, which are then bled at suitable (for example monthly) intervals. In addition, the colony is screened for pathogenic micro-organisms, including mycobacteria, fungi and mycoplasmas. The screening programme is designed to ensure that all animals are tested within a given period of time.

Any animal that dies is examined to determine the cause of death. If the presence of a causative infectious agent is demonstrated in the colony, the production of smallpox vaccine is discontinued.

At the time of kidney harvest, the animals are examined for the presence of abnormalities and, if any are noted, the animals are not used for vaccine production.

Each set of control cultures derived from a single group of animals used to produce a single virus harvest must remain identifiable as such until all testing, especially for extraneous agents, is completed.

VIRUS SEED LOT

The vaccinia virus isolate used for the master seed lot is identified by historical records that include information on its origin and the tests used in its characterisation.

Virus from the working seed lot must have the same characteristics as the strain that was used to prepare the master seed lot. The number of passages required to produce single harvests from the original isolate is limited and approved by the competent authority. Vaccine is produced from the working seed with a minimum number of intervening passages.

Since cell culture production and clonal selection (for example, plaque purification) may lead to altered characteristics of the virus, the master seed virus must be characterised as fully as possible, for example by comparing the safety profile and biological characteristics of the strain with that of the parental isolate. The characterisation shall include the following:

— antigenic analyses using specific antisera and/or monoclonal antibodies;

— biological studies such as infectivity titre, chorioallantoic membrane (CAM) assay, *in vitro* yield and *in vivo* growth characteristics in a suitable animal model;

— genetic analyses such as restriction mapping/southern blotting, PCR analyses and limited sequencing studies;

— phenotypic and genetic stability upon passage in the substrate;

— neurovirulence testing and immunogenicity studies.

The characterisation tests are also carried out on each working seed lot and on 3 batches of vaccine from the first working seed lot to verify genetic stability of the vaccine strain.

Only a virus seed that complies with the following requirements may be used for virus propagation.

Identification

Each working seed lot is identified as vaccinia virus using specific antibodies and molecular tests. Suitable tests are conducted to exclude the presence of variola virus and other orthopoxviruses.

Virus concentration

Determine by the CAM assay or by a suitable validated *in vitro* assay (plaque assay or $CCID_{50}$ assay). The virus concentration is the basis for the quantity of virus used in the neurovirulence test.

Extraneous agents *(2.6.16)*

If the working seed lot is produced in embryonated eggs, human diploid cells, or in a continuous cell line, it complies with the requirements for seed lots for virus vaccines. Seed lots produced in embryonated eggs and seed lots produced in primary cell cultures comply with the additional requirements described below.

Where the tests prescribed cannot be carried out because complete neutralisation of the seed virus is not possible, the seed lot may be diluted to a concentration equivalent to that of the dilution used as inoculum for production of vaccine prior to testing for extraneous viruses. Supplementary specific testing for extraneous viruses using validated nucleic acid amplification techniques *(2.6.21)* or immunochemical methods *(2.7.1)* may be envisaged. Where the indicator cell culture method for mycoplasma detection *(2.6.7)* cannot be carried out, nucleic acid amplification testing is performed instead.

Seed lots to be used for embryonated egg or cell culture production are in addition to be tested for carry-over of potential extraneous agents from the original seed. Given that the complete passage history of the original seed is unlikely to be known and that more than one species may have been used, this additional testing must at least cover important extraneous agents of concern.

The bioburden of master and working seed lots prepared in animal skins is limited by meticulous controls of facilities, personnel, and animals used for production, and by specific tests on the seeds. However, it may be difficult to ensure that seed lots produced in animal skins are totally free from extraneous agents, and consideration must be given to production procedures which remove or reduce them. Such lots must comply with the requirements indicated below. The absence of specific human pathogens is confirmed by additional testing procedures, for example, bacterial and fungal cultures, virus culture, nucleic acid amplification testings *(2.6.21)* for viral agents.

Neurovirulence

The neurovirulence of master and working seed lots is assessed using a suitable animal model, for example in monkeys or mice. The parental isolate is used as comparator. Where the original isolate is not available for this purpose, equivalent materials may be used.

VIRUS PROPAGATION AND HARVEST

Vaccine produced in living animals

Before inoculation the animals are cleaned and thereafter kept in scrupulously clean stalls until the vaccinia material is harvested. For 5 days before inoculation and during incubation the animals remain under veterinary supervision and must remain free from any sign of disease; daily rectal temperatures are recorded. If any abnormal rise in temperature occurs or any clinical sign of disease is observed, the production of vaccine from the group of animals concerned must be suspended until the cause has been resolved.

The inoculation of seed virus is carried out on such parts of the animal that are not liable to be soiled by urine and faeces. The surface used for inoculation is shaved and cleaned so as to achieve conditions that are as close as possible to surgical asepsis. If any antiseptic substance deleterious to the virus is used in the cleaning process it is removed by thorough rinsing with sterile water prior to inoculation. During inoculation the exposed surface of the animal not used for inoculation is covered with a sterile covering. By historical experience the ventral surface of female animals is appropriate for inoculation and inoculation of male animals is more appropriate on the flank.

Before the collection of the vaccinia material, any antibiotic is removed and the inoculated area is cleaned. The uninoculated surfaces are covered with a sterile covering. Before harvesting the animals are euthanised and exsanguinated to avoid heavy mixtures of the vaccinia material with blood. The vaccinia material from each animal is collected separately with aseptic precautions. All animals used in the production of vaccine are examined by autopsy. If evidence of any generalised or systemic disease other than vaccinia is found, the vaccinia material from that animal is discarded. If the disease is considered to be a communicable one, the harvest from the entire group of animals exposed must be discarded unless otherwise justified and authorised.

Vaccine produced in eggs

All processing of embryonated eggs is done under aseptic conditions in an area where no other infectious agents or cells are handled at the same time. After inoculation and incubation at a controlled temperature only living and suitable chick embryos are harvested. The age of the embryos at the time of virus harvest is reckoned from the initial introduction of the egg into the incubator and shall be not more than 12 days. After homogenisation and clarification by centrifugation, the extract of embryonic pulp is tested as described below and kept at - 70 °C or below until further processing. Virus harvests that comply with the prescribed tests may be pooled. No human protein is added to the virus suspension at any stage during production. If stabilisers are added, they shall have been shown to have no antigenic or sensitising properties for man.

Only a single harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Control eggs

Control eggs comply with the tests for extraneous agents (*2.6.16*). A sample of 2 per cent of uninoculated embryonated eggs (not less than 20 and not more than 50) from the batch used for vaccine production shall be incubated under the same conditions as the inoculated eggs. At the time of virus harvest the uninoculated eggs are processed in the same manner as the inoculated eggs.

Sterility *(2.6.1)*

It complies with the test for sterility, carried out using 10 ml for each medium.

VACCINE PRODUCED IN CELL CULTURES (PRIMARY CHICK EMBRYO CELLS, PRIMARY RABBIT KIDNEY CELLS, HUMAN DIPLOID CELLS OR CONTINUOUS CELL LINES)

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled at the same time during production. Suitable animal (but not human) serum may be used in the culture media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. On the day of inoculation with the virus working seed lot, not less than 5 per cent or 1000 ml, whichever is the least, of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells); special requirements, given below, apply to control cells when the vaccine is produced in primary rabbit kidney cell cultures.

After inoculation of the production cell culture with the working seed lot, inoculated cells are maintained at a suitable fixed temperature, and the virus suspension is harvested after a suitable incubation period.

Only a single harvest that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Control cells

The control cells of the production cell culture from which the virus harvest is derived comply with a test for identity and with the requirements for extraneous agents (*2.6.16*) or, where primary rabbit kidney cells cultures are used, with specific tests as mentioned hereafter. The test is invalid if more than 20 per cent of the control cell cultures have been discarded at the end of the observation period.

Extraneous agents *(2.6.16)*

The single harvest complies with the tests for extraneous agents. Complete neutralisation of vaccinia virus may be difficult to achieve at high virus concentration. In this case specific tests such as nucleic acid amplification *(2.6.21)* and immunochemical tests *(2.7.1)* can replace non-specific testing in cell culture or eggs. To save biological reagents such as vaccinia neutralising antisera, testing for extraneous agents may be performed on the final bulk instead of on the single harvests.

Vaccine prepared in primary chick embryo cells A sample of fluids pooled from the control cultures is tested for adenoviruses and for avian retroviruses such as avian leukosis virus. In addition, a volume of each neutralised virus pool equivalent to 100 human doses of vaccine or 10 ml, whichever is the greater, is tested in a group of fertilised eggs by the allantoic route of inoculation, and a similar sample is tested in a separate group of eggs by the yolk-sac route of inoculation. In both cases 0.5 ml of inoculum is used per egg. The virus pool passes the test if, after 3-7 days, there is no evidence of the presence of any extraneous agent.

Vaccine prepared in primary rabbit kidney cell cultures The following special requirements apply to virus propagation, harvest and testing. On the day of inoculation with virus working seed, a sample of at least 30 ml of the pooled fluid is removed from the cell cultures of the kidneys of each group of animals used to prepare the primary cell suspension. The pooled fluid is inoculated in primary kidney cell cultures in such a way that the dilution of the pooled fluid does not exceed 1 in 4. The cultures are incubated at a temperature of 34-36 °C and observed for a period of at least 4 weeks. During this observation period and after not less than 2 weeks of incubation, at least 1 subculture of fluid is made from each of these cultures and observed also for a period of 2 weeks. The test is invalid if more than 20 per cent of the cultures are discarded. If evidence is found of the presence of an extraneous agent, no cell cultures from the entire group may be used for vaccine production.

— *Control cell cultures*. Cultures prepared on the day of inoculation with the working virus seed lot from 25 per cent of the cell suspensions obtained from the kidneys of each group of animals are maintained as controls. These control cell cultures are incubated under the same conditions as the inoculated cultures for at least 2 weeks. The test is invalid if more than 20 per cent of the control cell cultures are discarded for non-specific reasons.

— *Test for haemadsorbing viruses*. At the time of harvest or not more than 4 days after the day of inoculation of the production cultures with the virus working seed, a sample of 4 per cent of the control cell cultures is tested for haemadsorbing viruses by addition of guinea-pig red blood cells.

— *Test for other extraneous agents*. At the time of harvest or not more than 7 days after the day of inoculation of the production cultures with the virus working seed, a sample of at least 20 ml of the pooled fluid from each group of control cultures is tested for other extraneous agents.

— *Tests of neutralised single harvest in primary rabbit kidney cell cultures*. Each neutralised single harvest is additionally tested in primary kidney cell cultures prepared from a different group of animals to that used for production.

POOLED HARVEST

Only a pooled harvest that complies with the following requirements and is within the limits approved for the product may be used in the preparation of the final lot.

Identity

The vaccinia virus in the pooled harvest is identified by serological methods, which may be supplemented by molecular methods. Molecular tests such as restriction fragment length polymorphism or partial sequencing, especially of terminal DNA sequences which show the greatest variation between vaccinia strains, may be useful.

Virus concentration

The vaccinia virus concentration of the pooled harvest is determined by chick egg CAM assay or in cell cultures. A reference preparation is assayed in the same system in parallel for validation of the pooled harvest titration. The virus concentration serves as the basis for the quantity of virus used in the neurovirulence test in mice.

Consistency of virus characteristics

Vaccinia virus in the pooled harvest or the final bulk is examined by tests that are able to determine that the phenotypic and genetic characteristics of the vaccinia virus have not undergone changes during the multiplication in the production system. The master seed or an equivalent preparation is used as a comparator in these tests and the comparator and the tests to be used are approved by the competent authority.

Neurovirulence

The neurovirulence of the pooled harvest is assessed versus a comparator original seed (or equivalent) by intracerebral inoculation into suckling mice. Other tests may be useful to discriminate between acceptable and unacceptable batches.

Residual DNA

For viruses grown in continuous cells the pooled harvest is tested for residual DNA. The production process demonstrates a level of cellular DNA of less than 10 ng per human dose.

Bacterial and fungal contamination

For vaccines other than those prepared on animal skins, the final bulk complies with the test for sterility (*2.6.1*) using 10 ml for each medium.

Mycoplasma (*2.6.7*)

For vaccines other than those prepared on animal skins, the final bulk complies with the test for mycoplasma, carried out using 10 ml.

FINAL BULK VACCINE

A minimum virus concentration for release of the product is established such as to ensure, in the light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Vaccine produced in living animals

The pooled harvest is centrifuged. If the vaccine is intended for issue in the liquid form, treatment to reduce the presence of extraneous agents may consist of the addition of glycerol or another suitable diluent, with or without an antimicrobial substance, and temporary storage at a suitable temperature. If the vaccine is intended for issue in the dried form, the treatment may consist of the addition of a suitable antimicrobial substance. The following special requirements apply to the bulk vaccine for vaccines produced in living animals.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Total bacterial count

For vaccines produced on animal skins only, maximum 50 per millilitre, determined by plate count using a suitable volume of the final bulk vaccine.

Escherichia coli

At least 1 ml samples of a 1:100 dilution of the final bulk vaccine is cultured on plates of a medium suitable for differentiating *E. coli* from other bacteria. The plates are incubated at 35-37 °C for 48 h. If *E. coli* is detected the final bulk is discarded or, subject to approval by the competent authority, processed further.

Haemolytic streptococci, coagulase-positive staphylococci or any other pathogenic micro-organisms which are known to be harmful to man by vaccination

At least 1 ml samples of a 1:100 dilution of the final bulk vaccine are cultured on blood agar. The plates are incubated at 35-37 °C for 48 h. If micro-organisms are detected, the final bulk vaccine is discarded.

Bacillus anthracis

Any colony seen on any of the plates that morphologically resembles *B. anthracis* is examined. If the organisms contained in the colony are non-motile, further tests for the cultural character of *B. anthracis* are carried out, including pathogenicity tests in suitable animals. If *B. anthracis* is found to be present, the final bulk vaccine and any other associated bulks are discarded. Additional validated molecular testing may be performed.

Clostridium tetani and other pathogenic spore-forming anaerobes

A total volume of not less than 10 ml of the final bulk vaccine is distributed in equal amounts into 10 tubes, each containing not less than 10 ml of suitable medium for the growth of anaerobic micro-organisms. The tubes are kept at 65 °C for 1 h in order to reduce the content of non-spore-forming organisms, after which they are anaerobically incubated at 35-37 °C for at least 1 week. From every tube or plate showing growth, subcultures are made on plates of a suitable medium. Tubes and plates are incubated anaerobically at the same temperature. All anaerobic colonies are examined and identified and if *C. tetani* or other pathogenic spore-forming anaerobes are present, the final bulk is discarded.

Vaccine produced in eggs

The pooled harvest is clarified and may be further purified.

Vaccine produced in cell cultures (primary chick embryos fibroblasts, human diploid cells or continuous cell lines)

The pooled harvest is clarified to remove cells and may be further purified.

FINAL LOT

Only a final lot that complies with the requirements for minimum virus concentration for release, with the following requirement for thermal stability and with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for antimicrobial preservative, protein content, bovine serum albumin and ovalbumin have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Thermal stability

Representative final containers of the vaccine are incubated at an elevated temperature for a defined period.

For liquid products, the conditions of the test and the requirements are approved by the competent authority.

For freeze-dried vaccines, maintain at least 3 vials of the final lot in the dry state at 37 ± 1 °C for 28 days. The total virus content in the 3 treated vials is determined as described under Assay in parallel alongside 3 vials of vaccine maintained at the recommended storage temperature. An appropriate reference preparation is also included to validate each assay. The vaccine complies with the test if the loss in titre after exposure at elevated temperature is not greater than 1.0 log infectious units per human dose and if the virus concentration after exposure at elevated temperature is not less than the minimum stated on the label.

IDENTIFICATION

The vaccinia virus is identified by an appropriate method.

TESTS

Antimicrobial preservative

Where applicable determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Phenol *(2.5.15)*

Maximum 0.5 per cent, if phenol is used.

Protein content

The protein content of each filling lot, if not done on the final bulk, is determined and is within the limits approved by the competent authority.

Bovine serum albumin

Maximum 50 ng per single human dose, determined by a suitable immunochemical method *(2.7.1)*, where bovine serum albumin is used during cell culture.

Ovalbumin

For vaccines produced in embryonated eggs, the ovalbumin content is within the limits approved by the competent authority.

Residual moisture

The residual moisture content of each final lot of freeze-dried vaccines is within the limits approved by the competent authority.

Bacterial count

For skin-derived vaccines, examine the vaccine by suitable microscopic and culture methods for micro-organisms pathogenic for man and, in particular, haemolytic streptococci, staphylococci, pathogenic spore-bearing organisms, especially *B. anthracis*, and *E. coli*. The vaccine is free from such contaminants. The total number of non-pathogenic bacteria does not exceed 50 per millilitre.

Sterility *(2.6.1)*

Except for skin-derived vaccines, the vaccine complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

The vaccine complies with the specification approved by the competent authority.

ASSAY

Reconstitue the vaccine if necessary and titrate for infectious virus using at least 3 separate vials of vaccine. Titrate 1 vial of an appropriate virus reference preparation in triplicate to validate each assay. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory. Calculate the individual virus concentration for each vial of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations, using the usual statistical methods (for example, *5.3*). The combined virus concentration for the 3 vials of vaccine is not less than 8.0 log pock-forming units per millilitre or the validated equivalent in plaque-forming units or 50 per cent cell culture infective doses, unless a lower titre is justified by clinical studies.

The assay is not valid if:

— the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than ± 0.5 log infectious units;

— the virus concentration of the reference preparation differs by more than 0.5 log infectious units from the established value.

The assay is repeated if the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.5 log infectious units; data obtained from valid assays only are combined by the usual statistical methods (for example, *5.3*) to calculate the virus concentration of the sample. The confidence interval ($P = 0.95$) of the combined virus concentration is not greater than ± 0.5 log infectious units.

Where justified and authorised, different assay designs may be used; this may imply the application of different validity and acceptance criteria. However, the vaccine must comply if tested as described above.

LABELLING

The label states:

— the designation of the vaccinia virus strain;

— the minimum amount of virus per millilitre;

— the substrate used for the preparation of the vaccine;

— the nature and amount of stabiliser, preservative or additive present in the vaccine and/or in the diluent.

Ph Eur

Tick-borne Encephalitis Vaccine, Inactivated

General Notices

(Tick-borne Encephalitis Vaccine (Inactivated), Ph Eur monograph 1375)

The label may state 'Tic/enceph'.

Ph Eur

DEFINITION

Tick-borne encephalitis vaccine (inactivated) is a liquid preparation of a suitable strain of tick-borne encephalitis virus grown in cultures of chick-embryo cells or other suitable cell cultures and inactivated by a suitable, validated method.

PRODUCTION

GENERAL PROVISIONS

Production of the vaccine is based on a virus seed-lot system. The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man. Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more passages from the master seed lot than the virus in the vaccine used in clinical trials.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in chick embryo cells prepared from eggs derived from a chicken flock free from specified pathogens *(5.2.2)* or in other suitable cell cultures *(5.2.3)*.

SEED LOTS

The strain of virus used is identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are stored at or below - 60 °C.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each seed lot is identified as containing the vaccine strain of tick-borne encephalitis virus by a suitable immunochemical method *(2.7.1)*, preferably using monoclonal antibodies.

Virus concentration

The virus concentration of each seed lot is determined by titration in suitable cell cultures to monitor consistency of production.

Extraneous agents *(2.6.16)*

Each seed lot complies with the requirements for extraneous agents in viral vaccines for human use. For neutralisation of the vaccine virus, the use of monoclonal antibodies is preferable.

VIRUS PROPAGATION AND HARVEST

If the virus has been passaged in mouse brain during preparation of the master seed lot, not fewer than 2 passages of the master seed virus in cell culture are made before inoculation of the production cell culture.

All processing of the cell cultures is performed under aseptic conditions in an area where no other cells are being handled. Serum and trypsin used in the preparation of cell suspensions and media used must be shown to be free from extraneous agents. The cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. At least 500 ml of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells).

Only a single harvest that complies with the following requirements may be used in the preparation of the inactivated harvest.

Identification

The single harvest is shown to contain tick-borne encephalitis virus by a suitable immunochemical method *(2.7.1)*, preferably using monoclonal antibodies, or by virus neutralisation in cell cultures.

Bacterial and fungal contamination *(2.6.1)*

The single harvest complies with the test for sterility, carried out using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

The single harvest complies with the test for mycoplasmas carried out using 1 ml for each medium.

Control cells

The control cells comply with the tests for extraneous agents *(2.6.16)*. If the vaccine is produced using a cell-bank system, the control cells comply with a test for identification.

Virus concentration

Determine the virus concentration by titration in suitable cell cultures to monitor consistency of production.

INACTIVATION

To avoid interference, viral aggregates are removed, where necessary, by filtration immediately before the inactivation process. The virus suspension is inactivated by a validated method; the method shall have been shown to be consistently capable of inactivating tick-borne encephalitis virus without destroying the antigenic and immunogenic activity; as part of the validation studies, an inactivation curve is plotted representing residual live virus concentration measured on not fewer than 3 occasions. If formaldehyde is used for inactivation, the presence of an excess of free formaldehyde is verified at the end of the inactivation process.

Only an inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Residual infective virus

Inoculate a quantity of the inactivated harvest equivalent to not less than 10 human doses of vaccine in the final lot into primary chicken fibroblast cell cultures, or other cells shown to be at least as sensitive to tick-borne encephalitis virus, with not less than 3 cm^2 of cell sheet per millilitre of inoculum. Incubate at 37 ± 1 °C for 14 days. No cytopathic effect is detected at the end of the incubation period. Collect the culture fluid and examine for the presence of infective tick-borne encephalitis virus by the following test in mice or by a validated *in vitro* method: inoculate 0.03 ml intracerebrally into each of not fewer than 10 mice about 4 weeks old. Observe the mice for 14 days. They show no evidence of tick-borne encephalitis virus infection.

PURIFICATION

Several inactivated single harvests may be pooled before concentration and purification by suitable methods, preferably by continuous-flow, sucrose density-gradient centrifugation.

Several purified inactivated harvests may be pooled.

Only a purified, inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility *(2.6.1)*

The purified, inactivated harvest complies with the test for sterility carried out using 10 ml for each medium.

Specific activity

Determine the antigen content of the purified, inactivated harvest by a suitable immunochemical method *(2.7.1)*. Determine the total protein content by a suitable method. The specific activity, calculated as the antigen content per unit mass of protein, is within the limits approved for the specific product.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more purified, inactivated harvests.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde, bovine serum albumin (where applicable) and pyrogens and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

IDENTIFICATION

The vaccine is shown to contain tick-borne encephalitis virus antigen by a suitable immunochemical method *(2.7.1)* using specific antibodies. The assay also serves to identify the vaccine.

TESTS

Aluminium *(2.5.13)*

Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde *(2.4.18)*

Maximum 0.1 g/l.

Bovine serum albumin

Maximum 50 ng per single human dose, determined by a suitable immunochemical method *(2.7.1)*, if bovine serum albumin has been used during production.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

Pyrogens *(2.6.8)*

The vaccine complies with the test for pyrogens. Inject into each rabbit, per kilogram of body mass, 1 dose of vaccine.

ASSAY

The potency is determined by comparing the dose necessary to protect a given proportion of mice against the effects of a lethal dose of tick-borne encephalitis virus, administered intraperitoneally, with the quantity of a reference preparation of tick-borne encephalitis vaccine necessary to provide the same protection. For this comparison an approved reference preparation and a suitable preparation of tick-borne encephalitis virus from an approved strain for use as the challenge preparation are necessary.

The following is cited as an example of a method that has been found suitable for a given vaccine.

Selection and distribution of test animals Use healthy mice weighing 11-17 g and derived from the same stock. Distribute the mice into not fewer than 6 groups of a suitable size to meet the requirements for validity of the test; for titration of the challenge suspension, use not fewer than 4 groups of 10 mice. Use mice of the same sex or distribute males and females equally between groups.

Determination of potency of the vaccine Prepare not fewer than 3 suitable dilutions of the vaccine to be examined and of the reference preparation; in order to comply with validity criteria 4 or 5 dilutions will usually be necessary. Prepare dilutions such that the most concentrated suspension is expected to protect more than 50 per cent of the animals and the least concentrated suspension less than 50 per cent. Allocate each dilution to a different group of mice and inject subcutaneously into each mouse 0.2 ml of the dilution allocated to its group. 7 days later make a second injection using the same dilution scale. 14 days after the second injection prepare a suspension of the challenge virus containing not less than 100 LD_{50} in 0.2 ml. Inject 0.2 ml of this virus suspension intraperitoneally into each vaccinated mouse. To verify the challenge dose, prepare a series of not fewer than 3 dilutions of the challenge virus suspension at not greater than one-hundredfold intervals. Allocate the challenge suspension and all of the dilutions, one to each of the groups of 10 mice, and inject intraperitoneally into each mouse 0.2 ml of the challenge suspension or the dilution allocated to its group. Observe the animals for 21 days after the challenge and record the number of mice that die in the period between 7 and 21 days after the challenge. Humane endpoints may be used to avoid unnecessary suffering of animals after the virulent challenge.

Calculations Calculate the results for an assay with quantal responses by the usual statistical methods *(for example, 5.3.)*.

Validity criteria The test is not valid unless:

— the concentration of the challenge virus is not less than 100 LD50,

— for both the vaccine to be examined and the reference preparation the 50 per cent protective dose (PD50) lies between the largest and smallest doses given to the mice,

— the statistical analysis shows a significant slope and no significant deviation from linearity and parallelism of the dose-response lines,

— the confidence limits (P = 0.95) are not less than 33 per cent and not more than 300 per cent of the estimated potency.

Potency requirement Include all valid tests to estimate the mean potency and the confidence limits ($P = 0.95$) for the mean potency; compute weighted means with the inverse of the squared standard error as weights. The vaccine complies with the test if the estimated potency is not less than that approved by the competent authority, based on data from clinical efficacy trials.

LABELLING

The label states:

— the strain of virus used in preparation;

— the type of cells used for production of the vaccine.

Ph Eur

Typhoid (Strain Ty 21a) Vaccine, Live (Oral)

General Notices

(Typhoid Vaccine (Live, Oral, Strain Ty 21a), Ph Eur monograph 1055)

The label may state 'Typhoid(live,oral)'.

Ph Eur

DEFINITION

Typhoid vaccine (live, oral, strain Ty 21a) is a freeze-dried preparation of live *Salmonella typhi* strain Ty 21a grown in a suitable medium. When presented in capsules, the vaccine complies with the monograph on *Capsules (0016).*

PRODUCTION

CHOICE OF VACCINE STRAIN

The main characteristic of the strain is the defect of the enzyme uridine diphosphate-galactose-4-epimerase. The activities of galactopermease, galactokinase and galactose-1-phosphate uridyl-transferase are reduced by 50 per cent to 90 per cent. Whatever the growth conditions, the strain does not contain Vi antigen. The strain agglutinates to anti-O:9 antiserum only if grown in medium containing galactose. It contains the flagellar H:d antigen and does not produce hydrogen sulphide on Kligler iron agar. The strain is nonvirulent for mice. Cells of strain Ty 21a lyse if grown in the presence of 1 per cent of galactose.

BACTERIAL SEED LOTS

The vaccine is prepared using a seed-lot system. The working seed lots represent not more than one subculture from the master seed lot. The final vaccine represents not more than four subcultures from the original vaccine on which were made the laboratory and clinical tests showing the strain to be suitable.

Only a master seed lot that complies with the following requirements may be used in the preparation of working seed lots.

Galactose metabolism

In a spectrophotometric assay, no activity of the enzyme uridine diphosphate-galactose-4-epimerase is found in the cytoplasm of strain Ty 21a compared to strain Ty 2.

Biosynthesis of lipopolysaccharide

Lipopolysaccharides are extracted by the hot-phenol method and examined by size-exclusion chromatography. Strain Ty 21a grown in medium free of galactose shows only the rough (R) type of lipopolysaccharide.

Serological characteristics

Strain Ty 21a grown in a synthetic medium without galactose does not agglutinate to specific anti-O:9 antiserum. Whatever the growth conditions, strain Ty 21a does not agglutinate to Vi antiserum. Strain Ty 21a agglutinates to H:d flagellar antiserum.

Biochemical markers

Strain Ty 21a does not produce hydrogen sulphide on Kligler iron agar. This property serves to distinguish Ty 21a from other galactose-epimerase-negative *S. typhi* strains.

Cell growth

Strain Ty 21a cells lyse when grown in the presence of 1 per cent of galactose.

BACTERIAL PROPAGATION AND HARVEST

The bacteria from the working seed lot are multiplied in a preculture, subcultured once and are then grown in a suitable medium containing 0.001 per cent of galactose at 30 °C for 13 h to 15 h. The bacteria are harvested. The harvest must be free from contaminating micro-organisms.

Only a single harvest that complies with the following requirements may be used for the preparation of the freeze-dried harvest.

pH

The pH of the culture is 6.8 to 7.5.

Optical density

The optical density of the culture, measured at 546 nm, is 6.5 to 11.0. Before carrying out the measurement, dilute the culture so that a reading in the range 0.1 to 0.5 is obtained and correct the reading to take account of the dilution.

Identification

Culture bacteria on an agar medium containing 1 per cent of galactose and bromothymol blue. Light blue, concave colonies, transparent due to lysis of cells, are formed. No yellow colonies (galactose-fermenting) are found.

FREEZE DRIED HARVEST

The harvest is mixed with a suitable stabiliser and freeze-dried by a process that ensures the survival of at least 10 per cent of the bacteria and to a water content shown to be favourable to the stability of the vaccine. No antimicrobial preservative is added to the vaccine.

Only a freeze-dried harvest that complies with the following tests may be used for the preparation of the final bulk.

Identification

Culture bacteria are examined on an agar medium containing 1 per cent of galactose and bromothymol blue. Light blue, concave colonies, transparent due to lysis of cells, are formed. No yellow colonies (galactose-fermenting) are found.

Number of live bacteria. Not fewer than 1×10^{11} live *S. typhi* strain Ty 21a per gram.

Water *(2.5.12)*

1.5 per cent to 4.0 per cent, determined by the semi-micro determination of water.

FINAL BULK VACCINE

The final bulk vaccine is prepared by aseptically mixing one or more freeze-dried harvests with a suitable sterile excipient.

Only a final bulk that complies with the following requirement may be used in the preparation of the final lot.

Number of live bacteria

Not fewer than 40 × 10^9 live *S. typhi* strain Ty 21a per gram.

FINAL LOT

The final bulk vaccine is distributed under aseptic conditions into capsules with a gastro-resistant shell or into suitable containers.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Number of live bacteria may be released for use, except that in the determination of the number of live bacteria each dosage unit must contain not fewer than 4 × 10^9 live bacteria.

IDENTIFICATION

Culture bacteria from the vaccine to be examined on an agar medium containing 1 per cent of galactose and bromothymol blue. Light blue, concave colonies, transparent due to lysis of cells, are formed. No yellow colonies (galactose-fermenting) are found.

TESTS

Contaminating micro-organisms *(2.6.12, 2.6.13)*

Carry out the test using suitable selective media. Determine the total viable count using the plate-count method. The number of contaminating micro-organisms per dosage unit is not greater than 10^2 bacteria and 20 fungi. No pathogenic bacterium, particularly *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and no salmonella other than strain Ty 21a are found.

Water *(2.5.12)*

1.5 per cent to 4.0 per cent, determined on the contents of the capsule or of the container by the semi-micro determination of water.

NUMBER OF LIVE BACTERIA

Carry out the test using not fewer than five dosage units. Homogenise the contents of the dosage units in a 9 g/l solution of *sodium chloride R* at 4 °C using a mixer in a cold room with sufficient glass beads to emerge from the liquid. Immediately after homogenisation prepare a suitable dilution of the suspension using cooled diluent and inoculate brain heart infusion agar; incubate at 36 ± 1 °C for 20 h to 36 h. The vaccine contains not fewer than 2 × 10^9 live *S. typhi* Ty 21a bacteria per dosage unit.

LABELLING

The label states:

— the minimum number of live bacteria per dosage unit,

— that the vaccine is for oral use only.

Ph Eur

Typhoid Polysaccharide Vaccine

General Notices

(Ph Eur monograph 1160)

The label may state 'Typhoid'.

Ph Eur

DEFINITION

Typhoid polysaccharide vaccine is a preparation of purified Vi capsular polysaccharide obtained from *Salmonella typhi* Ty 2 strain or some other suitable strain that has the capacity to produce Vi polysaccharide.

Capsular Vi polysaccharide consists of partly 3-*O*-acetylated repeated units of 2-acetylamino-2-deoxy-D-galactopyranuronic acid with α-(1→4) linkages.

PRODUCTION

The production of Vi polysaccharide is based on a seed-lot system. The method of production shall have been shown to yield consistently typhoid polysaccharide vaccines of adequate immunogenicity and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

BACTERIAL SEED LOTS

The strain of *S. typhi* used for the master seed lot shall be identified by historical records that include information on its origin and by its biochemical and serological characteristics. Cultures from the working seed lot shall have the same characteristics as the strain that was used to prepare the master seed lot.

Only a strain that has the following characteristics may be used in the preparation of the vaccine: (a) stained smears from a culture are typical of enterobacteria; (b) the culture utilises glucose without production of gas; (c) colonies on agar are oxidase-negative; (d) a suspension of the culture agglutinates specifically with a suitable Vi antiserum or colonies form haloes on an agar plate containing a suitable Vi antiserum.

Purity of bacterial strain used for the seed lot is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

CULTURE AND HARVEST

The working seed lot is cultured on a solid medium, which may contain blood-group substances, or a liquid medium; the inoculum obtained is transferred to a liquid medium which is used to inoculate the final medium. The liquid medium used and the final medium are semi-synthetic, free from substances that are precipitated by cetrimonium bromide and do not contain blood-group substances or high-molecular-mass polysaccharides, unless it has been demonstrated that they are removed by the purification process.

The bacterial purity of the culture is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

The culture is then inactivated at the beginning of the stationary phase by the addition of formaldehyde. Bacterial cells are eliminated by centrifugation; the polysaccharide is precipitated from the culture medium by addition of hexadecyltrimethylammonium bromide (cetrimonium bromide). The precipitate is harvested and may be stored at - 20 °C before purification.

PURIFIED VI POLYSACCHARIDE

The polysaccharide is purified, after dissociation of the polysaccharide/cetrimonium bromide complex, using suitable procedures to eliminate successively nucleic acids, proteins and lipopolysaccharides. The polysaccharide is precipitated as the calcium salt in the presence of ethanol and dried at 2-8 °C; the powder obtained constitutes the purified Vi polysaccharide. The loss on drying is determined by thermogravimetry *(2.2.34)* and is used to calculate the results of the chemical tests shown below with reference to the dried substance.

Only a purified Vi polysaccharide that complies with the following requirements may be used in the preparation of the final bulk.

Protein *(2.5.16)*

Maximum 10 mg per gram of polysaccharide, calculated with reference to the dried substance.

Nucleic acids *(2.5.17)*

Maximum 20 mg per gram of polysaccharide, calculated with reference to the dried substance.

O-Acetyl groups *(2.5.19)*

Minimum 2 mmol per gram of polysaccharide, calculated with reference to the dried substance.

Molecular size

Examine by size-exclusion chromatography *(2.2.30)* using *cross-linked agarose for chromatography R*. Use a column 0.9 m long and 16 mm in internal diameter equilibrated with a solvent having an ionic strength of 0.2 mol/kg and a pH of 7.0-7.5. Apply about 5 mg of polysaccharide in a volume of 1 ml to the column and elute at about 20 ml/h. Collect fractions of about 2.5 ml. Determine the point corresponding to $K_0 = 0.25$ and make 2 pools consisting of fractions eluted before and after this point. Determine *O*-acetyl groups on the 2 pools *(2.5.19)*. Not less than 50 per cent of the polysaccharide is found in the pool containing fractions eluted before $K_0 = 0.25$.

Identification

Carry out an identification test using a suitable immunochemical method *(2.7.1)*.

Bacterial endotoxins

The content of bacterial endotoxins determined by a suitable method *(2.6.14)* is within the limits approved for the specific product.

FINAL BULK VACCINE

One or more batches of purified Vi polysaccharide are dissolved in a suitable solvent, which may contain an antimicrobial preservative, so that the volume corresponding to 1 dose contains 25 µg of polysaccharide and the solution is isotonic with blood (250 mosmol/kg to 350 mosmol/kg).

Only a final bulk vaccine that complies with the following tests may be used in the preparation of the final lot.

Sterility *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable physicochemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended amount.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile tamper-proof containers that are then closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements prescribed below under Identification, Tests and Assay and with the requirement for bacterial endotoxins may be released for use. Provided the tests for free formaldehyde and antimicrobial preservative have been carried out on the final bulk vaccine, they may be omitted on the final lot.

Bacterial endotoxins

The content of bacterial endotoxins determined by a suitable method *(2.6.14)* is within the limit approved for the specific product.

CHARACTERS

Clear colourless liquid, free from visible particles.

IDENTIFICATION

Carry out an identification test using a suitable immunochemical method *(2.7.1)*.

TESTS

pH *(2.2.3)*

6.5 to 7.5.

O-Acetyl groups

0.085 (± 25 per cent) µmol per dose (25 µg of polysaccharide).

Test solution Place 3 ml of the vaccine in each of 3 tubes (2 reaction solutions and 1 correction solution).

Reference solutions Dissolve 0.150 g of *acetylcholine chloride R* in 10 ml of *water R* (stock solution containing 15 g/l of acetylcholine chloride). Immediately before use, dilute 0.5 ml of the stock solution to 50 ml with *water R* (working dilution containing 150 µg/ml of acetylcholine chloride). In 10 tubes, place in duplicate (reaction and correction solutions) 0.1 ml, 0.2 ml, 0.5 ml, 1.0 ml and 1.5 ml of the working dilution.

Prepare a blank using 3 ml of *water R*.

Make up the volume in each tube to 3 ml with *water R*. Add 0.5 ml of a mixture of 1 volume of *water R* and 2 volumes of *dilute hydrochloric acid R* to each of the correction tubes and to the blank. Add 1.0 ml of *alkaline hydroxylamine solution R* to each tube. Allow the reaction to proceed for exactly 2 min and add 0.5 ml of a mixture of 1 volume of *water R* and 2 volumes of *dilute hydrochloric acid R* to each of the reaction tubes. Add 0.5 ml of a 200 g/l solution of *ferric chloride R* in *0.2 M hydrochloric acid* to each tube, stopper the tubes and shake vigorously to remove bubbles.

Measure the absorbance *(2.2.25)* of each solution at 540 nm using the blank as the compensation liquid. For each reaction solution, subtract the absorbance of the corresponding correction solution. Draw a calibration curve from the corrected absorbances for the 5 reference solutions and the corresponding content of acetylcholine chloride and read from the curve the content of acetylcholine chloride in the test solution for each volume tested. Calculate the mean of the 2 values.

1 mole of acetylcholine chloride (181.7 g) is equivalent to 1 mole of *O*-acetyl (43.05 g).

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l.

Antimicrobial preservative

Where applicable, determine the amount of antimicrobial preservative by a suitable physicochemical method. The content is not less than the minimum amount shown to be effective and not more than 115 per cent of the content stated on the label. If phenol has been used in the preparation, the content is not more than 2.5 g/l *(2.5.15)*.

Sterility *(2.6.1)*

The vaccine complies with the test for sterility.

ASSAY

Determine Vi polysaccharide by a suitable immunochemical method *(2.7.1)*, using a reference purified polysaccharide. The estimated amount of polysaccharide per dose is 80 per cent to 120 per cent of the content stated on the label. The confidence limits (*P* = 0.95) of the estimated amount of polysaccharide are not less than 80 per cent and not more than 120 per cent.

LABELLING

The label states:

— the number of micrograms of polysaccharide per human dose (25 µg);

— the total quantity of polysaccharide in the container.

Ph Eur

Typhoid Vaccine, Freeze-dried

General Notices

(Ph Eur monograph 0157)

The label may state 'Typhoid'.

Ph Eur

DEFINITION

Freeze-dried typhoid vaccine is a freeze-dried preparation of inactivated *Salmonella typhi*. The vaccine is reconstituted as stated on the label to give a uniform suspension containing not less than 5×10^8 and not more than 1×10^9 bacteria (*S. typhi*) per human dose. The human dose does not exceed 1.0 ml of the reconstituted vaccine.

PRODUCTION

The vaccine is prepared using a seed-lot system from a suitable strain, such as Ty 2 [1], of *S. typhi*. The final vaccine represents not more than 3 subcultures from the strain on which were made the laboratory and clinical tests that showed it to be suitable. The bacteria are inactivated either by acetone or by formaldehyde or by heat. Phenol is not used in the preparation. The vaccine is distributed into sterile containers and freeze-dried to a moisture content favourable to the stability of the vaccine. The containers are then closed so as to exclude contamination.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)* modified as follows: inject 0.5 ml of the vaccine into each mouse and 1.0 ml into each guinea pig.

IDENTIFICATION

The vaccine reconstituted as stated on the label is identified by specific agglutination.

TESTS

Phenol *(2.5.15)*

If phenol has been used in the preparation, the concentration is not more than 5 g/l.

Antigenic power

When injected into susceptible laboratory animals, the reconstituted vaccine elicits anti-O, anti-H and, to a lesser extent, anti-Vi agglutinins.

Sterility *(2.6.1)*

The reconstituted vaccine complies with the test for sterility.

LABELLING

The label states:

- — the method used to inactivate the bacteria,
- — the number of bacteria per human dose,
- — that the vaccine should be used within 8 h of reconstitution.

Ph Eur

[1] *This strain is issued by the World Health Organisation Collaborating Centre for Reference and Research on Bacterial Vaccines, Human Serum and Vaccine Institute, Szallas Utea 5, H-1107, Budapest, Hungary.*

Typhoid Vaccine

General Notices

(Ph Eur monograph 0156)

The label may state 'Typhoid'.

Ph Eur

DEFINITION

Typhoid vaccine is a sterile suspension of inactivated *Salmonella typhi* containing not less than 5×10^8 and not more than 1×10^9 bacteria (*S. typhi*) per human dose. The human dose does not exceed 1.0 ml.

PRODUCTION

The vaccine is prepared using a seed-lot system from a suitable strain, such as Ty 2 [1], of *S. typhi*. The final vaccine represents not more than 3 subcultures from the strain on which were made the laboratory and clinical tests that showed it to be suitable. The bacteria are inactivated by acetone, by formaldehyde, by phenol or by heating or by a combination of the last 2 methods.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)* modified as follows: inject 0.5 ml of the vaccine into each mouse and 1.0 ml into each guinea pig.

IDENTIFICATION

It is identified by specific agglutination.

TESTS

Phenol *(2.5.15)*

If phenol has been used in the preparation, the concentration is not more than 5 g/l.

Antigenic power

When injected into susceptible laboratory animals, it elicits anti-O, anti-H and, to a lesser extent, anti-Vi agglutinins.

Sterility *(2.6.1)*

It complies with the test for sterility.

LABELLING

The label states:

— the method used to inactivate the bacteria,

— the number of bacteria per human dose.

Ph Eur

[1] This strain is issued by the World Health Organisation Collaborating Centre for Reference and Research on Bacterial Vaccines, Human Serum and Vaccine Institute, Szallas Utea 5, H-1107, Budapest, Hungary.

VACCINES

General Notices

(Vaccines for Human Use, Ph Eur monograph 0153)

Vaccines for human use comply with the requirements of the European Pharmacopoeia monograph for Vaccines for Human Use. These requirements are reproduced below.

Ph Eur

For a combined vaccine, where there is no monograph to cover a particular combination, the vaccine complies with the monograph for each individual component, with any necessary modifications approved by the competent authority.

DEFINITION

Vaccines for human use are preparations containing substances capable of inducing a specific and active immunity in man against an infecting agent or the toxin or the antigen elaborated by it. They shall have been shown to have acceptable immunogenic activity in man with the intended vaccination schedule. They may contain an adjuvant.

Vaccines for human use may contain: organisms inactivated by chemical or physical means that maintain adequate immunogenic properties; living organisms that are naturally avirulent or that have been treated to attenuate their virulence whilst retaining adequate immunogenic properties; antigens extracted from the organisms or secreted by them or produced by genetic engineering; the antigens may be used in their native state or may be detoxified by chemical or physical means and may be aggregated, polymerised or conjugated to a carrier to increase their immunogenicity.

Terminology used in monographs on vaccines for human use is defined in chapter *5.2.1*.

Bacterial vaccines are suspensions of various degrees of opacity in colourless or almost colourless liquids, or may be freeze-dried. The concentration of living or inactivated bacteria is expressed in terms of International Units of opacity or, where appropriate, is determined by direct cell count or, for living bacteria, by viable count.

Bacterial toxoids are prepared from toxins by diminishing their toxicity to a non-detectable level or by completely eliminating it by physical or chemical procedures whilst retaining adequate immunogenic properties. The toxins are obtained from selected strains of micro-organisms. The method of production is such that the toxoid does not revert to toxin. Toxoids may be liquid or freeze-dried. They may be purified and adsorbed. Adsorbed toxoids are suspensions of white or grey particles dispersed in colourless or pale yellow liquids and may form a sediment at the bottom of the container.

Viral vaccines are prepared from viruses grown in animals, in fertilised eggs, in suitable cell cultures or in suitable tissues or by culture of genetically engineered cells. They are liquids that vary in opacity according to the type of preparation or may be freeze-dried. Liquid preparations and freeze-dried preparations after reconstitution may be coloured if a pH indicator such as phenol red has been used in the culture medium.

PRODUCTION

General provisions

The production method for a given product must have been shown to yield consistently batches comparable with the batch of proven clinical efficacy and safety in man. Requirements for production including in-process testing are included in individual monographs. Where justified and authorised, certain tests may be omitted where it can be demonstrated, for example by validation studies, that the production process consistently ensures compliance with the test.

Unless otherwise justified and authorised, vaccines are produced using a seed-lot system. The methods of preparation are designed to maintain adequate immunogenic properties, to render the preparation harmless and to prevent contamination with extraneous agents.

Where vaccines for human use are manufactured using materials of human or animal origin, the general requirements of chapter *5.1.7*. *Viral safety* apply in conjunction with the more specific requirements relating to viral safety in this monograph, in chapters *5.2.2*. *Chicken flocks free from specified pathogens for the production and quality control of vaccines*, *5.2.3*. *Cell substrates for the production of vaccines for human use* and *2.6.16*. *Tests for extraneous agents in viral vaccines for human use,* and in individual monographs.

Unless otherwise justified and authorised, in the production of a final lot of vaccine, the number of passages of a virus, or the number of subcultures of a bacterium, from the master seed lot shall not exceed that used for production of the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

Vaccines are as far as possible free from ingredients known to cause toxic, allergic or other undesirable reactions in man. Suitable additives, including stabilisers and adjuvants may be incorporated. Penicillin and streptomycin are not used at any stage of production nor added to the final product; however, master seed lots prepared with media containing penicillin or streptomycin may, where justified and authorised, be used for production.

Consistency of production is an important feature of vaccine production. Monographs on vaccines for human use give limits for various tests carried out during production and on the final lot. These limits may be in the form of maximum values, minimum values or minimum and maximum tolerances around a given value. While compliance with these limits is required, it is not necessarily sufficient to ensure consistency of production for a given vaccine. For relevant tests, the manufacturer must therefore define for each product a suitable action or release limit or limits to be applied in view of the results found for batches tested clinically and those used to demonstrate consistency of production. These limits may be subsequently refined on a statistical basis in the light of production data.

Substrates for propagation

Substrates for propagation comply with the relevant requirements of the Pharmacopoeia *(5.2.2, 5.2.3)* or in the absence of such requirements with those of the competent authority. Processing of cell banks and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled. Serum and trypsin used in the preparation of cell suspensions shall be shown to be free from extraneous agents.

Seed lots

The strain of bacterium or virus used in a master seed lot is identified by historical records that include information on the origin of the strain and its subsequent manipulation. Suitable measures are taken to ensure that no micro-organism other than the seed strain is present in a seed lot.

Culture media

Culture media are as far as possible free from ingredients known to cause toxic, allergic or other undesirable reactions in man; if inclusion of such ingredients is necessary, it shall be demonstrated that the amount present in the final lot is reduced to such a level as to render the product safe. Approved animal (but not human) serum may be used in the growth medium for cell cultures but the medium used for maintaining cell growth during virus multiplication shall not contain serum, unless otherwise stated. Cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration although it is preferable to have a medium free from antibiotics during production.

Propagation and harvest

The seed cultures are propagated and harvested under defined conditions. The purity of the harvest is verified by suitable tests as defined in the monograph.

Control cells

For vaccines produced in cell cultures, control cells are maintained and tested as prescribed. In order to provide a valid control, these cells must be maintained in conditions that are essentially equivalent to those used for the production cell cultures, including use of the same batches of media and media changes.

Control eggs

For live vaccines produced in eggs, control eggs are incubated and tested as prescribed in the monograph.

Purification

Where applicable, validated purification procedures may be applied.

Inactivation

Inactivated vaccines are produced using a validated inactivation process whose effectiveness and consistency have been demonstrated. Where there are recognised potential contaminants of a harvest, for example in vaccines produced in eggs from healthy, non-SPF flocks, the inactivation process is also validated with respect to the potential contaminants. A test for effectiveness of the inactivation process is carried out as soon as possible after the inactivation process, unless otherwise justified and authorised.

Stability of intermediates

During production of vaccines, intermediates are obtained at various stages and are stored, sometimes for long periods. Such intermediates include:

- seed lots;
- live or inactivated harvests from bacterial or viral cultures;
- purified harvests that may consist of toxins or toxoids, polysaccharides, bacterial or viral suspensions;
- purified antigens;
- adsorbed antigens;
- conjugated polysaccharides;

— final bulk vaccine;

— vaccine in the final closed container stored at a temperature lower than that used for stability studies and intended for release without re-assay.

Except where they are used within a short period of time, stability studies are carried out on the intermediates in the intended storage conditions to establish the expected extent of degradation. For final bulk vaccine, stability studies may be carried out on representative samples in conditions equivalent to those intended to be used for storage. For each intermediate (except for seed lots), a period of validity applicable for the intended storage conditions is established, where appropriate in the light of stability studies.

Final bulk

The final bulk is prepared by aseptically blending the ingredients of the vaccine.

Adsorbents Vaccines may be adsorbed on aluminium hydroxide, aluminium phosphate, calcium phosphate or other suitable adsorbent; the adsorbents are prepared in special conditions which confer the appropriate physical form and adsorptive properties.

Antimicrobial preservatives Antimicrobial preservatives are used to prevent spoilage or adverse effects caused by microbial contamination occurring during the use of a vaccine. Antimicrobial preservatives are not included in freeze-dried products. For single-dose liquid preparations, inclusion of antimicrobial preservatives is not normally acceptable. For multidose liquid preparations, the need for effective antimicrobial preservation is evaluated taking into account likely contamination during use and the maximum recommended period of use after broaching of the container. If an antimicrobial preservative is used, it shall be shown that it does not impair the safety or efficacy of the vaccine. Addition of antibiotics as antimicrobial preservatives is not normally acceptable.

During development studies, the effectiveness of the antimicrobial preservative throughout the period of validity shall be demonstrated to the satisfaction of the competent authority.

The efficacy of the antimicrobial preservative is evaluated as described in chapter *5.1.3*. If neither the A criteria nor the B criteria can be met, then in justified cases the following criteria are applied to vaccines for human use: bacteria, no increase at 24 h and 7 days, 3 log reduction at 14 days, no increase at 28 days; fungi, no increase at 14 days and 28 days.

Final lot

For vaccines for parenteral administration, the final lot is prepared by aseptically distributing the final bulk into sterile tamper-proof containers which, after freeze-drying where applicable, are closed so as to exclude contamination. For vaccines for administration by a non-parenteral route, the final lot is prepared by distributing the final bulk under suitable conditions into sterile, tamper-proof containers.

Appearance

Each container (vial, syringe or ampoule) in each final lot is inspected visually or mechanically for acceptable appearance.

Degree of adsorption During development of an adsorbed vaccine, the degree of adsorption is evaluated as part of the consistency testing. A release specification for the degree of adsorption is established in the light of results found for batches used in clinical testing. From the stability data generated for the vaccine it must be shown that at the end of the period of validity the degree of adsorption will not be less than for batches used in clinical testing.

Stability During development studies, maintenance of potency of the final lot throughout the period of validity shall be demonstrated; the loss of potency in the recommended storage conditions is assessed and excessive loss even within the limits of acceptable potency may indicate that the vaccine is unacceptable.

Expiry date Unless otherwise stated, the expiry date is calculated from the beginning of the assay or from the beginning of the first assay for a combined vaccine. For vaccines stored at a temperature lower than that used for stability studies and intended for release without re-assay, the expiry date is calculated from the date of removal from cold storage. If, for a given vaccine, an assay is not carried out, the expiry date for the final lot is calculated from the date of an approved stability-indicating test or failing this from the date of freeze-drying or the date of filling into the final containers. For a combined vaccine where components are presented in separate containers, the expiry date is that of the component which expires first.

The expiry date applies to vaccines stored in the prescribed conditions.

Animal tests

In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. The criteria for judging tests in monographs must be applied in the light of this. For example, if it is indicated that an animal is considered to show positive, infected etc. when typical clinical signs or death occur then as soon as sufficient indication of a positive result is obtained the animal in question shall be either humanely destroyed or given suitable treatment to prevent unnecessary suffering. In accordance with the General Notices, alternative test methods may be used to demonstrate compliance with the monograph and the use of such tests is particularly encouraged when this leads to replacement or reduction of animal use or reduction of suffering.

TESTS

Vaccines comply with the tests prescribed in individual monographs including, where applicable, the following:

pH *(2.2.3)*

Liquid vaccines, after reconstitution where applicable, comply with the limits for pH approved for the particular preparation.

Adjuvant

If the vaccine contains an adjuvant, the amount is determined and shown to be within acceptable limits with respect to the expected amount (see also the tests for aluminium and calcium below).

Aluminium *(2.5.13)*

Maximum 1.25 mg of aluminium (Al) per single human dose where an aluminium adsorbent has been used in the vaccine, unless otherwise stated.

Calcium *(2.5.14)*

Maximum 1.3 mg of calcium (Ca) per single human dose where a calcium adsorbent has been used in the vaccine, unless otherwise stated.

Free formaldehyde *(2.4.18)*

Maximum 0.2 g/l of free formaldehyde is present in the final product where formaldehyde has been used in the preparation of the vaccine, unless otherwise stated.

Phenol ***(2.5.15)***

Maximum 2.5 g/l is present in the final product where phenol has been used in the preparation of the vaccine, unless otherwise stated.

Water ***(2.5.12)***

Maximum 3.0 per cent *m/m* for freeze-dried vaccines, unless otherwise stated.

Extractable Volume ***(2.9.17)***

Unless otherwise justified and authorised, it complies with the requirement for Extractable Volume.

STORAGE

Store protected from light. Unless otherwise stated, the storage temperature is 5 ± 3 °C; liquid adsorbed vaccines must not be allowed to freeze.

LABELLING

The label states:

- — the name of the preparation;
- — a reference identifying the final lot;
- — the recommended human dose and route of administration;
- — the storage conditions;
- — the expiry date;
- — the name and amount of any antimicrobial preservative;
- — the name of any antibiotic, adjuvant, flavour or stabiliser present in the vaccine;
- — the name of any constituent that may cause adverse reactions and any contra-indications to the use of the vaccine;
- — for freeze-dried vaccines:
  - — the name or composition and the volume of the reconstituting liquid to be added;
  - — the time within which the vaccine is to be used after reconstitution.

Ph Eur

Varicella Vaccine (Live)

General Notices

(Ph Eur monograph 0648)

The label may state 'Var(live)'.

Ph Eur

DEFINITION

Varicella vaccine (live) is a freeze-dried preparation of a suitable attenuated strain of *Herpesvirus varicellae*. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The production of vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently live varicella vaccines of adequate immunogenicity and safety in man. The virus in the final vaccine shall not have been passaged in cell cultures beyond the 38th passage from the original isolated virus.

The potential neurovirulence of the vaccine strain is considered during preclinical development, based on available epidemiological data on neurovirulence and neurotropism, primarily for the wild-type virus. In light of this, a risk analysis is carried out. Where necessary and if available, a test is carried out on the vaccine strain using an animal model that differentiates wild-type and attenuated virus; tests on strains of intermediate attenuation may also be needed.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)*.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in human diploid cells *(5.2.3)*.

VIRUS SEED LOT

The strain of varicella virus used shall be identified as being suitable by historical records that include information on the origin of the strain and its subsequent manipulation. The virus shall at no time have been passaged in continuous cell lines. Seed lots are prepared in the same kind of cells as those used for the production of the final vaccine. Virus seed lots are prepared in large quantities and stored at temperatures below - 20 °C if freeze-dried, or below - 60 °C if not freeze-dried.

Only a virus seed lot that complies with the following requirements may be used for virus propagation.

Identification

The master and working seed lots are identified as varicella virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration

The virus concentration of the master and working seed lots is determined as prescribed under Assay to monitor consistency of production.

Extraneous agents *(2.6.16)*

The working seed lot complies with the requirements for seed lots for live virus vaccines; a sample of 50 ml is taken for the test in cell cultures.

VIRUS PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled during the production. Suitable animal (but not human) serum may be used in the culture media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. 5 per cent, but not less than 50 ml, of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells). The infected cells constituting a single harvest are washed, released from the support surface and pooled. The cell suspension is disrupted by sonication.

Only a virus harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

The virus harvest contains virus that is identified as varicella virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration

The concentration of infective virus in virus harvests is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents *(2.6.16)*

Use 50 ml for the test in cell cultures.

Control cells

The control cells of the production cell culture from which the single harvest is derived comply with a test for identity and with the requirements for extraneous agents *(2.6.16)*.

FINAL BULK VACCINE

Virus harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bacterial and fungal contamination

Carry out the test for sterility *(2.6.1)* using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers and freeze-dried to a moisture content shown to be favourable to the stability of the vaccine. The containers are then closed so as to prevent contamination and the introduction of moisture.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

IDENTIFICATION

When the vaccine reconstituted as stated on the label is mixed with specific *Herpesvirus varicellae* antibodies, it is no longer able to infect susceptible cell cultures.

TESTS

Bacterial and fungal contamination

The reconstituted vaccine complies with the test for sterility *(2.6.1)*.

Bovine serum albumin

Not more than 0.5 µg per human dose, determined by a suitable immunochemical method *(2.7.1)*.

Water *(2.5.12)*

Not more than 3.0 per cent, determined by the semi-micro determination of water.

ASSAY

Titrate the vaccine for infective virus, using at least 3 separate vials of vaccine. Titrate 1 vial of an appropriate virus reference preparation in triplicate to validate each assay. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory. Calculate the individual virus concentration for each vial of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations, using the usual statistical methods (for example, *5.3*). The combined estimate of the virus concentration for the 3 vials of vaccine is not less than that stated on the label.

The assay is not valid if:

— the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than ± 0.3 log PFU;

— the virus concentration of the reference preparation differs by more than 0.5 log PFU from the established value.

The assay is repeated if the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 log PFU; data obtained from valid assays only are combined by the usual statistical methods (for example, *5.3*) to calculate the virus concentration of the sample. The confidence interval ($P = 0.95$) of the combined virus concentration is not greater than ± 0.3 log PFU.

Where justified and authorised, different assay designs may be used; this may imply the application of different validity and acceptance criteria. However, the vaccine must comply if tested as described above.

LABELLING

The label states:

- — the strain of virus used for the preparation of the vaccine;
- — the type and origin of the cells used for the preparation of the vaccine;
- — the minimum virus concentration;
- — that contact between the vaccine and disinfectants is to be avoided;
- — that the vaccine is not to be administered to pregnant women.

Ph Eur

Yellow Fever Vaccine, Live

General Notices

(Yellow Fever Vaccine (Live), Ph Eur monograph 0537)

The label may state 'Yel(live).'

Ph Eur

DEFINITION

Yellow fever vaccine (live) is a freeze-dried preparation of the 17D strain of yellow fever virus grown in fertilised hen eggs. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid.

PRODUCTION

The production of vaccine is based on a virus seed-lot system. The production method shall have been shown to yield consistently yellow fever vaccine (live) of acceptable immunogenicity and safety for man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use *(2.6.9)* modified as follows for the test in guinea-pigs: inject 10 human doses into each guinea-pig at 2 different injection sites and observe for 21 days.

Reference preparation In the test for neurotropism, a suitable batch of vaccine known to have satisfactory properties in man is used as the reference preparation.

SUBSTRATE FOR VIRUS PROPAGATION

Virus for the preparation of master and working seed lots and of all vaccine batches is grown in the tissues of chick embryos from a flock free from specified pathogens (SPF) *(5.2.2)*.

SEED LOTS

The 17D strain shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are prepared in large quantities and stored at a temperature below - 60 °C. Master and working seed lots shall not contain any human protein or added serum.

Unless otherwise justified and authorised, the virus in the final vaccine shall be between passage levels 204 and 239 from the original isolate of strain 17D. A working seed lot shall be only 1 passage from a master seed lot. A working seed lot shall be used without intervening passage as the inoculum for infecting the tissues used in the production of a vaccine lot, so that no vaccine virus is more than 1 passage from a seed lot that has passed all the safety tests.

Only a virus seed lot that complies with the following requirements may be used for virus propagation.

Identification

The master and working seed lots are identified as containing yellow fever virus by serum neutralisation in cell culture, using specific antibodies.

Extraneous agents ***(2.6.16)***

Each master seed lot complies with the following tests:

— test in guinea-pigs (as described in chapter *2.6.16* under Virus seed lot);

— bacterial and fungal sterility (as described in chapter *2.6.16* under Virus seed lot and virus harvests);

— mycoplasmas (as described in chapter *2.6.16* under Virus seed lot and virus harvests).

Avian leucosis viruses ***(2.6.24)***

Each master seed lot complies with the test for avian leucosis viruses.

Extraneous agents ***(2.6.16)***

Each working seed lot complies with the following tests:

— test in adult mice (intraperitoneal inoculation only) (as described in chapter *2.6.16* under Virus seed lot);

— test in guinea-pigs (as described in chapter *2.6.16* under Virus seed lot);

— bacterial and fungal sterility (as described in chapter *2.6.16* under Virus seed lot and virus harvests);

— mycoplasmas (as described in chapter *2.6.16* under Virus seed lot and virus harvests);

— mycobacteria (as described in chapter *2.6.16* under Virus seed lot and virus harvests);

— test in cell culture for other extraneous agents (as described in chapter *2.6.16* under Virus seed lot and virus harvests);

— avian viruses (as described in chapter *2.6.16* under Virus seed lot and virus harvests).

Avian leucosis viruses ***(2.6.24)***

Each working seed lot complies with the test for avian leucosis viruses.

Tests in monkeys

Each master and working seed lot complies with the following tests in monkeys for viraemia (viscerotropism), immunogenicity and neurotropism.

The monkeys shall be *Macaca* sp. susceptible to yellow fever virus and shall have been shown to be non-immune to yellow fever at the time of injecting the seed virus. They shall be healthy and shall not have received previously intracerebral or intraspinal inoculation. Furthermore, they shall not have been inoculated by other routes with neurotropic viruses or with antigens related to yellow fever virus. Not fewer than 10 monkeys are used for each test.

Use a test dose of 0.25 ml containing the equivalent of not less than 5000 mouse LD_{50} and not more than 50 000 mouse LD_{50}, determined by a titration for infectious virus and using the established equivalence between virus concentration and mouse LD_{50} (see under Assay). Inject the test dose into 1 frontal lobe of each monkey under anaesthesia and observe the monkeys for not less than 30 days.

Viraemia (Viscerotropism) Viscerotropism is indicated by the amount of virus present in serum. Take blood from each of the test monkeys on the 2nd, 4th and 6th days after inoculation and prepare serum from each sample. Prepare 1:10, 1:100 and 1:1000 dilutions from each serum and inoculate each dilution into a group of at least 6 cell culture vessels used for the determination of the virus concentration. The seed lot complies with the test if none of the sera contains more than the equivalent of 500 mouse LD_{50} in 0.03 ml and at most 1 serum contains more than the equivalent of 100 mouse LD_{50} in 0.03 ml.

Immunogenicity Take blood from each monkey 30 days after the injection of the test dose and prepare serum from each sample. The seed lot complies with the test if at least 90 per cent of the test monkeys are shown to be immune, as determined by examining their sera in the test for neutralisation of yellow fever virus described below.

It has been shown that a low dilution of serum (for example, 1:10) may contain non-specific inhibitors that influence this test; such serum shall be treated to remove inhibitors. Mix dilutions of at least 1:10, 1:40 and 1:160 of serum from each monkey with an equal volume of 17D vaccine virus at a dilution that will yield an optimum number of plaques with the titration method used. Incubate the serum-virus mixtures in a water-bath at 37 °C for 1 h and then cool in iced water; add 0.2 ml of each serum-virus mixture to each of 4 cell-culture plates and proceed as for the determination of virus concentration. Inoculate similarly 10 plates with the same amount of virus, plus an equal volume of a 1:10 dilution of monkey serum known to contain no neutralising antibodies to yellow fever virus. At the end of the observation period, compare the mean number of plaques in the plates receiving virus plus non-immune serum with the mean number of plaques in the plates receiving virus plus dilutions of each monkey serum. Not more than 10 per cent of the test monkeys have serum that fails to reduce the number of plaques by 50 per cent at the 1:10 dilution.

Neurotropism Neurotropism is assessed from clinical evidence of encephalitis, from incidence of clinical manifestations and by evaluation of histological lesions, in comparison with 10 monkeys injected with the reference preparation. The seed lot is not acceptable if either the onset and duration of the febrile reaction or the clinical signs of encephalitis and pathological findings are such as to indicate a change in the properties of the virus.

Clinical evaluation

The monkeys are examined daily for 30 days by personnel familiar with clinical signs of encephalitis in primates (if necessary, the monkeys are removed from their cage and examined for signs of motor weakness or spasticity). The seed lot is not acceptable if in the monkeys injected with it the incidence of severe signs of encephalitis, such as paralysis or inability to stand when stimulated, or mortality is greater than for the reference vaccine. These and other signs of encephalitis, such as paresis, incoordination, lethargy, tremors or spasticity are assigned numerical values for the severity of symptoms by a grading method. Each day each monkey in the test is given a score based on the following scale:

— grade 1: rough coat, not eating;

— grade 2: high-pitched voice, inactive, slow moving;

— grade 3: shaky, tremors, unco-ordinated, limb weakness;

— grade 4: inability to stand, limb paralysis or death (a dead monkey receives a daily score of 4 from the day of death until day 30).

A clinical score for a particular monkey is the average of its daily scores; the clinical score for the seed lot is the mean of the individual monkey scores. The seed lot is not acceptable if the mean of the clinical severity scores for the group of monkeys inoculated with it is significantly greater ($P = 0.95$) than the mean for the group of monkeys injected with the reference preparation. In addition, special consideration is given to any animal showing unusually severe signs when deciding on the acceptability of the seed lot.

Histological evaluation

5 levels of the brain are examined including:

— block I: the corpus striatum at the level of the optic chiasma;

— block II: the thalamus at the level of the mamillary bodies;

— block III: the mesencephalon at the level of the superior colliculi;

— block IV: the pons and cerebellum at the level of the superior olives;

— block V: the medulla oblongata and cerebellum at the level of the mid-inferior olivary nuclei.

Cervical and lumbar enlargements of the spinal cord are each divided equally into 6 blocks; 15 µm sections are cut from the tissue blocks embedded in paraffin wax and stained with gallocyanin. Numerical scores are given to each hemisection of the cord and to structures in each hemisection of the brain as listed below. Lesions are scored as follows:

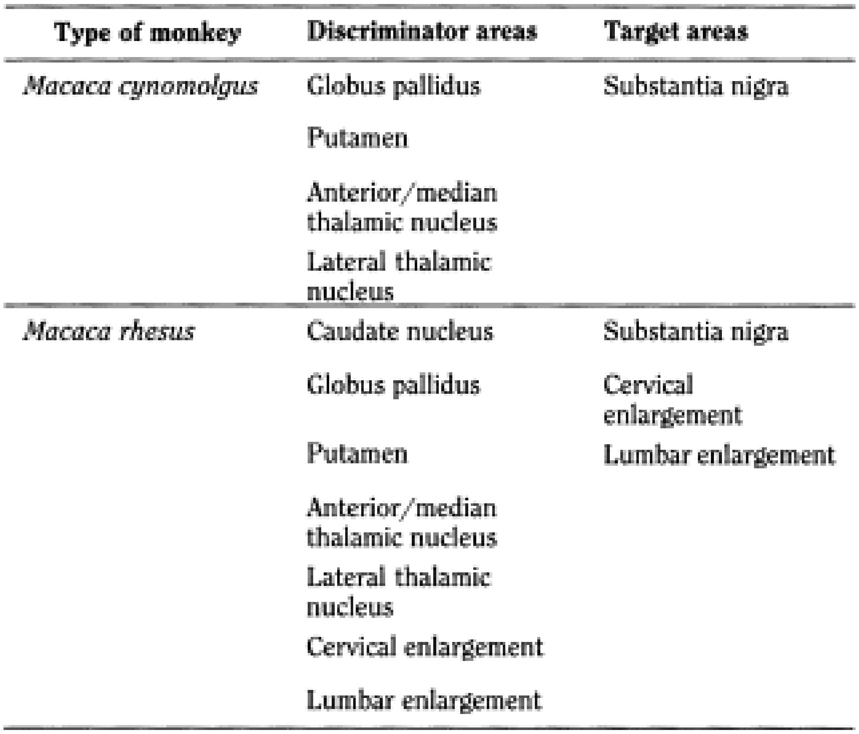
— grade 1 - minimal: 1 to 3 small focal inflammatory infiltrates; degeneration or loss of a few neurons;

— grade 2 - moderate: 4 or more focal inflammatory infiltrates; degeneration or loss of neurons affecting not more than one third of cells;

— grade 3 - severe: moderate focal or diffuse inflammatory infiltration; degeneration or loss of up to two thirds of the neurons;

— grade 4 - overwhelming: variable but often severe inflammatory reaction; degeneration or loss of more than 90 per cent of neurons.

It has been found that inoculation of yellow fever vaccine into the monkey brain causes histological lesions in different anatomical formations of the central nervous system with varying frequency and severity (I. S. Levenbook *et al., Journal of Biological Standardization*, 1987, 15, 305-313). Based on these 2 indicators, the anatomical structures can be divided into target, spared and discriminator areas. Target areas are those which show more severe specific lesions in a majority of monkeys irrespective of the degree of neurovirulence of the seed lot. Spared areas are those which show only minimal specific lesions and in a minority of monkeys. Discriminator areas are those where there is a significant increase in the frequency of more severe specific lesions with seed lots having a higher degree of neurovirulence. Discriminator and target areas for *Macaca cynomolgus* and *Macaca rhesus* monkeys are shown in the table below.



Type of monkey	Discriminator areas	Target areas
Macaca cynomolgus	Globus pallidus	Substantia nigra
	Putamen	
	Anterior/median thalamic nucleus	
	Lateral thalamic nucleus	
Macaca rhesus	Caudate nucleus	Substantia nigra
	Globus pallidus	Cervical enlargement
	Putamen	Lumbar enlargement
	Anterior/median thalamic nucleus	
	Lateral thalamic nucleus	
	Cervical enlargement	
	Lumbar enlargement	

Scores for discriminator and target areas are used for the final evaluation of the seed lot. The individual monkey score is calculated from the sum of individual target area scores in each hemisection divided by the number of areas examined. A separate score is calculated similarly for the discriminator areas.

Mean scores for the test group are calculated in 2 ways: (1) by dividing the sum of the individual monkey discriminator scores by the number of monkeys and (2) by dividing the sum of the individual monkey target and discriminator scores by the number of monkeys. These 2 mean scores are taken into account when deciding on the acceptability of the seed lot. The seed lot is not acceptable if either of the mean lesion scores is significantly greater ($P = 0.95$) than for the reference preparation.

PROPAGATION AND HARVEST

All processing of the fertilised eggs is done under aseptic conditions in an area where no other infectious agents or cells are handled at the same time. At least 2 per cent but not less than 20 and not more than 80 eggs are maintained as uninfected control eggs. After inoculation and incubation at a controlled temperature, only living and typical chick embryos are harvested. At the time of harvest, the control eggs are treated in the same way as the inoculated eggs to obtain a control embryonic pulp. The age of the embryo at the time of virus harvest is reckoned from the initial introduction of the egg into the incubator and shall be not more than 12 days. After homogenisation and clarification by centrifugation, the extract of embryonic pulp is tested as described below and kept at - 70 °C or colder until further processing. Virus harvests that comply with the prescribed tests may be pooled. No human protein is added to the virus suspension at any stage during production. If stabilisers are added, they shall have been shown to have no antigenic or sensitising properties for man.

Only a single harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

The single harvest contains virus that is identified as yellow fever virus by serum neutralisation in cell culture, using specific antibodies.

Bacterial and fungal contamination *(2.6.1)*

The harvest complies with the test for sterility, carried out using 10 ml for each medium.

Mycoplasmas *(2.6.7)*

The single harvest complies with the test for mycoplasma, carried out using 10 ml.

Mycobacteria *(2.6.2)*

A 5 ml single harvest sample is tested for the presence of *Mycobacterium spp*. by culture methods known to be sensitive for the detection of these organisms.

Embryonic pulp of control eggs

The extract of the control eggs shows no evidence of the presence of any extraneous agents in the tests described below.

Test in cell culture for other extraneous agents Inoculate a 5 ml sample of embryonic pulp of the control eggs into continuous simian kidney and human cell cultures as well as into primary chick-embryo-fibroblast cells. The cells are incubated at 36 ± 1 °C and observed for a period of 14 days. The embryonic pulp of the control eggs passes the test if there is no evidence of the presence of any extraneous agents. The test is not valid unless at least 80 per cent of the cell cultures remain viable.

Avian viruses Using 0.1 ml per egg, inoculate the embryonic pulp of control eggs: by the allantoic route into a group of 10 fertilised SPF eggs *(5.2.2)* (9 to 11 days old); into the yolk sac of a group of 10 fertilised SPF eggs *(5.2.2)*, 5 to 7 days old. Incubate for 7 days. The embryonic pulp lot of the control eggs complies if the allantoic and yolk sac fluids show no signs of haemagglutinating agents and if the embryos and chorio-allantoic membranes examined to detect any macroscopic pathology are typical. The test is not valid unless at least 80 per cent of the inoculated eggs survive during the 7 day observation period.

Virus concentration

In order to calculate the dilution for formulation of the final bulk, each single harvest is titrated as described under Assay.

FINAL BULK VACCINE

Single harvests that comply with the tests prescribed above are pooled and clarified again. A test for protein nitrogen content is carried out. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bacterial and fungal contamination *(2.6.1)*

The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

Protein nitrogen content

Maximum 0.25 mg per human dose before the addition of any stabiliser.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers and freeze-dried to a moisture content shown to be favourable to the stability of the vaccine. The containers are then closed so as to prevent contamination and the introduction of moisture.

Only a final lot that is satisfactory with respect to thermal stability and each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for ovalbumin has been performed with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability

Maintain samples of the final lot of freeze-dried vaccine in the dry state at 37 °C for 14 days. Determine the virus concentration as described under Assay in parallel for the heated vaccine and for unheated vaccine. The difference in the virus concentration between unheated and heated vaccine does not exceed 1.0 log and the virus concentration of the heated vaccine is not less than the number of plaque-forming units (PFU) equivalent to 3.0 log mouse LD_{50} per human dose.

IDENTIFICATION

When the vaccine reconstituted as stated on the label is mixed with specific yellow fever virus antibodies, there is a significant reduction in its ability to infect susceptible cell cultures.

TESTS

Ovalbumin

Maximum 5 µg of ovalbumin per human dose, determined by a suitable immunochemical method *(2.7.1)*.

Water *(2.5.12)*

Maximum 3.0 per cent.

Bacterial and fungal contamination *(2.6.1)*

The reconstituted vaccine complies with the test for sterility.

Bacterial endotoxins *(2.6.14)*

Less than 5 IU per single human dose.

ASSAY

Titrate for infective virus in cell cultures using at least 3 separate vials of vaccine. Titrate 1 vial of an appropriate virus reference preparation in triplicate to validate each assay. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory. Calculate the individual virus concentration for each vial of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations using the usual statistical methods (for example, *5.3*). The combined virus concentration for the 3 vials of vaccine is not less than the equivalent in PFU of 3.0 log mouse LD_{50} per human dose. The relationship between mouse LD_{50} and PFU is established by each laboratory and approved by the competent authority.

The assay is not valid if:

- — the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than ± 0.3 log PFU;
- — the virus concentration of the reference preparation differs by more than 0.5 log PFU from the established value.

The assay is repeated if the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 log PFU; data obtained from valid assays only are combined by the usual statistical methods (for example, *5.3*) to calculate the virus concentration of the sample. The confidence interval ($P = 0.95$) of the combined virus concentration is not greater than ± 0.3 log PFU.

Where justified and authorised, different assay designs may be used; this may imply the application of different validity and acceptance criteria. However, the vaccine must comply if tested as described above.

The method shown below, or another suitable technique, may be used to determine the mouse LD_{50}.

Suggested method for determination of the mouse LD_{50}

Mouse LD_{50} The statistically calculated quantity of virus suspension that is expected to produce fatal specific encephalitis in 50 per cent of mice of a highly susceptible strain, 4 to 6 weeks of age, after intracerebral inoculation.

Appropriate serial dilutions of the reconstituted vaccine are made in diluent for yellow fever virus (a 7.5 g/l solution of *bovine albumin R* in *phosphate buffered saline pH 7.4 R,* or any other diluent that has been shown to be equivalent for maintaining the infectivity of the virus).

Mice of a highly susceptible strain, 4 to 6 weeks of age, are injected intracerebrally under anaesthesia with 0.03 ml of the vaccine dilution. Groups of not fewer than 6 mice are used for each dilution; the series of dilutions is chosen so as to cover the range 0-100 per cent mortality of the mice. Injection of the mice is performed immediately after the dilutions have been made. The mice are observed for 21 days and all deaths are recorded.

Only survivors and deaths caused by typical yellow fever infections are counted in the computations. Mice paralysed on the 21st day of observation are counted as survivors.

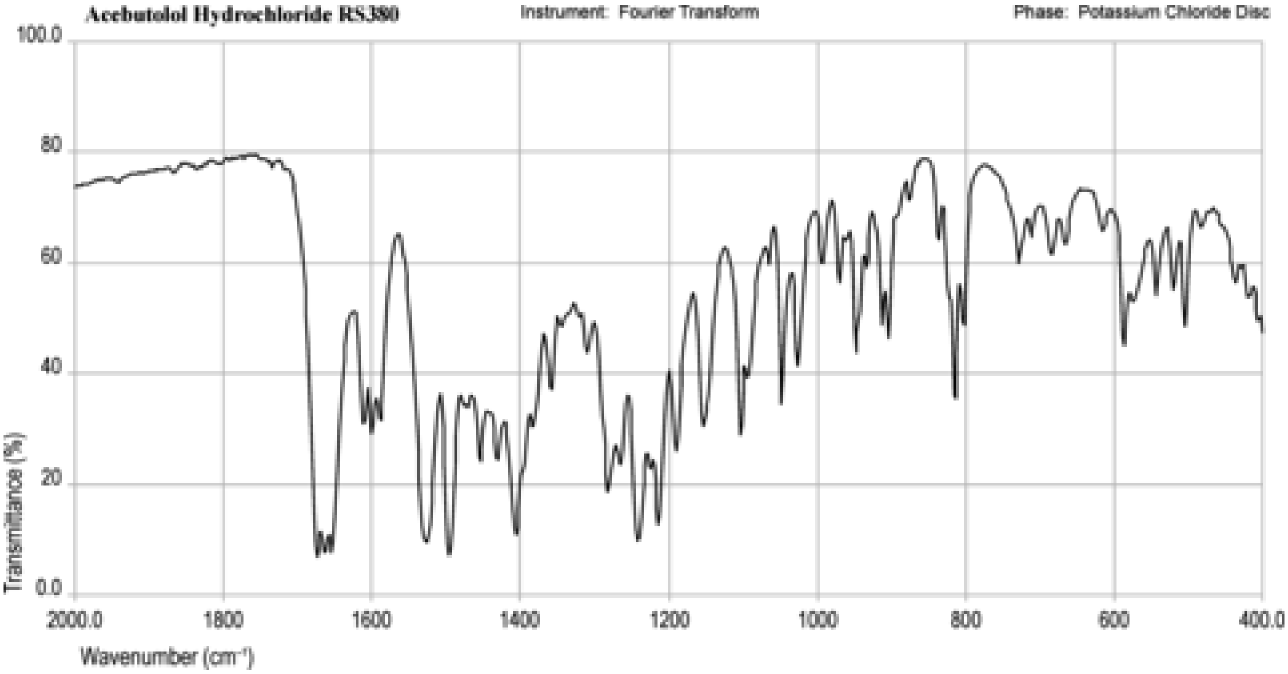
LABELLING

The label states:

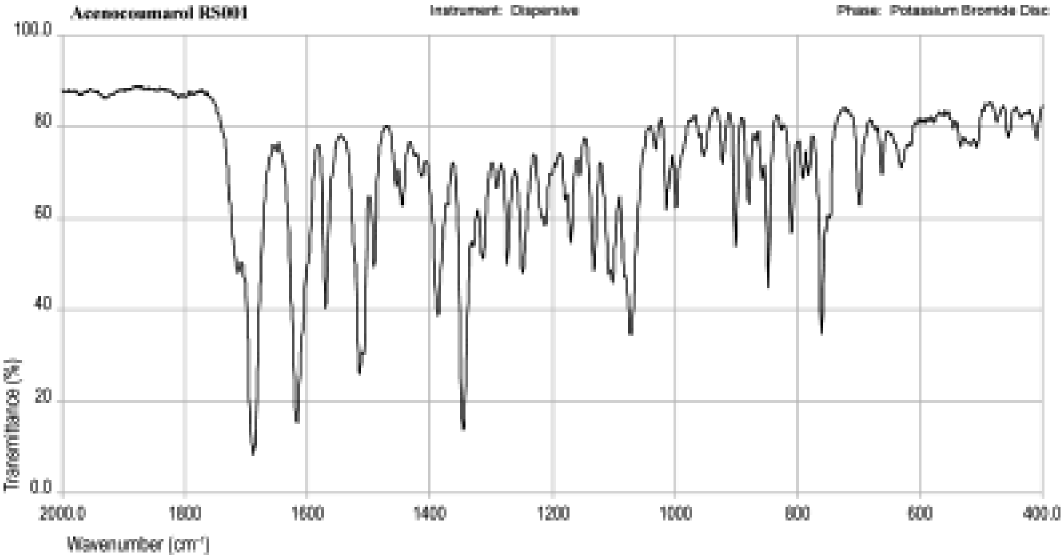
- — the strain of virus used in preparation of the vaccine;
- — that the vaccine has been prepared in chick embryos;
- — the minimum virus concentration;
- — that contact between the vaccine and disinfectants is to be avoided.

Ph Eur

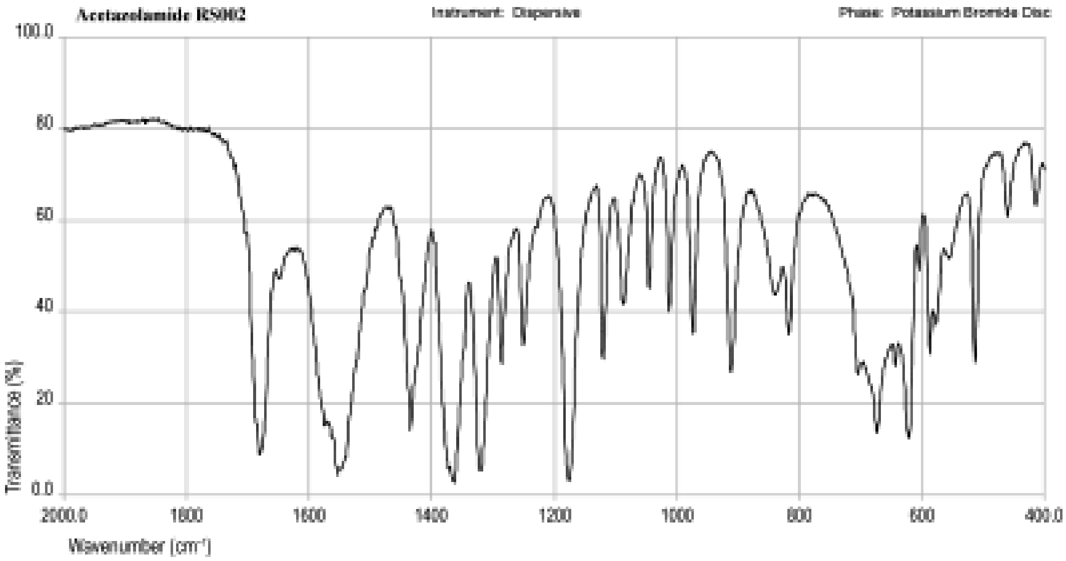
Acebutolol Hydrochloride



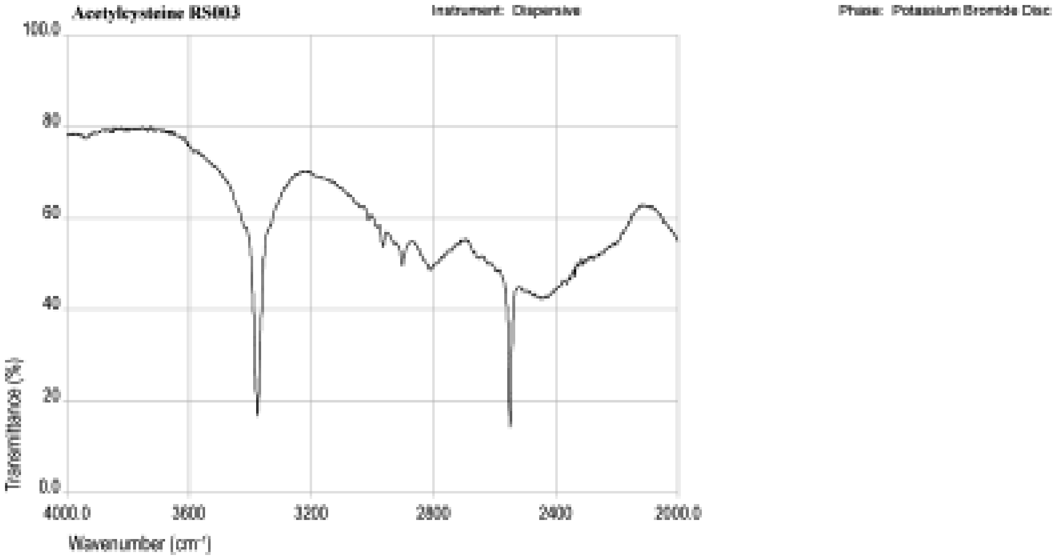
Acenocoumarol

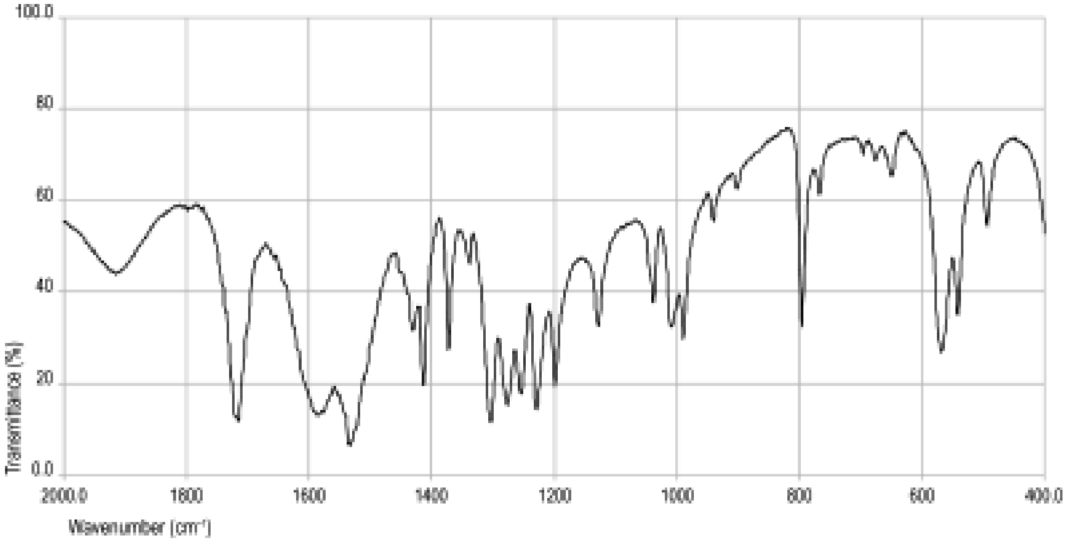


Acetazolamide

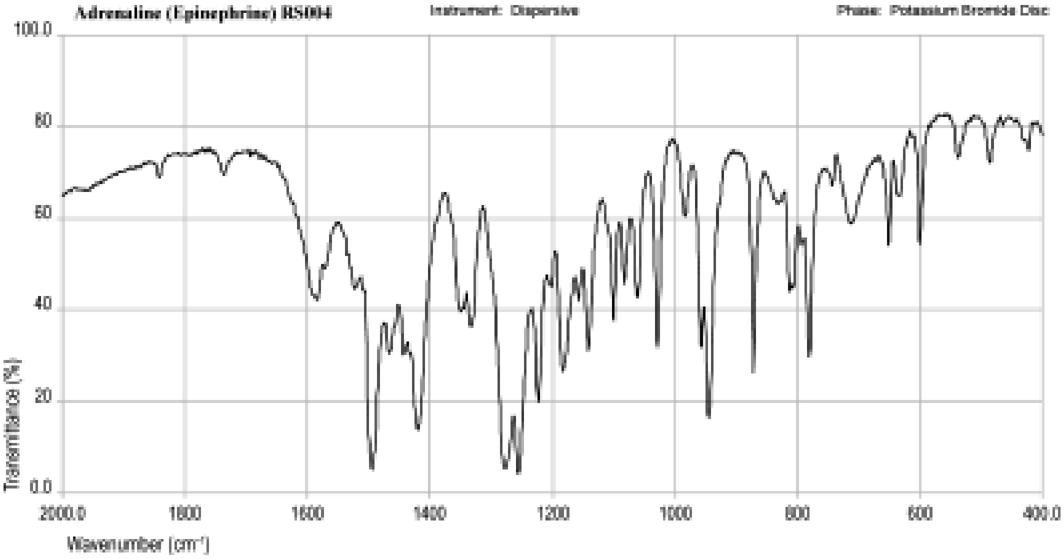


Acetylcysteine

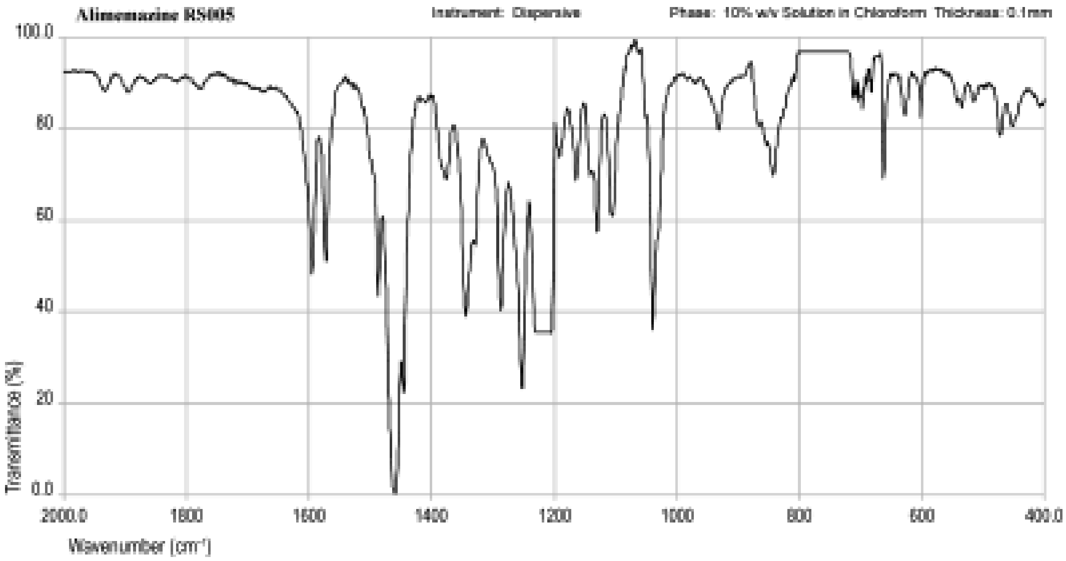




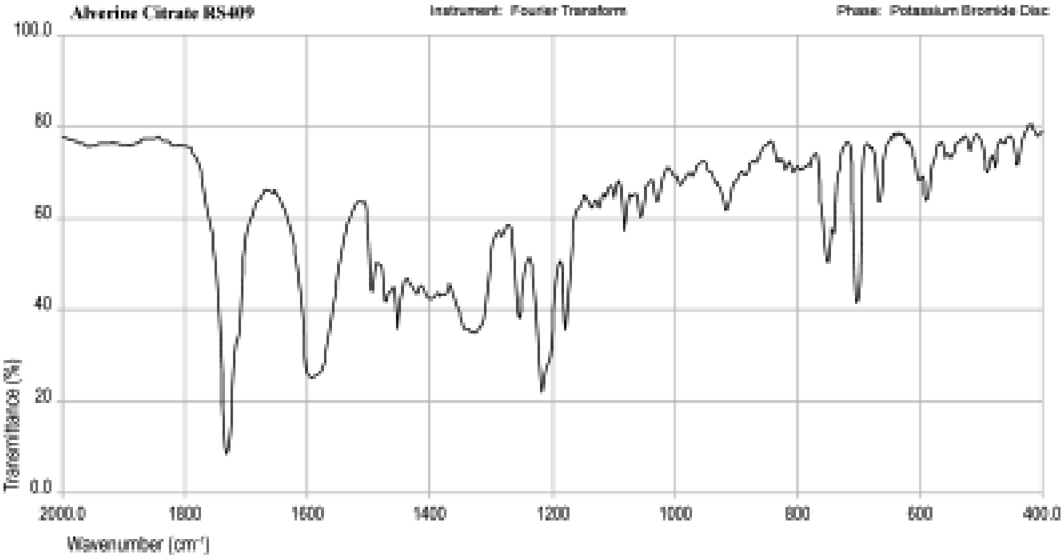
Adrenaline (Epinephrine)



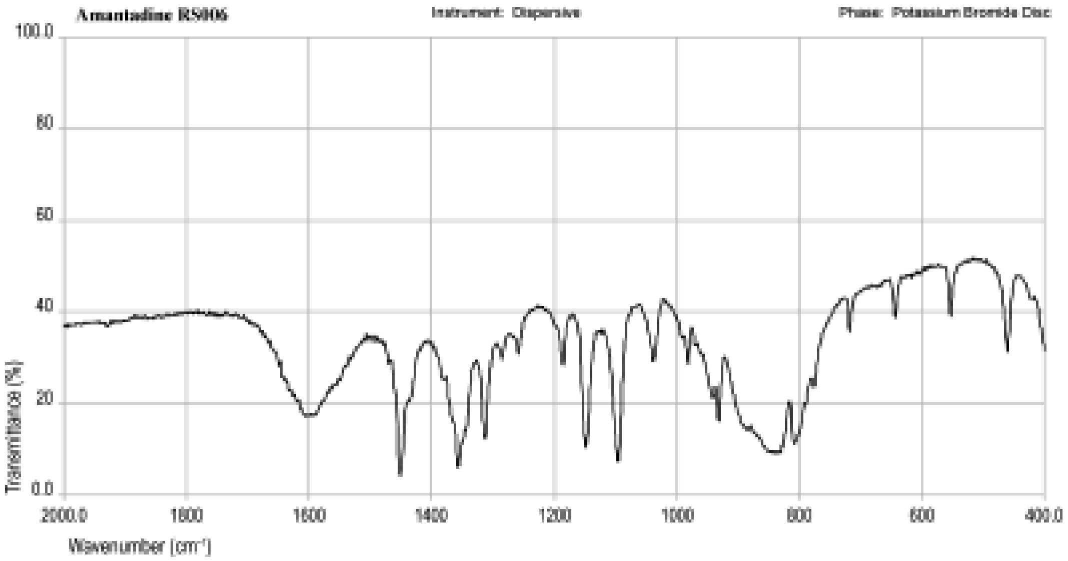
Alimemazine



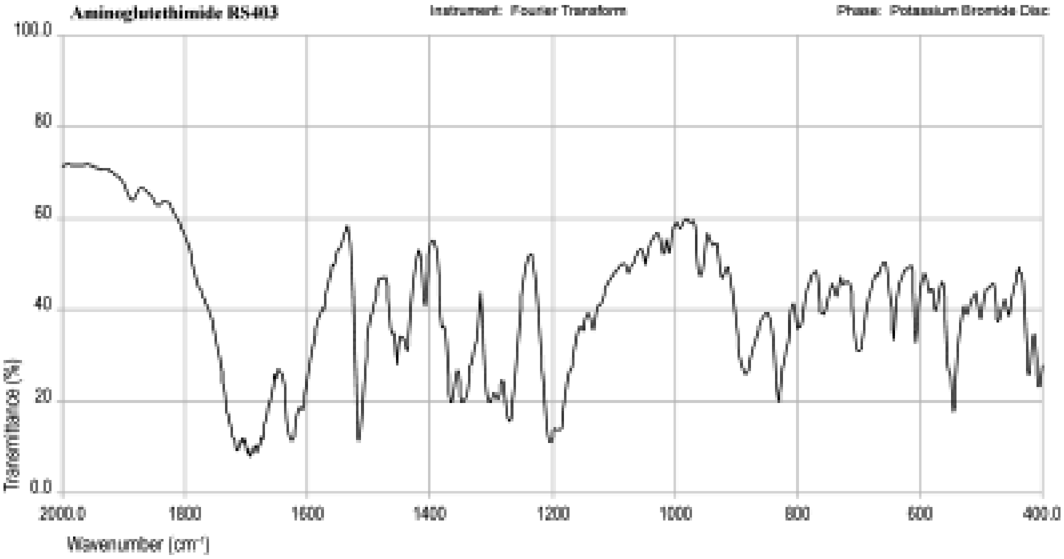
Alverine Citrate



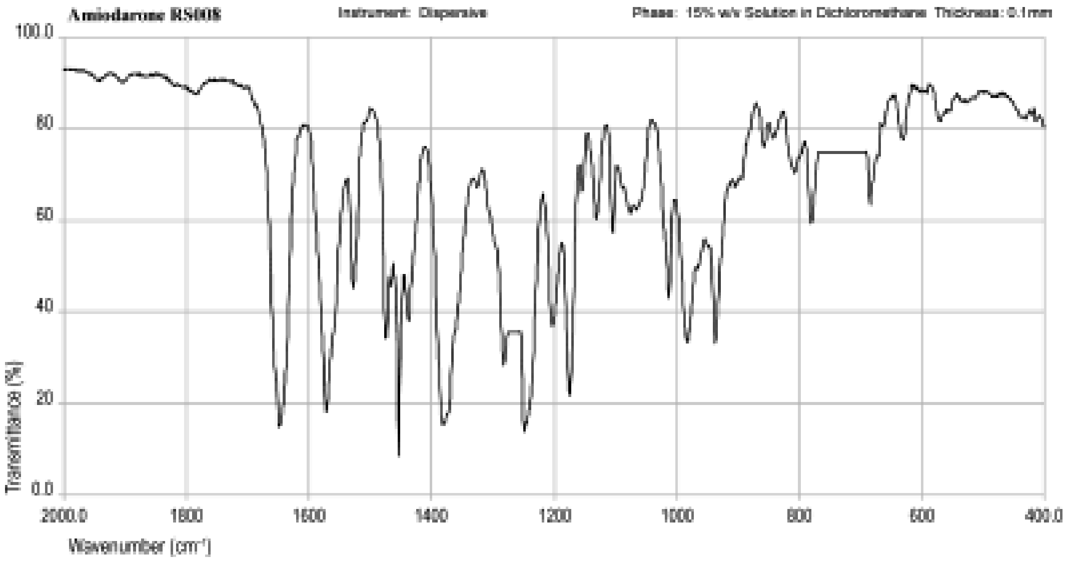
Amantadine


Amantadine RS006
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

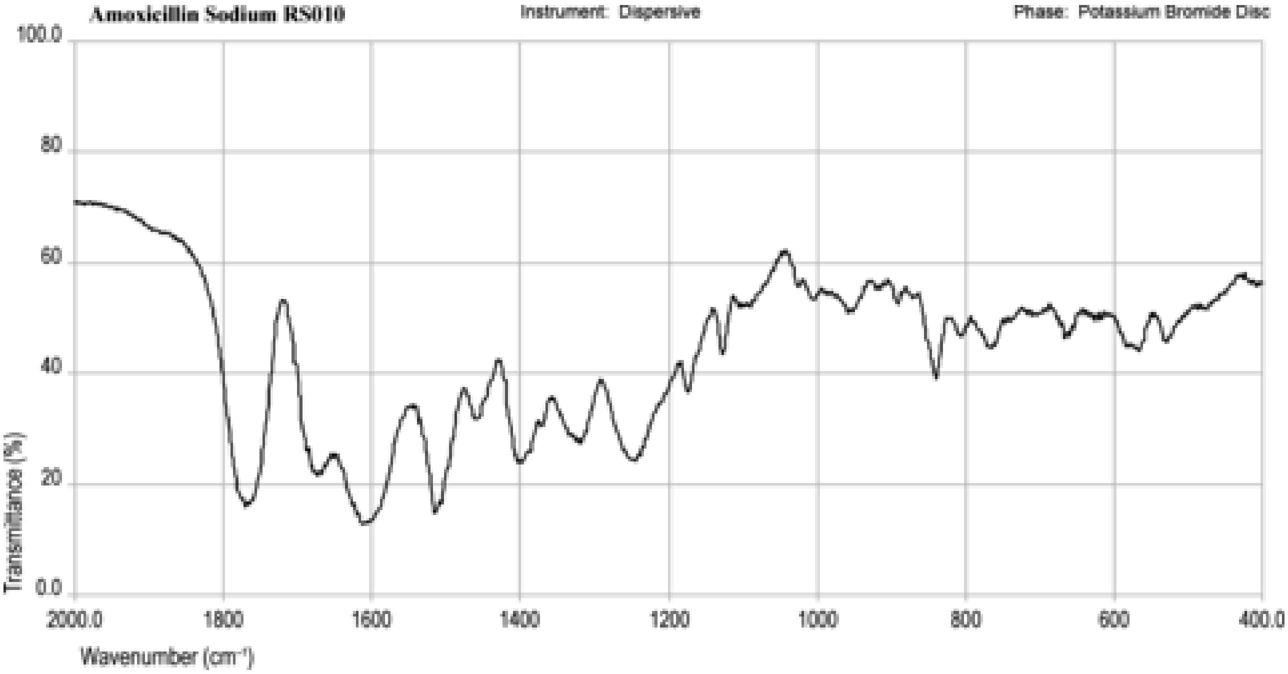
Aminoglutethimide



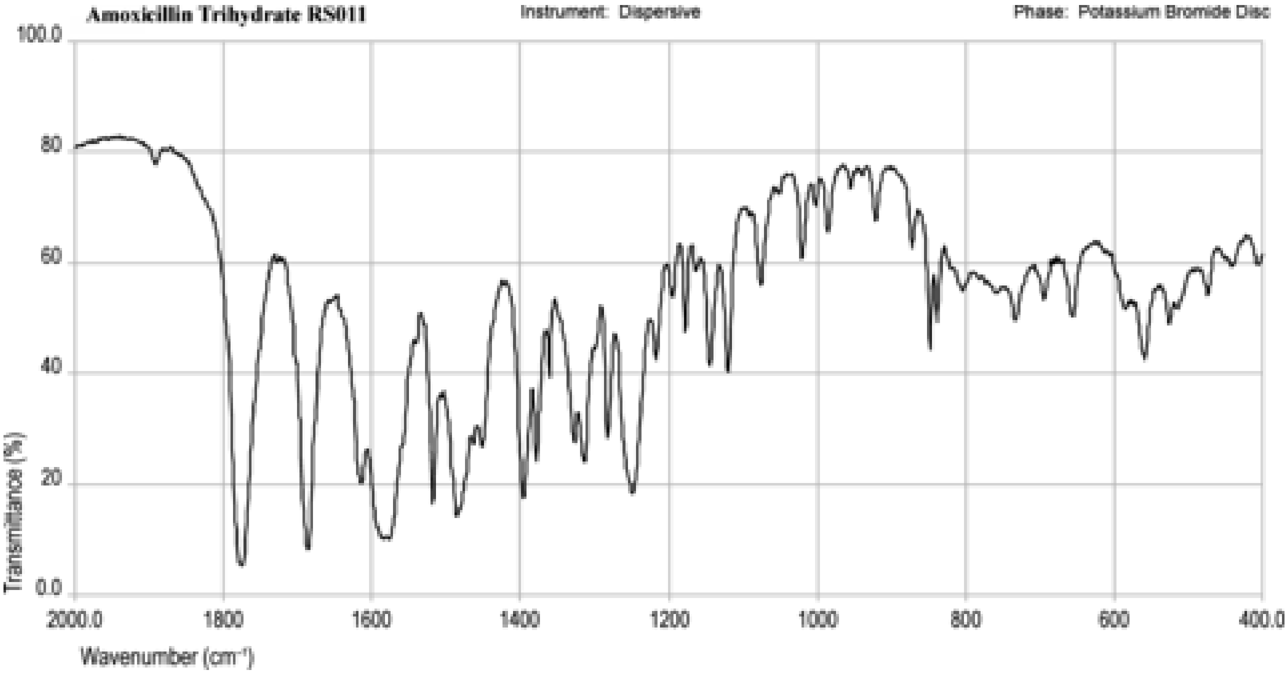
Amiodarone


Amiodarone RS008
Instrument: Dispersive
Phase: 15% w/v Solution in Dichloromethane Thickness: 0.1mm
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

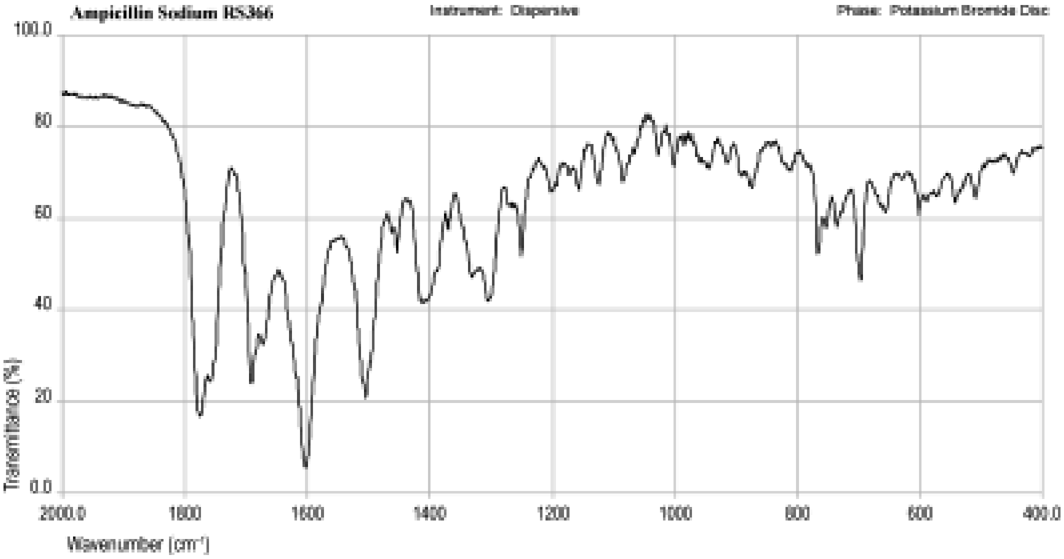
Amoxicillin Sodium



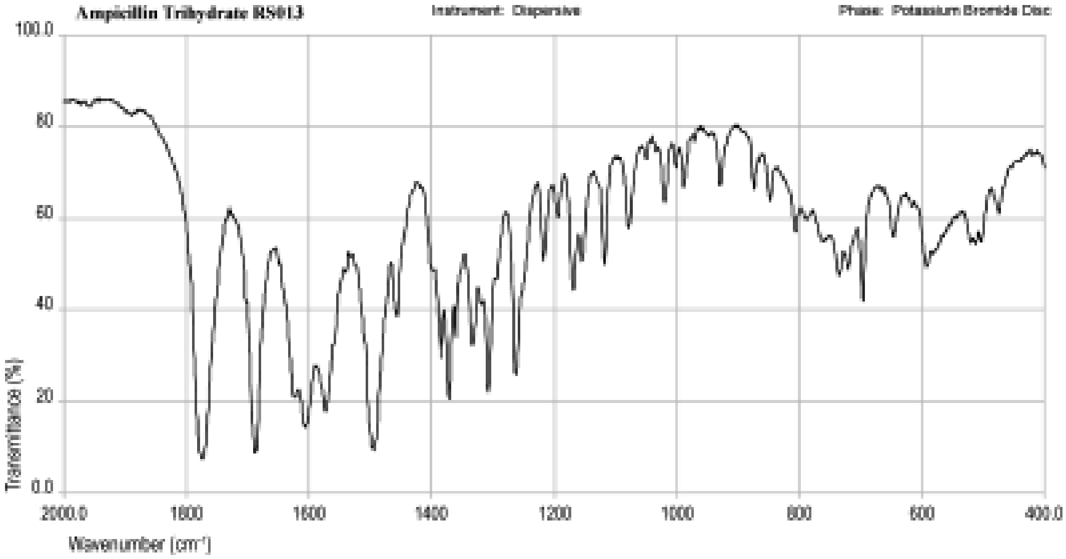
Amoxicillin Trihydrate



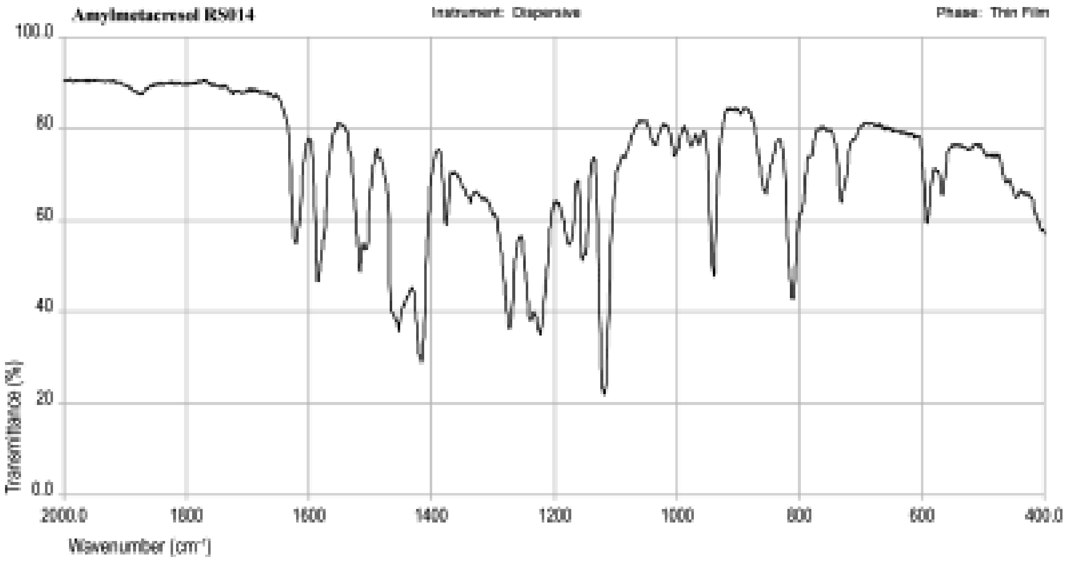
Ampicillin Sodium



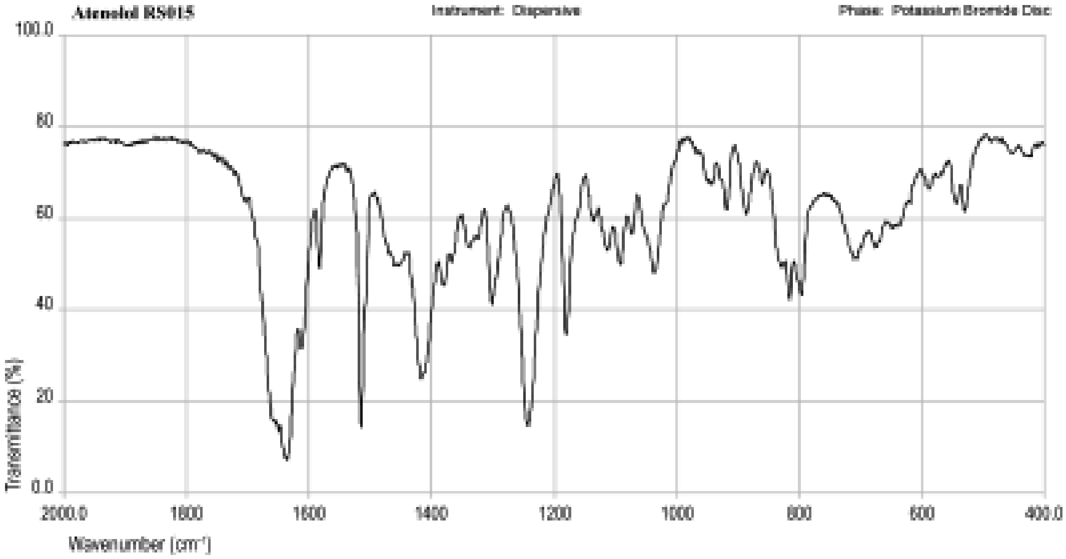
Ampicillin Trihydrate



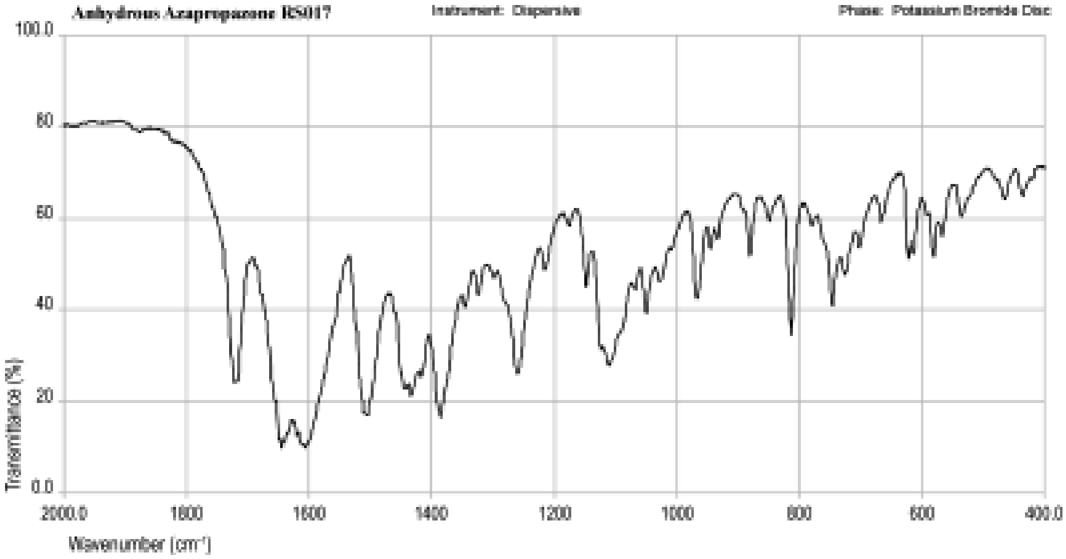
Amylmetacresol



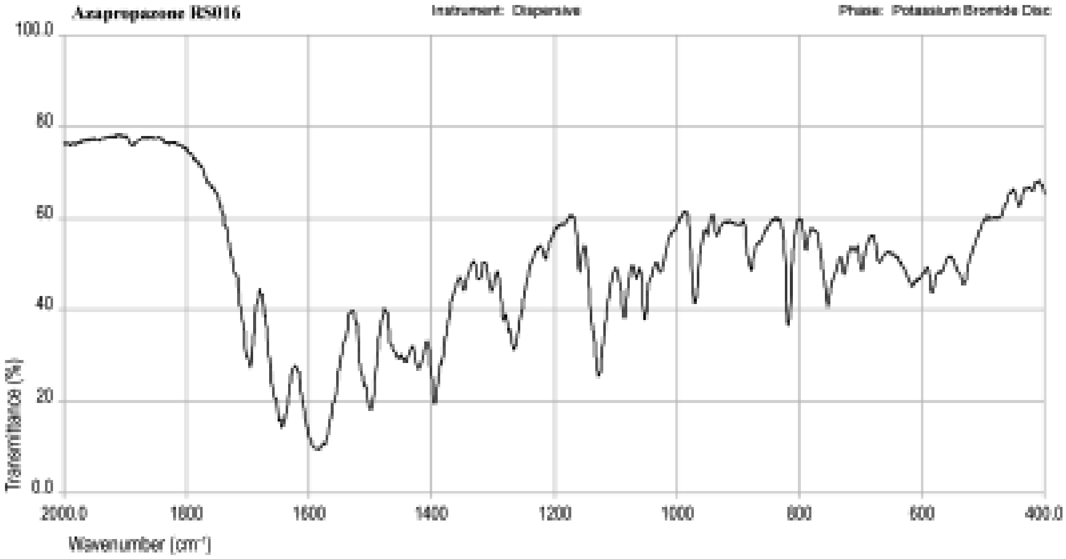
Atenolol


Atenolol RS015
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

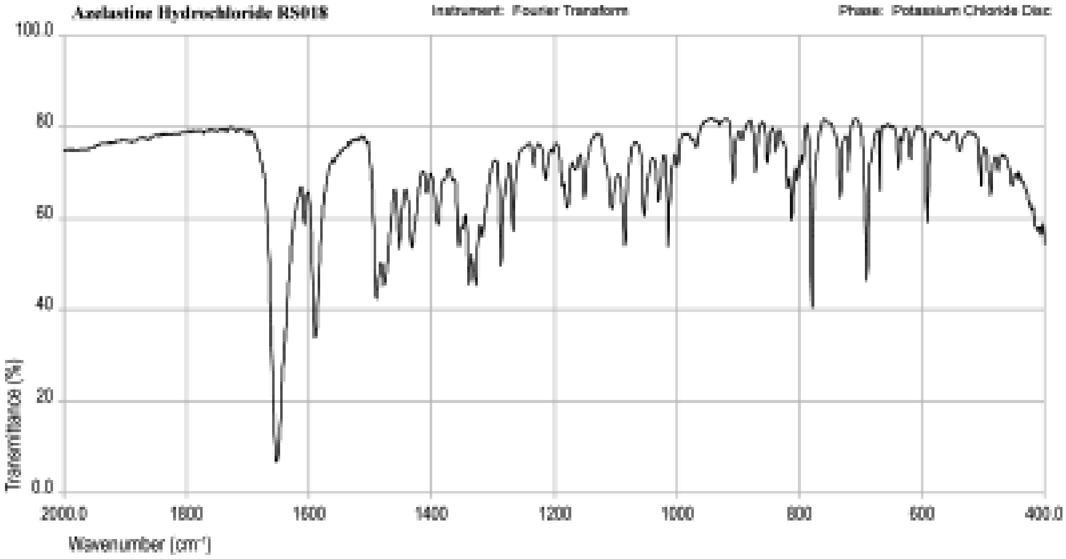
Azapropazone, Anhydrous



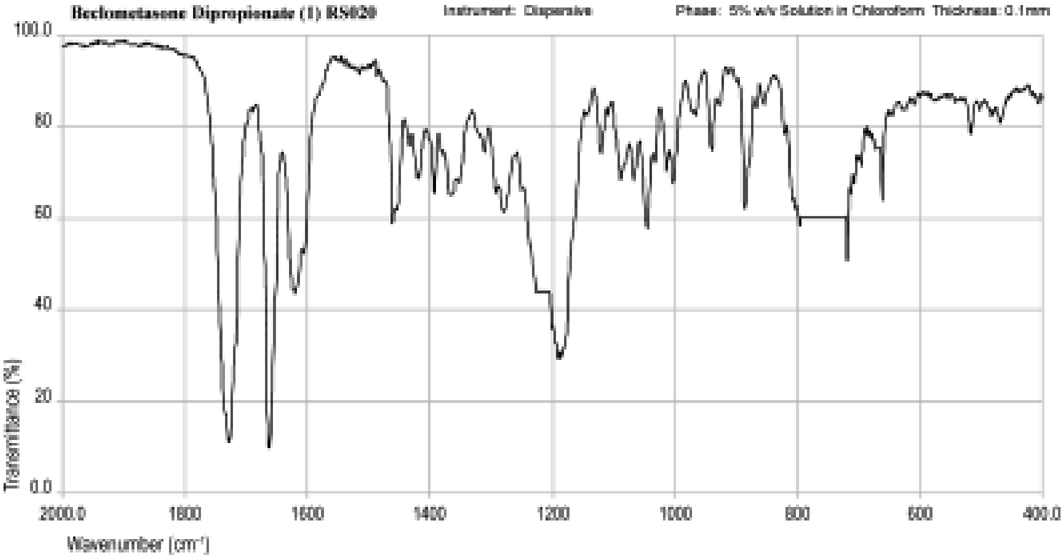
Azapropazone



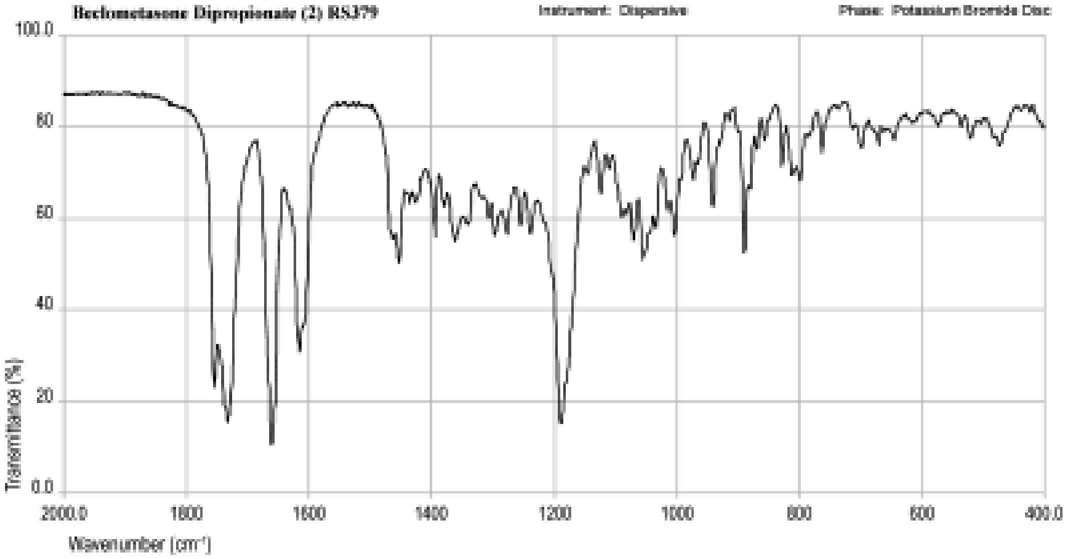
Azelastine Hydrochloride



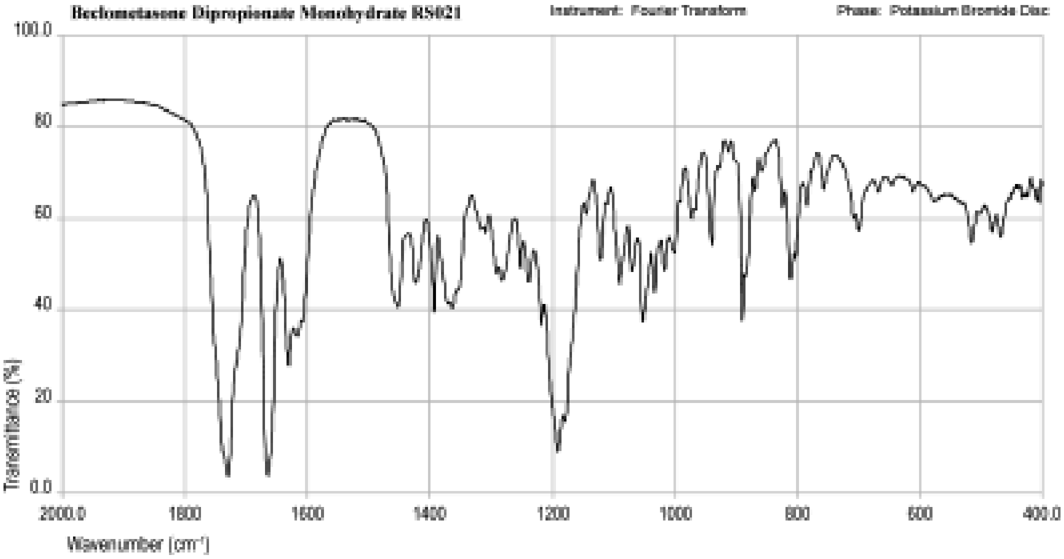
Beclometasone Dipropionate (1)



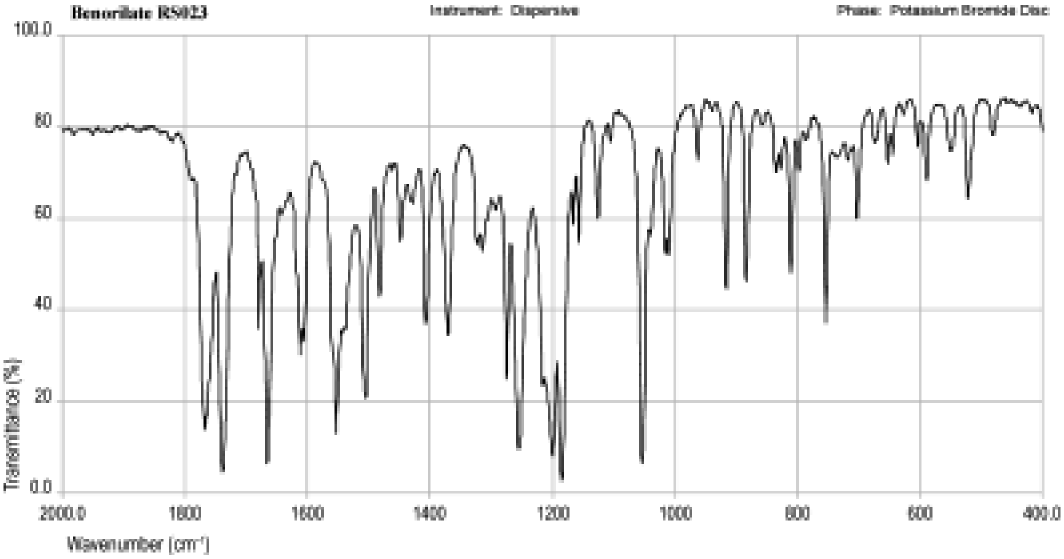
Beclometasone Dipropionate (2)



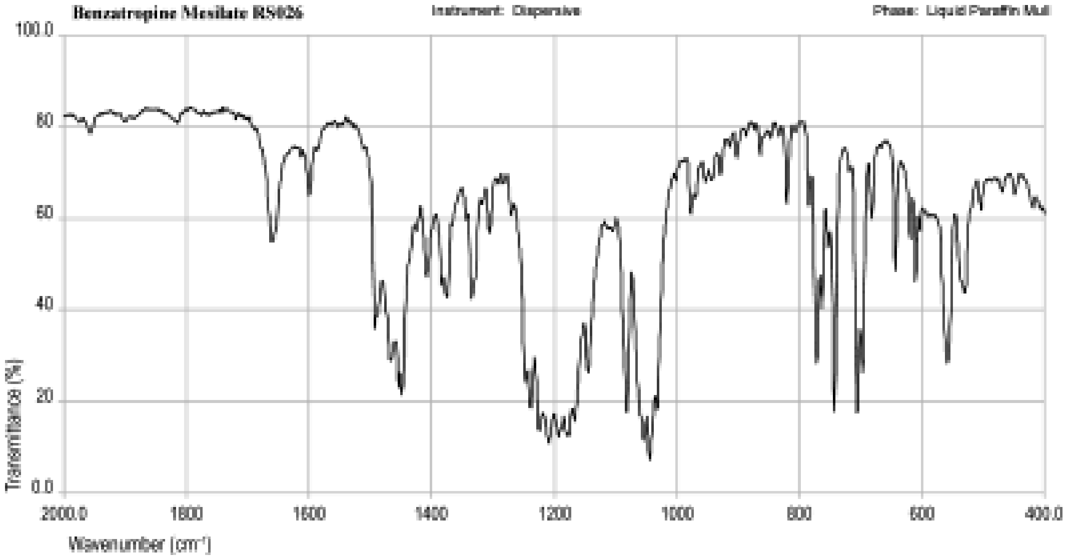
Beclometasone Dipropionate Monohydrate



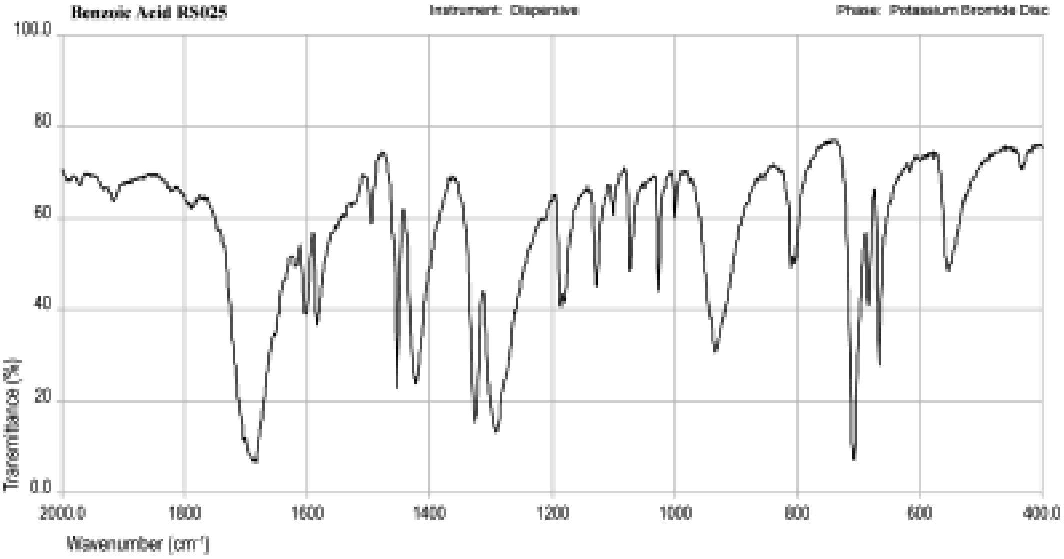
Benorilate


Benorilate RS023
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

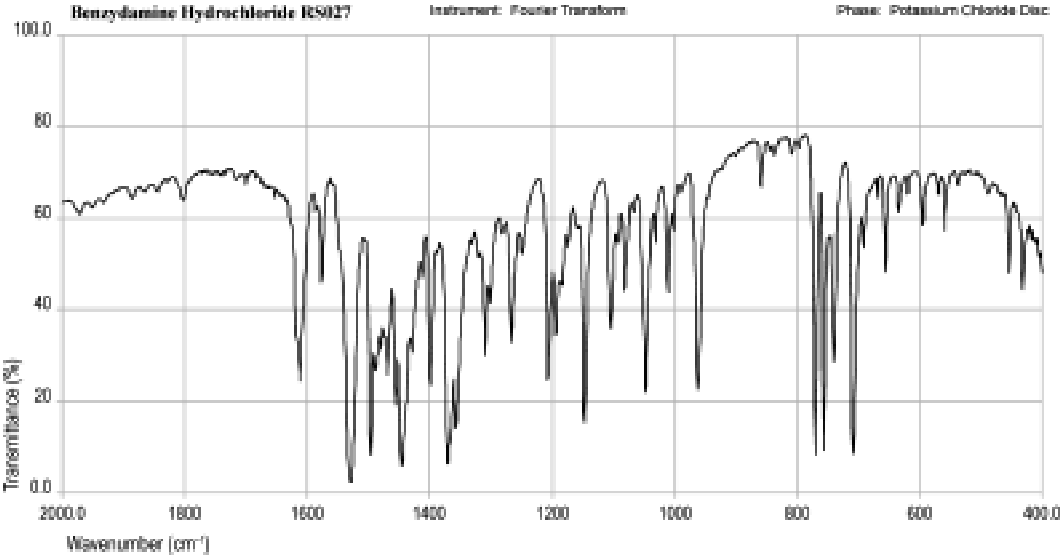
Benzatropine Mesilate



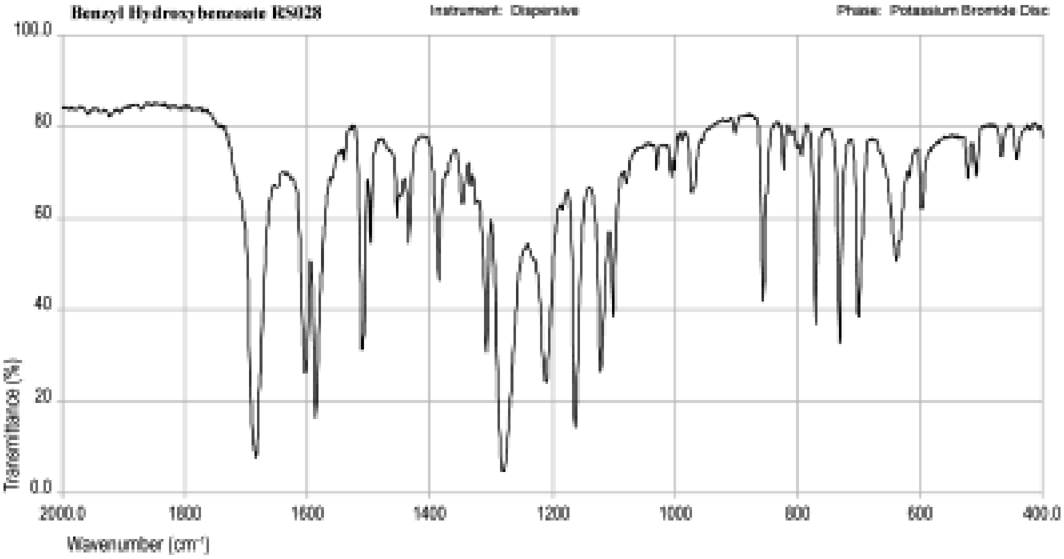
Benzoic Acid



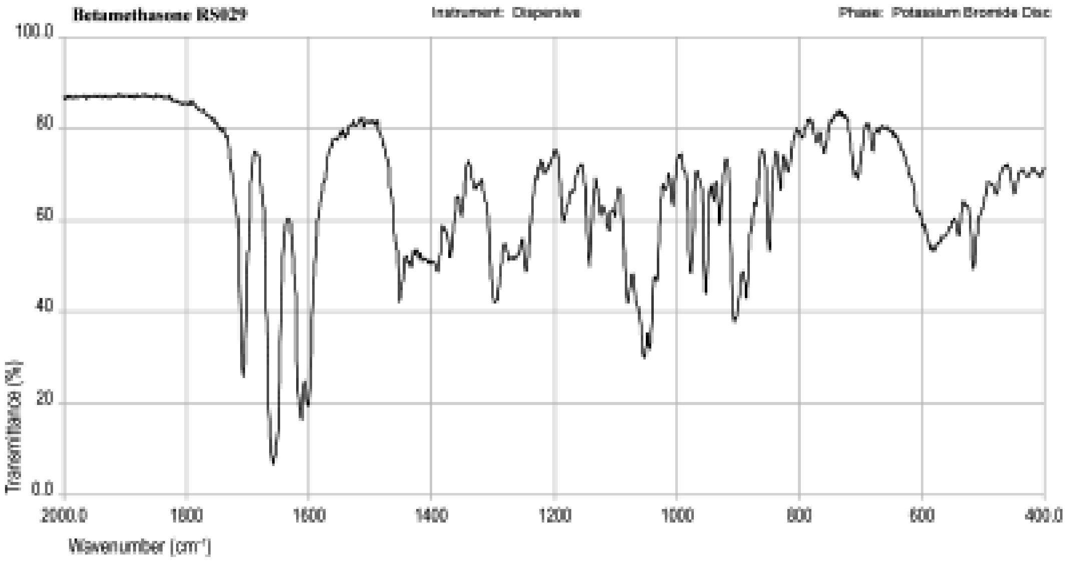
Benzydamine Hydrochloride



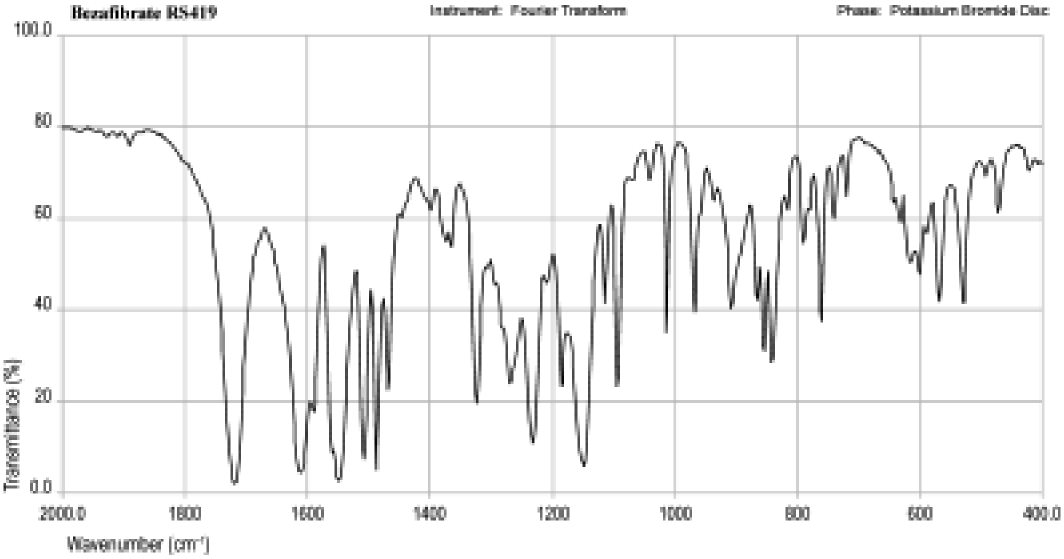
Benzyl Hydroxybenzoate



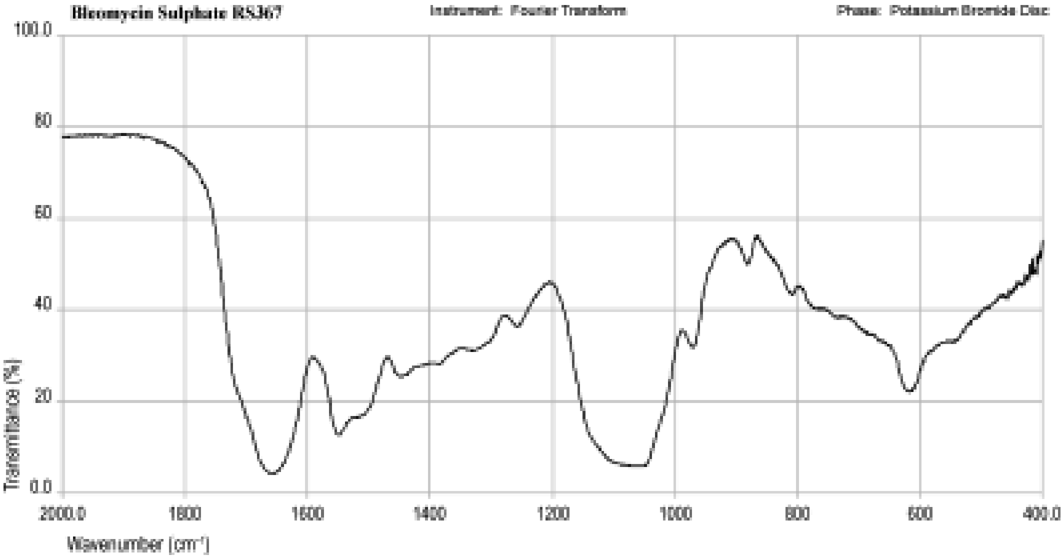
Betamethasone



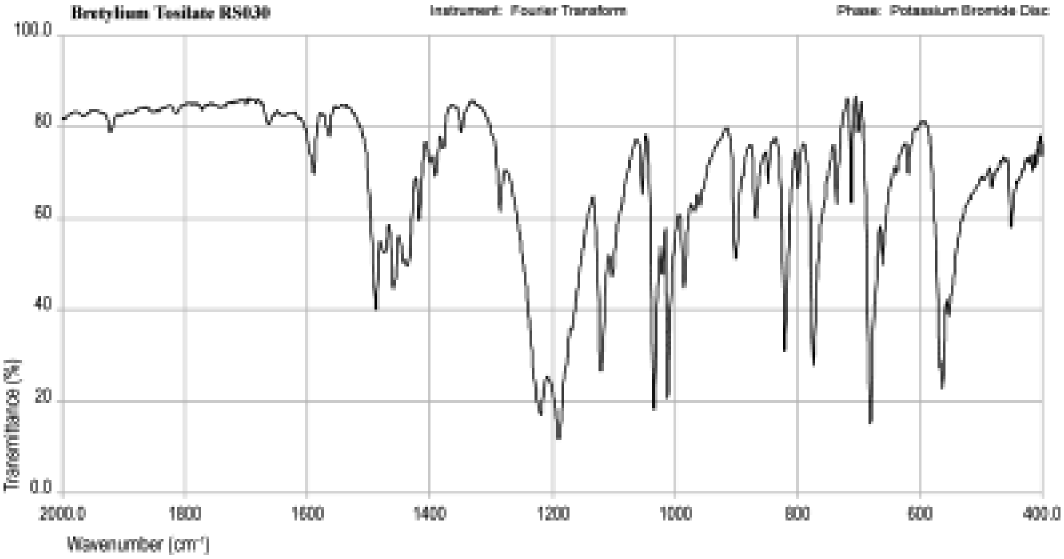
Bezafibrate



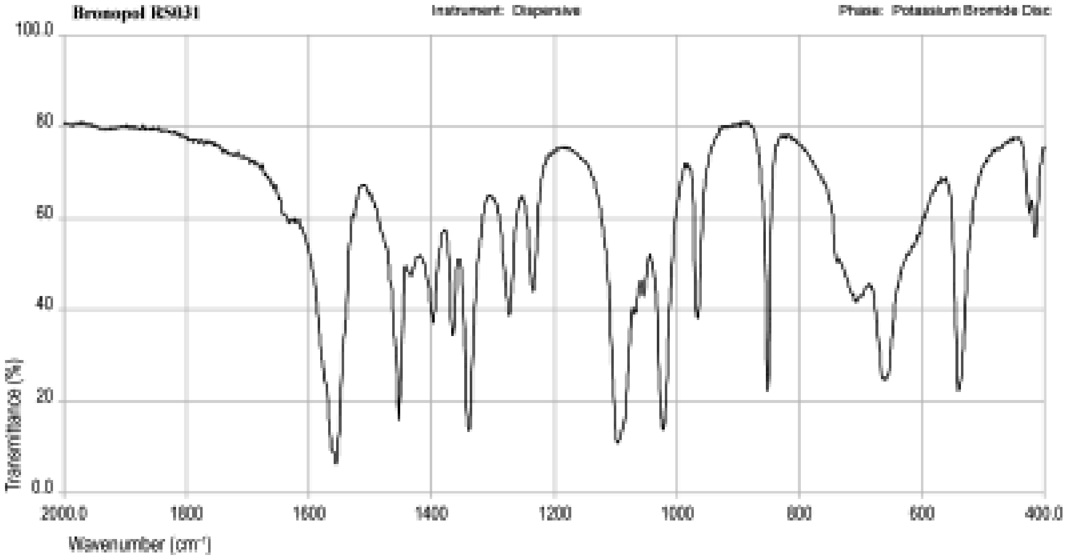
Bleomycin Sulphate



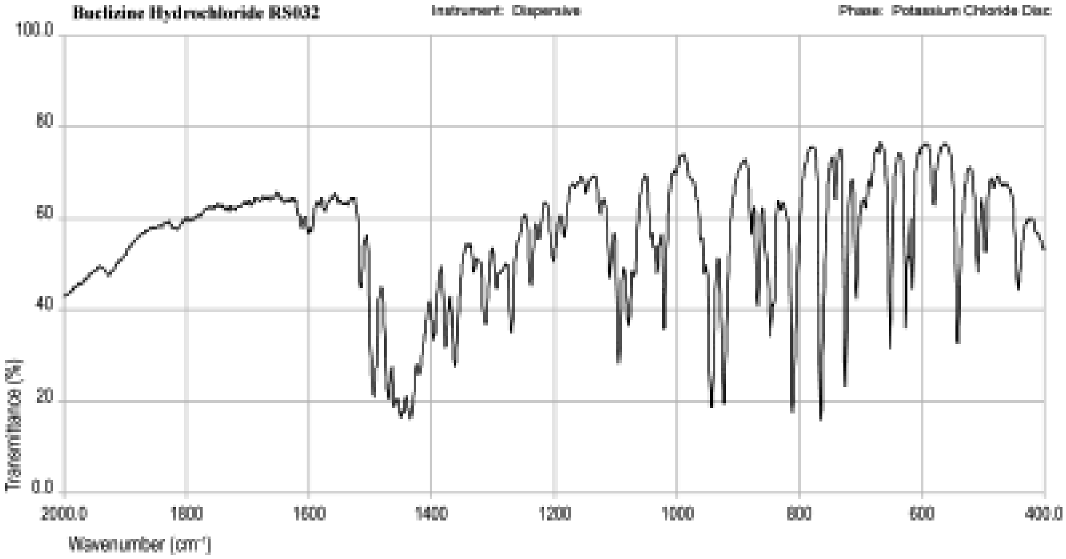
Bretylium Tosilate



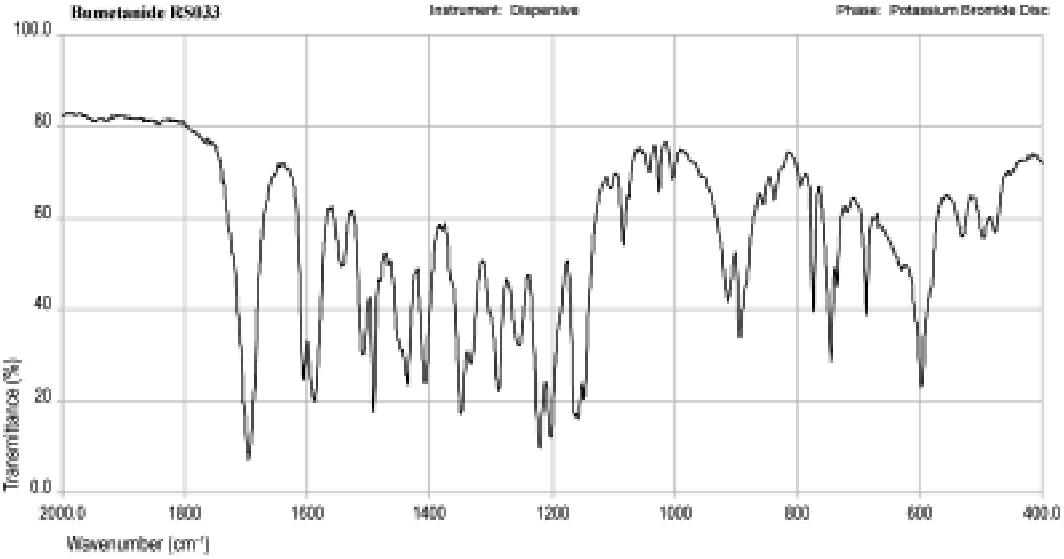
Bronopol


Bronopol R5031
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

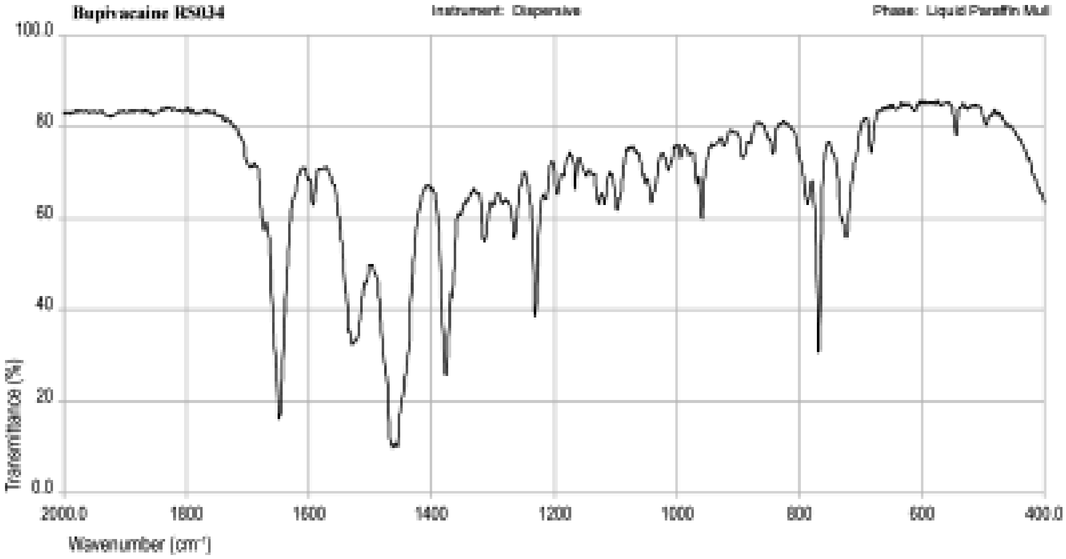
Buclizine Hydrochloride



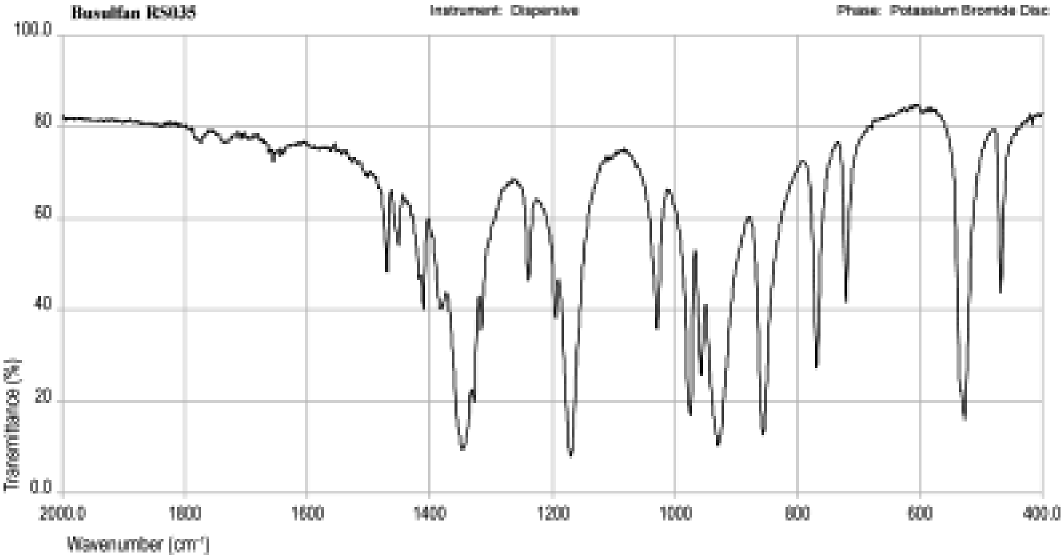
Bumetanide


Bumetanide RS033
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

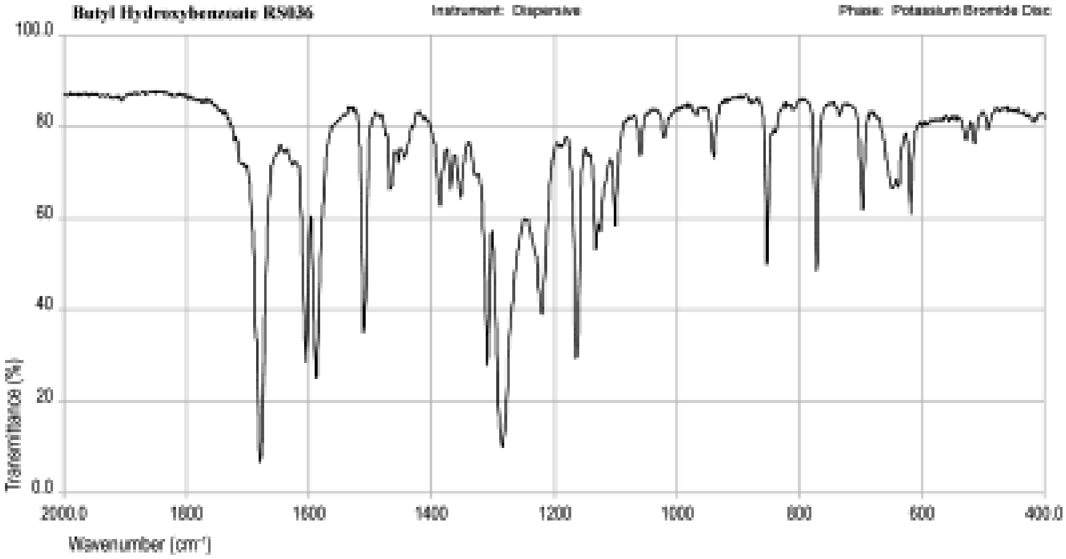
Bupivacaine



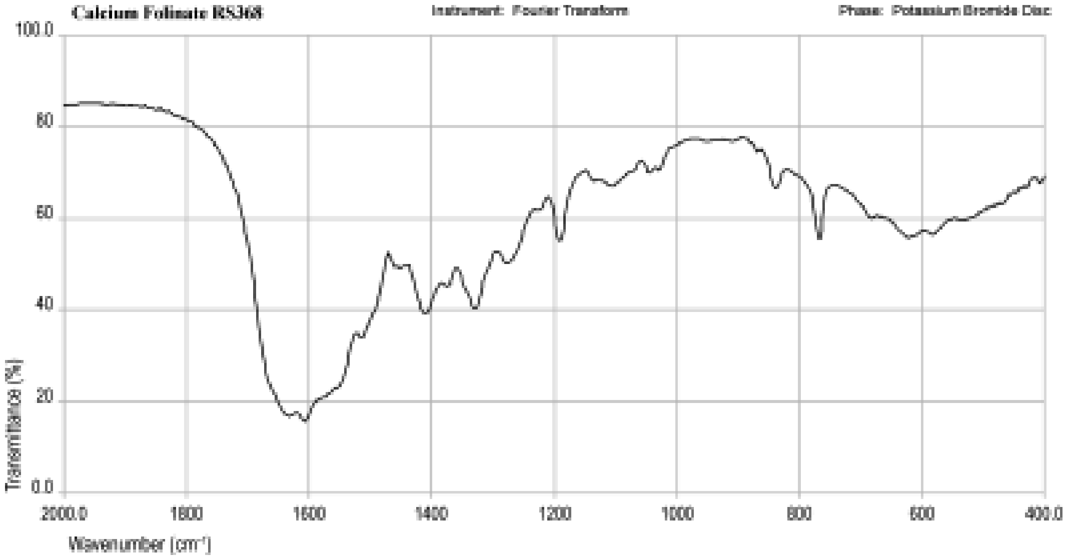
Busulfan


Busulfan RS035
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

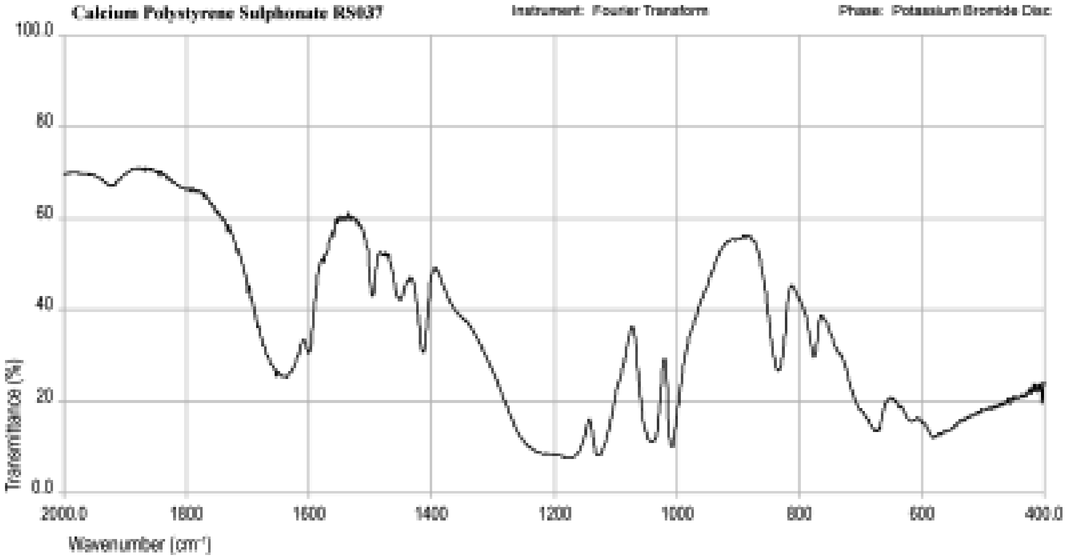
Butyl Hydroxybenzoate



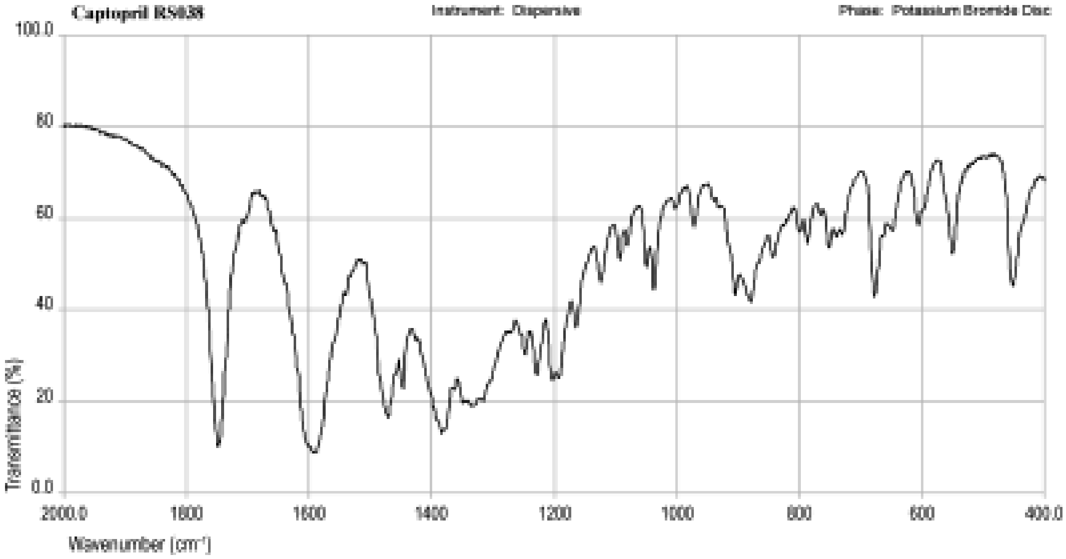
Calcium Folinate



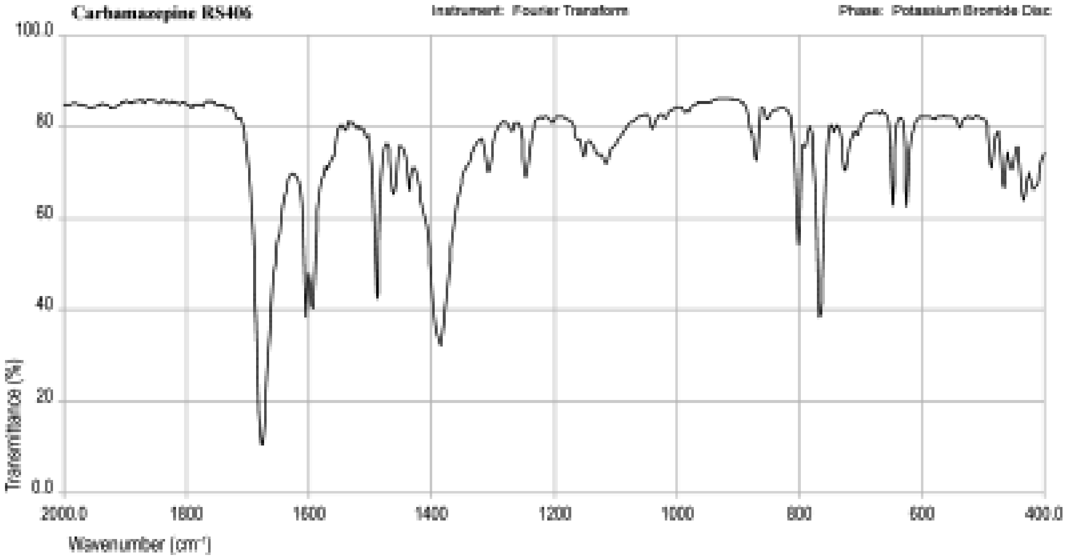
Calcium Polystyrene Sulphonate



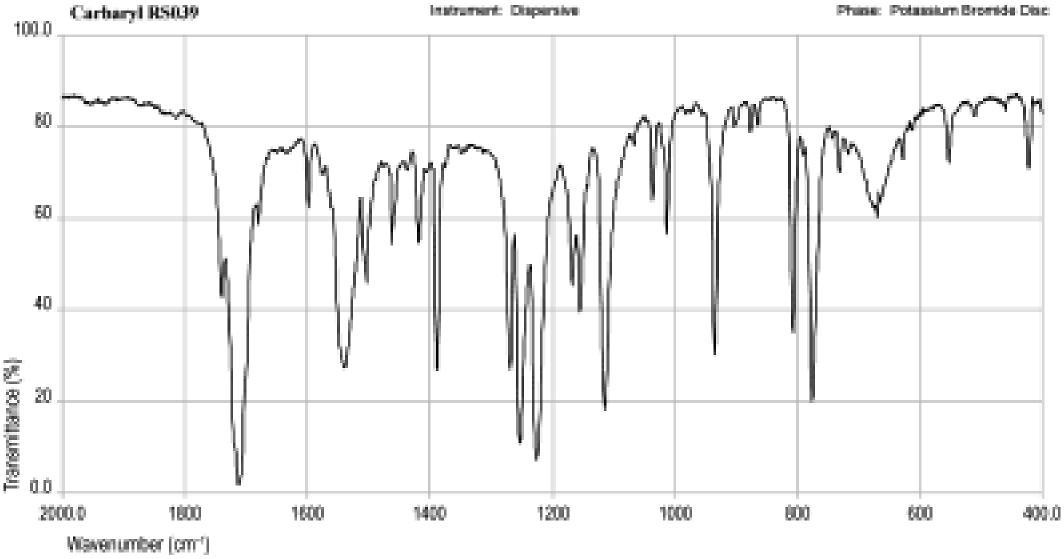
Captopril


Captopril RS038
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

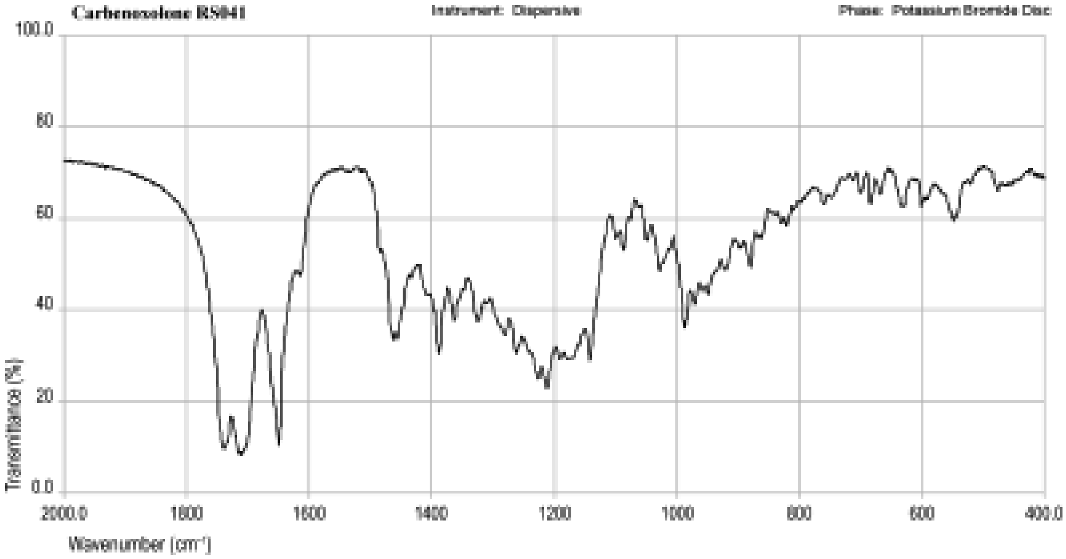
Carbamazepine



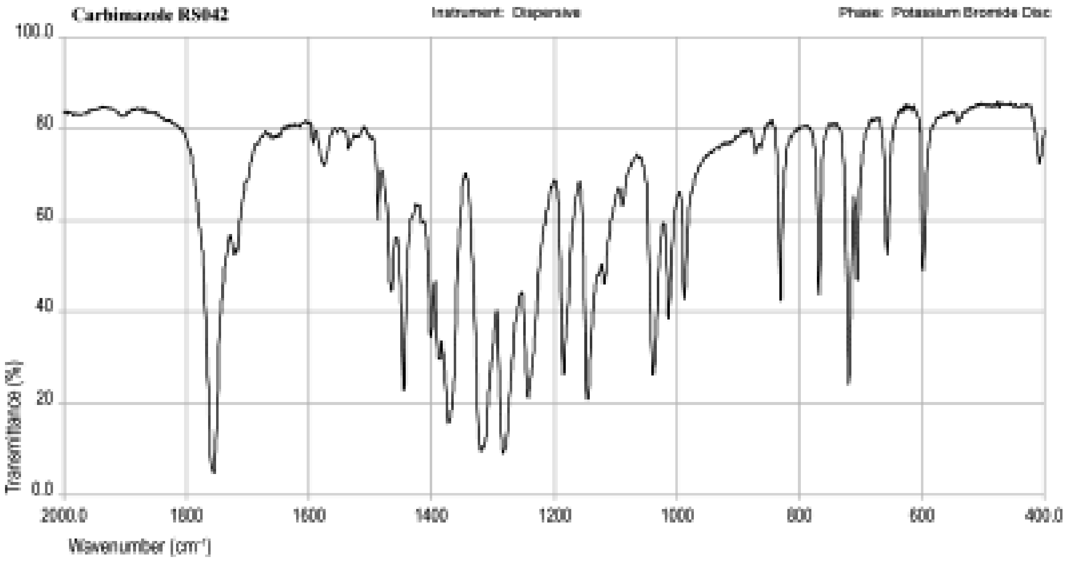
Carbaryl


Carbaryl RS039
Instrument: Dispersive
Phase: Potassium Bromide Disc
Transmittance (%)
100.0
80
60
40
20
0.0
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

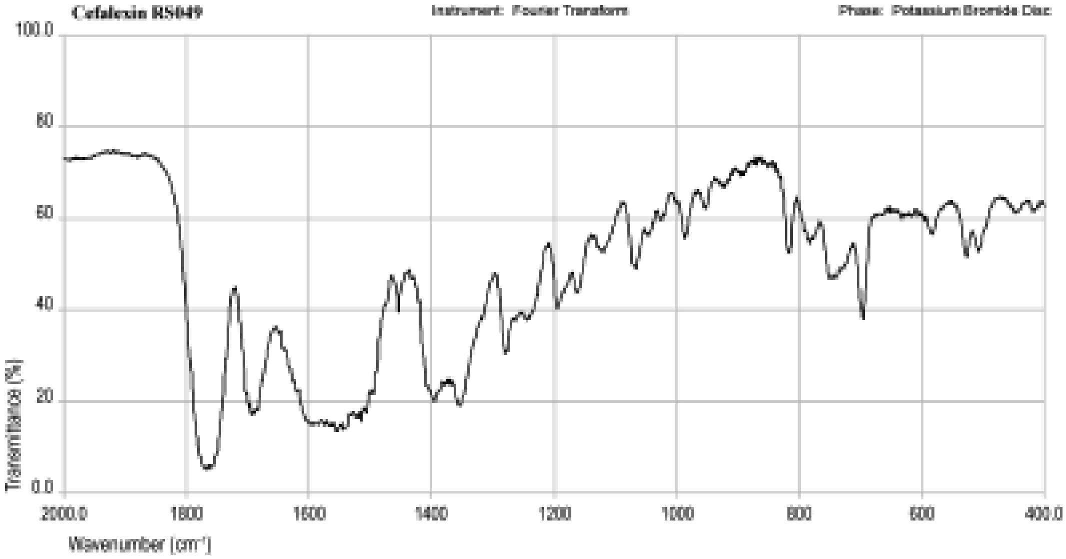
Carbenoxolone



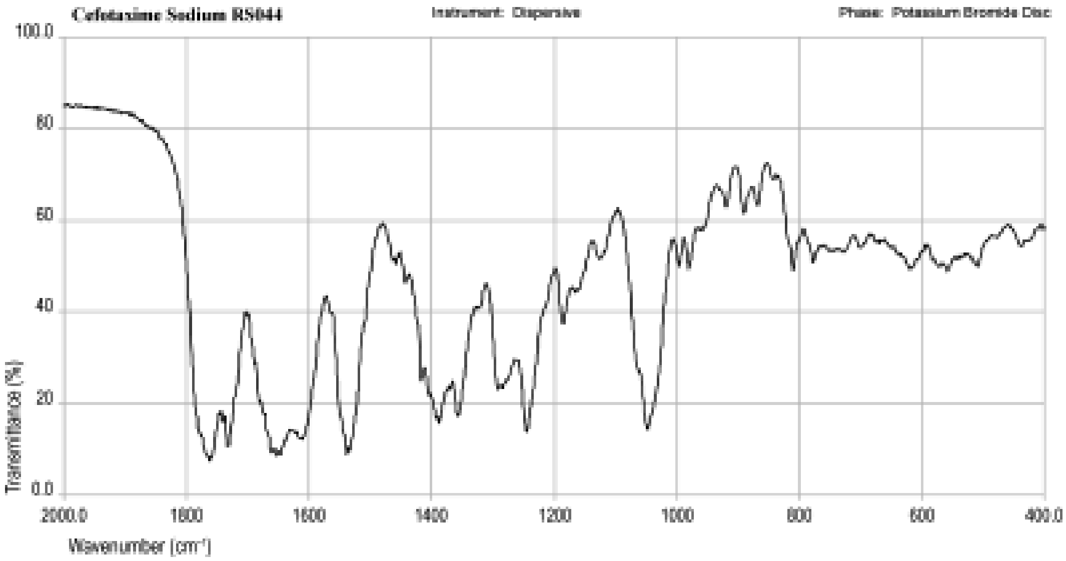
Carbimazole



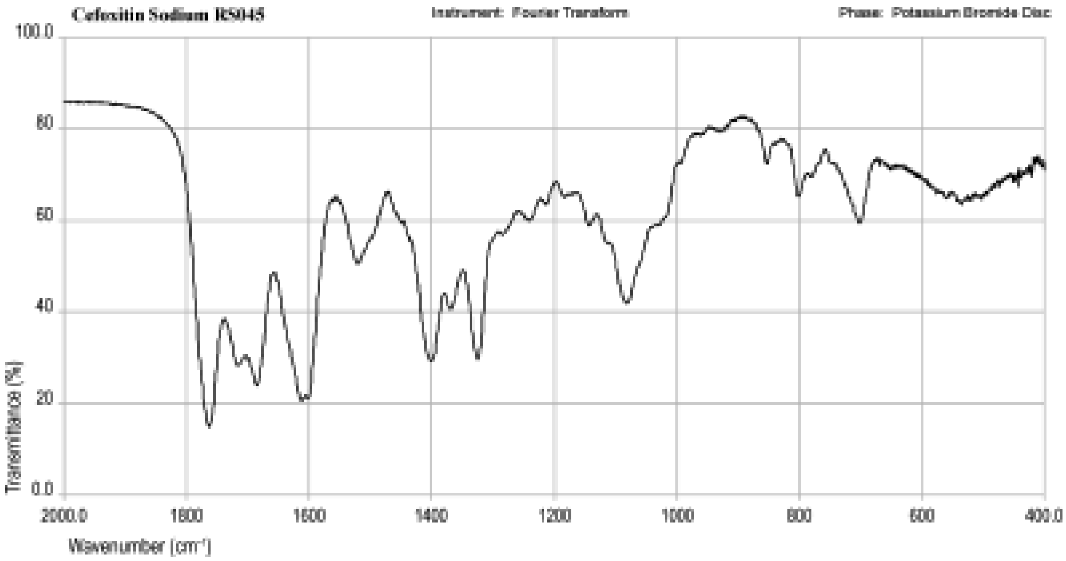
Cefalexin


Cefalexin RS049
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

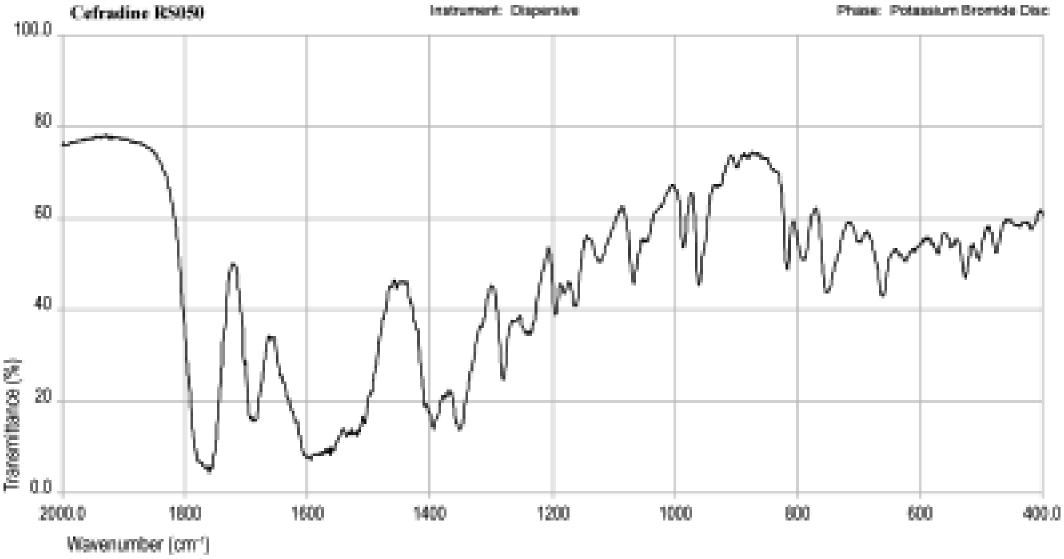
Cefotaxime Sodium



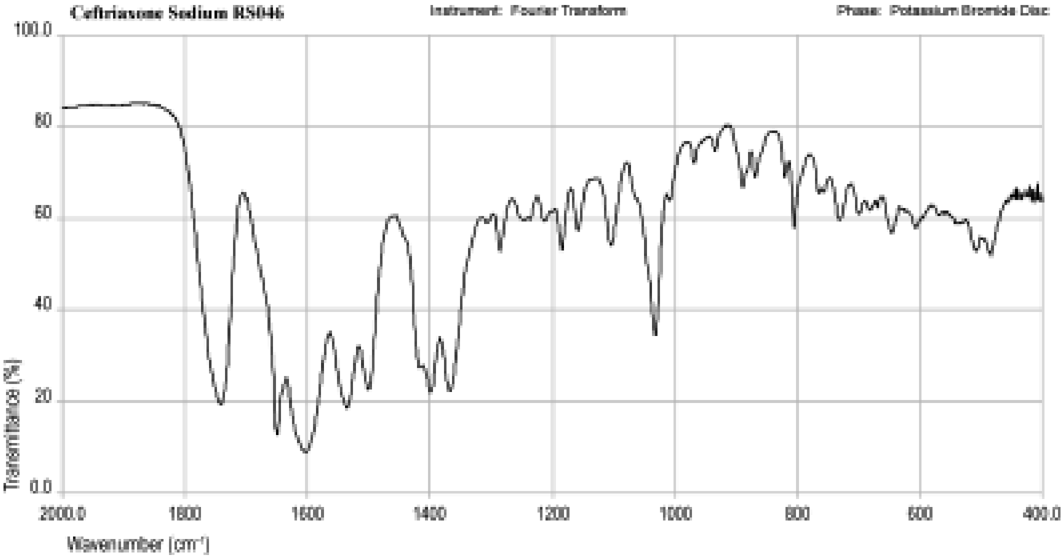
Cefoxitin Sodium



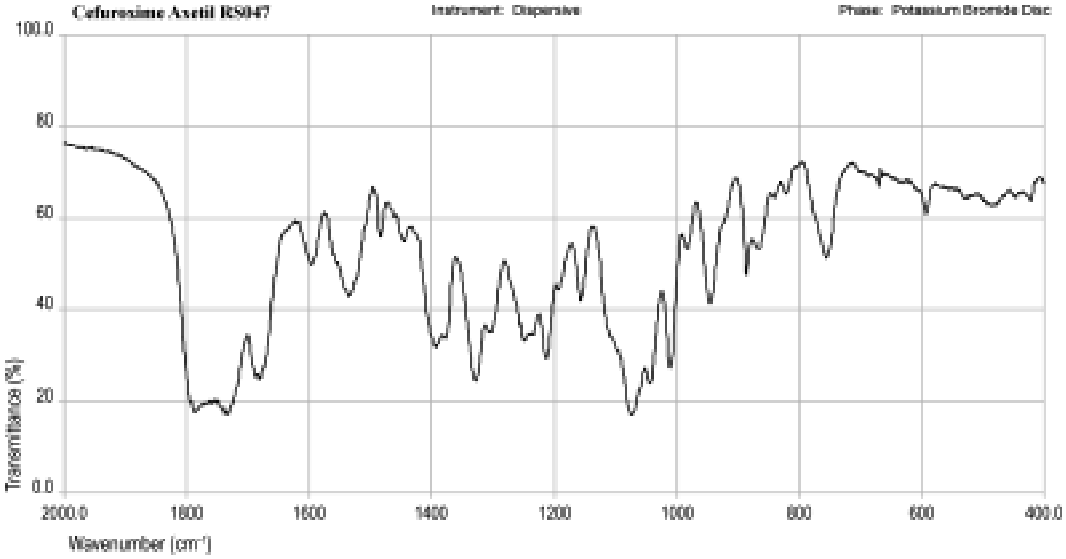
Cefradine


Cefradine RS050
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

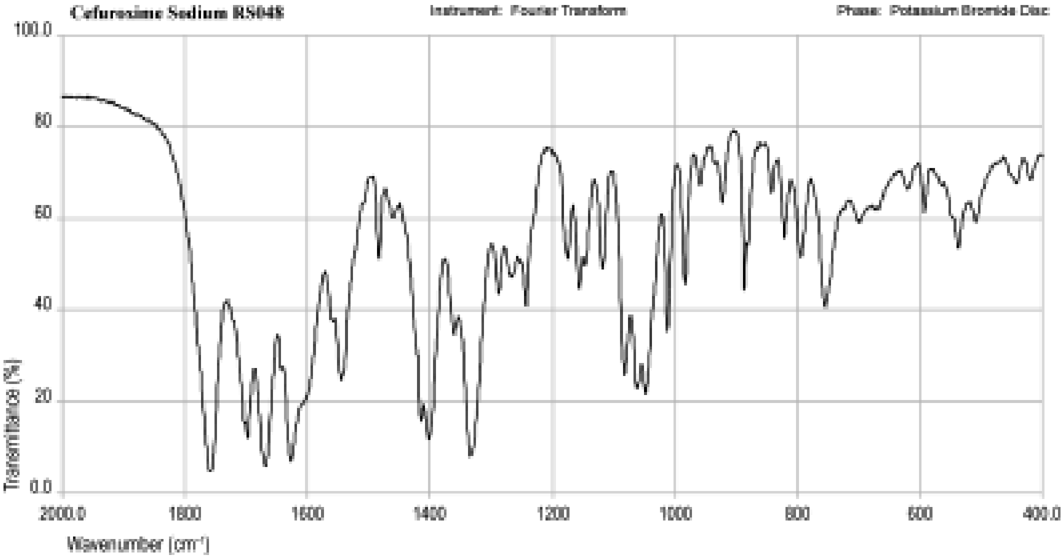
Ceftriaxone Sodium



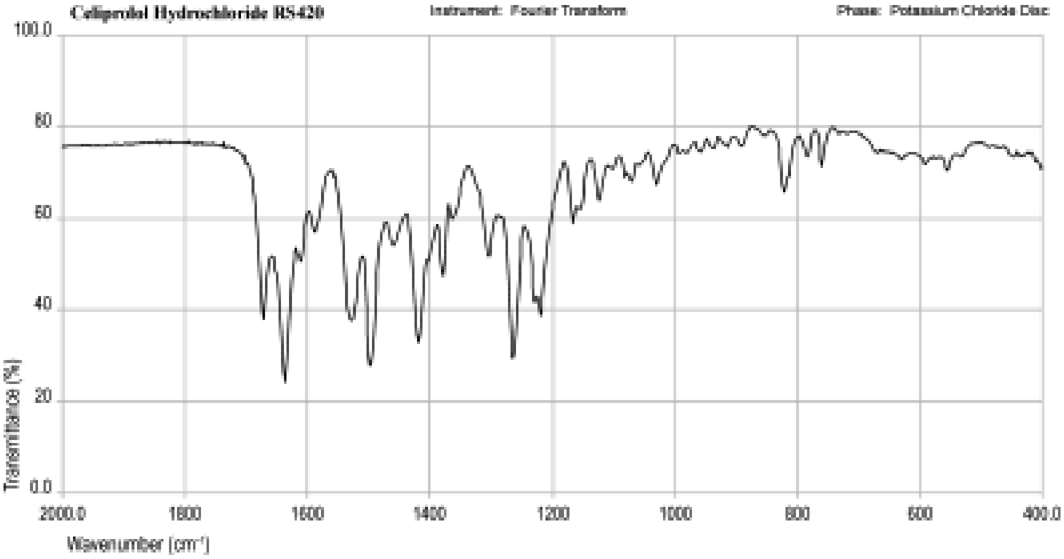
Cefuroxime Axetil



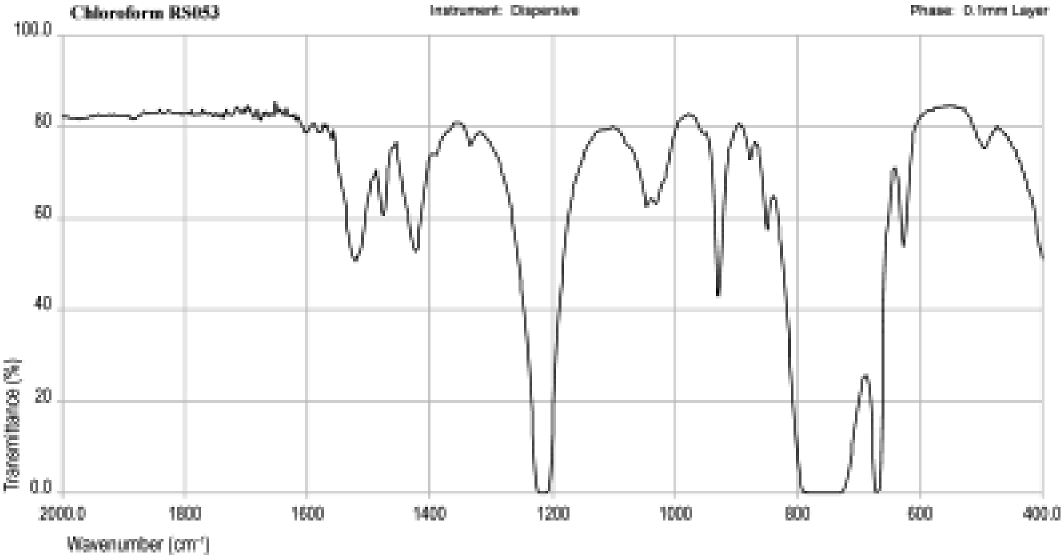
Cefuroxime Sodium



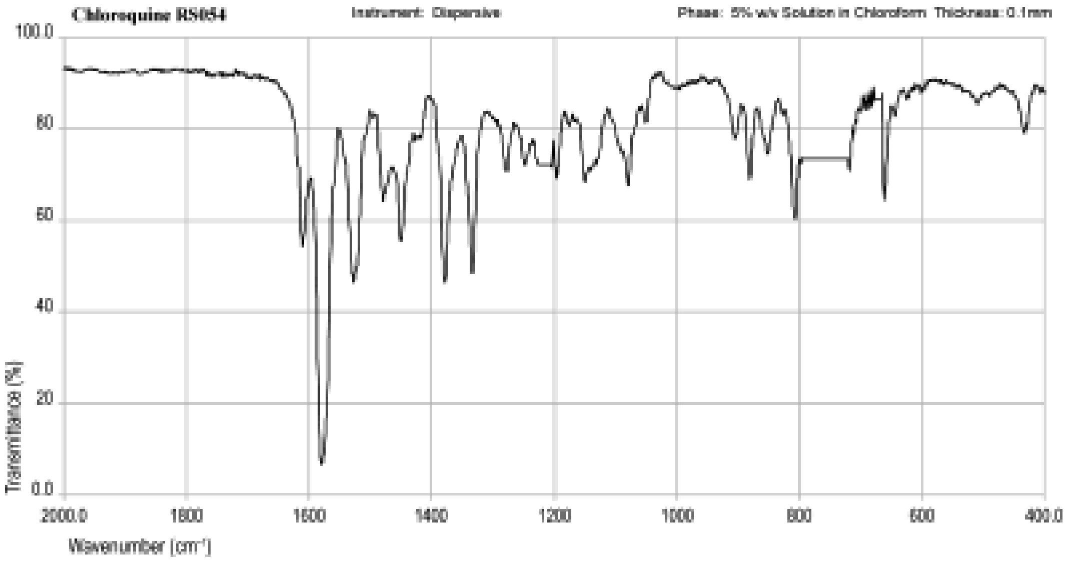
Celiprolol Hydrochloride



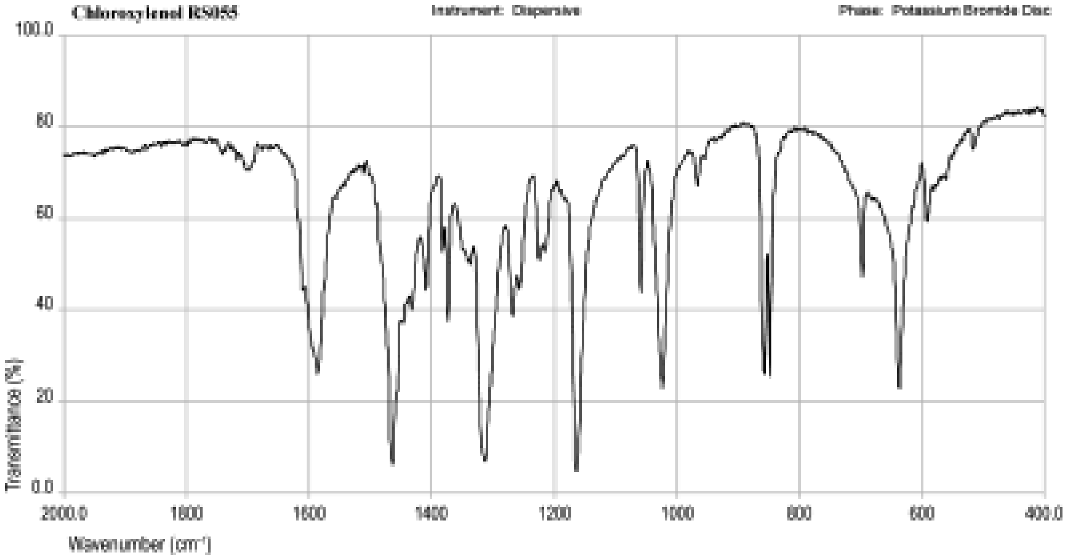
Chloroform


Chloroform RS053
Instrument: Dispersive
Phase: 0.1mm Layer
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

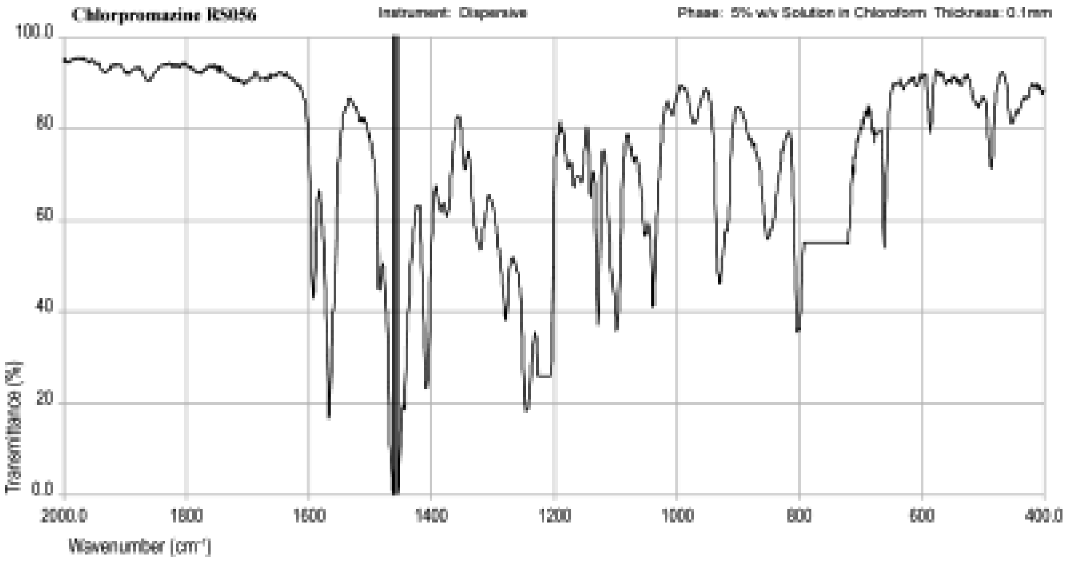
Chloroquine



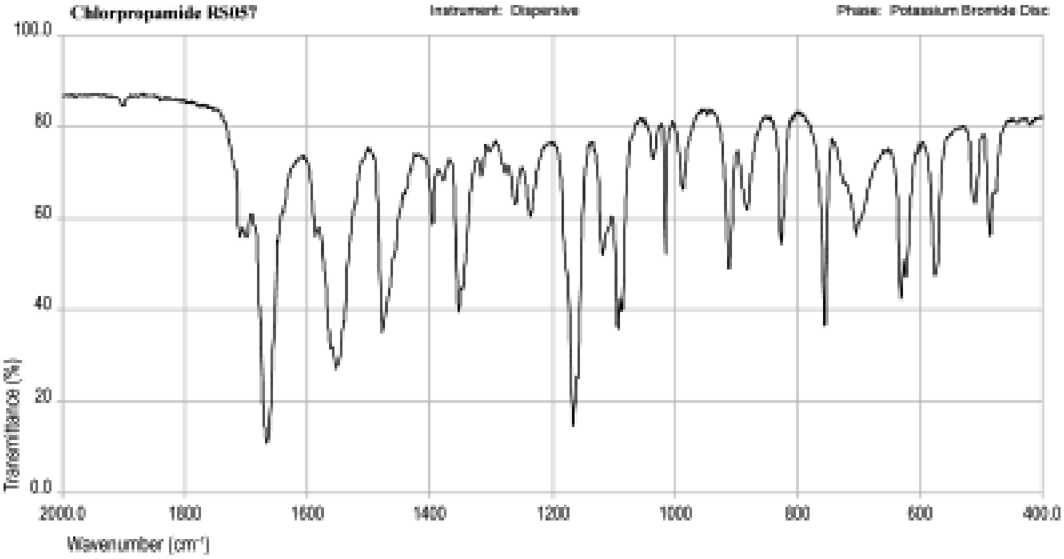
Chloroxylenol



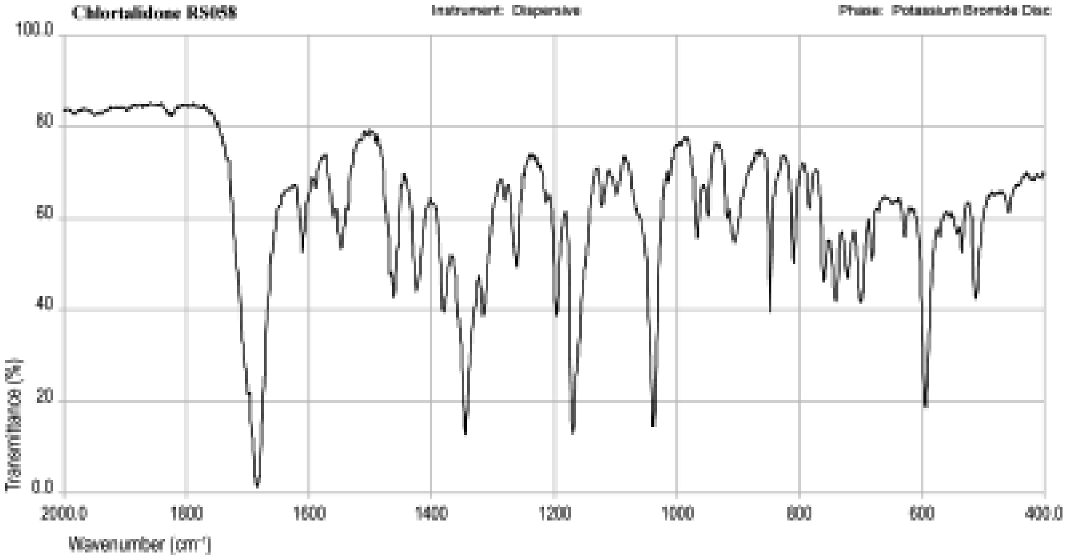
Chlorpromazine



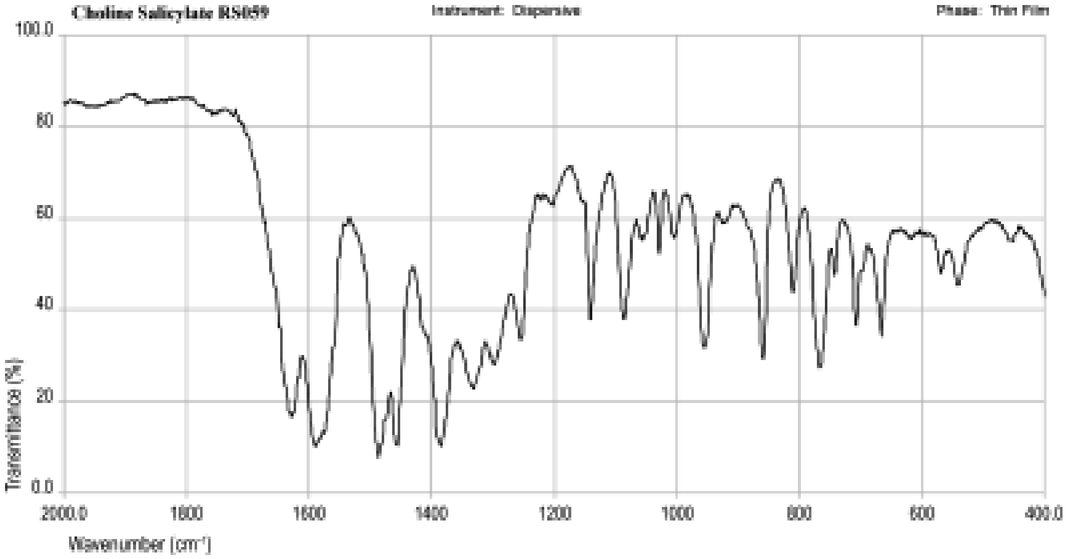
Chlorpropamide



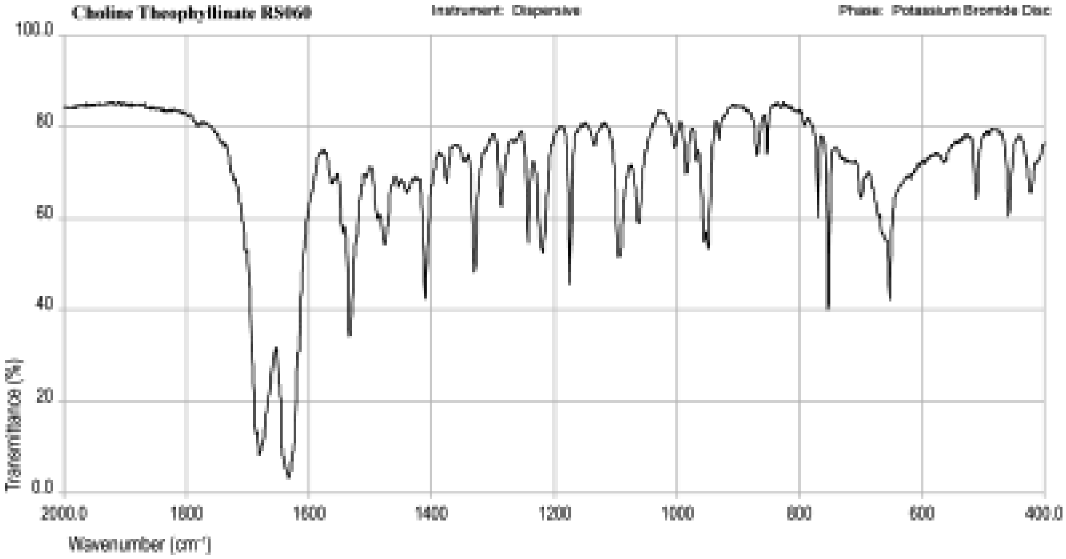
Chlortalidone



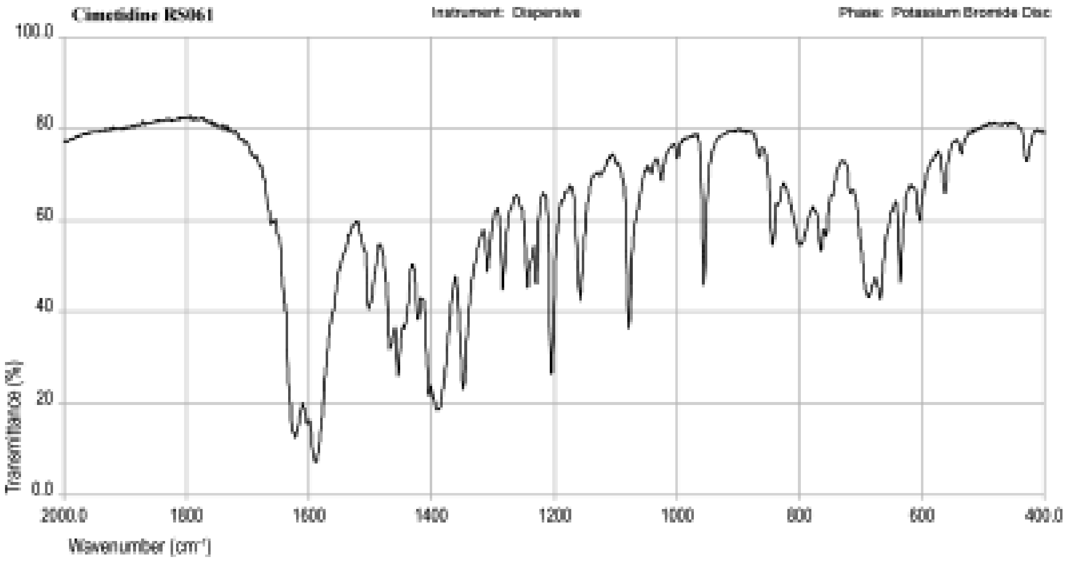
Choline Salicylate



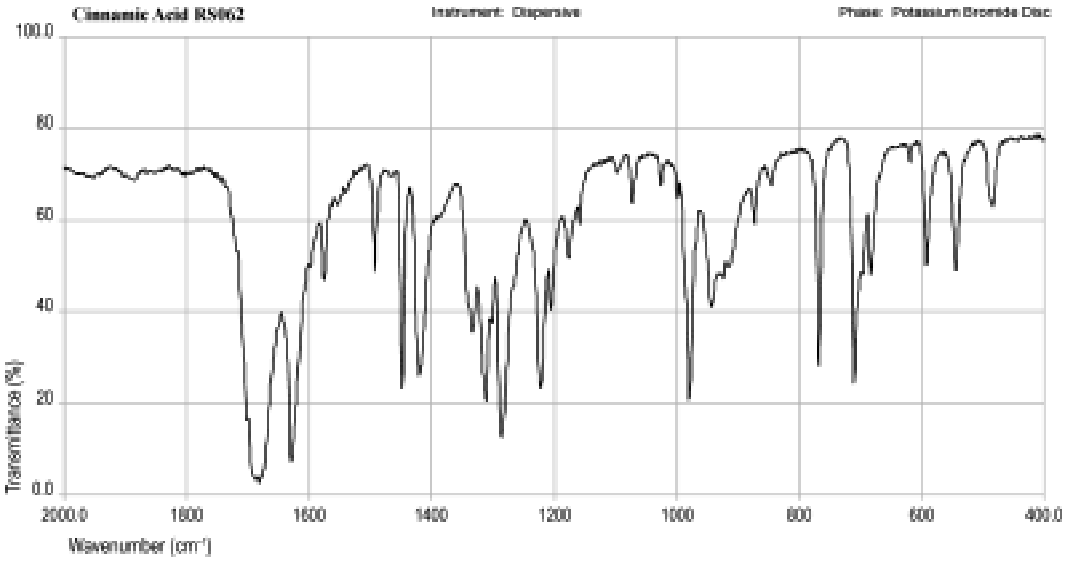
Choline Theophyllinate



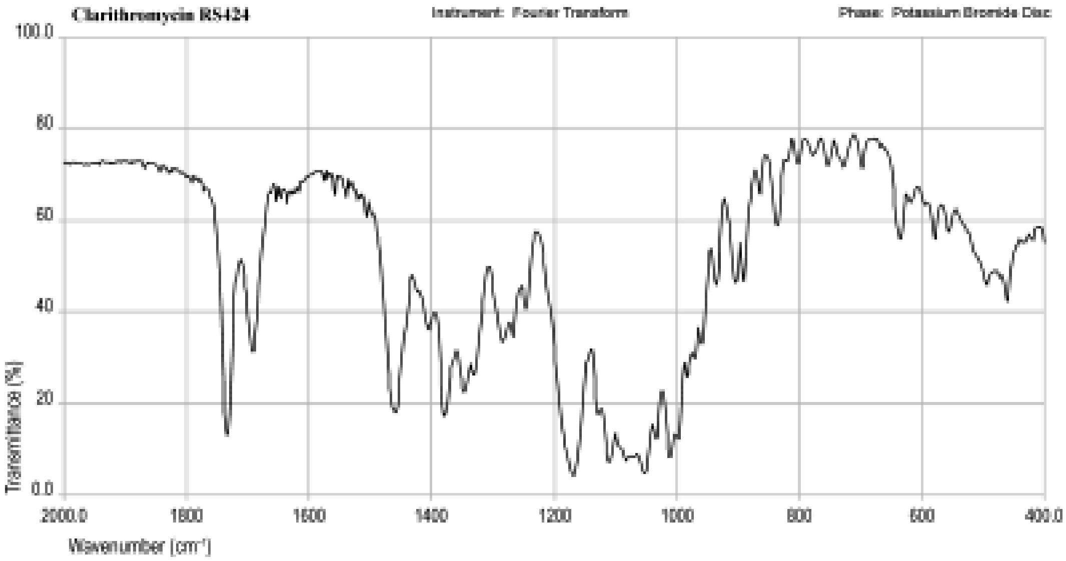
Cimetidine


Cimetidine RS061
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

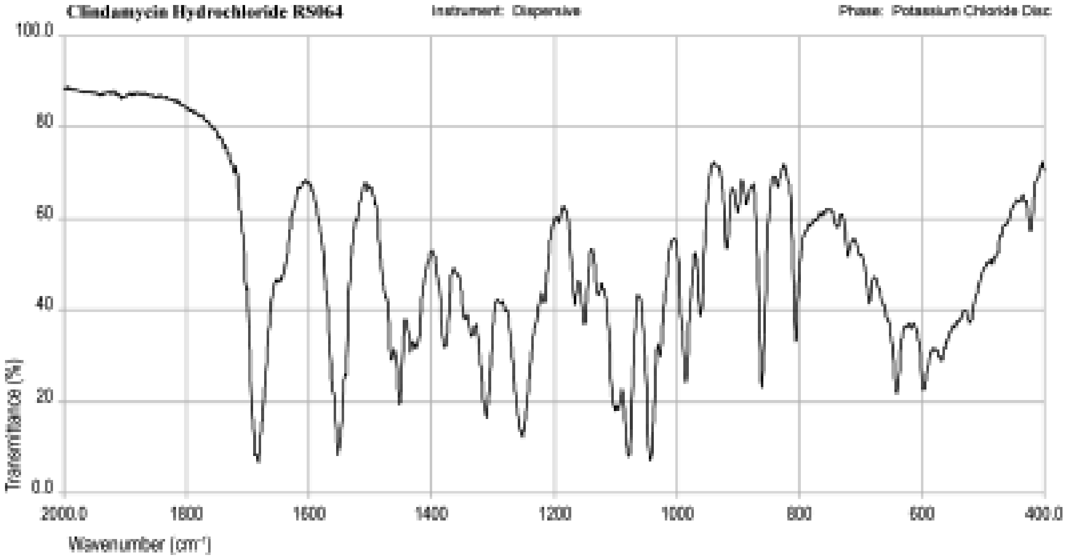
Cinnamic Acid



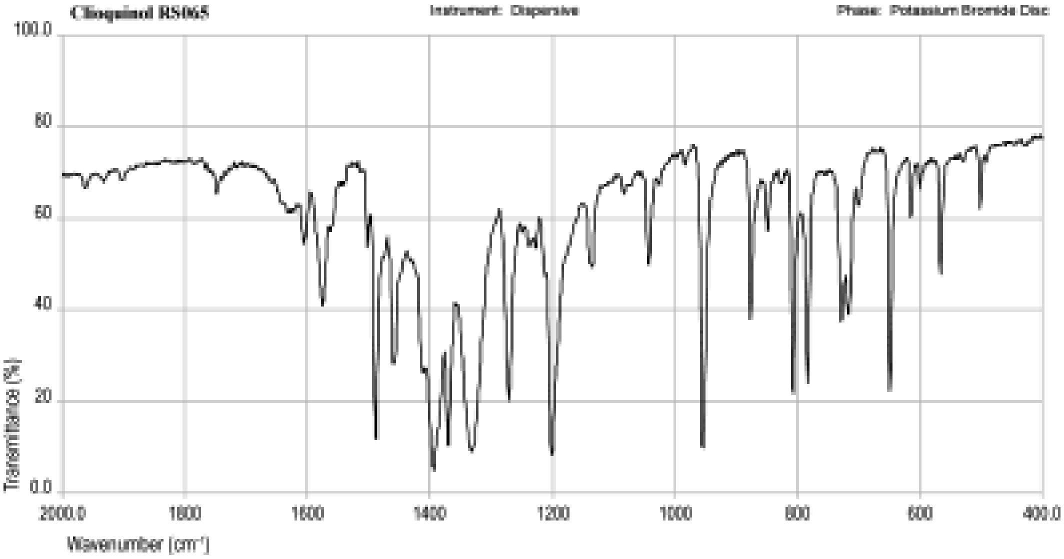
Clarithromycin



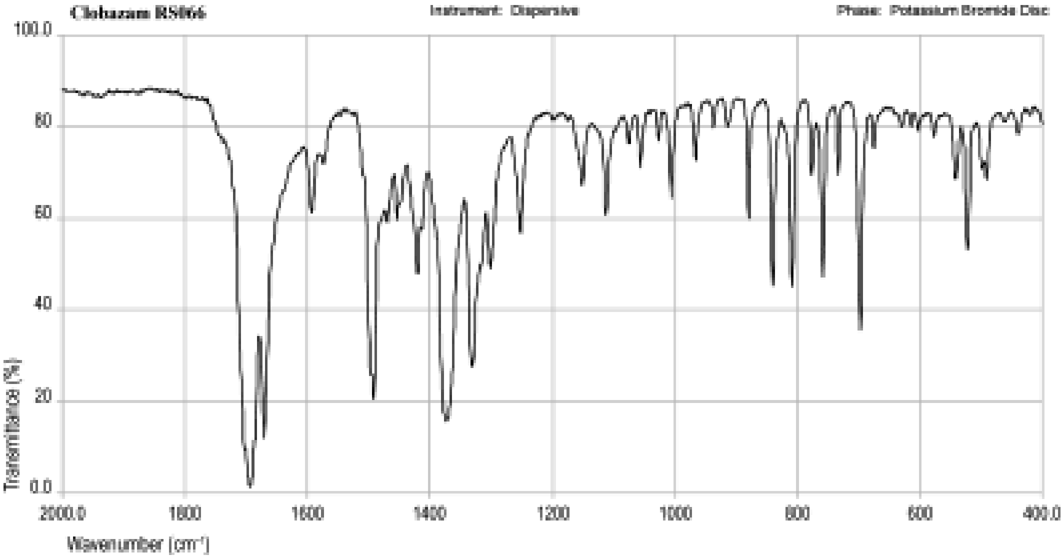
Clindamycin Hydrochloride



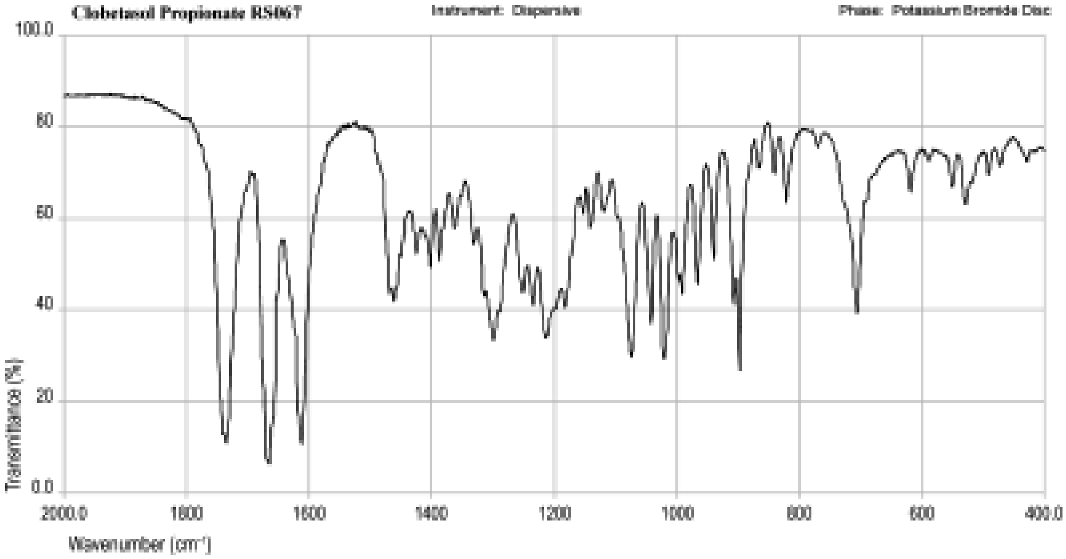
Clioquinol


Clioquinol RS065
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

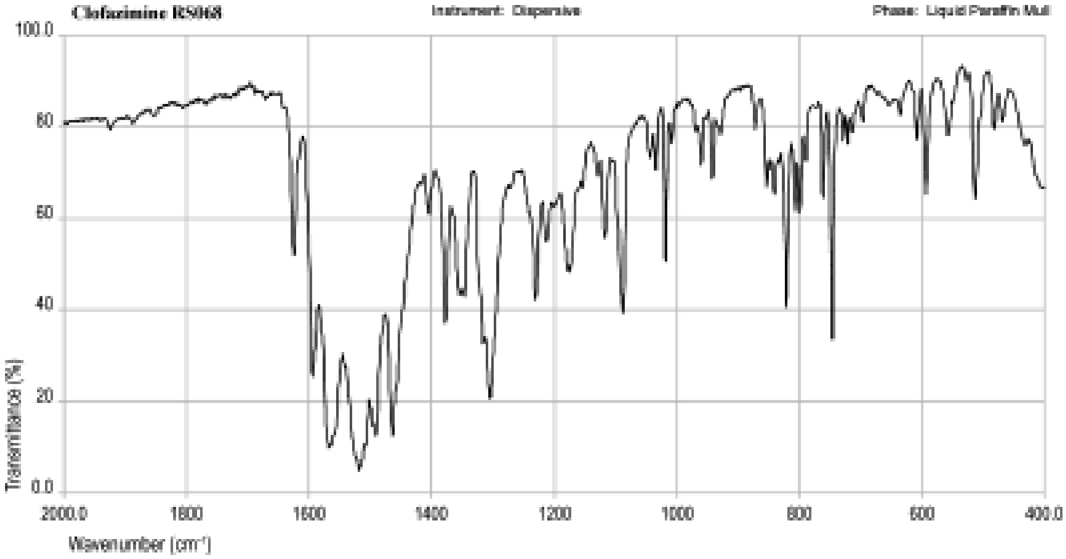
Clobazam


Clobazam RS066
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

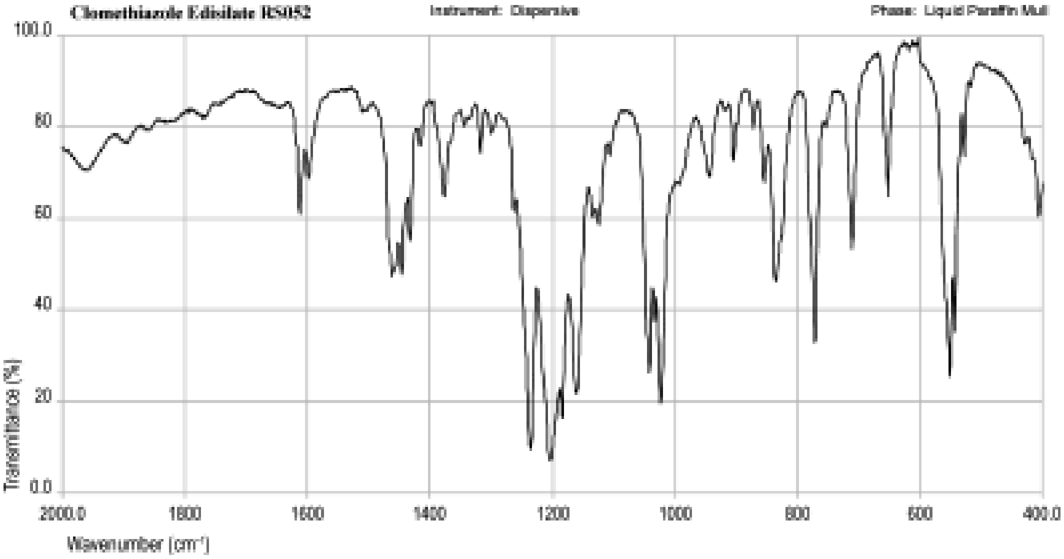
Clobetasol Propionate



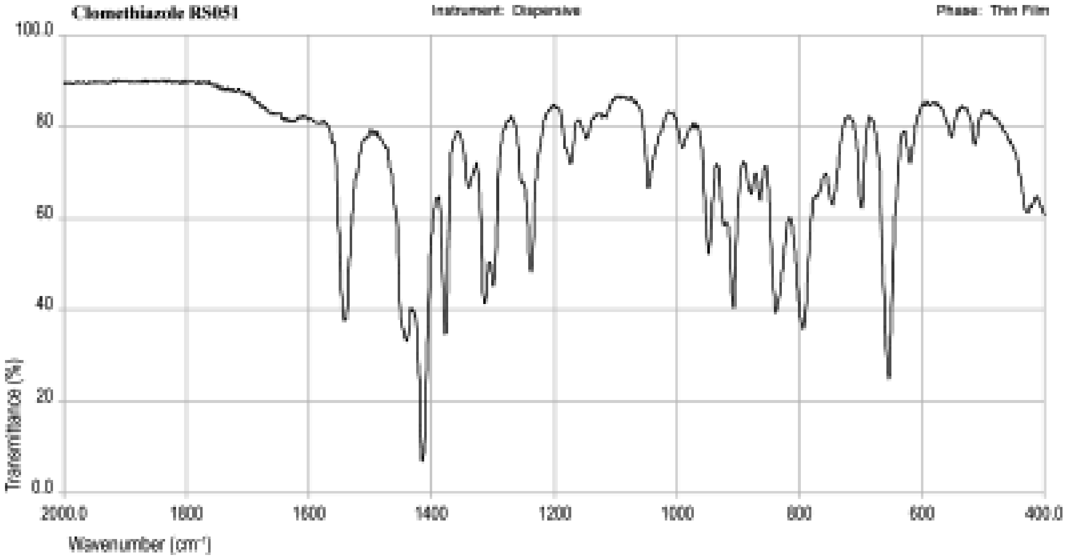
Clofazimine



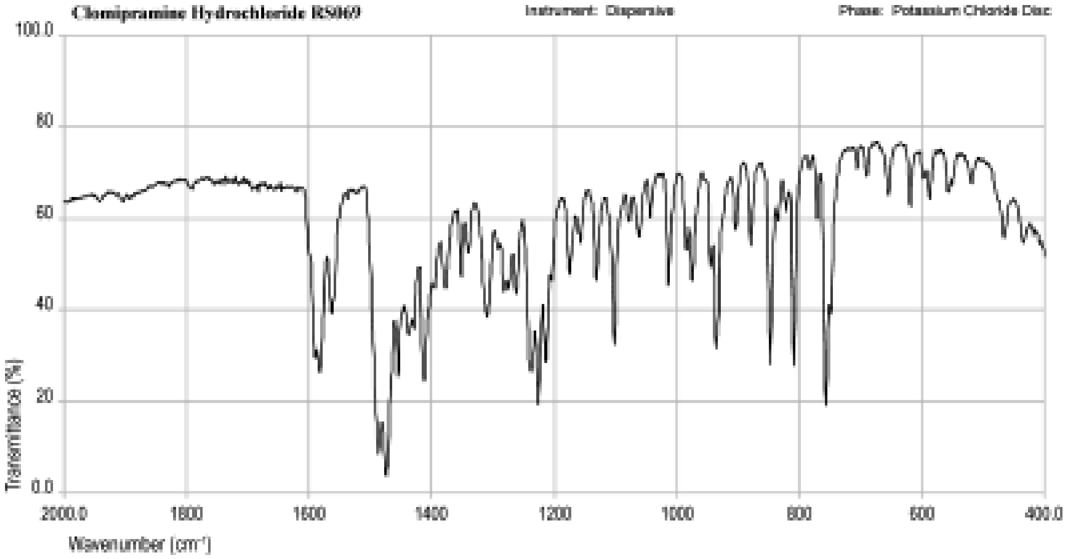
Clomethiazole Edisilate



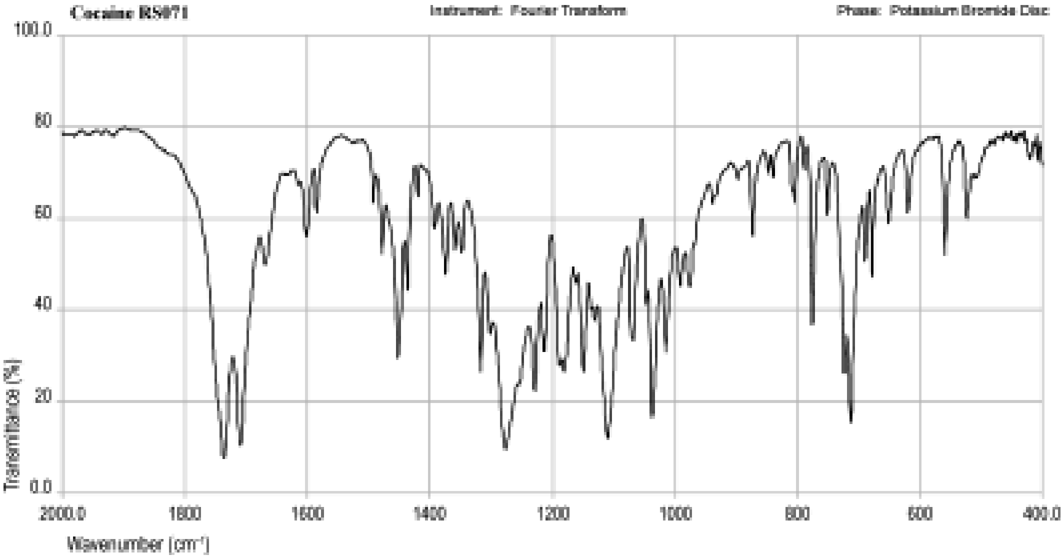
Clomethiazole



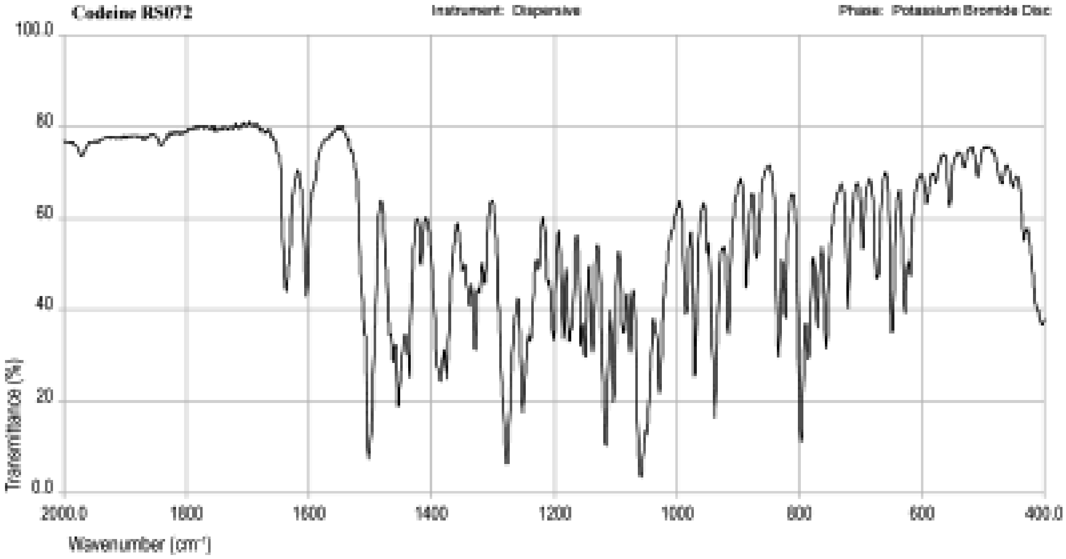
Clomipramine Hydrochloride



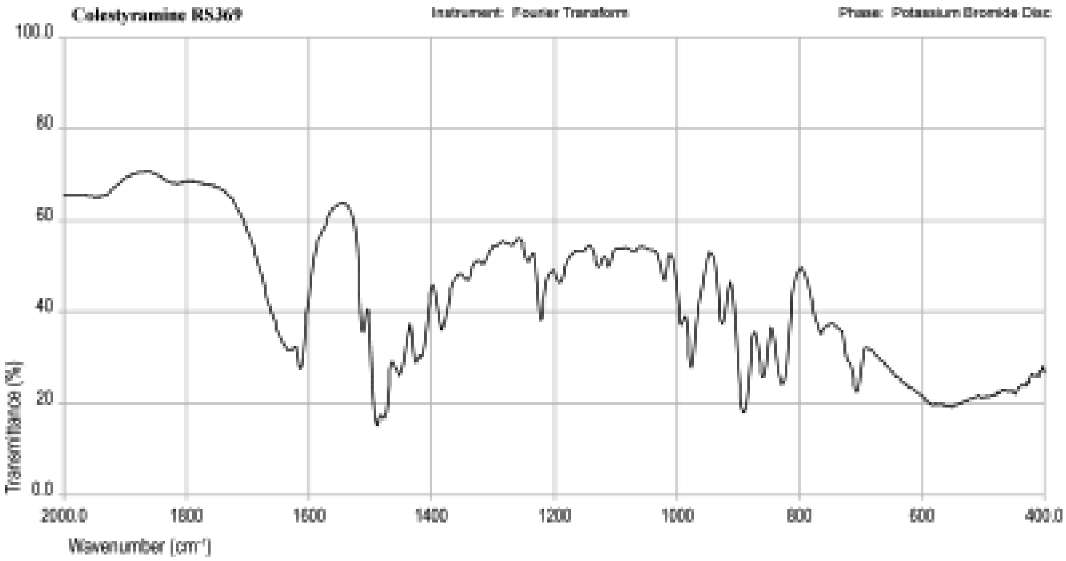
Cocaine


Cocaine RS071
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

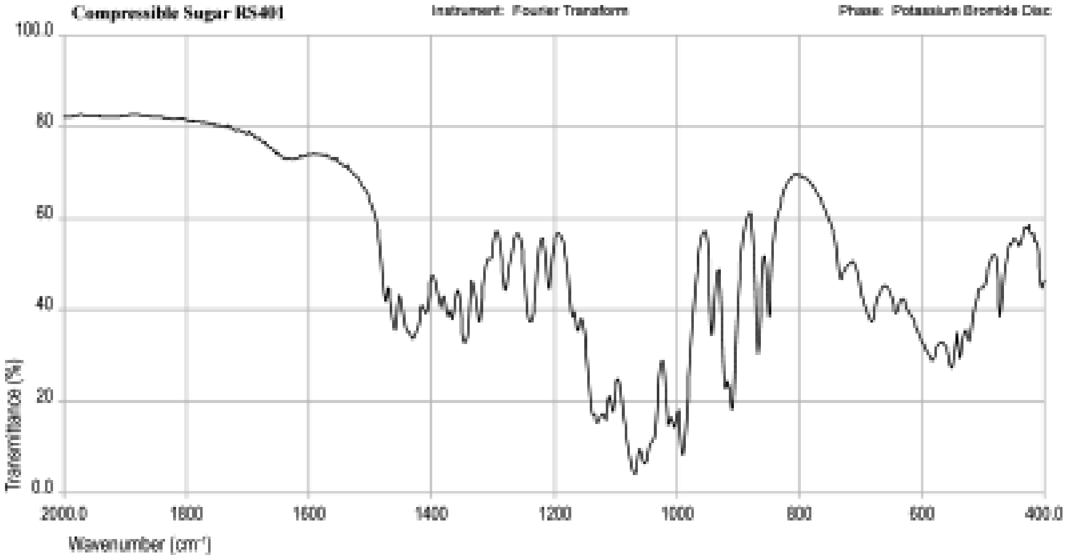
Codeine


Codeine RS072
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

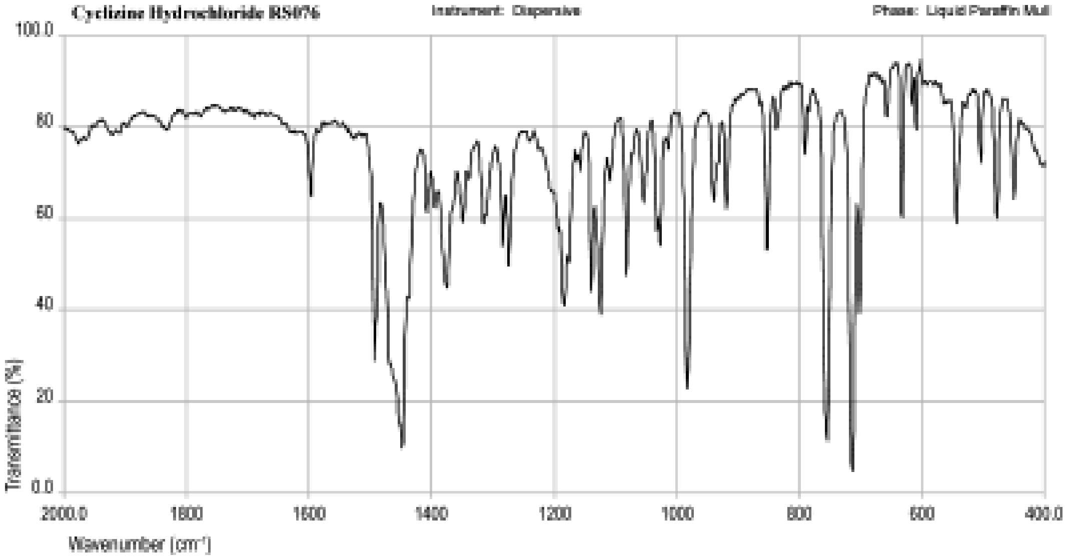
Colestyramine



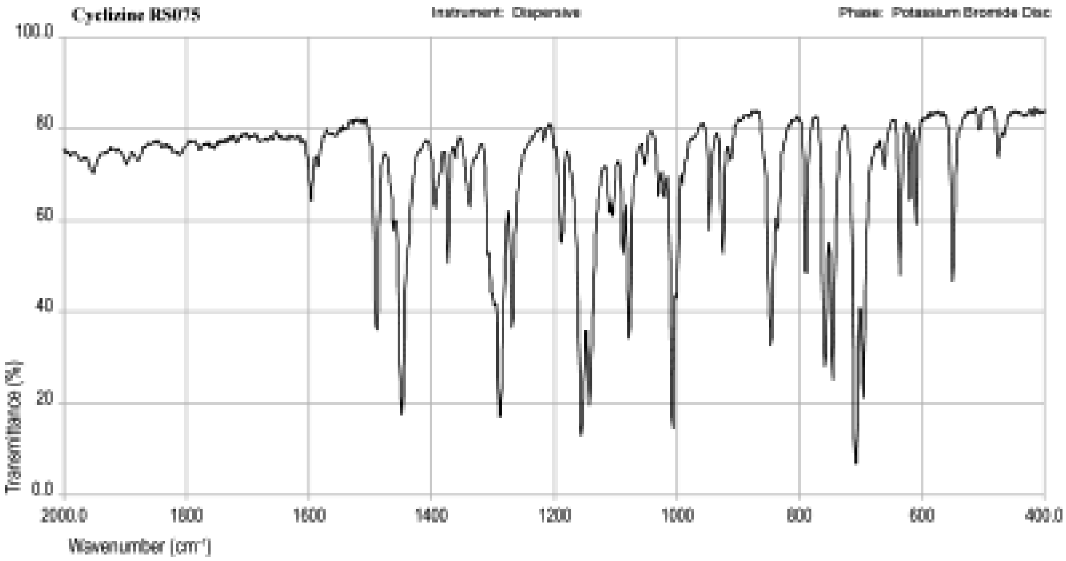
Compressible Sugar



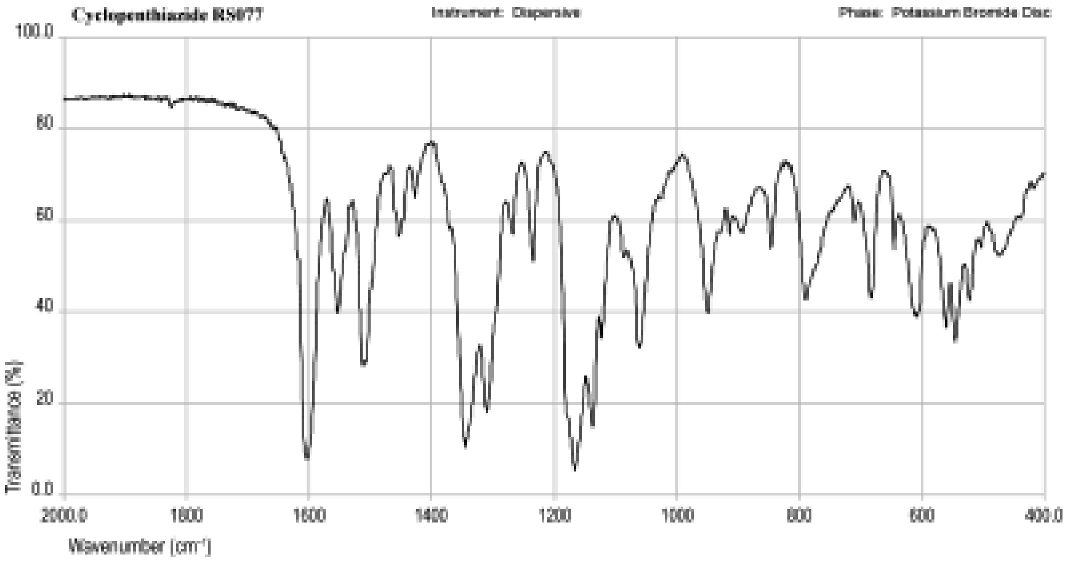
Cyclizine Hydrochloride



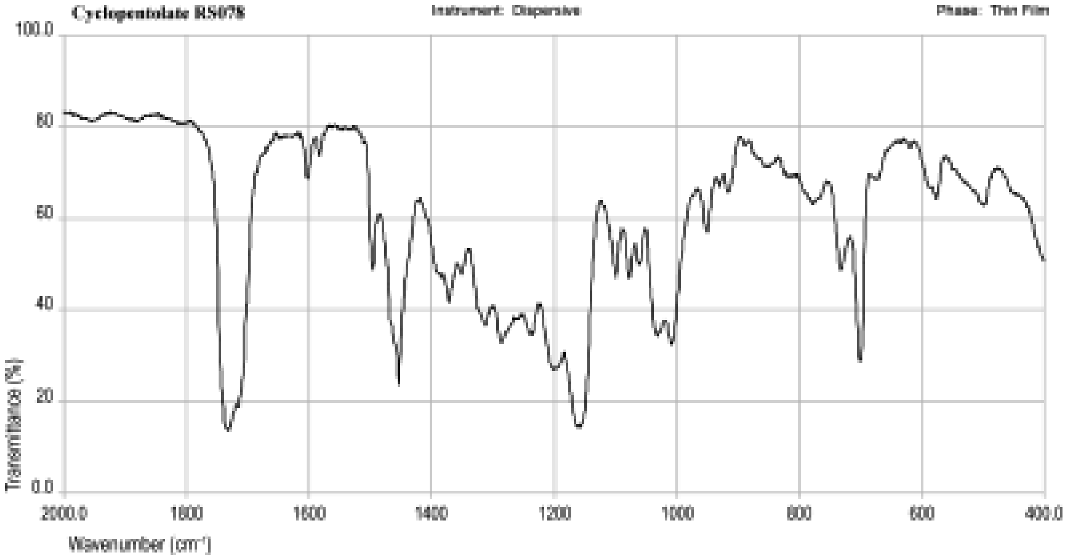
Cyclizine


Cyclizine RS075
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

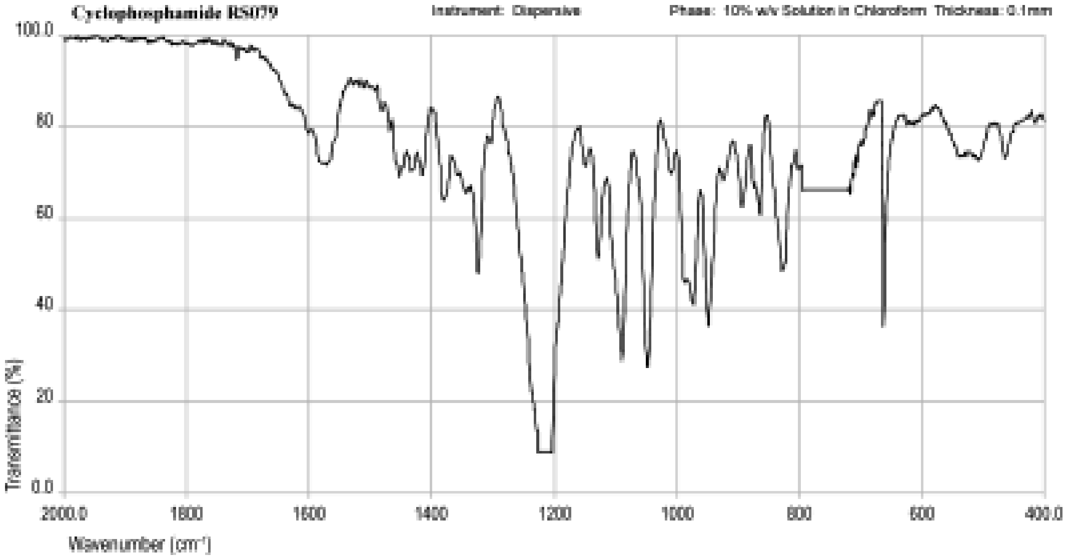
Cyclopenthiazide



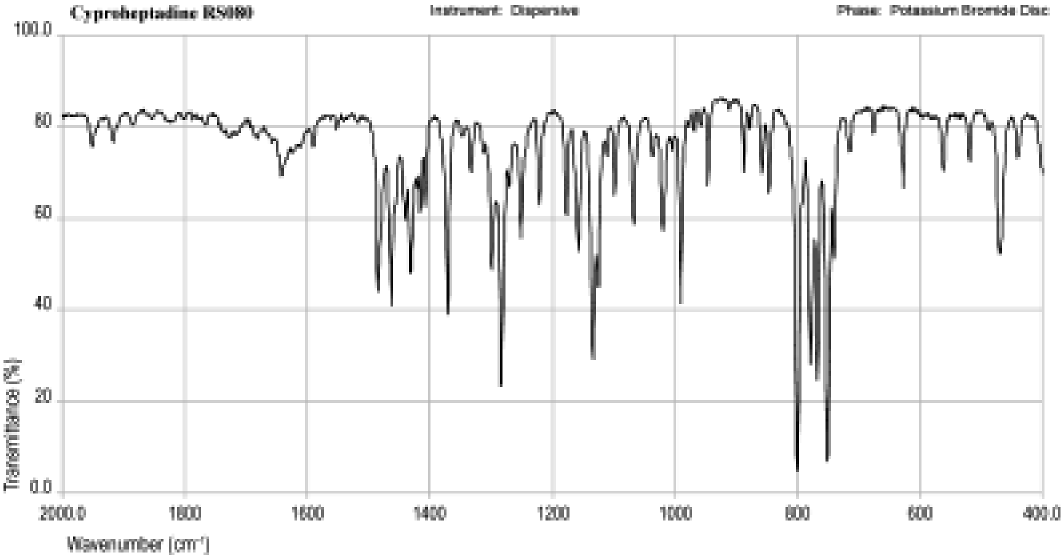
Cyclopentolate



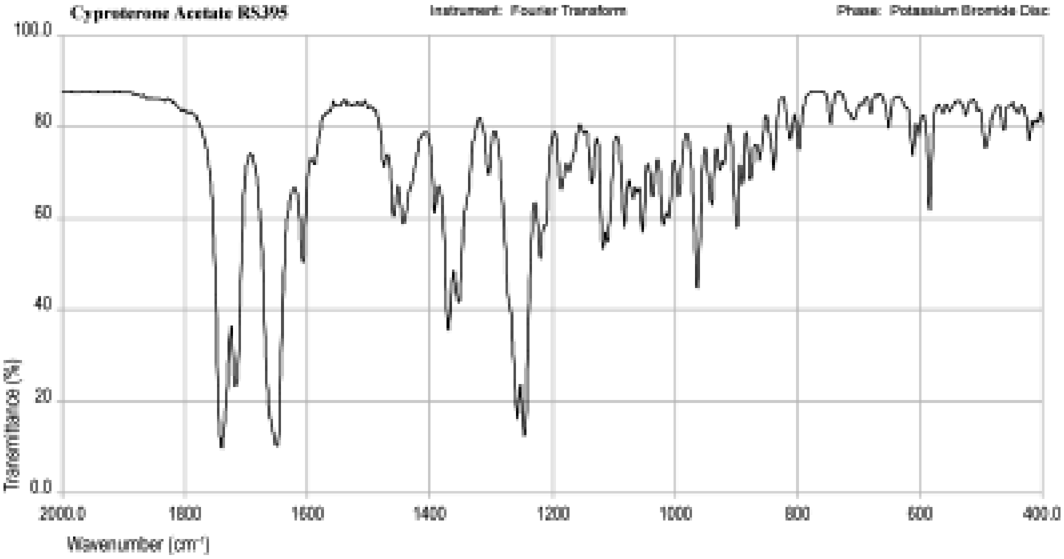
Cyclophosphamide



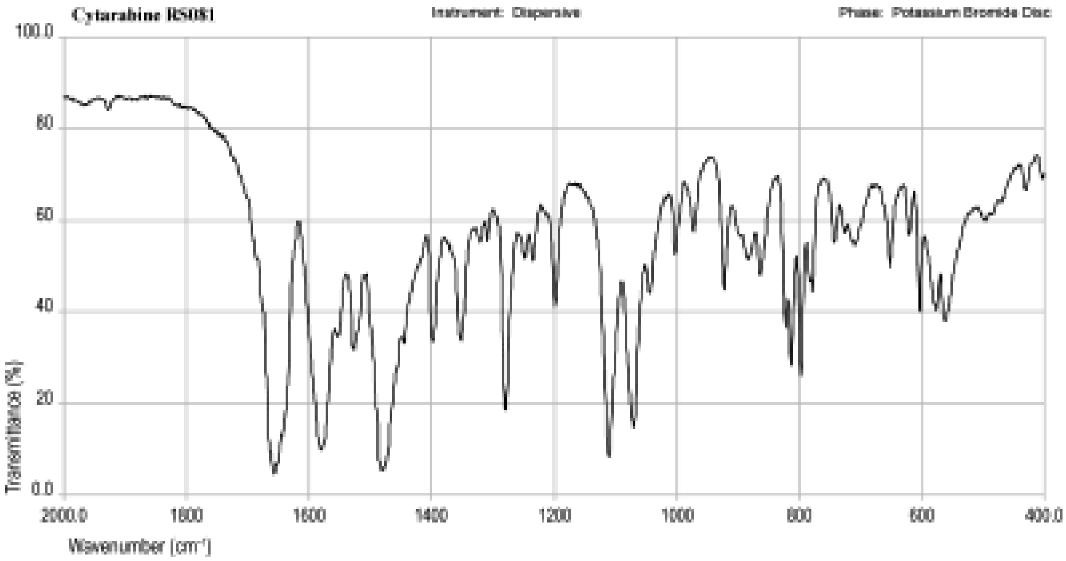
Cyproheptadine



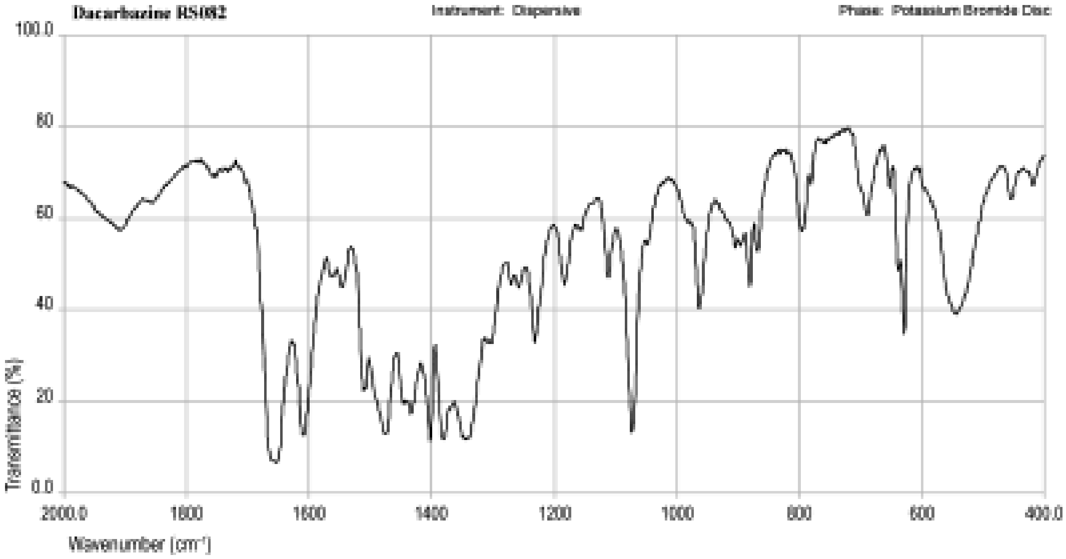
Cyproterone Acetate



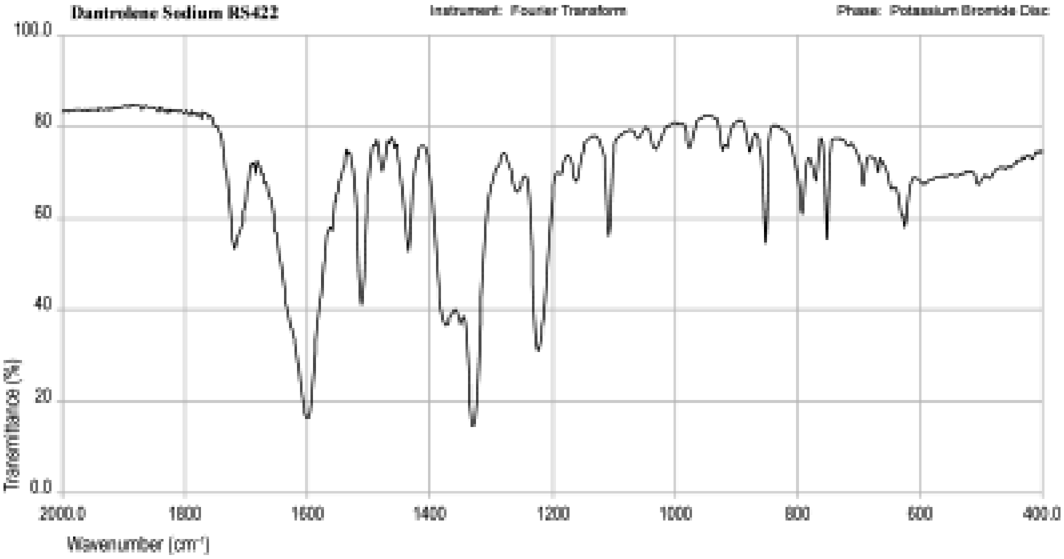
Cytarabine


Cytarabine RS081
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

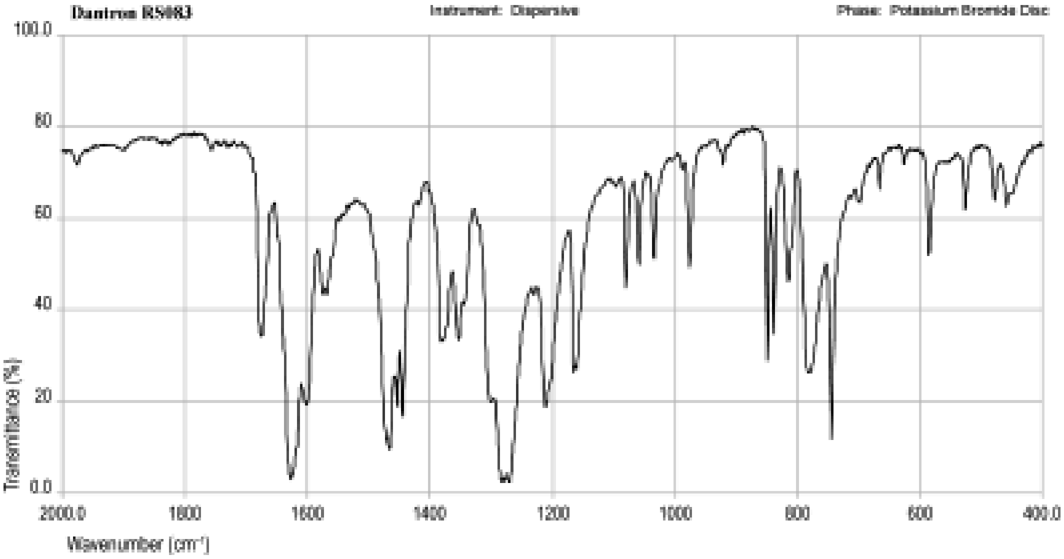
Dacarbazine



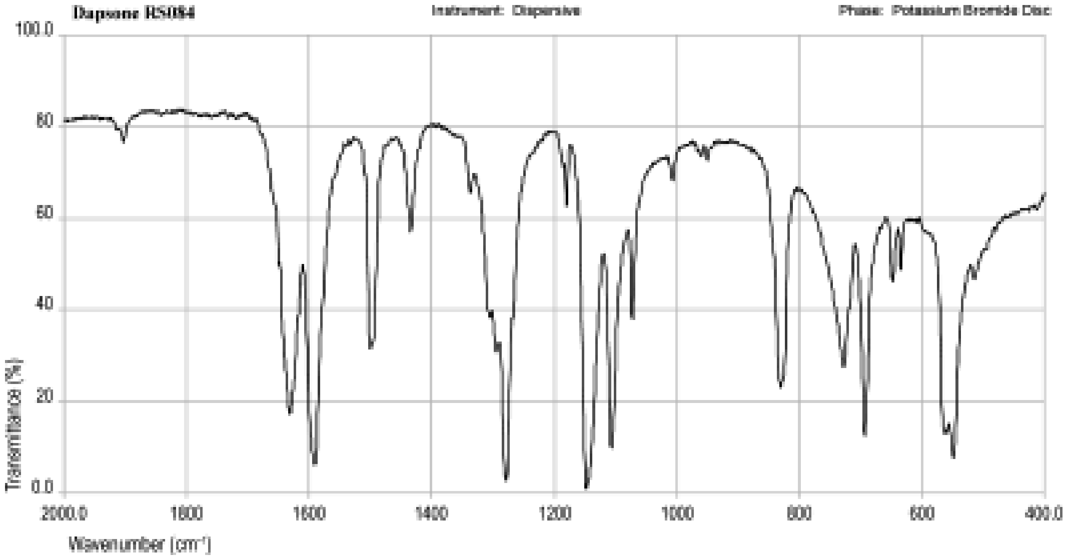
Dantrolene Sodium



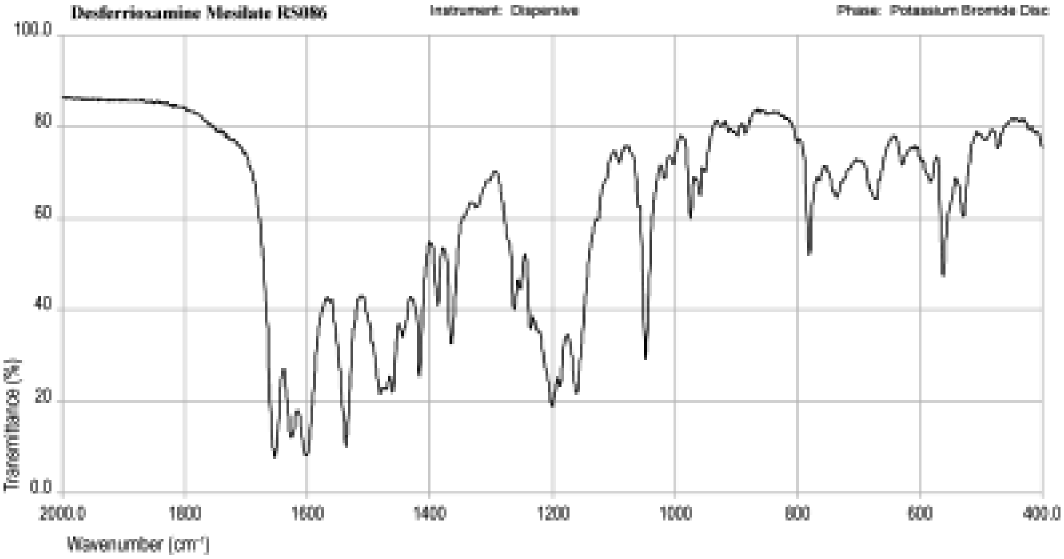
Dantron


Dantron RS083
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

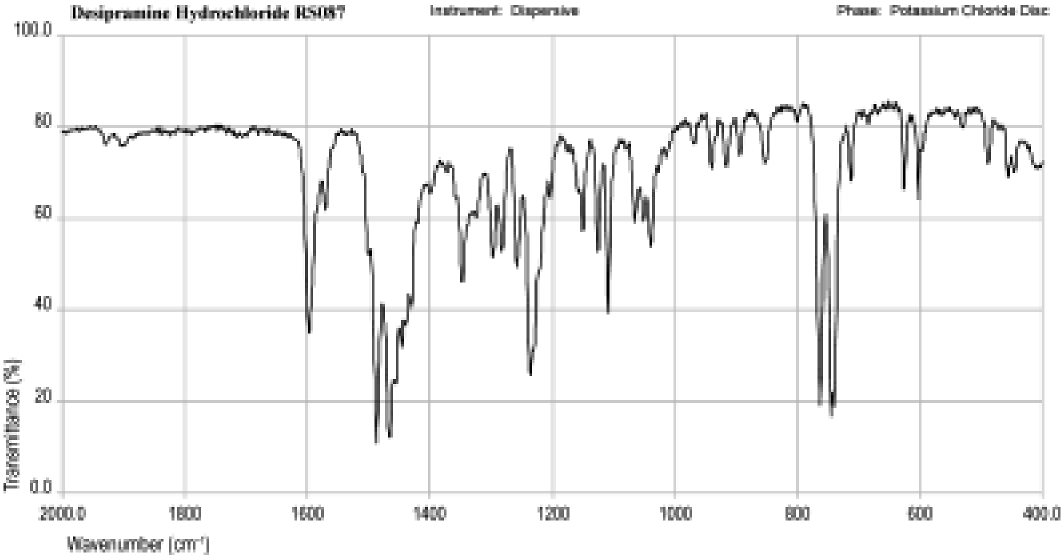
Dapsone


Dapsone RS084
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

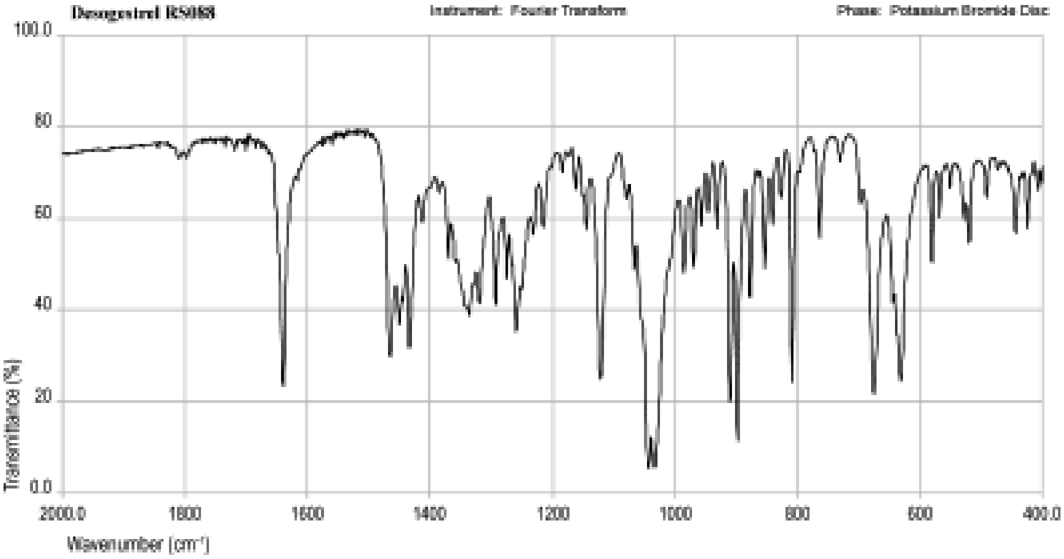
Desferrioxamine Mesilate



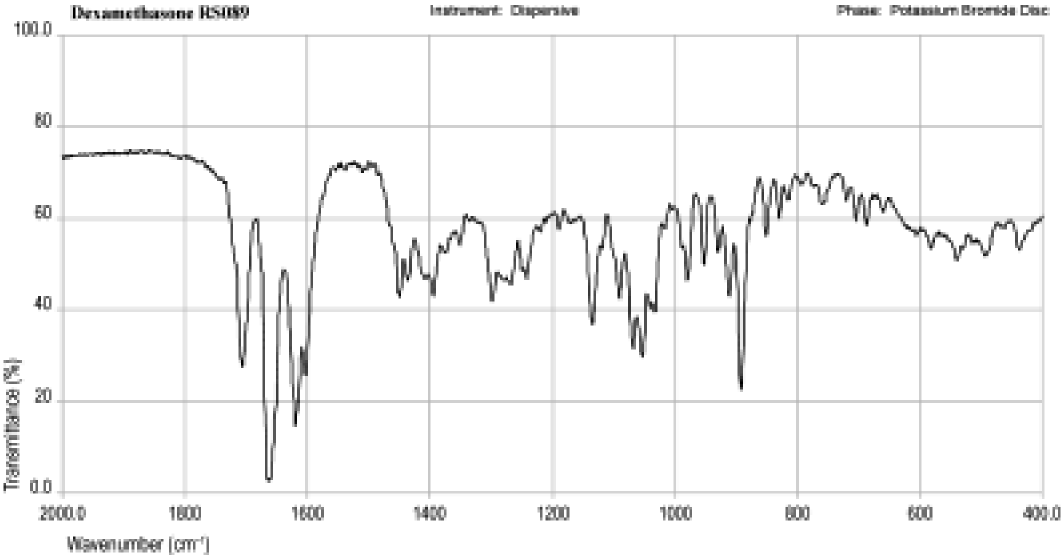
Desipramine Hydrochloride



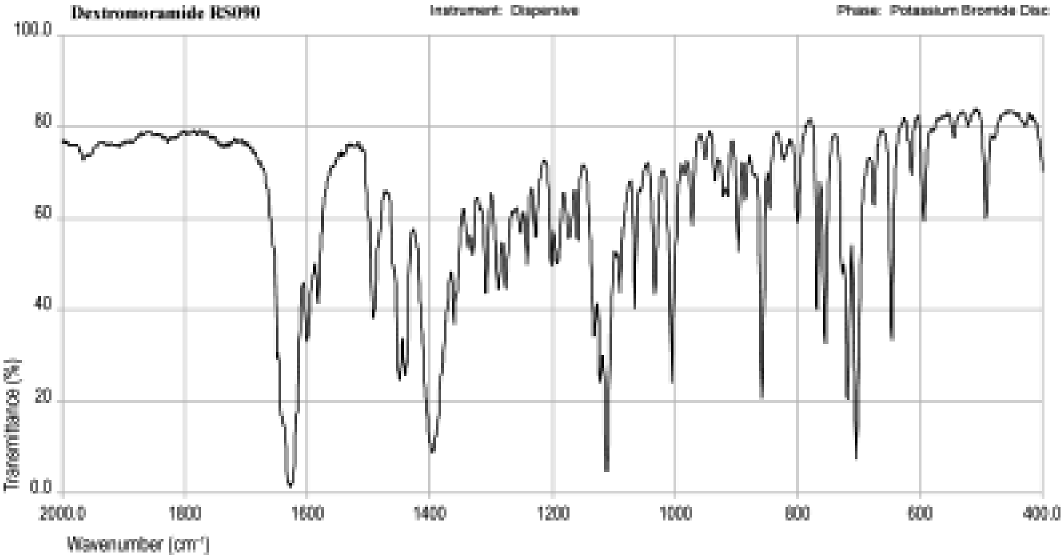
Desogestrel



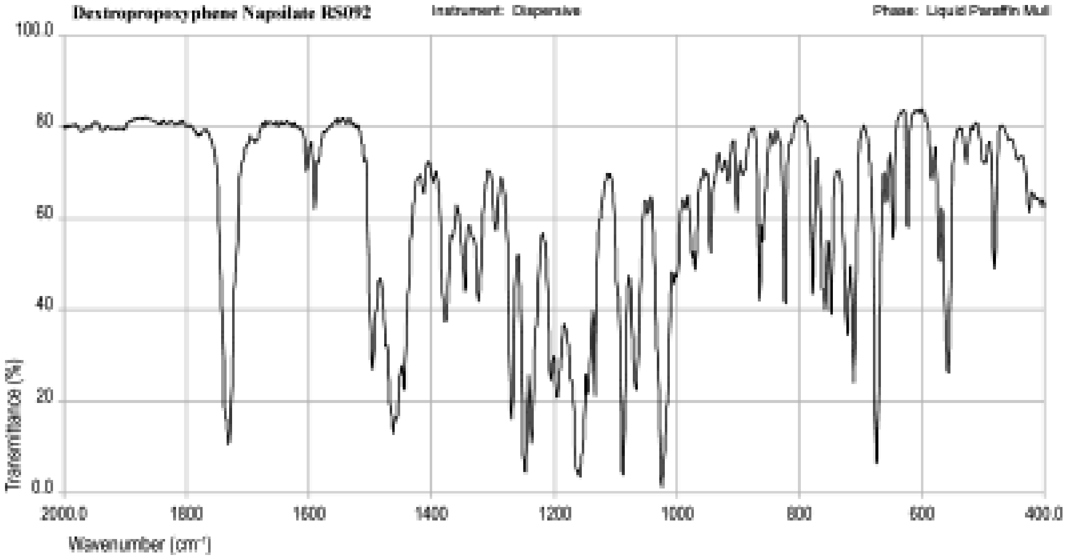
Dexamethasone



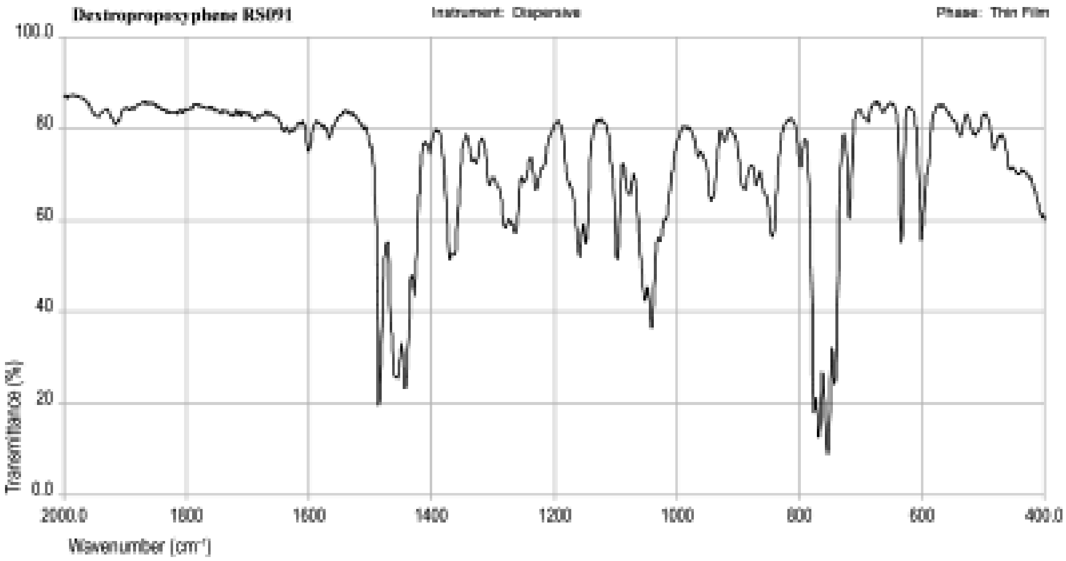
Dextromoramide



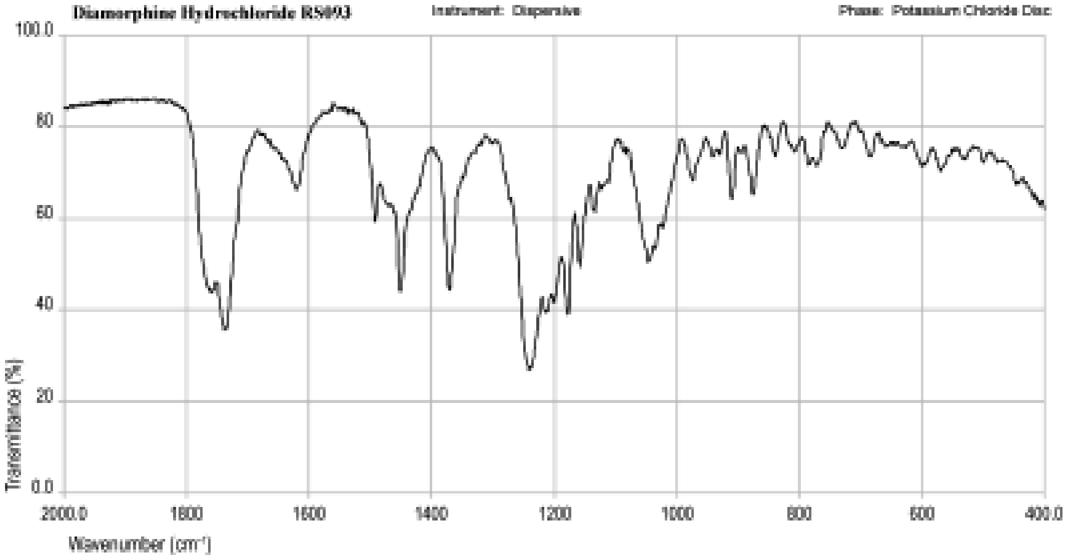
Dextropropoxyphene Napsilate



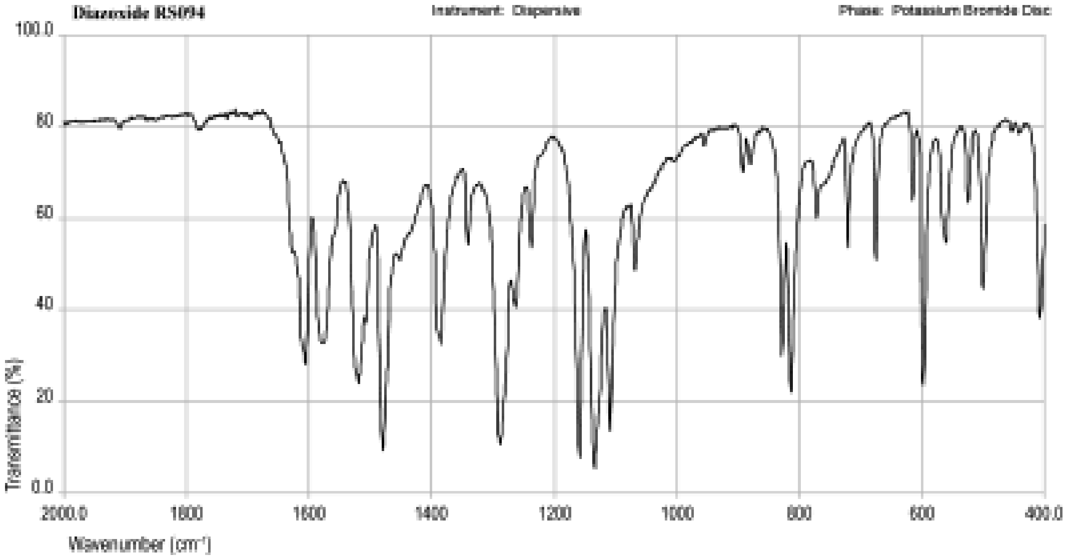
Dextropropoxyphene



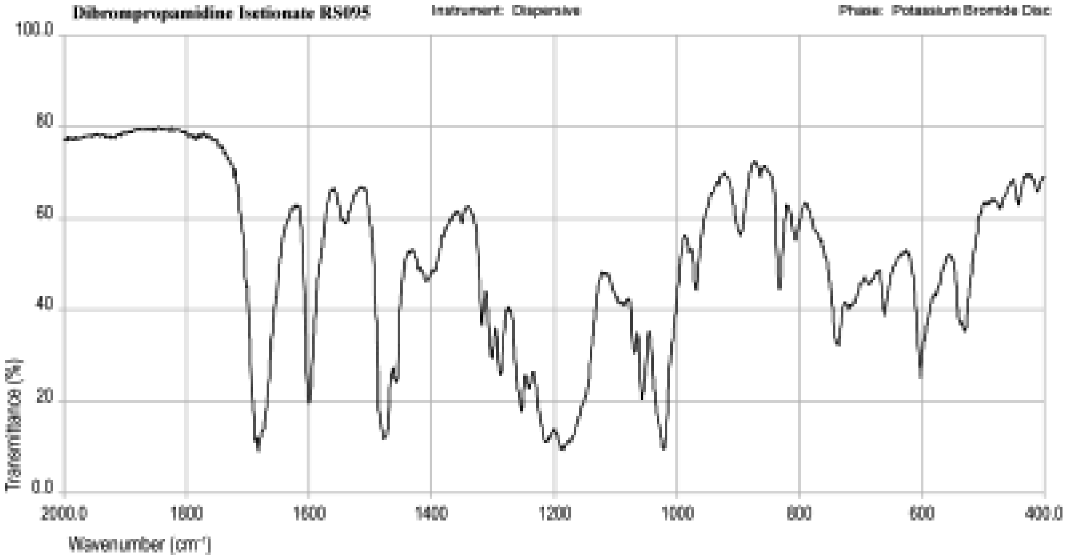
Diamorphine Hydrochloride



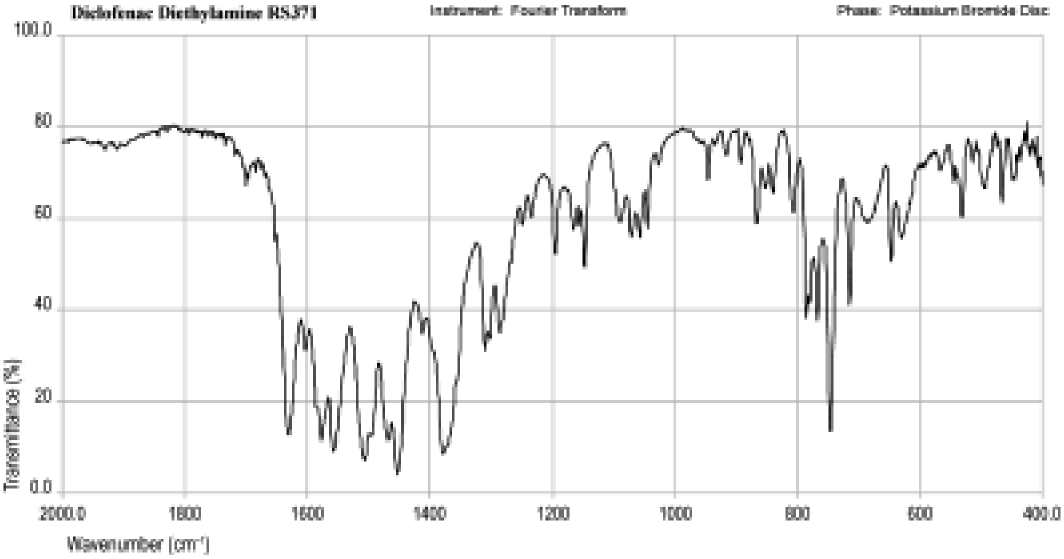
Diazoxide


Diazoxide RS094
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

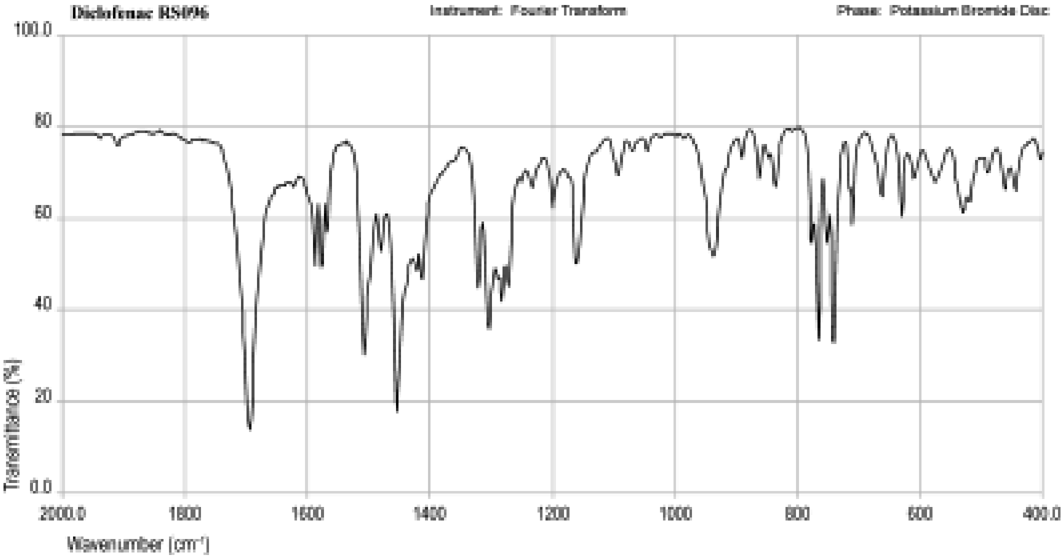
Dibrompropamidine Isetionate



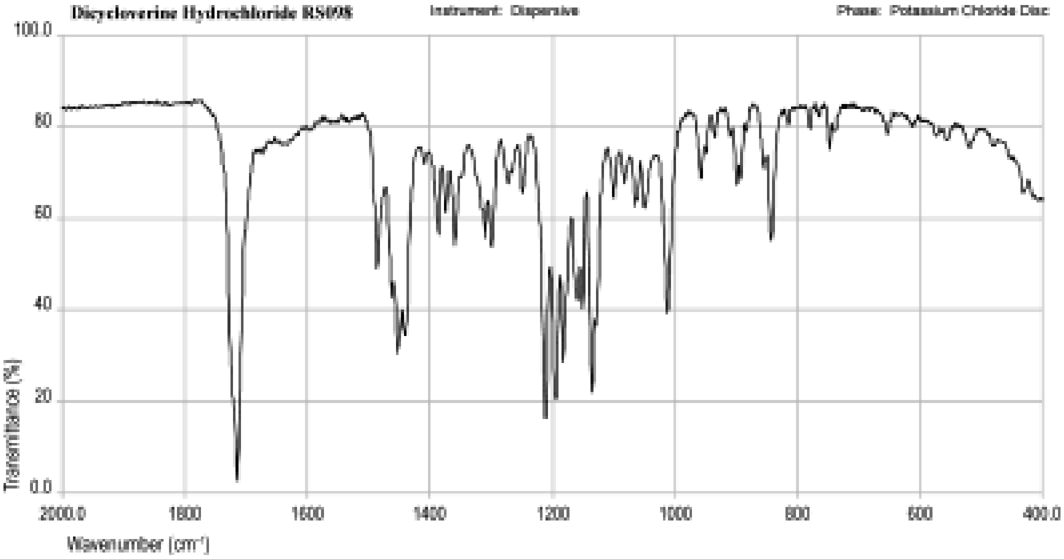
Diclofenac Diethylamine



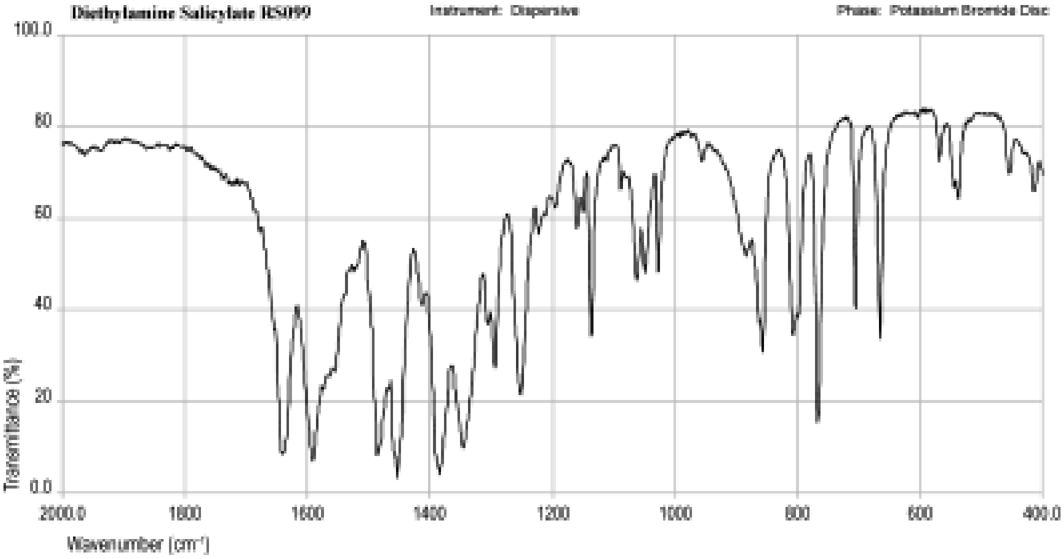
Diclofenac


Diclofenac RS096
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

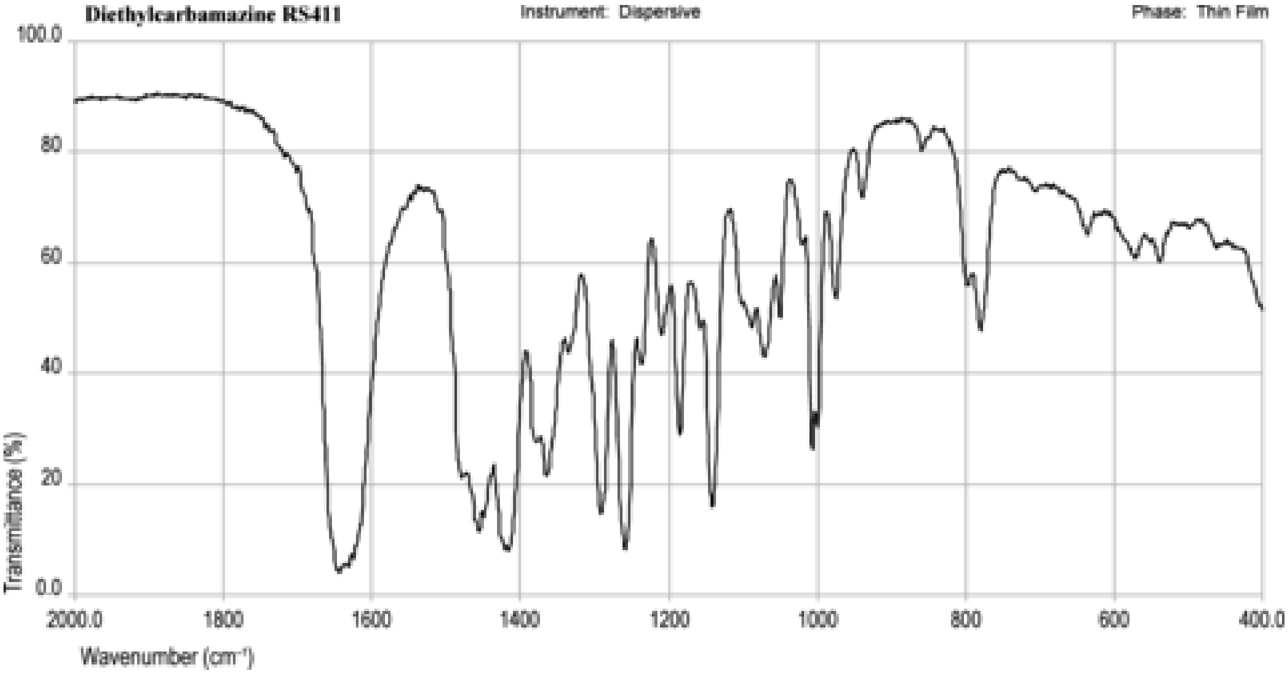
Dicycloverine Hydrochloride



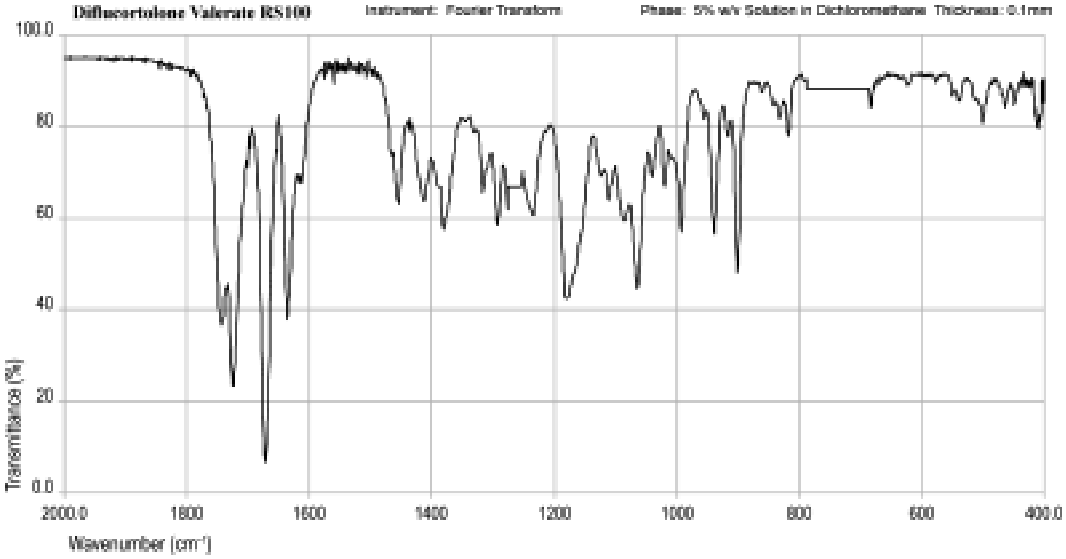
Diethylamine Salicylate



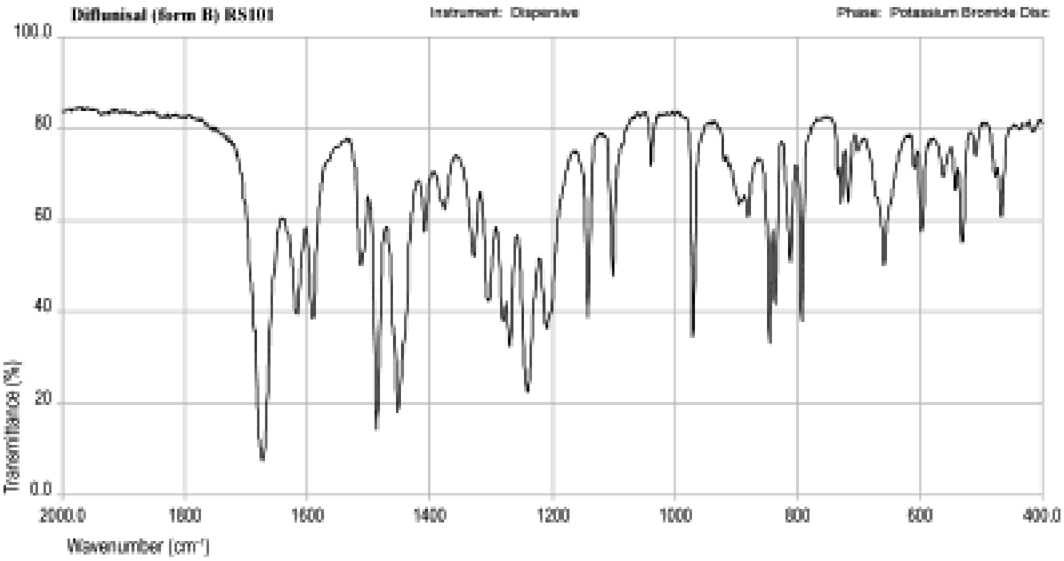
Diethylcarbamazine



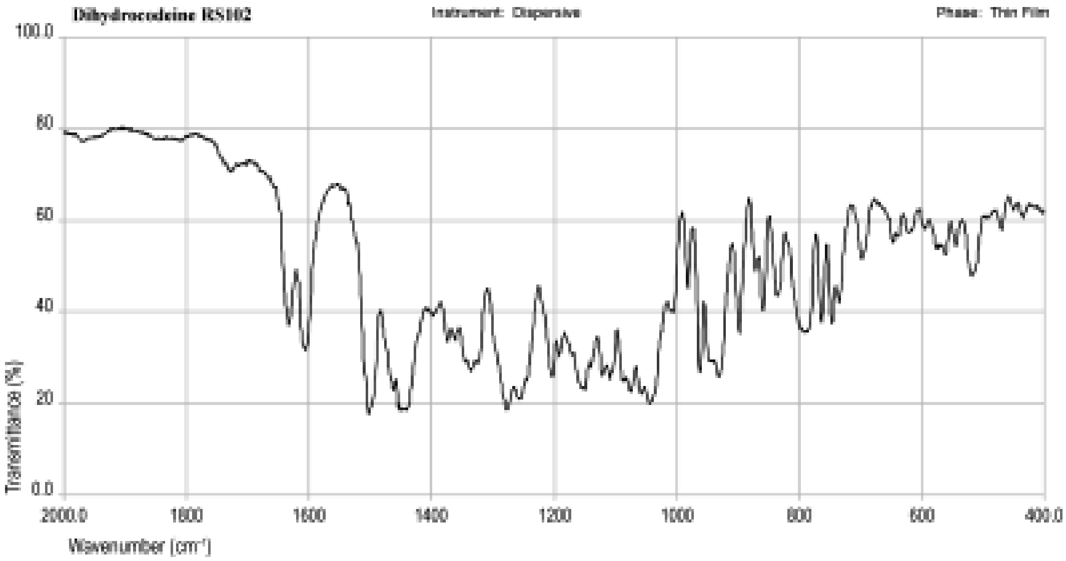
Diflucortolone Valerate



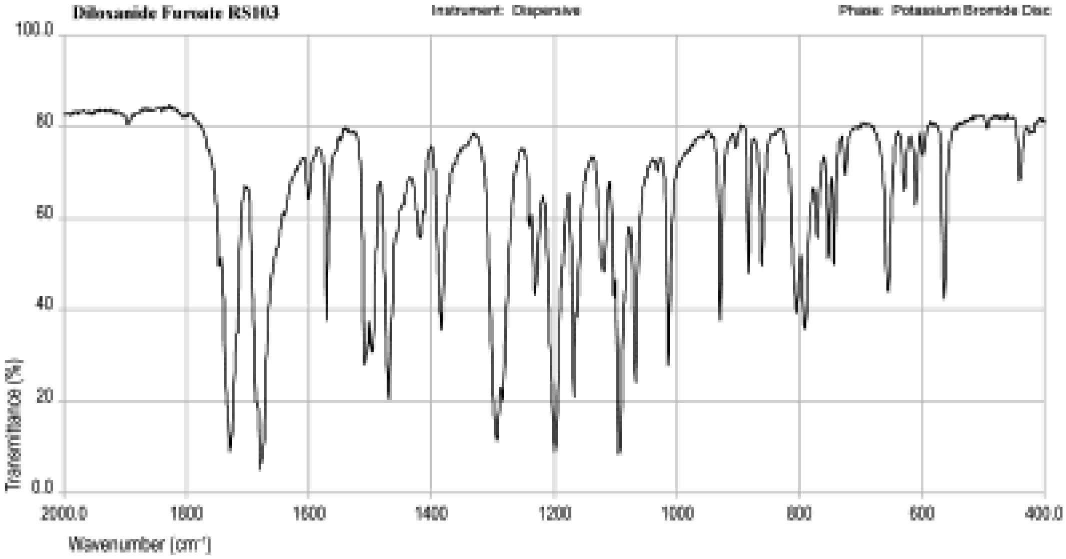
Diflunisal (form B)



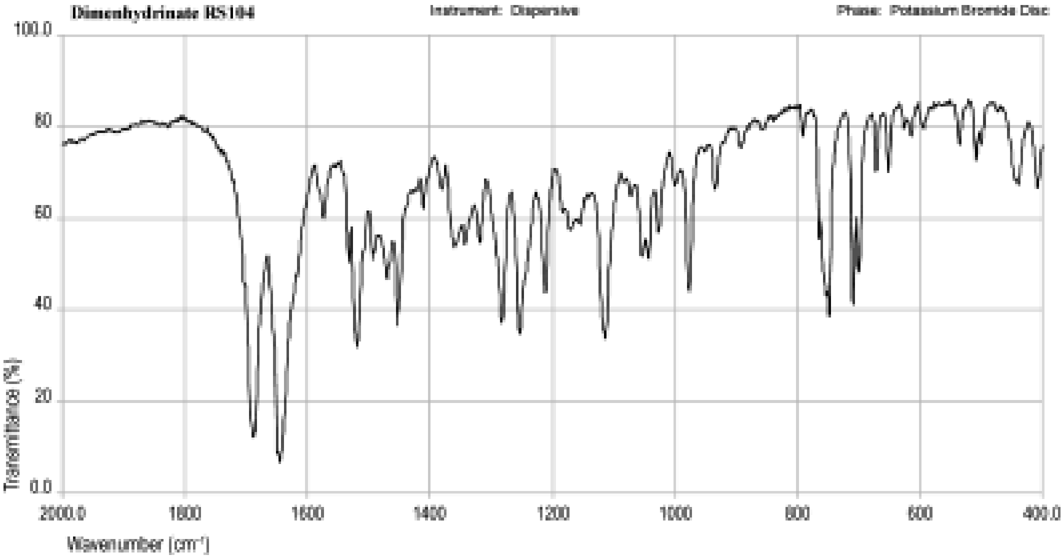
Dihydrocodeine



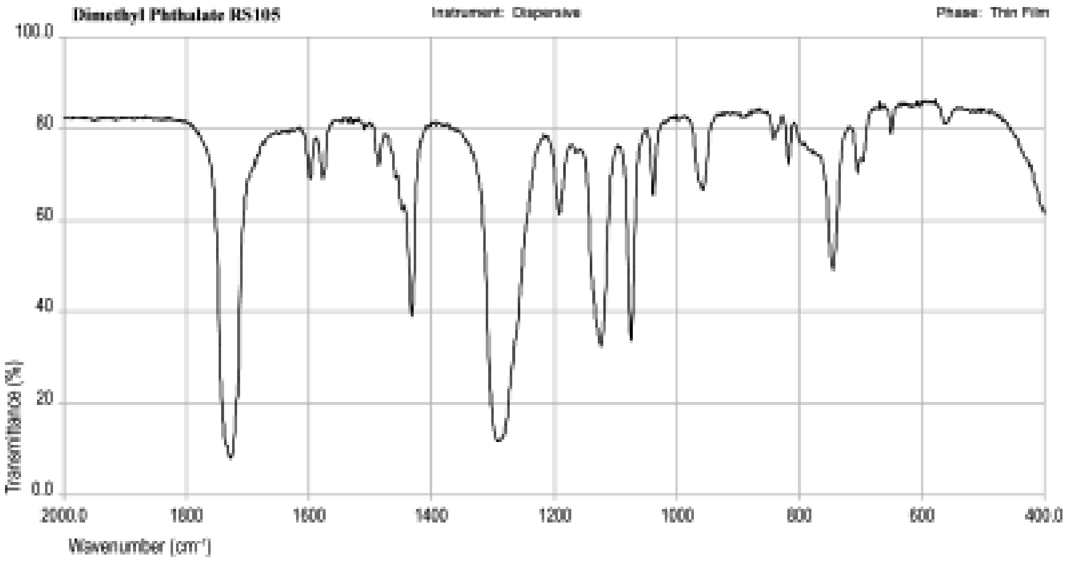
Diloxanide Furoate



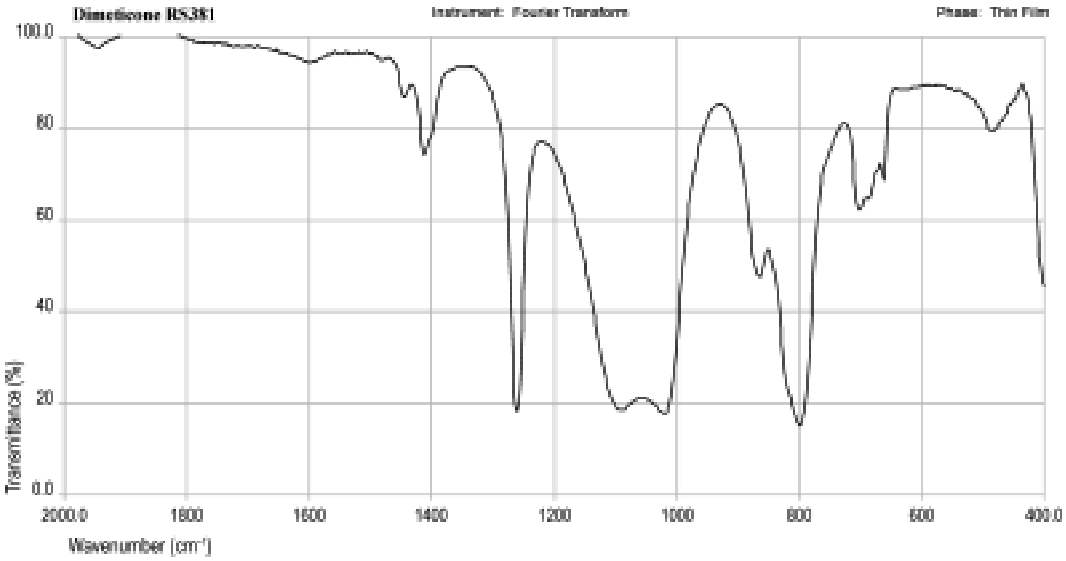
Dimenhydrinate



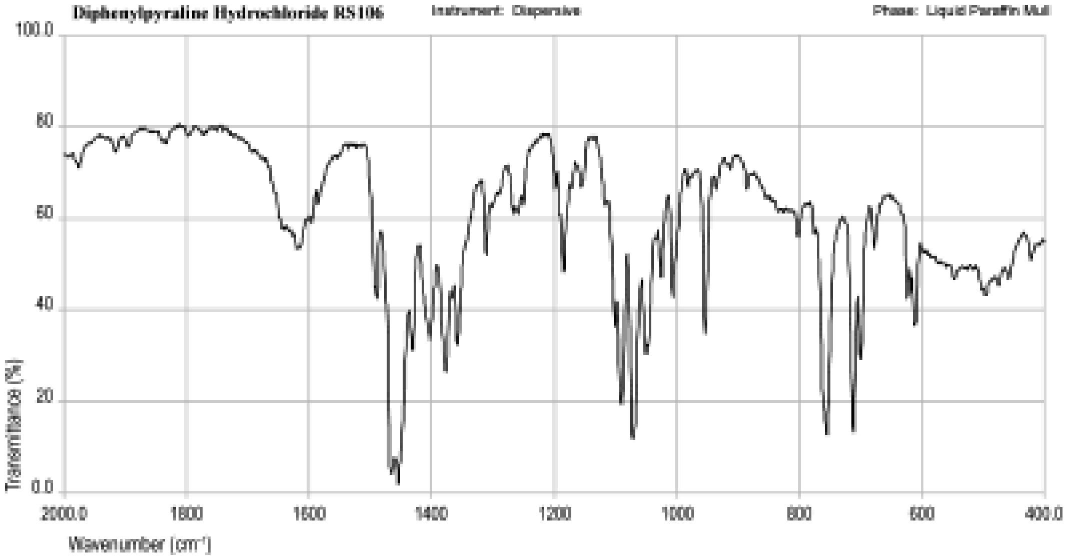
Dimethyl Phthalate



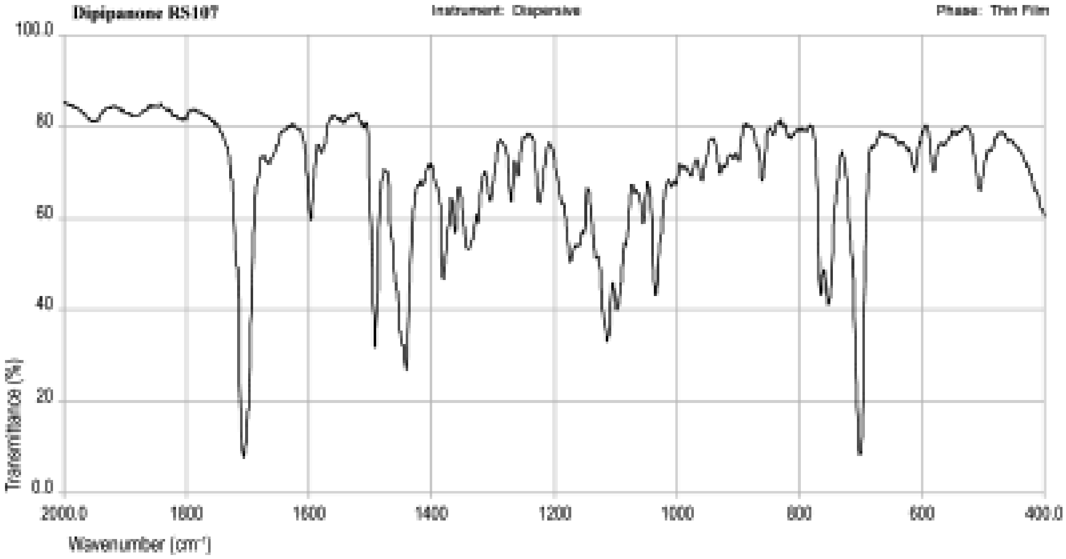
Dimeticone


Dimeticone RS381
Instrument: Fourier Transform
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

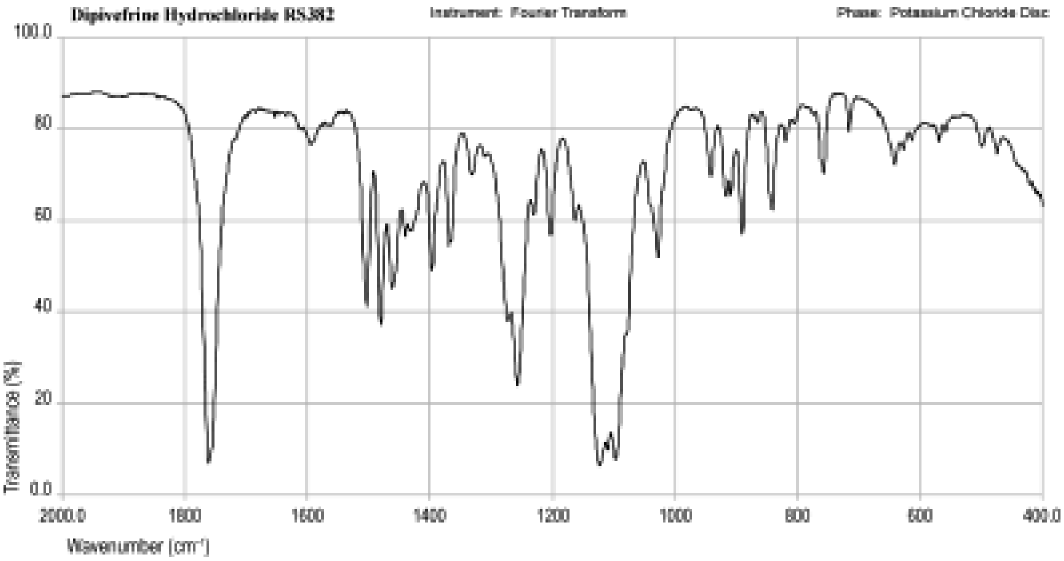
Diphenylpyraline Hydrochloride



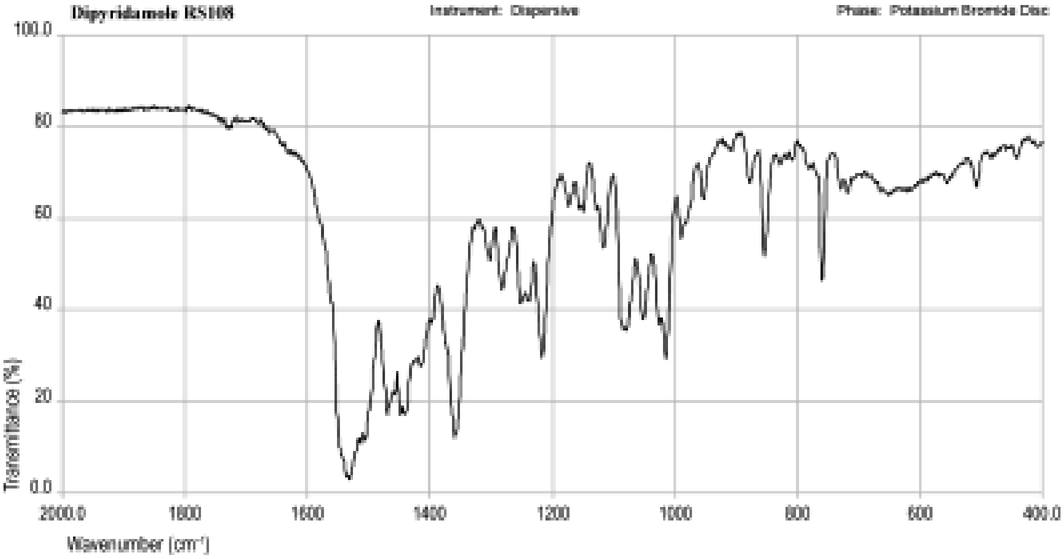
Dipipanone


Dipipanone RS107
Instrument: Dispersive
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

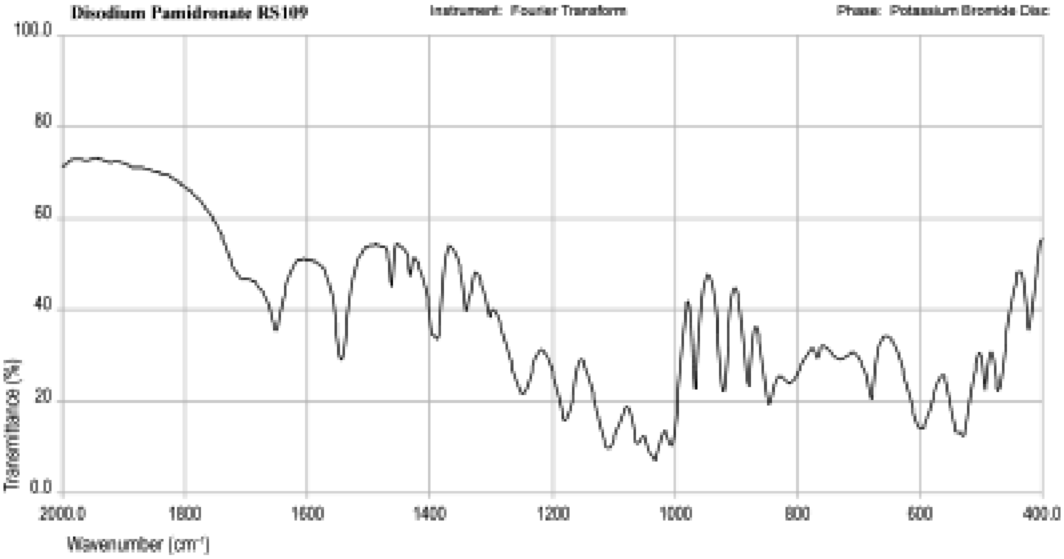
Dipivefrine Hydrochloride



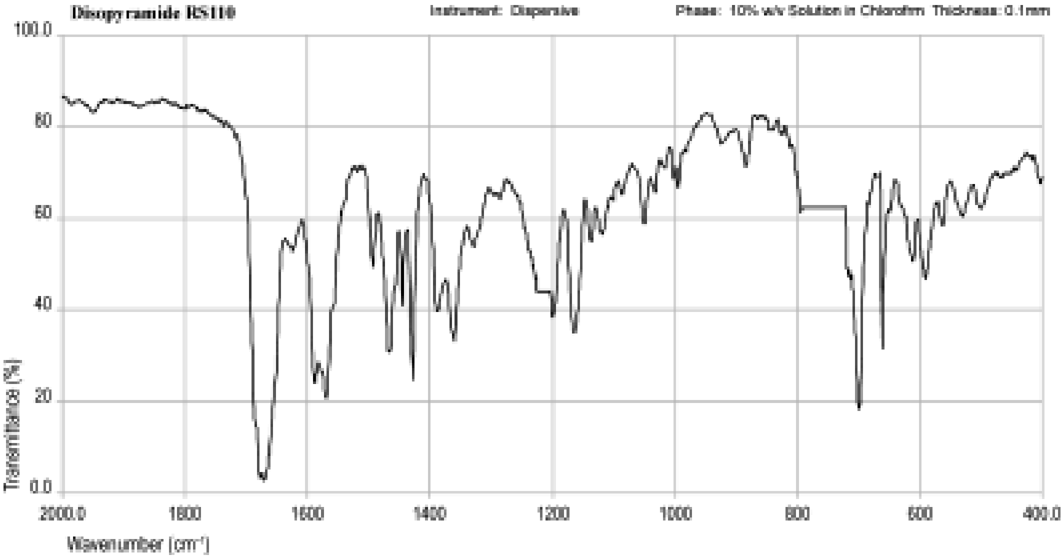
Dipyridamole



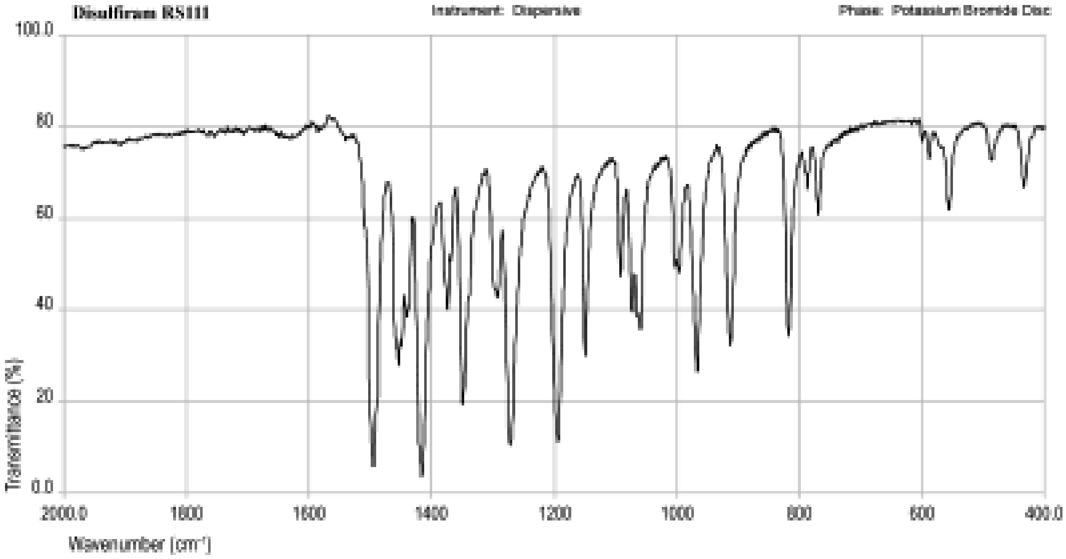
Disodium Pamidronate



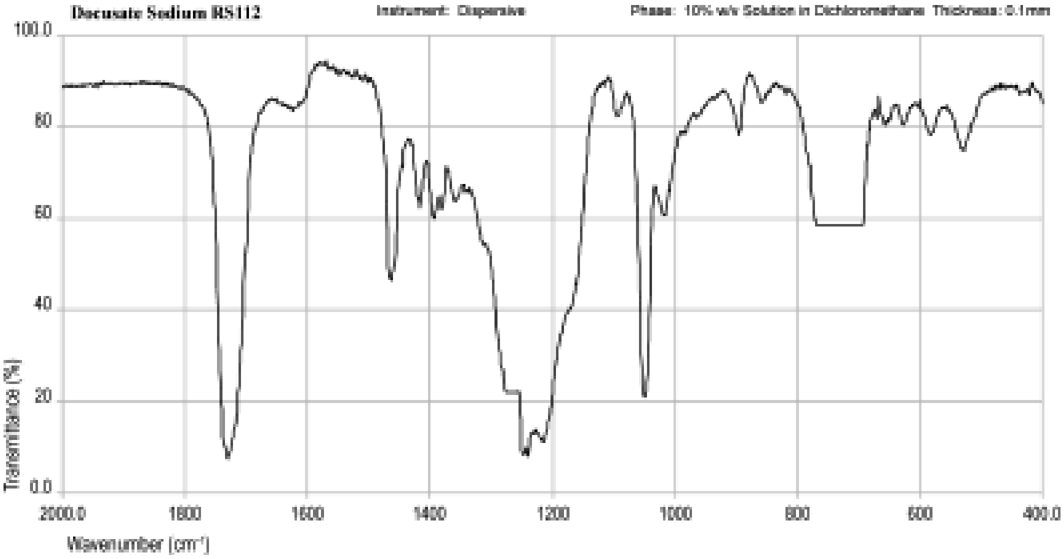
Disopyramide



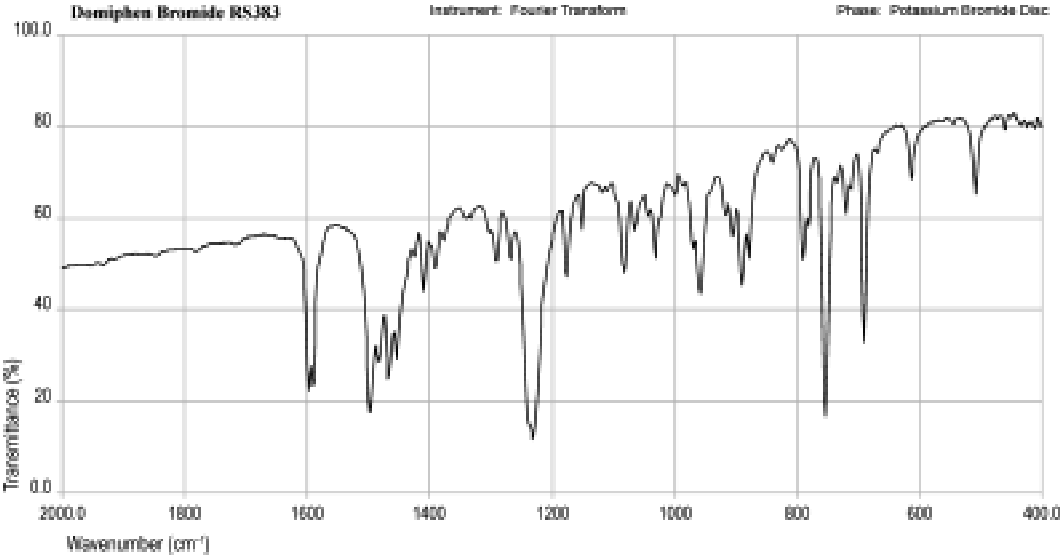
Disulfiram


Disulfiram RS111
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

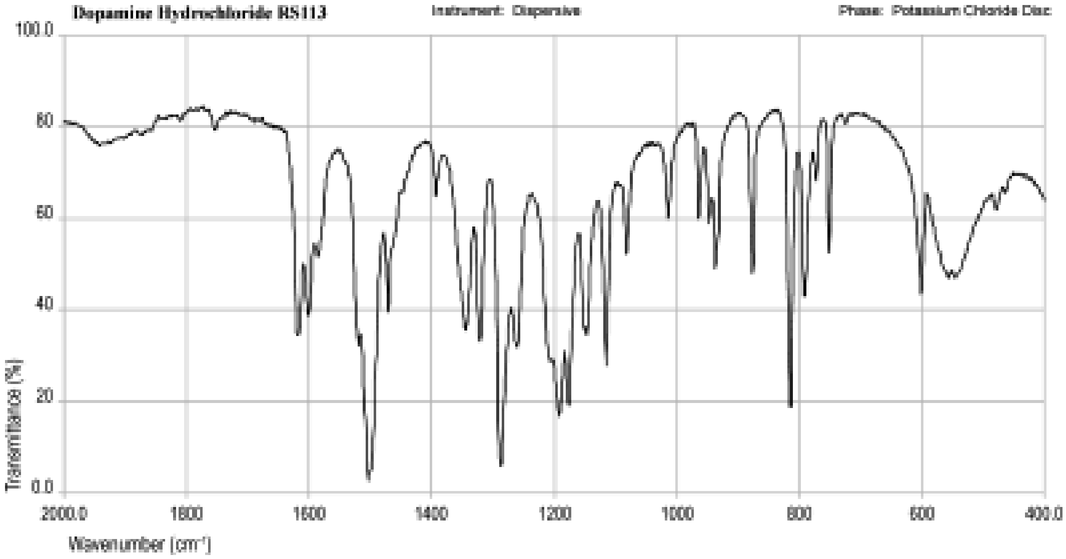
Docusate Sodium



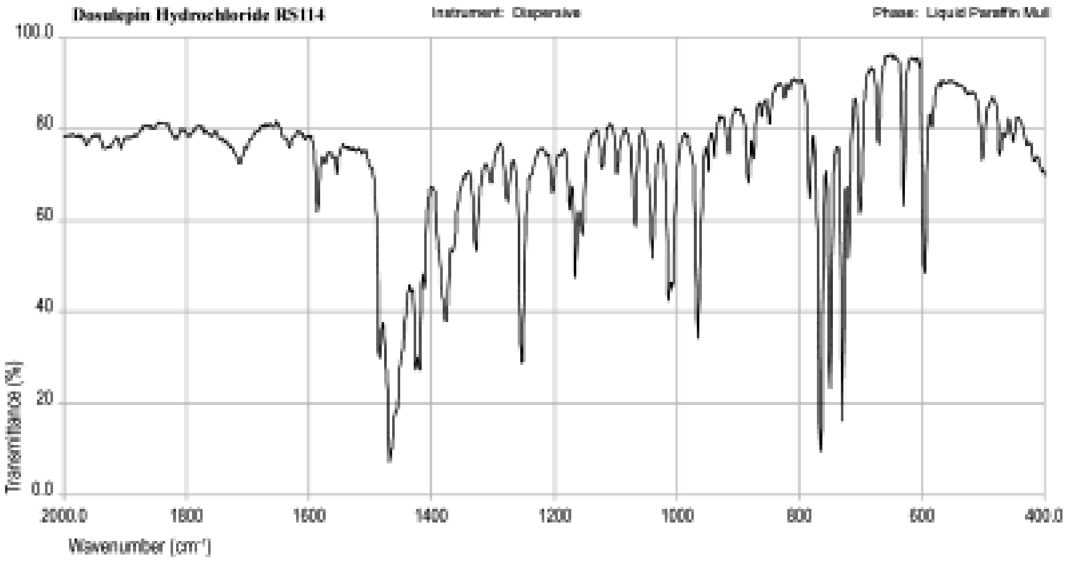
Domiphen Bromide



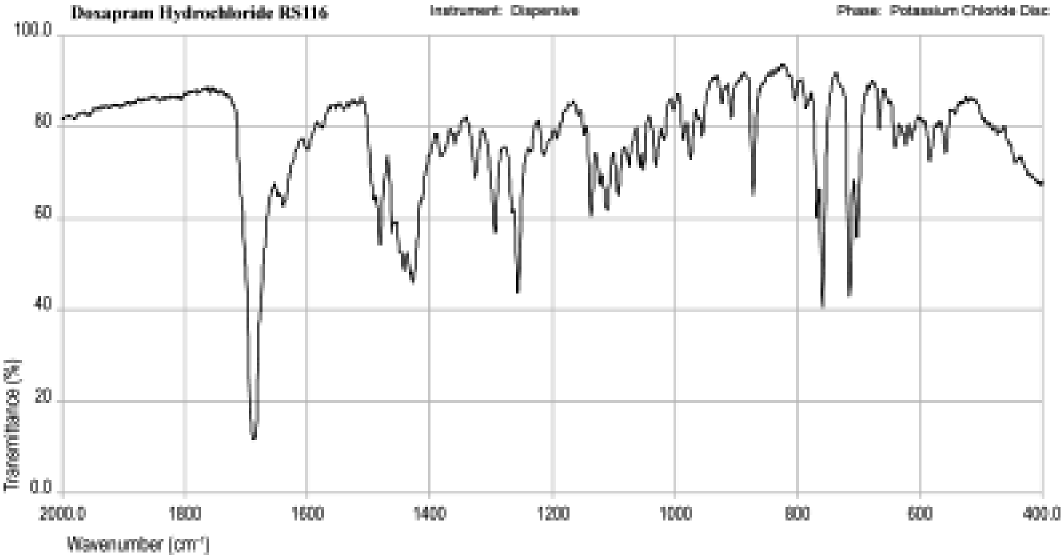
Dopamine Hydrochloride



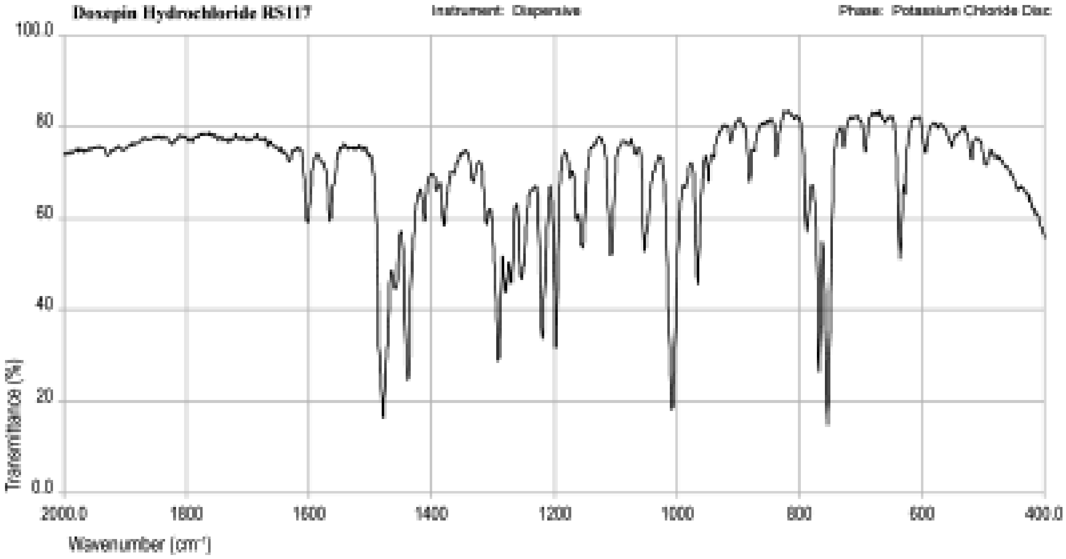
Dosulepin Hydrochloride



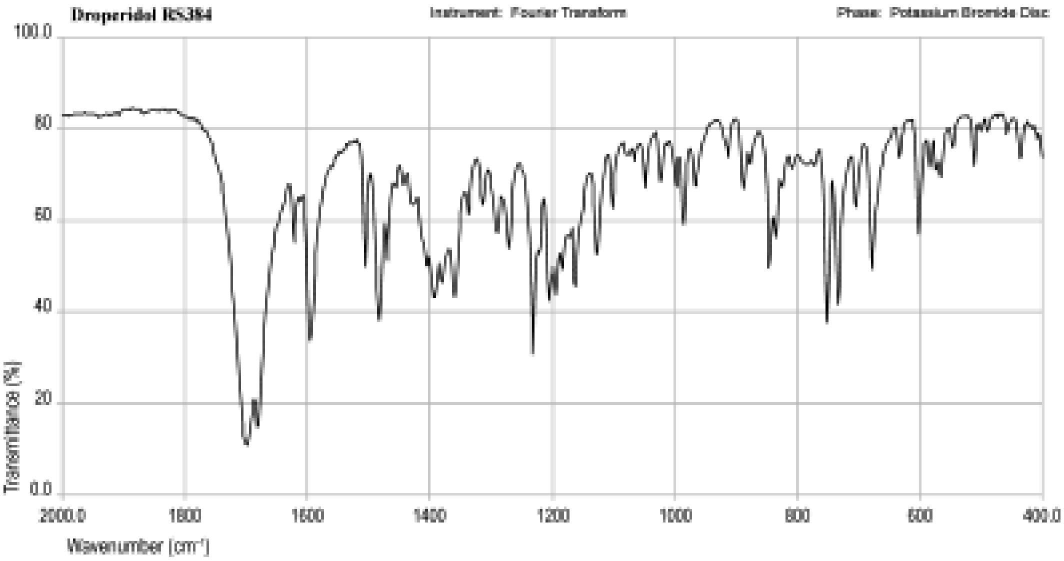
Doxapram Hydrochloride



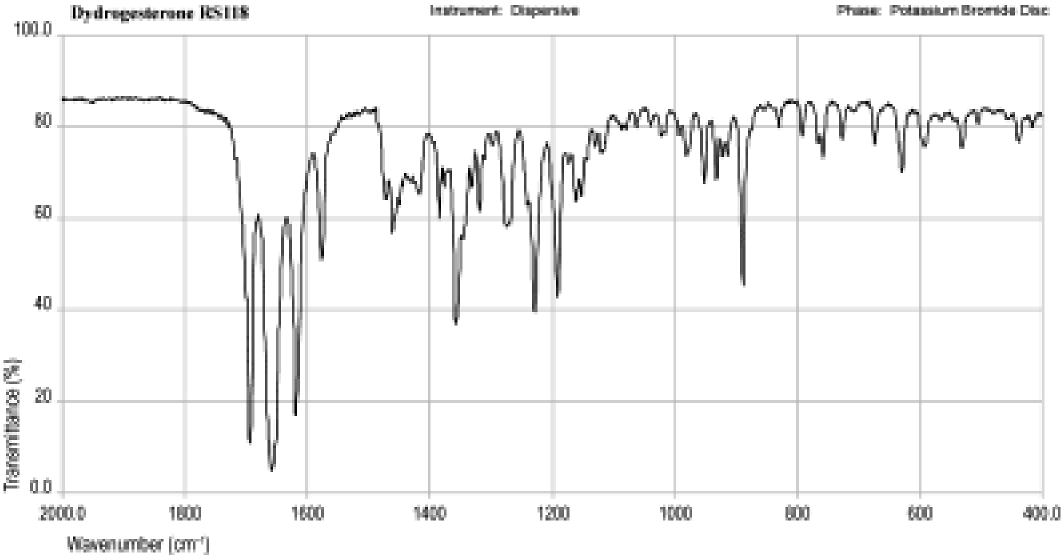
Doxepin Hydrochloride



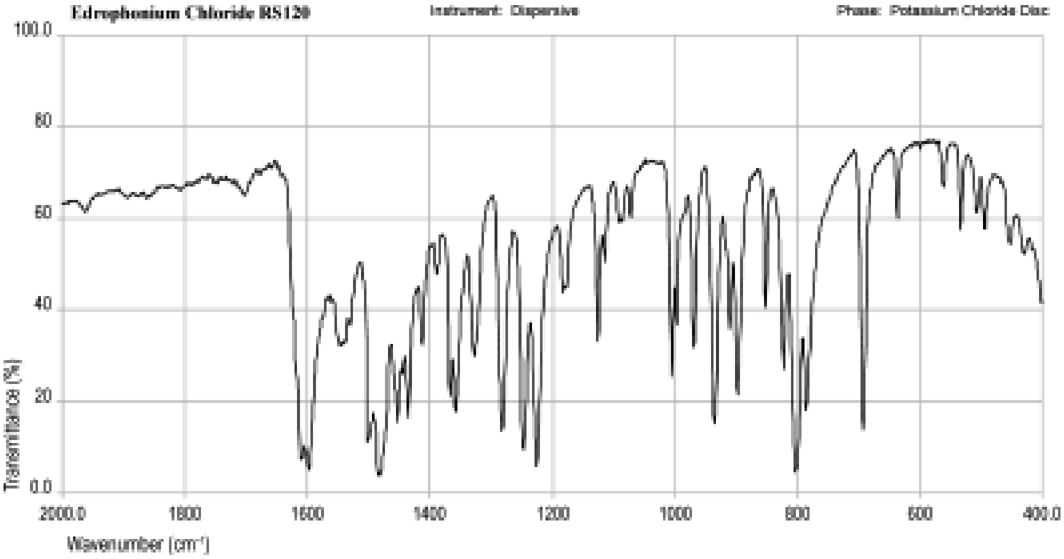
Droperidol


Droperidol RS384
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

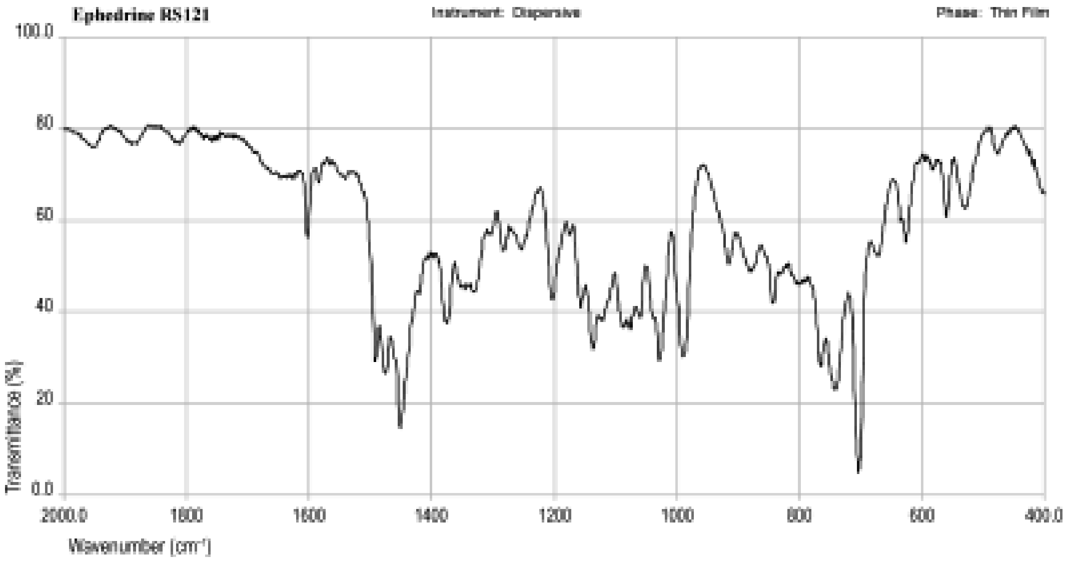
Dydrogesterone



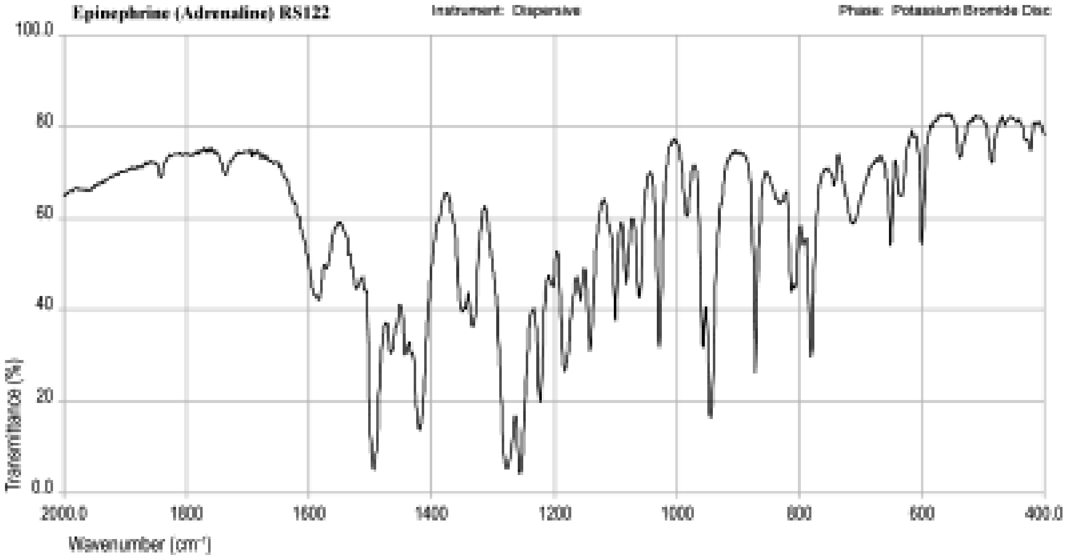
Edrophonium Chloride



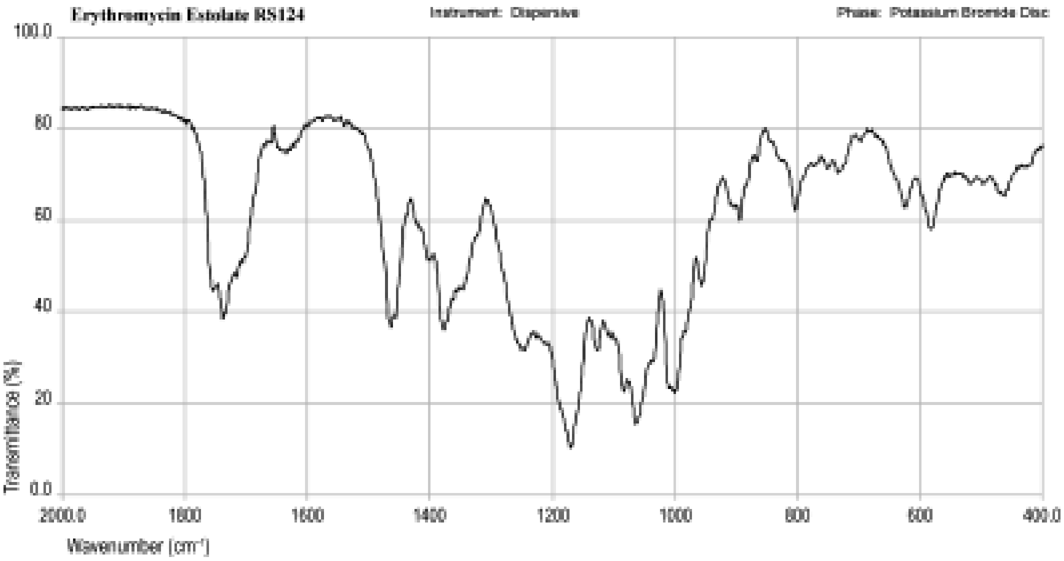
Ephedrine


Ephedrine RS121
Instrument: Dispersive
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

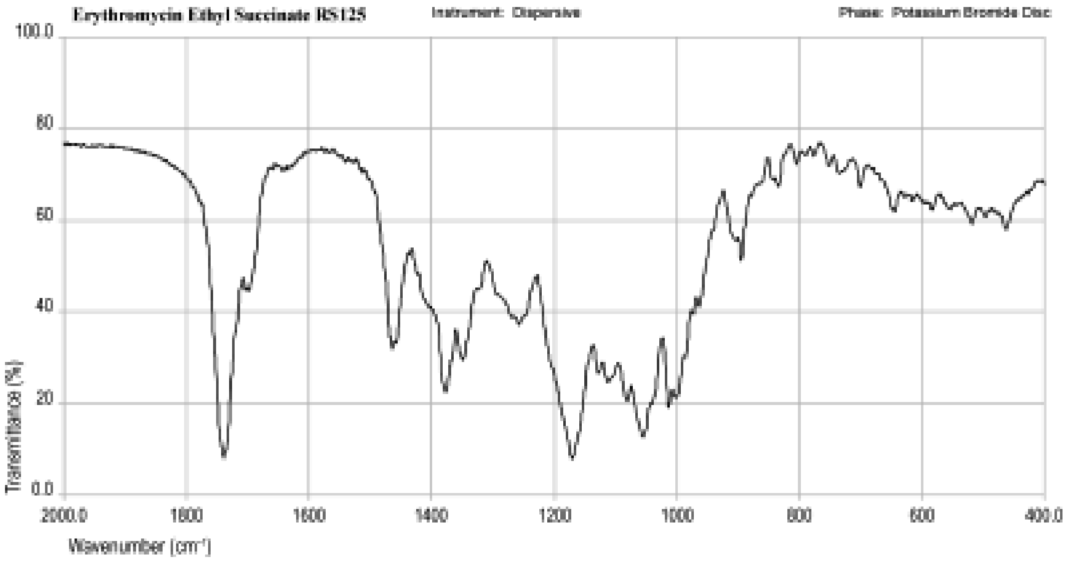
Epinephrine (Adrenaline)



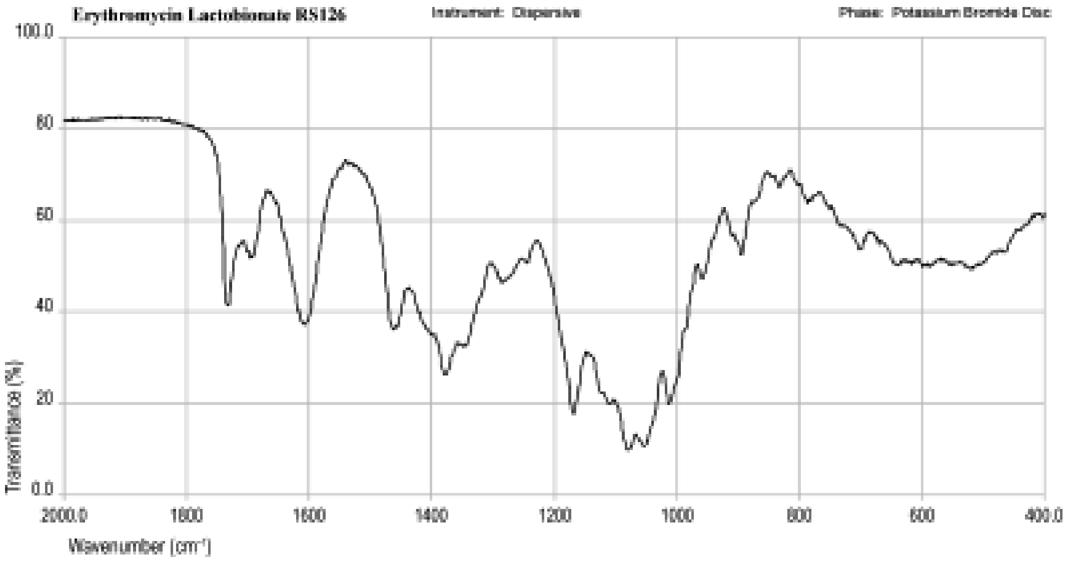
Erythromycin Estolate



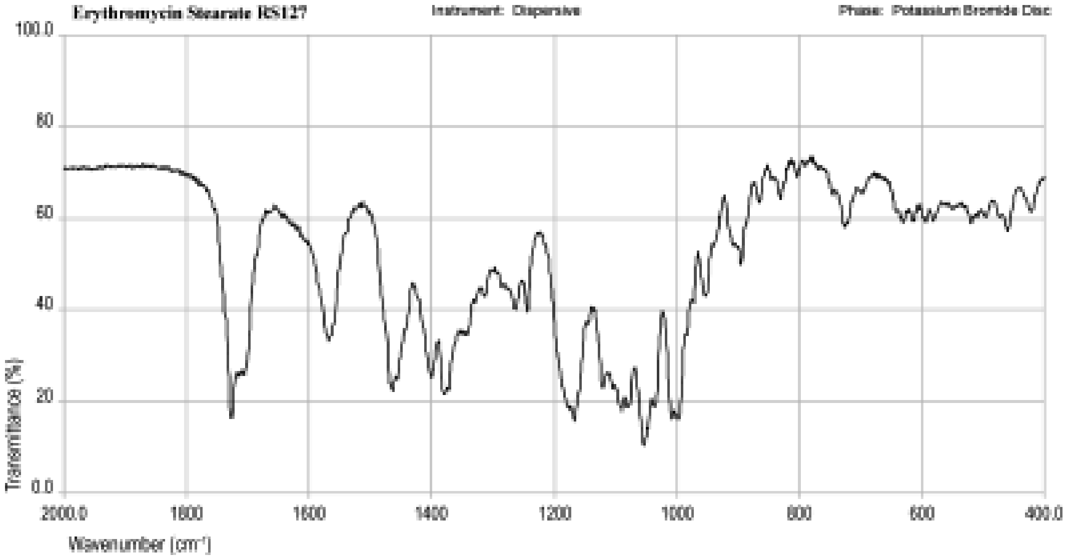
Erythromycin Ethyl Succinate



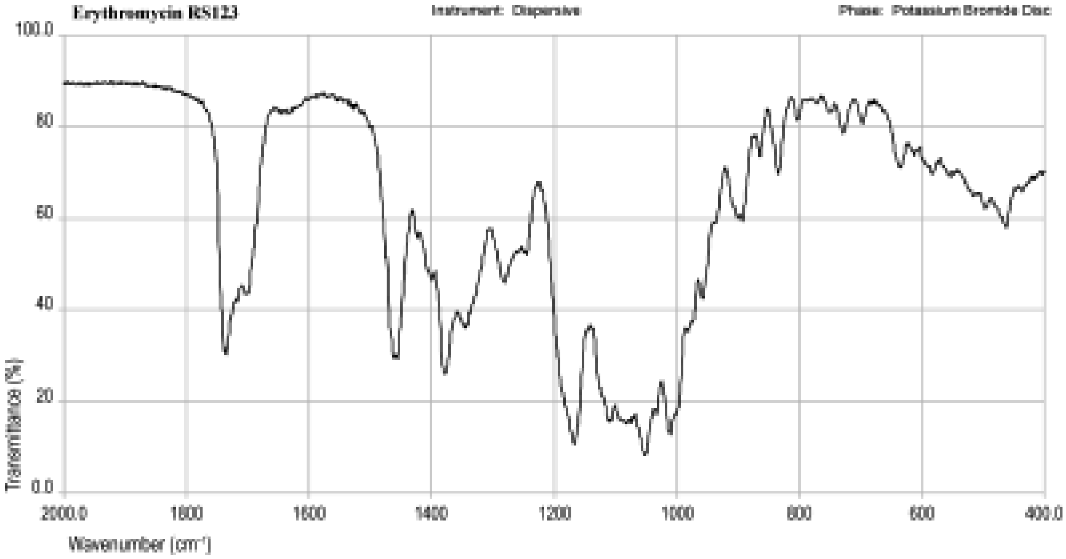
Erythromycin Lactobionate



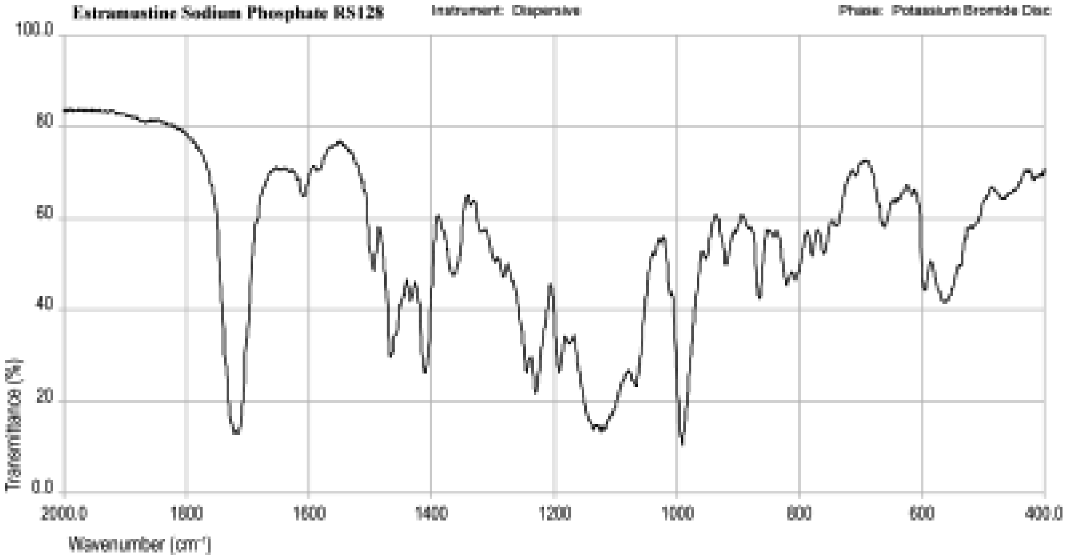
Erythromycin Stearate



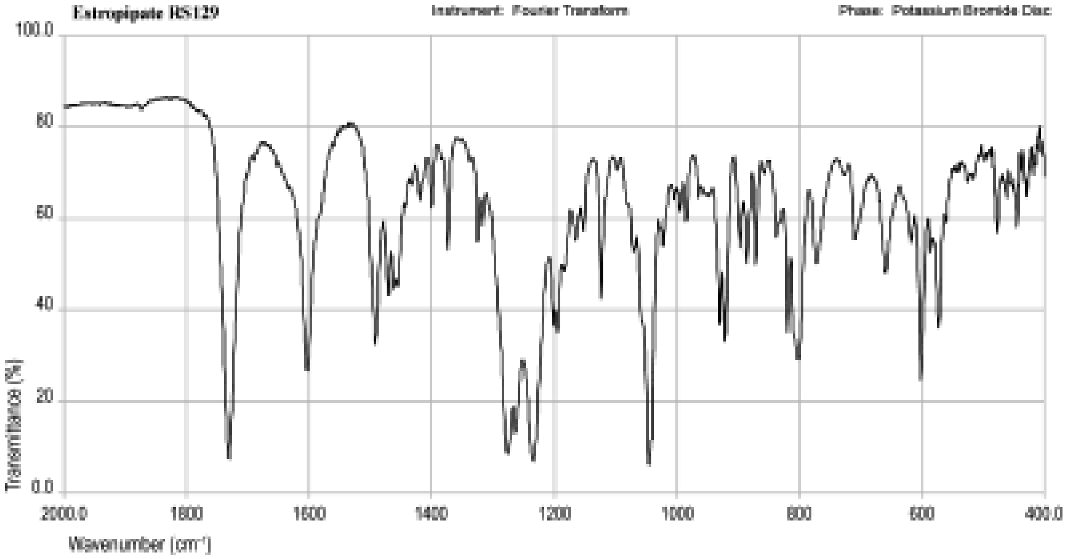
Erythromycin



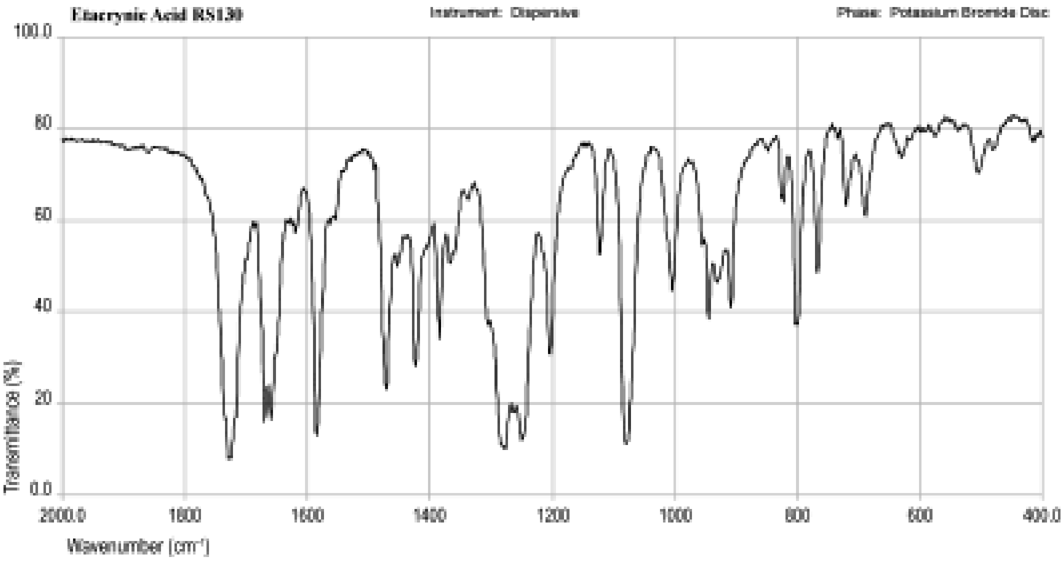
Estramustine Sodium Phosphate



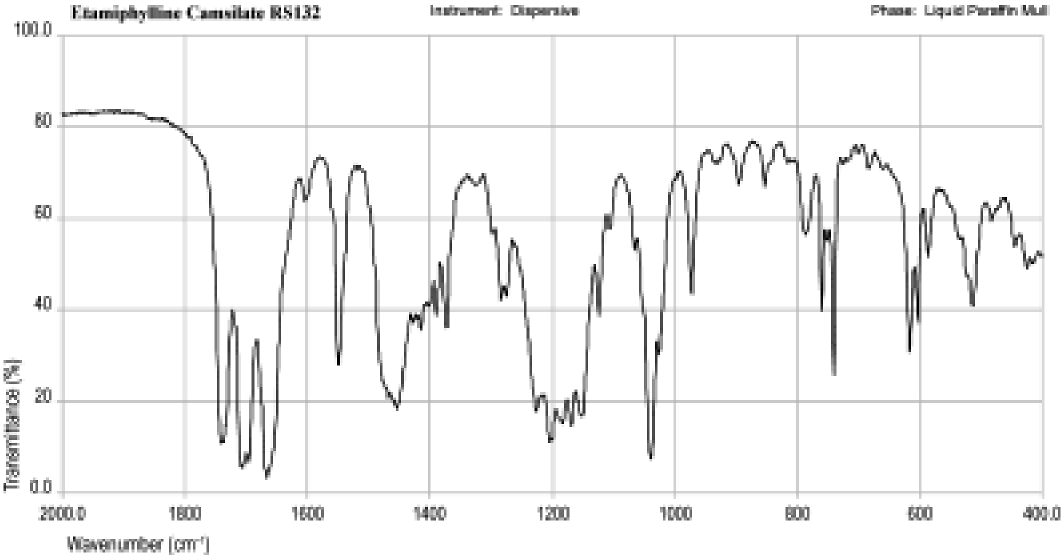
Estropipate



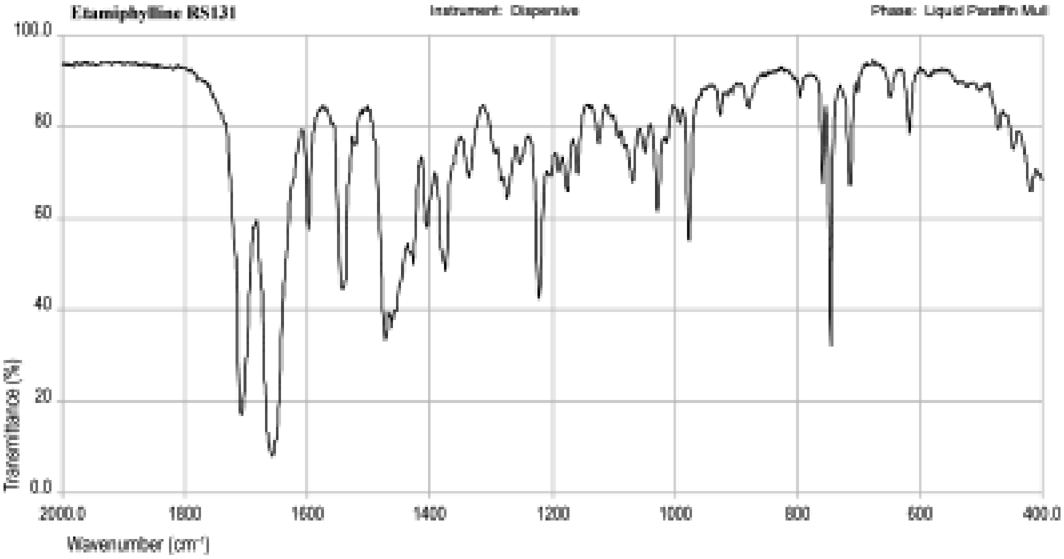
Etacrynic Acid



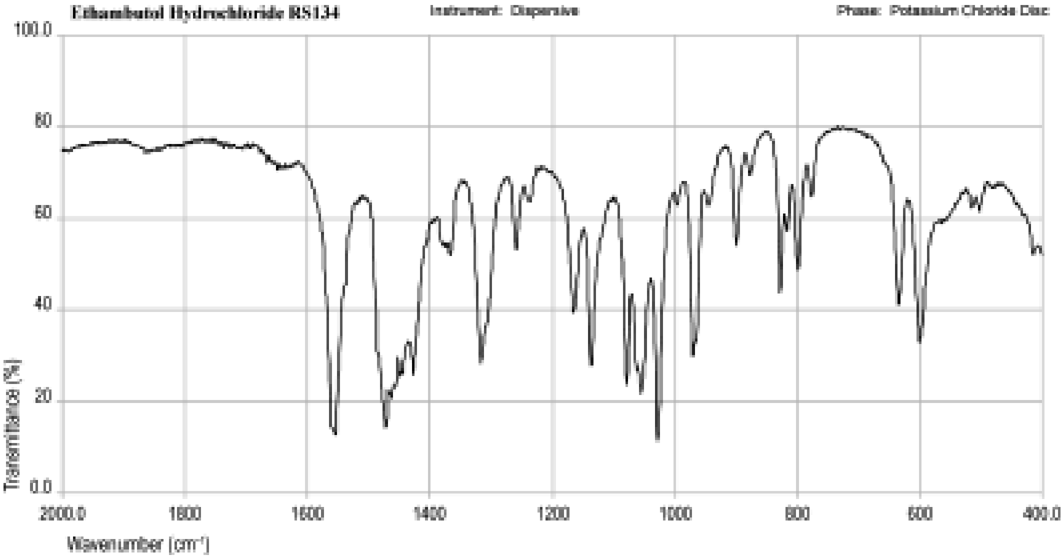
Etamiphylline Camsilate



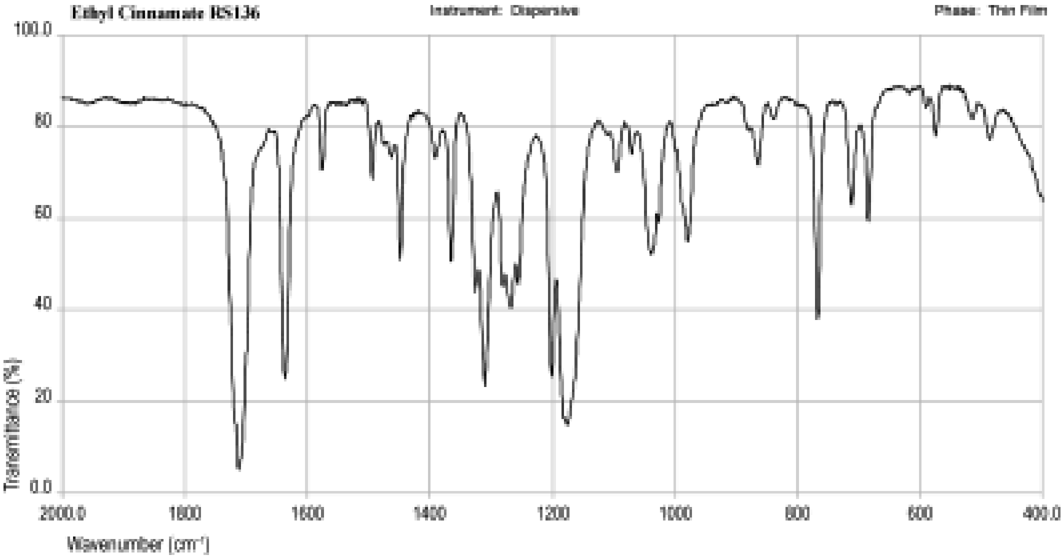
Etamiphylline



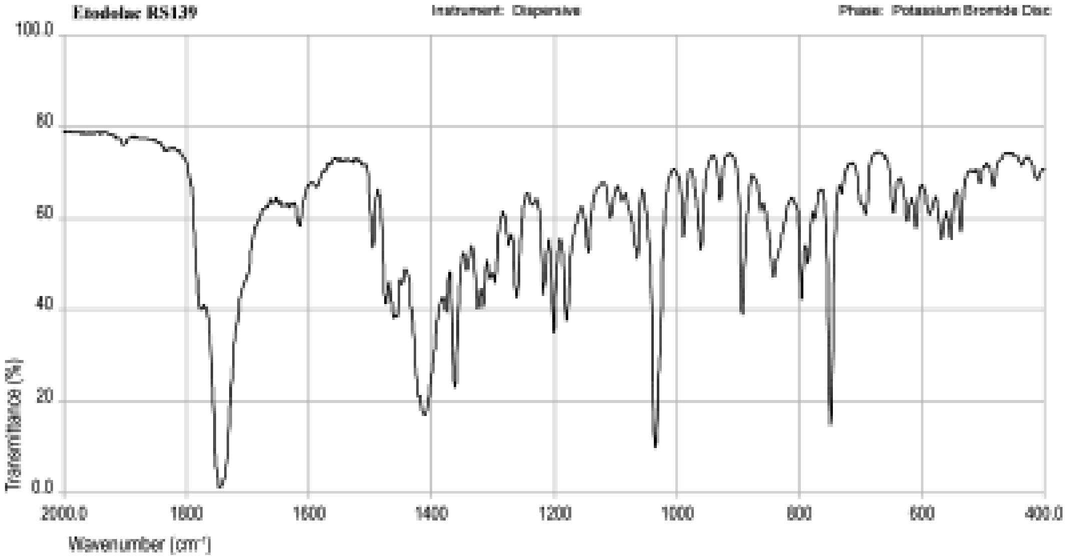
Ethambutol Hydrochloride



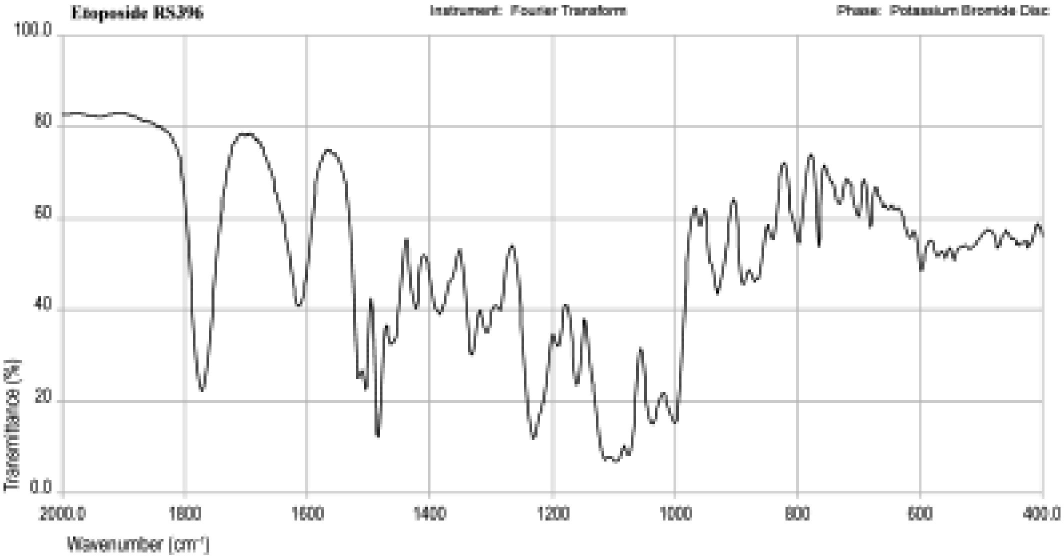
Ethyl Cinnamate



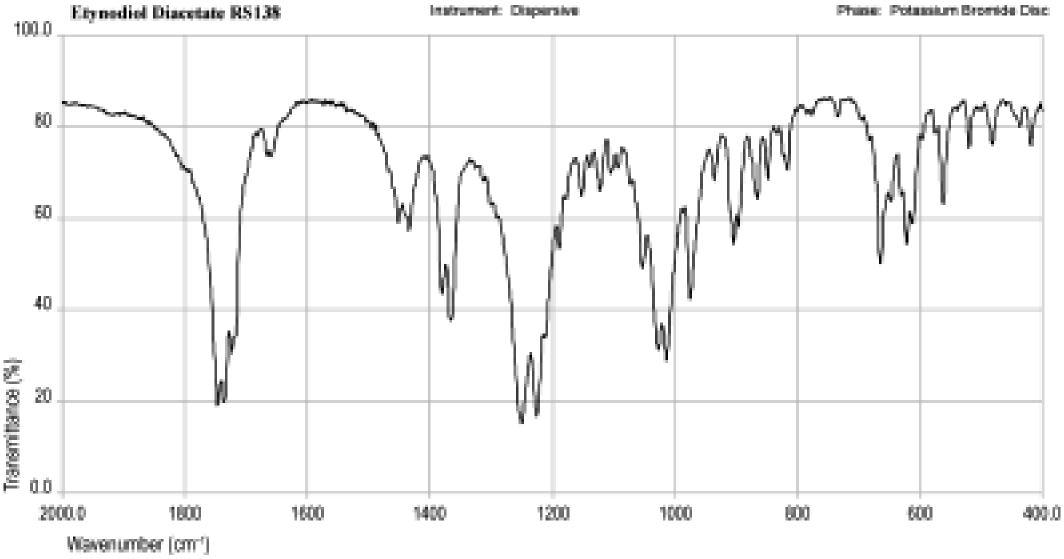
Etodolac


Etodolac RS139
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

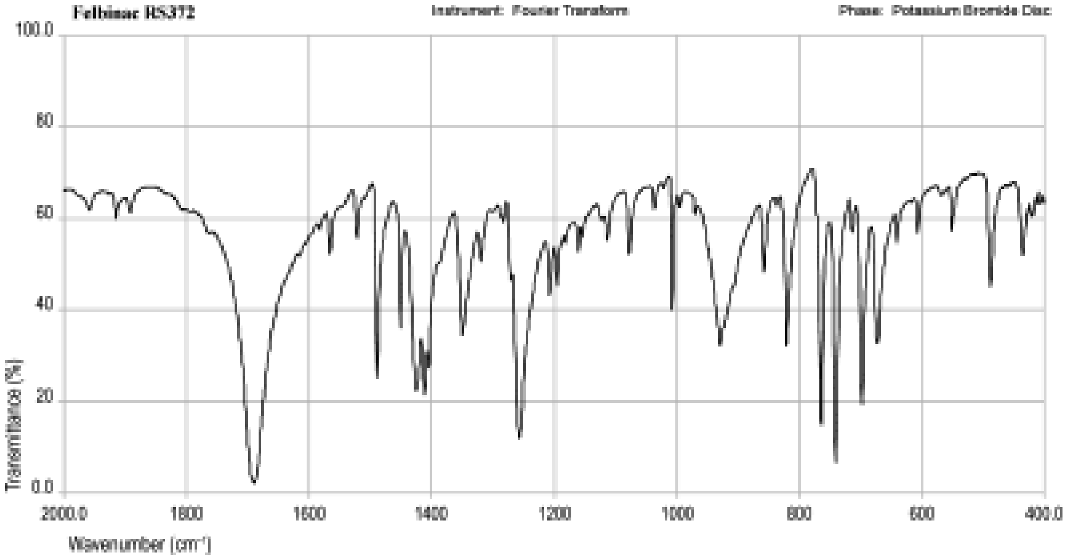
Etoposide


Etoposide RS396
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

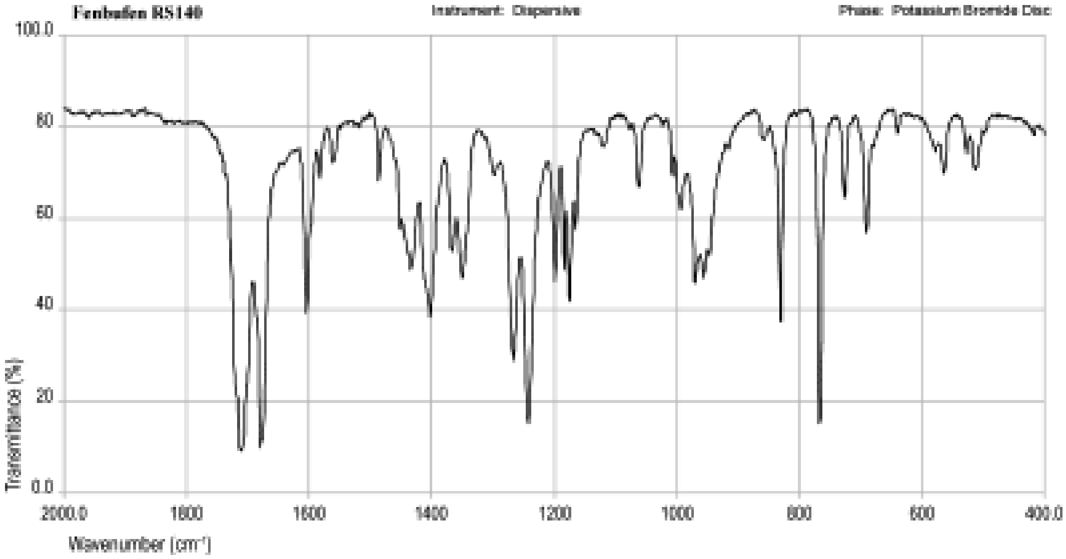
Etynodiol Diacetate



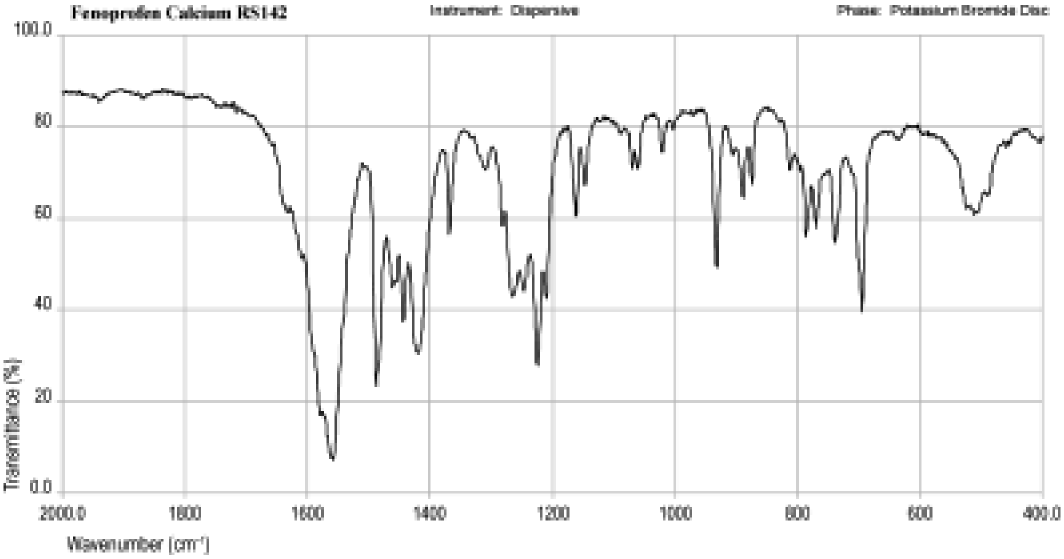
Felbinac


Felbinac RS372
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

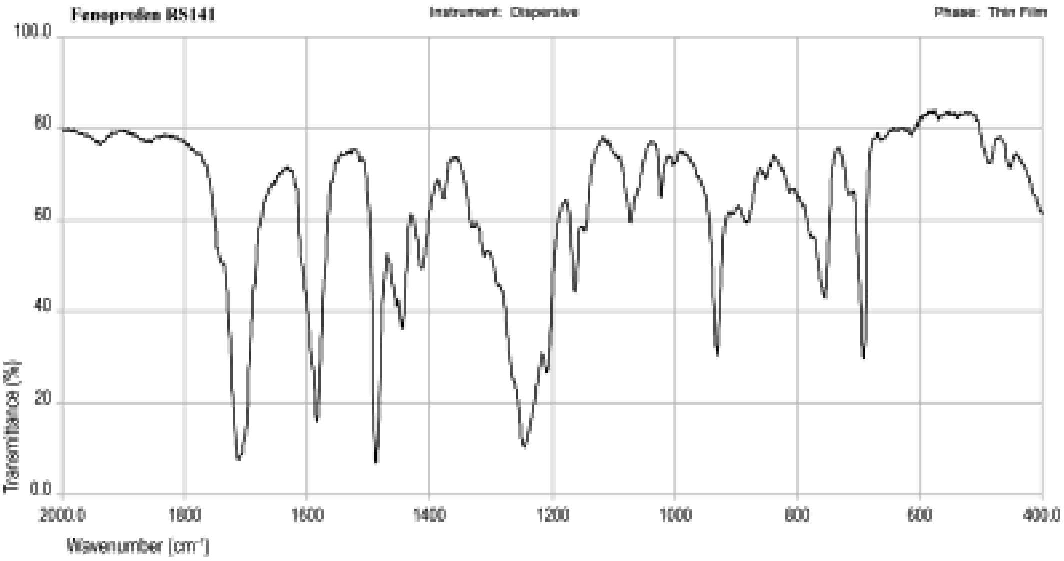
Fenbufen


Fenbufen RS140
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

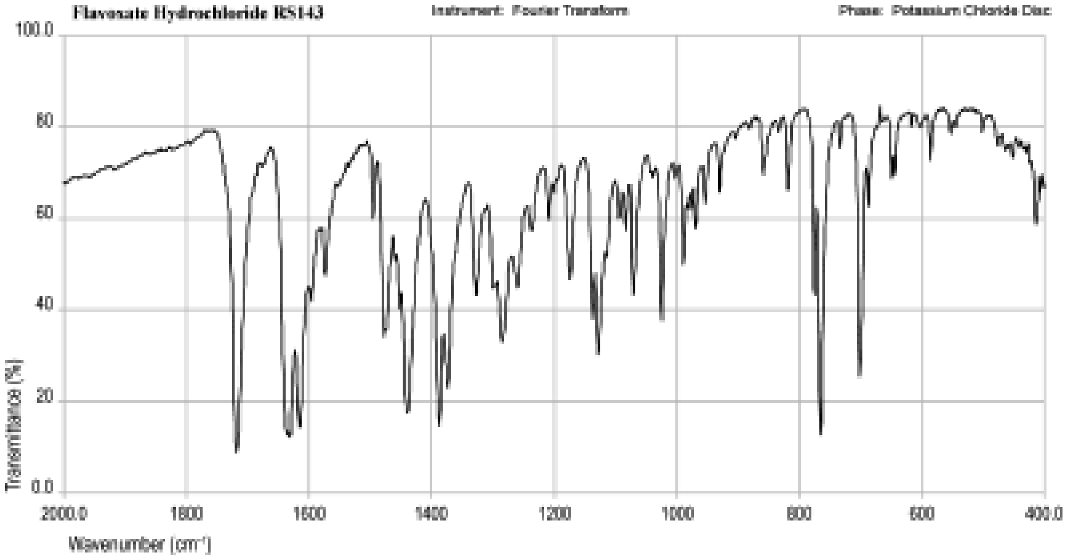
Fenoprofen Calcium



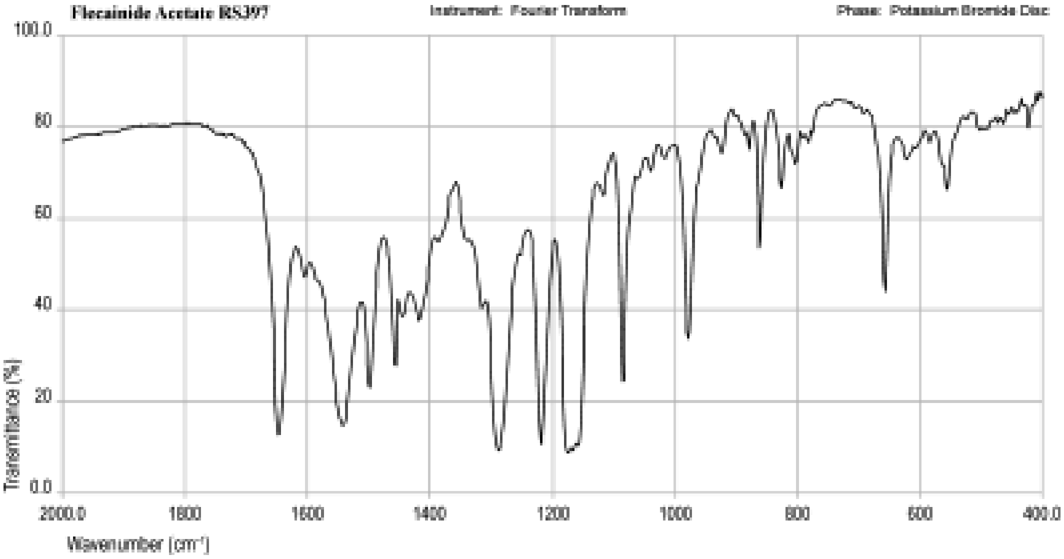
Fenoprofen


Fenoprofen RS141
Instrument: Dispersive
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

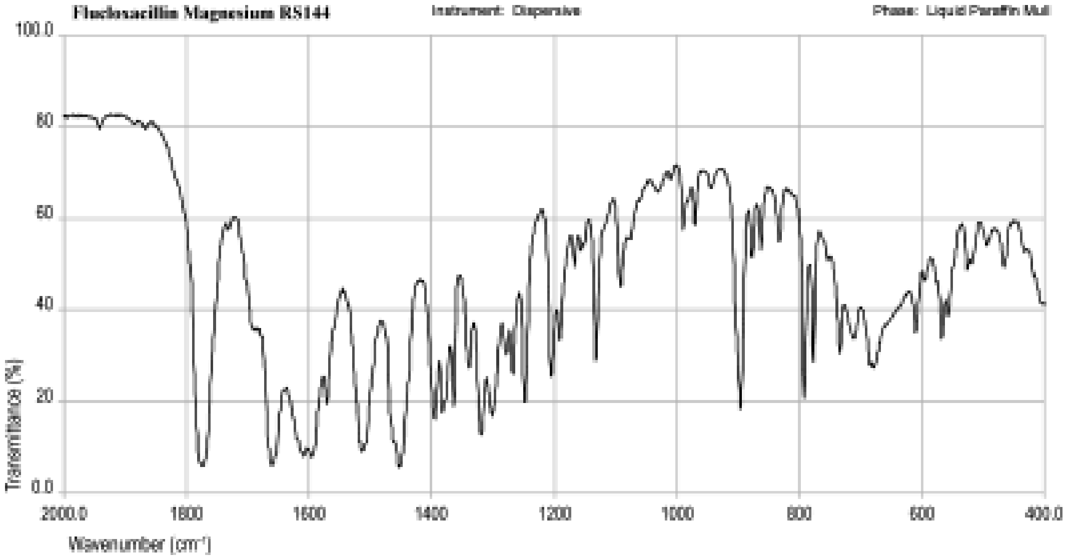
Flavoxate Hydrochloride



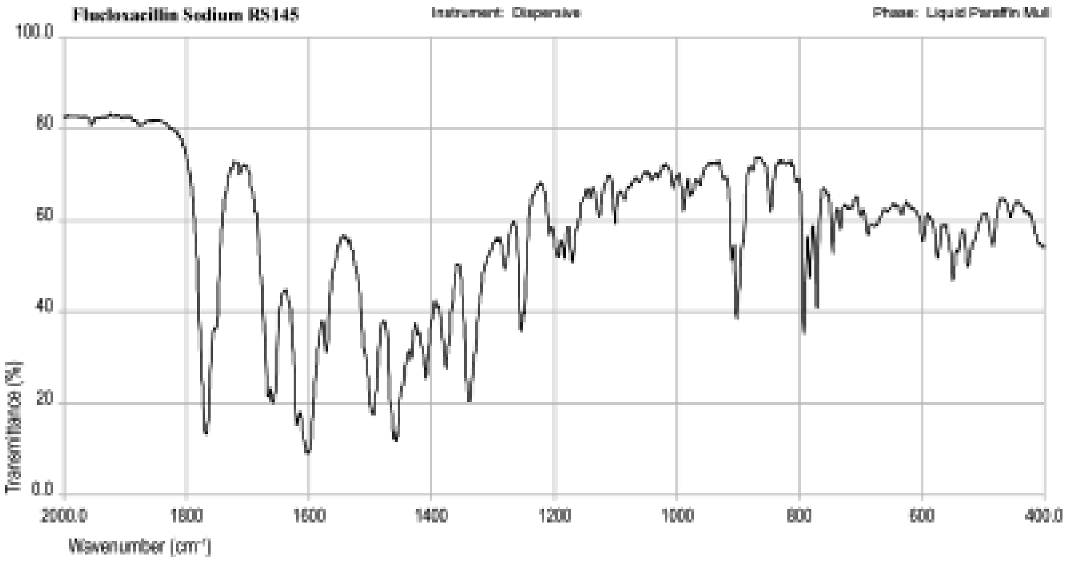
Flecainide Acetate



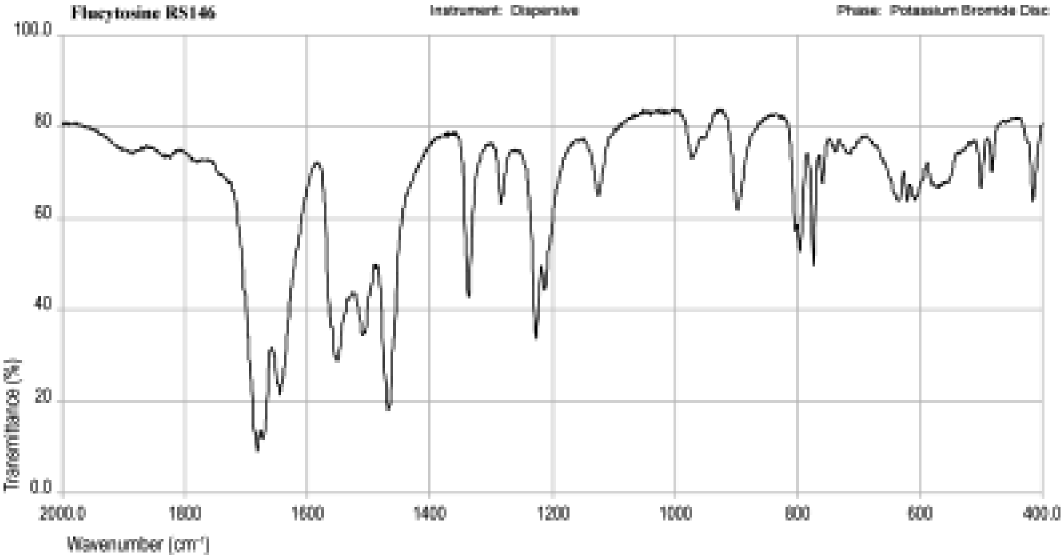
Flucloxacillin Magnesium



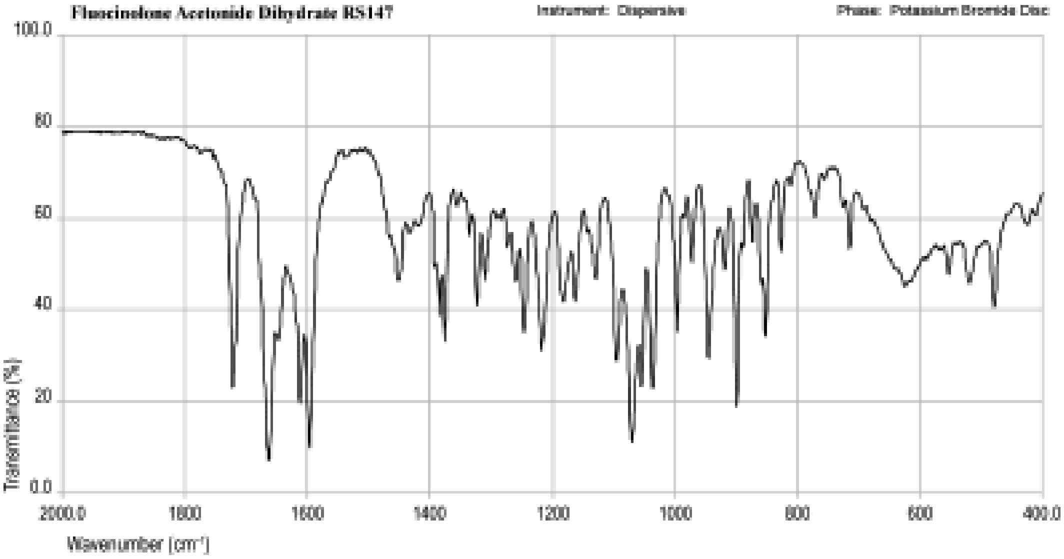
Flucloxacillin Sodium



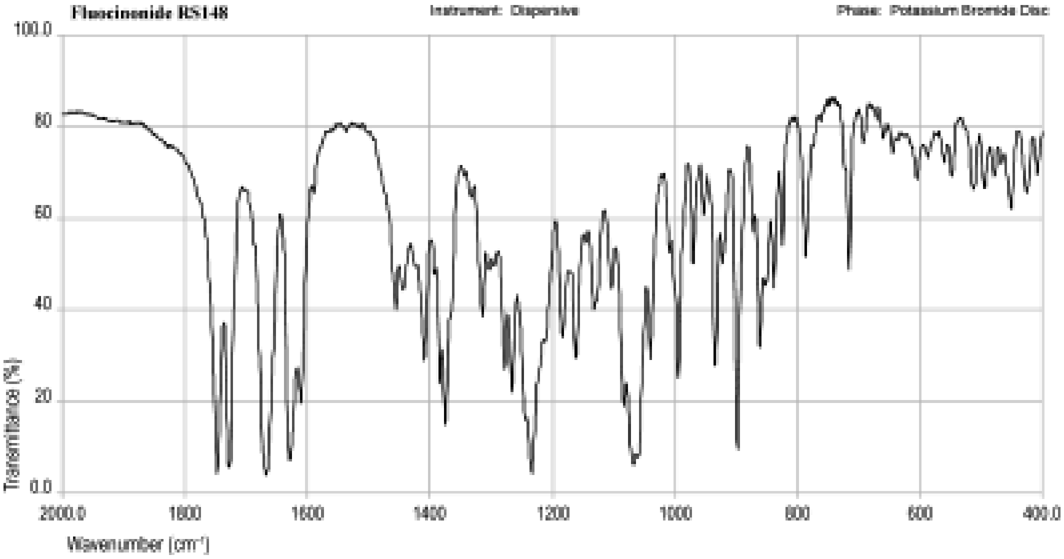
Flucytosine



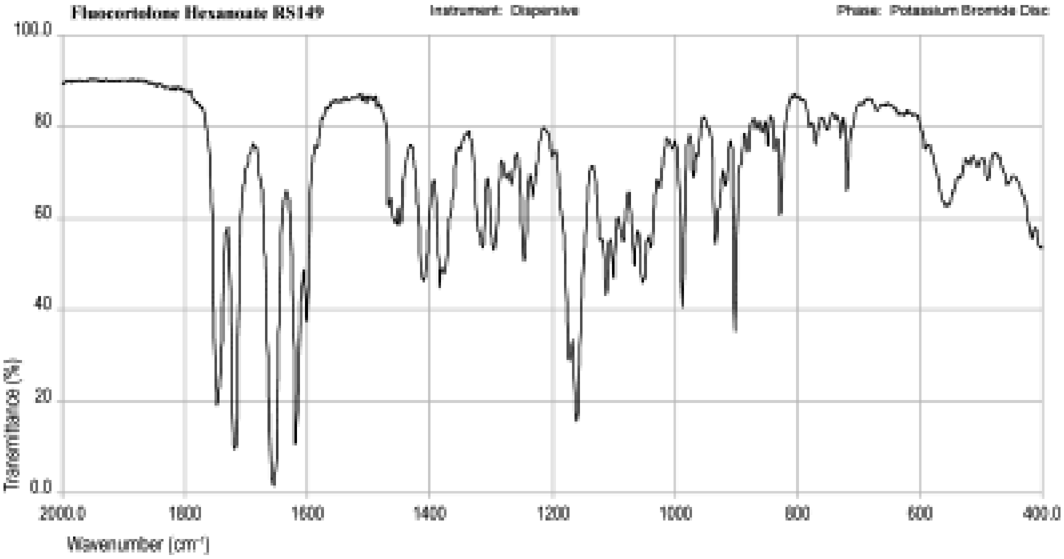
Fluocinolone Acetonide Dihydrate



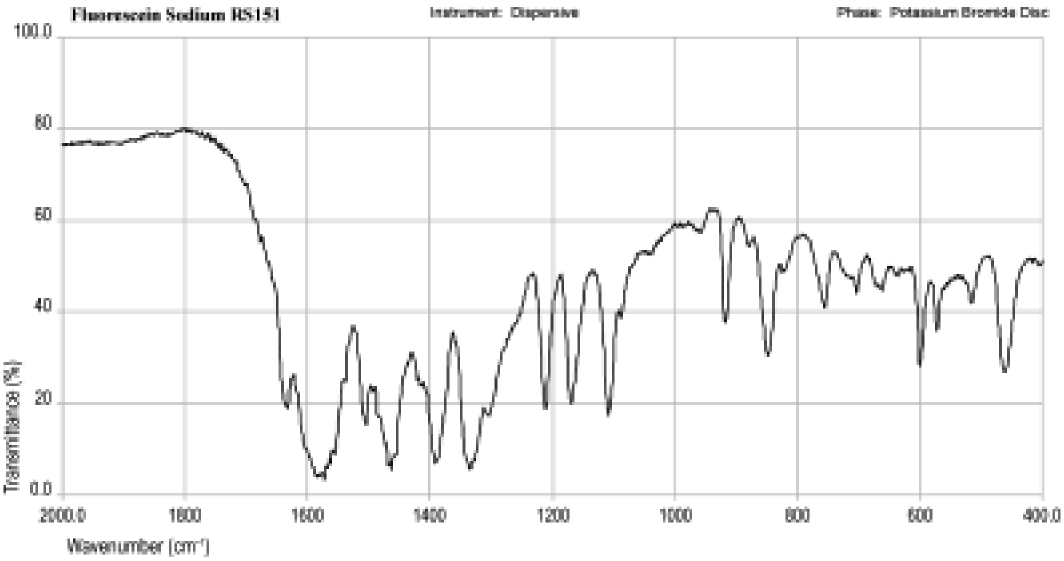
Fluocinonide



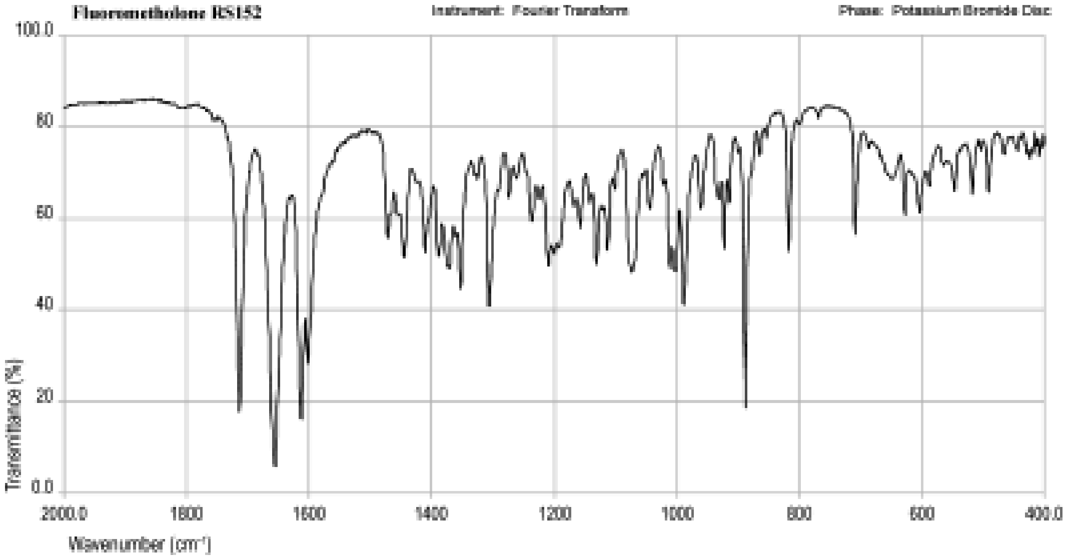
Fluocortolone Hexanoate



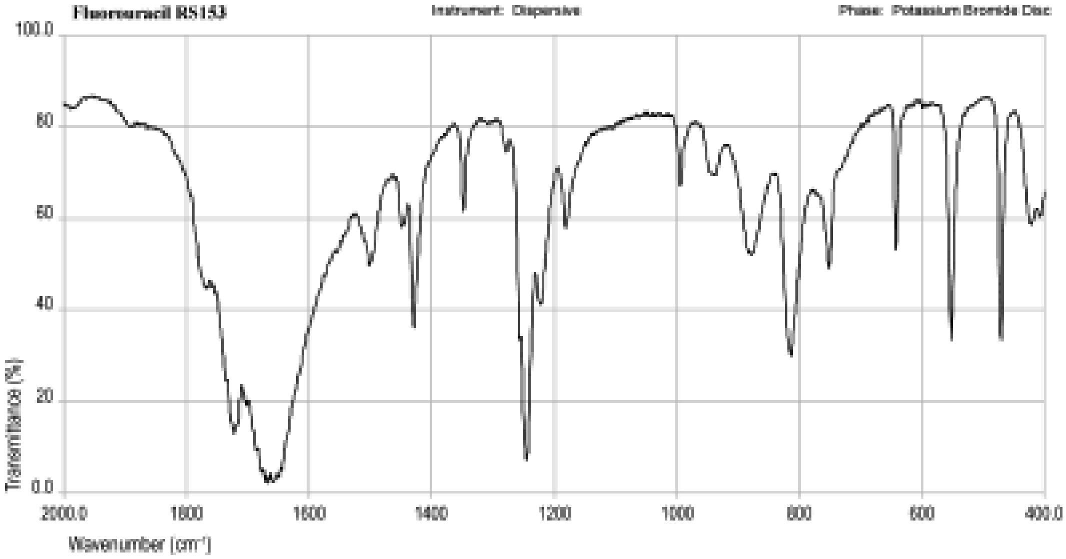
Fluorescein Sodium



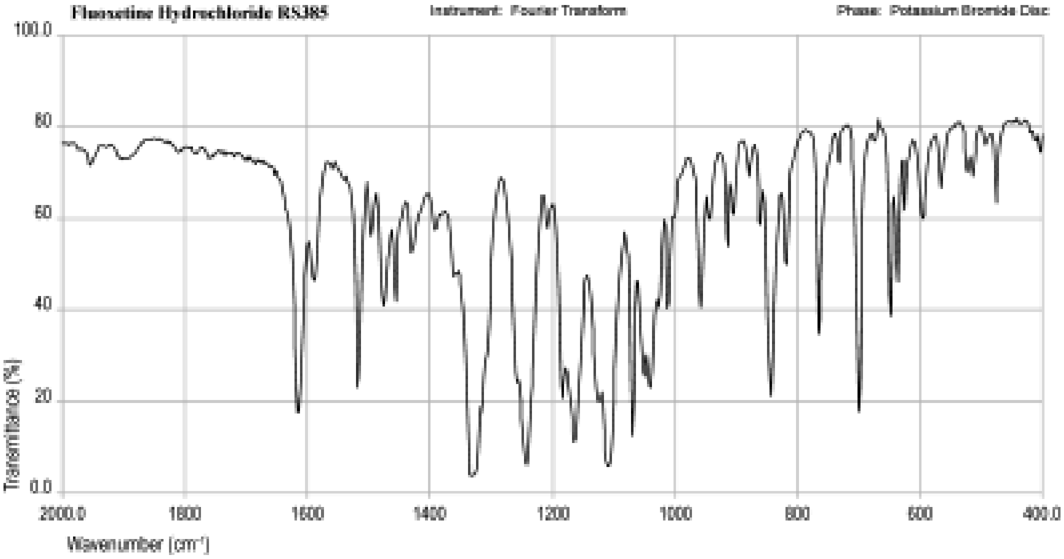
Fluorometholone



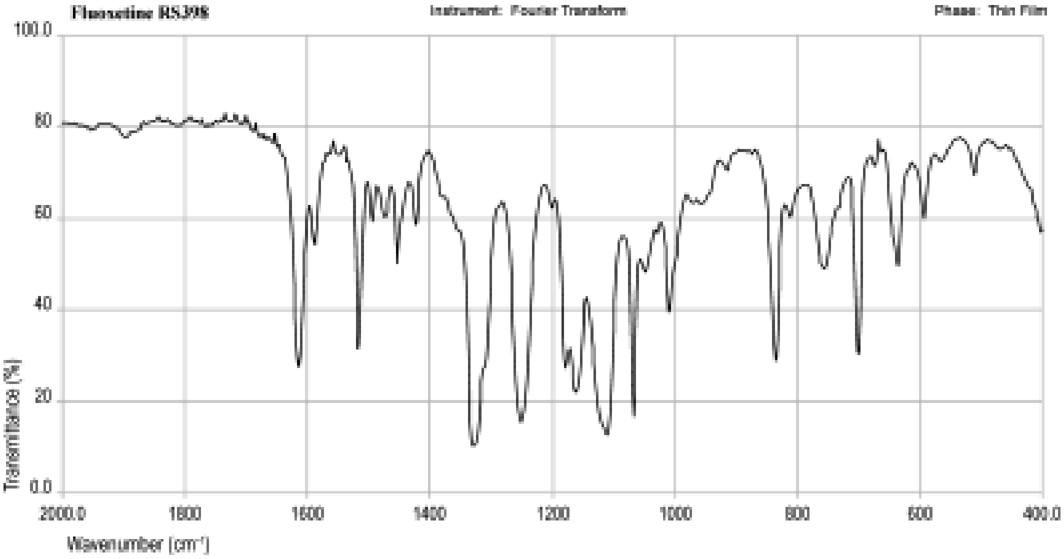
Fluorouracil



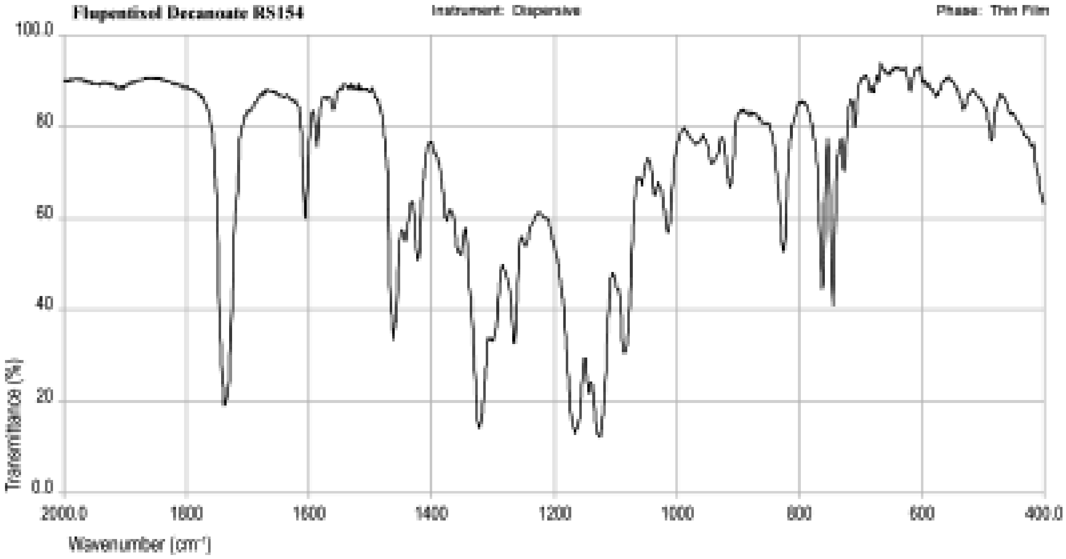
Fluoxetine Hydrochloride



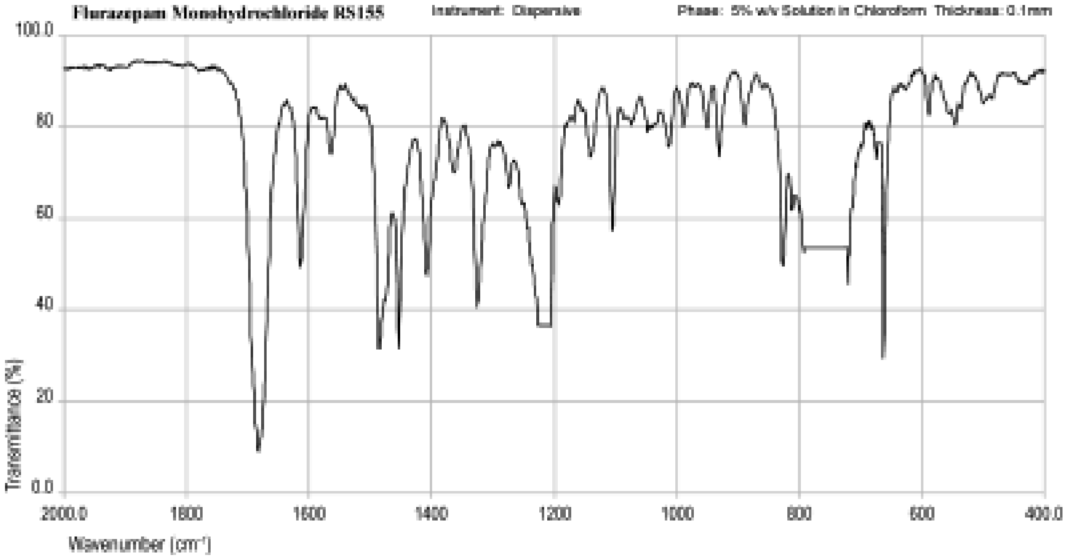
Fluoxetine


Fluoxetine RS398
Instrument: Fourier Transform
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

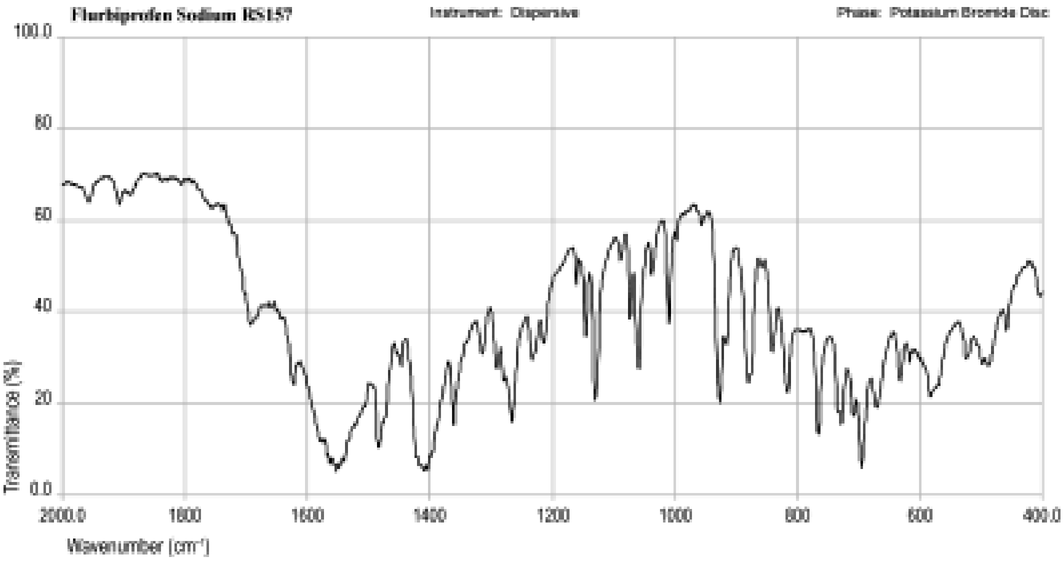
Flupentixol Decanoate



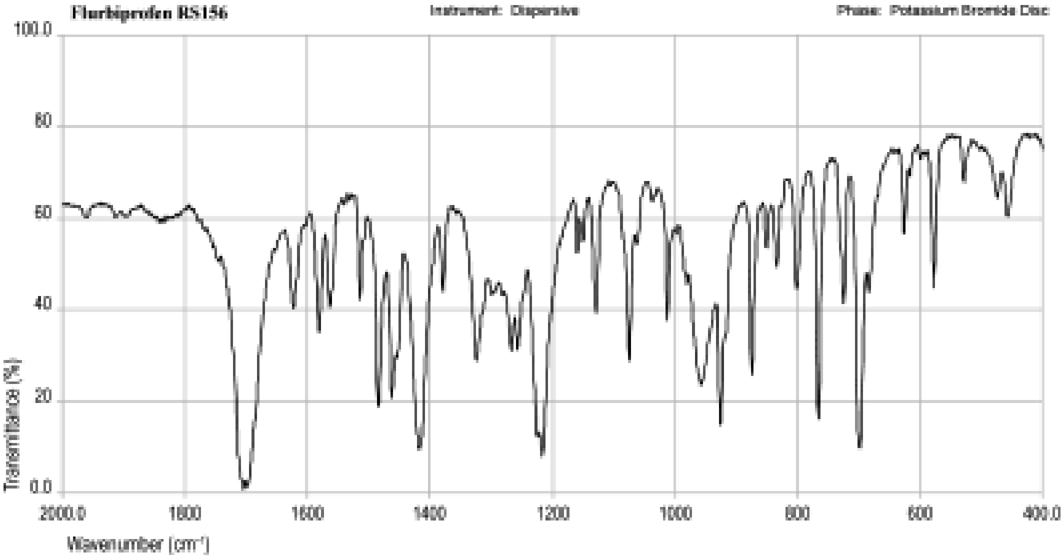
Flurazepam Monohydrochloride



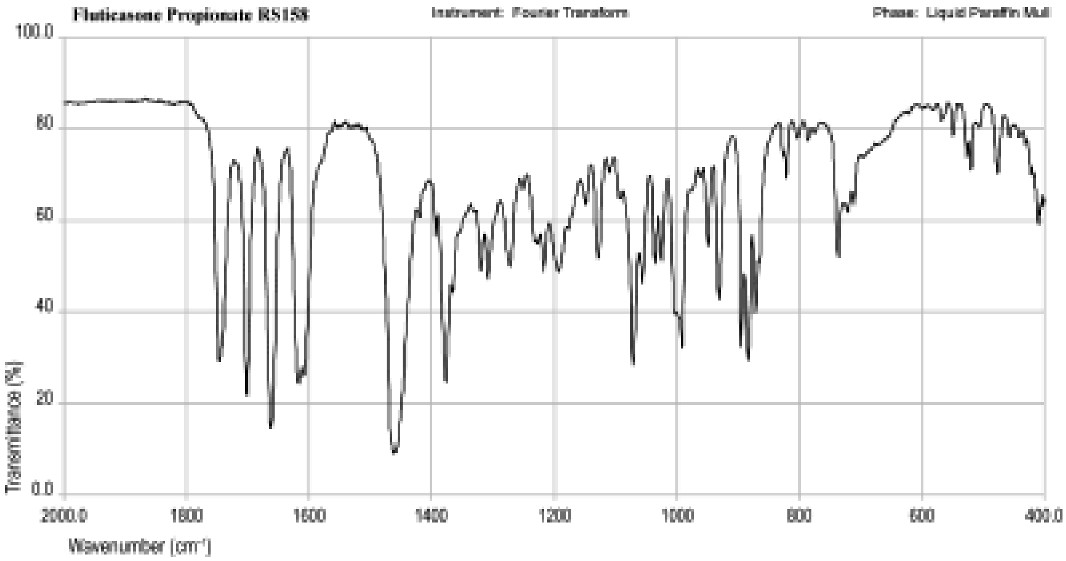
Flurbiprofen Sodium



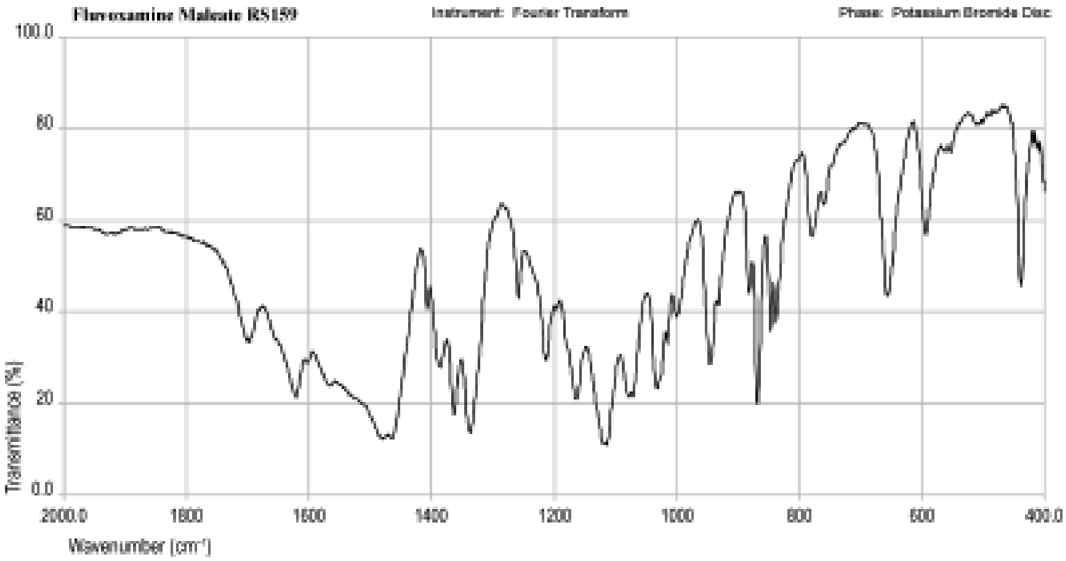
Flurbiprofen



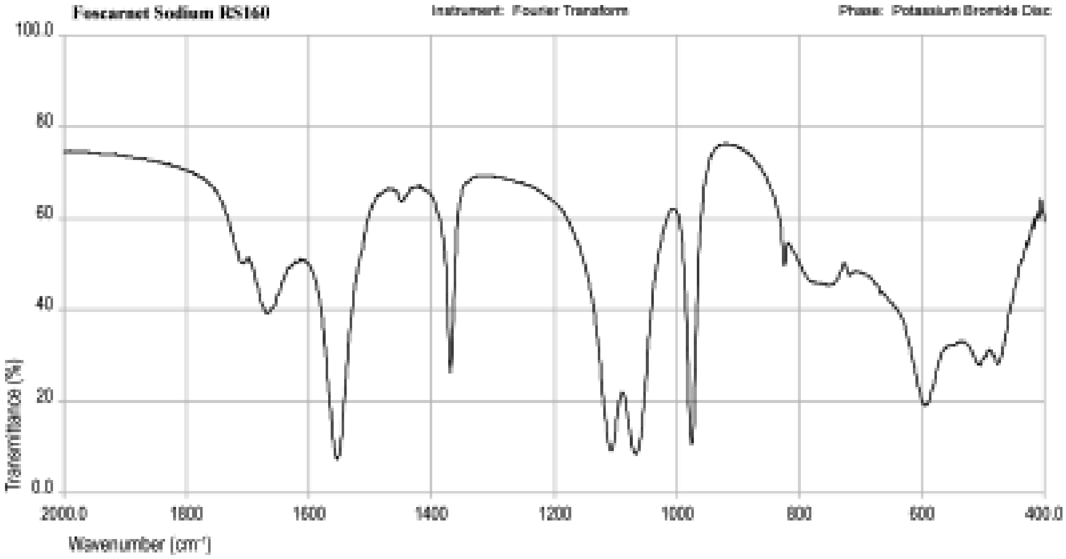
Fluticasone Propionate



Fluvoxamine Maleate



Foscarnet Sodium

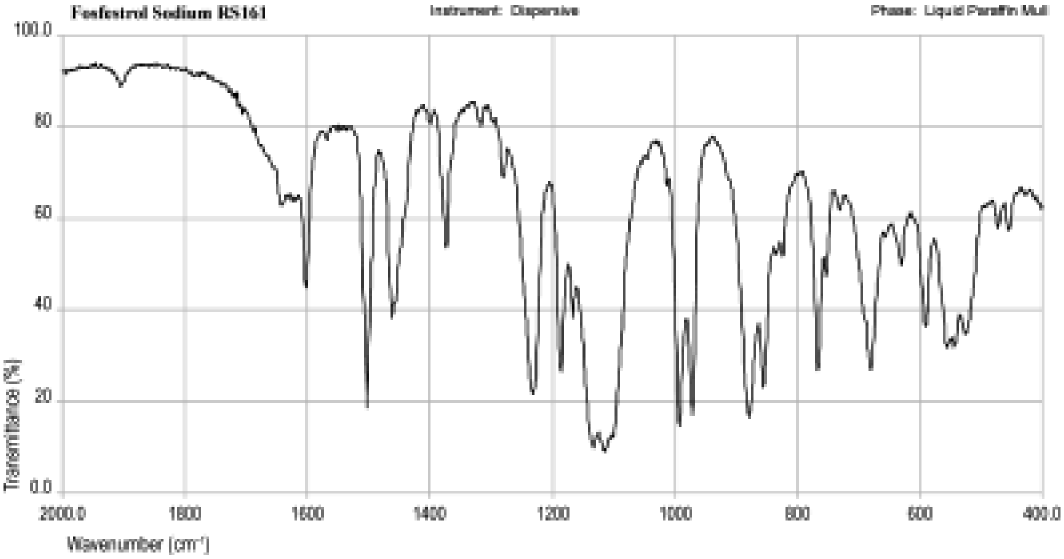


Foscarnet Sodium RS160

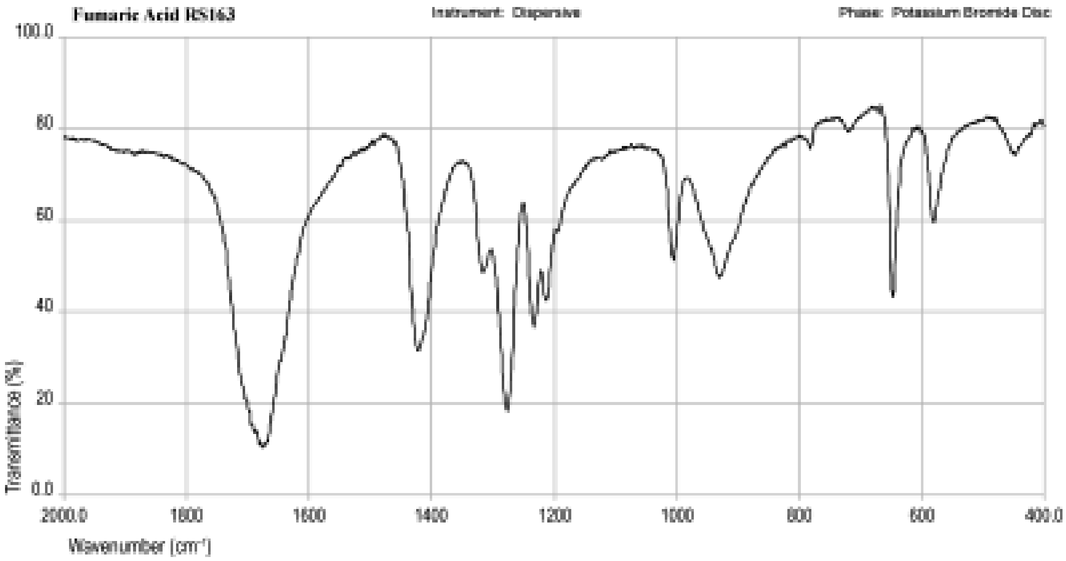
Instrument: Fourier Transform

Phase: Potassium Bromide Disc

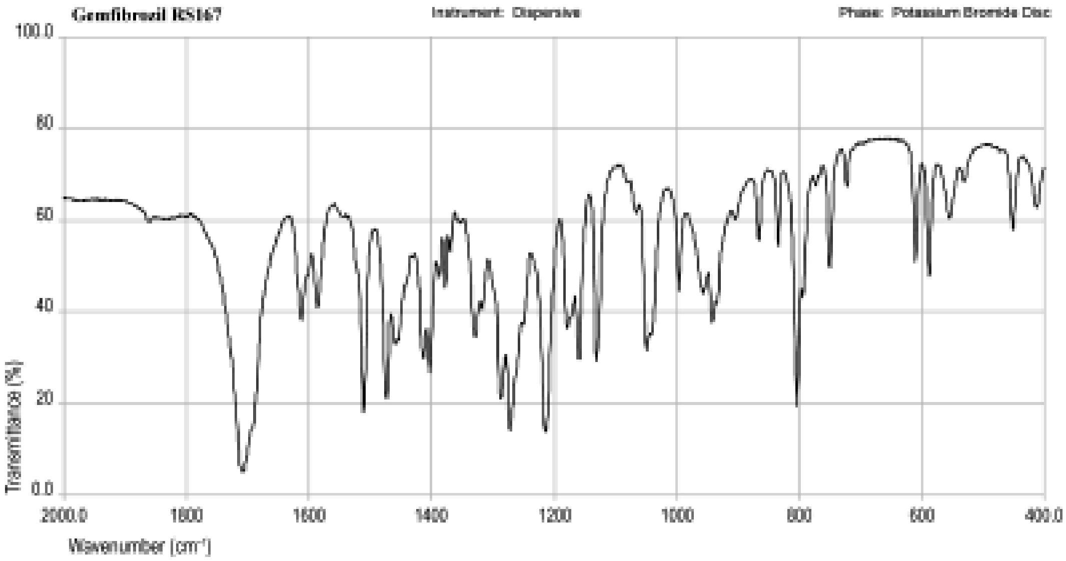
Fosfestrol Sodium



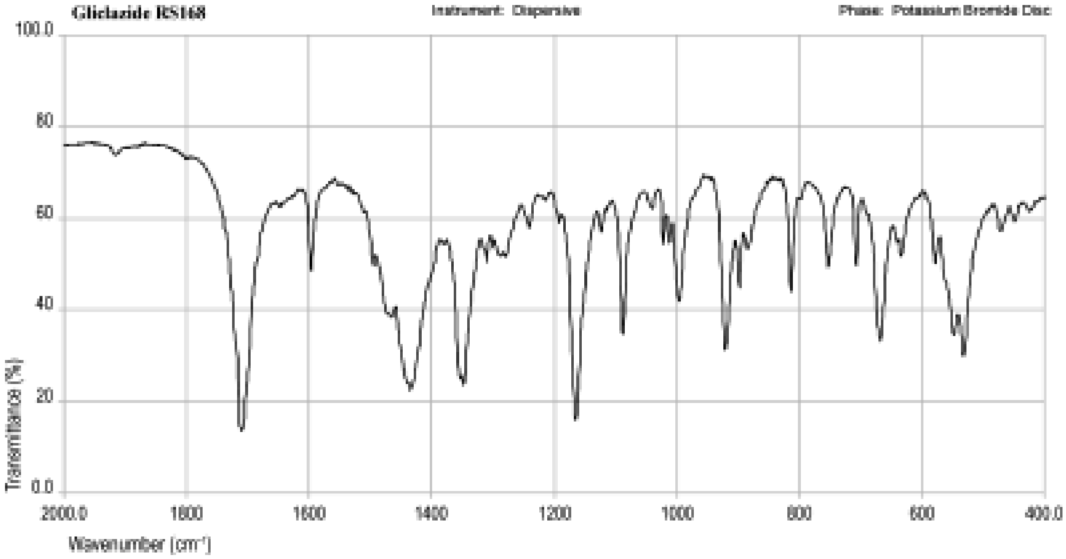
Fumaric Acid



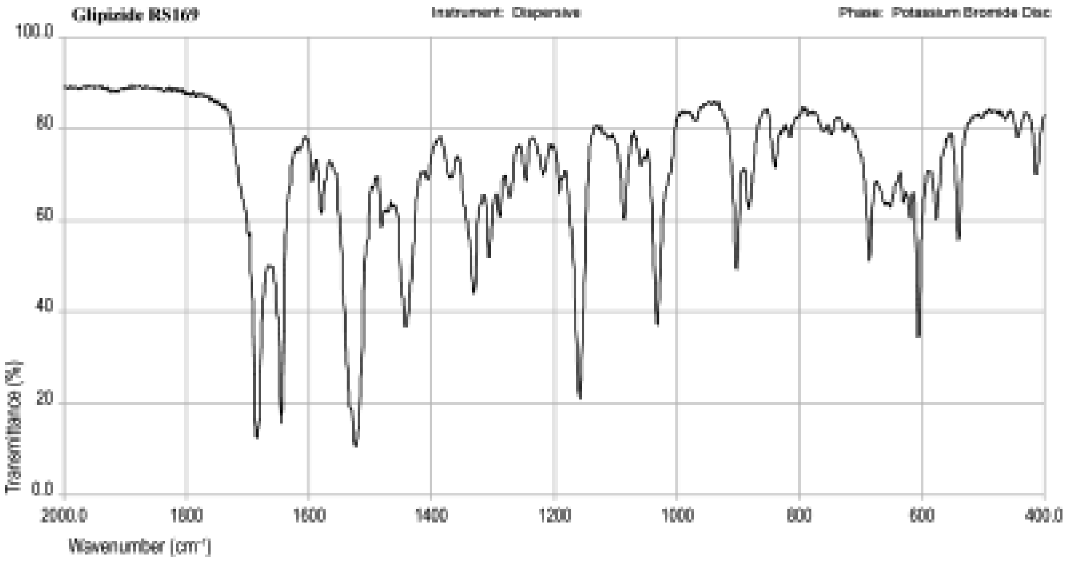
Gemfibrozil



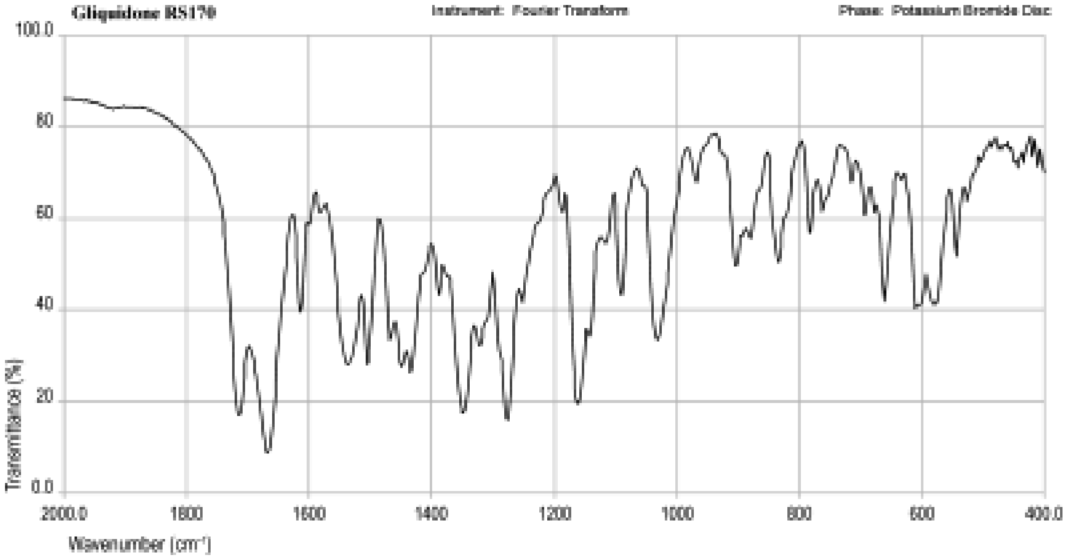
Gliclazide


Gliclazide RS168
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

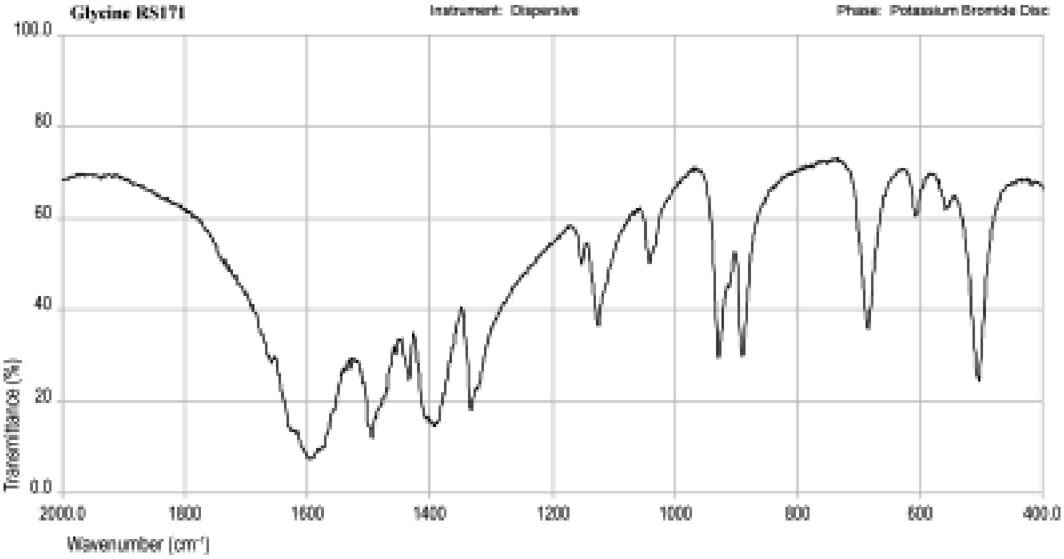
Glipizide


Glipizide RS169
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

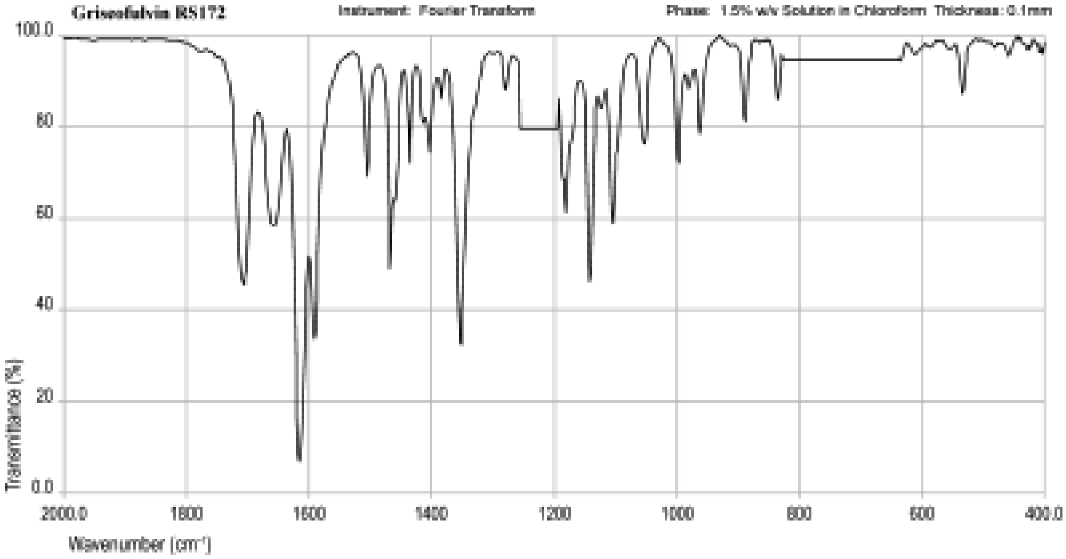
Gliquidone


Gliquidone RS170
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

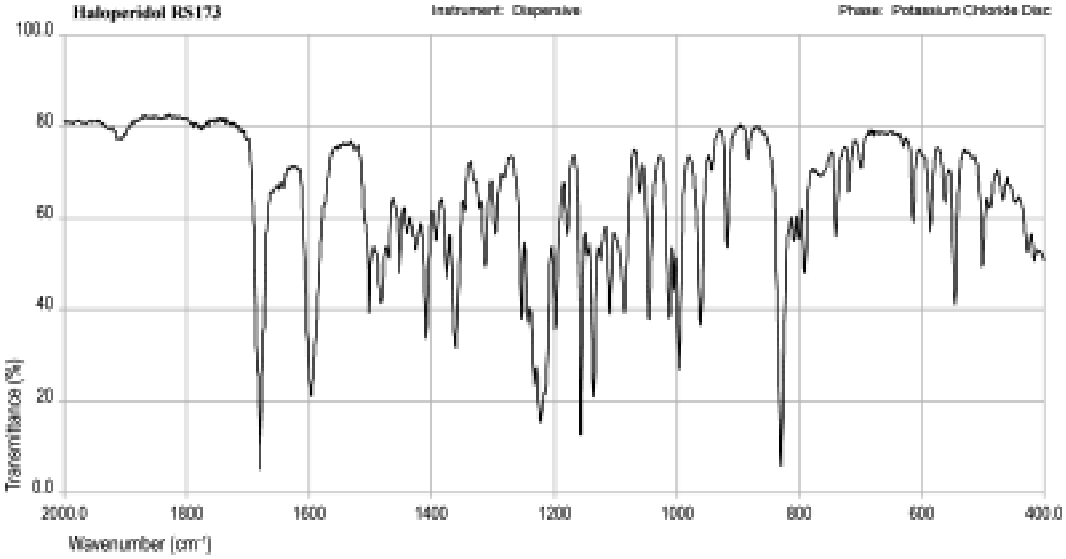
Glycine


Glycine RS171
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

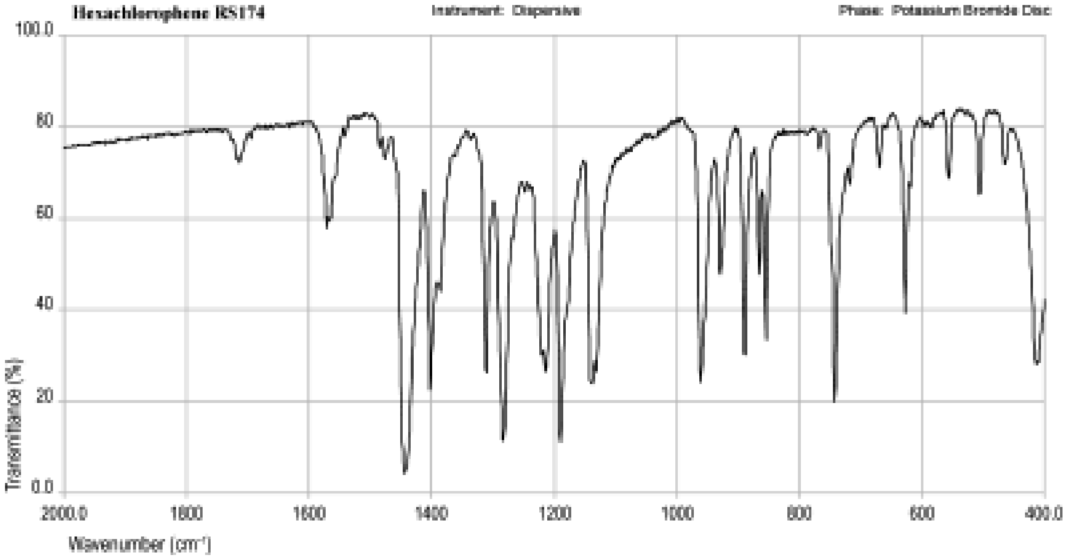
Griseofulvin



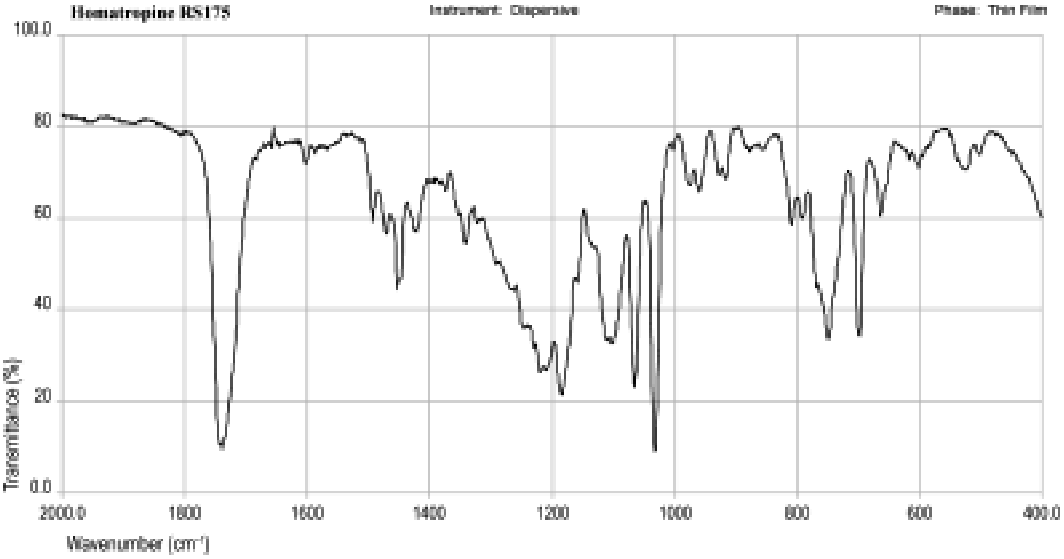
Haloperidol



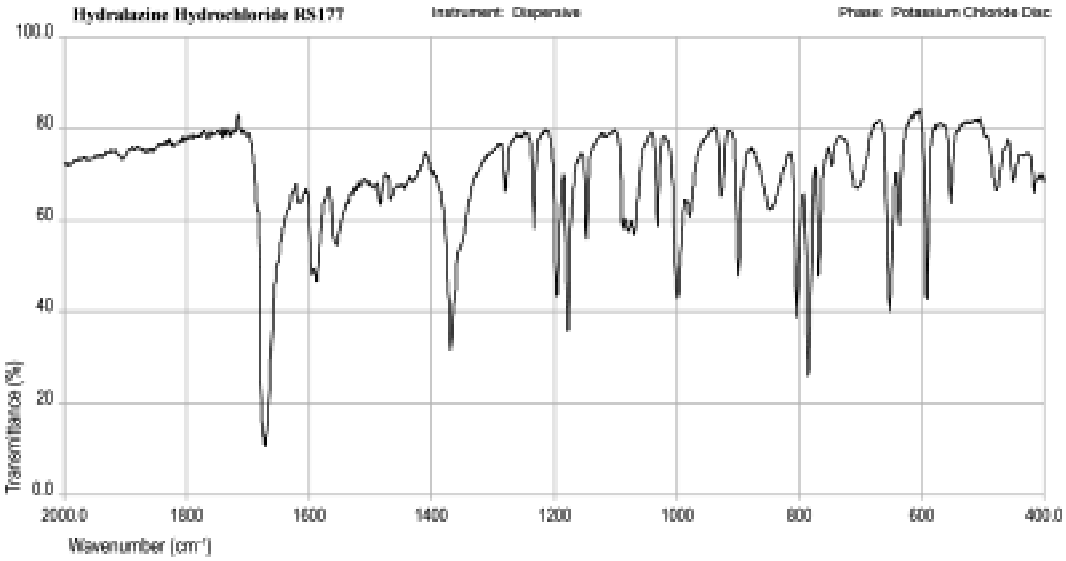
Hexachlorophene



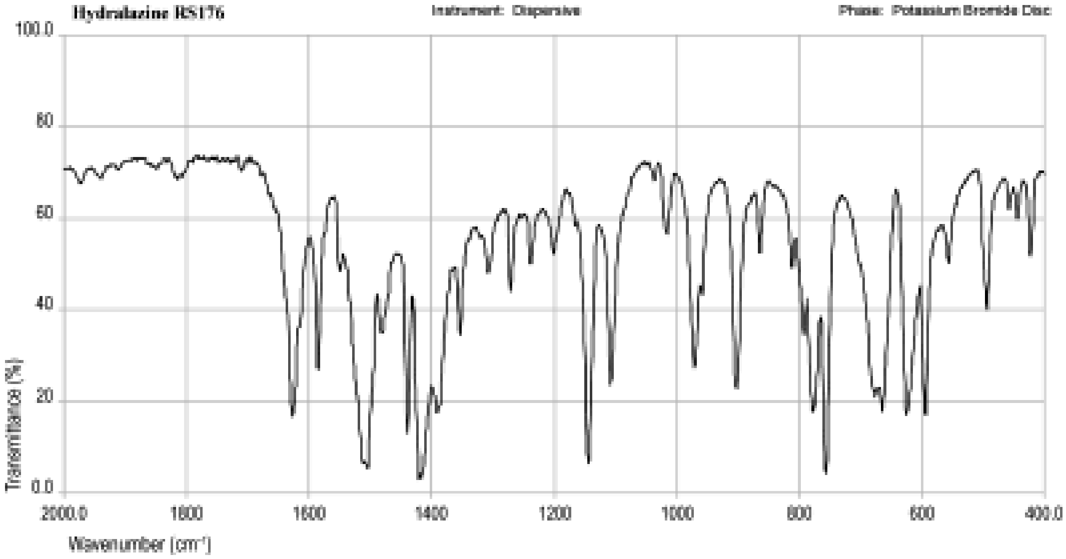
Homatropine



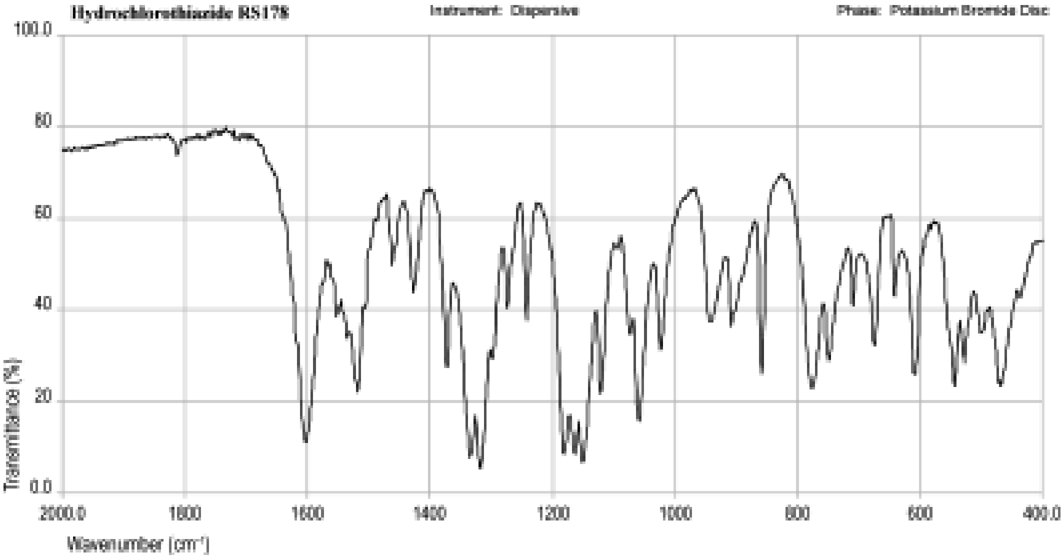
Hydralazine Hydrochloride



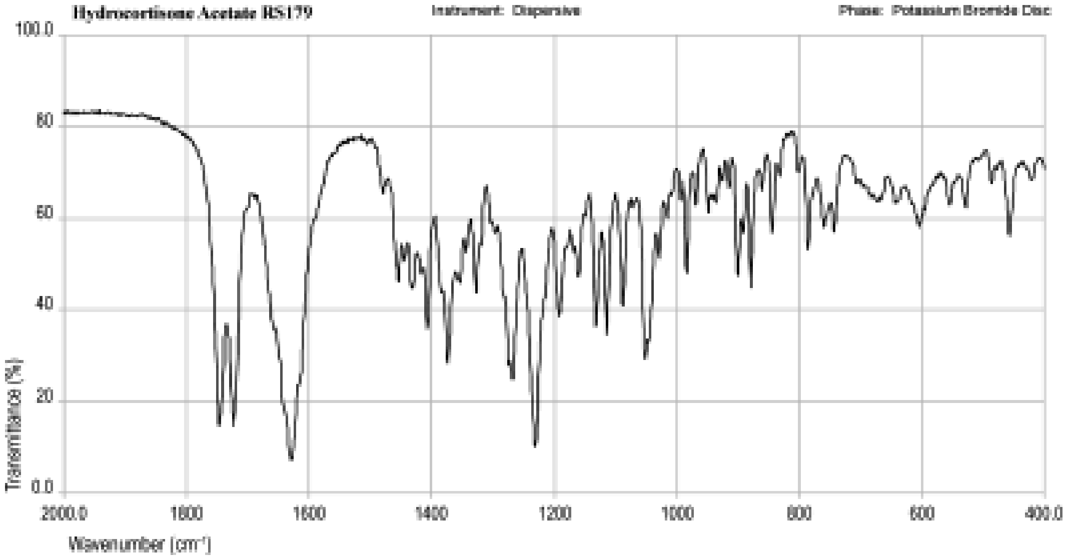
Hydralazine



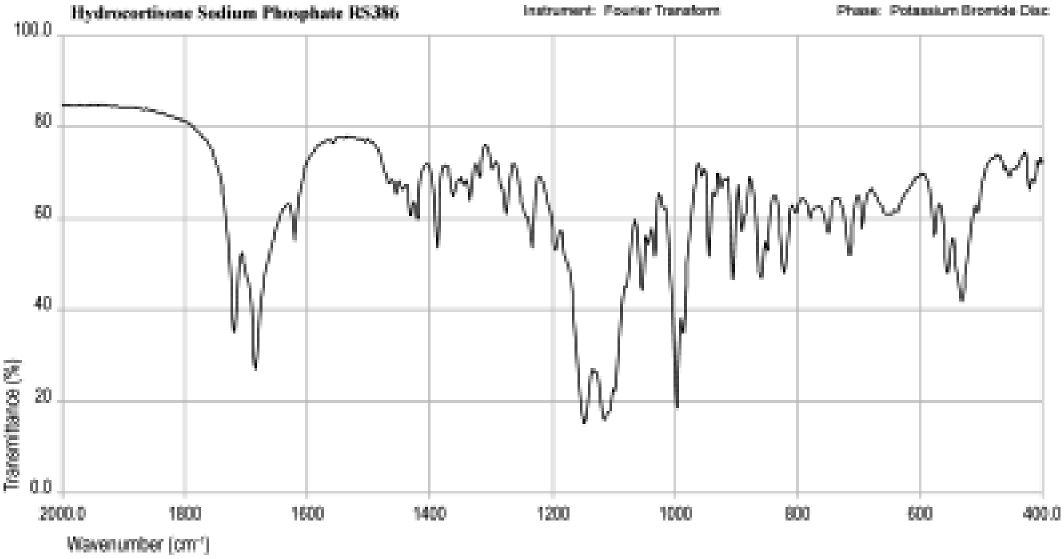
Hydrochlorothiazide



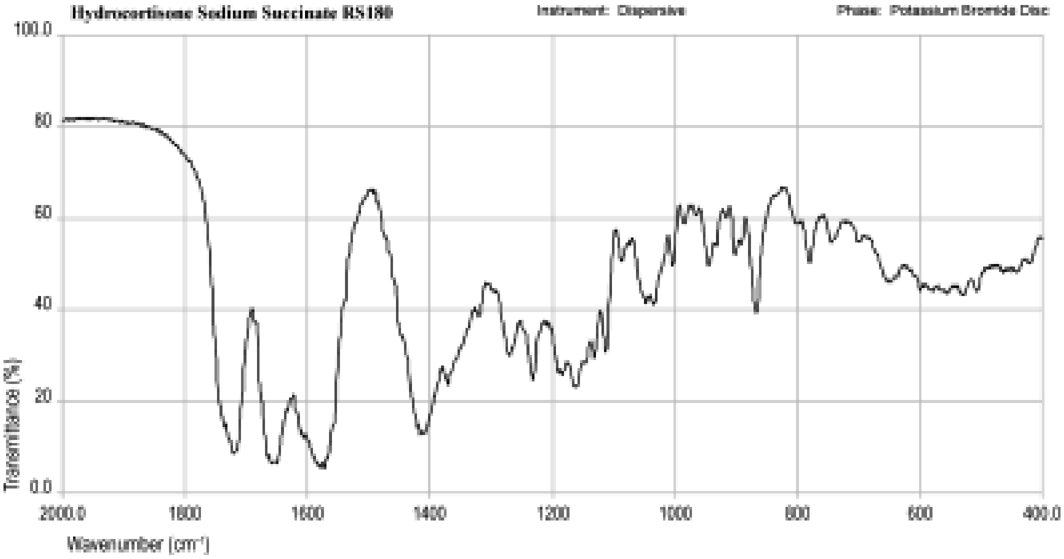
Hydrocortisone Acetate



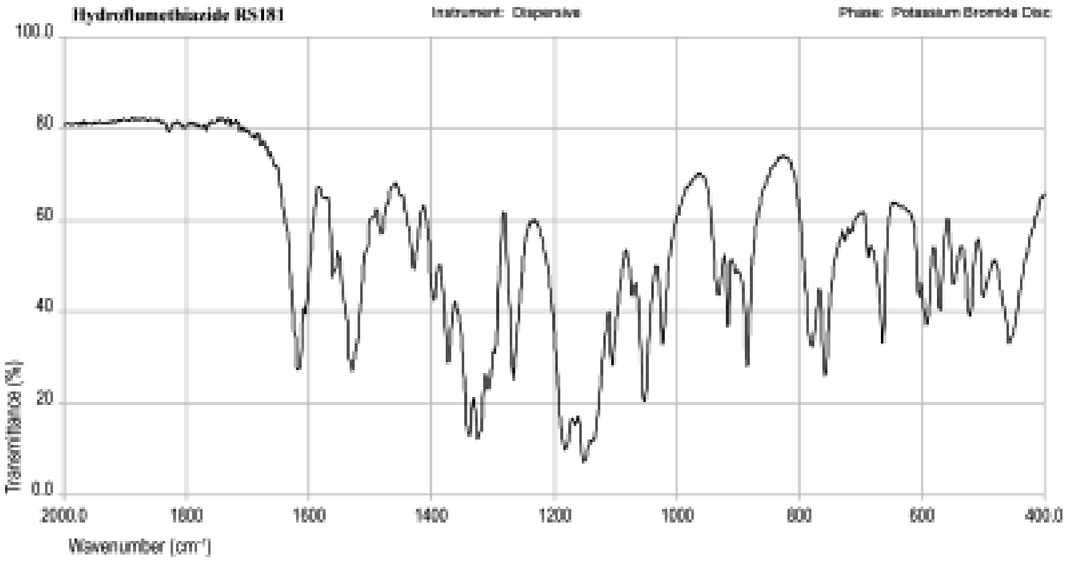
Hydrocortisone Sodium Phosphate



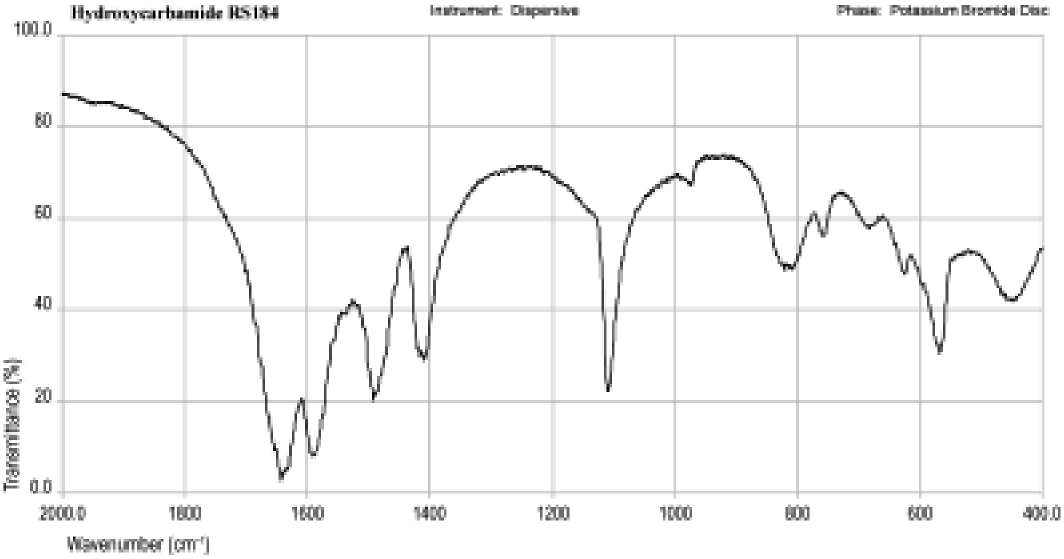
Hydrocortisone Sodium Succinate



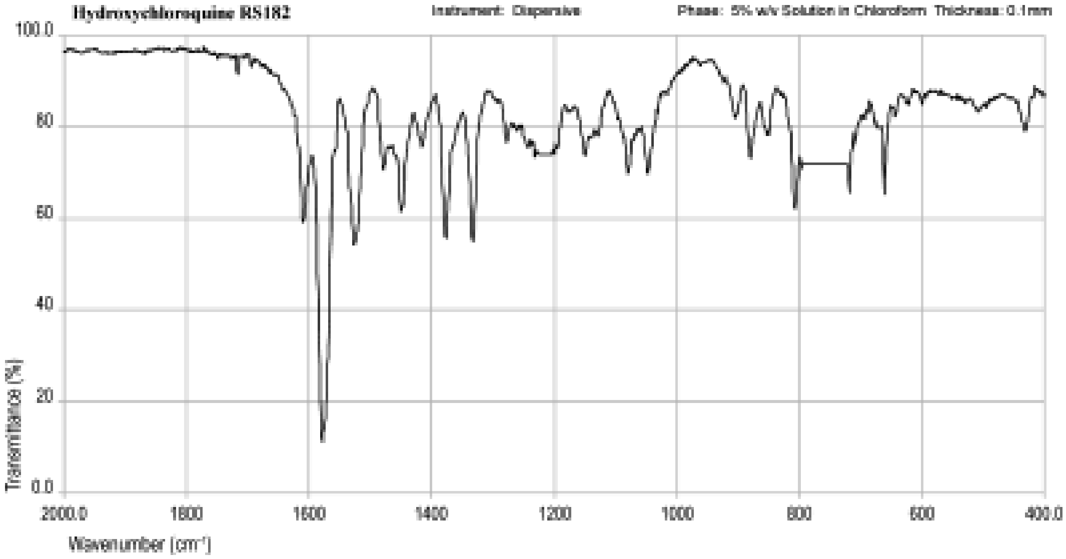
Hydroflumethiazide



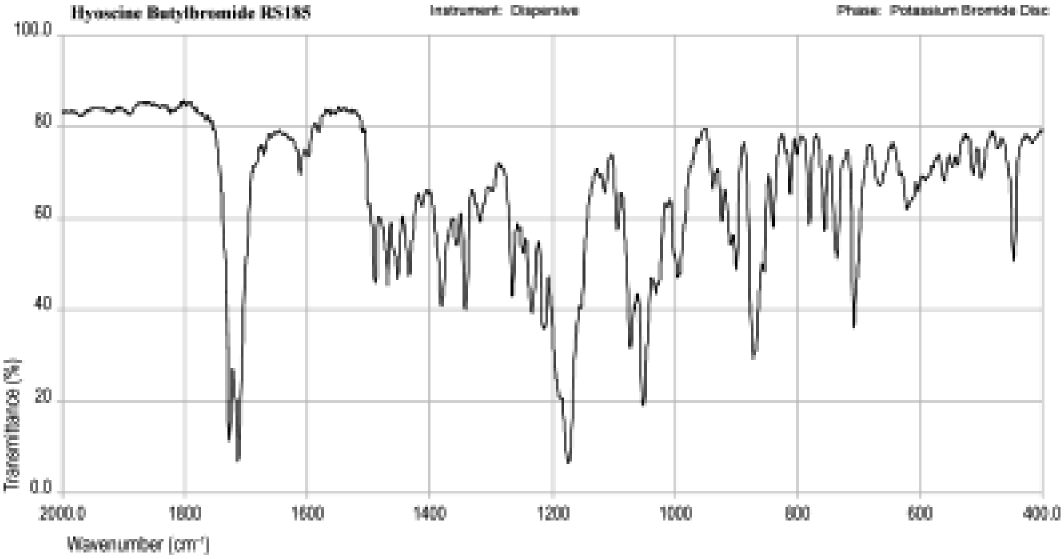
Hydroxycarbamide



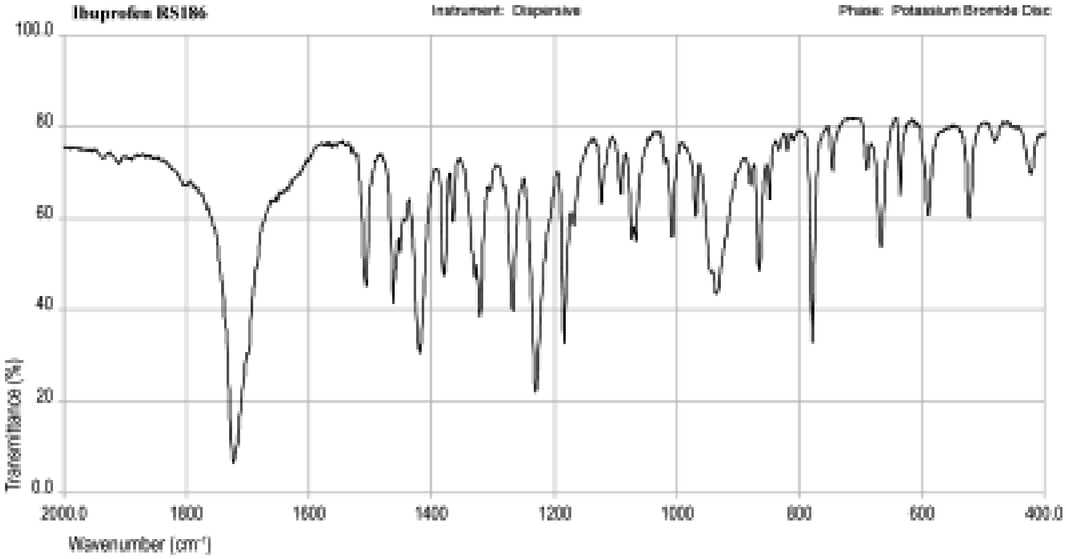
Hydroxychloroquine



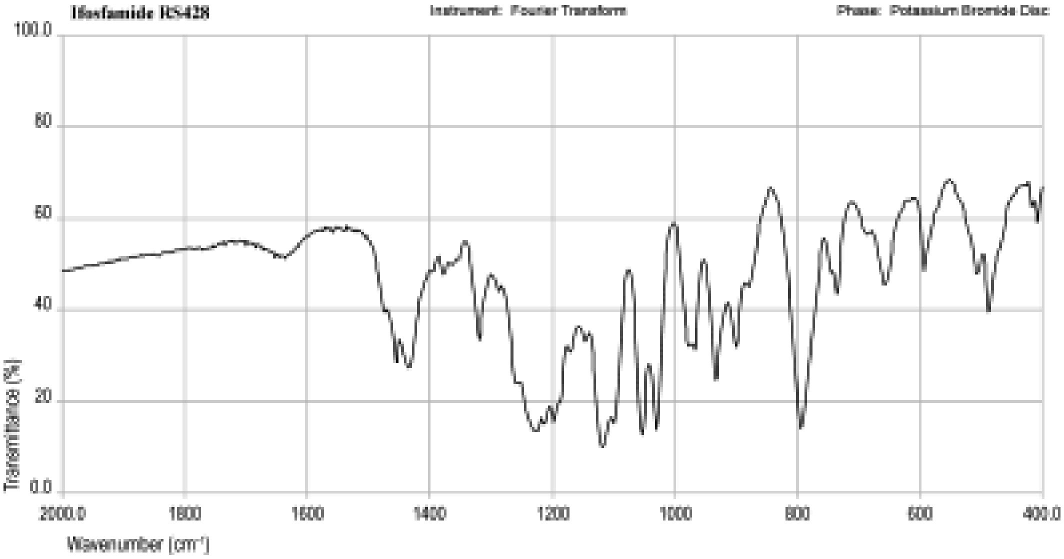
Hyoscine Butylbromide



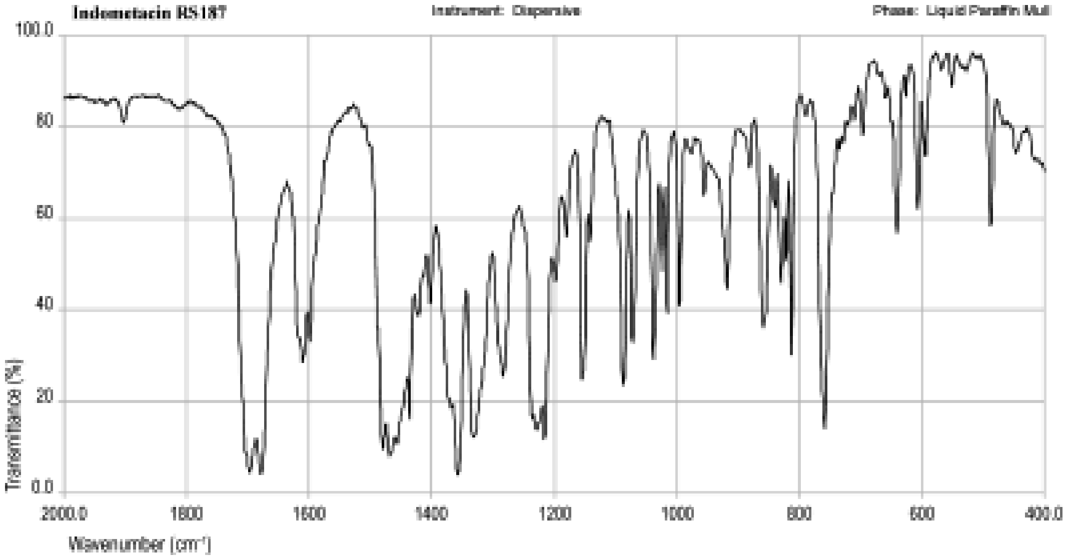
Ibuprofen


Ibuprofen RS186
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

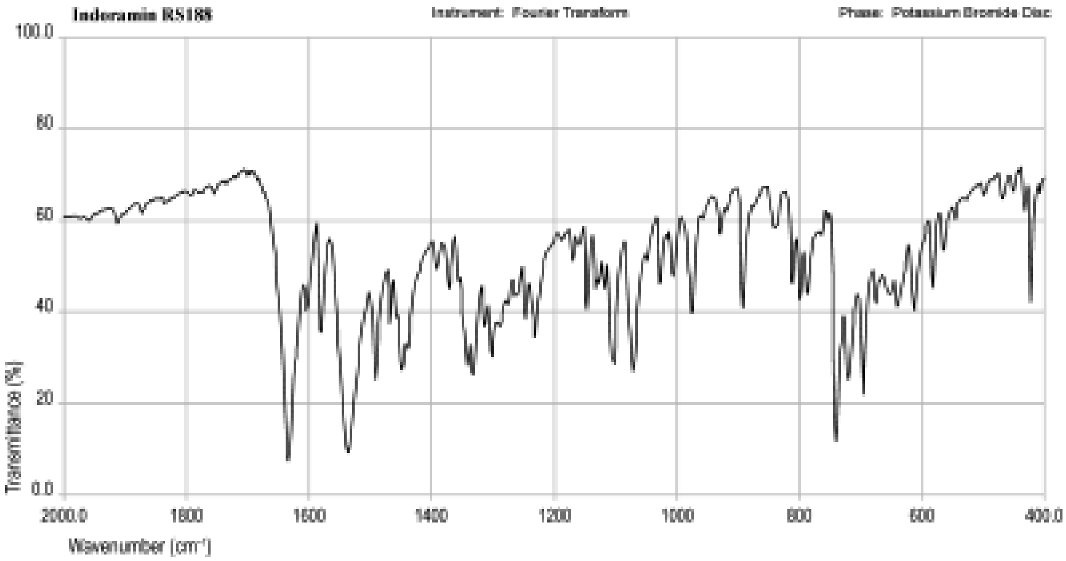
Ifosfamide


Ifosfamide RS428
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

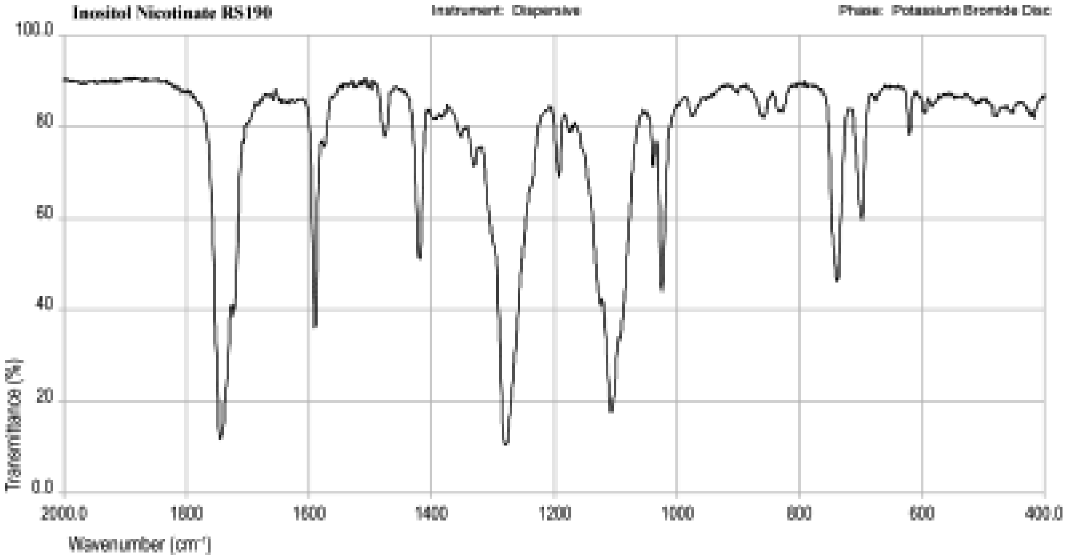
Indometacin



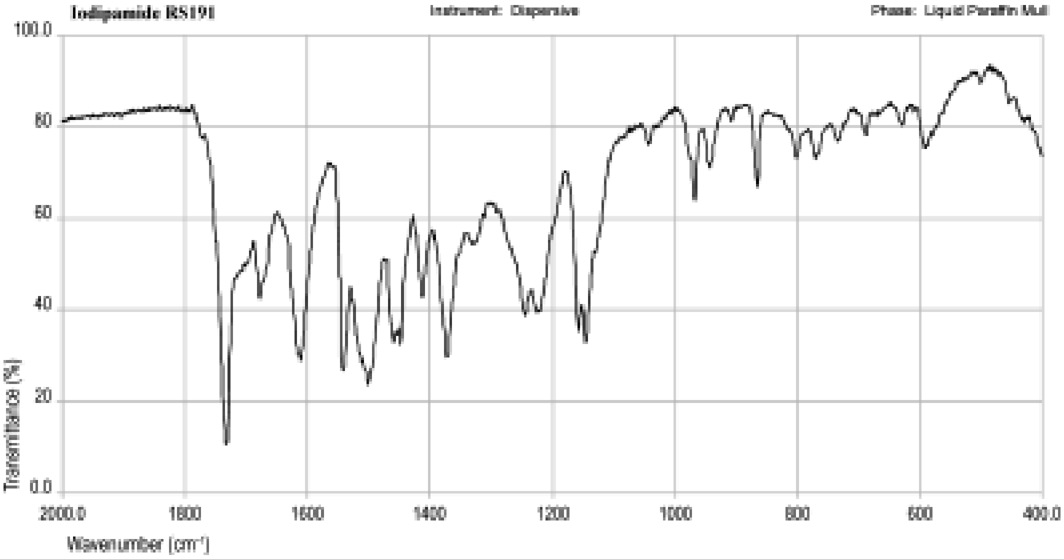
Indoramin


Indoramin RS188
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

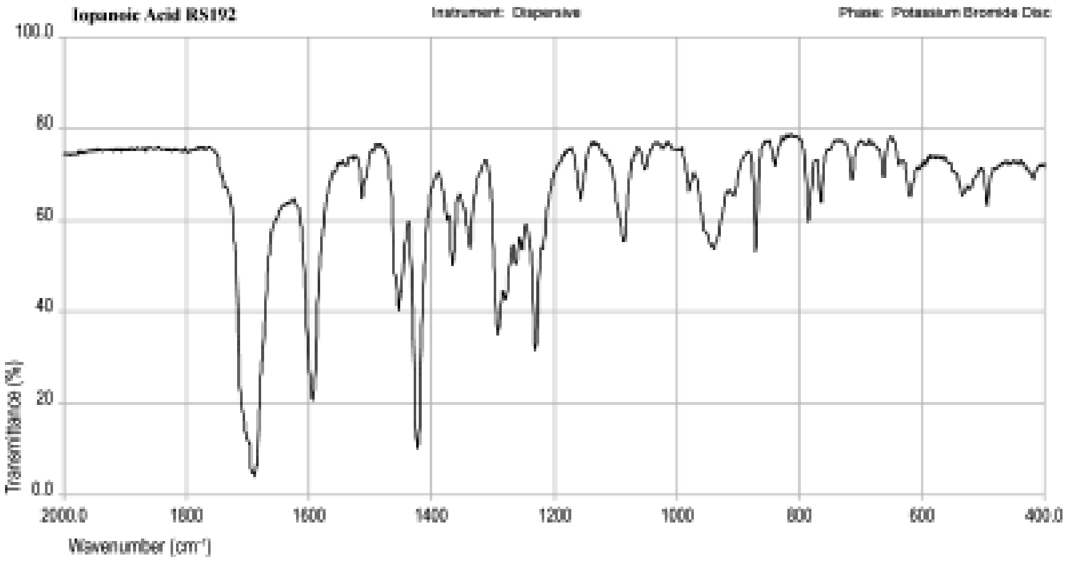
Inositol Nicotinate



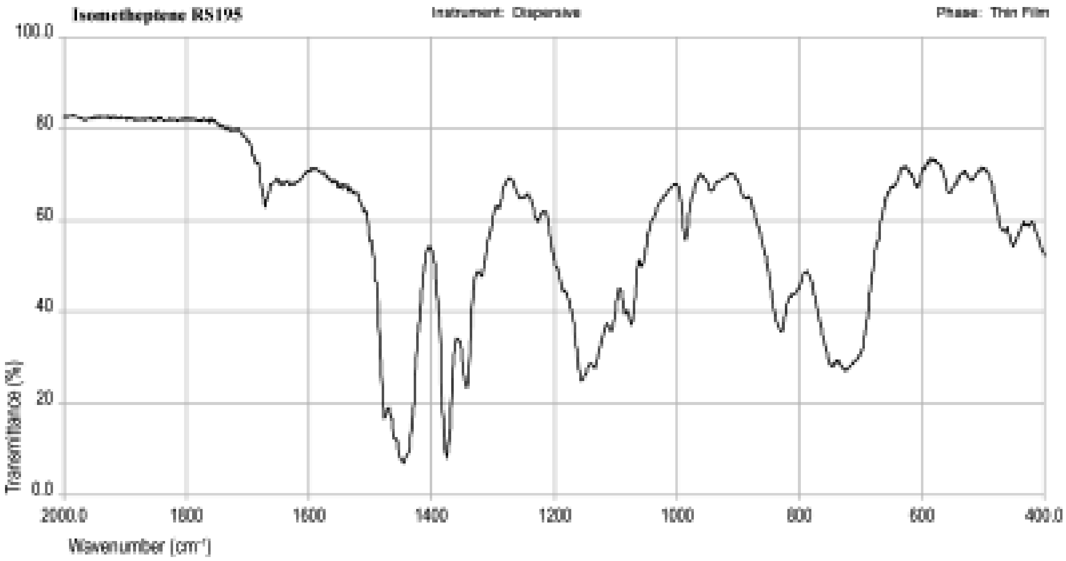
Iodipamide


Iodipamide RS191
Instrument: Dispersive
Phase: Liquid Paraffin Mull
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

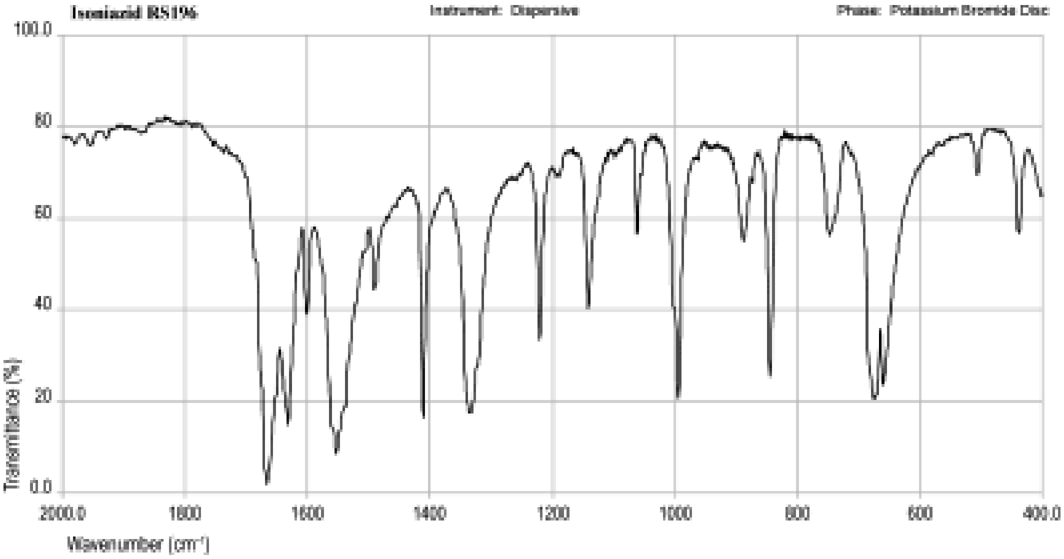
Iopanoic Acid



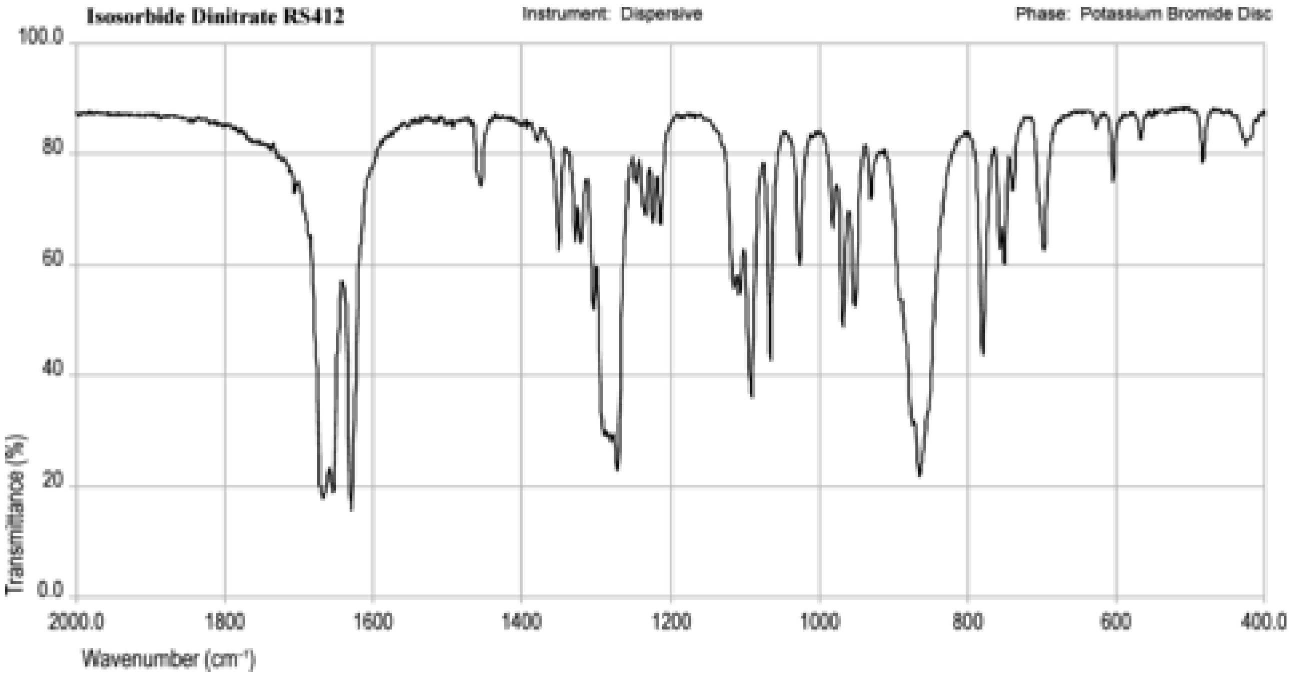
Isometheptene



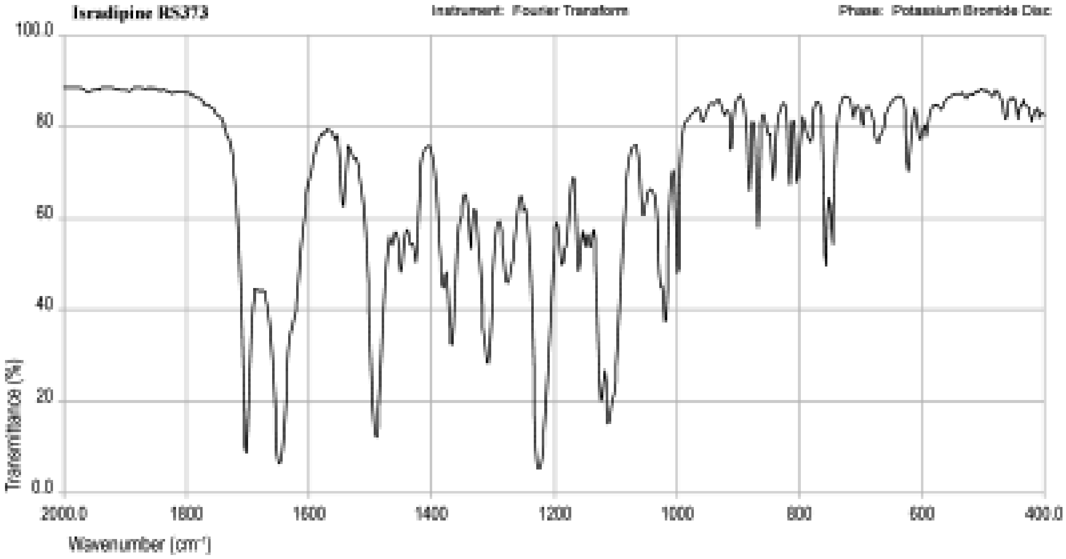
Isoniazid


Isoniazid RS196
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

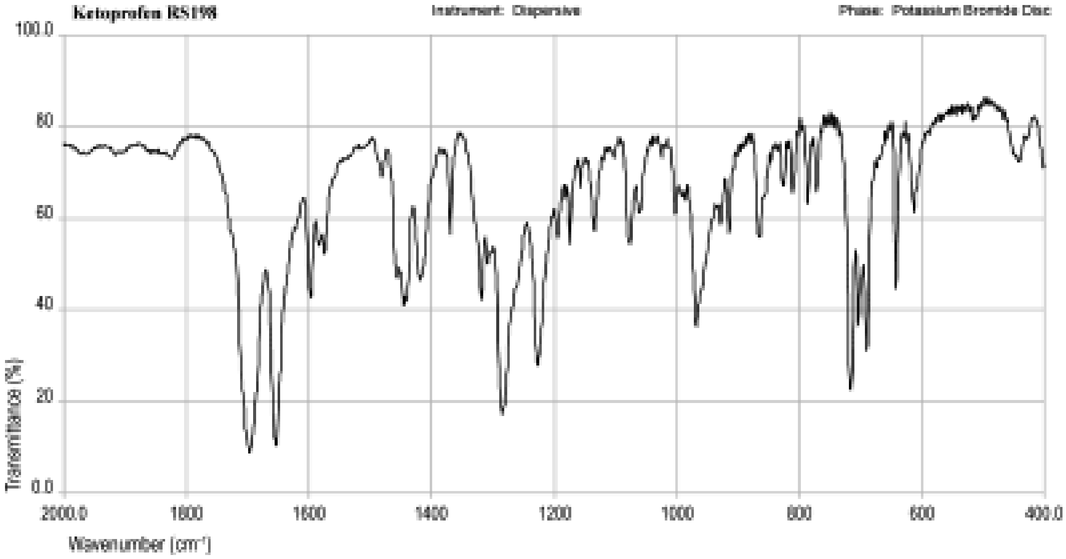
Isosorbide Dinitrate



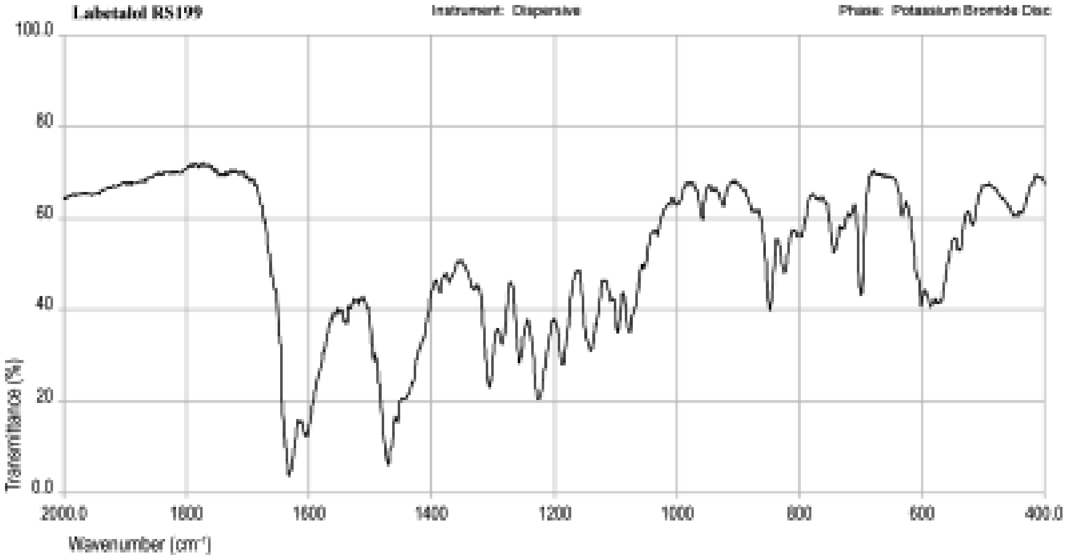
Isradipine


Isradipine RS373
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

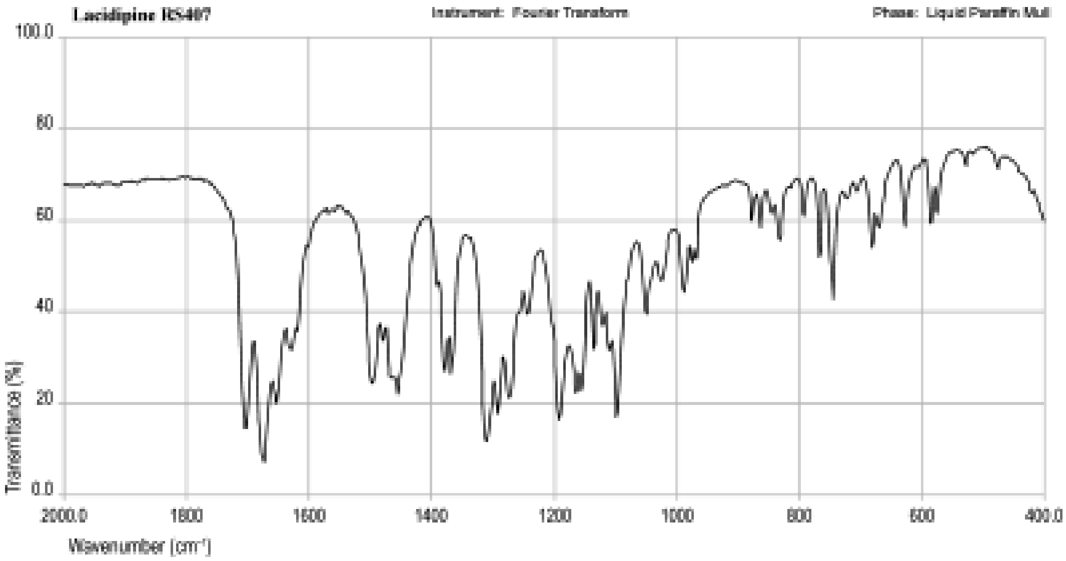
Ketoprofen


Ketoprofen RS198
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

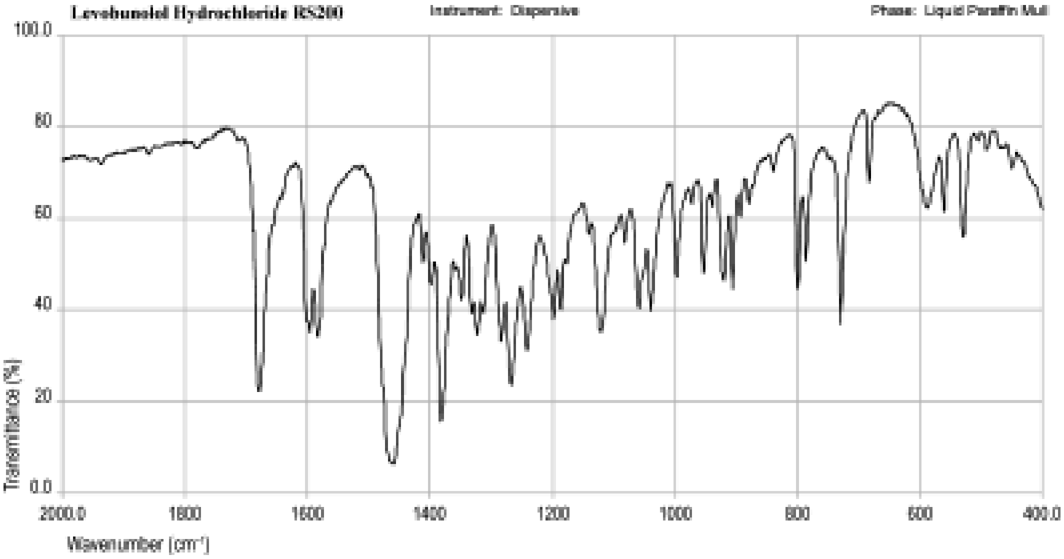
Labetalol


Labetalol RS199
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

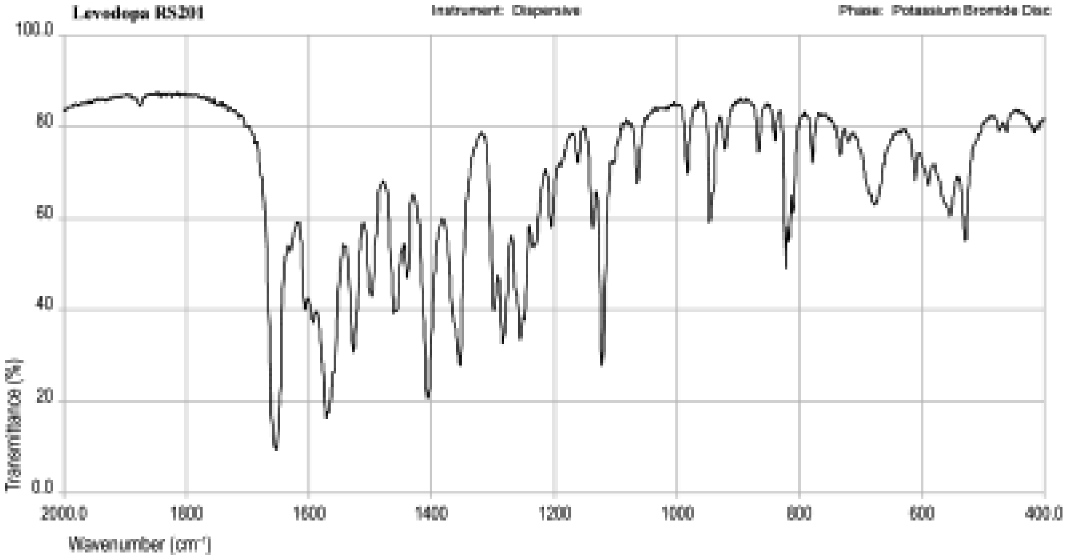
Lacidipine


Lacidipine RS407
Instrument: Fourier Transform
Phase: Liquid Paraffin Mull
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

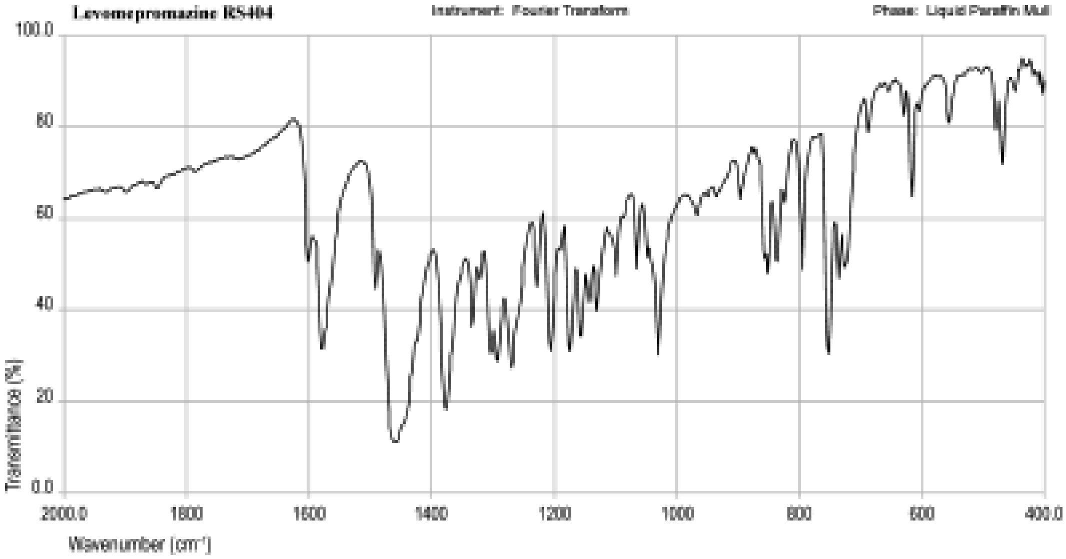
Levobunolol Hydrochloride



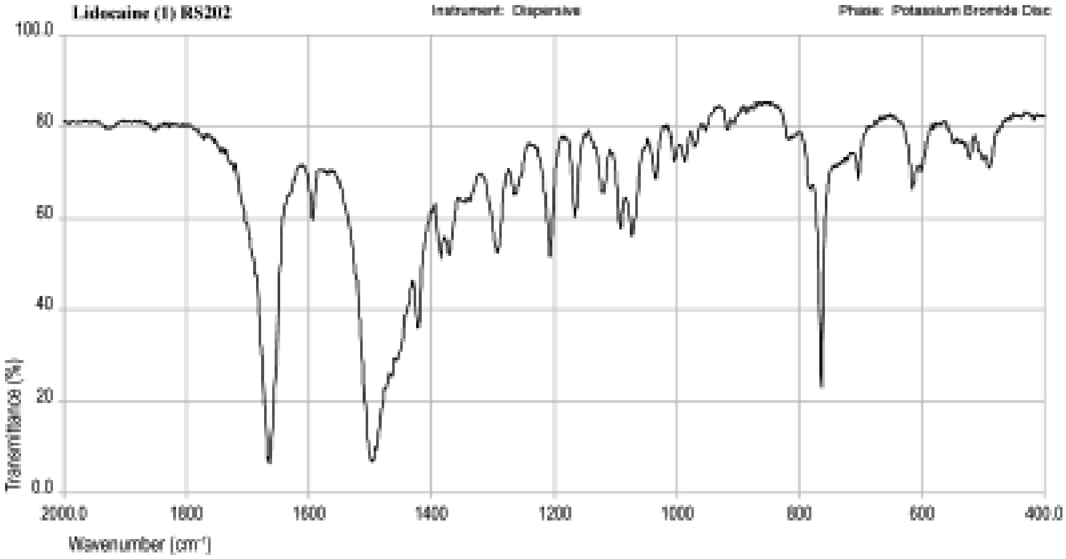
Levodopa


Levodopa RS201
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

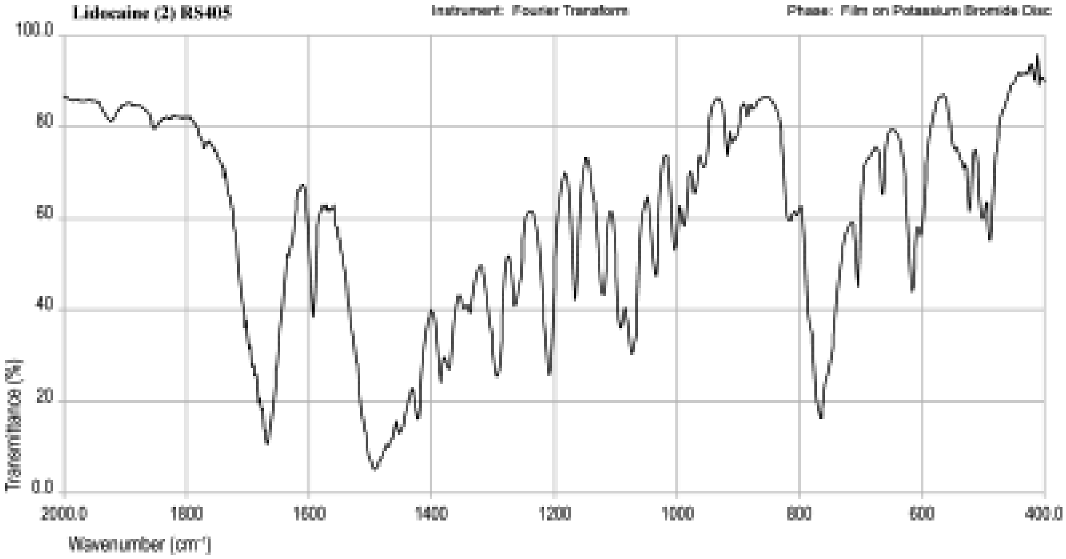
Levomepromazine



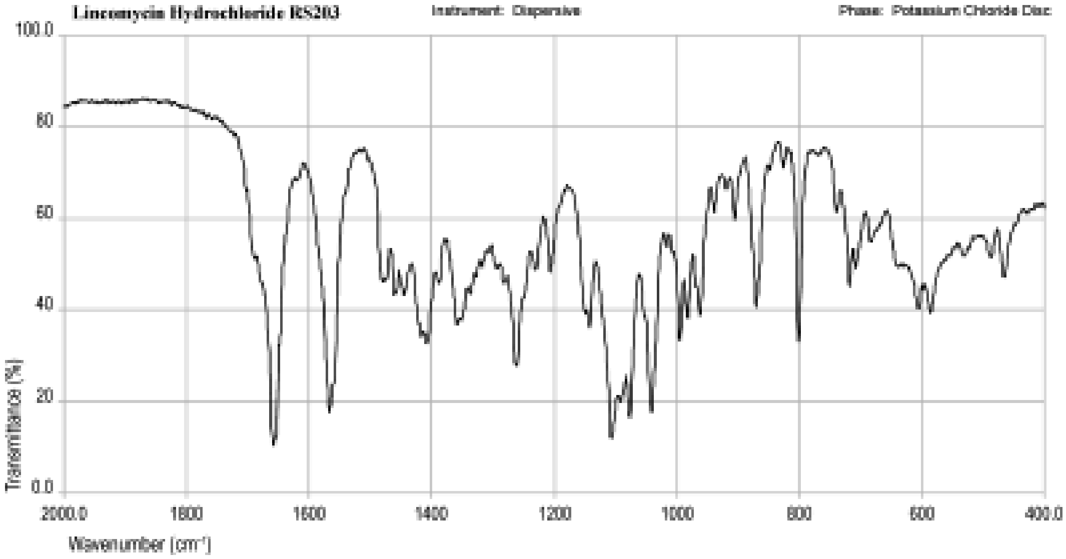
Lidocaine (1)


Lidocaine (1) RS202
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

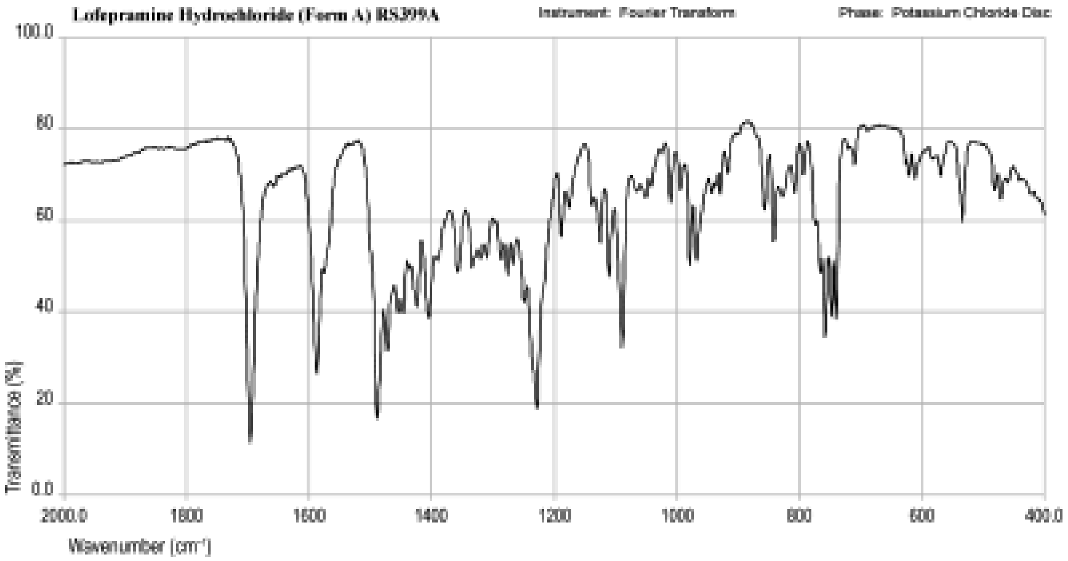
Lidocaine (2)


Lidocaine (2) RS405
Instrument: Fourier Transform
Phase: Film on Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

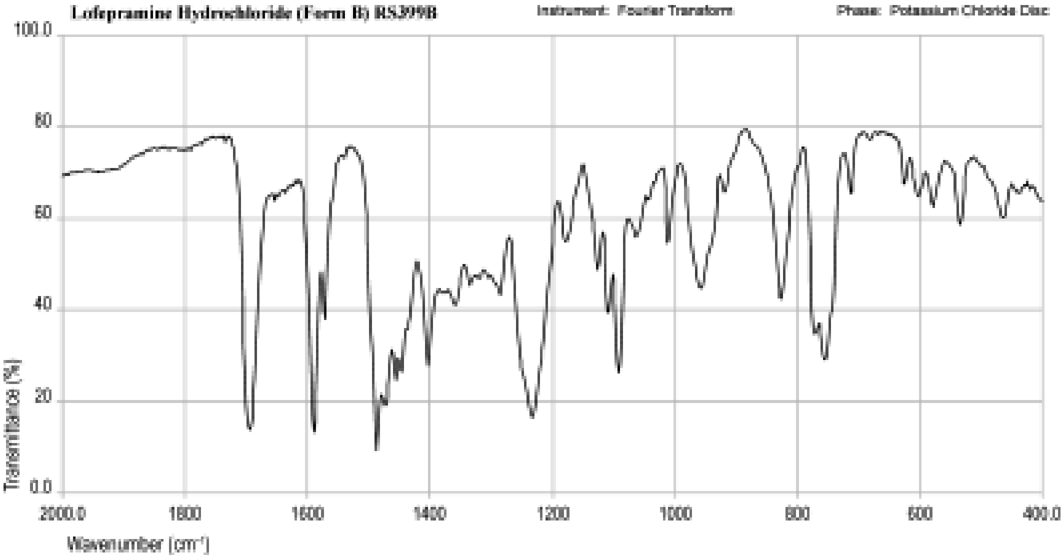
Lincomycin Hydrochloride



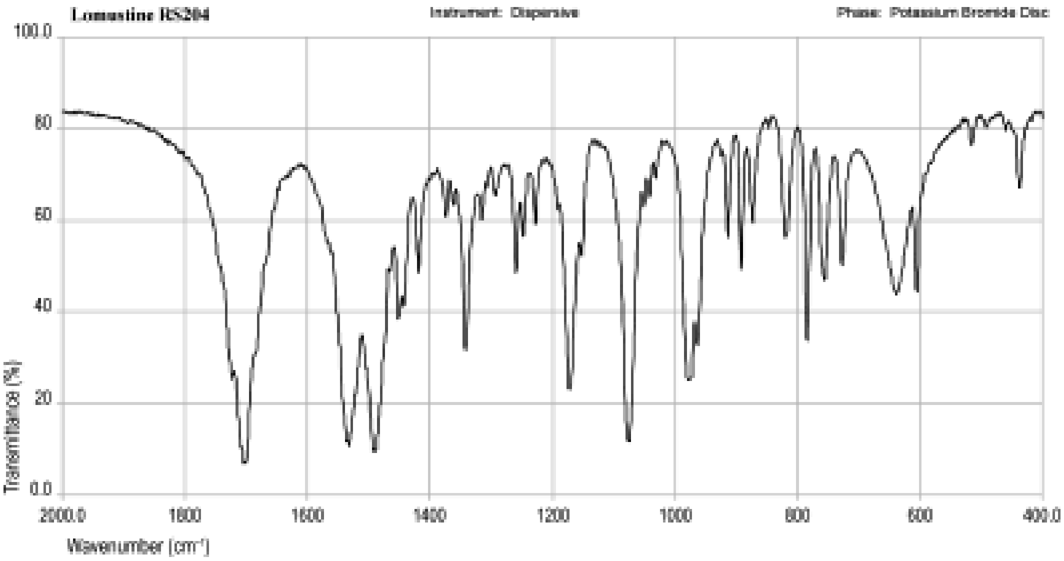
Lofepramine Hydrochloride (Form A)



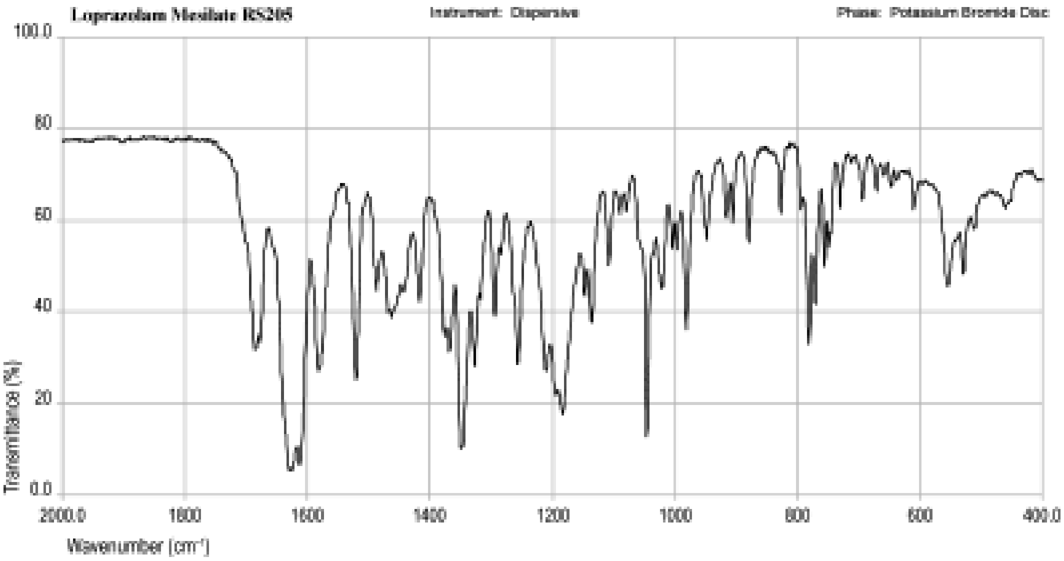
Lofepramine Hydrochloride (Form B)



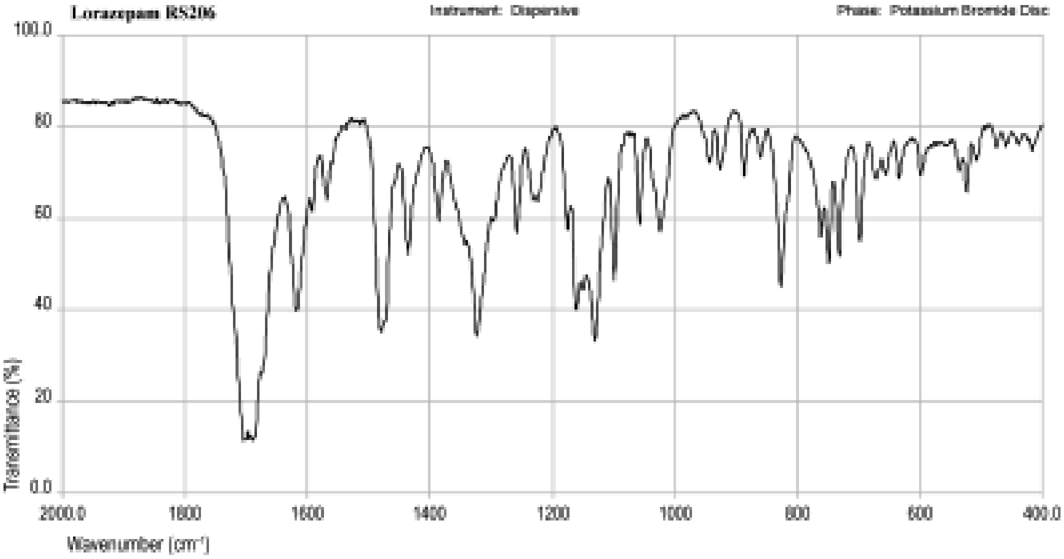
Lomustine


Lomustine RS204
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

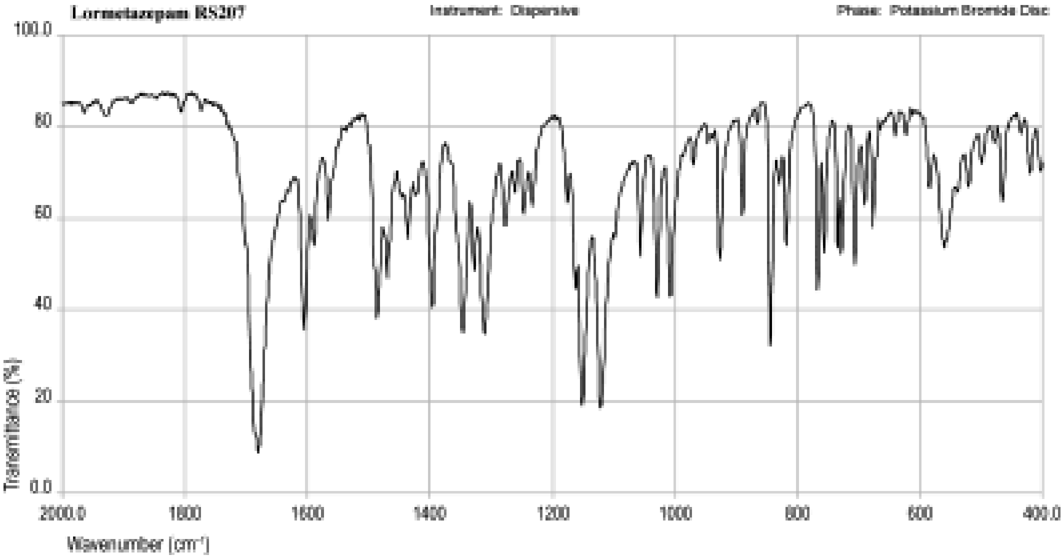
Loprazolam Mesilate



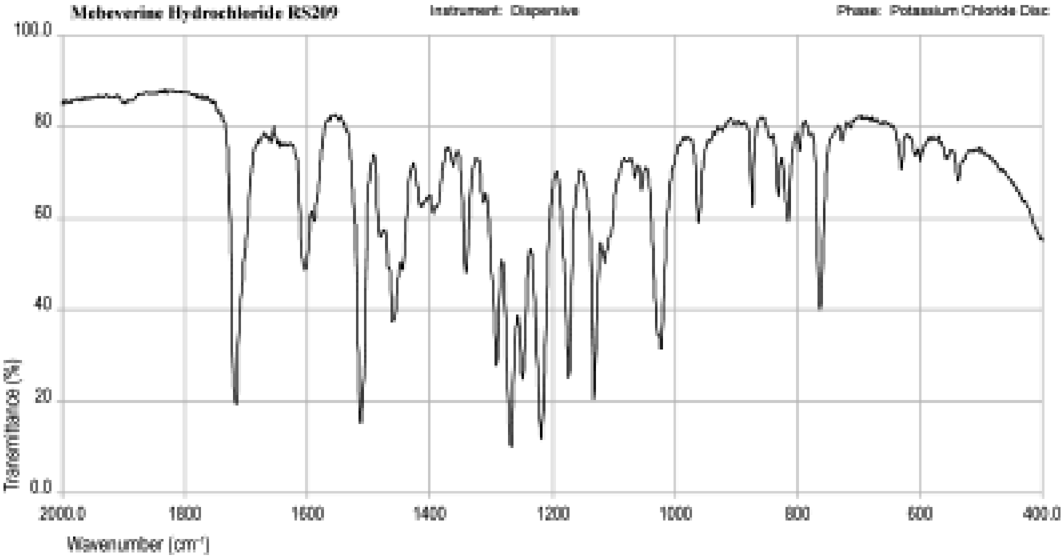
Lorazepam


Lorazepam RS206
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

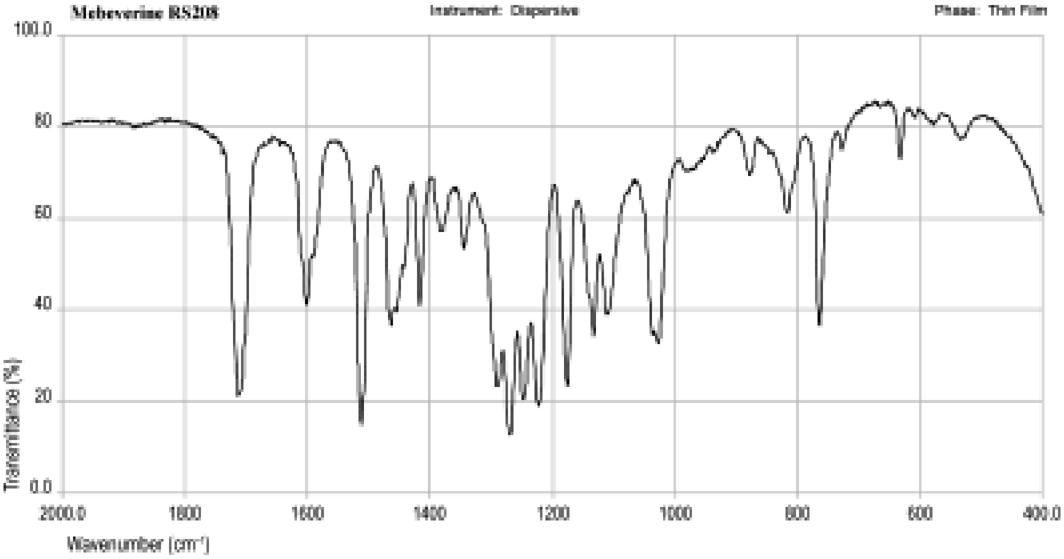
Lormetazepam



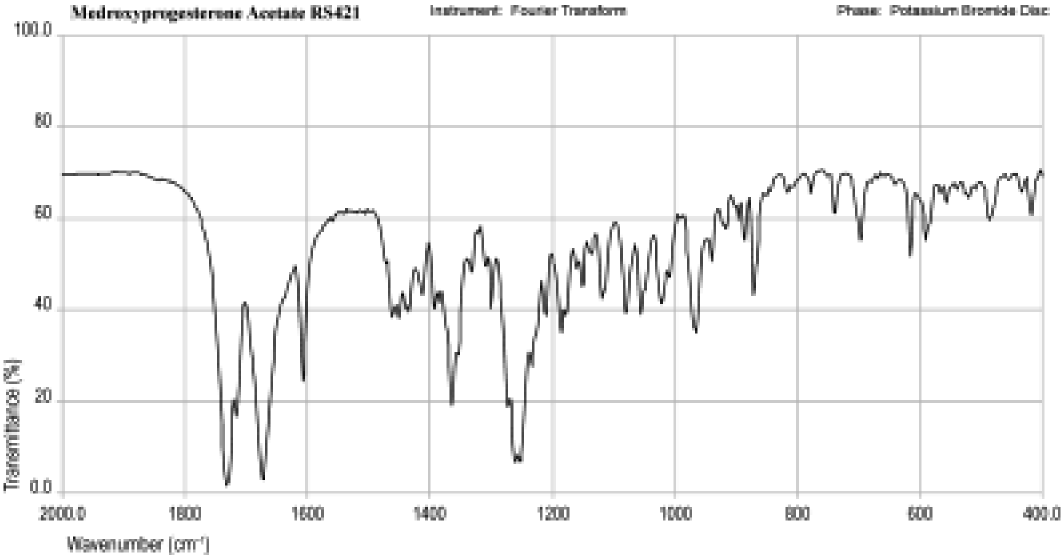
Mebeverine Hydrochloride



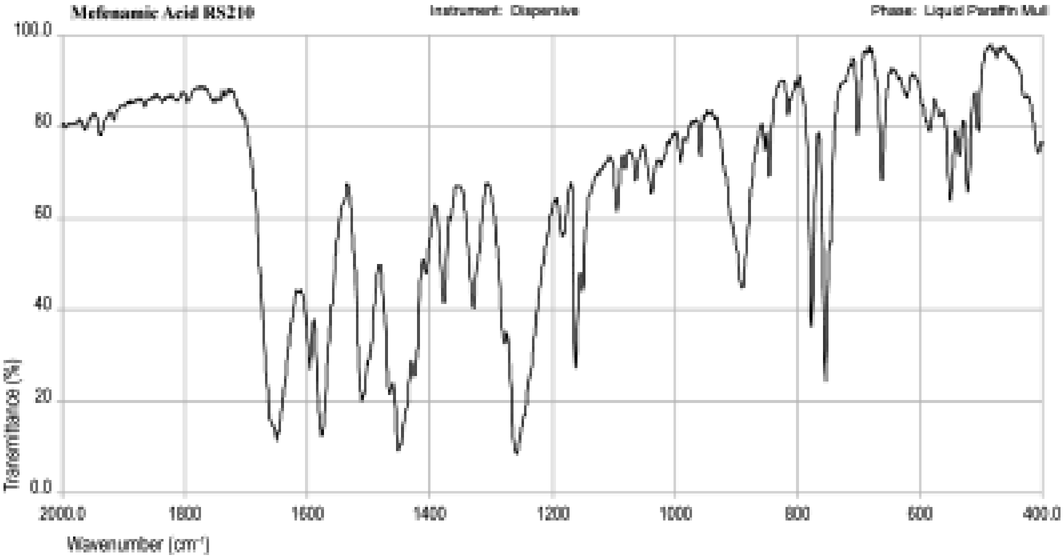
Mebeverine


Mebeverine RS208
Instrument: Dispersive
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

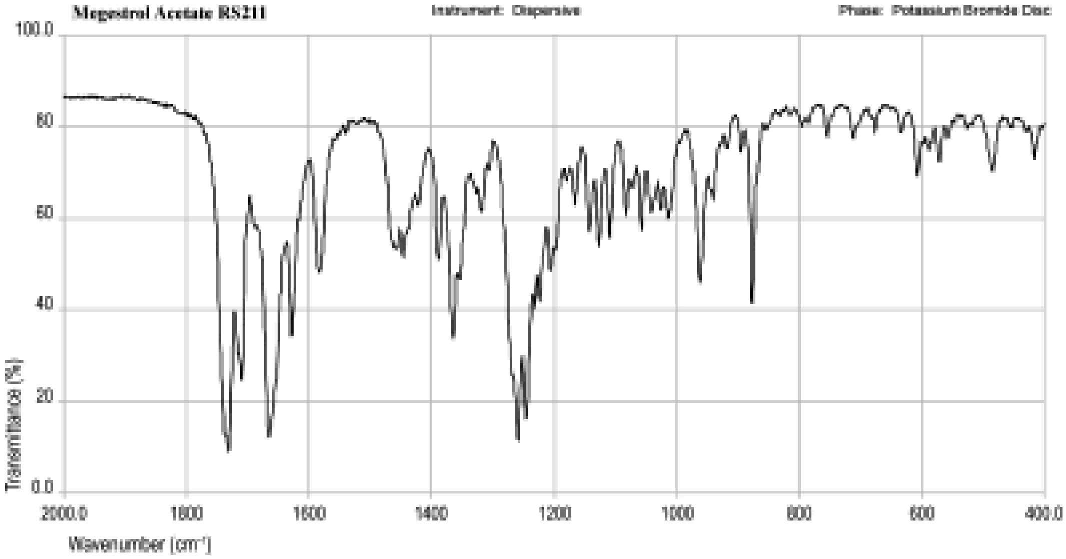
Medroxyprogesterone Acetate



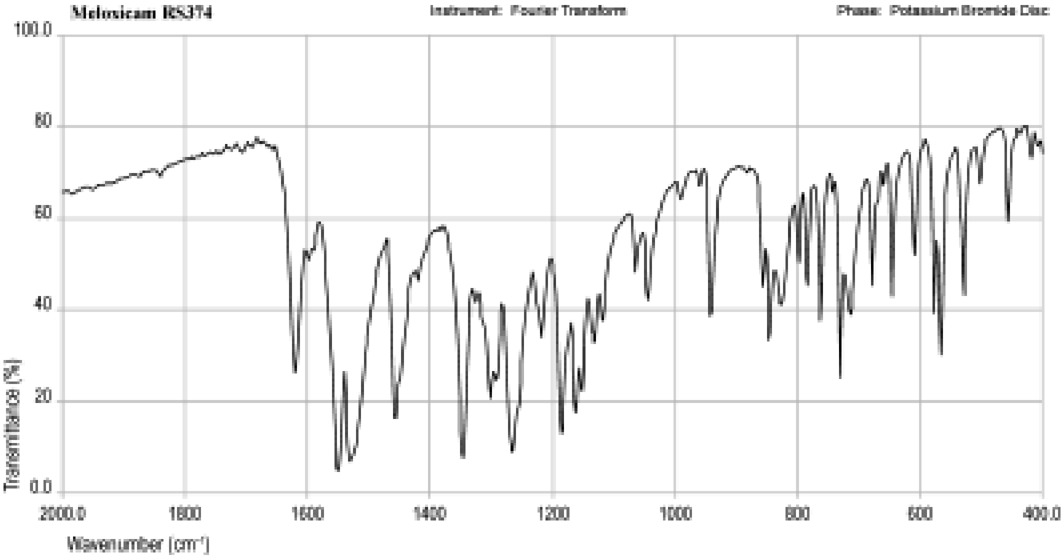
Mefenamic Acid



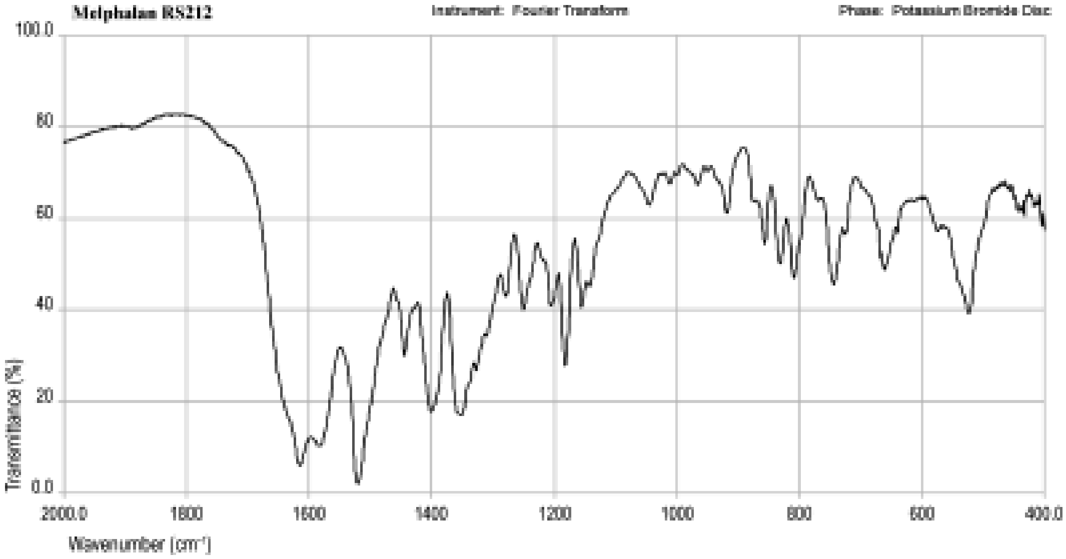
Megestrol Acetate



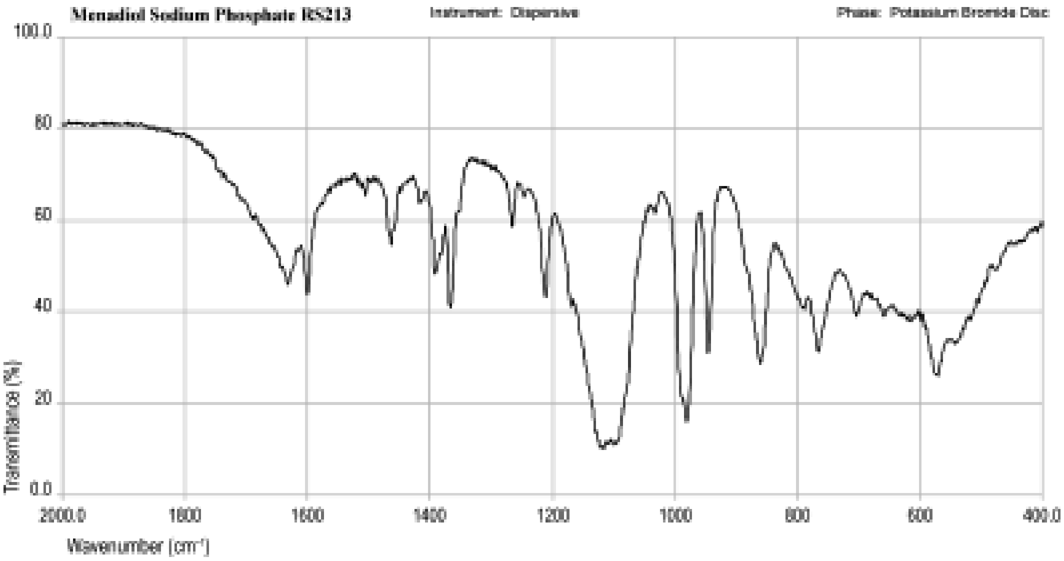
Meloxicam


Meloxicam RS374
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

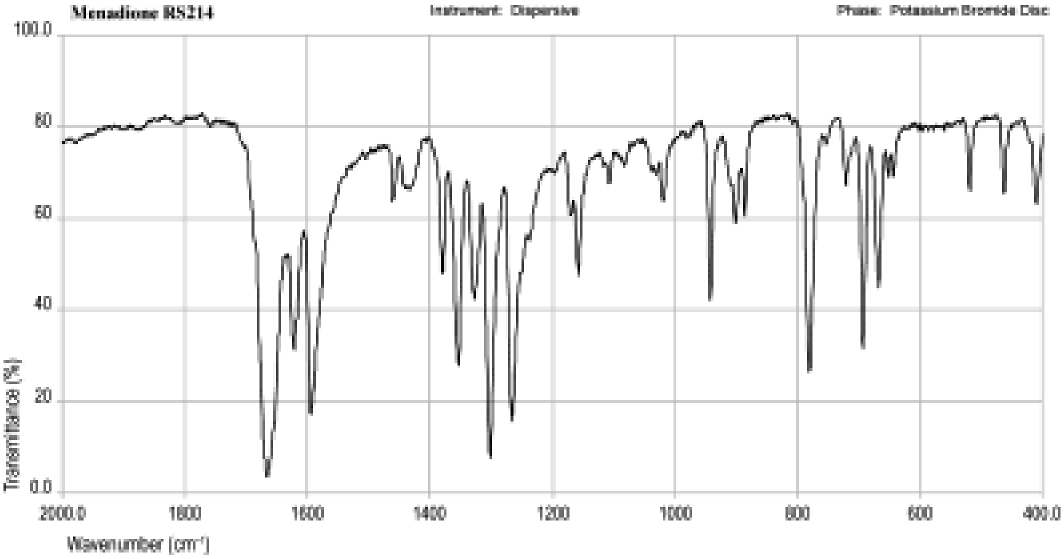
Melphalan


Melphalan RS212
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

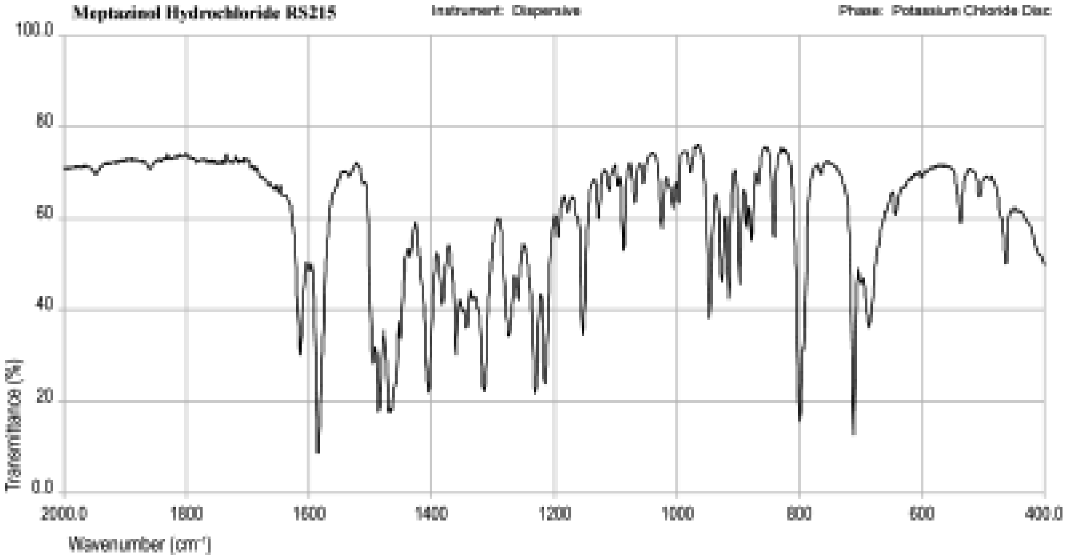
Menadiol Sodium Phosphate



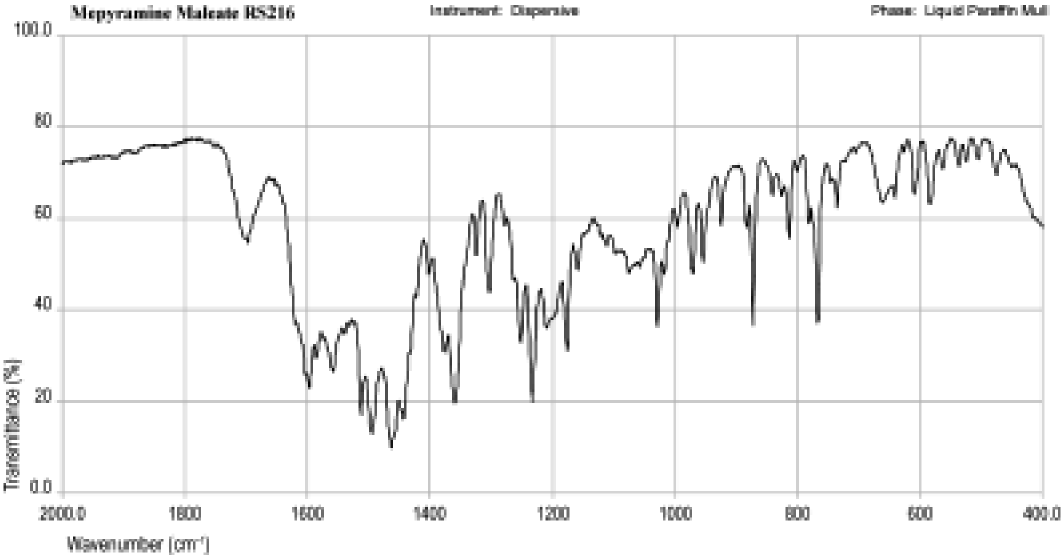
Menadione


Menadione RS214
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

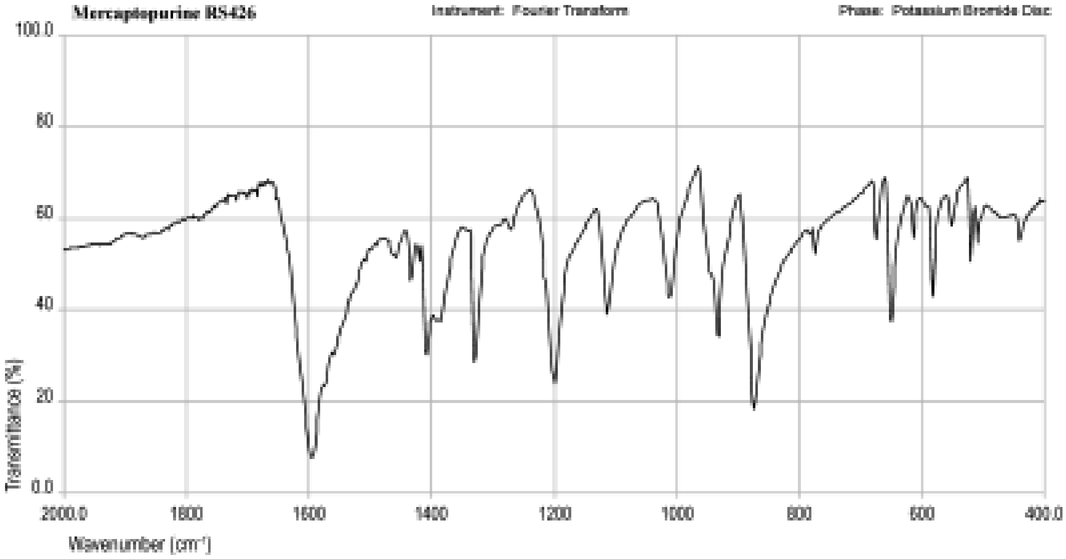
Meptazinol Hydrochloride



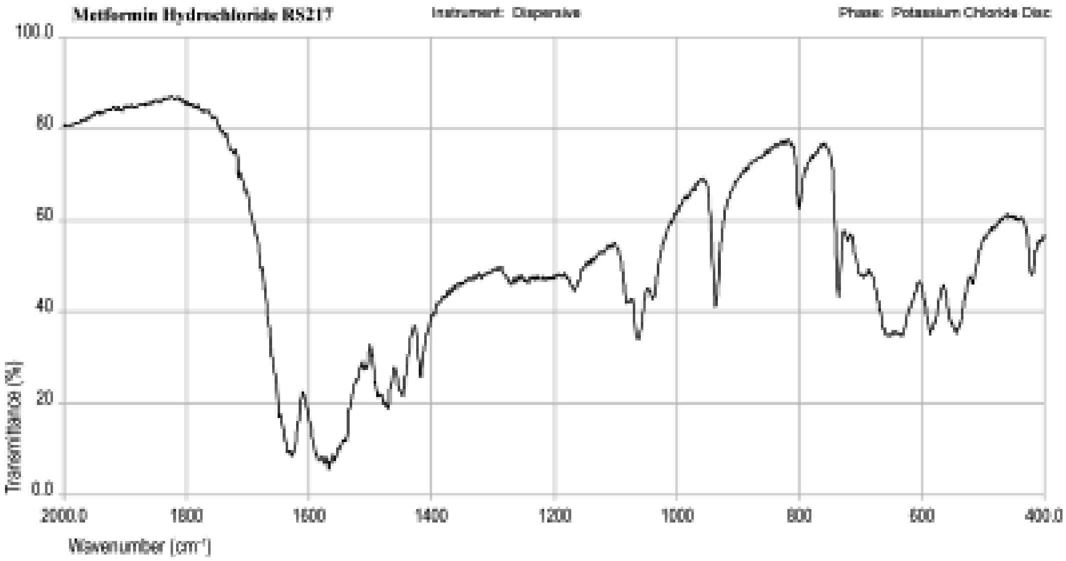
Mepyramine Maleate



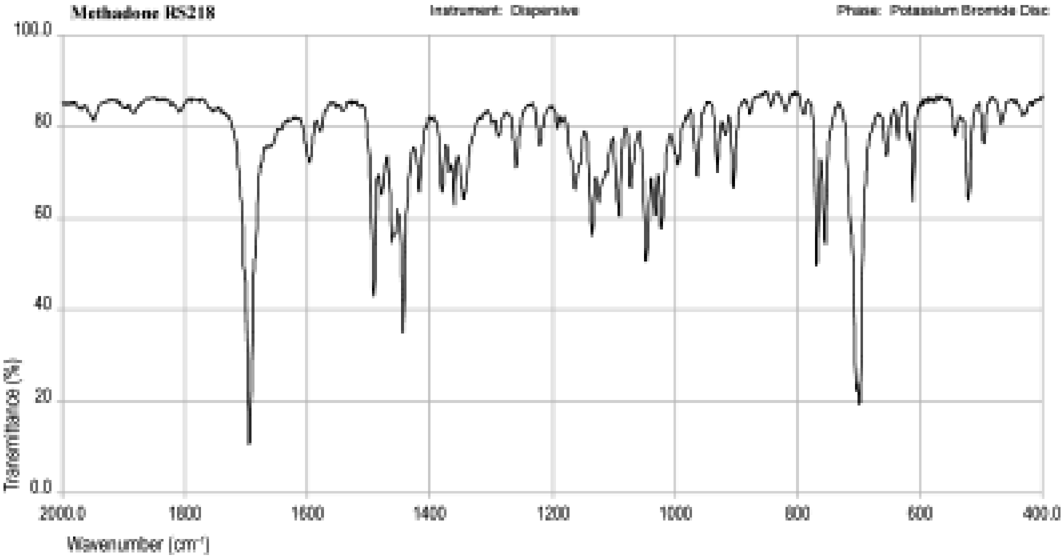
Mercaptopurine



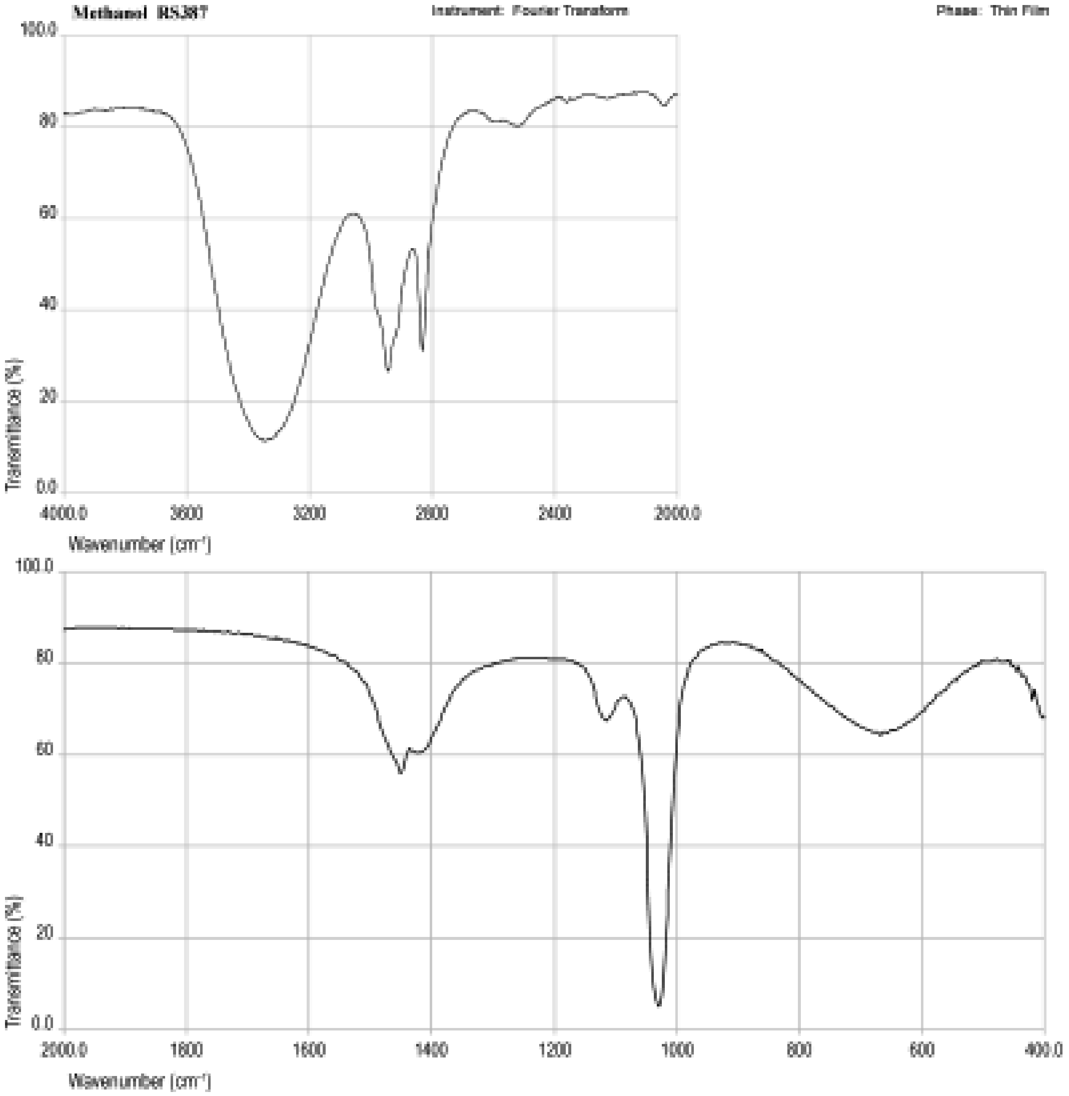
Metformin Hydrochloride



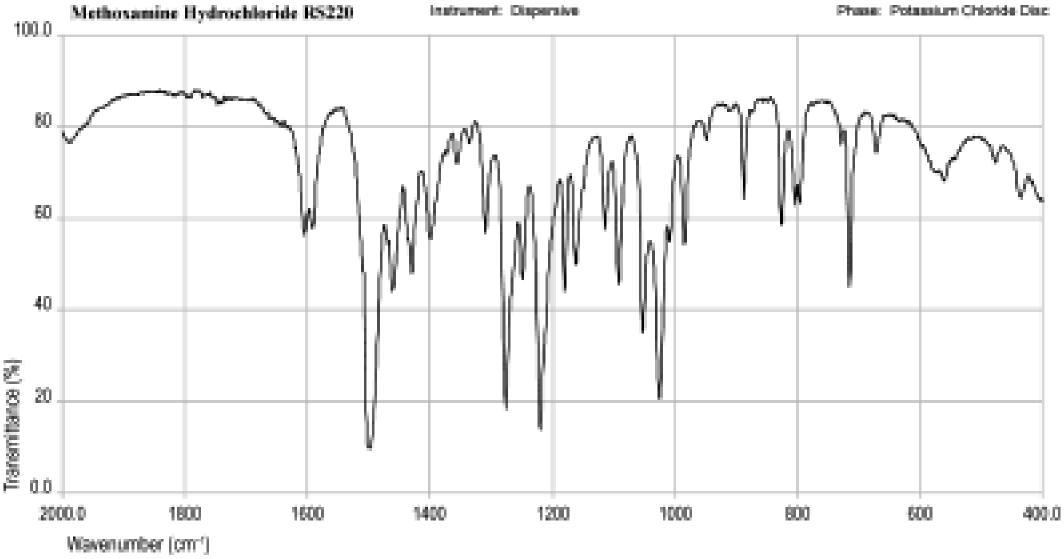
Methadone


Methadone RS218
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

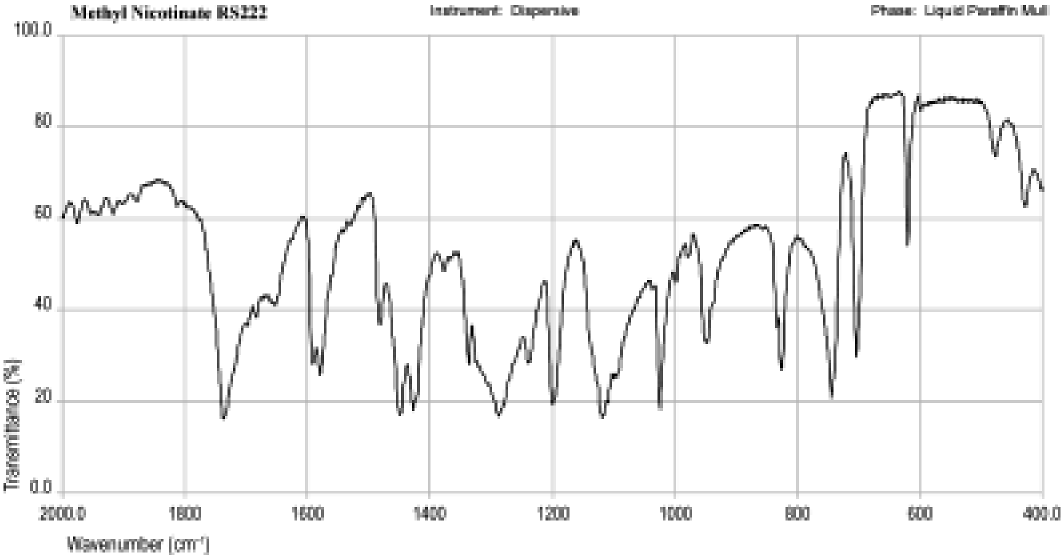
Methanol


Methanol RS387
Instrument: Fourier Transform
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
4000.0
3600
3200
2800
2400
2000.0
Wavenumber (cm⁻¹)
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

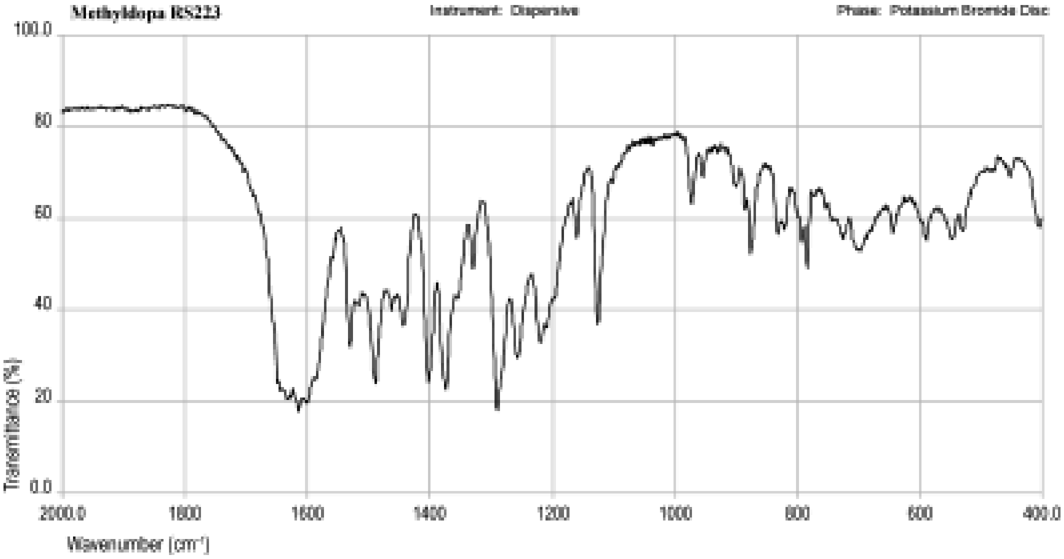
Methoxamine Hydrochloride



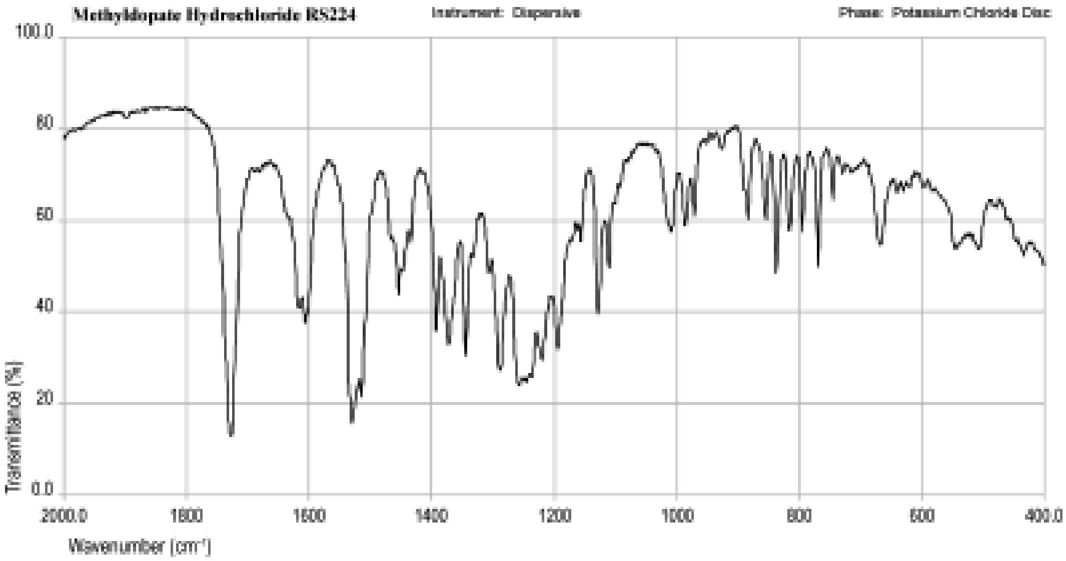
Methyl Nicotinate



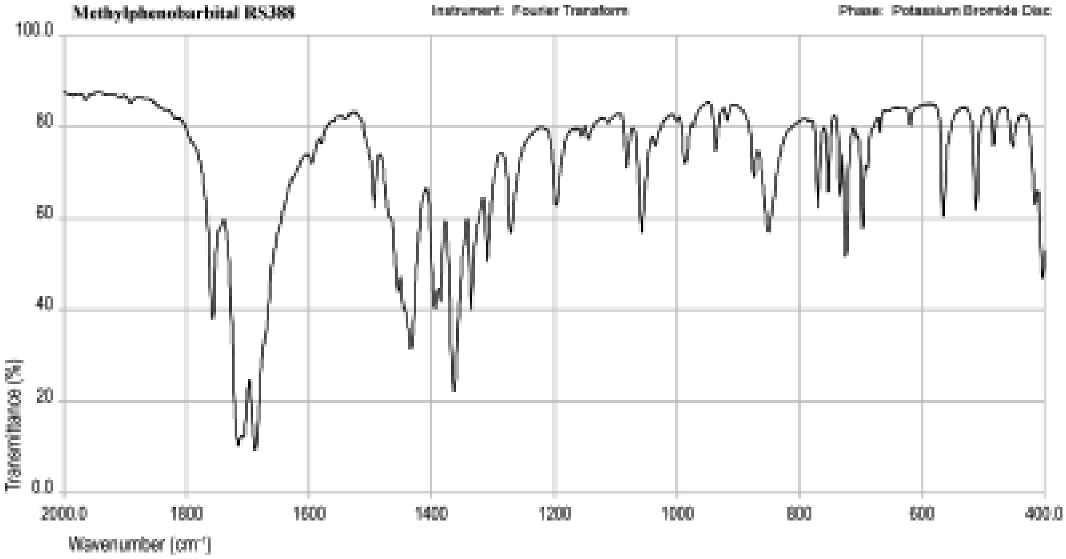
Methyldopa


Methyldopa RS223
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

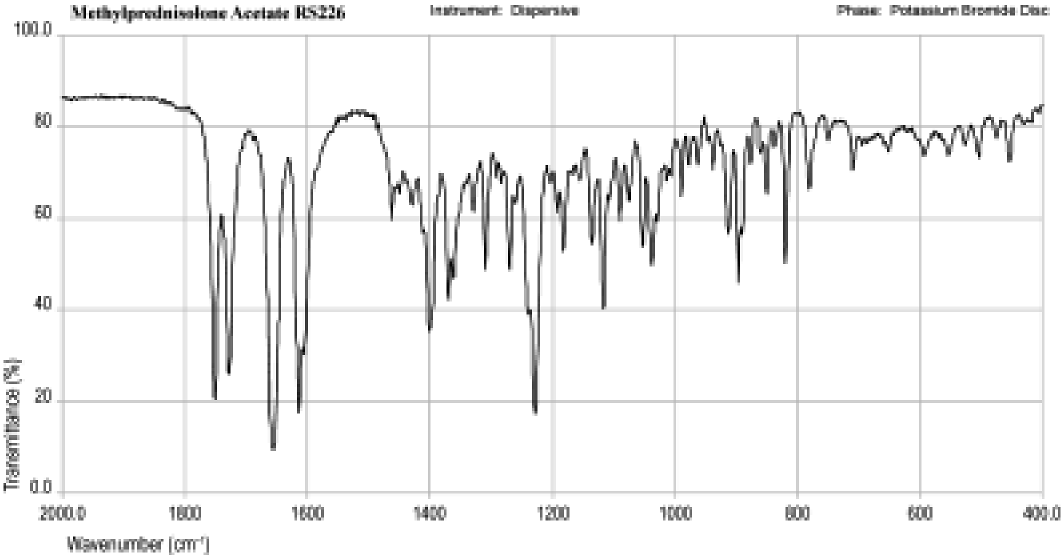
Methyldopate Hydrochloride



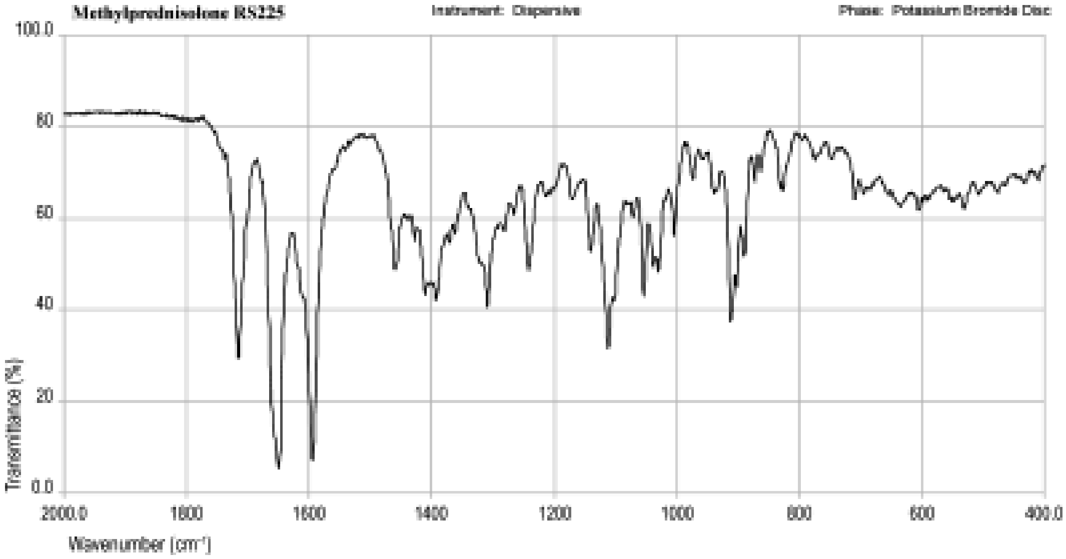
Methylphenobarbital



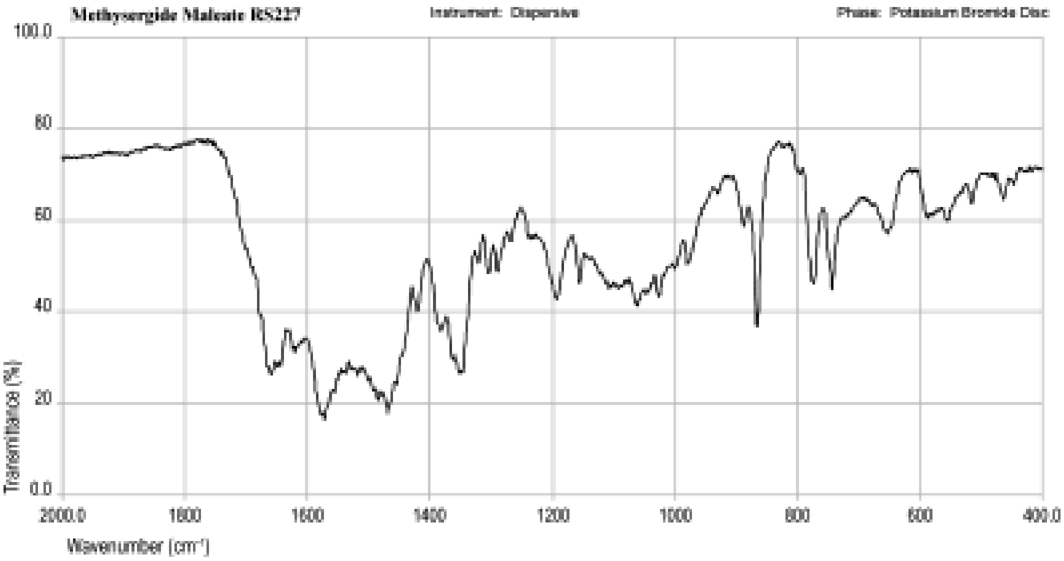
Methylprednisolone Acetate



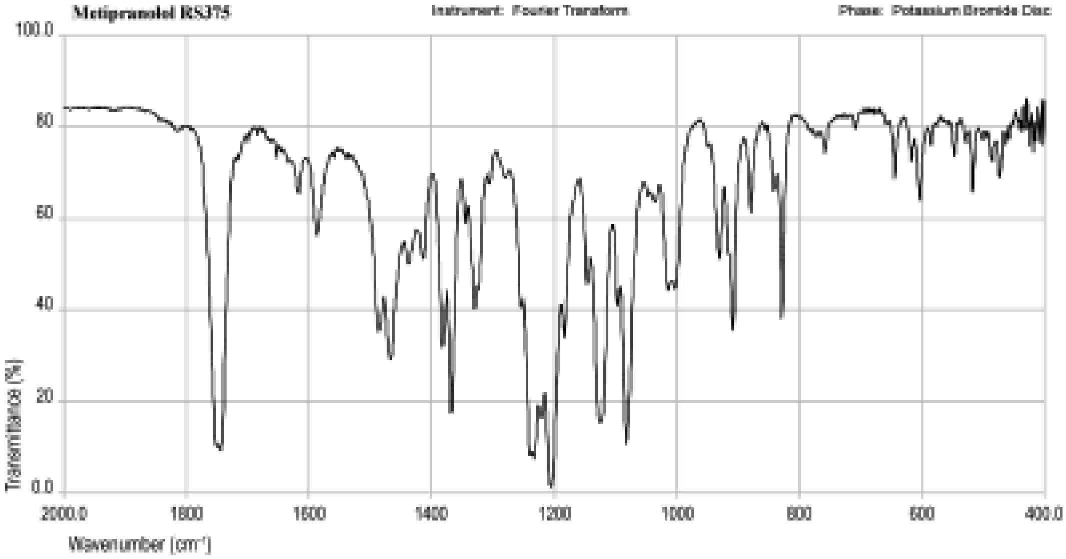
Methylprednisolone



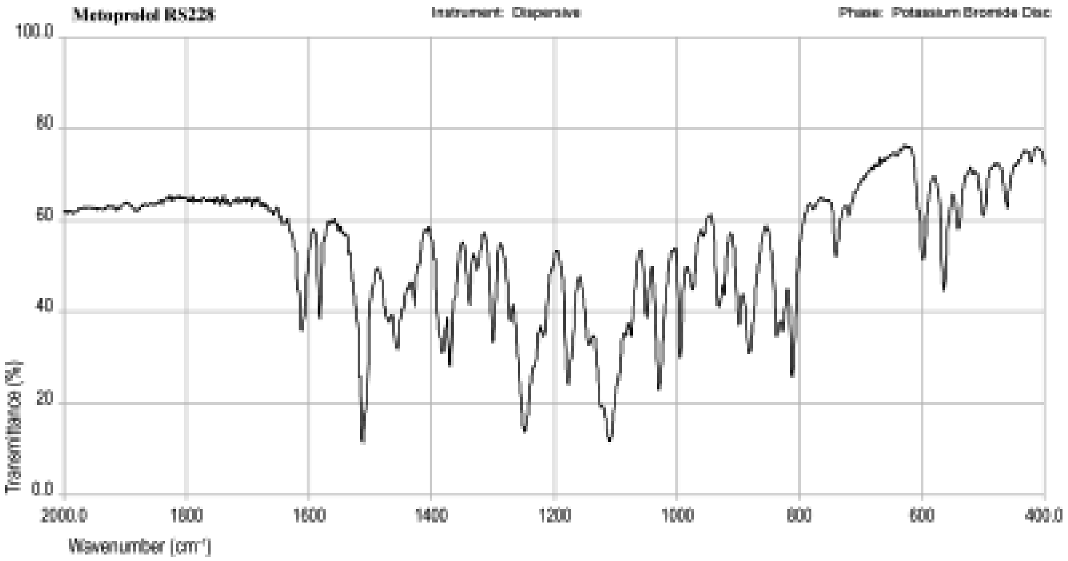
Methysergide Maleate



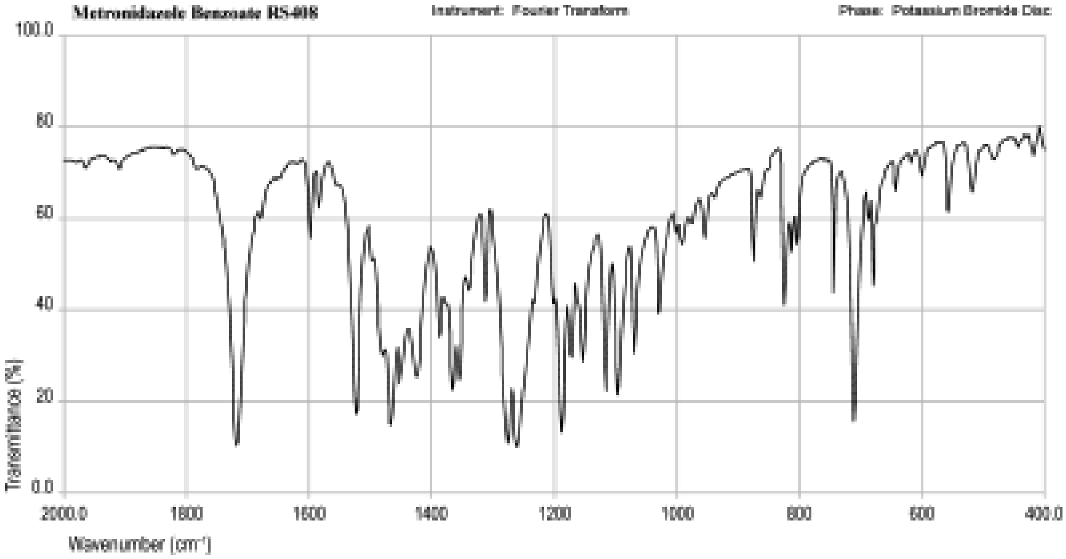
Metipranolol



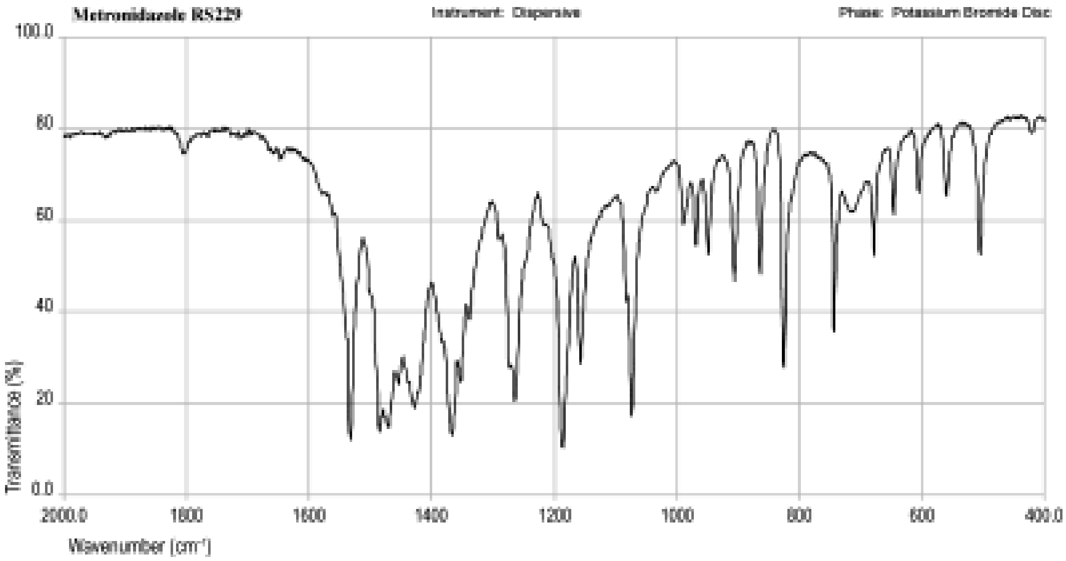
Metoprolol


Metoprolol RS228
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

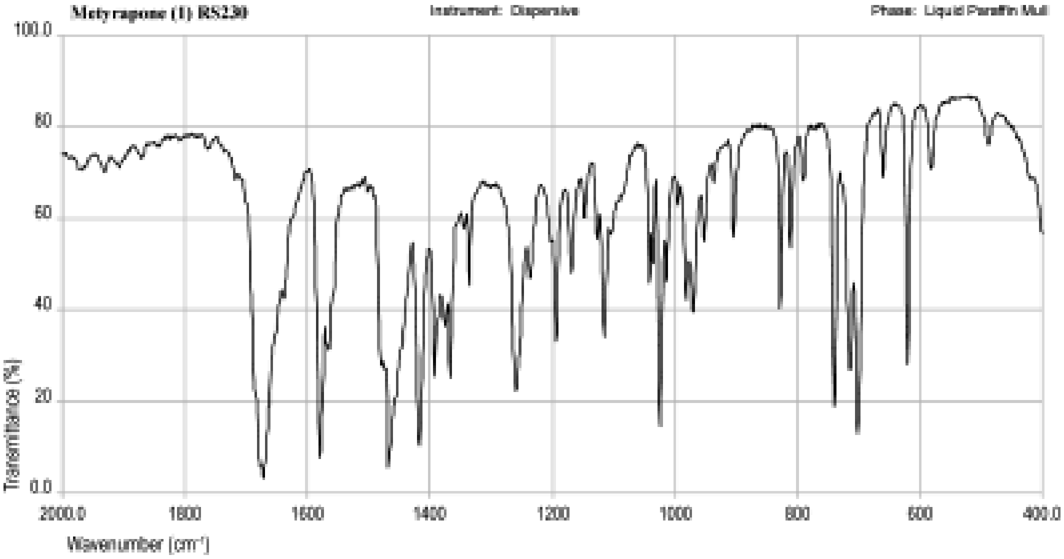
Metronidazole Benzoate



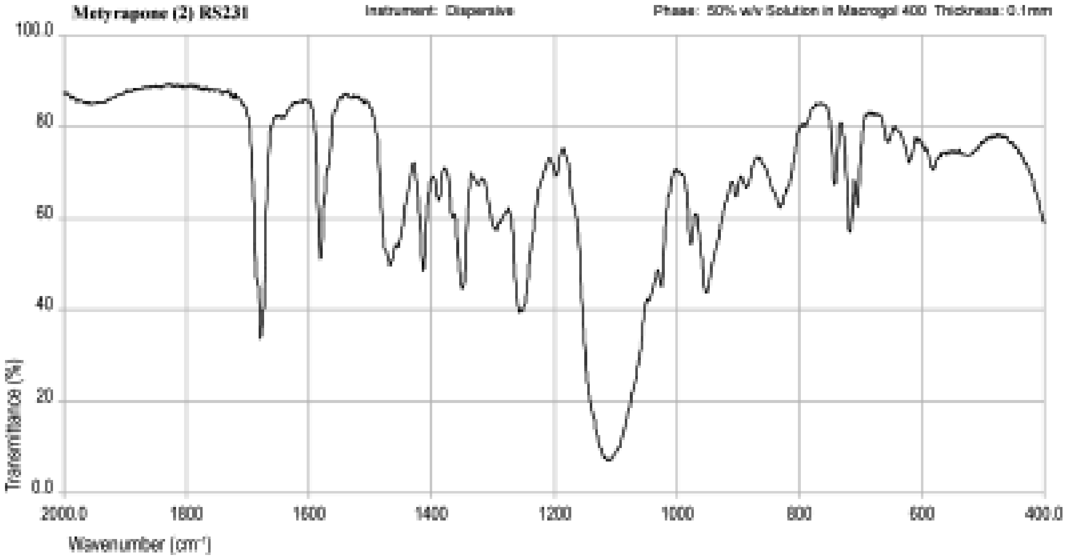
Metronidazole



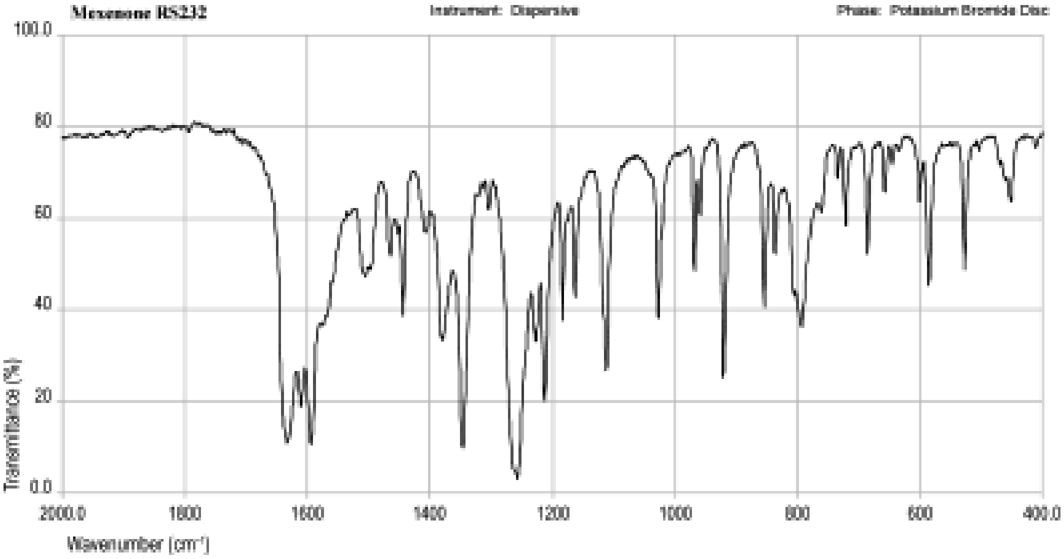
Metyrapone (1)



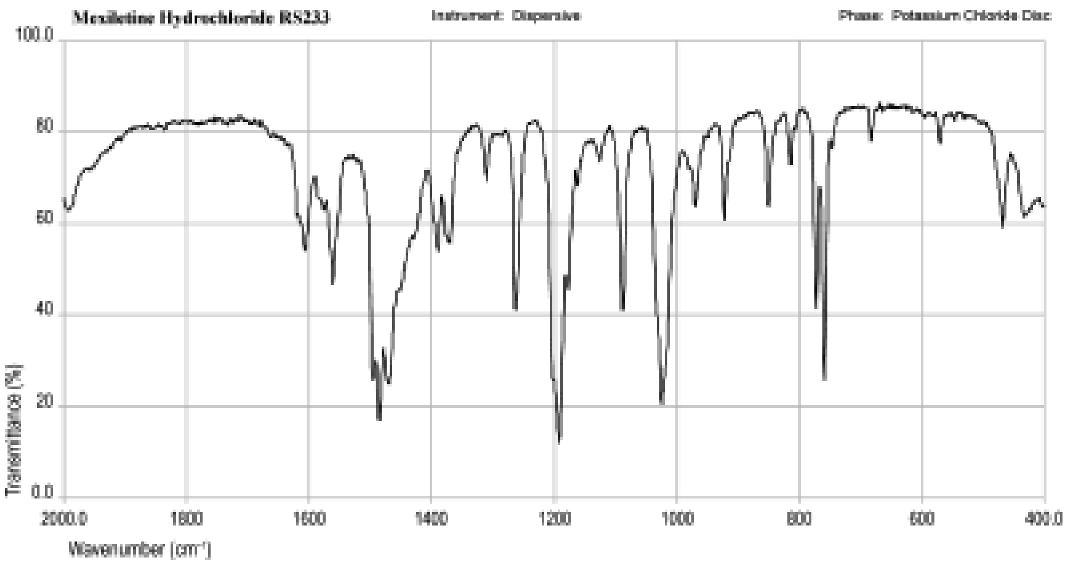
Metyrapone (2)



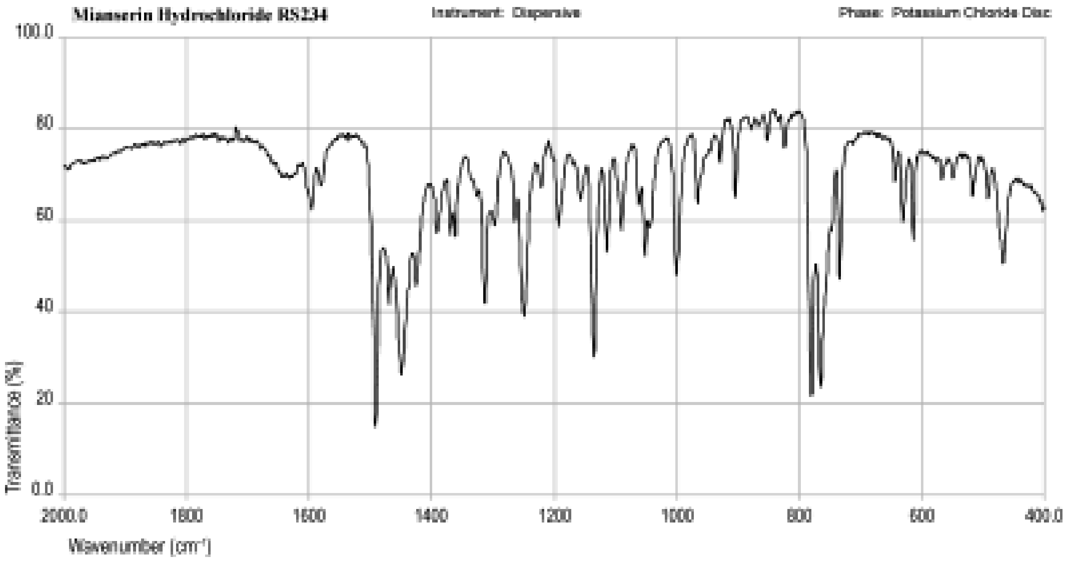
Mexenone


Mexenone RS232
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

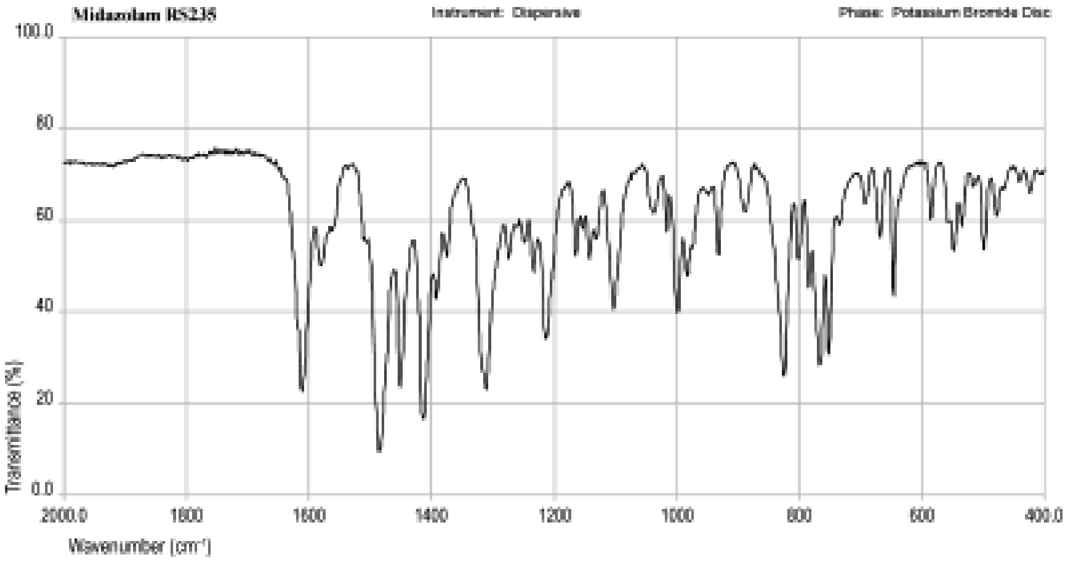
Mexiletine Hydrochloride



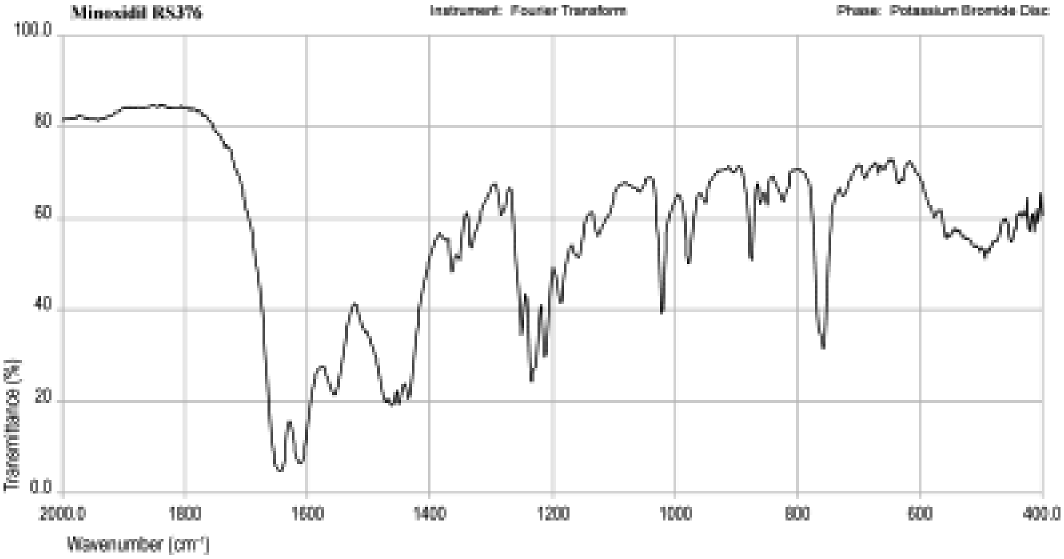
Mianserin Hydrochloride



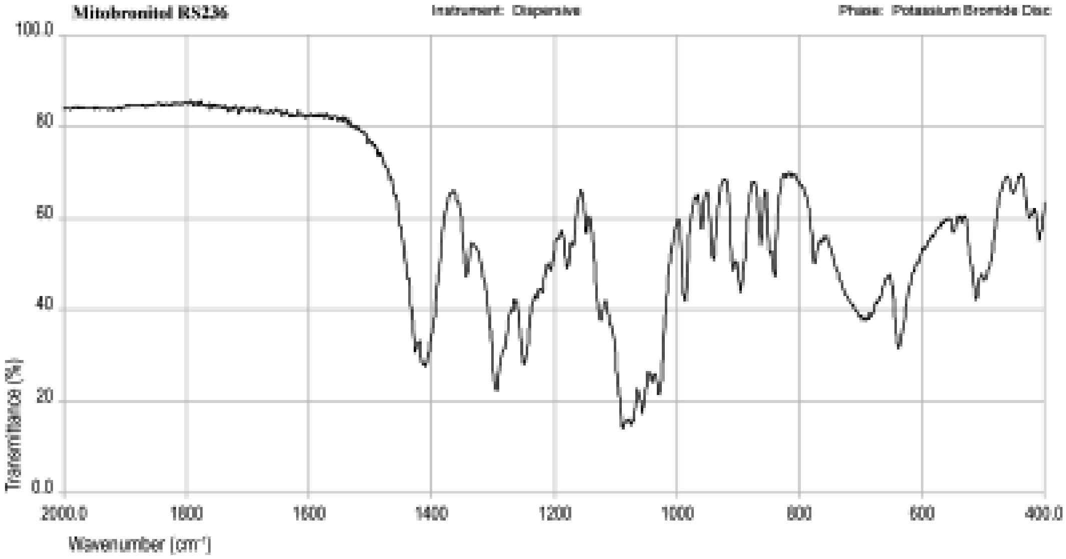
Midazolam


Midazolam RS235
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

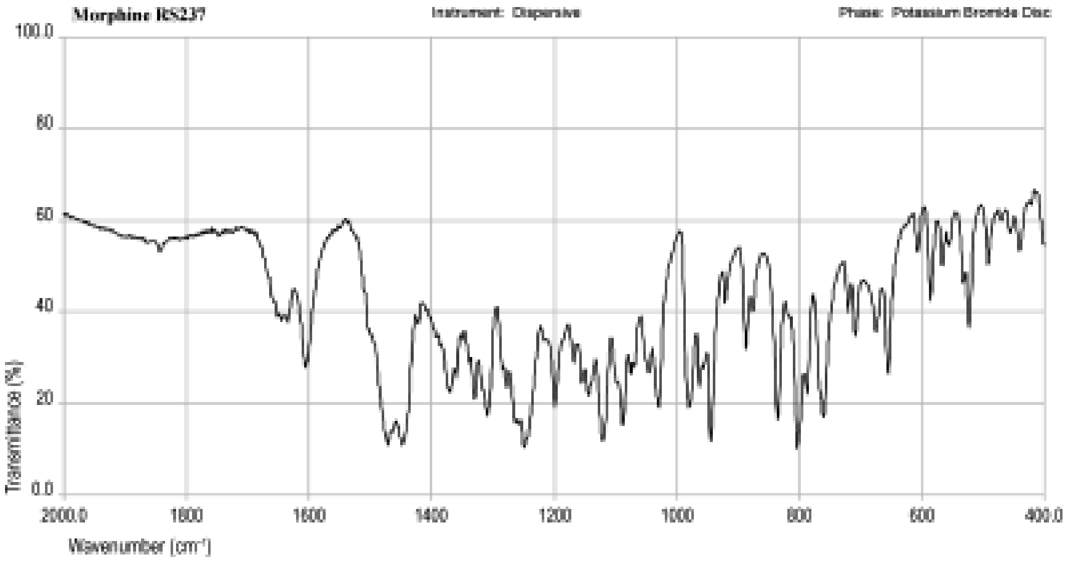
Minoxidil


Minoxidil RS376
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

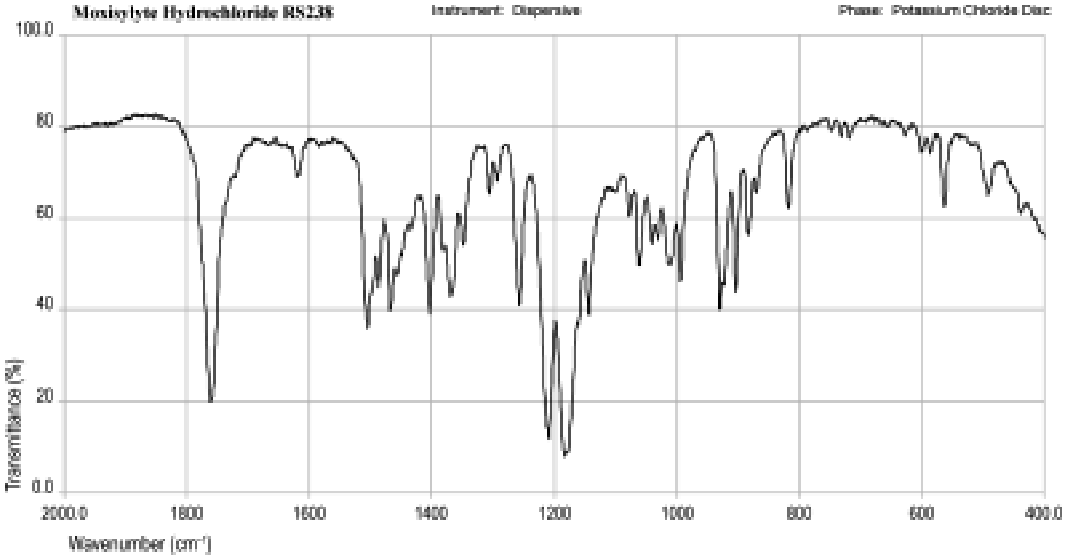
Mitobronitol



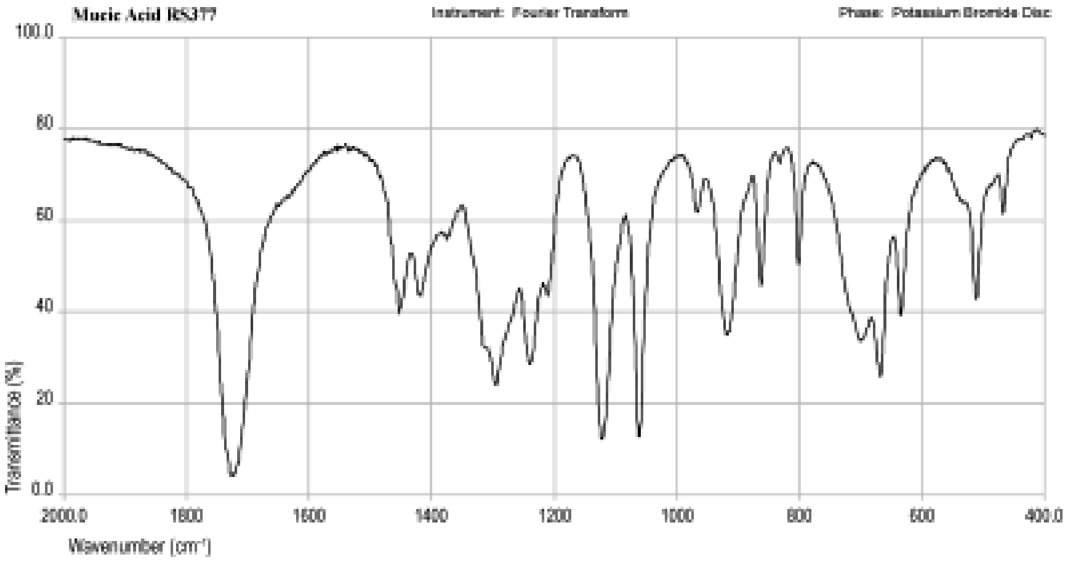
Morphine


Morphine RS237
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

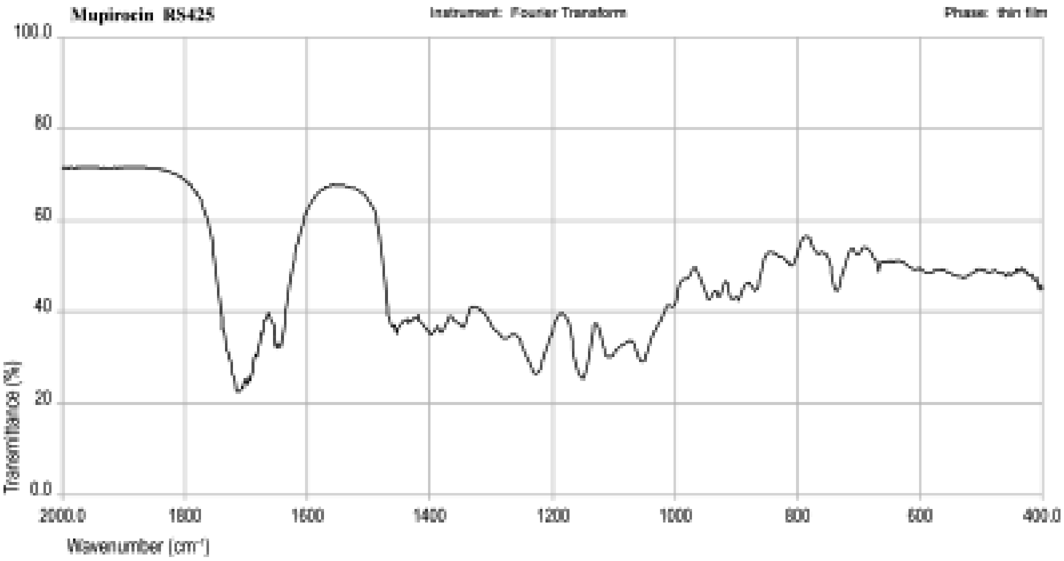
Moxisylyte Hydrochloride



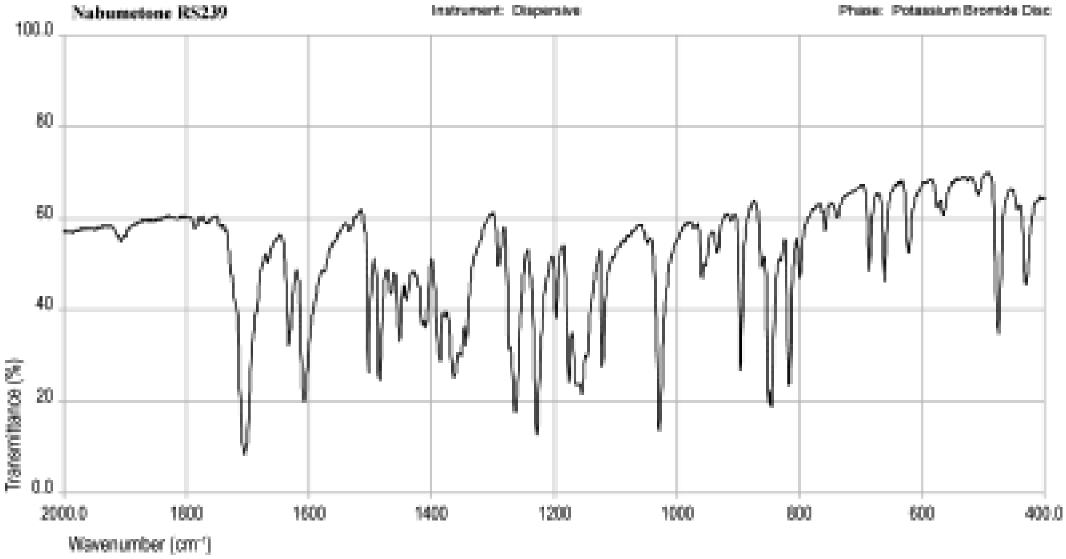
Mucic Acid


Mucic Acid RS377
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

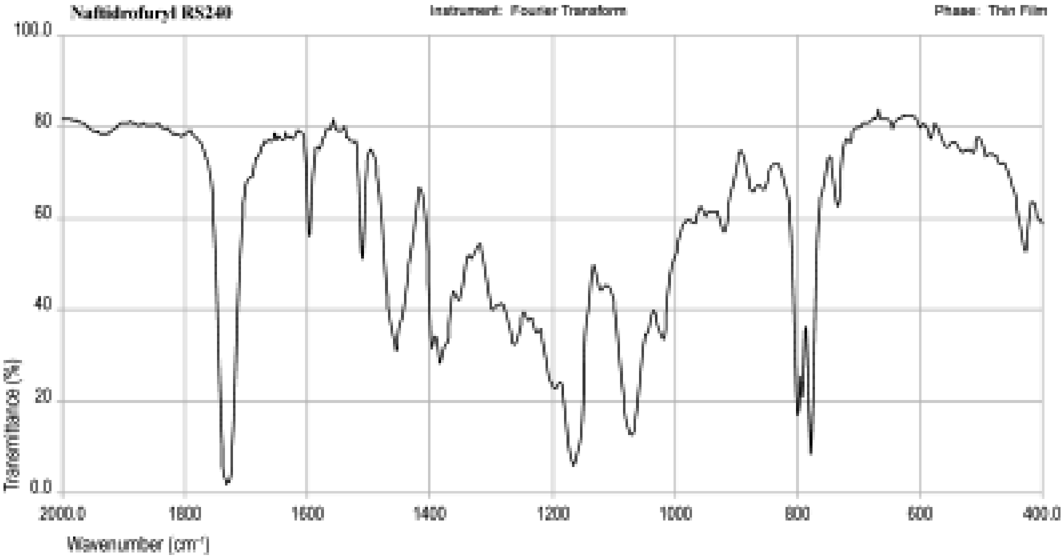
Mupirocin


Mupirocin RS425
Instrument: Fourier Transform
Phase: thin film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

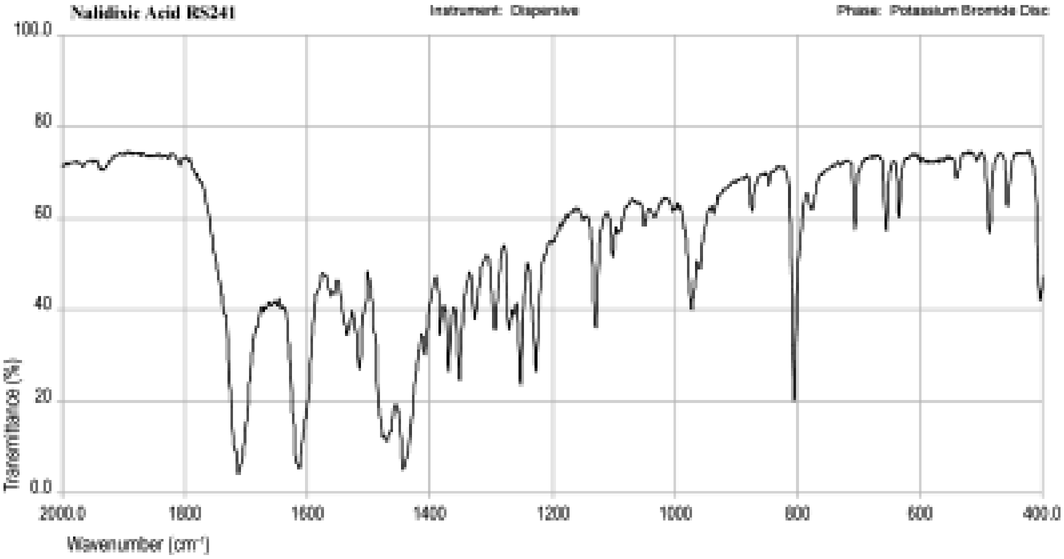
Nabumetone


Nabumetone RS239
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

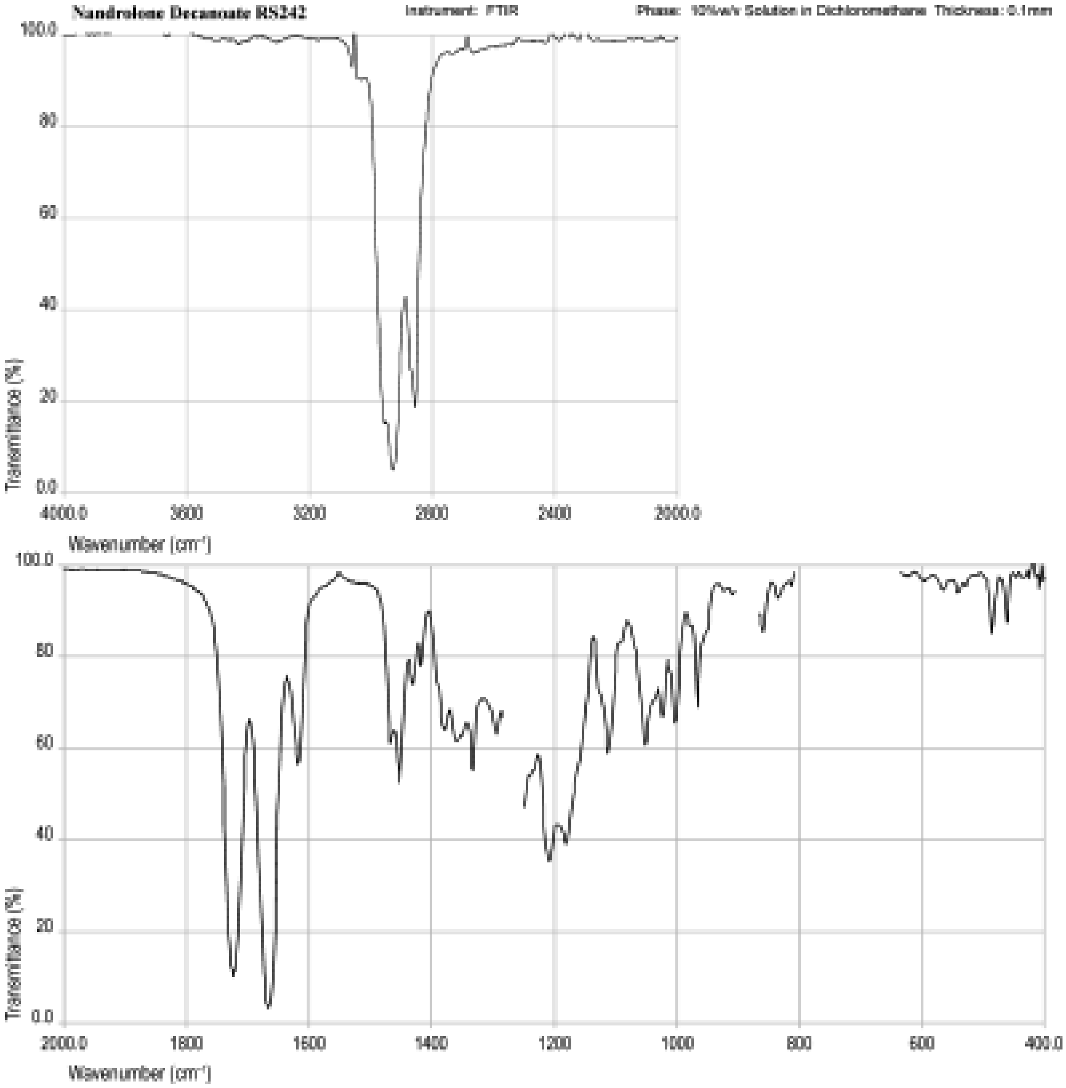
Naftidrofuryl



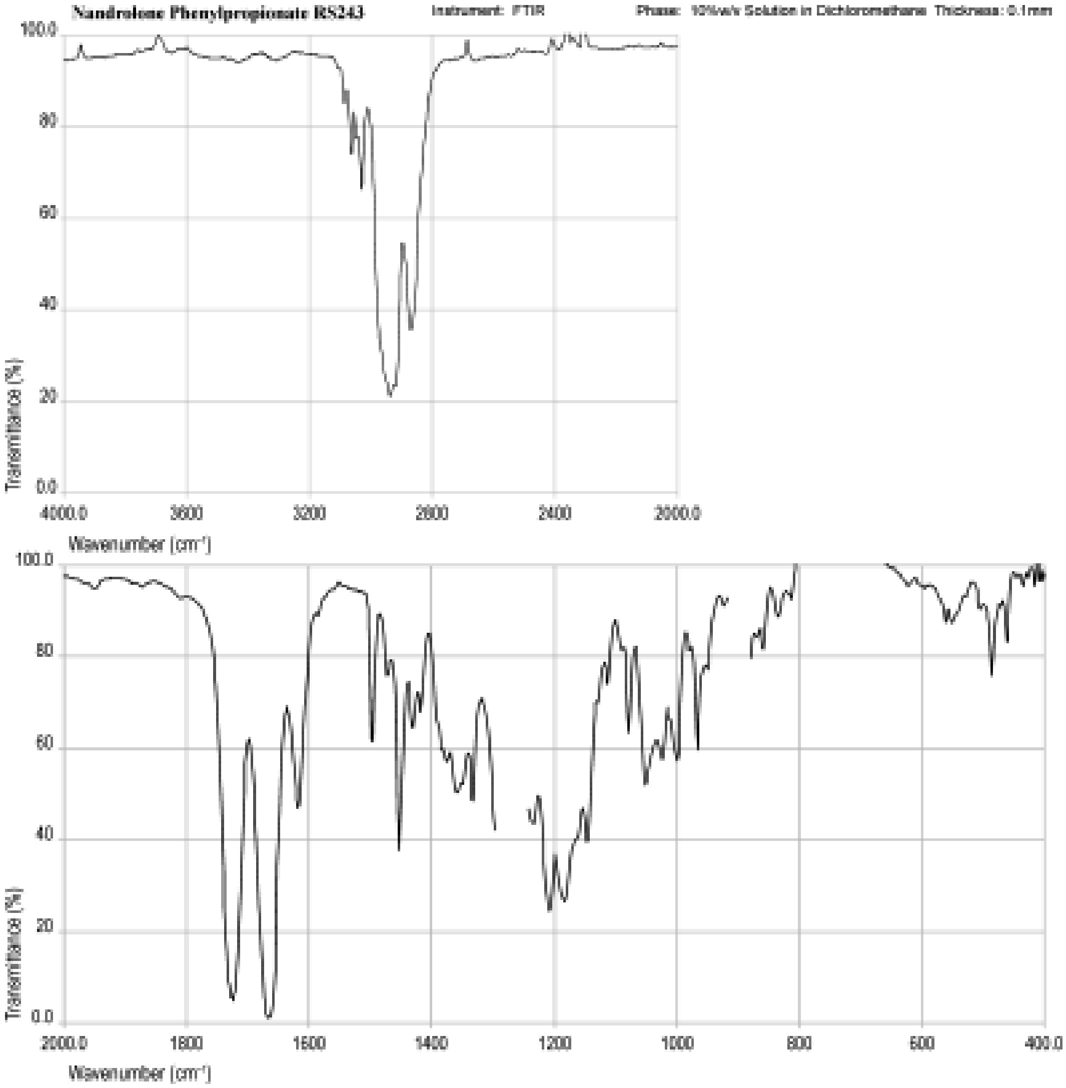
Nalidixic Acid



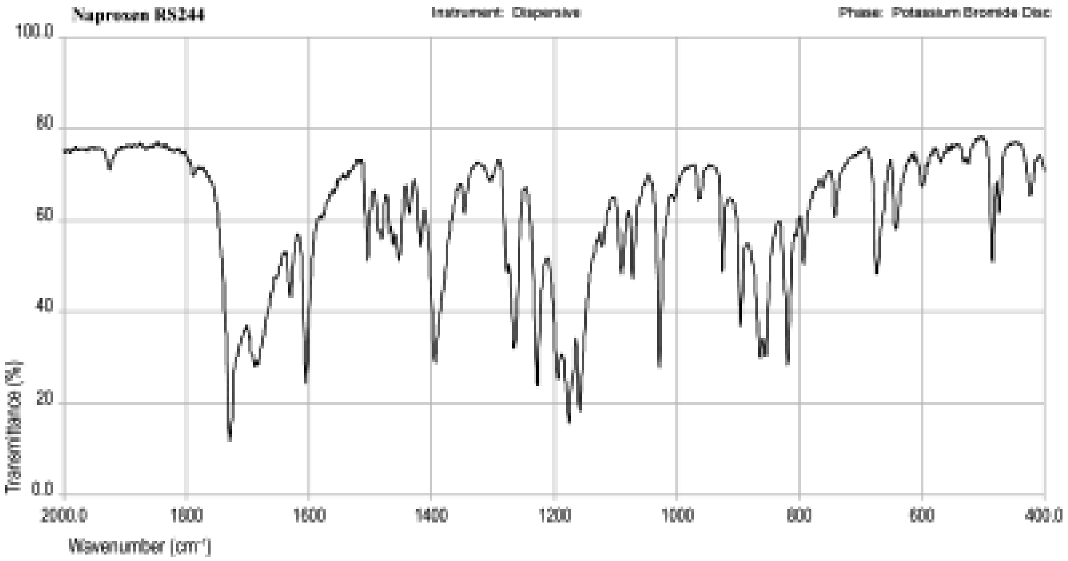
Nandrolone Decanoate



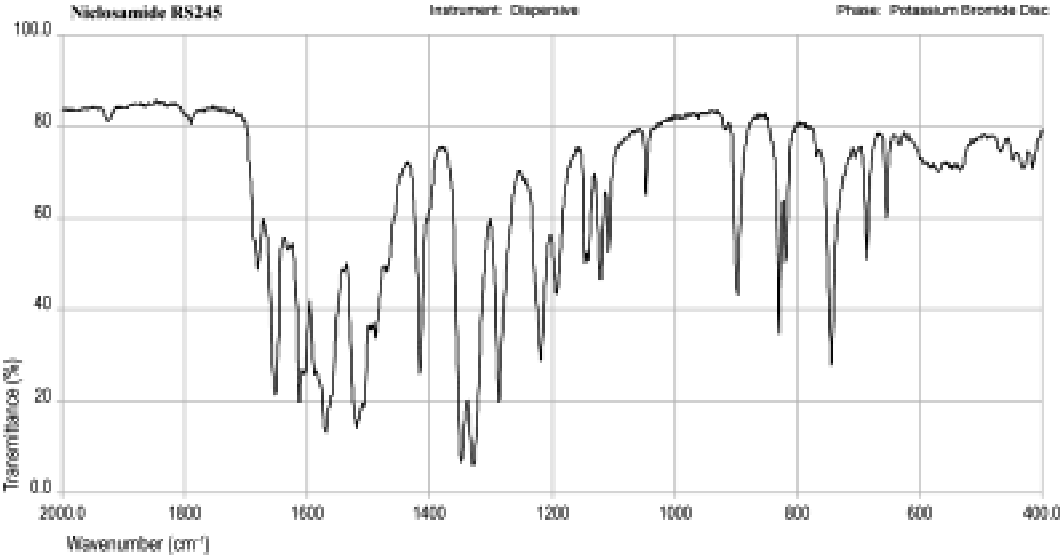
Nandrolone Phenylpropionate



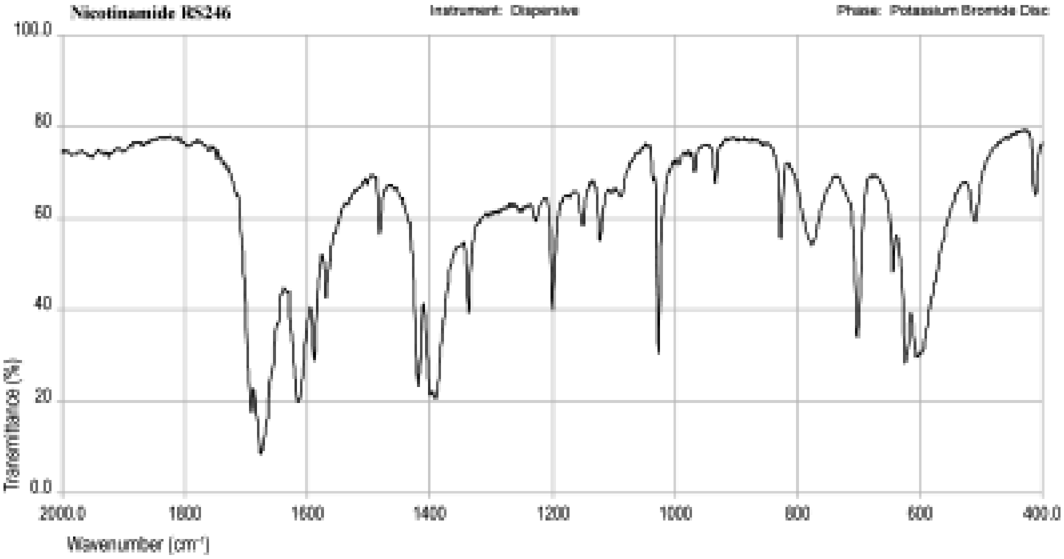
Naproxen


Naproxen RS244
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

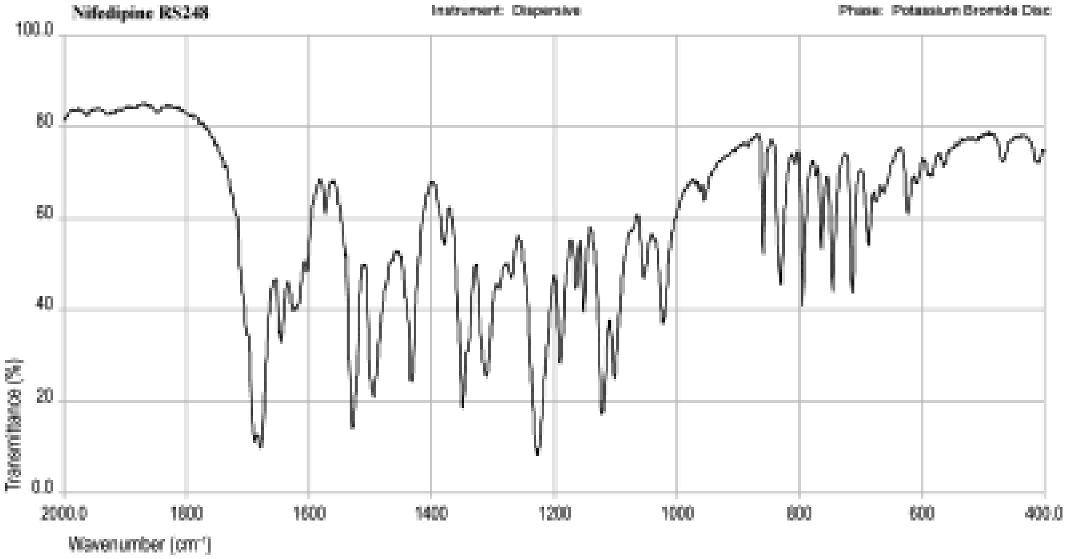
Niclosamide



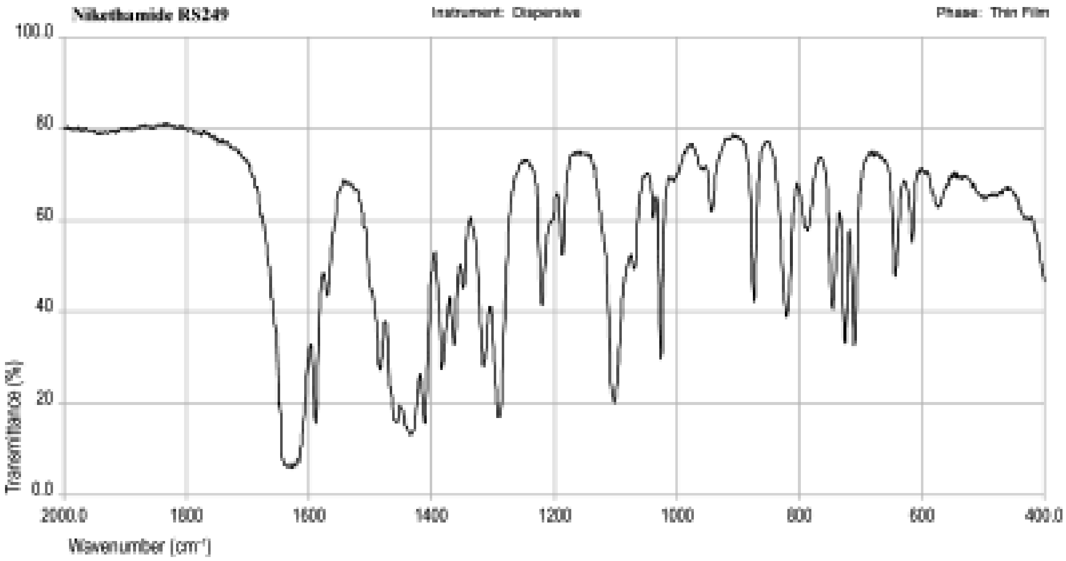
Nicotinamide



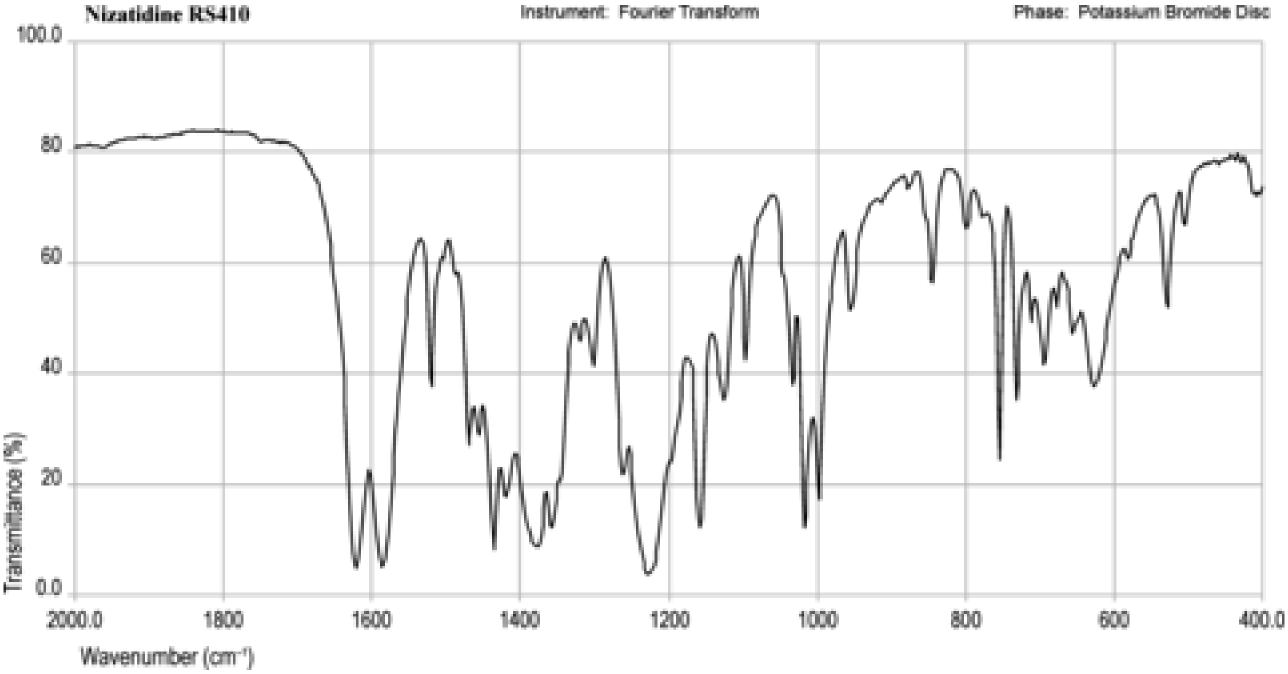
Nifedipine


Nifedipine RS248
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

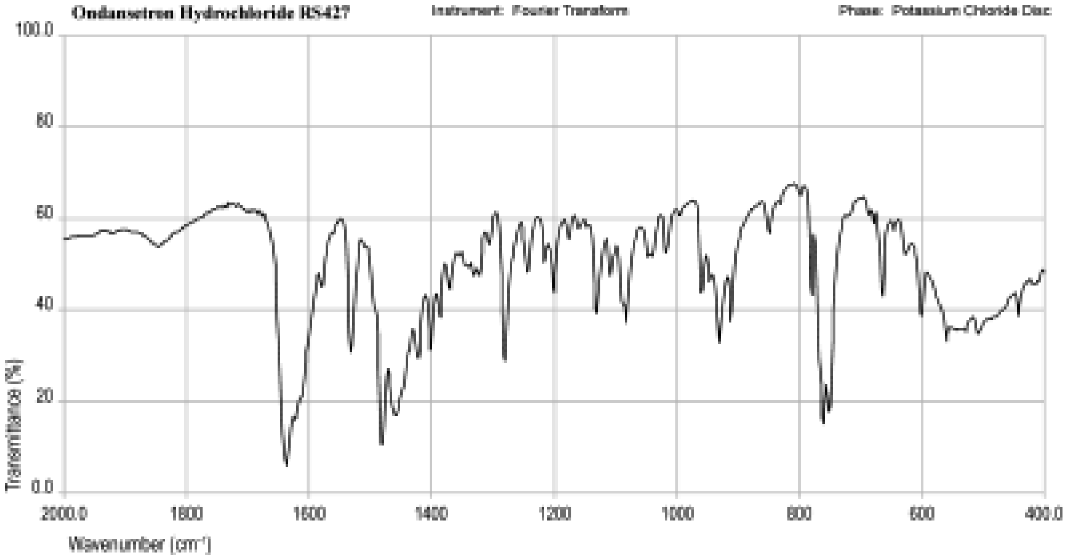
Nikethamide



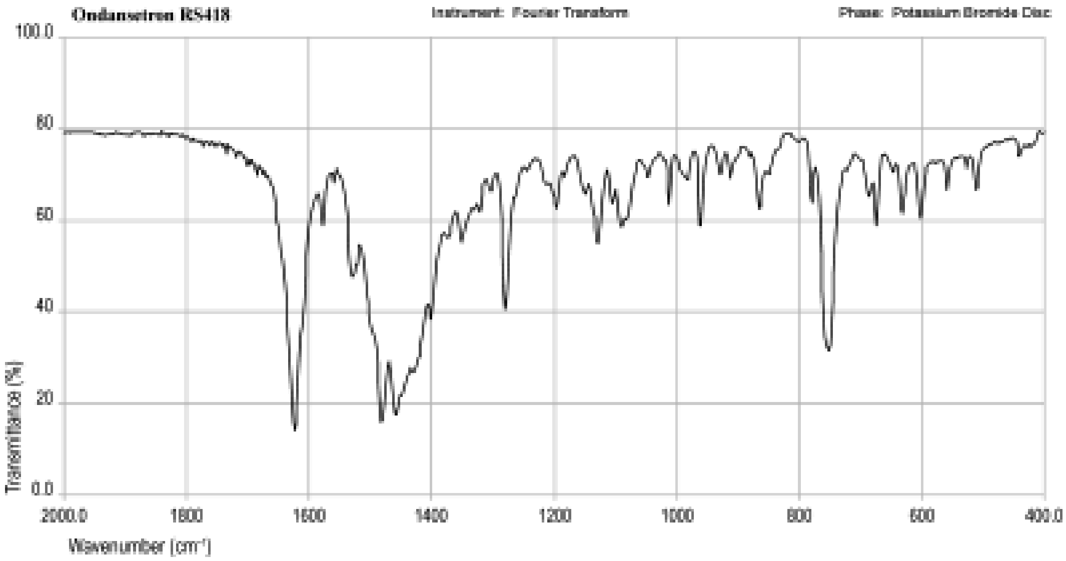
Nizatidine


Nizatidine RS410
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

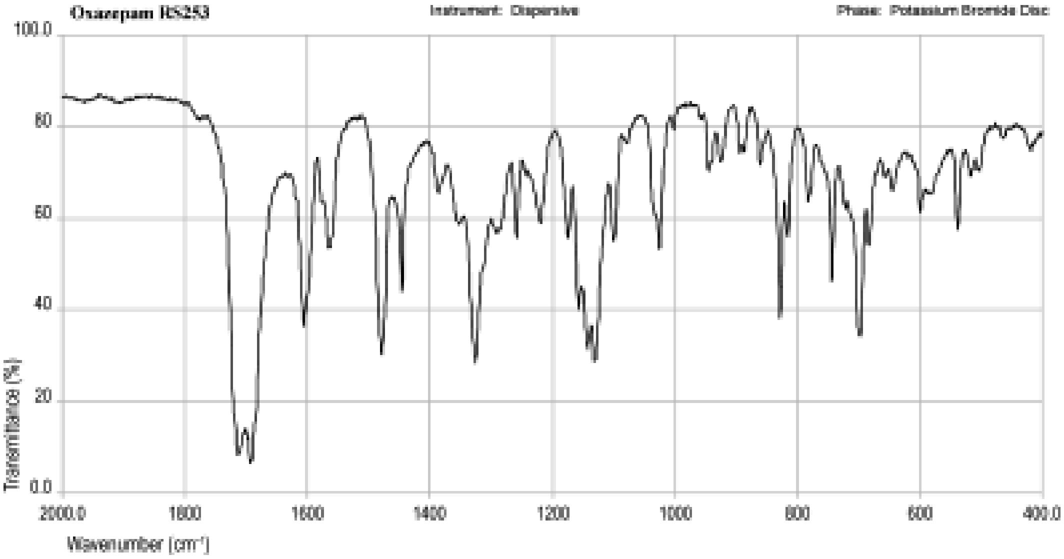
Ondansetron Hydrochloride



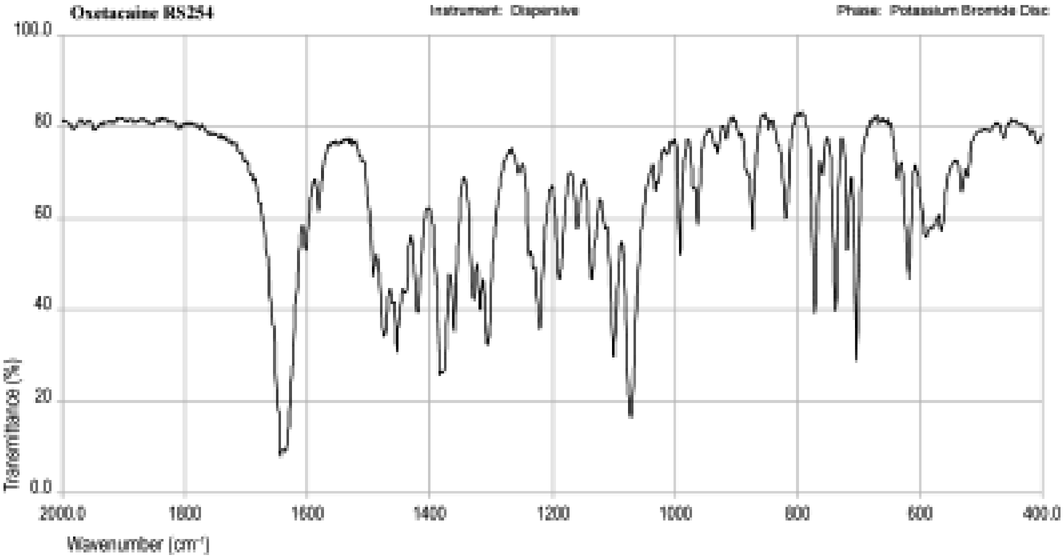
Ondansetron



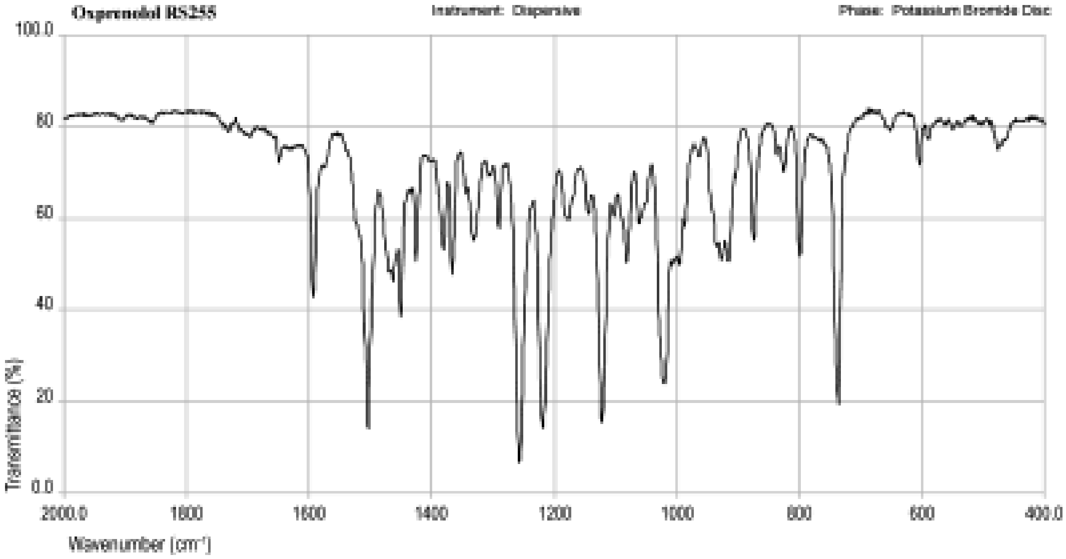
Oxazepam


Oxazepam RS253
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

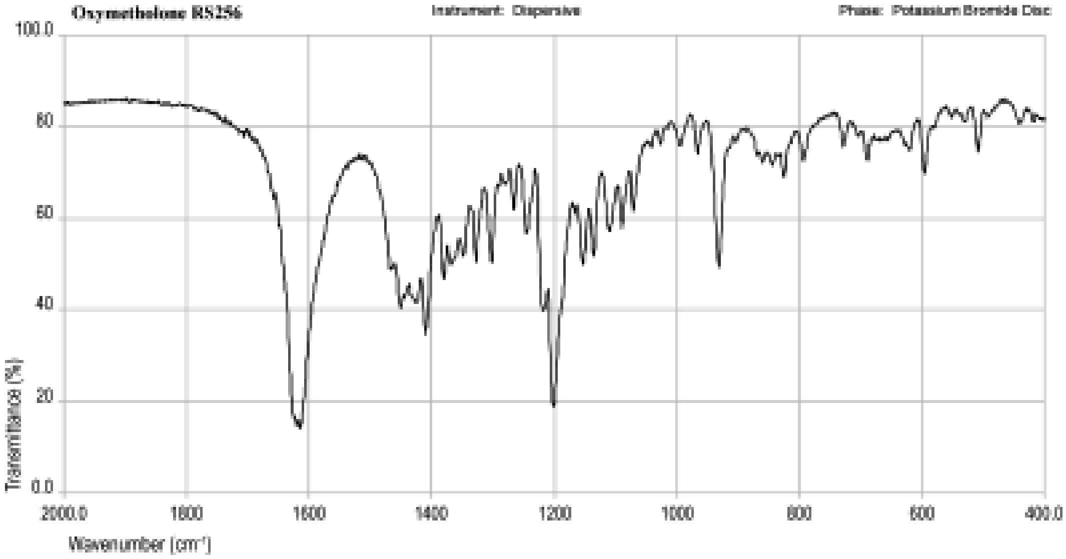
Oxetacaine


Oxetacaine RS254
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

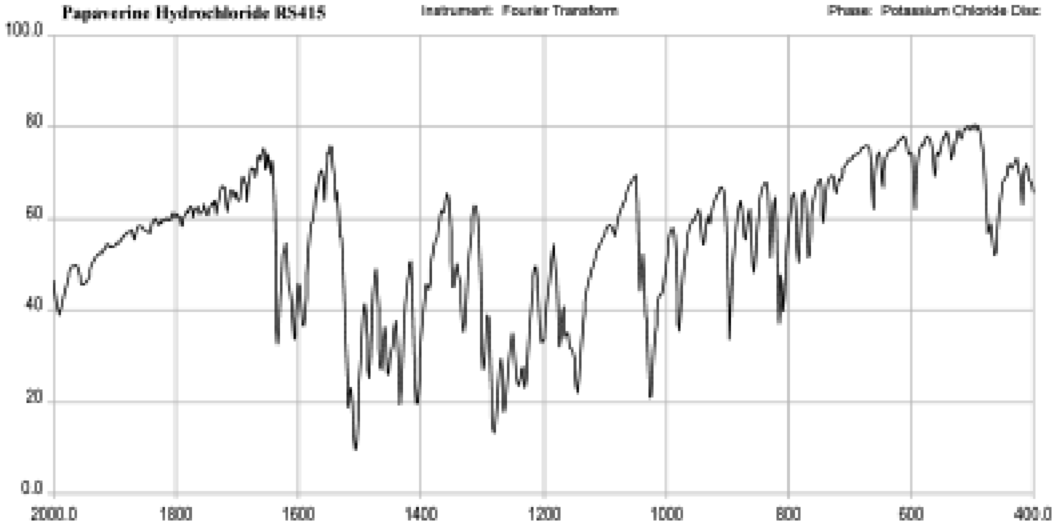
Oxprenolol


Oxprenolol RS255
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

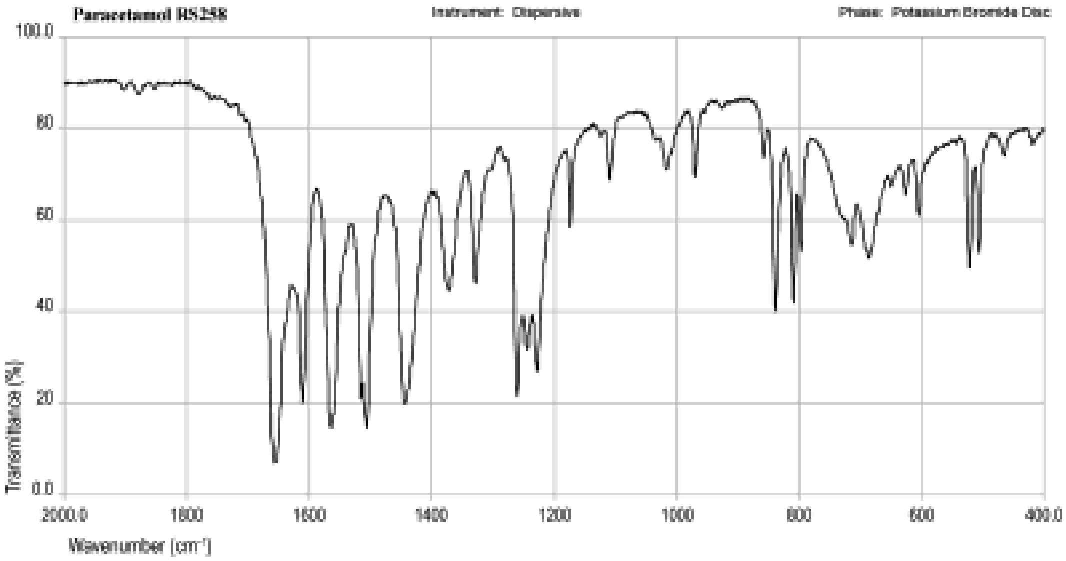
Oxymetholone



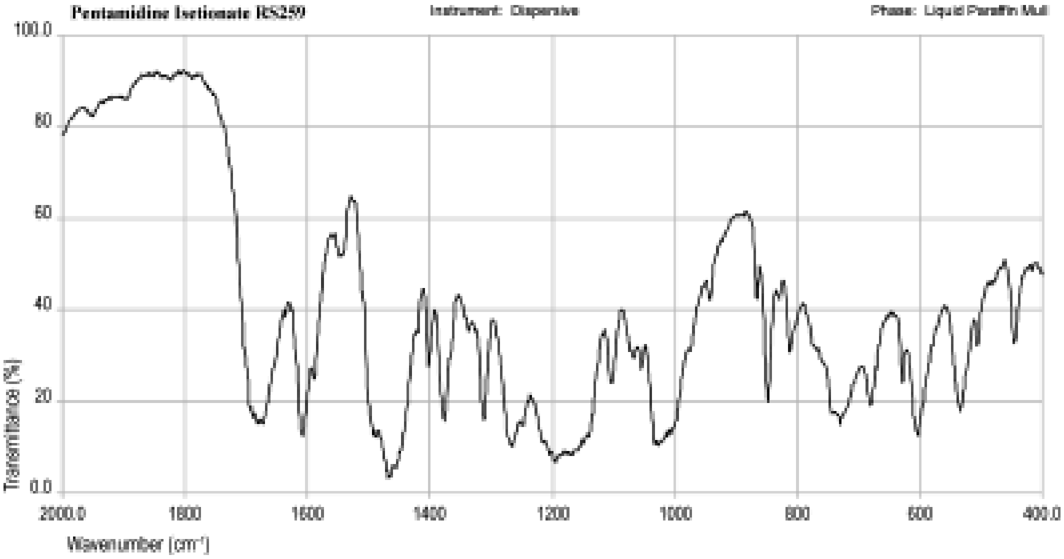
Papaverine Hydrochloride



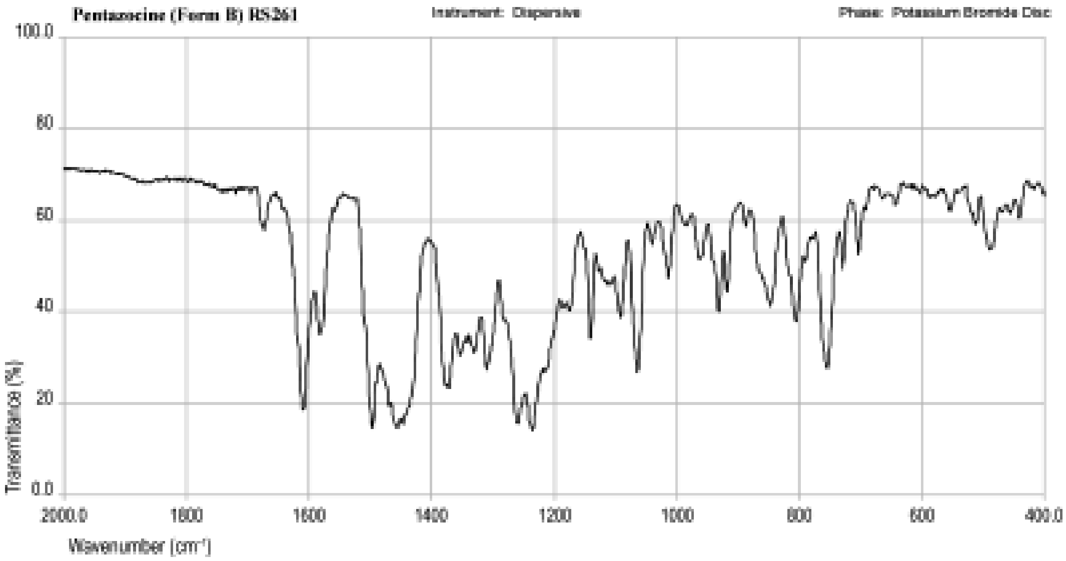
Paracetamol



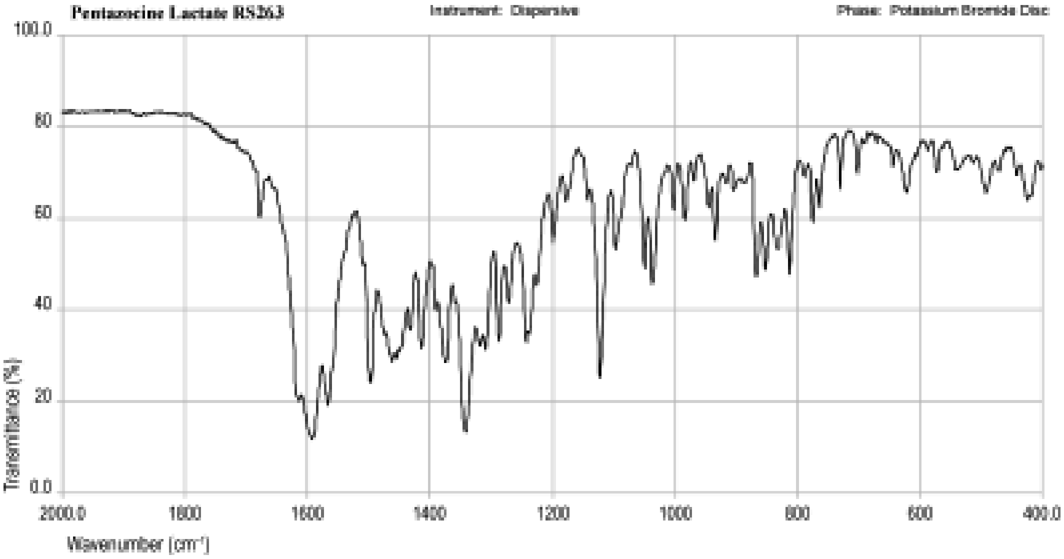
Pentamidine Isetionate



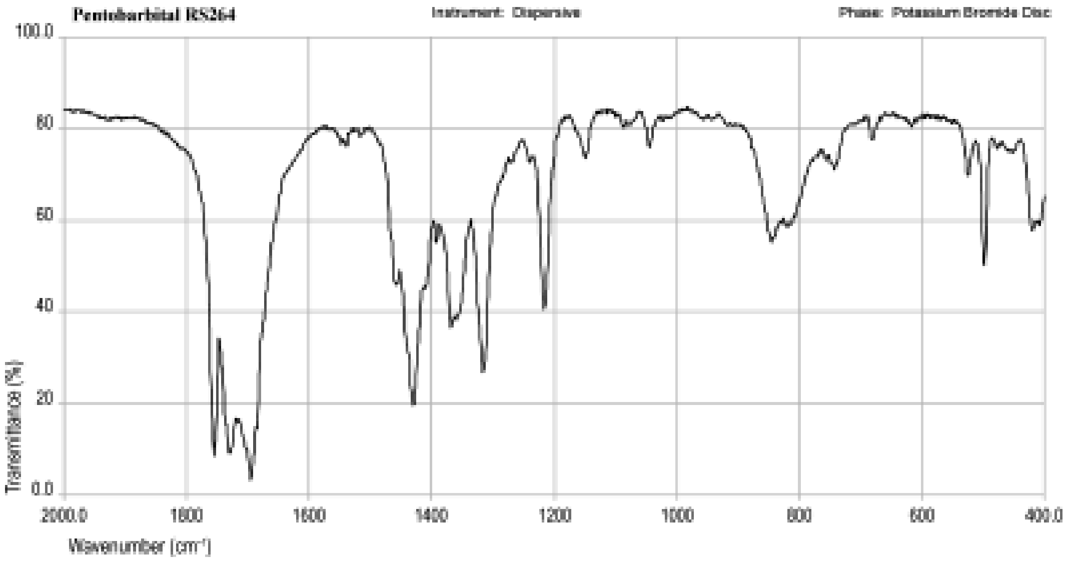
Pentazocine (Form B)



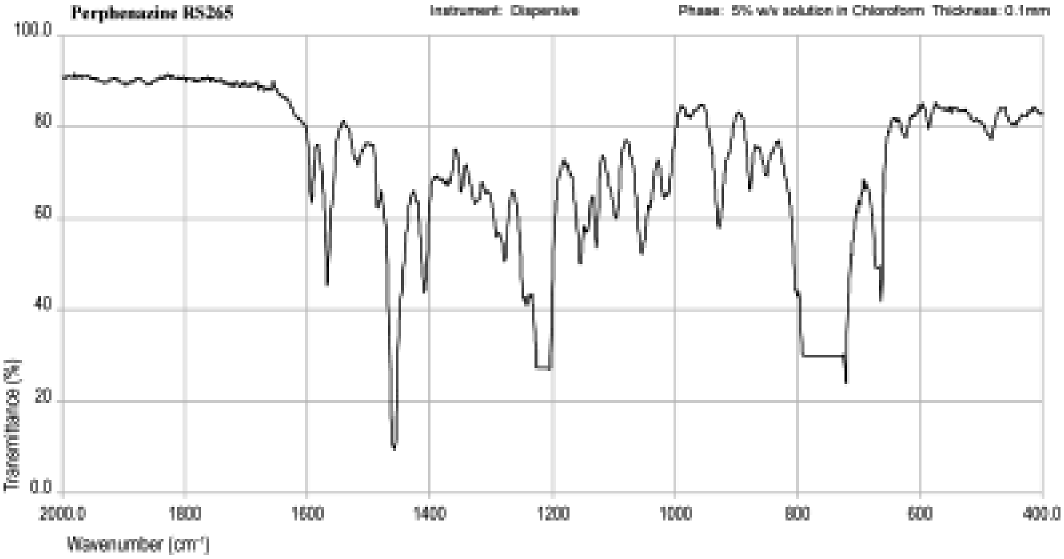
Pentazocine Lactate



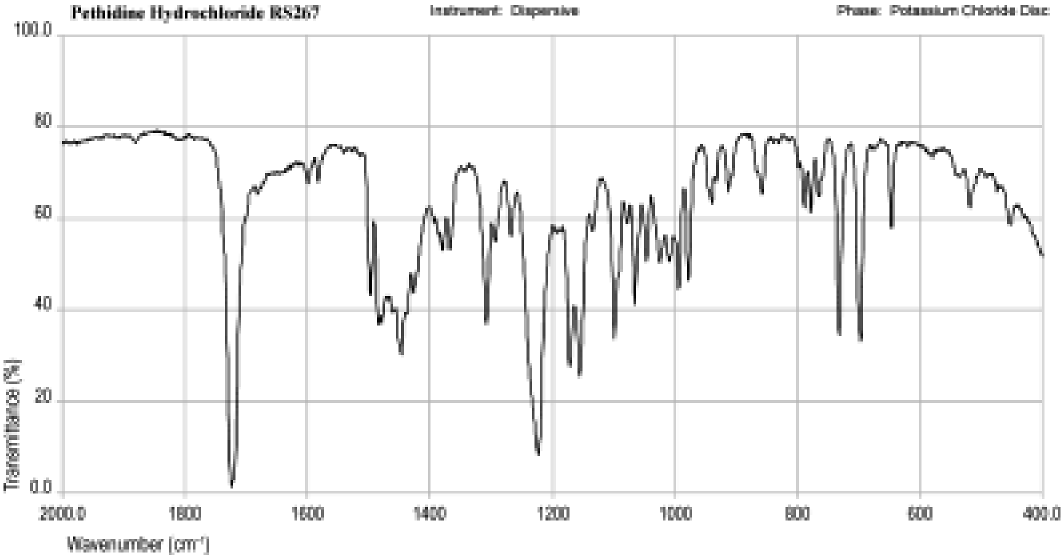
Pentobarbital



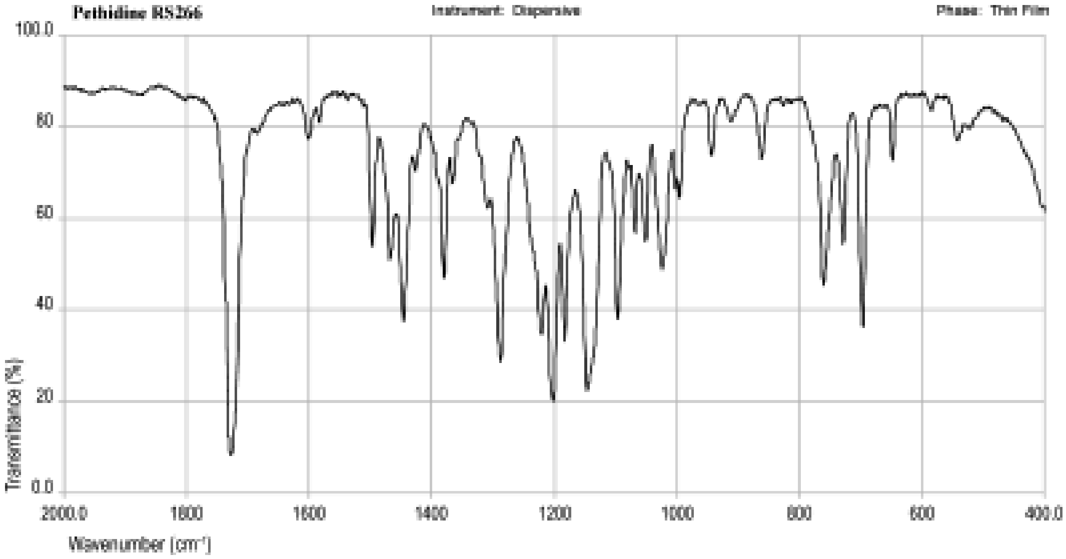
Perphenazine



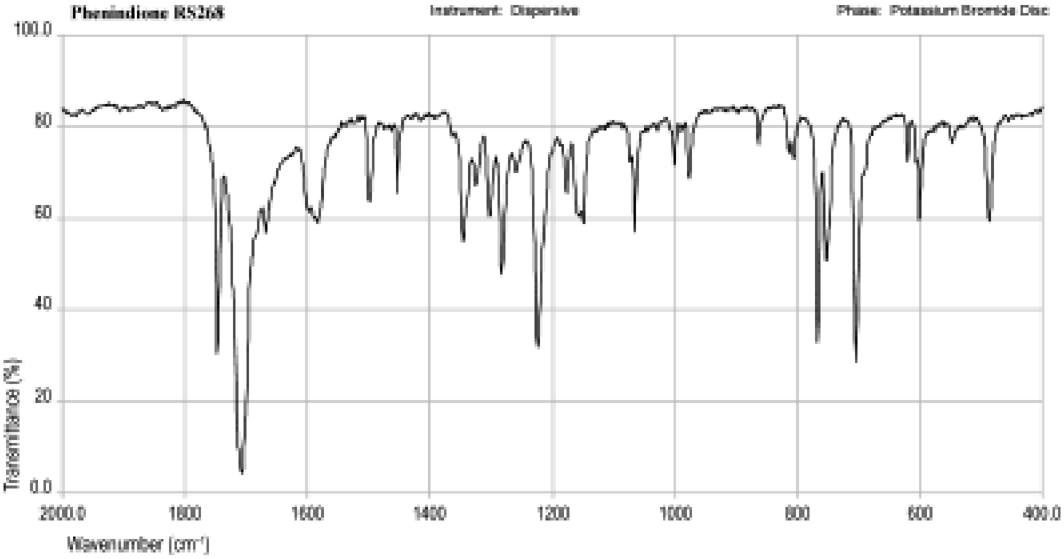
Pethidine Hydrochloride



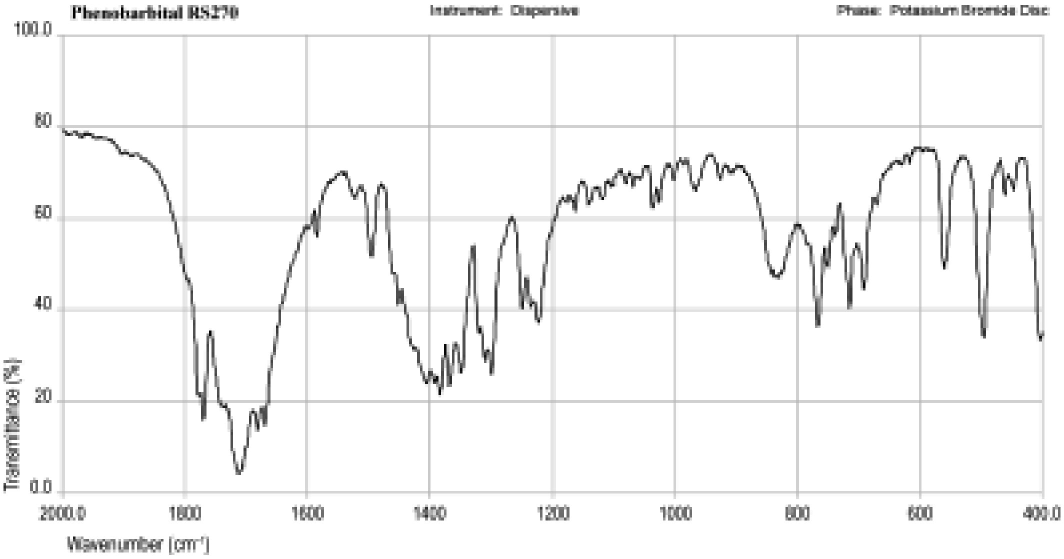
Pethidine


Pethidine RS266
Instrument: Dispersive
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

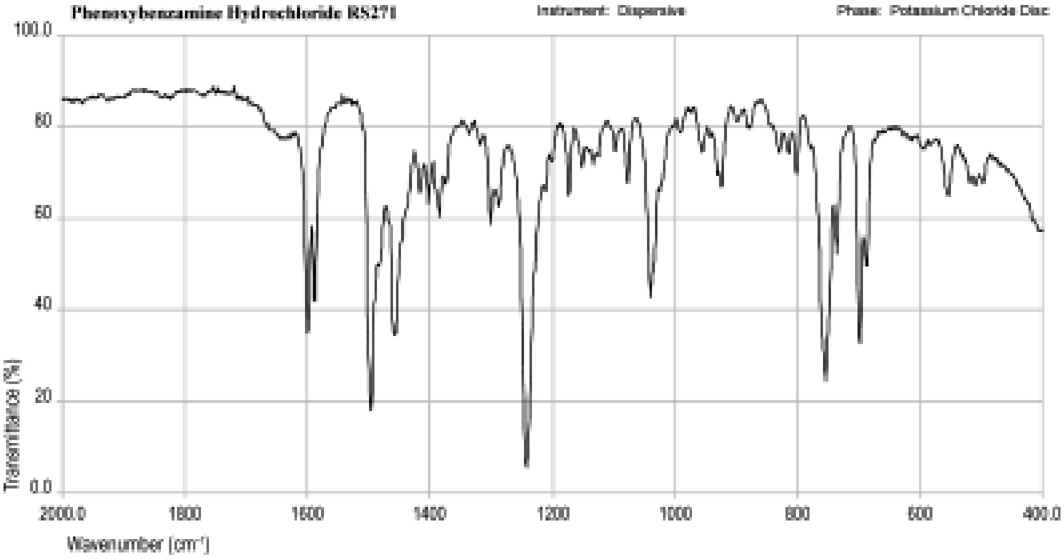
Phenindione



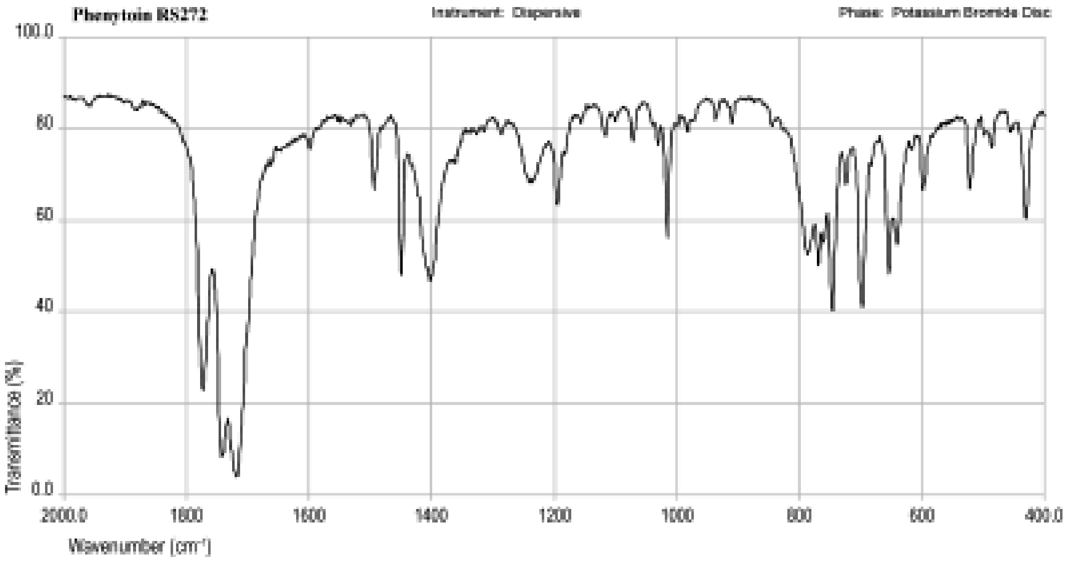
Phenobarbital



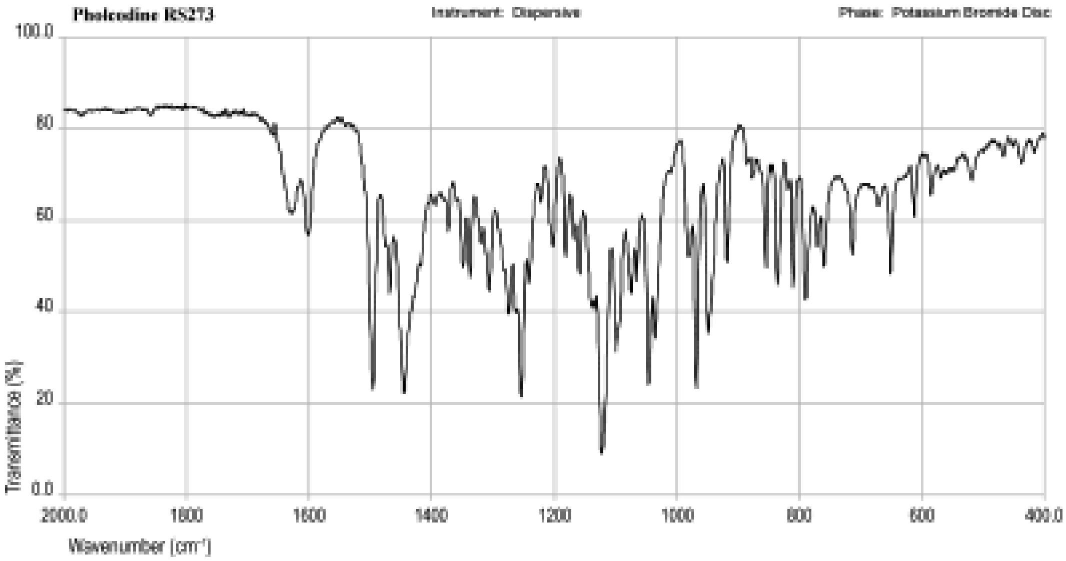
Phenoxybenzamine Hydrochloride



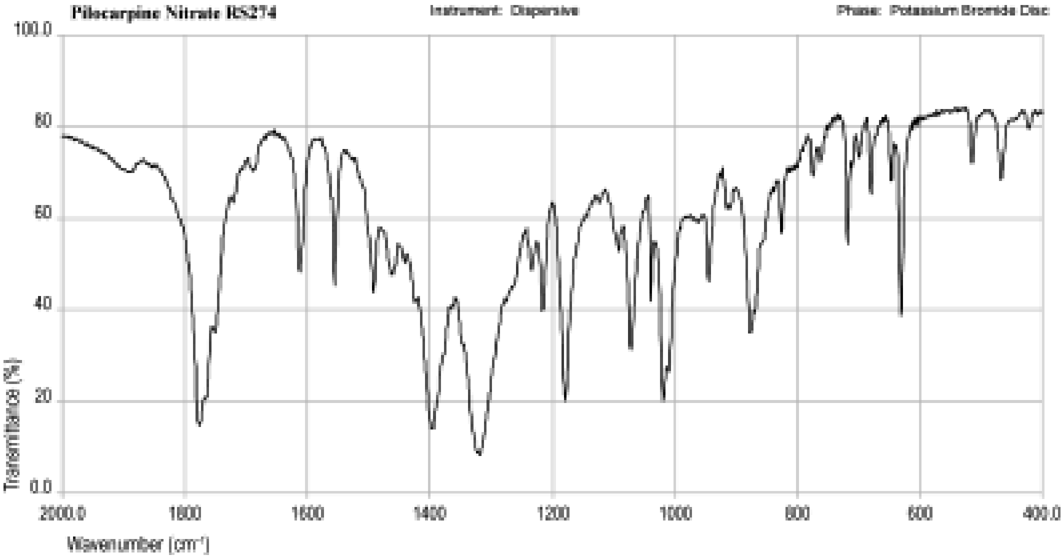
Phenytoin


Phenytoin RS272
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

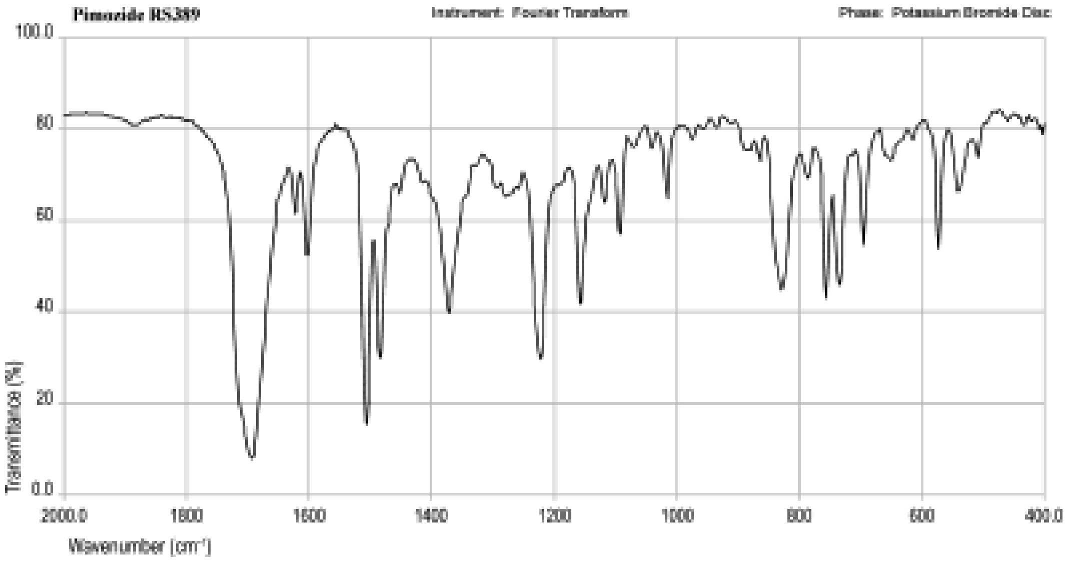
Pholcodine


Pholcodine RS273
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

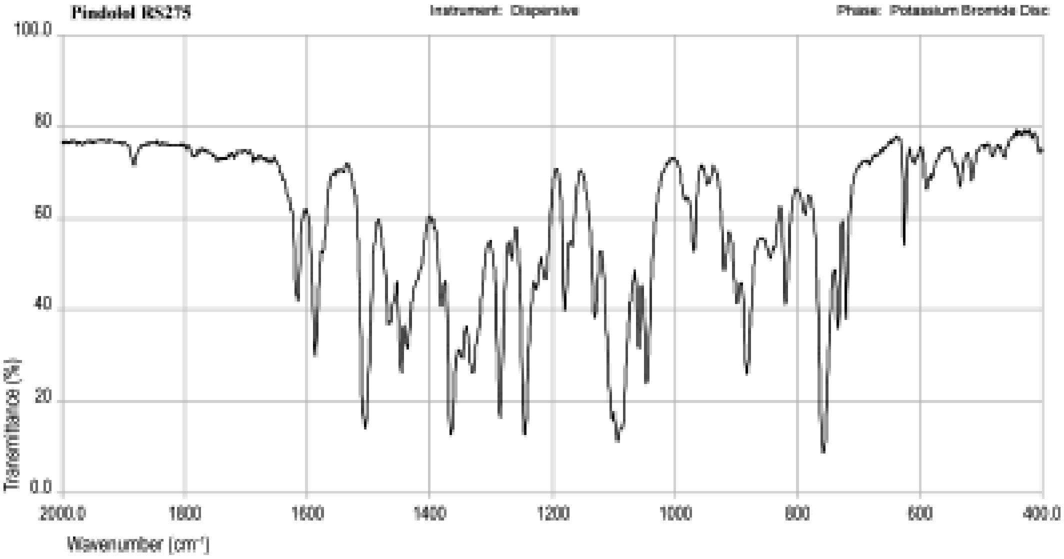
Pilocarpine Nitrate



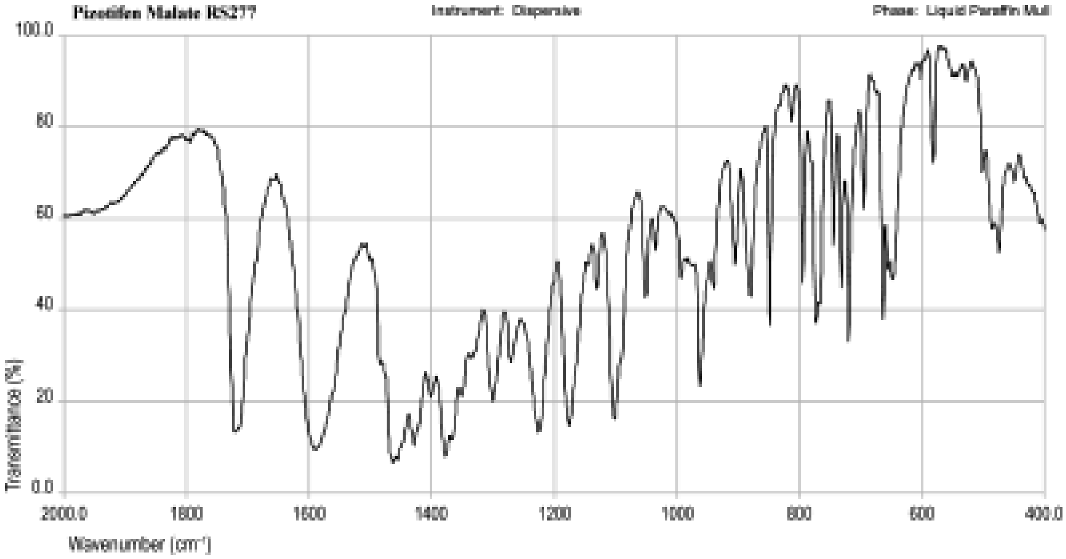
Pimozide


Pimozide RS389
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

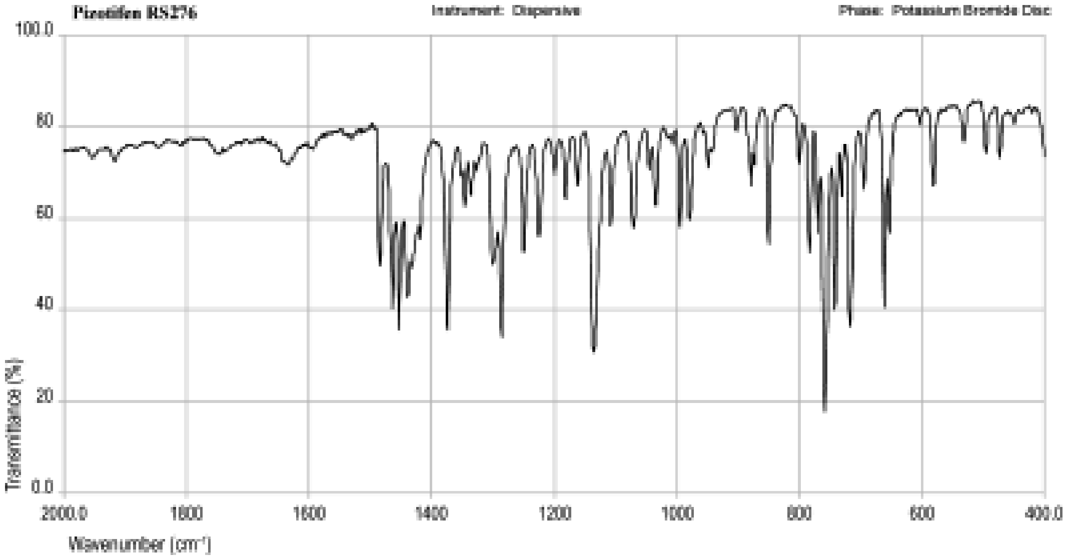
Pindolol


Pindolol RS275
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

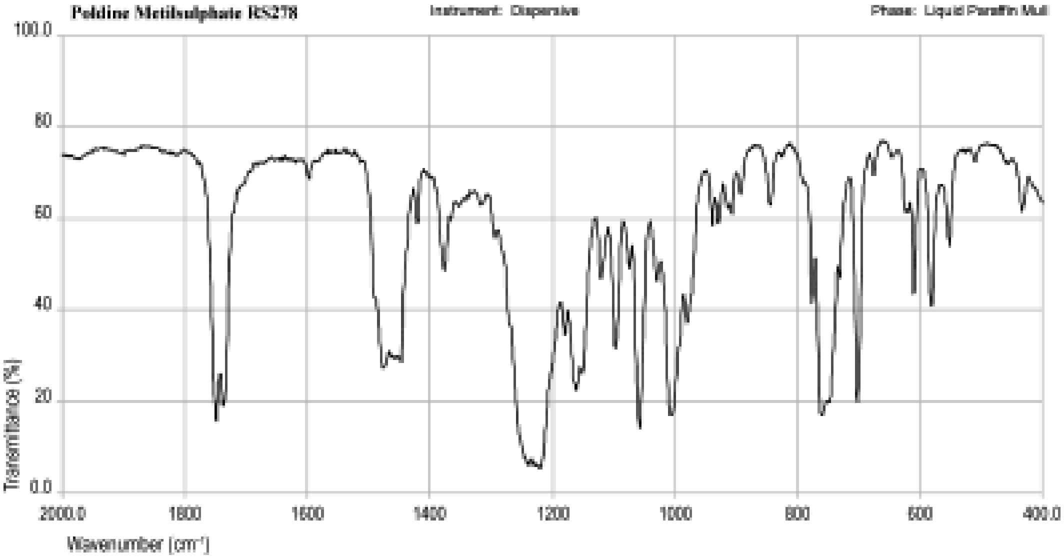
Pizotifen Malate



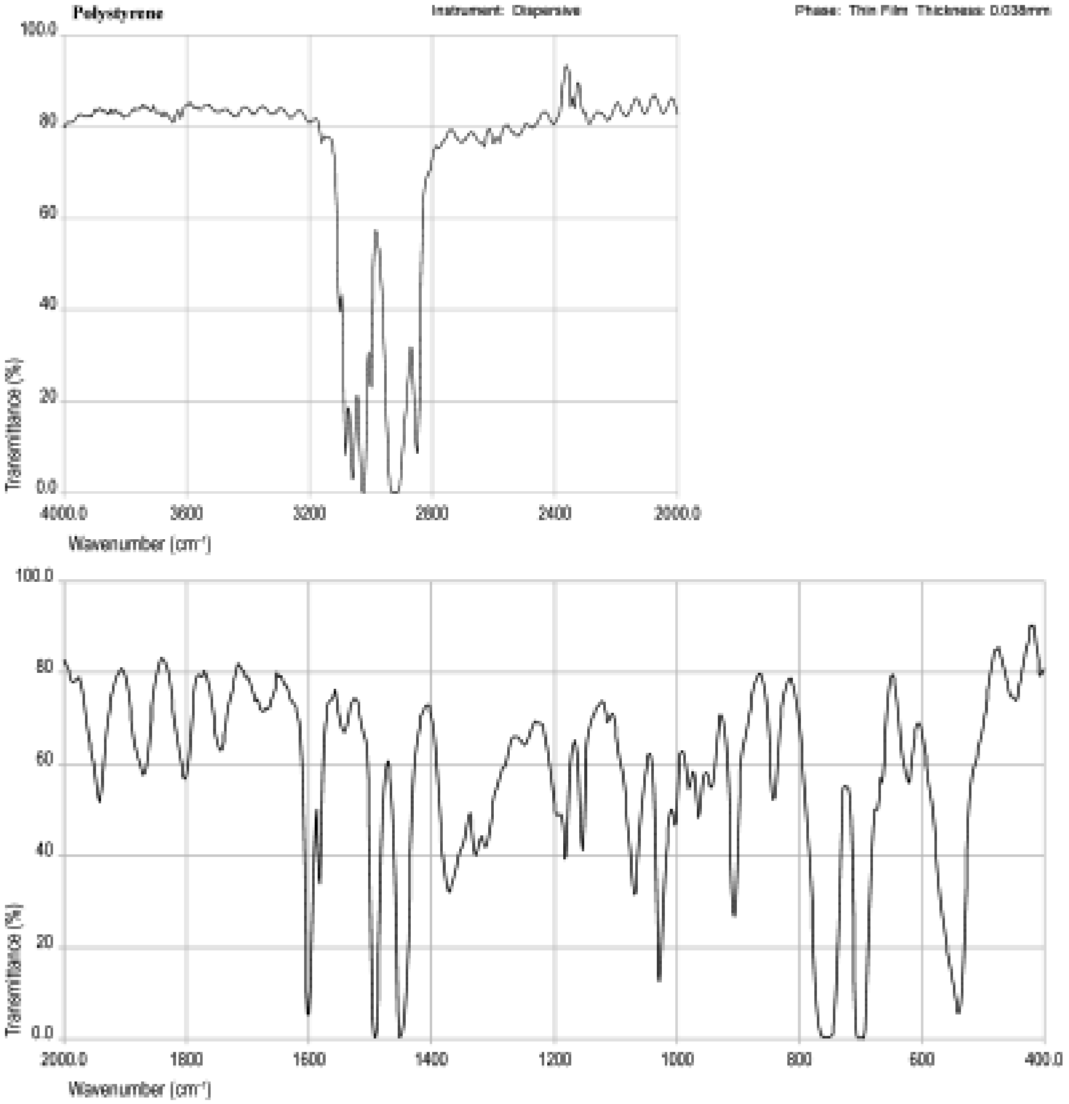
Pizotifen


Pizotifen RS276
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

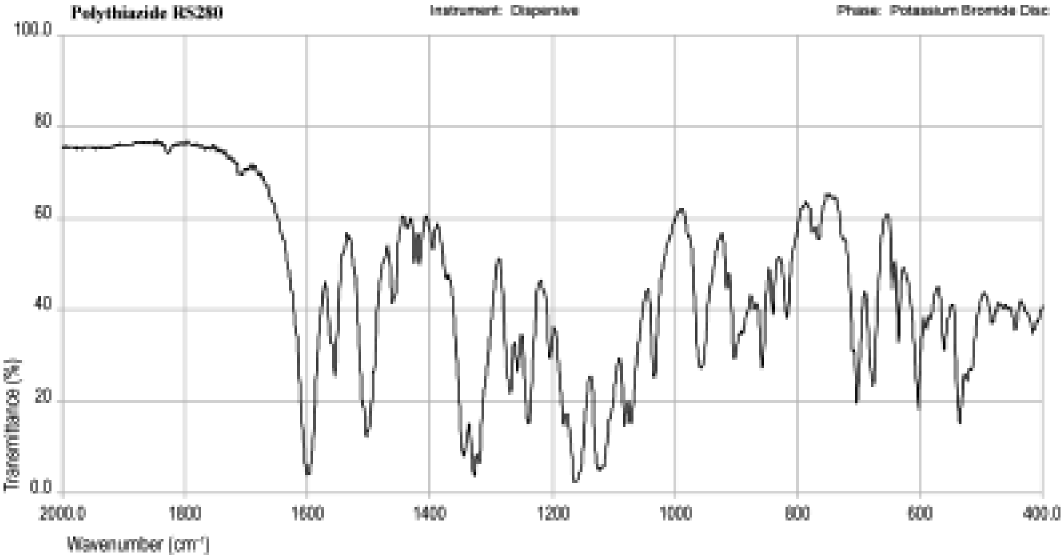
Poldine Metilsulphate



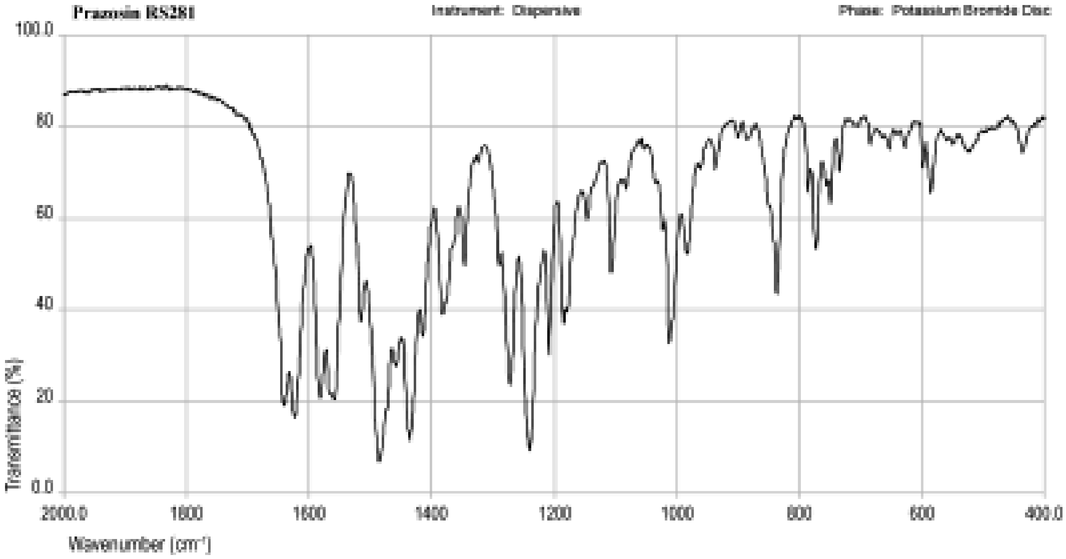
Polystyrene



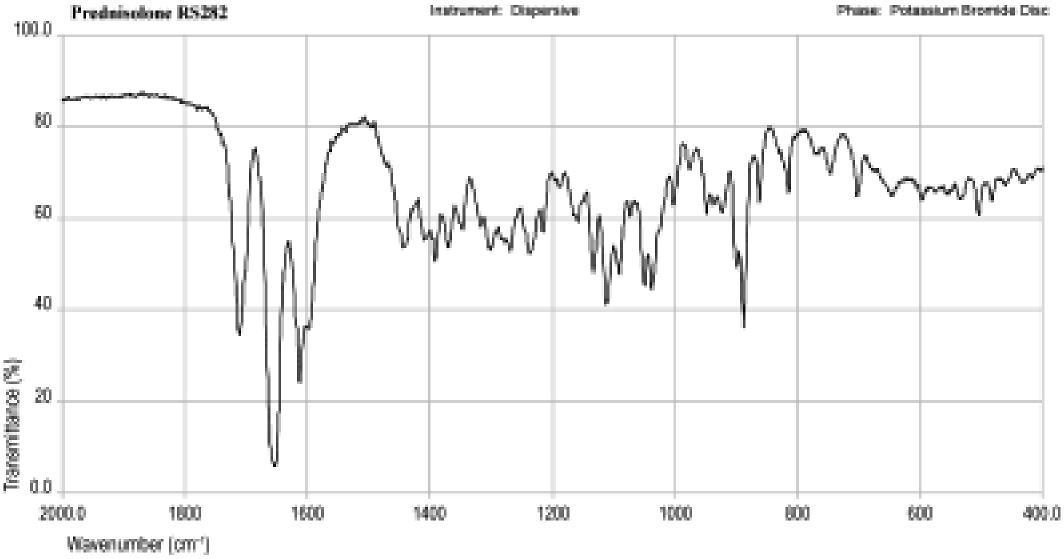
Polythiazide



Prazosin


Prazosin RS281
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

Prednisolone



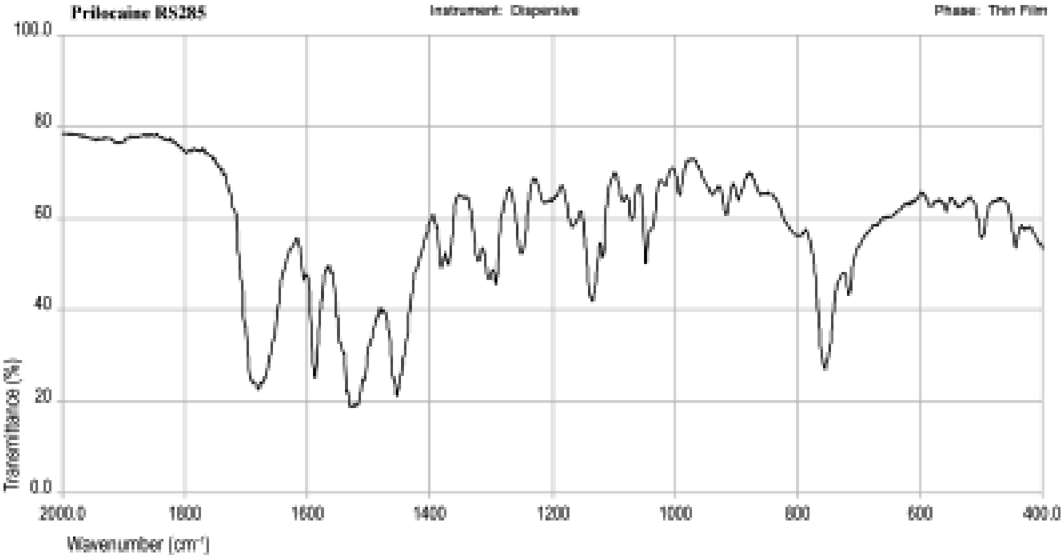
Preparation of Infrared Reference Spectra

All spectra presented in this section were recorded using either a Perkin-Elmer model 682 dispersive infrared spectrophotometer or a Perkin Elmer model 16PC Fourier transform infrared spectrophotometer.

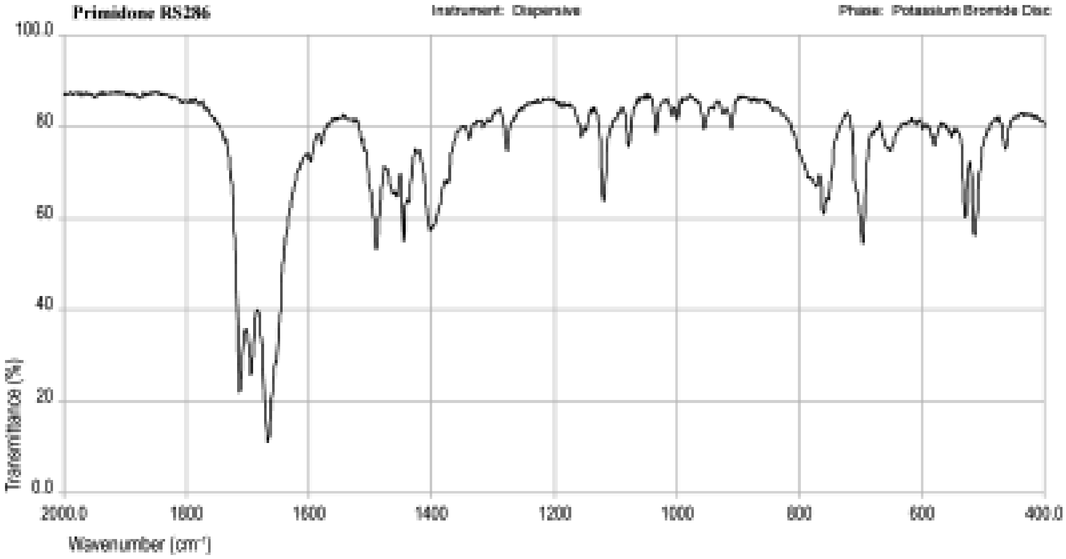
Pressed discs, 13 mm in diameter, were prepared using potassium bromide or potassium chloride. Liquid paraffin mulls and thin films were prepared between potassium bromide plates, and gas and solution spectra were prepared using cells with potassium bromide windows. Solution spectra were prepared against a solvent reference and all other spectra were recorded against air.

For solution spectra the regions of the spectrum within which the solvent shows strong absorption should be disregarded. Solvent 'cut-offs' in the reference spectra may be recorded as horizontal straight lines or may appear as blank regions on the spectrum.

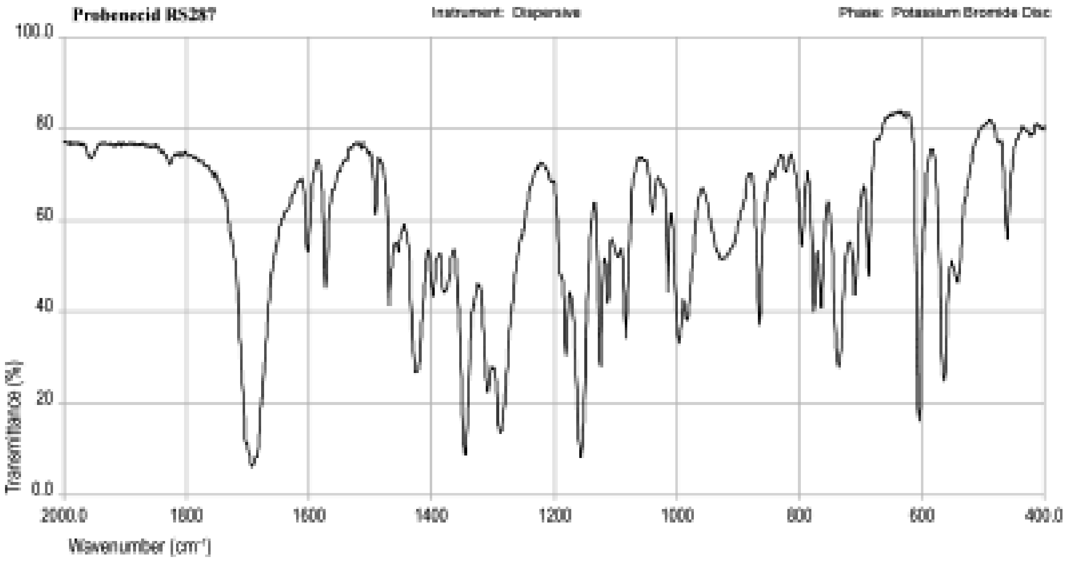
Prilocaine


Prilocaine RS285
Instrument: Dispersive
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

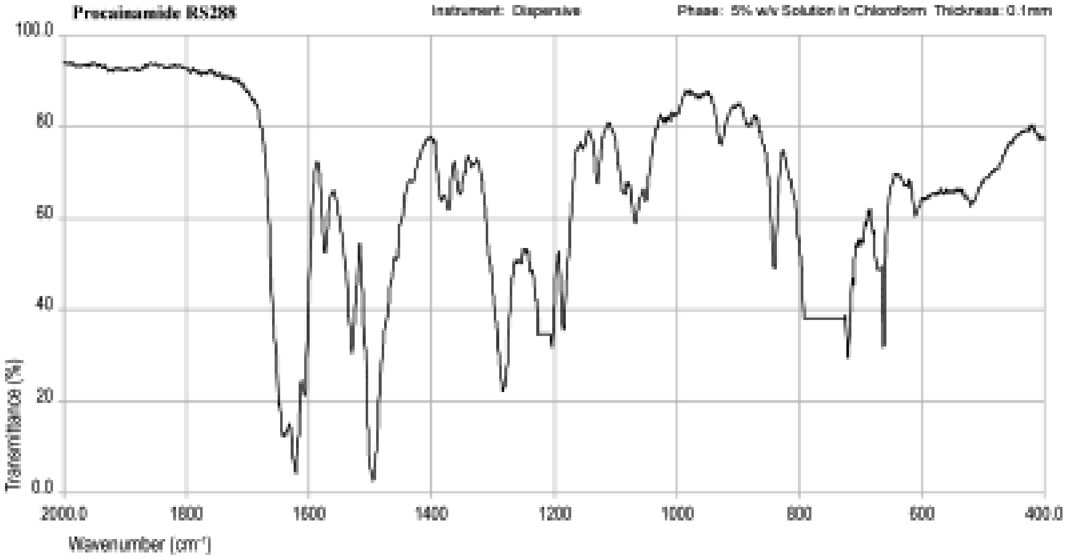
Primidone


Primidone RS286
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

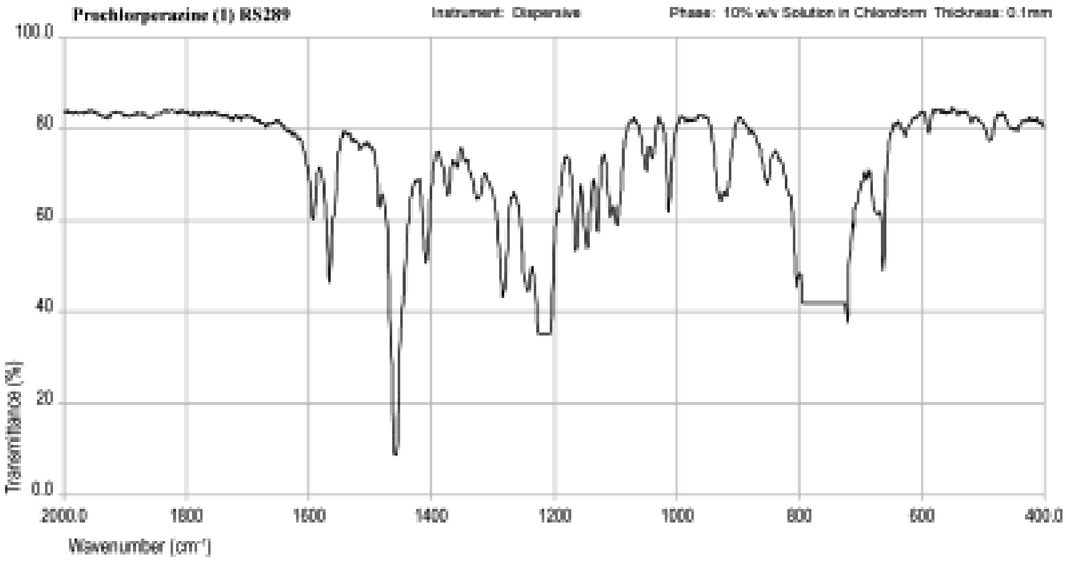
Probenecid


Probenecid RS287
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

Procainamide

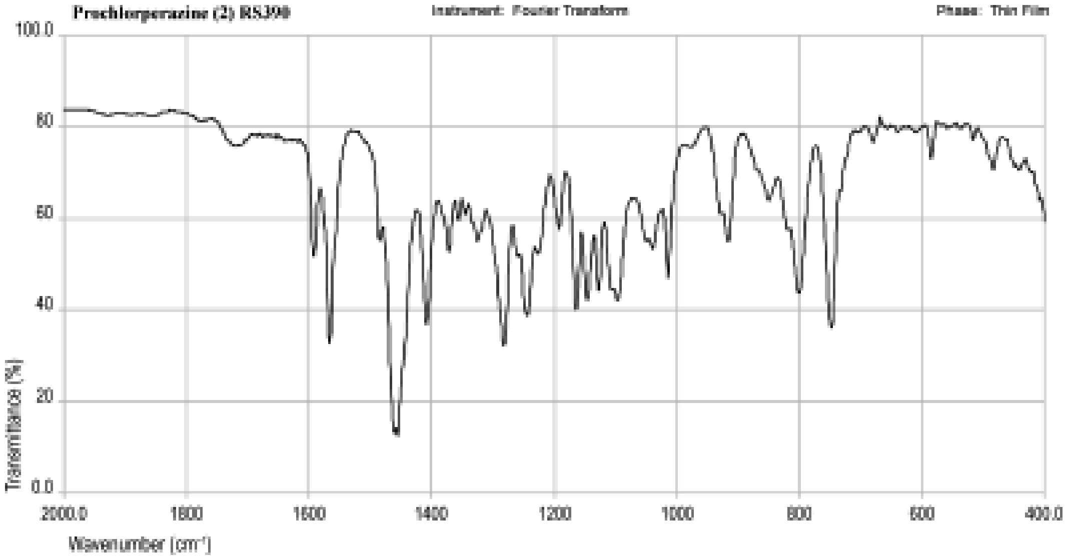


Prochlorperazine (1)

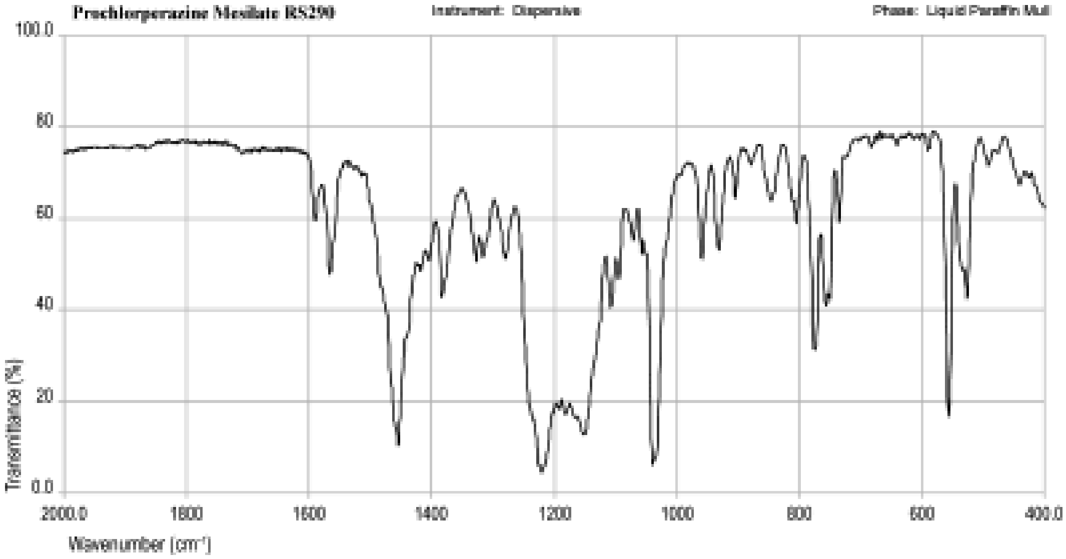


Prochlorperazine (1) RS289 Instrument: Dispersive Phase: 10% w/v Solution in Chloroform Thickness: 0.1mm

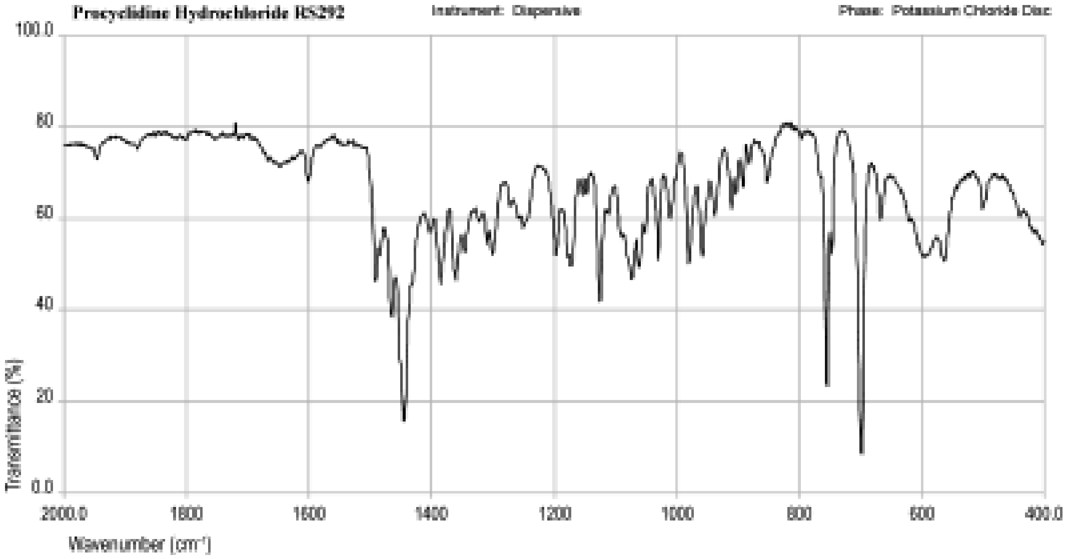
Prochlorperazine (2)



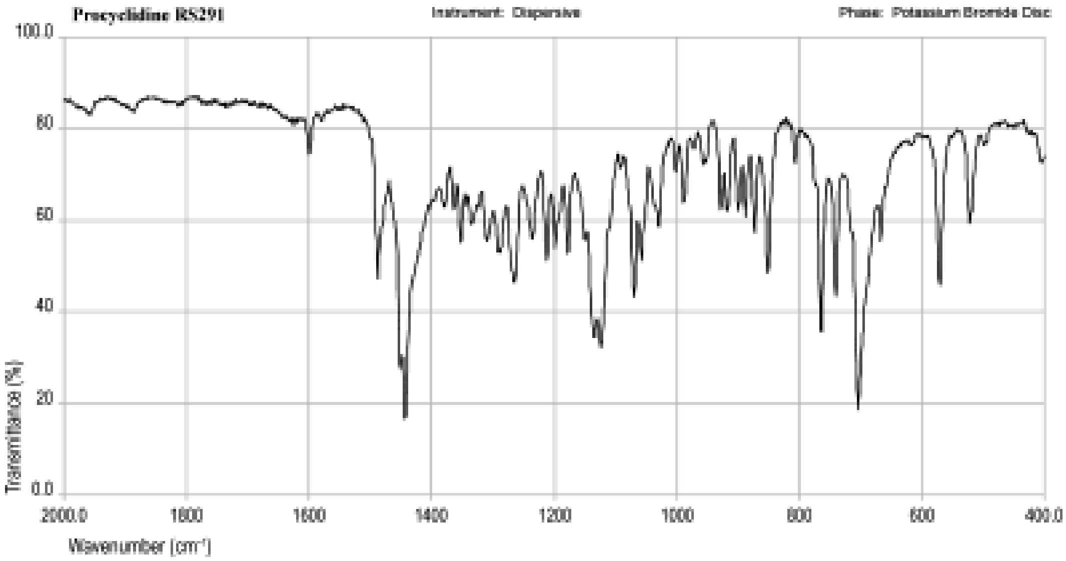
Prochlorperazine Mesilate



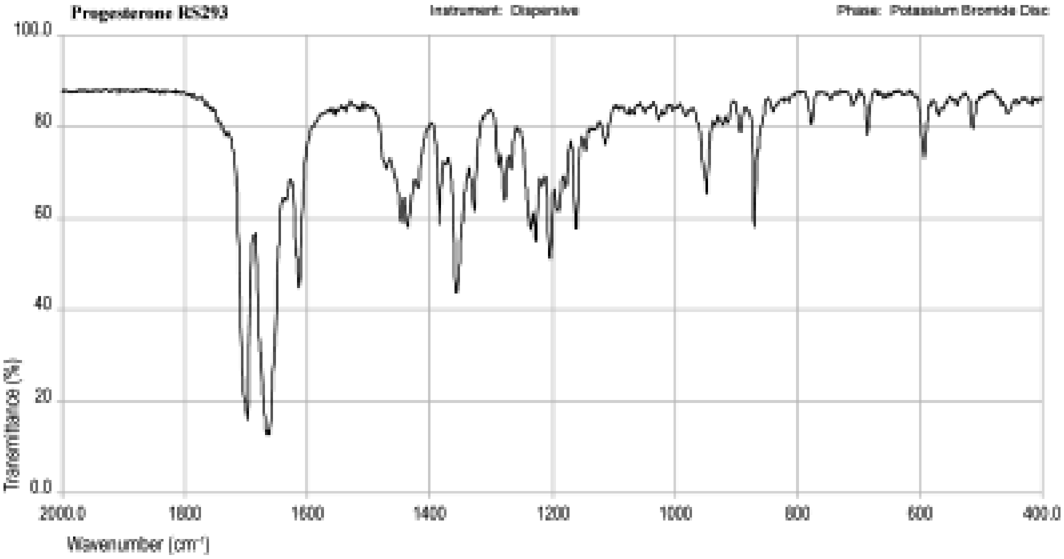
Procyclidine Hydrochloride



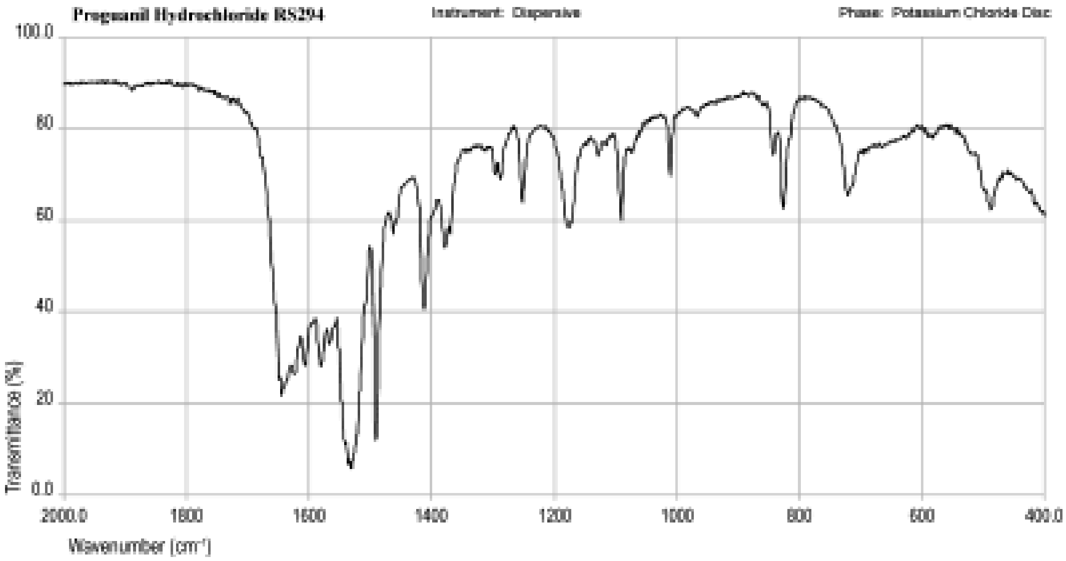
Procyclidine



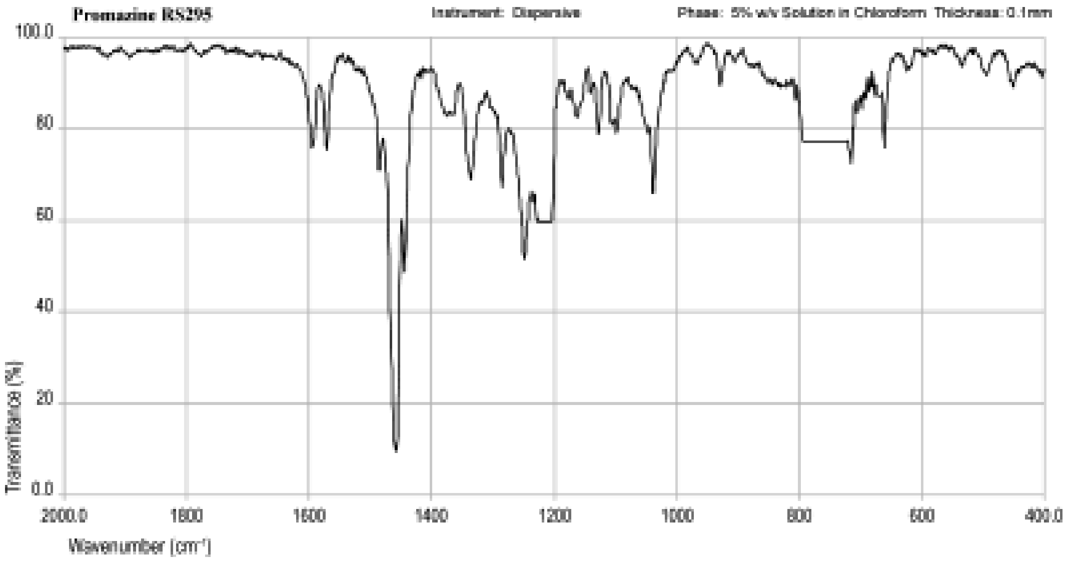
Progesterone



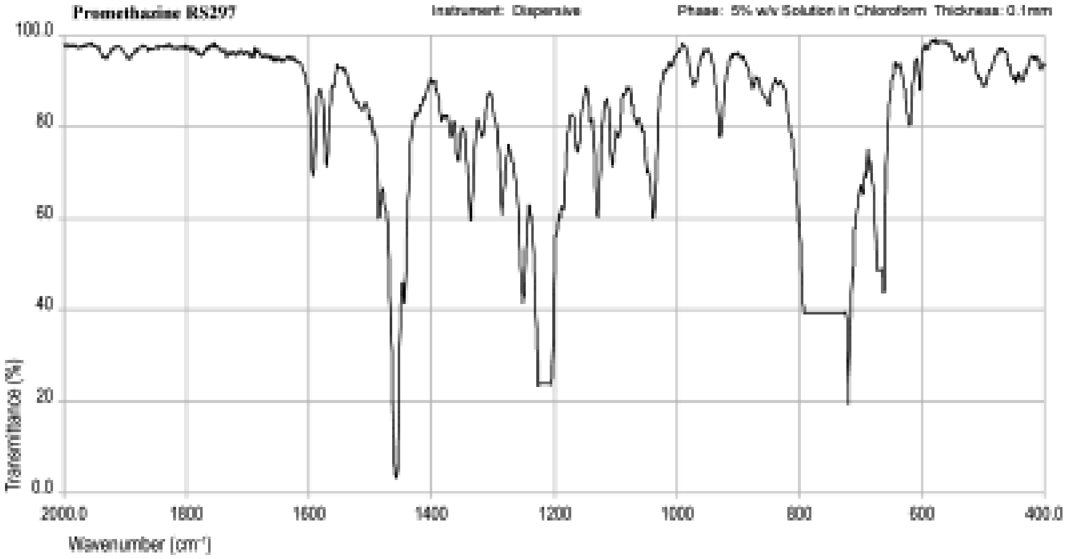
Proguanil Hydrochloride



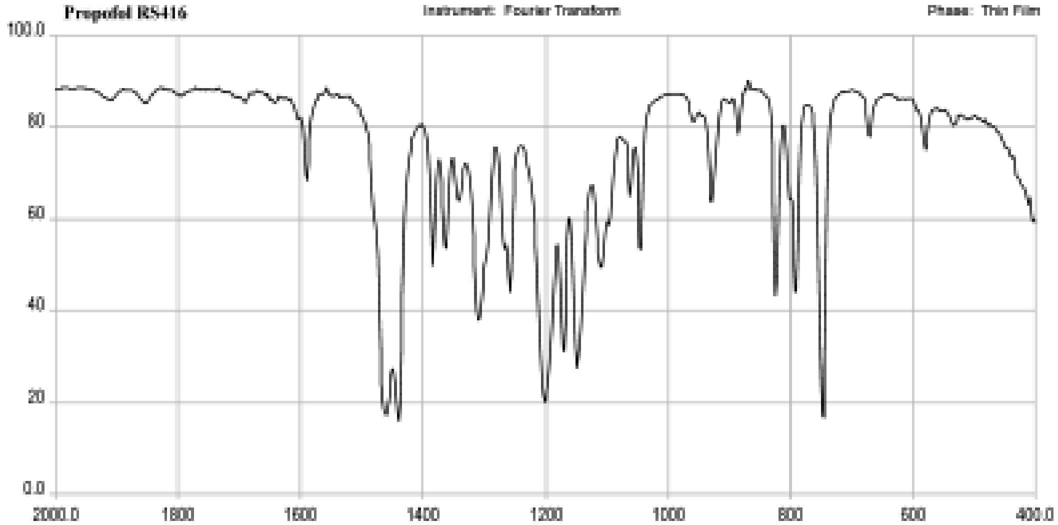
Promazine


Promazine RS295
Instrument: Dispersive
Phase: 5% w/v Solution in Chloroform Thickness: 0.1mm
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

Promethazine



Propofol


Propofol RS416
Instrument: Fourier Transform
Phase: Thin Film
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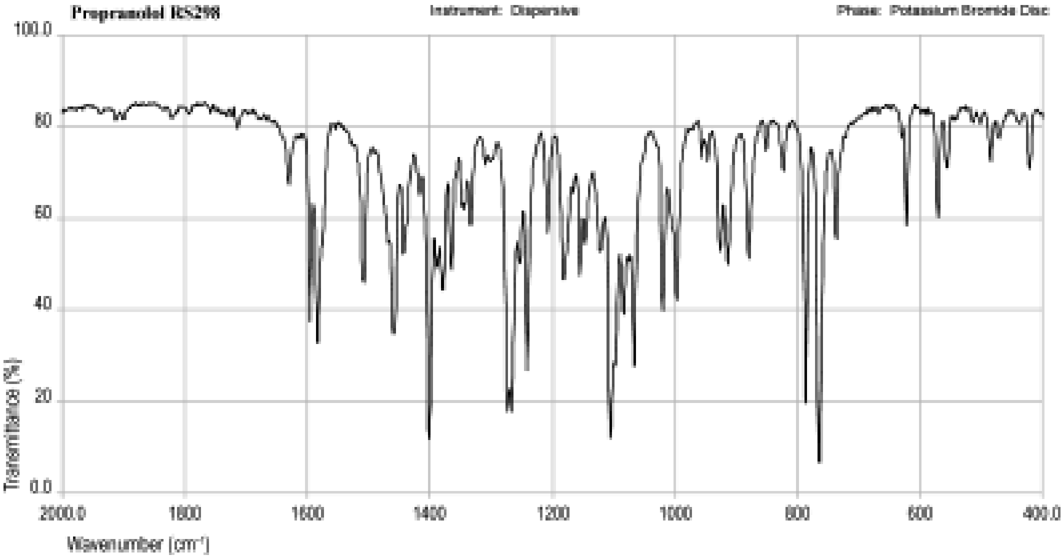
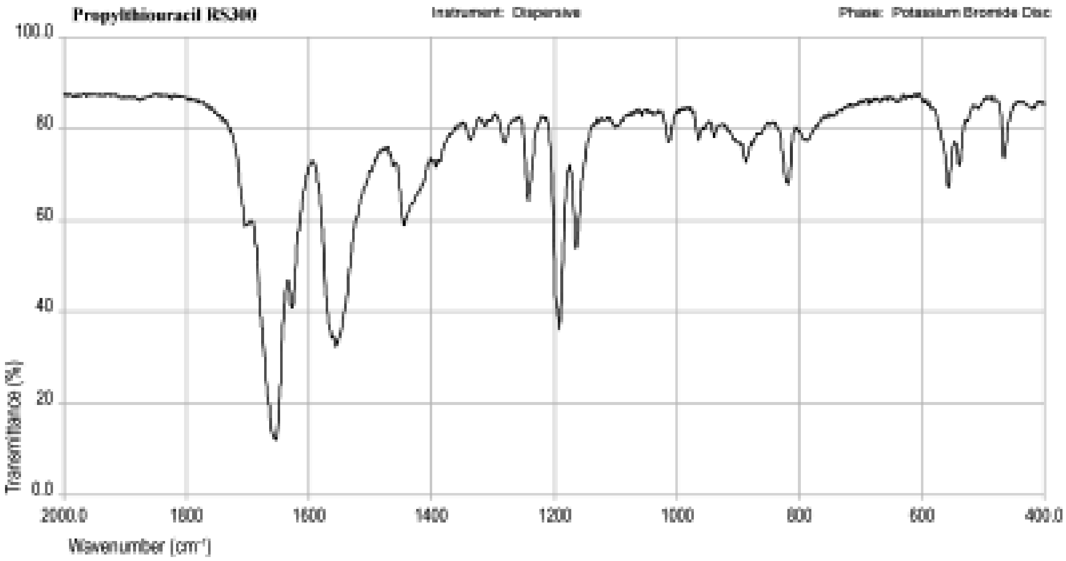
80
60
40
20
0.0
2000.0
1800
1600
1400
1200
1000
800
600
400.0

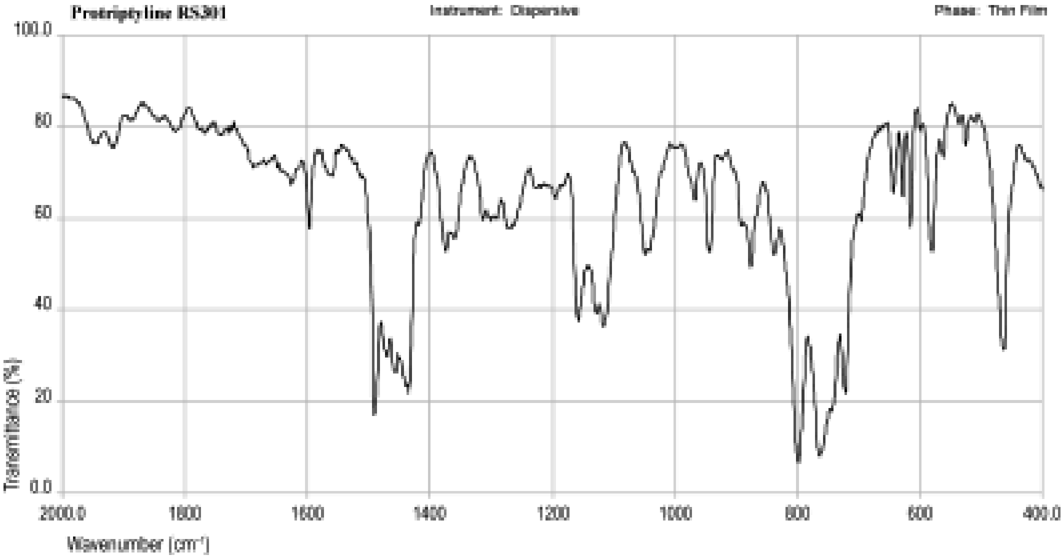
Propranolol



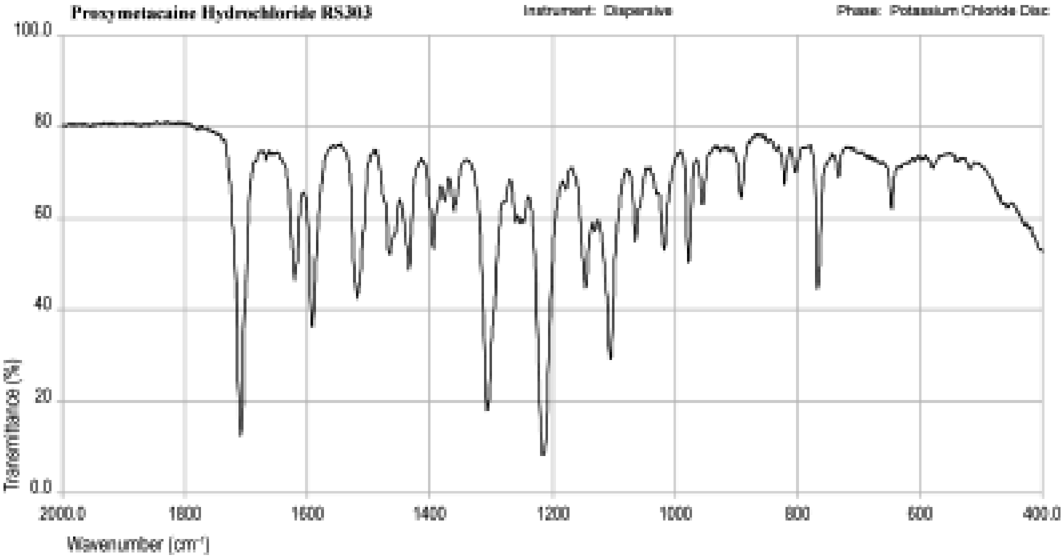
Propylthiouracil



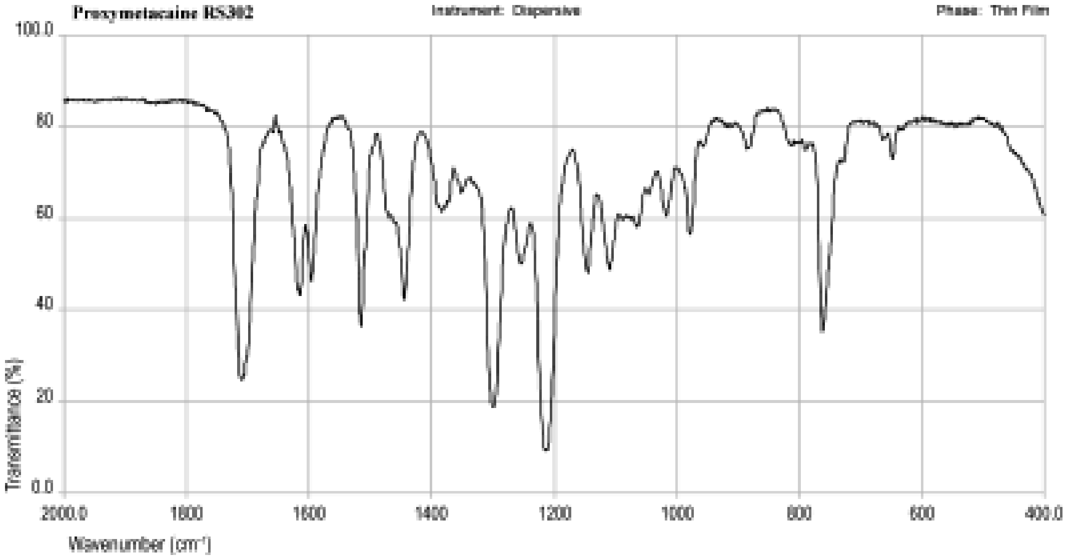
Protriptyline



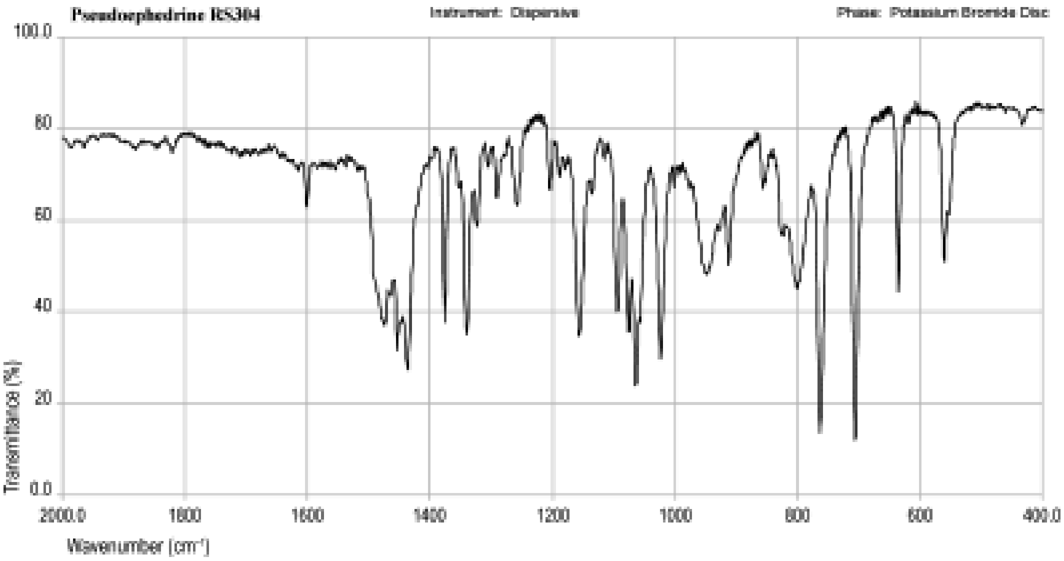
Proxymetacaine Hydrochloride



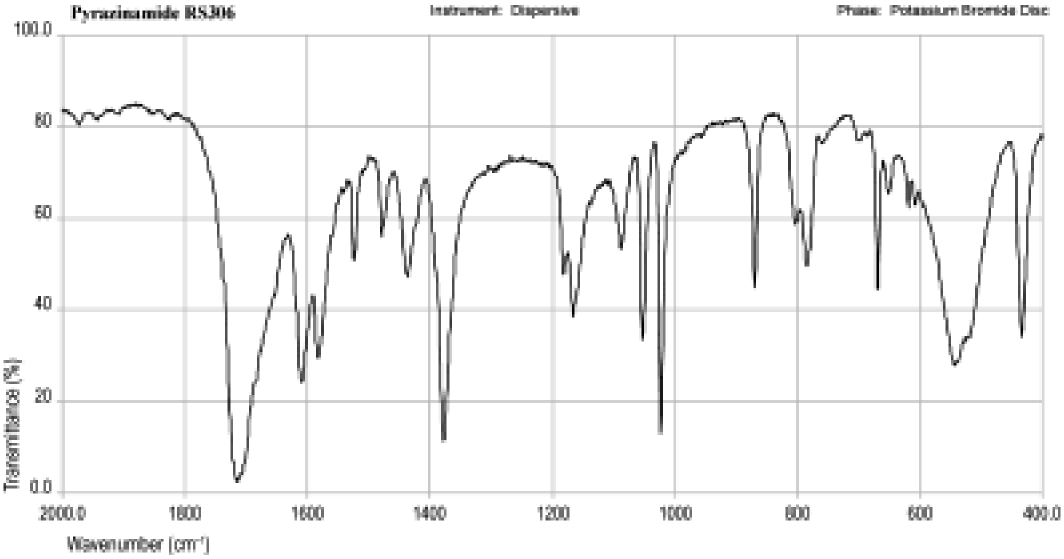
Proxymetacaine



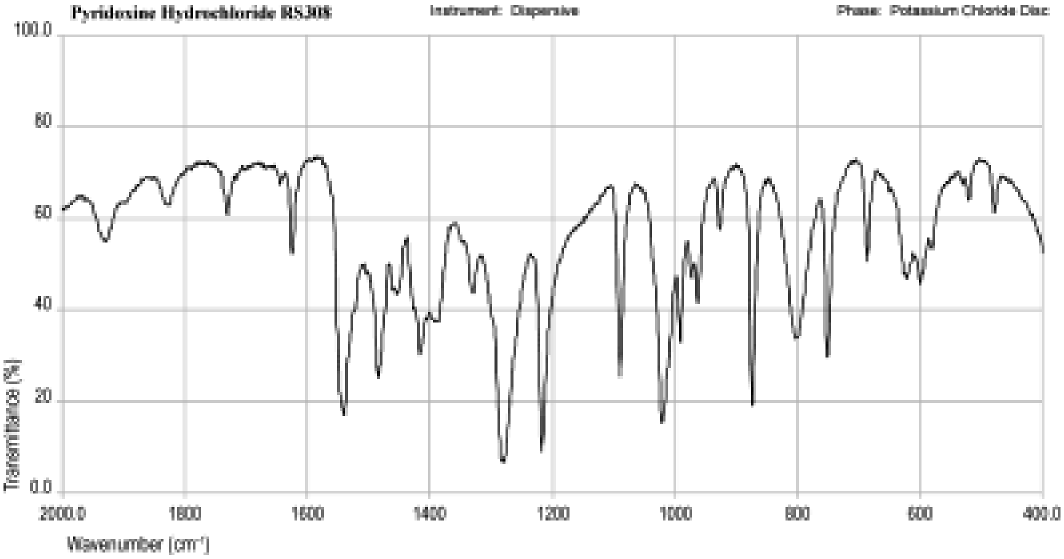
Pseudoephedrine



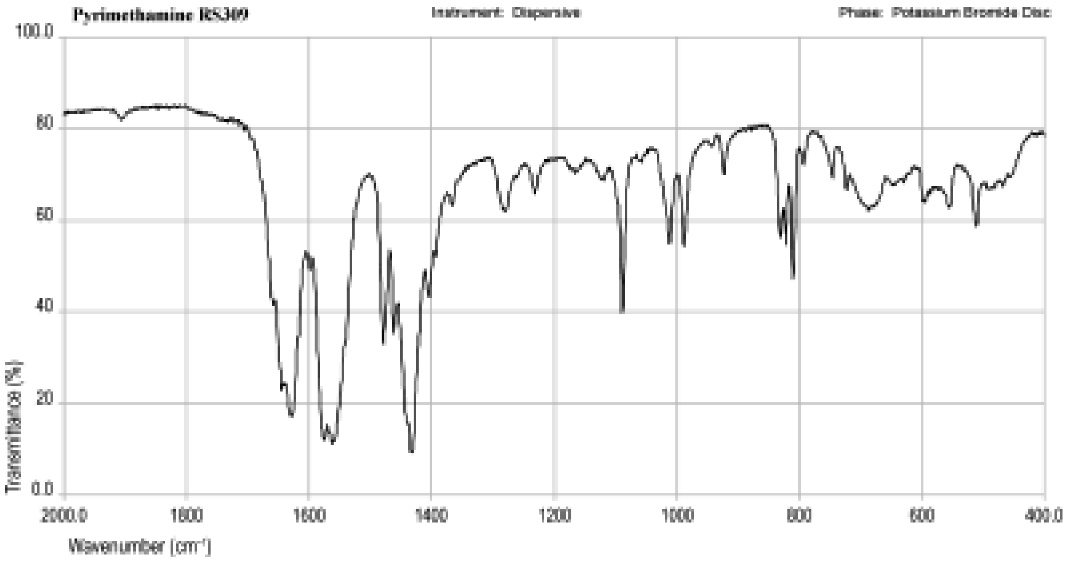
Pyrazinamide



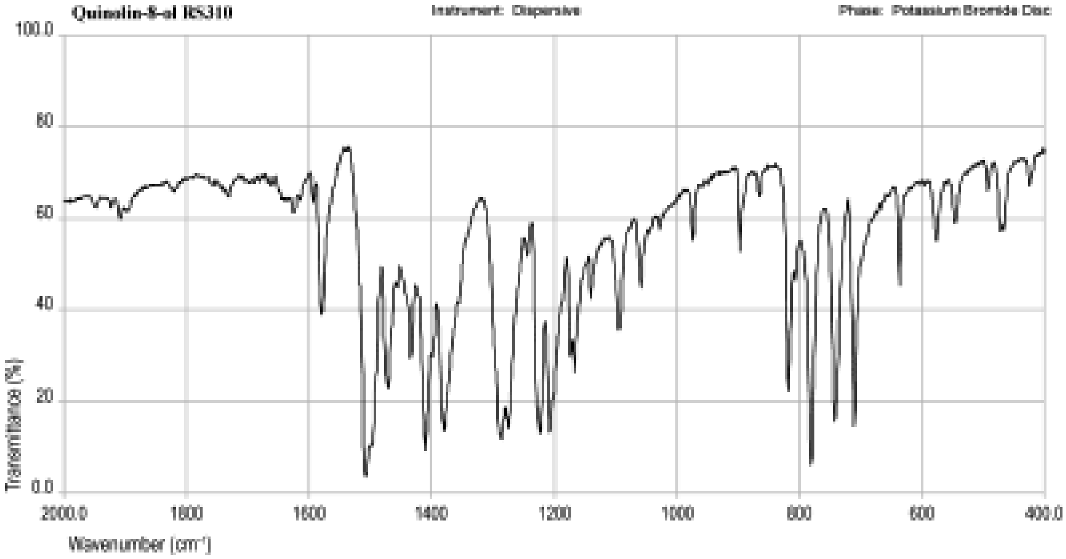
Pyridoxine Hydrochloride



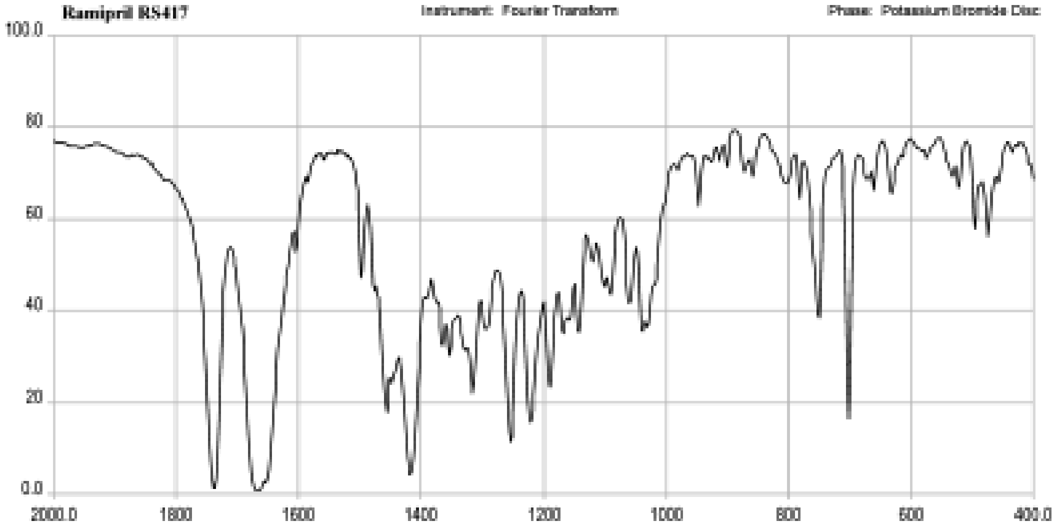
Pyrimethamine



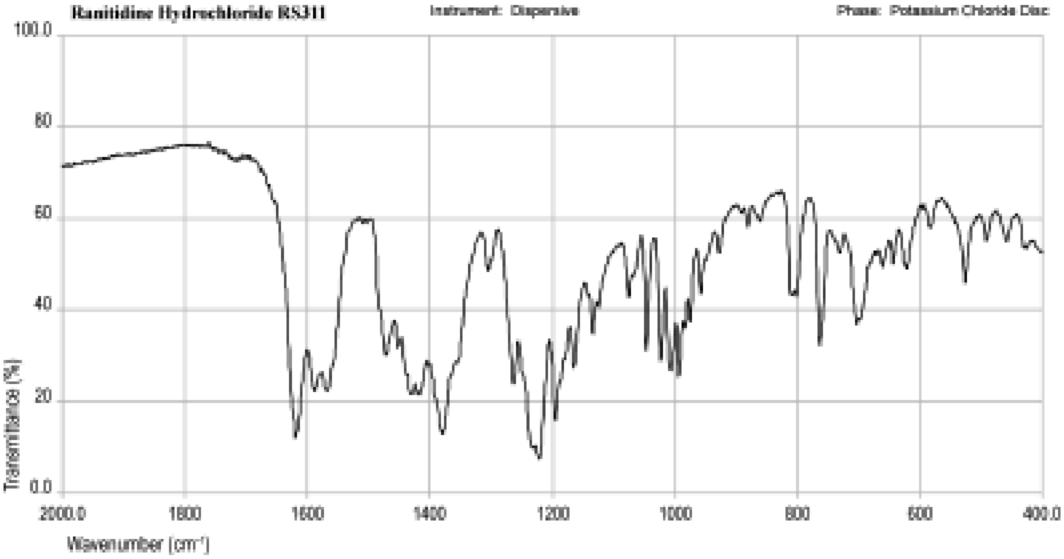
Quinolin-8-ol



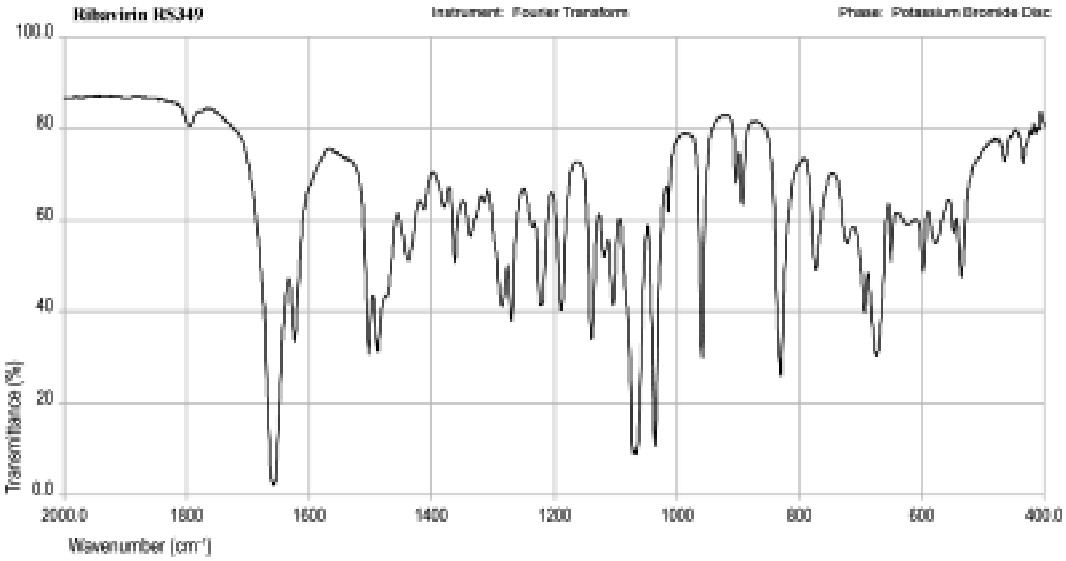
Ramipril


Ramipril RS417
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
2000.0
1800
1600
1400
1200
1000
800
600
400.0

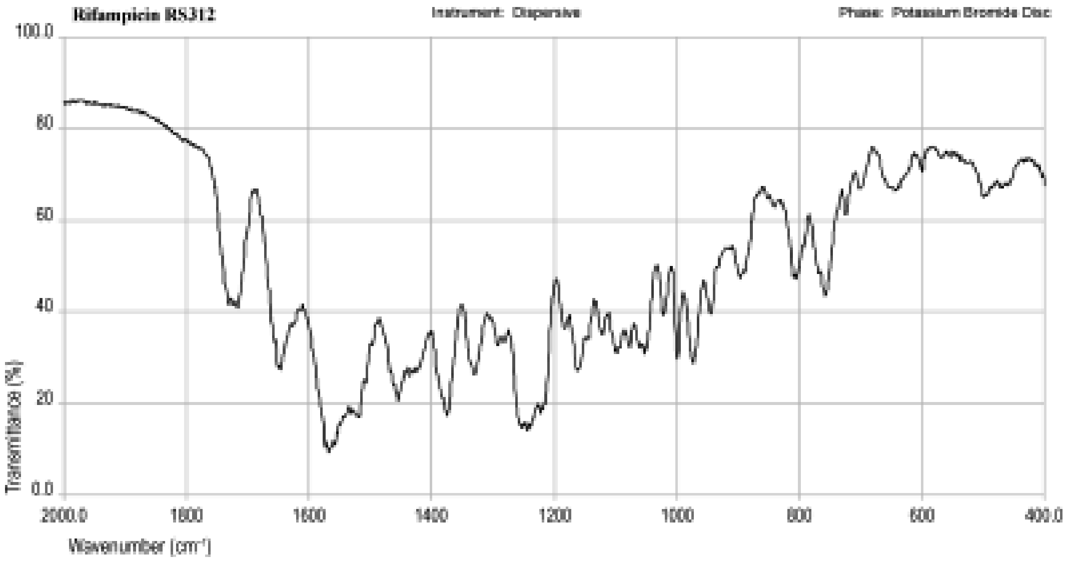
Ranitidine Hydrochloride



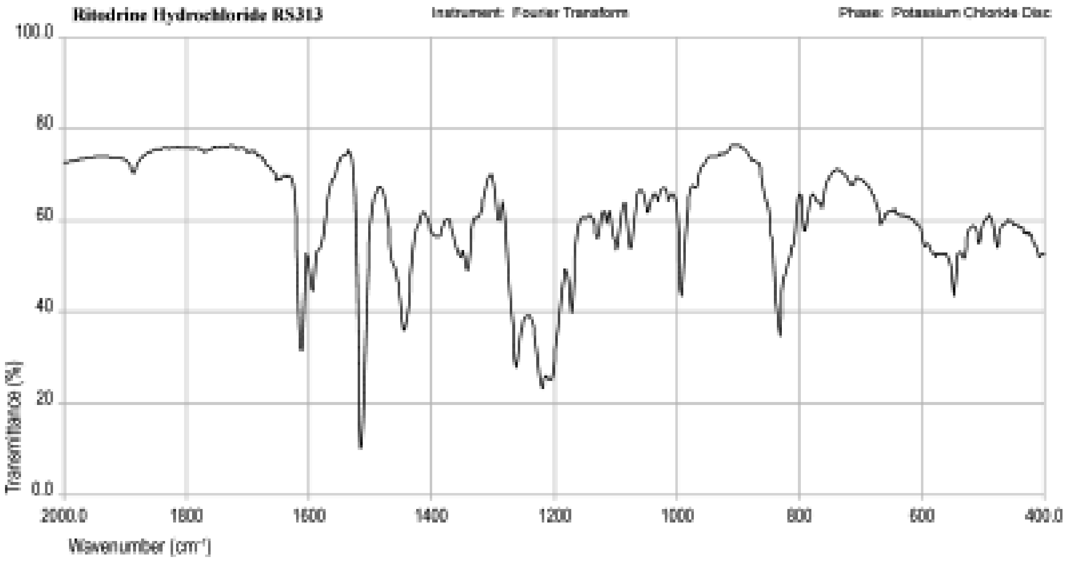
Ribavirin


Ribavirin RS349
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

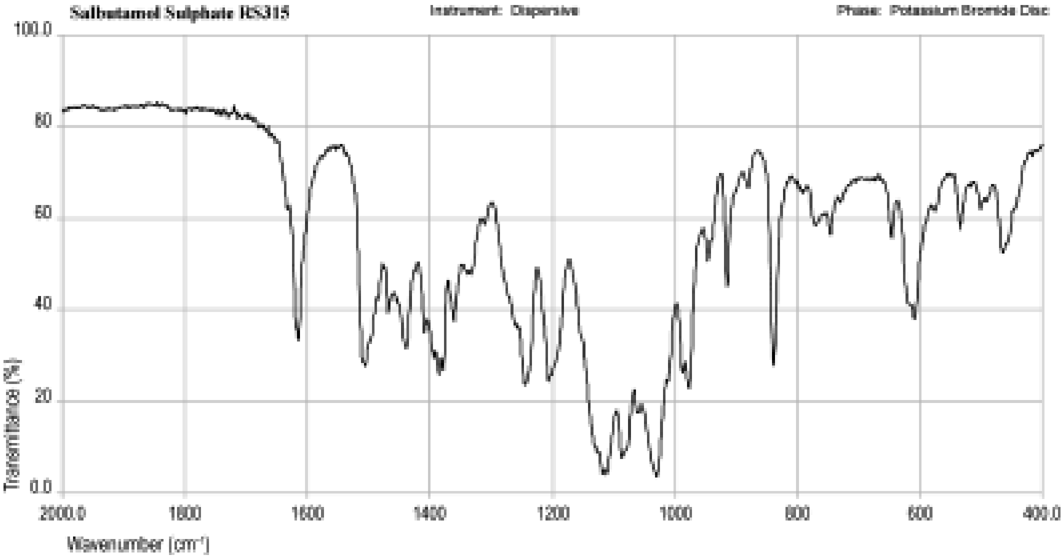
Rifampicin


Rifampicin RS312
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

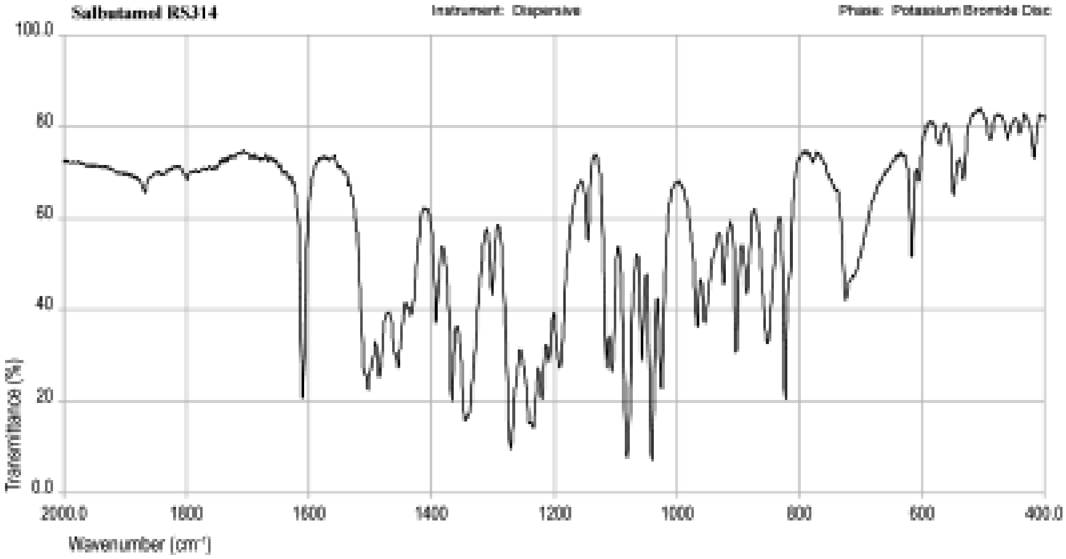
Ritodrine Hydrochloride



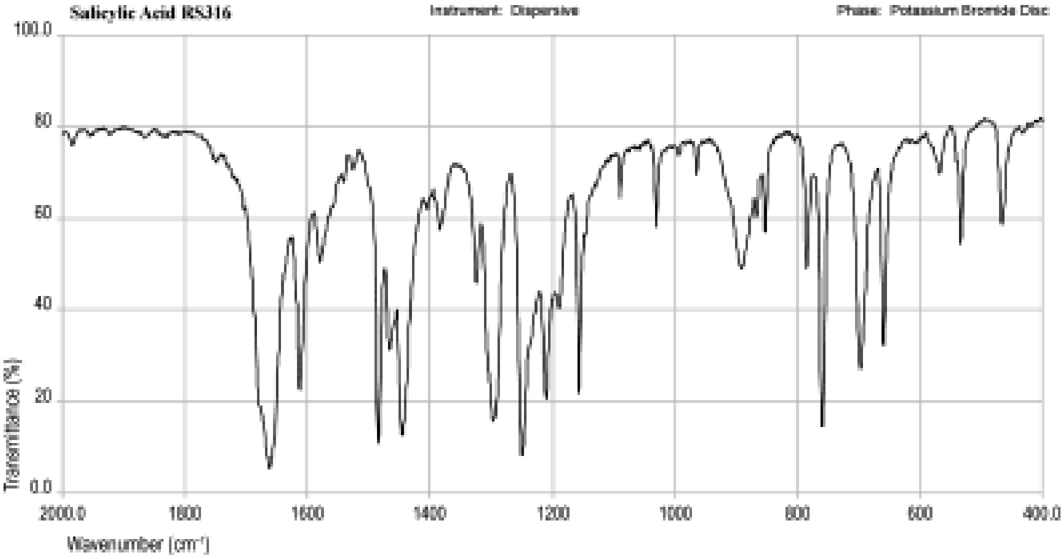
Salbutamol Sulphate



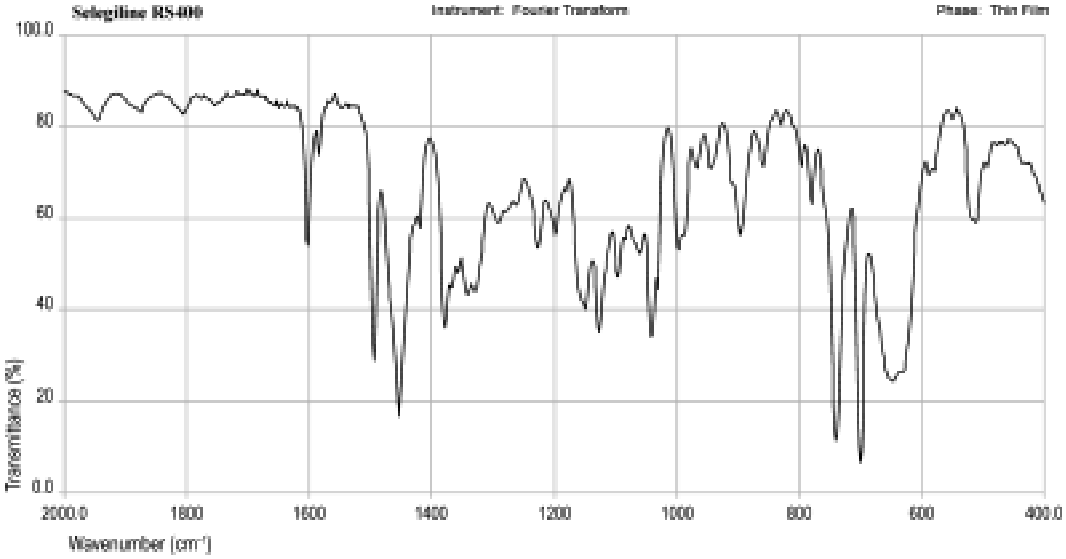
Salbutamol


Salbutamol RS314
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

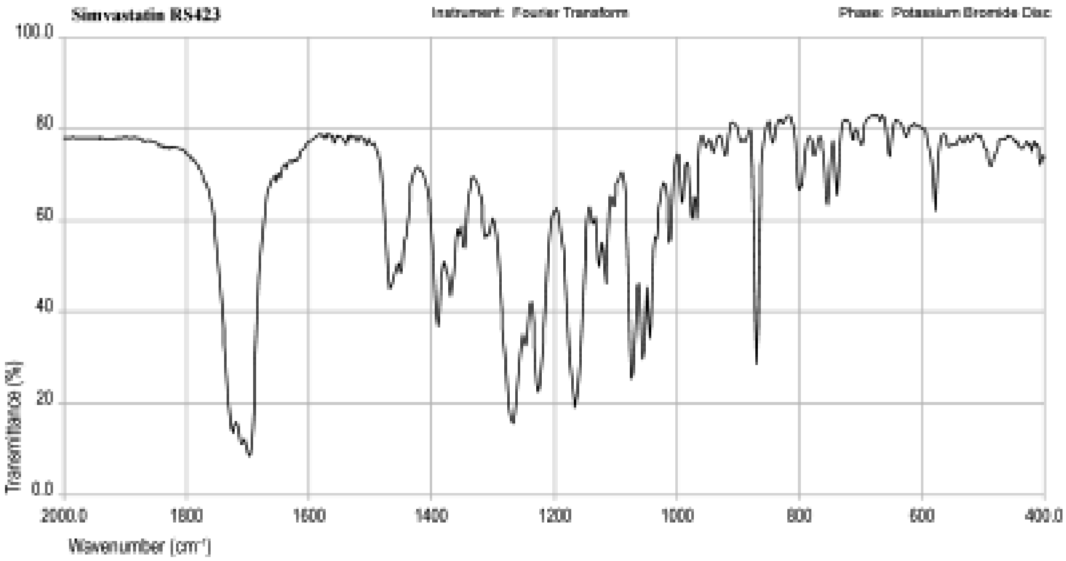
Salicylic Acid



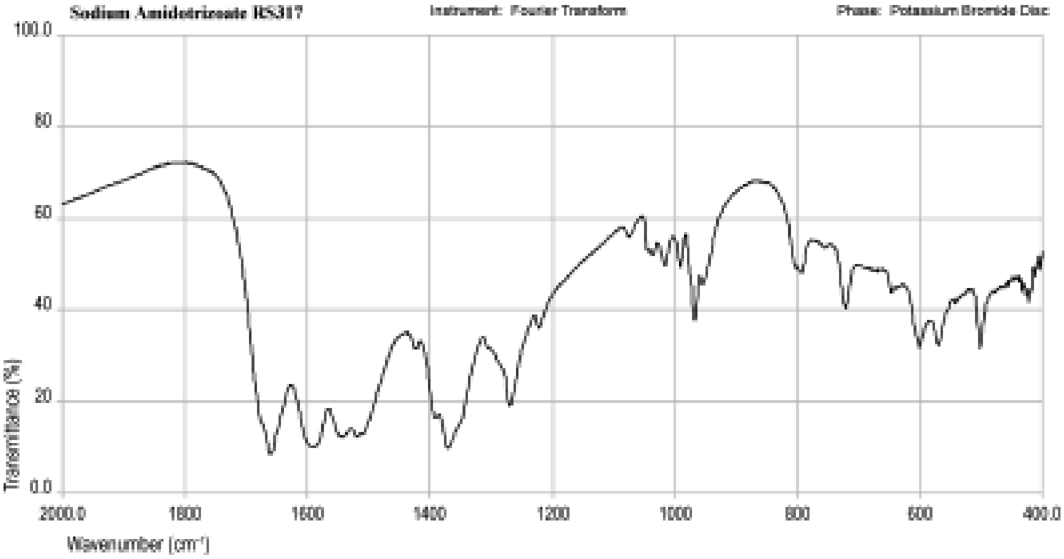
Selegiline


Selegiline RS400
Instrument: Fourier Transform
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

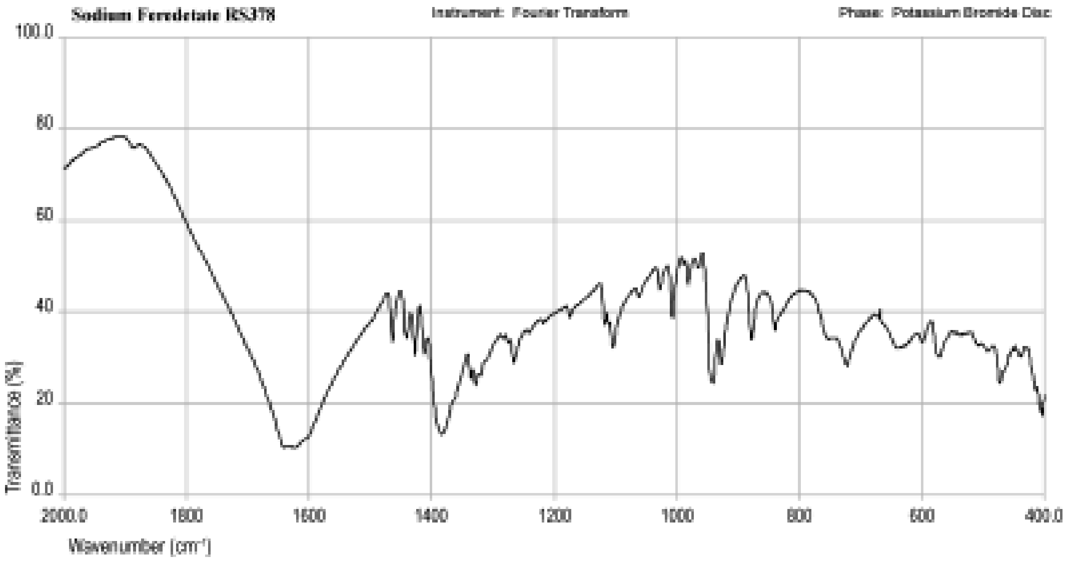
Simvastatin



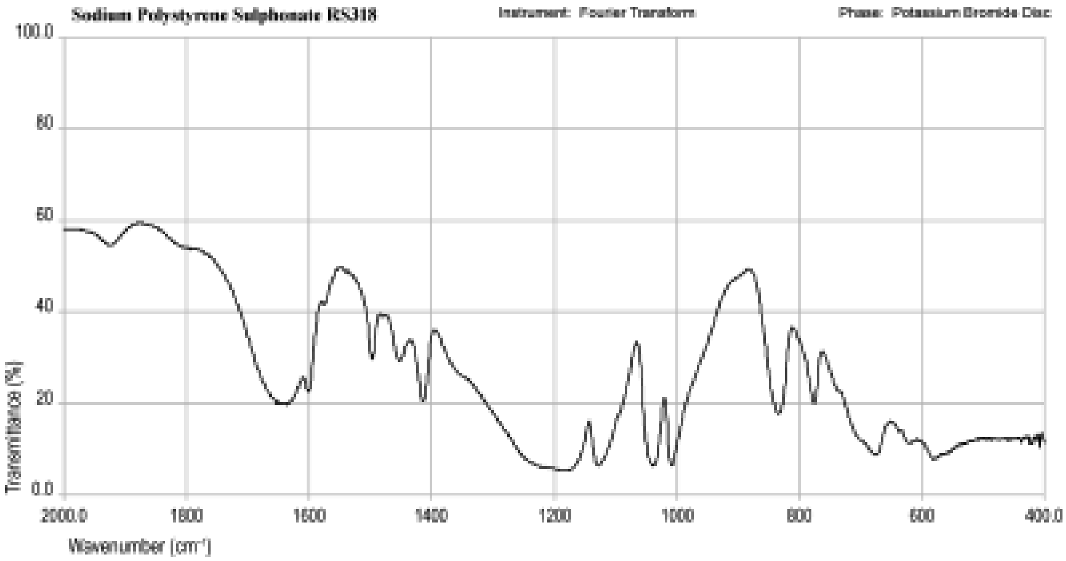
Sodium Amidotrizoate



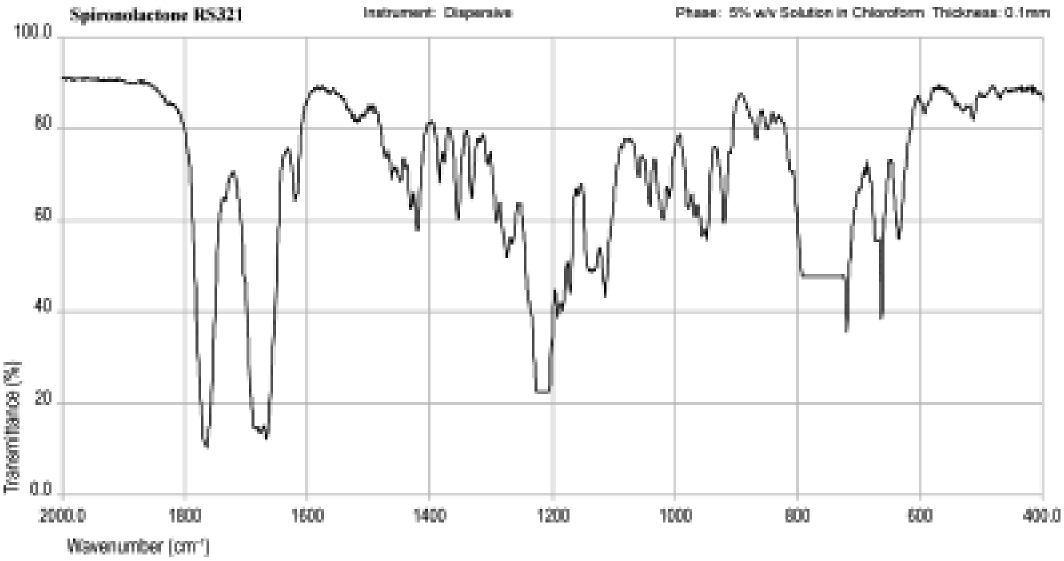
Sodium Feredetate



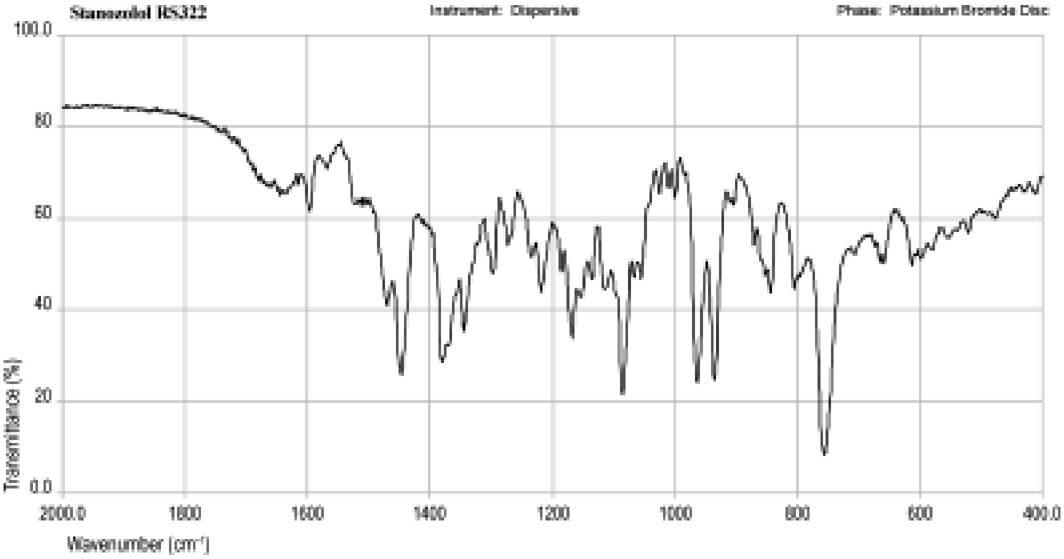
Sodium Polystyrene Sulphonate



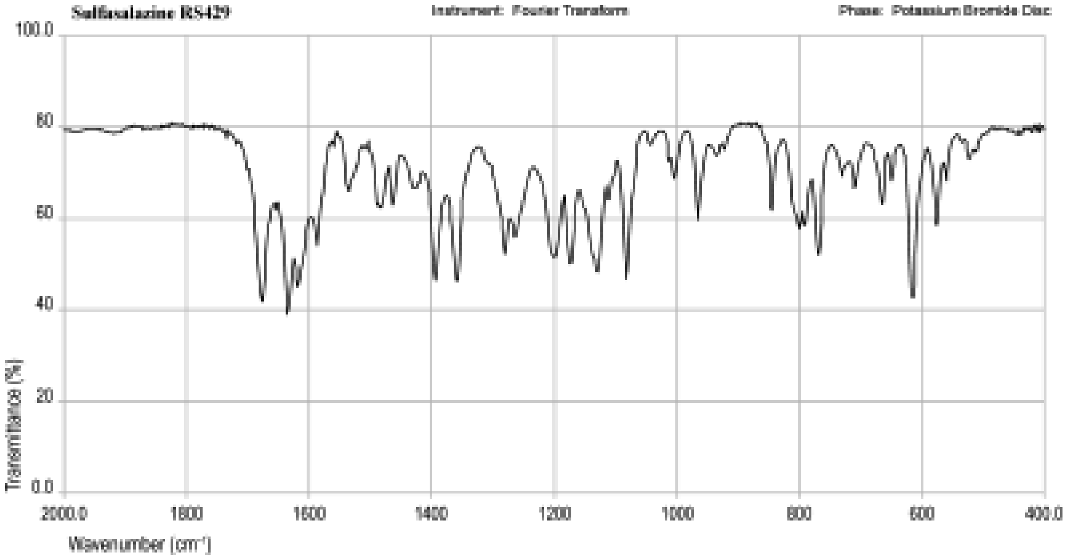
Spironolactone



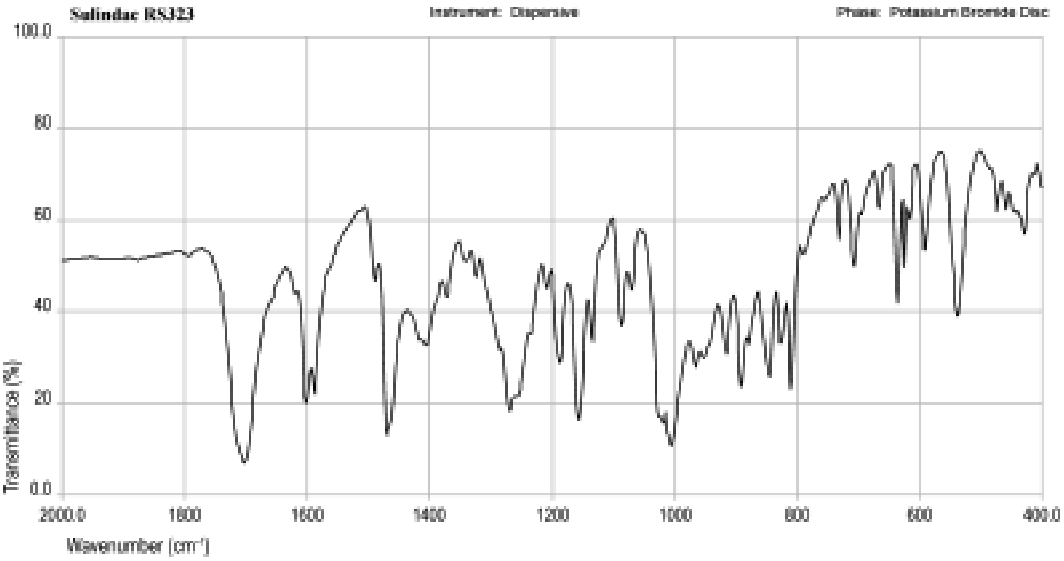
Stanozolol


Stanozolol RS322
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

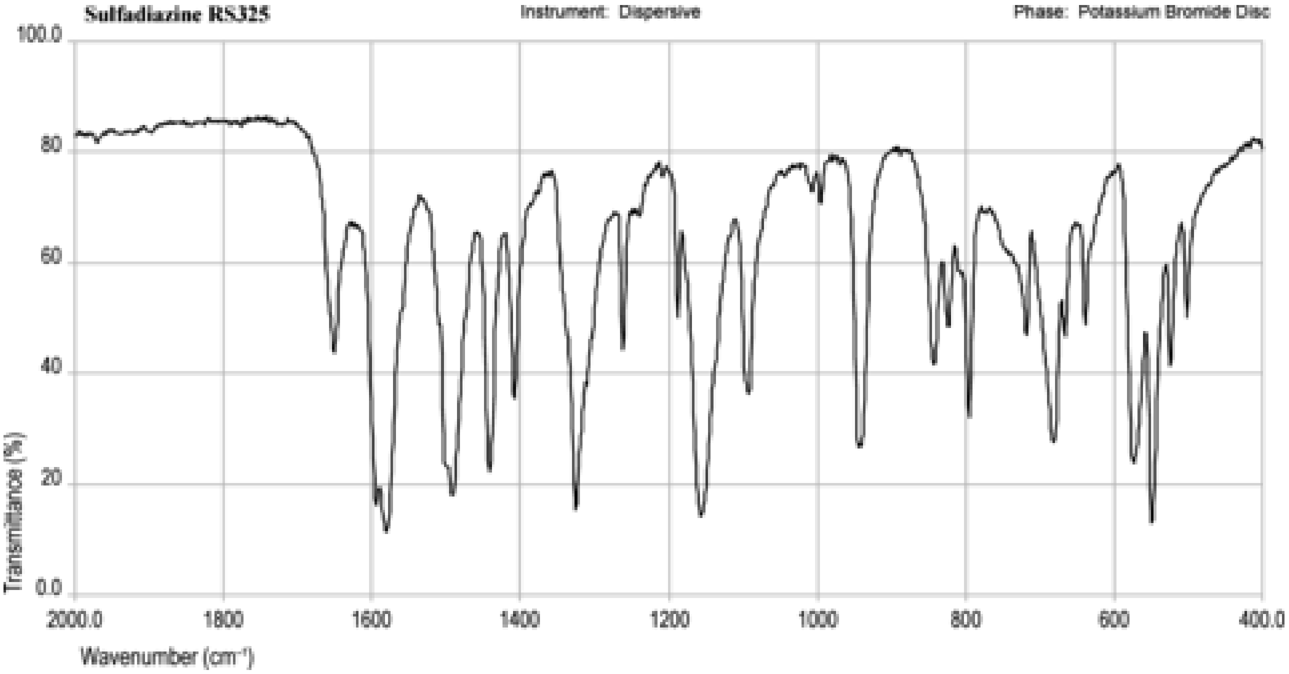
Sulfasalazine



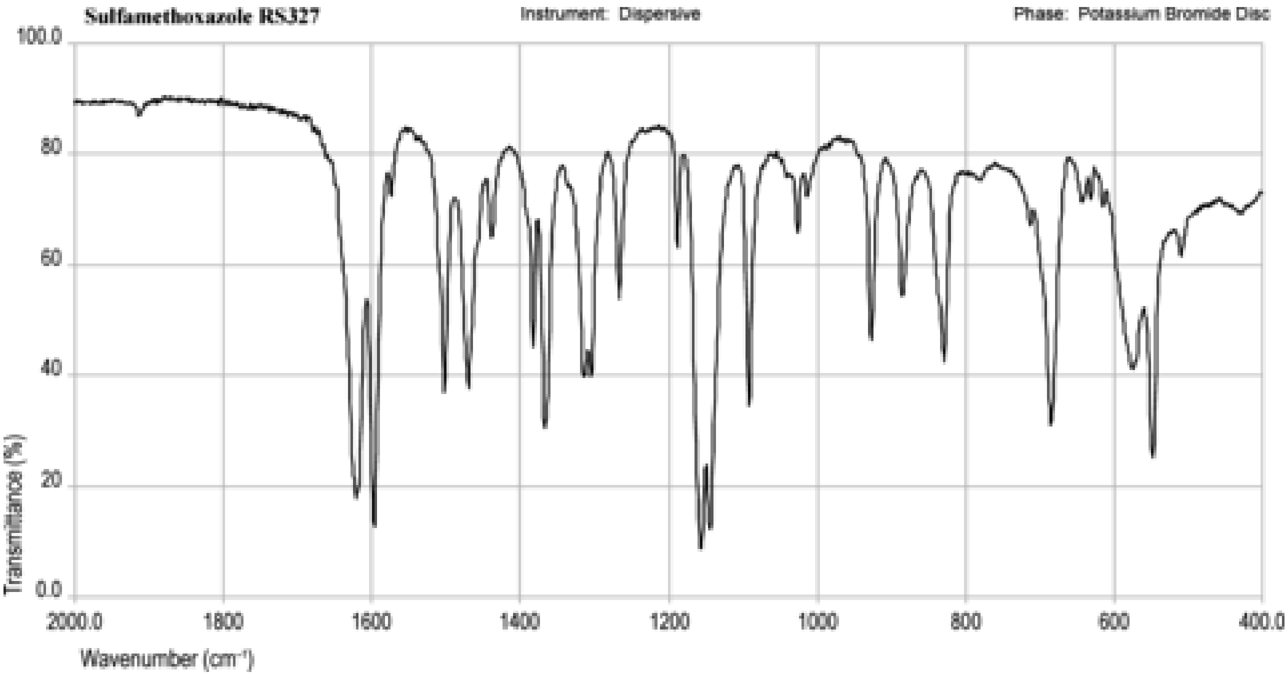
Sulindac


Sulindac RS323
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

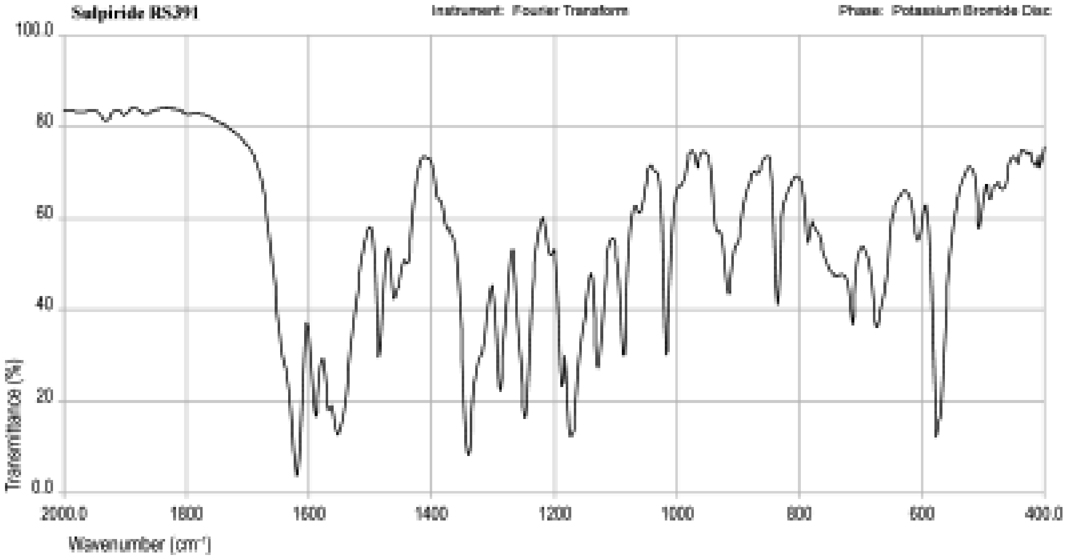
Sulphadiazine



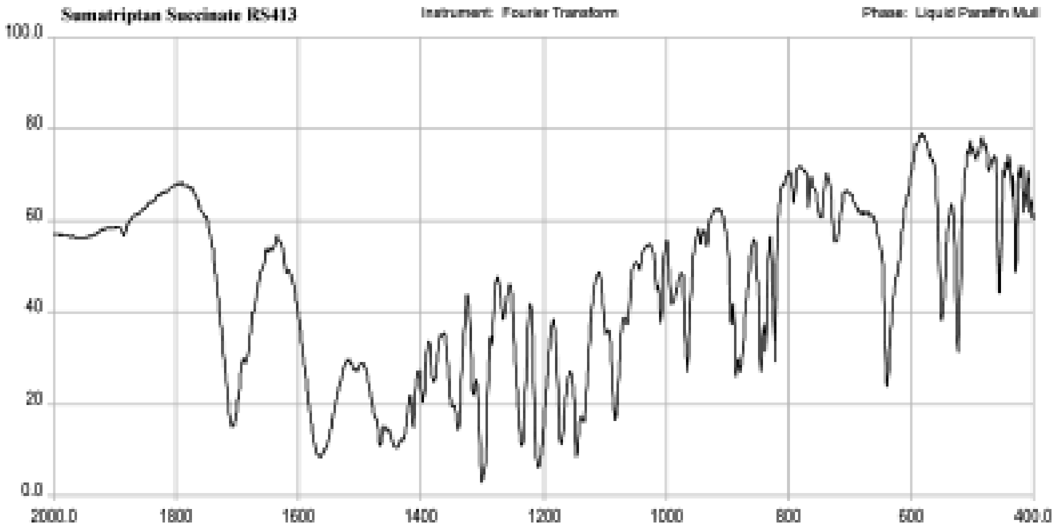
Sulphamethoxazole



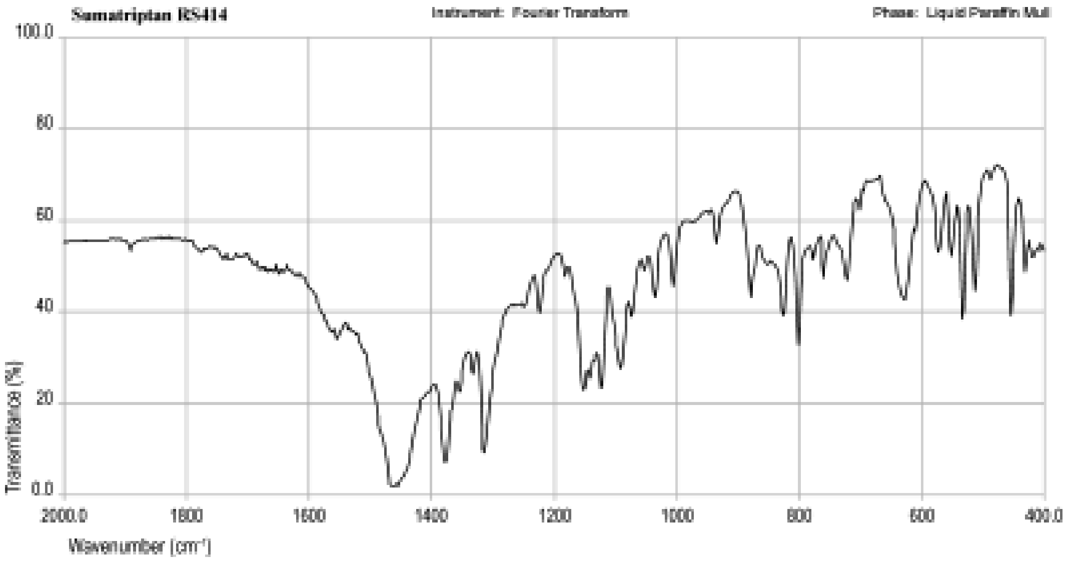
Sulpiride


Sulpiride RS391
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

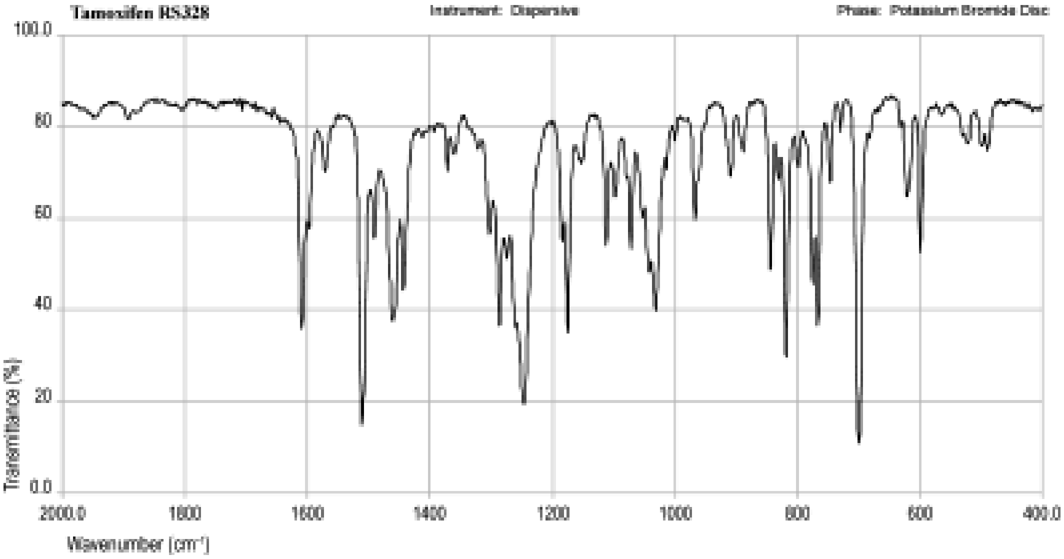
Sumatriptan Succinate



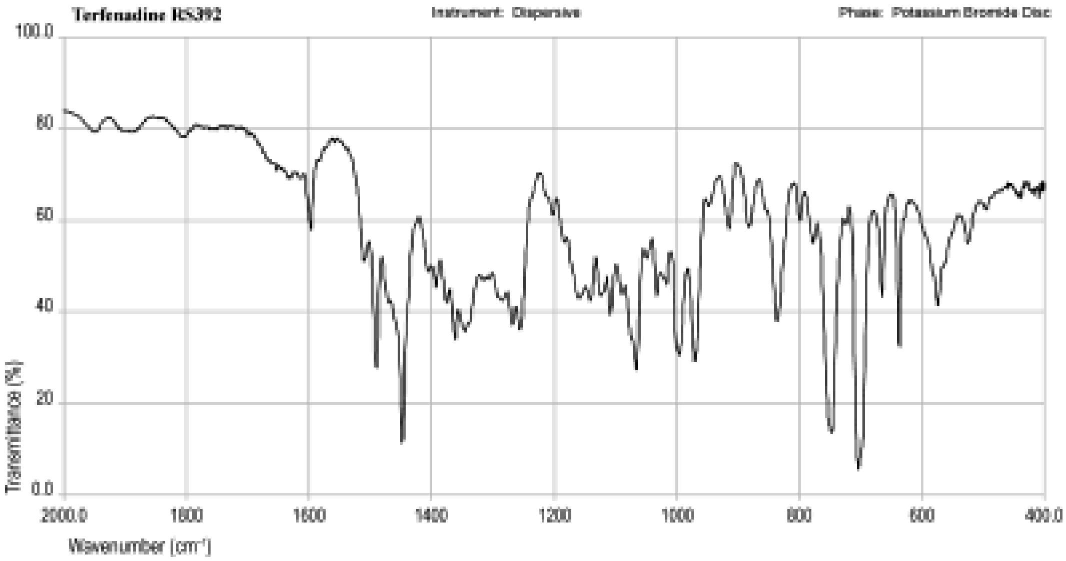
Sumatriptan



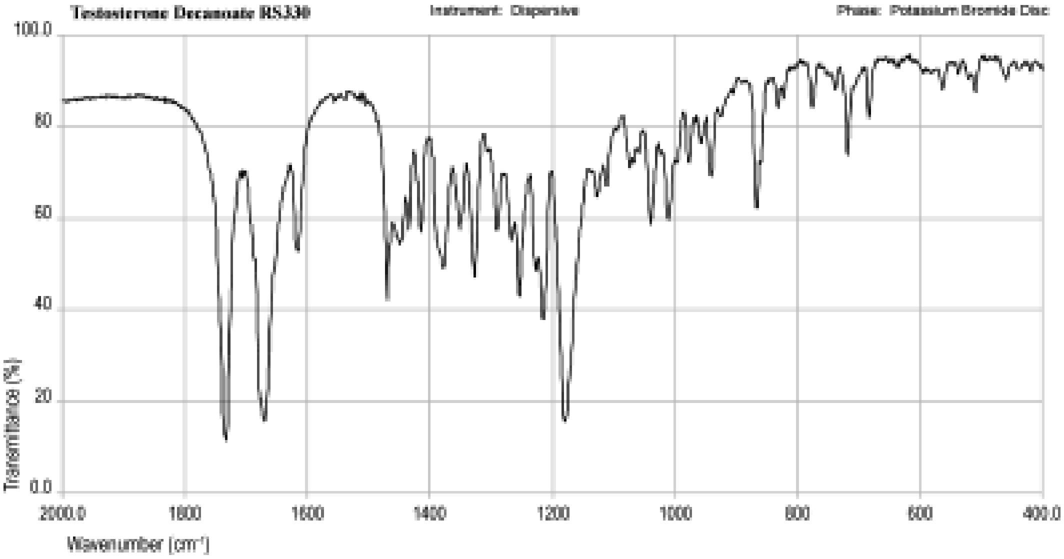
Tamoxifen


Tamoxifen RS328
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

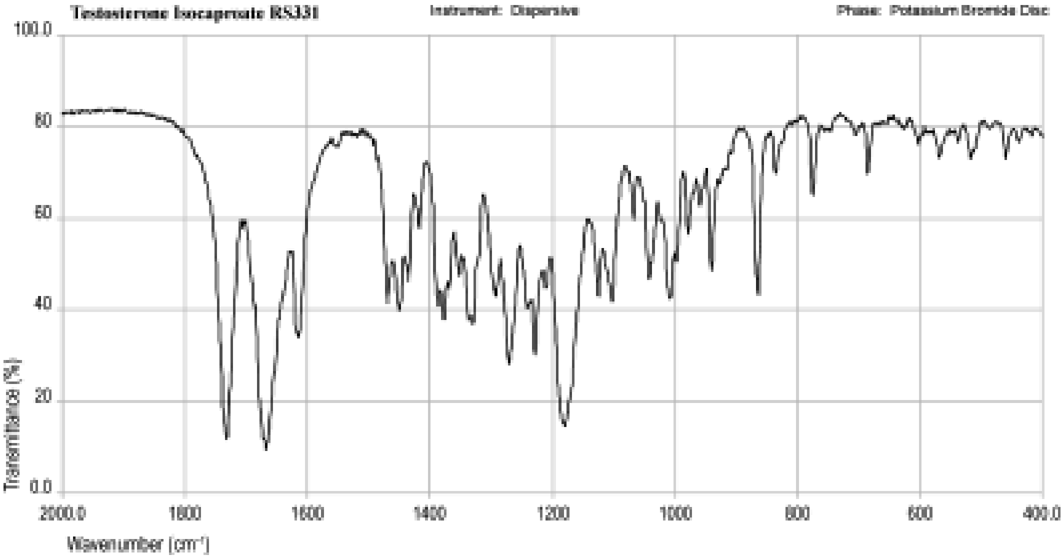
Terfenadine



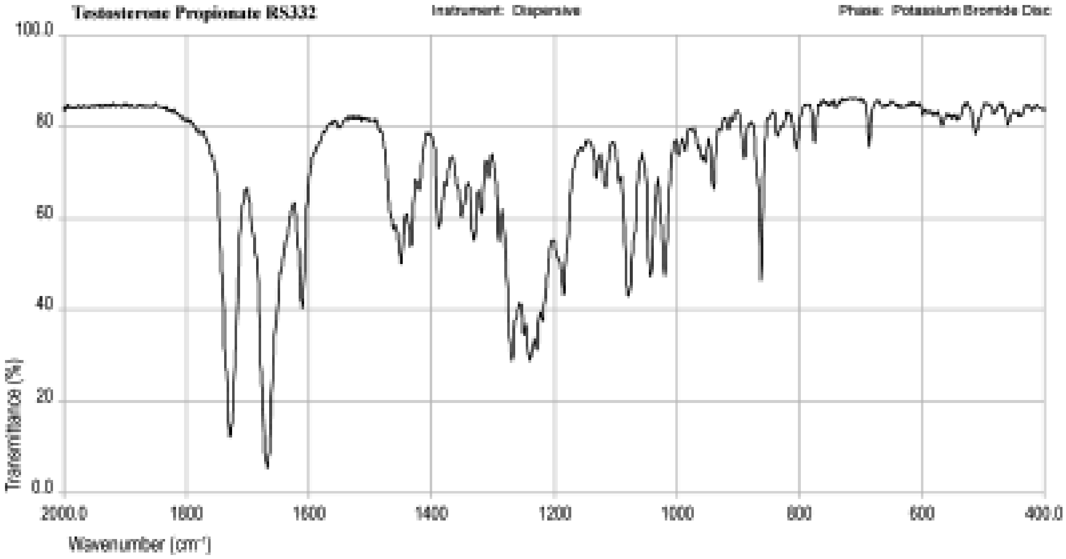
Testosterone Decanoate



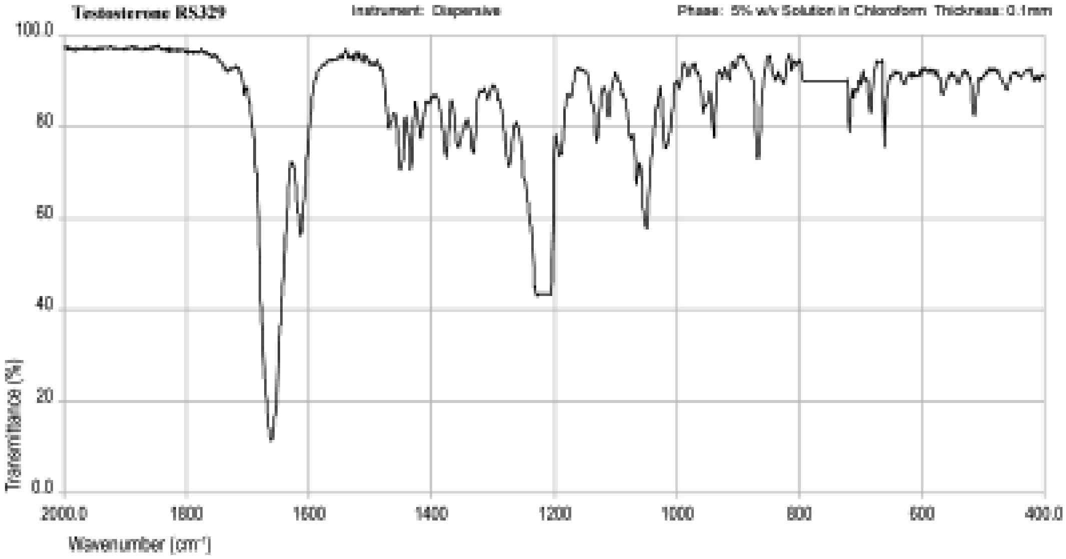
Testosterone Isocaproate



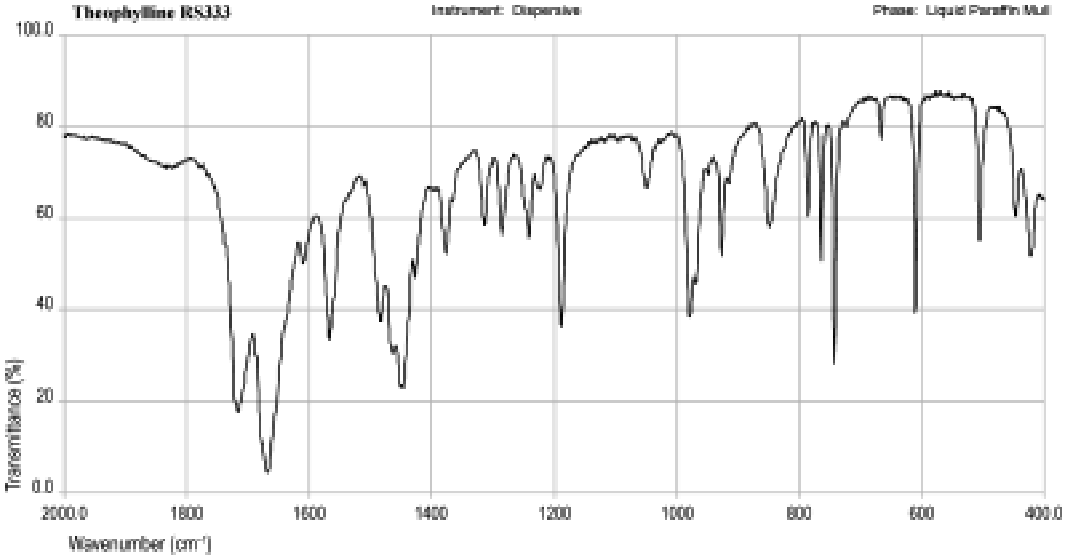
Testosterone Propionate



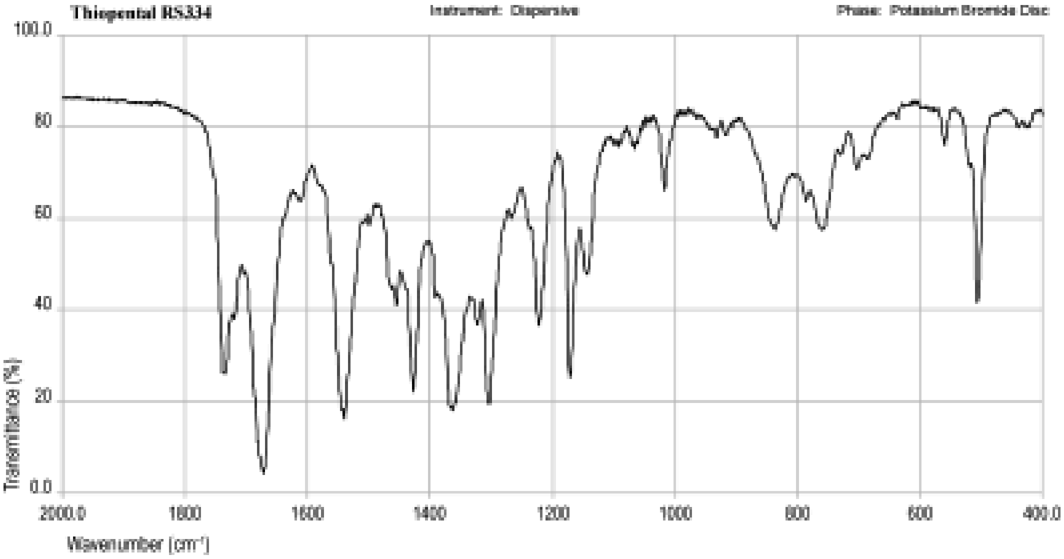
Testosterone



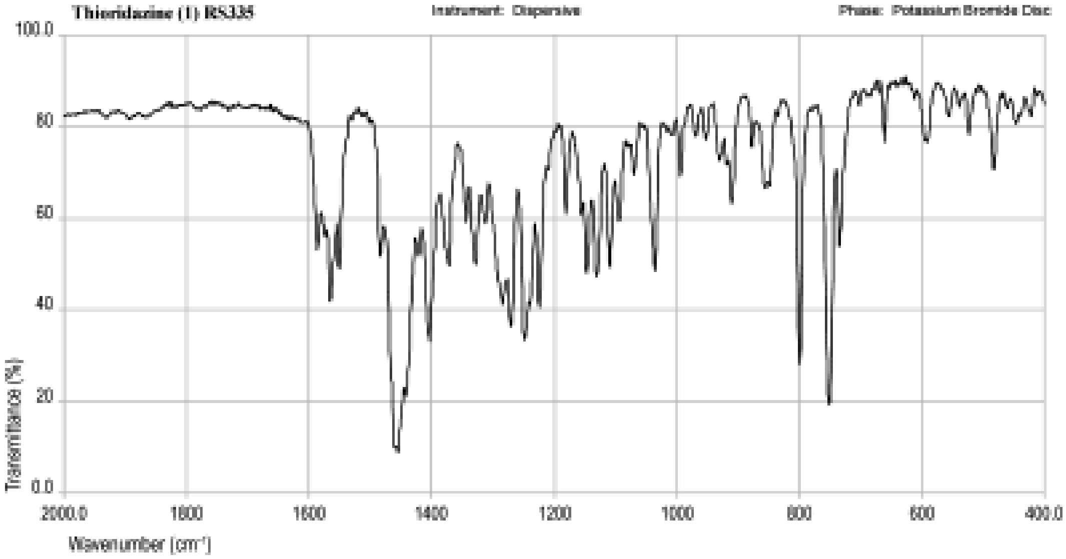
Theophylline



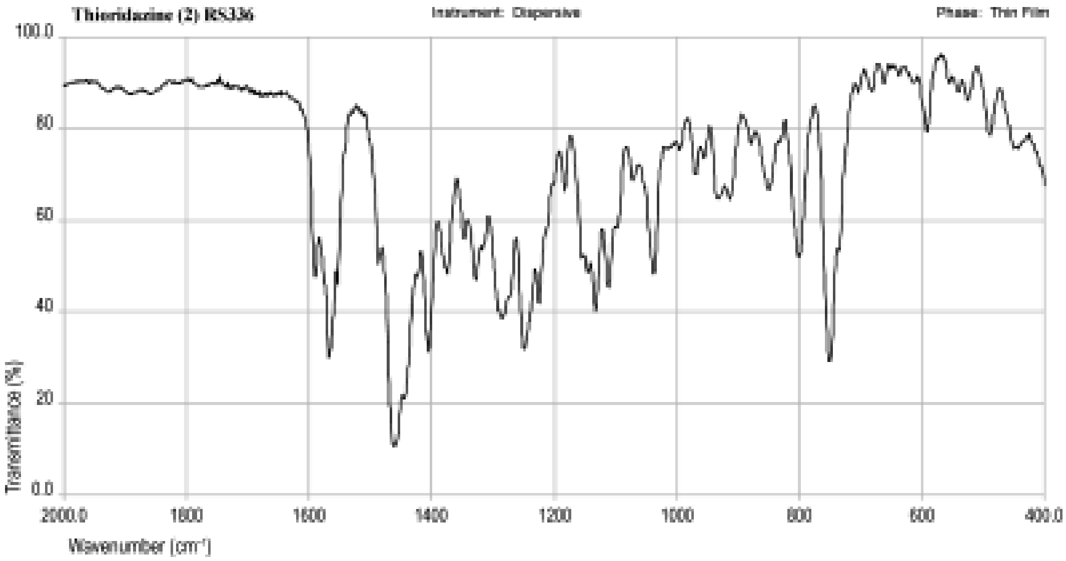
Thiopental


Thiopental RS334
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

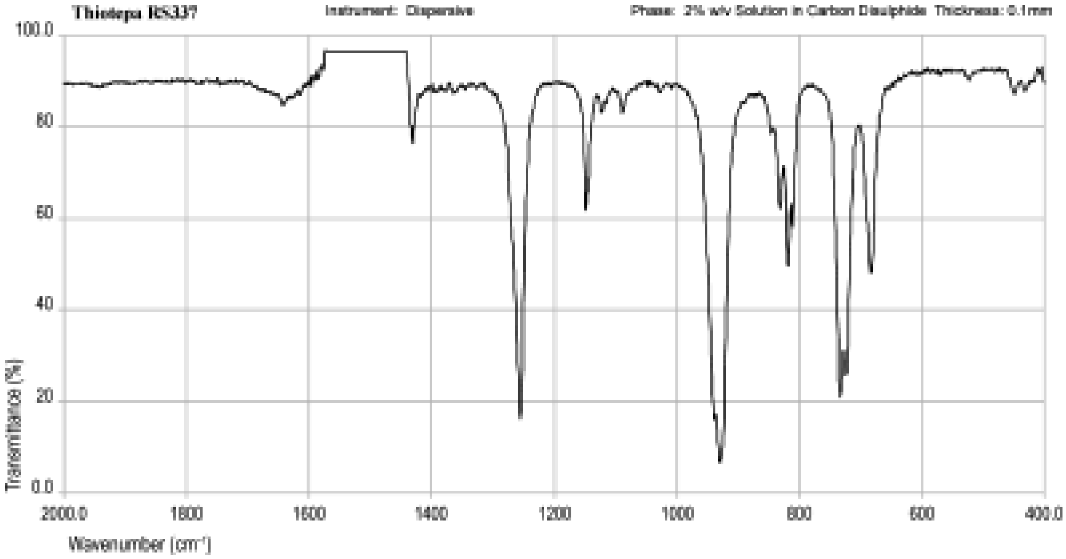
Thioridazine1



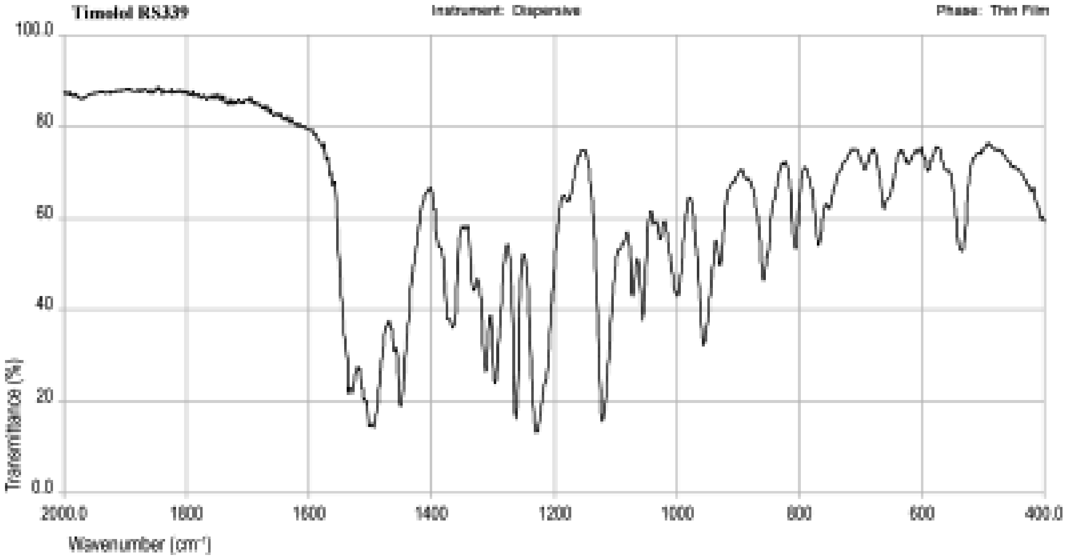
Thioridazine2



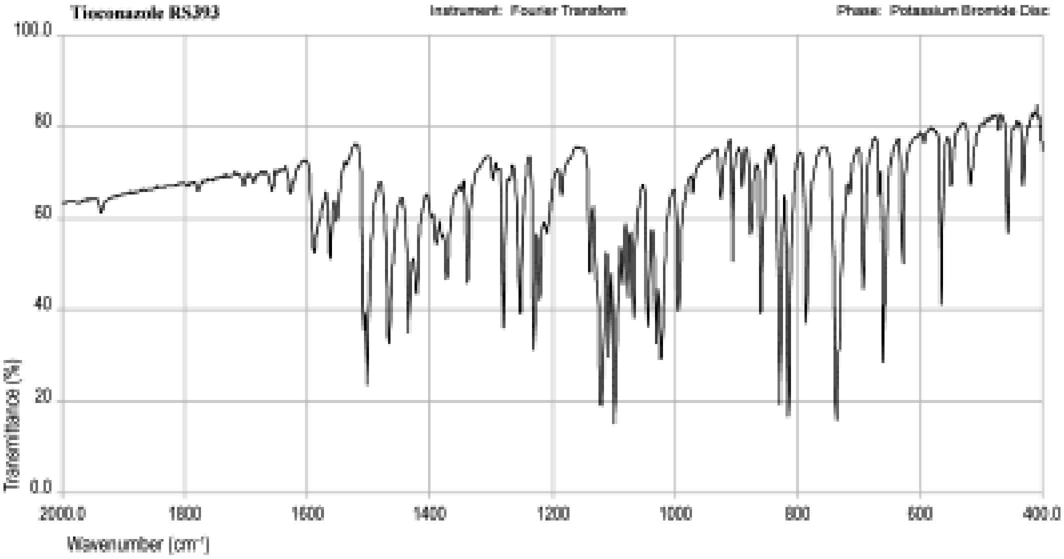
Thiotepa


Thiotepa RS337
Instrument: Dispersive
Phase: 2% w/v Solution in Carbon Disulphide Thickness: 0.1mm
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

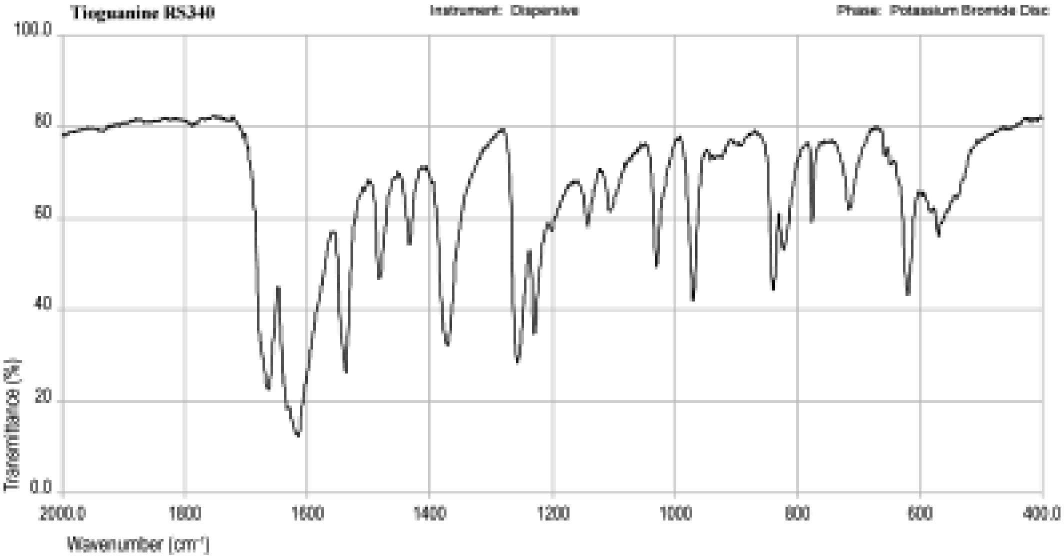
Timolol


Timolol RS339
Instrument: Dispersive
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

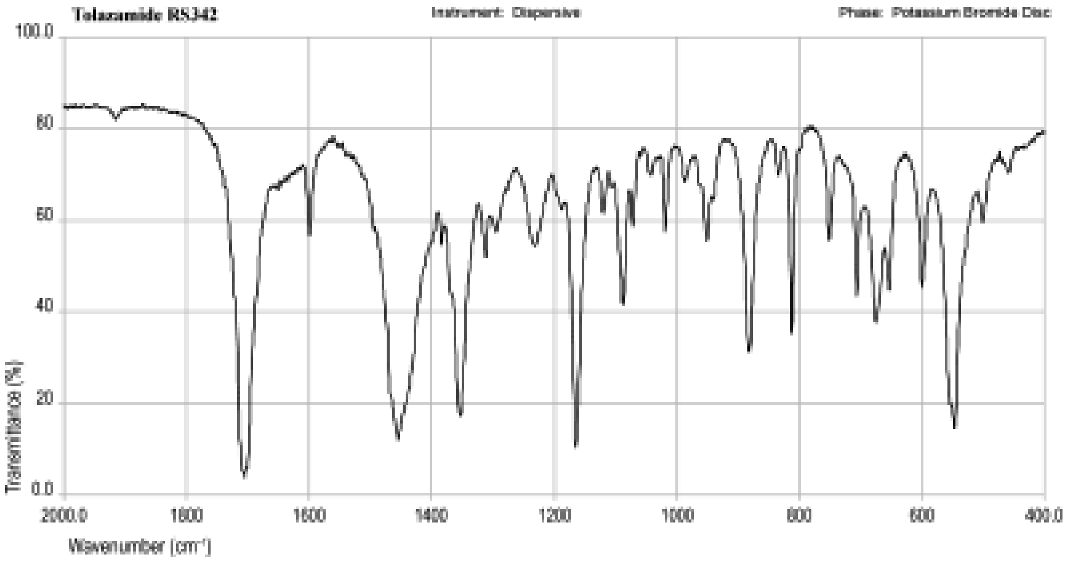
Tioconazole



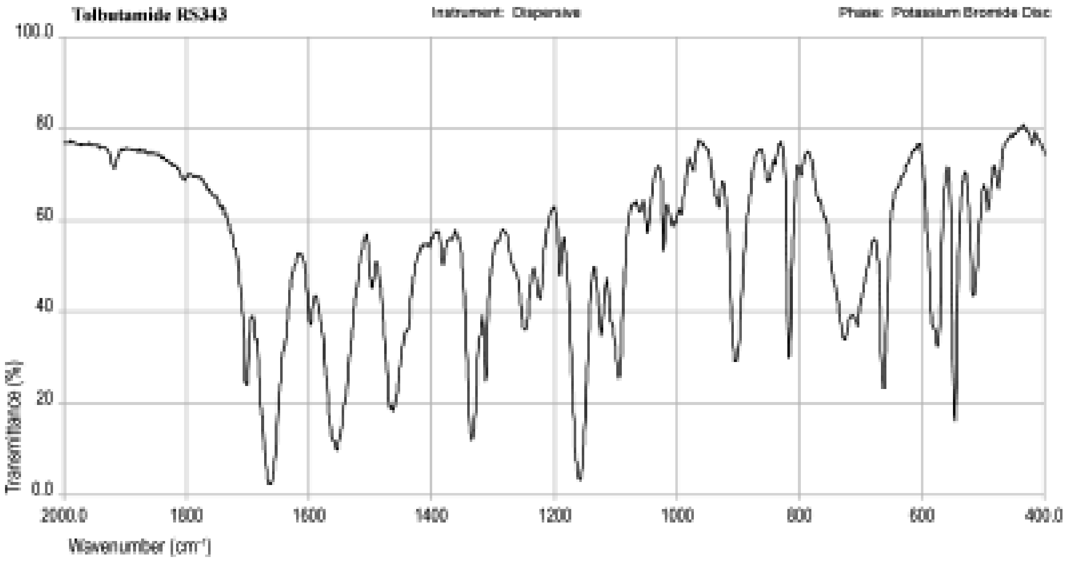
Tioguanine


Tioguanine RS340
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

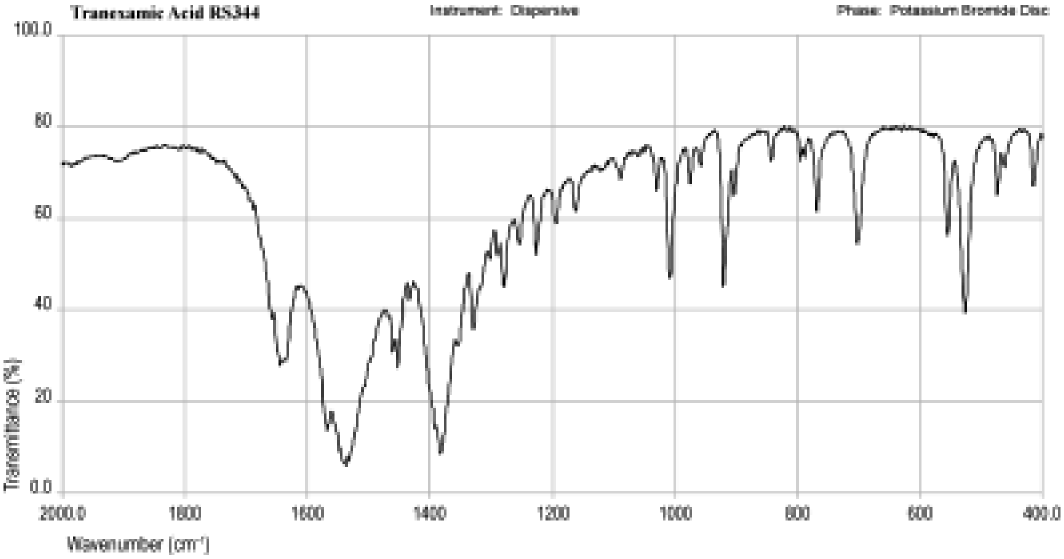
Tolazamide


Tolazamide RS342
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

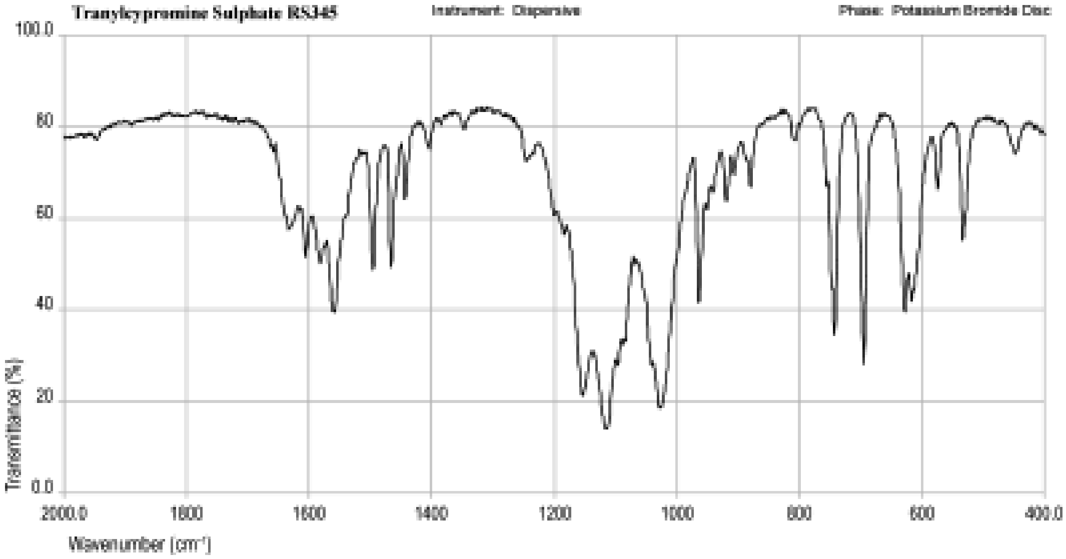
Tolbutamide



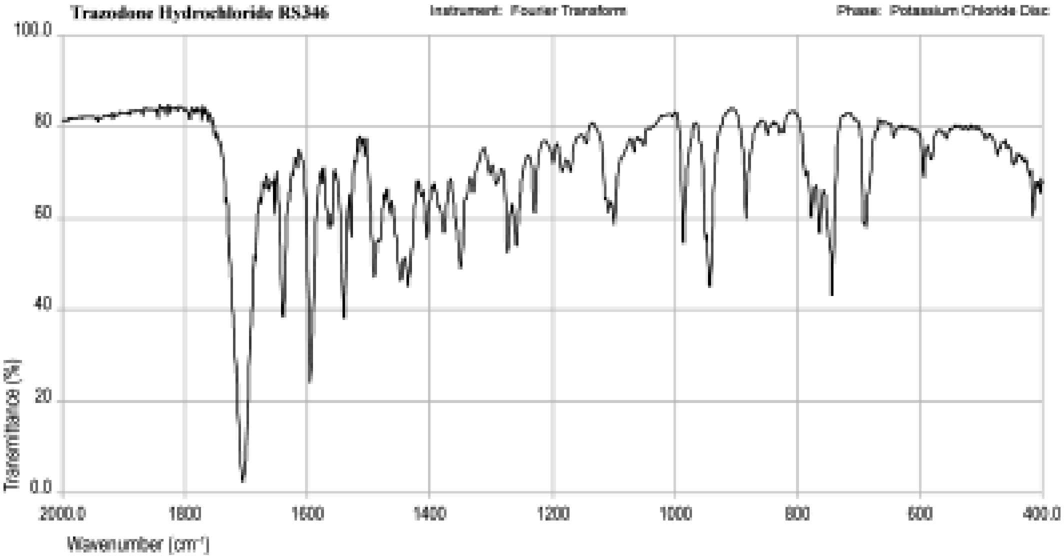
Tranexamic Acid



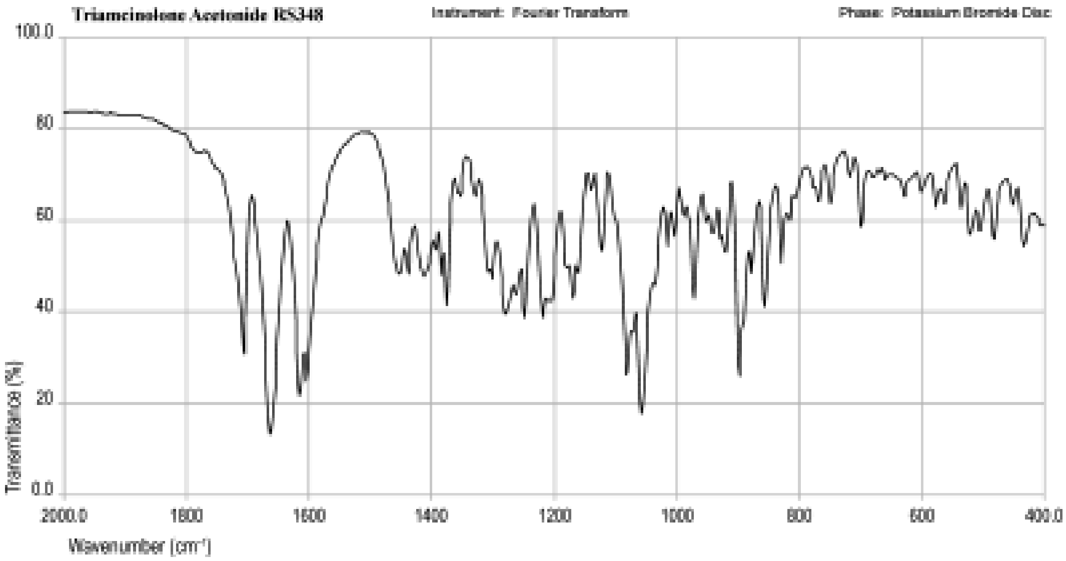
Tranylcypromine Sulphate



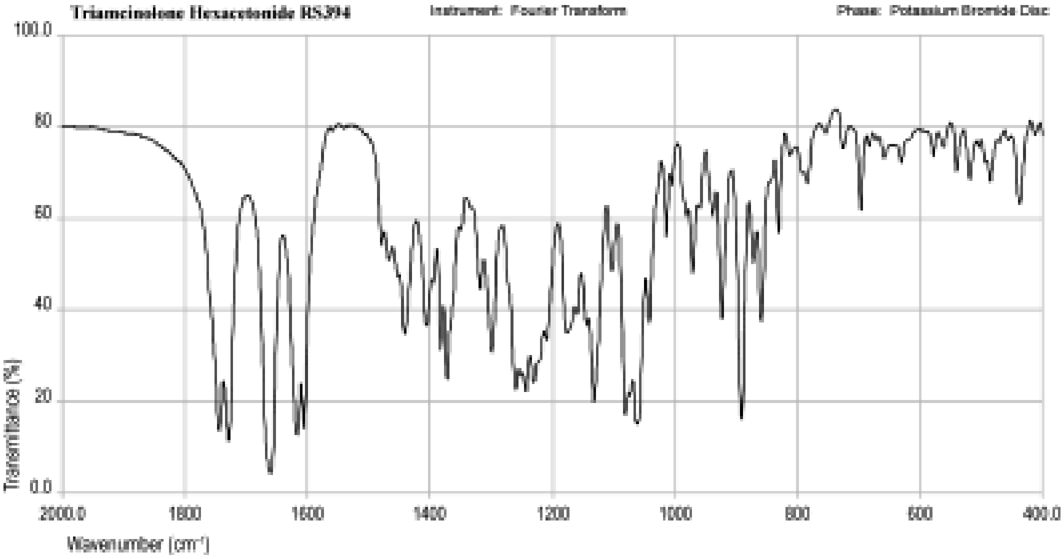
Trazodone Hydrochloride



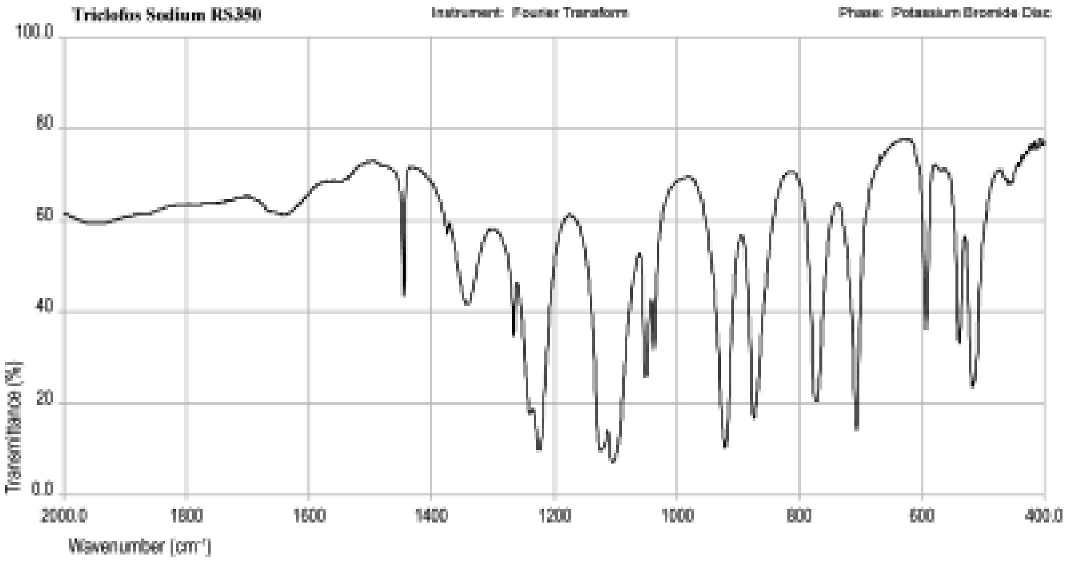
Triamcinolone Acetonide



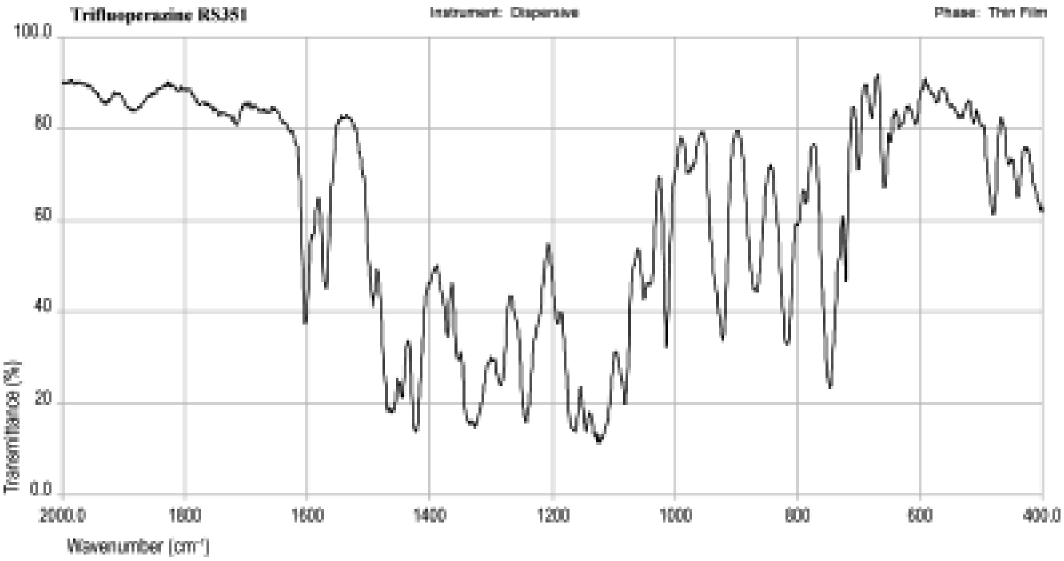
Triamcinolone Hexacetonide



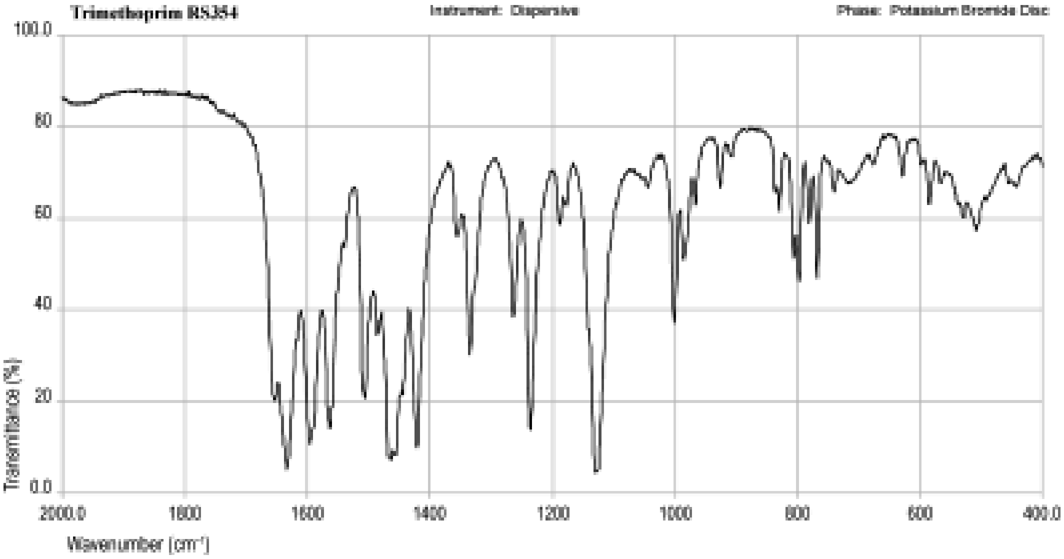
Triclofos Sodium



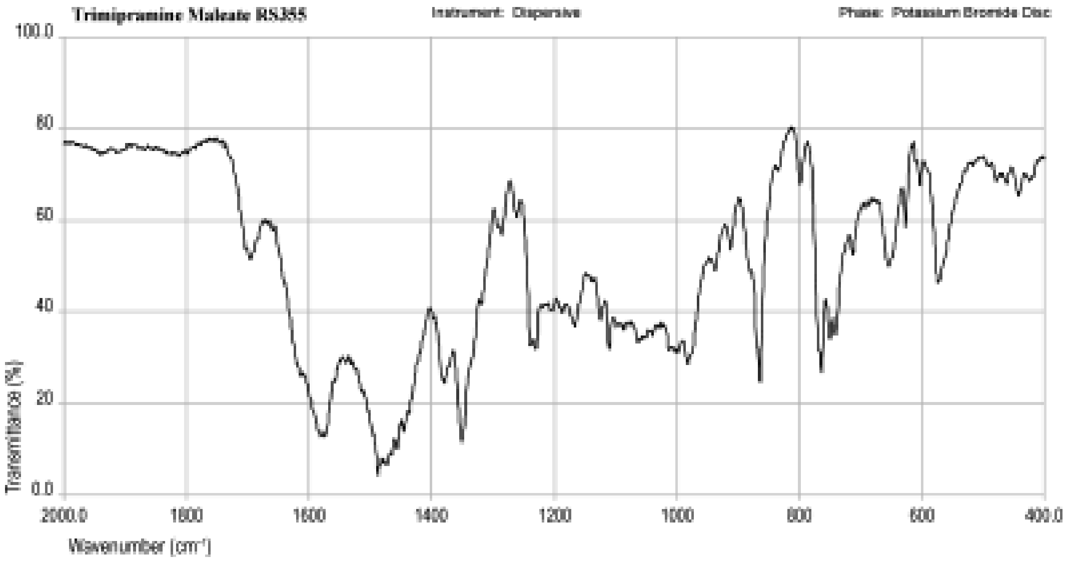
Trifluoperazine



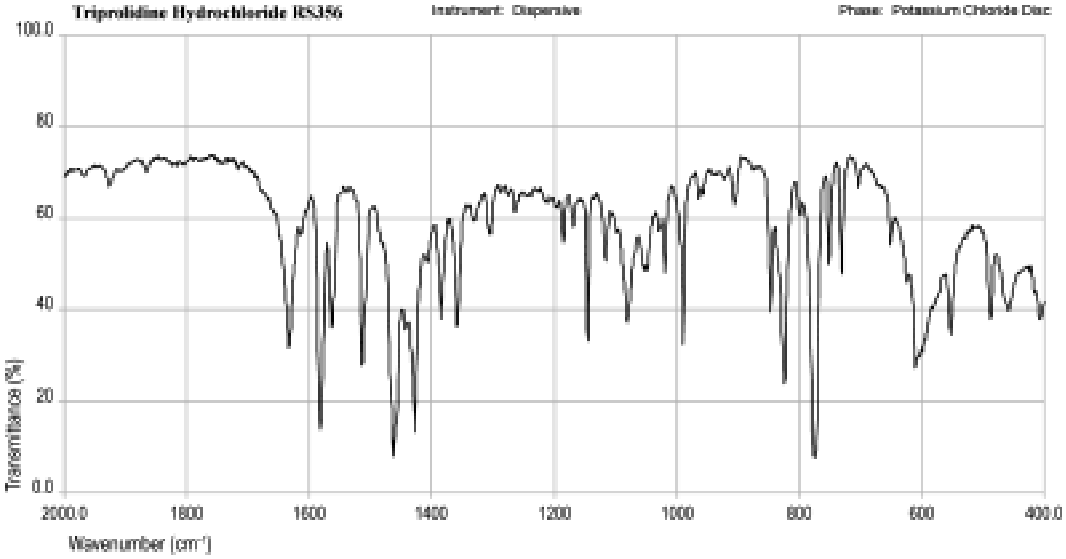
Trimethoprim



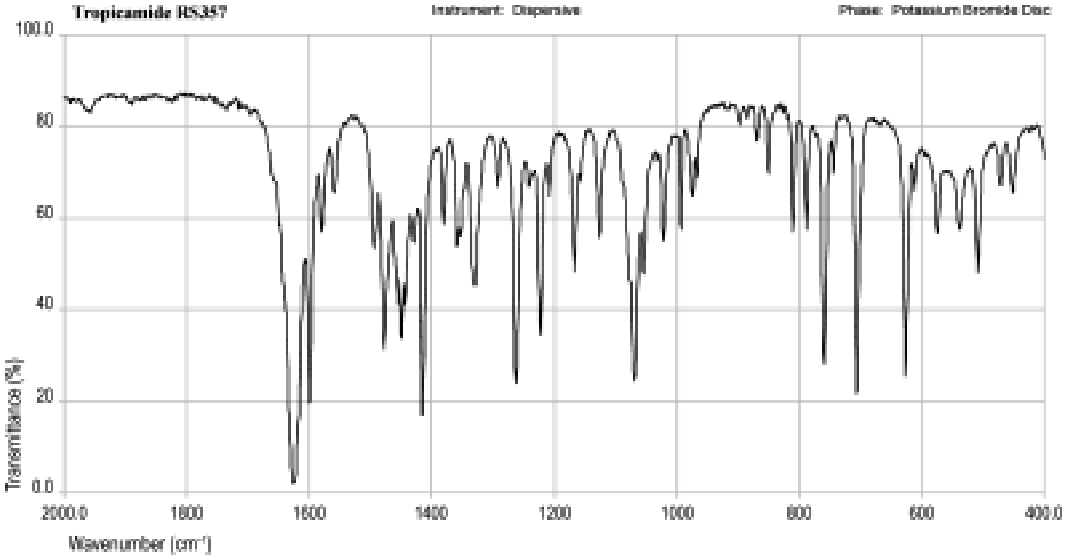
Trimipramine Maleate



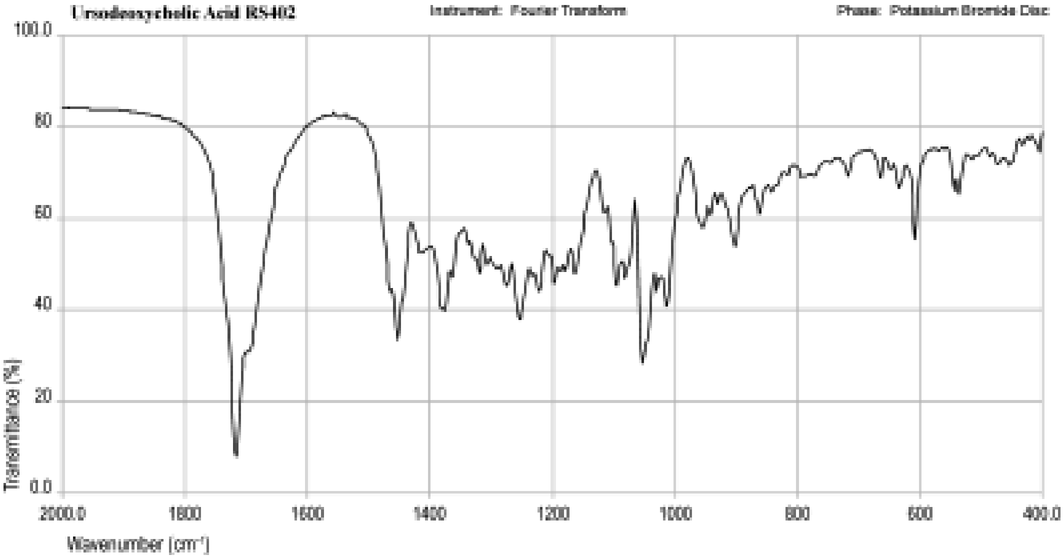
Triprolidine Hydrochloride



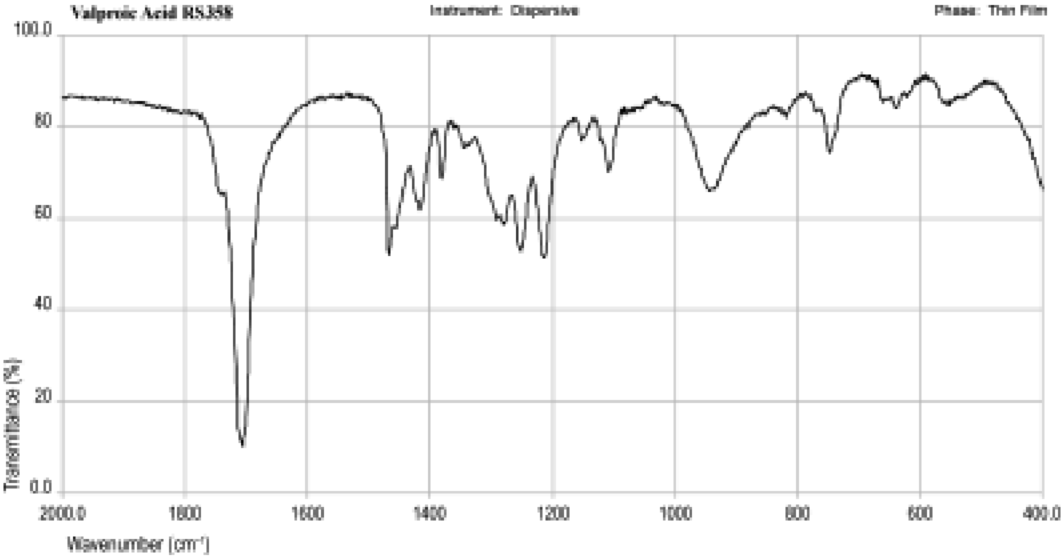
Tropicamide



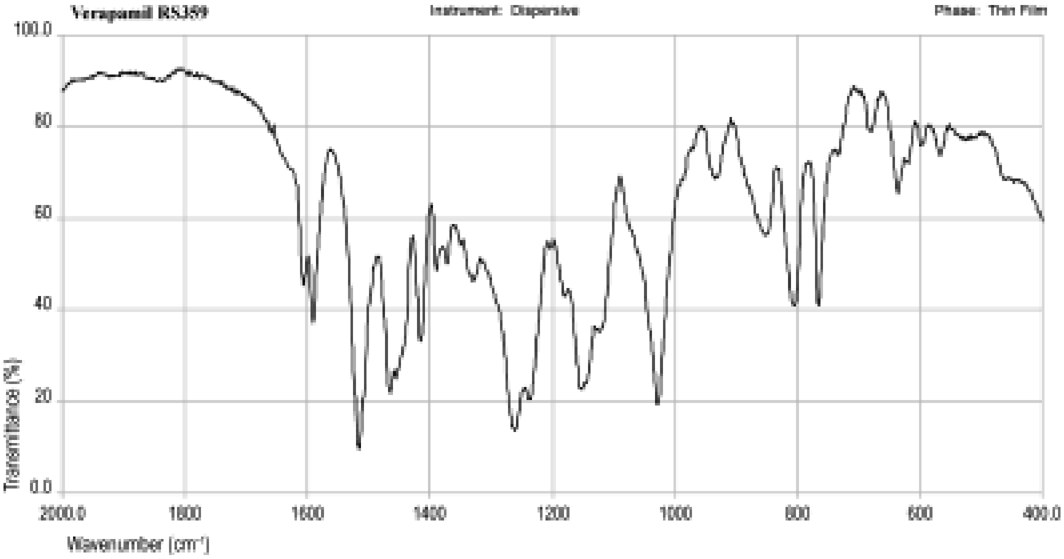
Ursodeoxycholic Acid



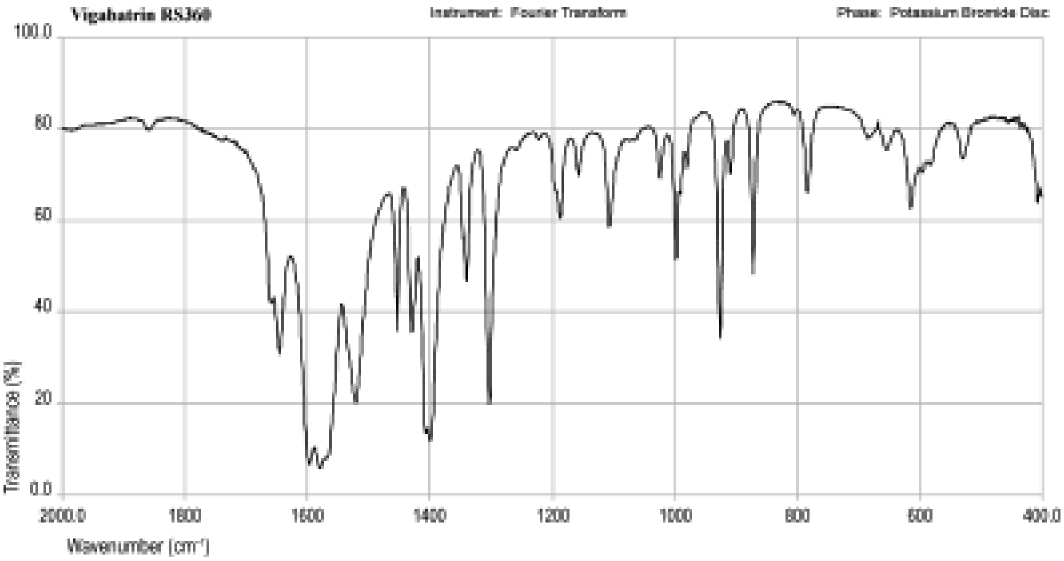
Valproic Acid



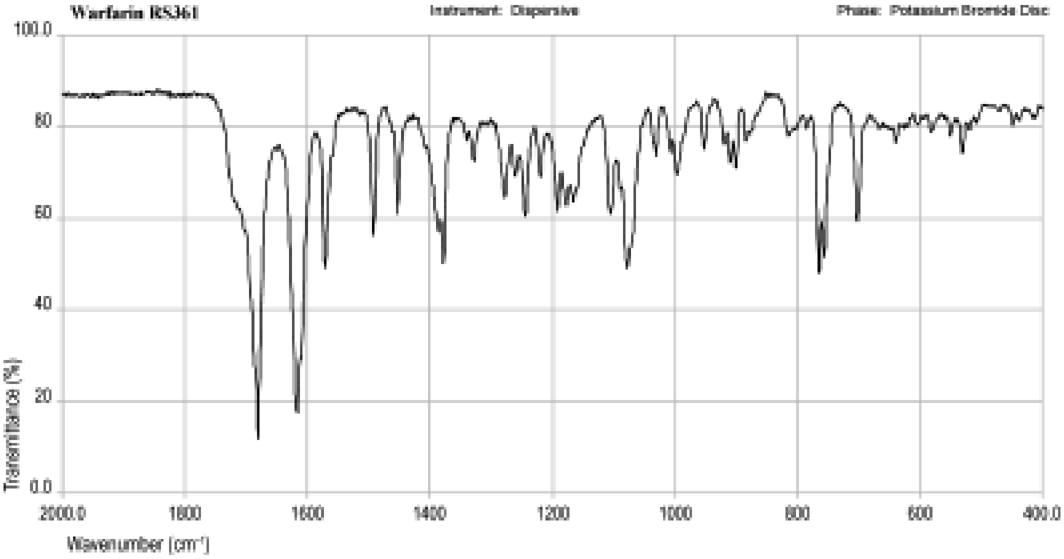
Verapamil


Verapamil RS359
Instrument: Dispersive
Phase: Thin Film
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

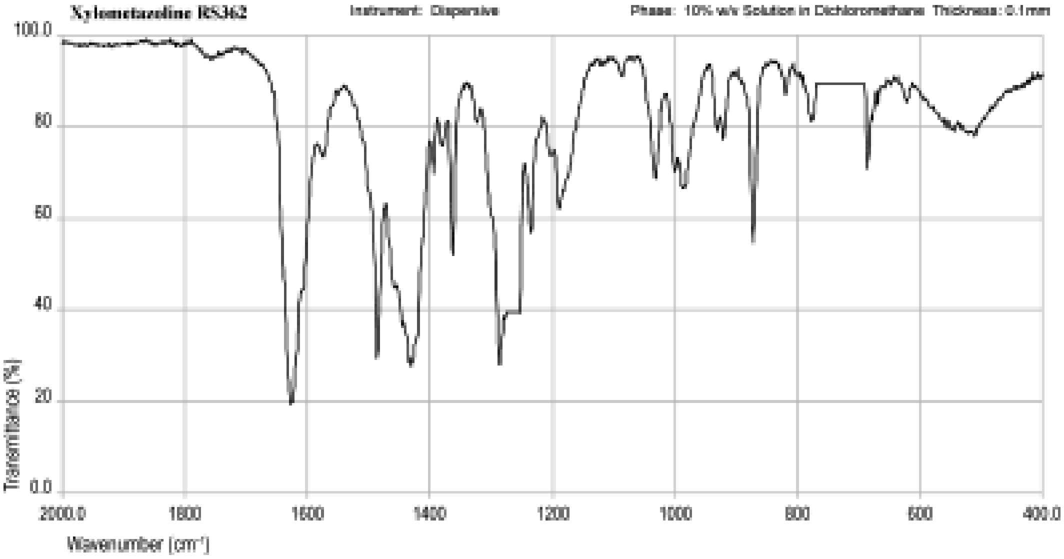
Vigabatrin


Vigabatrin RS360
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

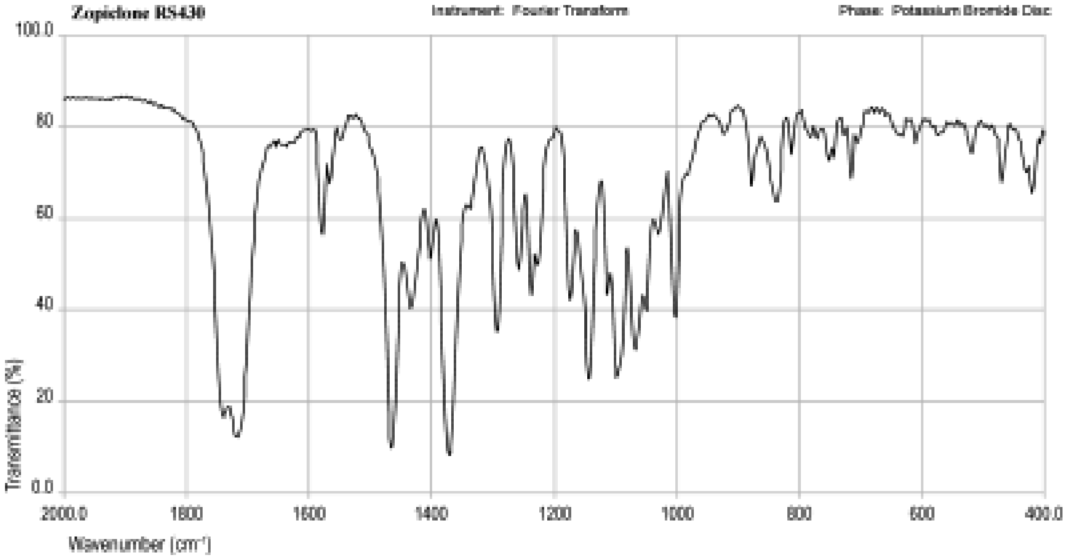
Warfarin


Warfarin RS361
Instrument: Dispersive
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

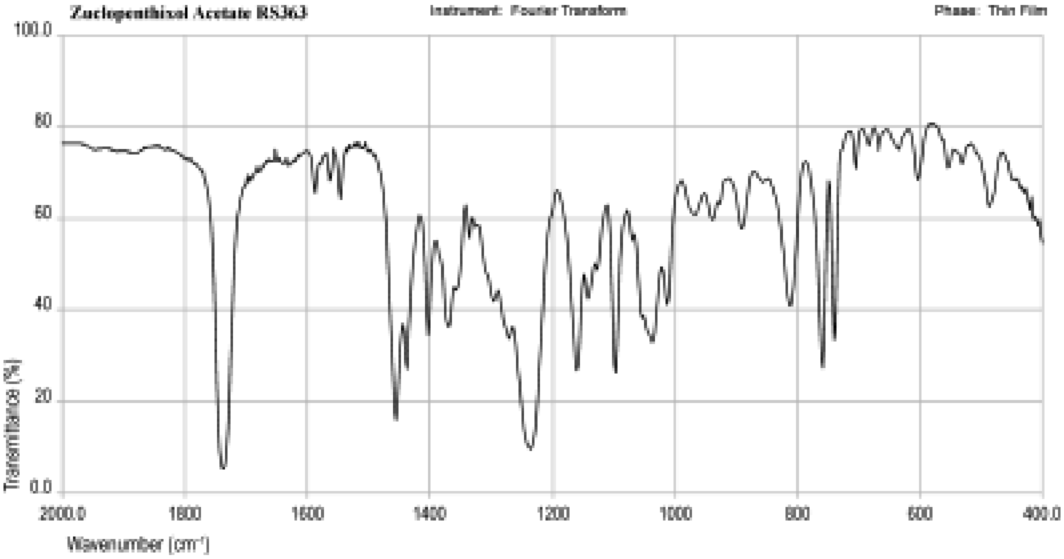
Xylometazoline



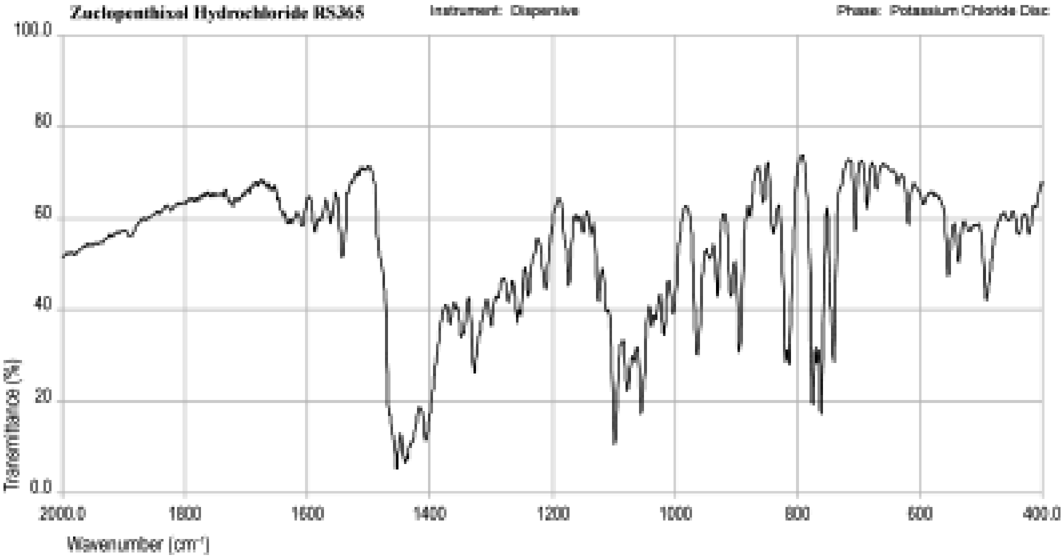
Zopiclone


Zopiclone RS430
Instrument: Fourier Transform
Phase: Potassium Bromide Disc
100.0
80
60
40
20
0.0
Transmittance (%)
2000.0
1800
1600
1400
1200
1000
800
600
400.0
Wavenumber (cm⁻¹)

Zuclopenthixol Acetate



Zuclopenthixol Hydrochloride



Dedicated

To My

Fellow pharmacists

Compiled by

John